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Synthesis, Molecular Docking and Antimicrobial Activity of Thiazolidine-2,4-dione Derivatives

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ABSTRACT

A novel series of thiazolidine-2,4-dione–naphthyridine hybrid derivatives was synthesized by reacting thiazolidine-2,4-dione with various naphthyridine carbaldehyde derivatives and evaluated for their *in vitro* antibacterial activity. The results revealed that all the synthesized compounds exhibited mild to good antibacterial activity against both Gram-positive and Gram-negative bacterial strains. Among them, compounds **5a** and **5e** demonstrated comparatively better antibacterial activity against the tested organisms. Compounds bearing halogen or other electron-withdrawing substituents at the C5 position of the thiazolidinedione ring displayed relatively lower antibacterial potency. Furthermore, all the synthesized compounds were subjected to molecular docking studies against dihydrofolate reductase (DHFR; PDB ID: 4DFR). The docking results indicated prominent interactions of these compounds with the active site residues of DHFR, comparable to those of the co-crystallized ligand.

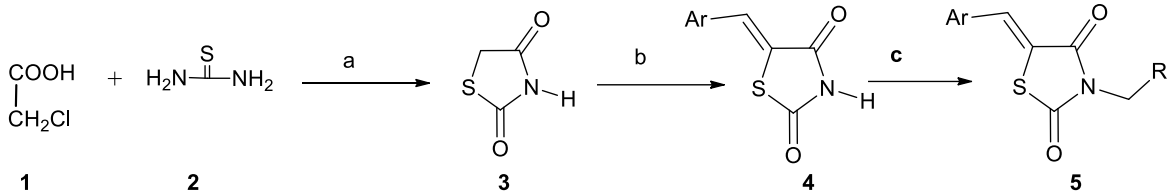
Introduction

Antibiotic resistance is a serious concern that has been recognized as one of the major health challenges of the 21st century and it is responsible for over 700,000 deaths annually around the world and it is one of the major focus for WHO[1, 2]. Antimicrobial resistance deaths are predicted to increase to above 10 million deaths per year by 2050.[3, 4] The global economic cost of such a rise in mortality and morbidity is estimated to be $100 trillion.[5] The discovery of new antibiotics are very much important because resistance will eventually develop for all currently generation antibiotics; it is only a matter of time.[6] That time can be deferred by cautious use of antibiotics, but it cannot be extended forever.

New antibacterial drug resistance mechanisms are emerging and spreading worldwide, threatening our ability to cure common infectious diseases, resulting in prolonged illness, disability, and death.[7] Without effective antibacterial drugs for prevention and treatment of various infections, medical procedures such as organ transplantation, cancer chemotherapy, diabetes and major surgery become very high

risk[8]. Antimicrobial drug resistance increases the cost of health care with prolonged stays in hospitals and more rigorous care required. The Antimicrobial drug resistance is putting the gains of the millennium development goals at risk and endangers accomplishment of the sustainable development[9].

Literature survey revealed that Thiazolidine-2,4-dione possess diverse chemotherapeutic activities such as antibacterial[10], antidiabetic[11], anti-fungal[12], anticancer[13, 14], and anticonvulsant[15]. There are very few antibacterial agents are in the pipeline, new therapeutic targets and molecules with novel mechanisms of action are needed to treat bacterial resistant strains. Hence, there is an urgent need for novel antibacterial agents which can overcome the antimicrobial resistance.



Scheme-1. Synthesis of Thiazolidine-2,4-dione Derivatives **5a-j**.

Reagents and conditions: a) Conc HCl, reflux, 12h; b) EtOH, Ar-CHO, CH_3COOH, reflux, 10 h; c) NaOH, EtOH, reflux, 24 h.

1. Chloroacetyl chloride; 2. Thiourea; 3. Thiadiazole-2,4,dione;

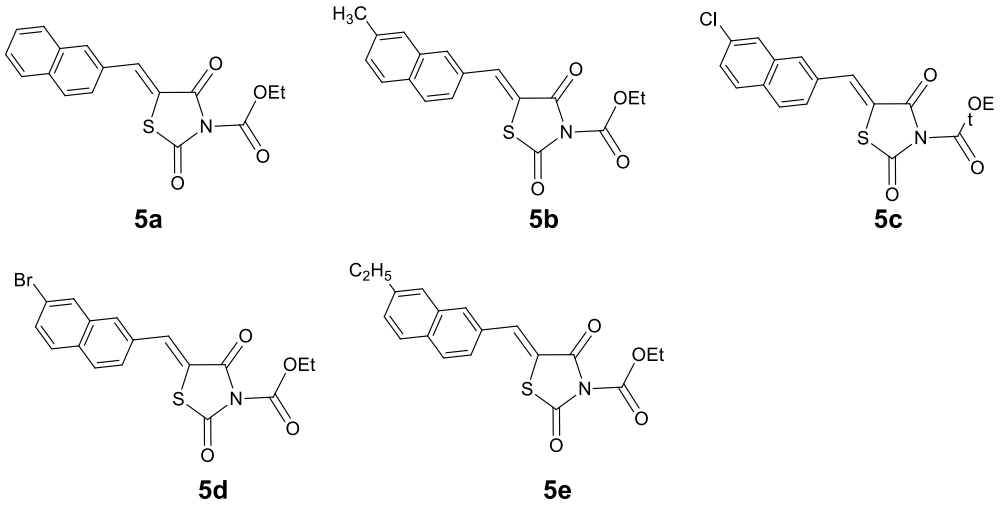


Figure-1: Molecular Library

Materials and Methods

Melting points (mp) were determined in open capillaries, using Toshniwal melting point apparatus, expressed in ^{o}C and are uncorrected. The IR spectra of the compounds were recorded on thermo Nicolet Nexus 670S series, FT-IR spectrometer using KBr disc.^{1}H NMR were recorded on a Avance-300 MHz instrument using TMS as an internal standard(chemical shifts in δ, ppm), mass spectra were recorded on a LC-MSD-Trap-SL. The purity of the compounds was checked on silica gel-coated aluminum sheets by thin-layer chromatography (TLC). TLC was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapor or by irradiation with ultraviolet light (short wave length, 254 nm). Column chromatography was performed by using Qualigen's silica gel for column chromatography (60–120 mesh).

Chemistry

Synthesis of TZD: The synthesis of TZD were carried out by the same procedure as we reported earlier from our lab.[16]

Synthesis of 5-(naphthalen-2-ylmethylene)thiazolidine-2,4-dione (4): 2-naphthaldehyde **2** and 2,4-thiazolidinedione **3** were dissolved in ethanol in a beaker. The reaction mixture was refluxed with stirring at 75 ^{o}C for 24 hr. The reaction mixture was allowed to cool at room temperature and the solid product thus formed was filtered and thoroughly washed with cold water, dried and recrystallized from ethanol

E*thyl 5-(naphthalen-2-ylmethylene)-2,4-dioxothiazolidine-3-carboxylate (5a)*

Yield: 91.4 %,; IR (KBr) ν cm^{-1}: 3094 (Ar-H), 2990 (C-H), 2885 (C-H), 1839, 1780, 1651, 1609 (C=O), 1455 (C-H), 929-878 (Ar-H); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): δ 7.93 – 7.86 (m, 3H), 7.84 (d, *J* = 8.22 Hz, 1H), 7.80 (s, 1H), 7.58 – 7.47 (m, 3H), 4.18 (q, *J* = 6.54 Hz, 2H), 1.27 (t, *J* = 6.59 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ (ppm): = 166.26, 165.75, 134.83, 133.97, 133.14, 131.64, 130.59, 129.15, 128.84, 128.15, 127.84, 127.17, 125.91, 121.08, 62.18, 42.18, 14.11.; Chemical Formula: $C_{17}H_{13}NO_4S$ (327.06).

Ethyl 5-((7-methylnaphthalen-2-yl)methylene)-2,4-dioxothiazolidine-3-carboxylate (5b): Yield: 78.48 %,; IR (KBr) ν cm^{-1}: 3096 (Ar-H), 2980 (C-H), 2891 (C-H), 1780, 1650, 1618 (C=O), 1480 (C-H), 955-807 (Ar-H); ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.87 – 7.82 (m, 1H), 7.85 – 7.78 (m, 3H), 7.62 (t, *J* = 1.97 Hz, 1H), 7.54 (dd, *J* = 8.27, 1.79 Hz, 1H), 7.39 – 7.33 (m, 1H), 4.18 (q, *J* = 6.54 Hz, 2H), 1.27 (t, *J* = 6.59 Hz, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 165.00, 164.15, 154.61, 135.77, 133.77, 133.76, 132.83, 130.46, 129.01, 128.28, 127.57, 127.43, 127.26, 127.17, 122.86, 62.19, 21.41, 14.48.. Chemical Formula: $C_{18}H_{15}NO_4S$ (341.07).

Ethyl 5-((7-chloronaphthalen-2-yl)methylene)-2,4-dioxothiazolidine-3-carboxylate (5c): Yield: 94.59 %,; IR (KBr) ν cm-1: 3109(Ar-H), 2940, 2894 (C-H), 1841, 1782, 1646, 1589 (C=O), 997-815 (Ar-H); ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.92 – 7.87 (m, 1H), 7.86 – 7.78 (m, 4H), 7.54 (dd, *J* = 8.41, 1.82 Hz, 1H), 7.36 (dd, *J* = 8.50, 2.00 Hz, 1H), 4.18 (q, *J* = 6.54 Hz, 2H), 1.27 (t, *J* = 6.59 Hz, 3H)^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 165.00, 164.15, 154.61, 134.46, 134.09, 133.51, 132.10, 130.42, 129.88, 129.44, 128.19, 127.73, 127.71,

127.49, 122.86, 62.19, 14.48. Chemical Formula $C_{17}H_{12}ClNO_4S$ (361.02).

***Ethyl 5-((7-bromonaphthalen-2-yl)methylene)-2,4-dioxothiazolidine-3-carboxylate* (5d):** Yield: 74.74 %, mp: 287-289 ˚C; IR (KBr) ν cm-1: 3105(Ar-H), 2987, 2889 (C-H), 1843, 1784, 1649, 1586 (C=O), 995-820 (Ar-H); ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.24 (t, *J* = 2.12 Hz, 1H), 7.94 (t, *J* = 1.93 Hz, 1H), 7.85 (dd, *J* = 8.89, 8.11 Hz, 1H), 7.83 – 7.78 (m, 2H), 7.63 (dd, *J* = 8.34, 1.91 Hz, 1H), 7.54 (dd, *J* = 8.27, 1.98 Hz, 1H), 4.18 (q, *J* = 6.54 Hz, 2H), 1.27 (t, *J* = 6.59 Hz, 3H ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 167.00, 166.44, 157.15, 134.36, 132.06, 130.44, 129.97, 129.51, 128.26, 127.72, 122.86, 121.03, 62.19, 14.48.. Chemical Formula: $C_{17}H_{12}BrNO_4S$ (404.97)

Ethyl 5-((7-ethylnaphthalen-2-yl)methylene)-2,4-dioxothiazolidine-3-carboxylate (5e): Yield: 86.48 %,; IR (KBr) ν cm^{-1}: 3092, 3057(Ar-H), 2984, 2994 (C-H), 1838, 1779, 1615, 15959 (C=O), 930-801 (Ar-H); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.86 (d, *J* = 2.01 Hz, 1H), 7.85 – 7.78 (m, 3H), 7.62 – 7.53 (m, 2H), 7.35 (dd, *J* = 8.46, 2.04 Hz, 1H), 4.18 (q, *J* = 6.54 Hz, 2H), 2.77 (q, *J* = 7.23 Hz, 2H), 1.30 – 1.23 (m, 6H). Chemical Formula: $C_{19}H_{17}NO_4S$ (355.09)

***In vitro* Antibacterial Activity**

Anti-bacterial activity of compounds studied by the Agar well-diffusion method[17] were tested against different bacterial pathogens such as *Bacillus Subtilis* and *Staphylococcus aureus* as gram positive, and *E. coli* as gram negative organisms. The plates were incubated at 37°C for 24 hrs and end of the experiment the diameter of the inhibition zone (mm) was measured.

Molecular Docking Study:

The crystal structure of DHFR (PDB ID: 4DFR) was downloaded from the RCSB Protein Data Bank[18]. Protein preparation included removal of co-crystallized ligands and waters, addition of polar hydrogens, assignment of appropriate charges, and conversion to PDBQT format for docking. Ligands were energy-minimized, assigned Gasteiger charges and converted into PDBQT format. Docking simulations were performed using AutoDock Vina. The grid box was centered at (X = 9.47, Y = 20.14, Z = 15.48 Å) and set to a dimension of 70 × 70 × 70 Å. An exhaustiveness parameter of 100 was used to ensure thorough sampling. Following docking, the resulting binding poses were analyzed and visualized using Discovery Studio 2016 Client to inspect ligand orientation, intermolecular interactions (H-bonds, hydrophobic contacts) and binding-site complementarity.

In silico ADMET Properties Prediction:

The drug-likeness quantitative estimation (QED) of the synthesized compounds was calculated using RDKit implemented on the UseGalaxy webserver. Molecular descriptors, including physicochemical and geometric parameters, are essential for understanding the structural characteristics that influence biological activity and pharmacokinetic behavior. In this study, the Lipinski parameters and QED scores were determined to assess the pharmacological suitability and drug-likeness of the synthesized molecules. Furthermore, toxicity predictions, including tumorigenic, mutagenic, reproductive, and irritant effects,

were evaluated using the DataWarrior software to ensure the safety profile of the compounds.[19]

Results

Chemistry

Novel TZD–naphthalene hybrids were designed and synthesized as illustrated in Scheme 1. The TZD scaffold was obtained by refluxing thiourea (**1**) with chloroacetic acid (**2**) in concentrated HCl for 12 hours to yield compound (**3**). The resulting intermediate underwent a Knoevenagel condensation with substituted naphthaldehydes in ethanol using piperidine and glacial acetic acid to provide compound (**4**). Subsequent alkylation of this intermediate with suitable alkyl halides in the presence of NaOH afforded the target compounds (**5a–5e**) in good yields. These hybrids were further evaluated for their biological potential in the present study.

Biological Evaluation

In vitro antibacterial Activity

The synthesized compounds 5a-5e were performed antibacterial activity by using *Bacillus subtilis* and *Staphylococcus aureus* which are gram positive and *E. coli* which is gram negative strain. The antibacterial activity was carried out by agar diffusion method. The standard used for the study is oxytetracycline. Nutrient agar media is used for the growth of the microorganisms. Each plates were incubated at 37^{o}C for 24 hrs. Zone of inhibition in diameter (mm) was measured for each organisms.

Table-1: in vitro Antibacterial Activity of synthesized compounds against various bacterial strains

Strain	Zone of Inhibition(mm)					
Concentration (25 µg/ml)	**5a**	**5b**	**5c**	**5d**	**5e**	**Standard**
Bacillus subtilis	24.2	17.2	14.4	12.5	22.7	28.7
S. aureus	22.1	10.2	12.3	11.5	20.9	20.7
E.coli	12.4	8.6	7.4	8.6	13.5	15.8

Among the tested compounds, **5a** showed the highest antibacterial activity across all strains, with zones of inhibition of 24.2 mm against *B. subtilis*, 22.1 mm against *S. aureus*, and 12.4 mm against *E. coli*. Compound **5e** also exhibited strong activity, particularly against *B. subtilis* (22.7 mm) and *S. aureus* (20.9 mm). Moderate to weak inhibition was observed for **5b**, **5c**, and **5d**, with *E. coli* showing the least susceptibility overall (Table-1). When compared with the standard drug (zone of inhibition ranging from 15.8–28.7 mm), compounds **5a** and **5e** displayed comparable activity, particularly against *B. subtilis* and *S. aureus*. However, all compounds showed reduced activity against *E. coli*, indicating lower efficacy toward Gram-negative bacteria.

Discussion

The results indicate that the synthesized compounds possess varying degrees of antibacterial potency, which appear to depend on their structural features. The consistently higher activity of **5a** and **5e** suggests that the presence of specific substituents in these molecules enhances their ability to penetrate bacterial cell walls or interact with essential microbial targets.

The overall lower inhibition zones observed against *E. coli* may be attributed to the outer membrane of Gram-negative bacteria, which serves as an additional permeability barrier, thereby reducing the uptake of antibacterial agents. In contrast, Gram-positive strains such as *B. subtilis* and *S. aureus* were more susceptible to these compounds. In summary, compounds **5a** and **5e** demonstrated promising antibacterial potential, comparable in some cases to the standard drug, and could serve as lead candidates for further optimization and structure–activity relationship (SAR) studies.

Molecular Docking study

The molecular docking studies were carried out for all the synthesized compounds against Dihydro Folate Reductase (DHFR) PDB ID: 4DFR. The docking simulation were carried out by using Pymol software with Dockpie plugin with Autodock vina configuration. Initially, we have validated the docking with extracted cocrystal ligand and performed redocking into the active site. The redocked pose exhibits same orientation and found to occupy the same binding site of DHFR as shown in Figure-1. The cocrystal ligand exhibits hydrogen bond interactions with Arg52, Arg57, Asp27, Ile5 and Ile94 residues. The hydrophobic interactions with Phe31, Ile50, Ile5, Ala74 residues with dock score value of -6.57 kcal/mol (Table-2).

Similarly, among the synthesized compounds **5a** and **5e** have shown good dock score values -8.02 and -7.71 kcal/mol respectively. Compounds **5a** and **5e** exhibits hydrogen bond interactions with Arg52 and hydrophobic interactions with Ile5, Trp30, Phe31 and Ala7 residues were observed, seen in Figure 2&3.

Table-2: Molecular Docking study of TZD analogues against DHFR

Compound	Dock Score
5a	-8.02
5b	-7.51
5c	-7.29
5d	-6.94
5e	-7.71

Cocrystal Ligand	-6.57

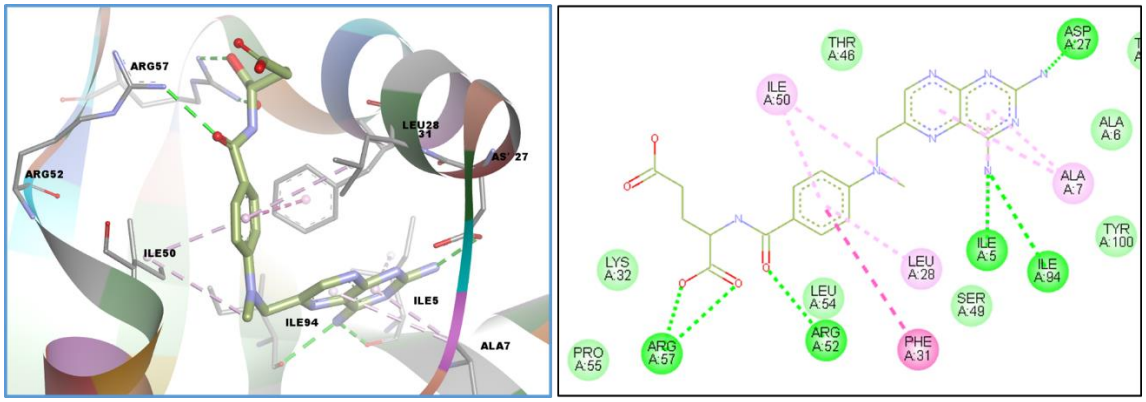


Figure 1. Binding Interactions and orientation of Methotrexate in the active site of DHFR (PDB ID: 4DFR)

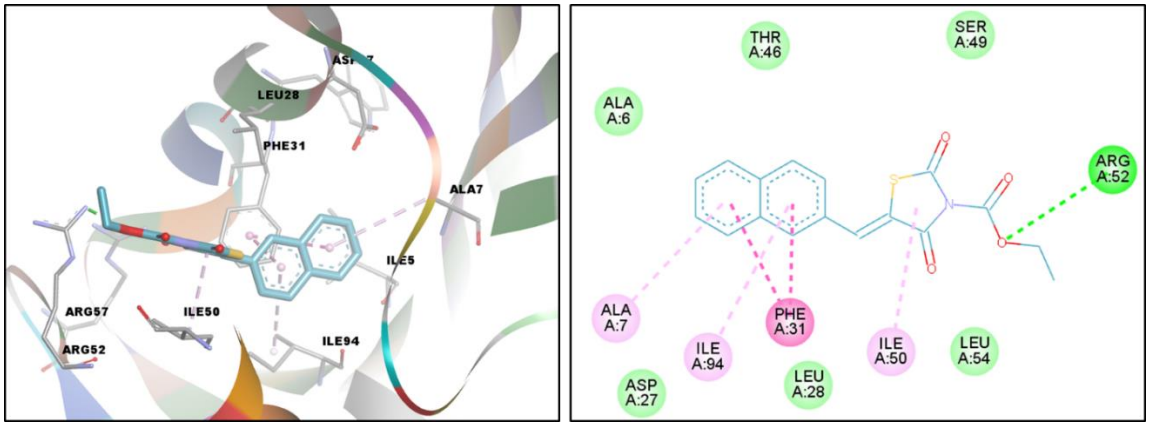


Figure 2. Interactions and orientation of **5a** in the active site of DHFR (PDB ID:4DFR)

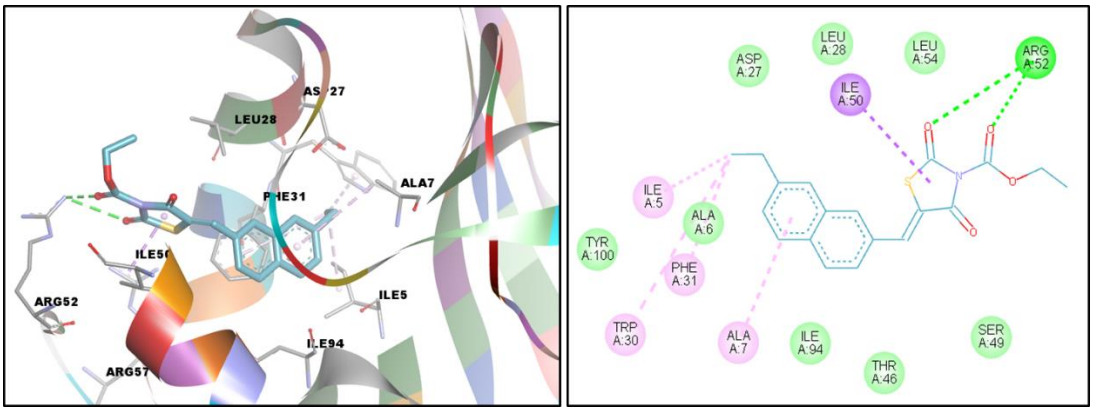


Figure 3. Binding Interactions and orientation of **5e** in the active site of DHFR (PDB ID:4DFR)

Drug-likeness quantitative estimation (QED) with RDKit:

The quantitative estimation of drug-likeness (QED) utilizing RDKit was carried out within the Galaxy Web Server. The physicochemical and drug-likeness parameters of the synthesized compounds (5a–5e) were evaluated using in silico analysis (Table 3). The molecular weight (MW) of all compounds ranged from 327.36 to 406.26 g/mol, which is within the acceptable limit of Lipinski's rule of five (≤500 g/mol), suggesting favorable drug-like properties. The ALOGP values varied between 4.03 and 4.79, indicating moderate lipophilicity suitable for membrane permeability without compromising solubility. All compounds showed five hydrogen bond acceptors (HBA) and no hydrogen bond donors (HBD), maintaining a balanced polarity profile. The polar surface area (PSA) remained constant at 63.68 Å^2 across the series, well below the threshold of 140 Å^2, implying good potential for oral bioavailability and passive membrane diffusion. The number of rotatable bonds (ROTB) ranged from 2 to 3, indicating limited conformational flexibility, which can favor binding stability with the target protein. Each compound possessed two aromatic rings (AROM = 2) and exhibited one structural alert, possibly due to the presence of reactive or conjugated moieties. However, the Lipinski's rule of five (LRo5) violations were zero for all compounds, confirming their compliance with major drug-likeness criteria. The quantitative estimate of drug-likeness (QED) values ranged from 0.638 to 0.734, where compound 5a showed the highest QED score (0.734), followed by 5b (0.716) and 5e (0.712). Compound 5d exhibited the lowest QED (0.638), primarily attributed to its higher molecular weight and lipophilicity. Overall, these findings suggest that compounds 5a–5c possess optimal physicochemical properties and superior drug-likeness profiles, making them promising candidates for further biological evaluation.

Table 3 presents the values of eight molecular descriptors, along with the QED score and the number of Lipinski's rules satisfied by each molecule.

Compound	MW	ALOGP	HBA	HBD	PSA	ROTB	AROM	ALERTS	LRo5	QED
5a	327.36	4.033	5	0	63.68	2	2	1	0	0.734
5b	341.39	4.341	5	0	63.68	2	2	1	0	0.716
5c	361.81	4.686	5	0	63.68	2	2	1	0	0.688
5d	406.26	4.795	5	0	63.68	2	2	1	0	0.638
5e	355.42	4.595	5	0	63.68	3	2	1	0	0.712

***In silico* Toxicity**:

The in silico toxicity assessment of compounds **5a–5e** was performed to evaluate their potential safety profiles (Table 4). All compounds were found to be non-tumorigenic, non-mutagenic, non-reproductive toxic, and non-irritant, indicating a favorable toxicological profile. The absence of tumorigenic and mutagenic effects suggests that these molecules do not possess functional groups or structural alerts typically associated with DNA damage or carcinogenic potential. Similarly, the lack of reproductive toxicity indicates that the compounds are unlikely to interfere with hormonal or developmental processes. The non-irritant nature of all derivatives further supports their safety for potential therapeutic use. Overall, these results demonstrate that compounds 5a–5e are predicted to be safe and well-tolerated, meeting key preliminary toxicity requirements for drug-like molecules and thus suitable for further pharmacological and biological evaluation.

Table-4: Toxicity data of synthesized TZD compounds

Compound	Tumorigenic	Mutagenic	Reproductive Effective	Irritant
5a	none	none	none	none
5b	none	none	none	none
5c	none	none	none	none
5d	none	none	none	none
5e	none	none	none	none

Conclusion

The present study involves the synthesis of TZD–naphthalene hybrids, as depicted in Scheme 1. All the final compounds were obtained in good yields and further purified using column chromatography. The compounds were characterized by NMR spectral analysis. All compounds were evaluated for antibacterial activity against *S. aureus* and *B. subtilis* as Gram-positive organisms, and *E. coli* as a Gram-negative organism. The compounds exhibited mild to moderate zones of inhibition. Furthermore, molecular docking studies were performed against DHFR, and the compounds showed significant interactions with the active site of DHFR. These compounds could serve as potential chemotherapeutic agents, and future studies will focus on exploring their mechanism of action.

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