**Design, Syntheses and Biological Evaluations of Nonpeptidic Caspase 3 Inhibitors**

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Caspase 3, a member of cysteine protease family, is well known as a major apoptosis effector and is involved in cell death as a result of ischemic diseases such as stroke and myocardial infarction, therefore the inhibition of caspase 3 may protect those apoptotic cell damages. During the high-throughput screening of the compounds from the Korea Chemical Bank, berberine derivatives (A and B), an isoquinoline alkaloid, have been identified as potential inhibitors for caspase 3. Based on this finding we carried out molecular modeling study to identify the pharmacophoric elements of berberine structure which interact with a substrate-recognition binding site of caspase 3 and came up with several novel scaffolds. In this report, we will discuss the molecular modeling, syntheses and the enzyme inhibitory activities of these novel compounds.

**Keywords:** Caspase 3, Nonpeptidic inhibitor, Apoptosis, Isoquinoline derivatives.

**Introduction**

Deregulated apoptosis can cause several diseases when it is either excessive or insufficient. Tissue damage following stroke or myocardial infarction is largely apoptotic, and there is growing evidence that the inhibition of apoptosis can lessen tissue damage and improve a patient’s prospects.1 Although a wide variety of molecular and cellular events are involved in apoptosis, most pathways converge onto a single family of enzymes, the caspases, leading to the breakdown of proteins in a proteolytic manner and ultimately cell death.2,3 Caspases (cysteinyl aspartate specific proteases) were first identified in mutational studies using Caenorhabditis elegans,9,10 and to date, 13 mammalian members of this family have been characterized including 11 members in human.11-14 They can be subdivided into three groups based on homology and substrate specificity: (1) caspases involved in inflammation (caspases 1, 4, 5, and 13), (2) initiator caspases which are found at the top of the signaling cascade (caspases 6 and 8-10), and (3) effector caspases which are activated in further downstream (caspases 2, 3, and 7).15 Caspase 3, a member of effector caspases, has been found to be activated in nearly every model of apoptosis, thus offers an attractive therapeutic target for the treatment of disorders involving apoptosis.16,17

Because nonspecific peptide inhibitors have been reported not to block apoptosis sufficiently, the prospect of caspase inhibitors as drug candidates may largely depend on the selectivity as well as potency.18-21 Recent studies on caspase structure, specificity, and catalytic mechanism have provided insight into the design of selective compounds. In this study, we have synthesized and biologically evaluated the putative caspase 3 inhibitors, aiming at the identification of novel scaffolds for caspase 3 inhibitors.

**Design of a Scaffold for Nonpeptidic Caspase 3 Inhibitors**

A high-throughput screening of the compounds from the Korea Chemical Bank in KRICT on the inhibition of caspase 3 using 96-well plate format identified berberine derivatives A and B showing 67% and 64% inhibition at 20 µM, respectively (Figure 1).

We carried out a docking analysis using FlexX program with these molecules and the crystal structure of caspase 3 obtained from PDB (code; 1GFW). All procedures were performed on Silicon Graphics workstation (Origin R1000, 256 Mbytes memory, 2 CPU, 180 MHz IP27 processor) using SYBYL (v. 6.7) (Tripos Associate Inc.). The final docked molecules with the lowest binding energies (A-1; -20.92 and A-2; -20.13 kcal) were shown in Figure 2.

Caspase 3 appeared to fit into the active site of caspase 3 by forming several interactions; (1) A-1; H-bonds with Ser63, Ser65, Thr62, and Phe250, (2) A-2; hydrophobic contact with His121, H-bonds with Arg64, Gln161, Ser120.

**Figure 1**

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and Tyr204. But those didn't look forming the H-bond with His121 or Cys163 at catalytic domain. Based on the docking model, we suggested to modify the berberine backbone structure for improved interaction with the enzyme; (1) removal of the ethylene bridge connecting the isoquinoline and catechol rings to make the rigid ring skeleton of the compound A more flexible (Figure 3), (2) the addition of N-Oxide which may increase the ionic interaction with Arg207 at the active site, (3) transposition of catechol ring from 3- to 1-position of isoquinoline for an additional hydrophobic interaction with Try206 and Phe256 at the hydrophobic pocket (S2), (4) removal of 7- and 8-OH in the isoquinoline

**Figure 2.** Docking the compound A to the crystal structure of caspase 3 by SYBYL.

**Figure 3.** De novo design of isoquinoline N-oxide derivatives and possible interaction sites with caspase 3.
moiety which may not interact with the active site, different from the compound A itself.

From this analysis we designed and subsequently synthesized various 1-phenylisoquinoline derivatives. Besides the compounds directly connecting benzene and isoquinoline rings, we prepared the compounds with an amide or ether linkage as a spacer between two rings.

Chemistry

1-Phenylisoquinoline derivatives. We synthesized a series of 1-phenylisoquinoline derivatives 8-15 by Suzuki coupling reaction between various phenyl boronic acids and 1-isoquinoline-O-triflate 7 using sodium carbonate as a base in toluene/ethanol/water (Scheme 1). Subsequently N-Oxides 16-23 and N-methyl iodide salts 27-34 were prepared using m-CPBA in CH₂Cl₂, and excess iodomethane, respectively. Demethylation reactions of methoxy substituted compounds (17-19, 28-30) in the benzene ring were carried out with boron tribromide in anhydrous CH₂Cl₂ to provide the corresponding hydroxy substituted compounds (24-26, 35-37).

Isoquinoline-1-carboxylic acid phenyl amide derivatives. Isoquinoline-1-carboxylic acid phenyl amides 38-43 were prepared by well established method from isoquinoline-1-carboxylic acid via acid chloride followed by the treatment with aniline derivatives (Scheme 2). The N-Oxides 44-48 and N-methyl iodide salts 49-51 were synthesized as described above. The oxidation to N-oxide of 4-nitrophenyl derivative 42, and N-methylation of 4-chloro-phenyl- (41) and 4-nitrophenyl derivatives (42) were turned out to be very resistant by unknown reasons.

1-Phenoxyisoquinoline derivatives. To obtain the ether type of compounds, we initially examined the Ullmann type of the reaction between isocarboxystyril and bromobenzenes, but the reaction failed to give the desired ether compounds presumably due to the lack of nucleophilicity of the hydroxy group in isocarboxystyril. Alternatively, a substitution reaction between substituted phenols and 1-bromoisoquinoline was tried and was successful to provide 1-phenoxyisoquinoline derivatives 52-57 (Scheme 3).

Results and Discussion

The inhibitory effects on caspase 3 were determined at 20 µM concentration of compounds (Table 1). As reference compounds, we used 5-nitroisatin derivatives, N-methyl C and N-benzyl D compounds (Figure 4) which were reported to be good inhibitors for caspase 3 with IC₅₀ of 1 and 0.25 µM. At 20 µM concentration, those compounds C and D represented 82% and 95% of inhibition on caspase 3, respectively.

Initially we prepared various 1-phenylisoquinoline derivatives 8-37 modified from the basic backbone of the compound A, which was identified as a caspase 3 inhibitor by the high throughput screening on samples from the Korea Chemical Bank in KRICT (Table 1). Among 3,4-Dimethoxy- (17, 28) and 3,4-dihydroxy (24, 35) substituted 1-phenylisoquinoline analogues with the same substitution pattern as the starting compound A, only compound 35 showed moderate inhibitory effect, 42%. While most of the disubstituted compounds didn’t show significant inhibitory activi-
ties, some of monosubstituted compounds represented relatively good inhibitory activities. 1-(p-Methoxyphenyl)isoquinoline 11, 1-(p-methoxyphenyl)isoquinoline N-oxide 19, 1-(p-hydroxyphenyl)isoquinoline N-oxide 26, and 1-(p-hydroxyphenyl)isoquinoline N-methylated compound 37 showed 69%, 60%, 59% and 62% of inhibition at 20 µM, respectively. Monomethoxy substituted isoquinoline N-oxide derivatives 18 (51%), 19 (60%) showed better inhibitory activity (51%, 60%) than the corresponding dimethoxy derivative 17 (19%). The relative location of methoxy group didn’t seem to influence the activity much as seen in 18 and 19.

The lower activities of the unsubstituted phenyl isoquinoline 8 (7%) and N-oxide 16 (31%) than the methoxy substituted analogues 11 (69%) and 19 (60%) indicate that the methoxy group might participate in the binding interaction with the enzyme.

In addition, we have prepared a series of compounds with an amide linkage between the benzene and isoquinoline ring (44-51). 3,4-Dimethoxyphenyl amide derivatives (39, 45, 50) showed generally better inhibitory activities (59%, 48%, 51%) than 3,4-dimethoxyphenylisoquinoline compounds (17 (19%), 28 (22%)) (Table 1).

We also synthesized another series of compounds 52-57 in which an ether linkage was used as a spacer and found that this series of compounds didn’t show any inhibitory activities on caspase 3.

Although few classes of compounds described in this report did not show prominent inhibitory activities compared to known isatin type of inhibitors, we identified several novel scaffolds. The finding that the compounds with different spacers between the benzene and isoquinoline rings exhibit a wide range of activities requires further molecular modeling study for establishing the relationship between structure and activity.

In conclusion we designed several novel putative caspase-3 inhibitors based on the active sites of the enzyme and evaluated their inhibitory activities. 1-(p-Methoxyphenyl)- and 1-(m-methoxyphenyl)-isoquinoline derivatives (10,11, 18-19, 29) showed significant inhibitory effects (>50%). Further modification for improved activity and the establishment of structure and activity relationship are subjects for future works.

Experimental Section

Chemistry. Melting points were determined on a capillary melting point apparatus and are uncorrected. Anhydrous
solvents were dried by conventional methods. Reagents of commercial quality were used from freshly opened containers unless otherwise stated. ¹H NMR spectra were recorded on a Varian Gemini 200 or a Bruker DRX-300 spectrometer. ¹³C NMR spectra were obtained on a Bruker AMX-300 spectrometer. Mass spectra were obtained with a JEOl JMS-DX 303 instrument by using electron impact or chemical ionization techniques.

**General procedure for the syntheses of phenyl boronic acid (1-6).** To a solution of bromobenzene (1.39 mmol) in THF (3 mL), n-BuLi (1.5 M in Hexane, 2.09 mmol) was added at -78 °C. After stirring at that temperature for 30 min, trimethyl borate (4.17 mmol) was added to the mixture. The reaction mixture was warmed up to room temperature and stirred for an additional hr, then quenched with water. The mixture was extracted with CH₂Cl₂, followed by the addition of a aqueous 2 M solution of sodium carbonate (0.3 mL). The reaction mixture was heated at reflux until TLC showed the completion of reaction (4 hr). After cooling, the reaction was washed with water and extracted with ethyl acetate. The organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure to remove all volatiles. The residue was purified by silica gel column chromatography (ethyl acetate : hexanes = 3 : 1) to give a desired compound.

1-Phenylisoquinoline (8) mp 190-191 °C; ¹H NMR (200 MHz, CDCl₃): δ 7.51-7.68 (m, 8H), 7.88 (d, 1H), 8.09 (d, 1H), 8.62 (d, 1H).

1-(3,4-Dimethoxyphenyl)isoquinoline (9) mp 109-111 °C; ¹H NMR (200 MHz, CDCl₃): δ 3.94 (s, 3H), 3.96 (s, 3H), 7.00 (d, 1H), 7.23-7.28 (m, 2H), 7.52-7.71 (m, 3H), 7.86 (d, 1H), 8.17 (d, 1H), 8.58 (1H, dd); MS 265 (M⁺).

1-(3-Methoxyphenyl)isoquinoline (10) mp 111-112 °C; ¹H NMR (200 MHz, CDCl₃): δ 3.85 (s, 3H), 7.05 (m, 1H), 7.30 (m, 2H), 7.4-7.7 (m, 4H), 7.84 (m, 1H), 8.16 (d, 1H), 8.6 (d, 1H).

1-(4-Methoxyphenyl)isoquinoline (11) mp 166-167 °C; ¹H NMR (200 MHz, CDCl₃): δ 3.86 (s, 2H), 7.04 (d, 2H), 7.40-7.76 (m, 5H), 7.80 (d, 1H), 8.17 (d, 1H), 8.56 (d, 1H).

1-p-Tolylisoquinoline (12) mp 170 °C; ¹H NMR (200 MHz, CDCl₃): δ 2.46 (s, 3H), 7.31-7.48 (m, 2H), 7.51-7.71 (m, 5H), 7.86 (d, 1H), 8.12 (d, 1H), 8.60 (dd, 1H).

1-(4-Chlorophenyl)isoquinoline (13) mp 293-294 °C; ¹H NMR (200 MHz, CDCl₃): δ 7.47-7.71 (m, 7H), 7.86 (d, 1H), 8.03 (d, 1H), 8.59 (d, 1H); HRMS (M⁺) 239.0502 calcd. for C₁₀H₁₂ClN, found 239.0500.

1-Isouquinolin-1-yl-benzoic acid (14) mp 179-180 °C; ¹H NMR (200 MHz, CDCl₃): δ 7.58 (m, 1H), 7.71-7.76 (m, 2H), 7.82 (m, 3H), 7.96 (m, 2H), 8.63 (d, 1H); MS 229 (M⁺).

1-Isouquinolin-1-yl-pentan-1-ol (15) mp 201-203 °C; ¹H NMR (200 MHz, CDCl₃): δ 0.88-0.91 (m, 3H), 1.31-1.44 (m, 4H), 1.75-1.86 (m, 2H), 3.45 (br, 1H), 4.71 (m, 1H), 7.26-7.45 (m, 2H), 7.47-7.50 (m, 1H), 7.60-7.62 (m, 3H), 7.64-7.67 (m, 1H), 7.85 (d, 1H), 8.07 (d, 1H), 8.55 (d, 1H); MS 291 (M⁺).

**General procedure for the syntheses of 2-oxy-1-phenylisoquinolines (16-23).** To a solution of 1-phenylisoquinoline (8) (2.50mmol) in CH₂Cl₂ (10 mL) was added m-CF₃BA (5.00 mmol), then the mixture was stirred vigorously for 3-10 hr. The reaction was washed with an aqueous solution of saturated NaHCO₃ and extracted with CH₂Cl₂. The organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure to remove all volatiles. The residue was purified by silica gel column chromatography (ethyl acetate : hexanes = 3 : 1) to give a desired compound.

2-Oxy-1-phenylisoquinoline (16) mp 153-154 °C; ¹H NMR (200 MHz, CDCl₃): δ 7.42-7.62 (m, 8H), 7.65 (d, 1H), 7.79 (d, 1H), 8.27 (d, 1H).

1-(3,4-Dimethoxyphenyl)-2-oxyisoquinoline (17) mp 121-123 °C; ¹H NMR (200 MHz, CDCl₃): δ 3.85 (s, 1H), 3.94 (s, 3H), 7.03 (dd, 1H), 7.11-7.21 (m, 2H), 7.60-7.77 (m, 3H), 7.97-8.03 (m, 2H), 8.30 (d, 1H).

1-(3-Methoxyphenyl)-2-oxyisoquinoline (18) mp 125-126 °C; ¹H NMR (200 MHz, CDCl₃): δ 3.83 (s, 3H), 7.04-7.08
(m, 3H), 7.45-7.56 (m, 4H), 7.61-7.77 (m, 4H), 8.04 (m, 1H), 8.24 (d, 1H), 8.54 (d, 1H), 9.13 (d, 1H).

1-(4-Methoxyphenyl)-2-methylisoquinolinium iodide (30) mp 178-180 °C; 1 H NMR (200 MHz, CDCl3): δ 3.95 (s, 3H), 4.43 (s, 3H), 7.20 (d, 2H, J = 8.9 Hz), 7.63 (2H, J = 8.9 Hz), 7.80 (m, 2H), 8.04-8.12 (m, 1H), 8.26 (d, 1H), 8.50 (d, 1H), 9.10 (d, 1H).

2-Methyl-1-phenylisoquinolinium iodide (31) mp 191-192 °C; 1 H NMR (200 MHz, CDCl3): δ 2.53 (s, 3H), 4.42 (s, 3H), 7.32-7.59 (m, 4H), 7.70-7.85 (m, 2H), 8.10 (m, 1H), 8.28 (d, 1H), 8.53 (d, 1H), 9.13 (d, 1H).

1-(4-Chlorophenyl)-2-methylisoquinolinium iodide (32) mp 181-182 °C; 1 H NMR (200 MHz, CDCl3): δ 3.45 (s, 3H), 7.59-7.80 (m, 6H), 8.05 (m, 1H), 8.24 (d, 1H), 8.52 (d, 1H), 9.00 (d, 1H).

1-(4-Cyanophenyl)-2-methylisoquinolinium iodide (33) mp 182-183 °C; 1 H NMR (200 MHz, CDCl3): δ 4.34 (s, 3H), 7.63 (d, 1H), 7.89-8.23 (m, 6H), 8.33 (d, 1H), 8.55 (d, 1H), 8.96 (d, 1H).

1-[4-(1-Hydroxypentyl)phenyl]-2-methylisoquinolinium iodide (34) mp 199-201 °C; 1 H NMR (300 MHz, CDCl3): δ 0.88-0.90 (m, 3H), 1.33-1.38 (m, 3H), 1.44-1.46 (m, 1H), 1.78-1.86 (m, 2H), 3.44 (m, 1H), 4.35 (s, 3H), 4.87 (br, 1H), 7.61 (d, 2H, J = 3.2 Hz), 7.68-7.72 (m, 3H), 7.81 (dd, 1H), 8.09 (dd, 1H), 8.27 (d, 1H), 8.50 (d, 1H), 9.00 (d, 1H).

1-(3,4-Dihydroxyphenyl)-2-methylisoquinolinium iodide (35) The compound 35 was prepared from the same procedure for the syntheses of the compounds 24-26 except using the compound 28 as a starting material; mp 190-192 °C; 1 H NMR (200 MHz, CDCl3): δ 1.00 (m, 3H), 1.33-1.38 (m, 3H), 1.44-1.46 (m, 1H), 1.78-1.86 (m, 2H), 3.44 (m, 1H), 4.35 (s, 3H), 4.87 (br, 1H), 7.61 (d, 2H, J = 3.2 Hz), 7.68-7.72 (m, 3H), 7.81 (dd, 1H), 8.09 (dd, 1H), 8.27 (d, 1H), 8.50 (d, 1H), 9.00 (d, 1H).

1-(3-Hydroxyphenyl)-2-methylisoquinolinium iodide (36). The compound 36 was prepared from the same procedure for the syntheses of the compounds 24-26 except using the compound 29 as a starting material; mp 231-232 °C 1 H NMR (200 MHz, CDCl3): δ 4.22 (s, 3H), 7.02-7.07 (m, 2H), 7.19 (m, 1H), 7.58 (m, 1H), 7.81-7.96 (m, 2H), 8.19 (m, 1H), 8.34 (d, 1H), 8.49 (d, 1H), 8.69 (1H, J = 6.8 Hz).

1-(4-Hydroxyphenyl)-2-methylisoquinolinium iodide (37). The compound 37 was prepared from the same procedure for the syntheses of the compounds 24-26 except using the compound 30 as a starting material; mp 229-230 °C 1 H NMR (200 MHz, CDCl3): δ 4.24 (s, 3H), 7.10-7.18 (m, 2H), 7.45-7.51 (m, 2H), 7.84-7.96 (m, 2H), 8.14-8.22 (m, 1H), 8.33 (d, 1H), 8.47 (d, 1H), 8.71 (d, 1H).

General procedure for the syntheses of 1-phenylcarboxamoylisooquinoline derivatives (38-42). To a solution of isoquinoline-1-carboxylic acid (1.73 mmol) in CH2Cl2 (6
ml), oxalyl chloride (8.66 mmole) was added during 5 min at room temperature. After vigorous stirring for 10 min at that temperature, all volatiles were removed under reduced pressure. The residue was dissolved in CH2Cl2 (3 mL), to which the mixture of an appropriate aniline (1.38 mmol) and triethylamine (8.66 mmol) in CH2Cl2 (3 mL) was added slowly. After stirring for 1 hr at rt, the reaction was washed with water and extracted with CH2Cl2. The organic layer was dried over MgSO4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 2 : 1) to give a desired compound.

1-Phenylcarbamoylisoquinoline (38)\(^{28}\) mp 156-157 °C; \(^1\)H NMR (200 MHz, CDCl3): \(\delta\) 7.11-7.18 (m, 1H), 7.35-7.43 (m, 2H), 7.63-7.85 (m, 6H), 8.47 (d, 1H), 7.69 (m, 1H), 10.23 (br, 1H).

1-(3,4-Dimethoxyphenylcarbamoyl)isoquinoline (39) mp 108-109 °C; \(^1\)H NMR (200 MHz, CDCl3): \(\delta\) 3.89 (s, 3H), 3.96 (s, 3H), 6.89 (d, 1H), 7.2 (dd, 1H), 7.6-7.9 (m, 5H), 8.5 (d, 1H), 9.72 (m, 1H), 10.27 (br, 1H); HRMS 308.1161 (M^+) calculated for C\(_{16}\)H\(_{12}\)N\(_2\)O\(_3\), found 308.1163.

1-Phenoxyisoquinoline (52) mp 190-201 °C; 1H NMR (200 MHz, CDCl3): \(\delta\) 3.84 (s, 3H), 7.03 (dd, 1H), 7.41-7.50 (m, 4H), 7.60-7.97 (m, 4H), 8.49 (d, 1H), 11.04 (br, 1H).

General procedure for the syntheses of 1-phenylcarbamoyl-2-oxoisquinoline derivatives (44-48). The compounds 44-48 were prepared from the same procedure for the syntheses of the compounds 16-23.

1-Phenylcarbamoyl-2-oxoisquinoline (44) mp 190-191 °C; \(^1\)H NMR (200 MHz, CDCl3): \(\delta\) 7.09 (m, 1H), 7.26-7.34 (m, 2H), 7.52-7.77 (m, 7H), 8.78 (d, 1H), 12.03 (br, 1H); HRMS 264.0899 (M^+) calculated for C\(_{16}\)H\(_{13}\)N\(_2\)O, found 264.0905.

1-(3,4-Dimethoxyphenylcarbamoyl)-2-methylisoquinolinium iodide (49) mp 112-114 °C; \(^1\)H NMR (200 MHz, CDCl3): \(\delta\) 3.84 (s, 3H), 4.53 (s, 3H), 6.94-6.96 (m, 2H), 7.94-7.98 (m, 3H), 8.09 (dd, 1H), 8.17 (d, 1H), 8.30 (d, 1H), 8.40 (d, 1H), 8.50 (d, 1H).

General procedure for the syntheses of phenyoxyquinolines (52-57). To a solution of an appropriate phenol (1.92 mmol) in anhydrous dimethyl sulfoxide (3 mL) was added potassium tert-butoxide (197 mg, 1.44 mmol) slowly. After stirring for 1 hr at rt, the reaction was washed with water and extracted with ethyl acetate. The organic layer was dried over MgSO4, filtered, and concentrated under reduced pressure to remove all volatiles. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 2 : 1) to give a desired compound.

1-Phenoxyisoquinoline (52) \(^{29}\) mp 120-121 °C; \(^1\)HNMR (200 MHz, CDCl3): 7.23-7.34 (m, 4H), 7.43-7.51 (m, 2H), 7.59-7.83 (m, 3H), 7.89 (d, 2H), 11.46 (br, 1H).

1-(4-Chlorophenylcarbamoyl)-2-methylisoquinolinium iodide (50). The compound 51 was prepared from the same procedure for the syntheses of the compounds 27-34 except using the compound 40 as a starting material; mp 219-221 °C; \(^1\)H NMR (200 MHz, CDCl3): \(\delta\) 3.90 (s, 3H), 4.53 (s, 3H), 6.94-6.96 (m, 2H), 7.94-7.98 (m, 3H), 8.09 (dd, 1H), 8.17 (d, 1H), 8.30 (d, 1H), 8.40 (d, 1H), 8.50 (d, 1H).

1-(4-Chlorophenylcarbamoyl)-2-methoxyisoquinolinium iodide (51). The compound 51 was prepared from the same procedure for the syntheses of the compounds 27-34 except using the compound 40 as a starting material; mp 219-221 °C; \(^1\)H NMR (200 MHz, CDCl3): \(\delta\) 3.84 (s, 3H), 4.53 (s, 3H), 6.94-6.96 (m, 2H), 7.94-7.98 (m, 3H), 8.09 (dd, 1H), 8.17 (d, 1H), 8.30 (d, 1H), 8.40 (d, 1H), 8.50 (d, 1H).
were conducted at 37 °C for 1 h. The amount of released AMC) and 20 µM of acetyl-Asp-Glu-Val-Asp-aminomethylcoumarin (Ac-DEVD-CPP32 (Medical & Biological Laboratories, Japan), 50 µM CHAPS, 10 mM DTT) containing 50 ng of active caspase-3.


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