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

Ali H. Alsadoon,[1*] Saif Sahib Radhi [2], Shaker A. Abdul Hussein[3]

[1] Assistant lecture, Department of Organic chemistry, College of pharmacy, University of Babylon, Iraq, Email: alialsoud1987@gmail.com , Orcid: 0000-0001-9832-6540
[2] Assistant lecture, Department of Organic chemistry, College of pharmacy, University of Babylon, Iraq, Email: pharm.saif.sahib@uobabylon.edu.iq
[3] lecture, Department of pharmaceutical chemistry, College of pharmacy, University of Babylon, Iraq, Email: phar.shaker.awad@uobabylon.edu.iq

* Corresponding Author: Ali H. Alsadoon, alialsoud1987@gmail.com
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ABSTRACT

Some plant chemicals might help fight diseases like cancer. To explore this, scientists made new versions of double-flavonoid molecules using a specific chemical reaction. Instead of combining parts directly, they heated them together - one flavonoid-like base, plus a carbon-rich additive, along with a drying agent, all stirred into a clear liquid solvent. Progress was tracked by spotting changes on small glass plates dipped lightly into solutions. After confirming formation, each substance got tested further through light absorption patterns and magnetic responses to map out its makeup clearly. Then came testing against tumor cells grown in lab dishes - specifically those from human breast tissue affected by cancer - measuring how well the newly formed substances slowed down harmful growth. Starting strong, these compounds showed clear toxicity to cells, suggesting they might help fight breast cancer when judged by IC50 levels. With that in mind, the lab-made biflavonoids appear effective against cancer, yet digging deeper into their healing traits could open doors across medicine.

1. Introduction

Scientists look closely at flavonoids because they show many health-related effects. These plant-based substances often fight cancer, reduce oxidative damage, slow inflammation. Among them, one modified form - 3-hydroxy-2-(4-dimethylaminophenyl) benzopyran-4-one - attracts special interest. Research continues on this molecule, adjusting its structure to boost how well it works inside the body [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. Production of scutellarin and its methylated

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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].

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

From trusted suppliers, pure substances were mixed carefully by weight. Inside a flask went two flavan-based compounds along with ethyl acetate and dry KCO powder, all suspended in twenty milliliters of acetone. Heating began - steady at sixty degrees - with constant stirring keeping things uniform throughout. Six to eight hours passed before progress checks started. A small sample got spotted on a plate each time, moving through hexane to reveal components slowly separating. Once done, warmth dropped away as the

solution settled down to room conditions. Paper filtration followed, using circular sheets cut precisely to fit the funnel shape. A vacuum pulled the mixture tighter. From there, crystals formed while a column cleaned the rest along the way.

2.2. Cytotoxicity Evaluation via MTT Assay

To check how toxic the new compounds were to MCF-7 breast cancer cells, researchers used the MTT assay. Inside each well of a 96-well plate, exactly 10,000 cells were placed. Afterward, they waited one full day so the cells could stick properly. Then came exposure: different strengths of the substances were added. For three more days, everything stayed warm, at body temperature, inside a controlled environment filled with carbon dioxide. Later on, every well got 28 microliters of MTT solution, two milligrams per milliliter in strength; then came a wait lasting two and a half hours. Once that time passed, out went the liquid, replaced by DMSO to dissolve the purple formazan made only by living cells. The microplate reader checked how much light hit 570 nanometers after passing through each sample. Compared to untouched wells, these results turned into percentages showing how many cells stayed alive.

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.

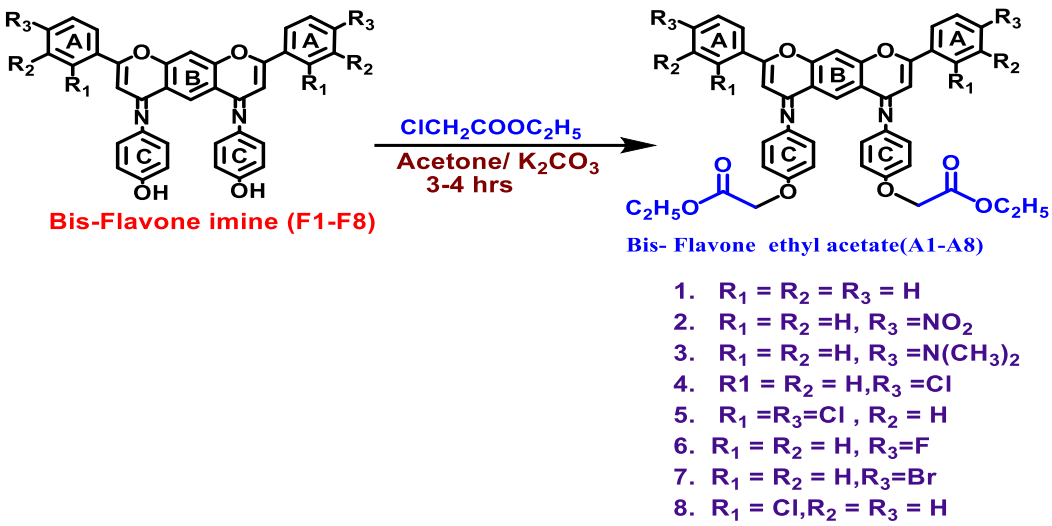

Fig. 1. The targeted bis-flavone ethyl acetate derivatives

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

Compounds A1, A4, and A8 showed strong activity against cancer, hitting hard on MCF-7 cells taken from human breast tissue. Low IC numbers gave clear proof - these substances pack a punch where it counts. Flavonoid-based work suddenly looks more valuable, thanks to how these versions behave under lab conditions. Instead of fading out, interest grows when you see what they might do down the road in medicine. Further

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digging into their actions feels less like guesswork now, more like stepping onto firmer ground toward better treatments.

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