RESEARCH ARTICLE

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Design, Synthesis and Biological Evaluation of Thiazolidine-2,4-dione-biphenyl Derivatives as Anticancer Agents

Udaya Sri Earati[1], Kiran Gangarapu[1]*, Mahendar Porika[2]

Abstract

Objective: A new library of Thiazolidine-2,4-dione-biphenyl Derivatives derivatives (10a-j) was designed and synthesized. All compounds were characterized by spectral data. Further, these were evaluated for their in vitro anticancer activity. **Methods:** The compounds were synthesized as planned in the Scheme-1 and compounds were screened against four human cancer cell lines like cervical cancer (Hela), prostate cancer (PC3), lung cancer cells and breast cancer cells (MDA-MB-231) by employing of MTT assay. Doxorubicin was chosen as positive control. **Result:** Most of the compounds showed moderate to good activity. Among them, these compounds 10b and 10d and displayed more potent activity. Predominantly, one compound 10d showed remarkable anticancer activity. Molecular docking studies with EGFR target (PDB ID: 1M17) exhibits compounds 10b and 10d showed desirable molecular interactions with the same residue similar to cocrystal ligand Erlotinib. **Conclusion:** This results indicate that these two compounds are well targeted the EGFR active sites and showed more inhibitory effects than the other compounds.

Keywords: Thiazolidine-2- 4-dione- biphenyl- napthyridine- anticancer activity

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Introduction

Cancer persists as a substantial global health concern, marked by elevated mortality rates, trailing only cardiovascular diseases as the second most prevalent cause of death worldwide [1]. Various factors contribute to the high mortality rate associated with cancer, including late-stage diagnosis, limited access to quality healthcare, and inadequate treatment options [2]. According to IARC report on 2020 about 20 million of new cancer cases and approximately globally 9.7 million deaths have reported [3, 4]. The advancement of potent anticancer agents is of paramount significance in medical research. These agents play a pivotal role in combating diverse neoplastic conditions, drawing upon a synergy of traditional and contemporary methodologies for their development [5]. Synthetic anticancer compounds have notably diversified the therapeutic armamentarium, empowering healthcare practitioners with a versatile array of interventions to address a spectrum of malignancies. Their evolution stands as a pivotal component of ongoing endeavors to refine cancer management protocols and elevate patient prognoses

One of the primary goals of medicinal chemist is to design, and discover, compounds with potential as human therapeutic agents. Heterocyclic compounds are regarded as fundamental substances in medicinal chemistry due to their various biological properties. Among these, thiazolidine-2,4-dione (TZD), characterized by their five-membered heteroaromatic structure, occupy a central position in pharmaceutical research and development due to their significant roles and applications [6, 7]. In 1982 a number of TZDs were intensively studied for their anti-diabetic activity. The first representative of this class was ciglitazone, whereas other derivatives like englitazone, pioglitazone and troglitazone followed soon. The thiazolidine-2,4-dione nucleus has been reported for being responsible for majority of their pharmacological actions. Henceforth, thiazolidine-2,4-dione derivatives have been studied extensively and found to have diverse chemical reactivity's and broad spectrum of biological activities. TZD are reported to have wide range of pharmacological activities viz., anticancer, antidiabetics, anti-inflammatory, anti-microbial, anti-viral, anti-tubercular, hypolipidemic, anti-convulsant, and neuroprotective activity [8-10]. TZDs can inhibit tumor angiogenesis, alter the cell cycle, induce cell differentiation, and promote apoptosis [11, 12]. They can also act as anticancer agents by targeting VEGFR-2 and reducing VEGF synthesis [13, 14]. TZDs are being tested in clinical trials for the treatment of human cancers that express high levels of PPARγ [15, 16]. The anticancer effects of TZDs are mediated by the activation of PPAR-γ. Biphenyl derivatives have shown various biological activities such as anticancer, antimicrobial, antihypertensive, antihyperlipidemic, anti-inflammatory, and anticonvulsant activities [17-19] (Figure 1).

*[1]School of Pharmacy, Anurag University, Venkatapur(V), Ghatkaser (M), Hyderabad, Telangana, India. [2]Department of Biotechnology, Kakatiya University, Warangal, Telangana, India. *For Correspondence: gkiran1905@gmail.com*

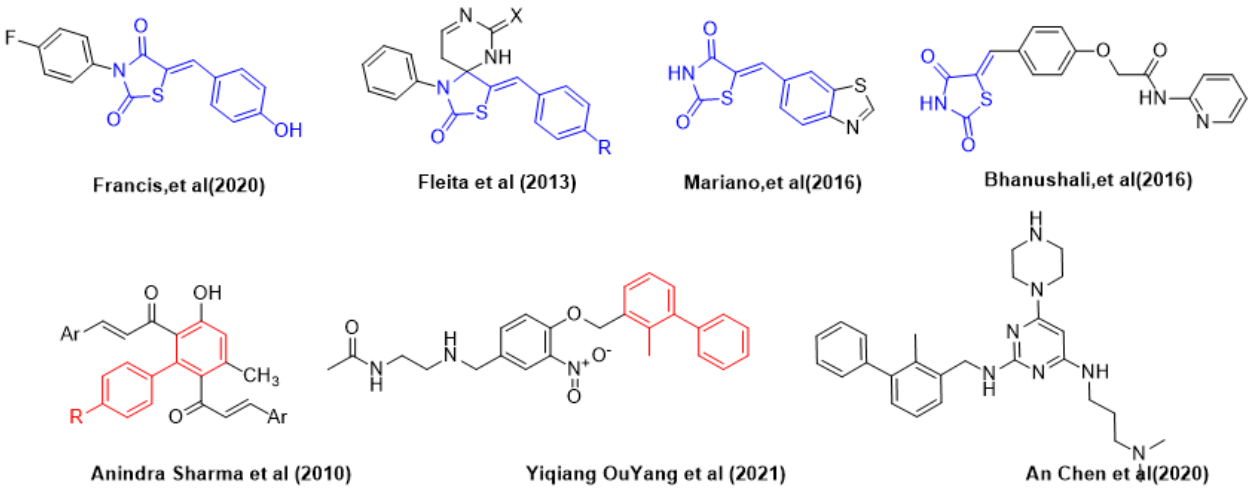


Figure 1. Examples of Agents Containing Thiazolidine-2,4-dione and Biphenyl Rings

The present work describe the TZD-biphenyl derivatives (10a-j) had synthesized and evaluated for their in vitro cytotoxicity against HELA, PC3, MDA-MB-231 and HepG2 cell lines. Doxorubicin was used as positive controls. Additionally, a molecular docking study were performed to predict the possible binding mode of these compounds in the active site of EGFR as the possible receptor.

Materials and Methods

All materials and solvents were procured from Merck and Sigma-Aldrich Chemical Co. were used without further purification. TLC was performed on silica gel (Merck) plates to monitor the progress of the reactions. Melting points were determined on a ThermoCal50 instrument and were uncorrected. The 1H-NMR and 13C-NMR spectra were recorded in CDCl3 on a Bruker 500 and 125 MHz spectrometer, respectively. Chemical shifts (δ) are reported in ppm scale toward tetramethylsilane (TMS) as an internal standard. Mass analyses were carried out with an Agilent Technologies (HP) mass spectrometer operating at an ionization potential of 70 eV.

Chemistry

Synthesis of TZD

6 mL aqueous solution of 5.6 g of chloroacetic acid was added to a 6 mL aqueous solution of 4.56 g of thiourea and stirred for 15 min, resulting in a white precipitate accompanied by considerable cooling. The resulting precipitate was treated with conc. HCl and refluxed for 10 h at 100–110 °C with stirring. The mixture was then cooled and the resulting white needles were filtered, washed with water and recrystallized from ethanol [20].

Synthesis of (Z)-5-([1,1'-biphenyl]-4-ylmethylene) thiazolidine-2,4-dione 8: [1,1'-biphenyl]-4-carbaldehyde 7 (3.2 mmol) and 2,4-thiazolidinedione 6 were dissolved in ethanol and piperidine (6.0 mmol) was added to the reaction vessel. The reaction mixture was refluxed with stirring at 75 oC for 24 hr. The reaction mixture was allowed to cool at room temperature and precipitate thus obtained were collected by filtration and further washed with water and allow to dry to afford the compound.

(Z)-5-([1,1'-biphenyl]-4-ylmethylene)-3-(pyridin-3-ylmethyl)thiazolidine-2,4-dione (10a)

Compound 8 (0.02 mol), 3-ethylpyridine (0.02 mol) and NaOH were dissolved in ethanol and was refluxed for 1 day. The mixture in the reaction flask was added ethyl acetate and organic layer was collected and dried. The final product was collected and purified by using column chromatography.

White solid; yield: 84.2 %, mp: 220-222 ˚C; IR (KBr) ν cm-1: 3100 (Ar-H), 1750, 1650, 1490 (C=O); 1H-NMR (500 MHz, CDCl3) δ (ppm): 4.93 (s, 2H, CH2), 7.29 (m, 1H, Ar-H), 7.40 (t, 1H, Ar-H), 7.48 (dd, 2H, Ar-H), 7.58 (d, 2H, Ar-H), 7.62 (dd, 2H, Ar-H), 7.71 (m, 2H, Ar-H), 7.79 (d, 1H, Ar-H), 7.96 (d, 1H, Ar-H), 8.58 (t, 1H, Ar-H), 8.74 (d, 1H, Ar-H). 13C-NMR (125 MHz, CDCl3) δ (ppm): 167.67, 166.05, 150.26, 149.99, 149.65, 148.65, 136.73, 134.54, 127.33, 126.85, 123.65, 118.60, 109.15, 102.0, 42.69 MS: m/z (%): 373.25 [M+1, 100%], 331.80 (20.48%), 325.70 (24.75), 322.0 (24.12). Chemical Formula: C22H16N2O2S (372.44).

(Z)-5-([1,1'-biphenyl]-4-ylmethylene)-3-pentylthiazolidine-2,4-dione (10b): White solid; yield: 79.52 %, mp: 241-243 ˚C; IR (KBr) ν cm-1: 3050(Ar-H), 1730, 1640, 1450 (C=O); 1H-NMR (500 MHz, CDCl3) δ (ppm): 0.86-0.88 (3H, CH2), 1.27-1.67 (m, 6H, CH2), 3.78 (s, 2H, CH2), 7.37 (m, 1H, Ar-H), 7.42 (t, 1H, Ar-H), 7.50 (d, 1H, Ar-H), 7.64 (dd, 2H, Ar-H), 7.70 (m, 2H, Ar-H), 7.73 (d, 1H, Ar-H), 7.93 (s, 1H, Ar-H). 13C-NMR (125 MHz, CDCl3) δ (ppm): 167.97, 166.57.26, 149.76, 148.59, 133.69, 129.78, 129.40, 127.56, 127.36, 127.25, 126.65, 119.17, 109.11, 101.94, 62.84, 41.85, 32.60, 30.19, 29.72, 27.73, 26.37, 25.74, 25.60, 25.41, 25.17. MS: m/z (%): 352.47 [M+1, 100%]. C21H21NO2S (351.46).

(Z)-5-([1,1'-biphenyl]-4-ylmethylene)-3-(3,5-dimethoxybenzyl)thiazolidine-2,4-dione(10c): White solid; yield: 81.14 %, mp: 271-272 ˚C; IR (KBr) ν cm-1: 3150(Ar-H), 1760, 1680, 1480 (C=O); 1H-NMR (500 MHz, CDCl3) δ (ppm): δ 3.73 (6H, s), 4.84 (2H, s), 6.38 (1H, t, J = 2.9 Hz), 6.80 (2H, t, J = 2.8 Hz), 7.42-7.64 (7H, 7.49 (dddd, J = 7.9, 7.2, 1.5, 0.5 Hz), 7.50 (tdd, J =

7.2, 1.6, 1.3 Hz), 7.56 (ddd, J = 8.6, 1.3, 0.4 Hz), 7.57 (ddd, J = 8.6, 2.5, 0.4 Hz)), 7.75 (2H, dtd, J = 7.9, 1.4, 0.5 Hz), 8.15 (1H, s). 13C-NMR (125 MHz, CDCl3) δ (ppm): 169.05, 168.07, 155.17, 152.19, 148.55, 133.59, 130.79, 129.02, 128.20, 127.82, 127.12, 106.69, 100.16, 77.26, 76.76, 55.39, 45.19. MS: m/z (%): 432.10 [M+1, 20%], 341.15 (20%), 320.65 (20%), 309.10 (20%), 261.15 (15%). C25H21NO4S (431.50).

(Z)-2-((5-([1,1'-biphenyl]-4-ylmethylene)-2,4-dioxothiazolidin-3-yl)methyl)benzonitrile (10d): White solid; yield: 74.74 %, mp: 287-289 ˚C; IR (KBr) ν cm-1: 3090 (Ar-H), 1700, 1610, 1490 (C=O); 1H-NMR (500 MHz, CDCl3) δ (ppm): 4.98 (s, 2H, CH2), 7.41 (m, 1H, Ar-H), 7.46 (m, 4H, Ar-H), 7.54 (m, 2H, Ar-H), 7.59 (dd, 4H, Ar-H), 7.64 (m, 2H, Ar-H), 7.71 (d, 3H, Ar-H), 7.76 (dd, 2H, Ar-H), 7.97 (s, 1H, C-H), 13C-NMR (125 MHz, CDCl3) δ (ppm): 168.08, 166.45, 133.87, 133.80, 132.86, 130.82, 130.06, 129.34, 129.19, 129.02, 128.21, 127.82, 127.71, 127.13, 121.01, 118.38, 114.47, 44.91. MS: m/z (%): 446.80 [M+, 20%]. C24H16N2O2S (396.46).

(Z)-ethyl 2-(5-([1,1'-biphenyl]-4-ylmethylene)-2,4-dioxothiazolidin-3-yl)acetate (10e): White solid; yield: 64.66 %, mp: 268-269 ˚C; IR (KBr) ν cm-1: 2918, 2853, (C-H), 1734, 1678, 1605, 1542 (C=O); 1H-NMR (500 MHz, CDCl3) δ (ppm): 1.31 (t, 3H, CH3), 4.26 (q, 2H, CH2), 4.50 (s, 2H, CH2), 7.44 (m, 3H, Ar-H), 7.62 (t, 4H, Ar-H), 7.73 (d, 2H, Ar-H), 7.98 (s, 1H, Ar-H). 13C-NMR (125 MHz, CDCl3) δ (ppm): 168.08, 166.45, 133.87, 133.80, 132.86, 130.82, 130.06, 129.34, 129.19, 129.02, 128.21, 127.82, 127.71, 127.13, 121.01, 118.38, 114.47, 44.91. MS: m/z (%): 368.78 [M+1, 80%]. Chemical Formula: C20H17NO4S (367.42).

(Z)-5-([1,1'-biphenyl]-4-ylmethylene)-3-(6-hydroxyhexyl)thiazolidine-2,4-dione (10f): White solid; yield: 71.24 %, mp: 253-235˚C; IR (KBr) ν cm-1: 3400 (O-H), 3100 (Ar-H), 1620, 1574, 1480 (C=O); 1H-NMR (500 MHz, CDCl3) δ (ppm): 1.25-1.29 (m, 6H, 3CH2), 1.38-1.88 (m, 2H, CH2), 3.42-3.80 (m, 4H, CH2), 7.26-7.37 (m, 3H, Ar-H), 7.40-7.48 (m, 4H, Ar-H), 7.50-7.62 (d, 2H, Ar-H), 7.98 (s, 1H, Ar-H). 13C-NMR (125 MHz, CDCl3) δ (ppm): 168.08, 166.45, 133.87, 133.80, 132.86, 130.82, 130.06, 129.34, 129.19, 129.02, 128.21, 127.82, 127.71, 127.13, 121.01, 118.38, 114.47, 44.91. MS: m/z (%): 382.78 [M+1, 100%]. Chemical Formula: C22H23NO3S (381.49).

(Z)-5-([1,1'-biphenyl]-4-ylmethylene)-3-methylthiazolidine-2,4-dione (10g): White solid; yield: 71.24 %, mp: 249-251-235˚C; IR (KBr) ν cm-1: 3090 (Ar-H), 1780, 1680, 1620 (C=O); 1H-NMR (500 MHz, CDCl3) δ (ppm): 1H NMR: δ 3.34 (3H, s), 7.42-7.64 (m, 7H, Ar-H), 7.75 (2H, m, Ar-H), 8.13 (1H, s). 13C-NMR (500 MHz, CDCl3) δ (ppm): 167.96, 166.53, 143.29, 139.71, 133.29, 132.12, 130.80, 129.86, 128.90, 127.82, 127.82, 127.31, 127.12, 127.08. 77.27, 77.01, 76.76, 27.96. MS: m/z (%): 296.30 [M+1, 20%], 290.50 (15%), 279.40(12%), 256.80(10%), 242(15%). Chemical Formula: C17H13NO2S (295.36).

(Z)-5-([1,1'-biphenyl]-4-ylmethylene)-3-(3-(trifluoromethyl)benzyl)thiazolidine-2,4-dione (10h): White solid; yield: 71.24 %, mp: 249-251-235˚C; IR (KBr) ν cm-1: 3394, 2935 (C-H). 1734, 1685, 1610 (C=O); 1H-NMR (500 MHz, CDCl3) δ (ppm): 1H NMR: δ 5.19 (2H, s), 7.14 (d, 1H, Ar-H), 7.41 (m, 2H, Ar-H), 7.50 (m, 3H, Ar-H), 7.63 (m, 4H, Ar-H), 7.72 (dd, 2H, Ar-H), 8.00 (s, 1H, C-H), 13C-NMR (500 MHz, CDCl3) δ (ppm): 168.09, 166.43, 143.47, 139.64, 136.0, 132.33, 131.97, 130.58, 129.33, 128.25, 127.85, 127.12, 125.77, 125.25, 120.69, 77.26, 44.72. MS: m/z (%): 440.24 [M+1, 30%], Chemical Formula: C24H16F3NO2S (439.45).

(Z)-5-([1,1'-biphenyl]-4-ylmethylene)-3-(2-(trifluoromethyl)benzyl)thiazolidine-2,4-dione (10i): White solid; yield: 65%, mp: 252-253˚C; IR (KBr) ν cm-1: 3375, 2935 (C-H). 1723, 1650, 1620 (C=O); 1H-NMR (500 MHz, CDCl3) δ (ppm): 1H NMR: δ 2.29 (s, 1H), 4.53 (s, 2H), 7.38-7.40 (m, 3H, Ar-H), 7.48-7.58 (m, 4H, Ar-H), 7.61-7.65 (dd, 2H, Ar-H), 7.99 (s, 1H, C-H); 13C-NMR (500 MHz, CDCl3) δ (ppm): 167.79, 166.03, 136.01, 134.73, 133.96, 132.33, 131.61, 130.30, 129.33, 128.42, 127.83, 127.17, 125.90, 125.80, 125.77, 125.25, 121.44; MS: m/z (%): 439.90 [M+1, 20%], Chemical Formula: C24H16F3NO2S (439.45).

(Z)-5-([1,1'-biphenyl]-4-ylmethylene)-3-(prop-2-yn-1-yl)thiazolidine-2,4-dione (10j): White solid; yield: 45%, mp: 284.286˚C; IR (KBr) ν cm-1: 3381, 2920 (C-H). 1743, 1683, 1600 (C=O); 1H-NMR (500 MHz, CDCl3) δ (ppm): 1H NMR: δ 4.96 (2H, s), 7.40 (m, 1H, Ar-H), 7.48 (t, 3H, Ar-H), 7.62 (m, 6H, Ar-H), 7.71 (m, 3H, Ar-H), 7.96 (s, 1H, C-H); 13C-NMR (500 MHz, CDCl3) δ (ppm): 169.9, 164.6, 138.9, 139.0, 138, 132, 128.4, 127.8, 127.2, 126.9, 125.7, 121.9, 79.3, 72.8, 42.9; MS: m/z (%): 320.19 [M+1, 20%], 320.19 (12%), 242.20(15%), 238.90(15%), Chemical Formula: C19H13NO2S (319.38).

In vitro cytotoxicity

MTT assay

Individual wells of a 96-well tissue culture micro titer plate were inoculated with 100 µL of complete medium containing 1×10^4 cells. The plates were incubated at 37 oC in a humidified 5% CO2 incubator for 18 hours prior to the experiment. After medium removal, 100 µL of fresh medium containing the test compounds and doxorubicin at different concentrations such as 0.5, 1, and 2 µM were added to each well and incubated at 37 oC for 24 hours. Then the medium was discarded and replaced with 10 µL MTT dye. Plates were incubated at 37 oC for 2 hours. The resulting formazan crystals were solubilized in 100 µL extraction buffer. The optical density (O.D) was read at 570 nm with micro plate reader (Multi-mode Varioskan Instrument-Themo Scientific). The percentage of DMSO in the medium never exceeded 0.25% [21].

Molecular Docking Study

The crystal structure of the EGFR target was downloaded from the RCSB protein data bank site (PDB ID: 1M17) [22]. AutoDock Vina was used to run the molecular docking procedure. The structure of compounds was minimized in terms of energy and converted to pdbqt format. A grid box of 70×70×70 Å and an exhaustiveness of 100 were set for docking analysis. To visualize the interaction and orientation of the compounds, the Discovery Studio 2016 client was used.

In silico ADMET Properties Prediction

The Drug-likeness quantitative estimation (QED) with RDKit was estimated in the usegalaxy webserver. Molecular descriptors, including physical-chemical and geometric descriptors, play a key role in determining a chemical structure's characteristics. Using Galaxy webserver, our study examined the pharmacological properties and drug-likeness of synthesized molecules by calculating Lipinski parameters and QED score. We also evaluated toxicity parameters, tumorigenic, mutagenic, reproductive effective, irritarnt by using the Datawarrior software [23].

Results

Chemistry

A new library of TZD ring combined biphenyl derivatives (10a-j) were designed, synthesized and depicted in Scheme 1. thiourea (4) was reacted with chloro acetic acid (5) in the presence of conc HCl at 80 oC for 12hours to give pure compound thiazolidine-2,4-dione (6). Compound 6 underwent Knoevenagel condensation reaction with substituted aromatic aldehyde derivatives 7 in presence of piperidine using ethanol solvent to give pure benzylidene intermediate 8. Further, alkylation of acidic imide of NH intermediate 8 in the presence of NaOH was refluxed to afford the final target compound 10a-j.

Biological Evaluation

In vitro cytotoxicity

A new library of TZD ring combined biphenyl derivatives (10a-j) was evaluated for their in vitro anticancer activity towards four human cancer cell lines like cervical cancer (Hela), prostate cancer (PC3), lung cancer (HepG2) and breast cancer (MDA-MB-231) by employing of MTT assay. Doxorubicin was chosen as positive control. From the results in Table 1, it indicated that the anticancer activity of title compounds exhibited moderate to good activity. Among them, these compounds 10a, 10b, 10d, 10e and 10j displayed more potent activity. Predominantly, one compound 10d was showed remarkable anticancer activity. Structure-activity relationship (SARs) examination of compounds revealed that the compound 10d having cyano moiety on phenyl group attaching to thiazolidine-2,4-dione ring showed good activity on all cell lines (Hela=32.38±1.8 µM; PC3=74.28±1.3 µM; MDA-MB-231= 148.55±3.2µM and HepG2=59.67±1.6µM). When compound 10b with pentyl group attaching to thiazolidine-2,4-dione ring displayed decreased activity (Hela=79.95±1.6µM; PC3=120.07±2.7µM; MDA-MB-231= 182.95±1.3µM and HepG2=55.62±2.8µM) to compare with 10d.

Molecular docking study

We had performed synthesized compounds for docking studies to understand the protein ligand interactions in to the binding cavity of EGFR target and to elucidate the mechanism of inhibition. Validation of docking was performed by extracting cocrystal ligand and performing the docking into the active site of EGFR with PDB ID 1M17 and RMSD of docking was found to be 1.85 Ao [22]. Redocking of the cocrystal ligand (Erlotinib) in to the binding site of 1M17 are shown in Figure 2. Hydrogen bond interactions with the residues of Lys721, Arg817, Asp831 and Leu764 and also alkyl and π-alkyl interactions with Cys773, Leu820, Ala719 and Met769 were observed, seen in Figure 2.

As it is shown in, Figure 3-4, based on docking results, common interactions such as pi-pi or pi-alkyl and hydrophobic interactions with Thr 766, Thr 830, Leu 764, Phe 699, Val 702, Gly 772, Ala 719, Leu 694 and Leu 820 were seen in all compounds In compounds 10a-j showed different interaction depend on substitution on TZD ring. Compounds 10b substituted with pentyl group at nitrogen atom TZD has shown hydrogen bond interactions with Gly772 and Thr766 and hydrophobic interactions with Leu694, Val702, Ala719, Leu820 and Leu764 residues. Similarly 10d, which had cyano substitution at ortho position on biphenyl moiety, they interacted through similar hydrogen bond interaction with Lys721 and Met742 and hydrophobic interactions with Leu694, Val702, Ala719, Lys721, and Leu764 residues. These

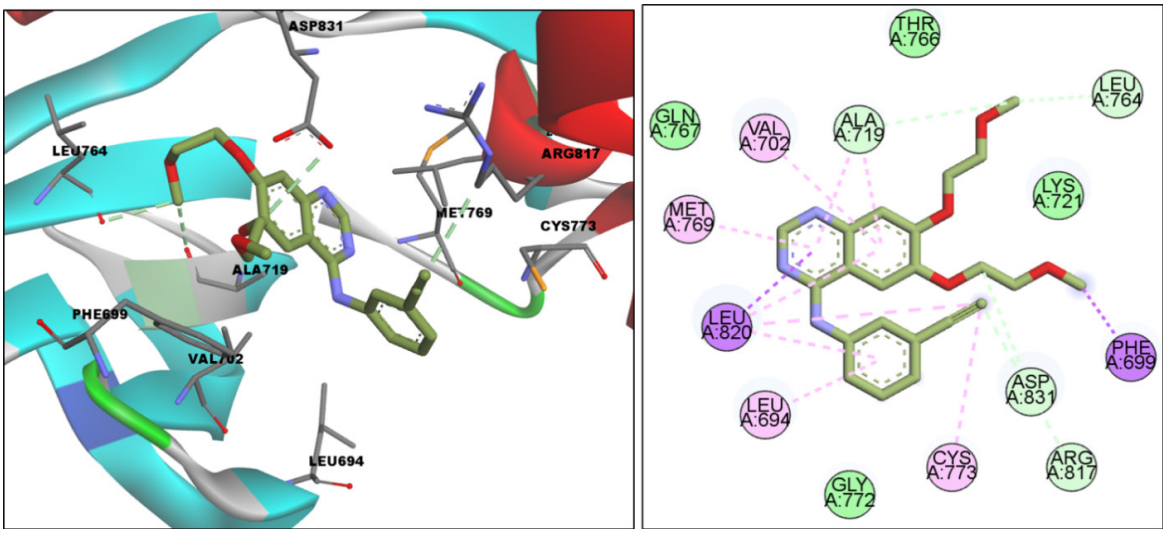
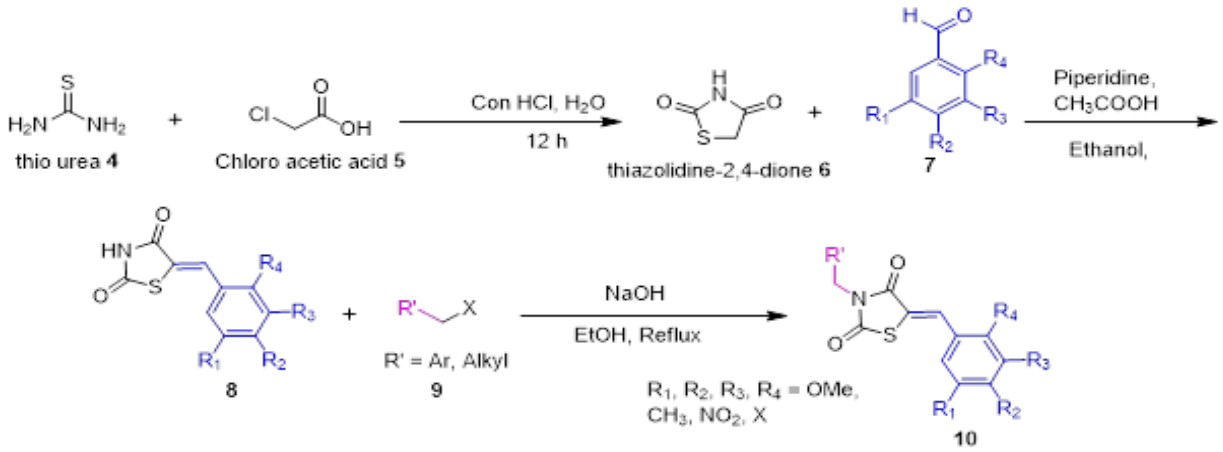


Figure 2. Interactions and Orientation of Erlotinib in the Active Site of 1M17



Scheme 1. Synthesis of Thiazolidine-2,4-dione-biphenyl Derivatives

Table 1. In vitro Cytotoxicity Studies of Synthesized Compounds against Cervical Cancer (Hela), Prostate Cancer (PC3), Lung Cancer (HepG2) and Breast Cancer (MDA-MB-231) Cancer Cell Lines IC_{50} (µM± SD)

COMPOUND (µM)	HELA	PC3	MDA-MB-231	HepG2
10a	97.73±2.1	139.08±1.8	196.27±1.5	87.53±1.7
10b	79.95±1.6	120.07±2.7	182.95±1.3	86.21±2.8
10c	83.20±1.9	70.68±2.6	214.37±1.7	55.62±2.0
10d	32.38±1.8	74.28±1.3	148.55±3.2	59.67±1.6
10e	146.15±2.8	132.55±1.7	265.09±2.8	51.44±1.2
10f	204.99±2.2	NA	NA	245.09±2.9
10g	68.73±2.5	133.74±1.1	NA	95.14±2.0
10h	42.79±1.4	55.53±1.9	88.76±2.5	204.60±1.4
10i	136.55±1.2	125.40±2.5	250.34.±3.0	NA
10j	124.30±1.4	111.78±2.8	180.98±2.5	66.69±1.1
DOX	2.20±0.6	1.47±0.5	2.58±0.8	1.84±0.7

common and extra strong and desirable interactions with EGFR target made the 10b and 10d as potential inhibitors of EGFR target among all the tested compounds. Amino acid residues Lys 721, Leu 694, Leu 820, Met 742, Ala719 are very important for the active conformation of Erlotinib.

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 investigation into the drug-like properties of bioactive compounds constitutes a critical endeavor in the realm of drug discovery. Subsequently, the drug-likeness parameters were computed for all synthesized compounds by evaluating adherence to the Lipinski Rule of Five (LRo5). LRo5, a widely recognized principle in drug discovery and development, serves to prognosticate

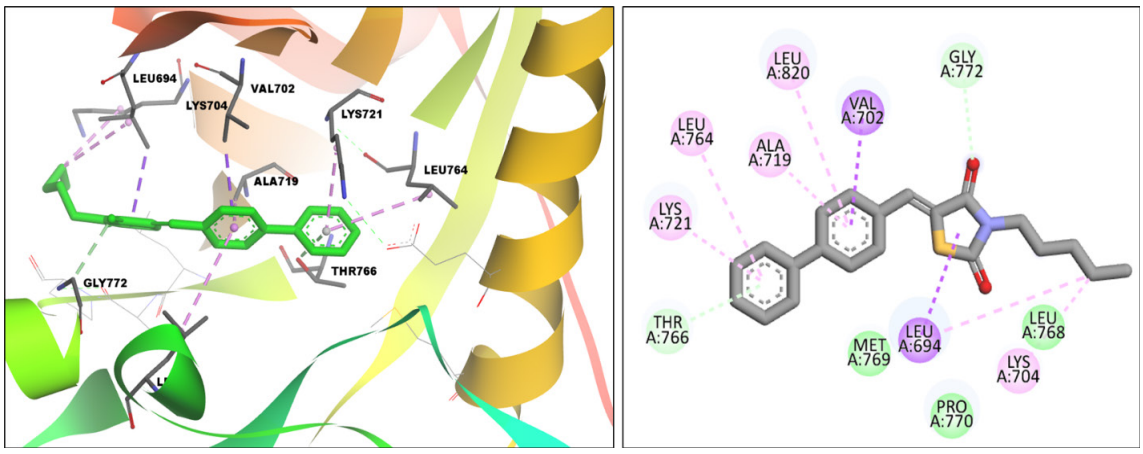


Figure 3. Interactions and Orientation of 10b in the Active Site of 1M17

Table 2. A Table Listing the Values of the Eight Features, the QED Score, and the Number of Lipinski Rules which the Molecule Obeys.

Compound	MW	ALOGP	HBA	HBD	PSA	ROTB	AROM	ALERTS	LRo5	QED
10a	372.45	4.99	4	0	50.27	4	3	1	0	0.61
10b	351.47	5.58	3	0	37.38	6	2	2	1	0.46
10c	431.51	5.61	5	0	55.84	6	3	1	1	0.5
10d	446.53	6.62	4	0	61.17	4	4	1	1	0.4
10e	341.39	3.44	5	0	63.68	4	2	2	0	0.55
10f	381.5	4.94	4	1	57.61	8	2	2	0	0.46
10g	295.36	4.02	3	0	37.38	2	2	1	0	0.74
10h	439.46	6.61	3	0	37.38	4	3	1	1	0.48
10i	439.46	6.61	3	0	37.38	4	3	1	1	0.48
10j	319.38	4.02	3	0	37.38	3	2	2	0	0.54

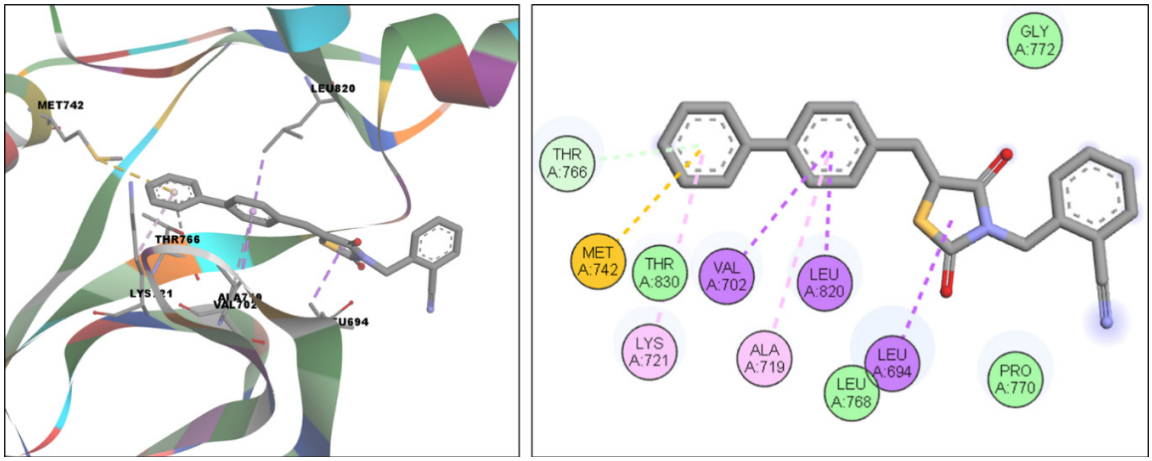


Figure 4. Interactions and Orientation of 10d in the Active Site of 1M17

a molecule's potential for oral activity based on its physicochemical characteristics. According to LRo5, a molecule is deemed more likely to exhibit oral activity if it satisfies specific criteria, including possessing no more than five hydrogen bond donors, ten or fewer hydrogen bond acceptors, a molecular weight below 500 daltons, and a partition coefficient (logP) below 5. For optimal oral bioavailability, LRo5 suggests a logP value below 2. Analysis of Table 2 reveals that the molecular weights of the tested compounds were below 500 daltons, their logP values were below 5, they had fewer than 5 hydrogen bond donors (HBD), and fewer than 10 hydrogen bond acceptors (HBA). All compounds conformed to Lipinski's rule, thereby indicating their potential for favorable oral bioavailability. The synthesized are also evaluated for in silico toxicity by using Datawarrior software. All the compounds are free from toxicity only 10f is shown high irritant effect it may due to the structure having more no of rotatable bonds. Thus all the synthesized compounds are capable for oral bioavailable (Table 3).

Table 3. Toxicity Data of Synthesized Compounds

Compound	Tumorigenic	Mutagenic	Reproductive Effective	Irritant
10a	none	none	none	none
10b	none	none	none	none
10c	none	none	none	none
10d	none	none	none	none
10e	none	none	none	none
10f	none	none	none	high
10g	none	none	none	none
10h	none	none	none	none
10i	none	none	none	none
10j	none	none	none	none

Discussion

The synthesis of the title compounds TZD ring combined biphenyl derivatives (10a-j) were planned accordingly as shown in Scheme 1. All the compounds have obtained with sufficient yields and the final compounds were recrystallized by suitable organic solvents and further purified by column chromatography. All the synthesized compounds were purified by using column chromatography and recrystallized and structures were confirmed by using FT-IR, NMR and Mass spectral data. The compounds were further evaluated by in vitro cytotoxicity activity against the human cancer cell lines like cervical cancer (Hela), prostate cancer (PC3), lung cancer (HepG2) and breast cancer (MDA-MB-231) by employing of MTT assay. The order of cytotoxicity activity against Hela cell line is found be 10d>10h>10b>10c>10a; for PC3 cell line is 10h>10c>10d>10j>10b; for MDA-MB-231 cell line is 10h>10d>10j>10b>10a; for HepG2 cell line is 10c>10d>10b>10a>10g. Among all the compounds, 10d having cyano moiety on phenyl group attaching to thiazolidine-2,4-dione ring showed good activity.

Compounds 10b and 10d also showed desirable docking interactions against EGFR target with the same residue similar to Erlotinib. This results indicate that these two compounds are well targeted the EGFR active sites and showed more inhibitory effects than the other compounds. All the compounds have evaluated for Drug-likeness quantitative estimation (QED) with RDKit and have obeyed the Lipinski Rule of Five (LRo5) which indicates the synthesized are compatible with oral bioavailability and also free from toxicity.

In conclusion, a new library of tetrazole ring incorporated oxazole-pyrimidine derivatives (10a-j) was designed and synthesized. All compounds were characterized by spectral data. Further, these were evaluated for their in vitro anticancer activity towards four human cancer cell lines like cervical cancer (Hela), prostate cancer (PC3), lung cancer (HepG2) and breast cancer (MDA-MB-231) by employing of MTT assay. Doxorubicin was chosen as positive control. Among them, these compounds 10a, 10b, 10d, 10e and 10j displayed more potent activity. Predominantly, one compound 10d was showed remarkable anticancer activity. Structure-activity relationship (SARs) examination of compounds revealed that the compound 10d having cyano group on phenyl moiety attaching to thiazolidine-2,4-dione ring showed good activity on all cell lines (Hela=15.3±1.8 µg/ml; PC3=35.1±1.3 µg/ml; MDA-MB-231= 70.2±3.2µg/ml and DU-145=28.2±1.6µg/ml). When compound 10b with pentyl group attaching to thiazolidine-2,4-dione ring displayed decreased activity (Hela=28.1±1.6µg/ml; PC3=42.2±2.7µg/ml; MDA-MB-231= 64.3±1.3µg/ml and DU-145=30.3±2.8µg/ml) to compare with 10d.

Full experimental details, spectral data of the products, 1H NMR and 13C NMR of all the new compounds can be found via the Supplementary Content section of this article's Web page.

Author Contribution Statement

All authors contributed equally in this study.

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Approval

The work is approved by the DRC of Anurag University for the Udaya Sri PhD thesis

How the ethical issue was handled (name the ethical committee that approved the research)

No ethical permission is required as the study carried out in vitro study

Any conflict of interest

The authors declare that they have no competing interests

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