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Synthesis and Evaluation Biological Activity of Bis-Flavones Imines Ethyl Acetate Derivatives

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ABSTRACT

Flavonoids and their derivatives are considered a source of therapeutic agents, particularly for cancer treatment. This research aimed to synthesize biflavonoid ethyl acetate derivatives via biflavonoid iminate alkylation and evaluate the anticancer properties of these compounds. The compounds were synthesized by heating a mixture of biflavonoid iminate, chloroethyl acetate, and anhydrous potassium carbonate in acetone, using thin-layer chromatography (TLC). FT-IR, ^{1}H-NMR, and ^{13}C-NMR techniques were employed to determine the chemical structure and characterize the resulting compounds. MTT assays were performed on the human breast cancer cell line MCF-7 to assess anticancer activity. The compounds exhibited significant cytotoxicity, indicating their potential therapeutic use in breast cancer treatment, based on IC50 values. Therefore, the synthesized biflavonoid derivatives offer promising anticancer activity, and further investigation into their other medicinal properties is important as a basis for therapeutic applications in various fields.

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1. Introduction

Flavonoids have garnered significant attention due to their wide range of biological activities. A diverse class of polyphenolic compounds, they are characterized by their anticancer, antioxidant, and anti-inflammatory properties. One important flavonoid derivative is 3-hydroxy-2-(4-dimethylaminophenyl) benzopyran-4-one, which has been the subject of extensive research and chemical modification to enhance its pharmacological efficacy [1, 2]. Numerous novel alkyl and ester derivatives have been synthesized through structural conversion using various alkylating and acylating agents, such as bromoacetyl coumarin, benzyl chloride, methyl iodide (CH_3I), allyl bromide, acetamide chloride ($CH_3ClCONH_2$), and chloroacetyl. The Scutellaria plant contains scutellarin, which has recently been identified as a potent cytotoxic agent against human leukemia cells.

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Production of scutellarin and its methylated derivatives [3, 4]. Recent studies have shown that scutellarin exerts its anticancer effect by modulating the PI3K/Akt/NFκB signaling pathway, significantly inhibiting the development of hepatocellular carcinoma [5].

Other research has also included the preparation a series of amino-alkylated flavones were prepared starting from 5-hydroxy-4′,7-dimethoxyflavone. The synthesized compounds were then assessed for their antiproliferative effects in vitro against three human cancer cell lines-HeLa, HCC1954, and SK-OV-3- utilizing the Cell Counting Kit-8 (CCK-8) assay [6]. Similarly, indole-substituted flavone derivatives have shown promising antiproliferative effects in MCF-7 and HCT-116 cells through Akt pathway inhibition, confirming their therapeutic potential [7].

In recent years, significant research efforts have been dedicated to the synthesis of 2(3)-substituted flavones and isoflavones, as well as 2,3-disubstituted chromones [8]. Notable advancements have been made in the development of chromone–pyrazole fused structures, azachromones, and azachromanones have been summarized [9, 10]. Furthermore, the construction of triazole-bridged flavonoid dimers has emerged as a promising approach for overcoming multidrug resistance, particularly through potent inhibition of breast cancer resistance protein (BCRP) at nanomolar concentrations, thus providing a novel scaffold for anticancer drug design [11, 12].

The amide process is crucial as a prerequisite for a high level of biological activity, as the acid itself and its ester have shown considerably less performance. Functional isoflavones have also demonstrated efficacy in preventing hyperlipidemia, type 2 diabetes, atherosclerosis, and non-alcoholic fatty liver disease. Other isoflavone-rich fractions extracted from fermented plants have exhibited selective cytotoxicity against HeLa cells, highlighting the ongoing importance of natural isoflavone structures in cancer therapy [13, 14]. Pyrannoisoflavones have been shown to act as butyrylcholinesterase inhibitors and thus could be used in the treatment of Alzheimer's disease. Methods for obtaining biologically active substances from isoflavones can rely not only on modifying functional groups but also on recycling the unstable pyron moiety [15, 16].

Despite the progress made in flavonoid research, a research gap remains in understanding and identifying all their anticancer properties, particularly with regard to diflavonoids. Therefore, this research aims to synthesize diflavonoid ethyl acetate derivatives through the alkylation of diflavonoid imines and to evaluate their anticancer properties, in order to enhance and develop current scientific knowledge and explore new approaches to cancer treatment.

2. Method

2.1. Sample Preparation

A concentration-controlled working mixture was prepared using high-purity (98%) chemicals from reliable commercial sources. The mixture contained diflavonoid imines (0.1 mmol), ethyl acetate (0.2 mmol), and anhydrous potassium carbonate (K_2CO_3, 0.45 g) in acetone (20 mL). The mixture was stirred continuously and heated at 60 °C for 6–8 hours. Thin-layer chromatography (TLC) was then used to monitor the reaction, which involved a mixture of acetates, with hexane as the mobile phase. After the reaction was complete, the

mixture was cooled and filtered using filter paper of a specified diameter. It was then concentrated under reduced pressure. Recrystallization and column chromatography were performed to obtain the crude compound.

2.2. Cytotoxicity Evaluation via MTT Assay

The MTT assay was employed to assess the cytotoxic activity of the synthesized compounds against the MCF-7 human breast cancer cell line. Cells were seeded in a 96-well plate at a density of 1×10^4 cells/well and incubated for 24 hours to allow for cell attachment. Following this, cells were treated with various concentrations of the test compounds and incubated for an additional 72 hours at 37 °C under 5% CO_2.

Subsequently, MTT solution (2 mg/mL) was added to each well (28 µL/well), and the plates were incubated for 2.5 hours. After incubation, the medium was removed, and formazan crystals formed by viable cells were solubilized using dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader, and cell viability was expressed as a percentage relative to untreated controls.

3. Results and Discussion

The targeted bis-flavone ethyl acetate derivatives (A1–A8) were synthesized through the alkylation of bis-flavone imines with chloroethyl acetate in acetone under reflux conditions. The structures of the synthesized compounds were confirmed by FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopic analyses, as shown in Fig. 1.

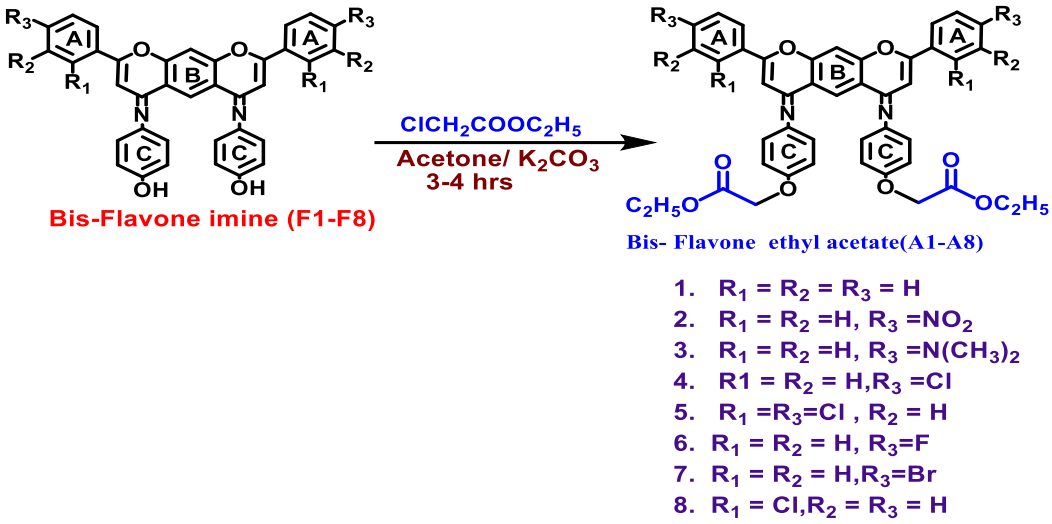


Fig. 1. The targeted bis-flavone ethyl acetate derivatives

The resulting compounds were characterized as follows:

- A1: 1H NMR (499 MHz, DMSO) δ 8.00 (s, 1H), 7.28-7.49 (m, 8H), 6.93-7.12 (m, 10H), 4.93 (s, 1H) proton at (C=C), 4.64 (s, 4H) (-CH2 of ether), 4.18 (q, 4H) (-CH2 ester), 1.23 (t, 6H) (-CH3 of ester). 13C NMR (126 MHz, DMSO) δ 168.28(C=O of ester), 161.99(C=N), 150.29(C-O of chromene), 145.91, 138.25, 133.48, 127.02, 125.29, 121.41, 116.98, 99.13(C=C), 66.25(-CH2 of ether), 61.42(-CH2 of ester), and 14.45(-CH3 of ester).

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- A2: 1H NMR (499 MHz, DMSO) δ 7.95 (s, 1H), 7.42-7.6 (m, 8H), 6.62-6.82(m, 10H), 5.66 (s, 1H) proton at (C=C), 5.04 (s, 4H) (-CH2 of ether), 4.77 (q, 4H) (-CH2 ester), 1.22 (t, 6H) (-CH3 of ester). 13C NMR (126 MHz, DMSO) δ 176.31(C=O of ester), 164.02 (C=N), 157.56 (C-O of chromene), 148.66, 142.83, 137.55, 135.43, 131.72, 125.15, 121.84, 119.84, 101.69, 88.55(C=C), 68.23(-CH2 of ether), 65.86(-CH2 of ester), and 22.47(-CH3 of ester).

- A3: 1H NMR (499 MHz, DMSO) δ 7.99 (s, 1H), 7.4-7.56 (m, 8H), 6.4-6.78(m, 10H), 5.09 (s, 1H) proton at (C=C), 4.94 (s, 4H) (-CH2 of ether), 4.37 (q, 4H) (-CH2 ester), 3.52[-N-CH3)2], 1.12 (t, 6H) (-CH3 of ester). 13C NMR (126 MHz, DMSO) δ 176.93(C=O of ester), 167.93 (C=N), 155.53 (C-O of chromene), 151.33, 144.40, 136.82, 133.18, 127.78, 123.88, 116.55, 112.26, 104.56, 88.26(C=C), 66.89(-CH2 of ether), 62.48(methylene of ester), and 16.74(methyl of ester).

- A4: 1H NMR (499 MHz, DMSO) δ 8.09 (s, 1H), 7.24-7.4 (m, 8H), 6.61-6.8 (m, 10H), 4.98 (s, 1H) proton at (C=C), 4.63 (s, 4H) (-CH2 of ether), 4.10 (q, 4H) (-CH2 ester), 1.20 (t, 6H) (-CH3 of ester).

- A5: 1H NMR (499 MHz, DMSO) δ 8.29 (s, 1H), 7.38-7.62 (m, 8H), 6.49-6.68 (m, 10H), 5.13 (s, 1H) proton at (C=C), 4.94 (s, 4H) (-CH2 of ether), 4.14 (q, 4H) (-CH2 ester), 1.17 (t, 6H) (-CH3 of ester). 13C NMR (126 MHz, DMSO) δ 174.58 (C=O of ester), 161.99 (C=N), 155.28 (C-O of chromene), 149.37, 140.94, 138.28, 133.45, 132.28, 127.09, 125.22, 121.49, 116.98, 101.08, 87.69 (C=C), 68.15(-CH2 of ether), 62.61(-CH2 of ester), and 17.05(-CH3 of ester).

- A6: 1H NMR (499 MHz, DMSO) δ 8.05 (s, 1H), 7.53-7.69 (m, 8H), 6.44-6.68 (m, 10H), 5.19 (s, 1H) proton at (C=C), 4.82 (s, 4H) (-CH2 of ether), 4.17 (q, 4H) (-CH2 ester), 1.20 (t, 6H) (-CH3 of ester). 13C NMR (126 MHz, DMSO) δ 171.81 (C=O of ester), 166.38 (C=N), 163.74 (C-F), 161.18 (C-O of chromene), 157.08, 146.37, 128.65, 124.81, 122.27, 116.52, 107.14, 102.64, 91.86(C=C), 62.50(-CH2 of ether), 56.50(-CH2 of ester), and 19.02(-CH3 of ester).

- A7: 1H NMR (499 MHz, DMSO) δ 7.96 (s, 1H), 7.08-7.3 (m, 8H), 6.3-6.52 (m, 10H), 5.71 (s, 1H) proton at (C=C), 5.09 (s, 4H) (-CH2 of ether), 4.13 (q, 4H) (-CH2 ester), 1.09 (t, 6H) (-CH3 of ester). 13C NMR (126 MHz, DMSO) δ 170.47 (C=O of ester), 167.93 (C=N), 155.53 (C-O of chromene), 143.93, 136.82, 133.12, 128.02, 123.91, 122.27, 116.52, 112.28, 104.56, 83.83 (C=C), 65.24 (-CH2 of ether), 61.62(-CH2 of ester), and 16.74 (-CH3 of ester).

- A8: 1H NMR (499 MHz, DMSO) δ 7.95 (s, 1H), 7.34-7.62 (m, 8H), 6.62-6.92 (m, 10H), 5.16 (s, 1H) proton at (C=C), 4.98 (s, 4H) (-CH2 of ether), 4.16 (q, 4H) (-CH2 ester), 1.17 (t, 6H) (-CH3 of ester). 13C NMR (126 MHz, DMSO) δ 172.69 (C=O of ester), 164.73 (C=N), 158.82 (C-O of chromene), 140.78, 135.57, 132.68, 129.40, 128.14, 125.06, 118.08, 114.16, 105.81, 86.96 (C=C), 66.26 (-CH2 of ether), 61.14(-CH2 of ester), and 21.20 (-CH3 of ester)

The FT-IR spectra of compounds A1–A8 exhibited characteristic absorptions corresponding to key functional groups. The stretching vibrations of the ester carbonyl group (C=O) appeared in the range 1644.46–1738.17 cm^{-1}, while the C=N imine stretching vibrations were observed at 1596.99–1633.31 cm^{-1}. Peaks associated with C=C stretching

vibrations of the aromatic rings were present in the range 1508.92–1604.05 cm^{-1}. Additionally, bands corresponding to the C–O stretching of ester groups appeared between 1301.81–1200.14 cm^{-1}, and those of the cyclic ether C–O were found within 1103.91–1213.95 cm^{-1}.

The 1HNMR chemical shifts of bis-flavone ethyl acetate (A1-A8) showed multiple signal peaks at 7.26-7.68 ppm of substituted aryl, and multiple signal peaks at 6.42-7.04 ppm chromene group, signal singlet peaks at 4.93-5.71ppm, signal singlet peaks at4.64-5.09 ppm methylene of ether group, quartet signal peaks at 4.13-4.77 ppm methylene of ester group, and triplet signal peaks at 1.09-1.23 ppm methyl of the ester group. The 13CNMR appears to peak at 168.28-176.93 ppm of (C=O) of the ester group, 161.99-166.38 ppm of the (C=N) group, 150.29-161.18 ppm of (-C-O) of chromene group, 83.83-99.13 (C=C) cyclic, 62.5-68.15(-CH2) of ether, 56.5-65.86ppm (-CH2) of the ester group, and 14.45-22.47 ppm (-CH3) of the ester group.

3.1. Anticancer activity

The IC50 values were computed in Table 1 and used to estimate the cytotoxic effect of bis-flavone ethyl acetate (A1-A8) at varied concentrations, for each cell line. Due to their anticancer properties. The results also demonstrated that A1, A4, and A8 are more limited against cancer than other synthetic compounds.

Table 1. Cytotoxicity (IC_{50}) of A1–A8 Compounds on Cancer Cell Line

Synthesized compounds		IC_{50} values µg/mL
A1	4,6-bis(1-((4-hydroxyphenyl)imino)-3-phenyl allyl)benzene-1,3-diol	2.49802
A2	4,6-bis(1-((4-hydroxyphenyl)imino)-3-(4-nitrophenyl)allyl)benzene-1,3-diol	8.79927
A3	4,6-bis(3-(4-(dimethyl amino)phenyl)-1-[(4 hydroxy phenyl) imino]allyl)benzene-1,3-diol	4.5504
A4	4,6-bis[3-(4-chlorophenyl)-1-((4-hydroxyphenyl)imino)allyl]benzene-1,3-diol	2.95968
A5	4,6-bis(3-(2,4-dichlorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	10.4406
A6	4,6-bis(3-(4-fluorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	7.04704
A7	4,6-bis(-3-(4-bromophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	10.3126
A8	4,6-bis(-3-(2-chlorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	3.71607

4. Conclusion

The resulting compounds A1, A4, and A8 exhibited potent anticancer activity, demonstrating significant cytotoxic effects against the human breast cancer cell line MCF-7. This was evidenced by their relatively low IC_{50} values, indicating promising potential for developing these compounds as anticancer agents. These findings highlight the importance of research targeting flavonoids as novel therapeutic sources, as these derivatives show

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potential therapeutic capabilities that could contribute to the development of effective cancer drugs. This study opens new avenues for future research, underscoring the need to further investigate the biological activities of these compounds to enhance their efficacy as oncological therapies.

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