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Computational identification of human targets of mitragynine – the main active compound of *Mitragyna speciosa*

Nipitpon Jaroenkit, Sirawit Ittisoponpisan*

Center for Genomics and Bioinformatics Research, Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla Thailand 90110

*Corresponding author, e-mail: sirawit.i@psu.ac.th

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ABSTRACT: Even though several studies have been conducted in attempts to find the potential medical applications of kratom (*Mitragyna speciosa*) extracts, the molecular understanding of its main active substance, mitragynine, remains to be further elucidated. In this study, we used bioinformatic approaches to identify putative protein targets of mitragynine and their binding associations. Human targets of mitragynine were identified using 2 methods: protein and drug similarity approaches. First, 155 homologous proteins of delta-opioid receptor were obtained through a BLASTP search. Second, 12 drugs similar to mitragynine were identified, many of which are used for treating hypertension and cognitive and psychotic disorders. From both approaches, the protein targets with available 3D structures were verified using docking simulations. Out of 48 candidates (39 from BLASTP search and 9 from drug similarity approach), 10 are known in the literature while the rest require further *in vitro* investigation. Examples of these targets are orexin/hypocretin receptor type 1, 5-hydroxytryptamine receptor 1B, and orexin receptor type 2, which had very strong predicted binding affinity of −9.3, −9.2, and −9.0 kcal/mol, respectively. Moreover, docking simulations suggest that mitragynine may replace commercial drugs in most of the receptor binding pockets, highlighting its potential application in drug repositioning. This *in silico* study provides insights into the molecular mechanism of mitragynine, which can help inform clinical researchers in developing safe and effective medical applications of kratom.

KEYWORDS: kratom, mitragynine, molecular, drug repurposing, homology

INTRODUCTION

Kratom (*Mitragyna speciosa*) is a plant in the coffee family (Rubiaceae) native to Southeast Asia and Thailand. It is regarded as a traditional medicinal plant and has been commonly used by locals in the south of Thailand mainly for relieving pain and stress [1, 2] as well as other conditions such as diarrhoea, abdominal pain, and cough [3]. Traditionally, kratom was used to treat diabetes and high blood pressure, despite very little scientific evidence to support such therapeutic properties [2, 4]. Some studies in rats suggested that intaking kratom extract could decrease appetite and suppress weight gain [5, 6].

The use of kratom for recreational purposes in Thailand has been a controversial issue for many decades due to the potential risk of addiction. As a result, the plant was banned in 1943 [7]. However, in recent years, the Thai government has revisited this decision in order to decriminalise the use of kratom in local communities as well as to comply with international protocols, culminating in its official legalisation in 2021 [8, 9]. This policy change has created an opportunity for further research to be conducted on its potential medical uses. Therefore, a comprehensive understanding of the mechanism of active compounds in kratom could potentially reveal its therapeutic efficacy and ensure its safe use.

Despite surveys and behavioural studies being conducted, there are limited molecular studies on kratom extracts, especially on mitragynine (chemically known as 9-methoxy-corynanthidine), the most abundant pharmacologically active compound. It has been shown that alkaloid extracts from kratom leaves exhibit opioid-like analgesic effects upon ingestion [3, 10]. Mitragynine is primarily known for its affinity to opioid receptors, including delta-, kappa-, and mu-types. Meanwhile, 7-hydroxymitragynine (which is found in kratom leaves at much lower concentrations) has a much stronger affinity to the mu-type opioid receptor (Fig. 1) [11].

The active compounds of kratom, particularly mitragynine and 7-hydroxymitragynine, are believed to be able to bind to multiple targets that are structurally related to the opioid receptors. Additionally, derivatives and analogues of mitragynine have been developed and demonstrated for their ability to activate opioid receptors, indicating potential therapeutic applications [12, 13]. However, apart from the known opioid receptors, further investigations are necessary to identify other possible protein targets and the binding associations between those targets and the active substances of kratom. Previous studies have demonstrated that kratom extract can bind to several receptors, including D1 dopamine [14], D2 dopamine, serotonin (5-HT2C and 5-HT7), and alpha-2 adrenergic receptors [15]. All of these receptors are also known as targets for antipsychotic drugs, which suggests possible indications of kratom extracts as antipsychotics and antidepressants [15]. An *in silico* study

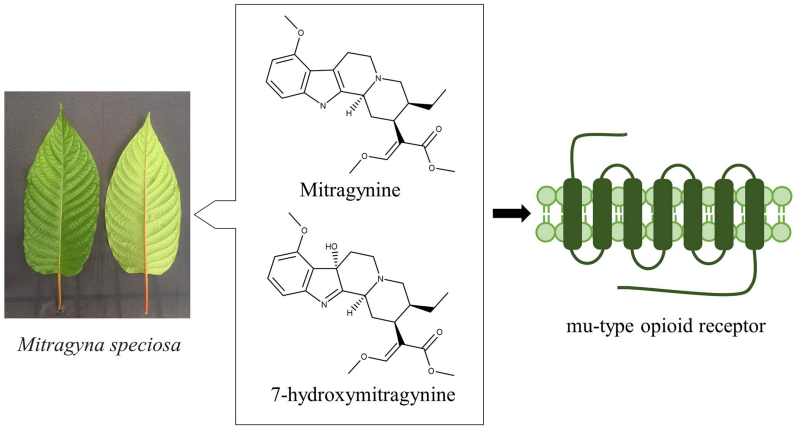

Fig. 1 Kratom (*Mitragyna speciosa*) and two of its main active compounds, mitragynine and 7-hydroxymitragynine. Both alkaloids have been known to have an affinity for mu-type opioid receptor.

showed that mitragynine and 7-hydroxymitragynine could be involved in several metabolic pathways [16]. Another recent study conducted through *in silico* virtual screening, docking simulation, and verification *in vitro* suggested that kratom extract had the potential to inhibit acetylcholinesterase (AChE), rendering itself an alternative drug candidate for treating Alzheimer's patients [17]. Thus, identification of the specific targets of kratom extracts could lead to the development of more effective and targeted therapies.

It has been well established that enzymes within the same family or with homologous structures can share highly similar 3D conformations, particularly in their active sites which are conserved and crucial for enzymatic activities [18, 19]. Therefore, identification of homologous proteins of opioid receptors (the main targets of mitragynine) through docking simulations could lead to the discovery of new potential protein targets for the active substances. Additionally, it has been suggested that structurally similar drugs could be repurposed for their new indications [20]. Thus, by identifying commercial drugs that share similar structures to mitragynine, some new pharmaceutical properties of kratom could be discovered.

In this paper, we aim to explore the binding targets of mitragynine, the main active compound of kratom extract, as well as its putative therapeutic properties using protein homology and drug similarity concepts. The identification of putative targets was conducted through a BLASTP search, and the binding associations between mitragynine and the candidate targets were predicted through molecular docking. Moreover, the structural comparisons of mitragynine with other commercial drugs found in an online database were performed to identify potential indications for these active compounds. Computational analysis of mitragynine could provide deeper insights into its structural and molecular basis, leading to proper medical applications of kratom and promoting the effective treatment of disease using local medicinal plants.

MATERIALS AND METHODS

The flow diagram depicting the process of selection and screening is presented in Fig. 2 with details provided in the following subsections and in the result section.

Retrieval of mitragynine and drug structures

We retrieved the mitragynine structure in SDF format from PubChem (PubChem ID: 3034396). Additionally, in order to determine drugs that are structurally similar to mitragynine, 11,172 drug structures in SDF format were retrieved from DrugBank online database [21].

Identification of homologous proteins of the mitragynine receptor

Mitragynine is best known for its binding affinity for delta-, kappa-, and mu-type opioid receptors. However, at the time of this study, the delta-type opioid receptor is the only human receptor whose 3D structure is available in high quality on Protein Data Bank (PDB) (ID: 4N6H, resolution: 1.80 Å). As the focus of this study is to structurally analyse the protein-ligand interaction, therefore, to identify putative homologous protein receptors, the FASTA sequence of the delta-type opioid receptor was used in a BLASTP search against the Reference Proteins (refseq_protein) database. The search was limited to human proteins only (taxonomy id: 9606), and all other parameters were kept at their default settings (0.05 for the E-value threshold and BLOSUM62 for the scoring matrix). If multiple isoforms were identified by BLASTP, only the first isoform would be chosen. Identified homologous proteins of which their full PDB structures were available were

then used in docking simulation to confirm their interactions with mitragynine. Partially available structures were excluded from the study. If there were multiple structures representing the same protein, the structure that had the best resolution would be selected.

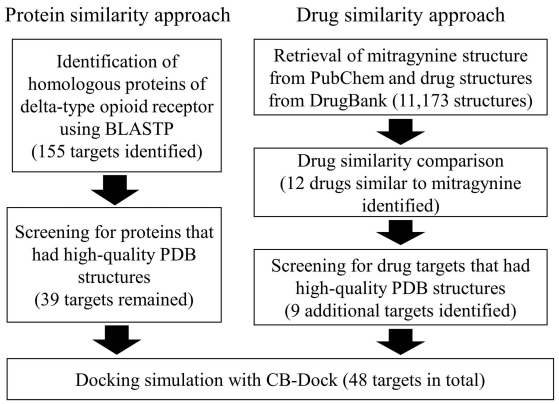

Fig. 2 The overall workflow of this study.

Docking simulation

We used CB-Dock, which was reported to have ∼70% accuracy [22], to simulate the docking between mitragynine (in SDF format) and its putative targets (in PDB format) identified through the BLASTP search. The CB-Dock algorithm begins by automatically scanning for the 5 largest surface pockets and will then attempt to dock the ligand into each identified pocket using the state-of-the-art AutoDock Vina algorithm [23]. DockRMSD web server [24]was also used to calculate the Root Mean Square Deviation (RMSD) between the poses of the docked ligand molecules and the original one found in crystal structures.

Drug similarity comparison

The similarities between the mitragynine and all the DrugBank structures were measured using Tanimoto coefficient, which ranges from 0 to 1 where 1 indicates an identical match between the 2 compounds [25]. The comparison was performed using Python programming in conjunction with the pre-defined script in the RDKIT python package (version 2021.09.01) (available at www.rdkit.org). The drugs that shared a high Tanimoto coefficient ($\geqslant 0.70$) were then selected for further analysis.

RESULTS AND DISCUSSION

Potential drug targets for mitragynine

By using the FASTA sequence of the delta-type opioid receptor as the query for the BLASTP search, we identified 155 human receptors that could be homologous to the delta-type opioid receptor (see Appendix: Supplementary data for the full list of proteins). The lowest identity was 22.83, yielded by neurotensin receptor type 1. Despite low sequence identity, the E-value of 1.00E-20 strongly suggests that this protein is still a homologue of the delta-type opioid receptor. However, among all the identified homologues, only 39 had their structures available in high quality (including the delta-type opioid receptor itself). The kappa-type opioid receptor was identified as the closest homologue of the delta-type receptor. Binding associations between mitragynine and 10 of its targets have been previously validated through experiments (Table 1). Notable confirmed targets include 5-hydroxytryptamine receptor 2A, 2B, 2C, adenosine receptor A2, alpha-2A, and alpha-2C adrenergic receptor. The other 29 receptors are novel mitragynine target candidates for further investigation.

Docking simulations of mitragynine and its potential receptors

CB-Dock was used to predict the binding between mitragynine and the 39 homologous receptors shown in Table 1. Some of the results with strong predicted vina scores are presented in Fig. 3 for further discussion. The vina scores when bound to delta-type and kappa-type opioid receptors were −6.9 kcal/mol and −8.4 kcal/mol, respectively (Fig. 3a,b). The predicted contacts between mitragynine and the opioid receptors were mostly hydrophobic interactions. It is worth noting that CB-Dock employs the NGL viewer as its default molecular viewer. In this viewer, hydrophobic interactions are defined as those between alkyl groups or between an alkyl group and a Pi group, within a 4 Å distance. H-bonds are displayed only when strong donor and acceptor atoms are within a 3.5 Å distance [26]. The oxygen atom in the methylester group of mitragynine was found to form an H-bond with the side chain of Y129 on delta-type opioid receptor (Fig. 3a). In the case of kappa-type opioid receptor, the 2 nitrogen atoms in mitragynine were responsible for forming H-bonds with the side chains of D138 and Y320 (Fig. 3b).

The receptor with the strongest binding to mitragynine was the 5-hydroxytryptamine receptor 2A (5-HT2A), exhibiting a binding affinity of −9.5 kcal/mol (Fig. 3c). 5-HT2A is known to be a target of numerous drugs [27] as well as serotonergic psychedelic drugs such as lysergic acid diethylamide (LSD) [28]. A study in mice showed that mitragynine acts as an antagonist blocking the stimulation of the 5-HT2A receptor [29]. Notably, our docking result showed that mitragynine made only hydrophobic interactions with the receptor at the binding site. The binding residues depicted in Fig. 3c are labelled in accordance with the binding site information from UniProt, and this labelling convention is maintained in subsequent figures where such information is available.

The D1 dopamine receptor displayed the sec-

Table 1 Proteins that are homologous to the delta-type opioid receptor and had PDB structures available. Targets that have been experimentally confirmed are marked with *.

Description	Per. ident	Accession	Vina score (Mit.)	Vina score (Lig.)	Cavity size (Å^3)	PDB	Res. (Å)	Ref.
*delta-type opioid receptor	100	NP_000902.3	–6.9	–11.5 (EJ4)	932	4N6H	1.80	[31]
*kappa-type opioid receptor	59.41	NP_000903.2	–8.4	–11.4 (JDC)	1842	4DJH	2.90	[31]
*5-hydroxytryptamine receptor 2A	24.26	NP_000612.1	–9.5	–9.9 (ZOT)	2634	6A94	2.90	[32]
*5-hydroxytryptamine receptor 2B	23.42	NP_000858.3	–8.1	–15.2 (ERM)	2384	4IB4	2.70	[33]
*5-hydroxytryptamine receptor 2C precursor	23.75	NP_000859.2	–8.3	–13.2 (E2J)	2992	6BQH	2.70	[15]
*adenosine receptor A2a	25.37	NP_000666.2	–7.8	–9.4 (ZMA)	919	5NM4	1.70	[34]
*alpha-2A adrenergic receptor	28.26	NP_000672.3	–7.7	–9.5 (E3F)	5686	6KUX	2.70	[35]
*alpha-2C adrenergic receptor	28.76	NP_000674.2	–7.6	–10.7 (E33)	7344	6KUW	2.80	[36]
*D1 dopamine receptor	23.57	NP_000785.1	–8.9	–8.9 (VFP)	839	7JOZ	3.80	[14]
*muscarinic acetylcholine receptor M5	26.91	NP_001307846.1	–8.2	–7.7 (0HK)	2479	6OL9	2.54	[37]
5-hydroxytryptamine receptor 1B	23.23	NP_000854.1	–9.2	–14.3 (ERM)	1643	4IAR	2.70	–
apelin receptor	28.99	NP_005152.1	–7.6	–	3061	5VBL	2.60	–
beta-2 adrenergic receptor	26.85	NP_000015.2	–7.6	–6.9 (JTZ)	6793	6PS2	2.40	–
C-C chemokine receptor type 2	28.48	NP_001116513.2	–7.6	–10.5 (73R)	1942	5T1A	2.81	–
C-C chemokine receptor type 5	27.56	NP_000570.1	–6.6	–	708	5UIW	2.20	–
C-C chemokine receptor type 7 precursor	30.93	NP_001829.1	–6.5	–8.1 (JLW)	2889	6QZH	2.10	–
C-C chemokine receptor type 9	27.87	NP_001373376.1	–8.8	–12.7 (79K)	1971	5LWE	2.80	–
C-X-C chemokine receptor type 4	26.88	NP_001008540.1	–7.8	–7.6 (ITD)	3199	3ODU	2.50	–
C5a anaphylatoxin chemotactic receptor 1	26.50	NP_001727.2	–6.9	–	219	6C1R	2.20	–
cysteinyl leukotriene receptor 1	28.47	NP_001269115.1	–8.1	–13.2 (ZLK)	2185	6RZ5	2.53	–
D3 dopamine receptor	25.34	NP_387512.3	–7.4	–8.2 (ETQ)	933	3PBL	2.89	–
endothelin receptor type B precursor	27.38	NP_000106.1	–7.2	–	295	6IGK	2.00	–
growth hormone secretagogue receptor type 1	25.60	NP_940799.1	–7.5	–7.3 (8QX)	1332	6KO5	3.30	–
leukotriene B4 receptor 1	28.37	NP_001137391.1	–7.9	–9.8 (VRJ)	2173	7K15	2.88	–
melatonin receptor type 1A	27.86	NP_005949.1	–3.6	–8.7 (JEV)	1241	6ME2	2.80	–
melatonin receptor type 1B	25.38	NP_005950.1	–5.1	–10.0 (JEY)	2556	6ME6	2.80	–
N-formyl peptide receptor 2	29.28	NP_001005738.1	–7.9	–	71351	6LW5	2.80	–
neuropeptide Y receptor type 1	29.04	NP_000900.1	–8.8	–11.8 (9AO)	2290	5ZBQ	2.70	–
nociceptin receptor	57.05	NP_000904.1	–6.7	–8.1 (DGV)	4089	5DHG	3.00	–
orexin receptor type 2	27.12	NP_001371201.1	–9.0	–9.0 (7MA)	1169	5WQC	1.96	–
orexin/hypocretin receptor type 1	24.86	NP_001516.2	–9.3	–9.4 (7MA)	4840	6TOD	2.11	–
oxytocin receptor	26.21	NP_000907.2	–7.7	–9.4 (NU2)	503	6TPK	3.20	–
P2Y purinoceptor 1	27.90	NP_002554.1	–7.4	–8.3 (BUR)	1564	4XNV	2.20	–
prostaglandin D2 receptor 2	31.63	NP_004769.2	–8.5	–10.2 (FT4)	1911	6D27	2.74	–
proteinase-activated receptor 1 precursor	26.83	NP_001983.2	–6.7	–14.5 (VPX)	3601	3VW7	2.20	–
proteinase-activated receptor 2	27.65	XP_016864712.1	–7.5	–6.5 (8TZ)	934	5NDD	2.80	–
substance-P receptor	29.07	NP_001049.1	–7.6	–12.0 (GAW)	1421	6HLP	2.20	–
type-1 angiotensin II receptor	29.97	NP_000676.1	–7.1	–	544	6OS2	2.70	–
type-2 angiotensin II receptor	31.29	NP_000677.2	–7.6	–11.7 (8ES)	11496	5UNF	2.80	–

Per. ident: percent identity between delta-type opioid receptor and the protein structure under investigation. Vina score (Mit.): docking score of mitragynine; Vina score (Lig.): docking score of original ligands found in the PDB structure (kcal/mol). Res.: resolution of the PDB structure. Ref.: references of experimental studies.

ond strongest binding affinity with mitragynine at −8.9 kcal/mol (Fig. 3d). This receptor plays a role in regulating memory, learning, and neuronal growth. Some drugs in pre-clinical models and clinical trials as selective D1 agonists were found to counteract Parkinson's symptoms in humans [30]. While most of the contacts were hydrophobic interactions, CB-Dock predicted that the methyl ester group on C16 of mitragynine forms H-bonds with the side chains of S189 and N292 as well as the main chain of L190 in the D1 dopamine receptor. Although the binding could be confirmed both *in silico* (this study) and *in vitro* [14], more studies are still needed to elucidate the binding effects.

To confirm the accuracy of the docking simulations, we also re-docked the original ligands present in the PDB structures and observed that all these ligands could be predicted to bind to their respective binding pockets with high affinity. Nearly half of them displayed scores ranging between −8 and −11 kcal/mol. The RMSD between the re-docked EJ4 and its original coordinates in the crystal structure of the delta-type opioid receptor was 0.184 Å, indicating a very good alignment with the original crystal structure.

While most ligands had binding scores relatively stronger than mitragynine, exceptions were observed for the D1 dopamine receptor and the muscarinic acetylcholine receptor M5. Notably, the ligand ergo-

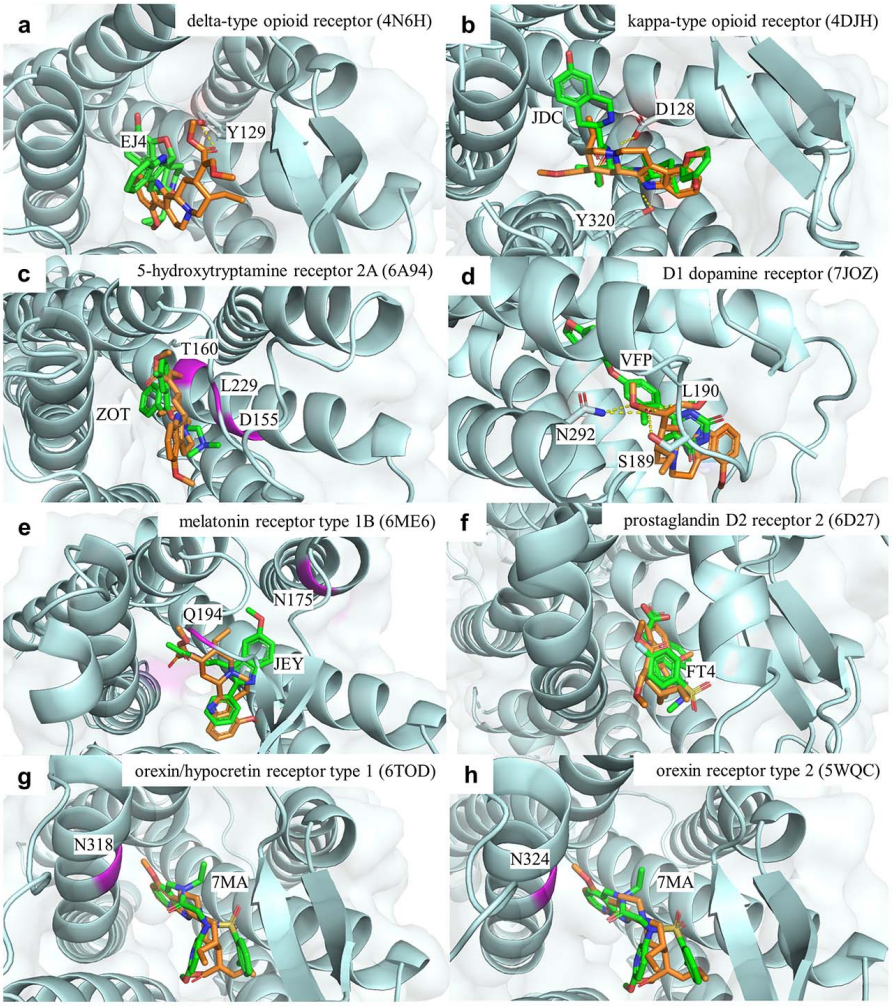

Fig. 3 Docking results for (a) delta-type opioid receptor (PDB: 4N6H), (b) kappa-type opioid receptor (PDB: 4DJH), (c) 5-hydroxytryptamine receptor 2A (PDB: 6A94), (d) D1 dopamine receptor (PDB: 7JOZ), (e) melatonin receptor type 1B (PDB:@ 6ME6), (f) prostaglandin D2 receptor 2 (PDB: 6D27), (g) orexin/hypocretin receptor type 1 (PDB: 6TOD), and (h) orexin receptor type 2 (PDB: 5WQC). Mitragynine molecules are depicted in orange, and ligands from the original PDB files are shown in green. H-bonds (where discussed) are represented by yellow dashes, and ligand binding residues, as identified from UniProt, are highlighted in magenta.

tamine (ERM) had the strongest binding affinity for the 5-hydroxytryptamine receptor 2B (−15.2 kcal/-mol). However, it is important to understand that the docking scores are influenced not only by the molecular complementarity but also by the size of the docked ligand. For instance, while the molecular weight of mitragynine is similar to that of VFP, the original ligand found in the D1 dopamine receptor structure (398 g/mol vs. 363 g/mol), leading to similar binding scores, it is significantly smaller than ergotamine (582 g/mol). Consequently, larger ligands might produce more negative binding scores compared to their smaller counterparts.

In our docking simulations across the 29 potential targets, a wide range of vina scores could be observed from −3.6 kcal/mol (for melatonin receptor type 1A) to −9.3 kcal/mol (for orexin/hypocretin receptor type 1). 5-hydroxytryptamine receptor 1B (5-HT1B) was the only 5-HT receptor that had not been experimentally proven for its affinity to mitragynine. Given its predicted binding affinity of −9.2 kcal/mol and considering that all other proteins within the same family have been confirmed for their binding associations with mitragynine, it is likely that 5-HT1B might also bind with mitragynine. Hence, further studies should be conducted *in vitro* to validate this finding.

Notable potential binding targets that are worth further investigation include melatonin receptor type 1B, prostaglandin D2 receptor 2, orexin/hypocretin receptor type 1, and orexin receptor type 2. The

docking simulation between mitragynine and melatonin receptor type 1B yielded a predicted vina score of −5.1 kcal/mol (Fig. 3e). Melatonin receptor is known to be responsible for sleep promotion and the synchrony of biological clocks [38]. Apart from being targeted by melatonin, the type 1B receptor is also a target of many other drugs such as ramelteon, agomelatine, and tasimelteon – receptor agonists designed for treating sleep disorders [39]. The predicted binding affinity between mitragynine and the melatonin receptor type 1B warrants further investigation conducted to confirm the effects of mitragynine on sleep mediation.

Prostaglandin D2 receptor 2 yielded a vina score of −8.5 kcal/mol (Fig. 3f). The activation of the prostaglandin D2 receptor is known for promoting inflammatory pathways. A previous study reported that mitragynine inhibits the mRNA expression of cyclooxygenase-2 (COX-2), also known as prostaglandin-endoperoxide synthase 2 (PTGS2). This suppression subsequently results in reduced prostaglandin E2 (PGE2) production [40]. When PGE2 binds to prostaglandin E2 receptor, it further exacerbates inflammation. The successful docking prediction of mitragynine with the prostaglandin D2 receptor (a homolog of prostaglandin E2 receptor) supports the hypothesis that mitragynine can play a role in inflammatory control by interfering with the interaction between the prostaglandin D2 receptor and its corresponding activator.

Orexin/hypocretin receptor type 1 yielded a very strong binding affinity (−9.3 kcal/mol) (Fig. 3g). Similarly, orexin receptor type 2 also exhibited a similar affinity of −9.0 kcal/mol (Fig. 3h). Drugs targeting these receptors are commonly used to address sleep disorders. The agonist binding to the orexin receptor results in elevated intracellular calcium levels, while its antagonism has been associated with the treatment of insomnia [41]. Although the exact nature of mitragynine interaction – whether agonistic or antagonistic – with this protein remains to be elucidated from docking simulations, these findings hint at a potential role for mitragynine in managing sleep disorders.

Drug similarity and reposition analysis

Even though mitragynine was not listed as a drug molecule on DrugBank, there were 12 drugs (among all drug structures in DrugBank analysed) that yielded high Tanimoto coefficients ($\geqslant 0.70$) (Table 2). All these drugs can be categorised as alkaloids. Among these, raubasine, also known as ajmalicine and delta-yohimbine, has a very high coefficient of 0.86. It is an alkaloid found naturally in various plants, including kratom but in very low concentration [42]. This alkaloid is used as an antihypertensive drug for treating high blood pressure [43]. This underscores the potential of mitragynine, a naturally occurring alkaloid, in offering unique structural properties that may have therapeutic implications.

From the 12 drugs identified, we surveyed their drug target information from both the DrugBank and the NCATS Inxight Drugs database (https://drugs.ncats.io/) to find potential proteins that might serve as novel targets for mitragynine. Drug targets that had high-quality X-ray structures available were used for docking simulations. Target structures that were available in small fractions were excluded. Table 3 lists the drug targets that met our screening criteria, their percent identities with the delta-type opioid receptor, and their vina scores from docking simulations with 1) their intended drugs and 2) mitragynine. From this approach, 9 additional potential mitragynine targets were identified (shown in bold). It can be noted that many drugs share the same targets and many of those targets are also listed in Table 1 as potential targets for mitragynine. This could be explained by the fact that similar ligands tend to bind to similar binding pockets, which tend to be conserved among related proteins. However, it has been demonstrated that in some cases unrelated proteins can share similar binding pockets too [44].

Raubasine targets both the alpha-2A and alpha-2C adrenergic receptors, and both receptors have been confirmed to have affinities for mitragynine. The docking simulation confirmed that both mitragynine and raubasine could bind to the same binding sites of both alpha-2A (Fig. 4a) and alpha-2C adrenergic receptors (Fig. 4b), suggesting that they could share related pharmacological properties.

In general, the binding energies yielded by mitragynine were about 1 kcal/mol weaker than those of the original drugs. However, a notable exception was observed in the docking simulation between mitragynine and the 5-hydroxytryptamine receptor 2A (5-HT2A), as depicted in Fig. 4c. In this simulation, mitragynine yielded −9.5 kcal/mol, stronger than that of the drug rescinnamine (−8.6 kcal/mol). According to DrugBank, rescinnamine is an alkaloid that can inhibit angiotensin-converting enzyme and is used as an antihypertensive drug [45]. Given these findings, there is a potential implication that mitragynine might be explored for its therapeutic benefits in managing high blood pressure.

Another type of receptor that could yield strong binding associations with mitragynine is the dopamine receptors. This finding is consistent with our previous receptor identification method using a homology approach with BLASTP. Fig. 4d illustrates an example docking interaction between the D1 dopamine receptor and mitragynine. Notably, mitragynine appears to bind to the same site as rescinamine with vina scores of −8.9 kcal/mol for mitragynine and −9.6 kcal/mol for rescinamine. Information from DrugBank and the NCATS Inxight Drugs database indicates that dopamine receptors are targeted by yohimbine (specif-

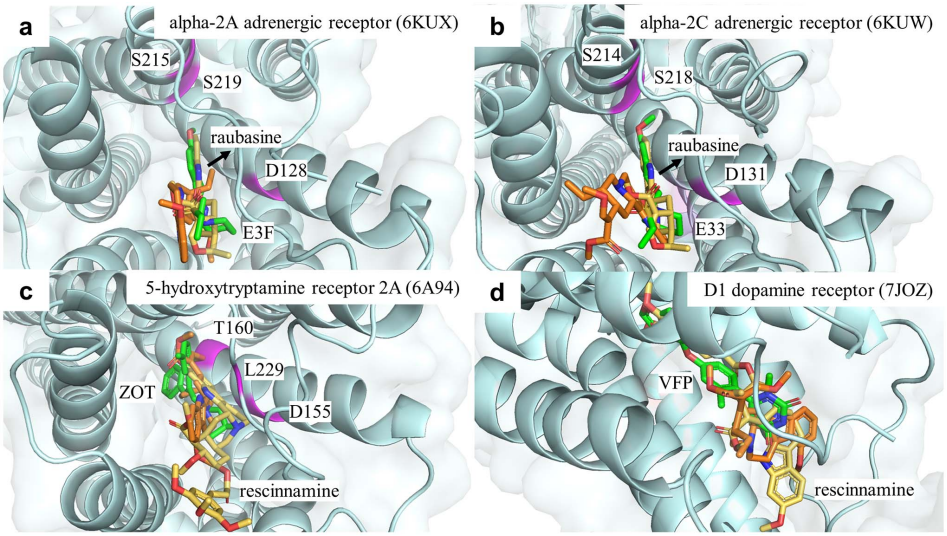

Fig. 4 Docking results for (a) alpha-2A adrenergic receptor (PDB: 6KUX), (b) alpha-2C adrenergic receptor (PDB: 6KUW), (c) 5-hydroxytryptamine receptor 2A (PDB: 6A94), and (d) D1 dopamine receptor (PDB: 7JOZ). Mitragynine molecules are depicted in orange, the drug molecules are in yellow, and the ligands from the original PDB files are in green. Ligand binding residues, as identified from UniProt, are highlighted in magenta.

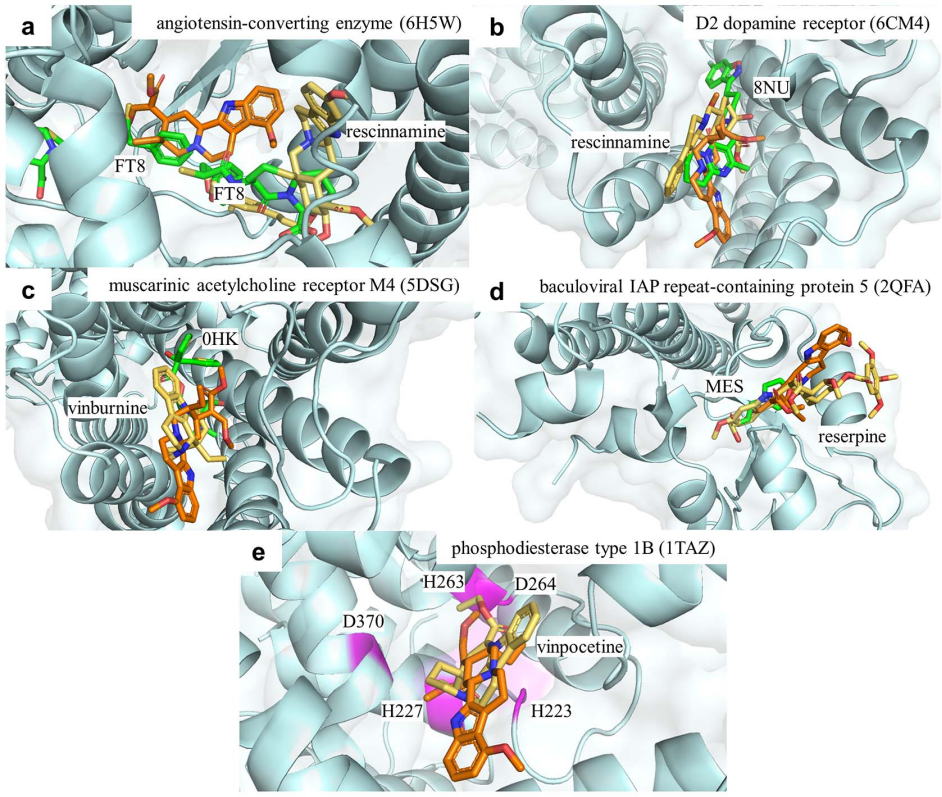

Fig. 5 Docking results for (a) angiotensin-converting enzyme (PDB: 6H5W), (b) D2 dopamine receptor (PDB: 6CM4), (c) muscarinic acetylcholine receptor M4 (PDB: 5DSG), (d) baculoviral IAP repeat-containing protein (PDB: 2QFA), and (e) phosphodiesterase type 1B (PDB: 1TAZ). Mitragynine molecules are depicted in orange. Drug molecules are shown in yellow, and ligands from the original PDB files are represented in green. Ligand binding residues, as identified from UniProt, are highlighted in magenta.

Table 2 Drugs that are similar to mitragynine (with Tanimoto coefficient ⩾ 0.70).

Drug name	DrugBank ID	Tanimoto coefficient	Main condition from Inxight Drugs
raubasine	DB15949	0.86	hypertension (approved) cognitive disorder (phase I)
metoserpate	DB11530	0.81	a sedative drug used in veterinary
rescinnamine	DB01180	0.79	hypertension (approved)
yohimbine	DB01392	0.79	erectile dysfunction (approved)
reserpine	DB00206	0.77	hypertension (approved) agitated psychotic state (approved)
methoserpidine	DB13631	0.76	used in the 1960s as an antihypertensive drug
deserpidine	DB01089	0.75	for treating hypertension and psychotic disorder
bietaserpine	DB13575	0.73	hypertension (approved)
(7as,12ar,12bs)-1,2,3,4,7a,12,12a,12b-octahydroindolo [2,3-a]quinolizin-7(6h)-one	DB02191	0.71	N/A
vinburnine	DB13793	0.71	Cerebrovascular disease (approved)
vinpocetine	DB12131	0.70	Vascular dementia (approved) Epilepsy (phase II) Acute ischemic stroke (phase III)
vincamine	DB13374	0.70	Cerebrovascular insufficiency (approved)

Table 3 Potential mitragynine targets and their docking scores.

Drug name	DrugBank ID	Tanimoto coefficient	Target name	Per. ident	PDB ID	Res. (Å)	Vina score (kcal/mol) (drug, mitragynine)
raubasine	DB15949	0.86	alpha-2A adrenergic receptor[b]	28.26	6KUX	2.70	–9.9, –7.7
			alpha-2C adrenergic receptor[b]	28.76	6KUW	2.80	–9.3, –7.6
rescinnamine	DB01180	0.79	5-hydroxytryptamine receptor 1B[b]	23.23	4IAR	2.70	–10.4, –9.2
			5-hydroxytryptamine receptor 2A[b]	24.26	6A94	2.90	–8.6, –9.5
			5-hydroxytryptamine receptor 2B[b]	23.42	4IB4	2.70	–9.9, –8.1
			5-hydroxytryptamine receptor 2C[b]	23.75	6BQH	2.70	–9.4, –8.3
			angiotensin-converting enzyme[ac]	28.26	6H5W	1.37	–9.8, –8.2
			D1 dopamine receptor[b]	23.57	7JOZ	3.80	–9.6, –8.9
			D2 dopamine receptor[b]	27.40	6CM4	2.87	–9.2, –8.1
			D3 dopamine receptor[b]	25.34	3PBL	2.89	–8.6, –7.4
			D4 dopamine receptor[b]	38.27	5WIU	1.96	–10.5, –9.2
yohimbine	DB01392	0.79	5-hydroxytryptamine receptor 1B	23.23	4IAR	2.70	–10.3, –9.2
			5-hydroxytryptamine receptor 2A[a]	24.26	6A94	2.90	–10.0, –9.5
			5-hydroxytryptamine receptor 2C[a]	23.75	6BQH	2.70	–9.0, –8.3
			5-hydroxytryptamine receptor 2B[a]	23.42	4IB4	2.70	–8.8, –8.1
			alpha-2A adrenergic receptor	28.26	6KUX	2.70	–8.6, –7.7
			alpha-2C adrenergic receptor	28.76	6KUW	2.80	–9.1, –7.6
			D2 dopamine receptor[a]	27.40	6CM4	2.87	–8.9, –8.1
			D3 dopamine receptor[a]	25.34	3PBL	2.89	–8.7, –7.4
reserpine	DB00206	0.77	baculoviral IAP repeat-containing protein 5[ac]	80.00	2QFA	1.40	–6.0, –5.4
vinburnine	DB13793	0.71	muscarinic acetylcholine receptor M1[b]	26.42	6WJC	2.55	–9.1, –7.4
			muscarinic acetylcholine receptor M2[b]	27.55	5ZKC	2.30	–9.5, –8.3
			muscarinic acetylcholine receptor M4[b]	27.83	5DSG	2.60	–9.3, –8.5
vinpocetine	DB12131	0.7	phosphodiesterase type 1B[bc]	23.21	1TAZ	1.77	–7.5, –7.1

[a] target found in DrugBank only. [b] target found in NCATS Inxight Drugs only. [c] target that had E-value > 0.05 upon pairwise alignment using BLASTP. Per. ident: percent identity between delta-type opioid receptor and the protein structure under investigation. Res.: resolution of the PDB structure.

ically D2 and D3) and rescinnamine (specifically D1, D2, D3, and D4). Docking simulations demonstrated that mitragynine interacts with these receptors in a manner comparable to that of yohimbine and rescinnamine.

New potential targets of mitragynine include angiotensin-converting enzyme (ACE) which had a low identity of 28.26% and a high E-value of 11 when compared to the delta-type opioid receptor. This explains why ACE was not detected in our initial BLASTP search. Interestingly, rescinnamine, an ACE-targeting drug, also interacts with several types of 5-hydroxytryptamine receptors and dopamine receptors, similarly to mitragynine. This could suggest the presence of similar binding pockets among these receptors. The docking result shows that mitragynine can bind to the same pocket as rescinnamine, although they did not align perfectly (Fig. 5a). This observation underscores the need for further *in vitro* verification. It is also worth noting that rescinnamine

targets the D2 and D4 dopamine receptors. These 2 dopamine receptors were not identified in our earlier BLASTP search due to lower query coverages and higher E-values. However, strong binding affinities were observed between mitragynine and these newly identified dopamine receptors. Docking simulations also supported these findings (an example is shown in Fig. 5b), suggesting that mitragynine might target multiple dopamine receptors.

We also identified muscarinic acetylcholine receptors M1, M2, and M4 as targeted by vinburnine – a drug for treating cerebrovascular disorders. These receptors are homologues of muscarinic acetylcholine receptor M5 which has been known to bind to mitragynine. As depicted in Fig. 5c, mitragynine can occupy the same binding pocket as vinburnine on muscarinic acetylcholine receptor M4. This suggests a need for further investigation into the interactions between mitragynine and various muscarinic acetylcholine receptors.

Two additional potential targets include baculoviral IAP repeat-containing protein 5 (BIRC5, targeted by reserpine) (Fig. 5d) and phosphodiesterase type 1B (PDE1B, targeted by vinpocetine) (Fig. 5e). When compared to the delta-type opioid receptor, both proteins exhibited very high E-values and much lower query coverages than other targets. Specifically, BIRC5 had an E-value of 176 and a query coverage of 4%, while E-value of PDE1B was 22 with a 29% query coverage. These metrics suggest a potential lack of evolutionary relationships with the delta-type opioid receptor. Notably, the high % identity observed for BIRC5 resulted from short sequence matches, spanning only 5–10 amino acid residues. Docking simulations for these proteins yielded relatively weaker vina scores compared to other receptors. However, for both proteins, the results suggest that mitragynine could bind to the same pockets as the respective targeting drugs. Thus, more research is needed to explore the potential benefit of mitragynine in the management of psychotic disorders and cerebrovascular diseases.

Overall, we have identified many potential new human protein targets for mitragynine, suggesting its potential therapeutic applications in various clinical conditions. Our approach primarily utilised docking simulations to evaluate the interaction between mitragynine and selected proteins. The binding affinity scores obtained from the simulations served as preliminary indicators, guiding us towards potential targets warranting further investigation. However, it should be noted that these docking simulations do not account for the dynamic interactions inherent in protein-ligand complexes. To enhance the reliability of our binding affinity scores, future work could incorporate molecular dynamics simulations. Such an approach would offer insights into conformational changes and stability of the protein-ligand complex, providing a more comprehensive understanding of their interaction dynamics. This dynamic analysis remains a limitation of our current study. To truly understand and validate the effects of mitragynine, it is imperative to complement our *in silico* findings with *in vitro* studies.

CONCLUSION

In this study, we utilized bioinformatic approaches to delve into the molecular and pharmaceutical properties of mitragynine. Our research has pinpointed several potential targets for mitragynine with many being substantiated through docking simulations. These targets play important roles in medical conditions such as cognitive disorders, sleep disturbances, and inflammation. Notable targets that exhibited strong binding affinity include 5-hydroxytryptamine receptors, dopamine receptors, and orexin receptors. Furthermore, our study revealed that mitragynine shares high similarity with drugs designed for hypertension and cognitive disorders, suggesting its potential for managing these conditions. Overall, our *in silico* study provides further evidence supporting the potential medical benefits of kratom and highlights its prospective utility in the field of medicine.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at: https://doi.org/10.7910/DVN/644ZH4.

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