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Design, Synthesis and Molecular Docking-Based Evaluation of Piperazine-Linked Triazine Derivatives as Antioxidant Agents

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Oxidative stress is a key driver of the pathogenesis of numerous chronic inflammatory and metabolic diseases emphasizing the requirement for the development of potent and selective antioxidant therapeutics. In present study, a series of novel piperazine-linked 1,3,5-triazine derivatives (**6a-h**) was rationally designed, synthesised and evaluated for antioxidant activity using integrated computational and experimental approaches. Molecular docking studies were carried out against myeloperoxidase (MPO; PDB ID: 1DNU), a heme-containing enzyme involved in reactive oxygen species (ROS) generation, to examine ligand–protein interactions and binding affinities. The synthesized compounds exhibited binding energies ranging from -3.816 to -4.987 kcal/mol. Among them, compound **6f**, bearing a *para*-fluorophenyl substituent, shows the highest binding affinity (-4.987 kcal/mol). The enhanced binding was attributed to the formation of two hydrogen bonds with ARG27 and LEU97 and exceeded the binding energy of the reference antioxidant, ascorbic acid (-4.690 kcal/mol). The antioxidant potential was assessed by the DPPH free radical scavenging assay. Compound **6f** displayed the strongest activity with an IC_{50} value of 14.15 ± 0.14 μM, which was comparable to that of ascorbic acid (IC_{50} = 14.06 ± 0.18 μM). Structure-activity relationship analysis indicated that the electron-withdrawing substituents at the *para*-position of aryl ring, together with nitrogen-containing heteroaromatic moieties, improve both binding affinity and free radical scavenging activity. The results identify piperazine-linked 1,3,5-triazine derivatives as promising antioxidant scaffolds and provide a strong basis for future myeloperoxidase inhibition studies and *in vivo* pharmacological evaluation.

Keywords: Piperazine, Triazine, Molecular docking, Antioxidant activity.

INTRODUCTION

In recent years, heterocyclic compounds have gained considerable attention due to their diverse biological activities and structural versatility. Among them, 1,3,5-triazine (*s*-triazine) derivatives represent an important class of compounds with a broad spectrum of pharmacological properties, such as antimicrobial, anticancer, anti-inflammatory and antioxidant activities [1,2]. The triazine core is particularly attractive due to its electron-deficient aromatic ring, which facilitates nucleophilic substitution reactions and allow the introduction of various functional groups to fine-tune biological activity. Also, the presence of multiple substitution sites enables the design of structurally diverse molecules with enhanced pharmacological profiles [3-6].

Piperazine is another privileged scaffold in drug design, frequently found in numerous clinically approved drugs. Its significance arises from its favourable pharmacokinetic properties, including improved solubility, bioavailability and the ability to interact with biological targets through hydrogen bonding and electrostatic interactions [7-10]. Incorporation of a piperazine moiety into heterocyclic frameworks has been shown to enhance biological activity, including antioxidant potential, by improving molecular flexibility and facilitating interactions with biomolecular targets [11,12].

The hybridisation of triazine and piperazine scaffolds into a single molecular framework is therefore a promising strategy for developing novel bioactive compounds. Moreover, the introduction of functional groups such as allyloxyamine moieties may contribute to enhanced radical scavenging activity due to their potential hydrogen-donating and electron-stabilising properties [13-15]. Structural modification of such hybrid molecules offers an opportunity to explore structure activity relationships (SAR) and optimize antioxidant efficacy [16,17].

Computational methods have become an integral part of contemporary drug discovery, complementing experimental strategies by facilitating the rapid identification and optimisation of bioactive molecules. Among these methods, molecular docking is extensively employed to predict the binding orientation, interaction profile and binding affinity of small molecules toward protein targets involved in oxidative stress. The technique reduces the need for exhaustive experimental screening while providing molecular-level insight into ligand–protein recognition. Docking studies involving enzymes associated with the generation and regulation of reactive oxygen species (ROS) are particularly valuable for prioritizing lead compounds and guiding structural optimization [18].

Myeloperoxidase (MPO) is a clinically significant heme-containing peroxidase that catalyses the formation of hypochlorous acid (HOCl) and other highly reactive oxidants. Elevated MPO activity contributes to oxidative tissue injury and has been linked to cardiovascular diseases, neurodegenerative disorders, and chronic inflammatory conditions. Owing to its well-characterized crystal structure (PDB ID: 1DNU) and established role in oxidative stress, MPO serves as a suitable molecular target for the evaluation of antioxidant and anti-inflammatory compounds.

The present work describes the rational design, synthesis and biological evaluation of a series of piperazine-linked 1,3,5-triazine derivatives as potential antioxidant agents. Antioxidant activity was assessed using a standard *in vitro* DPPH free radical scavenging assay, while molecular docking against MPO was employed to examine ligand–protein interactions and binding affinity. Experimental observations were compared with computational predictions to explore structure–activity relationships and identify structural features associated with enhanced antioxidant activity. The findings provide a basis for the future development of triazine-based molecules as therapeutic candidates for oxidative stress-related disorders [19].

EXPERIMENTAL

The solvents and chemical reagents needed for the synthetic processes were purchased from various commercial vendors like Merck Ltd., S.D. Fine Chemicals and Sigma-Aldrich Chemicals, India. Before being used, the reagent-grade compounds were purified by either distillation or recrystallisation after being purchased from commercial sources. The purity of the synthesized compounds was assessed by thin-layer chromatography (TLC). Melting points were measured uncorrected using an electrical digital melting point device VMP-PM-Digital Melting/Boiling point Apparatus. Using TMS as the internal standard, ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker Avance NEO 500 MHz spectrometer. The mass spectra were obtained using a Triple Quadrupole LC-MS/MS (LCMS-8060NX) spectrometer.

Synthesis of O-[(2*E*)-3-chloroprop-2-en-1-yl]hydroxylamine (2): In a 500 mL glass flask with a stirring apparatus, a mixture of methyl acetate (0.15 mol), hydroxylamine (0.15 mol) in water and 100 mL of water was prepared. Following this, (0.15 mol) of 4% NaOH solution was added in 60 min and after 2-3 h, a solution of 1,3-dichloropropene was slowly added dropwise while simultaneously adding (0.20 mol) of 4% NaOH solution. The reaction temperature was maintained at 0-5 ºC and the mixture was stirred for approximately 2-4 h until the starting material disappeared, as confirmed by TLC. Subsequently, the reaction mass was heated to 50 ºC and (0.45 mol) of conc. HCl solution was added. The mixture was stirred for about 5-6 h until the material disappeared, as confirmed by TLC examination. After that, 200 mL of CCl_4 was added and the pH was neutralised with 4% NaOH solution and the organic layer was separated. The CCl_4 was then evaporated under reduced pressure using a rotary evaporator to obtain compound **2**. Purified by column Distillation. Elemental analysis of C_3H_6ClNO (m.w.: 107.53); calcd. (found) %: C, 33.51 (33.53); H, 5.62 (5.60); Cl, 32.97 (32.99); N, 13.03 (13.01); O, 14.88 (14.85); ^{1}H NMR (100 MHz, $CDCl_3$, δ ppm): 4.26 (2H, d, *J* = 6.9 Hz), 5.75 (1H, dt, *J* = 14.0, 6.9 Hz), 6.22 (1H, d, *J* = 14.0 Hz); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 69.2 (1C, s), 119.3 (1C, s), 131.1 (1C, s).

Synthesis of 4,6-dichloro-*N*-{[(2*E*)-3-chloroprop-2-en-1-yl]oxy}-1,3,5-triazin-2-amine (3): *s*-Triazine (**1**) (0.05 mol), 4% NaOH solution (0.1 mol) and 100 mL toulene were fed into a 500 mL glass flask equipped with a stirrer. O-[(2*E*)-3-Chloroprop-2-en-1-yl]hydroxylamine (**2**) (0.05 mol) dissolved in 50 mL methylbenzene and the obtained solution was added dropwise into the flask. The reaction temperature was kept at 0-5 ºC and the mixture was stirred for about 2-4 h until the starting material disappear examined by TLC, the inorganic material was filtered off and the filtrate wash with water and separate out organic layer methylbenzene evaporated under reduced pressure by rota evaporator to give com-pound **3** (yield: 89%). Elemental analysis of $C_6H_5Cl_3N_4O$, (m.w.: 255.48); calcd. (found) %: C, 28.21 (28.19); H, 1.97 (1.99); Cl, 41.63 (41.65); N, 21.93 (21.91); O, 6.26 (6.28); IR (KBr, ν_{max}, cm^{-1}): 3373 (N-H), 3208 (C-H), 1565 (C=C), 1419 (C=N), 1171 (C-N), 752 (Cl-C); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 4.54 (2H, d, *J* = 6.4 Hz), 5.82 (1H, dt, *J* = 14.0, 6.4 Hz), 6.22 (1H, d, *J* = 14.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 69.2 (1C, s), 119.3 (1C, s), 131.1 (1C, s), 160.9 (2C, s), 162.8 (1C, s).

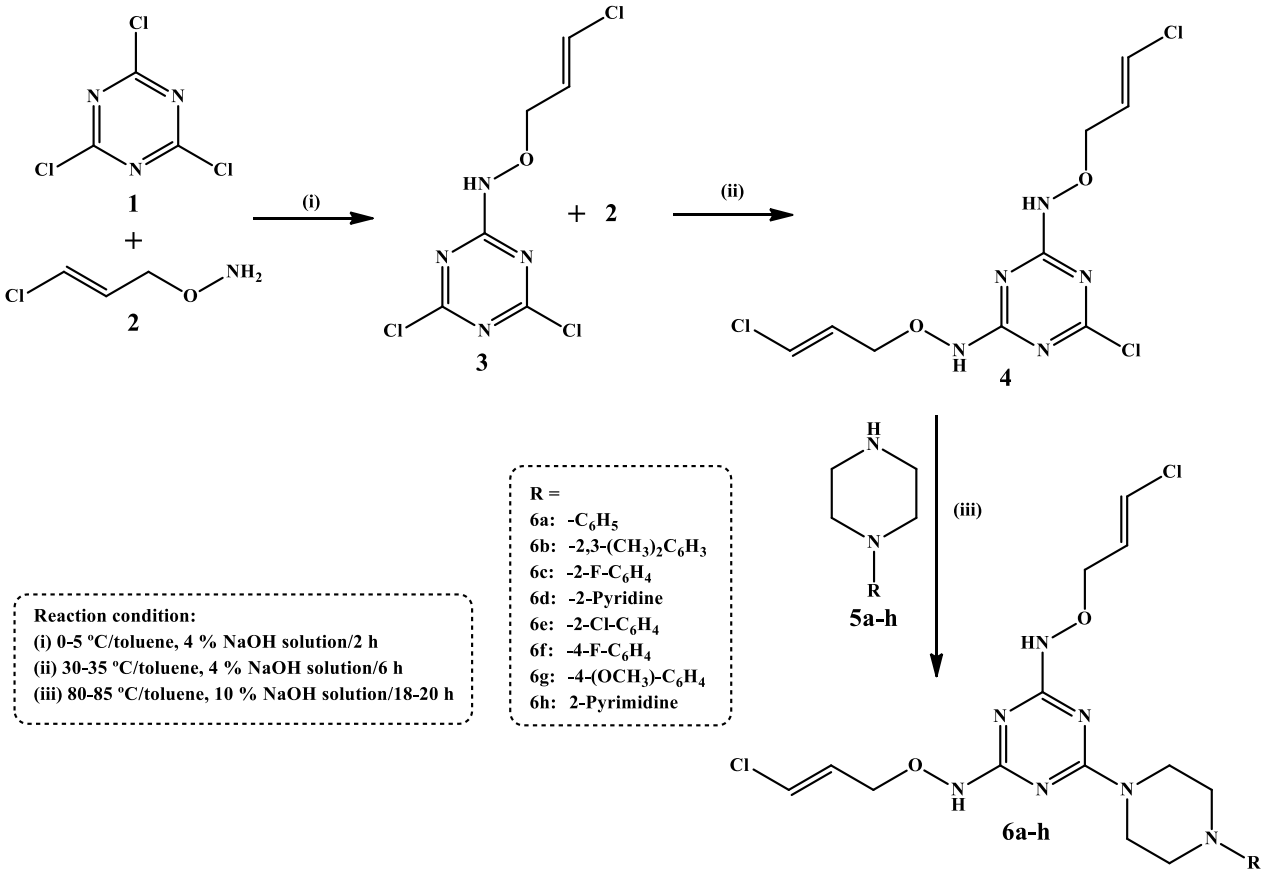
Synthesis of 6-chloro-N^2,N^4-*bis*{[(2E)-3-chloroprop-2-en-1-yl]oxy}-1,3,5-triazine-2,4-diamine (4): 4,6-Dichloro-*N*-{[(2*E*)-3-chloroprop-2-en-1-yl]oxy}-1,3,5-triazin-2-amine (**3**) (0.05 mol), 4% NaOH solution (0.1 mol) and 100 mL methylbenzene were fed into a 500 mL glass flask equipped with a stirrer. O-[(2*E*)-3-Chloroprop-2-en-1-yl]hydroxylamine (**2**) (0.05 mol) dissolved in 50 mL methylbenzene and the obtained solution was added dropwise into the flask. The reaction temperature was kept at 30-35 ºC and the mixture was stirred for about 8-10 h until the starting material disappeared examined by TLC, the inorganic material was filtered off and the filtrate wash with water and separate out organic layer methylbenzene evaporated under reduced pressure by rota evaporator to give compound **4** (yield: 93%). Elemental analysis of $C_9H_{10}Cl_3N_5O_2$ (m.w.: 326.56); calcd. (found) %: C, 33.10 (33.12); H, 3.09 (3.07); Cl, 32.57 (32.55); N, 21.45 (21.47); O, 9.80 (9.78); IR (KBr, ν_{max}, cm^{-1}): 3361 (N-H), 1670 (C=C), 1447 (C=N), 1290 (C-N), 761 (Cl-C); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 4.54 (4H, d, *J* = 6.4 Hz), 5.82 (2H, dt, *J* = 14.0, 6.4 Hz), 6.22 (2H, d, *J* = 14.0 Hz); ^{13}C NMR (100

MHz, $CDCl_3$, δ ppm): 69.2 (2C, s), 119.3 (2C, s), 131.1 (2C, s), 160.9 (1C, s), 162.8 (2C, s).

Synthesis of 4,6-*bis*((((*E*)-3-chloroallyl)oxy)amino)-*N*-cyclopropyl-1,3,5-triazin-2-amine derivatives (6a): In the three-necked 500 mL flask, a solution of compound **4** (0.05 mol) and substituted piperazine (**6a**) in methylbenzene (100 mL) and 10% NaOH solution (0.1 mol) were added, a solution of cyclopropylamine in methylbenzene (0.05 mol) in 50 mL methylbenzene was added dropwise to the mixture. The reaction mixture was stirred at reflux for 12-16 h till the solution become homogenous. Then, ice water (100 mL) was added to the semi-dry precipitate. The solid was collected by vacuum filtration, washed with water, dried and recrystallised from chloroform/*n*-hexane to give a colourless crystalline product of compound **6a** (yield: 85%) (**Scheme-I**). Elemental analysis of $C_{12}H_{16}Cl_2N_6O_2$ (m.w.: 347.2); calcd. (found) %: C, 41.51 (41.53); H, 4.65 (4.63); Cl, 20.42 (20.44); N, 24.21 (24.19); O (9.22%); IR (KBr, ν_{max}, cm^{-1}): 3029 (N-H), 3008 (C-H), 1558 (C=C), 1451 (C=N), 1262 (C-N), 803 (Cl-C); ^1H NMR (500 MHz, $CDCl_3$, δ ppm): 0.45 (4H, dddd, *J* = 8.1, 7.8, 7.5, 6.7 Hz), 2.97 (1H, tt, *J* = 8.1, 7.5 Hz), 4.54 (4H, d, *J* = 6.4 Hz), 5.82 (2H, dt, *J* = 14.0, 6.4 Hz), 6.22 (2H, d, *J* = 14.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 6.6 (2C, s), 23.3 (1C, s), 69.2 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 162.7 (1C, s), 162.8 (2C, s).

***N*,*N*′-(6-(4-Phenylpiperazin-1-yl)-1,3,5-triazine-2,4-diyl)-*bis*(O-((*E*)-3-chloroallyl)hydroxylamine) (6a):** Yield: 78%; m.p.: 112 ºC; Elemental analysis of $C_{19}H_{23}Cl_2N_7O_2$ (m.w.: 452.34); calcd. (found) %: C, 50.45 (50.48), H: 5.13 (5.10); N, 21.68 (21.67); O: 7.07 (7.06); IR (KBr, ν_{max}, cm^{-1}): 3262 (N-H), 2923 (C-H), 1667 (C=C), 1407 (C=N), 1340 (C-N) and at 803 (C-Cl); ^1H NMR (500 MHz, $CDCl_3$, δ ppm): 3.17 (4H, dd, *J* = 17.1, 6.7, Hz), 4.03 (4H, dd, *J* = 13.2, 6.7 Hz), 4.47 (4H, d, *J* = 6.4 Hz), 5.89 (2H, dt, *J* = 14.0, 6.4 Hz), 6.27 (2H, d, *J* = 14.0 Hz), 6.91-7.23 (3H, 6.91 (dd, *J* = 8.3, 1.2, Hz), 7.23 (2H, tt, *J* = 8.1, 1.2 Hz), 8.14 (2H, dd, *J* = 8.3, 8.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 45.1-48.3 (4C, 45.1 (s), 48.3 (s), 71.9 (2C, s), 116.4 (2C, s), 118.6 (2C, s), 120.5 (1C, s), 128.9 (2C, s), 129.3 (2C, s), 151.1 (1C, s), 164.5 (2C, s), 164.6 (1C, s).

***N*,*N*′-(6-(4-(2,3-Dimethylphenyl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)*bis*(O-((*E*)-3-chloroallyl)hydroxylamine (6b):** Yield: 68%; m.p.: 115 ºC; Elemental analysis of $C_{21}H_{27}Cl_2N_7O_2$ (m.w.: 480.39); calcd. (found) %: C, 52.51 (52.50), H: 5.67 (5.66), N: 20.41 (20.44), O: 6.66 (6.67); IR (KBr, ν_{max}, cm^{-1}): 3262 (N-H), 3011 (C-H), 1638 (C=C), 1493 (C=N), 1285 (C-N), 802 (C-Cl); ^1H NMR (500 MHz, $CDCl_3$, δ ppm): 2.15 (3H, s), 2.15 (3H, s), 3.47 (4H, dd, *J* = 17.2, 6.7, Hz), 4.03 (4H, dd, *J* = 6.7, 2.5 Hz), 4.47 (4H, d, *J* = 6.4 Hz), 5.90 (2H, dt, *J* = 14.0, 6.4 Hz), 6.27 (2H, d, *J* = 14.0 Hz), 6.56-6.82 (2H, 6.56 (dd, *J* = 7.9, 1.5 Hz), 6.82 (1H,dd, *J* = 7.6, 1.5 Hz), 8.14 (2H, dd, *J* = 7.9, 7.6 Hz); ^{13}C NMR (500 MHz, $CDCl_3$, δ ppm): 14.1 (1C, s), 19.9 (1C, s), 45.0 (2C, s), 50.1 (2C, s), 71.9 (2C, s), 115.6 (1C, s), 120.5 (2C, s), 123.6 (1C, s), 127.0 (1C, s), 128.1 (1C, s), 128.9 (2C, s), 138.6 (1C, s), 149.9 (1C, s), 164.5 (2C, s), 164.6 (1C, s).



Scheme-I: Synthetic route for the synthesis of piperazine-linked-triazine derivatives (**6a-h**)

***N*,*N*′-(6-(4-(2-Fluorophenyl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)*bis*(O-((*E*)-3-chloroallyl)hydroxylamine (6c):** Yield: 78%; m.p.: 135 ºC; Elemental analysis of $C_{19}H_{22}Cl_2FN_7O_2$ (m.w.: 470.33); C, 48.52 (48.51), H, 4.71 (4.72), N, 20.85 (20.87), O, 6.80 (6.78); IR (KBr, ν_{max}, cm^{-1}): 3253 (N-H), 3092 (C-H), 1620 (C=C), 1455 (C=N), 1354 (C-F), 1285 (C-N), 796 (C-Cl); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 3.02 (4H, dd, *J* = 17.2, 6.7 Hz), 4.02 (4H, dd, *J* = 13.9, 6.7 Hz), 4.47 (4H, d, *J* = 6.4 Hz), 5.92 (2H, dt, *J* = 14.0, 6.4 Hz), 6.30 (2H, d, *J* = 14.0 Hz), 6.96-8.14 (4H, 6.96, dd, *J* = 8.0, 1.7 Hz), 7.22 (dd, *J* = 8.1, 1.3 Hz), 7.32 (dd, *J* = 8.1, 7.5, Hz), 7.36 (dd, *J* = 8.0, 7.5 Hz), 8.14 (2H, dd, *J* = 8.3, 8.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 44.7 (2C, s), 45.0 (2C, s), 71.6 (2C, s), 109.8 (2C, s), 120.5 (2C, s), 128.6 (2C, s), 157.3 (2C, s), 161.3 (2C, s), 164.5 (2C, s), 164.6 (1C, s).

***N*,*N*′-(6-(4-(Pyridin-2-yl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)*bis*(O-((*E*)-3-chloroallyl)hydroxylamine (6d):** Yield: 77%; m.p.: 125 ºC; Elemental analysis of $C_{18}H_{22}Cl_2N_8O_2$ (m.w.: 470.33); C, 47.69 (47.68), H: 4.89 (4.87), N: 24.72 (24.74), O: 7.06 (7.07); IR (KBr, ν_{max}, cm^{-1}): 3256 (N-H), 3017 (C-H), 1638 (C=C), 1561 (C=N), 1284 (C-N), 766 (C-Cl); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 3.96 (4H, dd, *J* = 15.9, 6.7 Hz), 3.99 (4H, dd, *J* = 14.8, 6.7 Hz), 6.64 (4H, d, *J* = 6.4 Hz), 5.92 (2H, dt, *J* = 14.0, 6.4 Hz), 6.80 (2H, d, *J* = 14.0 Hz), 7.47 (1H, dd, *J* = 7.9, 4.8 Hz), 7.50 (1H, dd, *J* = 8.1, 1.3 Hz), 8.07 (1H, dd, *J* = 8.1, 7.9 Hz), 8.14 (1H, dd, *J* = 4.8, 1.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 45.0-45.9 (4C, 45.0 (s), 45.9 (s), 71.9 (2C, s), 110.9 (2C, s), 114.5(1C, s), 120.5 (1C, s), 128.9 (1C, s), 137.8 (2C, s), 148.4 (1C, s), 159.3 (1C, s), 164.5 (2C, s), 164.6 (1C, s).

***N*,*N*′-(6-(4-(2-Chlorophenyl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)*bis*(O-((*E*)-3-chloroallyl)hydroxylamine (6e):** Yield: 68%; m.p.: 138 ºC; Elemental analysis of $C_{19}H_{22}Cl_3N_7O_2$ (m.w.: 486.78); C, 46.88 (46.89); H, 4.56 (4.55); N, 20.14 (20.15); O: 6.57 (6.55); IR (KBr, ν_{max}, cm^{-1}): 3374 (N-H), 3112 (C-H), 1572 (C=C), 1466 (C=N), 1274 (C-N), 740 (C-Cl); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 3.09 (4H, dd, *J* = 17.3, 6.7 Hz), 3.71 (4H, dd, *J* = 13.8, 6.7 Hz), 4.54 (4H, d, *J* = 6.4 Hz), 5.82 (2H, dt, *J* = 14.0, 6.4 Hz), 6.23 (2H, d, *J* = 14.0 Hz), 6.57 (1H, dd, *J* = 8.2, 1.1 Hz), 6.85-7.05 (2H, 6.92, dd, *J* = 8.1, 7.5, Hz), 6.98 (dd, *J* = 8.2, 7.5 Hz), 7.40 (1H, dd, *J* = 8.1, 1.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 49.1 (2C, s), 53.9 (2C, s), 69.2 (2C, s), 119.9 (2C, s), 120.4 (1C, s), 123.6 (1C, s), 127.5 (1C, s), 128.8 (1C, s), 130.6 (1C, s), 131.1 (2C, s), 149.7 (1C, s), 162.8 (2C, s), 164.8 (1C, s).

***N*,*N*′-(6-(4-(4-Fluorophenyl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)*bis*(O-((*E*)-3-chloroallyl)hydroxylamine (6f):** Yield: 75%; m.p.: 129 ºC, Elemental analysis of $C_{19}H_{22}Cl_2N_7O_2$ (m.w.: 470.33); C, 48.52 (48.53); H, 4.71 (4.74); N, 20.85 (20.83); O, 6.80 (6.81); IR (KBr, ν_{max}, cm^{-1}): 3396 (N-H), 3115 (C-H), 1667 (C=C), 1465 (C=N), 1322 (C-F), 1236 (C-N), 742 (C-Cl); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 3.18 (4H, dd, *J* = 17.3, 6.7 Hz), 3.65 (4H, dd, *J* = 13.2, 6.7 Hz), 4.54 (4H, d, *J* = 6.4 Hz), 5.82 (2H, dt, *J* = 14.0, 6.4 Hz), 6.23 (2H, d, *J* = 14.0 Hz), 6.79-6.99 (4H, 6.85 (dd, *J* = 8.1, 1.7 Hz), 6.93 (dd, *J* = 8.1, 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 49.1-49.1 (4C, 49.1 (s), 49.1 (s), 69.2 (2C, s), 115.5 (2C, s), 117.8 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 147.8 (1C, s), 160.2 (1C, s), 162.8 (2C, s), 164.8 (1C, s).

***N*,*N*′-(6-(4-(4-Methoxyphenyl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)*bis*(O-((*E*)-3-chloroallyl)hydroxylamine (6g):** Yield: 83%; m.p.: 112 ºC; Elemental analysis of $C_{20}H_{25}Cl_2N_7O_3$ (m.w.: 482.36): C, 49.80 (49.81); H, 5.22 (5.20); N, 20.33 (20.30); O, 9.95 (9.93); IR (KBr, ν_{max}, cm^{-1}): 3373 (N-H), 3102 (C-H), 1567 (C=C), 1458 (C=N), 1252 (C-N), 740 (C-Cl); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 3.19 (4H, dd, *J* = 17.4, 6.7 Hz), 3.57-3.78 (7H, 3.65 dd, *J* = 13.2, 6.7 Hz), 3.73 (2H, s), 4.54 (4H, d, *J* = 6.4 Hz), 5.82 (2H, dt, *J* = 14.0, 6.4 Hz), 6.23 (2H, d, *J* = 14.0 Hz), 6.76-6.99 (4H, 6.83 (dd, *J* = 8.6, 2.8 Hz), 6.93, dd, *J* = 8.6, 2.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 49.1-49.1 (4C, 49.1 (s), 49.1 (s), 55.3 (1C, s), 69.2 (2C, s), 115.2 (2C, s), 116.1 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 145.6 (1C, s), 159.5 (1C, s), 162.8 (2C, s), 164.8 (1C, s);

***N*,*N*′-(6-(4-(Pyrimidin-2-yl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)*bis*(O-((*E*)-3-chloroallyl)hydroxylamine (6h):** Yield: 71%; m.p.: 109 ºC; Elemental analysis of $C_{17}H_{21}Cl_2N_9O_2$ (m.w.: 454.31): C, 44.94 (44.95); H, 4.66 (4.68); N, 27.75 (27.73); O, 7.04 (7.05); IR (KBr, ν_{max}, cm^{-1}): 3353 (N-H), 3098 (C-H), 1574 (C=C), 1468 (C=N), 1273 (C-N), 744 (C-Cl); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 3.36-3.63 (8H, 3.44, dd, *J* = 15.4, 6.7 Hz), 3.55 (dd, *J* = 15.1, 6.7 Hz), 4.54 (4H, d, *J* = 6.4 Hz), 5.82 (2H, dt, *J* = 14.0, 6.4 Hz), 6.23 (2H, d, *J* = 14.0 Hz), 6.53 (1H, t, *J* = 5.3 Hz), 8.43 (2H, dd, *J* = 5.3, 1.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 49.1-49.1 (4C, 49.1 (s), 49.1 (s), 69.2 (2C, s), 109.7 (1C, s), 119.9 (2C, s), 131.1 (2C, s), 157.6 (2C, s), 161.8 (1C, s), 162.8 (2C, s), 164.8 (1C, s).

Molecular docking studies: Molecular docking was performed using the Glide module (Schrödinger Suite, XP mode) against the crystal structure of human myeloperoxidase (MPO; PDB ID: 1DNU). The protein was prepared using the protein preparation wizard (hydrogen addition, bond order assignment, PROPKA pH 7.4, OPLS4 energy minimisation, RMSD 0.30 Å). Ligands were prepared *via* LigPrep/Epik at pH 7.4 ± 2.0. A receptor grid box (20 Å × 20 Å × 20 Å) was centered on the MPO substrate-binding channel encompassing key residues PHE 29, ARG 27, LEU 97, ASP 96, ASP 98 and HIS 95. Optimal poses were selected based on GScore and interaction analysis; binding free energies were refined by MM-GBSA calculations in Prime. Ascorbic acid served as the reference standard [20-22].

Antioxidant activity: The synthesised compounds **6a-h** were evaluated for their *in vitro* antioxidant activity using standard free radical scavenging assay. The antioxidant potential was assessed by determining the IC_{50} values. All synthesised compounds were tested for their ability to scavenge radicals using the DPPH technique to estimate their antiradical potential. The synthesized compounds (100 µL) were mixed with 2 mL of DPPH solution (12.5 mg/L) and the resulting mixture was incubated at room temperature for 30 min. Methanol was used as the blank and absorbance was recorded at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1900). Ascorbic acid was used as standard and the antioxidant activity of all synthetic compounds was expressed as percentage inhibition using the following formula [23,24].

$$\text{Inhibition of DPPH (\%)} = \frac{A - B}{A} \times 100$$

where A = absorbance of blank and B = absorbance of sample.

RESULTS AND DISCUSSION

A series of novel piperazine-linked 1,3,5-triazine derivatives (**6a-h**) was synthesised following the synthetic route outlined in **Scheme-I**. The synthesis commenced with the preparation of intermediate **2**, which subsequently underwent nucleophilic substitution with cyanuric chloride to afford intermediate **3**. Intermediate **3** was then reacted with O-[(2*E*)-3-chloroprop-2-en-1-yl]hydroxylamine to furnish intermediate **4**, followed by nucleophilic substitution with the corresponding cyclic amines to obtain the target compounds **6a-h**. The final step was carried out in methylbenzene under reflux in the presence of aqueous sodium hydroxide.

All the synthesised compounds were characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopy. In the ^{1}H NMR spectra, the N–H proton attached to the triazine-linked cyclic amine appeared as a broad singlet or in some cases, a broad doublet at δ 5.0-6.5 ppm (1H). Two additional exchangeable N–H protons originating from the allyloxyamine moiety were observed as broad resonances in the δ 7.5-8.5 ppm region (2H). Their assignments were confirmed by D_2O exchange experiments, in which the corresponding resonances disappeared after D_2O addition, confirming their exchangeable nature. The proton integration values are consistent with the proposed structures and account for all non-exchangeable and exchangeable protons.

Antioxidant activity: Among the synthesised compounds, compound **6d** bearing a 2-pyridyl substituent, exhibited moderate antioxidant activity with an IC_{50} value of 17.36 ± 0.09 μM. Compounds **6b**, **6c** and **6e** were less active, affording IC_{50} values between 21 and 23 μM. The phenyl-substituted analogue **6a** exhibited the lowest activity, with an IC_{50} value of 24.91 ± 0.27 μM (Table-1). Structure–activity relationship analysis revealed that electron-withdrawing substituents, particularly at the *para*-position of the aromatic ring, favour free radical scavenging activity. This observation was particularly evident for compound **6f**, which showed the strongest antioxidant activity among the synthesized compounds. The molecular docking study also supported these findings, with compound **6f** providing the most favourable docking score (-4.987 kcal/mol), in agreement with its higher *in vitro* antioxidant activity. The observed activity pattern suggests that the nature and position of substituents, along with the electronic properties of the aromatic ring, play an important role in determining the antioxidant activity of the newly synthesised piperazine-linked 1,3,5-triazine derivatives.

Molecular docking: Molecular docking analysis was carried out to examine the binding behaviour of compounds **6a-h** within the active site of myeloperoxidase (MPO; PDB ID: 1DNU), with ascorbic acid serving as the reference antioxidant. The docking scores together with the main ligand-protein interactions are presented in Fig. 1.

The MPO binding cavity consists of a predominantly hydrophobic pocket formed by PHE 29, VAL 30, TRP 32, LEU 22, LEU 97, PHE 99 and PRO 101. Polar residues, namely ARG 27, ASN 26, SER 25, THR 21 and HIS 95, surround this region, while ASP 94, ASP 96 and ASP 98 contribute negatively charged sites and ARG 27 and ARG 31 provide positively charged centres. All derivatives occupied the MPO binding cavity through varying contributions of hydrophobic interactions, hydrogen bonding and electrostatic contacts. Among the series, compound **6f** exhibit the most favourable docking score (-4.987 kcal/mol). The ligand formed two hydrogen bonds, one with the side chain of ARG 27 and another with the backbone of LEU 97, in addition to extensive hydrophobic contacts with PHE 29, VAL 30, TRP 32, ALA 28, and LEU 22. Additional polar interactions with SER 25, ASN 26, THR 21, and HIS 95 further stabilised the complex. These interactions agreed with its high antioxidant activity (IC_{50} = 14.15 ± 0.14 μM) and a stronger calculated binding affinity than the reference compound, ascorbic acid (-4.690 kcal/mol).

TABLE-1
ANTIOXIDANT ASSAY (FREE RADICAL SCAVENGING EFFECT BY DPPH) OF **6a-h** IN % INHIBITION

Compd.	Substituents (R)	IC_{50} ± SD (μM)[a]	Doc score (kcal/mol)
6a	-C_6H_5	24.91 ± 0.27	–4.277
6b	-2,3-$(CH_3)_2C_6H_3$	21.14 ± 0.19	–4.873
6c	-2-F-C_6H_4	23.19 ± 0.23	–4.531
6d	-2-Pyridine	17.36 ± 0.09	–4.048
6e	-2-Cl-C_6H_4	22.62 ± 0.34	–4.032
6f	-4-F-C_6H_4	14.15 ± 0.14	–4.987
6g	-4-(OCH_3)-C_6H_4	16.36 ± 0.12	–4.662
6h	2-Pyrimidine	15.01 ± 0.14	–3.816
Ascorbic acid	–	14.06 ± 0.18	–4.690

Compounds **6b** (-4.873 kcal/mol) and **6g** (-4.662 kcal/mol) yielded the next most favourable docking scores. Compound **6b** formed a hydrogen bond with the side chain of ARG 27 and interacted with the positively charged residues ARG 27 and ARG 31. Compound **6g** established a side-chain hydrogen bond with SER 25 while maintaining extensive hydrophobic contacts within the binding cavity, particularly with TRP 32. Their interaction patterns were in line with the strong antioxidant activities observed experimentally, although both compounds remained less active than **6f**.

Compound **6c** displayed a docking score of -4.531 kcal/mol and possessed the largest interaction network within the series, involving eleven hydrophobic residues together with a backbone hydrogen bond to PHE 99. Despite this extensive interaction profile, its antioxidant activity was only moderate (IC_{50} = 23.19 μM). The lower activity may be attributed to the steric and electronic effects of the *ortho*-fluoro substituent, which could influence ligand orientation within the binding cavity despite favourable docking interactions.

Compounds **6d** and **6e** recorded docking scores of -4.048 and -4.032 kcal/mol, respectively. Their binding modes were dominated by non-classical interactions rather than extensive hydrogen-bond formation. Compound **6d** formed a halogen bond between chlorine and PHE 29, whereas compound **6e** established two halogen bonds involving GLY 23 and THR 21 together with a backbone hydrogen bond to ARG 27. Although these interactions contributed to stable complex formation, their

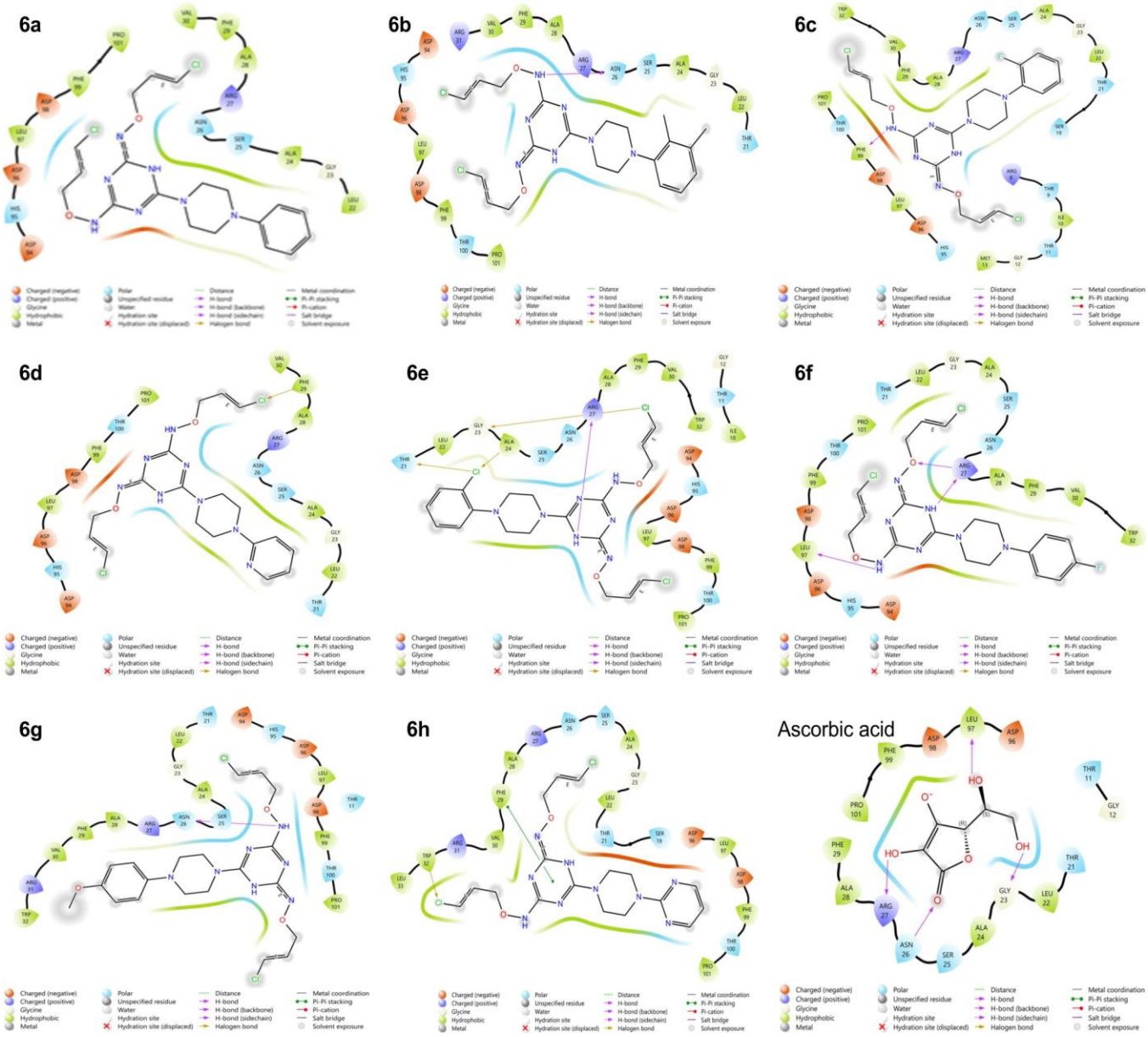


Fig. 1. Docking scores and key interactions of compounds **6a-h** against MPO (1DNU)

antioxidant activities were lower than those observed for the leading compounds in the series.

Compound **6h** showed the least favourable docking score (-3.816 kcal/mol). Despite this relatively weak calculated affinity, the ligand established π-π stacking interactions with PHE 29 together with a halogen bond involving TRP 32. Since such interactions are not always fully represented by docking scores, they may partly explain its comparatively strong antioxidant activity (IC_{50} = 15.01 ± 0.14 μM). In contrast, compound **6a** (-4.277 kcal/mol) did not form hydrogen bonds and relied mainly on hydrophobic interactions within the MPO active site. This limited interaction pattern was associated with its lower antioxidant activity (IC_{50} = 24.91 μM).

Ascorbic acid exhibited a docking score of -4.690 kcal/mol and formed four backbone hydrogen bonds with LEU 97, ARG 27, SER 25 and GLY 23. This interaction profile reflects the highly polar nature of the molecule and its established radical-scavenging behaviour. Although the reference compound formed a larger number of hydrogen bonds than **6f**, the side-chain hydrogen bonds present in **6f** may provide superior directional specificity and stronger stabilisation within the active site.

Comparison of the docking results with the DPPH assay data indicated a close association between binding affinity and antioxidant potency. Derivatives with more favourable docking scores generally produced lower IC_{50} values, with compound **6f** showing the closest agreement between computational and experimental findings. Structural variations also played an important role in determining biological activity. Electron-withdrawing substituents at the *para*-position improved both MPO binding and free-radical scavenging activity, while nitrogen containing heteroaromatic substituents, as present in compounds **6g** and **6h**, were also associated with enhanced antioxidant properties. These findings suggest that substitu-

tion on the terminal aromatic ring is an important factor governing the activity of this series of piperazine-linked 1,3,5-triazine derivatives.

Conclusions

A novel series of piperazine-linked 1,3,5-triazine derivatives (**6a-h**) was rationally designed, synthesised and evaluated for antioxidant activity using molecular docking against myeloperoxidase (MPO; PDB ID: 1DNU) and the DPPH free radical scavenging assay. All derivatives exhibited favourable interactions within the MPO active site through hydrophobic, polar and electrostatic contacts. Compound **6f**, bearing a *para*-fluorophenyl substituent, emerged as the most active derivative, affording the highest binding energy (-4.987 kcal/mol) and the lowest IC_{50} value (14.15 ± 0.14 μM), closely comparable to ascorbic acid (IC_{50} = 14.06 ± 0.18 μM; binding energy = -4.690 kcal/mol). Compounds **6g** and **6h** likewise displayed strong antioxidant activity, supported by favourable hydrogen-bonding, π-π stacking and halogen-bonding interactions. Structure–activity relationship analysis identified *para* electron-withdrawing substituents and nitrogen containing heteroaromatic rings as the structural features associated with enhanced MPO binding and free radical scavenging activity. The agreement between computational predictions and experimental results supports the utility of the piperazine-linked 1,3,5-triazine scaffold as a promising platform for antioxidant drug discovery.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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