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.

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**).

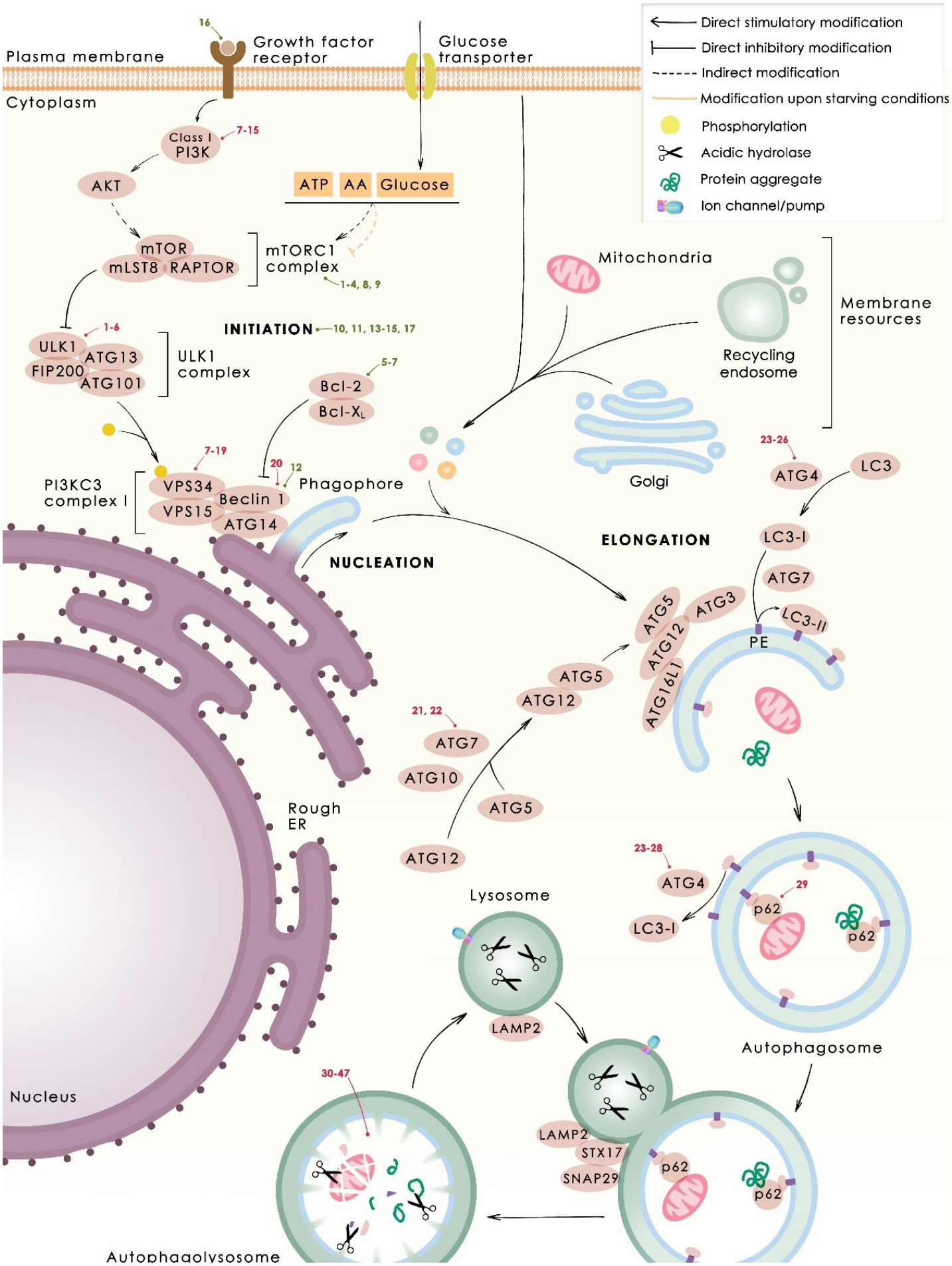
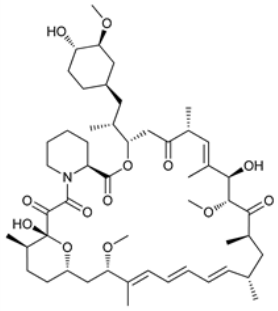
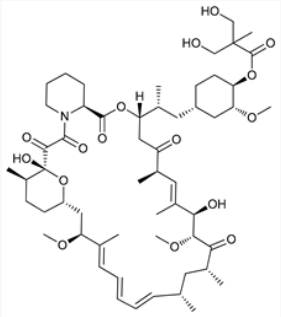
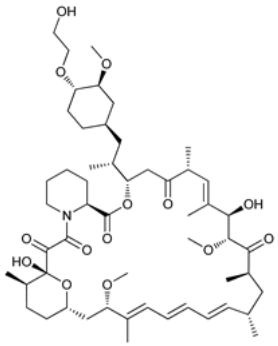

Direct stimulatory modification
Direct inhibitory modification
Indirect modification
Modification upon starving conditions
Phosphorylation
Acidic hydrolase
Protein aggregate
Ion channel/pump
Plasma membrane
Cytoplasm
16
Growth factor receptor
Glucose transporter
Class I PI3K
7-15
AKT
ATP
AA
Glucose
mTOR
mLST8
RAPTOR
mTORC1 complex
1-4, 8, 9
INITIATION
10, 11, 13-15, 17
ULK1
ATG13
FIP200
ATG101
ULK1 complex
1-6
Bcl-2
Bcl-XL
5-7
PI3KC3 complex I
VPS34
VPS15
ATG14
Beclin 1
7-19
20
12
Phagophore
NUCLEATION
Mitochondria
Recycling endosome
Membrane resources
Golgi
ELONGATION
23-26
ATG4
LC3
LC3-I
ATG7
LC3-II
PE
ATG5
ATG12
ATG3
ATG16L1
21, 22
ATG7
ATG10
ATG5
ATG12
Rough ER
Nucleus
Lysosome
LAMP2
23-28
ATG4
LC3-I
29
p62
p62
Autophagosome
30-47
LAMP2
STX17
SNAP29
p62
p62
Autophagolysosome

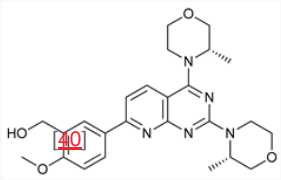
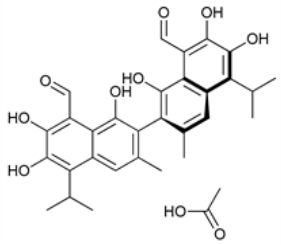
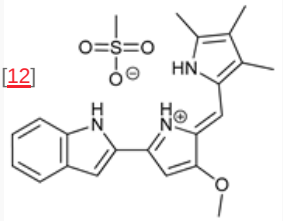
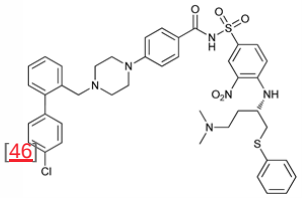
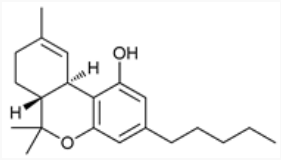
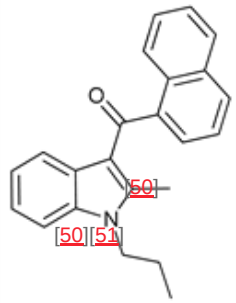
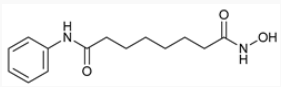
Figure 1. Mechanism of autophagy. The phases of the process (nucleation, elongation, maturation and degradation), with the main proteins that participate in each one, are depicted.Autophagy activators (green) and inhibitors (red) are marked where they interfere with the autophagy process. Numbers correspond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

2.1. Autophagy Stimulation for Cancer Treatment

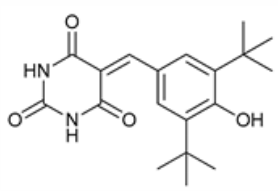
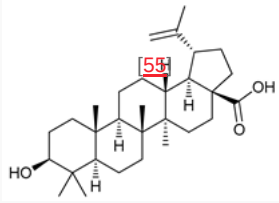
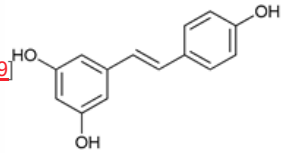
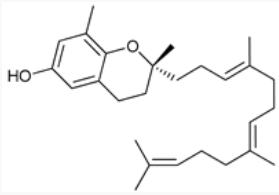
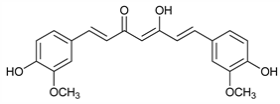
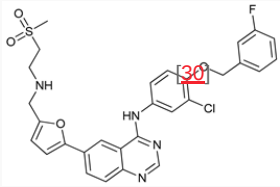
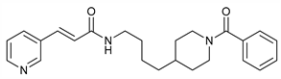
Induction of ACD has become an interesting alternative to overcome resistance to apoptosis and to exploit a caspase independent cell death for cancer treatment. In the following sections, compounds for which the mechanism of action is based on stimulating autophagy are described (**Table 1**).

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]

Mechanism of Action/Type	Name	Structure	Number in Figure 1	Refs.
	AZD8055		4	[16][17]
	(-)-gossypol (AT-101) [45]		5	[43][44] [18][19] [20][21]
BH3 Mimetics	Obatoclax (GX15-070) [13]		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]

survival, tosis, and netabolite ppressive selective ever, their signaling ng cancer shown to as a pro- rapalog gradation ncer cells its target omplexes t it is also her these a tumor esistance oteins [49], stimulate -w [50]. Its [21], but the noma [18], na [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]

Mechanism of Action/Type	Name	Structure[53]	Number in Figure 1	Refs.
Natural Products	MHY2256		11	[31]
	Betulinic acid		12	[32]
	Resveratrol		13	[33]
	δ-Tocotrienol		14	[34]
	Curcumin		15	[35]
Others	Lapatinib		16	[36][37]
	APO866		17	[38]

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].

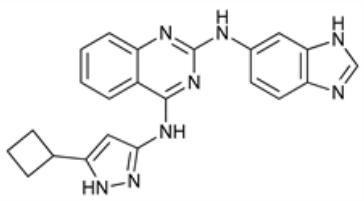
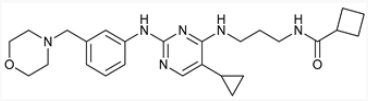
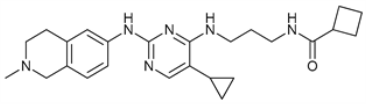
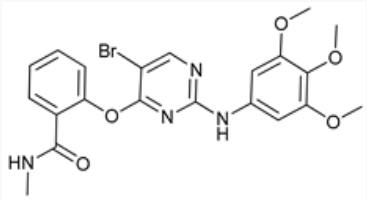
2.1.6. Others

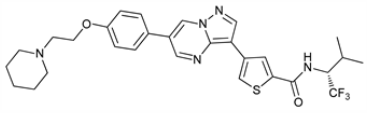
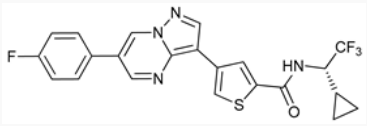
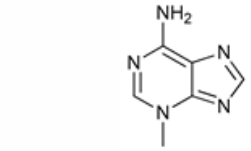
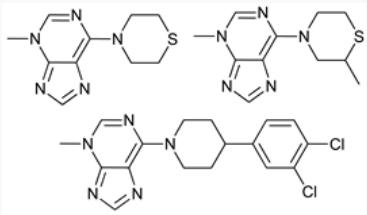
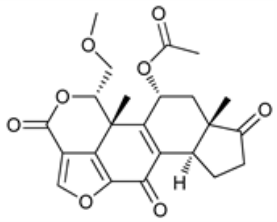
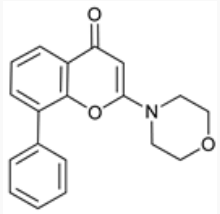
Other compounds have been reported to induce ACD in cancer. For example, lapatinib is a small molecule tyrosine kinase inhibitor, targeting epidermal growth factor receptors that is capable of inducing ACD in hepatocellular carcinoma [36] and in acute leukemia cell lines [37]. APO866 is an inhibitor 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

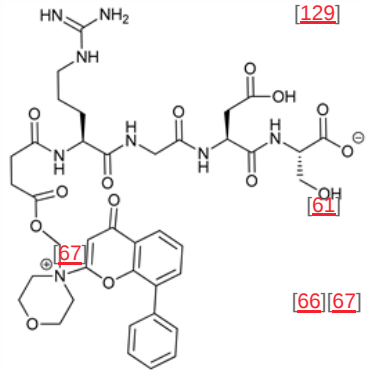
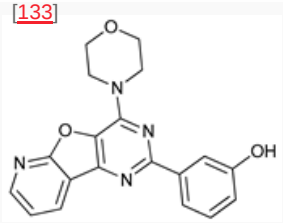
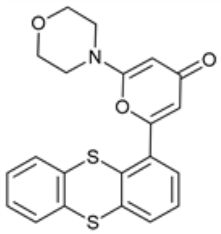
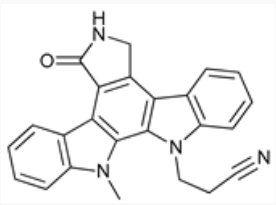
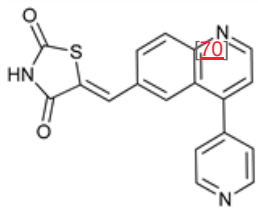
In several tumors, autophagy has a protective role; therefore, its inhibition could be an interesting approach for tumor treatment. There are several autophagy inhibitors that block the process of autophagy at different steps, which we detail below (**Table 2**).

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]

Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
Pan PI3k Inhibitors	ULK-100		5	[68]
	ULK-101		6	[68]
	3MA		7	[69][70][71]
	3 MA derivatives		8	[72]
	Wortmannin		9	[73][74]
	LY294002		10	[75]

2.2.1. ULK Inhibitors

Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
[125][126][127]	SF1126		11	[76][77]
[128] [129] [64][130][131][132] [65][66] [65] [66][67] [64]	PI103	 [133] [68]	12	[78]
	KU55933		13	[79]
[134] [135]	Gö6976		14	[79]
	GSK1059615	 [72]	15	[80][81] [71]

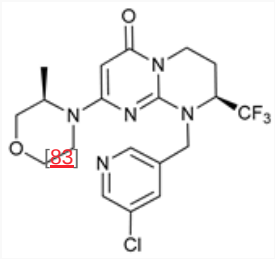
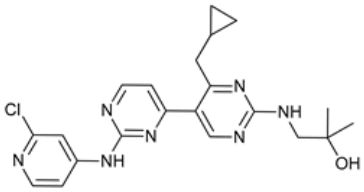
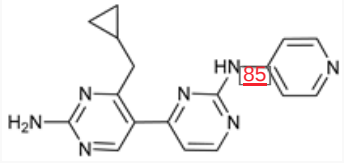
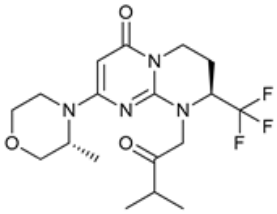
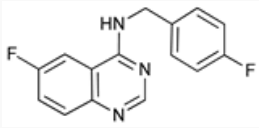
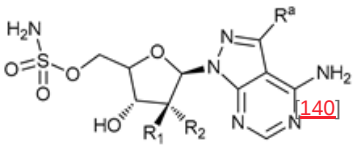
e role of ms to be bit ULK2 osis and owth and ng some 7 [62][63]. otosis in arcinoma e kinase 100 and ategy for erences, nTORC1 utophagy such as elopment different ibition of effect on , in the ed that it 3MA is on some alytic site

autophagy. Under starving presence of nutrients it pro reduces the expression of effective at high concentrat derivatives have been syntl [69] [70]

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

Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
VPS34 (PI3KC3) Inhibitors	SAR405		16	[78]
	VPS34-IN1		17	[78]
	PIK-III		18	[83]
	Compound 31		19	[84]
	Spautin-1		20	[85]
ATG Inhibitors	ATG7 inhibitor		21	WO2018/089786
	ATG7 inhibitor, miR154	UAGGUUAUCCGUGUUGCCUUCG	22	[86]

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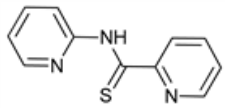
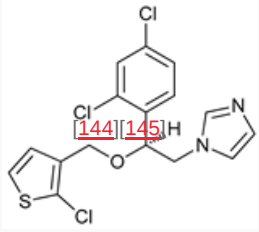
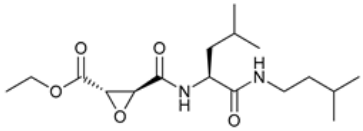
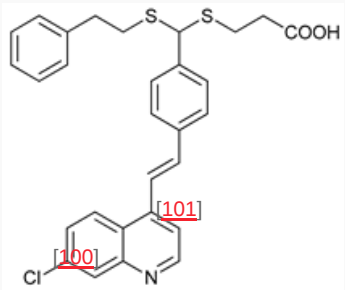
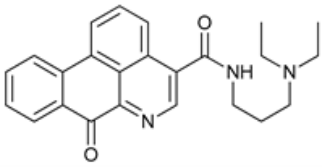
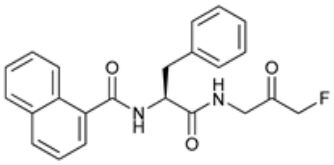
not only

ume of the autophagosomes, which is accompanied by suppression of tumor growth in eous 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

Mechanism of Action [143]	Name [142]	Structure	Number in Figure 1	Refs.
[96][98] [146]	NSC185058		23	[87]
	Tioconazol	 [144][145]	24	[88]
	[97]UAMC-2526		25	[89]
	LV320	 [101] [100]	26	[90]
+	S130		27	[99] [91]
	FMK-9a [149]		28	[92][93][94]

and not

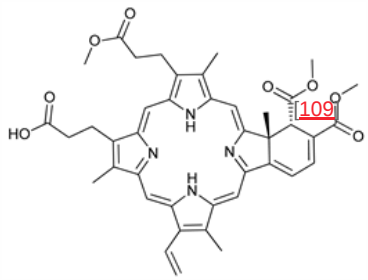
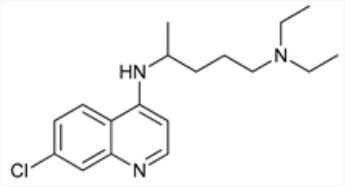
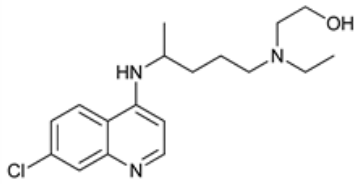
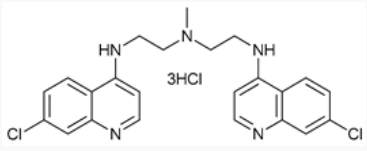
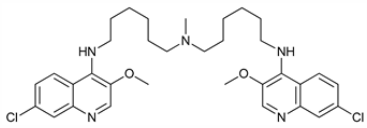
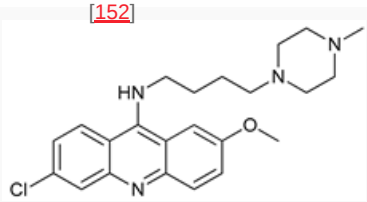
prevents [95]. One y for the rteporfin stem cell s [147]. It ensitizes

rade the s.

various form of the high HCQ are ctivity of

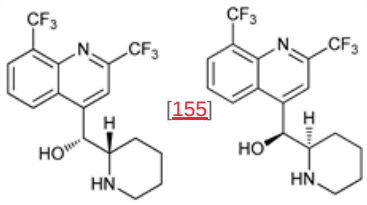
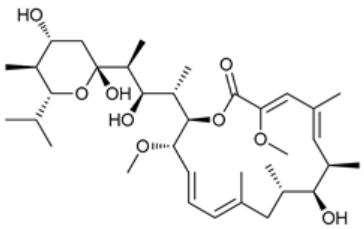
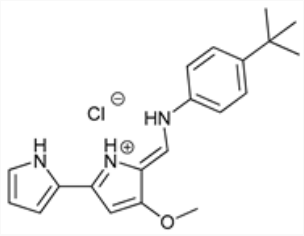
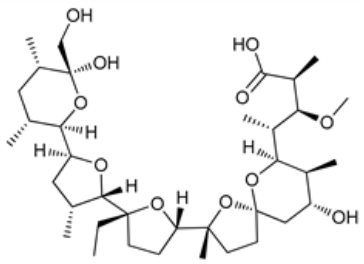
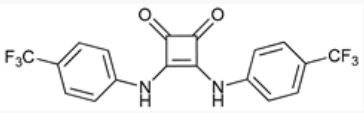
reatment in about reatment

has been considered safe, 7.5% of patients [148] and c [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].

Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
+ Autophagy Formation + 2+	Verteporfin		29	[109] [95][96][97][98] [111][112]
Lysosome Inhibitors Lysosomotropic Agents	Chloroquine		30	[99][100] [114]
	Hydroxychloroquine		31	[116] [101]
	Lys05		32	[102][103]
	DQ661		33	[104]
	VATG-027 [121]		34	[120] [105] [122]

lucidum ties [108]. recently lation of vacuolar- nting the uption of re anion ling and onensin, es [115]. nes with their pH. tophagic osomes, sosomal [117] [118] of which, mor cells hibit the nd E64d ated into [123] and [119] [124]. umors to [152]

gested that the anti-tumor effects of lysosomal inhibitors may be independent of hey 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].

Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
	Mefloquine [164]	 [155]	35 [169]	[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]

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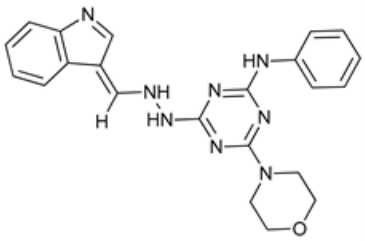
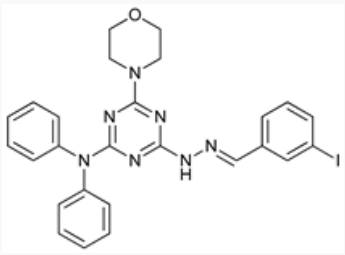
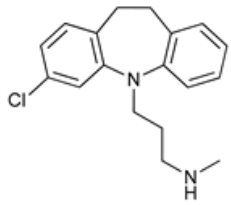
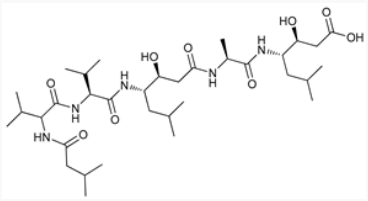
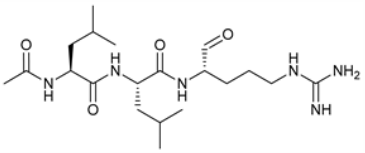
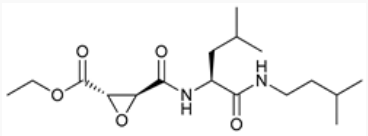
Mechanism of Action	Name	Structure	Number in Figure 1	Refs.
Inhibition of Autophagosome-Lysosome Fusion	WX8 family		41	[117]
	Vacuolin-1		42	[118]
	Desmethylclomipramine		43	[119]
Acid Protease Inhibitors	Pepstatin A		44	[120]
	Leupeptin		45	[120]
	E64d		46	[121]
Others	Nanoparticles		47	[122][123][124]

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