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Synthesis, docking and ADMET prediction of a new class of benzenesulfonate (BZS) clubbed 2, 4-thiazolidinedione (TZD) compounds as potent anticancer agents

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Abstract

The series of substituted (E)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-3-methoxyphenyl benzenesulfonate derivatives (BA-1 to BA-10) were synthesised using benzenesulfonate (BZS) (Int-1) and 2,4-thiazolidinedione (TZD) (Int-2) as intermediates. The structures of twenty-six new compounds were identified by using elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, and MS spectral data. The antineoplastic potential of synthesised compounds was investigated using Autodock 4.2 molecular docking studies with the human 5,10-Methenyltetrahydrofolate Synthetase enzyme (PDB ID: 3hy6) to determine receptor-ligand interactions. The ADMET prediction was investigated using Swiss ADME. Considering the docking scores and binding energies obtained from docking studies, it has been shown that BA-05 is an ideal candidate for further studies of its anticancer properties. The ADMET study suggests that further modifications are needed to get the ideal lead for future studies.

Keywords: Anticancer; Benzenesulfonate; Docking; ADMET; Thiazolidiones

Introduction

Thiazolidine2, 4-diones (TZD) are five-membered heterocyclic compounds with numerous industrial applications. TZD compounds are highly useful as analytical reagents and potential electroplating agents. TZD derivatives are important heterocyclic compounds that possess a variety of pharmacological properties such as antidiabetic [2, 3], anticancer [1], antimicrobial [9, 17], antitubercular [17], etc. on the other hand benezenesulfonate (BZS) also known for its vide array of medicinal properties including anticancer [8, 13, 14], antimicrobial [7, 21], antitubercular [15], antiviral [21], insecticidal [20], etc.

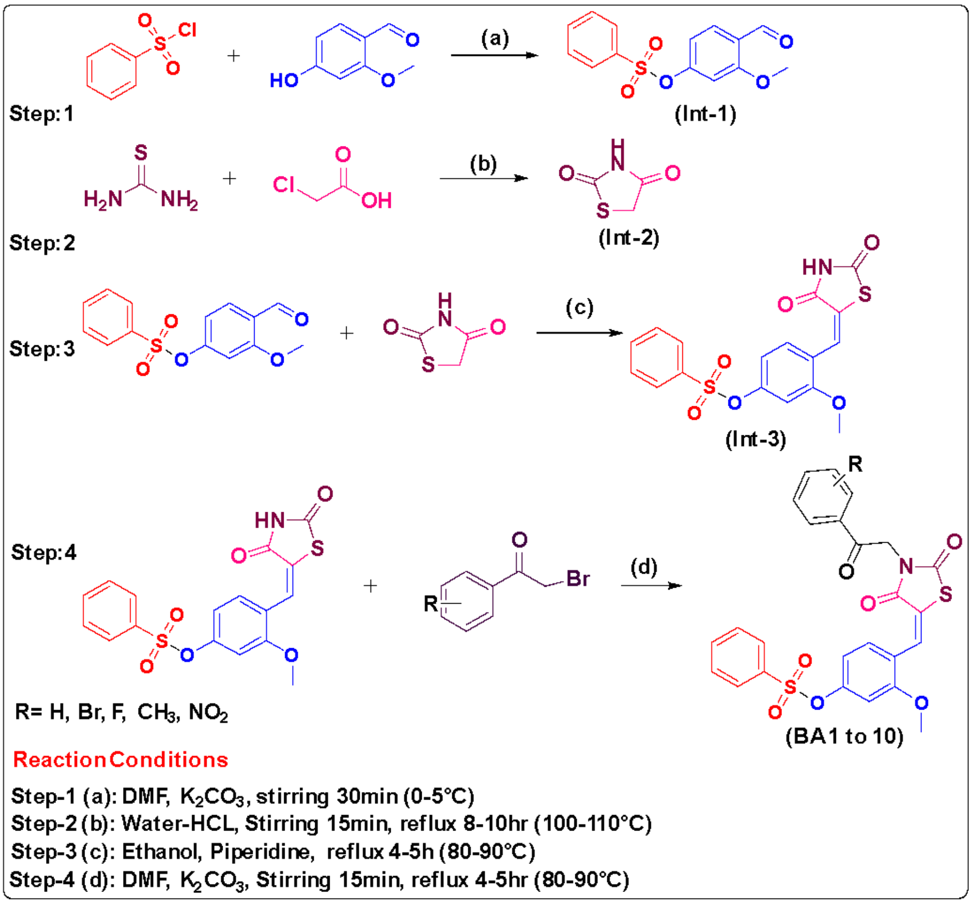
Cancer is the leading cause of death across the globe. According to GLOBOCAN-2020, worldwide estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths occurred in 2020 [16]. The number of cases and deaths is increasing day by day. In the normal mammalian cell, folates are not synthesized and they are supplemented as vitamins. In cell biology, folate is transformed into tetrahydrofolate (H_4folate) by Human Methenyltetrahydrofolate Synthetase (MTHFS) enzyme (PDB ID: 3hy6) in mitochondria. Transformed H_2folate acts as a coenzyme for the transfer, oxidation and reduction of single carbon units used for the biosynthesis of thymidylate, purines, methionine, serine, glycine and many other compounds [5, 6]. MTHFS activity in mitochondria is also required for the reintroduction of mitochondrial 5-formyltetrahydrofolate into mitochondrial folate pools. MTHFS inhibition resulted in blockage of the reentry of 5-formultetrahydrofolate and may be used as a potential target for the development of anticancer drugs.

The design, synthesis, and development of molecules having therapeutic value for humans are one of the primary aims of organic and medicinal chemistry. Recent research focused on the development of either TZD or BZS molecules as potential candidates for anticancer drugs. However, to the best of our knowledge, no researcher has combined both molecules and studied their anti-cancer activity. Our hypothesis says that combining TZD and BZS will make them work better together and could be a good candidate for a future drug to treat cancer.

Materials and Methods

Chemistry

All chemical, solvents, and reagents used in current experiments were analytical grade and purchased from local vendor. Monitoring of reaction was carried out with silica gel GF254 (Merck) TLC plates. Melting points were noted with the open glass capillary method. FT-IR spectra were recorded on a Shimadzu-8200 infinity IR spectrophotometer with the KBr pellet method, ^{1}H, and ^{13}C NMR spectra were obtained on a Bruker Avance-III 400MHz spectrometer, and TMS was used as an internal reference. Mass (EI) spectra were recorded on the Shimadzu QP-2010 mass spectrometer. General reaction scheme of proposed molecules was depicted in figure 1.



R= H, Br, F, CH_3, NO_2

Reaction Conditions

Step-1 (a): DMF, K_2CO_3, stirring 30min (0-5°C)
Step-2 (b): Water-HCL, Stirring 15min, reflux 8-10hr (100-110°C)
Step-3 (c): Ethanol, Piperidine, reflux 4-5h (80-90°C)
Step-4 (d): DMF, K_2CO_3, Stirring 15min, reflux 4-5hr (80-90°C)

Figure: 1 General reaction scheme

Synthesis of 4-formyl-3-methoxyphenyl benzenesulfonate. (Int-1)

In a round bottom flask charged with 4-hydroxy-2-methoxybenzaldehyde (1 eq.) and potassium carbonate (3 eq.), using DMF as a solvent, continue stirring for 30 min in an ice bath (5–10 °C). Following this, benzene sulfonyl chloride was added dropwise. The reaction mixture allowed for stirring and was monitored with TLC. After the completion of the reaction, the reaction mass was poured into crushed ice. To obtain a solid product, it was necessary to dry it under vacuum and recrystallize it with ethanol.

Synthesis of thiazolidine-2, 4-diones (Int-2)

Chloroacetic acid (0.1 mmol) in 10 ml of water and thiourea (0.1 mmol) dissolved in 10 ml of water: both solutions were mixed and stirred for 15 minutes until a white precipitate was obtained, then cooled. After that, 10 ml of hydrochloric acid, was added slowly in a reaction mixture with a dropping funnel. The flask was then connected to a reflux condenser, and gentle heat was applied. After that, the reaction mixture was stirred and refluxed for 8–10 h at 100–110 °C. The product was cooled, faltered, washed, and dried at room temperature, followed by recrystallization with a suitable solvent [19].

Synthesis of N′-(Substituted benzaldehyde)-2,4-thiazolidinedione (Int-3)

The solution of 2,4-thiazolidinedione (Int-2) (0.01 mmol) and substituted benzaldehyde (0.01 mmol) was suspended in ethanol with a catalytic amount of piperidine, and the mixture was shaken well for a few minutes and then refluxed at 80–90 °C for 4–5 h. TLC was used to monitor the progress of the reaction after the reaction mixture had been cooled to room temperature. The product precipitated out of ethanol, was separated by using a separating funnel, and dried under vacuum, followed by recrystallization with a suitable solvent.

Synthesis of substituted (E)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)-3-methoxyphenyl benzenesulfonate derivatives (BA-01 to BA-10)

Take dimethylformamide (DMF) as a solvent in a round-necked flask. After adding N′-(substituted benzaldehyde)2,4-thiazolidinedione (Int-3) (1 eq.) to the flask, add Potassium Carbonate (3 eq.) and shake well for 15 minutes after adding substituted phenacyl bromide (1 eq.) to the round bottom flask. After stirring the reaction mixture for 4-5 hours at a reflux temperature of 80-90°C and checking the reaction progression with TLC, the reaction mass was poured into ice, filtered, dried over the vacuum, washed with hexane, and recrystallized from ethanol.

In silico evaluation

Ligand preparation

All of the compounds in this study had their 3D structures drawn up in Chemdraw Ultra 12.0 and Chem3D Pro 12.0, and the resulting files were saved in the SDF format for easy analysis. Avogadro software was used to optimize the structures of all ligands before molecular docking was performed.

Receptor preparation

The RCSB Protein Data Bank (https://www.rcsb.org/) allowed us to retrieve the structure of 5,10-methenyltetrahydrofolate synthetase (PDB ID: 3HY6) in PDB format. PyMOL 2.5 was utilised for the protein's visual representation. Protein molecules had their water, ion, and ligand molecules removed by autodocktools 1.5.7. After that, AutoDock Tools 1.5.7 was used to give the receptors hydrogen atoms and electric charges. After making modifications to the protein structure, the results were recorded in Protein Data Bank, Partial Charge (Q), and Atom Type (T) formats for further study.

Molecular docking

Following the preparation of the ligand and the receptor, a molecular docking study was performed using AutoDock tools 1.5.7 and AutoDock 4 to ascertain the binding affinities and hydrogen bond interactions [10, 18, 19]. The grid box covering the entire protein was set up at X = 10.006, Y = 18.838, and Z = -0.125 for blind docking. Docking results were compared between the reference compound and the phytochemicals at the same protein binding sites using the same sized grid boxes to determine binding affinities and interactions. The binding energy (in kcal/mole) is presented for the docking results.

In silico pharmacokinetic study

The synthesised compounds' pharmacological and druglikeness evaluation was performed by uploading the ligand file in SDF format or canonical SMILES format to the Swiss ADME. The Swiss ADME predicts ADME parameters, druglikeness properties, lipophilicity, water solubility, and pharmacokinetic properties, and investigates the medicinal chemistry friendliness of one or more small molecules to aid in drug discovery. If ADME is estimated early in the discovery process, a lot of the loss caused by pharmacokinetics can be avoided in the clinical stages.

In silico Toxicity Prediction

With the help of the online tool pkCSM, the toxicological profiles of the synthesised compounds could be predicted [12]. It is one of the computer programmes used to predict whether a given molecule is toxic to humans or not. Maximum hepatotoxicity, AMES toxicity, hERG I and II inhibitors tolerable upper intake level, skin sensitization, toxicity to minnows, toxicity to rats when orally administered like lowest observed adverse-effect level (LOAEL), toxicity to rats when orally administered (LD50), and toxicity to Tetrahymena pyriformis are all testable.

3D and 2D visualization

Discovery Studio v21.1.0.20298 was used to analyze the 3D and 2D hydrogen-bond interactions of the complex receptor-ligand structure to determine which amino acid of the receptor interacted with the ligand.

Result and Discussion

Physical properties of the compounds are well described in table 1.

Table 1: Physical Properties of Synthesized derivatives

Code	R-	M.F.	M.W.	M.P. °C	Yield %	R_f
BA-1	H	$C_{25}H_{19}NO_7S_2$	509	168-170	88	0.55
BA-2	4-Br	$C_{25}H_{18}BrNO_7S_2$	587	155-157	72	0.51
BA-3	4-F	$C_{25}H_{18}FNO_7S_2$	527	172-174	83	0.61
BA-4	4-Me	$C_{26}H_{21}NO_7S_2$	523	138-140	77	0.57
BA-5	4-NO_2	$C_{25}H_{18}N_2O_9S_2$	554	186-189	74	0.48
BA-6	4-Cl	$C_{25}H_{18}ClNO_7S_2$	543	128-130	85	0.60
BA-7	3-F	$C_{25}H_{18}FNO_7S_2$	527	146-148	78	0.52
BA-8	3-Cl	$C_{25}H_{18}ClNO_7S_2$	544	142-144	80	0.62
BA-9	3-Br	$C_{25}H_{18}BrNO_7S_2$	588	158-160	68	0.50
BA-10	3-NO_2	$C_{25}H_{18}N_2O_9S_2$	554	142-144	76	0.56

Spectral Discussion of synthesized compounds (BA 1 to 10)

BA-1(H) (E)-4-((2,4-dioxo-3-(2-oxo-2-phenylethyl)thiazolidin-5-ylidene)methyl)-3-methoxyphenyl benzenesulfonate: **Colour:** Off-white**, Yield: 88%, MS (m/z)=** 509, **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 8.067 – 8.015 (m, 3H), 7.927 (s, 1H), 7.823 – 7.731 (m, 3H), 7.709 – 7.678 (m, 2H), 7.647-7.628 (d, *J* = 7.6 Hz, 1H), 7.548 – 7.509 (m, 3H), 7.054 (s, 1H), 5.742 (s, 1H), 3.882 (s, 3H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 188.05, 167.25, 164.62, 161.55, 151.05, 137.95, 135.10, 132.75, 131.31, 128.17, 125.07, 123.10, 119.55, 116.69, 105.02, 54.21, 47.05. **FT-IR (KBr, (υmax) cm^{-1})** 3595.43, 3333.1, 3078.49, 2947.33, 2576.98, 1905.73, 1689.7, 1589.4, 1504.53, 1381.08, 1280.78, 1188.19, 1087.89, 1018.45, 848.71, 748.41, 686.68, 547.8.

BA-2(4-Br)- (E)-4-((3-(2-(4-bromophenyl)-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)-3-methoxyphenyl benzenesulfonate: **Colour:** Cream Yellow**, Yield:72%, MS (m/z)**=587**, ^{1}H NMR (400 MHz, DMSO-*d*$_6$) ^{1}H NMR (400 MHz, DMSO-*d*$_6$)** 8.368-8.348 (d, *J* = 8 Hz, 2H), 7.923 (s, 1H), 7.902-7.864 (t, *J* = 7.6 Hz, 3H), 7.723-7.692 (m, 3H), 7.457 (s, 1H), 7.344-7.323 (m, 1H), 7.253-7.227 (m, 2H), 5.347 (s, 2H), 3.581 (s, 3H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 186.19, 164.37, 162.10, 152.76, 138.34, 136.45, 133.97, 133.13, 132.42, 131.07, 128.25, 127.84, 126.17, 124.44, 121.05, 121.95, 114.50, 54.28, 46.12. **FT-IR (KBr, (υmax) cm^{-1})** 3595.43, 3333.1, 3078.49, 2947.33, 2576.98, 1905.73, 1689.7, 1589.4, 1504.53, 1381.08, 1280.78, 1188.19, 1087.89, 1018.45, 848.71, 748.41, 686.68, 547.8.

BA-3(4-F)- (E)-4-((3-(2-(4-fluorophenyl)-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)-3-methoxyphenyl benzenesulfonate: **Colour:** light Cream**, Yield: 83 %, MS (m/z)**=527**, ^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 8.201 (s, 2H), 7.995 (s, 1H), 7.888-7.870 (d, *J*= 7.2 Hz, 3H), 7.716-7.698 (d, *J*= 7.2 Hz, 2H), 7.481-7.440 (t, *J*= 8 Hz, 2H), 7.408 (s, 1H), 7.347-7.326 (d, *J* = 8.4 Hz, 1H), 7.282-7.263 (d, *J* = 7.6 Hz, 1H), 5.359 (s, 2H), 3.571 (s, 3H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 190.25, 169.91, 167.12, 165.50, 162.10, 153.30, 140.65, 136.91, 133.38, 130.97, 129.17, 125.17, 120.39, 116.52, 114.20, 104.10, 54.21, 48.30. **FT-IR (KBr, (υmax) cm^{-1})** 3610.86, 3387.11, 3078.49, 2939.61, 2600.13, 2522.98, 2445.82, 1944.31, 1689.7, 1597.11, 1504.53, 1381.08, 1303.92, 1219.05, 1095.6, 1018.45, 848.71, 748.41, 547.8, 462.93.

BA-4(4-ME)- (E)-4-((2,4-dioxo-3-(2-oxo-2-(p-tolyl)ethyl)thiazolidin-5-ylidene)methyl)-3-methoxyphenyl benzenesulfonate: **Colour:** Cream**, Yield: 83 %, MS (m/z) =** 527, **^{1}H NMR (400 MHz, DMSO)** δ 8.154-8.131 (d, *J* = 9.2 Hz, 2H), 7.943 – 7.801 (m, 3H), 7.695-7.655 (t, *J* = 8.0 Hz, 3H), 7.464 – 7.217 (m, 5H), 5.356 (s, 2H), 3.516 (s, 3H), 2.465 (s, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 190.60, 165.93, 151.66, 145.14, 138.67, 134.94, 132.91, 131.26, 129.52, 128.27, 124.43, 121.99, 115.56, 55.78, 50.78, 45.65, 21.25. **FT-IR (KBr, (υmax) cm^{-1})** 3641.73, 3055.35, 2947.33, 2854.74, 2584.7, 1967.46, 1905.73, 1689.7, 1604.83, 1496.81, 1388.79, 1303.92, 1180.47, 1103.32, 1026.16, 848.71, 748.41, 563.23, 462.93.

BA-5(4-NO$_2$)- (E)-3-methoxy-4-((3-(2-(4-nitrophenyl)-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzenesulfonate: **Colour:** Reddish**, Yield: 74 %, MS(m/z)=** 554, **^{1}H NMR (400 MHz, DMSO)** δ 8.430 – 8.335 (m, 3H), 8.002 (s, 1H), 7.869 (m, 3H), 7.699 (m, 3H), 7.408 (s, 1H), 7.328-7.285 (m, 2H), 5.459 (s, 2H), 3.571 (s, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 190.18, 169.66, 167.26, 158.60, 152.24, 136.05, 134.46, 133.49, 133.13, 129.97, 129.19, 128.38, 126.37, 123.63, 118.55, 117.69, 104.02, 54.91, 48.05. **FT-IR (KBr, (υmax) cm^{-1})** 3618.58, 3348.54, 3078.49, 2939.61, 2862.46, 1959.74, 1689.7, 1519.96, 1365.65, 1195.91, 1103.32, 1018.45, 856.42, 748.41, 694.4, 586.38.

BA-6(4-Cl)- (E)-4-((3-(2-(4-chlorophenyl)-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)-3-methoxyphenyl benzenesulfonate: **Colour:** Cream yellow**, Yield: 85 %, MS(m/z)=** 543, **^{1}H NMR (400 MHz, DMSO-d_6)** δ 8.128-8.107 (d, *J* = 8.4 Hz, 2H), 7.988 (s, 1H), 7.887-7.849 (t, *J* = 7.6 Hz, 3H), 7.704-7.684 (m, 3H), 7.398 (s, 1H), 7.344-7.322 (d, *J* = 8.6 Hz, 1H), 7.278-7.231 (m, 2H), 5.352 (s, 2H), 3.574 (s, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 190.49, 166.77, 165.01, 151.67, 139.43, 138.69, 134.97, 134.94, 133.13, 132.77, 132.42, 130.27, 129.52, 129.48, 129.17, 128.16, 124.44, 122.00, 121.96, 115.60, 55.82, 47.92. **FT-IR (KBr, (υmax) cm^{-1})** 3618.58, 3371.68, 3078.49, 2939.61, 2862.46, 2584.7, 1913.45, 1689.7, 1581.68, 1504.53, 1381.08, 1296.21, 1195.91, 1095.6, 1018.45, 848.71, 748.41, 540.09, 462.93.

BA-07(3-F)- (E)-4-((3-(2-(3-fluorophenyl)-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)-3-methoxyphenyl benzenesulfonate: **Colour:** Light Cream**, Yield: 78%, MS(m/z)=** 527, **^{1}H NMR (400 MHz, DMSO-d_6)** δ 8.126 (s, 1H), 7.974 (s, 1H), 7.862 – 7.624 (m, 2H), 7.585–7.480 (m, 2H), 7.344 (m, 3H), 7.058 (m, 2H), 6.745-6.725 (d, *J* = 8 Hz, 2H), 5.675 (s, 2H), 3.924 (s, 3H).**^{13}C NMR (101 MHz, DMSO-d_6)** δ 192.50, 168.66, 163.62, 160.60, 152.24, 136.05, 133.49, 130.39, 129.97, 128.31, 126.04, 125.37, 121.63, 119.55, 116.69, 115.94, 105.02, 55.91, 48.68. **FT-IR (KBr, (υmax) cm^{-1})** 3610.86, 3387.11, 3078.49, 2939.61, 2600.13, 2522.98, 2445.82, 1944.31, 1689.7, 1597.11, 1504.53, 1381.08, 1303.92, 1219.05, 1095.6, 1018.45, 848.71, 748.41, 547.8, 462.93.

BA-08(3-Cl)- (E)-4-((3-(2-(3-chlorophenyl)-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)-3-methoxyphenyl benzenesulfonate: **Colour:** Cream yellow**, Yield: 80%, MS(m/z)=** 544, **^{1}H NMR (400 MHz, DMSO-d_6)** δ 8.327 (s, 2H), 7.956 (s, 1H), 7.894-7.883 (m, 3H), 7.763-7.744 (d, *J*= 7.6 Hz, 2H), 7.479-7.458 (m, 2H), 7.408 (s, 1H), 7.356-7.337 (d, *J* = 7.6 Hz, 1H), 7.265 (s, 1H), 5.352 (s, 2H), 3.215 (s, 3H).**^{13}C NMR (101 MHz, DMSO-d_6)** δ 189.21, 167.63, 166.16, 160.60, 152.24, 136.05, 134.45, 133.49, 131.66, 129.97, 128.25, 125.37, 121.63, 119.55, 115.55, 104.02, 55.91, 49.94. **FT-IR (KBr, (υmax) cm^{-1})** 3618.58, 3371.68, 3078.49, 2939.61, 2862.46, 2584.7, 1913.45, 1689.7, 1581.68, 1504.53, 1381.08, 1296.21, 1195.91, 1095.6, 1018.45, 848.71, 748.41, 540.09, 462.93.

BA-09(3-Br)- (E)-4-((3-(2-(3-bromophenyl)-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)-3-methoxyphenyl benzenesulfonate: **Colour:** Cream yellow, **Yield: 68%, MS(m/z)=** 588, **^{1}H NMR (400 MHz, DMSO-d_6)** δ 7.947 (s, 1H), 7.964 –7.904 (m, 2H), 7.831 –7.754 (m, 3H), 7.686 (s, 3H), 7.544-7.523 (d, *J* = 8.4 Hz, 1H), 7.396-7.287 (m, 1H), 7.098– 7.079 (m, 1H), 6.747-6.726 (d, *J* = 8.4 Hz, 1H), 5.881 (s, 2H), 3.685 (s, 3H).**^{13}C NMR (101 MHz, DMSO-d_6)** δ 189.11, 167.23, 162.60, 155.24, 137.50, 135.70, 134.39, 133.49, 131.92, 130.45, 129.97, 128.10, 124.37, 121.08, 117.25, 105.02, 53.23, 47.20. **FT-IR (KBr, (υmax) cm^{-1})** 3595.43, 3333.1, 3078.49, 2947.33, 2576.98, 1905.73, 1689.7, 1589.4, 1504.53, 1381.08, 1280.78, 1188.19, 1087.89, 1018.45, 848.71, 748.41, 686.68, 547.8.

BA-10 (3-NO$_2$)- (E)-3-methoxy-4-((3-(2-(3-nitrophenyl)-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenyl benzenesulfonate: **Colour:** Reddish, **Yield: 76%, MS(m/z)=** 554, **^{1}H NMR (400 MHz, DMSO-d_6)** δ 8.662-8.644 (t, J = 7.2 Hz, 1H), 8.437-8.209 (m, 3H), 7.971 (s, 1H), 7.842–7.729 (m, 1H), 7.714–7.637 (m, 3H), 7.547-7.529 (d, J = 7.2 Hz, 2H), 7.057 (s, 1H), 6.748 (m, 1H), 5.052 (s, 2H), 3.858 (s, 3H).**^{13}C NMR (101 MHz, DMSO-d_6)** δ 188.10, 167.61, 164.30, 161.20, 151.33, 148.10, 138.20, 136.27, 133.21, 131.49, 129.10, 128.95, 126.10, 125.20, 120.70, 118.25, 115.18, 104.11, 54.68, 46.12. **FT-IR (KBr, (υmax) cm^{-1})** 3618.58, 3348.54, 3078.49, 2939.61, 2862.46, 1959.74, 1689.7, 1519.96, 1365.65, 1195.91, 1103.32, 1018.45, 856.42, 748.41, 694.4, 586.38

In silico evaluation

All the synthesized compounds were screened for *in silico* anti-cancer activity to evaluate their hypothetical binding energy using X ray crystallographic structure of 3hy6. The docking score was calculated using autodock 4.2. The detail of docking score (Reference RMSD value), binding energy (Kcal/mol), inhibition constant, number of hydrogen bond and amino acid interacted are depicted in Table 2. BA05 showed the best lowest binding energy (-14.68 Kcal/Mol) followed by BA10 (-11.51 Kcal/Mol), BA06 (-10.47 Kcal/Mol), BA09 (-10.25 Kcal/Mol), BA02 (-10.21 Kcal/Mol), BA08 (-10.17 Kcal/Mol), BA03 (-9.35 Kcal/Mol), BA07 (-9.12 Kcal/Mol), BA01 (-9.04 Kcal/Mol) and BA04 (-8.83 Kcal/Mol) respectively. The 3D and 2D interactions of test compounds with MTHFS is well described in figure 2 and figure 3 respectively.

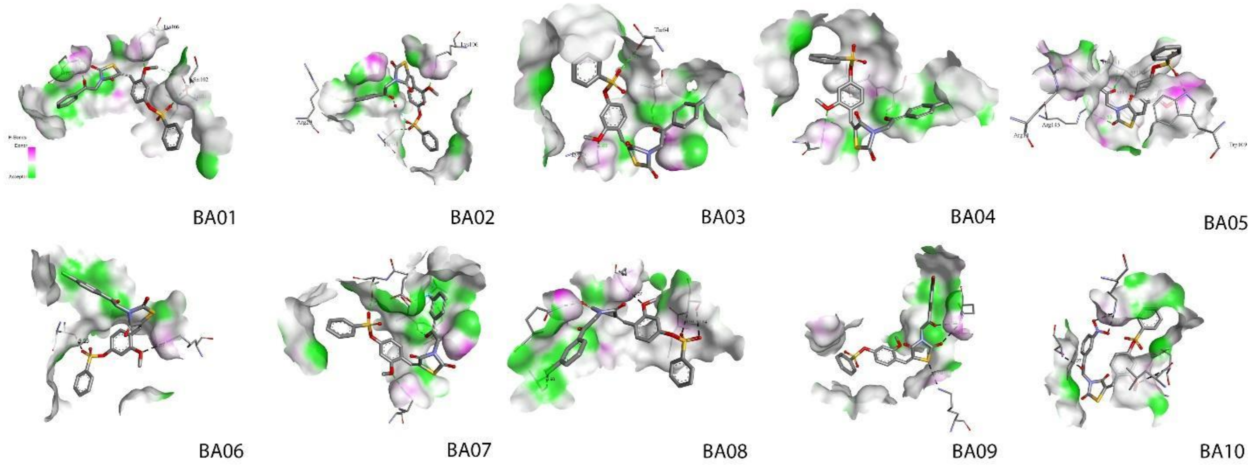

BA01 BA02 BA03 BA04 BA05
BA06 BA07 BA08 BA09 BA10

Figure 2: 3D presentation of best pose of BA01 to BA10 interacted in binding pocket of MTHFS

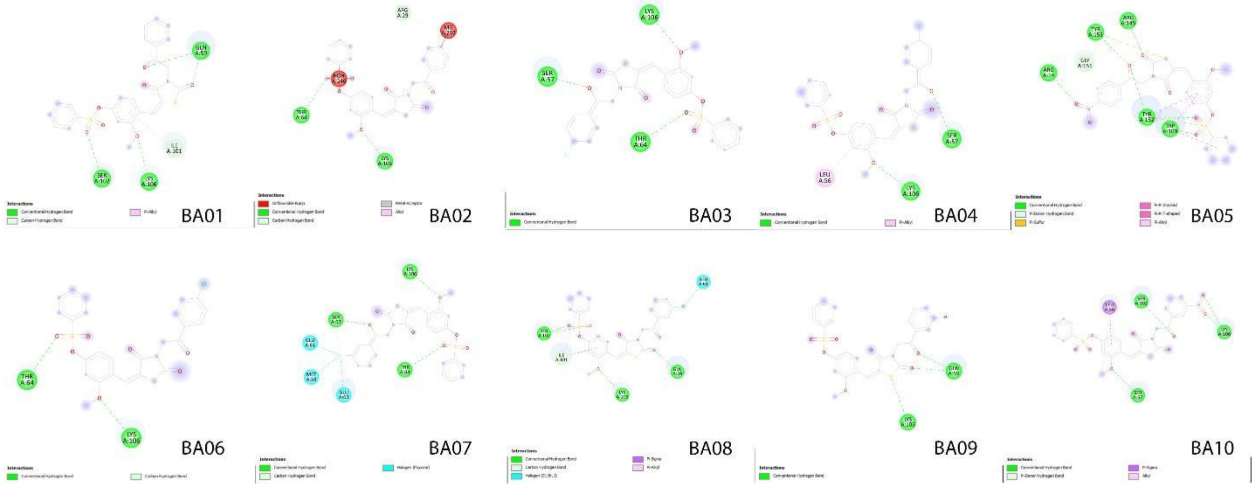

BA01 BA02 BA03 BA04 BA05
BA06 BA07 BA08 BA09 BA10

Figure 3: 2D presentation of best pose of BA01 to BA10 interacted in binding pocket of MTHFS

Table 2: Result of Molecular docking of synthesized compounds.

Sr. No	Protein	Compound	RMSD from reference cluster A	Binding Energy (Kcal/Mol)	Inhibition constant (Ki)	Number of H bond (ligand-protein)	Amino acid involved in interaction
1	3hy6	BA01	100.37	-9.04	237.58 nM	04	Gln59, Ser102 & Lys106
2		BA02	31.089	-10.21	32.84 nM	02	Thr64 & Lys106
3		BA03	33.428	-9.35	140.85 nM	03	Ser57, Thr64 & Lys106
4		BA04	31.998	-8.83	336.94 nM	02	Ser57 & Lys106
5		BA05	18.466	-14.68	17.25 pM	07	Arg14, Trp109, Arg145, Gly151, Tyr152 & Try153
6		BA06	32.433	-10.47	21.26 nM	02	Thr64 & Lys106
7		BA07	32.357	-9.12	207.45 nM	03	Ser57, Thr64 & Lys106 glu61*, Met58* & Glu63*
8		BA08	32.622	-10.17	35.27 nM	04	Gln59, Ser102 & Lys106 Glu61#
9		BA09	32.426	-10.25	30.51 nM	03	Gln59 & Lys106
10		BA10	31.770	-11.51	3.65 nM	04	Ser57, Ser102 & Lys106

*halogen bond with fluorine, # halogen bond with Cl, Br or I

ADME and Toxicity Analysis

After molecular docking, the Swiss ADME tool was used to find out more about the ADME properties of the compounds that were synthesized. Good solubility of any medicinal product is desirable. Result of the in silico solubility analysis is described in table 3.

Table 3: *In silico* water Solubility of synthesized compounds

	Water solubility								
	Log S (ESOL)			Log S (Ali)			Log S (SILICOS-IT)		
		Solubility (mg/ml)	Class		Solubility	Class		Solubility	Class
BA01	-5.91	6.22×10^{-4}	Moderately soluble	-7.55	1.44×10^{-5}	Poorly soluble	-7.47	1.71×10^{-5}	Poorly soluble
BA02	-6.83	8.77×10^{-5}	Poorly soluble	-8.27	3.19×10^{-5}	Poorly soluble	-8.23	3.48×10^{-5}	Poorly soluble
BA03	-6.08	4.41×10^{-4}	Poorly soluble	-7.65	1.17×10^{-5}	Poorly soluble	-7.73	9.78×10^{-6}	Poorly soluble
BA04	-6.22	3.18×10^{-4}	Poorly soluble	-7.92	6.25×10^{-6}	Poorly soluble	-7.84	7.50×10^{-6}	Poorly soluble
BA05	-5.99	5.68×10^{-4}	Moderately soluble	-8.34	2.56×10^{-6}	Poorly soluble	-6.80	8.81×10^{-5}	Poorly soluble
BA06	-6.51	1.67×10^{-4}	Poorly soluble	-8.20	3.40×10^{-6}	Poorly soluble	-8.05	4.86×10^{-6}	Poorly soluble
BA07	-6.08	4.41×10^{-4}	Poorly soluble	-7.65	1.17×10^{-5}	Poorly soluble	-7.73	9.78×10^{-6}	Poorly soluble
BA08	-6.51	1.67×10^{-4}	Poorly soluble	-8.20	3.40×10^{-6}	Poorly soluble	-8.05	4.86×10^{-6}	Poorly soluble

BA09	-6.83	8.77×10^{-5}	Poorly soluble	-8.27	3.19×10^{-6}	Poorly soluble	-8.23	3.48×10^{-6}	Poorly soluble
BA10	-5.99	5.68×10^{-4}	Moderately soluble	-8.34	2.56×10^{-6}	Poorly soluble	-6.80	8.81×10^{-5}	Poorly soluble

***In silico* lipophilicity of synthesized compounds**

Lipophilicity of drug candidate represent how efficiently drug is reached to target site. Lipophilicity of compound is represented as $LogP_{o/w}$. for the synthezied compounds lipophilicity was calculated using different online models namely iLOGP, XLOGP3, WLOPG, MLOGP, SILICOS-IT and sonsensus $LogP_{o/w}$ respectively. Detailed analysis of lipophilicity of synthesized compounds are well depicted in table 4.

Table 4: *In silico* lipophilicity of synthesized compounds

	Log $P_{o/w}$ (iLOGP)	Log $P_{o/w}$ (XLOGP3)	Log $P_{o/w}$ (WLOGP)	Log $P_{o/w}$ (MLOGP)	Log $P_{o/w}$ (SILICOS-IT)	Consensus Log $P_{o/w}$
BA01	3.34	4.86	4.97	2.57	3.5	3.85
BA02	4.02	5.55	5.74	3.14	4.18	4.53
BA03	3.74	4.96	5.53	2.94	3.93	4.22
BA04	386	5.22	5.28	2.77	4.03	4.23
BA05	3.04	4.69	4.88	1.75	1.36	3.14
BA06	3.86	5.49	5.63	3.04	4.15	4.43
BA07	3.68	4.96	5.53	2.94	3.93	4.21
BA08	3.9	5.49	5.63	3.04	4.15	4.44
BA09	4.03	5.55	5.74	3.14	4.18	4.53
BA10	3.13	4.69	4.88	1.75	1.36	3.16

In silico pharmacokinetics of synthesized compounds

Pharmacokinetics of drug candidates means, how much drug is absorbed via GI tract, whether the drug cross BBB or not, P-gp substrate or not, whether the drug is inhibiting any of the liver enzymes or not. Detailed in silico analysis of pharmacokinetics of all the compounds are described in table 5.

Table 5: *In silico* pharmacokinetics of synthesized compounds

	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (skin permeation)
BA01	Low	No	No	No	Yes	Yes	No	Yes	-5.96 cm/s
BA02	Low	No	No	Yes	No	Yes	No	Yes	-5.95 cm/s
BA03	Low	No	No	Yes	Yes	Yes	No	Yes	-6.00 cm/s
BA04	Low	No	No	Yes	Yes	Yes	No	Yes	-5.79 cm/s
BA05	Low	No	No	No	No	Yes	No	Yes	-6.35 cm/s
BA06	Low	No	No	Yes	Yes	Yes	No	Yes	-5.72 cm/s

BA07	Low	No	No	Yes	Yes	Yes	No	Yes	-6.00 cm/s
BA08	Low	No	No	Yes	Yes	Yes	No	Yes	-5.72 cm/s
BA09	Low	No	No	Yes	No	Yes	No	Yes	-5.95 cm/s
BA10	Low	No	No	No	No	Yes	No	Yes	-6.35 cm/s

In silico Toxicity of synthesized compounds

All the drug candidates must not toxic to human body. In silico toxicity evaluation was performed such as AMES toxicity, macimum tolerated dose in humans (mg/kg/day), hERG-I inhibition, hERG-II inhibition, oral rat acute toxicity (LD50, ml/kg) oral cronic toxicity (LOAEL, mg/kg/day), hepatotoxicity, skin sensitization, T. pyriformis toxicity (ug/L) and Min bow toxicity 9Log mM). Detailed toxicity data is described in table 6.

Table 6: In silico Toxicity data of synthesized compounds.

Compound code	**AMES toxicity**	**Max. tolerated dose (human) (mg/kg/ day)**	**hERG I inhibitor**	**hERG II inhibitor**	**Oral Rat Acute Toxicity (LD50) (mol/ kg)**	**Oral Rat Chronic Toxicity (LOAEL) (mg/kg/ day)**	**Hepatotoxicity**	**Skin Sensitization**	***T. pyriformis* toxicity (ug/L)**	**Minnow toxicity (log mM)**
BA01	No	0.676	No	Yes	2.559	1.114	Yes	No	0.285	-0.11
BA02	No	0.537	No	Yes	2.696	0.725	Yes	No	0.285	-1.142
BA03	No	0.569	No	Yes	2.786	0.85	Yes	No	0.285	-0.35
BA04	No	0.529	No	Yes	2.692	0.962	Yes	No	0.285	-0.778
BA05	No	0.549	No	Yes	2.49	0.924	Yes	No	0.285	-0.068
BA06	No	0.537	No	Yes	2.695	0.735	Yes	No	0.285	-0.996
BA07	No	0.6	No	Yes	2.746	0.852	Yes	No	0.285	0.004
BA08	No	0.577	No	Yes	2.695	0.774	Yes	No	0.285	-0.829
BA09	No	0.577	No	Yes	2.696	0.764	Yes	No	0.285	-0.975
BA10	Yes	0.5	No	Yes	2.5	0.708	Yes	No	0.285	-0.775

Discussion:

The synthesis was initiated by synthesizing Intermediate 1 and 3 as separate reactions. Combining Int. 1 and Int. 2 was resulted into Int. 3 which was further reacted with substituted phenacyl bromide in presence of DMF to obtained desired product. Reaction process is comparatively easy and less time consuming compared to reported methods. Purification of product was easy and relatively good amount of yield was obtained by adopting the proposed method. No further purification was required. Characterization was done by different spectroscopic methods such as IR, NMR and mass.

The number of cancer cases are increased day by day. Due to increase in prevalence of cancer all over the world, there is an urgent need of batter anticancer drug. Number of anticancer drugs already available in market, however, most of the drugs suffer one or other problem such as, high toxicity, poor pharmacokinetics, poor activity, etc. Numerous mechanisms are available to combat cancer cells. One of the important mechanism of action of anticancer agent is to inhibit MTHFS. MTHFS is directly involved in the unidirectional transformation of 5-formyltetrahydrofolate to 5,10-methenyltetrahydrofolate. Transformed 5,10-methenyltetrahydrofolate is directly involved in the biosynthesis of thymidylate, purines, methionine, serine, glycine and many other compounds. Blockage of MTHFS directly impacted on the synthesis of such bioilogically important compounds. Hence drugs inhibiting MTHFS resulted into a good candidate for the treatment of cancerous cells.

The goal of molecular docking, a computer process that tries to predict how macromolecules (like receptors and proteins) and small molecules (like ligands) will interact, is to figure out exactly how much energy these two groups need to stick together. It has been shown to be an incredibly useful method for screening a large number of compounds and locating new medicines against target proteins. Protein-ligand docking stands out from other kinds of docking because it could be used in the drug industry [4]. This research made use of two free, open-source programs for molecular docking simulations: AutoDock tools version 1.5.7 and AutoDock version 4.2. The Scripps Research Institute has created a very precise, user-friendly, and accessible piece of software [10, 18, 19]. MTHFS was selected as a target protein due to its involvement in the folate reduction. The docking score and the hydrogen bonds made with the amino acids from the group interaction atoms are used to predict how docked ligands will bind, how strongly they will bind, and how they will be oriented at the active site of the protein receptor. Docking study suggest that the BA05 showed the excellent binding with lowest energy. Molecular simulation showed that BA05 form 7 hydrogen bond with Arg14, Trp109, Arg145, Gly151, Tyr152 & Try153. However, BA05 showed moderate water solubility, poor lipophilicity, does not cross BBB, poor skin permeability and failed in Lipinski rule (Violation: MW>500, N or O>10). Further modification in the structure of BA05 are necessary.

BA10 showed the second lowest binding energy (-11.51 Kcal/Mol). With MTHFS, BA10 formed 04 hydrogen bond with Ser57, Ser102 and Lys106 aminoacids. BA10 is moderately soluble in water. Log $P_{o/w}$ value of BA10 is second lowest, GI absorbance is low and not crossing BBB. BA10 has the highest Log kp (skin permeation). AMES toxicity for the is only positive in BA10 compared to all 10 molecules.

BA06 showed third lowest good binding energy (-10.47 Kcal/Mol). Docking study revealed that it only formed two hydrogen bonds (Thr64 & Lys106). ADME study revealed that it is poorly water soluble (1.67×10^{-4} mg/ml) with -6.51 Log S (ESOL). Lipophilicity is good compared to other compounds. GI absorbance is poor and did not cross BBB. The findings are negative for AMES test however it may produce hepatotoxicity, however it did not produce and skin sensitization. BA06 may inhibit hERG II. The maximum calculated human dose is 0.537 mg/kg/day and in rat proposed oral acute toxic dose is 2.695 mol/kg.

BA09 showed the fourth lowest binding energy (-10.25 Kcal/Mol). Docking simulation showed that it bound with 2 amino acids. Two hydrogen bonds were formed with Gln59 and one hydrogen bond between Lys106 and Sulphur. ADME study revealed that it is poorly water soluble (8.77×10^{-5} mg/ml) with -6.83 Log S (ESOL). Lipophilicity is highest among all compouns. GI absorbance is poor and did not cross BBB. The findings are negative for AMES test however it may produce hepatotoxicity, however it did not produce and skin sensitization. BA09 may inhibit hERG II. The maximum calculated human dose is 0.577 mg/kg/day and in rat proposed oral acute toxic dose is 2.696 mol/kg.

BA02 showed the fifth lowest binding energy (-10.21 Kcal/Mol). Docking simulation showed that it formed hydrogen bond with 2 amino acids namely Thr64 and Lys106. ADME study revealed that it is poorly water soluble (8.77 × 10^{-5} mg/ml) with -6.83 Log S (ESOL). Lipophilicity is second highest among all compounds. GI absorbance is poor and did not cross BBB. The findings are negative for AMES test however it may produce hepatotoxicity, however it did not produce and skin sensitization. BA09 may inhibit hERG II. The maximum calculated human dose is 0.537 mg/kg/day and in rat proposed oral acute toxic dose is 2.696 mol/kg.

BA08 showed the fifth lowest binding energy (-10.17 Kcal/Mol). Docking simulation showed that it formed hydrogen bond with 4 amino acids. Total four conventional hydrogen bonds were formed, two hydrogen bond with Ser102 and one each with Lys106 and Gly59. ADME study revealed that it is poorly water soluble (1.67 × 10^{-4} mg/ml) with -6.51 Log S (ESOL). Lipophilicity is second highest among all compounds. GI absorbance is poor and did not cross BBB. The findings are negative for AMES test however it may produce hepatotoxicity, however it did not produce and skin sensitization. BA08 may inhibit hERG II. The maximum calculated human dose is 0.577 mg/kg/day and in rat proposed oral acute toxic dose is 2.695 mol/kg.

BA03 showed the sixth lowest binding energy (-9.35 Kcal/Mol). Docking simulation showed that it formed hydrogen bond with 3 amino acids namely Ser57, Thr64 and Lys106. ADME study revealed that it is poorly water soluble (4.41 × 10^{-4} mg/ml) with -6.08 Log S (ESOL). Lipophilicity is second highest among all compounds. GI absorbance is poor and did not cross BBB. The findings are negative for AMES test however it may produce hepatotoxicity, however it did not produce and skin sensitization. BA03 may inhibit hERG II. The maximum calculated human dose is 0.569 mg/kg/day and in rat proposed oral acute toxic dose is 2.786 mol/kg.

BA07 showed the sixth lowest binding energy (-9.12 Kcal/Mol). Docking simulation showed that it formed hydrogen bond with 3 amino acids namely Ser57, Thr64 and Lys106. ADME study revealed that it is poorly water soluble (4.41 × 10^{-4} mg/ml) with -6.08 Log S (ESOL). Lipophilicity is second highest among all compounds. GI absorbance is poor and did not cross BBB. The findings are negative for AMES test however it may produce hepatotoxicity, however it did not produce and skin sensitization. BA07 may inhibit hERG II. The maximum calculated human dose is 0.6 mg/kg/day and in rat proposed oral acute toxic dose is 2.746 mol/kg.

BA01 showed the sixth lowest binding energy (-9.04 Kcal/Mol). Docking simulation showed that it formed hydrogen bond with 3 amino acids and form 4 hydrogen bonds. Out of 4, 2 hydrogen bonds with gly59, and 1 hydrogen bond with each Ser102 and Lys106. ADME study revealed that it is poorly water soluble (6.22 × 10^{-4} mg/ml) with -5.91 Log S (ESOL). Lipophilicity is second highest among all compounds. GI absorbance is poor and did not cross BBB. The findings are negative for AMES test however it may produce hepatotoxicity, however it did not produce and skin sensitization. BA01 may inhibit hERG II. The maximum calculated human dose is 0.676 mg/kg/day and in rat proposed oral acute toxic dose is 2.559 mol/kg.

BA04 showed the sixth lowest binding energy (-8.83 Kcal/Mol). Docking simulation showed that it formed hydrogen bond with 2 amino acids namely Ser57 and Lys106. ADME study revealed that it is poorly water soluble (6.22 × 10^{-4} mg/ml) with -5.91 Log S (ESOL). Lipophilicity is second highest among all compounds. GI absorbance is poor and did not cross BBB. The findings are negative for AMES test however it may produce hepatotoxicity, however it did not produce and skin sensitization. BA04 may inhibit hERG II. The maximum calculated human dose is 0.529 mg/kg/day and in rat proposed oral acute toxic dose is 2.692 mol/kg.

Conclusion:

We have been focusesd on synthesis and spectral characterizations of active Thiazolidinediones (TZD) which are sulfur containing pentacyclic compounds that abundant in nature. Diverse therapeutic potentials in the medical area, including antidiabetic, antimicrobial, anti-inflammatory, anticancer, antioxidant, antitubercular, antiviral, anti-malarial, anti-HIV, and anticonvulsant activities, among many others make them attractive . Furthermore synthesized TZD compound undergoes for antineoplastic potential with the human 5,10-Methenyltetrahydrofolate Synthetase enzyme (PDB ID: 3hy6) to determine receptor-ligand interactions and the ADMET prediction was investigated using Swiss ADME and based on its favourable docking scores and binding energies from docking experiments, BA-05 is a promising compound for further investigation into its anticancer effects. In order to gather the best possible data for future investigations present investigation help the researcher to comeup with potential anticancer lead. The ADMET study advises more adjustments to acquire the best lead for future experiments. Further optimization of lead compound and in vitro and in vivo activities are needed for future experimentation.

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