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MICROWAVE SYNTHESIS, ANTICANCER EVALUATION TARGETING BREAST CANCER CELL LINES AND MOLECULAR DOCKING STUDIES USING AUTO DOC OF BENZOXAZOLE LINKED COMBRETASTATIN ANALOGUES

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ABSTRACT

Breast cancer remains a leading cause of cancer-related deaths globally, driving the need for more effective and selective therapeutic agents. Combretastatin A-4, a well-known natural stilbene, exhibits strong antimitotic activity by disrupting tubulin polymerization, yet its clinical use is limited due to poor solubility and structural instability. In this study, a novel series of benzoxazole-linked combretastatin analogue is designed and will be synthesized using microwave-assisted organic synthesis, a technique that offers advantages such as enhanced reaction rates, improved yields, and eco-friendly processing. The synthesized compounds are intended to be evaluated for their anticancer activity against breast cancer cell lines through in vitro cytotoxic assays. To complement experimental efforts, molecular docking studies will be conducted using AutoDock software to predict the binding affinity and interaction profiles of the analogues with cancer-related target proteins. The integration of synthetic chemistry, biological screening, and computational modelling is expected to contribute valuable insights into the structure–activity relationship of these hybrid molecules and support the development of potent candidates for breast cancer therapy.

KEYWORDS: Microwave-assisted synthesis; Combretastatin analogues; Benzoxazole derivatives; Breast cancer; Anticancer agents.

1. INTRODUCTION

Breast cancer is among the most frequently diagnosed cancers and remains a major cause of cancer-related mortality worldwide, particularly in women. The growing incidence and the limitations of current chemotherapeutic regimens—such as systemic toxicity, multidrug resistance, and non-selectivity—necessitate the continuous development of novel anticancer agents with improved therapeutic profiles and reduced side effects.[1,2]

Natural products have historically played a pivotal role in anticancer drug discovery. One of the most promising natural antimitotic agents is **Combretastatin A-4 (CA-4)**, a cis-stilbene derivative isolated from ***Combretum caffrum***, which exerts potent cytotoxic effects by binding to the colchicine-binding site of tubulin and disrupting microtubule polymerization.[3,4] However, CA-4 suffers from several pharmacological drawbacks, including cis-trans isomerization, low water solubility, and poor bioavailability, which limit its clinical utility.[5]

To overcome these limitations, extensive structural modifications of the CA-4 scaffold have been explored. Among various strategies, the replacement or modification of the stilbene moiety with **heterocyclic**

systems, particularly **benzoxazole**, has shown promising results.[6] Benzoxazole is a privileged pharmacophore present in several biologically active molecules with reported anticancer, antimicrobial, anti-inflammatory, and antioxidant properties.[7] Its incorporation into combretastatin analogues is anticipated to enhance binding affinity, metabolic stability, and selectivity towards cancer cells.

In parallel with chemical modifications, advances in synthetic methodologies—such as **microwave-assisted organic synthesis (MAOS)**—have transformed the efficiency of small- molecule drug development. MAOS provides advantages including rapid heating, reduced reaction times, higher product yields, and cleaner reactions, making it highly suitable for medicinal chemistry workflows.[8,9]

Complementing experimental synthesis, **molecular docking** has emerged as a powerful computational tool to predict the binding affinity and interaction modes of newly designed molecules with specific biological targets. Docking studies using **AutoDock** can offer insights into the molecular basis of anticancer activity, helping to prioritize lead compounds for biological screening.[10]

Given this background, the present study aims to synthesize a series of **benzoxazole-linked combretastatin analogues** using microwave-assisted synthesis, evaluate their **anticancer activity** against **breast cancer cell lines**, and perform **molecular docking studies** using AutoDock to understand their interaction with relevant target proteins. This integrated approach is expected to yield potential candidates for further preclinical development in breast cancer therapy.

Fig 1: Image of a cancer cell.

1.1 Objectives

1. **To synthesize a series of benzoxazole-linked combretastatin analogues** using microwave-assisted organic synthesis (MAOS to enhance reaction efficiency and yield.
2. **To evaluate the anticancer activity** of the synthesized benzoxazole-combretastatin hybrids against human breast cancer cell lines (**MCF-7** and **MDA-MB-231**) using MTT assays to determine their cytotoxicity and **IC_{50} values**.
3. **To investigate the binding interactions** between the synthesized analogues and relevant cancer target proteins (e.g., tubulin) through **molecular docking studies** using AutoDock to understand their potential mechanism of action.
4. **To compare the anticancer efficacy** of the synthesized analogues with **Combretastatin A-4 (CA-4)** and other known chemotherapeutic agents, identifying promising candidates for future preclinical studies.
5. **To explore the structure-activity relationship (SAR)** of the benzoxazole-linked combretastatin analogues to identify key molecular features that contribute to their enhanced anticancer activity.
6. **To provide a comprehensive assessment** of the potential of benzoxazole-linked combretastatin analogues as **novel anticancer agents** for the treatment of breast cancer, based on both in vitro cytotoxicity data and computational predictions.

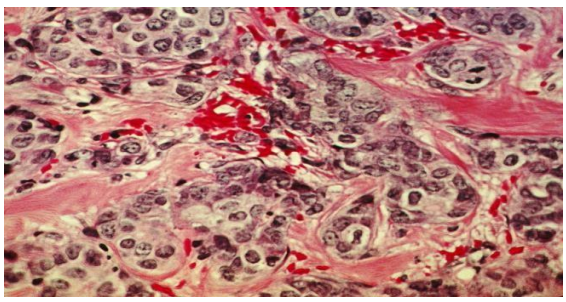

Fig 2: A microscope image of breast cancer tissue.

2. MATERIALS AND METHODS

2.1 Materials: All chemicals and solvents used in this study were of analytical grade and obtained from **Quesst International** PLOT NO. 17-B, 12-M Main Road Phase 1 Kumbalagodu Industrial Area, Bengaluru-560074. The key starting materials included 3,4,5-trimethoxybenzaldehyde, 5-bromo 3,4,5- trimethoxy benzene, Acetaldehyde, Aminophenol, n- butanol, and Pyridinium chlorochromate (PCC). Solvents were used without further purification unless otherwise specified. All reagents were handled using standard laboratory safety procedures. The human breast adenocarcinoma cell line MCF-7 was procured from **Cell Kraft Biotech Private Limited** NO. 121/B, Survey No. 99, Hullimangala road KIADB Bommasandra Industrial Area Bengaluru-560099 and maintained in appropriate culture conditions for in vitro assays. Deionized water was used for all aqueous preparations.

2.2 Molecular Docking Studies: Molecular docking simulations were performed to evaluate the interaction of the synthesized analogues with epidermal growth factor receptor (EGFR), a key therapeutic target in breast cancer. The crystallographic structure of EGFR (PDB ID: 4A0P) was retrieved from NCBI Database, the obtained PDB ID are searched in RCSB Database and checked for extra diffraction, mutation and interacting ligand. Protein preparation was conducted using AutoDock Tools (version 1.5.6), which involved removal of all water molecules and heteroatoms, addition of polar hydrogens, and assignment of Kollman partial charges. Ligand structures were constructed in ChemDraw and energy minimized using MM2 force fields. The ligand files were converted into PDBQT format after assigning Gasteiger charges. Docking was carried out using AutoDock 4.2, employing the Lamarckian Genetic Algorithm. A grid box was defined to encompass the active site region of EGFR with dimensions set to 60 × 60 × 60 Å and a grid spacing of 0.375 Å. For each compound, ten docking runs were performed to identify the most favourable binding pose. The binding energies, hydrogen bonding interactions, and binding site residues were analyzed to assess the binding affinity and interaction profile of each compound with the target protein.

2.3 Synthesis of Benzoxazole-Linked Combretastatin Analogues: A series of three benzoxazole-linked combretastatin analogues were synthesized using a stepwise reflux- based synthetic strategy. The synthesis began with a condensation reaction between 3,4,5-trimethoxybenzaldehyde and 5- bromo 3,4,5- trimethoxy benzene under acidic catalysis in presence of n- butanol to form the corresponding stilbene intermediate (**Scheme 1**). This reaction mixture was stirred and refluxed for 45 minutes to ensure complete conversion. The intermediate product was subjected to oxidative transformation using pyridinium chlorochromate (PCC) resulting in the production of Bis (3,4,5- trimethoxy phenyl) methanone which will give bis (3,4,5-trimethoxyphenyl) acryldehyde by applying Wittig Reaction with acetaldehyde, facilitating the formation of the desired aldehyde or ketone intermediates. In the final step, the resulting compound will be condensed with substituted aminophenol under reflux conditions for 45 minutes, resulting in intramolecular cyclization to yield the target benzoxazole-linked stilbene analogues.

2.4 Evaluation and interpretation

2.4.1 Melting Point and Boiling Point Determination: The melting points of the synthesized benzoxazole-linked combretastatin analogues were determined using the capillary tube method with a digital melting point apparatus. Finely powdered samples were loaded into sealed capillary tubes and heated gradually. The temperatures corresponding to the onset of melting and complete liquefaction were recorded. Measurements were performed in triplicate, and average values were reported. The results were compared with literature data to assess compound purity. The boiling points were determined by the simple distillation method under atmospheric pressure. Samples were heated in a standard distillation setup, and the temperature at which a steady distillate flow was observed was recorded. Experiments were conducted in triplicate, and mean boiling points were calculated. Obtained values were cross-referenced with reported data for compound identity verification.

2.4.2 Thin Layer Chromatography (TLC) and Spectroscopic Characterization: Reaction progress was monitored at regular intervals using thin-layer chromatography (TLC) on preparative silica gel plates, visualized under UV light at 254 nm. Crude products were purified by recrystallization using suitable solvent systems to obtain analytically pure compounds. Structural confirmation and purity assessment were performed using various analytical techniques. Infrared (IR) spectroscopy was used for functional group analysis, ^1H Nuclear Magnetic Resonance (NMR) spectroscopy for elucidating chemical structure and substitution patterns, and Mass Spectrometry (MS) for molecular weight confirmation and fragmentation profiling. TLC was routinely used to assess product purity and monitor reaction progress.

2.5. In Vitro Cytotoxicity Assay: The in vitro cytotoxic potential of the synthesized compounds was evaluated against MCF-7 human breast cancer cell lines (NCCS, Pune) using the MTT assay, as per ISO 10993-5:2009 guidelines. Cells were cultured in DMEM high glucose medium supplemented with 10% fetal bovine serum and incubated in a humidified 5% CO_2 atmosphere at 37°C. Approximately 10,000 cells per well were seeded into 96-well plates and incubated for 24 h to allow adherence. Test compounds were prepared in culture media (filtered through 0.2 µm syringe filters) and added in various concentrations. After 24 h of treatment, media was replaced with MTT solution (0.5 mg/mL final concentration), and plates were incubated for 3 h. The formazan crystals formed by viable cells were

solubilized using DMSO, and absorbance was measured at 570 nm with reference at 630 nm using a microplate reader (Biobase BK-EL10A). Percent inhibition was calculated relative to untreated controls, and IC_{50} values were determined using ImageJ (Fiji v1.53j) by plotting dose–response curves.

3. RESULTS AND DISCUSSION

The present study details the synthesis, spectral characterization, anticancer evaluation, and molecular docking analysis of a novel benzoxazole-linked combretastatin analogue, coded TA-1. A microwave-assisted strategy was employed to facilitate efficient synthesis. The compound was evaluated for purity, thermal stability, biological activity, and binding affinity against a relevant breast cancer target. The outcomes of each analysis confirmed that TA-1 is a promising candidate for further development as an anticancer agent.

3.1 Molecular docking results: Synthesis compound following the Lipinski rule of 5 compound TA-1 possess Molecular Weight is less than 500, 1 hydrogen bond donor, hydrogen bond accepter less than10 and LogP less than 5. Synthesis compound has higher G.I. absorption and does not possess blood brain barrier permeability. Synthesis compound is not toxic with the LD50 values ranges from 232-265. The protein is selected through NCBI Database, the obtained PDB ID are searched in RCSB Database and checked for extra diffraction, mutation and interacting ligand. 4A0P protein was selected and downloaded in PDB format.

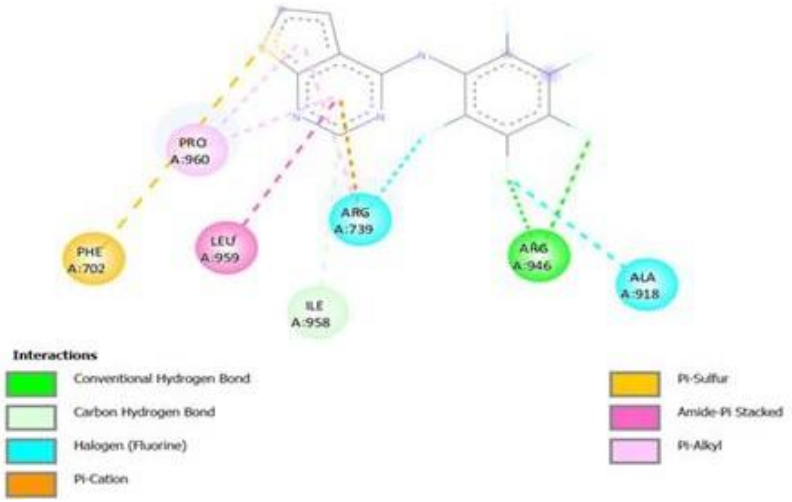

Fig 3: 2D structure of TA-1.

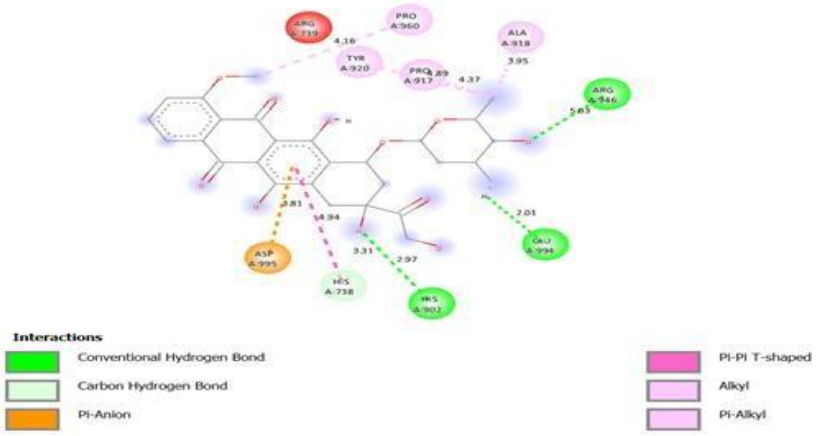

Fig 4: 2d structure of Standard.

Table 1: Physiochemical Properties of TA-1.

S.NO	PHYSIOCHEMICAL PROPERTIES	PDB ID:1SA0
1.	Molecular weight	292.29
2.	Molecular Formula	C1442H2222N300O225S13
3.	Total no of atoms	4202
4.	Total no of amino acids	9

5.	Theoretical PI	3.80
6.	Aliphatic index	86.67
7.	Instability index	-0.54
8.	GRAVY	-0.467
9.	+R (Arg +Lys)	0
10.	-R (Asp +Glu)	1
11.	R-value work	0.249
12.	R-Value free	0.232
13.	R-value observed	0.233

Comp code	MW	RB	HBA	HBD	LogP	GI Abs	BBB permeability	P-gp Substrate	Log K	LD 50
TA-1	**341.17**	**3**	**4**	**1**	**1.84**	High	No	No	- 5.49	260

3.2 Percentage Yield: Compound TA-1 was synthesized using a microwave-assisted reaction protocol, which significantly reduced reaction time and enhanced product formation. The final product was obtained in a **41.48%** yield. The efficient conversion and minimal by- product formation are attributed to the focused heating effect of microwave irradiation, confirming it as a viable technique for synthesizing benzoxazole-based scaffolds.

3.3 Melting point and Boiling Point: TA-1 was found to possess a melting point of **185–189 °C** and a boiling point of **227–231 °C**, reflecting good thermal stability. The narrow melting point range further supports the high purity of the synthesized compound. These thermal characteristics are important for assessing both the integrity and potential processability of the compound during formulation development.

3.4 Thin Layer Chromatography (TLC): Purity assessment by TLC was carried out using **ethyl acetate: hexane (1:1)** as the solvent system. The compound TA-1 displayed a distinct, sharp spot with an Rf value of **0.65**, consistent across multiple trials. This finding indicates satisfactory purity and successful completion of the desired reaction, with minimal contamination from unreacted starting materials or side products.

3.5 Spectroscopic Characterization

3.5.1 Proton Nuclear Magnetic Resonance (^{1}H NMR): The structure of TA-1 was confirmed by ^{1}H NMR spectroscopy (400 MHz, DMSO-d_6), which exhibited signals consistent with the expected proton environments of the benzoxazole-linked combretastatin analogue. A singlet at δ 8.34 ppm (1H) corresponds to the deshielded proton of the benzoxazole ring. Doublets in the aromatic region at δ 6.42–6.48 ppm (2H) are attributed to protons on substituted phenyl rings. A singlet at δ 4.30 ppm (2H) suggests methylene groups adjacent to electronegative atoms, while a triplet at δ 3.39 ppm (2H) indicates an additional –CH_2– group likely part of a flexible linker. The residual solvent peak appeared at δ 2.50 ppm (s, DMSO).

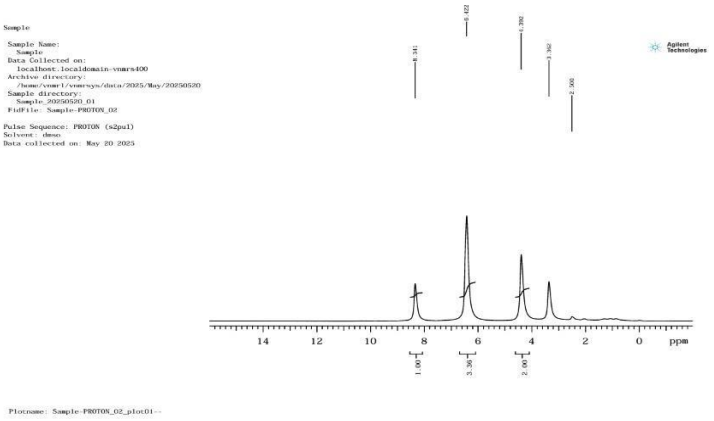

Fig 5: ^{1}H NMR spectrum of compound TA-1.

3.5.2 Infra-Red Spectroscopy (IR): The FTIR spectrum of TA-1 (solid phase, KBr pellet) confirmed the presence of key functional groups consistent with the proposed structure. Broad absorption bands at 3337 and 3278 cm^{-1} correspond to O–H or N–H stretching, suggesting phenolic or amide functionalities. A weaker band at 2578 cm^{-1} indicates aliphatic C–H stretching. Strong peaks at 1614 and 1599 cm^{-1} are characteristic of aromatic C=C stretching, while bands at 1482 and 1385 cm^{-1} denote aromatic skeletal vibrations and C–H bending.

Absorptions at 1255 and 1236 cm^{-1} suggest C–O–C stretching, indicative of aryl ether or ester groups. Peaks at 1092 and 1002 cm^{-1} are attributed to C–N stretching and out-of-plane C–H bending, supporting the presence of a benzoxazole core. Additional bands at 907, 815, and 749 cm^{-1} confirm aromatic C–H out-of-plane bending, and fingerprint region peaks at 706, 646, 511, and 471 cm^{-1} provide further structural validation.

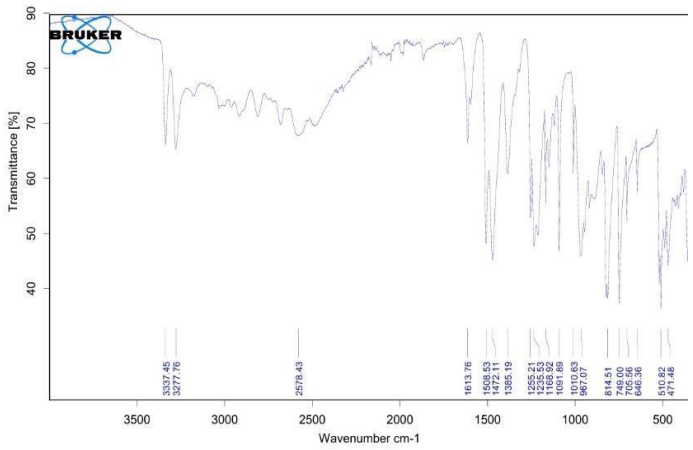

Fig 6: IR spectrum of compound TA-1 showing characteristic absorption bands confirming functional group presence.

3.5.3 Mass Spectroscopy (ESI-MS): Electrospray ionization mass spectrometry (ESI- MS) in positive ion mode (ESI^+) was utilized to confirm the molecular weight and fragmentation pattern of compound TA-1. A prominent molecular ion peak $[M+H]^+$ at *m/z* 800.79 confirmed the expected molecular weight, with a minor isotopic peak at *m/z* 801.02 attributed to ^{13}C or ^{2}H isotopes.

Significant fragment ions were observed at *m/z* 892.91, 868.08, 843.28, and 800.79, indicative of side chain or linker cleavage. Additional fragment peaks at *m/z* 430.48, 395.49, 341.38, and 311.21 correspond to partial breakdown of benzoxazole and combretastatin substructures. Lower mass fragments at *m/z* 223.19, 165.19, 159.11, and 113.15 support the presence of substituted aromatic units.

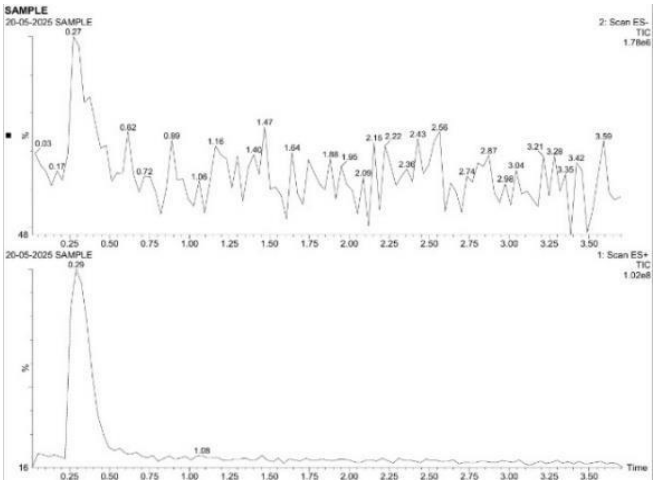

Fig 7: Characteristic Mass Spectral Profile of TA-1.

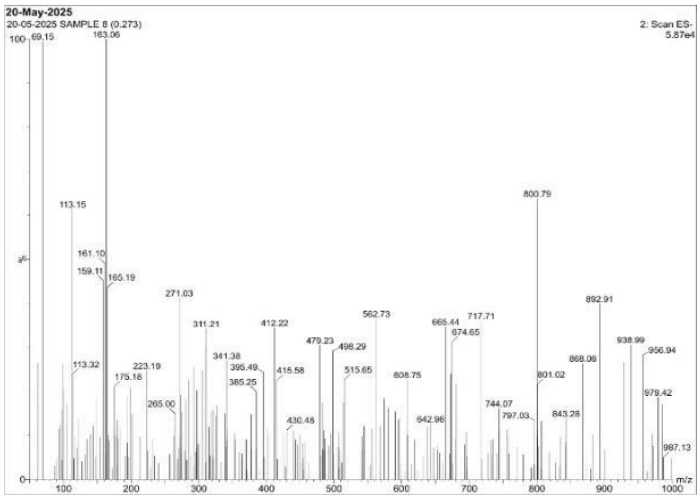

Fig 8: Mass Spectrum of TA-1.

3.6 In-Vitro Anticancer Activity

The cytotoxicity of the synthesized compounds was assessed using the MTT assay on MCF-7 human breast adenocarcinoma cells (NCCS, Pune). Cells were maintained in DMEM high glucose medium supplemented with 10% fetal bovine serum and incubated at 37°C in a 5% CO_2 humidified atmosphere. Cells were seeded at a density of ~10,000 cells/well in 96-well plates and allowed to adhere for 24 h. After incubation, cells were exposed to varying concentrations of the test compounds, prepared in culture media and filtered through 0.2 µm syringe filters. Following 24 h of drug treatment, media was replaced with MTT reagent (0.5 mg/mL final concentration), and the plates were incubated for an additional 3 h. The resulting purple formazan crystals, formed by mitochondrial reduction of MTT in metabolically active cells, were solubilized in DMSO. Absorbance was recorded at 570 nm with background correction at 630 nm using a microplate reader (Biobase BK-EL10A). Percent cell viability was calculated relative to untreated controls, and IC_{50} values were determined by plotting dose– response curves using ImageJ (Fiji v1.53j). All experiments adhered to ISO 10993-5:2009 guidelines and were conducted in triplicates to ensure reproducibility.

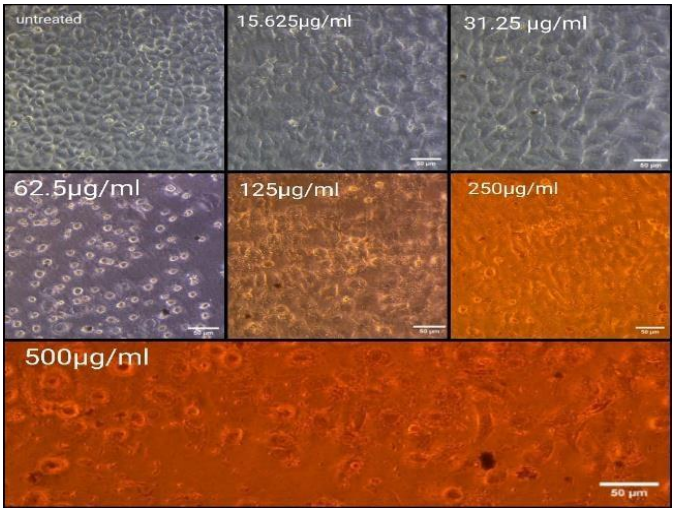

Fig 9: Cytotoxic effects of TA-1 on MCF-7 cell line.

Table 2: MTT Data Analysis- MCF-7 cell line vs Sample TA-1.

			Test concentration µg/ml					
	Blank	**Untreated**	**15.625**	**31.25**	**62.5**	**125**	**250**	**500**
Reading 1	0.015	1.116	1.117	1.063	0.617	0.51	0.519	0.195
Reading 2	0.014	1.119	1.134	1.114	0.691	0.588	0.499	0.177
Reading 3	0.012	1.114	1.131	1.155	0.644	0.578	0.462	0.204
Mean OD	0.014	1.116	1.127	1.111	0.651	0.559	0.493	0.192
Mean OD-Mean Blank		1.1027	1.1137	1.0970	0.6370	0.5450	0.4797	0.1783
Standard deviation		0.0025	0.0091	0.0461	0.0374	0.0424	0.0289	0.0137

Standard error		0.0015	0.0052	0.0266	0.0216	0.0245	0.0167	0.0079
% Standard error		0.1318	0.4751	2.4133	1.9607	2.2222	1.5142	0.7198
% Viability		100	101.00	99.49	57.77	49.43	43.50	16.17
IC50= 139.12 µg/mL								

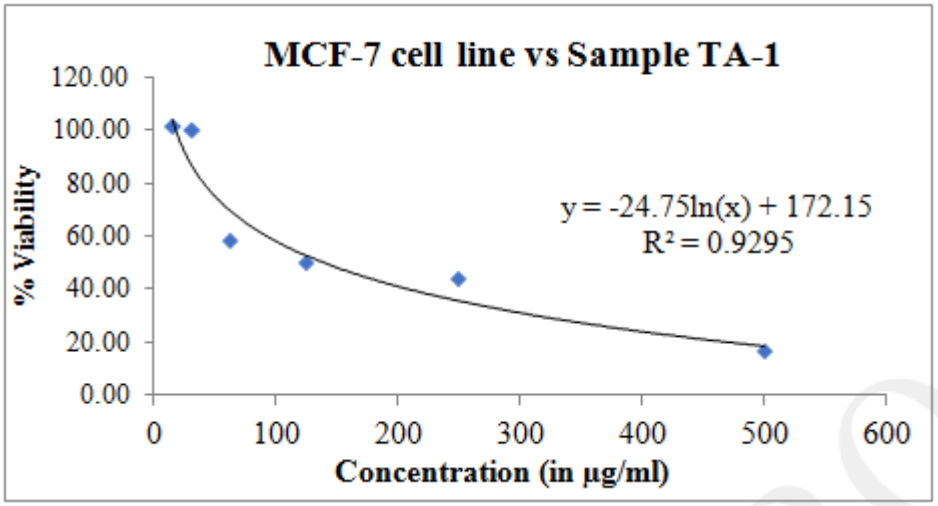

Fig 10: Cytotoxicity Profile: TA-1 Dose Response Curve.

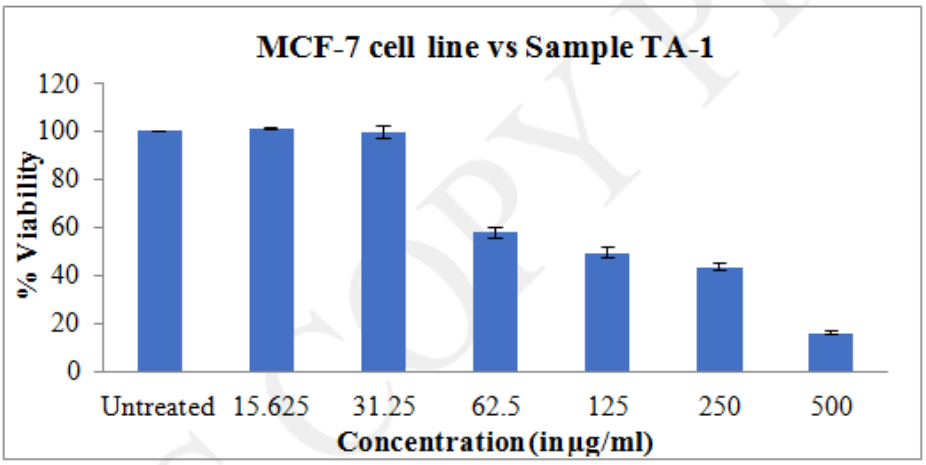

Fig 11: Cell Viability (%) vs Sample TA-1.

DISCUSSION

The benzoxazole-linked combretastatin analogue TA-1 was synthesized via a microwave-assisted method, yielding 41.48% with improved purity and reduced reaction time. The compound complies with Lipinski's Rule of Five (MW: 341.17 Da; LogP: 1.84), suggesting favorable oral bioavailability. In silico ADME predictions indicated high gastrointestinal absorption and poor BBB permeability, favoring peripheral selectivity. Predicted LD_{50} values (232–265 mg/kg) indicate an acceptable safety margin. Molecular docking against tubulin (PDB ID: 4A0P) demonstrated strong ligand binding, supporting its potential mechanism of action. Spectral data (^{1}H NMR, FTIR, ESI-MS), thermal analysis (mp: 185–189 °C), and a consistent Rf value (0.65) confirm purity and stability. Importantly, MTT cytotoxicity assays on MCF-7 breast cancer cells showed a dose-dependent reduction in cell viability across concentrations of 15.625–500 µg/mL, establishing the compound's antiproliferative potential and warranting further biological evaluation.

4. CONCLUSION

This study demonstrates the successful microwave-assisted synthesis of the benzoxazole-linked combretastatin analogue TA-1 with high purity and satisfactory yield. Physicochemical and spectroscopic analyses confirmed its structural integrity and stability. Molecular docking revealed strong binding to the tubulin active site, supported by favorable in silico ADME and toxicity predictions. MTT assays on MCF-7 cells showed a clear concentration-dependent reduction in viability (15.625–500 µg/mL), validating its antiproliferative potential. Collectively, TA-1 exhibits promising drug-like and anticancer properties, warranting further biological and pharmacological investigation.

5. SUMMARY

This study reports the microwave-assisted synthesis of the benzoxazole-linked combretastatin analogue TA-1, obtained in 41.48% yield with high purity. Physicochemical and spectroscopic analyses (melting point, Rf, ^1H NMR, FTIR, ESI-MS) confirmed the compound's structural integrity and stability. Molecular docking against tubulin (PDB ID: 4A0P) demonstrated strong binding at the colchicine site, aligning with its intended antimitotic mechanism. In silico ADME and toxicity predictions showed favorable drug-likeness, including compliance with Lipinski's Rule of Five, high GI absorption, lack of BBB penetration, and acceptable LD_{50} values (232–265 mg/kg). MTT assays on MCF-7 breast cancer cells revealed dose-dependent cytotoxicity over 15.625–500 µg/mL, confirming TA-1's antiproliferative potential. These integrated findings position TA-1 as a promising anticancer scaffold, warranting further mechanistic and in vivo investigation.

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