Caspases: Structure-Guided Design of Drugs to Control Cell Death

Irene T. Weber*, Bin Fang and Johnson Agniswamy

Department of Biology, Molecular Basis of Disease, Georgia State University, Atlanta, GA 30303, USA

Abstract: The structures of caspases reveal the mechanism of binding for non-peptide and protein inhibitors, and have been applied in the design of agents that either inhibit or activate caspases to control cell death in diverse diseases. Decreased cell death is desirable for treatment of stroke, nerve crush injury, myocardial infarction, neuromuscular and neurodegenerative diseases and several non-peptide caspase inhibitors have been developed. In contrast, activation of cell death would be advantageous in cancer therapy, and the strategy is to block the binding of inhibitory proteins to caspases. Recent preclinical studies are described.

Key Words: Cysteine protease, drug design, cell death, substrate recognition, apoptosis.

INTRODUCTION

Cysteinyl aspartate specific proteases (caspases) are cysteine proteases that promote apoptosis and inflammation by proteolytic cleavage of a number of protein substrates [1]. Caspase-1, -4, -5 and -11 are involved in inflammation, while caspase-2, -3, -6, -7, -8, -9, and -10 are initiators or effectors of apoptosis [2]. Many non-apoptotic processes are controlled by caspases, including cell differentiation, heart development, survival, and cytokine expression [3-5]. The proteins are expressed as inactive precursors, or procaspases, which are activated by proteolytic cleavage and/or dimerization [6]. The mammalian caspases differ in their sequences, N-terminal domains in the procaspase, regulation and substrate specificity. Regulation of caspase activity in cells is complex, including interactions with proteins containing caspase recruitment domains (CARD), and inhibitors of apoptosis proteins (IAPs), such as X-linked inhibitor of apoptosis protein (XIAP). Furthermore, many IAPs are regulated by protein inducers of apoptosis, such as second mitochondria-derived activator of caspases (SMAC).

The apoptotic caspases are potential targets for pharmacological modulation of cell death. Excessive cell death due to enhanced activity of caspases is associated with acute (nerve crush injury and stroke) and chronic diseases (Alzheimer's, Parkinson's and Huntington's) [7-10]. Caspase activity and cell loss were increased after spinal cord injury [11]; in Alzheimer's disease brains [12]; and in Huntington's disease [13,14]. It has been suggested that apoptosis is responsible for the death of cardiomyocytes during acute myocardial infarction as well as for progressive loss of surviving cells in failing hearts [15,16]. Consequently, inhibitors of caspases have promise for treatment of stroke, spinal cord injury, and neurodegenerative diseases. Alternatively, reduction or loss of caspase activity is observed in cancer. Inactivating caspase mutations, including deletion of caspase-8, have been reported in various cancers [17-19]. Moreover, some cancers are characterized by overexpression of IAPs which inhibit caspases and cause resistance to apoptosis [20]. Also, caspase activation was required for antiviral treatment response in chronic Hepatitis C virus infection [21]. Therefore, caspase activation has potential in therapy for cancer and infective diseases.

The molecular structures of caspases and their inhibitor complexes are valuable for the design of new therapies [7]. The crystal structures have been determined for unliganded caspases and several complexes with different peptidic, non-peptidic, or protein inhibitors (reviewed in [22]). The active enzyme consists of two small and two large subunits arising from cleavage of the procaspase, and the peptide inhibitors are bound within the active site grooves (Fig. (**1**)).

SUBSTRATE SPECIFICITY OF CASPASES

Knowledge of the substrate specificity of caspases is critical for defining the cellular pathways regulated by caspase-mediated proteolysis, and for the design of active site inhibitors. Caspases are specific for cleavage after P1 Asp, which is unique among known human and mouse proteases with the exception of granzyme B. Three major groups of caspases were defined by their substrate specificity from a positional scanning synthetic peptide library approach [23]. In general, caspases recognize tetrapeptide sequence motifs (P4-P1). The group I or inflammatory caspase-1, 4, 5, 13 and 14 cleave after the tetrapeptide WEHD, while caspase-2, 3 and 7 have a preference for DEXD, and caspase-6, 8, 9 and 10 prefer (L/V)EXD [7,23]. Caspase-2, 8, 9 and 10 are initiator caspases and they are classified as group II, while executioner caspase-3, 6 and 7 form group III [7]. Recent studies on the group III caspase-3 and -7 have shown more variation, although the best peptides have Asp at P4 and P1 [24,25].

Structural studies have provided the molecular basis for caspase specificity for peptides. The structural analysis of caspase-8 in complex with DEVD-CHO showed that both small hydrophobic Ile and acidic Asp residues are tolerated equally well at the P4 position, although LETD is the canonically preferred sequence [26]. Recently, a similar non-polar region for binding aliphatic P4 residues was observed in caspase-7 [27]. Structural studies on various tetrapeptide

*Address correspondence to this author at the Department of Biology, Georgia State University, P.O. Box 4010, Atlanta, GA 30302, USA; Tel: 404 413-5411; Fax: 404 413-5301; E-mail: iweber@gsu.edu

1389-5575/08 $55.00+.00

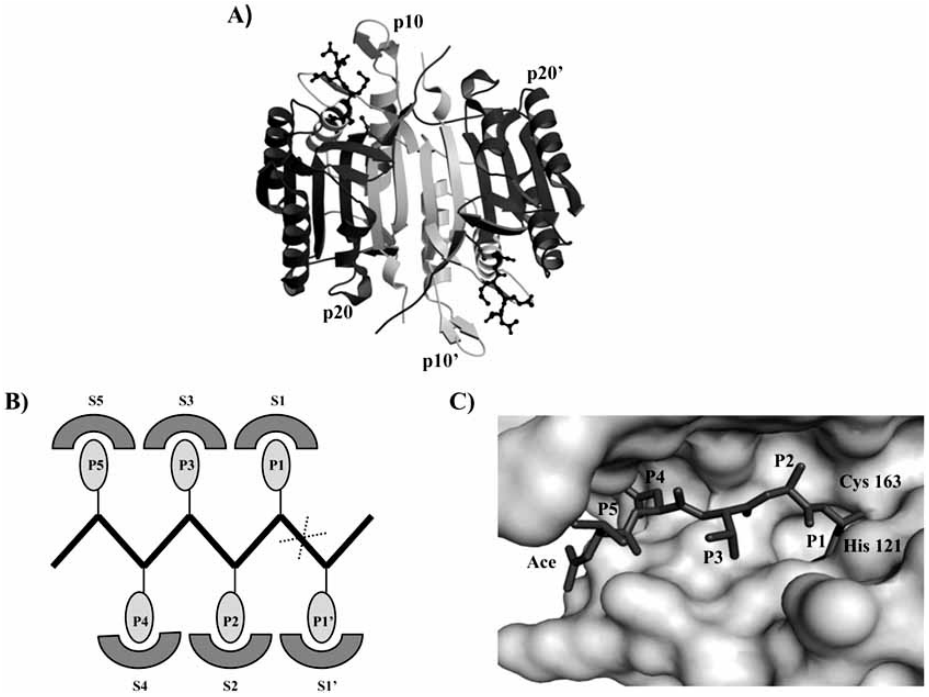

Fig. (1). Structure of caspases. (a) Ribbon diagram of caspase-7 tetrameric assembly from the protein data bank (PDB) code 2QL9. The large p20 subunit and the small p10 subunits are shown in dark and light grey, respectively. The black ball and stick model represents the Ac-DQMD-CHO inhibitor. (b) Schematic substrate binding site of caspases. The residues of peptide substrates or inhibitors, minimally P4-P1', where the scissile peptide bond is between P1 and P1', are bound within pockets S4-S1' formed by the caspase residues. Additional recognition of P5 within S5 subsite was observed for caspase-2 and -3. (c) The catalytic cleft of caspase-3 bound to the pentapeptide inhibitor Ac-VDVAD-CHO (PDB code 2H65). The caspase surface is shown in light grey and the inhibitor residues are shown in black. The catalytic residues of caspase-3 are labeled. The P1 residue is buried deep in the basic cleft next to catalytic cysteine, and the P2-P5 residues occupy defined specificity pockets.

inhibitors of caspase-7 have demonstrated the plasticity of the S2-S4 subsites in agreement with the observed cleavage at noncanonical motifs [27]. These discrepancies in the knowledge of substrate specificity will limit the successful design of selective caspase inhibitors.

Fewer studies have investigated residues outside of the P4-P1 region of substrates. Analysis of the P1' position showed a preference for small amino acids, glycine and serine [28,29]. The pentapeptide P5-P1 was the optimal recognition sequence for caspase-2 substrates [29]. The crystal structure of caspase-2 in its complex with pentapeptide inhibitor Ac-LDESD-CHO revealed the hydrophobic S5 specificity pocket suitable for hydrophobic P5 residues [30]. Similarly, structural and kinetic studies on caspase-3 identified a hydrophobic S5 site and preference for pentapeptides with a hydrophobic P5 (Fig. (**1c**)) [31]. In contrast, caspase-7 preferred the tetrapeptide compared to the pentapeptide substrate [31]. Structural analysis suggested that caspase-1, -7, -8 and -9 differ from caspase-2 and -3 in their selectivity for the P5 position of substrates.

Not all the cellular protein substrates of caspases have been identified, although around 400 have been reported. Recently, a searchable web based resource named the caspase substrate database homepage (CASBAH) (www.casbah.ie) was described that contains substrates of all known caspases [32]. Despite many studies with tetrapeptide substrate analogs, the recognition of the protein substrates in the cell remains less well understood. Inflammatory caspases appear to be very specific, and, unlike the apoptotic caspases with hundreds of proposed substrates, only two confirmed substrates of inflammatory caspases have been described so far [32]. Many known caspase substrates are cleaved at noncanonical sequences, which challenges the specificity deduced from *in vitro* studies with short peptides. Unlike synthetic peptides, cellular proteins are large and their predicted cleavage sites can be partially buried within the protein interior. Sometimes, perfectly accessible regions with canonical sequences are not hydrolyzed by caspases, implying a role for exosite binding in recognition of protein substrates [22]. However, no exosite has been identified yet for caspases. Therefore, not all the predicted caspase substrates with optimal cleavage sequences may be *in vivo* substrates. Rigorous investigation in cells and animals are required to define true caspase substrates [33].

CASPASE INHIBITORS AS POTENTIAL THERAPEUTIC AGENTS

The known inhibitors of caspases include substrate analog peptides, non-peptide compounds, and inhibitory proteins or protein domains. The cellular inhibitors of apoptosis proteins (IAPs) are large with multiple domains including the N-terminal baculoviral inhibitory repeat (BIR) domains that interact with caspases. The best characterized is XIAP, which is important for control of apoptosis in cancer. The BIR2 domain of XIAP inhibits caspase-3 and -7, while the

BIR3 domain inhibits caspase-9. The other IAPs are less well characterized and recent studies suggest they may not directly inhibit caspases [34]. These large protein inhibitors of caspases are less suitable than small molecules for pharmacological interventions.

Substrate analog inhibitors are usually peptides with ketone or aldehyde warheads. These peptide inhibitors are not attractive for therapeutic invention due to lack of specificity, inhibiting all caspases and other cysteine proteases, or poor cell penetration and metabolic stability. The mode of binding of peptide inhibitors has been studied in crystal structures [22,27,31,35-37], and many structure based designs have aimed to reduce the peptidyl features [38-43]. This strategy has led to the identification of several effective substitutions for P2-P4 residues, as summarized in an earlier review [43].

Recently, a number of new peptidomimetic caspase inhibitors were reported. One example is MX1153 (compound **1** in Fig. (**2**)) [39]. Although this inhibitor has fluoromethylketone as a warhead, it showed >5000 fold selectivity for caspase-3 over several other cysteine proteases and serine proteases, and reduced apoptosis in a mouse model. Another potent caspase-3 inhibitor M867 was synthesized recently [40] (compound **2** in Fig (**2**)). The P1 Asp was retained in this compound, while the P2-P4 backbone was replaced by an amino pyrazinone template. The hydrophilic furazanmethylamino showed the best binding affinity at P4, and at P2, the hydrophobic *t*-Bu displayed the optimal whole cell activity, consistent with the substrate specificity of caspase-3. At the P1' position, an N-methyl-N-hexyl group dramatically improved cell permeability. Overall, M867 was highly effective in the *in vivo* anti-apoptosis tests (IC_{50} 20-1200nM). Interestingly, M867 was shown to be about 60 fold more effective against recombinant caspase-3 versus caspase-7, and may facilitate the development of inhibitors selective for caspase-3 rather than caspase-7, which has been a difficult issue for many years.

Other novel compounds are the aza-peptide inhibitors [44,45]. These inhibitors carry P2-P4 peptide residues, but the P1 Asp is modified to aza-Asp (Cα was replaced by N). Ketone and aldehyde were substituted by Michael acceptors as warheads in these compounds, resulting in better selectivity against caspases over clan CA and other clan CD proteases, such as legumain, clostripain, and gingipain K [44]. The interactions between these aza-Asp inhibitors and caspases are similar to those of conventional tetrapeptide inhibitors at the P1-P4 positions, although the extension at P1' allows the exploration of the S1' site of caspases. Crystal structures showed that the S1' pocket of caspase-3 is surrounded by four loops forming an internal space of 900 $Å^3$ [44]. Thr166 and Tyr204 are located at one side of this pocket and Phe128 and Met61 are on the other side (Fig. (**2B**)). Analysis of kinetic data and crystal structures suggested that a bulky hydrophobic group can penetrate deeply into the S1' pocket of caspase-3 forming favorable interactions. Consequently, this inhibitor had enhanced binding affinities compared with compounds with small P1' groups [44,45]. In contrast, caspase-8 has a relatively small S1' site created by Leu254, Ile257 and Tyr324 (Fig. (**2B**)), and prefers small non-polar groups such as ethyl [44]. Enzyme inhibition assays suggested that caspase-7 has the same S1' se-

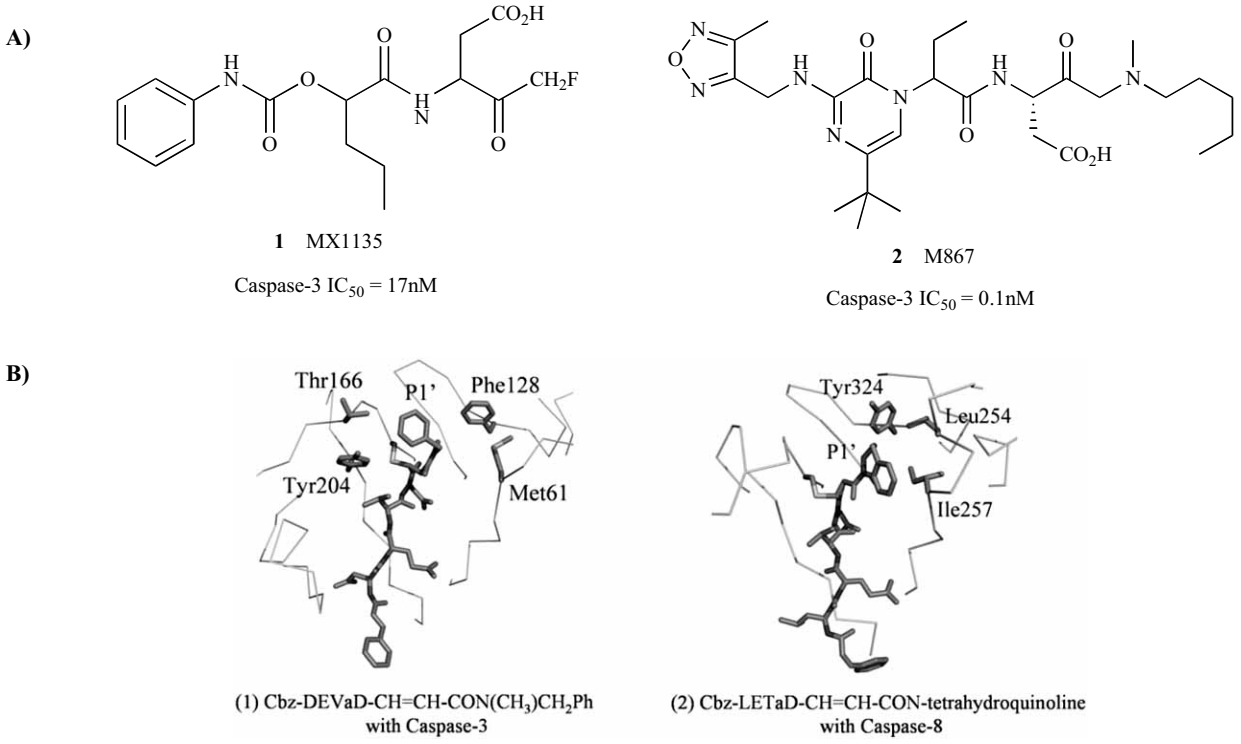

Fig. (2). Peptidomimetic inhibitors. **A.** MX1153 and M867 are two examples of peptidomimetic inhibitors of caspase-3. **B.** The binding of aza-peptide inhibitors in caspase-3 (PDB code 2C2M) and caspase-8 (PDB code 2C2Z). Side chains of caspase S1' residues are shown in stick format and labeled with residue names. The aza-peptide Michael acceptor inhibitors are shown in stick format and their P1' groups are labeled.

lectivity as caspase-3, perhaps due to their highly homologous sequences; esters were the best candidates at P1' for caspase-2; ethyl was the optimal P1' group for caspase-9 and 10; while there was no distinct S1' selectivity observed for caspase-6 [44].

Isatin Sulfonamide Analogs

Parallel to the modification of peptidic inhibitors, potential caspase inhibitors have been discovered by searching in the available chemical libraries. 5-nitroisatin was first discovered as a nonpeptide caspase-3 inhibitor by using a high-throughput screen in the SmithKline Beecham compound collection [46]. The modification of this compound led to the development of a series of potent isatin analog inhibitors of caspase-3/7, including compound **3** in Fig. (**3A**) with K_{iapp} values of 1.2 nM and 6 nM for caspase-3 and caspase-7, respectively [47]. Kinetic data and crystallographic analysis on the compound **5** in the complex with caspase-3 indicated that a reversible covalent, tetrahedral adduct was formed between the isatin carbonyl and the active site cysteine [46]. Unlike most peptidomimetic inhibitors, the isatin inhibitors lack a P1 Asp and the caspase S1 pocket is thus empty [46] Fig. (**3C**). The pyrrolidine rings interact with caspase-3/7 primarily in the hydrophobic S2 pocket formed by three aromatic residues: tyrosine, tryptophan, and phenylalanine (Fig. (**3D**)). Because caspases differ in their S2 pockets, this exclusive binding profile of these inhibitors results in 1000 fold higher selectivity for caspase-3/7 versus many other caspases (1, 2, 4, 6, and 8). This unique feature enables them to specifically inhibit caspase-3 and 7, which has been unachievable by peptidomimetic inhibitors to date. Surprisingly, a recent biochemical and biophysical study showed that compound **3** abolished caspase-3 activity upon binding to only one active site of the homodimer [42]. This interesting phenomenon, however, was not observed in the previous crystal structure [46]. Despite the ambiguities in the inhibition mechanism of isatin sulfonamide inhibitors, their effectiveness in reducing apoptosis has been clearly demonstrated [46,48]. N1-

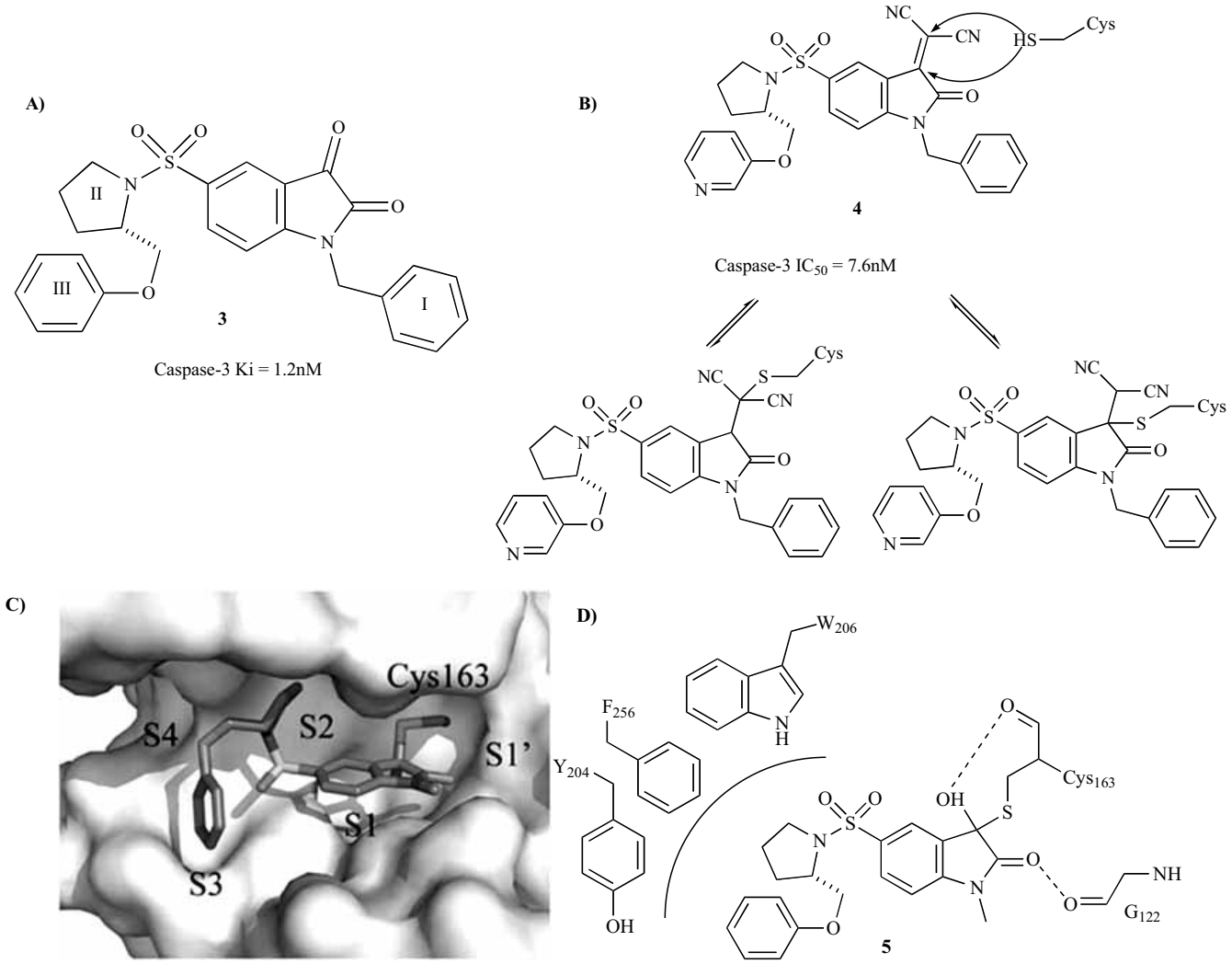

Fig. (3). Isatin sulfonamide analog inhibitors. **A.** Isatin sulfonamide analog inhibitors of caspase-3/7. I, II, and III indicate three groups modified in later studies (12,49). **B.** IMA inhibitor, and proposed reaction mechanism with caspases. **C.** Crystal structure of 1GFW shows the binding mode of compound **5** in caspase-3. Caspase-3 active groove is in a surface representation. S1'~S4 binding pockets are labeled. **D.** Schematic representation of interactions between inhibitor **5** and caspase-3. Hydrogen interactions are shown in dashed lines. Caspase-3 S2 residues having non-polar interactions with inhibitor 5 are labeled.

substituted 5-pyrrolidinylsulfonyl isatins have been shown to inhibit caspase processing in apoptotic endothelial cells [49]. For instance, the compound (s)-(+)-5- [1-(2-methoxymethyl-pyrrolidine)sulfonyl]isatin (MMPSI) reduced myocardial ischemic injury in an isolated rabbit heart model [48].

The isatin sulfonamide compounds, which are potent and selective reversible nonpeptide caspase inhibitors, have become good templates for the structure based drug design. Recently, modifications at three different regions of an isatin sulfonamide molecule were evaluated for caspase-3/7 inhibition (Fig. (**3A**)) [50]. In the region I, neither substitution of the para-position nor replacement with a pyridine ring significantly changed the inhibitory potency. Nevertheless, a 20 fold lower potency was observed when the phenoxymethyl moiety in this region was removed, indicating the hydrophobic group in the region I possibly binds in the non-polar S1' site of caspase-3/7. In the region II, the replacement of pyrrolidine ring with an azetidine ring did not remarkably improve the potency. In the region III, the substitution of the benzene ring with a pyridine ring increased the potency by 3-4 times against caspase-3/7, possibly due to the introduction of a hydrophilic interaction between the phenoxymethyl moiety and the S3 binding pocket of caspase-3/7 [50].

An earlier study claimed that the *in vivo* applications of isatin sulfonamide inhibitors may be limited because of the highly reactive nature of their ketone carbonyl groups toward nucleophiles [46]. A possible solution for this issue has been achieved recently by a new class of isatin sulfonamide analog compounds called isatin Michael acceptors (IMAs) [51]. They contain Michael addition acceptors as their warheads, which can be attacked by the thiol nucleophile of cysteine (Fig. (**3B**)). The binding affinities of certain IMAs, such as compound **4** in Fig. (**3B**), have reached the nanomolar range against caspase-3/7. Interestingly, all IMAs showed 10-fold higher potency for caspase-6 relative to their isatin sulfonamide analogs [51]. This potency may provide a valuable hint for the design of caspase-6 inhibitors, which has lagged far behind the development of caspase-3/7 inhibitors.

Quinoline Derivatives

1,3-dioxo-2,3-diydro-1H-pyrrolo[3,4-c]quinoline (Fig. (**4B**)) was recently discovered as a small molecule inhibitor of caspase-3/7. It represents a novel scaffold for non-peptide inhibitors of executioner caspases. A number of derivatives varying at the R1, R2 and R3 positions were synthesized and evaluated in caspase-3 *in vitro* inhibition assays [52-55]. Compound **6** with the combination of a morpholinesulfonyl moiety at position R1 and 1,3,5-trimethyl-1*H*-pyrazol-4-yl group at R2 was the lead compound among those tested when R3 was fixed to $-CH_3$ (Fig. (**4A**)) [55]. It showed an IC_{50} value of 4 nM against caspase-3, comparable to the commercial tetrapeptide inhibitor Ac-DEVD-CHO (IC_{50}=3.1 nM under the same conditions). The best R3 moiety was examined by fixing R1 and R2 groups. Methyl group and phenyl group displayed the best IC_{50} values of 23nM and 27nM, respectively [53]. Overall, the best combination of R1-R3 is shown in compound **8** in Fig. (**4A**), however, its activity remains to be determined.

The inhibitory mechanism of this class of inhibitors has been suggested to arise from nucleophilic attack of the caspase catalytic cysteine on the 'phthalimide' carbonyls [55] (Fig. (**4B**)). However, the mode of binding of these inhibitors has not been confirmed due to the absence of structural evidence. In addition, recent kinetic studies indicated that these compounds were noncompetitive reversible inhibitors for caspase-3 [53], implying that they do not bind in the active site groove of caspase-3 or interact with the catalytic cysteine. Possibly these compounds bind in another region of caspase-3, such as the allosteric binding site. Further insights into the mode of binding of these inhibitors could lead to the discovery of new inhibitory mechanisms.

Another subset of this class of inhibitors is the isoquinoline-1,3,4-trione (compound **9**) derivatives [56]. The best compound (compound **10**) showed a comparable potency against caspase-3 to that of the peptidic inhibitor Ac-DEVD-CHO. Although, most of these compounds demonstrated better activities against caspase-3/7 over other caspases, the differences were not sufficient to ensure selectivity. However, some isoquinoline-1,3,4-trione derivatives specifically inactivated caspase-1 [57]. Importantly, this class of compounds showed 50-1000 fold higher specificities against caspases relative to other cysteine and serine proteases [56]. The compounds were validated in PC21 cells and primary neuronal cells, where they effectively attenuated apoptosis induced by the amyloid-beta protein that is released by caspase-3 in the progression of Alzheimer's disease [58]. Enzyme kinetic studies indicated that these compounds performed as noncompetitive inhibitors [56] and they bound to caspases irreversibly in a slow-binding manner [58]. The binding mode of these compounds is unknown. Therefore, the future development of these promising quinoline derivatives would benefit greatly from structural analysis of the caspase-inhibitor complexes.

CASPASE ACTIVATORS AS POTENTIAL THERAPEUTIC AGENTS

Caspases are targets for selective induction of apoptosis in cancer cells [59,60]. Selective targeting of cancer cells overexpressing v-erb-b2 erythroblastic leukemia viral oncogene homolog-2/human epidermal growth factor receptor-2 (erbB2/HER2) has been achieved using chimeric caspases comprising a single chain anti-erbB2/HER2 antibody and a constitutively active caspase-3 or 6 [61,62]. An alternative, successful strategy to induce apoptosis targets XIAP, which is an important cellular inhibitor of caspase-3, -7 and -9 [63,64]. Antagonists of XIAP inhibition of caspase-3 have shown antitumor activity on a variety of cell types and sensitized cells to chemotherapy [65]. These caspase "activators" are essentially inhibitors of the XIAP interaction with caspases. Knowledge of the crystal structures of different complexes of XIAP has been valuable for drug design. The available complexes include the BIR2 domain of XIAP with caspase-3 and -7, the complex of the BIR3 domain with caspase-9, and the BIR3 domain with the human mitochondrial antagonist SMAC. The novel therapeutic agents under development include antisense oligonucleotides and small molecule inhibitors that bind to the BIR domains of XIAP. Small molecules in preclinical development include peptidomimetics, the natural product embelin, and polyphenylurea compounds.

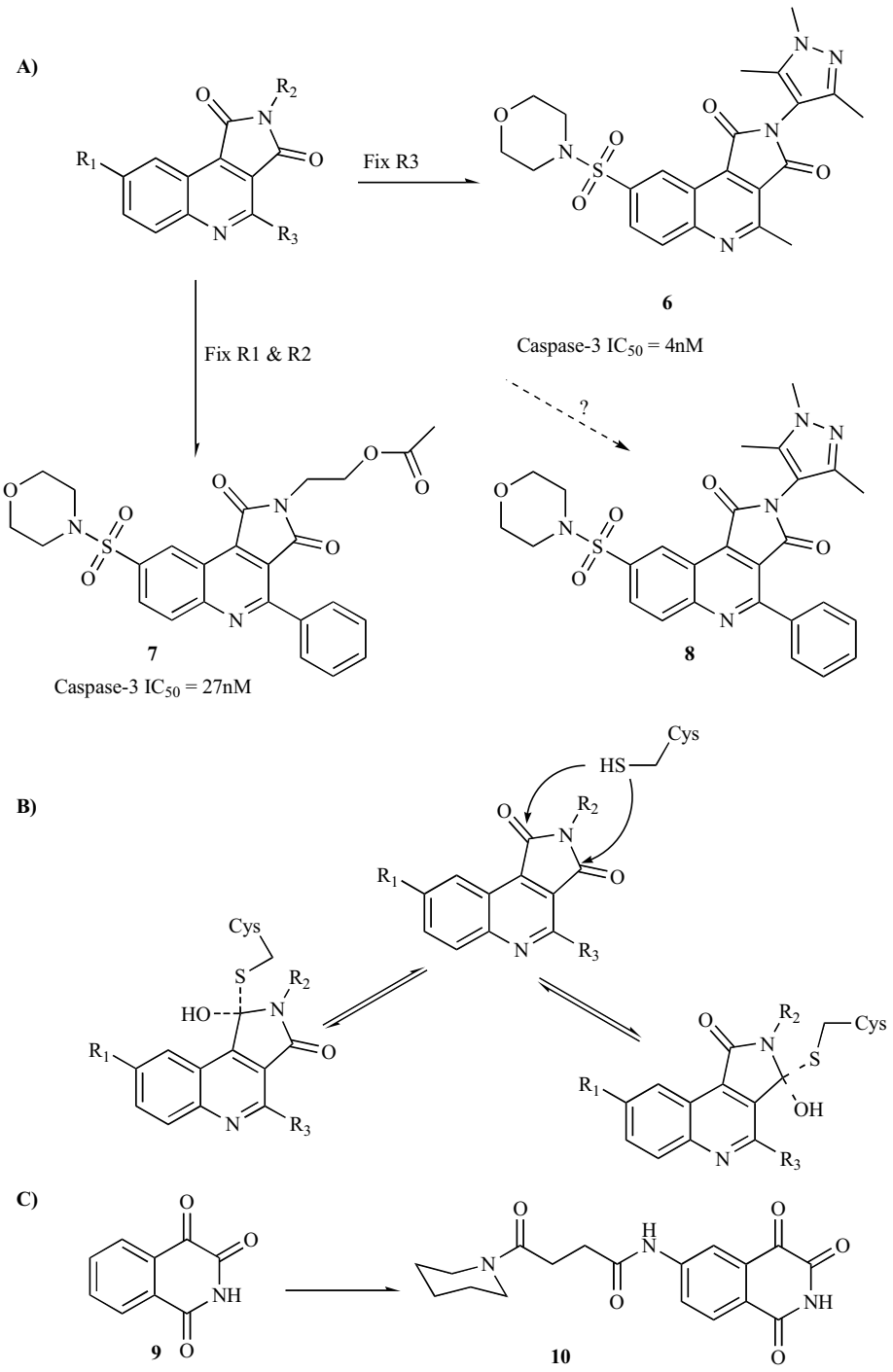

Fig. (4). Quinoline derivative inhibitors. **A**. Modification of a general quinoline derivative inhibitor led to the discovery of two potent caspase-3 inhibitors, compounds **6** and **7**. Compound **8** suggests a putative better inhibitor. **B**. Putative inhibitory mechanism of quinoline inhibitors against caspases. **C.** Compound **10** is an example of isoquinoline inhibitor derived from compound **9**.

The natural product embelin (2,5-dihydroxy-3- undecyl-cyclohexa-2,5-diene-1,4-dione) (Fig. (**5A**) compound **11**) was discovered through structure-based computational screening of a library of traditional herbal medicines [66]. Embelin was shown to interact with residues in the BIR3 domain of XIAP near the binding sites for SMAC and caspase-9. This compound is cell-permeable and induces apoptosis in prostate cancer cells expressing high levels of XIAP. Evaluation of embelin analogs has resulted in a promising lead compound with the K_i value of 180 nM for the BIR3 domain (Fig. (**5A**) compound **12**) [67].

Several groups have designed and tested peptidomimetic compounds based on the crystal structure of the SMAC peptide bound to the BIR3 domain of XIAP [68]. The SMAC residues AVPI interact with the BIR3 residues in the complex and provided the initial template for designs. Various SMAC mimics have been shown to enhance apoptosis in

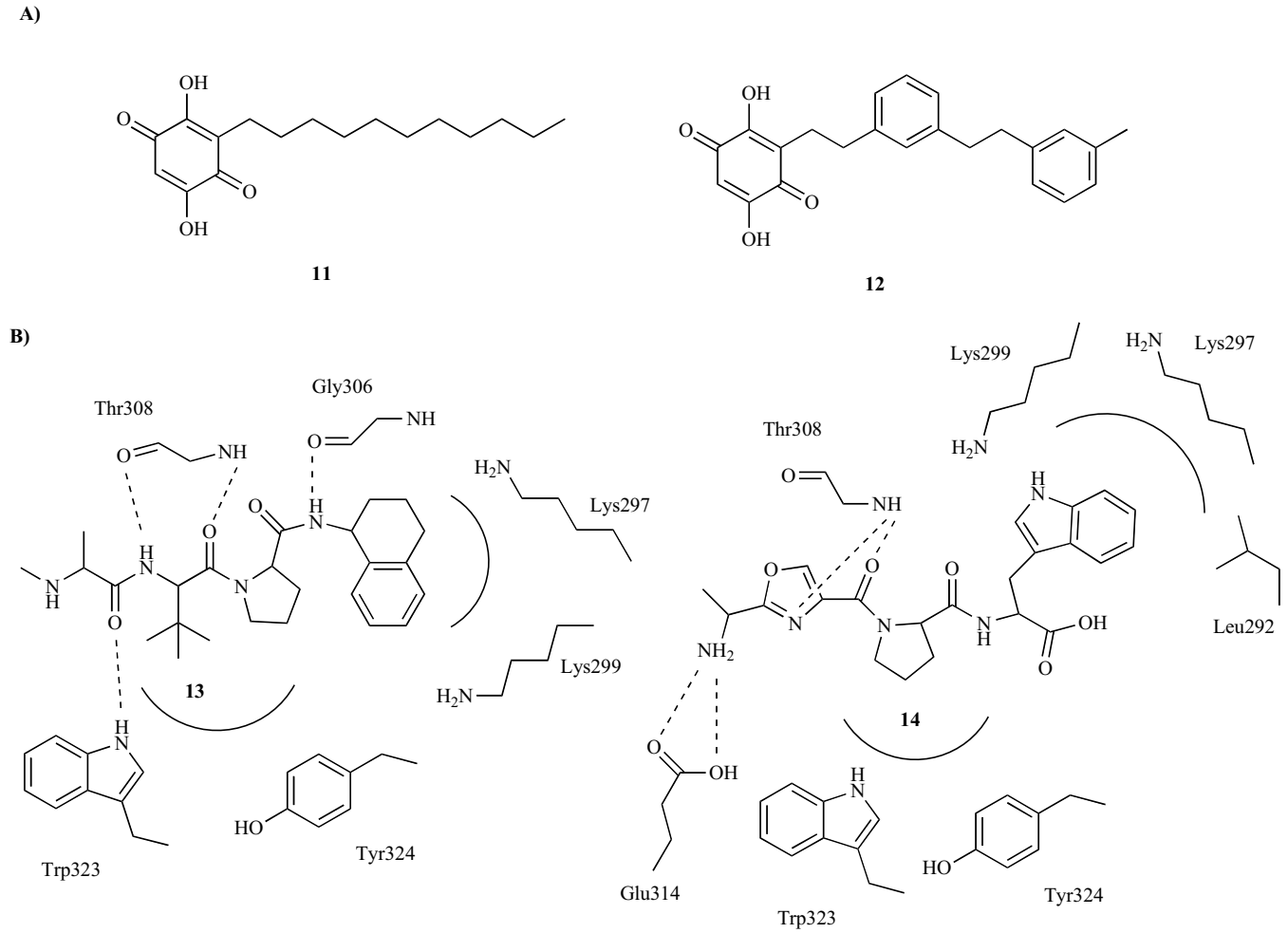

Fig. (5). A. Structure of embelin (**11)** and its lead analog inhibitor (**12)** against BIR3 of XIAP. **B.** Schematic representation of compound **13** bound to BIR3 domain of XIAP (left, PDB code 1TFQ) and compound **14** bound to the same target (right, PDB code 2OPY). Hydrogen bonds are indicated by dashed lines and non-polar binding pockets on the protein are represented by curves.

human cancer cells [69-71]. One series of peptidomimetic compounds were designed to vary the groups at the second and fourth positions [70]. The mode of binding for the compound with the low K_d of 0.012 μM for the BIR3 domain was verified by NMR spectroscopy (Fig. (**5B**) compound **13**). This compound, and its derivative with a cyclohexyl group substituting for the t-Bu group, were effective in inducing apoptosis in human cancer cells lines and potent in breast cancer xenograft models [70]. Another series of compounds used oxazole at the N-terminus and varied the fourth amino acid [71]. The derivative with Trp gave the best Kd of 30 μM for the BIR3 domain (Fig. (**5B**) compound **14**) and showed increased apoptosis in cells compared to the SMAC peptide, probably due to better cell permeability. Symmetric compounds with two peptidomimetic groups connected by a linker were designed to mimic the SMAC dimer [69]. Although this dimeric design is attractive, no structural verification has been reported yet. The best compound has a Ki of 120 nM, which is better than the measured Ki of 260 nM for the SMAC peptide AVPF. This compound showed promise in sensitizing non-small cell lung cancer cells and ovarian cancer cells for apoptosis in chemotherapy [72,73].

The polyphenylurea derivatives were identified by screening a combinatorial library [65]. They were shown to selectively displace caspase-3 and not caspase-9 from XIAP,

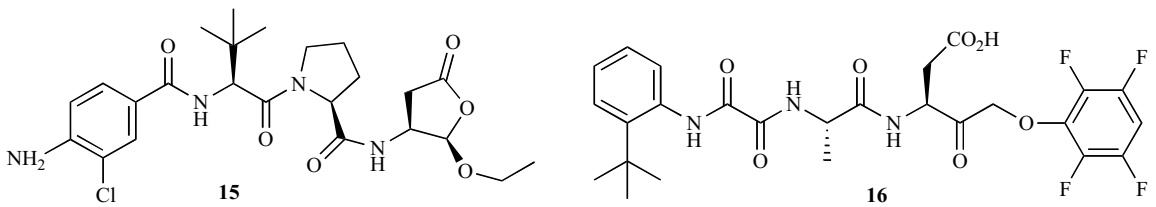

Fig. (6). Structure of VX-765 (**15)** and IDN-6556 (**16)** in clinical trials.

which suggests these compounds bind to the BIR2 domain [74]. However, the mode of binding has not been verified. The most promising compound is *N*-[(*5R*)-6-[(anilinocarbonyl) amino]-5-((anilinocarbonyl) {[(*2R*)-1-(4-cyclohexylbutyl) pyrrolidin-2-yl] methyl} amino) hexyl]-*N*-methyl-*N'*-phenylurea (1396-12), which is an XIAP antagonist that induces apoptosis in B-cell lymphomas and acute myelogenous leukemia cells [75,76].

CONCLUSIONS

Despite substantial efforts, relatively few compounds targeting caspases are currently in clinical trials. The caspase inhibitors VX-765 (Fig. (**6**) compound **15**), VX-740, and MX-1013 were reviewed previously [77]. Caspase-1 inhibitor VX-756 is in Phase II clinical trials for the treatment of inflammatory diseases [78]. VX-799, a small molecule pan caspase inhibitor developed by Vertex/Serono for septic organ failure, is in Phase I clinical trials. The pan caspase inhibitor IDN-6734 developed for the treatment of acute myocardial infarction is also in Phase I clinical trials [78]. IDN-6556 (Fig. (**6**) compound **16**) is an oxamyl dipeptide pancaspase inhibitor [43] with promising results in Phase I clinical trials [77] and in Phase II clinical trials in patients with liver transplants [79]. Also, IDN-6556 has promise as a potential drug for chronic hepatitis C [80].

The strategy of activating caspases by targeting XIAP has proven successful. The most advanced is the second generation antisense compound AEG35156, which is in Phase I/II clinical trials for solid tumors, leukemia and a variety of other types of cancer [81]. Also, small molecule inhibitors of XIAP are in preclinical development including analogs of the natural product embelin, peptidomimetics and polyphenylurea derivatives.

Information from crystal structures of caspase complexes has proven valuable in the design of more selective and potent caspase inhibitors such as the aza-peptides with Michael acceptors, and the non-peptide isatin derivatives, and for the design of SMAC mimics to activate caspases. In parallel, structure-based screening of chemical libraries has identified novel compounds to control caspase activity like the isatin derivative inhibitors and the activator embelin. Overall, the development of pharmacological agents to control caspase-mediated cell death has greatly benefited from structural studies, and several compounds are in clinical trials or preclinical development for treatment of various diseases.

REFERENCES

[1] Alnemri, E. S.; Livingston, D. J.; Nicholson, D. W.; Salvesen, G.; Thornberry, N. A.; Wong, W. W.; Yuan, J. *Cell,* **1996,** *87*, 171.
[2] Thornberry, N. A., Lazebnik, Y. *Science,* **1998,** *281*, 1312.
[3] Lakhani, S. A.; Masud, A.; Kuida, K.; Porter, G. A., Jr.; Booth, C. J.; Mehal, W. Z.; Inayat, I.; Flavell, R. A. *Science,* **2006,** *311*, 847.
[4] Sehra, S., Dent, A. L. *Crit Rev. Immunol.,* **2006,** *26*, 133.
[5] Siegel, R. M. *Nat.Rev.Immunol.,* **2006,** *6*, 308.
[6] Boatright, K. M., Salvesen, G. S. *Curr. Opin. Cell Biol.,* **2003,** *15*, 725.
[7] Lavrik, I. N.; Golks, A.; Krammer, P. H. *J. Clin. Invest ,* **2005,** *115*, 2665.
[8] Nicholson, D. W. *Nature,* **2000,** *407*, 810.
[9] Robertson, G. S.; Crocker, S. J.; Nicholson, D. W.; Schulz, J. B. *Brain Pathol.,* **2000,** *10*, 283.
[10] Philchenkov, A. *J. Cell Mol. Med.,* **2004,** *8*, 432.
[11] Takagi, T.; Takayasu, M.; Mizuno, M.; Yoshimoto, M.; Yoshida, J. *Neurol. Med. Chir (Tokyo),* **2003,** *43*, 20.
[12] Matsui, T.; Ramasamy, K.; Ingelsson, M.; Fukumoto, H.; Conrad, C.; Frosch, M. P.; Irizarry, M. C.; Yuan, J.; Hyman, B. T. *J. Neuropathol. Exp. Neurol.,* **2006,** *65*, 508.
[13] Hermel, E.; Gafni, J.; Propp, S. S.; Leavitt, B. R.; Wellington, C. L.; Young, J. E.; Hackam, A. S.; Logvinova, A. V.; Peel, A. L.; Chen, S. F.; Hook, V.; Singaraja, R.; Krajewski, S.; Goldsmith, P. C.; Ellerby, H. M.; Hayden, M. R.; Bredesen, D. E.; Ellerby, L. M. *Cell Death. Differ.,* **2004,** *11*, 424.
[14] Kim, Y. J.; Yi, Y.; Sapp, E.; Wang, Y.; Cuiffo, B.; Kegel, K. B.; Qin, Z. H.; Aronin, N.; DiFiglia, M. *Proc. Natl. Acad. Sci. U.S.A,* **2001,** *98*, 12784.
[15] Zidar, N.; Jera, J.; Maja, J.; Dusan, S. *Adv.Clin.Chem.,* **2007,** *44*, 1.
[16] Takemura, G., Fujiwara, H. *J. Cell Mol. Med.,* **2006,** *10*, 56.
[17] Kim, H. S.; Lee, J. W.; Soung, Y. H.; Park, W. S.; Kim, S. Y.; Lee, J. H.; Park, J. Y.; Cho, Y. G.; Kim, C. J.; Jeong, S. W.; Nam, S. W.; Kim, S. H.; Lee, J. Y.; Yoo, N. J.; Lee, S. H. *Gastroenterology,* **2003,** *125*, 708.
[18] Soung, Y. H.; Lee, J. W.; Kim, S. Y.; Sung, Y. J.; Park, W. S.; Nam, S. W.; Kim, S. H.; Lee, J. Y.; Yoo, N. J.; Lee, S. H. *Oncogene,* **2005,** *24*, 141.
[19] Soung, Y. H.; Lee, J. W.; Kim, S. Y.; Jang, J.; Park, Y. G.; Park, W. S.; Nam, S. W.; Lee, J. Y.; Yoo, N. J.; Lee, S. H. *Cancer Res.,* **2005,** *65*, 815.
[20] Vucic, D.; Stennicke, H. R.; Pisabarro, M. T.; Salvesen, G. S.; Dixit, V. M. *Curr. Biol.,* **2000,** *10*, 1359.
[21] Volkmann, X.; Cornberg, M.; Wedemeyer, H.; Lehner, F.; Manns, M. P.; Schulze-Osthoff, K.; Bantel, H. *Hepatology,* **2006,** *43*, 1311.
[22] Fuentes-Prior, P., Salvesen, G. S. *Biochem. J.,* **2004,** *384*, 201.
[23] Thornberry, N. A.; Rano, T. A.; Peterson, E. P.; Rasper, D. M.; Timkey, T.; Garcia-Calvo, M.; Houtzager, V. M.; Nordstrom, P. A.; Roy, S.; Vaillancourt, J. P.; Chapman, K. T.; Nicholson, D. W. *J. Biol. Chem.,* **1997,** *272*, 17907.
[24] Lien, S.; Pastor, R.; Sutherlin, D.; Lowman, H. B. *Protein J.,* **2004,** *23*, 413.
[25] Yoshimori, A.; Takasawa, R.; Tanuma, S. *BMC. Pharmacol.,* **2004,** *4*, 7.
[26] Blanchard, H.; Donepudi, M.; Tschopp, M.; Kodandapani, L.; Wu, J. C.; Grutter, M. G. *J. Mol. Biol.,* **2000,** *302*, 9.
[27] Agniswamy, J.; Fang, B.; Weber, I. T. *FEBS J.,* **2007,** *274*, 4752.
[28] Stennicke, H. R.; Renatus, M.; Meldal, M.; Salvesen, G. S. *Biochem. J.,* **2000,** *350 Pt 2*, 563.
[29] Talanian, R. V.; Quinlan, C.; Trautz, S.; Hackett, M. C.; Mankovich, J. A.; Banach, D.; Ghayur, T.; Brady, K. D.; Wong, W. W. *J. Biol. Chem.,* **1997,** *272*, 9677.
[30] Schweizer, A.; Briand, C.; Grutter, M. G. *J. Biol. Chem.,* **2003,** *278*, 42441.
[31] Fang, B.; Boross, P. I.; Tozser, J.; Weber, I. T. *J. Mol. Biol.,* **2006,** *360*, 654.
[32] Luthi, A. U., Martin, S. J. *Cell Death. Differ.,* **2007,** *14*, 641.
[33] Timmer, J. C., Salvesen, G. S. *Cell Death. Differ.,* **2007,** *14*, 66.
[34] Eckelman, B. P.; Salvesen, G. S.; Scott, F. L. *EMBO Rep.,* **2006,** *7*, 988.
[35] Blanchard, H.; Kodandapani, L.; Mittl, P. R.; Marco, S. D.; Krebs, J. F.; Wu, J. C.; Tomaselli, K. J.; Grutter, M. G. *Structure.,* **1999,** *7*, 1125.
[36] Mittl, P. R.; Di Marco, S.; Krebs, J. F.; Bai, X.; Karanewsky, D. S.; Priestle, J. P.; Tomaselli, K. J.; Grutter, M. G. *J. Biol. Chem.,* **1997,** *272*, 6539.
[37] Watt, W.; Koeplinger, K. A.; Mildner, A. M.; Heinrikson, R. L.; Tomasselli, A. G.; Watenpaugh, K. D. *Structure,* **1999,** *7*, 1135.
[38] Becker, J. W.; Rotonda, J.; Soisson, S. M.; Aspiotis, R.; Bayly, C.; Francoeur, S.; Gallant, M.; Garcia-Calvo, M.; Giroux, A.; Grimm, E.; Han, Y.; McKay, D.; Nicholson, D. W.; Peterson, E.; Renaud, J.; Roy, S.; Thornberry, N.; Zamboni, R. *J. Med. Chem.,* **2004,** *47*, 2466.
[39] Wang, Y.; Guan, L.; Jia, S.; Tseng, B.; Drewe, J.; Cai, S. X. *Bioorg. Med. Chem. Lett.,* **2005,** *15*, 1379.
[40] Han, Y.; Giroux, A.; Colucci, J.; Bayly, C. I.; Mckay, D. J.; Roy, S.; Xanthoudakis, S.; Vaillancourt, J.; Rasper, D. M.; Tam, J.; Tawa, P.; Nicholson, D. W.; Zamboni, R. J. *Bioorg. Med. Chem. Lett.,* **2005,** *15*, 1173.
[41] Han, Y.; Giroux, A.; Grimm, E. L.; Aspiotis, R.; Francoeur, S.; Bayly, C. I.; Mckay, D. J.; Roy, S.; Xanthoudakis, S.; Vaillancourt,

J. P.; Rasper, D. M.; Tam, J.; Tawa, P.; Thornberry, N. A.; Paterson, E. P.; Garcia-Calvo, M.; Becker, J. W.; Rotonda, J.; Nicholson, D. W.; Zamboni, R. J. *Bioorg. Med. Chem. Lett.,* **2004,** *14*, 805.

[42] Aulabaugh, A.; Kapoor, B.; Huang, X.; Dollings, P.; Hum, W. T.; Banker, A.; Wood, A.; Ellestad, G. *Biochemistry,* **2007,** *46*, 9462.

[43] O'Brien, T., Lee, D. *Mini. Rev. Med. Chem.,* **2004,** *4*, 153.

[44] Ekici, O. D.; Li, Z. Z.; Campbell, A. J.; James, K. E.; Asgian, J. L.; Mikolajczyk, J.; Salvesen, G. S.; Ganesan, R.; Jelakovic, S.; Grutter, M. G.; Powers, J. C. *J. Med. Chem.,* **2006,** *49*, 5728.

[45] Ganesan, R.; Jelakovic, S.; Campbell, A. J.; Li, Z. Z.; Asgian, J. L.; Powers, J. C.; Grutter, M. G. *Biochemistry,* **2006,** *45*, 9059.

[46] Lee, D.; Long, S. A.; Adams, J. L.; Chan, G.; Vaidya, K. S.; Francis, T. A.; Kikly, K.; Winkler, J. D.; Sung, C. M.; Debouck, C.; Richardson, S.; Levy, M. A.; DeWolf, W. E., Jr.; Keller, P. M.; Tomaszek, T.; Head, M. S.; Ryan, M. D.; Haltiwanger, R. C.; Liang, P. H.; Janson, C. A.; McDevitt, P. J.; Johanson, K.; Concha, N. O.; Chan, W.; Abdel-Meguid, S. S.; Badger, A. M.; Lark, M. W.; Nadeau, D. P.; Suva, L. J.; Gowen, M.; Nuttall, M. E. *J. Biol. Chem.,* **2000,** *275*, 16007.

[47] Lee, D.; Long, S. A.; Murray, J. H.; Adams, J. L.; Nuttall, M. E.; Nadeau, D. P.; Kikly, K.; Winkler, J. D.; Sung, C. M.; Ryan, M. D.; Levy, M. A.; Keller, P. M.; DeWolf, W. E., Jr. *J. Med. Chem.,* **2001,** *44*, 2015.

[48] Chapman, J. G.; Magee, W. P.; Stukenbrok, H. A.; Beckius, G. E.; Milici, A. J.; Tracey, W. R. *Eur. J. Pharmacol.,* **2002,** *456*, 59.

[49] Kopka, K.; Faust, A.; Keul, P.; Wagner, S.; Breyholz, H. J.; Holtke, C.; Schober, O.; Schafers, M.; Levkau, B. *J. Med. Chem.,* **2006,** *49*, 6704.

[50] Chu, W.; Zhang, J.; Zeng, C.; Rothfuss, J.; Tu, Z.; Chu, Y.; Reichert, D. E.; Welch, M. J.; Mach, R. H. *J. Med. Chem.,* **2005,** *48*, 7637.

[51] Chu, W.; Rothfuss, J.; d'Avignon, A.; Zeng, C.; Zhou, D.; Hotchkiss, R. S.; Mach, R. H. *J. Med. Chem.,* **2007,** *50*, 3751.

[52] Kravchenko, D. V.; Kysil, V. M.; Tkachenko, S. E.; Maliarchouk, S.; Okun, I. M.; Ivachtchenko, A. V. *Farmaco,* **2005,** *60*, 804.

[53] Kravchenko, D. V.; Kuzovkova, Y. A.; Kysil, V. M.; Tkachenko, S. E.; Maliarchouk, S.; Okun, I. M.; Balakin, K. V.; Ivachtchenko, A. V. *J. Med. Chem.,* **2005,** *48*, 3680.

[54] Kravchenko, D. V.; Kysil, V. M.; Tkachenko, S. E.; Maliarchouk, S.; Okun, I. M.; Ivachtchenko, A. V. *Eur. J. Med. Chem.,* **2005,** *40*, 1377.

[55] Kravchenko, D. V.; Kysil, V. V.; Ilyn, A. P.; Tkachenko, S. E.; Maliarchouk, S.; Okun, I. M.; Ivachtchenko, A. V. *Bioorg. Med. Chem. Lett.,* **2005,** *15*, 1841.

[56] Chen, Y. H.; Zhang, Y. H.; Zhang, H. J.; Liu, D. Z.; Gu, M.; Li, J. Y.; Wu, F.; Zhu, X. Z.; Li, J.; Nan, F. J. *J. Med. Chem.,* **2006,** *49*, 1613.

[57] Ma, X. Q.; Zhang, H. J.; Zhang, Y. H.; Chen, Y. H.; Wu, F.; Du, J. Q.; Yu, H. P.; Zhou, Z. L.; Li, J. Y.; Nan, F. J.; Li, J. *Biochem. Cell Biol.,* **2007,** *85*, 56.

[58] Zhang, Y. H.; Zhang, H. J.; Wu, F.; Chen, Y. H.; Ma, X. Q.; Du, J. Q.; Zhou, Z. L.; Li, J. Y.; Nan, F. J.; Li, J. *FEBS J.,* **2006,** *273*, 4842.

[59] Jiang, X.; Kim, H. E.; Shu, H.; Zhao, Y.; Zhang, H.; Kofron, J.; Donnelly, J.; Burns, D.; Ng, S. C.; Rosenberg, S.; Wang, X. *Science,* **2003,** *299*, 223.

[60] Nguyen, J. T., Wells, J. A. *Proc. Natl. Acad. Sci. U.S.A,* **2003,** *100*, 7533.

[61] Jia, L. T.; Zhang, L. H.; Yu, C. J.; Zhao, J.; Xu, Y. M.; Gui, J. H.; Jin, M.; Ji, Z. L.; Wen, W. H.; Wang, C. J.; Chen, S. Y.; Yang, A. G. *Cancer Res.,* **2003,** *63*, 3257.

[62] Xu, Y. M.; Wang, L. F.; Jia, L. T.; Qiu, X. C.; Zhao, J.; Yu, C. J.; Zhang, R.; Zhu, F.; Wang, C. J.; Jin, B. Q.; Chen, S. Y.; Yang, A. G. *J. Immunol.,* **2004,** *173*, 61.

[63] Reed, J. C. *Nat. Clin. Pract. Oncol.,* **2006,** *3*, 388.

[64] Schimmer, A. D.; Dalili, S.; Batey, R. A.; Riedl, S. J. *Cell Death.Differ.,* **2006,** *13*, 179.

[65] Schimmer, A. D.; Welsh, K.; Pinilla, C.; Wang, Z.; Krajewska, M.; Bonneau, M. J.; Pedersen, I. M.; Kitada, S.; Scott, F. L.; Bailly-Maitre, B.; Glinsky, G.; Scudiero, D.; Sausville, E.; Salvesen, G.; Nefzi, A.; Ostresh, J. M.; Houghten, R. A.; Reed, J. C. *Cancer Cell,* **2004,** *5*, 25.

[66] Nikolovska-Coleska, Z.; Xu, L.; Hu, Z.; Tomita, Y.; Li, P.; Roller, P. P.; Wang, R.; Fang, X.; Guo, R.; Zhang, M.; Lippman, M. E.; Yang, D.; Wang, S. *J. Med. Chem.,* **2004,** *47*, 2430.

[67] Chen, J.; Nikolovska-Coleska, Z.; Wang, G.; Qiu, S.; Wang, S. *Bioorg. Med. Chem. Lett.,* **2006,** *16*, 5805.

[68] Liu, Z.; Sun, C.; Olejniczak, E. T.; Meadows, R. P.; Betz, S. F.; Oost, T.; Herrmann, J.; Wu, J. C.; Fesik, S. W. *Nature,* **2000,** *408*, 1004.

[69] Li, L.; Thomas, R. M.; Suzuki, H.; De Brabander, J. K.; Wang, X.; Harran, P. G. *Science,* **2004,** *305*, 1471.

[70] Oost, T. K.; Sun, C.; Armstrong, R. C.; Al Assaad, A. S.; Betz, S. F.; Deckwerth, T. L.; Ding, H.; Elmore, S. W.; Meadows, R. P.; Olejniczak, E. T.; Oleksijew, A.; Oltersdorf, T.; Rosenberg, S. H.; Shoemaker, A. R.; Tomaselli, K. J.; Zou, H.; Fesik, S. W. *J. Med. Chem.,* **2004,** *47*, 4417.

[71] Wist, A. D.; Gu, L.; Riedl, S. J.; Shi, Y.; McLendon, G. L. *Bioorg. Med. Chem.,* **2007,** *15*, 2935.

[72] Checinska, A.; Hoogeland, B. S.; Rodriguez, J. A.; Giaccone, G.; Kruyt, F. A. *Exp. Cell Res.,* **2007,** *313*, 1215.

[73] Petrucci, E.; Pasquini, L.; Petronelli, A.; Saulle, E.; Mariani, G.; Riccioni, R.; Biffoni, M.; Ferretti, G.; Benedetti-Panici, P.; Cognetti, F.; Scambia, G.; Humphreys, R.; Peschle, C.; Testa, U. *Gynecol. Oncol.,* **2007,** *105*, 481.

[74] Wang, Z.; Cuddy, M.; Samuel, T.; Welsh, K.; Schimmer, A.; Hanaii, F.; Houghten, R.; Pinilla, C.; Reed, J. C. *J. Biol. Chem.,* **2004,** *279*, 48168.

[75] Carter, B. Z.; Gronda, M.; Wang, Z.; Welsh, K.; Pinilla, C.; Andreeff, M.; Schober, W. D.; Nefzi, A.; Pond, G. R.; Mawji, I. A.; Houghten, R. A.; Ostresh, J.; Brandwein, J.; Minden, M. D.; Schuh, A. C.; Wells, R. A.; Messner, H.; Chun, K.; Reed, J. C.; Schimmer, A. D. *Blood,* **2005,** *105*, 4043.

[76] Cillessen, S. A.; Reed, J. C.; Welsh, K.; Pinilla, C.; Houghten, R.; Hooijberg, E.; Deurhof, J.; Castricum, K. C.; Kortman, P.; Hess, C. J.; Ossenkoppele, G. J.; Meijer, C. J.; Oudejans, J. J. *Blood,* **2007**.

[77] Callus, B. A., Vaux, D. L. *Cell Death. Differ.,* **2007,** *14*, 73.

[78] Fischer, U., Schulze-Osthoff, K. *Cell Death. Differ.,* **2005,** *12 Suppl 1*, 942.

[79] Baskin-Bey, E. S.; Washburn, K.; Feng, S.; Oltersdorf, T.; Shapiro, D.; Huyghe, M.; Burgart, L.; Garrity-Park, M.; van Vilsteren, F. G.; Oliver, L. K.; Rosen, C. B.; Gores, G. J. *Am. J. Transplant.,* **2007,** *7*, 218.

[80] Pockros, P. J.; Schiff, E. R.; Shiffman, M. L.; McHutchison, J. G.; Gish, R. G.; Afdhal, N. H.; Makhviladze, M.; Huyghe, M.; Hecht, D.; Oltersdorf, T.; Shapiro, D. A. *Hepatology,* **2007,** *46*, 324.

[81] LaCasse, E. C.; Cherton-Horvat, G. G.; Hewitt, K. E.; Jerome, L. J.; Morris, S. J.; Kandimalla, E. R.; Yu, D.; Wang, H.; Wang, W.; Zhang, R.; Agrawal, S.; Gillard, J. W.; Durkin, J. P. *Clin. Cancer Res.,* **2006,** *12*, 5231.

Received: 07 November, 2007 Revised: 14 January, 2008 Accepted: 17 January, 2008