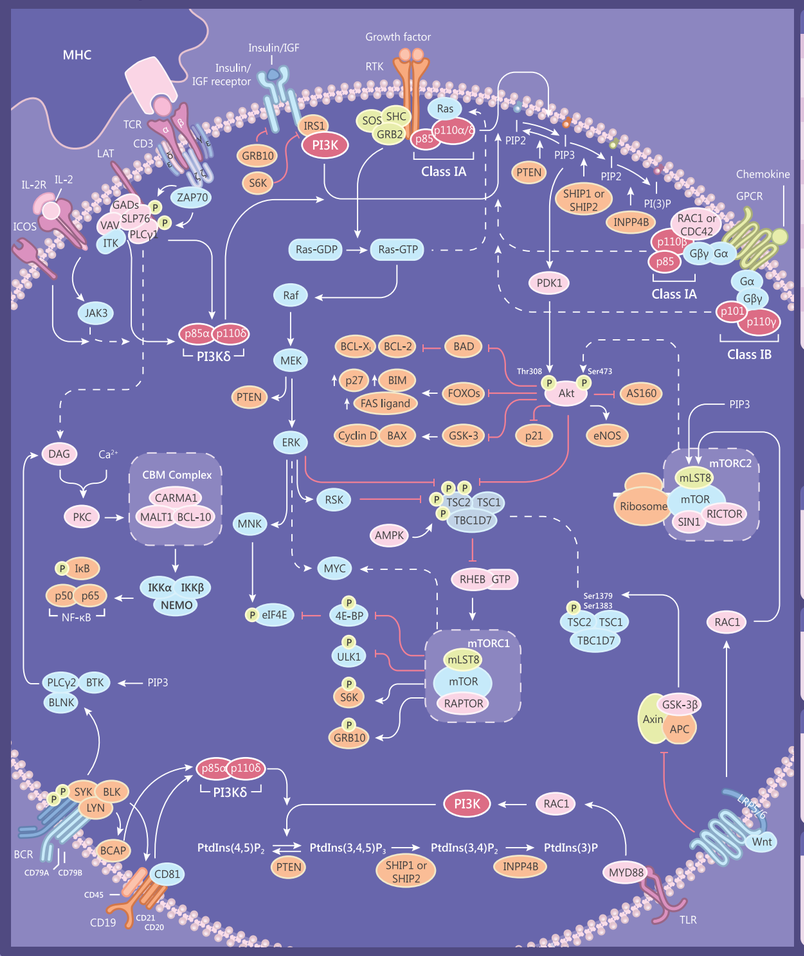
MedChemExpress Signaling Pathways --- Part Ia

PI3K (Phosphoinositide 3-kinase)



PI3K Pan Inhibitors
LY294002
Inhibits PI3Kα/PI3Kδ/PI3Kβ, also inhibits CK2.
Wortmannin
Inhibits PI3K irreversibly, also inhibits PLK and DNA-PK.
NVP-BKM120
Oral pan-class I PI3K inhibitor that penetrates the BBB.
3-Methyladenine
Inhibits Vps34 and PI3Kγ, also inhibits autophagy.
BEZ235
Dual pan-class I PI3K and mTOR inhibitor.

PI3Kα Selective Inhibitors	
BYL-719	HY-15244
A66	HY-13261
MLN1117	HY-12285
GDC-0326	HY-101272

PI3Kβ Selective Inhibitors	
GSK2636771	HY-15245
TGX-221	HY-10114
AZD 6482	HY-10344

PI3Kγ Selective Inhibitors	
IPI549	HY-100716
AS-252424	HY-13532
CZC24832	HY-15294
CAY10505	HY-13530

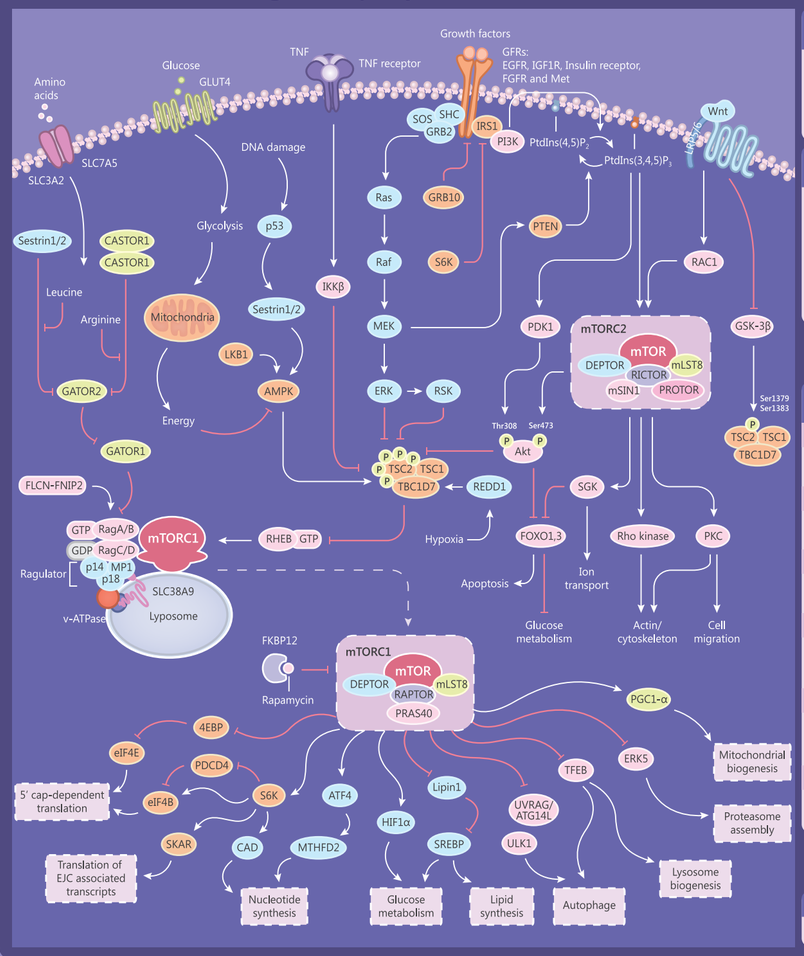
PI3Kδ Selective Inhibitors	
CAL-101	HY-13026
IPI-3063	HY-111510
TGR-1202	HY-12279
PI-3065	HY-12235

Phosphatidylinositol 3 kinases (PI3Ks) are a family of lipid kinases that integrate signals from growth factors, cytokines and other environmental cues, translating them into intracellular signals that regulate multiple signaling pathways. These pathways control many physiological functions and cellular processes, which include cell proliferation, growth, survival, motility and metabolism.

In the absence of activating signals, p85 interacts with p110 and inhibits p110 kinase activity. Following receptor tyrosine kinase (RTK) or G protein-coupled receptor (GPCR) activation, class I PI3Ks are recruited to the plasma membrane, where p85 inhibition of p110 is relieved and p110 phosphorylates PIP2 to generate PIP3. The activated insulin receptor recruits intracellular adaptor protein IRS1. Phosphorylation of IRS proteins on tyrosine residues by the insulin receptor initiates the recruitment and activation of PI3K. PIP3 acts as a second messenger which promotes the phosphorylation of Akt at Thr308 by PDK-1. RTK activation can also trigger Ras-Raf-MEK-ERK pathway. Activated Akt, ERK and RSK phosphorylate TSC2 at multiple sites to inhibit TSC1-TSC2-TBC1D7, which is the TSC complex that acts as a GTPase-activating protein (GAP) for the small GTPase RHEB. During inhibition of the TSC complex, GTP-loaded RHEB binds the mTOR catalytic domain to activate mTORC1. Glycogen synthase kinase 3β (GSK-3β) activates the TSC complex by phosphorylating TSC2 at Ser1379 and Ser1383. Phosphorylation of these two residues requires priming by AMPK-dependent phosphorylation of Ser1387. Wnt signaling inhibits GSK-3β and the TSC complex, and thus activates mTORC1. mTORC2 is activated by Wnt in a manner dependent on the small GTPase RAC1. Akt activation contributes to diverse cellular activities which include cell survival, growth, proliferation, angiogenesis, metabolism, and migration. Important downstream targets of Akt are GSK-3, FOXOs, BAD, AS160, eNOS, and mTOR. mTORC1 negatively regulates autophagy through multiple inputs, including inhibitory phosphorylation of ULK1, and promotes protein synthesis through activation of the translation initiation promoter S6K and through inhibition of the inhibitory mRNA cap binding 4E-BP1.

PI3Kδ is a heterodimeric enzyme, typically composed of a p85α regulatory subunit and a p110δ catalytic subunit. In T cells, the TCR, the costimulatory receptor ICOS and the IL-2R can activate PI3Kδ. In B cells, PI3Kδ is activated upon crosslinking of the B cell receptor (BCR). The BCR co-opts the co-receptor CD19 or the adaptor B cell associated protein (BCAP), both of which have YXXM motifs to which the p85α SH2 domains can bind. In lymphocytes, BTK and ITK contribute to the activation of PLCγ and promotes the generation of DAG and the influx of Ca^{2+}, which in turn activate PKC and the CARMA1-, BCL 10- and MALT1 containing (CBM) complex. The resulting NF-κB inhibitor kinase (IKK) activation leads to the phosphorylation and the degradation of IκB, and to the nuclear accumulation of the p50-p65 NF-κB heterodimer. MyD88 is an adapter protein that mediates signal transduction for most TLRs and leads to activation of PI3K.

mTOR (Mammalian target of rapamycin)



First Generation mTOR Inhibitors	
Rapamycin	HY-10219
Everolimus	HY-10218
Temsirolimus	HY-50910
Deforolimus	HY-50908

mTORC1/2 Selective Inhibitors	
INK-128	HY-13328
Torin 1	HY-13003
PP 242	HY-10474
AZD-8055	HY-10422
AZD2014	HY-15247
KU-0063794	HY-50710

mTOR and PI3K Dual-Specificity Inhibitors
PI-103
Inhibitor of DNA-PK, PI3K (p110α) and mTOR.
BEZ235
Dual pan-class I PI3K and mTOR inhibitor.
XL765
Inhibitor of class I PI3K isoforms, mTOR, and DNA-PK.
GDC-0980
Orally bioavailable inhibitor of pan-class I PI3K, mTORC1 and mTORC2.
Bimiralisib
Brain-penetrant, orally bioavailable inhibitor of pan-class I PI3K and mTOR.
LY3023414
Inhibitor of class I PI3K isoforms, mTOR, and DNA-PK.

mTOR Activator	
MHY1485	HY-B0795

The mTOR signaling pathway integrates both intracellular and extracellular signals and serves as a central regulator of cell metabolism, growth, proliferation and survival. mTOR is the catalytic subunit of two distinct complexes called mTORC1 and mTORC2. **mTORC1** comprises DEPTOR, PRAS40, RAPTOR, mLST8, mTOR, whereas **mTORC2** comprises DEPTOR, mLST8, PROTOR, RICTOR, mSIN1, mTOR. Rapamycin binds to FKBP12 and inhibits mTORC1 by disrupting the interaction between mTOR and RAPTOR. mTORC1 negatively regulates autophagy through multiple inputs, including inhibitory phosphorylation of ULK1 and TFEB. mTORC1 promotes protein synthesis through activation of S6K and inhibition of 4E-BP1, and regulates glycolysis through HIF1α. mTORC2 inhibits FOXO1,3 through SGK and Akt. The complex also regulates actin cytoskeleton assembly through PKC and Rho kinase.

Growth factors: Growth factors can signal to mTORC1 through both PI3K-Akt and Ras-Raf-MEK-ERK axis. For example, ERK and RSK phosphorylate TSC2, and inhibit it.

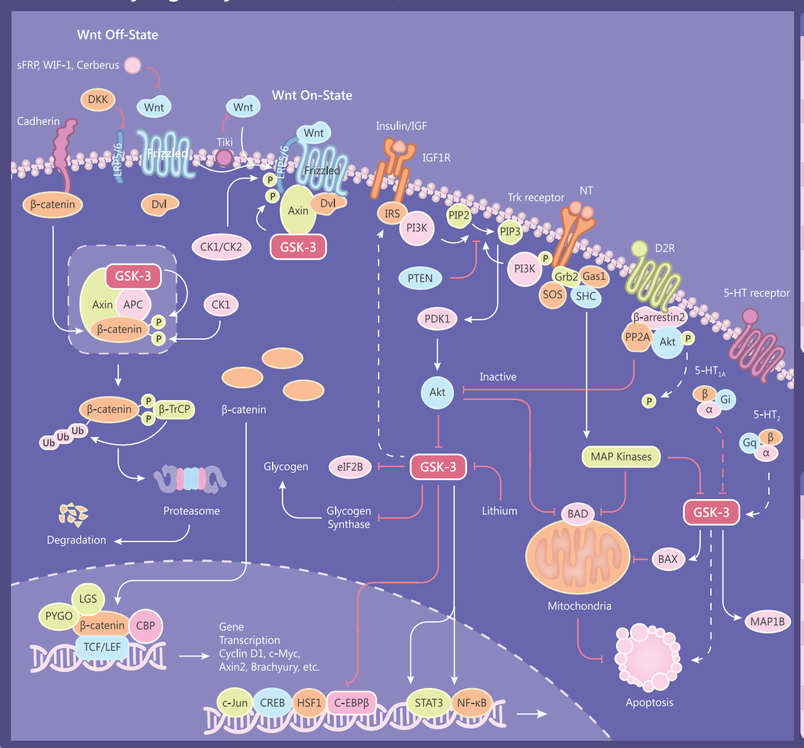
Insulin Receptor: The activated insulin receptor recruits intracellular adaptor protein IRS1. Phosphorylation on tyrosine residues by the insulin receptor initiates the activation of PI3K. PIP3 acts as a second messenger which promotes the phosphorylation of Akt and triggers the Akt-dependent phosphorylation of TSC2. TSC is a heterotrimeric complex comprised of TSC1, TSC2, and TBC1D7, and functions as a GTPase activating protein (GAP) for the small GTPase Rheb, which directly binds and activates mTORC1.

Wnt: The Wnt pathway activates mTORC1. GSK-3β acts as a negative regulator of mTORC1 by phosphorylating TSC2. mTORC2 is activated by Wnt in a GTPase RAC1-dependent manner .

Amino acids: Amino acids induce the movement of mTORC1 to lysosomal membranes, where the Rag proteins reside. A complex named Ragulator, interact with the Rag GTPases, recruits them to lysosomes through a mechanism dependent on the lysosomal v-ATPase, and is essential for mTORC1 activation. In turn, lysosomal recruitment enables mTORC1 to interact with GTP-bound RHEB, the end point of growth factor. Cytosolic leucine and arginine signal to mTORC1 through a distinct pathway comprised of the GATOR1 and GATOR2 complexes.

Stresses: mTORC1 responds to intracellular and environmental stresses that are incompatible with growth such as low ATP levels, hypoxia, or DNA damage. A reduction in cellular energy charge, for example during glucose deprivation, activates the stress responsive metabolic regulator AMPK, which inhibits mTORC1 through phosphorylation and activation of TSC2, or through the phosphorylation of RAPTOR. Sestrin1/2 are two transcriptional targets of p53 that are implicated in the DNA damage response, and they potentially activate AMPK, thus mediating the p53-dependent suppression of mTOR activity. During hypoxia, mitochondrial respiration is impaired, leading to low ATP levels and activation of AMPK.

GSK-3 (Glycogen synthase kinase 3)



GSK-3 Pan Inhibitors
CHIR-99021
Highly selective inhibitor of GSK-3α/β.
SB 216763
Selective ATP-competitive inhibitor of GSK-3α/β.
AR-A014418
Selective ATP-competitive inhibitor of GSK-3.
BIO
Specific, reversible and ATP -competitive GSK-3α/β inhibitor.
LY2090314
Selective GSK-3α/β inhibitor.

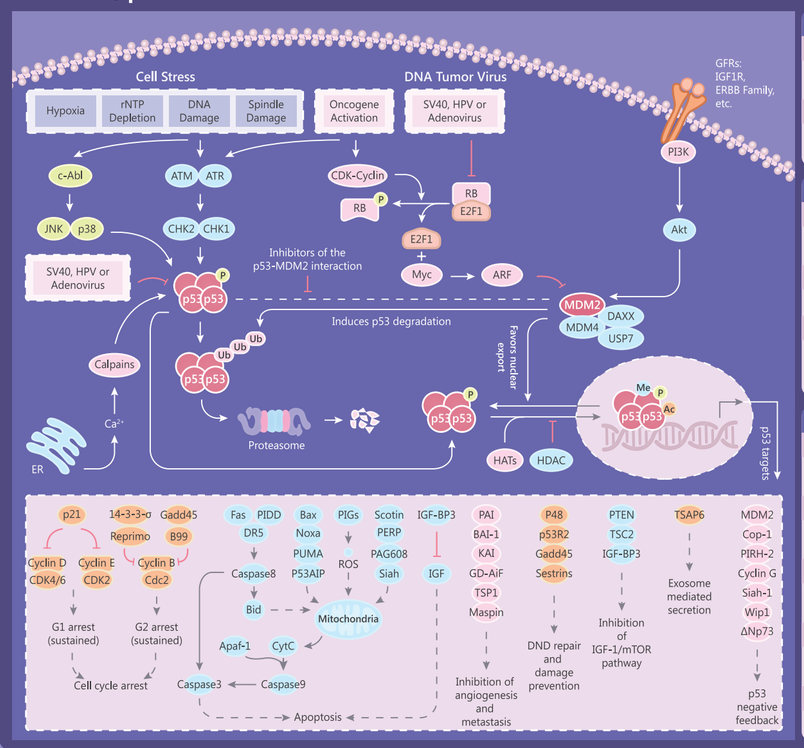
GSK-3β Selective Inhibitors
TWS119
Inhibits GSK-3β selectively. Induces neurogenesis in ESCs.
Tideglusib
Irreversible non-ATP-competitive GSK-3β inhibitor.
Indirubin-3′-monoxime
Inhibits GSK-3β and other kinases.
CP21R7
Selective GSK-3β inhibitor.

Glycogen synthase kinase 3 (GSK-3) is a multifunctional serine/threonine kinase found in all eukaryotes. GSK-3 is one of the few signaling mediators that play central roles in a diverse range of signaling pathways, including those activated by Wnt, PI3K, growth factors, cytokines, and ligands for G protein-coupled receptors. The **PI3K pathway** is known for regulating metabolism, cell growth, and cell survival. The PI3K activity is stimulated by diverse oncogenes and growth factor receptors. PI3K-mediated production of PIP3 leads to the activation of Akt. The activation of Akt leads to the phosphorylation of GSK-3, which is active in resting cells, but is inactivated by the phosphorylation. The GSK-3 has been linked to the regulation of an assembly of transcription factors, including β-catenin, NF-κB, c-Jun, CREB, and STAT. Thus, the altered activity of GSK-3 causes various effects on cytokine expression.

In the absence of **Wnt** signaling, β-catenin is phosphorylated by CK1 and GSK-3. This phosphorylation leads to recognition by β-TrCP, leading to the ubiquitylation of β-catenin and degradation by the proteasome. Upon binding of a lipid-modified **Wnt** protein to the receptor complex, a signaling cascade is initiated. LRP is phosphorylated by CK1/CK2 and GSK-3, and Axin is recruited to the plasma membrane. The kinases in the β-catenin destruction complex are inactivated and β-catenin translocates to the nucleus to form an active transcription factor complex with TCF, leading to transcription of a large set of target genes.

Some endogenous growth factors could bind to and activate the tyrosine kinase receptor. This facilitates the recruitment of other proteins (SHC, SOS), which results in the activation of the ERK-MAPK cascade and the inhibition of GSK-3. GSK-3 exerts many cellular effects: it regulates cytoskeletal proteins, and is important in determining cell survival/cell death. GSK-3 has also been identified as a target for the actions of lithium. GSK-3 can inhibit glycogen synthase, the enzyme that catalyzes the transfer of glucose from UDPG to glycogen.

MDM-2/p53



p53 Inhibitors
Pifithrin-α
Inhibitor of p53. Also an aryl hydrocarbon receptor agonist.
Pifithrin-μ
Inhibitor of binding of p53 to mitochondria. Inhibits HSP70.

p53 Activators
RITA
Binds to p53, blocks p53-HDM2 interaction and activates p53.
PRIMA-1
Re-activator of mutant p53.
Tenovin-1
p53 activator, protecting p53 from MDM2-mediated degradation.

MDM2 Inhibitors
RG7388
MDM2 inhibitor, blocking the binding of p53-MDM2.
Nutlin 3a
MDM2 antagonist. Active enantiomer of Nutlin-3.
AMG 232
Orally bioavailable piperidinone inhibitor of MDM2.
RG7112
Orally bioavailable MDM2 antagonist.
Serdemetan
HDM2 antagonist.

p53 is at the centre of biological interactions that translates stress signals into cell cycle arrest or apoptosis. Upstream signaling to p53 increases its level and activates its function as a transcription factor in response to a wide variety of stresses, whereas downstream components execute the appropriate cellular response.

Cell Stress: p53 induction by acute DNA damage begins when DNA double-strand breaks trigger activation of ATM, a kinase that phosphorylates the CHK2 kinase, or when stalled or collapsed DNA replication forks recruit ATR, which phosphorylates CHK1. p53 is a substrate for both the ATM and ATR kinases, as well as for CHK1 and CHK2, which coordinately phosphorylate p53 to promote its stabilization. These phosphorylation events are important for p53 stabilization, as some of the modifications disrupt the interaction between p53 and its negative regulators MDM2 and MDM4. MDM2 and MDM4 bind to the transcriptional activation domains of p53, thereby inhibiting p53 transactivation function, and MDM2 has additional activity as an E3 ubiquitin ligase that causes proteasome-mediated degradation of p53. Phosphorylation also allows the interaction of p53 with transcriptional cofactors, which is ultimately important for activation of target genes and for responses such as cell cycle arrest, DNA repair, apoptosis and senescence. Non-receptor tyrosine kinase c-Abl can also be activated by DNA damage. Then the JNK/p38 is activated and leads to p53 activation.

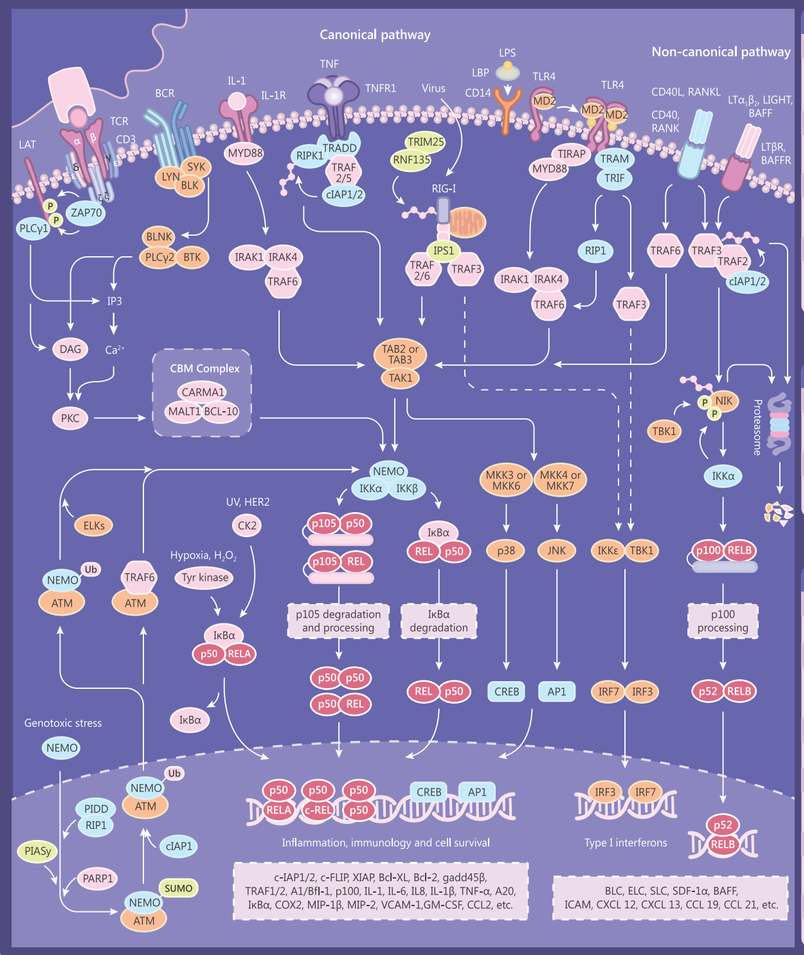
Oncogenic signaling: The response to oncogene activation depends on the binding of ARF to MDM2. ARF is normally expressed at low levels in cells. Inappropriately increased E2F or Myc signals, stemming from oncogene activation, leads to the increased expression of ARF, which inhibits MDM2 by blocking its E3 ubiquitin ligase activity, uncoupling the p53-MDM2 interaction, thereby segregating it from nucleoplasmic p53.

The PI3K-Akt pathway activates MDM2 and increases the ubiquitination of p53.

MedChemExpress Signaling Pathways --- Part Ib

MCE MedChemExpress

NF-κB



NF-κB Related Inhibitors
SN50
Cell permeable inhibitory peptide of NF-κB nuclear translocation .
PDTC
Inhibits NF-κB, and suppresses IL-1β-induced NOS expression.
QNZ
Inhibits NF-κB activation and TNF-α production.
JSH-23
Inhibits nuclear translocation of NF-κB without affecting IκB degradation.

IκB Related Inhibitors
BAY 11-7082
Inhibits TNF-α-induced IκBα phosphorylation irreversibly.
BAY 11-7085
Inhibits TNF-α-induced IκBα phosphorylation irreversibly.

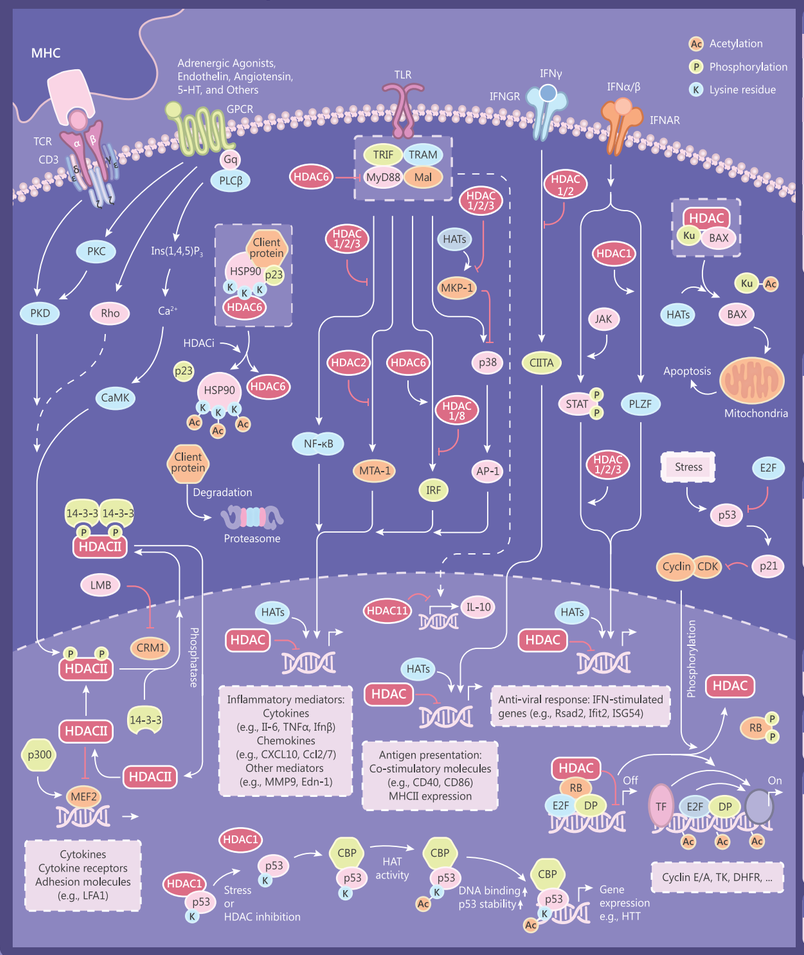
IKK Related Inhibitors
Amlexanox
Inhibitor of TBK1 and IKKε.
ACHP
Selective inhibitor for IKKβ and IKKα over NEMO, inhibiting NF-κB DNA binding activity.
TPCA-1
Selective inhibitor of IKKβ.
BMS-345541
Selective allosteric-site binding inhibitor of IKKβ and IKKα.
MLN120B
Novel, ATP competitive, and selective IKKβ inhibitor.
BI605906
Selective inhibitor of IKKβ.

NF-κB transcription factors are critical regulators of immunity, stress responses, apoptosis and differentiation. In mammals, there are five members of the transcription factor NF-κB family: RELA (p65), RELB and c-REL, and the precursor proteins NF-κB1 (p105) and NF-κB2 (p100), which are processed into p50 and p52, respectively. NF-κB transcription factors bind as dimers to κB sites in promoters and enhancers of a variety of genes and induce or repress transcription. NF-κB activation occurs via two major signaling pathways: the **canonical** and the **non-canonical** NF-κB signaling pathways.

The **canonical NF-κB pathway** is triggered by signals from a large variety of immune receptors, such as TNFR, TLR, and IL-1R, which activate TAK1. TAK1 then activates IκB kinase (IKK) complex, composed of catalytic (IKKα and IKKβ) and regulatory (NEMO) subunits, via phosphorylation of IKKβ. Upon stimulation, the IKK complex, largely through IKKβ, phosphorylates members of the inhibitor of κB (IκB) family, such as IκBα and the IκB-like molecule p105, which sequester NF-κB members in the cytoplasm. Iκ Bα associates with dimers of p50 and members of the REL family (RELA or c-REL), whereas p105 associates with p50 or REL (RELA or c-REL). Upon phosphorylation by IKK, IκBα and p105 are degraded in the proteasome, resulting in the nuclear translocation of canonical NF-κB family members, which bind to specific DNA elements, in the form of various dimeric complexes, including RELA-p50, c-REL-p50, and p50-p50. Atypical, IKK-independent pathways of NF-κB induction also provide mechanisms to integrate parallel signaling pathways to increase NF-κB activity, such as hypoxia, UV and genotoxic stress.

The **non-canonical NF-κB pathway** is induced by certain TNF superfamily members, such as CD40L, BAFF and lymphotoxin-β (LT-β), which stimulates the recruitment of TRAF2, TRAF3, cIAP1/2 to the receptor complex. Activated cIAP mediates K48 ubiquitylation and proteasomal degradation of TRAF3, resulting in stabilization and accumulation of the NFκB-inducing kinase (NIK). NIK phosphorylates and activates IKKα, which in turn phosphorylates p100, triggering p100 processing, and leading to the generation of p52 and the nuclear translocation of p52 and RELB.

HDAC (Histone deacetylase)



HDAC Pan Inhibitors
Trichostatin A (TSA)
Reversible inhibitor of class I and II mammalian HDACs.
Vorinostat (SAHA)
Orally bioavailable inhibitor of class I, II and IV HDACs.
Panobinostat
Oral pan-HDAC inhibitor.
Mocetinostat
Orally bioavailable inhibitor, targeting class I & IV HDAC.

Class I HDAC Inhibitors	
Entinostat	HY-12163
Romidepsin	HY-15149
CI-994	HY-50934
Valproic acid	HY-10585

Class IIa HDAC Inhibitors	
TMP195	HY-18361
TMP269	HY-18360
MC1568	HY-16914

HDAC3 Selective Inhibitor	
RGFP966	HY-13909

HDAC4 Allosteric Modulator	
Tasquinimod	HY-10528

HDAC6 Selective Inhibitors	
ACY-1215	HY-16026
Tubacin	HY-13428
Tubastatin A	HY-13271
CAY10603	HY-18613

HDAC8 Selective Inhibitor	
PCI-34051	HY-15224

TCR, GPCR and HDAC II interaction: Diverse agonists act through G-protein-coupled receptors (GPCRs) to activate the PKC-PKD axis, CaMK, Rho, or MHC binding to antigens stimulates TCR to activate PKD, leading to phosphorylation of class II HDACs. Phospho-HDACs dissociate from MEF2, bind 14-3-3, and are exported to the cytoplasm through a CRM1-dependent mechanism. CRM1 is inhibited by leptomycin B (LMB). Release of MEF2 from class II HDACs allows p300 to dock on MEF2 and stimulate gene expression. Dephosphorylation of class II HDACs in the cytoplasm enables reentry into the nucleus.

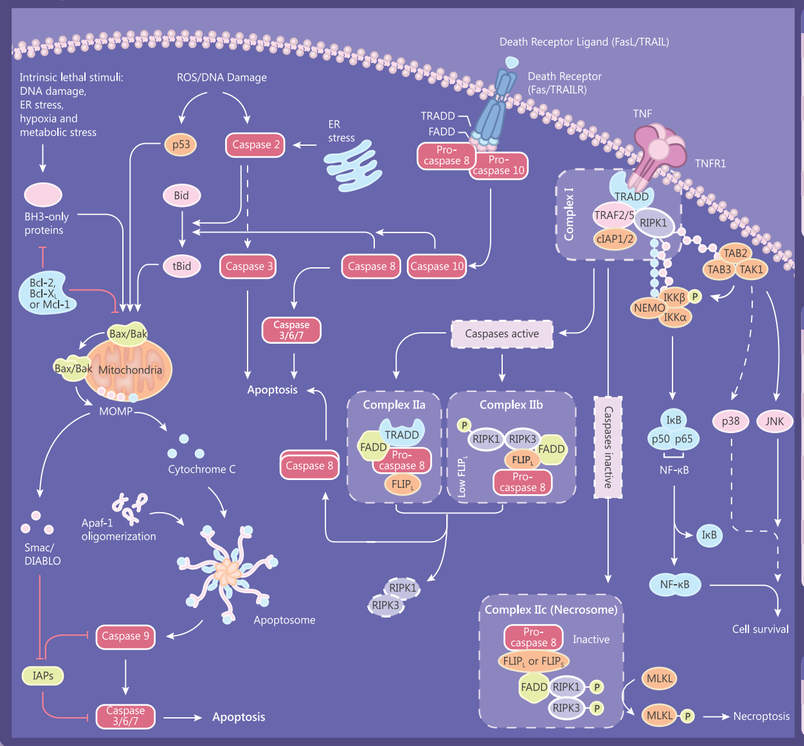
TLR: TLR signaling is initiated by ligand binding to receptors. The recruitment of TLR domain-containing adaptor protein MyD88 is repressed by HDAC6, whereas NF-κB and MTA-1 can be negatively regulated by HDAC1/2/3 and HDAC2, respectively. Acetylation by HATs enhance MKP-1 which inhibits p38-mediated inflammatory responses, while HDAC1/2/3 inhibits MKP-1 activity. HDAC1 and HDAC8 repress, whereas HDAC6 promotes, IRF function in response to viral challenge. HDAC11 inhibits IL-10 expression and HDAC1 and HDAC2 represses IFNγ-dependent activation of the CIITA transcription factor, thus affecting antigen presentation.

IRNAR: IFN-α/β induce activation of the type I IFN receptor and then bring the receptor-associated JAKs into proximity. JAK adds phosphates to the receptor, STATs bind to the phosphates and then phosphorylated by JAKs to form a dimer, leading to nuclear translocation and gene expression. HDACs positively regulate STATs and PZLF to promote antiviral responses and IFN-induced gene expression.

Cell cycle: In G1 phase, HDAC, Retinoblastoma protein (RB), E2F and polypeptide (DP) form a repressor complex. HDAC acts on surrounding chromatin, causing it to adopt a closed chromatin conformation, and transcription is repressed. Prior to the G1-S transition, phosphorylation of RB by CDKs dissociates the repressor complex. Transcription factors (TFs) gain access to their binding sites and, together with the now unmasked E2F activation domain. E2F is then free to activate transcription by contacting basal factors or by contacting histone acetyltransferases, such as CBP, that can alter chromatin structure.

The function of non-histone proteins is also regulated by HATs/HDACs. **p53:** HDAC1 impairs the function of p53. p53 is acetylated under conditions of stress or HDAC inhibition by its cofactor CREB binding protein (CBP) and the transcription of genes involved in differentiation is activated. **HSP90:** HSP90 is a chaperone that complexes with other chaperones, such as p23, to maintain correct conformational folding of its client proteins. HDAC6 deacetylates HSP90. Inhibition of HDAC6 would result in hyperacetylated HSP90, which would be unable to interact with its co-chaperones and properly lead to misfolded client proteins being targeted for degradation via the ubiquitin-proteasome system.

Caspase



Caspase Pan Inhibitors
Z-VAD(OMe)-FMK
Cell-permeable irreversible pan-Caspase peptide inhibitor.
Q-VD-OPh
Brain and cell permeable, irreversible pan-Caspase peptide inhibitor.
BOC-D-FMK
Cell-permeable irreversible pan-Caspase inhibitor.

Isoform Selective Inhibitors
VX-765
Orally bioavailable Caspase-1 inhibitor.
Z-DEVD-FMK
Cell-permeable irreversible inhibitor of Caspase-3.
Z-IETD-FMK
Cell-permeable Caspase-8 inhibitor.
Ac-DEVD-CHO
Inhibits Caspase-3 reversibly, less potent to other caspases.

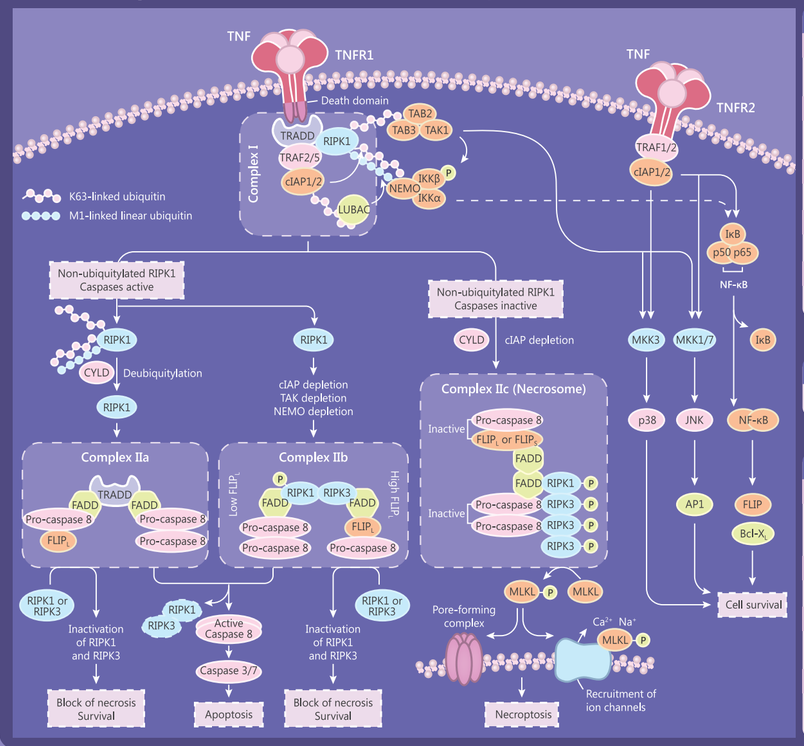
Procaspase Activator
PAC-1
Procaspase-3 activator.

Upon binding to their cognate ligand, death receptors such as Fas and TRAILR can activate initiator Caspases (Pro-caspase 8 and Pro-caspase 10) through dimerization mediated by adaptor proteins such as FADD and TRADD. Active Caspase 8 and Caspase 10 then cleave and activate the effector Caspase 3, 6 and 7, leading to apoptosis. ROS/DNA damage and ER stress trigger Caspase 2 activation. Active Caspase 2 cleaves and activates Caspase 3 and initiates apoptosis directly.

Caspase 2, 8 and 10 can also cleave Bid, stimulate mitochondrial outer membrane permeabilization (MOMP) and initiate the intrinsic apoptotic pathway. Following MOMP, mitochondrial intermembrane space proteins such as Smac and Cytochrome C are released into the cytosol. Cytochrome C interacts with Apaf-1, triggering apoptosome assembly, which activates Caspase 9. Active Caspase 9, in turn, activates Caspase 3, 6 and 7, leading to apoptosis. Mitochondrial release of Smac facilitates apoptosis by blocking the inhibitor of apoptosis (IAP) proteins.

Following the binding of TNF to TNFR1, TNFR1 binds to TRADD, which recruits RIPK1, TRAF2/5 and cIAP1/2 to form TNFR1 signaling complex I. Formation of the complex IIa and complex IIb is initiated either by RIPK1 deubiquitylation mediated by CYLD or by RIPK1 non-ubiquitylation due to depletion of cIAPs. The Pro-caspase 8 homodimer in complex IIa and complex IIb generates active Caspase 8. This active Caspase 8 in the cytosol then carries out cleavage reactions to activate downstream executioner caspases and thus induce classical apoptosis.

TNF Receptor (Tumor necrosis factor receptor)



TNF-α Related Products
Lenalidomide
TNF-α inhibitor. Cereblon binder.
Thalidomide
TNF-α inhibitor. Cereblon binder.
Pomalidomide
TNF-α and Cereblon inhibitor.
TIC10
TRAIL inducer.
C 87
TNF-α inhibitor.

TNF-α Receptor Antagonist	
R-7050	HY-110203

RIP Kinase Inhibitors
Necrostatin-1
ATP-competitive, allosteric inhibitor of RIPK1, blocking necroptosis .
GSK'872
Selective RIPK3 inhibitor.
GSK2982772
Orally bioavailable, ATP-competitive, RIPK1 inhibitor.
RIPA-56
Highly selective inhibitor of RIPK1 .

Following the binding of TNF to TNF receptors, TNFR1 binds to TRADD, which recruits RIPK1, TRAF2/5 and cIAP1/2 to form TNFR1 signaling complex I; TNFR2 binds to TRAF1/2 directly to recruit cIAP1/2. Both cIAP1 and cIAP2 are E3 ubiquitin ligases that add K63 linked polyubiquitin chains to RIPK1. The ubiquitin ligase activity of the cIAPs is needed to recruit the LUBAC, which adds M1 linked linear polyubiquitin chains to RIPK1. K63 polyubiquitylated RIPK1 recruits TAB2, TAB3 and TAK1, which activate signaling mediated by JNK, p38 and the IκB kinase complex. The IKK complex then activates NF-κB signaling, which leads to the transcription of anti-apoptotic factors-such as FLIP and Bcl-X$_L$-that promote cell survival.

The formation of TNFR1 complex IIa and complex IIb depends on non-ubiquitylated RIPK1. For the formation of complex IIa, ubiquitylated RIPK1 in complex I is deubiquitylated by CYLD. This deubiquitylated RIPK1 dissociates from the membrane-bound complex and moves into the cytosol, where it interacts with TRADD, FADD, Pro-caspase 8 and FLIP, to form complex IIa. By contrast, complex IIb is formed when the RIPK1 in complex I is not ubiquitylated owing to conditions that have resulted in the depletion of cIAPs. This non-ubiquitylated RIPK1 dissociates from complex I, moves into the cytosol, and assembles with FADD, Pro-caspase 8, FLIP, and RIPK3 (but not TRADD) to form complex IIb. For either complex IIa or complex IIb to prevent necroptosis, both RIPK1 and RIPK3 must be inactivated by the cleavage activity of the Pro-caspase 8-FLIP, heterodimer or fully activated caspase 8. The Pro-caspase 8 homodimer generates active Caspase 8, which is released from complex IIa and complex IIb. This active Caspase 8 then activate downstream executioner caspases and thus induce classical apoptosis.

Formation of the complex IIc (necrosome) is initiated either by RIPK1 deubiquitylation mediated by CYLD or by RIPK1 non-ubiquitylation due to depletion of cIAPs, similar to complex IIa and complex IIb formation. RIPK1 recruits numerous RIPK3 molecules. Activated RIPK3 phosphorylates and recruits MLKL, eventually leading to necroptosis.