



Research Article

Computational Assessment of Secondary Metabolites as Potential Inhibitors of Methylenetetrahydrofolate Dehydrogenase-2 (MTHFD2)

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Abstract:

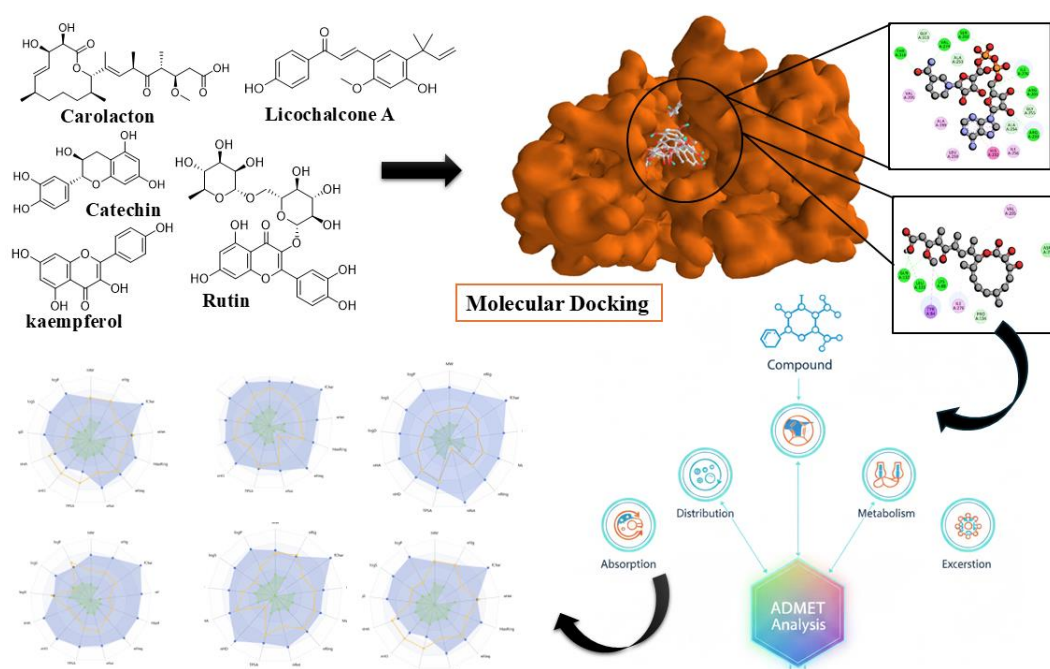
Methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) is a mitochondrial enzyme involved in one-carbon metabolism and is highly upregulated in rapidly proliferating cancer cells, making it a promising therapeutic target. In this study, five structurally diverse secondary metabolites—catechin, kaempferol, rutin, licochalcone A, and carolacton—were computationally evaluated as potential MTHFD2 inhibitors using an integrated molecular docking and ADMET approach. Docking simulations were performed using PyRx with AutoDock against the crystal structure of MTHFD2 (PDB ID: 6KG2), and interaction profiles were analyzed using Discovery Studio Visualizer. Catechin and kaempferol demonstrated the strongest binding affinities (−7.1 kcal/mol), exceeding that of the native ligand (−5.9 kcal/mol), and formed stable hydrogen bonding and hydrophobic interactions with key catalytic residues including TYR84, ILE276, and THR316. Licochalcone A and carolacton showed moderate binding potential, whereas rutin exhibited weaker affinity, likely due to steric hindrance from its glycosidic moiety. Comprehensive ADMET profiling using SwissADME and ADMETlab 3.0 indicated that catechin and kaempferol possess favorable physicochemical properties, good drug-likeness, and comparatively safer metabolic and toxicity profiles. Environmental toxicity predictions further suggested acceptable ecological safety across the evaluated compounds. Overall, catechin and kaempferol emerge as promising natural scaffolds for MTHFD2 inhibition. Further in vitro and in vivo studies are required to validate their therapeutic potential and translational applicability.

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Graphical Abstract



1. Introduction

Cancer and other proliferative disorders are characterized by extensive metabolic reprogramming that supports rapid cell growth, survival, and biosynthesis (1). One of the key metabolic adaptations observed in highly proliferative cells is the upregulation of one-carbon metabolism, which is a pathway essential for nucleotide synthesis, redox balance, and methylation reactions (2). Within this pathway, methylenetetrahydrofolate dehydrogenase-2 (MTHFD2) plays a central role by catalyzing the mitochondrial conversion of one-carbon units, thereby supporting de novo purine and thymidylate biosynthesis (3). Importantly, MTHFD2 is highly expressed in embryonic tissues and a wide range of cancers, whereas its expression remains low or absent in most normal adult tissues, making it an attractive and selective therapeutic target (4). Inhibition of MTHFD2 has emerged as a promising strategy for disrupting cancer cell metabolism and limiting tumor progression (5,6). Although several synthetic inhibitors have been reported, their clinical application has been limited by issues related to toxicity, metabolic instability, and off-target effects. Consequently, there is a growing interest in identifying safer and more effective MTHFD2 inhibitors from natural sources. Secondary metabolites, particularly phytochemicals such as flavonoids, chalcones, and other polyphenolic compounds, are known for their structural diversity, biological compatibility, and multi-target pharmacological potential (7,8). These compounds often exhibit anticancer, antioxidant, and enzyme-inhibitory activities, making them promising candidates for targeting metabolic enzymes (9,10).

To systematically explore the inhibitory potential of natural metabolites against MTHFD2, five structurally diverse phytochemicals, catechin, kaempferol, rutin, licochalcone A, and carolacton, were selected for this study. Catechins and kaempferols are well-characterized flavonoids known for their strong antioxidant, antiproliferative, and enzyme-modulating activities, along with favorable drug-likeness profiles (11,12). Rutin, a glycosylated flavonoid derivative, was used to evaluate the influence of increased molecular size and polarity on binding affinity and pharmacokinetic behavior (13). Licochalcone A, a chalcone derivative, possesses a distinct open-chain flavonoid structure that demonstrates notable anticancer and anti-inflammatory potential (14). Carolacton, a natural macrolide product, was selected because of its unique scaffold and bioactivity against proliferative pathways (15). The inclusion of these compounds enables a comparative evaluation across different phytochemical classes, structural frameworks, and physicochemical properties, thereby providing a rational basis for identifying promising MTHFD2 inhibitors from secondary metabolites. In this context,

integrating natural-product-based drug discovery with modern computational screening tools provides a rational strategy for identifying novel and selective MTHFD2 inhibitors.

Advances in computational drug discovery have significantly accelerated the early stages of lead identification by enabling the rapid screening of bioactive compounds against specific molecular targets. Molecular docking allows for the prediction of binding affinity, orientation, and key interactions between small molecules and target proteins, providing insights into their inhibitory potential at the molecular level. In parallel, *in silico* ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) analysis plays a crucial role in assessing pharmacokinetic behavior, drug-likeness, and safety profiles, thereby reducing the risk of late-stage drug development failure (16). Therefore, combining molecular docking with ADMET prediction enables comprehensive evaluation of candidate molecules prior to experimental validation.

In this context, the present study focused on the computational assessment of selected secondary metabolites as potential inhibitors of MTHFD2 (17). A structure-based molecular docking approach was employed to investigate the binding efficiency of these compounds within the active site of MTHFD2 and compare their performance with that of the native ligand (18,19). Furthermore, comprehensive *in silico* ADMET profiling was conducted to evaluate physicochemical properties, oral bioavailability, metabolic stability, and toxicity risks. By integrating molecular docking with pharmacokinetic and toxicity predictions, this study aimed to identify promising natural lead compounds that combine a strong target affinity with favorable drug-like characteristics. These findings are expected to provide a rational basis for further experimental validation and development of novel MTHFD2-targeted therapeutics derived from secondary metabolites.

2. Method Section

2.1 Molecular Docking

Molecular docking was performed to evaluate the binding affinities and interaction patterns of the selected phytochemical compounds with the target protein (Figure 1). Ligand structures were retrieved from the PubChem database in the SDF format and refined using ChemDraw to ensure correct stereochemistry. The ligands were energy-minimized and converted into three-dimensional formats suitable for docking (20) (21). The crystal structure of the target protein was obtained from the Protein Data Bank and was prepared by removing water molecules, adding hydrogen atoms, correcting bond orders, and optimizing the structure. Docking simulations were performed using PyRx integrated with AutoDock tools. Both proteins and ligands were converted into the PDBQT format, and the docking grid was centered at X = -63.106811, Y = 29.481838, and Z = -27.692730 to cover the active site region. Default docking parameters were applied and multiple binding poses were generated. Docking results were assessed based on the binding energy (kcal/mol), and the most stable ligand-protein complex was selected. Interaction analysis and visualization were performed using Discovery Studio Visualizer to identify key noncovalent interactions (22,23). Following the assessment of the binding affinity and interaction patterns through molecular docking, further pharmacokinetic and toxicity evaluations were conducted using *in silico* ADMET analysis to determine the drug development potential of the selected compounds.

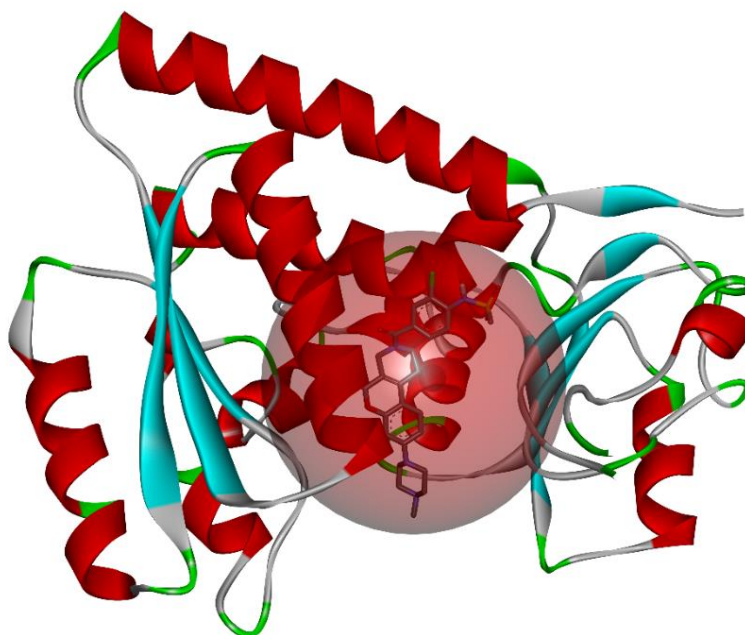


Figure 1. Active Cavity of Methylenetetrahydrofolate dehydrogenase 2 With native ligand

2.2 ADMET Analysis

In silico ADMET analyses were performed to evaluate the pharmacokinetic and drug-likeness properties of the selected compounds (24). Chemical structures were retrieved from the PubChem database and redrawn or verified using ChemDraw to ensure the correct structural and stereochemical accuracy. The finalized structures were exported in SMILES format for further analysis. SwissADME was used to predict key absorption and drug-likeness parameters, including gastrointestinal absorption, lipophilicity (LogP), water solubility, bioavailability score, blood–brain barrier permeability, compliance with Lipinski’s rule of five, and other drug-likeness filters (25,26). Comprehensive ADMET profiling was further conducted using ADMETlab 3.0, to assess the distribution, metabolism, excretion, and toxicity-related parameters. The predictions included human intestinal absorption, plasma protein binding, cytochrome P450 interactions, clearance, hERG inhibition, hepatotoxicity, mutagenicity, and carcinogenicity. This integrated computational approach enabled the identification of compounds with favorable pharmacokinetic behaviors and acceptable safety profiles for further experimental investigations.

3. Results and Discussion

3.1 Molecular Docking

Molecular docking studies were performed to evaluate the binding affinity, orientation, and interaction profiles of selected phytochemicals within the active site of Methylenetetrahydrofolate dehydrogenase 2 (MTHFD2; PDB ID: 6KG2), with particular emphasis on comparison with the native ligand. Docking scores and interaction details (Table 1) revealed clear differences in the binding strength and interaction quality among the tested compounds. Catechin and kaempferol exhibited the strongest binding affinities, with a docking score of -7.1 kcal/mol, which was notably stronger than that of the native ligand (-5.9 kcal/mol). Catechin establishes multiple conventional hydrogen bonds with key catalytic residues TYR84, VAL274, and ILE276, supported by hydrophobic π - σ interactions with ILE276 and π - π stacking with TYR84. This combination of strong polar and aromatic interactions indicated efficient anchoring and optimal positioning of catechin within the active pocket, surpassing the binding efficiency of the native ligand. Similarly, kaempferol forms stable hydrogen bonds with TYR84, THR316, and ILE276, along with π - π stacking interactions with TYR84. Its planar flavonoid scaffold likely promotes favorable alignment within the binding cavity, resulting in stronger binding than that of the

native ligand. Licochalcone A also demonstrated high binding affinity (−6.9 kcal/mol), outperforming the native ligand. It interacted through a conventional hydrogen bond with ASN87 and a carbon hydrogen bond with VAL274, whereas hydrophobic π - σ and π -alkyl interactions involving TYR84, THR316, and ILE276 further stabilized the complex. Carolacton showed a moderately strong docking score (−6.7 kcal/mol), again higher than native ligand, forming hydrogen bonds with LEU133 and GLN132 and additional hydrophobic interactions with TYR84, ILE276, and VAL205. Although slightly weaker than that of catechin and kaempferol, its interaction profile suggests a meaningful inhibitory potential. In contrast, the native ligand displayed a moderate binding affinity (−5.9 kcal/mol) despite forming an extensive interaction network across the binding pocket. It establishes a conventional hydrogen bond with ARG201, carbon hydrogen bonding with ALA254, and several hydrophobic and amide- π interactions involving HIS232, ARG233, ILE256, and LEU259. While these interactions indicated good accommodation within the active site, the overall binding energy was lower than that of most of the tested phytochemicals, suggesting comparatively reduced binding stability.

Rutin showed the weakest binding affinity (−5.1 kcal/mol), which was lower than that of both the native ligand and other phytochemicals. Although it formed multiple hydrogen bonds and π -interactions, its bulky glycosidic structure likely caused steric hindrance, limiting deep penetration into the active site and reducing the binding efficiency. Overall, compared with the native ligand, catechin, kaempferol, and licochalcone A demonstrated superior binding affinities and more favorable interaction patterns with crucial active-site residues, such as TYR84, ILE276, and THR316. These findings indicate that these phytochemicals may act as more effective MTHFD2 inhibitors than the native ligand, and merit further experimental validation through in vitro and in vivo studies. 2D and 3D binding interactions of the most potent compounds with methylenetetrahydrofolate dehydrogenase 2 are shown in Table 2.

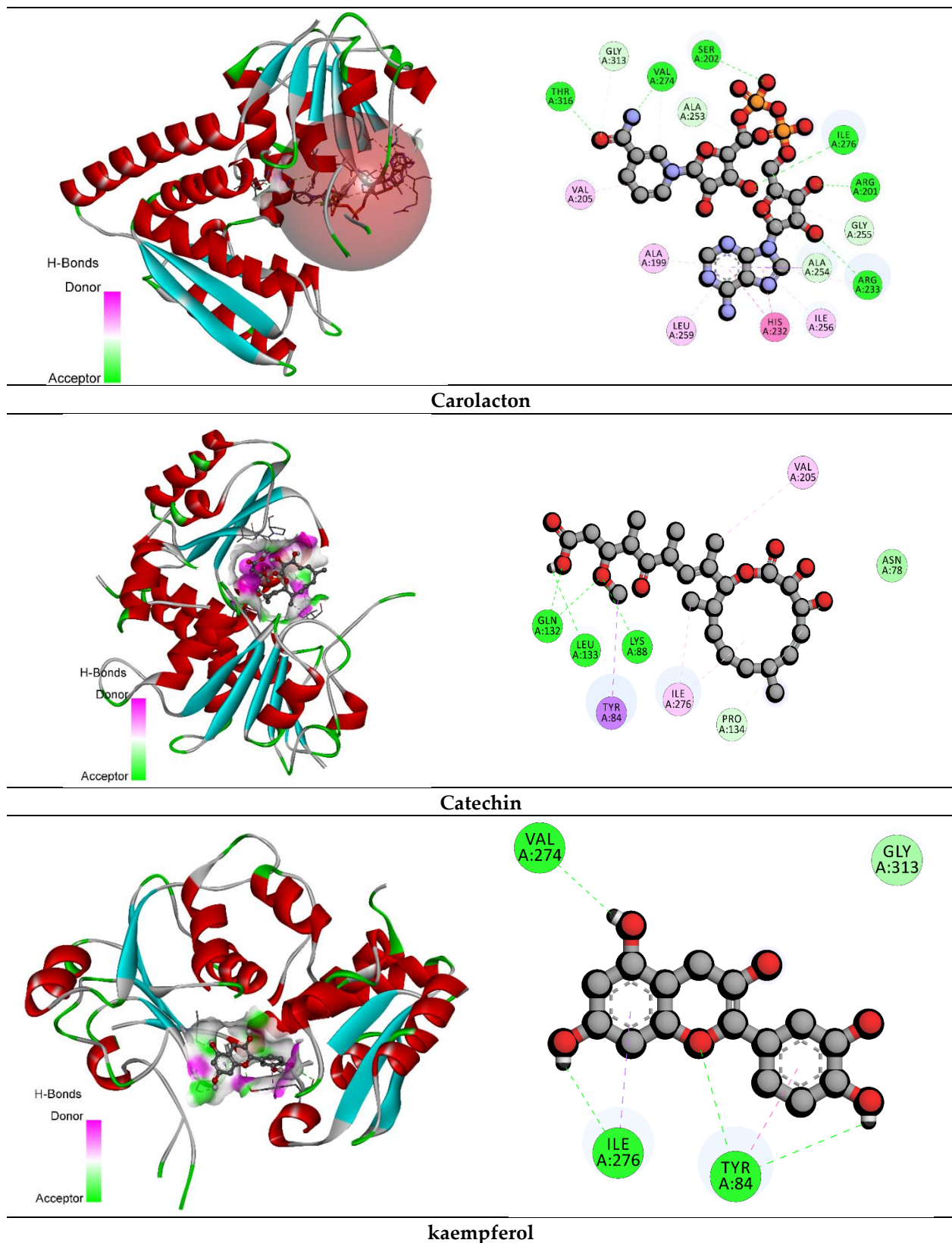
Table 1. Binding interaction of selected compounds With Methylenetetrahydrofolate dehydrogenase 2 (PDB ID 6KG2)

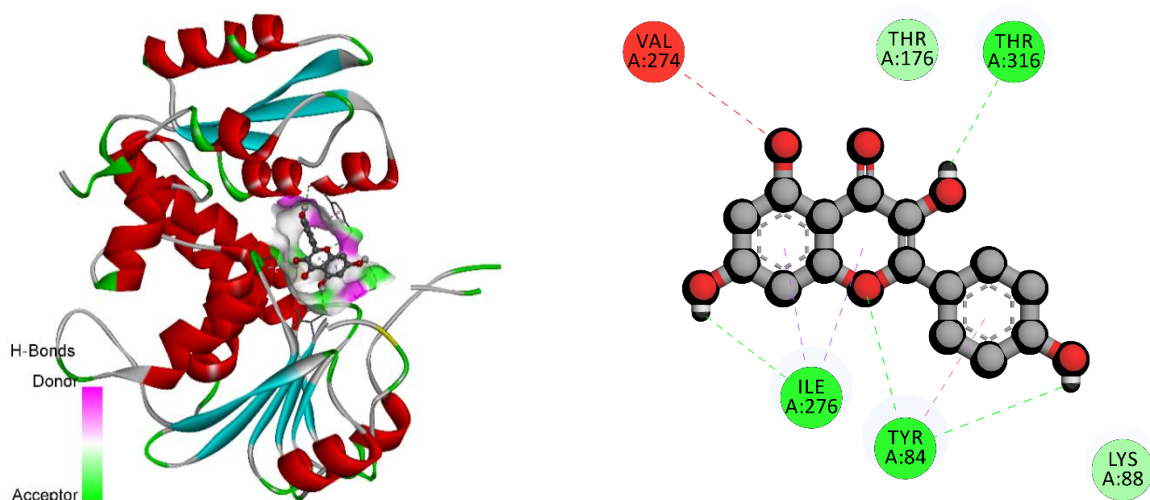
Amino Acid	Bond Length	Bond Type	Bond Category	Ligand Energy	Docking Score
				(Kcal/mol)	
Native Ligand					
ARG201	3.04907	Hydrogen Bond	Conventional Hydrogen Bond	1729.32	-5.9
SER202	2.48157				
ARG233	2.88061				
ILE276	2.88965				
THR316	2.64803				
NAD501	3.3197		Carbon Hydrogen Bond		
ALA254	3.31573				
GLY255	3.40896				
GLY313	3.69191				
ALA254	3.59952				
ALA253	3.17882	Hydrophobic	Pi-Sigma		
VAL274	2.95911				
NAD501	3.4395				
ALA254	3.70455				
ALA254	3.94172				
HIS232	3.68175		Amide-Pi Stacked		
ARG233	4.61768				
ILE256	5.31997				
ALA199	4.74451				
LEU259	5.26296				
Carolacton					

LEU133	2.35416	Hydrogen Bond	Conventional Hydrogen Bond	175.96	-6.7
GLN132	2.65358		Carbon Hydrogen Bond		
PRO134	3.47559		Pi-Sigma		
TYR84	3.7419	Hydrophobic	Alkyl	261.03	-7.1
ILE276	3.99393				
VAL205	4.53671				
ILE276	5.3558				
Catechin					
TYR84	2.64484	Hydrogen Bond	Conventional Hydrogen Bond	261.03	-7.1
VAL274	2.04089		Pi-Sigma		
ILE276	2.34838		Pi-Pi Stacked		
TYR84	2.51836	Hydrophobic		179.69	-7.1
ILE276	3.73962				
TYR84	3.96469				
kaempferol					
TYR84	2.79727	Hydrogen Bond	Conventional Hydrogen Bond	179.69	-7.1
THR316	2.45629		Pi-Sigma		
ILE276	2.62323		Pi-Pi Stacked		
TYR84	2.57006	Hydrophobic		282.09	-6.9
TYR84	4.00273				
Licochalcone_A					
ASN87	2.31382	Hydrogen Bond	Conventional Hydrogen Bond	282.09	-6.9
VAL274	3.36601		Carbon Hydrogen Bond		
TYR84	3.82547		Pi-Sigma		
THR316	3.94129	Hydrophobic	Pi-Alkyl	1639.66	-5.1
ILE276	5.09573				
TYR84	4.21587				
Rutin					
LEU133	3.07751	Hydrogen Bond	Conventional Hydrogen Bond	1639.66	-5.1
TYR84	3.00282		Pi-Pi Stacked		
LYS88	2.43838		Alkyl		
GLN132	2.17685	Hydrophobic		282.09	-6.9
TYR84	3.90356				
ILE276	3.53993				
LEU289	5.35278	Hydrophobic	Pi-Alkyl	1639.66	-5.1
ALA80	5.3782				
VAL205	5.45288				
ILE276	5.06566				

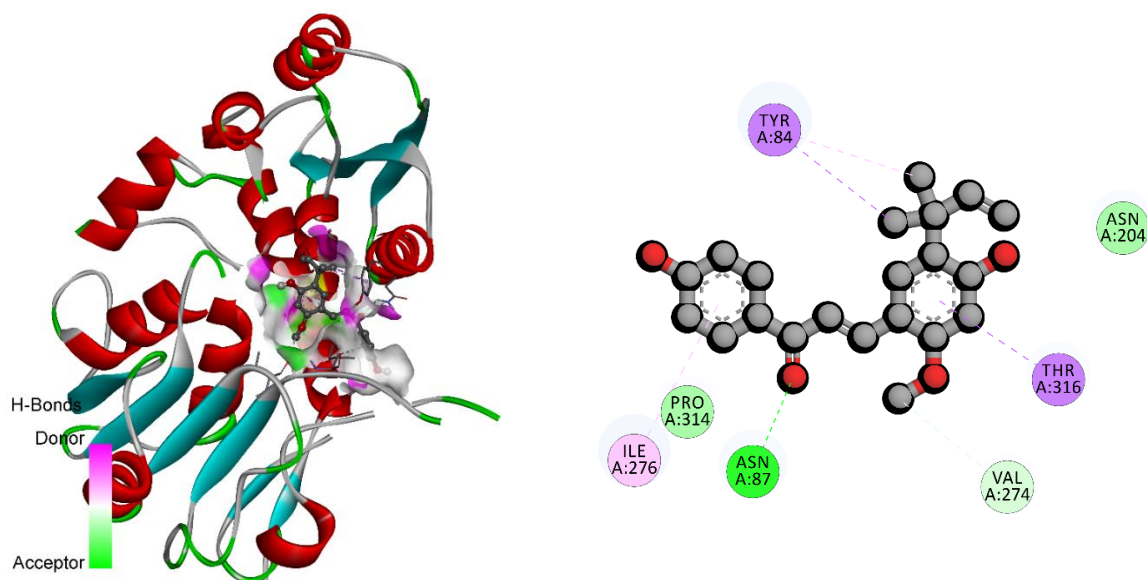
Table 2. 2D and 3D molecular interaction profiles of selected phytochemicals and native ligands within the active site of MTHFD2 (PDB ID: 6KG2) illustrate hydrogen bonding, hydrophobic interactions, π - π stacking, and key interacting amino acid residues contributing to binding stability.

3D Binding Interaction	2D Binding Interaction
NL_6KG2	

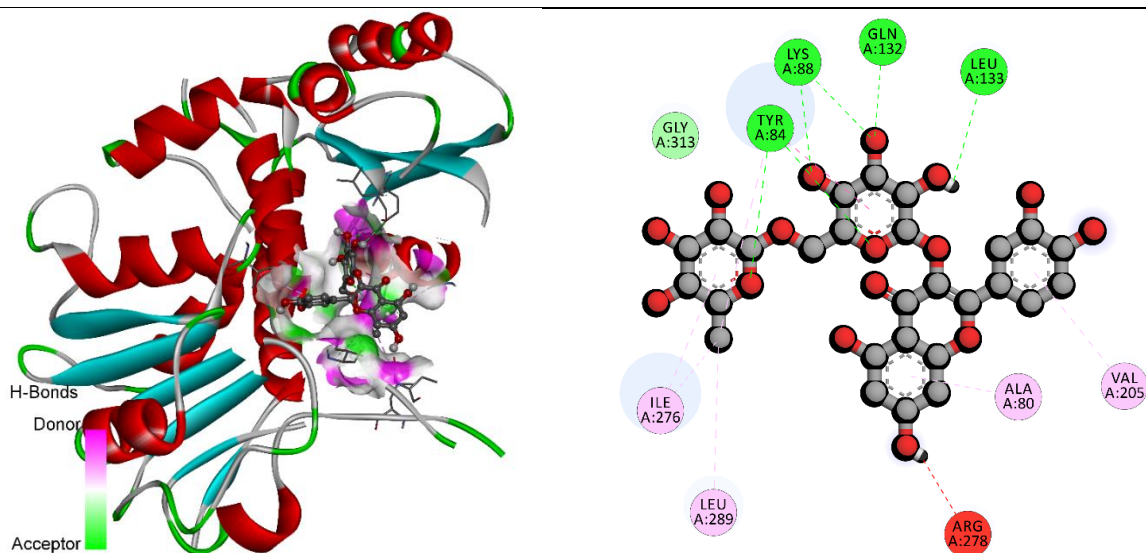




Licochalcone_A



Rutin



3.2 ADMET Analysis

ADMET analysis is a crucial early-stage evaluation that predicts the pharmacokinetic behavior, safety, and drug-likeness of bioactive compounds prior to experimental validation. It helps to identify potential limitations related to absorption, permeability, and bioavailability, thereby guiding the selection of promising lead molecules. The ADMET radar data for all selected compounds are shown in table 9.

The physicochemical characteristics of the selected compounds are summarized in Table 3. Molecular weight analysis revealed that kaempferol (286.05 Da) and catechin (290.08 Da) fell well within the optimal range for oral drugs, whereas NL-6KG2 (544.15 Da) and rutin (610.15 Da) exceeded the 500 Da threshold, which may negatively affect permeability and bioavailability. TPSA values varied widely, with licochalcone A exhibiting the lowest TPSA (66.76 Å²), indicative of favorable membrane permeability, while rutin showed an exceptionally high TPSA (269.43 Å²), suggesting poor intestinal absorption. Moderate TPSA values were observed for catechin (110.38 Å²) and kaempferol (111.13 Å²). LogP values ranged from 0.99 (rutin) to 3.82 (licoachalcone A), indicating acceptable lipophilicity for most compounds, although the higher logP of licoachalcone A may compromise solubility.

Table 3. Physicochemical properties of selected derivatives

Compounds	MW	Volume	Dense	nHA	nHD	nRot	nRing	TPSA	logS	logP
NL-6KG2	544.15	513.3999	1.059895	9	1	5	5	103.17	-3.15641	1.381934
Carolacton	468.27	489.5392	0.956553	8	3	8	1	130.36	-2.59848	1.62148
Catechin	290.08	279.2495	1.038784	6	5	1	3	110.38	-2.58137	1.172588
kaempferol	286.05	273.9766	1.044067	6	4	1	3	111.13	-3.64796	1.965317
Licochalcone A	338.15	366.092	0.923675	4	2	6	2	66.76	-4.81441	3.820301
Rutin	610.15	552.3177	1.104708	16	10	6	5	269.43	-2.39666	0.986122

Drug-like parameters are listed in Table 4. Quantitative estimation of drug-likeness (QED) scores ranged from 0.14 for rutin to 0.546 for kaempferol, with catechin (0.51) also demonstrating favorable drug-like characteristics. Lipinski rule analysis showed that rutin violated one rule, whereas all the other compounds complied fully. The natural product (NP) scores were positive for all phytochemicals, with catechin exhibiting the highest NP score (2.304), reflecting structural complexity. Licoachalcone A violated the Pfizer rule, whereas rutin triggered multiple alerts, including Lipinski, GSK, Golden Triangle, and Chelator rules, indicating reduced pharmaceutical suitability.

Table 4. Drug-likeness properties of designed derivatives

Compounds	QED	NP Score	Lipinski rule	Pfizer Rule	GSK Rule	GoldenTriangle	Chelator Rule
NL-6KG2	0.503	-1.445	0	0	1	1	0
Carolacton	0.366	1.862	0	0	1	0	0
Catechin	0.51	2.304	0	0	0	0	1
kaempferol	0.546	1.546	0	0	0	0	1
Licochalcone A	0.463	1.134	0	1	0	0	0
Rutin	0.14	2.015	1	0	1	1	1

The absorption-related properties are presented in Table 5. The predicted Caco-2 permeability values ranged from -6.55 (rutin) to -5.02 (licoachalcone A), indicating a generally low intestinal permeability across compounds (Table 5). The MDCK permeability followed a similar trend. However, the fraction absorbed values (F20–F50) were notably high for carolacton (F50 = 0.9996) and licoachalcone A (F50 = 0.9998), suggesting efficient absorption. In contrast, NL-6KG2 exhibited extremely poor absorption, with F20, F30, and F50 values of 0.0018, 0.0006, and 0.0056, respectively. P-glycoprotein analysis indicated that native ligands (Pgp substrate probability = 0.733) and rutin (0.699) were likely efflux substrates, potentially limiting oral bioavailability.

Table 5. Absorption parameter of selected compounds

Compounds	Caco-2 Permeability	MDCK Permeability	Pgp- inhibitor	Pgp- substrate	HIA	F20%	F30%	F50%
NL-6KG2	-5.72655	-4.97139	0.006986	0.73308	7.15E-07	0.00183	0.000638	0.005624
Carolacton	-5.45405	-5.10515	0.990079	3.51E-06	0.015499	0.974332	0.998901	0.999603
Catechin	-6.16859	-4.95279	0.00149	0.124462	0.016103	0.876646	0.994156	0.992989
kaempferol	-5.96934	-4.90902	0.14215	0.163041	0.015143	0.084482	0.715565	0.983332
Licochalcone A	-5.02306	-4.85602	0.388072	0.003546	0.00444	0.942095	0.986147	0.999828
Rutin	-6.54652	-5.02701	6.22E-08	0.699256	0.639735	0.772111	0.999751	0.999936

The distribution and metabolic characteristics of the selected compounds are summarized in Table 6, providing important insights into their systemic behavior and potential safety concerns. Plasma protein binding (PPB) values were consistently high across all compounds, ranging from 85.00% (rutin) to 97.88% (kaempferol and NL-6KG2). Such a high PPB suggests strong binding to circulating proteins, which may prolong systemic retention but simultaneously reduce the pharmacologically active free drug fraction. Consistent with this observation, the fraction unbound (Fu) values were relatively low for kaempferol (1.36) and NL-6KG2 (1.92), whereas catechin (17.10) and rutin (14.66) showed comparatively higher unbound fractions, potentially allowing better tissue availability. The volume of distribution (VD) values further distinguished these compounds. Kaempferol exhibited a negative VD (-0.81), suggesting limited tissue distribution, whereas licochalcone A (0.27) and catechin (0.06) showed moderate distribution. The blood-brain barrier (BBB) penetration probabilities were negligible for most compounds, particularly licochalcone A (0.00028) and carolactone (9.76E-09), indicating minimal central nervous system exposure and reduced risk of neurological side effects. CYP450 interaction profiling revealed marked differences in metabolic risk. Kaempferol showed strong inhibition of CYP1A2 (0.996) and CYP2C9 (0.799), and high interaction probabilities with CYP3A4, suggesting possible drug-drug interaction liability. Similarly, licochalcone A demonstrated a high inhibition probability for CYP1A2 (0.995), CYP2C19 (0.999), and CYP3A4 (0.999), raising metabolic concerns. In contrast, catechin and rutin displayed negligible inhibition and substrate probabilities across the major CYP isoforms, indicating a comparatively safer metabolic profile. Overall, catechins demonstrated the most balanced distribution and metabolic characteristics among the evaluated compounds.

Table 6. Distribution and metabolism parameter of selected molecules

Compo unds	Distribution				Metabolism									
	PPB %	VD	BBB	Fu	CYP1A2		CYP2C19		CYP2C9		CYP2D6		CYP3A4	
					Inhi bitor	Subs trate	Inhi bitor	Subs trate	Inhi bitor	Subs trate	Inhi bitor	Subs trate	Inhi bitor	Subs trate
NL- 6KG2	97.8 7039	0.03 5447	0.01 1336	1.92 4468	0.036 621	0.399 277	0.066 295	0.977 84	0.025 508	0.917 182	0.000 436	0.001 334	0.028 953	0.999 705
Carolact on	86.8 399	- 0.21 793	9.76 E-09	10.4 1131	3.13 E-08	1.12E -05	1.15 E-06	0.000 176	0.002 009	0.026 086	2.34 E-05	1.09E -06	3.49 E-05	0.219 104
Catechi n	89.2 8076	0.06 869	0.13 5322	17.1 0715	1.86 E-05	0.047 731	9.82 E-06	0.000 296	2.47 E-06	0.008 066	8.32 E-08	0.010 639	0.000 169	4.65E -05
kaempferol	97.8 8079	- 0.81 165	0.00 0951	1.36 0188	0.996 672	0.598 314	0.132 402	0.000 515	0.799 358	0.518 549	0.004 89	0.994 622	0.974 528	0.001 706
Licochalcone A	97.6 8781	0.27 0597	0.00 0281	2.43 2302	0.995 5	0.000 187	0.999 726	0.000 922	0.489 697	0.528 566	0.299 747	0.707 579	0.999 853	4.67E -07

Rutin	85.0 054	- 0.05 883	3.59 E-05	14.6 5911	0.000 1	0.003 64	1.44 E-07	1.07E -06	1.85 E-06	7.47E -05	2.26 E-06	1.48E -07	0.029 205	2.29E -09
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The excretion and toxicity profiles summarized in Table 7 further differentiate the therapeutic potential of the selected compounds. The plasma clearance (CL-plasma) values varied substantially, with catechin exhibiting the highest clearance (16.45), indicating rapid systemic elimination, whereas rutin showed the lowest clearance (1.61), suggesting prolonged systemic retention. This trend is supported by half-life (T_{1/2}) predictions, where rutin demonstrated the longest half-life (4.61), potentially increasing the accumulation risk, while NL-6KG2 showed the shortest half-life (0.56), indicating rapid elimination but possibly reduced therapeutic duration. The hepatotoxicity (H-HT) and drug-induced liver injury (DILI) probabilities revealed notable safety differences. NL-6KG2 (DILI: 0.998) and carolacton (0.993) showed very high predicted hepatotoxic risk, raising significant safety concerns. Rutin also exhibited a high probability of DILI (0.936), whereas catechin (0.222) and licochalcone A (0.399) demonstrated comparatively lower hepatotoxic potential. Kaempferol showed a moderate DILI risk (0.702), suggesting the need for cautious evaluation. Ames mutagenicity predictions were highest for rutin (0.756) and kaempferol (0.546), whereas licochalcone A displayed the lowest probability (0.205), indicating a relatively safer mutagenic profile. The carcinogenicity risk was most pronounced for NL-6KG2 (0.871) and kaempferol (0.716), whereas rutin (0.046) and catechin (0.226) showed lower probabilities. Importantly, the eye corrosion risks were negligible across all compounds, although moderate probabilities of eye irritation and respiratory toxicity were observed for several candidates. Overall, catechin demonstrates the most favorable balance among acceptable clearance, manageable half-life, and comparatively lower predicted toxicity, reinforcing its suitability for further development.

Table 7. Excretion and Toxicity parameters of selected compounds

Compounds	Excretion				Toxicity							
	CL-plasma	T1/2	H-HT	DILI	Ame s Toxi city	Rat Oral Acute Toxi city	FDA MD D	Skin Sensiti zation	Carcinog enicity	Eye Corro sion	Eye Irrita tion	Respir atory Toxicit y
NL-6KG2	3.476511	0.561104	0.976378	0.998622	0.450793	0.970186	0.951378	0.846844	0.871192	3.47E-12	2.44E-06	0.954736
Carolacton	5.175784	1.164534	0.931936	0.992568	0.449948	0.451093	0.281245	0.997736	0.363628	0.006827	0.547817	0.719376
Catechin	16.45525	2.44266	0.556749	0.222664	0.458893	0.271372	0.800951	0.969255	0.226083	0.061184	0.991463	0.646167
kaempferol	5.694431	1.38759	0.386179	0.702867	0.545969	0.487835	0.804589	0.621322	0.715984	0.575216	0.998204	0.712718
Licochalcone A	10.57622	1.08809	0.403453	0.399132	0.205781	0.358053	0.848935	0.703571	0.384914	0.15889	0.987892	0.864385
Rutin	1.610724	4.616005	0.406325	0.936922	0.756376	0.044139	0.137174	0.997444	0.046632	3.59E-05	0.904546	0.030272

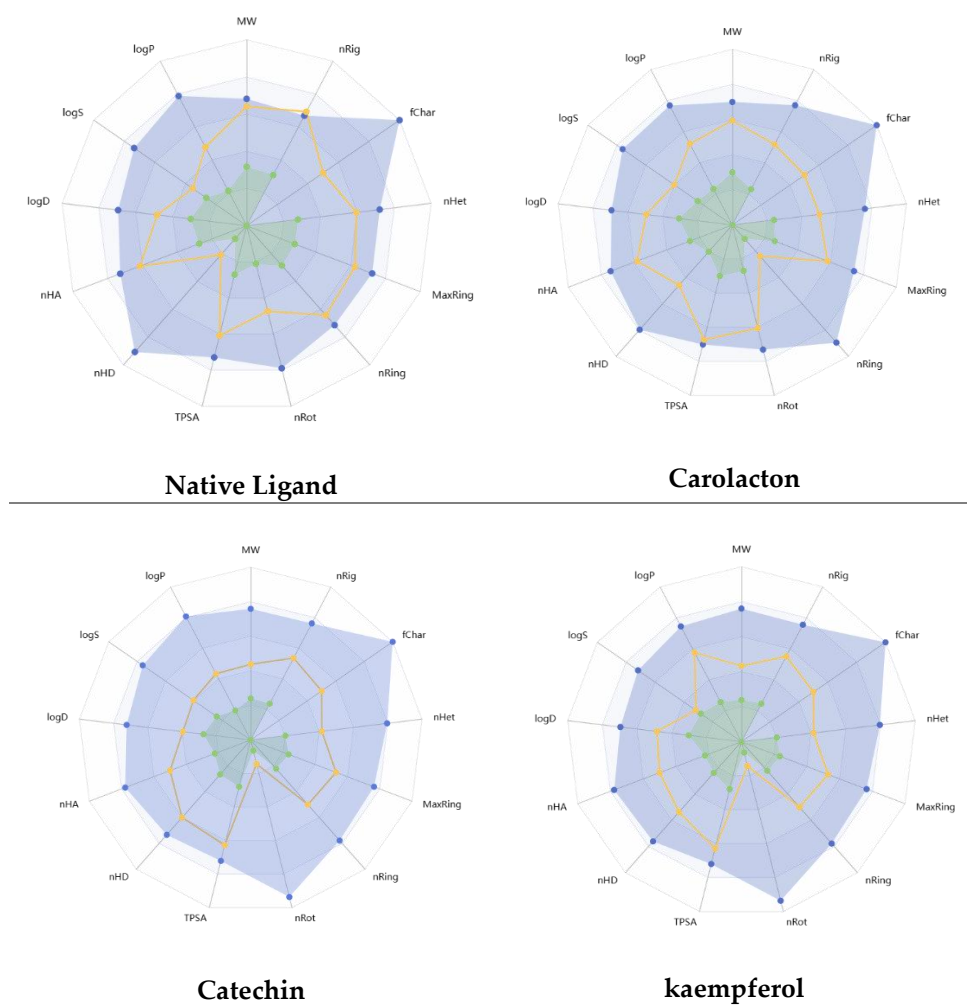
The environmental toxicity parameters listed in Table 8 indicate a generally low ecological risk for the designed molecules. Bioconcentration factor (BCF) values ranged from 0.44 (carolacton) to 1.48 (licochalcone A), suggesting minimal to moderate bioaccumulation. Compounds such as carolacton, rutin, and NL-6KG2 exhibited lower BCF values, indicating reduced environmental persistence, whereas licochalcone A and kaempferol exhibited comparatively higher but still acceptable values. Aquatic toxicity indicators (IGC50, LC50FM, and LC50DM) further supported moderate environmental safety. Licochalcone A displayed the highest IGC50 (4.34) and LC50 values (5.14–5.49), indicating lower aquatic toxicity. NL-6KG2 also demonstrated

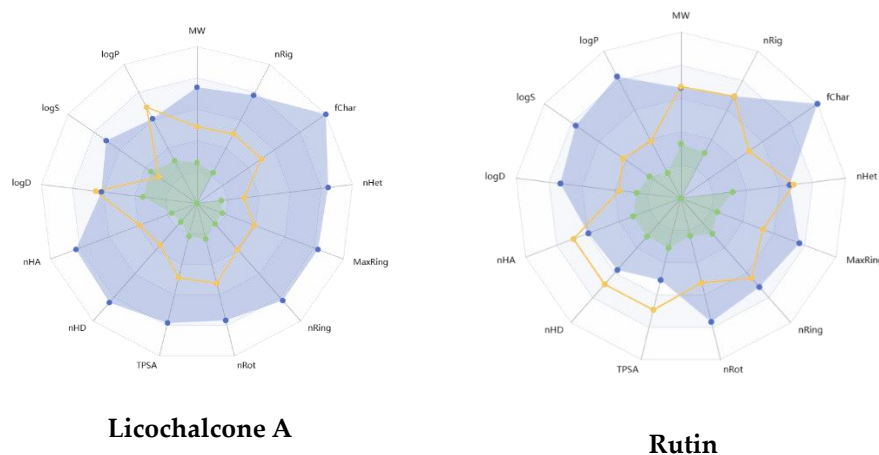
relatively favorable LC50 values (4.63–5.08). In contrast, catechins showed lower IGC50 (2.90) and LC50 values (3.77–4.13), suggesting comparatively higher aquatic sensitivity. Overall, the data in Table 6 suggest acceptable environmental profiles for all compounds, with licochalcone A demonstrating the most favorable ecological safety.

Table 8. Environmental toxicity profile of designed molecules

Compounds	BCF	IGC50	LC50FM	LC50DM
NL-6KG2	0.657131	3.526667	4.630615	5.076828
Carolacton	0.445624	3.065868	3.709297	4.428576
Catechin	0.750926	2.901756	3.768878	4.132019
kaempferol	1.241837	3.653071	4.00664	4.37644
Licochalcone A	1.480362	4.339439	5.14148	5.489758
Rutin	0.533413	3.180953	3.927314	4.680011

Table 9. ADMET radar of most potent compounds and Native ligand





4. Conclusion

This study systematically applied molecular docking and comprehensive in silico ADMET analyses to identify promising secondary metabolites as potential inhibitors of methylenetetrahydrofolate dehydrogenase 2 (MTHFD2). Among the evaluated compounds, catechin and kaempferol exhibited the strongest binding affinities, stable interactions with key catalytic residues (TYR84, ILE276, and THR316), and favorable binding orientations within the active site. Integrated pharmacokinetic profiling demonstrated acceptable physicochemical characteristics, compliance with drug-likeness criteria, and comparatively safer metabolic behavior, particularly for catechins. Toxicity and environmental assessments further indicated manageable safety profiles, thereby supporting their suitability for continued development. Collectively, these findings establish a robust computational foundation for the identification of natural MTHFD2 inhibitors. To translate these results into therapeutic applications, future investigations should include in vitro enzyme inhibition assays, cell-based antiproliferative studies in MTHFD2-overexpressing cancer models, and mechanistic evaluations of one-carbon metabolic disruption. Subsequent in vivo pharmacokinetic and toxicity studies are essential for confirming its efficacy and safety. Overall, catechin and kaempferol are promising natural scaffolds for the rational development of selective MTHFD2-targeted anticancer agents.

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None

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Authors contribution includes "Conceptualization, and methodology, software, validation, and formal analysis investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization, supervision, project administration, Siddiqui Fatima Sarah and Sunita Bhosole acquisition. All authors have read and agreed to the published version of the manuscript.

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