

ORIGINAL RESEARCH ARTICLE

Effect of Darjeeling black tea aromatics on central nervous system function: An *in silico* study of glutamate receptor–ligand interactions

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Abstract

Darjeeling tea (*Camellia sinensis* var. *sinensis*) is recognized for its unique aroma and taste, associated with mood and cognitive enhancement. However, the underlying neurochemical mechanisms remain unclear. The present study investigates the potential interaction of Darjeeling tea's volatile aroma compounds with glutamate receptors (GluRs), the predominant excitatory receptors in the central nervous system (CNS). We hypothesized that these compounds target GluRs to elicit their effects. An *in silico* approach was employed to analyze the physicochemical properties, bioactivity scores, and toxicity profiles of the aroma compounds. Subsequently, molecular docking simulations were performed using the retrieved 3D structures of relevant GluRs to predict the binding affinity of selected compounds exhibiting high bioactivity, drug-likeness, and bioavailability, and identify key amino acid residues within the receptor binding pockets. Our findings revealed α -ionone and safranal as prominent ligands exhibiting strong binding interactions. Among metabotropic GluRs, mGluR1 (IEWK), GluR5 (3FUZ), and GluR6 (3G3F) showed the highest affinity. Ionotropic receptor subtypes α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (2WJW) and N-methyl-D-aspartate receptor (7EOR) also displayed significant binding scores, where greater structural dynamics were found in metabotropic GluRs on ligand binding compared to ionotropic subtypes. Given that inhalation via the nasal passage is a primary exposure route and the presence of GluR-expressing cells along this pathway, the high bioavailability of α -ionone and safranal suggests their potential to interact with neuroglial cells and subsequently influence CNS neurons and microglia/macrophages. In conclusion, the identified binding of Darjeeling tea's aromatic ligands and GluRs offers a promising framework for elucidating the mechanisms underlying the tea's effects on mood, psychological states, and immune-physiological responses.

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1. Introduction

Black tea, particularly harvested and processed from the slopes of the Darjeeling Himalayan region, is famous for its rich flavor and is widely consumed worldwide

and is valued for its distinctive flavor. The Darjeeling tea (*Camellia sinensis* var. *sinensis*), famous for its rich aromatic properties, has been associated with mood-related effects and other bioactivities, including roles in metabolic regulation, atherosclerosis, inflammation, and cancer.^{1,2} Chemical analysis of the black tea processed using a specific extraction procedure shows a rich presence of polyphenol and catechin compounds. Theaflavins and thearubigins, in particular, contribute to its distinctive coloration upon brewing.^{3,4} Recent studies have reported anti-cancer activity of theaflavins and other compounds from black tea.⁴⁻⁶ In addition, tea polysaccharides are gaining attention for their potential in managing metabolic diseases. They are also noted for their prebiotic effects, skin-care benefits, and predicted anti-tumor activity.^{7,8}

However, the aroma compounds responsible for the characteristic flavor of Darjeeling black tea and their potential contributions to psychophysiological stress and mood responses have yet to be properly evaluated. In a comparison between Darjeeling and Assam tea, Yoto *et al.*⁹ showed that black tea aroma can attenuate stress-related changes in brain function by attenuating autonomic stress responses after inhalation, as reflected by reduced salivary chromogranin-A released from adrenal chromaffin cells and adrenergic neurons, thereby improving mood as observed in tension-anxiety score in Profile of Mood States. Odor stimuli derived from black tea are the aromatic volatile compounds that reach the olfactory tract and bulb, where olfactory nerve receptors bind the molecules and trigger corresponding signals to the brain.¹⁰ This indicates a receptor-ligand interaction between the aroma compounds and receptors on the neuronal projections. As the Darjeeling tea aroma has a mood-enhancing effect, we hypothesize that such compounds may bind to glutamate receptors (GluRs), as glutamate is the most abundant excitatory neurotransmitter found in the nervous system.¹¹ GluRs are widely expressed in neurons and glial cells and are broadly classified into metabotropic and ionotropic families. Metabotropic GluRs (mGluR1-8) are further subdivided into three groups, whereas ionotropic GluRs include α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) (GluR1-4) and N-methyl-D-aspartate receptor (NMDA).^{11,12} In addition, the olfactory nerve fibers around the olfactory bulb are accompanied by the immunoregulatory cells of the brain, that is, microglia, which are highly responsive and active protectors of the central nervous system (CNS) against any microenvironmental modifications in the tissue. GluRs are also highly expressed on these myeloid lineage cells.¹³ Therefore, it is likely that tea aroma compounds bind to

GluRs on both neurons and microglia or macrophages. If volatile aroma compounds from Darjeeling black tea bind to these receptors, they can trigger both neurophysiological and neuro-immune cascades, thereby relieving stress and improving mood.

In our investigation, we selected specific volatile aroma compounds associated with the characteristic Darjeeling black tea aroma, not necessarily containing a benzene ring, and validated their molecular properties as ligand candidates. Both metabotropic and ionotropic GluRs identified in neuronal cells, microglia, and macrophages were selected for their stable structural forms and subjected to *in silico* receptor-ligand binding and docking. This approach estimates the likelihood of receptor-ligand interactions of tea aroma compounds to GluRs, thereby providing valuable insights into potential mood-enhancement pathways and aroma-driven modulation of immune functions.

2. Materials and methods

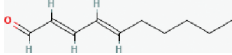
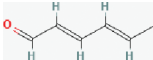
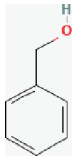
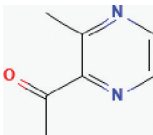
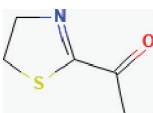
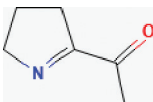
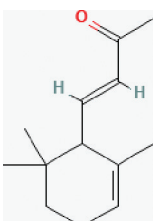
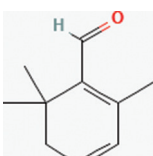
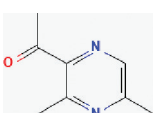
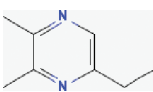
2.1. Selection and preparation of ligands

Available aroma compounds in Darjeeling black tea were screened and selected based on a literature search of online databases, including Web of Science, Scopus, ScienceDirect, PubMed, and others, with an emphasis on studies that analyzed black tea chemical composition and aromatic formation.^{3,14,15} We selected 10 specific fragrant compounds characterizing the Darjeeling tea aroma of the first and second flush harvests, regardless of whether they contain aromatic rings (Table 1). Molecules were retrieved from online chemical database: PubChem (www.pubchem.ncbi.nlm.nih.gov/) and later converted to their three-dimensional (3D) Protein Data Bank (PDB) format using Open Babel software (Version 2023). A PDBQT format file was created after adding hydrogens and defining rotatable bonds (torsions) to a PDB format file,¹⁶ and the 3D models of the molecules are shown in Figure 1.

2.2. Validation of ligands

The lack of pharmacokinetic studies is one of the main obstacles in the commercial implementation of plant-based products as therapeutic agents. Thus, we used computational methods to evaluate the physicochemical, pharmacokinetic, and drug-likeness properties. Physiologically significant properties of a ligand, designated by absorption, distribution, metabolism, and excretion (ADME), were assessed using the SwissADME website (<https://www.swissadme.ch/>).¹⁷ Ligand toxicity was predicted to assess potential systemic toxicity using molecular data of compounds with similar physical,

Table 1. Specific fragrant compounds characterizing the Darjeeling tea aroma of the first and second flush harvests

No.	Fragrant compounds	Characteristic fragrance (when exposed to air and matured/aged)	Two-dimensional chemical structure
1	(E, E)-2,4-decadienal	Citrus, on maturation fatty, fried odor	
2	(E, E)-2,4-hexadienal	Citrus, on maturation fatty, fried odor	
3	Benzyl alcohol	Fruity to almond-like burnt odor	
4	2-Acetyl-3-methylpyrazine	Roasted nutty and/or grain-roasted odor	
5	2-Acetyl-2-thiazoline	Nutty, popcorn-like roasted odor	
6	2-Acetyl-1-pyrroline	Cooked rice, popcorn-like odor	
7	α -Ionone	Hay-like, iris, violet, warm-woody, faint balsamic-floral odor	
8	Safranal	Spicy saffron-like note with herbaceous, tobacco facets and floral undertones	
9	2-Acetyl 3,5-dimethylpyrazine	Nutty, roasted hazelnut odor	
10	5-Ethyl 2,3-dimethylpyrazine	Nutty to deep roasted cocoa-like odor	

structural, and chemical properties from the online server PreADMET (<https://preadmet.bmdrc.kr/>). In the PreADMET server, the structural data files retrieved from the PubChem were converted to mol2 format using

Open Babel software and then submitted to the online server for toxicity prediction, including mutagenicity (Ames mutagenicity test), carcinogenicity, and human Ether-à-go-go-related gene (hERG) inhibition.

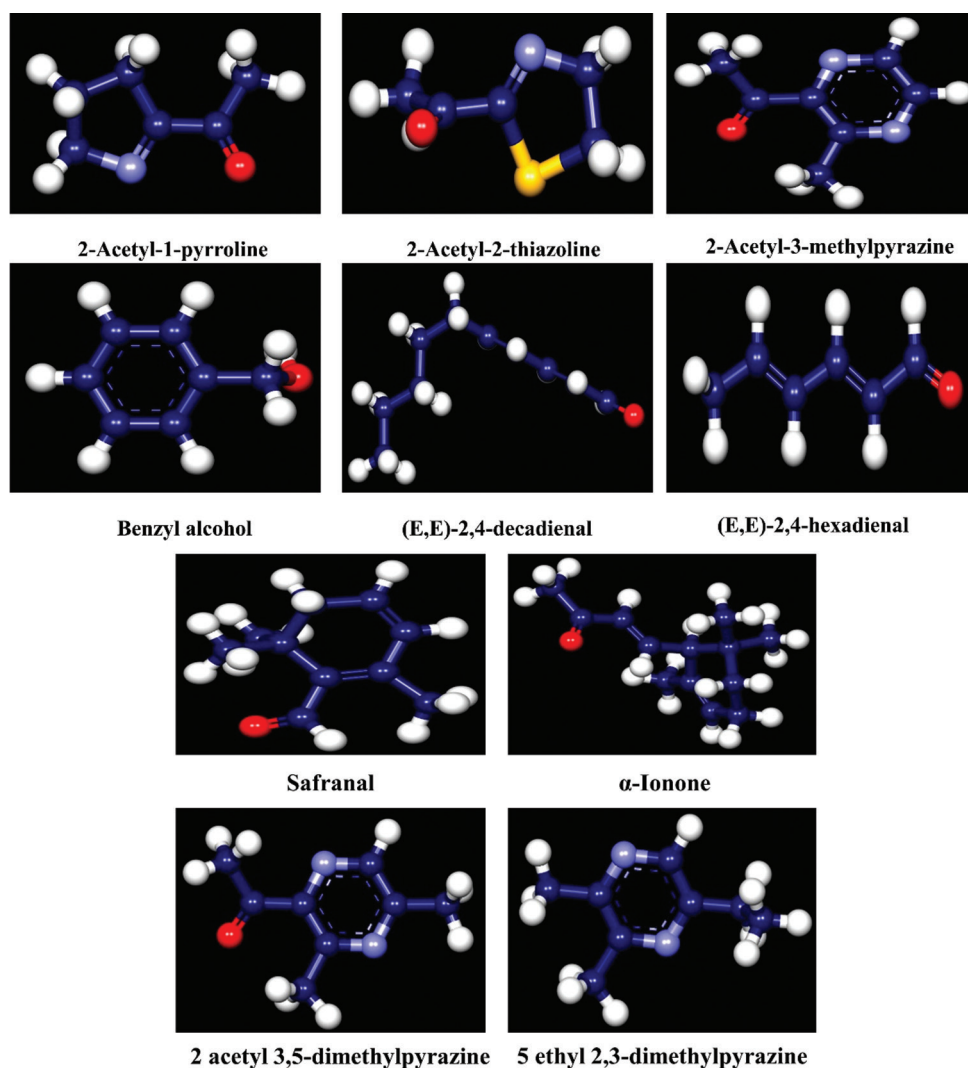


Figure 1. Three-dimensional structures of selected aromatic bioactive compounds

2.3. Receptor protein selection and validation

Based on the literature and database analysis, GluRs identified in both neuronal and non-neuronal cells in the CNS, particularly in microglia and CNS-associated macrophages, were selected. Both metabotropic and ionotropic GluRs are commonly expressed on neuronal and myelomonocytic cells in the nervous system (Table 2; Figure 2A and B). The 3D structures were retrieved from the PDB (<http://www.rcsb.org/>). Then, using BIOVIA Discovery Studio 2020 software (Version 20.1, Dassault Systèmes, USA) (<https://discover.3ds.com/discovery-studio-visualizer-download/>), the co-crystallized ligands, water molecules, and other non-protein components were removed to prepare receptors for docking. Then, the newly generated protein PDB structure underwent a series of quality analyses, including ERRAT and Procheck, using

SAVES 6.0 (<https://saves.mbi.ucla.edu/>) as per standard protocol.¹⁸

2.4. Molecular docking interaction in AutoDock Vina

AutoDock Vina software (Version 1.2.0, Scripps Research, CA, USA) (<http://vina.scripps.edu/>) was used for molecular docking and virtual screening to predict protein–ligand binding poses and estimate binding affinities.¹⁹ Docking was performed within a predefined grid box covering the binding site, and poses were ranked by the Vina scoring function. Kollman charges and other modifications were applied to the purified protein before proceeding to the final docking phase and converting it to a properly readable PDBQT file format. The ligand is similarly converted into a PDBQT file. A grid box was created around the protein's active residues, with various grid sizes and centers, but a

consistent grid spacing of 0.375 Å. Using the AutoDock Vina, binding affinity was predicted with an exhaustiveness value of 8. The final visualization of the docked structure was performed using BIOVIA Discovery Studio 2020.

2.5. Assessment of binding pockets, structural hotspots, and dynamics of receptor proteins

A systematic quantitative characterization of protein surface topography is available in the Computer Atlas Surface Topography of Protein (CASTp).²⁰ Active amino acid residues, structural hotspots, and different binding pockets of varied sizes on the receptor protein molecules were predicted using the CASTp 3.0 service. The iMod Server Prediction (<http://imods.chaconlab.org/>) application was used to assess the transition paths in internal coordinates

Table 2. Selected ionotropic and metabotropic receptors with their respective PDB IDs

Type	PDB ID	Description
Metabotropic	1EWK	Glutamate receptor subtype 1
	3FUZ	Glutamate receptor, GluR5
	3G3F	Glutamate receptor, GluR6
	3KS9	Glutamate receptor mGluR1
	6BSZ	Glutamate receptor mGlu8
Ionotropic	5L1F	AMPA subtype glutamate receptor GluA2 in complex with Perampanel
	2WJW	Glutamate receptor AMPA subtype GluA2
	5L1G	Glutamate receptor AMPA subtype GluA2 in complex with GYKI-Br
	6IRH	Glutamate receptor, GluN1/GluN2A NMDA subtype
	7EOR	Glutamate receptor, GluN1/GluN2A NMDA subtype

Abbreviations: AMPA: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; NMDA: N-methyl-D-aspartate receptor; PDB: Protein Data Bank.

naturally produced in collective functional motions within biomolecules,²¹ providing advanced visualization and an improved affinity-model-based representation of domain dynamics.

3. Results

3.1. SwissADME prediction of ligands

On submission of ligand structure in SMILES format, the SwissADME result was generated on the basis of ADME/toxicity analysis and Lipinski filter analysis (Table 3 and Figure 3). Various parameters, including physicochemical properties, lipophilicity, pharmacokinetics, and drug likeness, were included. Table 3 shows that all the compounds had molecular weights below the acceptable range (≤ 500 Da) and also met Lipinski's Rule of Five criteria (number of hydrogen bond acceptors ≤ 10 and number of hydrogen bond donors ≤ 5). This indicated that all the compounds could be easily absorbed, diffused, and transported.²² The number of rotatable bonds is a measure of molecular flexibility and is widely used as a filter during the drug discovery process. In this criterion, all compounds fell within the acceptable range (≤ 15), indicative of their potential permeability and oral bioavailability. Table 4 shows that all compounds had a calculated logarithm of the partition coefficient below 5, indicating favorable lipophilicity and potential for membrane permeability. According to Table 5, most compounds exhibited high potential for absorption across the gastrointestinal tract and the blood-brain barrier (BBB). However, 2-acetyl-2-thiazoline and 2-acetyl-1-pyrroline showed low BBB permeation (Figure 3). Penetration across the BBB is essential for compounds targeting the CNS, and 8 out of 10 selected compounds were capable. Metabolism prediction data for compounds against five cytochrome P450 isoforms is a main priority during the drug discovery process. All compounds in our study had negative LogKp

Table 3. Physicochemical properties of the selected aroma compounds of Darjeeling black tea

Compound name	Formula	Molecular weight	Number of rotatable bonds	Number of H acceptors	Number of H donors
(E, E)-2,4-decadienal	C ₁₀ H ₁₆ O	153.23 g/mol	6	1	0
(E, E)-2,4-hexadienal	C ₆ H ₈ O	96.13 g/mol	2	1	0
Benzyl alcohol	C ₇ H ₈ O	108.14 g/mol	1	1	1
2-Acetyl-3-methylpyrazine	C ₇ H ₈ N ₂ O	136.15 g/mol	1	3	0
2-Acetyl-2-thiazoline	C ₅ H ₇ NOS	129.18 g/mol	1	2	0
2-Acetyl-1-pyrroline	C ₆ H ₉ NO	111.14 g/mol	1	2	0
α -Ionone	C ₁₃ H ₂₀ O	192.30 g/mol	2	1	0
Safranal	C ₁₀ H ₁₄ O	150.22 g/mol	1	1	0
2-Acetyl-3,5-dimethylpyrazine	C ₈ H ₁₀ N ₂ O	150.18 g/mol	1	3	0
5-Ethyl-2,3-dimethylpyrazine	C ₈ H ₁₂ N ₂	136.19 g/mol	1	2	0

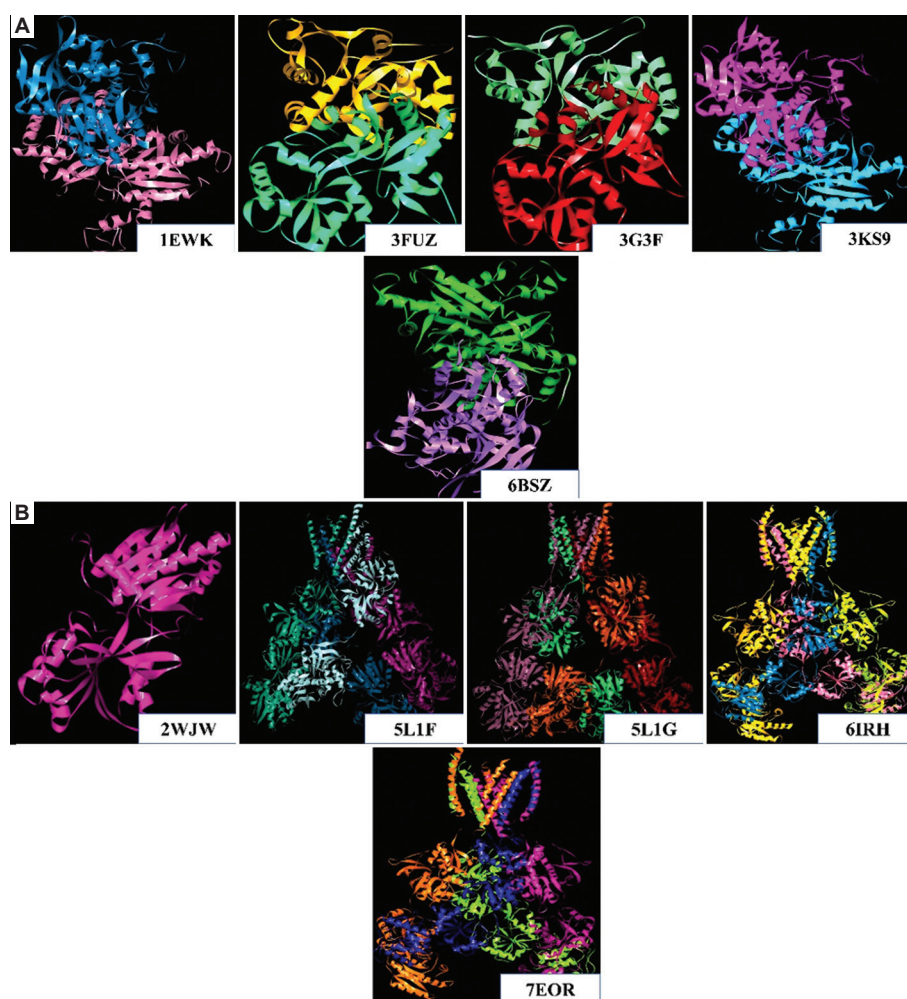


Figure 2. Three-dimensional structures of the (A) metabotropic and (B) ionotropic receptor proteins

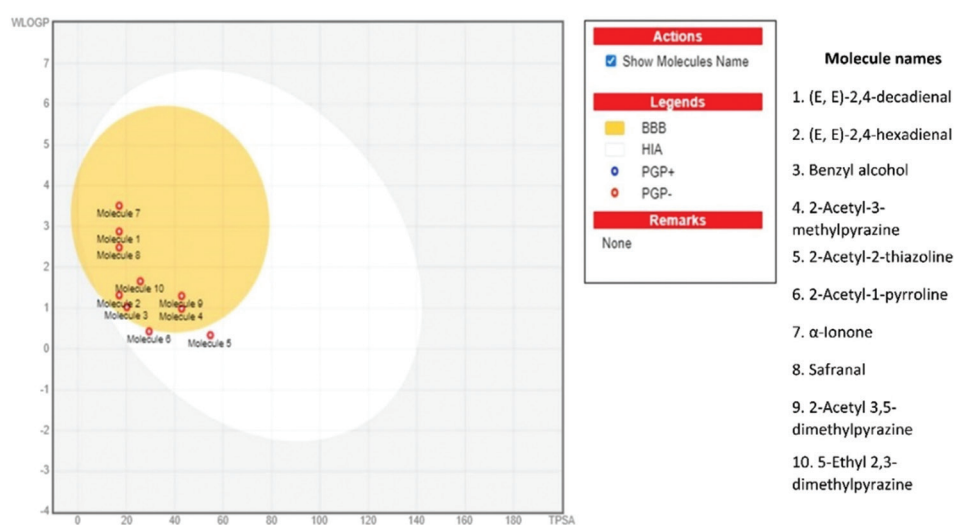


Figure 3. Boiled egg structure showing the permeability and other potential of the selected tea aroma compounds
Abbreviations: BBB: Blood–brain barrier; HIA: Human intestinal absorption; PGP: P-glycoprotein.

Table 4. Lipophilicity of the selected aroma compounds of Darjeeling black tea

Compound name	Log Pa/w (iLOGP)	Log Pa/w (XLOGP3)	Log Pa/w (WLOGP)	Log Pa/w (MLOGP)	Log Pa/w (SILICOS-IT)	Consensus log Pa/w
(E, E)-2,4-decadienal	2.67	3.25	2.88	2.49	2.96	2.85
(E, E)-2,4-hexadienal	1.63	1.19	1.32	1.18	1.23	1.31
Benzyl alcohol	1.66	1.10	1.03	1.54	1.74	1.41
2-Acetyl-3-methylpyrazine	1.50	0.20	0.99	-0.75	1.61	0.71
2-Acetyl-2-thiazoline	1.51	0.22	0.34	-0.42	2.21	0.77
2-Acetyl-1-pyrroline	1.07	-0.43	0.43	-0.04	2.17	0.64
α -Ionone	2.81	3.85	3.51	2.94	3.41	3.31
Safranal	2.13	2.14	2.49	2.10	2.62	2.30
2-Acetyl-3,5-dimethylpyrazine	1.29	0.60	1.30	-0.42	2.06	0.97
5-Ethyl-2,3-dimethylpyrazine	2.02	1.38	1.66	0.55	2.53	1.63

Table 5. Physiological properties of the selected aroma compounds of Darjeeling black tea

Compound name	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (skin permeation)
(E, E)-2,4-decadienal	High	Yes	No	No	No	No	No	No	-4.92 cm/s
(E, E)-2,4-hexadienal	High	Yes	No	No	No	No	No	No	-6.02 cm/s
Benzyl alcohol	High	Yes	No	Yes	No	No	No	No	-6.18 cm/s
2-Acetyl-3-methylpyrazine	High	Yes	No	No	No	No	No	No	-6.99 cm/s
2-Acetyl-2-thiazoline	High	No	No	No	No	No	No	No	-6.93 cm/s
2-Acetyl-1-pyrroline	High	Yes	No	No	No	No	No	No	-7.28 cm/s
α -Ionone	High	Yes	No	No	No	Yes	No	No	-4.74 cm/s
Safranal	High	Yes	No	No	No	No	No	No	-5.70 cm/s
2-Acetyl-3,5-dimethylpyrazine	High	Yes	No	No	No	No	No	No	-6.79 cm/s
5-Ethyl-2,3-dimethylpyrazine	High	Yes	No	No	No	No	No	No	-6.15 cm/s

Abbreviations: BBB: Blood-brain barrier; CYP: Cytochrome P450; GI: Gastrointestinal; Kp: Skin permeation coefficient; P-gp: P-glycoprotein.

values, indicating low predicted skin permeation. The drug-likeness results (Table 6) showed that all compounds met the criteria, with no parameter violations. Table 6 also shows the bioavailability scores that define the extent and rate at which compounds administered can enter systemic circulation and ultimately reach the targeted sites upon oral administration. Nine compounds showed a similar score of 0.55, except benzyl alcohol, scoring 0.85. This value indicates that the compounds adhered to Lipinski's Rule of Five and had a predicted oral bioavailability of 55% or higher.

3.2. Toxicity prediction of the ligands

During drug development, small-molecule toxicity testing is an essential step. Table 7 displays toxicological predictions from the PreADMET service, including mutagenicity, carcinogenicity, and hERG inhibition, where a negative prediction indicates carcinogenic

Table 6. Drug likeness of the selected aroma compounds of Darjeeling black tea

Compound name	Lipinski's rule satisfactory	Number of violations	Bioavailability score
(E, E)-2,4-decadienal	Yes	0	0.55
(E, E)-2,4-hexadienal	Yes	0	0.55
Benzyl alcohol	Yes	0	0.85
2-Acetyl-3-methylpyrazine	Yes	0	0.55
2-Acetyl-2-thiazoline	Yes	0	0.55
2-Acetyl-1-pyrroline	Yes	0	0.55
α -Ionone	Yes	0	0.55
Safranal	Yes	0	0.55
2-Acetyl-3,5-dimethylpyrazine	Yes	0	0.55
5-Ethyl-2,3-dimethylpyrazine	Yes	0	0.55

Table 7. Results of mutagenicity, carcinogenicity, and hERG inhibition of the selected aroma compounds of black tea

Compound name	Ames test	Carcinogenicity (mouse)	Carcinogenicity (rat)	hERG inhibition
(E, E)-2,4-decadienal	Mutagen	Negative	Positive	Medium risk
(E, E)-2,4-hexadienal	Mutagen	Positive	Positive	Medium risk
Benzyl alcohol	Mutagen	Negative	Negative	Medium risk
2-Acetyl-3-methylpyrazine	Mutagen	Negative	Negative	Low risk
2-Acetyl-2-thiazoline	Mutagen	Negative	Negative	Medium risk
2-Acetyl-1-pyrroline	Mutagen	Negative	Negative	Medium risk
α -Ionone	Mutagen	Positive	Negative	Low risk
Safranal	Mutagen	Negative	Negative	Low risk
2-Acetyl-3,5-dimethylpyrazine	Mutagen	Negative	Negative	Low risk
5-Ethyl-2,3-dimethylpyrazine	Mutagen	Negative	Negative	Low risk

Abbreviation: hERG: human Ether-à-go-go-related gene.

activity and a positive means the compound possesses no carcinogenic activity. The Ames test, based on bacterial mutagenicity, showed that all selected black tea aromatic molecules had mutagenic properties as ligand candidates. The hERG inhibition test, conducted to assess the effect of the molecules on the cardiac potassium channel,²³ showed moderate to low risk, with selected pyrazine, ionone, and safranal compounds having much lower risk to cardiac function. Probable carcinogenicity assessment in the PreADMET service indicated that most selected candidates were non-carcinogenic in mice and rats, except for the decadienal compound in rats, α -ionone in mice, and the hexadienal compound in both species (Table 7).

3.3. Validation of protein structures

The overall quality of all 3D protein PDB structures was assessed using the online tools (Figure S1). By confirming the protein PDB model against several quality criteria, the ideal protein structures were approved. The “overall quality factor” that ERRAT displayed indicated that proteins with higher scores are of greater quality. Most proteins had quality scores between 86% and 98%, except for 6IRH (43%), indicating that most are all well-modeled. The Ramachandran plots of most protein models showed that over 80% of residues were found in the most preferred regions, followed by the extra allowed, generously allowed, and banned regions, in accordance with the Procheck results. Together, these strong validations ensured the precision of molecular docking investigations by demonstrating that the interactions between ligands and proteins were faithfully reproduced.

3.4. Molecular docking interaction using AutoDock Vina

Docking analyses were conducted using AutoDock Vina. The overall binding affinities of all tea bioactive compounds

for the five metabotropic receptors were depicted pictorially (Figure 4). When the stability of the docking interactions was calculated, several specific receptor–ligand interactions emerged as the most potent and stable, with binding affinities around -6 kcal/mol or lower. α -ionone and safranal were the most prominent ligands, and mGluR1 (IEWK), GluR5 (3FUZ), and GluR6 (3G3F) were the most common metabotropic receptors, providing stable interactions. Table 8 shows the best interactions and their binding affinities. Based on the graphical view generated through BIOVIA Discovery Studio 2020, two-dimensional and 3D modes of binding interactions of top docked compounds are presented in Figure 5A-E, where the affinity score ranged between -5.7 and -7.1 kcal/mol.

For ionotropic GluRs, specific subtypes of AMPA (2WJW) and NMDA (7EOR) exhibited stronger binding affinities for α -ionone and safranal (Figure 6). Two-dimensional and 3D models of binding prediction using BIOVIA Discovery Studio 2020 showed binding pockets and molecular interactions with the ionotropic receptors (Figure 7A-E), with affinity scores ranging from -4.7 to -6.4 kcal/mol.

3.5. Assessment of binding pockets, active amino acid residues, and dynamics

Table 9 shows the results from the CASTp 3.0 online server for structural hotspots or active amino acid residues of the protein PDB structure. The best-fit receptor–ligand complexes with the highest binding affinity were used to identify the active amino acid residues during ligand binding. Numerous amino acid types distributed across different regions create the receptors’ structural pockets and are implicated in molecular interactions with ligands. The interaction showed that the most potential binding may occur between GluR6 metabotropic receptor (3G3F)

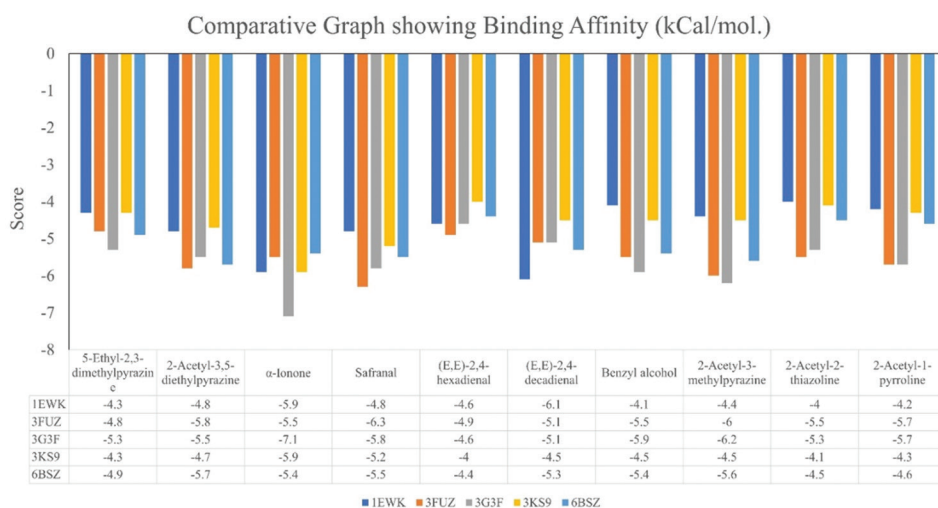


Figure 4. Graphical representation of binding affinity along with the score (kcal/mol) toward the target metabotropic receptors of glutamate by the respective compounds or ligands

Table 8. Results of maximum binding affinity scores in AutoDoc Vina between glutamate receptors and the best selected aroma compounds of black tea

Ranking	Receptor (common name)	Receptor (PDB ID)	Ligand	Affinity score (kcal/mol)
1	GluR6 metabotropic receptor	3G3F	α-Ionone	-7.1
2	GluN1/GluN2A NMDA ionotropic receptor subtype	7EOR	α-Ionone	-6.4
3	GluR5 metabotropic receptor	3FUZ	Safranal	-6.3
4	GluR-AMPA ionotropic receptor subtype	2WJW	Safranal	-6.2
5	GluR1 metabotropic receptor	1EWK	(E, E)-2,4-decadienal	-6.1
6	Glutamate Receptor mGlu8	6BSZ	2-Acetyl-3,5-dimethylpyrazine	-5.7
7	Glutamate Receptor AMPA Subtype	5L1G	α-Ionone	-4.7
8	GluR1 metabotropic receptor	3KS9	α-Ionone	-5.9
9	GluN1/GluN2A NMDA ionotropic receptor subtype	6IRH	α-Ionone	-5.9
10	AMPA Subtype Glutamate Receptor GluA2	5L1F	α-Ionone	-4.8

Abbreviations: AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; NMDA: N-methyl-D-aspartate receptor; PDB: Protein Data Bank.

and α-ionone, with an affinity scoring of -7.1 kcal/mol, where the ligand interacted with the Thr108 and Lys248 of B chain of the receptor through the hydrogen bond and alkyl bond, respectively, providing a stable interaction output (Figures 5, S1C, and S2VIII). Next significant docking and interaction occurred between the ionotropic GluN1/Glu2A NMDA receptor (7EOR) with α-ionone, having affinity scoring of -6.4 kcal/mol, where the α-ionone terminal oxygen of C=O interacted with B chain Ser700, a pi-alkyl bond formed between the benzene ring of ligand and Trp795 of C chain, and pi-sigma bond forms with same residue (Figure 7). Further analysis of the structure and dynamics showed a lower level of interaction favorability than in the previous one (Figures S1J and S2E). The next higher probability of structural interaction was observed with safranal and the GluR5 metabotropic

receptor (3FUZ), with a binding affinity score of -6.3 kcal/mol. In this interaction, safranal interacted with the adjacent Ser674 and Thr675 of the B chain of the receptor through hydrogen bonding, and the ligand benzene ring interacted with the receptor B chain Tyr474 through pi-alkyl and pi-sigma bonding in the binding pocket (Figure 5). Further protein structure analysis showed favorable structural stability and molecular dynamics of receptor-ligand interactions (Figures S1B and S2VII). Safranal also showed potential binding to the Glu-AMPA ionotropic receptor (2WJW), where it interacted in the binding pocket through hydrogen and alkyl bonds with the receptor A chain Arg129, Arg156, and Tyr295 (Figure 7), but this interaction was dynamic and unstable (Figure S2A). Other interactions showed similar or lesser docking potential and structural stability.

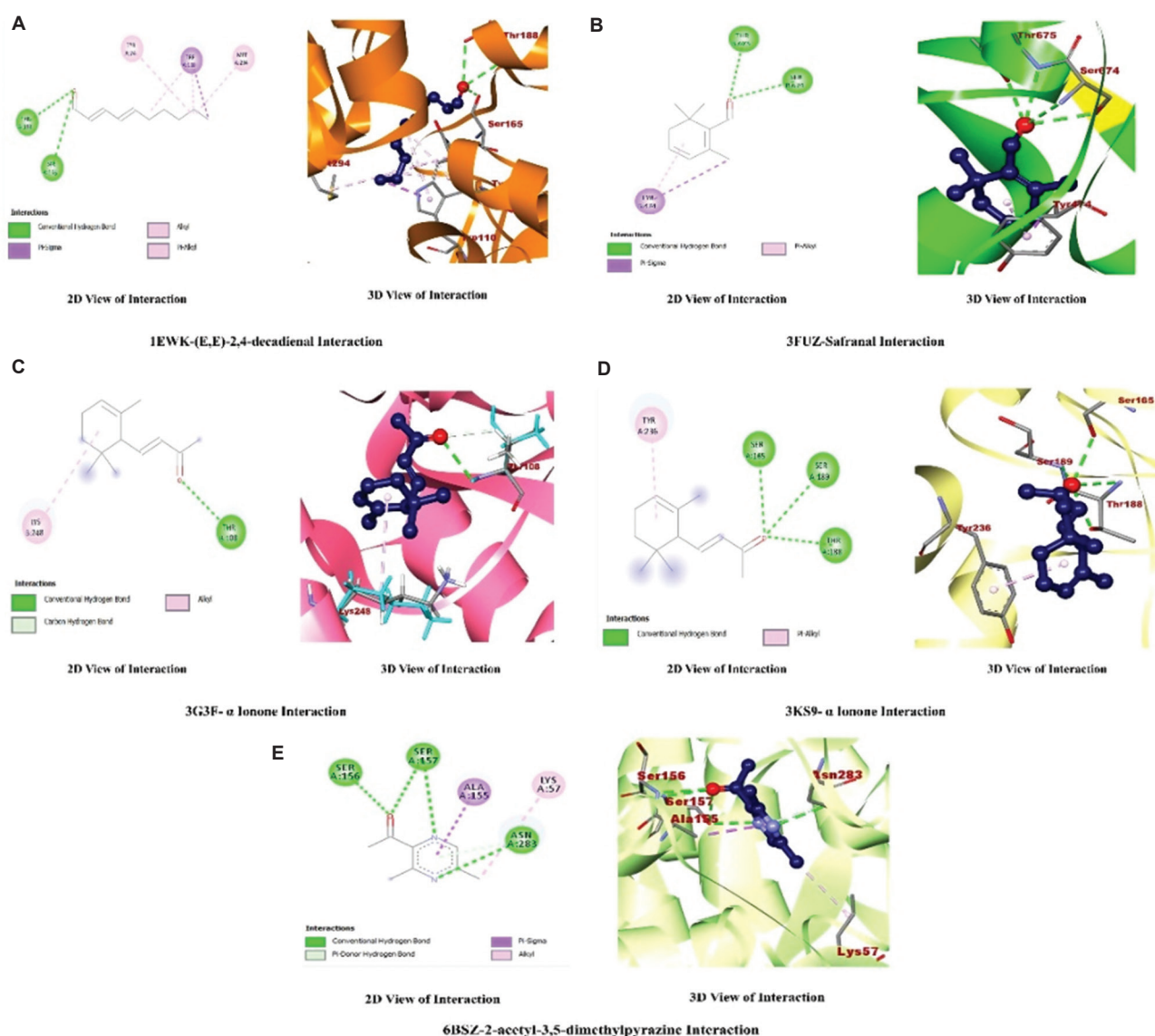


Figure 5. Complete representation and close insight into 2D and 3D interactions between best-scored metabotropic glutamate receptors and ligands. (A) 1EWK-(E,E)-2,4-decadienal interaction. (B) 3FUZ-safranal interaction. (C) 3G3F- α -ionone interaction. (D) 3KS9- α -ionone interaction. (E) 6BSZ-2-acetyl-3,5-dimethylpyrazine interaction.

Abbreviations: 2D: Two-dimensional; 3D: Three-dimensional.

Among the amino acid residues, polar uncharged serine and threonine, along with positively charged arginine and lysine, were most frequently observed within the binding pockets that interacted with α -ionone and safranal, indicating favorable receptor–ligand interactions. Other amino acids in the binding pockets were hydrophobic residues, such as tyrosine, tryptophan, valine, leucine, isoleucine, alanine, and methionine, which were available to interact with aromatic ligands. The iMOD server analysis showed specific eigenvalues of the receptor–ligand complex systems (eigenvectors)

as per the principal component analysis, where three out of five metabotropic receptors, namely, GluR type 1 (1EWK), GluR5 (3FUZ), GluR6 (3G3F), and only one out of five ionotropic receptors, namely, AMPA receptor (5LIG), showed high eigenvalue that signified a large variance or dynamics in the system. In general, upon ligand binding, metabotropic receptors are structurally dynamic; whereas ionotropic receptors are structurally stiffer, except for the AMPA receptor 5LIG (Figure S2). Overall, we observed that the most suitable binding occurred between α -ionone and 3G3F, as well

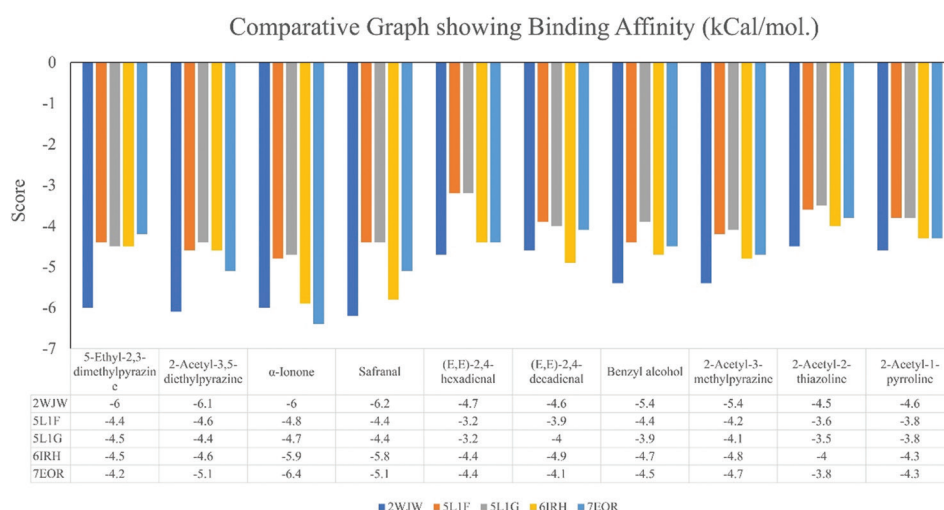


Figure 6. Graphical representation of binding affinity along with the score (kcal/mol) towards the target ionotropic receptors of glutamate by the respective compounds/ligands

Table 9. Active amino acid residues of the receptors using Computer Atlas Surface Topography of Protein 3.0 with best-fitted ligand aroma compounds derived from Darjeeling black tea

No.	Receptor (PDB ID)	Ligand	Active amino acid residues for binding
1	3G3F	α-Ionone	THR 108, LYS 248
2	7EOR	α-Ionone	SER 700, TRP 795
3	3FUZ	Safranal	TYR 474, SER 674, THR 675
4	2WJW	Safranal	ARG 129, ARG 156, TYR 295
5	1EWK	(E, E)-2,4-decadienal	TYR 74, TRP 110, SER 165, THR 188, MET 294
6	6BSZ	2-Acetyl-3,5-dimethylpyrazine	LYS 57, ALA 155, SER 156, SER 157, SER 283
7	5L1G	α-Ionone	ILE 664, ARG 675, LYS 761, LYS 765
8	3KS9	α-Ionone	SER 165, THR 188, SER 189, TYR 236
9	6IRH	α-Ionone	ASN 432, LYS 457, LEU 794, TRP 795
10	5L1F	α-Ionone	ARG 453, VAL 484, SER 654

Abbreviation: PDB: Protein Data Bank.

as between safranal and 3FUZ, which may have greater biological significance.

4. Discussion

Tea consumption is one of the most common cultural practices worldwide and has putative public health benefits. Depending on preparation, particularly for green and black tea, it releases wide variations of bioactive compounds and flavors in infusions, such as catechins,

flavonoids, polyphenols, L-theanine, and numerous aroma compounds, as well as their derivatives.^{3,14} Previous studies have shown that L-theanine, unique to green and black tea and some mushrooms, induces relaxation and improves attention in humans.^{1,24} Additionally, routine intake of tea has been reported to have a significant effect on mood and improve the restoration of cortisol at the basal level after stress, resulting in quicker stress recovery, as evidenced in various population-based studies.^{9,25,26}

There are a variety of aroma compounds that characterize different tea types for the world population. Among which, one of the best authentic aromatic tea varieties is Darjeeling tea, produced and processed in the cool, humid southern hill slopes of the Himalayas, at altitudes of 1,000 m–3,000 m, from Nepal to Bhutan, centered on the Darjeeling district of West Bengal, India. The tea leaves are harvested in spring and summer, selected, weathered, rolled, fermented-oxidized, and processed to produce signature Darjeeling black tea. In hot, aqueous infusion, it releases the flavor and taste for which it is widely recognized.^{14,15,27} In the current study, some of the prominent compounds associated with Darjeeling tea flavor were selected (Table 1) with their fragrance characteristics. When different physical and chemical parameters of the compounds, including BBB permeability and drug likeness, were tested, they were found to be highly absorbable through gastric endothelial tissue, and most were BBB permeable except 2-acetyl-2-thiazoline. However, all tested aroma compounds met Lipinski's criteria, demonstrating drug-likeness with persistent bioavailability scores of 0.55 and above, with a maximum score of 0.85 for benzyl alcohol (Tables 3-6). Most of these compounds were low

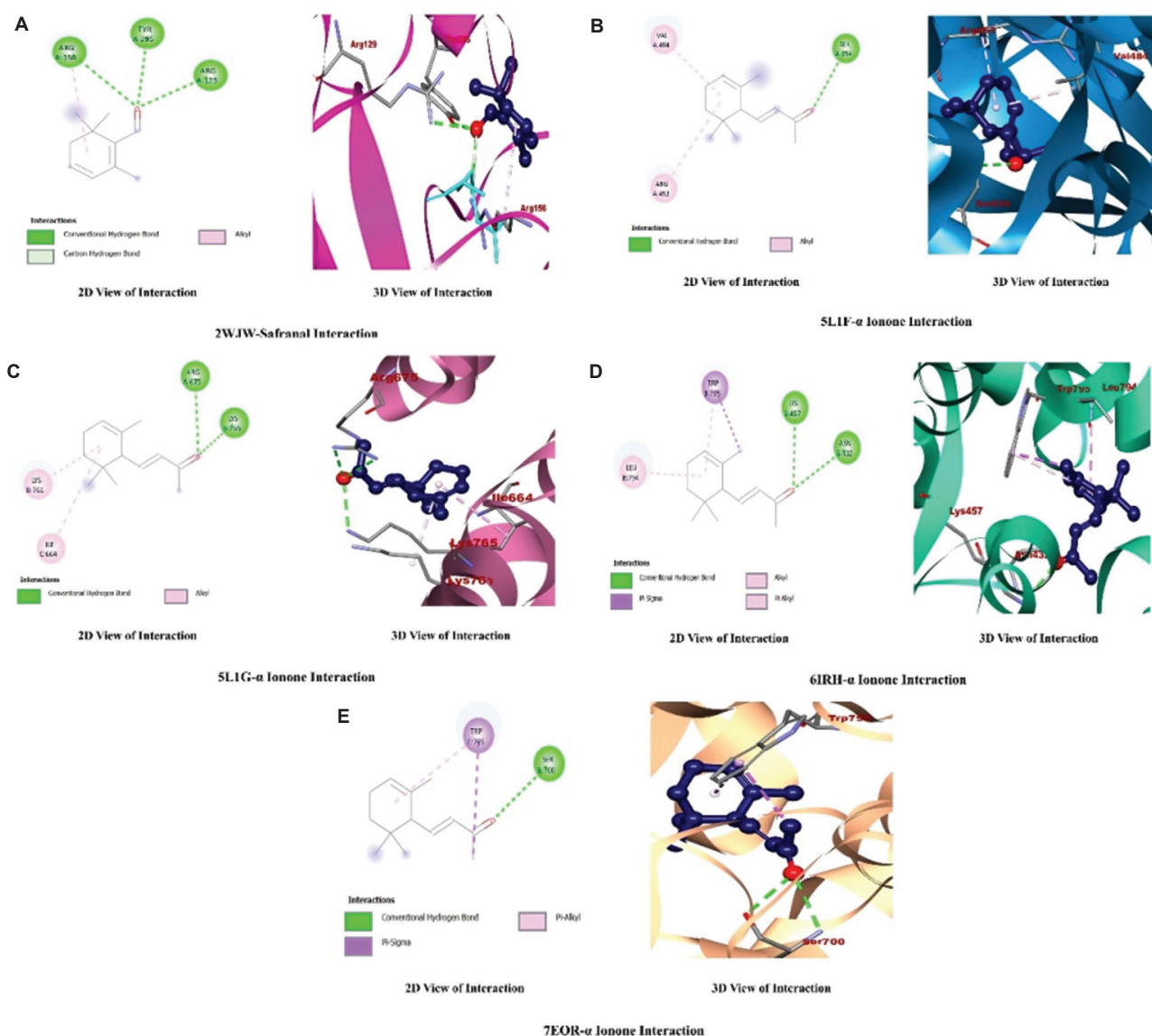


Figure 7. Complete representation and close insight into 2D and 3D interactions between the best-scoring ionotropic glutamate receptors and ligands. (A) 2WJW-safranal interaction. (B) 5L1F- α -ionone interaction. (C) 5L1G- α -ionone interaction. (D) 6IRH- α -ionone interaction. (E) 7EOR- α -ionone interaction.

Abbreviations: 2D: Two-dimensional; 3D: Three-dimensional.

to medium-risk mutagens, with most of them showing no carcinogenicity, thereby qualifying as effective ligands. Prominent metabotropic and ionotropic GluRs (Table 2) were subjected to the structural validation of the PDB models on which the ligand docking experiments were performed (Figure S1). The experiments showed binding affinities ranging from -7.1 to -4.8 kcal/mol for the top 10 receptor-ligand pairs, within the flexible-ligand docking range, indicating biological efficiency of this interaction.²⁸ Hence, interactions of metabotropic receptors—GluR1 (1EWK), GluR5 (3FUZ), and GluR6 (3G3F)—with

(E, E)-2,4-decadienal, safranal, or α -ionone, respectively, and ionotropic receptors—GluN1/GluN2A NMDA subtype (7EOR) and GluR-AMPA subtype (2WJW)—with α -ionone or safranal, respectively, were found to be the most promising and probable. Among the ligands, safranal and α -ionone were monoterpenoids with a characteristic aroma of the black tea variant well-known to tea lovers.^{15,27} Analysis through the iMOD server on the structural coordinates of the receptors showed that metabotropic receptors had higher eigenvalues and spread, indicating higher dynamics. Whereas ionotropic receptors involved in

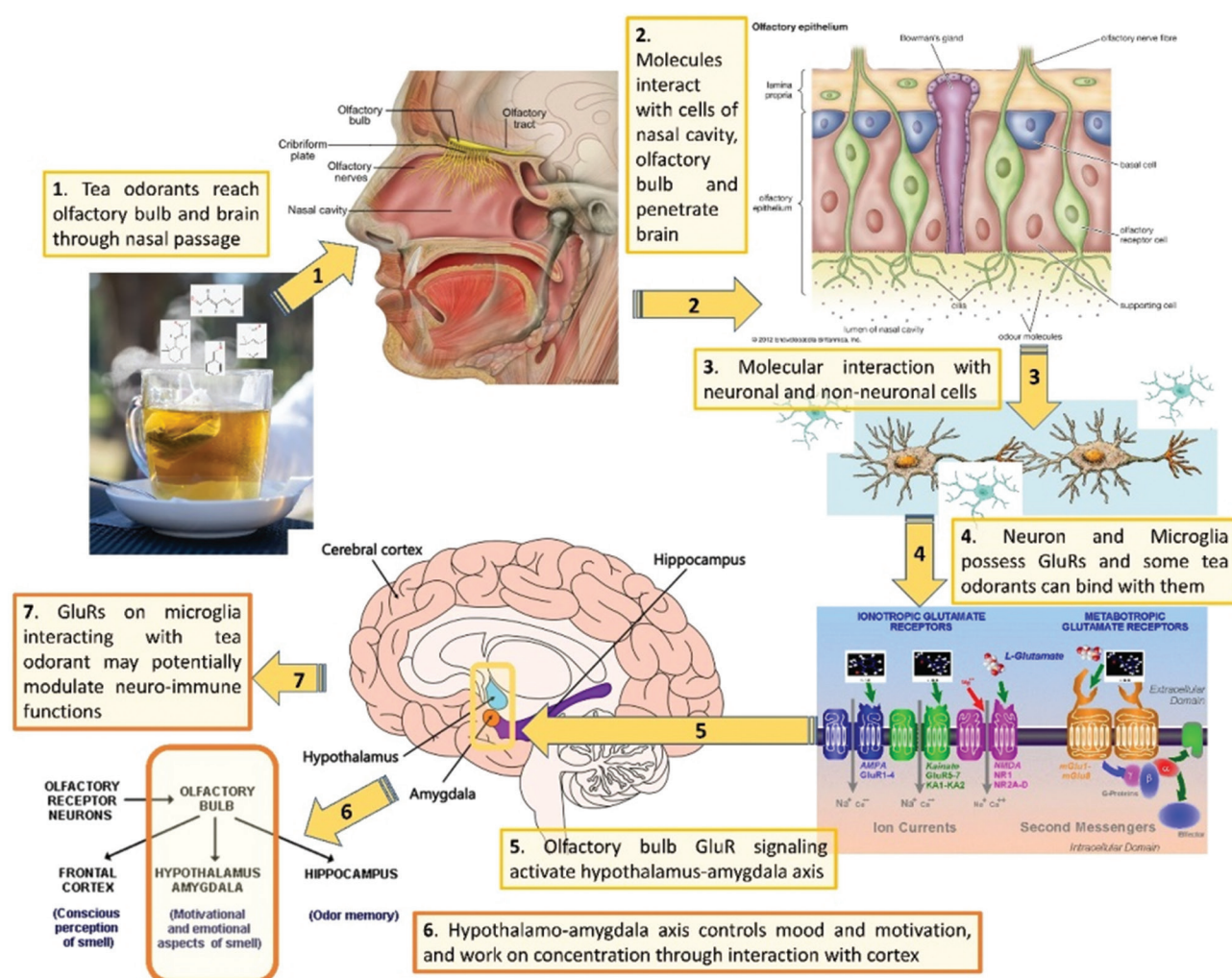


Figure 8. Concept diagram of the prospective mechanism of action of Darjeeling tea aromatics on mood, attention, and neuro-immune physiology

binding showed lower eigenvalues and spread, indicating structural stiffness (Figure S2).^{21,28} However, the most dynamic components were the metabotropic GluR5 and GluR6, and the ionotropic AMPA receptor also exhibits notable dynamicity. The top four ligand receptor bindings showed that the interactions between ligands (α -ionone and safranal) and receptors were occurring in between only 2–3 amino acid residues, either polar or hydrophobic in nature (Figures 5 and 7).

As found, the ligands were mostly capable of crossing the endothelial lining, absorbing through the gastrointestinal tract and BBB with sufficient bioavailability scores, and can be delivered via both oral and olfactory routes to reach their target receptors. Therefore, the black tea volatile compounds selected in this study had ample opportunity to interact with, dock onto, and exert subsequent effects on the neuroglial cells associated with this passage. In addition, their high absorption score through the gastrointestinal

tract, moderate to high bioavailability, and penetration capability through the BBB (except 2-acetyl-2-thiazoline) (Figure 3) ensure their systemic reach to the CNS matrix and capability to interact with the neuroglial population from regions of cerebral cortex to the hippocampus and the hypothalamus, thus supporting possibility that these compounds could influence neurophysiology, behavior, and neuroimmune signaling.

In a recent study, glutamate ionotropic AMPA and NMDA receptors, including GluA1, GluA2, GluN1, and GluN2A, were identified in the hypothalamus and showed their critical role in glutaminergic synaptic transmission.²⁹ Such signaling is significantly related to learning and memory, as well as neuroendocrine functions, and is associated with circadian, feeding, and behavioral disorders.^{30,31} In addition to the widespread presence of GluRs throughout the CNS, including the olfactory circuit, olfactory tissue exhibits a robust innate

immune defense. Immune cells migrate across the lamina propria into olfactory neurons, express ionized calcium-binding adapter molecule 1 and C-X3-C motif chemokine receptor 1, and release inflammatory mediators, such as inducible nitric oxide synthase, interleukin 6, and tumor necrosis factor alpha (TNF- α).³² These cells, particularly microglia, express a wide range of neurotransmitter receptors and membrane proteins, including cluster of differentiation (CD)200, CD22, and CD47, which facilitate interactions with neurons. Microglia can modulate neuronal function by releasing glutamate, ATP, ADP, reactive oxygen species, nitric oxide, prostaglandin E2, brain-derived neurotrophic factor, miRNAs, and various cytokines/chemokines.³³ Previous studies have shown that activation of Group III metabotropic GluRs (mGluR4/6/8) promotes a neuroprotective microglial phenotype, whereas activation of Group II receptors (mGluR2/3) is associated with a neurotoxic phenotype characterized by the release of TNFs and Fas receptor signaling, as well as R, Ca²⁺-wave-driven neuronal injury. NMDA expression in these cells follows nuclear factor κ B signaling and the release of nitric oxide, interleukin 1 β , and TNF- α , thereby mediating increased excitability of hippocampal cornu ammonis area 3 neurons.³³⁻³⁵ Evidence shows that microglia are present throughout the nasal epithelium and olfactory nerves, extending along the subventricular zone–olfactory bulb axis. These cells play a protective role against pathogenic insults and the development of olfactory dysfunctions, and can receive glutamate signals to modulate neuronal activity.^{36,37} As AMPA and NMDA receptors and metabotropic GluRs are expressed on both neurons and microglia, their reciprocal interactions and functional responses under glutamate release or storming are crucial for a wide range of neurophysiological conditions. In all these situations, safranal, α -ionone, and other aroma compounds derived from tea may play a pivotal role from multiple perspectives.

5. Conclusion

Our study explored the potential of several prominent Darjeeling tea aroma compounds to modulate neuroglial function, thereby influencing neurophysiology and behavior via GluRs (Figure 8). These compounds, prominently α -ionone, safranal, (E, E)-2,4-decadienal, are found to be sufficiently bioavailable to the olfactory bulb and CNS, and capable of interacting with group I metabotropic receptors (mGluR1 and mGluR5), as well as Group III metabotropic receptors (mGluR6 and mGluR8). As a Group I receptors are the moderators capable of increasing NMDA receptor activity; they can synergistically uplift the function of neuronal circuitry. In contrast, Group III metabotropic receptors are characterized by downregulation of secondary messenger-dependent signaling and by neurotoxic

effects.^{11,12} The same aroma compounds, such as safranal and α -ionone, can also bind to GluR-AMPA and GluN1/GluN2A NMDA ionotropic receptor subtypes, which are widespread on neuroglial cells throughout the naso-buccal cavity, cortex, and hypothalamus. These interactions may have broad functional implications, including activation of the hypothalamo–amygdala axis, as discussed previously. Simultaneously, they can bind to GluRs expressed on microglia and brain macrophages, thereby activating the neuro-immuno axis as previously discussed. This study highlights the novel potential of Darjeeling tea aroma compounds and suggests a new avenue for investigating their effects on neuroglial function.

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Conflict of interest

The authors declare that they have no competing interests.

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Formal analysis: Anirban Ghosh, Sirshendu Chatterjee

Investigation: All authors

Methodology: Moumita Saha

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Writing-review & editing: Anirban Ghosh, Sirshendu Chatterjee

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All data were obtained from the website/databank and derived from software or open platforms as described in the text.

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