**In Vitro and In Silico Characterization of Kurarinone as a Dopamine D₁A Receptor Antagonist and D₂L and D₄ Receptor Agonist**

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**ABSTRACT:** Alterations in the expression and/or activity of brain G-protein-coupled receptors (GPCRs) such as dopamine D₁R, D₂LR, D₃R, and D₄R, vasopressin V₁AR, and serotonin 5-HT₁AR are noted in various neurodegenerative diseases (NDDs). Since studies have indicated that flavonoids can target brain GPCRs and provide neuroprotection via inhibition of monoamine oxidases (hMAOs), our study explored the functional role of kurarinone, an abundant flavonolavandulatived flavonoid in *Sophora flavesens*, on dopamine receptor subtypes, V₁AR, 5-HT₁AR, and hMAOs. Radioligand binding assays revealed considerable binding of kurarinone on D₁R, D₂LR, and D₄R. Functional GPCR assays unfolded the compound’s antagonist behavior on D₁R (IC₅₀ 42.1 ± 0.35 μM) and agonist effect on D₂LR and D₄R (EC₅₀ 22.4 ± 3.46 and 71.3 ± 4.94 μM, respectively). Kurarinone was found to inhibit hMAO isoenzymes in a modest and nonspecific manner. Molecular docking displayed low binding energies during the intermolecular interactions of kurarinone with the key residues of the deep orthosteric binding pocket and the extracellular loops of D₁R, D₂LR, and D₄R, validating substantial binding affinities to these prime targets. With appreciable D₁R and D₄R agonism and D₁R antagonism, kurarinone might be a potential compound that can alleviate clinical symptoms of Parkinson’s disease and other NDDs.

**1. INTRODUCTION**

G-protein-coupled receptors (GPCRs), also known as seven-transmembrane receptors, represent the largest set of cell membrane receptors that mediate a wide range of physiological functions and are emerging molecular targets in the drug development process. Different GPCRs have been linked to the pathophysiology of a variety of diseases including metabolic disorders (diabetes and obesity), immunological, cancer, cardiovascular, and neurodegenerative diseases (NDDs), and thus, they become potential drug targets for therapy. Of 800 human GPCRs identified, over 90% of the nonsensory GPCRs occur in the central nervous system (CNS) and regulate mood, appetite, pain, immune system, cognition, and synaptic transmission. Alzheimer’s disease (AD), vascular dementia, frontotemporal dementia, Parkinson’s disease (PD), Huntington’s disease (HD), and autism are common NDDs in which alterations in the GPCR expression and/or activity lead to different pathological conditions such as PD, multiple sclerosis, schizophrenia, attention-deficit/hyperactivity disorder, Tourette syndrome, drug abuse, AD, and HD. Thus, DAR modulators (agonists and antagonists) find applications in the management of symptoms associated with these neurological illnesses. Serotonergic 5-HT₁A receptors (5-HT₁ARs), which are abundantly expressed in the hippocampus, were found to be reduced in the hippocampal fields and raphe nuclei, whereas an increase in 5-HT₁ARs at the caudal regions of striatum was observed in chronic parkinsonian monkeys. Postmortem studies also revealed a decline in 5-HT₁ARs in the brains of AD patients. Addition-
ally, 5-HT_{1A}Rs are also implicated in the pathogenesis of anxiety and depression. Different studies indicate the significance of targeting 5-HT_{1A}Rs for ameliorating various motor (extrapyramidal disorders), mood (anxiety and depression), and nonmotor symptoms (impairment in learning and memory) in NDDs.\textsuperscript{19} Thus, 5-HT_{1A}Rs are regarded as a therapeutic target for mitigating the symptoms of NDDs. Another GPCR target that plays a crucial role in learning and memory is a vasopressin V_{1A} receptor (V_{1A}R), which is widely distributed in the CNS (septum, cerebral cortex, hippocampus, and hypothalamus). Besides a fundamental role in fluid homeostasis and blood pressure regulation, arginine vasopressin (AVP), a neurohypophyseal peptide, plays a crucial role in social recognition, learning, memory, and anxiety-regulated behavior mediated via V_{1A}R. Studies conducted on vasopressin receptor (V_{1A}R) knockout mice reported a decrease in social recognition, impairment of spatial learning, and decreased anxiety-related behavior.\textsuperscript{10,11}

Monoamines such as DA, 5-HT, epinephrine, and norepinephrine are the major GPCR ligands which are metabolized by monoamine oxidases (MAOs). Of the two isoforms, MAO-A is found primarily in catecholaminergic neurons of the locus coeruleus, where it degrades 5-HT and norepinephrine. MAO-B is located in serotonergic neurons of the raphe nucleus and glial cells and selectively oxidizes phenylethylamine and benzylamine. Other brain bioamines such as DA, tyramine, and tryptamine are deaminated by both isoforms of MAOs. MAO-B accounts for about 80% of the overall MAO activity and major striatal DA oxidation compared to MAO-A. Both MAO-A and -B were found to be elevated in the postmortem brain of patients suffering from NDDs such as AD and PD. Therefore, the inhibitors of MAOs are used as an adjunct therapy in PD and AD, while the antidepressant action is associated with the inhibition of MAO-A.\textsuperscript{12}

Sophora flavesces (Fabaceae) occurs as a wild and cultivated perennial shrub in Northeast Asia. The roots of S. flavesces (Kushen) represent an important component in the traditional Chinese medication and offer a rich source of prenylated flavonoids such as sophoravonone G, kurarinone, kuriaranol, kushenol E, kushenol F, kuriaranid, xanthohumol, and others that are known to possess antioxidant, anti-inflammatory, anti-diabetic, cytotoxic, and MAO inhibitory activities.\textsuperscript{13−15} Recently, these prenylated flavonoids have drawn attention to their potential role in NDDS owing to multiple reported bioactivities such as inhibition of β-site APP cleaving enzyme 1 (BACE 1), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE);\textsuperscript{16} suppression of MAO;\textsuperscript{17} and protection of hippocampal cells against neurotoxic chemicals.\textsuperscript{17} Kurarinone (Figure 1), a 2',4',7'-trihydroxy-5-O-methoxy-3-lavandulated flavanone, is the most abundant flavonoid in S. flavesces radix\textsuperscript{18} with a moderate to potent inhibitory action on BACE 1 (IC_{50}: 24.50 ± 1.73 μM), BChE (IC_{50}: 5.29 ± 0.67 μM), and AChE (IC_{50}: 74.28 ± 7.85 μM).\textsuperscript{19} Kurarinone has shown a considerable reduction of CNS inflammation and demyelination and the suppression of the development of autoimmune encephalitis in a murine model of MS.\textsuperscript{19} These observations suggest that kurarinone might have beneficial effects in NDDs, which involve multiple underlying causes. Additionally, an HPLC-based activity profiling study by Yang et al. identified kurarinone as a new scaffold with a flavonoid moiety possessing GABA_A receptors modulating effect (EC_{50}: 8.1 ± 1.4 μM).\textsuperscript{20} Studies have revealed that the DARs are involved in the modulation of GABA_A receptors.\textsuperscript{21,22} Thus, our present study aims to disentangle the modulatory activity of kurarinone on DA (D_1, D_2, D_3, and D_4) receptors and also on V_{1A} and SHT_{1A} receptors, since these receptors are associated with pathophysiology of the CNS disorders. Receptor binding assays and GPCR functional assays were performed together with molecular docking studies to explore the pharmacological significance of kurarinone on the tested receptors. Additionally, we investigated the human recombinant MAO (hMAO) inhibition potential of kurarinone because previous studies have found that the prenylated flavonoids such as formononetin, kushenol F, and sophoraflavonone B inhibit MAO activity.\textsuperscript{15,23}

2. RESULTS

2.1. Binding Affinity of Kurarinone for Human DA and Vasopressin Receptors. Antagonist radioligand binding assays were carried out to examine the binding affinity of 50 μM kurarinone on DA D_1R, D_2LR, D_3R, and D_4R. V_{1A}R-binding property was determined by the agonist radioligand binding assay using [3H]AVP on the recombinant CHO−V_{1A}R membrane homogenates. Table 1 presents the receptor-binding data of kurarinone. The percentage (%) inhibition of control-specific binding indicates the binding characteristic of the compound. Significant binding (>70%) by 50 μM kurarinone was observed on recombinant CHO-D_1R, HEK-D_2LR, and CHO-D_4R with the highest binding on CHO-D_4R (99.1% inhibition of control specific binding). No significant displacement of radioligands was observed from CHO-D_3R and CHO-V_{1A}R, which indicated a weak affinity of kurarinone for D_3R and V_{1A}R.

![Figure 1. Chemical structure of kurarinone.](image-url)
behavior is indicated by % inhibition of control agonist response. On preliminary screening of functional activity, 100 μM kurarinone showed notable agonist response on D2LR and D4R with % stimulation of a control agonist response of 113 ± 28.9 and 71.7 ± 6.36%, respectively. It had a negligible agonist effect on D1R and 5HT1AR. In the antagonist mode

Table 1. DA D1, D2, D3, and D4 and Vasopressin V1A Receptor-Binding Data of Kurarinone

| receptors        | radioligand | % inhibition of control-specific binding (%) | reference antagonist     | reference IC50 |
|------------------|-------------|---------------------------------------------|--------------------------|----------------|
| D1               | [3H]SCH-23390 | 99.1                                        | SCH23390                 | 0.5            |
| D2L              | [3H]methy dịpiperone | 78.5                                      | (+)butaclamol             | 2.6            |
| D3               | [3H]methy distânciaperone | 49.2                                      | (+)butaclamol             | 4.6            |
| D4               | [3H]methy distanciaperone | 71.1                                      | (+)butaclamol             | 93             |
| V1A              | [3H]AVP     | 47.6                                        | [d(CH2)5,Tyr(Me)2]-AVP    | 1.4            |

Values are presented as the mean of percent inhibition of control-specific binding by 50 μM kurarinone performed in duplicate. Concentration producing 50% inhibition of the control-specific binding by the reference antagonists (nM). Agonist radioligand binding assay.

Table 2. Functional Effect (% Stimulation and % Inhibition) and Efficacy (EC50 and IC50) of Kurarinone on Human DA (hD1, hD2L, and hD4) and Serotonin (h5-HT1A) Receptors

| receptors | % stimulation (%) | EC50 (IC50) | reference agonist (reference antagonist) | reference EC50 (IC50) |
|-----------|-------------------|-------------|------------------------------------------|----------------------|
| hD1       | 0.25 ± 0.64% (98.5 ± 1.48)% | 42.1 ± 0.35 | DA (SCH-23390)                           | 44 (1.3)             |
| hD2L      | 113 ± 28.8% (43.8 ± 25.9)% | 22.4 ± 3.46 | DA (butaclamol)                          | 31 (26)              |
| hD4       | 71.7 ± 6.36% (−4.20 ± 2.55)% | 71.3 ± 4.94 | DA (clozapine)                           | 13 (520)             |
| h5-HT1A   | 17.8 ± 1.13% (30.3 ± 6.58)% |              | serotonin ((S)-WAY-100635)               | 2.9 (7.0)            |

% stimulation represent % stimulation of control agonist effect, by kurarinone at 100 μM. % inhibition represent % inhibition of control agonist effect, by kurarinone at 100 μM. Concentration required to exhibit 50% of control agonist effect (μM). Concentration required to inhibit 50% of control agonist effect (μM). Values denoted as mean ± S.D. from a duplicate experiment. Reference agonists for each assay. Reference antagonists for each assay. EC50 of the reference agonist (nM). IC50 of the reference antagonist (nM). AGO: the test compound induced at least 25% agonist or agonist-like effect at this concentration. Different letters indicate a significant difference of kurarinone’s effect on different receptors (p < 0.05, Duncan’s test). (−) Not tested.

Figure 2. Concentration-dependent percentage inhibition of control agonist response of kurarinone on hD1R (A). Concentration-dependent percentage of control agonist response of kurarinone on hD2R (B) and hD4R (C).

Figure 3. Molecular docking of kurarinone (yellow stick) to the hD1R model (A). Zoom-in view of the ligand-binding pocket of the hD1R model in complex with kurarinone obtained from docking simulation (B). The superscript refers to the Ballesteros-Weinstein numbering system for GPCR. Two-dimensional representation of the binding mode of kurarinone with key amino acid residues of hD1R (C).
assays, 100 μM kurarinone displayed a significant effect on D1R with the % inhibition of control agonist response of 98.5 ± 1.48%. On D2LR, it showed 43.8 ± 25.9% antagonist effect but with at least 25% agonist response. Thus, kurarinone cannot produce full antagonist response and results in an apparent inhibition only. Similarly, 100 μM compound inhibited the maximal control agonist response on 5HT1AR moderately with 30.3 ± 6.58% inhibition and had no antagonist effect on D4R. From these observations, kurarinone is detected to have a significant D1R antagonist effect and a D2LR and D4R agonist effect, measured at 100 μM.

Further functional evaluations were made using lower concentrations of kurarinone on D1, D2L, and D4 receptors which revealed its dose-dependent antagonist activity on D1R with an IC50 of 42.1 ± 0.35 μM and dose-dependent agonist effects on D2LR and D4R with an EC50 of 22.4 ± 3.46 and 71.3 ± 4.94 μM, respectively. Figure 2 shows the concentration-dependent modulatory effects of kurarinone on DAR subtypes along with the corresponding EC50 and IC50 values.

2.3. Molecular Docking of Kurarinone with DA D1, D2L, and D4 Receptors. To understand the interactions of kurarinone within the active sites of D1R, D2LR, and D4R, we conducted computational docking studies using the homology model for human D1R and the crystallographic structures of 6CM4 and 5WIV for hD2LR and hD4R, respectively. The binding energies and the ligand–receptor interactions obtained from the in silico analysis are tabulated in Table S1. The best docking poses (with the lowest binding energy) obtained for the kurarinone–hD1R, hD2LR, and hD4R complexes, along with the corresponding ligand–protein interactions, are shown in Figures 3–5, respectively.

Figure 4. Molecular docking of kurarinone (yellow stick) to the hD1R model (A). Zoom-in view of the ligand-binding pocket of the hD1R model in complex with kurarinone obtained from docking simulation (B). The superscript refers to the Ballesteros–Weinstein numbering system for GPCR. Two-dimensional representation of the binding mode of kurarinone with key amino acid residues of hD1R (C).

Figure 5. Molecular docking of kurarinone (yellow stick) to the hD2LR model (A). Zoom-in view of the ligand-binding pocket of the hD2LR model in complex with kurarinone obtained from docking simulation (B). The superscript refers to the Ballesteros–Weinstein numbering system for GPCR. Two-dimensional representation of the binding mode of kurarinone with key amino acid residues of hD2LR (C).
Additional H-bonding occurred with Asp103, Ser107, Ala195, and Ser199.

The docking study on DA hD2LR demonstrated that the chroman-4-one moiety of kurarinone was located in the deep binding pocket within the TM1, 3, 5, and 6, interacting with Asp115, Val111, Phe110, Ser193, Phe390, and Phe389. Through hydrophilic and hydrophobic mechanisms via different bonds, as shown in Figure 4C, whereas the dihydroxyphenyl and lavandulyl or 8-(5-methyl-2-prop-1-en-2-ylhex-4-enyl) substituents extended toward the extracellular dihydroxyphenyl and lavandulyl or 8-(5-methyl-2-prop-1-en-2-ylhex-4-enyl) substituents extended toward the extracellular part of TM7 and interacted with the residues defining the hD2LR extended binding pocket (D2-EBP) such as Trp110 (ECL1), Ile184 (ECL2), and Tyr408.63 and Tyr416.74 (TM7) via both H-bonds and nonpolar interactions. These interactions might have resulted in high affinity binding of kurarinone to the hD2LR (−7.46 kcal/mol). In our experiment, reference agonist (DA) and antagonist (butaclamol) did not engage with the ECL residues (Table S1).

As shown in Figure 5, the molecular docking of kurarinone with hD2LR presented a lower binding energy of −7.61 kcal/mol (strong binding) in comparison with DA but a higher energy requirement compared to the D2R antagonist, nemonapride (−10.5 kcal/mol). For D2R modulatory activity, specific interaction with a highly conserved residue Asp115 through H-bonding interactions such as a salt bridge and −OH bond was identified vital using agonist DA and antagonist nemonapride. Kurarinone displayed binding with Asp115 through H-bonding and electrostatic interactions through the 2’-OH group and the phenyl ring B, respectively. The lavandulyl substituent together with the 7-hydroxy-5-methoxychromen-4-one ring of kurarinone exhibited hydrophobic interactions with conserved orthosteric binding pocket (OBP) lined by residues such as Leu83, Val116, Leu118, Cys119, Trp407, Phe410, Phe411, Phe390, and Tyr438. Additionally, the 2,4-dihydroxyphenyl moiety extended to bind with extracellular conserved residues Cys185 and Leu187 of ECL2 via H-bonding and π–alkyl interaction with the 4’-OH group and phenyl ring B, respectively.

2.4. hMAO Inhibition by Kurarinone. Recombinant human MAO inhibitory activity of kurarinone was studied using a chemiluminescent assay in a 96-well opaque white plate using an MAO-Glo kit. On testing the compound at different concentrations (456, 228, 114, and 57 μM) against hMAO-A and hMAO-B, dose-dependent inhibition was observed, as shown in Figure 6. Kurarinone exhibited a very weak inhibitory effect on both hMAO-A and hMAO-B with IC₅₀ values of 186 ± 6.17 and 198 ± 12.66 μM, respectively (Table 3). In the kinetic analyses, kurarinone showed competitive inhibition of hMAO-A with Kᵢ values of 65.1 ± 3.53 μM, whereas it showed mixed inhibition of hMAO-B (Table S2).

Table 3. Recombinant hMAO Inhibitory Activity of Kurarinone and the Reference Drug

| compound       | hMAO-A IC₅₀a (μM) | hMAO-B IC₅₀b (μM) | SIb |
|----------------|-------------------|-------------------|-----|
| kurarinone     | 186 ± 6.17        | 198 ± 12.7        | 0.94|
| l-deprenyl-HCl | 15.6 ± 0.67       | 0.17 ± 0.01       | 91.94|

“*The 50% inhibition concentration (IC₅₀) values (μM) were calculated from a log dose inhibition curve and expressed as the mean ± SD of triplicate experiments. *The selectivity index (SI) was determined as the ratio of IC₅₀ for hMAO-A inhibition to IC₅₀ for hMAO-B inhibition. **Positive control, expressed as μM.**
interactions with the aromatic rings of critical catalytic tyrosine residues were missing in the complexes of kurarinone and hMAO-A/B.

2.5. In Silico Prediction of Drug-Likeliness and ADME. Table 4 presents the pharmacokinetic parameters assessed for kurarinone using the PreADMET application. Kurarinone followed Lipinski’s rule of five, thus indicating suitability for oral administration.29 Also, it fitted to the MDDR-like rule and was found to have drug-like characteristics. The log Po/w value was anticipated to be 5.5 for the test compound. Kurarinone exhibited >90% of plasma protein binding and high intestinal absorption (>70%). Permeability across Coca-2 and MDCK cells was expected to be 0.05 and 22.93 nm/s, respectively. For molecules targeting the CNS, they should pass readily through the blood—brain barrier (BBB). Kurarinone was estimated to have high permeability across the BBB as the ratio of concentration in the brain relative to the blood was 4.02. Overall, PreADMET results indicated kurarinone as a suitable candidate for drug development with the nonmutagenic property. However, these predicted results can vary from the experimental values.

3. DISCUSSION

Over the past decade, different flavonoids have been identified with a potential role in NDDs through multiple mechanisms such as anti-inflammatory, antioxidant, and anti-apoptosis. Experimental and clinical studies provide evidence on the improvement of cognition and learning by flavonoids through the modulation of neuronal signaling pathways, facilitating neurogenesis and inhibiting neurodegeneration and neuro-inflammation.31-33 Nevertheless, research on the modulatory activity of flavonoids on GPCRs is limited.34 In our previous work, we reported luteolin, a 3’,4’,5,7-tetrahydroxyflavone, to be a selective antagonist of V1AR and D4R and a selective hMAO-A inhibitor.35 Our present study found that kurarinone at 50 μM could significantly bind to the hD1R, hD2LR, and hD4R in the order of hD1R > hD2LR > hD4R. From the GPCR functional assay, kurarinone was identified to have an hD2LR agonist effect (EC50 22.4 ± 3.46 μM) and an antagonist effect on hD1R (IC50 42.1 ± 0.35 μM). It also displayed substantial binding to hD4R and stimulated the receptor with an EC50 of 71.3 ± 4.94 μM. No significant binding and activity were observed on SHT1A3R and V1A3R.

In silico docking was performed to understand the molecular interactions of kurarinone at the active sites of the hD1R, hD2LR, and hD4R. The molecular docking of DA with the hD1R model detected the H-bond formation between the aromatic hydroxyl groups of DA and Asp1033.32 (salt bridge), Ser2025.46, Ser1985.42, and Ser1995.43. Investigation of binding modes of D1-selective antagonist SCH23390 (R) to the model revealed H-bond interaction with Asp1035.32, Ala1955.39, and Ser1995.43, which were also present with the kurarinone-receptor association. Binding to Ser1995.43 of TM5 and Ser1075.36 (TM3), as well as to TM6 residues, has been reported liable for the higher binding affinities of D1R antagonists to the receptor.36,37 These molecular interactions might account for the notable antagonist behavior of kurarinone on hD1R. Likewise, kurarinone displayed interactions with the defined residues of D2LR-EBP in addition to that with the reactive loci of binding sites in D2LR. These interactions were similar to those observed with the inverse agonist risperidone–D2R complex.38 Generally, DA D2R antagonists bind tightly with serine residues Ser1933.42 and Ser1943.43 of TM5 via H-bonds together with Asp1143.32 in TM4 via salt bridge formation.39 These findings are consistent with the interaction observable in D2L–kurarinone binding, as the compound is anchored with Ser1933.42 and Asp1143.32 by H-bonds. Moreover, the D2LR binding was stabilized by alky1 and α-alkyl interactions with the highly conserved hydrophobic residue of ECL2, Ile184.38 In silico docking revealed that kurarinone binds to the conserved OPB and EBP residues of hD4R. Interactions of the compound with hD4R OPB residues such as Asp1153.31, Val1163.37, Cys1194.36, and Phe4114.32 are common to those found in the complexes of reference ligands (DA and nemonapride) and hD4R.

Even though earlier studies have reported hMAO inhibition potential of some prenylated flavonoids from S. flavescens, the ability of kurarinone to act on hMAO was unknown.38,39 Thus, we examined the activity of kurarinone on hMAO-A and hMAO-B and observed a modest and nonselective inhibition of hMAO isoenzymes. Formerly, a structurally similar flavanone but devoid of a 5-0-methoxy substituent, sophoravone G, had displayed selective hMAO-A inhibition with an IC50 value of 38.8 ± 1.1 μM.37 The docking study demonstrated that the lavundulyl substituent in the structure of kurarinone facilitated most of the hydrophobic interactions with the enzymes. However, the weak activity of the compound might be due to the hindrance by the same moiety to make aromatic sandwich interactions between the aromatic rings of kurarinone with the catalytic recognition sites of the enzymes (Tyr residues and FAD).

DAR modulators have been implicated under different neurological conditions that involve abnormalities in dopaminergic (DAergic) transmission and its receptors. For instance, the loss of DAergic neurons resulting in a reduced level of DA in basal ganglia is the pathophysiological abnormality of PD, and to treat this condition, DAR agonists and DA precursor L-dopa are used to compensate for the loss or lack of endogenously produced DA,8 whereas DAR antagonists are implicated in schizophrenia, where hyper-activation of the DAergic system occurs in mesolimbic pathways.40

Table 4. In Silico Prediction of Drug-Likeliness, ADME Characteristics, and Toxicity of Kurarinone

| compound | drug-likeness | ADME characteristics | toxicity |
|----------|---------------|----------------------|---------|
|          | MDDR-like rule | Lipinski’s rule | log Pw | PPB | HIA | MDCK cell permeability (nm/s) | Caco2 permeability (nm/s) | BBB penetration ([brain]/[blood]) | Ames test |
| kurarinone | drug-like | suitable | 5.55 | 99.3 | 92.3 | 0.05 | 22.9 | 4.02 | non-mutagen |

“Lipophilicity represented by the n-octanol–water partition coefficient. Plasma protein binding (<90% denotes weak binding and >90% denotes strong binding). Human intestinal absorption (0–20%: poorly absorbed; 20–70%: moderately absorbed; and 70–100% is well-absorbed). Permeability across Madin–Darby canine kidney (MDCK) cells. Permeability across human colorectal adenocarcinoma cells (0–10: low permeability; 10–100: medium permeability; and >100: high permeability). Permeability across the BBB (<0.1: low absorption; 0.1–2.0: middle absorption; and >2.0: high absorption).
PD is characterized by progressive damage to the nigrostriatal DAergic neurons involving reactive oxygen radical-mediated and iron-dependent lipid peroxidation that eventually leads to ischemia-induced brain injury. D1 receptor agonists such as pramipexole, bromocryptine, lisuride, pergolide, and ropinirole afforded prominent protection against neurotoxin-induced cell apoptosis. 41–43 Likewise, D1/D3R agonist and 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT) induced significant neurogenesis in the SNc of the adult rat brain along with the betterment of locomotor function and enhanced reward learning. 44,45 Stimulation of D1R has been related to novelty-seeking character, memory consolidation, and the striatal motor process. 46–48 A recent systematic review and meta-analysis by Pan and colleagues reported that the DA level was considerably lowered in AD patients along with the decrease in the levels of DARs. Clinical studies engaging pharmacological intervention by L-dopa and DAR agonists in AD patients have found improvement of cognitive ability and cortical plasticity. 49 Therefore, the benefits of DAR modulators and DA replacement therapy are not limited to PD.

D1R activation induces cAMP-dependent activation of protein kinase (PKA) and increases the level of intracellular calcium (Ca2+) levels via opening of L-type calcium channels. Lebel and colleagues reported that the D1R activation-induced physiological changes mediate phosphorylation of tau proteins. Their study also showed that neither the D3R agonism nor the reduction of cAMP levels affects tau-phosphorylation and cellular damage. 50 These observations suggest that kurarinone might play a plausible role in AD and other dementia via D1R antagonism, as tau-hyperphosphorylation has been known to cause neuronal dysfunction and implicated in AD pathogenesis. 51 Moreover, SCH23390 also blocked the neurotoxicity caused by DA-induced D1R signaling and autoxidation. 52 Increased D1R signaling has also been associated in the pathogenesis of L-dopa-induced dyskinesia (LID) by different preclinical studies. 53–55 Grondin et al. reported that selective D1R blockade reduces LID and, however, aggravates parkinsonism in parkinsonian monkeys. 56

ADMET assessment by PreADMET predicted kurarinone to be a drug-like molecule capable of crossing the BBB (>

4) (Table 4). Corroborating with the in silico prediction, previous in vivo studies have demonstrated high absorption and clearance and moderate bioavailability of kurarinone. 57,58 Despite a favorable ADMET profile, Jiang et al. 59 and Yu et al. 60 in independent studies have detected kurarinone as a hepatotoxic compound in the S. flavescens extract owing to the hepatic accumulation of the compound and inhibition of fatty acid β-oxidation, resulting in lipid accumulation in the liver. In contrast, another study by Nishikawa and colleagues found the induction of heme oxygenase-1 by kurarinone via activation of the KEAP1/Nrf2 pathway and Nrf2 activation plays a protective role against hepatotoxicity. 61 The metabolic study suggests that glucuronidation plays a vital role in detoxification by the formation of soluble compounds that can be eliminated readily from the body. High hepatic accumulation and toxicity of kurarinone may result when the activity of UDP-glucuronosyltransferase is limited and high concentrations of kurarinone occur in the liver. 62 Studies on the mechanism of hepatic injury by kurarinone and the possible derivatization of the compound to generate functional derivatives with reduced hepatotoxicity may be important to establish kurarinone as a suitable drug-like molecule for the treatment of different diseases.

4. CONCLUSIONS

Our study evaluates DARs, V1aR, and 5-HT1aR modulatory activities and hMAO inhibition capacities of kurarinone for the first time. The knowledge of the pharmacological importance of flavonoids on GCPR modulation is scarce. Functional GPCR screening showed that kurarinone possessed D1R and D3R agonist properties along with the ability to fully antagonize D2R. For multifactorial complexities associated with NDDs such as AD and PD, molecules with a multitarget nature and minimal toxicity are anticipated to be potential therapeutics. 63 Thus, multifunctional nature of kurarinone might be beneficial for targeting different underlying causes of NDDs and ameliorating the associated symptoms. Further in vivo studies are necessary to support the in vitro DAR modulatory effects and observe the efficacy of kurarinone in alleviating DAergic neurodegeneration and disease conditions in animal models.

5. MATERIALS AND METHODS

5.1. Chemicals and Reagents. Kurarinone was isolated from the ethyl acetate fraction of S. flavescens radix as reported in our earlier work. 16 Recombinant Chinese hamster ovary (CHO) and human embryonic kidney (HEK-293) cells were generated by Eurofins Discovery (Le Bois l’ Eveque, France). ThermoFisher Scientific (Madison, U.S.A.) provided cell-culture media such as Roswell Park Memorial Institute (RPMI-1641), Dulbecco’s modified Eagle medium buffer, Hank’s balanced salt solution (HBSS) buffer, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer. The MAO-A/B assay kit was acquired from Promega (Promega Cooperation, Madison, WI). The recombinant hMAO isoenzymes and the reference drugs: -deprenyl-HCl, DA, S-HT, AVP, clozapine, (+) butaclamol, SCH 23390, (S)-WAY-100635, and [d(CH2)5]-Tyr(Me)2]-AVP were supplied by Sigma-Aldrich (St. Louis, MO, USA). The remaining chemicals of reagent grade were procured from commercial suppliers.

5.2. Radioligand Binding Assays. The binding affinity of kurarinone to the membrane cloned with human DA and vasopressin receptors was characterized by the radioligand binding assays following the validated methods and standard procedures developed at the Eurofins Cerep (Le Bois l’ Eveque, France). The experimental methods for the binding assays were similar as described by Chen et al. and Gorbunov et al. 54,65 Human D1R, D2R, D3R, and V1aR binding was assessed using the membrane homogenates of the transfected CHO cells. For D1, D2, and D4 receptors, the respective membrane homogenates were suspended in buffer solution comprising 50 mM Tris–HCl (pH 7.4), 5 mM KCl, 5 mM CaCl2/120 mM NaCl, and 5 mM EDTA and incubated with 0.3 nM appropriate radioligands, [3H] SCH23390 (for D1R) and [3H] methylspiperone (for D2R and D3R), at 22 °C for 1 h in the presence or absence of test compounds. In the case of V1aR binding, the membrane homogenates were diluted in a

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binding buffer consisting of 50 mM Tris–HCl (pH 7.4), 5 mM MgCl₂, and 0.1% BSA and incubated with [³H] AVP at 22 °C for 1 h. D₂LR binding was evaluated using the plasma membrane homogenates from the transfected HEK-293 cells. The membrane homogenates of D₂LR expressing HEK-293 cells were suspended in binding buffer (50 mM Tris–HCl, 5 mM MgCl₂, 1 U/l mL ADA, 1 mM EDTA, 1 μg/mL leupeptin, 1 μg/mL pepstatin, and 10 μg/mL trypsin inhibitor, pH 7.4) and incubated with 0.3 nM [³H] methylspiperone for 1 h at 22 °C for 1 h. D₂LR binding was assessed by measuring Ca²⁺ ion mobilization (for D₂L and HT1A receptors). Likewise, crystal structures of L-deprenyl, clorgyline, harmine, and nemonapride were also acquired from PubChem database and subsequently used. The 3D chemical structure of kurarinone was attained using the FilterMax F5 Multimode Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). An enzyme kinetics experiment was performed using different concentrations of the hMAO substrate (80 to 240 μM for hMAO-A and 4–16 μM for hMAO-B) and kurarinone (0–400 μM). The inhibition constant (Kᵢ) was derived from the secondary plots obtained using SigmaPlot 12.0 TM software (SPCC Inc, Chicago IL, USA). Lineweaver–Burk and Dixon plots were used to determine the mode of enzyme inhibition.

5.4. Human Monoamine Oxidase-A and -B Inhibitory Assay and Enzyme Kinetics. The hMAO-A and -B inhibition potential of kurarinone was examined using an MAO-Glo assay kit in an opaque white 96-well plate following the manufacturer’s protocol as described in our earlier report. As this assay is based on measuring luminescence produced by luciferin at the end of the reaction of the enzyme with substrates and test samples. Briefly, the initial reaction system in each well of the plate consisted of 12.5 μL of substrate (beetle luciferin derivative, 40 and 4 μM for hMAO-A and hMAO-B, respectively), 12.5 μL of kurarinone or L-deprenyl solutions (of varying concentrations), and 25 μL of enzyme solution. The mixture was kept at 25 °C. Following incubation, the enzyme reaction was ceased by adding 50 μL of luciferin detection reagent. The reaction plate was incubated for an extra 20 min at 25 °C, and luminescence from the plate was measured using a FilterMax F5 Multimode Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). An enzyme kinetics experiment was performed using different concentrations of the hMAO substrate (80 to 240 μM for hMAO-A and 4–16 μM for hMAO-B) and kurarinone (0–400 μM). The inhibition constant (Kᵢ) was derived from the secondary plots obtained using SigmaPlot 12.0 TM software (SPCC Inc, Chicago IL, USA). Lineweaver–Burk and Dixon plots were used to determine the mode of enzyme inhibition.

5.5. Homology Modeling. The primary sequence for hD₃R was retrieved from the UniProt database having the IDs P21728 (DRD1_HUMAN). The template of the β₂ adrenergic receptor (β₂R) crystal structure with PDB ID 2RH1 was used for modeling of hD₃R using Swiss-Model server because high sequence similarity is found in the overall structure and binding sites between D₂R and β₂R. The homology model was refined using ModRefiner server.

5.6. Molecular Docking. AutoDock 4.2 program was run to conduct molecular docking and gain insights into the intermolecular interactions between kurarinone/reference ligands and receptors/enzymes. X-ray crystallographic structures with PDB IDs 6CM4, SWIV, 2BXR, and 2V60 for hD₂R, hD₃R, hMAO-A, and hMAO-B, respectively, were used. The 3D chemical structure of kurarinone was attained from the PubChem compound database with CID 11982640. Likewise, crystal structures of L-deprenyl, clorgyline, harmine, 7-[(3-chlorobenzoyl)oxy]-2-oxo-2H-chromene-4-carbaldehyde (C-17), DA, SCH23390, risperidone, butaclamol, and nemonapride were also acquired from PubChem database with CIDs S1945, 4380, 5280953, 16750123, 681, 5018, 5073, 37461, and 156333, respectively. Discovery Studio (v17.2, Accelrys, San Diego, USA) was used for enzyme/receptor preparation. AutoDock Tool was employed for docking simulation and analyzing Autodock dockings by adding necessary parameters such as H-bonds, Gasteiger charge, and rotatable bonds. Grid maps were calculated using AutoGrid.
The grid points were generated automatically by centering on ligands (co-crystallized/reference), and the grid box was 60 × 60 × 60. Initially, the co-crystallized ligands were redocked with the proteins, and at the lowest binding energy, their binding interactions were found similar to the original PDB protein—ligand interactions to verify the docking protocol. A total of 10 independent genetic algorithms were set as docking protocols for the rigid as well as flexible ligand docking. The docking results were evaluated by AutoDockTools and visualized using Discovery Studio. The generic residue numbers (Ballesteros-Weinstein numbers) for interacting residues of DARs were obtained from the GPCR database system (https://gpcrdb.org).72

5.7. Drug-Likeliness and ADME Prediction. The absorption, distribution, metabolism, elimination, toxicity, and drug-likeliness characters of kurarinone were estimated by employing PreADMET (http://preadmet.bmdrc.org). Pharmacokinetic parameters, viz., lipophilicity (log \( P \)), protein binding interactions were found similar to the original PDB with the proteins, and at the lowest binding energy, their 60 × numbers (Ballesteros-Weinstein numbers) for interacting residues of DARs were obtained from the GPCR database system (https://gpcrdb.org).72

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04109.

Binding energies and interacting residues of kurarinone and reference ligands in the binding site of human DA (hD1, hD2L, and hD4) receptors; kinetic constants and inhibition mode of kurarinone for the inhibition of hMAO-A and hMAO-B; binding energies and interacting residues of kurarinone and reference ligands in hMAO-A and hMAO-B; Lineweaver–Burk and Dixon plots for the inhibition of hMAO-A and hMAO-B by kurarinone, respectively, and secondary plots for the inhibition of hMAO-B by kurarinone; molecular docking of kurarinone (red stick), harmine (HRM, blue stick), and deprenyl (black stick) with hMAO-A; zoom-in view of the ligand-binding site of hMAO-A, two-dimensional representation of interactions of kurarinone with the catalytic site residues of hMAO-A, and structure of cofactor FAD is shown using a cyan blue stick; and molecular docking of kurarinone (red stick), C17 (pink stick), and deprenyl (black stick) with hMAO-B, zoom-in view of catalytic and allosteric binding sites of hMAO-B, and two-dimensional representation of interactions of kurarinone with the catalytic and allosteric site residues of hMAO-B (PDF)

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Notes
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