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REVIEW

Targeting the Autophagy–Apoptosis Axis in Osteosarcoma: Therapeutic Potential of Biocompounds: A Review

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ABSTRACT

Osteosarcoma is a prevalent form of cancer that develops in growing bones. The precise cause of this multifaceted disease remains uncertain. Yet, genetic, genomic and proteomic research has enhanced our understanding of the disruption in its molecular signaling pathways. In this review, we attempted to highlight the regulation of autophagy pathways by a range of biocompounds (chemical compounds of biological origin) in osteosarcoma. Autophagy is a degradation mechanism, maintaining cellular homeostasis under basal and stress conditions. However, the role of autophagy is variable in cancer. There is a delicate balance between autophagy and apoptosis, and disruption of this balance is one of the causes of tumor formation and development. Autophagy and apoptosis can reciprocally activate or inhibit each other. Thus, autophagy works synergistically or antagonistically with apoptosis. In osteosarcoma, different biocompounds involve in the regulation of autophagy and/or apoptosis, triggering cellular signaling pathways, convenient or inconvenient for growth, proliferation, invasion and metastasis of cancer cells in a tumor. Also, some of the biocompounds have been shown to affect the therapeutic response of osteosarcoma cells. Therefore, biocompounds are promising candidates for the regulation of autophagy and may play critical roles in the crosstalk between autophagy and apoptosis in osteosarcoma.

1 | Introduction

Osteosarcoma, an aggressive bone tumor, exhibits a metastasis rate of approximately 25%. These malignant cells tend to spread more prominently to the lungs. The standard treatment for metastatic patients involves surgical resection, but this approach enhances the survival of < 20% of patients over 5 years. While a combination of specific chemotherapy drugs, including methotrexate, doxorubicin, and cisplatin (MTX), has shown improved outcomes, around 30% of metastatic osteosarcoma cases remain resistant to this combined chemotherapy (Chou et al. 2008; Gorlick and Khanna 2010; He et al. 2014; Hengartner 2000; Longhi et al. 2006; Ritter and Bielack 2010; Sakamoto and Iwamoto 2008; Zhou et al. 2014). A significant portion of osteosarcoma patients are adolescents, indicating a potential anomaly during osteogenesis. In osteogenesis, the development of osteoblast cells from mesenchymal progenitor cells requires complex signaling pathways, and any defect in these signaling pathways leads to malignancy due to dysregulation of cell differentiation and proliferation (De Azevedo et al. 2019; Greenwood et al. 2025; Ivan et al. 2025). Consistently, various studies have demonstrated that (epi)genetic changes disrupt normal differentiation processes and lead to oncogenic transformation

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during the differentiation of mesenchymal progenitor cells into osteoblasts (Gorlick and Khanna 2010; Hengartner 2000; Ritter and Bielack 2010; Zhou et al. 2014). Hence, it is essential to comprehend the molecular mechanisms underlying osteosarcoma and devise therapeutic approaches that can modulate signaling pathways for effective treatment. Disruptions in cell growth and differentiation pathways, such as Wnt, Hedgehog, Janus kinase/signal transducers and activators of transcription (JAK/STAT), Notch and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/AKT, have been recognized as contributing factors to osteoblast development abnormalities leading to carcinogenesis. Another pathway that exhibits abnormal upregulation in osteosarcoma is nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). The suppression of Glycogen Synthase Kinase-3 Beta (GSK-3β) led to enhanced cell death in osteosarcoma, achieved by inhibiting the NF-κB pathway (Hoeflich et al. 2000). Efforts to improve the effectiveness of chemotherapy through the use of GSK-3β inhibitors and NF-κB inhibitors increased the efficacy of chemotherapeutic drugs in both in vitro and in vivo applications (Liu et al. 2012). Additionally, specific mutations in explicit tumor suppressor genes have been identified as associated with osteosarcoma. In 20%–40% of sporadic cases, aberrancy in the growth, differentiation, survival, and death of cells was reported to be connected with the Retinoblastoma protein (Rb1) inactivation (Zhou et al. 2014). In vitro and in vivo research revealed that the introduction of tumor protein p53 mutants induced abnormal growth and proliferation of cells as well as transformation into cancer (Hellwinkel et al. 2005).

Autophagy is a lysosome-dependent degradation mechanism in cells, maintaining cellular metabolism by recycling of the biomolecules under basal conditions. Under cellular stress, including nutrient limitation, oxidative stress, inflammation, etc., autophagy is activated as a protective mechanism that allows the cells to survive and overcome stress conditions. Nevertheless, autophagy interfaces multiple cellular signaling pathways that, in fact, make the role of autophagy in cancer contradictory, widely depending on the type and phase of cancer (Erbil-Bilir et al. 2019). In osteosarcoma, autophagy has dual functions as protective and lethal. During the early phases, autophagy inhibits tumorigenesis by suppressing osteosarcoma cell growth (Debnath et al. 2023; Ichimiya et al. 2020). Conversely, autophagy induces tumorigenesis in late phases by supporting the survival of tumor cells against hypoxia, nutrient limitation, etc., thereby promoting tumor cell growth, proliferation, invasion, and metastasis (Tedesco et al. 2024). On the other hand, in late phases of osteosarcoma, autophagy prevents apoptosis, a well-known cell death mechanism, causing tumor cells to develop drug resistance, which also promotes tumor cell invasion and metastasis (Ando et al. 2020; Ning et al. 2023). Consequently, autophagy regulation, as well as its interaction with apoptosis, should be well considered for effective treatment strategies in osteosarcoma. In this review, we tried to focus on diverse biocompounds (chemical compounds of biological origin) and their effect on autophagy and its interaction with apoptosis in osteosarcoma.

2 | Autophagy

In autophagy, macromolecules that could not be degraded by the proteasomal system are degraded and building blocks of the macromolecules are recycled for metabolic activities. Therefore, autophagy is a survival mechanism for the cells under basal and stress conditions. In contrast, excessive autophagy has been shown to contribute to cell death as well and therefore it is known as one of the programmed cell death mechanisms, called Type II autophagic cell death. However, it can kill the cells with a different molecular mechanism than by far the best-understood apoptotic (Apoptosis) cell death (Eberhart et al. 2014).

Apoptosis is a Type I form of programmed cell death that naturally occurs in the developmental and aging processes. It is morphologically characterized by apoptotic bodies formation, shrinkage of cells, fragmentation of DNA, and condensation of chromosomes (Elmore 2007). On the other hand, the most significant morphological change in autophagic cell death is the accumulation of double-membraned vesicles, called autophagosomes. At the molecular level, caspase activation and cytochrome-c release from mitochondria are major molecular alterations in apoptosis, while autophagic cell death is activated independently of caspases and requires the coordinated action of autophagy-related genes (ATGs). Although autophagy and apoptosis are individually well-controlled biological processes, numerous genes, proteins, and signaling pathways, such as Class III Phosphoinositide 3-kinases (PI3K), mTOR, MAPK/ERK, etc., are often involved in their complex interactions, varying with the physiological and pathological conditions in different cell types. Eventually, autophagy may induce or block apoptosis by altering the key apoptosis regulatory pathways, or two mechanisms can act in a cooperative manner to induce cell death (Kutlu et al. 2016; Liu, Liu, et al. 2024).

2.1 | Molecular Mechanism of Autophagy

Autophagy is the cellular event that engulfs and degrades cytoplasmic constituents (i.e., misfolded proteins, lipid droplets) and certain organelles (i.e., mitochondria, ribosomes, and endoplasmic reticulum) in double-membraned vesicles with the help of lysosomes. Development and differentiation strictly depend on autophagy, which is active even under basal conditions. Autophagy becomes more pronounced in response to specific stressors such as starvation, lack of growth signals, hypoxia, and exposure to toxic chemicals (Eisenberg-Lerner et al. 2009). Autophagy commences with the creation of a double-layered phagophore, also known as an isolation membrane, within the cytoplasm around the autophagic target. The membrane of the phagophore may originate from the cell membrane or organelles with membranes. The isolation membrane undergoes elongation and closure to develop into autophagosomes. When autophagosomes merge with lysosomes, they are then called autolysosomes. The molecules targeted within the autolysosomes undergo degradation within the acidic environment post-lysosomal fusion, and the resulting end products are recycled within the cell (Kutlu et al. 2016).

There are multiple protein complexes involved in the formation of the isolation membrane to initiate autophagosome formation. The initiation of isolation membrane formation is orchestrated by the unc-51-like kinase (ULK) complex, consisting of Unc-51-like autophagy-activating kinases 1 and 2 (ULK1/2), autophagy-related protein 13 (ATG13), FAK family-interacting protein of 200 kDa (FIP200), and autophagy-related protein

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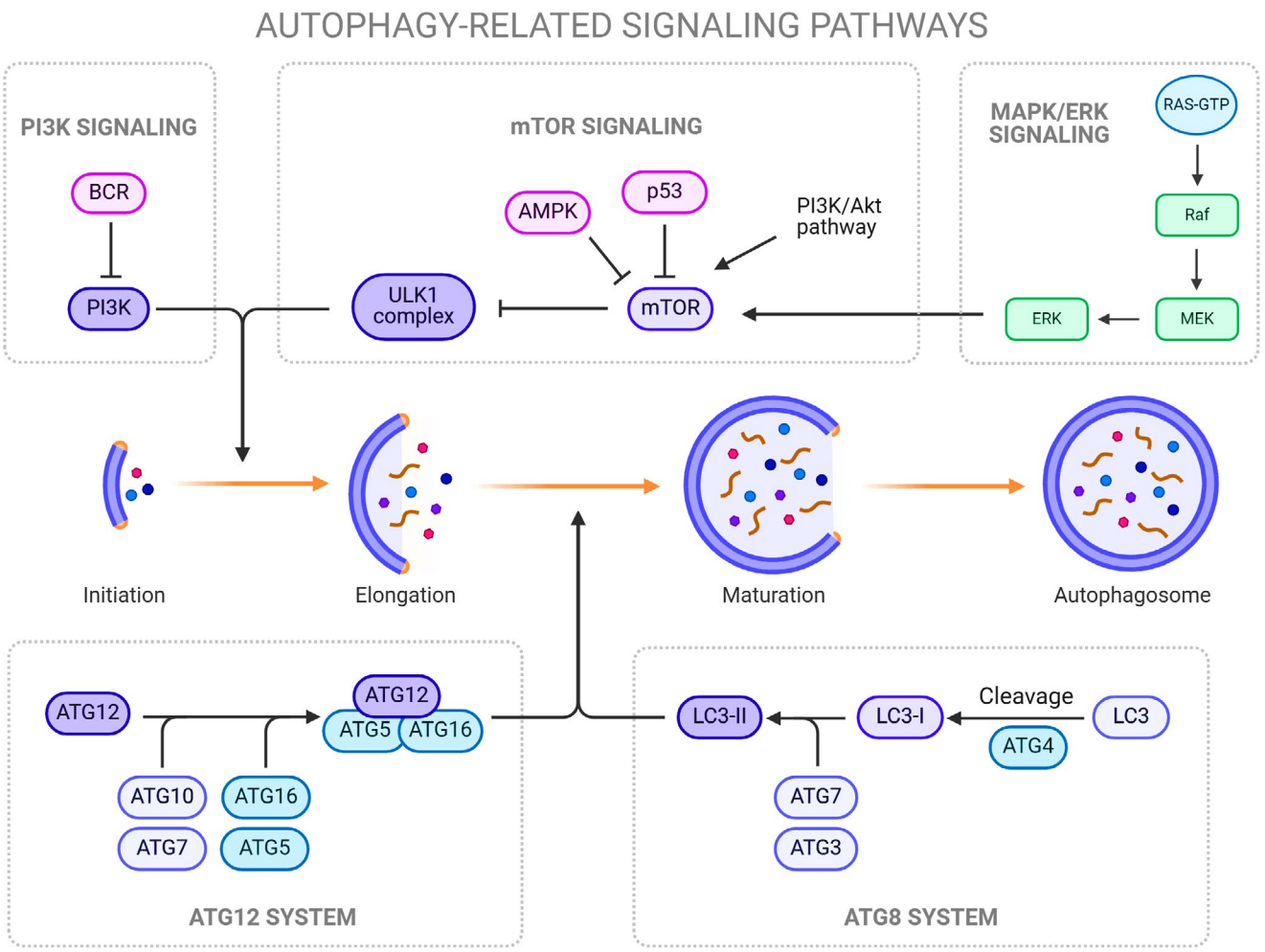


FIGURE 1 | Autophagy-related Signaling Pathways. Autophagy is initiated upon the inhibition of mTOR complex, which leads to activation of ULK1 complex. ULK1 complex and PI3K cooperate to stimulate the elongation step of autophagy. Atg12 system and Atg8 system mediate the maturation of autophagosomes. MAPK/ERK signaling is another important mechanism to suppress the proliferation of osteosarcoma cells via upregulation of autophagy *(This image is created by using BioRender).*

101 (ATG101), along with the Class III PI3K complex, comprising autophagy related 14 (ATG14L), Beclin-1, vacuolar protein-sorting 34 (Vps34), and vacuolar protein-sorting 15 (Vps15). During the elongation of the membrane, two functional conjugation systems come into play. Autophagy related protein 12 (ATG12)—Autophagy related protein 5 (ATG5)- Autophagy related protein 16 like 1 (ATG16L) complex ensures the proper lipidation of Microtubule-associated protein 1A/1B-light chain 3 (LC3), resulting in the formation of lipid-conjugated LC3-II. The lipidation of the LC3 protein, facilitated by the activity of autophagy related protein 4 (ATG4), autophagy related protein 3 (ATG3), and autophagy related protein 7 (ATG7), plays a crucial role in recruiting LC3-II to mature autophagosomes (Figure 1). Subsequently, lysosomes merge with mature autophagosomes, causing the autophagic cargo to degrade.

3 | Targeting Autophagy for Osteosarcoma Treatment

The role of autophagy in cancer is intricate. It can act as either a pro-tumorigenic or anti-tumorigenic factor, contingent on the tissue and the specific context. Autophagy and apoptosis may collaborate or counteract each other in the regulation of oncogenesis. In simpler terms, the induction of autophagy could either enhance or hinder apoptosis (Eisenberg-Lerner et al. 2009). Hence, the development of therapeutic strategies that manipulate cell death pathways to regulate oncogenesis requires careful consideration. To combat osteosarcoma, surgery, chemotherapy, or a combination of both approaches might be used. However, it is important to consider the limitations of current osteosarcoma treatment strategies such as low efficiency, high toxicity, drug resistance, and potential side effects, including fatigue, vascular injury, and demyelination. Table 1 summarizes the current treatment strategies in osteosarcoma, providing the advantages and the disadvantages of each approach.

Extensive research has proposed using biocompounds (chemical compounds from biological origin) for anti-tumor drug development because of their strong anti-tumor effectiveness and low toxicity to normal tissues or cells. Tumor cells undergo apoptosis, and multidrug resistance is reversed upon use of biocompounds. Furthermore, biocompounds can strengthen the anti-tumor effects of chemotherapy (Colobatiu et al. 2021) and make cancer cells more sensitive to radiation (Nisar et al. 2022).

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TABLE 1 | Current treatment strategies for osteosarcoma.

Drug name/ Combination regimens	Advantages	Disadvantages	References
MAP (methotrexate, doxorubicin, cisplatin)	Standard treatment regimen, long-term survival in more than 60% of patients with localized cases	Limited progress in survival rates, low efficacy in metastasis	Yu et al. (2019), Anninga et al. (2011)
Ifosfamide and etoposide	Some efficacy in relapsed/ refractory patients with unresectable cases	Increased toxicity and lack of significant contribution to survival	Goorin et al. (2002), Berrak et al. (2005)
Gemcitabine and docetaxel	Effective in some relapse cases	Low efficacy, recommended only in certain situations	Palmerini et al. (2016)
Lenvatinib	33% increase in 4-month progression-free survival in some relapsed osteosarcomas	Increased toxicity in combination with chemotherapy	Gaspar et al. (2018)
Regorafenib	Improved median progression-free survival in relapsed metastatic osteosarcoma	Low response rate and high toxicity rate in combination regimens	Davis et al. (2019)
Mifamurtide (muramyl tripeptide)	Promising as immunotherapy in metastasis, improved survival in localized cases	Approved by the EMA (European Medicines Agency) but not the FDA (U.S. Food and Drug Administration)	Meyers et al. (2008), Anderson et al. (2014)
Dinutuximab	Targeted therapy for osteosarcomas with high GD2 expression	Not yet proven efficacy in clinical studies	Hingorani et al. (2022)

4 | Biocompounds Mediated Autophagy in Osteosarcoma

Compounds originating from plants, animals and microorganisms, referred to as biocompounds, have become a valuable resource for cancer therapy. This is attributed to their ability to influence various molecular pathways, including the differentiation, proliferation, survival, and death of cells. This review aims to summarize the literature on how different biocompounds influence autophagy regulation in osteosarcoma. The articles that are summarized in this review illustrate the modulation of autophagy by different biocompounds (as shown in Figure 2 and Table 2).

4.1 | Biocompounds Acting on Multiple Autophagy-Related Signaling Pathways

4.1.1 | PI3K/AKT/ mTOR—ROS/JNK—MAPK Pathways

4.1.1.1 | **Capsaicin (CAP).** Capsaicin (CAP), extracted from red pepper, is another biocompound that has potential anti-tumorigenic effects. CAP was found to increase autophagy levels in human osteosarcoma cells. CAP-induced cell death was diminished upon co-administration with pan-caspase inhibitor Z-VAD-FMK, suggesting the contribution of apoptotic pathway in this context. Additionally, CAP therapy increased ERK phosphorylation, which triggers a caspase-independent pathway for cell death (Chien et al. 2013). In another study, combined use of capsaicin with cisplatin (DDP) induced autophagy, cell cycle arrest as well as apoptosis in human osteosarcoma cells. The overall effect of stimulation of apoptosis, cell cycle arrest, and autophagy restricted tumor growth. In addition, autophagy was found to be triggered through ROS/JNK and pAKT/mTOR pathway in osteosarcoma cells upon combined treatment when used in combination with cisplatin (Wang et al. 2018).

4.1.1.2 | **Honokiol (HNK).** Honokiol (HNK), a key component found in Magnolia, exhibits antiviral, antibacterial, and anti-inflammatory properties. Moreover, it can hinder tumor growth by promoting apoptosis and autophagy. When osteosarcoma cells were treated with HNK, p53 and BCL2 associated X, apoptosis regulator (Bax) protein were upregulated along with a downregulation in cyclin D1 protein levels. In addition, caspase-3 activation was triggered depending on the concentration of HNK. The LC3-II protein level was also elevated, while p-Akt, PI3K, and p-mTOR protein levels were reduced. Taken together, honokiol triggered autophagy by suppressing the PI3K/AKT/mTOR pathway (Li et al. 2018). Another report suggested that HNK might prevent proliferation of human osteosarcoma cells via stimulating apoptosis by suppressing the miR-21/Phosphatase and TENsin homolog deleted on chromosome 10 (PTEN)/PI3K/AKT signaling pathway (Yang et al. 2018). Also, HNK exhibited significant anti-metastatic activity and induced rapid cell death in vitro, which was independent of necrosis, apoptosis, or autophagy (Steinmann et al. 2012). In

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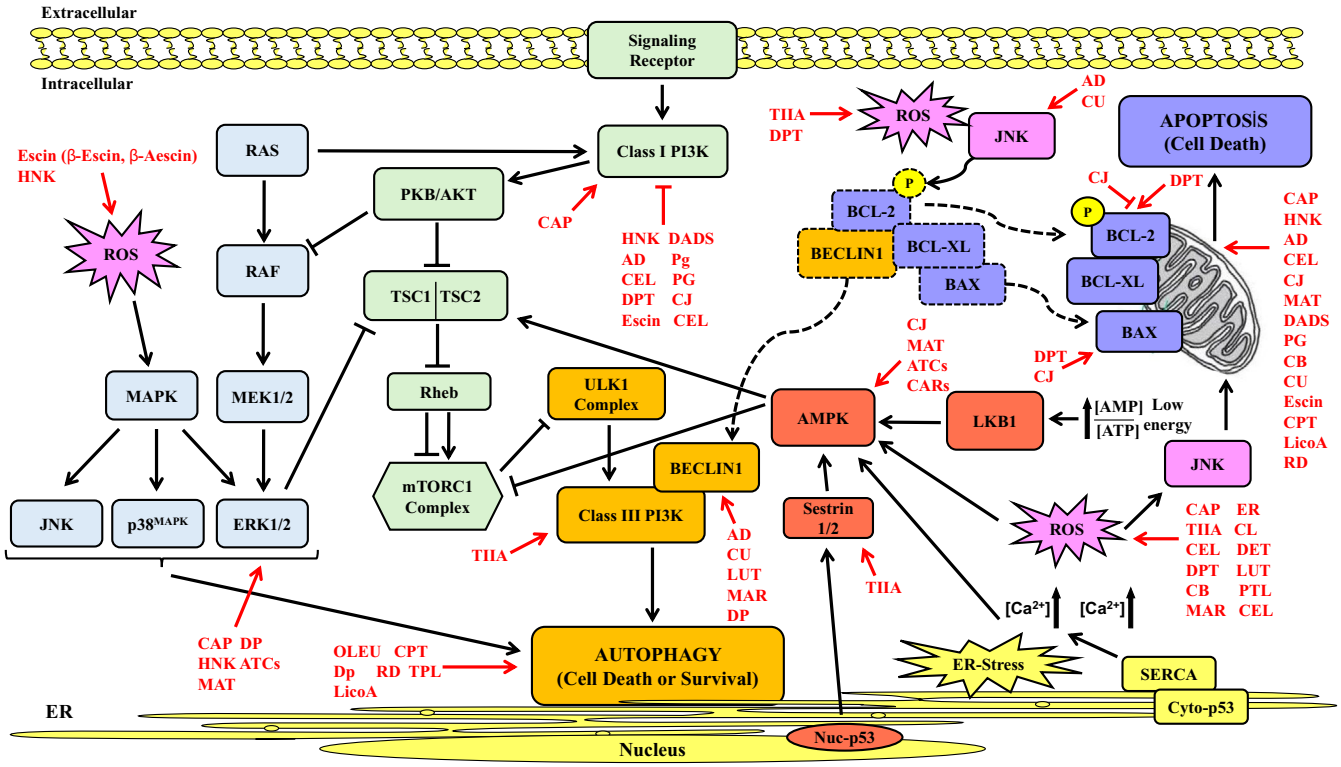


FIGURE 2 | Anti-cancer biocompounds acting through autophagy-related signaling pathways and apoptosis in osteosarcoma. Different colors represent major signaling pathways: PI3K/AKT/mTOR, green; MAPK, blue; ROS/JNK, pink; AMPK, red; Apoptosis, purple; Autophagy, orange. Abbreviations of the biocompounds: AD, Andrographolide; ATCs, Anthocyanins; CAP, Capsaicin; CARs, Carotenoids; CB, Cinobufagin; CEL, Celastrol; CJ, Chamaejasmine; CL, Carnosol; CPT, Camptothecin; CU, Curcumin; DADS, Diallyldisulfide; DET, Deoxyelephantopin; Dp, Delphinidin; DP, Dendropanoxide; DPT, Deoxypodophyllotoxin; ER, Erianin; Escin, β-Escin, β-Aescin; HNK, Honokiol; LicoA, Licochalcone A; LUT, luteolin; MAR, Marrubenol; MAT, Matrine; OLEU, Oleuropein; Pg, Pelargonidin; PG, Physakengose G; PTL, Parthenolide; RD, Riccardin; TIIA, Tanshinone IIA; TPL, Triptolide.

another study, ROS-mediated apoptosis and autophagy were stimulated upon HNK treatment in osteosarcoma cells. Moreover, ERK activation via ROS production partly participated in HNK-mediated cell death and upon HNK treatment, Atg5 and Atg7 protein levels were elevated. The authors concluded that HNK-induced autophagy was dependent on elevated Atg7 levels (Huang et al. 2018). Zhang et al. employed HNK-loaded liposomes altered with hyaluronic acid-phospholipid conjugates (HA-DOPE) for the treatment of osteosarcoma. Their results indicated that animals treated with HNK-loaded liposomes modified with HA-DOPE had smaller tumors without organ toxicity (Zhang et al. 2022).

4.1.2 | PI3K/AKT/mTOR—ROS/JNK—AMPK Pathways

4.1.2.1 | Tanshinone IIA (TIIA). Derived from a traditional Chinese herb *Salvia miltiorrhiza*, Tanshinone IIA (TIIA) has been found to alter both autophagy and apoptosis in several cancer cell types (Sohn and Park 2017). TIIA exerts its effect on autophagy via altering the levels of autophagy-related proteins. It inhibited the mTOR pathway in leukemia (Yun et al. 2014), upregulated Beclin-1, Atg3, LC3-II, Atg5, Atg7, and Atg12 in lung cancer cells (Sohn and Park 2017; Gao et al. 2015). In osteosarcoma cells, Beclin-1-mediated autophagy was upregulated due to high levels of ROS upon TIIA treatment (Ma, Zhang, Huang, Guo, and Hu 2016). A low dose and a short period of TIIA treatment caused induction of autophagy as a damage repair system. On the other hand, a higher dose and a longer period of TIIA treatment triggered autophagic cell death as well as apoptosis. This cytotoxic effect could be potentiated with ROS agonists (Ma, Zhang, Huang, Guo, and Hu 2016).

In another study, Sestrin 2 (SESN2) promoter recruitment by TIIA was demonstrated to activate SESN2 and AMPK-α, which in turn prevented the growth of osteosarcoma by upregulating autophagy via the hematopoietic progenitor kinase-germinal cell kinase-like kinase/stress-activated protein kinase/c-Jun N-terminal kinase (HGK/SAPK/JNK-Jun) signaling pathway (Yen et al. 2018). Treatment with TIIA considerably reduced the growth of human osteosarcoma cells both in vitro and in vivo. The viability of osteosarcoma cells was reduced by the use of TIIA via regulation of the Akt and class I PI3K pathways. The use of TIIA induced autophagy via a considerable increase in class III PI3K protein expression level. Besides, anchorage-dependent osteosarcoma cell growth was suppressed and the rate of tumor growth in NOD/SCID mice was attenuated upon TIIA treatment. Via HGK/JNK1, TIIA induced Jun and enhanced Jun recruitment to the activator protein-1 (AP-1) gene's SESN2 promoter-binding site. According to their research, TanIIA may have therapeutic potential (Ma, Zhang, Huang, Guo, and Hu 2016). According to a different study, TIIA treatment increased cell cycle arrest and apoptosis and inhibited the proliferation of U2-OS cells while having no effect on MG-63

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TABLE 2 | Biocompounds targeting different pathways that regulate autophagy in osteosarcoma.

Biocompounds	Targeting pathways	Effects	Ref.
Andrographolide (AD)	PI3K/AKT/mTOR ROS/JNK	Class I PI3K is inhibited ROS/JNK phosphorylation is induced	Liu, Zhang, et al. (2017), Huang et al. (2022)
Anthocyanins (ATCs)	AMPK	Intracellular ATP level is reduced ERK1/2 phosphorylation is upregulated	Shin et al. (2009), Reddivari et al. (2007), Lee et al. (2009), Longo et al. (2008), Choe et al. (2012)
Camptothecins (CPT)	Not identified	LC3-II protein level is increased The level of p62 protein is decreased	Grohar and Helman (2013), Hollomon et al. (2013), Zhao et al. (2020)
Capsaicin (CAP)	PI3K/AKT/mTOR ROS/JNK—MAPK	p-AKT, p-mTOR inhibited ROS level is increased ERK phosphorylation is stimulated	Chien et al. (2013), Wang et al. (2018)
Carnosol (CL)	ROS/JNK	ROS level is increased	Lo et al. (2017), Karadas et al. (2019)
CarotenoidS (CARs)	AMPK	Intracellular ATP level is downregulated AMPK pathway is upregulated	Russo et al. (2017, 2022)
Celastrol (CEL)	PI3K/AKT/mTOR ROS/JNK	PI3K/AKT/mTOR pathway is inhibited ROS production is increased	Li et al. (2015), Chen, Ou, et al. (2018), Cheng et al. (2023)
Chamaejasmine (CJ)	PI3K/AKT/mTOR AMPK	PI3K/AKT/mTOR pathway is inhibited AMPK pathway was induced	Yang et al. (2019), Zhang et al. (2013)
Cinobufagin (CB)	ROS/JNK	ROS production is increased	Ma, Zhang, Huang, Li, and Hu (2016), Geng et al. (2024)
Curcumin (CU)	ROS/JNK	ROS/JNK pathway is activated Beclin-1 protein level is increased	Chen (2015), Kanai (2014), Terlikowska et al. (2014), Aoki et al. (2007), Dhule et al. (2012), Zhang et al. (2017), Ge et al. (2022)
Delphinidin (Dp)	Not identified	LC3-II protein level is increased p62 protein level is decreased	Lee et al. (2018), Kang et al. (2018)
Dendropanoxide (DP)	MAPK	MAPK-ERK1/2 is activated Beclin-1 protein level is increased	Lee et al. (2013), Kim (2023)
Deoxyelephantopin (DET)	ROS/JNK	ROS production is increased	Zou et al. (2017), Jayaraman and Veeraraghavan (2025)
Deoxypodophyllotoxin (DPT)	PI3K/AKT/mTOR ROS/JNK	PI3K/AKT/mTOR pathway was inhibited ROS production is increased	Kim et al. (2017), Tang et al. (2025)
Diallyldisulfide (DADS)	PI3K/AKT/mTOR	PI3K/Akt/mTOR pathway is inhibited	Yue et al. (2019), Liu, Wang, et al. (2024)

(Continues)

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TABLE 2 | (Continued)

Biocompounds	Targeting pathways	Effects	Ref.
Escin (β-Escin, β-Aescin)	PI3K/AKT/mTOR ROS/JNK	PI3K/AKT/mTOR pathway is inhibited ROS production is stimulated	Zhu et al. (2017, 2018)
Erianin (ER)	ROS/JNK	ROS/JNK pathway is activated	Wang et al. (2016), Chen et al. (2020)
Honokiol (HNK)	PI3K/AKT/mTOR ROS/JNK MAPK	PI3K/AKT/mTOR pathway is inhibited ROS production is induced MAPK-ERK1/2 is activated	Li et al. (2018), Yang et al. (2018), Steinmann et al. (2012) Huang et al. (2018), Zhang et al. (2022)
Licochalcone A (LicoA)	Not identified	Autophagy is activated	Shen et al. (2019), Rossi et al. (2024)
Luteolin (LUT)	ROS/JNK	ROS production is istimulated Beclin-1 protein level is increased.	Ashrafizadeh et al. (2020), Zhang et al. (2015), Wang et al. (2015)
Marrubenol (MAR)	ROS/JNK	ROS level is elevated Beclin-1 protein level is increased	Zhang et al. (2018), Paunovic et al. (2016)
Matrine (MAT)	MAPK AMPK	MAPK-ERK1/2 is activated AMPK pathway is stimulated	Liu et al. (2006), Zhang (2010), Li et al. (2013), Wang, Gao, et al. (2013), Luo et al. (2007), Yixiang et al. (2010), Yu et al. (2009), Zhang et al. (2007), Liang et al. (2012), Ma et al. (2021), Ma, Huang, Guo, and Hu (2016)
Oleuropein (OLEU)	Not identified	Autophagy is activated	Ahmad Farooqi et al. (2017), Gorzynik-Debicka et al. (2018), Zheng et al. (2022), Przychodzen et al. (2019), Morana et al. (2016), Gioti et al. (2021)
Parthenolide (PTL)	ROS/JNK	ROS production is upregulated	Hwang et al. (2006), Yang et al. (2016), Zuch et al. (2012), Sun et al. (2010)
Pelargonidin (Pg)	PI3K/AKT/mTOR	PI3K/AKT/mTOR pathway is inhibited	Giampieri et al. (2015), Lee and Bae (2017), Kamenickova et al. (2013), Chen, Wang, et al. (2018), Tian et al. (2022), Li et al. (2022), Yuan et al. (2023), Shi et al. (2025), Zeng et al. (2023)
Physakengose G (PG)	PI3K/AKT/mTOR	PI3K/AKT/mTOR pathway is inhibited	Lin et al. (2018), Ji et al. (2023)
Riccardin D (RD)	Not identified	Autophagy is activated	Wang, Ji, et al. (2013), Li, Sun, et al. (2018)
Tanshinone II A (TIIA)	PI3K/AKT/mTOR ROS/JNK AMPK	Class III PI3K is activated ROS production is elevated SESN2/AMPK is activated	Sohn and Park (2017), Yun et al. (2014), Gao et al. (2015), Ma, Zhang, Huang, Guo, and Hu (2016), Yen et al. (2018), Hu et al. (2021)
Triptolide (TPL)	Not identified	Autophagy is activated LC3-II protein level is increased Beclin-1 protein level is increased	Kim et al. (2014), Zhou et al. (2012), Krosch et al. (2013), Mujumdar et al. (2010), Hsu et al. (2013), Zhao et al. (2016), Li, Lu, et al. (2018)

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cells lacking p53. Therefore, the authors concluded that TIIA might inhibit proliferation of osteosarcoma cells through non-dependent Src or P53 signaling pathways (Hu et al. 2021).

4.1.3 | PI3K/AKT/mTOR—ROS/JNK Pathways

4.1.3.1 | Andrographolide (AD). Andrographolide (AD), derived from *Andrographis paniculata*, stimulated cell death and prevented invasion and metastasis of human osteosarcoma cells. AD treatment caused a considerable decrease in cell viability in Saos-2, U-2 OS, and MG-63 cell lines as evidenced by MTT assay and clonogenic assay. To determine the cause of this reduction in cell viability, MG-63 and U-2 OS cells were treated with either the autophagy inhibitor 3-MA (3-methyladenine) or the caspase inhibitor Z-VAD-FMK (carbo benzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone). While the use of 3-MA led to a substantial rise in cell viability, the use of Z-VAD-FMK could not boost cell viability. AD treatment did not cause nuclear condensation or caspase 3 activation. Furthermore, the ratio of apoptotic cells was lower in cells treated with AD than in the positive control as evidenced by annexin-V/PI staining. These findings demonstrated that apoptosis was not triggered by AD treatment. To determine whether AD treatment upregulated autophagy, the levels of several autophagic proteins were checked in the study. The levels of ATG5 and Beclin-1 were found to be elevated after AD treatment. The LC3 lipidation was also boosted by AD treatment. Furthermore, the quantity of autophagosomes and autolysosomes was elevated in AD-treated osteosarcoma cell lines. Consistently, Beclin-1 knockdown reversed the effect of AD treatment on cell viability. These results suggested that autophagy was upregulated by AD treatment in osteosarcoma cell lines. To reveal the molecular mechanisms of AD-induced autophagy, the proteins involved in the phosphoinositide 3 kinase (PI3K)/Akt/mammalian (or mechanistic) target of rapamycin (mTOR) (PI3K/Akt/mTOR) pathway were examined. Upon AD treatment, phosphorylation of mTOR and Akt was hindered and while class III PI3K protein level was upregulated, class I PI3K protein level was downregulated. These results suggested that the PI3K/Akt/mTOR pathway was inactivated upon AD treatment. Mitogen-activated protein kinase (MAPK) pathway was also checked in the same study. In AD-treated osteosarcoma cells, phosphorylation of extracellular signal-regulated kinase (ERK) and Jun N-terminal kinase (JNK) was induced whereas phosphorylation of p38 was hindered. Moreover, the combined use of JNK inhibitor and AD diminished the levels of ATG5, LC3-II, and Beclin-1 implying an impairment in autophagy. On the other hand, treatment with AD and ERK inhibitor did not change the levels of these autophagic proteins. These results indicated that activation of the JNK pathway was also responsible for the autophagic induction by AD treatment in osteosarcoma cells. Another effect of AD was impairment in cell migration and metastasis, and this effect was reversed in Beclin-1 siRNA. Taken together, the induction of autophagy upon andrographolide treatment occurred through activation of JNK pathway and inactivation of PI3K/Akt/mTOR signaling (Liu, Zhang, et al. 2017).

In another study performed with various osteosarcoma cell lines, treatment with AD suppressed the proliferation, invasion, and migration of cells. In addition, apoptosis was stimulated in these cell lines. In vivo studies indicated that the growth and metastasis of osteosarcoma cells were hindered upon AD treatment. Wnt/β-catenin, PI3K/AKT, and NF-κB were all downregulated after using andrographolide. Additionally, osteosarcoma cell lines were unable to proliferate, migrate, or invade when AD and inhibitors of these three pathways were combined (Huang et al. 2022).

4.1.3.2 | Celastrol (CEL). Celastrol (CEL) is extracted from *Tripterygium wilfordii* root. CEL was found to be less cytotoxic against human fibroblasts, despite suppressing the growth of various human osteosarcoma cells (Li et al. 2015). Upon CEL treatment, HOS and MG-63 osteosarcoma cell lines were arrested in G2/M phase and the expressions of cyclin B1, cyclin-dependent kinase inhibitor 1 (p21), checkpoint kinase 2 (Chk2), phospho-cell division control 2 (Cdc2), phospho-Chk2 and phospho-cell division cycle 25C (Cdc25C) were elevated whereas the levels of Cdc2 and Cdc25C were attenuated. Flow cytometry, Hoechst33258 staining and transmission electron microscopy (TEM) analysis indicated the activation of the intrinsic (mitochondrial) pathway of apoptosis with CEL treatment in HOS and MG-63 cell lines. According to western blot analysis, CEL treatment resulted in a dose- and time-dependent accumulation of cleaved forms of caspase-3, -8, -9, and cleaved PARP. Caspase activity test gave coherent results with western blot analysis, demonstrating the involvement of the intrinsic pathway of apoptosis in CEL-induced apoptosis. Although the protein level of death receptor 5 (DR5) was increased, Fas, Fas ligand (FasL), death receptor 4 (DR4) and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) protein levels were barely modulated by CEL treatment. Collectively, the data indicate CEL activates caspase-dependent apoptosis. The use of Z-VAD-FMK led to a decrease in CEL-induced cell death, which demonstrated the involvement of other types of cell death. Moreover, apoptosis-inducing factor (AIF) and endonuclease G (EndoG) were found to be minimally released following CEL treatment. Therefore, the researchers checked the autophagy levels and found out that autophagy levels were increased after CEL treatment as assessed by LC3-II accumulation and microscopy pictures of autophagosomes. To identify the molecular pathway involved in CEL-induced cell death, ROS/JNK pathway was checked. CEL was found to trigger JNK phosphorylation and induce ROS generation. The production of ROS was found to initiate CEL-triggered apoptosis and cell cycle arrest. Furthermore, the use of apoptosis and autophagy blockers demonstrated that inhibition of apoptosis triggered autophagy whereas inhibition of autophagy resulted in a decline in apoptosis levels. In addition, CEL had a similar cell-death promoting effect in primary cell lines and in vivo. CEL functioned as an anti-tumorigenic agent in the mouse xenograft model. Therefore, the authors concluded that CEL might be a promising antitumor biocompound for osteosarcoma (Li et al. 2015).

In another study by Chen et al., endoplasmic reticulum (ER) was swollen after CEL treatment and the levels of ER-stress related proteins such as endoplasmic reticulum oxidoreductin-1α (Ero1-Lα), Inositol-requiring transmembrane kinase/endoribonuclease 1α (IRE1α), protein disulfide isomerase (PDI), binding immunoglobulin protein (Bip), Calnexin and p-Protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) as well as

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apoptosis related proteins cleaved caspase-3, cleaved caspase-12 and C/EBP homogous protein (CHOP) were elevated whereas PERK protein level was diminished upon CEL treatment. Moreover, protein levels of Bcl-2 associated X-protein (Bax) and cytochrome-c, indicators of intrinsic pathway of apoptosis were found to be upregulated whereas B-cell leukemia/lymphoma 2 (Bcl-2) protein levels were downregulated after CEL treatment. Autophagy and apoptosis were elevated after pretreatment with the ER-stress inhibitor tauroursodeoxycholic acid (TUDCA). Pre-treatment with a PERK inhibitor, GSK2656157, caused apoptosis levels to rise and autophagy levels to fall (Chen, Ou, et al. 2018).

In a recent study, through the production of ROS, a magnesium alloy loaded with CEL suppressed the growth and migration of osteosarcoma cells. The potential of the mitochondrial membrane was reduced, and apoptosis was triggered by the production of ROS. On one hand, upregulation of autophagy inhibited apoptosis at the beginning. On the other hand, the PI3K/Akt/mTOR pathway was blocked, and both autophagy and apoptosis were upregulated in the prolonged culture of osteosarcoma cells, indicating that CEL-loaded Mg alloy might cure osteosarcoma (Cheng et al. 2023).

4.1.3.3 | Deoxypodophyllotoxin (DPT). Deoxypodophyllotoxin (DPT), isolated from *Anthriscus sylvestris*, is a biocompound with possible antiproliferative and antitumorigenic functions on several types of cancer. DPT inhibited the PI3K/AKT/mTOR pathway and stimulated autophagy in human osteosarcoma cells. It also enhances the formation of ROS. On the other hand, inhibition of ROS formation led to an elevation in cell number, and inhibition of autophagy by 3-metyhladenine (3-MA) enhanced apoptosis. Considering these results, DPT treatment favored pro-oncogenic autophagy over anti-oncogenic apoptosis (Kim et al. 2017).

DPT decreased the survival of osteosarcoma cell viability and suppressed tumor xenograft growth. It stimulated mitochondrial dysfunction via increasing BCL2-associated X protein (Bax) and B-cell CLL/lymphoma 2 (Bcl-2) levels. The cytotoxic effect of DPT on cancer cells was weakened by overexpression of Grp78, as evidenced by suppression of caspase signaling. The loss of Grp78 expression resulted in downregulation of autophagy-related genes, whereas DPT treatment upregulated autophagy via the PI3K/AKT/mTOR pathway, and this autophagic induction led to an inhibition in the apoptotic process (Tang et al. 2025).

4.1.3.4 | Escin (β-Escin, β-Aescin). Escin (β-Escin, β-Aescin) is extracted from horse chestnut, and it has apoptosis- and autophagy-inducing effects on four different human osteosarcoma cell lines. Treatment with varying concentrations of escin resulted in anti-proliferative effects on these cell lines. The elevated levels of cleaved caspases and reduced levels of anti-apoptotic proteins indicated the induction of apoptosis upon escin treatment. In addition, microscopy results further demonstrate the stimulation of apoptosis after treatment with escin. Elevation in LC3, ATG5, ATG12, and Beclin-1 protein levels was associated with increased autophagosome formation, and the activation of autophagy was found to occur through the activation of the ROS/p38/MAPK pathway. Exposure to escin after pre-treatment with the p38 MAPK inhibitor SB203580, the ROS inhibitor NAC, the apoptosis inhibitor Z-VAD-FMK, or the autophagy inhibitor 3-MA demonstrated that escin-stimulated ROS initiates apoptosis and autophagy through the ROS/p38 MAPK pathway (Zhu et al. 2017).

The therapeutic effects of escin have also been reported in other types of cancer. In a recent study, co-treatment of lung cancer cells with escin and sorafenib was found to be more effective than escin alone. In this study, ATG5 was knocked down to suppress autophagy, which led to cell-cycle arrest at the G0/G1 phase and induction of late apoptosis. In vivo experiments further demonstrated that tumor load was decreased and serum biochemical parameters were improved. The authors concluded that the combined use of escin and sorafenib enhanced the effectiveness of sorafenib, and that this effect might be mediated through autophagy-dependent apoptosis (Hussain et al. 2023). The same group knocked down Atg5 to suppress autophagy to understand the mechanism underlying the synergistic effect of escin and sorafenib in lung cancer. Knocking down Atg5 gave similar results to the combined use of escin and sorafenib. Cellular ROS levels and mitochondrial membrane potential were checked. In addition, Annexin V staining was used to evaluate apoptosis, cell cycle was analyzed by flow cytometry, and the levels of autophagy proteins were determined by western blotting. The combined treatment diminished serum α-fetoprotein, a hepatocellular carcinoma (HCC) marker, significantly, decreased the liver weight as well as the number and size of tumors. The findings of this latter study were in line with the previous study, reporting that cotreatment of escin with sorafenib induced apoptosis due to suppression of autophagy. Furthermore, these studies suggest that escin may have the potential to enhance the effectiveness of chemotherapy in different types of cancer (Hussain et al. 2024).

In a study done by, treatment with escin decreased U-2 OS cell viability in a dose-dependent fashion. Besides, escin treatment caused a considerable elevation in reactive oxygen species (ROS) levels and a significant reduction in mitochondrial membrane potential in a dose-dependent manner. Monodansylcadaverine (MDC) is an in vivo marker of autophagic and lysosomal vesicles. Immunofluorescence studies indicated elevated levels of MDC and LC3 staining. The elevation in GFP-LC3 dots was further corroborated by increased LC3-II protein levels. Moreover, LC3-II protein level was elevated by both increased concentration and duration of escin treatment. In another study, escin inhibited osteosarcoma cell proliferation in a dose- and time-dependent manner. Treatment with escin also promoted apoptotic cell death by regulating the expression of caspase-3, caspase-8, caspase-9, Bax, and Bcl-2 in various OS cell lines and in an established osteosarcoma xenograft model. The combined use of escin with several chemotherapeutic agents further enhanced the suppression of cell proliferation. Moreover, escin treatment led to inactivation of the PI3K/Akt signaling pathway, as evidenced by reduced levels of p-PI3K, p-Akt, and p-mTOR. Collectively, these findings suggest that the anti-proliferative effect of escin on osteosarcoma cells is, at least in part, mediated through the inhibition of the PI3K/Akt pathway (Zhu et al. 2018).

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4.1.4 | PI3K/AKT/mTOR—AMPK Pathways

4.1.4.1 | Chamaejasmine (CJ). Chamaejasmine (CJ) represents a key biologically active component of *Stellera chamaejasmine L*. Four human osteosarcoma cell lines showed growth inhibition from CJ treatment, with the effect varying with time and concentration. CJ also stimulated apoptosis as evidenced by increased levels of caspase-3, -8, and -9. Additionally, Bcl2 protein levels were diminished while Bax protein levels were upregulated upon treatment with increasing concentrations of chamaejasmine. CJ was also found to trigger autophagy, as evidenced by increased LysoTracker staining and increased levels of autophagic proteins, autophagy-related protein 7 (ATG7), Beclin-1, and LC3-II. The pro-autophagic and anti-tumorigenic effect of CJ was associated with the induction of AMPK and suppression of mTOR (Yang et al. 2019).

In another study performed by three human osteosarcoma types along with five other cancer cell lines, both CJ B and neo-CJ C, isolated from *Stellera chamaejasmine* L., prevented the proliferation of all tested cell lines. Of note, the anti-proliferative effect of CJ B was more prominent. In the most sensitive cell lines, KHOS and A549, cell cycle arrest and DNA damage were notable (Zhang et al. 2013).

4.1.5 | MAPK—AMPK Pathways

4.1.5.1 | Matrine (MAT). Matrine (MAT) displays a potent anti-tumor effect in several cancer types, such as hepatic cancer and leukemia (Liu et al. 2006; Zhang 2010). MAT also stimulates protective autophagy in hepatocellular and gastric cancer (Li et al. 2013; Wang, Gao, et al. 2013). MAT prevents tumor progression via stimulating apoptosis and preventing cell proliferation in distinct cancer types such as lung, stomach, breast, cervical cancers, along with leukemia, hepatocellular carcinoma, and multiple myeloma (Liu et al. 2006; Luo et al. 2007; Yixiang et al. 2010; Yu et al. 2009; Zhang et al. 2007).

MAT can prevent tumor growth by regulating the levels of the genes that are related to apoptosis and/or proliferation, such as p53, apoptosis protease activating factor 1 (Apaf-1), and caspases (Luo et al. 2007; Zhang et al. 2007). MAT upregulated the cleavage of caspase-3, -8, and -9 and stimulated apoptosis in different osteosarcoma cell lines. Additionally, MAT treatment downregulated Bcl-2 protein and upregulated Bax protein. When combined, these findings imply that MAT promotes apoptosis by inhibiting the Bcl-2/Bax ratio, triggering Fas/FasL activation, and cleaving caspase-3, -8, and -9 (Liang et al. 2012).

MAT treatment led to a significant suppression of U2-OS and MG-63 cell proliferation in the 0.5–15 μM concentration range. In matrine (15 μM) treated MG-63 and U2-OS cells, the apoptosis ratio was upregulated to 58.92% and 61.76%, respectively, at 48 h. Cleaved caspases-3 as well as Bax levels were markedly elevated, and Bcl-2 was suppressed in MAT-treated cells. Treatment with MAT caused a marked elevation in phosphorylation of AMPK-α and a decrease in the levels of Enhancer of Zeste homolog 2 (EZH2), SUZ12 polycomb repressive complex 2 subunit (SUZ12), and embryonic ectoderm development (EED), as well as H3K27me3 expression. Taken together, MAT prevented osteosarcoma cell viability via induction of apoptosis. It also stimulates AMPK and downregulates Histone H3 lysine 27 trimethylation (Ma et al. 2021).

In a different study, MAT treatment inhibited osteosarcoma cell growth in a way that was dependent on both time and dose. MAT also triggered autophagy as indicated by enhanced GFP-LC3 dot formation and LC3 lipidation. MAT-triggered autophagy was found to be dependent on ERK. Moreover, inhibition of autophagy by CQ improved the cytotoxic effect of MAT, suggesting the cytoprotective role of matrine-induced autophagy (Ma, Huang, Guo, and Hu 2016).

4.2 | Biocompounds Acting on Single Autophagy-Related Signaling Pathway

4.2.1 | PI3K/AKT/mTOR

4.2.1.1 | Diallyldisulfide (DADS). Diallyldisulfide (DADS) is a biocompound extracted from garlic and scallion. The colony formation assay demonstrated cell cycle arrest and a decrease in cell viability following DADS treatment. Moreover, LC3 lipidation was potentiated and the levels of Bax and cleaved caspase 3 proteins were elevated, indicating induction of both autophagy and apoptosis. After treatment with the autophagic inhibitor 3-MA, DADS-induced apoptosis was attenuated. Moreover, suppression of the PI3K/Akt/mTOR pathway triggered DADS-induced autophagy and apoptosis. Overall, the study found out that DADS stimulated G2/M arrest, autophagy, and apoptosis by impeding the PI3K/Akt/mTOR pathway (Yue et al. 2019).

In a recent study, Diallyl trisulfide (DATS), a derivative compound structurally related to DADS, was shown to suppress epithelial-mesenchymal transition (EMT), proliferation, and migration of osteosarcoma cells. Furthermore, its ability to promote autophagy and apoptosis was found to be related to inactivation of the EGFR/PI3K/AKT/mTOR signalling (Liu, Wang, et al. 2024).

4.2.1.2 | Pelargonidin (Pg). Pelargonidin (Pg) is extracted from natural foods such as strawberries and blueberries (Giampieri et al. 2015). It has antitumor, antioxidant, and anti-inflammatory effect (Lee and Bae 2017). Besides, in colorectal and liver cancer, it had an antitumor impact via the aryl hydrocarbon receptor (AhR)-CYP1A1 signaling pathway (Kamenickova et al. 2013). Pg showed an anticancer effect in human osteosarcoma cells by suppressing the PI3K/AKT/mTOR pathway, thereby resulting in loss of mitochondrial integrity, cell cycle arrest and autophagy (Chen, Wang, et al. 2018). In human osteosarcoma cells, Pg exhibited an anticancer effect by suppressing the PI3K/AKT/mTOR pathway, leading to loss of mitochondrial integrity, cell cycle arrest, and induction of autophagy (Chen, Wang, et al. 2018). Pg has been reported to similarly suppress the PI3K/AKT/mTOR signaling in brain gliomas. In these cells, Pg treatment reduced both metastasis and angiogenesis (Tian et al. 2022). Considering the pivotal role of ROS in carcinogenesis, Pg's antioxidant ability (Li et al. 2022) has been related to both the reduction

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of ROS-induced DNA damage and the modulation of autophagy. A recent study showed that in a mouse osteoarthritis model, Pg treatment decreased IL-6, TNF-α, COX-2, and iNOS levels in IL-1β-stimulated chondrocytes, while also attenuating ECM degradation, inflammation, and cartilage degeneration through suppression of NF-κB signaling. Given that NF-κB signaling and ECM remodeling are of crucial importance in osteosarcoma (Yuan et al. 2023; Shi et al. 2025), these findings suggest that Pg may similarly regulate tumor growth, invasion, and the tumor microenvironment in osteosarcoma cells (Zeng et al. 2023).

4.2.1.3 | Physakengose G (PG). Physakengose G (PG), isolated from *Physalis alkekengi*, is another potential therapeutic biocompound for osteosarcoma. PG treatment inhibited the phosphorylation of Epidermal Growth Factor Receptor (EGFR) and disrupted the PI3K/AKT/mTOR pathway. Moreover, treatment with PG disrupted lysosomal acidification by altering LAMP1 expression. Overall, PG was found to decrease the proliferation of osteosarcoma cells via upregulation of apoptosis and downregulation of autophagy (Lin et al. 2018). EGFR pathway is upregulated in osteosarcoma, inducing proliferation, migration and invasion of cancer cells via the PI3K/AKT/mTOR and ERK signaling pathways. In addition, upregulation of the EGFR pathway was found to be associated with resistance to gemcitabine. Reported that PG suppressed the EGFR/mTOR pathway and disrupted lysosomal acidification in OS cells, PG might be used as a way to overcome gemcitabine resistance in osteosarcoma therapy (Ji et al. 2023).

4.2.2 | ROS/JNK Pathway

4.2.2.1 | Cinobufagin (CB). Prostate, hepatocellular, cervical, lung, leukemia, and colon cancer are among the many cancer types that can be treated with cinobufagin (CB), which is isolated from the skin and parotid venom glands of *Bufo bufo gargarizans* Cantor. In osteosarcoma cell line, it reduced cell viability in a manner that was dependent on both time and dose. The stimulation of caspase-dependent apoptosis via CB treatment was notable as demonstrated by increased levels of caspase-3, -8 and -9 proteins along with cleaved PARP. Moreover, CB treatment promoted autophagy as assessed by decreased levels of p62, elevated ratio of LC3-II to LC3-I, and increased GFP-LC3 staining. ROS fluorescence was also found to be increased by CB treatment. CB-induced autophagy and apoptosis occurred through phosphorylated JNK and p38 because of ROS production. Of note, chemical- or siRNA-mediated blockage of autophagy resulted in decreased levels of apoptosis (Ma, Zhang, Huang, Li, and Hu 2016). In another study, osteogenic differentiation was upregulated, and osteoclast differentiation was downregulated in CB-treated exosomes from RAW264-7 cells. Alox15 was found to be the target of MiR-3102-5p and the reason for reduced lipid peroxidation in osteoblasts (Geng et al. 2024).

4.2.2.2 | Curcumin (CU). Curcumin (CU), extracted from *Curcuma longa* (turmeric), was found to trigger either autophagy or apoptosis in several cancers including prostate, pancreatic, breast, and malignant glioma (Chen 2015; Kanai 2014; Terlikowska et al. 2014). CU-treated malignant glioma cells were found to have high autophagic activity due to suppressed Akt/mTOR/p70S6K pathway and upregulated ERK1/2 pathway. These two pathways are main regulators of starvation-induced autophagy, while mTOR is a suppressor and ERK1/2 is an inducer of autophagy. The results were further confirmed in a xenograft model, suggesting CU as a therapeutic reagent for glioblastoma (Aoki et al. 2007).

Induction of autophagy by CU treatment was also observed in KHOS osteosarcoma cell line. In a recent study conducted with different CU formulations, DMSO-CU caused an elevation in Beclin-1 levels as well as induced perinuclear localization of the protein in osteosarcoma cell line. These alterations are indicative of stimulated autophagy. On the other hand, the liposomal form of CU triggered apoptosis in the same cell line (Dhule et al. 2012). In another study, CU increased the level of both apoptosis and autophagy in the MG-63 osteosarcoma cell line. Blockage of apoptosis led to a more significant elevation in autophagy through stimulation of c-Jun N-terminal kinase (JNK) signaling. However, autophagic inhibition with JNK-inhibitor with or without 3-methyladenine (3-MA) resulted in CU-triggered apoptosis increasing even more, indicating that autophagy and apoptosis interact in cancer (Zhang et al. 2017).

A nanocarrier named CUR-BMS1166@ZIF-8@PEG-FA (CBZP), responsive to pH changes, was employed to deliver CU for enhancing the immunotherapeutic response of PD-1 (Programmed Death-1)/PD-L1 (Programmed Death-Ligand 1) blockade. This was achieved by triggering immunogenic cell death through autophagic cell death and simultaneously inhibiting the PD-1/PD-L1 interaction with BMS1166. The combination aimed to boost tumor immunogenicity and sensitize antitumor T cell immunity. Upon arriving at tumor cells, the pH-responsive nanoparticles stimulated autophagy, lowering intracellular pH and facilitating CU release to elevate autophagic activity. In mouse models of osteosarcoma, CBZP demonstrated potent antitumor activity and provided enduring protection against the recurrence of tumors. The study underscores the efficacy of combining immunogenic cell death induction with checkpoint blockade through CBZP, presenting a promising approach for clinical therapy in osteosarcoma by controlling autophagy (Ge et al. 2022).

4.2.2.3 | Erianin (ER). Erianin (ER), derived from *Dendrobium chrysotoxum*, has exhibited preventive effects on oncogenesis in various malignancies, such as promyelocytic leukemia, melanoma, and hepatocarcinoma (Wang et al. 2016). The impact of ER includes the suppression of cell proliferation due to cell cycle arrest. Increased levels of pro-apoptotic proteins suggest the induction of apoptosis following ER treatment. FACS analysis further demonstrated the accumulation of late apoptotic cells with ER treatment. Enhanced autophagy was confirmed by a rise in LC3 lipidation, along with elevated Beclin-1 and p62 protein levels. The involvement of the ROS/JNK pathway was revealed using the ROS inhibitor NAC or the JNK inhibitor SP600125, showing that the increased levels of autophagy and apoptosis with ER treatment were a result of stimulation in the ROS/JNK pathway. Additionally, inhibiting autophagy led to a more significant increase in apoptosis, indicating a pro-oncogenic role of autophagy in ER-treated osteosarcoma cells (Geng et al. 2024).

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ER-regulated apoptosis and autophagy pathway was also reported in oral squamous cell carcinoma (OSCC) cells (Chen et al. 2020). Similar to the study (Wang et al. 2016), erianin was found to be cytotoxic to OSCC cells leading to G2/M cell cycle arrest. Intrinsic apoptotic pathway was induced as evidenced by elevated levels of Bax and Bak, and reduced levels of Bcl-2 and Bcl-xL along with alteration in mitochondrial membrane potential upon erianin treatment. Elevated protein levels of both LC3-I and LC3-II, diminished p62 expression, along with the presence of distinct autophagosomes, indicated the induction of autophagy upon ER treatment, similar to what has been found in osteosarcoma cells (Wang et al. 2016). In addition, ERK1/2 and JNK1/2 inhibitors suppressed apoptosis and autophagy whereas the p38 inhibitor did not have a significant effect on ER-stimulated apoptosis (Wang et al. 2016).

4.2.2.4 | Carnosol (CL). Carnosol (CL) is extracted from rosemary. Treatment with CL elevated the levels of ROS and triggered autophagy, apoptosis, and cell cycle arrest in human osteosarcoma cells. These changes were associated with decreased levels of cell viability. Moreover, carnosol elevated ROS levels, and pre-treatment with the ROS scavenger N-acetylcystein (NAC) diminished the changes caused by CL treatment. Moreover, co-treatment of the autophagy inhibitor and carnosol had a similar effect, indicating the anti-tumorigenic effect of autophagy on osteosarcoma cells. Collectively, treatment with carnosol restricted tumor growth by upregulation of autophagy and apoptosis via elevated levels of ROS in human osteosarcoma cells (Lo et al. 2017).

In another study, the cytotoxic and antimicrobial effects of both carnosol and carnosic acid were observed, and it was found that carnosol may be used as an anticancer and antimicrobial agent to enhance therapeutic approaches for osteosarcoma (Karadas et al. 2019).

4.2.2.5 | Deoxyelephantopin (DET). Deoxyelephantopin (DET) is extracted from *Elephantopus scaber*. Based on 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay results, DET treatment inhibited cell proliferation. Moreover, it elevated ROS levels, altered the potential of mitochondrial membrane, and stimulated apoptosis. In addition, DET triggered autophagy in human osteosarcoma cells as indicated by elevated LC3 lipidation. The use of a ROS scavenger NAC blocked DET-induced autophagy, indicating that DET-induced autophagy is a ROS-dependent process (Zou et al. 2017).

In another study done with oral cancer cell lines, DET suppressed cell proliferation, increased oxidative stress accompanied by downregulation of superoxide dismutase (SOD) and catalase activities. Upregulation of pro-apoptotic genes and downregulation of anti-apoptotic genes revealed the inhibition of the mTOR pathway (Jayaraman and Veeraraghavan 2025).

4.2.2.6 | Luteolin (LUT). Natural flavonoid component luteolin (LUT) (3′,4′,5,7-tetrahydroxyflavone) is isolated from several traditional Chinese remedies, such as *Ajugadecumbens Thunb, Schizonepetatenuifolia*, and *Lonicerajaponica* (Ashrafizadeh et al. 2020; Zhang et al. 2015). The pharmacological actions of LUT, including angiogenesis, anti-inflammatory activity, suppression of cell proliferation, trigger of apoptosis and metastasis, are associated with its anticancer property (Ashrafizadeh et al. 2020). According to earlier research, LUT can also be considered an antioxidant or a tumor cell apoptosis sensitization agent, as it prevents tumor cell proliferation via suppressing angiogenesis and upregulating apoptosis (Wang et al. 2015). Beclin-1 and LC3-II protein levels were upregulated upon LUT treatment. Additionally, the combined treatment of LUT with doxorubicin exhibited a stronger effect on autophagic induction, indicating that LUT sensitized cells to doxorubicin treatment. Moreover, this combined treatment also led to the disruption of mitochondrial integrity and a notable rise in reactive oxygen species (ROS) production (Zhang et al. 2015). LUT shows potential to enhance the anticancer effect of doxorubicin, but further studies involving animal models are imperative.

4.2.2.7 | Marrubenol (MAR). Marrubenol (MAR) is extracted from *Marrubium vulgare*. It was revealed that MAR prevented colony formation and triggered autophagy in human osteosarcoma cell line. Upregulation of autophagy was demonstrated by elevated Beclin-1 and LC3-II levels, decreased levels of p62 and vacuolar protein-sorting 34 (Vps34), and an increase in the number of autophagosomes. Moreover, MAR increased the level of ROS and decreased the mitochondrial membrane potential. These findings imply that this substance may turn out to be a crucial lead molecule for osteosarcoma treatment (Zhang et al. 2018).

In a different study, the effect of ethanolic extract of *Marribium vulgare* (MVE) on melanoma and glioma cell lines was examined. Upon MVE treatment, both apoptosis and autophagy were stimulated. MVE treatment led to cell cycle arrest at both S and G2/M phases. In addition, MVE induced the intrinsic pathway of apoptosis, upregulated the levels of pro-apoptotic genes, and downregulated the levels of anti-apoptotic genes. The increase in several autophagy-related genes such as Atg5, Atg7, Atg12, Beclin-1, and p62, and the increased ratio of LC3-II to LC3-I confirmed the induction of autophagy. However, the use of autophagic inhibitors strengthened the cytotoxic effect of MVE, suggesting that MVE-stimulated autophagy is cytoprotective, not cytotoxic (Paunovic et al. 2016).

4.2.2.8 | Parthenolide (PTL). Parthenolide (PTL), a sesquiterpene lactone found in *Tanacetum parthenium*, possesses anti-microbial, anti-inflammatory as well as anti-cancer properties (Hwang et al. 2006). PTL induced both autophagy and mitophagy in osteosarcoma cells. The increased translocation of PINK1 and Parkin to mitochondria was an indicator of induction of mitophagy. Besides, increased levels of autophagy-related proteins were an indicator of induction of autophagy. The stimulation of autophagy by PTL was linked to a rise in ROS levels and the use of ROS antioxidant N-acetylcysteine (NAC) diminished PTL-induced autophagy (Yang et al. 2016). PTL also enhances the sensitivity of radioresistant osteosarcoma cells to radiotherapy (Zuch et al. 2012). Of note, PTL does not exert a cytotoxic effect on normal cells (Sun et al. 2010).

4.2.3 | MAPK Pathway

4.2.3.1 | Dendropanoxide (DP). Dendropanoxide (DP) is extracted from *Dendropanax morbifera Leveille*. Its therapeutic

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use has been associated with diabetes, skin, and infectious diseases (Lee et al. 2013). Recently, its anti-cancer properties are the focus of research.

In MG-63 human osteosarcoma cells, DP treatment reduced the viability of cells and triggered apoptosis as demonstrated by AnnexinV/PI staining, cytochrome-c release in the cytosol as well as immunoblotting of caspases (Chen et al. 2020). Additionally, the amount of the autophagic proteins Beclin-1 and ATG7 was increased, along with LC3 lipidation, indicating that autophagic activity is stimulated by DP treatment. Increased GFP-LC3 staining and autophagic vacuole accumulation were also detected by DP treatment. The combined use of autophagic inhibitors 3-MA or Wortmannin with DP triggered apoptosis as evidenced by AnnexinV/Propidium Iodide (PI) staining. Inhibition of the ERK1/2 pathway led to a reduction in DP-stimulated autophagy. On the other hand, chemical inhibition of autophagy led to a boost in apoptosis, indicating the pro-oncogenic role of autophagy. Combining DP with autophagy repressors may make treating osteosarcoma simple, according to this study (Lee et al. 2013).

On the other hand, treatment of hepatocellular carcinoma cells with *Dendropanax morbifera* extract (DME) stimulated the intrinsic pathway of apoptosis as revealed by increased levels of pro-apoptotic protein Bax and decreased levels of anti-apoptotic proteins such as Bcl-2, caspase-9, and caspase-3. DME also regulated the autophagy levels as revealed by downregulation of PI3K/AKT/mTOR/p70S6K signalling and upregulation of p-p38 and p-FOXO3a levels. Overall, DME treatment of hepatocellular carcinoma cells reduced cell proliferation, cell migration, and invasiveness by inducing both autophagy and apoptosis (Kim 2023).

4.2.4 | AMPK Pathway

4.2.4.1 | Anthocyanins (ATCs). Anthocyanins (ATCs) are water-soluble vacuolar pigments in fruits and vegetables that might change their color depending on pH. Induction of apoptosis in human colon cancer, prostate, leukemia, and leukemia by anthocyanin treatment has been reported (Shin et al. 2009; Reddivari et al. 2007; Lee et al. 2009). Furthermore, hepatocellular carcinoma cells underwent apoptosis when autophagy was inhibited through ATC treatment (Longo et al. 2008).

In a study done by Choe et al. (2012), ATCs extracted from black soybean were found to activate apoptosis after 24 h in U-2 OS cells. Subsequently, scientists examined if ATC treatment also triggered autophagy. Following 6 h of ATC treatment, puncta formation of GFP-LC3 was observed in U-2 OS-GFP-LC3 cells. The finding that U-2 OS and U-2 OS-GFP-LC3 cells had an increased LC3 lipidation after 6 h of ATC treatment lends credence to these conclusions. These results indicated that ATC initiated autophagy in U-2 OS cells before apoptosis. To clarify the signaling process underlying autophagic induction, the phosphorylation of several proteins associated with ATC-induced autophagy was examined. Phosphorylated levels of p38 MAPK, AKT, JNK, and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) proteins were upregulated with ATC treatment in a dose-dependent manner. Nonetheless, GFP-LC3 dot formation and LC3 lipidation were not suppressed by the use of AKT, p38 MAPK, MEK, and JNK inhibitors, excluding the involvement of these pathways in ATC-induced autophagy. Therefore, the authors wanted to examine whether adenosine monophosphate-activated protein kinase (AMPK) was upregulated in ATC-treated U-2 OS cells. Phosphorylated mTOR levels were upregulated in U-2 OS cells treated with different concentrations of ATC, indicating the activation of the AMPK pathway, whereas phosphorylated AMPKα1 and acetyl CoA carboxylase levels were downregulated. Consistently, pretreatment with an inhibitor of AMPK suppressed both LC3 lipidation and GFP-LC3 dot formation. Moreover, the use of siRNA against AMPKα1 decreased LC3 lipidation. In addition, treatment with ATC led to a reduction in intracellular ATP levels, and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an analog of AMP, triggered autophagy in U-2 OS cells. These results allowed the researchers to suggest that the AMPK pathway was involved in ATC-induced autophagy potentially by resulting from ATP depletion. As the last step in the study, siRNAs against downstream target proteins of AMPK, which are involved in the stimulation of autophagy, were used. ULK1 siRNA did not prevent the upregulation of either autophagy or apoptosis upon ATC treatment. The use of siRNA against (forkhead box O3) FOXO3a diminished GFP-LC3 dot formation and the lipidation of LC3 but did not change the levels of cleaved poly-ADP ribose polymerase (PARP). On the other hand, siRNA against cyclin-dependent kinase inhibitor 1B ($p27^{Kip1}$) did not alter the lipidation of LC3 but increased ATC-induced PARP cleavage. Collectively, their findings implied that the induction of AMPK by ATCs might stimulate autophagy through FOXO3a and suppress apoptosis via $p27^{Kip1}$ (Choe et al. 2012).

4.2.4.2 | Carotenoids (CARs). Carotenoids (CARs), extracted from pumpkin, delayed the growth of human osteosarcoma cell line via inducing cytotoxic autophagy and autophagic induction was mediated via activation of AMPK pathway. In the study, nanoemulsions with CAR-enriched extract prevented cell growth in SAOs cell line (Russo et al. 2017). The authors observed the presence of intracellular vacuoles under microscopy and decided to check autophagic activity. Compared to untreated or control-nanoemulsion treated cells, carotenoid-enriched nanoemulsion (CEN) treated SAOs cells showed a significantly higher number of autophagosomes. Furthermore, the use of all-trans retinoic acid (ATRA), an mTOR inhibitor and positive regulator of autophagy, had a similar effect to CEN, resulting in accumulation of autophagosomes but to a lesser extent. The induction of autophagy with CEN was also evidenced with LC3-II accumulation. Next, the researchers wanted to determine whether the autophagy stimulated by CEN was protective or non-protective. In the case of induction of non-protective autophagy, co-treatment with CEN and an autophagy inhibitor versus treatment with CEN alone gave similar results in terms of cell growth since suppression of non-protective autophagy does not change drug sensitivity. Cell viability was found not to be altered by co-treatment with CEN and autophagy inhibitors compared to CEN monotreatment which indicated the block of autophagic flux by CEN. Further analysis with CEN showed that intracellular ATP levels were significantly downregulated after 24 h of CEN treatment in SAOs cells and this reduction was mediated by AMPK activation (Russo et al. 2017).

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In the latter study of the same group, incubation of radio-resistant SAOS400 cell line with CEN elevated the level of autophagic proteins and cells were arrested at G2/M phase (Ashrafizadeh et al. 2020). Incubation of γ-irradiated SAOS cells with CEN induced autophagic cell death. SAOS400 cells, which contain high levels of senescent cells, upregulated autophagy and stopped cell division to compensate for cellular damage. Autophagic upregulation was assessed by staining and counting the autophagosomes as well as LC3-II accumulation. Treatment with CEN resulted in a cell cycle arrest in SAOS400 cells. CEN was found to trigger a cytoprotective form of autophagy as evidenced using chloroquine (CQ) as an autophagy blocker. When CEN was used in combination with CQ, cell viability was decreased compared to CEN alone and CQ alone. In addition to autophagy, CEN also regulated senescence. CEN treatment resulted in a decrease in protein levels of the senescence marker $p16^{INK4}$. Irradiation (IR), CEN and combined treatment did not alter apoptosis levels as shown by the caspase-3 enzymatic assay. IR or CEN treatment of SAOS400 cells raised the number of autophagosomes. In addition, when the cells were first irradiated then exposed to CEN, the number of autophagosomes was amplified further. The protein levels of the autophagic marker Beclin-1 were upregulated and the senescence marker $p16^{INK4}$was downregulated in SAOS400 cells cotreated with CEN and IR. On the contrary, irradiation alone led to a downregulation in protein levels of Beclin-1 and p62. Cotreatment with CEN and γ (gamma)-rays showed a synergetic effect and caused a statistically significant level of cell death. When Baf-A1 was added to irradiated or CEN treated cells, BafA1 caused a statistically significant reduction in cell viability which is an indicator of induction of a protective form of autophagy. On the other hand, SAOS400 cells cotreated with CEN and IR showed signs of a lethal form of autophagy since after the addition of Bafilomycin A1 (BafA1), cell viability was upregulated. Next, the authors wanted to check AMPK activity. Although IR alone did not alter intracellular ATP levels, CEN and CEN plus IR cotreatment significantly downregulated intracellular ATP levels, and CEN increased the activity of AMPK (Russo et al. 2022).

4.3 | Biocompounds Acting on Other/Unidentified Signaling Pathway

4.3.1 | Camptothecin (CPT)

Camptohecin (CPT) is an inhibitor of topoisomerase I and causes DNA damage and cell death (Grohar and Helman 2013). Hollomon et al. (2013) used three cell lines, metastatic murine osteosarcoma cell lines K7M3, DLM8, and MC3T3, a non-transformed murine osteoblast cell line to analyze the effect of CPT on autophagy and apoptosis levels in these cell lines. Cell viability was diminished with CPT treatment in a concentration-dependent fashion in DLM8 and K7M3 cell lines. In K7M3 cells, autophagic inhibition by shRNA against ATG5 caused a significant decline in metabolic activity as well as cell growth. CPT treatment was found to stimulate apoptosis, as revealed by higher levels of cleaved caspase-3 and cleaved PARP levels. Caspase-3 activation was impeded by pre-treatment with pan-caspase inhibitor. CPT-induced cell death was reversed in the DLM8 cell line but not in the K7M3 cell line using pan-caspase inhibitor. It appears that in addition to inducing apoptosis, CPT also triggered autophagy, as indicated by the accumulation of acidic vesicular organelles and LC3-II. Moreover, there was a decline in the p62 protein level, which is a substrate protein involved in autophagy. This suggests that CPT treatment leads to the activation of autophagy, which may contribute to its cytotoxic effects alongside apoptosis induction. The use of Bafilomycin A1, which is an inhibitor of fusion of autophagosomes with lysosomes, led to a rise in LC3-II protein levels suggesting that autophagic flux was active in DLM8 and K7M3 cell lines. The DLM8 and K7M3 cell lines seemed to respond differently to ATG5 protein knockdown in terms of CPT-induced cell death. While CPT-induced cell death in K7M3 cells increased when ATG5 was knocked down, it decreased in DLM8 cells. In comparison to DLM8 cells and a non-transformed osteoblast cell line, K7M3 cells had higher baseline levels of autophagy. These findings suggested that K7M3 cells rely more on autophagy for maintaining metabolic homeostasis. The levels of phosphorylation of p53 at Ser15 upon CPT treatment were similar in autophagy-competent and autophagy-inhibited DLM8 cells, suggesting comparable levels of DNA damage (Hollomon et al. 2013).

In a more recent study, the resistance of osteosarcoma cells to CPT was attributed to DEAD-box helicase 5 (DDX5)-mediated regulation of the DNA repair protein NONO (Zhao et al. 2020). Silencing DDX5 protected 143B cells from CPT-induced cell death and DNA damage, leading to improved proliferative capacity. In addition, the interaction between DDX5 and NONO was found to modulate the activity of NONO. Upon CPT treatment, the degradation of DDX5 was induced, which led to the disruption of the DDX5-NONO complex and increased resistance to CPT. These findings highlight DDX5 as a potential therapeutic target for improving the clinical efficacy of CPT-based treatments in osteosarcoma (Zhao et al. 2020).

4.3.2 | Delphinidin (Dp)

Delphinidin (Dp) is commonly found in various fruits and vegetables. In both time- and dose-dependent ways, Dp suppressed the proliferation of U-2 OS cells (Lee et al. 2018). When the cells were pre-treated with ROS inhibitor NAC (N-Acetyl-L-Cystine), the decrease in cell viability upon Dp treatment was not detected, implying the anti-carcinogenic role of Dp that is unrelated to its antioxidant capacity. Autophagy was found to be promoted upon Dp treatment, as evidenced by the elevated autophagosome count, reduced levels of p62 protein, and increased LC3 lipidation. However, the combined use of Dp and NAC resulted in a reduction in LC3 lipidation. Moreover, cotreatment with Dp and inhibitors of autophagy suppressed U-2 OS cell growth, indicating the cytoprotective function of autophagy in the development of cancer. Analysis of cell cycle progression revealed that upon Dp treatment, cells were arrested and the percentage of cells that underwent apoptosis was increased (Lee et al. 2018).

In another study, treatment of osteosarcoma cells with delphinidin reduced cell viability and colony formation due to upregulation of apoptosis via inhibition of the MAPK pathway; however, autophagy was not checked in this study and the authors suggest that inhibition of the MAPK pathway might have an effect on modulation of autophagy (Kang et al. 2018).

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4.3.3 | Oleuropein (OLEU)

Oleuropein (OLEU) content is high in plants belonging to the Oleaceae, Cornales, and Gentianales family (Ahmad Farooqi et al. 2017; Gorzynik-Debicka et al. 2018; Zheng et al. 2022). OLEU, either used alone or in combination with a chemotherapeutic agent, 2-Mercaptoethanol (2-ME), was found to upregulate autophagy and restrain the growth of highly metastatic osteosarcoma cell line 143B. Treatment with OLEU also limited the migration capability of this highly metastatic osteosarcoma cell line (Przychodzen et al. 2019). Additionally, OLEU demonstrated substantial cell-killing effects on human osteosarcoma cell lines MG-63 and Saos2 in a way that was dependent on both dose and time (Morana et al. 2016).

In a more recent report, OLEU administered either alone or in combination with the chemotherapeutic agent Adriamycin (ADR) inhibited the proliferation of the MG-63 osteosarcoma cell line. The antiproliferative effect of ADR was more pronounced in the presence of OLEU. Treatment with OLEU upregulated the expression of autophagy-related genes, including BNIP3L (BCL2 Interacting Protein 3 Like), ULK1 (Unc-51 Like Autophagy Activating Kinase 1), and AMBRA1 (Activating Molecule in BECN1-regulated Autophagy Protein 1). However, the induction of autophagy upon OLEU treatment was neutralized upon combined application with ADR. Notably, OLEU administration was associated with reduced LC3-I and LC3-II levels and an increase in p62, indicating regulation of autophagy. Collectively, these findings suggest that OLEU modulates ADR-induced autophagy, potentially contributing to improved therapeutic responses in osteosarcoma (Gioti et al. 2021).

4.3.4 | Licochalcone A (LicoA)

Licochalcone A (LicoA), derived from licorice root, is another compound known for inducing autophagy. LicoA treatment caused cell cycle arrest and consequent inhibition of the viability and growth of osteosarcoma cells. The treatment also prompted apoptosis through ataxia-telangiectasia mutated 2 (ATM2)-checkpoint kinase 2 (Chk2) activation. In addition, LicoA increased the activated forms of PARP, caspase 3, and caspase 8, while reducing the activated forms of caspase 9 and Bax. It also downregulated certain inhibitors of apoptosis protein (IAP) family members and Bcl-2. Taken together, these findings suggested that LicoA triggered apoptosis through caspase 8 and caspase 3. Besides, LicoA triggered cytotoxic autophagy. The use of an autophagy inhibitor caused a rise in the viability of cells due to the suppression of both apoptosis and autophagy. These findings suggest that the stimulation of both ATM-Chk2 and autophagy was responsible for the anti-proliferative effects triggered by LicoA in osteosarcoma cell lines (Shen et al. 2019). In a recent study, LicoA was found to trigger both autophagy and apoptosis and prevented cell invasion in 2D and 3D models of osteosarcoma (Rossi et al. 2024).

4.3.5 | Riccardin D (RD)

Riccardin D (RD), derived from liverwort, has anticancer properties in various types of cancer. In human osteosarcoma cell line, autophagy as well as apoptosis were triggered with RD treatment. The induction of apoptosis was found to be related to increasing the expression of p21. RD treatment triggered autophagic cell death associated with an increase in autophagic flux. In fact, the effect of RD was similar to that of Rapamycin, an mTOR inhibitor (Wang, Ji, et al. 2013). Although the target of RD was not identified in the study, the increase in autophagic flux implies that RD acts as a positive regulator of upstream pathways of autophagic membrane nucleation.

On the other hand, RDD648, a synthetic derivative of the natural compound Riccardin D, was found to exert strong anticancer activity by stimulating lysosome-dependent cell death in breast cancer. RDD648 triggers STAT3 nuclear translocation. As a result of the interaction between nuclear STAT3 and TFEB, lysosomal turnover was disrupted, and cell death was upregulated. Overall, the authors concluded that lysosome-mediated cytotoxicity was observed in breast cancer cells upon RDD648 treatment (Li, Sun, et al. 2018).

4.3.6 | Triptolide (TPL)

The stimulation of apoptosis and the suppression of cancer cell proliferation are linked to the antitumorigenic properties of triptolide (TPL), an extract from *Tripterygium wilfordii* (Kim et al. 2014). The antitumorigenic role of TPL against distinct cancer types such as cholangiocarcinoma, melanoma, gastric, and breast cancer has been confirmed both in vitro and in vivo (Zhou et al. 2012).

TPL-induced autophagy is observed in pancreatic cancer cells and in two different neuroblastoma cell lines (Krosch et al. 2013; Mujumdar et al. 2010). In pancreatic cancer cells, the autophagy triggered by TPL treatment has a pro-death effect and is dependent on Atg5 or Beclin-1. TPL treatment led to the inhibition of the Akt/mTOR/p70S6K1 pathway and the stimulation of the ERK pathway (Mujumdar et al. 2010). In neuroblastoma cell lines, triptolide stimulates both apoptosis and autophagy and led to a decrease in NF-κB activity. Another study showed that by inhibiting HSP70 overexpression with TPL pretreatment, the positive effects of mild heat preconditioning (averting apoptosis, heat-induced cell damage, and autophagy) were considerably diminished in cardiomyocytes (Hsu et al. 2013).

Another research revealed that TPL could diminish the viability of osteosarcoma cells by downregulation of mitogen-activated protein kinase phosphatase-1 (MKP-1) and Hsp70 expression (Zhao et al. 2016). A later study reported that TPL induced autophagy via suppression of Wnt/β–catenin signaling in osteosarcoma cells. The autophagic induction was associated with elevated LC3 lipidation and Beclin-1 expression. Additionally, TPL downregulated vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1α (HIF-1α), consequently inhibiting angiogenesis (Li, Lu, et al. 2018).

5 | Future Perspective and Conclusion

Today, it is known that the source of many drugs used in cancer treatment is biological compounds. This paper emphasizes that treatment of osteosarcoma by several biocompounds shows

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promising data. However, most of the studies are conducted under in vitro conditions and there is a lack of in vivo studies. Therefore, both in vivo and clinical studies are imperative to establish the therapeutic efficacy of biocompounds on osteosarcoma. Indeed, the intricate crosstalk and interplay between different pathways pose challenges, as the anti-tumorigenic effect of biocompounds may interfere with other pathways in the organism. Modulating a pathway indirectly affected by biocompounds could lead to challenging side effects. The combined impact of potential biocompounds with other cancer drugs should also be carefully considered. In summary, conducting more in vivo studies is crucial for identifying anti-tumorigenic biocompounds, and a thorough examination of side effects and potential interactions with other drugs is essential.

Author Contributions

O.B.E., S.B.: writing – original draft. A.A.F.: writing – original draft. S.E.: preparation of Figure 1 and Table 1. D.G.: conceptualization. O.K.: supervision, conceptualization, writing, review and editing, preparation of Figure 2 and Table 2. All authors: review and editing; final approval of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

No datasets were generated or analyzed during the current study.

References

Ahmad Farooqi, A., S. Fayyaz, A. Silva, et al. 2017. "Oleuropein and Cancer Chemoprevention: The Link Is Hot." *Molecules* 22: 705.

Anderson, P. M., P. Meyers, E. Kleinerman, et al. 2014. "Mifamurtide in Metastatic and Recurrent Osteosarcoma: A Patient Access Study With Pharmacokinetic, Pharmacodynamic, and Safety Assessments." *Pediatric Blood & Cancer* 61: 238–244.

Ando, T., J. Ichikawa, T. Fujimaki, N. Taniguchi, Y. Takayama, and H. Haro. 2020. "Gemcitabine and Rapamycin Exhibit Additive Effect Against Osteosarcoma by Targeting Autophagy and Apoptosis." *Cancers* 12: 3097.

Anninga, J. K., H. Gelderblom, M. Fiocco, et al. 2011. "Chemotherapeutic Adjuvant Treatment for Osteosarcoma: Where Do We Stand?" *European Journal of Cancer* 47: 2431–2445.

Aoki, H., Y. Takada, S. Kondo, R. Sawaya, B. B. Aggarwal, and Y. Kondo. 2007. "Evidence That Curcumin Suppresses the Growth of Malignant Gliomas In Vitro and In Vivo Through Induction of Autophagy: Role of Akt and Extracellular Signal-Regulated Kinase Signaling Pathways." *Molecular Pharmacology* 72: 29–39.

Ashrafizadeh, M., Z. Ahmadi, T. Farkhondeh, and S. Samarghandian. 2020. "Autophagy Regulation Using Luteolin: New Insight Into Its Anti-Tumor Activity." *Cancer Cell International* 20: 537.

Berrak, S. G., M. Pearson, S. Berberoğlu, İ. E. İlhan, and N. Jaffe. 2005. "High-Dose Ifosfamide in Relapsed Pediatric Osteosarcoma: Therapeutic Effects and Renal Toxicity." *Pediatric Blood & Cancer* 44: 215–219.

Chen, Q.-H. 2015. "Curcumin-Based Anti-Prostate Cancer Agents." *Anti-Cancer Agents in Medicinal Chemistry* 15: 138–156.

Chen, Y., Y. Ou, Y. Tao, et al. 2018. "Effect and Mechanisms of Celastrol on the Apoptosis of HOS Osteosarcoma Cells." *Oncology Reports* 40: 2260–2268.

Chen, Y., S. Wang, B. Geng, and Z. Yi. 2018. "Pelargonidin Induces Antitumor Effects in Human Osteosarcoma Cells via Autophagy Induction, Loss of Mitochondrial Membrane Potential, G2/M Cell Cycle Arrest and Downregulation of PI3K/AKT Signalling Pathway." *Journal of the Balkan Union of Oncology* 23: 735–740.

Chen, Y.-T., M.-J. Hsieh, P.-N. Chen, C.-J. Weng, S.-F. Yang, and C.-W. Lin. 2020. "Erianin Induces Apoptosis and Autophagy in Oral Squamous Cell Carcinoma Cells." *American Journal of Chinese Medicine* 48: 183.

Cheng, S., H. Shao, D. Yin, et al. 2023. "Molecular Mechanism Underlying the Action of a Celastrol-Loaded Layered Double Hydroxide-Coated Magnesium Alloy in Osteosarcoma Inhibition and Bone Regeneration." *ACS Biomaterials Science & Engineering* 9: 4940–4952.

Chien, C.-S., K.-H. Ma, H.-S. Lee, et al. 2013. "Dual Effect of Capsaicin on Cell Death in Human Osteosarcoma G292 Cells." *European Journal of Pharmacology* 718: 350–360.

Choe, Y.-J., T. J. Ha, K.-W. Ko, S.-Y. Lee, S. J. Shin, and H.-S. Kim. 2012. "Anthocyanins in the Black Soybean (Glycine Max L.) Protect U2OS Cells From Apoptosis by Inducing Autophagy via the Activation of Adenosyl Monophosphate-Dependent Protein Kinase." *Oncology Reports* 28: 2049–2056.

Chou, A. J., D. S. Geller, and R. Gorlick. 2008. "Therapy for Osteosarcoma: Where Do We Go From Here?" *Pediatric Drugs* 10: 315–327.

Colobatiu, L., L. Gavrilas, and A. Mocan. 2021. "Natural Compounds as Chemosensitizers: A Lesson From Plants." In *pH-Interfering Agents as Chemosensitizers in Cancer Therapy*, 147–165. Elsevier.

Davis, L. E., V. Bolejack, C. W. Ryan, et al. 2019. "Randomized Double-Blind Phase II Study of Regorafenib in Patients With Metastatic Osteosarcoma." *Journal of Clinical Oncology* 37: 1424–1431.

De Azevedo, J., T. Fernandes, J. Fernandes, et al. 2019. "Biology and Pathogenesis of Human Osteosarcoma (Review)." *Oncology Letters* 19: 1099–1116.

Debnath, J., N. Gammoh, and K. M. Ryan. 2023. "Autophagy and Autophagy-Related Pathways in Cancer." *Nature Reviews. Molecular Cell Biology* 24: 560–575.

Dhule, S. S., P. Penfornis, T. Frazier, et al. 2012. "Curcumin-Loaded γ-Cyclodextrin Liposomal Nanoparticles as Delivery Vehicles for Osteosarcoma." *Nanomedicine: Nanotechnology, Biology, and Medicine* 8: 440.

Eberhart, K., O. Oral, and D. Gozuacik. 2014. "Induction of Autophagic Cell Death by Anticancer Agents." In *Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging*, 179–202. Elsevier.

Eisenberg-Lerner, A., S. Bialik, H.-U. Simon, and A. Kimchi. 2009. "Life and Death Partners: Apoptosis, Autophagy and the Cross-Talk Between Them." *Cell Death and Differentiation* 16: 966–975.

Elmore, S. 2007. "Apoptosis: A Review of Programmed Cell Death." *Toxicologic Pathology* 35: 495–516.

Erbil-Bilir, S., D. Gozuacik, and O. Kutlu. 2019. "Autophagy as a Physiological Response of the Body to Starvation." In *Handbook of Famine, Starvation, and Nutrient Deprivation*, edited by V. R. Preedy and V. B. Patel, 2067–2081. Springer International Publishing.

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Gao, H., W. Sun, W. Zhao, et al. 2015. "Total Tanshinones-Induced Apoptosis and Autophagy *via* Reactive Oxygen Species in Lung Cancer 95D Cells." *American Journal of Chinese Medicine* 43: 1265.

Gaspar, N., M. Casanova, F. J. B. Sirvent, et al. 2018. "Single-Agent Expansion Cohort of Lenvatinib (LEN) and Combination Dose-Finding Cohort of LEN + Etoposide (ETP) + Ifosfamide (IFM) in Patients (Pts) Aged 2 to ≤25 Years With Relapsed/Refractory Osteosarcoma (OS)." *Journal of Clinical Oncology* 36: 11527.

Ge, Y.-X., T.-W. Zhang, L. Zhou, et al. 2022. "Enhancement of Anti-PD-1/PD-L1 Immunotherapy for Osteosarcoma Using an Intelligent Autophagy-Controlling Metal Organic Framework." *Biomaterials* 282: 121407.

Geng, Z., T. Sun, J. Yu, et al. 2024. "Cinobufagin Suppresses Lipid Peroxidation and Inflammation in Osteoporotic Mice by Promoting the Delivery of miR-3102-5p by Macrophage-Derived Exosomes." *International Journal of Nanomedicine* 19: 10497.

Giampieri, F., T. Y. Forbes-Hernandez, M. Gasparrini, et al. 2015. "Strawberry as a Health Promoter: An Evidence Based Review." *Food & Function* 6: 1386.

Gioti, K., A. Papachristodoulou, D. Benaki, et al. 2021. "Assessment of the Nutraceutical Effects of Oleuropein and the Cytotoxic Effects of Adriamycin, When Administered Alone and in Combination, in MG-63 Human Osteosarcoma Cells." *Nutrients* 13: 354.

Goorin, A. M., M. B. Harris, M. Bernstein, et al. 2002. "Phase II/III Trial of Etoposide and High-Dose Ifosfamide in Newly Diagnosed Metastatic Osteosarcoma: A Pediatric Oncology Group Trial." *Journal of Clinical Oncology* 20: 426–433.

Gorlick, R., and C. Khanna. 2010. "Osteosarcoma." *Journal of Bone and Mineral Research* 25: 683–691.

Gorzynik-Debicka, M., P. Przychodzen, F. Cappello, et al. 2018. "Potential Health Benefits of Olive Oil and Plant Polyphenols." *International Journal of Molecular Sciences* 19: 686.

Greenwood, A. C., R. D. Arora, and H. Shaikh. 2025. *StatPearls*. StatPearls Publishing.

Grohar, P. J., and L. J. Helman. 2013. "Prospects and Challenges for the Development of New Therapies for Ewing Sarcoma." *Pharmacology & Therapeutics* 137: 216–224.

He, H., J. Ni, and J. Huang. 2014. "Molecular Mechanisms of Chemoresistance in Osteosarcoma (Review)." *Oncology Letters* 7: 1352–1362.

Hellwinkel, O. J. C., J. Müller, A. Pollmann, and H. Kabisch. 2005. "Osteosarcoma Cell Lines Display Variable Individual Reactions on Wildtype p53 and Rb Tumour-Suppressor Transgenes." *Journal of Gene Medicine* 7: 407–419.

Hengartner, M. O. 2000. "The Biochemistry of Apoptosis." *Nature* 407: 770–776.

Hingorani, P., M. Krailo, A. Buxton, et al. 2022. "Phase 2 Study of Anti-Disialoganglioside Antibody, Dinutuximab, in Combination With GM-CSF in Patients With Recurrent Osteosarcoma: A Report From the Children's Oncology Group." *European Journal of Cancer* 172: 264–275.

Hoeflich, K. P., J. Luo, E. A. Rubie, M.-S. Tsao, O. Jin, and J. R. Woodgett. 2000. "Requirement for Glycogen Synthase Kinase-3beta in Cell Survival and NF-kappaB Activation." *Nature* 406: 86–90.

Hollomon, M. G., N. Gordon, J. M. Santiago-O'Farrill, and E. S. Kleinerman. 2013. "Knockdown of Autophagy-Related Protein 5, ATG5, Decreases Oxidative Stress and Has an Opposing Effect on Camptothecin-Induced Cytotoxicity in Osteosarcoma Cells." *BMC Cancer* 13: 500.

Hsu, S.-F., C.-M. Chao, W.-T. Huang, M.-T. Lin, and B.-C. Cheng. 2013. "Attenuating Heat-Induced Cellular Autophagy, Apoptosis and Damage in H9c2 Cardiomyocytes by Pre-Inducing HSP70 With Heat Shock Preconditioning." *International Journal of Hyperthermia* 29: 239–247.

Hu, C., X. Zhu, T. Zhang, et al. 2021. "Tanshinone IIA Inhibits Osteosarcoma Growth Through a Src Kinase-Dependent Mechanism." *Evidence-Based Complementary and Alternative Medicine* 2021: 1–15.

Huang, H., Q. Lu, X. Yuan, et al. 2022. "Andrographolide Inhibits the Growth of Human Osteosarcoma Cells by Suppressing Wnt/β-Catenin, PI3K/AKT and NF-κB Signaling Pathways." *Chemico-Biological Interactions* 365: 110068.

Huang, K., Y. Chen, R. Zhang, et al. 2018. "Honokiol Induces Apoptosis and Autophagy via the ROS/ERK1/2 Signaling Pathway in Human Osteosarcoma Cells In Vitro and In Vivo." *Cell Death & Disease* 9: 157.

Hussain, Y., J. Singh, A. Meena, R. A. Sinha, and S. Luqman. 2023. "Escin Enhanced the Efficacy of Sorafenib by Autophagy-Mediated Apoptosis in Lung Cancer Cells." *Phytotherapy Research* 37: 4819–4837.

Hussain, Y., J. Singh, A. Meena, R. A. Sinha, and S. Luqman. 2024. "Escin-Sorafenib Synergy Up-regulatesLC3-IIand p62 to Induce Apoptosis in Hepatocellular Carcinoma Cells." *Environmental Toxicology* 39: 840–856.

Hwang, D.-R., Y.-S. Wu, C.-W. Chang, et al. 2006. "Synthesis and Anti-Viral Activity of a Series of Sesquiterpene Lactones and Analogues in the Subgenomic HCV Replicon System." *Bioorganic & Medicinal Chemistry* 14: 83–91.

Ichimiya, T., T. Yamakawa, T. Hirano, et al. 2020. "Autophagy and Autophagy-Related Diseases: A Review." *International Journal of Molecular Sciences* 21: 8974.

Ivan, A., E. Cojocaru, P. D. Sirbu, et al. 2025. "Clinical and Pathological Profile of Children and Adolescents With Osteosarcoma." *Diagnostics (Basel, Switzerland)* 15: 266.

Jayaraman, S., and V. P. Veeraraghavan. 2025. "Deoxyelephantopin Induces Cell Death in Oral Cancer Cells via the Downregulation of AKT1-mTOR-Mediated Mechanisms." *Journal of Oral and Maxillofacial Pathology* 29: 193–205.

Ji, Z., J. Shen, Y. Lan, Q. Yi, and H. Liu. 2023. "Targeting Signaling Pathways in Osteosarcoma: Mechanisms and Clinical Studies." *MedComm* 4: e308.

Kamenickova, A., E. Anzenbacherova, P. Pavek, et al. 2013. "Pelargonidin Activates the AhR and Induces CYP1A1 in Primary Human Hepatocytes and Human Cancer Cell Lines HepG2 and LS174T." *Toxicology Letters* 218: 253–259.

Kanai, M. 2014. "Therapeutic Applications of Curcumin for Patients With Pancreatic Cancer." *World Journal of Gastroenterology* 20: 9384–9391.

Kang, H., B. Park, H. Kang, H. Park, S. Yu, and I. Kim. 2018. "Delphinidin Induces Apoptosis and Inhibits Epithelial-to-Mesenchymal Transition via the ERK/p38 MAPK-Signaling Pathway in Human Osteosarcoma Cell Lines." *Environmental Toxicology* 33: 640.

Karadas, O., G. Mese, and E. Ozcivici. 2019. "Cytotoxic Tolerance of Healthy and Cancerous Bone Cells to Anti-Microbial Phenolic Compounds Depend on Culture Conditions." *Applied Biochemistry and Biotechnology* 188: 514–526.

Kim, G. D. 2023. "Induction of Hepatocellular Carcinoma Cell Cycle Arrest and Apoptosis byDendropanax morbiferaLeveille Leaf Extract via the PI3K/AKT/mTOR Pathway." *Journal of Cancer Prevention* 28: 185–193.

Kim, H.-J., K. Ravichandran, A. Ozkok, et al. 2014. "The Water-Soluble Triptolide Derivative PG490-88 Protects Against Cisplatin-Induced Acute Kidney Injury." *Journal of Pharmacology and Experimental Therapeutics* 349: 518–525.

17470285, 2026, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/cbdd.70257 by Koc University, Wiley Online Library on [16/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Kim, S.-H., K.-M. Son, K.-Y. Kim, et al. 2017. "Deoxypodophyllotoxin Induces Cytoprotective Autophagy Against Apoptosis via Inhibition of PI3K/AKT/mTOR Pathway in Osteosarcoma U2OS Cells." *Pharmacological Reports* 69: 878–884.

Krosch, T. C. K., V. Sangwan, S. Banerjee, et al. 2013. "Triptolide-Mediated Cell Death in Neuroblastoma Occurs by Both Apoptosis and Autophagy Pathways and Results in Inhibition of Nuclear Factor–Kappa B Activity." *American Journal of Surgery* 205: 387–396.

Kutlu, O., Y. Akkoç, O. Bayraktar, and D. Gozuacik. 2016. "Physiological and Pathological Significance of the Molecular Cross-Talk Between Autophagy and Apoptosis." *Histology and Histopathology* 31: 479–498.

Lee, D.-Y., Y.-J. Park, S.-C. Hwang, K.-D. Kim, D.-K. Moon, and D.-H. Kim. 2018. "Cytotoxic Effects of Delphinidin in Human Osteosarcoma Cells." *Acta Orthopaedica et Traumatologica Turcica* 52: 58–64.

Lee, I.-. C., and J.-. S. Bae. 2017. "Suppressive Effects of Pelargonidin on PolyPhosphate-Mediated Vascular Inflammatory Responses." *Archives of Pharmacal Research* 40: 258.

Lee, J.-W., K.-S. Kim, H.-K. An, C.-H. Kim, H.-I. Moon, and Y.-C. Lee. 2013. "Dendropanoxide Induces Autophagy Through ERK1/2 Activation in MG-63 Human Osteosarcoma Cells and Autophagy Inhibition Enhances Dendropanoxide-Induced Apoptosis." *PLoS One* 8: e83611.

Lee, S. H., S. M. Park, S. M. Park, et al. 2009. "Induction of Apoptosis in Human Leukemia U937 Cells by Anthocyanins Through Down-Regulation of Bcl-2 and Activation of Caspases." *International Journal of Oncology* 34: 1077–1083.

Li, H., C. Zhang, Z. Deng, B. Zhang, and H. Li. 2022. "Antioxidant Activity of Delphinidin and Pelargonidin: Theory and Practice." *Journal of Food Biochemistry* 46: 46.

Li, H.-Y., J. Zhang, L.-L. Sun, et al. 2015. "Celastrol Induces Apoptosis and Autophagy via the ROS/JNK Signaling Pathway in Human Osteosarcoma Cells: An In Vitro and In Vivo Study." *Cell Death & Disease* 6: e1604.

Li, L., B. Sun, Y. Gao, H. Niu, H. Yuan, and H. Lou. 2018. "STAT3 Contributes to Lysosomal-Mediated Cell Death in a Novel Derivative of Riccardin D-Treated Breast Cancer Cells in Association With TFEB." *Biochemical Pharmacology* 150: 267–279.

Li, X., Q. Lu, W. Xie, Y. Wang, and G. Wang. 2018. "Anti-Tumor Effects of Triptolide on Angiogenesis and Cell Apoptosis in Osteosarcoma Cells by Inducing Autophagy via Repressing Wnt/β-Catenin Signaling." *Biochemical and Biophysical Research Communications* 496: 443–449.

Li, Y., J. Zhang, H. Ma, et al. 2013. "Protective Role of Autophagy in Matrine-Induced Gastric Cancer Cell Death." *International Journal of Oncology* 42: 1417–1426.

Li, Z., H. Dong, M. Li, et al. 2018. "Honokiol Induces Autophagy and Apoptosis of Osteosarcoma through PI3K/Akt/mTOR Signaling Pathway." *Molecular Medicine Reports* 17: 2719–2723. https://doi.org/10.3892/mmr.2017.8123.

Liang, C. Z., J. K. Zhang, Z. Shi, B. Liu, C. Q. Shen, and H. M. Tao. 2012. "Matrine Induces Caspase-Dependent Apoptosis in Human Osteosarcoma Cells In Vitro and In Vivo Through the Upregulation of Bax and Fas/FasL and Downregulation of Bcl-2." *Cancer Chemotherapy and Pharmacology* 69: 317–331.

Lin, H., C. Zhang, H. Zhang, et al. 2018. "Physakengose G Induces Apoptosis via EGFR/mTOR Signaling and Inhibits Autophagic Flux in Human Osteosarcoma Cells." *Phytomedicine* 42: 190–198.

Liu, K., F. Luo, S. Xie, et al. 2012. "Glycogen Synthase Kinase 3β Inhibitor (2′Z,3′E)-6-Bromo-Indirubin-3′-Oxime Enhances Drug Resistance to 5-Fluorouracil Chemotherapy in Colon Cancer Cells." *Chinese Journal of Cancer Research* 24: 116.

Liu, S., C. Liu, Y. Wang, et al. 2024. "The Role of Programmed Cell Death in Osteosarcoma: From Pathogenesis to Therapy." *Cancer Medicine* 13: e7303.

Liu, X., N. Wang, Z. He, et al. 2024. "Diallyl Trisulfide Inhibits Osteosarcoma 143B Cell Migration, Invasion and EMT by Inducing Autophagy." *Heliyon* 10: e26681.

Liu, X.-S., J. Jiang, X.-Y. Jiao, Y.-E. Wu, and J.-H. Lin. 2006. "Matrine-Induced Apoptosis in Leukemia U937 Cells: Involvement of Caspases Activation and MAPK-Independent Pathways." *Planta Medica* 72: 501–506.

Liu, Y., Y. Zhang, J. Zou, et al. 2017. "Andrographolide Induces Autophagic Cell Death and Inhibits Invasion and Metastasis of Human Osteosarcoma Cells in an Autophagy-Dependent Manner." *Cellular Physiology and Biochemistry* 44: 1396–1410.

Lo, Y.-C., Y.-C. Lin, Y.-F. Huang, et al. 2017. "Carnosol-Induced ROS Inhibits Cell Viability of Human Osteosarcoma by Apoptosis and Autophagy." *American Journal of Chinese Medicine* 45: 1761.

Longhi, A., C. Errani, M. De Paolis, M. Mercuri, and G. Bacci. 2006. "Primary Bone Osteosarcoma in the Pediatric Age: State of the Art." *Cancer Treatment Reviews* 32: 423–436.

Longo, L., F. Platini, A. Scardino, O. Alabiso, G. Vasapollo, and L. Tessitore. 2008. "Autophagy Inhibition Enhances Anthocyanin-Induced Apoptosis in Hepatocellular Carcinoma." *Molecular Cancer Therapeutics* 7: 2476–2485.

Luo, C., Y. Zhu, T. Jiang, et al. 2007. "Matrine Induced Gastric Cancer MKN45 Cells Apoptosis via Increasing Pro-Apoptotic Molecules of Bcl-2 Family." *Toxicology* 229: 245–252.

Ma, H., Y. Meng, Y. Wu, et al. 2021. "Inhibition of Osteosarcoma Cell Proliferation by Matrine (MT) via Targeting Histone H3 Lysine 27 Trimethylation and AMPK Activation." *Die Pharmazie – An International Journal of Pharmaceutical Sciences* 76, no. 1: 38–41. https://doi.org/10.1691/ph.2021.0718.

Ma, K., M.-Y. Huang, Y.-X. Guo, and G.-Q. Hu. 2016. "Matrine-Induced Autophagy Counteracts Cell Apoptosis via the ERK Signaling Pathway in Osteosarcoma Cells." *Oncology Letters* 12: 1854.

Ma, K., C. Zhang, M.-Y. Huang, Y.-X. Guo, and G.-Q. Hu. 2016. "Crosstalk Between Beclin-1-Dependent Autophagy and Caspasedependent Apoptosis Induced by Tanshinone IIA in Human Osteosarcoma MG-63 Cells." *Oncology Reports* 36: 1807–1818.

Ma, K., C. Zhang, M.-Y. Huang, W.-Y. Li, and G.-Q. Hu. 2016. "Cinobufagin Induces Autophagy-Mediated Cell Death in Human Osteosarcoma U2OS Cells Through the ROS/JNK/p38 Signaling Pathway." *Oncology Reports* 36: 90–98.

Meyers, P. A., C. L. Schwartz, M. D. Krailo, et al. 2008. "Osteosarcoma: The Addition of Muramyl Tripeptide to Chemotherapy Improves Overall Survival—A Report From the Children's Oncology Group." *Journal of Clinical Oncology* 26: 633–638.

Morana, J. M., O. Leal-Hernande, M. L. Canal-Macías, et al. 2016. "Antiproliferative Properties of Oleuropein in Human Osteosarcoma Cells." *Natural Product Communications* 11: 491–492.

Mujumdar, N., T. N. Mackenzie, V. Dudeja, et al. 2010. "Triptolide Induces Cell Death in Pancreatic Cancer Cells by Apoptotic and Autophagic Pathways." *Gastroenterology* 139: 598.

Ning, B., Y. Liu, T. Huang, and Y. Wei. 2023. "Autophagy and Its Role in Osteosarcoma." *Cancer Medicine* 12: 5676–5687.

Nisar, S., T. Masoodi, K. S. Prabhu, et al. 2022. "Natural Products as Chemo-Radiation Therapy Sensitizers in Cancers." *Biomedicine & Pharmacotherapy* 154: 113610.

Palmerini, E., R. L. Jones, E. Marchesi, et al. 2016. "Gemcitabine and Docetaxel in Relapsed and Unresectable High-Grade Osteosarcoma and Spindle Cell Sarcoma of Bone." *BMC Cancer* 16: 280.

Paunovic, V., M. Kosic, S. Djordjevic, et al. 2016. "Marrubium Vulgare Ethanolic Extract Induces Proliferation Block, Apoptosis, and Cytoprotective Autophagy in Cancer Cells In Vitro." *Cellular and Molecular Biology (Noisy-le-Grand, France)* 62: 108–114.

17470285, 2026, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/cbdd.70257 by Koc University, Wiley Online Library on [16/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Przychodzen, P., R. Wyszkowska, M. Gorzynik-Debicka, T. Kostrzewa, A. Kuban-Jankowska, and M. Gorska-Ponikowska. 2019. "Anticancer Potential of Oleuropein, the Polyphenol of Olive Oil, With 2-Methoxyestradiol, Separately or in Combination, in Human Osteosarcoma Cells." *Anticancer Research* 39: 1243–1251.

Reddivari, L., J. Vanamala, S. Chintharlapalli, S. H. Safe, and J. C. Miller. 2007. "Anthocyanin Fraction From Potato Extracts Is Cytotoxic to Prostate Cancer Cells Through Activation of Caspase-Dependent and Caspase-Independent Pathways." *Carcinogenesis* 28: 2227.

Ritter, J., and S. S. Bielack. 2010. "Osteosarcoma." *Annals of Oncology* 21: vii320.

Rossi, M., C. Pellegrino, M. M. Rydzyk, et al. 2024. "Chalcones Induce Apoptosis, Autophagy and Reduce Spreading in Osteosarcoma 3D Models." *Biomedicine & Pharmacotherapy* 179: 117284.

Russo, M., S. Moccia, S. Bilotto, et al. 2017. "A Carotenoid Extract From a Southern Italian Cultivar of Pumpkin Triggers Nonprotective Autophagy in Malignant Cells." *Oxidative Medicine and Cellular Longevity* 2017: 7468538.

Russo, M., S. Moccia, C. Spagnuolo, I. Tedesco, and G. L. Russo. 2022. "Carotenoid-Enriched Nanoemulsions and γ-Rays Synergistically Induce Cell Death in a Novel Radioresistant Osteosarcoma Cell Line." *International Journal of Molecular Sciences* 23: 15959.

Sakamoto, A., and Y. Iwamoto. 2008. "Current Status and Perspectives Regarding the Treatment of Osteosarcoma: Chemotherapy." *Reviews on Recent Clinical Trials* 3: 228–231.

Shen, T.-S., Y.-K. Hsu, Y.-F. Huang, H.-Y. Chen, C.-P. Hsieh, and C.-L. Chen. 2019. "Licochalcone A Suppresses the Proliferation of Osteosarcoma Cells Through Autophagy and ATM-Chk2 Activation." *Molecules* 24: 2435.

Shi, S., X. Ou, C. Liu, R. Li, Q. Zheng, and L. Hu. 2025. "NF-κB Signaling and the Tumor Microenvironment in Osteosarcoma: Implications for Immune Evasion and Therapeutic Resistance." *Frontiers in Immunology* 16: 1518664.

Shin, D. Y., W. S. Lee, J. N. Lu, et al. 2009. "Induction of Apoptosis in Human Colon Cancer HCT-116 Cells by Anthocyanins Through Suppression of Akt and Activation of p38-MAPK." *International Journal of Oncology* 35: 1499–1504.

Sohn, E. J., and H. T. Park. 2017. "Natural Agents Mediated Autophagic Signal Networks in Cancer." *Cancer Cell International* 17: 110.

Steinmann, P., D. K. Walters, M. J. E. Arlt, et al. 2012. "Antimetastatic Activity of Honokiol in Osteosarcoma." *Cancer* 118: 2117–2127.

Sun, Y., D. K. St. Clair, Y. Xu, P. A. Crooks, and W. H. St. Clair. 2010. "A NADPH Oxidase–Dependent Redox Signaling Pathway Mediates the Selective Radiosensitization Effect of Parthenolide in Prostate Cancer Cells." *Cancer Research* 70: 2880.

Tang, X., L. Luo, W. Zhou, et al. 2025. "Deoxypodophyllotoxin Mediates Autophagy Death Through Inhibition of GRP78 in Human Osteosarcoma." *Current Cancer Drug Targets* 25: 1118–1133.

Tedesco, G., M. Santarosa, and R. Maestro. 2024. "Beyond Self-Eating: Emerging Autophagy-Independent Functions for the Autophagy Molecules in Cancer (Review)." *International Journal of Oncology* 64: 57.

Terlikowska, K., A. Witkowska, and S. Terlikowski. 2014. "Curcumin in Chemoprevention of Breast Cancer." *Postępy Higieny i Medycyny Doświadczalnej* 68: 571–578.

Tian, Z., C. Sun, and J. Liu. 2022. "Pelargonidin Inhibits Vascularization and Metastasis of Brain Gliomas by Blocking the PI3K/AKT/mTOR Pathway." *Journal of Biosciences* 47: 64.

Wang, H., T. Zhang, W. Sun, et al. 2016. "Erianin Induces G2/M-Phase Arrest, Apoptosis, and Autophagy via the ROS/JNK Signaling Pathway in Human Osteosarcoma Cells In Vitro and In Vivo." *Cell Death & Disease* 7: e2247.

Wang, L., C. Gao, S. Yao, and B. Xie. 2013. "Blocking Autophagic Flux Enhances Matrine-Induced Apoptosis in Human Hepatoma Cells." *International Journal of Molecular Sciences* 14: 23212–23230.

Wang, Y., X. Deng, C. Yu, et al. 2018. "Synergistic Inhibitory Effects of Capsaicin Combined With Cisplatin on Human Osteosarcoma in Culture and in Xenografts." *Journal of Experimental & Clinical Cancer Research* 37: 251.

Wang, Y., Y. Ji, Z. Hu, et al. 2013. "Riccardin D Induces Cell Death by Activation of Apoptosis and Autophagy in Osteosarcoma Cells." *Toxicology In Vitro* 27: 1928.

Wang, Y., D. Kong, X. Wang, X. Dong, Y. Tao, and H. Gong. 2015. "Molecular Mechanisms of Luteolin Induced Growth Inhibition and Apoptosis of Human Osteosarcoma Cells." *Iranian Journal of Pharmaceutical Research* 14: 531–538.

Yang, C., Q. O. Yang, Q.-J. Kong, W. Yuan, and Y.-P. Ou Yang. 2016. "Parthenolide Induces Reactive Oxygen Species-Mediated Autophagic Cell Death in Human Osteosarcoma Cells." *Cellular Physiology and Biochemistry* 40: 146.

Yang, D., H. Zhang, J. Wu, et al. 2019. "The Role of Chamaejasmine in Cellular Apoptosis and Autophagy in MG-63 Cells." *Bioscience Reports* 39: BSR20181707.

Yang, J., Y. Zou, and D. Jiang. 2018. "Honokiol Suppresses Proliferation and Induces Apoptosis via Regulation of the miR-21/PTEN/PI3K/AKT Signaling Pathway in Human Osteosarcoma Cells." *International Journal of Molecular Medicine* 41: 1845–1854.

Yen, J.-H., S.-T. Huang, H.-S. Huang, et al. 2018. "HGK-Sestrin 2 Signaling-Mediated Autophagy Contributes to Antitumor Efficacy of Tanshinone IIA in Human Osteosarcoma Cells." *Cell Death & Disease* 9: 1003.

Yixiang, H., Z. Shenghui, W. Jianbo, et al. 2010. "Matrine Induces Apoptosis of Human Multiple Myeloma Cells via Activation of the Mitochondrial Pathway." *Leukemia & Lymphoma* 51: 1337–1346.

Yu, D., S. Zhang, A. Feng, et al. 2019. "Methotrexate, Doxorubicin, and Cisplatinum Regimen Is Still the Preferred Option for Osteosarcoma Chemotherapy." *Medicine (Baltimore)* 98: e15582.

Yu, P., Q. Liu, K. Liu, K. Yagasaki, E. Wu, and G. Zhang. 2009. "Matrine Suppresses Breast Cancer Cell Proliferation and Invasion via VEGF-Akt-NF-κB Signaling." *Cytotechnology* 59: 219–229.

Yuan, Z., Y. Li, S. Zhang, et al. 2023. "Extracellular Matrix Remodeling in Tumor Progression and Immune Escape: From Mechanisms to Treatments." *Molecular Cancer* 22: 48.

Yue, Z., X. Guan, R. Chao, et al. 2019. "Diallyl Disulfide Induces Apoptosis and Autophagy in Human Osteosarcoma MG-63 Cells Through the PI3K/Akt/mTOR Pathway." *Molecules* 24: 2665.

Yun, S., J. H. Jung, S. Jeong, E. J. Sohn, B. Kim, and S. Kim. 2014. "Tanshinone IIA Induces Autophagic Cell Death via Activation of AMPK and ERK and Inhibition of mTOR and p70 S6K in KBM-5 Leukemia Cells." *Phytotherapy Research* 28: 458–464.

Zeng, Z., H. Li, C. Luo, W. Hu, T. Weng, and F. Shuang. 2023. "Pelargonidin Ameliorates Inflammatory Response and Cartilage Degeneration in Osteoarthritis via Suppressing the NF-κB Pathway." *Archives of Biochemistry and Biophysics* 743: 109668.

Zhang, B., X. Yu, and H. Xia. 2015. "The Flavonoid Luteolin Enhances Doxorubicin-Induced Autophagy in Human Osteosarcoma U2OS Cells." *International Journal of Clinical and Experimental Medicine* 8: 15190–15197.

Zhang, C., S. Zhou, L. Feng, et al. 2013. "In Vitro Anti-Cancer Activity of Chamaejasmenin B and Neochamaejasmin C Isolated From the Root of Stellera Chamaejasme L." *Acta Pharmacologica Sinica* 34: 262–270.

Zhang, J.-Q. 2010. "Antitumor Effect of Matrine in Human Hepatoma G2 Cells by Inducing Apoptosis and Autophagy." *World Journal of Gastroenterology* 16: 4281–4290.

17470285, 2026, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/cbdd.70257 by Koc University, Wiley Online Library on [16/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Zhang, L., T. Wang, X. Wen, et al. 2007. "Effect of Matrine on HeLa Cell Adhesion and Migration." *European Journal of Pharmacology* 563: 69–76.

Zhang, X., H. Chen, Y. Zhang, et al. 2022. "HA-DOPE-Modified Honokiol-Loaded Liposomes Targeted Therapy for Osteosarcoma." *International Journal of Nanomedicine* 17: 5137–5151.

Zhang, Y., P. Chen, H. Hong, L. Wang, Y. Zhou, and Y. Lang. 2017. "JNK Pathway Mediates Curcumin-Induced Apoptosis and Autophagy in Osteosarcoma MG63 Cells." *Experimental and Therapeutic Medicine* 14: 593–599.

Zhang, Y., R. Ma, S. Cheng, and G. Gu. 2018. "Marrubenol Inhibits Osteosarcoma Cancer Cell Growth by Inducing Autophagic Cell Death and Inhibiting Cancer Cell Migration and Invasion." *Journal of the Balkan Union of Oncology* 23: 729–734.

Zhao, L., B. Jiang, D. Wang, et al. 2016. "Triptolide Reduces the Viability of Osteosarcoma Cells by Reducing MKP-1 and Hsp70 Expression, 2010." *Experimental and Therapeutic Medicine* 11: 2005.

Zhao, X., M. Bao, F. Zhang, and W. Wang. 2020. "Camptothecin Induced DDX5 Degradation Increased the Camptothecin Resistance of Osteosarcoma." *Experimental Cell Research* 394: 112148.

Zheng, Y., Z. Liu, X. Yang, L. Liu, and K. S. Ahn. 2022. "An Updated Review on the Potential Antineoplastic Actions of Oleuropein." *Phytotherapy Research* 36: 365–379.

Zhou, W., M. Hao, X. Du, K. Chen, G. Wang, and J. Yang. 2014. "Advances in Targeted Therapy for Osteosarcoma." *Discovery Medicine* 17: 301–307.

Zhou, Z.-L., Y.-X. Yang, J. Ding, Y.-C. Li, and Z.-H. Miao. 2012. "Triptolide: Structural Modifications, Structure–Activity Relationships, Bioactivities, Clinical Development and Mechanisms." *Natural Product Reports* 29: 457.

Zhu, J., W. Yu, B. Liu, et al. 2017. "Escin Induces Caspase-Dependent Apoptosis and Autophagy Through the ROS/p38 MAPK Signalling Pathway in Human Osteosarcoma Cells In Vitro and In Vivo." *Cell Death & Disease* 8: e3113.

Zhu, M., J. Ying, C. Lin, et al. 2018. "β-Escin Inhibits the Proliferation of Osteosarcoma Cells *via* Blocking the PI3K/Akt Pathway." *RSC Advances* 8: 29637.

Zou, J., Y. Zhang, J. Sun, et al. 2017. "Deoxyelephantopin Induces Reactive Oxygen Species-Mediated Apoptosis and Autophagy in Human Osteosarcoma Cells." *Cellular Physiology and Biochemistry* 42: 1812.

Zuch, D., A. Giang, Y. Shapovalov, et al. 2012. "Targeting Radioresistant Osteosarcoma Cells With Parthenolide." *Journal of Cellular Biochemistry* 113: 1282–1291.

17470285, 2026, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/cbdd.70257 by Koc University, Wiley Online Library on [16/04/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License