Synergistically Anti-Multiple: Flavonoid, Non-Flavonoid Polyphenols, and Bortezomib

Subjects: Oncology

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Multiple myeloma (MM) is a clonal plasma cell tumor originating from a post-mitotic lymphoid B-cell lineage. When two or more medications are combined to improve their therapeutic effects, this is referred to as drug synergism. For various diseases, including MM, appropriate drug combinations can reduce drug resistance or maximize efficacy. Bortezomib (BTZ), as a clinically used protease inhibitor, is a cornerstone of Velcade, Revlimid, and Dexamethasone (VRD). Flavonoids and non-flavonoid polyphenols are potential supportive therapies to address some of the challenges faced in BTZ treatment.

multiple myeloma bortezomib flavonoids non-flavonoid polyphenols

1. Mechanisms of Multiple Myeloma

Multiple myeloma (MM) ’s onset, progression, metastasis, and osteolytic destruction are associated with the regulation of multiple signaling pathways (**Figure 1**). Throughout the development of MM, rapid cell proliferation was mainly regulated by PI3K/Akt, Ras/Raf/MEK/Erk, JAK/STAT, Wnt/β-catenin, and RANK/RANKL/OPG signal pathways [1]. Uncontrolled cell proliferation was attributed to the inhibition of normal cell cycle regulation (e.g., FOXO1 and Cyclin D1), and the upregulation of downstream key anti-apoptotic factors (e.g., Bcl2, Bcl-xl, Mcl-1, and Caspase) [2]. Angiogenesis, mainly promoted by VEGF, provides oxygen and nutrients for tumor proliferation. Additionally, the activation of the Wnt/-catenin and RANK/RANKL/OPG signal pathways is primarily responsible for osteolytic bone disease in MM, which is brought on by an increase in osteoclast activity [1].

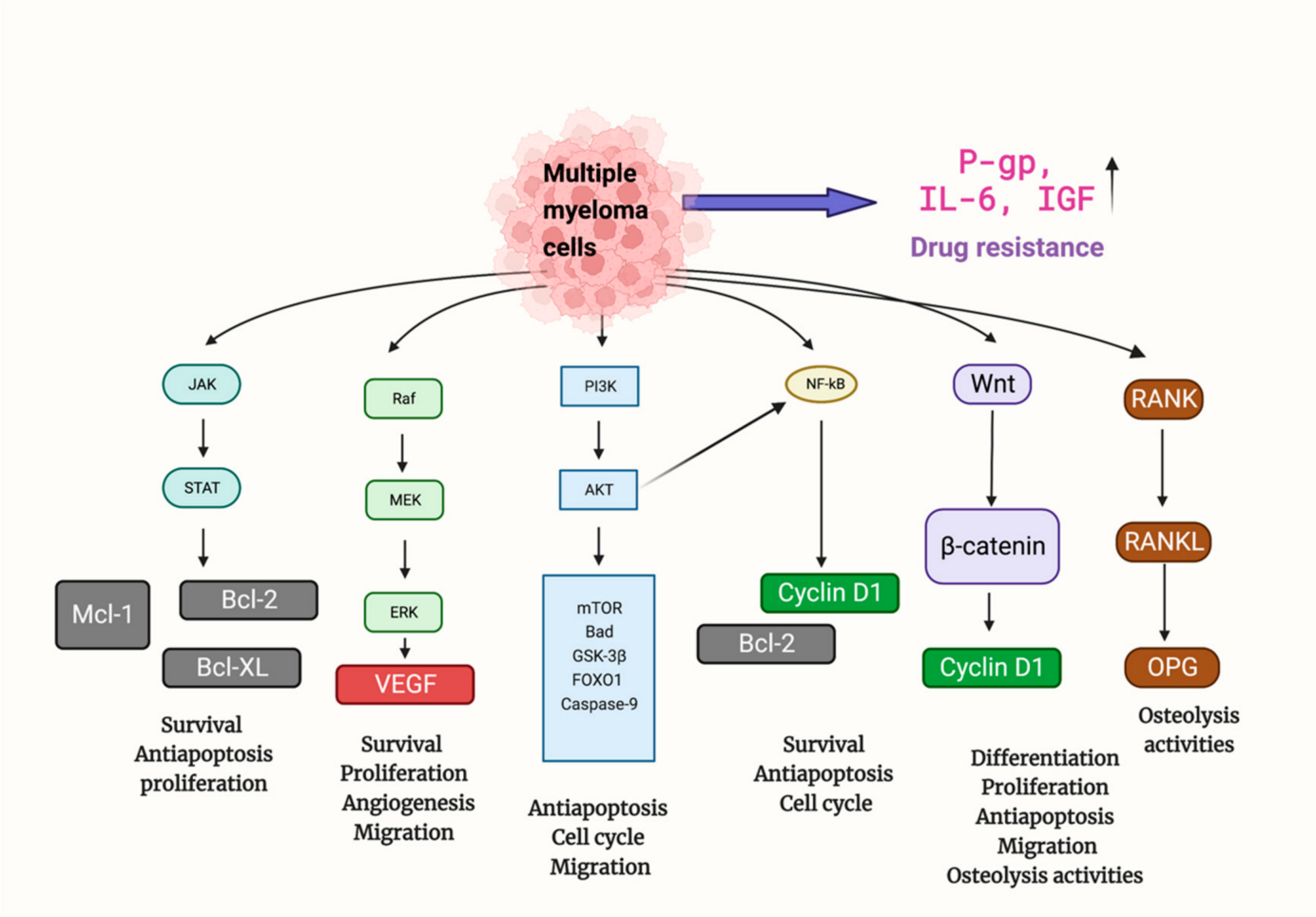

Figure 1. Multiple signaling pathways are involved in the tumor formation and progression of MM. Mechanisms promoting anti-apoptosis, proliferation, angiogenesis, migration, and drug resistance were upregulated, while mechanisms related to apoptosis and normal cell cycle regulation were suppressed. Abbreviations: Bad: BCL2 associated agonist of cell death; Bcl2: B-cell lymphoma 2; Bcl-xl: B-cell lymphoma-extra-large; FOXO1: Forkhead box protein O1; GSK-3β: Glycogen synthase kinase-3 beta; JAK/STAT: Janus kinases/signal transducer and activator of transcription proteins; Mcl-1: Myeloid cell leukemia 1; MEK/Erk: MAP kinase–ERK kinase/extracellular-signal-regulated kinases; TOR: mammalian target of rapamycin; PI3K/Akt: Phosphoinositide 3-kinases/Protein Kinase B; RANK/RANKL/OPG: Receptor activator of nuclear factor κB/Receptor activator of nuclear factor kappa-B ligand/Osteoprotegerin; Wnt: Wingless and Int-1.

Finally, MM cells can further develop drug resistance, and this may be related to mechanisms such as the upregulation of permeability-glycoprotein(P-gp) (leading to reduced drug aggregation in vivo). IL-6 and insulin-like growth factor (IGF), two cytokines produced by bone marrow mesenchymal stem cells, also encourage drug resistance in MM cells(activating specific signaling pathways that lead to drug resistance) [3].

2. Bortezomib in MM Therapy

An important class of medications for the treatment of MM is protease inhibitors. Based on targeted inhibition of the 26S proteasome, protease inhibitors are crucial in the pathogenesis and proliferation of MM [4]. Bortezomib (BTZ), the first protease inhibitor, approved for clinical use in 2003, has resulted in gains in overall survival, progression-free survival, and remission rates in patients with MM (**Figure 2**) [5]. Its main anti-cancer mechanism is the inhibition of the chymotrypsin-like site of the 20S protein hydrolysis core within the 26S proteasome, which induces cell cycle arrest and apoptosis [6]. The main signaling mechanisms of BTZ-induced apoptosis in MM cells are NF-κB blockade and JNK activation. In addition, BTZ stabilizes various tumor suppressor proteins, such as P53, inhibiting MM cell cycle progression [7]. Currently, the drug is commonly used in MM patients in first-line, relapsed, and/or refractory settings [8]. However, drug resistance in MM therapy is the main challenge currently faced when using BTZ [3].

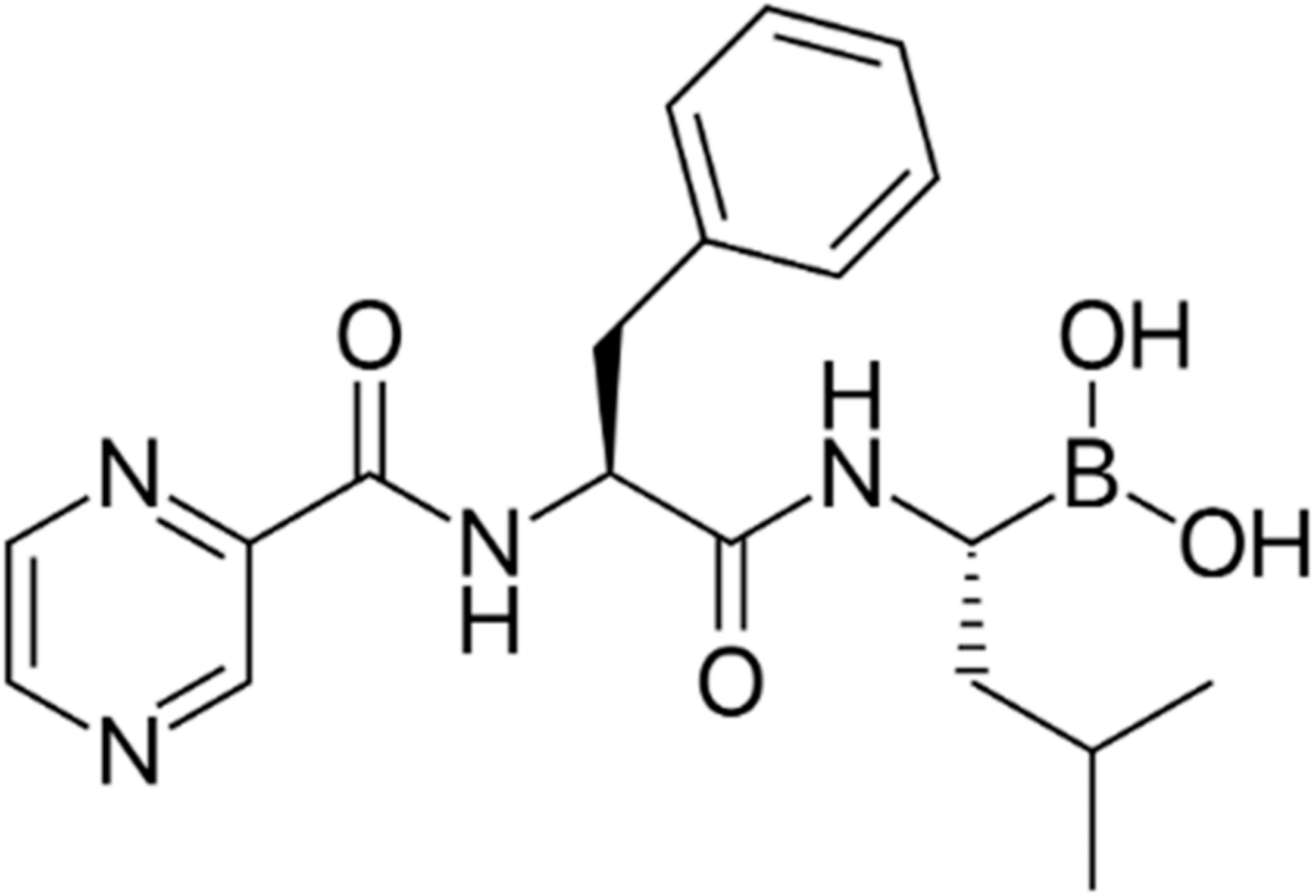

Figure 2. Chemical structure of bortezomib, an anti-MM Protease inhibitor.

3. Flavonoids and Non-Flavonoid Polyphenols in MM Therapy

Polyphenols are a class of phytochemicals divided into flavonoids and non-flavonoids. A group of compounds known as flavonoids consists of two benzene rings connected by phenolic hydroxyl groups by a central three-

carbon atom. They are secondary metabolites in fruits, vegetables, and other herbal plants. The main flavonoid subgroup includes flavones, flavonols, flavan-3-ols, anthocyanins, isoflavones, and chalcones (**Figure 3**) [9]. The main non-flavonoids contained phenolic, hydroxycinnamic, lignans, stilbenes, and tannins [10]. Various polyphenols, including flavonols(icariin, icariside II), flavan-3-ols((-)-epigallocatechin-3-gallate), flavone(scutellarein, wogonin, morin), isoflavone(formononetin, daidzin), plant extracts rich in flavonoids(punica granatum juice) and non-flavonoid polyphenols(silibinin, resveratrol, curcumin, caffeic acid) exerted anti-MM synergistic effects in combination with BTZ in vivo and/or in vitro (**Figure 4**).

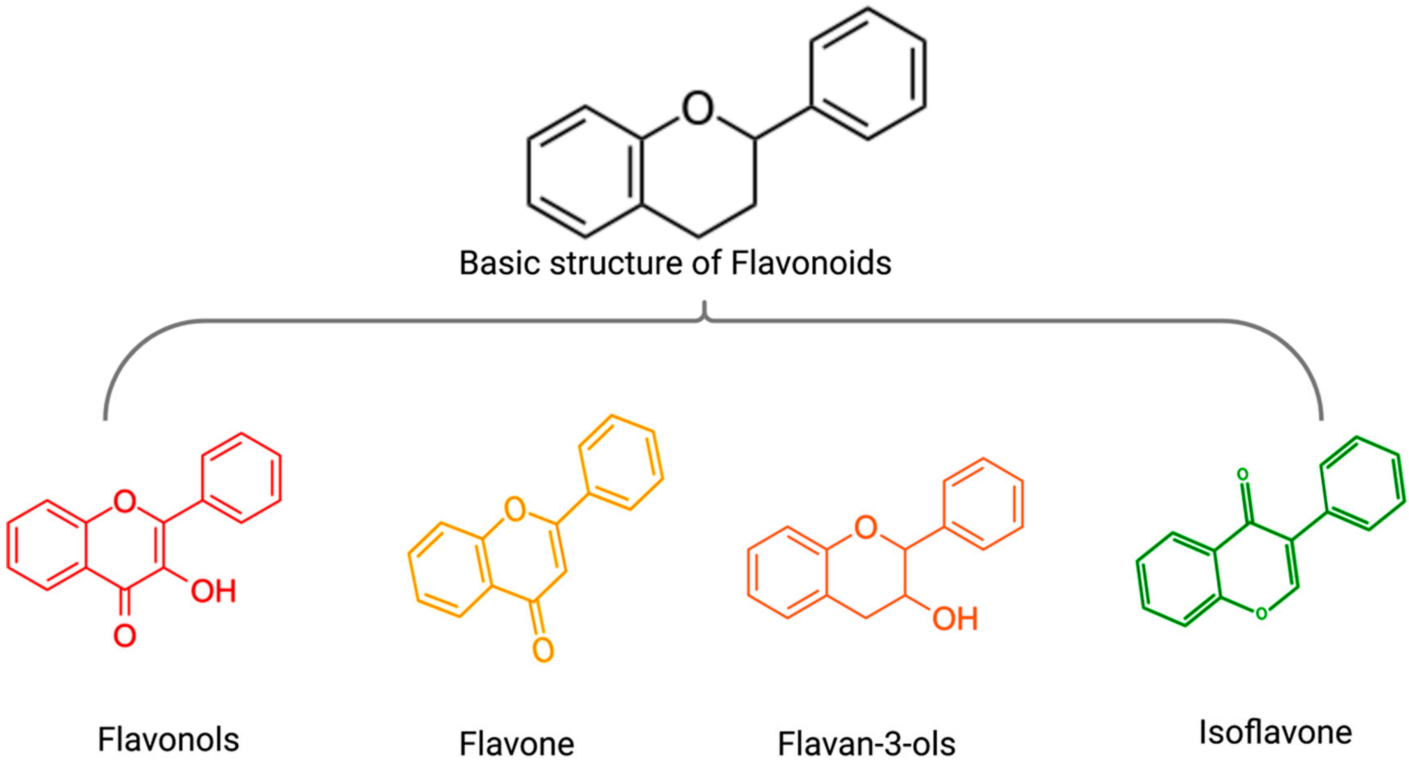

Figure 3. The basic structure of flavonoids(black) and the general structure of flavonols (red), flavone (yellow), flavan-3-ols (orange), isoflavone (green).

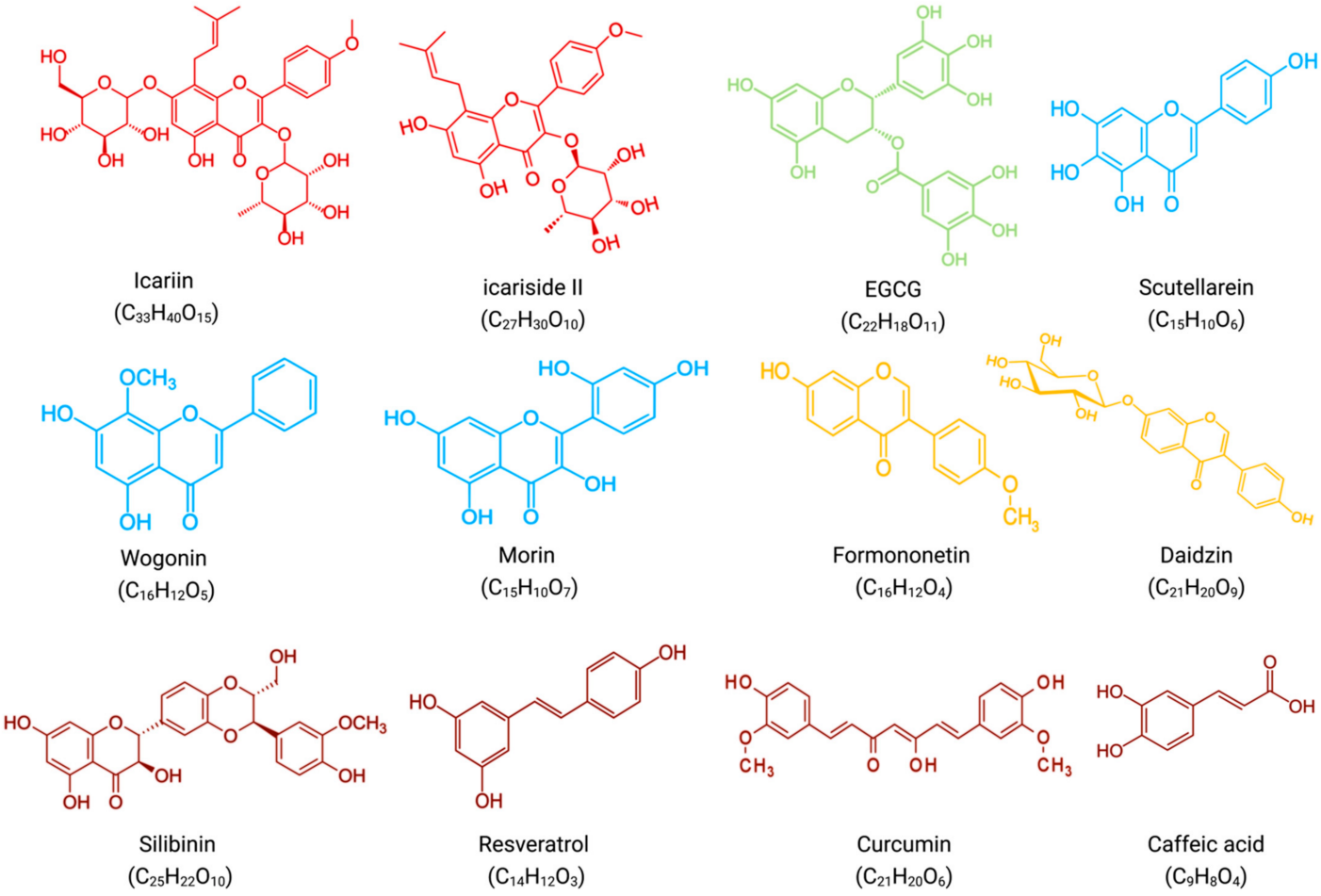

Figure 4. Molecular formulae and chemical structures of flavonoid compounds and non-flavonoid polyphenolic compounds(excluding PGJ, a plant extract rich in flavonoids). Flavonols(icariin, icariside II) have a red chemical formula. Flavan-3-ol (EGCG) is green in color. The formula of flavones (scutellarein, wogonin, morin) is blue. The chemical formula of isoflavones (formononetin, daidzin) is yellow. Non-flavonoid polyphenols (silibinin, resveratrol, curcumin, caffeic acid) have a brown chemical formula.

4. Synergistic Effects of Flavonoids and Bortezomib in Anti-MM

Nine flavonoids (icariin, icariside II, EGCG, scutellarein, wogonin, morin, formononetin, daidzin) plus a flavonoid-rich plant extract (*PGJ*) synergize with BTZ in anti-MM by regulating proliferation, apoptosis, and drug resistance-related signaling pathways (**Figure 5**).

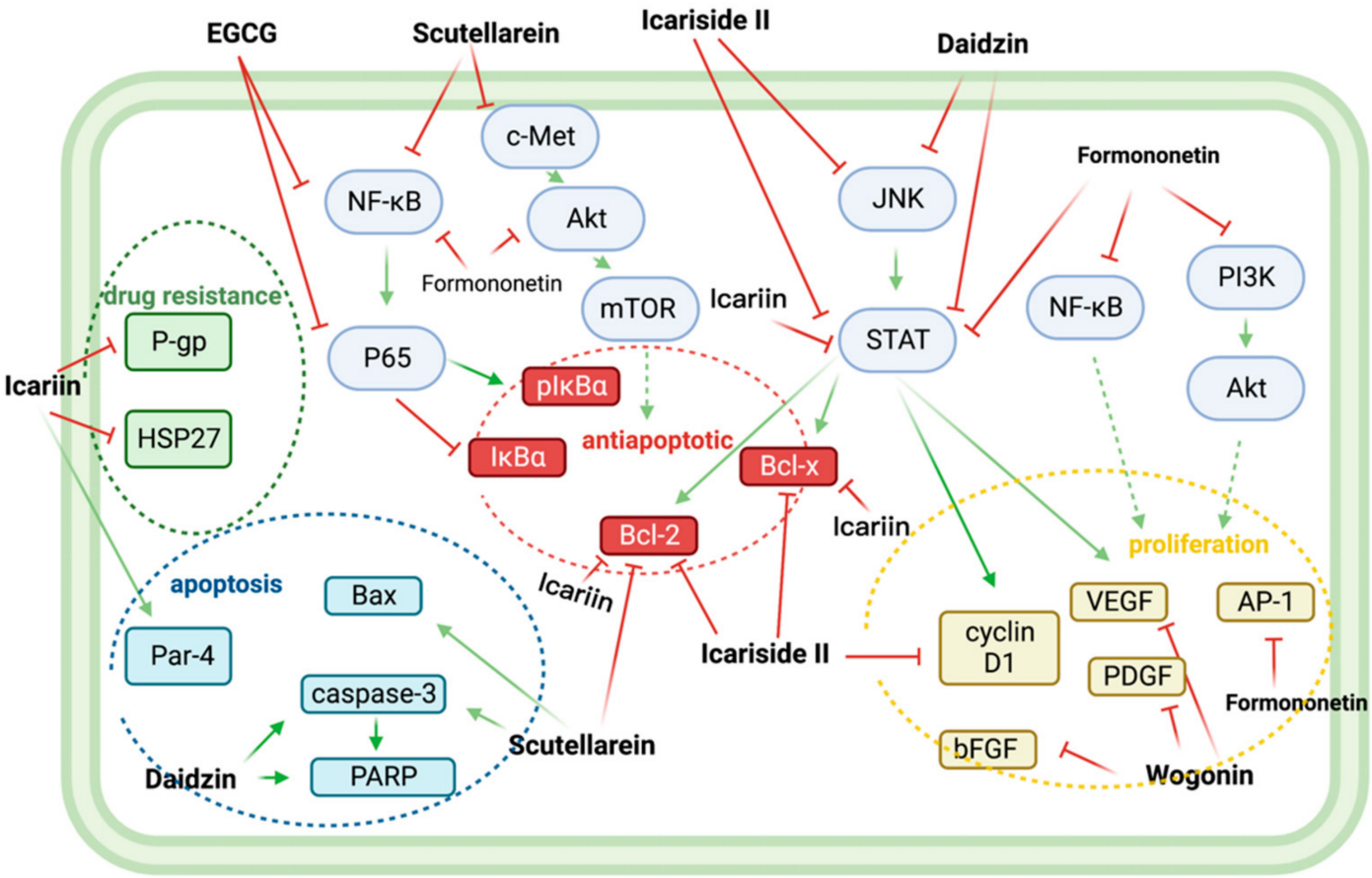

Figure 5. Nine flavonoids, icariin, icariside II, EGCG, scutellarein, wogonin, morin, formononetin, daidzin, and isoginkgetin, together exerted multiple synergistic BTZ anti-MM effects. Icariin synergistically improved the drug sensitivity of MM to BTZ by reducing the expression of drug resistance proteins HSP27 and P-gp. EGCG, scutellarein, icariin, icariside II, and formononetin down-regulated the downstream anti-apoptotic proteins Bcl2, BclXl, pIκBα by inhibiting NF-κB, c-Met/Akt/mTOR, and JAK/STAT pathways, thereby synergistically reversing anti-apoptosis. Icariside II, formononetin, and Wogonin, with BTZ synergistically, exerted antiproliferative effects by downregulating VEGF, PDGF, bFGF, cyclin D1, and AP-1. Finally, scutellarein, Daidzin, and Icariin enhanced BTZ-related apoptosis by upregulating the expression of Par-4, Bax, caspase-3, and PARP. Abbreviations: AP-1: Activator protein 1; Bax: bcl-2-like protein 4; Bcl2: B-cell lymphoma 2; Bcl-xl: B-cell lymphoma-extra-large; bFGF: basic fibroblast growth factor; c-Met: tyrosine-protein kinase Met; NF-κB/P56: Nuclear factor-kappa-B/p65; HSP27: Heat shock protein 27; JAK/STAT: Janus kinases/signal transducer and activator of transcription proteins; IκBα: nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; TOR: mammalian target of rapamycin; PARP: Poly (ADP-ribose) polymerase; Par-4: Prostate apoptosis response-4; PDGF: Platelet-derived growth factor; P-gp: P-glycoprotein; PI3K/Akt: Phosphoinositide 3-kinases/Protein Kinase B.

In KM3/BTZ-resistant cells, icariin increased the sensitivity of KM3/BTZ cells to BTZ and partially reversed the drug resistance. Its effect on changing drug resistance may be mediated by upregulating the expression of pro-apoptotic cytokine Par-4, decreasing the expression of drug resistance proteins HSP27 and P-gp [11]. Another study found that the combination of icariin and BTZ promoted apoptosis in U266 cells by blocking the JAK/STAT pathway and

lowering the expression of anti-apoptotic proteins like Bcl-2, Bcl-xl, and Survivin. This combination also delayed the cell cycle progression, as shown by the result that more U266 cells were in the G0/G1 phase [12].

While icariside II enhanced the apoptotic effect of BTZ in U266 cells, it also inhibited the JAK/STAT pathway and down-regulated the expression of STAT3 target genes Bcl-2, Bcl-xl, Survivin, cyclin D1, COX-2, and VEGF, beneficially inhibiting proliferation and promoting apoptosis [13].

EGCG, a catechin in green tea, and BTZ synergistically inhibited KM3 cell growth and induced apoptosis by inhibiting NF-κB/P65 expression, down-regulating pIκBα, and up-regulating IκBα expression [14].

In vitro, scutellarein alone time-dependently reduced cell viability and significantly induced apoptosis in MM.1R and IM-9 cells. In vivo, dual intervention with scutellarein and BTZ significantly reduced xenograft tumor burden in nude mice with no significant effect on mouse body weight. In parallel, protein expression levels of some apoptotic markers were altered, such as active caspase-3 (upregulated), Bax (upregulated), and Bcl-2 (downregulated) [15].

Wogonin and BTZ synergistically inhibited the secretion levels of pro-angiogenic factors VEGF, PDGF, and bFGF in RPMI 8226 cells. The angiogenesis-inhibitory effect of wogonin may be related to its inhibition of the c-Myc/HIF-1α signaling axis [16].

Morin potentiates BTZ-induced apoptosis in U266 cells, from 18.7% to 51.2%, by inhibiting the STAT3 pathway, leading to downregulation of STAT3-dependent gene expressions, such as XIAP, cFLIP, Mcl-1, Survivin, Bcl2, BclXl, and c-IAP-2. And morin's inhibition of STAT3 phosphorylation was more pronounced than other flavonols (galangin, kaempferol, quercetin, and myricetin) with different positions and numbers of its hydroxyl groups on the B-loop [17].

In vitro, formononetin and BTZ enhanced STAT3 inhibition and promoted the death of U266 cells [18]. Subsequently, a follow-up study by the same research group found that formononetin and BTZ exert synergistic enhancement of anti-proliferation and pro-apoptosis by blocking the activation of NF-κB, PI3K/AKT, and AP-1 [19].

Daidzin synergistically increased the apoptotic and cytotoxic effects of BTZ by inhibiting the activation of STAT3 and its upstream kinases (JAK1, JAK2, and c-Src). In addition, this drug combination increased caspase-3 activation and PARP cleavage, leading to the downregulation of the expression of various oncogenic apoptotic proteins [20].

In vitro, PGJ inhibited angiogenesis, microvascular growth outside of aortic rings, cell migration, and invasion in MM cells. In addition, After BTZ exposure, PGJ intervention increased the cytotoxic effects on U266 cells [21].

5. Synergistic Effects of Non-Flavonoid Polyphenols and Bortezomib in Anti-MM

In combination with low concentrations of BTZ, silibinin increased the cytotoxic effect of BTZ by increasing the expression of activated caspases, which promoted apoptosis [22].

In vitro, resveratrol induced apoptosis in MM144 cells by upregulating the Fas/CD95 signaling pathway and caspase-8 and caspase-10. Moreover, when used in combination, resveratrol, and BTZ highly enhanced U266 cell apoptosis [23].

Curcumin enhances the apoptotic effect of BTZ on MM cells by regulating multiple signaling pathways. The bone marrow microenvironment, in which bone marrow stromal cells (BMSCs) interact with MM, influences the survival and growth of MM cells. In vitro, curcumin inhibited the activation of JAK/STAT and MAPK pathways in U266 cells after treatment with BMSCs cell supernatant. Additionally, curcumin and BTZ co-treatment efficiently prevented IL-6-induced STAT3 and Erk phosphorylation, increased PARP cleavage, and decreased pro-caspase-3 levels. Through these mechanisms mentioned above, this combination inhibited the growth of U266 cells and promoted apoptosis [24].

In vitro, caffeic acid alone inhibits NF-κB-binding activity and IL-6 levels, which are closely linked to apoptosis and growth of tumor cells. Moreover, the combination of caffeic acid and BTZ synergistically increased cytotoxic and anti-proliferative effects on ARH-77 cells [25].

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