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DESIGN, MOLECULAR DOCKING, SYNTHESIS, AND *In-vitro* EVALUATION OF SEMICARBAZIDE THIAZOLIDINONE DERIVATIVES AS ACETYLCHOLINESTERASE AND TYROSINASE INHIBITORS

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ABSTRACT

In this study, we report the design, molecular docking, synthesis, and *in vitro* evaluation of a novel series of Schiff base-derived semicarbazide thiazolidinones as anti-cholinesterase and antityrosinase agents for the treatment of Alzheimer's disease. Schrödinger software was used in conjunction with *in silico* tools for molecular docking studies targeting acetylcholinesterase, tyrosinase, and peroxiredoxin, as well as for determining binding free energy. The acetylcholinesterase inhibitory activity of thiazolidinones was evaluated using Ellman's test, tyrosinase inhibition assays, and antioxidant activity assessments. All derivatives displayed good docking scores and higher acetylcholinesterase and tyrosinase inhibitory action. The designed and synthesised Schiff base-derived semicarbazide thiazolidinone derivatives are potent multitargeted anti-cholinesterase and anti-tyrosinase agents.

Keywords: Molecular Docking, Schiff Base, Thiazolidinones, Anti-Alzheimer's Agent, Acetylcholinesterase, Ellman's Test, Tyrosinase.

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative condition characterized by cognitive decline, including memory and reasoning impairments. Its pathology involves amyloid plaques, neurofibrillary tangles, and synaptic dysfunction.[1] A key feature is the reduction of acetylcholine (ACh), essential for cognitive function, alongside lower acetylcholinesterase (AChE) activity. Enzymes like tyrosinase also contribute to oxidative stress and neuroinflammation, accelerating disease progression.[2] Tyrosinase, known for producing melanin, also contributes to oxidative stress and neuro inflammation in Alzheimer's disease (AD). Increased tyrosinase activity generates free radicals, worsening brain cell damage. Oxidative stress plays a key role in AD pathology, and antioxidants may offer potential treatment by neutralizing harmful molecules, protecting brain cells, and reducing amyloid plaques and neuroinflammation. The combination of cholinergic dysfunction and oxidative stress accelerates AD progression, complicating treatment.[3] Schiff bases (C=N) and thiazolidinones, known for their biological activities such as enzyme inhibition and anticancer effects, show promise in targeting neurodegenerative diseases. Combining these compounds may lead to novel therapies addressing both cholinergic dysfunction and oxidative stress in Alzheimer's disease.[4,5] This study evaluates Schiff base-derived semicarbazide thiazolidinones as dual inhibitors of acetylcholinesterase (AChE) and tyrosinase. Through structure-based drug design, promising compounds were identified and tested, with the potential to develop multitarget therapies for Alzheimer's disease.

EXPERIMENTAL

In-silico Studies

Molecular docking with ligands and targeting proteins has proven to be an effective approach. In this study, all computational analyses were conducted using Schrodinger Maestro software, which included various measures like Glide XP docking, LigPrep, and MMGBSA (Schrodinger Release 2020-4).[6] Three proteins were chosen to target Alzheimer's disease: acetylcholinesterase (PDB ID: 6O4W), tyrosinase (PDB ID: 5I38), and peroxiredoxin (PDB ID: 1HD2).[7-11] Prime MMGBSA determined the total free energy in dGbind

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(kcal/mol) using the Prime module.[12] The Schrodinger software's QikProp function is useful for determining ligand molecules' physicochemical characteristics and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties.[13-14]

Synthesis of Compounds

All the reactions were undertaken per standard laboratory conditions (Fig.-1). The study was carried out by using available laboratory reagents from Sigma Aldrich and Loba Chemie and suitable types of equipment. Schiff bases were synthesized from equimolar quantities of aldehydes and semicarbazides.[15-16] Thiazolidinones were obtained from Schiff bases reacting with N, N-dimethyl formamide, and thioglycolic acid. Silica gel-G-coated TLC plates monitored the progress of the reactions. TLC solvent system- hexane: ethyl acetate 2:8.The melting point was determined using a digital melting point instrument using the capillary method and was uncorrected. The infrared spectra of synthesised compounds were recorded using a BRUKER II FT-IR, and frequencies are expressed in cm^{-1}. The NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer. All spectra were obtained in DMSO. Chemical shift values are reported as values in ppm relative to TMS (δ=0) as an internal standard. The mass spectra were recorded on a JEOL SX-102/DA-6000 Mass spectrometer using Argon/Xenon (6 kV, 10 mA) as the FAB gas.

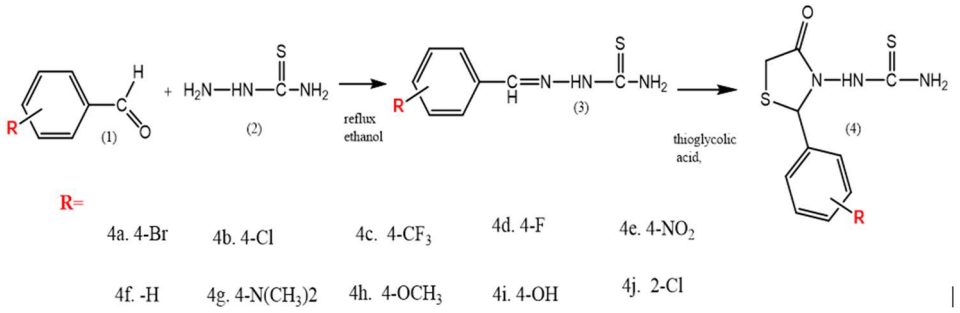

Fig.-1: Scheme for Synthesis of Thiazolidinones

1-(2-(4-bromophenyl)-4-oxothiazolidin-3-yl) urea (4a): Yield: 73%; Cream crystalline solid. Melting point 207°C - 208°C, Rf value 0.66. FT-IR (cm^{-1}): 1586.66 (C=O cyclic), 823.39 (C-S-C), 3104.66 (aromatic C-H stretching), 1633.15 aromatic C=C stretching, 3464.77 (NH stretching), 751.17 (C-Br). ^{1}H NMR (400MHz, DMSO, d/ppm): 7.84-7.89 (m, Ar-H), 6.52(s, NH_2), 3.77-3.39 (m, CH) ^{13}C NMR (100MHz, DMSO, d/ppm) 158.76-128.29 (Ar-CH), 38.33-36.94 (CH), Mass (m/z) (M+1) 316.

I-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl) urea (4b): Yield: 75%; Light yellow crystalline solid. Melting point 134°C -136°C, Rf value 0.73. FT-IR (cm^{-1}): 1589.60 (C=O cyclic), 821.49 (C-S-C), 3102.64 (aromatic C-H stretching), 1623.05 aromatic C=C stretching, 3462.75 (NH stretching), 651.07 (C-Cl). ^{1}H NMR (400MHz, DMSO, d/ppm): 7.81-7.88 (m, Ar-H), 6.51(s, NH_2), 3.78-3.38 (m, CH) ^{13}C NMR (100MHz, DMSO, d/ppm) 156.75-128.24(Ar-CH), 39.34-35.94 (CH), Mass (m/z) (M+1) 271.

1-(4-oxo-2-(4-(trifluoromethyl) phenyl) thiazolidin-3-yl) urea (4c): Yield: 86%; Orange crystalline solid. Melting point 144ºC-145ºC, Rf value 0.78. FT-IR (cm^{-1})1600.23(C=O cyclic), 755.75 (C-S-C), 3155.27 (aromatic C-H stretching), 1681.64 aromatic C=C stretching, 3457.24 (NH stretching), 1012.11(C-F). ^{1}H NMR (400MHz, DMSO, d/ppm): 8.11-7.70 (m, Ar-H), 6.59 (m, NH_2), 3.39-2.50 (m, CH) ^{13}C NMR (100MHz, DMSO, d/ppm) 156.65-122.89 (Ar-CH), 40.14-39.72 (CH) Mass (m/z) (M+1) 305.

1-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl) urea (4d): Yield: 78%; Yellow crystalline solid. Melting point 204°C -206°C, Rf value 0.68. FT-IR (cm^{-1}): 1588.60 (C=O cyclic), 825.79 (C-S-C), 3112.54 (aromatic C-H stretching), 1625.06 aromatic C=C stretching, 3463.78 (NH stretching), 851.07 (C-F). ^{1}H NMR (400MHz, DMSO, d/ppm): 6.81-7.68 (m, Ar-H), 6.65(s, NH_2), 3.75-2.38 (m, CH) ^{13}C NMR (100MHz, DMSO, d/ppm) 150.07-124.09(Ar-CH), 32.34-39.90 (CH), Mass (m/z) (M+1) 255.

1-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl) urea (4e): Yield: 70%; Light yellow crystalline solid. Melting point 203°C -204°C, Rf value 0.73. FT-IR (cm^{-1}): 1580.61 (C=O cyclic), 821.49 (C-S-C), 3102.64

(aromatic C-H stretching), 1623.05 aromatic C=C stretching, 3462.75 (NH stretching). ^{1}H NMR (400MHz, DMSO, d/ppm): 6.88-8.08 (m, Ar-H), 6.56(s, NH_2), 3.74-2.88 (m, CH) ^{13}C NMR (100MHz, DMSO, d/ppm) 151.71-121.11 (Ar-CH), 40.04-45.34 (CH), Mass (m/z) (M+1) 282.

1-(4-oxo-2-phenylthiazolidin-3-yl) urea (4f): Yield: 71%; Brown crystalline solid. Melting point 193°C - 195°C, Rf value 0.76. FT-IR (cm^{-1}): 1597.99 (C=O cyclic), 870.38 (C-S-C), 3106.50 (aromatic C-H stretching), 1646.25 aromatic C=C stretching, 3554.14 (NH stretching). ^{1}H NMR (400MHz, DMSO, d/ppm): 6.01-7.50 (m, Ar-H), 6.57(s, NH_2), 3.76-2.83 (m, CH) ^{13}C NMR (100MHz, DMSO, d/ppm) 151.35-123.22(Ar-CH), 32.48-45.14 (CH), Mass (m/z) (M+1) 237.

1-(2-(4-(dimethylamino) phenyl)-4-oxothiazolidin-3-yl) urea (4g): Yield: 80%; Light red crystalline solid, Melting point 150ºC-152ºC, Rf value 0.80. FT-IR (cm^{-1})1601.87 (C=O cyclic), 760.49 (C-S-C), 2012.73 (aromatic C-H stretching), 1363.13 (aromatic C=C stretching), 3458.67 (NH stretching).^{1}H NMR (400MHz, DMSO, d/ppm): 8.49-7.673 (m, Ar-H), 6.72-6.75 (m, NH_2), 3.64 (m, N-CH) 3.04-3.03 (m, CH). ^{13}C NMR (100MHz, DMSO, d/ppm) 129.53-111.72 (Ar-CH), 40.14-39.73 (CH). Mass (m/z) (M+1) 280.

1-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl) urea (4h): Yield: 67%; Light brown crystalline solid, Melting point 208ºC-210ºC, Rf value 0.84. FT-IR (cm^{-1}): 1590.59 (C=O cyclic), 823.48 (C-S-C), 3112.54 (aromatic C-H stretching), 1620.15 aromatic C=C stretching, 3464.74 (NH stretching). ^{1}H NMR (400MHz, DMSO, d/ppm): 7.41-7.90 (m, Ar-H), 6.54(m, NH_2), 3.78-2.33 (m, CH) ^{13}C NMR (100MHz, DMSO, d/ppm) 160.05-120.24(Ar-CH), 30.30-39.94 (CH), Mass (m/z) (M+1) 267.

1-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-yl) urea (4i): Yield: 80%; Yellow crystalline solid, Melting point 210ºC-212ºC, Rf value 0.73. FT-IR (cm−1): 1598.79 (C=O cyclic), 833.88 (C-S-C), 3013.54 (aromatic C-H stretching), 1590.19 aromatic C=C stretching, 3500.74 (NH stretching). 1H NMR (400MHz, DMSO, d/ppm): 6.72-8.48 (m, Ar-H), 6.51(s, NH_2), 3.78-2.34 (m, CH) ^{13}C NMR (100MHz, DMSO, d/ppm) 155.53-120.24 (Ar-CH), 32.50-45.04 (CH), Mass (m/z) (M+1) 253.

1-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl) urea (4j): Yield: 88%; Light cream crystalline solid, Melting point 219ºC-220ºC, Rf value 0.87. FT-IR (cm^{-1}): 1691.99 (C=O cyclic), 873.28 (C-S-C), 3002.50 (aromatic C-H stretching), 1622.11 aromatic C=C stretching, 3444.77 (NH stretching), 661.07 (C-Cl). ^{1}H NMR (400MHz, DMSO, d/ppm): 7.58-7.80 (m, Ar-H), 6.55(s, NH_2), 3.78-3.00 (m, CH) ^{13}C NMR (100MHz, DMSO, d/ppm) 154.65-133.44 (Ar-CH), 31.74-43.16 (CH), Mass (m/z) (M+1) 271.

In-vitro Studies

Acetylcholinesterase Inhibitory Test

AChE inhibitory activity of thiazolidinones was evaluated by Ellman's and its absorbance was measured at 412 nm using a microplate reader (LISA Plus Thermo Scientific). IC_{50} represented the inhibitory effects.[17]

Tyrosinase Inhibitory Test

Tyrosinase inhibition activity of thiazolidinones was assessed by measuring dopachrome production at 492 nm in the presence of a known tyrosinase inhibitor.[17]

Hydrogen Peroxide Free Radical Scavenging Effects

The antioxidant activity of thiazolidinones was assessed using a hydrogen peroxide scavenging assay. The absorbance of hydrogen peroxide at 230 nm was measured against a blank solution.[18]

DPPH Assay

The DPPH method was employed to evaluate the *in vitro* antioxidant activity of the synthesised compounds. The absorbance of the resulting solution was measured at 517 nm.[18]

RESULTS AND DISCUSSION

Molecular Docking

Schiff base-derived thiazolidinone inhibitors were chosen for docking investigations against acetylcholinesterase (6O4W), tyrosinase (5I38), and peroxiredoxin (1HD2) targets. The derivatives of thiazolidinones (4a to 4j) demonstrated notable binding affinity compared to their co-crystals (Table-1).

Compound 4c has shown excellent binding with 6O4W with a docking score of -8.984 kcal/mol. Compound 4c forms hydrophobic bond interactions with amino acids Trp286, Leu289, Phe297, Phe295, Val294, Phe338, Tyr341, Tyr124, and Tyr72; polar interactions with Ser293; and hydrogen bond interactions with Arg296 and Phe295 in the active site of peroxiredoxins (Table-1 and Fig.-2a).

Table-1: Docking Score, Molecular Interactions, and Binding Free Energy with 1HD2, 5I38, and 6O4W

Comp. code	PDB ID	Docking score (kcal/mol)	Hydrophobic interactions	Hydrogen bonds	MMGBSA dG Bind
4a	1HD2	-5.078	Ile119, Phe120, Pro40, Leu149, Pro45, Cys47	Arg127, Cys47	-42.81
	5I38	-5.383	Phe197, Pro201, Met61, Val218, Val217, Met215	Glu195, Asn205	-61.03
	6O4W	-7.705	Phe297, Trp286, Leu289, Phe295, Val294, Phe338, Tyr124, Tyr341, Tyr72, Leu76	Arg296, Phe295	-57.80
4b	1HD2	-5. 236	Ile119, Phe120, Pro40, Leu149, Pro45, Cys47	Arg127, Cys47	-43.94
	5I38	-5.245	Phe197, Pro201, Met61, Val218, Val217, Met215	Glu195, Asn205	-60.46
	6O4W	-6.743	Phe297, Phe295, Tyr124, Tyr72, Val73, Trp86, Tyr341, Phe338, Tyr337	Ser125, Phe295	-56.06
4c	1HD2	-5.530	Ile119, Phe120, Pro40, Pro45, Cys47, Leu149	Arg127, Cys47	-41.37
	5I38	-5.644	Phe197, Val217, Val218, Pro201, Met61	Glu195, Asn205	-59.65
	6O4W	-8.984	Trp286, Leu289, Phe297, Phe295, Val294, Phe338, Tyr341, Tyr124, Tyr72	Arg296, Phe295	-47.92
4d	1HD2	-4.976	Ile119, Phe120, Pro40, Pro45, Cys47, Leu149	Arg127, cys47	-39.10
	5I38	-5.483	Phe197, Met61, Met215, Val217, Val218, Ala221, Phe227	Asn205	-52.53
	6O4W	-8.668	Tyr124, Tyr341, Phe338, Val294, Phe295, Phe297, Leu289, Trp286, Tyr72, Leu76	Arg296, Phe295	-51.53
4e	1HD2	-4.986	Phe120, Ile119, Pro40, Pro45, Cys47, Leu149	Arg127, Cys47	-40.63
	5I38	-4.973	Phe197, Pro201, Met61, Met215, Val217, Val218	Glu195, Asn205	-56.38
	6O4W	-8.344	Leu289, Trp286, Tyr72, Leu76, Phe338, Tyr124, Val294, Tyr341, Phe295, Phe297	Arg296, Phe295, Tyr72	-54.41
4f	1HD2	-4.707	Leu116, Pro40, Ile119, Phe120, Pro45, Cys47, Leu149	Cys47, Arg127	-38.69
	5I38	-5.055	Phe197, Phe65, Met215, Val217, Val218, Ala221, Phe227, Met61	Glu195, Asn205	-48.27
	6O4W	-8.401	Trp286, Leu289, Phe297, Phe295, Val294, Phe338, Tyr341, Tyr124, Tyr72	Arg296, Phe295	-49.01
4g	1HD2	-5.111	Ile119, phe120, pro40, pro45, cys47, leu149	Arg127, cys47	-44.37
	5I38	-5.327	Phe197, Pro201, Met61, Met215, Val217, Val218	Asn205	-62.44
	6O4W	-8.765	Trp286, Leu289, Phe297, Phe295, Val294, Phe338, Tyr341, Tyr72, Tyr124, Leu76	Arg296, Phe295	-57.87
4h	1HD2	-5.050	Pro40, ile119, phe120, pro45, cys47, leu149	Arg127, Cys47	-41.65
	5I38	-5.083	Val218, Val217, Met215, Met61, Pro201, Phe197	Asn205, Glu195	-59.10

	6O4W	-7.455	Trp286, Leu289, Phe295, Val294, Phe297, Phe338, Tyr341, Tyr124, Tyr72	Arg296, Phe295, Tyr72	-52.66
4i	1HD2	-4.996	Pro40, Ile119, Phe120, Leu149, Pro45, Cys47	Arg127, cys47	-38.50
	5I38	-4.539	Phe197, Met61, Met215, Val217, Val218, Phe227, Ala221, Pro222, Phe227	Asn205	-50.12
	6O4W	-8.493	Tyr124, Tyr341, Phe338, Val294, Phe295, Phe297, Leu286, Trp286, Tyr72, Leu76	Arg296, Phe295, Tyr72	-51.18
4j	1HD2	-4.890	Ile119, Phe120, Pro40, Leu149, Pro45, Cys47	Arg127, Cys47	-43.94
	5I38	-4.846	Phe197, Val217, Val218, Pro201, Met61	Glu195, Asn205	-54.63
	6O4W	-7.393	Tyr124, Tyr341, Phe338, Tyr337, Trp86, Phe297, Phe295, Ala204	Ser203	-49.15
Donepezil	6O4W	-9.54	Phe338, Tyr337, Ile451, Tyr133, Trp86, Tyr 124, Tyr341, Phe297, Tyr72, Trp286, Leu289, Val294, Phe295,	Phe295	-25.44
Benzoic acid	1HD2	-5.506	Leu116, Ile119, Phe120, Leu112, Pro120, Cys47, Pro45	Arg127, Cys47, Thr44, Gly46	-40.17
Kojic acid	5I38	-5.508	Met215, Val217, Val218, Ala221, Phe65, Phe227	Gly216, His60	-27.37

Compound 4c has shown excellent binding with 5I38 with a docking score of -5.644 kcal/mol. Compound 4c forms hydrophobic bond interactions with amino acids Phe197, Val217, Val218, Pro201, and Met61; polar interactions with His208, Asn205, His204, and His60; hydrogen bond interactions with Glu195 and Asn205; pi-pi stacking with Phe197 in the active site of tyrosinase. All compounds have good binding capacity and docking score with target proteins. (Table-1 and Fig.-2b). Compound 4c has shown excellent binding with 1HD2 with a docking score of -5.530 kcal/mol, followed by 4b -5.236 kcal/mol. Co-crystal is benzoic acid; the docking score was -5.506 kcal/mol. Compound 4c forms hydrophobic bond interactions with amino acids Ile119, Phe120, Pro40, Leu149, Pro45, and Cys47; polar interactions with Thr147 and Thr44; hydrogen bond interactions with Arg127, and Cys47; pi-pi stacking with Phe120 in the active site of peroxiredoxins. (Table-1 and Fig.-2c). All compounds have good binding capacity and docking score with target proteins.

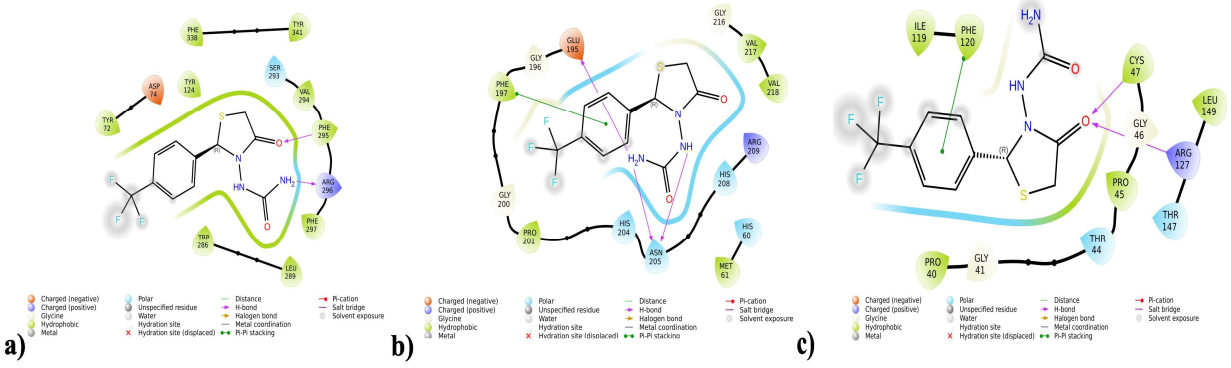

Fig.-2: 2D Interaction of Compound 4c with (a) 6O4W, (b) 5I38, (c) 1HD2

Binding Free Energy Calculation

The extra-precision docking results are validated by accurately ranking the ligands according to their binding free energies using the MMGBSA methodology to determine the binding free energy of the ligand-protein complexes. The Acetylcholinesterase (6O4W) enzyme has ΔG bind values between -47.92 and -

57.80 kcal/mol. The best-interacted chemical compound is 4c -47.92 kcal/mol, and the co-crystal donepezil -40.17 kcal/mol. Tyrosinase (5I38) enzyme has ΔG bind values between -48.27 to -62.44 kcal/mol. The best-interacted chemical compound, 4c -59.65 kcal/mol, and co-crystal, kojic acid -40.17 kcal/mol, were lower than all derivatives. Human peroxiredoxin (1HD2) enzymes have ΔG bind values between -38.50 to 44.37 kcal/mol. The best-interacted chemical compound 4c (-39.10 kcal/mol) and co-crystal benzoic acid -27.37 kcal/mol (Table-1).

Physicochemical and ADMET Properties

Using QikProp, we were able to ascertain the physicochemical parameters. It determines the compound's druglikeness property, and all compounds follow the Lipinski rule of five. The molecular weight was between the suggested range (237.276 – 316.172) given by Qikprop. The lipophilicity QPlogPo/w ranged from -0.035 to 1.589, falling within –2.0 to 6.5, the acceptable limit. The Van der Waals surface area of polar nitrogen and oxygen atoms was correlated with the polar surface area (PSA), which ranged from -69.24 Å to 122.01 Å. All derivatives fell inside the typical range. The predicted number of donors and acceptors for hydrogen bonds (0.0–6.0 donors and 2.0–20 acceptors) was within the allowed range. It has been discovered that all derivatives adhere to Lipinski's rule. Overall, it is implied that all compounds qualify as a drug-like molecule (Table-2). QikProp was utilised to ascertain the pharmacokinetic characteristics. QPlogBB assessed the accessibility of the central nervous system. Each derivative showed a CNS value ranging from -2 to +2, and all of them could pass through the blood-brain barrier. The highest docking score compounds 4b, 4c, and 4g have a CNS range of -1. Plasma protein binding to human serum albumin (log KHSA) is best measured between -1.5 and 1.5. All derivatives will be more easily accessible to the target site because they all fall within the recommended range and are likely to enter the bloodstream freely. All chemicals will undergo metabolism beyond eight, with metabolic rates falling between the recommended ranges of one to eight processes. *Potassium (hERG K+) channel blockage associated with the human ether-a-go-go gene*. The human ether-a-go-go-related gene (hERG) protein plays an important role in cardiac action potential. Every compound has an hERG K+ value that is less than the allowed range of -5 (Table-2).

Table-2: Physicochemical and ADMET Properties of Thiazolidinones

Comp. Code	Physicochemical properties					ADMET properties						
	Molecular weight	Log P	Donor HB	Acceptor HB	PSA	% Oral Absorption	# metab	QPlog hERG	QPP Caco	CNS	QP logB B	QPlog Khsa
4a	316.17	1.20	2.25	4.25	94.63	72.82	3	-2.74	147.97	-1	-0.60	-0.40
4b	271.72	1.12	2.25	4.25	94.59	72.41	3	-2.69	148.33	-1	-0.60	-0.42
4c	305.27	1.58	2.25	4.25	94.65	75.08	3	-2.78	147.79	-1	-0.53	-0.30
4d	255.26	0.89	2.25	4.25	94.51	71.12	3	-2.62	150.10	-1	-0.63	-0.48
4e	282.27	-0.03	2.25	5.25	139.67	48.90	4	-2.97	17.32	-2	-1.79	-0.54
4f	237.27	0.63	2.25	4.25	93.59	69.67	4	-2.93	150.82	-1	-0.77	-0.53
4g	280.34	1.05	2.25	5.25	98.20	71.63	4	-3.04	141.95	-1	-0.96	-0.37
4h	267.30	0.74	2.25	5.00	102.87	70.11	4	-2.87	147.61	-1	-0.89	-0.50
4i	253.27	-0.08	3.25	5.00	117.32	55.94	4	-2.82	44.33	-2	-1.34	-0.66
4j	271.72	1.03	2.25	4.25	94.83	80.64	4	-2.75	160.48	-1	-0.61	-0.45
Donepezil	379.49	4.38	0.00	5.50	50.55	100.00	6	-2.23	832.60	1	0.07	0.60
Kojic acid	142.11	-0.63	2.00	4.95	81.68	66.03	2	-2.12	245.99	-1	-0.93	-0.86
Benzoic acid	122.12	1.86	1.00	2.00	50.37	80.64	0	-1.70	244.92	-1	-0.30	-0.68

***In-vitro* Studies**

Acetylcholinesterase Inhibitory Test

The standard drug, donepezil, showed an IC_{50} value of 5.79 ± 1.08 µM. *In vitro,* results revealed that the thiazolidinone derivative 4c has shown an IC_{50} of 6.54 ± 1.09 µM acting as a potent AChE inhibitor (Table-3).

Tyrosinase Inhibitory Test

The compound 4c (IC_{50} 23.45 ± 1.06µM) recorded the inhibitory action against the tyrosinase enzyme. Kojic acid was used as the standard (22.73 ± 1.11). All synthesised compounds are suitable tyrosinase inhibitors (Table-3).

Hydrogen Peroxide Scavenging Assay

The synthesised compound 4c obtained an IC_{50} value of 20.83±1.01 µM and a standard ascorbic acid value IC_{50} value of 20.81±0.91µM, which predicts that they are potent peroxide scavengers, according to the results of *in vitro* tests (Table-3).

DPPH Assay

Ascorbic acid was used as the standard, with an IC_{50} value of 20.81±0.98 µM. 4c was the potent enzyme inhibitor, with an IC_{50} value of 21.17±0.92 µM, closer to the standard value (Table-3).

Table-3: Acetylcholinesterase and Tyrosinase Inhibitory Test and Antioxidant Activity Assay

Compound code	Acetylcholinesterase Inhibitory Test	Tyrosinase inhibitory Test	Hydrogen peroxide scavenging assay	DPPH assay
	IC_{50} (µM)			
4a	6.09 ± 5.72	27.52 ± 1.78	25.76 ± 1.55	24.45 ± 1.32
4b	8.59 ± 1.02	24.46 ± 1.02	21.63 ± 0.97	21.34 ± 0.93
4c	6.54 ± 1.09	23.45 ± 1.06	20.83 ± 1.01	21.17 ±0.92
4d	9.39 ± 2.52	29.32 ± 1.78	28.42 ± 0.54	28.87 ± 0.54
4e	9.91 ± 1.35	36.73 ± 2.87	31.44 ± 0.78	34.78 ± 1.37
4f	9.67 ± 2.10	33.65 ± 2.11	34.59 ± 1.83	45.78 ± 1.32
4g	7.45 ± 1.00	25.21 ± 1.00	21.69 ± 1.01	25.00 ± 1.07
4h	15.53 ± 1.78	41.44 ± 2.37	35.87 ± 1.56	42.78 ± 0.34
4i	14.21 ± 1.97	35.72 ± 1.47	39.69 ± 2.54	36.45 ± 1.45
4j	13.32 ± 1.54	32.48 ± 1.78	43.54 ± 0.88	51.78 ± 2.34
Donepezil	5.79 ± 1.08	-	-	-
Kojic acid	-	22.73 ± 1.11	-	-
Ascorbic acid	-	-	20.81 ± 0.91	20.81 ± 0.91

CONCLUSION

Semicarbazide-derived thiazolidinones were synthesised and carried out *in silico* and *in vitro* studies to screen for their enzyme inhibitory action against acetylcholinesterase (6O4W), tyrosinase (5I38), and peroxiredoxin (1HD2) enzymes. Based on their physicochemical and ADMET features, designed compounds can be categorised as druglike molecules. From *in silico* studies, the highest docking score compound 4c showed -8.984 kcal/mol for 6O4W, -5.644 kcal/mol for 5I38, and -5.530 kcal/mol for 1HD2. The compounds' inhibitory activity against the chosen target proteins indicates that they could be useful for treating Alzheimer's disease. The synthesised compound 4c showed IC_{50} values of 6.54 ± 1.09 µM for acetylcholinesterase inhibitory activity, 23.45 ± 1.06 µM for tyrosinase inhibitory activity, 20.83 ± 1.01 µM for hydrogen peroxide scavenging assay, and 21.17± 0.92 µM for DPPH assay. The structural analysis reports that the presence of a trifluoro substituent is the reason for its higher potency. The results point to a possible direction for further study and development, suggesting that these derivatives of thiazolidinone might act as multitargeted medications for Alzheimer's disease.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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