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Original Article

2,4-Substituted Oxazolones: Antioxidant Potential Exploration

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ABSTRACT

Background: This study focuses on the development of a novel and environmentally friendly synthetic methodology for the production of a series of six 4-benzylidene-2-phenyloxazol-5(4H)-one derivative (M1-M6). The approach involves a one-pot procedure utilizing hippuric acid, fused sodium acetate and various substituted aromatic aldehydes in the presence of acetic anhydride. **Purpose:** The primary objective is to explore the potential antioxidant properties of the synthesized compounds and contribute to the understanding of azlactones as promising antioxidants. The study integrates experimental synthesis with *in silico* methodologies to comprehensively characterize the chemical and biological properties of the derivatives. **Materials and Methods:** The synthesis process employed a combination of hippuric acid, fused sodium acetate and substituted aromatic aldehydes in a one-pot procedure. The chemical structures of the derivatives were characterized and validated through *in silico* techniques, including docking studies, drug-likeness assessments, bioactivity predictions, ADME profiling and toxicity evaluations. **Results:** The *in silico* analyses provided insights into the molecular interactions, pharmacokinetic properties and safety profiles of the synthesized compounds. *In vitro* antioxidant potential was systematically investigated using the DPPH method, with compounds M_3 and M_5 demonstrating significant antioxidant activity at a concentration 40 µg/mL showing (88% inhibition) and (85.7% inhibition) respectively, surpassing ascorbic acid as the reference standard. **Conclusion:** This study successfully explores azlactones as potential antioxidants, combining experimental synthesis and *in silico* methodologies to characterize the chemical and biological properties of the synthesized derivatives. The notable antioxidant activity of compounds M_3 and M_5 positions them as promising candidates for further investigation. The findings establish a foundation for future research and development of these compounds in potential antioxidant-related therapeutic interventions.

Keywords: Azlactones, DPPH, Hydroxy radical, Molecular Docking, Oxazolidinones.

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INTRODUCTION

Oxidative stress plays a role in the development of various chronic illnesses, such as cancer, atherosclerosis and neurodegenerative conditions. Lipid peroxidation that occurs during periods of oxidative stress results in the formation of lipid electrophiles and free radicals which can modify a multitude of proteins in the cell. The generation of free radicals is considered a primary factor contributing to various diseases such as heart disease and cancer. Reactive Oxygen Species (ROS), on the other hand, primarily oxidize the cell membrane due to their high concentration of unsaturated fatty acids in the lipid components. Membrane lipids cause lipid peroxidation by ROS leading to the formation of lipid hydroperoxide that can further decompose to aldehydes such as malondialdehyde, 4-hydroxynonenal, Isoprotans, cyclic endoperoxides, etc.[1-3] This lipid peroxidation results in the cross-linking of membrane proteins, altered membrane fluidity, forming lipid DNA and lipid-protein adduct, which may be disastrous to the cell function. Certain amino acid residues of protein side chains like tryptophan, cysteine and methionine are permitted for oxidation by ROS. Protein oxidation products are carbonyl compounds that have direct and indirect damage by alteration in the tertiary structure. Cross-linkages between proteins, proteolytic degradation and fragmentation. Even though DNA is a well-sheltered molecule, ROS can interact and cause various damages such as alteration of DNA base pairs, strand breaks, loss of Nitrogenous bases, damage to the sugar moiety, DNA protein cross-linkages and damage to DNA repair mechanisms. Sugar moiety is also susceptible to attack by free radicals leading to sugar peroxyl radicals and eventually resulting in strand breakage. The modification of genetic material as a cause of DNA damage results in cell death, mutagenesis and

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ageing.[4,5] Chronic and cumulative oxidation process results in drastic changes in DNA, proteins and lipids.

In this context, antioxidants play a crucial role in counteracting the harmful effects of free radicals by donating electrons, thereby neutralizing them and preventing further electron donation. This mechanism effectively safeguards tissues and cells from reactive oxygen species-mediated damage. Antioxidants are often described as substances that "swab off" free radicals by neutralizing them and inhibiting the pickup of electrons by nucleophiles.

Mechanochemical synthesis is one of the green chemistry techniques that involves grinding the crystals together, using different reagents in a mortar and pestle with the formation of local heat that induces a reaction between two materials in the presence of a small amount of liquid phase.[6-9] Heterocyclic Chemistry has attracted chemists to achieve the synthesis of several heterocyclic systems such as pyrazolines, pyrimidines, etc., via greener technique. This method possesses short reaction time, higher yields and products formed at normal room temperature. Moreover, it was successful and selective only for solid-state reactions rather than the liquid phase. Because the molecules are more firmly held and arranged regularly in a crystal lattice than liquid components. Based on the advantages and highlights of the MHC approach, it is envisaged that it can provide superficial access to azlactones in a more efficient, economical and greener way than conventional methods.

Azlactones are a class of small five-membered heterocycles recognized as essential pharmacophores with excellent pharmaceutical properties. They are regarded as keto derivatives of oxazolines and serve as a useful intermediate in the synthesis of alpha-amino acids, alpha-keto acids, amides, peptides, amino alcohols and polyfunctional compounds. It can also be readily prepared from N-protected amino acids by dehydration. It also possesses several biological activities like immune modulatory,[10] pesticidal,[11] antidiabetic activity,[12] antitubercular[13] anti-cancer,[14] anti-HIV,[15] anticonvulsant,[16] anti-angiogenic,[17] analgesic and anti-inflammatory activity.[18] Recently research has explored all these biological activities and believed that most of the reported diseases would be occurring only because of a demand for antioxidants in the human system. Therefore, antioxidants have attracted considerable attention to oxidative stress, reducing free radicals and the prophylaxis of cancer and longevity. Therefore, this research paper aims to investigate the role of azlactones in antioxidant activity as well as to assess the antioxidant potential of azlactones and determine their effectiveness in scavenging free radicals and preventing oxidative damage.

MATERIALS AND METHODS

Experimental Section

Materials

4-Benzylidene-2-phenyl-substituted-4(5)-oxazolone derivatives were prepared using various aromatic aldehydes and Hippuric acid using acetic anhydride and sodium acetate as catalyst. The reactant hippuric acid, acetic anhydride and sodium acetate was available at college; various aromatic aldehydes like benzaldehyde, 3,5-dimethoxy-4-hydroxy benzaldehyde, were purchased from Southern India Scientific Corporation Limited Chennai.

Methods

By using a different technique like Grind stone method, 4-Benzylidene-2-phenyl substituted-4(5)-oxazolone derivatives were synthesized. The melting points were determined using Open capillary tube method. The chemicals purity was justified using thin layer chromatographic Technique. Docking was performed for the designed compounds using Autodock version 4.2. The compounds were sketched using ChemDraw version 8.0 and geometry optimization was done by using AvogadroV1.2.0. The protein was preprocessed by UCSF Chimera, Kollmann charges were assigned, grid points were generated and it was subjected to molecular docking to analyze the active site binding affinity and its interactions which resulted in rank by energy. Visualization of the docked complexes was done by using the Biovia molecular discovery studio visualizer.[19] Based on the experimental evidence and the validation report like resolution, Ramachandran outliers and side chain outliers, the pdb 2X08 was found to be selective. Moreover, the co-crystallized ligand present in the protein was found to be ascorbate which is an excellent standard that can be comparatively studied against the synthesized molecules. The compounds that ranked excellent were subjected to various *in silico* analysis viz., drug-likeness by Molsoft, bioactivity by Molinspiration,[20,21] ADME parameters by SWISS ADME[22] and toxicity analysis by VNN-ADMET software.[23,24]

Experimental work

Scheme: Synthesis of 2, 4- substituted oxazolone derivatives

Synthetic procedure: A mixture of hippuric acid (1.2 mmol; 0.210 g), fused sodium acetate (1.5 mmol; 0.123 g) and appropriate aromatic aldehyde (1.0 mmol) was mixed in a porcelain mortar and pestle in the presence of a few drops of acetic anhydride (Figure 1). Grinding was carried out for different time intervals at room temperature. The completion of the reaction was determined by TLC and the reaction mixture turned to a yellow solid. To this, 15 mL of ethanol was added and the mixture was kept in the refrigerator overnight.[25-30] The resulting solid product was collected by filtration, washed with cold water and recrystallized from ethanol to give the desired azlactones. All the synthesized compounds were subjected to *in*

vitro antioxidant activity by DPPH method and Hydroxy radical scavenging method using ascorbic acid as standard.

RESULTS

Docking and *in silico* analysis

The docking results of title compounds were presented in Table 1 with their binding energies expressed in kcal/mol. All the compounds showed excellent binding affinity towards the receptor 2X08. The physicochemical properties of the synthesized compounds were tabulated in Table 2 Drug likeness is an important criterion to justify the compound's potential to be a drug candidate by correlating the molecular properties and structural features and its results were tabulated in Table 3. The designed derivatives were subjected to bioactivity against regular human receptors which is necessary for the development of drugs. The bioactivity scores of the compounds were calculated and reported in Table 4 for their GPCR ligand, kinase inhibitor, protease inhibitor, ion channel inhibitor and nuclear receptor ligand and enzyme inhibitor activities. The toxicity profile of the docked compounds was predicted using VNN-ADMET software and reveals results in terms of membrane transporters along with other properties like Herg blockers, MMP, AMES and MRTD values shown in Table 5.

Characterization

Each compound is assigned a unique identifier for reference throughout the study (M_1-M_6), with corresponding information about its molecular weight (in grams per mole), the time taken for synthesis (in min), the melting point (in degrees Celsius) and the percentage yield of the reaction of various 4-Benzylidene-2-phenyl substituted-4(5)-oxazolone derivatives were given in Table 5.

Spectral Data

Synthesis of(Z)-4-([1,1'-biphenyl]-4-ylmethylene)-2-phenyloxazol-5(4H)-one (M_1)

M.P.165°C, yield 88%; FT-IR (KBr,cm^{-1}): 1506 (C=C), 1202 (C-C),1220 (C=O) (lactone), 1613 (C-N); 3014 (C-H);^{1}H NMR (400 MHz, DMSO): δ 7.33 and δ 7.60 (m, 5H,Ar-H), δ7.91(s, 1H,CH) δ 7.52 and δ 7.95 (m, 5H,Ar-H); MS (ESI) m/z:250 [M]+1; Anal. found C,76.8%; H, 4.0%,N, 5.61%, O, 12.82%;Calculated values: C, 77.02%, H, 4.41%, N, 5.62%, O, 12.84%.M.F:$C_{16}H_{11}NO_2$.

Synthesis of(Z)-4-((4'-hydroxy-3',5'-dimethoxy-[1,1'-biphenyl]-4-yl) methylene)-2-phenyloxazol-5(4H)-one (M_2)

M.P.150°C, yield 90%; IR (KBr,cm^{-1}): 1512 (C=C), 1214 (C-C), 1750 (C=O) (lactone), 1610 (C-N), 3612 (phenolic OH), 1350 (C-O-C); 3016 (C-H); ^{1}H NMR (400 MHz, DMSO): δ7.33 and δ7.60(m, 5H,Ar-H),δ7.91(s, 1H,CH) δ 7.52 and δ7.95 (m,

Table 1: Docking results and Amino acid interactions.

Sl. No.	Compound Code	Binding energy (K/Cal.)	Name of the amino acids and Type of interaction
1	M_1	-7.54	ARG A: 48, MET A:172 (Pi- Sulfur), PHE A: 191, TRY A: 51, LEU A: 171 (Pi-Pi Stacked), ALA A:174 (Pi-Sigma), HIS A: 175(Conventional Hydrogen bond).
2	M_2	-8.72	TRP A: 51, LEU A: 171 (Pi-Pi Stacked), ALA A: 174, ARG A: 48 (Pi- Sigma), LYS A: 179 Conventional Hydrogen Bond, MET A: 172 Vander Waals.
3	M_3	-8.9	PRO A: 145, ALA A: 174, (Pi-Alkyl), HIS A: 52, ARG A: 48 (Conventional Hydrogen Bond), HIS A: 175 (Pi-Cation).
4	M_4	-8.87	PHE A: 191, LEU A: 171, TRP A:51(Pi-Pi Stacked), ARG A: 48 (Pi- Cation), MET A: 172 (Pi- Sulfur), ALA A: 174 (Pi-Sigma).
5	M_5	-10.04	ALA A:174, VAL A:47, PRO A: 145 (Pi- Alkyl), ARG A:48, TRP A:51 (Conventional Hydrogen Bond)
6	M_6	-8.5	TRP A: 51, LEU A: 171, PHE A: 191 (Pi-Pi Stacked), ARG A: 48 (Pi- Alkyl), META: 172 (Pi- Sulfur), ALA A:174 (Pi-Sigma), LYS A: 179 Carbon Hydrogen Bond.
7	Ascorbic acid	-7.36	ILE A:274, GLY A: 273, ASN A: 272 (Conventional Hydrogen Bond), LYS A: 268 (Carbon Hydrogen Bond).

Table 2: **Physiochemical properties of the synthesized compounds.**

Sl. No.	Compound code	Molecular weight (g/mol)	Time Taken (min)	Melting point (°C)	% Yield
1	M_1	249.26	25	165	88
2	M_2	325.32	45	150	90
3	M_3	265.26	35	135	96
4	M_4	283.71	40	189	89
5	M_5	294.26	40	238	93
6	M_6	279.29	45	166	86

Table 3: **Drug likeness of synthesized compounds.**

Compound code	LogP	TPSA (Å²)	MW (g/mol)	HBA	HBD	N Viol	ROTB	Drug likeness score
M_1	2.75	38.66	249.26	3	0	0	2	0.55
M_2	2.85	77.35	325.32	6	1	0	4	0.55
M_3	2.85	58.89	265.26	4	1	0	2	0.55
M_4	3.81	38.66	283.71	3	0	0	2	0.55
M_5	2.51	84.48	294.26	5	0	0	3	0.55
M_6	3.26	47.89	279.29	4	0	0	3	0.55

Table 4: **Bioactivity scores of Designed title derivatives.**

Sl. No.	Compound code	GPCR Ligand	Ion channel inhibitor	Kinase Inhibitor	Nuclear Receptor Ligand	Protease inhibitor	Enzyme inhibitor
1	M_1	-0.93	-1.28	-0.37	-0.86	-1.09	-0.34
2	M_2	-0.68	-1.07	-0.18	-0.56	-0.79	-0.24
3	M_3	-0.80	-1.18	-0.28	-0.59	-0.99	-0.26
4	M_4	-0.85	-1.23	-0.34	-0.80	-1.05	-0.36
5	M_5	-0.90	-1.19	-0.39	-0.77	-1.01	-0.44
6	M_6	-0.82	-1.25	-0.31	-0.72	-0.97	-0.36

Table 5: **Toxicity studies of Designed title derivatives by using VNN-ADMET.**

Compound Code	Liver Toxicity		Membrane Transporters			Others			
	DILI	Cytotoxicity	BBB	P-gp inhibitor	P-gp substrate	Herg blocker	MMP	AMES	MRTD (mg/day)
M_1	Yes	No	Yes	Yes	No	No	No	No	135
M_2	Yes	No	Yes	Yes	Yes	No	No	No	166
M_3	Yes	No	Yes	Yes	Yes	No	No	No	133
M_4	Yes	No	Yes	No	No	No	No	No	133
M_5	Yes	Yes	No	No	No	No	No	Yes	99
M_6	No	No	Yes	Yes	Yes	No	No	No	142

5H,Ar-H); δ 6.72(s, 2H,Ar-H), δ 3.83(s, 6H,O-CH_3), δ 7.91(s, 1H,CH),δ 7.52 and 7.95 (m, 5H,Ar-H),δ 5.35(s, 1H, OH); MS (ESI) m/z:326 [M]+1; Anal. found C,66.39%; H, 4.62%, N, 4.30%, O, 24.56%; Calculated values: C, 66.46%, H, 4.65V N, 4.31%, O, 24.59%. M.F: $C_{18}H_{15}NO_5$.

Synthesis of(Z)-4-((3'-hydroxy-[1,1'-biphenyl]-4-yl) methylene)-2-phenyloxazol-5(4H)-one (M_3)

M.P.135°C,yield 96%; IR (KBr,cm^{-1}): 1508 (C=C),1220 (C-C),1602 (C=O) (lactone), 1400 (C-N), 3010 (C-H); 3600 (OH); ^{1}H NMR(400 MHz, DMSO): δ 7.16(d1H,Ar-H), δ 7.53(t,1H,Ar-H) δ 6.83(s, 1H,Ar-H), δ 5.35(s, 1H,Ar-H), δ 6.70(s, 1H,Ar-H), δ 7.91(s, 1H,CH), δ 7.52 and 7.95 (m, 5H,Ar-H); MS (ESI) m/z:266 [M]+1; Anal. found C, 72.28%; H, 4.14%; N, 5.27%, O, 18.06%; Calculated values: C, 72.45%, H, 4.18%, N, 5.28%, O, 18.09%, M.F: $C_{16}H_{11}NO_3$.

Synthesis of (Z)-4-((3'-hydroxy-[1,1'-biphenyl]-4-yl) methylene)-2-phenyloxazol-5(4H)-one(M_4)

M.P.189°C, yield 96%; IR (KBr,cm^{-1}) 1512(C=C),1214(C-C),1285 (C=O) (lactone),1606 (C-N), 787 (C-Cl), 3011 (C-H);^{1}H NMR (400 MHz, DMSO): δ 7.68 and 7.44(d,4H,Ar-H), δ7.91(s, 1H,CH),δ 7.95(d, 2H,Ar-H), δ 7.52(m, 3H,Ar-H);MS (ESI) m/z: 284 [M]+1; Anal. found C, 67.67%, H, 3.52%, N, 4.93%, O, 11.26%, Cl 12.33%; Calculated values: C, 67.74%, H, 3.55%, N, 4.94%, O, 11.28%, Cl, 12.52%. M.F $C_{16}H_{10}ClNO_2$.

Synthesis of (Z)-4-((3'-nitro-[1,1'-biphenyl]-4-yl) methylene)-2-phenyloxazol-5(4H)-one (M_5)

M.P.238°C, yield 93%; IR (KBr,cm^{-1}):1514 (C=C), 1210(C-C),1274 (C=O) (lactone),1610 (C-N), 1562 (NO_2); 3015 (C-H);^{1}H NMR (400 MHz, DMSO): δ 7.99(d, 1H,Ar-H), δ 7.66(t, 1H,Ar-H), δ 8.14(d,1H,Ar-H), δ 8.31(s, 1H,Ar-H), δ 8.02(s, 1H,OH), δ 7.95(d, 2H,Ar-H) and δ 7.52(m, 3H,Ar-H);MS (ESI) m/z:295 [M]+1; Anal. found C, 65.24%, H, 3.39%, N, 9.51%, O, 21.72%; Calculated values: C, 65.31%, H, 3.43%, N,9.52%, O, 21.75%. M.F: $C_{16}H_{10}N_2O_4$.

Synthesis of (Z)-4-((4'-methoxy-[1,1'-biphenyl]-4-yl) methylene)-2-phenyloxazol-5(4H)-one (M_6)

M.P.166°C, yield 86%; IR (KBr,cm^{-1}): 1519 (C=C), 3014 (C-H),1204 (C-C), 1274 (C=O)(lactone), 1612 (C-N), 1242 (C-O-C); ^{1}H NMR (400 MHz, DMSO):δ 7.62(d, 2H,Ar-H) δ 6.94(d, 2H,Ar-H), δ 3.83(s, 3H,O-CH3),δ 7.91(s, 1H,CH),δ 7.95(d, 2H,Ar-H) and δ 7.52(m, 3H,Ar-H);MS (ESI) m/z:280 [M]+1; Anal. found C, 73.04%, H, 4.65%, N, 5.01%, O, 17.18%; Calculated values: C, 73.11%, H, 4.69%, N, 5.02%, O, 17.19%. M.F: $C_{17}H_{13}NO_3$.

Biological Evaluation

Antioxidant activity

Antioxidants plays a pivotal role in the neutralization of all free radicals generated out of different metabolic reactions. Every organism constantly produces nitrogen species and reactive oxygen species during their physiological activities. However,

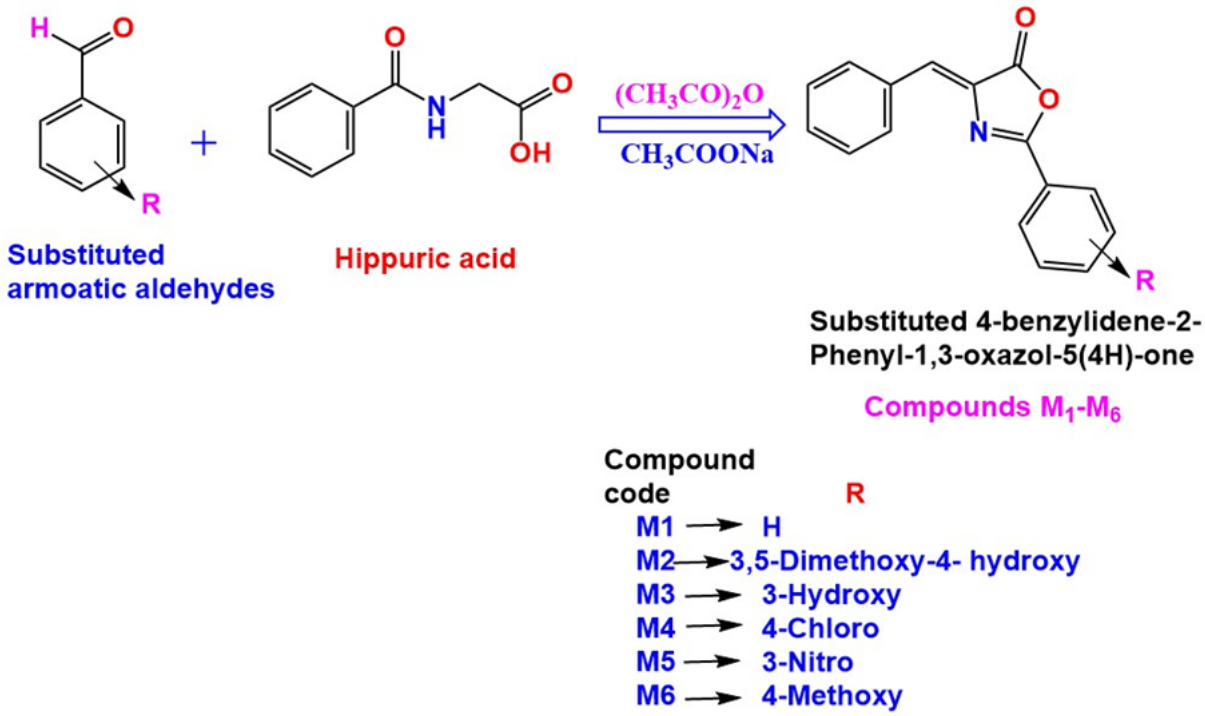

Figure 1: Synthetic route for 4-benzylidene-2-phenyloxazol-5(4*H*)-ones (M_1-M_6).

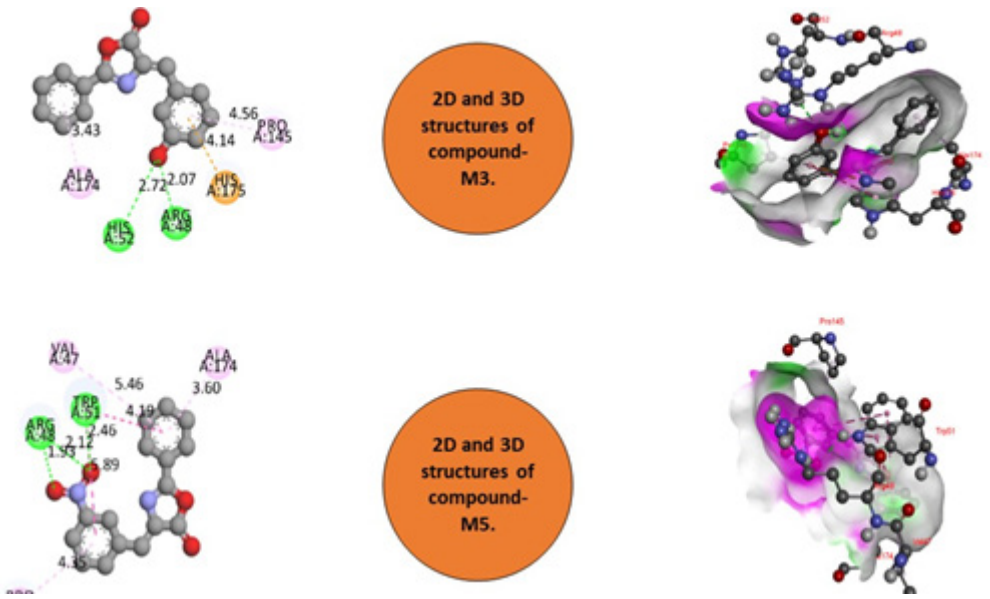

Figure 2: Docking interactions of the potent compounds.

Table 6: ***In vitro*** **Antioxidant activity of synthesized compounds.**

Sl. No.	Compound code	Concentration / % inhibition			
		10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL
1	M_1	12.9%	42%	56%	74%
2	M_2	53%	67.2%	75.2%	83%
3	M_3	36%	51.2%	65%	88%
4	M_4	34.5%	24%	63.5%	86%
5	M_5	33%	35%	44%	87%
6	M_6	58%	79%	85%	85.7%
7	Ascorbic acid	46%	61.3%	77.5%	90%

their level drastically increases in different pathological conditions as a result of dysfunction of pro-oxidative systems. In such cases their production and trapping should be counterbalanced only by natural anti-oxidant system. A disruption in this extends to oxidative stress causing irreversible damage to cell and their components leading to different diseased conditions. Inspite of ascorbic acid as standard, synthetic compounds are also being tested to identify the potential antioxidant capacity. Therefore, all the synthesized compounds were subjected to *in vitro* antioxidant activity by DPPH method and hydroxy radical scavenging method using ascorbic acid as standard.

DISCUSSION

All the six compounds were subjected to molecular docking to identify the ligand's affinity on the receptor through different amino acid interactions. The compounds were docked through the validated target 2X08 cytochrome C oxidase and it is exhibiting different amino acid interactions proving the ligand efficiency. Compounds M_3 and M_5 showed good interaction with the amino acids by illustrating less binding energy. The 2-dimensional and 3-dimensional images with interactions of compounds M3 and M5 are shown in Figure 2. Molecular Weight (g/mol) indicates the molecular weight of each compound, providing insight into its mass and chemical composition. All compound's molecular weights are in between the range of 249.26 to 325.32. The time required for the synthesis of each compound is documented in Time Taken (min), reflecting the duration of the chemical reactions involved in the process in between 25 to 45 min. The Melting Point is crucial for understanding the compound's physical properties. All compound's percentage yields are obtained in between the range of 86 to 96 which represents the efficiency of the synthesis process and higher percentage yield suggests a more efficient and successful synthesis process. All the compounds fall within the range of 2.5 to 4Å for their log P values, indicating favorable oral bioavailability according to Lipinski's rule of five, which establishes suitable log P values. The compounds satisfy the criterion where the Total Polar Surface Area (TPSA) should be less than 140Å. With TPSA values ranging from 35-85Å, all compounds have TPSA values below 90Å, indicating enhanced penetration. Furthermore, the compounds have molecular

weights between the ranges of 249.26 to 325.32 classifying them as small molecule inhibitors since their molecular weights are less than 500 Daltons. Table 3 demonstrates that the compounds adhere to the Lipinski rule, as the number of hydrogen bond donors and acceptors fall within the specified range. Additionally, the number of rotatable bonds in the compounds is less than 5, indicating favorable flexibility of the molecules. As a general rule, the larger the bioactivity score, the higher the probability that the investigated compound will be active. Therefore, a molecule having a bioactivity score of more than 0.00 is most likely to possess considerable biological activities, while values -0.50 to 0.00 are expected to be moderately active and if the score is less than -0.50 it is presumed to be inactive. The obtained results revealed that the investigated novel compounds are biologically active molecules and will produce the physiological effect by interacting with GPCR ligands, nuclear receptor ligands, protease inhibitors and other enzymes. GPCR lies between -0.95 to -0.65, showing Nuclear Receptor (-0.55 to -0.90). Protease inhibitor from -0.55 to -0.90 and enzyme inhibitor depicted from -0.25 to -0.45. For drug-induced liver toxicity, all M_1-M_5 showed toxicity except compound M_6. Furthermore, cytotoxicity was only exhibited by compound M_5, the remaining all did not indicate any toxicity. In conclusion, only compound M_6 did not depict any toxicity. All the compounds cross the blood-brain barrier, except compound M_5. Additionally, for PgP inhibitors only compounds M_4 and M_5 were not inhibited remaining all were inhibited. Similarly, with the PgP Substrate, compoundsM_1, M_4 and M_5 are not the substrate which does not aid in the elimination of the toxins from the cells, whereas M_2, M_3 and M_6 show the activity eventually acting as PgP substrates. Compound M_5 did not produce any activity whereas compound M_6 showed the activity for all the transporters. None of the drugs indicated Herg blocking activity as well as MMPS. On the other hand, the Ames test showed that only compound M_5 had mutagenicity, whereas the other four compounds were devoid of special toxicities. Likewise, MRTD was calculated least for compound M_5 and maximum for compound M_6 at 99 mg/day and 142 mg/day respectively. The structure activity relationship of 4- Benzylidene-2-Substituted oxazolones was exhibited diverse in activity. Compound M_3 substituted with hydroxyl group showed higher antioxidant activity and compound M_5 substituted with nitro group showed increase in activity whereas compounds substituted with electron withdrawing groups like 3,5-dimethoxy-4-hydroxy, 4-methoxy and 4-chloro showed substantial decrease in activity. The findings from the anti-oxidant activity reveals that the compound M_3 and M_5 showed excellent antioxidant activity against the standard ascorbic acid, whereas other compounds M_2, M_4 and M_6 exhibited moderate antioxidant activity which is closer to the values of M_3 and M_5. Therefore, compounds with excellent antioxidant activity can be further investigated for their related potential biological activities.

SAR analysis

4-phenylidene-2-Substituted oxazolones were exhibited diverse in activity. Compound M_3 substituted with hydroxyl group showed higher antioxidant activity and compound M5 substituted with nitro group showed increase in activity whereas compounds substituted with electron withdrawing groups like 3,5-dimethoxy-4-hydroxy, 4-methoxy and 4-chloro showed substantial decrease in activity.

CONCLUSION

In the current research investigation, we established a simple, efficient and eco-friendly method to synthesize azlactones without solvents. This approach offers advantages such as higher yields and rapid workup compared to traditional methods. After synthesis, we conducted a comprehensive *in silico* evaluation, including docking studies, drug-likeness assessments, bioactivity predictions, ADME profiling and toxicity analyses, affirming the rationale and potential applicability of compounds M_1-M_6. Compounds M_3 and M_5 showed significant *in vitro* antioxidant activity, surpassing ascorbic acid in the DPPH assay. The binding affinity was too excellent towards the target for M_3 and M_5 exhibiting -8.9 and -10.04 kcal and the percentage of inhibition was found to be 88% and 89% respectively. The *in silico* findings from the docking analysis were well correlated with the *in vitro* antioxidant activity justifying the potency. Their substantial efficacy suggests potential therapeutic use against oxidative stress-related disorders. These findings provide a strong foundation for further exploration and development of these compounds as promising therapeutic agents, bridging efficient synthesis with detailed computational assessments for potential applications in addressing oxidative stress-related conditions.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FT-IR: Fourier transform Infrared spectroscopy; **NMR:** Nuclear magnetic resonance spectroscopy; **MS:** Mass spectroscopy; **KBr:** Potassium bromide; **MMP:** Mitochondrial membrane potential; **MRTD:** Maximum Recomended Therapeutic Dose; **PgP:** P-glycoprotein**, Log P:** Lipophilicity; **TPSA:** Topological Surface Area; **MW:** Molecular Weight; **HBA:** Hydrogen bond acceptors; **HBD:** Hydrogen bond donors; **N Viol:** Number of Violations; **ROTB:** Number of Rotatable Bonds; **GPCR:** Guanidine Protein Coupled receptor; **VNN-ADMET:** Variable Nearest Neighbour Adsorption, Distribution, Metabolism, Excretion; **TLC:** Thin Layer Chromatography; **DNA:** Deoxy ribonucleic acid; **HIV:**

Human immuno virus; **ROS:** Reactive oxygen species; **%:** Percentage; **µg/mL:** Microgram per milliliter; **MHz**; Mega hertz; **DMSO:** Dimethyl sulfoxide; **Anal:** Analytical; **M.F:** Molecular Formula; **ESI:** Electron Spin Ionization; **mmol:** Millimoles; **DPPH:** 2,2- DiPhenyl-1- Picryl Hydrazyl.

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