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Structure-Based Computational Screening of Indonesian Spice-Derived Compounds Targeting the Neuropeptide Y1 Receptor

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HIGHLIGHTS

- cis-Theaspirone is considered the best ligand for modulating NPY1R
- cis-Theaspirone has blood-brain barrier permeability to modulate NPY1R
- Targeting NPY1R regulates blood glucose levels by modulating appetite and altering gene expression

ABSTRACT

The neuropeptide Y1 receptor (NPY1R) is a promising therapeutic target for type 2 diabetes mellitus (T2DM) due to its role in regulating appetite and insulin secretion. Indonesian spices contain diverse bioactive compounds with potential metabolic effects, yet their interaction with NPY1R remains largely unexplored. This study aimed to identify potential natural allosteric modulators of NPY1R using structure-based virtual screening and pharmacokinetic analysis. A total of 17,356 compounds from the NCBI PubChem database were pre-filtered, yielding 860 ligands for blind molecular docking. Of these, 97 showed stronger predicted binding affinities than the reference antagonist BMS-193885. Further screening based on physicochemical properties, toxicity, and ADMET parameters identified six top candidates. Among them, cis-theaspirone demonstrated the most favorable profile, including binding to an allosteric pocket (–10.0 kcal/mol; RMSD 2.658 Å), high gastrointestinal absorption, blood–brain barrier permeability, and acceptable solubility. Molecular dynamics simulations over 50 ns confirmed stable ligand–receptor interactions. Overall, cis-theaspirone emerges as a potential NPY1R modulator, highlighting the promise of Indonesian spice-derived compounds for further investigation in metabolic disease research.

INTRODUCTION

Global burden of type 2 diabetes and emerging opportunities from Indonesian natural products

Type 2 diabetes mellitus (T2DM) represents a growing global health crisis, with prevalence rates steadily increasing worldwide. In Indonesia, the rise in obesity has paralleled this trend, contributing significantly to the nation's diabetes burden. 41 million children under the age of five are overweight or obese, contributing to an escalation of chronic diseases, including T2DM, in adulthood (World Health Organization, 2019). This shift in health trends imposes a significant burden on healthcare systems, with the economic loss due to obesity in Indonesia alone estimated at IDR 78.5 trillion annually (Wulansari et al., 2016). The link between obesity and diabetes is well-documented, as obesity exacerbates the risk of developing insulin resistance and T2DM through complex metabolic pathways.

In parallel with advances in pharmacology, natural products continue to provide valuable chemical diversity for drug discovery. Indonesian spices represent a particularly rich yet underexplored source of bioactive compounds with potential metabolic regulatory properties. Indonesia's biodiversity includes traditional medicinal plants such as turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), clove (*Syzygium aromaticum*), and nutmeg (*Myristica fragrans*), which have historically been used to manage inflammation, digestive disorders, and metabolic conditions. Phytochemicals derived from these spices encompass diverse structural classes, including terpenoids, phenolics, and volatile compounds, many of which demonstrate antioxidant, anti-inflammatory, and metabolic-modulating activities in experimental studies. Despite this potential, systematic evaluation of Indonesian spice-derived compounds against emerging molecular targets relevant to metabolic disease remains limited. Computational approaches offer an efficient strategy to prioritize candidate molecules from large natural product libraries and explore their potential interactions with biologically relevant receptors.

NPY1R: A key regulator in glucose metabolism

Among emerging therapeutic targets, the neuropeptide Y1 receptor (NPY1R) has attracted increasing attention due to its central role in regulating energy balance and glucose metabolism. NPY1R is a G protein-coupled receptor (GPCR) within the neuropeptide Y (NPY) signaling system, which integrates appetite regulation, energy expenditure, and metabolic homeostasis. NPY, predominantly produced in hypothalamic neurons, influences feeding behavior and metabolic regulation by modulating neuronal signaling pathways. Elevated NPY activity has been associated with increased food intake and reduced energy expenditure, partly through suppression of brown adipose tissue thermogenesis (Shi et al., 2013).

NPY1R is highly expressed in key hypothalamic regions, including the arcuate nucleus and paraventricular nucleus, which coordinate appetite regulation and energy balance (Sousa et al., 2023; Deem et al., 2021). Beyond the central nervous system, NPY1R is also present in peripheral tissues such as adipose tissue, liver, and kidneys, suggesting a broader role in systemic metabolic regulation (Sigorsky et al., 2025). Dysregulation of NPY1R signaling has been linked to insulin resistance and impaired glucose metabolism, indicating that modulation of this receptor may represent a promising strategy for addressing both obesity and T2DM (Yang et al., 2018).

Challenges in targeting NPY1R and the potential of allosteric modulation

Despite its therapeutic promise, the development of pharmacological modulators targeting NPY1R has progressed relatively slowly. Current treatments for T2DM primarily focus on insulin sensitizers and secretagogues, which may be associated with adverse effects and variable long-term efficacy. Synthetic ligands, including compounds such as BIBO 3304, have demonstrated activity toward NPY1R; however, their

development often involves complex and resource-intensive synthesis processes (Xiao et al., 2024). These limitations motivate exploration of alternative strategies, including natural product-based discovery.

One promising approach involves targeting allosteric sites rather than orthosteric ligand-binding regions. Allosteric modulators interact with distinct receptor pockets and may fine-tune receptor signaling without directly competing with endogenous ligands. This mechanism can potentially enhance selectivity, reduce receptor desensitization, and enable modulation of signaling bias. Within GPCR pharmacology, allosteric modulation provides opportunities for improved therapeutic specificity, including modulation of orthosteric ligand affinity or efficacy, enhanced receptor subtype selectivity, and the possibility of biased signaling pathways (Zacarías et al., 2018). Computational predictions in the present study suggest that certain Indonesian spice-derived compounds, including cis-Theaspirone, may interact with predicted allosteric regions of NPY1R; however, such interactions require experimental validation.

Despite growing interest in NPY1R as a therapeutic target, the identification of natural product-derived allosteric modulators remains limited, particularly among compounds associated with Indonesian spices. Furthermore, the structural basis for potential allosteric binding and interaction stability within NPY1R has not been systematically explored using integrated computational workflows. Therefore, this study aims to computationally prioritize Indonesian spice-derived compounds as putative allosteric modulators of NPY1R through structure-based virtual screening, pharmacokinetic profiling, and molecular dynamics simulations. We hypothesize that selected natural compounds may exhibit stable predicted interactions within allosteric regions of NPY1R, providing a rational foundation for future experimental investigation.

MATERIALS AND METHODS

Study design and computational framework

This study employed a structure-based computational drug discovery design to identify and prioritize Indonesian spice-derived compounds as putative modulators of the neuropeptide Y1 receptor (NPY1R). The research was conducted entirely *in silico* and followed a sequential workflow consisting of compound library curation, receptor preparation, binding pocket identification, molecular docking-based virtual screening, pharmacokinetic (ADMET) prediction, and molecular dynamics simulation for dynamic stability assessment. Computational analyses were carried out between June 2023 and September 2025 at Calvin Institute of Technology. The primary variables studied included predicted binding affinity, ligand–receptor interaction profiles, pharmacokinetic parameters, and structural stability metrics derived from molecular dynamics trajectories (e.g., RMSD and interaction persistence). Software and platforms used in this study included PyRx with AutoDock Vina for molecular docking, PyMOL for structural visualization, Allosite 2.0 for pocket prediction, LigPlot+ for interaction analysis, SwissADME and pkCSM for pharmacokinetic prediction, and the SiBioLead platform, which employs the OPLS/AA force field for molecular dynamics simulation. Statistical analysis was primarily descriptive and comparative, focusing on relative ranking and consistency across computational outputs rather than hypothesis-testing statistics, reflecting the exploratory nature of computational screening workflows.

Structure data collection and compound library curation

To construct the compound library for *in silico* screening, Indonesian spice-derived phytochemicals were collected through systematic literature mining and database retrieval. Publications from the past 15 years that report bioactive compounds associated with Indonesian medicinal spices and their antidiabetic activity were reviewed to identify candidate molecules. Chemical structures were obtained from public

repositories, primarily the PubChem database (NCBI), using structured keyword queries combining plant species names and known phytochemical classes. Retrieved compounds were exported together with canonical SMILES strings, database identifiers, and available three-dimensional structures. Library curation was conducted to ensure reproducibility and chemical consistency, including removal of duplicate entries based on canonical SMILES, normalization of salt forms to parent structures, standardization of tautomeric states, and assignment of protonation states corresponding to physiological pH conditions (~7.4). Compounds with undefined or ambiguous structural information were excluded. Custom Python scripts developed in PyCharm IDE were used to automate data retrieval, preprocessing, and file organization. The final curated dataset comprised the compounds used for subsequent docking analysis, and full compound identifiers are provided in the supplementary materials to facilitate reproducibility.

Allosteric binding site identification

Binding site analysis of the Neuropeptide Y1 receptor (NPY1R) was conducted to identify orthosteric, allosteric, and G-protein coupling regions. The three-dimensional structure of NPY1R was retrieved from the Protein Data Bank and visualized using PyMOL (v3.1). Potential allosteric pockets were predicted using the Allosite 2.0 server (https://mdl.shsmu.edu.cn/AST/), which integrates structural features, pocket geometry, and machine learning-based scoring. The identified binding domains were mapped and compared in PyMOL to ensure spatial distinction between orthosteric and allosteric regions, providing a foundation for targeted ligand docking.

Ligand and receptor preparation

Ligands and receptor structures were prepared following standardized computational protocols to ensure structural consistency prior to molecular docking and simulation analyses. The three-dimensional structure of the neuropeptide Y1 receptor (NPY1R), a class A G protein-coupled receptor (GPCR), was obtained from experimentally resolved structural data representing a stabilized inactive-like conformation suitable for antagonist or modulator screening. The selected structural state was chosen to enable identification of putative inhibitory or non-orthosteric binding modes rather than agonist-induced activation. Engineered mutations and missing segments inherent to crystallized GPCR constructs were carefully assessed, and unresolved residues or atoms were repaired where feasible using standard modeling procedures. Water molecules not directly involved in structural stabilization were removed, polar hydrogens were added, and protonation states were assigned under physiological pH assumptions. Partial atomic charges were calculated using the Gasteiger method.

Ligand structures derived from Indonesian spice compounds were similarly prepared by removing nonessential solvent molecules, assigning Gasteiger charges, and optimizing geometry prior to docking. All structures were converted into PDBQT format using OpenBabel to ensure compatibility with AutoDock-based workflows. Ligands were compiled into a single batch file to facilitate automated docking execution and consistent parameter application across all candidates.

Molecular docking and binding affinity screening

Blind molecular docking was performed using PyRx v0.8 to evaluate the binding potential of ligand candidates across the full surface of the neuropeptide Y1 receptor (NPY1R). Prior to docking, all ligand structures underwent energy minimization to reduce steric hindrance and optimize geometry. The use of blind docking allowed exploration of both orthosteric and allosteric binding sites, enabling unbiased ligand placement and broader interaction assessment.

Each compound was subjected to eight independent docking runs using AutoDock Vina to account for ligand flexibility and to ensure consistency in predicted binding affinities. The resulting binding poses were ranked based on predicted binding free energy (kcal/mol). Ligands demonstrating stronger binding affinity than the reference antagonist BMS-193885 were shortlisted for further evaluation. Visual inspection of docking orientations was also conducted to confirm favorable spatial conformation and key interaction profiles within the binding pocket. This combined approach enabled the identification of promising candidates with potential allosteric binding preference and high-affinity receptor engagement.

Binding interaction visualization

Detailed interaction analysis of top-ranked ligands was performed using LigPlot+ v2.2.8 to visualize hydrogen bonding networks and hydrophobic contacts within the receptor environment. The lead compound, cis-Theaspirone, was compared with both the endogenous ligand (NPY) and the reference antagonist (BMS-193885) to assess interaction similarity and potential non-agonistic binding characteristics. Interaction mapping focused on residue-level engagement within predicted non-orthosteric regions to evaluate whether binding patterns were consistent with putative modulatory mechanisms rather than orthosteric activation. These analyses were used solely to support structural hypotheses and not to claim functional inhibition.

Pharmacokinetic characterization

Pharmacokinetic and toxicity profiles were predicted using SwissADME (http://www.swissadme.ch/) and pkCSM (https://biosig.lab.uq.edu.au/pkcsm/) web servers. Ligand structures were submitted in defined molecular representations, including stereochemically accurate neutral forms under physiological conditions, to ensure consistency across prediction platforms. SwissADME was employed to evaluate physicochemical properties, gastrointestinal absorption, blood–brain barrier (BBB) permeability, and drug-likeness criteria such as Lipinski's rule of five. pkCSM provided quantitative predictions for absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters, including $CaCO_2$ permeability, volume of distribution (VDss), cytochrome P450 interactions, AMES toxicity, LD_{50} estimates, and clearance rates.

Interpretation of BBB permeability predictions was framed within the context of intended therapeutic application. Because modulation of NPY1R may involve central or peripheral mechanisms, BBB penetration was evaluated as a pharmacological property rather than an inherently advantageous feature. ADMET outputs were standardized into consistent units for comparative analysis across compounds.

Molecular dynamics

Molecular dynamics (MD) simulations were conducted using the SiBioLead platform (https://sibiolead.com) with the OPLS/AA force field to investigate stability and dynamic behavior of selected receptor–ligand complexes. Systems were solvated using the SPC (Simple Point Charge) water model within a triclinic periodic simulation box, and ionic strength was adjusted by adding NaCl to a concentration of 0.15 M to approximate physiological conditions. Ligand parameterization followed the force field specifications integrated within the simulation environment.

Energy minimization was performed for 5000 steps to remove steric clashes and optimize geometry prior to equilibration. Two equilibration phases were conducted: constant volume and temperature (NVT), followed by constant pressure and temperature (NPT), maintaining a temperature of 310°K and pressure of 1.0 bar. Each phase lasted 500 ps. Long-range electrostatic interactions were treated using particle mesh Ewald methods, and standard constraints were applied to maintain bond stability during simulation.

Production simulations were run for 50 ns to capture structural stability and interaction persistence under near-physiological conditions.

Trajectory analysis focused on root mean square deviation (RMSD), root mean square fluctuation (RMSF), and interaction stability metrics. While the simulation duration provides initial insights into complex stability, it is acknowledged that longer simulations or replicate runs would further strengthen conclusions for GPCR systems; therefore, results are interpreted as preliminary dynamic assessments rather than definitive energetic validation.

RESULTS

Virtual screening of Indonesian spice compounds and molecular docking

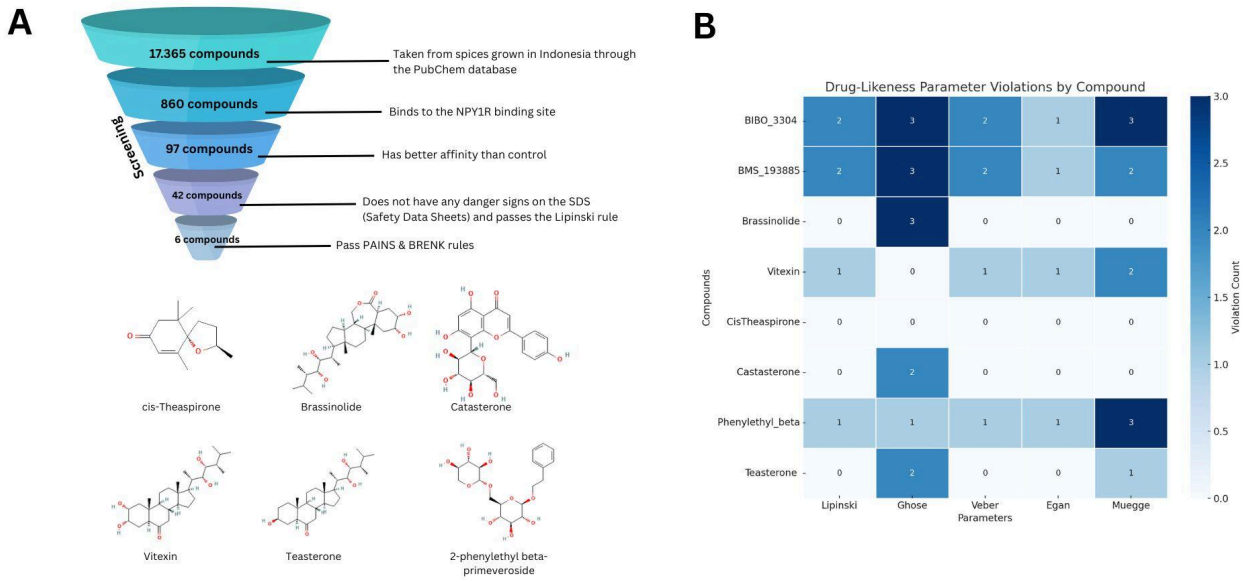

Figure 1. (A) Compounds screening process and (B) Drug-Likeness rule violations testing results across selected compounds

A multi-stage virtual screening workflow was applied to identify potential NPY1R modulators derived from Indonesian spice-associated compounds. A total of 17,356 chemical compounds and secondary metabolites were retrieved from the NCBI PubChem database and subjected to structure-based screening. Initial site mapping enabled unbiased exploration of ligand-accessible cavities across the NPY1 receptor (NPY1R), allowing identification of ligands localized within orthosteric and non-orthosteric regions without predefining binding pockets.

Following blind docking simulations, approximately 860 ligands demonstrated predicted binding poses within receptor-accessible regions. These compounds were further evaluated using a hierarchical prioritization strategy integrating docking score distribution, pose clustering consistency, physicochemical feasibility, and interaction plausibility rather than relying solely on absolute docking scores. This approach acknowledges that small differences in predicted binding energies may fall within the intrinsic uncertainty of empirical scoring functions and should therefore be interpreted as relative prioritization rather than definitive ranking.

Among the docked compounds, 97 ligands exhibited predicted binding energies comparable to or lower than the reference antagonist BMS-193885. These candidates were retained for subsequent filtering and structural evaluation. Root-mean-square deviation (RMSD) values reported at this stage refer

specifically to internal clustering metrics generated during docking pose analysis, reflecting structural similarity among predicted ligand conformations after alignment within the receptor binding cavity (Ramirez & Caballero, 2018). RMSD values were calculated over heavy atoms of the ligand after alignment to the receptor coordinate frame. Importantly, these RMSD values do not represent molecular dynamics-derived stability or experimental structural deviation and were therefore used only to identify recurring binding orientations across independent docking runs. Thresholds commonly used for structural validation (e.g., 2–3 Å RMSD) are not directly applicable to blind docking pose clustering and were not used as strict selection criteria (Fadil et al., 2023).

Pharmacokinetic and toxicity filtering

From the 97 prioritized docking candidates, additional filtering was applied to refine compounds based on drug-likeness and safety considerations. 72 ligands were classified as non-toxic according to hazard annotations available in the NCBI PubChem database. These compounds were subsequently evaluated using SwissADME to assess pharmacokinetic properties and compliance with Lipinski's rule of five, resulting in 42 ligands demonstrating physicochemical profiles consistent with oral drug-like characteristics (≤1 rule violation) (Cordero et al., 2024)

To reduce the risk of false-positive bioactivity and chemical reactivity, Pan-Assay Interference Compounds (PAINS) and Brenk structural filters were applied as orthogonal prioritization criteria. This filtering stage aimed to remove molecules with known assay-interfering substructures or unstable chemical motifs, thereby increasing the likelihood that retained candidates represent chemically tractable and biologically plausible scaffolds.

Rather than defining a single "best" compound based solely on docking results, the screening funnel was designed to iteratively reduce the chemical space using complementary criteria encompassing predicted binding potential, chemical liability, and pharmacokinetic feasibility. This integrated prioritization strategy allowed the identification of a refined subset of candidates for further structural and dynamic analysis.

Putative non-orthosteric binding site analysis

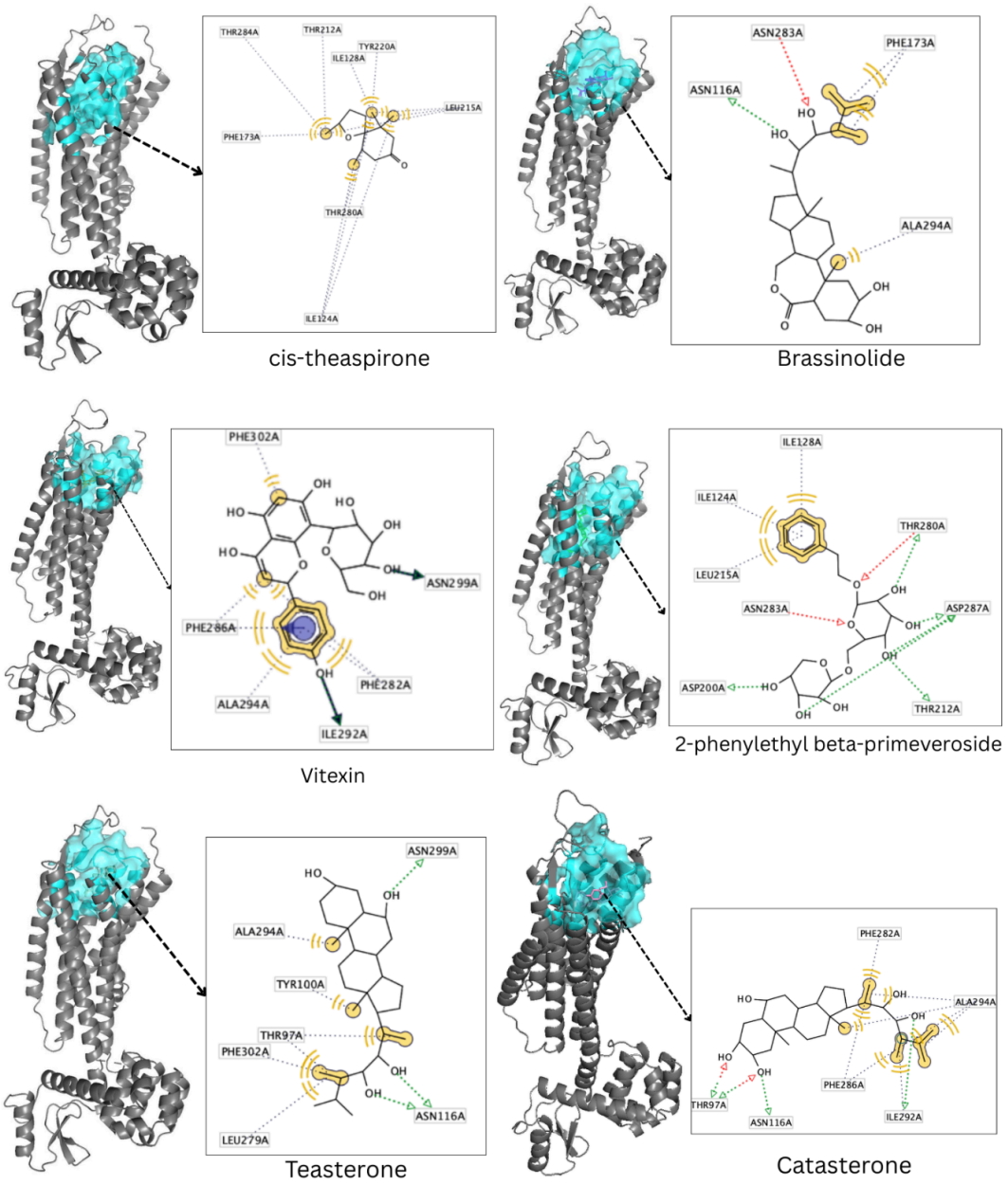

Figure 2. Molecular docking simulation results of selected ligands with the target protein. The binding interactions between the target protein (displayed as a gray ribbon structure) and six ligands: cis-Theaspirone, brassinolide, vitexin, 2-phenylethyl β-primeveroside, teasterone, and catasterone. The predicted binding pocket is highlighted in cyan, indicating the active site region where ligand interaction occurs. Each inset shows a 2D interaction diagram of the ligand within the binding site.

This tiered filtering process yielded six top ligand candidates: brassinolide, castasterone, cis-Theaspirone, teasterone, 2-phenylethyl beta-primeveroside, and vitexin. Among them, cis-Theaspirone emerged as the most promising allosteric modulator of NPY1R. It satisfied all Lipinski criteria, including molecular weight ≤ 500 Da, lipophilicity (MLOGP) ≤ 4.15, hydrogen bond acceptors (≤10), and hydrogen bond donors (≤5) (Ekawasti et al., 2021). The blue-highlighted section indicated the allosteric binding site with the amino acid that is involved in the interaction, which can be further analyzed as drug specificity sites (Salahudeen & Nishtala, 2017).

Table 1. Interaction of the best ligand candidates with NPY1R

Compound	RMSD (A)	Binding Affinity (kcal/mol)	Binding Sites (Residue)	Orthosteric Overlap	Allosteric Overlap
cis-Theaspirone	2,658	-10	Ile128, Tyr220, Thr212, Thr284, Thr280, Phe 173, Leu215	None	Ile128, Phe173
Castasterone	2,100	-9.2	Thr97, Asn116, Ala294, Phe282, Ile292	Thr97	None
Teasterone	3,085	-9.1	Asn299, Ala294, Tyr100, Thr97, Phe 302A, Asn116, Leu279	Thr97, Phe302	None
2-Phenylethyl beta-primeveroside	2,100	-9.9	Ile124, Ile128, Leu215, Asn283, Asp287, Thr280, Asp200, Thr212	Asp200	Ile124, Ile128
Brassinolide	1,973	-9.2	Ala294, Asn116, Phe Phe173,Asn283	None	None
Vitexin	3.766	-9.8	Ile292, Phe282, Ala294, Phe286, Phe302	Phe302	None

Among the six lead ligands, cis-Theaspirone exhibited the strongest binding affinity (–10.0 kcal/mol) and an RMSD of 2.658 Å. It interacted with residues localized in pocket 2 (Ile128, Phe173) without overlapping with the orthosteric site, suggesting selective allosteric binding. In contrast, castasterone and teasterone were found to interact with canonical orthosteric residues (Thr97, Phe302), indicating potential competitive binding. 2-Phenylethyl beta-primeveroside exhibited dual binding behavior, interacting with both orthosteric (Asp200) and allosteric (Ile124, Ile128) residues. Brassinolide and vitexin did not engage any known allosteric residues, although vitexin showed interaction with orthosteric residue Phe302.

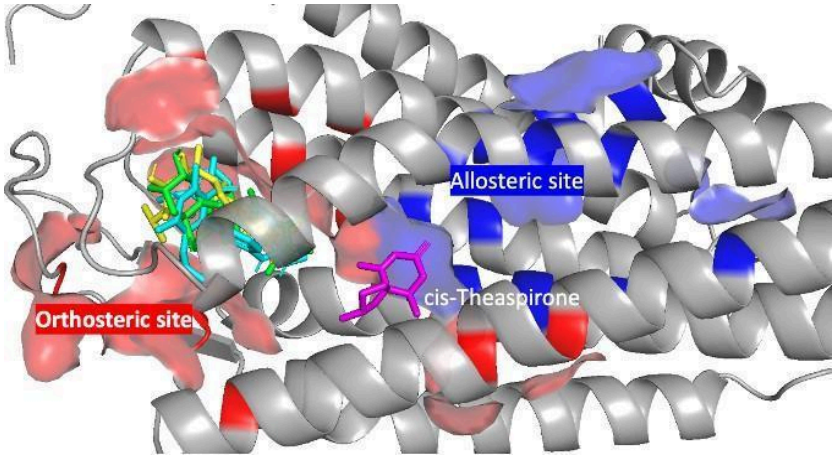

Figure 3. Predicted binding poses of the top ligand candidates on NPY1R, highlighting the orthosteric and allosteric binding sites

Rather than interpreting docking poses as definitive evidence of functional modulation, these analyses provide structural hypotheses regarding potential binding regions. The visualization of docking poses (**Figure 3**) illustrates the spatial separation between the orthosteric and putative non-orthosteric cavities. The orthosteric site (red) aligns with known native ligand interactions, whereas pocket 2 (blue) represents a geometrically distinct cavity identified through structural analysis and pocket prediction tools. The cis-Theaspirone binding pose (magenta) localized within pocket 2 without predicted orthosteric overlap under the defined criteria.

To complement static interaction diagrams, preliminary interaction persistence was evaluated by examining recurring contact residues across multiple docking poses and subsequent molecular dynamics sampling. Residues Ile128 and Phe173 exhibited repeated interaction patterns with cis-Theaspirone across independent conformations, suggesting potential stability of ligand positioning within the putative non-orthosteric cavity. However, these findings should be interpreted as computational hypotheses requiring experimental validation.

Pharmacokinetic characterization

The pharmacokinetic profiles of the six lead compounds—cis-Theaspirone, castasterone, teasterone, 2-phenylethyl beta-primeveroside, brassinolide, and vitexin—were evaluated using *in silico* tools pkCSM and SwissADME. All compounds demonstrated varying degrees of oral absorption, distribution, and blood–brain barrier (BBB) permeability. cis-Theaspirone, castasterone, and teasterone exhibited high intestinal absorption (>70%), suggesting favorable oral bioavailability, while 2-phenylethyl beta-primeveroside and vitexin showed markedly lower absorption values (<50%), indicating limited uptake via the gastrointestinal tract.

Table 2. Predicted pharmacokinetic parameters of selected lead compounds using pkCSM

Parameter	cis-Theaspirone	Catasterone	Teasterone	2-Phenylethyl beta-primeveroside	Brassinolide	Vitexin	Unit
Water solubility	-2.957	-4.721	-5.263	-2.284	-4.717	-2.845	Log mol/L
$CaCO_2$ permeability	1.471	0,445	0,534	0,225	0,446	-0.956	Log Papp in 10-6 cm
Intestinal absorption (human)	99.558	71.892	92.796	34.437	68.118	46.695	% Absorbed
Skin Permeability	-2.428	-3.424	-3.436	-2.736	-3.348	-2.735	Log Kp

Parameter	cis-Theaspirone	Catasterone	Teasterone	2-Phenylethyl beta-primeveroside	Brassinolide	Vitexin	Unit
P-glycoprotein substrate	Yes	Yes	Yes	Yes	Yes	Yes	Categorical (Yes/No)
P-glycoprotein inhibitor	No	Yes	Yes	No	Yes	No	Categorical (Yes/No)
P-glycoprotein II inhibitor	No	Yes	Yes	No	Yes	No	Categorical (Yes/No)
VDss (human)	0.245	-0.792	-0.356	0,275	-0.918	1.071	Log L/kg
Fraction unbound (human)	0.304	0.045	0	0,461	0.063	0,168	Fu
BBB permeability	00.41	-0.573	-0.425	-0.939	-0.682	-1.449	Log BB
CNS permeability	-2558	-2.511	-2.122	-4.305	-3.115	-3.834	Log PS
CYP2D6 substrate	No	No	No	No	No	No	Categorical (Yes/No)
CYP3A4 substrate	No	Yes	Yes	No	Yes	No	Categorical (Yes/No)
CYP1A2 inhibitor	Yes	No	No	No	No	No	Categorical (Yes/No)
CYP2C19 inhibitor	No	No	No	No	No	No	Categorical (Yes/No)
CYP2C9 inhibitor	No	No	No	No	No	No	Categorical (Yes/No)
CYP2D6 inhibitor	No	No	No	No	No	No	Categorical (Yes/No)

Parameter	cis-Theaspirone	Catasterone	Teasterone	2-Phenylethyl beta-primeveroside	Brassinolide	Vitexin	Unit
CYP3A4 inhibitor	No	No	No	No	No	No	Categorical (Yes/No)
Total clearance	0.633	0,438	0,0423	1.094	0,420	0,308	Log ml/min/kg
Renal OCT2 substrate	No	No	No	No	No	No	Categorical (Yes/No)
AMES toxicity	No	No	No	No	No	No	Categorical (Yes/No)
Max. tolerated dose (human)	0.484	-1.15	-1.043	0,252	-1.071	0,400	Log mg/kg/day
hERG I inhibitor	No	No	No	No	No	No	Categorical (Yes/No)
hERG II inhibitor	No	No	No	No	No	No	Categorical (Yes/No)
Oral rat acute toxicity (LD_{50})	2.282	2.762	2.563	1.907	2.777	2.595	mol/kg
Oral rat chronic toxicity (LOAEL)	1.697	0.239	1.824	3.231	1.942	4.635	Log mg/kg_bw / day
Hepatotoxicity	No	No	No	No	No	No	Categorical (Yes/No)
Skin sensitization	Yes	No	No	No	No	No	Categorical (Yes/No)
T. pyriformis toxicity	0.436	0.227	0,325	0,197	0,207	0,197	Log ug/L

Parameter	cis-Theaspirone	Catasterone	Teasterone	2-Phenylethyl beta-primeveroside	Brassinolide	Vitexin	Unit
Minnow toxicity	1.483	0.468	0,078	4.852	0,501	4.897	Log mM
Plants Source	*Camellia sinensis*	*Camellia sinensis, Nutmeg, Clove*	*Camellia sinensis*	*Ficus fistulosa*	*Camellia sinensis*	*Acanthus ilicifolius*	

In terms of permeability, cis-Theaspirone had the highest $CaCO_2$ permeability, suggesting a strong membrane-crossing potential, whereas vitexin displayed a negative value, suggesting poor passive permeability. BBB and CNS permeability also varied significantly. Cis-Theaspirone was the only compound predicted to cross the BBB according to SwissADME (**Table 3**), while the others were largely restricted, supported by negative log BB and log PS values in pkCSM (**Table 2**). This suggests a limited potential for CNS activity in most of the compounds, except for cis-Theaspirone.

Regarding metabolic interactions, most compounds were non-substrates of CYP2D6 and CYP3A4, reducing the likelihood of rapid metabolism or drug–drug interactions. However, some variation was seen in CYP inhibition, notably with teasterone and catasterone showing potential inhibition of P-glycoprotein and multiple CYP isoforms. Distribution profiles based on VDss and fraction unbound were heterogeneous; for example, vitexin showed a high VDss and low Fu, which could affect tissue accumulation and free drug levels in plasma.

Table 3. SwissADME prediction of BBB permeability, GI bioavailability, and solubility of bioactive compounds found in nutmeg, clove, and tea

Compound	BBB Permeability	Gastrointestinal Bioavailability	Water Solubility (mg/ml)
cis-Theaspirone	Yes	High	15.2 (High)
Castasterone	No	High	67.5 (High)
Teasterone	No	High	39.1 (High)
2-Phenylethyl beta-primeveroside	No	Low	99 (High)
Brassinolide	No	High	0.000719 (Low)
Vitexin	No	Low	0.116 (Moderate)

Collectively, these findings reveal cis-Theaspirone and teasterone as possessing the most balanced PK characteristics, whereas 2-phenylethyl beta-primeveroside and vitexin may be limited by poor absorption and CNS permeability, despite some strengths in solubility or clearance. This comparative analysis provides a basis for prioritizing candidates for further pharmacological and *in vivo* evaluation.

Molecular dynamic simulations

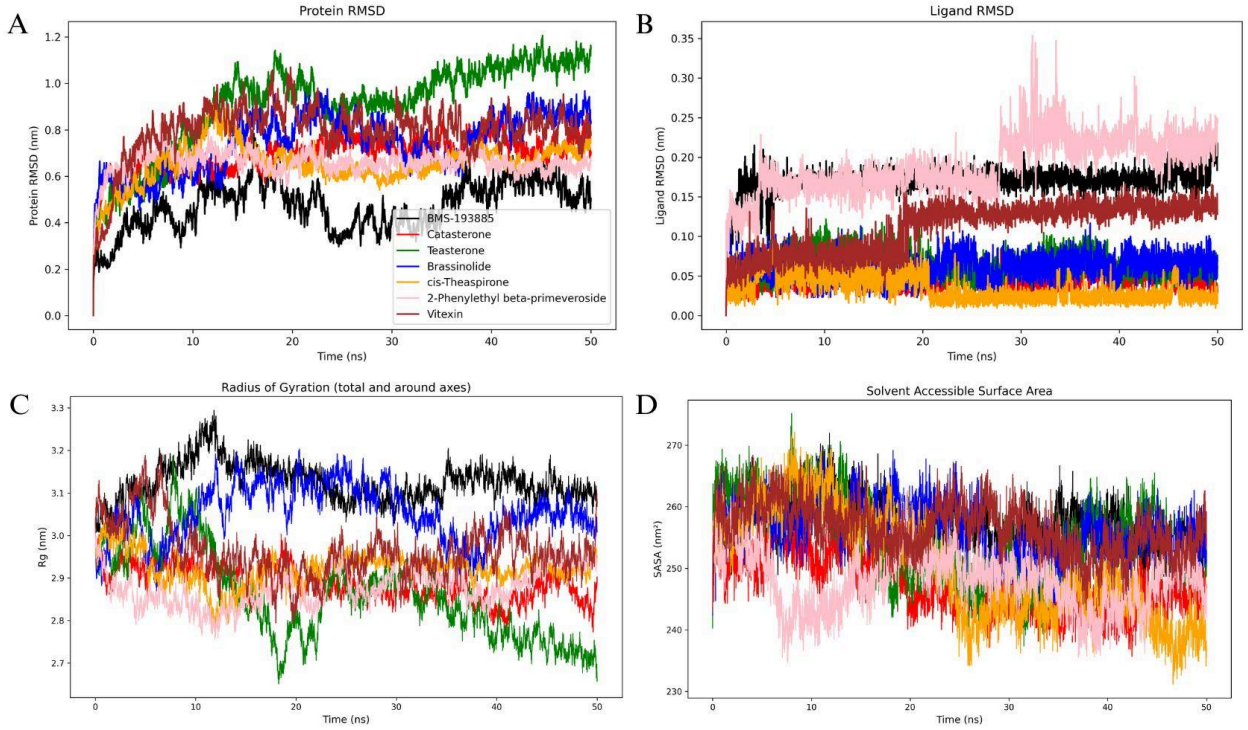

Figure 4. Molecular dynamics (MD) simulation profiles of NPY1R–ligand complexes. Time-dependent structural and interaction analyses of NPY1R in complex with six candidate ligands and the control (BMS-193885) over a 50 ns simulation are presented. (A) Protein root mean square deviation (RMSD) showing backbone stability of the receptor. (B) Ligand RMSD indicating positional stability of ligands within the binding pocket. (C) Radius of gyration (Rg) representing overall compactness of the protein structure. (D) Solvent-accessible surface area (SASA) reflects changes in protein surface exposure to the solvent.

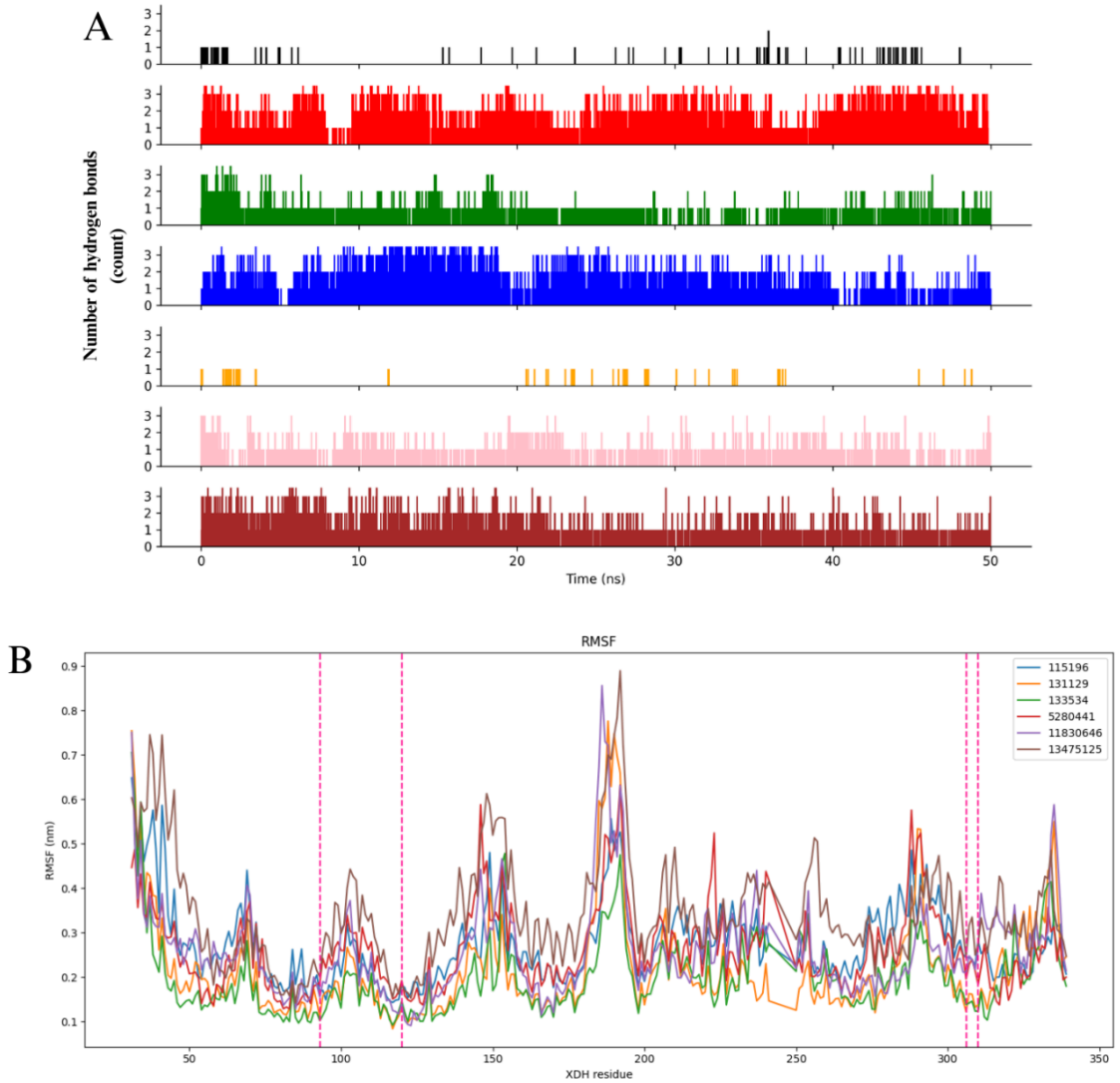

Figure 5. Molecular dynamics (MD) simulation profiles of NPY1R–ligand complexes. Time-dependent structural and interaction analyses of NPY1R in complex with six candidate ligands and the control (BMS-193885) over a 50 ns simulation are presented. (A) Number of hydrogen bonds formed between ligands and NPY1R over time, indicating interaction persistence. (B) Root mean square fluctuation (RMSF) showing residue-level flexibility across the receptor, with dashed regions highlighting residues associated with the binding site.

Based on the molecular dynamics simulation results, the six lead compounds identified through structure-based virtual screening were further evaluated for their dynamic stability and interaction with the NPY1 receptor (NPY1R), with BMS-193885 serving as the control. The analysis of the radius of gyration (Rg) revealed that catasterone and teasterone contributed to a more compact receptor structure, which may support protein stabilization, while brassinolide and the control compound BMS-193885 led to a slightly more expanded conformation, indicating increased protein flexibility (**Figure 4c**). Ligand RMSD analysis highlighted cis-Theaspirone and brassinolide as the most stable within the NPY1R binding pocket, suggesting strong and consistent interactions over the 50 ns simulation period. In contrast, 2-phenylethyl beta-primeveroside exhibited high fluctuations, indicating weaker binding affinity (**Figure 4b**). These results were further supported by hydrogen bond analysis, where catasterone and brassinolide formed the most persistent interactions with the receptor, and cis-Theaspirone also demonstrated moderate but stable hydrogen bonding (**Figure 5a**).

The root mean square fluctuation (RMSF) profiles showed that catasterone and cis-Theaspirone minimized residue-level flexibility, especially around functionally relevant regions of the receptor, indicating structural stability (**Figure 5b**). Protein RMSD analysis confirmed that BMS-193885 maintained the most rigid protein backbone, consistent with its role as a known NPY1R inhibitor. Among the candidate compounds, teasterone caused the highest structural deviation, suggesting potential destabilizing effects on the receptor (**Figure 4a**). Solvent-accessible surface area (SASA), ligand pockets, and hydrogen bond occupancy analysis indicated that catasterone and cis-Theaspirone maintained or slightly reduced surface exposure, further supporting their roles in stabilizing the receptor conformation (**Figure 4d**).

DISCUSSION

Therapeutic potential analysis of cis-Theaspirone

According to previously mentioned criteria, overall, cis-Theaspirone emerged as the most promising compound, displaying strong binding stability, moderate hydrogen bonding, and limited structural disruption. These dynamic characteristics align with initial docking predictions and support cis-Theaspirone's potential as a lead natural compound for NPY1R modulation. Its favorable profile suggests that it could be further developed into a safer and more effective therapeutic candidate for type 2 diabetes mellitus, with potential benefits in appetite regulation and weight control, thus preventing metabolic disorders due to diabetes complications through targeted modulation of the NPY signaling pathway (Marcos & Coveñas, 2022; Młynarska et al., 2025).

The present computational analysis identifies cis-Theaspirone as a prioritized ligand candidate predicted to interact with a putative non-orthosteric pocket of the neuropeptide Y1 receptor (NPY1R). Importantly, docking and molecular dynamics simulations provide structural hypotheses regarding ligand–receptor engagement but do not establish functional modulation or pharmacological directionality. Accordingly, the mechanistic interpretation presented here is framed as a working hypothesis rather than a definitive conclusion.

Based on its predicted localization outside the canonical orthosteric site and absence of direct overlap with known endogenous ligand residues, cis-Theaspirone is hypothesized to function as a putative negative allosteric modulator (NAM) of NPY1R signaling. Negative allosteric modulation could theoretically attenuate receptor signaling by altering receptor conformational dynamics or reducing orthosteric ligand efficacy without direct competitive binding. However, this interpretation remains speculative because computational modeling alone cannot determine whether ligand binding results in receptor inhibition, potentiation, or silent stabilization. Future functional assays, including β-arrestin recruitment, G-protein signaling assays, and mutagenesis studies, will be required to determine the true pharmacological behavior of cis-Theaspirone.

Implications of non-orthosteric targeting in GPCR drug discovery

Targeting non-orthosteric pockets in G protein-coupled receptors (GPCRs) represents an emerging strategy to improve selectivity and modulation of receptor signaling bias. Unlike orthosteric ligands that compete directly with endogenous peptides, non-orthosteric ligands may fine-tune receptor activity by stabilizing specific conformational states. The predicted interaction of cis-Theaspirone within pocket 2 suggests a potential mechanism for modulating receptor signaling without fully blocking endogenous ligand binding. Nevertheless, pocket prediction identifies geometric cavities rather than validated regulatory sites; therefore, the designation of this region as an allosteric site should be interpreted cautiously pending experimental confirmation.

Relevance of Indonesian spice-derived natural products

A central focus of this study is the systematic investigation of spice-derived compounds from Indonesia as a reservoir of structurally diverse bioactive molecules. Several prominent plant species, including *Camellia sinensis* (tea), vanilla (*Vanilla planifolia*), and purple passion fruit (*Passiflora edulis*), are recognized for contributing characteristic aroma and flavor profiles through their complex phytochemical compositions (Sun et al., 2023). Indonesia's exceptional biodiversity, combined with its longstanding ethnopharmacological traditions, provides access to a wide spectrum of phytochemicals possessing unique chemical scaffolds that differ substantially from those typically represented in synthetic drug libraries. These natural products often arise from specialized secondary metabolic pathways and exhibit significant structural complexity and stereochemical diversity. For example, cis-Theaspirone, a norisoprenoid compound, originates from plant-derived secondary metabolites commonly associated with aromatic spices and traditional herbal preparations, including clove (*Syzygium aromaticum*) and nutmeg (*Myristica fragrans*). Such compounds highlight the potential of Indonesian spice plants as a valuable source of novel lead structures for drug discovery and bioactivity-guided research.

The integration of natural product chemistry with computational screening offers an efficient strategy to prioritize candidates with novel structural features that may interact with unconventional receptor pockets. Importantly, this approach does not assume therapeutic efficacy but instead highlights Indonesian spices as a valuable reservoir for hypothesis-driven drug discovery pipelines.

ADMET profile and therapeutic considerations

Predicted pharmacokinetic properties suggest that cis-Theaspirone possesses favorable oral bioavailability and potential blood–brain barrier permeability. According to previous research, besides central nervous system (CNS) exposure, which could be advantageous for modulating hypothalamic NPY signaling, it may also introduce safety considerations, including effects on appetite regulation or neurobehavioral pathways. Therefore, BBB permeability should not be interpreted as inherently yet directly beneficial but rather as a property that requires careful evaluation within a defined therapeutic context. The current ADMET predictions should be considered preliminary, as *in silico* tools provide probabilistic estimates rather than experimentally validated pharmacokinetic behavior.

Subtype selectivity and off-target considerations

A critical limitation of the present analysis is the absence of cross-target evaluation against related receptors within the neuropeptide Y receptor family (NPY2R, NPY4R, and NPY5R) or broader GPCR panels. Given the structural similarity among NPY receptor subtypes and their overlapping physiological roles, subtype selectivity represents a major challenge in developing safe modulators. Off-target binding to related GPCRs could lead to unintended physiological effects, particularly if CNS exposure occurs. Future work should include cross-docking against related receptor subtypes, ligand-based similarity screening, or experimental selectivity assays to evaluate potential off-target interactions (Nestler & Lüscher, 2019; Qiu & Fu, 2025).

Limitations and future validation strategy

Several limitations inherent to computational studies must be acknowledged. First, docking scores provide relative estimates that may fall within the uncertainty range of scoring functions, and therefore should not be interpreted as definitive measures of binding affinity. Second, GPCRs are highly dynamic proteins, and simulations based on a single receptor conformation may not fully capture conformational

ensembles relevant to ligand binding. Third, ADMET predictions rely on statistical models that may not accurately reflect biological complexity (Lafferty et al., 2024).

To address these limitations, future validation should include (1) functional assays to determine modulation directionality, (2) mutagenesis studies targeting predicted pocket residues to confirm binding regions, (3) extended molecular dynamics simulations with replicate trajectories and energetic analysis, and (4) experimental evaluation of subtype selectivity and off-target interactions.

CONCLUSION

This study computationally prioritizes cis-Theaspirone as a potential allosteric modulator of the Neuropeptide Y1 Receptor (NPY1R), a biologically relevant target associated with appetite regulation and type 2 diabetes mellitus (T2DM). The investigated compound library was derived from phytochemical constituents reported in Indonesian spices, including secondary metabolites traditionally present in culinary and medicinal plants widely used in Indonesian ethnobotanical practices. By leveraging the chemical diversity inherent to spice-derived natural products, this work explores an underutilized reservoir of bioactive molecules for modern structure-based drug discovery.

Through an integrated *in silico* workflow combining virtual screening, molecular docking, molecular dynamics simulation, and ADMET prediction, an initial dataset of more than 17,000 spice-associated compounds was systematically refined into a small subset of computationally prioritized candidates. Among these, cis-Theaspirone demonstrated the most favorable predicted interaction profile at a putative allosteric site distinct from the orthosteric NPY-binding region. Simulation analyses suggested stable receptor–ligand interactions with limited conformational disruption, while pharmacokinetic predictions indicated favorable absorption characteristics and potential central nervous system accessibility. These properties highlight the value of spice-derived phytochemicals as structurally diverse scaffolds capable of modulating metabolically relevant receptors.

Experimental validation remains essential to verify binding mechanisms, functional modulation of NPY1R signaling, and therapeutic relevance. Nonetheless, this work highlights Indonesian spice-derived phytochemicals as an underexplored resource in drug discovery and establishes a computational framework for identifying novel allosteric modulators targeting metabolic pathways. cis-Theaspirone, therefore, represents a promising starting point for subsequent *in vitro* and *in vivo* investigation.

AUTHOR CONTRIBUTIONS

BW: Conceptualization, Investigation, Writing Original – draft, Writing – Review & editing. **JA**: Conceptualization, Data curation, Writing Original – draft, Writing – Review & editing. **MD**: Methodology, Software/validation, Formal analysis. **KK**: Conceptualization, Supervision/project administration, Writing – review.

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COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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