

Dopamine docking studies of biologically active metabolites from *Curcuma longa* L.

Estudos de docagem molecular de dopamina de metabólitos biologicamente ativos de *Curcuma longa* L.

Estudios de acoplamiento de dopamina de metabolitos biológicamente activos de *Curcuma longa* L.

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Abstract

The dopaminergic system is involved in a wide range of neuropsychiatric and neurodegenerative disorders. The lack of receptor subtype specificity is related to several pharmacological side effects that are observed during therapy among parkinsonian and schizophrenic patients. It is of paramount importance to search for new compounds that act on dopamine receptors with therapeutic potential, higher clinical effectiveness, and fewer adverse effects. In the present study, we performed a molecular docking study of D₂, D₃, and D₄ receptor interactions with 92 metabolites from *Curcuma longa* using an *in silico* approach. We sought to identify compounds for possible drug development. A virtual library of compounds was built using molecules that were identified in the phytochemical characterization of *C. longa*. Protocols that were validated by redocking were applied to a virtual scan of this library using the Autodock-

v4.2.3, Autodock Vina, and Molegro-v6.0 Virtual Docker programs, with four repetitions each. The three-dimensional structures of D₂, D₃, and D₄ receptors in complex with risperidone, eticlopride, and nemonapride were obtained from the Protein Data Bank. Four compounds—stigmasterol, β -sitosterol, cholest-5-en-3-one, and cholestan-3-ol,2-methylene-(3 β , 5 α)—were the most likely to bind D₂, D₃, and D₄ dopamine receptors, suggesting their potential for possible drug development.

Keywords: Stigmasterol; β -sitosterol; Cholest-5-en-3-one; Cholestan-3-ol,2-methylene- (3 β , 5 α); Dopamine receptors; *Curcuma longa*.

Resumo

O sistema dopaminérgico está envolvido em uma ampla gama de doenças neuropsiquiátricas e neurodegenerativas. A falta de especificidade do subtipo de receptor está relacionada a vários efeitos colaterais farmacológicos que são observados durante a terapia entre pacientes parkinsonianos e esquizofrênicos. É de suma importância a busca por novos compostos que atuem nos receptores da dopamina com potencial terapêutico, maior eficácia clínica e menor número de efeitos adversos. No presente estudo, realizamos um estudo de docagem molecular das interações dos receptores D₂, D₃ e D₄ com 92 metabólitos da *Curcuma longa* usando uma abordagem *in silico*. Procuramos identificar compostos para possível desenvolvimento de drogas. Uma biblioteca virtual de compostos foi construída a partir de moléculas que foram identificadas na caracterização fitoquímica de *C. longa*. Os protocolos que foram validados por redocagem e foram aplicados a uma varredura virtual dessa biblioteca usando os programas Autodock-v4.2.3, Autodock Vina e Molegro-v6.0 Virtual Docker, com quatro repetições cada. As estruturas tridimensionais dos receptores D₂, D₃ e D₄ em complexo com risperidona, eticloprida e nemonaprida foram obtidas no Protein Data Bank. Quatro compostos - estigmasterol, β -sitosterol, colest-5-en-3-ona e colestán-3-ol, 2-metileno- (3 β , 5 α) - foram os mais propensos a se ligar aos receptores de dopamina D₂, D₃ e D₄, sugerindo seu potencial para possível desenvolvimento de drogas.

Palavras-chave: Estigmasterol; β -sitosterol; Colest-5-en-3-ona; Colestán-3-ol, 2-metileno- (3 β , 5 α); Receptores de dopamina, *Curcuma longa*.

Resumen

El sistema dopaminérgico está involucrado en una amplia gama de trastornos neuropsiquiátricos y neurodegenerativos. La falta de especificidad del subtipo de receptor está relacionada con varios efectos secundarios farmacológicos que se observan durante la terapia entre pacientes parkinsonianos y esquizofrênicos. Es de suma importancia buscar nuevos compuestos que actúen sobre los receptores de dopamina con potencial terapéutico, mayor efectividad clínica y menos efectos adversos. En el presente estudio, realizamos un estudio de acoplamiento molecular de las interacciones de los receptores D₂, D₃ y D₄ con 92 metabolitos de *Curcuma longa* utilizando un enfoque *in silico*. Buscamos identificar compuestos para el posible desarrollo de fármacos. Se construyó una biblioteca virtual de compuestos utilizando moléculas que se identificaron en la caracterización fitoquímica de *C. longa*. Los protocolos que fueron validados por redocking se aplicaron a un escaneo virtual de esta biblioteca usando los programas Autodock-v4.2.3, Autodock Vina y Molegro-v6.0 Virtual Docker, con cuatro repeticiones cada uno. Las estructuras tridimensionales de los receptores D₂, D₃ y D₄ en complejo con risperidona, eticloprida y nemonaprida se obtuvieron del Protein Data Bank. Cuatro compuestos (estigmasterol, β -sitosterol, colest-5-en-3-ona y colestán-3-ol, 2-metileno-(3 β , 5 α)) fueron los más propensos a unirse a los receptores de dopamina D₂, D₃ y D₄, lo que sugiere su potencial para el posible desarrollo de fármacos.

Palabras clave: Estigmasterol; β -sitosterol; Colest-5-en-3-ona; Colestán-3-ol, 2-metileno- (3 β , 5 α); Receptores de dopamina; *Curcuma longa*.

1. Introduction

Dopamine (3-hydroxytyramine) modulates several physiological functions, including reward, cognition, emotion, motor control, and blood depuration by the kidneys (Miyoshi et al., 2002; Da Cunha et al., 2009a; Da Cunha et al., 2009b; Wietzikoski et al., 2012; Fontoura et al., 2017; Alfio et al., 2020; Fernandes et al., 2020; Gildea et al., 2019). The dopaminergic system is involved in a wide range of psychiatric disorders (e.g., schizophrenia, depression, bipolar disorder, attention-deficit/hyperactivity disorder, anorexia nervosa, Tourette's syndrome, addiction, and autism), neurological disorders (e.g., Parkinson's disease [PD], Alzheimer's disease, dyskinesia, and dementia with Lewy bodies), and cardiovascular diseases (e.g., hypertension; Badgaiyan, 2016; Broft et al., 2015; Dong et al., 2020; Galaj et al., 2020; Horga et al., 2016; Li et al., 2020; Martel and McArthur, 2020; Martinez et al., 2020; Nakamura et al., 2010; Steeves et al., 2010; Wooten et al., 2015; Yatham et al., 2002, Zhai et al., 2019).

Brain dopaminergic neurons are mainly located in the mesencephalon (i.e., substantia nigra and ventral tegmental

area) and project throughout four main pathways: nigrostriatal, mesocortical, mesolimbic, and tuberoinfundibular (Alfio et al., 2020; Conn et al., 2020; Hou et al., 2014). After being released into the synaptic cleft, dopamine binds to dopamine receptors. All dopamine receptors are seven-transmembrane domain metabotropic receptors that are divided into two main families, D₁-like receptors (D₁ and D₅) and D₂-like receptors (D₂, D₃, and D₄), that couple to G_s and G_i proteins, respectively (Dong et al., 2020; Martinez et al., 2020). Substances that can interact with dopaminergic receptors have promising therapeutic potential, either by acting as receptor agonists (e.g., pramipexol) or antagonists (e.g., haloperidol).

Dopamine receptor agonists can be used for the symptomatic treatment of patients with early PD and restless leg syndrome (Grimes et al., 2019; Liu et al., 2016). Parkinson's disease is a neurodegenerative disorder that is characterized by the progressive loss of dopaminergic neurons in the substantia nigra (Fontoura et al., 2017; Li et al., 2020; Zhai et al., 2019). Dopamine replacement therapy can mainly alleviate motor symptoms of the disease. However, levodopa (L-DOPA) and dopamine receptor agonists can cause diverse side effects, such as dyskinesias, motor fluctuations, sleepiness, and hallucination (Kim et al., 2015; Paus et al., 2003; Yun et al., 2017).

Dopamine receptor antagonists are widely used antipsychotic medications that are used for the treatment of schizophrenia, bipolar disorder, and other psychotic conditions (Moncrieff et al., 2020). However, antipsychotics are associated with various side effects, such as sedation, weight gain, metabolic disturbances, sexual dysfunction, gastrointestinal distress, and extrapyramidal movement disturbances (Hynes et al., 2020).

It is of paramount importance to search for new compounds that act on dopamine receptors with therapeutic potential, higher clinical effectiveness, and fewer adverse effects. The drug discovery process is particularly challenging because it is both time- and resource-consuming. However, the recent use of newer technologies and information management has led to the optimization of drug development and design (Makhouri & Ghasemi, 2018).

One promising natural product for the development of pharmacological agents is *Curcuma longa* L. (Zingiberaceae). This herbaceous and perennial species originated in Asia and is distributed throughout the tropics. It is a rhizome-like pseudostem plant, popularly known as turmeric (Bortolucci et al., 2020). *C. longa* is commonly used in the food industry as a food coloring, seasoning, and condiment (Wang et al., 2014). The main classes of compounds that are identified in rhizomes of this species are curcuminoids (curcumin, demethoxycurcumin, and bisdesmethoxycurcumin) and oxygenated sesquiterpenes (Marchi et al., 2020). Its pharmacological properties, identified by preclinical studies, include antiinflammatory, gastroprotective, hepatoprotective, antiparasitic, antibacterial, antiviral, antifungal, anti-cancer, antiarthritic, antioxidant, hypolipidemic, and anti-human immunodeficiency virus effects (Araujo & Leon, 2001; Bastos et al., 2009; Krup et al., 2013; Yu et al., 2002). Furthermore, the species also has actions on the immune system, sedative properties, and neuroprotective activity (Kim et al., 2014).

To identify potential dopaminergic ligands, the present study performed a molecular docking investigation of D₂, D₃, and D₄ receptor interactions with active metabolites from *C. longa* using an *in silico* approach. We sought to identify possible compounds for drug development, following the good standards of scientific research methodology (Pereira et al., 2018).

2. Methodology

2.1 Extract preparation and virtual library of compounds

Rhizomes of *Curcuma longa* were collected in June and July 2016 at the Paranaense University Medicinal Garden (S23°46'11.3"-W53°16'41.2"; voucher specimen no. 2000). The extract preparation and phytochemical characterization were previously described (Marchi et al., 2020).

2.2 Virtual library

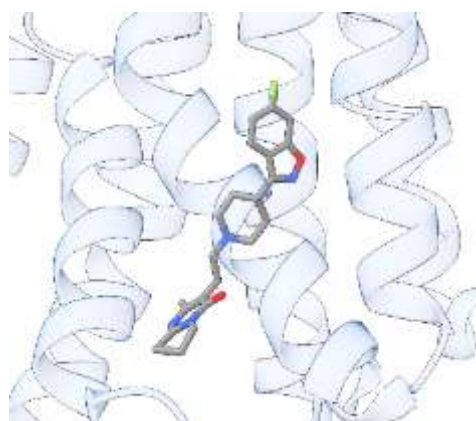
A virtual library of compounds was built using molecules that were identified in the phytochemical characterization of *C. longa* by gas chromatography coupled with mass spectrometry and high-performance liquid chromatography (Marchi et al. 2020). The three-dimensional structures were obtained from the PubChem (Kim et al., 2019) and Zinc15 (Sterling and Irwin, 2015) databases. A total of 92 molecules were obtained. The protocols that were validated by redocking were applied to the virtual screening of this library using three different programs, with four repetitions each.

2.3 Biological targets selection

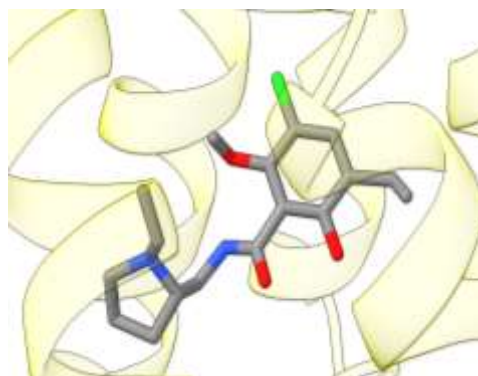
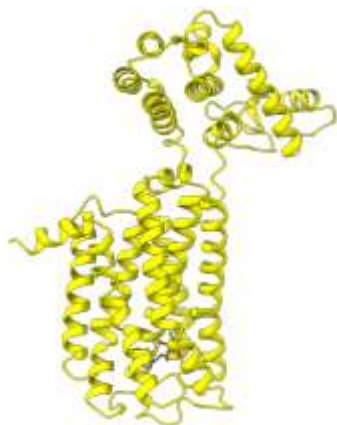
The three-dimensional structures of D₂, D₃, and D₄ receptors in complex with risperidone (RPN; Fig. 1A), eticlopride (ETC; Fig. 1B), and nemonapride (NMP; Fig. 1C) were obtained from the Protein Data Bank (PDB ID: 6cm4, 3pbl, and 5wiu, respectively). All small molecules and ions that were present in the solvent were then removed from the coordinates, except the 2112 structural water molecule in the D₂ receptor structure.

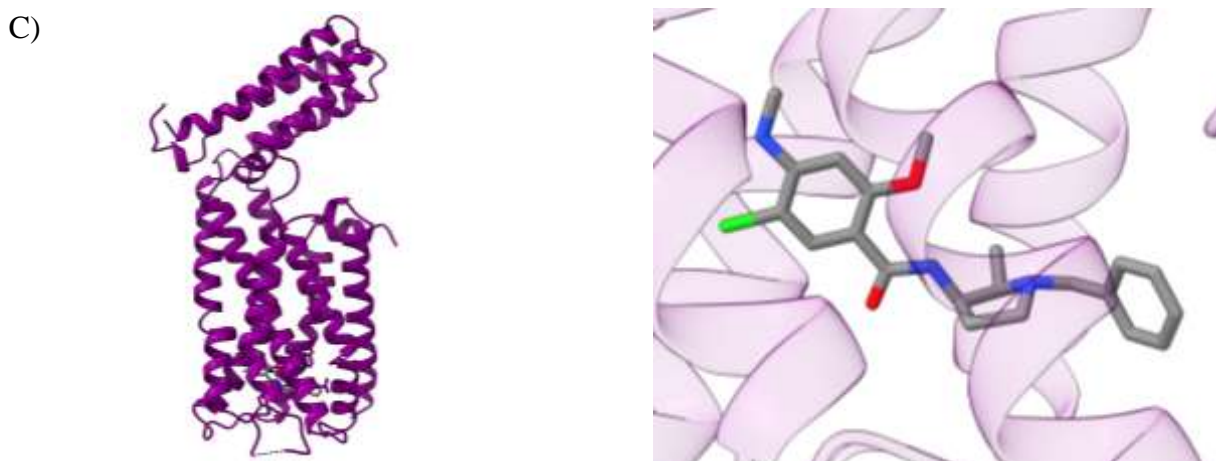
Figure 1. (A) Three-dimensional structure of the D₂ receptor in complex with RPN (PDB ID: 6cm4). (B) Three-dimensional structure of the D₃ receptor in complex with ETC (PDB ID: 3pbl). (C) Three-dimensional structure of the D₄ receptor in complex with NMP (PDB ID: 5wiu).

A)



B)





Source: Authors.

2.4 Molecular docking analysis

To perform the docking simulations, three programs were used: Autodock-v4.2.3 (Morris et al., 2009) and Autodock Vina (Trott & Olson, 2010), both implemented in the PyRx-0.9 graphical interface (Dallakyan & Olson, 2015), and Molegro-v6.0 Virtual Docker (Thomsen & Christensen, 2006). The parameters of each program were selected from the redocking of crystallographic ligands in their respective receptors. The protocols were considered validated when redocking returned poses with a root mean square deviation (RMSD) below 3 Å relative to the original conformation of the receptor-ligand complex in four repetitions. In Autodock, the search box was centered on the crystallographic ligand at x, y, z coordinates of 7, 8, -9 for x, 0, -14, 11 for y, and -18, 15, -16 for z (D₂, D₃, and D₄ receptors, respectively). For all three receptors, grid dimensions of 50, a resolution of 0.375 Å, 25 runs, and 2,500,000 energy evaluations were used. In Vina, the same coordinates and dimensions that are described above were used, and the exhaustiveness parameter was changed to 16, and the modes parameter was changed to 25. In Molegro for the D₂ receptor, Plants Score (grid) and Moldock Optimizer were used as ranking and search algorithms, with 10 runs and a 15 Å search radius, respectively. For D₃ and D₄ receptors, the Iterated Simplex search algorithm was selected, and the other parameters were the same as those described above for the D₂ receptor.

3. Results and Discussion



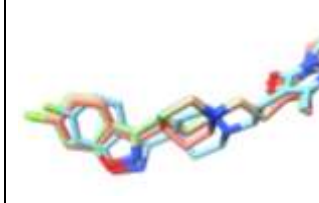






Molecular docking is an important tool that is useful for the rational design of drugs based on structures that can predict how a receptor interacts with a small-molecule ligand to form a stable complex (Raghu et al., 2018). This method has been useful for developing several drugs without expending excessive effort or investment in research, and these drugs subsequently underwent preclinical studies and clinical trials for validation (Chaurasiya et al., 2016; Shruthy & Shakkela, 2014). In the present study, molecular docking was used to simulate *in silico* interactions between biologically active metabolites from *C. longa* with D₂, D₃, and D₄ receptors to identify possible new therapeutic targets, which appears to be a common link between neurodegenerative and neuropsychiatric disorders.

Dopamine has been intensively studied as a key neurotransmitter in the central nervous system. Many studies have sought to understand the physiological processes and define the mechanisms of several pathologies, including PD, schizophrenia, substance dependence, and attention-deficit/hyperactivity disorder (Pan et al., 2019; Yang & Tsai, 2017). Subtypes of DA receptors have high amino acid sequence homogeneity and differ in their distribution, expression, affinity, and functional properties, thus creating challenges in developing receptor subtype-selective agonists and antagonists (Bueschbell et

al., 2019; Missale et al., 1998; Neve et al., 2004; Yang et al., 2020). The lack of subtype specificity is related to several side effects that are commonly observed in the treatment of parkinsonian and schizophrenic patients (Briggs et al., 2008; Peschanski et al., 1994; Reichmann, 2016). For some diseases, such as PD and schizophrenia, the therapeutic arsenal only provides symptomatic relief, without significantly altering the underlying pathophysiology (Cuny, 2012; Hou et al., 2019). Thus, it is urgent to search for new compounds from many sources, including drug synthesis, existing drugs, and natural products, that act on pathways that are involved in neurodegenerative and neuropsychiatric disorders and are safer and more effective for the treatment of these diseases (Durães et al., 2018).

The present study employed redocking validation with risperidone, eticlopride, and nemonapride and D₂, D₃, and D₄ receptor binding sites, respectively, to determine whether the docking protocol is acceptable (Table 1). The repetitions returned poses with an RMSD below 3 Å, thereby indicating that the protocols were validated and could be applied to screening the virtual library.

Table 1. Redocking study of risperidone (RPN), eticlopride (ETC), and nemonapride (NMP) at D₂, D₃, and D₄ binding sites, respectively.

	Autodock-v4.2.3	Autodock Vina	Molegro-v6.0
RPN redocking at D ₂ receptor			
RMSD Å	2.56 ± 0.03	0.56 ± 0.05	0.63 ± 0.17
ETC redocking at D ₃ receptor			
RMSD Å	0.58 ± 0.09	1.21 ± 0.00	0.96 ± 0.22
NMP redocking at D ₄ receptor			
RMSD Å	0.64 ± 0.02	0.82 ± 0.01	0.95 ± 0.01

Source: Authors.

D₂ receptors are highly expressed in striatopallidal medium spiny neurons in the external globus pallidus, nucleus accumbens core, ventral tegmental area, and substantia nigra pars compacta. They are expressed in lower concentrations in the

amygdala, cerebral cortex, hippocampus, and pituitary (Mishra et al., 2018; Surmeier et al., 2007). Two isoforms of D₂ receptors, D₂ long and D₂ short, are expressed mainly postsynaptically and presynaptically, respectively. Postsynaptic D₂ receptors mediate behavioral and extrapyramidal activity. Presynaptic D₂ receptors decrease dopamine release, resulting in a decrease in locomotor activity (Hisahara & Shimohama, 2011). The hyperactivity of dopamine at D₂ receptors in the mesolimbic pathway is related to manifestations of positive symptoms of schizophrenia (Brisch et al., 2014).

D₃ receptors are abundantly distributed in mesolimbic areas, including the nucleus accumbens, the olfactory tubercle, the amygdala, islands of Calleja, and the striatum (Gurevich & Joyce, 1999; Jaber et al., 1996), and their activation or inhibition is related to symptoms of neurodegenerative and neuropsychiatric diseases, including emotional, behavioral, motivational, and memory fluctuations (Yang et al., 2020).

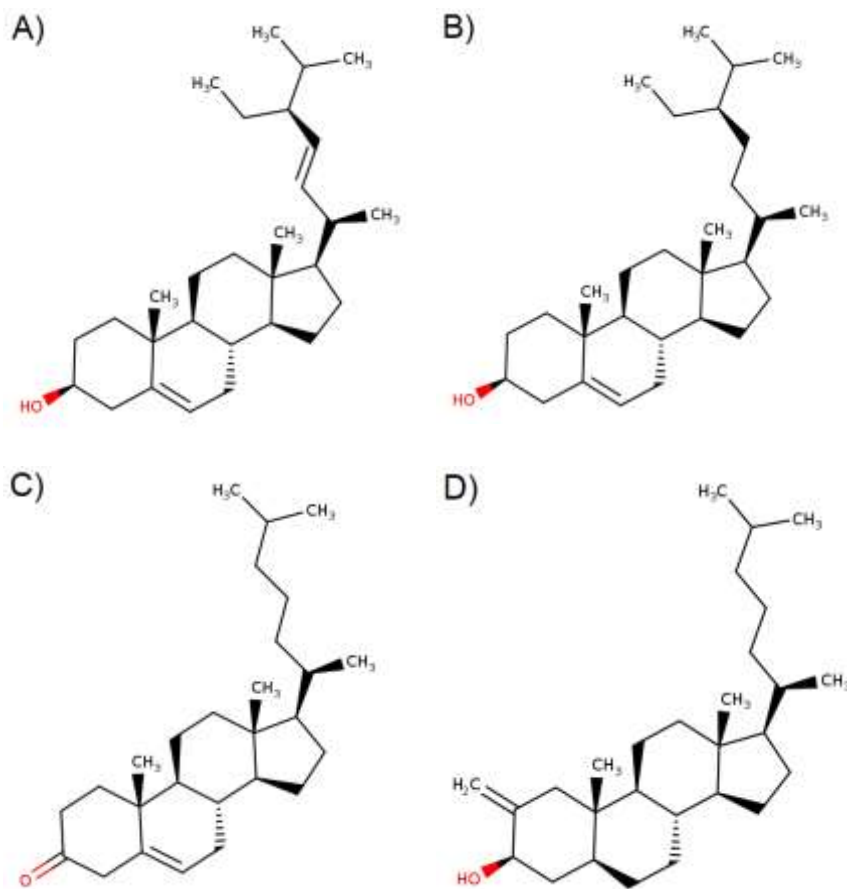
D₄ receptors are primarily expressed on pyramidal neurons and interneurons in the prefrontal cortex, but their localization is also found on medium spiny neurons in the basal ganglia (striatum and nucleus accumbens core), throughout the limbic system, and in the thalamus in rodents (Gan et al., 2004; Mrzljak et al., 1996; Tarazi et al., 2004).

Dopamine receptors have long been considered critical players in PD and schizophrenia. In PD, the loss of dopaminergic neurons in the substantia nigra pars compacta leads to dopamine deficiency in the putamen and caudate nucleus, producing classic motor symptoms of PD, such as tremors, trouble moving, and fatigue (Alexander, 2004). Treatment for PD mainly involves dopaminergic-based strategies, which currently remain the best symptomatic treatment to improve quality of life (Migueluez et al., 2020). However, these therapies are limited by various side effects. New alternative treatment strategies, alone or combined with currently available synthetic drugs, are needed.

Schizophrenia is associated with the hyperactivity of dopaminergic systems (Brunelin et al., 2013). Currently available medications focus on the blockade of overstimulated dopamine receptors. For the therapeutic care of schizophrenia patients, these medications are generally effective for treating positive symptoms, but they have strong side effects (Grace, 2012; Miodownik et al., 2019).

C. longa is widely studied because curcumin has been shown to have diverse molecular mechanisms that underlie its beneficial effects in neurological diseases (Farooqui & Farooqui, 2019; Ji & Shen, 2014; Mythri & Bharath, 2012; Miodownik et al., 2019). It contains several phytochemicals with potential biological activity. In the present study, of the 92 molecules that were analyzed, four were consistently ranked among those with the highest affinity for the three dopamine receptors (Supplementary Tables S1, S2, and S3). These molecules included stigmasterol (Fig. 2A), β -sitosterol (Fig. 2B), cholest-5-en-3-one (Fig. 2C), and cholestan-3-ol,2-methylene-(3 β , 5 α) (Fig. 2D).

Figure 2. (A) Stigmasterol. (B) β -sitosterol. (C) Cholest-5-en-3-one. (D) Cholestan-3-ol,-2-methylene-($3\beta,5\alpha$).



Source: Authors.

Tables 2 show the docking results of stigmasterol, β -sitosterol, cholest-5-en-3-one, and cholestan-3-ol,2-methylene-($3\beta, 5\alpha$) ligands at the D_2 receptor.

Table 2. Virtual screening of stigmasterol, β -sitosterol, cholest-5-en-3-one, and cholestan-3-ol,2-methylene-($3\beta, 5\alpha$) ligands at the D_2 receptor.

PubChem CID	Name	Autodock-v4.2.3	Autodock Vina	Molegro-v6.0
	risperidone	-13.03 \pm 0.01	-103.98 \pm 3.70	-11.8 \pm 0.00
5280794	stigmasterol	-12.85 \pm 0.23	-82.40 \pm 1.16	-8.70 \pm 0.00
222284	β -sitosterol	-12.69 \pm 0.21	-86.07 \pm 0.65	-8.90 \pm 0.05
9908107	cholest-5-en-3-one	-12.53 \pm 0.08	-79.94 \pm 1.41	-8.75 \pm 0.06
22213932	cholestan-3-ol,2-methylene-($3\beta,5\alpha$)	-12.45 \pm 0.01	-82.34 \pm 0.30	-8.23 \pm 0.05

Source: Authors.

Tables 3 show the docking results of stigmasterol, β -sitosterol, cholest-5-en-3-one, and cholestan-3-ol,2-methylene-($3\beta, 5\alpha$) ligands at the D_3 receptor.

Table 3. Virtual screening of stigmasterol, β -sitosterol, cholest-5-en-3-one, and cholestan-3-ol,2-methylene-(3 β , 5 α) ligands at the D₃ receptor.

PubChem CID	Name	Autodock-v4.2.3	Autodock Vina	Molegro-v6.0
	eticlopride	-9.42 \pm 0.07	-85.14 \pm 0.04	-8.40 \pm 0.00
5280794	stigmasterol	-13.39 \pm 0.11	-91.97 \pm 0.02	-10.00 \pm 0.00
222284	β -sitosterol	-13.30 \pm 0.14	-96.71 \pm 0.19	-9.33 \pm 0.13
9908107	cholest-5-en-3-one	-12.63 \pm 0.02	-91.98 \pm 0.15	-9.58 \pm 0.52
22213932	cholestan-3-ol,2-methylene-(3 β ,5 α)	-12.47 \pm 0.01	-92.22 \pm 0.00	-9.35 \pm 0.19

Source: Authors.

Tables 4 show the docking results of stigmasterol, β -sitosterol, cholest-5-en-3-one, and cholestan-3-ol,2-methylene-(3 β , 5 α) ligands at the D₄ receptor.

Table 4. Virtual screening of stigmasterol, β -sitosterol, cholest-5-en-3-one, and cholestan-3-ol,2-methylene-(3 β , 5 α) ligands at the D₄ receptor.

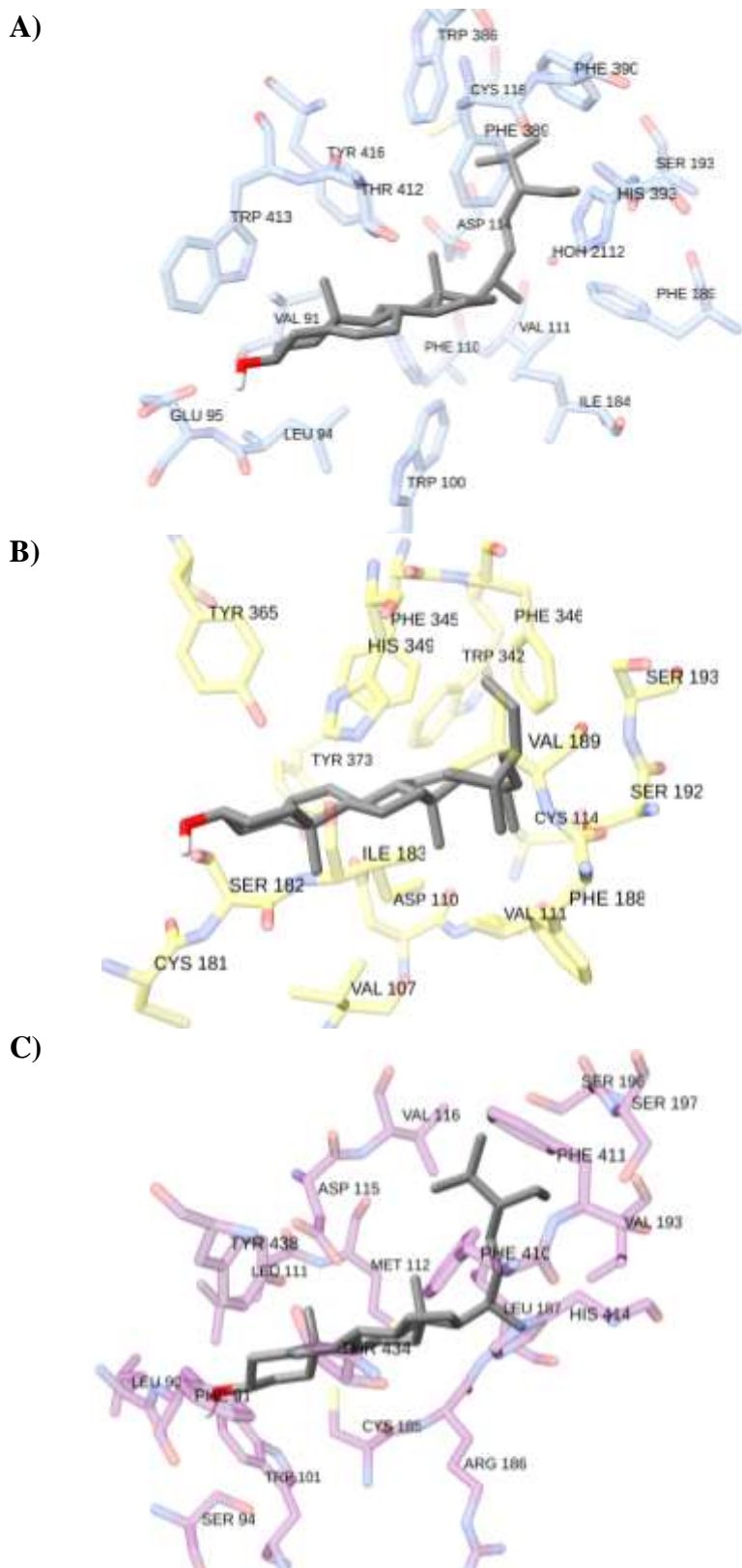
PubChem CID	Name	Autodock-v4.2.3	Autodock Vina	Molegro-v6.0
	nemonapride	-11.30 \pm 0.02	-90.12 \pm 0.05	-10.20 \pm 0.00
5280794	stigmasterol	-12.78 \pm 0.08	-93.39 \pm 1.16	-10.20 \pm 0.00
222284	β -sitosterol	-12.49 \pm 0.14	-92.05 \pm 0.21	-9.68 \pm 0.13
9908107	cholest-5-en-3-one	-12.38 \pm 0.05	-85.44 \pm 0.05	-9.48 \pm 0.10
22213932	cholestan-3-ol,2-methylene-(3 β ,5 α)	-12.55 \pm 0.04	-94.35 \pm 0.01	-10.00 \pm 0.00

Source: Authors.

Stigmasterol is a phytoestrogen that is naturally found in many herbs, including *Akebia quinata*, *Gypsophila oldhamiana*, *Emilia sonchifolia*, *Eucalyptus globules*, *Aralia cordata*, *Emilia sonchifolia*, *Theobroma cacao* L., and *C. longa* (Marchi et al., 2020; Awad et al., 2009; Kaur et al., 2011; Yadav et al., 2018). Stigmasterol has been reported to possess many pharmacological activities (Ayati et al., 2019).

The neurological effects of stigmasterol *in vitro* and *in vivo* have been reported. Haque et al. (2019) investigated the role of stigmasterol in neuroprotection and neuronal viability in hypoxia reoxygenation-induced injury in hippocampal cultures. The authors found that stigmasterol exerted a neuroprotective effect at a concentration of 20 μ M. Another study found that stigmasterol (10 mg/kg) promoted cognitive improvements in animals that were subjected to a passive avoidance task and the Morris water maze (Park et al., 2012). In this study, stigmasterol interacted with D₂, D₃, and D₄ receptors. Formation of the most stable binding complex with D₃ and D₄ receptors featured neuroactive potential for improving brain functions. In Fig. 3, residues can be seen at least 4 Å away from stigmasterol for D₂, D₃, and D₄ receptors.

Figure 3. Stigmasterol docking at (A) D₂ receptor (residues 4 Å away from the ligand), (B) D₃ receptor (residues 4 Å away from the ligand), and (C) D₄ receptor (residues 4 Å away from the ligand).



Source: Authors.

The molecular docking study also supported the binding of β -sitosterol, a plant sterol, with D₂, D₃, and D₄ receptors. Several studies reported various neuroprotective and antioxidant effects of β -sitosterol (Vivancos & Moreno, 2005; Baskar et al., 2012; Muhammad et al., 2017). Additionally, the antidepressant activity of β -sitosterol was evaluated in the tail suspension test and forced swim test in mice. The authors concluded that β -sitosterol exhibits antidepressant-like effects that are mediated by the serotonergic, dopaminergic, and γ -aminobutyric acid-ergic systems (Yin et al., 2018). Cholest-5-en-3-one and cholestan-3-ol,2-methylene-(3 β , 5 α) were other compounds that were identified in the *C. longa* extract and had affinity for D₂, D₃, and D₄ receptors. These findings should prompt further *in vitro* and *in vivo* studies to prove their biological activity.

Finally, the current pharmaceutical armamentarium against neurodegenerative (Youdim & Buccafusco, 2005) and neuropsychiatric (Miodownik et al., 2019) diseases remains limited in terms of both treatment outcome and disease modification. The present results demonstrate that four *C. longa* compounds interact with dopamine receptors that are involved in the pathophysiology of several neurodegenerative and neuropsychiatric disorders.

4. Conclusion

Stigmasterol, β -sitosterol, cholest-5-en-3-one, and cholestan-3-ol,2-methylene-(3 β , 5 α) are metabolites of *C. longa* that had the highest probability of binding dopamine D₂, D₃, and D₄ receptors. Our findings may contribute to further drug development.

The present study has limitations. We performed only a virtual throughput screening. Further high-throughput screening is required, in addition to studies with animal models and clinical trials to further confirm that *C. longa* metabolites may be potential drug candidates.

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Supplementary Tables

Table S1. Virtual screening of secondary metabolites isolated from *Curcuma longa* L. at the D2 receptor (RMSD Å). Values expressed as mean \pm S.D.M. for four repetitions.

	PubChem CID	Autodock-v4.2.3	Autodock Vina	Molegro-v6.0
0	risperidone	-13.415 \pm 0.114	-11.800 \pm 0.000	-103.979 \pm 3.695
1	5280794	-12.852 \pm 0.275	-8.700 \pm 0.000	-82.399 \pm 1.156
2	222284	-12.687 \pm 0.213	-8.875 \pm 0.050	-86.074 \pm 0.647
3	9908107	-12.527 \pm 0.076	-8.750 \pm 0.058	-79.940 \pm 1.406
4	22213932	-12.448 \pm 0.005	-8.225 \pm 0.050	-82.343 \pm 0.299
5	173058	-10.585 \pm 0.034	-9.000 \pm 0.000	-76.482 \pm 0.118
6	163263	-10.200 \pm 0.043	-7.600 \pm 0.000	-76.107 \pm 1.733
7	13858190	-10.012 \pm 0.073	-7.225 \pm 0.050	-75.137 \pm 2.930
8	72326	-9.937 \pm 0.142	-9.100 \pm 0.000	-74.643 \pm 0.000
9	102582	-9.707 \pm 0.029	-7.450 \pm 0.058	-69.353 \pm 0.000
10	5281437	-9.527 \pm 0.015	-7.100 \pm 0.000	-60.050 \pm 0.003
11	61125	-9.483 \pm 0.025	-6.800 \pm 0.000	-57.693 \pm 0.016
12	5366016	-9.345 \pm 0.201	-8.575 \pm 0.171	-73.614 \pm 1.151
13	522296	-9.228 \pm 0.015	-7.000 \pm 0.000	-60.683 \pm 1.468
14	6431015	-9.123 \pm 0.005	-6.925 \pm 0.050	-61.517 \pm 4.580
15	11947764	-9.120 \pm 0.020	-6.600 \pm 0.000	-60.054 \pm 1.773
16	91753606	-9.002 \pm 0.055	-7.275 \pm 0.050	-59.205 \pm 0.004
17	19725	-8.992 \pm 0.015	-6.800 \pm 0.000	-60.444 \pm 0.004
18	5321293	-8.958 \pm 0.046	-6.500 \pm 0.000	-64.312 \pm 0.235

19	14632996	-8.902 ± 0.123	-8.125 ± 0.050	-68.920 ± 1.892
20	196216	-8.880 ± 0.041	-7.925 ± 0.096	-67.508 ± 1.664
21	6432447	-8.863 ± 0.013	-5.900 ± 0.000	-53.450 ± 0.002
22	494439	-8.860 ± 0.060	-7.600 ± 0.000	-65.373 ± 0.000
23	11736155	-8.852 ± 0.013	-8.175 ± 0.050	-66.479 ± 0.925
24	65575	-8.828 ± 0.005	-6.600 ± 0.000	-58.463 ± 2.551
25	10583	-8.818 ± 0.005	-6.900 ± 0.000	-65.537 ± 0.003
26	442343	-8.815 ± 0.031	-7.950 ± 0.058	-70.196 ± 3.980
27	6365122	-8.803 ± 0.010	-8.050 ± 0.100	-71.501 ± 6.022
28	12311096	-8.730 ± 0.020	-6.200 ± 0.000	-57.911 ± 1.736
29	78265163	-8.730 ± 0.020	-6.200 ± 0.000	-57.161 ± 1.508
30	6427078	-8.710 ± 0.020	-6.800 ± 0.000	-60.699 ± 0.003
31	12315492	-8.695 ± 0.033	-8.050 ± 0.058	-70.182 ± 5.966
32	13213649	-8.678 ± 0.091	-7.500 ± 0.000	-68.839 ± 2.071
33	521253	-8.655 ± 0.039	-8.075 ± 0.050	-65.789 ± 0.636
34	92776	-8.640 ± 0.067	-8.100 ± 0.000	-69.850 ± 5.710
35	167812	-8.630 ± 0.080	-7.400 ± 0.000	-59.801 ± 0.004
36	6432312	-8.617 ± 0.005	-6.575 ± 0.050	-60.664 ± 2.287
37	5281515	-8.598 ± 0.065	-6.400 ± 0.000	-57.938 ± 0.235
38	5365976	-8.595 ± 0.013	-6.725 ± 0.126	-65.297 ± 1.263
39	6429302	-8.578 ± 0.010	-7.700 ± 0.000	-61.295 ± 0.266
40	6857681	-8.570 ± 0.043	-7.800 ± 0.000	-62.884 ± 0.935
41	91746582	-8.557 ± 0.055	-6.400 ± 0.000	-58.731 ± 0.001
42	12304273	-8.543 ± 0.010	-8.000 ± 0.000	-71.467 ± 4.423
43	72503	-8.532 ± 0.005	-6.600 ± 0.000	-62.190 ± 0.002
44	5352653	-8.518 ± 0.005	-7.350 ± 0.058	-68.011 ± 4.752
45	1268142	-8.512 ± 0.015	-6.975 ± 0.050	-65.269 ± 0.003
46	6436348	-8.460 ± 0.020	-6.100 ± 0.000	-54.702 ± 1.933
47	10104370	-8.435 ± 0.013	-7.900 ± 0.000	-70.113 ± 5.956
48	21576980	-8.435 ± 0.030	-6.000 ± 0.000	-58.182 ± 0.638
49	15601436	-8.410 ± 0.020	-7.100 ± 0.000	-63.921 ± 0.004
50	6429350	-8.395 ± 0.024	-7.100 ± 0.000	-64.438 ± 0.001
51	160512	-8.370 ± 0.008	-8.100 ± 0.000	-67.701 ± 2.055
52	221071	-8.360 ± 0.020	-8.000 ± 0.000	-61.797 ± 0.003
53	5315469	-8.350 ± 0.047	-8.400 ± 0.000	-69.456 ± 1.293
54	5281531	-8.348 ± 0.035	-7.400 ± 0.000	-65.999 ± 0.251
55	14350	-8.320 ± 0.020	-6.800 ± 0.000	-59.130 ± 0.004
56	5367608	-8.320 ± 0.787	-7.200 ± 0.000	-66.755 ± 0.002
57	10955174	-8.320 ± 0.014	-5.800 ± 0.000	-53.837 ± 0.001
58	5365659	-8.262 ± 0.116	-7.175 ± 0.050	-71.320 ± 1.550

59	5281520	-8.252 ± 0.005	-7.300 ± 0.000	-59.159 ± 0.032
60	534856	-8.223 ± 0.005	-6.000 ± 0.000	-55.961 ± 0.004
61	519545	-8.218 ± 0.005	-6.400 ± 0.000	-59.089 ± 0.002
62	5370837	-8.203 ± 0.013	-7.800 ± 0.000	-72.030 ± 4.067
63	92139	-8.197 ± 0.010	-8.100 ± 0.000	-71.202 ± 3.719
64	5322111	-8.183 ± 0.015	-6.700 ± 0.000	-59.504 ± 0.002
65	519762	-8.160 ± 0.045	-6.400 ± 0.000	-61.690 ± 0.002
66	14014430	-8.102 ± 0.013	-8.000 ± 0.000	-71.493 ± 3.572
67	6440942	-8.100 ± 0.073	-6.250 ± 0.100	-59.153 ± 0.254
68	5367460	-8.092 ± 0.089	-7.125 ± 0.096	-66.460 ± 1.791
69	5281519	-7.992 ± 0.042	-6.700 ± 0.000	-56.452 ± 0.752
70	6466	-7.930 ± 0.320	-7.100 ± 0.000	-66.159 ± 0.001
71	15560276	-7.840 ± 0.000	-7.000 ± 0.000	-60.206 ± 0.006
72	5282184	-7.817 ± 0.063	-6.775 ± 0.263	-63.914 ± 3.628
73	5470187	-7.803 ± 0.005	-6.925 ± 0.050	-60.705 ± 0.003
74	64971	-7.797 ± 0.252	-9.300 ± 0.000	-74.363 ± 0.001
75	445070	-7.785 ± 0.051	-7.225 ± 0.150	-67.667 ± 1.094
76	494329	-7.748 ± 0.005	-7.500 ± 0.000	-62.089 ± 0.031
77	15559499	-7.742 ± 0.005	-6.900 ± 0.000	-58.696 ± 2.525
78	6430550	-7.655 ± 0.044	-6.800 ± 0.115	-62.962 ± 2.383
79	5281517	-7.575 ± 0.066	-7.300 ± 0.082	-70.236 ± 7.362
80	6440940	-7.547 ± 0.041	-6.500 ± 0.000	-59.310 ± 0.002
81	1752	-7.543 ± 0.062	-7.450 ± 0.058	-69.273 ± 2.976
82	92812	-7.485 ± 0.117	-7.100 ± 0.000	-59.749 ± 0.002
83	11996452	-7.440 ± 0.040	-6.900 ± 0.000	-59.535 ± 0.002
84	526687	-7.388 ± 0.055	-7.600 ± 0.000	-62.280 ± 0.002
85	12304985	-7.372 ± 0.010	-6.900 ± 0.000	-60.927 ± 0.001
86	2355	-7.348 ± 0.015	-6.550 ± 0.058	-56.770 ± 0.003
87	66514	-7.173 ± 0.030	-6.825 ± 0.050	-67.951 ± 0.022
88	14109432	-7.168 ± 0.049	-6.000 ± 0.000	-53.787 ± 0.186
89	91354	-7.120 ± 0.000	-7.525 ± 0.050	-62.498 ± 0.001
90	10910653	-7.080 ± 0.020	-7.400 ± 0.000	-60.735 ± 0.002
91	5364174	-6.988 ± 0.162	-7.125 ± 0.050	-66.183 ± 0.427
92	8181	-6.973 ± 0.182	-6.250 ± 0.129	-62.279 ± 2.038
93	460	-4.855 ± 0.006	-5.800 ± 0.000	-62.156 ± 0.001

Table S2. Virtual screening of secondary metabolites isolated from *Curcuma longa* L. at the D3 receptor (RMSD Å). Values expressed as mean \pm S.D.M. for four repetitions.

	PubChem CID	Autodock-v4.2.3	Molegro-v6.0	Autodock Vina
0	eticlopride	-9.417 \pm 0.067	-85.143 \pm 0.037	-8.400 \pm 0.000
1	5280794	-13.390 \pm 0.110	-91.968 \pm 0.017	-10.000 \pm 0.000
2	222284	-13.295 \pm 0.142	-96.711 \pm 0.185	-9.325 \pm 0.126
3	9908107	-12.633 \pm 0.022	-91.975 \pm 0.145	-9.575 \pm 0.519
4	22213932	-12.465 \pm 0.013	-92.216 \pm 0.001	-9.350 \pm 0.191
5	13858190	-9.920 \pm 0.059	-75.674 \pm 0.010	-8.125 \pm 0.050
6	163263	-9.420 \pm 0.008	-72.764 \pm 0.003	-7.600 \pm 0.000
7	173058	-9.350 \pm 0.061	-69.112 \pm 0.002	-8.450 \pm 0.058
8	5367608	-8.782 \pm 0.017	-71.859 \pm 0.003	-8.500 \pm 0.000
9	19725	-8.780 \pm 0.000	-70.344 \pm 0.001	-7.400 \pm 0.000
10	494329	-8.738 \pm 0.005	-66.514 \pm 0.000	-8.600 \pm 0.000
11	5365976	-8.630 \pm 0.008	-75.854 \pm 0.094	-7.100 \pm 0.000
12	526687	-8.560 \pm 0.000	-72.213 \pm 0.000	-8.500 \pm 0.000
13	196216	-8.523 \pm 0.022	-78.884 \pm 0.013	-7.000 \pm 0.294
14	494439	-8.510 \pm 0.000	-66.080 \pm 0.003	-7.000 \pm 0.000
15	91354	-8.490 \pm 0.000	-70.445 \pm 0.001	-8.175 \pm 0.050
16	13213649	-8.480 \pm 0.038	-77.324 \pm 0.585	-7.550 \pm 0.100
17	5470187	-8.480 \pm 0.000	-66.310 \pm 0.001	-7.500 \pm 0.000
18	5352653	-8.457 \pm 0.010	-75.730 \pm 0.002	-7.500 \pm 0.000
19	61125	-8.440 \pm 0.000	-60.531 \pm 0.001	-7.700 \pm 0.000
20	1268142	-8.390 \pm 0.012	-65.832 \pm 0.001	-7.425 \pm 0.050
21	5281437	-8.390 \pm 0.000	-63.626 \pm 0.001	-7.400 \pm 0.000
22	5322111	-8.380 \pm 0.000	-69.239 \pm 0.001	-7.100 \pm 0.000
23	6429302	-8.335 \pm 0.010	-76.170 \pm 0.017	-7.300 \pm 0.000
24	6440942	-8.330 \pm 0.008	-67.409 \pm 0.002	-6.800 \pm 0.000
25	6440940	-8.303 \pm 0.017	-65.927 \pm 0.002	-7.000 \pm 0.000
26	14632996	-8.277 \pm 0.026	-75.573 \pm 0.028	-7.100 \pm 0.231
27	5364174	-8.277 \pm 0.005	-71.072 \pm 0.005	-8.600 \pm 0.000
28	6857681	-8.265 \pm 0.070	-78.213 \pm 0.054	-7.300 \pm 0.000
29	519545	-8.250 \pm 0.000	-60.496 \pm 0.001	-7.300 \pm 0.000
30	12315492	-8.242 \pm 0.098	-77.232 \pm 1.035	-7.100 \pm 0.200
31	10104370	-8.240 \pm 0.116	-78.037 \pm 0.375	-6.975 \pm 0.150
32	91753606	-8.240 \pm 0.000	-73.865 \pm 0.000	-7.900 \pm 0.000
33	5366016	-8.223 \pm 0.196	-91.473 \pm 2.217	-7.875 \pm 0.096
34	21576980	-8.200 \pm 0.000	-63.146 \pm 0.001	-6.900 \pm 0.000
35	6431015	-8.190 \pm 0.000	-63.833 \pm 0.001	-7.300 \pm 0.000
36	521253	-8.188 \pm 0.064	-76.842 \pm 0.004	-7.200 \pm 0.000
37	12304985	-8.180 \pm 0.000	-66.230 \pm 0.001	-7.000 \pm 0.000

38	11736155	-8.178 ± 0.028	-75.699 ± 0.066	-7.350 ± 0.058
39	6432447	-8.170 ± 0.000	-63.576 ± 0.001	-6.800 ± 0.000
40	6365122	-8.113 ± 0.026	-78.455 ± 0.005	-7.600 ± 0.000
41	92776	-8.092 ± 0.113	-77.713 ± 0.021	-6.975 ± 0.126
42	5281531	-8.080 ± 0.041	-74.384 ± 0.007	-6.900 ± 0.082
43	6427078	-8.060 ± 0.000	-58.647 ± 0.002	-7.100 ± 0.000
44	15601436	-8.053 ± 0.052	-64.159 ± 0.001	-7.300 ± 0.000
45	221071	-8.040 ± 0.000	-63.569 ± 0.001	-7.700 ± 0.000
46	6429350	-8.022 ± 0.015	-63.450 ± 0.003	-6.900 ± 0.000
47	11947764	-8.007 ± 0.005	-59.970 ± 0.001	-7.200 ± 0.000
48	6466	-8.005 ± 0.013	-59.430 ± 0.001	-8.225 ± 0.150
49	5281515	-8.000 ± 0.000	-65.167 ± 0.001	-7.100 ± 0.000
50	11996452	-7.998 ± 0.005	-61.880 ± 0.001	-7.000 ± 0.000
51	160512	-7.995 ± 0.021	-77.025 ± 0.278	-7.400 ± 0.000
52	522296	-7.990 ± 0.000	-63.989 ± 0.001	-7.100 ± 0.000
53	167812	-7.980 ± 0.000	-67.883 ± 0.001	-7.000 ± 0.000
54	92812	-7.970 ± 0.000	-63.494 ± 0.002	-7.100 ± 0.000
55	12304273	-7.965 ± 0.064	-77.357 ± 0.159	-7.100 ± 0.000
56	5281520	-7.940 ± 0.000	-66.214 ± 0.000	-6.900 ± 0.000
57	10910653	-7.930 ± 0.000	-62.856 ± 0.001	-6.700 ± 0.000
58	65575	-7.925 ± 0.006	-61.449 ± 0.001	-6.825 ± 0.050
59	14350	-7.900 ± 0.000	-64.148 ± 0.002	-7.050 ± 0.058
60	5315469	-7.885 ± 0.082	-75.387 ± 0.038	-7.150 ± 0.100
61	5281519	-7.850 ± 0.000	-60.464 ± 0.001	-6.700 ± 0.000
62	72326	-7.845 ± 0.013	-67.369 ± 0.003	-6.625 ± 1.242
63	92139	-7.802 ± 0.039	-76.202 ± 0.124	-7.175 ± 0.189
64	5321293	-7.795 ± 0.006	-69.239 ± 0.020	-7.000 ± 0.000
65	442343	-7.697 ± 0.080	-72.852 ± 0.082	-7.375 ± 0.050
66	72503	-7.695 ± 0.006	-61.449 ± 0.002	-6.700 ± 0.000
67	10583	-7.683 ± 0.005	-69.298 ± 0.004	-6.700 ± 0.000
68	5367460	-7.660 ± 0.411	-95.294 ± 2.130	-6.875 ± 0.150
69	102582	-7.652 ± 0.021	-69.046 ± 0.020	-7.700 ± 0.000
70	5370837	-7.645 ± 0.044	-77.026 ± 0.378	-6.975 ± 0.096
71	534856	-7.630 ± 0.000	-56.924 ± 0.001	-6.900 ± 0.000
72	10955174	-7.620 ± 0.014	-55.620 ± 0.001	-6.700 ± 0.000
73	519762	-7.607 ± 0.013	-65.960 ± 0.001	-6.900 ± 0.000
74	5365659	-7.572 ± 0.214	-99.910 ± 0.604	-6.750 ± 0.058
75	91746582	-7.570 ± 0.000	-56.760 ± 0.001	-7.125 ± 0.150
76	12311096	-7.535 ± 0.006	-60.491 ± 0.003	-6.500 ± 0.000
77	78265163	-7.535 ± 0.006	-60.490 ± 0.004	-6.500 ± 0.000

78	15559499	-7.502 ± 0.005	-58.956 ± 0.001	-6.850 ± 0.058
79	64971	-7.485 ± 0.021	-68.269 ± 0.001	-6.625 ± 0.550
80	14014430	-7.385 ± 0.066	-77.131 ± 0.077	-7.075 ± 0.050
81	6432312	-7.325 ± 0.037	-64.630 ± 0.001	-6.450 ± 0.058
82	15560276	-7.320 ± 0.000	-57.703 ± 0.000	-6.900 ± 0.000
83	14109432	-7.305 ± 0.006	-54.613 ± 0.001	-7.000 ± 0.000
84	5281517	-7.240 ± 0.103	-83.292 ± 0.466	-6.675 ± 0.096
85	5282184	-7.213 ± 0.152	-94.124 ± 2.684	-6.675 ± 0.096
86	6430550	-7.205 ± 0.073	-81.518 ± 1.038	-6.350 ± 0.058
87	1752	-7.100 ± 0.127	-82.516 ± 0.881	-6.550 ± 0.058
88	445070	-7.092 ± 0.179	-85.743 ± 0.496	-6.825 ± 0.096
89	6436348	-7.010 ± 0.000	-55.921 ± 0.000	-6.875 ± 0.050
90	2355	-6.580 ± 0.000	-55.602 ± 0.001	-6.975 ± 0.050
91	8181	-6.385 ± 0.285	-89.345 ± 2.220	-5.875 ± 0.126
92	66514	-6.343 ± 0.036	-73.825 ± 0.009	-6.275 ± 0.050
93	460	-4.723 ± 0.015	-53.961 ± 0.002	-4.975 ± 0.050

Table S3. Virtual screening of secondary metabolites isolated from *Curcuma longa* L. at the D4 receptor (RMSD Å). Values expressed as mean ± S.D.M. for four repetitions.

	PubChem CID	Autodock-v4.2.3	Molegro-v6.0	Autodock Vina
0	nemonapride	-11.302 ± 0.019	-90.119 ± 0.037	-9.800 ± 0.000
1	5280794	-12.777 ± 0.078	-93.388 ± 1.162	-10.200 ± 0.000
2	22213932	-12.555 ± 0.039	-94.345 ± 0.010	-10.000 ± 0.000
3	222284	-12.490 ± 0.141	-92.053 ± 0.213	-9.680 ± 0.126
4	9908107	-12.380 ± 0.048	-85.438 ± 0.049	-9.480 ± 0.096
5	64971	-12.087 ± 0.005	-73.133 ± 0.002	-7.780 ± 0.050
6	72326	-11.783 ± 0.068	-76.399 ± 0.002	-9.000 ± 0.000
7	13858190	-9.668 ± 0.042	-76.155 ± 0.393	-8.130 ± 0.050
8	173058	-9.588 ± 0.029	-70.589 ± 0.007	-8.800 ± 0.000
9	163263	-9.527 ± 0.010	-76.350 ± 0.010	-7.900 ± 0.000
10	494329	-9.450 ± 0.020	-70.861 ± 0.016	-9.200 ± 0.000
11	5367608	-9.010 ± 0.029	-66.392 ± 0.107	-8.300 ± 0.000
12	6432447	-8.825 ± 0.006	-63.830 ± 0.001	-7.200 ± 0.000
13	6466	-8.645 ± 0.019	-70.020 ± 0.002	-9.030 ± 0.050
14	5366016	-8.495 ± 0.199	-91.725 ± 2.435	-7.930 ± 0.150
15	5470187	-8.490 ± 0.000	-61.274 ± 0.001	-7.380 ± 0.050
16	534856	-8.490 ± 0.000	-62.552 ± 0.001	-7.100 ± 0.000
17	494439	-8.455 ± 0.006	-74.611 ± 0.002	-8.200 ± 0.000
18	91354	-8.440 ± 0.000	-72.191 ± 0.000	-8.400 ± 0.000
19	526687	-8.430 ± 0.000	-70.561 ± 0.001	-8.300 ± 0.000
20	102582	-8.378 ± 0.030	-70.059 ± 0.082	-8.300 ± 0.000

21	5281515	-8.350 ± 0.000	-65.940 ± 0.001	-8.100 ± 0.000
22	5281437	-8.330 ± 0.000	-63.829 ± 0.006	-8.000 ± 0.000
23	6431015	-8.320 ± 0.000	-68.620 ± 0.002	-8.000 ± 0.000
24	19725	-8.305 ± 0.013	-68.874 ± 0.001	-7.100 ± 0.000
25	11947764	-8.295 ± 0.006	-64.166 ± 0.001	-7.300 ± 0.000
26	65575	-8.293 ± 0.005	-60.239 ± 0.002	-7.600 ± 0.000
27	6429350	-8.290 ± 0.041	-72.221 ± 0.002	-7.630 ± 0.050
28	221071	-8.290 ± 0.000	-65.134 ± 0.002	-8.180 ± 0.050
29	522296	-8.280 ± 0.000	-67.087 ± 0.001	-7.700 ± 0.000
30	5322111	-8.257 ± 0.005	-64.876 ± 0.001	-7.200 ± 0.000
31	91753606	-8.207 ± 0.060	-65.539 ± 0.001	-7.500 ± 0.000
32	11996452	-8.182 ± 0.005	-70.436 ± 0.001	-7.900 ± 0.000
33	6427078	-8.160 ± 0.000	-65.349 ± 0.002	-7.330 ± 0.050
34	12304985	-8.160 ± 0.000	-64.420 ± 0.001	-7.500 ± 0.000
35	91746582	-8.160 ± 0.000	-64.826 ± 0.001	-7.900 ± 0.000
36	92812	-8.150 ± 0.000	-67.337 ± 0.001	-7.700 ± 0.000
37	6440942	-8.150 ± 0.000	-66.985 ± 0.003	-6.700 ± 0.000
38	10910653	-8.140 ± 0.000	-68.245 ± 0.001	-7.500 ± 0.000
39	15560276	-8.140 ± 0.000	-66.077 ± 0.001	-7.400 ± 0.000
40	12311096	-8.133 ± 0.112	-57.781 ± 0.002	-6.700 ± 0.000
41	61125	-8.090 ± 0.000	-60.607 ± 0.002	-7.200 ± 0.000
42	78265163	-8.085 ± 0.083	-57.781 ± 0.002	-6.700 ± 0.000
43	15601436	-8.080 ± 0.000	-67.030 ± 0.004	-7.200 ± 0.000
44	14350	-8.070 ± 0.000	-70.316 ± 0.002	-7.530 ± 0.050
45	1268142	-8.055 ± 0.006	-67.577 ± 0.002	-7.600 ± 0.000
46	167812	-8.050 ± 0.000	-62.609 ± 0.001	-7.800 ± 0.000
47	72503	-8.047 ± 0.013	-62.939 ± 0.004	-7.000 ± 0.000
48	5365659	-8.030 ± 0.321	-97.150 ± 2.938	-7.030 ± 0.050
49	6857681	-7.960 ± 0.080	-70.926 ± 0.743	-6.830 ± 0.096
50	14109432	-7.958 ± 0.005	-57.123 ± 0.001	-6.200 ± 0.000
51	6429302	-7.947 ± 0.135	-72.202 ± 0.010	-6.980 ± 0.050
52	5364174	-7.947 ± 0.013	-68.080 ± 0.128	-8.400 ± 0.000
53	5281531	-7.940 ± 0.022	-73.655 ± 0.054	-6.880 ± 0.050
54	519545	-7.897 ± 0.022	-60.860 ± 0.003	-7.300 ± 0.000
55	6440940	-7.895 ± 0.006	-62.125 ± 0.004	-7.200 ± 0.200
56	21576980	-7.890 ± 0.120	-57.999 ± 0.001	-6.600 ± 0.000
57	13213649	-7.878 ± 0.030	-78.236 ± 1.216	-7.580 ± 0.050
58	5281520	-7.842 ± 0.005	-62.109 ± 0.002	-7.400 ± 0.000
59	5365976	-7.827 ± 0.215	-75.414 ± 0.215	-7.200 ± 0.000
60	10955174	-7.810 ± 0.000	-53.005 ± 0.000	-6.800 ± 0.000

61	519762	-7.787 ± 0.202	-67.816 ± 0.004	-6.800 ± 0.000
62	15559499	-7.670 ± 0.000	-57.660 ± 0.001	-6.900 ± 0.000
63	6436348	-7.640 ± 0.000	-55.393 ± 0.004	-6.800 ± 0.000
64	196216	-7.623 ± 0.255	-72.533 ± 0.244	-7.300 ± 0.000
65	92776	-7.618 ± 0.149	-75.811 ± 0.506	-6.880 ± 0.150
66	12304273	-7.548 ± 0.051	-75.589 ± 1.204	-7.250 ± 0.058
67	11736155	-7.535 ± 0.291	-74.205 ± 0.573	-7.300 ± 0.000
68	5367460	-7.532 ± 0.155	-92.173 ± 3.264	-6.730 ± 0.222
69	5281519	-7.530 ± 0.000	-65.343 ± 0.001	-6.700 ± 0.000
70	14632996	-7.510 ± 0.195	-74.036 ± 0.221	-7.380 ± 0.150
71	6365122	-7.498 ± 0.050	-74.148 ± 1.818	-7.400 ± 0.000
72	6432312	-7.492 ± 0.005	-68.493 ± 0.000	-7.000 ± 0.000
73	5352653	-7.467 ± 0.026	-72.686 ± 0.074	-6.900 ± 0.000
74	5321293	-7.450 ± 0.203	-68.854 ± 0.013	-6.800 ± 0.000
75	521253	-7.438 ± 0.029	-74.116 ± 0.464	-7.400 ± 0.000
76	10583	-7.420 ± 0.008	-69.888 ± 0.013	-6.800 ± 0.000
77	5315469	-7.397 ± 0.170	-77.334 ± 1.148	-7.250 ± 0.129
78	12315492	-7.388 ± 0.099	-75.391 ± 0.356	-7.080 ± 0.250
79	160512	-7.345 ± 0.087	-76.563 ± 1.127	-7.530 ± 0.050
80	92139	-7.342 ± 0.244	-76.282 ± 0.035	-7.250 ± 0.100
81	10104370	-7.327 ± 0.088	-75.869 ± 0.438	-7.400 ± 0.000
82	442343	-7.202 ± 0.053	-78.148 ± 0.027	-6.800 ± 0.000
83	5282184	-7.143 ± 0.196	-92.085 ± 4.888	-6.350 ± 0.129
84	5370837	-7.002 ± 0.189	-78.169 ± 0.147	-7.180 ± 0.050
85	14014430	-6.895 ± 0.084	-76.892 ± 0.072	-7.180 ± 0.050
86	2355	-6.788 ± 0.005	-63.458 ± 0.003	-7.430 ± 0.050
87	1752	-6.723 ± 0.180	-80.090 ± 0.717	-6.500 ± 0.141
88	6430550	-6.695 ± 0.337	-77.300 ± 1.046	-6.280 ± 0.189
89	5281517	-6.613 ± 0.075	-79.940 ± 1.218	-6.350 ± 0.173
90	445070	-6.560 ± 0.125	-79.926 ± 0.969	-6.700 ± 0.115
91	8181	-6.085 ± 0.074	-84.469 ± 1.915	-5.730 ± 0.206
92	66514	-5.912 ± 0.186	-70.527 ± 1.328	-6.150 ± 0.058
93	460	-4.558 ± 0.065	-56.751 ± 0.002	-5.500 ± 0.000
