

Synthesis, C-N/N-N bond conformational analysis and evaluation of naphtho[2,3-*d*][1,2,3]triazole-4,9-dione tethered *N*-acyl hydrazones as α -amylase inhibitors: Insights from molecular modeling and ADMET analysis

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ABSTRACT

In an effort to expand the repertoire of potent α -amylase inhibitors, we sought to develop novel inhibitors by combining 1,4-naphthoquinone, 1,2,3-triazole, and *N*-acyl hydrazone scaffolds in a single matrix. To achieve this, twelve novel naphtho[2,3-*d*][1,2,3]triazole-4,9-dione tethered *N*-acyl hydrazones were synthesized through condensation reaction of 2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-1-yl)acetohydrazide with various substituted aryl aldehydes. Structural elucidation for all the compounds was performed using 1D, 2D-NMR, FTIR, and mass spectral analyses. The synthesized molecules were evaluated for their ability to inhibit α -amylase activity using acarbose as the standard drug. All the derivatives exhibited potent inhibition of α -amylase, with IC₅₀ values ranging between 17.26 $\pm$ 0.07 to 25.62 $\pm$ 0.03 μ g/mL. Notably, compound **9c** possessing *-meta* substituted -NO₂ group displayed the highest activity (IC₅₀ = 17.26 $\pm$ 0.07 μ g/mL) among the series. Structure-activity relationship (SAR) revealed the pivotal role of aryl ring substitutions in determining inhibitory efficacy. To validate these findings and to assess the binding stability of **9c** within the catalytic site α -amylase derived for *A. oryzae* (PDB ID: 7TAA), *in silico* studies were performed. The compound **9c** effectively occupies the enzyme's active pocket, with minimal RMSD fluctuations observed over 100 ns simulation, indicating stable protein-ligand complex. ADMET predictions suggested favorable drug-like properties, underscoring the potential of these compounds as novel α -amylase inhibitors for managing type 2 diabetes mellitus

1. Introduction

Diabetes mellitus (DM) is a widespread, potentially life-threatening disease affecting about 537 million people globally in 2021. The International Diabetes Federation (IDF) projects this number to rise to 783 million by 2045 [1,2]. This disorder is caused by persistently high blood glucose due to inadequate insulin action or secretion, influenced by both genetic and environmental factors [3]. Insulin, a peptide hormone produced by the pancreas, helps the body in sugar utilisation for meeting energy demands. When the pancreas produces insufficient insulin or the

body can't use it effectively, blood sugar levels rise [4,5]. Dysregulation in insulin production or use disrupts carbohydrate, fat, and protein metabolism, leading to persistently high blood sugar and potential hyperglycemia. Without sufficient insulin, glucose cannot enter the cells, accumulating in the bloodstream and potentially causing diabetes [6,7]. DM encompasses several types, including type-1 (insulin-dependent), type-2 (non-insulin-dependent), type-3c (secondary or pancreatogenic diabetes), gestational (diabetes in pregnant ladies), and prediabetes [8–10]. Amongst, type-2 DM, affecting 90–95 % of diabetic patients, represents a significant global health burden [11,12], associated with

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severe complications such as blurred vision, polydipsia, nephrotoxicity, hypertension, renal syndrome, and cardiovascular diseases [13–16].

Considering the chronic nature of diabetes, proper management strategies for the treatment of DM have become a major global health challenge in recent years. Enzymes that regulate gluconeogenesis and glycogenolysis are critical targets for therapeutic interventions [17]. Literature revealed that strategies for managing the absorption and maintenance of glucose level largely relies on the inhibition of hydrolytic enzymes, specifically α -amylase and α -glucosidase [18–22]. There are many commercially available inhibitors of these enzymes like acarbose, miglitol, and voglibose. However, despite their efficacy, these inhibitors often cause gastrointestinal issues such as diarrhea, abdominal pain, flatulence, stomach distress, nausea, indigestion, and hepatotoxicity [23–25]. To address these challenges, the molecular hybridization, which combines multiple structures into a single framework, has gained importance in developing compounds with multi-target biological potential [26]. In this scenario, heterocyclic compounds exhibit a strong affinity for a widespread array of biological targets. Among these, the 1,2,3-triazole framework stands out in medicinal chemistry for its diverse range of biological activities. This structural motif has demonstrated significant potential as an anti-diabetic, anti-cancer, anti-malarial, anti-oxidant, and anti-tubercular agent [27–31]. Recent studies have explored the potential of naturally occurring naphthoquinones, such as plumbagin and lawsone, as inhibitors of enzymes involved in carbohydrate metabolism, including α -amylase and glucosidase [32–34]. The compound rhinacanthin-C, containing 1,4-naphthoquinone, demonstrated beneficial effects in nicotinamide-streptozotocin-induced diabetic rats, alleviating hypolipidemic, hypoglycemic, and pancreatic dysfunction [35, 36]. Many synthetically derived compounds featuring a 1,4-naphthoquinone skeleton have demonstrated notable potential as inhibitors of α -amylase [37–39]. Furthermore, *N*-acyl hydrazones have also shown potent inhibitory activity against α -amylase [40–44]. Researchers have continuously studied and explored these structural motifs in numerous investigations to regulate the α -amylase inhibitory activity [45–48].

In the quest to develop safer and more successful therapeutics, *in silico* practices such as molecular docking, molecular dynamics (MD) simulations, and Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) analysis, have revolutionized modern drug design and discovery by leveraging computational tools and simulations to

rationalize and enhance the drug development process. Docking studies offer critical insights into the specific binding sites, and strength of the interaction and visualize how a small molecule interacts with its target protein that is involved in insulin signaling pathways and glucose metabolism [49,50]. Meanwhile, MD simulations provide a dynamic perspective on ligand-receptor complexes, revealing conformational fluctuations and inter-molecular interactions over time [51,52]. In later stages of drug development, ADMET studies are crucial for prioritizing the compounds with favorable pharmacokinetic profiles, overall effectiveness and safety while minimizing the adverse side effects and reducing the possibility of late-stage failures [53].

Based on our previous research exploring naphtho[2,3-*d*]imidazole-4,9-dione linked *N*-acyl hydrazones for their α -amylase inhibition [41] and driven by our goal to broaden the chemical diversity of α -amylase inhibitors [54–61], we have designed a novel target structure. This structure integrates 1,4-naphthoquinone, 1,2,3-triazole, and an *N*-acyl hydrazone backbone using a molecular hybridization strategy (Fig. 1). The present study outlines the design rationale, synthetic approach, and spectral evaluation of naphtho[2,3-*d*][1,2,3]triazole-4,9-dione tethered *N*-acyl hydrazone derivatives, along with their *in vitro* and *in silico* evaluations as α -amylase inhibitors. These findings contribute to the development of therapeutic strategies aimed at managing glucose metabolism.

2. Experimental

2.1. Materials and methods

The chemicals used in the synthesis and biological assay were sourced from Avra, Sigma Aldrich and Merck chemical companies, ensuring purity levels exceeding 95 %. Melting points of all compounds were determined on a Popular India digital melting point tool taking compounds in open capillaries, and the outcomes were reported without correction. Thin layer chromatography (TLC) was conducted on Aluminium TLC plates coated with 60-F₂₅₄ silica gel obtained from Merck, and the plates were visualized under UV light for analysis.

NMR spectra were recorded in deuterated DMSO ((CD₃)₂SO) solvent using tetramethylsilane (TMS) as an internal standard, on a Jeol Delta NMR instrument. Chemical shifts (δ) and coupling constants (*J*) were reported in parts per million (ppm) and Hertz (Hz), respectively.

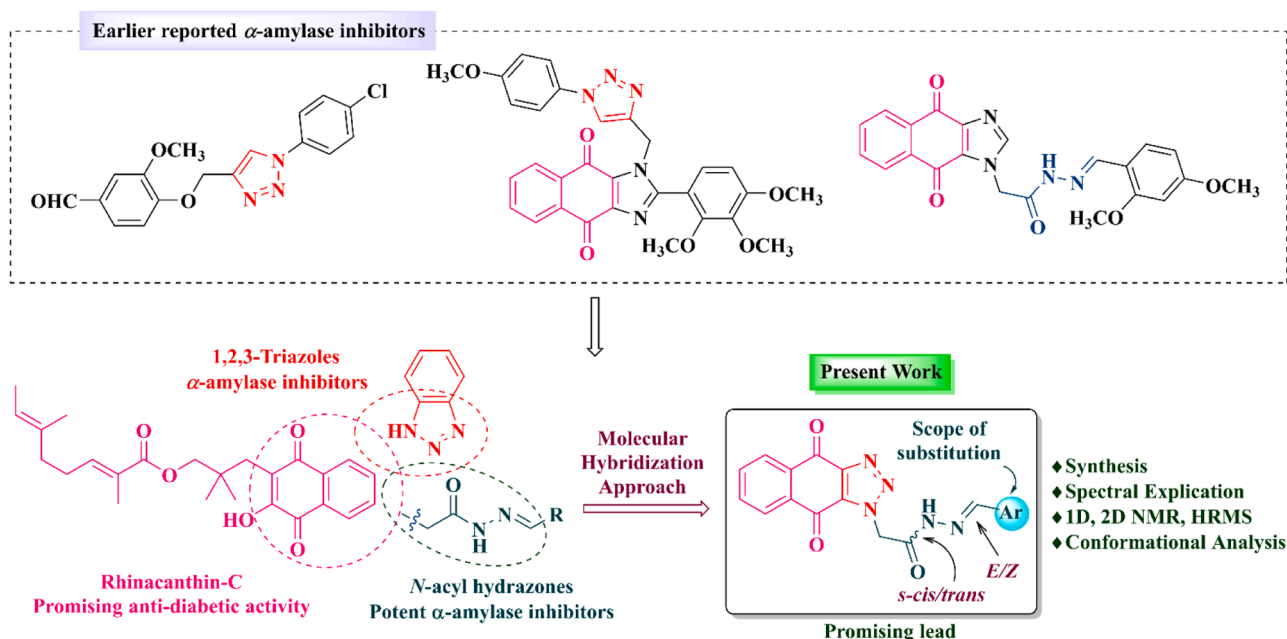


Fig. 1. Design rationale for naphtho[2,3-*d*][1,2,3]triazole-4,9-dione tethered *N*-acyl hydrazones.

Infrared (IR) absorption spectra were acquired using a Perkin Elmer FTIR spectrophotometer, and the resultant values were presented as $\nu_{\max}$ values (cm^{-1}).

High-resolution mass spectrometry (HRMS) analysis was performed using an LCMS system on the SCIEX Triple TOF 5600+, operating in positive scan mode with a resolution of 120,000 FWHM (Full Width at Half Maximum), covering a mass range of 100–1000 m/z . This comprehensive analytical approach ensured accurate characterization and validation of the synthesized compounds and their properties.

2.2. Synthesis and characterization

2.2.1. Synthetic procedure for 2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (7)

Firstly, 2,3-diamino-1,4-naphthoquinone (1.00 mmol) (1) was treated with aqueous sodium nitrite (1.2 mmol) (2) in glacial acetic acid under cold conditions. This reaction yielded light brown crystalline material, which was characterised as 1H-naphtho[2,3-d][1,2,3]triazole-4,9-dione (3). Subsequently, compound 3 (1.0 mmol) was alkylated at the N—H site of the triazolyl group with ethyl bromoacetate (4) (1.2 mmol) using K_2CO_3 as a base in acetone at room temperature. The reaction was examined by TLC (pet ether: ethyl acetate (60:40, v/v)). After 2 h, the reaction was quenched by adding its content to an ice-water mixture. The resulting solution was neutralized with dilute acetic acid. The solid product (compound 5) was isolated by filtration, thoroughly washed with an ice-water mixture, and then recrystallized using methanol and THF (1:1, v/v) mixture to afford the purified compound.

Further, a mixture of compound 5 (1.0 mmol) and hydrazine hydrate (6, 1.1 equivalents) in ethanol was refluxed for 6 h. This reaction yielded 2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (7) in 85 % yield. The reaction progress was monitored by TLC (petroleum ether: ethyl acetate; (40:60, v/v)) at different intervals. Upon complete consumption of reactants, the mixture was cooled and transferred into an ice-water mixture, resulting into greenish-yellow precipitates. The precipitated solid (compound 7) was separated by filtration, thoroughly washed with copious amounts of cold water, and subsequently rinsed with chilled ethanol to ensure purity. After these purification steps, the purified compound 7 was obtained.

1H-Naphtho[2,3-d][1,2,3]triazole-4,9-dione (3)

Dark brown solid; Yield: 88 %; M.pt.: > 320°C; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$): δ 14.05 (s, 1H, -NH), 8.04–7.98 (m, 2H, $\text{C}_{5/8}$ -H), 7.80–7.75 (m, 2H, $\text{C}_{6/7}$ -H); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): δ 179.95, 144.34, 134.10, 132.26, 126.56; Elemental Analysis: $\text{C}_{10}\text{H}_5\text{N}_3\text{O}_2$ (199.169) Calc.: C, 66.31; H, 2.53; N, 21.10 %; Found: C, 66.29; H, 2.52; N, 21.12 %.

Ethyl 2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetate (5)

Yellow solid; Yield: 89 %; M.pt.: 137.8–139.5°C; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$): δ 8.11–8.07 (m, 2H, $\text{C}_{5/8}$ -H), 7.99–7.91 (m, 2H, $\text{C}_{6/7}$ -H), 5.89 (s, 2H, -CH₂, C_{14} -H), 4.26 (q, J = 7.2 Hz, 2H, -CH₂, C_{17} -H), 1.22 (t, J = 7.2 Hz, 3H, -CH₃, C_{18} -H); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): δ 177.79, 167.87, 147.52, 134.98, 133.42, 129.26, 62.18, 49.32, 16.12; Elemental Analysis: $\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_4$ (285.259) Calc.: C, 58.95; H, 3.89; N, 14.73 %; Found: C, 58.96; H, 3.87; N, 14.74 %.

2-(4,9-Dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (7)

Greenish yellow solid; Yield: 85 %; M.pt.: > 320°C; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$): δ 9.16 (s, 1H, -NH, 84.26 %), 8.87 (s, 1H, -NH, 15.74 %), 8.08–8.01 (m, 2H, $\text{C}_{5/8}$ -H), 7.87–7.79 (m, 2H, $\text{C}_{6/7}$ -H), 5.70 (s, 2H, -CH₂, C_{14} -H, 16.08 %), 5.07 (s, 2H, -CH₂, C_{14} -H, 83.96 %), 4.61 (s, 2H, -NH₂, 16.57 %), 4.57 (s, 2H, -NH₂, 83.43 %); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): δ 177.69, 163.92, 146.93, 135.11, 133.24, 127.79, 48.21; Elemental Analysis: $\text{C}_{12}\text{H}_9\text{N}_5\text{O}_4$ (287.235) Calc.: C, 50.18; H, 3.16; N, 24.38 %; Found: C, 50.16; H, 3.19; N, 24.39 %.

2.2.2. Synthesis of (E/Z)-N'-(arylbenzylidene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazides (9a-9l)

To prepare compounds (9a-9l), an ethanolic solution of compound 7 (1.0 mmol) and substituted aryl or heteroaryl aldehydes (8a-8l) (1.0 mmol) was stirred using dilute acetic acid as catalyst at 70 °C for 6–8 h. The progress of the reaction was monitored by TLC (petroleum ether and ethyl acetate (40:60, v/v)). Upon completion of the reaction, as indicated by TLC, the mixture was poured into an ice-water mixture. The resulting solid product was then filtered, rinsed with copious amounts of cold water, and dried in a desiccator to afford the crude product (9a-9l). Finally, the crude product was recrystallized from a 1:2 (v/v) mixture of dimethyl sulfoxide (DMSO) and methanol (MeOH) to afford the purified final product (9a-9l).

(E/Z)-N'-(4-Methoxybenzylidene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9a)

Cream solid; Yield: 89 %; M.pt.: 286.3–289.1°C; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$): δ 11.88 (s, 1H, -NH, 14.81 %), 11.85 (s, 1H, -NH, 85.19 %), 8.20–8.15 (m, 2H, $\text{C}_{5/8}$ -H), 7.97 (s, 1H, -CH, C_{18} -H), 7.94–7.90 (m, 2H, $\text{C}_{6/7}$ -H), 7.68–7.62 (m, 2H, $\text{C}_{20/24}$ -H), 6.99–6.65 (m, 2H, $\text{C}_{21/23}$ -H), 6.05 (s, 2H, -CH₂, 81.28 %), 5.57 (s, 2H, -CH₂, 18.72 %), 3.76 (s, 3H, OCH₃, C_{25} -H); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): δ 176.53, 167.86, 161.42, 146.84, 144.92, 143.49, 134.88, 134.47, 133.30, 132.94, 129.13, 127.16, 126.97, 126.59, 114.91, 55.87; Elemental Analysis: $\text{C}_{20}\text{H}_{15}\text{N}_5\text{O}_4$ (389.371) Calc.: C, 61.69; H, 3.88; N, 17.99 %; Found: C, 61.68; H, 3.85; N, 17.99 %.

(E/Z)-N'-(4-Dimethylamino)benzylidene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9b)

Brick red solid; Yield: 79 %; M.pt.: 272.6–274.7°C; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$): δ 11.69 (s, 1H, -NH, 20.21 %), 11.66 (s, 1H, -NH, 79.79 %), 8.21–8.14 (m, 2H, $\text{C}_{5/8}$ -H), 8.07 (s, 1H, -CH, C_{18} -H, 21.05 %), 7.94–7.90 (m, 2H, $\text{C}_{6/7}$ -H), 7.89 (s, 1H, -CH, C_{18} -H, 78.95 %), 7.53–7.46 (m, 2H, $\text{C}_{20/24}$ -H), 6.72–6.65 (m, 2H, $\text{C}_{21/23}$ -H), 6.01 (s, 2H, -CH₂, 77.13 %), 5.54 (s, 2H, -CH₂, 22.87 %), 2.93 (s, 6H, -CH₃, $\text{C}_{25/26}$ -H); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): δ 177.53, 166.12, 156.88, 152.09, 146.31, 145.95, 142.83, 135.25, 134.32, 129.23, 128.99, 127.58, 121.56, 112.24, 57.93; Elemental Analysis: $\text{C}_{21}\text{H}_{18}\text{N}_6\text{O}_3$ (402.414) Calc.: C, 62.68; H, 4.51; N, 20.88 %; Found: C, 62.66; H, 4.48; N, 20.88 %.

(E/Z)-N'-(3-Nitrobenzylidene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9c)

Yellow solid; Yield: 78 %; M.pt.: 291.8–293.3°C; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$): δ 12.24 (s, 1H, -NH, 21.43 %), 12.18 (s, 1H, -NH, 78.57 %), 8.54 (t, J = 8.8 Hz, 1H, C_{22} -H, 77.78 %), 8.52 (t, J = 8.8 Hz, 1H, C_{22} -H, 22.22 %), 8.37 (s, 1H, -CH, C_{18} -H, 22 %), 8.30 (d, J = 8.1 Hz, 1H, C_{20} -H), 8.26–8.12 (m, 4H, $\text{C}_{5/8/18/24}$ -H, 78 %), 7.75–7.65 (m, 2H, $\text{C}_{6/7}$ -H), 7.50 (t, J = 7.7 Hz, 1H, C_{21} -H), 6.16 (s, 2H, -CH₂, 79.18 %), 5.67 (s, 2H, -CH₂, 20.82 %); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): δ 176.97, 167.59, 148.83, 143.26, 140.49, 137.02, 136.72, 136.21, 133.90, 130.92, 130.35, 127.54, 127.01, 124.98, 123.35, 122.23, 122.00, 57.68; Elemental Analysis: $\text{C}_{19}\text{H}_{12}\text{N}_6\text{O}_5$ (404.342) Calc.: C, 56.44; H, 2.99; N, 20.78 %; Found: C, 56.42; H, 2.97; N, 20.79 %.

(E/Z)-N'-(2,3,4-Trimethoxybenzylidene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9d)

Pale yellow solid; Yield: 84 %; M.pt.: 255.3–257.7°C; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$): δ 11.96 (s, 1H, -NH, 19.79 %), 11.83 (s, 1H, -NH, 80.21 %), 8.38 (s, 1H, -CH, 22.22 %), 8.20 (s, 1H, -CH, 77.78 %), 8.18–8.13 (m, 2H, $\text{C}_{5/8}$ -H), 7.93–7.87 (m, 2H, $\text{C}_{6/7}$ -H), 7.60 (d, J = 8.9 Hz, 1H, C_{20} -H, 23.30 %), 7.52 (d, J = 8.9 Hz, 1H, C_{20} -H, 76.70 %), 6.88 (d, J = 9.0 Hz, 1H, C_{21} -H, 22.77 %), 6.85 (d, J = 9.0 Hz, 1H, C_{21} -H, 77.33 %), 6.04 (s, 2H, -CH₂, 80 %), 5.56 (s, 2H, -CH₂, 20 %), 3.82 (s, 6H, -OCH₃, $\text{C}_{25/27}$ -H, 16.66 %), 3.81–3.79 (m, 6H, -OCH₃, $\text{C}_{25/27}$ -H, 83.34 %), 3.73 (s, 3H, -OCH₃, C_{26} -H, 16.62 %), 3.72 (s, 3H, -OCH₃, C_{26} -H, 83.38 %); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): δ 177.54, 166.54, 156.04, 155.82, 153.25, 153.13, 146.02, 145.97, 144.55, 142.00, 141.32, 135.26, 134.31, 131.73, 127.58, 121.39, 121.25, 120.37, 109.15, 62.31, 61.02, 57.96, 56.55; Elemental Analysis: $\text{C}_{22}\text{H}_{19}\text{N}_5\text{O}_6$ (449.423) Calc.:

C, 58.80; H, 4.26; N, 15.58 %; Found: C, 58.78; H, 4.23; N, 15.59 %.

(E/Z)-N'-Benzylidene-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9e)

Off-white solid; Yield: 83 %; M.pt.: 301.5–303.8 °C; ¹H NMR (400 MHz, (CD₃)₂SO): δ 12.00 (s, 1H, -NH, 18.28 %), 11.97 (s, 1H, -NH, 81.78 %), 8.24 (s, 1H, -CH, C₁₈-H, 21.21 %), 8.19–8.14 (m, 2H, C_{5/8}-H), 8.03 (s, 1H, -CH, C₁₈-H, 78.79 %), 7.93–7.87 (m, 2H, C_{6/7}-H), 7.75–7.67 (m, 2H, C_{20/24}-H), 7.45–7.37 (m, 3H, C_{21/22/23}-H), 6.08 (s, 2H, -CH₂, 80.83 %), 5.61 (s, 2H, -CH₂, 19.17 %); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 177.52, 166.88, 161.38, 149.06, 145.99, 145.50, 135.25, 134.30, 130.98, 130.76, 129.33, 127.86, 127.70, 127.58, 58.30, 57.98; **Elemental Analysis:** C₁₉H₁₃N₅O₃ (359.345) Calc.: C, 63.51; H, 3.65; N, 19.49 %; Found: C, 63.49; H, 3.62; N, 19.49 %.

(E/Z)-N'-(2-Chlorobenzylidene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9f)

Cream solid; Yield: 84 %; M.pt.: 275.8–278.1 °C; ¹H NMR (400 MHz, (CD₃)₂SO): δ 12.25 (s, 1H, -NH, 16.49 %), 12.13 (s, 1H, -NH, 83.51 %), 8.60 (s, 1H, -CH, C₁₈-H 18.75 %), 8.39 (s, 1H, -CH, C₁₈-H, 81.25 %), 8.19–8.13 (m, 2H, C_{5/8}-H), 8.04 (dd, *J* = 7.6, 1.9 Hz, 1H, C₂₀-H), 7.93–7.88 (m, 2H, C_{6/7}-H), 7.51–7.45 (m, 1H, C₂₂-H), 7.43–7.34 (m, 2H, C_{21/23}-H), 6.11 (s, 2H, -CH₂, 82.91 %), 5.62 (s, 2H, -CH₂, 17.09 %); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 177.51, 167.11, 161.62, 145.99, 144.97, 141.48, 135.26, 134.28, 133.89, 133.67, 132.47, 132.20, 131.53, 130.42, 128.27, 128.10, 127.79, 127.58, 58.27, 58.00; **Elemental Analysis:** C₁₉H₁₂ClN₅O₃ (393.787) Calc.: C, 57.95; H, 3.07; N, 17.78 %; Found: C, 58.00; H, 3.05; N, 17.81 %; **ESI-MS:** 394.07 [*M* + *H*]⁺.

(E/Z)-N'-(2,5-Dimethoxybenzylidene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9g)

Pale yellow solid; Yield: 84 %; M.pt.: 298.2–301.4 °C; ¹H NMR (400 MHz, (CD₃)₂SO): δ 12.02 (s, 1H, -NH, 15.62 %), 11.91 (s, 1H, -NH, 84.38 %), 8.52 (s, 1H, -CH, C₁₈-H, 17.35 %), 8.30 (s, 1H, -CH, C₁₈-H, 82.65 %), 8.19–8.12 (m, 2H, C_{5/8}-H), 7.93–7.87 (m, 2H, C_{6/7}-H), 7.39 (d, *J* = 2.9 Hz, 1H, C₂₃-H, 82.83 %), 7.02–7.91 (m, 2H, C_{21/22}-H), 6.09 (s, 2H, -CH₂, 83.92 %), 5.57 (s, 2H, -CH₂, 16.08 %), 3.77 (s, 3H, -OCH₃, C₂₅-H, 17.55 %), 3.75 (s, 3H, -OCH₃, C₂₅-H, 82.45 %), 3.70 (s, 3H, -OCH₃, C₂₆-H, 82.08 %), 3.68 (s, 3H, -OCH₃, C₂₆-H, 17.92 %); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 177.49, 166.84, 153.74, 152.72, 145.95, 144.36, 140.84, 135.22, 134.28, 127.56, 122.78, 118.49, 118.61, 113.72, 110.34, 109.72, 58.30, 58.07, 56.80, 56.69, 56.02; **Elemental Analysis:** C₂₁H₁₇N₅O₅ (419.397) Calc.: C, 60.14; H, 4.09; N, 16.70 %; Found: C, 60.12; H, 4.06; N, 16.70 %.

(E/Z)-N'-(2,4-Dichlorobenzylidene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9h)

Cream solid; Yield: 86 %; M.pt.: 305.7–308.2 °C; ¹H NMR (400 MHz, (CD₃)₂SO): δ 12.20 (s, 1H, -NH, 100 %), 8.55 (s, 1H, -CH, C₁₈-H, 18 %), 8.34 (s, 1H, -CH, C₁₈-H, 82 %), 8.19–8.14 (m, 2H, C_{5/8}-H), 8.07 (d, *J* = 8.6 Hz, 1H, C₂₀-H, 82 %), 7.94–7.89 (m, 3H, C_{6/7/20}-H, 18 %), 7.69 (d, *J* = 2.1 Hz, 1H, C₂₁-H, 22.22 %), 7.68 (d, *J* = 2.1 Hz, 1H, C₂₁-H, 77.88 %), 7.49–7.44 (m, 1H, C₂₃-H), 6.12 (s, 2H, -CH₂, 82.74 %), 5.63 (s, 2H, -CH₂, 17.26 %); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 177.52, 167.19, 146.02, 140.42, 135.83, 135.26, 134.39, 134.31, 130.69, 129.91, 129.02, 128.45, 127.57, 58.28, 57.99; **Elemental Analysis:** C₁₉H₁₁Cl₂N₅O₃ (428.229) Calc.: C, 53.29; H, 2.59; N, 16.35 %; Found: C, 53.24; H, 2.57; N, 16.35 %.

(E)-N'-(4-Fluorobenzylidene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9i)

Bright yellow solid; Yield: 83 %; M.pt.: 312.8–315.1 °C; ¹H NMR (400 MHz, (CD₃)₂SO): δ 12.02 (s, 1H, -NH, 20 %), δ 11.98 (s, 1H, -NH, 80 %), 8.23 (s, 1H, -CH, 20.79 %, C₁₈-H), 8.20–8.12 (m, 2H, C_{5/8}-H), 8.02 (s, 1H, -CH, 79.21 %, C₁₈-H), 7.94–7.87 (m, 2H, C_{6/7}-H), 7.82–7.73 (m, 2H, C_{20/24}-H), 7.28–7.21 (m, 2H, C_{21/23}-H), 6.09 (s, 2H, -CH₂, 81 %), 5.60 (s, 2H, -CH₂, 19 %); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 177.54, 166.93, 164.96, 162.49, 147.95, 145.99, 144.35, 135.27, 134.31, 130.96, 129.99, 129.90, 127.59, 116.51, 116.29, 57.97; **Elemental Analysis:** C₁₉H₁₂FN₅O₃ (377.335) Calc.: C, 60.48; H, 3.21;

N, 18.56 %; Found: C, 60.42; H, 3.18; N, 18.55 %.

(E/Z)-N'-(Pyridin-2-ylmethylene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9j)

Grey solid; Yield: 83 %; M.pt.: 280.4–283.7 °C; ¹H NMR (400 MHz, (CD₃)₂SO): δ 12.23 (s, 1H, -NH, 20 %), 12.17 (s, 1H, -NH, 80 %), 8.60–8.54 (m, 1H, C₂₃-H), 8.23 (s, 1H, -CH, C₁₈-H, 19 %), 8.19–8.14 (m, 2H, C_{5/8}-H), 8.05 (s, 1H, -CH, C₁₈-H, 81 %), 8.02 (d, *J* = 8.0 Hz, 1H, C₂₀-H, 82 %), 7.93–7.88 (m, 3H, C_{6/7/20}-H, 18 %), 7.84 (td, *J* = 7.7, 1.9 Hz, 1H, C₂₁-H), 7.41–7.36 (m, 1H, C₂₂-H), 6.13 (s, 2H, -CH₂, 82.32 %), 5.65 (s, 2H, -CH₂, 17.68 %); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 177.54, 166.93, 164.96, 162.49, 147.95, 145.99, 144.35, 135.27, 134.31, 130.96, 129.99, 129.90, 127.59, 116.51, 116.29, 57.97; **Elemental Analysis:** C₁₈H₁₂N₆O₃ (360.333) Calc.: C, 60.00; H, 3.36; N, 23.32 %; Found: C, 60.01; H, 3.35; N, 23.30 %.

(E/Z)-N'-(Pyridin-4-ylmethylene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9k)

Off white solid; Yield: 85 %; M.pt.: 286.4–289.1 °C; ¹H NMR (400 MHz, (CD₃)₂SO): δ 12.31 (s, 1H, -NH, 23.40 %), 12.28 (s, 1H, -NH, 76.60 %), 8.66 (d, *J* = 5.6 Hz, 2H, C_{21/23}-H), 8.28 (s, 1H, -CH, C₁₈-H, 19.56 %), 8.26–8.18 (m, 2H, C_{5/8}-H), 8.07 (s, 1H, -CH, 81.44 %), 8.00–7.91 (m, 2H, C_{6/7}-H), 7.74 (d, *J* = 5.2 Hz, 2H, C_{20/24}-H, 82.41 %), 7.68 (d, *J* = 5.2 Hz, 2H, C_{20/24}-H, 17.59 %), 6.18 (s, 2H, -CH₂, 84.02 %), 5.75 (s, 2H, -CH₂, 15.98 %); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 177.58, 167.46, 150.82, 146.09, 143.14, 141.51, 135.32, 134.38, 127.64, 121.67, 58.03; **Elemental Analysis:** C₁₈H₁₂N₆O₃ (360.333) Calc.: C, 60.00; H, 3.36; N, 23.32 %; Found: C, 60.01; H, 3.35; N, 23.30 %.

(E/Z)-N'-(2,6-Dichlorobenzylidene)-2-(4,9-dioxo-4,9-dihydro-1H-naphtho[2,3-d][1,2,3]triazol-1-yl)acetohydrazide (9l)

Cream solid; Yield: 84 %; M.pt.: 300.5–303.7 °C; ¹H NMR (400 MHz, (CD₃)₂SO): δ 12.30 (s, 1H, -NH, 15.96 %), 12.24 (s, 1H, -NH, 84.04 %), 8.42 (s, 1H, -CH, C₁₈-H, 15 %), 8.32–8.27 (m, 3H, -CH, C_{5/8/18}-H), 8.20–8.13 (m, 2H, C_{5/8}-H, 89.5 %), 7.95–7.87 (m, 2H, C_{6/7}-H, 88.11 %), 7.73–7.67 (m, 2H, C_{6/7}-H, 11.89 %), 7.56–7.47 (m, 2H, C_{21/23}-H), 7.44–7.37 (m, 1H, C₂₂-H), 5.97 (s, 2H, -CH₂, 87.30 %), 5.66 (s, 2H, -CH₂, 12.70 %); ¹³C NMR (100 MHz, (CD₃)₂SO): δ 177.51, 167.10, 146.02, 140.71, 135.28, 134.61, 134.31, 132.06, 131.90, 129.98, 129.80, 129.59, 127.60, 57.89; **Elemental Analysis:** C₁₉H₁₁Cl₂N₅O₃ (428.229) Calc.: C, 53.29; H, 2.59; N, 16.35 %; Found: C, 53.24; H, 2.57; N, 16.35 %.

2.3. α-Amylase inhibition assay

The synthesized compounds were evaluated for their α-amylase inhibition potential using a modified DNSA (3,5-dinitrosalicylic acid) assay procedure [54–58]. This assay was conducted on a Systronics spectrophotometer 169. Acarbose, a commercially available drug was used as the reference. The solution without the test sample served as the control. Detailed steps for conducting inhibition studies are provided in the supplementary data.

All experiments were executed in triplicate to validate the consistency of the findings, and the results were calculated as a percentage of inhibition using the following equation;

$$\% \text{ Inhibition} = (A_{\text{Control}} - A_{\text{Test}}) / A_{\text{Control}} \times 100$$

Here, A_{Control} stands for the absorbance of the control solution and A_{Test} refers to the absorbance value of the test sample. The % inhibition data, obtained from triplicate experiments, was used to determine the IC₅₀ values. IC₅₀ represents the concentration of the compound that inhibits 50 % of α-amylase activity and was calculated in μg/mL.

2.4. Molecular docking

In our current study, we utilized the iGEMDOCKv2.1 (<http://gemdock.life.nctu.edu.tw/dock>) for docking simulations to explore binding interactions between synthesized compounds and the biological

receptor *A. oryzae* α -amylase (PDB ID: 7TAA). The 3D structure of the target receptor was retrieved from the RCSB Protein Data Bank (<http://www.rcsb.org/>), while ligand structures were built and optimized using ChemAxon's Marvin Sketch software (<http://www.chemaxon.com>). The types of interactions for the best-docked poses were visualised using DS visualizer [62], allowing for a detailed examination of key interactions between ligands and the receptor. A detailed, step-by-step procedure for the complete docking process is provided in the supplementary data.

2.5. Molecular dynamics

A 100 ns molecular dynamics (MD) simulation was conducted using Schrodinger's Desmond 2019-4 (<https://www.schrodinger.com/products/desmond>) molecular dynamics system to study the docking complexes of the most active and least active derivatives with the target protein *A. oryzae* α -amylase (PDB: 7TAA) [63]. The acquired trajectory data was investigated using the Maestro graphical interface [64] to examine the dynamics and interactions of the docked complexes over the simulation period. A detailed procedure for the MD simulation process is provided in the supplementary data.

2.6. ADMET analysis

A web-based tool, SwissADME (<http://www.swissadme.ch/>), was employed to predict the ADME parameters of the synthesized molecules [65]. This tool provides valuable insights into several critical aspects of a compound's pharmacokinetic properties, which are essential for drug development including passive GI absorption, permeability and blood-brain barrier (BBB) permeability through BOILED-Egg diagram, plasma protein binding, inhibition of Cytochrome P₄₅₀, and predictions of P-glycoprotein (P-gp) substrate. The toxicity endpoints were predicted using the ProTox-3.0 web tool (<https://tox.charite.de/protox3/>).

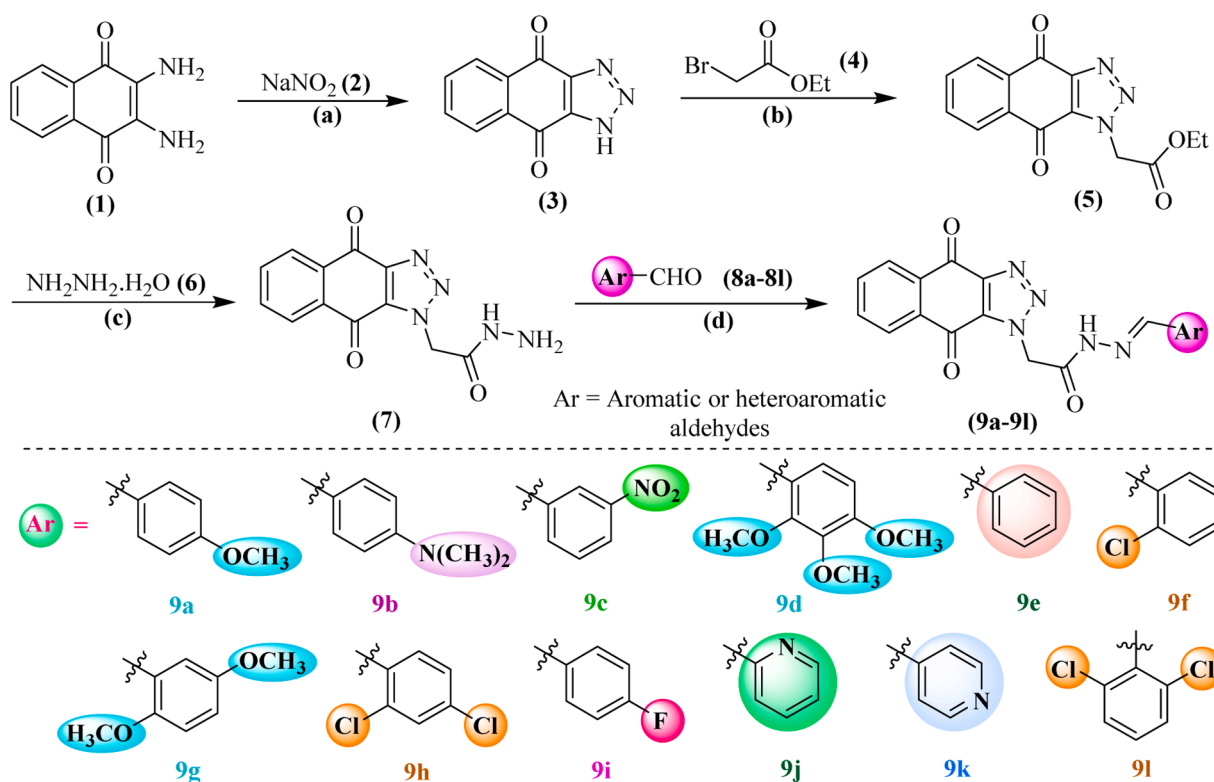
3. Results and discussion

3.1. Chemistry

The complete synthetic procedure followed for the development of targeted molecules (*E/Z*)-*N'*-(arylbenzylidene)-2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-1-yl)acetohydrazide derivatives (9a-9l) is outlined in Scheme 1.

The designed naphtho[2,3-*d*][1,2,3]triazole-4,9-dione *N*-acyl hydrazones (9a-9l) were synthesized from 2,3-diamino-1,4-naphthoquinone (1). Compound 1 was synthesized from 2,3-dichloro-1,4-naphthoquinone using previously described method [66–68]. 2,3-Diamino-1,4-naphthoquinone (1) was reacted with aqueous sodium nitrite (2) in glacial acetic acid under cold conditions. The reaction mixture was poured over ice-cold water, the precipitated solid was collected in buchner funnel by suction and washed with plenty of water to obtain 1*H*-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione (3). The next step involved the alkylation of compound 3 at the N–H of the 1,2,3-triazole moiety with ethyl bromoacetate (4) in the presence of K₂CO₃ at normal temperature. Following this, the compound was reacted with hydrazine hydrate (6) to yield 2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-1-yl)acetohydrazide (7). The final step involved the condensation of compound 7 with various aromatic or heteroaromatic aldehydes (8a-8l) in ethanol, using a catalytic amount of acetic acid and heating at 60 °C. Upon completion of the reaction, as indicated by TLC (petroleum ether: ethyl acetate; 40:60, v/v), the contents of the round-bottom flask were poured over crushed ice. The resulting product was isolated by filtration, thoroughly rinsed with water and then with cold ethanol to afford pure *N'*-(arylbenzylidene)-2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-1-yl)acetohydrazide derivatives (9a-9l) in moderate to excellent yields.

The final structural elucidation of the titled molecules was established through detailed analysis of NMR (1D & 2D) as well as HRMS



Scheme 1. Synthetic route of *N'*-(arylbenzylidene)-2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-1-yl)acetohydrazides. Reaction Conditions: (a) 2 (1.2 mmol), 0°C, Acetic acid, 10 min; (b) 4 (1.2 mmol), K₂CO₃ (1.1 mmol), acetone, rt, 2 h; (c) 6 (1.6 mmol), EtOH, 60°C, 6 h; (d) 8a-8l (1.0 mmol), AcOH (cat.), EtOH, 70°C, 5–7 h.

spectroscopic data. Following reaction optimization, the influence of different substituents on the rate of reaction and yield of the compound was also studied. It was observed that condensation reactions involving aldehydes bearing electron-withdrawing groups (e.g., 3-NO₂) proceeded at a significantly faster rate compared to reactions with other aldehydes. Moreover, these reactions yielded the desired products in excellent yields. This observation highlights the pronounced effect of electronic factors on the reaction rate and efficiency in the synthesis of the targeted molecular hybrids.

3.2. Spectral and conformational analysis

The structural confirmation of all synthesized molecular hybrids (**9a-9l**) was finalized using spectroscopic data including FT-IR, NMR, and HRMS. In the FT-IR spectra, signals for –NH stretching were identified in the range of 3334–3212 cm^{−1}, while amidic –C=O stretching signals appeared between 1682 and 1646 cm^{−1}, confirming the presence of *N*-acyl hydrazone backbone in the synthesized compounds. Due to the presence of restricted rotation about the imine (–C=N) bond and partial double-bond behavior of the hydrazido (–N–C(O)) bond, *N*-acyl hydrazones are expected to exhibit four isomeric forms. These include two geometrical isomers (*E/Z*) concerning the –C=N linkage and two rotational isomers (*s-cis/s-trans*) concerning the hydrazido bond. The *E* (*trans*) isomer has larger groups located on opposite sides of the imine bond, while the *Z* (*cis*) isomer has them on the same side. Rotational isomerism involves the *s-cis* orientation where the –C=N bond is *cis* to the hydrazido group, and the *s-trans* orientation where it is *trans* to this group as shown in Fig. 2.

Literature findings on *N*-acyl hydrazones indicate that those derived from aryl aldehydes tend to adopt a planar structure. The restricted rotation about the imine linkage strongly favors the occurrence of the geometrical *E*-isomer relative to the –C=N bond. This structural rigidity suggests that, in many cases, only the *E*-isomer is predominantly formed due to the stable arrangement of substituents on opposite sides of the imine bond [69–72]. This observation was corroborated by analytical data, with two distinct peaks in ¹H NMR data confirming the existence of both *E* (geometric) and *s-cis/s-trans* (rotational) conformers in (CD₃)₂SO.

As established by the literature findings, we prioritized the formation of the *E*-conformers (*E, s-cis* and *E, s-trans*) isomers only. Further, in the ¹H NMR spectra of all *N*-acyl hydrazone derivatives, the existence of *E*-conformers (*s-cis* and *s-trans*) led to two distinct peaks for the –CH₂ (δ 6.16–5.54 ppm), –N=CH (δ 8.60–7.89 ppm), –CONH (δ 12.25–11.66 ppm) groups. Following literature guidelines, the downfield signals correspond to the *s-trans* isomer, while the upfield signals are attributed

to the *s-cis* isomers; the inverse pattern was observed for the methylene protons. Additionally, protons of the aryl or heteroaryl ring system exhibited paired NMR peaks in the δ 8.57–6.65 ppm region. However, due to the overlapping of signals in the NMR spectrum, the multiplicity of these peaks could not be clearly assigned. Instead, the abundance ratio of both the isomers was determined by integrating the NMR peaks associated with *E, s-cis* and *E, s-trans* isomer for methylene (–CH₂), amidic (–CONH), and imine (–N=CH) protons at 25 °C (Table S1 in supplementary file). The percentage ratio of both the conformers was determined and a higher ratio is assigned to *s-cis* conformation. Analysis of the 1D-NMR spectra of compounds **9a-9l** indicated that these compounds predominantly exist in the *E, s-cis* and *E, s-trans* conformers at an approximate ratio (75 % *s-cis* while 25 % of *s-trans*) in (CD₃)₂SO with slight variation from compound to compound. The *s-cis* isomers ranged from 78.57 % to 85.19 %, while the *s-trans* isomers ranged from 15.62 % to 22.87 %.

Compound **9f** was chosen as the model compound for comprehensive structural characterization, including 1D-NMR, 2D-NMR, mass spectrometry, and FT-IR data analysis. In the FT-IR spectrum, an absorption band was observed in the region of 3316–3218 cm^{−1} for –NH stretching, whereas the stretching signals for –C=O of the amide group appeared between 1682 and 1645 cm^{−1}. To obtain detailed structural information, 1D-NMR experiments (DEPT-45, DEPT-90, and DEPT-135) and 2D-NMR experiments (¹H–¹H COSY, ¹H–¹³C HMBC, and ¹H–¹³C HSQC) were performed. The different correlations detected during the detailed analysis of 2D-NMR spectra of compound **9f** are tabularized in Table 1, while the characteristic signal assignments for all protons and carbons are illustrated in Fig. 3.

In the ¹H NMR spectrum of compound **9f**, two resonating singlets for the –OCH₂ protons were observed at δ 5.62 (17.09 %, for *E, s-trans* isomer) and 6.11 ppm (82.91 %, for *E, s-cis* isomer). Similarly, for –N=CH proton, two singlets appeared at δ 8.60 ppm (18.75 %, for *E, s-trans* isomer) and 8.39 ppm (81.25 %, for *E, s-cis* isomer) in approximately same ratio. The proton of the –CONH group resonated at δ 12.25 ppm (16.49 %, for *E, s-trans* isomer) and 12.13 ppm (83.51 %, for *E, s-cis* isomer). Furthermore, in the ¹³C NMR spectrum, two discrete sets of peaks were identified for the carbon atoms of –CONH (167.11 and 161.62 ppm) and –N=CH (141.48 and 144.99 ppm) (Fig. 3). The relative intensities of these signals indicate the predominance of the *E, s-cis* isomer over the *E, s-trans* isomer. The 1°, 2°, and 3° carbons were distinguished with the help of 1D-DEPT spectrum analysis. The nine protonated –CH carbons appeared at δ 144.97, 141.48, 135.26, 132.20, 130.42, 128.10, 127.79, 127.58 ppm, and one –CH₂ resonated at δ 58.27 & 58.00 ppm. The residual 3° carbons resonated at δ 145.99, 134.28,

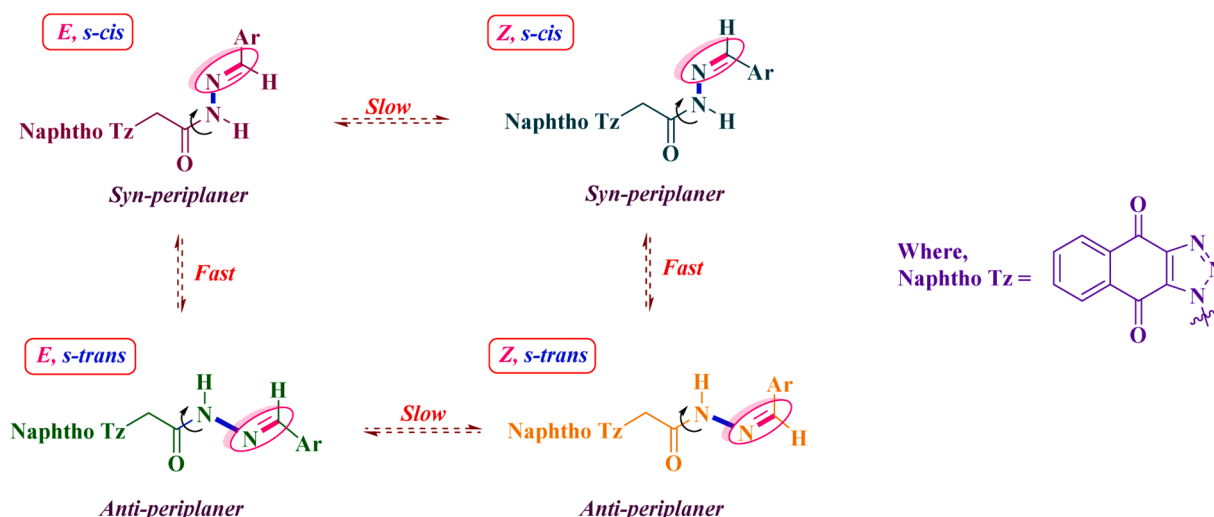


Fig. 2. Possible Stereoisomers of *N*-acyl hydrazones.

Table 1
1D and 2D-NMR analysis of compound **9f**.

Positions	^1H - ^1H COSY	^1H - ^{13}C HSQC	^1H - ^{13}C HMBC	δ_{H} (ppm)	δ_{C} (ppm), Type
4	–	–	5	–	177.51, CO
5	6, 7	5	4, 6, 12	8.18–8.13, <i>m</i>	127.58, CH
6	5, 8	6	5, 12	7.92–7.87, <i>m</i>	135.26, CH
7	5, 8	7	8, 13	7.92–7.87, <i>m</i>	135.26, CH
8	6, 7	8	7, 9, 13	8.18–8.13, <i>m</i>	127.58, CH
9	–	–	8	–	177.51, CO
10	–	–	–	–	145.99, C
11	–	–	–	–	145.99, C
12	–	–	5, 6	–	134.28, C
13	–	–	7, 8	–	134.28, C
14	–	14	15	6.11 and 5.62, <i>s</i>	58.27, 58.00, CH ₂
15	–	–	14, NH	–	167.11, 161.62, CO
16 (NH)	–	–	14, 15, 18	12.25 and 12.13, <i>s</i>	–
18	–	18	19, 20, 24, NH	8.60 and 8.39, <i>s</i>	141.48, 144.97, CH
19	–	–	18, 21, 22, 23	–	131.56, C
20	21, 23	20	18, 22, 24	8.04, <i>dd</i> (<i>J</i> = 7.6, 1.9 Hz)	127.79, CH
21	–	21	19, 20, 23, 24	7.39–7.34, <i>m</i>	128.10, CH
22	21, 23	22	19, 23, 24	7.43–7.40, <i>m</i>	132.20, CH
23	22	23	20, 24	7.50–7.45, <i>m</i>	130.46, CH
24	–	–	18, 20, 21, 22, 23	–	133.67, C

133.67 and 131.53 ppm. Additionally, mass spectral analysis of compound **9f** revealed an $[M + H]^+$ peak at m/z value of 394.07 in the ESI-MS spectrum.

3.3. α -Amylase inhibition evaluation

In this section, the synthesized *N*-acyl hydrazones derived from naphtho[2,3-*d*][1,2,3]triazole-4,9-dione (**9a–9l**) were screened for their inhibitory activity against the α -amylase enzyme. A total of twelve derivatives were tested using *in vitro* α -amylase assay at five different concentrations: 200, 100, 50, 25, and 12.5 $\mu\text{g/mL}$. The IC_{50} values for all examined molecules were determined in $\mu\text{g/mL}$, as shown in Table 2 and compared with the IC_{50} value of the reference drug, Acarbose. The detailed analysis of the enzymatic assay results showed that the tested derivatives exhibited substantial inhibitory activity against *A. oryzae* α -amylase, with IC_{50} values in the range of 17.26 ± 0.07 to 25.62 ± 0.03 $\mu\text{g/mL}$. Among the examined compounds, four compounds, **9a** ($\text{IC}_{50} = 19.99 \pm 0.10$ $\mu\text{g/mL}$), **9c** ($\text{IC}_{50} = 17.26 \pm 0.07$ $\mu\text{g/mL}$), **9d** ($\text{IC}_{50} = 19.03 \pm 0.03$ $\mu\text{g/mL}$) and **9l** ($\text{IC}_{50} = 19.05 \pm 0.01$ $\mu\text{g/mL}$) displayed better inhibition than acarbose ($\text{IC}_{50} = 20.14 \pm 0.06$ $\mu\text{g/mL}$). Compound **9c**,

possessing *–meta* substituted $-\text{NO}_2$ group displayed excellent inhibition ($\text{IC}_{50} = 17.26 \pm 0.07$ $\mu\text{g/mL}$). A comparative graph of IC_{50} values of examined molecules and acarbose is shown in Fig. 4.

3.3.1. Structure-activity relationship (SAR) evaluation

A focused SAR analysis was conducted to determine the key structural features that influence the inhibitory potency of the tested molecules. The presence of aryl/heteroaryl rings and various chemical entities attached to the aryl ring of *N*-acyl hydrazones exerted a significant influence on their inhibitory activity. Based on this analysis, a graphical SAR summary is presented in Fig. 5, illustrating the influence of structural modifications on the effectiveness of these molecules.

Based on the ring structures and their substituents, the following key analyses were identified:

- Derivatives having $-\text{OCH}_3$ and $-\text{NO}_2$ groups showed higher inhibitory response over others.
- The presence of $-\text{NO}_2$ at the *–meta* position exhibited greater activity than *–ortho* and *–para* positions.
- Compounds with unsubstituted aryl rings or heteroaryl substitutions demonstrated lower activity than those with substituted aromatic rings.
- The derivative substituted with 2,6-dichloro displayed higher activity than those substituted with 4-chloro and 2,4-dichloro.

3.4. Molecular docking

To establish the experimentally observed inhibitory response of most promising compound (**9c**) and least active derivative (**9h**), docking simulations were conducted within the receptor active site using iGEMDOCK 2.1 program. Fig. 6 illustrates the most stable docked poses of both derivatives, highlighting their respective interactions with the

Table 2

Inhibitory potential of naphtho[2,3-*d*][1,2,3]triazole-4,9-dione tethered *N*-acyl hydrazones (**9a–9l**).

No.	Compound (9a–9l)	Ar	IC_{50} ($\mu\text{g/mL}$)
1.	9a	4- $\text{CH}_3\text{OC}_6\text{H}_4$	19.99$\pm$0.09
2.	9b	4- $(\text{N}(\text{CH}_3)_2\text{C}_6\text{H}_4)$	21.14 $\pm$ 0.05
3.	9c	3- $\text{O}_2\text{NC}_6\text{H}_4$	17.26$\pm$0.07
4.	9d	2,3,4- $(\text{CH}_3\text{O})_3\text{C}_6\text{H}_2$	19.03$\pm$0.03
5.	9e	$-\text{C}_6\text{H}_5$	23.82 $\pm$ 0.08
6.	9f	2- ClC_6H_4	21.17 $\pm$ 0.08
7.	9g	2,5- $(\text{CH}_3\text{O})_2\text{C}_6\text{H}_3$	20.36 $\pm$ 0.12
8.	9h	2,4- $\text{Cl}_2\text{C}_6\text{H}_3$	25.62 $\pm$ 0.03
9.	9i	4- FC_6H_4	21.78 $\pm$ 0.08
10.	9j	2-Pyridyl	22.51 $\pm$ 0.09
11.	9k	4-Pyridyl	23.88 $\pm$ 0.25
12.	9l	2,6- $\text{Cl}_2\text{C}_6\text{H}_3$	19.05$\pm$0.01
13.	Acarbose	–	20.14 $\pm$ 0.06

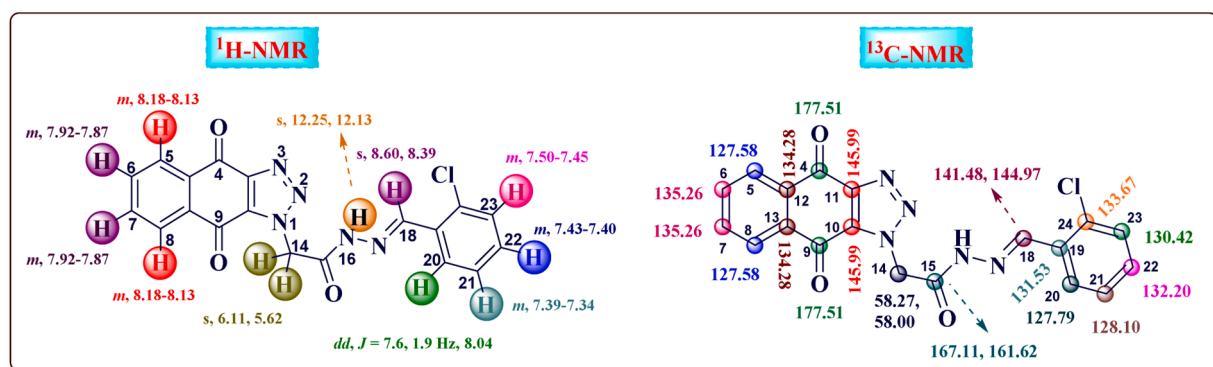


Fig. 3. NMR Spectral analysis of compound **9f**.

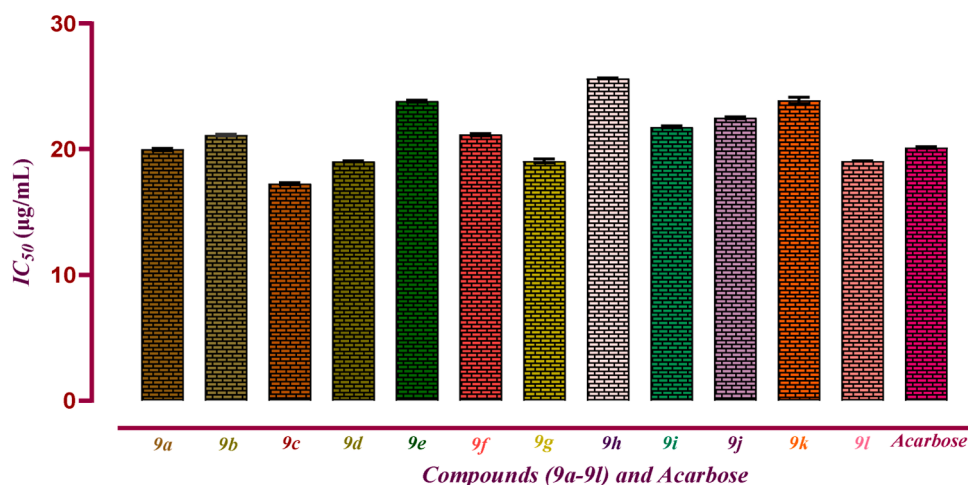


Fig. 4. Comparison of IC₅₀ values of examined molecules (9a-9l) with acarbose.

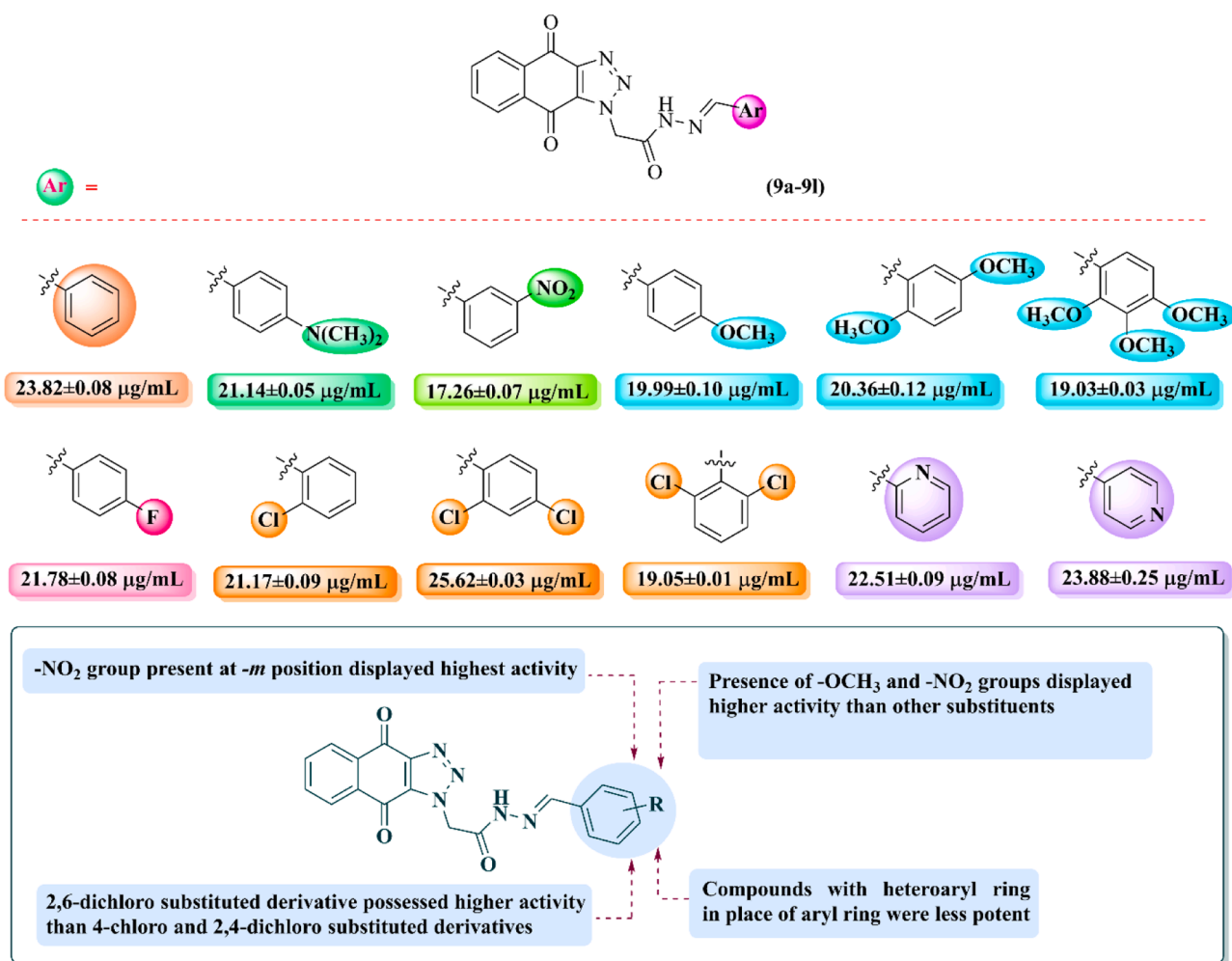


Fig. 5. SAR analysis of naphtho[2,3-d][1,2,3]triazole-4,9-dione linked N-acyl hydrazones (9a-9l).

active site residues of the receptor protein.

Table S2 (in supplementary file) provides details of the various interactions prevailing in the protein-ligand complexes. The comprehensive docking analysis revealed that compound 9c effectively fits within the binding pocket of *A. oryzae* α -amylase (PDB ID: 7TAA). The 9c-7TAA complex was stabilised by electrostatic, three conventional

hydrogen bonds, three C—H bonds, a π -sigma, a π -donor hydrogen, a π - π T-shaped, and three π - π stacking interactions. Similarly, compound 9h exhibited five conventional hydrogen bonds, five π - π stacked interactions, two C—H bonds, and one π -alkyl interaction. Further details of these interactions are provided below:

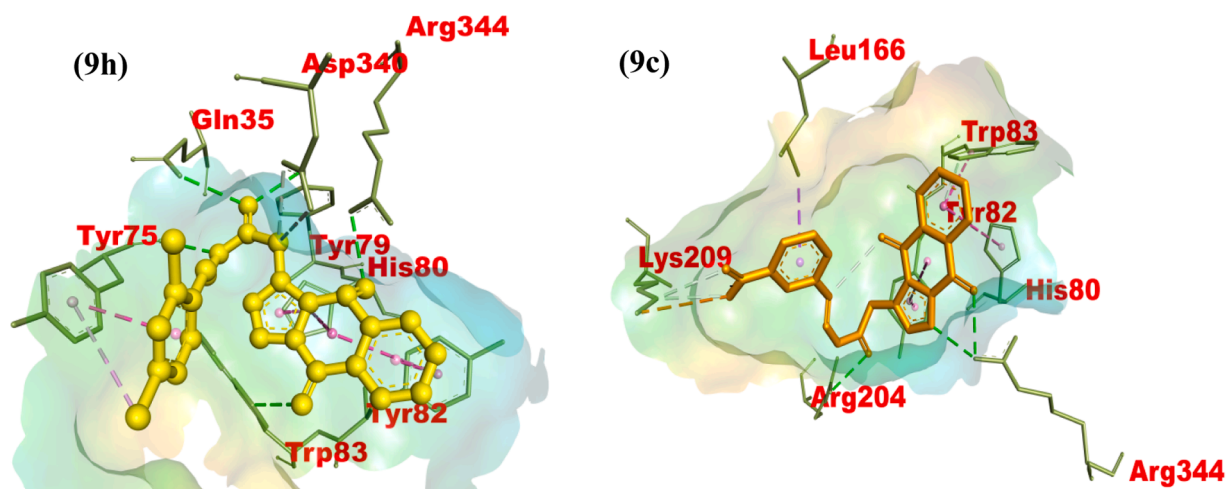


Fig. 6. Various binding interactions identified in the docked poses of **9h** and **9c**.

■ The -NO_2 substituent present at the 3rd position of the phenyl portion significantly influenced the binding stability of compound **9c** with the receptor, as it displayed electrostatic interaction and two hydrogen bonds with LEU²⁰⁹. Additionally, the π -orbitals of the aryl portion of the naphthoquinone framework contributed to a π - π T-shaped interaction and a π -donor H-bond with TRP⁸³ residue, as well as π - π stacked interactions with TYR⁸² and HIS⁸⁰ residues. Additionally, π -orbitals of the phenyl moiety were engaged with LEU¹⁶⁶ amino acid residue through π -sigma type hydrophobic interactions. Furthermore, the N atoms present in the triazole ring and the carbonyl group of naphthoquinone displayed interactions through conventional H-bonding interactions with ARG³⁴⁴ residues, whereas the oxygen of the -CONH linkage displayed conventional H-bonding with ARG²⁰⁴.

■ The least potent compound, **9h**, exhibited similar interactions as observed for **9c**. These interactions included a π -alkyl hydrophobic interaction, two C—H bonds, five π - π stacked interactions, and five conventional H-interactions. Specifically, the carbonyl part on the naphthoquinone moiety formed conventional H-bonds with ARG³⁴⁴ and TRP⁸³. π -Orbitals from both the naphthoquinone and imidazole ring were engaged in π - π stacked bonding with HIS⁸⁰ & TYR⁸² amino acid residues and π -orbitals of phenyl ring interacted in a π - π stacked manner with TYR⁷⁵, while the -OCH_3 substituent at the 3rd place of the phenyl moiety displayed a π -alkyl interaction with TYR⁷⁵. TYR⁷⁵

also participated in conventional H-bonding with the amidic -NH . The oxygen of the hydrazide backbone displayed conventional H-bonds with GLN³⁵ and TYR⁷⁹. Additionally, the -CH_2 group linked to the imidazole ring displayed C—H interactions with ASP³⁴⁰.

■ Based on the above observations, it was concluded that the presence of -NO_2 substituent on the phenyl moiety significantly enhanced the activity of compound **9c** compared to **9h**. The binding energies of compounds **9c** and **9h** in the enzyme's inhibitory pocket were -147.927 kcal/mol and -128.946 kcal/mol, respectively. The greater binding energy value for **9c** as compared to compound **9h** correlates with the observed higher activity of compound **9c**.

3.5. Molecular dynamics (MD)

The molecular docking analysis of compounds **9c** and **9h** provided insights into their interaction potential within the receptor's binding pocket. To further validate these findings and assess the stability of the ligand-protein complexes, 100 ns molecular dynamics (MD) simulations were conducted using Schrodinger's Desmond 2019–4 software. Fig. 7 displays root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) plots for both most potent and the least potent compounds, illustrating their stability profiles during the simulation.

The RMSD plot indicated that **9c** exhibited minimal fluctuations throughout the MD simulation, whereas **9h** showed higher RMSD

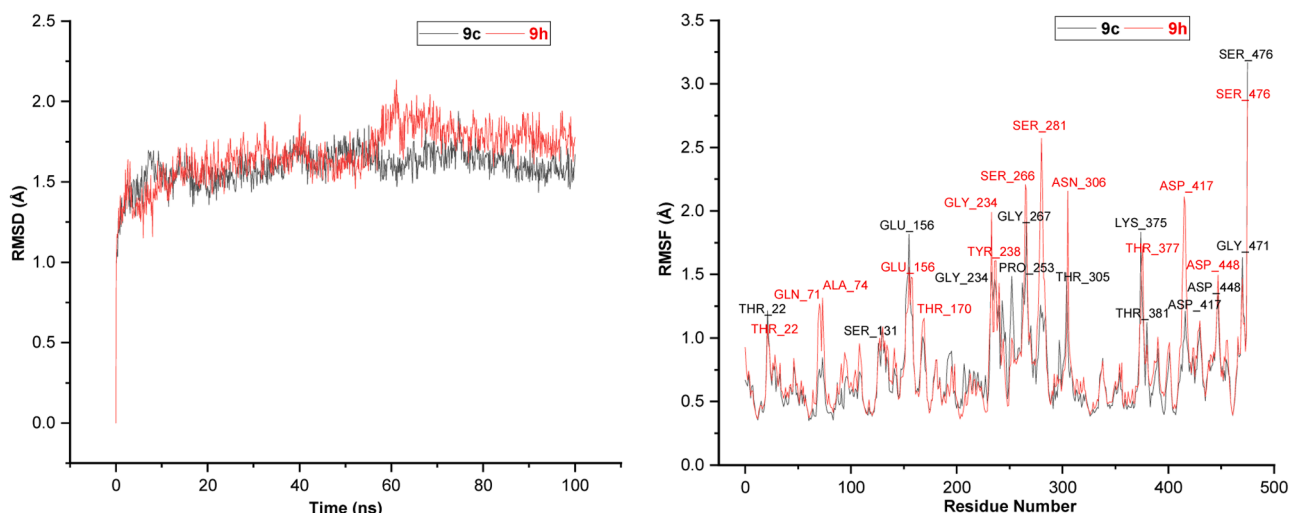


Fig. 7. Graphs depicting the changes in RMSD and RMSF for ligand-protein complexes of **9c** and **9h**.

fluctuations between 55 and 70 ns of the time frame. This suggests that the **9c** ligand-protein complex with *A. oryzae* α -amylase exhibits superior intermolecular stability. In a similar way, the RMSF values revealed minimal fluctuations for **9c**, while **9h** showed greater RMSF fluctuations.

Additionally, the binding strength of both compounds with protein residues was evaluated, and Figs. 8 and 9 depicts the fraction of interactions with amino acid residues. Compound **9c** exhibited a greater number of hydrogen bonds and ionic interactions compared to **9h**.

In conclusion, based on the docking results, RMSD and RMSF analyses, it is evident that compound **9c** exhibits superior binding stability and stronger interactions with *A. oryzae* α -amylase compared to **9h**.

3.6. ADMET properties

The predicted pharmacokinetic properties, including lipophilicity, polarity, and drug-likeness behavior of naphtho[2,3-*d*][1,2,3]triazole-4,9-dione tethered *N*-acyl hydrazones (compounds **9a-9l**), are summarized in Table 3. Analysis of Table 3 reveals that the majority of these compounds adhere to Lipinski's rule of five, typically with at most one violation. The molecular weights of the synthesized compounds range from 359.34 to 449.42 Da, all falling below the 500 Da threshold. The partition coefficient (LogP) values range from 1.59 to 2.89, indicating moderate lipophilicity. The number of hydrogen bond acceptor (HBA) groups ranges from 6 to 9, consistently fewer than 10. Additionally, each molecule has only one hydrogen bond donor (HBD), which is well below the threshold of five.

The physicochemical properties of the active compounds (**9a**, **9c**, **9d** and **9l**) were assessed using the bioavailability radar, depicted in Fig. 10. This radar chart delineates six key characteristics: size, solubility, polarity, lipophilicity, flexibility and saturation. The optimal range for these pharmacokinetic attributes is highlighted by the pink area on the radar plot. This area highlights the desirable values for size, flexibility, and lipophilicity, indicating where the compounds **9a**, **9c**, **9d** and **9l** fall within these parameters.

The BOILED-Egg graphical method, illustrated in Fig. 11, was employed to evaluate the polarity and lipophilicity properties of the molecules under study. The majority of the molecules were situated in the white area of the BOILED-Egg diagram, indicating favorable gastrointestinal (GI) absorption without potential permeation across the blood-brain barrier (BBB). Notably, compound **9c** was an exception, residing outside this white region.

Additionally, the BOILED-Egg graph indicated that all studied molecules, except for **9c**, were not P-glycoprotein (PGP) substrates, as

represented by the red dots. This suggests that these molecules are unlikely to be effluxed from the central nervous system (CNS) by PGP, reducing the risk of CNS depression or drowsiness.

Furthermore, the impact on cytochrome P450 (CYP) metabolism was evaluated, revealing that all the tested compounds were non-inhibitors of CYP1A2, with the exception of compounds **9h** and **9l**. Similarly, compounds **9d** and **9g** were found to inhibit CYP3A4, while none inhibited CYP2D6. Mixed effects were observed for other CYP isoforms.

In summary, the BOILED-Egg method provided insights into the pharmacokinetic properties of the compounds, highlighting their GI absorption, PGP non-substrate status, and varied effects on CYP enzyme inhibition across different isoforms.

For the designed derivatives, the *in silico* toxicity predictions on various toxicity endpoints, such as LD₅₀, predicted toxicity class, cytotoxicity, immunotoxicity, and Peroxisome Proliferator-Activated Receptor Gamma (PPAR- γ) was obtained using ProTox-3.0 web tool (<https://tox.charite.de/prottox3/>). The predictions resulted into negative toxicity data indicating lower toxicity of designed compounds, especially the most active **9c** having predicted LD₅₀ of 1000 mg kg⁻¹. The details about various toxicity parameters are given in Table 4.

4. Conclusion

In this manuscript, we focus on developing α -amylase inhibitors with enhanced potency as a promising strategy to address diabetes. Our aim is to expand the pool of potential therapeutic agents for diabetes management, contributing to the advancement of treatment options for this widespread and challenging disease. The present study details the synthesis and spectral analysis of novel *N'*-(arylbenzylidene)-2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-1-yl)acetohydrazide derivatives. These compounds were prepared via a straight forward multistep synthetic route, and their structural assignments were made using various spectroscopic techniques. NMR spectral analysis revealed duplicated signals for the (*E/Z*)-*N'*-(arylbenzylidene)-2-(4,9-dioxo-4,9-dihydro-1*H*-naphtho[2,3-*d*][1,2,3]triazol-1-yl)acetohydrazides, indicating the presence of two conformers related to the amide bond. The agreement of our ¹H and ¹³C NMR data with literature reports suggests that these compounds rapidly reach *s-cis/s-trans* equilibrium around the amide bond in (CD₃)₂SO solution. At room temperature, the ratio of *s-cis/s-trans* isomers was determined from the NMR spectra. The *s-cis* isomer is more stable and predominates over the *s-trans* isomer due to its ability to form favorable intermolecular interactions with the polar (CD₃)₂SO solvent. Subsequently, the titled compounds were evaluated for their ability to inhibit α -amylase activity. All derivatives exhibited

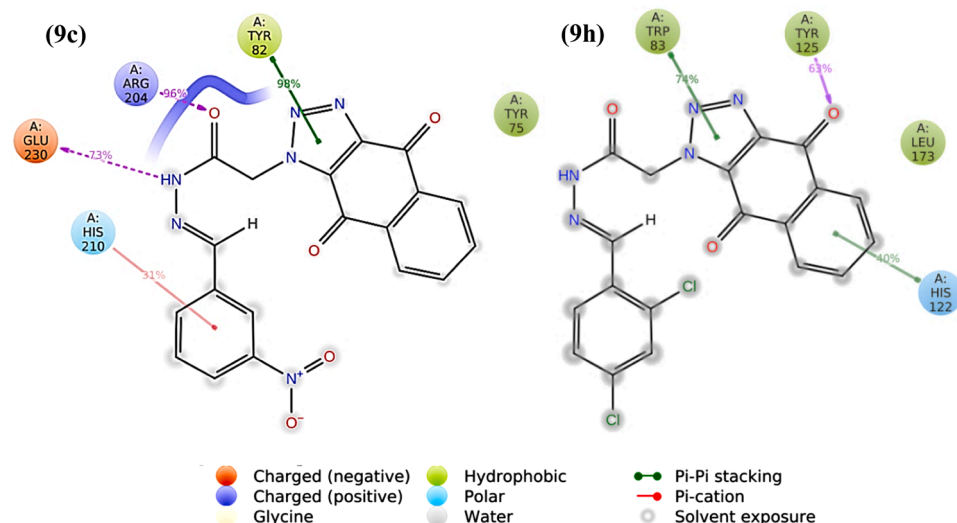


Fig. 8. Ligand-protein interactions observed for compounds **9c** and **9h**.

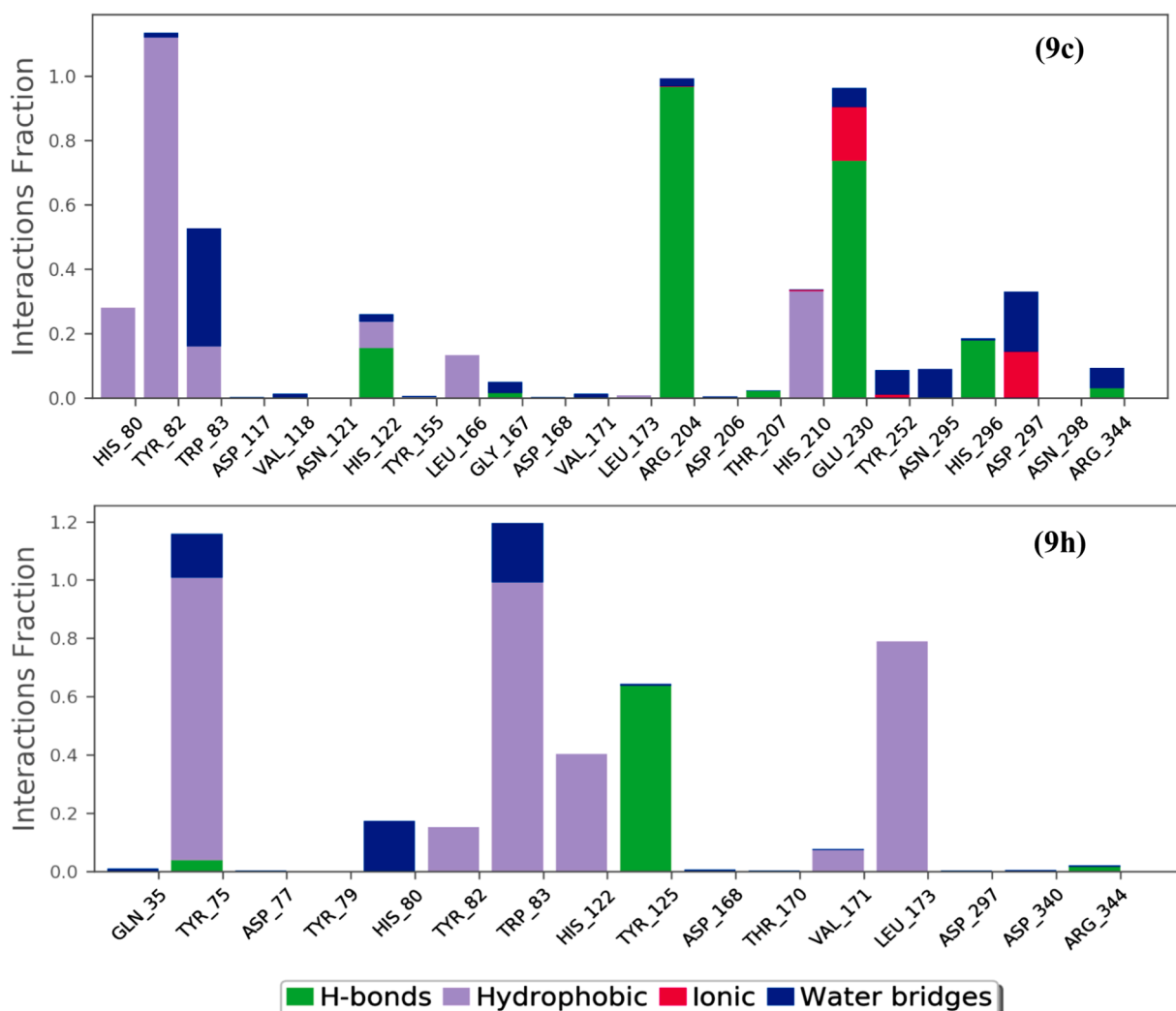


Fig. 9. Bar graph depicting fraction of interactions with different amino acid residues for compounds **9c** and **9h**.

Table 3

ADME parameters of the examined compounds (**9a-9l**).

Molecules	Physicochemical Parameters										Drug-likeness		
	M.Wt.	MLogP	HBA	HBD	RB	GIA	BBBP	PGP	TPSA	LogK _p	Lipinski	Muegge	Veber
9a	389.36	2.32	7	1	6	High	No	No	115.54	-7.01	0	0	0
9b	402.41	2.35	6	1	6	High	No	No	109.55	-6.98	0	0	0
9c	404.34	1.61	8	1	6	Low	No	No	152.13	-7.20	1	1	1
9d	449.42	2.81	9	1	8	High	No	Yes	134.00	-7.42	1	0	0
9e	359.34	1.84	6	1	5	High	No	No	106.31	-6.80	0	0	0
9f	393.78	2.33	6	1	5	High	No	No	106.31	-6.56	0	0	0
9g	419.39	2.62	8	1	7	High	No	No	124.77	-7.21	0	0	0
9h	428.23	2.37	6	1	5	High	No	No	106.31	-6.33	0	0	0
9i	377.33	2.06	7	1	5	High	No	No	106.31	-6.84	0	0	0
9j	360.33	1.92	7	1	5	High	No	No	119.20	-7.33	0	0	0
9k	360.33	1.59	7	1	5	High	No	No	119.20	-7.57	0	0	0
9l	428.23	2.21	6	1	5	High	No	No	106.31	-6.33	0	0	0

Molecular weight: M.Wt. = (≤ 500 Da), Log of octanol/water partition coefficient: MLOGP = (< 5), No. of hydrogen bond acceptor(s): HBA = (≤ 10), No. of hydrogen bond donor(s): HBD = (≤ 5), No. of rotatable bonds: RB = (≤ 10), Total polar surface area: TPSA = ($< 140 \text{ \AA}^2$), Gastrointestinal absorption: GIA, Blood brain barrier permeability: BBBP, P-glycoprotein substrates: PGP, Log of skin permeability: log K_p (cm/s) = (-8.0 to -1.0 cm/s).

potent α -amylase inhibition, with IC₅₀ values in the range of 17.26 ± 0.07 to 25.62 ± 0.03 $\mu\text{g/mL}$. Notably, compound **9c** possessing *-meta* substituted $-\text{NO}_2$ group displayed highest activity (IC₅₀ = 17.26 ± 0.07 $\mu\text{g/mL}$) among the series. The SAR study revealed the influence of substituent variation on biological outcome. A docking study of the most potent derivative, **9c**, indicated its strong potential with stabilising

bonding interactions within the active site of the protien. Molecular dynamics (MD) simulation of 100 ns further strengthened the outcomes of docking studies, as a stable protien-ligand complex with an RMSD value consistently below $2 \text{ \AA}$ was observed. Additionally, ADMET predictions suggest that these designed molecules have potential as novel α -amylase inhibitors for the treatment of T2DM.

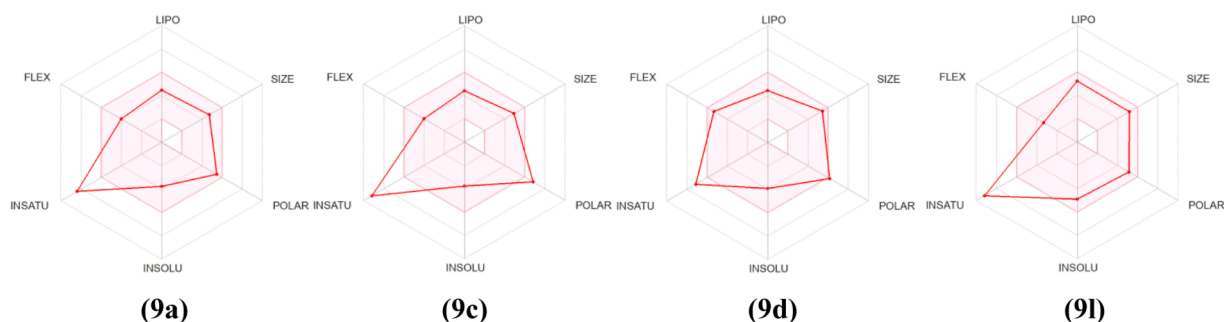


Fig. 10. Assessment of drug-likeness of active compounds with bioavailability radar.

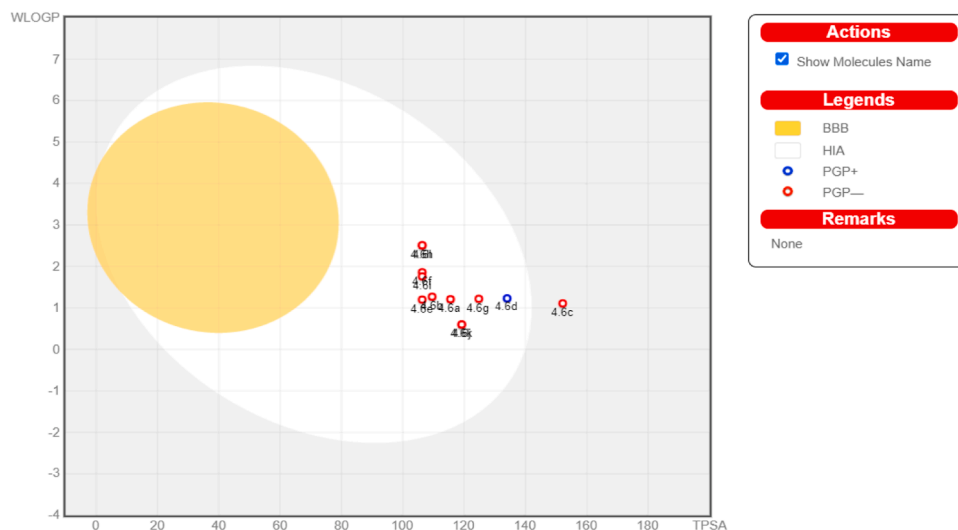


Fig. 11. BOILED-Egg diagram for the prediction of GI absorption and BBB properties of compounds (9a-9l).

Table 4

Predicted toxicity endpoints for the examined compounds (9a-9l).

Molecules	9a	9b	9c	9d	9e	9f	9g	9h	9i	9j	9k	9l
Predicted LD ₅₀ (mg kg ⁻¹)	500	600	1000	500	500	980	500	980	500	980	980	980
Predicted toxicity class	4	4	4	4	4	4	4	4	4	4	4	4
Cytotoxicity	–	–	–	–	–	–	–	–	–	–	–	–
Immunotoxicity	+	–	+	+	–	–	+	–	–	–	–	–
PPAR-γ	–	–	–	–	–	–	–	–	–	–	–	–

Conflicts disclosure

The authors declare that they have no known personal or financial interests associated with the research presented in this study.

CRediT authorship contribution statement

Meena Devi: Writing – original draft, Investigation, Data curation. **Jayant Sindhu:** Writing – review & editing, Software, Data curation. **Rahul Singh:** Writing – review & editing, Formal analysis. **Sohan Lal:** Writing – review & editing, Software. **Ashwani Kumar:** Writing – review & editing, Software. **Devender Singh:** Writing – review & editing, Resources. **Harish Kumar:** Writing – review & editing, Formal analysis. **Parvin Kumar:** Writing – review & editing, Visualization, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial

interests.

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Supplementary materials

Supplementary material associated with this article can be found, in

the online version, at [doi:10.1016/j.molstruc.2024.140390](https://doi.org/10.1016/j.molstruc.2024.140390).

Data availability

Data will be made available on request.

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