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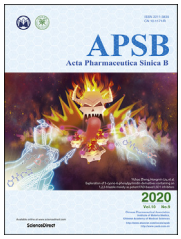

REVIEW

Targeting necroptosis in anticancer therapy: mechanisms and modulators

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Abstract Necroptosis, a genetically programmed form of necrotic cell death, serves as an important pathway in human diseases. As a critical cell-killing mechanism, necroptosis is associated with cancer progression, metastasis, and immunosurveillance. Targeting necroptosis pathway by small molecule modulators is emerging as an effective approach in cancer therapy, which has the advantage to bypass the apoptosis-resistance and maintain antitumor immunity. Therefore, a better understanding of the mechanism of necroptosis and necroptosis modulators is necessary to develop novel strategies for cancer therapy. This review will summarize recent progress of the mechanisms and detecting methods of necroptosis. In particular, the relationship between necroptosis and cancer therapy and medicinal chemistry of necroptosis modulators will be focused on.

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1. Introduction

A dynamic balance among cell death, proliferation and differentiation is of great significance in maintaining tissue homeostasis[1,2]. For a long time, apoptosis has been considered as the only pathway in the programmed cell death (PCD), which is characterized by condensed nuclei, shrinking but integral membrane, and rapid elimination by phagocytosis[3,4]. In contrast, necrosis refers to the process that cells swell, rupture and then release cellular contents and proinflammatory molecules in response to the overwhelming stress (*e.g.*, heat)[5–7]. Necrosis is regarded as an uncontrollable process, and therefore it is highly challenging to identify small molecules that can interfere with this process[5–7]. In the late 1980s, cells under the treatment of tumor necrosis factor (TNF) were found to be characterized with either apoptotic or necrotic features in a cell type-dependent manner[8]. In 2004, Thompson et al.[9] discovered that alkylating agent *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (**1**, Fig. 1) initiated cell necrosis depending on the expression of poly ADP-ribose polymerase (PARP), suggesting that necrosis could be regulated by small molecules. In 2005, the concept "necroptosis", a combination of "necrosis" and "apoptosis", was proposed by Yuan's group[10], and Nec-1 (**2**, Fig. 1) was reported to be able to reverse cell death in this pathway. Since apoptosis-resistant cases were reported in clinical cancer therapy[11], it was urgent and important to search for a new pathway to induce the death of cancer cells, while necroptosis is a promising alternative[12,13]. Therefore, intensive attention and efforts have been paid to investigate the mechanism of necroptosis and clarify its relationship and effectiveness to cancer therapy[14–16]. Recent studies indicated that necroptosis could be initiated by activation of specific receptors on cell membrane and regulated by cytokines and small molecules[17–22]. While the relationship between necroptosis and cancer has not been clarified explicitly, both efforts are being focused on the factors relevant to the induction and inhibition of necroptosis[23–25]. Thus, a better understanding of the mechanism of necroptosis and necroptosis modulators is important to develop novel strategies for cancer therapy. From a medicinal chemist point of view, the present review will summarize recent progress of the mechanisms and detecting methods of necroptosis. In particular, the relationship between necroptosis and cancer therapy, and the discovery and biological activity of necroptosis modulators will be focused on.

2. Mechanism of necroptosis

2.1. *Initiation of necroptosis*

Necroptosis is initiated under several circumstances, including the combination of TNF cytokines and membrane receptors, virus infection and interferon stimulation or chemical compounds (Fig. 2)[17–22,26]. TNF cytokines include TNF-α, TNF-related apoptosis-inducing ligand, factor-associated suicide ligand (FASL), etc.[27] When TNF-α binds to the membrane receptor, it can promote the formation of a plasma membrane-associated complex which is consisted of adaptor proteins tumor necrosis factor receptor 1 (TNFR1)-associated death domain protein (TRADD), receptor-interacting serine-threonine kinase 1 (RIPK1), TNFR-associated factor 2/5 (TRAF2/5), cellular

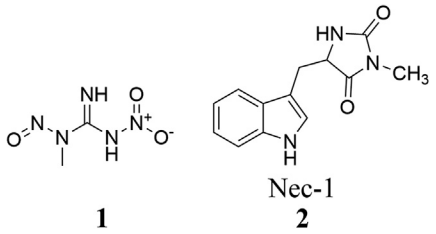


Figure 1 Chemical structures of necroptosis modulators **1** and **2**.

inhibitor of apoptosis protein 1/2 (cIAP1/2) and the linear ubiquitin chain assembly complex (LUBAC complex)[18]. Subsequently, the complex results in RIPK1 polyubiquitination which is responsible for the activation of NF-κB pathway and mitogen-activated protein kinases (MAPKs)[17–19]. Inhibition of cIAPs or deubiquitination of RIPK1 by cylindromatosis (CYLD) can block the activation of NF-κB pathway, leading to the dissociation of RIPK1 and TRADD from the plasma membrane-associated complex[28]. Then RIPK1 and TRADD bind to FAS-associated death domain protein (FADD) and procaspase-8 to form a complex called cytosolic death-inducing signaling complex (DISC) which is able to activate caspase-8 and initiate apoptosis[29], while caspase-8 activation can be inhibited by the cellular FLIP long isoform protein (cFLIPL). When receptor-interacting serine/threonine-protein kinase 3 (RIPK3) is overexpressed, it can be connected to RIPK1 through their own RIP homotypic interaction motif (RHIM) to form a complex called necrosome[30]. Then both or either of RIPK1 and RIPK3 are phosphorylated, resulting in recruitment and subsequent phosphorylation of mixed lineage kinase domain-like protein (MLKL), and activation of necroptosis[31]. It was reported that caspase-8 in the necrosome was able to cleave RIPK1−RIPK3 complex, and therefore, cFLIPL could promote necroptosis[32].

Other necroptotic stimulus, including FASL, lipopolysaccharide (LPS), double-stranded RNA (dsRNA), double-stranded DNA (dsDNA), interferon-γ (IFN-γ) and some chemical compounds, can be recognized by their corresponding receptors located on the cell membrane or sensors outside or inside the cell[20,21,26,33]. For example, the combination of FAS to FASL or combination of TRAIL to DR4/5 subsequently binds to FADD through intercellular DD domain, leading to the formation of DISC directly[33]. Then the downstream pathway is the same to that of TNF-α-induced necroptosis. In addition, LPS can activate Toll-like receptor 4 (TLR4)[20], while viral dsRNA is able to activate TLR3[21]. Their combination enables recruitment of Toll/IL-1 receptor domain-containing adaptor to elicit inflammatory cytokines and type 1 IFN responses. Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF•TICAM-1) is the sole adaptor protein for TLR3[22], while either TRIF or myeloid differentiation primary response gene (Myd88) can mediate TLR4[20]. TRIF contains the RHIM motif that links RIPK1 and RIPK3, and then induces necroptosis[34]. Apart from these two pathways, DNA virus can trigger the DNA-dependent activator of interferon-regulatory factors (DAI) pathway[35]. DAI is a cytosolic DNA sensor that activates IRF3 and NF-κB pathways to elicit cytokines and type1 IFN responses[35]. Like TRIF, it also contains the RHIM motif which can link RIPK1 and RIPK3, and induce necroptosis[35]. Radiation was supposed to be an invitation to necroptosis in endocrine cancers, but the mechanism remained to be clarified[26].

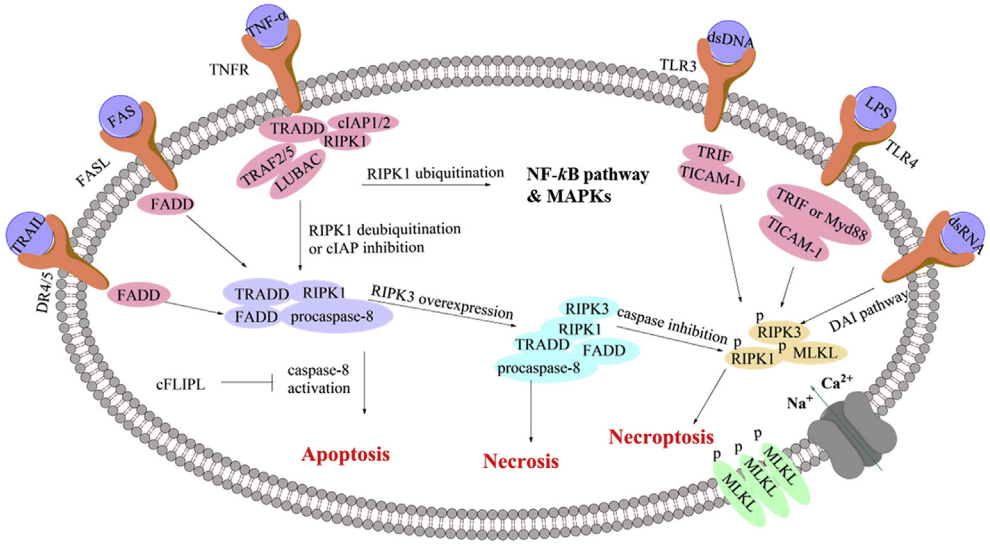


Figure 2 A brief pathway of necroptosis.

2.2. *Execution of necroptosis*

The functional necrosome consists of RIPK1, RIPK3 and MLKL. Inactivated MLKL is a monomer in the cytoplasm[36]. It turns into the form of trimer once phosphorylated by RIPK3 at the kinase-like domain of MLK. Afterwards, the trimers bind to phosphatidylinositol phosphates (PIPs) and cardiolipin (CL) which are mitochondria-specific, leading to their translocation to membrane rich in PIPs and CL[36]. There is evidence that MLKL in the plasma membrane binds to the ion channel transient receptor potential melastatin related 7 (TRMP7) and then leads to the influx of Ca^{2+} ions and subsequent cell death[36]. Another study revealed that MLKL in the membrane promoted the influx of Na^{+} and then increased the osmotic pressure, causing the rupture of plasma membrane[37]. Papatriantafyllou et al.[38] proposed that phosphoglycerte mutase family member 5 (PAGM5) was another target of necrosome. PGAM5 recruited the mitochondrial fission factor dynamin-related protein 1 (Drp1) and activated its GTP-binding proteins (GTPase) activity, leading to mitochondrial fragmentation which is an early and indispensible step for necrosis execution[38]. Lysosomal permeability transition (LMT) involved the increase of Ca^{2+} concentration in response of FAS and TNF, lysosome enlargement and lysosome membrane permeabilization[39]. Then, the leakage of proteolytic enzyme into the cytoplasm degraded proteins and activated other hydrolases participated in the necroptosis[39].

Reactive oxygen species (ROS)[40], adenine nucleotide translocase (ANT)[41], reactive nitrogen species (RNS)[42] are important regulators in the execution of necroptosis. RIPK3 activates the activity of glycogen phosphorylase (PYLG), glutamate ammonia ligase (GLUL) and glutamate dehydrogenase (GLUD1), which promotes the production of ROS. Then the increasing amount of ROS activates the pro-necrotic effect of c-Jun NH_2-terminal kinase (JNK), which can suppress TNF-α-induced apoptosis[40,43], leading cells to undergo necrotic or necroptotic pathways. ANT, an ADP/ATP carrier located in the inner mitochondrial membrane, is essential for the synthesis of mitochondrial ATP[41]. In case that the ability of ANT to transport cytoplasmic ADP is blocked, mitochondrial ATP will be in shortage, and then necrotic or necroptotic pathways are triggered[41]. Another study indicated that excessive nitric oxide induced the production of RNS which played an important role in lipid and protein oxidation and peroxidation[42]. In addition, nitration also could induce RIPK1- and RIPK3-dependent necroptosis[44]. Nitration of NDUFB8, a vital component of mitochondrial supercomplex, resulted in the dissociation of mitochondrial supercomplex and mitochondrial depolarization, which were associated with necroptosis.

2.3. *Propagation of necroptosis*

Previous studies confirmed that necroptosis was an invitation to inflammation[33]. Damage-associated molecule pattern (DAMP), including cytokines and other chemo-attractants, are capable of recruiting primary immune cells to the necroptotic cells, followed by phagocytosis and termination of cell death signaling[45]. Furthermore, inflammation is inclined to tumorgenesis[46] and promotes cancer progression and metastasis, attenuating adaptive immune responses and increasing drug resistance[47].

There is another assumption that poly ADP-ribose polymerase 1 (PARP-1) participates in the disposal of necroptotic cells[48]. PARP-1 is a nuclear enzyme that is essential in repairing DNA damage[49]. PARP-1 overexpression induced by DNA-strand breakage results in NAD^{+} and ATP consumption, and the release of apoptosis-inducing factor (AIF) that binds to DNA from mitochondria to nucleus, causes chromatin condensation and DNA fragmentation, and induces necrosis[50]. Compound **2** is able to inhibit the effects of PARP-1 and block the release of AIF from mitochondria to nucleus, whereas direct inhibition of PARP-1 fails to protect cells from necroptosis[48]. Thus, PARP-1-induced necrosis may be associated with the downstream pathway of necroptosis.

Necroptotic cells are cleared from the immune system through pinocytosis, or cellular drinking, which is mediated by macropinosome, a subcellular component of macrophages[51]. The process of propagation of necroptosis is irreversible; therefore, more attention should be paid to discover inhibitors to block the process of initiation and execution of necrosis.

3. Detection of necroptosis

Since the mechanism of necroptosis has been clarified quite clearly, several methods have been developed to detect necroptosis based on the morphological and biochemical characteristics (Fig. 3). It should be noted that these methods were often used in combination rather than alone since there are many interference factors in the experiments.

3.1. Direct observation of necroptosis cells by morphology change

Direct observation of cell morphology by transmission electron microscopy (TEM) provides a preliminary judgment to distinguish apoptosis, necrosis and necroptosis[52–54]. Apoptosis is characterized by cell shrinkage, nuclear condensation and organelle fragmentation[52,55], which is apparently distinct from necrosis and necroptosis because they are characterized by cell swelling and membrane rupture[53]. For necroptosis, it can be distinguished from necrosis that necroptotic cells retain integral nucleus while necrotic cells do not[54]. Chen et al.[56] clearly observed necroptotic cell morphology including the changes of endoplasmic reticula, mitochondria, cytomembrane and cell nucleus compared with normal cells by TEM.

3.2. Identifying necroptotic cells by flow cytometry

Flow cytometry is able to distinguish necroptotic cells. Compared with the control group, an increase in annexin V^-/propidium $iodide^+$ or annexin V^+/propidium $iodide^+$ in treated cells is the indicative of primary necrosis or secondary necrosis, respectively, while a decrease in annexin V^-/propidium $iodide^+$ is the indicative of apoptosis[57]. Notably, this method is not enough to accurately identify necroptosis, which should be applied in combination with other methods.

3.3. Detecting necroptotic cells by content variation and phosphorylation status of proteins

Expression of important proteins involved in necroptosis, such as RIPK1, RIPK3, and MLKL, can be detected by Western blot or immunofluorescence. Their overexpression and phosphorylation are commonly the indicative of necroptosis. There are several antibodies that can be applied to verify whether the proteins are activated at the phosphorylation sites. For example, anti-phospho-S14/15 RIPK1 antibody and anti-phospho-S166 hRIPKl antibody were used to detect the activation of RIPK1[58,59]. Anti-phospho-S227 hRIPK3 antibody[60] and anti-phospho-S232 mRIPK3 antibody[61] were used to detect the activation of RIPK3, while anti-phospho-T357/S358 hMLKL antibody[62] was often used in detecting phosphorylation of MLKL.

3.4. Detection of necrosome formation

Necrosome formation can be detected by RIPK1/RIPK3 interactions in FADD or caspase-8 pull-down experiments. For RIPK3/MLKL or complex formations, immunoprecipitation or other methods are commonly used[62–64].

3.5. Cell death inhibition in RIPK1, RIPK3 or MLKL deficient transgenic models

If cells are killed by necroptosis, knockdown of RIPK1, RIPK3 or MLKL can rescue cells, leading to a higher IC_{50} value or higher cell viability compared to the normal models. The mechanism is similar to that of necroptosis inhibitors. For example, knockdown of RIPK3 or MLKL through siRNA in NP cells showed decreased cytotoxicity compared with untransfected cells under the same circumstances, indicating the untransfected cells were killed through necroptosis[56].

3.6. Rescuing cells from necroptosis by chemical inhibitors

Recently, the development of the new screening methods facilitated the discovery of a number of necroptosis inhibitors[65]. Combination use with necroptosis inhibitors would rescue necroptotic cells from the single treatment, leading to higher IC_{50} values or higher cell viability[66]. Classification and activity of necroptosis inhibitors will be introduced in the following sections (Figs. 4–7).

4. Inhibitors of necroptosis

Release of DMAPs during necroptosis could induce inflammation, which is known to promote tumorgenesis and cancer metastasis[67]. The mechanism will be introduced in the following sections.

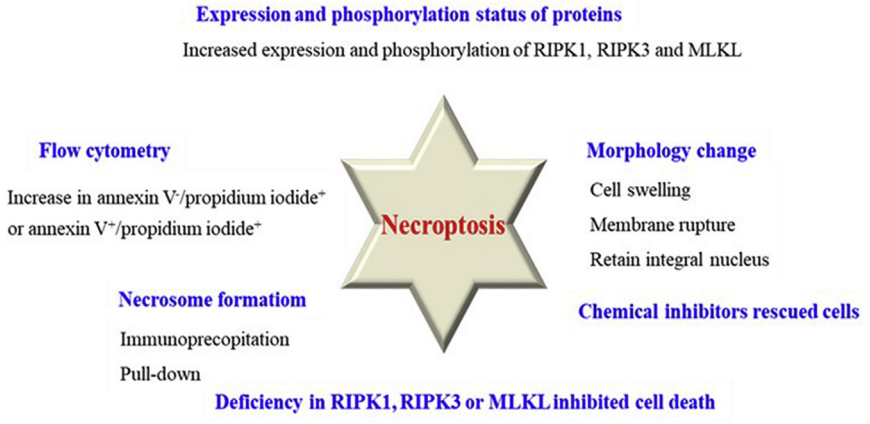


Figure 3 Methods of detection of necroptosis.

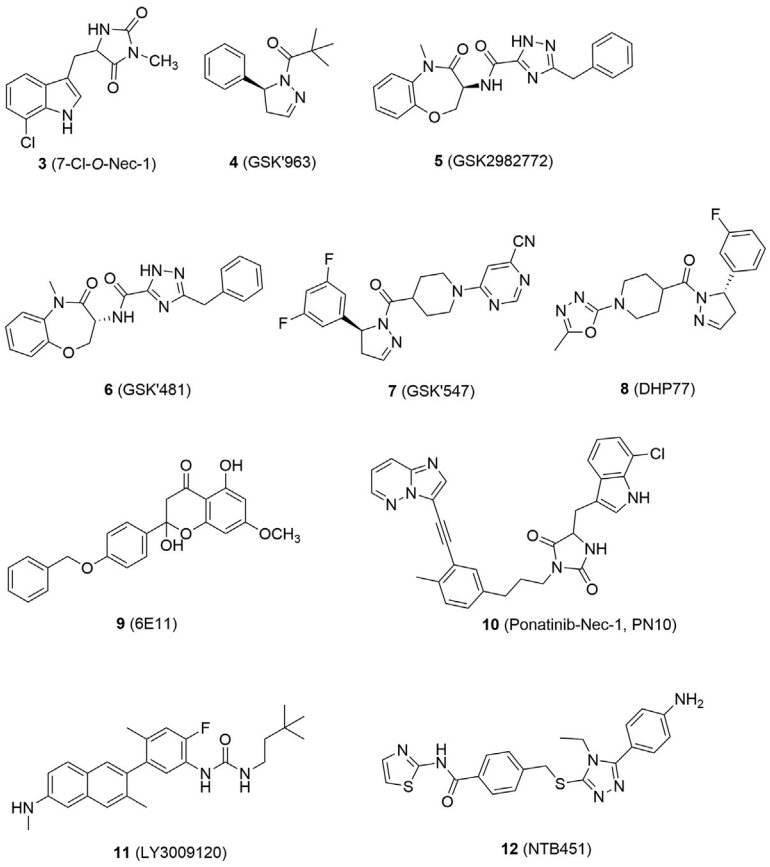


Figure 4 Chemical structures of RIPK1 inhibitors.

Therefore, inhibiting necroptosis may be helpful in cancer treatment.

4.1. RIPK1 inhibitors

Compound **2** was a RIPK1 inhibitor (RIPK1 IC_{50} = 1.98 μmol/L) and has been widely used as a tool compound in the mechanism studies of necroptosis[68]. However, it was relatively toxic because it also inhibited indoleamine 2,3-dioxygenase (IDO), resulting in the interference in inflammation-associated tumorgenesis to break tumor immune-tolerance and sensitize tumors to cell death[68]. In contrast, its analog 7-Cl-*O*-Nec-1 (**3**, RIPK1 IC_{50} = 0.91 μmol/L, Fig. 4) is less toxic because it did not show any inhibition towards IDO[68].

GSK′963 (**4**, RIPK1 IC_{50} = 29 nmol/L) was discovered by screening GSK compound collections and exhibited higher inhibitory activity than compound **2** at molecular and cellular levels[69]. It was verified as a selective RIPK1 inhibitor because it was inactive against more than 100 kinases[69]. At the dose of 2 mg/kg, compound **4** could inhibit 90% RIPK1 activity and protect mice from shock even at a lower dosage (0.2 mg/kg), while its enantiomer was inactive so that it can be used to validate on-target effects[69].

GSK2982772 (**5**, RIPK1 IC_{50} = 16 nmol/L) is the first orally-active RIPK1 inhibitor and is currently undergoing phase II clinical studies for treatment of ulcerative colitis, psoriasis and rheumatoid arthritis[70]. Compound **5** showed excellent activity in rats and monkeys with a wide therapeutic window (dosage range: 2−1000 mg/kg)[70,71]. It also demonstrated low toxicity such as no inhibition towards recombinant human cytochrome P450 (CYP450s), weak inhibition towards human Ether-a-go-go-related gene (hERG, IC_{50} = 195 μmol/L) and weak activation of human Pregnane X receptor (hPXR, EC_{50} = 13 μmol/L)[70,71].

GSK′481 (**6**, RIPK1 IC_{50} = 10 nmol/L) was identified by screening GSK's DNA-encoded small-molecule libraries, and was considered as a promising lead compound due to its high selectivity and potency[72]. GSK′547 (**7**) is also a highly selective RIPK1 inhibitor against other kinases with favorable pharmacokinetic properties in mice, whose oral exposure was 400 times higher than that of compound **4**[73]. In the model of type C1 Niemann-Pick disease (NPC1), treatment with compound **7** could significantly extend the life span of mice[74].

Robert's group[75] discovered DHP77 (**8**, RIPK1 IC_{50} = 2.0 nmol/L) as an orally active RIPK1 inhibitor through high-throughput screening and hit optimization. Compound **8** is a potent and selective dihydropyrazole RIPK1 inhibitor and with good pharmacokinetic and pharmacodynamic profiles in several animal models, such as sclerosis and retinitis pigmentosa[75].

Natural product derivative 6E11 (**9**, RIPK1 IC_{50} = 90 nmol/L) inhibited necroptosis induced by TNF-α or TRAIL, but failed to inhibit TRAIL-induced apoptosis, thus providing a novel structural type for RIPK1 inhibitors[76].

Derived from pharmacophore fusion of ponatinib (**29**, dual RIPK1 and RIPK3 inhibitor) and compound **2**, PN10 (**10**, RIPK1 IC_{50} = 90 nmol/L) was discovered as a potent and specific RIPK1 inhibitor and showed excellent *in vivo* activity in treatment with TNF-α[77]. LY3009120 (**11**), a pan-RAF inhibitor, was able to inhibit necroptosis by blocking phosphorylation of RIPK1 and subsequent

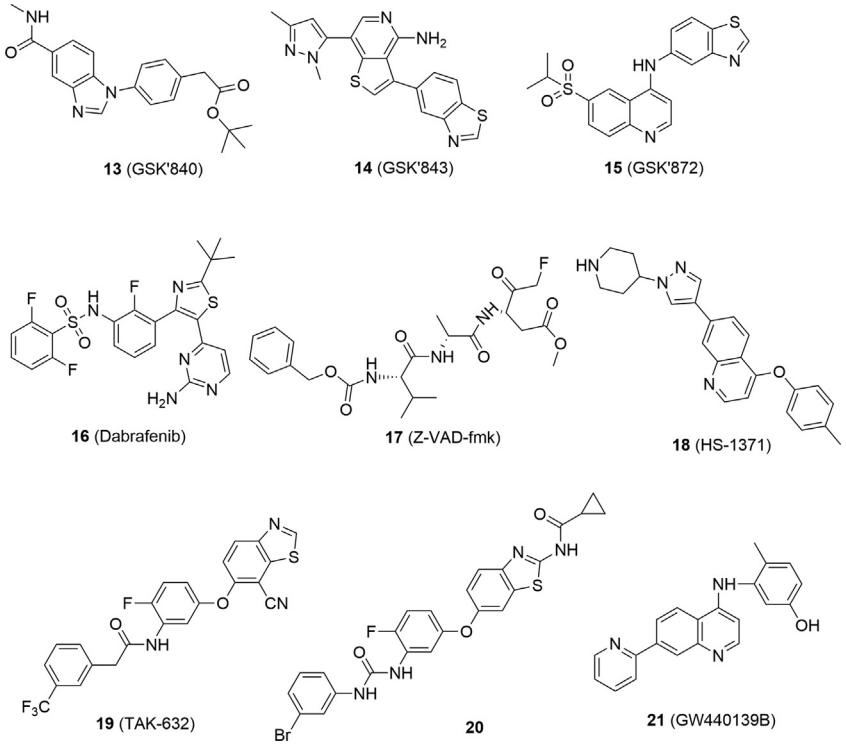


Figure 5 Chemical structures of RIPK3 inhibitors and caspase inhibitor **17**.

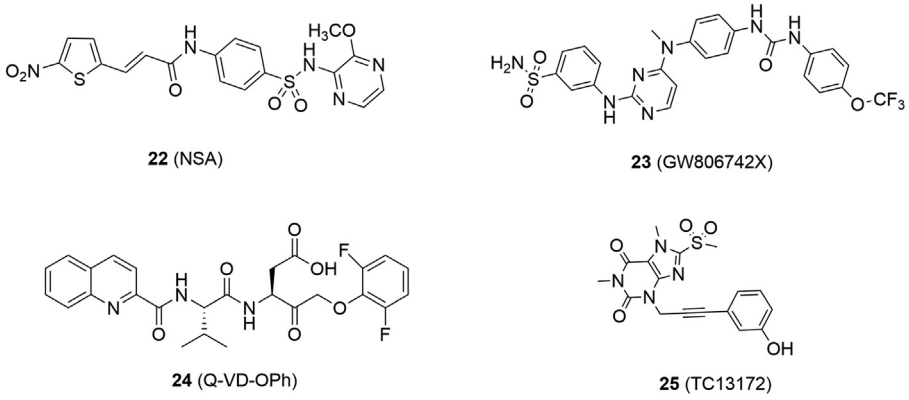


Figure 6 Chemical structures of MLKL inhibitors and caspase inhibitor **24**.

phosphorylation of RIPK3 and MLKL[78]. NTB451 (**12**) was confirmed to be a specific RIPK1 inhibitor (IC_{50} = 7.9 μmol/L) through molecular docking, molecular dynamics simulation and drug affinity-responsive target stability (DARTS).

Furthermore, DNL747 and DNL758 were validated as highly efficacious RIPK1 inhibitors, whose chemical structures remain undisclosed. DNL747 is a blood−brain barrier (BBB) penetrable compound and has entered clinical trials for the treatment of Alzheimer's disease (AD)[79], while DNL758 was unable to penetrate BBB and was supposed to be used in the treatment of systemic inflammatory[79].

4.2. *RIPK3 inhibitors*

GSK′840 (**13**, RIPK3 IC_{50} = 0.3 nmol/L), GSK′843 (**14**, RIPK3 IC_{50} = 6.5 nmol/L) and GSK′872 (**15**, RIPK3 IC_{50} = 1.3 nmol/L) are classic and potent RIPK3 inhibitors identified by screening compound libraries (Fig. 5)[80]. Dabrafenib (**16**, RIPK3 IC_{50} = 250 nmol/L), a clinical drug for the treatment of $Braf^{V600E}$ melanoma, was also validated to target RIPK3, which showed excellent selectivity compared to its inhibition against RIPK1, RIPK2 and RIPK5[81]. TNF-α, Smac mimetic and caspase inhibitor z-VAD-fmk (**17**, TSZ) are widely accepted to be able to induce RIPK3-mediated necroptosis[82]. Dabrafenib rescued cells from TSZ-induced cell death in RIPK3-expressed HT29 cells, in which morphology changes were apparently observed[81]. Dabrafenib had no influence on the apoptosis induced by TNF-α and Smac mimetic in HT29 cells, suggesting that it had little impact on apoptosis[81]. Dabrafenib was proven to be able to bind to RIPK3 through molecular docking and the surface plasma resonance assay[81]. Further analysis indicated that it disrupted the RIPK3−MLKL interaction rather than RIPK1 and RIPK1−RIPK3 interaction[81].

HS-1371 (**18**) was discovered to be a RIPK3 inhibitor (IC_{50} = 20.8 nmol/L) by screening kinase-targeted chemical

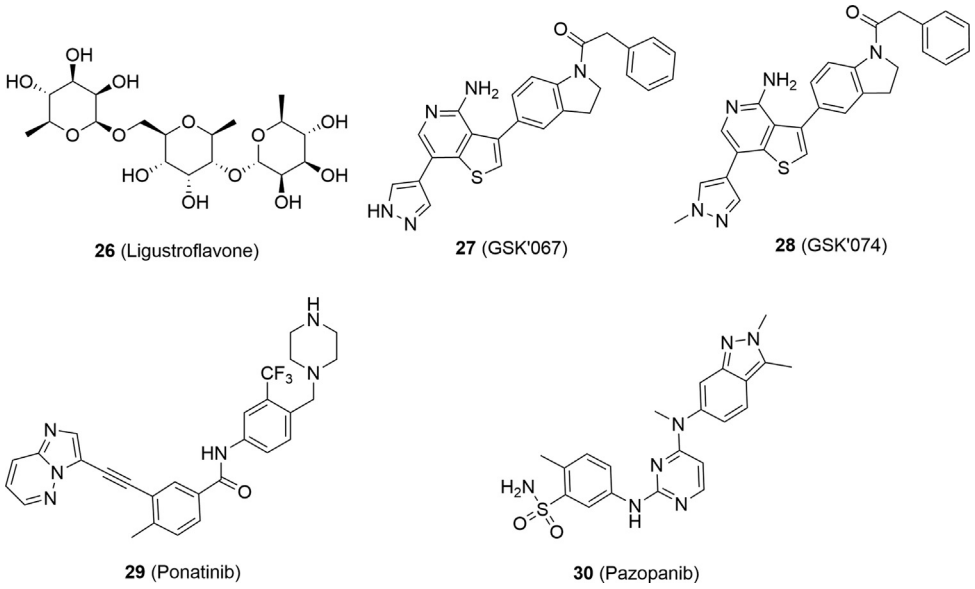


Figure 7 Chemical structures of multi-targeting necroptosis inhibitors.

libraries[83]. Molecular docking studies demonstrated that HS-1371 directly bound to the ATP binding pocket of RIPK3[83]. ATP competition assay showed that HS-1371 inhibited RIPK3 in a time-dependent and ATP-competitive manner[83]. HS-1371 declined phosphorylation of RIPK3, thus blocking the interaction of RIPK3 and MLKL[83]. Furthermore, HS-1371 was able to rescue cells from TNF-induced necroptosis rather than apoptosis[83]. Pretreatment or post-treatment of cells with HS-1731 both inhibited RIPK3-mediated necroptosis[83].

Structural optimization of dual RIPK1 and RIPK3 inhibitor TAK632 (**19**) led to a selective RIPK3 inhibitor (**20**, RIPK3 K_d = 81 nmol/L), which showed more than 60-fold selectivity against RIPK3 than RIPK1[84]. Molecular docking studies showed that compound **20** could form $\pi-\pi$ stacking and hydrogen bonding interactions with RIPK3 rather than RIPK1, accounting for the high selectivity[84]. Compound **20** rescued cells from necroptosis rather than apoptosis, indicating it is a selective necroptosis inhibitor[84]. It blocked the phosphorylation of RIPK3 and MLKL while having no influence on the phosphorylation of RIPK1[84]. Compound **20** also displayed favorable pharmacokinetic properties in rats with an oral bioavailability of 25.2% without obvious side effects[84].

Green's group[85] previously confirmed the significant role of the phosphorylation of Ser345 in necroptosis, and then designed a selective monoclonal antibody for detection of necroptosis. Using this antibody to detect their capability to inhibit necroptosis, GW4401398 (**21**, EC_{50} = 73.6 nmol/L) was identified by screening 8904 compounds[85]. GW4401398 was proven to inhibit TNF- and TRIF-mediated necroptosis independent of RIPK1−RIPK3 interaction[85]. It directly inhibited RIPK3-mediated MLKL phosphorylation, thus inhibiting necroptosis[85].

4.3. *MLKL inhibitors*

Necrosulfonamide (NSA, **22**, Fig. 6) was identified by screening compound libraries and blocked necroptosis induced by different stimulus[86]. Target validation investigations by synthesizing biotin-based probes and pull-down experiments further confirmed that MLKL was a specific target for NSA[86].

By screening a library of 367 compounds using thermal shift assay, GW806742X (**23**, RIPK3 K_d = 9.3 μmol/L) was validated to bind to the MLKL pseudokinase domain in an ADP or ATP dependent manner[87]. It dose-dependently inhibited necroptosis of wild-type mouse dermal fibroblasts stimulated with TSQ consisting of TNF, Smac-mimetic, and caspase inhibitor Q-VD-OPh (**24**)[87].

Yan et al.[88] identified TC13172 (**25**) as a novel MLKL inhibitor with an EC_{50} value of 2 nmol/L through phenotype-based screen and subsequent structure−activity relationship study, which was inactive against RIPK1 and RIPK3. It was confirmed to bind to MLKL in cell lysates at the site of Cys-86 by a biotin-tag and pull-down experiments[88]. Further mechanism studies indicated that compound **25** inhibited the translocation of MLKL to cell membrane rather than the phosphorylation of MLKL to block cell necroptosis[88].

4.4. *Multi-targeting necroptosis inhibitors*

Ligustroflavone (**26**, Fig. 7), a natural product extracted from privet, was verified to possess anti-inflammation activity[89]. Upon the treatment of ligustroflavone, RIPK3 and MLKL were significantly decreased and the interaction between RIPK3 and RIPK1 or MLKL was reduced, while RIPK1 remained unaffected[89]. Thus, ligustroflavone was considered as a triple RIPK1/RIPK3/MLKL inhibitor and blocked necroptosis[89].

GSK′067 (**27**) and GSK′074 (**28**) were RIPK1 and RIPK3 dual inhibitors identified by screening compound libraries[90]. Further mechanism studies indicated that compound **28** blocked the formation of RIPK1−RIPK3 complex and phosphorylation of MLKL without affecting the expression levels of RIPK1 and RIPK3[90]. It blocked necroptosis with an IC_{50} value of 3 nmol/L and showed high binding affinity to RIPK1 (K_d = 12 nmol/L) and RIPK3 (K_d = 130 nmol/L) without activity to other kinases, indicating its selectivity to RIPK1 and RIPK3[90].

Fauster et al.[91] screened 268 U.S. Food and Drug Adminstration (FDA) approved drugs for their ability to inhibit TNF-α-induced necroptosis, and identified ponatinib (**29**) and pazopanib (**30**) as necroptosis inhibitors. Ponatinib is a potent and orally available antitumor agent targeting BCR-ABL, VEGFR2 and FGFR1, while pazopanib is also a multi-targeting kinase inhibitor with inhibitory activity against VEGFR1, VEGFR2, VEGFR3, PDGFR, FGFR, c-Kit and c-Fms[91]. Ponatinib and pazopanib both displayed potent inhibitory activity against necroptosis with an EC_{50} of 89 and 254 nmol/L, respectively[91]. Further mechanism studies demonstrated that they blocked necroptosis induced by TNF-α, TRAIL and FasLs[91]. Ponatinib declined the phosphorylation of RIPK1, RIPK3 and MLKL, and inhibited the interaction between RIPK3 and MLKL[91]. Furthermore, thermal shift assay confirmed that RIPK3 was a target of ponatinib[91]. These data indicated that ponatinib directly targeted RIPK1 and RIPK3[91]. Similarly, pazopanib showed relatively lower affinity towards RIPK1 and RIPK3. However, it had no influence on the interaction between RIPK3 and MLKL[91].

5. Necroptosis in cancer therapy

5.1. *Necroptosis is important in cancer treatment*

5.1.1. *Cancerous cells undergoing necroptosis is immunogenic*

When the necroptotic cells are co-cultured with BMDCs (mouse bone marrow-derived dendritic cells), they can be phagocytized, resulting in the phenotypic maturation of BMDCs[92]. Meanwhile, necroptotic cancer cells in the form of vaccination effectively cross-primed cytotoxic $CD8a^+$ T cells *in vivo* and induced strong CT26 tumor antigen-specific production of IFN-γ *ex vivo*, which was independent of the extent of NF-κB activation[93]. Thus, necroptotic cancer cells were considered as potent inducers of adaptive immune response and could mediate efficient antitumor immunity[92]. Additionally, there was evidence that compound **2** could prolong the life span of activated primary macrophages, suggesting that necroptosis could modulate the amount of active macrophages during infection[92]. Thus, necroptosis may serve as a cellular defense mechanism against the invasion of foreign organisms.

5.1.2. *Necroptosis influences the metastasis of cancer cells*

Metastasis is the primary cause of morbidity and mortality in cancer patients, which includes dissociation of malignant cells in the primary tumor, local invasion, angiogenesis, intravasation of invading cells into the vasculature or lymphatic systems, survival in these channels, extravasation, and proliferation at a distant site[94]. It was reported that shikonin (**37**) dramatically reduced the metastasis of osteosarcoma C6 and U87 glioma cells due to its induction of necroptosis[95,96]. Besides, ROS plays a significant role in the migration and invasion of cells through regulation of cytoskeleton dynamics and adhesion molecules, such as controlling expression levels of essential extracellular matrix-degrading enzymes (*e.g.*, uPA and uPAR) in cell metastasis[96,97]. As mentioned above, overproduction of ROS promotes the execution of necroptosis. In other studies, RIPK3 was supposed to modulate the ROS level in case of necroptosis[97]. Thus, necroptosis has a close relationship with the metastasis of cancer cells.

5.1.3. *Necroptosis serves as a back-up way for apoptosis*

Before the concept of necroptosis was suggested, a prevailing trend in cancer chemotherapy is to discover molecules that can induce apoptosis[98]. However, apoptosis inducers cannot work in some circumstances, such as drug resistance due to the activation of alternative compensatory pathways, upregulation of drug transporters and multidrug resistance[98]. For example, proapoptotic agents, such a TRAIL and BCL-2 inhibitors, can induce necroptosis in cancer cells when apoptosis is blocked[99]. When a diphtheria-based fusion toxin or Smac mimetic applied with demethylating agents (*e.g.*, 5-azacytidine or 5-aza-20-deoxycytidine), it can synergistically trigger cancer cell death and overcome apoptosis resistance by inducing necroptosis[98]. Trastuzumab, a humanized recombination monoclonal antibody, induces necroptosis to overcome the resistance of tumor cells against antibody treatment[100]. Treatment of L929 cells with caspase-inhibitor **17** (Fig. 4) mimicked the invasion of virus involved in innate immunity. As a result, such cells were proven to commit suicide through necroptosis[92]. The underlying mechanism was associated with the fact that apoptosis relied on the activation of caspases whereas necroptosis is independent of the activation of caspases[92]. Therefore, necroptosis can serve as an alternative way in cancer therapy in replace of various pathways of apoptosis.

5.2. *Necroptosis may be conducive to cancer progression*

Necroptotic cells are characterized with cell membrane leakage and release of intracellular damage-associated molecular patterns (*e.g.*, IL-1 family cytokines, nucleic acids, ribonucleoproteins, histones, HMGB family members, and heat-shock proteins) which result in inflammatory responses and related side effects[45]. Subsequently, they might facilitate tumor progression, promote tumor cell proliferation and survival, as well as tumor angiogenesis, invasion and metastasis, leaving a detrimental effect on the tissue which is not favorable for the treatment[101]. In turn, the damaged tissue may also promote the metastasis of cancer cells[101].

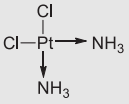
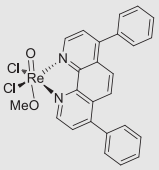
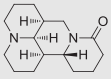
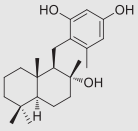
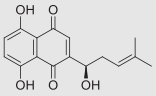
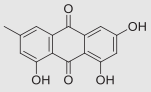
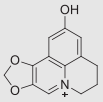
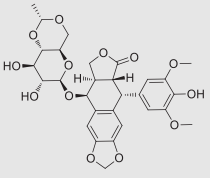
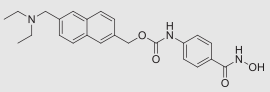
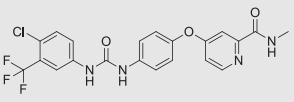
5.3. *Necroptosis is not universally sensitive in tumor cell lines*

Different cancer cell lines vary in terms of their sensitivity to necroptosis due to different environments such as the availability of oxygen or nutrients[102]. For example, human colorectal carcinoma cells are more sensitive to necroptosis under the hypoxic circumstances or in the absence of glucose compared to that in presence of abundant oxygen or glucose, possibly because of pyruvate scavenging of mitochondrial free radicals[103]. Also, the expression level of RIPK3 is fundamental for inducing necroptosis[102]. Numerous cancer cell lines, such as human breast adenocarcinoma cell line MCF-7, human hypotriploid cell line Hek293 and human cervix adenocarcinoma cell line HeLa, are unsuitable for necroptosis-based therapy due to low level of RIPK3 expression[102]. Therefore, necroptosis-based chemotherapy is only effective in limited tumor cell lines.

6. Necroptosis inducers

Recently, a series of compounds were reported to be capable of inducing tumor necroptosis in different pathways (Table 1)[95,104–129]. Several of them have been previously known to act by typical

Table 1 Necroptosis-inducing agents and their therapeutic applications.

Necroptosis-inducing agent		Type of cells	Dosage	Ref.
Inorganic salts and metal complexes				
$CoCl_2$	**31** (Cobalt chloride)	HT-29 (human colon carcinoma cells)	300 mol/L	104
LiCl	**32** (Lithium chloride)	Human primary Schwannoma cells	20 mmol/L	105
	**33** (Cisplatin)	L929 cells (mouse fibroblast cells)	100 mol/L	106
	**34** (Rhenium(V) oxo complexes)	A549 cells (human lung cancer cells)	20 mol/L	107
Natural products				
	**35** (Matrine)	Mz-ChA-1 and QBC939 (human cholangiocarcinoma cells)	1.5 mg/mL	108
	**36** (Neoalbaconol)	C666-1 and HK1 cells (human nasopharyngeal carcinoma cells)	40 mol/L	109
	**37** (Shikonin)	C6 and U87 (human glioma cells)	3 μmol/L	95
	**38** (Emodin)	U251(human glioblastoma cells)	20 μmol/L	110
	**39** (Ungeremine)	CCRF-CEM (human leukemia cells)	4.89 μmol/L	111
Antitumor agents				
	**40** (Etoposide)	MDA-MB-231 (human breast cancer cells) HT1080 (human sarcoma cells)	20 mmol/L	112
	**41** (Givinostat)	HDLM-2 and L-540 HL cells (human Hodgkin lymphoma cells)	100 μmol/L	113
	**42** (Sorafenib)		5 μmol/L	

(*continued on next page*)

Table 1 (*continued*)

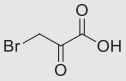
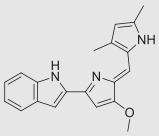
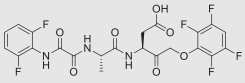
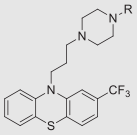
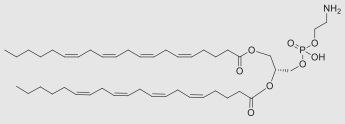
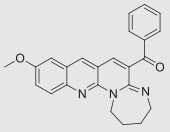
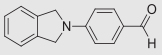
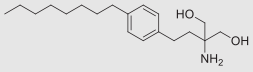
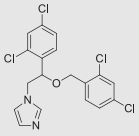
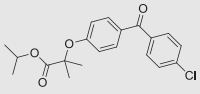
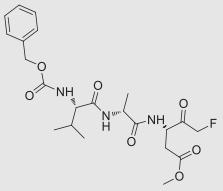
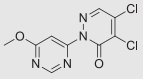
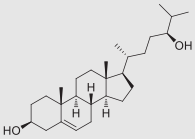
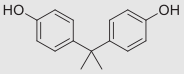
Necroptosis-inducing agent		Type of cells	Dosage	Ref.
	**43** (3-Bromopyruvate)	MDA-MB-435 cells (human breast cancer cells)	40 μmol/L	114
	**44** (Obatoclax)	TE671 and RMS13 cells (human bone marrow transverse muscle carcinoma cells)	200 nmol/L	115
	**45** (5-Fluorouracil)	CRC cells (human colorectal cancer cells)	50 μg/mL	116
	**46** (IDN-7314)			
	**47** (Methyl methanesulfonate)	A549 cells (human lung cancer cells)	100 μmol/L	117
	**48** (ZZW-115)	MiaPaCa-2 (human pancreatic cells)	15 μmol/L	118
	**49** (Diarachidonoylphosphoethanolamine)	NCI-H28 cells (human malignant pleural mesothelioma cells)	100 μmol/L	119
	**50** (3u)	A375 cells (human melanoma cells)	8 μmol/L	120
	**51** (673A)	SKOV3 (human ovarian cells)	12.5 μmol/L	121
Non-antitumor agents				
	**52** (FTY720)	U251MG, U87MG cells (human glioblastoma cells)	15 μmol/L	122
	**53** (Miconazole)	MDA-MB-231 (human breast cancer cells)	40 μmol/L	123
	**54** (Fenofibrate)	Hep3B (human hepatoma cells)	50 μmol/L	124
Other necroptosis inducers				
	**17** (Z-VAD-fmk)	Classically activated macrophages	20 μmol/L	125
	55 (Furosine)	Primary hepatocytes	100 μg/mL	126

Table 1 (*continued*)

Necroptosis-inducing agent		Type of cells	Dosage	Ref.
	**56** (LGH00168)	A549 cells (human lung cancer cells)	3.26 μmol/L	127
	**57** (24(*S*)-Hydroxycholesterol)	SH-SY5Y cells (human neuroblastoma cells)	50 μmol/L	128
	**58** (Bisphenol A)	SH-SY5Y (human neuroblastoma cells)	1 nmol/L	129

antitumor mechanisms, whose new role in necroptosis aid to draw a comprehensive mechanism diagram of these antitumor agents.

6.1. Inorganic salts and metal complexes as necroptosis inducers

Cobalt chloride ($CoCl_2$, **31**) was able to induce necrosis through direct observation by TEM. While it was verified to elevate the level of hypoxia-inducible factor-1α (HIF-1α) and induce necroptosis at 300 μmol/L with increased levels of RIPK1, RIPK3 and MLKL proteins when caspase activity was suppressed by compound **17** in HT-29 cells[104].

Lithium chloride (LiCl, **32**) was confirmed to induce cell death through activating TNF-α and inducing necroptosis[105]. The effect could be reversed by the treatment of necroptosis inhibitor **2**, ROS inhibitor *N*-acetyl-L-cysteine (NAC), and inhibitors of the AKT/mTOR pathway in schwannoma cells, suggesting that LiCl-induced necroptosis was associated with ROS production[105].

Cisplatin (**33**) is conventionally considered to induce cell apoptosis[130]. Chen et al.[106] found that combinational treatment of cisplatin and pan-caspase inhibitor **17** increased the death of L929 cells compared to single treatment with cisplatin, suggesting there is additional pathway in cisplatin-induced cell death. Moreover, the addition of necroptosis inhibitor **2** dramatically rescued almost all of the cells. Further studies revealed that L929 cells treated with cisplatin secreted TNF-α which promoted the formation and translocation of RIPK1/RIPK3/MLKL complex, followed by the mitochondrial permeability transition and release of ROS, which finally led to the necroptosis[106].

Rhenium(V) oxo complexes (**34**) effectively killed cancer cells by triggering necroptosis observed by fluorescence microscopy with cell membrane disintegration and PI uptake[107]. They also induced mitochondrial membrane rupture which is a downstream of ROS generation[107]. ROS generation induced by Rhenium(V) oxo complexes could be downregulated by co-treatment with necroptosis inhibitor **2**, suggesting that RIPK1 is essential in ROS production, which is indicative of necroptotic prosperities[107].

6.2. Natural products as necroptosis inducers

Matrine (**35**), an alkaloid extracted from traditional Chinese herb *Sophora flavescens*, was reported to induce necroptosis in cholangiocarcinoma cells, which was different from its classic apoptosis-inducing effects in other cancer cell lines[108]. Mz-ChA-1 and QBC939 cells (cholangiocarcinoma cells) treated with matrine were characterized with extensive organelle, plasma membrane rupture and integral nuclei which coincided with the morphology of necroptotic cells[108]. Additionally, cells died due to the treatment of matrine could not be rescued by the addition of pan-caspase inhibitor **17**, but they could be rescued by necroptosis inhibitor **2**[108]. Further study verified that matrine induced necroptosis through the formation of RIPK1/RIPK3/MLKL complex[108]. Osmotic pressure and release of ROS also facilitated the necroptotic process[108]. More interestingly, matrine was able to upregulate the expression of RIPK3 in Mz-ChA-1 cells, making it possible to treat cholangiocarcinoma with a low expression of RIPK3[108]. This work potentially provided a new strategy to deal with the apoptosis-resistance in cholangiocarcinoma therapy[108].

Neoalbaconol (NA, **36**), extracted from *Albatrellus confluens*, was reported to trigger several kinds of cell death[109]. NA downregulated E3 ubiquitin ligases, resulting in reduced ubiquitination of RIPK1. Thus, an elevated expression level of RIPK1 was observed, which also activated the transcription of TNF-α[109]. Moreover, NA caused RIPK3-mediated ROS generation which contributed to cell death[109]. Thus, it can be concluded that NA was able to induce necroptosis through activation of TNF-α and RIPK3-dependent production of ROS[109].

Shikonin (**37**), extracted from the traditional Chinese herb "Zicao", was originally used to treat wound healing because of its anti-inflammatory and antimicrobial properties[131]. It was reported to kill tumor cells by inducing apoptosis[131]. Huang et al.[95] found that lost in plasma membrane integrity and intact nuclear membrane in glioma cells were observed in cells treated with shikonin directly by electronic transmission microscopy. Shikonin-induced C6 and u87 glioma cells can be rescued by necroptosis inhibitor **2** but unaffected by treatment with the caspase inhibitor **17**. Also, an increased expression of RIPK1 was observed after treatment with shikonin[95]. All these facts indicated that shikonin-induced necroptosis through RIPK1 activation.

Emodin (**38**), an anthraquinone derivative extracted from traditional Chinese medicine *Rheum palmatum*, has been used to treat various diseases due to its antitumor, anti-inflammation, anti-metastasis and immunosuppressive effects, but its mechanism of action remained unclear[132]. Zhou et al.[110] found that cells treated with emodin showed increased levels of RIPK1, RIPK3 and TNF-α. Moreover, combinational use of emodin with necroptosis inhibitor **2** or **15** reduced the release of lactate dehydrogenase (LDH)[110]. Hematoxylin-eosin (H&E) staining of tumor tissues separated from mice treated with emodin demonstrated obvious necrosis effect[110]. Further studies indicated that TNF-α, RIPK1,

RIPK3 and MLKL in tumor tissues were upregulated when treated with emodin, indicating that it could inhibit glioma growth *in vivo* through necroptosis[110].

Ungeremine (**39**), an alkaloid extracted from *Ungernia minor*, showed potent cytotoxicity in drug-resistant cancer cell lines and upregulated RIPK3 level[111]. Co-treatment with necroptosis inhibitor **2** decreased the cytotoxicity of ungeremine, and flow cytometry analysis indicated that 13.1% cells were necoptotic[111].

6.3. *Antitumor agents as necroptosis inducers*

Etoposide (**40**), a topoisomerase II inhibitor, has been used for the treatment of several various cancers, such as small cell lung cancer, lymphomas and testicular cancer[133]. Meier's group[112] reported that etoposide (20 mmol/L) promoted the binding of caspase-8 to RIPK1 and FADD in cancer cells independent of TNF, TRAIL death receptors and mitochondria, while the effect could not be observed in normal HUVEC cells.

Combinational treatment of HDAC inhibitor givinostat (**41**) and kinase inhibitor sorafenib (**42**) showed synergistic activity in triggering cell death, ROS generation and mitochondrion disruption[113]. Further studies indicated that the combinational use induced cell necrosis three times higher than the single dosage[113]. Treatments with necroptosis inhibitor **2** or the ROS inhibitor resulted in cell survival, which indicated combinational use of givinostat and sorafenib-induced necroptotic cell death[113].

3-Bromopyruvate (3-BrPA, **43**), an alkylating agent, was reported to induce cell death in various ways through inhibiting hexokinase II activity which is a key enzyme of glycolysis overexpressed in cancer cells[134]. The addition of NSA reversed T24 cell (bladder carcinoma cell line) death caused by 3-BrPA, however radically treatment combined with necroptosis inhibitor **2** could rescue cells from death, indicating that 3-BrPA induced necroptosis in a RIPK1-independent but RIPK3-dependent pathway[114].

Obatoclax (**44**), a small-molecule inhibitor of BCL-2 proteins, was verified to trigger cell death *via* autophagy[115]. Recently, it was reported to stimulate the interaction between Atg 5 (a constituent of autophagosomal membranes) and components of the necrosome (*e.g.*, FADD, RIPK1 and RIPK3) through co-immunoprecipitation assay[115]. Knockdown of RIPK1 or treatment of necroptosis inhibitor **2** both suppressed obatoclax-induced cell death[115]. Moreover, knockdown of RIPK3 also partially reversed cell death[115]. Knockdown of RIPK1 and RIPK3 failed to affect the cells undergoing autography, indicating that necroptosis induced by obatoclax is the downstream of autography[115]. *In vivo* study was performed in RIPK1 wide-type and RIPK1 knockdown models, and the results showed that RIPK1 knockdown significantly suppressed the tumor inhibition caused by obatoclax[115].

5-Fluorouracil (5-FU, **45**) is a thymidylate synthetase inhibitor and is used as the standard chemotherapy for colorectal cancer[135]. However, drug resistance of 5-FU is a huge challenge in clinical studies[116]. Recently, it was reported that pan-caspase inhibitors were able to sensitize 5-FU to cell death in drug resistant cells[116]. *In vivo* studies showed that the combination of pan-caspase inhibitor IDN-7314 (**46**) and 5-FU showed synergistic effects in inhibiting tumor growth of HT29 xenografts[116]. Further mechanism study showed that the combinational use could suppress caspase-8 mediated cleavage of RIPK1 and stabilize RIPK1-containg complexes and the component of necrosome was observed. Thus, 5-FU induced necroptosis in the presence of pan-caspase inhibitors in drug-resistant models[116].

Methyl methanesulfonate (**47**), an alkylating agent, was reported to induce cell death through apoptosis[136]. Recently, Jiang et al.[117] found that high dosage (400 and 800 μmol/L) of compound **47** induced cell death through necroptosis rather than apoptosis. Co-treatment of compound **47** with necroptosis inhibitor **2** could reverse cell death, while co-treatment with caspase inhibitor **17** failed[117]. Necroptosis biomarkers, such as leakage of LDH, HMGB1, ROS and RIPKs, were upregulated, while apoptosis biomarkers caspase-3 and caspase-9 were not influenced, suggesting that compound **47** was able to induce necroptosis in A549 cells (human lung cancer cells)[117].

Trifluoperazine, an antipsychotic agent, possesses antitumor activity[137]. However, its application in cancer therapy is limited by significant side effects on central nervous system[137]. Based on the structure−activity relationship study, trifluoperazine derivative ZZW-115 (**48**) was found to induce cell death mainly by necroptosis without neurological effects[118]. Co-treatment with necroptosis inhibitor **2** or caspase inhibitor **19** could partially reverse cell death induced by compound **48**, indicating that it induced necroptosis and apoptosis through independent mechanisms[118].

Diarachidonoylphosphoethanolamine (DAPE, **49**) was verified to induce apoptosis and necrosis through flow cytomety. Later, it was found that the cell death induced by NCI-H28 cells could be partially reversed by necroptosis inhibitor **2** or knockdown of RIPK1, indicating that DAPE could induce necroptosis[119]. Moreover, mitochondrial membranes were disrupted and ROS was released, which could be reversed by cyclophilin D inhibitors. Therefore, DAPE induced necroptosis through activation of RIPK1- and RIPK3-dependent production of ROS[119].

Naphthyridine derivatives are heterocycles widely used in anti-tumour therapy[138]. Kong et al.[120] discovered a potent naphthyridine derivative (**50**) through screening 1,3-diazaheterocycle fused naphthyridine derivatives. Low dosage (4 and 8 μmol/L) of compound **50**-induced cell death could not be clearly rescued by caspase inhibitor **17**, while rescue effects were observed at high dosage (12, 16 and 20 μmol/L), suggesting that there was another pathway to induce cell death when compound **50** was used at low dosages. Western blot studies showed that RIPK1 and MLKL were upregulated at low concentrations while cleaved by caspase-8 at high concentrations, suggesting that necroptosis was induced at low concentration and inhibited at high concentrations[120].

Aldehyde dehydrogenase (ALDH) is associated with antitumor drug resistance and is a potential target for cancer therapy[139]. Pretreatment of cells with the caspase inhibitor **17** before ALDH inhibitor 673 A (**51**) did not promote cell viability, indicating that compound **51** induces cell death in a non-apoptotic way[121]. Compound **51**-treated cells stained with DAPI led to nuclear swelling and loss of nuclear content, which was consistent with TEM, indicating that cells are necroptotic[121]. Downregulation of RIPK1 levels failed to rescue necroptotic cells trigger by compound **51**, and similar results were observed for the necroptosis inhibitor **2** treatment[121]. Low dose of MLKL inhibitor NSA could protect cells from necroptotic, suggesting that compound **51** induced necroptosis is RIPK3 dependent[121].

6.4. *Non-antitumor agents as necroptosis inducers*

FTY720 (**52**) is an immunosuppressive drug that was approved for the treatment of multiple sclerosis[140]. The levels of RIPK1 and RIPK3 were upregulated in human glioblastoma cells when

treated with compound **52**, indicating that it induced RIPK1/RIPK3-mediated necroptosis[122]. Cells treated with compound **52** and necroptosis inhibitor **2** rescued most of the cells, while knockdown of RIPK3 also resulted in rescuing cells from death[122].

Antifungal agent miconazole (**53**) induced both apoptosis and necroptosis in MDA-MB-231 cells, which could be directly observed by TEM[123]. Treatment with caspase inhibitor **17** or necroptosis inhibitor **2** could partially rescue cells, indicating that micronazole induced both apoptosis and necroptosis[123]. Furthermore, upregulated RIPK3 levels, MLKL levels and ROS generation were also observed as necroptotic properties[123].

Fenofibrate (**54**) was widely used to reduce high cholesterol and high triglyceride levels in blood[141]. Hep3B cells (human hematoma cells) treated with fenofibrate for 24 h underwent nuclear morphology changes such as chromatin condensation, nuclear fragmentation and irregularly shaped nucleus[124]. Analysis of the effect of fenofibrate on the expression level of caspases and necroptotic markers showed that active form of caspase-3 and caspase-8 were decreased while RIPK1, RIPK3 and MLKL were upregulated, indicating that fenofibrate induced necroptosis in Hep3B cells[124].

6.5. *Other necroptosis inducers*

As described above, compound **17** is a pan-caspase inhibitor and is commonly used to sensitize resistant cells through inducing necroptosis[125]. When it was used alone, the expression level of phosphorylated MLKL was upregulated in LPS-activated macrophages (CAMs)[125]. Furthermore, co-treatment with necroptosis inhibitor **2** partially inhibited LDH release and phosphorylation of MLKL in CAMs, while co-treatment with TNF-α antagonist had no influence on compound **17** induced cell death, indicating that it induced necroptosis is TNF-α independent[125].

Furosine (**55**) was reported to activate the expression of RIPK1, RIPK3, MLKL and TNF-α in hepatocytes, suggesting that it induced the necroptosis pathway and subsequent inflammatory reactions[126].

C/EBP homologous protein (CHOP) played a significant role in endoplasmic reticulum (ER) stress-induced apoptosis[142]. A549 cells treated with CHOP activator LGH00168 (**56**) were regarded as necroptotic due to rupture of plasma membrane and lysosomal membrane permeabilization and caspase-8 inhibition[127]. Moreover, it could generate ROS in a dose-dependent manner and led to rupture of the plasma membrane, which was a symbol of necrosis. However, it can be effectively attenuated by ROS scavenger NAC, which indicated that necroptosis rather than necrosis played an essential role in inducing cell death[127]. Co-treatment with necroptosis inhibitor **2** rescued cells from death and reduced PI uptake, suggesting that the induced necroptosis was in a RIPK1-dependent manner[127].

24(*S*)-Hydroxycholesterol (24*S*−OHC, **57**) is enzymatically produced in the brain to maintain cholesterol homeostasis[143]. In T-lymphoma Jurkat cells, it triggered caspase-independent cell death, which was suppressed by additional treatment of necroptosis inhibitor **2** or knockdown of RIPK3, suggesting its necroptosis inducing roles[128].

Bisphenol A (BPA, **58**) is an endocrine disruptor with estrogenic and obesogenic activity[42]. SH-SY5Y cells (human neuroblastoma cell line) treated with compound **58** underwent apoptosis within 48 h, then necrosis was occurred and increased gradually[129]. When necroptosis inhibitor **2** was added in combination treatment with compound **58**, necroptosis was replaced by apoptosis, indicating that it induced necroptosis[129].

7. Necroptosis and other diseases

Apart from the relationship with tumor therapy, necroptosis has been reported to be related to cerebral ischemia, neurodegenerative diseases and immune diseases. Dabrafenib, a potent RIPK3 inhibitor, was proven to be able to block LPS-induced activation of TNF-α and significantly reduce infarct lesion size, indicating that inhibiting necroptosis could be neuroprotective and attenuate cerebral ischemia[144]. Necroptosis could mediate neuronal loss in neurogenerative diseases. Either inhibition of RIPK1 or the knockdown of RIPK3 and MLKL was able to decrease degeneration, suggesting inhibiting necroptosis was favorable in treating neurodegenerative diseases[145]. Overexpression of RIPK3 was observed in human Paneth cells and increased necroptosis in Crohn's disease, suggesting potential therapeutic effect of necroptosis inhibition[146].

8. Conclusions and perspectives

In summary, the mechanism of necroptosis and its applications in cancer therapy were reviewed. Necroptosis of cancer cells could be initiated by various stimuli and through different pathways. RIPK1, RIPK3 and MLKL played an important role in the process of necroptosis, resulting in membrane leakage and release of cytokines. Necroptosis is associated with tumor metastasis which is the major cause of morbidity and mortality in cancer patients in clinic. Therefore, induction of necroptosis is an effective strategy in clinical cancer therapy. Necroptotic cells could potentially induce intrinsic and adaptive immune response and thus mediate efficient antitumor immunity. In addition, necroptosis can serve as a back-up way for apoptosis-resistant circumstances, which are capable of overcoming the obstacle of drug-resistance which is common but intricate in clinical cancer therapy. For those apoptosis-resistant cases, necroptosis inducers could sensitize tumor cells to death, which provides another way to overcome drug resistance when common therapy is failed. Thus, drug development of necroptosis inducers deserves to be paid more attention. However, the release of cytokines also induces inflammation which would be harmful to the tissue and in turn tissue damage could facilitate the metastasis of tumor cells. However, necroptosis is accompanied by the release of cytokines and induce inflammation, which is harmful to the tissue and in turn tissue damage can facilitate the metastasis of tumor cells. Also, it should be noted that necroptosis is not widely sensitive in cancer cells in that apoptosis is the main cause of cancer death. Therefore, the necroptosis inducers are mostly favorable in apoptosis-resistant cases in clinical cancer therapy. Currently, several antitumor agents have been verified to act as necroptosis inducers while more medicinal chemistry efforts are required to discover new inducers with drug-like properties.

So far, a series of necroptotic inducers have been identified, paving the pathway for investigating the new mode of cancer

death and providing novel therapeutic tools. However, most studies are carried out by *in vitro* experiments, and the *in vivo* efficacy of necroptosis inducer and their selectivity in killing tumors remain to be further explored. Also, there is still lack of *in vivo* necroptotic markers. Thus, it is highly desirable to discover new necroptotic markers and investigate their effects on the selectivity of cancer cells. With better understanding of the mechanism of necroptosis in cancer cells, targeting necroptosis will be an effective strategy for cancer therapy.

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Author contributions

Chunquan Sheng and Guoqiang Dong proposed the idea and revised the manuscript. Ying Wu and Guoqiang Dong wrote the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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