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Synthesis and structure–activity relationships of oxamyl dipeptide caspase inhibitors developed for the treatment of liver disease

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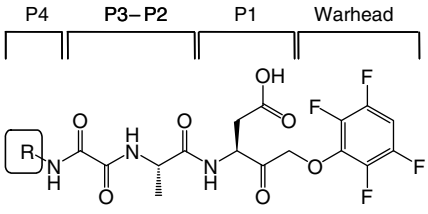
ABSTRACT

The P4 region of a series of oxamyl dipeptide caspase inhibitors was optimized by the combination of anti-apoptotic activity in the Jurkat/Fas (JFas) cellular assay and membrane permeability in the PAMPA assay. Two highly potent anti-apoptotic agents with moderate membrane permeability, **29** and **36**, showed strong in vivo efficacy in a murine model of α-Fas-induced liver injury.

Apoptosis, or programmed cell death, is a highly regulated biological process involved in maintaining normal tissue homeostasis.[1–4] Caspases are a family of cysteine proteases with strict substrate specificity requiring aspartic acid as P1 amino acid.[5] Caspases can be divided into two groups. One group, represented by caspase-1, plays an important function in cytokine maturation.[6] The other group, including caspase-3, -8, and -9, plays a critical role in apoptosis by cleaving numerous important proteins.[7] Because of the important function of caspases in both inflammation and apoptosis, the discovery and development of caspase inhibitors could result in novel anti-inflammatory and anti-apoptotic drugs for the treatment of a variety of diseases.[8]

Many dipeptide caspase inhibitors have been designed and synthesized based on substrate specificity of caspases.[9–11] Among them, oxamyl dipeptide derivatives were identified by Idun Pharmaceuticals as irreversible pan-caspase inhibitors for the treatment of liver diseases.[12,13] They reported that the 2-substituted phenyl or halogenated phenyl oxamides such as compounds **1**, **2**, and **3** showed potent caspase inhibitory activities and anti-apoptotic activity in the Jurkat/Fas (JFas) cellular assay (Fig. 1).[14] However, some of the oxamyl dipeptide derivatives showed only weak in vivo efficacy in a murine model of α-Fas-induced liver injury in spite of their potent caspase inhibitory activity and cellular



1 (IDN-7314): R=2,6-di-F-Ph
2 (IDN-7568): R=2-Cl-Ph
3 (IDN-6734): R=2-CF_3-Ph
29: R=2,5-di-Cl-PhCH_2
36: R=2,5-di-Me-PhCH_2CH_2

Figure 1. Oxamyl dipeptide caspase inhibitors.

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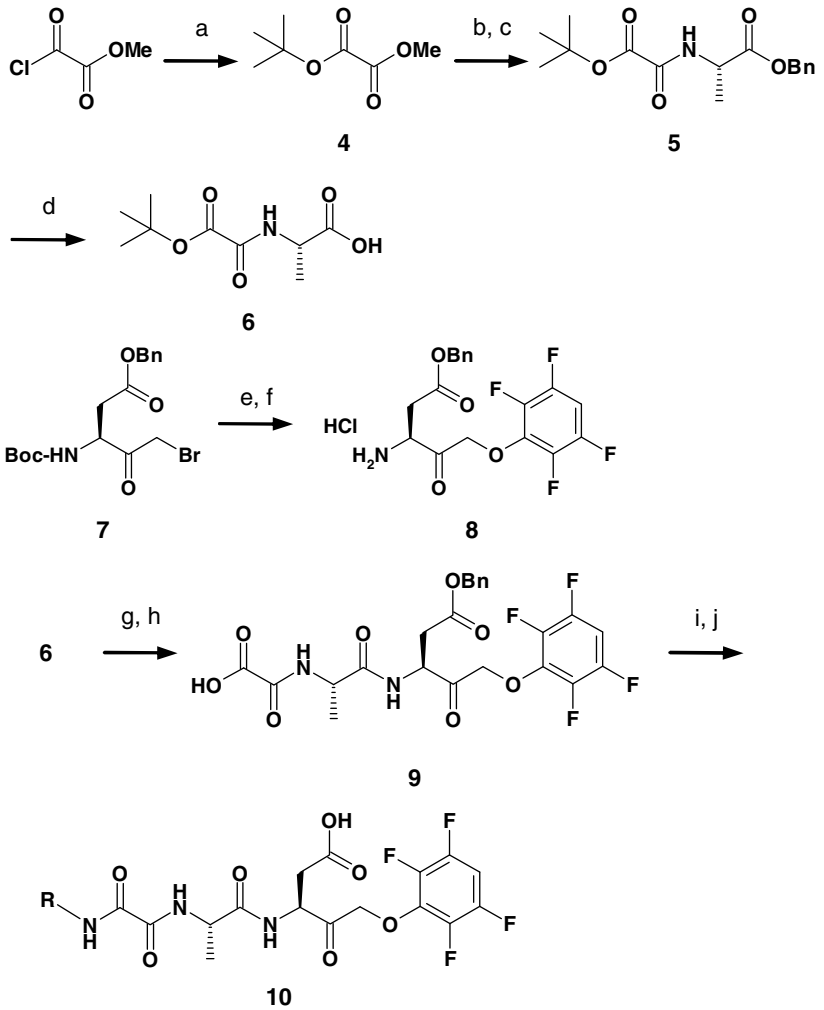
activity.[15] Therefore, further optimization at the P4 region of the oxamyl dipeptides has been conducted in order to improve in vivo efficacy of caspase inhibitors.

Synthesis of the various P4 derivatives of the oxamyl dipeptides was carried out as outlined in Scheme 1. Commercially available methyl chlorooxoacetate was converted to the *tert*-butyl methyl oxalate **4** by treatment with *tert*-BuOH in the presence of pyridine. Hydrolysis of the methyl ester **4** with aqueous potassium hydroxide followed by coupling with H-Ala-OBzl·HCl using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) gave the compound **5**. Removal of the benzyl group of **5** by catalytic hydrogenation yielded the P2–P3 segment **6**. The P1-warhead segment **8** was prepared from the bromomethylketone **7**[16] by treatment with potassium 2,3,5,6-tetrafluorophenolate[12] followed by removal of the Boc group. The carboxylic acid **6** was coupled with compound **8** using HATU followed by removal of the *tert*-butyl group affording the key intermediate **9**. Preparation of the P4 derivatives was achieved by coupling of compound **9** with various amines followed by removal of the benzyl group.

The relevance of the caspase inhibitory activities and the JFas cellular activity of the P4 derivatives were initially analyzed. The results revealed that the cellular activity was correlated with the caspase-8 inhibitory activity (Fig. 2). However, a large variation in caspase inhibitory activity (k_3/K_i values: 10,000–1,000,000) was observed for the compounds exhibiting potent JFas cellular activities (<20 nM). In the case of these derivatives, the potency of the cellular activity tended to depend on inhibitory activities against other caspases in addition to caspase-8. Ultimately the derivatives with a broad spectrum of caspases inhibition against caspase-3, caspase-8, and caspase-9 turned out to have potent cellular activity (Table 1). Notably the substituted benzyl derivative **11** and the phenethyl derivative **12** showed potent JFas cellular activity comparable to **1** (IDN-7314) (see Table 2).

The in vivo activity of compounds **11** and **12** was evaluated in a murine model of α-Fas-induced liver injury[15,17] (single dose oral administration) and the ED_{50} values were 0.7 and 0.2 mg/kg, respectively. Although the cellular activities of the compounds **14** and **16** were weaker than those of **11** and **12**, these compounds were more efficacious than compound **1** by comparing their ED_{50} values. In order to explain these results, key factors such as cell permeability that have an effect on the in vivo efficacy have been investigated. Using a parallel artificial membrane permeability assay (PAMPA[18]), effective permeability (Pe) of **11** and **12** was found to be low (Pe: $<1.7 \times 10^{-6}$ cm/s). In contrast, compounds **14** and **16** had moderate permeability (Pe: $>3.1 \times 10^{-6}$ cm/s). These results suggested that the in vivo activity of compounds could be predicted to some extent by both the cellular activity and permeabil-



Scheme 1. Synthetic route of the P4 derivatives of the oxamyl dipeptides. Reagents and conditions: (a) *t*-BuOH, pyridine, Et_2O, rt (70%); (b) KOH, CH_3CN, H_2O, rt; (c) H-Ala-OBz HCl, HATU, DIEA, CH_2Cl_2, DMF, rt (85%, 2 steps); (d) H_2, 10% Pd-C, AcOEt, rt (92%); (e) potassium 2,3,5,6-tetrafluorophenolate, NaI, acetone, rt (90%); (f) 4 N HCl-AcOEt, rt (95%); (g) **8**, HATU, DIEA, CH_2Cl_2, DMF, rt (50%); (h) TFA, CH_2Cl_2, rt (90%); (i) **R**-NH_2, HATU, DIEA, DMA, rt; (j) H_2, 10% Pd-C, THF, 50 °C or HCO_2NH_4, 10% Pd-C, MeOH, THF, 40 °C.

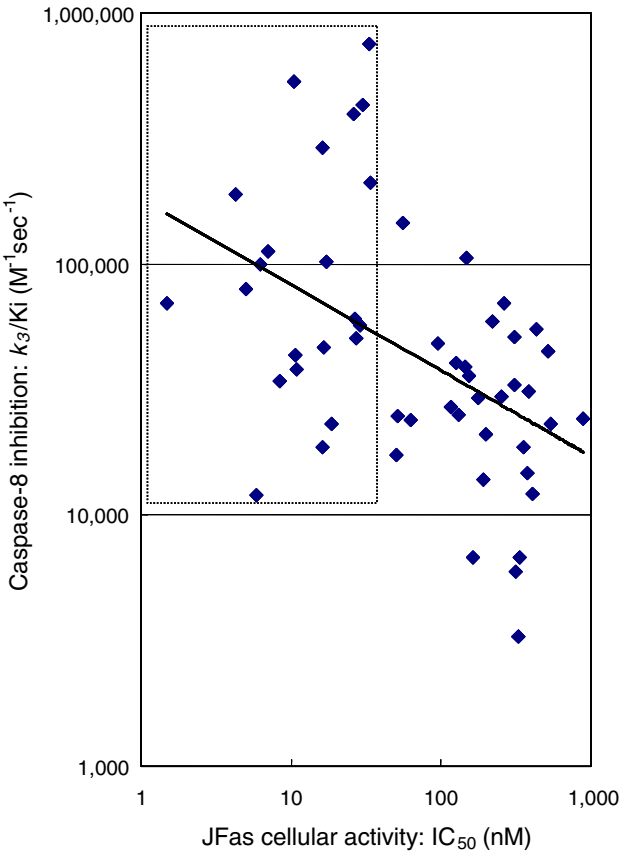
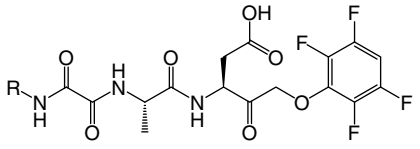


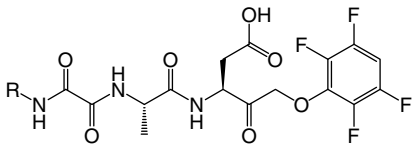
Figure 2. Scatter plot of caspase-8 inhibition and JFas cellular activity.

Table 1
Pan-caspase inhibitory activity and JFas cellular activity.



Compound	R	Enzyme assays[14] k_3/K_i (M^{-1} s^{-1})			JFas cellular assay[14]
		Caspase-3	Caspase-8	Caspase-9	IC_{50} (nM)
1 (IDN-7314)	2,6-di-F-Ph	37,400	100,000	264,000	6.3
11	4-Cl-$PhCH_2$	12,200	34,300	117,000	8.4
12	$PhCH_2CH_2$	18,600	79,900	205,000	5.0
13	2-Cyclopentylethyl	9470	38,100	122,000	11
14	Cyclohexylmethyl	9870	23,000	133,000	19
15	2-(Dimethylamino)-3-pyridinyl	4880	60,200	98,600	27
16	(1-Phenylcyclo-propyl)methyl	9720	50,700	125,000	27

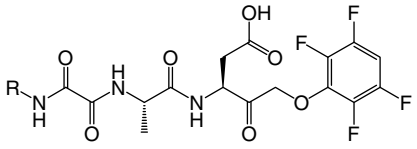
Table 2
Results of JFas cellular assay, in vivo assay and PAMPA.



Compound	R	Enzyme assay caspase-8 k_3/K_i (M^{-1} s^{-1})	JFas cellular assay IC_{50} (nM)	In vivo assay[a,15,17,*] ED_{50} (mg/kg)	PAMPA[18] Pe ($\times 10^{-6}$ cm/s)
1 (IDN-7314)	2,6-di-F-Ph	100,000	6.3	0.7	<0.9
11	4-Cl-$PhCH_2$	34,300	8.4	0.7	<1.7
12	$PhCH_2CH_2$	79,900	5.0	0.2	<1.0
14	Cyclohexylmethyl	23,000	19	0.1	3.1
16	(1-Phenylcyclo-propyl)methyl	50,700	27	0.3	3.7

[a] Single dose, p.o. administration.
[*] All experimental procedures used in this study were approved by the local ethics committee (ACUP) based on international guidelines (IACUC) and adherence to the Pfizer policy.

Table 3
Results of JFas cellular assay and PAMPA.

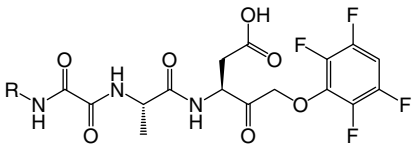


Compound	R	JFas cellular assay IC_{50} (nM)	PAMPA Pe ($\times 10^{-6}$ cm/s)
1 (IDN-7314)	2,6-di-F-Ph	6.3	<0.9
17	2-Me-$PhCH_2$	52	1.3
18	3-Me-$PhCH_2$	127	1.2
19	4-Me-$PhCH_2$	50	1.1
20	2-F-$PhCH_2$	207	0.3
21	3-F-$PhCH_2$	106	0.3
22	4-F-$PhCH_2$	197	0.4
23	2,3-di-Me-$PhCH_2$	102	3.0
24	2,4-di-Me-$PhCH_2$	>10,000	4.0
25	2,5-di-Me-$PhCH_2$	11	4.2
26	3,4-di-Me-$PhCH_2$	>10,000	3.7
27	2,3-di-Cl-$PhCH_2$	76	3.1
28	2,4-di-Cl-$PhCH_2$	165	2.7
29	2,5-di-Cl-$PhCH_2$	4.3	3.2
30	2,6-di-Cl-$PhCH_2$	110	4.6
31	3,5-di-Cl-$PhCH_2$	23	2.6
32	3,5-di-F-$PhCH_2$	16	<0.3
33	2-Me-$PhCH_2CH_2$	44	1.6
34	3-Me-$PhCH_2CH_2$	40	1.7
35	2,4-di-Me-$PhCH_2CH_2$	84	7.4
36	2,5-di-Me-$PhCH_2CH_2$	7.1	4.3
37	3,4-di-Me-$PhCH_2CH_2$	63	6.2
13	2-Cyclopentylethyl	11	3.9
14	Cyclohexylmethyl	19	3.1

ity. Therefore, further optimization around the benzyl, phenethyl and cycloalkylmethyl derivatives was carried out to confirm this hypothesis.

As shown in Table 3, the di-substituted benzyl derivatives **23**–**31** and the di-substituted phenethyl derivatives **35**–**37** showed moderate permeability (Pe: 2.6–7.4 $\times$ 10^{-6} cm/s) except for the di-fluoro derivative **32**. In contrast, the permeability of the mono-substituted benzyl derivatives **17**–**22** or phenethyl derivatives **33** and **34** was relatively low. In terms of the cellular activity, the substituent effect appears prominent in the di-substituted benzyl and di-substituted phenethyl derivatives. Namely, the 2,5-di-substituted benzyl (**25** and **29**), 3,5-di-substituted benzyl (**31** and **32**) and 2,5-dimethyl-phenethyl derivative **36** showed significantly improved cellular activity compared with the other di-substituted derivatives. Among these derivatives, the compounds **25**, **29**, and **36** showed potent JFas cellular activity (IC_{50}: < 11 nM) with moderate permeability (Pe: 3.2–4.3 $\times$ 10^{-6} cm/s). In addition, the 2-cyclopentylethyl derivative **13** also had a good profile comparable

Table 4
Results of JFas cellular assay and PAMPA for representative derivatives.



Compound	R	Enzyme assays k_3/K_i (M^{-1} s^{-1})			JFas celluar assay IC_{50} (nM)	In vivo assay ED_{50} (mg/kg)	PAMPA Pe ($\times 10^{-6}$ cm/s)
		Caspase-3	Caspase-8	Caspase-9			
1 (IDN-7314)	2,6-di-F-Ph	37,400	100,000	264,000	6.3	0.7	<0.9
29	2,5-di-Cl-PhCH_2	26,800	190,000	380,000	8.4	<0.01	3.2
36	2,5-di-Me-PhCH_2 CH_2	20,600	112,000	184,000	5.0	0.02	4.3
13	2-Cyclopentylethyl	9470	38,100	122,000	11	0.01	3.9

to compounds **25**, **29**, and **36**. Therefore, the in vivo activity of the representative pan-caspase inhibitors **29**, **36**, and **13** was evaluated and, as expected, these compounds showed potent in vivo activity (ED_{50}: <0.01–0.02 mg/kg) in a murine model (Table 4).

In conclusion, the P4 region of the oxamyl dipeptide caspase inhibitors was optimized by the combination of anti-apoptotic activity in the JFas cell and membrane permeability. Among the P4 derivatives, the compounds which showed pan-caspase inhibitory activity turned out to have potent cellular activity. Ultimately highly potent anti-apoptotic compounds with improved membrane permeability, **29**, **36**, and **13**, were identified. These compounds showed strong in vivo efficacy in a murine model of α-Fas-induced liver injury as predicted. Thus these compounds are expected to be useful for the treatment of liver disease. We intend to apply this study results to the SAR study for oxamyl dipeptide with valine at P2 region as oxamyl dipeptides with valine at P2 region have already shown potent activities in cellular models of apoptosis.[12]

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