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Small molecule probes for cellular death machines

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Abstract

The past decade has witnessed a significant expansion of our understanding about the regulated cell death mechanisms beyond apoptosis. The application of chemical biological approaches had played a major role in driving these exciting discoveries. The discovery and use of small molecule probes in cell death research has not only revealed significant insights into the regulatory mechanism of cell death but also provided new drug targets and lead drug candidates for developing therapeutics of human diseases with huge unmet need. Here, we provide an overview of small molecule modulators for necroptosis and ferroptosis, two non-apoptotic cell death mechanisms, and discuss the molecular pathways and relevant pathophysiological mechanisms revealed by the judicious applications of such small molecule probes. We suggest that the development and applications of small molecule probes for non-apoptotic cell death mechanisms provide an outstanding example showcasing the power of chemical biology in exploring novel biological mechanisms.

Introduction

Apoptosis has been established as an evolutionarily conserved programmed cell death mechanism involved in sculpting the development of metazoan [1–3]. On the other hand, necrosis had been traditionally considered to be an uncontrolled process triggered by overwhelming environmental stress or acute injury. In the past decade, this opinion has been challenged by the identification of molecular mechanisms involved in regulating necrotic cell death [4–7]. In this regard, the development of small molecule modulators of necrotic cell death has played major roles in the discovery of cellular machinery that controls cell death beyond apoptosis. The combination of chemical biology and traditional genetics has made it possible to vigorously define novel cell death mechanisms as well as establish their physiological and pathological significance. Here we will provide a critical review of the

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field and discuss future prospective and applications in understanding the molecular mechanisms of cell death beyond apoptosis.

Small molecules are not only core players in pharmaceutical industry for drug development, but also have become powerful tools for investigating the function of proteins and biological mechanisms in academic research. Small molecules provide several advantages for exploring biological functions in mammalian systems: easy to be applied in cell culture systems, accessible to precise temporal control and possibility to be tested in animal models of human diseases. Furthermore, small molecules provide the possibility to selectively target one particular function of a multifunctional protein, which used to be only possible in traditional genetic model systems such as fruit fly *Drosophila* and nematode *C. elegans*. In this review, we will discuss two non-apoptotic forms of cell death mechanisms, of which the discovery and development were tightly connected with the application of small molecule tools.

Necroptosis: a programmed necrotic cell death

The ability of TNF α , a very important pro-inflammatory cytokine, to induce necrotic cell death of certain cultured cell lines was noted at late 1980's and early 1990's [8,9]. In this regard, Vercammen et al. demonstrated that inhibition of caspases not only did not inhibit, as one would expect if caspases mediated this type of cell death, but sensitized the death of L929 cells treated with TNF α [8]. Various mechanisms were speculated as to how TNF α might be able to induce necrosis, including the induction of ROS after mitochondrial damage, or aggregated TNF α to punch holes directly on cell membrane. Holler et al. extended these studies to demonstrate that activated primary T cells and Jurkat cells, a T cell line, could also undergo this type of necrotic cell death when stimulated by FasL with inhibition of caspases. By complementing RIPK1 deficient Jurkat cells with a kinase inactive mutant, Holler et al. showed that the kinase activity of RIPK1 was required for Jurkat cells to die when induced by FasL, TNF α and TRAIL under caspase deficient condition [10]. However, the scope of this study was limited as it was unclear if this mechanism applied to any other cell types or in vivo, as which would require making RIPK1 kinase dead mutations in each cell line and in animal models. Without such evidence, one cannot establish a common necrotic cell death mechanism regulated by RIPK1.

Degterev et al. conducted a phenotypic high throughput screen for small molecule inhibitors of U937 cell necrosis induced by TNF α and zVAD.fmk, a pan caspase inhibitor [4], which led to the identification of necrostatin-1 (Nec-1). Nec-1 was able to inhibit a wide array of necrosis induced by ligands of death receptors in 10 different cell cultured models of necrosis including that induced by the dimerization of RIPK1 kinase domain. The ability of Nec-1 to inhibit this diverse list of necrotic cell death models made it possible to propose the existence of a common regulated necrotic cell death mechanism termed "necroptosis" [4]. Nec-1 was shown to be an inhibitor of RIPK1 [11]. The ability of Nec-1 to inhibit diverse examples of necrosis induced by ligands of death receptor family demonstrated the critical role of RIPK1 kinase activity in mediating necroptosis as a common regulated necrosis mechanism [12,13].

The ease of applying a small molecule RIPK1 inhibitor, Nec-1, made it possible to widely test the role of RIPK1 and necroptosis in many cell culture systems and animal models as a tool to study the involvement of necroptosis [12,13]. The discovery and application of Nec-1 provided a prominent example of the power of chemical biology in exploring unknown biological mechanisms and has led to the human clinical trials for developing RIPK1 inhibitors as treatments of human inflammatory and neurodegenerative diseases from colitis and rheumatoid arthritis to amyotrophic lateral sclerosis and Alzheimer's disease. The success of Nec-1 also stimulated subsequent chemical biological studies of necroptosis and led to the development of not only inhibitors of RIPK1, but also RIPK3 and MLKL [14–16] (Figure 1).

The rapid development in the past decade has led to the understanding of the molecular mechanism that mediates necroptosis [17]. When cells are stimulated by TNF α under caspase deficient condition, the intracellular domain of TNFR1 recruits a complex that includes RIPK1 to promote its activation. Activated RIPK1 in turn promotes necroptosis by interacting with RIPK3 to lead to its activation. Activated RIPK3 then mediates the phosphorylation of MLKL, a catalytically inactive pseudokinase [14,15,18] to promote cell lysis [16,19–22]. Emerging in vitro and in vivo evidence has demonstrated necroptosis as a common intrinsic cell death mechanism in response to the activation of death receptors, toll-like receptors [23,24] and viral infections [25,26] in various cell and animal models. Necroptosis has been established as a form of regulated cell death involved in ischemia-reperfusion injury [27,28], inflammatory diseases [29], pathogen infection [30,31], tumor metastasis [32] and neurodegenerative diseases [33,34].

RIPK1 kinase inhibitors

The definitive evidence for RIPK1 as the target of Nec-1 and its improved analog Nec-1s (also called 7N-1) was provided by Degterev et al. [11] (Table 1). Although the original isolate Nec-1, methyl-thiohydantoin-Trp (MTH-Trp), has been noted to be a low affinity inhibitor of indoleamine 2,3-dioxygenase (IDO) (IC₅₀~100 μ M), an improved analogue of necrostatin-1, 7-Cl-O-Nec-1(=Nec-1s or 7N-1) (5-(7-chloro-1H-indol-3-yl)methyl)-3-methylimidazolidine-2,4-dione[35,36], has no IDO inhibitory activity. Nec-1s is a highly specific and CNS permeable inhibitor of RIPK1 [37,38]. Since the kinase activity of RIPK1 is involved in mediating most, if not all, of the deleterious functions downstream of TNFR1 upon stimulation by TNF α , inhibition of RIPK1 may provide much of the beneficial effects of anti-TNF α therapy without the side effects associated with removing TNF α . Thus, the ability of small molecule inhibitors of RIPK1 such as Nec-1s to block its kinase activity without affecting its scaffolding function may selectively block the deleterious effects of TNF α without removing TNF α [4]. This prediction is supported by the normal physiology of mice carrying genetic knockin mutations that inactivate RIPK1, including D138N and K45A, and their resistance against systemic inflammatory syndrome induced by TNF α as well as animal models of neurodegeneration [38–41].

RIPK1 kinase has been recognized as an important therapeutic target for the treatment of inflammatory and degenerative human diseases. GlaxoSmithKline (GSK) has identified a set of RIPK1 inhibitors using fluorescence polarization (FP) assay. Different from Nec-1, which

occupies a hydrophobic pocket in close proximity to the activation loop of RIPK1 kinase as type III inhibitor[42], these GSK compound series (1-aminoisoquinolines, pyrrolo[2,3-b]pyridines and furo[2,3-d]pyrimidines, represented by Cpd27) bind to the DLG-out inactive conformation as typical type-II inhibitors [43]. GSK has also developed a type III RIPK1 inhibitor GSK2982772, an optimized derivative of benzoxazepinone, and advanced this compound into human clinical trials targeting psoriasis, rheumatoid arthritis, and ulcerative colitis [44,45]. In addition, Denali Therapeutics has advanced a RIPK1 inhibitor into Phase I human clinical trial targeting ALS and AD.

Multi-targeted type II receptor tyrosine kinase inhibitors Ponatinib, Rebastinib and Pazopanib were reported as pan-RIP family kinase inhibitors and blocked necroptotic cell death efficiently. A hybrid molecule named PN10 designed as a fusion of the scaffold of ponatinib and Nec-1s robustly and selectively inhibited RIPK1 kinase and protected against necroptosis in cellular and mouse model [46]. Exploring novel chemical space on RIPK1 may be an important future direction for developing potent RIPK1 inhibitors.

RIPK3 kinase inhibitors

RIPK3 is a homolog of RIPK1 and contains a N-terminal kinase domain like other members of RIP family, but lacks a C-terminal death domain as that of RIPK1. The kinase activity of RIPK3 is important for mediating TNF α induced necroptosis [15,47]. The RHIM domain in activated RIPK1 interacts with the RHIM motif in RIPK3 to form necrosome to transmit downstream necroptosis signaling [48]. RIPK3 was postulated to be a highly specific mediator of necroptosis when it was first identified. Surprisingly, while both D138N and K45A RIPK1 kinase dead knockin mutant mice as well as RIPK3 deficient mice are normal and resistant to TNF α mediated inflammatory syndrome and mouse models of neurodegeneration [38–41], the RIPK3 kinase dead D161N knockin mice were shown to be embryonic lethal due to excessive apoptosis mediated by caspase-8 [49]. In addition, inducible inhibition of RIPK3 kinase activity by RIPK3 D161N mutation in adult mice also led to weight loss, intestinal degeneration with caspase-8-dependent apoptosis and lethality [49]. Consistently, a series of small-molecule RIPK3 kinase inhibitors, such as GSK'840, GSK'843 and GSK'872, developed by GSK can suppress necroptosis but promote apoptosis [24,50] (Table 1). It is still not clear how the kinase activity of RIPK3 controls the activation of caspase-8.

MLKL inhibitors

The pseudokinase MLKL was identified as a mediator of necroptosis using a biotinylated small-molecule probe, Necrosulfonamide (NSA), that can form covalent bond with its targets [16] (Table 1). Although NSA can form covalent linkages with multiple proteins, the use of NSA in combination with anti-RIPK3 immunoprecipitation allowed the identification of hMLKL, due to the covalent modification of Necrosulfonamide (NSA) through Michael addition reaction with MLKL at Cys86 to allow pulldown by biotinylated-NSA. The binding of NSA prevents the oligomerization of N-terminus four-helical bundle domain (4HB) and propagation of necroptosis. However, Cys86 is not conserved between human and mouse MLKL, and that impedes the application of NSA in mouse models.

GW806742X, also known as SYN-1215, was shown to compete with ATP to the nucleotide binding site in mouse MLKL pseudokinase domain, sequentially block the conformational change induced by RIPK3 kinase [20]. The working mechanism of GW806742X should be further investigated as it is originally reported as a VEGFR2 inhibitor, and may even have off-targets on other kinases such as RIPK1.

HSP90 inhibitors

The key mediators of necroptosis, including RIPK1 [51,52], RIPK3 [53,54] and MLKL [55,56], have all been reported as clients of Hsp90 and its co-chaperon cdc37. The Hsp90/cdc37 complex assists the folding of RIPK1/RIPK3/MLKL into proper conformation and promotes the assembly of necrosome. Genetic or pharmacological interference with Hsp90 function destabilizes RIPK1, RIPK3 and MLKL, resulting in their degradation by the proteasome pathway. However, since Hsp90 has a diverse array of client proteins, some of which are critical for cell survival, inhibiting Hsp90 would affect cell survival as well as necroptosis.

Ferroptosis: cell death triggered by the accumulation of lipid peroxidation

Ferroptosis is a form of iron-dependent but caspase-independent regulated cell death, driven by depletion of cellular antioxidant defenses such as glutathione (GSH) and overwhelming lethal lipid peroxidation [5]. GSH depletion leads to an increase in the intracellular labile iron pool to promote ferroptosis (Figure 2). The morphology of ferroptosis is distinct from that of apoptosis and necroptosis, but the underlying biochemical mechanism of ferroptotic cell death remains unclear.

Ferroptosis was first defined also in a phenotypic screen by a set of small molecules (RAS-selective lethal = RSL) able to selectively induce cell death in isogenic tumors carrying a mutant form of RAS [57]. Induction of ferroptosis by small molecule erastin (ERA) was shown to be mediated by the inhibition of system x_c^- [5]. System x_c^- is a cystine–glutamate antiporter which is important to provide adequate levels of cystine (the oxidized form of cysteine) for the synthesis of the tripeptide glutathione (GSH). GSH is involved in inhibiting ferroptosis by maintaining the function of GSH peroxidase 4 (GPx4), which is critical for preventing detrimental phospholipid oxidation. GPx4, a widely expressed selenium protein in brain, exists as a membrane anchored glycoprotein and mediates the reduction of complex hydroperoxy lipids and various thiols. Consistent with the role of GPx4 in ferroptosis, RSL3, a ferroptosis inducing compound, can directly inactivate GPx4 to induce the accumulation of lipid peroxides independent of GSH [5,57]. Other inducers of ferroptosis including Buthioninesulfoximine (BSO) [58], Lanperisone [59], Sorafenib [60,61] and Artesunate [62] may induce similar synthetic lethality by indirectly dysregulating the GPx4 pathway (Table 1). While various upstream stimuli may converge on the same lipid peroxidation downstream signal pathway to induce necroptosis, the biochemical mechanism by which cell death occurs in ferroptosis needs to be established.

On the other hand, since oxidative stress and depletion of GSH has long been established as a major cause of tissue injury and cell death, resistance to chemotherapy and neurodegenerative diseases [63–66], the activation of ferroptosis has been proposed to be

involved in a wide range of human diseases including cancer [5,58], acute renal injury [67,68], ischemia/reperfusion heart injury [69], retinal degeneration [70], neuronal cell death after intracerebral hemorrhage (ICH) [71], Parkinson's disease [72], Huntington's disease (HD) [73] and other neurodegenerative diseases [74]. However, we would like to note that since the biochemical mechanism of ferroptosis remains to be provided and oxidative stress and depletion of GSH has been known to be associated with other forms of cell death, including apoptosis [75], the presence of oxidative stress and depletion of GSH per se in cell death is not sufficient to indicate ferroptosis.

Lipophilic antioxidants

Disruption of redox homeostasis is a key feature of ferroptosis, therefore, common antioxidants such as trolox, α -tocopherol, butylated hydroxytoluene (BHT), β -carotene, glutathione peroxidase mimetic-ethylselen (Ebs) can all protect cells against ferroptosis [5,76].

Ferrostatins, a class of arylalkylamine, were identified as lipophilic antioxidants, which can specifically inhibit RSL-induced ferroptosis. The lipophilic N-cyclohexyl moiety of ferrostatin-1 (Fer-1) anchors it in biological membrane, and the o-phenylenediamine moiety acts to prevent radical chain reactions as a reducing agent [5,73]. The ability to eliminate radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH) under cell-free condition [5] suggests Fer-1 may work as a ROS scavenger, but interestingly, it has no effect on rotenone induced-mitochondrial superoxide, staurosporine induced-mitochondrial cardiolipin peroxidation, or hydrogen peroxide induced lysosome membrane permeabilization [73]. Such selectivity entitles Fer-1 to be used as a ferroptosis probe for investigating its biological roles in a variety of contexts. Currently, a third-generation Ferrostatin SRS-16-86 with improved plasma and metabolic stability has been developed for in vivo studies [68].

Liproxstatins, a class of spiroquinoxalinamine derivatives were also reported to efficiently inhibit ferroptosis and related biochemical changes induced by ferroptosis-inducing agents (FINs) pharmacologically or GPx4 knockout genetically [67]. These compounds may work as lipophilic antioxidants similar to ferrostatin based on their chemical structures, although the exact working mechanism has not yet been defined.

Lipoxygenases (LOX) inhibitors

LOXs are a family of iron-containing enzymes contributing to the production of lipid hydroperoxides, such as metabolizing arachidonic acid (AA) to hydroperoxy-eicosatetraoic acid (HpETE). The expression levels of LOX-5/-12/-15 are elevated in GPx4-knockout cells and supplement of specific LOX substrate AA accelerated cell death [77]. NDGA (general LOX inhibitor), Zileuton (LOX-5), AA861 (LOX-5, LOX12/15 inhibitor), BW A4C (LOX5, LOX15), PD 146176 (LOX12/15), but not MK886 (LOX-5 inhibitor) rescue GPx4-knockout cells, suggest that LOX12/15 might be important for ferroptosis [67,77] (Table 1).

Iron chelators

Iron metabolism is recognized as another critical mediator of ferroptosis. Iron is known to serve as a catalyst in Fenton reaction for intracellular free radicals generation, and a cofactor of LOX, haem peroxidase or other enzymes for catalyzing oxidative reaction. The

ferroptosis-sensitive Ras^{G12V} mutant cells show elevated levels of cellular labile iron, and preventing cellular iron overload by the addition of iron chelators such as ciclopirox olamine (CPO), deferoxamine (DFO), deferasirox or deferiprone reduces cell death [5,78,79] (Table 1).

Acyl-CoA synthetase long-chain family member 4 (ACSL4) modulators

ACSL4, a member of the long-chain fatty-acid-coenzyme A ligase family, preferentially converts arachidonate and eicosapentaenoate into acyl-CoA esters and thereby plays a key role in lipid metabolism. Knockout of ACSL4 lowers the levels of acyls-arachidonoyl (AA)- and adrenoyl (AdA)-containing PE species and promotes the resistance to lipid oxidation and ferroptosis without affecting GSH level or GPx4 activity. A group of thiazolidinedione peroxisome proliferator-activated receptor- γ (PPAR γ) agonists, including rosiglitazone (ROSI), pioglitazone (PIO) and troglitazone (TRO), were found to directly suppress ACSL4 activity[80] (Table 1). Pharmacologically modulating ACSL4 activity by these PPAR γ agonists alleviate ferroptosis independent of PPAR γ both *in vitro* and *in vivo* [81]. Since ACSL4 promotes the esterification of AA and AdA into PEs which are sensitive to oxidation by lipoxygenases (LOXs), lipid oxidation in ferroptosis can be suppressed by tocopherols and tocotrienols (vitamin E), inhibitors of LOXs [82].

Conclusion and perspectives

The development and application of small molecule probes in phenotypic cell death screens has greatly accelerated our understanding of the biological significance and the underlying mechanisms of necroptosis and ferroptosis, two distinct non-apoptotic cell death pathways. Necroptosis is an orderly necrotic cell death pathway primarily triggered by death receptor activations under pathological conditions. By contrast, mechanistic characterizations of structurally diverse ferroptosis inducers and inhibitors reveal that ferroptosis mainly results from the inactivation of cellular glutathione (GSH)-dependent antioxidant defenses, leading to the accumulation of toxic lipid ROS. Therefore, the signaling propagation in necroptosis and ferroptosis is totally distinct. The transmission of necroptosis signaling resembles canonical signaling pathways and relies on various post-translational modifications of key proteins; whereas a series of metabolic perturbations might constitute the major pathway for ferroptosis. Although we have obtained substantial molecular insights into the mechanism of necroptosis and obtained the congruent results from genetic and pharmacological manipulations, substantial gaps in our knowledge in the pathway remain. Additional small molecule screens with target-specific readouts may help us fill in these gaps. On the other hand, despite significant advances in ferroptosis, molecular components of ferroptosis are still not well defined and the precise roles of iron remain elusive.

Exciting opportunities for pharmacological modulation of RIPK1 are already being explored in human clinical trials as therapeutics for major diseases. Since TNF α is involved in mediating a plethora of human diseases, the inhibition of RIPK1 may benefit patients beyond the indications that are currently being tested. The discovery and development of RIPK1 inhibitors from a phenotypic cell-based screen without the guidance from X-ray structural insights exemplifies the power of chemical biology from exploring novel

biological mechanisms as well as drug development. We may expect that future application of chemical biology in exploring novel biological mechanisms may lead to the discovery and development of additional exciting new drug candidates, including inhibitors of ferroptosis, for the treatment of human diseases.

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Highlights

- Phenotypic screens for non-apoptotic cell death led to the identifications of necroptosis and ferroptosis.
- Small molecule inhibitors of RIPK1 played an important role in establishing necroptosis as a regulated necrotic cell death mechanism.
- Ferroptosis is caused by iron-dependent accumulation of lethal lipid peroxidation.

Figure 1.

The necroptosis pathway and small molecule inhibitors of necroptosis. The binding of TNF α to its cognate receptor TNFR1 triggers the assembly of a membrane associated complex composed of TRADD, RIPK1, TRAF2, cIAPs and CYLD, which promotes the activation of RIPK1. Under apoptosis deficient conditions, activated RIPK1 binds to RIPK3 and stimulates its phosphorylation, which in turn phosphorylates a pseudo-kinase MLKL. Phosphorylated MLKL promotes its oligomerization and translocation from cytosol to cytoplasmic and internal membrane compartments to execute necroptosis. The Hsp90/Cdc37 complex promotes necroptosis by facilitating the folding and maturation of three core components of necroptotic machine, RIPK1, RIPK3 and MLKL. Nec-1 and its improved analog Nec-1s, the clinical candidate GSK2982772, a hybrid molecule PN10 consisting of pharmacophores of Nec-1s and ponatinib, and the type II RIPK1 inhibitor Cpd27 from GSK all inhibit the kinase activity of RIPK1, thereby preventing necroptosis. Additional necroptosis inhibitors include RIPK3 kinase inhibitors GSK'840, GSK'843 and GSK'872,

the covalent inhibitor of MLKL NSA, the ATP competitive inhibitor of MLKL GW806742X, and Hsp90 inhibitors 17-AAG and Kongensin A.

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Figure 2.

The ferroptosis pathway and the inducers and inhibitors of ferroptosis. Ferroptosis is induced by iron-dependent accumulation of lethal lipid peroxidation products due to the disruption of the essential cellular antioxidative defense mechanism. Under normal conditions, the uptake of cystine through system x_c⁻ and the subsequent reduction of cystine to cysteine are critical for the synthesis of the major antioxidant GSH. GPx4, the major enzyme that detoxifies lipid ROS, converts GSH to oxidized glutathione and reduces lipid peroxides to their corresponding hydroxyl derivatives. On the other hand, ACSL4 (acyl-CoA synthetase long-chain family member 4) promotes the production of polyunsaturated fatty acids which may be converted to phospholipids sensitive to oxygenation by lipoxygenases (LOX12/15). The import and export of iron is mediated by TfR1 and Ferroportin respectively. Changes in iron transport can lead to iron overload that induces ferroptosis. Excessive iron in its labile form can catalyze the Fenton reaction to produce free radicals or act as cofactors for LOXs to promote ferroptosis through the Fenton reaction to generate lipid ROS. A series of ferroptosis inducers has been shown to interfere with the anti-oxidant network at different upstream events. Erastin, Glutamate, sorafenin and lanperisone inhibit

cytstine uptake through system x_c^- , BSO blocks the biosynthesis of GSH, and RSL3 inactivate GPx4. Ferroptosis can also be inhibited by a variety of ferroptosis inhibitors. CPO, DFO, Deferasirox and deferiprone inhibit ferroptosis by chelating irons. NDGA, Zileuton, AA861, BW A4C and PD 146176 inhibit LOX 12/15. ROSI, PIO and TRO, known PPAR γ agonists, inhibit ACSL4 and alleviate ferroptosis independent of PPAR γ . Ferrostatins, Liproxstatins, trolox, α -tocopherol, BHT, β -carotene and Ebs function as antioxidants to protect cells from ferroptosis.

Table 1

Small molecule modulators of necroptosis and ferroptosis.

Necroptosis Inhibitors		Ferroptosis Inducers		Ferroptosis Inhibitors	
RIPK1 inhibitors		System x_c^- inhibitors		Lipid ROS scavengers	
Necrostatin-1 (Nec-1)		Erastin		Ferrostatin-1	
				Liproxstatin-1	
GSK2982772		Glutamate		Antioxidants	
PN10		Sorafenib		Trolok	
Cpd27		Lanperisone		α -tocopherol	
RIPK3 inhibitors		γ -glutamylcysteine synthetase inhibitor		Butylated hydroxytoluene (BHT)	
GSK'843		Buthionine Sulfoximine (BSO)		β -carotene	
GSK'840		GPx4 inhibitors		Ebselen (Ebs)	
GSK'872		RSL3		Iron chelators	
MLKL inhibitors		LOX Inhibitors		Ciclopirox (CPO)	
				Deferoxamine (DFO)	

Necroptosis Inhibitors		Ferroptosis Inducers		Ferroptosis Inhibitors	
Necrosulfonamide(NSA)		Nordihydroguaiaretic acid (NDGA)		Deferiprone
GW806742X		Zileuton		Deferasirox
HSP90 inhibitors				ACSL4 modulators	
Kongensin A		AA861		Rosiglitazone (ROSI)
17-AAG		BW A4C		Pioglitazone (PIO)
		PD 146176		Troglitazone (TRO)