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Original Research Article

Design and characterization of core scaffold pyrazolone fused thiazolidinone analogues as potent anticancer agents

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Abstract

Purpose: *To synthesize novel pyrazolone fused thiazolidinone analogues and evaluate their efficiency as potent HER2 and EGFR inhibitors in human breast adenocarcinoma cells for anti-cancer activity.*
Method: *In this study, several pyrazolone fused thiazolidinone analogues were synthesized, and characterized by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy, as well as tested for their in vitro cytotoxicity against breast cancer cell line (MCF-7) by MTT assay. A correlation study of the cytotoxicity was performed to obtain the Docking score using Schrodinger (Maestro) Version 9.6 Glide XP software.*
Result: *A total of 10 compounds were synthesised and analysed for their physiochemical, spectral, and cytotoxic activity against breast cancer cell lines (MCF-7). Among the synthesised compounds, compound 4B5 showed significantly higher ($p < 0.05$) anticancer properties against MCF-7 cell lines with docking score of -6.614, and half-maximal concentration (IC_{50}) value of 001.17 M compared to other synthesized compounds of the same categories.*
Conclusion: *Novel pyrazolone-fused thiazolidinone analogues have been successfully synthesized. The synthesised compounds possess anti-cancer activity against the MCF-7 cell lines. This could potentially lead to the development of new anti-breast cancer agents.*

Keywords: *Pyrazolone, Thiazolidinone scaffolds, HER2, EFGR inhibitors, GLIDE XP, In vitro cytotoxicity, MCF-7 cell line*

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INTRODUCTION

Pyrazolone fused thiazolidinone analogue is gaining interest in the pharmaceutical arena as it possesses multiple biological activities such as antimalarial, antiviral, anti-inflammatory, antidiabetic, antitumor, and antimicrobial properties [1]. According to the Indian Council of Medical Research report and the Bengaluru-based National Centre for Disease Informatics and Research, in India, cancer affects both men and women commonly to a wider extent, the total number of cancer cases in men was estimated to be 679,421 in 2020, and it is estimated to reach

763,575 in 2025, whereas in women, breast cancer is the most prevalent cancer with a projected score of 712,758 cases in 2020 and 806,218 cases in 2025 [2]. Apart from breast cancer, lung cancer and mouth cancer are expected to be the most common cancers among Indian women, which is estimated to be 111,328 and 90,060 cases in 2025, respectively [2]. Breast cancer represents the number one cancer among Indian females with an age-adjusted rate of 25.8 per 100,000 women and a mortality rate of 12.7 % [3]. Earlier studies have proven that the novel dual inhibitors of Human epidermal growth factor receptor-2 (HER2) and Epidermal growth factor receptor (EGFR) inhibitors designed and synthesised using pyrazolone fused thiazolidinone analogues possess the potential anticancer property for breast cancer treatment [4]. The majority of advanced breast cancer patients are treated with combination therapies. For example, the paclitaxel plus gemcitabine combination was commonly used to treat early breast cancers. Pyrazoline is chemically a 'five-membered nitrogen-containing heterocyclic' compound that possesses numerous biological activities. It exhibits analgesic (propylphenazone, phenazone, metamizole, etc.), anti-cancer (TELIN - a catalytic inhibitor of telomerase enzyme), anti-ischemic (edaravone), and anti-anxiolytic properties. The 4-thiazolidinone scaffold has a variety of therapeutic effects, including anti-diabetic, anti-microbial, anticonvulsant, anti-tubercular, anti-tumor, antiviral, anti-parkinsonism, anti-arthritic, analgesic, and anti-inflammatory properties [4]. The aim of this study was to examine the dual receptor inhibitors for HER2 and EGFR acting as core scaffold pyrazolone fused thiazolidinone analogues in the MCF-7 cell line.

EXPERIMENTAL

All the reagents used in the current study were analytical grade and purchased from industrial sources. The synthesis of the designed compounds is described below in 4 steps. The universal scheme of synthesis is presented in Figure 1.

Scheme for the synthesis of some pyrazolone fused carboxymethyl thiazolidinone derivatives

Step 1: Synthesis of 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one

Phenylhydrazine (0.01 mol), ethyl acetoacetate (0.01 mol), and a few drops of acetic acid were refluxed until the orange-colored solution transformed into a thick liquid. Later, 15 mL of ether was added to the reactants after they had been allowed to cool to room temperature. The formed yellow colour precipitate was filtered, dried and recrystallized from ethanol [6].

Step 2: Synthesis of benzoyl derivative of 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol- 3-one

The 0.1 mol of 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one was dissolved with 80 mL of dioxane, 0.175 mol of calcium hydroxide and 9.9 mL of benzoyl chloride. The mixture was refluxed for 30 min. The Buchner funnel was used to collect the crystals. They were re-crystallized from methanol or n-hexane to produce vibrant yellow colour crystalline substances [6,7].

Step 3: Synthesis of Schiff base derivatives of 5-methyl-2-phenyl-2, 4-dihydro-3H-pyrazol-3-one

Equimolar amines (0.1 mol) and their corresponding acylpyrazolones (0.1 mol) were dissolved in ethanol (30 mL) while being stirred occasionally for three to four hours and in the presence of a few drops of acetic acid. The formed coloured precipitate was washed, filtered, and recrystallized with ethanol [8-11].

Step: 4 Preparation of carboxy methyl thiazolidinone analogues from Schiff base ($4B_1$-$4B_{10}$)

Schiff base (0.01 mol) and thiomallic acid (0.01 mol) were introduced in the presence of anhydrous zinc chloride (0.5 g) in dimethylformamide (DMF) at 160 ^oC for 2 h. The product formed was dissolved in a sodium bicarbonate solution and filtered. The product was reprecipitated with dilute HCl, then crystallized from methanol [12-14].

Melting point determination

The melting points of the synthesised compounds were determined by using the open capillary technique. Samples were placed in the capillary tubes which were heated in the NSAW-1420 Melting Point Apparatus at a heating rate of 1 ^oC/min and the temperature at which the sample started to melt was noted.

Identification of the synthesised compounds

TLC and IR

Thin layer chromatography (TLC) was used to identify the synthesised compounds and

determine their purity and the progress of the reactions. Infrared (IR) spectra were recorded with the aid of the use of KBr disk on Shimadzu FTIR-8400S to identify the functional groups present.

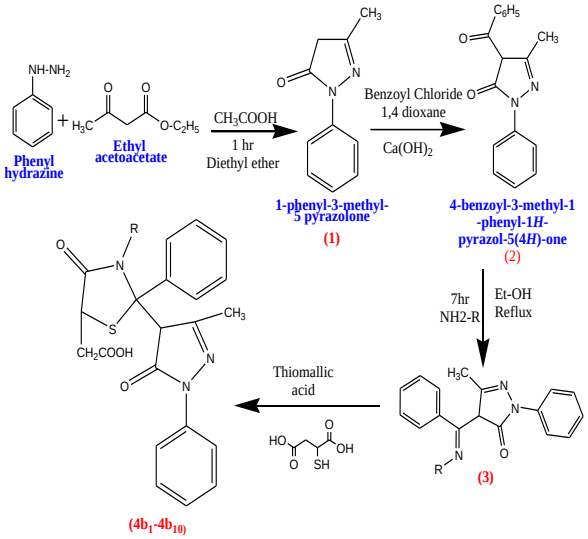

Figure 1: General scheme for the synthesis of selected core pyrazolone fused carboxymethyl thiazolidinone derivatives

Proton and carbon-13 (^{1}H and ^{13}C) NMR

The ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL AL300 FTNMR 300 MHz spectrophotometer using tetramethylsilane as an internal standard. The values of chemical shift (δ) were recorded in ppm.

Mass spectroscopy

Mass spectra was carried out using a Waters Micromass Q-Tof Micro Mass spectrometer geared up with electrospray ionization (ESI).

Molecular docking studies

Protein preparation

The HER2 enzyme with co-crystallized ligand (PDB ID: 3RCD, 2.3 Å) was obtained from the protein data bank. Protein practise Wizard Module of Schrodinger suite 2016 was utilized to set up the protein. Water molecules were removed to make computations easier and clear the binding pocket, as the presence of water molecules would distort the pose search.

Receptor grid generation

The centroid of the dynamic site was formerly represented in docking by a drift field. The protein planning wizard's gem shape was used to hold a co-crystallized ligand, which was then employed to build the receptor lattice. Grid boxes were created using the Glide Grid Era Wizard. The drift grid field measurements for the protein have been set at 14 Å x 14 Å x 14 Å.

Ligand preparation

The ligand shape was created using a 2D sketcher and uncovered to the Schrodinger suite 2016's ligand instruction module. The chiralities were corrected, and the ligands were converted from 2D to 3D using stereo concoction and ionisation techniques. Using the Epik module, the ionisation and tautomeric enumeration were done in a pH range of 6.7 - 7.1. Mixtures were limited using optimum potentials for liquid simulations (OPLS-3) in the final stage of ligand preparation until a root indicating a rectangular deviation of 1.8 Å was reached. Ligands were streamlined and one low vitality ring affirmation per ligand was generated.

Glide ligand docking

All the ligands (4B1-4B10) were docked into the synergist pocket of HER2 protein (PDB ID: 3RCD). The best-docked ligands were chosen based on the total glide score. The best gathering among the ligands and the receptor were scored using the float ligand docking module. Extra precision XP visualizer of flow module was utilized to analyze the results. The upgrading parameters and the diminishing motion were cited concurrently to achieve better outcome in the glide-ligand docking. The list of synthesised compounds were compiled.

Assessment of *in vitro* anticancer activity

Antitumor activities of the selected compounds were evaluated using MCF-7 (Breast cancer) cell line by the MTT assay method. The cell lines used in this study were procured from National Centre for Cell Science (NCCS), Pune, India. The analyses of anticancer activity of the compounds were performed according to the procedure described in earlier studies [15-17].

The cell lines were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10 % inactivated FBS and incubated at 37 °C in a humidified incubator of 5 % CO_2.

Cells were placed into 96-well flat-back plates at a density of 1 × 10^4 cells/mL and incubated for 24 h at 37 °C in a humid incubator (5 %, CO_2) before being exposed to multiple doses of the test substances for 48 h. The MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium

bromide) reagent was then added to a single well, agitated for 4 h, and then measured. The supernatant was removed, and 150 µL of dimethyl sulphoxide (DMSO) was added to the mixture to properly dissolve the MTT- formazan crystals. Finally, the absorbance was measured at 570 nm using a microplate reader and DMSO as a blank to determine the cell growth inhibition. The percentage surviving cells or cell growth inhibition (S) was calculated using Eq 1.

S (%) = (AT/AC)100 ……. (1)

where AT = Absorbance of test compound, and AC = absorbance of negative control.

Statistical analysis

Data are analysed using Graph Pad Prism (Version 5.1). The ANOVA and post-hoc test were computed by Dunnett's Multiple Comparisons for the study of anticancer properties against MCF-7 cell lines and *p*-value < 0.05 was considered significant.

RESULTS

The present study examines the dual receptor inhibitors for HER2 and EGFR acting as core scaffold pyrazolone fused thiazolidinone analogues in the MCF-7 cell line. There were 10 compounds synthesised and were named 4B1 - 4B10. The physicochemical properties of the synthesised compounds are presented in Table 1, and the structures of the compounds are presented in Figure 2. Physical characterization of synthesized compounds shows that compound 4B10 has a higher molecular weight with a melting point of 110 and an Rf value of 0.6 compared to other synthesised compounds. The details are presented in Table 1.

The FT-IR spectra of all the synthesized compounds confirmed that a strong, extensive absorption band for the pyrazolone CH_3 group at 2960 - 3059 cm^{-1}. Another strong absorption signal occurred for C=O at 1577-1617cm^{-1}, and a absorption characteristic band for aromatic stretching at 1499 - 1470 cm^{-1}. The details of IR spectral analysis are presented in Table 2.

Table 1: Physical characterization of synthesized compounds (4B1 to 4B10)

Compound Code	Molecular formula	Molecular weight	Melting point (°C)	R_f value	Yield (%)	Colour	Log P
4B1	$C_{27}H_{23}N_3O_4S$	485.54	135	0.2	86	Lime yellow	5.43
4B2	$C_{27}H_{22}ClN_3O_4S$	519.99	145	0.8	96	Brownish yellow	6.02
4B3	$C_{27}H_{22}ClN_3O_4S$	519.99	130	0.5	90	Lime yellow	6.02
4B4	$C_{27}H_{22}FN_3O_4S$	503.54	135	0.6	89	yellow	5.48
4B5	$C_{27}H_{22}FN_3O_4S$	503.54	125	0.3	90	Brown	5.48
4B6	$C_{28}H_{25}N_3O_4S$	499.58	150	0.3	91	Yellow	5.89
4B7	$C_{28}H_{25}N_3O_4S$	499.58	110	0.6	86	Yellow	5.89
4B8	$C_{28}H_{25}N_3O_5S$	515.58	130	0.7	90	Black	5.24
4B9	$C_{28}H_{25}N_3O_5S$	515.58	85	0.5	88	Yellow	5.34
4B10	$C_{28}H_{24}N_4O_7S$	560.57	110	0.6	90	Brown	5.38

Table 2: IR spectral data of the synthesized compound

Compound Code	IR ABSORPTION BAND (KBR cm^{-1}) or wave number(cm^{-1})
4B1	3503(O-H Stretching), 2922(C-CH_3 Stretching), 1601(C=O Stretching), 1480 (Aromatic Stretching), 1377(CH_2 Bending)
4B3	3453(O-H Stretching), 3057(C-CH_3 Stretching), 1582(C=O Stretching), 1374(CH_2 Bending), 753(C- Cl Stretching)
4B5	3488(O-H Stretching), 3042(C-CH_3 Stretching), 1608(C=O Stretching), 1487(C-F Stretching), 1291(CH_2 Bending)
4B7	3491(O-H Stretching), 3044(C-CH_3 Stretching), 2924 (C-CH_3 Stretching), 1612 (C=O Stretching), 1481(CH_2 Bending)
4B9	3466(O-H Stretching), 2928(O-CH_3 Stretching), 1586(C=O Stretching), 1383(CH_2 Bending)
4B10	3487(O-H Stretching), 1589 (C=O Stretching), 1510(N-O Stretching), 1416(CH_2 Bending)

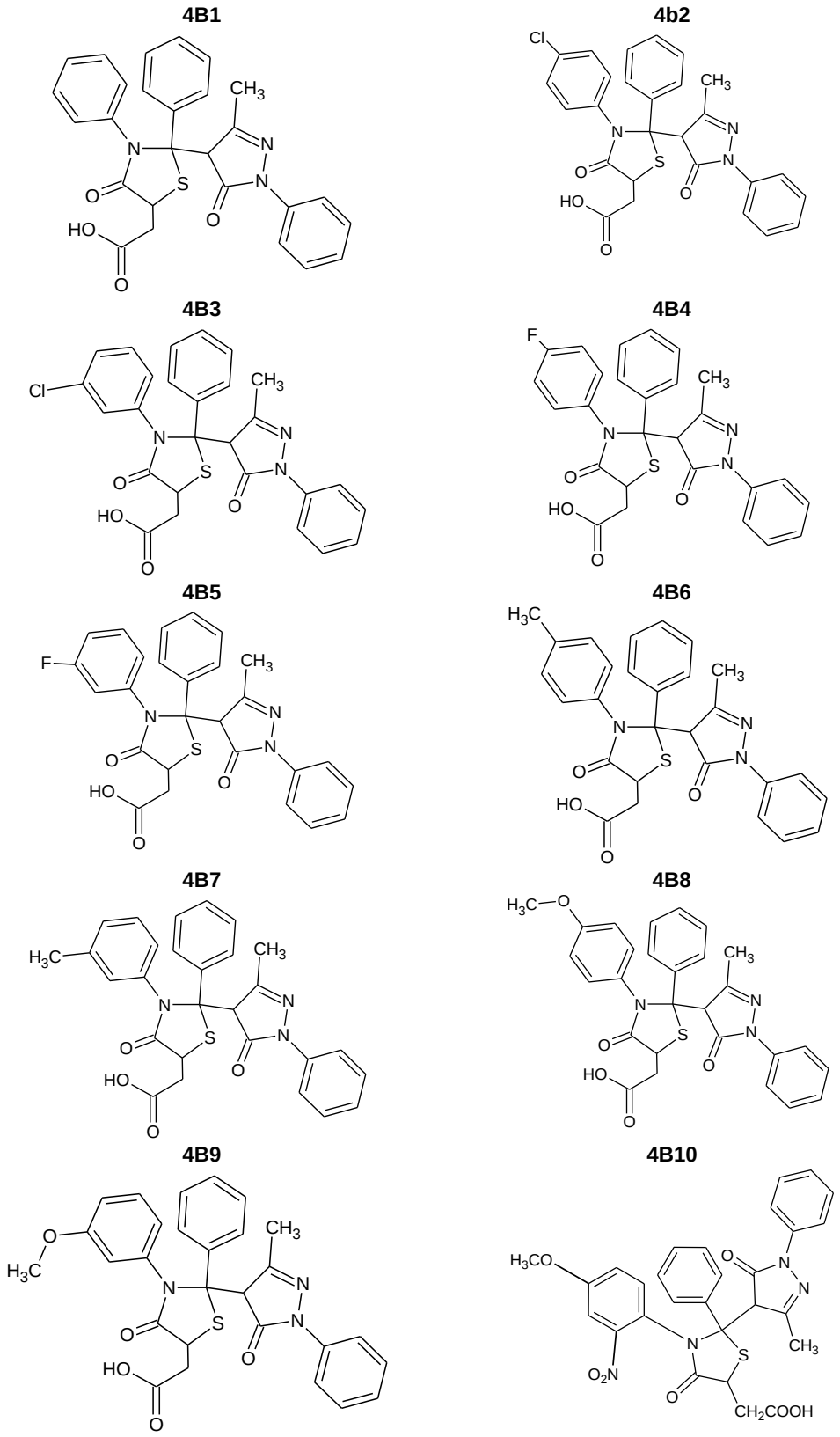

Figure 2: Chemical structures of synthesised compounds

^{1}H NMR spectra of all the synthesized compounds showed a characteristic signal of one doublet at 3.33 - 3.34 δ ppm, indicating the presence of CH_2 of thiazolidinone protons, one singlet at 3.7 δ ppm for the presence of CH of pyrazolone proton and one triplet at 0.9 δ ppm

confirming the CH_3 of pyrazolone proton. The details of ^1H NMR spectral analysis are presented in Table 3.

^{13}C NMR data of all the synthesized compounds further supported these results and revealed the existence of CH-C carbon of Pyrazolone linked Thiazolidinone at 55.12 - 55.23 ppm. The details of ^{13}C NMR spectral analysis are presented in Table 4.

Table 5 shows the ESI mass spectral data for the synthesized compounds. The Mass spectra exhibited consecutive intense molecular ion peak m/z $(M + H)^+$ of the titled compounds. The details of Mass spectral analysis are presented in Table 5.

The results of elemental analysis data of synthesized compounds are shown in Table 6. The results of the elemental analysis were in close agreement with those calculated and founded values.

Table 5: ESI mass spectra data for the synthesized compounds

Compound code	M/z values
4B1	486.7 $(M+H)^+$
4B3	520.1 $(M+H)^+$
4B5	503.2 (M^+)
4B7	501.2 $(M+H)^+$
4B9	516.7 $(M+H)^+$
4B10	561.3 $(M+H)^+$

Comparison of docking results with actual cytotoxic activity

The cytotoxic activity of the synthesised compounds was carried out using MCF-7 cell lines using the MTT assay method, and the results of the cytotoxic studies were compared with the docking results of the synthesised compounds. The data are presented in Table 7.

Table 3: ^1H NMR spectral data of synthesized compounds

Compound code	Chemical shift (δ) ppm
4B1	0.9δ (t, 3H, CH_3), 3.07δ (d, 2H, CH_2 of acetic acid), 3.7δ (s, 1H, CH of pyrazolone), 3.8δ (s, 1H, CH of thiazolidinone), 6.81δ-8.01δ (m, 15H, ArH), 11.0δ (s, 1H, OH of acetic acid)
4B3	0.9δ (t, 3H, CH_3), 3.07δ (d, 2H, CH_2 of acetic acid), 3.7δ (s, 1H, CH of pyrazolone), 3.8δ (s, 1H, CH of thiazolidinone), 6.77δ-8.0δ (m, 14H, ArH), 11.0δ (s, 1H, OH of acetic acid)
4B5	0.9δ (t, 3H, CH_3), 2.9δ (d, 2H, CH_2 of acetic acid), 3.7δ (s, 1H, CH of pyrazolone), 3.8δ (s, 1H, CH of thiazolidinone), 7.29δ-7.86δ (m, 14H, ArH), 11.3δ (s, 1H, OH of acetic acid)
4B7	0.9δ (t, 3H, CH_3), 2.16δ (t, 3H, $ArCH_3$), 3.37δ (d, 2H, CH_2 of acetic acid), 3.7δ (s, 1H, CH of pyrazolone), 3.8δ (s, 1H, CH of thiazolidinone), 6.67δ-8.02δ (m, 14H, ArH), 11.1δ (s, 1H, OH of acetic acid)
4B9	0.9δ (t, 3H, CH_3), 3.07δ (d, 2H, CH_2 of acetic acid), 3.55δ (t, 3H, methoxy), 3.7δ (s, 1H, CH of pyrazolone), 3.8δ (s, 1H, CH of thiazolidinone), 6.29δ-8.01δ (m, 14H, ArH), 11.1δ (s, 1H, OH of acetic acid)
4B10	1.2δ (t, 3H, CH_3), 3.07δ (d, 2H, CH_2 of acetic acid), 3.55δ (t, 3H, methoxy), 3.77δ (s, 1H, CH of pyrazolone), 3.8δ (s, 1H, CH of thiazolidinone), 6.73δ-8.02δ (m, 13H, ArH), 11.1δ (s, 1H, OH of acetic acid)

Table 4: ^{13}C NMR spectral data of synthesized compounds

Compound code	^{13}CNMR (100 MHz, (∂) ppm, CDCl, DMSO-d_6, 90:10)
4B1	16.12, 55.12, 101.44, 119.44, 123.87, 124.64, 126.06, 128.56, 128.87, 128.96, 130.56, 131.55, 137.54, 162.26, 165.76, 192.20
4B3	16.13, 55.12, 102.07, 119.40, 121.72, 123.72, 124.77, 125.99, 128.46, 128.89, 129.17, 130.02, 130.87, 161.74, 165.70, 192.20
4B5	15.93, 55.12, 103.67, 120.94, 126.83, 127.6, 128.51, 129.21, 131.97, 137.26, 148.11, 161.54, 192.20
4B7	15.92, 16.11, 21.27, 55.12, 101.32, 119.40, 120.59, 124.46, 126.79, 128.51, 128.78, 128.85, 128.93, 130.51, 139.12, 162.15, 165.73, 192.20
4B9	16.13, 55.23, 101.54, 108.80, 112.30, 115.94, 119.42, 124.65, 128.49, 128.87, 129.04, 129.80, 130.59, 131.71, 138.66, 138.91, 148.20, 159.94, 161.93, 15.71, 192.20
4B10	15.89, 15.96, 16.02, 16.23, 55.24, 55.91, 56.04, 106.23, 109.42, 120.23, 126.84, 128.49, 128.86, 128.96, 140.08, 150.81, 192.20

Table 6: Elemental analysis data of synthesized compounds

Compound code	(% Calculated)							(% Found)				
	C	H	N	O	S	Cl	F	C	H	N	O	S
4B1	66.79	4.77	8.65	13.18	6.60	-	-	66.59	4.52	8.55	13.01	6.44
4B3	62.36	4.26	8.08	12.31	6.17	6.82	-	62.25	4.13	7.91	12.11	6.09
4B5	64.40	4.40	8.34	12.71	6.37	-	3.77	64.20	4.21	8.26	12.51	6.21
4B7	67.32	5.04	8.41	12.81	6.42	-	-	67.12	4.88	8.32	12.61	6.22
4B9	65.23	4.8	8.19	15.52	6.22	-	-	65.12	4.71	8.01	15.31	6.11
4B10	59.99	4.32	9.99	19.98	5.72	-	-	59.80	4.15	9.88	9.80	5.60

Table 7: Comparison of docking results with actual cytotoxic activity of synthesised compounds

Compound code	Docking score	IC_{50} (µM)
4B1	-3.816	1234.00±15.56
4B2	-4.304	142.95±10.25
4B3	-4.581	259.30±11.31
4B4	-4.511	559.80±11.17
4B5	-6.614	001.17±00.21
4B6	-4.369	134.20±07.92
4B7	-4.571	256.70±09.90
4B8	-3.429	217.00±07.78
4B9	-4.333	2979.00±94.75
4B10	-4.486	3952.50±331.63
Standard (Tamoxifen)	-7.893	40.78±00.48

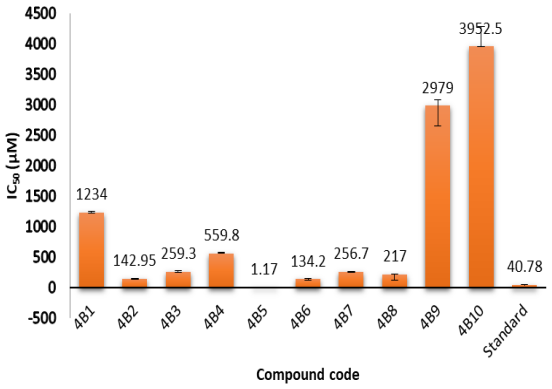

Figure 3: IC_{50} Values of the synthesised 4B1-4B10 series compounds by MTT assay

Docking study was carried out for comparing the cytotoxic activity of the designed compounds with synthesized compounds. The preferred ligands 4B Series (4B5) had a docking score value of -6.614. The docking score of standard cytotoxic agent Tamoxifen was comparatively lesser than their analogues with a scoring of -7.893 (Table 7). The lowest quantity of energy required for the configuration of a complex between ligand and receptor suggests tremendous binding resemblance. Very low energy shows that the ligand is protected in the cavity of the receptor. Analysis of docking results, in 4B Series (4B1, 4B2, 4B3, 4B4, 4B5, 4B6, 4B7, 4B8, 4B9 and 4B10) with the acetic acid in the fifth function of thiazolidinone rings suggest hydrogen bond with hydroxyl crew at Asp 863 and oxygen atom at Lys753.

All the synthesized compounds were screened for their *in vitro* cytotoxic activity against MCF-7 cell line (Human breast cancer) using the MTT assay. Tamoxifen was used as a reference drugs. The IC_{50} Values of the synthesized compounds (Figure 1) are presented in Table 7.

The influence of the different chemical groups on the observed cytotoxic activity against MCF-7 cell line was due to the incorporation of the m-Fluro phenyl group at the 3rd position of the thiazolidinone ring (Compound 4B5) which resulted in a potent inhibition of MCF7 cell line at 1.71 µM concentration. *In vitro* cytotoxicity analysis revealed that compound 4B5 has much stronger ($p < 0.05$) cytotoxic activity against the MCF-7 breast cancer cell line than other synthesised compounds (IC_{50} = 001.17 ± 00.21).

DISCUSSION

The synthesized Schiff bases 4B1-4B10 were subjected to solubility and melting point determination. The structures of the compounds (4B1, 4B3, 4B5, 4B7, 4B9 and 4B10) were established on the basis of FT-IR, ^{1}H NMR, ^{13}C NMR, and Mass spectral data. Results of cytotoxic activity are supported by docking study reports. The 4B Series (4B5) compounds revealed that these compounds are well accommodated in the energetic active site of enzyme and binding pattern of compounds. The 4B Series (4B5) proved that they had a strong interaction with the active site of the human epidermal growth factor receptor (3RCD).

The present studies confirmed through the results that these thiazolidinone compounds

synthesized from Schiff base (4B5) were effective and had the capability for inhibiting the growth of breast cancer MCF-7 cell by 50 % thereby exhibiting a potent degree of inhibition. The 4B5 compounds revealed that the docking score was equivalent to an *in vitro* cytotoxic activity which showed that these compounds were strongly accommodated in the energetic active site of enzyme and binding pattern of compounds. The compound (4B5) had a strong interaction with the active site of the Human Epidermal Growth factor receptor (3RCD).

CONCLUSION

The synthesised thiazolidinone compound has a potent cytotoxic activity against breast cancer cells. The strongest anti-proliferation result against MCF-7 cells is exhibited by compound 4B5 which produced more apoptosis than the other compounds. Thus, 4B5 possesses the potential for further development into an effective anticancer agent.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Author Sumathy Arunachalam framed the scheme for the study, synthesized and characterised the inhibitor, and performed the biochemical experiments and antimicrobial assays. Authors Suresh Ramalingam and Srinivasan Nagarajan managed the analyses of the results. Author Gowrishankar Narayanasamy Lachmanan reviewed and commented on the manuscript. All the authors have read and approved the manuscript.

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