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Chalcones, inhibitors for topoisomerase I and cathepsin B and L, as potential anti-cancer agents

Seok-Ho Kim [a,†], Eunyoung Lee [b,†], Kyung Hye Baek [b], Han Byeol Kwon [b], Hyunjung Woo [b], Eung-Seok Lee [c], Youngjoo Kwon [b,*], Younghwa Na [a,*]

[a] College of Pharmacy, CHA University, Pocheon 487-010, Republic of Korea
[b] College of Pharmacy, Ewha Global Top 5 Program, Ewha Womans University, Seoul 120-750, Republic of Korea
[c] College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Republic of Korea

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ABSTRACT

In order to diversify the pharmacological activity of chalcones and extend the scaffold of topoisomerase and cathepsins B and L inhibitors, we have designed and synthesized total 18 chalcone compounds and tested their biological activity. In the topoisomerase inhibition test, most analogues in group III and IV except compound **11** exhibited more efficient topoisomerase I inhibitory activity than camptothecin at 20 μM. Compounds **15**, **16** and **18** in group IV showed significant cathepsin B and L inhibitory activity. Among the compounds, compound **15** was most active with IC_{50} values of 1.81 ± 0.05 μM on cathepsin B and 3.15 ± 0.07 μM on cathepsin L, respectively. Compound **15** also showed most potent cytotoxic activity against T47D and SNU638 cells with IC_{50} values of 1.37 ± 0.05 μM and 0.62 ± 0.01 μM, respectively. Overall, although more compounds should be tested and analyzed for clear SAR against topoisomerase I and cathepsin B and L, compound **15** showed consistent inhibitory ability on the tested assays, which can implicate the cytotoxic activity of compound **15** on topoisomerase I and cathepsin B and L inhibitory pathways.

Topoisomerase enzymes are one of the key factors solving the topological hurdles during transcription and replication. The enzymes use transient cleavage and religation mechanism to relieve the difficulties of transcription and replication process. Topoisomerases are categorized into two types, type I and II. Type I enzyme introduces one strand cleavage and rejoining of single strand of DNA duplex of supercoiled chromosomes and type II uses two strand breakage during process.[1] It was known that topoisomerase II enzyme was required to separate replicated molecules.[2] Camptothecin is well-known anti-cancer drug working on topoisomerase I function and doxorubicin and etoposide are clinically important anticancer drugs targeting topoisomerase II, which attracted a number of researchers to develop active compounds inhibiting topoisomerase enzymes.

Cathepsins belonging to cysteine proteases are a class of proteolytic enzymes, which are highly expressed in malignant cells.[3] In tumor progression process, cathepsins are released into extracellular system and exert diverse roles.[4–6] For this reasons, cathepsins emerged as a potential therapeutic target for anticancer treatment area.[7] Among the 11 human cathepsin family reported so far, cathepsins B and L are reported to be implicated in human pancreatic cancers.[8,9] In the RIPI-Tag2 mouse model study, cathepsins B and L knockouts reduce cell proliferation and tumor growth, which suggest that cathepsins B and L can be potential therapeutic target for anti-cancer drug.[8,10]

Chalcones display a various pharmacological effects, including anti-proliferative, anticancer, antioxidant, anti-inflammatory, or anti-infective activities.[11] Chalcone derivatives have shown potential as lead compounds for the new drug discovery area due to their promising biological activity and safety profiles.

Some natural cyclohexyl chalcones panduratin A and nicolaioidesin C showed cytotoxic activity against prostate cancer cells and also cathepsin inhibitory activity in vitro.[12]

* Corresponding authors. Tel.: +82 2 3277 4653; fax: +82 2 3277 3051 (Y.K.); tel.: +82 2 8017 9896; fax: +82 2 8017 9420 (Y.N.).
E-mail addresses: ykwon@ewha.ac.kr (Y. Kwon), yna7315@cha.ac.kr (Y. Na).
† These two authors contributed equally to this work.

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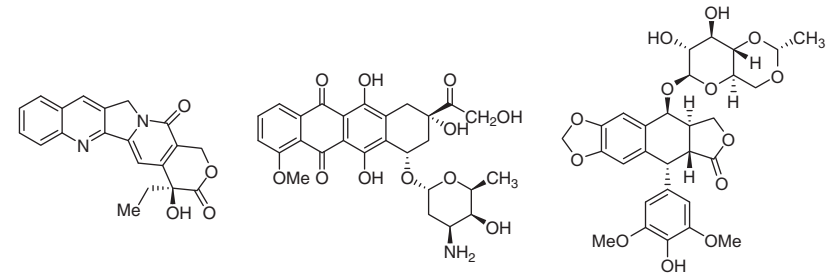
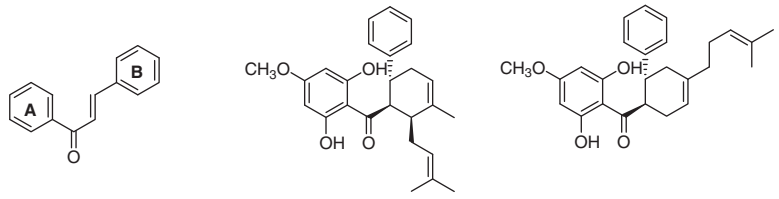
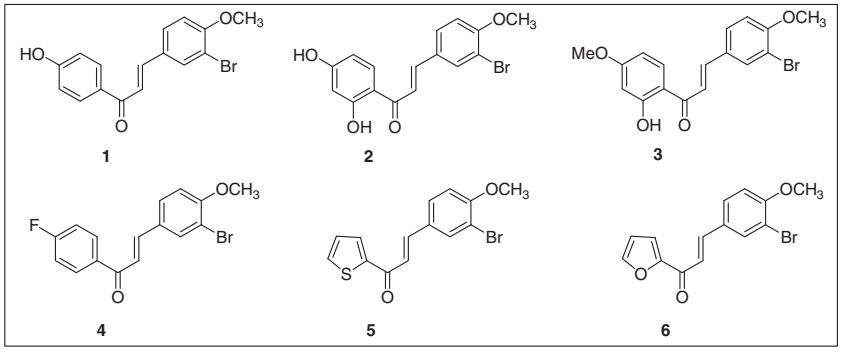


Camptothecin **Doxorubicin** **Etoposide**



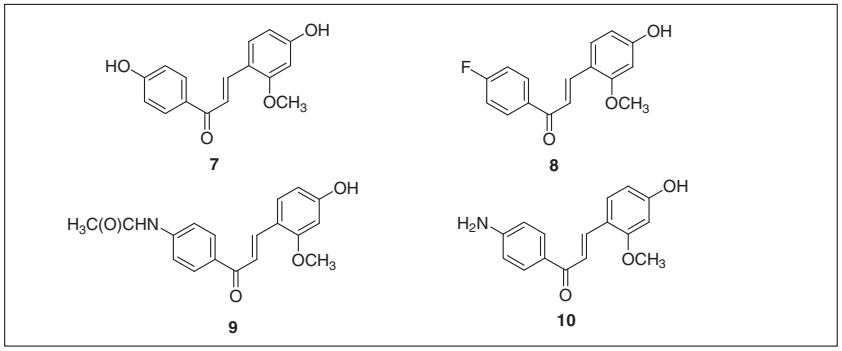
Chalcone **Panduratin A** **Nicolaioidesin C**

Group I



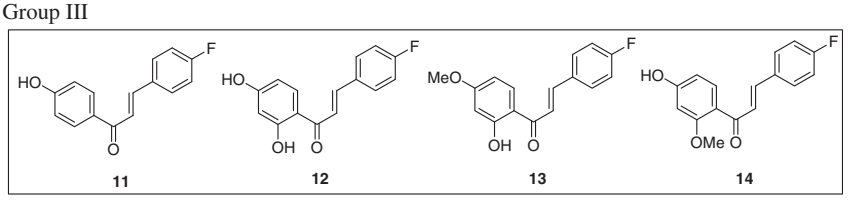
1 **2** **3** **4** **5** **6**

Group II



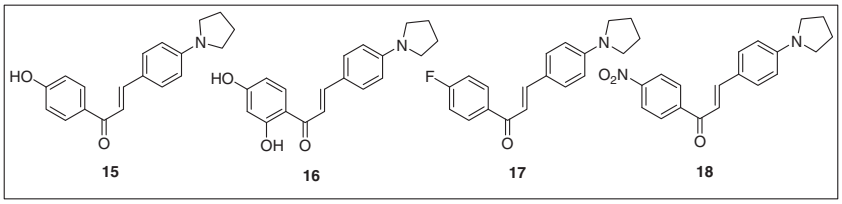
7 **8** **9** **10**

Group III



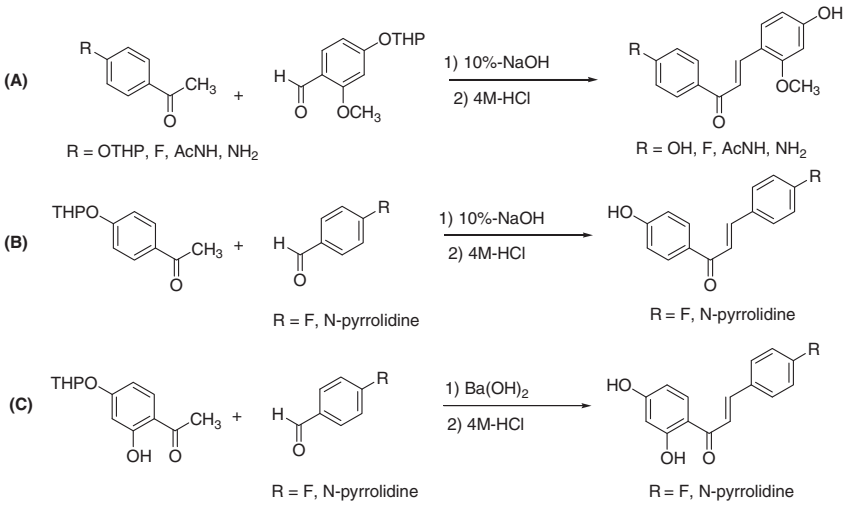
11 **12** **13** **14**

Group IV



15 **16** **17** **18**

Figure 1. Structures of prepared chalcone compounds.



Scheme 1. Representative synthetic methods for prepared chalcone compounds.

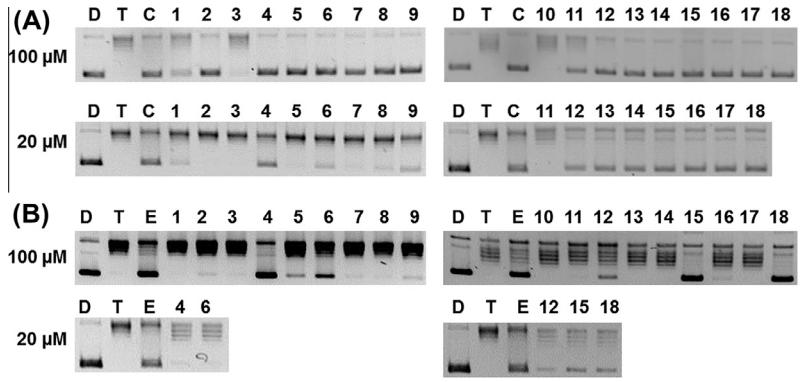


Figure 2. Topoisomerase I (A) and II (B) inhibitory activities of compounds. Compounds were examined in a final concentration of 20 and 100 μM, respectively as designated. (A) Lane D: pBR322 only, Lane T: pBR322 + Topoisomerase I, Lane C: pBR322 + Topoisomerase I + camptothecin, Lanes **1**–**18**: pBR322 + Topoisomerase I + compounds (**1**–**18**) in designated concentrations. (B) Lane D: pBR322 only, Lane T: pBR322 + Topoisomerase II, Lane E: pBR322 + Topoisomerase II + etoposide, Lanes **1**–**18**: pBR322 + Topoisomerase II + compounds (**1**–**18**) in designated concentrations.

In order to explore the pharmacological activity of chalcones and extend the scaffold of topoisomerase and cathepsins B and L inhibitors, we have designed and synthesized four different groups, I–IV (Fig. 1) based on the substituents on the B ring of chalcone structures. Substituents on the B ring were randomly selected considering the electronic property of oxygen, nitrogen, and fluorine atoms. We intended to compare the pharmacological activities of four groups to understand and identify the effect of substituents on the chalcone skeleton. A total of 18 chalcone derivatives were prepared and representative synthetic methods[11,13] are described in Scheme 1.

Claisen–Schmidt reaction without protection of the phenolic OH group at 4-position on the acetyl aryl and aryl aldehyde gave unsatisfactory results, which was solved by introduction of tetrahydropyranyl (THP) protecting group to 4-phenolic OH. Representative synthetic methods are described in the Ref. 14. In the ^{1}H NMR spectrum, two doublet signals appeared at around δ 7.40 and 8.00 coupling to each other with J values of 15.2–15.6 Hz, which confirmed the *E*-geometry of olefin in chalcone compounds. The reactions provided expected products in low to moderate reaction yields. All the spectral data are consistent with the expected structures.[14]

Compounds prepared were evaluated for the topoisomerase I and II, cathepsin B and L inhibitory activity and cytotoxicity. First, topoisomerases relaxation inhibitory activities were assayed using human topoisomerase I and II (Topogen) with camptothecin and etoposide as positive controls.[15] Data were analyzed and evaluated with LabWork 4.5 Software to calculate the inhibition ratio. The result was indicated in Figure 2 and Table 1. Although compounds **15** and **18** showed superior inhibitory activity to etoposide at 100 μM, most compounds were weak or insensitive to topoisomerase II function at 20 μM. But, interestingly, compounds in group III and IV showed potent inhibitory activity on topoisomerase I. Most analogues in group III and IV except compound **11** were more efficient topoisomerase I inhibitor than camptothecin at tested concentrations. Observation implied that 4-fluoro and 4-*N*-pyrrolidino group on ring B of chalcone instead of hydroxyl or methoxy group are proper functional group for topoisomerase I inhibition. Cathepsins B and L inhibitory activities were also tested with the known method[16] using CA-074 and Z-FF-FMK as cathepsin B and L inhibitor, respectively. The results are described in Table 2. Compounds **15**, **16** and **18** in group IV showed significant cathepsin B and L inhibitory activity. Among the compounds, compound **15** was most active with IC_{50} values of 1.81 ± 0.05 μM on cathepsin B and

Table 1
Inhibitory activities of compounds on topoisomerase I and topoisomerase II

Compounds	% Inhibition[a] of topo I		% Inhibition[a] of topo II	
	100 μM	20 μM	100 μM	20 μM
Camptothecin	70.5	37.2	—	—
Etoposide	—	—	63.0	38.5
1	24.8	6.6	1.2	NT[b]
2	73.2	8.2	2.4	NT
3	5.0	3.8	1.0	NT
4	76.5	20.1	48.7	0.0
5	74.4	2.1	5.8	NT
6	92.0	8.8	24.7	0.0
7[13a]	88.9	2.1	1.6	NT
8	95.8	9.5	0.7	NT
9	100.0	17.3	0.0	NT
10	0.8	NT	0.0	NT
11	50.9	18.0	0.0	NT
12	80.2	56.7	42.2	12.6
13	81.2	60.0	0.0	NT
14	86.1	61.6	0.0	NT
15	74.7	60.0	96.8	19.1
16	87.1	64.4	0.0	NT
17	90.6	73.1	0.0	NT
18	91.1	77.6	96.1	15.3

[a] The value of % inhibition was expressed as the mean of triplicate experiments.
[b] NT: non-tested.

Table 2
Inhibitory activities of compounds on cathepsin B and cathepsin L

Compound	IC_{50}[a] (μM)	
	Cathepsin B	Cathepsin L
CA074	0.0036 ± 0.0003	>100
ZFF-FMK	0.0656 ± 0.0029	0.0837 ± 0.0008
1	>100	29.81 ± 0.15
2	>100	67.51 ± 0.12
3	>100	45.27 ± 0.28
4	>100	>100
5	>100	>100
6	>100	>100
7	57.68 ± 0.82	30.34 ± 0.83
8	95.09 ± 1.23	73.22 ± 1.05
9	46.81 ± 0.40	39.31 ± 0.27
10	76.47 ± 0.49	32.85 ± 0.39
11	>100	>100
12	>100	>100
13	>100	98.68 ± 0.37
14	>100	91.17 ± 0.15
15	1.81 ± 0.05	3.15 ± 0.07
16	28.67 ± 0.18	4.72 ± 0.08
17	54.29 ± 0.25	56.51 ± 0.08
18	28.95 ± 0.93	9.42 ± 0.07

[a] The IC_{50} value was expressed as the mean ± standard deviation of 50% inhibitory concentrations of triplicate experiments.

3.15 ± 0.07 μM on cathepsin L, respectively. Compound **16** was also a good inhibitor for cathepsin L with IC_{50} value of 4.72 ± 0.08 μM. From this result, we suspect that 4-hydroxy group on ring A and 4-*N*-pyrrolidino group on ring B of chalcones seem to be important for the inhibition of cathepsin B and L function. Finally, cytotoxic activity of the compounds was tested against two human cancer cell lines, T47D (Human breast ductal carcinoma cell line) and SNU638 (human gastric cancer cell line), using adriamycin, etoposide and camptothecin as references. Test was conducted with typical MTT assay procedure.[15] The result was shown in Table 3. Most compounds tested showed effective cytotoxic activity on the cancer cell lines. Especially, compound **15** showed most potent cytotoxic activity with IC_{50} value of 1.37 ± 0.05 μM on T47D cell and IC_{50} value of 0.62 ± 0.01 μM on SNU638 cell.

Table 3
Cytotoxicity of compounds

Compds/cells	IC_{50}[a] (μM)	
	T47D[b]	SNU638[c]
Adriamycin[d]	2.91 ± 0.14	0.84 ± 0.04
Etoposide[e]	4.98 ± 0.19	2.57 ± 0.08
Camptothecin[f]	0.07 ± 0.004	0.28 ± 0.01
1	NT[g]	NT
2	1.05 ± 0.01	2.89 ± 0.07
3	NT	NT
4	>25	>25
5	21.88 ± 0.49	16.62 ± 0.32
6	10.9 ± 0.53	4.29 ± 0.06
7	12.11 ± 0.29	10.41 ± 0.44
8	8.51 ± 0.29	6.3 ± 0.19
9	12.33 ± 0.33	17.41 ± 0.28
10	NT	NT
11	13.83 ± 0.38	5.54 ± 0.19
12	8.31 ± 0.18	9.43 ± 0.27
13	21.88 ± 0.46	18.41 ± 0.12
14	>25	>25
15	1.37 ± 0.05	0.62 ± 0.01
16	6.99 ± 0.21	5.77 ± 0.25
17	>25	>25
18	21.3 ± 0.96	>25

[a] Each data point represents mean ± SD from three different experiments performed in triplicate.
[b] Human breast ductal carcinoma cell line (T47D).
[c] Human gastric cancer cell line (SNU638).
[d] Adriamycin: positive control for cytotoxicity.
[e] Etoposide: positive control for topoisomerase II and cytotoxicity.
[f] Camptothecin: positive control for topoisomerase I and cytotoxicity.
[g] NT: non-tested.

In conclusion, total 18 chalcone compounds were synthesized and tested for pharmacological activity. In the topoisomerase I and II inhibition test, most compounds except compounds **15** and **18** did not show significant topoisomerase II inhibitory activity, but compounds in group III and IV were potent topoisomerase I inhibitors. In the cathepsin B and L inhibition assay, compound **15** showed highly efficient inhibitory activity on both enzymes with IC_{50} values of 1.81 ± 0.05 μM on cathepsin B and 3.15 ± 0.07 μM on cathepsin L, respectively. In the cytotoxicity test, compound **15** was most cytotoxic on tested human cancer cell lines. Judging on the observed pharmacological activity used for this study, compound **15** showed consistent inhibitory ability on the tested assays, which may implicate the cytotoxic activity of compound **15** on topoisomerase I and cathepsin B and L inhibitory pathways. Although more compounds should be tested and analyzed for clear SAR against topoisomerase I and cathepsin B and L, our findings reflect that more elaborate modification of the chalcone structure can provide useful information on the development of new topoisomerase I and cathepsin B and L inhibitor.

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14. *Representative synthetic procedure*: Method 1. A mixture of THP protected 4-hydroxyacetophenone analogue, benzaldehyde analogue and NaOH or $Ba(OH)_2$ base in ethanol was stirred (4–24 h) at room temperature. To the mixture was added 4 M-HCl and the reaction mixture was stirred for more 20–40 min. After adding water, the reaction mixture was extracted with ethyl acetate and organic layer was washed with water. Organic layer was dried over anhydrous $MgSO_4$ and solvent was removed under reduced pressure. (1) The residue was treated with EtOAc and hexane, filtered and collected to give product, or (2) The residue was applied to silica gel column chromatography to give desired compound.
Method 2. A mixture of acetophenone analogue (or acetyl 5-membered heteroaromatic analogue), benzaldehyde analogue and NaOH or $Ba(OH)_2$ base in ethanol was stirred at room temperature. (1) To the mixture was added water and solid formed was filtered and dried on the air. Solid was triturated with mixed solvent, filtered and dried to give desired compound. or (2) After adding water, the reaction mixture was extracted with ethyl acetate and organic layer was washed with water. Organic layer was dried over anhydrous $MgSO_4$ and solvent was removed under reduced pressure. The residue was applied to silica gel column chromatography to give desired compound.
Compound ***1***: Yellow solid (39.7%). R_f 0.11 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 3.91 (s, 3H), 6.87 (d, J = 8.8 Hz, 2H), 6.90 (d, J = 8.4 Hz, 1H), 7.39 (d, J = 15.6 Hz, 1H), 7.50 (dd, J = 2.0, 8.4 Hz, 1H), 7.63 (d, J = 15.6 Hz, 1H), 7.84 (d, J = 2.0 Hz, 1H), 7.92 (d, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) 56.5, 112.0, 112.4, 115.6, 120.9, 129.3, 129.8, 130.1, 131.3, 132.7, 142.4, 157.6, 162.0, 189.2 ppm. *Compound* ***2***: Yellow solid (72.6%). R_f 0.77 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 3.89 (s, 3H), 6.32 (d, J = 2.0 Hz, 1H), 6.39 (dd, J = 2.0, 8.8 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 7.40 (d, J = 15.6 Hz, 1H), 7.50 (dd, J = 1.2, 8.4 Hz, 1H), 7.68 (d, J = 15.6 Hz, 1H), 7.76 (d, J = 8.8 Hz, 1H), 7.83 (d, J = 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) 56.5, 103.2, 108.6, 112.0, 112.5, 113.6, 119.4, 129.0, 130.0, 132.0, 132.7, 142.4, 157.8, 165.0, 166.0, 191.6 ppm. *Compound* ***3***: Yellow solid (11.7%). R_f 0.48 (Ethyl acetate:*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 3H), 3.97 (s, 3H), 6.49 (d, J = 2.0 Hz, 1H), 6.50 (dd, J = 2.0, 8.8 Hz, 1H), 6.95 (d, J = 8.8 Hz, 1H), 7.46 (d, J = 15.2 Hz, 1H), 7.55 (dd, J = 2.0, 8.8 Hz, 1H), 7.79 (d, J = 15.2 Hz, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.91 (d, J = 2.0 Hz, 1H), 13.5 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 55.8, 56.7, 101.3, 108.0, 112.1, 112.7, 114.3, 119.4, 129.1, 130.2, 131.4, 132.9, 142.8, 158.0, 166.5, 166.9, 191.8 ppm. *Compound* ***4***: Off-white solid (89.0%). R_f 0.51 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (s, 3H), 6.95 (d, J = 8.8 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H), 7.40 (d, J = 15.2 Hz, 1H), 7.55 (dd, J = 2.0, 8.8 Hz, 1H), 7.73 (d, J = 15.2 Hz, 1H), 7.90 (d, J = 2.0 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 112.1, 112.8, 116.0 (d, J = 21.5 Hz), 120.6, 129.1, 130.1, 131.2, 131.3 (d, J = 8.9 Hz), 132.9, 143.5, 153.0,158.0, 188.7 ppm. *Compound* ***5***: Weak yellow solid (91.4%). R_f 0.47 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (s, 3H), 6.94 (d, J = 8.4 Hz, 1H), 7.20 (dd, J = 3.8, 4.8 Hz, 1H), 7.31 (d, J = 15.6 Hz, 1H), 7.55 (dd, J = 2.0, 8.4 Hz, 1H), 7.69 (d, J = 4.8 Hz, 1H), 7.75 (d, J = 15.6 Hz, 1H), 7.88 (dd, J = 1.2, 3.8 Hz, 1H), 7.90 (d, J = 2.0 Hz. 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 112.1, 112.7, 120.7, 128.5, 129.0, 130.1, 132.0, 132.9, 134.1, 142.5, 145.8, 158.0, 182.3 ppm. *Compound* ***6***: Ivory solid (70.8%). R_f 0.37 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (s, 3H), 6.60–6.62 (m, 1H), 6.93 (d, J = 8.4 Hz, 1H), 7.33 (d, J = 3.2 Hz, 1H), 7.34 (d, J = 15.6 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.66 (s, 1H), 7.77 (d, J = 15.6 Hz, 1H), 7.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 56.6, 112.1, 112.6, 112.8, 117.6, 120.3, 129.1, 130.2, 132.9, 142.4, 146.7, 153.9, 157.9, 178.0 ppm. *Compound* ***8***: Orange solid (70.8%). R_f 0.14 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (s, 3H), 5.46 (s, 1H), 6.47–6.49 (m, 2H), 7.16 (d, J = 8.8 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 15.6 Hz, 1H), 7.52 (d, J = 8.8 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 8.05 (d, J = 8.8 Hz, 1H), 8.06 (d, J = 15.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 55.8, 99.4, 108.1, 115.8 (d, J = 21.6 Hz), 117.2, 120.2, 131.2 (d, J = 8.9 Hz), 131.4, 135.0, 141.0, 146.8, 159.5, 160.9, 189.9 ppm. *Compound* ***9***: Brown solid (15.3%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.08 (s, 3H), 3.84 (s, 3H), 6.45 (dd, J = 2.0, 8.4 Hz, 1H), 6.48 (d, J = 2.0 Hz, 1H), 7.67 (d, J = 16.0 Hz, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 16.0 Hz, 1H), 8.05 (d, J = 8.8 Hz, 2H), 10.3 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 24.2, 55.5, 99.1, 108.3, 114.4, 117.8, 118.2, 129.5, 130.2, 132.7, 138.5, 143.3, 160.2, 161.9, 168.9, 187.5 ppm. *Compound* ***10***: Dark brown solid (31.2%). R_f 0.78 (Methanol/Chloroform = 1:4); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (s, 3H), 6.46 (dd, J = 2.0, 8.4 Hz, 1H), 6.54 (d, J = 2.0 Hz, 1H), 6.72 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.4 Hz, 1H), 7.61 (d, J = 15.6 Hz, 1H), 7.87 (d, J = 15.6 Hz, 1H), 7.88 (d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 55.7, 98.9, 108.6, 114.2, 114.7, 116.9, 118.3, 129.8, 130.0, 130.6, 137.0, 159.8, 163.7, 186.8 ppm. *Compound* ***11***: Weak yellow solid (64.9%). R_f 0.23 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 6.87 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 8.4 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 7.44 (d, J = 15.6 Hz, 1H), 7.59 (d, J = 8.8 Hz, 1H), 7.60 (d, J = 8.8 Hz, 1H), 7.70 (d, J = 15.6 Hz, 1H), 7.92 (d, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) 115.6, 116.2 (d, J = 21.5 Hz), 121.8, 130.0, 130.4 (d, J = 8.2 Hz), 131.3, 131.5, 142.8, 162.1, 164.8, 189.3 ppm. *Compound* ***12***: Orange solid (73.9%). R_f 0.24 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 6.35 (d, J = 2.0 Hz, 1H), 6.40 (dd, J = 2.0, 8.4 Hz, 1H), 7.07 (d, J = 8.8 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 7.47 (d, J = 15.6 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 8.8 Hz, 1H), 7.78 (d, J = 15.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) 103.3, 108.6, 113.6, 116.3 (d, J = 22.3 Hz), 120.4, 130.6 (d, J = 8.4 Hz), 131.3, 132.1, 142.9, 165.1, 165.5, 166.1, 191.6 ppm. *Compound* ***13***: Yellow solid (4.0%). R_f 0.60 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 3H), 6.49 (d, J = 2.8 Hz, 1H), 6.50 (dd, J = 2.8, 8.4 Hz, 1H), 7.13 (dd, J = 8.8, 8.8 Hz, 2H), 7.51 (d, J = 15.6 Hz, 1H), 7.66 (dd, J = 8.8, 8.8 Hz, 2H), 7.83 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 15.6 Hz, 1H), 13.4 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 55.9, 101.3, 108.1, 114.3, 116.4 (d, J = 21.5 Hz), 120.3, 120.4, 130.7 (d, J = 9.0 Hz), 131.3, 131.4, 165.6, 166.5, 167.0, 191.9 ppm. *Compound* ***14***: Yellow solid (76.5%). R_f 0.11 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (s, 3H), 6.49 (d, J = 2.4 Hz, 1H), 6.50 (dd, J = 2.4, 8.0 Hz, 1H), 6.66 (s, 1H), 7.09 (dd, J = 8.4, 8.4 Hz, 2H), 7.46 (d, J = 15.6 Hz, 1H), 7.59 (dd, J = 8.4, 8.4 Hz, 2H), 7.67 (d, J = 15.6 Hz, 1H), 7.70 (d, J = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 56.0, 99.5, 108.3, 116.2 (d, J = 21.6 Hz), 121.7, 126.9, 127.0, 130.4 (d, J = 8.9 Hz), 131.8, 133.3, 161.1, 161.6, 165.3, 191.0 ppm. *Compound* ***15***: Red solid (3.8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.95–1.99 (m, 4H), 3.29–3.33 (m, 4H), 6.58 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 7.57 (d, J = 16.0 Hz, 1H), 7.61 (d, J = 16.0 Hz, 1H), 7.66 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 24.9, 47.3, 111.7, 115.2, 115.6, 121.7, 129.7, 130.6, 130.7, 114.1, 149.2, 161.7, 186.7 ppm. *Compound* ***16***: Red solid (17.7%). R_f 0.20 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.05–2.07 (m, 4H), 3.37–3.41 (m, 4H), 6.43 (s, 1H), 6.57 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 14.8 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 8.4 Hz, 2H), 7.84 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 14.8 Hz, 1H), 13.77 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 25.7, 47.8, 103.9, 107.6, 112.0, 114.0, 122.2, 125.0, 131.1, 146.1, 150.0, 152.3, 162.5, 166.5, 190.6 ppm. *Compound* ***17***: Orange solid (38.4%). R_f 0.53 (Ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03–2.07 (m, 4H), 3.35–3.40 (m, 4H), 6.57 (d, J = 8.8 Hz, 2H), 7.16 (dd, J = 8.6, 8.6 Hz, 2H), 7.29 (d, J = 15.6 Hz, 1H), 7.55 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 15.6 Hz, 1H), 8.04 (dd, J = 8.6, 8.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 25.7, 47.8, 112.0, 115.7 (d, J = 21.5 Hz), 115.9, 122.2, 130.9, 131.0 (d, J = 9.0 Hz), 135.6, 135.7, 146.6, 149.9, 164.2, 166.7, 189.2 ppm. *Compound* ***18***: Red solid (31.8%). R_f 0.45 (Ethyl acetate:*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.08–2.12 (m, 4H), 3.41–3.45 (m, 4H), 6.61 (d, J = 8.8 Hz, 2H), 7.29 (d, J = 15.6 Hz, 1H), 7.60 (d, J = 8.8 Hz, 2H), 7.87 (d, J = 15.6 Hz, 1H), 8.16 (d, J = 8.8 Hz, 2H), 8.37 (d, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 25.7, 47.9, 112.1, 115.5, 121.7, 123.9, 129.4, 131.4, 144.7, 148.4, 150.4, 189.1 ppm.
15. Cho, H.-J.; Jung, M.-J.; Woo, S.; Kim, J.; Lee, E.-S.; Kwon, Y.; Na, Y. *Bioorg. Med. Chem.* **2010**, *18*, 1017.
16. (a) Kang, D. H.; Jun, K. Y.; Lee, J. P.; Pak, C. S.; Na, Y.; Kwon, Y. *J. Med. Chem.* **2009**, *52*, 3093; Cathepsin B and L inhibitory activities were assayed in the reaction buffer (50 mM NaOAc–HCl, 2 mM dithiothreitol (DTT), 2 mM EDTA, pH 5.5 for cathepsin B and 0.1 M NaOAc–HCl, 1 mM EDTA, 0.1% β-mercaptoethanol, pH 5.5 for cathepsin L) with 20 μM substrate and 1.5 nM cathepsin B or 4 nM cathepsin L. Cathepsin B and L were obtained from Calbiochem (Darmstadt, Germany). The substrates used were RR-AMC (Sigma, USA) for cathepsin B and Z-FR-AMC (Sigma, USA) for cathepsin L. The cathepsins were reductively activated by preincubation in assay buffer at 37 °C for 30 min, prior to initiating the reaction by substrate and compounds. Afterwards, the reaction mixture was incubated at room temperature for 30 min. Fluorescence intensities were determined by using 360 nm excitation and 450 nm emission wavelengths. CA-074, Z-FF-FMK, and nimodipine were used as cathepsin B inhibitor, cathepsin L inhibitor, calcium channel blocker, respectively, and all were purchased from Sigma Chemical Co., (USA).