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Synthesis of 6-Arylaminoflavones via Buchwald–Hartwig Amination and Its Anti-Tumor Investigation

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Abstract

A new series of 6-arylaminoflavones was synthesized via the Buchwald–Hartwig cross-coupling reaction, aiming to functionalize the flavone core efficiently. Reaction optimization revealed that $Pd_2(dba)_3$/XantPhos with Cs_2CO_3 in toluene provided the best yields, with isolated yields ranging from 8% to 95%, depending on the arylamine structure. Steric hindrance and electron-withdrawing groups at the arylamine ring impacted the reaction outcomes. Cytotoxicity assays in different human cancer cell lines indicated that substitution patterns at both the arylamine and B-rings strongly impacted biological activity. In particular, compounds bearing a 3,4-dimethoxy substitution at the B-ring and a trifluoromethyl (**13c**) or chlorine (**13g**) group at the aniline moiety exhibited enhanced cytotoxicity. These findings provide insights into the structure–activity relationship of 6-arylaminoflavones while contributing to the development of synthetic methodologies for functionalized flavones.

Keywords: aminoflavones; Buchwald-Hartwig amination; drug design; cytotoxicity assays

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1. Introduction

Flavonoids represent a diverse group of heterocyclic molecules featuring oxygen in their structure. They are categorized into six main subclasses and are commonly found in plant-based foods and beverages including fruits, vegetables, cocoa, cereals, tea, and wine [1]. Among these, flavones consist of two aromatic rings (A and B) and a heterocyclic pyranone ring (C), forming the framework of 2-phenyl-4*H*-chromen-4-one (Figure 1) [2,3]. In particular, aminoflavones are flavone derivatives that have been modified to include one or more amino groups (-NH_2) attached to various positions on the flavone core structure. These amino groups can also be further substituted, enhancing the diversity and potential biological activity of the compounds. It is also observed that the 3′ and 4′ positions are often mono- or di-substituted by electron-donating groups such as methoxy groups [4].

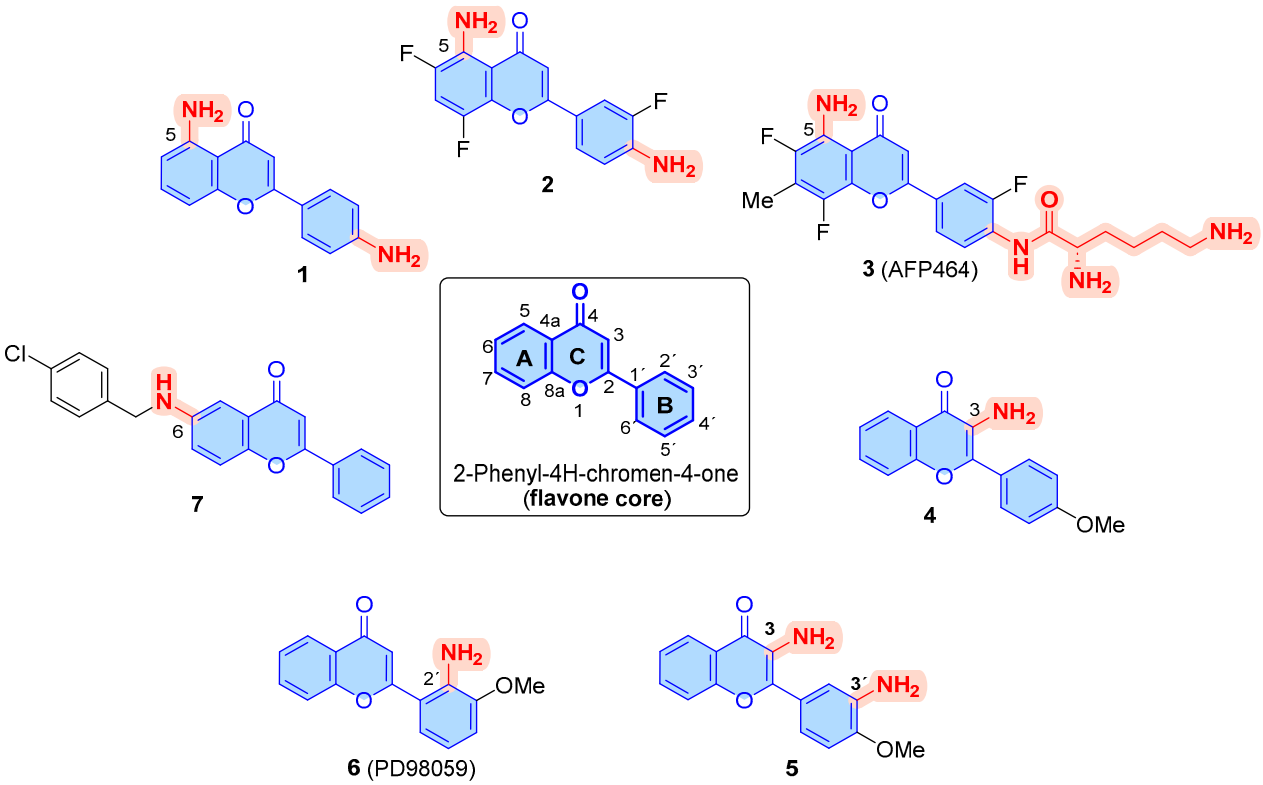


Figure 1. Structure of some cytotoxic aminoflavones and their core structure.

The 5-aminoflavones **1**,**2** (Figure 1) showed antiproliferative activity against human breast cancer (MCF-7), with potencies of 7.2 and 1.2 nM, respectively. Compound **2** also suppressed tumor growth in mice, highlighting its potential as a chemotherapeutic agent [5,6]. Compound **3** (AFP464), a synthetic lysyl prodrug of **2**, has demonstrated efficacy against breast and renal cancer cell lines [7,8]. Compound **3** has progressed to phase II clinical trials against solid tumors; however, its development was discontinued due to pulmonary toxicity [9]. The 3-aminoflavones **4**,**5** (Figure 1), have demonstrated considerable cytotoxicity against the murine lymphocytic leukemia cell line L1210, with IC_{50} values of 22 μM and 10 μM, respectively [10]. The 2′-aminoflavone **6** (PD98059) (Figure 1) is a selective MEK1 (IC_{50} = 2–7 μM) and MEK2 (IC_{50} = 50 μM) inhibitor that blocks the MAPK/ERK pathway [11], with antiproliferative effects at 50 μM in breast cancer (MCF-7 and MDA-MB-231) [12]. The 6-benzylaminoflavone **7** (Figure 1) exhibited anticancer activity against MCF-7 (IC_{50} = 9.4 μM), and inhibited topoisomerase II (IC_{50} = 12 μM) [13]. Despite significant advancements in the synthesis of aminoflavones, the selective functionalization of positions 6 and 7 are particularly underexplored relative to other positions. This highlights an opportunity to explore the 6-position with different amines and observe its impacts on tumor cell proliferation.

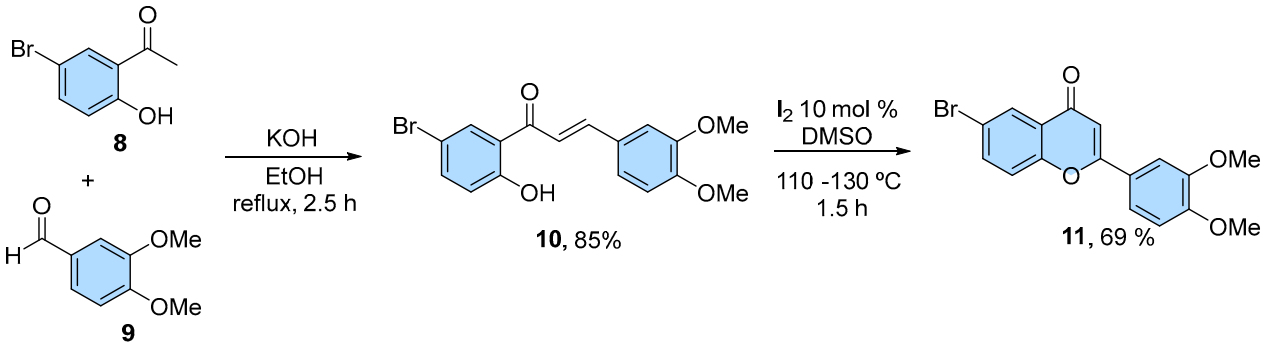
In this context, the present study aims to synthesize 6-arylaminoflavone derivatives and evaluate their cytotoxic activities against different cancer cell lines. The introduction of various arylamino groups at the 6-position of flavones was explored to understand the influence of these modifications on the biological activity of the compounds. This approach is expected to not only reveal new candidates for anticancer agents but to also provide valuable insights into the structure–activity relationship (SAR) of these flavone derivatives.

2. Results and Discussion

Motivated by the scarcity of 6-arylaminoflavone derivatives reported in the literature and their promising antitumor potential, we designed a novel series of 6-arylaminoflavones

featuring 3,4-dimethoxy substitutions on the aromatic ring B, along with diverse substituents at the R_3 position of the aniline ring. This study aimed to evaluate the influence of these substituents on biological activity, thereby contributing to the elucidation of the structure–activity relationship (SAR).

The synthetic pathway was accomplished in three key steps: the first two stages were dedicated to the construction of the flavone core, while the final step focused on the introduction of diverse arylamino groups at the 6-position of this ring. For the synthesis of the flavone core, firstly, a Claisen–Schmidt condensation [14] was performed between 5′-bromo-2′-hydroxyacetophenone **8** and 3,4-dimethoxybenzaldehyde **9** (Scheme 1). This reaction was carried out under reflux conditions in ethanol, using potassium hydroxide (KOH) as the base, yielding the corresponding α,β-unsaturated chalcone **10** with an 85% yield. Subsequently, an oxidative cyclization step was conducted using dimethyl sulfoxide (DMSO) and iodine (I_2) as reagents [15]. In this step, DMSO was a dual function agent—a solvent and oxidizing agent—while iodine acted as an electrophilic species [16], enabling the efficient formation of the 6-bromoflavone intermediate **11** with a 69% yield.



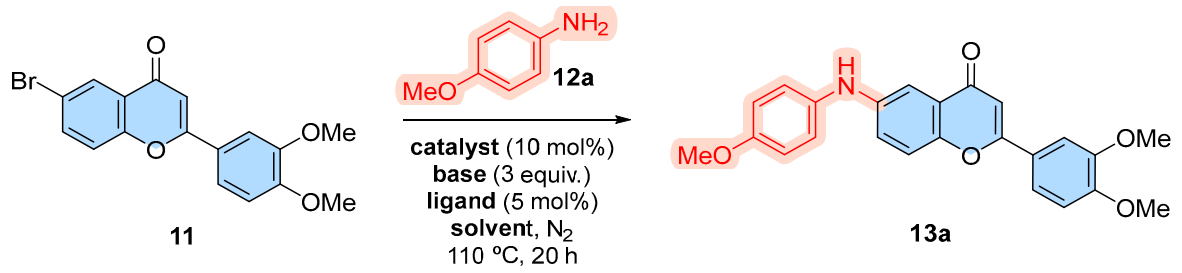
Scheme 1. Synthetic route of the flavone core.

The final step was dedicated to the synthesis of 6-arylaminoflavone derivatives employing a Buchwald–Hartwig coupling reaction. To optimize the reaction conditions, strategic exploratory experiments were performed using classical conditions [17], employing flavone **11** and 4-methoxyaniline **12a** as model substrates. The influence of the palladium source, phosphine ligand, base, and solvent were evaluated at 110 °C to assess their impact on the reaction outcome (Table 1).

The optimization of the reaction conditions for the synthesis of 6-arylaminoflavone via Buchwald–Hartwig coupling reactions revealed significant differences in the efficiency of the employed conditions [18]. Initially, the coupling reaction was performed using $Pd_2(dba)_3$ as the catalyst, XantPhos as the ligand, and toluene as the solvent, while evaluating four different bases (entries 1–4, Table 1). In the first experiment, CsF was used as the base, but no reaction was observed (entry 1). Kónya and co-workers (2015) investigated the use of the stronger base, NaOtBu, in the Buchwald–Hartwig coupling for the synthesis of 6-arylaminoflavones derivatives [19]. The authors suggested that steric hindrance from the amine was a key factor limiting the overall yields, and that benzylamines were generally more suitable substrates. In our case, however, the use of NaOtBu resulted in only trace amounts of product (entry 2). In contrast, the desired compound **13a** was successfully obtained when alkali metal carbonates such as K_2CO_3 and Cs_2CO_3 were employed (entries 3–4). Notably, although using a different substrate (6-tosyloxyflavone), Yuen and co-workers [20] also reported the synthesis of 6-aminoflavones via palladium-catalyzed cross-coupling using K_2CO_3 as the base. In our study, the optimal conditions were achieved with Cs_2CO_3, affording compound **13a** in 77% yield (entry 4). The Cs_2CO_3 proved to be superior to other bases tested, reinforcing the need for moderately strong bases to efficiently

activate the nucleophilic amine and subsequently form the C-N bond [21]. Subsequently, an alternative ligand—DavePhos—was employed (entry 5); however, it yielded results comparable to those obtained with XantPhos. Nevertheless, XantPhos, being a bidentate ligand, likely offers enhanced chelation and steric stabilization, resulting in a marginally superior yield [22]. Other palladium catalysts, such as $PdCl_2(PPh_3)_2$, were also evaluated; however, the reactions did not yield superior results compared to those obtained with $Pd_2(dba)_3$ (20%; entry 6). Finally, replacing toluene with THF as the solvent (entry 7) resulted in reduced yields of the coupling product. This decrease in efficiency can be attributed to the coordination of polar solvents, such as THF, with the palladium center, which likely disrupts the catalytic cycle and compromises the reaction's overall performance [23]. The screening of reaction conditions was limited to selected combinations of bases, ligands, and solvents. A more extensive optimization may further improve yields for challenging substrates and will be explored in future studies. Besides that, with optimized conditions established, which included flavone **11** (1.0 equiv), amine (1.2 equiv), $Pd_2(dba)_3$ (10 mol %), XantPhos (5 mol %), Cs_2CO_3 (3.0 equiv), and toluene at 110 °C for 20 h (entry 4), we investigated the scope of the Buchwald–Hartwig coupling reactions (Scheme 2).

Table 1. Optimization of the Buchwald–Hartwig reaction conditions. [a]

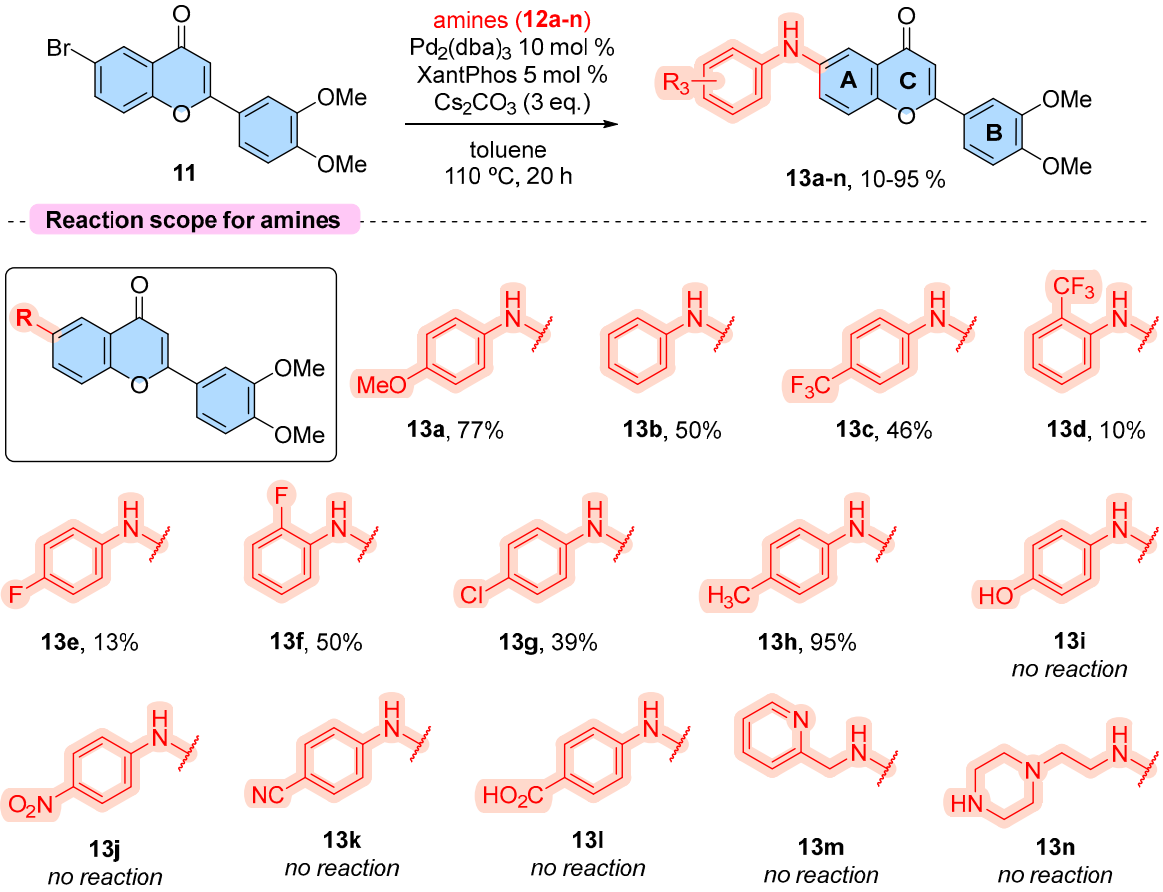


Entry	Catalyst	Ligand	Base	Solvent	Yield (%) [b]
1	$Pd_2(dba)_3$	XantPhos	CsF	toluene	No reaction
2	$Pd_2(dba)_3$	XantPhos	NaOtBu	toluene	Trace
3	$Pd_2(dba)_3$	XantPhos	K_2CO_3	toluene	32
4	$Pd_2(dba)_3$	XantPhos	Cs_2CO_3	toluene	77
5	$Pd_2(dba)_3$	DavePhos	Cs_2CO_3	toluene	75
6	$PdCl_2(PPh_3)_2$	XantPhos	Cs_2CO_3	toluene	20
7	$Pd_2(dba)_3$	XantPhos	Cs_2CO_3	THF	35

[a]: Reaction conditions: **11** (1 mmol) and **12a** (1.2 mmol) at reflux in a sealed tube. [b]: Yields refer to isolated and purified compounds.

As illustrated in Scheme 2, the formation of 6-arylaminoflavones is markedly influenced by the substituents on the arylamine ring, with electronic and steric factors playing a critical role in determining the reaction yields. Better yields were obtained when anilines substituted with electron-donating groups were employed (**13a** and **13h**; 77% and 95%). When an unsubstituted aniline was employed, the desired product **13b** was obtained only in moderate yield (50%). Electron-withdrawing groups such as trifluoromethyl and fluorine afforded the 6-arylaminoflavone products **13c** and **13f** in moderate yields, comparable to unsubstituted aniline (**13b**). In contrast, other strongly electron-withdrawing groups, such as cyano, nitro, or carboxyl (**13j–13l**), resulted in no reaction, possibly due to their ability to hinder the formation of the C–N bond under the conditions of the Buchwald–Hartwig coupling [24,25]. Substituent positioning was not systematically varied in this study; however, future investigations involving meta-substituted anilines may help clarify

positional influences on reactivity. Regarding compounds **13m–n**, which are aliphatic amines, these reactions were designed with the aim of improving the solubility of the 6-arylaminoflavones. However, no product was obtained, likely due to the nature of the groups involved, which were not suitable for the desired reaction pathway.



Scheme 2. Substrate scope of 6-arylaminoflavone derivative syntheses via Buchwald–Hartwig coupling.

To assess the cytotoxic effects of the compounds **13a–h**, we initially conducted an MTT assay using four tumorigenic cell lines (A172, HEK293T, MDA-MB-231, and PC3) [26]. The first screening was performed at a concentration of 100 µM to identify compounds exhibiting cytotoxicity at a high concentration (Figure 2). We observed that substituents in the 6-arylamine group strongly impacted cellular viability when compared to the vehicle control (DMSO). Comparing **13a** with **13b**, we found that the 4-methoxy substituent appeared to reduce the basal toxic effects, as **13a** was non-toxic to the tested cell lines. Of note, **13c** (4-trifluoromethyl) exhibited preferential toxicity towards PC3 (prostate adenocarcinoma) cells compared to the other cell lines, whereas, shifting the trifluoromethyl from the *para*-position in **13c** to the *ortho*-position in **13d**, almost abolished the toxic effect on tumor cells. On the other hand, this effect was inverted when the 4-fluoro group (**13e**) was replaced by a 2-fluoro (**13f**). Furthermore, switching the 4-fluoro (**13e**) to a 4-chloride (**13g**) regained toxicity towards A172, MDA-MB-231, and, especially, PC3 cells as observed in **13c**, whereas a 4-methyl (**13h**) substitution abolished this effect.

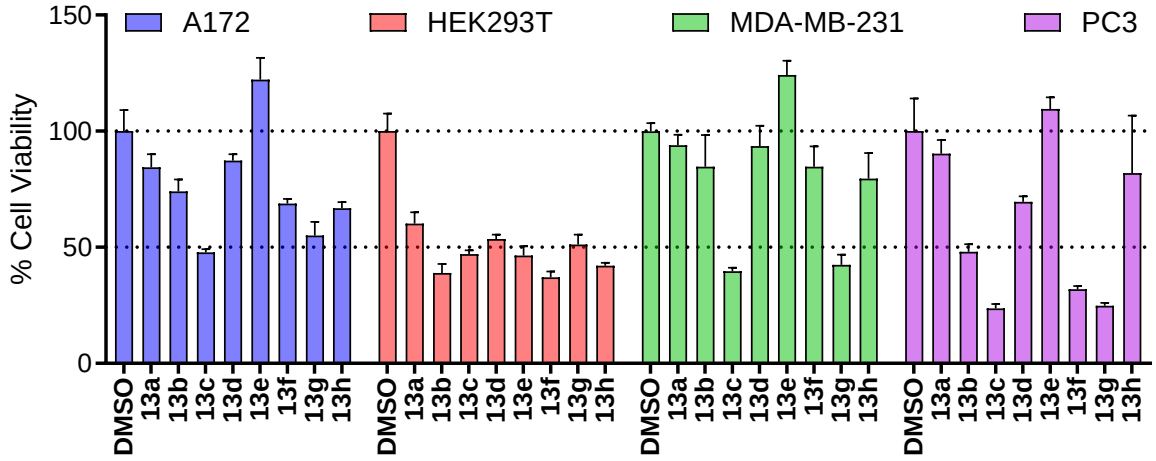


Figure 2. Cellular toxicity of 6-arylaminoflavones **13a**–**h**. Results are expressed as viable cells relative to vehicle control (DMSO). Data are expressed as mean + standard deviation of one experiment performed in quadruplicate (n = 4).

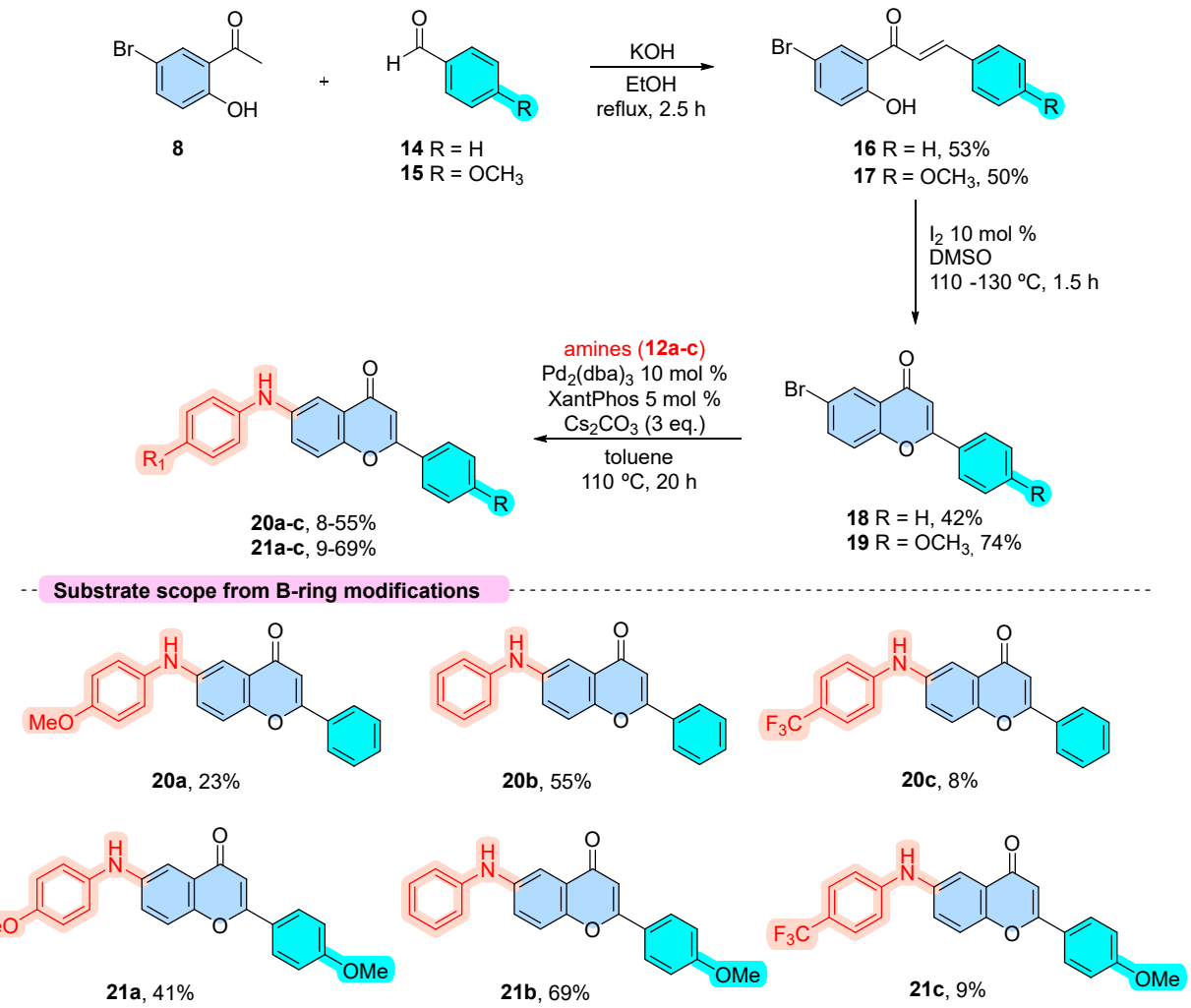
Based on these findings, we sought to further explore the influence of B-ring modifications on cytotoxicity by synthesizing six new analogs (**20a**–**c**, **21a**–**c)** under the same previously described conditions (Scheme 3). The synthesis followed a three-step approach, as detailed below.

The Claisen–Schmidt condensation was performed with **8** and benzaldehyde **14** or 4-methoxybenzaldehyde **15** to afford the corresponding α,β-unsaturated chalcone **16**–**17** with 53% and 50% yield, respectively. The oxidative cyclization gave the 6-bromoflavone intermediates **18**–**19** with a 42–74% yield. Finally, the previous optimized condition for the synthesis of **13a** was used to achieve the final compounds. Noteworthy, this procedure proved less efficient for 6-arylaminoflavone bearing a benzene (**20a**–**c**) or 4-methoxybenzene (**21a**–**c**) substituents in the B-ring, particularly when the 6-substitution was a 4-trifluoromethyl group. It is important to note that the optimized synthesis of 6-arylamino flavones bearing a benzene B-ring has been reported elsewhere [19,20]. However, the synthetic route had to be optimized each time using different conditions than the ones applied here for **13a**. Nonetheless, we successfully obtained the desired derivatives of **13a**–**c** bearing the modification at the B-ring for cytotoxic evaluation.

The comparative analysis of compounds **13a**–**c**, **20a**–**c** and **21a**–**c** across tumor cell lines A172, MDA-MB-231, and PC3 revealed a consistent structure–activity relationship. The aniline precursors **13a**–**c**, bearing two methoxy groups at the B ring, exhibited the highest cytotoxicity among the series. The corresponding flavones **20a**–**c**, which lack B-ring substitution, showed markedly reduced activity (Figure 3). The introduction of a single methoxy group in compounds **21a**–**c** led to a modest increase in cytotoxicity but not sufficient to reach the potency of the parent anilines (Figure 3). Of note was that compound **21a** demonstrated decreased selectivity due to higher activity in HEK293T cells, suggesting that partial methoxylation at the B ring may reduce the tumor selectivity observed in the **13a**–**c** series.

Within this context, the evaluation of compounds **13c**, **20c**, and **21c**—each containing a trifluoromethyl group at the *para*-position of the aniline ring—provides further insight into the contribution of substituents to biological activity. While **13c** maintained strong cytotoxicity across tumor cell lines, its corresponding analogs **20c** and **21c** were significantly less active. The complete loss of the B-ring substitution in **20c** was associated with minimal activity, while the introduction of a single methoxy group in **21c** led to a partial recovery. These results suggest that the trifluoromethyl group alone does not compensate for the

absence of electron-donating groups on the B ring, and that the observed activity in **13c** likely results from a synergistic effect between both regions of the molecule.



Scheme 3. Synthetic route to obtain 6-arylaminoflavone derivatives (**20a–c**, **21a–c**) with modification in the B-ring.

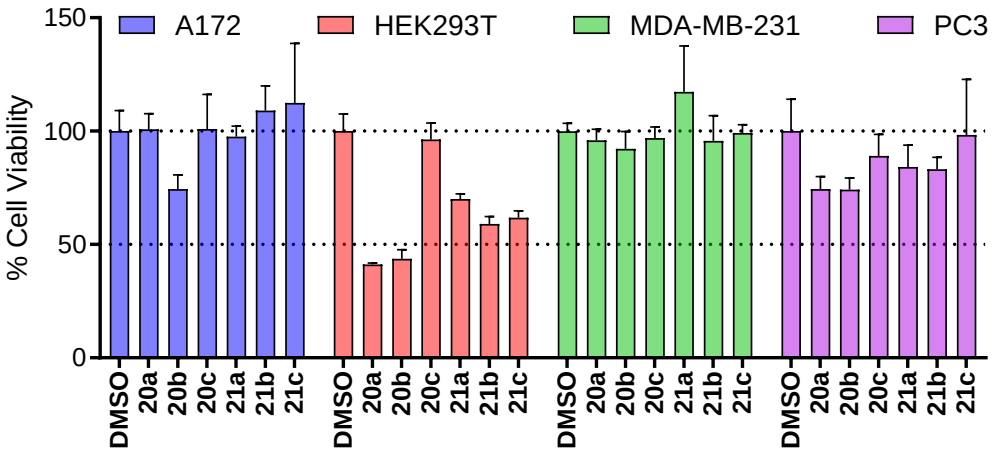


Figure 3. Cellular toxicity of 6-arylaminoflavones **20a–c** and **21a–c**. Results are expressed as viable cells relative to vehicle control (DMSO). Data are expressed as mean + standard deviation of one experiment performed in quadruplicate (n = 4).

To contextualize these findings, a comparison with the aminoflavones previously reported in the literature (compounds **4**–**7**, Figure 1) was considered. Compound **7**, a 6-benzylaminoflavone without a B-ring substitution, shares structural features with **20a**–**c** and similarly displays low cytotoxicity. Compounds **4** and **5**, although substituted differently—with amino groups at the 3-position and methoxylation at the B ring—highlight the relevance of electron-donating groups in modulating biological response. Compound **6**, with an amino substitution at the B ring itself, further supports this trend. While these reference compounds were evaluated under different biological conditions, their structural profiles support the conclusion that B-ring substitution plays a central role in defining cytotoxic activity in flavone-based scaffolds.

3. Conclusions

This study highlights the significant potential of 6-arylaminoflavone in medicinal chemistry, owing to their diverse biological activities and structural versatility. A series of compounds was synthesized via Buchwald–Hartwig coupling, using optimized conditions that afforded products in moderate to high yields, depending on the steric and electronic properties of the aniline precursors. Cytotoxicity assays revealed that the presence of a 3,4-dimethoxy substitution on the B ring, combined with electron-withdrawing groups at the 6-arylamino position—such as 4-trifluoromethyl (**13c**) and 4-chloro (**13g**)—was associated with increased activity, particularly against prostate and breast cancer cell lines. In contrast, analogs with absent or limited methoxy substitution on the B ring **(20a**–**c** and **21a**–**c**) displayed significantly lower cytotoxicity, underscoring the cooperative role of both the B ring and the 6-substituent in modulating biological response. These findings provide valuable insights into the structure–activity relationships of 6-arylaminoflavone, suggesting that careful design and modification of these compounds can enhance their therapeutic potential.

4. Materials and Methods

4.1. General Information

Reactions were monitored by thin layer chromatography (TLC) with silica gel 60 GF_{254} (Merck®, Darmstadt, Germany), 0.20 mm thick. The revelations were carried out by inspection in a chamber with emission of ultraviolet light (UV) with a length of λ = 254 and 366 nm, and subsequent use of a developing chamber with iodine and vanillin solution. Silica gel 60 (230–400 mesh) was used for column chromatography. Solvents were purified by distillation, except for HPLC quality products. Anhydrous solvents were conditioned in the presence of 3 Å molecular sieves. Reactions sensitive to humidity and air were carried out in tubes under an inert atmosphere (N_2). FTIR data were obtained using an Agilent Technologies Cary 630 (Santa Clara, CA, USA), and melting points were measured using a Buchi B-545 melting point apparatus (Buchi, Flawil, Switzerland). Structural analyses of ^{1}H (300–800 MHz), ^{13}C (75–200 MHz), and ^{19}F (282 MHz) Nuclear Magnetic Resonance (NMR) spectra were performed in a Bruker (Billerica, MA, USA), Advanced DPX-300 and AIII 800 MHz models. Chemical shifts (δ) were expressed in parts per million (ppm), being referenced when compared with DMSO-d_6 at 2.55 ppm (^{1}H) and 39.52 ppm (^{13}C) as well as at 77.16 ppm (^{13}C) when using $CDCl_3$; the coupling constant (*J*) is expressed in Hertz (Hz). ^{19}F NMR spectra obtained DMSO-d_6, using trifluoroacetic acid as internal standard.

The purity of molecules was analyzed by High-Performance Liquid Chromatography (HPLC), using Shimadzu Proeminence® (Shimadzu, Kyoto, Japan) chromatograph, with a Zorbax SB-Phenyl® (Agilent Technologies, Inc., Santa Clara, CA, USA) analytical column (5 μm, 150 × 4.6 mm). Samples were diluted to 0.25 mg/mL. Detection was conducted by ultraviolet (UV) light at 264 nm. The injection volume was 7 μL. Deionized water and ace-

tonitrile (ACN) were used as the eluent system with 0.1% trifluoroacetic acid (TFA), with a flow rate of 1 mL/min. The chromatographic run started with 5% of the ACN/TFA mixture; after 10 min, it became 100% of the mixture. After 15 min, the ACN/TFA concentration was reduced to 50%; in 20 min it dropped to 25%, ending in 25 min with 0%. The measurement of the mass/charge ratios of the 6-arylaminoflavones was obtained using Bruker Daltonics® (Bremen, Germany) microOTOF QII/ESI-TOF. They were analyzed using high-performance liquid chromatography (Prominence liquid chromatography, Shimadzu®) coupled to a Q-TOF mass spectrometer, a compact model from Bruker Daltonics GmbH®, with an electrospray ionization interface. The compounds were dissolved in DMSO, separated using a Luna C18 column (3 µm, 100 Å, 150 × 3 mm, Phenomenex® brand, Torrance, CA, USA) using 0.1% formic acid in deionized water (A) and 0.1% of formic acid in acetonitrile (B) with a flow rate of 0.4 mL/min. The gradient program was: 30% B at the start, 100% B between 3 and 6 min, returning to 30% B in 6.01 min, and finishing the race with a total time of 11 min. The injection volume was 20 µL, and the column temperature was maintained at 40 °C. The Q-TOF/MS instrument was operated in positive mode using the following parameters: ionic gas source temperature (N_2) of 200 °C; nebulizer pressure of 45 psi; and capillary voltage of 2800 V. The mass spectrometer was operated in MS scan mode. Sodium formate was used as the internal mass calibration.

4.2. Synthetic Methods

4.2.1. General Procedure A: Claisen–Schmidt Condensation

A solution of 5′-bromo-2′-hydroxyacetophenone **8** (1 mmol, 1 eq.); aldehydes **9**, **14**, or **15** (1.1 mmol, 1.1 eq.); and KOH (2 mmol, 2 eq.) in ethanol (6 mL) was heated at reflux for 2.5 h. The reaction mixture was quenched with water and extracted with AcOEt (3× 30 mL). The combined organic phases were collected, dried over anhydrous MgSO4, filtered, and concentred under reduced pressure. The crude product was purified by recrystallization with methanol.

(E)-1-(5-bromo-2-hydroxyphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**10**). Yield: 85%; yellow solid; mp. 177.1–178.3 °C (Lit. mp. 178–179 °C [27]). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.87 (s, 1H, OH), 8.02 (s, 1H), 7.92 (d, *J* = 15 Hz, 1H, C=C), 7.57 (d, *J* = 9 Hz, 1H), 7.41 (d, *J* = 15 Hz, 1H, C=C), 7.30 (d, *J* = 9 Hz, 1H), 7.19 (s, 1H), 6.94 (d, *J* = 9 Hz, 2H), 4.00 (s, 3H, O-CH_3) 3.97 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.7 (C=O), 162.6, 152.3, 149.6, 147.0, 138.8, 131.8, 127.5, 124.1, 121.5, 120.7, 117.2, 111.4, 110.7, 110.4, 56.2 (2C; O-CH_3).

(E)-1-(5-bromo-2-hydroxyphenyl)-3-phenylprop-2-en-1-one (**16**). Yield: 53%; yellow solid; mp. 129.7–131.6 °C (Lit. mp. 130–132 °C [28]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.75 (s, 1H, OH), 8.03 (s, 1H), 7.97 (d, *J* = 15 Hz, 1H, C=C), 7.70 (s, 1H), 7.58 (m, 1H), 7.52 (d, *J* = 15 Hz, 1H, C=C), 6.96 (d, *J* = 9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.9 (C=O), 162.7, 146.7, 139.1, 134.5, 132.0, 131.4, 129.2, 129.0, 127.7, 121.4, 120.8, 119.6, 110.6.

(E)-1-(5-bromo-2-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**17**). Yield: 50%; yellow solid; mp. 100.3–102.1 °C (Lit. mp. 102–104 °C [29]). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.88 (s, 1H, OH), 8.02 (s, 1H), 7.94 (d, *J* = 15 Hz, 1H, C=C), 7.67 (d, *J* = 9 Hz, 2H), 7.57 (d, *J* = 9 Hz, 1H), 7.45 (d, *J* = 15 Hz, 1H, C=C), 7.00–6.93 (m, 3H), 3.90 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.8 (C=O), 162.6, 162.5, 146.7, 138.8, 131.9, 131.0, 127.3, 121.6, 120.7, 117.0, 114.8, 110.5, 55.6 (O-CH_3).

4.2.2. General Procedure B: Oxidative Cyclization

In a round bottom flask, chalcone **10**, **16**, or **17** (1 mmol), iodine I_2 (10 mol %), and anhydrous DMSO (5 mL) were added. The reaction was kept under constant stirring and in an inert atmosphere for 1.5 h at a temperature between 110 and 130 °C. After the completion of the reaction, the mixture was extracted with saturated $Na_2S_2O_3$ and EtOAc (3x 30 mL),

dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (eluent: ethyl acetate/hexane 8:2).

6-Bromo-2-(3,4-dimethoxyphenyl)-4H-chromen-4-one (**11**). Yield: 69%; light-yellow solid; mp. 204.1–206.3 °C (Lit. mp. 206–208 °C [30]). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.67 (dd, *J* = 9 Hz, 2 Hz, 1H), 7.45 (dd, *J* = 9 Hz, 2 Hz, 1H), 7.37 (d, *J* = 9 Hz, 1H), 7.28 (s, 1H), 6.90 (d, *J* = 9 Hz, 1H), 6.66 (s, 1H), 3.90 (s, 3H, O-CH_3), 3.89 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.3 (C=O), 163.1, 152.0, 151.0, 149.3, 142.4, 141.1, 124.9, 124.5, 124.1, 122.0, 120.0, 119.1, 118.5, 111.2, 110.8, 108.8, 105.9, 56.2 (2C; O-CH_3).

6-Bromo-2-phenyl-4H-chromen-4-one (**18**). Yield: 42%; white solid; mp. 187.4–188.1 °C (Lit. mp. 188–190 °C [31]). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.96 (d, *J* = 6 Hz, 2H), 7.83 (d, *J* = 9 Hz, 1H), 7.60–7.58 (m, 3H), 7.52 (d, *J* = 9 Hz, 1H), 6.90 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.1 (C=O), 163.9, 155.1, 136.9, 132.0, 131.5, 129.3, 128.5, 126.5, 125.4, 120.2, 118.8, 107.6.

6-Bromo-2-(4-methoxyphenyl)-4H-chromen-4-one (**19**). Yield: 74%; white solid; mp. 192.2–193.4 °C (Lit. mp. 185–187 °C [31]). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, *J* = 3 Hz, 1H), 7.77 (d, *J* = 9 Hz, 2H), 7.66 (dd, *J* = 9 Hz, 2.4 Hz, 1H), 7.35 (d, *J* = 9 Hz, 1H), 6.93 (d, *J* = 9 Hz, 2H), 6.64 (s, 1H), 3.81 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.0 (C=O), 163.8, 162.8, 155.0, 136.6, 128.5, 128.2, 125.4, 123.7, 120.0, 118.6, 114.7, 106.2, 55.7 (O-CH_3).

4.2.3. General Procedure C: Buchwald–Hartwig Amination

A flame-dried pressure tube was charged with flavone **11**, **18**, or **19** (1 mmol), $Pd_2(dba)_3$ (10 mol %), XantPhos (5 mol %), Cs_2CO_3 (3 mmol, 3 eq.), and correspondent amine* (1.1 mmol). The tube was capped with septum, evacuated for 1 h with vacuum, heated at approximately 40–50 °C, and then filled with nitrogen twice. Subsequently, anhydrous toluene (5 mL) was added using a syringe, and the mixture was stirred at 110 °C for 20 h. The resulting solids were removed by vacuum filtration and the filtrate was then concentrated under reduced pressure. The crude material was purified by flash column chromatography (eluent: hexane/dichloromethane (9:1), to 100% dichloromethane, and concluding with dichloromethane/methanol (99:1)). *Liquid amines were previously degassed and added with the solvent.

2-(3,4-dimethoxyphenyl)-6-((4-methoxyphenyl)amino)-4H-chromen-4-one (**13a**). Yield: 77%; light-yellow solid; mp. 204.6–205 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.55 (d, *J* = 6 Hz, 1H), 7.45–7.39 (m, 2H), 7.29–7.14 (m, 4H), 6.98 (d, *J* = 9 Hz, 1H), 6.90 (d, *J* = 6 Hz, 2H), 6.75 (s, 1H), 3.99 (s, 3H, O-CH_3), 3.97 (s, 3H, O-CH_3), 3.82 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.3 (C=O), 163.2, 152.1, 150.5, 149.4, 124.8, 124.6, 123.0, 122.6, 120.1, 119.1, 115.0, 111.3, 109.1, 108.4, 105.7, 56.21 (O-CH_3), 56.20 (O-CH_3), 55.7 (O-CH_3). IR (ν, cm^{-1}): 1566 e 1544 (C=O), 2902 (Ar-CH), 3205 (N-H). HRMS (ESI-TOF) *m*/*z* calcd for $C_{24}H_{21}NO_5$ 404.1497 $[M+H]^+$, found 404.1483. Analytical HPLC retention time: 12.1 min; purity: 98.2%.

2-(3,4-dimethoxyphenyl)-6-(phenylamino)-4H-chromen-4-one (**13b**). Yield: 50%; yellow solid; mp. 197.2–197.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.56 (d, *J* = 6 Hz, 1H), 7.48 (t, *J* = 9 Hz, 2H), 7.39 (s, 1H), 7.35–7.30 (m, 2H), 7.15 (d, *J* = 6 Hz, 2H), 7.03–6.98 (m, 2H), 6.74 (s, 1H), 6.10 (s, 1H), 4.00 (s, 3H, O-CH3), 3.98 (s, 3H, O-CH3). 13C NMR (75 MHz, CDCl3) δ 178.3 (C=O), 163.1, 152, 151, 149.3, 142.4, 141.1, 129.7, 124.9, 124.5, 124.1, 122, 120, 119.1, 118.5, 111.2, 110.8, 108.8, 105.9, 56.2 (O-CH3). IR (ν, cm−1): 1564 e 1546 (C=O), 3019 (Ar-CH), 3203 (N-H). HRMS (ESI-TOF) *m*/*z* calcd for $C_{23}H_{19}NO_4$ 374.1392 [M+H]+, found 374.1393. Analytical HPLC retention time: 12.2 min; purity: 99.1%.

2-(3,4-dimethoxyphenyl)-6-((4-(trifluoromethyl)phenyl)amino)-4H-chromen-4-one (**13c**). Yield: 46%; yellow solid; mp. 221.4–221.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.50–7.42

(m, 5H), 7.32 (s, 1H), 7.19 (s, 1H), 7.04 (d, *J* = 9 Hz, 2H), 6.92 (d, *J* = 9 Hz, 1H), 6.72 (s, 1H), 3.91 (s, 3H, O-CH_3), 3.90 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178 (C=O), 163.8, 152.5, 152.1, 149.6, 146.2, 139.3, 127 (q, *J* = 7,5 Hz, C-F), 126.1, 124.9, 124.3, 120.3, 119.5, 116.1 (2C), 113.9, 111.4, 109.1, 106.0, 56.3 (2C; O-CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ −60.6. IR (ν, cm^{-1}): 1559 e 1546 (C=O), 3024 (Ar-CH), 3173 (N-H). HRMS (ESI-TOF) *m*/*z* calcd for $C_{24}H_{18}F_3NO_4$ 442.1266 $[M+H]^+$, found 442.1264. Analytical HPLC retention time: 12.7 min; purity: 99.1%.

2-(3,4-dimethoxyphenyl)-6-((2-(trifluoromethyl)phenyl)amino)-4H-chromen-4-one (**13d**). Yield: 10%; yellow solid; mp. 214.8–215.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.55–7.46 (m, 3H), 7.36–7.28 (m, 4H), 6.99–6.90 (m, 2H), 6.77 (s, 1H), 3.91 (s, 3H, O-CH_3), 3.89 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.9 (C=O), 163.9, 152.5, 152.0, 149.5, 141.2, 140.0, 133.2, 127.2 (q, *J* = 7.5 Hz, C-F), 126.2, 124.7, 124.3, 121.4, 120.4, 119.5, 119.3, 119.1, 118.9, 113.5, 111.4, 109.1, 105.8, 56.3 (2C; O-CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ −60.6. IR (ν, cm^{-1}): 1564 e 1547 (C=O), 2969 (Ar-CH), 3183 (N-H). HRMS (ESI-TOF) *m*/*z* calcd for $C_{24}H_{18}F_3NO_4$ 442.1266 $[M+H]^+$, found 442.1261. Analytical HPLC retention time: 12.6 min; purity: 98.8%.

2-(3,4-dimethoxyphenyl)-6-((4-fluorophenyl)amino)-4H-chromen-4-one (**13e**). Yield: 13%; yellow solid; mp. 190.7–191.6 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.41 (s, 1H), 7.65 (d, *J* = 6 Hz, 2H), 7.55 (d, *J* = 9 Hz, 2H), 7.41 (d, *J* = 6 Hz, 1H), 7.17–7.09 (m, 5H), 6.93 (s, 1H), 3.89 (s, 3H, O-CH_3), 3.84 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.7 (C=O), 162.1, 157.0 (d, *J* = 237.3 Hz, C-F), 151.7, 149.5, 149.0, 141.9, 138.9, 138.9, 124.0, 123.6, 123.2, 120.0 (d, *J* = 7.9 Hz, C-F), 119.5 (d, *J* = 9.6 Hz, C-F), 115.9 (d, *J* = 22.4 Hz, C-F), 111.7, 109.3, 106.6, 104.9, 55.8 (O-CH_3), 55.6 (O-CH_3). ^{19}F NMR (282 MHz, DMSO-d_6) δ −125.9. IR (ν, cm^{-1}): 1566 e 1534 (C=O), 3020 (Ar-CH), 3192 (N-H). HRMS (ESI-TOF) *m*/*z* calcd for $C_{23}H_{18}FNO_4$ 392.1298 $[M+H]^+$, found 392.1279. Analytical HPLC retention time: 12.3 min; purity: 98.1%.

2-(3,4-dimethoxyphenyl)-6-((2-fluorophenyl)amino)-4H-chromen-4-one (**13f**). Yield: 50%; dark brown solid; mp. 172.9–173.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.58–7.51 (m, 2H), 7.44–7.39 (m, 2H), 7.16–6.93 (m, 5H), 6.75 (s, 1H), 6.05 (s, 1H), 4.00 (s, 3H, O-CH_3), 3.98 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2 (C=O), 163.2, 153.5 (d, *J* = 241.8 Hz, C-F), 152.1, 151.4, 149.3, 140, 130.8 (d, *J* = 11.1 Hz, C-F), 124.8, 124.7, 124.7, 124.4, 121.9 (d, *J* = 7.3 Hz, C-F), 120.0, 119.3, 118.2, 118.2, 115.8 (d, *J* = 19.2 Hz, C-F), 111.4 (d, *J* = 29.8 Hz, C-F), 108.8, 106.0, 56.2 (2C; O-CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ −129.6. IR (ν, cm^{-1}): 1583 e 1551 (C=O), 2935 (Ar-CH), 3164 (N-H). HRMS (ESI-TOF) *m*/*z* calcd for $C_{23}H_{18}FNO_4$ 392.1298 $[M+H]^+$, found 392.1282. Analytical HPLC retention time: 12.2 min.; purity: 98.9%.

6-((4-chlorophenyl)amino)-2-(3,4-dimethoxyphenyl)-4H-chromen-4-one (**13g**). Yield: 39%; yellow solid; mp. 201.3–201.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.58–7.50 (m, 2H), 7.40 (m, 2H), 7.27 (d, *J* = 9 Hz, 2H), 7.07 (d, *J* = 6 Hz, 2H), 7.00 (d, *J* = 9 Hz, 1H), 6.74 (s, 1H), 6.09 (s, 1H), 4.01 (s, 3H, O-CH_3), 3.99 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2 (C=O), 163.3, 152.1, 151.2, 149.4, 141.2, 140.7, 129.6 (2C), 126.6, 124.9, 124.4, 124.3, 120.1, 119.6, 119.3 (2C), 111.3, 111.2, 108.8, 105.9, 56.2 (O-CH_3). IR (ν, cm^{-1}): 1574 e 1553 (C=O), 3015 (Ar-CH), 3181 (N-H). HRMS (ESI-TOF) *m*/*z* calcd for $C_{23}H_{18}ClNO_4$ 408.1003 $[M+H]^+$, found 408.1022. Analytical HPLC retention time: 12.7 min; purity: 99.7%.

2-(3,4-dimethoxyphenyl)-6-(p-tolylamino)-4H-chromen-4-one (**13h**). Yield: 95%; pale-yellow solid; mp. 187.1–187.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.41 (d, *J* = 9 Hz, 1H), 7.30–7.26 (m, 2H), 7.02–6.94 (m, 5H), 6.85 (d, *J* = 9 Hz, 1H), 6.60 (s, 1H), 3.87 (s, 3H, O-CH_3), 3.85 (s, 3H, O-CH_3), 2.21 (s, 3H, Ar-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2 (C=O), 163.0, 152.0, 150.6, 149.3, 142.0, 139.7, 131.8, 130.1 (2C), 124.7, 124.5, 123.4, 119.9, 119.5 (2C), 118.9, 111.2, 109.6, 108.9, 105.7, 56.11 (O-CH_3), 56.10 (O-CH_3), 20.8 (Ar-CH_3). IR

(ν, cm^{-1}): 1564 e 1544 (C=O), 2987 (Ar-CH), 3211 (N-H). HRMS (ESI-TOF) m/z calcd for $C_{24}H_{21}NO_4$ 388.1549 $[M+H]^+$, found 388.1520. Analytical HPLC retention time: 12.5 min; purity: 98.4%.

6-((4-methoxyphenyl)amino)-2-phenyl-4*H*-chromen-4-one (**20a**) Yield: 23%; orange solid; mp. 166.9–167.4 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.20 (s, 1H), 8.07 (m, 2H), 7.65–7.58 (m, 4H), 7.43 (s, 1H), 7.38–7.35 (m, 1H), 7.12 (d, J = 9 Hz, 2H), 6.95–6.92 (m, 3H), 3.75 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.9 (C=O), 161.9, 154.6, 149.0, 143.5, 135.2, 131.5, 131.4 (2C), 129.0 (2C), 126.1, 124.1, 122.6, 121.6 (2C), 119.4, 114.7 (2C), 105.9, 104.9, 55.2 (O-CH_3). IR (ν, cm^{-1}): 1568 e 1553 (C=O), 3023 (Ar-CH), 3179 (N-H). HRMS (ESI-TOF) m/z calcd for $C_{22}H_{17}NO_3$ 344.1286 $[M+H]^+$, found 344.1280. Analytical HPLC retention time: 12.5 min; purity: 99.7%.

2-phenyl-6-(phenylamino)-4H-chromen-4-one (**20b**). Yield: 55%; yellow solid; mp. 193.1–193.9 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.10 (m, 2H), 7.78–7.74 (m, 2H), 7.60–7.58 (m, 7H), 7.23 (d, J = 6 Hz, 2H), 6.99 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.9 (C=O), 162.4, 150.8, 146.8, 139.6, 131.8, 131.3, 129.2 (2C), 126.74 (2C), 126.70 (2C), 126.3, 125.9, 124.11, 119.9, 115.5 (2C), 110.8, 106.3. IR (ν, cm^{-1}): 1566 e 1540 (C=O), 3019 (Ar-CH), 3175 (N-H). HRMS (ESI-TOF) m/z calcd for $C_{21}H_{15}NO_2$ 314.1181 $[M+H]^+$, found 314.1170. Analytical HPLC retention time: 12.5 min; purity: 99.1%.

2-phenyl-6-((4-(trifluoromethyl)phenyl)amino)-4H-chromen-4-one (**20c**). Yield: 8 %; yellow solid; mp. 241.3–241.7 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.00 (s, 1H), 8.10 (m, 2H), 7.77–7.75 (m, 2H), 7.60–758 (m, 6H), 7.23 (d, J = 6 Hz, 2H), 7.00 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.7 (C=O), 162.3, 150.7, 146.7, 139.5, 131.7, 131.2, 129.1 (2C), 126.6 (q, J = 7.5 Hz, C-F), 126.2 (2C), 125.8, 124.0, 123.0, 122.9, 119.8, 119.7, 119.3, 115.4 (2C), 110.7, 106.2. ^{19}F NMR (282 MHz, DMSO-d_6) δ -58.2. IR (ν, cm^{-1}): 1559 e 1544 (C=O), 3021 (Ar-CH), 3166 (N-H). HRMS (ESI-TOF) m/z calcd for $C_{22}H_{14}F_3NO_2$ 382.1055 $[M+H]^+$, found 382.1058. Analytical HPLC retention time: 12.9 min; purity: 98.1%.

2-(4-methoxyphenyl)-6-((4-methoxyphenyl)amino)-4H-chromen-4-one (**21a**). Yield: 41%; yellow solid; mp. 196.4–196.8 °C. ^{1}H NMR (800 MHz, DMSO-d_6) δ 8.20 (s, 1H), 8.04 (d, J = 9.0 Hz, 2H), 7.62 (d, J = 9.0 Hz, 1H), 7.41 (d, J = 3.0 Hz, 1H), 7.34 (dd, J = 9.0 Hz, 3.0 Hz, 1H), 7.11 (m, 4H), 6.94 (d, J = 9.0 Hz, 2H), 6.84 (s, 1H), 3.86 (s, 3H, O-CH_3), 3.75 (s, 3H, O-CH_3). ^{13}C NMR (200 MHz, DMSO-d_6) δ 176.8 (C=O), 162.0, 161.9, 154.6, 149.0, 143.3, 135.2, 128.0 (2C), 124.1, 123.6, 122.5, 121.6 (2C), 119.4, 114.7 (2C), 114.5 (2C), 105.0, 104.6, 55.5 (O-CH_3), 55.2 (O-CH_3). IR (ν, cm^{-1}): 1562 e 1542 (C=O), 3017 (Ar-CH), 3183 (N-H). HRMS (ESI-TOF) m/z calcd for $C_{23}H_{19}NO_4$ 374.1392 $[M+H]^{+\cdot}$ found 374.1361. Analytical HPLC retention time: 12.4 min; purity: 99.6%.

2-(4-methoxyphenyl)-6-(phenylamino)-4H-chromen-4-one (**21b**). Yield: 69%; yellow solid; mp. 176.7–177.1 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.03 (d, J = 9 Hz, 2H), 7.67–7.62 (m, 3H), 7.48 (d, J = 9 Hz, 1H), 7.30 (t, J = 9 Hz, 1H), 7.16–7.10 (m, 4H), 6.92 (t, J = 6 Hz, 1H), 6.86 (s, 1H), 3.86 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.7 (C=O), 162.2, 161.9, 149.6, 142.6, 141.4, 129.3 (2C), 128.0 (2C), 124.0, 123.8, 123.5, 120.7, 119.4, 117.6 (2C), 114.5 (2C), 107.5, 104.6, 55.5 (O-CH_3). IR (ν, cm^{-1}): 1560 e 1546 (C=O), 3033 (Ar-CH), 3175 (N-H). HRMS (ESI-TOF) m/z calcd for $C_{22}H_{17}NO_3$ 344.1286 $[M+H]^+$, found 344.1276. Analytical HPLC retention time: 12.6 min; purity: 96.8%.

2-(4-methoxyphenyl)-6-((4-(trifluoromethyl)phenyl)amino)-4H-chromen-4-one (**21c**). Yield: 9%; yellow solid; mp. 200.3–201.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.95 (s, 1H), 8.00 (d, J = 9 Hz, 1H), 7.72–7.67 (m, 2H), 7.56 (m, 3H), 7.21 (d, J = 6 Hz, 2H), 7.08 (d, J = 9 Hz, 2H), 6.86 (s, 1H), 3.83 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.5 (C=O), 162.3, 162, 150.6, 146.8, 139.2, 128.0 (2C), 126.6 (d, J = 7.5 Hz, C-F), 125.5, 124, 123.3, 123, 119.6, 119.2, 115.2 (2C), 114.4 (2C), 110.9, 104.8, 55.4 (O-CH3). ^{19}F NMR (282 MHz, DMSO-d_6) δ −58.2. IR (ν, cm^{-1}): 1553 e 1542 (C=O), 3091 (Ar-CH), 3192 (N-H). HRMS (ESI-TOF) m/z calcd for

$C_{23}H_{16}F_3NO_3$ 412.1160 $[M+H]^+$, found 412.1147. Analytical HPLC retention time: 13.9 min; purity: 95.4%.

4.3. Cytotoxicity Assays

To assess the cytotoxic effects of the 6-arylaminoflavones, a viability assay was conducted using four different human cell lines: HEK293T (human embryonic kidney, ATCC CRL-11268), PC3 (prostate adenocarcinoma, BCRJ 0269), A172 (glioblastoma, ATCC CRL-1620), and MDA-MB-231 (breast adenocarcinoma, BCRJ Code 0164) were obtained from the American Type Culture Collection (ATCC, USA) or from the Banco de Células do Rio de Janeiro (BCRJ, Brazil). All cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, adjusted to pH 7.2, and maintained at 37 °C in a 5% CO_2 atmosphere. Cells were seeded in 96-well plates at a density of 1×10^5 cells per well in 100 µL of culture medium. Untreated control cells received only medium and solvent (0.1% DMSO as the final concentration). The cells were incubated with compounds (100 µM) for 48 h. Then, 10 µL of a freshly prepared MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) solution (5 mg/mL, in PBS) was added to each well, followed by incubation for 3 h at 37 °C. Subsequently, 100 µL of DMSO was added to each well to solubilize the formazan, and the absorbance was measured in a plate reader at 540 nm. Cell viability was calculated using the following formula: Viability (%) = [100 × (sample absorbance)/(control absorbance)]. The numerical data was obtained from one experiment performed in quadruplicates.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/reactions6030042/s1, Figure S1: ^1H, ^{13}C and ^{19}F NMR spectra; Figure S2: Infrared, Mass Spectrometry, and HPLC; Table S1: Supplementary Cell Viability Results.

Author Contributions: K.E.P. and M.R.C. contributed equally to this work. K.E.P. and M.R.C. performed the main experiments and data analysis. G.A.M. conducted the biological studies. N.M.A.H. contributed to the analysis of compounds. K.B.W. supervised the biological studies. M.F.Z.J.T. and R.P.-F. assisted in writing and reviewing the final manuscript. K.B.M. and R.P.-F. conceived and supervised the study. All authors have read and agreed to the published version of the manuscript.

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