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Graphical Abstract

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Synthesis and activity evaluation of the cyclic dipeptides arylidene *N*-alkoxydiketopiperazines

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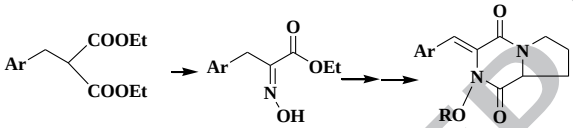

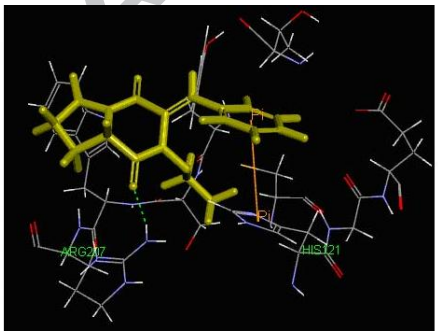

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Synthesis and activity evaluation of the cyclic dipeptides arylidene *N*-alkoxydiketopiperazines

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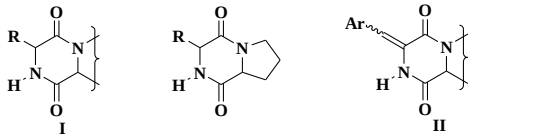
ABSTRACT

A series of arylidene *N*-alkoxydiketopiperazines was designed and stereoselectively synthesized *via* oxime-ether formation and intramolecular acylation. Possible cyclization and acid-catalyzed rearrangement-fragmentation mechanisms were discussed. The crystal structure of the novel diketopiperazine further confirmed the rearrangement mechanism. Most compounds exhibited antitumor activity. Several compounds were more potent against caspase-3. Specifically, compounds **6e**, **6g**, and **6f** inhibited caspase-3 at IC_{50} values lying within the low micromolar range and demonstrated good selectivity. The binding modes of alkoxydiketopiperazines in the active center of caspase-3 were also discussed based on the molecular docking results.

1. Introduction

Diketopiperazines (DKPs) (I) are the smallest cyclic peptides isolated from microorganisms and sponges, as well as from a variety of tissues and body fluids [1–3]; they represent an important class of biologically active natural products that have been used as versatile intermediates in asymmetric synthesis of amino acid derivatives and related natural products [4, 5]. These heterocyclic compounds exhibit remarkable biological and pharmacological activities, such as antimicrobial, antitumor, antiviral, and plant growth regulation [6, 7]. Moreover, investigations show that many DKP derivatives are cytotoxic against an array of human cancer cells [8]. Different proline-containing DKPs exhibit a number of bioactivities, such as inhibition of *Serratia marcescens* and *Vibrio anguillarum* and antihyperglycemic activity [9–11]. Among DKPs, ylidene DKPs II are natural products often produced by fungi, such as *Actinomyces* strains and *Penicillium* species. Some of these compounds possess antibacterial activity and inhibit tumor growth in mice [12].



diketopiperazines **arylidene diketopiperazines**

Phenylahistin (PLH) [12, 13], an arylidene DKP produced by *Aspergillus ustus*, is a cell cycle inhibitor. (−)PLH demonstrates antitumor activity against eight tumor cell lines at IC_{50} values that ranged from 1.8×10^{-7} M to 3.7×10^{-6} M. It also shows antitumor activity against P388 leukemia and Lewis lung cancinoma cells *in vivo*. Total synthesis of PLH was investigated [14] to understand its precise biological functions and develop more potent antitumor agents based on DKP structures. In addition, a dehydro product of (−)-PLH CDP dehydropenylahistin (△ (−)-PLH) demonstrates potent cytotoxic activity higher than that of the known anticancer drugs taxol, vinblastine, and vincristine, indicating that this novel compound is a candidate anticancer drug; in addition, △ (−)-PLH demonstrates an inhibitory activity toward the first cleavage of sea urchin embryos by more than 1000 times that of (−)-PLH

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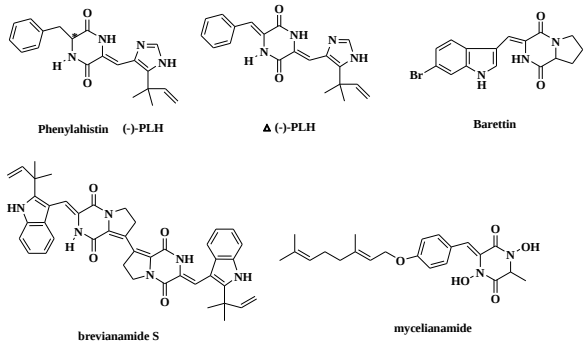
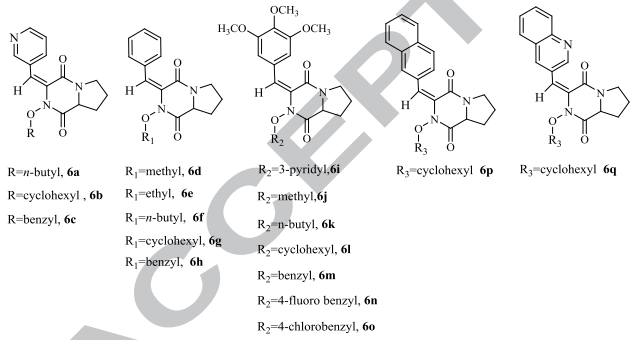


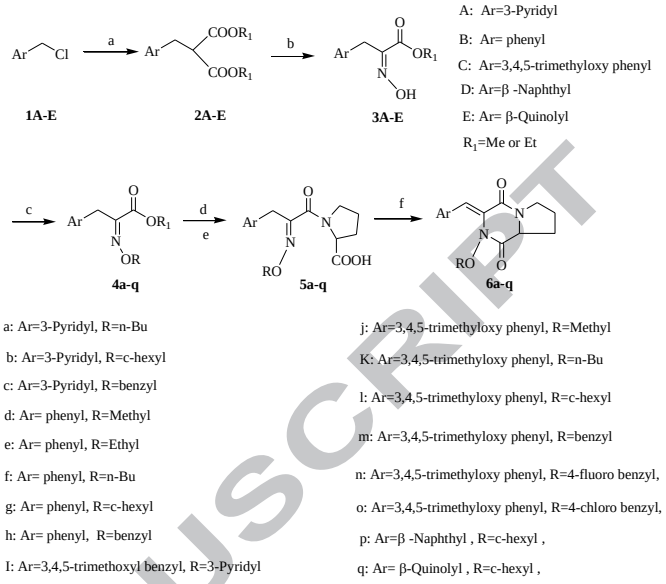
Synthesis of ylidene DKPs as versatile building blocks of bicyclic *N*-heterocycles has attracted a considerable amount of attention. The various reactive functional groups of ylidene DKPs allow reactions to occur at different positions of the ring and at the exocyclic carbon atom of C=C. Therefore, ylidene DKPs have been synthesized following various substituent patterns in the non-chiral, racemic, and optically active series *via* different synthetic pathways. Liebscher et al. [16, 17] reviewed the synthesis, properties, and applications of 3-ylidene-DKPs; other synthesis methods have also been reported [18–20]. Proline-containing arylidene bicyclo-DKPs, such as brevianamides and barettin, have been found in natural products, and they exhibit antibacterial and antiviral activities [21, 22]. The characteristic structure of *N*-hydroxyl DKPs found in mycelianamide, which is isolated from *Penicillium griseofulvum* mycelium [23], caught our interest. To determine the bioactivity of arylidene bicyclo-DKPs that contain proline and the *N*-alkoxy structure fragment, we investigated a novel, efficient, and stereoselective synthesis and the relative chemical characteristics of *N*-alkoxy derivatives of *E*-arylidene bicyclo-DKPs [24]. In this work, we synthesized several new *E*-arylidene bicyclo-DKPs and investigated their bioactivity against certain tumor cell lines and their inhibitory effect against caspase-3.



2. Results and discussion

2.1. Synthesis

E-Arylidene bicyclo-DKPs were synthesized according to the general methods described in Scheme 1. We initially used benzylic halides 1 as raw material. The benzylic halides 1 was dropped into *N*,*N*-dimethylformamide (DMF) dilution of diethyl malonate and nano-K_2CO_3, producing the substitute product **2** at 75% yield. However, nucleophilic substitution reaction occurs in different bases when the aromatic ring varies. Treatment of **2** with EtONO at 0 °C formed a high yield (>90%) of α-oximino esters **3** in the presence of nano-K_2CO_3. The subsequent formation of oxime ether bond with RX by using K_2CO_3 produced compound **4**. The oxime or oxime ether was an excellent substrate to form an amino group *via* reduction reaction. Therefore, we used oxime ether as a masked amine group.



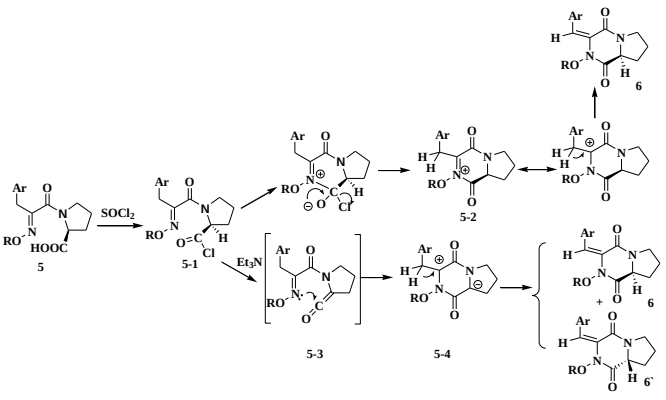
Scheme 1 Synthesis of *E*-arylidene bicyclo-DKPs

(a) $CH_2(CO_2Et)2$, nano-K_2CO_3; (b) EtONO, nano-K_2CO_3; (c) RX, K_2CO_3; (d) (1) NaOH, H_2O; (2) $(C_2H_5)_3N$, HBTU, DMF, proline methyl ester, (e) LiOH, THF:H_2O; and (f) $SOCl_2$

The oxime ether ester of **4** is hydrolyzed with NaOH followed by coupling with proline methyl ester in the presence of HBTU and Et_3N. The resulting products were deprotected with LiOH in THF, and H_2O produced pseudodipeptides **5** containing free carboxyl group. Compound **5** was treated with $SOCl_2$ to produce acyl chloride, which directly underwent intramolecular *N*-acylation with the oxime ether to stereoselectively generate compound **6** at good overall yields.

2.2. Chemistry

The method described above to prepare cyclic dipeptides involves six synthetic steps. In steps 2, 4, and 5, the resulting products **3** and **5** were used in the subsequent reaction without purification. Rearrangement cyclization (step 6) is a key step in the synthesis process. The mechanism of cyclization of the pseudodipeptides was studied. First, the carboxyl of pseudodipeptides **5** react with $SOCl_2$, producing acyl chloride **5-1**. A nucleophile was subsequently added into the carbonyl C with the N of oxime ether as nucleophilic reagent, and the leaving group chloride was eliminated to produce the ring-closing product iminium intermediate **5-2**. The hydrogen of



Scheme 2. Possible cyclization reaction

benzylic group can then be fragmented to produce the cyclic dipeptides **6a**–**6q** that contain exocyclic double bond, and the stereochemistry of L-proline was maintained. During cyclization, the configuration of proline depended on the reaction conditions. In the presence of Et_3N, the acyl chloride, which was prepared through reaction of compound **5** with $SOCl_2$, can be converted into ketene **5-3**. Finally, mixtures of compounds **6** and **6′** were produced by losing the configuration of proline in the cyclization products (Scheme 2).

The configurations of C=C in the final products were confirmed by single-crystal X-ray diffraction (XRD). The XRD studies revealed that the dipeptide **6b** crystallized in the orthorhombic space group P212121. The conformation of the molecule is illustrated in the stereo diagram in Fig. 1. Figure 1 shows that E-configuration of the exocyclic C(1)=C(14) in compound **6b** is formed. In addition to the conformation of the DKP ring, the conformation of the pyrrolidine ring is interesting. In L-proline, the five-membered ring of the pyrrolidine displays the envelope conformation. The substituted cyclohexyl group on nitrogen atom N(2) exhibited a chair conformation. Otherwise, the similar chemical shifts of the H(14) singlet in ^{1}H NMR suggest the same configuration for the other compounds. Investigation on the molecular structure further confirmed that this method can stereoselectively synthesize arylidene *N*-alkoxy DKPs.

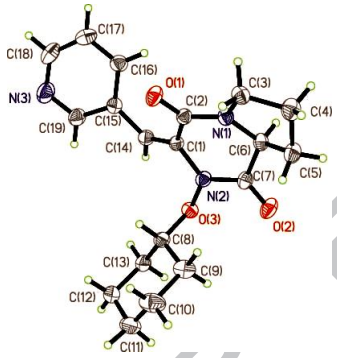
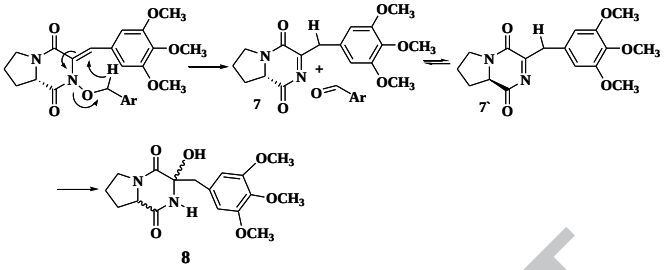


Fig.1. Structure of **6b** with ellipsoid displacement drawn at 30% probability level.

The chemistry of *N*-alkoxy *E*-arylidene bicyclo-DKPs was also investigated. Under acidic condition, the rearrangement-fragmentation and addition products of **6i**, **6j**, **6n**, and **6o** are similar to those reported in reference [14], whereas **6a**, **6c**, **6e**, **6p**, and **6q** were stable under the same condition. When the alkyl in *N*-alkoxy groups was arylmethyl, the rearrangement-fragmentation obviously strongly depended on Ar structure in the *N*-alkoxy *E*-arylidene bicyclo-DKPs. The electron-rich Ar, such as trimethyloxy phenyl 6i-6o, is favorable for these reactions. The rearrangement-fragmentation proceeds through a concerted *retro-ene* reaction mechanism contained heteroatom, which occurred between aryl formaldehyde and substituted imine (**Scheme 3)**. Tautomerism of the intermediate **7** cyclic acyl imine easily occurs to form the balance with **7′**, and the configuration of proline is lost. Thus, the reactions of the rearrangement-fragmentation of **6i**, **6j**, **6n**, and **6o** and H_2O addition produced a mixture containing four isomers of **8**.



Scheme 3. Possible mechanism of rearrangement-fragmentation reaction

The molecular structure of dipeptide **8** was determined through single-crystal XRD analysis. The XRD studies revealed that the dipeptide **8** crystallized in the triclinic space group P-1. The conformation of the molecule is illustrated in the stereo diagram in Figure 2. The diketopiperazine ring of **8** also exists in the boat conformation (β = −17°) in solid state with axial dispositions for the O_4 oxygen atoms. The dihedral angle between the average plane of the DKP ring and the plane of the benzyl residue is 78.4°. The hydroxy group on C_{11} and the H atoms on C_{16} are in the axial positions and located on the same side of the DKP ring, whereas the aromatic rings of the side chains are in quasi-equatorial positions.

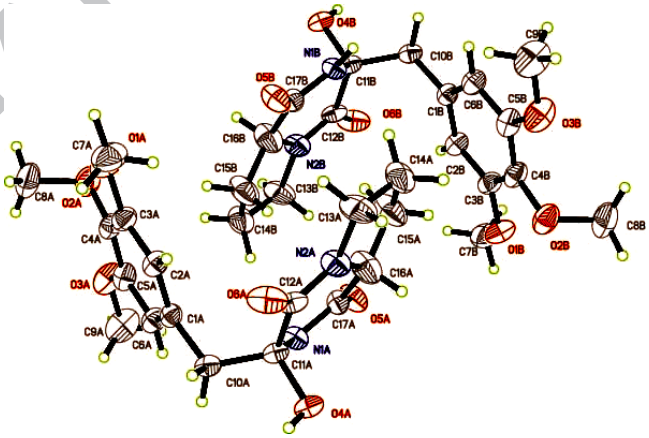


Fig. 2. Structure of **8** with displacement ellipsoids drawn at 30% probability level

2.3. Biological evaluations

2.3.1 Antitumor activities

To characterize the biological potential of *E*-arylidene bicyclo-DKPs, we determined the antitumor activities of some compounds against the cell lines K562-1, HCT-15, HT-29, HCT-8, HePG2, MDA-MB-231, A549, and Bre-04. The results presented in Table 1 show that most of the DKPs at 20 μg/mL concentration inhibited the cell lines K562-1, HCT-15, HT-29, HCT-8, and HePG2, and the activity against MDA-MB-231, A549, and Bre-04 was weak. These results indicate that the group on N atom played a critical role in antiproliferative potency. When the alkyl on nitrogen atom is the same, such as in **6b**, **6g** and **6l**; **6f** and **6k; 6h** and **6m**, the compounds containing different aryl showed varying antiproliferative activities against the cell lines. Compared with other compounds, the compound **6f** shows a high activity in the K562-1, HCT-15, and A549 cell lines (Table 1); **6b** demonstrates a high activity in the HT-29 and HCT-8 cell lines; and **6m** displays a high activity in the HePG2 and MDA-MB-231 cell lines. When the aryl group is trimethyloxy phenyl, the chlorinated analogue **6o** was more

potent than the corresponding nonhalogenated **6m** against the K562-1, HCT-15, A549, and Bre-04 cancer cells. The compounds **6d**, **6f**, and **6o** are worth investigating as potential agents against the K562-1 and HCT-15 cells. At 10 μM concentration, the inhibitory effects of **6d** and **6f** toward K562-1, HCT-15, HT-29, and A549 were lower than 50%, whereas the activities of **6l** against K562-1 and that of **6o** against K562-1 and HCT-15 remained higher than 50% (Table 2). The IC_{50} values of the four compounds against K562-1, HCT-15, HT-29, and A549 cells are shown in Table 3. The compound **6o** is more potent against K562-1, HCT-15, and A549 cells, with corresponding IC_{50} values of 7.3, 8.6, and 11.4 μM.

Table 1 *In vitro* % inhibition of compound **6** (20 μg mL^{-1}) against tumor cell lines

Compound	K562-1	HCT-15	HT-29	HCT-8	HePG2	MDA-MB-231	A549	Bre-04
6b	69.3±2.1[a]	62.1±3.5[a]	70.3±2.6[a]	80.1±3.8[a]	60.4±1.5[a]	16.1±4.1[a]	35.2±3.2[a]	35.3±2.9[b]
6d	90.4±2.7[a]	88.8±1.5[a]	65.4±4.1[a]	78.8±2.4[a]	62.2±1.9[a]	23.5±3.2[a]	26.6±4.0[a]	24.5±3.5[b]
6e	42.6 ±3.1[b]	-	62.8±1.1[b]	-	-	-	25.3±3.7[b]	21.9±4.6[b]
6f	96.2±1.9[a]	90.6±2.0[a]	64.9±2.8[a]	68.4±3.2[a]	59.9±2.2[a]	16.3±2.6[a]	50.8±1.7[a]	-
6g	78.2±2.2[a]	72.3±1.8[a]	69.4±5.1[a]	76.3±3.4[a]	68.5±6.1[a]	17.3±5.1[a]	33.8±5.3[a]	-
6h	65.6±2.1[a]	69.7±2.1[a]	76.2±2.1[a]	66.8±2.1[a]	69.2±2.1[a]	32.6±2.1[a]	41.3±2.1[a]	25.0±2.1[b]
6i	-	78.7±4.4[b]	30.7±3.8[b]	-	-	-	27.1±2.6[b]	-
6k	64.4±6.1[a]	61.6±4.3[a]	61.6±1.1[a]	60.6±2.3[a]	64.3±3.6[a]	45.6±4.6[a]	43.4±3.9[a]	-
6l	80.1±1.2	67.9±2.2[a]	60.5±2.8[a]	75.2±6.1[a]	76.1±3.1[a]	42.5±6.3[a]	26.4±1.1[a]	-
6m	73.2±2.8[a]	74.2±1.7[a]	70.1±4.0[a]	79.1±3.1[a]	75.0±4.5[a]	43.2±1.5[a]	8.9±0.9[a]	
6o	95.7±1.0[b]	92.6±3.3[b]	53.2±2.4[b]	-	-	-	76.6±3.6[b]	50.3±2.3[b]

[a]: reference[24] [b] this work

Table 2. Cytotoxic activities of some compounds against tumor cell lines

Compounds	Dose	Cytotoxic activities (%)			
	(μM)	K562-1	HCT-15	HT-29	A549
6d	100	98.6±2.1	96.5±1.9	88.6±4.4	50.5±3.1
	50	87.3±1.5	82.6±2.4	56.4±3.5	22.4±1.2
	20	55.4±3.0	60.5±3.6	34.9±1.1	-
	10	36.2±2.8	46.8±1.7	28.1±1.6	-
	5	24.4±2.0	37.3±4.1	-	-
6f	100	99.6±3.1	99.1±4.1	80.6±6.0	91.1±4.3
	50	92.7±4.3	87.5±1.1	61.6±4.1	48.4±3.9
	20	46.5±3.2	78.3±4.0	39.8±3.5	16.2±2.8
	10	39.2±5.1	48.8±3.7	21.2±2.7	-
	5	30.2±2.7	4.04±0.9	-	-
6l	100	96.4±3.1	89.7±4.6	88.5±6.1	-
	50	83.6±5.4	69.3±2.1	56.6±5.8	33.4±6.0
	20	61.3±1.6	47.6±1.7	42.9±3.9	20.5±4.1
	10	50.7±3.5	39.2±2.4	48.8±2.6	-
	5	30.2±2.2	40.4±2.9	-	-
6o	100	97.8±3.1	98.6±3.9	86.8±8.3	91.7±3.0
	50	96.5±1.6	94.4±2.6	55.7±5.1	81.8±3.6
	20	73.9±3.6	81.6±1.1	39.2±5.7	63.4±4.2
	10	58.1±2.9	60.5±4.3	24.2±4.5	48.1±2.1
	5	36.7±1.5	42.4±3.2	-	4.03±0.5

Table **3**. Antiproliferative activity of **6d**, **6f**, **6l**, and **6o** against three human tumor cell lines

Compounds	IC_{50} μM		
	K562-1	HCT-15	A549
6d	17.6±2.1	14.1± 2.8	-
6f	27.3±3.5	11.8±1.5	-
6l	9.8±2.3	23.7±3.4	-
6o	7.3±2.6	8.6±2.7	11.4±3.1

2.3.2 Inhibitory activity of capase-3

During high-throughput screening of enzyme inhibiting activities, **6e**, **6f**, and **6j** exhibited good inhibition activities against caspase-3. The corresponding inhibition rates at 50 μM concentration were 93.7%, 77.3%, and 85.6% (Figure 3). However, when the concentration was reduced to 20 μM, **6e** still

showed a high inhibitory activity against caspase-3 at approximately 89% inhibition ratio, whereas the activities of **6f** (25.5%**)** and **6j** (34.3%)were lower than 50%. The dose-dependent activity of **6e** against viable caspase-3 was further evaluated, and the result shows that the IC_{50} was 7.6 μM (Table 4).

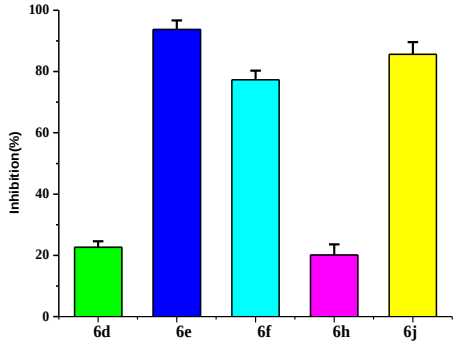


Fig. 3. Caspase-3 inhibitory activity of some compounds (50 μM)

Table 4. Inhibitory activities of **6e** against caspase-3

Dose (μM)	inhibition for caspase-3 (%)
100	96.2
50	93.7
20	88.7
10	57.4
5	40.6

To obtain some insight into the binding mode of cyclic dipeptide, we docked **6e** into the active site of caspase-3. Computer docking was performed with the X-ray crystal structure of caspase-3 (PDB identification code: 3H0E) by using CDOKER from Discovery Studio version 3.5 (Accelrys, San Diego, USA). The molecular surface of the caspase-3 catalytic domain was generated from the surrounding residues of the co-crystallized inhibitor within 5 Å. The -CDOCKER energies of the top10 docked conformations were ranked, and the lowest one was recorded.

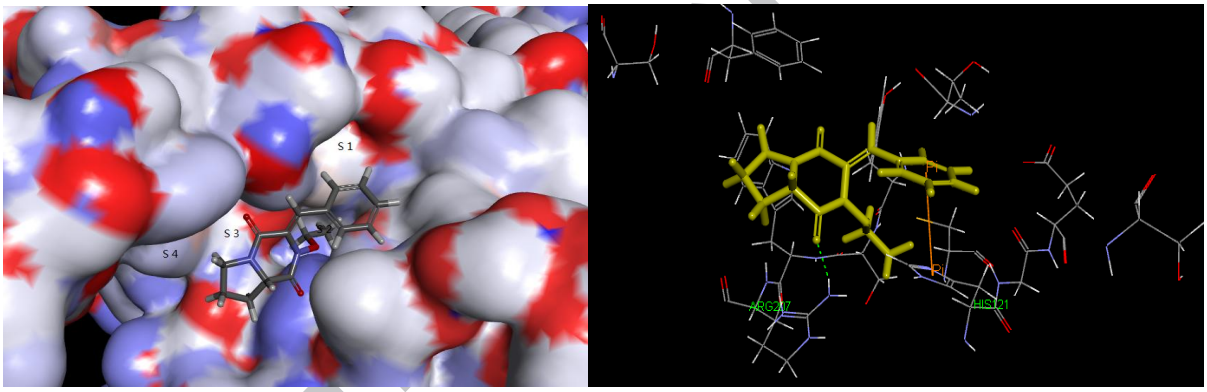


Fig.4. (a) Proposed binding mode of **6e** to caspase-3 enzyme based on the X-ray co-crystal structure of caspase-3 complexed with a small molecule inhibitor (left). (b) Interaction of the chain cyclic peptide **6e** with adjacent amino acid residues (right).

The cyclic dipeptide **6e** can interact with S1, S2, and S3 pockets of caspase-3. One hydrogen bond formed between the NH of guanidyl of Arg 207 and the O of the amide group. π–π interaction between phenyl ring and HIS 121 formed in the S1 pocket. In addition, the hydrophobic side chain of **6e** plunges into the S2 pocket.

3. Conclusion

DKPs are important in drug discovery because they contain constrained amino acids embedded within their structures. In this work, we designed and synthesized a series of arylidene *N*-alkoxyDKPs (**6**) bearing various substituted chains. Different DKPs (**6a–6q**) demonstrate some activities against different tumor cells. **6d, 6f, 6l and 6o** showed higher anti-tumor activity, wherein, the compound **6o** is more potent against K562-1, HCT-15, and A549 cells, with corresponding IC_{50} values of 7.3, 8.6, and 11.4 μM. In addition, **6e**, **6f**, and **6j** show moderate and good caspase-3 inhibitory activities, and **6e** at 50μM concentration shows an inhibition rate of 93.7%. Further experiments are currently conducted to better investigate the mechanism of action of such compounds and to confirm the improvement in their biological activity.

4. Experimental

4.1. Chemistry

5.1.1. Materials and measurements

^{1}H NMR spectra were obtained using a Bruker Advance II 500 instrument in $CDCl_3$ solution, with tetramethylsilane as internal reference and operated at 500 for 1 h. Elemental analyses were performed on a Perkin-Elmer 2400 C instrument. Infrared spectra were recorded on a Shimadzu Bio-Rad FTS 135 instrument. Most of the reagent-grade chemicals were commercially available and used without further purification unless otherwise noted. DMF was dried over CaH_2 for 2 days and then distilled under a reduced pressure

prior to use. Ethanol was refluxed over sodium turnings and then distilled fractionally. Flash chromatography was performed using silica gel (200–400 mesh).

4.1.2 Synthesis

General procedure to prepare substituent diethyl malonate 2 [25]**:**

Nano-K_2CO_3 (0.65 mol, 0.90 g), diethyl malonate solution (0.5 mol, 80.1 g), and benzylic halide **1** (1.1 mol) in absolute ethanol (250 ml) were added into a round-bottomed flask equipped with a water-cooled reflux condenser and a thermometer. The mixture was heated to 65 °C on oil bath and stirred for 8 h. The reaction was monitored by TLC. The mixture was filtered, and the solvents were removed *in vacuo*. The residue obtained was added into an equal volume of the mixture of water and ice, and the organic layer was separated. Water layer was extracted with ethyl acetate (4 × 30 ml), mixed with organic the layer, and dried over $MgSO_4$. Concentration under reduced pressure followed by column chromatography over silica gel produced the title compound **2.**

3-Pyridine methyl malonic acid diethyl ester 2A: **2A** was obtained as colorless oil at 75% yield (94.2g) after purification through column chromatography (EtOAc/hexane, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25 (t, *J*=7.0Hz, 6H), 3.22 (d, *J*=7.5Hz, 2H), 3.36 (t, *J*=7.5Hz, 1H), 4.17 (q, *J*=7.0Hz, 4H), 7.56 (d, *J*=7.5Hz, 1H), 8.47 (d, *J*=7.5Hz, 1H), 8.49 (d, *J*=7.5Hz, 2H), ppm; IR (KBr) ν 1732, 1576, 1479, 1445 cm^{-1}; Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.32; H, 7.01; N, 5.46. HR-MS (ESI, m/s) calcd. for $C_{13}H_{17}NO_4$ $(M+1)^+$: 252.1230, found: 252.1243

Benzyl malonic acid diethyl ester 2B: **2B** was obtained as colorless oil at 90% yield (112.6 g) after purification through column chromatography (EtOAc/hexane, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25 (t, *J*=7.0Hz, 6H), 3.22 (d, *J*=8.5Hz 2H), 3.63 (t, *J*=8.5Hz 1H), 4.14 (q, *J*=7.0Hz, 4H), 7.45 (m, 5H) ppm; IR (KBr) ν 1733, 1606, 1585, 1490, 1466 cm^{-1}; Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25; Found: C, 67.22; H, 7.19. HR-MS (ESI, m/s) calcd. for $C_{14}H_{18}O_4$ $(M+1)^+$: 251.1278, found: 251.1260

3-(3,4,5-trimethyloxy)benzylmalonic acid diethyl ester 2C: 2C was obtained as white solid at 80% yield (136.1 g) (m.p. 77 °C–78 °C) after purification through column chromatography (EtOAc/hexane, 5:1) ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22 (t, *J*=7.0Hz, 3H), 1.27 (t, *J*=7.0Hz 3H), 3.79 (t, *J*=8.5Hz, 2H), 3.86(s, 9H), 4.12 (d, *J*=8.5Hz, 1H), 4.16 (q, *J*=7.0Hz, 4H), 6.64 (s, 2H) ppm; IR (KBr) ν 1734, 1568, 1479, 1485 cm^{-1}; Anal. Calcd for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11; Found: C, 59.78; H, 7.26. HR-MS (ESI, m/s) calcd. for $C_{17}H_{24}O_7$ $(M+1)^+$:341.1595, found: 341.1622

2-Naphthylmethyl malonic acid dimethyl ester 2D: 2D was obtained as viscous liquid at 75% yield(102.0g) after purification through column chromatography (EtOAc/hexane, 3:1) ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.70 (s, 6H), 4.23 (d, *J*=9.0Hz 2H), 4.40 (t, *J*=9.0Hz, 1H), 7.44 (m, 3H), 7.76 (m, 4H) ppm; IR (KBr) ν 1738, 1564, 1480, 1486 cm^{-1}; Anal. Calcd for $C_{16}H_{16}O_4$: C, 70.57; H, 5.92; Found: C, 70.53; H, 5.98. HR-MS (ESI, m/s) calcd. for $C_{16}H_{16}O_4$ $(M+1)^+$: 273.1121, found: 273.1102

2-(3-Quinolylmethyl malonic acid dimethyl ester 2E: 2E was obtained as colorless oil at 71% yield(97.0g) after purification through column chromatography (EtOAc/hexane, 3:1), ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.61 (d, *J*=7.5Hz, 2H), 3.75 (s, 6H), 4.36 (t, *J*=7.5Hz, 1H), 7.30 (d, *J*=8.5Hz, 1H), 7.47 (t, *J*=7.5Hz, 1H), 7.66 (t, *J*=7.5Hz, 1H), 7.77 (d, *J*=8.5Hz, 1H), 7.99 (d, *J*=8.5Hz, 1H), 8.07 (d, *J*=8.5Hz, 1H) ppm; IR (KBr) ν 1738, 1564, 1480, 1486 cm^{-1}; Anal. Calcd for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N,5.13; Found: C, 65.88; H, 5.5050; N, 5.20. HR-MS (ESI, m/s) calcd. for $C_{17}H_{24}O_7$ $(M+1)^+$: 274.1074, found:274.1088

General procedure to prepare ethyl 2-alkyloxyimine-3-arylpropionate 4:

Diethyl or dimethyl arylmethylmalonate (0.1 mol) was added into a 250 ml reaction flask equipped with a mechanical stirrer, reflux condenser with drying tube, and dropping funnel. After cooling to −15 °C, ethyl nitrite (0.105 mol) was added into the reaction solution. Subsequently, EtONa/EtOH solution (0.1 mol) was slowly added dropwise under mechanical stirring, and the mixture was refrigerated for 12 h. The solution was concentrated, and the residue obtained was added into equal volume of water. The pH of the mixture was adjusted to 6 with diluted hydrochloric acid, extracted with ethyl acetate (4 × 50 ml), and dried over anhydrous Na_2SO_4. After the solvent was removed under reduced pressure, the crude product **3A–E** was obtained without purification (yield >90%).

The above crude product (0.05 mol), 50 ml of acetone, and anhydrous K_2CO_3 (0.055 mol) were added into a 250 ml reaction flask equipped with a mechanical stirrer, reflux condenser with dry tube, and dropping funnel. Alkyl halide (0.1 mol) was subsequently added dropwise into the reaction solution. The mixture was stirred for 4 h at 35 °C–40 °C. After the precipitates were filtered, the solution was concentrated to produce a residue, which was purified through column chromatography over silica gel to obtain the title compound **4.**

Ethyl 2-butyloxyimine-3-β-pyridylpropionate 4a: 4a was obtained (with n-BuBr) at 81% yield (10.7g) according to general procedure after purification through column chromatography (EtOAc/hexane, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) 0.94 (t, *J*=7.5Hz, 3H), 1.33 (t, *J*=7.0Hz, 3H), 1.33 (m, 2H), 1.35 (m, 2H), 3.91 (s, 2H), 3.91 (t, *J*=7.5Hz 2H), 4.32 (q, *J*=7.0Hz, 2H), 7.20 (m, 1H), 7.66 (d, *J*=7.0Hz, 1H), 8.43 (d, *J*=4.5Hz, 1H), 8.53 (d, *J*=2.0Hz, 1H) ppm; IR (KBr) ν 1716, 1603, 1576, 1477 cm^{-1}; Anal. Calcd for $C_{14}H_{20}N_2O_3$: C, 63.62; H, 7.63; N, 10.60; Found: C, 63.75; H, 7.41; N, 10.73. HR-MS (ESI, m/s) calcd. for $C_{14}H_{20}N_2O_3$ $(M+1)^+$: 265.1552, found: 265.1538

Ethyl 2-benzyloxyimine-3-β-pyridylpropionate 4c: 4c was obtained (with benzyl chloride) at 85% yield (12.7g) according to general procedure after purification through column chromatography (EtOAc/hexane, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) 1.33 (t, *J*=7.0Hz, 3H), 3.92 (s, 2H), 4.31 (q, *J*=7.0Hz, 2H), 5.33 (s, 2H), 7.15 (m, 1H), 7.53 (m, 1H), 8.44 (m, 1H), 8.51 (s, 1H), 7.32–7.37 (m, 5H) ppm; IR (KBr) ν 1715, 1574, 1496 cm^{-1}; Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39; Found: C, 68.22; H, 6.23; N, 9.50. HR-MS (ESI, m/s) calcd. for $C_{17}H_{18}N_2O_3$ $(M+1)^+$: 299.1396, found: 299.1411

Ethyl 2-ethyloxyimine-3-phenyl propionate 4e: 4e was obtained (with C_2H_5Br) at 80% yield(9.4g) according to general procedure after purification through column chromatography (EtOAc/hexane, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) 1.30 (t, *J*=7.0Hz, 6H), 3.93 (s, 2H), 4.20 (q, *J*=7.0Hz, 2H), 4.28 (q, *J*=7.0Hz, 2H), 7.14–7.16 (m, 2H), 7.23–7.25 (m, 3H) ppm; IR (KBr) ν 1717, 1578, and 1495 cm^{-1};

Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95; Found: C, 66.48; H, 6.13; N, 6.16. HR-MS (ESI, m/s) calcd. for $C_{13}H_{17}NO_3$ $(M+1)^+$:236.1287, found: 236.1305

Ethyl 2-(3-pyridylmethyloxyimine-3-(3,4,5-trimethoxy)phenyl propionate 4i: 4i was obtained(with 3-pyridylmethyl chloride) (at 79% yield(15.3g) according to general procedure after purification through column chromatography (EtOAc/hexane, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) 1.29 (t, *J*=7.0Hz, 3H), 3.63 (s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 4.03 (s, 2H), 4.28 (q, *J*=7.0Hz, 2H), 5.31 (s, 2H), 6.41 (s, 2H), 7.26 (m, 1H), 7.60 (m, 1H), 8.55 (d, *J*=7.5Hz, 1H), 8.59 (s, 1H) ppm; IR (KBr) ν 1719, 1592, 1568, 1484 cm^{-1}; Anal. Calcd for $C_{20}H_{24}N_2O_6$: C, 61.68; H, 6.47; N, 7.19; Found: C, 61.45; H, 6.52; N, 7.60. HR-MS (ESI, m/s) calcd. for $C_{20}H_{24}N_2O_6$ $(M+1)^+$:389.1713, found:389.1736

Ethyl 2-(methyloxyimine-3-(3,4,5-trimethoxy)phenyl prop-ionate 4j: 4j (yellow liquid) was obtained (with CH_3I) at 65% yield(10.1g) according to general procedure after purification through column chromatography (EtOAc/hexane, 1:5). ^{1}H NMR (500 MHz, $CDCl_3$) 1.33 (t, *J*=7.0Hz, 3H), 3.81 (s, 3H) 3.82 (s, 6H), 3.87 (s, 2H), 4.11 (s, 3H), 4.31 (q, *J*=7.0Hz 2H), 6.49 (s,2H) ppm; IR (KBr) ν 2941, 1718, 1591, 1507, and 1460 cm^{-1}; Anal. Calcd for $C_{15}H_{21}NO_6$: C, 57.87; H, 6.80; N,4.50; Found: C, 57.35; H, 7.15; N,4.81. HR-MS (ESI, m/s) calcd. for $C_{15}H_{21}NO_6$ $(M+1)^+$:312.1447, found:312.1425

Ethyl 2-(4-fluorobenzyloxy imine)-3-(3,4,5-trimethyloxy) phenyl propionate 4n: 4n: (yellow liquid) was obtained (with 4-fluorobenzyl chloride) at 65% yield(13.2g) according to general procedure after purification through column chromatography (EtOAc/hexane, 1:5). ^{1}H NMR (500 MHz, $CDCl_3$) 1.20 (t, J=7.0Hz, 3H), 3.52 (s, 3H), 3.75 (s, 3H), 3.77 (s, 3H), 3.98 (s,2H), 4.20 (q, J=7.0Hz, 2H), 5.15 (s,2H), 6.28 (s, 2H), 7.10 (m, 2H), 7.19 (m, 2H) ppm; IR (KBr) ν 2960,1718, 1597, 1575, 1478 cm^{-1}; Anal. Calcd for $C_{21}H_{24}FNO_6$: C, 62.21, H, 5.97; N, 3.45; Found: C, 62.18; H, 5.92; N, 3.52. HR-MS (ESI, m/s) calcd. for $C_{21}H_{24}FNO_6$ $(M+1)^+$:406.1666, found:406.1688

Ethyl 2-(4-chlorobenzyloxy imine)-3-(3,4,5-trimethyloxy) phenyl propionate 4o: 4o (yellow liquid) was obtained (with 4-chlorobenzyl chloride)at 70% yield(14.7g) according to general procedure after purification through column chromatography (EtOAc/hexane, 1:5). ^{1}H NMR (500 MHz, $CDCl_3$) 1.20 (t, J=7.0Hz, 3H), 3.52 (s, 3H), 3.75 (s, 3H), 3.77 (s, 3H), 3.98 (s, 2H), 4.20 (q, J=7.0Hz, 2H), 5.15 (s, 2H), 6.28 (s, 2H), 7.10 (m, 2H), 7.19 (m, 2H) ppm; IR (KBr) ν 1718, 1597, 1575, 1478 cm^{-1}; Anal. Calcd for $C_{21}H_{24}ClNO_6$: C, 62.21, H, 5.97; N, 3.45; Found: C, 62.22; H, 5.99; N, 3.49. HR-MS (ESI, m/s) calcd. for $C_{21}H_{24}ClNO_6$ $(M+1)^+$:422.1370, found:422.1359

Methyl 2-cyclohexyloxyimine-3-β-Naphthylpropionate 4p: 4p was obtained(with cyclohexyl bromide) at 72% yield(11.7g) according to general procedure after purification through column chromatography (EtOAc/hexane, 1:5). ^{1}H NMR (500 MHz, $CDCl_3$) 1.28–1.37 (m, 3H), 1.51–1.56 (m, 3H), 1.72 (m, 2H), 1.98 (m, 2H), 3.56(s,3H), 3.94 (s, 2H), 4.34 (m, 1H), 7.25–7.45 (m, 3H), and 7.72–7.79 (m, 4H) ppm; IR (KBr) ν 1718, 1597, 1575, and 1478 cm^{-1}; Anal. Calcd for $C_{21}H_{23}NO_3$: C, 73.82, H, 7.12; N, 4.30; Found: C, 74.35; H, 7.38; N, 4.20. HR-MS (ESI, m/s) calcd. for $C_{21}H_{23}NO_3$ $(M+1)^+$:326.1756, found:326.1790

Methyl 2-cyclohexyloxyimine-3-β-Quinolyl propionate 4q: **4q** was obtained(with cyclohexyl bromide) at 72% yield(11.7g) according to the general procedure after purification through column chromatography (EtOAc/hexane, 1:5). ^{1}H NMR (500 MHz, $CDCl_3$), 1.16–1.20 (m, 1H), 1.24–1.32 (m, 2H), 1.40–1.47 (m, 3H), 1.58–1.61 (m, 2H), 1.86–1.92 (m, 2H), 3.85 (s, 3H), 4.34 (m, 1H), 4.34 (s, 2H), 7.37 (d, *J*=8.5Hz, 1H), 7.51 (t, *J*=7.5Hz, 1H), 7.69 (t, *J*=7.5Hz,1H), 7.78 (d, *J*=8.0Hz, 1H), and 8.03 (m, *J*=8.0Hz,1H), 8.10 (m, *J*=8.5Hz, 1H) ppm; IR (KBr) ν 1717, 1600, 1574, 1478 cm^{-1}; Anal. Calcd for $C_{19}H_{22}N_2O_3$: C, 69.92, H, 6.79; N, 8.58; Found: C, 70.51; H, 7.17; N, 8.27. HR-MS (ESI, m/s) calcd. for $C_{19}H_{22}N_2O_3$ $(M+1)^+$:327.1709, found:327.1732

General procedure to prepare *N*-(2-alkyloxyimine-3-arylpropionyl)proline 5

The above preparation product **4** (0.02 mol) and 60 ml of 2*N* NaOH were placed in a 100 ml reaction flask equipped with a mechanical stirrer, reflux condenser, and a thermometer. The mixture was stirred for 2 h at 95 °C. After cooling, the solution was acidified with hydrochloric acid to pH 3. The mixture was subsequently extracted with EtOAc (5 × 30 ml), and the extract was dried over $MgSO_4$. The solution was concentrated under reduced pressure to produce the corresponding crude product carboxylic acid**,** which was used in the subsequent reaction without purification.

$(C_2H_5)_3N$ (1.5 ml) and HBTU (2.6 g) were added into DMF solution (30 ml, 6.5 mmol) to the above corresponding product under stirring, and methyl proline hydrochlorinate (6.8 mmol) was subsequently added dropwise. The mixture was stirred for 24 h at 35 °C–40°C, then 10 ml of H_2O was added. The pH of the mixture was adjusted to 6 with diluted hydrochloric acid and then extracted with CH_2Cl_2 (2 × 50 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated to produce solid residue. The crude product was purified through flash chromatography on silica gel to produce coupling products.

LiOH (5 eq) was added under stirring in a 100 ml reaction flask containing 0.1 mol of the above reaction products and solvent (THF:H_2O = 7:1). The mixture was stirred for 12 h at room temperature. The solution was concentrated, and then equal volume of water was added. The pH of the solution was adjusted to 3.0 with hydrochloric acid and then extracted with ethyl acetate (30 ml × 5). Organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to produce the crude product **5 (5a-5q)** (yield >95%), which was used in the subsequent reaction without purification.

General procedure to prepare *N*-alkyloxy DKP 6

In a 100 ml reaction flask, 0.1 mol compound **5(a–q)** and 40 ml of anhydrous benzene were added and cooled to 0 °C. $SOCl_2$ (0.15 ml) was added dropwise under stirring. The reaction lasted for 4 h at room temperature. The solution was concentrated *in vacuo* to produce ropy residue and then equal volume of water was added under stirring. The pH of solution was adjusted to 8 with hydrochloric acid and then extracted with CH_2Cl_2 (30 ml × 5). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to produce crude product, which was purified through flash chromatography on silica gel to produce the title compound **6.** The racemic product **6** were obtained , if equivalent Et_3N was added to reaction system, after adding $SOCl_2$

***Cyclo*[*N*-butyloxy-2-[(β-pyridyl) methylene]glycyl-prolyl] 6a: 6a** was obtained at 80% yield according to general procedure after purification through flash chromatography (EtOAc/hexane, 1:2). IR (KBr) ν 1701, 1665, 1628, 1586, 1475 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 0.67–0.70 (t, *J*=7.5Hz，3H), 0.92–0.97 (m, 2H), 1.02–1.16 (m, 2H), 1.97–2.03 (m, 1H), 2.11–2.19 (m, 2H), 2.49 (m, 1H), 3.47–3.51 (q, *J*=7.0Hz, 1H), 3.66–3.78 (m, 3H), 4.31 (q, *J*=7.0Hz, 1H), 7.11 (s, 1H), 7.27 (dd, *J*=5.0Hz, *J*=3.0Hz, 1H), 7.75 (d, *J*=8.0 Hz, 1H), 8.51 (dd, *J*=6.5 Hz, *J*=3.0Hz, 1H), 8.65 (s, 1H) ppm; Anal. Calcd for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32; Found: C, 64.81; H, 6.73; N, 13.35. HR-MS (ESI, m/s) calcd. for $C_{17}H_{21}N_3O_3$ $(M+1)^+$:316.1661, found:316.1653

***Cyclo*[*N*-cyclohexyloxy-2-[(β-pyridyl)methylene]glycyl-prolyl] 6b**

The product **6b** was obtained as a white solid according to the general procedure in 88% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1:2). Crystals suitable for single crystal X-ray analysis were grown by slow evaporation from $CHCl_3$. Mp 129.5~131 ◦C; IR (KBr) ν 1712, 1666, 1631, 1586,1480 cm^{-1}; 1H NMR (500 MHz, $CDCl_3$) 0.90–1.16 (m, 6H), 1.38–1.41 (m, 1H), 1.47–1.49 (m, 1H), 1.59–1.62 (m, 1H), 1.67–1.69 (m,1H), 1.97–2.02 (q, *J*=8.5 Hz, 1H), 2.09–2.17 (m, 2H), 2.50–2.54(m, 1H), 3.66–3.76 (m, 4H), 4.30 (q, *J*= 7.0 Hz, 1H), 7.05 (s, 1H),7.76 (d, *J*= 9.0 Hz, 1H), 8.50 (d, *J*=5.0 Hz, 1H), 8.65 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) 162.1, 157.8, 151.9, 150.1, 137.0,128.6, 128.3, 121.4, 114.0, 81.9, 57.5, 44.5, 29.0, 28.9, 27.7, 24.1,22.8, 22.6, 20.0; Anal. Calcd for $C_{19}H_{23}N_3O_3$: C, 66.84; H, 6.79; N, 12.31; Found: C, 66.88; H, 6.85; N, 12.33. HR-MS (ESI, m/s) calcd. for $C_{19}H_{23}N_3O_3$ $(M+1)^+$:342.1818, found:342.1829

***Cyclo*[*N*-benzyloxy-2-[(β-pyridyl)methylene]glycyl-prolyl] 6c: 6c** was obtained at 83% yield according to the general procedure after purification through flash chromatography (EtOAc/hexane, 1:2). IR (KBr) ν 1705, 1671, 1631, 1589, 1479 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$), 1.69 (s, 1H), 1.96–2.08 (m, 2H), 2.10 (m, 1H), 2.44 (m, 1H), 3.67 (m, 2H), 4.28 (q, *J* = 7.0 Hz, 1H), 4.52 (d, J = 9.5 Hz, 1H), 4.63(d, *J* = 9.5 Hz, 1H), 6.88 (d, *J* = 7.5 Hz, 2H), 7.14–7.2 (m, 3H), 7.26 (d, *J* = 7.0 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 8.46 (d, *J* = 4.5 Hz, 1H), and 8.70 (d, *J* = 1.5 Hz, 1H) ppm; Anal. Calcd for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03; Found: C, 68.68; H, 5.45; N, 12.08. HR-MS (ESI, m/s) calcd. for $C_{20}H_{19}N_3O_3$ $(M+1)^+$:350.1505, found:350.1531

***Cyclo*[*N*-methyloxy-2-(phenylmethylene)glycyl-prolyl] 6d**

The product **6d** was obtained according to the general procedure in 87% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1:2). Chiral HPLC analyses of the product showed amajor peak for the (*S*)-enantiomer at R_f 14.5min (99.3% *e.e.*) compared to racemic **6d** (synthesized by using racemic **5d**) which showed two equal peaks with R_fs of 14.5 min and 15.5 min for the (*S*)- and (*R*)-enantiomers respectively. IR (KBr) ν 1698, 1674, 1622, 1578, 1506 cm^{-1}; ^1H NMR (500 MHz, CDCl3) 1.85–2.02 (m, 1H), 2.11–2.21 (m, 2H), 2.45–2.49 (m, 1H), 3.39 (s, 3H),3.67–3.72 (m, 2H), 4.29 (q, *J*=7.0 Hz, 1H), 7.21 (s, 1H), 7.31–7.33(m, 3H), 7.45 (d, *J*=7.0Hz, 2H) ppm; ^{13}C NMR(125 MHz, $CDCl_3$)162.1, 159.4, 132.5, 130.7 (2), 128.6, 127.6 (2), 126.0, 120.3, 61.5, 58.1, 45.6, 28.2, 22.4; Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H,5.92; N, 10.29; Found: C,66.20; H, 5.97; N, 10.33. HR-MS (ESI, m/s) calcd. for $C_{15}H_{16}N_2O_3$ $(M+1)^+$:273.1239, found:273.1256.

***Cyclo*[*N*-ethyloxy-2-(phenylmethylene)glycyl-prolyl] 6e: 6e** was obtained at 85% yield according to the general procedure after purification through column chromatography (EtOAc/hexane, 1:2). ^1H NMR (500 MHz, $CDCl_3$) 1.01 (t, *J* = 7.0 Hz, 3H), 1.87–1.98 (m, 1H), 2.11–2.20 (m, 2H), 2.46–2.48 (m, 1H), 3.47 (q, *J* = 7.0 Hz, 2H), 3.67–3.72 (m, 2H), 4.29 (q, *J* = 7.0 Hz, 1H), 7.20 (s, 1H), 7.30–7.33 (m, 3H), 7.44 (d, *J* = 7.0 Hz, 2H) ppm; IR (KBr) ν 1702, 1678, 1620, 1577, and 1505 cm^{-1}; Anal. Calcd for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78; Found: C,67.25; H, 6.25; N, 9.83. HR-MS (ESI, m/s) calcd. for $C_{16}H_{18}N_2O_3$ $(M+1)^+$:287.1396, found:287.1412.

***Cyclo*[*N*-butyloxy-2-(phenylmethylene)glycyl-prolyl] 4f**

The product **4f** was obtained according to the general procedure in 80% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1 : 2). IR (KBr) ν 1705, 1680, 1618, 1577, 1503 cm^{-1}; ^1H-NMR (500 MHz, CDCl3) 0.65 (t, *J*=7.0 Hz, 3H), 0.91–0.95 (m, 2H), 1.03–1.13 (m, 2H), 1.96–1.99 (m, 1H), 2.10–2.18 (m,2H), 2.46–2.48 (m, 1H), 3.49 (q, *J*=7.0 Hz, 1H), 3.66–3.74 (m, 3H), 4.29 (q, *J*=6.5 Hz, 1H), 7.18 (s, 1H), 7.28–7.33 (m, 3H), 7.44 (d, *J*=7.0 Hz, 2H) ppm; ^{13}C NMR (125 MHz, CDCl3) 162.2, 159.3, 132.8, 130.7(2), 128.5, 127.5(2), 126.6, 120.0, 74.1, 58.1, 45.6, 29.0, 28.3, 22.4, 18.5, 13.6; Anal. Calcd for $C_{18}H_{22}N_2O_3$: C, 68.77; H, 7.05; N, 8.91; Found: C,68.81; H, 7.09; N, 8.94. HR-MS (ESI, m/s) calcd. for $C_{18}H_{22}N_2O_3$ $(M+1)^+$:315.1709, found:315.1688.

***Cyclo*[*N*-cyclohexyloxy-2-(phenylmethylene)glycyl-prolyl] 6g**

The product **6g** was obtained according to the general procedure in 85% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1 : 2). IR (KBr) ν 1701, 1670, 1624, 1585, 1503 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 0.88–1.08 (m, 5H), 1.18–1.20(m, 1H), 1.36–1.39 (m, 1H), 1.46–1.47 (m, 1H), 1.63–1.66 (m, 2H),1.97–2.00 (m, 1H), 2.10–2.15 (m, 2H), 2.59–2.51 (m, 1H), 3.63–3.74 (m, 3H), 4.29 (q, *J*=6.5 Hz, 1H), 7.12 (s, 1H), 7.28–7.33 (m,3H), 7.43 (d, *J*=7.0 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$):163.2, 159.6, 132.7, 130.9(2), 128.4, 127.4(2), 119.8, 82.2, 58.4,45.4, 30.0, 29.5, 28.7, 25.2, 23.8, 23.6, 22.6, 22.4; Anal. Calcd for$C_{20}H_{24}N_2O_3$: C, 70.56; H, 7.11; N, 8.23; Found: C, 70.59; H, 7.15;N, 8.26. HR-MS (ESI, m/s) calcd. for $C_{20}H_{24}N_2O_3$ $(M+1)^+$:341.1865, found:341.1842

***Cyclo*[*N*-benzyloxy-2-(phenylmethylene)glycyl-prolyl] 6h**

The product **6h** was obtained according to the general procedure in 88% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1 : 2). IR (KBr) ν 1706, 1681, 1618, 1589, 1577, 1511, 1503 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.92–2.08 (m, 3H), 2.39–2.44 (m, 1H), 3.63–3.66 (m, 2H), 4.26 (q, *J*=6.5 Hz, 1H), 4.56(s, 2H), 6.83–6.85 (m, 2H), 7.14–7.17 (m, 2H), 7.23–7.25 (m, 2H),7.30–7.32 (m, 3H), 7.48–7.49 (m, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) 162.4, 159.2, 132.7, 132.5, 131.0(2), 130.1(2), 128.9, 128.7, 128.1(2), 127.7(2), 126.5, 120.4, 75.8, 58.0, 45.6, 29.5, 22.4; Anal. Calcd for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.79; N, 8.04; Found: C,72.44; H, 5.82; N, 8.08. HR-MS (ESI, m/s) calcd. for $C_{21}H_{20}N_2O_3$ $(M+1)^+$:349.552, found:349.1568

***Cyclo*[*N*-(3-pyridylmethyloxy)-2-[(3,4,5-trimethyloxy) phenyl -methylene] glycyl-prolyl] 6i: 6i** was obtained at 75% yield according to the general procedure after purification through column chromatography (EtOAc/hexane, 1:1). IR (KBr) ν 1696, 1668, 1622, 1590,

1569, 1518, 1490 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$), 1.95 (m, 1H), 2.10 (m, 2H), 2.41 (m, 1H), 3.67 (m, 2H), 3.85 (s, 3H), 3.89 (s, 3H), 3.90 (s, 3H), , 4.27 (q, *J*=6.5 Hz, 1H), 4.63(s, 2H) 6.75 (s, 2H),7.14(s,1H), 7.28 (m, 1H), 7.58 (m, 1H), 8.52 (d, *J*=7.5Hz, 1H), 8.60 (s, 1H) ppm; Anal. Calcd for $C_{23}H_{25}N_3O_6$: C, 62.86; H, 5.73; N, 9.56; Found: C, 62.79; H, 5.76; N, 9.64. HR-MS (ESI, m/s) calcd. for $C_{23}H_{25}N_3O_6$ $(M+1)^+$:440.1822, found:440.1806

***Cyclo*[*N*-methyloxy-2-[(3,4,5-trimethyloxy) phenylmethylene] glycyl-prolyl] 6j: 6j** was obtained at 86% yield according to the general procedure after purification through flash chromatography (EtOAc/hexane, 1:2). IR (KBr) ν 1697, 1670, 1624, 1580, 1505 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.99 (m, 1H), 2.13 (m, 1H), 2.22 (m, 1H), 2.46(m,1H), 3.48 (s, 3H), 3.68(m,2H) 3.88 (s, 3H), 3.89 (s, 3H), 3.91 (s, 3H), 4.29 (q, *J*=6.5Hz, 1H), 6.75 (s, 2H), 7.13(s, 1H) ppm; Anal. Calcd for $C_{18}H_{22}N_2O_6$: C, 64.12; H, 5.81; N, 9.84; Found: C, 64.10; H, 5.78; N, 9.92. HR-MS (ESI, m/s) calcd. for $C_{18}H_{22}N_2O_6$ $(M+1)^+$:363.1556, found:363.1570

***Cyclo*[*N*-butyloxy-2-[(3,4,5-trimethyloxy)phenylmethylene] glycylprolyl] 6k**

The product **6k** was obtained as a white solid according to the general procedure in 85% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1:2). Mp. 138.5~141.5 ◦C; IR(KBr) ν 1701, 1676, 1608, 1583, 1099 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 0.71 (t, *J*=7.0 Hz, 3H), 0.96–1.05 (m, 2H), 1.19–1.28 (m, 2H), 1.99–2.05 (m, 1H), 2.11–2.18 (m, 2H), 2.44–2.47 (m, 1H), 3.55 (dd, *J*=7.0 and *J*=7.5 Hz, 1H), 3.67–3.70 (m, 2H), 3.77–3.80(m, 1H), 3.85 (s, 9H), 4.27–4.31 (m, 1H), 6.69 (s, 2H), 7.10 (s, 1H) ppm; Anal. Calcd for $C_{21}H_{28}N_2O_6$: C, 62.36; H, 6.98; N, 6.93; Found: C, 62.39; H, 7.02; N, 6.98. HR-MS (ESI, m/s) calcd. for $C_{21}H_{28}N_2O_6$ $(M+1)^+$:405.2026, found:405.2019

***Cyclo*[*N*-cyclohexyloxy-2-[(3,4,5-trimethyloxy)phenyl methyl-ene]glycyl-prolyl] 6l**

The product **6l** was obtained according to the general procedure in 83% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1:2). IR (KBr) ν 1693, 1678, 1621, 1583, 1503,1099 cm^{-1}; 1H-NMR (500 MHz, $CDCl_3$): 1.01–1.14 (m, 4H), 1.19 (br, 1H), 1.28–1.29 (m, 2H), 1.42 (br, 1H), 1.65–1.72 (m, 2H),1.99–2.01 (m, 1H), 2.13–2.19 (m, 2H), 2.49–2.51 (m, 1H), 3.69–3.77 (m, 3H), 3.86–3.90 (m, 9H), 4.29 (q, *J* 7.0 Hz, 1H), 6.77 (s,2H), 7.04 (s, 1H) ppm; ^{13}C NMR(125 MHz, $CDCl_3$) 163.2, 159.9, 152.2, 138.8, 127.9, 126.9, 119.9, 108.5(2), 82.28, 65.9, 61.0, 58.4, 56.3(2), 45.5, 30.8, 30.1, 28.6, 25.2, 23.8, 23.6, 22.5; Anal. Calcd for $C_{23}H_{30}N_2O_6$: C, 64.17; H, 7.02; N, 6.51; Found: C, 64.21; H, 7.07; N, 6.48. HR-MS (ESI, m/s) calcd. for $C_{23}H_{30}N_2O_6$ $(M+1)^+$:431.2182, found:431.2155

Cyclo[*N*-benzyloxy-2-[(3,4,5-trimethyloxy)phenylmethylene] glycyl-prolyl] 6m:The product **6m** was obtained according to the general procedure in 87% yield after purification by silica gel flash chromatography (EtOAc/hexane, 1 : 2). IR (KBr) ν 1698, 1677, 1621, 1608, 1583, 1503, 1099 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.67 (s, 2H), 1.95-1.98 (m, 1H), 2.05–2.09 (m, 2H), 2.41–2.43 (m, 1H), 3.75 (s, 6H),3.87(s, 3H), 4.25–4.28 (m, 1H), 4.58 (d, *J*=9.0 Hz, 1H), 4.68 (d,*J*=9.0 Hz, 1H), 6.77 (s, 2H), 6.95 (d, *J*=7.5 Hz, 2H), 7.16 (s, 1H),7.19–7.26 (m, 3H) ppm; Anal. Calcd for $C_{24}H_{26}N_2O_6$: C, 65.74; H,5.98; N, 6.39; Found: C, 65.75; H, 6.01; N, 6.42. HR-MS (ESI, m/s) calcd. for $C_{24}H_{26}N_2O_6$ $(M+1)^+$:439.1869, found:439.1888

***Cyclo*[*N*-4-fluorobenzyoxyl-2-[(3,4,5-trimethyloxy) phenyl-methylene]glycyl-prolyl] 6n:** The product **6n** was obtained according to the general procedure in 80% yield after purification by flash chromatography (EtOAc/hexane, 1:2). IR (KBr) ν 1736, 1688, 1632, 1591, 1510, 1457 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.96 (m, 1H), 2.08 (m, 2H), 2.42 (m, 1H), 3.65 (m, 2H), 3.84 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 4.12 (m, 1H), 5.14 (s, 2H), 6.75 (s, 2H), 7.16 (s, 1H), 7.36 (m, 2H), 7.58 (m, 2H) ppm; Anal. Calcd for $C_{24}H_{25}FN_2O_6$: C, 63.15; H, 5.52; N, 6.14; Found: C, 63.18; H, 5.55; N, 6.22. HR-MS (ESI, m/s) calcd. for $C_{24}H_{25}FN_2O_6$ $(M+1)^+$:457.1775, found:457.1763

***Cyclo*[*N*-4-clorobenzyoxyl-2-[(3,4,5-trimethyloxy) phenyl-methyl-ene]glycyl-prolyl] 6o: 6o** was obtained at 83% yield according to the general procedure after purification through flash chromatography (EtOAc/hexane, 1:2). IR (KBr) ν 1739, 1686, 1635, 1590, 1507, 1461 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 2.00 (m, 1H), 2.16 (m, 2H), 2.42 (m, 1H), 3.64 (m, 2H), 3.84 (s, 3H) 3.86 (s, 3H), 3.88 (s, 3H), 4.13 (m, 1H), 5.12(s, 2H), 6.75 (s, 2H), 7.36 (m, 2H), 7.16 (s, 1H), 7.58(m, 2H) ppm; Anal. Calcd for $C_{24}H_{25}ClN_2O_6$: C, 63.15; H, 5.52; N, 6.14; Found: C, 63.09; H, 5.51; N, 6.20. HR-MS (ESI, m/s) calcd. for $C_{24}H_{25}ClN_2O_6$ $(M+1)^+$:473.1479, found:473.1482

***Cyclo*[*N*-cyclohexyloxy-2-(β-naphthylmethylene)glycyl-prolyl] 6p: 6p** was obtained at 75% yield according to the general procedure after purification through flash chromatography (EtOAc/hexane, 1:2). IR (KBr) ν 1715, 1668, 1628, 1586, 1509, 1480 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 0.86–0.91 (m, 4H), 1.00–1.05 (m, 1H), 1.13–1.18 (m, 1H), 1.31–1.38 (m, 2H), 1.59–1.67 (m, 2H), 1.85 (m, 1H), 2.04–2.11 (m, 2H), 2.48–2.52 (m, 1H), 3.61–3.66 (m, 1H), 3.69–3.73 (m, 2H), 4.28(m, 1H), 7.12 (s, 1H), 7.44–7.47(m, 3H), 7.71–7.84 (m, 4H) ppm. Anal. Calcd for $C_{23}H_{26}N_2O_3$: C, 73.82; H, 6.71; N, 7.17; Found: C, 73.77; H, 6.80; N, 7.25. HR-MS (ESI, m/s) calcd. for $C_{23}H_{26}N_2O_3$ $(M+1)^+$:391.2022, found:391.2009

***Cyclo*[*N*-cyclohexyloxy-2-(β-quinolylmethylene)glycyl-prolyl] 6q: 6q** was obtained at 70% yield according to the general procedure after purification through flash chromatography (EtOAc/hexane, 1:2). IR (KBr) ν 1710, 1670, 1624, 1583, 1500, 1475 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 0.85–0.91 (m, 4H), 1.26–1.35 (m, 3H), 1.57–1.60 (m, 2H), 1.85 (m, 1H), 2.00–2.10 (m, 2H), 2.44–2.50 (m, 1H), 3.59–3.64 (m, 1H), 3.66–3.70 (m, 2H), 4.29 (q, *J* = 7.0 Hz, 1H), 7.15 (s, 1H), 7.33 (dd, *J* = 3.0 Hz, *J* = 5.5 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 1H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.93 (t, *J* = 9.0 Hz, 1H), 8.03 (dd, *J* = 3.0 Hz, *J* = 5.5 Hz, 4H). Anal. Calcd for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73; Found: C, 70.63; H, 6.37; N, 10.81. HR-MS (ESI, m/s) calcd. for $C_{23}H_{25}N_3O_3$ $(M+1)^+$:392.1974, found:392.1960.

4.2. Molecular modeling

Computer docking was performed using the X-ray crystal structure of human caspase-3 (PDB Identification Code: 3H0E) by utilizing DOKER of the Discovery Studio version 3.5 (Accelrys, San Diego, USA). The molecular surface of the caspase-3 catalytic domain was generated from the surrounding residues of co-crystallized inhibitor within 5 Å. The Docker energies of the top 10 docked conformations were ranked, and the lowest energy was recorded.

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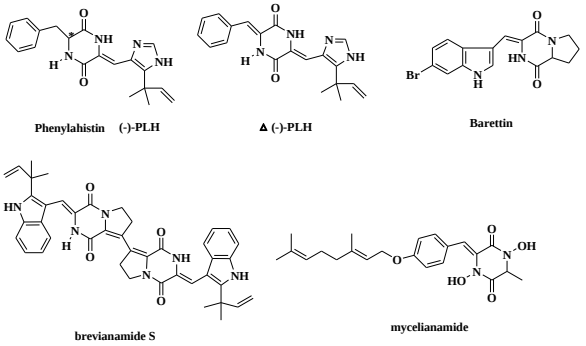
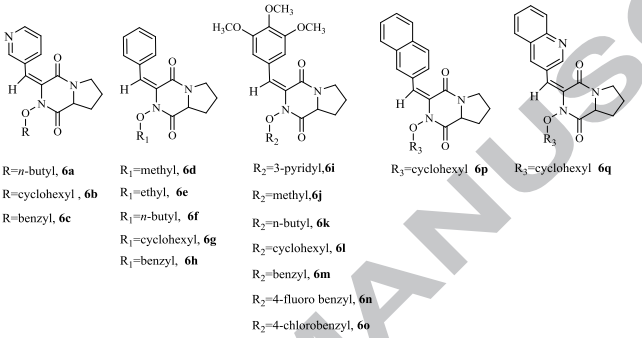
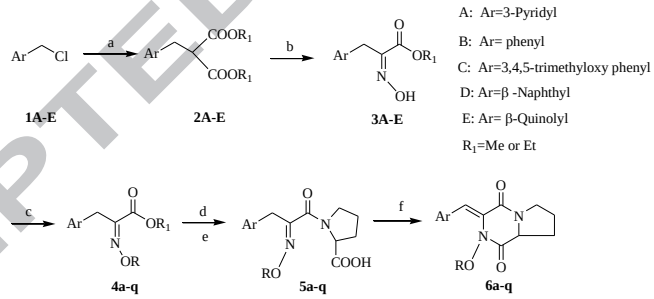
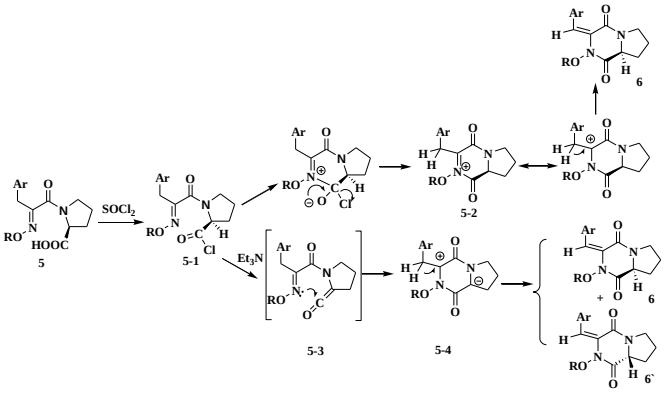






a: Ar=3-Pyridyl, R=n-Bu
b: Ar=3-Pyridyl, R=c-hexyl
c: Ar=3-Pyridyl, R=benzyl
d: Ar= phenyl, R=Methyl
e: Ar= phenyl, R=Ethyl
f: Ar= phenyl, R=n-Bu
g: Ar= phenyl, R=c-hexyl
h: Ar= phenyl, R=benzyl
I: Ar=3,4,5-trimethoxyl benzyl, R=3-Pyridyl
j: Ar=3,4,5-trimethyloxy phenyl, R=Methyl
K: Ar=3,4,5-trimethyloxy phenyl, R=n-Bu
l: Ar=3,4,5-trimethyloxy phenyl, R=c-hexyl
m: Ar=3,4,5-trimethyloxy phenyl, R=benzyl
n: Ar=3,4,5-trimethyloxy phenyl, R=4-fluoro benzyl,
o: Ar=3,4,5-trimethyloxy phenyl, R=4-chloro benzyl,
p: Ar=β -Naphthyl , R=c-hexyl ,
q: Ar= β-Quinolyl , R=c-hexyl ,

Scheme 1 Synthesis of *E*-arylidene bicyclo-DKPs



Scheme 2. Possible cyclization reaction

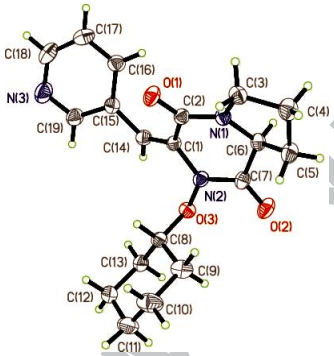


Fig.1. Structure of **6b** with ellipsoid displacement drawn at 30% probability level.

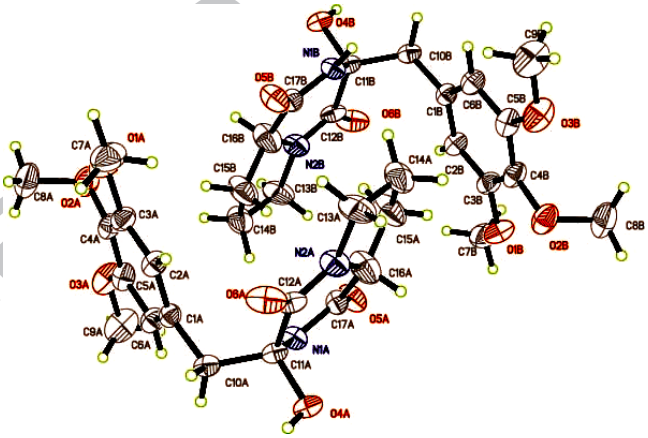
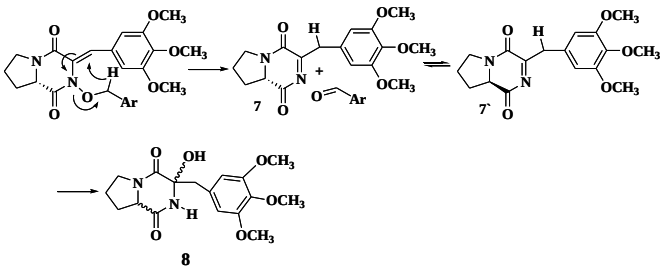


Fig. 2. Structure of **8** with displacement ellipsoids drawn at 30% probability level



Scheme 3. Possible mechanism of rearrangement-fragmentation reaction

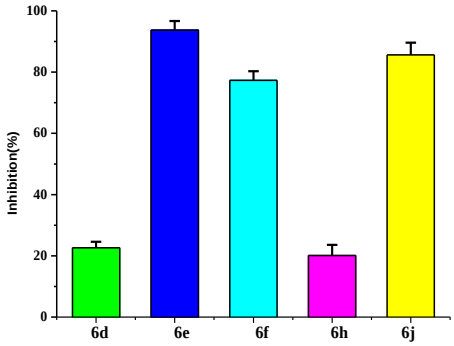


Fig. 3. Caspase-3 inhibitory activity of some compounds (50 μM)

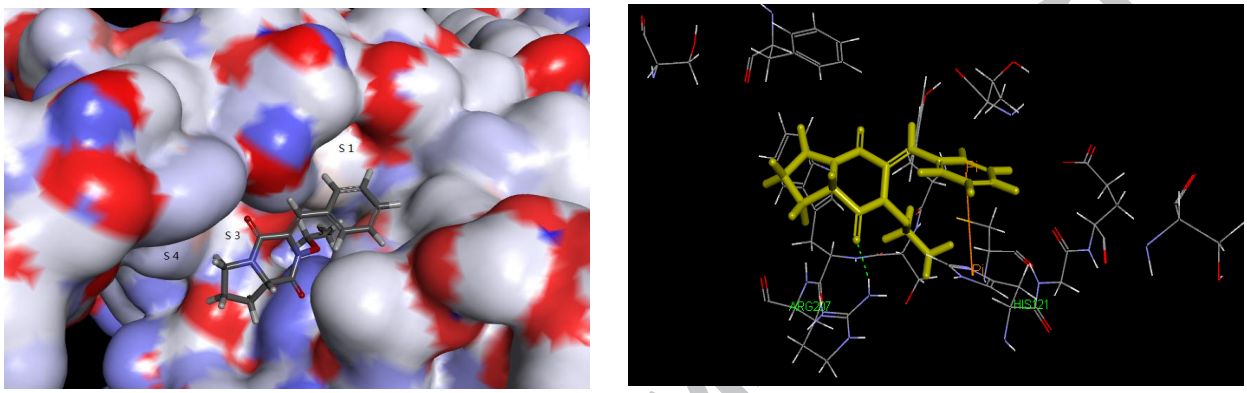


Fig.4. (a) Proposed binding mode of **6e** to caspase-3 enzyme based on the X-ray co-crystal structure of caspase-3 complexed with a small molecule inhibitor (left). (b) Interaction of the chain cyclic peptide **6e** with adjacent amino acid residues (right).