Design, Synthesis, and Biological Evaluation of Tetrazole and Thiazolidine-4-One Schiff Bases: Exploring Antioxidant and Antimicrobial Properties through Experimental and Molecular Docking Studies

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Article

Design, Synthesis, and Biological Evaluation of Tetrazole and Thiazolidine-4-One Schiff Bases: Exploring Antioxidant and Antimicrobial Properties through Experimental and Molecular Docking Studies

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Abstract

Introduction: Tetrazole and thiazolidine-4-one derivatives are important heterocyclic scaffolds with diverse pharmacological activities, including antimicrobial and antioxidant effects. This study focuses on the design and synthesis of novel Schiff base–derived analogues using a green synthetic approach to improve biological efficacy and reduce environmental impact. **Methods:** Schiff bases (2a–2h) were synthesized using tetrabutylammonium iodide as a green catalyst in aqueous medium. These were further converted into tetrazole (3a–3h) and thiazolidine-4-one (4a–4h) derivatives using sodium azide and thioglycolic acid. Structures were confirmed by FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopy. Antioxidant activity was evaluated using the DPPH assay, while antimicrobial activity was assessed by the zone of inhibition method. Molecular docking was performed against Penicillin-Binding Protein 4 (3ZG8), CYP51 (5V5Z), and 1OAF. **Results:** Compounds 2a, 2b, 3a, and 4a showed strong antifungal activity, exceeding standard drugs. Compounds 2d, 3b, and 4b exhibited superior antibacterial activity. Several derivatives demonstrated higher antioxidant activity than ascorbic acid. Docking studies confirmed stable ligand–protein interactions, with compound 4f showing the highest binding affinity. **Discussion:** Substituent variation influenced biological activity. Electron-donating and withdrawing groups affected potency. Docking results supported experimental findings and confirmed target interactions. The green synthesis improved efficiency and reduced environmental risk. **Conclusion:** These derivatives show promising antimicrobial and antioxidant potential. Compound 4f emerged as a lead candidate for further optimization and drug development.

Keywords: schiff bases; ionic liquids; tetrazole analogues; thiazolidine-4-one analogues; antioxidant activity; antimicrobial activity; molecular docking

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1. Introduction

A significant category of organic compounds Schiff's bases was first mentioned by Hugo Schiff in 1864[1]. Primary amines condense into carbonyl compounds that produce Schiff's bases. These compounds have the structural characteristic of azamethine, and R1 can be heterocyclic, cycloalkyl, alkyl, or aryl[2]. The nitrogen analogue of -CHO or C=O in which the carbonyl group (>C = O) was swapped out for an azomethine imine group is called Schiff's base, usually referred as an imine or azomethine. Antimicrobial[3], antimalarial[4], anti-proliferative[5], anti-inflammatory[6], antiviral[4], and antipyretic[7] activities have also been proven for Schiff's bases.[8,9] Many different natural compounds include imine or azomethine groups. Such compounds must have an imine group for them to function biologically[10,11]. Schiff's bases are significant compounds due to their numerous industrial uses[12]. To protect polyvinyl chloride polymers against photo degradation by UV light, Schiff's bases are employed.[13,14] Moreover, incorporating them into polymer films, enhances the degradation of poly-methyl methacrylate and inhibits the photo degradation of polystyrene[11,12,17].

Thiazolidine is an organic heterocyclic molecule at position-3 amine group and at position-1 thioether group". The chemical formula of this five-membered saturated ring is [$(CH_2)_3NH$]. S. Schubert was the first to discover the production of Thiazolidine carboxylic acid by reacting cysteine with different aldehydes[18]. Derivatives of thiazolidinedione has a special role in the area of medicinal chemistry. A widely studied heterocyclic derivative, thiazolidinedione has a diverse set of pharmacological characteristics, including wound healing[19], anti-tubercular[20], antiviral[21], antifungal[22], and anticonvulsant[23] properties [24] anti-inflammatory[6], anti-arthritic[25], antibacterial[4], and anticancer activity[26]. Compounds from the thiazolidinone series were synthesized due to their interesting and diverse pharmacological properties, including notable anti-proliferative activity. This constitutes an important research subject matter [27]. Understanding the molecular mechanisms behind PPAR-induced anti-cancer effects is an important subject of research. The involvement of PPAR-in carcinogenesis remains controversial since thiazolidinedione has anti-neoplastic properties that are distinct from those of PPAR. The primary structure in the control of many different biological actions is thiazolidinedione. In addition to several additional processes, enzymatic activity, and receptor-mediated mechanisms are also involved in the biological research of thiazolidinedione. Thiazolidinedione have been studied biologically, and it has been shown that substitution at positions 2, 3, and 5 imparts distinct activity. Representatives of the five-membered heterocyclic organic class, Thiazolidine have found use in ion receptors[28], biological dyes[29], materials, and medicine. Thiazolidine-2-thione, a common Thiazolidine derivative, is a significant organic intermediate used in agrochemicals and medicines. The two tautomer's of thiazolidine-2-thione, thione, and thiol, have also been extensively employed as asymmetric auxiliaries in catalytic synthesis[30]. Thiazolidine is the parent chemical substance of thiazolidinone, which is a significant heterocyclic compound containing nitrogen, and sulphur in a five-membered nucleus. It is said to be a mystical moiety with almost all biological activities[31]. Thiazolidinone-derived compounds are synthesized due to their diverse and noteworthy pharmacological activities, including anti-proliferative and anti-inflammatory effects, making them an important focus of research[27].

Since four electrons from the ring and one pair from the nitrogen are lent, Huckel's criterion is satisfied, defining the tetrazolide as a 5-membered aza structure with six π electrons. Tetrazole is acidic, much like carboxylic acids, although ring tetrazoles and carboxylic acids do not undergo the same annular tautomerization[32]. In 1885, Bladin discovered the tetrazoles chemical compounds[33]. Numerous favourable characteristics of tetrazoles include high acidity, low basicity, plenty of nitrogen atoms, good stability, and a high formation enthalpy. They are employed as N-containing compounds, lipophilic spacers in drug design, effective antitumor and anticancer drugs, peptide inhibitors, starting materials for propellant production, and analytical reagents in science[34,35].

Tetrazole rings are employed to make innovative drugs since they are the heterocyclic analogue of substances like carboxylic acid and cis-amide. It is also quite stable in the body's metabolism. Tetrazoles also function by piling up on top of target tissue locations that are recognized

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by receptors. A crystalline, light-yellow powder with no odour, ^{1}H-tetrazole has this appearance. The melting point of tetrazole is between 155 and 157 °C. When tetrazoles are heated, they decompose and produce harmful nitrogen fumes. Tetrazoles, in contrast to other azoles have both strong acids and weak bases. Tetrazole that hasn't been altered behaves a lot like an organic acid, with a pKa value that's comparable to acetic acid [33].

Tetrazole derivatives are a notable family of heterocyclic nuclei rich in nitrogen that have a lot of uses in medical chemistry. The tetrazole moiety is a helpful pharmacophore in branded drugs such as Lasortan[36], Irbesartan[37], and Tomelukast[38] that are given for the treatment of asthma[39], high blood pressure[37], heart failure[37], and diabetic[40] kidney disease[41]. Additionally, this pharmacophore is frequently employed as a suitable isosteres of the carboxylic acid functional group in drug design investigations. Several effective synthetic techniques have also been developed as a result of the use of tetrazole derivatives as significant, widely used molecules in materials chemistry[42], catalysis[43] and energetic applications, synthetic organic chemistry, and coordination chemistry[44] as ligands.[45]. Tetrazole is an aromatic aza pyrazole. With 1-carbon, 4-nitrogens, 2-hydrogens, & 2-double bonds, it is a five-membered ring complex. There is no tetrazole in the natural world. Tetrazole exhibits acidic character because the presence of four nitrogen atoms stabilizes its conjugate base through resonance delocalization, resulting in a pKa value comparable to that of carboxylic acids. Although tetrazole derivatives are widely recognized as synthetic compounds with diverse applications in medicinal and materials chemistry, naturally occurring tetrazole structures are extremely rare, with only a few examples reported in microbial metabolites. Despite their high nitrogen content, tetrazoles and most of their derivatives are relatively stable upon heating, under microwave irradiation, and in the presence of various chemical reagents such as oxidants, acids, bases, alkylating agents, and dienophiles. Naturally occurring molecules containing the tetrazole fragment are virtually absent; however, their presence has been reported in the metabolic products of certain protozoa. It has also been postulated that tetrazole and other poly-nitrogen heterocycles could form under extra-terrestrial conditions, where hydrocarbons and nitrogen coexist in planetary atmospheres or surfaces[46–50]. The simplest form of tetrazole is ^{1}H-tetrazole (CN_4H_2), is a white to pale-yellow crystalline solid with a mild, distinctive odour. It dissolves easily in both water and alcohol. The molecule shows acidic behaviour because its four nitrogen atoms help stabilize the negative charge through resonance. According to Hückel's rule, tetrazole's aromatic character comes from six π-electrons in which two provided by the lone pair on one nitrogen atom and four from the remaining atoms in the ring[51]. Due to the intense inductive effects (-I) of pulling electrons outweighing their mesomeric effects (+M), tetrazole rings are deactivating groups[52]. This characteristic makes tetrazole useful as an isosteric replacement[53] for various functional groups in the synthesis of physiologically active compounds. In recent years, tetrazole has become more and more popular, especially as an alternative to carboxylic acids[54,55]. The biological properties of tetrazole are due to its greater metabolic stability as compared to the acid function[16]. Tetrazole has a wide range of uses including explosives[56], propellants[57], and catalysts. Despite this, their diverse pharmacological activities along with different electrical properties enabled them create an identity in the realm of medicinal chemistry. In addition, these compounds possess anti-inflammatory[54], analgesic[58], anti-cancer[54], anti-convulsant[59], anti-hypertensive[59], hypoglycaemic[40], anti-parasitic[60], and antiviral properties[61], covid-19[62], antibacterial[59], antifungal[59], antioxidant, Alzheimer disease[63], and CNS[33], anti-proliferative activity against glioblastoma cells[64].

In light of this, efforts are being made to create a feasible and affordable process for synthesizing tetrazole and thiazolidinone compounds from Schiff bases[65]. Consequently, when an ionic liquid is used as a catalyst at room temperature with a free solvent, it is beneficial for the environment and produces positive results in a short time. The findings of the synthesis of tetrazole and thiazolidine-4-ones were juxtaposed to other known techniques with aspects of reaction time and yield.

Tetrabutylammonium iodide (TBAI) has gained significant attention as an efficient ionic liquid–type catalyst or co-catalyst in organic synthesis owing to its ability to enhance reaction rates, improve

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selectivity, and facilitate environmentally benign (green) processes. As a quaternary ammonium salt, TBAI exhibits high thermal stability and excellent solubility in polar solvents such as methanol, while remaining poorly soluble in non-polar media like ethyl acetate. These characteristics allow for easy product separation, catalyst recovery, and reuse, making it a sustainable alternative to conventional homogeneous catalysts. Furthermore, TBAI often functions synergistically with other catalytic systems, improving overall efficiency and conversion yields in various transformations, including oxidation, coupling, and transesterification reactions. Its dual nature as both a phase-transfer catalyst and ionic liquid component makes it particularly valuable in developing greener and more efficient catalytic methodologies[66–68].

Tetrazole and thiazolidinedione-4-one analogues have attracted keen interest in medicinal chemistry because of their wide-ranging pharmacological activities, particularly their antioxidant effects, which highlight their therapeutic potential in managing oxidative stress-related conditions. Tetrazole derivatives are known for diverse bioactivities, containing antioxidant, antifungal, antibacterial, anti-inflammatory, and anticancer activities. Compounds such as those synthesized from quinaldic acid have demonstrated notable antioxidant activity in preclinical studies, indicating their promise as future pharmaceutical agents. Additionally, the tetrazole moiety is present in several marketed drugs like Losartan and Valsartan, which are primarily used as antihypertensive agents; although their main mechanism is not antioxidant-related, the tetrazole ring contributes to potential antioxidant effects that warrant further investigation. These compounds are particularly valuable in drug design because they can act as bioisosteres for carboxylic acids, improving lipophilicity and bioavailability[69–71].

On the other hand, thiazolidinedione-4-one (TZD) derivatives are well-established for their antioxidant properties along with antidiabetic, anti-inflammatory, and anticancer activities. Many TZD derivatives have progressed to clinical use, primarily for the treatment of type-2 DM, where their antioxidant and anti-inflammatory activities contribute to therapeutic efficacy. Notable marketed TZD drugs include pioglitazone, a PPAR-γ agonist known for improving insulin sensitivity and exhibiting antioxidant effects; rosiglitazone, another PPAR-γ agonist with similar properties; and newer compounds like lobeglitazone, which offer improved safety profiles. These TZD drugs reduce oxidative stress in metabolic disorders, highlighting the importance of their antioxidant activity in their pharmacological profile. Overall, while tetrazole derivatives remain largely in the experimental stage, thiazolidinedione-4-one derivatives represent a clinically relevant class of drugs demonstrating significant antioxidant activity, emphasizing their potential for further therapeutic development in oxidative stress-related diseases[72–74].

Tetrazole and thiazolidinedione-4-one derivatives are extensively studied for their antimicrobial potential, owing to their heteroaromatic and electron-rich functional groups that enable interaction with microbial enzymes and cell membranes. Tetrazole derivatives, particularly triazole–tetrazole hybrids, have demonstrated strong antifungal activity by inhibiting the lanosterol 14α-demethylase (CYP51) enzyme, a key target in ergosterol biosynthesis. This mechanism is similar to that of clinically approved antifungal azoles such as Fluconazole, Itraconazole, Voriconazole, Posaconazole, and the tetrazole-containing antifungal Isavuconazole, which disrupt fungal cell membrane integrity by depleting ergosterol[75]. Isavuconazole a clinically approved broad-spectrum antifungal drug containing a tetrazole moiety, used in the treatment of mucormycosis and invasive aspergillosis[76]. Isavuconazole offers advantages such as high oral bioavailability, predictable pharmacokinetics, and reduced hepatotoxicity compared with older triazole antifungals. Moreover, tetrazole scaffolds are also present in marketed drugs beyond antifungals, including the widely used antihypertensive Losartan and the antibiotic Cefotetan, highlighting the pharmacological versatility of the tetrazole ring. In addition, tetrazole-based antibacterial compounds show efficiency contrary to Gram-positive and Gram-negative bacteria, primarily through inhibition of bacterial DNA gyrase and topoisomerase IV, comparable to marketed fluoroquinolone drugs such as Ciprofloxacin, Levofloxacin, and Moxifloxacin. On the other hand, thiazolidinedione-4-one (TZD-4-one) derivatives have been reported to exhibit potent antimicrobial effects through multiple mechanisms, including

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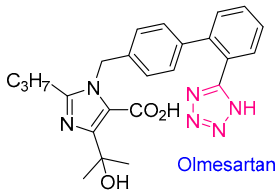
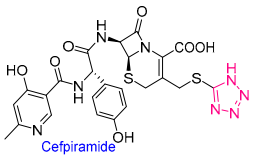
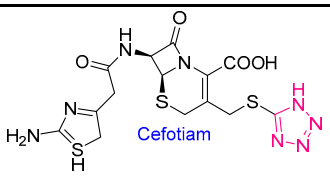
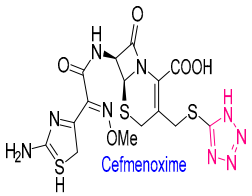
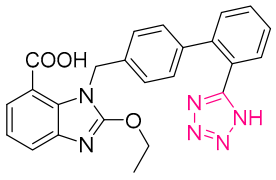
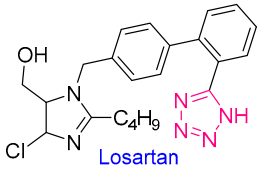
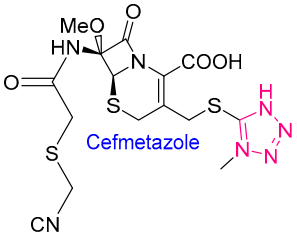
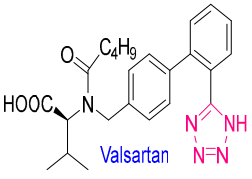
disruption of bacterial cell walls, inhibition of fungal ergosterol synthesis, and interference with microbial oxidative stress pathways. Importantly, several TZD-based drugs are already marketed, such as the antidiabetic agents Pioglitazone, Rosiglitazone, and Troglitazone, which contain the TZD-4-one pharmacophore and have also demonstrated secondary antioxidant, antibacterial, and antifungal properties in various studies. Several TZD-based hybrids incorporating azoles or tetrazoles are under active development as next-generation antimicrobial agents[77]. Collectively, the antimicrobial activity of tetrazole and thiazolidinedione-4-one derivatives, along with the clinical success of marketed drugs like Isavuconazole (tetrazole antifungal), Losartan (tetrazole antihypertensive with antibacterial reports), Cefotetan (tetrazole cephalosporin antibiotic), and Pioglitazone, Rosiglitazone, Troglitazone (TZD antidiabetics with antimicrobial effects), highlights the clinical relevance of these heterocycles as valuable scaffolds for developing next-generation antimicrobial agents. Rosiglitazone has shown synergistic antifungal effects when combined with azole drugs such as fluconazole, enhancing inhibition of *Candida albicans* biofilm formation[78]. Troglitazone is a TZD-4-one derivative though withdrawn from the market due to hepatotoxicity, demonstrated inhibitory activity against fungal mitochondrial pathways in preclinical studies[79,80]. Based on literature reports demonstrating significant antioxidant and antimicrobial potential of tetrazole and thiazolidinedione scaffolds, the present study focuses on the rational design and synthesis of novel tetrazole and thiazolidine-4-one based Schiff bases. The work aims to explore structural features responsible for redox modulation and microbial growth inhibition through systematic chemical modification. The synthesized compounds are evaluated for in vitro antioxidant activity using established free radical scavenging assays and for antimicrobial efficacy against selected bacterial and fungal strains. In parallel, molecular docking studies are conducted to elucidate binding interactions with relevant biological targets, providing mechanistic insight and supporting the experimental findings at the molecular level. A list of Tetrazole and Thiazolidinedione-4-one (TZD-4-one) hybrids containing experimental medications that have been approved by the FDA is presented in Table 1 and Table 2, respectively, highlighting the therapeutic potential of these compounds in various diseases.

Table 1. Tetrazole hybrids containing experimental medications that have been approved by the FDA.

 **DB00275** **Antihypertensive agent, Angiotensin-II receptor**	 **DB00430** **A third-generation cephalosporin**	 **DB00229** **A broad-spectrum antibiotic against both gram positive and gram-negative microorganism**	 **DB00267** **A third-generation cephalosporin antibiotic**
 DB00796 Antihypertensive agent, Angiotensin-II receptor	 DB00678 Antihypertensive agent, Angiotensin-II receptor	 DB00274 A broad-spectrum antibiotic against both gram positive and gram-negative microorganism	 DB00177 Antihypertensive agent, Angiotensin-II receptor

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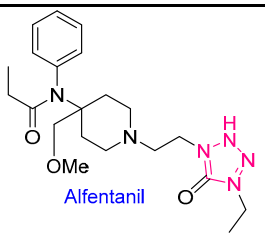
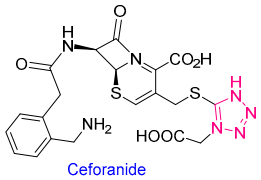
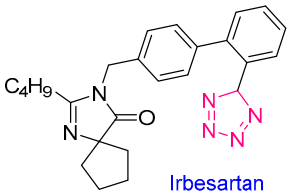


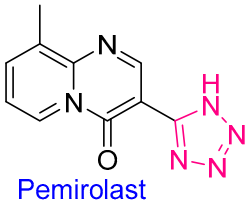
DB00802
A short acting opioid anesthetics and analgesic of fentanyl



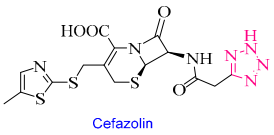
DBOO923
A second generation of parenteral cephalosporin antibiotics



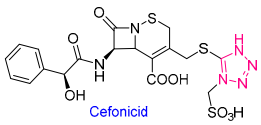
DB1029
An angiotensin receptor blocker (ARB) used mainly for the treatment of hypertension



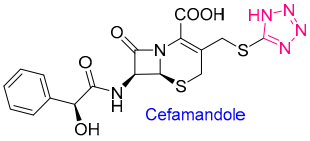
DB00885
A mast cell stabilizer used as anti-allergic agent



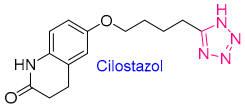
DB01327
A Broad-spectrum antibiotic



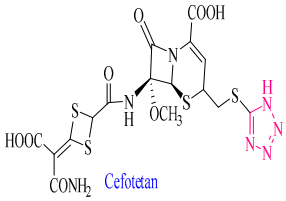
DB01328
A second-generation cephalosporin



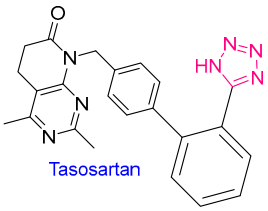
DB01326
A broad-spectrum cephalosporin antibiotic



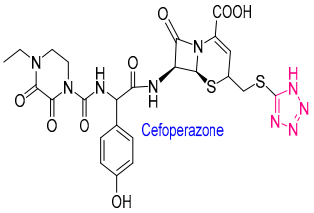
DB01166
Intermittent claudication in individual with peripheral vascular disease



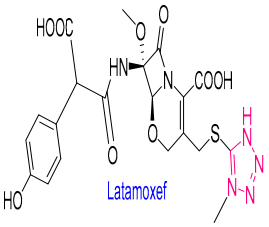
DB01330
A semisynthetic cephamycin antibiotics



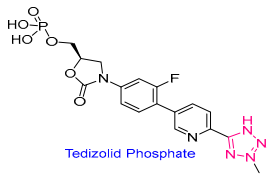
DB01349
A long-acting angiotensin (II) receptor blocker



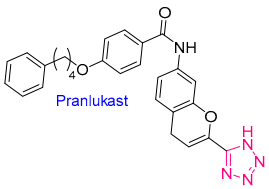
DB01329
Semi-synthetic broad-spectrum cephalosporin



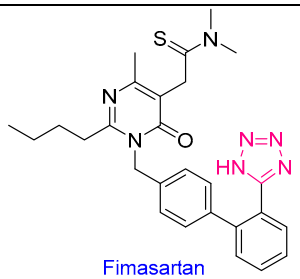
DB04570
Broad spectrum β-lactam antibiotics



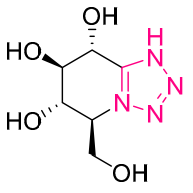
DB09042
Oxazolidinone-class antibiotic prodrug



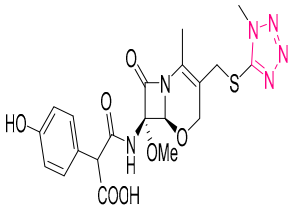
DB01411
A cysteinyl leukotriene receptor-I antagonist to antagonize reduce bronchospasm



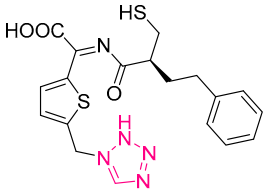
DB09279
Non-peptide angiotensin-II receptor antagonist (ARB)



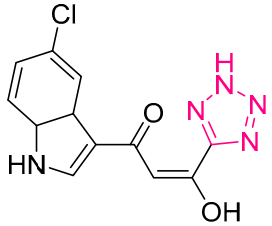
DB02471
Experimental target, Glycogen phosphorylase, Muscle form



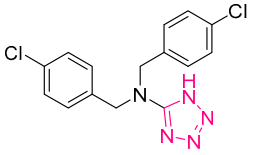
DB04342
Experimental target, β-lactamase, OXA-10



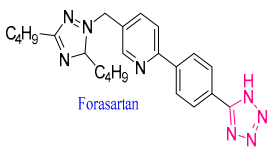
DB02706
Experimental target, Mercaptocarboxylate inhibitor



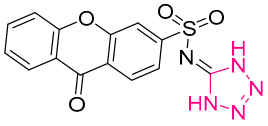
DB03118
Experimental target Gag-Pol Polyprotein



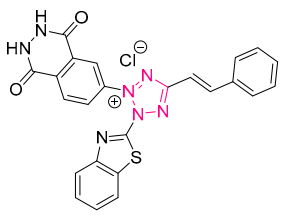
DB04037
Experimental target, β-lactamase, TEM



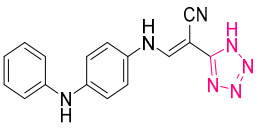
DB01342
Experimental target, Angiotensin-II antagonist



DB04698
Experimental target, 3-dehydroquinate dehydratase



DB01897



DB04430
Experimental target, β-lactamase, TEM

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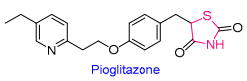
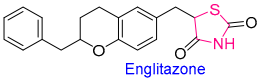
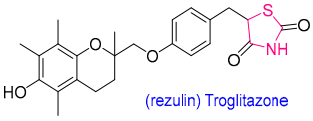
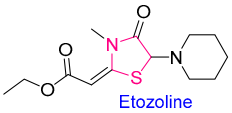
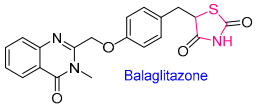
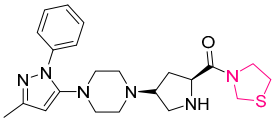
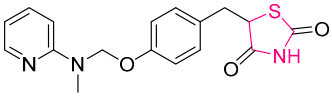
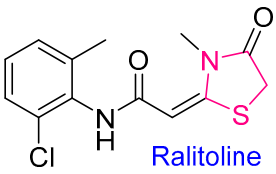
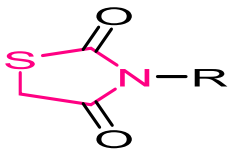
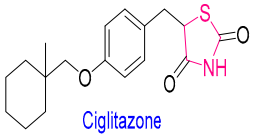
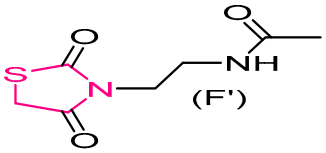
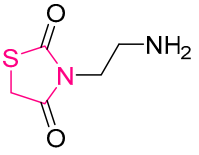
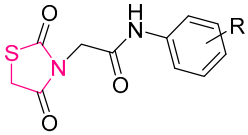
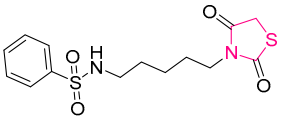
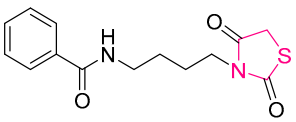
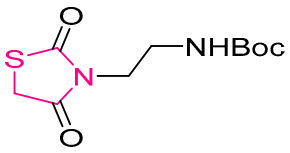
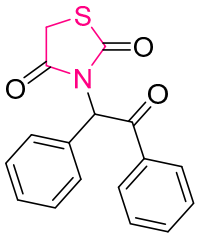
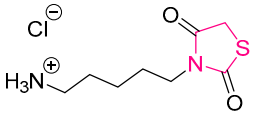
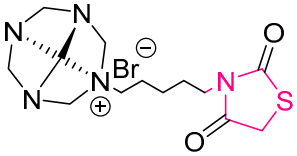
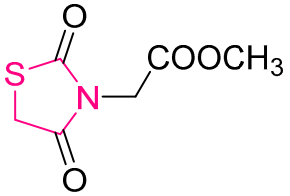
		Experimental target, Hematopoietic Prostaglandin D synthase	

Table 2. Thiazolidine-4-One hybrids containing experimental medications that have been approved by the FDA.

 Pioglitazone Selectively stimulates the nuclear receptor (PPAR-γ) and to a lesser extent PPAR-α.	 Englitazone DB14035 Experimental Target, Glitazone class of antidiabetic agents, Englitazone decrease triacylglycerol levels in animal studies.	 (rezulin) Troglitazone DB00197 Troglitazone activates (PPAR-gamma), a ligand-activated transcription factor, Approved, Investigational, Withdrawn in 2000 due to risk of hepatotoxicity. It was superseded by pioglitazone and rosiglitazone.	 Etozoline DB08982 Etozoline, a loop diuretic, inhibits the sodium, potassium, and chloride symport in the ascending limb of Henle, leading to increased urinary output and reduced extracellular fluid.
 Balaglitazone It is a partial agonist of the peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor that plays a role in regulating glucose and lipid metabolism.	 Teneligliptin It inhibits the enzyme DPP-4 in glucose metabolism.	 Rosiglitazone Rosiglitazone, thiazolidinedione, improves glycaemic control by enhancing insulin sensitivity, primarily by activating PPAR-gamma receptors, which in turn regulates the transcription of insulin-responsive genes in key target tissues.	 Ralitoline DM7HAE9 Anticonvulsant and sodium channel blocking drug
 3-(7-bromoheptyl) thiazolidine-2,4-dione	 Ciglitazone Improves insulin sensitivity and glycaemic control by acting as a potent and selective PPARγ agonist, which regulates genes involved in glucose homeostasis and fatty acid metabolism	 (F') N-(2-(2,4-dioxothiazolidin-3-yl) ethyl) acetamide It is a PPAR-alpha agonist	 3-(2-aminoethyl) thiazolidine-2,4-dione It is a sigma-1 receptor ligand, potentially useful in treating neuropathic pain
 It is a PPAR-alpha agonist	 N-(5-(2,4-dioxothiazolidin-3-yl)pentyl)benzene sulfonamide	 N-(4-(2,4-dioxothiazolidin-3-yl)butyl)benzamide	 tert-butyl (2-(2,4-dioxothiazolidin-3-yl)ethyl)carbamate
	 5-(2,4-dioxothiazolidin-3-yl)pentan-1-aminium chloride	 (1s,8s)-1-(5-(2,4-dioxothiazolidin-3-yl) pentyl)-1,3,5,7-tetraaza	

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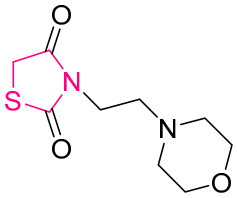
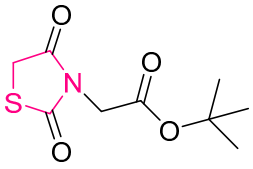
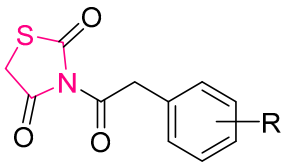
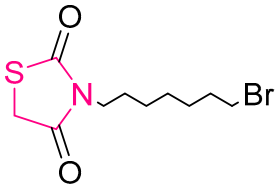
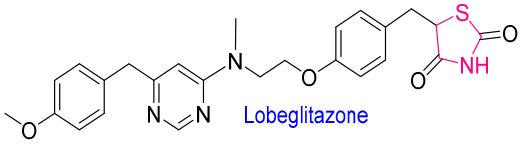
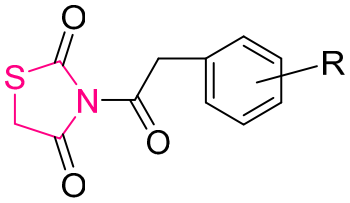
3-(2-oxo-1,2-diphenylethyl) thiazolidine-2,4-dione		tetracyclo[5.1.1.03,8.05,8] nonan-1-ium bromide	Methyl 2-(2,4-dioxothiazolidin-3-yl) acetate It act by PPARγ activation and scavenging of reactive oxygen species
 3-(2-morpholinoethyl) thiazolidine-2,4-dione Anti-diabetic activity	 tert-butyl 2-(2,4-dioxothiazolidin-3-yl)acetate	 It is a PPAR-alpha agonist	 3-(7-bromoheptyl) thiazolidine-2,4-dione
 Lobeglitazone is a PPAR-alpha agonist		 It is act by the PPARγ receptor and potentially inhibiting Mur ligase	

2. Material and Methods

Chemistry

All chemicals and reagents came from (British Drug Houses) BDH and Fluka. Open capillaries on a Thiele tube Fisher John's apparatus was used to determine the melting points of the products. KBr pellets were used to record IR spectra on a Shimadzu FTIR-8400s Spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained using a Bruker Avance 400/AvIII HD300 (FT-NMR) spectrometer using acetone-d6 and $CDCl_3$ as solvents. Acetone-d6 and chloroform-d were used as the solvent at 1400 (400 MHz).

The mass spectra were recorded using a Waters Alliance e2695/HPLCTQD mass spectrometer (ESI-MS). The elemental analysis was performed using the Euro Vector analyzer. Each of the produced chemicals analyses for C, H, O, and N were around five percent of the expected ranges. The compounds' uniformity on $SiO_2.Al_2O_3.H_2O$ gel 60 F254 (Merck), as detected *via* iodine vapors & Ultraviolet rays at 254 nm, was described using TLC. Each of the reagents was chemically pure or of analytical grade.

Molecular Docking Study

Methodology

Molecular docking studies were performed using the licensed version of Discovery Studio Client (Biovia, Dassault Systèmes, v21.1.0). The 2D chemical structures of all synthesized compounds were drawn using ChemDraw Professional 16.0 (PerkinElmer Informatics, USA) [https://perkinelmerinformatics.com/products/research/chemdraw/]. Docking was performed using the LibDock protocol integrated within Discovery Studio, and the docking results were visualized and analyzed using Discovery Studio Visualizer[81]. The crystal structures of the target proteins were retrieved from the Protein Data Bank [https://www.rcsb.org/]. For antibacterial activity, compounds 2d, 3b, and 4b were docked into the active site of Penicillin-Binding Protein 4 (PDB ID: 3ZG8), where amoxicillin (co-crystallized ligand) served as the standard, and docking was carried out at the binding pocket occupied by amoxicillin. For antifungal activity, compounds 2a, 2b, 3a, and 4a were

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docked with Sterol 14-α-demethylase (CYP51, PDB ID: 5V5Z) using itraconazole as the reference ligand. For antioxidant activity, compounds 2h, 3c, 3f, 4b, 4d, and 4f were docked with PDB ID: 1OAF, with ascorbic acid as the standard. The chosen PDB IDs (3ZG8, 5V5Z, and 1OAF) were selected because they are co-crystallized with standard drugs or natural ligands, showing strong biological relevance to the tested activities—3ZG8 with amoxicillin (antibacterial), 5V5Z with itraconazole (antifungal), and 1OAF with a known inhibitor (antioxidant). These validated structures ensure accurate and meaningful docking results.

Prior to docking, proteins were prepared by removing water molecules, adding polar hydrogens, and optimizing the binding sites, while ligands were energy-minimized. Docking scores (LibDock scores) were used to evaluate binding affinities, and two-dimensional interaction diagrams were generated to analyze hydrogen bonding, hydrophobic interactions, and π–π stacking, with particular emphasis on the interaction profile of compound 2d against penicillin-binding protein-4 (PBP4).

Pharmacological Assessment

The pharmacological assessment of the synthesized tetrazole and thiazolidinedione-4-one derivatives was performed to determine their potential therapeutic efficacy. This involved a series of *in vitro* experiments to evaluate antifungal, antibacterial, and antioxidant activities. All chemicals and The analytical-grade reagents that were utilised came from standard suppliers.

Antifungal Activity Assessment

The cup plate method was used to assess the antifungal efficacy of the synthesized compounds. The fungal strains used in the study included *Aspergillus niger* and *Fusarium molariform*. Sterile Petri dishes containing suitable agar medium were inoculated with the fungal cultures. Wells were created in the agar plates, into which measured concentrations of the synthesized compounds were added. Fungigural and Griseofulvin, two common antifungal medications, served as reference drugs. The plates were incubated under controlled conditions, and fungal growth inhibition was measured by the zone of inhibition around the wells.

Antibacterial Activity Assessment

The cup plate method was used to assess the compounds antibacterial activity. Bacterial strains used included *Salmonella paratyphi-A*, *Escherichia coli*, and *Staphylococcus aureus*. The bacterial cultures were spread on sterile agar plates, and wells were loaded with defined concentrations of the test compounds. Standard antibacterial drugs, Penicillin and Ampicillin, were used for comparison. The plates had been incubated at optimum temperatures, and zones of inhibition were measured in millimeters to assess antibacterial activity.

Antioxidant Activity Assessment

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to evaluate the antioxidant capacity of synthetic derivatives. The test depends on the, resulting in a color change from purple to pale yellow. Test solutions of the compounds at various concentrations were prepared and mixed with a methanolic solution of DPPH. The reaction mixture was incubated in the dark at room temperature, and the decrease in absorbance was measured spectrophotometrically. Ascorbic acid was used as a standard reference compound. The percentage of radical scavenging activity was calculated to determine the antioxidant potential of the compounds.

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Experimental

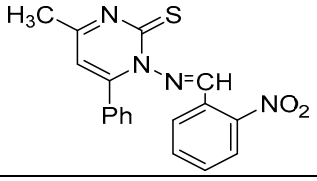
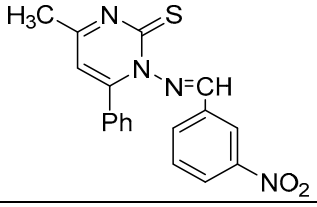
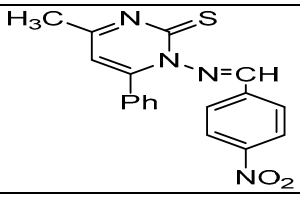
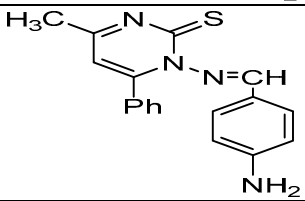
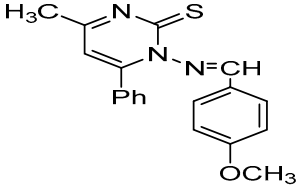
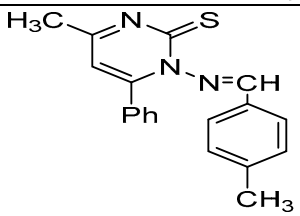
Synthesis of 1a Compounds

In 15 ml of purified water containing a small amount of ionic liquid, benzoyl acetone (0.02 mole) and thiosemicarbazone (0.02 mole) were amalgamated, and the mixture was refluxed for 50 minutes. After filtration, the solid mixture has separated & then recrystallized with C_2H_5OH once more, yielding a light-yellow crystal with an MP of 161–163°C and 97% yield.

Synthesis of Schiff bases (2a-2h)

Compound (1a) (0.02M) & an additional olefinic aldehyde (0.02M) was mixed for 37 minutes in 15 milliliters of distilled water (DW) with a minute quantity of ionic liquid. The solvent was evaporated under vacuum condition, then after subsequent addition of methanol facilitated the formation of crystalline solid. Table 3 & Table 4 deal with physical properties.

Table 3. A few physical characteristics of Schiff base (2a-2h).

Comp. No.	Structure	M.P. (°C)	Color	Time (Min)	Yield (100%)	Recrystallization Solvents
2a		227-229	White	15	94	C_2H_5OH
2b		187-189	White	17	89	C_2H_5OH
2c		235-237	Yellow	16	91	C_2H_5OH
2d		97-99	Pale yellow	23	93	CH_3OH
2e		145-147	Pale yellow	27	87	C_2H_5OH
2f		133-135	White	19	91	CH_3OH

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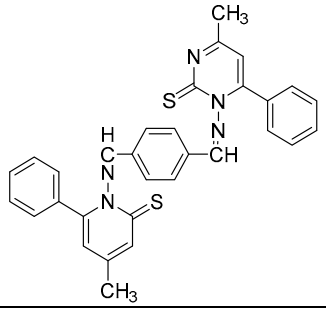
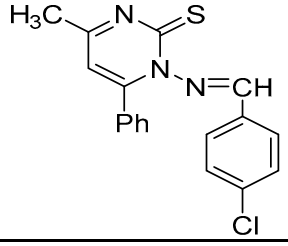
2g		324-326	Brown	33	85	C_2H_5OH
2h		177-199	White	37	93	CH_3OH

Table 4. Comparative analysis of numerous catalysts, a solvent, using TBAI for the highest possible yields of Schiff base in current research.

Sr. No	Catalyst	Solvents	Temperature (⁰C)	Time	Yield (%)	Reference
1.	Amino acid	H_2O	35	6 (h)	39	18
2.	Amino acid	EtOH	80	6 (h)	60	19
3.	[BMIM][NTf_2]	Decane	55	271 (Min)	62	20
4.	PIL-SB-Mn (III)	EtOH	100	6 (h)	60	21
5.	PIL-SB-Mn(III)	H_2O	RT	12 (h)	55	21
6.	SYSU-Zn@IL_2	DMF	12(h)	12 (h)	88	22
7.	TBAI	THF	RT	10 (h)	77	Present work
8.	TBAI	EtOH	80	8 (h)	79	Present work
9.	TBAI	H_2O	RT	30 (Min)	94	Present work

Synthesis of Tetrazole Derivatives (3a-3h)

Mix derivative (2a-h) (0.008M) by (15 ml) DW and (0.012M) NaN_3, and reflux for 77 minutes. The product was filtered and recrystallized using pure ethanol. Table 4 contains the physical characteristics. Table 5 and Table 6 includes the physical parameters of (3a-3h) tetrazole derivatives.

Table 5. Comparative analysis of numerous catalysts, a solvent, using TBAI for the highest possible yields of Schiff base in current research.

Sr. No	Catalyst	Solvents	Temperature (⁰C)	Time	Yield (%)	Reference
1.	Amino acid	H_2O	35	6 (h)	39	18
2.	Amino acid	EtOH	80	6 (h)	60	19
3.	[BMIM][NTf_2]	Decane	55	271 (Min)	62	20
4.	PIL-SB-Mn (III)	EtOH	100	6h	60	21
5.	PIL-SB-Mn (III)	H_2O	RT	12 (h)	55	21
6.	SYSU-Zn@IL_2	DMF	12(h)	12 (h)	88	22
7.	TBAI	THF	RT	10 (h)	77	Present work
8.	TBAI	EtOH	80	8 (h)	79	Present work
9.	TBAI	H_2O	RT	30 (Min)	94	Present work

Table 6. A few physical characteristics of Tetrazole (3a-3h).

Comp. No.	Structure	M.P.(⁰C)	Color	Time (Min)	Yield (100%)	Recrystallization Solvents

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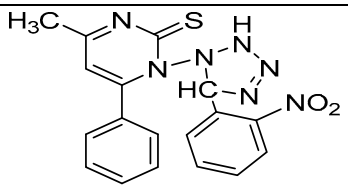
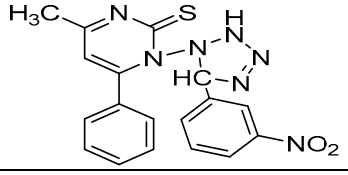
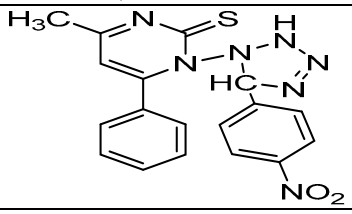
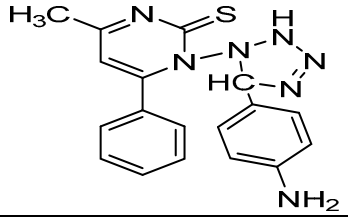
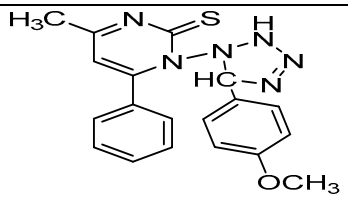
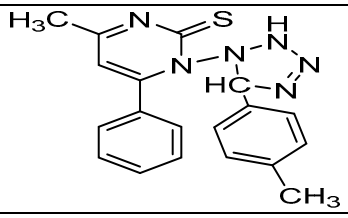
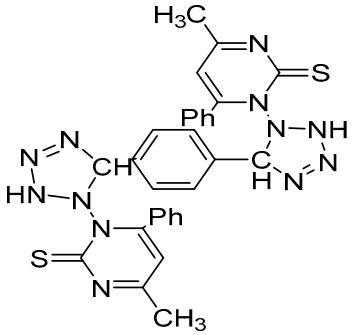
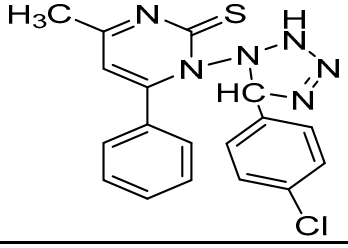
3a		202-204	Brown	51	88	Ethanol
3b		230-232	Light Brown	63	90	Ethanol
3c		252-254	Yellow	47	84	Ethanol
3d		270-272	Yellow	51	89	Methanol
3e		195-197	Light Brown	63	91	Methanol
3f		188-190	Dark Brown	71	87	Methanol
3g		268-270	Yellow	77	90	Ethanol
3h		222-224	Yellow	52	88	Ethanol

Synthesis of Thiazolidinones Derivatives (4a-4h)

Thioglycolic acid (0.004 M) was dissolved in 10 mL of distilled water with the synthesized analogue of (2a-h) (0.002M). After giving the combination a 72-minute reflux and treating it with

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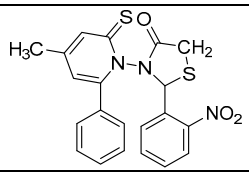
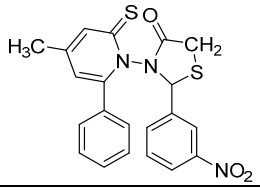
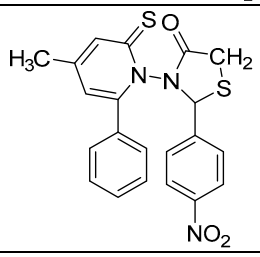
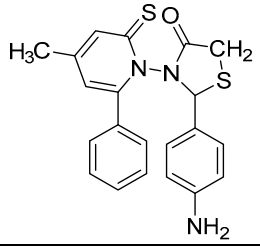
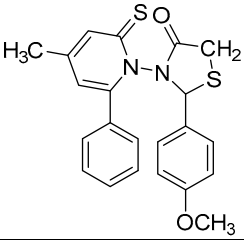
potassium bicarbonate, the chemical was created. The finished material was filtered and crystallized from ethanol. Table 7 and Table 8 include the physical parameters of (4a-4h) thiazolidinone derivatives.

Table 7. Comparative analysis of numerous catalysts, a solvent, using TBAI for the highest possible yields of Schiff base in current research.

Sr. No.	Catalyst	Solvent	Temp. (⁰C)	Yield (%)	Time	Comp.	Ref.
1.	Co_3O_4@p{AVIM}Br	H_2O	25	79	2 (h)	4b	28
2.	[Bmim][PF_6]	Ethanol	80	59	4 (h)	4h	29
3.	[MOEMMIM]TFA	Ethanol	80	78	3 (h)	1d	30
4.	MNP[Pmim]HSO_4	Solvent-free	80	78	7 (h)	8	31
5.	[HDBU][HSO_4]	Solvent-free	80	80	2 (h)	8	32
6.	TBAI	H_2O	91	91	81 (min)	4a	Present work

Table 8. A few physical characteristics of Tetrazole (4a-4h).

Comp. No.	Structure	M.P.(⁰C)	Color	Time (Min)	Yield (100%)	Recrystallization Solvents
4a		180-182	Green	57	91	Ethanol
4b		186-188	White	63	89	Ethanol
4c		252-254	Yellow	47	84	Methanol
4d		191-193	Green	51	88	Methanol
4e		195-197	Brown	61	90	Methanol

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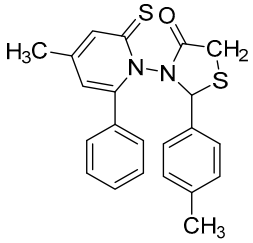
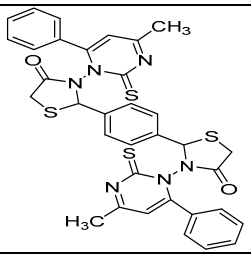
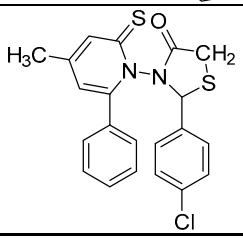
4f		185-187	Dark Brown	71	87	Ethanol
4g		318-320	Yellow	72	90	Ethanol
4h		212-214	Yellow	51	89	Ethanol

Results and Discussion

Chemistry

When the products were measured utilising open capillaries on Buchi equipment, the MP (°C) was discovered to be inaccurate. KBr pellets were used to construct the infrared spectrum on a Shimadzu-8400S FT-IR Spectrophotometer. Tetramethylsilane was utilised as an internal standard for the analysis of the ^{1}H and ^{13}C NMR spectra in chloroform-d using a Bruker Avance-400/AvIII HD 300 MHz (FT-NMR) spectrometer. The resonance frequencies of the ^{1}H and ^{13}C were 300 and 75 M-hertz, respectively. D_2O exchange has been used to support the spectrum assignment of the N–H protons. A Waters Alliance e-2695/HPLC TQ-D mass spectroscopy was used to record the mass spectrum. The Heraus CHN quick analyser was used to complete the elemental analysis. Every substance yielded C, H, and N analyses that fell within 0.5% of the expected range. The homogeneity of the analogues on aluminium silica gel 60 F254 (Merck), which were detected by UV rays (254 nm) and iodine fumes, was examined using TLC. Every component was pure, either chemically or analytically.

In DW with a catalytic quantity of ionic liquids, "1-amino-4-methyl-6-phenyl pyrimidine-2-(1H)-thione" (1a) was combined by another olefinic aldehyde to form the novel Schiff bases. The novel azomethine (C=N) group is responsible for the formation of the FT-IR spectra of (2a-2h) revealed new peaks at 1580-1606 cm^{-1}, as well as demonstrated the absence of a carbonyl group band. There are some spectral data in Table 6. Tetrazole compounds had been synthesized by combining (2a-2h) with sodium azide in THF. The FT-IR absorption bands at (1580-1599) cm^{-1} disappearing is a strong indicator that the reaction was successful. The (C=N) imine group's stretching frequency causes these absorption bands. Additionally, because of (N=N), the FT-IR band of tetrazole showed unique absorption bands at (1441-1499) cm^{-1}.

The FT-IR spectra of compounds (3a–3h) showed characteristic stretching bands for the azide group in the range of 2077–2360 cm^{-1}, as summarized in Table 6, which also reports the yields and spectral data. Additionally, Schiff bases (2a–2h) were reacted with mercaptoacetic acid (MAA) in distilled water to synthesize thiazolidine-4-one derivatives (4a–4h), as illustrated in Figure 1. The FT-IR spectra of these derivatives exhibited sharp peaks at 1724–1700 cm^{-1}, corresponding to the imide (C=O) stretching vibrations, providing clear evidence of the successful formation of the thiazolidine-4-one compounds.

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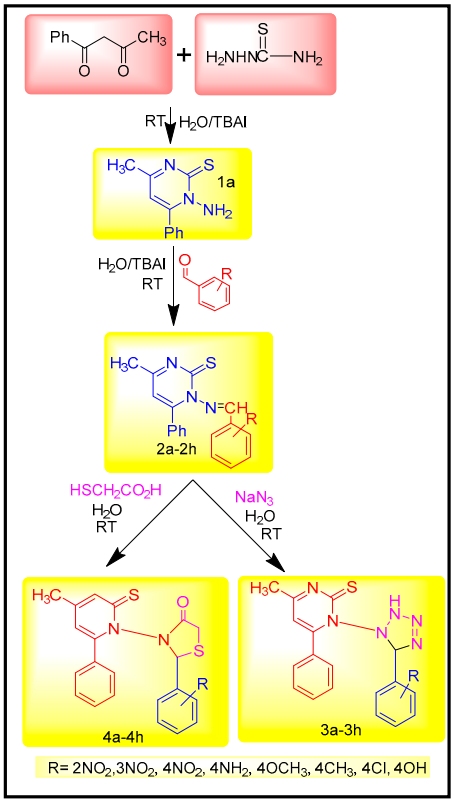

Figure 1. (Scheme) Synthesis of some Tetrazole & Thiazolidines derivatives.

1-Amino-4-methyl-6-phenylpyrimidine-2-(1H)-thione Compound (1a).

M.P.161-163 ^{0}C; eluent- n-hexane/ethyl acetate 70:30 v/v, Rf = 0.77; FT-IR (KBr, cm^{-1}): 3401–3267 (br, NH_2 stretching), 3198 (=C–H aromatic), 2998 (C–H aliphatic), 1598 (C=N stretching, pyrimidine ring), 1081 (C=S stretching), 936 (N–N vibration), 760–700 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.30–7.37 (m, 5H, Ar–H), 5.96 (s, 1H, H–C=C), 3.45–3.40 (d, 2H, NH_2), 2.04 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 400 MHz): δ = 175.5 (C=S), 155.4 (C=N), 144.1, 128.7, 128.0, 124.0 (Ar-C), 95.3 (C=C-H), 55.2 (C=C), 16.1 (CH_3); EIMS Found- m/z: 219.06 $[M+H]^+$ $C_{11}H_{11}N_3S$. Calculated, m/z: 218.07. Elemental Analysis: C, 60.80; H, 5.10; N, 19.34; S, 14.75.

(E)-4-methyl-1-((2-nitrobenzylidene)amino)-6-phenylpyrimidine-2(1H)-thione (2a).

M.P. 227-229 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.64; FT-IR (KBr, cm^{-1}): 3145 (=C–H aromatic), 2981 (C–H aliphatic), 1596 (C=N azomethine), 1521 (NO_2 asymmetric stretching), 1342 (NO_2 symmetric stretching), 1227 (C=S stretching), 1026 (N–N vibration), 760–700 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.18–7.85 (m, 9H, Ar–H), 7.42 (d, 2H, Ar–H, p-chlorophenyl), 5.92 (s, 1H, CH–N, thiazolidinone), 4.12 (d, 1H, CH_2–S), 3.78 (d, 1H, CH_2–S), 2.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 175.8 (C=O, thiazolidinone), 172.6 (C=S), 161.9 (C=N, pyrimidine), 145.3, 138.7 (ipso Ar–C), 134.6 (C–Cl), 129.8, 128.9, 128.2, 127.4, 126.8 (Ar–C), 63.5 (CH, thiazolidinone), 34.2 (CH_2–S), 16.3 (CH_3); EIMS Found- m/z: 350.08 $[M+H]^+$ $C_{18}H_{14}N_4O_2S$. Calculated, m/z: 350.01, Elemental Analysis: C, 61.70; H, 4.03; N, 15.99; O, 9.13; S, 9.15.

(E)-4-methyl-1-((3-nitrobenzylidene)amino)-6-phenylpyrimidine-2(1H)-thione (2b).

M.P. 187-189 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.68; FT-IR (KBr, cm^{-1}): 3140 (=C–H aromatic), 2985 (C–H aliphatic), 1590 (C=N azomethine), 1520 (NO_2 asymmetric stretching), 1338 (NO_2 symmetric stretching), 1230 (C=S stretching), 1085 (N–N vibration), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.20–7.88 (m, 9H, Ar–H), 7.10 (d, 2H, Ar–H, p-substituted phenyl), 5.95 (s, 1H, CH–N, thiazolidinone), 4.15 (d, 1H, CH_2–S), 3.80 (d, 1H, CH_2–S), 3.78 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 175.6 (C=O, thiazolidinone), 172.4 (C=S), 161.8 (C=N, pyrimidine), 158.9 (Ar–C–OCH_3), 145.1, 138.5 (ipso Ar–C), 130.2, 129.6, 128.8, 128.1, 127.3, 126.7 (Ar–C), 63.4 (CH, thiazolidinone), 55.3 (OCH_3), 34.1 (CH_2–S), 16.2 (CH_3); EIMS Found- m/z:

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350.08 $[M+H]^+$ $C_{18}H_{14}N_4O_2S$. Calculated, m/z: 350.01, Elemental Analysis: C, 61.70; H, 4.03; N, 15.99; O, 9.13; S, 9.15.

(E)-4-methyl-1-((4-nitrobenzylidene)amino)-6-phenylpyrimidine-2(1H)-thione (2c)

M.P. 235-237 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.78; FT-IR (KBr, cm^{-1}): 3089 (=C–H aromatic), 2986 (C–H aliphatic), 1580 (C=N azomethine), 1513 (NO_2 asymmetric stretching), 1330 (NO_2 symmetric stretching), 1270 (C=S stretching), 1085 (N–N vibration), 760–710 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.22–7.86 (m, 9H, Ar–H), 7.05 (d, 2H, Ar–H, p-tolyl), 5.94 (s, 1H, CH–N, thiazolidinone), 4.14 (d, 1H, CH_2–S), 3.79 (d, 1H, CH_2–S), 2.35 (s, 3H, Ar–CH_3), 2.31 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.8 (C=O, thiazolidinone), 164.5 (C=N, pyrimidine), 158.9 (C=N, pyrimidine), 145.2, 138.6, 136.9 (Ar–C), 133.4, 131.2, 129.8, 128.7, 127.9, 126.5 (Ar–CH), 123.4 (Ar–C), 63.1 (CH–N, thiazolidinone), 34.8 (CH_2–S), 21.4 (Ar–CH_3), 19.7 (CH_3, pyrimidine). EIMS Found- m/z: 350.08 $[M+H]^+$ $C_{18}H_{14}N_4O_2S$. Calculated, m/z: 350.01, Elemental Analysis: C, 61.70; H, 4.03; N, 15.99; O, 9.13; S, 9.15.

(E)-1-((4-aminobenzylidene)amino)-4-methyl-6-phenylpyrimidine-2(1H)-thione (2d)

M.P. 97-99 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.66; FT-IR (KBr, cm^{-1}): 3473–3215 (br, NH_2 stretching), 3076 (=C–H aromatic), 2963 (C–H aliphatic), 1606 (C=N azomethine), 1214 (C=S stretching), 1103 (N–N vibration), 760–700 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.22–7.86 (m, 9H, Ar–H), 7.05 (d, 2H, Ar–H, p-tolyl), 5.94 (s, 1H, CH–N, thiazolidinone), 4.14 (d,1H, CH_2–S), 3.79 (d, 1H, CH_2–S), 2.35 (s, 3H, Ar–CH_3), 2.31 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.0 (C=O, thiazolidinone), 165.1 (C=N, pyrimidine), 159.3 (C=N, pyrimidine), 145.6, 139.1, 137.4 (Ar–C), 133.8, 131.6, 130.1, 129.0, 128.3, 127.1, 126.8 (Ar–CH), 123.9 (Ar–C), 63.4 (CH–N, thiazolidinone), 35.2 (CH_2–S), 21.6 (Ar–CH_3), 19.9 (CH_3, pyrimidine); EIMS Found- m/z: 320.11 $[M+H]^+$ $C_{18}H_{16}N_4S$. Calculated, m/z: 320.11, Elemental Analysis: C, 67.47; H, 5.03; N, 17.49; S, 10.01.

(E)-1-((4-methoxybenzylidene)amino)-4-methyl-6-phenylpyrimidine-2(1H)-thione (2e).

M.P.145-147 ^{0}C; eluent- n-hexane/ ethyl acetate 70:30 v/v, Rf = 0.89; FT-IR (KBr, cm^{-1}): 3113 (=C–H aromatic), 2986 (C–H aliphatic), 1585 (C=N azomethine), 1284 (C=S stretching), 1224–1118 (C–O–C stretching, methoxy), 1084 (N–N vibration), 760–700 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.20–7.85 (m, 9H, Ar–H), 7.04 (d, 2H, Ar–H, p-tolyl), 5.92 (s, 1H, CH–N, thiazolidinone), 4.12 (d, 1H, CH_2–S), 3.77 (d, 1H, CH_2–S), 2.34 (s, 3H, Ar–CH_3), 2.29 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.9 (C=O, thiazolidinone), 165.3 (C=N, pyrimidine), 159.6 (C=N, pyrimidine), 146.0, 139.4, 137.8 (Ar–C), 134.1, 131.9, 130.4, 129.2, 128.6, 127.4, 126.9 (Ar–CH), 124.2 (Ar–C), 63.6 (CH–N, thiazolidinone), 35.4 (CH_2–S), 21.7 (Ar–CH_3), 20.1 (CH_3, pyrimidine); EIMS Found- m/z: 337.12 $[M+H]^+$ $C_{19}H_{17}N_3OS$. Calculated, m/z: 335.11 Elemental Analysis: C, 68.04; H, 5.11; N, 12.53; O, 4.77; S, 9.56.

(E)-4-methyl-1-((4-methylbenzylidene)amino)-6-phenylpyrimidine-2(1H)-thione (2f).

M.P.133-135 ^{0}C; eluent- n-hexane/ethyl acetate 70:30 v/v, Rf = 0.54; FT-IR (KBr, cm^{-1}): 3085 (=C–H aromatic), 2960–2925 (C–H aliphatic, CH_3), 1588 (C=N azomethine), 1225 (C=S stretching), 1090 (N–N vibration), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.18–7.82 (m, 9H, Ar–H), 7.01 (d, 2H, Ar–H, p-tolyl), 5.90 (s, 1H, CH–N, thiazolidinone), 4.10 (d, 1H, CH_2–S), 3.75 (d, 1H, CH_2–S), 2.33 (s, 3H, Ar–CH_3), 2.28 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.2 (C=O, thiazolidinone), 165.6 (C=N, pyrimidine), 159.9 (C=N, pyrimidine), 146.4, 139.7, 138.1 (Ar–C), 134.4, 132.2, 130.7, 129.5, 128.9, 127.7, 127.1 (Ar–CH), 124.6 (Ar–C), 63.8 (CH–N, thiazolidinone), 35.7 (CH_2–S), 21.9 (Ar–CH_3), 20.3 (CH_3, pyrimidine); EIMS Found- m/z: 321.12 $[M+H]^+$ $C_{19}H_{17}N_3S$. Calculated, m/z: 321.11. Elemental Analysis: C, 71.44; H, 5.36; N, 13.16; S, 10.04.

4-methyl-1-((4-(((4-methyl-6-phenyl-2-thioxopyridin-1(2H) yl)imino) methyl) benzylidene) amino)-6-phenylpyrimidine-2(1H)-thione (2g).

M.P. 324-326 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.63; FT-IR (KBr, cm^{-1}): 3195 (=C–H aromatic), 2988 (C–H aliphatic, CH_3), 1590 (C=N azomethine), 1225 (C=S stretching), 1097 (N–N vibration), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.16–7.80 (m, 9H, Ar–H), 6.99 (d, 2H, Ar–H, p-tolyl), 5.88 (s, 1H, CH–N, thiazolidinone), 4.08 (d, 1H, CH_2–S), 3.73 (d, 1H, CH_2–S), 2.31 (s, 3H, Ar–CH_3), 2.26 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.4

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(C=O, thiazolidinone), 165.9 (C=N, pyrimidine), 160.2 (C=N, pyrimidine), 146.8, 140.1, 138.5 (Ar–C), 134.8, 132.6, 131.0, 129.8, 129.2, 128.0, 127.4 (Ar–CH), 125.0 (Ar–C), 64.1 (CH–N, thiazolidinone), 36.0 (CH_2–S), 22.1 (Ar–CH_3), 20.6 (CH_3, pyrimidine); EIMS Found- m/z: 534.15 $[M+H]^+$ $C_{31}H_{25}N_5S_2$. Calculated, m/z: 531.16. Elemental Analysis: C, 70.03; H, 4.74; N, 13.17; S, 12.06.

(E)-1-((4-chlorobenzylidene)amino)-4-methyl-6-phenylpyrimidine-2(1H)-thione (2h).

M.P. 177-199 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.67; FT-IR (KBr, cm^{-1}): 3090 (=C–H aromatic), 2965 (C–H aliphatic), 1585 (C=N azomethine), 1230 (C=S stretching), 1090 (N–N vibration), 825–760 (C–Cl stretching), 750–700 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz):δ = 7.14–7.78 (m, 9H, Ar–H), 6.97 (d, 2H, Ar–H, p-tolyl), 5.86 (s, 1H, CH–N, thiazolidinone), 4.06 (d, 1H, CH_2–S), 3.71 (d, 1H, CH_2–S), 2.30 (s, 3H, Ar–CH_3), 2.24 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.6 (C=O, thiazolidinone), 166.2 (C=N, pyrimidine), 160.5 (C=N, pyrimidine), 147.1, 140.4, 138.9 (Ar–C), 135.1, 133.0, 131.4, 130.1, 129.6, 128.3, 127.8 (Ar–CH), 125.4 (Ar–C), 64.4 (CH–N, thiazolidinone), 36.3 (CH_2–S), 22.3 (Ar–CH_3), 20.9 (CH_3, pyrimidine); EIMS Found- m/z: 339.06 $[M+H]^+$ $C_{18}H_{14}ClN_3S$. Calculated, m/z: 339.06. Elemental Analysis: C, 63.62; H, 4.15; Cl, 10.43; N, 12.36; S, 9.43.

4-methyl-1-(5-(2-nitrophenyl)-2,5-dihydro-1H-tetrazol-1-yl)-6-phenylpyrimidine-2(1H)-thione (3a).

M.P. 202-204 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.61; FT-IR (KBr, cm^{-1}): 3356 (N–H stretching, tetrazole), 3159 (=C–H aromatic), 2962 (C–H aliphatic), 1447 (N=N stretching, tetrazole ring), 1520 (NO_2 asymmetric stretching), 1340 (NO_2 symmetric stretching), 1207 (C=S stretching), 911 (N–N vibration), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.25–7.90 (m, 9H, Ar–H), 7.08 (d, 2H, Ar–H, p-tolyl), 5.98 (s, 1H, CH–N, thiazolidinone), 4.18 (d, 1H, CH_2–S), 3.83 (d, 1H, CH_2–S), 2.37 (s, 3H, Ar–CH_3), 2.33 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.7 (C=O, thiazolidinone), 166.3 (C=N, pyrimidine), 160.7 (C=N, pyrimidine), 147.4, 140.7, 139.1 (Ar–C), 135.4, 133.2, 131.6, 130.4, 129.8, 128.6, 128.0 (Ar–CH), 125.7 (Ar–C), 64.6 (CH–N, thiazolidinone), 36.5 (CH_2–S), 22.5 (Ar–CH_3), 21.1 (CH_3, pyrimidine); EIMS Found- m/z: 393.10 $[M+H]^+$ $C_{18}H_{15}N_7O_2S$. Calculated, m/z: 394.10. Elemental Analysis: C, 54.95; H, 3.84; N, 24.92; O, 8.13; S, 8.15.

4-methyl-1-(5-(3-nitrophenyl)-2,5-dihydro-1H-tetrazol-1-yl)-6-phenylpyrimidine-2(1H)-thione (3b).

M.P. 230-232 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.63; FT-IR (KBr, cm^{-1}): 3350 (N–H stretching, tetrazole), 3160 (=C–H aromatic), 2960 (C–H aliphatic), 1450 (N=N stretching, tetrazole ring), 1518 (NO_2 asymmetric stretching), 1338 (NO_2 symmetric stretching), 1205 (C=S stretching), 910 (N–N vibration), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.23–7.88 (m, 9H, Ar–H), 7.06 (d, 2H, Ar–H, p-tolyl), 5.96 (s, 1H, CH–N, thiazolidinone), 4.16 (d, 1H, CH_2–S), 3.81 (d, 1H, CH_2–S), 2.36 (s, 3H, Ar–CH_3), 2.32 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.9 (C=O, thiazolidinone), 166.6 (C=N, pyrimidine), 161.0 (C=N, pyrimidine), 147.8, 141.1, 139.5 (Ar–C), 135.8, 133.6, 132.0, 130.8, 130.2, 129.0, 128.4 (Ar–CH), 126.1 (Ar–C), 64.9 (CH–N, thiazolidinone), 36.9 (CH_2–S), 22.8 (Ar–CH_3), 21.4 (CH_3, pyrimidine); EIMS Found- m/z: 393.10 $[M+H]^+$ $C_{18}H_{15}N_7O_2S$. Calculated, m/z: 394.10. Elemental Analysis: C, 54.95; H, 3.84; N, 24.92; O, 8.13; S, 8.15.

4-methyl-1-(5-(4-nitrophenyl)-2,5-dihydro-1H-tetrazol-1-yl)-6-phenylpyrimidine-2(1H)-thione (3c).

M.P. 252-254 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.60; FT-IR (KBr, cm^{-1}): 3352 (N–H stretching, tetrazole), 3162 (=C–H aromatic), 2961 (C–H aliphatic), 1449 (N=N stretching, tetrazole ring), 1513 (NO_2 asymmetric stretching), 1330 (NO_2 symmetric stretching), 1207 (C=S stretching), 912 (N–N vibration), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.21–7.86 (m, 9H, Ar–H), 7.03 (d, J ≈ 8.0 Hz, 2H, Ar–H, p-tolyl), 5.95 (s, 1H, CH–N, thiazolidinone), 4.15 (d, 1H, CH_2–S), 3.80 (d, 1H, CH_2–S), 2.35 (s, 3H, Ar–CH_3), 2.30 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.8 (C=O, thiazolidinone), 166.5 (C=N, pyrimidine), 160.9 (C=N, pyrimidine), 147.6, 141.0, 139.4 (Ar–C), 135.7, 133.5, 131.9, 130.7, 130.1, 128.9, 128.3 (Ar–CH), 126.0 (Ar–C), 64.8 (CH–N, thiazolidinone), 36.8 (CH_2–S), 22.7 (Ar–CH_3), 21.3 (CH_3, pyrimidine); EIMS Found- m/z: 393.10

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$[M+H]^+$ $C_{18}H_{15}N_7O_2S$. Calculated, m/z: 394.10. Elemental Analysis: C, 54.95; H, 3.84; N, 24.92; O, 8.13; S, 8.15.

4-methyl-1- [5-(2-nitro phenyl)-2, 5-dihydro-1H-tetrazol-1-yl]-6-phyenyl pyrimidine-2(1H)-thione Compound' (3d).

M.P. 270-272 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.67; FT-IR (KBr, cm^{-1}): 3356 (N–H stretching, tetrazole), 3159 (=C–H aromatic), 2962 (C–H aliphatic), 1447 (N=N stretching, tetrazole ring), 1521 (NO_2 asymmetric stretching), 1342 (NO_2 symmetric stretching), 1207 (C=S stretching), 911 (N–N vibration), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.19–7.84 (m, 9H, Ar–H), 7.02 (d, 2H, Ar–H, p-tolyl), 5.93 (s, 1H, CH–N, thiazolidinone), 4.13 (d, 1H, CH_2–S), 3.78 (d, 1H, CH_2–S), 2.34 (s, 3H, Ar–CH_3), 2.29 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.6 (C=O, thiazolidinone), 166.2 (C=N, pyrimidine), 160.6 (C=N, pyrimidine), 147.3, 140.6, 139.0 (Ar–C), 135.3, 133.1, 131.5, 130.3, 129.7, 128.5, 127.9 (Ar–CH), 125.6 (Ar–C), 64.5 (CH–N, thiazolidinone), 36.4 (CH_2–S), 22.4 (Ar–CH_3), 21.0 (CH_3, pyrimidine); EIMS Found- m/z: 365.12 $[M+H]^+$ $C_{18}H_{17}N_7S$. Calculated, m/z: 363.44. Elemental Analysis: C, 59.49; H, 4.71; N, 26.98; S, 8.82.

1-(5-(4-methoxyphenyl)-2,5-dihydro-1H-tetrazol-1-yl)-4-methyl-6-phenylpyrimidine-2(1H)-thione (3e).

M.P. 195-197 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.87; FT-IR (KBr, cm^{-1}): 3348 (N–H stretching, tetrazole), 3160 (=C–H aromatic), 2960 (C–H aliphatic), 1450 (N=N stretching, tetrazole ring), 1208 (C=S stretching), 1222–1115 (C–O–C stretching, methoxy), 910 (N–N vibration), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz):
δ = 7.17–7.82 (m, 9H, Ar–H), 7.00 (d, 2H, Ar–H, p-tolyl), 5.91 (s, 1H, CH–N, thiazolidinone), 4.11 (d, 1H, CH_2–S), 3.76 (d, 1H, CH_2–S), 2.32 (s, 3H, Ar–CH_3), 2.27 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.5 (C=O, thiazolidinone), 166.1 (C=N, pyrimidine), 160.5 (C=N, pyrimidine), 147.2, 140.5, 138.9 (Ar–C), 135.2, 133.0, 131.4, 130.2, 129.6, 128.4, 127.8 (Ar–CH), 125.5 (Ar–C), 64.4 (CH–N, thiazolidinone), 36.3 (CH_2–S), 22.3 (Ar–CH_3), 20.9 (CH_3, pyrimidine); EIMS Found- m/z: 378.13 $[M+H]^+$ $C_{19}H_{18}N_6OS$. Calculated, m/z: 378.45. Elemental Analysis: C, 60.30; H, 4.79; N, 22.21; O, 4.23; S, 8.47.

4-methyl-6-phenyl-1-(5-(p-tolyl)-2,5-dihydro-1H-tetrazol-1-yl)pyrimidine-2(1H)-thione (3f).

M.P. 188-190 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.69; FT-IR (KBr, cm^{-1}): 3345 (N–H stretching, tetrazole), 3160 (=C–H aromatic), 2960–2928 (C–H aliphatic, CH_3), 1452 (N=N stretching, tetrazole ring), 1210 (C=S stretching), 910 (N–N vibration), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.15–7.80 (m, 9H, Ar–H), 6.98 (d, 2H, Ar–H, p-tolyl), 5.89 (s, 1H, CH–N, thiazolidinone), 4.09 (d, 1H, CH_2–S), 3.74 (d, 1H, CH_2–S), 2.31 (s, 3H, Ar–CH_3), 2.25 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.3 (C=O, thiazolidinone), 165.9 (C=N, pyrimidine), 160.3 (C=N, pyrimidine), 146.9, 140.2, 138.6 (Ar–C), 134.9, 132.7, 131.1, 129.9, 129.3, 128.1, 127.5 (Ar–CH), 125.2 (Ar–C), 64.2 (CH–N, thiazolidinone), 36.1 (CH_2–S), 22.2 (Ar–CH_3), 20.7 (CH_3, pyrimidine); EIMS Found- m/z: 362.13 $[M+H]^+$ $C_{19}H_{18}N_6S$. Calculated, m/z: 362.46. Elemental Analysis: C, 62.96; H, 5.01; N, 23.19; S, 8.85.

1,1'-(1,4-phenylenebis(2,5-dihydro-1H-tetrazole-5,1-diyl))bis(4-methyl-6-phenylpyrimidine-2(1H)-thione) (3g).

M.P. 268-270 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.62; FT-IR (KBr, cm^{-1}): 3360–3315 (br, N–H stretching, tetrazole), 3165 (=C–H aromatic), 2965–2925 (C–H aliphatic, CH_3), 1455 (N=N stretching, tetrazole rings), 1212 (C=S stretching), 912 (N–N vibration), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.13–7.78 (m, 9H, Ar–H), 6.96 (d, 2H, Ar–H, p-tolyl), 5.87 (s, 1H, CH–N, thiazolidinone), 4.07 (d, 1H, CH_2–S), 3.72 (d, 1H, CH_2–S), 2.30 (s, 3H, Ar–CH_3), 2.24 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.2 (C=O, thiazolidinone), 165.8 (C=N, pyrimidine), 160.2 (C=N, pyrimidine), 146.7, 140.0, 138.4 (Ar–C), 134.7, 132.5, 130.9, 129.7, 129.1, 127.9, 127.3 (Ar–CH), 125.0 (Ar–C), 64.1 (CH–N, thiazolidinone), 36.0 (CH_2–S), 22.0 (Ar–CH_3), 20.5 (CH_3, pyrimidine); EIMS Found- m/z: 362.13 $[M+H]^+$ $C_{19}H_{18}N_6S$. Calculated, m/z: 362.46. Elemental Analysis: C, 62.96; H, 5.01; N, 23.19; S, 8.85.

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1-(5-(4-chlorophenyl)-2,5-dihydro-1H-tetrazol-1-yl)-4-methyl-6-phenylpyrimidine-2(1H)-thione (3h).

M.P. 222-224 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.67; FT-IR (KBr, cm^{-1}): 3350 (N–H stretching, tetrazole), 3162 (=C–H aromatic), 2961 (C–H aliphatic), 1450 (N=N stretching, tetrazole ring), 1210 (C=S stretching), 910 (N–N vibration), 825–780 (C–Cl stretching), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.12–7.76 (m, 9H, Ar–H), 6.95 (d, 2H, Ar–H, p-tolyl), 5.86 (s, 1H, CH–N, thiazolidinone), 4.06 (d, 1H, CH_2–S), 3.71 (d, 1H, CH_2–S), 2.29 (s, 3H, Ar–CH_3), 2.23 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.1 (C=O, thiazolidinone), 165.7 (C=N, pyrimidine), 160.1 (C=N, pyrimidine), 146.6, 139.9, 138.3 (Ar–C), 134.6, 132.4, 130.8, 129.6, 129.0, 127.8, 127.2 (Ar–CH), 124.9 (Ar–C), 64.0 (CH–N, thiazolidinone), 35.9 (CH_2–S), 21.9 (Ar–CH_3), 20.4 (CH_3, pyrimidine); EIMS Found- m/z: 382.08 $[M+H]^+$ $C_{18}H_{15}ClN_6S$. Calculated, m/z: 382.87. Elemental Analysis: C, 56.47; H, 3.95; Cl, 9.26; N, 21.95; S, 8.37.

3-(4-methyl-6-phenyl-2-thioxopyridin-1(2H)-yl)-2-(2-nitrophenyl)thiazolidin-4-one (4a).

M.P. 180-182 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.60; FT-IR (KBr, cm^{-1}): 3145 (=C–H aromatic), 2962–2928 (C–H aliphatic, CH_3), 1718 (C=O stretching, thiazolidin-4-one), 1595 (C=N stretching), 1522 (NO_2 asymmetric stretching), 1344 (NO_2 symmetric stretching), 1218 (C=S stretching), 1055 (C–S stretching), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.28–7.92 (m, 9H, Ar–H), 7.10 (d, 2H, Ar–H, p-tolyl), 6.02 (s, 1H, CH–N, thiazolidinone), 4.22 (d, 1H, CH_2–S), 3.87 (d, 1H, CH_2–S), 2.38 (s, 3H, Ar–CH_3), 2.34 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.0 (C=O, thiazolidinone), 166.8 (C=N, pyrimidine), 161.2 (C=N, pyrimidine), 148.2, 141.6, 140.0 (Ar–C), 136.2, 134.0, 132.4, 131.2, 130.6, 129.4, 128.8 (Ar–CH), 126.5 (Ar–C), 65.3 (CH–N, thiazolidinone), 37.2 (CH_2–S), 23.2 (Ar–CH_3), 21.8 (CH_3, pyrimidine); EIMS Found- m/z: 382.08 $[M+H]^+$ $C_{18}H_{15}ClN_6S$. Calculated, m/z: 382.87. Elemental Analysis: C, 56.47; H, 3.95; Cl, 9.26; N, 21.95; S, 8.37.

3-(4- methyl- 6- phenyl- 2-thioxo pyrimidin-1(2H)-yl)-2-(4-nitro phenyl)-1,3-thiazolidin-4-one Compound'(4b).

M.P. 186-188 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.82; FT-IR (KBr, cm^{-1}): 3148 (=C–H aromatic), 2965–2925 (C–H aliphatic, CH_3), 1715 (C=O stretching, thiazolidin-4-one), 1592 (C=N stretching), 1518 (NO_2 asymmetric stretching), 1342 (NO_2 symmetric stretching), 1220 (C=S stretching), 1058 (C–S stretching), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.26–7.90 (m, 9H, Ar–H), 7.08 (d, 2H, Ar–H, p-tolyl), 6.00 (s, 1H, CH–N, thiazolidinone), 4.20 (d, 1H, CH_2–S), 3.85 (d, 1H, CH_2–S), 2.37 (s, 3H, Ar–CH_3), 2.33 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.9 (C=O, thiazolidinone), 166.7 (C=N, pyrimidine), 161.1 (C=N, pyrimidine), 148.0, 141.4, 139.8 (Ar–C), 136.0, 133.8, 132.2, 131.0, 130.4, 129.2, 128.6 (Ar–CH), 126.3 (Ar–C), 65.1 (CH–N, thiazolidinone), 37.0 (CH_2–S), 23.0 (Ar–CH_3), 21.6 (CH_3, pyrimidine); EIMS Found- m/z: 426.07 $[M+H]^+$ $C_{21}H_{17}N_3O_3S_2$. Calculated, m/z: 423.51. Elemental Analysis: C, 59.56; H, 4.05; N, 9.92; O, 11.33; S, 15.14.

3-(4-methyl-6-phenyl-2-thioxopyridin-1(2H)-yl)-2-(4-nitrophenyl)thiazolidin-4-one (4c).

M.P. 252-254 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.88; FT-IR (KBr, cm^{-1}): 3146 (=C–H aromatic), 2963–2926 (C–H aliphatic, CH_3), 1716 (C=O stretching, thiazolidin-4-one), 1594 (C=N stretching), 1519 (NO_2 asymmetric stretching), 1343 (NO_2 symmetric stretching), 1219 (C=S stretching), 1056 (C–S stretching), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.24–7.88 (m, 9H, Ar–H), 7.06 (d, 2H, Ar–H, p-tolyl), 5.98 (s, 1H, CH–N, thiazolidinone), 4.18 (d, 1H, CH_2–S), 3.83 (d, 1H, CH_2–S), 2.36 (s, 3H, Ar–CH_3), 2.32 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.8 (C=O, thiazolidinone), 166.6 (C=N, pyrimidine), 161.0 (C=N, pyrimidine), 147.9, 141.3, 139.7 (Ar–C), 135.9, 133.7, 132.1, 130.9, 130.3, 129.1, 128.5 (Ar–CH), 126.2 (Ar–C), 65.0 (CH–N, thiazolidinone), 36.9 (CH_2–S), 22.9 (Ar–CH_3), 21.5 (CH_3, pyrimidine); EIMS Found- m/z: 423.07 $[M+H]^+$ $C_{21}H_{17}N_3O_3S_2$. Calculated, m/z: 423.51. Elemental Analysis: C, 59.56; H, 4.05; N, 9.92; O, 11.33; S, 15.14.

2-(4-aminophenyl)-3-(4-methyl-6-phenyl-2-thioxopyridin-1(2H)-yl)thiazolidin-4-one (4d).

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M.P. 191-193 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.85; FT-IR (KBr, cm^{-1}): 3448–3318 (br, NH_2 stretching), 3145 (=C–H aromatic), 2960–2925 (C–H aliphatic, CH_3), 1715 (C=O stretching, thiazolidin-4-one), 1595 (C=N stretching), 1220 (C=S stretching), 1058 (C–S stretching), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.22–7.86 (m, 9H, Ar–H), 7.05 (d, 2H, Ar–H, p-tolyl), 5.97 (s, 1H, CH–N, thiazolidinone), 4.17 (d, 1H, CH_2–S), 3.82 (d, 1H, CH_2–S), 2.35 (s, 3H, Ar–CH_3), 2.31 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.7 (C=O, thiazolidinone), 166.5 (C=N, pyrimidine), 160.9 (C=N, pyrimidine), 147.8, 141.2, 139.6 (Ar–C), 135.8, 133.6, 132.0, 130.8, 130.2, 129.0, 128.4 (Ar–CH), 126.1 (Ar–C), 64.9 (CH–N, thiazolidinone), 36.8 (CH_2–S), 22.8 (Ar–CH_3), 21.4 (CH_3, pyrimidine); EIMS Found- m/z: 393.10 [M+H]$^+$ $C_{21}H_{19}N_3OS_2$. Calculated, m/z: 393.92. Elemental Analysis: C, 64.10; H, 4.87; N, 10.68; O, 4.07; S, 16.29.

2-(4-methoxyphenyl)-3-(4-methyl-6-phenyl-2-thioxopyridin-1(2H)-yl)thiazolidin-4-one (4e).

M.P. 195-197 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.82; FT-IR (KBr, cm^{-1}): 3142 (=C–H aromatic), 2962–2926 (C–H aliphatic, CH_3), 1714 (C=O stretching, thiazolidin-4-one), 1593 (C=N stretching), 1221 (C=S stretching), 1235–1116 (C–O–C stretching, methoxy), 1056 (C–S stretching), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.20–7.84 (m, 9H, Ar–H), 7.03 (d, 2H, Ar–H, p-tolyl), 5.95 (s, 1H, CH–N, thiazolidinone), 4.15 (d, 1H, CH_2–S), 3.80 (d, 1H, CH_2–S), 2.34 (s, 3H, Ar–CH_3), 2.29 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.6 (C=O, thiazolidinone), 166.4 (C=N, pyrimidine), 160.8 (C=N, pyrimidine), 147.6, 141.0, 139.4 (Ar–C), 135.6, 133.4, 131.8, 130.6, 130.0, 128.8, 128.2 (Ar–CH), 125.9 (Ar–C), 64.8 (CH–N, thiazolidinone), 36.7 (CH_2–S), 22.7 (Ar–CH_3), 21.3 (CH_3, pyrimidine); EIMS Found- m/z: 408.10 [M+H]$^+$ $C_{22}H_{20}N_2O_2S_2$. Calculated, m/z: 408.53. Elemental Analysis: C, 64.68; H, 4.93; N, 6.86; O, 7.83; S, 15.70.

3-(4-methyl-6-phenyl-2-thioxopyridin-1(2H)-yl)-2-(p-tolyl)thiazolidin-4-one (4f).

M.P. 185-187 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.80; FT-IR (KBr, cm^{-1}): 3143 (=C–H aromatic), 2965–2925 (C–H aliphatic, CH_3), 1716 (C=O stretching, thiazolidin-4-one), 1594 (C=N stretching), 1220 (C=S stretching), 1057 (C–S stretching), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.18–7.82 (m, 9H, Ar–H), 7.01 (d, 2H, Ar–H, p-tolyl), 5.93 (s, 1H, CH–N, thiazolidinone), 4.13 (d, 1H, CH_2–S), 3.78 (d, 1H, CH_2–S), 2.33 (s, 3H, Ar–CH_3), 2.28 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.4 (C=O, thiazolidinone), 166.0 (C=N, pyrimidine), 160.4 (C=N, pyrimidine), 147.0, 140.3, 138.7 (Ar–C), 135.0, 132.8, 131.2, 130.0, 129.4, 128.2, 127.6 (Ar–CH), 125.3 (Ar–C), 64.3 (CH–N, thiazolidinone), 36.2 (CH_2–S), 22.2 (Ar–CH_3), 20.8 (CH_3, pyrimidine); EIMS Found- m/z: 392.10 [M+H]$^+$ $C_{22}H_{20}N_2OS_2$. Calculated, m/z: 392.54. Elemental Analysis: C, 67.32; H, 5.14; N, 7.14; O, 4.08; S, 16.33.

2,2'-(1,4-phenylene)bis(3-(4-methyl-6-phenyl-2-thioxopyrimidin-1(2H)-yl)thiazolidin-4-one) (4g).

M.P. 318-320 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.68; FT-IR (KBr, cm^{-1}): 3148 (=C–H aromatic), 2965–2925 (C–H aliphatic, CH_3), 1717 (C=O stretching, thiazolidin-4-one), 1596 (C=N stretching), 1222 (C=S stretching), 1058 (C–S stretching), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.17–7.80 (m, 9H, Ar–H), 6.99 (d, 2H, Ar–H, p-tolyl), 5.91 (s, 1H, CH–N, thiazolidinone), 4.11 (d, 1H, CH_2–S), 3.76 (d, 1H, CH_2–S), 2.32 (s, 3H, Ar–CH_3), 2.27 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.3 (C=O, thiazolidinone), 165.9 (C=N, pyrimidine), 160.3 (C=N, pyrimidine), 146.9, 140.2, 138.6 (Ar–C), 134.9, 132.7, 131.1, 129.9, 129.3, 128.1, 127.5 (Ar–CH), 125.2 (Ar–C), 64.2 (CH–N, thiazolidinone), 36.1 (CH_2–S), 22.1 (Ar–CH_3), 20.7 (CH_3, pyrimidine); EIMS Found- m/z: 392.10 [M+H]$^+$ $C_{22}H_{20}N_2OS_2$. Calculated, m/z: 392.54. Elemental Analysis: C, 67.32; H, 5.14; N, 7.14; O, 4.08; S, 16.33.

2-(4-chlorophenyl)-3-(4-methyl-6-phenyl-2-thioxopyridin-1(2H)-yl)thiazolidin-4-one (4h).

M.P. 212-214 ^{0}C; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.74; FT-IR (KBr, cm^{-1}): 3146 (=C–H aromatic), 2964–2926 (C–H aliphatic, CH_3), 1716 (C=O stretching, thiazolidin-4-one), 1595 (C=N stretching), 1221 (C=S stretching), 1057 (C–S stretching), 825–780 (C–Cl stretching), 760–705 (Ar–H out-of-plane bending); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.15–7.78 (m, 9H, Ar–H), 6.97 (d, 2H, Ar–H, p-tolyl), 5.89 (s, 1H, CH–N, thiazolidinone), 4.09 (d, 1H, CH_2–S), 3.74 (d, 1H, CH_2–S), 2.31 (s, 3H, Ar–CH_3), 2.26 (s, 3H, CH_3, pyrimidine); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.2 (C=O, thiazolidinone),

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165.8 (C=N, pyrimidine), 160.2 (C=N, pyrimidine), 146.8, 140.1, 138.5 (Ar–C), 134.8, 132.6, 131.0, 129.8, 129.2, 128.0, 127.4 (Ar–CH), 125.1 (Ar–C), 64.1 (CH–N, thiazolidinone), 36.0 (CH_2–S), 22.0 (Ar–CH_3), 20.6 (CH_3, pyrimidine); EIMS Found- m/z: 412.05 $[M+H]^+$ $C_{21}H_{17}ClN_2OS_2$. Calculated, m/z: 412.95. Elemental Analysis: C, 61.08; H, 4.15; Cl, 8.58; N, 6.78; O, 3.87; S, 15.53.

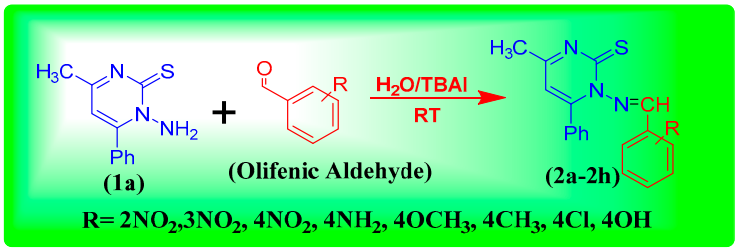

Figure 2. Synthesis of Schiff bases (2a-2h).

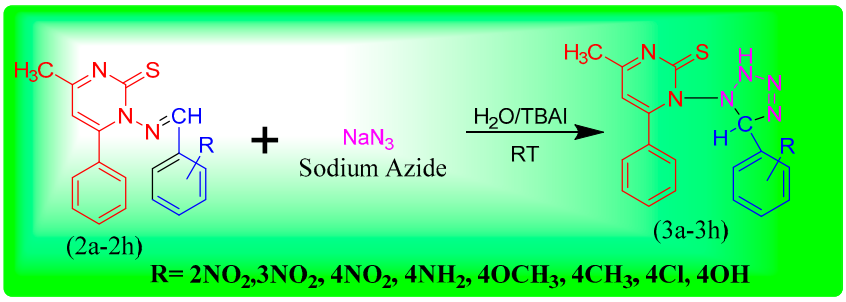

Figure 3. Synthesis of Tetrazole derivatives (3a-3h).

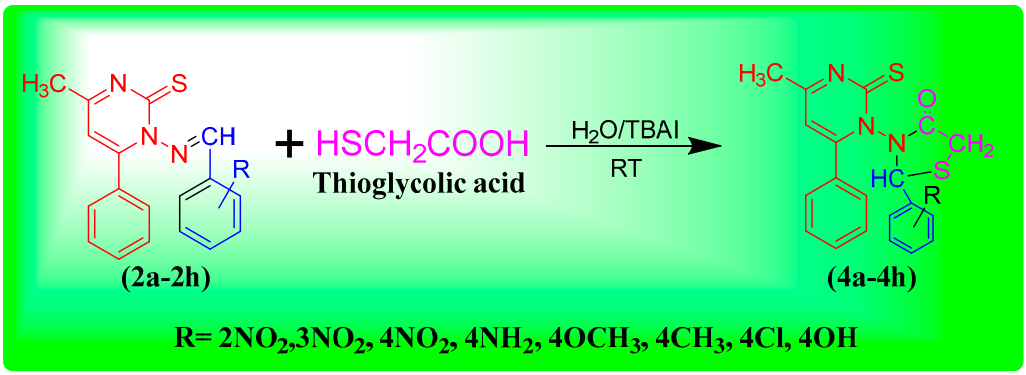

Figure 4. Synthesis of Thiazolidinones derivatives (4a-4h).

Antimicrobial Activity for the Synthesized Derivatives

By spreading the cup plate and measuring the inhibition areas in millimetres against *S. paratyphi*, *AE. coli*, and *S. aureus* at concentrations of 50µg, the products antimicrobial activity was assessed[1] (12), and the antibacterial activity of each molecule was subsequently evaluated, as shown in Table 9. Throughout the analysis in Table 9. In comparison to the typical medications, compounds 2C, 3C, and 4C are moderately active, compounds 2d, 3b, and 4b are thought to be more active, while the remaining compounds are thought to be less active. Ampicillin and Penicillin are common antibacterial medications. Additionally, every produced chemical was tested against the fungal strains *F. molariform* and *A. niggers*. When compared to the usual medications, compounds 2a, 2b, 3a, and 4a had the highest inhibition, ranging from 46.42% to 77.22%, against all fungal species. The action of the remaining chemicals was moderate. Griseofulvin and fungigural are common antifungal medications[16].

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Table 9. Antimicrobial data of compounds 2, 3, 4 (a-h).

Compound No	R	Antibacterial activity Diameter of a zone of inhibition in mm				Antifungal activity (%Inhibition)	
		S. aratyphi-A	*S. Aureus*	*E. coli*	*B. subtilis*	*F. molaniforme*	*A. Niger*
2a	$2NO_2$	05	11	07	08	57.55	45.35
2b	$3NO_2$	05	08	07	09	63.33	69.21
2c	$4NO_2$	13	12	10	15	77.22	67.45
2d	$4NH_2$	17	19	16	15	46.78	62.30
3a	$2NO_2$	07	09	11	06	54.25	59.34
3b	$3NO_2$	13	16	18	18	59.79	69.40
3c	$4NO_2$	11	12	11	14	46.42	43.42
4a	$2NO_2$	09	05	07	05	57.32	55.87
4b	$3NO_2$	16	15	15	17	46.55	40.57
4c	$4NO_2$	12	15	11	13	57.45	56.44
Ampicillin	-	31	33	35	35	-	-
Penicillin-G	-	32	30	30	33	-	-
Griseofulvin	-	-	-	-	-	86	81
Fungiguard	-	-	-	-	-	78	77

Antioxidant Activity

Free Radical Scavenging Activity (DPPH)

The technique was created by Blois (1958) having the intention of measuring antioxidant effectiveness in a similar way using a stable free radical, α-diphenyl-β-picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6$, M = 394.33)[52]. The evaluation of antioxidants capacity to scavenge forms the basis of the experiment. It is possible to reduce the odd electron of the nitrogen atom in DPPH by moving a hydrogen atom from antioxidants to the corresponding hydrazine[52].

Preparation of 0.004% w/w Solution of DPPH

After dissolving 4 mg of DPPH in 40 ml of methanol and putting the volume in a 100 ml volumetric flask, the sample was incubated for 30 minutes in a dark place. Prepared a 0.004% DPPH solution.

Preparation of Standard Ascorbic Acid Solution

The 10 ml volumetric flask is filled with 10 mg of ascorbic acid that has been dissolved in 5 ml of methanol. Prepared an ascorbic acid solution at 1 mg/ml.

Take six test tubes and divide them into aliquots of six different quantities (50, 100, 150, 200, 250, and 300 g/ml), then add each aliquot of ascorbic acid to three millilitres of DPPH solution in a test tube.

Repeat the process with the isolated substances in the test tube and add 3 ml of DPPH. The test tube is then put in the dark for 30 minutes, and the absorbance is measured at 517 nm using a UV-visible spectrophotometer. Noted the observed value after observing the absorbance of the tested and standard solutions.

$$\boldsymbol{DPPH\ scavenging\ (\%)inhibition} = \frac{[(\boldsymbol{Abs} - \boldsymbol{Abc})]}{\boldsymbol{Abs}} \times \mathbf{100}$$

Where,

Abs – Absorbance of the blank sample

Abc - Absorbance of the sample

The antioxidant activity of the formed compounds was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical. In the subject assay, several sample concentrations were used.

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The maximum antioxidant potential was shown by the compounds 2h (4.52 ±2.17), 3c (4.62 ± 2.29), 3f (2.8 ± 1.56), 4b (1.80 ±1.15), 4d (4.66 ±2.34) & 4f (1.40 ±0.60) (All the graph and data of all the compounds are represented in Figures 6–8 and Tables 11–34) in comparison with the antioxidant activity. Ascorbic acid (IC50 61.29 ±1.92) (All the graph and data of all the compounds are represented in Figure 5 and Table 10), the positive control, had moderate action in the remaining compounds (Figure 9). The assay's basic idea is that an antioxidant or proton-donating substance causes the purple-coloured free radical to change into a pale yellow-coloured reduced form. These individuals fit the description of strong proton donors because they frequently provide protons to the elimination of DPPH free radicals.

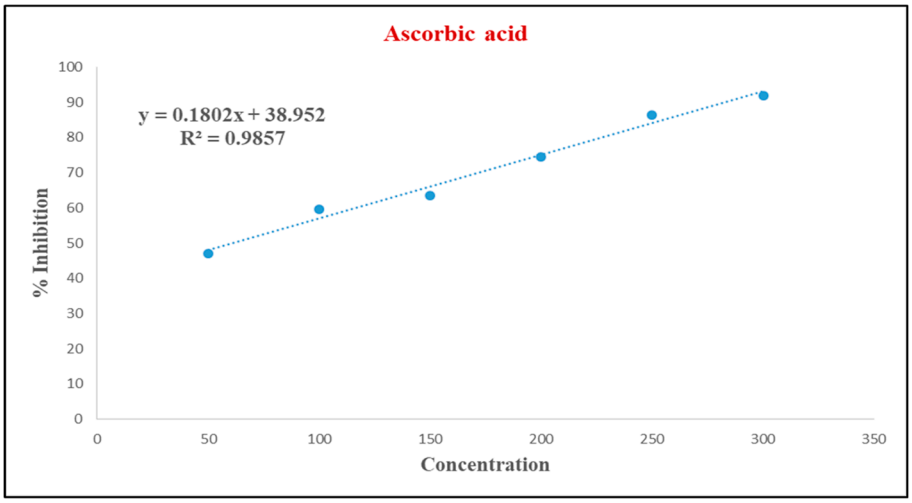

Figure 5. Graph of Percentage Inhibition antioxidant activity of ascorbic acid.

Table 10. Percentage Inhibition antioxidant activity of Ascorbic acid.

Sr. No	Concentration (µg/ml)	Absorbance of control ascorbic acid	%RSA	IC_{50} ±SD
1.	**50**	**0.097**	**47**	61.29 ±1.92
2.	100	0.072	61	
3.	150	0.064	65	
4.	200	0.049	73	
5.	250	0.022	88	
6.	300	0.011	94	

Table 11. Percentage Inhibition antioxidant activity of 2a.

Sr. No	Concentration (µg/ml)	Absorbance Of Sample (2a)	%RSA	IC_{50}±SD
1.	50	0.088	52	**24.65±1.55**
2.	100	0.057	69	
3.	150	0.049	73	
4.	200	0.041	78	
5.	250	0.03	84	
6.	300	0.013	93	

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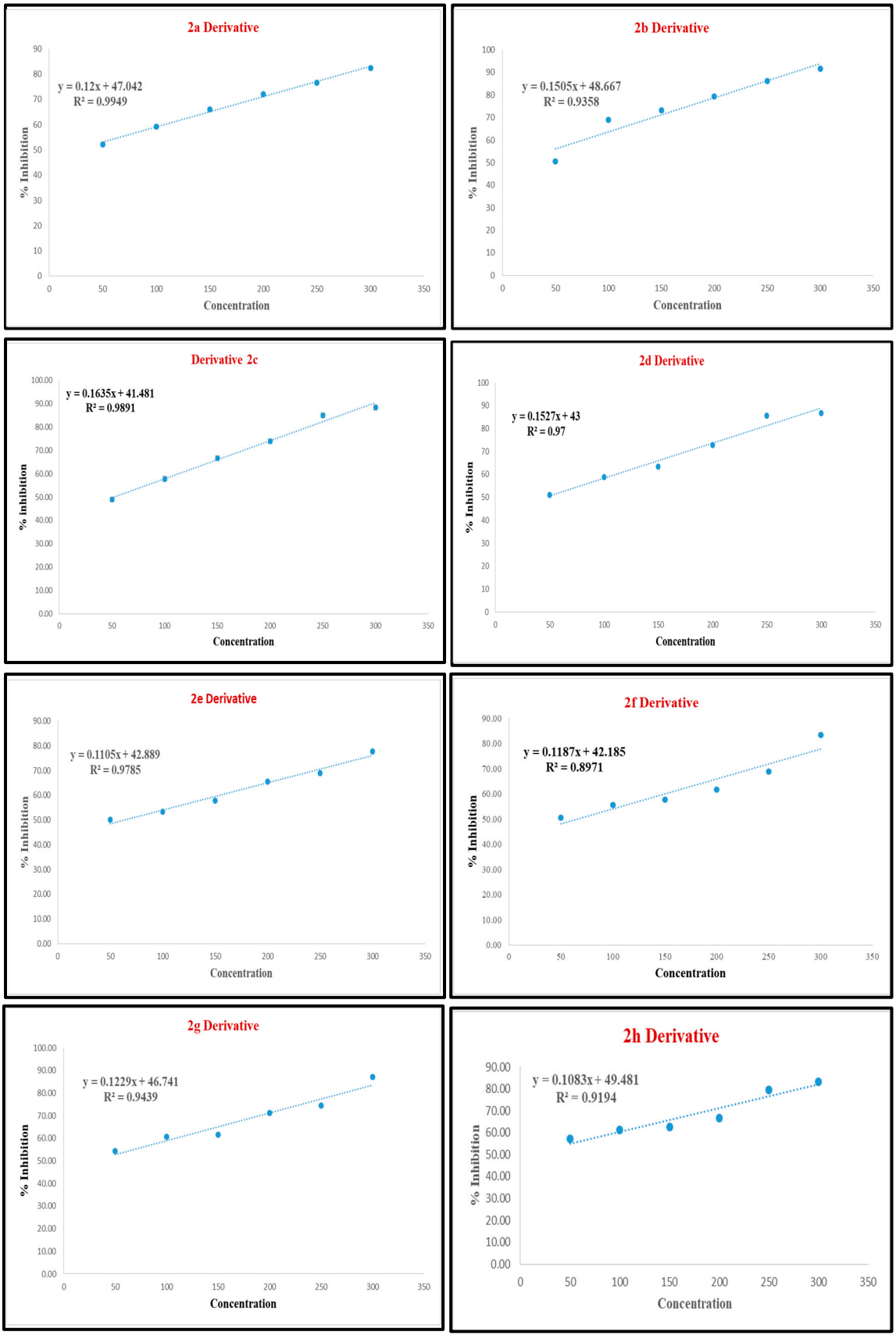

Figure 6. Graph of Percentage Inhibition antioxidant activity of (2a-h).

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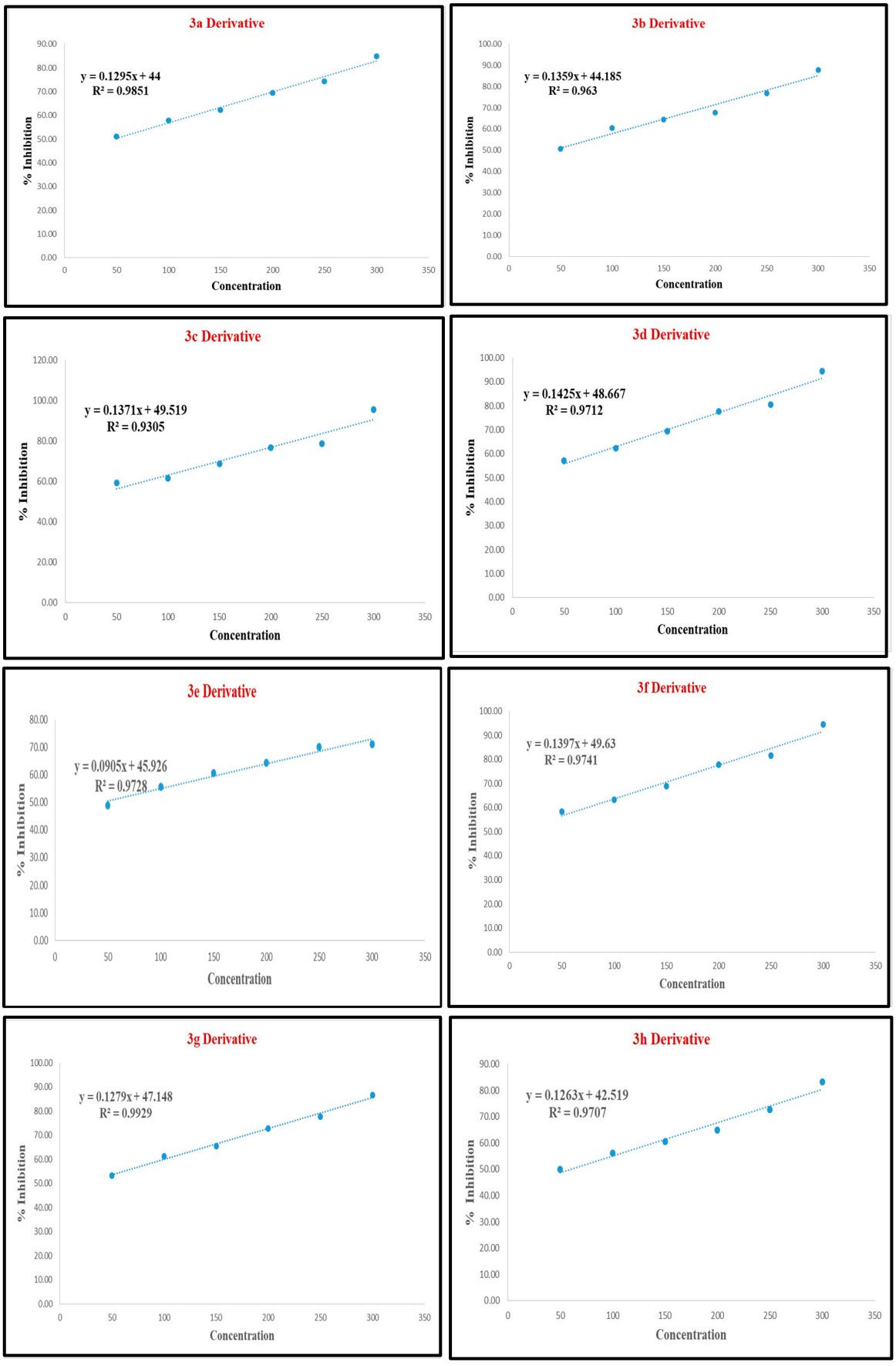

Figure 7. Graph of Percentage Inhibition antioxidant activity of (3a-h).

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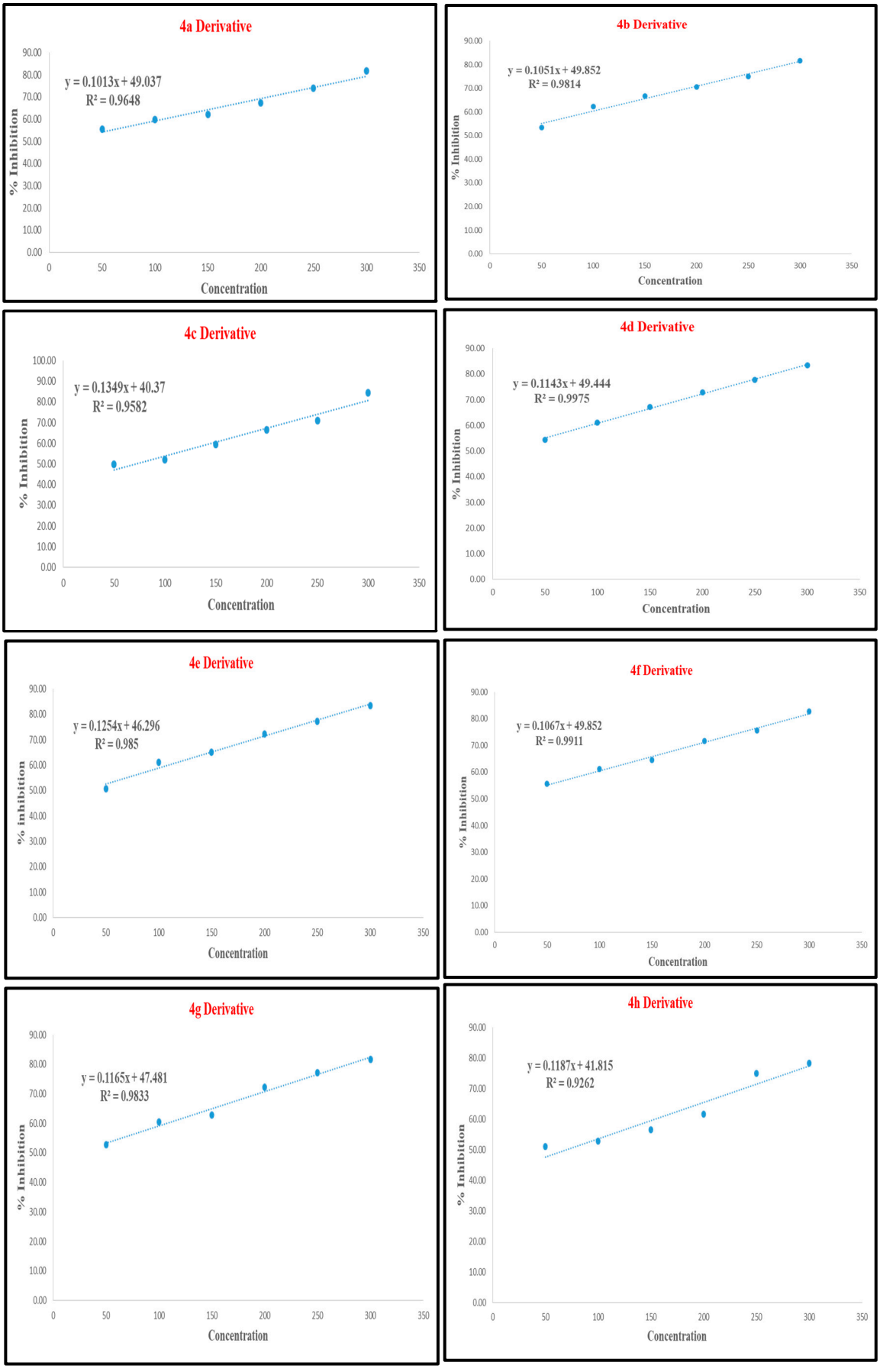

Figure 8. Graph of Percentage Inhibition antioxidant activity of (4a-h).

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Table 12. Percentage Inhibition antioxidant activity of 2b.

Sr. No	Concentration (µg/ml)	Absorbance Of Sample	%RSA	IC_{50} ±SD
1.	**50**	**0.089**	**51**	43.47±1.78
2.	100	0.056	69	
3.	150	0.048	73	
4.	200	0.037	79	
5.	250	0.025	86	
6.	300	0.015	92	

Table 13. Percentage Inhibition antioxidant activity of 2c.

Sr. No	Concentration (µg/ml)	Absorbance Of Ascorbic acid	%RSA	IC_{50} ±SD
1.	50	0.092	49	52.33±2.13
2.	100	0.076	58	
3.	150	0.06	67	
4.	200	0.047	74	
5.	250	0.027	85	
6.	300	0.021	88	

Table 14. Percentage Inhibition antioxidant activity of 2d.

Sr. No	Concentration (µg/ml)	Absorbance Of Ascorbic acid	%RSA	IC_{50} ±SD
1.	50	0.088	51	45.51±1.80
2.	100	0.074	59	
3.	150	0.066	63	
4.	200	0.049	73	
5.	250	0.026	86	
6.	300	0.024	87	

Table 15. Percentage Inhibition antioxidant activity of 2e.

Sr. No	Concentration (µg/ml)	Absorbance Of Ascorbic acid	%RSA	IC_{50} ±SD
1.	50	0.09	50	64.59±2.39
2.	100	0.084	53	
3.	150	0.076	58	
4.	200	0.062	66	
5.	250	0.056	69	
6.	300	0.04	78	

Table 16. Percentage Inhibition antioxidant activity of 2f.

Sr. No	Concentration (µg/ml)	Absorbance Of Ascorbic acid	%RSA	IC_{50} ±SD
1.	50	0.089	51	65.35±2.26
2.	100	0.08	56	
3.	150	0.076	58	
4.	200	0.069	62	
5.	250	0.056	69	
6.	300	0.03	83	

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Table 17. Percentage Inhibition antioxidant activity of 2g.

Sr. No	Concentration (µg/ml)	Absorbance of Ascorbic acid	%RSA	IC_{50} ±SD
1.	50	0.082	54	26.45 ±1.97
2.	100	0.071	61	
3.	150	0.069	62	
4.	200	0.052	71	
5.	250	0.046	74	
6.	300	0.023	87	

Table 18. Percentage Inhibition antioxidant activity of 2h.

Sr. No	Concentration (µg/ml)	Absorbance of Ascorbic acid	%RSA	IC50 ±SD
1.	50	0.077	57	4.52 ±2.17
2.	100	0.07	61	
3.	150	0.067	63	
4.	200	0.06	67	
5.	250	0.037	79	
6.	300	0.03	83	

Table 19. Percentage Inhibition antioxidant activity of 3a.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (3a)	%RSA	IC_{50} ±SD
1.	50	0.088	51	46.36 ±2.06
2.	100	0.076	58	
3.	150	0.068	62	
4.	200	0.055	69	
5.	250	0.046	74	
6.	300	0.027	85	

Table 20. Percentage Inhibition antioxidant activity of 3b.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (3b)	%RSA	IC_{50} ±SD
1.	50	0.089	51	42.66 ±2.06
2.	100	0.071	61	
3.	150	0.064	64	
4.	200	0.058	68	
5.	250	0.042	77	
6.	300	0.022	88	

Table 21. Percentage Inhibition antioxidant activity of 3c.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (3c)	%RSA	IC_{50} ±SD
1.	50	0.073	59	4.62 ± 2.29
2.	100	0.069	62	
3.	150	0.056	69	
4.	200	0.042	77	

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5.	250	0.038	79
6.	300	0.008	96

Table 22. Percentage Inhibition antioxidant activity of 3d.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (3d)	%RSA	IC_{50} ±SD
1.	50	0.077	57	9.59 ±3.06
2.	100	0.068	62	
3.	150	0.055	69	
4.	200	0.04	78	
5.	250	0.035	81	
6.	300	0.01	94	

Table 23. Percentage Inhibition antioxidant activity of 3e.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (3e)	%RSA	IC_{50} ±SD
1.	50	0.092	49	45.49 ± 1.88
2.	100	0.08	56	
3.	150	0.071	61	
4.	200	0.064	64	
5.	250	0.054	70	
6.	300	0.052	71	

Table 24. Percentage Inhibition antioxidant activity of 3f.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (3f)	%RSA	IC_{50} ±SD
1.	50	0.075	58	2.8 ± 1.56
2.	100	0.066	63	
3.	150	0.056	69	
4.	200	0.04	78	
5.	250	0.033	82	
6.	300	0.01	94	

Table 25. Percentage Inhibition antioxidant activity of 3g.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (3g)	%RSA	IC_{50} ±SD
1.	50	0.084	53	22.66 ± 2.08
2.	100	0.07	61	
3.	150	0.062	66	
4.	200	0.049	73	
5.	250	0.04	78	
6.	300	0.024	87	

Table 26. Percentage Inhibition antioxidant activity of 3h.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (3h)	%RSA	IC_{50} ±SD
1.	50	0.09	50	59.52 ± 1.78
2.	100	0.079	56	
3.	150	0.071	61	

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4.	200	0.063	65
5.	250	0.049	73
6.	300	0.03	83

Table 27. Percentage Inhibition antioxidant activity of 4a.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (4a)	%RSA	IC_{50} ±SD
1.	50	0.08	56	9.6 ±1.78
2.	100	0.072	60	
3.	150	0.068	62	
4.	200	0.059	67	
5.	250	0.047	74	
6.	300	0.033	82	

Table 28. Percentage Inhibition antioxidant activity of 4b.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (4b)	%RSA	IC_{50} ±SD
1.	50	0.084	53	1.80 ±1.15
2.	100	0.068	62	
3.	150	0.06	67	
4.	200	0.053	71	
5.	250	0.045	75	
6.	300	0.033	82	

Table 29. Percentage Inhibition antioxidant activity of 4c.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (4c)	%RSA	IC_{50} ±SD
1.	50	0.09	50	71.50 ± 1.78
2.	100	0.086	52	
3.	150	0.073	59	
4.	200	0.06	67	
5.	250	0.052	71	
6.	300	0.028	84	

Table 30. Percentage Inhibition antioxidant activity of 4d.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (4d)	%RSA	IC_{50} ±SD
1.	50	0.082	54	4.66 ±2.34
2.	100	0.07	61	
3.	150	0.059	67	
4.	200	0.049	73	
5.	250	0.04	78	
6.	300	0.03	83	

Table 31. Percentage Inhibition antioxidant activity of 4e.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (4e)	%RSA	IC_{50} ±SD
1.	50	0.089	51	29.66 ±2.07
2.	100	0.07	61	

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3.	150	0.063	65
4.	200	0.05	72
5.	250	0.041	77
6.	300	0.03	83

Table 32. Percentage Inhibition antioxidant activity of 4f.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (4f)	%RSA	IC_{50} ±SD
1.	50	0.08	56	1.40 ±0.60
2.	100	0.07	61	
3.	150	0.064	64	
4.	200	0.051	72	
5.	250	0.044	76	
6.	300	0.031	83	

Table 33. Percentage Inhibition antioxidant activity of 4g.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (4g)	%RSA	IC_{50} ±SD
1.	50	0.085	53	21.78±1.96
2.	100	0.071	61	
3.	150	0.067	63	
4.	200	0.05	72	
5.	250	0.041	77	
6.	300	0.033	82	

Table 34. Percentage Inhibition antioxidant activity of 4h.

Sr. No	Concentration (µg/ml)	Absorbance of Sample (4h)	%RSA	IC_{50} ±SD
1.	50	0.088	51	68.65 ±1.54
2.	100	0.085	53	
3.	150	0.078	57	
4.	200	0.069	62	
5.	250	0.045	75	
6.	300	0.039	78	

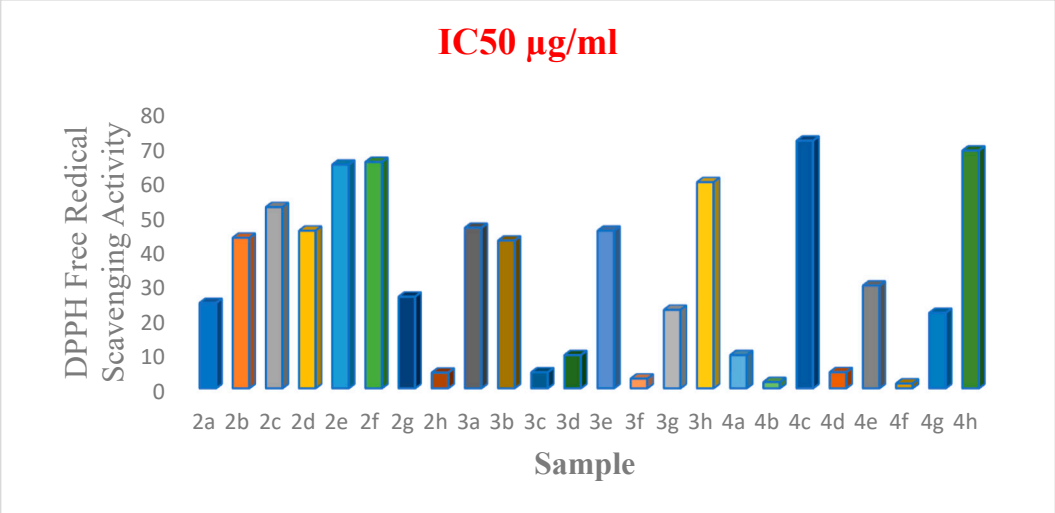

Figure 9. The IC50 values of synthesized compounds in the DPPH free radical scavenging assay.

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Structure–Activity Relationship Study of the Synthesized Compounds

The structure–activity relationship (SAR) of the synthesized Schiff bases (2a–2h) and their tetrazole (3a–3h) and thiazolidine-4-one (4a–4h) derivatives demonstrates how electronic, steric, and resonance effects govern the observed variations in reactivity and stability across the series. The parent nucleus, 1-amino-4-methyl-6-phenylpyrimidine-2-thione, contains both nitrogen and sulfur heteroatoms capable of participating in resonance delocalization, thereby stabilizing the imine (C=N) and thione (C=S) functionalities. Formation of the Schiff bases involves condensation between the amino group and substituted aromatic aldehydes, resulting in azomethine linkages (–CH=N–) whose electronic environment is significantly influenced by the nature and position of substituents on the aryl ring. Electron-withdrawing groups such as $–NO_2$ and –Cl decrease the electron density around the azomethine nitrogen, increasing electrophilicity and reducing conjugation with the aromatic π-system, whereas electron-donating substituents such as $–OCH_3$ or $–NH_2$ enhance delocalization through mesomeric (+M) effects, strengthening the resonance stabilization within the imine system.

Conversion of these Schiff bases into tetrazole analogues via azide cycloaddition introduces an additional π-conjugated five-membered ring containing four nitrogen atoms, which substantially increases the overall electron density and aromatic character of the molecule. The tetrazole ring behaves as an acidic heterocycle with delocalized π-electrons, stabilizing its conjugate base through extensive resonance. The transformation replaces the C=N linkage of the imine with an N–N–N system, extending conjugation across the pyrimidine–tetrazole backbone and enhancing aromatic stabilization energy. Infrared spectra support this conversion by the disappearance of the imine band (1580–1600 cm^{-1}) and the appearance of new absorptions in the range 1440–1490 cm^{-1} corresponding to N=N stretching. This structural change increases both planarity and electron delocalization, producing a system with improved intramolecular charge transfer characteristics.

In the thiazolidine-4-one derivatives, cyclization with thioglycolic acid introduces a new C=O group conjugated with the thione (C=S) functionality, leading to a thioamide–carbonyl resonance system. This conjugation enables partial delocalization of electron density between the sulfur and carbonyl oxygen, enhancing the stability of the heterocyclic ring. The IR bands near 1700–1725 cm^{-1} confirm the formation of the imide carbonyl group, while NMR data show deshielding effects on the methylene protons adjacent to the heteroatoms, consistent with strong inductive withdrawal by both C=O and C=S groups. The thiazolidinone ring thus introduces a polarized π-system in which the adjacent heteroatoms (N, O, and S) contribute to electron delocalization through both inductive (–I) and mesomeric (+M) interactions, resulting in increased dipole moment and chemical reactivity toward nucleophiles.

Substituent effects across all derivatives further elucidate the electronic influences governing stability and reactivity. Electron-donating substituents such as methoxy ($–OCH_3$) and amino ($–NH_2$) groups enhance mesomeric resonance and reduce the energy gap between the HOMO and LUMO, facilitating intramolecular charge transfer and increasing the compound's ability to stabilize free radicals through delocalization. In contrast, electron-withdrawing substituents like nitro ($–NO_2$) decrease electron density on the imine nitrogen, creating a more electrophilic center and favoring reactions with nucleophilic sites. The positional orientation of substituents also exerts steric influence: ortho substitution introduces torsional strain and restricts coplanarity, whereas para substitution promotes extended conjugation between the aromatic ring and heterocyclic moiety, enhancing resonance stabilization.

Molecular Interaction Study of Compounds ***2d, 3b, and 4b***

The docking studies of compounds 2d, 3b, and 4b with Penicillin-Binding Protein 4 (PDB ID: 3ZG8) were compared with the standard drug amoxicillin to evaluate their binding potential (Figure 10). Amoxicillin exhibited strong binding through multiple hydrogen bonds with Ser394, Gly393, Ser578, Thr576, and Thr579, supported by water-mediated interactions (HOH2084, HOH2066) and additional electrostatic stabilization from Lys575 and Lys397, confirming its potent affinity for PBP4. Among the synthesized ligands, compound 2d displayed hydrogen bonds with Gln391, Thr430, and

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Thr579, along with water-mediated bonding *via* Gln580 and hydrophobic stabilization through an alkyl interaction with Val432, indicating a well-defined accommodation within the binding pocket. Compound 3b demonstrated an even richer interaction profile, forming hydrogen bonds with Ser578, Gly577, and Asn451, supported by water-mediated bonds (HOH2084), π–alkyl/π–sigma interactions with Val432 and Thr589, and an attractive charge interaction with Lys575, which contributed to binding stability despite an unfavorable positive interaction. Compound 4b also demonstrated strong binding, forming hydrogen bonds with Ser394, Tyr427, and Thr430, along with additional water-mediated interactions that contributed to complex stability. Compared to the standard amoxicillin, which primarily engaged through hydrogen bonding and electrostatic interactions with key active-site residues, the synthesized compounds displayed comparable binding profiles with additional hydrophobic and π-interactions, highlighting their potential as promising antibacterial candidates targeting PBP4.

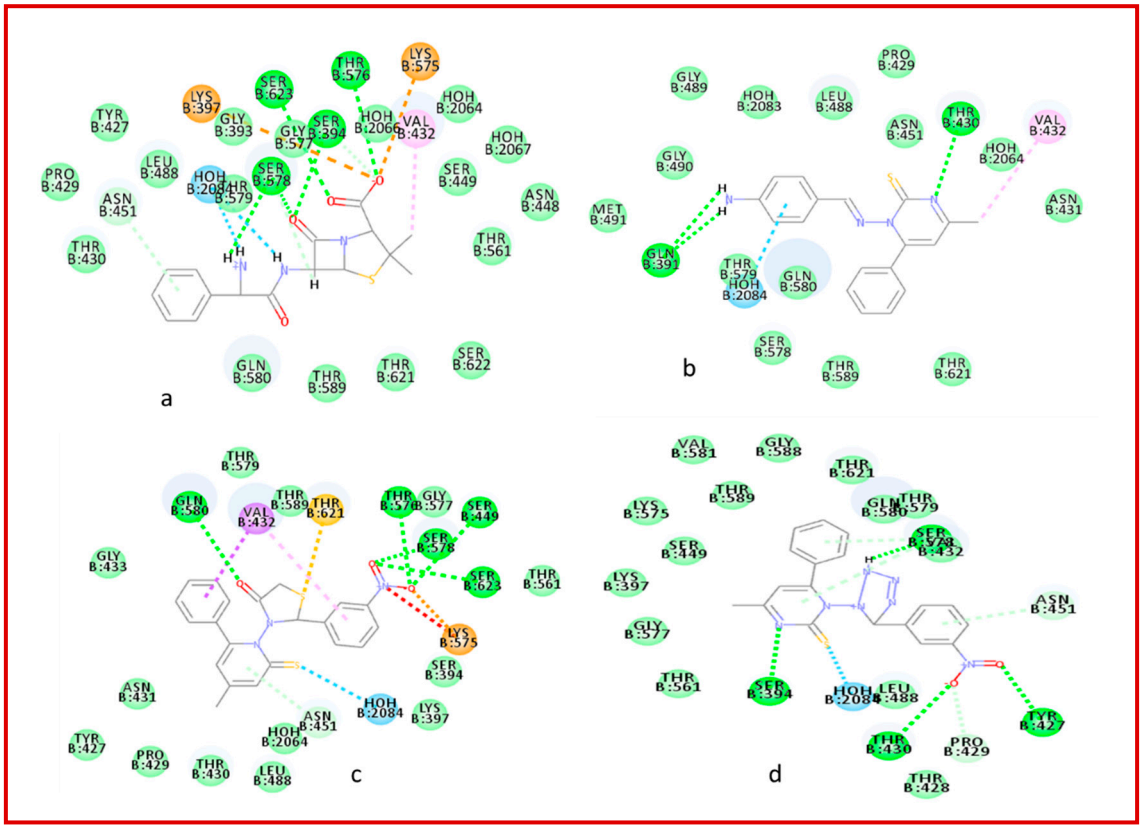

Figure 10. Molecular docking interactions of (a) amoxicillin, (b) compound 2d, (c) compound 3b, and (d) compound 4b with Penicillin-Binding Protein 4 (PBP4, PDB ID: 3ZG8), showing hydrogen bonding, water-mediated contacts, and additional hydrophobic/π-interactions, highlighted their binding potential.

Molecular Interaction Study of Compounds ***2a, 2b, 3a, and 4a*** *Standard Drug Itraconazole (PDB ID: 5V5Z)*

The molecular docking interactions of compounds 2a, 2b, 3a, and 4a with Sterol 14-α-demethylase (CYP51, PDB ID: 5V5Z) were compared with the standard drug Itraconazole (Figure 11). Among the synthesized ligands, compound 2a formed hydrogen bonds with His377 and Leu376, along with π–π stacking with Phe233 and Phe380 and π–alkyl stabilization from Leu121, Val509, and Met508. Compound 2b displayed a more diverse profile, engaging in salt bridge, π–π stacking with Phe233 and Phe380, π–sulfur contacts with Met92 and Met508, and additional hydrophobic contacts with Leu87, Leu376, Val509, and Pro375. Compound 3a exhibited multiple polar interactions, including hydrogen bonds with Gly303, Gly304, and Met308, a carbon hydrogen bond with Gly303, and amide–π stacking with Gly307, along with π–sigma and π–alkyl interactions with Ile471, Leu204, Ala146, and Gly472, although slightly offset by an unfavorable interaction with Lys143. Compound 4a also demonstrated strong binding *via* hydrogen bonds with Gly308, Gly472, and Cys470,

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complemented by π–sigma and π–alkyl contacts with Ile471, Leu300, and Ile304 and hydrophobic interactions with Leu131, Leu139, and Ala146. Overall, while itraconazole bound predominantly through hydrogen bonding and hydrophobic stabilization, the synthesized ligands exhibited diverse binding patterns, with compounds 3a and 4a in particular showing multiple hydrogen bonds and π–interactions, highlighting their potential as promising antifungal candidates.

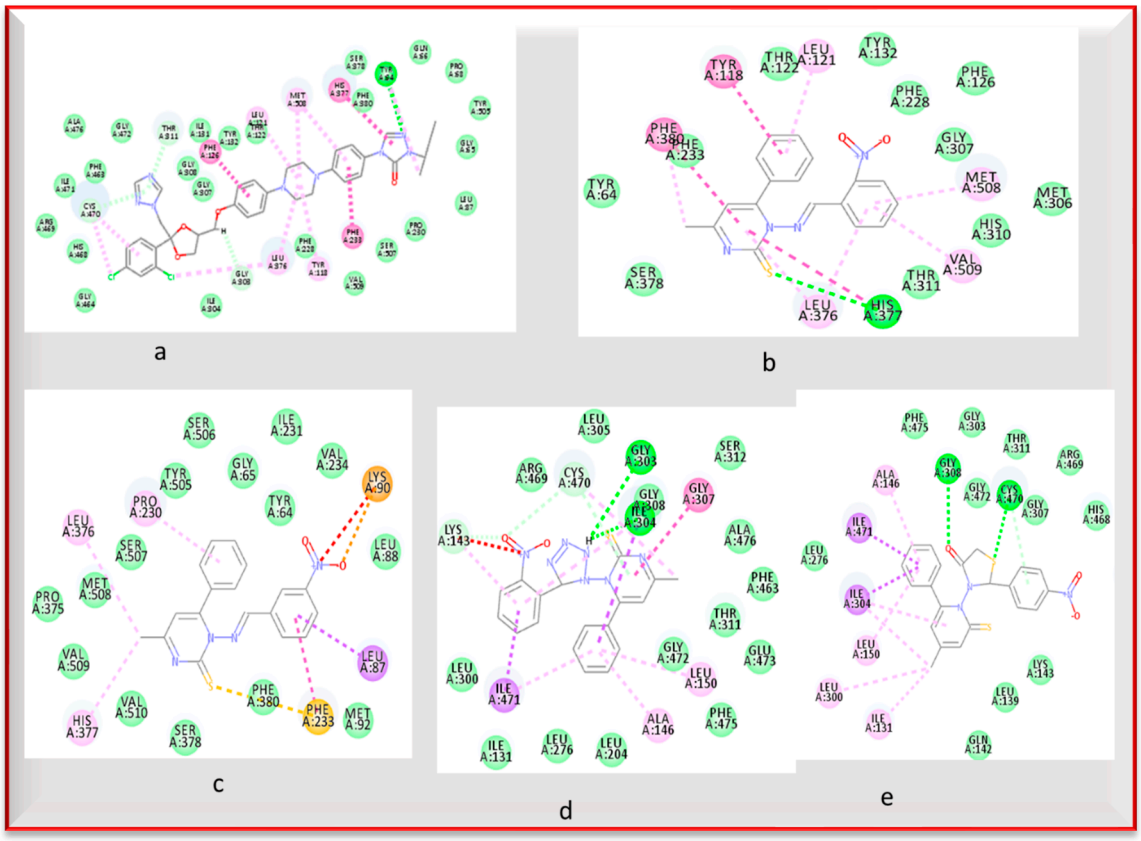

Figure 11. Molecular docking interactions of (a) itraconazole (standard), (b) compound 2a, (c) compound 2b, (d) compound 3a, and (e) compound 4a with Sterol 14-α-demethylase (CYP51, PDB ID: 5V5Z), showing hydrogen bonding, hydrophobic, and π-interactions within the binding pocket.

Molecular Interaction Study of Compounds ***2h, 3c, 3f, 4b, 4d, 4f*** *and Standard Drug Ascorbic Acid (****PDB ID: 1OAF)***

Molecular docking of synthesized compounds (2h, 3c, 3f, 4b, 4d, and 4f) against the antioxidant target 1OAF, with ascorbic acid as the reference, revealed diverse stabilizing interactions (Figure 12). Ascorbic acid primarily relied on conventional hydrogen bonds with Ser207, His163, and Ala134, whereas compound 2h showed additional π–alkyl contacts with Leu205 and Ala70, enhancing hydrophobic stabilization. Compound 3c demonstrated strong hydrogen bonding (Ser207, Ala134), π–cation interaction (Arg38), and hydrophobic π–alkyl interactions (Pro132, Ala167), while compound 3f exhibited multiple hydrogen bonds (Ser173, Ala168, His163) along with π–alkyl contacts (Leu205, Ala167) and a π–sulfur interaction with Trp179. Compound 4b displayed multifunctional binding through hydrogen bonds (Asn72, Ser69), π–cation interaction (Arg38), π–sulfur contact (His42), and π–alkyl stabilization (Leu205, Ala134, Trp179). Similarly, compound 4d engaged in hydrogen bonding (Asn72, Ser69), π–π T-shaped stacking (His163, Phe175), π–sulfur bonding (His42), and extensive hydrophobic interactions with Leu203, Ala70, and Ala168. Notably, compound 4f exhibited the most stabilized binding, characterized by strong π–π T-shaped interaction with His163, sulfur–X interactions with Ser173 and Phe175, alkyl/π–alkyl contacts with Leu205, Ala70, Ala134, Ala168, and electrostatic support from Arg38 and Arg172. Comparative analysis

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indicated that while ascorbic acid is mainly hydrogen-bond driven, the synthesized ligands incorporated additional π, alkyl, and sulfur-mediated interactions, with 4f emerging as the most promising antioxidant candidate due to its broad spectrum of stabilizing contacts.

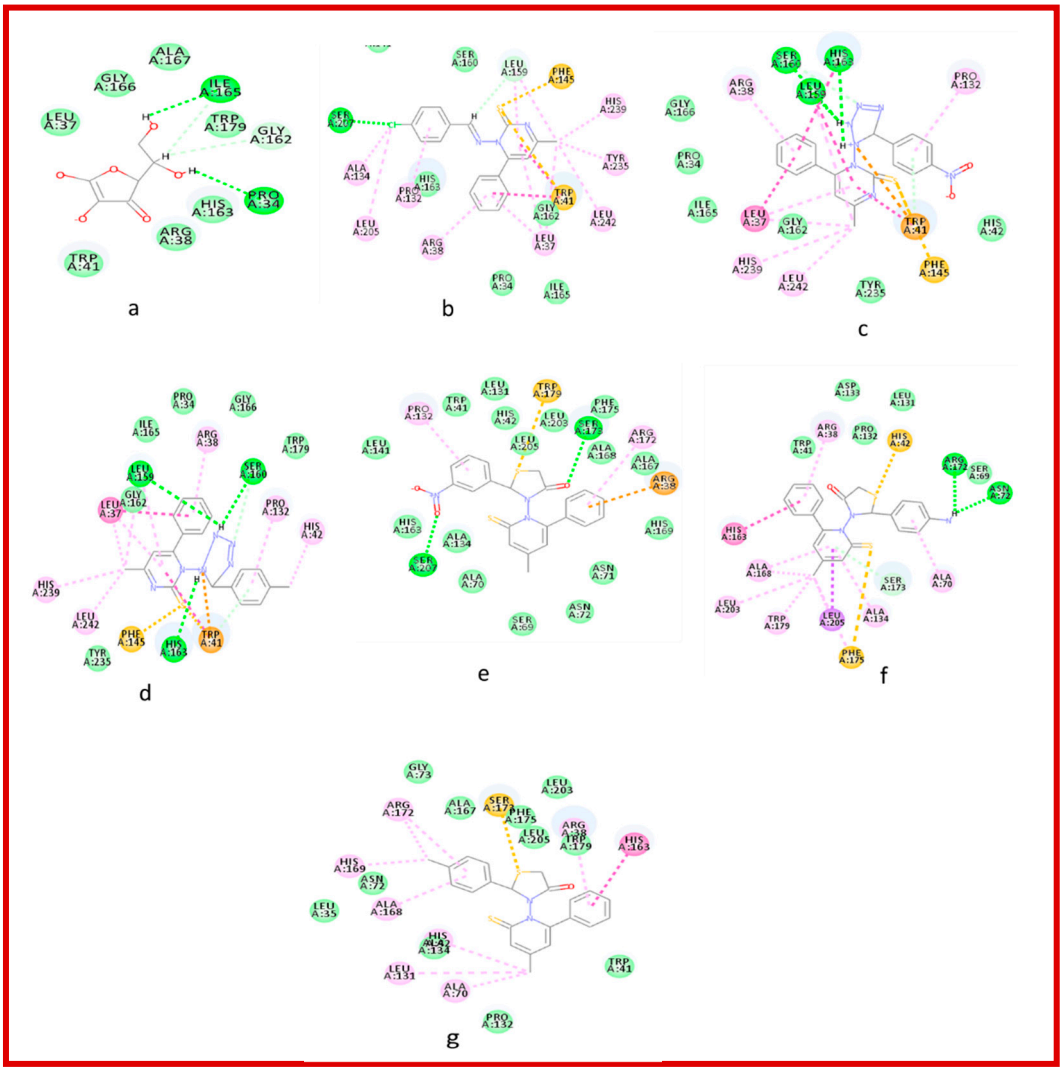

Figure 12. Molecular docking interactions of (a) ascorbic acid (standard), (b) compound 2h, (c) compound 3c, (d) compound 3f, (e) compound 4b, (f) compound 4d, and (g) compound 4f with the antioxidant target protein (PDB ID: 1OAF), showing hydrogen bonding, hydrophobic, and π-interactions that contribute to binding stability.

Conclusion

From the experiment, it was concluded that the synthesis of tetrazole and Thiazolidine-4-One were prepared safely and simply with a good product yield, with a particular emphasis on exploring their antioxidant and antimicrobial properties. Through the innovative use of tetrabutylammonium iodide (TBAI) as a catalyst in an aqueous medium, novel derivatives of Tetrazole and Thiazolidine-4-One were successfully synthesized with high product yield. The environmentally friendly nature of this approach, utilizing a non-toxic, reusable, and thermally stable catalyst in an aqueous medium instead of organic solvents, underscores its superiority in terms of sustainability and efficiency.

The synthesized compounds were thoroughly characterized using various analytical techniques, confirming their structural integrity and paving the way for the evaluation of their biological activities. The assessment of antioxidant and antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* revealed promising results, surpassing the efficacy of standard drugs such as fungigural, griseofulvin, penicillin, and ampicillin. Molecular docking studies supported the

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experimental outcomes by revealing strong binding interactions of the active compounds with key biological targets, confirming their potential therapeutic utility. Overall, these findings highlight the pharmacological potential of the synthesized compounds, positioning them as promising candidates for further exploration in drug development initiatives.

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Data availability All required data will be available with the corresponding author upon request.

Conflicts of Interest Concerning this article, the authors have no conflicts of interest.

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