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Article

Novel Mechanistic Insights into the Anticancer Potential of Methoxyflavones Analogs:A Review

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Abstract: 2-phenylchromen-4-one, commonly known as flavone, plays multifaceted roles in biological response, largely due to its abundance in natural sources. The methoxy group in naturally occurring flavones promotes cytotoxic activity in various cancer cell lines by targeting protein markers, in facilitating ligand-protein binding mechanisms and activating cascading downstream signaling pathways leading to cell death. However, the lipophilic nature of these analogs is a key concern as it impacts drug membrane transfer. While lipophilicity is crucial for drug efficacy, excessive effects in flavonoids can reduce water solubility and hinder drug transport to target sites. Recent in vitro studies suggest that the incorporation of polar hydroxyl groups which can form hydrogen bonds and stabilize free radicals may help overcome the challenges associated with methoxy groups while maintaining their essential lipophilic properties. Naturally coexisting with the methoxyflavones, this review explores the synergistic role of hydroxy and methoxy moieties through hydrogen bonding capacity in maximizing cytotoxicity against cancer cell lines. The physicochemical analysis revealed the potential intramolecular interaction and favorable electron delocalization region between both moieties to improve cytotoxicity levels. Together, the analysis provides a useful strategy for structural-activity relationship (SAR) of flavonoid analogs in distinct protein markers, suggesting optimal functional group positioning to achieve balanced lipophilicity, effective hydrogen bonding, and simultaneously minimized steric hindrance in targeting specific cancer cell types.

Keywords: Medicinal plants; methoxyflavones; physicochemical properties; structural-activity relationship; anticancer mechanisms

1. Introduction

Flavones, a prominent subclass within the flavonoid family, exhibit a multi-target pharmacological profile owing to their intricate structural system. This structure features a highly soluble chromone core (1-benzopyran-4-one) with a hydrophobic phenyl ring attached to the ortho position of pyran ring [1]. The unique pharmacological properties of natural flavonoid, particularly flavones contribute to its diverse bioactivities in both traditional therapies and modern medicine [2–5]. The physicochemical properties of these flavones are largely attributed to two major group that coexist, predominantly the methoxy and hydroxy substituents in natural [6,7]. Both groups exhibit distinct characteristics and work synergistically in an electron donor-acceptor mechanism, influencing electron density distribution depending on their position in rings A, B, and C of the flavone structure [8–10]. As a result, the flavones pharmacophores significantly influence the bioavailability of flavones, one of the primary focus in discovering potential therapeutic drugs (Moldoveanu & David, 2022) [11]. Drug discovery originating from medicinal plants has fruitfully developed new potent therapeutics, such as morphine, an alkaloid derived from *Papaver somniferum*

(opium poppy) [12]. Similarly, the discovery of taxol, a terpenoid compound from *Taxus brevifolia* (pacific yew tree) led to the commercialization of paclitaxel as an antitumor drug [13]. Despite the reported biological efficacy of flavones, a comprehensive systematic review authored by Xu et al. highlighted significant challenges in developing anti-tumor drugs based on flavones aglycones. These challenges are often due to physical and structural limitations that affect the bioavailability. Thus, efforts to delve into the chemistry of highly lipophilic flavones were enormous, particularly focusing on the hydrophobic region such as the unsaturated double bond between C2 and C3 on ring C and phenyl ring B.

The distinct physicochemical attributes of methoxy and hydroxy moieties add a prospect to the synergistic relationship between both functional groups in enhancing biological responses. Therefore, the in-depth analysis of the physicochemical properties, including lipophilic effects, intramolecular interactions and hydrogen bonding capacity will be useful in the structural-activity relationship (SAR) investigations of methoxyflavones' analogs to determine its target of interest and eventually contribute to its distinct biological activities [15–17]. An optimal lipophilic capacity of a lead compound is part of the major limitation of flavones in anticancer drug discovery [18,19]. The partition coefficient, LogP is one of the parameters to predict hydrophobicity and lipophilicity and has been validated on various flavone scaffolds [20]. The lipid-water partitioning of flavones is crucial given its influence on the uptake and delivery of flavones to the target sites. In plant species, methoxy moieties within the flavones scaffold strengthen the hydrophobic interaction through methylation, possessing hydrogen bonding acceptor (HBA) capabilities and initiating a stabilized ligand-protein binding energy [17,21]. Despite its greater binding and interaction with the active sites, the methoxylated group is a metabolic liability to a compound that leads to biotransformation through the *O*-demethylation metabolic pathway [22,23]. Although not reported for every naturally isolated and synthesized methoxyflavones scaffold, methoxylation could alter bioavailability, solubility, and pharmacology in flavones [24–26]. Nevertheless, the presence of the hydroxy group within the methoxyflavones scaffolds could offset the drawbacks by altering the extreme lipophilicity of flavones through intramolecular interaction and greater electron delocalization region in stabilizing the free radicals and established strong interaction with the target binding sites as hydrogen bond donor (HBD) (**Figure 1**) [27,28]. However, the incompatible position of the highly polar hydroxyl group within the flavones' pharmacophores may activate unfavourable thermodynamic properties and desolvation penalties which pose significant challenges in medicinal chemistry [29,30].

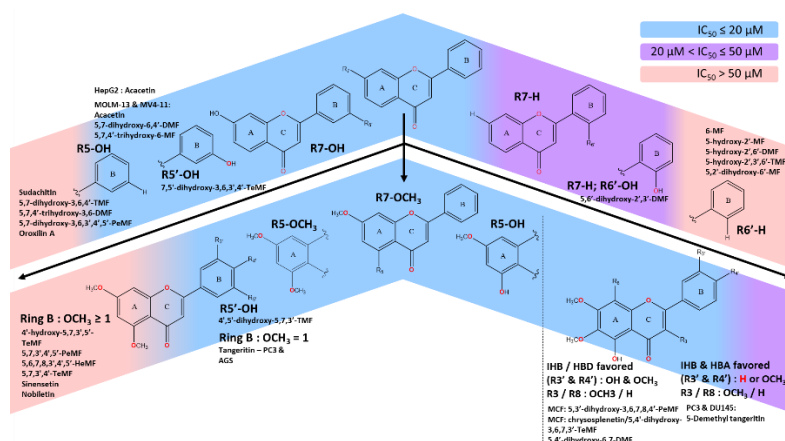


Figure 1. Schematic diagram on anticancer activity of methoxyflavones scaffold.

**This diagram illustrates the relationship between the methoxyflavones scaffolds and their effect on the IC₅₀. The C7 position plays a key role in the cytotoxic activity on cancer cell lines. The strong effect of C7-OH scaffold was contributed by at least one methoxy moieties on ring A and -OH moieties on the C5 or C5' position. C6'-OH could exert a similar effect if the pharmacophore of C7-OCH₃ or -OH was preserved. The C7-OCH₃ was widely studied and contributed the most in yielding strong IC₅₀ (<20 μM), with few exception:

- With C5-OCH₃, stronger IC₅₀ could be achieved by either the presence of optimal polar region from -OH moieties or HBA strength of at least one -OCH₃ on ring B. Having multiple methoxy moieties on ring B significantly deteriorates the IC₅₀ of the scaffold on numerous cancer cell lines.
- C5-OH, in general, was the most favored to induce strong IC₅₀, with a few exclusions. The established methoxylated backbone on ring A (C6,7 or 8) with the addition of both methoxy and hydroxy moieties in neighboring positions (C3' & C4') strengthens the IHB, HBD and HBA effect. Otherwise, the absence of hydroxy moieties on ring B could be offset by the single methoxylated substitution on the same ring. The C3-OCH₃ could coalesce, dependent on cell types.

As illustrated in **Figure 1**, the subtle repositioning of hydroxy and methoxy substituents on the flavone ring greatly affects the biological activity and the application of the flavones' analogs either in the hydrophobic or polar aqueous region [31]. The coexistence of a hydroxy functional group may expand the polar region through hydrogen bonding interactions, though this effect depends on its position (Veiko et al., 2021) [32]. The hydroxy group in flavonoids including flavones has been extensively researched for its ability to scavenge free radicals, diminish ROS levels, and contribute to its potency as antioxidant, anticancer, and anti-inflammatory agents [33–37]. Hydrogen bonding of the hydroxyl group is notably robust and crucial for acting as a hydrogen bond donor. Nevertheless, an enormous polar region might accelerate drug hydrolysis and impair lipid membrane permeation (Hawthorne et al., 2022) [20]. Despite challenges related to metabolic stability and lipophilicity, methoxy moieties in flavones analogs have shown selectively potent anti-inflammatory effects, enhanced by the C4'-methoxyl group and an increased anti-proliferative effect from C7-methoxy substitution [16]. In addition, the existence of both methoxy and hydroxy functional groups in adjacency and its impact on the stereospecificity of flavones merits further exploration [16]. These interactions could promote the formation of an intramolecular interaction between the two atoms, serving as a double-edged sword by improving the stability of the parent structure and potentially blocking polar interaction in favor of a lipophilic environment through the shielding effect of intramolecular hydrogen bonding (IHB) [39,40]. The IHB between a hydroxyl group adjacent to the carbonyl group in various aromatic and polyphenolic compounds has been reported in computational studies [41–43]. For instance, the C5-OH substituent within the flavone skeleton interacts with C4 carbonyl group through intramolecular hydrogen bonding, with multiple studies showing that the associated increase in lipophilic capacity enhances cytotoxic effects on cancer cell lines more than C5-OCH₃. In addition, the methoxy group acting as a hydrogen bond acceptor (HBA), may also form intramolecular hydrogen bonds in optimally positioned flavones analogs, particularly when ortho to hydroxy moieties. The presence of C3'-OCH₃ lowers the hydrogen bonding acidity of C4'-OH by 20%, suggesting a stronger potential for stable IHB formation between the OH and OCH₃ groups, thereby increasing lipophilic character [44,45]. Nevertheless, stable IHB formation can restrict the hydrogen donor capacity of C4'-OH and reduce its affinity for target proteins, unless compensated for by the external interaction with protein markers. In flavones, the intramolecular hydrogen bonding with the adjacent atoms predominantly occurs in rings A and B. Given their distinct roles in polar-hydrophobic interactions, a balanced relationships between these moieties is crucial for maximizing the pharmacological potential of flavones, particularly in anticancer applications.

2. SAR and Mechanism of Anticancer Activity of Methoxyflavones Derivatives

2.1. Breast Cancer

Compound 5,3'-dihydroxy-3,6,7,8,4'-PeMF, isolated from *Glycosmis ovoidea* demonstrated potent cytotoxic effects on breast cancer cells, MCF-7 with IC₅₀ values of 49.46, 17.56 & 3.71 μ M [46]. Signs of apoptosis such as cell membrane blebbing, shrinkage, and fragmentation were observed. A loss of mitochondrial membrane potential at concentrations of 2 & 20 μ M was noted, indicating that intrinsic apoptosis had occurred [47].

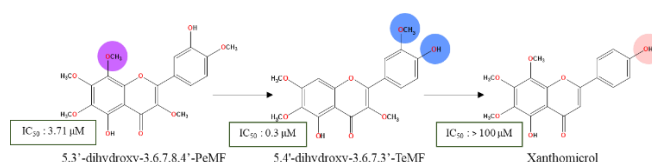


Figure 2. The influence of the position of both functional groups in ring B on the IC_{50} value in MCF-7 in 72-hour treatment duration.

**This figure illustrates the effect of pharmacophore changes on ring B. The presence of both moieties on neighboring positions (C3' & C4') is a crucial factor for stronger IC_{50} of 5-OH methoxyflavones analogs. The C8- OCH_3 plays a crucial role in stabilizing the lipophilic capacity of ring A flavones. Given the presence of C6 and C7- OCH_3 , the reduction of single C8- OCH_3 will not significantly affect the IC_{50} of these scaffolds. For xanthomicrol, the absence of methoxy adjacent to the C4'-OH was a single factor in the expected weak cytotoxicity profile.

By swapping the hydroxy and methoxy groups' positions from C4' to C3', and removing of C8- OCH_3 , chrysosplenetin (5,4'-dihydroxy-3,6,7,3'-TeMF) was isolated and effectively induced cancer cell death on MCF-7 in 72-hour treatment with stronger IC_{50} value of 0.3 μM [48]. Another methoxyflavones, xanthomicrol, 5,4'-dihydroxy-6,7,8-TMF was inactive with an IC_{50} exceeding 100 μM [49]. Based on the schematic diagram (Figure 1), the 3,6,7-TMF scaffold is the backbone that preserves the lipophilic character, acts as a potential hydrogen bond acceptor, and facilitates hydrophobic interaction with the target proteins. The delocalization region of electron density from C5-OH free radicals (Figure 3) enable a highly stabilized intramolecular hydrogen bond (IHB) with the adjacent carbonyl group. This IHB between C5 and C4 substituents elevates the lipophilic capacity of the flavones' analogs enhancing drug-lipid transfer and cytotoxic activity [44,50]. Interestingly, the interchanging effect of neighboring moieties on ring B significantly influences the cytotoxicity in MCF-7 reducing the IC_{50} from 3.71 to 0.3 μM , which highlights the favorable positioning of the C4' hydroxy group over C3'. Regardless, both positions are within the ideal region for maximizing the potential of the hydroxy group to intensify the anticancer effect of methoxyflavones analogs. Similarly, compared to C5-OH, C4'-OH free radicals generate a stronger resonance effect than the C3' attributed to the extended pi-conjugation from C2-C3 unsaturated bond up to carbonyl ring C (Figure 3) [27]. The existence of stabilized hydrogen bonding between C5-OH and the neighboring carbonyl group, along with the resonance effect from C4' free radicals assist in interaction with C3'- OCH_3 , preserving the conformation and shielding the polar surface on ring B against specific target [51–54]. As a result, the absence of a neighboring methoxy group next to C4'-OH (xanthomicrol) leads to poor bioactivity. Thus, greater reduction potential from the resonance effect paves the way for strong hydrogen bonding with protein markers. The removal of C8- OCH_3 unexpectedly preserved the biological effect of the scaffold. Due to the limited data available to predict the roles of C8- OCH_3 , it is hypothesized that for specific cell lines, the methoxylation of at least three positions within the AC-ring system from C5-OH methoxyflavones would be accommodative in balancing its physicochemical properties. C8- OCH_3 may not necessarily contribute to the cytotoxic effect against MCF-7, even with the presence of heavily substituted C3,6,7- OCH_3 in the methoxyflavones scaffold.

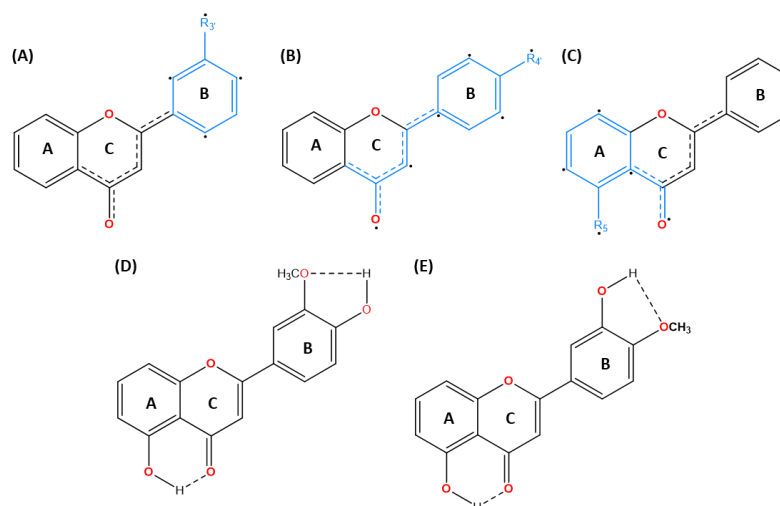


Figure 3. Lewis's resonance structures illustrating electron delocalization in (A) C3', (B) C4', (C) C5 free radicals. Ring AC and BC are not conjugated. According to a 2013 study, a strong IHB between C5-OH and the C4 oxygen atom of the carbonyl group facilitates the formation of IHB's involving (D) C4'-OH and (E) C3'-OH with an adjacent methoxy groups.

Focusing on triple-negative breast cancer cell lines, a similar compound 5,3'-dihydroxy-3,6,7,8,4'-PeMF was compared with nobletin, 5,6,7,8,3',4'-HeMF for cytotoxicity activity against MDA-MB-231 cell lines. As anticipated, compound 5,3'-dihydroxy-PeMF significantly reduced cell viability with an IC_{50} of 21.27 μ M, compared to nobletin which showed ~25% reduction at 10 μ M, with no IC_{50} reported after 72-hour treatment. A marginal increase in IC_{50} in MDA-MB-231 cells compared to MCF-7 may be attributed to the highly invasive and metastatic nature. Several factors contribute to nobletin inactivity including, though not restricted to (i) extreme lipophilic effect of ring B, (ii) exacerbated steric hindrance, (iii) and absence of IHB that stabilize the pharmacophore, leading to compound degradation. Despite the similarities in functional groups between the two compounds except for C3 hydroxy group, the role of hydroxy groups in the antitumor activity of methoxyflavones was noteworthy. The direct apple-to-apple comparison may be premature to definitively claim that hydroxy flavones are the key markers in flavones analogs' effectiveness as opposed to the methoxy groups, reports indicate that hydroxyflavone analogs' cytotoxicity against MDA-MB-231 cell lines is considerably lower with IC_{50} values around 200 μ M for treatments ranging from 24 to 72-hour, compared to nobletin. This reduced effectiveness is attributed to the extensive polar surface of polyhydroxylated flavones [55–57]. Thus, strategically optimizing the placement of hydroxy moieties within the methoxyflavones pharmacophore could balance the extreme lipophilic and polar region features, enhancing their synergistic interaction.

OH methoxyflavones scaffolds, influenced by the C5'-OH. As summarized in **Figure 1**, excessive methoxylated effect on ring B yielding negative IC_{50} result on cancer cell lines.

The polymethoxyflavones were screened against human epidermal growth factor receptor 2 positive (Her-2+) HCC1954 breast cancer cell lines in vitro by [58]. Compound 4',5'-dihydroxy-5,7,3'-TMF exhibited strong cytotoxicity against these cancer cells with an IC_{50} value of 8.58 μ M (Figure 4). The cytotoxicity of the treatment was marginally weaker compared to the previous study that had been discussed due to the more invasive and metastatic nature of the HCC1954 relative to MCF-7 [59]. Surprisingly, dehydroxylated moieties on ring B increased cytotoxicity activity. The formation of IHB between C4' and C5' hydroxy groups, which was found to be active and greatly stabilized the scaffold and preserved the lipophilic properties of the flavones [27]. However, modifying the skeleton by substituting C5'-hydroxy to C5'-methoxy group (4'-hydroxy-5,7,3',5'-TeMF) significantly weakened the cytotoxic effect, resulting in an IC_{50} exceeding 100 μ M. The comparison between both compounds highlights several challenges including the bulkiness of the C5'-OCH₃ group that hindered IHB formation with the carbonyl group and the presence of multiple methoxylated

substitution on ring B (C3' and C5') which were unfavorable. Without C5-OH, the C4'-OH in compound 4'-hydroxy-5,7,3',5'-TeMF exhibited unexpectedly poor cytotoxicity, triggered by an increased potential for resonance effect arising from C4'-OH free radicals to the carbonyl group as both AC- and BC-ring electron delocalization were not in conjugated system [60,61]. The C4'-hydroxy regions of flavone pharmacophores are known for having the weakest hydrogen bonding energy in ring B and are the most active radicals, that readily distribute electron density with greater active region rise from the ring B up to carbonyl group of ring C from resonance and conjugation effects [62]. Thus, an excessive polar surface significantly weakens the bioactivity of the scaffold. In fact, fully methoxylated flavones, 5,7,3',4',5'-PeMF demonstrated a better IC₅₀ at 53.84 μ M, although the range was considered weak as the IC₅₀ exceeded the threshold of 50 μ M. With a limited polar surface, the massive lipophilic capacity from C3',4',5'-trimethoxy substitution may enhance membrane transfer but will restrict strong hydrogen bonding interaction with the protein target markers. As a result, the addition of two more methoxy groups on C-6 and C-8 (5,6,7,8,3',4',5'-heptamethoxyflavone) resulted in weak cytotoxic effects on the cell lines with an IC₅₀ > 100 μ M. We hypothesize that high methoxy substituents on ring A of the flavones structure, with the absence of a polar region worsens the active region of methoxyflavones, thus suppressing the antiproliferative effect on HCC1954 breast cancer cell lines. Nevertheless, the SAR analysis revealed that the C5'-OH contributes to enhancing the antiproliferative effect on HCC1954 cell lines. An in-depth investigation into the incorporation of C5'-OH with the methoxyflavones scaffold may be required as current data is limited to validate the strength of the C5' position.

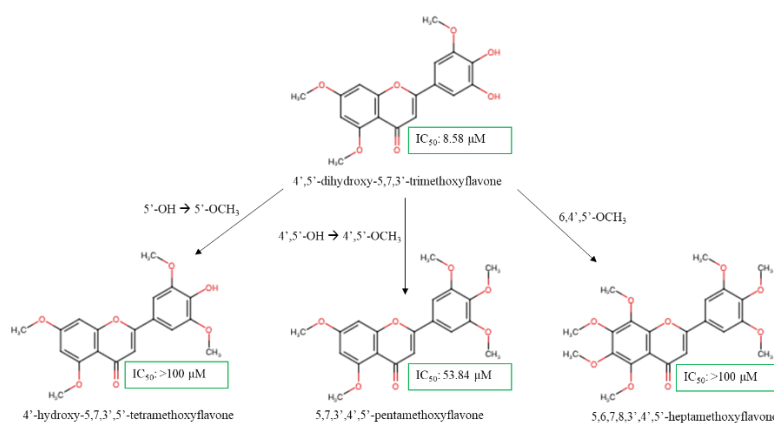


Figure 4. Cytotoxic effects governed by dihydroxylated moieties on ring B highlighting the impact of C5' scaffold composition. The presence of C5-OCH₃ shows enhanced cytotoxicity similar to the C5-

2.2. Prostate Cancer

The anticancer effects of methoxylated flavones have been extensively studied against various prostate cancer cell lines in vitro, including PC3, VCaP, LNCaP, and DU145. Recent research involving six methoxyflavones across five studies revealed that all flavones' derivatives bearing C6-OCH₃ incorporated into their skeleton structure (**Figure 1**). The variations in the antiproliferative effects against different cell lines can be thoroughly analyzed, given the similarities in parent structural structures to determine the future potential target of these flavone derivatives and may postulate the crucial role of C6-OCH₃ in combatting prostate cancer.

In studies targeting PC3 prostate cancer cell lines, both polymethoxyflavones, tangeritin (5,6,7,8,4'-PeMF) and 5-demethyltangeritin (5-hydroxy-6,7,8,4'-TeMF) exerted strong cytotoxicity in a concentration-controlled manner [63]. Within 48-hour treatment, both compounds significantly reduced PC3 cell viability with IC₅₀ values of 17.2 and 11.8 μ M, respectively (**Table 1**). The increase in logP magnitude correlates with enhanced hydrophobic capacity, facilitates strong intramolecular hydrogen bonding (IHB) between C5-OH and the neighboring C4 carbonyl group. While IHB was absent with C5-OCH₃, this group likely interacts with the target site as HBA, enabling polar interactions with the carbonyl group and the binding target [64]. The SAR analysis suggests that both

hydroxylation and methoxylation at the C5 position significantly contribute to the antiproliferative effects on PC3 cell lines. Conversely, another study on similar cancer cell lines showed minimal bioactivity of nobiletin (5,6,7,8,3',4'-HeMF) in a concentration-dependent manner with IC₅₀ values around 80 μM for the same treatment duration [65]. A comparison of cell viability between the two studies confirmed the inactive anti-proliferative effect of nobiletin. Treatment of 20 μM tangeritin reduced cell viability to below 50%, whereas a similar concentration of nobiletin maintained a 100% cell viability over the same treatment period. Interestingly, the only structural difference between tangeritin and nobiletin is the addition of C3'-OCH₃ to nobiletin's structure. The increased bulkiness of ring B in nobiletin, due to multiple methoxy substitutions at adjacent positions (C3' & C4'), enhances steric hindrance and elevates both the partition coefficient (logP), and distribution coefficient (logD) (**Figure 5**) [66]. Therefore, the presence of an optimal polar surface provided by hydroxy moieties at ring B introduces an amphipathic characteristic, essential for a more potent cytotoxic effect on cancer cell lines.

Table 1. Comparative Antiproliferative Effects of Methoxyflavones against Prostate Cancer Cell Lines.

Prostate cancer cell lines	C ₃	C ₅	C ₇	C ₈	C _{3'}	C _{4'}	IC ₅₀ (μM) / Cell viability (%)	Reference
PC3	H	OMe	OMe	OMe	H	OMe	17.2	[63]
	H	OH	OMe	OMe	H	OMe	11.8	
	H	OMe	OMe	OMe	OMe	OMe	80.0	
VCaP	H	OMe	OMe	OMe	OMe	OMe	> 80% (120 μM)	[65]
	H	OH	OH	H	H	OH	>50% (50 μM)	[67]
DU145	OMe	OH	OMe	H	OH	OMe	< 80% (50 μM)	[68]
	OMe	OH	OMe	OMe	H	OH	235.0	
LNCaP	OMe	OH	OMe	OMe	H	OH	116.5	[69]

**This table illustrates pharmacophore changes on ring B flavones (C3' and C4') flavones significantly influenced the IC₅₀ of the analogs. Green indicates favorable moieties and red signifies unfavorable position.

Besides PC3 cell lines, another androgen-independent DU145 cell line is known for its metastatic and invasive behavior due to its non-reliance on hormones for growth [70,71]. Compared to the vehicle group (0.5% DMSO), casticin (5,3'-dihydroxy-3,6,7,4'-TeMF) induced a weak cytotoxic effect maintaining over 80% cell viability at concentrations of 20-50 μM during 48-hour treatment [68]. Structural modifications on rings A and B specifically shifting C4' > C8-OCH₃ and C3'-OH to a more favorable hydroxy position at C4'-OH resulted in the creation of calycopterin (5,4'-dihydroxy-3,6,7,8-TeMF). However, the cytotoxic potential of calycopterin remained low with an IC₅₀ value of 235 μM in 48-hour treatment [69]. Nevertheless, when comparing IC₁₀ values of both compounds, casticin (IC₁₀: 2.5 μM) was significantly more potent than the calycopterin (IC₁₀: 20 μM). SAR analysis suggested similar cytotoxic behavior in both hormone-independent PC3 and DU145 cells upon treatment with these derivatives. The C6,7-OCH₃ group was identified as the backbone for the potent antiproliferative effect through lipophilic capacity and pi-alkyl hydrophobic interactions. Meanwhile, the absence of at least one methoxy group (C3' or C5') adjacent to the C4'-OH adversely reversed the potency (**Figure 5**). Multiple anticancer studies on both cell lines depicted comparable cytotoxic capacities across different samples (**Table 2**).

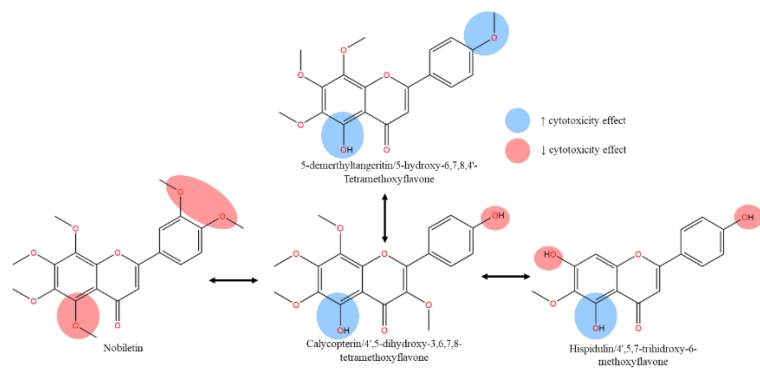


Figure 5. SAR analysis of methoxyflavones analogs and their cytotoxic effects on prostate cancer cell lines.

** This diagram illustrates the structural modifications between various flavone derivatives and their associated cytotoxic effects. Blue highlights indicate regions of the molecule that enhance cytotoxicity, while red highlights denote regions that reduce it. The arrow directions show the transformation from one compound to another, detailing the specific chemical changes like the addition or removal of hydroxyl (OH) and methoxy (OMe) groups. For example, the transformation from nobiletin to 5-demethyltangeritin shows the replacement of methoxy groups with hydroxyl groups, correlated with an increase in cytotoxic activity.

Table 2. Comparison of IC₅₀ value of various compounds on hormone-independent prostate cancer cell lines (PC3 and DU145).

Sample	IC ₅₀ (μM) / cell viability (%)		Reference
	PC3	DU145	
Auraptene	<50% (120 μM)	<50% (120 μM)	[72]
Curcumin	<60% (50 μM)	<60% (50 μM)	[73]
Cyclosporine-A	8.80 μM	12.14 μM	[74]
Demethylzeylasteral (T-96)	13.10 μM	11.47 μM	[75]

** This table validate the similarities on cytotoxicity activity of some natural and synthetic compounds on both hormonal-dependent prostate cancer cell lines.

VCaP and LNCaP are androgen-dependent cell lines with weak metastatic potential. Interestingly, owing to the structural similarities of flavonoid families with reproductive hormones, these compounds demonstrate active interactions with androgen receptors [76,77]. The list of phytoestrogenic and phytoandrogenic compounds known to be polyphenolics which demonstrate strong radical scavengers [78]. Thus, we hypothesize that an increased polar region on ring B of methoxyflavones may induce stronger cytotoxic activity on hormone-dependent cell lines by targeting hormone receptors. Supporting this hypothesis, calycopterin exhibited potent cytotoxicity on LNCaP cells (IC₅₀: 116.5 μM) compared to DU145 cells (235.0 μM) in 48-hour treatment [69], although, the IC₅₀ still exceeded 100 μM. Similar to observations in PC3 cell lines, the influence of C4'-OH in enhancing free radicals scavenging capabilities was suppressed by the IHB formation between C5-OH and the carbonyl group, further limited by the absence of IHB in ring B resulting in a smaller region of electron delocalization (Figure 5) [27]. To support the hypothesis, cell viability remained above 80% (100 μM) after 48-hour treatment with nobiletin on VCaP cells, significantly weaker compared to treatment on hormone-independent PC3 cells [65]. Thus, the lack of a polar region within the aromatic ring B leads to an increased lipophilic capacity, that hinders the hydrogen donating capability. Likewise, the enormous hydroxylation in the flavone's lipophilic backbone at ring A in C5,7,4'-OH (hispidulin / 5,7,4'-trihydroxy-6-methoxyflavone) failed to improve the cytotoxic effect, proving that the lipophilic effect of methoxy group on ring A is crucial in creating hydrophobic interactions, with hydroxy moieties on ring B to elevate its cell death effects [67,79]. We can conclude that both methoxylated and hydroxylated groups play a crucial and synergistic role in their

respective region on rings A and B of the flavone structure, maximizing cytotoxic effects on both hormone-dependent and -independent cell lines.

2.3. Colon Cancer

The compound 5,7,3',4'-TeMF (60 μ M) slightly reduced HCT116 cell viability to approximately 75% upon treatment for 72-hour [80]. Meanwhile, another tetramethoxyflavones, compound 7,8,3',4'-TeMF reduced viability marginally to 90% under the same conditions. The rearrangement of the methoxy group from C5 to C8 did not significantly enhance the cytotoxic effect of the TeMF derivatives on HCT116 cell lines (**Figure 6**). The strong hydrophobic effect from the methoxylated substituents, combined with a lack of polar interaction sites on ring B, may contribute to the impotent effect. Although the rearrangement of C5 to C8-OCH₃ reduced the cytotoxic effect, it is hypothesized that the absence of C5-OCH₃ or -OH plays a greater role in influencing the bioactivity of methoxyflavones analogs. Based on the behavior of C8-OCH₃ on cancer cell lines, these moieties have little to no effect on anticancer efficacies. Nevertheless, despite higher cell viability, both tetramethoxyflavones (TeMF) reduced cell migration and invasion, consistently decreasing cell motility, and the expression of vimentin, and axin2 mRNA, key components of the Wnt- β -catenin signaling pathway in HCT116 cells [80].

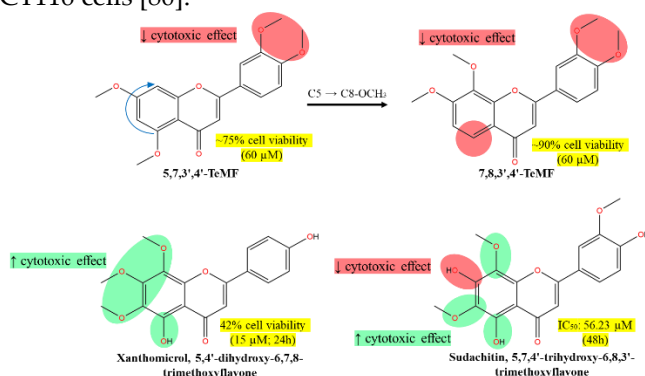


Figure 6. SAR analysis on cytotoxicity activity of methoxyflavones analogs on HCT116 colon cancer cell lines.

**This diagram illustrates the molecular structures of various methoxyflavones and their respective impacts on cell viability. Key features include:

- 5,7,3',4'-TeMF at 60 μ M resulting in 75% cell viability, highlighting its moderate cytotoxic effect.
- 7,8,3',4'-TeMF showing a slight reduction in cytotoxic efficacy with about 90% cell viability at the same concentration.
- Xanthomicrol (5,4'-dihydroxy-6,7,8-TMF) and sudachitin (5,7,4'-trihydroxy-6,8,3'-TMF) demonstrating enhanced cytotoxic effects with 42% cell viability at 15 μ M and an IC₅₀ of 56.23 μ M at 48 hours, respectively.
- The arrows indicate the structural transitions between compounds, noting the shift from C5 to C8-OCH₃ and the associated impact on cytotoxicity. Highlighted green regions emphasize the structural components correlated with increased cytotoxic effects, whereas red highlights denote areas associated with decreased effects. This visualization aids in understanding how specific structural modifications influence anticancer activity against HCT116 cells.

In vitro treatment of xanthomicrol, 5,4'-dihydroxy-6,7,8-TMF for 24 hours on HCT116 colon cancer cell lines potently reduced the cell viability to 42% at 15 μ M and to 3% at higher concentrations (21 μ M) [81]. Meanwhile, sudachitin, 5,7,4'-trihydroxy-6,8,3'-TMF moderately suppressed cell viability in HCT-116 and HT-29 colorectal cell lines in 48-hour treatment of yielding IC₅₀ values of 56.23 μ M and 37.07 μ M, respectively [82]. The screening portrays hydroxy group at position C5 and C4' in xanthomicrol were potent against HCT116, while the additional C7-OH in sudachitin disrupted the methoxyflavone scaffold, which moderately impacting the efficacy, owing to stabilized

IHB between C3'-OCH₃ and C4'-OH (**Figure 6**). As highlighted in our schematic diagram, the disproportionate substitution of the methoxy group, particularly on ring B (C3' and C4') of both tetramethoxyflavones leads to steric hindrance, that inhibits the formation of intermolecular bond and reducing the antiproliferative effects. Xanthomicrol and sudachitin demonstrate a perfect hypothesis whereby the weak cytotoxic effect by the extra bulkiness of the methoxylated substituent on ring A and absence of polar region on ring B were offset by hydroxylation of C4', contributing to stronger bioactivity. The presence of methoxy and hydroxy moieties on position C5 ring A did not significantly influence the bioactivity of flavones derivatives, as both exert similar lipophilic characteristics through different mechanisms - methylation and electron delocalization. Nevertheless, the unexpected cytotoxicity of xanthomicrol on HCT116 gained our interest due to the deviation from the proposed SAR schematic in **Figure 1**. With the formation of IHB between C5-OH with carbonyl group C4, and the lack of methoxy group on ring B, we are expecting a moderate effect resulting from the exposure of C4'-OH polar region. For instance, xanthomicrol reported weak activity against HeLa and MCF-7 with IC₅₀ exceeding 100 μ M in 24- and 72-hour, respectively [49,83]. Meanwhile, calycopterin, with a similar ring B scaffold to xanthomicrol showed a weaker activity against prostate cancer cell [12]. Therefore, we suggest that the HCT116 viable cells may be selectively suppressed by the polar surface of C4'-OH in xanthomicrol indicating a role in cell death induction. The overwhelming cell death induced by xanthomicrol was due to high cell accumulation at the G2/M phase (15 μ M) and G1/G0 phase (21 μ M) through early and late apoptosis [81]. We believe that C4'-OH may be a critical substituent that triggering the apoptotic cell death pathway, evidenced by its potent IC₅₀ values. These findings suggest that increased methoxylation on the flavone skeleton does not correlate to elevated cytotoxicity on cancer cells. Instead, cytotoxicity appears to depend on the specific positioning of substituents and the strategic placement of hydroxyl groups within the flavone structure.

2.4. Liver Cancer

Demethylated nobiletin's derivatives revealed significant antiproliferative potential against HepG2 liver cancer cell lines [84]. Compound 5,3'-didemethylnobiletin demonstrates the strongest IC₅₀ (41.37 μ M), followed by 5,3',4'-tridemethylnobiletin (46.18 μ M), 5-demethylnobiletin (47.31 μ M) and 5,4'-didemethylnobiletin (54.46 μ M) when treated for 24-hour.

** This diagram maps out the structural modifications of 5-demethylnobiletin and their corresponding IC₅₀ values, indicating cytotoxic potency against HepG2 cells. Each derivative (1-4) shows a different substitution pattern on ring B methoxyflavones structure, highlighted in the blue circle. The IC₅₀ values adjacent to each structure illustrate the effect of these modifications on cytotoxic efficacy. Notably, derivative 2 with a hydroxyl group at position R_{3'} shows the lowest IC₅₀ value of 41.37 μ M, suggesting increased efficacy compared to other derivatives.

Demethylation of C3' and C4' in compounds (1) to (4) significantly enhances cytotoxic effects on HepG2 cells as depicted in **Figure 7**. Hydroxylation at C5, together with C3' or C4' has been shown to improve cell death effects with C4' displaying slightly superior potential, arguably. Nevertheless, based on our proposed figure, the cytotoxic potential between C3'- and C4'-OH derivatives narrows down to the target cell death markers, preferable of the polar surface region on ring B and lipophilic capacity of the target compound. Although C4'-OH flavone derivatives have demonstrated strong cytotoxic activities across various cancer cell lines as compared to C3'-OH with the presence of C5-OH, it may not be the case for HepG2's anti-cancer mechanisms. In principle, as illustrated in the breast cancer section and multiple studies, the potent effect of C4'-OH is contributed by the greater electron delocalization within rings B and C, which further stabilizes free radicals within the conjugated system and boosts electron donor capability. As a result, the potential of IHB interaction between the adjacent group intensified [44]. To add, C3' and C4' interaction with protein targets through respective electron donor and acceptor capability, could induce more potent cytotoxic effects. A highly stable conformation preserves the molecular structure, nonetheless, may hinder interactions with the target site, which is coherent with the multiple reports that additional hydroxylation on ring B does not significantly correlate with the potency effect on cancer cells.

Interestingly, the interaction that exists between the hydroxy group at C3' and C4' on ring B (P4) achieved comparable IC_{50} values to its methoxylated counterpart (P1). To conclude, all four compounds show strong potency despite a variety of functional groups, especially the catechol moiety in ring B, which is commonly found in naturally isolated flavones [85]. Thus, we hypothesize the demethylation of C5, alongside 6,7,8-trimethoxylated substituent on ring A is crucial for anticancer activities on HepG2 cell lines.

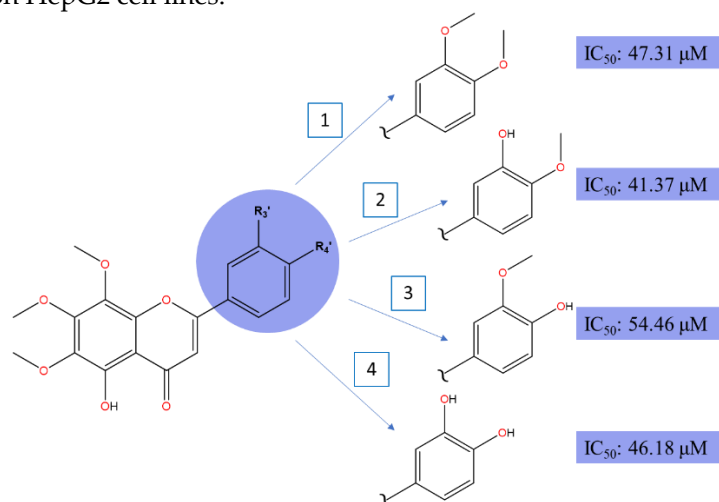


Figure 7. SAR analysis on cytotoxicity activities of 5-demethylnobiletin derivatives on HepG2 liver cancer cell lines.

** This diagram displays the structural variations among three flavonoid derivatives and correlates these changes with their cytotoxic effectiveness. Key features highlighted:

- **Favored moieties** (green) are indicated on structures where modifications have led to improved cytotoxic activity.
- **Unfavored moieties** (blue) are shown on structures where modifications have reduced cytotoxic effectiveness.
- Each compound's impact on cell viability is quantified next to the respective molecular structure, with IC_{50} values provided:
 - 5-demethylnobiletin: 50% cell viability at 47.31 μ M over 24 hours.
 - Sinensetin: 70-80% cell viability at 50 μ M over 24 hours.
 - Sudachitin: 50% cell viability at 49.32 μ M over 48 hours.

The arrows indicate structural transformations and their direct impact on cytotoxic efficacy, illustrating how specific modifications in the flavonoid skeleton influence cell death pathways in HepG2 cells. This visual representation aids in understanding the critical chemical groups responsible for the compounds' varying levels of potency against cancer cells.

To validate our hypothesis, results were compared with an earlier study by Kim et al. [86]. Disturbance on ring A, by removal of methoxy and hydroxy group on C8 and C5, respectively led to a significant drop in cytotoxic effect. In a similar treatment duration (24-hour), 50 μ M of sinensetin (5,6,7,3',4'-PeMF) reduced cell viability by only 30%, with no IC_{50} reported. Despite a longer treatment period (48h) was employed, it's worth discussing the cytotoxicity effect of sudachitin due to its unique conformation [87]. While it retained the 5-hydroxy group, justifying a strong IC_{50} (49.32 μ M), and hydroxylation was also observed on position C7 and C4' on rings A and B, respectively. Note that as 24-hour treatment was not reported, we assumed the activity of sudachitin might be lower if the cytotoxic effect was in a time-controlled condition. The result, however, appeals to our interest in exploring the activity of C7-OH given its strong cytotoxicity activity on par with the C7-OCH₃ methoxylated flavone analogs (**Figure 8**). Our analysis shows that current C7-OH methoxylated flavones demonstrate varied cytotoxicity strength against cancer cell lines [67,88]. Acacetin, 5,7-dihydroxy-4'-methoxyflavone was one of the earlier mono-methoxyflavones studied in anticancer that may untangle the potency of C7-OH. Acacetin reduced cell viability with an estimated IC_{50} of 25 μ M after 24-hour treatment [89]. The variation may exist due to the preferential of specific moieties

and the pathway that leads to the reduction of cell viability. Interestingly, the slight variation in IC_{50} values of the 5-demethylnobiletin may attributed to the different pathways leading to cell death. The strongest IC_{50} was observed with 5,3'-didemethylnobiletin which induced apoptotic cell death via cleavage on caspase-3 and cell cycle arrest at the G2/M phase [84]. In contrast, its methoxylated counterpart, sinensetin reduced cell viability by inducing autophagic cell death [86]. With muted expression of pro- and anti-apoptotic markers with early indicator of apoptotic cell death was not detected, Increased levels of the autophagic marker, beclin-1, and LC3B-II, along with downregulation of p62, confirmed the cell death mechanism of sinensetin. Simultaneous activation of the upstream autophagic marker, p-AMPK, and weakened p-mTOR by sinensetin treatment initiate autophagy cell death.

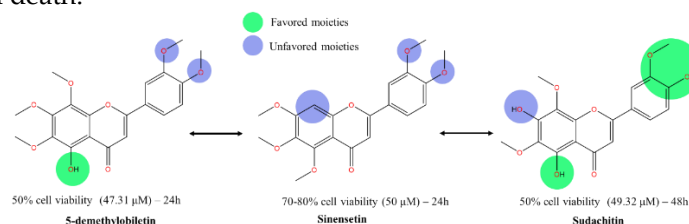


Figure 8. SAR analysis on cytotoxicity activities of 5-demethylnobiletin, sinensetin, and sudachitin analogs on HepG2 colon cancer cell lines.

2.5. Acute & Chronic Myeloid Leukemia

FMS-like tyrosine kinase 3 (FLT3) is notable overexpressed in AML, making the inhibition of this kinase a critical focus in leukemia therapy [90,91]. FLT3 activation facilitated through its extracellular ligand-binding domain, triggers downstream protein cascades that stimulate cell proliferation and differentiation. However, it is a challenge in AML due to the aggressive nature of this cancer. Given the high FLT3 levels, the exploration of flavone derivatives as potential inhibitors is vital. FLT3-internal tandem duplication (FLT3-ITD) positive cell lines such as MOLM-13 and MV4-11 serve as principle models to investigate drug resistance mechanism against tyrosine kinase inhibitors [92]. An in-vitro study by Yen et al. identified four flavone derivatives with potent cytotoxic activity in these cell lines over 72-hour treatment period:

- 5,7-dihydroxy-4'-MF (IC_{50} : 9.1 & 6.8 μ M)
- 5,4'-dihydroxy-6,7-DMF (IC_{50} : 2.6 & 2.6 μ M),
- 5,7-dihydroxy-6,4'-DMF (IC_{50} : 5.9 & 7.9 μ M) and
- 5,7,4'-trihydroxy-6-MF/Hispidulin (IC_{50} : 7.0 & 6.8 μ M),

The compound 5,4'-dihydroxy-6,7-dimethoxyflavone demonstrated the strongest cytotoxic effect, notably due to the C7-OCH₃ (Table 3). Likewise, substituting the hydroxy group on C7 disrupts the lipophilic capacity with rich electron delocalization region, which is crucial for ring A. Upon C7 demethylation, pharmacophore changes on C6 and C4's position are incapable of reversing the cytotoxicity potential.

2.5.1. Acute and Chronic Myeloid Leukemia: C5-OH as a Promising Methoxyflavones' Anticancer Backbone

C5-OH has showcased strong evidence as a backbone for an anticancer marker within the flavone class. Inevitably, researchers are keenly focused on the demethylation of C5 in nobiletin structure to develop targeted anticancer strategies. According to Chen et al. [82], 5-demethyl nobiletin exhibited outstanding antiproliferative effects against multiple acute myeloid leukemia (AML) cell lines including HL-60, THP-1, U-937, and HEL as well as the chronic myeloid leukemia (CML) cell line, K562. When treated for 48 hours, this compound demonstrated IC_{50} values of 85.7, 32.3, 30.4, and 65.3 μ M, respectively against all AML cell lines, compared to a vehicle control (0.1% DMSO).

2.5.2. Diverse Mechanisms in Different Cell Lines

A comprehensive review by Skopek et al. [93], pointed out that the cell death pathway of HL-60 is primarily driven by the apoptotic proteins [94]. In contrast, the survival of THP-1 and U-937 cells is largely controlled by the inflammatory markers [95], while proliferation in HEL cell lines is stimulated by the JAK-STAT3-ERK1/2 signaling pathway [96,97]. These pathways highlight the multi-target of flavones which contribute to strong IC₅₀ values across all cancerous cells.

2.5.3. Comparative Efficacy and Binding Preferences

Notably, similar treatment on K562 CML cell lines yielded a slightly weaker IC₅₀ value of 91.5 μ M. Astonishingly, an earlier study by Yen et al. demonstrated a stronger IC₅₀ value of 82.49 μ M for nobiletin under comparable conditions, suggesting that selective binding associated with C5-OCH₃ could be advantageous as observed in studies involving triple-negative breast cancer, pancreatic (MIA paca-2), and skin cancer cell lines. With limited studies on flavone families, methoxy moieties were reported to be highly favored compared to other cancer cell lines with greater lipophilic character and antiproliferative effect on K562 cell lines [99,100]. Both studies highlighted the contribution of the lipophilic region in facilitating the transport of active compounds to target proteins.

2.5.4. Casticin's Distinct Cytotoxic Profile

Nevertheless, 5,3'-dihydroxy-3,6,7,4'-TeMF, casticin, was investigated for its cytotoxic effects on WEHI-3 mouse leukemia cell lines where it reduced cell viability moderately to 53.4% at a concentration of 1.0 μ M for 48-hour treatment, corresponding to an IC₅₀ less than 1 μ M, markedly more potent than its effects observed on DU145 prostate cancer cell lines [101]. The inhibitory activity reflects the selective characteristic of the flavones compound, which requires high specificity to induce a potent anti-tumor effect by targeting cell death protein markers.

2.6. Gastric Cancer

Three polymethoxyflavones; nobiletin, 5-demethyl nobiletin, and tangeritin have exhibited potent antiproliferative activity against several gastric cancer cell lines including AGS, BGC-823, and SGC-7901 (**Table 3**) [102]. In 48-hour treatment, tangeritin emerged as the most effective, reducing AGS cell viability to below 20% at an estimated concentration of 33.57 μ M. However, its efficacy was moderate on the same cell lines maintaining about to ~30% viability at a concentration of 62.13 μ M. In contrast, both nobiletin, 5-demethyl nobiletin showed no activity at concentrations up to 100 μ M on both BGC-823 and SGC-7901 cell lines, and 5-demethyl nobiletin also was inactive on all tested cell lines.

2.6.1. Physicochemical and SAR Insights

Physicochemical and structure-activity relationship (SAR) analysis indicates that the C5-OH group is generally unfavorable against all tested gastric cancer cell lines although showing strong activity in other cancerous cell lines. This inconsistency underscores the need for further pharmacophore studies to fully understand the interactions. Interestingly, C5-OCH₃ appears to maintain stable hydrogen bond interactions with protein markers, enhancing the cytotoxic potential of tangeritin significantly.

2.6.2. Molecular Interactions and Anticancer Effects

Based on our schematic diagram analysis, the heavy substitution of the methoxy group on ring B does diminish the cytotoxicity capacity of nobiletin compared to tangeritin. In addition, the presence of C5-OCH₃ may facilitate the interaction of the polar carbonyl surface with the target site through an electron transfer/hydrogen acceptor mechanism [103], which is crucial for stronger binding with protein markers. This mechanism demonstrates how specific functional groups significantly affect the binding strength and overall anticancer efficacy of these compounds.

2.7. Skin Cancer

Among eight methoxyflavones isolated from *Gardenia oudiepe* (**Table 3**), only 2 PMF displayed a strong antiproliferative effect against A2058 melanoma cell lines [104]. Treatment with compounds 5,7-dihydroxy-3,6,4'-TMF (**P1**) and 7,5'-dihydroxy-3,6,3',4'-TeMF (**P2**) at 10 μ M for 72-hour significantly reduced cancer cell growth by 66.52% and 42.86%, respectively. Meanwhile, both compounds 5,7,4'-trihydroxy-3,6-DMF (**P3**) and 5,7-dihydroxy-3,6,3',4',5'-PeMF (**P4**) showed the least cytotoxic potential due to amphipathicity disruption on ring B. The A2058 melanoma cell lines are highly invasive and metastasized, and having extra lipophilic and hydrophobic characteristics on ring B was expected to improve the cytotoxic effect of the methoxyflavones analogs.

The substitution of the methoxy group (**P1**) with hydroxy group (**P4**) at position C4' reduced the hydrophobic strength on ring B and diminished its growth inhibition effect significantly from 66.52% to 18.55% under the same concentration and treatment duration. Nevertheless, the neighboring effect of a methoxy group (C4') and a hydroxyl group (C5') may triggered the formation of IHB, stabilizing the compound and improving its hydrophobic capacity with the addition of C3'-OCH₃ (**P2**) despite the intense bulkiness within ring B. On another note, additional C5' methoxy (5,7-dihydroxy-3,6,3',4',5'-PeMF) replacing the hydroxy group reversed the cytotoxic effect and significantly reduced its effectiveness with >90% cancer cell viability. The massive hydrophobic effect introduced by the addition of a trimethoxy group on ring B without possible interaction from adjacent functional group is the main contributing factor in its reduced efficacy. However, both compounds **P1** and **P2** induced potent inhibitory effect with strong IC₅₀ values of 3.92 and 8.18 μ M, respectively through apoptotic cell death mechanism involving the cleavage of caspase-3.

2.8. Oral Cancer

Five methoxyflavone compounds isolated from *Hottonia palustris* were tested on SCC-25 squamous carcinoma cell line over 24- and 48-hour demonstrating distinct antiproliferative activity [105]. Of these, only one isolated PMF (5,6'-dihydroxy-2',3'-DMF) showed potent cytotoxic effects with IC₅₀ values of 78.2 and 40.6 μ M, on respective duration treatment. Meanwhile, 5-hydroxy-2'-MF, 5-hydroxy-2',6'-DMF, 5-hydroxy-2',3',6'-TMF and 5,2'-dihydroxy-6'-MF were ineffective with IC₅₀ values exceeding 200 μ M (**Table 3**).

2.8.1. Structural Insights and SAR Analysis

Limited SAR analysis suggests that a balance between both hydrophobic and polar interactions across rings A and B is crucial. Based on our screening, these compounds are notable among flavones derivatives for having methoxy or hydroxy groups bonded to the C6' position on ring B. Based on the cytotoxic effect, it's interesting to highlight the structural similarities between 5,6'-dihydroxy-2',3'-DMF (IC₅₀: 40.6 μ M) and 5-hydroxy-2',3',6'-TMF (IC₅₀ > 200 μ M). Position C6' was hypothesized to significantly influence the bioactivity of the isolated PMF on SCC-25 cell lines. Early screening demonstrates the favorable C6'-OH position, however, more SAR analysis is required to confirm the pharmacophore that contributes to a strong cytotoxicity effect.

2.8.2. Hydroxylation and its Impact

Unlike the active compound 5,6'-dihydroxy-2',3'-DMF, hydroxylation on ring B was not observed in the other screened compounds, which is a crucial moiety for C5-OH methoxyflavones scaffold. Further investigation will assist in determining if the C6'-OH has a distinct feature particularly as the position C2',4', 6'-OH align within the golden triangle, optimizing free radicals electron delocalization. This configuration potentially contributes to IHB and enhances the polar surface region's interaction with target proteins.

2.9. Bile duct and Pancreatic Cancer

In bile duct cancer, sudachitin (5,7,4'-trihydroxy-6,8,3'-TMF) significantly reduces cancer cell viability with lower IC₅₀ values of HuCCT1 (53.21 μ M) and RBE (24.1 μ M) in 48-hour treatment

(Table 3). As discussed previously, sudachitin is well known for its potent activity in various cancer types, including colon and pancreatic cancers. Nevertheless, the detailed cell death mechanisms have remained scarce and have not been investigated up to the recent studies. In contrast, nobiletin (5,6,7,8,3',4'-HeMF) unable to reduce cell viability in both TFK1 and RBE cell lines, even at concentrations up to 100 μ M in both 24- and 48-hour treatments [106]. As previously discussed, inactivity of nobiletin against certain cancer cell lines, including breast and prostate cancer cell lines is attributed to its massive lipophilic effect and selective cell death pathways. The GSK3 β pathway is identified as contributing to nobiletin's antiproliferative effects based on western blot analysis. Resembling the bile duct cancer, sudachitin shows potent activity against MIA PaCa-2 and PANC-1 pancreatic cancer cell lines, in 48-hour treatment (IC₅₀: 43.35 & 32.73 μ M) [87]. Meanwhile, nobiletin displays weak antiproliferative effects on these cells with PANC-1 cell viability remaining above 80% even after treatment at the highest concentration (120 μ M) and longest duration treatment (72-hour) [107]. Nonetheless, nobiletin shows significant cytotoxicity effects of MIA PaCa-2 cells after 72-hour treatment, reducing cell viability to less than 20% at 80 μ M. The selective cytotoxicity of nobiletin may be attributed to the heterogeneity of the cells, with PANC-1 being reported to be more invasive and have a greater metastatic effect than MIA PaCa-2 cells.

2.10. Cervical and Ovarian cancer

2.10.1. Cervical Cancer and HPV Infection

The growth of cervical cancer cell lines has been associated with human papillomaviruses (HPVs) infections, notably its subgroup, HPV18+ (Hela) and HPV16+ (C33A), meanwhile, SiHa cells are HPV negative (HPV-) [108]. The HPV oncoproteins E6 and E7 are known to facilitate malignant tumor conversion by disrupting the cancer cell death markers, which is evident in HPV18+ Hela cells [109,110]. In both studies reported by Zhang et al. [111,112], compound 6-methoxyflavone exhibited potent cytotoxicity against cervical cancer cells. Over a 72-hour treatment period, this compound reduced Hela cell viability the most (IC₅₀:55.31 μ M), followed by C33A (109.57 μ M and SiHa (208.53 μ M) (Table 3). Although compound 6-methoxyflavone was the most potent to Hela, its IC₅₀ value was considered moderate, compared to the other cancer cell lines. Conversely, another methoxyflavones, xanthomicrol, 5,4'-dihydroxy-6,7,8-TMF was less potent against Hela cells with an IC₅₀ exceeding 100 μ M in a 72-hour treatment [83]. Due to the distinct pharmacophore, SAR predictions on methoxyflavone selectivity against cervical cancer cell lines based on recent studies were found to be inadequate.

2.10.2. Ovarian Cancer and Treatment Efficacy

With limited studies utilizing methoxyflavones scaffold, the SAR profiling is limited on targeting ovarian cancer cell lines. Based on Table 3, treatment with wogonin on all cisplatin sensitive (SKOV3 and OV2008) and its respective resistance counterparts (SKOV3/DDP and C13*) ovarian cancer cell lines for 72 hours at doses up to 20 μ M demonstrated a weak antiproliferative effect with cell viability remaining above 80% [113]. Nonetheless, at the highest concentration (160 μ M), wogonin significantly reduced all cell viabilities to 20%. The induction of apoptotic cancer cell death was proposed by the presence of DNA laddering and confirmed through the downregulation of Akt phosphorylation.

Table 3. Cytotoxicity assay of methoxyflavones analogs.

Natural sources	Cancer	Cells	Treatment (hr)	IC ₅₀ / %		References
				cell viability (%)	μ M	
5,3'-dihydroxy-3,6,7,8,4'-PeMF	Breast	MCF-7	24	49.46	μ M	[46]
			48	17.56	μ M	

Nobiletin	<i>Glycosmis ovoidea</i> Pierre	MDA-MB-231	72	3.71 μM	[102]			
			24	49.46 μM				
			48	49.46 μM				
			72	21.27 μM				
		AGS		>20% (62.13 μM)				
			Gastri c	BGC-823		48	>90% (100 μM)	
				SGC-7901			>90% (100 μM)	
			CML	K562		48	82.49 μM	
	<i>Citrus sinensis</i> / <i>Citrus reticulata</i>	Prostate	VCaP	48		> 80% (100 μM)	[98]	
			PC3	48		80 - 100 μM	[65]	
			MIA	72		80% (120 μM)	[63]	
			Pancreas	PaCa-2		72	20% (80 μM)	[107]
		PANC-1	72	20% (80 μM)				
			Breast	MDA-MB-231		72	70% (10.04 μM)	[114]
			Bile duct	TFK1		48	Inactive	[82]
				RBE		48	Inactive	
5-demethylnobiletin	<i>Citrus reticulata</i>	Compound	Liver	HepG2	24	47.31 μM	[84]	
				HL-60		85.7 μM		
			AML	THP-1		32.3 μM		
				U-937		30.4 μM	[98]	
			HEL		65.3 μM			
				CML	K562		91.5 μM	
			AGS	48	>90% (100 μM)			
				Gastri c	BGC-823		>90% (100 μM)	[102]
				SGC-7901		>90% (100 μM)		
					Colon	HCT116	48	56.23 μM
	HT-29	48			37.07 μM			
Liver	HepG2	48			49.32 μM			
5,7,4'-trihydroxy-6,8,3'-TMF / sudachitin	<i>Citrus sudachi</i>	Bile duct	HuCC1	48	53.21 μM	[87]		
				RBE	48		24.1 μM	
				MIA	48		43.35 μM	
			Pancreas	PaCa-2	48		32.73 μM	
5,6'-dihydroxy-2',3'-DMF	<i>Hottonia palustris</i>	Oral	SCC-25	24	78.2 μM	[105]		
					48		40.6 μM	
					48		Inactive	
					48		Inactive	

5-hydroxy-2',3',6'-TMF							Inactive	
5,2'-dihydroxy-6'-MF							Inactive	
5,7-dihydroxy-4'-MF / acacetin	Robinia pseudoacacia						9.1 μM	
5,4'-dihydroxy-6,7-DMF	Quercus incana		MOLM-13				2.6 μM	
5,7-dihydroxy-6,4'-DMF / Pectolinarigenin	Artemisia capillaris						5.9 μM	
5,7,4'-trihydroxy-6-MF/ hispidulin	Artemisia capillaris						7.0 μM	
5,7,4'-trihydroxy-6-MF/hispidulin	Artemisia capillaris			72			6.8 μM	[88]
5,4'-dihydroxy-6,7-DMF	Quercus incana		MV4-11				2.6 μM	
5,7-dihydroxy-6,4'-DMF / Pectolinarigenin	Artemisia capillaris						7.9 μM	
5,7-dihydroxy-4'-MF	Robinia pseudoacacia						6.8 μM	
5,7-dihydroxy-3,6,4'-TMF				72			66.52% (10 μM)	
7,5'-dihydroxy-3,6,3',4'-TeMF	Gardenia oudiepe (Rubiaceae)	Skin	A2058	72			42.86% (10 μM)	[104]
5,7,4'-trihydroxy-3,6-DMF				72			Inactive	
5,7-dihydroxy-3,6,3',4',5'-PeMF				72			Inactive	
5,7-dihydroxy-8-MF / wogonin	Scutellaria baicalensis	Ovarian	SKOV3 OV2008 SKOV3/DDP C13*	72			80% (20 μM)	[113]
4',5'-dihydroxy-5,7,3'-TMF							8.58 μM	
4'-hydroxy-5,7,3',5'-TeMF							>100 μM	
5,7,3',4',5'-PeMF	Synthetic compound	Breast	HCC1954	NA			53.84 μM	[58]
5,6,7,8,3',4',5'-heptamethoxyflavone		Prostate	PC3	48			>100 μM	
5,6,7,8,4'-PeMF / tangeritin	Citrus reticulata	Gastric	AGS BGC-823 SGC-7901	48			<20% (33.57 μM)	
				48			>90% (100 μM)	[102]
5,4'-dihydroxy-6,7,8-TMF/ xanthomicrol	Achillea erba-rotta subsp. moschata	Colon	HCT116	24			42% (15 μM)	[81]
		Breast	MCF7	72			> 100 μM	[49]
		Cervical	Hela	72			> 100 μM	[83]
6-MF	Pimelea decora	Cervical	Hela C33A SiHa	72			55.31 μM	
				72			109.57 μM	[111,112]
				72			208.53 μM	

5,3'-didemethylnobiletin				24	41.37 μM	
5,4'-didemethylnobiletin	Citrus	Liver	HepG2	24	54.46 μM	[84]
5,3',4'-tridemethylnobiletin	reticulata			24	46.18 μM	
5,7,3',4'-TeMF	Kaempferia			72	75% (60 μM)	[80]
7,8,3',4'-TeMF	parviflora	Colon	HCT116	72	90% (60 μM)	
	Citrus					
	reticulata					
5,4'-dihydroxy-3,6,7,8-TeMF/ calycopterin	D. kotschy	Prosta	DU145	48	235 μM	[69]
	Boiss	te	LNCaP	48	116.5 μM	
5,4'-dihydroxy-6,7-DMF / cirsimaritin	Quercus			24	26.23 μM	
5,3'-dihydroxy-6,7,4'-TMF / eupatorin	incana	Lung	NCI-H460	24	37.50 μM	[115]
5,7-dihydroxy-6-MF / acacetin	Robinia			24	25 μM	[89]
5,6,7,3',4'-PeMF / Sinensetin	pseudoacacia	Liver	HepG2	24	80% (50 μM)	[86]
	O. aristatus					
	leaves					
5,7-dihydroxy-6-MF/ Oroxylin A	Scutellaria	Breast	4T1	24	~90% (40 μM)	[116]
	baicalensis					
5,7,4'-trihydroxy-6-MF	Artemisia	Prosta	VCaP	48	NA	[67]
	capillaris	te				
5,4'-dihydroxy-3,6,7,3'-TeMF / chrysosplenetin	Artemisia	Breast	MCF	72	0.3 μM	[48]
	annua L.					
5-hydroxy-6,7,8,4'-TeMF / 5-Demethyltangeritin	Citrus	Prosta	PC3	48	11.8 μM	[63]
	reticulata	te				
5,3'-dihydroxy-3,6,7,4'-TeMF / casticin	Dracocephalu	Prosta	DU145	48	>80%	[68]
	m kotschy	te				
	Boiss					

****This table illustrates the cytotoxicity of all methoxyflavones analogs on various cancer cell lines.** The cytotoxicity effect of methoxyflavones was centered on Nobiletin and its demethylation derivatives. Bolded IC₅₀ indicates strong cytotoxicity activity, aligned with the proposed schematic diagram **Figure 1**.

3. Mechanism of Methoxy and Hydroxy Flavones Derivatives

3.1. Flavones modulate the apoptotic cell death pathway

Briefly, recent studies on the antitumor mechanism of flavones derivatives depict a multi-strategy approach. These compounds leverage the versatile positions available for hydroxy and methoxy substituents in flavones' molecular structure, influencing various carcinogenic pathways. Apoptosis or programmed cell death has been a hallmark mechanism employed by flavones to prevent oncogenesis through both extrinsic and intrinsic mitochondrial pathways. The activation of mitochondrial outer membrane permeabilization (MOMP) is crucial in stimulating the expression of apoptotic proteins. Caspase-3, a critical player in apoptosis is often due to its central role in both mitochondrial pathways that leading to cell death [40,117–119]. For instance, 6-methoxyflavone induces apoptosis via the extrinsic or death receptor pathways, activating receptors such as Fas and tumor necrosis factor (TNF). Prior to caspase-3 cleavage, this is followed by the upregulation of the tumor suppressor p53 in the intrinsic mitochondrial pathway occurred due to mutation in the Tp53 gene, given its primary roles in cell cycle arrest, DNA repair, and apoptosis. Flavones derivatives are known to activate the Bcl-2 family's protein, which comprises pro-apoptotic, anti-apoptotic, and BH3-only proteins, controlled by the expression level of p53 [81,111,112,115,120]. Elevated levels of pro-apoptotic proteins such as BID, BAX and BAK alongside the downregulation of anti-apoptotic proteins like MCL-1, Bcl-2, and Bcl-xL in the intrinsic mitochondrial pathway resulted in activation

of caspase-3, -8 and -9, prior to cell death [46,63,101,102]. In some cases, the variation of pro- and anti-apoptotic protein expression levels induced the release of cytochrome C, subsequently inhibiting APAF-1 mRNA expression before caspase cleavage [111,112].

3.2. Flavones-Induced Cell Cycle Arrest

Flavones not only induce apoptosis but also impact cell cycle regulation through the tumor suppressor protein p53. The transcription factor p53 is a central tumor-blocking protein that activates dozens of downstream genes that modulate cell cycles. Numerous studies have illustrated the affiliation of p53 with its transcriptional target, p21^{Waf1/CIP1} in suppressing cyclins and cyclin-dependent kinase (CDK) expression by forming inhibitory complexes, that lead to cell cycle arrest [121,122]. Flavone derivatives such as 5,4'-dihydroxy-6,7,8-TMF and 5-demethyltangeritin by activation of p21^{Waf1/CIP1} via transcriptional target of P53 arrest cell cycle at the G0/G1 phase, consequently inhibiting cyclin D and CDK4 expression [81,106]. Nobiletin is known for its antitumor mechanism as it also induces cell cycle arrest. Concurrently, Lotfizadeh et al. and Yen et al. depict similar behavior of calycopterin and nobiletin, respectively by inducing cell cycle arrest at the G1 phase, with nobiletin specifically activating p21 and p27 expression, subsequently suppressed the cyclin D mRNA expression. Nevertheless, modifying nobiletin by replacing methoxy group with hydroxy at the C5 position (5-demethylnobiletin) notably influences the cell cycle arrest, shifting the cell cycle arrest towards the G1/S phase with weaker expression of cyclin E and A mediated by p21 [82]. Meanwhile, 6-methoxyflavone depicts cell cycle arrest at the S phase through p21^{Waf1/CIP1} activation resulting in suppression of cyclin A and CDK2 activities [111,112].

3.3. Mitogen-activated protein kinase (MAPK) pathway

The mitogen-activated protein kinase (MAPK) signalling pathway, centred around the RAS oncogene GTP-binding protein has been targeted for colorectal and pancreatic cancer therapies since the late 1970s [123,124]. The pathway involves the inhibition of RAS upstream novel target, SOS-1, a guanine nucleotide exchange factor. This inhibition prevents the conversion of GDP to GTP by RAS assisted by GRB2 (growth factor receptor bound protein 2), thus blocking RAF dimerization and subsequent downstream signalling [125]. Over 85% of cancers are marked by abnormal activity of MAPK, emphasizing the need for targeted intervention by suppressing the mitogen-activated proteins. The activation of SOS-1 triggers a series of interactions among RAF, MEK, and ERK1/2, resulted in the abnormal activation of the MAPK pathway [126]. Casticin has been shown to inhibit both SOS-1 and GRB2, reducing phosphorylation levels of ERK1/2, a downstream component of the MAPK pathway, indicating suppression of MAPK signalling [65,68]. Within the cytosol, casticin, has been shown inhibit both SOS-1 and GRB2, reducing phosphorylation levels of ERK1/2 level, a downstream component of the MAPK pathway, indicating suppression of MAPK signalling [65,68]. This inhibition also leads to suppression of c-myc, a transcription factor with positive correlation with this pathway [127]. Moreover, while regulating MAPK signalling, RAS and GRB2 are well-known to activate PI3K/Akt pathway, contributing to oncogenic behavior and cancer progression [128]. Stimulation of both oncogenes-induced RAS-activated PI3K together with the amplification of mutation on its major effector, AKT and mTOR drives the malignant resistance and tumor progression (Glaviano et al., 2023). Methoxyflavones derivatives like wogonin and casticin are noted for inhibiting angiogenesis by impacting these critical pathways, highlighting their potential in therapeutic strategies against cancer [68,113].

3.4. NF-κB Signaling

NF-κB, a key transcription factor plays a critical role in inflammation and cancer by promoting tumorigenesis through the activation of various genes including IκB, p65, and p50. This pathway, involved in both canonical nor non-canonical pathways, is crucial for DNA repair, drug resistance, inhibition of apoptosis, and promotion of cell proliferation [130,131]. Various studies consistently link

NF- κ B to cancer progression, notably its role in resisting TNF- α -induced apoptosis by enhancing ROS levels and antioxidant enzyme activities, thereby preventing cell death [132].

Methoxyflavones derivatives have been identified as anti-inflammatory agents that can moderate the activation of NF- κ B triggered by LPS- and TNF- α as demonstrated by numerous studies [133,134]. Concurrent investigation on the activity of PARP-1 nuclear enzyme was important given its roles in DNA repair and reversing cancer cell death by triggering NF- κ B expression [135]. Thus, the inhibition of PARP-1 have been shown to promote apoptosis, effectively leading to cancer cell death as discussed by Chen et al. [82]. For instance, phosphorylated NF- κ B (p-p65) were markedly downregulated in LPS-induced cancer cells while TNF- α -induced NF- κ B expression was suppressed with upregulation of I κ B α [114]. Nonetheless, treatment with flavones has been associated with an overexpression of PARP-1 which suggests potential unique mechanism of action. This overexpression leads to an increase in Poly(ADP-ribosyl)ation (PAR) synthesis during the early apoptosis, subsequently activating caspase-3 and initiating the proteolysis of PARP-1, which contributes to the strong cytotoxic effects observed in Hela cell viability [136].

3.5. Matrix Metalloproteinase (MMP) cascades signaling

Cancer-associated fibroblast (CAF) is one of the largest groups in the matrix component and part of the tumor microenvironment and plays crucial role in cancer progression [116]. These cells, when activated from normal fibroblast within adipose tissue notably by cancer cells such as 4T1 breast cancer cell line, exhibited increased mRNA expression of fibroblast biomarker, alpha-smooth muscle actin (α -sma), vimentin, and others [116]. This interaction between CAFs and tumor cells contributes to the micro-interaction between the CAF and tumor cell, contributes to the development of highly metastatic and invasive cancer cells.

Research has shown that PMF, specifically oroxylin A (5,7-dihydroxy-6-methoxyflavone), successfully reduced the mRNA expression of α -sma, vimentin, and fibronectin without significant toxicity in killing normal cell L-929 and normal primary cell, NBF, indicating selective efficacy [116]. Furthermore, CAF activation leads to higher invasiveness in the 4T1 cells, which PMFs have been potently downregulating the mRNA expression of MMP-2, MMP-9, and MMP-14 in a dose-controlled manner. The inactivation of CAF by PMF is facilitated by targeting ACTN1 through interaction at ASP-781, as revealed by molecular docking analysis. This interaction leads to a significant reduction in the phosphorylation of downstream signaling pathway, including focal adhesion kinase (FAK), janus kinase 2 (JAK2), the mammalian target of rapamycin (mTOR), and signal transducer and activator of transcription 3 (STAT3), thereby inhibiting key process that promote cancer cell migration and invasion.

3.6. Autophagy in Cancer

Autophagy, a self-degradation process essential for removing damaged organelles and other intracellular components, involves the formation of autophagosomes that fuse with lysosomes for decomposition and recycling [137]. In cancer, autophagy plays a dual indefinite roles: it suppresses cancer growth in the earlier stage by eliminating defective components that could promote malignancy, and it may support tumor growth at later stages by aiding cancer cell survival under stress [138].

One of the critical functions of autophagy in cancer is its ability to prevent the accumulation of ROS that leads to oxidative stress and DNA damage. This prevention is achieved by blocking the lipidation of LC3 protein, converting it from LC3-I to LC3-II via ATG4, an enzyme essential for autophagosome formation [139,140]. An extensive review by Zhao et al. discussed the regulatory roles of mTOR-ATG4- LC3-II pathway in regulating ROS levels and autophagy. A study by Wang et al. explored the potential antiproliferative effects of hispidulin, a compound known to influence this pathway, on DU145 and VCaP prostate cancer cell lines. Hispidulin potency in reduced cell migration and invasion after 48-hour treatment, suggesting its potential to elevate autophagy in these cancer cells. Evidence of increased rate of autophagy flux, characterized by the conversion of LC3 I to LC3

II, and elevated PPAR γ expression, in tandem with weak phosphorylation of mTOR and S6K1, supports the hypothesis that hispidulin can modulate autophagy to suppress tumor progression.

3.7. ER-Stress-Induced Apoptosis By Flavone

The progression of malignant tumors is often influenced by the stress induced within the cells intrinsically and extrinsically resulting to disruption within the tumor microenvironment [142]. One significant source of cellular stress is the endoplasmic reticulum (ER), where irregularities and disruptions in protein folding can activate stress-induced signalling pathways. These pathways not only promote cancer cell growth but also enhance metastatic potential [143,144]. A key component in the ER stress response is the PERK signalling pathway. PERK plays vital role in mediating apoptosis through the mitochondrial pathway via BAX/Bcl-2 regulation or through triggering autophagy by activating downstream markers. Recent studies have shown that flavone derivatives can induce autophagy through elevated mRNA expression of downstream proteins such as PERK, EIF2 α , IRE1 α , ATF4, and CHOP [111,112]. This enhanced autophagic response to ER stress presents a significant mechanism by which flavones may exert their anticancer effects, providing a dual approach to controlling tumor progression through both apoptosis and autophagy.

3.8. Targeting Topoisomerase Enzymes In Highly Expressed Cancer Cells

Topoisomerases I and II are critical enzymes in DNA replication and transcription, and they are often highly expressed in various cancers including breast cancer [145]. Methoxyflavones derivatives have been identified as potent inhibitors of these enzymes exploiting their ability to interfere with unwinding process of DNA, leading to cell death [146]. For instance, chrysosplenetin, a specific methoxyflavones derivative has shown a strong inhibitory effect on topoisomerase I [48]. The strong potency of the compound may be attributed to the targeting inhibition of the topoisomerase enzyme. The strong inhibitory effect of chrysosplenetin toward topoisomerase I leads to a higher form I percentage in concentration concentration-dependent manner. The Form I of pBR322 plasmid DNA increased from 31.0% to 69.0%, whereas the percentage of Forms II and III declined. Meanwhile, topoisomerase II was inhibited by the compound in a similar concentration with the form I accumulation increased to 96.9% at the highest concentration. These findings highlight the potent anticancer properties of methoxyflavones derivatives through their targeted action against topoisomerase enzymes providing a promising avenue for therapeutic intervention in cancers with high topoisomerase expression.

3.9. Wnt- β -Catenin Pathway

The Wnt- β -catenin signaling pathway is critically involved in uncontrolled cancer cell proliferation, especially noted in colorectal cancer cells. Abnormalities in this pathway contribute to the cancer's invasive and metastatic characteristics [147]. Dysregulation of the key signaling cofactor β -catenin is known to influence the development of a cancer stem cell phenotype, characterized by high levels of cyclin D and c-myc, which are crucial for tumorigenesis [148]. The activation of this pathway leads to the elevated expression of downstream Wnt target genes which includes axin2 and vimentin which drive the cancer progression [149]. Studies using the RT-PCR have shown that tetramethoxyflavone specifically 5,7,3',4'-TeMF & 7,8,3',4'-TeMF can downregulate the mRNA expression of c-Myc, axin2, and vimentin in HCT116 colorectal cells at a concentration of 30 μ M [80].

4. Conclusions

The multi-target of methoxyflavones has contributed to various biological activities in recent in-vitro studies. Being naturally abundant, the flavonoid subclass could exist in a variety range of scaffolds with distinct features. Our review indicates major parts of the methoxyflavones scaffold have not been fully explored with limited findings. The pharmacophore of the anticancer effect of methoxyflavones was centered on ring A, strengthened by C3' and C4' ring B, with few compounds bearing C2', C5' and C6' moieties. The superiority of the anticancer effect of methoxyflavones against

other natural flavones such as hydroxyflavones and the flavones glycosides was established. The methoxy group governed the lipophilic capacity and facilitated drug-membrane transfer, thus preserving the bioavailability of the scaffolds. The lipophilic character of methoxyflavones was the main contribution to a remarkable activity against various cancer cell lines, based on our analysis. Additionally, critical investigation summarized the methoxyflavones themselves could not sustain a stronger cytotoxic effect of IC₅₀ less than 20 µM without the presence of the hydroxy group. The preservation of lipophilic properties on ring A flavones, with a balance capacity between a polar surface and lipophilic effect on ring B is the pathway to achieve stronger IC₅₀. Generally, methoxy moieties occupied at least two out of three positions from the C6, 7, and 8 positions, and the addition of hydroxy group on position C5 was fundamental to stronger anticancer activity. Next, having hydroxy moieties alongside with methoxy group on either C3' or C4' arrangement could amplify the cytotoxic capacity. Both methoxy or hydroxy groups in the respective position on ring B could achieve similarly strong IC₅₀, yet it came down to the conformational changes on rings A & B and on the cell types. Some C5-OCH₃ derivatives could induce strong IC₅₀, with few exceptions such as a maximum of single hydroxy or methoxy group on ring B. To maximize interaction with the target protein marker, balancing between the methoxy and hydroxy moieties in terms of the number and position of both groups in ring A and B flavones is crucial. Additionally, the collaborative efficacy of both groups will maximize the resonance effect and electron delocalization of free radical flavones and initiate the formation of IHB. Concurrently, both interaction mechanisms stabilized and preserved the lipophilicity while simultaneously offering an optimal polar surface for stronger interaction with the cancer cell death marker. The review solely focused on the pharmacophore specificity of methoxyflavones analogs in-vitro, with other factors such as drug metabolism, bioavailability, and toxicity, which may influence the resultant efficacy. The review may narrow down future research for targeting and isolating active natural methoxyflavones and assisting in the synthetic designing of flavones scaffolds with favorable positions of both methoxy and hydroxy moieties.

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Abbreviations

SAR; structural-activity relationship; HBA; hydrogen bond acceptor; HBD; hydrogen bond donor; OCH₃; methoxy group; OH; hydroxy group; IHB; intramolecular hydrogen bond; MF; methoxyflavone; DMF; dimethoxyflavone; TMF; trimethoxyflavone; TeMF; tetramethoxyflavone; PeMF; pentamethoxyflavone; HeMF; hexamethoxyflavone; Her-2+, epidermal growth factor receptor 2; AML; acute myeloid leukaemia; CML; chronic myeloid leukaemia; logP; lipid partition coefficient; logD; distribution coefficient; TNFR; tumor necrosis factor receptor; MCL-1; Myeloid cell leukemia-1; BID; BH3 interacting-domain death agonist; BAX; Bcl-2-associated X protein; APAF-1; Apoptotic protease activating factor-1; CDK; cyclin-dependent kinase; MAPK; mitogen-activated protein kinase; GDP; guanosine diphosphate; GTP; guanosine triphosphatase; RAF; rapidly accelerated fibrosarcoma; MEK; mitogen-activated protein kinase kinase; ERK1/2; extracellular signal-regulated kinase 1/2; GRB2; Growth Factor Receptor-bound protein 2; PI3K;

phosphatidylinositol 3-kinase; AKT; protein kinase B; mTOR; mammalian target of rapamycin; NF- κ B; nuclear factor kappa B; kappaB kinase; LPS; lipopolysaccharide; TNF- α ; tumor necrosis factor alpha; PARP-1; Poly (ADP-ribose) polymerase 1; CAF; cancer-associated fibroblast; α SMA ; alpha smooth muscle actin; MMP; matrix metalloproteinase; FAK; focal-adhesion kinase; JAK2; janus kinase 2; STAT3; signal transducer and activator of transcription 3; ACTN1; alpha-actinin-1; LC3; microtubule-associated protein light chain 3; PPAR γ ; peroxisome proliferator-activated receptor-gamma; ER; endoplasmic reticulum; PERK; protein kinase R-like endoplasmic reticulum kinase; EIF2 α . eukaryotic translation initiation factor 2 alpha; IRE1 α ; inositol-requiring enzyme 1 alpha; ATF4; activating transcription factor 4; CHOP; C/EBP homologous protein

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