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Article

Microwave-Assisted Synthesis and Characterization of Flavone–Thiazole–Aryl Hybrids with Potential Anticancer and Antiparasitic Activity

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Abstract

Flavone–thiazole–aryl hybrid molecules based on 6-aminoflavone and 5-arylidene-4-aminothiazol-2(5*H*)-ones were synthesized and subjected to physicochemical and biological studies. Microwave-assisted synthesis was performed in two steps. First, an aminolysis reaction of isorhodanine with 6-aminoflavone was carried out to achieve the corresponding hybrid flavone-thiazole **3**, which was later subjected to a Knoevenagel condensation with selected aromatic aldehydes, yielding 5-arylidene derivatives **5a**–**5i**. The resulting hybrids were purified and characterized by UV–Vis, NMR, and HR-MS (ESI). In the UV–Vis spectra of all compounds, two characteristic bands were noted. The UV–Vis spectra in DMF of the studied flavone–thiazole–aryl hybrids consist of two major bands with maxima appearing at 280–288 nm, corresponding to band II and 383–399 nm, corresponding to band I, which clearly distinguish them from the large group of modified flavonoids. Among the compounds tested on human bladder cancer 5637 cells, (5*Z*)-5-[(4-hydroxyphenyl)methylene]-4-[(4-oxo-2-phenyl-chromen-6-yl)amino]thiazol-2-one (**5b**) exhibited interesting micromolar activity (IC_{50} 2.37 μM). In addition, four of the tested compounds (**3**, **5f**, **5d**, and **5b**) presented noteworthy antiplasmodial activity against *P. falciparum* in the low micromolar range (IC_{50} 1.90–4.90 μM). The obtained group of flavone–thiazole–aryl hybrid molecules constitutes valuable starting points for further structural optimisation, which could usher in future novel active pharmaceutical ingredients and pave the way for novel therapeutic strategies.

Keywords: cancer; flavonoids; Knoevenagel condensation; thiazoles; microwave; bladder cancer cell line

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1. Introduction

Among many groups of natural compounds, flavonoids are of great importance. All flavonoids share a similar chemical structure, in which two benzene rings are joined by a three-carbon bridge that usually fuses into a benzopyran. Depending on the structural specifics of their general structure, flavonoids can be divided into different subclasses, like flavones, flavanones, flavonols, flavanols, flavanonols, isoflavones, and chalcones [1,2]. Flavonoids present many interesting medical applications. Especially interesting are flavones—2-phenyl-chromen-4-ones, known for their anticancer, antimutagenic, anti-inflammatory, antiviral, antibacterial, antifungal, antidiabetic, and antiparasitic properties [3–5]. What is especially important for our study is that aminoflavones, aminochalcones, and their derivatives exhibit interesting anticancer, antimicrobial, antimalarial, antiviral, and anti-inflammatory properties (Figure 1a,b) [6–8].

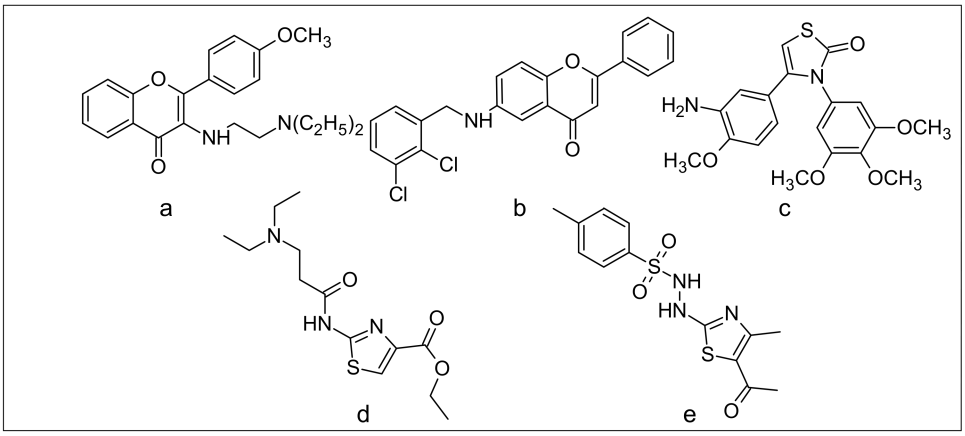


Figure 1. Selected biologically and medically active aminoflavone and thiazole derivatives (**a**–**e**).

When considering potential modifications of flavonoids with peripheral heterocyclic moieties, a very interesting heterocycle, thiazole, was employed. The structure of thiazoles is based on a five-membered aromatic cycle, which includes two heteroatoms—sulphur in the first position, and nitrogen in the third position. The thiazole structure has been found in many biological substances and serves as the basis for various pharmacological agents (Figure 1c–e) [9–12]. According to studies, the thiazole motif is responsible for various therapeutic effects, including antiparasitic, anti-inflammatory, anticancer, antimicrobial, antifungal, and antidiabetic [13–16]. Nowadays, thiazole derivatives constitute unique scaffolds in the development of new anticancer molecules. Thus, among the thiazole derivatives were identified PI3K [17], PARP1/2 [18], aurora kinase [19], tubulin [20], and Bcr-Abl/histone deacetylase dual inhibitors [21]. In addition, the thiazole derivative serves as the basis for the FDA-approved anticancer drug dasatinib [22]. Additionally, selected bioactive flavone, chalcone, or coumarin hybrids with various heterocycles showed promising anticancer and antimicrobial activity [23–26].

Modifying the thiazole core structure may alter the mechanisms of antitumor activity. Substituted 2-aminothiazol-2(5*H*)-ones are of special interest in modern medicinal chemistry for potential anticancer design. It is worth noting that the impact of a 5-ylidene fragment and 2-arylamino substituent in the thiazole structure can completely change the antitumor profile of the molecule and increase selectivity. Accordingly, 5-ylidene-2-(aryl)aminothiazol-2(5*H*)-ones demonstrated cytotoxicity against leukemic and breast cancer cells [27,28]. It is important to note that research data on 4-aminothiazol-2(5*H*)-ones, which are isomeric with 2-aminothiazol-2(5*H*)-one, is limited. Nevertheless, the various 4-aminothiazol-2(5*H*)-one derivatives also deserve attention [29]. Among this chemotype, prospective hits with anticancer activity against leukemic and breast tumour cell lines, with low toxicity towards pseudonormal cells, were identified [30].

The design of hybrid molecules constitutes one of the modern approaches in medicinal chemistry. Hybrid molecules are chemical compounds with two or more structural domains that have different biological functions, dual activity, and act as two different pharmacophores. This approach is quite successful for 2/4-aminothiazolones. Given the above, the goal of this work is the design of flavone–thiazole hybrid molecules based on 5-arylidene-4-aminothiazol-2(5*H*)-ones as a key scaffold.

2. Materials and Methods

2.1. General Experimental Details

Microwave syntheses were performed on an Anton-Paar Monowave 400 (Anton-Paar, Graz, Austria). All solvents and reagents were obtained from commercial suppliers (Merck, Rahway, NJ, USA) and used without further purification. The starting 4-thioxo-2-thiazolidinone (isorhodanine) was obtained according to previously described methods [31]. Melting point (m.p.) values were obtained on a "Stuart" Bibby apparatus (Bibby Scientific Ltd., Stone, Staffordshire, UK) and are uncorrected. The NMR spectra (^{1}H NMR, ^{13}C NMR) were recorded using a full-fledged, two-channel 400 MHz Bruker AvanceCore NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). All spectra were taken at 25 °C. Chemical shifts (d) are quoted in parts per million (ppm) and are referred to as residual solvent peaks. Coupling constants (*J*) are quoted in Hertz (Hz). The abbreviations s, d, dd, t, q and m refer to singlet, doublet, doublet of doublets, triplet, quintet, and multiplet, respectively. Thin-layer chromatography (TLC) was performed on silica gel Merck Kieselgel 60 F254 plates and DC Kieselgel 60 RP-18 F254 and visualised with UV illumination (λmax 254 or 365 nm). UV–Vis spectra were recorded on a Hitachi UV–Vis U-1900 spectrometer (Hitachi High-Technologies Corporation, Tokyo, Japan); λ_{max} (log ε), nm. HRMS spectra were recorded on Bruker Compact QTOF (Bruker Daltonics GmbH & Co. KG, Bremen, Germany); monoisotopic mass values were calculated with ChemCalc (https://www.chemcalc.org/, URL accessed on 4 September 2025) [32].

2.2. General Synthetic Procedures

2.2.1. General Procedure for the Synthesis of the Flavone–Thiazole Precursor—4-[(4-Oxo-2-phenyl-chromen-6-yl)amino]-5H-thiazol-2-one (**3**)

6-Aminoflavone **1** (712 mg, 3 mmol) and 4-thioxo-2-thiazolidinone **2** (400 mg, 3 mmol) were dissolved in glacial acetic acid (2 mL) in a round-bottom microwave reaction vial (2–5 mL, G10) and sealed in an inert gas (N_2) environment. The mixture was then transferred to a reactor and stirred under microwave irradiation at 120 °C for 10 min. The resulting precipitate was filtered with solvents in the following order: water, methanol, and diethyl ether. After filtering off the solvent residues and drying, a pale-yellow solid was formed. Compound 3 was obtained as a pale-yellow solid (750 mg, 74% yield): m.p. 268–270 °C, decomp.; R_f 0.472 (DCM/MeOH 20:1); UV–Vis (DMF): λ_{max}, nm (logε) 288 (4.53). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30 (s, 1H), 8.51 (d, *J* 2.7 Hz, 1H), 8.15–8.07 (m, 3H), 7.87 (d, *J* 9.1 Hz, 1H), 7.67–7.55 (m, 3H), 7.07 (s, 1H), 4.59 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 185.7, 177.3, 177.2, 163.1, 152.9, 136.4, 132.4, 131.6, 129.6, 127, 126.8, 124.1, 120, 115.6, 115.4, 107.1. HRMS ESI (pos) m/z calcd for $C_{18}H_{13}N_2O_3S$: 337.0647 [M+H]$^+$, found 337.0640, calcd for $C_{18}H_{12}N_2O_3SNa$: 359.0466 [M+Na]$^+$, found 359.0455.

2.2.2. General Procedure for the Synthesis of the Flavone–Thiazole–Aryl Hybrids (**5a–i**)

Flavone–thiazole precursor **3** (168 mg, 0.5 mmol) and corresponding aromatic aldehydes **4a–i** (0.5 mmol) were dissolved with sodium acetate (41 mg, 0.5 mmol) in glacial acetic acid (2 mL) in a round-bottom microwave reaction vial (2–5 mL, G10) and sealed in an inert gas (N_2) environment. The mixture was then transferred to a reactor and stirred under microwave irradiation at 140 °C for 10 min. The resulting precipitate was filtered with solvents in the following order: water, methanol, and diethyl ether. After filtering off the solvent residues and drying, a yellow solid was formed.

(5Z)-5-[(4-Methoxyphenyl)methylene]-4-[(4-oxo-2-phenyl-chromen-6-yl)amino]thiazol-2-one (**5a**)

p-Anisaldehyde **4a** (61 µL, 0.5 mmol) was applied as the benzaldehyde for condensation. Compound **5a** was obtained as a yellow solid (196 mg, 86% yield): m.p. 289–291 °C, decomp.; R_f 0.107 (DCM/MeOH 50:1); UV–Vis (DMF): λ_{max}, nm (logε) 390 (4.38), 282 (4.49). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 8.53 (d, *J* 2.7 Hz, 1H), 8.35 (dd, *J* 9.1, 2.8 Hz, 1H), 8.20–8.10 (m, 3H), 7.93 (d, *J* 9.1 Hz, 1H), 7.69–7.57 (m, 5H), 7.21–7.13 (m, 2H), 7.10 (s, 1H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 178.5, 176.9, 170.5, 162.7, 161.0, 155.8, 152.8, 136.3, 135.8, 132.0, 131.1, 129.7, 129.2, 128.3, 126.8, 126.4, 123.5, 119.4, 117.0, 115.1, 106.7, 55.5. HRMS ESI (pos) *m*/*z* calcd for $C_{26}H_{19}N_2O_4S$: 455.1065 [M+H]$^+$, found 455.1059.

(5Z)-5-[(4-Hydroxyphenyl)methylene]-4-[(4-oxo-2-phenyl-chromen-6-yl)amino]thiazol-2-one (**5b**)

4-Hydroxybenzaldehyde **4b** (61 mg, 0.5 mmol) was applied as the benzaldehyde for condensation. Compound **5b** was obtained as a yellow solid (143 mg, 65% yield): m.p. 295–297 °C, decomp.; R_f 0.434 (DCM/MeOH 20:1); UV–Vis (DMF): λ_{max}, nm (logε) 394 (4.37), 283 (4.48). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 10.36 (s, 1H), 8.52 (d, *J* 2.8 Hz, 1H), 8.34 (dd, *J* 9.1, 2.8 Hz, 1H), 8.18–8.11 (m, 3H), 7.92 (d, *J* 9.1 Hz, 1H), 7.67–7.56 (m, 3H), 7.53 (d, *J* 8.8 Hz, 2H), 7.10 (s, 1H), 6.98 (d, *J* 8.8 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 178.6, 176.9, 171.9, 170.5, 164.3, 162.7, 159.9, 154.5, 152.8, 137.5, 133.7, 132.3, 131.9, 130.2, 129.2, 128.3, 126.4, 125.5, 124.9, 119.3, 116.9, 116.5, 106.7. HRMS ESI (pos) *m*/*z* calcd for $C_{25}H_{16}N_2O_4SNa$: 463.0728 [M+Na]$^+$, found 463.0728.

(5Z)-5-[(3,5-Dimethoxyphenyl)methylene]-4-[(4-oxo-2-phenyl-chromen-6-yl)amino]thiazol-2-one (**5c**)

3,5-Dimethoxybenzaldehyde **4c** (83 mg, 0.5 mmol) was applied as the benzaldehyde for condensation. Compound **5c** was obtained as a yellow solid (178 mg, 73% yield): m.p. 275–277 °C, decomp.; R_f 0.125 (DCM/MeOH 50:1); UV–Vis (DMF): λ_{max}, nm (logε) 385 (4.19), 284 (4.45). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 8.53 (d, *J* 2.8 Hz, 1H), 8.35 (dd, *J* 9.1, 2.8 Hz, 1H), 8.17–8.10 (m, 3H), 7.93 (d, *J* 9.1 Hz, 1H), 7.68–7.57 (m, 3H), 7.09 (s, 1H), 6.77 (d, *J* 2.2 Hz, 2H), 6.66 (t, *J* 2.2 Hz, 1H), 3.83 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 178.5, 177.3, 170.8, 163.2, 161.3, 153.4, 136.5, 132.4, 131.6, 130.3, 129.9, 129.6, 128.8, 126.9, 124.0, 119.9, 117.6, 108.0, 107.2, 102.7, 55.9. HRMS ESI (pos) *m*/*z* calcd for $C_{27}H_{21}N_2O_5S$: 485.1171 [M+H]$^+$, found 485.1166.

(5Z)-5-[(3,5-Dihydroxyphenyl)methylene]-4-[(4-oxo-2-phenyl-chromen-6-yl)amino]thiazol-2-one (**5d**)

3,5-Dihydroxybenzaldehyde **4d** (69 mg, 0.5 mmol) was applied as the benzaldehyde for condensation. Compound **5d** was obtained as a yellow solid (177 mg, 78% yield): m.p. > 300 °C; R_f 0.49 (toluene/DMF 4:1); UV–Vis (DMF): λ_{max}, nm (logε) 383 (4.27), 283 (4.49). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 9.69 (s, 2H), 8.52 (d, *J* 2.8 Hz, 1H), 8.34 (dd, *J* 9.1, 2.8 Hz, 1H), 8.17–8.10 (m, 2H), 8.03 (s, 1H), 7.92 (d, *J* 9.1 Hz, 1H), 7.69–7.57 (m, 3H), 7.09 (s, 1H), 6.56 (d, *J* 2.1 Hz, 2H), 6.34 (t, *J* 2.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 179.0, 177.3, 170.9, 163.2, 159.4, 153.4, 136.2, 136.1, 132.4, 131.6, 130.6, 129.7, 128.9, 128.8, 126.9, 123.9, 119.8, 117.7, 108.5, 107.2, 105.3. HRMS ESI (pos) *m*/*z* calcd for $C_{25}H_{16}N_2O_5SNa$: 479.0678 [M+H]$^+$, found 479.0681.

(5Z)-4-[(4-Oxo-2-phenyl-chromen-6-yl)amino]-5-[(2,4,6-trimethoxyphenyl)methylene]thiazol-2-one (**5e**)

2,4,6-Trimethoxybenzaldehyde **4e** (98 mg, 0.5 mmol) was applied as the benzaldehyde for condensation. Compound **5e** was obtained as a yellow solid (195 mg, 76% yield): m.p. 284–286 °C, decomp.; R_f 0.107 (DCM/MeOH 50:1); UV–Vis (DMF): λ_{max}, nm (logε) 389 (4.10), 280 (4.27). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.53 (d, *J* 2.8 Hz, 1H), 8.32 (dd, *J* 9.1, 2.8 Hz, 1H), 8.14 (dd, *J* 7.8, 1.9 Hz, 2H), 7.99 (s, 1H), 7.89 (d, *J* 9.1 Hz, 1H), 7.68–7.56 (m, 3H), 7.08 (s, 1H), 6.35 (s, 2H), 3.87 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 179.7, 177.3, 170.8, 164.0, 163.1, 159.4, 153.1, 136.5, 132.4, 131.6, 130.4, 129.7, 128.7, 126.9, 124.8, 123.9, 119.7, 117.2, 107.1, 104.8, 91.4, 56.1, 56.1. HRMS ESI (pos) *m*/*z* calcd for $C_{28}H_{23}N_2O_6S$: 515.1277 [M+H]$^+$, found 515.1268.

(5Z)-4-[(4-Oxo-2-phenyl-chromen-6-yl)amino]-5-[(3,4,5-trimethoxyphenyl)methylene]thiazol-2-one (**5f**)

3,4,5-Trimethoxybenzaldehyde **4f** (98 mg, 0.5 mmol) was applied as the benzaldehyde for condensation. Compound **5f** was obtained as a yellow solid (200 mg, 78% yield): m.p. 278–280 °C, decomp.; R_f 0.089 (DCM/MeOH 50:1); UV–Vis (DMF): λ_{max}, nm (logε) 391 (4.35), 284 (4.51). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.53 (d, *J* 2.7 Hz, 1H), 8.35 (dd, *J* 9.1, 2.8 Hz, 1H), 8.16–8.08 (m, 3H), 7.90 (d, *J* 9.1 Hz, 1H), 7.68–7.56 (m, 3H), 7.07 (s, 1H), 6.91 (s, 2H), 3.87 (s, 6H), 3.76 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 178.7, 177.3, 172.5, 170.7, 163.2, 153.7, 153.3, 139.8, 136.2, 132.4, 131.5, 130.2, 130.0, 129.6, 128.6, 128.5, 126.9, 123.9, 119.8, 117.4, 107.7, 107.1, 60.7, 56.4. HRMS ESI (pos) *m*/*z* calcd $C_{28}H_{23}N_2O_6S$ for 515.1277 [M+H]$^+$, found 515.1265.

(5Z)-5-[(2,4-Dichlorophenyl)methylene]-4-[(4-oxo-2-phenyl-chromen-6-yl)amino]thiazol-2-one (**5g**)

2,4-Dichlorobenzaldehyde **4g** (88 mg, 0.5 mmol) was applied as the benzaldehyde for condensation. Compound **5g** was obtained as a yellow solid (196 mg, 79% yield): m.p. 260–262 °C, decomp.; R_f 0.161 (DCM/MeOH 50:1); UV–Vis (DMF): λ_{max}, nm (logε) 399 (4), 286 (4.36). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 8.51 (d, *J* 2.7 Hz, 1H), 8.33 (dd, *J* 9.1, 2.8 Hz, 1H), 8.25 (s, 1H), 8.18–8.09 (m, 2H), 7.93 (d, J = 9.2 Hz, 1H), 7.86 (d, *J* 2.2 Hz, 1H), 7.74 (d, *J* 8.5 Hz, 1H), 7.68–7.56 (m, 4H), 7.11 (s, 1H); ^{13}C NMR (101 MHz, DMSO) δ 178.0, 177.3, 170.1, 163.2, 153.5, 135.9, 135.7, 135.3, 134.9, 132.6, 132.4, 131.5, 131.1, 130.2, 129.7, 129.2, 128.7, 126.9, 126.6, 124.9, 123.9, 119.9, 118.1, 107.2. HRMS ESI (pos) *m*/*z* calcd for $C_{25}H_{14}N_2O_3SCl_2Na$: 515.0000 [M+Na]$^+$, found 515.0006.

(5Z)-5-[(3-Hydroxy-4-methoxy-phenyl)methylene]-4-[(4-oxo-2-phenyl-chromen-6-yl)amino]thiazol-2-one (**5h**)

3-Hydroxy-4-methoxybenzaldehyde **4h** (76 mg, 0.5 mmol) was applied as the benzaldehyde for condensation. Compound **5h** was obtained as a yellow solid (193 mg, 82% yield): m.p. > 300 °C; R_f 0.49 (toluene/DMF 4:1); UV–Vis (DMF): λ_{max}, nm (logε) 398 (4.07), 283 (4.24). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.87 (s, 1H), 9.56 (s, 1H), 8.52 (d, *J* 2.8 Hz, 1H), 8.34 (dd, *J* 9.1, 2.8 Hz, 1H), 8.17–8.11 (m, 2H), 8.09 (s, 1H), 7.91 (d, *J* 9.1 Hz, 1H), 7.68–7.56 (m, 3H), 7.14 (d, *J* 4.6 Hz, 3H), 7.08 (s, 1H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 179.1, 177.3, 170.9, 163.2, 153.2, 150.5, 147.5, 136.3, 132.4, 131.6, 130.5, 129.7, 128.8, 127.1, 126.9, 125.9, 123.9, 123.6, 119.8, 117.4, 116.5, 113.0, 107.1, 56.1. HRMS ESI (pos) *m*/*z* calcd for $C_{26}H_{18}N_2O_5SNa$: 493.0934 [M+Na]$^+$, found 493.0965.

(5*Z*)-4-[(4-Oxo-2-phenyl-chromen-6-yl)amino]-5-[(2,3,4-trimethoxyphenyl)methylene]thiazol-2-one (**5i**)

2,3,4-Trimethoxybenzaldehyde **4i** (98 mg, 0.5 mmol) was applied as the benzaldehyde for condensation. Compound **5i** was obtained as a yellow solid (220 mg, 86% yield): m.p. 287–289 °C, decomp.; R_f 0.107 (DCM/MeOH 50:1); UV–Vis (DMF): λ_{max}, nm (logε) 391 (4.26), 284 (4.44). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1H), 8.52 (d, 1H), 8.35 (dd, *J* 9.1, 2.7 Hz, 1H), 8.23 (s, 1H), 8.16–8.09 (m, 2H), 7.89 (d, *J* 9.1 Hz, 1H), 7.67–7.57 (m, 3H), 7.39 (d, *J* 8.8 Hz, 1H), 7.03 (t, *J* 4.5 Hz, 2H), 3.93 (d, *J* 12.4 Hz, 6H), 3.84 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 178.9, 177.2, 171.0, 163.3, 156.1, 153.6, 153.5, 142.5, 136.4, 132.3, 131.7, 129.6, 129.3, 128.6, 126.9, 125.3, 124.4, 124.0, 121.6, 119.6, 118.1, 109.1, 107.3, 62.4, 61.0, 56.7. HRMS ESI (pos) *m/z* calcd ($C_{28}H_{23}N_2O_6S$): 515.1277 $[M+H]^+$, found 515.1270.

2.3. Cell Viability Evaluation After Treatment with Compounds

2.3.1. Cell Culture

Human bladder carcinoma 5637 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in RPMI-1640 medium supplemented with 10% (*v*/*v*) foetal bovine serum and 1% (*v*/*v*) penicillin–streptomycin and L-glutamine solution (containing 200 mM L-glutamine, 10,000 units of penicillin and 10 mg/mL of streptomycin) at 37 °C in a humidified atmosphere with 5% CO_2.

2.3.2. Reagents

RPMI-1640 was purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). The following reagents were obtained from Sigma-Aldrich (Burlington, MA, USA): foetal bovine serum (FBS), penicillin–streptomycin and L-glutamine solution, Dulbecco's phosphate-buffered saline (DPBS), trypsin-EDTA, dimethyl sulfoxide (DMSO), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), sulforhodamine B, Tris base, trichloroacetic acid (TCA). The acetic acid was obtained from Avantor Chemicals (Gliwice, Poland).

2.3.3. Tested Compounds

Compounds were dissolved in DMSO and stored at −20 °C while being protected from light. Table 1 presents the stock concentration and concentrations used for preliminary experiments to verify their biological activity.

Table 1. The concentration of stock solutions used in biological experiments.

Compound	Stock Concentration	Tested Concentration in Screening Experiments
3	10 mM	1 µM, 10 µM
5a	1 mM	0.5 µM, 1 µM
5b	5 mM	1 µM, 5 µM
5c	3 mM	1 µM, 3 µM
5d	20 mM	1 µM, 10 µM
5e	10 mM	1 µM, 10 µM
5f	5 mM	1 µM, 5 µM
5g	5 mM	1 µM, 5 µM
5h	2 mM	1 µM, 2 µM
5i	2 mM	1 µM, 2 µM

Cells were seeded at a density of 15,000 cells per well in 96-well plates and allowed to adhere overnight. In preliminary experiments, cells were treated with the tested compounds at two concentrations, depending on their solubility in DMSO, for 24 or 48 h. Stock solutions of compounds **3** and **5a–5i**, as well as their final concentrations in the culture medium, are presented in Table 1. Control cells received DMSO at a final concentration of 0.1% (v/v) in the culture medium.

Initial cytotoxicity was assessed using the MTT assay; however, interference between some compounds and the MTT reagent resulted in inconsistent measurements. Therefore, cell viability was determined using the sulforhodamine B (SRB) assay. The SRB assay was performed according to the protocol published by Vichai and Kirtikara [33]. Following treatment, cells were washed with DPBS, fixed with cold 10% (w/v) trichloroacetic acid at 4 °C for 1 h, washed four times with ultrapure water, and stained with 100 µL of 0.057% (w/v) SRB per well for 30 min at room temperature. Excess dye was removed with 1% (v/v) acetic acid, and the protein-bound dye was solubilised in 10 mM Tris base (200 µL per well). Absorbance was measured at 490 nm using a microplate reader (ELx800, BioTek Instruments, Winooski, VT, USA).

To determine the IC_{50} value, 5637 cells were treated with compound **5b** at concentrations of 0.5, 1, 2, 3, 4, and 5 µM. DMSO at a final concentration of 0.1% (v/v) in the culture medium was used as a negative control. The SRB assay was performed as described above, following 24 and 48 h of incubation. Dose–response curves were generated using GraphPad Prism 8 software (GraphPad Software, Inc., La Jolla, CA, USA). All experiments were conducted in three independent biological replicates.

2.4. In Vitro Antiplasmodial Assay on Plasmodium Falciparum

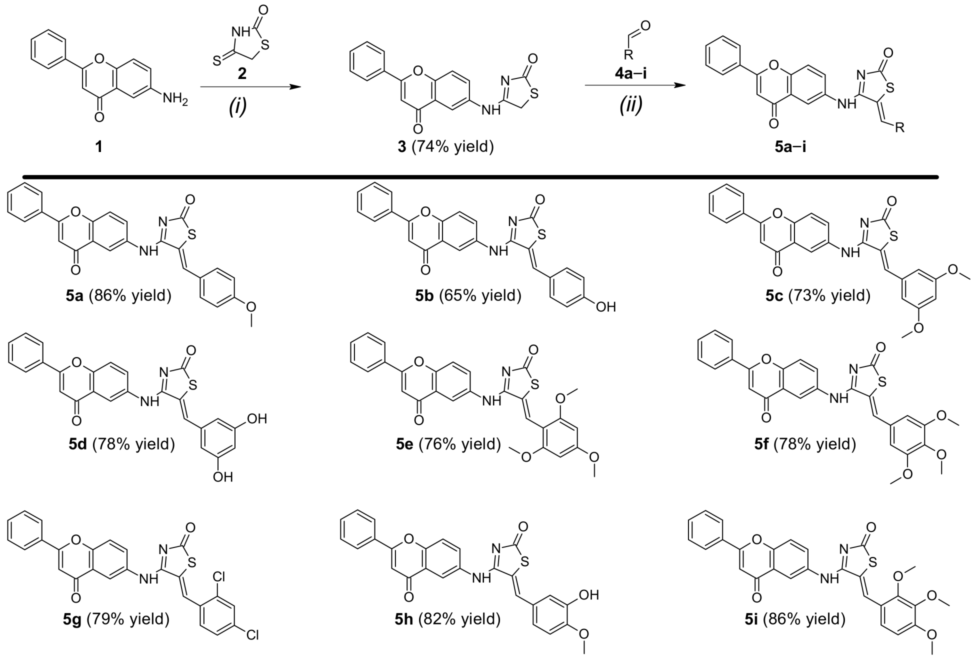
The chloroquine-resistant *Plasmodium falciparum* strain FcB-1 (originating from Colombia) was cultured in vitro on O^+ human erythrocytes supplied by the Etablissement Français du Sang. Cultures were maintained in RPMI-1640 medium supplemented with 0.5% Albumax-II at 37 °C under 5% CO_2, following the procedure described by Grellier et al. [34]. Parasite synchronisation was routinely achieved using 5% *D*-sorbitol treatment [35]. Antiplasmodial activity was assessed in 96-well plates as previously reported [36]. Stock solutions of the tested compounds were prepared in pure DMSO and serially diluted two-fold in plates containing 100 µL of culture medium per well. Subsequently, 100 µL of synchronised ring-stage parasite culture (1% parasitemia, 2% haematocrit) was added to each well. Artesunate served as a positive control, while DMSO was used as a negative control, showing no inhibitory effect up to 0.5%. After 48 h incubation at 37 °C under 5% CO_2, plates were frozen at −20 °C. Following thawing, 100 µL of each well was transferred to a new plate and mixed with 80 µL of lysis buffer (20 mM Tris pH 7.4, 5 mM EDTA, 0.008% w/v saponin, 0.08% v/v Triton X-100). Plates were incubated for 20 min, and autofluorescence was measured using a BioTek Cytation 1 multimode reader (Agilent™, Santa Clara, CA, USA) with excitation/emission filters at 485/20 and 528/20 nm (510 nm cut-off). Next, 20 µL of lysis buffer containing Thiazole Green (SYBR® Green I, Biotium, Fremont, CA, USA) was added, and plates were incubated for an additional 40 min at 37 °C before fluorescence recording. Autofluorescence values were subtracted from Thiazole Green readings. Artesunate treatment resulted in 100% growth inhibition. The IC_{50} (drug concentration causing 50% parasite growth inhibition) was calculated using the IC_{50} Calculator (AAT Bioquest®, Pleasanton, CA, USA, https://www.aatbio.com/tools/ic50-calculator, accessed on 4 September 2025) [37].

3. Results and Discussions

3.1. Chemical Synthesis

The synthetic process leading to flavone–thiazole–aryl hybrid molecules can be divided into two steps (Scheme 1). First, aminolysis of isorhodanine (**2**) with 6-aminoflavone

(**1**) was carried out to obtain imine **3** [29]. For this purpose, an equimolar mixture of reagents together with 2 mL of glacial acetic acid was sealed in a round-bottom microwave reaction vial under nitrogen. The reaction was carried out in a microwave reactor at 120 °C for 10 min. The resulting pale-yellow precipitate after cooling was washed on a filter under vacuum with the following solvent sequence: water, methanol, and diethyl ether. After drying, compound **3** was introduced into the next step of the synthesis, which involved its Knoevenagel condensation with aromatic aldehydes **4a–i**. The imine, aldehyde, and sodium acetate [38] in a ratio of 1:1:1 were immersed in 2 mL of glacial acetic acid, sealed under nitrogen in a round-bottom microwave reaction vial, and placed in a reactor for 10 min at a temperature of 140 °C. The resulting hybrids **5a–i** were isolated from the reaction mixture in a manner similar to the previous product—washing on a filter with water, methanol, and ether.



Scheme 1. Reagents and conditions: (i) acetic acid, microwave irradiation, 120 °C, 10 min; (ii) acetic acid, sodium acetate, microwave irradiation, 140 °C, 10 min.

The resulting hybrids were characterised by UV–Vis, ^{1}H and ^{13}C NMR, and HR-MS (ESI) (see Supporting Information). Based on the NMR data, it is worth adding that the location of the CH= group signal (singlet) at near 7.10 ppm confirms the formation of compounds with a cis position of the arylidene residue (*Z*-configuration) in flavone–thiazole–aryl hybrid molecules **5a–i** (see literature data [29]).

The UV–Vis spectra of the obtained flavone–thiazole–aryl hybrid molecules were carefully studied (Figure 2). Generally, in the absorption spectra of flavonoids, two bands can be found: band I in the range 300–380 nm and band II in the range 240–295 nm. Only in selected flavonoids does the absorption tail to 400–450 nm [39]. In the Digital PhotochemCAD^{TM} database, 42 flavone records were found, which serve as the basis for the herein-discussed hybrids [40]. The most structurally relevant for comparison were the structures recorded in MeOH for flavone with maxima at 251 and 295 nm; 6-methoxyflavone with maxima at 270 and 302 nm, and 6-hydroxyflavone with maxima at 270 and 304 nm [39]. What is interesting is that even the presence of an aromatic substituent in the chemical structure of 6-phenylflavone did not bathochromatically shift the maxima, as they were observed at 273 and 303 nm [39,41]. The UV–Vis spectra in DMF of the studied flavone–thiazole–aryl hybrids **5a–i** consist of two major bands with maxima appearing at 280–288 nm, corresponding to band II and 383–399 nm, corresponding to band I.

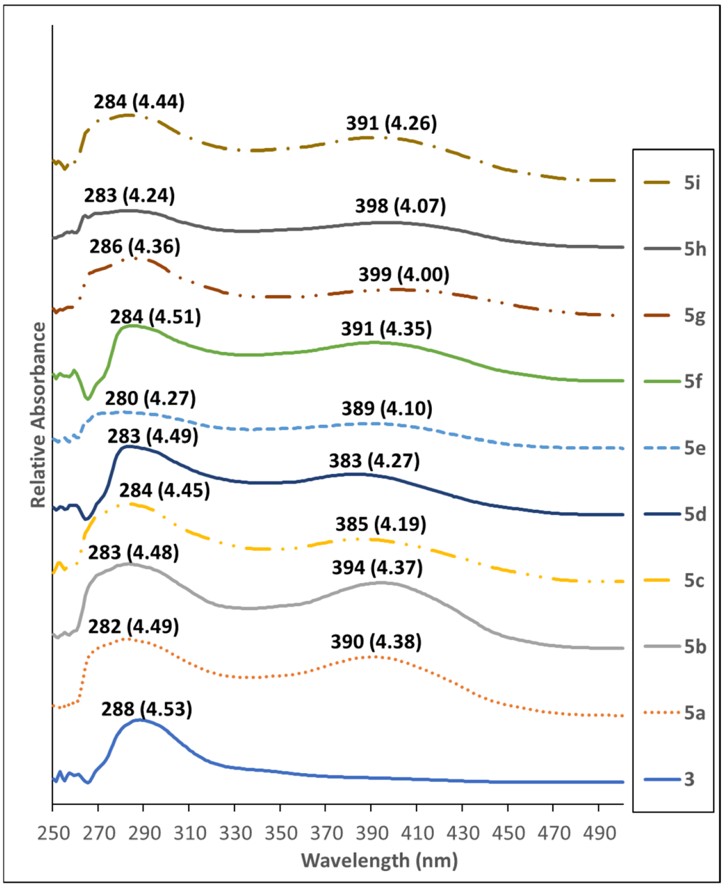


Figure 2. UV–Vis spectra recorded for DMF for the obtained compounds **3** and **5a**–**i**. The λ(logε) values are plotted on the course of individual spectra at concentrations ca. 10^{-5} mol/L.

3.2. The Antiproliferative Activity of the Tested Compounds

Compounds incorporating the 5-arylidene-4-aminothiazol scaffold have gained interest in medicinal chemistry for many years [19,42]. Accumulating evidence indicates that this class of derivatives exerts anticancer activity predominantly through the inhibition of tubulin polymerization, associated with binding to the colchicine-binding site, thereby inducing mitotic arrest [20]. Furthermore, these compounds have been reported to trigger apoptosis and suppress cancer cell proliferation. In addition, literature data suggest that such derivatives may act as multi-target agents, modulating diverse cellular pathways, including cell cycle progression, kinase activity, and apoptotic signalling cascades [42,43]. Furthermore, Geronikaki and co-workers have demonstrated that 2-thiazolylimino/heteroarylimino-5-arylidene-4-thiazolidinones may act as SHP-2 inhibitors [44]. In this study, we initially used the MTT assay to evaluate the antiproliferative activity of the compounds; however, potential interference between the compounds and the MTT reagent was observed. Although cell morphology remained unaffected, absorbance values were markedly reduced, suggesting that the compounds may decrease metabolic activity, possibly by inhibiting mitochondrial dehydrogenases. To avoid false-positive results, the SRB assay was subsequently performed to verify the biological activity of the tested compounds. The SRB assay relies on the electrostatic binding of sulforhodamine B to cellular proteins under acidic conditions, enabling quantification of cell biomass as an indirect measure of cell proliferation [33].

In preliminary screening, only two compounds, **5b** and **5h**, demonstrated inhibitory activity against cancer cell proliferation (Figures 3 and 4). Compound **5h** decreased cell viability to 73% and 66% at a concentration of 2 µM following 24 and 48 h of incubation, respectively, indicating moderate antiproliferative activity (Figure 4).

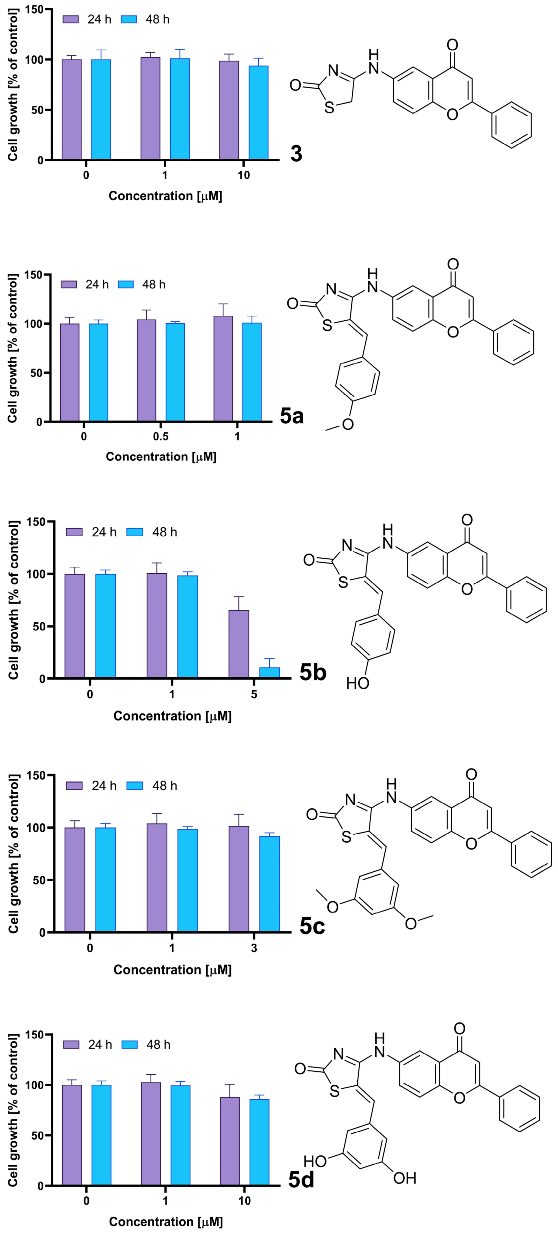


Figure 3. Antiproliferative effects of compounds **3** and **5a**–**5d** in human bladder cancer 5637 cells. Cells were treated with the tested compounds at two selected concentrations and incubated for 24 and 48 h. Antiproliferative activity was assessed using the SRB assay. Data are presented as mean ± SD from two independent experiments.

In contrast, compound **5b** exhibited markedly stronger cytotoxic effects (Figure 3), reducing cell viability to 10.8% at the highest tested concentration (5 µM). Consequently, this compound was selected for further evaluation to determine its IC_{50} value. Following 24 h incubation, compound **5b** reduced cancer cell growth to 52% at 5 µM, precluding accurate IC_{50} determination at this time point, although a clear antiproliferative effect was already evident. After extended incubation (48 h), the IC_{50} value was determined to be 2.37 ± 0.9 µM (Figure 5).

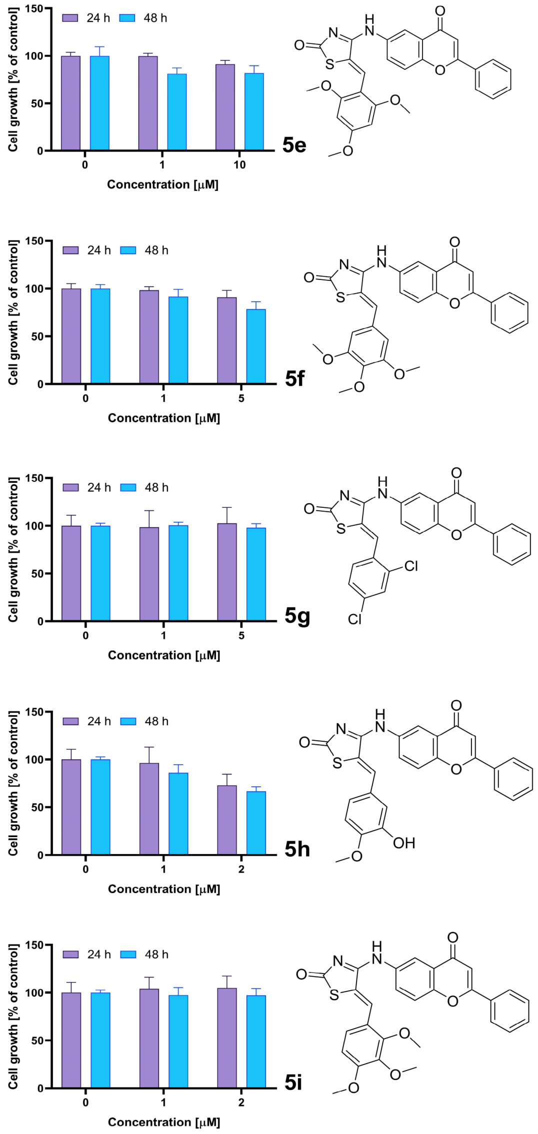


Figure 4. Antiproliferative effects of compounds **5e**–**5i** in human bladder cancer 5637 cells. Cells were treated with the tested compounds at two selected concentrations and incubated for 24 and 48 h. Antiproliferative activity was assessed using the SRB assay. Data are presented as mean ± SD from two independent experiments.

Ali et al. reported a series of novel anticancer compounds obtained by combining two pharmacophores, tetrahydrocarbazole (THC) and 5-arylidene-4-thiazolinone. They evaluated their antiproliferative activity against human cancer cell lines using the SRB assay [42]. The studied compounds showed selective antiproliferative activity, particularly against HCT116, Jurkat, and U937 cell lines. SAR analysis showed that 6-chloro derivatives were more active than non-chlorinated analogues, and electron-donating substituents on the arylidene moiety enhance activity, whereas excessive methoxylation reduces potency. Among the tested compounds, (*Z*)-5-(4-(dimethylamino)benzylidene)-, (*Z*)-5-(2-hydroxybenzylidene)- and (*Z*)-5-(4-methoxybenzylidene)- derivatives exhibited the highest activity against Jurkat leukaemia cells, with IC_{50} values of 3.11 µM, 4.53 µM, and 5.13 µM, respectively, after 48 h incubation [42]. In our study, only the compound bearing a *para*-hydroxy substituent on the arylidene moiety (4-hydroxybenzylidene) exhibited antiproliferative activity against cancer cells. Notably, compound **5a**, which shares the same core structure as **5b** but contains a methoxy group instead of a hydroxy group, was inactive. Although both hydroxy and methoxy substituents are electron-donating groups, the hydroxy group provides additional hydrogen-bonding capability, which may enhance target binding. In contrast, the methoxy group increases lipophilicity and may improve cellular uptake. However, in this case, the presence of the hydroxy group appears to be more favourable for antiproliferative activity against bladder cancer cells.

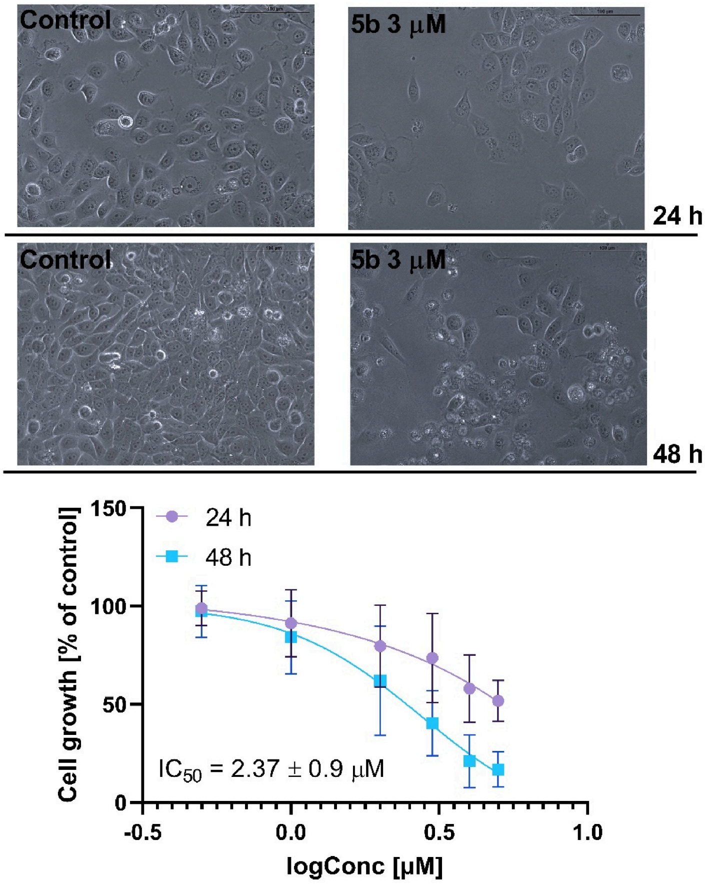


Figure 5. Dose–response curves for compound **5b** in 5637 cells following 24 and 48 h of incubation. Cell growth was determined using the SRB assay. Data are presented as mean ± SD from three independent experiments. The upper panel shows cell morphology after treatment with compound **5b** at a concentration of 3 μM. Images were acquired using a Nikon Eclipse T100 microscope. Scale bars represent 100 μm.

3.3. Results of the Antiplasmodial Assay

Four of the tested compounds (**3**, **5f**, **5d**, and **5b**) exhibited noteworthy antiplasmodial activity in the low micromolar range (see Table 2 and Figure 6). Although compounds **3** and **5f** are the most active within the series, their IC$_{50}$ values (1.9 μM) remain substantially higher than those of nanomolar reference drugs such as artesunate (IC$_{50}$ 4.4 nM). Other frontline drugs, including mefloquine, atovaquone–proguanil, and quinine, also show substantially lower IC$_{50}$ values compared with most tested polyphenol and flavonoid derivatives. Consequently, although compounds such as **3** and **5f** exhibit promising in vitro activity, their overall efficacy remains modest when benchmarked against current therapeutic standards. Nevertheless, their unique chemical scaffolds and natural origin could provide alternative mechanisms of action and represent valuable starting points for structural optimisation. Such efforts may help to overcome resistance issues associated with existing antimalarial drugs and pave the way for novel therapeutic strategies and nutraceutical approaches.

Table 2. Antiplasmodial activity of the tested compounds. IC_{50} values ± standard deviation (SD) in [μM] against *Plasmodium falciparum*.

Compound	*Plasmodium falciparum*	
	IC_{50} [μM]	SD [μM]
3	1.9	0.4
5a	>200	
5b	4.9	1.0
5c	30	0.9
5d	4.8	0.6
5e	50.2	12.8
5f	1.9	0.3
5g	51.1	2.7
5h	18.4	2.2
5i	64	9.4
Artesunate	0.0044	0.0002

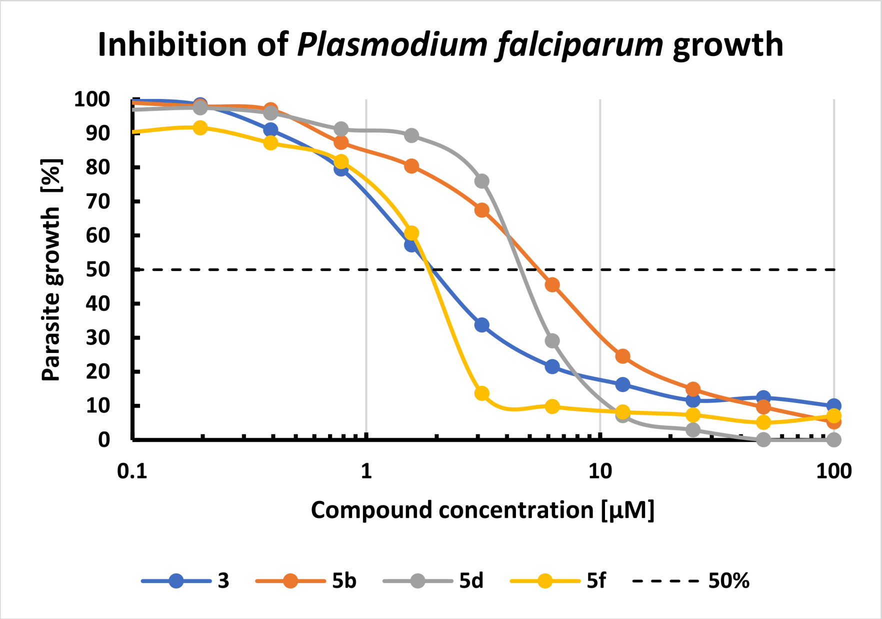


Figure 6. The dose–response curves plotted for the most active compounds **3**, **5b**, **5d**, and **5f** against *Plasmodium falciparum*.

A preliminary structure–antiplasmodial activity analysis (Figure 7) confirmed the general trend for 5-arylidene thiazolidinones. Thus, the strength and specificity of the biological effect depend on the structure of the arylidene moiety [45]. The anti-plasmodial effect was the highest for the 5-unsubstituted hybrid flavone–thiazole **3** and its 3,4,5-trimethoxybenzylidene derivative **5f** (IC_{50} 1.9 μM for both compounds). It is interesting to note that the 5-(4-methoxybenzylidene) derivative **5a** did not show any effect, but the introduction of additional methoxy groups positively contributed to the manifestation of the antiplasmodial activity (compounds **5f**, **5i**, **5c** and **5e**). On the other hand, the additional hydroxyl group in the *m*-position of the aromatic nucleus (compound **5h**) led to the appearance of a moderate effect in comparison with **5a** (IC_{50} 18.4 μM). Moreover, as in the case of the antitumor activity of synthesized compounds, the presence of hydroxyl groups is also positive for the antiplasmodial effect (compounds **5d**, **5b** and **5h**). Another important feature of the obtained compounds is the combination of antitumor and antiplasmodial effects observed for hits **5b** and **5h**, which warrants further investigation.

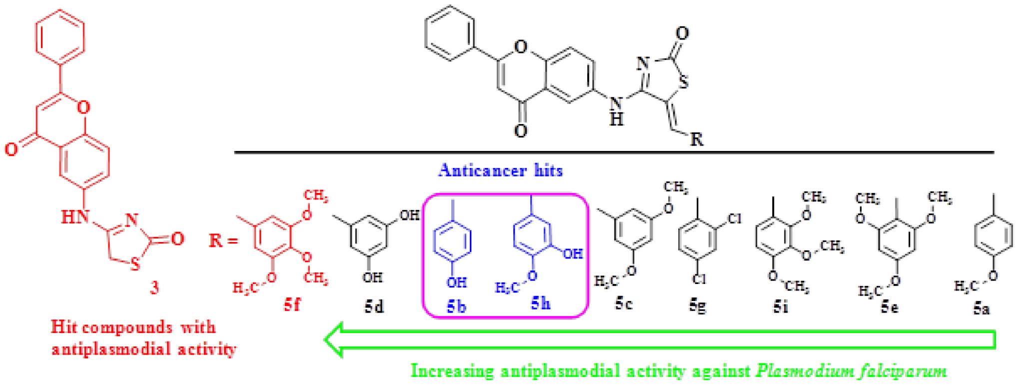


Figure 7. Structure–activity relationship for the synthesised hybrids.

4. Conclusions

The design of hybrid molecules is a modern approach in medicinal chemistry, but it has proven to be a major challenge. We noted that this approach could be promising for 5-arylidene-4-aminothiazol-2(5*H*)-ones, which were successfully used to design flavone–thiazole hybrid molecules.

Microwave-assisted synthesis becomes a very rational approach for the synthesis of flavone–thiazole–aryl hybrids and was successful when applied. Among the compounds tested on human bladder cancer 5637 cells, one hybrid molecule exhibited interesting micromolar activity. In addition, four of the tested compounds presented noteworthy antiplasmodial activity against *P. falciparum* in the low micromolar range.

The obtained group of flavone–thiazole–aryl hybrid molecules constitute valuable starting points for further structural optimisation, which could yield novel active pharmaceutical ingredients. It seems that unique chemical scaffolds and natural origin could provide alternative mechanisms of action. Such efforts may help to overcome resistance issues associated with existing anticancer and antimalarial drugs and pave the way for novel therapeutic strategies.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/scipharm94020049/s1.

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References

1. Tang, S.; Wang, B.; Liu, X.; Xi, W.; Yue, Y.; Tan, X.; Bai, J.; Huang, L. Structural Insights and Biological Activities of Flavonoids: Implications for Novel Applications. *Food Front.* **2025**, *6*, 218–247. [CrossRef]
2. Berga, M.; Logviss, K.; Lauberte, L.; Paulausks, A.; Mohylyuk, V. Flavonoids in the Spotlight: Bridging the Gap between Physicochemical Properties and Formulation Strategies. *Pharmaceuticals* **2023**, *16*, 1407. [CrossRef] [PubMed]
3. Hădărugă, D.I.; Hădărugă, N.G. Flavones: Structure, Properties, Sources and Food Applications. In *Handbook of Food Bioactive Ingredients*; Springer International Publishing: Cham, Switzerland, 2023; pp. 111–158.
4. Leonte, D.; Ungureanu, D.; Zaharia, V. Flavones and Related Compounds: Synthesis and Biological Activity. *Molecules* **2023**, *28*, 6528. [CrossRef]
5. Kerboeuf, D.; Riou, M.; Guegnard, F. Flavonoids and Related Compounds in Parasitic Disease Control. *Mini-Rev. Med. Chem.* **2008**, *8*, 116–128. [CrossRef] [PubMed]
6. Stompor-Gorący, M.; Bajek-Bil, A.; Potocka, N.; Zawlik, I. Therapeutic Perspectives of Aminoflavonoids—A Review. *Int. J. Mol. Sci.* **2025**, *26*, 2014. [CrossRef]
7. Dauzonne, D.; Folléas, B.; Martinez, L.; Chabot, G. Synthesis and in Vitro Cytotoxicity of a Series of 3-Aminoflavones. *Eur. J. Med. Chem.* **1997**, *32*, 71–82. [CrossRef]
8. Thorat, N.M.; Sarkate, A.P.; Lokwani, D.K.; Tiwari, S.V.; Azad, R.; Thopate, S.R. N-Benzylation of 6-Aminoflavone by Reductive Amination and Efficient Access to Some Novel Anticancer Agents via Topoisomerase II Inhibition. *Mol. Divers.* **2021**, *25*, 937–948. [CrossRef] [PubMed]
9. Liu, Z.-Y.; Wang, Y.-M.; Li, Z.-R.; Jiang, J.-D.; Boykin, D.W. Synthesis and Anticancer Activity of Novel 3,4-Diarylthiazol-2(3*H*)-Ones (Imines). *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5661–5664. [CrossRef]
10. Arora, P.; Narang, R.; Nayak, S.K.; Singh, S.K.; Judge, V. 2,4-Disubstituted Thiazoles as Multitargated Bioactive Molecules. *Med. Chem. Res.* **2016**, *25*, 1717–1743. [CrossRef]
11. Zaharia, V.; Ignat, A.; Palibroda, N.; Ngameni, B.; Kuete, V.; Fokunang, C.N.; Moungang, M.L.; Ngadjui, B.T. Synthesis of Some *p*-Toluenesulfonyl-Hydrazinothiazoles and Hydrazino-Bis-Thiazoles and Their Anticancer Activity. *Eur. J. Med. Chem.* **2010**, *45*, 5080–5085. [CrossRef]
12. Ali, S.H.; Sayed, A.R. Review of the Synthesis and Biological Activity of Thiazoles. *Synth. Commun.* **2021**, *51*, 670–700. [CrossRef]
13. Petrou, A.; Fesatidou, M.; Geronikaki, A. Thiazole Ring—A Biologically Active Scaffold. *Molecules* **2021**, *26*, 3166. [CrossRef]
14. Arshad, M.F.; Alam, A.; Alshammari, A.A.; Alhazza, M.B.; Alzimam, I.M.; Alam, M.A.; Mustafa, G.; Ansari, M.S.; Alotaibi, A.M.; Alotaibi, A.A.; et al. Thiazole: A Versatile Standalone Moiety Contributing to the Development of Various Drugs and Biologically Active Agents. *Molecules* **2022**, *27*, 3994. [CrossRef]
15. Singh, A.; Malhotra, D.; Singh, K.; Chadha, R.; Bedi, P.M.S. Thiazole Derivatives in Medicinal Chemistry: Recent Advancements in Synthetic Strategies, Structure Activity Relationship and Pharmacological Outcomes. *J. Mol. Struct.* **2022**, *1266*, 133479. [CrossRef]
16. Rouf, A.; Tanyeli, C. Bioactive Thiazole and Benzothiazole Derivatives. *Eur. J. Med. Chem.* **2015**, *97*, 911–927. [CrossRef]
17. Xie, X.-X.; Li, H.; Wang, J.; Mao, S.; Xin, M.-H.; Lu, S.-M.; Mei, Q.-B.; Zhang, S.-Q. Synthesis and Anticancer Effects Evaluation of 1-Alkyl-3-(6-(2-Methoxy-3-Sulfonylaminopyridin-5-yl)benzo[d]thiazol-2-yl)urea as Anticancer Agents with Low Toxicity. *Bioorg. Med. Chem.* **2015**, *23*, 6477–6485. [CrossRef]
18. Ivasechko, I.; Yushyn, I.; Roszczenko, P.; Senkiv, J.; Finiuk, N.; Lesyk, D.; Holota, S.; Czarnomysy, R.; Klyuchivska, O.; Khyluk, D.; et al. Development of Novel Pyridine-Thiazole Hybrid Molecules as Potential Anticancer Agents. *Molecules* **2022**, *27*, 6219. [CrossRef]
19. Bathula, S.; Sankaranarayanan, M.; Malgija, B.; Kaliappan, I.; Bhandare, R.R.; Shaik, A.B. 2-Amino Thiazole Derivatives as Prospective Aurora Kinase Inhibitors against Breast Cancer: QSAR, ADMET Prediction, Molecular Docking, and Molecular Dynamic Simulation Studies. *ACS Omega* **2023**, *8*, 44287–44311. [CrossRef]
20. Rehulka, J.; Subtelna, I.; Kryshchyshyn-Dylevych, A.; Cherniienko, A.; Ivanova, A.; Matveieva, M.; Polishchuk, P.; Gurska, S.; Hajduch, M.; Zagrijtschuk, O.; et al. Anticancer 5-arylidene-2-(4-hydroxyphenyl)Aminothiazol-4(5*H*)-ones as Tubulin Inhibitors. *Arch. Pharm.* **2022**, *355*, 2200419. [CrossRef]
21. Chen, X.; Zhao, S.; Wu, Y.; Chen, Y.; Lu, T.; Zhu, Y. Design, Synthesis and Biological Evaluation of 2-Amino-N-(2-Aminophenyl)Thiazole-5-Carboxamide Derivatives as Novel Bcr-Abl and Histone Deacetylase Dual Inhibitors. *RSC Adv.* **2016**, *6*, 103178–103184. [CrossRef]
22. Fauziya; Gupta, A.; Nadaf, A.; Ahmad, S.; Hasan, N.; Imran, M.; Sahebkar, A.; Jain, G.K.; Kesharwani, P.; Ahmad, F.J. Dasatinib: A Potential Tyrosine Kinase Inhibitor to Fight against Multiple Cancer Malignancies. *Med. Oncol.* **2023**, *40*, 173. [CrossRef]
23. Sivakumar, K.K.; Naveen Kumar, A.; Munirathinam, M.; Ponnilavarasan, I.; Preedeep, A.; Muthu Priya, A.S. Design, conventional and microwave assisted synthesis of molecular hybrids of thiazol-2-amine containing flavone/chalcone moieties possessing antimicrobial properties. *Eur. J. Biomed. Pharm. Sci.* **2018**, *5*, 246–256.

24. Bozdağ-Dündar, O.; Verspohl, E.J.; Daş-Evcimen, N.; Kaup, R.M.; Bauer, K.; Sarıkaya, M.; Evranos, B.; Ertan, R. Synthesis and Biological Activity of Some New Flavonyl-2,4-Thiazolidinediones. *Bioorg. Med. Chem.* **2008**, *16*, 6747–6751. [CrossRef]
25. Babu, R.S.; Palaniappan, S.; Kathiravan, M.K. Synthesis and Anticancer Activity of Novel Thiazolo-Coumarin Derivatives. *Asian J. Chem.* **2023**, *35*, 187–193. [CrossRef]
26. Hazai, L.; Zsoldos, B.; Halmai, M.; Keglevich, P. Flavone Hybrids and Derivatives as Bioactive Agents. *Appl. Sci.* **2024**, *14*, 1039. [CrossRef]
27. Subtelna, I.; Kryshchyshyn-Dylevych, A.; Jia, R.; Lelyukh, M.; Ringler, A.; Kubicek, S.; Zagrijtschuk, O.; Kralovics, R.; Lesyk, R. 5-Arylidene-2-(4-hydroxyphenyl)Aminothiazol-4(5*H*)-ones with Selective Inhibitory Activity against Some Leukemia Cell Lines. *Arch. Pharm.* **2021**, *354*, 2000342. [CrossRef]
28. Dudchak, R.; Podolak, M.; Sydorenko, I.; Czarnomysy, R.; Gornowicz, A.; Karpenko, O.; Holota, S.; Bielawska, A.; Bielawski, K.; Lesyk, R. 5-Ene-2-Arylaminothiazol-4(5H)-Ones Induce Apoptosis in Breast Cancer Cells. *Cells* **2025**, *14*, 861. [CrossRef]
29. Kaminskyy, D.; Subtel'na, I.; Zimenkovsky, B.; Karpenko, O.; Gzella, A.; Lesyk, R. Synthesis and Evaluation of Anticancer Activity of 5-Ylidene-4-Aminothiazol-2(5H)-One Derivatives. *Med. Chem.* **2015**, *11*, 517–530. [CrossRef] [PubMed]
30. Yushyn, I.; Holota, S.; Finiuk, N.; Senkiv, J.; Karpenko, O.; Vovchuk, O.; Kozak, Y.; Ivasechko, I.; Pinyazhko, R.; Gzella, A.; et al. Synthesis and Anticancer Activity Evaluation of New Linked/Fused Bioisosteric Pyrazole-Thiazole-Bearing Hybrid Molecules. *Bioorg. Med. Chem.* **2025**, *128*, 118291. [CrossRef] [PubMed]
31. Komaritsa, I.D.; Baranov, S.N.; Grishuk, A.P. 4-Thiazolidines, Derivatives and Analogs. *Chem. Heterocycl. Compd.* **1967**, *3*, 533–534. [CrossRef]
32. Patiny, L.; Borel, A. ChemCalc: A Building Block for Tomorrow's Chemical Infrastructure. *J. Chem. Inf. Model.* **2013**, *53*, 1223–1228. [CrossRef] [PubMed]
33. Vichai, V.; Kirtikara, K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat. Protoc.* **2006**, *1*, 1112–1116. [CrossRef]
34. Sow, C.; Laughhunn, A.; Girard, Y.A.; Lanteri, M.C.; Amar El Dusouqui, S.; Stassinopoulos, A.; Grellier, P. Inactivation of Plasmodium Falciparum in Whole Blood Using the Amustaline and Glutathione Pathogen Reduction Technology. *Transfusion* **2020**, *60*, 799–805. [CrossRef]
35. Lambros, C.; Vanderberg, J.P. Synchronization of Plasmodium Falciparum Erythrocytic Stages in Culture. *J. Parasitol.* **1979**, *65*, 418–420. [CrossRef]
36. Cordeiro, T.d.M.; Borghetti, F.; Caldas Oliveira, S.C.; Bastos, I.M.D.; de Santana, J.M.; Grellier, P.; Charneau, S. Brazilian Cerrado Qualea Grandiflora Mart. Leaves Exhibit Antiplasmodial and Trypanocidal Activities In Vitro. *Pharmacogn. Mag.* **2017**, *13*, 668–672. [CrossRef]
37. AAT Bioquest, Inc. IC50 Calculator [Online Tool]. AAT Bioquest. 2026. Available online: https://www.aatbio.com/tools/ic50-calculator (accessed on 22 April 2026).
38. Zhai, X.; Li, W.; Chen, D.; Lai, R.; Liu, J.; Gong, P. Design and Synthesis of 2-Iminothiazolidin-4-one Moiety-Containing Compounds as Potent Antiproliferative Agents. *Arch. Pharm.* **2012**, *345*, 360–367. [CrossRef]
39. Taniguchi, M.; LaRocca, C.A.; Bernat, J.D.; Lindsey, J.S. Digital Database of Absorption Spectra of Diverse Flavonoids Enables Structural Comparisons and Quantitative Evaluations. *J. Nat. Prod.* **2023**, *86*, 1087–1119. [CrossRef]
40. PhotochemCAD. Available online: https://www.photochemcad.com/ (accessed on 31 March 2026).
41. Matsumura, H.; Tsuchiya, T.; Takeda, T.; Imafuku, K. Synthesis of 6- and 8-Phenyl-Substituted Flavonoids. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2037–2043. [CrossRef]
42. Ali, B.S.; Mohammed, A.F.; Kariuki, B.M.; El-Awady, R.; H. M. Abdu-Allah, H. Tetrahydrocarbazoles Incorporating 5-Arylidene-4-Thiazolinones as Potential Antileukemia and Antilymphoma Targeting Tyrosine Kinase and Tubulin Polymerase Enzymes: Design, Synthesis, Structural, Biological and Molecular Docking Studies. *Bioorg. Chem.* **2024**, *153*, 107817. [CrossRef]
43. Türe, A.; Ergül, M.; Ergül, M.; Altun, A.; Küçükgüzel, İ. Design, Synthesis, and Anticancer Activity of Novel 4-Thiazolidinone-Phenylaminopyrimidine Hybrids. *Mol. Divers.* **2021**, *25*, 1025–1050. [CrossRef] [PubMed]
44. Geronikaki, A.; Eleftheriou, P.; Vicini, P.; Alam, I.; Dixit, A.; Saxena, A.K. 2-Thiazolylimino/Heteroarylimino-5-Arylidene-4-Thiazolidinones as New Agents with SHP-2 Inhibitory Action. *J. Med. Chem.* **2008**, *51*, 5221–5228. [CrossRef] [PubMed]
45. Kaminskyy, D.; Kryshchyshyn, A.; Lesyk, R. 5-Ene-4-Thiazolidinones—An Efficient Tool in Medicinal Chemistry. *Eur. J. Med. Chem.* **2017**, *140*, 542–594. [CrossRef]

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