Therapeutic agents targeting apoptosis pathways with a focus on quinazolines as potent apoptotic inducers

Raghad M. Nowar[1], Essam Eldin A. Osman[2], Sahar M. Abou-Seri[2], Samir M. El Moghazy[2], Dalal A. Abou EL Ella[3]

[1]Department of Chemistry, Faculty of Pharmacy, Heliopolis University for Sustainable Development, 11777 Cairo, Egypt
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ain Shams University, 11566 Cairo, Egypt
raghad.nowar@gmail.com

Abstract: Intrinsic and extrinsic apoptotic mechanisms are significant targets for cancer treatments, Therapeutic agents target different pro-apoptotic proteins involved in the apoptotic process, since many of the proteins involved in apoptosis have redundant functions, selective blockers of these pathways may not be enough to induce apoptosis. Both apoptotic pathways converge to caspase-3, thus different caspases inducers also promotapoptosis. Novel heterocyclic compounds based on quinazoline scaffold are believed to be promising agents as potent caspases activators and Bcl2 inhibitors, which can be used for developing precise therapeutic agents and target-specific drugs that target apoptosis and/or anoikis in different fields.

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I. Introduction

Apoptosis, or programmed cell death, is a main mechanism by which cells die if DNA damage is not repaired.[1, 2]Apoptosis is also important in controlling proliferation. It is also necessary for the elimination of self-reactive lymphocytes.[3] Some types of cancers, such as B-cell chronic lymphocytic leukemia (CLL) and follicular lymphoma are characterized by apoptotic failings producing immortal copies of cells.[4] Other malignancies have defects in the apoptotic regulatory pathways such as p53, the nuclear factor kappa B (NFκB), or phosphatidylinositol 3-Kinase (PI3K)/Akt leading to apoptotic defects.[5] Current cancer therapies, mainly exert their antitumor effect by triggering apoptosis in cancer cells.[6]Apoptosis is characterized by typical morphological and biochemical indications, including cell shrinkage, nuclear DNA fragmentation and membrane blebbing.[6] The essential mechanisms for initiation of apoptosis upon cytotoxic therapy may depend on the individual stimulus and have not been identified. However, damage to DNA or to other critical molecules is considered as a common initial event which is then propagated by the cellular stress response.[6] Proteolytic enzymes such as caspases are keymolecules in apoptosis.[6]Activation of caspases in response to anticancer chemotherapy can be initiated through activation of the extrinsic pathway or at the mitochondria by stimulating the intrinsic pathway.[6] The antiapoptotic mechanisms regulating cell death have also been implicated in conferring drug resistance to tumor cells.[7]

However, the concept that apoptosis represents the major mechanism by which cancer cells are eliminated may not be generally applied, where caspase independent apoptosis or other modes of cell death are also to be considered as cellular response to anticancer therapy.[8]

This review will discuss the current knowledge about the apoptotic pathways and some treatments that target them, with broad review about quinazolines as synthetic activators of caspases, and their involvement in extrinsic and intrinsic apoptotic pathways.

II. Different pathways of apoptosis.

There are two major mechanisms of cell death, necrosis and apoptosis. Cells that are damaged by external injury undergo necrosis, while cells that are induced to commit programmed suicide because of internal or external stimuli undergo apoptosis. Although understanding of the detailed signaling pathways that trigger apoptosis is incomplete, this process is controlled by a number of complex proteins, which are activated by various triggers and arranged in sequential signaling modules. Apoptosis occurs through two main pathways. The first, referred to as the extrinsic or cytoplasmic pathway, is triggered through the Fas death receptor, a member of the tumor necrosis factor (TNF) receptor super family.[9]The

second pathway is the intrinsic or mitochondrial pathway that when stimulated leads to the release of cytochrome-c from the mitochondria and activation of the death signal.[10]Both pathways join to a final common pathway involving the activation of a cascade of proteases called caspasesthat cleave regulatory and structural molecules, leading to the cell death.[12] **(Figure 1)**. Over expression of Bcl-2 in the intrinsic pathway may lead to the inhibition of extrinsic mediated apoptosis.[11]

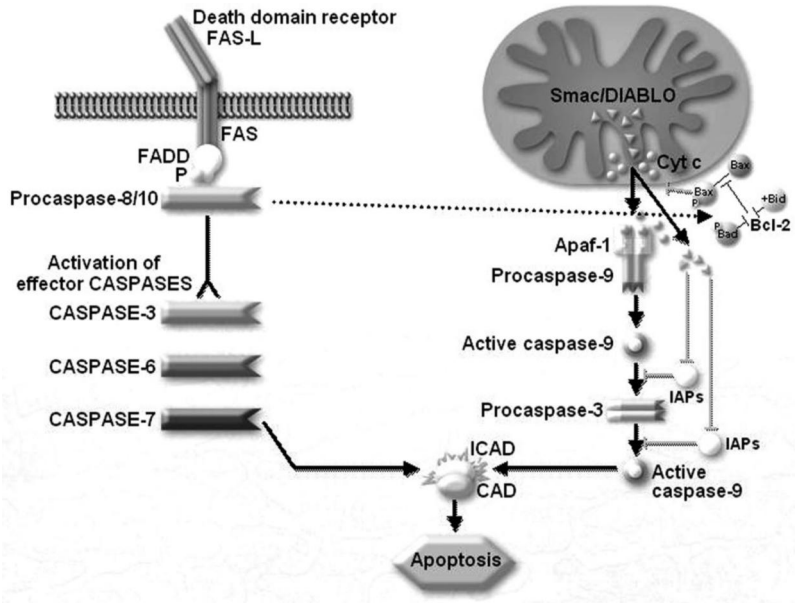


Figure 1. The extrinsic & intrinsic pathways of apoptosis.[12]

II.1. The Extrinsic Pathway: Fas

Fas is a member of the tumor necrosis factor receptor superfamily and is also called Apo-1 or CD95.[9] This pathway contains several protein members including the death receptors, the membrane-bound Fas ligand, the Fas complexes, the Fas-associated death domain, and caspases 8 and 10, which ultimately activate the rest of the downstream caspases leading to apoptosis.[7]**(Figure1)**. Activation of the extrinsic pathway is initiated with the ligation of cell surface receptors called death receptors (DRs).[9]

The Fas ligand (FasL)-Fas system is mainly recognized for its death-related functions, but it is also involved in several proliferative and inflammatory signaling pathways that are not well defined.[13] When a death stimulus triggers the pathway, the membrane-bound FasL interacts with the inactive Fas complexes and forms the death-inducing signaling complex.[15] The Fas death-inducing signaling complex contains the adaptor protein Fas-associated death domain protein and caspases 8 and 10 and leads to activation of caspase 8, which then activates the rest of the downstream caspases. In some cells, the activation of caspase 8 may be the only requirement to execute death, while in other cell types, caspase 8 interacts with the intrinsic apoptotic pathway by cleaving Bid (a proapoptotic member of the Bcl-2 family), leading to the subsequent release of cytochrome-c.[14]

Several pathways and proteins regulate the activation of the extrinsic pathway. Dys regulation of these modulators may also lead to malignant transformation, as mutations or deletions of the Fas gene have been found in some hematologic malignancies.[15, 16]

Other inhibitors of the pathway include FAP-1, Fas-associated-death-domain-protein like interleukin-1β-converting enzyme-like inhibitory protein, and the soluble decoy receptors such as TRAIL antagonize the stimulation of Fas by FasL though competition with the ligand.[17, 18]

II.2 The Intrinsic Pathway

One of the most important regulators of this pathway is the Bcl-2 family of proteins. The *bcl-2* gene was originally identified at the chromosomal breakpoint of the translocation of chromosome 18 to 14 in follicular NHL (non-Hodgkin lymphoma).[19]

The Bcl-2 family are key regulators of apoptosis and are overexpressed in many malignancies even without the presence of the (14; 18) chromosomal translocations. Increased expression of Bcl-2 causes resistance to chemotherapeutic drugs and radiation therapy, while decreasing Bcl-2 expression may promote apoptotic responses to anticancer drugs. Also over-expressing Bcl-2 may result in accumulation of cells in the G_0 phase of cell cycle division and contribute to chemoresistance.[20]

The Bcl-2 family includes proapoptotic members such as Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim, and Hrk, and antiapoptotic members such Bcl-2, Bcl-X_L, Bcl-W, Bfl-1, and Mcl-1.[21] Antiapoptotic Bcl-2 members act as repressors of apoptosis through blockage of the release of cytochrome-c, where the proapoptotic members act as promoters, giving that these effects are more dependent on the balance between Bcl-2 and Bax than on Bcl-2 quantity alone.[22]

Following a death signal, proapoptotic proteins undergo posttranslational modifications that include dephosphorylation and cleavage resulting in their activation and translocation to the mitochondria leading to apoptosis.[23]In response to apoptotic stimuli, the outer mitochondrial membrane becomes permeable, leading to the release of cytochrome-c and second mitochondria-derived activator of caspase (also called direct IAP-binding protein). Cytochrome-c, once released in the cytosol, interacts with Apaf-1 (Apoptotic protease activating factor-1), leading to the activation of caspase-9 proenzymes. Active caspase-9 then activates caspase-3, which subsequently activates the rest of the caspase cascade and leads to apoptosis. Activated caspases lead to the cleavage of nuclear lamins and breakdown of the nucleus through caspase-3.[22]

II.3. The Final Pathway: Caspases

The final pathway that leads to execution of the death signal is the activation of a series of proteases termed caspases.[12] Not all caspases are involved in apoptosis. The caspases that have been well described are caspases-3, -6, -7, -8, and-9 where the intrinsic and extrinsic apoptotic pathways converge to caspase-3, which cleaves the inhibitor of the caspase-activated deoxyribonuclease, and the caspase-activated deoxyribonuclease becomes active leading to nuclear apoptosis.[24] The upstream caspases that converge to caspase-3 are caspase-9 in the intrinsic pathway and caspase-8 in the extrinsic pathway.[25] Caspases also affect cytoskeletal structure, cell cycle regulation, and signaling pathways, ultimately leading to the morphologic manifestations of apoptosis, such as DNA condensation and fragmentation.[12]

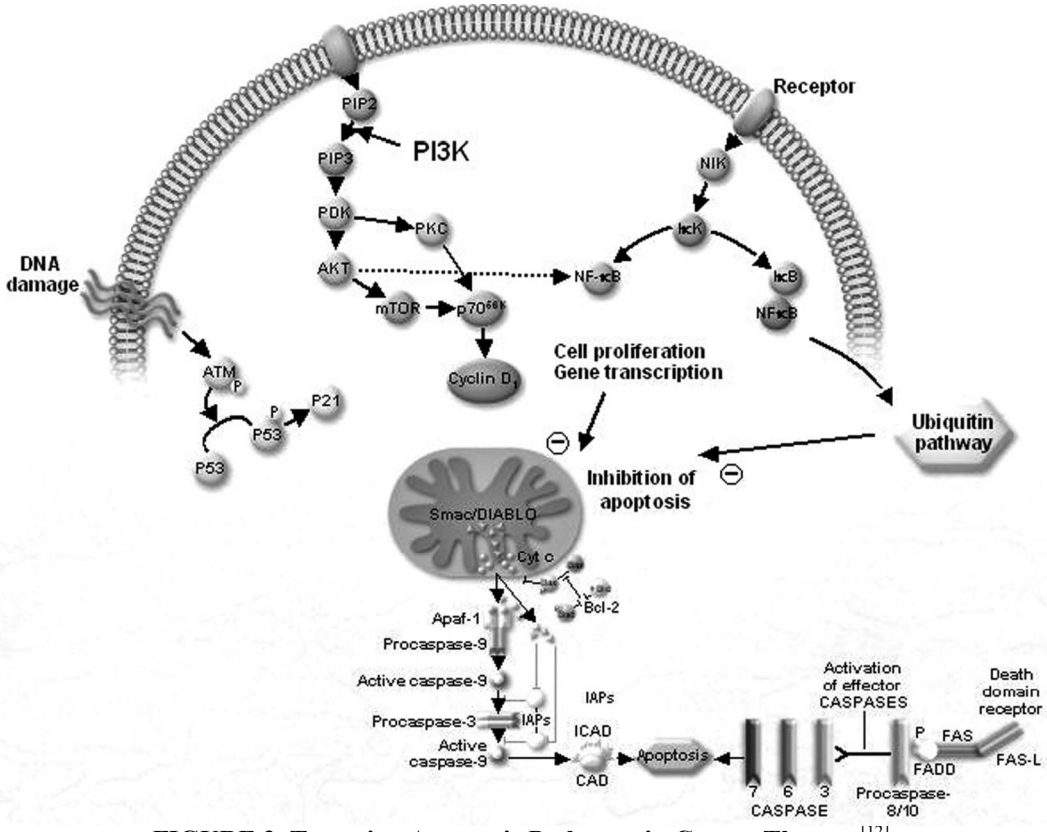


FIGURE 2. Targeting Apoptosis Pathways in Cancer Therapy[12]

III. Regulation of proteins involved in apoptosis

The extrinsic and intrinsic apoptotic pathways are regulated by proteins such as the p53, NFκB, the ubiquitin proteosome system, and the PI3K pathway **(Figure 2)**. These will be briefly described as they are relevant to the treatments discussed in this review.

III.1. p53

p53 functions as a transcription factor regulating downstream genes important in cell cycle arrest, DNA repair, and apoptosis. The mechanism by which p53 promotes apoptosis is still not fully understood.[12] The critical role that p53 plays is evident by the large number of tumors that bear a mutation in this gene.[1]Loss of p53 in many cancers leads to genomic instability, impaired cell cycle regulation, and inhibition of apoptosis, where after the DNA is damaged, p53 holds the cell at a checkpoint until the damage is repaired.[26] If the damage is irreversible, apoptosis is triggered. [27]

III.2. NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells)

NFκB is a nuclear transcription factor that regulates expression of a large number of genes involved in the regulation of apoptosis, viral replication, tumorigenesis, inflammation, and many autoimmune diseases.[28]

It is activated by different stimuli that cause phosphorylation of IκB, which is followed by its degradation, which results in translocation of the molecule to the nucleus where it binds with the consensus sequence of various genes and thus activates their transcription.[28]It has been shown to have both anti- and proapoptotic functions that may be determined by the nature of the death stimulus rather than by the origin of the tissue.[29]

III.4. The Ubiquitin/Proteosome System

The ubiquitin/proteosome system is composed of a large proteinase complex that is responsible for the turnover of most intracellular proteins and consequently regulates cell growth and apoptosis.[30] In addition, many of the Bcl-2 family members are substrates of the ubiquitin/proteosome. [31]

The induction of apoptosis by proteosome inhibitors leads to an initial accumulation of proteins such as p53, p27, proapoptotic Bad or Bax, or activation of the stress kinase, which leads to the release of cytochrome-c and the activation of the intrinsic apoptosis pathway.

III.5. PI3K (phosphoinositide-3-kinase)

PI3K is a kinase that plays a central role in signaling pathways important to cell survival, proliferation, motility, and tissue neovascularization. PI3K is upregulated in many cancers.[32] Phosphatidylinositol 4,5-bisphosphate 3 signals activate the kinase 3-phosphoinositide-dependent protein kinase-1, which in turn leads to phosphorylation of certain proteins that lead to cell survival.[33]

IV. Therapeutic Agents

This section is discussing some treatments considered as apoptotic agents, and are view about quinazolines, and their activity as synthetic inducers of caspases. The regulation of apoptotic proteins and their pathways was simply described.

Many of the proteins involved in apoptosis have redundant functions, and many pathways include signals that are involved in both the extrinsic and intrinsic pathways. Thus, selective blockers of these pathways may not be enough to induce apoptosis.[12]

IV.1. Agents That Target the Extrinsic Pathway

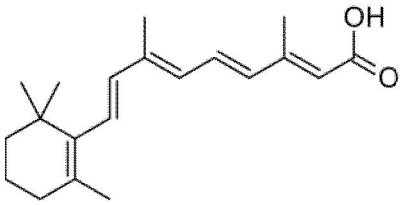
These include recombinant human TRAIL, monoclonal antibodies agonistic to Dr4 and Dr5, and all trans retinoic acid (ATRA).

IV.1.1 TRAIL (TNF-related apoptosis-inducing ligand)

TRAIL is a cytokine that is produced and secreted by most normal tissue cells. It causes apoptosis primarily in tumor cells. IT has been introduced as an extrinsic pathway inducer that does not have the toxicities of Fas and TNF, it induces apoptosis in a variety of tumor cell types and suppresses the growth of colon and breast xenografts..[12] Monoclonal antibodies with agonist function at the Dr4 (TRAIL R1) and Dr5 (TRAIL R2) receptor sites may also induce apoptosis and caspase activation and have shown tumor regression in colon tumor xenograft models.[36] The antibodies are being studied as single agents and in combination with cytotoxic chemotherapy.[12]

IV.1.2. ATRA (Tretinoin)

It is a liposomal form of tretinoin that is administered intravenously. ATRA induces differentiation in acute promyelocytic leukemia(APL) by modulation of the PML-RARα protein(acute promyelocytic leukemia-specific fusion protein).[34] It promotes apoptosis through the extrinsic pathway by activating the paracrine release of membrane-associated TRAIL, which leads to apoptosis in ATRA-treated APL cells and in adjacent non-ATRA responsive and non-APL cells.[35]



Tertinoin

IV.2. Agents That Target the Intrinsic Pathway

These include the agents that act directly on the mitochondrial inner membrane, agents that antagonize the antiapoptotic members of the Bcl-2 protein family, and agents that complements the activity of the proapoptotic members of the Bcl-2 family of proteins such as Bax.[36]

IV.2.1. Agents That Target the Mitochondrial Inner Membrane

IV.2.1.1 Arsenic Trioxide

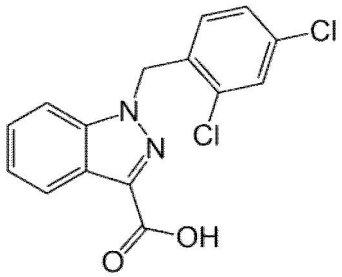
It is FDA approved for the treatment of APL (Acute promyelocytic leukemia).[37]

APLis characterized by a chromosomal translocation involving the retinoic acid receptor alpha (*RARα* or *RARA*) gene and is distinguished from other forms of AML (Acute myeloid leukemia) by its responsiveness to all-*trans* retinoic acid (ATRA; also known as tretinoin) therapy.[38]

Arsenic trioxide targets the PML-RARα protein which At higher concentrations, it promotes apoptosis of the leukemic cells by interrupting the mitochondrial inner membrane potential, which inhibits Bcl-2 expression and elevation of caspase-3.[39]

IV.2.1.2. Lonidamine

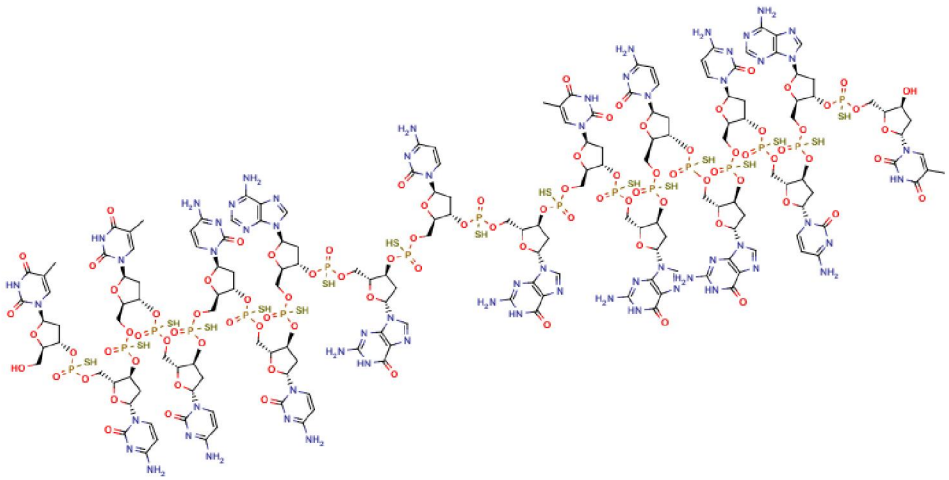
It is a derivative of indazole-3-carboxylic acid that acts on the mitochondria to induce apoptosis through the disruption of the intrinsic transmembrane potential.[12]



Lonidamine

IV.2.2. Agents That Target Bcl-2

This antiapoptotic protein is very important clinically. Modulation of its expression is of great interest. Agents that can affect the Bcl-2 protein include antisense oligonucleotides such as G3, 139 (oblimersen sodium), small molecules that recognize the surface pocket of Bcl-2 or Bcl-x_L, and antisense Bcl-x_L which is in preclinical development. The effect of G3,139 is dose dependent and leads to degradation of *bcl-2* mRNA and thus inhibition of Bcl-2 protein expression.[40]



G3,139 (oblimersen sodium)

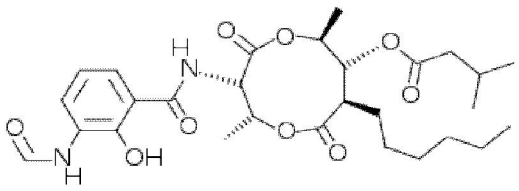
IV.2.2.1 Antisense BCl-xL and Antisense Clusterin

Bcl-xLhas large sequence homology areas with Bcl-2 but has a different biological role than that of Bcl-2. These two proteins can be coexpressed in many tumors.[41]

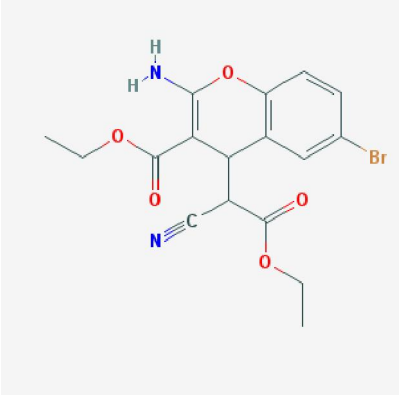
The inhibition of cluster in by sequence-specific antisense oligonucleotides has been showed to enhance chemotherapy effects in hormone refractory prostate cancer in vitro and in vivo.[12]

IV.2.2.2. Small Molecules That Recognize the Surface Pocket of Bcl-2 or Bcl-x_L

There are natural products and synthetic organic peptides that identifies the surface pocket of Bcl-2 and Bcl-X_L. The natural products such as Antimycin-A.[42] They are important templates for the development of Bcl-2 inhibitors or other proteins in the intrinsic pathway. [22] For example, Bcl-2 inhibitor, HA14-1, which binds to a surface pocket of the Bcl-2 protein, induces apoptosis including cells morphological changes.[12]



Antimycin-A



Bcl-2 inhibitor: HA14-1

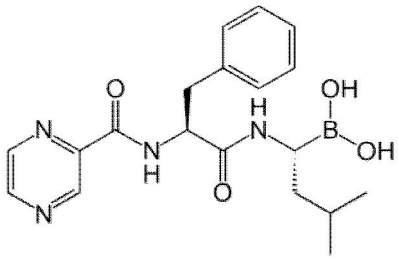
IV.2.3. Agents Modulating the Proapoptotic Proteins Bax and Bcl-xs

The activation of the proapoptotic Bax protein can be activated by gene therapy through the delivery of Bax vectors, and this approach has been successful in inducing apoptosis in cancer cell lines. The use of Bcl-xs gene therapy can be promising in tumor regression induction.[43]

IV.3. Agents targeting the Modulators of the Apoptotic Pathways

These include proteosome inhibitors, mTOR inhibitors, and p53 inhibitors.

IV.3.1. Proteosome Inhibitors



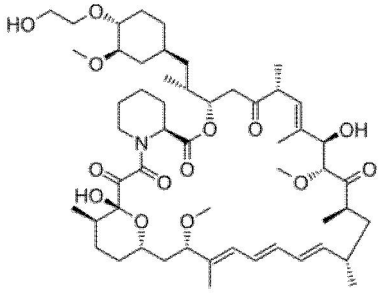
Bortezomib

Proteosome inhibitors showed to promote apoptosis in cell lines in vitro, even in cell lines that are resistant to chemo-therapy, they also sensitize cells to apoptosis, inhibit angiogenesis and metastases in vivo.[12] The effects of these agents may be selective to cancer cells because they induce apoptosis in transformed cells or those with high proliferation rate.[44]

IV.3.2. mTOR Inhibitors (Mammalian target of rapamycin)

It is a serine/threonine kinase, which belong to phosphatidylinositol-3 kinase (PI3K) related kinases (PIKKs) family.

Tumors that depend on the activation of the PI3K/Akt pathway may be more prone to the effect of this targeted therapy.[12] Rapamycin is a natural product that has antimicrobial, immunosuppressive, and anticancer properties.[45] RAD001 (everolimus) is another rapamycin analog that is available in orally.[12]



Everolimus(RAD001)

IV.3.3. P53 Inhibitors

Gene therapy is one of the trials to target p53, such as **ONYX-015and INGN201** which are adenoviruses modified selectively to replicate in and kill cells that harbor p53 mutations.[46] As well as antisense therapy to the protein that controls p53 activity as**MDM2,**[12]which are identified as target genes of p53 that has the ability to bind and suppress its activity.[47]

IV.4. Agents targeting Common Pathway: Caspases Activators

These include synthetic activators of caspases, Apoptin, and IAP(Inhibitors of apoptosis proteins) targets such as survivin.

IV.4.1. Apoptin

It is a caspase-inducing agent derived from chicken anemia virus that can result in selective apoptosis in malignant cells and not in normal cells. This selectivity may be due to the nuclear localization of the protein in tumor cells that is necessary for its activation.[12]

IV.4.2. Targeting IAP (Inhibitors of apoptosis proteins)**, Survivin**

Survivin is a member of the IAP family that plays a dual role in suppressing apoptosis and regulating cell division.[48]

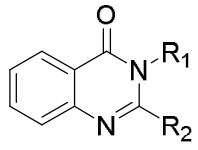
IV.5. Quinazolines as activators of caspases.

Caspase-activating agents, also named death switches, can be produced by the fusion of one or more chemically inducible dimerization domains. These drugs lead to protein aggregation inside the cell that induces caspase activation and downstream apoptosis. The activation of caspases 1 and 3 may be sufficient to induce death in the cells.[12] One of these agents are novel heterocyclic compounds based on quinazoline scaffold. They are a class of fused heterocycles that are of noticeable interest because of their several biological properties.

The use of combinatorial synthesis, microwave-enhanced processes and new catalytic methodologies in the preparation of 4(3H)-Quinazolinones**(1)** and related quinazolines**(2)** is a clear indication that

significant progress has been made in recent years.[49, 50]

Connolly et al[51]compiled more than one hundred scheme for preparation of quinazolines and different derivatives in their vast, diverse review about quinazolines and quinazolinones synthesis. Many of the synthetic methods of this simple ring structure are time consuming, exhausting and often low yielding.[51]



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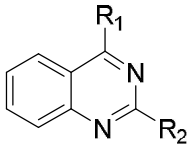
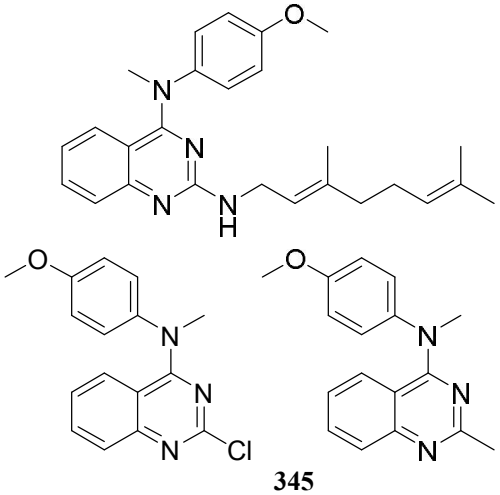


2

The mechanism of apoptosis involves a cascade of initiator and effector caspases that are activated sequentially. Caspase-3 is one of the key effector caspases that cleaves multiple protein substrates in cells, leading to cell death. A lot of clinically useful cytotoxic agents was found to promote apoptosis in cancer cells. For example, the proapoptotic chemotherapeutic agents targeting tubulin, including taxanesas Taxotere and Taxol. Also vinca alkaloids such as vinblastine, vincristine, are considered of the most successful anticancer therapies.[2]

Recently, It was reported the discovery of N4-(4-methoxyphenyl)-N4-methyl-N2-((E)-3,7-dimethylocta-2,6-dienyl)quinazoline-2,4-diamine **(3)** as a potent apoptosis inducer using cell- and caspase-based HTS assay, and SAR studies which led to the identification of 2-chloro-N-(4- methoxyphenyl)-N-methylquinazolin-4-amine **(4)**,[52] and N-(4-methoxyphenyl)-N,2-dimethylquinazolin-4-amine **(5)**,[52] as potent apoptosis inducers with activity in multi-drug-resistant cancer cell lines, high BBB penetration, good pharmacokinetics and high efficacies in vivo anticancer xenograft models.[52]



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Mark and co-workers claim the synthesis of a series of 4-arylamino-quinazolines as caspase activators and apoptosis promotors in solid tumor cells. In addition, these compounds are also found to be potent inhibitors of tubulin and topoisomerase-II. One of the compounds2-chloro-N-(4-methoxyphenyl)-N-methylquinazolin-4-amine(4) and its analogs have been tested against MCF-7, NCI/ADRRES,P388 and P2388/ADR cancer cell lines (IC50 = 1.12 --2.9 nM) and identified as potent caspase cascade activators and inducers of apoptosis.[53]

Terazosin and doxazosin

These two quinazoline-based drugs have shown to facilitate anoikis in prostate cells by death receptor mediated mechanisms involving death-inducing signaling complex formation/caspase-8 activation and inhibition of Akt survival signaling, consequential to the disruption of cell attachment to the extracellular matrix via targeting integrins.[54]

They have also been shown to α1-independently down regulate the vascular endothelial growth factor in prostate tissue, thus impairing angiogenesis in malignant prostate tumors.[55]

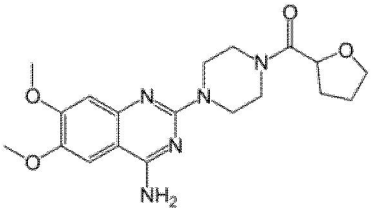
In another studies, Cubedo, et al, studied symmetrical derivatives with anticancer activity, and searched for new compounds able to induce a selective proapoptotic mechanism in cancer cells. The potential antitumor activity of several quinazoline derivatives was evaluated in vitro against human breast, colon and bladder cancer cell lines.

JRF12 (2,4-dibenzylaminoquinazoline) **(6)**was chosen as the best candidate and its mechanism of action was extensively studied. A time dependent evaluation of apoptosis was performed in the three cancer cell lines, followed by an evaluation of the cell cycle regulation involvement that showed a decrease of cells in G1 phase and increase of cells in G2 phase before cell death, concluding that 2,4-dibenzylaminoquinazoline is a promising anticancer drug showing cytostatic and apoptotic effects mainly in a transcription independent manner.[56]

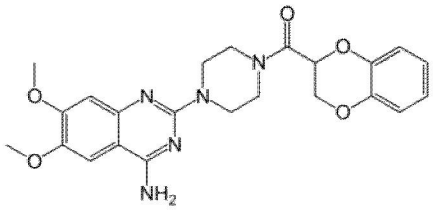
Data indicate that 2,4-dibenzylaminoquinazoline induced cell cycle arrest in G2 and apoptosis may result from the activation of the same signal transduction pathways. For further understanding of the mechanism by which JRF12 promotes the apoptotic pathway, the effect of caspase inhibition was evaluated. In all the cell lines tested the caspase inhibitors partially inhibit cell death. Caspase-8 inhibition was greater than caspase-9 indicating that JRF12 cell death is mainly activated by caspase-8.[56]

In the apoptotic cascade, caspase-8 is the most upstream enzyme mediating death receptor apoptosis pathways, and its activation propagates the apoptotic signal by activating executioner caspases such as caspase-3. Caspase-8, in addition to activation of

caspase-3, has the ability to cleave BH3 interacting domain death agonist (Bid).[57]

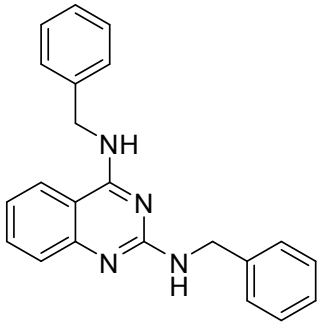


Terazosin



Doxazosin

The truncated Bid interacts with the proapoptotic protein Bax, inducing a conformational change of Bax that promotes the release of cytochrome c from mitochondria to cytosol, therefore activating caspase-9, which activates executioner caspases.[56]



6: The structural formula of JRF12 (2,4-dibenzylaminoquinazoline)[56]

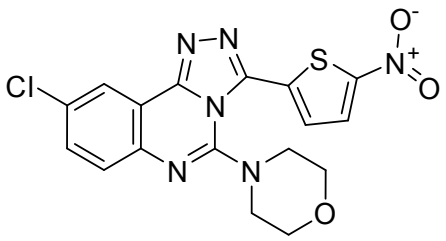
Caspase-3 is believed to act as a general mediator and is early activated during apoptosis. It is considered as a very important executioner in apoptosis because of its ability to cleave a vast array of proteins.

Cubedo, et al. used a colorimetric enzymatic assay to measure changes of caspase-3 activity between extracts prepared from control and JRF12 treated cells. Cells were treated with their corresponding IC50 at different times. JRF12 stimulates caspase-3 like activity in the three cell lines. In a time course study, at the IC50 dose of JRF12 caspase-3 activity reached a peak after 18 h of treatment in T24, while in HT29 this peak appeared at 24 h and after 10 hours in MDA-MB-231, which correlated with the inmunofluorescence results. The above results indicate that caspase-3- like proteases were activated in response to treatment with JRF12. To confirm that the obtained signal was due to caspase-3-like protease activity, caspase-3 inhibition by DEVD-CHO was evaluated showing a complete decrease of activation.[56]

The data reported here also demonstrate that 2,4-dibenzylaminoquinazoline is able to induce an apoptotic response with caspase-3 activation and DNA degradation in at least two cell lines.

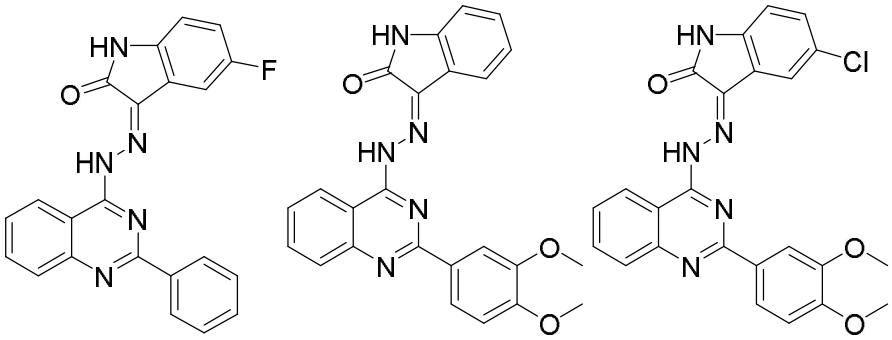
In another approach concerning the effect of 3-(5-nitro-2-thienyl)-9-chloro-5-morpholin-4-yl[1,2,4]triazolo[4,3-c]quinazoline**(7)** on cell growth, cell cycle, induction of DNA fragmentation, and activity of caspase 3 in murine leukemia L1210 cells and fibroblast NIH-3T3 cells, Jantova et al. Monitored the cytotoxic effect of 3-(5-nitro-2-thienyl)-9-chloro-5-morpholin-4-yl[1, 2,4]triazolo [4,3-c]quinazoline in two more cell lines, i.e., murine leukemia L1210 cells and murine fibroblast NIH-3T3 cells.[58] Caspase 3 activity assay which showed that the addition of quinazoline to the medium reduced L1210 and 3T3 viable cell number. The cell cycle analysis and the agarose gel electrophoresis showed that a quinazoline concentration of 12 mM induced apoptotic cell death of L1210 cells. Quinazoline-treated cells had altered cell morphology, had undamaged cytoplasmic membrane.[58]

In another study, using a molecular hybridization approach, a new series of is atin-quinazoline hybrids was designed and synthesized, and their anticancer activity was evaluated in vitro against the liver HepG2, breast MCF-7 and colon HT-29 cancer cell line.



7

A distinctive selective growth inhibitory effect was observed towards the HepG2 cancer cell line. Compounds **(8)**, **(9)** and **(10)** displayed the highest potency, with IC50 values ranging from 1.0 (±) 0.2 to 2.4 (±) 0.4mM, and they were able to induce apoptosis in HepG2 cells, as evidenced by enhanced expression of the pro-apoptotic protein Bax and reduced expression of the anti-apoptotic protein Bcl-2, in addition to increased caspase-3 levels.[59]



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Many studies have investigated the various biological activities of quinazoline derivatives, and their cytotoxic effects.

Bilbro et al. related their anticancer effects to both induction of classical apoptosis and reversal of anoikis resistance. Recent drug optimization efforts have generated several novel compounds with quinazoline-derived chemical structure that exert potent anti-tumor activity via anoikis.[60]

Conclusion

In this review we have compiled and discussed apoptosis and different apoptotic mechanisms that are targeted for cancer treatment, as well as detailed discussion about quinazolines and their involvement in apoptosis as caspases activators.

This could provide a guide for medicinal chemists forprecise and target-specific information for development of quinazoline-based drugs that target apoptosis and/or anoikis.

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