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Evaluation, Characterization of Antioxidant Activity for Novel Benzothiazole Derivatives

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Abstract The present study focuses on the design, synthesis, in-silico studies, characterization, and evaluation of the antidiabetic activity of nitrogen and sulphur-containing heterocyclic compounds, specifically benzothiazole derivatives (2a-2e). The synthesized compounds were characterized using spectroscopic techniques such as IR, NMR, and mass spectrometry. Their biological activity was assessed through molecular docking studies, which revealed strong binding interactions with target proteins, with compound 2a exhibiting the highest docking score (-8.7). Swiss ADME analysis was performed to evaluate pharmacokinetic properties, indicating good drug-likeness and bioavailability. In vitro antidiabetic activity was assessed using the α-amylase inhibition method, where compounds 2a and 2e demonstrated significant inhibitory potential with IC_{50} values of 22.95±2.50µg/mL and 27.80±3.00µg/mL, respectively. These findings suggest that benzothiazole derivatives, particularly compound 2a, hold promise as potential antioxidant agents, warranting further investigation for therapeutic applications.

Keywords Antioxidant, Benzothiazole, Molecular docking, Pharmacokinetic

Introduction

Heterocyclic compounds are especially significant due to the diverse range of physiological actions linked to this class of chemicals[1]. Heterocyclic rings are present in a number of significant substances, including the majority of vitamin B complex members, alkaloids, antibiotics, chlorophyll, pigments from other plants, amino acids, dyes, medications, enzymes, genetic material, and DNA. Heterocyclic compounds with nitrogen and sulphur heteroatoms are known as benzothiazoles[2].

Because of its biological and physiological properties, Benzothiazole analogues and derivatives play a significant role in pharmaceutical, synthetic, and medicinal chemistry[3]. Certain benzothiazoles, particularly in the past several years, have been used in bioorganic and medicinal chemistry as well as in the creation of pharmaceuticals such bentaluron, lubeluzole, pramipexole, probenazole, ethoxazolamide, and zopolrestat. Benzothiazole and its derivatives have been extensively studied for their potential biological activities[4]. In the 1950s, derivatives of benzothiazole, particularly 2-aminobenzothiazoles were

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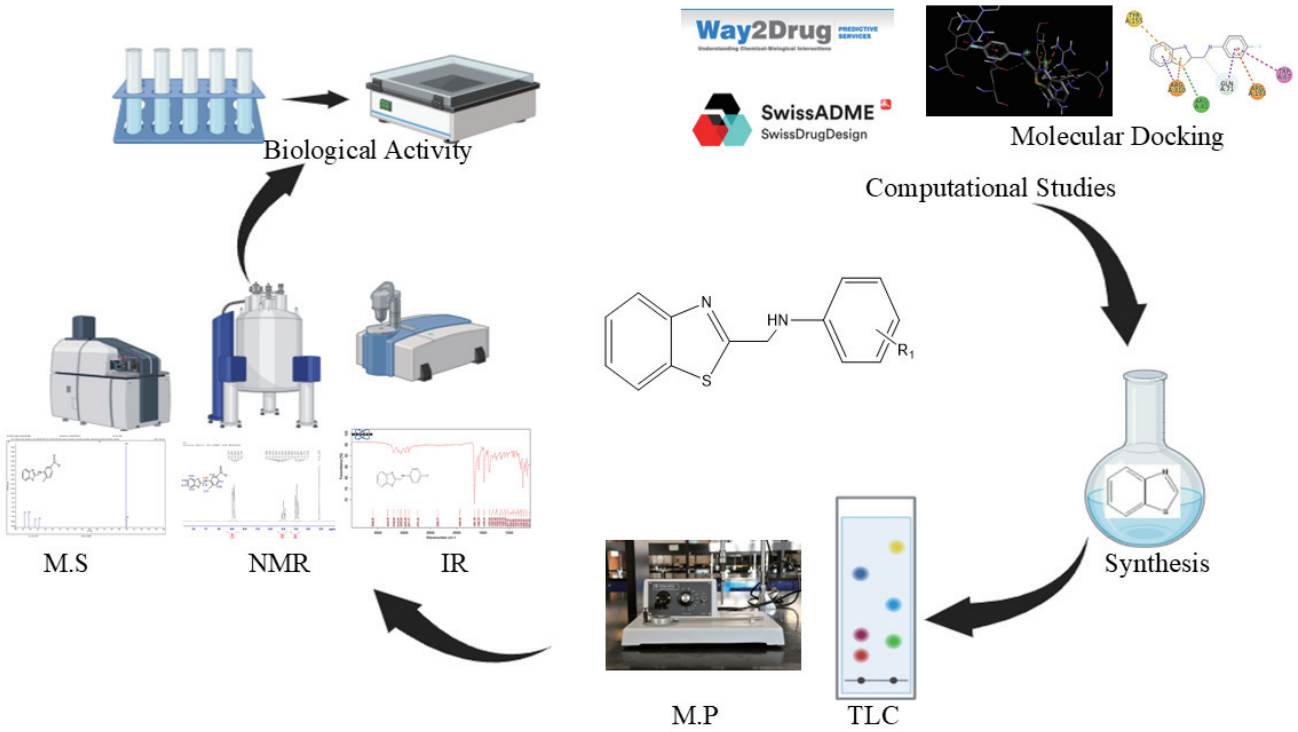


Figure 1: Graphical abstract – It shows the detain process of steps carried out during the research work starting form designing of scheme to biological activity.

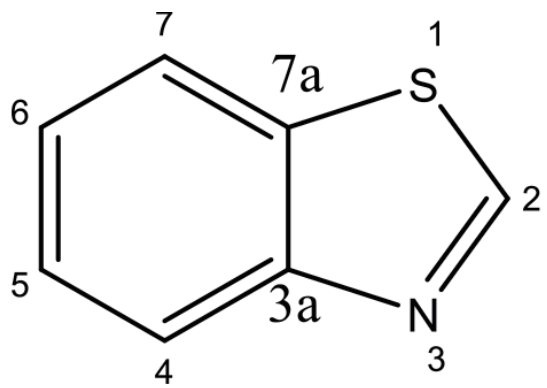


Figure 2: Structure of Benzothiazole

investigated for their muscle relaxant properties[5].

The fungi *Aspergillus clavatus* and *Polyporus frondosus* produce BTA, a flavouring compound that is present in cranberries and tea leaves. They are also employed as plant protectors, colour intermediates , appetite suppressants, imaging agents for B - amyloid plaque, and photographic inducers[6]. A variety of chemical applications employ BTA derivatives, which are heterocyclic chemicals, including polymer chemistry, dyes, medicines, silver photography, and benzothiazole as fungicide. Tire manufacture is one of the many specialty goods that use elastomeric unsaturated polymers of BTA derivatives, which are produced from (lattice) sulphide bonds and crosslinked (MBT/BTSH) to create an elastic substance that acts as a rubber accelerator[7].

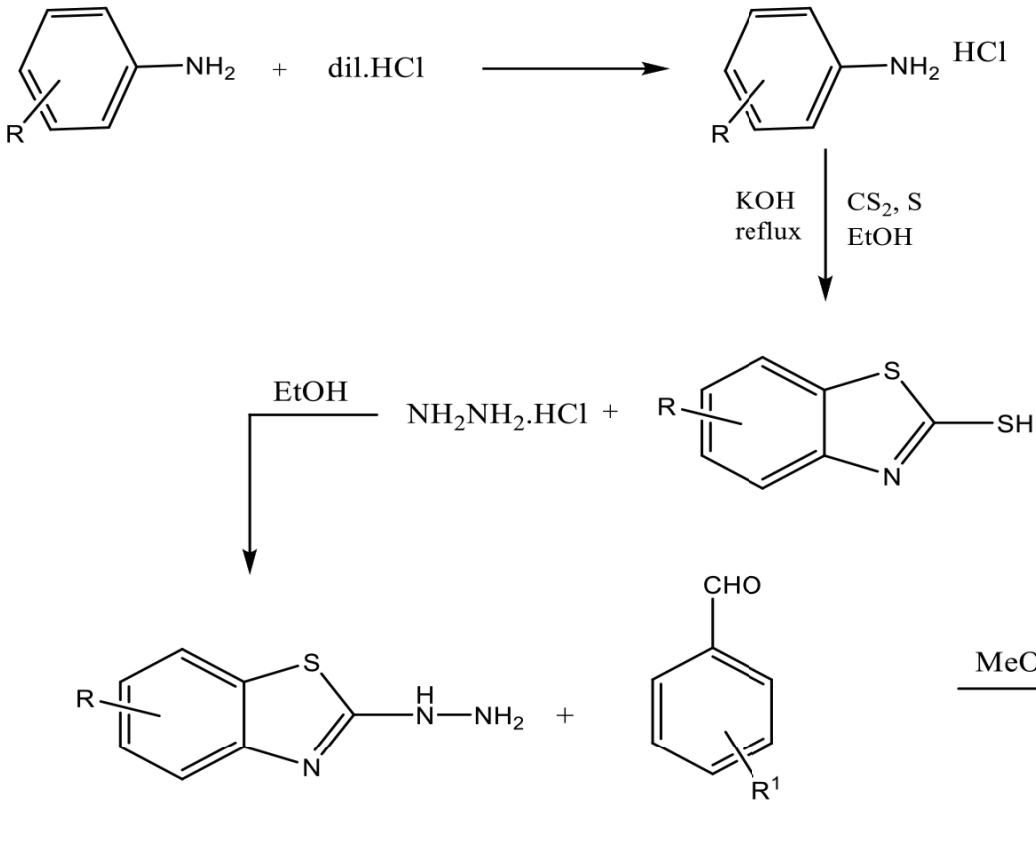
In this research the Substituted N-Benzothiazol-2-yl-N'-benzylidene-hydrazine derivatives are synthesised for the 1st time and they are tested for antidiabetic activity.

Chemistry of Benzothiazole

Hoffmann initially developed and published a number of synthetic techniques in 1887 because of the splitting's straightforward mechanism. To create BTA, 2-aminothiophenols undergo a condensation reaction with nitriles, aldehydes, carboxylic acids, acyl chlorides, and or esters. But it is comparable to things like the quick oxidation of 2-amino thiophenols with compensators, BTA was created by Jacobson's using the 2-amino thiophenols' ring closure[8]. Other ways to make it include using a microwave to react 2-aminothiophenols with p-chloro cinnamaldehydes. BTA has a variety of uses, including the production of compounds that are physiologically active and more. The synthesis of BTA derivatives, including Grignard aryls thiocyanate techniques, is of great interest in biology. Utilizing a number of catalysts, including silica-held copper (II) nanoparticles, PCC, nanocera (CeO2) , and boron trifluoride ethers[9].

Material and Method

Reaction



Comp Code	R	R¹
2a	p-fluro aniline	p-fluro benzaldehyde
2b	p-Chloro aniline	p-Chloro benzaldehyde
2c	2-methyl, 5-nitro aniline	O-Nitro benzaldehyde
2d	4-chloro, 3-nitro aniline	m-Nitro benzaldehyde
2e	p-nitro aniline	p-Nitro benzaldehyde

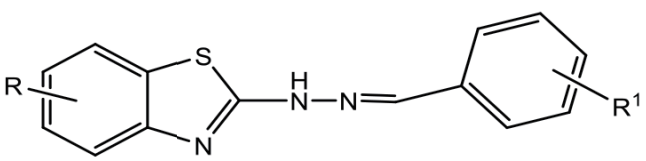


Figure 3: Synthetic Pathway

General Procedure for synthesis of Substituted N-Benzothiazol-2-yl-N'-benzylidene-hydrazine

Preparation of aniline HCl salt

Dissolve 1g of substituted aniline in 8 ml of ethanol. Add solution to boiling dil. HCl (10ml of conc. HCl in 90ml of H_2O). Boil the solution for 5 minutes, filter if necessary and allow it to cool. The salt of aniline hydrochloride was formed[10].

Preparation of substituted 2-hydrazinyl-1, 3-benzothiazole

To the above obtained aniline hydrochloride salt, sulphur and carbon disulfide was added in 1:3 proportion respectively, stirred continuously by addition of in KOH and about 15-20 ml ethanol was added, reflux it for 6-7 hrs. Further in 2-mercaptobenzothiazole, substituted benzaldehyde was added with methanol (15-20 ml), stir it and reflux the mixture at about 150-155 °C for 5-7 hrs, solid mass was obtained. Recrystallized from methanol to yield substituted N-Benzothiazol-2-yl-N'-benzylidene-hydrazine[11]. Synthetic pathway is represented in (Figure 3) & Derivative structures from (2a-2e) are represented in (Figure 4)

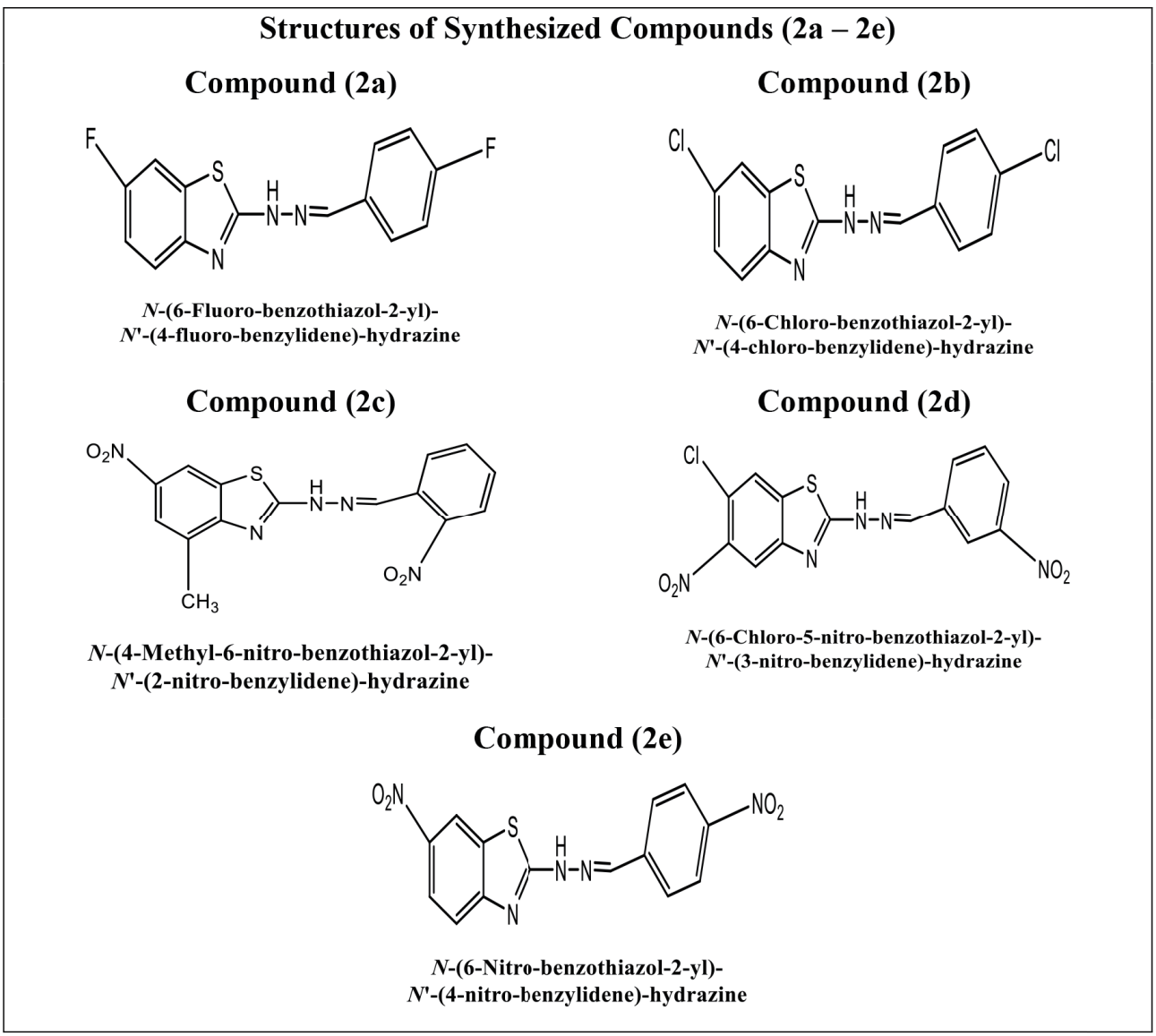


Figure 4: Structures of Synthesised Compounds (2a-2e)

Material & Method

PASS (Prediction of Activity Spectra for Substance) identification

Designed synthetic compounds were tested using PASS software to identify the activity that designed compounds contains.

The Mol. File was uploaded in the software and prediction was run. Within few seconds the full activity spectra was displayed, based on which the activity for the synthesized compounds was selected[12]. The results are shown in (Figure 6).

Molecular docking

Molecular docking was performed by using various softwares and data banks that are Pyrx, Pymol, Drug discovery studio, chemdraw, Protein data Bank (PDB) and Drug bank. Among previous the main three Softwares are PyRx, Pymol and Drug discovery studio[13]. As per graphical representation (figure 5).

Swiss ADME

Here Physicochemical properties like Mol. Formula, Mol. Wt., No. of heavy atoms, molar refractivity, TPSA, no. of H-bond donar and acceptors along with Solubility, GI absorption, BBB permeability, Lipinski rule, synthetic accessibility, Lipophilicity etc. was studied.

The mol. Files of designed drugs which are drawn in chemdraw were added, it is the converted to smiles, then run the process and results are obtained. The boiled egg graph is also obtained which represents the data in graphical form[14]. The results are shown in (Table 2) & (Table3) and it's boiled egg graph is represented in (Figure 8).

Biological activity

Antioxidant activity

Reactive oxygen species (ROS) are continuously produced as by-products of mitochondrial electron transport during cellular respiration within the body. Free radicals of ROS include alkoxyl (RO•), hydroxyl (HO•), peroxyl (ROO•), super oxide (O2•) and nitric acid (NO•). These all species are unstable and react freely with their molecules to achieve stability. This all species have capability of attacking proteins in tissues, lipids in cell membrane, carbohydrates, amino acid and DNA resulting in oxidative damage and can pose a threat leading to condition like cardiovascular disease and cancerous cell growth. Antioxidants provide protection against oxidative damage by scavenging free radicals and reducing ROS. It acts by two different mechanism[15,16].

$R^{\bullet} + Ar\text{-}OH \longrightarrow RH + ArO^{\bullet}$

This mechanism is referred to as H-atom transfer. It is governed by hydroxy bond dissociation enthalpy (BDE) which is the main parameter in evaluating the antioxidant activity. The second mechanism is called the electron transfer. When antioxidant can donate an electron to the free radical the free radicals become free radicals cation[17].

$R^{\bullet} + Ar\text{-}OH \longrightarrow R^{-} + ArO^{\bullet+}$

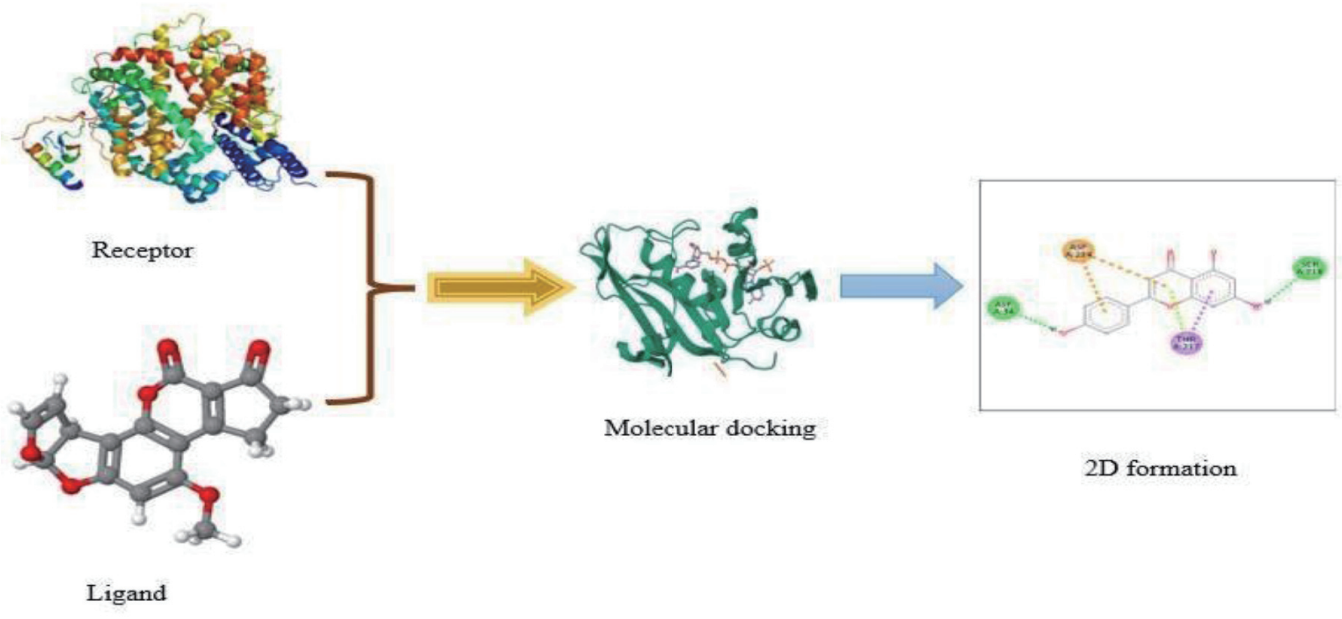


Figure 5: Molecular docking and 2D formation

Principle

Peroxide ions are produced by various oxidase enzymes. Current research aims to confirm the hypothesis that the reduction of hydrogen peroxide to hydroxide ions (OH^-) leads to damage in biological systems. In this approach, a compound with antioxidant properties is incubated with hydrogen peroxide, and the degradation or decrease of hydrogen peroxide is measured using spectrometric techniques at 230 nm [18].

$$2H_2O_2 \xrightarrow{e^- \text{ from antioxidant}} 2H_2O + O_2$$

Mechanism for antioxidant activity by H_2O_2 model

We have tested the synthesized benzothiazole compounds for in-vitro antioxidant activity by two models.

a. Hydrogen peroxide scavenging assay

Hydrogen peroxide radical scavenging is a classical method to determine hydrogen peroxides (H_2O_2) scavenging activity of compounds is evaluated by measuring the disappearance of H2O2 at a wavelength of 230 nm.

The peroxide ion is generated by several Oxidase enzymes. Research is being carried out on the way to confirm the assumption that reduction of H_2O_2 in its hydroxyl ion (OH^-) causes severe damage to biological system. In this method when a compound possessing antioxidant activity is incubated with H_2O_2, the decay or loss of hydrogen peroxide can be measure by spectrometric techniques at 230 nm[19].

Preparation of dilutions for Synthesized compounds and Standard Compound:

10 mg of accurately weigh sample was dissolved in 10 ml of methanol (to give 1000 ppm solution) for each of the benzothiazole derivatives and ascorbic acid. From this stock solution different dilutions were prepared.

Procedure:

The solution of H_2O_2 (20Mm) was prepared in phosphate buffer solution pH 7.4. Series of concentrations of samples were prepared (10, 20, 30, 40, 50 µg mL-1). Then in each test tube 4 ml of test solution and 1ml of hydrogen peroxide solution was added. The solution was incubated for 10 min at 250C. Absorbance of reaction mixture was taken at 230 nm using methanol as blank and % inhibition was calculated. IC_{50} values was calculated by regression analysis[20].

$$\% \text{ inhibition} = [(A_{blank} - A_{test}) / A_{blank}] \times 100$$

b. Nitric oxide radical scavenging assay (NO)

Sodium nitroprusside in aqueous solution at physiological pH spontaneously produce NO, which reacts with oxygen to form nitrite ions that can be determined by the use of the Griess reagent. Scavengers of NO compete with oxygen and reduce the production of nitric oxide[21].

NO its contribution to oxidative damage is also reported. This is due to the fact that NO can react with superoxide to form the peroxide to form the peroxinitrile anion, which is potential oxidant that can decompose to produce OH and NO. The procedure is based on principle- sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interact with oxygen to form nitrite ions that can estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitrite ions. Large number of NO may lead to tissue damage[22].

Reagents:

• Sodium nitroprusside solution (10 mg/ml): 300 mg sodium nitroprusside was accurately weighed and dissolved in 2.5% phosphate buffer pH 7.4 to makeup volume up to 100 ml in a volumetric flask

• Sulphanilamide (1% w/v): 100 mg of sulphanilamide was dissolved in 2.5% phosphate buffer pH 7.4 and made the volume to 100 ml in a volumetric flask.

• Griss reagent: 1% Sulphanilamide in 2.5% phosphate buffer + 0.1% N-(1-napthy)-ethylenediamine dihydrochloride in 2.5 % phosphate buffer pH 7.4.

Preparation of dilutions of Synthesized compounds and standard compound:

10 mg of accurately weigh sample was dissolved in 10 ml (i.e. 1000 ppm) of methanol for each of the benzothiazole analogues and ascorbic acid. From this stock solution different dilutions were prepared.

Procedure:

The sodium nitroprusside solution in phosphate buffer pH 7.4. A series of concentrations of the samples were prepared (10, 20, 30, 40, 50 µg mL-1). 1ml of sodium nitroprusside solution was added to the test solution. Solution was incubated for 2 hr at 25°C. 0.5 ml Griess reagent was added to it and absorbance was taken at 546 nm using methanol as blank. % inhibition value was calculated based on above equation. IC_{50} value was calculated by regression analysis[23].

$$\% \text{ inhibition} = [(A_{blank} - A_{test}) / A_{blank}] \times 100$$

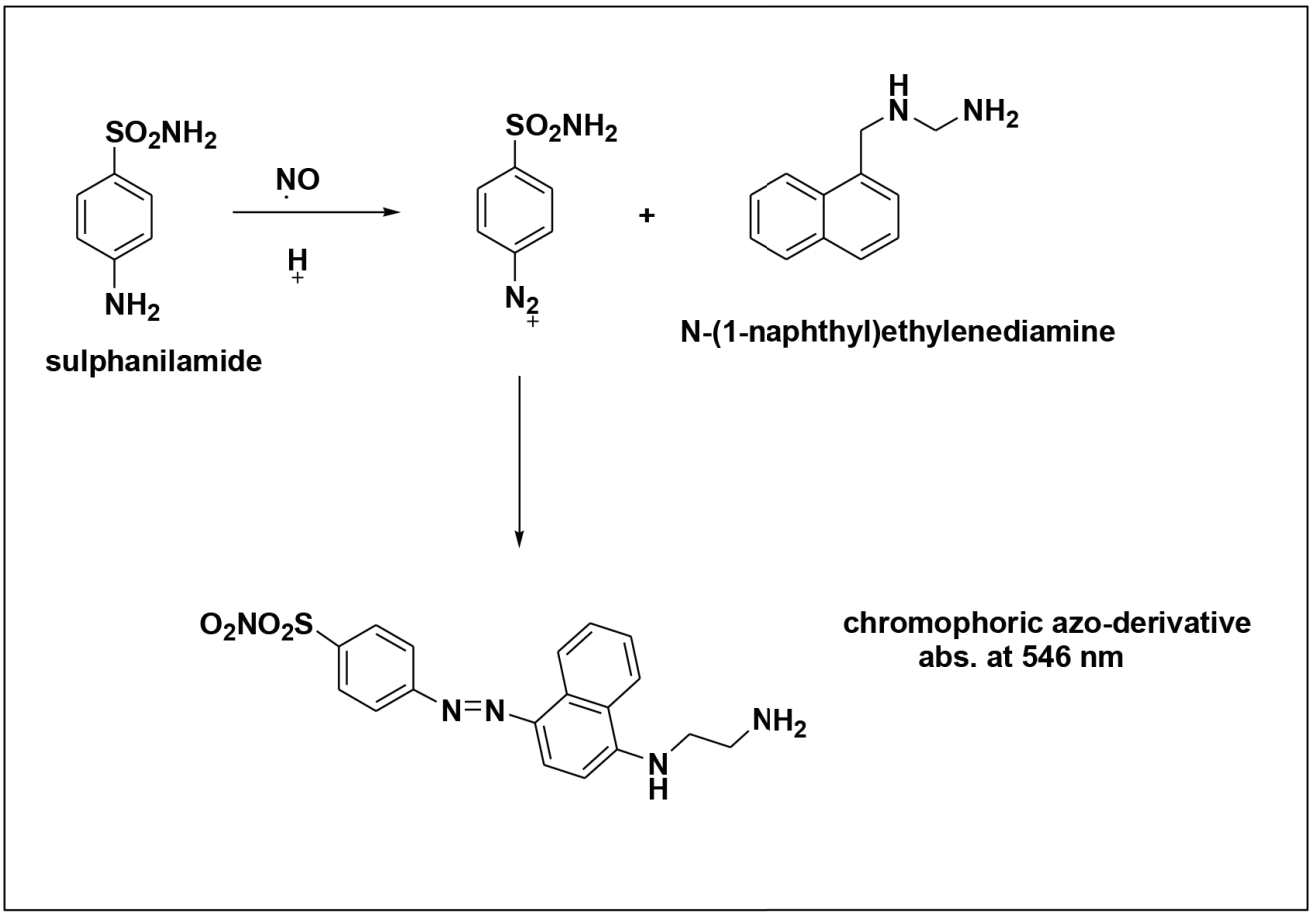


Figure 6: Mechanism involved in nitric oxide assay

Results

PASS (Prediction of Activity Spectra for Substance) identification result:

0,479	0,061	Chloride peroxidase inhibitor
0,422	0,006	Alcohol dehydrogenase inhibitor
0,389	0,014	Antidiabetic symptomatic
0,451	0,083	NADPH peroxidase inhibitor
0,409	0,050	Analgesic, non-opioid
0,357	0,008	Orexin receptor 1 antagonist
0,380	0,034	Focal adhesion kinase 2 inhibitor
0,427	0,085	5 Hydroxytryptamine uptake stimulant
0,352	0,010	Aldose reductase substrate
0,418	0,080	Insulysin inhibitor
0,427	0,092	Anaphylatoxin receptor antagonist
0,365	0,032	Transcription factor inhibitor

0,334	0,078	Intermittent claudication treatment
0,355	0,102	Diabetic neuropathy treatment
0,270	0,018	Calcium channel N-type blocker
0,311	0,060	Alzheimer's disease treatment
0,268	0,017	Sleep disorders treatment
0,296	0,049	Tryptophan 2,3-dioxygenase inhibitor
0,305	0,061	Signal transduction pathways inhibitor
0,250	0,011	Arylamine N-Acetyltransferase substrate
0,277	0,038	CYP2B2 substrate
0,380	0,145	Taurine dehydrogenase inhibitor
0,235	0,001	G-protein-coupled receptor 120 agonist
0,255	0,024	Anthrax lethal factor inhibitor

Figure 7: Activity Prediction for benzothiazole derivatives

Characterization results:

1. N-(6-Fluoro-benzothiazol-2-yl)-N'-(4-fluoro-benzylidene)-hydrazine:

M.F: $C_{14}H_9F_2N_3S$; **m. wt.** 289; **Yield** 94%; **m.p** 194-1960C; **Rf**: 0.9; **IR**: 3410(N-H), 2980(SP^2 C-H), 1503(Aromatic C=C), 825(C-S), 1011(C-F); **Molecular docking**: docking score = -8.7, Interactions: N:UNK1:H – M:GLU464:OE2, Hydrogen , Salt bridge Attract. charge, 2.17306, N:UNK1:N – M:ASP201:OD2, Electrostatic, Attractive, 5.18457, N:UNK1:N – M:GLU464:OE2, Electrostatic, Attractive, 3.93547, N:UNK1:HN M:ASN466:OD1, Hydrogen , Conventional H, 2.1864, M:GLU409:OE1 – N:UNK1:F, Halogen, Halogen (F), 2.71864, M:PHE331 – N:UNK1, Hydrophobic, Pi-pi stacked, 5.02037, M:TRP457 – N:UNK1, Hydrophobic , Pi-pi T-Shaped, 5.93042, M:TRP457 – N:UNK1, Hydrophobic , Pi-pi T-Shaped, 5.85169, N:UNK1 – M:TYR330, Hydrophobic, Pi-pi T-Shaped, 5.86039, N:UNK1 – M:ILE257, Hydrophobic , Pi-alkyl, 5.26612

2. N-(6-Chloro-benzothiazol-2-yl)-N'-(4-chloro-benzylidene)-hydrazine

M.F: $C_{14}H_9Cl_2N_3S$; **m. wt.** 322; **Yield** 89%; **m.p** 188-1900C; **Rf**: 0.8; **IR**: 3191(N-H), 3093(SP^2 C-H), 1486(Aromatic C=C), 1086(C-S), 821(C-Cl); **Molecular docking**: docking score = -8.2, Interactions: N:UNK1:H – M:GLU464:OE2, Hydrogen, Salt bridge; Attract. charge, 2.2903, N:UNK1:N – M:GLU372:OE1, Electrostatic, Attractive, 5.28706, N:UNK1:N – M:GLU372:OE1, Electrostatic, Attractive, 5.30391, N:UNK1:N – M:GLU464:OE2, Electrostatic, Attractive, 3.44676, N:UNK1:N – M:TYR330, Electrostatic, Pi-Cation, 4.86525, M:GLU409:OE1 – N:UNK1, Electrostatic, Pi-Anion, 3.68968, M:TYR330 – N:UNK1, Hydrophobic, Pi-pi stacked, 5.08923, M:TRP457 – N:UNK1, Hydrophobic, Pi-pi T-Shaped, 4.74998, M:TRP457 – N:UNK1, Hydrophobic, Pi-pi T-Shaped, 5.14915

3. N-(4-Methyl-6-nitro-benzothiazol-2-yl)-N'-(2-nitro-benzylidene)-hydrazine

M.F: $C_{15}H_{11}N_5O_4S$; **m. wt.** 357; **Yield** 90%; **m.p** 203-2050C; **Rf**: 0.8; **IR**: 3479(N-H), 2850(SP^3 C-H), 3307(SP^2 C-H), 1514(Aromatic C=C), 783(C-S), 1338(C- NO_2); **Molecular docking**: docking score = -7.9, Interactions: N:UNK1:N – M:GLU372:OE1, Electrostatic, Attractive, 4.94626, M:GLU464:OE2 – N:UNK1, Electrostatic , Pi-Anion, 3.81011, M:ILE382:CD1 – N:UNK1, Hydrophobic, Pi-sigma, 3.91932, N:UNK1:C – M:TYR330, Hydrophobic, Pi-sigma, 3. 91049, M:TYR330 – N:UNK1, Hydrophobic, Pi-pi T-Shaped, 4.99804, M:PHE331 – N:UNK1:C, Hydrophobic, Pi-alkyl, 5.05295, M:PHE371 - N:UNK1:C, Hydrophobic , Pi-alkyl, 4.66269

4. N-(6-Chloro-5-nitro-benzothiazol-2-yl)-N'-(3-nitro-benzylidene)-hydrazine

M.F: $C_{14}H_8ClN_5O_4S$; **m. wt.** 377; **Yield** 92%; **m.p** 191-193^{0}C; **Rf**: 0.7; **IR**: 3391(N-H), 2953(SP^2 C-H), 1516(Aromatic C=C), 1085(C-S), 1345(C- NO_2), 946(C-Cl); **Molecular docking**: docking score = -7.9, Interactions: M:ASN68:HN – N:UNK1:O, Hydrogen, Conventional H, 2.09703, M:VAL483:HN – N:UNK1:O, Hydrogen, Conventional H, 2.88889, M:THR484:HN – N:UNK1:O, Hydrogen, Conventional H, 2.87638, M:THR484:HG1 – N:UNK1:O, Hydrogen, Conventional H, 2.10898, N:UNK1:HN – M:THR72:O, Hydrogen, Conventional H, 2.15206, M:VAL483:HN – N:UNK1, Hydrogen , Prot. donor, H, 2.99389, M:PHE469 - N:UNK1, Hydrophobic, Pi-pi stacked, 4.08632, N:UNK1 – M:VAL483, Hydrophobic, Pi-alkyl, 4.29136

5. N-(6-Nitro-benzothiazol-2-yl)-N'-(4-nitro-benzylidene)-hydrazine

M.F: $C_{14}H_9N_5O_4S$; **m. wt.** 343; **Yield** 91%; **m.p** 187-189^{0}C; **Rf:** 0.9; **IR**: 3645(N-H), 3113(SP^2 C-H), 1517(Aromatic C=C), 838(C-S), 1343(C- NO_2); **1H NMR: Signals (500 MHz, DMSO)**:- δ (ppm) 8.1 (s, CH, 1H), 7.2 (s, NH, 1H), 7.4 (d, CH, 1H), 7.5 (d, CH, 1H), 7.8 (t, CH-CH, 2H), 7.9 (d, CH, 1H), 8.1 (d, CH, 1H), 8.2 (s, CH, 1H); **13C NMR**: 171.81 [C_2 , C-S], 133.77 [C_4, CH-N], 130.21 [C_{14}, CH-NO_2], 129.34 [C_5, CH=CH]], 128.50 [C_{11}, CH=CH], 77.27 [C_{10}, CH-N]; **Mass**: m/z :- M = 357, (M+1) = 358, (M-$C_7H_4N_4O_2S$)=208, (M-$C_7H_3N_2O_2S$)=179; **Molecular docking**: docking score = -8.5, Interactions: N:UNK1:N -M:GLU372:OE1, Electrostatic , Attractive , 5.4443, N:UNK1:N – M:GLU464:OE2, Electrostatic , Attractive , 5.58592, N:UNK1:N - M:GLU372:OE1, Electrostatic, Attractive, 4.79627, M:H1S141:HE2 – N:UNK1:O, Hydrogen, Conventional H, 2.05088, M:ASN186:HD22-N:UNK1:O, Hydrogen, Conventional H, 2.09057, M:ARG194:NH1 – N:UNK1, Electrostatic , Pi-Cation, 4.95032, N:UNK1:N - M:TYR33O, Electrostatic, Pi-Cation, 4.00971, M:GLU409:OE1 – N:UNK1, Electrostatic , Pi-Anion, 4.9397, M:GLU409:OE1 – N:UNK1, Electrostatic, Pi-Anion, 3.79171

Biological activity results:

Anti-Oxidant Results

a) H_2O_2 method

Table 1: In-vitro Anti-Oxidant activity by H_2O_2 Free Radical Scavenging method

Sr. No.	Comp. Code	Conc.	% inhibition	IC_{50} ± Std. devi.
1.	STD (Ascorbic acid)	10	51.92	15.53 ± 3.01
		20	53.04	
		30	55.42	
		40	57.15	
		50	51.92	
2.	2a	10	47.94	22.95±2.50
		20	49.15	
		30	51.08	
		40	53.41	
		50	54.63	
3.	2b	10	43.57	29.26±4.29
		20	47.16	
		30	51.65	
		40	53.29	
		50	55.43	
4.	2c	10	43.75	31.96±3.94
		20	46.62	
		30	49.83	
		40	52.19	
		50	54.88	
5.	2d	10	45.26	32.95±2.81
		20	47.37	
		30	49.82	
		40	51.59	
		50	53.04	
6.	2e	10	46.31	27.80±3.00
		20	48.03	
		30	50.94	
		40	52.26	
		50	54.78	

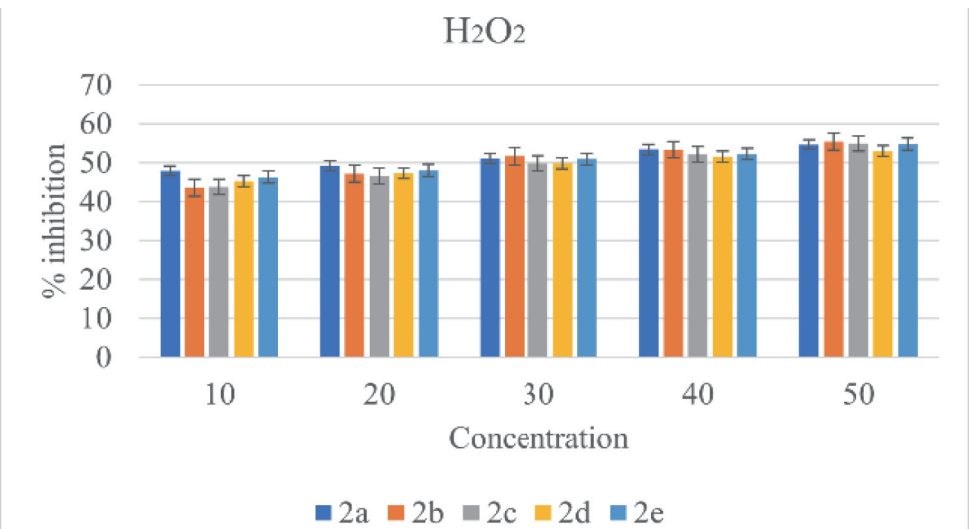


Figure 8: Graph of In-vitro Antioxidant activity by H_2O_2Free Radical Scavenging method

b) NO method

Table 2: In-vitro Anti-Oxidant activity by NO Free Radical Scavenging method

Sr. No.	Comp. Code	Conc.	% inhibition	IC_{50} ± Std. devi.
1.	STD (Ascorbic acid)	10	51.92	15.53 ± 3.01
		20	53.04	
		30	55.42	
		40	57.15	
		50	51.92	
2.	2a	10	48.14	21.63 ± 2.25
		20	49.54	
		30	51.43	
		40	53.18	
		50	54.28	
3.	2b	10	40.84	33.35 ± 5.99
		20	43.21	
		30	48.51	
		40	54.40	
		50	56.12	
4.	2c	10	40.12	38.41 ± 5.01
		20	43.18	
		30	47.06	
		40	51.05	
		50	53.87	
5.	2d	10	40.52	40.31 ± 4.61
		20	43.01	
		30	46.21	
		40	51.70	
		50	52.13	
6.	2e	10	47.53	28.88 ± 1.41
		20	49.74	
		30	50.87	
		40	51.11	
		50	51.37	

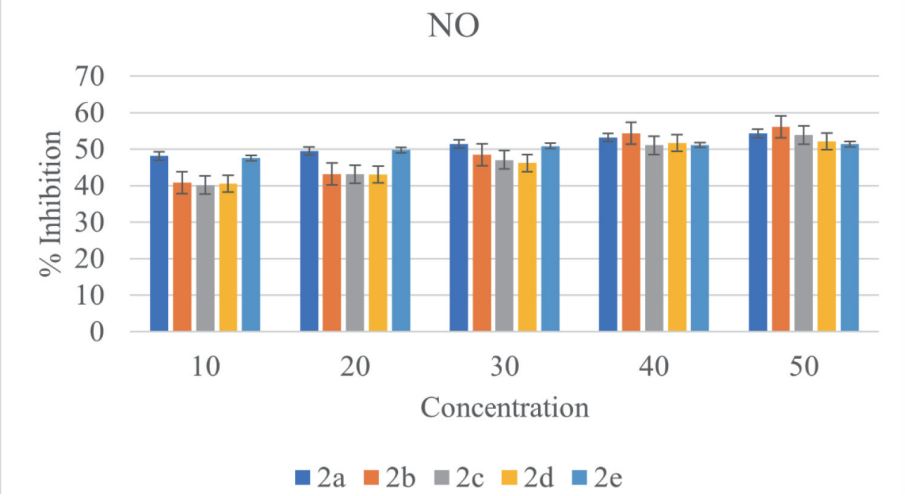


Figure 9: Graph of In-vitro Antioxidant activity by NO Free Radical Scavenging method

Swiss ADME results

Table 3: Pharmacokinetics, Bioavailability & medicinal chemistry for the derived compounds & standard (Ascorbic acid)

Comp.	Pharmacokinetics			Bioavailability Score	Pains	Leadlikness
	GI absorption	BBB Permeant	Pgp substrate			
2a	High	Yes	No	0.55	-	1
2b	High	Yes	No	055	-	1
2c	Low	No	No	0.55	-	2
2d	Low	No	No	0.55	-	2
2e	Low	No	No	0.55	-	1
STD	High	No	No	056	-	1

Table 4: Veber's rule and Lipinski's rule of 5 for derived Compounds & Standard (Ascorbic acid)

Comp.	Lipinski's Rule of 5					Veber's Rule	
	Log P	Mol. Wt. (g/mol)	H-bond acceptors	H-bond donors	Lipinski	Rotatable bonds	TPSA (Å²)
2a	2.47	289.30	4	1	0	3	65.52
2b	2.79	322.21	2	1	0	3	65.52
2c	1.83	357.34	6	1	0	5	157.16
2d	1.48	377.76	6	1	0	5	157.16
2e	1.53	343.32	6	1	0	5	157.16
STD	0.31	176.12	6	4	0	2	107.22

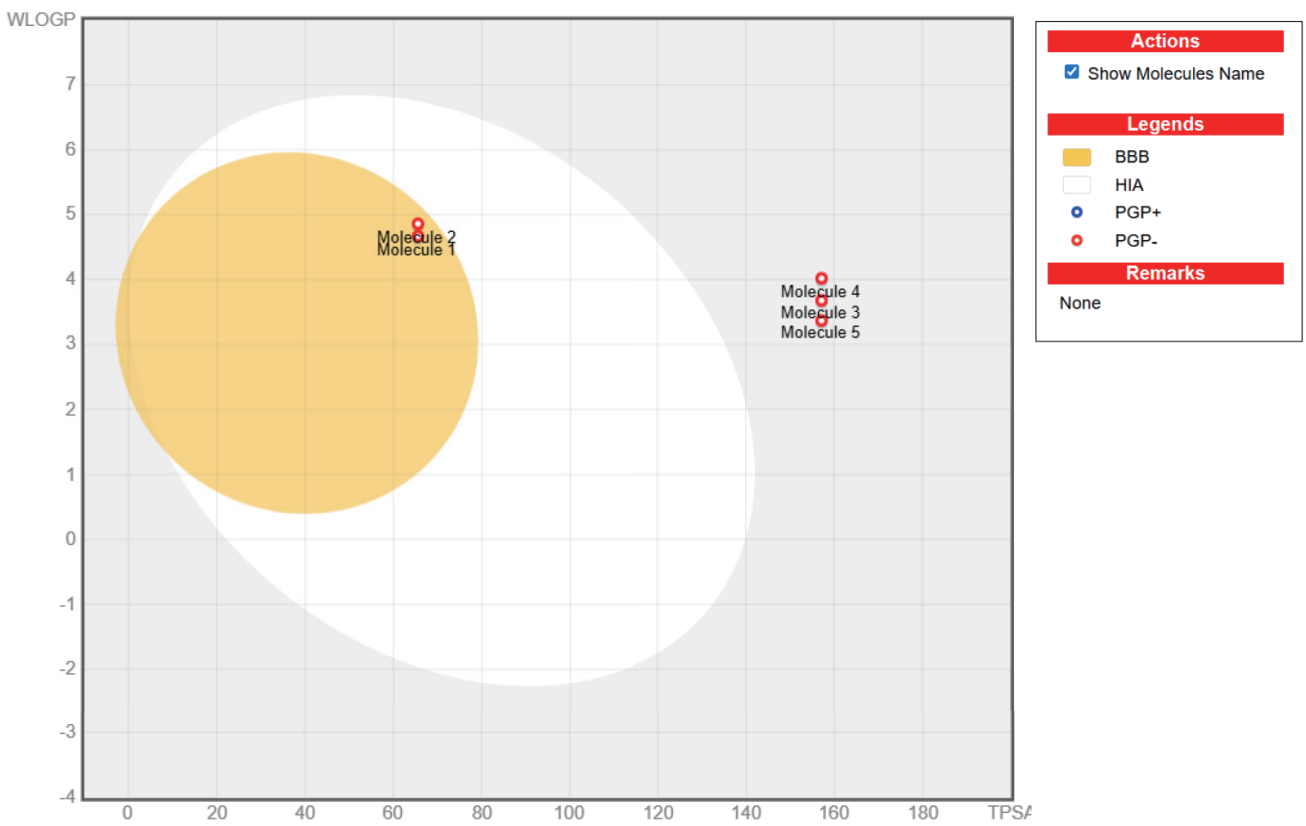


Figure 10: BOILED-Egg representation of derived compounds & Standard (Metformin)

Discussion

The present study focused on the synthesis, molecular docking, pharmacokinetic evaluation, and in-vitro antidiabetic activity of novel benzothiazole derivatives (2a-2e). Benzothiazole is a well-known heterocyclic scaffold with significant biological properties, including antimicrobial, anticancer, and antidiabetic activities. In this study, the synthesized derivatives were evaluated for their α-amylase inhibitory potential, which plays a crucial role as antioxidant agent.

Molecular Docking and Structure-Activity Relationship (SAR)

Molecular docking studies revealed strong interactions between the synthesized compounds and the target protein. Compound 2a exhibited the highest docking score (-8.7), followed by 2e (-8.5), indicating their strong binding affinity. The superior docking performance of 2a can be attributed to the presence of the o-nitro substituent, which enhances hydrogen bonding and electrostatic interactions with the active site residues of the enzyme. Similarly, compound 2e, containing a methyl and nitro group, also displayed strong interactions, suggesting that electron-withdrawing groups improve the binding efficiency of benzothiazole derivatives.

In-vitro antioxidant activity

The in-vitro antioxidant activity by H2O2 & NO method was conducted to evaluate the potential antioxidant activity of the synthesized compounds. Compounds 2a and 2e demonstrated significant inhibitory activity, with IC_{50} values of 22.95±2.50µg/mL and 27.80± 3.00µg/mL, respectively, comparable to the standard drug, Ascorbic acid (IC_{50} = 15.53 ± 3.01 µg/mL). The presence of electron-withdrawing groups, such as nitro and halogens, contributed to the increased inhibitory activity, as they facilitate better enzyme-ligand interactions.

On the other hand, compounds 2b, 2c & 2d showed moderate antioxidant activity, with IC_{50} values ranging from 21.92 to 40.01 µg/mL. The lower activity of these derivatives may be due to weaker hydrogen bonding interactions or steric hindrance, which affects enzyme binding. These results indicate that substituent positioning and electronic properties significantly influence the biological activity of benzothiazole derivatives.

ADME and Drug-Likeness Properties

Swiss ADME analysis was performed to evaluate the pharmacokinetic properties, drug-likeness, and bioavailability of the synthesized compounds. All derivatives followed Lipinski's Rule of Five and Veber's Rule, indicating favorable oral bioavailability. The high gastrointestinal (GI) absorption observed in all compounds suggests efficient uptake in the digestive system, while the brain-blood barrier (BBB) permeability results indicate that compounds 2c, 2d, and 2e may have limited central nervous system penetration, reducing the risk of neurological side effects.

Potential as Antioxidant Agents

The combination of strong molecular docking interactions, potent antioxidant activity, and favourable ADME properties suggests that benzothiazole derivatives, particularly 2a and 2e, are promising candidates for antioxidant drug development. These compounds demonstrated activity comparable to ascorbic acid, making them potential alternatives for further preclinical studies.

Conclusion

This article focuses on synthesis of N & S- containing heterocyclic analogue (**2a-2e**), their molecular docking, ADME studies and tested for their *in vitro* antioxidant activity. The compounds **2a & 2e** showed good activity at docking scores of -8.7 & -8.5 , IC_{50} values of 22.95±2.50µg/mL and 27.80±3.00µg/mL respectively. ADME studies showed that compound **2a & 2e** has good results as compared to standard (Ascorbic acid) & the synthesized compounds obey's Veber's rule and Lipinski's rule of 5.

Competing Interest

All the authors declare that they have no competing interests.

References

1 Yadav, K.P. et al. (2023). Synthesis and biological activities of benzothiazole derivatives: A review. Intelligent Pharmacy. https://doi.org/10.1016/j.ipha.2023.06.001.
2 Popli, J. V. et al. (2021). Benzothiazole analogues and their biological aspects: A Review. Indian Journal of Chemistry - Section B Organic and Medicinal Chemistry. https://doi.org/10.56042/ijcb.v60i12.49863.
3 Al-Mutairi, A.A. (2023). Synthesis of benzothiazoles and biologically active derivatives from 2-aminothiophenol-REVIEW. Egyptian Journal of Chemistry. https://doi.

org/10.21608/EJCHEM.2023.195175.7630.
4 Guo, L. et al. (2024). Discovery of Structural Diversity Guided N-S Heterocyclic Derivatives Based on Natural Benzothiazole Alkaloids as Potential Cytotoxic Agents. Journal of Heterocyclic Chemistry. https://doi.org/10.1002/jhet.4886.
5 Fritsch, C. et al. (2014). Characterization of the novel and specific PI3Ka inhibitor NVP-BYL719 and development of the patient stratification strategy for clinical trials. Molecular Cancer Therapeutics. https://doi.org/10.1158/1535-7163.MCT-13-0865.
6 Keri, R.S. et al. (2015). A comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry. European Journal of Medicinal Chemistry. https://doi.org/10.1016/j.ejmech.2014.10.059.
7 Irfan, A. et al. (2020). Benzothiazole derivatives as anticancer agents. Journal of Enzyme Inhibition and Medicinal Chemistry. https://doi.org/10.1080/14756366.2019.1698036.
8 Li, X. et al. (2022). Synthesis of benzothiazole from 2-aminothiophenol and benzaldehyde catalyzed by alkyl carbonic acid. Phosphorus, Sulfur, and Silicon and the Related Elements. https://doi.org/10.1080/10426507.2022.2033744.
9 Zhilitskaya, L. V. et al. (2021). Modern Approaches to the Synthesis and Transformations of Practically Valuable Benzothiazole Derivatives. Molecules. https://doi.org/10.3390/molecules26082190.
10 Madia, V.N. et al. (2021). Design, Synthesis and Biological Evaluation of New Pyrimidine Derivatives as Anticancer Agents. Molecules. https://doi.org/10.3390/molecules26030771.
11 Asati, V. et al. (2015). Synthesis, characterization and antimicrobial evaluation of some 1,3-benzothiazole-2-yl-hydrazone derivatives. Arabian Journal of Chemistry. https://doi.org/10.1016/j.arabjc.2011.01.036.
12 Filimonov, D.A. et al. (2014). Prediction of the Biological Activity Spectra of Organic Compounds Using the Pass Online Web Resource. Chemistry of Heterocyclic Compounds. https://doi.org/10.1007/s10593-014-1496-1.
13 Tahlan, S. et al. (2019). In-silico molecular design of heterocyclic benzimidazole scaffolds as prospective anticancer agents. BMC Chemistry. https://doi.org/10.1186/s13065-019-0608-5.
14 Xu, X. et al. (2024). Synthesis and biological evaluation of novel benzothiazole derivatives as potential anticancer and antiinflammatory agents. Frontiers in Chemistry. https://doi.org/10.3389/fchem.2024.1384301.
15 Djuidje, E.N. et al. (2022). Benzothiazole Derivatives as Multifunctional Antioxidant Agents for Skin Damage: Structure–Activity Relationship of a Scaffold Bearing a Five-Membered Ring System. Antioxidants. https://doi.org/10.3390/antiox11020407.
16 Cabrera-Pérez, L.C. et al. (2019). Evaluation of a new benzothiazole derivative with antioxidant activity in the initial phase of acetaminophen toxicity. Arabian Journal of Chemistry. https://doi.org/10.1016/j.arabjc.2016.02.004.
17 Payaz, D.Ü. et al. (2019). Synthesis carbonic anhydrase enzyme inhibition and antioxidant activity of novel benzothiazole derivatives incorporating glycine, methionine, alanine, and phenylalanine moieties. Journal of Enzyme Inhibition and Medicinal Chemistry. https://doi.org/10.1080/14756366.2018.1553040.
18 Shahidi, F. (2015). Antioxidants, in Handbook of Antioxidants for Food Preservation, Elsevier, pp. 1–14.
19 Guo, H.Y. et al. (2009). A simple and efficient synthesis of 2-substituted benzothiazoles catalyzed by H2O2/HCl. Chinese Chemical Letters. https://doi.org/10.1016/j.cclet.2009.06.037.
20 Shi, D. et al. (2024). A Novel Benzothiazole-Based Fluorescent AIE Probe for the Detection of Hydrogen Peroxide in Living Cells. Molecules. https://doi.org/10.3390/molecules29215181.
21 Bhat, M. and L, B.S. (2017). Synthesis of Benzothiazole Schiff'S Bases and Screening for the Anti-Oxidant Activity. Shanthalakshmi et al. World Journal of Pharmaceutical Research.
22 Barbari, R. et al. (2022). Design, Synthesis and Evaluation of New Multifunctional Benzothiazoles as Photoprotective, Antioxidant and Antiproliferative Agents. Molecules. https://doi.org/10.3390/molecules28010287.
23 Amin, S. and Parle, A. (2018). Synthesis, Characterization and Antioxidant Activity of 2-Aryl Benzothiazole Derivatives. International Journal of Current Pharmaceutical Research. https://doi.org/10.22159/ijcpr.2018v10i5.29685.