

Bryophyllum pinnatum extract mediated Biogenic ZnCuFe₂O₄ nanoparticles catalyzed benzoxazole/benzothiazole synthesis: Biological and Computational Insights

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***Bryophyllum pinnatum* extract mediated Biogenic ZnCuFe₂O₄ nanoparticles catalyzed benzoxazole/benzothiazole synthesis: Biological and Computational Insights**

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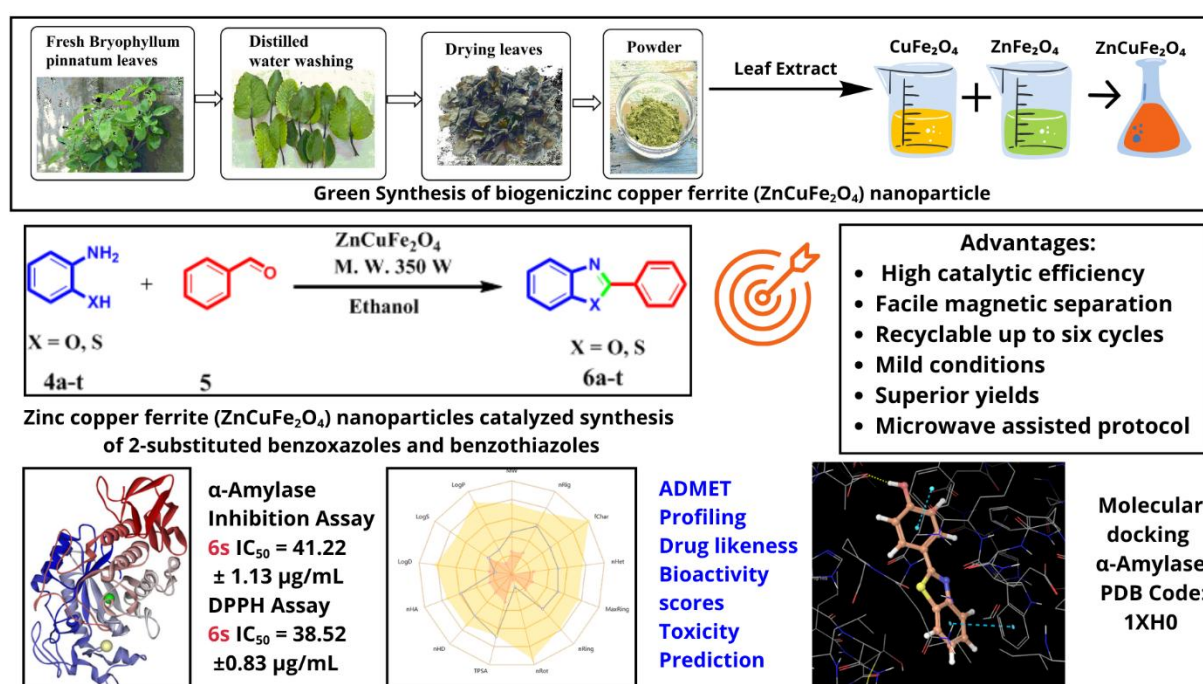
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Graphical abstract



Abstract

Zinc copper ferrite nanoparticles ($\text{ZnCuFe}_2\text{O}_4$) have been prepared by green synthesis of biogenic zinc copper ferrite nanoparticles using aqueous leaf extract of *Bryophyllum pinnatum* which served as robust heterogeneous catalyst for the synthesis of biologically active 2-substituted benzoxazoles and benzothiazoles. The designed synthetic protocol furnished desired products (**6a-t**) in superior yields under mild reaction conditions under microwave irradiation. All the synthesized compounds demonstrated moderate to excellent anti-diabetic activity employing α -amylase assay inhibition with IC_{50} values ranging 41.22 ± 1.13 to 48.74 ± 1.01 $\mu\text{g/mL}$ compared to standard drug acarbose (IC_{50} values = 34.10 ± 0.90). The compounds **6s** is found to be the most potent with IC_{50} value of 41.22 ± 1.13 $\mu\text{g/mL}$ followed by **6t**, **6q** and **6r** with IC_{50} values 44.52 ± 0.98 $\mu\text{g/mL}$, 45.93 ± 1.00 $\mu\text{g/mL}$ and 48.74 ± 1.01 $\mu\text{g/mL}$ respectively. Moreover, the compounds exhibited significant antioxidant activity with IC_{50} values for active compounds ranging 38.52 ± 0.03 to 47.20 ± 1.40 $\mu\text{g/mL}$ compared to standard ascorbic acid (IC_{50} value = 32.08 ± 0.70 $\mu\text{g/mL}$). The ADMET profiling studies revealed that all the synthesized compounds exhibited superior pharmacological characteristics, interactions with biological targets and minimal violations from Lipinski's rule of five. The molecular docking analysis confirmed the mode of action of target compounds *via* inhibition of human pancreatic α -amylase.

Keywords Zinc copper ferrite nanoparticles, benzoxazole, benzothiazole, α -amylase, anti-diabetic, biogenic

1. Introduction

In the current century, human beings are still fighting to conquer both lifestyle and infectious diseases. Before the COVID-19 pandemic, human beings were assumed to have conquered infectious diseases, and major clinical challenges were considered to be lifestyle disorders [1]. Diabetes mellitus (DM) is a pathological condition characterized by hyperglycaemia encountered in younger and adults as a result of genetic risks, insulin resistance, sedentary life style, poor nutrition choices, and autoimmune damage. The current treatments for DM involve lifestyle changes along with anti-diabetic medications and insulin therapy in chronic stages [2]. The marketed/available anti-diabetic drugs include metformin, DPP-4 inhibitors such as sitagliptin and SGLT-2 inhibitors such as empagliflozin, sulfonylureas, thiazolidinediones, injectables GLP-1 receptor agonists such as semaglutide [3]. The currently used anti-diabetic drugs suffer from several drawbacks such as gastrointestinal problems, hypoglycaemia, lactic acidosis leading to kidney damage, weight gain or loss, urinary disorders, etc [4]. Owing to this, there is a surge to search for new scaffolds with promising anti-diabetic potential.

Benzoxazoles and benzothiazoles are medicinally valuable classes of benzo-fused azoles which have demonstrated a broad array of elegant pharmacological activities by virtue of their unique chemical architecture [5]. Both benzoxazoles and benzothiazoles are encountered in bioactive scaffolds of natural and synthetic origin and serve as intermediates and synthons, as

shown in **Fig. 1** [6]. Benzoxazoles and benzothiazoles are known to exhibit a wide spectrum of activities such as anti-microbial [7], anti-fungal [8], anti-diabetic [9], anti-oxidant [10], anti-inflammatory [11], anti-tumor [12], anti-tubercular [13], anti-platelet [14] and anthelmintic activities [15]. Additionally, they are used for making optical brighteners, detergents, probes for gas sensing application and serves as core skeleton of marketed drugs like Flunoxaprofen – nonsteroidal anti-inflammatory drug (NSAID), tafamidis – effective against amyloid diseases [16], chlorzoxazone – muscle relaxant, calcimycin – antibiotic [17], nocarbenzoxazole G – anti-cancer [18], pesudopteroxazole–anti-tubercular [19], riluzole – glutamate antagonist, pramipexole – anti- parkinson’s disease [20], ethoxzalamide-carbonic anhydrase inhibitor used as diuretic, against ulcers and dithiazanine – anthelmintic [21].

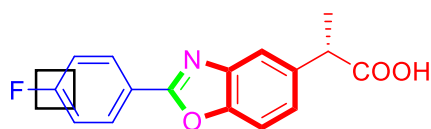
The traditional reaction between aromatic aldehydes and 2-aminophenol/2-amino thiophenol to furnish 2-substituted benzoxazoles/benzothiazoles has been extensively studied for developing efficient and eco-benign protocols [22]. Recent protocols involved the use of ionic liquids [23], ionic liquid phase supported catalysts [24], transition metals [25] Samarium(III) triflate [26], metal nanoparticles [27], gel catalysis [28], acid catalyst [29], surface functionalized magnetic nanoparticles [30] and *N*-heterocyclic carbene (NHC) complexes [31]. The scrutiny of the literature revealed that ferrites are being underexplored for their catalytic potential for the synthesis of bioactive heterocycles [32].

The plant *Bryophyllum pinnatum* is a member of family- Crassulaceae and commonly found in India and China [33]. The plant leaves is a rich source of diverse secondary metabolites such as glycosides, phenolic acids, alkaloids, triterpenes, steroids, flavonoids, terpenoids, fatty acids, proteins/peptides and vitamins [34]. These phytochemicals provide green medium, alkaline pH for preparation of nanoparticles and also served as stabilizer and capping agents. To the best of our knowledge, there is no report on green synthesis of biogenic zinc copper ferrite

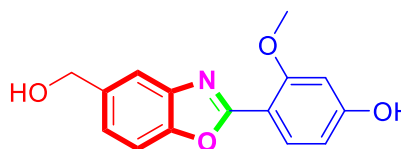
nanoparticles using *Bryophyllum pinnatum*, their structural, magnetic and morphological characterization and catalytic application.

Ferrite nanoparticles are promising factor as heterogeneous catalysts due to their good catalytic activity, high surface area, thermal stability, and magnetic properties [35]. Ferrites also have broad potential uses in catalysis, electronics, magnetic separation, sensors, and environmental remediation. Their distinctive magnetic and physicochemical features also enable them to be potential materials for energy-related and biomedical applications [36].

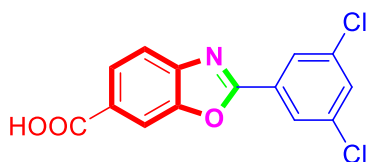
In view of above-mentioned discussion and in continuation of our work on rational design of bioactive scaffolds, we here in report preparation and characterisation of zinc copper ferrite ($\text{ZnCuFe}_2\text{O}_4$), synthesis of benzoxazoles and benzothiazoles using $\text{ZnCuFe}_2\text{O}_4$ nanoparticles, in vitro α -amylase inhibitory and anti-oxidant activities, and in silico ADMET and molecular docking against the crystal structure of human pancreatic α -amylase [37].



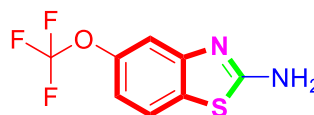
Flunaproxen
(anti-inflammatory)



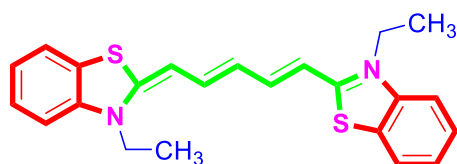
Nocarbenzoxazole G
(Anti-cancer)



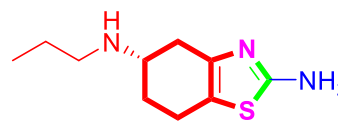
Tafamidis
(Deadly neurodegenerative disease)



Riluzole
(glutamate antagonist)



Dithiazanine
(anthelmintic)



pramipexole
(anti-parkinson's disease)

Fig 1: Examples of marketed drug containing benzoxazoles and benzothiazoles

2. Materials and methods

2.1. Materials

The chemicals required Zinc acetate dihydrate [$\text{Zn}(\text{CH}_3\text{COOH})_2 \cdot 2\text{H}_2\text{O}$], cupric nitrate trihydrate [$\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$], iron chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), zinc ferrite (ZnFe_2O_4), copper ferrite (CuFe_2O_4), aromatic aldehydes, 2-aminophenol, 2-aminothiophenol, and ethyl acetate were obtained from Spectrochem Pvt. Ltd. and were used without further purification. The alkali (NaOH , 2 M) was used as a precipitant. Deionized water was utilized throughout the preparation, and ethanol was employed for washing steps to ensure minimal contamination and to remove residual ions and organic impurities. The other reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH), alpha-amylase enzyme, soluble starch, 3,5-dinitrosalicylic acid (DNSA), and sodium phosphate buffer, were procured from a local supplier. All the chemicals were used as received without further purification.

2.2 Methods

The leaves of *B. pinnatum* were collected from the local area of place Sangola (District Solapur) of Maharashtra state in the month of June 2025 and shade dried. The plant was authenticated by botanists. The dried leaves were coarsely powdered (passed through sieve no. 40). Approximately 5 g of powdered leaf material was extracted by using Soxhlet extraction apparatus with 100 ml of distilled water. The crude extract obtained was evaporated at 45 °C,

then dried and stored in airtight container. All other chemicals, reagents and solvents were procured from local supplier and employed without further purification.

The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz and 100 MHz spectrometer, respectively, using TMS as an internal standard while using CDCl_3 as solvent. FTIR spectra were recorded using KBr pellets on a Bruker ALPHA II spectrometer. Mass spectra were recorded using a Thermo TSQ Quantum Discovery Max spectrometer. The UV-Vis spectra were recorded using UV-vis – BioSpectrometer fluorescence. SW-Version: 4.2.3.0. The X-ray diffractograms were obtained employing a Rigaku Ultima IV diffractometer. The SEM analysis was performed on SEM - JSM-IT200, and TEM analysis was done using TEM - TEM-HT7800 Series (120 kV) Hitachi. The VSM studies were done on a vibrating sample magnetometer - Quantachrome v5.25. The BET surface analysis was carried out on a Quantachrome BET Instrument v5.25. The mass spectra were scanned by a Waters ACQUITY QDa Mass spectrometer.

2.3 Preparation of zinc ferrite, copper ferrite and zinc copper ferrite nanoparticles

2.3.1 Synthesis of zinc ferrite nanoparticles (ZnFe_2O_4 NPs)

Zinc ferrite (ZnFe_2O_4) nanoparticles were synthesized by a simple co-precipitation using zinc acetate dihydrate [$\text{Zn}(\text{CH}_3\text{COOH})_2 \cdot 2\text{H}_2\text{O}$] and Iron chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) as precursors. Stoichiometric amounts of zinc salt (2.195 g in 100 mL distilled water) and Iron salt (2.78 g in 100 mL distilled water), in a 1:1 molar ratio. The mixed solution was treated with 0.5 M NaOH in 30 mL *B. pinntaum* aqueous extract at 70 °C and stirred for 2 h to obtain the brownish precipitate. The precipitate was centrifuged, washed with deionized water and 70 %, ethanol and dried at 120 °C for 8 h. The dried powder was calcined at 400 °C for 4 h to obtain ZnFe_2O_4 nanoparticles as shown in **Fig. 2** [38].

2.3.2 Synthesis of copper ferrite nanoparticles (CuFe₂O₄ NPs)

Copper ferrite (CuFe₂O₄) nanoparticles were synthesized by co-precipitation using cupric nitrate trihydrate [Cu(NO₃)₂·3H₂O] and iron chloride hexahydrate (FeCl₃·6H₂O) as precursors. Stoichiometric amounts of copper salt (2.42 g in 100 mL distilled water) and iron salt (2.78 g in 100 mL distilled water), in a 1:1 molar ratio. The mixed solution was treated with 0.5 M NaOH in 30 mL *B. pinntaum* aqueous extract at 70 °C and stirred for 2 h to obtain the blackish precipitate. The precipitate was centrifuged, washed with deionized water and 70 % ethanol, and dried at 120 °C for 8 h. The dried powder was calcined at 400 °C for 4 h to obtain CuFe₂O₄ nanoparticles as shown in **Fig. 2** [39].

2.3.3 Synthesis of Zinc Copper ferrite nanoparticles (ZnCuFe₂O₄ NPs)

Zinc Copper ferrite (ZnCuFe₂O₄) nanoparticles were synthesized using co-precipitation by using zinc acetate dihydrate [Zn(CH₃COOH)₂·2H₂O], cupric nitrate trihydrate [Cu(NO₃)₂·3H₂O], and iron chloride hexahydrate (FeCl₃·6H₂O) as precursors. Stoichiometric amounts of zinc salt (2.195 g in 100 ml distilled water), copper salt (2.42 g in 100 ml distilled water) and iron salt (2.78 g in 100 ml distilled water), maintaining a molar ratio of Zn:Cu:Fe to 1:1:1. The mixed solution was treated with 0.5 M NaOH in 30 mL *B. pinntaum* aqueous extract at 70 °C and stirred for 2 h to obtain the blackish precipitate. The precipitate was centrifuged, washed with deionized water and 70 % ethanol, and dried at 120 °C for 8 h. The dried powder was calcined at 400 °C for 4 h to obtain ZnCuFe₂O₄ nanoparticles as shown in **Fig. 2** [40].

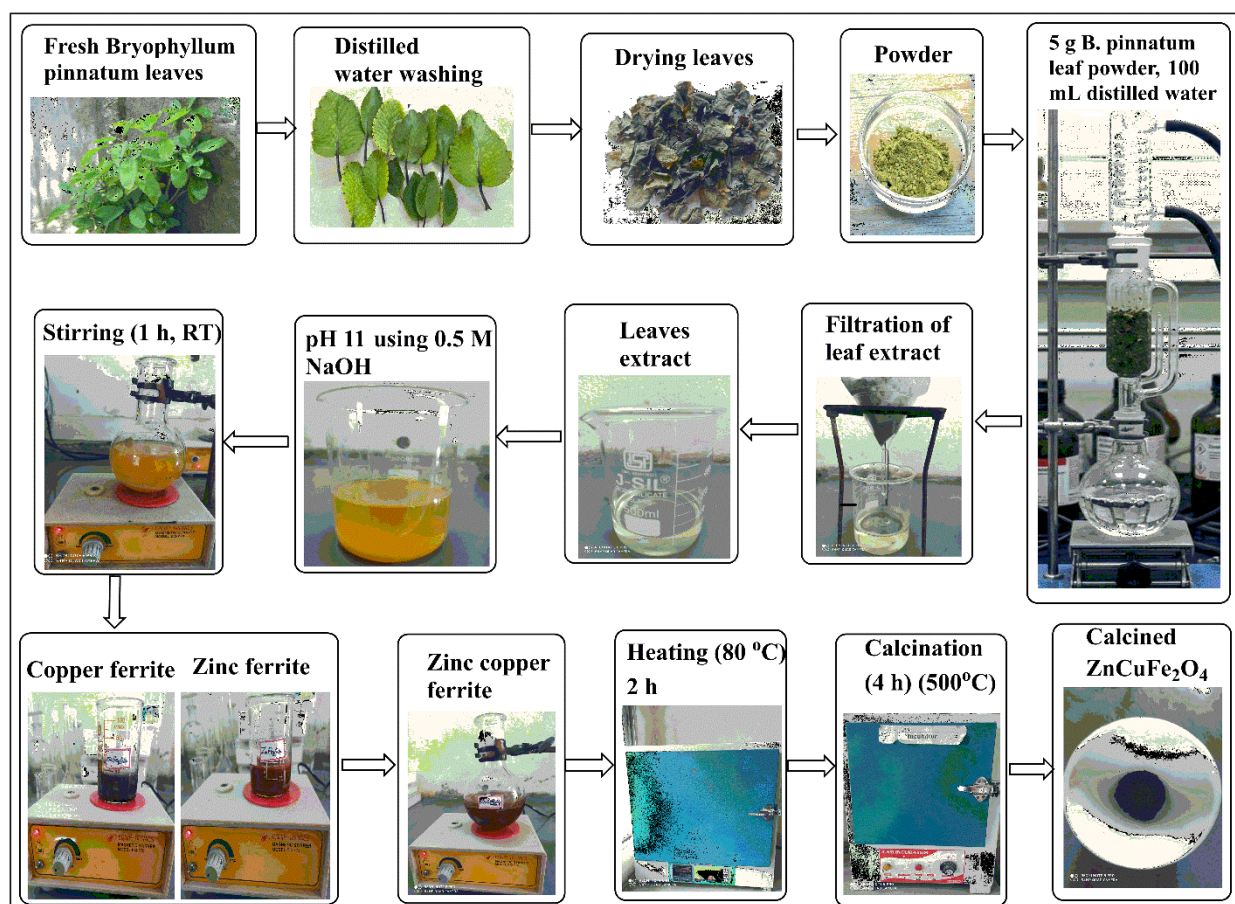


Fig 2: Flowsheet for preparation of biogenic zinc copper ferrite ($\text{ZnCuFe}_2\text{O}_4$) nanoparticles using using aqueous leaf extract of *Bryophyllum pinnatum*

2.3 General procedure for the synthesis of benzoxazoles and benzothiazoles (6a-t)

In a clean and oven-dried 50 mL capacity round-bottom flask, aromatic aldehydes (5 mmol) and 2-aminophenol/2-aminothiophenol (5 mmol) were added, followed by 100 mg $\text{ZnCuFe}_2\text{O}_4$ in ethanol. The reaction mixture was subjected to microwave irradiation at a power of 350 W and allowed to proceed until reaction completion, as monitored by thin layer chromatography (TLC). After completion of the reaction, the $\text{ZnCuFe}_2\text{O}_4$ nanoparticles were separated from the reaction matrix via magnetic separation using an external magnet. The reaction mixture was then cooled, poured onto crushed ice, and stirred vigorously to facilitate the formation of a solid

benzoxazole/benzothiazole. The crude product was then collected by filtration under vacuum, washed with cold water, and recrystallized from hot ethanol to obtain the pure benzoxazoles and benzothiazoles [31].

2.4 Spectral data of newly synthesized benzoxazoles and benzothiazoles (6a-t)

[1] 2-(3-nitrophenyl)benzo[d]oxazole (**6a**) [41]

M.F.: C₁₃H₈N₂O₃; Yield: 119 mg, 93 %; Colour: Yellow solid; M.P.: 145 °C

FTIR (KBr) ν (cm⁻¹): 3075, 1585, 1529, 1478, 1375, 1148; ¹H NMR (CDCl₃, 400 MHz): δ 8.79-8.25 (m, 4H, Ar), 7.71-6.92 (m, 4H, Ar) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 153.9, 152.6, 148.7, 137.4, 134.4, 134.0, 130.1, 129.9, 125.7, 123.3, 120.3, 115.9, 115.5 ppm; Elem. Anal.: C, 65.00 %; H, 3.36 %; N, 11.66 %; O, 19.98 %; found: C, 65.00 %; H, 3.35 %; N, 11.65 %; O, 19.97 %.

[2] 2-(anthracen-9-yl)benzo[d]oxazole (**6b**)

M.F.: C₂₁H₁₃NO; Yield: 143 mg, 91 %; Colour: Yellow solid; M.P.: 173 °C

FTIR (KBr) ν (cm⁻¹): 3052, 1658, 1486, 1161; ¹H NMR (CDCl₃, 400 MHz): δ 7.89 - 7.72 (m, 2H, Ar), 7.45 - 7.38 (m, 7H, Ar), 7.02 - 6.88 (m, 4H, Ar) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 193.0, 156.6, 152.4, 136.5, 135.2, 115.2, 115.8, 120.2, 123.5, 124.6, 124.7, 125.5, 125.7, 127.2, 127.4, 129.1, 129.3, 130.7, 131.0, 131.0, 131.3, 132.1, 135.2, 136.5, 152.4, 156.6 ppm; Elem. Anal.: C, 85.40 %; H, 4.44 %; N, 4.74 %; O, 5.42 %; found: C, 85.39 %; H, 4.43 %; N, 4.73 %; O, 5.41 %.

[3] 2-phenylbenzo[d]oxazole (**6c**) [42]

M.F.: C₁₃H₉NO; Yield: 098 mg, 90 %; Colour: White solid; M.P.: 106 °C

FTIR (KBr) ν (cm⁻¹): 2919, 1626, 1483, 1250; ¹H NMR (CDCl₃, 400 MHz): δ 7.93 (dd, J = 1.6, 2.8 Hz, 2H, Ar), 7.50 (dd, J =1.6, 1.2 Hz, 2H, Ar), 7.32-6.90 (m, 5H, Ar) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 157.1, 152.3, 135.8, 135.4, 131.6, 128.9, 128.8, 128.8, 120.1, 115.8, 114.9, 29.7 ppm; Elem. Anal.: C, 79.98 %; H, 4.65 %; N, 7.17 %; O, 8.20 %; found: C, 79.97 %; H, 4.63 %; N, 7.15 %; O, 8.19 %.

[4] *(Z)*-2-styrylbenzo[d]oxazole (**6d**) [43]

M.F.: C₁₅H₁₁NO; Yield: 113 mg, 92 %; Colour: Yellow solid; M.P.: 107 °C

FTIR (KBr) ν (cm⁻¹): 3042, 1584, 1484, 1153; ¹H NMR (CDCl₃, 400 MHz): δ 7.57-7.35 (m, 5H, Ar), 7.26-7.22 (m, 2H, Ar), 7.20-7.11 (m, 2H, Ar), 7.01 (t, 1H, olefinic), 6.99-6.87 (m, 1H, olefinic) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 158.2, 152.3, 144.0, 135.7, 135.5, 129.7, 128.9, 128.8, 128.5, 127.5, 120.0, 115.5, 114.9 ppm; Mass (EI) m/z [M⁺] Calculated 221, Observed 224; Anal.: C, 81.43 %; H, 5.01 %; N, 6.33 %; O, 7.23 %; found: C, 81.42 %; H, 5.00 %; N, 6.31 %; O, 7.22 %, found: C, 81.40 %; H, 5.00 %; N, 6.30 %; O, 7.20 %.

[5] 2-(pyridin-2-yl)benzo[d]oxazole (**6e**) [44]

M.F.: C₁₂H₈N₂O; Yield: 109 mg, 96 %; Colour: Brown solid; M.P.: 96 °C

FTIR (KBr) ν (cm⁻¹): 3055, 1589, 1512, 1487, 1182; ¹H NMR (CDCl₃, 400 MHz): δ 8.23-7.37 (m, 4H, Ar), 7.35-7.05 (m, 4H, Ar) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 192.8, 186.0, 179.1, 175.4, 165.6, 157.7, 150.1, 116.0, 67.1, 61.5, 60.0 ppm; Mass (EI) m/z [M⁺] Calculated 196, Observed 199; Elem. Anal.: C, 73.46 %; H, 4.11 %; N, 14.28 %; O, 8.15 %; found: C, 73.44 %; H, 4.10 %; N, 14.26 %; O, 8.14 %.

[6] 2-(thiophen-2-yl)benzo[d]oxazole (**6f**) [45]

M.F.: C₁₁H₇NOS; Yield: 112 mg, 93 %; Colour: Black solid; M.P.: 113 °C

FTIR (KBr) ν (cm⁻¹): 3097, 1592, 1426, 1174, 809; ¹H NMR (CDCl₃, 400 MHz): δ 7.51 (d, J =4 Hz, 2H, Ar), 7.29 (s, 1H, Ar), 7.20-7.00 (m, 4H, Ar) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 152.1, 149.7, 142.8, 135.0, 132.5, 130.7, 128.8, 127.9, 120.1, 115.7, 115.0 ppm; Elem. Anal.: C, 65.65 %; H, 3.51 %; N, 6.96 %; O, 7.95 %, S, 15.93%; found: C, 65.64 %; H, 3.50 %; N, 6.95 %; O, 7.94 %, S, 15.92 %.

[7] *(E)*-2-styrylbenzo[d]oxazole (**6g**) [46]

M.F.: C₁₅H₁₁NO; Yield: 116 mg, 94 %; Colour: Brown solid; M.P.: 93 °C

FTIR (KBr) ν (cm⁻¹): 3042, 1623, 1484, 1152; ¹H NMR (CDCl₃, 400 MHz): δ 7.57-7.39 (m, 5H, Ar), 7.38-6.87 (m, 4H, Ar), 6.95 (d, J =32 Hz, 1H, olefinic), 6.88 (d, J =8.0 Hz, 1H, olefinic) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 158.2, 152.3, 144.0, 135.7, 135.5, 129.7, 129.6, 129.1, 128.9, 128.8, 128.5, 127.5, 120.1, 120.0, 115.5, 114.9 ppm; Elem. Anal.: C, 81.43 %; H, 5.01 %; N, 6.33 %; O, 7.23 %; found: C, 81.42 %; H, 5.00 %; N, 6.32 %; O, 7.22 %.

[8] 2-(4-(benzyloxy)phenyl)benzo[d]oxazole (**6h**) [25]

M.F.: C₂₀H₁₅NO₂; Yield: 149 mg, 93 %; Colour: White solid; M.P.: 135 °C

FTIR (KBr) ν (cm⁻¹): 2918, 1636, 1576, 1260, 1165; ¹H NMR (CDCl₃, 400 MHz): δ 7.84 (d, J = 8.8 Hz, 4H, Ar), 7.44 -7.02 (m, 5H, Ar), 7.08 (d, J = 8.8 Hz, 4H, Ar), 5.15 (s, 2H, -CH₂) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 190.7, 163.7, 135.9, 132.0, 128.7, 128.3, 127.4, 115.1, 70.2 ppm; Elem. Anal.: C, 79.72 %; H, 5.02 %; N, 4.65 %; O, 10.62 %; found: C, 79.71 %; H, 5.01 %; N, 4.64 %; O, 10.61 %.

[9] 2-(4-chlorophenyl)benzo[d]oxazole (**6i**) [47]

M.F.: C₁₃H₈ClNO; Yield: 112 mg, 90 %; Colour: Brown solid; M.P.: 137 °C

FTIR (KBr) ν (cm^{-1}): 3046, 1585, 1490, 1198, 791; ^1H NMR (CDCl_3 , 400 MHz): δ 7.86 (d, $J=8.4$ Hz, 2H, Ar), 7.47 (t, 2H, Ar), 7.23 (d, $J=1.6$ Hz, 2H, Ar), 7.20 (d, $J=1.2$ Hz, 1H, Ar), 7.03-6.93 (m, 1H, Ar) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ 155.5, 152.3, 137.6, 135.1, 134.3, 129.9, 129.2, 129.2, 120.1, 115.8, 115.1 ppm; Elem. Anal.: C, 67.99 %; H, 3.51 %; Cl, 15.44 %; N, 6.10 %; O, 6.97 %; found: C, 67.98 %; H, 3.50 %; Cl, 15.43 %; N, 6.09 %; O, 6.95 %.

[10] 2-(ferrocenyl)benzo[d]oxazole (**6j**) [48]

M.F.: $\text{C}_{17}\text{H}_{13}\text{FeNO}$; Yield: 306 mg, 95 %; Colour: Black solid; M.P.: 150 °C

FTIR (KBr) ν (cm^{-1}): 2920, 1586, 1457, 1106, 492; ^1H NMR (CDCl_3 , 400 MHz): δ 7.77-7.36 (m, 4H, Ar), 4.80 (s, 2H, Ar), 4.62 (s, 2H, Ar), 4.28-4.16 (m, 5H, Ar) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ 178.9, 149.5, 136.6, 116.8, 101.1, 86.1, 69.9 ppm; Elem. Anal.: C, 67.36 %; H, 4.32 %; Fe, 18.42 %; N, 4.62 %; O, 5.28 %; found: C, 67.35 %; H, 4.31 %; Fe, 18.41 %; N, 4.61 %; O, 5.27 %.

[11] 2-(4-(benzyloxy)phenyl)benzo[d]thiazole (**6k**) [49]

M.F.: $\text{C}_{20}\text{H}_{15}\text{NOS}$; Yield: 162 mg, 90 %; Colour: White solid; M.P.: 150 °C

FTIR (KBr) ν (cm^{-1}): 3061, 2850, 1578, 1473, 1244, 747; ^1H NMR (CDCl_3 , 400 MHz): δ 7.49-7.33 (m, 7H, Ar), 7.03 – 6.94 (m, 5H, Ar), 6.93-6.76 (m, 1H, Ar), 5.07 (s, 2H, $-\text{CH}_2$) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ 167.8, 161.0, 159.1, 154.2, 146.2, 136.7, 136.3, 134.8, 133.8, 129.1, 128.6, 128.6, 128.3, 128.2, 128.0, 127.9, 127.5, 127.4, 126.7, 126.6, 126.2, 125.4, 124.8, 122.8, 121.6, 121.5, 120.6, 115.2, 114.9, 109.6, 70.1, 10.0, 69.9 ppm; Mass (EI) m/z [M^+] Calculated 317, Observed 318; Elem. Anal.: C, 75.68 %; H, 4.76 %; N, 4.41 %; O, 5.04 %; S, 10.10 %; found: C, 75.67 %; H, 4.75 %; N, 4.40 %; O, 5.03 %; S, 10.09 %.

[12] 2-(anthracen-9-yl)benzo[d]thiazole (**6l**) [50]

M.F.: C₂₁H₁₃NS; Yield: 162 mg, 92 %; Colour: Yellow solid; M.P.: 174 °C

FTIR (KBr) ν (cm⁻¹): 3059, 1582, 1474, 749; ¹H NMR (CDCl₃, 400 MHz): δ 8.25-8.05 (m, 2H, Ar), 7.55-7.45 (m, 1H, Ar), 7.53-7.50 (m, 2H, Ar), 7.14-6.70 (m, 4H, Ar), 6.70-6.57 (m, 4H, Ar) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 148.6, 136.8, 131.6, 128.5, 126.8, 125.4, 118.7, 118.2, 115.2 ppm; Mass (EI) m/z [M⁺] Calculated 311, Observed 312; Elem. Anal.: C, 81.00 %; H, 4.21 %; N, 4.50 %; S, 10.30 %; found: C, 81.00 %; H, 4.20 %; N, 4.49 %; S, 10.29 %.

[13] 2-(1*H*-indol-3-yl)benzo[*d*]thiazole (**6m**) [51]

M.F.: C₁₅H₁₀N₂S; Yield: 132 mg, 91 %; Colour: Brown solid; M.P.: 184 °C

FTIR (KBr) ν (cm⁻¹): 3120, 3056, 1552, 1442, 786; ¹H NMR (CDCl₃, 400 MHz): δ 8.45 (d, *J*=7.6 Hz, 1H, NH), 7.97 (d, *J*=52.8 Hz, 1H, Ar), 7.50 (t, 2H, Ar), 7.19-7.09 (m, 3H, Ar), 6.72 (d, *J*=3.2 Hz, 3H, Ar) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 185.4, 163.2, 153.7, 148.6, 136.8, 136.5, 133.8, 131.6, 126.5, 126.1, 124.9, 124.3, 124.2, 123.4, 122.3, 122.0, 121.8, 121.3, 120.9, 118.7, 118.3, 115.3, 112.2, 111.8, 29.6 ppm; Elem. Anal.: C, 71.97 %; H, 4.03 %; N, 11.19 %; S, 12.81 %; found: C, 71.96 %; H, 4.02 %; N, 11.18 %; S, 12.80 %.

[14] (*Z*)-2-styrylbenzo[*d*]thiazole (**6n**) [52]

M.F.: C₁₅H₁₁NS; Yield: 129 mg, 91 %; Colour: Brown solid; M.P.: 134 °C

FTIR (KBr) ν (cm⁻¹): 1646, 1569, 1313, 956, 698; ¹H NMR (CDCl₃, 400 MHz): δ 8.02 (d, *J*=8 Hz, 4H, Ar), 7.88-7.61 (t, 2H, Ar), 7.60-7.36 (m, 3H, Ar), 7.32-7.12 (m, 2H, olefinic) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 167.0, 153.8, 148.6, 137.7, 136.8, 135.3, 134.3, 131.6, 129.4, 129.1, 128.9, 128.8, 128.6, 128.5, 128.5, 128.4, 128.3, 127.7, 127.6, 127.4, 126.3, 125.4, 122.9, 122.1, 121.5, 118.2, 115.2, 57.6, 51.5, 49.9, 42.3, 36.0, 35.5 ppm; Mass (EI) m/z [M⁺] Calculated 237, Observed 238; Elem. Anal.: C, 75.92 %; H, 4.67 %; N, 5.90 %; S, 13.51 %; found: C, 75.94 %; H, 4.65 %; N, 5.88 %; S, 13.50 %.

[15] 2-(ferrocenyl)benzo[d]thiazole (**6o**) [53]

M.F.: C₁₇H₁₃FeNS; Yield: 309 mg, 91 %; Colour: Black solid; M.P.: 112 °C

FTIR (KBr) ν (cm⁻¹): 3092, 1570, 1446, 744, 489; ¹H NMR (CDCl₃, 400 MHz): δ 7.98-7.10 (m, 4H, Ar), 4.89-4.16 (m, 9H, Ar) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 160.3, 151.6, 143.8, 142.4, 131.4, 127.2, 124.0, 115.5, 110.5, 97.3, 92.4, 88.9, 72.9, 71.8, 70.7, 70.3, 69.6, 69.5, 68.7, 61.7, 59.7, 41.0, 29.1, 20.1, 14.1 ppm; Mass (EI) m/z [M⁺] Calculated 319, Observed 320; Elem. Anal.: C, 63.97 %; H, 4.11 %; Fe, 17.50 %; N, 4.39 %; S, 10.04 %; found: C, 63.96 %; H, 4.10 %; Fe, 17.49 %; N, 4.38 %; S, 10.03 %.

[16] 2-(thiophen-2-yl)benzo[d]thiazole (**6p**) [45]

M.F.: C₁₁H₇NS₂; Yield: 125 mg, 90 %; Colour: Pale Yellow solid; M.P.: 138 °C

FTIR (KBr) ν (cm⁻¹): 3097, 1579, 1543, 1462, 827, 754; ¹H NMR (CDCl₃, 400 MHz): δ 8.03 (dd, *J*=0.4, 0.4 Hz, 1H, Ar), 7.87 (dd, *J*=0.4, 0.8 Hz, 1H, Ar), 7.85-7.66 (m, 1H, Ar), 7.66-7.48 (m, 2H, Ar), 7.39-6.93 (m, 2H, Ar) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 161.4, 153.6, 146.0, 145.3, 137.3, 134.6, 129.3, 128.6, 128.0, 126.6, 126.5, 126.4, 126.1, 125.6, 125.3, 125.2, 122.9, 121.7, 121.4, 121.1, 110.3, 65.5 ppm; Mass (EI) m/z [M⁺] Calculated 217, Observed 218; Elem. Anal.: C, 60.80 %; H, 3.25 %; N, 6.45 %; S, 29.51%; found: C, 60.79 %; H, 3.24 %; N, 6.44 %; S, 29.50 %.

[17] (E)-2-styrylbenzo[d]thiazole (**6q**) [54]

M.F.: C₁₅H₁₁NS; Yield: 132 mg, 93 %; Colour: Brown solid; M.P.: 126 °C

FTIR (KBr) ν (cm⁻¹): 1569, 1498, 1313, 956, 690; ¹H NMR (CDCl₃, 400 MHz): δ 8.03-7.88 (t, 3H, Ar), 7.88-7.86 (t, 2H, Ar), 7.61-7.14 (m, 4H, Ar), 7.19 (s, 2H, olefinic) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ 167.0, 153.8, 148.6, 137.7, 136.8, 135.3, 134.3, 131.6, 129.4, 128.9,

128.8, 128.6, 128.4, 128.4, 128.3, 127.7, 127.6, 127.4, 126.8, 126.4, 126.3, 126.3, 125.9, 125.4, 124.8, 122.9, 122.5, 122.1, 122.0, 121.5, 118.2, 115.2, 36.0, 35.5 ppm; Mass (EI) m/z [M^+] Calculated 237, Observed 238; Elem. Anal.: C, 75.92 %; H, 4.67 %; N, 5.90 %; S, 13.51 %; found: C, 75.91 %; H, 4.66 %; N, 5.89 %; S, 13.50 %.

[18] 2-(3-nitrophenyl)benzo[d]thiazole (**6r**) [55]

M.F.: $C_{13}H_8N_2O_2S$; Yield: 132 mg, 89 %; Colour: Pale Yellow solid; M.P.: 156 °C

FTIR (KBr) ν (cm^{-1}): 3086, 1610, 1581, 1529, 1346, 760; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.80 (dd, $J=2$, 196 Hz, 1H, Ar), 8.41-8.32 (m, 3H, Ar), 8.32-7.94 (m, 1H, Ar), 7.89-7.44 (m, 3H, Ar) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.9, 153.9, 148.7, 135.2, 135.1, 133.0, 130.1, 126.8, 126.0, 125.1, 123.7, 122.3, 121.8, 118.2 ppm; Mass (EI) m/z [M^+] Calculated 256, Observed 257; Elem. Anal.: C, 60.93 %; H, 3.15 %; N, 10.93 %; O, 12.49 %; S, 12.51%; found: C, 60.92 %; H, 3.14 %; N, 10.92 %; O, 12.48 %; S, 12.50 %.

[19] 4-(benzo[d]thiazol-2-yl)phenol (**6s**) [56]

M.F.: $C_{13}H_9NOS$; Yield: 124 mg, Colour: Pale yellow solid; M.P.: 163 °C

FTIR (KBr) ν (cm^{-1}): 3054, 2919, 1514, 1481, 1166, 756; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.01 (s, 1H, -OH), 7.99-7.11 (m, 4H, Ar), 7.02-6.57 (m, 4H, Ar) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.2, 159.1, 148.6, 136.8, 136.4, 134.7, 131.6, 130.1, 129.9, 129.3, 126.2, 124.7, 122.6, 121.5, 118.7, 118.4, 118.2, 116.0, 115.5, 115.2, 114.8, 39.1, 29.7 ppm; Mass (EI) m/z [M^+] Calculated 227, Observed 228; Elem. Anal.: C, 68.70 %; H, 3.99 %; N, 6.16 %; O, 7.04 %; S, 14.11%; found: C, 68.69 %; H, 3.98 %; N, 6.17 %; O, 7.02 %; S, 14.10%.

[20] 2-(4-chlorophenyl)benzo[d]thiazole (**6t**) [57]

M.F.: $C_{13}H_8ClNS$; Yield: 136 mg, Colour: Green solid; M.P.: 150 °C

FTIR (KBr) ν (cm^{-1}): 3055, 1588, 1473, 828, 755; ^1H NMR (CDCl_3 , 400 MHz): δ 8.04 (t, 2H, Ar), 8.02-7.90 (m, 1H, Ar), 7.42 (d, $J=1.2$, 2H, Ar), 7.27-7.04 (m, 3H, Ar) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ 166.6, 154.0, 148.8, 137.3, 137.1, 137.1, 137.0, 135.0, 132.0, 132.0, 129.2, 128.7, 128.7, 128.3, 126.5, 125.4, 123.2, 121.6, 118.5, 116.9, 110.6, 46.9 ppm; Mass (EI) m/z [M^+] Calculated 245, Observed 246; Elem. Anal.: C, 63.54 %, H, 3.28 %, Cl, 14.43 %, N, 5.70 %, S, 13.05 %; found: C, 63.53 %, H, 3.27 %, Cl, 14.41 %, N, 5.68 %, S, 13.03 %.

2.5 Anti-diabetic activity by α -amylase inhibition assay

All the synthesized 2-substituted benzoxazoles and benzothiazoles (**6a-t**) were screened for α -amylase inhibitory activity using the DNSA colorimetric method. α -amylase enzyme (0.5 %) in 0.02 M sodium phosphate buffer (pH 6.9) containing 6 mM NaCl was prepared. Five concentrations of enzyme solution ranging from 20 to 100 $\mu\text{g/mL}$ were pre-incubated with different concentrations of **6a-t** at 37 $^\circ\text{C}$ for 10 min. After the pre-incubation step, 250 μL of 1 % soluble starch solution in phosphate buffer (as substrate) was quickly added, and the solution was incubated at 37 $^\circ\text{C}$ for 10 min. The reaction was ended by adding 1.0 mL of DNSA reagent, followed by a 5-min heating of the mixture in a boiling water bath for 5 min. After cooling, the final mixture was diluted with 5 mL of distilled water, and the absorbance was recorded at 540 nm employing a UV-Visible spectrophotometer. A control reaction without inhibitor was run simultaneously. Acarbose was used as the standard anti-diabetic drug. All experiments were performed in triplicate, and results were expressed as mean $\pm$ SD [58]. The percentage inhibition of α -amylase activity was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

2.6 Anti-oxidant activity by DPPH radical scavenging assay

The anti-oxidant potential of synthesized 2-substituted benzoxazoles and benzothiazoles (**6a-t**) was examined using the DPPH assay. A freshly prepared 5 mM DPPH solution in ethanol was used for the assay. The different solutions of **6a-t** with five concentrations ranging from 20 to 100 µg/mL were prepared, and to them 1.0 mL of DPPH solution was added. The reaction mixture was incubated in the absence of light for 30 min to avoid photodegradation. Post-incubation, the absorbance was recorded at 517 nm employing a UV-Visible spectrophotometer (Shimadzu UV-1800) against ethanol as a blank. Ascorbic acid was used as the reference anti-oxidant. All experiments were carried out in triplicate, and the results were expressed as mean ± standard deviation (SD) [559].

The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\% \text{ of inhibition} = \frac{[(A_0 - A_T) \times 100]}{(A_0)}$$

Where, A₀ is the absorbance of control sample and A_T is absorbance of test sample.

2.7 In silico assessment of bioactivity scores and pharmacokinetic parameters of synthesized benzoxazoles and benzothiazoles (6a-t)

ChemDraw Pro 2016 was used to generate 2-D molecular structures and obtain Simplified Molecular Input Line Entry System (SMILES) strings for the newly synthesized benzoxazoles and benzothiazoles (**6a-t**). These structures were fed to monolinspiration (<https://www.molinspiration.com/>) - to calculate molecular properties, predict bioactivity scores, and assess the drug-likeness of chemical compounds, ADMET Lab 3.0 (<https://admetlab3.scbdd.com/>) - to predict and evaluate the pharmacokinetics, drug-likeness, and toxicity of chemical compounds and Swiss ADMT (<http://www.swissadme.ch>) – for pharmacokinetic and drug-likeness profiling [60] and Schrödinger software

(<https://www.schrodinger.com/life-science/learn/white-papers/docking-and-scoring/>) - to predict bioactivity scores and estimate key molecular descriptors [61]. ProTox-III (https://tox.charite.de/protox3/index.php?site=compound_input) – for in silico toxicity prediction [62].

All the synthesized benzoxazoles and benzothiazoles (**6a-t**) were assessed for their absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling employing QikProp version 4.3 (Schrödinger LLC) [63]. The 2D structures of ligands were imported into the QikProp panel in Maestro using the descriptor calculation method. The prediction of key physicochemical and pharmacokinetic descriptors was carried out which served as a basis for assessing the drug-likeness of molecules [64]. All the synthesized benzoxazoles and benzothiazoles (**6a-t**) were evaluated for their key physicochemical and pharmacokinetic parameters, including molecular weight, hydrogen-bond donors and acceptors, lipophilicity (QPlogPo/w), blood–brain barrier distribution (QPlogBB), and Caco-2 cell permeability (QPPCaco).

The artificial intelligence (AI) and machine learning (ML) based in silico toxicity prediction tool, GeinTox module of Geinforce software, is used for the evaluation of toxicity studies [65].

2.8 Molecular docking

Molecular docking studies were performed using the Glide-dock module of Maestro version 13.4 (Schrödinger) to investigate the binding affinity and molecular interactions of the designed compounds **6a-t** against human pancreatic α -amylase. The crystal structure of human pancreatic α -amylase complexed with the inhibitor acarbose (PDB ID: 1XH0) was selected as the target protein. The structure contains the N298S mutation and was determined by X-ray crystallography at a resolution of approximately 2.1 Å. The enzyme consists of a single

polypeptide chain (Chain A) and exhibits the characteristic $(\beta/\alpha)_8$ TIM-barrel fold of α -amylases. The protein structure was prepared using the Protein Preparation Wizard of Schrödinger. The co-crystallized ligand, acarbose, was used to identify the catalytic binding pocket and define the receptor grid for subsequent molecular docking. Particular attention was given to the catalytically and structurally important residues Asp197, Glu233, Asp300, His201, His299, Tyr62, Trp58, and Trp59, which contribute to catalysis and ligand stabilization within the α -amylase active site.

The prepared ligands were docked into the acarbose-binding pocket using the Glide Standard Precision (SP) flexible docking protocol. The resulting ligand poses were ranked according to their docking scores, and the binding modes were further examined based on hydrogen bonding, π - π stacking, salt-bridge interactions, ligand orientation, Glide energy, and Glide E-model values. Glide predicts favourable ligand-protein binding poses using an empirical scoring function known as the Glide score (G-score), which incorporates different energetic and structural contributions. The scoring function can be represented as:

$$\text{G-Score} = 0.065 \times \text{vdW} + 0.130 \times \text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}$$

3. Result and discussion

3.1 Characterization and Characterization of biogenic $\text{ZnCuFe}_2\text{O}_4$ nanoparticles

Our initial efforts were devoted to the preparation of zinc copper ferrite ($\text{ZnCuFe}_2\text{O}_4$) from zinc ferrite (ZnFe_2O_4) and copper ferrite (CuFe_2O_4) using a green synthesis method employing aqueous leaf extract of *Bryophyllum pinnatum*. Encouraged by the scrutiny of literature on the effectiveness of transition metal catalysts in the synthesis of medicinally valuable 2-substituted benzoxazoles and benzothiazoles [66], we sought to explore the potential of prepared biogenic $\text{ZnCuFe}_2\text{O}_4$ nanoparticles as a heterogenous catalyst. The structural and morphological

confinement of the prepared catalyst and precursors was performed by employing U.V. Visible spectroscopy, FTIR spectroscopy, X-ray diffraction (XRD), vibrating sample magnetometer (VSM), scanning electron microscopy (SEM), energy-dispersive x-ray analysis (EDS), Brunauer-Emmett-Teller (BET) isotherms, and transmission electron microscopy (TEM).

3.2 UV-Visible absorption spectroscopic study

The UV-Visible absorption spectra of ZnFe_2O_4 , CuFe_2O_4 , and $\text{ZnCuFe}_2\text{O}_4$ nanoparticles were recorded in the wavelength range of 250-550 nm as shown in **Fig. 3**. ZnFe_2O_4 showed the highest absorbance and intensity, pointing to strong electronic transitions in the UV region. CuFe_2O_4 showed relatively lower absorption, while $\text{ZnCuFe}_2\text{O}_4$ displayed intermediate behaviour with a broad absorption band around 350-400 nm. The change in absorption intensity is attributed to the difference in metal ion composition (Zn^{2+} and Cu^{2+}) and electronic interactions within the spinel ferrite lattice [67].

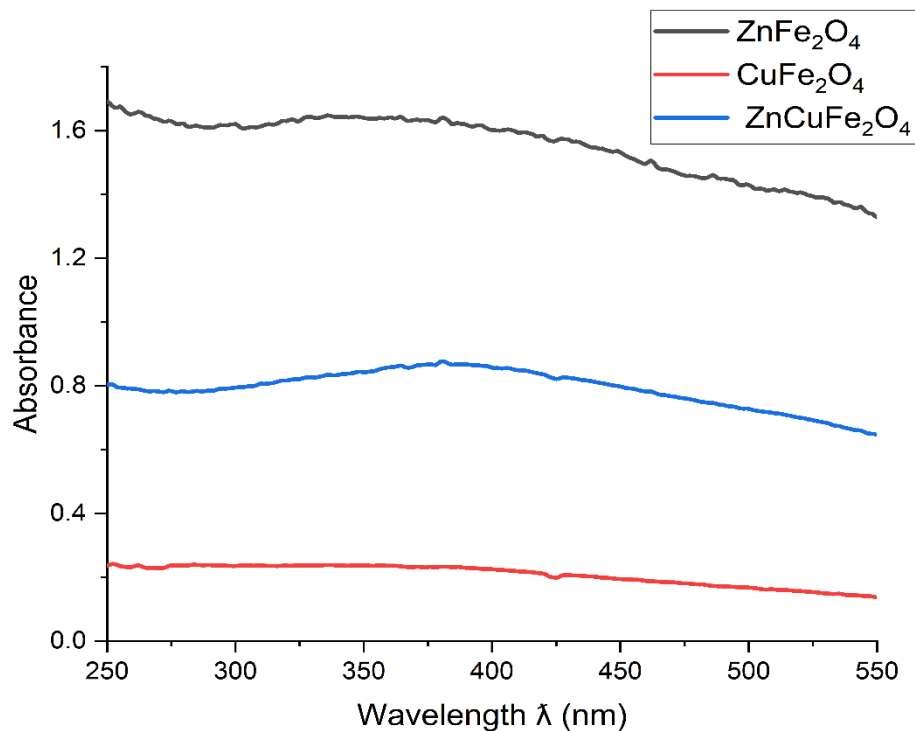


Fig 3: UV-visible spectra of ZnCuFe₂O₄ nanoparticles

3.2 Fourier Transform Infra-red (FTIR) spectroscopic study

The FTIR spectra of ZnFe₂O₄, CuFe₂O₄, and ZnCuFe₂O₄ nanoparticles were recorded in the range 4000-400 cm⁻¹, which suggests the formation of the spinel ferrite structure. The distinctive absorption bands identified below 600 cm⁻¹ are assigned to the metal-oxygen vibrations in the ferrite lattice. The peaks around 550 cm⁻¹ correspond to the stretching vibrations of Fe-O bands in ZnFe₂O₄ and CuFe₂O₄, respectively. A wide band identified in the region of 3200-3500 cm⁻¹ is assigned to the O-H stretching vibrations of adsorbed water molecules or surface hydroxyl groups, as shown in **Fig. 4**. Moreover, minor shifts in the peak sites among the three spectra indicate the integration of Zn²⁺ and Cu²⁺ ions into the ferrite lattice, suggesting the successful formation of ZnCuFe₂O₄ ferrite nanoparticles [68].

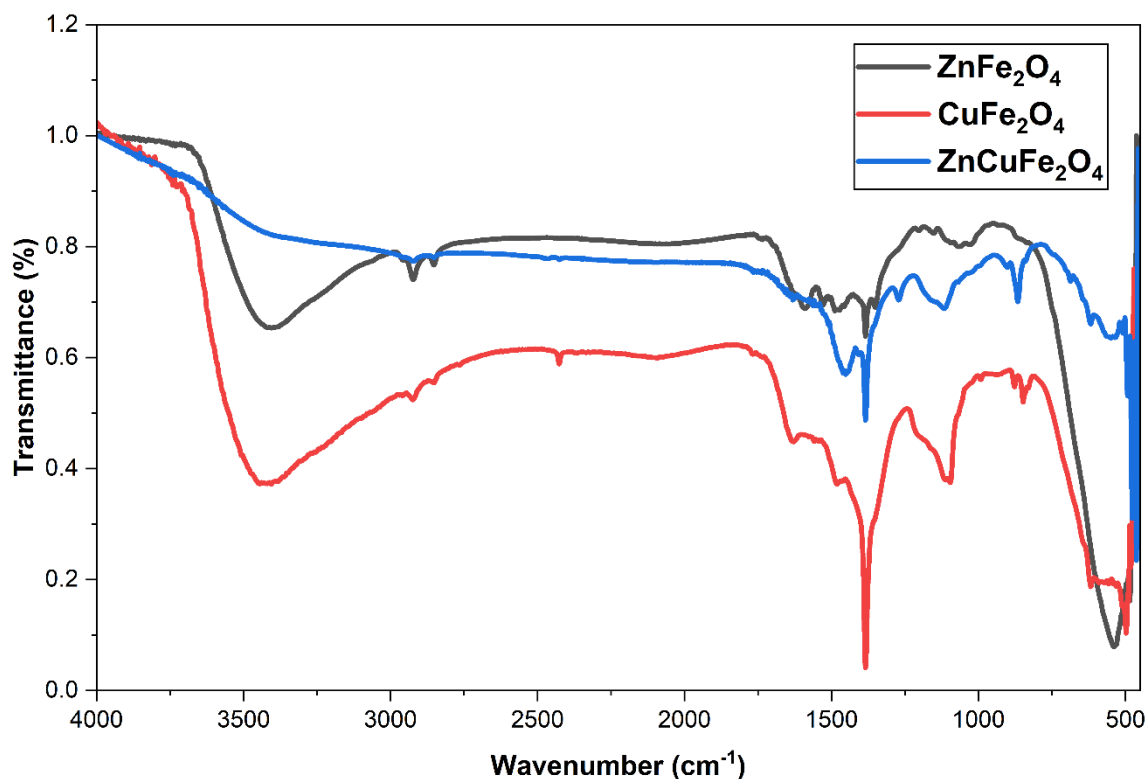


Fig 4: FT-IR spectrum of ZnFe₂O₄, CuFe₂O₄ and ZnCuFe₂O₄ nanoparticles

3.3 X-ray diffraction (XRD) study

The XRD profile of ZnFe_2O_4 , CuFe_2O_4 , and $\text{ZnCuFe}_2\text{O}_4$ nanoparticles is displayed in **Fig. 5** with the associated peak positions. The prepared CuFe_2O_4 nanoparticles showed a well-defined crystal structure with a distinct peak associated with the (103), (211), (004), (220), (321), and (413) planes corresponding to the 33° , 35° , 42° , 45° , 58° , 76° angles. The prepared CuFe_2O_4 nanoparticles show all diffraction peaks are in good agreement with the standard JCPDS card no. 48-1548. The peaks noted for the as-prepared ZnFe_2O_4 were assigned to the (311), (222), (400), (511), and (440) lattice planes of spinel ZnFe_2O_4 corresponds to the 35° , 36° , 56° , 61° angles. The analysis of the corresponding peak intensities in the XRD pattern showed good agreement with the typical features of ZnFe_2O_4 , as reported in the JCPDS card no. 74-2397. The XRD peaks of $\text{ZnCuFe}_2\text{O}_4$ corresponding to planes (220), (311), (400), (422), (511), (440) with 31° , 35° , 42° , 53° , 56° , 61° angles. This is in good agreement with the values of JCPDS card no. 82-1042; hence, the formation of $\text{ZnCuFe}_2\text{O}_4$ confirmed. A minor shift and variation in the intensity of the peaks can be identified while examining XRD patterns for ZnFe_2O_4 , CuFe_2O_4 , and $\text{ZnCuFe}_2\text{O}_4$ nanoparticles. These variations occur due to the substitution of Zn^{2+} and Cu^{2+} ions in the ferrite lattice, which slightly changes the lattice parameters and crystallinity. The sharp and well-defined peaks in the patterns reveal the good crystallinity of the synthesized nanoparticles [69].

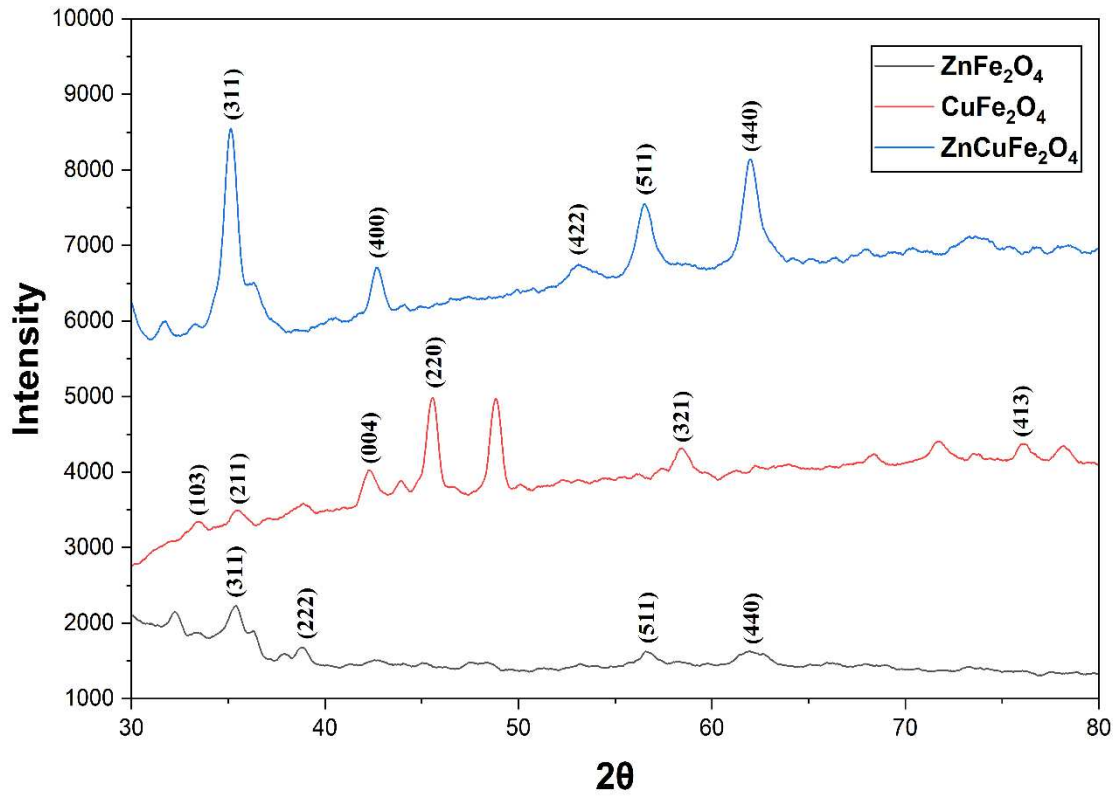


Fig 5: X-ray diffractogram (XRD) of ZnFe_2O_4 , CuFe_2O_4 and $\text{ZnCuFe}_2\text{O}_4$ nanoparticles

3.4 Vibrating Sample magnetometry (VSM) study

The magnetic characteristics of ZnFe_2O_4 , CuFe_2O_4 , and $\text{ZnCuFe}_2\text{O}_4$ nanoparticles were investigated by employing a vibrating sample magnetometer (VSM) analysis. The saturation magnetization (M_s) values of ZnFe_2O_4 , CuFe_2O_4 , and $\text{ZnCuFe}_2\text{O}_4$ are found to be 8.58, 23.91, and 82.5 emu g^{-1} , respectively. The M_s value for CuFe_2O_4 is greater than that of $\text{ZnCuFe}_2\text{O}_4$, as shown in **Fig. 6**. The reduced magnetization is attributed to the substitution of Zn^{2+} ions revealing the presence of Zn^{2+} ions in tetrahedral sites of the spinel lattice, which weakens the Fe-O-Fe super-exchange interactions. In addition, both the ferrites displayed small coercivity and remanent magnetization, suggesting their moderate magnetic behaviour [70].

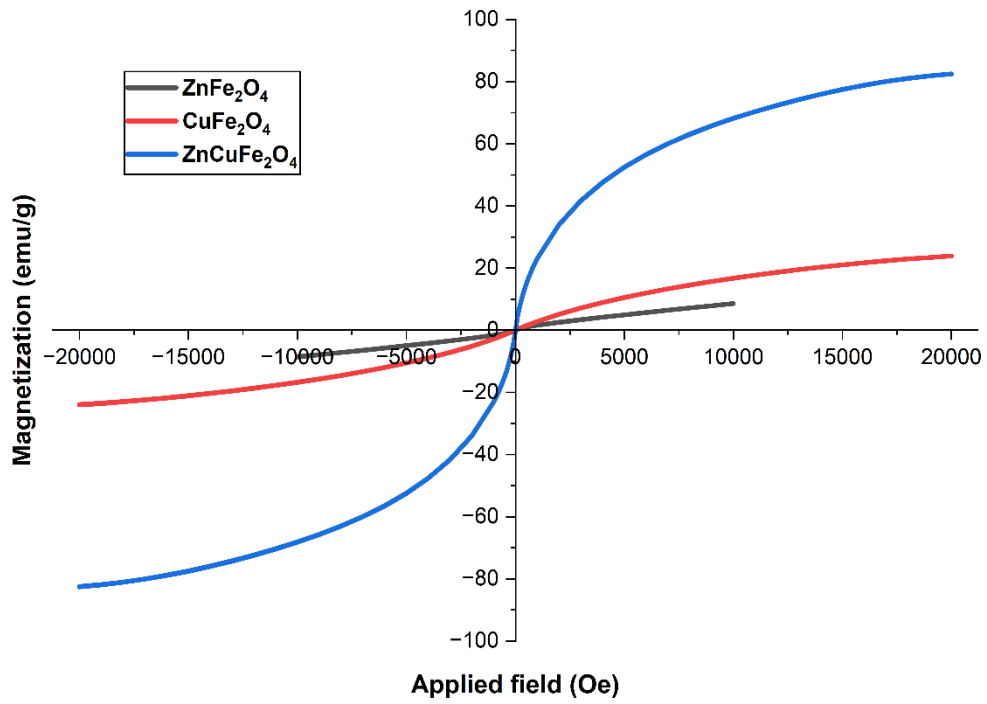


Fig 6: Vibrating sample magnetometer (VSM) analysis of the ZnCuFe₂O₄ nanoparticles

3.5 Scanning electron microscopy (SEM)

The SEM micrograph revealed that the synthesized ZnCuFe₂O₄ nanoparticles exhibited an irregular-shaped morphology with minor aggregation, which is a typical phenomenon due to magnetic interactions among the prepared nanoparticles. The ZnCuFe₂O₄ nanoparticles consist of irregular particle sizes with rough surfaces. The prepared nanoparticles displayed a particle size distribution ranging from 15 nm to 1 μ m indicating a wide size distribution, as shown in **Fig. 7** [71].

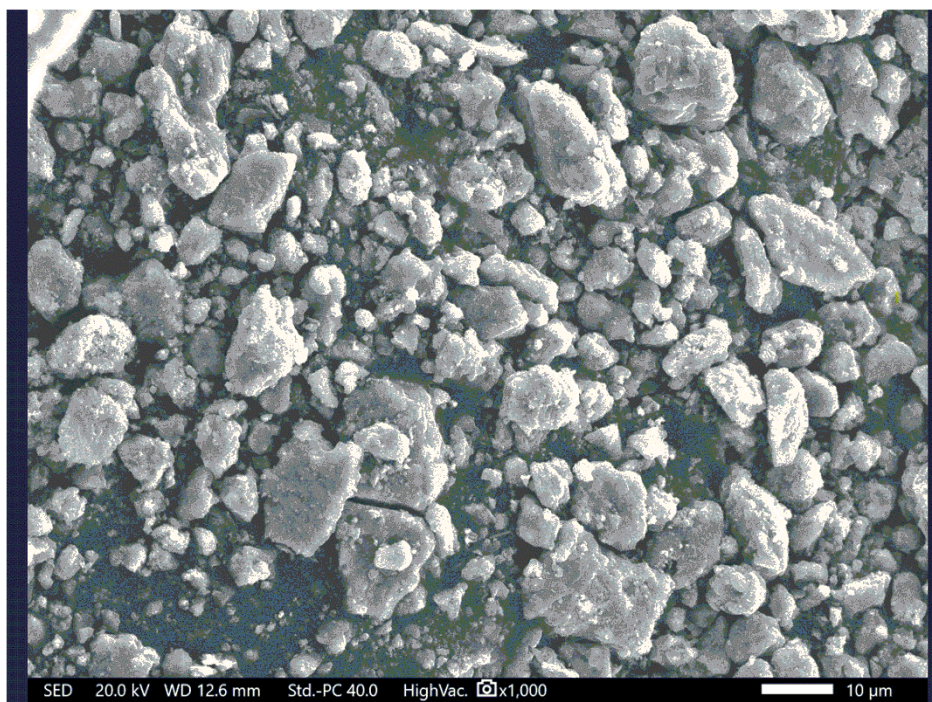


Fig 7: Scanning electron micrograph (SEM) of ZnCuFe₂O₄ nanoparticles

3.6 Energy-dispersive X-ray spectroscopy (EDX)

EDX analysis was used to estimate the chemical composition of the synthesized ZnCuFe₂O₄ nanoparticles, as shown in **Fig. 8**. The EDAX spectrum of the ZnCuFe₂O₄ compounds reveals the detection of Zn (13.44 %), Cu (15,00 %), Fe (17.34 %), and O (54.21 %), indicating the formation of desired ZnCuFe₂O₄ nanoparticles, as shown in **Table 1** [71].

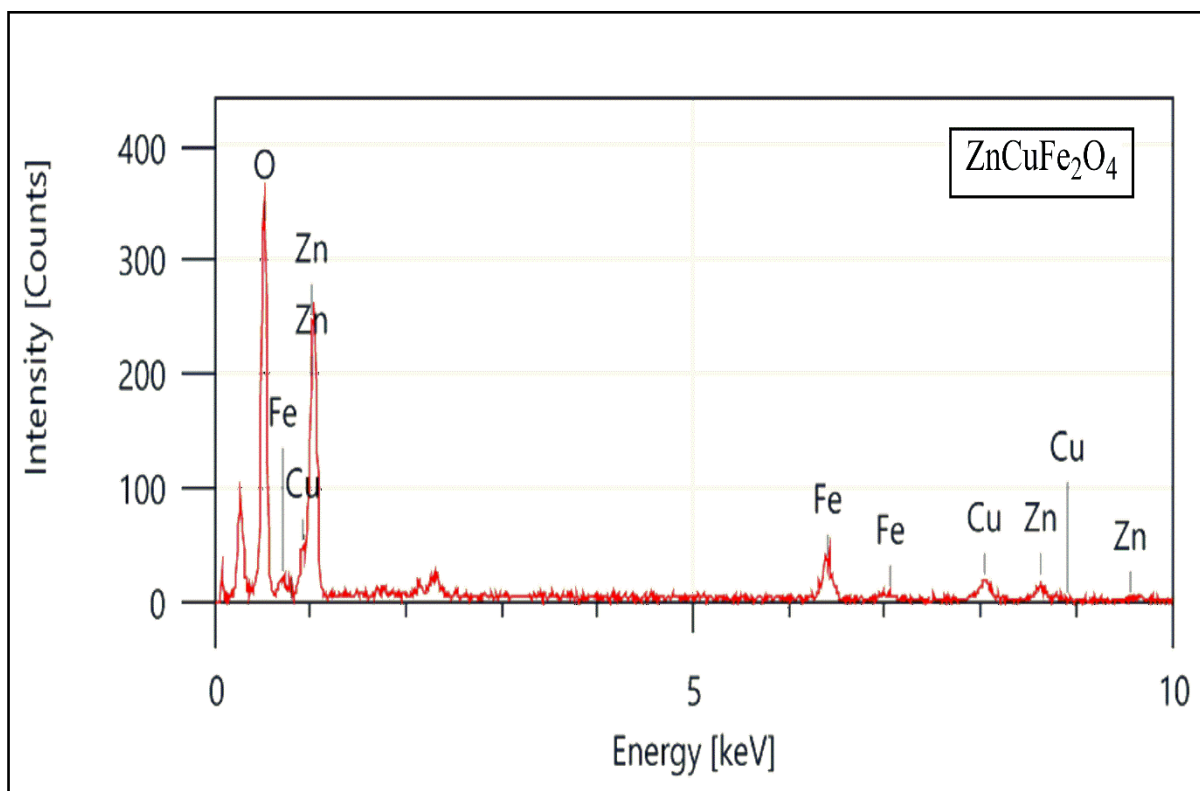


Fig 8: EDX analysis of ZnCuFe₂O₄ nanoparticles

Table 1: Elemental analysis of the ZnCuFe₂O₄ nanoparticles

Element	Line	Mass%
O	K	54.21±1.19
Fe	K	17.34±1.01
Cu	K	15.00±1.34
Zn	K	13.44±1.43

3.7 Transmission electron microscopy (TEM)

The particle size distribution and morphology of prepared ZnCuFe₂O₄ nanoparticles were examined by employing transmission electron microscopy (TEM). TEM micrograph of ZnCuFe₂O₄ nanoparticles revealed spherical-shaped nanoparticles of sizes ranging between 5 to 30 nm, as shown in **Fig. 9a-b**. The average particle size, calculated from the particle size

histogram, was found to be 17.12 ± 5.36 nm, as shown in **Fig. 9c**. A selective area electron diffraction pattern displayed two annotated rings at 2.262 Å and 3.270 Å corresponding to fringes on the surface of $\text{ZnCuFe}_2\text{O}_4$ nanoparticles. The central region of the SAED pattern clearly suggested a periodic lattice structure, as shown in **Fig. 9d** [68].

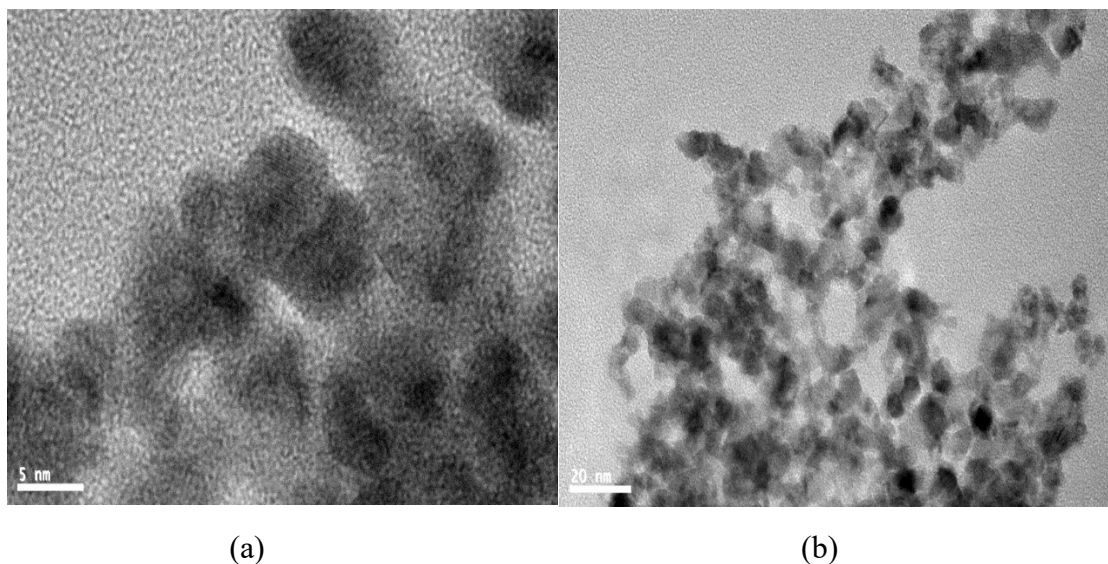


Fig 9 a &b: TEM micrographs of $\text{ZnCuFe}_2\text{O}_4$ nanoparticles

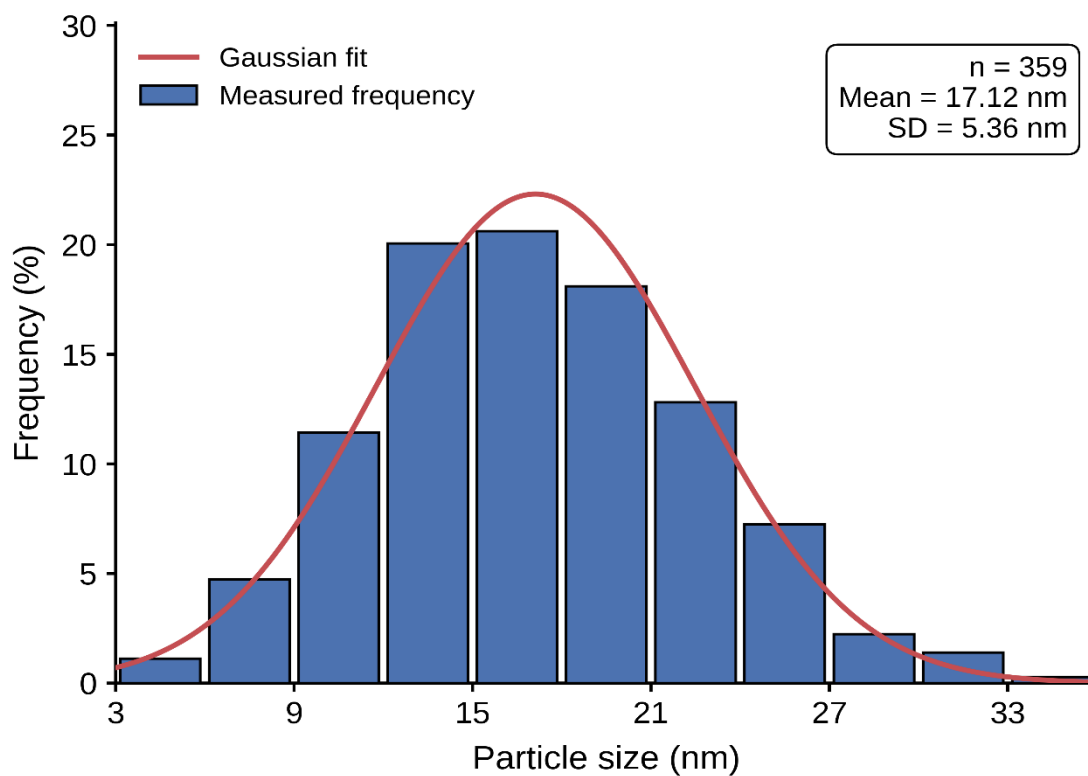


Fig 9c: Particle size distribution of $\text{ZnCuFe}_2\text{O}_4$ nanoparticles

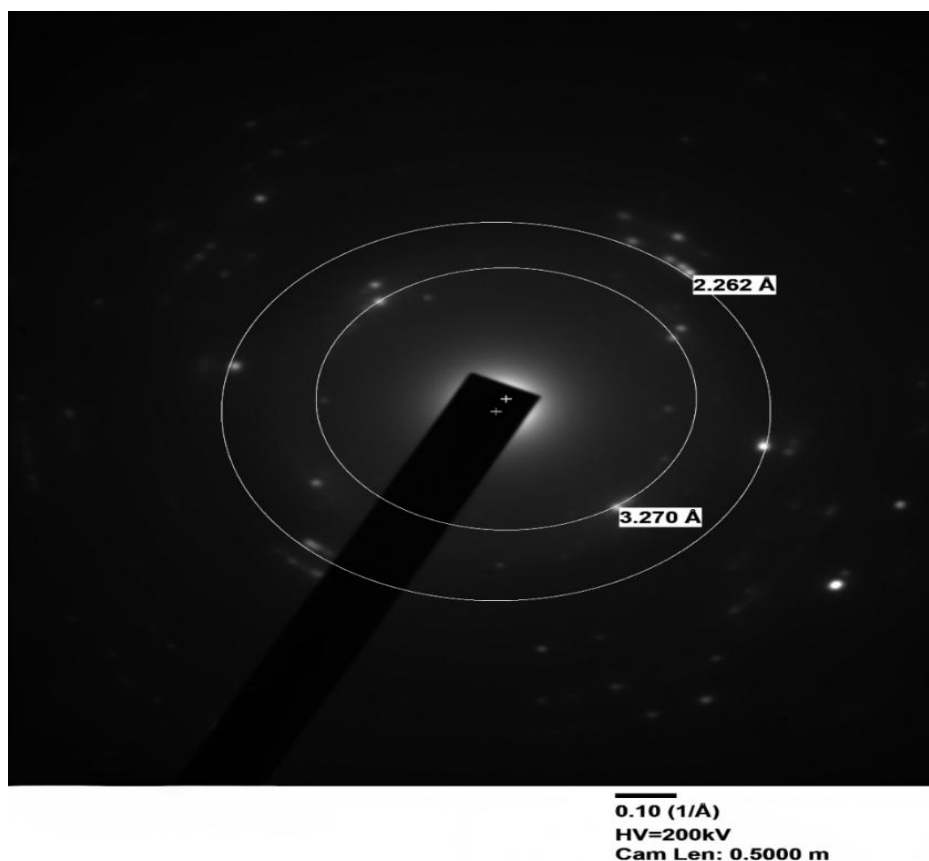


Fig 9d: SAED pattern of ZnCuFe₂O₄ nanoparticles

3.8 BET analysis (Brunauer–Emmett–Teller analysis)

The N₂ adsorption-desorption isotherm of the ZnCuFe₂O₄ catalyst represented a typical type IV profile with a hysteresis loop revealing the mesoporous nature of the prepared catalyst. The BET surface area was calculated from the linear part of the isotherm and was found to be 32.736 m²/g, as shown in **Fig. 10**. From BET analysis, it was evident that the prepared ZnCuFe₂O₄ exhibited a moderate surface area with a porous appearance for catalytic application [72].

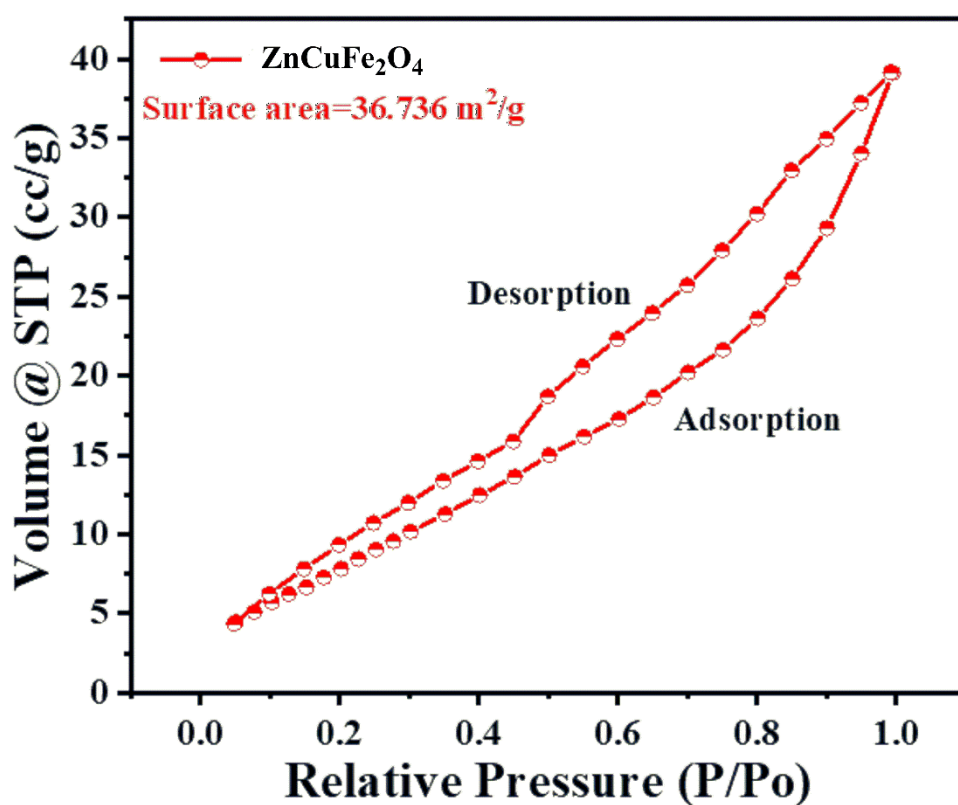
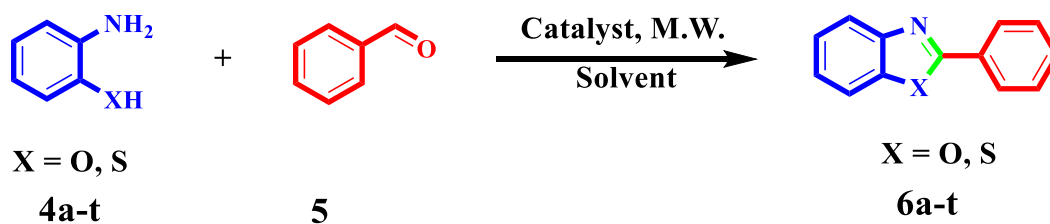


Fig 10: Nitrogen adsorption-desorption isotherm of ZnCuFe₂O₄ nanoparticles

Table 2: Optimization of reaction conditions



Entry	Catalyst	Solvent	Temp (°C)	Time (min)	Yield (%)
1	CuFe ₂ O ₄ (50 mg)	Ethanol: H ₂ O	80	240	65
2	CuFe ₂ O ₄ (100 mg)	Xylene	130	270	67
3	CuFe ₂ O ₄ (200 mg)	Dimethylformamide (DMF)	150	330	55
4	CuFe ₂ O ₄ (300 mg)	Solvent Free	R.T.	300	58

5	CuFe ₂ O ₄ (100 mg)	Dichloromethane	R.T.	270	75
6	CuFe ₂ O ₄ (100 mg)	Ethanol	75	240	78
7	ZnFe ₂ O ₄ (50 mg)	Ethanol: H ₂ O	80	240	60
8	ZnFe ₂ O ₄ (100 mg)	Xylene	130	270	72
9	ZnFe ₂ O ₄ (200 mg)	Dimethylformamide (DMF)	150	330	66
10	ZnFe ₂ O ₄ (300 mg)	Solvent Free	R.T.	300	68
11	ZnFe ₂ O ₄ (100 mg)	Dichloromethane	R.T.	270	72
12	ZnFe ₂ O ₄ (100 mg)	Ethanol	75	240	74
13	CuCl (0.05 mol %)	Butanol (BuOH)	100	210	63
14	CuI (0.05 mol %)	Butanol (BuOH)	100	240	67
15	CuBr (0.05 mol %)	Butanol (BuOH)	100	270	71
16	CuBr (0.05 mol %)	Xylene	130	270	63
17	CuBr (0.05 mol %)	Dimethylformamide (DMF)	150	330	62
18	ZnCuFe ₂ O ₄ (50 mg)	Ethanol: H ₂ O	80	240	73
19	ZnCuFe ₂ O ₄ (50 mg)	Xylene	130	270	65
20	ZnCuFe ₂ O ₄ (100 mg)	Dimethylformamide (DMF)	150	330	70
21	ZnCuFe ₂ O ₄ (200 mg)	Solvent Free	R.T.	300	75
22	ZnCuFe ₂ O ₄ (100 mg)	Dichloromethane	R.T.	270	79
23	ZnCuFe ₂ O ₄ (100 mg)	Ethanol	350 W	5	94

^a Reaction condition: Aldehyde (1 mmol), 2-aminophenol (1 mmol) stirred in 5 mL solvent in presence of catalyst under heating/microwave reflux.

^b All products are characterized FTIR, ¹H, ¹³C NMR spectroscopy and by mass spectrometry.

^c Isolated yields after recrystallisation.

After the successful formation of the zinc copper ferrite (ZnCuFe₂O₄) catalyst, our next plan was to investigate the catalytic potential of ZnCuFe₂O₄ for the synthesis of 2-substituted benzoxazoles and benzothiazoles. With this view in mind, the reaction between 2-aminophenol (1 mmol) and benzaldehyde (1 mmol) was selected as a model reaction in order to optimize the

reaction parameters. The effect of catalyst loading (50 mg, 100 mg, 200 mg and 300 mg) on the progress of the model reaction was evaluated as shown in **Table 2**.

Table 3: Loading of ZnCuFe₂O₄ nanoparticles

Entry	Catalyst	Amount of catalyst (mg)	Reaction time (min)	Yield (%)
1	ZnCuFe ₂ O ₄	50	20	65
2	ZnCuFe ₂ O ₄	100	5	94
3	ZnCuFe ₂ O ₄	200	20	68
4	ZnCuFe ₂ O ₄	300	20	71

Reaction conditions: 2-aminophenol (1 mmol), benzaldehyde (1 mmol), ZnCuFe₂O₄, ethanol (5 mL)

The progress of the model reaction was monitored by increasing the catalyst amount from 50-100 mg (**Table 3 entries 1 and 2**). It was evident that the desired product 2-phenyl benzoxazole was obtained in a maximum yield of 94 % at 100 mg, and no further significant effect on the product yield and reaction time was observed by increasing the catalyst amount (**Table 3 entries 3 and 4**).

Next, we explored the effect of various solvents such as ethanol, ethanol/water (1:1), xylene, DMF, dichloromethane, butanol, and also a solvent-free approach on the model reaction. Among the screened solvents, ethanol was found to be the most suitable solvent, furnishing an excellent yield of the desired product in the shortest reaction time under microwave irradiation at 350 MHz.

3.9 Recyclability study

The catalyst was recovered from the reaction matrix using an external magnet and reused for assessing recyclability on a model reaction under optimised reaction conditions. During the first four runs, the $\text{ZnCuFe}_2\text{O}_4$ nanoparticles gave an excellent yield of the desired product (90-96 %). A slight decline in catalytic activity was observed during the fifth and sixth runs (88 % - 85 %). Therefore, it was observed that the prepared $\text{ZnCuFe}_2\text{O}_4$ nanoparticles served as a robust, heterogeneous catalyst for the synthesis of 2-substituted benzoxazoles and benzothiazoles without significant loss of activity up to six cycles, as shown in **Fig. 11**

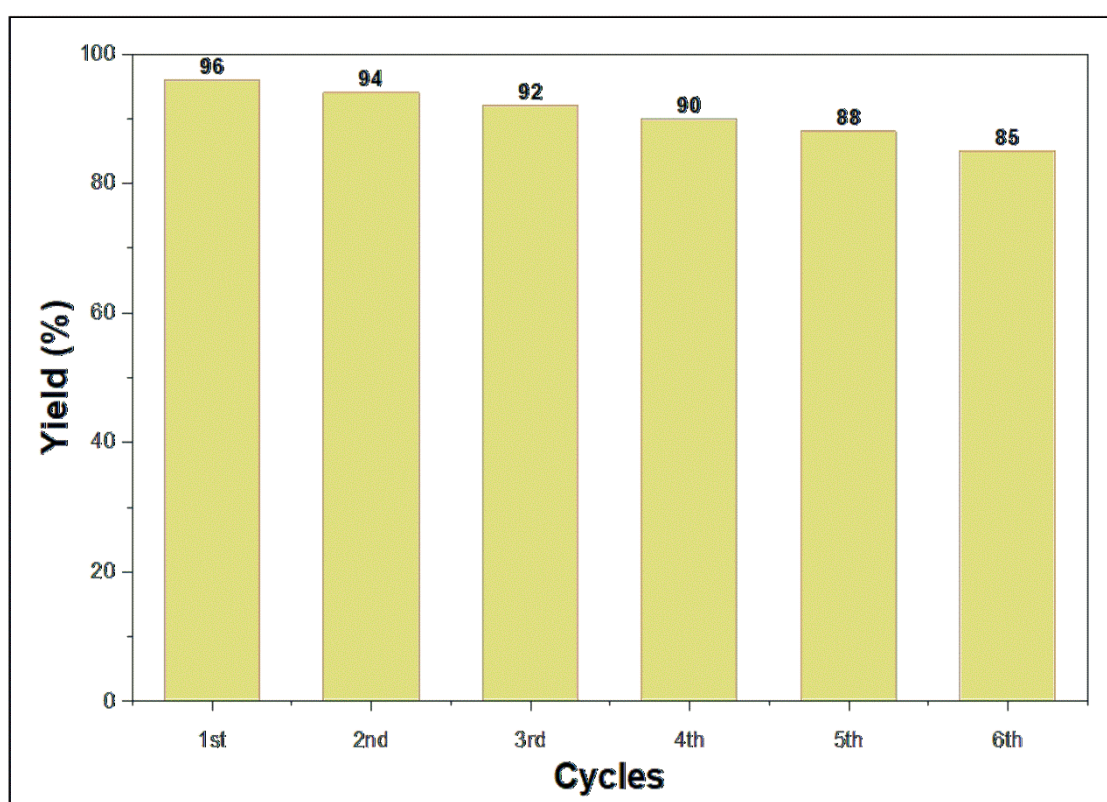
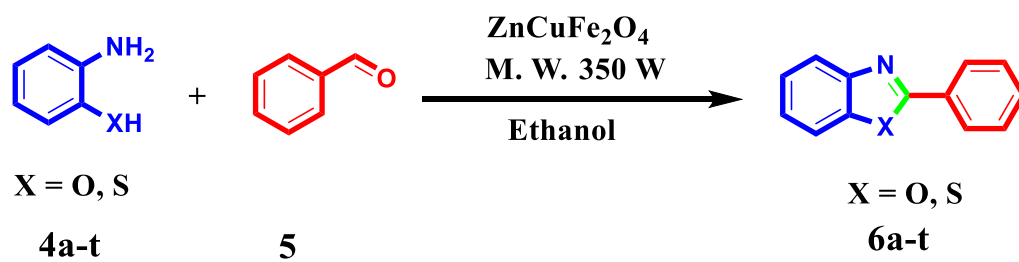
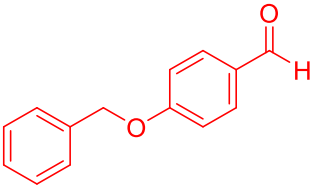
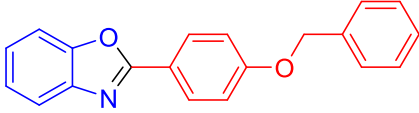
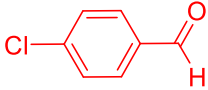
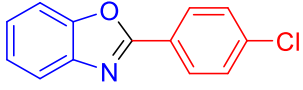
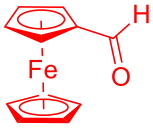
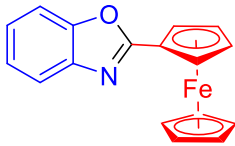
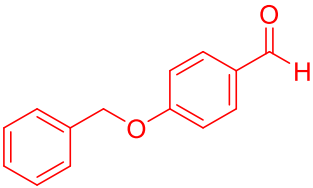
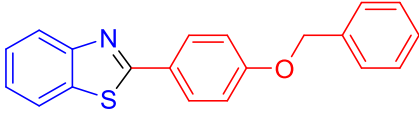
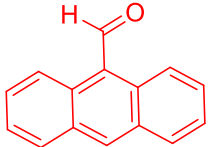
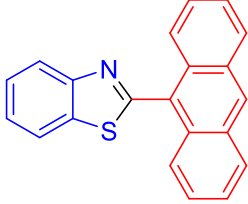
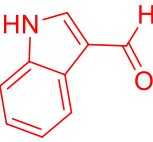
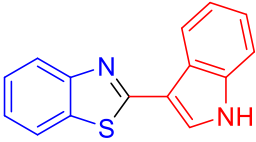
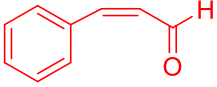
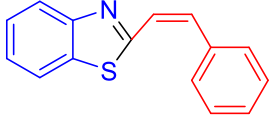
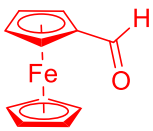
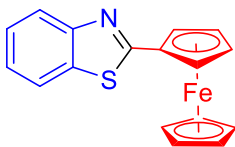
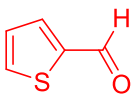
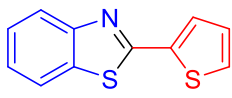
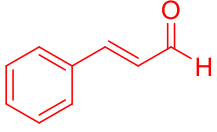
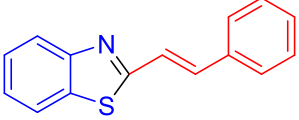


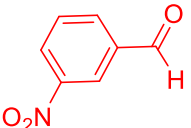
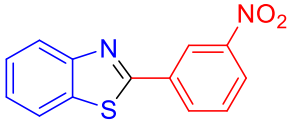
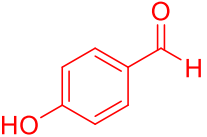
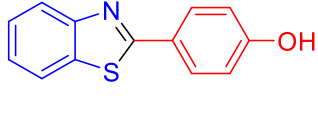
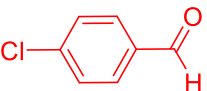
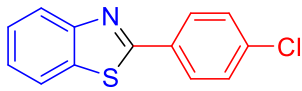
Fig 11: Recycling of the $\text{ZnCuFe}_2\text{O}_4$ nanoparticles

Table 4: Zinc copper ferrite catalyzed multicomponent synthesis of new benzoxazoles and benzothiazoles (**6a-t**)



Entry	Aldehyde	Product	Time (Min)	Yield	M. P. (°C)
6a			35	93	135
6b			120	91	173
6c			40	90	106
6d			60	92	107
6e			55	96	96
6f			90	93	113
6g			60	94	93

6h			50	93	145
6i			45	90	137
6j			75	95	150
6k			50	90	150
6l			120	92	174
6m			45	91	184
6n			60	91	134
6o			75	91	122
6p			60	90	138
6q			60	93	126

6r			35	89	156
6s			60	92	163
6t			45	92	150

^aReaction condition: Aldehyde (5 mmol), 2-aminophenol/ 2-aminothiophenol and ZnCuFe₂O₄ in ethanol (20 mL) under microwave at 350 W.

^bAll synthesized compounds are characterized by FTIR, ¹H NMR, ¹³C NMR spectroscopy and mass spectroscopy.

^cIsolated yields after recrystallisation.

Table 5: Comparison of model reaction for the synthesis of new benzoxazoles and benzothiazoles (**6a-t**) in the presence of the catalyst ZnCuFe₂O₄ in terms of time and yield with recent protocols.

Entry	Catalyst	Conditions		Time	Yield (%)	References
1	Anhydrous BiCl ₃	MeCN	RT	48 min	78	73
2	N-MnO ₂ , air	Solvent free	25 °C	1 h	87	74
3	Ni-SiO ₂	EtOH	RT	2.5 h	98	75
4	MIL-101(Cr) (10 mg)	Xylene	120 °C	9 h	87	76
5	BAIL gel (10 mg)	Solvent free	130 °C	5-7 h	98	28
6	[bmim][FeCl ₄]	EtOH	Reflux	90 min	94	77
7	Phosphonium acidic IL	Solvent free	120 °C	90 min	92	78
8	TiO ₂ NPs	Solvent free	50 °C	27 min	97	79
9	Fe ₃ O ₄ -Bis[Imine-Pyridine]-Cu(OAc) ₂ (20 mg)	DMF	Reflux	8 h	97	80

10	Magnetic CuFe ₂ O ₄ NPs (20 mg)	Water	Reflux	90 min	96	81
11	LAIL@MNP (4 mg)	Solvent free	70 °C	0.5 h	82	82
12	[choline Cl] [Imidazole]2 (35 % mol)		120 °C	6 h	78	83
13	PPC-SO ₃ H@MNP	H ₂ O	Reflux	45 min	87	84
14	Ag@Fe ₂ O ₃	H ₂ O/EtOH	RT	7 min	92	85
15	Fe ₃ O ₄ @SiO ₂ -SO ₃ H (0.03 g)	Solvent free	50 °C	0.7 h	92	46
16	FeMImeNHC-Ag	Ethanol	350 W	150 min	88	12

The UV-visible absorption spectra of all the synthesized benzoxazoles and benzothiazoles (**6a-t**) were recorded in ethanol (**Fig 12a-b**), and the UV-Vis absorption characteristics (λ max values) are provided in **Table 6**. The UV- Visible spectra for (**6a-t**) exhibited absorption peaks in the range 205-432 nm due to $\pi \rightarrow \pi^*$ electronic transition the conjugated absorption bond was detected at 327 nm for **6j** and **6o**, attributed the d-d transition the ferrocene moiety [86].

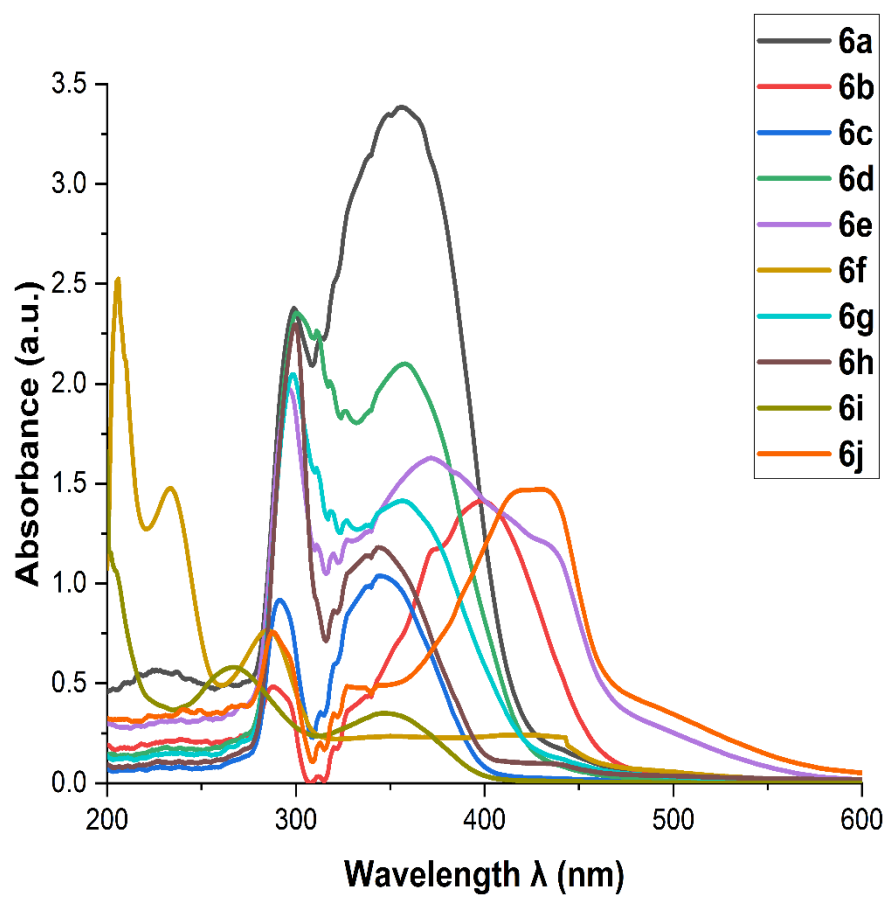


Fig 12 a: UV-visible absorption spectra of synthesized benzoxazoles and benzothiazoles (**6a-j**)

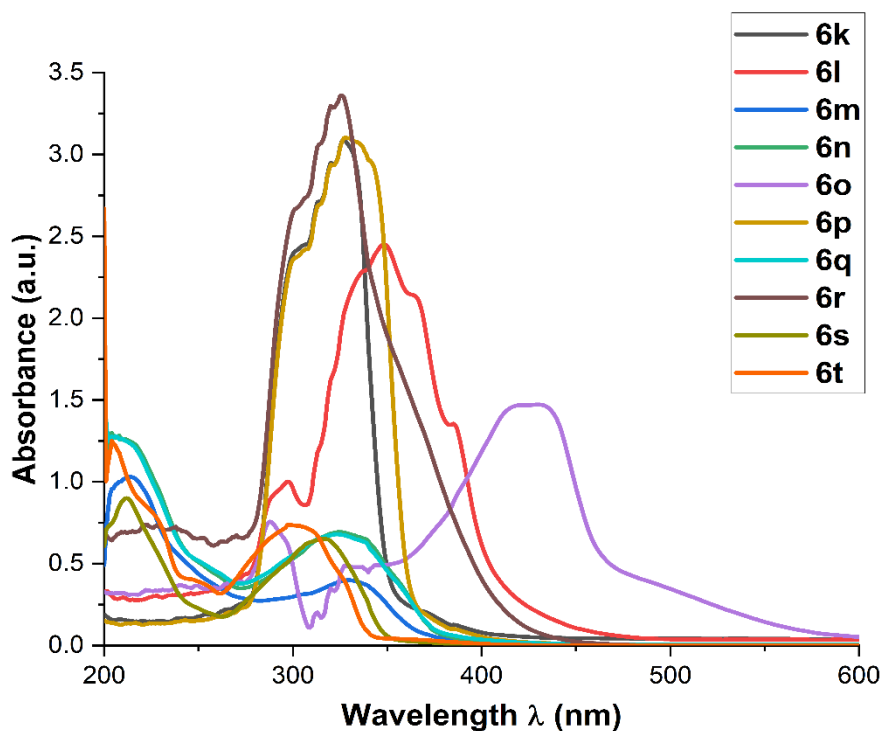


Fig 12 b: UV-visible absorption spectra of synthesized benzoxazoles and benzothiazoles (**6k-t**)

Table 6: The UV-visible maximum absorption wavelength (λ_{max}) values of synthesized benzoxazoles and benzothiazoles (**6a-t**) in ethanol.

Compound	λ_{max} for π - π^* transition (nm)	Compound	λ_{max} for π - π^* transition (nm)
6a	298, 356	6k	326
6b	398, 288	6l	348, 296
6c	291, 345	6m	215, 331
6d	300, 356	6n	327
6e	296, 372	6o	327, 288, 432
6f	206, 233, 285	6p	328
6g	299, 357	6q	206, 324
6h	299, 345	6r	325
6i	266, 349	6s	211, 314

The FTIR spectrum of the representative compound 2-(3-nitrophenyl)benzo[d]oxazole (**6a**) displayed a characteristic adsorption band at 3075 cm⁻¹ due to C-H stretching of the aromatic ring, bands at 1585 cm⁻¹ and 1478 cm⁻¹ due to C=N stretching of benzoxazole and C=C stretching of the benzene ring, respectively. The spectrum also displayed bands at 1529 cm⁻¹ and 1375 cm⁻¹ for N=O stretching of nitrobenzene, while bands at 1148 cm⁻¹ were attributed to C-O-C stretching vibration of the benzoxazole ring. The ¹H NMR spectrum of **6a** displayed a multiplet in the range 8.79 - 8.25 ppm for aromatic protons of the 3-nitrophenyl ring. The downfield value of the chemical shift is due to the presence of strongly electron-withdrawing nature of the nitro group. The four aromatic protons of the benzoxazole ring appeared as a multiplet in the range 7.71 – 6.92 ppm. The ¹³C NMR spectrum of **6a** displayed four downfield singlets at 153.9, 152.6, 148.7, 137.4 and 134.4 for the 3-nitrophenyl ring and signals at 134.0, 130.1, 129.9, 125.7, 123.3, 120.3, 115.9, 115.5 ppm. The mass spectrum of **6a** displayed an abundant ion peak at m/z=243 (M⁺), which represents the successful formation of **6a**.

3.10 Anti-diabetic activity

Benzoxazole and benzothiazoles are known to exert significant anti-diabetic potential via inhibition of the vital enzyme α -amylase involved in carbohydrate digestion [87]. The scrutiny of the literature has revealed that these scaffolds have the ability to control postprandial glucose by targeting α -amylase and α -glucosidase through the activation of PPAR- γ receptor [88] and AMPK [89].

Owing to the above discussion, we aimed at screening the synthesized benzoxazoles and benzothiazoles (**6a-t**) for their *in vitro* anti-diabetic activity employing an α -amylase inhibition assay. The IC₅₀ values for **6a-t** are presented in Table 7. It is noteworthy that all the synthesized benzoxazoles and benzothiazoles (**6a-t**) displayed moderate to significant activity. All the

synthesized compounds (**6a-t**) demonstrated a gradual increase in α -amylase inhibitory activity with increasing concentration ranging from 20-100 $\mu\text{g/mL}$. Among the synthesized series, **6s** was found to be the most potent compound with an IC_{50} value of $41.22 \pm 1.13 \mu\text{g/mL}$ compared to the standard as shown in **Fig 13**. It was observed that at a low concentration range of 20 $\mu\text{g/mL}$, **6s** exhibited 26-28 % inhibition, but at a gradually increasing concentration of 40 $\mu\text{g/mL}$, it showed almost 50 % inhibition. The significant α -amylase inhibitory activity was observed to be 79-80 % at a higher concentration of 100 $\mu\text{g/mL}$. This clearly demonstrated that compound **6s** has a high potential to interact with the receptor pocket of α -amylase. This might be due to the presence of elements like O, N, and OH groups for favourable hydrogen bonding.

In a similar manner, compounds **6m** and **6n** also showed appreciable inhibitory activity with IC_{50} values of $44.52 \pm 0.98 \mu\text{g/mL}$ and $45.93 \pm 1.00 \mu\text{g/mL}$, respectively. The consistent increase in inhibition across concentrations suggests that **6m** and **6n** were also effective in interacting with the α -amylase. This behavior may be attributed to the presence of conjugated groups and electron-rich moieties in the molecular structures of **6m** and **6n**, which might assist in stabilizing the interaction with the active site of α -amylase.

Moreover, compounds such as **6k** and **6c** also showed notable inhibitory potential, with IC_{50} values of $48.74 \pm 1.01 \mu\text{g/mL}$ and $50.26 \pm 1.00 \mu\text{g/mL}$, respectively. Though slightly less active than **6s**, their activity is still significant and indicates that structural features such as aromaticity and conjugation play an important role in enhancing enzyme inhibition. On the other hand, compounds like **6i**, **6b**, **6d**, **6a** and **6p** showed moderate activity, with IC_{50} values in the range of $77.04 \pm 1.50 \mu\text{g/mL}$, $81.80 \pm 2.60 \mu\text{g/mL}$, $85.41 \pm 3.62 \mu\text{g/mL}$, $79.21 \pm 1.91 \mu\text{g/mL}$ and $76.80 \pm 2.65 \mu\text{g/mL}$ as presented in **Table 7**. These compounds may have partial structural features required for activity, but lack the optimal arrangement necessary for strong interaction with the enzyme.

Table 7: Anti-diabetic activity of benzoxazoles and benzothiazoles (**6a-t**)

Entry	IC ₅₀ value (µg/mL)	Entry	IC ₅₀ value (µg/mL)
6a	79.21 ± 1.91	6k	48.74 ± 1.01
6b	81.80 ± 2.60	6l	216±9.33
6c	50.26 ± 1.00	6m	44.52 ± 0.98
6d	85.41 ± 3.62	6n	45.93± 1.00
6e	142.79 ± 6.37	6o	238.21± 12.51
6f	60.90 ± 1.40	6p	76.80 ±2.65
6g	119.13 ± 5.25	6q	56.16 ±1.44
6h	256.48 ±11.21	6r	236.00 ±10.90
6i	77.04± 1.50	6s	41.22 ±1.13
6j	236.1 ± 10.8	6t	153.5 ±7.12
		Acarbose	34.10 ± 0.90

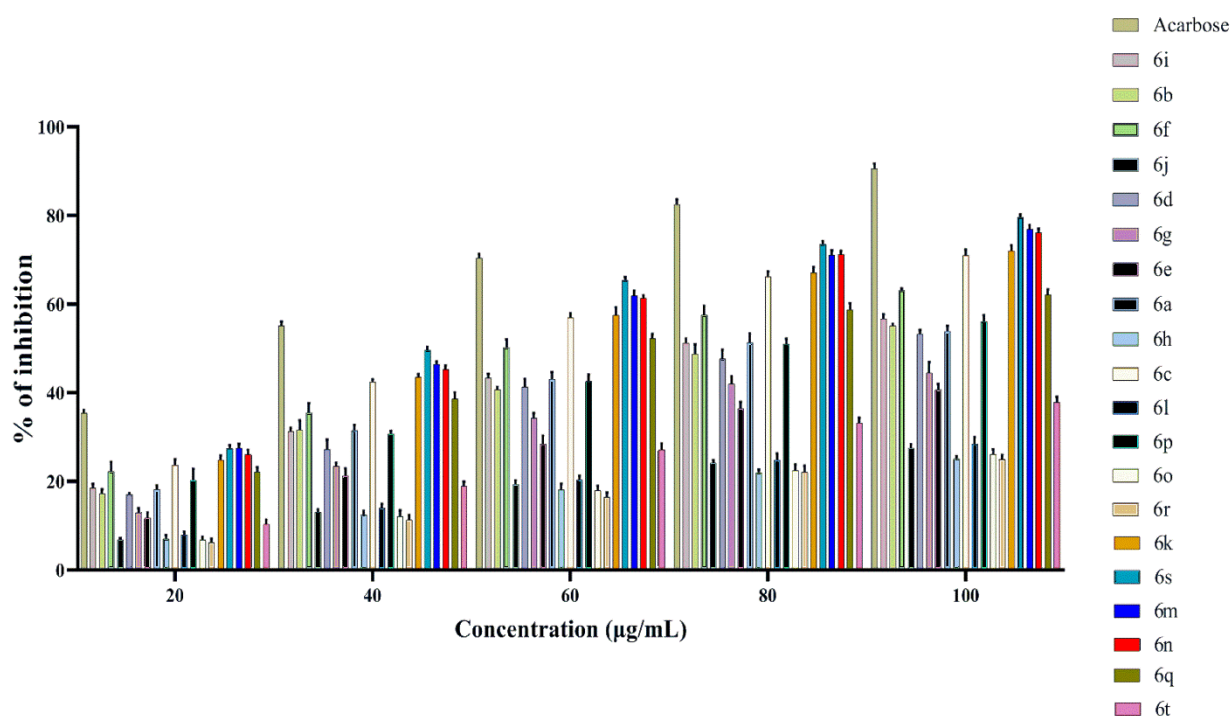


Fig 13: Anti-diabetic activity using α -amylase inhibitory activity (% inhibition) of benzoxazoles and benzothiazoles at 20–100 $\mu\text{g/mL}$ (mean $\pm$ SD, $n = 3$).

3.11 DPPH Anti-oxidant activity

A literature review revealed that substitution of specific functionalities in the core skeleton of benzoxazoles and benzothiazoles was observed to enhance the anti-oxidant activity of the parent heterocyclic motif [90]. The modified scaffolds can serve as radical scavengers there by inhibiting lipid peroxidation and protecting cells from oxidative stress [91]. Owing to this, we screened all the synthesized benzoxazoles and benzothiazoles (**6a-t**) for their in vitro anti-oxidant activity using the DPPH assay over a concentration range of 20-100 $\mu\text{g/mL}$ as shown in **Fig 14**.

Among the synthesized series, **6s** exhibited significant anti-oxidant activity with an IC_{50} value of $38.5 \pm 0.8 \mu\text{g/mL}$. The significant anti-oxidant activity of **6s** may be due to the presence of a phenolic hydroxyl group, which acts as an efficient hydrogen donor and stabilizes free radicals.

Compounds **6n** and **6m** also showed promising anti-oxidant activity with IC₅₀ value of 41.0 ± 1.75 µg/mL and 42.18 ± 1.68 µg/mL, respectively, which may be due to the presence of α, β-unsaturated conjugated systems and indole moieties, respectively, facilitating effective electron delocalization and radical stabilization. Additionally, compounds **6k** and **6c** exhibited good anti-oxidant activity with IC₅₀ values of 43.2 ± 1.3 µg/mL and 44.3 ± 2.3 µg/mL, respectively. The activity may be due to their aromatic ring structure and conjugated systems, which can cause effective delocalization of π-electron density and improve interaction with DPPH free radicals. Moreover, moderate antioxidant activity was observed for compounds **6i**, **6b**, **6d**, **6a** and **6p** with IC₅₀ values 54.1 ± 2.6 µg/mL, 55.8 ± 2.2 µg/mL, 59.5 ± 1.7 µg/mL, 57.2 ± 1.8 µg/mL and 56.1 ± 1.9 µg/mL, respectively. All these compounds have substituents that provide limited electron-donating ability or partial resonance stabilization. The rest of the compounds, including **6j**, **6h**, **6l**, **6o**, and **6r**, exhibited IC₅₀ values greater than 100 µg/mL, suggesting weak anti-oxidant activity. This reduced activity may be attributed to the presence of electron-withdrawing groups, lack of conjugation, or absence of effective hydrogen-donating functionalities, which limit their ability to scavenge free radicals. The ascorbic acid was used as a standard drug, which showed the lowest IC₅₀ value of 32.8 ± 0.7 µg/mL as presented in **Table 8**. The results clearly demonstrated that structural features like the presence of phenolic hydroxyl groups, heteroaromatic systems, and extended conjugation significantly enhance the anti-oxidant activity of benzoxazoles and benzothiazoles, while electron-withdrawing substituents and non-conjugated systems were associated with weak radical scavenging activity.

Table 8: Anti-oxidant activity of benzoxazoles and benzothiazoles (**6a-t**)

Entry	IC ₅₀ value (µg/mL)	Entry	IC ₅₀ value (µg/mL)
6a	57.2 ± 1.80	6k	43.24 ± 1.31

6b	55.80 ± 2.20	6l	>100
6c	44.30 ± 2.30	6m	42.18 ±1.68
6d	59.5± 1.70	6n	41.0 ±1.75
6e	78.50 ± 1.80	6o	>100
6f	47.20 ± 1.40	6p	56.12 ±1.85
6g	69.80 ± 2.30	6q	50.03 ±1.83
6h	>100	6r	>100
6i	54.10±0.70	6s	38.52 ±0.83
6j	>100	6t	76.40 ±2.74
		Ascorbic acid	32.08 ± 0.70

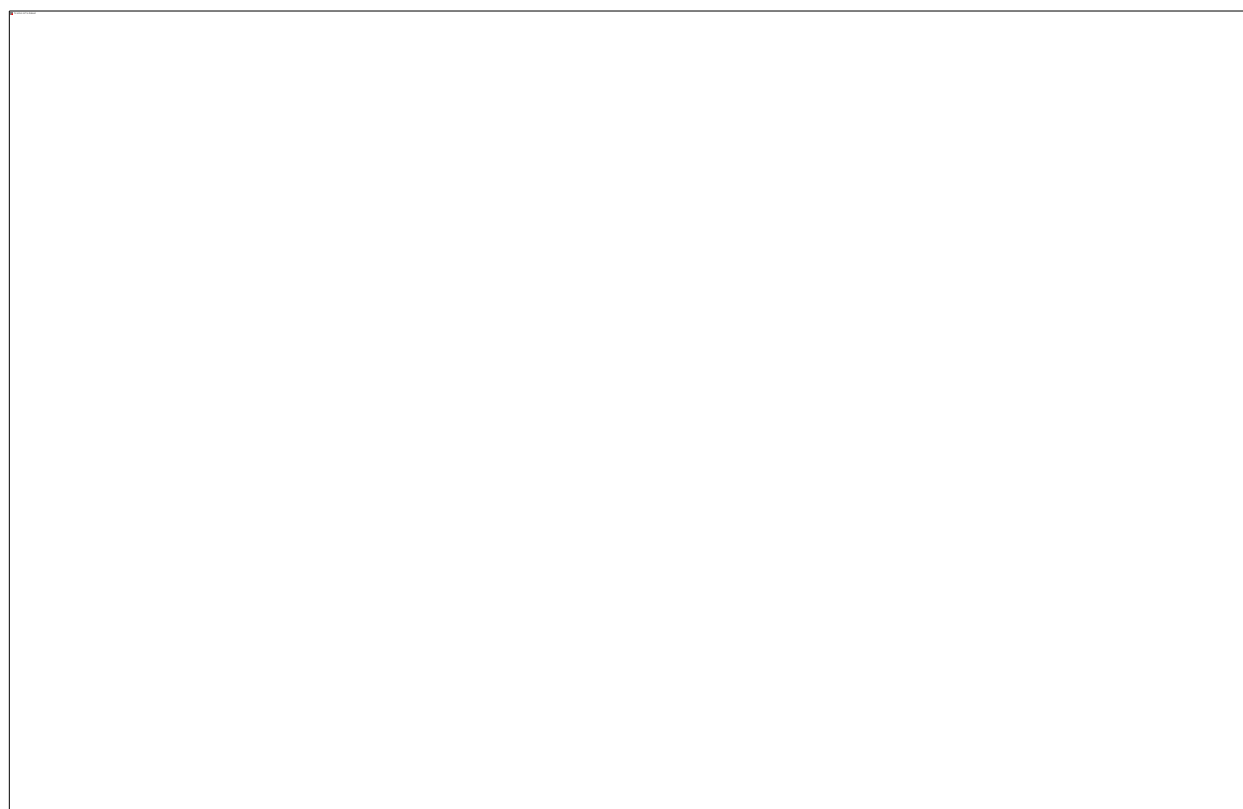


Fig 14: DPPH radical scavenging activity (% inhibition) of benzoxazoles and benzothiazoles and ascorbic acid at 20–100 µg/mL (mean ± SD, n = 3).

In contrast, compounds **6j**, **6h**, **6l**, **6o** and **6r** exhibited very low inhibitory activity, with IC_{50} values greater than 200 $\mu\text{g/mL}$. This reduced activity can be correlated with the absence of effective functional groups, lack of conjugation, or the presence of electron-withdrawing substituents, which may hinder their binding with the enzyme. The standard drug acarbose showed the highest inhibitory activity with an IC_{50} value of $34.10 \pm 0.9 \mu\text{g/mL}$. This confirms that the experimental method used is reliable and the obtained results are comparable with standard reference values. Although none of the synthesized compounds showed higher activity than acarbose, compounds like **6s**, **6m**, and **6n** exhibited promising results. Owing to this, it can be concluded from the present study that the presence of conjugated systems, aromatic rings, and electron-donating groups enhances the α -amylase inhibitory activity, whereas the absence of these features leads to a decrease in activity. These observations are useful for designing more effective antidiabetic molecules in future studies.

3.12 In silico assessment of bioactivity scores, pharmacological parameters and toxicity

In silico assessment using modern ADMET profiling and bioactivity evaluation software assists in the evaluation of pharmacological parameters relevant to absorption, distribution, metabolism, excretion, and toxicity. Recent literature showed that poor pharmacokinetic properties are a significant reason for the inadequacy of new drug candidates [92]. The bioactivity score prediction and pharmacological profile of the newly synthesized benzoxazoles and benzothiazoles (**6a-t**) were measured in order to analyze their drug-likeness, and the results are as presented in **Table 9**, which sheds light on a convincing overview of their potential to become drug candidates. The oral bioavailability of benzoxazoles and benzothiazoles (**6a-t**) was determined and analyzed according to Lipinski's rule of five. All the newly synthesized benzoxazoles and benzothiazoles (**6a-t**) showed violations of 0-1 of Lipinski's rule, suggesting their likelihood for oral activity. The molecular weights of the synthesized compounds ranged

from 195.2-319.2, indicating that these compounds may readily diffuse, be absorbed, or be transported across cell membranes. The calculated logarithmic partition coefficient (LogP), obtained based on partitioning between n-octanol and water, was within the optimal range, indicating a well-balanced ratio of lipophilic and hydrophilic properties.

Moreover, the topological polar surface area (TPSA) parameters of the synthesized benzoxazoles and benzothiazoles were observed to be within the optimal range, signifying their potential to participate in hydrogen bonding. The TPSA parameters of compounds (**6a–t**) ranged from 12.8-71.8 Å², which are well below the standard cutoff value of 160 Å² and are comparable to the TPSA of 329.0 Å². These results also support the desirable bioavailability of the synthesized compounds. In addition, all the synthesized benzoxazoles and benzothiazoles exhibited promising pharmacological profiles with respect to hydrogen-bonding properties, with hydrogen-bond donors ($n\text{-OHNH} \leq 5$) and acceptors ($n\text{-ON} \leq 10$) within the ideal limits. Overall, minimal violations of Lipinski's rule were observed, as presented in **Table 9**.

Table 9: ADMET properties of synthesized compounds of benzoxazoles and benzothiazoles using ADMET lab 3.0 software.

Compound	m log P	TPSA	n-atoms	n-ON	n-OHNH	n-violation	n-rot	MW
6a	3.58	71.86	18	5	0	0	2	240.05
6b	5.94	26.03	23	2	0	1	1	295.34
6c	3.65	26.03	15	2	0	0	1	195.22
6d	-	-	-	-	-	-	-	221.08
6e	2.50	38.92	15	3	0	0	1	196.06
6f	3.43	26.03	14	2	0	0	1	201.02
6g	4.35	12.89	17	2	0	0	2	237.06
6h	5.30	35.26	23	3	0	1	4	301.11

6i	4.33	26.03	16	2	0	0	1	229.03
6j	1.91	26.03	20	2	0	0	3	303.14
6k	5.94	22.12	23	2	0	1	4	317.09
6l	6.58	12.89	23	1	0	1	1	311.08
6m	4.44	28.68	18	2	1	0	1	250.06
6n	-	-	-	-	-	-	-	237.06
6o	2.05	12.89	20	1	0	0	3	319.21
6p	4.07	12.89	14	1	0	0	1	217.0
6q	4.48	12.89	17	1	0	0	2	237.06
6r	4.22	56.03	18	4	0	0	2	256.03
6s	3.79	33.12	16	2	1	0	1	227.04
6t	4.97	12.89	16	1	0	0	1	245.01

^aLogarithm of partition coefficient between n-octanol and water (mlogP)

^bTopological Polar Surface Area (TPSA)

^cNumber of atoms (n-atoms)

^dNumber of hydrogen bond acceptors (n-ON)

^eNumber of hydrogen bond donors (n-OH/NH)

^fMolecular weight (MW)

The ADMET analysis and drug-likeness properties of synthesized benzoxazoles and benzothiazoles (**6a-t**) were evaluated using the QikProp module of Schrödinger. QikProp was employed to predict important pharmacokinetic parameters related to absorption, distribution, metabolism, and excretion, along with compliance with Lipinski's Rule of Five. The analysis included key descriptors such as molecular weight, hydrogen-bond donors and acceptors, QPlogPo/w, QPlogBB, and QPPCaco. The predicted properties were used to assess the oral drug-likeness and pharmacokinetic suitability of the designed derivatives. **Table 10** summarizes the predicted ADMET properties of **6a-t** and compares them with the standard α -amylase inhibitor acarbose.

Table 10: ADME/ SAR and pharmacological parameter prediction for the newly synthesized benzoxazoles and benzothiazoles (**6a-t**) using ADME Lab – 3.0 software.

Compound	PlogBBB ^a	Pcaco ^b	LogHIA ^c	PLogS ^d
6a	0.309	- 4.749	0.0	- 4.902
6b	0.997	- 4.855	0.0	- 7.402
6c	0.978	- 4.823	0.0	- 4.745
6d	0.987	- 4.873	0.0	- 4.30
6e	0.971	- 4.614	0.002	- 2.84
6f	0.945	- 4.572	0.01	- 4.492
6g	0.597	- 4.558	0.001	- 5.588
6h	0.833	- 4.9	0.003	- 6.09
6i	0.991	- 4.526	0.0	- 5.782
6j	-	-	-	-
6k	0.782	- 4.778	0.007	- 6.097
6l	0.972	- 4.65	0.0	- 7.649
6m	0.966	- 4.857	0.002	- 5.465
6n	0.898	- 4.893	0.001	- 5.286
6o	-	-	-	-
6p	-	- 4.486	-	- 5.334
6q	-	- 4.558	-	- 5.588
6r	0.309	- 4.641	0.0	- 5.608
6s	0.562	- 5.072	0.001	- 4.816
6t	0.875	- 4.478	0.003	- 6.193

PlogBBB^a Predicted blood brain barrier partition coefficient (1-high penetration, 2-medium penetration and 3- low penetration).

PCaco^b Predicted Caco- 2 cell permeability in nm/s (acceptance range -1 is poor, +1 great),

log HIA^c Predicted human intestinal absorption in nm/s (acceptable range 0 is poor, >1 is great).

PlogS^d Predicted aqueous solubility.

The ADMET analysis of the synthesized benzoxazoles and benzothiazoles (**6a-t**) demonstrated generally favourable drug-like and pharmacokinetic characteristics compared with acarbose. The molecular weights of **6a-t** ranged from 195.22 to 319.20 g/mol, remaining well below the Lipinski limit of 500 g/mol, whereas acarbose showed a substantially higher molecular

weight of 645.611 g/mol. The compounds **6a-t** possessed 0–1 hydrogen-bond donors and 1.5–3 hydrogen-bond acceptors, indicating favourable hydrogen-bonding characteristics. In contrast, acarbose exhibited considerably higher values of 13 H-bond donors and 31.4 H-bond acceptors, contributing to its poor compliance with Lipinski's criteria.

The predicted QPlogPo/w values of the **6a-t** ranged from 2.154 to 5.677, suggesting moderate to relatively high lipophilicity. Most of the compounds from the series showed acceptable lipophilicity, although **6k** (5.481), **6l** (5.677), **6h** (5.142), and **6b** (5.069) exceeded the conventional Lipinski logP limit of 5 and consequently showed one Rule-of-Five violation each. The rest of the compounds exhibited zero violations, indicating better overall compliance with Lipinski's criteria. In comparison, acarbose showed a very low QPlogPo/w value of –7.14 and three Rule-of-Five violations, reflecting its highly hydrophilic character and poor conventional oral drug-likeness.

The QPlogBB values of **6a-t** ranged from –0.531 to 0.707, whereas acarbose showed a much lower value of –6.182. These results suggest markedly better predicted blood–brain barrier distribution for all the synthesized benzoxazoles and benzothiazoles relative to acarbose, although QPlogBB alone should not be interpreted as evidence of clinically meaningful CNS penetration. The predicted QPPCaco values were also considerably higher for **6a-t** ranging between 792.311–8047.98, than for acarbose (0.025), indicating substantially greater predicted Caco-2 cell permeability and, consequently, potentially better intestinal absorption.

Overall, the ADMET results indicated that the majority of the compounds exhibited favourable physicochemical properties, high predicted intestinal permeability, and good compliance with Lipinski's rule of five. It is worth mentioning that sixteen compounds from the series showed no rule-of-five violations, while **6k**, **6l**, **6h**, and **6b** showed only one violation each. These findings suggest that the **6a-t** derivatives have a substantially more favourable

predicted oral drug-like profile than acarbose and support their further investigation as potential lead compounds.

In order to examine the comparative toxicity profile, the safety profiles of all the synthesized benzoxazoles and benzothiazoles (**6a-t**) were evaluated using the AI-based GeinTox module of the Geinforce tool. The predicted toxicity profiles of **6a-t** were compared with acarbose, which was used as the standard α -amylase inhibitor. The analysis was performed to identify compounds with favourable predicted safety characteristics and to assess their suitability for further development as potential α -amylase inhibitors. The comparative toxicity profiles of **6a-t** and acarbose are summarized in **Table 11**.

Table 11: ADMET properties of synthesized compounds of benzoxazoles and benzothiazoles (**6a-t**) using Schrodinger LLC QikProp version 4.3.

Sr. No.	Compound	MW	donor HB	accept HB	QPlogPo/w	QPlogBB	QPP Caco	Violation (Rule of five)
1	Acarbose.	645.611	13	31.4	-7.14	-6.182	0.025	3
2	6a	240.218	0	3	2.414	-0.531	792.311	0
3	6b	295.34	0	2	5.069	0.39	6566.676	1
4	6c	195.22	0	2	3.234	0.404	6610.087	0
5	6d	221.258	0	2	3.776	0.3	8047.98	0
6	6e	196.208	0	3	2.387	0.211	4032.983	0
7	6f	201.242	0	2	3.149	0.51	6306.667	0
8	6g	221.258	0	2	3.795	0.211	6533.529	0
9	6h	301.344	0	2.75	5.142	0.194	6607.32	1
10	6i	229.665	0	2	3.643	0.575	6610.384	0
11	6j	303.14	0	2	2.154	0.456	6852.072	0
12	6k	317.405	0	2.25	5.481	0.336	7285.994	1

13	6l	311.4	0	1.5	5.677	0.512	7190.732	1
14	6m	250.317	1	1.5	4.003	0.339	4491.346	0
15	6n	237.319	0	1.5	4.299	0.271	6396.936	0
16	6o	319.20	0	1.5	4.012	0.315	6542.021	0
17	6p	217.303	0	1.5	3.567	0.627	7063.512	0
18	6q	237.319	0	1.5	4.52	0.363	7254.301	0
19	6r	256.278	0	2.5	2.994	-0.401	871.761	0
20	6s	227.28	1	2.25	3.021	-0.006	2207.437	0
21	6t	245.726	0	1.5	4.456	0.707	7286.995	0

mol MW: molecular weight in g/mol

donor HB: Donor Hydrogen bond (≤ 5)

accept HB: Acceptor Hydrogen bond (≤ 10)

QPlogPo/w: octanol/water partition coefficient (-2.0 to 6.5)

QPlogBB: brain/blood partition coefficient (-3.0 to 1.2)

QPPCaco: Caco₂ cell permeability (>25 poor, >500 great)

(%) Oral absorption: percent human oral absorption (>80% high)

Violation (Rule of five): Lipinski's rule of five violations by molecules (≤ 1)

The comparative toxicity analysis using the GeinTox tool revealed that the **6a-t** derivatives generally exhibited an acceptable predicted toxicity profile, as shown in **Table 12**. All **6a-t** compounds were predicted to be inactive for hepatotoxicity and respiratory toxicity, suggesting a lower probability of liver- and respiratory-related toxicity. Most derivatives were also predicted to be inactive for carcinogenicity; however, **6r** and **6a** showed active carcinogenicity predictions with a probability of 0.53, indicating that these two compounds may require additional safety evaluation. Most **6a-t** exhibited an LD₅₀ of 300 mg/kg, toxicity class 3, and a high predicted risk level. BBB penetration was predicted to be active for most compounds.

Table 12: Comparative toxicity profile: design molecules vs. standard acarbose using GeinTox tool

Compo und code	LD₅₀ (mg/kg)	Toxicity class	Hepatotoxicity	Carcinogenicity	Respiratory toxicity	BBB penetration	Risk level
6a	300.0	3	Inactive (0.39)	Active (0.53)	Inactive (0.10)	Active (0.85)	High
6b	423.8	4	Inactive (0.23)	Inactive (0.41)	Inactive (0.10)	Active (0.85)	Moderate
6c	300.0	3	Inactive (0.14)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6d	300.0	3	Inactive (0.16)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6e	300.0	3	Inactive (0.12)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6f	300.0	3	Inactive (0.34)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6g	300.0	3	Inactive (0.16)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6h	423.8	4	Inactive (0.20)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	Moderate
6i	300.0	3	Inactive (0.17)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6j	300.0	3	Inactive (0.18)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6k	423.8	4	Inactive (0.22)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	Moderate
6l	423.8	4	Inactive (0.24)	Inactive (0.41)	Inactive (0.10)	Inactive (0.15)	Moderate
6m	300.0	3	Inactive (0.18)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6n	300.0	3	Inactive (0.18)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6o	423.8	4	Inactive (0.20)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	Moderate
6p	300.0	3	Inactive (0.36)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6q	300.0	3	Inactive (0.18)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6r	300.0	3	Inactive (0.40)	Active (0.53)	Inactive (0.10)	Active (0.85)	High
6s	300.0	3	Inactive (0.15)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
6t	300.0	3	Inactive (0.18)	Inactive (0.10)	Inactive (0.10)	Active (0.85)	High
Acarbose	23829.8	6	Inactive (0.12)	Inactive (0.10)	Inactive (0.10)	Inactive (0.15)	Low

Among the investigated derivatives, **6k**, **6l**, **6o**, **6h**, and **6b** demonstrated comparatively better predicted safety profiles, with an LD₅₀ of 423.8 mg/kg, toxicity class 4, and moderate risk levels. Notably, **6l** was predicted to be inactive for BBB penetration, whereas the majority of the **6a-t** showed active BBB penetration. In comparison, the standard drug acarbose exhibited the most favourable overall toxicity profile, with an LD₅₀ of 23,829.8 mg/kg, toxicity class 6, and a low predicted risk level. Acarbose was also predicted to be inactive for hepatotoxicity, carcinogenicity, respiratory toxicity, and BBB penetration, indicating a superior predicted safety profile compared with the designed **6a-t**.

3.13Molecular docking

3.13.1 Protein Preparation

The three-dimensional crystal structure of human pancreatic α -amylase complexed with acarbose (PDB ID: 1XH0) was selected for molecular docking investigation. The structure was obtained by X-ray crystallography at approximately 2.1 Å resolution and contains the N298S (Asparagine-to-Serine at position 298) mutation. Despite this mutation, the active-site geometry is conserved, making the structure suitable for inhibitor-binding and molecular docking studies.

Human pancreatic α -amylase is a glycoside hydrolase (EC 3.2.1.1) responsible for the hydrolysis of α -1,4-glycosidic bonds in dietary starch, generating smaller carbohydrate products. The enzyme possesses a characteristic (β/α)₈ TIM-barrel architecture, with the catalytic site located within a well-defined substrate-binding cleft.

The 1XH0 structure contains the clinically used α -amylase inhibitor acarbose in the catalytic active-site cleft. Acarbose is a pseudo-tetrasaccharide and acts as a competitive inhibitor of α -amylase. The presence of the co-crystallized inhibitor provides a structurally defined binding pocket and makes 1XH0 particularly useful for comparative molecular docking and analysis of newly designed α -amylase inhibitors.

The catalytic region contains several important amino acid residues. In particular, Asp197 acts as the catalytic nucleophile, Glu233 functions as a proton donor, and Asp300 participates in stabilization during catalysis. Other residues, including His201, His299, Tyr62, Trp58, and Trp59, contribute to ligand recognition and stabilization. The aromatic residues Trp58 and Trp59 are especially important for stacking interactions with carbohydrate-like substrates and inhibitors.

The crystal structure also contains Ca^{2+} and Cl^- ions, which contribute to structural stability and enzymatic function, respectively. The protein preparation procedure was therefore carried out to obtain an optimized receptor structure suitable for reliable ligand–protein interaction analysis [93].

3.13.2 Receptor grid generation

The receptor grid was generated around the co-crystallized acarbose-binding site of human pancreatic α -amylase (PDB ID: 1XH0). Acarbose was used as the reference ligand to identify the biologically relevant catalytic pocket and to define the centre of the receptor grid. XYZ coordinates of generated grid box are $\mathbf{X}=-24.62 \text{ \AA}$, $\mathbf{Y}=32.53 \text{ \AA}$, $\mathbf{Z}=13.28 \text{ \AA}$

The selected binding region encompasses the major catalytic and ligand-stabilizing residues, particularly Asp197, Glu233, Asp300, His201, His299, Tyr62, Trp58, and Trp59. These residues are important for substrate recognition, catalytic activity, hydrogen-bond formation, and aromatic interactions within the α -amylase active site.

The receptor grid was constructed to adequately cover the acarbose-binding pocket and allow the designed **6a-t** sufficient conformational freedom during docking. Selection of the co-crystallized ligand-binding region provides a biologically relevant docking environment for evaluating the potential of the designed compounds as α -amylase inhibitors [94].

3.13.3 Interpretation of molecular docking study

The molecular docking study demonstrated that all the synthesized benzoxazoles and benzothiazoles (**6a-t**) exhibited favourable binding interactions with the target protein, with docking scores ranging from -4.136 to -5.992 kcal/mol as presented in **Table 13**.

Table 13: Glide SP docking scores and high interacting amino acids

Compound Code	Binding Affinity (Docking Score)	Glide Energy	Glide E-model	Bond/Interaction Type	Interacting Amino Acid	Bond Distance (Å)
6s	-5.992	-32.875	-46.154	π - π stacking	TRP59	4.18
				π - π stacking	TYR62	4.02
				H-bond	ASP197	1.54
Acarbose	-5.956	-67.874	-94.404	H-bond	GLU240	2.01
				H-bond	GLU240	2.15
				H-bond	LEU237	2.77
				H-bond	ASP197	2.11
				H-bond	ASP300	2.06
				Salt bridge	ASP300	3.88
				H-bond	ARG195	2.32
				H-bond	LYS200	2.60
6m	-5.286	-33.442	-45.023	π - π stacking	TRP58	5.36
				H-bond	ASP300	2.12
6t	-4.810	-30.282	-39.733	π - π stacking	TRP59	4.16
6i	-4.799	-28.666	-37.576	π - π stacking	TRP59	4.22
6b	-4.752	-31.956	-41.723	π - π stacking	HIS305	5.42
				H-bond	HIS305	2.07
6c	-4.719	-26.988	-35.274	π - π stacking	TRP59	4.19
6f	-4.707	-27.221	-35.664	π - π stacking	TRP59	4.20

6p	-4.672	-29.235	-38.095	π - π stacking	TRP59	4.16
6l	-4.632	-32.144	-41.732	π - π stacking	HIS305	5.43
				H-bond	HIS305	2.28
6o	-4.631	-32.568	-40.060	—	—	—
6q	-4.624	-30.375	-39.415	—	—	—
6e	-4.523	-27.527	-35.758	π - π stacking	TRP59	4.29
6d	-4.514	-29.920	-38.324	—	—	—
6n	-4.489	-30.774	-39.648	—	—	—
6k	-4.454	-36.737	-46.920	π - π stacking	HIS201	4.55
6h	-4.413	-36.586	-46.596	π - π stacking	TRP59	4.39
6g	-4.377	-28.697	-36.683	—	—	—
6a	-4.292	-33.601	-41.614	H-bond	ILE235	1.83
				H-bond	LYS200	2.75
				Salt bridge	GLU233	3.36
				π - π stacking	HIS201	4.76
6r	-4.210	-35.093	-43.459	H-bond	ILE235	1.84
				Salt bridge	GLU233	3.35
				H-bond	LYS200	2.72
				π - π stacking	HIS201	4.72
				π - π stacking	TYR62	5.01
6j	-4.136	-30.846	-38.223	π - π stacking	TRP59	4.21

Among the investigated compounds, **6s** showed the most favourable docking score (-5.992 kcal/mol), slightly better than the standard drug acarbose (-5.956 kcal/mol). **6s** was stabilised within the binding pocket through π - π stacking interactions with TRP59 and TYR62 at 4.18 and 4.02 Å, respectively, along with a strong hydrogen bond with ASP197 at 1.54 Å. These interactions indicate favourable accommodation of **6s** within the active site.

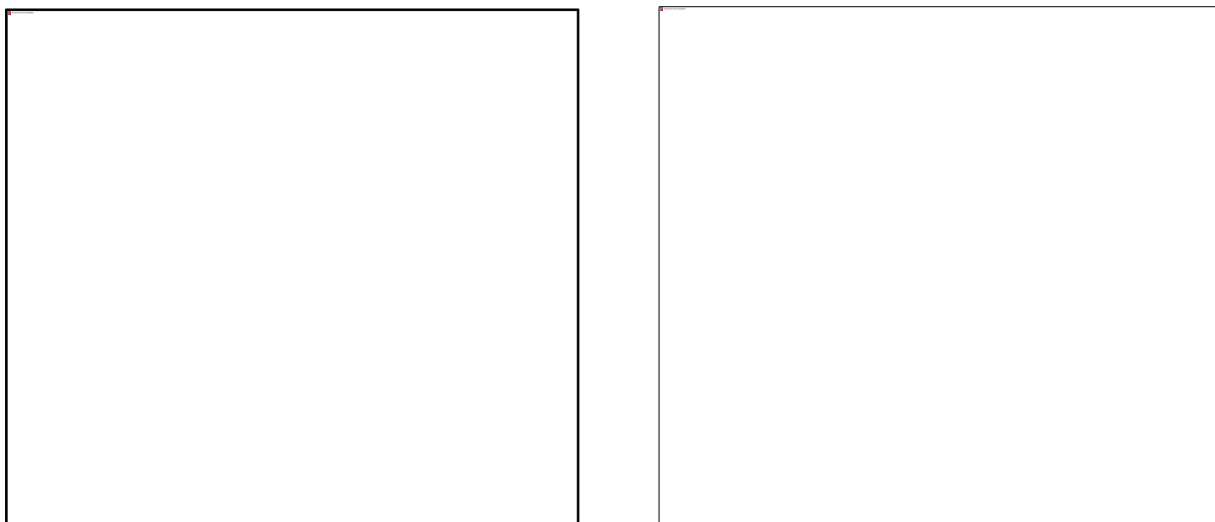


Fig 15: 2D and 3D interactions of **6s** with α -amylase

The standard drug acarbose exhibited a docking score of -5.956 kcal/mol and formed an extensive interaction network involving hydrogen bonds with GLU240, LEU237, ASP197, ASP300, ARG195, and LYS200, together with a salt bridge involving ASP300. Acarbose also displayed substantially more negative Glide energy (-67.874 kcal/mol) and Glide E-model score (-94.404) than **6a-t**, indicating strong overall energetic stabilisation of its docked pose. Therefore, although **6s** showed a marginally better docking score, acarbose exhibited stronger overall interaction energetics. Subsequently, **6m** displayed the next most favourable docking score of -5.286 kcal/mol and formed a π - π stacking interaction with TRP58 (5.36 Å) and a hydrogen bond with ASP300 (2.12 Å).

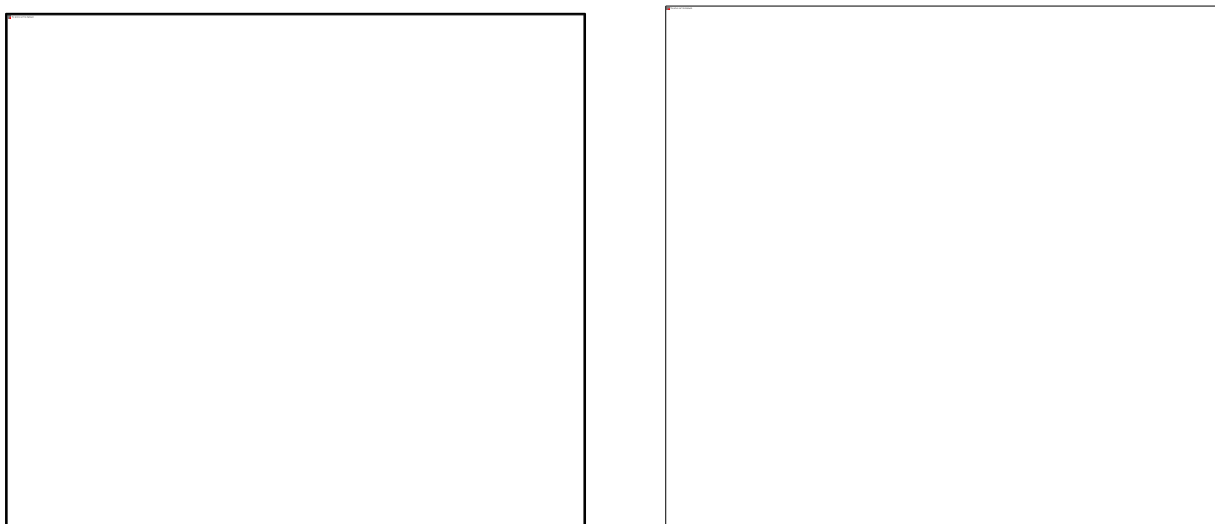


Fig 16: 2D and 3D interactions of acarbose with α -amylase

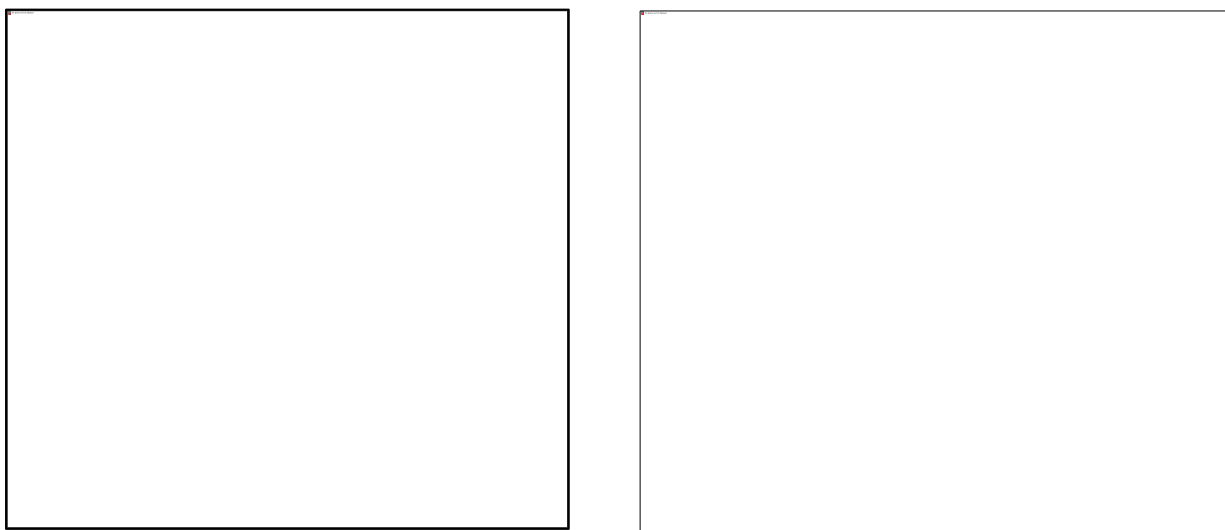


Fig 17: 2D and 3D interactions of **6m** with α -amylase

Compounds **6t**, **6i**, **6c**, **6f**, **6p**, **6e**, **6h**, and **6j** predominantly formed π - π stacking interactions with TRP59, suggesting that this aromatic residue plays an important role in ligand recognition and stabilisation. **6b** and **6l** interacted with HIS305 through both π - π stacking and hydrogen bonding. Notably, **6a** and **6r** displayed multiple interactions despite their comparatively moderate docking scores of -4.292 and -4.210 kcal/mol, respectively. **6a** formed hydrogen bonds with ILE235 and LYS200, a salt bridge with GLU233, and π - π stacking with HIS201. Similarly, **6r** interacted with ILE235, LYS200, GLU233, HIS201, and TYR62, indicating extensive active-site engagement. In contrast, **6o**, **6q**, **6d**, **6n**, and **6g** showed no specific hydrogen-bonding, salt-bridge, or π - π stacking interactions in the reported docking poses.

Overall, the docking results identify **6s** as the most promising compound based on docking score, showing a binding affinity comparable to and marginally better than acarbose while establishing key interactions with TRP59, TYR62, and ASP197 as shown in **Fig. 15-17**. The

recurring involvement of TRP59, ASP197, ASP300, HIS201, HIS305, and other active-site residues across the ligand series supports favourable binding of several **6a-t** within the target pocket. However, the markedly more favourable Glide energy and E-model values of acarbose indicate that the standard retains stronger overall energetic stabilisation, and therefore the docking score should be interpreted together with the interaction profile and energy terms.

3.14 Structure activity relationship (SAR)

The basic scaffold of all the synthesized 2-aryl substituted benzoxazoles and benzothiazoles (**6a-t**) was considered as the model compound for the anti-diabetic and anti-oxidant activities, as shown in **Fig 18**. The planar nature of the aromatic phenyl ring of the benzoxazole and benzothiazole skeletons promotes hydrophobic and π - π interactions with biological targets. The heteroatoms, especially nitrogen and oxygen or sulphur, can take part in hydrogen-bonding and other molecular interactions. The substituents on the aryl ring can markedly influence biological activity by altering the electronic, lipophilic and hydrogen-bonding properties of the molecules. The most potent compound **6s**, containing an electron-donating group such as -OH, may enhance anti-oxidant activity, particularly when phenolic -OH groups are present, because they can contribute to radical-scavenging through hydrogen or electron donation. In contrast, electron-withdrawing groups such as -NO₂ and halogens can modify molecular polarity and target-binding interactions and may improve activity depending on their position and the biological target. Halogen substitution may additionally enhance hydrophobic interactions and metabolic stability. For anti-diabetic activity, appropriate substitution on the 2-aryl ring may improve interactions with enzymes or proteins involved in glucose metabolism, including α -amylase. Overall, the biological activity of 2-aryl benzoxazoles and benzothiazoles is determined by the combined effects of the heterocyclic core, 2-aryl substitution, substituent position and electronic characteristics, and an optimum balance between hydrophilicity and

lipophilicity appears important for achieving enhanced anti-oxidant and anti-diabetic activity [95].

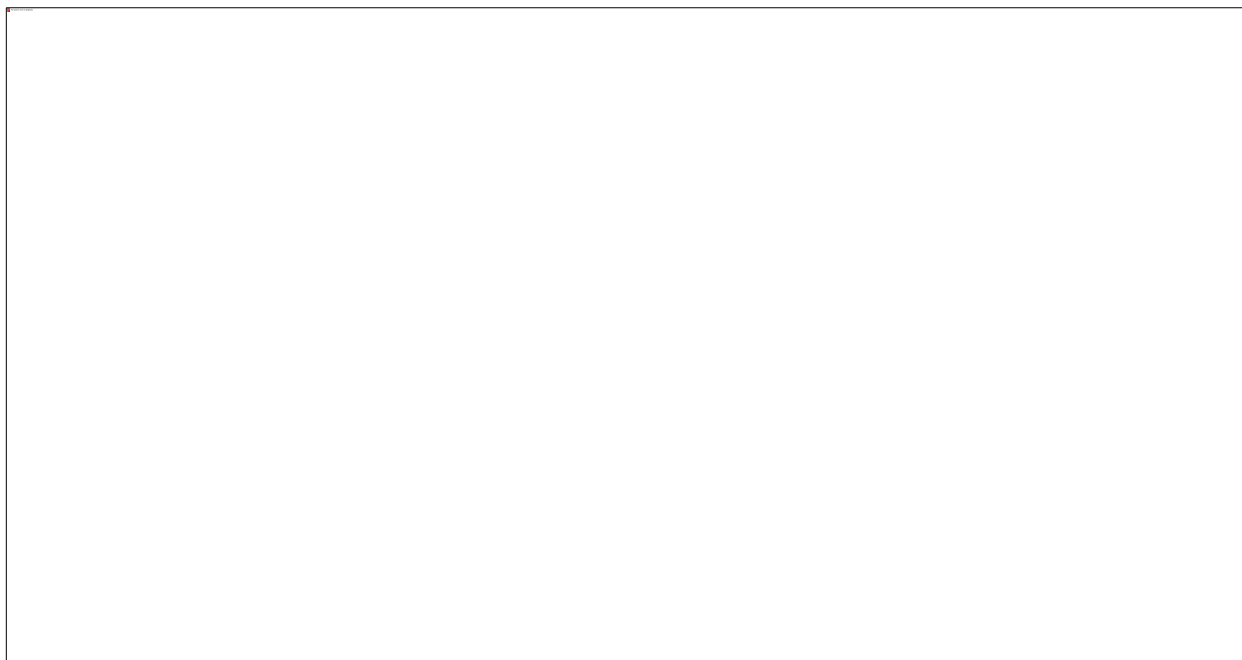


Fig 18: Summary of SAR studies of benzoxazoles and benzothiazoles (**6a-t**)

4. Conclusion

The $\text{ZnCuFe}_2\text{O}_4$ nanoparticle catalyst was easily prepared by green synthesis of biogenic zinc copper ferrite nanoparticles using *Bryophyllum pinnatum* leaf extract, their structural and morphological characterization was carried out using various analytical techniques. A new, rapid, and facile protocol for the synthesis of 2-substituted benzoxazoles/benzothiazoles starting from aromatic aldehydes and 2-aminophenol/2-aminothiophenol was reported using the prepared biogenic $\text{ZnCuFe}_2\text{O}_4$ nanoparticles as a robust heterogeneous catalyst. A small library of twenty new/reported benzoxazoles and benzothiazoles was synthesized using the developed protocol. The synthesized compounds demonstrated promising anti-diabetic and anti-oxidant activities. The ADMET profiling, bioactivity, and toxicity predictions using AI-ML-based tools revealed that the screened molecules exhibit drug-like properties and strong interactions with biological

receptors. The molecular docking analysis revealed the mode of action of target compounds *via* inhibition of α -amylase. The present research highlights the preliminary scope of target compounds in the design of new antidiabetic scaffolds after in-depth preclinical and clinical studies.

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Data availability All data produced or examined in the course of this study are presented within this article. Additional materials can be found in the separately provided Supplementary Information file.

Declarations

Conflict of interest The authors state that there are no financial interests or personal relationships that could have influenced the work presented in this paper.

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