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Biology and Medicinal Chemistry Approaches Towards Various Apoptosis Inducers

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Abstract: Apoptosis is a genetically in-built process whereby organisms remove unwanted cells. Apoptosis can serve as a regulatory and defense mechanism in the formation of the shape and size of the human body and also to eradicate surplus amount of cells. The regulation of apoptosis is relevant and differentiates between a normal cells of body and cancer cells by loss of control. Apoptosis being an intricate process regulated by much more than just a biological mechanism. The induction of the apoptosis manifests the control on the tumour size and number of tumour cells hence establishing the application of apoptotic inducers as vital components in the treatment of cancer. During apoptosis, cells die in a controlled and regulated fashion which makes apoptosis distinct from necrosis (uncontrolled cell death). Protein components and regulators for apoptosis signaling pathways can involve the mitochondria (intrinsic pathway) or signal through death receptors (extrinsic pathway). Many different drug and gene therapy approaches are being tested for initiating apoptosis. Resistance to apoptosis is considered a hallmark of cancer. Therapeutic approaches attempted to date include traditional small molecules, antisense oligonucleotides, monoclonal antibodies, recombinant proteins and several classes of chemical compounds discussed in this review. These compounds may serve as precursor molecules for more effective drugs, all aimed at developing clinically effective therapeutics, targeting key apoptosis regulatory mechanism. This review will discuss the current understanding of apoptosis induced by various chemical agents and highlighting the role of apoptosis inducing agents as emerging opportunities for cancer therapy.

Keywords: Apoptosis, DNA damage, Caspase, Apoptosis inducers, Anticancer agents.

INTRODUCTION

Apoptosis is a programmed cell death in the human body which occurs in response to certain stimuli. The term itself is derived from Greek and refers to the "dropping of the leaves from a tree" [1]. Apoptosis is a natural phenomenon in which cells commit "cellular suicide" only to be replaced by new cells. Apoptosis, being genetically encoded in each cell, serves as a self-regulatory mechanism to prevent abnormal proliferation and maintain tissue homeostasis in the human body. It has specific characteristics such as: cell size shrinkage, membrane blebbing, nuclear fragmentation and chromatin condensation [2]. Apoptosis occurs during embryonic development and tissue homeostasis, and it is induced by pathological signals [3]. Apoptosis occurs as a defense mechanism in immune reactions or when cells are damaged by disease. These are preparations for the suicidal death in which the entire cell is terminated into smaller particles which are then phagocytized by phagocytes [4]. A typical apoptosis can result in various pathological conditions ranging from stroke, liver failure and also including the prevalent crisis of cancer [5]. It is initiated by shifting the homeostatic balance between pre-apoptotic and anti-apoptotic factors [6]. This requires the over expression of pre-apoptotic factors which can be stimulated under stressful conditions for either regeneration or destruction of cells. It allows limb and digits separation in cells of fetus and also on the other side results in pathological conditions such as stroke or diabetes mellitus I [7]. Cellular suicide process also plays a role in generation of auto-immune diseases or neurodegenerative diseases. Alterations in the pathways of apoptosis can lead to deregulated growth of cells resulting in tumours. Thus, apoptosis is a beneficial technique along with chemotherapy in targeting the carcinogenic cells of tumour in order to reduce their size and number. The main advantage of apoptosis is that there are no destructive after-effects on the tissue or surrounding cells if the entire process is programmed in the cell [8]. Apoptosis can be initiated by either one of the two pathways: intrinsic (mitochondrial) pathway or extrinsic (death receptor) pathway. Mitochondrial pathway is instigated in accordance to DNA damage or withdrawal of cell survival factors whereas the death receptor pathway begins with death ligand binding to the death receptors. The initiation is followed by the activation of caspase cascade in which the family of caspase enzymes is activated [9-11]. Apoptosis is recognized to play a vital role in tumor biology; induction of apoptosis in tumour has significance for cancer therapy [12, 13]. It is evident that most of the anticancer agents are apoptosis inducer. During the past decade many compounds were identified as apoptosis inducer [14]. Many anticancer drugs like camptothecins, such as topotecan and irinotecan, [15] and vinca alkaloids, such as vincristine and vinblastine, [16] kill tumors to some extent through induction of apoptosis [17]. In the present communication, we have reviewed literature pertaining biology and medicinal chemistry of apoptosis inducer as potential anticancer agents.

MECHANISM OF APOPTOSIS

Apoptosis occurs by mitochondrial pathway [18], death receptor ligand pathway [19] and DNA damage. The former two pathways lead to activation of caspase enzymes directly while the DNA damage pathway initiates apoptosis through the mitochondrial pathway. The newly hypothesized pathway of apoptosis is the T-cell mediated cytotoxicity and perforin-granzyme dependent killing of cell [20]. The perforin-granzyme pathway is capable of inducing apoptosis by granzyme A or granzyme B. The mitochondria, death receptors and granzyme B lead to the activation of caspase enzymes while the exception being granzyme A. Granzyme A commences apoptosis by DNA damage of only one strand of the double helix which is caspase-independent cell death [21, 22]. Thus, the activation of caspase cascade leads to the formation of the apoptosome and results in the complete cell degradation.

A. Intrinsic Pathway

The intrinsic pathway is regulated at inter-membrane space of the mitochondria which involves the release of cytochrome *c*. The loss of mitochondrial trans-membrane potential results in the release of cytochrome *c* into the cytoplasm. The intrinsic signaling pathways direct DNA damage, hypoxia and survival factor deprivation resulting in apoptosis [23].

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Bcl-2 Protein Family: Cell Suicide Administrators

The family of Bcl-2 proteins consists of pro-apoptotic and anti-apoptotic proteins. The pro-apoptotic proteins are Bak, Bax and Bik. They have the same Bcl-2 homology or BH domains which are required for their dimerization to occur during apoptosis. The BH1, BH2 and BH4 are essential in order to suppress the function of anti-apoptotic proteins while BH3 is required for instigation of death of pro-apoptotic proteins [24]. The major communication channel of these proteins is the dimerization or oligomerization which commences the mitochondrial pathway [25]. Family of Bcl-2 proteins is regulated by a number of cellular processes including alternative slicing of mRNA transcript and post-translational modifications. For example, phosphorylation on the serine amino acid on the prototype Bcl-2 protein by JNK diminishes its anti-apoptotic capability. Conversely, the phosphorylation of Bad inactivates it. The positive regulation of Bcl-2 proteins and their expression leads to the MOMP (Fig. **1**). MOMP cannot occur without the two critical factors Bak and Bax. They are triggered on by the BH3-only proteins and so are called activators of MOMP [26].

Mitochondrial Death Induction: Intrinsic Pathway

Mitochondrion is a complex organelle consisting of outer-membrane, inner-membrane and inter-membrane space. The inter-membrane space contains pro-apoptotic factors such as AIF, SMAC/ DIABLO and cytochrome *c* along with nucleases, proteases and caspase activators [27]. Release of cytochrome *c* from mitochondria results in formation of apoptosome. Apoptosome, being the large molecule of seven fold symmetry, is responsible for the activation of caspase cascade. Cytochrome *c* binds to Apaf-1 immediately after its release by WD-40 repeating units of Apaf-1 [28]. This attachment causes a major change in the conformation of the molecule which pushes CARD of Apaf-1 outwards, allowing for communication with pro-caspase 9. This network leads to formation of holoenzyme which is capable of activating caspases-3 and -7. These caspases in turn cause proteolytic cleavage of pro-caspases to active the caspases -2, -6,-8 and -10 which are responsible for the establishment of the caspase cascade [29].

DNA Damage: Catastrophic Effects of p53

The p53 protein is the basis for disruption of normal cell cycle as it causes activation by transcription. p53 is required to be stabilized by phosphorylation and acetylation in order to enhance its transcriptional ability. It causes inhibition of the cell cycle by activating p21 cyclin kinase gene [30]. Additionally, p53 can hinder the expression of anti-apoptotic Bcl-2 family proteins like Bcl-2 and Bcl-xL and also augment the expression of Apaf-1 and several caspases such -1,-6,-8 and pro-apoptotic serine protease HTRA2. p53, being a major regulatory protein, can also cause apoptosis through the extrinsic pathway [31]. The protein is capable of expressing Fas, DR4 and DR5, all of which serve as ligands for the death receptor pathway [32].

B. Extrinsic Pathways

Death Receptors

A total of six death receptors are identified and distinguished by their intracellular death domain of 80 amino acids positioned in α helices in anti-parallel direction. Fas (CD95 or APO-1), TNF-R1 (p55 or CD120a), TRAIL-R1 (DR4), TRAIL-R2 (DR5, APO-2, KILLER), death receptor 3 (DR3, APO-3, TRAMP, WSL-1, LARD) and death receptor 6 (DR6) are identified as the death receptors. Each receptor is recognized with its own signaling

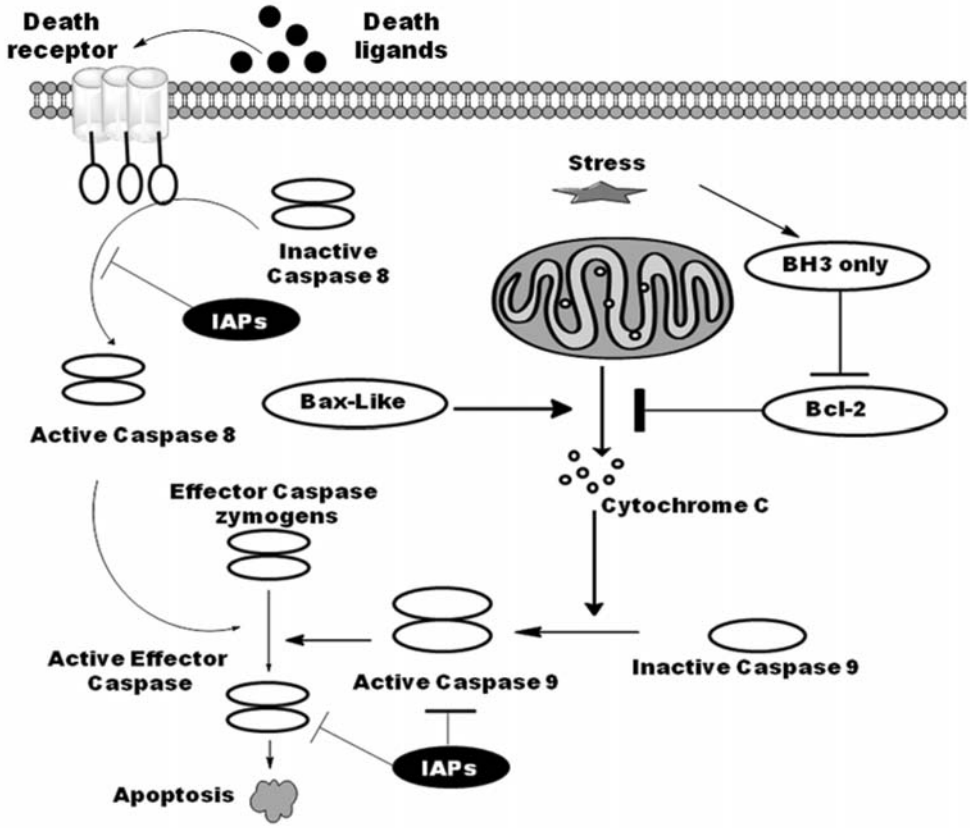


Fig. (1). Pathways involved in apoptosis. Figure depicts the scheme of death receptor and the mitochondrial apoptosis pathways. Ligand-induced death receptor oligomerization results in DISC formation which allows for recruitment and activation of the initiator caspase-8 and -10. Final activation of effector caspase zymogens results in apoptosis. Mitochondrial apoptosis pathway includes cytochrome *c* release from the mitochondrion which leads to apoptosome formation by caspase-9 activation.

system by specific ligands [33]. Fas is a glycosylated type I transmembrane receptor which upon binding by FasL attracts FADD and results in DISC formation [34, 35]. This route proceeds by the mitochondrial pathway as Bid is truncated to t-Bid and initiates MOMP and hence leading to the formation of apoptosome. The other commonly involved receptors are DR4, DR5 and TNF. The DR4 and DR5 share a common ligand called TRAIL [36] which also serves to bind with the "decoy receptors", TRAIL-R3 and TRAIL-R4. TNF stimulation results in formation of two complexes [37]. Complex I consists of the molecules TNF-R1, RIP1, TRADD, TRAF2. It activates NF-κβ pathway and leads to formation of complex II (traddosome). The establishment of complex II indicates positive route for apoptosis [38]. Therefore, death receptor pathway operates in response to stimuli received from the external environment.

Cytotoxic Cell Death

NK cells produce signaling molecules or secrete toxic substances like pore forming C9 (complement factor 9) causing the cell to instigate suicide. The killers produce cell surface markers like CD16 and CD2 cause apoptotic stimuli [39]. Cytotoxicity mediators like perforin (cytolysin) and granzymes (granule-associated serine esterases) direct the process. Perforin is required for regulation of itself and granzymes, however it does not produce typical DNA degradation in the cell [40]. The granzymes either truncate Bid to act by mitochondrial pathway or activate caspases directly towards apoptosis. Thus, the marginal fact being the release of lytic substances which is generated by the physical contact between CTL and its target serves as the incentive for lysis of the cell.

THE PATH OF ION HOMEOSTASIS TO APOPTOSIS

Ion homeostasis plays an important role in the generation of apoptosis [41] (Fig. **2**). Imbalance in the ion levels such as calcium, potassium and chloride are regarded as a significant step in apoptosis. Intracellular elevated calcium concentration can cause cell death by more than one route [42, 43]. The rise in the intracellular calcium levels, by influx of Ca^{+2} ions or release from endoplasmic reticulum, is sufficient to initiate apoptosis by allowing mitochondria to uptake the ions and cause MOMP [44]. Chances of cell survival can be enhanced by K^+ induced activation of voltage-gated calcium channels on the exterior side of the cell as potassium efflux is one of the major motives of apoptosis. The intracellular K^+ ion inhibit the initiation of cell death by mitochondria by suppression of Bcl-2 factors [45]. K^+ loss through Na^+/K^+-ATPase is the cause for apoptosis by Fas ligands of death receptor pathway in Jurkat cells [46]. Chloride ion efflux occurs simultaneously with K^+ ion efflux thus causing a drastic change in the osmolarity of the intracellular environment. The resulting water loss causes rapid decrease in the volume of the cell and thus serving as stimuli for cell death by unknown mechanism.

CASPASE CASCADE

The caspase family contains two types of caspase enzymes called executioner and initiator caspases. The executioner caspases are activated by proteolytic cleavage whereas the initiator caspases are activated indirectly by proximity-induced activation pathway. Caspase-3 particularly is highly capable of interacting with other caspases upstream (-8, and -9) and downstream (-3, -6 and -7) [47]. The executioner caspases -3 and -6 inactivate DNA repair enzymes

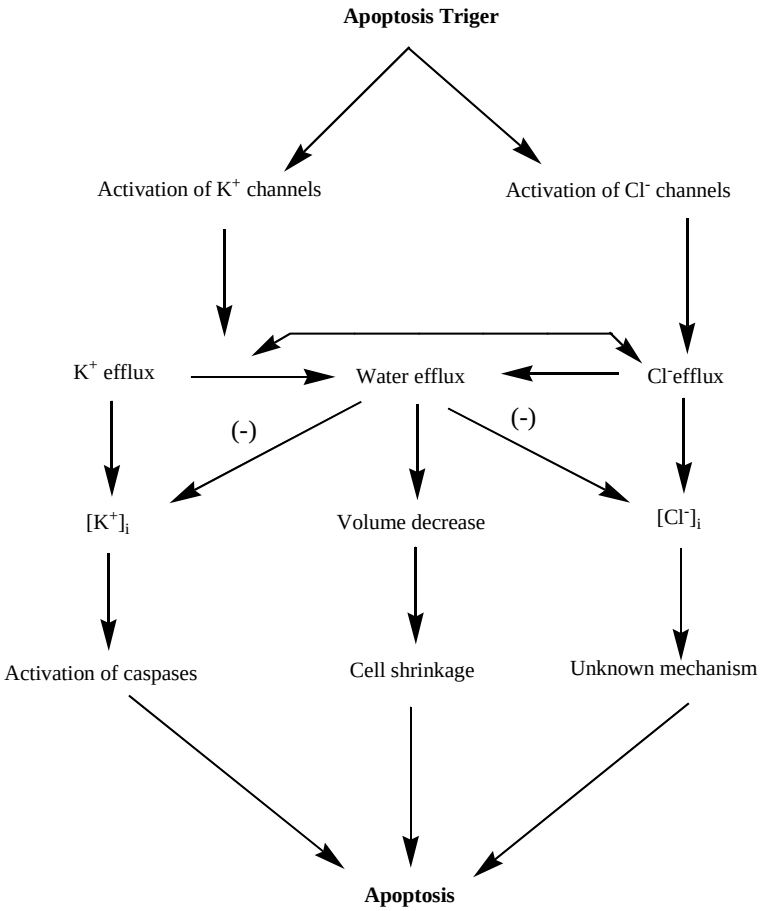


Fig. (2). Apoptotic triggers may activate K^+ or Cl^- channels, leading to K^+ and Cl^- efflux, water efflux and cell body shrinkage. K^+ efflux and Cl^- efflux are mutually reinforcing, due to charge effects. If K^+ or Cl^- efflux occurs to a greater extent than water efflux, $[K^+]_i$ or $[Cl^-]_i$ will fall. Decreases in $[K^+]_i$, or $[Cl^-]$ volume may each independently promote apoptosis.

like protein kinase C and PARP which may be able to correct DNA damage. Caspase-6 more specifically begins structural nuclear protein destruction of lamins. Also, caspase-3 cleaves ICAD (inhibitor of CAD) from CAD (caspase activated DNase) to activate CAD which initiates DNA fragmentation, the final stage of apoptosis [48]. Various consequences of substrate processing by the executioner caspases during the final phase of apoptosis are shown in Fig. (**3**). Six representative caspases substrates are shown, but it is important to note that hundreds of proteins are cleaved by the executioner caspases during this phase [49].

APOPTOSIS AND NECROSIS

Necrosis is recognized as unnatural death which occurs from a different route than apoptosis. Although in both types of death shrinkage of genetic material as well as blebbing of the cell membrane occurs, the significance and application of each vary drastically. Both processes are result of MPT but depending on the imbalance of ATP regeneration the fate of the cell is decided [50]. In apoptosis, the cell initiates its death program in full awareness whereas necrosis is a forcible course of cell death which occurs in response to destructive environmental factors like hypoxia. On the other hand, a cell undergoing necrosis appears to have no purpose in carrying out the metabolic functions and thus ends the production of proteins and ATP molecules, depriving the cell of nutrients and energy [51]. The process of apoptosis can be both beneficial by allowing new cell growth. Conversely, necrosis serves no purpose of such kind except to eliminate cells entirely without the possibility of regeneration and scarring the tissue region entirely. It may occur in response to stimuli such as hypoxia, oxidative stress, endotoxins or ATP depletion. Necrotic cells do not undertake any such task of providing protection to the neighboring cells [52, 53].

APOPTOSIS: THE CAUSE AND CURE OF CANCER

Cancer is a condition in which failure of apoptosis occurs, cells are programmed for death but they do not complete the entire process and instead rebound, proliferate rapidly [54]. In the view of the fact that the cure comes from the condition itself, apoptosis can be used as a protective method for cancerous body. Most of the anticancer agents are apoptosis inducers, whether they range from natural products such as quercetin to hormones and antibodies [55, 56]. In many of the cancer models, over expression of anti-apoptotic molecules such as IAPs, protein kinase Akt and PI3k was observed along with post-translational modifications of caspases [57]. More specifically higher levels of survivin, an IAP containing baculovirus are associated with carcinomas. Survivin impedes both the intrinsic and the extrinsic pathways leading to apoptosis by an unknown physical interaction between caspases [58]. Furthermore, the relationship between apoptosis and cancer is integrated so that the mechanisms which enhance proliferation of cancer cells also potentiate apoptosis [59]. For example, mitogenic oncogenes (myc E1A, E2F-1,cdc25, and ras) activate the suppressed p53 gene in tumour cells however, in some cases oncogenes can be blocked by Bcl-2 proteins [60].

MEDICINAL CHEMISTRY OF APOPTOSIS INDUCERS

In the year 2006 Cai, *et al.* [61] reviewed the concept of cell- and caspase-based apoptosis assay for the discovery of apoptosis inducers. The authors described the development of apoptosis inducers as potential anticancer agents and their related SAR studies. Gambogic acid and various derivatives of *N*-phenyl nicotinamides, indole-2-carboxylic acid benzylidene-hydrazides**,** 4-aryl-4*H*-chromenes and 3-aryl-5-aryl-1,2,4-oxadiazoles etc., was reported along with their related potency values as apoptosis inducers. Establishment of cell- and caspase-based anti-cancer screening apoptosis program (ASAP) led to easy identification of many novel vascular disrupting agents. Discovery of 4-Aryl-4*H*-chromenes as potent apoptosis inducers and SAR study on the same was reported earlier depicting the importance of ASAP in filtering the compounds having apoptosis inducing activity [62]. This review focuses on the literature pertaining to apoptosis inducers as potential vascular disrupting (anticancer) agents and their medicinal chemistry. Cai, *et al.* at Maxim Pharmaceuticals, San Diego USA were the first to discover gambogic acid as apoptosis inducer [63]

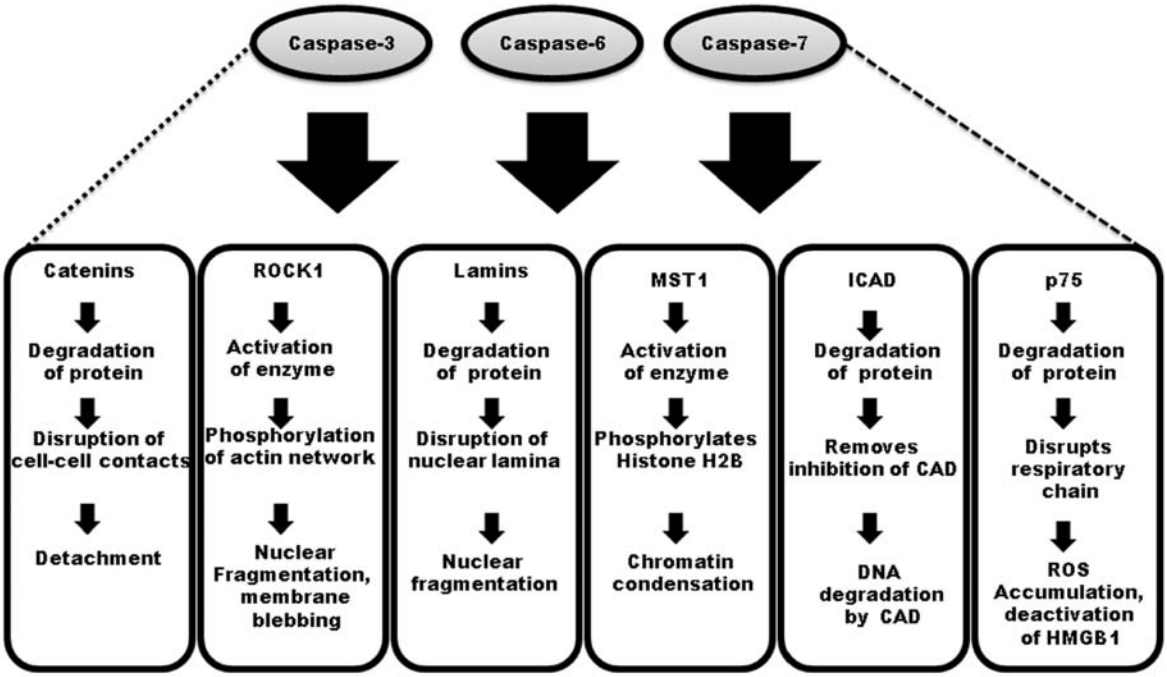
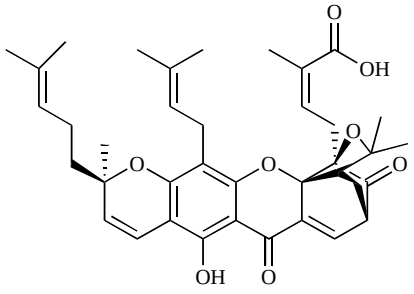


Fig. (3). Substrate processed by the executioner caspases. The figure depicts the consequences involved after the caspase cascade activation. During the demolition phase of apoptosis, substrate processing is done by the executioner caspases. More than 600 proteins are cleaved by the executioner caspases during this phase. To our convenience only six representative caspases substrates are shown.



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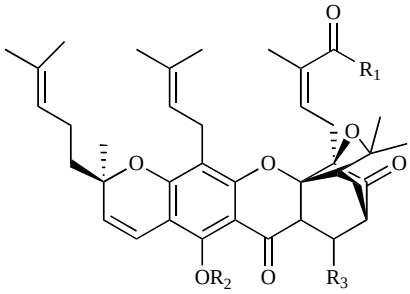
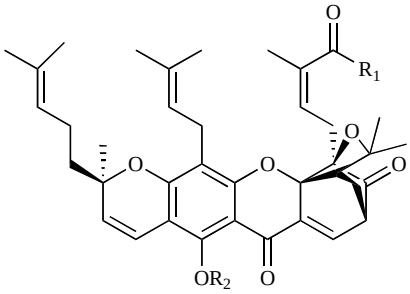


5. R_1 = OH; R_2 = H; R_3 = H; EC_{50} >10μM

6. R_1 = OH; R_2 = H; R_3 =  ;EC_{50} > 10μM



2. R_1 = OMe; R_2 = Me; EC_{50} = 0.80 μM

3. R_1 =  ; R_2 = Me; EC_{50} = 0.42μM

4. R_1=OH ;R_2 = Ac; EC_{50} = 0.51μM

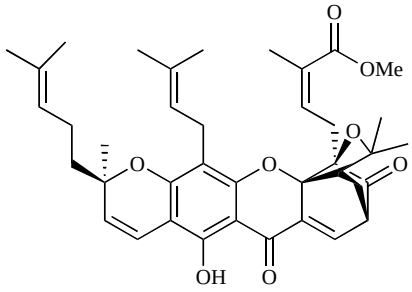


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Fig. (4). Chemical structures and activity of gambogic acid and its derivatives.

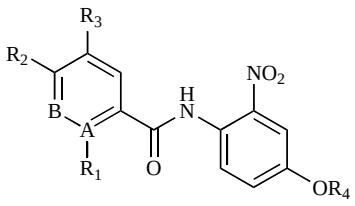
and transferrin receptor was identified as its molecular target [64]. This review has stressed a number of key points which include mechanism of apoptosis and identification of many compounds that activate the apoptosis signalling pathways. The compounds with their related potency on various cell lines such as breast cancer cells -T47D, HCT116, RKO, SNU398 using cell- and caspase based high throughput screening and flow cytometric analysis assay [65, 66] was reported.

A. Gambogic Acid as Apoptosis Inducers

Cai, *et al.* [67] isolated a natural product gambogic acid (**1**) (Fig. **4**) from the resin of *Garcinia hurburyi*. Based on cell- and caspase-based HTS assays gambogic acid was discovered as a potent apoptosis inducer. Gambogic acid was found to induce apoptosis and activate caspases in T47D cells (EC_{50} = 0.78 μM). Apoptosis-inducing activity of gambogic acid was also characterized by flow cytometry and it was found that apoptosis induced by gambogic acid is not preceded by arrest in any specific phase of the cell cycle. The authors synthesized derivatives **(2-4**) of gambogic acid with modification of the 6-hydroxy group by either methylation or acylation. Compounds **2-4** possess similar activity as the corresponding 6-hydroxy compound. SAR studies suggested that the 9, 10 carbon–carbon double bond of the α,β-unsaturated ketone (**5**, **6**) is important for biological activity, while the 6-hydroxy and 30-carboxy group (**7**) can tolerate a variety of modifications.

B. *N*-phenyl Nicotinamide Derivatives

A series of *N*-phenyl nicotinamides were identified as apoptosis inducer [67]. 6-Methyl-*N*-(4-ethoxy-2-nitrophenyl)pyridine-3-carboxamide (**8**) (Fig. **5**), showed EC_{50} of 0.082 μM in caspase activation assay in T47D breast cancer cells. *N*-phenyl nicotinamides were also found active in growth inhibition assay (**8**, GI_{50} = 0.21 μM**)**. *In vitro* studies reported that these derivatives act by inhibiting microtubule polymerization. SAR study revealed that substitution at the 6-position of the pyridine ring by –Cl (**9**) resulted in better potency and suggesting the presence of a binding pocket in that position. 6-Chloro in the pyridine ring with 4-ethoxy group at phenyl ring (**10**) showed good activity (Fig. **5**). It was concluded from SAR study that 3-pyridyl is an important group for the activity and substitution at the 6-position by a -chloro or methyl group resulted in increased potency. Compound **10** was also found more potent as an inducer of apoptosis in the flow cytometric assay.



8. R_1 = H; R_2 = Me; R_3 = H; R_4 = Et; A = C; B = N; EC_{50} = 0.082 μM

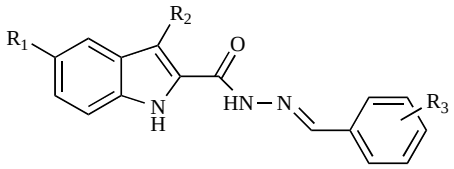
9. R_1 = H; R_2 = Cl; R_3 = H; R_4 = Me; A = C; B = N; EC_{50} = 0.69 μM

10 R_1 = H; R_2 = Cl; R_3 = H; R_4 = Et; A = C; B = N; EC_{50} =0.24 μM

Fig. (5). Chemical structures and activity of *N*-phenyl nicotinamides.

C. Indole-2-carboxylic Acid Benzylidene-hydrazides

Indole-2-carboxylic acid benzylidene-hydrazides were identified as a new class of potent apoptosis inducer [68]. 5-Chloro-3-methyl-indole-2-carboxylic acid-(4-nitrobenzylidene)-hydrazide (**11**) (Fig. **6**), was found to arrest T47D cells in G_2/M phase and to induce apoptosis. SAR study revealed that substitution at the 3-position of the indole ring (5-chloro-3-phenyl-indole-2-carboxylic acid-(4-nitrobenzylidene)-hydrazide (**12**) was found important for apoptotic activity.



11: R_1 = Cl; R_2 = Me; R_3 = *p*-NO_2 (EC_{50} 2.2 ± 0.2 μM)

12: R_1 = Cl, R_2 = Ph, R_3 = *p*-NO_2 (EC_{50} 0.1 ± 0.06 μM)

Fig. (6). Chemical structures and activity of indole-2-carboxylic acid benzylidene-hydrazides.

D. Substituted N'-(2-oxoindolin-3-ylidene)benzohydrazides

A series of substituted *N*′-(2-oxoindolin-3-ylidene)benzohydrazides exhibited potent apoptosis inducing action [69]. Compound **13** and **14** (Fig. **7**) were found as the most active compound. Compound **13** has an EC_{50} value of 0.24 μM and GI_{50} value of 0.056 μM on HCT116 cells. Compound **14** incorporating a methyl piperazine moiety was found to have EC_{50} values of 0.17 μM in HCT116, 0.088 μM in SNU398 cells and 0.14 μM in RKO cells.

E. 3-Aryl-5-aryl-1,2,4-oxadiazoles

5-(3-Chlorothiophen-2-yl)-3-(4-trifluoromethylphenyl)1,2,4-oxadiazole (**15**) [70] showed an EC_{50} value of 1.21μM on H47D cell line, and found inactive against several other cancer cell lines. Flow cytometry assay revealed that the compound **15** (Fig. **8**) was able to arrest cells in the G_1 phase, followed by induction of apoptosis. SAR studies of **15** revealed that the 3-phenyl ring can be replaced by a pyridyl ring, and a substituted five-member ring at the 5-position is important for activity. 5-(3-Chlorothiophen-2-yl)-3-(5-chloropyridin-2-yl) 1,2,4-oxadiazole (**16**) was found active *in vivo* in a MX-1 tumour model.

F. Substituted Pyrimidine Based Apoptosis Inducers

4-Anilino-2-(2-pyridyl) pyrimidine derivatives were discovered as potent inducers of apoptosis [71]. Compound **17** (Fig. **9**) showed an EC_{50} value of 0.018 μM in the caspase activation assay on T47D cell lines. SAR studies of anilino group showed an importance of *m*-CH_3O group for apoptosis-inducing activity (**18,** EC_{50} = 0.018μM). In pyrimidine ring replacement of 2-pyridyl group by other aryl groups (3-pyridyl, 4-pyridyl and 2-pyrazinyl group) accounts for better activity [72]. Compound **19** was found active against T47D, HTC116 and SNU398 cell lines with IC_{50} value of 0.5 μM. N-((benzo[*d*][1,3]dioxol-5-yl)methyl)-6-phenylthieno[3,2-d]pyrimidin-4-amine (**20**) [73] was active against T47D but not against HTC116 and SNU398 cell lines. Compounds **21** and **22** showed an EC_{50} value of 0.008 and 0.004 μM, respectively in T47D cells.

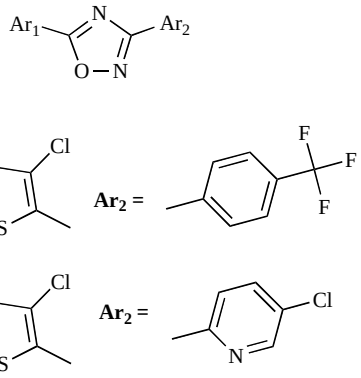


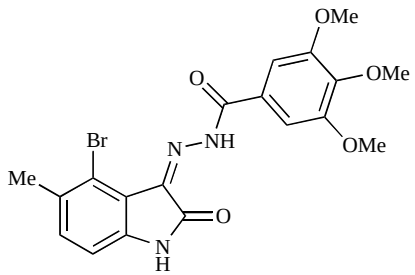
Fig. (8). Chemical structures of 3-aryl-5-aryl-1,2,4-oxadiazoles.

G. Thiazepine Derivatives

A potent apoptosis inducer, 5-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-7-(4-methylphenyl)-(*E*)-2,3,6,7-tetrahydro-1,4-thiazepine (**23**) (Fig. **10**) with an EC_{50} value of 1.19 μM on T47D cells was discovered using HTS assay [74]. 5-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-7-(2-methoxy-4-(methylthio)phenyl)-(E)-2,3,6,7-tetrahydro- 1,4-thiazepine (**24**) was identified with an EC_{50} value of 0.08 μM on T47D cells. Compound **24** apart from inhibiting tubulin polymerisation was found to be highly active in a growth inhibition assay with a GI_{50} value of 0.05 μM.

H. Substituted Quinolin-4-amines Derivatives

N-(3-acetylphenyl)-2,3-dihydro-1*H*-cyclopenta[*b*]quinolin-9-amine (**25**) was discovered [75] through cell- and caspase based HTS assays (Fig. **11**). This series of compounds was active against cancer cell lines derived from several human solid tumors with EC_{50} values ranging from 400-700 nM. SAR study of hit **25** led to the discovery of *N*-phenyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-amines as a potent apoptosis inducers.1,3-Dimethyl-*N*-(propionylphenyl)-1*H*-



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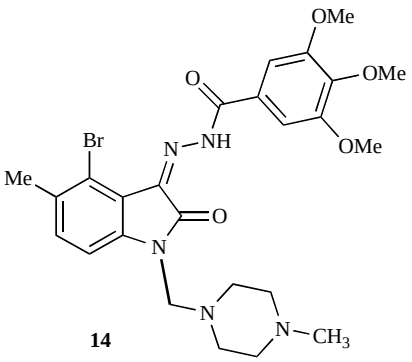

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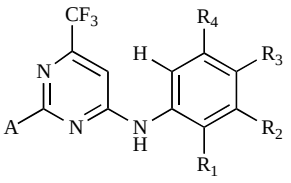
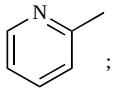
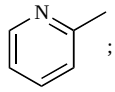
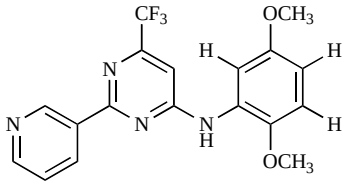
Fig. (7). Chemical structures of *N*'-(2-oxoindolin-3-ylidene) benzohydrazides.



17: A=  ; R1 = H; R_2 = OMe ; R_3 = H ; R_4 = H; EC_{50} = 0.36 ± 0.17 μM

18: A=  ; R_1=OMe ; R_2=H ; R_3=H ; R_4=OMe; EC_{50} = 0.018 ± 0.001 μM



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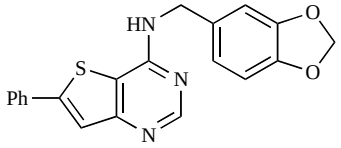
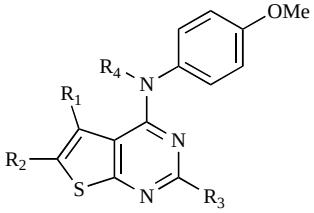


20

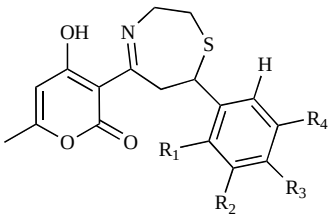


21: R_1 = H; R_2 = H; R_3 = Me; R_4 = Me

22: R_1 = Me; R_2 = H; R_3 = Me; R_4 = Me

Fig. (9). Chemical structures and activity of pyrimidine based apoptosis inducers.

pyrazolo[3,4-*b*]quinolin-amine (**26,** EC_{50}= 0.071 μM on T47D, EC_{50}=0.064 μM in HCT116 and EC_{50} = 0.034 μM on SNU398).



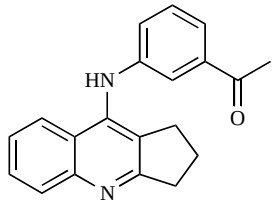
23: R_1 = H; R_2 = H; R_3 = Me; R_4 = H

24: R_1 = OMe; R_2 = H; R_3 = SMe; R_4 = H

Fig. (10). Chemical structures of thiazepine derivatives.

I. 4-Anilinoquinazoline Derivatives

2-Chloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4 amine (**27)** (Fig. **12**) was found highly active in T47D cells (EC_{50} for caspase activation is 2 nM) and as a potent inhibitor of cell proliferation (GI_{50} = 2 nM) [76]. SAR revealed that the methyl group at the nitrogen linker was essential for the apoptosis-inducing activity. Replacement of 2-Cl in lead compound **27** by other functional groups, resulted in the identification of *N*-(4-methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (**28**, EC_{50} = 2 nM) as a potent apoptosis inducer [77]. In another work the authors [78] explored substitution at the 5-, 6- and 7-positions of the quinazoline ring (**29** and **30**). SAR study revealed that substitution



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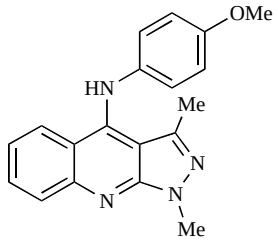
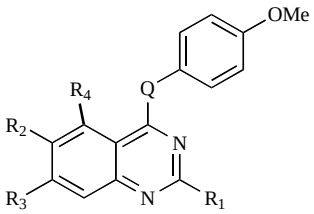


26

Fig. (11). Chemical structures of quinolin-4-amines derivatives.



27: Q = N-Me; R_1 = Cl; R_2 = H; R_3 = H; R_4= H ($T47D\ EC_{50}$ = 0.002 μM)

28: Q = N-Me; R_1 = Me; R_2 = H; R_3 = H; R_4= H ($T47D\ EC_{50}$ = 0.002 μM)

29: Q = N-Me; R_1 = Me; R_2 = H; R_3 = H; R_4= OMe ($T47D\ EC_{50}$ = 0.004 μM)

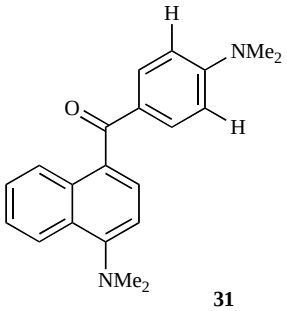
30: Q = N-Me; R_1 = Me; R_2 = NH_2; R_3 = H; R_4= H ($T47D\ EC_{50}$ = 0.008 μM)

Fig. (12). Chemical structures and activity of 4-anilinoquinazoline derivatives.

at the 6-position with a small group like an amino (**30**) was preferred for apoptosis inducing activity.

J. Phenyl-naphthalene Derivatives

(1-(Dimethylamino)naphthalen-4-yl)(4-(dimethylamino)phenyl) methanone (**31**) [79] (Fig. **13**) was found as potent inducers of apoptosis with EC_{50} values of 37, 49 and 44 nM in T47D, HCT116 and SNU398 cells, respectively. Compound **31** was highly active in a growth inhibition assay with a GI_{50} value of 34 nM in T47D cells.



31

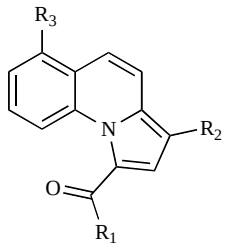
Fig. (13). (1-(Dimethylamino)naphthalen-4-yl)(4-(dimethylamino)phenyl) methanone.

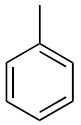
K. Substituted Quinoline Derivatives

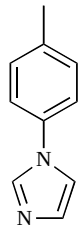
1-Benzoyl-3-cyanopyrrolo[1,2-α]quinoline (**32**) was discovered as an inhibitor of tubulin polymeristaion (Fig. **14**) [80]. SAR studies of **32** revealed that substitution at the 4-position of the 1-benzoyl group and 3-cyano group is essential for activity. 4-Substituted analogs such as 1-(4-(1*H*-imidazol-1-yl)benzoyl)-3-cyanopyrrolo [1,2-α]quinoline (**33**) were found highly active in the caspase activation as well as in the cell growth inhibition assay. Compound **33** was found to inhibit tubulin polymerization with an IC_{50} value of 5 μM, indicating that certain substituents at the 4-position of the 1-benzoyl group can affect the potency. Substitutions at the 4-, 5-, 6-, 7- and 8-positions of pyrrolo[1,2-α]quinoline were examined for apoptotic inducing activity [81]. SAR studies revealed that substitution at the 6-position by -Cl resulted in potent compound (**34**). Compound **35** was also found highly active in growth inhibition assay with a GI_{50} value of 0.018 μM against T47D cells.

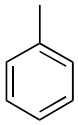
L. *N*-aryl-9-oxo-9*H*-fluorene-1-carboxamides

N-(2-Methylphenyl)-9-oxo-9*H*-fluorene-1-carboxamide (**36**) (Fig. **15**) was discovered [82] as potent apoptosis inducer. Substitution at the 2-position with a phenyl or pyrazolyl group resulted in potent compounds. The most potent compounds **37** and



32: R_1 =  ; R_2 = CN; R_3 = H ($T_{47}D\ EC_{50}$=0.078)

33: R_1 =  ; R_2 = CN; R_3 = H; ($T_{47}D\ EC_{50}$ = 0.034)

34: R_1 =  ; R_2 = CN; R_3 = Cl; ($T_{47}D\ EC_{50}$ = 0.083)

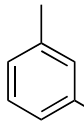
35: R_1 =  ; R_2= CN; R_3= Cl; ($T_{47}D\ EC_{50}$ = 0.080)

Fig. (14). Quinoline derivatives **Fig. (14).** Quinoline derivatives.

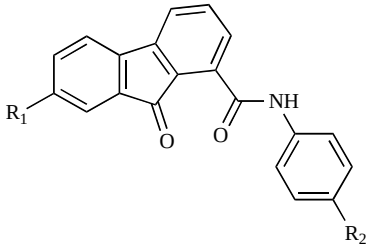
38 were synthesized by replacement of 2-methyl phenyl with aryl group. Compound **36** is 10 times less active than vinblastine and paclitaxel [83]. However, compound **38** was found active in tubulin inhibition assay, which suggests a different pathway of action from **37**. The authors extended their research by exploring modifications of the 9-oxo-9*H*-fluorene-1-carboxamides [84]. SAR studies at the 7-position of the 9-oxo-9*H*-fluorene ring led to compounds **39** (7-nitro), **40** (7-amino) and **41** (7-azido), which were more potent than **36**. Compounds **39** and **40** were found active in a tubulin inhibition assay, suggested a different mechanism of action from that of compound **36**.

M. Substituted Guanidine Derivatives

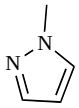
1-(2-(2,5-Dimethoxyphenylthio)benzylidene)semicarbazide (**42**) (Fig. **16**) was discovered as a potent apoptosis inducer with little aqueous solubility [85]. Later on a more aqueous soluble analogue (2-(2,5-dimethoxyphenylthio)-6-methoxybenzylideneamino)guanidine (**43**) was discovered based on the SAR study with EC_{50} value of 60 nM in the caspase activation assay and GI_{50} value of 62 nM in the growth inhibition assay.

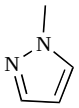
N. 4-Aryl-4*H*-chromenes

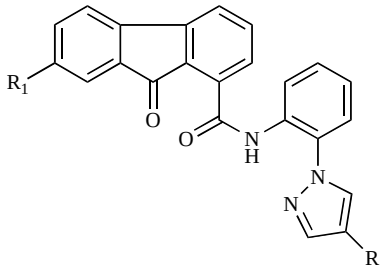
Among all the apoptosis inducer hits identified from HTS assay, 2-amino-3-cyano-7-dimethylamino-4-(3-methoxy-4,5-methylenedioxyphenyl)-4*H*-chromene (**44**) was identified as the most potent compound. 4-Aryl-4*H*-chromenes were reported to have apoptosis inducing action against T47D cell lines. Compound **43** was reported with an EC_{50} of 0.019 μM [86]. Modifications at the 5-, 6-, 7- and 8-positions showed that, a small hydrophobic



36:R_1 = H; R_2 = Me; EC_{50}(μM) =0.98 (T47D), 1.1(HCT116), 0.64 (SNU398)

37:R_1 = H; R_2 =  ; EC_{50}(μM) =1.4 (T47D), 1.3 (HCT116), 1.1 (SNU398)

38:R_1 = NO_2; R_2=  ; EC_{50}(μM) = 0.29 (T47D), 0.22 (HCT116), 0.15 (SNU398)



39: R = H; R_1 = NO_2; EC_{50}(μM) = 0.29 (T47D), 0.22 (HCT116), 0.15 (SNU398)

40: R = H; R_1 = NH_2; EC_{50}(μM) = 0.97 (T47D), 0.37 (HCT116), 0.15 (SNU398)

41: R = H; R_1 = N_3; EC_{50}(μM) = 0.47 (T47D), 0.29 (HCT116), 0.15 (SNU398)

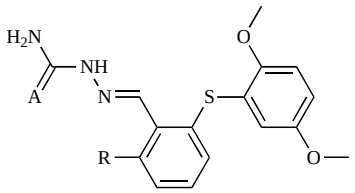
Fig. (15). Chemical structures of carboxamides.

group (-NMe_2, -NH_2, -NHEt, and -OMe) are preferred at the 7-position. Di-substitution at either 5- and 7-positions or 6- and 7-positions resulted in decrease of potency. Di-substitution at 7- and 8-positions, in general, was found to result in potent compounds. 7-NMe_2, 7-NHEt, 7-OMe and 7,8-di-NH_2 analogues were found to have similar SAR for the 4-aryl group, and several 7-substituted and 7,8-di-substituted analogs (**46-57**) (Fig. **17**) were found to have similar potencies as the lead compound **42**. It was also found that replacement of the 3-cyano group by an ester, including methyl and ethyl ester resulted in reduction of activity [87]. Conversion of the 2-amino group into an amide or urea resulted in 4- to 10-fold decrease in activity. Similarly, converting the 2-amino group into hydrogen resulted in 4- to 10-fold reduction of activity. Compound **55** was highly active with an EC_{50} value of 29 nM and a GI_{50} value of 6 nM in T47D cells. The 2-H analogue **58** was found much more stable as compared to the 2-NH_2 under acidic conditions, suggesting that 2-H analogue have better bioavailability than the 2-NH_2 analogue. Highly potent compound has an amino substitution at 2-position (**59**) [88]. Compound **59** was reported as a highly potent apoptosis inducer with an EC_{50} value of 2 nM and a highly potent inhibitor of cell growth with a GI_{50} value of 0.3 nM. It was found that, removal of the chiral center at 4-position and replacement of the 2-amino group with a 2-oxo group were tolerated and 4-aryl-2-oxo-2*H*-chromenes exhibited SAR similar to 4-aryl-2-amino-4*H* chromenes [89]. 4-Aryl-2-oxo-2*H*-chromenes with a *N*-methyl pyrrole fused at the 7,8-positions were highly active, compound **60** having an EC_{50} value of 13 nM in T47D cells. It was found that -OMe group was preferred at the 7-positon.

O. Resveratrol-based Derivatives

Resveratrol (**61**) (Fig. **18**), a plant polyphenol compound found in grapes and red wine has reported to have diverse effects on signaling molecules, activation of the apoptotic mechanisms and induction of cell cycle arrest. Nilesa, *et al.* [90] demonstrated the effectiveness of resveratrol in stimulating apoptosis in amelanotic cell line of A375 (IC_{50} = 30μM) and SK-mel28 (IC_{50} = 100μM) of 24 hr of treatment. Resveratrol induces phosphorylation of ERK1/2 in A375 cell line. Derivatives of resveratrol called 2,3′,4,5′-tetramethoxy-trans-stilbene (TMS) (**62**) and 3,4,4′,5,-tetramethoxy-trans-stilbene (MR-4) (**63**) [91, 92] showed sufficient potency to initiate apoptosis in colon cancer cell lines. The authors discovered a hybrid molecule of TMS and MR-4 called 2,3′,4,4′,5′-pentamethoxy-trans-stilbene (**64**) (PMS), capable of suppressing tumor progression in colon cancer xenograft model. PMS (**64**) causes apoptosis by PARP cleavage and DNA fragmentation which results in inhibition of mitosis and initiation of caspase cascade. Resveratrol analogues, nitrovinylstilbenes exhibited apoptotic activity. Compounds having a nitrovinyl side chain attached to the aromatic ring (β-nitrostyrenes) were reported as pro-apoptotic

anticancer agents (**65-68**) (Fig. **18**), and the nitrovinyl moiety was identified as the pharmacophore for the activity [93].



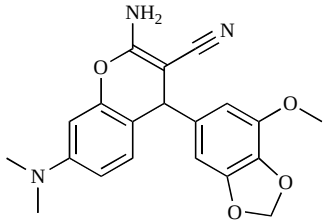
42: R = H; A = O (little aqueous solubility, EC_{50} = 0.052 ± 0.003 μM)

43:R = OMe; A = NH (more aqueous solubility, EC_{50} = 0.060 ± 0.003 μM)

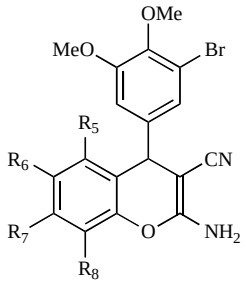
Fig. (16). Chemical structures and activity of guanidine derivatives.

P. Symmetrical Derivatives of Ether, Carbamate, Amide, Amine and Urea

Palop, *et al.* [94] synthesized and evaluated the biological activity of ether, carbamate, amide, amine and urea derivatives (Fig **19**). The most potent compounds were amide and urea derivatives of which compounds **69** (amine derivative), **70** (amide derivative, IC_{50} = 4.2μm (MD-MBA 231), 29.5μm (HT-29) 23.5μm (T-24)), and **71** (urea derivative) showed reasonably acceptable levels of apoptosis. The anticancer activity of **71** was measured on MD-MBA 231 and T-24 with the highest IC_{50} found on mama cells of 68.2μm and 18.6μm on T-24 cell line. SAR study revealed that the maximum amount of apoptotic activity is observed in the compounds which have central aromatic nucleus of W (Fig. **19**) in the general structure. R-Groups are required to be aliphatic chains whereas Y groups (ethers, amides, carbamates, amines) should be



44



45: R_5 = H; R_6 = H; R_7 = NMe_2; R_8 = H

46: R_5 = H; R_6 = H; R_7 = NH_2; R_8 = H

47: R_5 = H; R_6 = H; R_7 = NHEt; R_8 = H

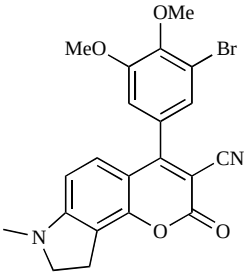
48: R_5 = H; R_6 = H; R_7 = OMe; R_8 = H

49: R_5 = H; R_6 = H; R_7 = NH_2; R_8 = NH_2

50: R_5 = H; R_6 = H; R_7 = NH_2; R_8 = Me

51: R_5 = H; R_6 = H; R_7 = Me; R_8 = Me

52: R_5 = H; R_6 = H; R_7 = OH; R_8= NH_2



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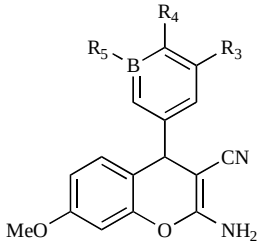


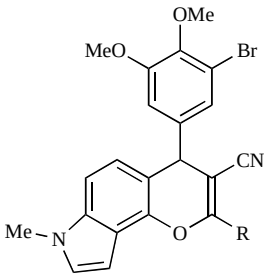
53: B = C; R_3 = OMe; R_4 = OMe; R_5 = OMe

54: B = C; R_3 = OMe; R_4 = H; R_5 = OMe

55: B = C; R_3 = Cl; R_4 = OMe; R_5 = OMe

56: B = C; R_3 = I; R_4 = OMe; R_5 = OMe

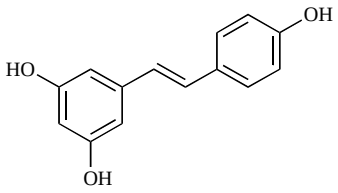
57: B = C; R_3 = Br; R_4 = OH; R_5 = OMe



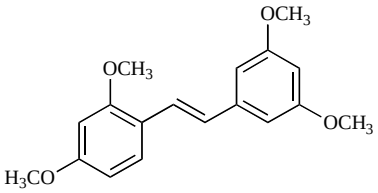
58: R = H

59: R = NH_2

Fig. (17). Chemical structures of 4-aryl-4*H*-chromenes.



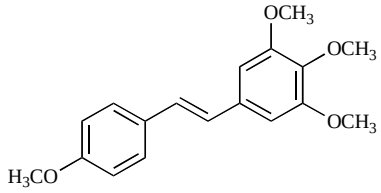
61



62:2,3',4,5'-tetramethoxy-trans-stilbene (TMS)

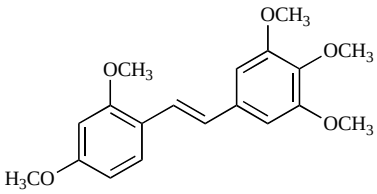
IC_{50} = 100μM (Caco-2), 24.9μM (HT-29)

and 37.0μM (SW1116)



63:3,4,4',5,-tetramethoxy-trans-stilbene (MR-4)

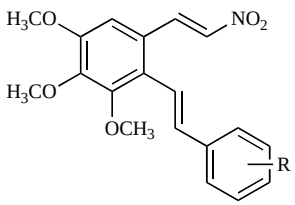
IC_{50} = 32.1μM (Caco-2), 20μM (HT-29)



64: 2,3',4,4',5'-pentamethoxy-trans-stilbene (PMS)

IC_{50} = 65.5μM (Caco-2), 14.7μM (HT-29)

and 27.2μM (SW1116)



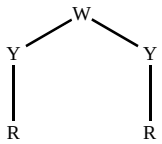
65: R = 4-F; IC_{50} =19.0 pM

66: R= 4-Cl; IC_{50} =25.7 pM)

67: R= 4-CH_3; IC_{50} = 42.5 pM)

68: R= 4-Br; IC_{50}=14.6 pM)

Fig. (18). Resveratrol-based derivatives.



W	Y	R
66: 4,4'-Methylenebisphenyl	–NH–	Benzyl
70: 2,2'-Methylenebis-4-chlorophenyl	–O–CO–NH–	4-Nitrophenyl
71: 4,4'-Methylenebisphenyl	–NH–CO–NH–	Propyl

Fig. (19). Derivatives of ether, carbamate, amide, amine and urea.

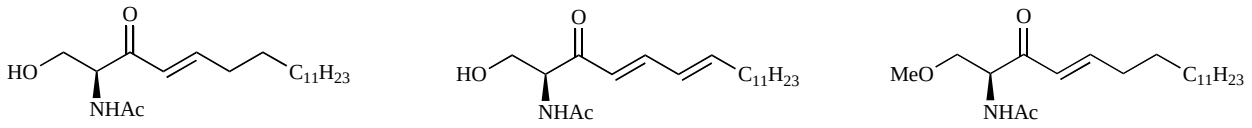
capable of building hydrogen bonds to provide electrostatic charges.

Q. 3-Ketoceramide Derivatives

Ijichi, *et al.* [95] synthesized short chain 3-ketoceramides called 2-acetylamino-3-oxo-4-octadecen-1-ol (**72**), 2-acetylamino-3-oxo-4,6-octadecadien-1-ol (**73**) (Fig. **20**), and 2-acetylamino-1-methoxy-3-oxo-4-octadecene (**74**). Among these compounds **72** and **74** are monoenoic compounds which showed maximum potency in HL-60 cells (human leukemia). The potency of these compounds is due to their capability to cause DNA fragmentation and release of cytochrome *c* from mitochondria for caspase activation.

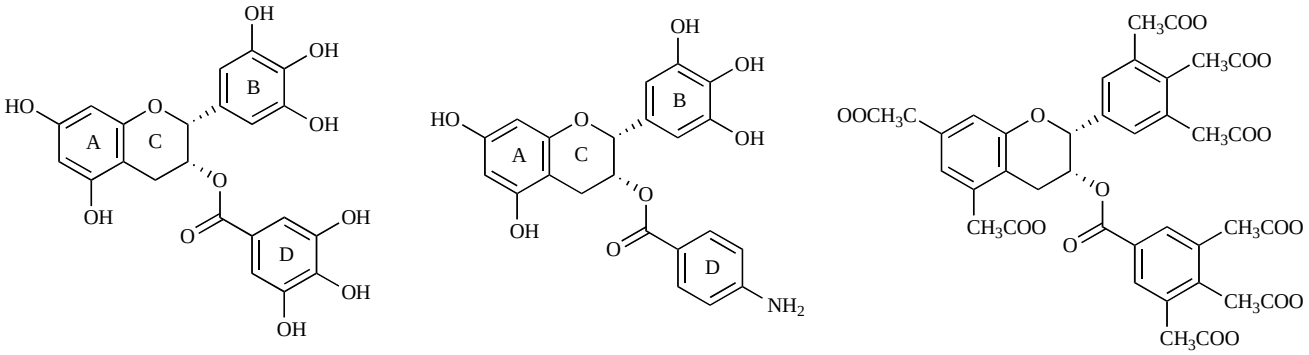
R. Polyphenol Epigallocatechin-3-gallate Derivatives

(–)-Epigallocatechin-3-gallate [(–)-EGCG] (**75**) (Fig. **21**) a major constituent discovered in green tea, is physiologically active



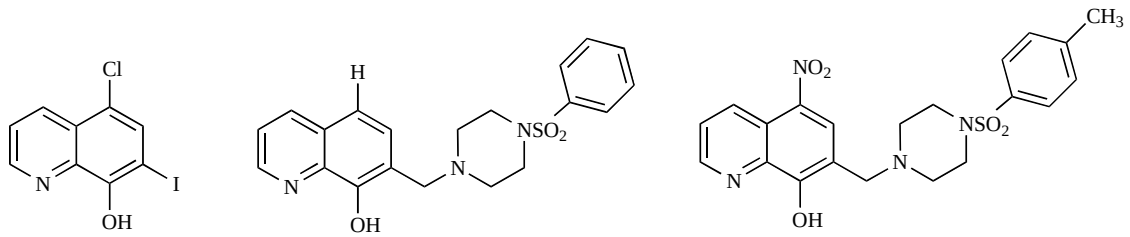
72 **73** **74**

Fig. (20). Chemical structures of ketoceramide derivatives.



75 **76** **77**

Fig. (21). Chemical structures of epigallocatechin-3-gallate derivatives.



78 **79** **80**

Fig. (22). Chemical structures of clioquinol derivatives.

green tea polyphenol (GTP). (–)-EGCG has been reported to possess anticancer activity and thus its analogs containing para-amino group on the D-ring (**76**) instead of the hydroxyl group were synthesized by Kumi, *et al.* [96]. These compounds inhibit only chymotrypsin-like activity of proteosome which has been linked with tumor cell apoptosis. Compound **76** showed an IC_{50} of 0.84μM. A prodrug of (–)-EGCG has been designed, known as Pro-E (EGCG peracetate) (**77**), which is easily converted to its active form with good bioavailability by esterases. Its inhibitory activity leads to increase in proteosome target proteins like Bax which may be responsible for triggering apoptosis in tumor cells.

S. Clioquinol Derivatives

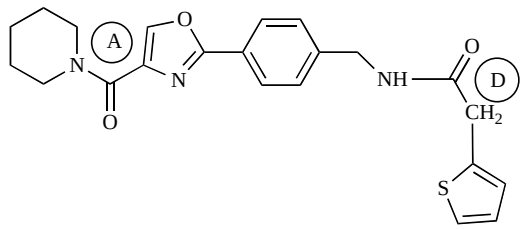
Clioquinol (**78**) (Fig. **22**) was discovered to have growth inhibitory effects on cancer cells as it functions as an ionophore and boosts levels of zinc. Arthur, *et al.* [97] synthesized clioquinol derivatives as inducers of cell death by Mannich reaction. The maximum activity of clioquinol on HeLa (cervical epitheliod carcinoma) cell line was measured as GI_{50} of 18.6 μM. Clioquinol derivatives with improved activity were synthesized by adding 7-methyl arylsulfonyl piperazine moiety and 5-nitro group on quinoline ring (**79,80**). Compound **79** showed GI_{50} value of 6.8μM while compound **80** has 26 times more potency than clioquinol (GI_{50} = 0.7μM). Clioquinol and mannich bases transport metal ions into the cell after binding with cell.

T. 2-Phenyl-oxazole-4-carboxamide Derivatives

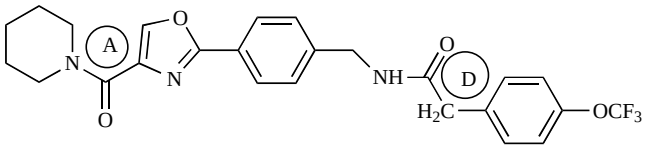
Vincent, *et al.* [98] discovered 2-phenyl-oxazole-4-carboxamides as apoptosis inducer. SAR study suggested the importance of van der Waals volume of the molecules for caspase activation around position-A (Fig. **23**). At the position D, one methylene group is essential to maintain distance between aryl/heteroaryl group and the amide group as shown in compound **81** with EC_{50} of 59 nM. Among the series of compounds synthesized, compound **82** (Fig. **23**) showed the maximum potency by causing cleavage of PARP and DNA fragmentation. Compound **81** was selected for caspase activation assay, and it was further analyzed for proliferation assay, solubility and pharmacokinetic profile in human tumor xenograft models. As a result, compound **82** has proved to be a potent apoptosis inducer with an EC_{50} value of 270 nM.

U. Hybrid Molecules of Benzo-imidazo-thiadiazole and α-bromoacryloyl Moieties

Romeo, *et al.* [99] synthesized hybrid molecules by bringing together two separate types of molecules known as benzo[4,5]imidazo[1,2-*d*][1,2,4]thiadiazole and benzoheterocyclic α-bromoacryloyl amides. Although, benzo[4,5]imidazo[1,2-*d*][1,2,4]thiadiazole lacks reactivity and even α-bromoacryloyl groups are not officially cytotoxic, it was their combination which produces cytotoxic compounds. Compounds **83** (IC_{50} = 0.29μM)

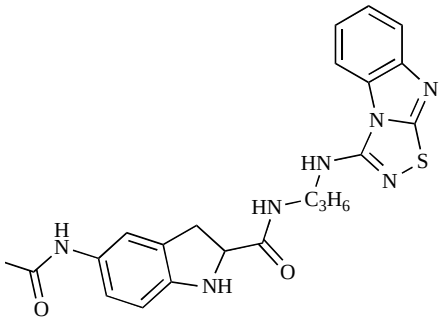


81

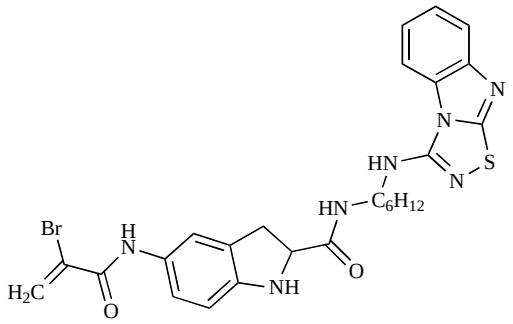


82

Fig. (23). Phenyl-oxazole-4-carboxamide derivatives.

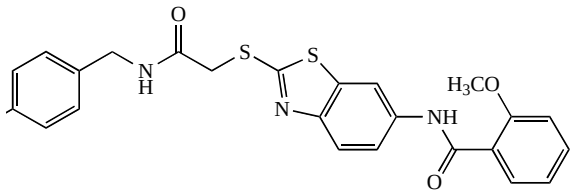


83

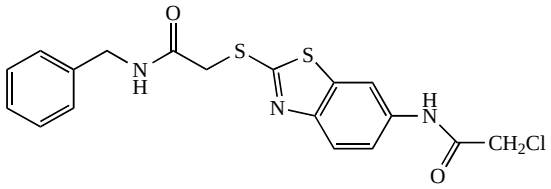


84

Fig. (24). Substituted benzo-imidazo-thiadiazole and α-bromoacryloyl.



85: IC_{50} = 0.6μM (HepG2); IC_{50} = 1.1μM (MCF-7)



86: IC_{50} =1.1μM (HepG2); IC_{50} = 4.2μM (MCF-7)

Fig. (25). Chemical structures and activity of benzothiazole-2-thiol derivatives.

and **84** (IC_{50} = 0.55μM) were found active on HL-60 (human leukemia cells) (Fig. **24**). Compound **83** and **84** cause release of cytochrome *c* from mitochondrial membrane, caspase-3 activation and thus DNA fragmentation leading to apoptosis.

V. Benzothiazole-2-thiol Derivatives

Luo-Ting, *et al.* [100] disclosed a series of benzothiazole-2-thiol derivatives as anti-cancer agents. Compound **85** and **86** (Fig. **25**) were found most potent and their pro-apoptotic activities were examined on cell lines such as HepG2 and MCF-7. Acceptable levels of anti-proliferative activity along with excellent apoptosis inducing activity were observed. 2-Methoxyphenyl group of **85** proves to be more potent that chloromethyl group as substituent on **86**. Compound **85** successfully provided the evidence of DNA fragmentation and chromatin condensation demonstrating the pro-apoptotic activity.

W. 5-Hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone

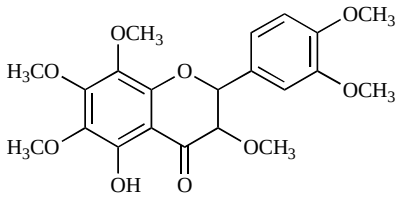
Polymethoxy flavones (PMFs) are a class of flavonoids which expressively act as anticancer molecules by initiating apoptosis in tumour cells. Structurally similar molecules to that of PMFs called 5-hydroxy- 3,6,7,8,3′,4′-hexamethoxyflavone (5-OH-HxMF) (**87**) [101] (Fig. **26**) which stimulate apoptosis by generation of ROS in HL-60 cell line. Cells treated with 5-OH-HxMF showed permanent DNA damage, increase in pro-apoptotic factors such as Bax and endonuclease G, all of which lead to programmed cell death. The SAR studies showed that C-5 hydroxyl group is required to be substituted on the flavone ring for production of ROS. Furthermore, the demethylation of methoxy group present on C-5 in 3,5,6,7,8,3′,4′-hepamethoxyflavone **(88**) to hydroxyl group proves the necessity of C-5 hydroxyl group.

X. Retinoids

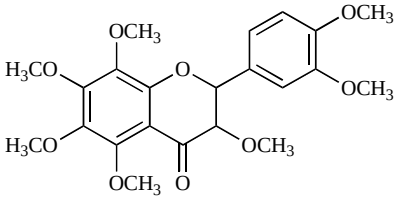
Zunino, *et al.* [102] identified a novel retinoid compound which lacks the ability to stimulate retinoid acid receptors however; it is capable of inducing DNA damage for apoptotic events. *E*-3-(4'-hydroxy-3'-adamantylbiphenyl-4-yl)acrylic acid (**89**) (Fig. **27**) was found as potent antiproliferative agent. According to the SAR study, the hydroxyl group, lipophilic adamantyl moiety and carboxylic groups are assumed essential for the activity.

Y. N-Lactylsphingosine and N-Lactyldihydrosphingosine

N-(*R*)- and *N*-(*S*)- conformation of lactylsphingosine molecules, also called (*R*)-Lac-Cer (**90**) and (*S*)-Lac-Cer (**91**), their dihydro-sphingosine derivatives (**92, 93**) (Fig. **28**) were synthesized and evaluated for the anticancer activities by MTT assay against HL-60 cells [103]. The *S* enantiomer showed significantly higher potency than *R* enantiomer. The potency of dihydrosphingospine derivatives



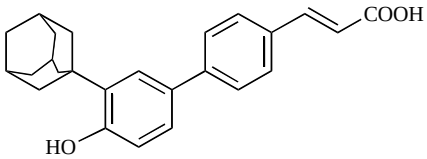
87



88

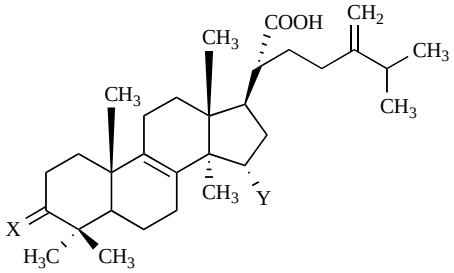
Fig. (26). Chemical structure of polymethoxy flavones.

of *N*-lactylsphingospine was found to be 19-20% and 17-20%, respectively of their R and S enantiomers.



89

Fig. (27). *E*-3-(4'-hydroxy-3'-adamantylbiphenyl-4-yl)acrylic acid.



94: X= O; Y= OH

95 X= Beta-OAc; Y= H

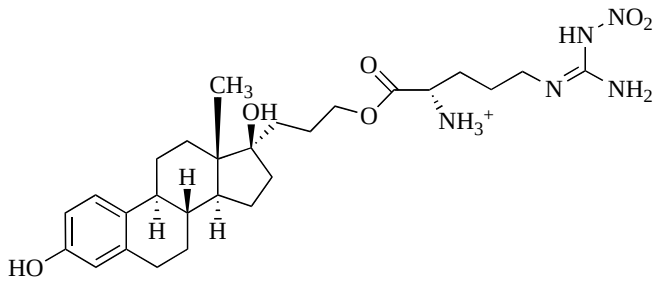
Fig. (29). Chemical structure of lanostanoid triterpenes.

Z. Lanostanoid Triterpenes

Novel lanostanoid triterpene molecules called 3-oxosulfurenic acid (**94**) (Fig. **29**) were isolated from the fruit bodies of *Laetiporus sulphureus* and found to inhibit progression of human HL-60 myleoid leukemia cells [104]. Compound **95** obtained by acetylation of **94,** were found to be the most active inhibitors. The evidence provided for apoptosis induction by these compounds was DNA fragmentation, caspase-3 activation, cleavage of PARP and also release of cytochrome *c* from mitochondria of treated cells.

AA. Estradiol-linked Nitro-L-arginine

The authors developed a hybrid molecule estrogen-linked L-nitro-arginine molecule (ESAr) by conjugating estradiol with N-monomethyl-L-arginine (L-NAME) (Fig. **30**) for inciting apoptotic effect in melanoma cells [105]. Anticancer effect of ESAr was studied on A375 and B16F10 cell lines as they have reasonable amount of expression of estrogen receptors. ESAr produces more post-apoptotic bodies, located by flow cytometric study, L-NAME thereby confirming the process of apoptosis.

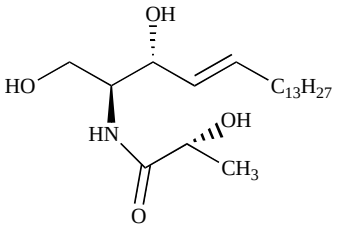


Estrogen-linked L-nitro arginine (ESAr)

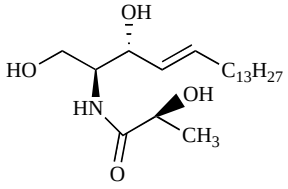
Fig. (30). Chemical structure of estradiol-linked nitro-L-arginine.

AB. Adamantyl-4-hydroxyphenyl-3-chlorocinnamic Acid Derivatives

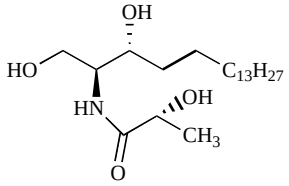
The authors suggested that (*E*)-4-[30-(1-adamantyl)-40-hydroxyphenyl]-3-chlorocinnamic acid (3-Cl-AHPC) (**96**) (Fig. **31**) arrests cell cycle and resulting in apoptosis of cancer cells [106]. Structural modifications in 3-Cl-AHPC were made and tested, which includes nitrogen atoms into the cinnamyl ring and replacement of *E*-double bond with XCH_2 (X = O, N, and S). 2,5-Disubstituted pyridine analogue **97** showed equal solubility profile



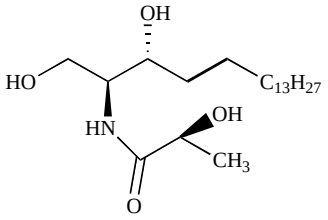
90: *R*- Lactylsphingosine



91: *S*- Lactylsphingosine

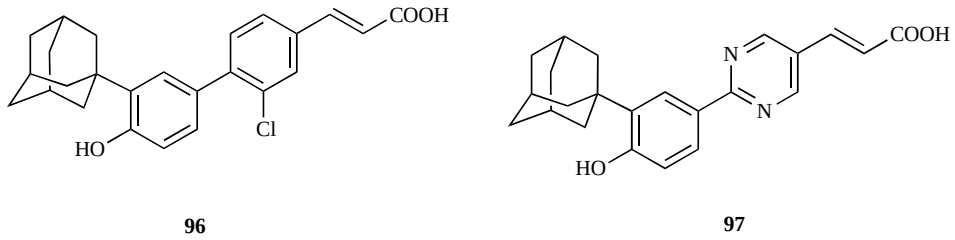


92 :*R*-Lac-DHCer



93: *S*-Lac-DHCer

Fig. (28). *N*-Lactylsphingosine and *N*-lactyldihydrosphingosine derivatives.



96 **97**

Fig. (31). Adamantyl-4-hydroxyphenyl-3-chlorocinnamic acid derivatives.

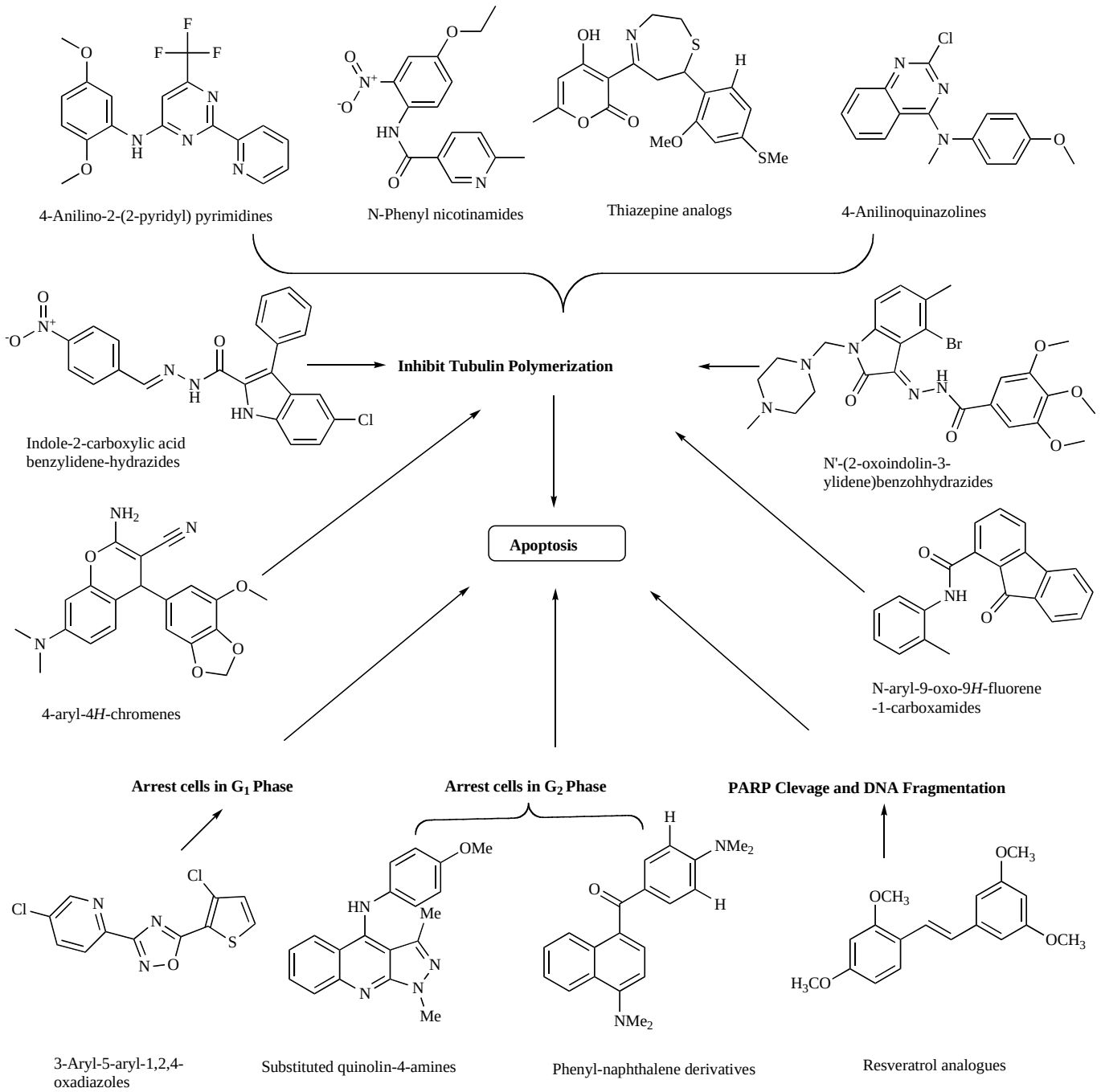


Fig. (32) Simplified scheme of induction of apoptosis by various chemical agents.

Table 1. Summary of Apoptosis-inducing Agents

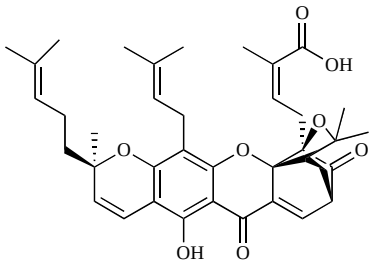
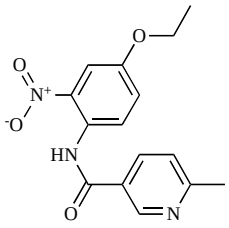
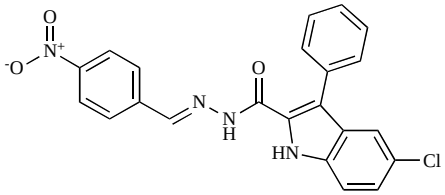
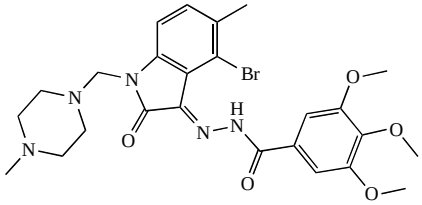
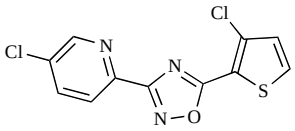
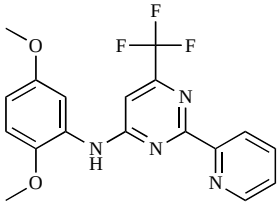
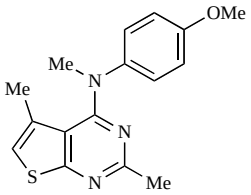
Category	Name and Chemical Structure of Active Compound	Ref.
Gambogic acid and its derivatives	 Gambogic acid	[62]
N-Phenyl nicotinamides	 6-methyl-*N*-(4-ethoxy-2-nitrophenyl)pyridine-3-carboxamide	[67]
Indole-2-carboxylic acid benzylidene-hydrazides	 5-Chloro-3-phenyl-indole-2-carboxylic acid-(4-nitrobenzylidene)-hydrazide	[68]
Substituted N′-(2-oxoindolin-3-ylidene)benzohhydrazides	 (E)-N'-(4-bromo-5-methyl-1-((4-methylpiperazin-1-yl)methyl)-2-oxoindolin-3-ylidene)-3,4,5-trimethoxybenzohydrazide	[69]
3-Aryl-5-aryl-1,2,4-oxadiazoles	 5-Chloro-2-(5-(3-chlorothiophen-2-yl)-1,2,4-oxadiazol-3-yl)pyridine	[70]
4-Anilino-2-(2-pyridyl) pyrimidines	 N-(2,5-dimethoxyphenyl)-2-(pyridin-2-yl)-6-(trifluoromethyl)pyrimidin-4-amine	[71,72]
Substituted pyrimidin-4-amine analogs	 N-(4-methoxyphenyl)-N,2,5-trimethylthieno[2,3-d]pyrimidin-4-amine	[73]

Table 1. contd….

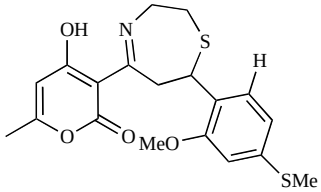
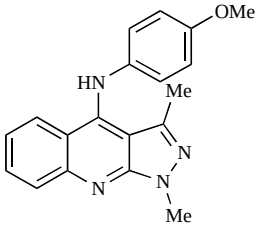
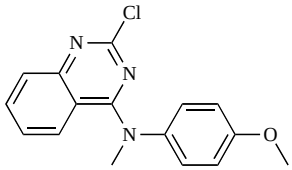
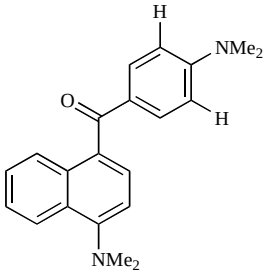
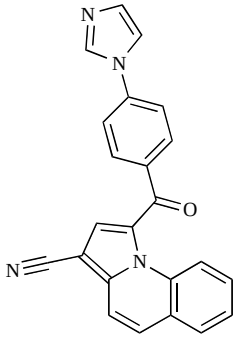
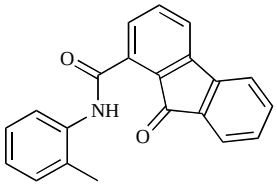
Category	Name and Chemical Structure of Active Compound	Ref.
Thiazepine analogs	 (E)-4-hydroxy-3-(7-(2-methoxy-4-(methylthio)phenyl)-2,3,6,7-tetrahydro-1,4-thiazepin-5-yl)-6-methyl-2H-pyran-2-one	[74]
Substituted quinolin-4-amines	 N-(4-methoxyphenyl)-1,3-dimethyl-1H-pyrazolo[3,4-b]quinolin-4-amine	[75]
4-anilinoquinazolines	 2-chloro-N-(4-methoxyphenyl)-N-methylquinazolin-4-amine	[76-78]
Phenyl-naphthalene derivatives	 (4-(dimethylamino)naphthalen-1-yl)(4-(dimethylamino)phenyl)methanone	[79]
Substituted pyrrolo (1,2-α)quinoline	 1-(4-(1H-imidazol-1-yl)benzoyl)pyrrolo[1,2-a]quinoline-3-carbonitrile	[80,81]
N-aryl-9-oxo-9*H*-fluorene-1-carboxamides	 9-oxo-N-o-tolyl-9H-fluorene-1-carboxamide	[82]

Table 1. contd….

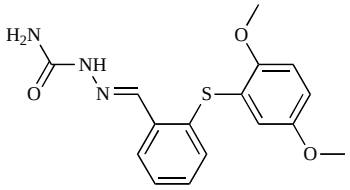
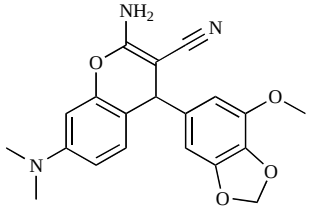
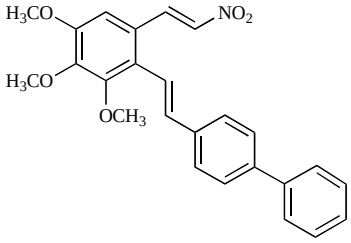
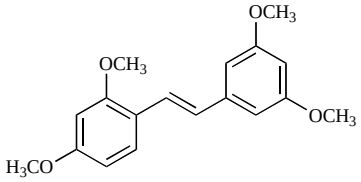
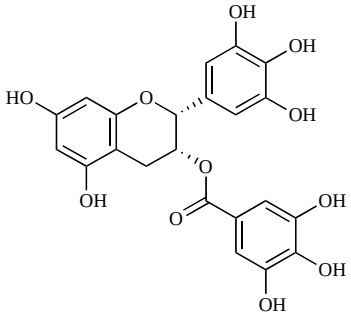
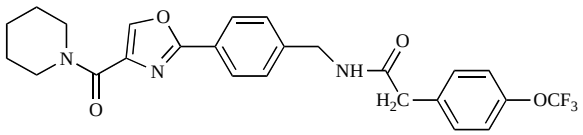
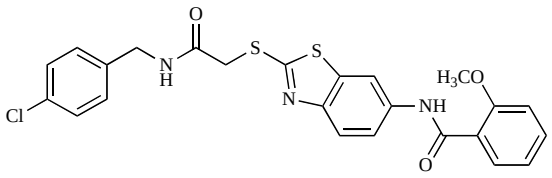
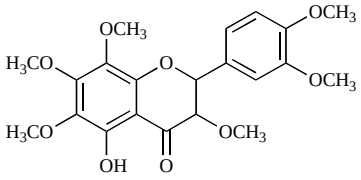
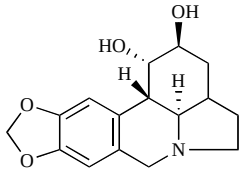
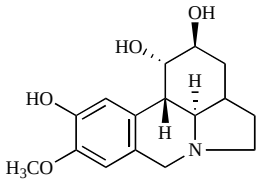
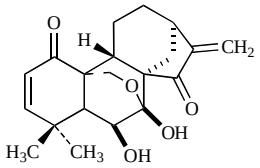
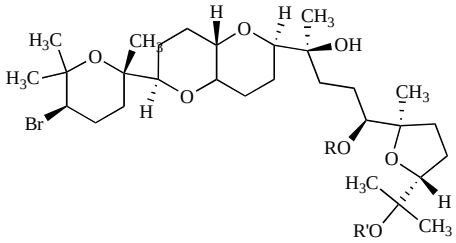
Category	Name and Chemical Structure of Active Compound	Ref.
Guanidine derivatives	 (E)-1-(2-(2,5-dimethoxyphenylthio)benzylidene)semicarbazide	[85]
4-aryl-4*H*-chromenes	 2-amino-3-cyano-7-dimethylamino-4-(3-methoxy-4,5-methylenedioxyphenyl)-4*H*-chromene	[86-89]
Resveratrol analogues	 Resveratrol  2,3',4,5'-tetramethoxy-trans-stilbene (TMS)	[90-93]
Epigallocatechin-3-gallate derivatives	 (*2R,3R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate	[96]
2-Phenyl-oxazole-4-carboxamide	 N-(4-(4-(piperidine-1-carbonyl)oxazol-2-yl)benzyl)-2-(4-(trifluoromethoxy)phenyl)acetamide	[98]
Benzothiazole-2-thiol derivatives	 N-(2-(2-(4-chlorobenzylamino)-2-oxoethylthio)benzo[d]thiazol-6-yl)-2-methoxybenzamide	[100]

Table 1. contd….

Category	Name and Chemical Structure of Active Compound	Ref.
Polymethoxy flavones (PMFs)	 5-Hydroxy- 3,6,7,8,3′,4′-hexamethoxyflavone	[101]
Alkaloids	 Lycorine  Pseudolycorine	[107]
Isodon eriocalyx (Chinese herb)	 Eriocalyxin B	[108]
Protein Phosphatase 2A inhibitor	 Thyrsiferyl 23-Acetate	[109]

and increased apoptotic activity. Computational studies were performed using the homology model form the ultraspiracle (USP) and docking of various energy-minimized conformers of the heterosubstituted analogues, which was compared to 3-Cl-AHPC. This study revealed that analogues with nitrogen atoms in heterocyclic rings corresponding to the cinnamyl phenyl ring showed higher biological activities.

CONCLUSION

Cancer, being an overgrown mass of cells called tumour, occurs due disruption of normal cell life cycle. Tumour can arise due to the negligent condition or inability of the cell to initiate apoptosis even where it undergoes serious trauma. The possibilities of malfunctioning of this inbuilt system may arise anywhere from the intrinsic mitochondrial pathway or extrinsic death receptor pathway, DNA damage on p53 or even from abnormality in ion homeostasis. Since, tumour arises due to unrestrained growth of the cells the treatment of inducing apoptosis is the rationale. Several apoptosis inducers with potent activity are reviewed in this subject of research, some of which are currently undergoing clinical trials. The functional groups in these specialized apoptosis promoters are capable of producing sufficient DNA damage or caspase activation by one route or another. Several compounds (Table **1**) including naturally derived compounds are reviewed along with their potencies and SAR studies in the present communication. The underlying principle of apoptosis inducers is to cause damage to the cell in many ways (Fig. **32**) and to promote toxic conditions which prove to be difficult for the cell life. Hence, rendering the cell incapable of growth inhibits its cellular division and also the size of the tumour. The pro-apoptotic compounds reviewed are specific indeed for each type of tumours. Nevertheless, with the capacity of apoptosis induction cure for tumours can be forecasted. Several compounds in the past decade showed a broad therapeutic success to induce programmed cell death for cancer therapy. Among many small molecules, 4-aryl-4*H*-chromenes were discovered as potent inducers of apoptosis in cell- and caspase-based ASAP HTS assay and found to inhibit tubulin polymerization. SAR studies on this series further led to the discovery of EPC2407 as a lead candidate with excellent efficacy. EPC2407 is in phase I clinical trials as a novel anticancer vascular disrupting agent. Hence, the compounds

of various structural classes described in this review will be useful as templates for the design and discovery of new anticancer agents.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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ABBREVIATIONS

AIF	=	apoptosis inducing factor
Apaf-1	=	apoptotic protease activating factor 1
ASAP HTS	=	anticancer screening apoptosis program high-throughput screening
Bcl	=	B cell lymphoma
BH domain	=	Bcl-2 homology domain
CAD	=	caspase-3-activated DNase
CARD	=	caspase recruitment domains
CD95, CD16, CD2 2	=	cluster of differentiation markers 95, 16, and
cFLIP	=	cellular FLICE inhibitory protein
CTLs	=	cytotoxic T cells
DED	=	death effector domain
DISC	=	death induced signaling complex
DR4, DR5	=	death receptors 4 and 5
FADD	=	Fas-associated death domain
FasL	=	Fas ligand
HTS	=	high-throughput screening
IAPs	=	inhibitors of apoptosis
JNK	=	jun N-terminal kinase
MOMP	=	mitochondrial outer membrane permeabilization
MPT	=	mitochondrial permeability transition
MTP	=	membrane transition permeabilization
NK	=	natural killer
PARP	=	poly ADP-ribose polymerase
RIP1	=	receptor interacting protein 1
ROS	=	reactive oxygen species
SMAC/ DIABLO	=	second mitochondria derived activator of caspase
TRADD	=	TNF receptor associated death domain
TRAF2	=	TNF receptor associated factor 2
TRAIL	=	TNF related apoptosis inducing ligand

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