RJC

RASĀYAN *J. Chem.*
Vol. 18 | No. 3 |1662-1670| July-September | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

DESIGN, SYNTHESIS OF BIS-TRIAZOLE BASED OXIME ANALOGUES AS ANTIOXIDANT AGENTS AND INSILICO DOCKING STUDIES

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ABSTRACT

A set of bis-triazole-based oxime analogues was designed and synthesized using an efficient click chemistry approach. The *in vitro* antioxidant potential of these anew synthesized titled compounds was assessed through DPPH and hydrogen peroxide, radical scavenging assays, by means of results were expressed as IC_{50} values and percentage inhibition. Among the tested derivatives, 4-chloro aryl substituted bis-triazole based oximes exhibited potent antioxidant activity, as shown in both assays, and demonstrated their IC_{50} values of 2.46 ± 0.22 µM and 2.41 ± 0.29 µM, respectively. Additionally, in silico molecular docking studies were carried out using PDB ID: 2CDU, against the crystal structure of NADPH oxidase. All the synthesized target compounds showed notably favorable docking scores, like binding affinities and H-bond interactions, compared to the reference drug.

Keywords: Synthesis, Oximes, Bis-Triazole, Antioxidant, Docking Studies.

RASĀYAN *J. Chem.*, Vol. 18, No. 3, 2025

INTRODUCTION

Free radicals are produced in the human body through both external (exogenous) sources, such as polluted air or water, alcohol[2] intake, exposure to heavy or transition metals, cigarette smoking, and internal (endogenous) processes, including psychological stress[3], immune responses, inflammation[4], and routine metabolic activities. Most oxygen-based free radicals are produced in the human body. These reactive species, commonly known as ROS (Reactive Oxygen Species), are primarily produced through interactions involving molecular or cellular water. ROS contain free radicals, for example, hydroxyl (•OH), and superoxide ($O_2^{\bullet -}$), as well as non-radicals like hydrogen peroxide (H_2O_2) and peroxy nitrite ($ONOO^-$), which can further contribute to radical formation. Furthermore, ROS, the body also generates RNS (Reactive Nitrogen Species), such as nitrogen dioxide (NO_2•) and nitric oxide (NO•). The human body employs a sophisticated antioxidant defense system to regulate and neutralize reactive species. However, when the production of ROS and RNS exceeds the body's defensive capacity, oxidative stress occurs.[6] This imbalance leads to the oxidation of essential cellular macromolecules, including nucleic acids, proteins, and lipids. The resulting oxidative modifications, such as the formation of secondary radicals like alkyl peroxy radicals (ROO•), compromise cellular integrity and function.[8] Prolonged oxidative stress is strongly associated with the onset and progression of various chronic disorders, with neurodegenerative diseases like Parkinson's and Alzheimer's, cardiovascular diseases, several types of cancer, and accelerated aging indicating consequences undermining overall health and vitality.[9] Regions of the body with high oxygen demand, such as the brain, are particularly susceptible to increased free radical production. In addition to elevated oxygen consumption, the brain contains high concentrations of polyunsaturated fatty acids (PUFAs), which are particularly prone to oxidative damage through lipid peroxidation mediated by ROS.[11] This oxidative stress contributes significantly to the development of neurodegenerative diseases. Therefore,

http://doi.org/10.31788/RJC.2025.1839370

additional compounds that combat oxidative stress are often necessary.[12] Antioxidant molecules help neutralize free radicals and inhibit their formation, thereby protecting cells from oxidative damage. Many natural antioxidants are derived from dietary sources such as fruits, vegetables, grains, and nuts, and include vitamins A, C, and E. [13] Phenolic compounds, a class of antioxidants, exhibit their activity primarily due to specific structural characteristics that enable them to neutralize free radicals. Their antioxidant function arises through mechanisms such as hydrogen atom donation, electron transfer, or indirect action by chelating metal ions that catalyze free radical formation. These interactions help convert reactive radicals into more stable, non-reactive species. Furthermore, the effectiveness of a phenolic antioxidant largely depends on the stability and low reactivity of the radical formed after the antioxidant action. Interestingly, synthetic compounds have been reported to possess quantitatively enhanced antioxidant properties[7] in contrast to natural sources of antioxidants, such as vitamins and carotenoids obtained through diet.[14] Despite the availability of various antioxidants, there remains a significant demand for compounds that exhibit rapid and effective antioxidant activity, supported by extensive and well-controlled large-scale studies.[15] Additionally, Antioxidants are used as food preservatives, particularly in food products composed of lipids, unsaturated fatty acids, or long aliphatic chains, to resist rancidification and thereby enhance shelf life[16], and maintain quality without causing adverse effects.[17] Oxime motif possesses an imine-alcohol structural unit, and molecules which contain this pharmacophore (Fig.-1) hold remarkable applications as drug candidates, *viz.*, as an antidote for organophosphate poisoning, and as agents with antibiotic, anti-inflammatory, anticancer, antioxidant, etc. In plants, oximes occur and are involved in the growth as well as defensive agents by generating hydrogen cyanide. They are key intermediates in the synthesis of many valuable organic products and have notable commercial value. For instance, oximes are the intermediates in the synthesis of lactams, which in turn are used in the synthesis of antibiotics like penicillin, cephalosporin, and nylon-6. They gain importance in organic synthesis because the oxime group can be transformed to other functional groups like amines, nitriles, nitro compounds, and heterocyclic compounds. Moreover, Oximes[18–20] are used as ligands in forming metal complexes, to enable the determination of metals.

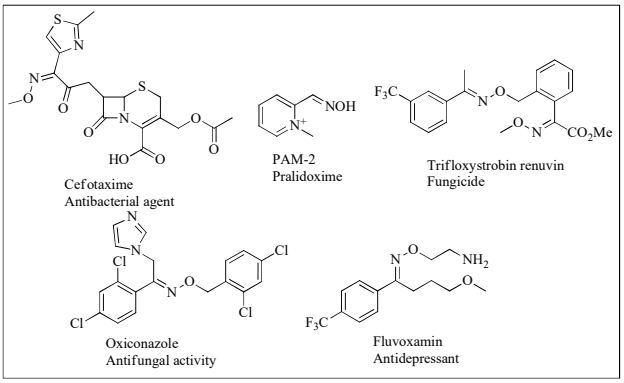


Fig.-1: Representation of Drug Molecules with Oxime Pharmacophore

Likewise, triazole has been reported to be a versatile scaffold with numerous medicinal applications.[21] Given the promising potential of both 1,2,3-triazoles and oximes, their conjugates are envisioned as promising bioactive molecules. Encouraged by these findings and the ongoing need for effective antioxidants, we aimed to design and synthesize a series of bis-triazole oxime derivatives.

EXPERIMENTAL

Materials and Methods

Melting points of all newly synthesized compounds were recorded by utilizing on SCICBMPA-01 apparatus. Reaction growth was observed by TLC, with spots visualized under UV light at 254 nm. Crude compounds were purified by column chromatography with silica gel (100–200 mesh). ^{1}H-NMR and ^{13}C-NMR (nuclear magnetic resonance) spectra were carried out on Bruker AV 400MHz, in solvents $CDCl_3$ and DMSO-d_6 with TMS as an internal standard, at 500 MHz and 126 MHz, correspondingly. Chemical shifts (δ) values are expressed in ppm, and coupling constants (J) are reported in Hz. An Agilent

SA/DA/INS/015 mass spectrometer instrument was used to confirm the Electronspray ionization (ESI) mass spectra. All the reagents and solvents were commercially available and procured from suppliers such as SD Fine, TCI, Spectrochem, and Avra Chemicals, and utilized without further purification.

Synthesis of 1-(2,4-bis(prop-2-yn-1-yloxy) phenyl) ethan-1-one (2)
To a solution of 1-(2,4-dihydroxyphenyl) ethenone (5g, 32.3 mmol) and potassium carbonate (1.25g, 32.3 mmol) was dissolved in 30 mL of dry DMF and allowed to stir for 15 minutes. After that, 3-bromoprop-1-yne (4.2g, 36.18 mmol) was subsequently slowly added to the reaction mixture with stirring, keeping the temperature at 0 °C. The reaction was then allowed to stir at room temperature (25 °C) for 4-5 hours. After completing the reaction, the solvent was removed under reduced pressure, the mixture was transferred into ice-cooled water (150 mL), neutralized with dilute HCl, and extracted with excess EtOAc (3×25mL). The organic layer was dried over Na_2SO_4, filtered, concentrated with a rotary evaporator, and purified by column chromatography to obtain compound **2** as a brown solid in 85% yield.

Synthesis of 1-(2,4-bis((1-phenyl-1H-1,2,3-triazol-4-yl) methoxy) phenyl) ethan-1-one 4(a-e)
The compound **2** (0.87 mmol), sodium ascorbate (10 mol%), and catalytic amounts of $CuSO_4 \cdot 5H_2O$ (5 mol%) were dissolved in a 1:1 ratio of *tert*-butanol and water mixture (15 mL). Subsequently, aryl azides **3(a–e)** (1.044 mmol) were added individually to the reaction mixture and allowed to stir overnight at 0 °C. Upon completion, the reaction was then poured onto crushed ice water, followed by extraction with EtOAc (3×25 mL). The combined organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Finally, the crude mass was then purified by column chromatography on silica gel (100-200 mesh) *via* EtOAc and *n*-hexane as the eluents to obtain the corresponding bis-triazole derivatives **4(a-e)** as white solids in good yields (75-85%).

Synthesis of 1-(2,4-bis((1-phenyl-1H-1,2,3-triazol-4-yl) methoxy) phenyl) ethan-1-one oxime (5a)
A solution of bis-triazole (4a) (0.215 mmol) and hydroxylamine hydrochloride (0.258 mmol) suspended in 10 mL of MeOH was treated with the addition of sodium acetate (1.2 mmol) and a catalytic amount of glacial acetic acid between 6-10 °C, over 10 minutes. The resulting mixture was then stirred at ambient temperature (25 °C) for 2 hours. The progress of the reaction was observed by TLC. This end the reaction mixture was then diluted with ice-cold water (50 mL) and extracted with EtOAc/petroleum ether (3×15 mL). The organic layer was washed with brine solution (15mL), dried over Na_2SO_4, and concentrated using a rotary evaporator. The resulting crude mass was purified by column chromatography (silica gel, 100-200 mesh), using 5% EtOAc in petroleum ether as the eluate to yield the desired bis-triazole-based oxime (**5a**) as a white solid. MP:141-143°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ, ppm; 10.88 (s, 1H, OH), 9.00 (s, 1H, triazole-H), 8.94 (s, 1H, triazole-H), 7.92 (d, J = 5.7 Hz, 4H, Ar-H), 7.69 – 7.57 (m, 4H, Ar-H), 7.52 (d, J = 7.2 Hz, 2H, Ar-H), 7.18 (d, J = 8.3 Hz, 1H, Ar-H), 7.04 (s, 1H, Ar-H), 6.71 (d, J = 7.2 Hz, 1H, Ar-H), 5.30 (s, 4H, $2CH_2$), 2.01 (s, 3H, CH_3): ^{13}CNMR (125Hz, $CDCl_3$) δ, ppm; 163.05, 159.69, 137.08, 133.06, 130.06, 129.25, 122.27, 121.60, 121.48, 120.86, 107.34, 100.44, 62.61, 62.27, 32.12. Mass (ESI mass) m/z $[M+H]^+$ 482.6, CHN analysis: Calc. for $C_{26}H_{23}N_7O_3$: C 71.93, H 5.39, N 8.99 % Found: C 71.95, H 5.40, N 8.97%.

1-(2,4-bis((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)ethan-1-one oxime (5b): White solid, Yield (%) 89, MP 158-161°C; ^{1}H NMR (500 MHz, DMSO) δ, ppm; 10.87 (s, 1H, OH), 8.89 (s, 1H, triazole-H), 8.83 (s, 1H, triazole-H), 7.82 (dd, J = 9.1, 3.1 Hz, 4H, Ar-H), 7.17 (d, J = 8.4 Hz, 1H, Ar-H), 7.14 (dd, J = 9.1, 3.0 Hz, 4H, Ar-H), 7.03 (d, J = 2.2 Hz, 1H, Ar-H), 6.70 (dd, J = 8.4, 2.2 Hz, 1H, Ar-H), 5.28 (s, 4H, $2CH_2$), 3.84 (s, 3H, OMe), 3.83 (s, 3H, OMe), 2.50 (s, 3H, CH_3). ^{13}CNMR (125Hz, $CDCl_3$) δ, ppm; 162.87, 160.00, 159.51, 143.81, 143.74, 132.79, 130.28, 122.26, 122.00, 121.47, 121.35, 114.82, 107.11, 100.18, 62.39, 62.05, 55.64, 31.93. Mass (ESI mass) m/z $[M+H]^+$ 542, CHN analysis: Calc. for $C_{28}H_{27}N_7O_5$: C 61.55, H 4.43, N 7.69 % Found: C 61.53, H 4.41, N 7.67%.

1-(2,4-bis((1-(3,5-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)ethan-1-one oxime(5c): Off White solid, Yield (%) 87, MP 160-162 °C; ^{1}H NMR (500 MHz, DMSO) δ, ppm; 10.87 (s, 1H, OH), 8.91 (s, 1H, triazole-H), 8.85 (s, 1H, triazole-H), 7.72 (dd, J = 4.5, 2.0 Hz, 2H, Ar-H), 7.66-7.57 (m, 2H, Ar-H), 7.35 (dd, J = 8.2, 2.6 Hz, 2H, Ar-H), 7.18 (d, J = 8.4 Hz, 1H, Ar-H), 7.02 (d, J = 2.2 Hz, 1H, Ar-H), 6.70

(dd, J = 8.4, 2.2 Hz, 1H, Ar-H), 5.28 (s, 4H, $2CH_2$), 2.32 (s, 6H, $2CH_3$), 2.29 (s, 6H, $2CH_3$), 2.01 (s, 3H, CH_3).^{13}CNMR (125Hz, $CDCl_3$) δ, ppm; 162.84, 159.48, 138.41, 137.77, 134.72, 132.74, 130.62, 121.91, 121.72, 117.86, 106.97, 100.15, 62.36, 62.04, 31.92, 19.86, 19.46. Mass (ESI mass) m/z $[M+H]^+$ 538; CHN analysis: Calc. for $C_{30}H_{31}N_7O_3$: C 67.00, H 4.82, N 8.37 % Found: C 66.99, H 4.81, N 8.56%.

1-(2,4-bis((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)ethan-1-one oxime (5d): Off White solid, Yield (%) 82, MP 165-167°C; ^{1}H NMR (500 MHz, DMSO) δ, ppm; 10.87 (s, 1H, OH), 9.02 (s, 1H, triazole-H), 8.96 (s, 1H, triazole-H), 7.97 (dd, J = 8.9, 2.5 Hz, 4H, Ar-H), 7.69 (dd, J = 8.8, 3.4 Hz, 4H, Ar-H), 7.18 (d, J = 8.4 Hz, 1H, Ar-H), 7.02 (d, J = 2.0 Hz, 1H, Ar-H), 6.71 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 5.30 (s, 4H, $2CH_2$), 2.01 (s, 3H, CH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ 162.68, 159.32, 144.28, 144.24, 135.27, 134.85, 132.83, 129.99, 122.06, 121.74, 121.58, 121.08, 107.14, 100.16, 62.26, 61.91, 31.80 Mass (ESI mass) m/z $[M+H]^+$ 550; CHN analysis: Calc. for $C_{26}H_{21}Cl_2N_7O_3$: C 65.04, H 4.52, N 7.85 % Found: C 65.03, H 4.50, N 7.83%.

1-(2,4-bis((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)ethan-1-one oxime (5e): Pale yellow solid, Yield(%) 79, MP 136-138°C ^{1}H NMR (500 MHz, DMSO) δ, ppm; 10.85 (s, 1H, OH), 8.32 (s, 1H, triazole-H), 8.27 (s, 1H, triazole-H), 7.42-7.26 (m, 10H, Ar-H), 7.12 (d, J = 8.4 Hz, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 6.62 (d, J = 8.4 Hz, 1H, Ar-H), 5.62 (s, 4H, $2CH_2$), 5.17 (s, 4H, $2CH_2$), 1.95 (s, 3H, CH_3).^{13}C NMR (125 MHz, $CDCl_3$) δ, ppm; 162.81, 159.44, 143.63, 143.57, 134.36, 134.28, 132.62, 129.15, 128.85, 128.12, 127.97, 122.90, 121.83, 106.89, 100.13, 62.39, 62.04, 54.28, 31.25. Mass (ESI mass) m/z $[M+H]^+$ 510; CHN analysis: Calc. for $C_{28}H_{27}N_7O_3$: C 65.62, H 4.72, N 10.93 % Found: C 65.61, H 4.71, N 10.91%.

Antioxidant Activity Procedure

DPPH Assay

This method is used to measure antioxidant activity using a spectrophotometer. DPPH, a stable free radical, has a deep purple color appearance with a significant absorption at 517 nm. When antioxidants are added, they donate hydrogen atoms or electrons to DPPH, converting it into a stable, non-radical form (DPPH-H), which appears yellow. As a result, the absorbance decreases. The color change reflects the antioxidant's free radical scavenging ability. In general, the antioxidant activity is shown as a percentage of inhibition [24]. To 3 mL of test and standard solution at various concentrations (10, 20, 40, 60, 80, and 100 µg/mL), 1 mL of DPPH 0.1mM (0.40 mg of DPPH in 12 mL of MeOH) was added. At the same time, for each concentration, a corresponding blank sample (without the adding solution 0.1mM of DPPH solution) was prepared by adding an equivalent volume of methanol instead of the DPPH solution. Additionally, a control was prepared by mixing 0.1 mM DPPH (1 ml) with methanol (3 ml), without any test or standard sample. For evaluation, Ascorbic acid was utilized as a standard. All test samples were incubated in the dark for 20 min, and absorbance or optical density was measured at 517 nm. Each test was repeated 3 times for accuracy. Subsequently, the concentration of the test sample required to decrease 50% percentage of the DPPH free radicals is defined as the IC_{50}**.** The IC_{50} values of the synthesized compounds, expressed in µg/mL, are summarized in Table-**1**. All samples were incubated for 20 minutes in a dark place, and their optical density was measured at 517 nm. Each test was frequent 3 times for accuracy. The IC_{50} values were defined for the sample concentration essential to decrease 50% of the DPPH free radicals. The IC_{50} values, expressed in µg/mL, for all synthesized compounds and summarized in Table-1.

Table-1: Antioxidant Activity of Target Compounds 5(a-e) by DPPH Method

Entry	% of inhibition	IC_{50} value ±standard error mean
5a	60.65	3.16±0.35
5b	62.53	3.8±0.58
5C	63.67	3.4±0.41
5d	71.76	2.46±0.22
5e	58.69	3.84±0.89
Ascorbic acid	74.79	1.46±0.52

Hydrogen Peroxide Assay

A mixture of 1.5 mL of the test or standard solution at the various concentrations (10, 20, 40, 60, 80, and 100 µg/mL) and 0.7 mL of 42 mM hydrogen peroxide was prepared and allowed to stand for 15 minutes.

The absorbance or optical density of each sample was then measured at 235 nm. Ascorbic acid was used as a standard drug for evaluation. The percentage inhibition and IC_{50} values of the synthesized target bis-triazole-based oxime are summarized in Table-2.

Table-2: Antioxidant Activity of Target Compounds 5(a-e) by Hydrogen Peroxide Method

Entry	% of inhibition	IC_{50} value ±standard error mean
5a	61.54	3.0±0.37
5b	61.67	3.7±0.25
5C	64.74	3.5±0.58
5d	71.89	2.41±0.29
5e	59.43	3.8±0.8
Ascorbic acid	74.79	1.46±0.52

Insilco Molecular Docking

In this investigation, molecular docking was performed using the open-source software Auto Dock Vina, integrated within the PyRx platform.[25-28] Auto Dock Vina assesses the binding energy of protein-ligand complexes using an empirical scoring function.[29] The Protein Data Bank (www.rcsb.org) provided the PDB ID: 2CDU of the crystal structure of NADPH oxidase to prepare it for docking. Polar hydrogen atoms were added to the macromolecule after water molecules and heteroatoms were removed. CHE Sketch was used to sketch ligand structures (.mol) format in MDL (https://www.acdlabs.com/). The ligands were energy-minimized and changed to PDBQT format after the target protein and ligand files were input into the PyRx interface. Docking imitations were carried out by defining a 3D grid box centered at coordinates x = 10.4336, y = –1.0584, and z = –1.6597, with dimensions of x = 47.6750, y = 51.8502, and z = 45.3411, ensuring coverage of the active site region. The exhaustiveness parameter was set to 8. Following docking, ligand conformations were ranked based on binding energy, with the conformation exhibiting the lowest binding energy considered the most favorable. Visualization and analysis of docking score were performed by means of PyMOL and BIOVIA Discovery Studio Visualizer.

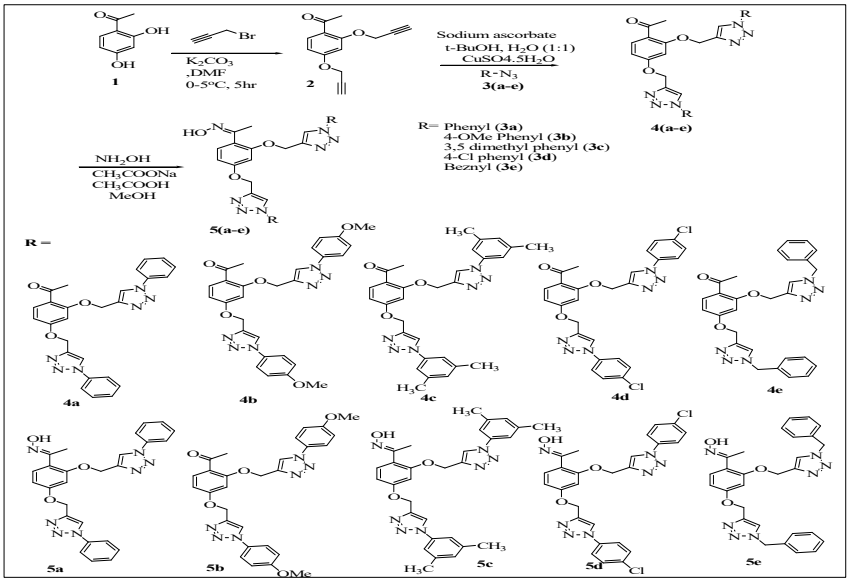
RESULTS AND DISCUSSION

Chemistry

The present work, the synthesis of bis-triazole based oximes, was strategically planned to the synthesis of intermediate compounds from commercially available initial chemicals illustrated in Scheme-1. First, the compound 1-(2,4-bis(prop-2-ynyloxy) phenyl) ethenone derivative 2 was synthesized in excellent yield *via* a nucleophilic substitution reaction between 1-(2,4-dihydroxyphenyl) ethenone 1 and 3-bromoprop-1-yne, using potassium carbonate (K_2CO_3) as a base at 0-5 °C.[22]

Further, click chemistry encompasses a set of highly efficient and versatile chemical reactions that are used to form covalent bonds. In this context, compound 2 underwent [3+2] cycloaddition with various substituted aromatic azides 3(a–e) in the presence of sodium ascorbate and $CuSO_4 \cdot 5H_2O$, yielding the bis-triazole derivatives 4(a–e) in excellent yields[10,23] with minimal by-products. Finally, a nucleophilic addition followed by an elimination reaction between bis-triazole derivatives 4(a–e) with hydroxylamine hydrochloride (NH_2OH) under acidic conditions yielded the target bis-triazole-based oxime derivatives 5(a–e) in good to moderate yields.[5] All bis-triazole-based oxime derivatives 5(a-e) were obtained in good yields, and their structures were validated using spectroscopic data. The ^1H NMR spectrum of bis-triazole base oxime (5a) displayed a singlet at δ 10.88, corresponding to the Oxime OH proton, and two singlet peaks at *δ* 9.00 and 8.94 ppm, showed to the bis-1,2,3-triazole protons. Thirteen aromatic protons were detected as a multiplet in the range of δ 7.92-6.70 ppm. The signal at δ 5.30 ppm was observed for the two methylene protons, whereas the signal at δ 2.01 ppm, attributed to the methyl protons.

The ^{13}C NMR spectrum of bis-triazole base oxime 5a showed peaks at δ 163.05 and 159.69 ppm, conforming to the two C-O carbons and the oxime carbon, respectively. Aromatic carbon signals were detected between δ 137.08 and 100.44 ppm. The signals at δ 62.61 and 62.27 ppm corresponded to the methylene carbons, while the peak at δ 32.12 ppm was attributed to the methyl carbon. Mass spectrometric analysis of 5a exhibited a molecular ion peak at m/z 482.6 ($[M+H]^+$), consistent with the molecular formula $C_{26}H_{23}N_7O_3$.



Scheme-1: Synthesis of Novel Oxime-Based Bis-Triazole Analogues

Antioxidant Activity

The antioxidant activity of the synthesized bis-triazole base oxime derivatives 5(a–e) was assessed using DPPH and H_2O_2, radical scavenging assays, with ascorbic acid as the standard reference drug. The results, presented in Table-1 and Table-2, were consistent athwart both assays. Antioxidant activity was expressed as the % of inhibition and IC_{50} values. Notably, compound 5d exhibited exceptional antioxidant activity, showing percentage inhibition values of 71.76% (IC_{50} = 2.46 ± 0.22 µM) and 71.89% (IC_{50} = 2.41 ± 0.29 µM) in the respective assays. In comparison, ascorbic acid demonstrated a percentage inhibition of 74.79% (IC_{50} = 1.46 ± 0.52 µM) in both tests. Compounds 5a, 5b, and 5c showed moderate activity, while compound 5e exhibited the lowest antioxidant potential among the series relative to ascorbic acid. Structure–activity relationship (SAR) analysis indicates that antioxidant activity is influenced by the electronic nature of the substituted aryl ring. The existence of electron-withdrawing groups at the para-position of the aryl ring significantly enhances antioxidant potential, suggesting that such substitutions are promising for further development. Similarly, compounds with +M effect substituents and unsubstituted phenyl rings exhibited moderate to good activity. However, replacing aryl substituents with a benzyl group markedly reduced antioxidant activity, indicating this structural modification is unfavorable for enhancing antioxidant properties. Both assay methods revealed similar polarization effects. The antioxidant activities and IC_{50} values of the synthesized bis-triazole-based oxime derivatives are summarized in Table-1 and Table-2, respectively. In particular, compound 5d recorded the lowest IC_{50} value, indicating its potential as a highly efficient antioxidant agent.

Molecular Docking Study

In silico docking is a valuable computer-aided technique used to predict the binding interactions between the most favorable conformation of a ligand and its target receptor, to identify a stable ligand-receptor complex. This approach is widely recognized in drug discovery for being reliable, cost-effective, and time-efficient. To examine the binding interactions between the synthesized bis-triazole-based oxime derivatives 5a-e and their target receptor, molecular docking simulations were achieved using PDB ID: 2CDU of the crystal structure of NADPH oxidase.[1] NADPH oxidase (NO) was selected as the antioxidant target due to its role in generating reactive oxygen species (ROS), which are crucial for immune defense against pathogens. However, the overexpression of NO has been associated with the development of several neurological disorders and cancers. All newly synthesized ligands demonstrated significantly better binding affinity values related to the ascorbic acid standard reference. The docking results of the synthesized oxime-based triazole derivatives ranged from -10.9 to -12.6 kcal/mol, whereas ascorbic acid showed a docking score of only -6.5 kcal/mol. The Insilco docking results and detailed binding interactions of oxime-based bis-triazole derivatives 5(a–e) are shown in Table-3. All newly synthesized oxime-based bis-triazole

Analogues demonstrated significantly stronger binding affinities than the reference compound, ascorbic acid. The docking scores of the oxime-based bis-triazole Analogues ranged from -10.9 to -12.6 kcal/mol, whereas ascorbic acid demonstrated a comparatively low score of -6.5 kcal/mol. The docking results and detailed binding interactions of oxime-based triazoles 5(a-e) are summarized in Table-3.

Table-3: Binding Affinities of Bis-Triazole Based Oxime Analogues and Interacting with Amino Acids of NADPH oxidase (PDB ID: 2CDU)

Compound	Binding Energy (Kcal/mol)	Interacting amino acids	
		H-bond	Hydrophobic
5a	-10.9	His10, Thr112, Thr9, Gly114, Lys134	Met33, Ile44, Cys133, Ile160, Asp282, Ala303
5b	-11	Thr9, Ala11, Thr113, Asp282, Pro298	His10, Ala11, Glu32, Met33, Thr113, Leu251, Asp282, Pro298, Ala303
5c	-12.6	Thr9, Thr112	Val6, Ala11, Glu32, Met33, Val81, Thr113, Lys134, Ile160, Phe245, Leu251, Pro298
5d	-11	Gly7, Thr9, His10, Glu32	Ala11, Met33, Ile44, Thr113, Cys133, Ile160, Asp282, Ala300, Ala303
5e	-11.2	Ser41, Gly114, Ser115, Lys134, Asn248	Thr9, His10, Ala11, Ile44, Thr113, Cys133, Lys134, Ile160, Asp282, Ala300, Ala303,
Ascorbic acid	-6.5	Thr9, His10, Ala11, Gly12	-

Binding Interactions Analysis

The binding interactions of the bis-triazole-based oxime analogues 5(a-e) at the active site of NADPH oxidase involve key amino acid residues such as Thr9, His10, and Ala11, which are also involved in the interaction with the standard compound, ascorbic acid. The majority of the synthesized compounds formed hydrogen bond (H-bond) interactions, which are essential for strong binding affinity and potential biological activity. For example, compound 5c, which recorded the maximum binding energy value of -12.6 kcal/mol, formed H-bond relations with Thr9 and Thr112, as well as hydrophobic interactions with Val6, Ala11, Glu32, Met33, Val81, Thr113, Lys134, Ile160, Phe245, Leu251, and Pro298 of NADPH oxidase (Fig.-2). Similarly, the reference compound ascorbic acid formed hydrogen bond relations with residues Thr9, His10, Ala11, and Gly12 within the binding pocket of NADPH oxidase (Fig.-3). The Insilco docking results and detailed binding interactions indicate that the bis-triazole-based oxime analogues 5(a-e) effectively occupy the active site pocket of NADPH oxidase, suggesting their potential as promising antioxidant agents.

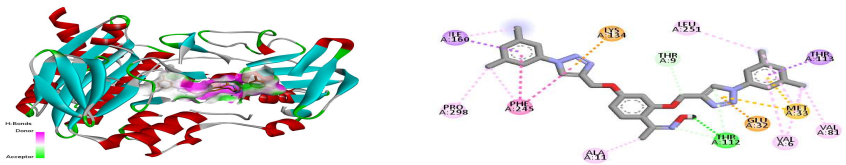


Fig.-2: (a) Docking Pose and (b) Binding Interactions of Compound 5c in the Cavity of NADPH Oxidase

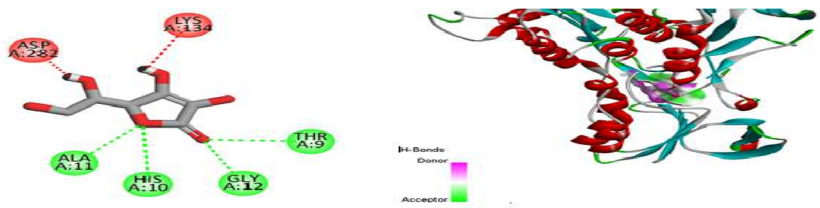


Fig.-3: (a) Docking Pose and (b) Binding Interactions of Ascorbic Acid in the Cavity of NADPH Oxidase

CONCLUSION

In conclusion, a series of bis-triazole-based oxime analogues 5(a-e) were successfully synthesized and evaluated for their antioxidant activity using both DPPH and H_2O_2 radical scavenging methods. The

developed hybrids exhibited promising results, particularly compound 5d, which showed significant activity. The remaining compounds demonstrated moderate to good antioxidant effects. These findings suggest that the combination of two bioactive structural motifs can enhance overall efficacy and warrants further investigation. Additionally, in silico screening revealed favorable binding interactions and binding energies compared to standard references, further supporting the potential of these compounds for continued research.

ACKNOWLEDGMENTS

The author thanks the Head Department of Chemistry, Osmania University, Hyderabad, for providing laboratory facilities. The authors also acknowledge the analytical support provided by the Central Facilities for Research and Development (CFRD), Osmania University, Hyderabad, India.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to SDG-9: *Industry, Innovation and Infrastructure*. 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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[RJC-9370/2025]