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Review Article

Comparison of Nobiletin and 5-Demethylnobiletin as Cancer Chemopreventive Agents

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Natural chemicals have been considered as promising molecules in cancer treatment because of their broad spectrum of activities. Flavonoids show high affinity against key molecular targets associated with developing cancer, and tumor cells exhibit an inability to resist flavonoid treatment. Flavonoids are natural molecules synthesized during secondary plant metabolism that has protective activities against biotic and abiotic factors (animals, bacteria, and fungi) and are resistant to ultraviolet light, temperature, and solid minerals and contaminants. They have been purified from plants and synthetic molecules and have an antioxidant, antitumor, and cardioprotective activities in humans. The large flavonoid family includes polymethoxyflavones, which are extracted from the peels of citrus fruits such as *Citrus nobilis*, from which nobiletin (NOB) is obtained. The compounds derived from this promising anticancer chemical include 5-demethylnobiletin (5-DMN). These compounds inhibit a large number of targets that regulate the hallmarks of cancer by inducing apoptosis, cell cycle arrest, inhibition of proliferation, epithelial–mesenchymal transition, limiting migration, and angiogenesis. 5-DMN has exhibited potency in the regulation of anticancer activities. This review was conducted to summarize and compare the effects of NOB and 5-DMN on different types of cancer.

Keywords: 5-demethylnobiletin; biological effects; cancer; chemopreventive; nobiletin; polymethoxyflavones

1. Introduction

Flavonoids comprise a group of polyphenolic compounds that are synthesized by plants as secondary metabolites and give plants particular coloration and flavors. They also provide plants with protection against ultraviolet rays and parasites and resistance to extreme temperatures. In humans, flavonoids have antiseptic, antifungal, re-epithelializing, healing, and anti-inflammatory activities, which their therapeutic effects depending on the dosage [1–6]. Flavonoids have a benzo ɣ pyran (C3-C6-C3) structure, of which up to 5000 different molecules have been identified. The great diversity of flavonoids is due to substituents in their molecules, which induce hydroxylation, acylation, prenylation, glycosylation, and methoxylation. Flavonoids are classified as anthocyanidins, flavanols, flavanones, flavonols, flavones, and isoflavones [1–4]. Polymethoxylated flavones (PMFs) are present in several varieties of the *Citrus* genus, particularly *Citrus sinensis* (orange), *Citrus reticulata* (Valencia orange), and *Citrus nobilis* (tangerine). Prolonged storage, after consumption, nobiletin (NOB) is degraded by autolysis or gastric juices into 5-demethylnobiletin (5-DMN). In this review, we evaluated the anticancer effects of these two PMFs.

2. Methods

2.1. Search Strategy. The PubMed, Web of Science, and Scopus databases were searched using the terms "nobiletin and cancer," "5-demethylnobiletin and cancer," "polymethoxyflavones and cancer," and "nobiletin and derivatives and cancer."

2.2. Extraction of Results. The search yielded a total of 330 articles and subtraction was performed for each PMF. For NOB and cancer, we found a total of 220 articles; for 5-DMN, a total of 19 articles; and for polymethoxyflavones and cancer, 91 articles. Afterward, we proceeded to group them according to the type of cancer we found that for NOB, results of research: 7 manuscripts, bioinformatics; 9, breast cancer; 1, bladder cancer; 14, colon and colorectal cancer; 2, fibrosarcoma; 9, gastric cancer; 4, glioma; 2, kidney cancer; 4, leukemia; 5, liver cancer; 12, lung cancer; 3, oral cancer; 1, osteosarcoma; 6, ovarian cancer; 8, prostate cancer; 3, renal cancer; 15, reviews; 1, skin cancer; 2, synergistic effects; and 2, thyroid cancer; for 5-DMN, results of research: 10, colon and colorectal cancer; 2, gastric cancer; 1, leukemia; 2, liver cancer; 5, lung cancer; 1, melanogenesis; and a review 1; and for articles on PMFs, the search yielded results similar to those for NOB, 15 manuscripts, and for 5-DMN, 3 and 15 reviews.

To compare the effectiveness of NOB and 5-DMN, we used research on four cancer types: colon and colorectal cancer, gastric cancer, leukemia, and lung cancer (Figure 1).

3. Results

3.1. PMF Characterization. NOB has the molecular formula of $C_{21}H_{22}O_8$, molecular weight of 402.399 g/mol, and Log *p* value of 3.02. It is a flavone with methoxy substituents, and the name according to the International Union of Pure and Applied Chemistry (IUPAC) is -(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxychromen-4-one and as 5,6,7,8,30,40-hexamethoxyflavone or 2-(3,4-dimethoxyphenyl)-5,6,7,8,30,40-tetramethoxychromen-4-one dimethoxyphenyl)-5,6,7,8-tetramethoxy-4H-1-benzopyran-4-ona (Figure 2(a)) [7]. 5-DMN is formed from the degradation of NOB by autolysis or gastric juices after consumption [8]. 5-DMN has a molecular weight of 388.47 g/mol and Log *p* value of 2.78 and has been designated by the IUPAC, as 4H-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy. In contrast to NOB, 5-DMN has a hydroxyl group at Position 5 (Figure 2(b)). 5-DMN is extracted from citrus peels, and its most abundant source is *C. reticulata* [9].

The biotransformation of NOB begins in the intestine, where it arrives after oral administration or intravenous or peritoneal injection. It is metabolized by the intestinal microbiota into products with higher activity, including 5-DMN [10, 11]. After absorption, NOB is demethylated by hepatic reductases such as CYP2C11, CYP2C12, CYP2D1, CYP3A1, and CYP3A2 at Positions 3, 6, and 7, and by CYP1A1 and CYP1A2 at Positions 3 and 4 [12]. This demethylation begins as soon as NOB enters the oral cavity, and small amounts of NOB have been identified in urinary metabolites [13, 14]. The metabolites 3′DMN, 4′DMN, and 3′4′DMN have been identified in mice (Figure 3) [14, 15]. Wu et al. [16] demonstrated that the anticarcinogenic effects of NOB are associated with metabolites. The physiological effects associated with NOB and 5-DMN are summarized in Figure 4. In humans, PMFs have been found to confer neuroprotection [17], inhibit osteoclastogenesis [18], have antioxidant [19, 20] and anti-inflammatory [21, 22] effects, stimulate melanogenesis [23], have anticancer [24, 25] and antiatherogenic [26] effects, and confer vascular protection (Figure 4) [21]. In this review, we compared the anticancer activities of NOB and 5-DMN.

3.2. Anticancer Activity. In 2011, Hanahan and Weinberg [27] published a review of the competencies that cells acquire during tumor development and the characteristics of neoplastic disease, concluding that signaling is maintained to promote proliferation, the expression of growth suppressor molecules, resistance to cell death, angiogenesis, and the stimulation of invasion and metastasis, including energy metabolism and the evasion of immune destruction [27]. These events have been studied to identify molecular markers that can be targeted for the elimination of specific cancer types. Likewise, new drugs that prevent tumor cells from acquiring resistance without the generation of adverse events are being sought. PMFs have been studied due to their relevant properties, and we address the potential anticancer roles of NOB and 5-DMN.

Relative to those of 5-DMN, the effects of NOB have been studied for more cancer types. The effects of both PMFs on colon cancer, gastric cancer, leukemia, liver cancer, and lung cancer have been studied and are compared here (Figure 4).

3.2.1. Colon Cancer. Colorectal cancer had the third greatest incidence among cancers worldwide in 2020, with >1.9 million cases, and it continues to be the second most common cause of cancer-related mortality despite having a better survival rate. Surgery is the main treatment, and chemotherapy is recommended for Stage III colon cancer. The risk of colon cancer increases with age, and most cases occur around the age of 50 years. Risk factors include high processed meat consumption, low vegetable intake, obesity, alcohol and tobacco consumption, and sedentary behavior [28].

3.2.1.1. Effects of NOB on Colon Cancer. NOB has been found to inhibit matrix metalloproteinase-7 (MMP-7) expression in rectal adenocarcinoma (HT-29 cell line) in dose (50 μM)- and time (24 h)-dependent manners [29]. In *in vivo* studies of azoxymethane (AOM) and dextran sulfate sodium (DSS) induced colon carcinogenesis in mice, NOB (100 ppm) decreased the incidence of adenocarcinoma; in HT-29 cells, NOB (10–100 μM) inhibited leptin expression mediated by kinase/extracellular signal-regulated protein kinase (MEK-1) [30]. In HT-29 and SW480 cells, NOB (100 μM) potentiates the inhibitory effects of oxaliplatin, inhibiting proliferation and inducing apoptosis by increasing Bax and caspase-3 expression and downregulating B-cell lymphoma 2 (Bcl-2), via the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway [31]. NOB (100 ppm) suppresses the early stages of carcinogenesis in obese mice treated with AOM [32], but it did not suppress the formation of the aberrant crypt stage in AOM-treated rats [33]. NOB (20–50 μM) inhibits the proliferation and promotes the cycle

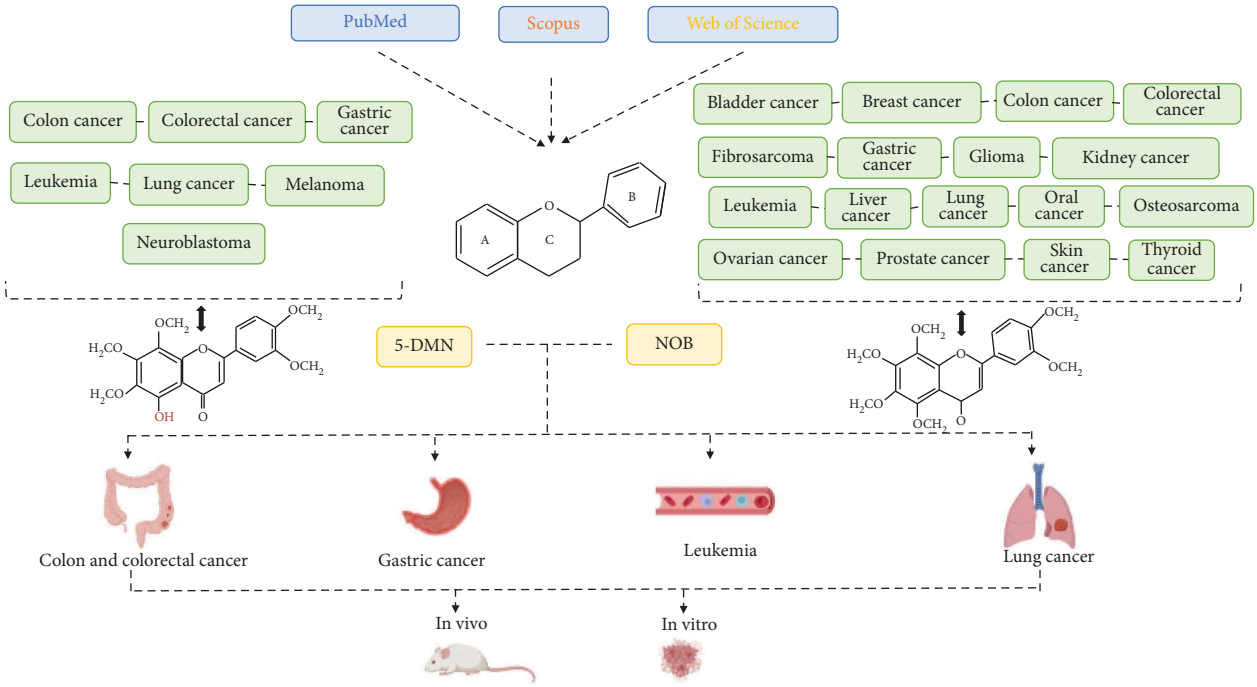

Figure 1: Nobiletin (NOB) and 5-demethylnobiletin (5-DMN) article search diagram. Common NOB and 5-DMN cancers.

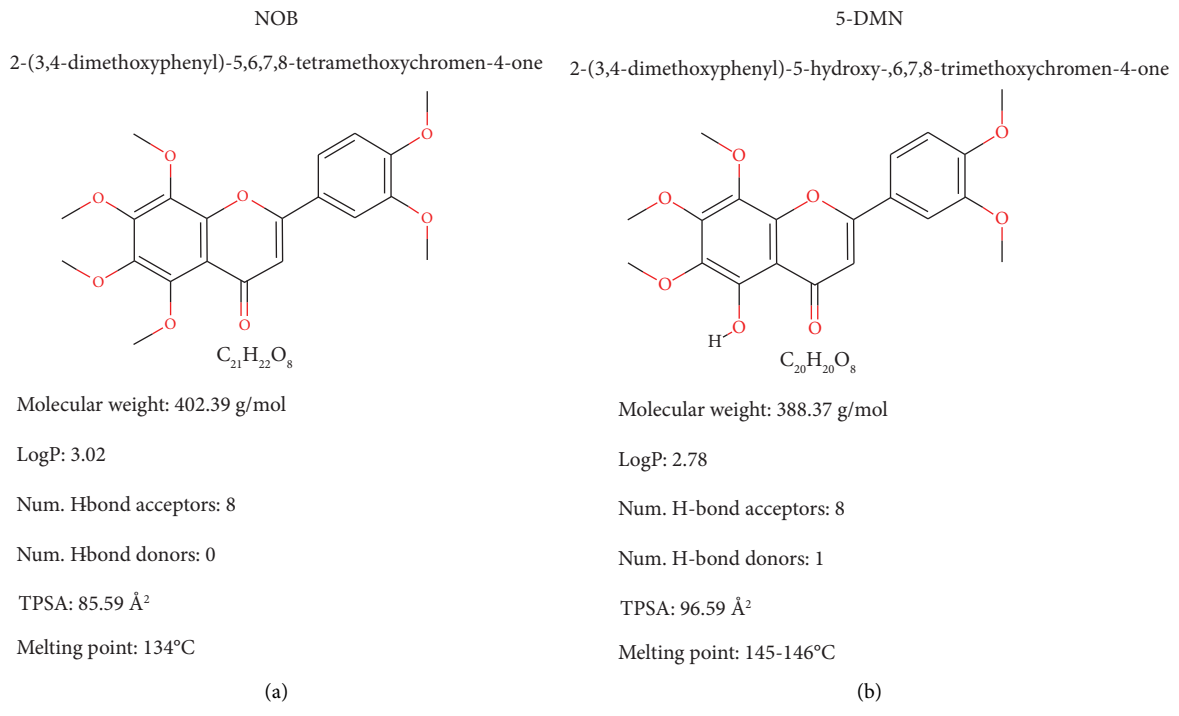

Figure 2: (a) Chemical structure of nobiletin (NOB) is a polymethoxyflavone with a chromatic core that extracted from citrus peels. The International Union of Pure and Applied Chemistry (IUPAC) designates it as 2-(3,4-dimethoxyphenyl)-5.6,7,8-tetramethoxychromin-4-one. NOB is a very lipophilic molecule with a high LogP o/w with no hydrogen bond donor group. (b) Chemical structure of 5-demethylnobiletin (5-DMN). 5-DMN is formed by the autolysis of NOB, involving demethylation and hydroxyl substitution at carbon 5. The IUPAC designates it as 2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxychromine-4-one. The polarity of the hydroxyl at Position 5 reduces the compound's LogP o/w. The main structural difference between NOB and 5-DMN is the hydrogen bond donor group contributed by NOB autolysis, which results in the transformation from a flavone to a flavonol structure.

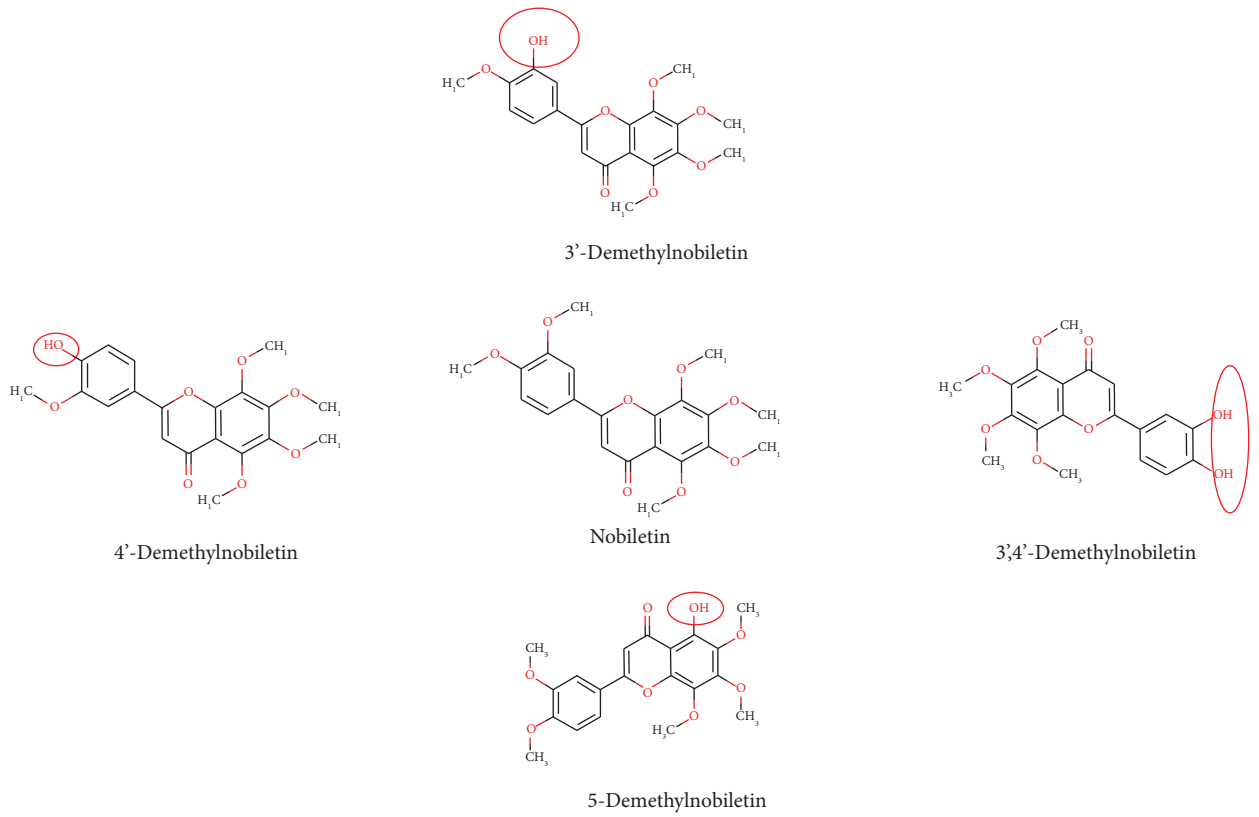

Figure 3: Metabolites of nobiletin (NOB) and 5-demethylnobiletin (5-DMN) molecules can be demethylated, at the 3′ and 4′ carbon positions. After the ingestion of NOB or 5-DMN, mouse urine contains the metabolites 3′-desmethylnobiletin (M1), 4′-desmethylnobiletin (M2), and 3′4′-didemethylnobiletin (M3).

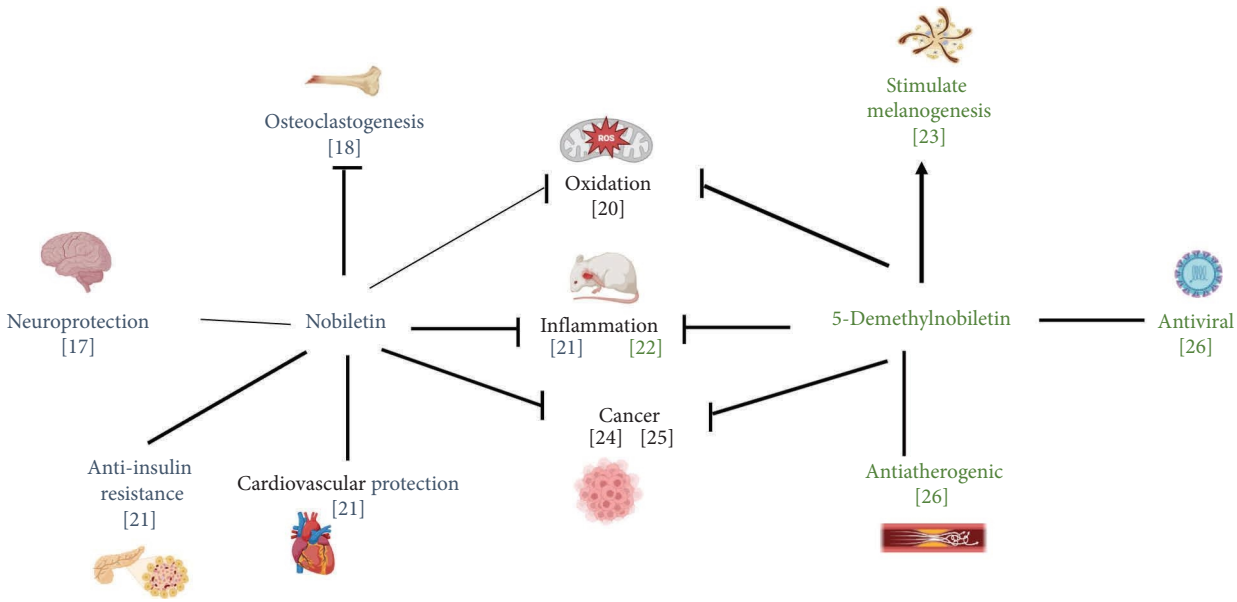

Figure 4: Pharmacological activity of nobiletin (NOB) and 5-demethylnobiletin (5-DMN).

arrest and apoptosis of HTC-116 and HT-29 cells, but demethylated molecules showed greater inhibitory effects [23]. NOB inhibits the proliferation of COLO320, SW480, and Caco-2 cells, with half-maximal inhibitory concentrations (IC_{50}s) of 40, 305, and 605 μM, respectively. In COLO320 cells, NOB (200 μM) induces apoptosis, DNA fragmentation, and the inhibition of DNA synthesis (Figure 5(a)) [34]. In AOM/DSS-treated mice, NOB decreases incidence and multiplicity of colonic tumors, inhibiting proliferation, and stimulating apoptosis and anti-

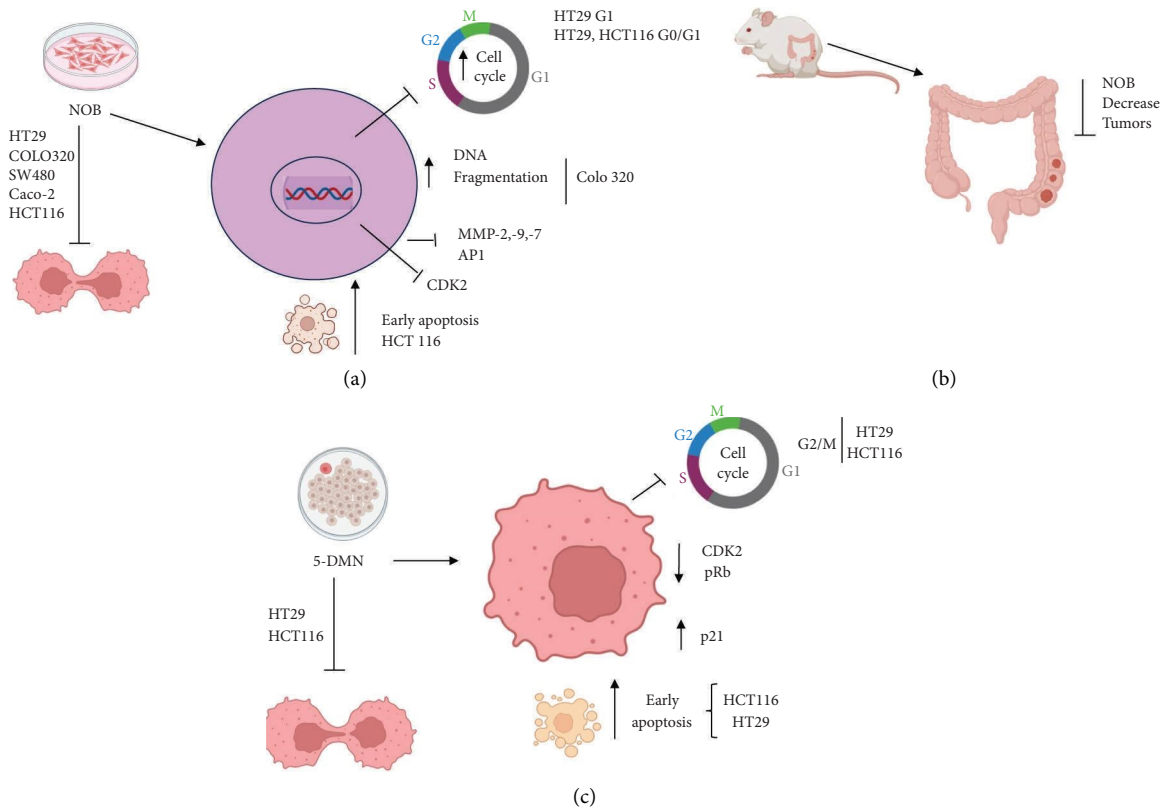

Figure 5: Effects of nobiletin (NOB) and 5-demethylnobiletin (5-DMN) on colon cancer. Effects of NOB in vitro (a) and in vivo (b). Effects of 5-DMN in vitro (c).

inflammatory activity (Figure 5(b)) [34]. High levels of 3′-demethylnobiletin, 4-demethylnobiletin, and 3,4-didemethylnobiletin were also found in the colonic mucosa of these mice, suggesting that these metabolites have higher NOB activity [34]. Synergistically with atorvastatin, NOB (1% in diet) induces cell cycle arrest and apoptosis [35, 36]. The combination of NOB, 4′-demethylnobiletin, and atorvastatin synergistically induces cell cycle arrest and apoptosis in HT-29 cells, and the most abundant metabolite in the intestinal mucosa was found to be 4′-demethylnobiletin [37]. Conversely, spheroids of HT-29 cells show increased aldehyde dehydrogenase 1 (ALDH1) activity relative to cell cultures and CD44, PROM1, SOX9, and SNAI1 gene expression, and their incubation with orange peel extract (1 mg/mL) inhibits cell proliferation; modulates cancer growth and self-renewal capacity, and reduces colony formation, ALDH1 activity, and the expression of the cell polarity protein kinase sodium polyoxotungstate and leucine-rich repeat-containing G protein–coupled receptor 5. NOB inhibits the expression of PROM1 and SNAI1, which are involved in epithelial–mesenchymal transition (EMT). However, tangeretin and scutellarein inhibit EMT-related markers more strongly. These PMFs interact synergistically with 5-fluorouracil to regulate antiproliferative activity [38]. NOB obtained from *C. sinensis* and xanthohumol from *Humulus lupulus* improve the efficacy of chemotherapy [39]. A meta-analysis of the pharmacological mechanisms of Chinese herbs showed that NOB use together with chemotherapy prolongs the survival of patients with metastatic colorectal cancer by regulating genes associated with the PI3K/AKT pathway (Table 1) [40].

3.2.1.2. Effects of 5-DMN on Colon Cancer. A study of the biotransformation of 5-DMN in mice showed that the metabolite 5,3-demethylnobiletin most effectively inhibited SW620 cell proliferation, with IC_{50}S of 0.12, 5.5, and 4.2 M, respectively [41]. In rodent studies, 5-DMN biotransformation was found to form the metabolites 5,3′-didemethylnobiletin, 5,4-didemethylnobiletin, and 5,3,4-tridemethylnobiletin. The effects of these metabolites on SW620 cell proliferation are represented by IC_{50}S of 0.12, 5.5, and 4.2 μM, respectively, lower than that of 5-DMN (10 μM), which reflects 37% inhibition of proliferation, suggesting higher activity of 5-DMN [41]. In lysogenic variants of HCT116 colon cancer cells, 5-DMN and 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone were found to induce apoptosis via p53-and Bax-associated mechanisms (Table 2) [42, 43, 46]. 5-DMN isolated from orange peel reduces HCT116 cell proliferation (IC_{50} = 8.7 μM), increases p21 expression, promotes Rb phosphorylation, and cleaves caspases 8 and 3 and poly-ADP ribose polymerase (PARP) (Figure 5(c)) [23, 44, 45].

In conclusion, in colon and colorectal carcinoma, NOB and 5-DMN inhibit cell proliferation at similar doses and promote cell cycle arrest and apoptosis.

Table 1: Effects of NOB on colon and colorectal cancer.

Cell line source	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
Not used NOB (97%) from Citrus peel	ND	In vivo Male Sprague Dawley rats NBT 0.01%	Experimental diet 10 weeks Azoxymethane (AOM) (15 mg/kg) injected at 3 and 4 weeks.	NS	ND	NBT = AOM treatment in High multiplicity of aberrant crypt foci. Colonocyte proliferation Number apoptotic cells NOS and COX2 expression in supernatants extracted from the colonic mucosal homogenates.	Leonardi, et al. 2010 [33].
3T3-L1 mouse preadipocytes Adipocyte differentiation was induced by treatment with a mixture of methyl isobutyl xanthine (0.5 mM), dexamethasone (1 M), and insulin (10 g/mL) NOB (98%) Synthetic	ND	In vivo In male db/db mice with obesity and diabetic phenotypes. In vivo Given 3 weeks intraperitoneal injections of AOM In vitro NBT 10, 100 μM	3 weeks	NS	In vivo ↓ PI3K/AKT ↓ERK	In vivo ↓ ACF	Miyamoto, et al. 2010 [32].
HCT116 HT29 NOB from orange peel	NOB 37 μM 5-DMN 22 μM	In vitro NOB and 5-DMN 10–50 μM Apoptosis 5-DMN 3 μM	Cell viability 48 h Cell cycle arrest 24 h Apoptosis 48 h	DMSO 0.1%	ND	Cell cycle arrest G0/G1 5DMN ↑ apoptosis ↓ p-Rb ↑ Mcl-1 ↑ PARP ↓ CDK2	Qiu, et al. 2010 [23].
COLO320 Swiss 480 Caco-2 NOB Nondetermined	COLO320 40.4 ± 9.1 μM Swiss 480 245 ± 9.1 μM Caco-2 305.6 ± 41.9 μM	In vitro 25–300 μM	Cell cycle 48 h DNA fragmentation 48 h	DMSO	ND	↓Cell viability Sensitive COLO320 >sw480 > Caco-2 ↑ DNA fragmentation	Zheng, et al. 2002 [34].

Table 1: Continued.

Cell line source	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
3T3-L1 mouse preadipocytes HCT116 HT29 NOB (98%) Synthetic	ND	In vivo Male mice Crj; CD-1 (ICR) treated with nobiletin Colon carcinogenesis was induced with azoxymethane (AOM) and dextran sulfate sodium (DSS) Five-week-old ICR mice were given a single intraperitoneal injection of AOM followed by 1% DSS in drinking water for 7 days. NOB (0.05 wt% in diet) starting at 1 week after the AOM injection In vivo Diet (100 p.p.m) for 17 weeks. NOB (10 or 100 μM) + leptin (1 μM)	Cell viability 24 h	DMSO	↓ Raf ↓ MEK 1/2 ↓ pi-ERK 1/2	In vivo ↓ Obesity ↓ Leptin ↓ IL-6 ↓ TNF-α In vitro NOB no effect on cell proliferation.	Miyamoto, et al. 2008 [30].
HTC116 colon cancer cells RAW 264.7 Macrophages NOB (98%) Synthetic	ND	In vivo Dimethyl sulfoxide (DMSO) was used as the vehicle to deliver NOB and its metabolites (NOB: M1: M2: M3 at a ratio of 1: 1.65: 12.05: 6) to the cells. Derivates 3′-demethylnobiletin (M1), 4′-demethylnobiletin (M2), and 3′,4′-didemethylnobiletin (M3). In vitro OBT: M1: M2: M3 at a ratio of 1: 1.65: 12.05: 6	Cell cycle progression 24h. Cell cycle proteins 24h	DMSO 0.01%	ND	In vivo NBT + metabolites: ↓ iNOS In vitro NBT + metabolites: ↓ LPS-induced NOS-1 expression RAW cells. ↓ NRF2 nuclear localization induced by LPS ↑ Levels of HO-1 and NQO1 in LPS-treated macrophages. HTC 116 cells NBt + metabolites: ↑ cell cycle arrest G0/G1	Wu, et al. 2017 [36].

Table 1: Continued.

Cell line source	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
HCT116 HT29 NOB (98%) Synthetic	ND	In vivo Intraperitoneal injection of AOM (12 mg/kg body weight) after 1 week, 1.5% DSS (molecular weight: 36,000–50 000). NOB (0.05 wt% in diet) starting at 1 week after the AOM injection until the end of study. In vitro NOB 10–50 μM	Cell cycle arrest 24 h	NS	ND	In vivo ↓ Incidence and multiplicity of colonic tumors in AOM/DSS-treated mice. NOB ↑ p21 In vitro ↓ Cell proliferation ↓Proinflammatory molecules/ IL-1β, IL-6, TNF-α ↑Apoptosis ↓ PCNA ↑ Cleaved caspase-3 M2 is must abundant metabolite. NBT, M1, M2, M3 ↓ Cell proliferation. M3 more active. ↑ Cell cycle arrest M2 and NBT at G0/G1 M1 and M3 at G2/M.	Wu, et al. 2015 [16].
HT-29 RAW 264.7 NOB (98%) Synthetic	ND	An azoxymethane (AOM) was used to induce colon carcinogenesis. In vivo Cotreatment NBT (0.1% w/w) Atorvastatin (ATST) (0.04%).	NS	ND	NBT ATST, NBT + ATST ↓ Cyclin D, cyclin E, cdK2, CDK4.	In vivo ↓ NBT tumor incidence ↓↓↓ tumor incidence NBT + AZT In vitro ↑ Cleaved caspase 3 ↑ Cleaved PARP ↓ VEGF ↓ MMP9	Wu, et al. 2017 [35].
HT-29 NOB Synthetic	ND	In vivo Twelve male F344 rats (5 weeks of age) fed NBT (0.05% w/w, in diet) + ATST (0.02% w/w, in diet). In vitro Cell viability 7.2, 14.4, 21.6, 28.8, 36 μM. Cell cycle progression of 4DN (36 μM) ATST (18 μM) (4DN at 18 μM + ATST at 9 μM) Apoptosis of 4DN (36 μM), ATST (18 μM) (4DN at 18 μM + ATST at 9 μM	In vitro Cell viability 72 h Cell cycle progression 24 h Apoptosis 24 h	DMSO 0.1%	4DN/ATST cotreatment (4DN at 18 μM + ATST at 9 μM) showed the stronger effects on p21 and CDK4 than 4DN or ATST alone.	In vivo NBT increases concentration of 4′-demethylnobiletin in colonic tissues Cotreatments of 4DN/ATST at 2: 1 concentration ratio produced much stronger growth inhibitory effect on human colon cancer. In vitro ↓Cell growth NBT + ATST > 4DN = ATST cotreatment synergistically induced G0/G1 cell cycle arrest	Wu, et al. 2018 [37].

Table 1: Continued.

Cell line source	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
CR-CSC (Untreated) CR-CSphC (CRC spheroid cells). CRC cells were treated with 5-fluorouracil + oxaliplatin Normal cell: HUVEC HS-5 HCT116 RKO NOB from *Citrus sinensis var.*	ND	In vitro 5, 10 years 25 μg/mL 5-fluorouracil and oxaliplatin (FOX) [0.1, 0.5, 1, 2.5, and 10 μM). Nobiletin and xanthohumol (XCF) [5, 10, and 25 μg/mL]. Nobiletin [12.5, 25, and 40 μg/mL]	24 or 48 h 3 h 48 h	DMSO	Wnt/β-catenin	Synergy with 5-fluorouracil and oxaliplatin: ↓ Viability (CCR). CR-CSphC showed resistance in viability. Nobiletin and xanthohumol: HS-5 and HUVEC showed no toxicity. Nobiletin, XCF, and mix: ↓ proliferation (CR-CSphCs) ↓ proliferation (CRC). Synergistic effects of nobiletin and mix plus FOX. Nobiletin and XCF: ↑ Apoptosis of CR-CSphCs. Cell cycle arrest (G0/G1 phase) of CR-CSphCs. Expression of proapoptotic genes (CR-CSphC): . ↑ ATG3 ↑ ATG5 ↑ ATG12 ↑ B2M ↑ CD40 ↑ CYLD ↑ FAS ↑ GADD45A Anticancer effect of nobiletin and XCF (CR-CSC): ↓ Formation of spheres ↓ Clonogenic potential of CR-CSphC. ↓ Expression of CD44v6	Turdo, et al. 2021 [39].
HT29 SW480 NOB from Citrus peel	ND	In vitro Nobiletin [25, 50, 100, and 200 μM]. Oxaliplatin [5 μM]. Oxaliplatin [5 μM] + nobiletin [100 and 200 μM].	24 h	NS	PI3K/Akt/mTOR	↓ Viability oxaliplatin and nobiletin acted synergistically. ↓ Proliferation Nobiletin: ↑ Apoptosis oxaliplatin + nobiletin: ↑ Bax ↑ Caspase-3 ↓ Bcl-2 Nobiletin promotes chemosensitization.	Li, et al. 2019 [31].

Table 1: Continued.

Cell line source	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
HT29 NOB from orange peel	ND	In vitro OPE [0.30, 0.60, 1,20 years 2,40 mg/mL] 5-fluorouracilo (5-FU) [(0.15, 0.30, 0,60 years 1,20 mg/L] Nobiletin [14.70, 29.39, 58.78, 117.56 μM] OPE [1,00 mg/mL] PMF (nobiletin—49.11 μM, sinensetin—46.62 μM, tangeretin—10.41 μM, and scutellarein tetramethyl ether—31.55 μM).	72 h 24 h	DMSO	ND	HT29 spheroids (3 D): Mesenchymal features: ↑ SNAI1 ↓ ZEB1 ↓ LGR5 ↑ PROM1 ↑ CD44 ↑ SOX9 ↑ ALDH + OPE and PNF (N–S–T–Sc). Antiproliferative effect. Apoptosis: ↓ BIRC5 expression. Cell cycle: ↑ CDKN1A ↓ CCNA2 G2/M cell cycle arrest. OPE. Metastatic potential: ↓ PROM1 ↓ LGR5 Mesenchymal markers: ↑ SNAI1 ↑ ZEB1 Epithelial marker: ↑ CDH1 Inhibition of spheroid self-renewal: ↓ Colony formation OPE, PMF, and 5-FU. ↑ Antiproliferative effect. Synergistic effect.	Pereira, et al. 2019 [38]

Abbreviations: ND, not determined; NS, not specified.

Table 2: Effects of 5-DMN on colon and colorectal cancer.

Cell line	IC50	Biological processes dose	Experiment duration	Vehicle	Pathway	Results	References
SW480 SW620 Not used 5-Demethylnobiletin (94%) Synthetic	ND	In vitro SW480: 4 μM SW620: 10 μM In vivo The CF-1 male mice. 5-DMN: 3 mg	48 h For 2 weeks. Urine samples were collected from each group in metabolic cages for 24 h.	NS NS	ND ND	↓ Proliferation Identification of metabolites: 5.3′-didemethylnobiletin	Zheng et al. 2013 [41].
HT29 HCT116 NOB from *Citrus depressa*	ND	In vitro HT29: 20 μM HCT116: 8 μM	48 h	DMSO	ND	G2/M cell cycle phase arrest. Inhibition of the DNA replication. ↓ Synthesis of cellular proteins.	Zhang et al. 2016 [42].
HCT-116 HT-29 COLO205 NOB Synthetic	ND	In vitro 5–40 μM	24 h	<0.05% DMSO	ND	↓ Proliferation COLO205 > HCT-116 > HT-29 ↑ Apoptosis ↑ PARP expression	Chiou et al. 2018 [7].
COLO205 NOB Synthetic	ND	In vivo Xenograft Male BALB/cAn.Cg-Foxnlnu/CrINarl mice. 50 and 100 mg/kg	21 days Cells were subcutaneously injected into the ventral flank. 5-DMN via intraperitoneal injection.	DMSO	ND	Antitumor effect ↓ Tumor growth ↓ Tumor weights ↑ PARP expression ↑ LC3I and LC3II ↑ p53 ↓ COX-2 ↓VEGF	Chiou et al. 2018 [7].
HCT116 HT-29	HCT116: 8.7 μM HT-29: 22 μM	In vitro HCT116: 4 μM and 8 μM. HT-29: 4 μM and 36 μM.	48 h	NS	ND	↓ Proliferation Induced G2/M phase arrest. ↑ p21 and Rb expression. ↓ CDK2 ↓pRb ↑ Early apoptotic ↑ Caspase 8, caspase 3, and PARP expression. Antiangiogenesis ↓ VEGF expression Antitumor effect ↑ p53 expression Autophagy induction ↑ LC3 expression Anti-inflammatory effect ↑ COX-2 expression	Goh et al. 2019 [43].
COLO205 NOB from *citrus depressa*	ND	In vivo Colorectal cancer xenograft mouse model. 5-DMN 50 mg/kg and 100 mg/kg	For 3 weeks. Intraperitoneal injection.	NS	ND		

Table 2: Continued.

Cell line	IC50	Biological processes dose	Experiment duration	Vehicle	Pathway	Results	References
HCT116 Not used	13.5 μM	In vitro 5-DMN: 10–20 μM	72 h	NS	ND	↓ Cell proliferation G2/M phase cell cycle arrest. ↑ Early apoptotic ↑ p21 and p27 expression. ↓ Levels of cyclin A2 and cyclin B1. ↑ Caspase-9, caspase-7, caspase-3, and PARP expression. ↑ p53 ↓ Tumor incidence, multiplicity, and burden. ↓ Ki67 and PCNA ↓ iNOS ↓ Levels of IL-1β, IL-6, and TNF-α	Song et al. 2020 [44]
NOB (99%) Synthetic	ND	In vivo Male CD-1 mice. 5-DMN: 0.05%	Intraperitoneal injection of AOM (12 mg per kg body weight). Dextran sulfate sodium (DSS): 1.5% was administered in the drinking water for 4 days.	Saline solution	ND		
HepG2 Not used	ND	In vitro 5-DMN: 15 and 20 μM.	24-h BaP (Benzo[a] pyrene): 10 μM.	DMSO: less than 0.5% of the total volume.	ND	↓ Carcinogen biotransformation (Phase I enzymes). ↓ CYP1B expression—CYP1A1 expression Preventing mutagenicity ↓ CYP1B1 expression–CYP1A1 expression ↑ GST and UGT Antimetastatic ↓ Number of polyps ↓ Adenomas and adenocarcinomas in the colonic sections.	Chou et al. 2021 [45].
Not used	ND	In vivo Five-week-old male ICR mice. 5-DMN: 0.02% in diet.	Diet for 1 week. BaP 125 mg/kg/day. Orally gavage for 5 days. Livers were collected and stored until RNA extraction.	NS	ND		
NOB Synthetic	ND	In vivo Male BALB/c mice. 5-DMN: 40 mg/kg/day	BaP 125 mg/kg/day was administered for 5 days, followed by two cycles of 2.5% (w/v) DSS in drinking water for 7 days. 5-DMN by oral gavage for 15 weeks. Collection of colon tissues	NS	ND		

Abbreviations: ND, not determined; NS, not specified.

Table 3: Effects of NOB on gastric cancer.

Cell line	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
AGS NOB from Citrus peel	ND	In vitro 1, 1.5, and 2 μM	24 h 48 h	0.2% DMSO	FAK/PI3K/Akt	↓ Viability ↓ Cell Adhesion Changed morphology: Elongated, spindle-shaped, and shrunken. ↓ Invasion ↓ Migration ↓MMP-2 and MMP-9 ↓ Ras ↓ c-Raf ↓ Rac-1 ↓ Cdc42 ↓ RhoA ↑ RhoB ↓ NF-κB DNA binding activity ↓ Cytoplasmic and nuclear level of NF-κB ↓ IκBα ↓ Akt	Lee, et al. 2011 [25].
TMK-1 MKN-45 MKN-74 KATO-III NOB Synthetic	134.8 μM 226.3 μM 189.7 μM 146.3 μM	In vitro 20, 60, and 200 μM	48 h	0.2% DMSO	ND	↓ Proliferation TMK-1 ↑ Apoptosis G1 cell cycle arrest. TMK-1 and MKN-45 NOB + CDDP: synergistic effect. Antitumor effect.	Yoshimizu, et al. 2004 [49].
N87	18.8 μM	In vitro 4, 20, and 100 μM	48 h	NS	ND	↓ Viability ↓ The adhesion of *H. pylori* ↓ CD74 expression.	Sekiguchi, et al. 2008 [48].
TMK-1 St-4 MKN-45	156 μM 655 μM	In vitro 16–64 μM	24 and 48 h	0.05% DMSO	ND	TMK-1 ↓ Proliferation ↓ Pro-MMP-9.	Minagawa, et al. 2001 [47].
NOB Synthetic	494	In vivo Five-week-old male mice. 21 mg/kg/day for 2 weeks by subcutaneously.	Three weeks. TMK-1 cells were injected intraperitoneally.	DMSO	ND	↓ MMP-9 Antimetastatic ↓ Formation of peritoneal dissemination nodules. ↓ weight of the peritoneal dissemination nodules.	

Table 3: Continued.

Cell line	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
SNU-16 NOB Synthetic	ND	In vitro 50 μM Chloroquine [40 μM]: Autophagy inhibitor and NOB [25 μM].	24 h 2 h	DMSO	Intracellular ER stress-mediated protective autophagy.	↓ Viability Cell death: ↑ RhoGDI (Rho GDP dissociation inhibitor 1). Cell survival: ↓ TXNDC5 ↓ COMD9 ↓ EIF4E ↓ PRDX3 Apoptosis: ↑ IRE1-α ↑ ATF-4 ↑ CHOP ↑ GRP78 ↑Caspase-4 ↓ Procaspase-4 ↑ XBP-1 autophagy: ↓ Akt and mTOR ↑ LC3B II/LC3B I ↓ p62 ↓ Proliferation Cell cycle arrest (sub-G1 phase) ↑PARP	Moon, et al. 2016 [51].

Abbreviations: ND, not determined; NS, not specified.

Table 4: Effects of 5-DMN on gastric cancer.

Cell line	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
AGS BGC-823 SGC-7901 NOB Synthetic	ND	In vitro AGS and BGC-823: 12.5, 25, 50, 100, and 200 mg/L SGC-7901: 25, 50, 100, and 200 mg/L	48 h	0.1% DMSO	ND	↓ Proliferation ↑ Apoptosis ↑ RARβ ↓ Procaspase-3 ↓ Procaspase-9 ↓ Pro-PARP1 expression ↑ Caspase-3 ↑Caspase-8 ↑Caspase-9 ↑PARP1 expression.	Wang et al. 2020 [54]

Abbreviation: ND, not determined.

Table 5: Effects of NOB on leukemia.

Cell line	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
KHYG-1 NOB Synthetic	ND	In vitro 0.5 and 1 mM 30 and 100 μM XPO1 inhibitor LMB [0.1, 0.3 and 1 nM]. NF-κB inhibitor BAY 11-7082 [0.3 μM].	24 h	DMSO	XPO1	Improves cytolysis: ↑ GrB ↑ IFN-γ ↓ XPO1 ↑ p53 Cell cycle arrest (G0/G1 phase). ↑ NF-κB (65 and 50) Inhibitor BAY 11-7082 + nobiletin ↓ GrB ↓ IFN-γ	Saito, et al. 2020 [57].
K562 NOB Synthetic	82.49 μM	In vitro 10, 20, 40, 80, and 100 μM 40, 80, and 100 μM Imatinib [0.2 μM] + nobiletin [40 or 80 μM]	24 and 48 h	0.1% DMSO	MAPK/ ERK	↓ Viability ↓ Proliferation cell cycle regulator: G1 phase arrest ↑ p21 ↑ p27 ↓ Cyclin D2 Megakaryocytic differentiation: ↑ CD61 ↑ CD41 ↑ CD42 Erythrocytic marker: ↓ Glycophorin A ↓ Hemoglobin α formation of cells with multilobulated nuclei. ↑ EGR1 ↑ERK1/2 Synergistic effect: ↓ Viability	Yen, et al. 2020 [58].
HL-60 OCI-AML3 MV4-11 AML U937 THP-1 NOB (98%) Synthetic	HL-60 40~80 μM	In vitro 40 μM	24 h	0.5% DMSO	p38 MAPK	↓ Proliferation HL-60 Cell cycle arrest: G0/G1 phase. ↓ ERK ↑ Apoptosis ↑ Caspase-8 ↑ Caspase-9 ↑ Caspase-3 ↑ PARP ↓ ERK ↑ p38	Hsiao, et al. 2014 [55].
K562 K562/ADM (adriamycin-resistant) KB/WT KB/VJ-300 (vincristine-resistant) NOB Synthetic	ND	In vitro 5.15 μM	120 min	0.5% DMSO	ND	Without nobiletin: K562/ADM ↑ P-gp K562 ↓ P-gp KB/VJ-300 ↑ P-gp KB/WT ↓ P-gp K562/ADM treated with nobiletin. ↓ [3H] vincristine resistance.	Ikegawa, et al. 2000 [56].

Abbreviation: ND, not determined.

Table 6: Effects of 5-DMN on leukemia.

Cell line	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
HL-60 THP1 U937 HEL K562 5-DMN Synthetic	85.7 Mm 32.3 μM 30.4 μM 65.3 μM 91.5 μM	In vitro 20, 40, and 80 μM.	48 h	0.01% DMSO	ND	↓ Proliferation THP-1 > U-937 > HEL > HL-60 > K562 No toxicity in PBMC (peripheral blood mononuclear cells). ↑ Apoptosis Cell cycle arrest in S-phase (THP1) ↓ Cyclin E1 and A1 ↑p21 (CDK) ↑ Cleaved caspase-3 and PARP1 ↓ Antiapoptotic Mcl1 ↑ CD11b mRNA expression ↑ Differentiation in AML cells ↑ Stanniocalcin 2 (STC2) gene expression ↓Phosphorylated levels of TNF-α	Chen et al. 2022 [59].

Abbreviation: ND, not determined.

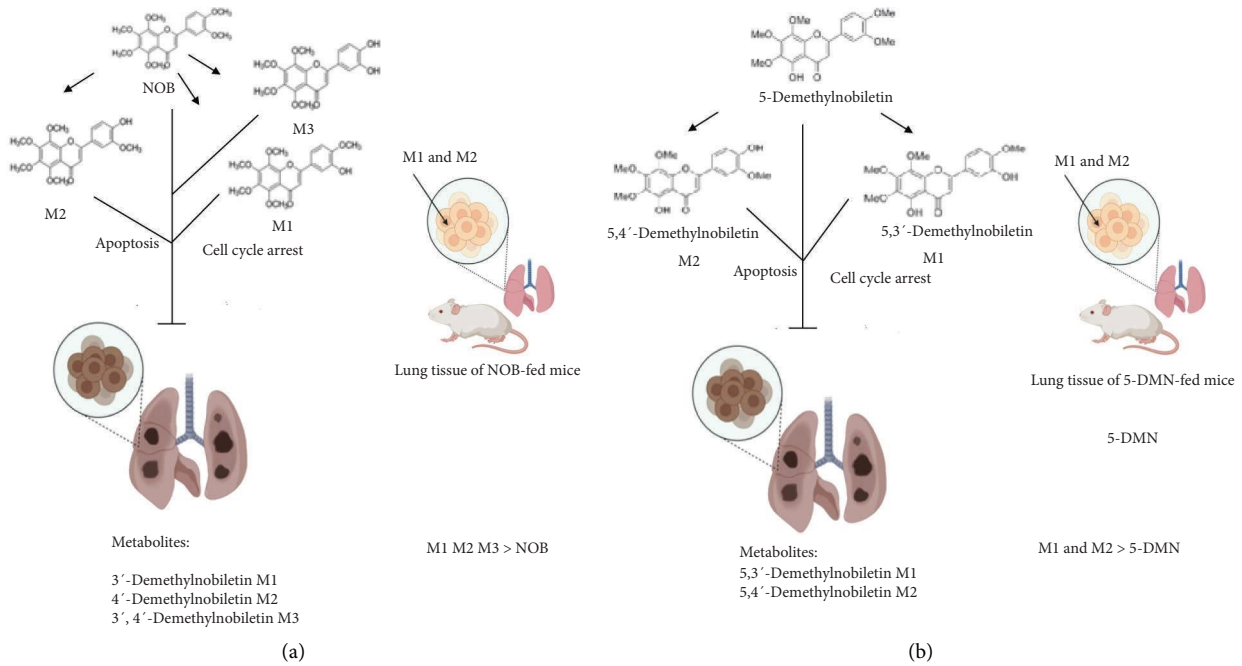

Figure 6: Effects of nobiletin (NOB), 5-demethylnobiletin (5-DMN), and metabolites on lung cancer. Effects of NOB and metabolites in vivo (a). Effects of 5-DMN and metabolites in vivo (b).

3.2.2. Gastric Cancer. The third most common stomach cancer is an adenocarcinoma that originates in the glands of the gastric mucosa or lymphoid tissue of the stomach muscles.

Gastric cancers are adenomas that originate in the glands of the gastric mucosa. In addition to adenocarcinomas, other types of cancer can arise from the stomach lymphoid tissue (of the mucosa and muscles) and muscles. Adenocarcinomas are classified into two groups: those associated with *Helicobacter pylori* infection and diffuse gastric cancers originating from pangastritis without atrophy. Symptoms are abdominal pain, abdominal bloating after eating, feeling full after eating small amounts of food, not being hungry when one expects to be, heartburn, indigestion, nausea, vomiting, and unintentional weight loss. Risk factors include the reflux of stomach acid into the esophagus, high-salt diet, low fruit and vegetable intakes, *H. pylori* infection tobacco use, and alcohol consumption. Treatment consists of surgery, radiotherapy, chemotherapy, and immunotherapy [47].

3.2.2.1. Effects of NOB on Gastric Cancer. In the TMK-1 human gastric carcinoma cell line and a severe combined immunodeficiency disease mouse model, NOB was found to inhibit the expression of MMP-9 and the formation of peritoneal dissemination nodules from TMK-1 [47]. Reverse-transcriptase polymerase chain reaction and enzyme-linked immunosorbent assays showed that NOB (4–100 μM) induced the downregulation of CD74, a protein expressed on gastric epithelial cells that is key for *H. pylori* binding, in NCI-N87 cells; the flavonoid luteolin, however, was more effective than NOB in this respect [48]. NOB (1–45 μM) inhibits the proliferation, adhesion, migration, and invasion of ACS cells in a PI3K/AKT pathway-dependent manner; at 24–48 h, it inhibits the expression of Ras, c-Raf, Rac-1, Cdc42, and RhoA and the phosphorylation of inhibitor of kappa B alpha (IkBα) and MMP-2/9 [25, 49]. NOB inhibits the proliferation and induces the apoptosis of SNU-16 cells by increasing the Bax/Bcl-2 ratio and has synergistic effects with 5-fluorouracil [50]. The treatment of SNU-16 cells with NOB (50 μM), reduced cell viability, and induced the expression of glucose-regulated protein 78 kDa and apoptosis, via pathways involving intracellular stress-mediated protective autophagy [51]. NOB reduces reactive oxygen species levels in *H. pylori*–infected GES-1 cells and inhibits inflammation and apoptosis, mediated by mitogen-activated proteins, potentially preventing *H. pylori* infection [51]. In a preliminary bioinformatics and network pharmacology, study of common herbal treatments for gastric cancer in China, quercetin, kaempferol, baicalein, NOB, and luteolin were identified as effective therapeutic agents with effects including cell cycle arrest, the promotion of apoptosis, and the inhibition of proliferation, migration, and invasiveness [52]. The Zhizhu pill, formulated form *Aurantii Fructus Immaturus* (zhishi) and *Atractylodes macrocephala* Koidz (baizhu) and originating from Shang Han, is used in traditional Chinese medicine to treat gastroesophageal reflux disease (a gastric cancer risk factor) and was shown to contain the flavonoids such as NOB, luteolin, didymin, and naringenin. Pharmacological network validated by molecular docking showed that NOB regulates the following gastroesophageal reflux-related genes: peroxisome proliferator-activated receptor gamma, MMP-9, JUN, and TP53 (Table 3) [53].

3.2.2.2. Effects of 5-DMN on Gastric Cancer. 5-DMN (24–200 mg/L) in Ougan (*C. reticulata*) extract was found to

Table 7: Effects of NOB on lung cancer.

Cell line	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
A549 H460 NOB Synthetic	ND	In vitro 2.5 μM	48 h	NS	WNT/β-catenin signaling (miR-15-5p) -Negative regulators: NKD1, AXIN2, and WIF1. -Positive regulators: WNT6 and Jun.	↓ Colony formation ↑ Apoptosis (PARP and caspase-3). ↓ Cell migration and invasion	Han et al. 2021 [60].
H460 H1299	78.44 μM	In vitro 50 μM	24 h 48 h	NS	ND	Cell cycle arrest (G0/G1). ↓ Cyclin D1, CDK1, and CDK6. ↑ Apoptosis ↑ Bax ↓ Tumorigenesis ↓ Proliferation	Sun et al. 2019 [63].
NOB (>98%) Synthetic	54.33 μM	In vivo Female A/J mice. 0.05% w/w in diet.	16 weeks AIN-76A diet for 1 week. Intraperitoneal injection of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK): 100 mg/kg body weight.	Saline solution	PCNA		
A549 H292 H460 NOB Synthetic	200 μM	In vitro 100 μM 200 μM	48 h Cells were treatment with EGF [25 ng/mL] and EGFR-tyrosine kinase inhibitor (AG1478) [50 μM] with or without nobiletin	NS	EGFR/JAK2/STAT3	↓ proliferation Immunosuppression effect: ↓ PD-L1 ↓ PD-1 ↓ pEGFR ↑ EFGR ↓ pJAK2 ↓ pSTAT3 ↓ p53	Sp, et al. 2021 [66].
A549		In vitro 10, 20, and 40 μg/mL	24 h	NS	ND	↓ Proliferation ↓ Colony formation ↑ Apoptosis: Nuclear condensation. DNA fragmentation G2/M cell cycle phase arrest. ↓ Bcl-2 ↑ Bax ↑ p53 ↓ Smad Antitumoral ↓ Tumor growth	Lou, et al. 2008 [62]
NOB Synthetic	23.82 μg/mL	In vivo Six-week-old male nude mice. 300 mg/kg, 200 mg/kg, and 100 mg/kg.	20 days A549 cells were inoculated (5 × 106 cells in 200 μL). Control group was treated with saline. The positive control group treated with cyclophosphamide (60 mg/kg). It was administered intraperitoneally only on the first day. Nobiletin was administered orally once a day for 15 days.	0.5% Carboxymethyl cellulose.	ND		

Table 7: Continued.

Cell line	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
A549 H1299 NOB No determined	ND	In vitro 10 and 20 μM In vivo Five-week-old nude mice. 20 and 40 mg/kg.	24 h Cells were treated with TGF-β1 [5 ng/mL]. 42 days Mice were injected with A549 cells via vein (4 × 105 cells in 0.2 mL PBS). Nobiletin was orally administered once daily. Cyclophosphamide [20 mg/kg] was the positive control, injected intraperitoneally every 3 days. Saline was administered orally every day for the control group.	NS	FAK/Src pathway	Inhibits EMT ↑ E-Cadherin ↓ N-Cadherin ↓ Snail ↓ Slug ↓ ZEB1 ↓ Twist inhibits metastasis: ↓ migration ↓MMP-2, MMP-9. ↓ Transcriptional activity of Smad 3 ↓ Invasion and adhesion	Da, et al. 2016 [64].
		Nude mice C57BL/6 mice.	42 days Mice were implanted subcutaneously with A549-Luc cells. 15 days Metastatic model: Mice were injected with Lewis cells via the tail vein (8 × 105 cells in PBS).	NS	ND	Metastasis: ↓ metastatic nodules tumor growth	
A549	0.32 μM	In vitro ADR: 0.5 μM NBT: 50 μM	6, 12, 24,and 48 h	NS	A549/ADR Akt/ GSK3β/ β-catenin/ MYCN	A549 (Only ADR) ↓ Proliferation Sub-G1 cell cycle phase arrest ↑Bax ↓ Bcl-2 A549/ADR Synergistic effect of NBT with ADR: ↓ Neuroblastoma-derived MYC (MYCN). ↓ Multidrug resistance–associated protein 1 (MRP1). ↓ Akt ↓ GSK3β ↓ β-catenin ↑ intracellular accumulation of ADR ↓ Viability ↑ Apoptosis ↑ Bax ↓ Bcl-2 ↓ Bcl-Xl ↓ Survivin ↑ Caspase 3 ↑ PARP Synergistic effect of NBT with ADR: ↓ Tumor volume and weight	Moon, et al. 2018 [67].
A549/ADR NOB Undetermined	0.5 μM	In vivo Mice BALB/c female nude mice. 40 mg/kg	35 days Mice were 1 × 106 A549/ADR cells resuspended in Matrigel and subcutaneously inoculated. ADR: 10 mg/kg	PBS	ND		

Table 7: Continued.

Cell line	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
H460 H1299 A549 NOB Synthetic	76.3 μM 54.8 μM	In vitro 50 μM	72 h 24 h	0.1% DMSO	ND	↓ Viability H460 ↓ Colony formation H1299 G0/G1 cell cycle phase arrest A549 ↓ Tumorsphere formation	Song et al. 2016 [68].
H1299 H441 H460 NOB from orange peel	50 μM	In vitro 25 μM	48 h	0.1% DMSO	ND	↓ Viability	Xiao, et al. 2009 [65].
A549 H1299 NOB from orange peel	100 μM	In vitro 10, 20, 40, 60, 80, and 100 μM IL-1β [1 ng/mL]	24 h 30 min 24 h	DMSO	ND	↓ Viability ↓ IL-1β-induced COX-2 protein expression.	Chen et al. 2007 [61].

Abbreviations: ND, not determined; NS, not specified.

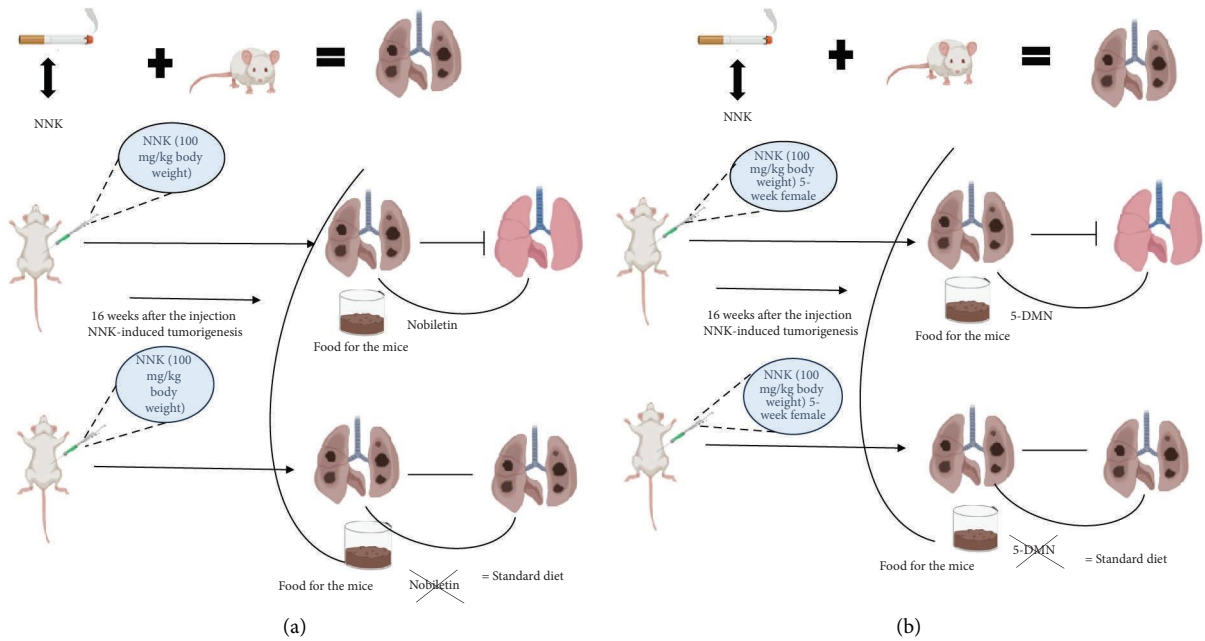

Figure 7: Effects of nobiletin (NOB) and 5-demethylnobiletin (5-DMN) on lung cancer. Effects of NOB in vivo (a). Effects of 5-DMN in vivo (b).

inhibit the proliferation of AGS, BGC-823, and SGC-7901 gastric cancer cells in a dose-dependent manner and to induce the formation of apoptotic bodies and cleaved caspase-8, but tangeretin and NOB from the same extract had greater inhibitory and early and late, respectively, apoptotic effects [54]. NOB and tangeretin also induced RARB gene expression and the inactivation of caspase-8 and caspase-9, and 5-DMN did not induce the activation of caspase-8, suggesting that these PMFs induce apoptosis by different mechanisms (Table 4) [54].

Further studies with 5DMN should be performed to establish their effect on cell migration, invasion, and cell arrest.

In relation to gastric cancer, NOB and 5DMN inhibit cell proliferation and apoptosis in similar doses.

3.2.3. Leukemia. Leukemia is characterized by the transformation of hematopoietic progenitors and diffuse infiltration of the bone marrow. The types of leukemia include acute lymphoblastic leukemia, and acute and chronic myeloid leukemia. Acute leukemia is most common in pediatric patients, and its incidence is 20–35 cases per 1 million inhabitants. The incidence is higher in Mexico (45 cases/1 million inhabitants). The symptoms of leukemia include fatigue, decreased platelet counts, weight loss, and lumpiness in the neck, armpits, and groin.

3.2.3.1. Effects of NOB on Leukemia. NOB (10–160 μM) inhibits the proliferation of HL-60, U937, THP-1, OCI-AML3, and MV4-11 cells in a dose-dependent manner, with HL-60 cells showing greater sensitivity to NOB treatment ($IC_{50} > 40$ μM) than the other four cell lines (IC50 = 40–80 μM). NOB (40 μM) also promotes G0/G1 cell cycle arrest in these cells. Extracellular signal-regulated kinase 1/2 (ERK 1/2) is involved in the cytotoxic and apoptotic effects of NOB [55]. p-Glycoprotein (p-GP) is implicated in multidrug resistance, as it transports lipophilic molecules from the cytosol against the gradient concentration, and NOB was found to inhibit the activity of this protein in K562 cells and their adriamycin-resistant variant K562/ADM cells [56]. In KHYG-1 cells, NOB inhibits the nuclear export factor exportin-1 [57]. NOB (10–100 μM) also inhibits the proliferation of K562 cells, upregulating p21, p27, cyclin D2, and G1 cell cycle arrest and inducing the expression of CD61, CD41, and CD42, markers of megakaryocytic differentiation (Table 5) [58].

3.2.3.2. Effects of 5-DMN on Leukemia. 5-DMN (20–80 μM) reduces human leukemia cell viability with different degrees of effectiveness (THP-1 cells ≈ U-937 cells > HEL cells > HL-60 cells > K562 cells). 5-Demethyl NOB (20 and 40 μM) modulated the cell cycle through the regulation of p21, cyclin E1, and cyclin A1 expression and induced S-phase arrest (Table 6) [59].

In conclusion, the effects of NOB on leukemia have been more extensively documented in comparison with 5DMN, and both polymethoxyflavones inhibit proliferation, induce cell cycle arrest, and promote apoptosis.

3.2.4. Lung Cancer. Lung cancer is the leading malignancy among men and women worldwide, and it also has the highest mortality rate. The majority (85%) of cases are caused by tobacco use and are detected at advanced stages, when treatment options are limited.

3.2.4.1. Effects of NOB on Lung Cancer. The A459 human lung adenocarcinoma cell line shows sensitivity to NOB, with dose-dependent (20–80 μg/mL) and time-dependent

Table 8: Effects of 5-DMN on lung cancer.

Cell line	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
A549 CL1-5 5-DMN Synthetic	ND	In vitro 12.5 and 25 μM	24 h	25 mM DMSO	JNK	↓ Proliferation ↑ Apoptosis (JNK) G2/M cell cycle phase arrest–dependent p53. ↓ cdc25 and pcdc2 Promote the formation of polymerized tubulin. ↑ Caspase-3 and PARP expression ↑BCL2 phosphorylation mediated by JNK ↓ Cyclin B1-cdc2 kinase ↑ p53 expression. A549 expresses wild-type p53 CL1-5 expresses mutant p53 ↑ Polymerized tubulin ↑ Autophagy ↑ LC3-I and LC3-II	Chen et al. 2015 [70].
CL1-5	ND	In vivo NOD/SCID mice 3 mg/kg/d	Intraperitoneal injection for 28 days cells were injected via subcutaneous route into both flanks of mice and tumors were allowed to grow for 10 days.	10% DMSO	ND	Antitumoral ↓ Tumor growth ↓ Expression of Ki67 ↑ Caspase 3 expression ↑ LC3B-II	
H1299 H460 5-DMN Synthetic	H1299 and H460: 12.2 μM	In vitro 3–10 μM	72 h	0.1% DMSO	ND	↓ Proliferation ↓ Colony formation ↑ Early apoptotic ↑ Bax and caspase-3 expression ↓ Cyclin D1 ↓ CDK6 ↓ CDK4 ↓ Tumorsphere formation	Song et al. 2016 [68].

Table 8: Continued.

Cell line	IC50	Biological processes Dose	Experiment duration	Vehicle	Pathway	Results	References
A549 CL13 5-DMN Synthetic	A549 and CL13: 21.8 μM	In vitro 10, 15, and 20 μM	72 h	DMSO	ND	↓ Proliferation G1/G0 and G2/M cell cycle phase arrest ↑ Early and late apoptotic ↑ p21, p21 Cip1/Waf1, and p53 expression ↑ Caspase 3 and PARP expression	Song et al. 2017 [71]
Not used	ND	In vivo Female A/J mice. 0.05% w/w in diet.	16 weeks AIN-76A diet for 1 week. Intraperitoneal injection of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK): 100 mg/kg body weight.	Saline solution	ND	↓ Proliferation ↑ p21 ↓ Tumorigenesis ↓ Tumor volume ↓NNK tumor inducer ↓PCNA	
A549/ PTX	ND	In vitro 5-DMN: 5 μg/mL PTX: 5 μg/mL PTX + DMN	72 h	NS	ND	↓ Proliferation Synergistic effect Cellular uptake efficiency Antitumor effect	Guo et al. 2019 [73].
A549/ PTX DMN synthetic	ND	In vivo Female BALB/c nude mice. 5-DMN: 10 mg/kg	Administered intravenously: PTX/DMN: 5 mg/kg/5 mg/kg PTX: 10 mg/kg	NS	ND	↓ Tumor growth Accumulation of the drug in the tumor.	
CL1–5 5-DMN	5-DMN: 12.8 μM	In vitro 5-DMN: 1.56, 3.1, 6.3, and 12.5 μM PTX: 10 nM 10 μM 5-DMN combined with 3.1, 6.3, 12.5, or 25.0 nM PTX.	48 h	NS	ND	↓ Proliferation No cytotoxic effect in BEAS-2B. Synergistic effect ↑ Apoptosis Cell cycle arrest: sub-G1 phase (PTX+ 12.5 μM 5-DMN).	Tan et al. 2019 [72].
Synthetic CL1–5	Paclitaxel (PTX): 28.4 nM PTX+ 5-DMN: 4.1 μM ND	In vivo Nude mice 5-DMN: 3 mg/kg PTX: 10 mg/kg PTX+5-DMN	The cells were subcutaneously injected into the back of each nude mouse, and the tumors were allowed to grow for 8 days. Administered intraperitoneally daily for 24 days (days 7–31) until day 32.	10% DMSO	ND	↑ Caspase-3, caspase-8, caspase-9, and c-PARP expression. Antitumor effect. ↓ Tumor sizes	

Abbreviations: ND, not determined; NS, not specified.

(2, 24, and 48 h) cytotoxic effects observed (IC_{50}s of 23.82 and 85 μg/mL at 24 and 48 h, respectively) [60]. Incubation with NOB (40 μg/mL) for 24 h induced apoptosis, DNA fragmentation, G2/M cell cycle arrest, and the alteration of the Bax/Bcl-2 ratio, but had no effect on colony formation. In vivo studies, NOB was found to inhibit tumor size (Figure 6) [61]. It reduces the proliferation of H1299, H441, and H460 cells; increases the expression of nitric oxide synthase (NOS), cyclooxygenase 2 (COX-2), myeloid cell leukemia 1 (Mcl-1), and K-RAS; and induces apoptosis, as evidenced by the activation of caspase-3 and cleavage of PARP with increased 5-DMN activity [62–64]. In A549 cells, incubation with interleukin-1β (IL-1β) (1–10 pM) induced COX-2 expression in a dose-dependent (1–10 pM) and time-dependent (3–24 h) manner, with tangeretin having a better inhibitory effect than NOB [65–67]. NOB inhibited the hypoxia-induced EMT, migration, and invasion of H1299 cells; decreased Twist1, Snail1, and zinc finger E box–binding homeobox 1/2 (ZEB1/2) expression; and increased tumor-suppressive microRNA-200b expression (Table 7) [68, 69].

3.2.4.2. Effects of 5-DMN on Lung Cancer. Chen et al. [70] reported that 5-DMN inhibits the proliferation of CL1-5 and A549 cell in a dose-dependent manner (3.125, 6.25, 12.5, and 25 μM) after 24-h treatment, showing inhibitory effect from 6.25 μM to 12.5 μM dose, and it shows inhibitory effect on cell viability from 12 to 72 h of treatment. They also found that 5-DMN promotes programmed cell death by autophagy in a Jun N-terminal kinase (JNK)–dependent manner and promotes G2/M cell cycle arrest and tubulin polymerization [70]. This group observed no effect of 5-DMN on tumor shrinkage, but noted that in vivo evaluation was necessary [70].

Song et al. [71] reported that the dietary administration of 5-DMN reduced the size and volume of (methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)–induced tumors in mice mediated by the induction of apoptosis and cell cycle arrest (Figure 7). Tan et al. [72] observed a synergistic effect of 5-DMN and paclitaxel leading to the reduction of cell viability and induction of apoptosis with concomitant activation of caspase-3, caspase-8, and caspase-9 [72]. Guo et al. [73] further demonstrated the synergistic effect of 5-DMN, paclitaxel, and cetuximab co-loaded on lipid nanostructures, with the inhibition of tumor proliferation and size. Other research indicates that metabolites of 5-DMN are more potent than 5-DMN for the inhibition of cell growth and induction of cell cycle arrest and apoptosis (Table 8) [71].

Finally in lung cancer, NOB has been most extensively studied and has been determined to both NOB and 5DMN in apoptosis, migration, and invasion.

4. Conclusion

NOB is a polymethoxyflavone, which has been extensively studied in the fight against various types of cancer. At low doses, it shows anti-inflammatory activity by regulating the expression of cytokines and reactive oxygen species. It also acts by inducing cell cycle arrest and is a potent inhibitor of cell proliferation, promoting programmed cell death and blocking angiogenesis. Its actions are exerted with half minimal (50%) inhibitory concentration (IC_{50}) in a broad spectrum ranging from 10 μM to 1×10^{-3} M doses showing different IC_{50} even among the same type of cancer. On the other hand, some authors point out that the demethylated forms of NOB show higher biological activity. For this reason, we decided to investigate the effects of 5-DMN. As a result of the bibliographic research carried out, we found that 5-DMN has been studied in fewer types of cancer compared to NOB, and although it shows the same spectrum of biological activities, we did not find greater effectiveness in IC_{50} and its biological activities are still limited. On the other hand, we found that both flavones show differences in their biological activity depending on the source of extraction or synthetic forms. For all these reasons, there are still investigations to be carried out in order to conclude the effectiveness of the biological activity between NOB and 5-DMN.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Writing the manuscript and making figures, Gloria Gutiérrez-Venegas, and making tables and figures, Marisol Rosas-Martínez.

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