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Synthesis and *in vitro* anticancer activity assessment of bis-1,3,4-thiadiazol-2-amino flavone derivatives

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ABSTRACT

2-Amino-1,3,4-thiadiazole is a heterocyclic scaffold associated with a wide range of pharmacological activities, including anticancer, antibacterial, antifungal, antiviral, antiparasitic, anticonvulsant, anticoagulant, antidiabetic, and antitumor effects. This structural motif has attracted considerable interest due to its therapeutic potential. Although several synthetic approaches have been proposed for this class of compounds, most are neither straightforward, economical, nor environmentally friendly. Given the established biosynthetic pathway of pyrimidines, it is reasonable to hypothesize that thiadiazoles may possess significant therapeutic value. The presence of a sulfur atom in the thiadiazole ring enhances liposolubility, while the mesoionic nature of these compounds facilitates cellular membrane penetration. In this study, novel 1,3,4-thiadiazole derivatives of bis-flavone imine were synthesized from bis-flavone imine ethyl acetate derivatives using a convergent synthetic strategy, yielding satisfactory product quantities. The chemical structures of the synthesized compounds were characterized using Fourier-transform infrared (FT-IR) spectroscopy and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. *In vitro* anticancer assays on MCF-7 human breast cancer cells have revealed that compound T8 can achieve a 50% inhibition of cell viability (IC_{50}) at a concentration of 29.23 μg/mL. Compound T4 has exhibited a comparable anticancer effect, with an IC_{50} value of 30.02 μg/mL.

1. Introduction

Breast cancer remains a major global health concern, affecting approximately 14% of women worldwide and ranking as the most frequently diagnosed malignancy among females[1]. Standard treatment modali-

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Table 1. A comparison of the *in vitro* cytotoxic effects of the synthesized compounds T1–T8 on the MCF-7 human breast cancer cell-line.

Synthesized compounds		**IC_{50}** (μg/mL)
T1	5,5'-(((((2,8-diphenyl-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene)) bis(4,1-phenylene))bis(methylene))bis(oxy))bis(1,3,4-thiadiazol-2-amine)	113.29
T2	5,5'-(((((2,8-bis(4-(dimethylamino)phenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))bis(4,1-phenylene))bis(methylene))bis(oxy))bis(1,3,4-thiadiazol-2-amine)	102.04
T3	5,5'-[(((((2,8-bis(4-nitrophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene)]bis(1,3,4-thiadiazol-2-amine)	145.45
T4	5,5'-(((((2,8-bis(4-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis (methylene)) bis (1,3,4-thiadiazol-2-amine)	30.02
T5	5,5'-(((((2,8-bis(2,4-dichlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	119.10
T6	5,5'-(((((2,8-bis(4-fluorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis (methylene))bis(1,3,4-thiadiazol-2-amine)	154.66
T7	5,5'-(((((2,8-bis(4-bromophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis (methylene))bis(1,3,4-thiadiazol-2-amine)	48.91
T8	5,5'-(((((2,8-bis(2-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis (methylene))bis(1,3,4-thiadiazol-2-amine)	29.23

ties include surgery, radiotherapy, and chemotherapy, each tailored to the individual patient. However, a persistent challenge in the pharmacological management of breast cancer is the rapid emergence of drug resistance. This underscores the urgent need for the development of novel anticancer agents[2].

A series of new glucoside derivatives incorporating the 1,3,4-thiadiazole moiety were synthesized from D-glucose and 5-amino-1,3,4-thiadiazole-2-thiol using a convergent synthetic strategy, yielding promising results[3]. The anticancer potential of amide derivatives of imidazo[2,1b][1,3,4]thiadiazoles has been evaluated *in vitro* against four human cancer cell lines, including MCF-7 and MDA-MB-231; both associated with breast cancer[4].

Additional 1,3,4-thiadiazole derivatives were synthesized from α-methyl cinnamic acid[5]. Thiadiazoles are well-documented for their antitumor activity. Four isomeric forms of thiadiazole exist: 1,3,4-thiadiazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, and 1,2,5-thiadiazole; among these, 1,3,4-thiadiazole derivatives are considered the most promising for therapeutic applications[6].

The antitumor activity of 1,3,4-thiadiazole derivatives includes both fused-ring systems and simple 2,5-disubstituted structures[7]. These compounds exert their effects through various molecular targets, such as carbonic anhydrase IX, Src and Abl kinases, and topoisomerase II[8]. Under diverse reaction conditions, substituted carboxylic acids condense with thiosemicarbazide to yield 2-amino-5-substituted 1,3,4-thiadiazoles[9].

2. Methodology

The melting point (m.p.) of each compound was determined using an electrothermal melting point

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apparatus (Model 200D / MP-200D-HR). Fourier-transform infrared (FT-IR) spectra of all synthesized compounds were recorded using a Bruker spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained at the University of Tehran, Iran, using a Bruker Avance III 500 spectrometer (499 MHz for ^{1}H-NMR), with dimethyl sulfoxide (DMSO) as the solvent. All reagents were purchased from Sigma-Aldrich (Munich, Germany) and used without further purification.

The MCF-7 breast cancer cell line (ATCC HTB-22) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Bis-1,3,4-thiadiazol-2-amino flavones were synthesized by gradually adding imine flavone ethyl acetate (1 mmol), phosphoryl chloride (3 mmol), and thiosemicarbazide (2 mmol) to a reaction flask under stirring at room temperature for 2–3 hours. The mixture was then refluxed at 90°C for 8 h. Upon completion, the reaction mixture was poured into ice-cold water and neutralized with ammonium hydroxide solution.

Cell viability was assessed using the MTT assay (Sigma-Aldrich). Cells were harvested, trypsinized, and seeded at a density of 1×10^4 cells/well in 96-well plates containing 200 µL of fresh medium per well. After 24 h of incubation, a monolayer was established and cells were treated with the test compounds for 48 h at 37°C in a humidified atmosphere containing 5% CO_2. Five serial dilutions were tested, ranging from 100 to 6.25 µg/mL. After 24 h of treatment, the supernatant was removed and 200 µL/well of MTT solution (0.5 mg/mL in phosphate-buffered saline) was added. Plates were incubated for an additional 4 h at 37°C. The supernatant was then discarded, and 100 µL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 570 nm using an ELISA reader (Model Wave XS2, BioTek, USA) in order to quantify cell viability[10].

3. Results and Discussion

The chemical structures of the synthesized compounds were confirmed by FT-IR and ^{1}H-NMR spectroscopy. Detailed compound names are presented in Table 1, and key data are summarized below.

Compound T1: orange solid; yield: 68.7%; m. p.: 331°C–337.2°C, ^{1}H-NMR spectrum (499 MHz, DMSO) peaks δ 7.50 (s, 1H), 7.23 (m, 10H), 6.78 (m, 8H), 6.54 (s, 1H) proton at (-NH_2), 6.24 (s, 1H), 5.87(s, 1H), 5.42 (s, 4H) (-CH_2).

Compound T2: green solid; yield: 68.7%; m. p.: 381°C–385.5°C; ^{1}H-NMR spectrum (499 MHz, DMSO) peaks δ 7.95 (s, 1H), 7.61 (m, 10H), 7.20 (m, 8H), 6.83 (s, 1H) proton at (-NH_2), 6.41 (s, 1H), 5.95 (s, 1H), 5.18 (s, 4H) (-CH_2).

Compound T3: white solid; yield: 77%; m. p.: 387.2°C–389.5°C; ^{1}H-NMR spectrum (499 MHz, DMSO) peaks δ 7.75 (s, 1H), 7.43 (m, 10H), 7.16 (m, 8H), 6.76 (s, 1H) proton at (-NH_2), 6.38 (s, 1H), 5.86 (s, 1H), 5.53 (s, 4H) (-CH_2), and 3.30 (s, 12H) proton of [-$N(CH_3)_2$].

Compound T4: dark yellow solid; yield: 74%; m. p.: 367.2°C–380.5°C; ^{1}H-NMR spectrum (499 MHz, DMSO) peaks δ 7.75 (s, 1H), 7.36 (m, 10H), 7.00 (m, 8H), 6.62 (s, 1H) proton at (-NH_2), 6.33 (s, 1H), 5.88 (s, 1H), 5.42 (s, 4H) (-CH_2).

Compound T5: violet solid; yield: 78.4%; m. p.: 392.4°C–395.7°C; ^{1}H-NMR spectrum (499 MHz, DMSO) peaks δ 7.88 (s, 1H), 7.63 (m, 10H), 7.14 (m, 8H), 6.70 (s, 1H) proton at (-NH_2), 6.28 (s, 1H), 5.92 (s, 1H), 5.57 (s, 4H) (-CH_2).

Compound T6: brownish-yellow solid; yield: 58.7%; m. p.: 395.3°C–397.7°C; ^{1}H-NMR spectrum (499 MHz, DMSO) peaks δ 7.95 (s, 1H), 7.41 (m, 10H), 7.08 (m, 8H), 6.75 (s, 1H) proton at (-NH_2), 6.47 (s, 1H), 5.96 (s, 1H), 5.52 (s, 4H) (-CH_2).

Compound T7: dark brown crystals; yield: 54.3%; m. p.: 388.3°C–392.8°C; ^{1}H-NMR spectrum (499 MHz, DMSO) peaks δ 7.65 (s, 1H), 7.34 (m, 10H), 6.85 (m, 8H), 6.52 (s, 1H) proton at (-NH_2), 6.26 (s, 1H), 5.94 (s, 1H), 5.43 (s, 4H) (-CH_2).

Compound T8: faint red solid; yield: 61.7%; m. p.: 348°C–353.7°C; ^{1}H-NMR spectrum (499 MHz, DMSO) peaks δ 7.84 (s, 1H), 7.40 (m, 10H), 7.08 (m, 8H), 6.74 (s, 1H) proton at (-NH_2), 5.35 (s, 1H), 5.03 (s, 1H), 4.43 (s, 4H) (-CH_2).

The FT-IR spectra of the bis-1,3,4-thiadiazol-2-amino flavones (T1–T8) revealed character-

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istic peaks: symmetric and asymmetric stretching of the amino group (–NH_2) at 3416.92–3471.32 cm^{-1}, stretching of the imine group (–C=N) at 1612.08–1692.68 cm^{-1}, aromatic C=C stretching at 1484.50–1629.18 cm^{-1}, ether C–O stretching at 1164.10–1251.87 cm^{-1}, and cyclic ether C–O stretching at 1034.77–1177.20 cm^{-1}.

The ^{1}H-NMR spectra (499 MHz, DMSO) have revealed multiple signals: aromatic protons at δ 7.23–7.63 ppm; substituted aromatic protons at δ 7.00–7.85 ppm; singlet signals for amine protons at δ 6.52–6.83 ppm; cyclic C=C protons at δ 5.03–6.38 ppm; and methylene (–CH_2) protons of ether groups at δ 4.43–5.57 ppm.

The assessment of the *in vitro* anticancer activity has revealed that compound T8 inhibited 50% of cell viability (IC_{50}) at a concentration of 29.23 μg/mL. Compound T4 has exhibited comparable activity with an IC_{50} of 30.02 μg/mL, compound T7 has exhibited a moderate activity (IC_{50}: 48.91 μg/mL), while the remaining compounds had IC_{50} values exceeding 100 μg/mL, as summarized in Table 1.

4. Conclusion

Novel thiosemicarbazide and 1,3,4-thiadiazole derivatives were successfully synthesized and evaluated for their *in vitro* anticancer activity against a human breast cancer cell line (MCF-7). Structural characterization was performed using FT-IR and ^{1}H-NMR spectroscopy. The IC_{50} values of these compounds against the MCF-7 cells ranged from 29.23 to 154.66 μg/mL; notably, compounds T4 and T8 demonstrated significant cytostatic activity against the MCF-7 cells.

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Conflicts of interest

None exist.

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References

1. Balasubramanian R., Rolph R., Morgan C., Hamed H. Genetics of breast cancer: management strategies and risk-reducing surgery. *Br. J. Hosp. Med. (Lond.)* 80(12), 720–725, 2019. DOI: 10.12968/hmed.2019.80.12.720
2. Costa B., Amorim I., Gärtner F., Vale N. Understanding breast cancer: from conventional therapies to repurposed drugs. *Eur. J. Pharm. Sci.* 151, 105401, 2020. DOI: 10.1016/j.ejps.2020.105401
3. Chen M., Zhang X., Lu D., Luo H., Zhou Z., Qin X., *et al.* Synthesis and bioactivities of novel 1,3,4-thiadiazole derivatives of glucosides. *Front. Chem.* 9, 645876, 2021. DOI: 10.3389/fchem.2021.645876
4. Sridhar G., Palle S., Vantikommu J., Gangarapu K. Design, synthesis, and biological evaluation of amide derivatives of imidazo[2,1-b][1,3,4]thiadiazole as anticancer agents. *Synth. Commun.* 50(21), 3221–3233, 2020. DOI: 10.1080/00397911.2020.1797814
5. Gür M., Şener N. Synthesis and characterization of some new 1,3,4-thiadiazole compounds derived from α-methyl cinnamic acid and their energetics and spectral analyses based on density functional theory calculations. *Anadolu Univ. J. Sci. Technol. A Appl. Sci. Eng.* 18(5), 939–950, 2017. DOI: 10.18038/aubtda.310899
6. Janowska S., Paneth A., Wujec M. Cytotoxic properties of 1,3,4-thiadiazole derivatives – a review. *Molecules* 25(18), 4309, 2020. DOI: 10.3390/molecules25184309

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7. Rashdan H.R.M., Farag M.M., El-Gendey M.S., Mounier M.M. Toward rational design of novel anti-cancer drugs based on targeting, solubility, and bioavailability exemplified by 1,3,4-thiadiazole derivatives synthesized under solvent-free conditions. *Molecules* 24(13), 2371, 2019. DOI: 10.3390/molecules24132371
8. Esteves-Souza A., Rodrigues-Santos C.E., Del Cistia Cde N., Silva D.R., Sant'anna C.M., Echevarria A. Solvent-free synthesis, DNA-topoisomerase II activity and molecular docking study of new asymmetrically N,N'-substituted ureas. *Molecules* 17(11), 12882–12894, 2012. DOI: 10.3390/molecules171112882
9. Kaviarasan L., Gowramma B., Kalirajan R., Mevithra M., Chandralekha S. Molecular docking studies and synthesis of a new class of chroman-4-one fused 1,3,4-thiadiazole derivatives and evaluation for their anticancer potential. *J. Iran. Chem. Soc.* 17, 2083–2094, 2020. DOI: 10.1007/s13738-020-01913-2
10. Gornowicz A., Szymanowska A., Mojzych M., Bielawski K., Bielawska A. The effect of novel 7-methyl-5-phenyl-pyrazolo[4,3-e]tetrazolo[4,5-b][1,2,4]triazine sulfonamide derivatives on apoptosis and autophagy in DLD-1 and HT-29 colon cancer cells. *Int. J. Mol. Sci.* 21(15), 5221, 2020. DOI: 10.3390/ijms21155221

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