Targeting Autophagy for Cancer Treatment

Subjects: Oncology

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Autophagy is a tightly regulated catabolic process that facilitates nutrient recycling from damaged organelles and other cellular components through lysosomal degradation. Deregulation of this process has been associated with the development of several pathophysiological processes, such as cancer and neurodegenerative diseases. In cancer, autophagy has opposing roles, being either cytoprotective or cytotoxic. Thus, deciphering the role of autophagy in each tumor context is crucial. Moreover, autophagy has been shown to contribute to chemoresistance in some patients. In this regard, autophagy modulation has recently emerged as a promising therapeutic strategy for the treatment and chemosensitization of tumors, and has already demonstrated positive clinical results in patients.

Keywords: autophagy ; anticancer therapy ; autophagy inhibitors ; autophagic cell death ; chemoresistance ; chemosensitization

1. Introduction

Cellular homeostasis is crucial for cell survival and refers to all processes involved in the maintenance of an internal steady state at the level of the cell. Autophagy is one of the main catabolic mechanisms that contributes to cellular homeostasis, through the degradation and recycling of cytoplasmic components and organelles in the lysosomes [1][2]. This process confers the ability to adapt to environmental stresses, preventing cellular damage, and promoting cell survival, even in starving conditions, thus having a main physiologic cytoprotective role. It is a process tightly regulated and its dysfunction has been related to several pathologies, such as neurodegeneration, cancer, or aging [3]. Hence, autophagy modulation is emerging as a promising new therapeutic strategy to treat these malignancies [4]. Indeed, more than 120 clinical trials related to the process of autophagy were initiated to date. The majority of those target autophagy for cancer treatment, already showing promising results, for instance, using chloroquine or hydroxychloroquine as single agents or in combination therapies [5][6]. Nevertheless, the role of autophagy in cancer is somewhat controversial. Cytotoxic or cytoprotective roles have been reported depending on the cellular context [7]. Therefore, the deep understanding of autophagy regulation and the identification of its role in each cellular context is crucial for the selection of an appropriate therapeutic intervention involving autophagy modulation in cancer.

2. Therapeutic Strategies Targeting Autophagy

Modulation of autophagy has emerged as a promising therapeutic option for cancer treatment. Due to the dual role of autophagy in cancer cells, activators as well as inhibitors have been described as feasible chemotherapeutic agents.

In this section, we compiled different therapeutic interventions targeting autophagy, either for its stimulation or for its inhibition (**Figure 1**).

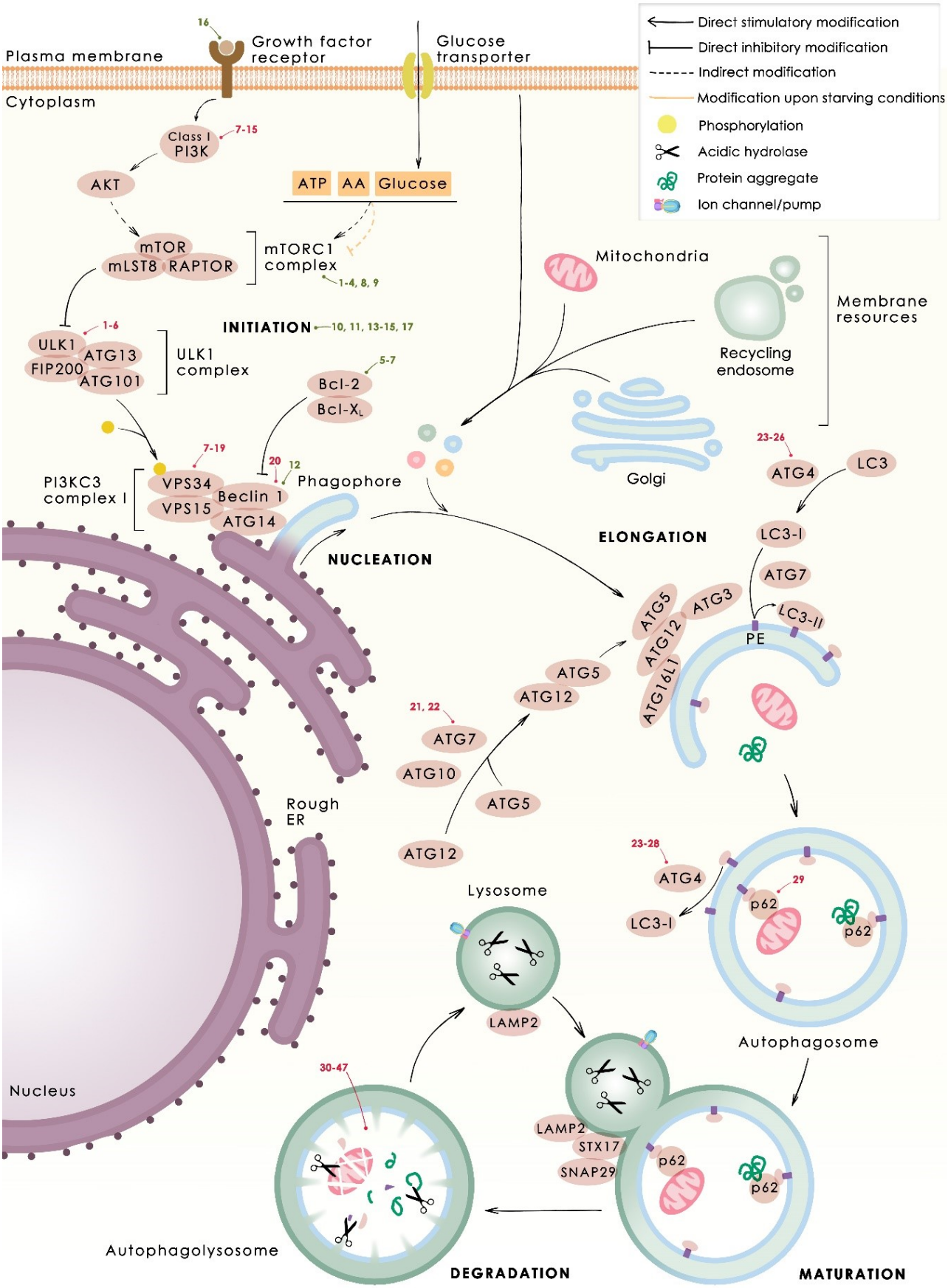
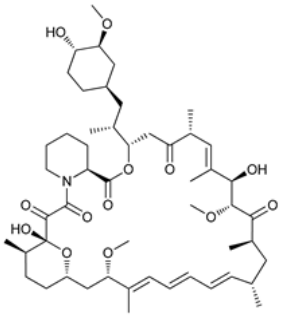
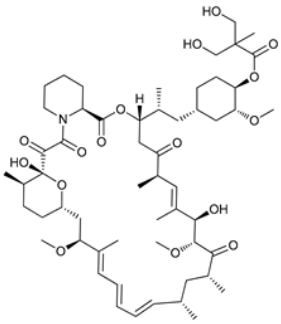
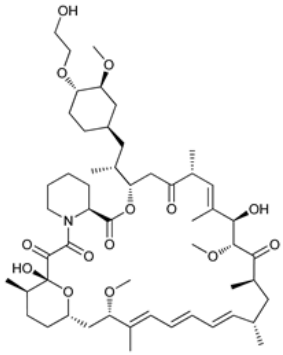
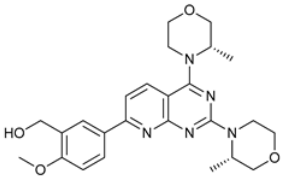


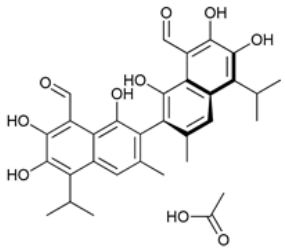
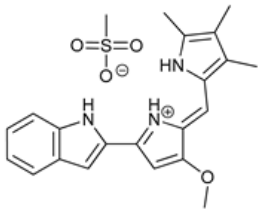
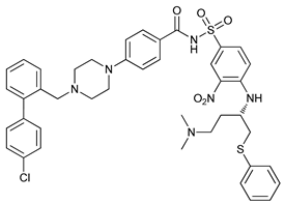
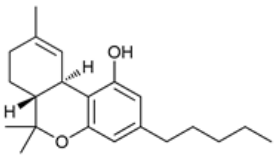
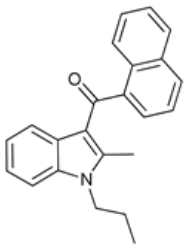
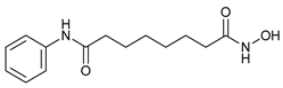
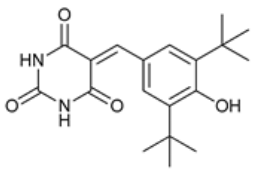
Figure 1. Mechanism of autophagy. The phases of the process of autophagy (nucleation, elongation, maturation and degradation), with the main proteins that participate in each one, are depicted.Autophagy activators (green) and inhibitors (red) are marked where they interfere with the autophagy process. Numbers correspond to those compounds listed in table 1 and 2, respectively.

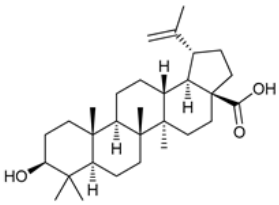
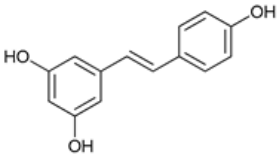
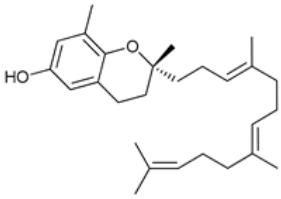
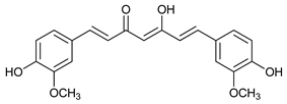
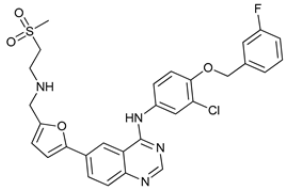
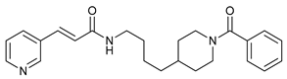
2.1. Autophagy Stimulation for Cancer Treatment

Induction of ACD has become an interesting alternative to overcome resistance to apoptosis and to exploit a caspase independent cell death for cancer treatment. In the following sections, compounds for which the mechanism of action is based on stimulating autophagy are described (**Table 1**).

Table 1. Autophagy activators.

Mechanism of Action/Type	Name	Structure	Number in Figure 1	Refs.
mTOR Inhibitors	Rapacmycin		1	[8][9][10] [11]
	Temsirolimus (CCI779)		2	[12][13]
	Everolimus (RAD001)		3	[14][15]
	AZD8055		4	[16][17]

Mechanism of Action/Type	Name	Structure	Number in Figure 1	Refs.
BH3 Mimetics	(-)-gossypol (AT-101)		5	[18][19][20][21]
	Obatoclax (GX15-070)		6	[22][23][24]
	ABT-737		7	[25]
Cannabinoids	Δ9-Tetrahydrocannabinol (THC)		8	[26][27][28]
	JWH-015		9	[29]
Histone Deacetylase Inhibitors	Suberoylanilide hydroxamic acid (SAHA, Vorinostat)		10	[30]
	MHY2256		11	[31]

Mechanism of Action/Type	Name	Structure	Number in Figure 1	Refs.
Natural Products	Betulinic acid		12	[32]
	Resveratrol		13	[33]
	δ-Tocotrienol		14	[34]
	Curcumin		15	[35]
Others	Lapatinib		16	[36][37]
	APO866		17	[38]

2.1.1. mTOR Inhibitors

The mTOR is a protein kinase that participates in multiple cellular processes such as cell growth, survival, metabolism, and immunity. Thus, mTOR regulates several cellular mechanisms including cell cycle, apoptosis, and autophagy [39], inhibiting the initiation of the latter process [40]. Rapamycin (sirolimus), a secondary metabolite isolated from *Streptomyces hygroscopicus,* showed potent antifungal, antitumor, and immunosuppressive properties [41][42]. Rapamycin and its semi-synthetic analogues, known as rapalogs, are allosteric selective inhibitors of mTORC1 affecting downstream targets, including the activation of autophagy [43][44]. However, their efficacy inhibiting tumor growth is limited due to lack of inhibition of mTORC2 and other compensatory signaling pathways that promote cell survival [45].

Rapamycin has shown to inhibit proliferation and induce ACD in murine sarcoma [8], neuroblastoma [9], lung cancer [10], and osteosarcoma [11]. Conversely, the rapalog temsirolimus or cell cycle inhibitor-779 (CCI779), has shown to inhibit tumor growth in vitro in adenoid cystic carcinoma [12] but has also shown to stimulate autophagy as a pro-survival mechanism in renal-cell carcinoma [13]. Additionally, everolimus (or RAD001), a derivative rapalog developed for oral administration, has shown to induce cell cycle arrest through autophagy-mediated degradation of cyclin D1 in breast cancer cells [14], but promotes autophagy in aromatase inhibitor-resistant breast cancer cells as a mechanism of resistance [15].

Other types of mTOR inhibitors are compounds that compete with ATP, impeding phosphorylation of its target proteins, resulting in a more efficient inhibition of mTOR [46]. Among them, AZD8055 inhibits both mTOR complexes and has shown to inhibit tumor growth [16] and induce ACD in hepatocellular carcinoma cell lines [17], but it is also capable of limiting tumor growth through induction of apoptosis and cell cycle arrest [47]. Taken together these findings suggest that mTOR inhibitors may act through different mechanisms to induce cell death in a tumor context dependent manner, which makes them suitable for combined therapies to overcome cancer cell resistance [48].

2.1.2. BH3 Mimetics

BH3 (Bcl-2 homology 3) mimetics are a group of small molecules that mimic interactions of BH3-only proteins [49], which are a sub-group of pro-apoptotic proteins in the Bcl-2 family [50]. In general, BH3 mimetics may stimulate autophagy by liberating Beclin-1 from Bcl2 and Bcl-X_L inhibition [50][51].

Gossypol is a BH3 mimetic isolated from cotton that has a high affinity for Bcl-2, Bcl-X_L, Mcl-1, and Bcl-w [50]. Its orally available enantiomeric form (-)-gossypol (AT-101) has shown to induce ACD in malignant glioma [21], but the induced autophagy has also been accompanied by apoptosis in head and neck squamous cell carcinoma [18], malignant mesothelioma [19], and colon cancer cells [20]. Obatoclax (GX15-070) is another BH3 mimetic that has shown autophagic-mediated necroptosis in oral squamous cell carcinoma [22], rhabdomyosarcoma cells [23], and acute lymphoblastic leukemia cells [24]. Moreover, obatoclax induced autophagy in adenoid cystic carcinoma [52] and Beclin-1 independent autophagy inhibition in colorectal cancer cells [53]. Finally, ABT-737 has shown effectivity in vitro for hepatocellular carcinoma cells in a Beclin-1-dependent autophagy manner [25].

2.1.3. Cannabinoids

Cannabinoids are a group of more than 60 lipophilic ligands for specific cell-surface cannabinoid receptors ($CB_{1\ y\ 2}$) present in the plant cannabis sativa, with Δ^9-Tetrahydrocannabinol (THC) being the main psychoactive compound [54]. Cannabinoids have shown potent anticancer effects related to autophagy, but they have also shown cytoprotective effects depending on cell type and cannabinoid used [55]. THC has shown to activate non-canonical autophagy-mediated apoptosis in melanoma cells [26] and induce ACD in glioma cells through mTORC1 inhibition and autolysosome permeabilization with the consequent release of cathepsins and posterior induction of apoptosis [27][28]. JWH-015 is a synthetic cannabinoid CB_2 receptor-selective agonist that has shown to inhibit tumor growth through an autophagy-dependent mechanism in hepatocellular carcinoma cells and in vivo models through inhibition of Akt/mTORC1-pathway via AMPK activation [29].

2.1.4. Histone Deacetylase Inhibitors (HDACIs)

The HDAC family includes four classes (I-IV) of transcriptional repressors that alter the structure of chromatin (via deacetylation) [56] and have been studied as anticancer compounds based on their potential to regulate gene expression [57]. Although apoptosis has been referred to as the main route for HDACIs-induced cancer cell death, autophagy stimulation has also been implicated, being the inactivation of PI3K/Akt/mTOR signaling the most described pathway [58].

Suberoylanilide hydroxamic acid (SAHA, Vorinostat) (a pan HDAC inhibitor) was the first HDACI approved by the FDA for the treatment of cutaneous T-cell lymphoma [59] that has shown to inhibit tumor growth through autophagy stimulation via activation of Cathepsin B in breast cancer cells in vitro [30]. Finally, MHY2256 (a synthetic class III HDAC inhibitor) has shown to induce ACD, cell cycle arrest and apoptosis in endometrial cancer cells in both in vitro and in vivo [31].

2.1.5. Natural Products

Some natural compounds have shown promising anticancer activities based on autophagy stimulation. Betulinic acid is a pentacyclic triterpenoid derived from widespread plants that has shown to induce ACD in multiple myeloma cells with high levels of Bcl-2 expression. This derivative acts as an attenuator for mitochondrial-mediated apoptosis, promoting ACD by inducing Beclin-1 phosphorylation [32]. Resveratrol, a polyphenol compound widely found in plants, has been shown to inhibit cell proliferation in breast cancer stem-like cells via suppressing the Wnt/b-catenin signaling pathway [33]. This pathway, which regulates critical genes in tissue development and homeostasis, is aberrantly activated in many cancers and its inhibition has been reported to be related with autophagy processes [33][60]. δ-Tocotrienol is one of the four isomers that comprises vitamin E that has shown cytotoxic effects against prostate cancer cells in vitro through autophagy activation via ER stress [34]. Curcumin is a major constituent of *Curcuma longa* (turmeric) that induces autophagy, which has been shown to elicit a dual role protecting or leading to cell death depending on the duration of the treatment and concentration used [35].

2.1.6. Others

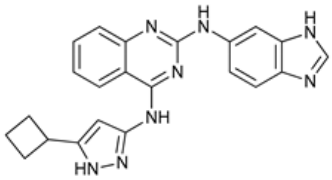
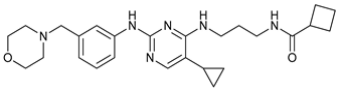
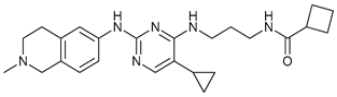
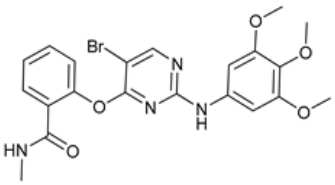
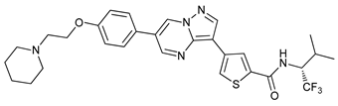
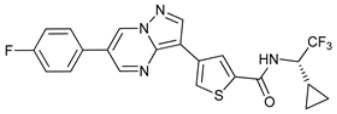
Other compounds have been reported to induce ACD in cancer. For example, lapatinib is a small molecule tyrosine kinase inhibitor, targeting epidermal growth factor receptors that is capable of inducing ACD in hepatocellular carcinoma [36] and in acute leukemia cell lines [37]. APO866 is an inhibitor of nicotinamide adenine dinucleotide (NAD) biosynthesis that has shown anticancer activity through induction of ACD in cells from hematological malignancies [38].

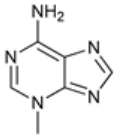
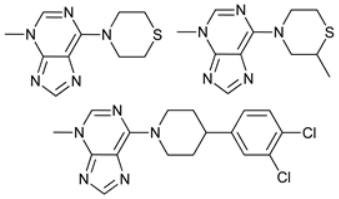
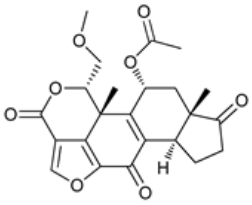
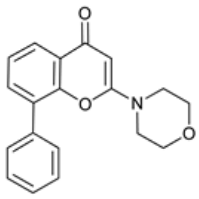
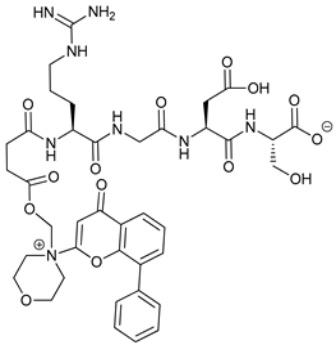
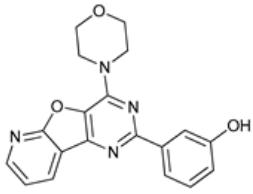
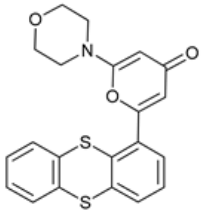
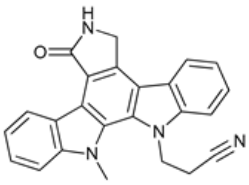
2.2. Autophagy Inhibition for Cancer Treatment

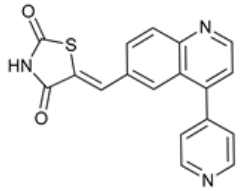
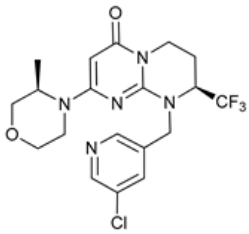
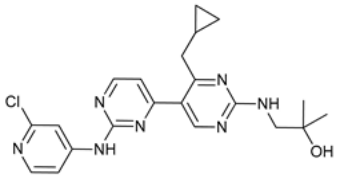
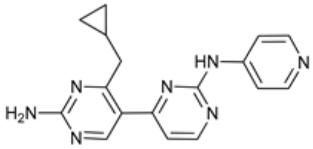
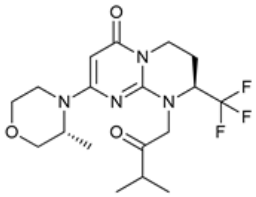
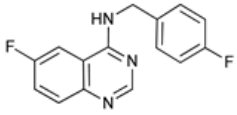
In several tumors, autophagy has a protective role; therefore, its inhibition could be an interesting approach for tumor treatment. There are several autophagy inhibitors that block the process of autophagy at different steps, which we detail

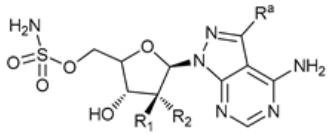
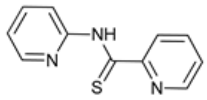
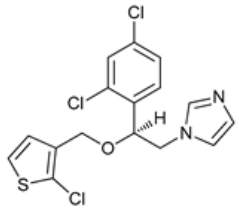
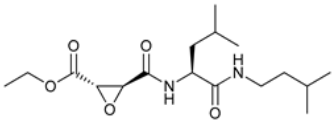
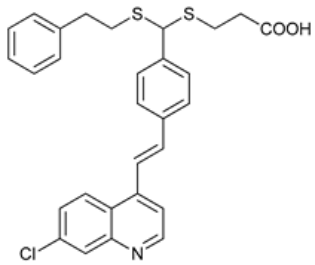
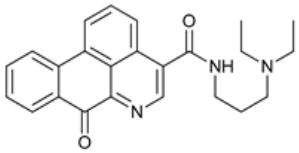
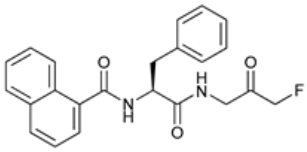
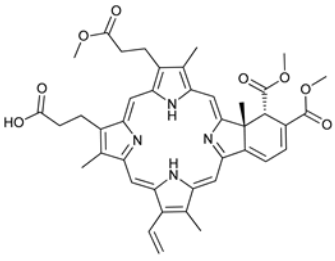
below (**Table 2**).

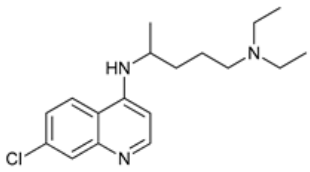
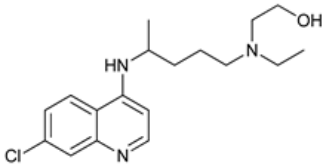
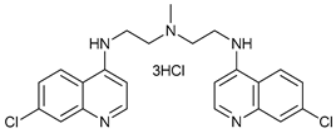
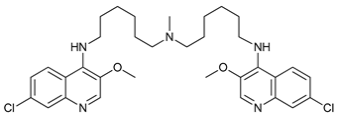
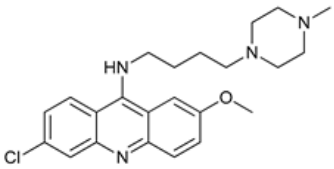
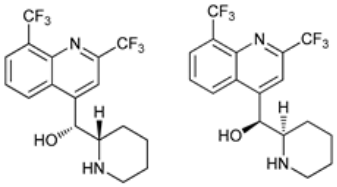
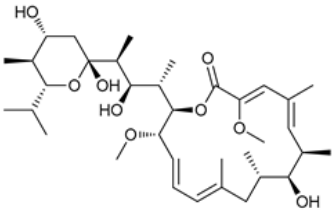
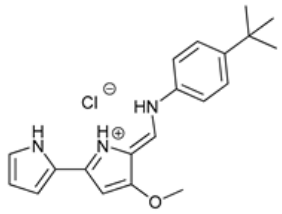
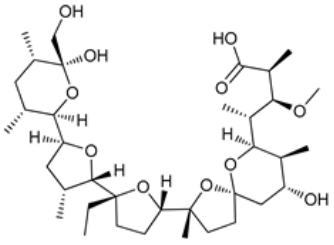
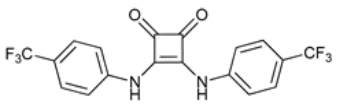
Table 2. Autophagy inhibitors.

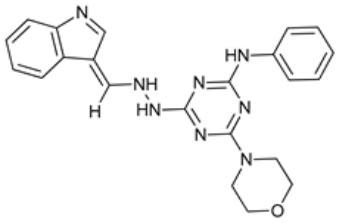
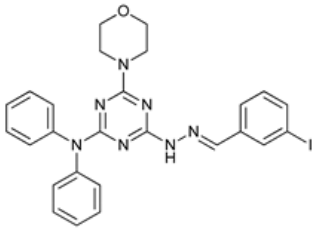
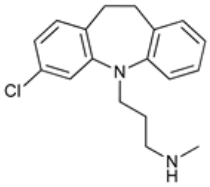
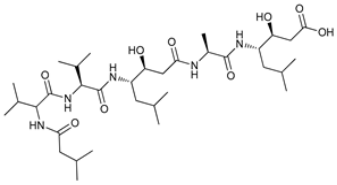
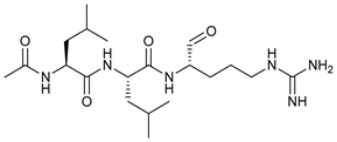
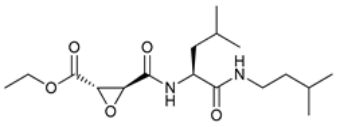
Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
ULK Inhibitors	Compound 6		1	[61]
	MRT68921		2	[62][63]
	MRT67307		3	[62][63]
	SBI-0206965		4	[64][65][66][67]
	ULK-100		5	[68]
	ULK-101		6	[68]

Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
Pan PI3k Inhibitors				
	3MA		7	[69][70][71]
	3 MA derivatives		8	[72]
	Wortmannin		9	[73][74]
	LY294002		10	[75]
	SF1126		11	[76][77]
	PI103		12	[78]
	KU55933		13	[79]
	Gö6976		14	[79]

Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
VPS34 (PI3KC3) Inhibitors	GSK1059615		15	[80][81]
	SAR405		16	[78]
	VPS34-IN1		17	[82]
	PIK-III		18	[83]
	Compound 31		19	[84]
	Spautin-1		20	[85]

Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
ATG Inhibitors	ATG7 inhibitor		21	WO2018/089786
	ATG7 inhibitor, miR154	UAGGUUAUCCGUGUUGCCUUCG	22	[86]
	NSC185058		23	[87]
	Tioconazol		24	[88]
	UAMC-2526		25	[89]
	LV320		26	[90]
	S130		27	[91]
	FMK-9a		28	[92][93][94]
Autophagy Formation	Verteporfin		29	[95][96][97][98]

Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
Lysosome Inhibitors	Chloroquine		30	[99][100]
	Hydroxychloroquine		31	[101]
	Lys05		32	[102][103]
Lysosomotropic Agents	DQ661		33	[104]
	VATG-027		34	[105]
	Mefloquine		35	[106][107]
	Ganoderma lucidum polysaccharide (GLP)		36	[108][109][110]
Vacuolar H^+ ATPase Inhibitors	Bafilomycin A1		37	[111][112][113]
	Tambjamines		38	[114]
Ionophores	Monensin		39	[115]
	Squaramides		40	[116]

Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
Inhibition of Autophagosome-Lysosome Fusion	WX8 family		41	[117]
	Vacuolin-1		42	[118]
	Desmethylclomipramine		43	[119]
Acid Protease Inhibitors	Pepstatin A		44	[120]
	Leupeptin		45	[120]
	E64d		46	[121]
Others	Nanoparticles		47	[122][123][124]

2.2.1. ULK Inhibitors

ULKs are a family of serine/threonine protein kinases that form complexes with multiple regulator units. The role of ULK1 is essential for the initiation of autophagy [125][126][127], however the role of ULK2 in autophagy seems to be cell type dependent [128]. Due to the homology between ULK1 and ULK2 [129], inhibitors of ULK1 also inhibit ULK2 [129]. ULK1 has been shown to be upregulated in several cancers, which correlated with poor prognosis and treatment resistance [64][130][131][132]. Inhibition of ULK1 has been shown to induce a decrease in tumor growth and induction of apoptosis [65][66]. This has led to the search for compounds that inhibit this kinase activity finding some molecules that compete with the ATP-binding site, such as compound 6 [61], MRT68921, and MRT67307 [62][63]. Besides them, SBI-0206965 is the most studied [67], which inhibits autophagy and induces apoptosis in neuroblastoma cell lines [65], non-small cell lung cancer (NSCLC) cells [66][67], and in clear cell renal carcinoma cells [64]. Moreover, it has also been reported to be a direct inhibitor of AMPK, which is a serine/threonine kinase that activates the ULK complex, among other roles [133]. Recently more ULK inhibitors, such as ULK100 and ULK101, have been described [68], which supports that the idea that blocking ULK1 may be a good strategy for cancer therapy.

2.2.2. Pan PI3K Inhibitors

The family of phosphoinositide 3-kinases (PI3Ks) is divided into three classes with different substrate preferences, which define their functions. The role of class II on autophagy is unclear. However, class I activates mTORC1 through the PI3K/Akt pathway and consequently inhibits autophagy, while class III (VPS34) activates autophagy [134]. PI3K pathways

have been associated with cancer due to their participation in tumorigenic processes such as cell proliferation, survival, migration, and angiogenesis. Therefore, they are a good target for therapy development [135]. Most of the studied PI3K inhibitors are not selective for a specific class of PI3K, hence, they affect different cellular processes, not only autophagy, and consequently their effect cannot be only attributed to inhibition of autophagy. However, due to their therapeutic relevance, we describe briefly some of them below.

3-Methyladenine (3MA) was one of the first inhibitors of autophagy described [69]. It exerts a dual effect on autophagy. Under starving conditions it suppresses autophagy through PI3KC3 inhibition. However, in the presence of nutrients it promotes autophagy by inhibition of PI3KC1 [70]. Additionally, it has been reported that it reduces the expression of drug efflux transporters, overcoming taxol and doxorubicin resistance [71]. 3MA is effective at high concentrations, although presents solubility problems. In order to overcome this limitation some derivatives have been synthetized [72]. Wortmannin is a fungal metabolite that binds irreversibly to the catalytic site of PI3Ks [73][74]. LY294002 is a synthetic small molecule [75] with poor solubility and short half-life. A conjugate analog of LY294002, named SF1126, was designed to accumulate in integrin expressing tissues, improving LY294002 solubility and pharmacokinetic, favoring its accumulation in the tumor site and showing antitumor and antiangiogenic properties in mouse models [76][77]. Other non-selective Pan PI3K inhibitors are PI103 [78], KU55933, Gö6976 [79], and GSK1059615 [80][81][136].

2.2.3. VPS34 (PI3KC3) Complex Inhibitors

VPS34 is a PI3KC3 that transforms PI to PI3P. VPS34 forms a complex with several subunits needed for its activation, such as VPS15 (also known as p150), ATG14, and Beclin-1. Autophagy can be blocked by inhibition of VPS34 activity; SAR405 is one compound of the (2S)-tetrahydropyrimido-pyrimidinones series with kinase inhibitor activity by strong competition for ATP site. However, it is highly selective for PI3KC3, compared to class I and II, and more than 200 protein kinases and 15 lipid kinases. SAR405 inhibits autophagy induced either by starvation or mTOR inhibition [78]. VPS34-IN1 is a bipyrimidinamine that inhibits PI3KC3 selectively, compared with more than 300 protein kinases analyzed [82]. Additionally, PIK-III, a bisaminopyrimidine, binds to a hydrophobic pocket unique in VPS34 that cannot be found in other related kinases [83]. Compound 31 is a small molecule selective against protein and other lipid kinases [84]. All these four inhibitors are selective for PI3KC3, but it should be noted that VPS34 can form different complexes with other subunits that lead to a different localization and function, participating also in vesicle trafficking [137]. Thus, inhibitors of VPS34 can also have an effect on endosomal trafficking, as the case of SAR405 that prevents the activity of both VPS34 complexes [78]. Therefore, it may also affect cellular secretion [138].

On the other hand, autophagy can be also inhibited blocking PI3KC3 complex formation; Spautin-1 indirectly inhibits the activity of VPS34 by proteosomal degradation of proteins that form VPS34 complexes through reduction of Beclin-1 deubiquitination mediated by USP10 and USP13 [85].

2.2.4. ATG inhibitors

Membrane PI3P produced by VPS34 leads to the recruitment of PI3P-binding ATG proteins and additional factors, resulting in the formation of complexes that participate in the elongation of the phagophore. Inhibition of autophagy can be achieved by impeding the formation of these complexes.

ATG7 participates in the formation of the complex ATG12-ATG5 and the conjugation of PE to LC3 and GABARAP. Recently, some inhibitors of ATG7 (WO2018/089786) have been designed and it has extended the use of micro RNAs that target ATG7 gene such as miR-154 that inhibits blade cancer progression [86].

On the other hand, ATG4B cleaves LC3, activating it for its conjugation with PE [139] necessary for the expansion of the autophagosome and its recognition. Additionally, it participates in LC3-PE deconjugation, which is important for LC3 recycling and for the fusion of the autophagosome with the lysosome [140]. Therefore, ATG4B could be a good target to inhibit autophagy more selectively, thus, a large number of ATG4B possible inhibitors have been screened in the last years [141]. NSC185058 is a small compound that docked at the active site of ATG4B inhibiting not only autophagy but also the volume of the autophagosomes, which is accompanied by suppression of tumor growth in an osteosarcoma subcutaneous mouse model [87]. Tioconazole is an antifungal drug that binds to the active site of ATG4 blocking autophagy flux reducing cell viability and sensitizing tumor cells to doxorubicin in a xenograft mouse model [88]. Other ATG4B inhibitors that suppress autophagy in cell lines and in vivo inhibiting cell proliferation are UAMC-2526, a derivative of benzotropolones stable in plasma [89], and LV-320, a styrylquinoline [90].

It should be noticed that the roles of ATG4B in cancer are not well understood and some of the ATG4 inhibitors showed only inhibition in LC3-PE delipidation, but not in the autophagosome formation such as S130 [91] and FMK-9a [92][93][94].

Additionally, some studies are focused on the evaluation of different markers that may predict the effectiveness of those inhibitors [142]. For instance, ATG4B inhibition is effective only in Her-2 positive cells and not in those negative [143].

2.2.5. Autophagosome Formation Inhibition

Verteporfin is a benzoporphyrin derivative used in the clinic in photodynamic therapy. Interestingly, it prevents autophagosome formation induced by glucose and serum deprivation, but not by mTOR inhibition [95]. One possible mechanism of action of verteporfin is the blockade of p62 oligomerization, a protein necessary for the sequestration of ubiquitinated targets into autophagosomes [144][145]. Additional to autophagy inhibition, verteporfin reduces [96][98] transcriptional co-activators that regulate the Hippo pathway, implicated in cell growth and stem cell function [146]. Verteporfin inhibits cell proliferation, angiogenesis, and migration, and induces apoptosis [147]. It inhibits autophagy in vivo but has no effect as a single agent in tumor growth. However, it moderately sensitizes tumor cells to cytotoxic agents [97].

2.2.6. Lysosome Inhibitors

The last step in autophagy is the fusion of autophagosomes with lysosomes, whose hydrolases degrade the autophagosome content. The inhibition of autophagy at this point consists of the use of lysosomal inhibitors.

Chloroquine (CQ) and its analog hydroxychloroquine (HCQ) [101] are drugs used for the treatment of various diseases, such as malaria and more recently cancer [100]. They are weak bases and the unprotonated form of CQ/HCQ can diffuse through cell membranes and enter into organelles such as lysosomes, where the high concentration of H^+ induces their protonation and consequently increases lysosomal pH [99]. Once CQ/HCQ are protonated, they are trapped in the lysosomes producing an increase of their volume, and inhibiting the activity of lysosomal enzymes.

CQ and HCQ are the only autophagy inhibitors approved for clinical use. Although short-term CQ/HCQ treatment has been considered safe, retinopathy has been reported produced by long-term treatment with HCQ in about 7.5% of patients [148] and cardiotoxicity [149]. The prevalence depends on the dosage and the duration of treatment [150]. This toxicity limitation, along with inconsistencies in the results obtained in the clinic, have led to the study of new and more potent autophagy inhibitors [151]. Thus, CQ analogs that exert more potent autophagy inhibitory activity have been synthetized. Lys05 is a dimeric analog of CQ that accumulates within acidic organelles, including lysosomes, more potently than HCQ [102]. DQ661, a dimeric quinacrine (DQ), not only inhibits lysosomal catabolism, including autophagy, but also targets palmitoyl-protein thioesterase-1, resulting in the inhibition of mTORC1 signaling. DQ661 has shown effects on tumor mouse models alone and it also overcame resistance to gemcitabine [104]. Another antimalaria compound found to inhibit autophagy with antitumoral properties is VATG-027 [105]. On the other hand, mefloquine is also accumulated in lysosomes disrupting autophagy, it induces apoptosis and inhibits multidrug resistance protein1 (MDR1) being effective in multidrug-resistant tumor cells [107]. Mefloquine sensitizes chronic myeloid leukemia (CML) cells derived from patients in chronic phase to TK inhibitors showing selectivity for stem/progenitor tumoral cells to normal cells [106].

CQ and its derivatives are not the only drugs that target lysosomes to inhibit autophagy; GLP (ganoderma lucidum polysaccharide) is a polysaccharide from the fungus *Ganoderma lucidium* with multiple antitumoral properties [108]. GLP induces apoptosis in cancer cell lines [110] and reduces tumor growth in mouse models [109]. It has recently been seen that GLP impairs autophagy flux by reduction of lysosome acidification and the accumulation of autophagosomes has suggested to be the cause of apoptosis induction [109]. Bafilomycin A (BafA) is a vacuolar-H^+ ATPase inhibitor that disrupts the acidification of lysosomes, vesicles, and vacuoles [111][112] by preventing the entry of H^+ into these organelles. BafA also inhibits the fusion of autophagosomes with lysosomes, by disruption of Ca^{2+} gradients implied in this process [113].

Ionophores can also disrupt lysosomal pH, impairing the autophagy process. Tambjamine analogues are anion selective ionophores derived from the naturally occurring tambjamines and induce mitochondrial swelling and autophagy blockade with cytotoxic effects in lung cancer cells and cancer stem cells (CSCs) [114]. Monensin, nigericin, and lasalocid are cation ionophores, but only monensin presents selectivity for lysosomes [115]. Squaramides are synthetic chloride transporters that also induce cell death by apoptosis [116].

On the other hand, the WX8-family comprises five chemical analogs that disrupt the fusion of lysosomes with autophagosomes, lysosomes fission, and sequestration of molecules into the lysosomes without altering their pH. These compounds bind to PIKFYVE phosphoinositide kinase and present potent antitumoral effects on autophagic dependent cells [117]. Vacuolin-1 activates RAB5A blocking the fusion of the autophagosomes with lysosomes, however it also inhibits the fusion of endosomes with lysosomes, resulting in a general endosomal-lysosomal degradation defective [118].

Clomipramine (CM) is a FDA-approved prodrug for the treatment of psychiatric disorders the metabolite of which, desmethylclomipramine (DCMI), impairs autophagic flux blocking lysosomal degradation that sensitizes tumor cells to

cancer treatment [119]. DCMI also affects lung CSCs [152]. Additionally, protease inhibitors can also inhibit the lysosomal degradation, such as pepstatin A (aspartyl proteases; cathepsin D and E), Leupeptin [120] and E64d (cysteine proteases; cathepsin B, H, and L) [121]. On the other hand, nanoparticles are usually accumulated into lysosomes by endocytosis internalization, which may cause lysosome impairment [122]. Gold nanoparticles [123] and nanodiamonds have shown to inhibit autophagy by disruption of lysosomal function, which sensitizes tumors to arsenical base therapy [124].

Several studies have suggested that the anti-tumor effects of lysosomal inhibitors may be independent of autophagy inhibition since they also interfere in other cellular mechanisms producing non-autophagy related effects [153][154][155][156][157][158][159][160]. Remarkably, disruption of the lysosomes not only blocks autophagy, but lysosomal permeabilization releases proteases such as cathepsins that are active at cytosolic pH and participate in apoptosis and apoptosis-like and necrosis-like cell death [161][162][163]. Additionally, lysosomes also participate in tumor invasion, hence, these inhibitors have shown to be effective against metastasis [103][164][165][166], targeting cancer stem cells [167], and inducing tumor vessel normalization [168].

As mentioned above, there are efforts to find genetic determinants to sensitivity or resistance to these lysosomal inhibitors. Metastatic cells are more vulnerable to CQ and BafA, suggesting that patients with metastasis could benefit from those treatments [164]. Morgan and coworkers also showed a relationship between the expression of ID4 and metastatic potential. Additionally, overexpression of helicase-like transcription factor (HLTF) seems to be related with the resistance to HCQ, Lys05 and BafA treatment [155] and tumors with the V600E mutation in BRAF (v-Raf murine sarcoma viral oncogene homolog B) present cytoprotective autophagy [169].

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