

First-in-Class Pan Caspase Inhibitor Developed for the Treatment of Liver Disease

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Abstract: A series of oxamyl dipeptides were optimized for pan caspase inhibition, anti-apoptotic cellular activity and in vivo efficacy. This structure–activity relationship study focused on the P4 oxamides and warhead moieties. Primarily on the basis of in vitro data, inhibitors were selected for study in a murine model of α -Fas-induced liver injury. IDN-6556 (**1**) was further profiled in additional in vivo models and pharmacokinetic studies. This first-in-class caspase inhibitor is now the subject of two Phase II clinical trials, evaluating its safety and efficacy for use in liver disease.

Apoptosis, or programmed cell death, is a highly regulated biological process involved in maintaining normal tissue homeostasis.¹ Dysregulation of apoptosis can lead either to pathological loss of cells (stroke, neuronal degeneration, myocardial infarction, liver disease) or to uncontrolled cell survival and excessive accumulation of cells (cancer, chronic inflammatory conditions, autoimmunity).^{1c,d} A family of cysteine proteases known as caspases² (cysteine aspartate-specific proteases) are responsible for the disassembly and phagocytosis of an apoptotic cell.³ The enzymatic cascade leading to apoptosis can be triggered through an extrinsic (extracellular) pathway, or an intrinsic (intracellular) pathway. The extrinsic pathway is driven by the cytokines Fas or TNF- α , and subsequent receptor-mediated activation of caspase-8. The intrinsic pathway is activated during normal tissue homeostasis and is governed at the level of the mitochondria. Internal damage to the cell leads to loss of integrity of the mitochondrial membrane, leakage of cytochrome C and depletion of ATP. Cytochrome C is a critical cofactor for the activation of caspase-9 via the apoptosome complex. Caspases 8 and 9 are commonly referred to as initiator or apical caspases, since these proteases activate downstream caspases (3, 6, and 7) that degrade structural proteins and execute the apoptotic program.

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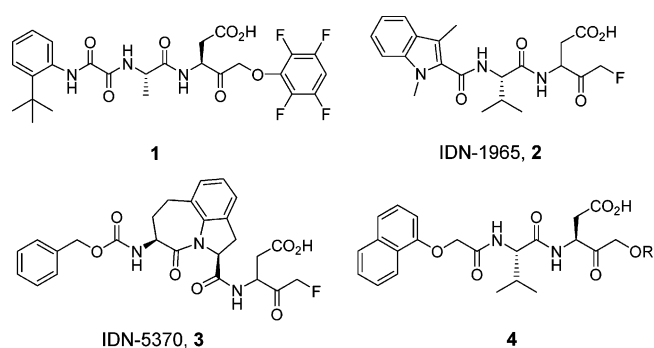
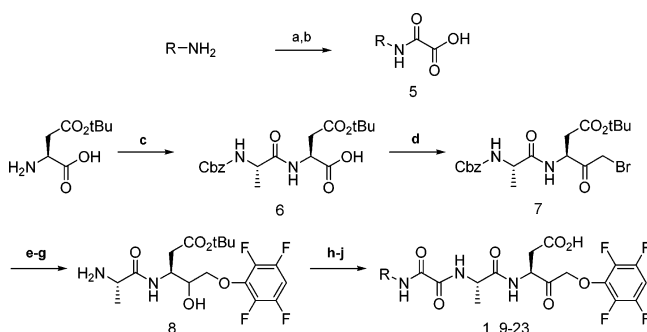


Figure 1.

The majority of early discovery programs targeting caspases centered on the development of reversible inhibitors of caspase-1.⁴ This caspase is not directly involved in apoptosis per se, but is involved in the processing of the zymogen pro-IL1 β to the active cytokine, IL-1 β . Numerous studies have been published targeting apoptotic mechanisms using caspase-3 selective or pan caspase inhibitors (PCIs),⁵ and several reviews concerning the therapeutic utility of regulating apoptosis via caspase inhibition have been written.⁶

This letter describes structure–activity relationships, lead optimization, and selected in vivo data that led to the identification of IDN-6556 (**1**). Compound **1** is the first irreversible PCI to enter clinical development. This compound is currently in Phase II clinical trials for the treatment of liver disease.

Our work in developing PCIs has evolved from early leads taken from tetrapeptide inhibitors, indolyl dipeptide (**2**)⁷ and oxo-azepino indole aspartyl inhibitors (**3**),⁸ to acyloxy dipeptides (**4**),⁹ and ultimately to an irreversible¹⁰ oxamyl dipeptide¹¹ inhibitor (**1**, Figure 1). After extensive evaluation, our data suggested that potent, irreversible inhibition of caspases was very successful in achieving effective anti-apoptotic activity.¹² Although these are irreversible inhibitors, they were shown to be selective for caspases, demonstrating high micromolar activity against related cysteine and serine proteases such as calpain, cathepsin B, chymotrypsin, papain, etc. (unpublished results). In addition to highly potent pan caspase inhibitory activity, high potency in a functional cell-based apoptosis assay was deemed to be critical for compound progression. We established a variety of such models that were useful for this purpose. In particular, a cellular assay utilizing an anti-Fas antibody-stimulated Jurkat E6.1 cell lymphoma cell line was a key part of our flowchart¹³ and was useful in discriminating among potent compounds in vitro from those that possessed acceptable functional activity. This assay is quite revealing in that the challenged Jurkat cells trigger the extrinsic apoptotic pathway, exhibiting all the standard hallmarks of apoptosis. These caspase inhibitors prevented apoptosis in the challenged cells and also maintained their basic cellular morphology. Making use of the same mechanism, these data can also be reconciled with in vivo activity observed in a murine model of α -Fas induced liver damage,¹⁴ using ALT levels (alanine aminotransferase) and histology as endpoints. This model is particularly relevant in the setting of liver

Scheme 1^a

^a (a) Methyl chlorooxoacetate (1.1 equiv), triethylamine (TEA) (1.1 equiv), 0 °C–room temperature (rt) 1 h; (b) 1 N LiOH (1.2 equiv), 1,4-dioxane, rt, 1 h; (c) bis(trimethylsilyl)-trifluoroacetamide (2 equiv), rt 30 min, Z-Ala-OSu ester (1 equiv), DMF, rt, 24 h, 82%; (d) i. isobutyl chloroformate (1.5 equiv), *N*-methylmorpholine (NMM) (1.6 equiv), THF, –10 °C, 20 min; ii. CH₂N₂/Et₂O (2.5 equiv), 0 °C, 15 min; iii. HBr (aq 48%) THF, 0 °C–rt, 30 min, 52%; (e) 2,3,5,6-tetrafluorophenol (1.2 equiv), KF (4 equiv), DMF, rt, 16 h, used crude; (f) NaBH₄ (4 equiv) EtOH, 0 °C, 1 h, 78%; (g) H₂ (1 atm), 10% Pd/C, MeOH, rt, 2 h, 70%; (h) oxamic acid, **5** (1 equiv), HOBT (1.05 equiv), EDCI (1.4 equiv), NMM (1.05 equiv), CH₂Cl₂, rt, 16 h; (i) iodobenzene diacetate (3.8 equiv), TEMPO (0.2 equiv), CH₂Cl₂, rt, 16 h; (j) TFA/CH₂Cl₂/anisole (66:33:1), rt, 1 h.

disease, where α -Fas is the major cytokine responsible for liver damage. Studies on **1** in other animal models will be discussed, as well as early clinical data.

The synthesis of compounds **1** and **9–23** is outlined in Scheme 1.¹¹ The oxamic acid (**5**) was prepared using methyl chlorooxoacetate and the amine of choice, followed by ester hydrolysis. The dipeptide fragment (**6**) is prepared by coupling the carbobenzyloxy alanine hydroxysuccinimide with the β -protected aspartic acid using bis(trimethylsilyl)-trifluoroacetamide. Conversion of **6** to its bromomethyl ketone derivative (**7**), followed by displacement with 2,3,5,6-tetrafluorophenol provided the tetrafluorophenoxy methyl ketone. After reduction of the ketone to the alcohol and hydrogenolysis of the protected amine, the requisite oxamic acid (**5**) is then coupled. Oxidation of the alcohol and subsequent *tert*-butyl ester hydrolysis provides the P4 oxamide dipeptide analogues in Table 1.

Preparation of the warhead analogues (**28–35**) is outlined in Scheme 2. Coupling of the requisite oxamic acid to the *p*-tosylate salt of amine (**24**), followed by basic hydrolysis, gave the oxamide dipeptide (**25**). Three-step conversion to the bromomethyl ketone employing diazomethane afforded a key intermediate, compound **26**. This bromomethyl ketone could then be reacted with the nucleophile of choice in the presence of potassium fluoride, followed by acidic hydrolysis of the aspartyl *tert*-butyl ester to yield the inhibitors of the general structure **27** found in Scheme 2.

The P4 oxamide SAR observed with alanine at P2 is very similar to that seen with valine,¹⁵ in that the P4 oxamide region has a very tolerant SAR with respect to caspase activity (Table 1). However, when evaluating both enzyme and cell data together, namely following potency in the Jurkat/Fas (JFas) assay, 2-substituted phenyl oxamides in general show the best overall activity (**1**, **14**, **17**, and **19**).¹⁵ Other examples include use of basic functionalities, such as the 2-pyridyl oxamide (**11**) having moderate JFas activity, and the 2-pyrrole phenyl oxamide (**18**) being among the more potent analogues tested in this assay.

Noting this tolerant P4 oxamide SAR, it was believed that the warhead would be critical in evaluating the overall potency and cellular activity of these inhibitors, as previously shown in a survey of various methyl ketone warheads on a naphthoxy dipeptide backbone^{9c} and a publication involving the use of irreversible caspase inhibitors in the context of apoptosis.¹² A subset of the P4 groups was selected for an SAR study, comparing the more active warhead moieties based on related SAR studies: tetrafluorophenoxy, dichlorobenzoyloxy, and diphenylphosphoryloxy methyl ketones. These were shown to be more potent than a standard fluoromethyl ketone on both the acyl dipeptide backbone^{9c} and the oxamyl valinyl backbone.¹¹ Comparing the activities of these three warhead analogues on four oxamyl-alanyl backbones (**28–35**) demonstrated an overall trend that tetrafluorophenoxy warhead generally showed better caspase inhibitory activity, and generally gave more potent results in the JFas cellular assay.

Activity seen in the α -Fas-induced Jurkat cellular assay prompted *in vivo* screening of these PCIs in an α -Fas-mediated liver model.¹⁴ Tetrafluorophenoxy and diphenylphosphoryloxy methyl ketone warhead analogues of selected P4-ala-asp backbones were studied. This model of acute liver damage is associated with marked hepatocellular apoptosis, elevated plasma ALT activities and lethality within 6 h.¹⁵ These endpoints were dose-dependently reduced by pan caspase inhibitors as demonstrated by the ED₅₀s shown in Table 1. In general, the tetrafluorophenoxy methyl ketones were consistently more potent in this model than other classes of inhibitors. Using various routes of administration, compound **1** was further evaluated in the α -Fas model; ip, iv, po, and im. ED₅₀ values of 0.08 (0.06–0.12) mg/kg, 0.38 (0.11–1.27) mg/kg, 0.31 (0.24–0.42) mg/kg, and 0.04 (0.02–0.07) mg/kg were observed, respectively.¹⁵

To discriminate between warheads, compounds **1** and **28** were studied in a second and complementary model of liver injury, a murine D-Gln/LPS model.¹⁵ After *i.p.* and *i.v.* administration of **1**, ED₅₀ values were 0.17 and 0.09 mg/kg, respectively, as compared to less potent results with compound **28** (0.97 and 5.03 mg/kg). Oral administration of compound **1** in two independent experiments provided an ED₅₀ determined to be less than 0.01 mg/kg.

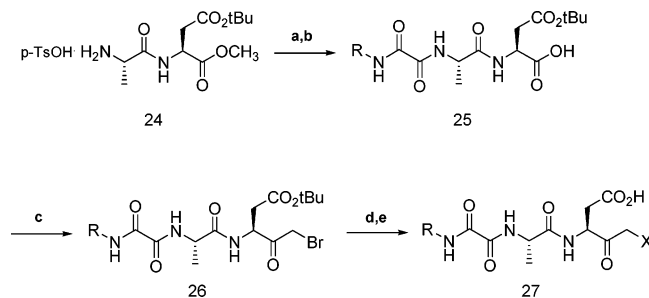
Noting the oral efficacy of **1** compared to other routes of administration, a pharmacokinetic study was conducted in rats.¹⁵ After a single bolus administration (*iv*, *ip*, or *sc*) **1** had terminal half-lives of 51, 47, and 46 min, respectively. The oral bioavailability of compound **1** (10 mg/kg, fasted rats) was low, ranging from 2.7 to 4%. This is in contrast to the systemic bioavailability of 49 and 70% achieved following *ip* and *sc* administration, respectively. Portal and systemic concentrations were compared in an attempt to reconcile the low systemic oral bioavailability of **1** in the rat with the observed similarity in efficacy between *ip* and *po* administration, also in the rat.¹⁵ After oral administration, AUC_{inf} and MRT_{inf} of **1** in the portal vein were 5.9–5.3-fold higher, respectively, than in the systemic compartment, suggesting a marked first-pass effect.

Compound **1** was studied in the bile duct ligated (BDL) mouse, a standard model of cholestatic liver

Table 1. P4 Oxamide and Warhead SAR

compd	R	X	enzyme assays ^a k_3/K_i ($M^{-1} s^{-1}$)				cellular assays ^a IC_{50} (μM)		
			mCsp-1 ^b	Csp-3	Csp-6	Csp-8	JFas	THP-1	a-Fas liver, IP, ED_{50} (mg/kg)
2	Figure 1	–	10100	19000	98000	34400	1.3	10	–
3	Figure 1	–	12000000	960000	25000	98000	0.51	0.3	–
9	cyclohexyl.	O(2,3,5,6-F ₄ -Ph)	390000	21000	268	2800	0.15	0.5	–
10	phenyl	O(2,3,5,6-F ₄ -Ph)	127000	25400	31700	65300	0.67	0.14	–
11	2-pyridyl	O(2,3,5,6-F ₄ -Ph)	468000	44000	23700	201000	1.04	0.2	–
12	benzyl	O(2,3,5,6-F ₄ -Ph)	674000	35500	37100	32483	0.3	0.16	–
13	2-F Ph	O(2,3,5,6-F ₄ -Ph)	1140000	70000	19800	114000	0.16	0.16	–
15	2-Br Ph	O(2,3,5,6-F ₄ -Ph)	1340000	186000	25200	542000	0.075	0.05	–
16	2-CF ₃ Ph	O(2,3,5,6-F ₄ -Ph)	9950000	309000	45600	332000	0.061	0.19	–
18	2-pyrrole Ph	O(2,3,5,6-F ₄ -Ph)	1520000	12000	7750	1200000	0.056	>32	–
20	2-Bn Ph	O(2,3,5,6-F ₄ -Ph)	1970000	44400	33600	617000	0.01	0.54	–
21	2-phenoxy Ph	O(2,3,5,6-F ₄ -Ph)	960000	51000	5900	154000	0.53	0.8	–
22	1-naphthyl	O(2,3,5,6-F ₄ -Ph)	1700000	57000	18500	460000	0.086	0.06	–
23	5-indanyl	O(2,3,5,6-F ₄ -Ph)	270000	42000	31900	204000	0.2	0.12	–
1	2-tBu Ph	O(2,3,5,6-F ₄ -Ph)	689000	75700	58700	2940000	0.025	0.27	0.08 (0.06–0.12) ^d
28	2-tBu Ph	OPOPPh ₂	2158000	99100	52100	2010000	0.081	9.09	0.14
29	2-tBu Ph	OCO(2,6-Cl ₂ -Ph)	8330000	236000	105000	4300	0.143	11.8	45.8
17	2,5 ditBu Ph	O(2,3,5,6-F ₄ -Ph)	16300000	38200	15400	365000	0.062	1.84	0.05 (0.02–0.11)
30	2,5 ditBu Ph	OPOPPh ₂	2710000	18300	7640	970000	0.109	5.87	0.5
31	2,5 ditBu Ph	OCO(2,6-Cl ₂ -Ph)	4025000	30000	6400	68	1.38	3.13	–
14	2-Cl Ph	O(2,3,5,6-F ₄ -Ph)	13400000	219000	26000	228000	0.064	0.54	0.05 (0.02–0.16)
32	2-Cl Ph	OPOPPh ₂	3960000	211000	37100	1410000	0.068	1.04	0.04 (0.03–0.05)
33	2-Cl Ph	OCO(2,6-Cl ₂ -Ph)	8312000	256000	44000	109	1.17	0.32	–
19	2-Ph Ph	O(2,3,5,6-F ₄ -Ph)	323000	3400	5900	154000	0.13	0.5	0.14 (0.08–0.23)
34	2-Ph Ph	OPOPPh ₂	5000000	28400	22700	1570000	3.94	4.12	0.44
35	2-Ph Ph	OCO(2,6-Cl ₂ -Ph)	7280000	29000	61000	342000	0.39	4.36	–

^a Assay conditions are described in ref 12. ^b Murine caspase-1 enzyme. Other enzyme data are from recombinant human. ^c Assay conditions are described in ref 15. ^d Confidence interval.

Scheme 2^a

^a (a) **5** (1 equiv), *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) (1.05 equiv), DIEA (3 equiv), *N*-methylpyrrolidinone (NMP)/CH₂Cl₂ (1:1); rt, 2 h; (b) 1 N LiOH (1.02 equiv), 1,4-dioxane, rt, 1 h; (c) i. isobutyl chloroformate (1.1 equiv), NMM (1 equiv), THF, –10 °C, 30 min; ii. CH₂N₂/Et₂O (2.5 equiv), 0 °C, 15 min; iii. HBr (aq 48%) THF, 0 °C, 15 min; (d) HX (1.2 equiv), KF (2 equiv), DMF, rt, 16 h; (e) TFA/CH₂Cl₂/anisole, (66:33:1), rt, 1 h.

disease.²¹ The results demonstrated that a pan caspase inhibitor reduces hepatocyte apoptosis, assessed by the terminal deoxynucleotidyl transferase dUTP nick-end labeling assay (TUNEL) and immunofluorescence of active caspases 3 and 7. A reduction in liver injury was observed by histopathology and the lowering of serum alanine aminotransferase levels. Further, it was noted that **1** reduced mRNA expression for markers of HSC activation, as well as a reduction of collagen expression and deposition.

Phase I clinical data evaluating compound **1** showed the drug to be safe and well tolerated in a clinical study involving 76 normal adults and patients with mild liver impairment, including individuals with stable HCV infection.¹⁷ Statistically significant and

clinically relevant improvements in ALTs and ASTs were seen in those patients with mild liver impairment. Compound **1** demonstrated dose-proportional pharmacokinetics, having a terminal half-life of 1.7–3.1 h. There was no evidence of accumulated drug over the 7-day dosing period following multiple i.v. doses. In general, its pharmacokinetic profile demonstrated it was quickly cleared from the venous circulation after infusion.

Given the clinical observations stated above, a 2-week oral dosing ranging study was initiated in a population of HCV patients refractory to currently approved HCV treatments (interferon- α and ribavirin). This study²⁴ demonstrated that compound **1** was well-tolerated, normalized liver enzymes (ALT/AST), and importantly had no adverse effect on viral load.

In summary, compound **1** has been demonstrated to be a very potent, irreversible pan caspase inhibitor, having potent anti-apoptotic activity in the Jurkat-fas cellular assay. This potency translates to its in vivo activity in murine models of liver injury and fibrosis. The oral efficacy of this compound is very intriguing when one considers that a cursory evaluation would characterize it as having poor oral pharmacokinetics. Comparisons of portal and systemic concentrations suggest a marked first-pass effect, targeting oral administration of the drug to the liver. Although reversible aldehyde and ketone inhibitors were examined early in this program, none were capable of fully inhibiting caspases in order to affect the relevant apoptotic pathway. The selective nature of these pan caspase inhibitors has enabled them not only to be highly potent and efficacious in both acute and chronic animal models, but

more importantly demonstrate safety in a clinical setting. Further successful clinical trials with compound **1** will open the door to the use of caspase inhibitors for chronic liver disease and prompt research for their use in other indications.

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Supporting Information Available: Experimental procedures for enzyme and cellular screening, murine α -Fas liver model, and synthesis of compound **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Ellis, R. E.; Yuan, J.; Horvitz, H. R. Mechanisms and Functions of Cell Death. *Annu. Rev. Cell Biol.* **1991**, *7*, 663–698. (b) Nicholson, D. W. From bench to clinic with apoptosis-based therapeutic agents. *Nature* **2000** *407*, 810–816. (c) Galle, P. R. Apoptosis in liver disease. *J. Hepatol.* **1997**, *27*, 405–412. Reed, J. Apoptosis based therapies. *Nat. Rev. Drug Discovery* **2002** *1*, 111–121.
- (2) (a) For nomenclature of the caspase family, see: Alnemri, E. S.; Livingston, D. J.; Nicholson, D. W.; Salvesen, G.; Thornberry, N. A.; Wong, W. W.; Yuan, J.-Y. Human ICE/ced-3 protease nomenclature. *Cell* **1996** *87*, 171. (b) Black, R. A.; Kronheim S. R.; Sleath, P. R. Activation of interleukin-1 β by a co-induced protease. *FEBS Lett.* **1989**, *247*, 386–390. (c) Thornberry, N. A. Caspases: Key mediators of apoptosis. *Chem. Biol.* **1998** *5*, R97–103. (d) Fuentes-Prior, P.; Salvesen, G. S. The protein structures that shape caspase activity, specificity, activation, and inhibition. *Biochem. J.* **2004**, *384*, 201–232.
- (3) Earnshaw, W. C.; Martins, L. M.; Kaufmann, S. H. Mammalian caspases: structure, activation, substrates and function during apoptosis. *Annu. Rev. Biochem.* **1999**, *68*, 383–424.
- (4) (a) Talanian, R. V.; Brady, K. D.; Cryns, V. L. Caspases as targets for antiinflammatory and anti-apoptotic drug discovery. *J. Med. Chem.* **2000**, *43*, 3351–3371. (b) Ashwell, S. Caspases: Recent advances in small molecule inhibitors. *Exp. Opin. Ther. Pat.* **2001**, *11*, 1593–1603.
- (5) (a) Cai, S. X.; Guan, L.; Jia, S.; Wang, Y.; Yang, W.; Tseng, B.; Drewe, J. Dipeptidyl aspartyl fluoromethylketones as potent caspase inhibitors: SAR of the N-protecting group. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5295–5300. (b) Wang, Y.; Guan, L.; Jia, S.; Tseng, B.; Drewe, J.; Cai, S. X. Dipeptidyl aspartyl fluoromethylketones as potent caspase inhibitors: peptidomimetic replacement of the P2 α -amino acid by an α -hydroxy acid. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1379–1383. (c) Isabel, E.; Black, W. C.; Bayly, C. I.; Grimm, E. L.; Janes, M. K.; McKay, D. J.; Nicholson, D. W.; Rasper, D. M.; Renaud, J.; Roy, S.; Tam, J.; Thornberry, N. A.; Vaillancourt, J. P.; Xanthoudakis, S.; Zamboni, R. Nicotinylnyl aspartyl ketones as inhibitors of caspase-3. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2137–2140. (d) Colucci, J.; Giroux, A.; Zhu, Y.; Bouche, M.; Pikounis, B.; Xanthoudakis, S.; Roy, S.; Rigby, M.; Zamboni, R.; Robertson, G. S.; Ng, G. Y.; Nicholson, D. W.; Fluckiger, J. P.; Toulmond, S.; Tang, K.; Bureau, Y.; Ashdown, H.; Degen, S.; O'Donnell, R.; Tam, J.; Han, Y. Neuroprotective effects of M826, a reversible caspase-3 inhibitor, in the rat malonate model of Huntington's disease. *Br. J. Pharmacol.* **2004** *141*, 689–697. (e) 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. Discovery of novel aspartyl ketone dipeptides as potent and selective caspase-3 inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 805–808. (f) 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. Novel pyrazinone mono-amides as potent and reversible caspase-3 inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1173–1180.
- (6) (a) Graczyk, P. P. Caspase inhibitors as antiinflammatory and antiapoptotic agents. *Prog. Med. Chem.* **2002**, *39*, 1–72. (b) O'Brien, Tom; Lee, Dennis. Prospects for caspase inhibitors. *Mini-Rev. Med. Chem.* **2004**, *4*, 153–165. (c) Linton, S. D. Caspase inhibitors: A pharmaceutical industry perspective. *Curr. Topics Med. Chem.*, in press.
- (7) (a) Bai, X.; Karanewsky, D. S. C-terminal modified (N-substituted)-2-indolyl dipeptides as inhibitors of the ICE/ced-3 family of cysteine proteases. US Patent 5,877,197, Feb 9, 1999. (b) Bai, X.; Karanewsky, D. S. C-terminal modified (N-substituted)-2-indolylcarbonyl dipeptides as inhibitors of the ICE/ced-3 family of cysteine proteases. US Patent 6,184,244, Feb 6, 2001.
- (8) Deckwerth, T. L.; Adams, L. M.; Wiessner, C.; Allegrini, P. R.; Rudin, M.; Sauter, A.; Hengerer, B.; Sayers, R. O.; Rovelli, G.; Aja, T.; May, R.; Nalley, K.; Linton, S. D.; Karanewsky, D. S.; Wu, J. C.; Roggo, S.; Schmitz, A.; Contreras, P. C.; Tomaselli, K. J. Long-term protection of brain tissue from cerebral ischemia by peripherally administered peptidomimetic caspase inhibitors. *Drug Dev. Res.* **2001**, *52*, 579–586. Indoless: A new class of potent broad spectrum caspase inhibitors with in vivo activity in the pMCAO model. *Abstracts of Papers*, 221st American Chemical Society National Meeting, San Diego, CA, United States, April 1–5, 2001.
- (9) (a) Linton, S. D.; Karanewsky, D. S.; Ternansky, R. J.; Wu, J. C.; Pham, B.; Kodandapani, L.; Smidt, R.; Diaz, J. L.; Fritz, L. C.; Tomaselli, K. J. Acyl dipeptides as reversible caspase inhibitors. Part 1: Initial lead optimization. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2969–2971. (b) Linton, S. D.; Karanewsky, D. S.; Ternansky, R. J.; Chen, N.; Guo, X.; Jahangiri, K. G.; Kalish, V. J.; Meduna, S. P.; Robinson, E. D.; Ullman, B. R.; Wu, J. C.; Pham, B.; Kodandapani, L.; Smidt, R.; Diaz, J. L.; Fritz, L. C.; Krosigk, U.; Roggo, S.; Schmitz, A.; Tomaselli, K. J. Acyl dipeptides as reversible caspase inhibitors. Part 2: Further optimization. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2973–2975. (c) Ullman, B. R.; Aja, T.; Deckwerth, T. L.; Diaz, J. L.; Herrman, J.; Kalish, V. J.; Karanewsky, D. S.; Meduna, S. P.; Nalley, K.; Robinson, E. D.; Roggo, S. P.; Sayers, R. O.; Schmitz, A.; Ternansky, R. J.; Tomaselli, K. J.; Wu, J. C. Structure–activity relationships within a series of caspase inhibitors: effect of leaving group modifications. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3623–3626.
- (10) (a) Brady, K. D. Bimodal inhibition of caspase-1 by aryloxymethyl and acyloxymethyl ketones. *Biochemistry* **1998**, *37*, 8508–8515. (b) Brady, K. D.; Giegel, D. A.; Grinnell, C.; Lunney, E.; Talanian, R. V.; Wong, W.; Walker, N. A catalytic mechanism for caspase-1 and for bimodal inhibition of caspase-1 by activated aspartic ketones. *Bioorg. Med. Chem.* **1999**, *7*, 621–631.
- (11) Linton, S. D.; Aja, T.; Allegrini, P. R.; Deckwerth, T. L.; Diaz, J. L.; Hengere, B.; Herrman, J.; Jahangiri, K. G.; Kallen, J.; Karanewsky, D. S.; Meduna, S. P.; Nalley, K.; Robinson, E. D.; Roggo, S.; Rovelli, G.; Sauter, A.; Sayers, R. O.; Schmitz, A.; Smidt, R.; Ternansky, R. J.; Tomaselli, K. J.; Ullman, B. R.; Wiessner, C.; Wu, J. C. Oxamyl dipeptide caspase inhibitors developed for the treatment of stroke. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2685–2691.
- (12) Wu, J. C.; Fritz, L. C. Irreversible caspase inhibitors: tools for studying apoptosis. *Methods: A Companion Methods Enzymol.* **1999**, *17*, 320–328.
- (13) Armstrong, R. C.; Aja, T.; Xiang, J.; Gaur, S.; Krebs, J. F.; Hoang, K.; Bai, X.; Kormsmeier, S. J.; Karanewsky, D. S.; Fritz, L. C.; Tomaselli, K. J. Fas-induced activation of the cell death-related protease CPP32 is inhibited by Bcl-2 and by ICE family protease inhibitors. *J. Biol. Chem.* **1996**, *271*, 16850–16855.
- (14) Kunstle, G.; Leist, M.; Uhlig, S.; Revesz, L.; Feifel, R.; MacKenzie, A.; Wendel, A. ICE-protease inhibitors block murine liver injury and apoptosis caused by CD95 or by TNF- α . *Immunol. Lett.* **1997**, *55*, 5–10.
- (15) Hoglen, N. C.; Chen, L. S.; Fisher, C. D.; Hirakawa, B. P.; Groessl, T.; Contreras, P. C. Characterization of IDN-6556 (3-{2-[(2-*tert*-Butyl-phenylamino)oxy]amino}propionylamino}-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid): a liver-targeted caspase inhibitor. *J. Pharm. Exp. Ther.* **2003**, *309*, 634–640.
- (16) Canbay, A.; Feldstein, A.; Baskin-Bey, E.; Bronk, S. F.; Gores, G. J. The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse. *J. Pharm. Exp. Ther.* **2004**, *308*, 1191–1196.
- (17) Valentino, K. L.; Gutierrez, M.; Sanchez, R.; Winship, M. J.; Shapiro, D. A. First clinical trial of a novel caspase inhibitor: anti-apoptotic caspase inhibitor, IDN-6556, improves liver enzymes. *Int. J. Clin. Pharm. Ther.* **2003**, *41*, 441–449.
- (18) Schiff, E. R.; Pockros, P.; Shiffman, M. L.; McHutchinson, J.; Gish, R.; Afdhal, N. H.; Makhviladze, M.; Huyghe, M.; Hecht, D.; Oltersdorf, T.; Shapiro, D. Oral IDN-6556, an anti-apoptotic caspase inhibitor, lowers aminotransferases in HCV patients. *Gastroenterology* **2004**, *126*, Suppl. 2: A-668.