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Review

Natural and Synthetic Coumarins as Tumor Microenvironment-Oriented Immunotherapeutic Agents: A Review

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Abstract: The tumor microenvironment (TME) plays a major role in cancer progression, immune evasion, and resistance to immunotherapy. Coumarins, a structurally diverse class of natural and synthetic compounds, have attracted increasing attention because of their anticancer, anti-inflammatory, antioxidant, and immunomodulatory properties, suggesting potential applications in TME-oriented cancer immunotherapy. This review aimed to summarize the immunotherapeutic potential of natural and synthetic coumarin derivatives in modulating TME and enhancing anticancer immune responses. A narrative review of the literature was conducted focusing on the effects of coumarin derivatives on immune-cell regulation, immune checkpoint signaling, angiogenesis, extracellular matrix remodeling, metabolic reprogramming, and hypoxia-related pathways within TME. Evidence from preclinical studies involving natural and synthetic coumarins was critically evaluated. Preclinical findings indicate that coumarin derivatives can modulate several components of TME by promoting M1 macrophage polarization, activating dendritic cells and natural killer cells, enhancing T-cell–mediated immunity, and suppressing immunosuppressive cell populations such as regulatory T cells and M2 macrophages. Several coumarins also demonstrated the ability to inhibit tumor glycolysis, attenuate hypoxia-associated immune resistance, normalize angiogenesis, and regulate inflammatory signaling pathways. Synthetic coumarins showed additional promise through structural optimization and targeted delivery approaches, while their potential complementary effects with immune checkpoint inhibitors remain to be established experimentally. However, available evidence remains limited, and further mechanistic and translational investigations are required. Natural and synthetic coumarins represent promising multifunctional immunomodulators capable of reshaping TME and improving anticancer immune responses. Their diverse biological activities support their future development as adjuncts or enhancers of cancer immunotherapy.

Keywords: Coumarins; Tumor Microenvironment; Cancer Immunotherapy; Immunomodulatory Agents; Metabolic Reprogramming

1. Introduction

An overview of tumor microenvironment (TME)-targeted immunotherapy is presented with particular emphasis on the potential roles of natural and synthetic coumarin derivatives. This review summarizes the fundamental characteristics of TME and the cancer–immunity cycle, highlighting the functions of major immune components, including T lymphocytes, dendritic cells (DCs), macrophages, and natural killer cells (NKs), together with the immune checkpoints that regulate antitumor responses. In addition, coumarin derivatives with the potential to influence immune signaling, intercellular communication, hypoxia, angiogenesis, and extracellular matrix (ECM) remodeling are critically discussed.

Cancer remains the second leading cause of mortality worldwide despite major advances in diagnosis and treatment. In recent years, immunotherapy has emerged as one of the most promising therapeutic strategies because it can produce more durable and selective antitumor responses than conventional approaches such as chemotherapy and radiotherapy [1]. However, the effectiveness of immunotherapy is strongly influenced by TME, which can either support immune-mediated tumor elimination or promote immune evasion and therapeutic resistance. Consequently, considerable attention has been directed toward developing strategies capable of overcoming TME-associated immunosuppressive barriers and restoring effective immune surveillance [2].

Within this context, coumarins have attracted increasing scientific interest. These naturally occurring or synthetically modified oxygen-containing bicyclic aromatic compounds represent one of the largest and most structurally diverse classes of phytochemicals [3]. Numerous studies have demonstrated that coumarins possess a broad spectrum of biological and pharmacological activities, including anti-inflammatory [4], antioxidant [5], anticancer [6], anti-hyperlipidemia [7], anti-obesity [8], and immunomodulatory effects [9]. Several coumarin derivatives have been shown to regulate immune responses by modulating the activity and distribution of key immune cell populations, and specific structural characteristics appear to contribute to these immunological effects [10]. Furthermore, some coumarins exhibit prolyl hydroxylase inhibitory activity, which may alleviate hypoxia-driven immunosuppression within TME and thereby enhance the efficacy of immunotherapeutic interventions [11]. Accordingly, this review examines selected natural and synthetic coumarins that may influence immune activation, promote immune cell–cell interactions, regulate angiogenesis and hypoxic signaling, or modify ECM dynamics. The available evidence is interpreted in light of current diagnostic and therapeutic concepts related to immune checkpoint regulation and tumor-associated immunostimulatory or immunosuppressive mechanisms.

Literature Search Strategy and Study Selection

A narrative literature search was conducted to identify relevant studies investigating the immunomodulatory and anticancer properties of natural and synthetic coumarin derivatives, with particular emphasis on their effects within the tumor microenvironment and their potential relevance to cancer immunotherapy. The literature search was performed using PubMed/MEDLINE, Scopus, and Web of Science databases. Publications available from database inception until March 2026 were considered.

The search strategy included combinations of keywords and Medical Subject Headings terms related to coumarins, tumor biology, and immune regulation. The main search terms included “coumarin”, “coumarin derivatives”, “natural coumarins”, “synthetic coumarins”, “tumor microenvironment”, “cancer immunotherapy”, “immune checkpoint”, “immune modulation”, “macrophage polarization”, “dendritic cells”, “natural killer cells”, “T cells”, “angiogenesis”, “hypoxia”, “metabolic reprogramming”, and “extracellular matrix remodeling”. Additional relevant publications were identified through manual examination of reference lists from selected articles.

Studies were selected based on their relevance to the objectives of this review. Included studies comprised experimental investigations evaluating the effects of coumarin-based compounds on cancer-associated immune mechanisms, tumor-associated immune cells, immune signaling pathways, or other components of the TME. Both *in vitro* and *in vivo* preclinical studies were considered, together with available clinical evidence when relevant. Studies unrelated to cancer immunology, publications lacking mechanistic insights, duplicate records, and non-English articles were excluded. The identified literature was critically analyzed and organized according to the major mechanisms through which coumarins may influence tumor immunity, including immune-cell modulation, immune checkpoint regulation, angiogenesis, ECM remodeling, hypoxia-associated pathways, and metabolic reprogramming.

To ensure consistent interpretation of the graphical summaries, common terminology, abbreviations, symbols, and visual conventions were applied across the figures. Where mechanistic relationships are depicted, experimentally supported effects are distinguished from proposed or indirect pathways. Solid connections denote relationships supported by the experimental evidence discussed in the text, whereas dashed connections indicate mechanistically plausible or hypothesis-generating relationships that require further validation.

2. Conceptual Framework: TME and Immunotherapy

TME, as illustrated in **Figure 1**, has reshaped the traditional view of tumors as isolated masses of malignant cells. Instead, tumors are now recognized as dynamic and highly interactive ecosystems in which cancer cells contin-

uously communicate with stromal, vascular, and immune components through metabolic, molecular, and inflammatory signaling networks. These reciprocal interactions critically influence whether tumor immunogenicity is sufficient to initiate and sustain the cancer-immunity cycle [12]. Accordingly, coumarin derivatives have attracted growing attention because of their ability to modulate multiple cellular and molecular components within TME [13]. Their immunomodulatory, anti-inflammatory, antioxidant, and signaling-regulatory properties suggest that they may serve as promising agents for remodeling TME and enhancing the efficacy of cancer immunotherapy [14]. Understanding the dual roles of tumor-promoting and tumor-restraining elements within TME is therefore essential for evaluating the therapeutic potential of coumarins in the context of immune-oriented anticancer strategies [15].

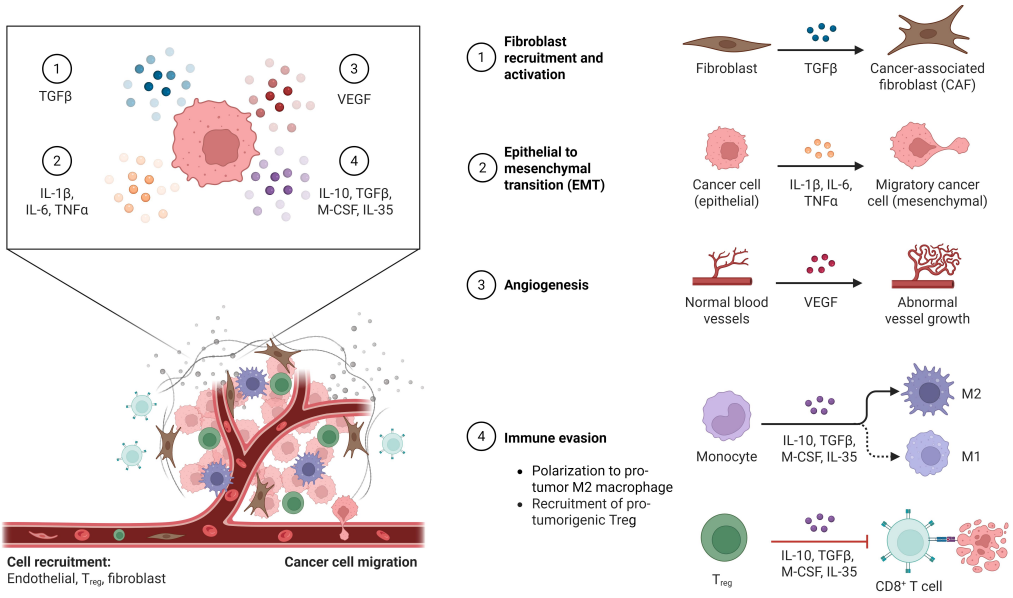

Figure 1. Overview of TME and its cellular interactions in cancer progression and antitumor immunity.

The cancer-immunity cycle describes the sequential events required for an effective antitumor immune response, beginning with the release of tumor-associated antigens and culminating in the recognition and elimination of malignant cells by cytotoxic immune effectors [16]. However, tumors frequently evade immune destruction through the activation of immune checkpoint pathways and the establishment of an immunosuppressive TME [17]. Numerous natural and synthetic coumarin derivatives have been reported to influence immune checkpoint signaling, cytokine production, oxidative stress, and inflammatory pathways, thereby supporting their potential role as modulators of different stages of the cancer-immunity cycle [18]. Importantly, coumarins may also contribute to overcoming TME-associated barriers that limit immune cell infiltration, activation, and persistence. In this regard, TME often represents a major obstacle to effective anticancer immunity, despite the intrinsic capacity of the immune system to recognize malignant cells [19].

Within TME, immune cells can exert either tumor-promoting or tumor-suppressive functions, as displayed in **Figure 2**, depending on their phenotype, activation status, and local signaling conditions. Antitumor immune populations, including $CD8^+$ cytotoxic T lymphocytes, NKs, DCs, and M1-polarized macrophages, contribute to tumor elimination through direct cytotoxicity, antigen presentation, and the production of pro-inflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). In contrast, protumor immune components—including regulatory T cells (Tregs), myeloid-derived suppressor cells, M2-polarized macrophages, and tolerogenic DCs—promote immune evasion, angiogenesis, tumor growth, and metastatic progression by suppressing effector immune responses and generating an immunosuppressive cytokine milieu [20]. The balance between these opposing immune populations largely determines whether TME remains immunologically "hot" and responsive to therapy or becomes "cold" and resistant to immune-mediated destruction [21], as shown in **Figure 3**. Emerging evidence suggests that coumarin derivatives may influence this balance by suppressing immunosuppressive cell populations while enhancing the activity of cytotoxic and antigen-presenting immune cells, thereby supporting a

more favorable antitumor microenvironment [22].

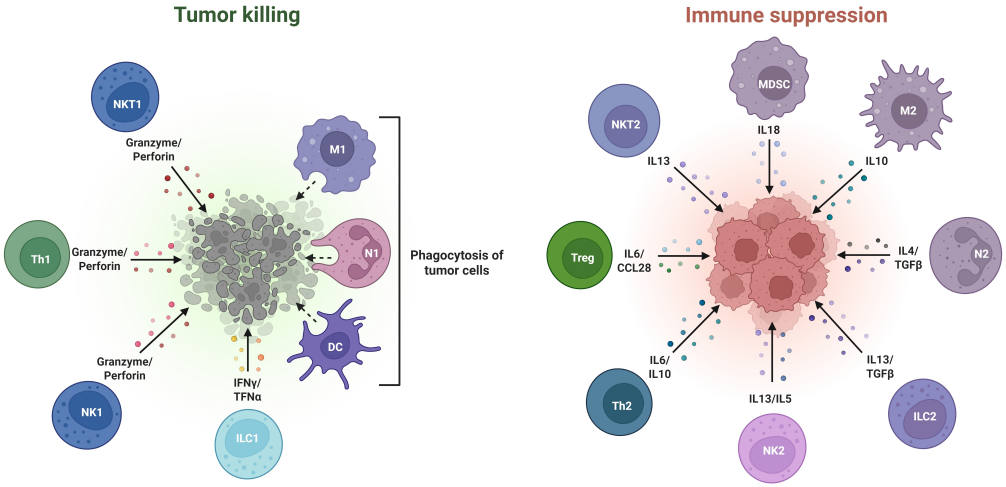

Figure 2. Protumor and antitumor immune cell populations within TME.

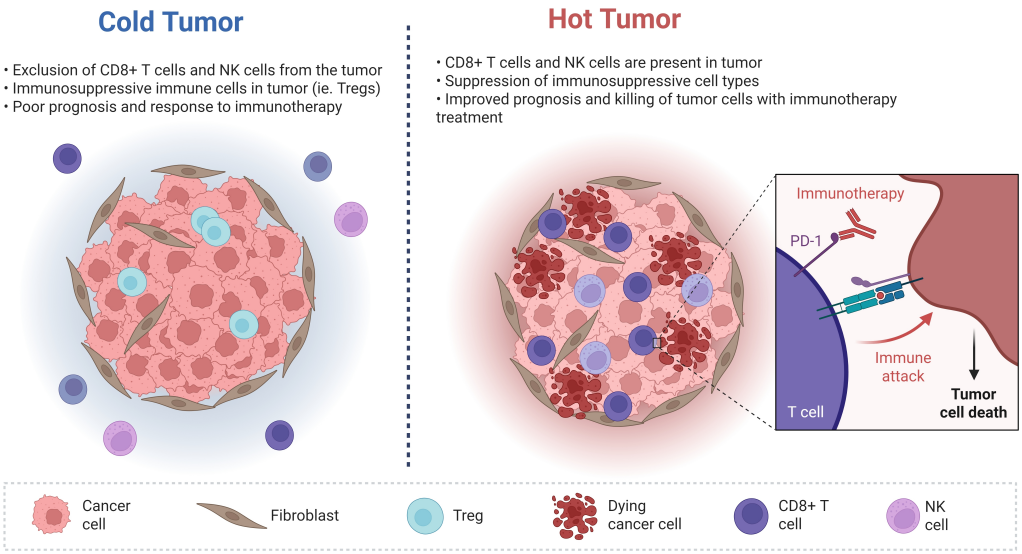

Figure 3. Characteristics of hot and cold tumors based on TME cellular features.

To provide a coherent framework for evaluating these multifaceted effects, the subsequent discussion is organized across complementary chemical, biological, and translational dimensions. Natural coumarins are first considered according to their chemical diversity and biological properties, followed by synthetic derivatives with emphasis on structure–activity relationships and pharmacological optimization. Coumarin-mediated TME regulation is then examined by distinguishing direct effects on immune-cell populations from modulation of non-immune components, including tumor metabolism, hypoxia, angiogenesis, and extracellular matrix remodeling. Finally, delivery and potential combination strategies are integrated with a critical appraisal of preclinical evidence, safety, pharmacokinetic limitations, and the principal barriers to clinical translation.

3. Chemical Classes and Biological Diversity of Natural Coumarins

Coumarins are a diverse group of naturally occurring compounds widely distributed in flowering plants and characterized by remarkable structural variability and broad biological activity [23]. Simple coumarin scaffolds are commonly biosynthesized by lower plants, whereas structurally more elaborate coumarins bearing multiple

substitutions are found in both lower and higher plant species [24]. In contrast, coumarins produced by fungi and microorganisms are generally less abundant but often possess greater structural complexity [25]. Pharmacologically, coumarins exhibit a wide spectrum of activities, including anticancer [26], antifungal [27], antiviral [28], anti-Alzheimer's [29], anti-inflammatory [30], and antibacterial [31] effects. Nevertheless, growing attention has been directed toward their immunological properties, particularly their roles as immunomodulatory agents, immune-supportive compounds, and therapeutic adjuvants [32]. In the context of TME-oriented immunotherapy, naturally occurring coumarins can be broadly classified into three principal categories: simple coumarins, hydroxylated coumarins, and those containing more complex substitution patterns [33].

Many natural coumarin derivatives have demonstrated considerable potential in restoring immune balance and enhancing antitumor immune responses. Evidence indicates that they can improve the functional activity of DCs, NKs, T lymphocytes, and macrophages, while also modulating the expression of immune checkpoint proteins and enhancing vaccine-mediated immunity [34]. In addition, coumarins may facilitate the activation and maturation of both T-cell and B-cell responses, thereby strengthening the tumor immunity–cancer cycle [35]. Collectively, these findings highlight coumarins as valuable molecular templates for the development of synthetic immunotherapeutic agents. Their structural diversity provides a robust platform for rational drug design and systematic exploration of chemical modifications aimed at optimizing coumarin-based immunomodulatory activity within the TME [36].

3.1. Simple Natural Coumarins

These compounds are characterized by a benzopyran-2-one core structure and share biosynthetic origins with flavonoids through the broader phenylpropanoid pathway, although their downstream biosynthetic routes diverge [37]. Among the naturally occurring immunomodulatory coumarins, scopoletin and umbelliferone, with chemical structures displayed in **Figure 4**, are considered two of the most prominent bioactive representatives. Scopoletin primarily exerts its immunomodulatory effects within the macrophage–tumor cell interaction axis [38], whereas umbelliferone has been associated with the regulation of T-cell–mediated antitumor responses [39]. In contrast, simple coumarins arise from alternative branches of coumarin metabolism and are associated with distinct biological activities [40]. Recent studies have demonstrated that the simple coumarin derivatives 4-hydroxycoumarin and hymecromone (**Figure 4**) can induce apoptosis in senescent T cells through activation of caspase-3- and caspase-9-dependent pathways. This pro-apoptotic effect is believed to involve complex interactions between senescence-associated secretory phenotype mediators and immune regulatory signaling networks [41]. By facilitating the elimination of dysfunctional senescent T cells that release immunosuppressive and tumor-promoting soluble factors, these coumarins may help attenuate immune evasion within TME [42]. Such findings further support the potential application coumarin and its related derivatives as adjuvant agents in immune checkpoint–based cancer immunotherapy.

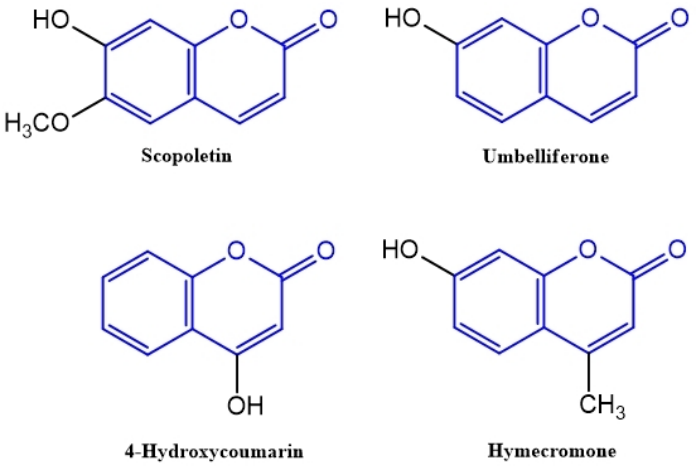

Figure 4. Chemical structures of selected natural immunoactive simple coumarins, highlighting the coumarin core scaffold in blue.

3.2. Mechanisms of Action Relevant to TME

Coumarins can influence multiple interconnected pathways within TME, extending their biological effects beyond direct tumor-cell cytotoxicity. Available evidence encompasses immune-cell regulation, inflammatory signaling, tumor metabolism, vascular remodeling, hypoxia-associated pathways, and stromal organization. These mechanisms are considered systematically in the subsequent sections according to their direct immune, non-immune microenvironmental, and translational relevance [43–45].

The antitumor effects of coumarins are closely associated with their capacity to influence key components of TME, including immune checkpoint pathways, stromal and vascular elements, metabolic networks, and inflammatory signaling cascades. Through these interactions, coumarins have demonstrated the capacity to influence immune recognition pathways and effector immune functions in experimental models; however, whether these molecular effects translate into clinically meaningful enhancement of antitumor immunity remains to be determined [46]. The diverse molecular targets affected by coumarins highlight their ability to address multiple barriers that limit effective immune-mediated tumor elimination. Consequently, coumarin-based modulation of TME represents a promising and multifaceted strategy for the development of next-generation anticancer therapeutics [47].

Despite these promising observations, the interpretation of coumarin-mediated TME modulation requires careful consideration of experimental context. Many reported immunological effects have been obtained using isolated immune-cell systems, inflammatory models, or cancer cell lines rather than intact immune-competent tumor models. Therefore, enhancement of immune-related signaling pathways or alteration of cytokine profiles does not necessarily indicate a clinically meaningful improvement in antitumor immunity. Differences in experimental design, including compound concentration, exposure duration, cellular background, and selection of immune endpoints, may contribute to variability among reported findings. Consequently, the therapeutic relevance of individual coumarin derivatives should be evaluated according to the strength and translational relevance of the supporting evidence [48].

To facilitate interpretation of this heterogeneous evidence base, **Table 1** summarizes the experimental context supporting each proposed TME-related effect, including the compound type, experimental system or tumor model, pharmacological exposure where available, major immune or mechanistic endpoints, combination treatment, and level of evidence. Importantly, therapeutic relevance is distinguished from experimentally demonstrated treatment benefit; effects inferred from isolated immune-cell systems, cancer cell lines, or pathway-based studies are therefore described as mechanistic or hypothesis-generating rather than as established immunotherapeutic efficacy.

Table 1. Experimental evidence supporting TME–relevant immunomodulatory effects of selected coumarin derivatives.

TME-Related Mechanism	Immunological Effect(s)	Coumarin Derivatives	Therapeutic Significance	Evidence Level/Model	Ref.
M1 macrophage polarization	Promotes pro-inflammatory macrophage phenotype and enhances antitumor cytokine production	Scopoletin, esculetin, and osthole	Enhances tumoricidal immune activity and reduces immunosuppressive macrophage populations	Stimulated macrophage-based systems; direct immunomodulatory evidence, with limited validation in tumor-associated models	Jayasingam et al. [49], Ouyang et al. [50], Chen et al. [51]
DC activation and maturation	Increases antigen presentation and T-cell priming through upregulation of MHC-II and co-stimulatory molecules	Umbelliferone and hymecromone	Improves initiation of the cancer-immunity cycle and strengthens adaptive immunity	DC culture systems and engineered delivery platforms; direct immunomodulatory/preclinical evidence, with limited tumor-associated validation	Kim et al. [52], Li et al. [53]
NK stimulation	Enhances NK cell cytotoxicity and secretion of IFN-γ and TNF-α	Umbelliferone and imperatorin	Promotes direct elimination of tumor cells and amplifies innate immune surveillance	NK-cell functional assays and immune-cell activation studies; direct immunomodulatory evidence	Kornicka et al. [54], Rodrigues et al. [55]
Treg cell suppression	Reduces immunosuppressive signaling within TME	4-Hydroxycoumarin	Restores effector T-cell activity and improves responsiveness to immunotherapy	T-cell activation and immune-signaling studies; mechanistic/direct immunomodulatory evidence	Bajaj et al. [56]
Th1/Th17 immune polarization	Favors pro-inflammatory T-cell responses over tolerogenic pathways	Esculetin, daphnetin, and fraxetin	Enhances cytotoxic immune responses against tumor cells	Cellular immune models; direct immunomodulatory evidence, with limited tumor-associated validation	Chen et al. [57], Zhang et al. [58], Mazumder et al. [59]
Induction of senescent T-cell apoptosis	Activates caspase-3 and caspase-9 pathways in dysfunctional T cells	4-Hydroxycoumarin and hymecromone	Eliminates senescent immunosuppressive cells and limits tumor-promoting inflammation	Senescent T-cell experimental systems; mechanistic/direct cellular evidence	Liu et al. [60], Hosseini et al. [61]
Immune checkpoint modulation	Alters immune checkpoint signaling and associated cytokine networks	7-Geranyloxycoumarin	Provides a mechanistic rationale for possible combination with immune checkpoint modulation; direct combination benefit has not been established	Mechanistic studies and limited preclinical observations; hypothesis-generating evidence	Hosseini et al. [61]
Inhibition of tumor glycolysis	Reduces glucose consumption and lactate production in tumor cells	5-Methoxy-6-hydroxy-7-hydrosulfonylcoumarin and 3-hydroxymethylcoumarin derivatives	Counteracts the Warburg effect and alleviates metabolic immunosuppression	Cancer-cell metabolic models; mechanistic/pathway evidence without direct demonstration of immune-mediated tumor control	Ji et al. [62], Zhou et al. [63]

Table 1. *Cont.*

TME-Related Mechanism	Immunological Effect(s)	Coumarin Derivatives	Therapeutic Significance	Evidence Level/Model	Ref.
Regulation of hypoxia signaling	Attenuates hypoxia-associated immune resistance and HIF-mediated pathways	Esculetin	Enhances immune-cell infiltration and improves antitumor immune persistence	Cancer-cell and pathway-based models; mechanistic/pathway evidence	Yang et al. [64]
Angiogenesis normalization	Modulates endothelial-cell proliferation and vascular remodeling	Osthole, fraxetin, and 7-ethoxycoumarin derivatives	Improves immune-cell access to tumors and reduces aberrant neovascularization	Endothelial-cell assays and angiogenesis models; mechanistic/preclinical evidence	Yao et al. [65], Guo et al. [66], Ionescu et al. [67]
ECM remodeling	Influences MMP activity and stromal organization	Psoralen and bergapten	Facilitates immune-cell infiltration and restricts metastatic progression	ECM-remodeling and metastasis-related models; mechanistic/preclinical evidence	Fan et al. [68], Du et al. [69]
Modulation of inflammatory signaling pathways	Regulates NF-κB, PI3K/Akt, ERK1/2, and JNK signaling	Scopoletin, esculetin, daphnetin, and auraptene	Coordinates immune activation and suppresses tumor-supportive inflammation	Inflammatory and cancer-signaling models; mechanistic/direct immunomodulatory evidence	Sakthivel et al. [70], Ma et al. [71], Wei et al. [72]

Note: Evidence classification reflects the current level of experimental validation. "Direct immunomodulatory evidence" refers to studies evaluating immune-cell function or immune-associated processes. "Preclinical evidence" includes tumor models or engineered immune–tumor systems. "Mechanistic/pathway evidence" refers to molecular observations that provide biological rationale but have not yet been demonstrated to produce clinically meaningful immunotherapeutic effects. MHC-II: Major histocompatibility complex class II, IFN-γ: Interferon-gamma, HIF: Hypoxia-inducible factor, PI3K/Akt: Phosphoinositide 3-kinase/protein kinase B signaling pathway, ERK1/2: Extracellular signal-regulated kinase 1/2, JNK signaling: c-Jun N-terminal kinase signaling pathway, PD-1: Programmed cell death protein 1, MMP: Matrix metalloproteinase.

3.3. Immunomodulatory Effects in Preclinical Models

The combined use of naturally derived coumarins with complementary immunomodulatory properties, administered over prolonged periods alongside therapeutic cancer vaccines or oncolytic viruses, may strengthen antitumor immune activation in preclinical models [73]. Improved therapeutic outcomes have been associated with increased infiltration of $CD4^+$ and $CD8^+$ T lymphocytes, enhanced polarization of macrophages toward the pro-inflammatory M1 phenotype [74], reduced populations of immunosuppressive M2 macrophages, and decreased expression of transforming growth factor-β within tumor tissues [75]. In addition, strategies aimed at selectively targeting TME can further improve T-cell effector activity. For example, engineered exosomal delivery systems incorporating a coumarin-coated zeolitic imidazolate framework-8 prodrug and α-lipoic acid achieved high local drug accumulation and enhanced the efficacy of cancer immunotherapy [76].

Coumarins also exhibit the capacity to regulate DC polarization toward an immunogenic M1-like phenotype, highlighting their potential utility as immunoadjuvant agents. Many natural coumarin derivatives possessing platelet-activating factor receptor agonistic activity have been shown to markedly influence the growth, morphology, and lipid composition of *Mycobacterium bovis* [77]. Furthermore, suppression of the immunoregulatory cytokines IL-10 and transforming growth factor-β promoted Th1-mediated immune responses, whereas certain coumarins strongly enhanced Th2-associated immune activity [78]. Beyond cytokine modulation, coumarins can influence several intracellular signaling cascades involved in T-lymphocyte function, including the ERK1/2, JNK, and PI3K/Akt pathways, all of which are critical for lymphocyte development, activation, and immune regulation [79].

Several structurally distinct coumarin derivatives have also demonstrated direct effects on immune cell survival and proliferation. Simple coumarins containing an exocyclic double bond significantly inhibited the activity of a mitochondrial porin associated with T-cell apoptosis, an effect that could be reversed by *m*-bromobenzylamine [80]. In another study, flavonoid-like coumarins bearing a benzo[c]chromene-4-one scaffold displayed marked cytotoxic activity against lymphocytes and DCs in a trinitrophenyl-fluorescein isothiocyanate-induced mouse model [81]. Conversely, an unsymmetrically substituted coumarin derivative enhanced splenic lymphocyte proliferation, primarily through activation of the transcription factors activator protein-1 and NF-κB, both of which are central regulators of immune-cell activation and inflammatory signaling [82].

4. Synthetic Coumarins: Structure–Activity Relationships, Optimization, and Therapeutic Strategies

Synthetic coumarin derivatives have been extensively developed and refined as immunotherapeutic agents targeting TME, either as standalone therapies or in combination with other anticancer approaches [83]. Considerable attention has been directed toward understanding the structure–activity relationships of these compounds, allowing researchers to identify specific substituents, linker systems, and pharmacophoric features that enhance their immunomodulatory properties [84]. Several coumarins that demonstrated promising antitumor and TME–modulating activities in preclinical studies are believed to exert at least part of their effects through immune-related mechanisms. Initially, however, the development of these molecules primarily focused on their influence on other

hallmarks of cancer, rather than on direct immune modulation [85]. More recent studies have shifted toward the intentional design of synthetic coumarins capable of activating or reprogramming immune responses within TME. In this context, structure–activity relationship analyses have become valuable tools for guiding the rational development of coumarin-based immunomodulators [86]. Such compounds may represent candidates for further development as monotherapies or adjunctive agents with immune checkpoint inhibitors; however, their therapeutic efficacy and clinical applicability remain to be established [87].

4.1. Structure–Activity Relationships in the Context of Immune Modulation

The rational design of coumarin-based immunomodulators depends on a systematic understanding of their structural characteristics and corresponding biological activities [88]. In particular, both the position and electronic properties of substituents on the coumarin scaffold play critical roles in regulating immune responses [89]. These modifications can influence cytokine production and release by immune cells, as well as the expression of co-stimulatory and immunoregulatory molecules in DCs and macrophages [90]. Coumarin derivatives containing a secondary amino group within the linker region often exhibit enhanced immunomodulatory effects, suggesting that this structural feature contributes positively to biological activity [91]. In addition, the length and composition of the connecting chain significantly affect pharmacological behavior. This has been demonstrated in several 3-benzodioxole-coumarin analogs with varying linker sizes, which display characteristic pharmacophoric arrangements [92]. Such arrangements commonly include an N-acetyl or N-carboxamide moiety attached to a coumarin aromatic ring through a three-carbon spacer, or alternatively, a substituted glucose-like side chain [93]. Collectively, these structural parameters provide valuable guidance for the development and optimization of coumarin-derived immunotherapeutic agents.

4.2. Delivery Approaches and Pharmacokinetic Considerations

Drug delivery strategies play a pivotal role in shaping the pharmacokinetic behavior of synthetic coumarins, ultimately influencing their tissue distribution and local concentrations within TME [94]. These characteristics are critical for regulating immune cell activity and enhancing antitumor immune responses. From a metabolic perspective, the therapeutic performance of coumarin derivatives largely depends on improving bioavailability and achieving selective tumor targeting while minimizing systemic and off-target exposure [95]. Such objectives can be achieved through the development of prodrug systems [96] and nanocarrier-based formulations [97]. In addition, increasing the lipophilicity of coumarin molecules or conjugating them with tumor-targeting ligands can further enhance tumor accumulation and prolong retention at the disease site [98].

Several coumarin-based prodrug systems have demonstrated promising therapeutic potential. One example involves a coumarin derivative incorporating the 2,5-bis(trifluoroamido)-1-benzothiazole-4-sulfonamide pharmacophore. This compound functioned as an efficient prodrug, remaining pharmacologically inactive in its original form but undergoing metabolic conversion *in vitro* to release the active therapeutic moiety [99]. Another notable derivative was generated by introducing a lipoic acid amide at the C3 position of the coumarin scaffold through an ester linkage. Intravenous administration of this compound significantly improved the efficacy of immune checkpoint modulation therapy in a murine B16F10 melanoma model [100]. Furthermore, biodistribution studies in healthy mice and whole-body autoradiography demonstrated markedly greater tumor accumulation and retention compared with the parent coumarin compound [101].

4.3. Potential Combination with Existing Immunotherapies

Combining coumarin derivatives with established immunotherapies represents a mechanistically plausible but insufficiently validated therapeutic strategy. Coumarins can influence several processes that determine responsiveness to immune checkpoint modulation, including T-cell activation, antigen presentation, macrophage polarization, inflammatory signaling, tumor metabolism, hypoxia, and immune-cell infiltration [102]. These activities provide a rationale for investigating coumarins as complementary agents alongside immune checkpoint inhibitors. However, the evidence identified in the literature reviewed here does not establish formal pharmacological synergy between coumarin derivatives and anti-PD-1, anti-PD-L1, or anti-CTLA-4 therapy. Most available observations concern modulation of checkpoint-associated pathways or other components of the tumor microenvironment rather than direct evaluation of coumarin–checkpoint inhibitor combinations [103]. Accordingly, the current evidence

should be interpreted as supporting a potential complementary effect or possible combination benefit rather than demonstrated synergy. Dedicated studies incorporating coumarin monotherapy, checkpoint inhibitor monotherapy, and combination-treatment arms will be required to determine whether such interactions are additive, synergistic, or potentially antagonistic [104]. Notably, no convincing study identified in the literature reviewed here formally demonstrated pharmacological synergy between a coumarin derivative and anti-PD-1, anti-PD-L1, or anti-CTLA-4 therapy.

Optimizing the delivery of coumarin-based compounds can further strengthen their immunomodulatory efficacy. For instance, coumarin conjugates containing mannosamine derivatives, as illustrated in **Figure 5**, have been developed to improve selective targeting of DCs, thereby enhancing antigen presentation and immune activation [105]. Similarly, adenylated isocoumarin derivatives have demonstrated improved immunogenic properties through activation of Toll-like receptor 7 [106]. In addition, several coumarin analogs have been engineered to maximize localized immunostimulatory effects while minimizing systemic toxicity and unintended immunosuppressive actions [107]. These strategies may be particularly beneficial during the early phases of tumor development, when the spatial separation between stromal and tumor compartments permits more selective therapeutic intervention. Conversely, certain coumarin-derived molecules that exhibit concentration-dependent immunomodulatory behavior—shifting from immunosuppressive to immunostimulatory activity at higher concentrations—could be advantageous in advanced stages of tumor progression [108]. Such properties may help restore the cancer–immunity cycle, enhance immune-mediated tumor clearance, and ultimately contribute to reducing tumor burden.

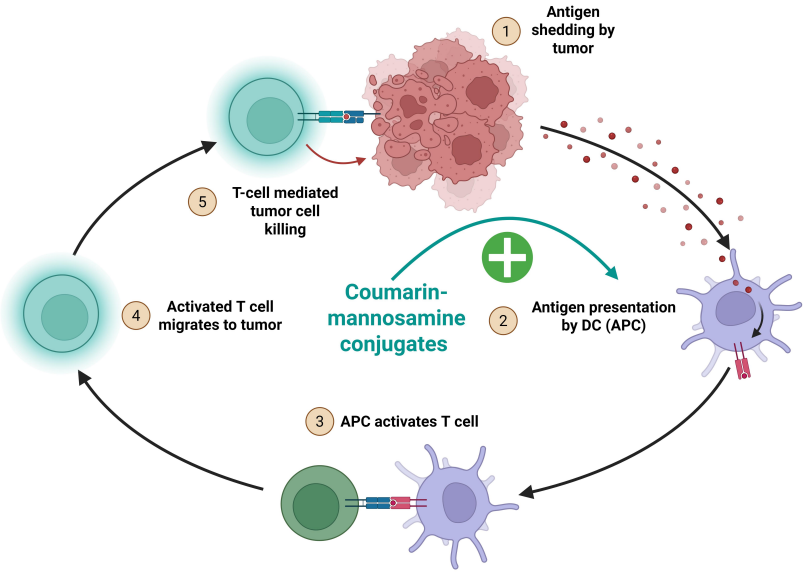

Figure 5. Coumarin-derived immunomodulatory conjugates targeting DC activation and TME remodeling in cancer immunotherapy.

Importantly, the concept of combining coumarin derivatives with immune checkpoint inhibitors remains largely hypothetical and is supported mainly by mechanistic rationale and limited preclinical observations. Current evidence does not establish coumarins as clinically validated checkpoint modulators or immunotherapy sensitizers. Therefore, reported effects on checkpoint-related pathways should be interpreted as preliminary molecular observations that require confirmation in immunocompetent tumor models, pharmacokinetic–pharmacodynamic studies, and ultimately well-designed clinical trials. Future investigations should also determine whether coumarin-mediated immune modulation provides additive or synergistic benefit when combined with approved immunotherapeutic strategies.

4.4. Comparative Pharmacological and Translational Profiles of Natural and Synthetic Coumarins

Although natural and synthetic coumarins share the benzopyrone scaffold and can influence overlapping components of the TME, their pharmacological and translational profiles are not equivalent (**Table 2**). Natural co-

umarins provide substantial chemical diversity generated through biosynthetic substitution, hydroxylation, prenylation, methoxylation, and ring fusion, and several members exhibit pleiotropic activity across inflammatory, metabolic, vascular, and immune-regulatory pathways. This multitarget behavior may be advantageous for remodeling the complex TME; however, naturally occurring compounds are generally constrained by the pharmacokinetic properties of their native structures. Variable aqueous solubility, absorption, metabolic transformation, and systemic exposure may therefore limit the extent to which promising *in vitro* immunomodulatory effects can be reproduced *in vivo*.

Table 2. Comparative pharmacological and translational characteristics of natural and synthetic coumarins for TME-oriented immunotherapy.

Comparative Feature	Natural Coumarins	Synthetic Coumarins	Translational Implication
Structural diversity	Extensive biosynthetic diversity, including hydroxylated, methoxylated, prenylated, and fused-ring derivatives	Structural space can be deliberately expanded through scaffold substitution, linker modification, conjugation, and pharmacophore incorporation	Natural compounds provide lead scaffolds; synthetic chemistry enables systematic optimization
Target selectivity	Frequently pleiotropic, with activity across inflammatory, metabolic, immune, and signaling pathways	Selectivity can potentially be enhanced through structure–activity-guided optimization	Synthetic derivatives may permit more precise targeting, whereas natural compounds may favor multitarget TME modulation
Bioavailability	May be limited by intrinsic solubility, absorption, metabolism, and physicochemical characteristics	Can be optimized through structural modification, prodrug design, and formulation strategies	Pharmacokinetic optimization may improve systemic exposure and therapeutic consistency
Metabolic stability	Determined by the native scaffold and substitution pattern; metabolic transformation may limit exposure	Can potentially be improved by modifying metabolically susceptible structural features	Rational modification may prolong effective exposure but requires metabolite characterization
Tumor accumulation	Generally dependent on intrinsic biodistribution unless incorporated into an engineered delivery system	Can be enhanced through increased lipophilicity, tumor-targeting ligands, prodrugs, or nanocarriers	Synthetic design provides greater opportunities for spatially controlled TME exposure
Delivery potential	Can serve as active compounds or payloads within engineered delivery platforms	Highly adaptable to conjugation, targeted delivery, prodrug, and nanocarrier strategies	Synthetic derivatives offer greater flexibility for cell- or tumor-directed delivery
Toxicity	Natural origin does not guarantee safety; toxicity remains structure-, dose-, metabolism-, and exposure-dependent	Structural optimization may reduce off-target effects but may also introduce new toxicological liabilities	Both groups require systematic pharmacological and toxicological evaluation
Therapeutic window and off-target exposure	Therapeutic window and off-target exposure	Therapeutic window and off-target exposure	Therapeutic window and off-target exposure
Translational feasibility	Attractive as biologically validated lead scaffolds, but many candidates remain supported primarily by preclinical evidence	Greater scope for medicinal-chemistry optimization and formulation, but increased development complexity may accompany structural modification	Neither group currently demonstrates sufficient clinical evidence to establish translational superiority

Synthetic coumarins offer a complementary development strategy in which the coumarin scaffold can be deliberately modified to optimize target engagement and drug-like behavior. Alteration of substituent position, electronic properties, linker architecture, lipophilicity, and pharmacophoric groups provides opportunities to improve potency, target selectivity, metabolic stability, and pharmacokinetic performance. Synthetic derivatives can also be incorporated into prodrugs, ligand-directed conjugates, and nanocarrier systems designed to increase tumor accumulation or selectively deliver immunomodulatory cargo to particular components of the TME. Indeed, the delivery strategies discussed above illustrate how chemical engineering of the coumarin scaffold can extend its function beyond intrinsic pharmacological activity toward spatially controlled immunomodulation [109].

Nevertheless, synthetic optimization does not inherently confer superior translational potential. Increasing structural complexity may introduce off-target activity, metabolic liabilities, formulation challenges, or unexpected toxicity, while extensive chemical modification can increase the burden of preclinical safety characterization. Conversely, the natural origin of a coumarin should not be interpreted as evidence of greater safety, because toxicity is determined by molecular structure, dose, exposure, metabolism, and target profile rather than source alone. Importantly, neither class currently possesses sufficient clinical evidence to establish superiority for TME-oriented immunotherapy. Natural coumarins may therefore be viewed primarily as biologically diverse scaffolds and sources

of mechanistic leads, whereas synthetic derivatives provide opportunities for rational optimization and targeted delivery. Future head-to-head pharmacokinetic, pharmacodynamic, biodistribution, toxicity, and efficacy studies in immunocompetent tumor models will be necessary to determine whether these theoretical differences translate into meaningful therapeutic advantages [110].

5. Immune and Non-Immune Modulation of the TME by Coumarins

Coumarins and related bioactive molecules have emerged as promising modulators of TME that plays a central role in determining both intrinsic tumor immunogenicity and the efficacy of anticancer interventions, particularly immune checkpoint inhibitors [111]. Effective activation of the cancer–immunity cycle depends on the coordinated recruitment and activation of antitumor immune cells while limiting the accumulation of immunosuppressive cell populations that promote tumor growth and immune evasion [112]. Understanding how coumarins influence these interconnected processes may provide valuable opportunities for the development of more effective immunotherapeutic strategies. Increasing evidence from both natural and synthetic coumarin derivatives demonstrates their ability to enhance pro-immunogenic signaling and support immune effector functions within TME [113].

Coumarins have been shown to regulate multiple aspects of TME architecture and activity. Experimental studies indicate that these compounds can alter macrophage polarization by suppressing the tumor-promoting M2 phenotype while favoring the antitumor M1 phenotype. In addition, coumarins can influence the balance between Th1 and Th2 immune responses, thereby shaping cytokine production and immune activation within tumor tissues [114]. Several coumarin derivatives also modulate the expression and activity of MMPs, contributing to reduced ECM degradation and diminished metastatic potential [115]. Moreover, these compounds have demonstrated the ability to promote vascular normalization, improve the infiltration and cytotoxic activity of tumor-infiltrating lymphocytes, and interfere with the metabolic adaptations that sustain tumor survival and immune suppression [116].

Metabolic reprogramming represents another important target of coumarin-mediated TME modulation. Studies suggest that coumarins can affect pathways associated with oxygen consumption, lactate transport, and glycolytic dependency in both cancer cells and stromal compartments. These effects may help reverse the acidic and hypoxic conditions that typically impair antitumor immunity [117]. In this context, HIFs, particularly HIF-1α and HIF-2α, appear to be important molecular targets influenced by coumarin treatment. Through simultaneous regulation of immune, vascular, and metabolic pathways, coumarins exhibit considerable potential as multifunctional agents capable of enhancing tumor immune surveillance and improving the overall effectiveness of cancer immunotherapy [118].

Lactate accumulation within TME has a profound influence on the behavior and function of immune cells, as illustrated in **Figure 6**. Rapid glycolysis in cancer cells leads to excessive lactate production, creating an acidic microenvironment that suppresses the activity of cytotoxic T lymphocytes and NKs while promoting the expansion of immunosuppressive populations such as Treg cells and M2-like tumor-associated macrophages [119]. Elevated lactate levels can also impair DC maturation and antigen-presenting capacity, thereby weakening antitumor immune responses. Consequently, metabolic reprogramming driven by lactate not only supports tumor survival and invasion but also contributes to immune evasion and resistance to immunotherapy [120].

5.1. Direct Immune-Cell Modulation: Macrophages, Dendritic Cells, T Cells, and NK Cells

Tumor-associated inflammatory cells are key regulators of cancer development and progression, exerting either tumor-suppressive or tumor-promoting effects depending on their functional phenotype within TME [121]. Among these immune populations, macrophages have attracted particular attention because of their remarkable plasticity and their ability to adopt either pro-inflammatory or anti-inflammatory states [122]. Although the M1/M2 framework is useful for describing experimentally induced macrophage phenotypes, tumor-associated macrophages exist along a continuum of activation states; accordingly, the M1 and M2 designations are used here as operational descriptors rather than as strictly discrete macrophage populations.

Natural and synthetic coumarins have emerged as promising bioactive molecules capable of modulating the polarization of THP-1 cell-derived macrophages stimulated by IFN-γ or IL-4. Through this regulatory activity, coumarins can influence the balance between M1 and M2 macrophage phenotypes and consequently alter the inflam-

matory status of TME [123]. M1 macrophages generally promote anti-tumor immunity by stimulating neighboring T lymphocytes and enhancing cytotoxic immune responses. In contrast, M2-polarized macrophages suppress T-cell activity, facilitate tumor growth and metastasis, and are frequently associated with poor clinical outcomes [124]. In breast cancer, the expression of CD68 and CD163 has been extensively investigated as markers of macrophage infiltration and polarization. CD68 is commonly associated with infiltrating macrophages, whereas CD163 is considered a characteristic marker of M2 macrophages [125]. In addition, CD16 expression is linked to the antitumor functions of NKs, including the production of IFN-γ and TNF-α as well as the release of perforins and granzymes. THP-1 cells also possess the capacity to differentiate into dendritic cells or dendritic cell-like populations with important immunoregulatory properties [126].

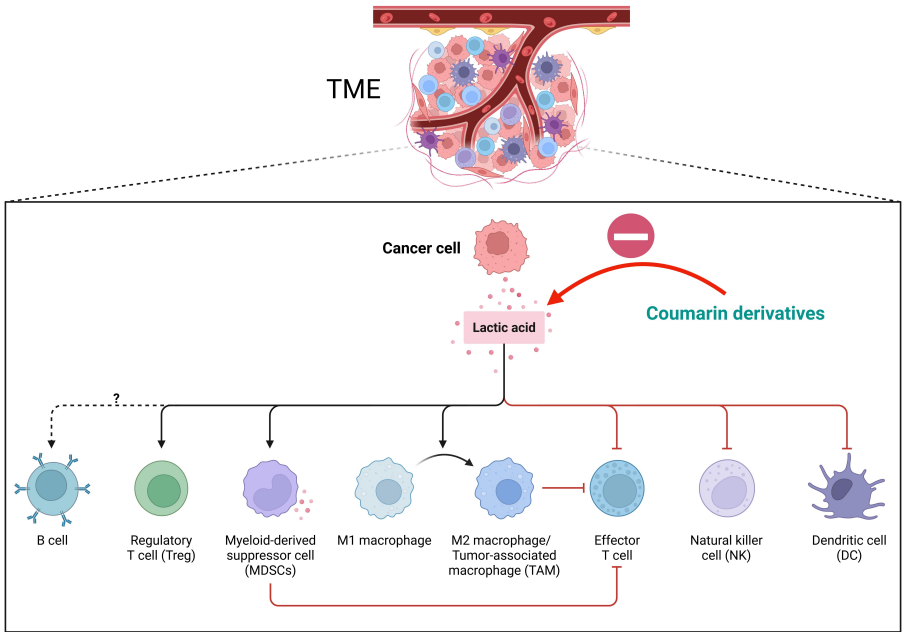

Figure 6. Metabolic reprogramming is mediated by coumarin derivatives in TME through the inhibition of lactate accumulation.

Note: Solid arrows indicate relationships supported by the experimental evidence discussed in the text, whereas dashed arrows indicate proposed or indirect mechanistic relationships that require further validation. Arrowheads indicate activation or promotion, whereas blunt-ended lines indicate inhibition.

The infiltration of cytotoxic T lymphocytes and other immune effector cells into TME represents a critical stage of the cancer–immunity cycle and is essential for the initiation of effective antitumor immune responses. Under pro-inflammatory conditions, DCs expressing MHC class II molecules together with co-stimulatory markers such as CD80 and CD83 activate T lymphocytes and NK cells, thereby promoting their maturation and cytotoxic activity against malignant cells [127]. Natural coumarins, particularly those capable of upregulating intercellular adhesion molecule-1 and MHC class II expression in DCs, may enhance DC maturation and subsequently support the activation of tumor-specific T cells [128]. Previous studies have demonstrated that immature DCs, which express relatively low levels of CD80, CD83, CD86, and CD40 and produce limited amounts of IL-12, tend to suppress T-cell activation and immune responsiveness. Conversely, mature DCs exhibit enhanced antigen-presenting capacity and play a crucial role in stimulating robust anticancer immune responses [129].

5.2. Non-Immune TME Modulation: ECM Remodeling and Angiogenesis

A growing body of evidence has examined the influence of both natural and synthetic coumarins on endothelial cell behavior, highlighting their potential roles in the regulation of angiogenesis and ECM remodeling [130]. These bioactive compounds have attracted considerable interest because of their ability to modulate key components of the TME, particularly pathways involved in neovascularization and ECM turnover [131]. Aberrant deposition and degradation of ECM components are hallmark events in the initiation and progression of cancer and fibrotic disorders [132]. MMPs, which are central regulators of ECM remodeling, are frequently dysregulated under pathological

conditions, contributing to abnormal angiogenesis and lymphangiogenesis [133]. Several naturally derived compounds, including coumarin-based molecules, have demonstrated the capacity to either suppress or enhance MMP activity depending on the biological context [134].

Coumarin derivatives have also been reported to interfere with tumor-associated neovascularization, as shown in **Figure 7**, through multiple mechanisms. In endothelial cells, certain coumarins promote apoptotic signaling, whereas others modulate pathways involved in endothelial migration, proliferation, and tube formation without directly triggering cell death [135]. Experimental studies have shown that these compounds can influence distinct stages of the angiogenic cascade, thereby affecting vascular development within TME [136]. Overall, coumarins and related bioactive molecules appear to regulate angiogenesis through modulation of both pro-angiogenic and anti-angiogenic mediators, ultimately influencing endothelial cell survival, functionality, and ECM dynamics [137]. These findings support the potential therapeutic value of coumarin derivatives as modulators of tumor progression and tissue remodeling processes.

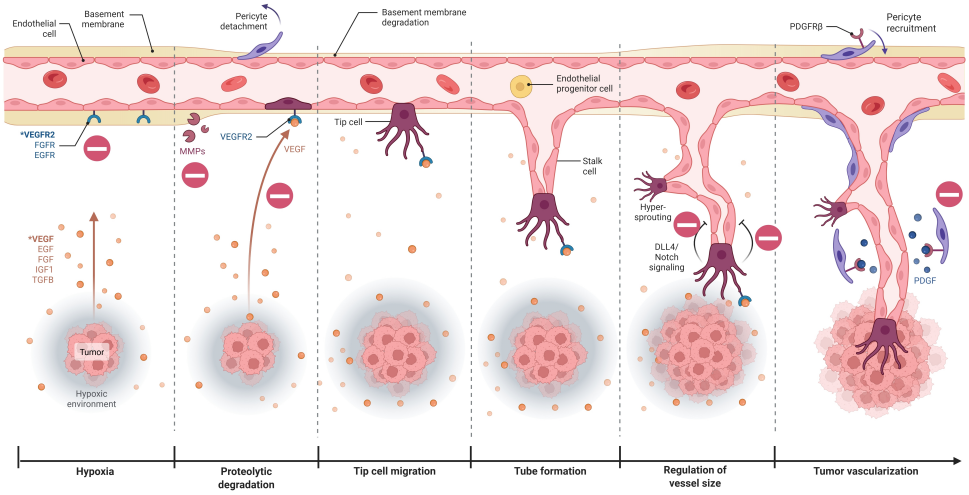

Figure 7. Cellular mechanisms through which coumarin derivatives modulate tumor-associated neovascularization.

Note: Arrowheads indicate activation or promotion, whereas blunt-ended lines indicate inhibition. Solid connections represent experimentally supported relationships discussed in the text, while dashed connections indicate proposed or indirect mechanistic relationships requiring further validation.

5.3. Non-Immune TME Modulation: Metabolic Reprogramming and Hypoxia

Cancer cells frequently undergo profound metabolic reprogramming that distinguishes them from normal tissues. One of the most recognized alterations is the preferential reliance on glycolysis for energy production even under oxygen-rich conditions, a phenomenon known as the Warburg effect. This shift in metabolism promotes acidification of the TME through excessive lactate accumulation, which in turn suppresses both innate and adaptive antitumor immune responses. At the same time, lactate and pyruvate released by tumor cells may be reutilized by surrounding stromal and immune cells to sustain oxidative phosphorylation and anabolic processes. Such metabolic interactions can favor the recruitment and maintenance of immunosuppressive cell populations, including M2-like macrophages and immune cells characterized by reduced IFN-γ secretion [138,139].

In recent years, several coumarin derivatives have attracted attention for their potential to counteract these tumor-associated metabolic alterations. Certain simple coumarins containing a hygroscopic hydroxymethyl substituent at the C-3 position have demonstrated the ability to sensitize different tumor cell types to oxidative stress. This activity appears to result from interference with the enzymatic function and expression of glutathione peroxidase family proteins [140]. The cytotoxic effects of these compounds are believed to be linked to suppression of glycolytic metabolism accompanied by enhancement of oxidative phosphorylation. Evidence supporting this mechanism includes reduced glucose uptake, decreased lactate production, and downregulation of genes involved in glycolysis [141]. Similarly, treatment with 5-methoxy-6-hydroxy-7-hydrosulfonylcoumarin (**Figure 8**) has been reported to decrease the expression of glucose transporters GLUT1 and GLUT3 in lung cancer cells, thereby limiting glucose availability required for rapid tumor growth [142].

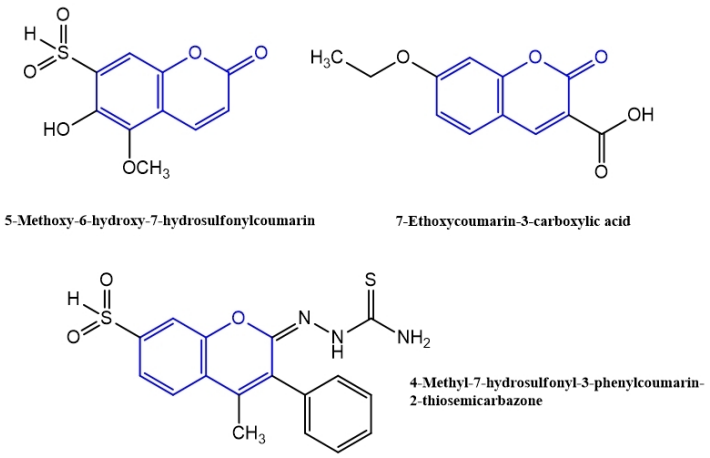

Figure 8. Chemical structures of selected coumarin derivatives capable of counteracting tumor-associated metabolic alterations.

Targeting tumor glycolysis has also emerged as a promising strategy to improve the efficacy of established chemotherapeutic agents such as cisplatin and doxorubicin [143]. In this context, 7-substituted carboxycoumarin derivatives, as well as transition-metal complexes of 4-methyl-7-hydrosulfonyl-3-phenylcoumarin-2-thiosemicarbazone (**Figure 8**), have demonstrated the capacity to interfere with tumor-associated metabolic processes, including monocarboxylate transporter-mediated lactate flux [144, 145]. These findings suggest that coumarin-based molecules may contribute to cancer therapy not only through direct cytotoxicity but also by reshaping tumor metabolism and immunosuppressive TME.

6. Preclinical Evidence and Translational Barriers

A wide variety of preclinical models have been utilized to evaluate TME-oriented immunotherapeutic potential of both natural and synthetic coumarins. These models include xenograft systems, syngeneic tumor models, and chemically induced carcinogenesis models, with immunological assessments covering multiple stages of the cancer–immunity cycle and experimental durations extending up to 26 weeks [146]. In parallel, coumarin-based therapeutic strategies, including TME-targeted neovaccines, are currently being investigated in clinical settings [147]. Emerging evidence indicates that integrating coumarin-related structure–activity relationships with specific TME-dependent mechanisms may enhance immune modulation, particularly in the context of immune checkpoint–based therapies [148].

Despite these encouraging advances, the current body of evidence remains limited and at an early stage of development. Only a small number of coumarins have been thoroughly investigated as TME-directed immunomodulators, and relatively few signaling pathways and therapeutic applications have been explored. In addition, many preliminary studies have been conducted under highly restricted experimental conditions, while the isolation and large-scale availability of naturally occurring coumarins continue to present practical challenges. Furthermore, the influence of microenvironmental factors on coumarin-mediated immune regulation remains insufficiently understood. Expanding research toward structurally diverse coumarins and exploring their applications in tumor growth suppression and cancer–immunity-cycle modulation could substantially broaden the current therapeutic landscape. Incorporating stromal-derived coumarin components may also improve the expression or biological activity of these compounds. Ultimately, repositioning coumarins within therapeutic strategies targeting primary tumor progression and treatment may further establish their value as promising preclinical immunomodulators and potential neovaccine candidates.

An important consideration in interpreting current evidence is the heterogeneity of experimental systems used to evaluate coumarin-mediated immunomodulation. While some studies directly investigate tumor-associated immune responses using immune-competent models, others rely on isolated immune cells, cancer cell lines, or non-cancer inflammatory models. Therefore, mechanistic similarity does not necessarily indicate equivalent immunotherapeutic relevance. Future studies employing syngeneic tumor models, spatial immune profiling, and clin-

ically relevant pharmacological exposures are required to determine whether these molecular effects translate into meaningful therapeutic benefit.

6.1. Critical Appraisal of Experimental Evidence

Current evidence supporting coumarin-mediated immunomodulation varies substantially in experimental rigor. Although some studies employ tumor-bearing animal models with immune characterization, many investigations rely on simplified systems, including cancer cell lines, isolated immune populations, or inflammatory disease models. These approaches are valuable for identifying molecular mechanisms but may not accurately reproduce the spatial and cellular complexity of TME. Furthermore, differences in compound dose, bioavailability, administration route, and treatment duration complicate direct comparison among studies. Future investigations should incorporate standardized pharmacological approaches, immune profiling, appropriate controls, and clinically relevant exposure levels to establish whether observed immunomodulatory effects translate into meaningful therapeutic benefit.

6.2. *In vivo* Models and Endpoints

The *in vivo* immunotherapeutic efficacy of coumarins remains insufficiently investigated. This limitation represents a significant challenge for clinical translation, as findings derived from simplified *in vitro* systems often fail to accurately predict the complex biological interactions occurring within TME [149]. Recent evidence suggests that coumarin derivatives may exert broad immunomodulatory effects by targeting dynamic interactions among tumor cells, stromal components, infiltrating immune populations, vascular networks, and tumor-associated metabolic pathways. Because TME plays a central role in cancer progression and therapeutic responsiveness, modulation of these interconnected compartments may substantially improve immunotherapeutic outcomes [150]. A growing number of *in vitro* studies have demonstrated that both natural and synthetic coumarin derivatives can selectively stimulate or suppress distinct immune-cell subsets, supporting their potential as immunoregulatory agents [151]. However, preclinical investigations examining their effects within intact TME remain scarce. Furthermore, many existing animal studies lack comprehensive immunological assessments, limiting the current understanding of how coumarins influence immune dynamics during tumor progression and treatment [152]. Consequently, additional well-designed *in vivo* studies are required to clarify their mechanisms of action and establish their translational value as adjuncts or enhancers of cancer immunotherapy.

6.3. Biomarkers of Response and Resistance

Numerous candidate biomarkers associated with therapeutic response and resistance have been identified in preclinical investigations, including molecular, cellular, and imaging-based parameters. These forms of evidence should be critically evaluated when determining the translational applicability of emerging therapeutic strategies [153]. Driven by the growing expectations surrounding immunotherapy and the pressure to achieve clinical success, the number of clinical trials has expanded substantially. However, many of these studies proceed without a sufficiently robust scientific foundation or adequate preliminary evidence [154]. Importantly, clinical assays cannot effectively address hypotheses that have not first been established through rigorous preclinical research. Comprehensive evaluation of potential treatment response and resistance mechanisms, together with the identification of patients most likely to benefit, is essential for selecting appropriate candidates for therapy and determining whether combination approaches may provide superior outcomes [155]. To ensure effective clinical management, patient stratification strategies must be carefully designed and supported by well-structured and scientifically rigorous preclinical studies.

6.4. Safety, Toxicity, Pharmacokinetics, and Off-Target Effects

The translational relevance of coumarin-mediated immunomodulation cannot be determined solely from the magnitude of an immune response, because therapeutic benefit ultimately depends on whether biologically effective concentrations can be achieved within the tumor without producing unacceptable systemic toxicity or disrupting protective immunity. This distinction is particularly important for compounds capable of bidirectional or concentration-dependent immune regulation. An exposure that modifies inflammatory or immune signaling within

the TME may be therapeutically advantageous, whereas excessive or prolonged systemic exposure could suppress immune-cell function or affect signaling pathways required for normal immune homeostasis [156]. Consequently, dose–response relationships should be interpreted together with pharmacokinetic parameters, tissue distribution, duration of exposure, and pharmacodynamic immune endpoints rather than considering immunostimulatory activity in isolation.

The safety profile of coumarins is strongly influenced by molecular structure, dose, route of administration, metabolism, and duration of exposure, and natural origin should not be considered synonymous with low toxicity. Natural coumarins may undergo extensive metabolic transformation, and differences in absorption, first-pass metabolism, clearance, and metabolite formation can substantially alter systemic exposure and biological activity. In addition, many natural products can limit the initiation and progression of pathological conditions, which has encouraged their investigation in the management of chronic infections and malignancies [157]. Several synthetic coumarin derivatives have also demonstrated promising antitumor activity in experimental models; however, inappropriate dosing may lead to undesirable immunosuppressive effects [158].

Certain coumarin derivatives, particularly diphenyl- and nitrogen-containing analogs, have been associated with significant toxicity and immune suppression in animal studies [159]. Conversely, some simple coumarins exhibit protective or immunoregulatory properties. Structural modifications can markedly influence biological safety; for example, the introduction of a C7-pivaloyl substituent was associated with notable toxicity, producing approximately 37% lethality in mice at doses ranging from 0.06 to 6 g/kg [160]. Similarly, several coumarin-quinolizidine derivatives reduced important immunobiological parameters, including the antiphagocytic index of blood, nonspecific reactivity index, and phagocytic index, although these compounds did not induce tumor formation [161]. Overall, toxicological investigations of natural and synthetic coumarins highlight the delicate balance between therapeutic benefit and harmful effects. These findings emphasize the importance of careful dose optimization and the rational design of less toxic derivatives during drug development [162]. Fine-tuning the nature and position of substituents on the benzene and pyran rings of coumarin core may enhance the immunomodulatory potential of these compounds while minimizing toxicity [163].

6.5. Evidence Strength and Translational Challenges

The current evidence supporting coumarin-mediated modulation of tumor immunity should be interpreted according to experimental hierarchy. Direct effects on immune cells, cytokine production, metabolic pathways, and tumor-associated signaling have primarily been demonstrated in cellular and animal models. Proposed mechanisms involving immune checkpoint regulation, enhancement of immunotherapy response, or clinical application remain hypothesis-generating and require further validation. Therefore, coumarins should currently be considered investigational immunomodulatory compounds rather than established immunotherapeutic agents.

7. Challenges, Gaps, and Future Directions

The diverse hypotheses surrounding coumarins and the wide range of biological activities attributed to these compounds make it difficult to draw definitive conclusions regarding their immunomodulatory roles. In many cases, coumarin derivatives exhibit contrasting effects depending on their chemical structure, target cell type, or biological context. For instance, modulation of inflammatory signaling may produce beneficial antitumor immune activation in one biological context but may promote immune suppression or tissue-protective responses in another. Similarly, the same coumarin derivative may exhibit different effects depending on concentration, cellular differentiation status, and the presence of tumor-associated signals. Such context dependency highlights the necessity of interpreting coumarin activity within specific biological systems rather than assuming universal immunostimulatory properties. Although studies investigating the immunomodulatory properties of coumarins remain relatively limited, the steadily increasing body of evidence highlights the importance of exploring their influence within TME. This includes understanding the complex interactions between coumarin derivatives, inflammatory mediators, and immune cell populations, as well as establishing rational design strategies for coumarin-based agents capable of modulating TME-associated pathways.

Several important knowledge gaps still need to be addressed before the therapeutic potential of coumarins can be fully realized. These include comprehensive characterization of immune responses *in vivo*, determination

of optimal and safe dosing regimens, identification of predictive biomarkers, and evaluation of coumarin activity in preclinical cancer models beyond conventional TME-focused systems. In addition, combining coumarin derivatives with established immunotherapeutic approaches may provide synergistic benefits and represent an attractive direction for future research. Structural modification of coumarins, particularly through the development of synthetic derivatives, represents a promising and rapidly expanding area of investigation. Incorporation of additional biosynthetic or pharmacophoric features may strengthen their interactions with inflammation-related pathways involved in tumor initiation and progression. However, despite encouraging preliminary findings, substantial work is still required before these compounds can be translated into clinically applicable therapies.

Overall, while major advances have been achieved in cancer immunotherapy, effective immunomodulation still depends on a deeper understanding of the molecular and cellular mechanisms governing communication between tumors and the host immune system. Both natural and synthetic coumarins possess several pharmacological characteristics that support their potential as modulators of TME, although this field remains insufficiently explored and warrants further systematic investigation.

8. Conclusion

Natural and synthetic coumarins represent a chemically diverse group of compounds with considerable yet underexplored potential for TME–directed immunomodulation. Their structural diversity arises from distinct biosynthetic origins and synthetic modifications, resulting in multiple subclasses with different signaling properties and biological activities within TME. Recent advances in the development of immunomodulatory agents have expanded interest in coumarin-based scaffolds, particularly for designing compounds with immune checkpoint–modulating characteristics. Preclinical investigations have demonstrated that coumarins can influence immune cell infiltration and stimulate stromal interactions while also regulating key tumor microenvironmental processes, including hypoxia, angiogenesis, extracellular matrix remodeling, and metabolic reprogramming. The complexity and heterogeneity of immune checkpoint regulation create opportunities for both natural and synthetic immunomodulators to act synergistically with current immunotherapies or to reshape TME in ways that enhance antitumor immune activation and sustain immune responsiveness.

Owing to their favorable structural and pharmacological properties, coumarins are promising candidates for such applications. Nevertheless, further research is required to clarify their interactions with established immunotherapeutic strategies and to identify optimal combinational approaches. In addition, the selection of appropriate experimental tumor models that accurately reflect specific immune checkpoint landscapes remains a critical factor for translating coumarin-based immunomodulators into clinically relevant cancer therapies. Importantly, future studies should prioritize standardized experimental designs incorporating immune-competent models, clinically achievable dosing strategies, and validated immunological endpoints to determine whether promising mechanistic findings translate into therapeutic efficacy.

Despite the encouraging immunomodulatory activities reported for natural and synthetic coumarins, several critical challenges currently limit their clinical translation. Most available evidence remains derived from *in vitro* studies and selected preclinical models, whereas comprehensive *in vivo* validation within clinically relevant TMEs is still insufficient. In addition, pharmacokinetic properties, including bioavailability, metabolic stability, tissue distribution, and optimal dosing strategies, remain incompletely characterized for many coumarin derivatives. Safety assessment represents another essential consideration, particularly regarding potential systemic toxicity, off-target immune modulation, and long-term effects during combination with established immunotherapies. Furthermore, clinical evidence supporting coumarin-based immunotherapeutic approaches remains scarce, highlighting the need for well-designed translational studies and early-phase clinical trials to determine their therapeutic feasibility, efficacy, and safety profiles.

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References

1. Ling, S.P.; Ming, L.C.; Dhaliwal, J.S.; et al. Role of Immunotherapy in the Treatment of Cancer: A Systematic Review. *Cancers* **2022**, *14*, 5205.
2. Zhang, M.; Liu, C.; Tu, J.; et al. Advances in cancer immunotherapy: historical perspectives, current developments, and future directions. *Mol. Cancer* **2025**, *24*, 136.
3. Zeki, N.M.; Mustafa, Y.F. 6,7-Coumarin-heterocyclic hybrids: A comprehensive review of their natural sources, synthetic approaches, and bioactivity. *J. Mol. Struct.* **2024**, *1303*, 137601.
4. Younes, A.H.; Mustafa, Y.F. Novel coumarins from green sweet bell pepper seeds: Their isolation, characterization, oxidative stress-mitigating, anticancer, anti-inflammatory, and antidiabetic properties. *J. Mol. Struct.* **2024**, *1312*, 138629.
5. Todorov, L.; Saso, L.; Kostova, I. Antioxidant Activity of Coumarins and Their Metal Complexes. *Pharmaceuticals* **2023**, *16*, 651.
6. Mustafa, Y.F. Triple coumarin-based 5-fluorouracil prodrugs, their synthesis, characterization, and release kinetics. *J. Mol. Struct.* **2024**, *1301*, 137415.
7. Zhang, J.; Yuan, Y.; Gao, X.; et al. Scopoletin ameliorates hyperlipidemia and hepatic steatosis via AMPK, Nrf2/HO-1 and NF-κB signaling pathways. *Biochem. Pharmacol.* **2025**, *231*, 116639.
8. Sharif, N.; Jabeen, H. Natural sources for coumarins and their derivatives with relevance to health-promoting properties: A systematic review. *Trends Phytochem. Res.* **2024**, *8*, 1–16.
9. Mustafa, Y.F. Natural immune boosters: Coumarins as emerging potentiators of immunity in drug discovery. *Phytomedicine Plus* **2025**, *5*, 100880.
10. Li, Y.; Wang, G.Q.; Li, Y.B. Therapeutic potential of natural coumarins in autoimmune diseases with underlying mechanisms. *Front. Immunol.* **2024**, *15*, 1–15.
11. Kubrak, T.P.; Makuch-Kocka, A.; Aebisher, D. Coumarins in anticancer therapy: Mechanisms of action, potential applications and research perspectives. *Pharmaceutics* **2025**, *17*, 595.
12. Liu, S.; Liu, C.; He, Y.; et al. Benign non-immune cells in tumor microenvironment. *Front. Immunol.* **2025**, *16*, 1561577.
13. Mustafa, Y.F. Biocompatible chlorocoumarins from harmful chlorophenols, their synthesis and biomedicinal evaluation. *J. Mol. Struct.* **2024**, *1309*, 138193.
14. Zeki, N.M.; Mustafa, Y.F. Annulated Heterocyclic[g]Coumarin Composites: Synthetic Approaches and Bioactive Profiling. *Chem. Biodivers.* **2024**, *21*, e202301855.
15. Zhao, Y.; Jing, L.; Han, L.; et al. Coumarins in the tumor-immune application: From molecular mechanisms to therapeutic innovations. *Front. Immunol.* **2025**, *16*, 1681892.
16. Mellman, I.; Chen, D.S.; Powles, T.; et al. The cancer-immunity cycle: Indication, genotype, and immunotype. *Immunity* **2023**, *56*, 2188–2205.
17. Kim, S.K.; Cho, S.W. The Evasion Mechanisms of Cancer Immunity and Drug Intervention in the Tumor Microenvironment. *Front. Pharmacol.* **2022**, *13*, 868695.

18. Shahbaz, M.; Perween, A.; Momal, U.; et al. Recent Perspectives on Anticancer Potential of Coumarin Against Different Human Malignancies: An Updated Review. *Food Sci. Nutr.* **2025**, *13*, e4696.
19. Tiwari, A.; Trivedi, R.; Lin, S.Y. Tumor microenvironment: Barrier or opportunity towards effective cancer therapy. *J. Biomed. Sci.* **2022**, *29*, 83.
20. Erasha, A.M.; El-Gendy, H.; Aly, A.S.; et al. The Role of the Tumor Microenvironment (TME) in Advancing Cancer Therapies: Immune System Interactions, Tumor-Infiltrating Lymphocytes (TILs), and the Role of Exosomes and Inflammasomes. *Int. J. Mol. Sci.* **2025**, *26*, 2716.
21. Peña-Romero, A.C.; Orenes-Piñero, E. Dual Effect of Immune Cells within Tumour Microenvironment: Pro- and Anti-Tumour Effects and Their Triggers. *Cancers* **2022**, *14*, 1681.
22. Bahrami, M.; Haji Molla Hoseini, M.; Rezaei, M.; et al. Umbelliprenin Increases the M1/M2 Ratio of Macrophage Polarization and Improves the M1 Macrophage Activity in THP-1 Cells Cocultured with AGS Cells. *Evid.-Based Complement. Altern. Med.* **2021**, *2021*, 9927747.
23. Zeki, N.M.; Mustafa, Y.F. Natural linear coumarin-heterocyclic conjugates: A review of their roles in phytotherapy. *Fitoterapia* **2024**, *175*, 105929.
24. Younes, A.H.; Mustafa, Y.F. Plant-Derived Coumarins: A Narrative Review of Their Structural and Biomedical Diversity. *Chem. Biodivers.* **2024**, *21*, e202400344.
25. Tsivileva, O.M.; Koftin, O.V.; Evseeva, N.V. Coumarins as Fungal Metabolites with Potential Medicinal Properties. *Antibiotics* **2022**, *11*, 1156.
26. Abdulaziz, N.T.; Mustafa, Y.F. Antibacterial and Antitumor Potentials of Some Novel Coumarins. *Int. J. Drug Deliv. Technol.* **2022**, *12*, 239–247.
27. Nogueira, P.L.; da Nóbrega Alves, D.; Queiroga Gomes da Costa, P.C.; et al. Derivative of 7-hydroxycoumarin has antifungal potential against Candida species and low cytotoxicity against human cells: In silico studies and biological evaluation. *Fungal Biol.* **2023**, *127*, 1451–1465.
28. Mishra, S.; Pandey, A.; Manvati, S. Coumarin: An emerging antiviral agent. *Heliyon* **2020**, *6*, e03217.
29. Orioli, R.; Belluti, F.; Gobbi, S.; et al. Naturally Inspired Coumarin Derivatives in Alzheimer's Disease Drug Discovery: Latest Advances and Current Challenges. *Molecules* **2024**, *29*, 3514.
30. Jibroo, R.N.; Mustafa, Y.F.; Al-Shakarchi, W. Synthesis and evaluation of linearly fused thiadiazolocoumarins as prospects with broad-spectrum bioactivity. *Results Chem.* **2024**, *7*, 101494.
31. Citarella, A.; Vittorio, S.; Dank, C.; et al. Syntheses, reactivity, and biological applications of coumarins. *Front. Chem.* **2024**, *12*, 1362992.
32. Hassanein, E.H.M.; Sayed, A.M.; Hussein, O.E.; et al. Coumarins as modulators of the Keap1/Nrf2 /ARE signaling pathway. *Oxid. Med. Cell. Longev.* **2020**, *2020*, 1675957.
33. Nan, Z.D.; Shang, Y.; Zhu, Y.D.; et al. Systematic review of natural coumarins in plants (2019–2024): Chemical structures and pharmacological activities. *Phytochemistry* **2025**, *235*, 114480.
34. Mustafa, Y.F.; Bashir, M.K.; Oglah, M.K.; et al. Bioactivity of Some Natural and Semisynthetic Coumarin Derived Compounds. *NeuroQuantology* **2021**, *19*, 129–138.
35. Aqib, M.; Khatoon, S.; Ali, M.; et al. Exploring the anticancer potential and mechanisms of action of natural coumarins and isocoumarins. *Eur. J. Med. Chem.* **2025**, *282*, 117088.
36. Zeki, N.M.; Mustafa, Y.F. Coumarin hybrids for targeted therapies: A promising approach for potential drug candidates. *Phytochem. Lett.* **2024**, *60*, 117–133.
37. Stefanachi, A.; Leonetti, F.; Pisani, L.; et al. Coumarin: A Natural, Privileged and Versatile Scaffold for Bioactive Compounds. *Molecules* **2018**, *23*, 250.
38. Gao, X.Y.; Li, X.Y.; Zhang, C.Y.; et al. Scopoletin: a review of its pharmacology, pharmacokinetics, and toxicity. *Front. Pharmacol.* **2024**, *15*, 1268464.
39. Aslantürk, Ö.S.; Aşkin Çelik, T. Anticancer effect of umbelliferone on MKN-45 and MIA PaCa-2 cell lines. *Toxicol. In Vitro* **2023**, *93*, 105694.
40. Venugopala, K.N.; Rashmi, V.; Odhav, B. Review on natural coumarin lead compounds for their pharmacological activity. *Biomed. Res. Int.* **2013**, *2013*, 963248.
41. Hacholli, V.B.; R., S.M.; H., P.B.; et al. Coumarin Derivatives as Anticancer Agents: Mechanistic Landscape with an Emphasis on Breast Cancer. *Molecules* **2025**, *30*, 4167.
42. Liu, X.; Hoft, D.F.; Peng, G. Senescent T cells within suppressive tumor microenvironments: Emerging target for tumor immunotherapy. *J. Clin. Invest.* **2020**, *130*, 1073–1083.
43. Jasim, S.F.; Mustafa, Y.F. New fused-coumarin composites: Synthesis, anticancer and antioxidant potentials evaluation. *Eurasian Chem. Commun.* **2022**, *4*, 607–619.
44. Basak, U.; Sarkar, T.; Mukherjee, S.; et al. Tumor-associated macrophages: an effective player of the tumor

microenvironment. *Front. Immunol.* **2023**, *14*, 1295257.
45. Wu, Y.; Xu, J.; Liu, Y.; et al. A Review on Anti-Tumor Mechanisms of Coumarins. *Front. Oncol.* **2020**, *10*, 592853.
46. Qian, R.; Ge, J.; Fan, N.; et al. Natural Products in Cancer Prevention and Therapy: Current Challenges and Future Directions. *MedComm* **2026**, *7*, e70585.
47. Pisani, L.; Catto, M.; Muncipinto, G.; et al. A twenty-year journey exploring coumarin-based derivatives as bioactive molecules . *Front. Chem.* **2022**, *10*, 1002547.
48. Srirangan, P.; Rathnasooriya, P.S.A.; Ranganathan, A.; et al. Unveiling the Potential of Esculetin in Cancer Models and Chemotherapy-Induced Toxicity: Mechanistic Insights From Preclinical Evidence. *J. Biochem. Mol. Toxicol.* **2026**, *40*, e70740.
49. Jayasingam, S.D.; Citartan, M.; Thang, T.H.; et al. Evaluating the Polarization of Tumor-Associated Macrophages Into M1 and M2 Phenotypes in Human Cancer Tissue: Technicalities and Challenges in Routine Clinical Practice. *Front. Oncol.* **2020**, *9*, 1512.
50. Ouyang, X.; Zhao, Z.; Hou, Y.; et al. Scopoletin alleviates cognitive deficits in 5xFAD mice via suppressing microglial inflammatory response. *Phytomedicine* **2026**, *153*, 157981.
51. Chen, F.; Wang, N.; Liao, J.; et al. Esculetin rebalances M1/M2 macrophage polarization to treat sepsis-induced acute lung injury through regulating metabolic reprogramming. *J. Cell. Mol. Med.* **2024**, *28*, e70178.
52. Kim, D.J.; Jung, M.S.; Jin, H.U.; et al. Anti-Melanogenic Effects of Umbelliferone: In Vitro and Clinical Studies. *Molecules* **2024**, *29*, 5571.
53. Li, M.; Wang, J.; Huo, B.; et al. Umbelliferone Enhances Immune Function in Cyclophosphamide-Induced Immunosuppressed Mice via Histidine and Purine Metabolism Regulation. *Curr. Drug Metab.* **2025**, *25*, 695–705.
54. Kornicka, A.; Balewski, Ł.; Lahutta, M.; et al. Umbelliferone and Its Synthetic Derivatives as Suitable Molecules for the Development of Agents with Biological Activities: A Review of Their Pharmacological and Therapeutic Potential. *Pharmaceuticals* **2023**, *16*, 1732.
55. Rodrigues, V.D.; Detregiachi, C.R.P.; dos Santos Bueno, M.; et al. Imperatorin: A Furanocoumarin with Potential in Combating Cancer Development and Progression—A Comprehensive Review. *Pharmaceuticals* **2026**, *19*, 436.
56. Bajaj, P.; Kumar, A.; Singh, B.; et al. Immunomodulatory Phytochemicals in Cancer: Mechanistic Pathways and Translational Potential. *Chem. Biodivers.* **2026**, *23*, e02737.
57. Chen, Y.; Zhang, Q.; Liu, H.; et al. Esculetin Ameliorates Psoriasis-Like Skin Disease in Mice by Inducing CD4+Foxp3+ Regulatory T Cells. *Front. Immunol.* **2018**, *9*, 2092.
58. Zhang, Z.; Long, S.; Huang, Z.; et al. Regulatory effect of daphnetin on the balance of Th17 and Treg cells in the peripheral blood mononuclear cells from patients with unexplained recurrent pregnancy loss. *Cent. Eur. J. Immunol.* **2021**, *45*, 403–408.
59. Mazumder, A.; Lee, J.Y.; Talhi, O.; et al. Hydroxycoumarin OT-55 kills CML cells alone or in synergy with imatinib or Synribo: Involvement of ER stress and DAMP release. *Cancer Lett.* **2018**, *438*, 197–218.
60. Liu, K.; Huang, H.; Zhang, M.; et al. When therapy-induced senescence meets tumors: A double-edged sword: A review. *Medicine* **2025**, *104*, e42886.
61. Hosseini, F.; Ahmadi, A.; Sarvi, Z.N.; et al. 7-Geranyloxycoumarin modulated metastatic potential of osteosarcoma cells via interaction with MMPs and JAK1/2. *Naunyn Schmiedebergs Arch. Pharmacol.* **2025**, *398*, 10139–10153.
62. Ji, H.; Tan, Y.; Gan, N.; et al. Synthesis and anticancer activity of new coumarin-3-carboxylic acid derivatives as potential lactate transport inhibitors. *Bioorg. Med. Chem.* **2021**, *29*, 115870.
63. Zhou, R.; Yu, Y.H.; Kim, H.; et al. Synthesis of coumarin derivatives and investigation of their inhibitory effects on lung cancer cell motility. *Sci. Rep.* **2022**, *12*, 21635.
64. Yang, J.; Han, J.; Li, Y.; et al. Esculetin inhibits the apoptosis in H9c2 cardiomyocytes via the MAPK signaling pathway following hypoxia/reoxygenation injury. *Biomed. Pharmacother.* **2017**, *88*, 1206–1210.
65. Yao, F.; Zhang, L.; Jiang, G.; et al. Osthole attenuates angiogenesis in an orthotopic mouse model of hepatocellular carcinoma via the downregulation of nuclear factor-κB and vascular endothelial growth factor. *Oncol. Lett.* **2018**, *16*, 4471–4479.
66. Guo, Y.; Xiao, Y.; Guo, H.; et al. The anti-dysenteric drug fraxetin enhances anti-tumor efficacy of gemcitabine and suppresses pancreatic cancer development by antagonizing STAT3 activation. *Aging* **2021**, *13*, 18545–18563.
67. Ionescu, C.; Oprea, B.; Ciobanu, G.; et al. The Angiogenic Balance and Its Implications in Cancer and Cardio-

vascular Diseases: An Overview. *Medicina* **2022**, *58*, 903.
68. Fan, Z.; Gao, J.; Chen, Y.; et al. Psoralen remodels the articular cartilage microenvironment by pharmacologically regulating the Nrf2 pathway in osteoarthritis treatment. *Int. Immunopharmacol.* **2025**, *149*, 114221.
69. Du, W.; Xia, X.; Hu, F.; et al. Extracellular matrix remodeling in the tumor immunity. *Front. Immunol.* **2024**, *14*, 1340634.
70. Sakthivel, K.M.; Vishnupriya, S.; Chandramani, L.; et al. Modulation of multiple cellular signalling pathways as targets for anti-inflammatory and anti-tumorigenesis action of Scopoletin. *J. Pharm. Pharmacol.* **2022**, *74*, 147–161.
71. Ma, J.; Zhang, M.; Mulati, S.; et al. Esculetin inhibits the PI3K/Akt/mTOR pathway and enhances anti-colorectal cancer activity via binding to ENO1. *Front. Pharmacol.* **2025**, *16*, 1627114.
72. Wei, Z.; Wei, N.; Su, L.; et al. The molecular effects underlying the pharmacological activities of daphnetin. *Front. Pharmacol.* **2024**, *15*, 1407010.
73. Abd, M.A.; Asiri, M.; Farhan, S.R.; et al. Update on Application of Oncolytic Virus-Based Combination Therapy in the Treatment of Hepatocellular Carcinoma. *J. Biochem. Mol. Toxicol.* **2025**, *39*, e70327.
74. Aden, D.; Zaheer, S.; Zaheer, S. Unlocking Cancer Immunity: Deciphering the Role of Tumor-Associated Macrophages in Modulating Immunosuppressive Tumor Microenvironment. *Cell Biol. Int.* **2026**, *50*, e70156.
75. He, L.; Tam, P.K.H.; Deng, C.X. Orchestration of Tumor-Associated Macrophages in the Tumor Cell-Macrophage-CD8+ T Cell Loop for Cancer Immunotherapy. *Int. J. Biol. Sci.* **2025**, *21*, 4098–4116.
76. Huang, L.; Wu, E.; Liao, J.; et al. Research Advances of Engineered Exosomes as Drug Delivery Carrier. *ACS Omega* **2023**, *8*, 43374–43387.
77. Ventura, T.; Silva, D.; Lassounskaia, E.; et al. Coumarine Analogues with Antimycobacterial and Immunomodulatory Activity. *Curr. Bioact. Compd.* **2015**, *11*, 109–115.
78. Di Stasi, L.C. Coumarin derivatives in inflammatory bowel disease. *Molecules* **2021**, *26*, 422.
79. Al-Attabi, A.; Mohammed, M.S.; Al-Khazali, N.A.Y.; et al. Nettle (Urtica Dioica L.) Modulates Angiogenesis by Targeting the PI3K/AKT/eNOS Pathway in Prostate Cancer. *Middle East J. Cancer* **2025**, *16*, 228–237.
80. Mustafa, Y.F. 4-Chloroskimmetine-based derivatives as potential anticancer and antibacterial prospects: Their synthesis and in vitro inspections. *Results Chem.* **2024**, *7*, 101511.
81. Leung, K.N.; Leung, P.Y.; Kong, L.P.; et al. Immunomodulatory effects of esculetin (6,7-dihydroxycoumarin) on murine lymphocytes and peritoneal macrophages. *Cell. Mol. Immunol.* **2005**, *2*, 181–188.
82. Al-Rashidi, R.R.; Noraldeen, S.A.M.; Kareem, A.K.; et al. Malignant function of nuclear factor-kappaB axis in prostate cancer: Molecular interactions and regulation by non-coding RNAs. *Pharmacol. Res.* **2023**, *194*, 106775.
83. Al-Warhi, T.; Sabt, A.; Elkaeed, E.B.; et al. Recent advancements of coumarin-based anticancer agents: An up-to-date review. *Bioorg. Chem.* **2020**, *103*, 104163.
84. Thakur, A.; Singla, R.; Jaitak, V. Coumarins as anticancer agents: A review on synthetic strategies, mechanism of action and SAR studies. *Eur. J. Med. Chem.* **2015**, *101*.
85. Rawat, A.; Vijaya Bhaskar Reddy, A. Recent advances on anticancer activity of coumarin derivatives. *Eur. J. Med. Chem. Rep.* **2022**, *5*, 100038.
86. Koley, M.; Han, J.; Soloshonok, V.A.; et al. Latest developments in coumarin-based anticancer agents: Mechanism of action and structure–activity relationship studies. *RSC Med. Chem.* **2024**, *15*, 10–54.
87. Ghemrawi, R.; Abuamer, L.; Kremesh, S.; et al. Revolutionizing Cancer Treatment: Recent Advances in Immunotherapy. *Biomedicines* **2024**, *12*, 2158.
88. Singh, A.; Singh, K.; Kaur, K.; et al. Coumarin as an Elite Scaffold in Anti-Breast Cancer Drug Development: Design Strategies, Mechanistic Insights, and Structure–Activity Relationships. *Biomedicines* **2024**, *12*, 1192.
89. Patra, I.; Ansari, M.J.; Saadoon, N.; et al. Insights into the Electronic Properties of Coumarins: A Comparative Study Photocatalytic Degradation of Methylene Blue. *Phys. Chem. Res.* **2023**, *11*, 437–447.
90. Xiujuan, Z.; Hengli, Y.; Jinye, W.U.; et al. Synthesis and anti-inflammatory activity of three series of coumarin-based derivatives. *J. China Pharm. Univ.* **2025**, *56*, 40–48.
91. Flores-Morales, V.; Villasana-Ruíz, A.P.; Garza-Veloz, I.; et al. Therapeutic Effects of Coumarins with Different Substitution Patterns. *Molecules* **2023**, *28*, 2413.
92. Mohamed-Ezzat, R.A.; Elgemeie, G.H. Synthetic strategies towards benzothiazole-based compounds of therapeutic potency: Experimental, investigational and approved drugs. *RSC Adv.* **2025**, *15*, 41724–41832.
93. Kumari, S.; Carmona, A.V.; Tiwari, A.K.; et al. Amide Bond Bioisosteres: Strategies, Synthesis, and Successes. *J. Med. Chem.* **2020**, *63*, 12290–12358.
94. Atia, Y.A.; Bokov, D.O.; Zinnatullovich, K.R.; et al. The role of amino acid functionalization for improvement

of adsorption Thioguanine anticancer drugs on the boron nitride nanotubes for drug delivery. *Mater. Chem. Phys.* **2022**, *278*, 125664.
95. Chavez Alvarez, A.C.; Moreau, E. Coumarin-Based Prodrugs: Therapeutic Promise or Still Confined to Preclinical Exploration? *Pharmaceutics* **2026**, *18*, 341.
96. Brion, A.; Chaud, J.; Léonard, J.; et al. Red Light-Responsive Upconverting Nanoparticles for Quantitative and Controlled Release of a Coumarin-Based Prodrug. *Adv. Healthc. Mater.* **2023**, *12*, e2201474.
97. Javed, M.; Saade, A.; Jasim, A.; et al. Anticancer Drug-Loading Capacity of Green Synthesized Porous Magnetic Iron Nanocarrier and Cytotoxic Effects Against Human Cancer Cell Line. *J. Clust. Sci.* **2022**, *4*, 44–47.
98. Zeki, N.M.; Mustafa, Y.F. Digital alchemy: Exploring the pharmacokinetic and toxicity profiles of selected coumarin-heterocycle hybrids. *Results Chem.* **2024**, *10*, 101754.
99. Rubab, L.; Afroz, S.; Ahmad, S.; et al. An Update on Synthesis of Coumarin Sulfonamides as Enzyme Inhibitors and Anticancer Agents. *Molecules* **2022**, *27*, 1604.
100. Chen, L.; Lv, Q.; Cai, J.; et al. Design, synthesis and anticancer activity studies of 3-(coumarin-3-yl)-acrolein derivatives: Evidenced by integrating network pharmacology and vitro assay. *Front. Pharmacol.* **2023**, *14*, 1141121.
101. Zhang, X.; Yu; Zhang, W.Q.; et al. The antitumor activity of a doxorubicin loaded, iRGD-modified sterically-stabilized liposome on B16-F10 melanoma cells: in vitro and in vivo evaluation. *Int. J. Nanomed.* **2013**, *8*, 2473.
102. Fusco, M.; Picón, C.R.; Diaz, M.A.; et al. 4-Methylumbelliferone Modulates CAIX to Mitigate Hypoxia-Driven Dysregulation and Enhance PD-1 Immunotherapy in Lung Cancer. *Int. J. Mol. Sci.* **2025**, *26*, 10427.
103. Anupindi, K.; Malachowski, J.; Hodson, I.; et al. The next innovations in chimeric antigen receptor T cell immunotherapies for cancer. *Cytotherapy* **2025**, *27*, 795–811.
104. Abdelnaby, R.M.; Rateb, H.S.; Ali, O.; et al. Dual PI3K/Akt Inhibitors Bearing Coumarin-Thiazolidine Pharmacophores as Potential Apoptosis Inducers in MCF-7 Cells. *Pharmaceuticals* **2022**, *15*, 428.
105. Kumari, S.; Sharma, A.; Yadav, S. Pharmacological Potential of Coumarin-Based Derivatives: (A Comprehensive Brief Review). *Orient. J. Chem.* **2023**, *39*, 568–576.
106. Pallathadka, H.; Khaleel, A.Q.; Zwamel, A.H.; et al. Multi-Drug Resistance and Breast Cancer Progression via Toll-Like Receptors (TLRs) Signaling. *Cell Biochem. Biophys.* **2024**, *82*, 3015–3030.
107. Saadati, F.; Modarresi Chahardehi, A.; Jamshidi, N.; et al. Coumarin: A natural solution for alleviating inflammatory disorders. *Curr. Res. Pharmacol. Drug Discov.* **2024**, *7*, 100202.
108. Song, B.; Wang, Z.; Liu, Y.; et al. Immunosuppressive Activity of Daphnetin, One of Coumarin Derivatives, Is Mediated through Suppression of NF-κB and NFAT Signaling Pathways in Mouse T Cells. Rengarajan, J., Ed. *PLoS One* **2014**, *9*, e96502.
109. Ahmad Bhat, A.; Kaur, G.; Tandon, N.; et al. Current advancements in synthesis, anticancer activity, and structure–activity relationship (SAR) of coumarin derivatives. *Inorg. Chem. Commun.* **2024**, *167*, 112605.
110. Lin, H.; You, Q.; Wei, X.; et al. Osthole, a Coumarin from Cnidium monnieri: A Review on Its Pharmacology, Pharmacokinetics, Safety, and Innovative Drug Delivery Platforms. *Am. J. Chin. Med.* **2024**, *52*, 1397–1425.
111. Maashi, M.S.; Al-Mualm, M.; Al-Awsi, G.R.L.; et al. Apigenin alleviates resistance to doxorubicin in breast cancer cells by acting on the JAK/STAT signaling pathway. *Mol. Biol. Rep.* **2022**, *49*, 8777–8784.
112. Budi, H.S.; Younus, L.A.; Lafta, M.H.; et al. The role of miR-128 in cancer development, prevention, drug resistance, and immunotherapy. *Front. Oncol.* **2023**, *12*, 1067974.
113. Thomas, V.; Giles, D.; Basavarajaswamy, G.; et al. Coumarin Derivatives as Anti-inflammatory and Anticancer Agents. *Anticancer Agents Med. Chem.* **2017**, *17*, 415–423.
114. Lv, J.; Ibrahim, Y.S.; Yumashev, A.; et al. A comprehensive immunobiology review of IBD: With a specific glance to Th22 lymphocytes development, biology, function, and role in IBD. *Int. Immunopharmacol.* **2024**, *137*, 112486.
115. Hosseini, F.; Ahmadi, A.; Hassanzade, H.; et al. Inhibition of melanoma cell migration and invasion by natural coumarin auraptene through regulating EMT markers and reducing MMP-2 and MMP-9 activity. *Eur. J. Pharmacol.* **2024**, *971*, 176517.
116. Giercuszkiewicz-Haśnik, K.; Morak-Młodawska, B.; Jeleń, M. Molecularly Targeted Therapies in Oncology: Mechanisms, Resistance, and Combination Strategies. *Molecules* **2026**, *31*, 1195.
117. Aden, D.; Sureka, N.; Zaheer, S.; et al. Metabolic Reprogramming in Cancer: Implications for Immunosuppressive Microenvironment. *Immunology* **2025**, *174*, 30–72.
118. Abdelaal, H.I.; Mohamed, A.R.; Abo-Ashour, M.F.; et al. Mitigating the resistance of MCF-7 cancer cells to Doxorubicin under hypoxic conditions with novel coumarin based carbonic anhydrase IX and XII inhibitors.

Bioorg. Chem. **2024**, *152*, 107759.
119. Jasim, S.A.; Khalaf, O.Z.; Alshahrani, S.H.; et al. An in vitro investigation of the apoptosis-inducing activity of corosolic acid in breast cancer cells. *Iran. J. Basic Med. Sci.* **2023**, *26*, 453–460.
120. Jin, X.; Zhang, N.; Yan, T.; et al. Lactate-mediated metabolic reprogramming of tumor-associated macrophages: Implications for tumor progression and therapeutic potential. *Front. Immunol.* **2025**, *16*, 1573039.
121. Mergia Terefe, E.; Catalan Opulencia, M.J.; Rakhshani, A.; et al. Roles of CCR10/CCL27–CCL28 axis in tumour development: Mechanisms, diagnostic and therapeutic approaches, and perspectives. *Expert Rev. Mol. Med.* **2022**, *24*, e37.
122. Jin, R.; Neufeld, L.; McGaha, T.L. Linking macrophage metabolism to function in the tumor microenvironment. *Nat. Cancer* **2025**, *6*, 239–252.
123. Singh, A.; Pillai, L.; Danes, D.; et al. 4-fluorophenylacetamide acetyl coumarin induces pro-inflammatory M1 macrophage polarization and suppresses the immunosuppressive M2 phenotype through PI3k/AKT/NF-κB modulation. *Mol. Biol. Rep.* **2025**, *52*, 415.
124. Rannikko, J.H.; Hollmén, M. Clinical landscape of macrophage-reprogramming cancer immunotherapies. *Br. J. Cancer* **2024**, *131*, 627–640.
125. Jamiyan, T.; Kuroda, H.; Yamaguchi, R.; et al. CD68- and CD163-positive tumor-associated macrophages in triple negative cancer of the breast. *Virchows Arch.* **2020**, *477*, 767–775.
126. Redavid, A.R.; Bigliardi, M.; Ciarrocchi, A.; et al. Transcriptional networks weaving natural killer anti-tumor immune response. *Mol. Cancer* **2026**, *25*, 113.
127. Mukherjee, S.; Ghosh, A.; Chakraborty, S.; et al. Tumor-infiltrating lymphocytes and tumor-associated macrophages in cancer immunoediting: A targetable therapeutic axis. *Front. Immunol.* **2025**, *16*, 1655176.
128. Long, Z.; Yang, Q.; Wu, Y.; et al. From Demyelination to Intervention: Natural Compounds as Emerging Therapeutic Targets in Multiple Sclerosis Neuroinflammation. Gurumallesh, P., Ed. *J. Immunol. Res.* **2026**, *2026*, 6333512.
129. Troise, D.; Infante, B.; Mercuri, S.; et al. Dendritic Cells: A Bridge between Tolerance Induction and Cancer Development in Transplantation Setting. *Biomedicines* **2024**, *12*, 1240.
130. Emam, S.H.; Hassan, R.A.; Osman, E.O.; et al. Coumarin derivatives with potential anticancer and antibacterial activity: Design, synthesis, VEGFR-2 and DNA gyrase inhibition, and in silico studies. *Drug Dev. Res.* **2023**, *84*, 475–499.
131. Kim, S.O.; Hwangbo, H.; Hong, S.H.; et al. Esculetin Attenuates the Migration and Invasion of Human Hepatocellular Carcinoma Cells by Attenuating Matrix Metalloproteinase Activity and Strengthening Tight Junctions. *J. Cancer Prev.* **2025**, *30*, 111–117.
132. Al-Hawary, S.I.S.; Alhajlah, S.; Olegovich, B.D.; et al. Effective extracellular vesicles in glioma: Focusing on effective ncRNA exosomes and immunotherapy methods for treatment. *Cell Biochem. Funct.* **2024**, *42*, e3921.
133. Cabral-Pacheco, G.A.; Garza-Veloz, I.; Castruita-De la Rosa, C.; et al. The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases. *Int. J. Mol. Sci.* **2020**, *21*, 9739.
134. Kao, S.J.; Su, J.L.; Chen, C.K.; et al. Osthole inhibits the invasive ability of human lung adenocarcinoma cells via suppression of NF-κB-mediated matrix metalloproteinase-9 expression. *Toxicol. Appl. Pharmacol.* **2012**, *261*, 105–115.
135. Majnooni, M.B.; Fakhri, S.; Smeriglio, A.; et al. Antiangiogenic Effects of Coumarins against Cancer: From Chemistry to Medicine. *Molecules* **2019**, *24*, 4278.
136. Liu, Z.L.; Chen, H.H.; Zheng, L.L.; et al. Angiogenic signaling pathways and anti-angiogenic therapy for cancer. *Signal Transduct. Target. Ther.* **2023**, *8*, 198.
137. Irfan, D.; Ahmad, I.; Patra, I.; et al. Stem cell-derived exosomes in bone healing: focusing on their role in angiogenesis. *Cytotherapy* **2023**, *25*, 353–361.
138. Schiliro, C.; Firestein, B.L. Mechanisms of Metabolic Reprogramming in Cancer Cells Supporting Enhanced Growth and Proliferation. *Cells* **2021**, *10*, 1056.
139. Rodrigues, P.; Bangali, H.; Ali, E.; et al. The mechanistic role of NAT10 in cancer: Unraveling the enigmatic web of oncogenic signaling. *Pathol. Res. Pract.* **2024**, *253*, 154990.
140. Tanimoto, A.; Witaicenis, A.; Caruso, Í.P.; et al. 4-Methylesculetin, a natural coumarin with intestinal anti-inflammatory activity, elicits a glutathione antioxidant response by different mechanisms. *Chem. Biol. Interact.* **2020**, *315*, 108876.
141. Puiggros, A.; Collado, R.; Calasanz, M.J.; et al. Patients with chronic lymphocytic leukemia and complex karyotype show an adverse outcome even in absence of TP53/ATM FISH deletions. *Oncotarget* **2017**, *8*, 54297–

54303.
142. Pliszka, M.; Szablewski, L. Glucose Transporters as a Target for Anticancer Therapy. *Cancers* **2021**, *13*, 4184.
143. Li, S.; Gong, J.; Kang, B.; et al. Targeting Glycolytic Metabolism in Cancer Therapy: Current Approaches and Future Perspectives. *Cells* **2026**, *15*, 362.
144. Draoui, N.; Schicke, O.; Fernandes, A.; et al. Synthesis and pharmacological evaluation of carboxycoumarins as a new antitumor treatment targeting lactate transport in cancer cells. *Bioorg. Med. Chem.* **2013**, *21*, 7107–7117.
145. Mosa, A.I.; Emara, A.A.A.; Yousef, J.M.; et al. Novel transition metal complexes of 4-hydroxy-coumarin-3-thiocarbohydrazone: Pharmacodynamic of Co(III) on rats and antimicrobial activity. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2011**, *81*, 35–43.
146. Wang, Y.; Shelton, S.E.; Kastrunes, G.; et al. Preclinical models for development of immune–oncology therapies. *Immuno-Oncol. Insights* **2022**, *3*, 396–398.
147. Kenoosh, H.A.; Pallathadka, H.; Hjazi, A.; et al. Recent advances in mRNA-based vaccine for cancer therapy; bench to bedside. *Cell Biochem. Funct.* **2024**, *42*, e3954.
148. Yadav, A.K.; Maharjan Shrestha, R.; Yadav, P.N. Anticancer mechanism of coumarin-based derivatives. *Eur. J. Med. Chem.* **2024**, *267*, 116179.
149. Gupta, R.; Radhakrishnan, R.; Kabekkodu, S.P.; et al. Application of in vitro and in vivo cancer models to study the impact of the tumor immune microenvironment on anticancer therapy. *Biochim. Biophys. Acta Rev. Cancer* **2026**, *1881*, 189504.
150. Wu, S.; Hu, Y.; Sui, B. Promotion Mechanisms of Stromal Cell-Mediated Lung Cancer Development within Tumor Microenvironment. *Cancer Manag. Res.* **2025**, *17*, 249–266.
151. Zamani Taghizadeh Rabe, S.; Iranshahi, M.; Mahmoudi, M. In vitro anti-inflammatory and immunomodulatory properties of umbelliprenin and methyl galbanate. *J. Immunotoxicol.* **2016**, *13*, 209–216.
152. Jung, H.S.; Park, Y.J.; Gu, B.H.; et al. Coumarin derivatives ameliorate the intestinal inflammation and pathogenic gut microbiome changes in the model of infectious colitis through antibacterial activity. *Front. Cell. Infect. Microbiol.* **2024**, *14*, 1362773.
153. Kim, S.; Abikenari, M.; Bergsneider, B.; et al. Biomarkers for predicting immunotherapy response and resistance in glioblastoma. *Front. Immunol.* **2026**, *17*, 1823338.
154. Passaro, A.; Al Bakir, M.; Hamilton, E.G.; et al. Cancer biomarkers: Emerging trends and clinical implications for personalized treatment. *Cell* **2024**, *187*, 1617–1635.
155. Holder, A.M.; Dedeilia, A.; Sierra-Davidson, K.; et al. Defining clinically useful biomarkers of immune checkpoint inhibitors in solid tumours. *Nat. Rev. Cancer* **2024**, *24*, 498–512.
156. Petroni, G.; Buqué, A.; Zitvogel, L.; et al. Immunomodulation by targeted anticancer agents. *Cancer Cell* **2021**, *39*, 310–345.
157. Gangopadhyay, A. Plant-derived natural coumarins with anticancer potentials: future and challenges. *J. Herb. Med.* **2023**, *42*, 100797.
158. Hanh, N.T.; Van, C.A.; Son, N.T. Recent advances in bioactive coumarin scoparone: A comprehensive review. *Fitoterapia* **2026**, *188*, 106966.
159. Lake, B.G.; Evans, J.G.; Lewis, D.F.V.; et al. Studies on the acute effects of coumarin and some coumarin derivatives in the rat. *Food Chem. Toxicol.* **1994**, *32*, 357–363.
160. Olatunde, A.; Tijjani, H.; Aremu, A.O.; et al. Editorial: Chemistry, toxicity, synthesis, biological and pharmacological activities of coumarins and their derivatives: Recent advances and future perspectives. *Front. Pharmacol.* **2024**, *15*.
161. Di Stasi, L.C. Natural Coumarin Derivatives Activating Nrf2 Signaling Pathway as Lead Compounds for the Design and Synthesis of Intestinal Anti-Inflammatory Drugs. *Pharmaceuticals* **2023**, *16*, 511.
162. Mohamed, F.A.M. A current summary of coumarin-based compounds and their therapeutic applications. *Egypt. J. Chem.* **2024**, *67*, 1417–1437.
163. Niinivehmas, S.; Pentikäinen, O. Coumarins as Tool Compounds to Aid the Discovery of Selective Function Modulators of Steroid Hormone Binding Proteins. *Molecules* **2021**, *26*, 5142.