Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Synthesis, PASS Prediction, Molecular Docking and Pharmacokinetic Studies of Newer 1,3,4-Thiadiazole Hybrids Of P-Thymol as Antidiabetic Agents

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Cite this paper as: Dattatraya S. Kale, Rahul T. Bhoi, Ganesh R. Borse, Santosh B. Katariya, Sanjay B. Sonawale, (2025) Synthesis, PASS Prediction, Molecular Docking and Pharmacokinetic Studies of Newer 1,3,4-Thiadiazole Hybrids Of P-Thymol as Antidiabetic Agents. *Journal of Neonatal Surgery*, 14 (32s), 2339-2353.

ABSTRACT

A novel series of amide analogs of p-thymol, clubbed with 1,3,4-thiadiazole (8a-8l) was developed and synthesized. This was achieved by attaching the phenolic group of the naturally occurring p-thymol core to 1,3,4-thiadiazole, followed by coupling with various substituted acyl chloride moieties through an amino linker, resulting in a good yield (83-91 %). The identities of all the synthesized compounds were confirmed using spectroscopic methods, including ^{1}H NMR, ^{13}C NMR, mass spectrometry, and FT-IR. In this study, the biological activity profile of the designed analogs was predicted using the PASS prediction tool, indicating their potential antidiabetic activity. The compounds were synthesized, and their activities were experimentally assessed at a concentration of 62.5 500 µL. The observed experimental activity was aligned with the predictions made by PASS. Molecular docking studies were conducted to determine the binding free energies of all the compounds at the active site of isomaltase from S. cerevisiae (PDB ID: 3A47). Compounds **8a** and **8d** exhibited excellent docking scores. The synthesized compounds were evaluated for their in vitro antidiabetic activity and showed moderate-to-good results. Notably, compounds **8a**, **8e**, **8j**, and **8l** exhibited significant antidiabetic activity compared with the positive standard acarbose. A comparative analysis of Lipinski's parameters and compound activity showed that all compounds adhered to Lipinski's rule of five.

Keywords: *1,3,4-thiadiazole, Para-thymol, α-amylase inhibitor, Pharmacokinetic study, Antidiabetic.*

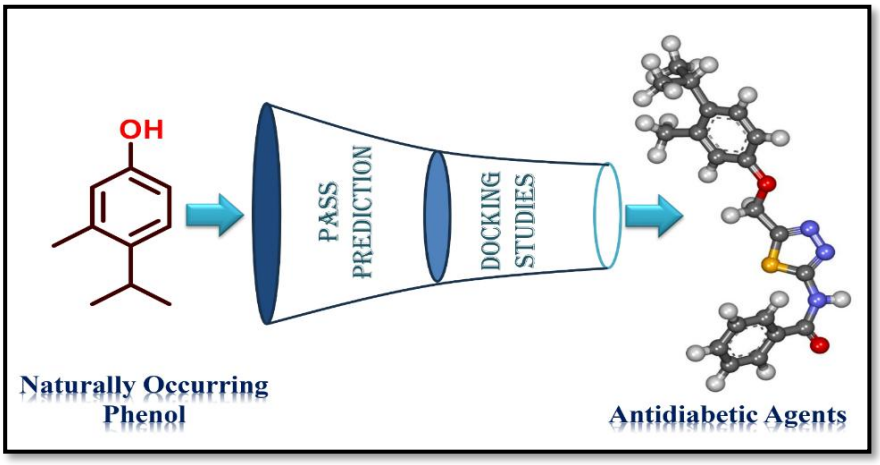
1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to insufficient insulin production or ineffective insulin utilization [1,2]. The global prevalence of diabetes has increased significantly, with projections indicating an increase from 382 million in 2013 to 592 million by 2035 [2]. This epidemic is largely attributed to sedentary lifestyles, high-calorie dietary habits, and increased obesity rates [3,4]. Interestingly, while type-2 diabetes (T2DM) has traditionally been associated with middle-aged and elderly adults, its incidence has been increasing in children and young adults due to obesity and inadequate nutrition [1]. Additionally, the duration of obesity has been shown to be an important factor in developing T2DM, with a longer duration increasing the risk regardless of the final BMI [5]. In conclusion, diabetes mellitus, particularly T2DM, has become a major public health concern owing to its increasing prevalence and associated complications. The disease is closely linked to lifestyle factors, emphasizing the importance of promoting healthy habits and early screening to effectively prevent or manage diabetes [1,3]. Understanding the complex interplay between obesity, insulin resistance, and diabetes is crucial for the development of targeted interventions and treatments [5,6].

Synthetic oral hypoglycemic medications such as acarbose and miglitol are commercially available; however, they are associated with adverse effects including abdominal discomfort, flatulence, meteorism, and diarrhea, which frequently result in treatment discontinuation [7]. These adverse effects can significantly impact patients' quality of life and adherence to treatment, potentially compromising glycemic control. As a result, there is growing interest in exploring alternative therapies, including natural compounds and herbal remedies, which may offer similar blood glucose-lowering effects with fewer side effects [8]. The World Health Organization advocates the identification of a safe, efficacious, and non-toxic natural

antidiabetic agent [9]. Although ethnobotanical studies of traditional herbal remedies have identified over 1200 plants with hypoglycemic properties, they cannot be directly utilized as pharmaceutical drugs [10].

Graphical Abstract



Terpenes or terpenoids constitute the most diverse group of naturally occurring compounds and are primarily found in essential oils extracted from plants. They are categorized as mono-, di-, tri-, tetra-, or sesquiterpenes. Among the natural products offering medicinal benefits to organisms, terpenes play a significant and diverse role [11]. Monoterpenes are the most structurally diverse group of terpenoids [12]. Terpenes and terpenoids exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties [13].

The structural diversity of terpenes allows them to interact with various biological targets, making them valuable for the development of new pharmaceuticals and natural therapies [14]. Research on the therapeutic potential of terpenes continues to expand, with ongoing investigations into their potential applications in the treatment of various diseases and disorders. Para-thymol specifically functions as an antimicrobial agent and is used as an animal feed additive [15,16]. The structural diversity of terpenes not only contributes to their wide range of biological activities but also allows for their potential synergistic effects when combined with other compounds [17]. This synergy can enhance the overall therapeutic efficacy of terpene-based treatments, opening new avenues for drug development and natural medicine [18].

In the pursuit of structures with predicted pharmacological activities through computer-aided programs, such as the prediction of the activity spectrum of substances (PASS), the most probable activity predicted for the synthesized compounds was experimentally evaluated. We focused on developing molecules by combining various active heterocyclic compounds with phenolic monoterpenoids, potentially leading to the creation of compounds with enhanced α-amylase inhibitory activity. In this study, we designed and synthesized a new series of 1,3,4-thiadiazole hybrids of p-thymol and assessed their *in vitro* antidiabetic activities. Computational studies, including docking simulations and ADME prediction, were conducted for all compounds.

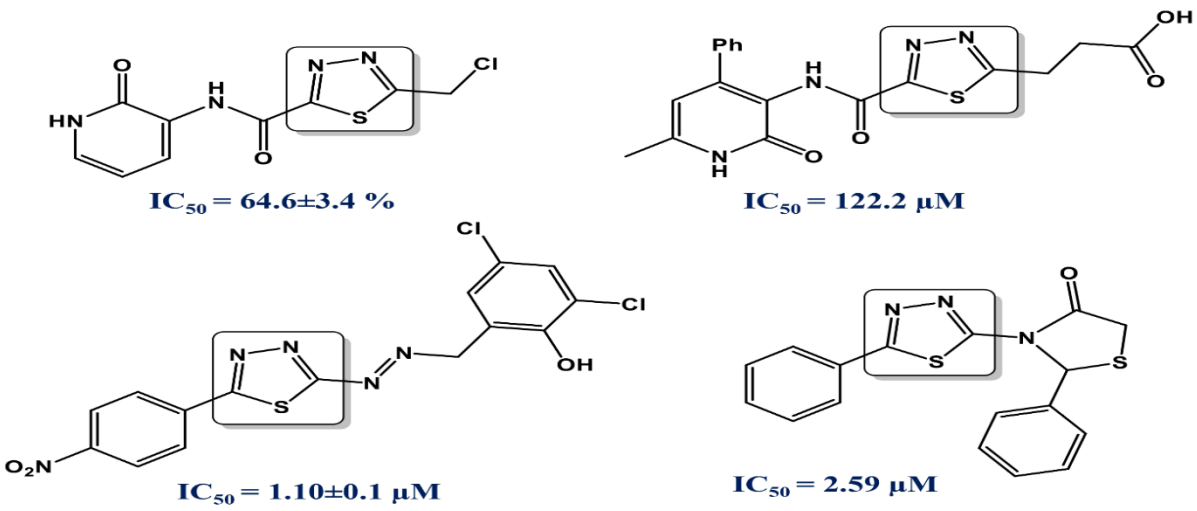


Fig.1. Some reported examples of potent antidiabetic thiadiazoles

2. MATERIALS AND METHODS

For this investigation, commercially available chemicals and reagents were procured from Sigma-Aldrich and TCI and utilized as received without further purification. Prior to utilization, solvents were distilled and dehydrated. The reaction progress was monitored by thin-layer chromatography (TLC) (Merck, silica gel 60 F254) and visualized under ultraviolet light. The synthesized compounds were analyzed using various spectroscopic techniques, including ^{1}H NMR, ^{13}C NMR, FT-IR, and Mass spectrometry (Q-TOF Micromass; Waters). NMR spectra were recorded on a Bruker AC-500 MHz spectrophotometer using $CDCl_3$ or DMSO-*d6* as solvents, with tetramethylsilane (TMS) serving as an internal reference standard. IR spectra were obtained using a Shimadzu FT-IR spectrometer, with samples prepared as anhydrous KBr pellets. The melting points of the synthesized 1,3,4-thiadiazole derivatives were determined utilizing an open capillary method.

Experimental

Synthesis of ethyl 2-(4-isopropyl-3-methylphenoxy)acetate *(2)*

A mixture of 4-isopropyl-3-methylphenol (p-thymol) (1.5 g, 10 mmol), ethanol (25 mL), anhydrous K_2CO_3 (1.67 g, 12 mmol), and TBAB (0.32 g, 1 mmol) in 100 mL round bottom flask was heated to reflux for 1 h. Upon cooling to room temperature and drop wise addition of ethyl chloroacetate (1.36 g, 11 mmol) was carried out during 1 h, refluxing was continued for 4 h. The excess of solvent was recovered by distillation, and the residue was quenched with crushed ice. Further, the contents were stirred for 0.5 h and extracted with DCM. The organic layer was washed with water and dried over anhydrous sodium sulfate (Na_2SO_4. The solvent was recovered under vacuum to obtain yellowish oil, Yield: 90-92 %, the product was used in the next step without purification.

Synthesis of 2-(4-isopropyl-3-methylphenoxy)acetic acid (3)

Acetate of 4-isopropyl-3-methylphenol (**2**) (2.36 g, 10 mmol) and 20 % aq. NaOH (20 g, 100 mmol) in ethanol (2 mL) was added to a 100 mL round-bottom flask and refluxed for 2 h. The progress of the reaction was monitored by TLC. The resulting clear solution was cooled to room temperature. and the mass was neutralized using a dil. HCl. The resultant product separated was filtered, washed with cold water, dried, and recrystallized from ethanol, white solid, Yield: 94-95 %.

Synthesis of 5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-amine (6)

The mixture of **3** (2.1 g, 10 mmol) and phosphorus oxychloride (4.6 g, 30 mmol) was stirred at 25-30 °C for 2 h. Thiosemicarbazide (1.1 g, 12 mmol) was then added to the resulting clear brown solution. The reaction mixture was heated at 80-82 °C for 4 h. Water was then added to the reaction mass and refluxed for 1 h, and the mass was cooled and neutralized using a NaOH solution. Filtered the solid and the product was recrystallized from ethanol to obtain white solid, Yield 76-78 %.

General procedure for synthesis of 1,3,4-thiadizole derivatives of p-thymol (8a-8l)

The mixture of **6** (0.53 g, 2 mmol) and triethylamine (0.22 g, 2.2 mmol) in dioxane (5 mL) was charged to a 50 mL round bottom flask and added dropwise substituted acyl chlorides (2 mmol) at 28-30 °C. The reaction was monitored using thin-layer chromatography (TLC). The obtained solids were filtered and washed with dioxane. The isolated product was recrystallized from ethanol to obtain pure product. (Scheme 1)

PASS prediction of the target compounds

A computational program designated PASS was used to predict the biological activities of the target compounds. Table 1 presents the predicted activities with Pa>0.5 for each target compound (**8a-8l**).

Biological screening

In vitro α-amylase inhibition

Several reports have highlighted antidiabetic activity of 1,3,4-thiadiazole hybrids (**Fig.1**), Palamarchuk, I. V. et al. have been synthesized 1,3,4-thiadiazole based amide derivatives and investigated for their *in vitro* α-amylase activity with IC_{50} 64.6 ±3.4 % and 122.2 µM [19]. The cyclic amides of thiadiazole were synthesized by Gummidi, L. et al. and elucidated with their *in vitro* α-amylase activity with IC_{50} 2.59 µM [20]. Ali, Z. et al. reported potent antidiabetic newer Schiff bases of 1.3.4-thiadiazoles with an IC_{50} 1.10 ± 0.1µM [21].

The newly synthesized compounds, incorporating 1,3,4-thiadiazole, amide, and amidine moieties, are shown in Fig.2. These structures exhibited structural similarities to previously identified lead candidates (Fig.1), which necessitated an investigation into the antidiabetic potential of these hybrid frameworks. It is noteworthy that the synthetic compounds were designated as (**8a-8l**).

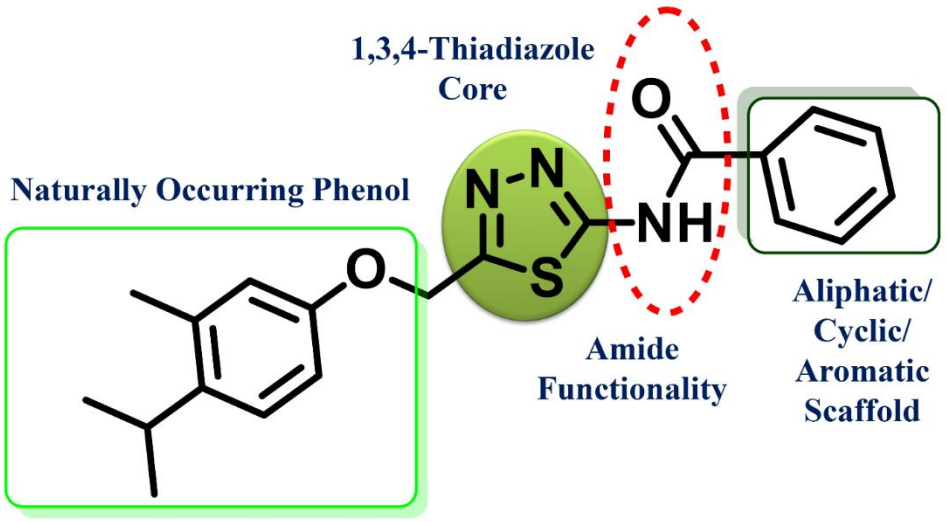


Fig.2. Design strategy for p-thymol mediated amides of 1,3,4-thiadiazoles

The α-amylase inhibitory activity was evaluated utilizing a modified version of the method delineated in the Worthington Enzyme Manual [22]. The procedure entailed combining 500 µL of the compound (8a-8l) with an equivalent volume of α-amylase solution (1.0 U/mL) in 0.02 M sodium phosphate buffer (pH 6.9, containing 0.006 M NaCl). This mixture underwent incubation at 25 °C for 10 minutes. Subsequently, 500 µL of 1 % starch solution in the aforementioned buffer was introduced. The resultant mixture was further incubated at 25 °C for an additional 10 minutes. To terminate the reaction, 1.0 ml of DNS color reagent was added. The test tubes were then heated in boiling water for 5 minutes and allowed to equilibrate at room temperature. The mixture was diluted with 10 ml of distilled water, and the absorbance was measured at 540 nm. The α-amylase inhibitory activity was determined using the following equation:

Inhibition (%) = $[(A_C - A_T) / A_C] X 100$

Where, A_C is the absorbance of the control (without compound)

A_T is the absorbance in the presence of compound

3. RESULTS AND DISCUSSION

Chemistry

Intermediate 2-amino-1,3,4-thiadiazole of p-thymol (**6**) was synthesized in several steps. In the cyclization step, $POCl_3$ was used to prepare acyl chloride **4**, and thiosemicarbazide was added to form uncyclized intermediate **5**. After the formation of **5**, cyclization occurred under acidic condition to achieve **6**. The synthesized intermediate (**6**) was further used for the preparation of its amide derivatives in dioxane, and triethylamine was used as an acid scavenger. All the hybrids of p-thymol clubbed 1,3,4-thiadiazole were successfully synthesized in excellent yields.

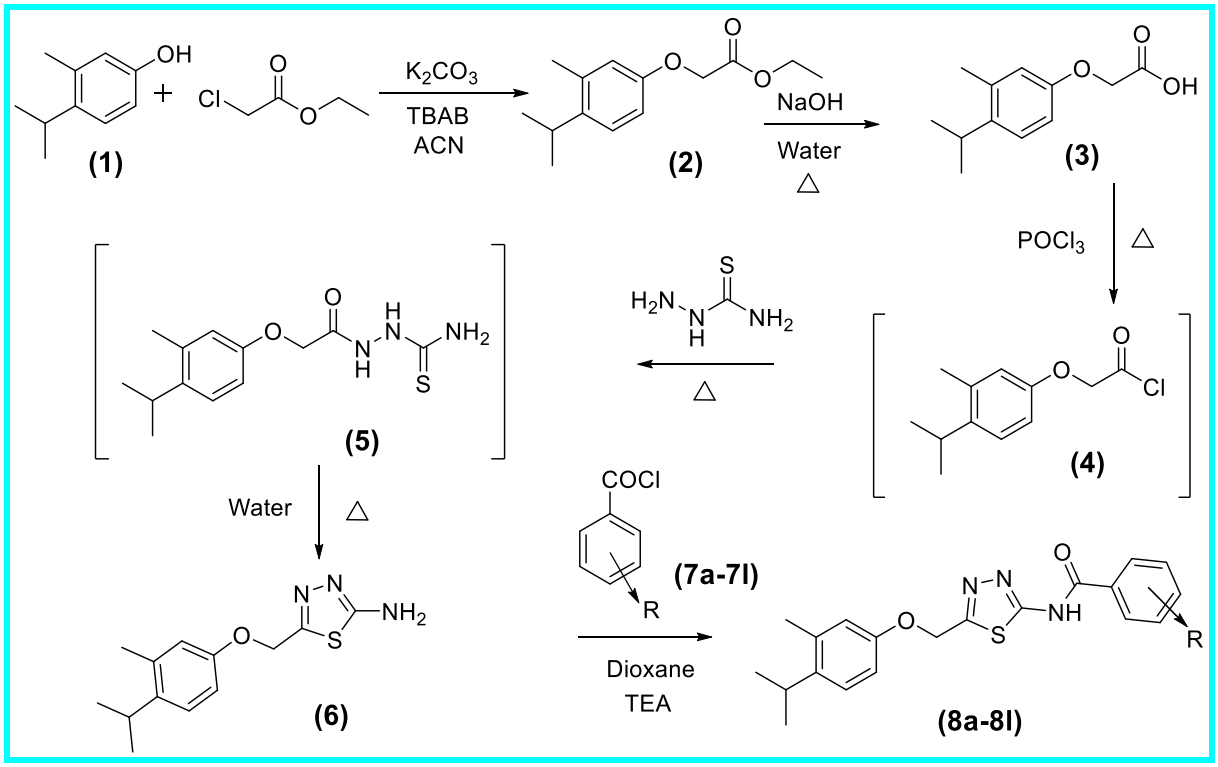
The structures of the synthesized hybrids (**8a-8l**) were confirmed using ^{1}H-NMR, ^{13}C NMR, FT-IR, and mass spectroscopic techniques. The spectral data of the compounds are briefly presented in the Experimental section of this article. The IR spectrum of the compound **8a** showed a broad stretching peak at 3219 cm^{-1} and bending frequency observed at 1597 cm^{-1} which confirming the presence of the-NH group of amides. Stretching at 2945 and 1674 cm^{-1} for the C-H and C=O bonds of the desired compound [23]. In ^{1}H NMR spectrum of compound **8a** a labile proton of –NH- appeared in the deshielded region at δ 13.08 with a lower intensity due to strong intramolecular hydrogen bonding. Two singlets around at δ 5.48 confirmed the aliphatic -CH_2- bridge between p-thymol and 1,3,4-thiadiazole. The six protons of the isopropyl group appeared as a doublet at approximately δ 1.165-1.179 (J = 6.9 Hz). A singlet at δ 2.26 corresponds to the three protons of the methyl group, and a multiplet observed at around δ 2.78-2.83 is related to a proton of the isopropyl group in all the synthesized thiadiazoles. The ^{13}C NMR spectrum provided additional evidence for the appearance of peaks around δ 21.1, 23.7, 33.3, and 63.8, clearly indicating the presence of aliphatic carbons in the resulting scaffolds. The peak corresponding to the carbonyl carbon of the amide moiety was detected at approximately δ 160.8. The peak observed in the ESI-MS spectrum at 390.0522 [M+Na] the molecular ions provided additional evidence for the formation of compound **8a**. The spectroscopic data were satisfactory and reliable for the structures of all the synthesized 1,3,4-thiadiazole derivatives.

Predicted biological activities of 1,3,4-thiadiazole derivatives by PASS

PASS predicts the pharmacological effects and potential side effects of molecules by analyzing structure-activity relationships in a training database. This database currently comprises of over 46,000 experimentally tested drugs, drug candidates, and lead compounds. For each activity, two probabilities were calculated: the probability of the compound being active (Pa) and inactive (Pi). These values range from 0.000 to 1.000, with Pa and Pi typically summing to less than 1, as they are calculated independently. PASS predictions can be interpreted in various ways: (i) activities where Pa exceeds Pi are considered possible; (ii) Pa values above 0.7 indicate a high likelihood of experimental confirmation; (iii) Pa values between 0.5 and 0.7, suggest a lower probability of experimental confirmation, but the compound may be distinct from known pharmaceuticals; and (iv) Pa values below 0.5, indicate an even lower probability of experimental confirmation, but a higher likelihood of discovering a novel chemical entity (NCE) [24,25]. A web-based version of PASS is now available on the developer's website, with a reported prediction accuracy of 85 %. The predicted activity spectra of the synthesized compounds (Table 1) demonstrated that most compounds had a high probability of exhibiting antidiabetic activity. Compounds with amidine and thiadiazole-like moieties attached to p-thymol as phenoxy rings were predicted to have stronger antidiabetic activity. Furthermore, aryl or cyclic amides were found to be more potent than compounds with aliphatic amide groups.

Table 1: PASS predicted activity spectrum of the targeted compounds (8a-8l)

Compounds	Antidiabetic activity		Compounds	Antidiabetic activity	
	P_a	P_i		P_a	P_i
8a	**0.557**	0.016	**8g**	0.237	0.124
8b	0.501	0.023	**8h**	0.358	0.057
8c	0.432	0.035	**8i**	0.520	0.020
8d	0.502	0.023	**8j**	**0.546**	0.017
8e	**0.545**	0.018	**8k**	0.467	0.028
8f	0.492	0.024	**8l**	**0.587**	0.014



Scheme 1: The synthesis pathway of the 1,3,4-thiadiazole derivatives (8a-8l)

Spectroscopic Characterizations and Physical Properties of Synthesized Derivatives

5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-amine (6)

White solid; Yield 76-78 %;

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.21 (d, *J* = 6.95 Hz, 6H, -CH(CH3)2), 2.30 (s, 3H, Ar-CH3), 2.81-2.84 (m, 1H, -CH(CH3)2), 5.26 (s, 2H, -CH2-), 5.34 (s, 2H, -NH2), 6.62 (s, 1H, Ar-H), 6.63 (d, 1H, Ar-H), 6.69 (d, 1H, Ar-H);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.6, 23.9, 34.1, 64.9, 110.4, 112.7, 121.0, 139.5, 150.7, 157.7, 158.4, 169.0;

MS m/z calculated $C_{13}H_{17}N_3OS$: 263.36, found: 264.15 [M+H].

N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)benzamide (8a)

White solid; Yield 90-91 %; m.p.250-252 °C;

IR (vmax, KBr): 3219 (-NH stretching), 2945 (C-H stretching), 1674 (C=O), 1597 (N-H bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (d, *J* = 6.9 Hz, 6H, -CH(CH3)2), 2.26 (s, 3H, Ar-CH3), 2.78-2.83 (m, 1H, -CH(CH3)2), 5.48 (s, 2H, -CH2-), 6.69 (s, 1H, Ar-H), 6.72 (d, *J* = 7.57 Hz, 2H, Ar-H), 7.54-7.57 (t, *J* = 7.67 Hz, 2H, Ar-H), 7.64-7.67 (t, *J* =7.37 Hz, 1H, Ar-H), 8.10 (d, *J* = 7.45 Hz, 2H, Ar-H), 13.08 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 23.7, 33.3, 63.8, 110.1, 112.7, 120.3, 128.3, 128.6, 131.4, 132.9, 138.8, 150.0, 157.4, 160.8, 165.2;

MS m/z calculated $C_{20}H_{21}N_3O_2S$: 367.4670, found: 390.0522 [M + Na].

N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)acetamide (8b)

White solid; Yield 91-92 %; m.p.320-321 °C;

IR (vmax, KBr): 3173 (-NH stretching), 2945 (C-H stretching), 1683 (C=O), 1571 (N-H bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15 (d, *J* = 6.55 Hz, 6H, -CH(CH3)2), 2.18 (s, 3H, N-C(O)-CH3), 2.24 (s, 3H, Ar-CH3), 2.77-2.82 (m, 1H, -CH(CH3)2), 5.43 (s, 2H, -CH2-), 6.67 (s, 1H, Ar-H), 6.68 (d, *J* = 8.55 Hz, 1H, Ar-H), 6.72 (d, *J* = 8.5 Hz, 1H, Ar-H), 12.54 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 22.3, 23.7, 33.3, 63.8, 110.1, 112.7, 120.3, 138.8, 150.0, 157.4, 159.4, 160.4, 168.7;

MS m/z calculated $C_{15}H_{19}N_3O_2S$: 305.3960, found: 328.0406 [M + Na].

(E)-N'-(5-chloro-2-hydroxybenzylidene)-2-((5-((4-isopropyl-3-methylphenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetohydrazide (8c)

Off white solid; Yield 88-89 %; m.p.185-187 °C;

IR (vmax, KBr): 3261 (-NH stretching), 2953 (C-H stretching), 1695 (C=O), 1527 (N-H bending), and 1313 (C-F stretching) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (d, *J* = 6.75 Hz, 6H, -CH(CH3)2), 2.25 (s, 3H, Ar-CH3), 2.77-2.81 (m, 1H, -CH(CH3)2), 3.97 (s, 3H, -N-CH$_3$), 5.45 (s, 2H, -CH2-), 6.65 (s, 1H, -CHF2-), 6.68 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.25 (s, 1H, Ar-H), 8.53 (s, 1H, -Ar-H), 12.86 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 23.7, 33.4, 63.8, 64.1, 109.6, 111.4, 112.7, 120.2, 134.4, 138.7, 145.9, 150.0, 154.3, 157.5, 160.0, 161.1, 169.7;

MS m/z calculated $C_{28}H_{28}ClN_5O_3S$: 421.4668, found: 444.0454 [M + Na].

N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)nicotinamide (8d)

Off white solid; Yield 89-90 %; m.p.247-248 °C;

IR (vmax, KBr): 3263 (-NH stretching), 2954 (C-H stretching), 1689 (C=O), and 1587 (N-H bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15 (d, *J* = 6.95 Hz, 6H, -CH(CH3)2), 2.24 (s, 3H, Ar-CH3), 2.79-2.80 (m, 1H, -CH(CH3)2), 5.46 (s, 2H, -CH2-), 6.65 (s, 1H, Ar-H), 6.68 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.25 (s, 1H, Ar-H), 8.54 (s, 1H, Ar-H), 8.57-8.58 (t, *J* = 6.56 Hz, 1H, Ar-H), 12.76 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 23.7, 33.4, 63.8, 109.6, 111.4, 112.7, 120.2, 120.7, 134.4, 138.7, 145.9, 150.0, 154.3, 157.5, 160.0, 161.1, 169.7;

MS m/z calculated $C_{19}H_{20}N_4O_2S$: 368.4550, found: 403.1160 [M+Cl].

4-chloro-N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)benzamide (8e)

Off white solid; Yield 91-92 %; m.p.228-230 °C;

IR (vmax, KBr): 3251 (-NH stretching), 2949 (C-H stretching), 1687 (C=O), 1525 (N-H bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15 (d, *J* = 6.9 Hz, 6H, -CH(CH3)2), 2.24 (s, 3H, Ar-CH3), 2.76-2.81 (m, 1H, -CH(CH3)2), 5.45 (s, 2H, -CH2-), 6.65 (s, 1H, Ar-H), 6.68 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.25 (s, 1H, Ar-H0), 7.55 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.94 (d, *J* =7.4 Hz, 1H, Ar-H), 8.53 (s, 1H, Ar-H), 12.77 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 23.7, 33.3, 63.8, 110.1, 112.7, 120.3, 128.7, 130.3, 131.1, 137.8, 138.7, 150.0, 154.3, 157.5, 159.9, 161.1, 169.6;

MS m/z calculated: $C_{20}H_{20}ClN_3O_2S$: 401.9090, found: 401.1251 [M^+].

N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)propionamide (8f)

White solid; Yield 87-88 %; m.p.251-252 °C;

IR (vmax, KBr): 3157 (-NH stretching), 2945 (C-H stretching), 1695 (C=O), 1577 (N-H bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.07-1.10 (t, *J* =7.5 Hz, 3H, -NH-C(O)-CH_2-CH_3), 1.16 (d, *J* = 6.8 Hz, 6H, -CH(CH3)2), 2.24 (s, 3H, Ar-CH3), 2.47-2.49 (q, *J* =8.05 Hz, 2H, -NH-C(O)-CH_2-CH_3), 2.78-2.82 (m, 1H, -CH(CH3)2), 5.43 (s, 2H, -CH2-), 6.68 (s, 1H, Ar-H), 6.71 (d, *J* = 7.5 Hz, 2H, Ar-H), 12.50 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 8.9, 21.1, 23.7, 28.1, 33.3, 63.8, 110.1, 112.7, 120.3, 138.8, 150.0, 157.4, 159.4, 160.3, 172.3;

MS m/z calculated $C_{16}H_{21}N_3O_2S$: 319.4230, found: 318.1573 [M^+].

2,2-dichloro-N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)acetamide (8g)

Off white solid; Yield 85-86 %; m.p.231-233 °C;

IR (vmax, KBr): 3265 (-NH stretching), 2953 (C-H stretching), 1712 (C=O), 1591 (N-H bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (d, *J* = 6.9 Hz, 6H, -CH(CH3)2), 2.24 (s, 3H, Ar-CH3), 2.76-2.81 (m, 1H, -CH(CH3)2), 5.22-5.47 (s, 2H, -CH2-), 6.65 (s, 1H, Ar-H), 6.68 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.25 (s, 1H, -$CHCl_2$), 12.58 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 23.7, 33.3, 63.8, 66.3, 110.1, 112.8, 120.2, 120.5, 138.7, 149.9, 154.3, 157.3, 169.6;

MS m/z calculated $C_{15}H_{17}Cl_2N_3O_2S$: 374.2800, found: 374.0618 [M^+].

4-chloro-N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)butanamide (8h)

Off white solid; Yield 87-88 %; m.p.240-242 °C;

IR (vmax, KBr): 3263 (-NH stretching), 2947 (C-H stretching), 1693 (C=O), 1573 (N-H bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (d, *J* = 6.9 Hz, 6H, -CH(CH3)2), 2.02-2.03 (t, *J* =6.5 Hz, 2H, -CH_2-), 2.62-2.65 (t, *J* =7.3 Hz, 2H, -CH_2-), 3.66-3.69 (t, *J* =6. Hz, 2H, -CH_2-), 2.24 (s, 3H, Ar-CH3), 2.76-2.81 (m, 1H, -CH(CH3)2), 5.43 (s, 2H, -CH2-), 6.65 (s, 1H, Ar-H), 6.71 (d, *J* = 7.50 Hz, 2H, Ar-H), 8.53 (d, *J* = 7.45 Hz, 2H, Ar-H), 12.60 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 23.7, 27.2, 32.0, 33.3, 44.6, 63.8, 110.1, 112.7, 120.3, 138.7, 150.0, 154.3, 157.4, 160.5, 169.6;

MS m/z calculated $C_{17}H_{22}ClN_3O_2S$: 367.8920, found: 392.2711 [M + Na].

N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)pivalamide (8i)

Off white solid; Yield 84-85 %; m.p.218-220 °C;

IR (vmax, KBr): 3255 (-NH stretching), 2949 (C-H stretching), 1693 (C=O), 1579 (N-H bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15 (d, *J* = 6.95 Hz, 6H, -CH(CH3)2), 1.24 (s, 3H, -C(O)-C(CH3)3), 2.24 (s, 3H, Ar-CH3), 2.76-2.82 (m, 1H, -CH(CH3)2), 5.44 (s, 2H, -CH2-), 6.65 (d, *J* = 7.5 Hz, 2H, Ar-H), 6.65 (s, 1H, Ar-H), 12.75 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 23.7, 26.4, 33.3, 63.7, 64.0, 110.0, 112.7, 120.3, 138.8, 150.0, 154.3, 157.5, 160.2, 169.7;

MS m/z calculated $C_{18}H_{25}N_3O_2S$: 347.4770, found: 346.1923 [M^+].

2,2,2-trifluoro-N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)acetamide (8j)

Off white solid; Yield 84-85 %; m.p.214-216 °C;

IR (vmax, KBr): 3172 (-NH stretching), 2953 (C-H stretching), 1662 (C=O), 1585 (N-H bending), and 1168 (C-F bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (d, *J* = 6.9 Hz, 6H, -CH(CH3)2), 2.24 (s, 3H, Ar-CH3), 2.76-2.82 (m, 1H, -CH(CH3)2), 5.23 (s, 1H, -CH2-), 5.44 (s, 1H, -CH2-), 6.65 (d, *J* = 7.5 Hz, 2H, Ar-H), 6.73 (s, 1H, Ar-H), 12.65 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 23.7, 33.3, 63.9, 110.1, 112.7, 115.4, 120.3, 138.8, 150.0, 154.4, 157.4, 159.9, 168.6;

MS m/z calculated: $C_{15}H_{16}F_3N_3O_2S$: 359.3672, found: 358.1146 [M^+].

N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)isobutyramide (8k)

Off white solid; Yield 86-87 %; m.p.216-218 °C;

IR (vmax, KBr): 3151 (-NH stretching), 2947 (C-H stretching), 1689 (C=O), 1573 (N-H bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.12 (d, *J* = 6.9 Hz, 6H, -CH(CH3)2), 1.15 (d, *J* = 6.9 Hz, 6H, -C(O)-CH(CH3)2), 2.24 (s, 3H, Ar-CH3), 2.49-2.50 (m, 1H, -C(O)-CH(CH3)2), 2.76-2.81 (m, 1H, -CH(CH3)2), 5.43 (s, 2H, -CH2-), 6.68 (d, *J* = 7.57 Hz, 2H, Ar-H), 6.71 (s, 1H, Ar-H), 12.52 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 18.9, 21.1, 23.7, 33.3, 33.8, 63.7, 110.1, 112.7, 120.3, 138.8, 150.0, 157.4, 159.9, 161.1, 175.4;

MS m/z calculated $C_{17}H_{23}N_3O_2S$: 333.4500, found: 332.1712 [M^+].

N-(5-((4-isopropyl-3-methylphenoxy)methyl)-1,3,4-thiadiazol-2-yl)-1-methylcyclohexane-1-carboxamide (8l)

Off white solid; Yield 83-84 %; m.p.240-242 °C;

IR (vmax, KBr): 3265 (-NH stretching), 2949 (C-H stretching), 1695 (C=O), 1591 (N-H bending) cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15 (d, *J* = 6.9 Hz, 6H, -CH(CH3)2), 1.29 (s, 3H, -C(O)-CCH3), 1.45 (q, *J* = 6.9 Hz, 2H, -CH2(CH2)CH2-), 1.53 (q, *J* = 6.9 Hz, 4H, -CH2(CH2)CH2-), 1.66 (t, *J* = 6.85 Hz, 4H, -CH2(CH2)CH2-), 2.24 (s, 3H, Ar-CH3), 2.77-2.81 (m, 1H, -CH(CH3)2), 5.22 (s, 1H, -CH2-), 5.45 (s, 1H, -CH2-), 6.66 (d, *J* = 7.50 Hz, 2H, Ar-H), 6.71 (s, 1H, Ar-H), 12.68 (s, 1H, Ar-CO-NH-);

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 22.2, 23.7, 33.4, 34.2, 35.4, 45.2, 63.8, 64.1, 110.1, 112.7, 120.2, 138.8, 150.0, 154.3, 157.5, 160.0, 169.7;

MS m/z calculated $C_{21}H_{29}N_3O_2S$: 387.5420, found: 410.2355 [M + Na].

Biological screening

***In vitro* α-amylase inhibition**

The in vitro antidiabetic activity of all newly synthesized compounds (**8a-8l**) and the positive control (acarbose) was evaluated using the α-amylase assay at concentrations ranging from 62.5 to 500 µg/mL. Among these, compounds **8a, 8e, 8j,** and **8l** exhibited significant antidiabetic properties when compared with the positive control. Table 2 presents the results of this assay, with the findings expressed as the IC_{50} values.

Table 2: *In vitro* α-amylase activity of synthesized thiadiazoles (8a-8l)

Compound	α-amylase Inhibition	Compound	α-amylase Inhibition
	IC_{50} ± S.D. (µg/mL)		IC_{50} ± S.D. (µg/mL)
8a	**29.96 ± 0.015**	**8h**	52.17 ± 0.142
8b	37.99 ± 0.374	**8i**	36.26 ± 0.214
8c	43.13 ± 0.123	**8j**	**31.86 ± 0.334**
8d	36.18 ± 0.034	**8k**	38.38 ± 0.312

8e	**31.75 ± 0.471**	**8l**	**29.25 ± 0.183**
8f	39.66 ± 0.097	**Acarbose**	25.78 ± 0.136
8g	66.48 ± 0. 844		

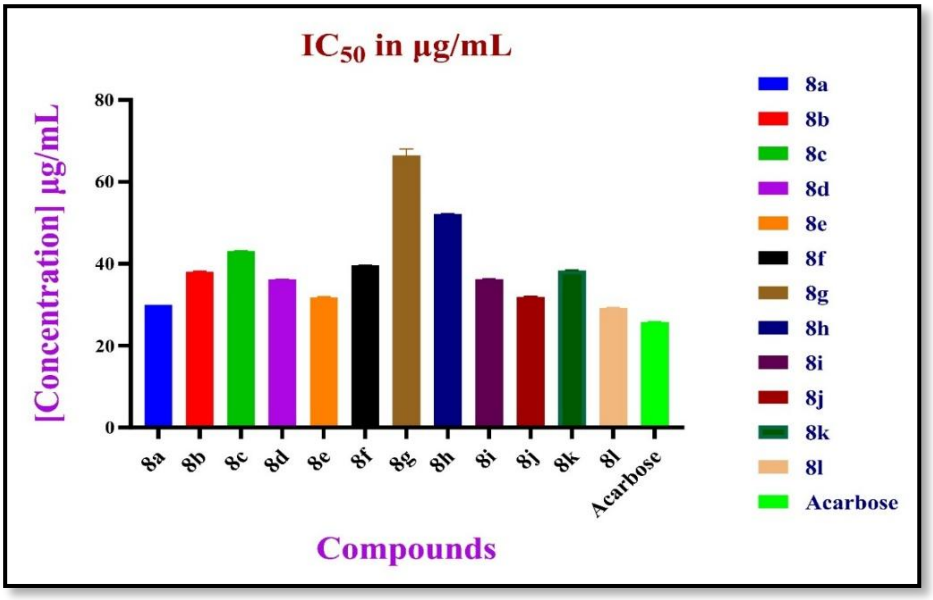
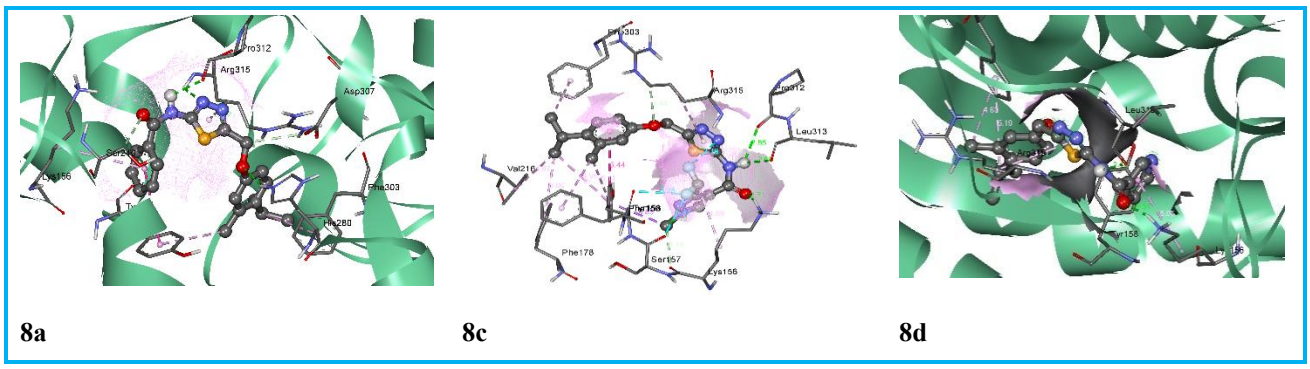


Fig.3. Graphical representation of Antidiabetic activity IC_{50} values in µg/mL.

Molecular docking

AutoDock4.2 software [26] was employed to conduct molecular docking simulations on S. cerevisiae isomaltase (PDB ID: 3A47). The protein was prepared for docking by removing ligand residues and water molecules and, then incorporating polar hydrogens and Kollman charges. The prepared protein was stored in the pdbqt format for subsequent grid generation. A grid box was established with dimensions of 40, 40, and 40 points for the x, y, and z-axes, respectively, utilizing 1 Å spacing. The center of the grid box was positioned at 25.879, -0.282, and 12.152 Å (x, y, and z, respectively). Each molecule underwent 10 runs, with up to 10 conformers per ligand. Marvin sketch [27] was utilized to develop and optimize the 3D structures of the ligands to their lowest energy conformers. Autodock_vina_1_1_2_win32 calculated the docking results as binding energy. The DS visualizer tool by Biovia [28] was employed to examine the interactions between 1,3,4-thiadiazole derivatives and 3A47.

The active site of the receptor revealed binding residues Arg315, Pro312, His280, Phe303, and Tyr158. Several compounds from this study exhibited strong conventional hydrogen bonds with Arg315, His280, and Phe303, as well as carbon hydrogen bonds with Asp307. Compounds **8a** and **8d** demonstrated the highest binding energy at -9.7 kcal/mol, while the remaining compounds exhibited binding energies ranging from -8.2 − -9.5 kcal/mol. The docked images are presented in **Fig.4** and **Fig.5**.



8a **8c** **8d**

Fig.4. Representation of binding interactions of 8a, 8c, and 8d in active site of isomaltase from S. cerevisiae enzyme (PDB ID: 3A47).

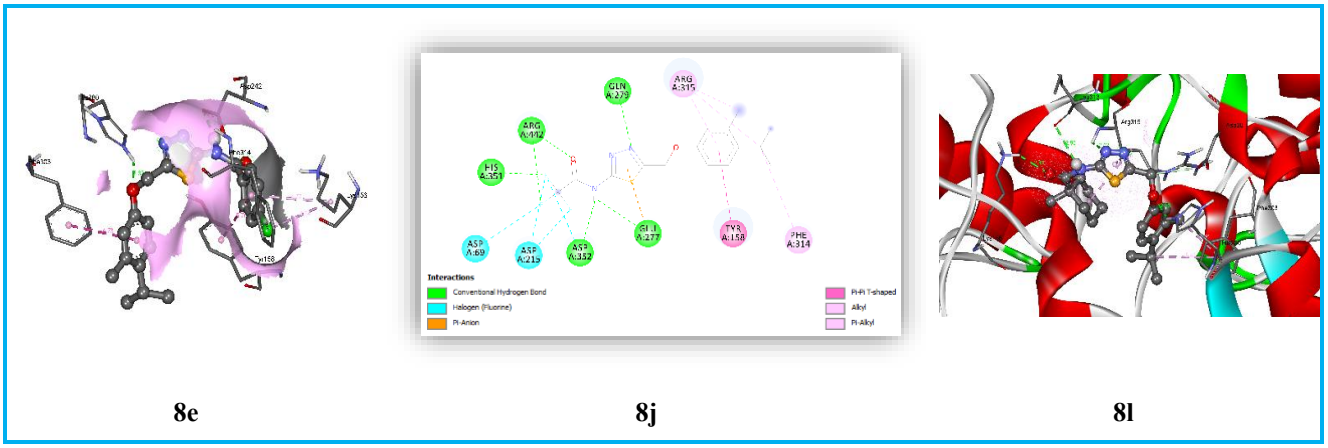


Fig.5. 3D binding interactions representation of 8e, 8j, and 8l in active site of isomaltase from S. cerevisiae enzyme (PDB ID: 3A47).

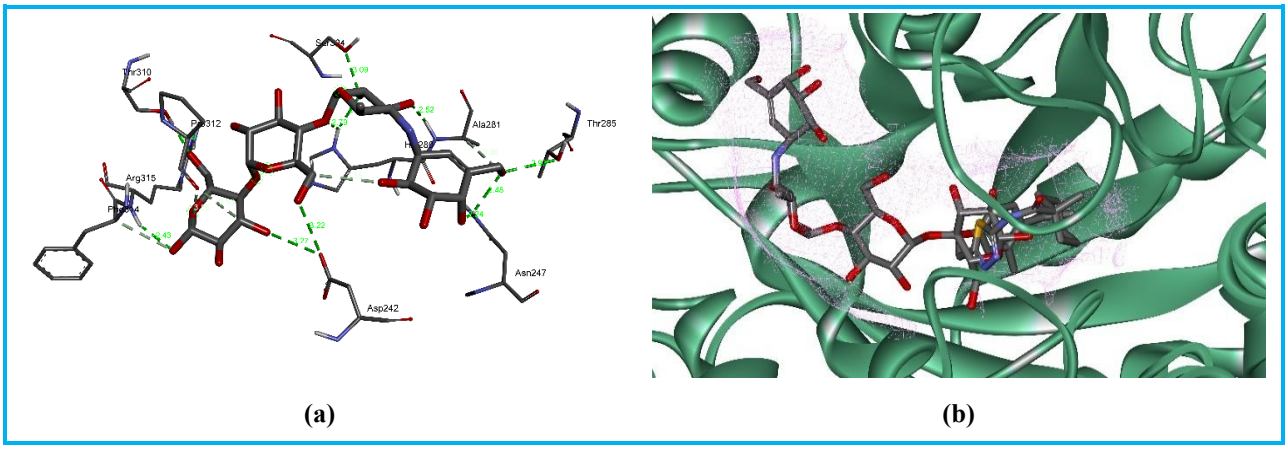


Fig.6. (a) 3D binding interactions representation of Acarbose in binding pocket (b) 3D overlay of docked poses of the compound 8l and the reference standard Acarbose in the active site of isomaltase from S. cerevisiae enzyme (PDB ID: 3A47).

Several compounds (**8a, 8c, 8d, 8e, 8j,** and **8l**) exhibited significant in vitro antidiabetic activities. Molecular docking studies of these compounds revealed two to five conventional and carbon-hydrogen bond interactions. *In silico* analysis confirmed that the amide group of 1,3,4-thiadiazole ligands effectively inhibited isomaltase from S. cerevisiae. Consequently, comprehensive docking investigations identified 1,3,4-thiadiazole of p-thymol as a promising candidate for potent antidiabetic agents. A summary of molecular docking results is presented in Table 3.

Table 3. Molecular docking results of synthesized thiadiazole compounds (8a-8l)

Compound	Binding Energy (Kcal/mol)	No. of H-bonds	H-Bond distance (Å)	Binding Residues
8a	**-9.7**	5	2.43, 2.68, 2.79, 3.44, 3.63	Arg315, Pro312, His280, Asp307, Ser240, Phe303, Tyr158, Lys156
8b	-8.2	4	2.27, 2.39, 2.47, 2.73	Arg315, Arg442, Asp352, Phe303, Phe314, Tyr316, Glu277, Gln279
8c	**-9.2**	5	2.66, 2.71, 2.85, 3.15, 3.16	Arg315, Pro312, Ser157, Phe303, Phe159, Phe178, Tyr158, Lys156, Leu313, Val216

8d	**-9.7**	2	2.31, 2.42	Arg315, Phe303, Tyr158, Lys156, Leu313
8e	**-9.5**	2	2.27, 2.33	His280, Asp242, Phe303, Phe314, Tyr158, Lys156
8f	-8.3	2	1.95, 2.03	Asp242, Ser241, Phe159, Phe303, Tyr158, Lys156
8g	-8.7	3	2.19, 2.40, 2.68	Arg315, Arg442, Phe303, Tyr316, Glu277, Gln279
8h	-8.3	4	2.35, 2.42, 2.45, 2.72	Arg315, His280, Phe303, Tyr158, Lys156, Leu313
8i	-8.5	4	2.14, 2.57, 2.60, 2.97	Arg315, Arg442, His351, Asp352, Phe178, Tyr72, Tyr316, Glu277, Gln279
8j	**-9.1**	5	2.10, 2.12, 2.45, 2.48, 2.57, 2.66, 2.96	Arg315, Arg442, His351, Asp352, Asp215, Asp69, Phe314, Tyr158, Glu277, Gln279
8k	**-9.3**	3	2.24, 2.60, 2.75	Arg315, Arg442, His351, Asp352, Phe178, Tyr72, Tyr316, Glu277
8l	**-9.5**	5	2.26, 2.49, 2.62, 2.93, 3.53	Arg315, Asp307, His280, Phe303, Lys156, Leu313
Acarbose	**-9.2**	10	2.24, 2.37, 2.20, 2.43, 2.48, 2.19, 2.52, 2.79, 2.93, 3.22, 3.27, 3.09, 3.35, 3.58	Arg315, Ser304, Asn247, His280, Asp242, Ala281, Phe314, Thr285, Thr310, Pro312

Physicochemical, pharmacokinetic and ADME predictions

Computational studies were conducted utilizing the SwissADME online tool (www.swiss.adme.ch) [29] to evaluate the physicochemical, pharmacokinetic, and ADME properties. The results of the computational analyses are presented in Table 4. These findings indicate that all the synthesized thiadiazole compounds exhibit moderate to favorable physicochemical characteristics. Furthermore, none of the compounds violated Lipinski's Rule of Five. The molecular weights of all the synthesized thiadiazole are between 305.4-421.46 g/mol and the TPSA range from 92.35 to 110.17 Å and the attained TPSA values are used to calculate percentage of absorption (% Abs) according to the reported equation (% Abs = 109 − [0.345 × TPSA]) [30]. The newly synthesized thiadiazole compounds exhibited promising physicochemical properties for effective oral absorption, with percentage absorption (% ABS) values ranging from 70.99 to 77.14 %. Computational analysis of the synthesized thiadiazoles revealed their favorable pharmacokinetic characteristics [31].

Table 4. *In silico* physicochemical and pharmacokinetic parameters for good oral bioavailability of synthesized thiadiazole derivatives (8a-8l)

Entry	MW[a]	H-bond acceptors[b]	H-bond donors[c]	MLogP[d]	Lipinski violations[e]	TPSA[f]	% ABS[g]	Rotatable bonds	Bioavailability score
Rule	**<500**	**≤10**	**≤5**	**≤4.15**	**0**	**<160**	**100 %**	**≤10**	
8a	367.46	4	1	3.06	0	92.35	77.1393	7	0.55
8b	305.4	4	1	1.43	0	92.35	77.1393	6	0.55
8c	421.46	7	1	2.57	0	110.17	70.9914	8	0.55
8d	368.45	5	1	2.02	0	105.24	72.6922	7	0.55
8e	401.91	4	1	3.56	0	92.35	77.1393	7	0.55

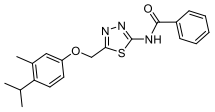
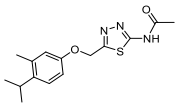
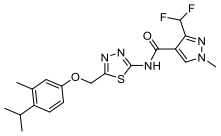
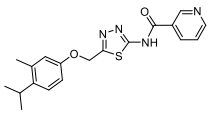
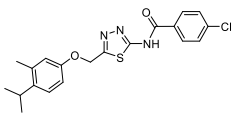
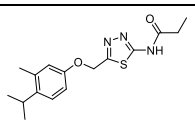
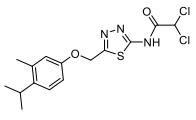
8f	319.42	4	1	2.09	0	92.35	77.1393	7	0.55
8g	374.29	4	1	2.33	0	92.35	77.1393	7	0.55
8h	367.89	4	1	2.57	0	92.35	77.1393	9	0.55
8i	347.48	4	1	2.57	0	92.35	77.1393	7	0.55
8j	359.37	7	1	2.21	0	92.35	77.1393	7	0.55
8k	333.45	4	1	2.33	0	92.35	77.1393	7	0.55
8l	387.54	4	1	3.25	0	92.35	77.1393	7	0.55

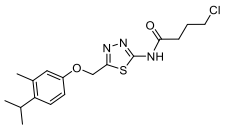
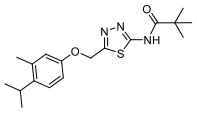
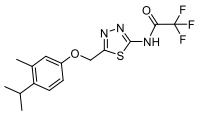
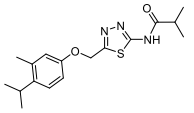
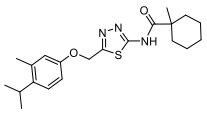
a Molecular weight; **b** Number of Hydrogen Bond Acceptors; **c** Number of Hydrogen Bond Donors; **d** Calculated Lipophilicity (MLog Po/w); **e** Violations from Lipinski's Rule; **f** Topological polar surface area; **g** Percentage of absorption (% Abs = 109 − [0.345 × TPSA]).

In silico toxicity prediction

The preADMET online platform (http://preadmet.bmdrc.org/) was employed to conduct *in silico* toxicity analysis [32]. These assessments indicated that, with the exception of 8h, all the synthesized thiadiazole compounds exhibited mutagenic properties. Furthermore, while compound 8c was predicted to be carcinogenic in rats, all other synthesized thiadiazole compounds, except **8g** and **8j**, were found to be non-carcinogenic in mice. The study also revealed that the majority of the synthesized compounds demonstrated a minimal risk of cardiotoxicity, with the exceptions being **8a**, **8c**, **8d**, and **8e**.

Table 5. *In silico* toxicity analysis of synthesized thiadiazoles (8a-8l)

Sr. No.	Compounds code	Structure	Ames test	Carcino Mouse	Carcino Rat	hERG inhibition
1)	**8a**		Mutagen	Negative	Negative	Medium risk
2)	**8b**		Mutagen	Negative	Negative	Low risk
3)	**8c**		Mutagen	Negative	Positive	Medium risk
4)	**8d**		Mutagen	Negative	Negative	Medium risk
5)	**8e**		Mutagen	Negative	Negative	Medium risk
6)	**8f**		Mutagen	Negative	Negative	Low risk
7)	**8g**		Mutagen	Positive	Negative	Low risk

8)	**8h**		Non-mutagen	Negative	Negative	Low risk
9)	**8i**		Mutagen	Negative	Negative	Low risk
10)	**8j**		Mutagen	Positive	Negative	Low risk
11)	**8k**		Mutagen	Negative	Negative	Low risk
12)	**8l**		Mutagen	Negative	Negative	Low risk

Carcino: Carcinogenicity; hERG: human ether-a-gogo-related gene

4. CONCLUSION

This investigation focuses on the development of novel thiadiazole hybrids derived from natural monoterpenoids, specifically p-thymol, to mitigate these adverse effects. Researchers have designed and synthesized a newer series of 1,3,4-thiadiazole p-thymol hybrids utilizing established methodologies. Molecular docking analyses revealed strong interactions between compounds **8a, 8c, 8d, 8e, 8j, 8l,** and acarbose and the active site of the S. cerevisiae enzyme. These interactions occur through conventional hydrogen bonds with Arg315, His280, and Phe303, as well as carbon-hydrogen bonds with Asp307. Based on these findings, all the synthesized thiadiazoles underwent *in vitro* antidiabetic activity screening via an α-amylase inhibition assay. The investigation determined that compounds **8a, 8e, 8j,** and **8l** exhibited exceptional activities. The research concluded that the synthesized 1,3,4-thiadiazole hybrids demonstrate significant potential as candidates for the future development of oral chemotherapeutic agents, as supported by *in silico* physicochemical, pharmacokinetic, and ADME predictions.

CRediT authorship contribution statement

Dattatraya S. Kale: Writing-original draft, Methodology, editing, Formal analysis, Conceptualization. **Rahul T. Bhoi:** Methodology, Data curation, Computational studies. **Ganesh R. Borse:** Writing-review & editing, Investigation. **Santosh B. Katariya:** Validation and investigation. **Sanjay B. Sonawale:** Supervision, Resources, Project Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

Data availability

We have shared all the data in the attached supporting file

Acknowledgments

The authors are grateful to the Chattrapati Shahu Maharaj National Research Fellowship-2023 (SARTHI) and the DES's Kirti M. Doongursee College, Mumbai University, Dadar (W), Mumbai, Maharashtra.

Funding information

Funding source CSMNRF-2023/2024-25/1773 (**SARTHI Fellowship)**

Word count: 5,488 words, excluding references

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