Published in final edited form as:

Trends Pharmacol Sci. 2018 December ; 39(12): 1021–1032. doi:10.1016/j.tips.2018.10.004.

Autophagy in Cancer: Regulation by Small Molecules

Allison S. Limpert^{#1}, Lester J. Lambert^{#1}, Nicole A. Bakas¹, Nicole Bata¹, Sonja N. Brun², Reuben J. Shaw², and Nicholas D. P. Cosford^{1,*}

¹NCI Designated Cancer Center, Sanford-Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA

²Department of Molecular and Cell Biology, The Salk Institute for Biological Studies, San Diego, La Jolla, CA, USA

[#] These authors contributed equally to this work.

Abstract

During times of stress, autophagy is a cellular process that enables cells to reclaim damaged components by a controlled recycling pathway. This mechanism for cellular catabolism is dysregulated in cancer with evidence indicating that cancer cells rely on autophagy in the hypoxic and nutrient-poor microenvironment of solid tumors. Mounting evidence suggests that autophagy plays a role in the resistance of tumors to standard-of-care therapies. There is therefore significant interest in the discovery of small molecules that can safely modulate autophagy. In this review we describe recent advances in the identification of new pharmacological compounds that modulate autophagy with a focus on their mode of action, value as probe compounds and validation as potential therapeutics.

Keywords

autophagy; autophagosome; cancer; kinase; oncology

The Modulation of Autophagy with Small Molecules

Restoring energy balance during periods of varying nutrient availability is a fundamental ability of eukaryotic cells [1]. This evolutionarily-conserved catabolic process is called macroautophagy (herein **autophagy**; see Glossary). During autophagy, cells recycle macromolecules and whole organelles by forming a compartment called an **autophagosome** which sequesters and traffics its contents for controlled fusion with the lysosome (Figure 1). Autophagy can act as either a pro-death or a pro-survival process and is both tissue and microenvironment specific [2–6]. Evidence for this contextual complexity comes both from observation of the physiological consequences of genetic impairment of specific autophagy

*Correspondence: ncosford@sbpdiscovery.org (N. D. P. Cosford).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

genes and the observed phenotypes in targeted knockout experiments [7, 8]. Furthermore, some autophagy proteins have non-canonical functions in cellular pathways that control other processes [9, 10]. However, autophagy is often activated in malignant cancers and as a consequence plays an important role in tumorigenesis, tumor evasion and resistance [11]. Given the importance of these pathological mechanisms there is intense interest in the development of specific therapeutic agents that control autophagy [12].

The repurposed autophagy inhibitors chloroquine (CQ) and hydroxychloroquine (HCQ) are clinically approved for the treatment of malaria. These compounds deacidify the lysosome, blocking fusion with the autophagosome. HCQ synergizes with standard-of-care (SOC) therapeutic agents but its anticancer effects are comingled with mechanisms independent of their effect on autophagy [3]. A series of small clinical trials combining high doses of HCQ with SOC have been conducted but these afforded only modest inhibition of autophagy in patients and yielded promising but inconsistent results [13, 14]. Significant limitations in these trials include the lack of potency in modulating autophagy and off-target toxicity, which restricts the therapeutic window [15, 16].

Herein we review recent progress in the discovery, development and therapeutic application of small molecules that modulate autophagic pathways with an emphasis on oncology. The scope is confined to conserved components of macroautophagic flux but it is important to point out that xenophagy and mitophagy have distinct macromolecular components and are also the subject of pharmacological research. Other recent reviews have focused on biological and clinical studies around autophagy whereas this review highlights recent advances in early drug discovery [3, 17–24].

Target: ULK1/2 Inhibition

Among critical factors in the formation of the yeast autophagy preinitiation complex is the apical serine/threonine kinase, ATG1 [25]. There are five mammalian homologues of ATG1, the unc-51-like kinases (**ULK**) 1–4 and serine/threonine kinase 36 (STK36). Of these, only ULK1 and ULK2 have been identified to be integral to the signaling that drives autophagy [26]. While most research to date has focused on the role of ULK1, including the discovery of small molecule ligands of ULK1, it is important to recognize that ULK1 and ULK2 are functional homologues and modulation of both kinases may be critical for autophagy regulation [27]. Like ATG1, ULK1/2 are the recipients of both negative and positive phosphorylation by the metabolic sensor proteins mammalian target of rapamycin kinase (**mTOR**) and 5' adenosine monophosphate-activated protein kinase (**AMPK**) [28–31]. During nutrient deprivation stress, mTOR is inactivated while AMPK becomes active (Figure 1), resulting in the activation of ULK1/2 bound in a complex consisting of **ATG13**, **ATG101**, and **FIP200** [32]. The activated state of ULK1/2 kinase promotes the phosphorylation of several autophagy initiating targets including **Beclin-1** and vacuolar protein sorting 34 (**VPS34**).

From a functional standpoint, the ULK1/2 dependent phosphorylation of components in the initiation complex is a key regulatory step in the nascent formation of the isolation membrane/phagophore and is therefore a target of interest for drug discovery.

a. ULK1/2 Inhibitor SBI-0206965

To discover small molecules that can inhibit autophagy at the point of initiation, Egan *et al.* used target-based reverse pharmacology to develop a small molecule inhibitor specific for ULK1/2 [33]. Starting with a focal adhesion kinase (**FAK**) inhibitor that also inhibited ULK1, analogues were tested using ULK1 *in vitro* kinase assays before progressing to cell-based assays to analyze the phosphorylation state of a consensus sequence in VPS34. This medicinal chemistry strategy based on structure-activity relationship (SAR) data enabled the development of the potent ULK1/2 inhibitor, SBI-0206965 (Table 1). In kinetic inhibition assays, this compound displayed nanomolar **IC₅₀** values for both ULK1 and its homologue ULK2.

Assays to test for target engagement in cells determined that SBI-0206965 inhibits the phosphorylation of VPS34 and Beclin-1, while also eliminating the gel mobility shifts of ATG13 in a manner consistent with a reduction in the ULK1/2 phosphorylation of these proteins. SBI0206965 promoted apoptosis in tumor cells dependent on autophagy and reduced autophagy in cancer cell lines following mTOR inhibition. Importantly when combined with mTOR inhibitors, SBI-0206965 induced robust cell death in apoptotic assays.

The utility of the chemical probe SBI-0206965 was explored by a separate team of researchers [34]. Tang *et al.* demonstrated that non-small cell lung cancer (NSCLC) cell lines have elevated levels of ULK1 and upregulated autophagy, which is linked to poor patient outcome. They showed that downregulation of ULK1 expression in NSCLC cells reduces cellular viability and increases sensitivity to cisplatin, a common NSCLC treatment. They also demonstrated that cisplatin treatment itself upregulates ULK1 expression in NSCLC cells and that short hairpin RNA (shRNA) knock down of ULK1 increases cisplatin-induced cell death [34]. Finally, SBI-0206965 was shown to destabilize the pro-survival proteins BCL2 and BCLXL, suggesting that apoptotic pathway activation is a significant factor in the activity of SBI-0206965 and that ULK1 is a promising therapeutic target for NSCLC treatment [34].

b. ULK1/2 inhibitors MRT67307 and MRT68921

Petherick *et al.* performed an *in vitro* screen of known kinase inhibitors against ULK1 and ULK2 [35]. This led to the discovery of MRT67307 and MRT68921, previously identified as inhibitors of TANK binding protein 1 (**TBK1**, a component of the innate immune response system), that exhibited **IC₅₀** values against both ULK1 and ULK2 in the mid to low nanomolar range (Table 1). Following amino acid starvation to induce ULK1 activity, treatment of mouse embryonic fibroblasts (MEFs) with either MRT67307 or MRT68921 reduced serine 318 phosphorylation on ATG13, an established phosphorylation site for ULK1 [36]. Treatment with either MRT67307 or MRT68921 reduced the processing of microtubule-associated protein 1A/1B-light chain (**LC3**)-I to the lipidated LC3-II phosphatidylethanolamine isoform that is associated with active autophagy (Figure 1) [37]. Unsurprisingly, *in vitro* profiling indicated that MRT67307 and MRT68921 display significant off-target effects at TBK1 and AMPK-related kinases. To determine if the reduction in **autophagic flux** was due to engagement with these kinases, MEFs with genetic

deletions in either TBK1 or liver kinase B1 (LKB1), a regulator of AMPK, were tested under comparable conditions. In these modified cells, the MRT compounds reduced LC3-I processing, suggesting that neither TBK1 nor AMPK were required for compound-dependent autophagy inhibition. Petherick *et al.* also showed that in MEFs MRT68921 blocked ATG flux in wild type but not a drug resistant mutant form of ULK1 (M92T) suggesting that MRT68921 blocks ATG through inhibition of ULK1 [35]. This work exemplifies the context dependence of autophagic modulation, since the off-target inhibition of TBK1 would be expected to have a significant effect on the closely related process of xenophagy.

c. ULK1/2 inhibitors Compounds 1/3

To obtain structural information on the kinase domain of ULK1, Lazarus *et al.* performed an *in vitro* screen for small molecules that could stabilize the ULK1 protein [38, 39]. This process led to the pyrazolylquinazoline derivative Compound 1 (as referenced in 33), which both stabilizes and inhibits ULK1 (Table 1). A crystal structure of ULK1 was solved with Compound 1 bound in the ATP binding site of the active conformation of the kinase. Although equipped with this structural information, chemistry optimization around Compound 1 did not provide an analogue selective enough for ULK1 to merit use in cells [38].

Lazarus *et al.* next explored pyrimidine derivatives identified in the original kinase screen [39]. The compound BX-795 (Table 1), a pyruvate dehydrogenase kinase I (**PDK1**) inhibitor, was the inspiration for chemistry focused on improving selectivity for ULK1, which led to the identification of Compound 3 (as referenced in 33) (Table 1) [39]. Compound 3 was co-crystallized with ULK1 to obtain a high-resolution crystal structure, which confirmed binding to the ATP binding site. In cells treated with Compound 3, a reduction in LC3-I processing was suggestive of autophagy inhibition. This work is significant because it is the first report of high resolution crystal structures of inhibitors bound to ULK1.

d. ULK1/2 inhibitors SR-17398 and SR-20295

Using the ULK1 crystal structure from the work of Lazarus *et al.* [38], Wood *et al.* initiated an *in silico* screen for inhibitors that prioritized hydrogen bonding interactions in the hinge region of the ATP binding pocket [40]. The *in vitro* phosphorylation of ATG13 was used to test predicted hits resulting in the identification of indazole SR-17398 (Table 1) [41]. Molecular modeling guided the synthesis of analogues, with high potency achieved with SR-20295, a nanomolar range ULK1 inhibitor (Table 1). Assessment of the drug-like properties of SR-20295 indicate that this compound exhibits few features that would lead to *in vivo* liabilities [40].

Target: ATG4B

ATG4B is a cysteine protease that cleaves the C-terminus of proLC3, a structural component of the autophagosome membrane (Figure 1). Upon exposure of the C-terminal glycine, **ATG7** activates LC3-I for delivery to **ATG3** which conjugates LC3-I to

phosphatidylethanolamine [42]. The lipidated LC3-II is anchored to the nascent autophagosome and promotes membrane growth and amplification of autophagy [43]. ATG4B is therefore a requisite enzymatic component of the autophagic pathway and a viable target for inhibition.

a. ATG4B Inhibitor NSC185058

The role of ATG4B in osteosarcoma Saos-2 cells was validated in a series of shRNA knockdown experiments described by Akin *et al.* [43]. Loss of the ATG4B protease resulted in a reduction of LC3-II driven formation of autophagosomes with a concomitant weaker capacity for survival in cell culture models of amino acid starvation and reduced growth of osteosarcoma tumors *in vivo*. Based on the existing crystal structure of ATG4B, Akin *et al.* performed *in silico* docking to identify small molecules that bind the active site [43]. Further tests of potential hits in cellular models of autophagy led to the ATG4B inhibitor NSC185058, which blocked amino acid-induced autophagy and suppressed the lipidation of LC3-I (Table 1). NSC185058 was demonstrated to have little effect on the autophagy regulatory pathways governed by mTOR and phosphatidylinositol indicative of selectivity. In a pivotal experiment, NSC185058 was determined to block the formation of autophagosomes. Importantly NSC185058 inhibited growth in mouse osteosarcoma xenograft models while being well tolerated.

The potential value of NSC185058 and ATG4B inhibitors in the treatment of cancer was investigated in a separate effort by Huang *et al.* who identified ATG4B as a direct substrate of mammalian STE20-like protein kinase 4 (**MST4**), which phosphorylates ATG4B on serine 383 [44]. Huang *et al.* linked elevated levels of MST4 expression with glioblastoma progression and reduced patient survival. Suppression of MST4 with shRNA reduced the growth of glioma stem-like cells in culture and lowered the tumor forming ability of these cells in mice. Huang *et al.* determined that the mechanism of MST4-induced tumorigenicity was through its ability to phosphorylate and activate ATG4B allowing for increased autophagic flux.

b. ATG4B Inhibitor UAMC-2526

Interest in ATG4B as a potential target for autophagy modulation *in vivo* prompted studies by Kurdi *et al.* who identified a benzotropolone derivative that significantly inhibits ATG4B [45]. This compound, UAMC-2526, attenuated autophagy in nutrient-deprived mice and was used in xenograft models of colorectal cancer (Table 1). When administered in combination with the SOC drug oxaliplatin, UAMC-2526 enhanced inhibition of tumor growth [45]. However, treatment of animals with UAMC-2526 was limited by unfavorable properties including poor solubility.

Target: VPS34

First identified in yeast to have dual roles in endosomal trafficking and autophagy, VPS34 is the most conserved member of the phosphoinositide 3-kinase (**PI3K**) family of proteins and is the only class III PI3K [46]. The requirement of VPS34 kinase activity for autophagy induction in mammals has been further corroborated in knockout studies [47, 48]. VPS34 is

a vital component of the autophagy initiation complex, along with autophagy regulators Beclin-1, ATG14 and VPS15/PIK3R4. Through its lipid kinase activity, VPS34 converts phosphatidylinositol to phosphatidylinositol-3-phosphate (PI3P) which is incorporated into autophagosomal precursors and are essential for autophagy initiation.

a. VPS34 Inhibitor SAR405

Using an innovative phenotypic high throughput screen (HTS) with HeLa cells stably expressing EGFP-LC3, Ronan *et al.* identified SAR405, a potent inhibitor of VPS34 (Table 1) [49, 50]. Confirmation of its binding mode and selectivity were provided by crystal structure data and a kinase panel indicating specificity for VPS34 over other PI3Ks. SAR405 treated tumor cells were unable to form autophagosomes in response to starvation or mTOR inhibition and SAR405 treatment prevented upregulation of autophagy in response to targeted mTOR inhibition. Interestingly SAR405 synergized with everolimus, a Food and Drug Administration approved mTOR allosteric inhibitor, to block renal and lung tumor cell proliferation *in vitro*. Consistent with these findings, a number of studies have now demonstrated synergy of SAR405 autophagy inhibition with chemotherapeutic agents across multiple cancer types [51–53]. Together, these data establish a therapeutic mechanism that VPS34 inhibition dampens upregulation of a pro-survival autophagy pathway in response to chemotherapeutic agents and prevents resistance.

b. VPS34 Inhibitor PIK-III

Dowdle *et al.* performed a HTS for VPS34 inhibitors to identify a bisaminopyrimidine hit that was chemically optimized into the VPS34 inhibitor, PIK-III (Table 1) [54]. To understand the basis for selectivity over other PIKs, a crystal structure of the VPS34/PIK-III complex was determined. Showing its utility, PIK-III was the tool compound used to demonstrate that VPS34 activity is essential for *de novo* lipidation of LC3-I and proper autophagy. In a pivotal finding, cells engineered to express an inducible dominant negative (DN) ATG4B to completely halt LC3-I processing were treated with PIK-III upon removal of DN ATG4B to directly assess the effects of VPS34 inhibition on the conversion to LC3-II. High levels of LC3-II were observed in control cells after removal of DN ATG4B expression, but no LC3-II was detected in cells treated with PIK-III, clearly showing that inhibition of VPS34 prevents the conversion of LC3-I to LC3-II. Recently, Honda *et al.* reported that a derivative of PIK-III, compound 19, prevents degradation of autophagy substrates in cells, has favorable PK characteristics and elicited LC3-II accumulation in RKO colon cancer flank xenografts [55]. While these results show promise chronic dosing studies with compound 19 and similar inhibitors will be critical to establish the efficacy of VPS34 inhibition *in vivo*.

c. VPS34 Inhibitor VPS34-IN1

A closely related analogue of PIK-III, VPS34-IN1, was the subject of work described by Bago *et al.* (Table 1) [56]. VPS34-IN1 was used as a chemical probe to explore a mechanism for translocation of late endosomes and lysosomes to the cellular periphery which contributes to mTOR activation. Additionally, VPS34-IN1 was used to demonstrate that VPS34 regulates activity and endosomal localization of SG3K, a PX domain-containing kinase, through binding to VPS34 generated PI3P pools on the surface of endosomal

membranes. While VPS34 modulators have demonstrated potential as autophagy regulators, the importance of VPS34 for normal tissue development and function may limit the therapeutic benefit of VPS34 inhibitors in cancer treatment [47, 48, 57, 58].

Other Autophagy Targets

Autophagy is a multistep process that is regulated by various signals during early initiation through to the late lysosomal stages. Autophagy can be modulated by targeting proteins that regulate the pathway, such as ULK1/2, and/or assemble it, such as VPS34, which directly supplies autophagosomal precursors. Alternatively, non-specific and indirect autophagy-mediating cellular components could be effective targets for therapy. This section will focus on indirect targets that regulate the lysosomal degradation pathway by the induction of Beclin-1 degradation or p62 oligomerization as well as non-specific targets that inhibit autophagosome and lysosomal fusion.

a. p62 Inhibitor Verteporfin

The multifunctional scaffold protein p62 engages with a number of signaling intermediates in the mTOR regulatory pathways that play a role in the stress response [59]. Serving as an adaptor protein for ubiquitinated proteins, p62 binds directly to the autophagosome bound LC3-II in a role that enables sequestration of targeted proteins [60]. Verteporfin was identified as an inhibitor of autophagosome accumulation in an automated microscopy screen (Table 1) [61]. Verteporfin is a mixture of two light activated **regioisomers**, and is employed clinically in photodynamic therapy to treat macular degeneration [62]. In the absence of red light, verteporfin was shown to be nontoxic and inhibited the sequestration of cytoplasmic material into the autophagosome *in vitro* and *in vivo* [61, 63]. In the presence of light verteporfin directly targets and modifies p62, which causes the formation of covalently crosslinked p62 oligomers by a nonspecific mechanism involving low-level highly reactive singlet oxygen production that proximally crosslinks proteins. Interestingly crosslinked p62 oligomers retain their ability to bind to LC3-I [60]. In nutrient rich conditions verteporfin appears to have no effect on cell viability, however, it substantially decreased cell survival in nutrient-deprived MCF-7 breast tumor cells. Furthermore, *in vivo* xenograft studies showed that verteporfin inhibits tumorigenesis in PC-3 prostate tumor cells [64].

b. Ubiquitin Specific Peptidase 10 (USP10) Inhibitor Spautin

A mechanistically distinct way to regulate autophagy is illustrated by Spautin-1, a small molecule that targets the Beclin-1 subunit of the VPS34 complex (Table 1) [65]. Spautin-1 is derived from the phosphodiesterase inhibitor MBCQ and was originally identified as an agent that reduced the spot size and intensity of LC3-GFP puncta in an image based screen [65]. Beclin-1 is not the direct target of Spautin-1, rather the compound acts as an inhibitor of the deubiquitination peptidases USP10 and USP13 that control the degradation of Beclin-1 in the VPS34 complex and has the net effect of reducing the activity of the PI-3K complex. The inhibition of autophagy by Spautin-1 has been tested as a means to enhance apoptosis in chronic myeloid leukemia (CML) in combination with the BCR-ABL tyrosine kinase inhibitor imatinib by restricting autophagy-associated resistance [66].

c. Lysosomal Inhibitors ROC-325, Lys05 and DQ661

Compounds that disrupt lysosomal function, similar to chloroquine or hydroxychloroquine would arguably escape the complex pathway crosstalk of autophagy initiation [67]. Adopting the strategy of improving the potency of lysosome targeting compounds, Carew *et al.* synthesized bivalent chemotherapeutics that link the acetylcholinesterase inhibitor lucanthone with HCQ [68]. This led to ROC-325, a well-characterized analogue that resulted from exploration of the SAR around HCQ (Table 1). ROC-325 was more cytotoxic than HCQ in 12 different tissue-derived cell lines and standard measures of autophagy, including deacidification of the lysosome and autophagosome buildup, indicating reduced autophagic flux [69]. In renal cell carcinoma xenograft models ROC325 showed a measurable improvement in potency over HCQ and was well tolerated [69]. In related work, the exploration of a bivalent form of HCQ called Lys05 (Table 1) has led to new insights into the mechanism of HCQ [70]. With the aim of potency improvement by systematic examination of linker properties, Rebecca *et al.* have brought under control disparate properties of HCQ [71, 72]. Methylation of the central nitrogen in their linker enabled “trapping” of their compounds in the acidic lysosome whereas des-methyl forms act as autophagy inducers, possibly by accumulation in the nucleus and induction of DNA damage [73]. Rebecca *et al.* made use of this sequestration in the lysozyme to probe for molecular targets of their compounds. A molecular probe based on a variant DQ661 (Table 1) led to the identification a new molecular target, palmitoyl-protein thioesterase 1 (**PPT1**) [74]. This is a novel autophagy target, as the inhibition of PPT1 has the interesting effect of destabilizing recruitment of mTOR to the lysosome [70, 75]. Establishment of the link between initiation and the end point of autophagy offers new possibilities in understanding the consequence of HCQ induced deacidification. Given that low potency leading to dose-limiting adverse events is the chief limitation in clinical trials for HCQ, reimagination of the CQ class of compounds has the potential for therapeutic benefit.

Concluding Remarks and Future Perspectives

Autophagy has been implicated in a number of human diseases and has emerged as an attractive target for therapeutic development. Recent phase I/II clinical trials with combinations of HCQ and SOC cancer therapeutics showed mixed results and varying levels of efficacy, highlighting the need to develop additional selective and potent agents [15, 16].

The discovery of small molecule ULK1/2 inhibitors has been undertaken by several groups using various approaches. Several compounds have been identified that display high potency for ULK1/2 inhibition *in vitro*, however many studies lack cellular confirmation of ULK1/2 activity or evaluation of ULK1/2 inhibitor efficacy in disease models. To date no studies utilizing ULK1/2 inhibitors in animal models have been reported.

ATG4B has a clear role in both the autophagy pathway and in tumor progression. Data support the use of small molecule ATG4B inhibitors in cancer treatment and indicate their use in combination with SOC drugs. ATG4B inhibitors identified to date have inadequate properties for patient treatment, thus identification of additional compounds targeting ATG4B are needed.

VPS34 is essential for both autophagy and endosomal trafficking. Its role in reversing autophagy upregulation in response to chemotherapeutic agents makes it a promising candidate for targeted treatment. Recent studies suggest that combination therapy with VPS34 inhibitors and SOC drugs may have therapeutic benefit in cancer patients and prevent resistance. However, the importance of VPS34 in normal tissues may limit the use of VPS34-targeting drugs.

In oncology the majority of autophagy-targeting compounds discovered so far inhibit autophagy pathways. However, in a context specific manner, excessive levels of autophagy have been shown to promote autophagy-dependent cell death. As a consequence, there is still much to be learned from the discovery of novel chemical probes that either activate or inhibit specific autophagy targets and allow us to tease out the subtleties of tumor-dependency and ultimately deliver effective cancer therapeutics (see Outstanding Questions).

Acknowledgements

This work was supported by the Epstein Foundation; additionally by NCI CCSG P30CA030199, NCI CCSG P30CA014195, NCI P01CA120964, and NCI R35CA220538. We thank Douglas J. Sheffler, Ph.D. for assisting with manuscript editing, and Mara Bosnak Lambert for figure design.

Glossary

5' Adenosine monophosphate-activated protein kinase (AMPK)

an enzyme that has a crucial role in regulating ATP production and maintaining cellular homeostasis.

Autophagic flux

a measure of autophagic degradation activity as determined by a defined methodology.

Autophagosome

key component of autophagy, which consists of double-membraned vesicles that enclose and isolate cellular material to be degraded by autophagy.

Autophagy

the degradation of intracellular proteins and damaged organelles to maintain cellular homeostasis.

Autophagy-related protein 13 (ATG13)

an autophagy protein target of the mTOR signaling pathway that regulates autophagosome formation through phosphorylation.

Autophagy-related protein 3 (ATG3)

an ubiquitin-conjugating enzyme E2 homologue that mediates the conjugation of LC3-I to phosphatidylethanolamine after LC3 activation by ATG7.

Autophagy-related protein 4B (ATG4B)

a cysteine peptidase enzyme, homologue of ATG4, involved in the cleavage of terminal residues of proLC3 that activates LC3-I to allow phospholipid conjugation and promote autophagosome formation. It also cleaves phosphatidylethanolamine from LC3-II.

Autophagy-related protein 7 (ATG7)

an ubiquitin-activating enzyme E1 homologue that binds to and activates LC3 for conjugation.

Beclin-1

an important PI3K protein that interacts with BCL-2 or PI3K and has a crucial role in the activation and regulation of autophagy and apoptosis.

FIP200

a component of the ULK1/2 complex that regulates early stage induction of autophagosome formation.

Focal adhesion kinase (FAK)

a non-receptor tyrosine kinase that has a crucial role in adhesion-mediated signaling in cells and is important for autophagy.

Half maximal inhibitory concentration (IC₅₀)

the concentration of an inhibitor in which a response is reduced by half.

Mammalian STE20-like protein kinase 4 (MST4)

a serine/threonine protein kinase that functions to phosphorylate ATG4B and initiate ATG4B activity.

Mammalian target of rapamycin (mTOR)

a serine/threonine protein kinase of the PIKK kinase family that regulates various cellular processes including metabolism, cell growth, and autophagy.

Microtubule-associated proteins 1A/1B light chain 3B (LC3)

an ubiquitin-like protein (LC3I) that conjugates to the phospholipid phosphatidylethanolamine to form LC3-II and is involved in the formation of autophagosomes.

Phosphoinositide 3-kinase (PI3K)

a class of signal transducer enzyme within cells that regulates cellular function by phosphorylation of phosphatidylinositol (PI) related lipids.

p62

a protein kinase C-interacting protein that is involved in the signaling and activation of the mTOR pathway. Degraded by autophagy.

Palmitoyl-protein thioesterase 1 (PPT1)

a lysosomal thioesterase that cleaves acyl thioester modified proteins in the lysosome.

Pyruvate dehydrogenase kinase 1 (PDK1)

phosphorylates the pyruvate dehydrogenase (PDH) complex, which is one of the major enzymes responsible for the regulation of homeostasis of carbohydrate fuels in mammals.

Regioisomers

A type of structural isomer in which two molecules with the same molecular formula contain a functional group or substituent with varying bonding arrangements.

TANK binding kinase 1 (TBK1)

a serine/threonine kinase involved in the innate immune response system.

Unc-51-like autophagy activating kinase 1/2 (ULK1/2)

are two similar serine/threonine kinase homologues of ATG1, which are crucial for autophagy signaling.

Vacuolar protein sorting protein 34 (VPS34)

a class III PI3K protein which functions to phosphorylate phosphatidylinositol to generate phosphatidylinositol (3)-phosphate (PI3P) necessary for autophagosome formation.

References:

1. Green DR and Levine B, To be or not to be? How selective autophagy and cell death govern cell fate. *Cell*, 2014 157(1): p. 65–75. [PubMed: 24679527]
2. Kimmelman AC and White E, Autophagy and tumor metabolism. *Cell metabolism*, 2017 25(5): p. 1037–1043. [PubMed: 28467923]
3. Amaravadi R, Kimmelman AC, and White E, Recent insights into the function of autophagy in cancer. *Genes & development*, 2016 30(17): p. 1913–1930. [PubMed: 27664235]
4. Nyfeler B and Eng CH, Revisiting autophagy addiction of tumor cells. *Autophagy*, 2016 12(7): p. 1206–1207. [PubMed: 27097231]
5. White E, The role for autophagy in cancer. *The Journal of clinical investigation*, 2015 125(1): p. 42–46. [PubMed: 25654549]
6. Galluzzi L, et al., Autophagy in malignant transformation and cancer progression. *The EMBO journal*, 2015 34(7): p. 856–880. [PubMed: 25712477]
7. Mainz L and Rosenfeldt MT, Autophagy and cancer—insights from mouse models. *The FEBS journal*, 2018 285(5): p. 792–808. [PubMed: 28921866]
8. van Beek N, Klionsky DJ, and Reggiori F, Genetic aberrations in macroautophagy genes leading to diseases. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 2018.
9. Mizushima N, Yoshimori T, and Ohsumi Y, The role of Atg proteins in autophagosome formation. *Annual review of cell and developmental biology*, 2011 27: p. 107–132.
10. Bestebroer J, et al., Hidden behind autophagy: the unconventional roles of ATG proteins. *Traffic*, 2013 14(10): p. 1029–1041. [PubMed: 23837619]
11. Kroemer G, Autophagy: a druggable process that is deregulated in aging and human disease. *The Journal of clinical investigation*, 2015 125(1): p. 1–4. [PubMed: 25654544]
12. Amaravadi R, Kimmelman AC, and White E, Recent insights into the function of autophagy in cancer. *Genes Dev*, 2016 30(17): p. 1913–30. [PubMed: 27664235]
13. Levy JMM, Towers CG, and Thorburn A, Targeting autophagy in cancer. *Nature Reviews Cancer*, 2017 17(9): p. 528–542. [PubMed: 28751651]
14. Boone BA, et al., Safety and biologic response of pre-operative autophagy inhibition in combination with gemcitabine in patients with pancreatic adenocarcinoma. *Annals of surgical oncology*, 2015 22(13): p. 4402–4410. [PubMed: 25905586]

15. Malhotra J, et al., Modulation of autophagy with hydroxychloroquine in patients with advanced non-small cell lung cancer (NSCLC): A phase Ib study. 2018, American Society of Clinical Oncology.
16. Wolpin BM, et al., Phase II and pharmacodynamic study of autophagy inhibition using hydroxychloroquine in patients with metastatic pancreatic adenocarcinoma. *The oncologist*, 2014 19(6): p. 637–638. [PubMed: 24821822]
17. Santana-Codina N, Mancias JD, and Kimmelman AC, The role of autophagy in cancer. 2017.
18. Vakifahmetoglu-Norberg H, Xia H.-g., and Yuan J, Pharmacologic agents targeting autophagy. *The Journal of clinical investigation*, 2015 125(1): p. 5–13. [PubMed: 25654545]
19. Chude CI and Amaravadi RK, Targeting autophagy in cancer: update on clinical trials and novel inhibitors. *International journal of molecular sciences*, 2017 18(6): p. 1279.
20. Rubinsztein DC, Codogno P, and Levine B, Autophagy modulation as a potential therapeutic target for diverse diseases. *Nature reviews Drug discovery*, 2012 11(9): p. 709. [PubMed: 22935804]
21. Wang C, Hu Q, and Shen H-M, Pharmacological inhibitors of autophagy as novel cancer therapeutic agents. *Pharmacological research*, 2016 105: p. 164–175. [PubMed: 26826398]
22. Gump JM and Thorburn A, Autophagy and apoptosis: what is the connection? *Trends in cell biology*, 2011 21(7): p. 387–392. [PubMed: 21561772]
23. Jiang X, Overholtzer M, and Thompson CB, Autophagy in cellular metabolism and cancer. *The Journal of clinical investigation*, 2015 125(1): p. 47–54. [PubMed: 25654550]
24. He S, et al., Design of Small Molecule Autophagy Modulators: A promising druggable strategy. *Journal of medicinal chemistry*, 2017 61(11): p. 4656–4687. [PubMed: 29211480]
25. Tsukada M and Ohsumi Y, Isolation and characterization of autophagy defective mutants of *Saccharomyces cerevisiae*. *FEBS letters*, 1993 333(1–2): p. 169–174. [PubMed: 8224160]
26. Chan EY, mTORC1 phosphorylates the ULK1-mAtg13-FIP200 autophagy regulatory complex. *Sci. Signal*, 2009 2(84): p. pe51–pe51. [PubMed: 19690328]
27. Alers S, et al., The incredible ulks. *Cell Communication and Signaling*, 2012 10(1): p. 7. [PubMed: 22413737]
28. Egan D, et al., The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. *Autophagy*, 2011 7(6): p. 643–4. [PubMed: 21460621]
29. Egan DF, et al., Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science*, 2011 331(6016): p. 456–461. [PubMed: 21205641]
30. Kim J, et al., AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol*, 2011 13(2): p. 132–41. [PubMed: 21258367]
31. Kang SA, et al., mTORC1 phosphorylation sites encode their sensitivity to starvation and rapamycin. *Science*, 2013 341(6144): p. 1236566. [PubMed: 23888043]
32. Ganley IG, et al., ULK1- ATG13- FIP200 complex mediates mTOR signaling and is essential for autophagy. *Journal of Biological Chemistry*, 2009 284(18): p. 12297–12305. [PubMed: 19258318]
33. Egan DF, et al., Small Molecule Inhibition of the Autophagy Kinase ULK1 and Identification of ULK1 Substrates. *Mol Cell*, 2015 59(2): p. 285–97. [PubMed: 26118643]
34. Tang F, et al., SBI0206965, a novel inhibitor of Ulk1, suppresses non-small cell lung cancer cell growth by modulating both autophagy and apoptosis pathways. *Oncology reports*, 2017 37(6): p. 3449–3458. [PubMed: 28498429]
35. Petherick KJ, et al., Pharmacological inhibition of ULK1 kinase blocks mammalian target of rapamycin (mTOR)-dependent autophagy. *J Biol Chem*, 2015 290(18): p. 11376–83. [PubMed: 25833948]
36. Joo JH, et al., Hsp90-Cdc37 chaperone complex regulates Ulk1-and Atg13-mediated mitophagy. *Molecular cell*, 2011 43(4): p. 572–585. [PubMed: 21855797]
37. Tanida I, Ueno T, and Kominami E, LC3 and Autophagy, in *Autophagosome and Phagosome*. 2008, Springer p. 77–88.
38. Lazarus MB, Novotny CJ, and Shokat KM, Structure of the human autophagy initiating kinase ULK1 in complex with potent inhibitors. *ACS Chem Biol*, 2015 10(1): p. 257–61. [PubMed: 25551253]

39. Lazarus MB and Shokat KM, Discovery and structure of a new inhibitor scaffold of the autophagy initiating kinase ULK1. *Bioorganic & medicinal chemistry*, 2015 23(17): p. 5483–5488. [PubMed: 26275681]
40. Wood SD, et al., In Silico HTS and Structure Based Optimization of Indazole-Derived ULK1 Inhibitors. *ACS Med Chem Lett*, 2017 8(12): p. 1258–1263. [PubMed: 29259744]
41. Rosenberg LH, et al., Development of an HTS-compatible assay for the discovery of Ulk1 inhibitors. *Journal of biomolecular screening*, 2015 20(7): p. 913–920. [PubMed: 25851035]
42. Tanida I, Ueno T, and Kominami E, Human light chain 3/MAP1LC3B is cleaved at its carboxyl-terminal Met121 to expose Gly120 for lipidation and targeting to autophagosomal membranes. *Journal of Biological Chemistry*, 2004 279(46): p. 47704–47710. [PubMed: 15355958]
43. Akin D, et al., A novel ATG4B antagonist inhibits autophagy and has a negative impact on osteosarcoma tumors. *Autophagy*, 2014 10(11): p. 2021–2035. [PubMed: 25483883]
44. Huang T, et al., MST4 Phosphorylation of ATG4B Regulates Autophagic Activity, Tumorigenicity, and Radioresistance in Glioblastoma. *Cancer Cell*, 2017 32(6): p. 840–855 e8. [PubMed: 29232556]
45. Kurdi A, et al., ATG4B inhibitors with a benzotropolone core structure block autophagy and augment efficiency of chemotherapy in mice. *Biochem Pharmacol*, 2017 138: p. 150–162. [PubMed: 28642033]
46. Marsh T and Debnath J, Ironing out VPS34 inhibition. *Nature cell biology*, 2015 17(1): p. 1. [PubMed: 25679028]
47. Parekh VV, et al., Impaired autophagy, defective T cell homeostasis, and a wasting syndrome in mice with a T cell-specific deletion of Vps34. *The Journal of Immunology*, 2013: p. 1202071.
48. Jaber N, et al., Class III PI3K Vps34 plays an essential role in autophagy and in heart and liver function. *Proceedings of the National Academy of Sciences*, 2012 109(6): p. 2003–2008.
49. Ronan B, et al., A highly potent and selective Vps34 inhibitor alters vesicle trafficking and autophagy. *Nature chemical biology*, 2014 10(12): p. 1013. [PubMed: 25326666]
50. Peppard J, et al., Identifying small molecules which inhibit autophagy: a phenotypic screen using image-based high-content cell analysis. *Current chemical genomics and translational medicine*, 2014 8(Suppl-1): p. 3. [PubMed: 24596680]
51. Young CD, Arteaga CL, and Cook RS, Dual inhibition of Type I and Type III PI3 kinases increases tumor cell apoptosis in HER2+ breast cancers. *Breast Cancer Research*, 2015 17(1): p. 148. [PubMed: 26637440]
52. Schlütermann D, et al. Targeting urothelial carcinoma cells by combining cisplatin with a specific inhibitor of the autophagy-inducing class III PtdIns3K complex in Urologic Oncology: Seminars and Original Investigations. 2018 Elsevier.
53. Zhou P, et al., Autophagy inhibition enhances celecoxib-induced apoptosis in osteosarcoma. *Cell Cycle*, 2018(just-accepted).
54. Dowdle WE, et al., Selective VPS34 inhibitor blocks autophagy and uncovers a role for NCOA4 in ferritin degradation and iron homeostasis in vivo. *Nature cell biology*, 2014 16(11): p. 1069. [PubMed: 25327288]
55. Honda A, et al., Potent, selective, and orally bioavailable inhibitors of VPS34 provide chemical tools to modulate autophagy in vivo. *ACS medicinal chemistry letters*, 2015 7(1): p. 72–76. [PubMed: 26819669]
56. Bago R, et al., Characterization of VPS34-IN1, a selective inhibitor of Vps34, reveals that the phosphatidylinositol 3-phosphate-binding SGK3 protein kinase is a downstream target of class III phosphoinositide 3-kinase. *Biochemical Journal*, 2014 463(3): p. 413–427. [PubMed: 25177796]
57. Zhou X, Takatoh J, and Wang F, The mammalian class 3 PI3K (PIK3C3) is required for early embryogenesis and cell proliferation. *PloS one*, 2011 6(1): p. e16358. [PubMed: 21283715]
58. Zhou X, et al., Deletion of PIK3C3/Vps34 in sensory neurons causes rapid neurodegeneration by disrupting the endosomal but not the autophagic pathway. *Proceedings of the National Academy of Sciences*, 2010 107(20): p. 9424–9429.
59. Duran A, et al., p62 is a key regulator of nutrient sensing in the mTORC1 pathway. *Molecular cell*, 2011 44(1): p. 134–146. [PubMed: 21981924]

60. Donohue E, et al., Induction of covalently crosslinked p62 oligomers with reduced binding to polyubiquitinated proteins by the autophagy inhibitor verteporfin. *PloS one*, 2014 9(12): p. e114964. [PubMed: 25494214]
61. Donohue E, et al., Inhibition of autophagosome formation by the benzoporphyrin derivative verteporfin. *Journal of Biological Chemistry*, 2011 286(9): p. 7290–7300. [PubMed: 21193398]
62. Schmidt-Erfurth U and Hasan T, Mechanisms of action of photodynamic therapy with verteporfin for the treatment of age-related macular degeneration. *Survey of ophthalmology*, 2000 45(3): p. 195–214. [PubMed: 11094244]
63. Donohue E, et al., The autophagy inhibitor verteporfin moderately enhances the antitumor activity of gemcitabine in a pancreatic ductal adenocarcinoma model. *Journal of Cancer*, 2013 4(7): p. 585. [PubMed: 24069069]
64. Wang L, et al., P62 as a therapeutic target for inhibition of autophagy in prostate cancer. *The Prostate*, 2018 78(5): p. 390–400. [PubMed: 29368435]
65. Liu J, et al., Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. *Cell*, 2011 147(1): p. 223–234. [PubMed: 21962518]
66. Shao S, et al., Spautin-1, a novel autophagy inhibitor, enhances imatinib-induced apoptosis in chronic myeloid leukemia. *International journal of oncology*, 2014 44(5): p. 1661–1668. [PubMed: 24585095]
67. Amaravadi RK and Winkler JD, Lys05: a new lysosomal autophagy inhibitor. *Autophagy*, 2012 8(9): p. 1383–1384. [PubMed: 22878685]
68. Carew JS and Nawrocki ST, Drain the lysosome: Development of the novel orally available autophagy inhibitor ROC-325. *Autophagy*, 2017 13(4): p. 765–766. [PubMed: 28118053]
69. Carew JS, et al., Disruption of autophagic degradation with ROC-325 antagonizes renal cell carcinoma pathogenesis. *Clinical Cancer Research*, 2016.
70. McAfee Q, et al., Autophagy inhibitor Lys05 has single-agent antitumor activity and reproduces the phenotype of a genetic autophagy deficiency. *Proceedings of the National Academy of Sciences*, 2012 109(21): p. 8253–8258.
71. Rebecca VW, et al., A unified approach to targeting the lysosome's degradative and growth signaling roles. *Cancer discovery*, 2017.
72. Towers CG and Thorburn A, Targeting the Lysosome for Cancer Therapy. *Cancer discovery*, 2017 7(11): p. 1218–1220. [PubMed: 29097619]
73. Bakkenist CJ and Kastan MB, DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature*, 2003 421(6922): p. 499. [PubMed: 12556884]
74. Nicastrì MC, et al., Dimeric quinacrine as chemical tools to identify PPT1, a new regulator of autophagy in cancer cells. *Molecular & cellular oncology*, 2018 5(1): p. e1395504. [PubMed: 29404393]
75. Bagh MB, et al., Misrouting of v-ATPase subunit V0a1 dysregulates lysosomal acidification in a neurodegenerative lysosomal storage disease model. *Nature communications*, 2017 8: p. 14612.
76. Hong Z, et al., PtdIns3P controls mTORC1 signaling through lysosomal positioning. *J Cell Biol*, 2017: p. jcb. 201611073.
77. Nawrocki ST, et al., Development of ROC-325: A Novel Small Molecule Inhibitor of Autophagy with Promising Anti-Leukemic Activity. *Blood*, 2016 128(22): p. 525–525.

Outstanding Questions:

- Within the core autophagy pathways which targets are optimal for pharmacologic intervention to modulate autophagic flux?
- What degree of selectivity across autophagy targets is optimal for chemical probes and therapeutic agents?
- For pharmacological impact, is it better to disrupt initiation, autophagosomal assembly or lysosomal integrity?
- For the treatment of cancer in what context should autophagy be activated versus inhibited for the optimal patient outcome?
- Will improved chemical probes and improved biomarkers for autophagy resolve these complexities?

Highlights

- Autophagy maintains cellular homeostasis during periods of stress.
- Dysregulation of autophagy plays a crucial role in cancer and tumor growth.
- There are several control points in the autophagic pathway that can be targeted to modulate the process.
- Small molecules that modulate autophagy by way of inhibition or activation of the autophagic pathway have potential in oncology.

Trends in Pharmacological Sciences

Figure 1: Overview of the Modulation of Autophagy.

The formative stages of autophagy, protein components, and points of modulation by small molecules are indicated. Under conditions of nutrient deprivation mTOR is inactivated and AMPK is activated. These metabolic sensor proteins phosphorylate negative and positive regulatory sites on ULK1/2 kinase in the context of the “preinitiation complex”. The preinitiation complex activates the “initiation complex” or the “class III PI-IIIK complex” via phosphorylation of VPS34 and Beclin-1. Regulation of the initiation complex has a direct effect on the production of cellular pools of phosphatidylinositol3-phosphate (PI3P) from the precursor phosphatidylinositol (PI) needed for nucleation of the isolation membrane. Cellular concentrations of the initiation complex are also under the control of an ubiquitination cascade regulated by the deubiquitination peptidases USP10 and USP13. Expansion of nascent precursor vesicles relies on the autophagosome protein LC3. Critical for this process is the phosphatidylethanolamine (PE) conjugated form of the LC3 protein called LC3-II. The generation of LC3-II is derived from the “LC3 elongation sequence” of modifying enzymes. LC3-I is the product of proteolytic cleavage of proLC3 by the protease ATG-4B. LC3-I is then lipidated by a series of conjugating enzymes to the LC3-II form which stably associates with mature autophagosomal membranes. The versatile scaffold protein p62 plays a role in the trafficking of proteins to the autophagosome and is an important component of apoptosis pathways. In autophagy, p62 stably binds to the LC3-II protein in the context of assembling autophagosomes.

Table 1:

Selected compounds that modulate autophagy.

Compound	Chemical Structure	Target	Novel Features	Potency/ Selectivity	Ref
SBI-0206965		ULK1 & ULK2	<ul style="list-style-type: none"> • Selective inhibitor. • Pyrimidine scaffold. • Suppresses ULK1 downstream phosphorylation of VPS34 and Beclin-1. • Induces apoptosis in NSCLC cells by destabilizing Bcl2 and Bclx1. 	ULK1 IC ₅₀ of 108 nM, ULK2 IC ₅₀ of 711 nM	[33, 34]
MRT67307		ULK1 & ULK2	<ul style="list-style-type: none"> • <i>In vitro</i> inhibitor. • Pyrimidine scaffold. • Also targets TBK1 and AMPK-related kinases. 	ULK1 IC ₅₀ of 45 nM, ULK2 IC ₅₀ of 38 nM	[35]
MRT68921		ULK1 & ULK2	<ul style="list-style-type: none"> • <i>In vitro</i> inhibitor. • Pyrimidine scaffold. • Also targets TBK1 and AMPK-related kinases. 	ULK1 IC ₅₀ of 2.9 nM, ULK2 IC ₅₀ of 1.1 nM	[35]
Compound 1		ULK1 & ULK2	<ul style="list-style-type: none"> • Inhibitor. • Pyrazole amino quinazoline scaffold. • Crystal structure obtained with ULK1. 	ULK1 IC ₅₀ of 5.3 nM, ULK2 IC ₅₀ of 13 nM, PDK1 IC ₅₀ of 420 nM.	[39]
BX-795		PDK1	<ul style="list-style-type: none"> • Inhibitor of PDK1. • Also shown to inhibit ULK1, ULK2 and IKKe • Pyrimidine scaffold. 	ULK1 IC ₅₀ of 87 nM, ULK2 IC ₅₀ of 310 nM, PDK1 IC ₅₀ of 65 nM.	[39]
Compound 3		ULK1	<ul style="list-style-type: none"> • Inhibitor • Pyrimidine scaffold • Crystal structure obtained with ULK1 	ULK1 IC ₅₀ of 120 nM, ULK2 IC ₅₀ of 360 nM, PDK1 IC ₅₀ of 710 nM.	[39]
SR-17398		ULK1	<ul style="list-style-type: none"> • Indazole derived inhibitor. • Mixture of four stereoisomers. 	ULK1 IC ₅₀ of 22 μM	[40]
SR-20295		ULK1	<ul style="list-style-type: none"> • Indazole derived inhibitor. 	ULK1 IC ₅₀ of 45 nM, <i>In vitro</i> microsome stability half-life of 225 minutes.	[40]
NSC185058		ATG4B	<ul style="list-style-type: none"> • Inhibitor/antagonist. • Targets autophagosome formation and suppresses the activation and lipidation of LC3. 	ATG4B IC ₅₀ of 51 μM	[43, 44]

Compound	Chemical Structure	Target	Novel Features	Potency/ Selectivity	Ref
UAMC-2526		ATG4B	<ul style="list-style-type: none"> • Inhibitor. • Benzotropolone scaffold. • Targets autophagosome formation. • Inhibits starvation-induced autophagy <i>in vivo</i>. 	Plasma half-life of 126 min, and 70% metabolism after 30 minutes.	[45]
SAR405		VPS34	<ul style="list-style-type: none"> • Selective inhibitor. • Tetrahydropyrimidopyrimidinones scaffold. • Dose dependent inhibition • Targets autophagosome formation. • Crystal structure obtained with VPS34. 	VPS34 IC ₅₀ of 1.2 nM and K _D of 1.5 nM	[49, 51, 76]
PIK-III		VPS34	<ul style="list-style-type: none"> • Selective and orally bioavailable inhibitor of VPS34. • Pyrimidine scaffold • Inhibits autophagy and LC3 lipidation. 	VPS34 IC ₅₀ of 18 nM mTOR IC ₅₀ of >9.1 μM	[54, 55]
VPS34-IN1		VPS34	<ul style="list-style-type: none"> • Selective cell permeable inhibitor. • Pyrimidine scaffold. • Selectively inhibits class III PI3K. 	VPS34 IC ₅₀ of 25 nM <i>in vitro</i> .	[56]
Verteporfin		ATG	<ul style="list-style-type: none"> • Concentration-dependent inhibition. • Benzoporphyrin scaffold. • Targets autophagosome formation and accumulation when co-treated with chloroquine. • Targets p62- prevents autophagy induced degradation of p62 in nutrient-deprived conditions. 	Chloroquine/verteporfin EGFP-LC3 cell IC ₅₀ of 1 μM. Plasma concentrations after single IP dose of 45 mg/kg- 122 μM at 2 h, 3.9 μM at 24 h.	[60, 61, 63]
Spautin-1		ATG	<ul style="list-style-type: none"> • Autophagy inhibitor. • Fluoroquinazoline scaffold. • USP10 and USP13 inhibitor-promoting ubiquitination and causing decreased levels of Beclin-1. • Targets autophagosome formation when co-treated with imatinib mesylate. • Spautin-1 alone has no activity. 	Co-treatment with Spautin-1 improved imatinib mesylate induced cytotoxicity of K562 leukemia cells- IC ₅₀ from 1.03 μM to 0.45 μM.	[66]
ROC-325		ATG	<ul style="list-style-type: none"> • Orally bioavailable inhibitor. • Chloroquinoline scaffold. • Targets lysosomal function and autophagosome accumulation. • About 10-fold more potent than hydroxychloroquine. • Exhibits significant anticancer activity against a broad range of tumor types. 	AML cell IC ₅₀ range of 0.7 – 2.2 μM. A498 renal cell IC ₅₀ of 4.9 μM.	[68, 69, 77]

Compound	Chemical Structure	Target	Novel Features	Potency/ Selectivity	Ref
Lys05		ATG	<ul style="list-style-type: none"> • Autophagy inhibitor. • Dimeric chloroquinoline scaffold. • Targets lysosomal function. 	LN229 (glioma), 1205Lu (melanoma), c8161 (melanoma), HT-29 (colon) cell IC ₅₀ range of 4–8 μM.	[67, 70]
DQ661		ATG	<ul style="list-style-type: none"> • Inhibitor of autophagy and mTOR by targeting PPT1. • Dimeric quinacrine scaffold. • <i>In vivo</i> activity against melanoma, pancreatic cancer and colorectal cancer tumor growth in mice. • Can be used in combination with chemotherapy. 	Estimated A375P melanoma cell IC ₅₀ of approx. 0.1 μM.	[71, 74]