Organic & Biomolecular Chemistry

Supporting Information

High Selective Azadipeptide Nitrile Inhibitors for Cathepsin K: Design, Synthesis and Activity Assay

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Table of Contents

1. Inhibition Assays

1.1. Inhibition Assay of Cathepsin K

Human cathepsin K (Merck 219461, His•Tag, Human, Recombinant, *E. coli.*, Germany) was assayed at FLx800 Fluorescence Microplate Reader (BioTek) using 96-well plates. The wavelength for excitation was 360 nm and that for emission was 440nm. Assay buffer was 100 mM MES-NaOH solution (pH = 5.5) containing 2.5 mM EDTA, 2.5 mM DTT and 9% DMSO. A stock solution of 25 μg enzyme in 500 mM NaCl, 200 mM sodium acetate, 25 mM Tris-HCl, 10 mM trehalose, 5 mM DTT, 5 mM EDTA, 50% glycerol, pH 4.5 was diluted to 10 nM with assay buffer containing 2.5 mM DTT. Stock solutions of inhibitor were prepared in DMSO. A 10 mM stock solution of the chromogenic substrate Z-Phe-Arg-AMC (ALX-260-131, Alexis, ≥98% (HPLC)) was prepared with DMSO and diluted with assay buffer. The final concentration in enzymatic assay of DMSO was 10%, and those of the substrate and cathepsin K was 20 μM and 1 nM, respectively. Into a well containing 39 μL assay buffer, 1 μL inhibitor solution (or DMSO) and 10 μL diluted solution of cathepsin K were added and mixed thoroughly, and then incubated at 37 ºC for 30 min. The reaction was initiated by adding 50 μL diluted solution of substrate and the fluorescence intensity (I) at 440 nm was measured (**Figure S1**). Experiments were performed in triplicate with at least five different concentrational inhibitor and three controls.

1.2. Inhibition Assay of Cathepsin L

Human cathepsin L (Merck 219402, Human Liver, Germany) was assayed at FLx800 Fluorescence Microplate Reader (BioTek) using 96-well plates. The wavelength for excitation was 360 nm and that for emission was 440 nm. Assay buffer was 100 mM MES-NaOH solution (pH = 5.5) containing 2.5 mM EDTA, 2.5 mM DTT and 9% DMSO. A stock solution of 25 μg enzyme in 400 mM NaCl, 20 mM malonate buffer, 1 mM EDTA, pH 5.5 was diluted to 20 nM with assay buffer containing 2.5 mM DTT. Stock solutions of inhibitor were prepared in DMSO. A 10 mM stock solution of the chromogenic substrate Z-Phe-Arg-AMC (ALX-260-131,

Alexis, ≥98% (HPLC)) was prepared with DMSO and diluted with assay buffer. The final concentration in enzymatic assay of DMSO was 10%, and those of the substrate and cathepsin L was 20 μM and 2 nM, respectively. Into a well containing 39 μL assay buffer, 1 μL inhibitor solution (or DMSO) and 10 μL diluted solution of cathepsin L were added and mixed thoroughly, and then incubated at 37 ºC for 30 min. The reaction was initiated by adding 50 μL diluted solution of substrate and the fluorescence intensity (I) at 440 nm was measured. Experiments were performed in triplicate with at least five different concentrational inhibitor and three controls.

1.3. Inhibition Assay of Cathepsin S

Human cathepsin S (Merck 219343, Human, Recombinant, E. coli., Germany) was assayed at FLx800 Fluorescence Microplate Reader (BioTek) using 96-well plates. The wavelength for excitation was 360 nm and that for emission was 440 nm. Assay buffer was 100 mM MES-NaOH solution (pH = 6.5) containing 2.5mM EDTA, 2.5 mM DTT , 0.001% BSA and 9% DMSO. A stock solution of 50 μg enzyme in 35 mM potassium phosphate, 35 mM Sodium acetate, 2 mM DTT, 2 mM EDTA, 50% ethylene glycol, pH 6.5 was diluted to 50 nM with assay buffer containing 2.5 mM DTT. Stock solutions of inhibitor were prepared in DMSO. A 10 mM stock solution of the chromogenic substrate Z-VVR-AMC (Biomol BML-P199-0010, 99%) was prepared with DMSO and diluted with assay buffer. The final concentration in enzymatic assay of DMSO was 10%, and those of the substrate and cathepsin S was 20 μM and 5 nM, respectively. Into a well containing 39 μL assay buffer, 1 μL inhibitor solution (or DMSO) and 10 μL diluted solution of cathepsin S were added and mixed thoroughly, and then incubated at 37 ºC for 30 min. The reaction was initiated by adding 50 μL diluted solution of substrate and the fluorescence intensity (I) at 440 nm was measured. Experiments were performed in triplicate with at least five different concentrational inhibitor and three controls.

1.4. Inhibition Assay of Cathepsin B

Human cathepsin B (Merck 219362, Human Liver, Germany) was assayed at

FLx800 Fluorescence Microplate Reader (BioTek) using 96-well plates. The wavelength for excitation was at 360 nm and that for emission was 440 nm. Assay buffer was 100 mM MES-NaOH solution (pH = 6.0) containing 2.5 mM EDTA, 2.5 mM DTT, 0.001% Tween20 and 9% DMSO. A stock solution of 50 μg enzyme in 50 mM sodium acetate buffer, 1 mM EDTA, pH 5.0 was diluted to 50 nM with assay buffer containing 2.5 mM DTT. Stock solutions of inhibitor were prepared in DMSO. A 10 mM stock solution of the chromogenic substrate Z-Phe-Arg-AMC (ALX-260-131, Alexis, ≥ 98% (HPLC)) was prepared with DMSO and diluted with assay buffer. The final concentration of DMSO in enzymatic assay was 10%, and those of the substrate and cathepsin B was 20 μM and 5 nM, respectively. Into a well containing 39 μL assay buffer, 1 μL inhibitor solution (or DMSO) and 10 μL diluted solution of cathepsin B were added and mixed thoroughly, and then incubated at 37 ºC for 30 min. The reaction was initiated by adding 50 μL diluted solution of substrate and the fluorescence intensity (I) at 440 nm was measured. Experiments were performed in triplicate with at least five different concentrational inhibitor and three controls.

2. Representative Plots and IC_{50} Values

2.1 Determination of IC_{50} for Cathepsin K, B, L and S

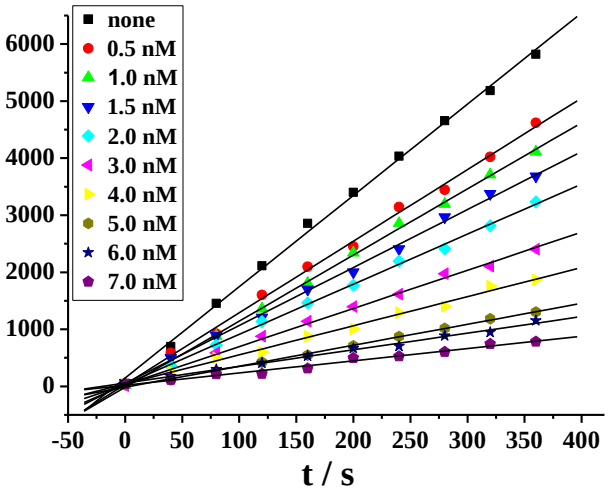
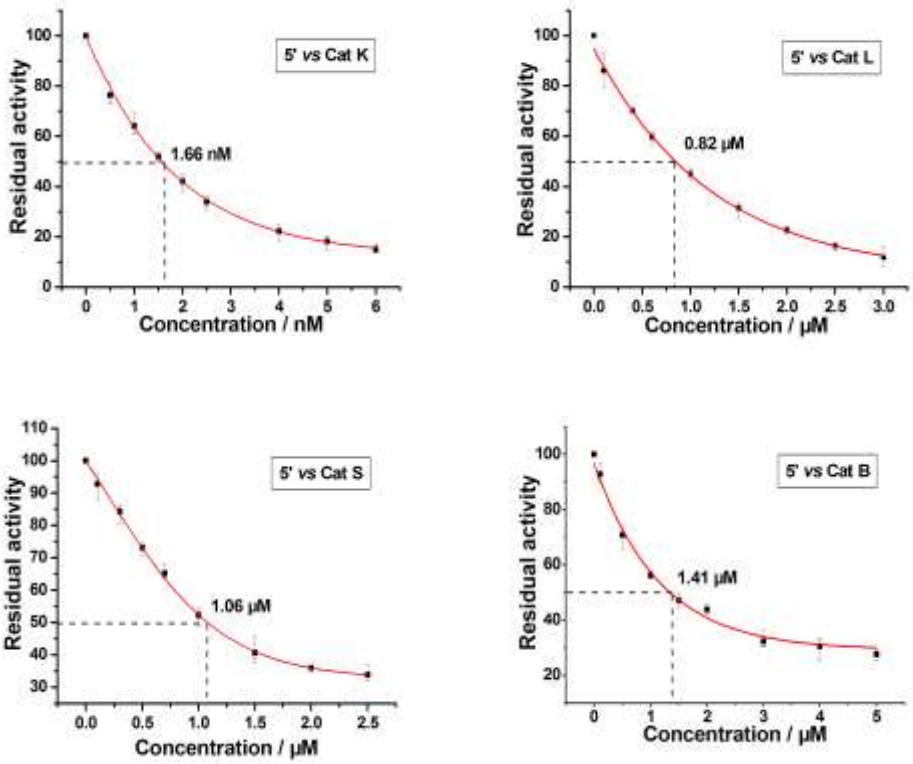


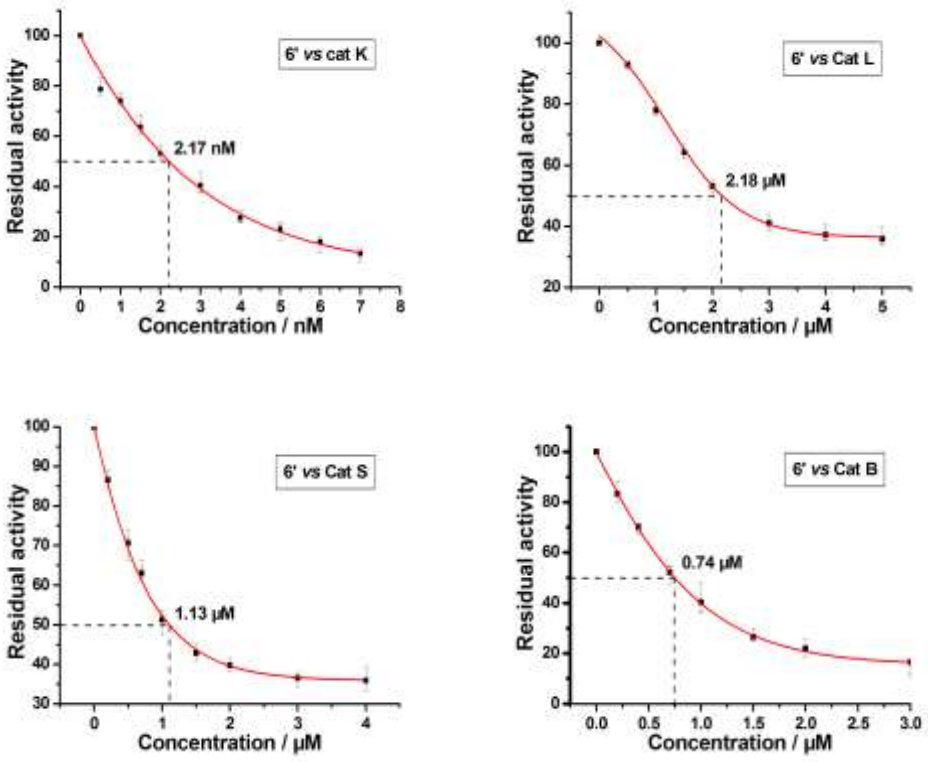
Figure S1. Monitoring of the human cathepsin K-catalyzed hydrolysis of Z-Phe-Arg-AMC (20 μM) in the presence of increasing concentrations of the

azadipeptide nitrile inhibitor **6′** (■, none; ●, 0.5 nM; ▲, 1.0 nM; ▼, 1.5 nM; ◆, 2.0 nM; ◂, 3.0 nM; ▸, 4.0 nM; ⬢, 5.0 nM; ★, 6.0 nM; ⬟,7.0 nM). The reaction was initiated by the addition of substrate and the fluorescence intensity (I) at 440 nm was measured under the excitation of 360 nm. Fluorescence units (FU) were corrected for background fluorescence.

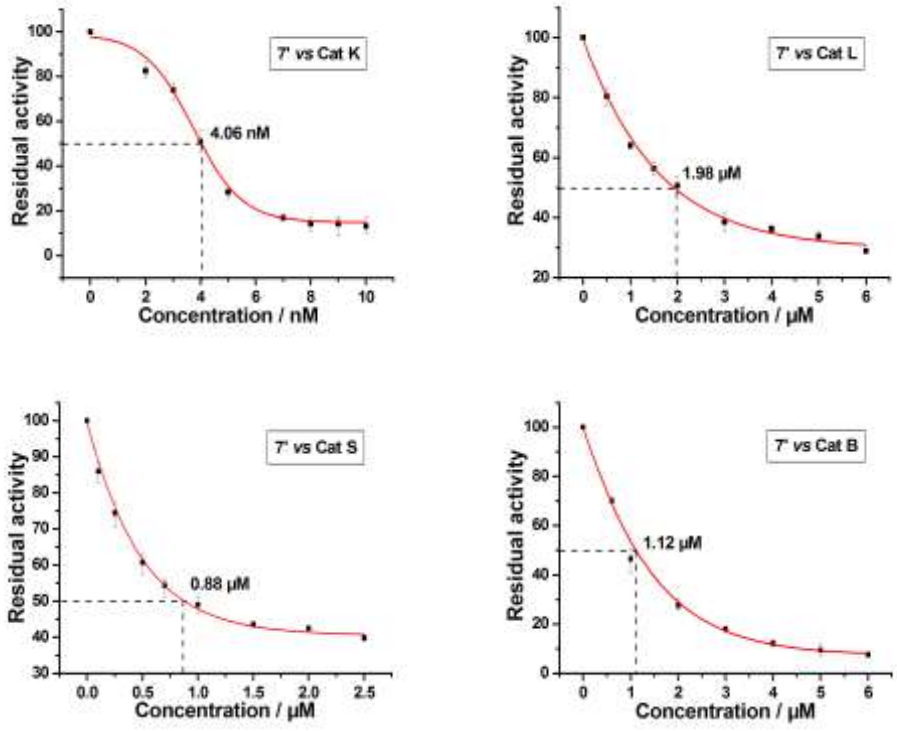
2.2 IC_{50} of Compound 5′ against Cathepsin K, L, S and B



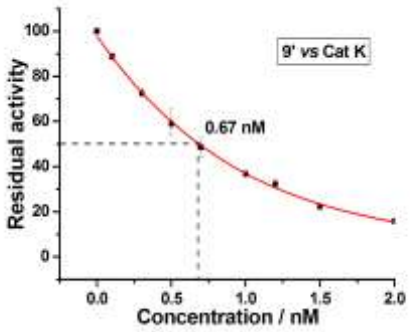
2.3 IC_{50} of Compound 6′ against Cathepsin K, L, S and B

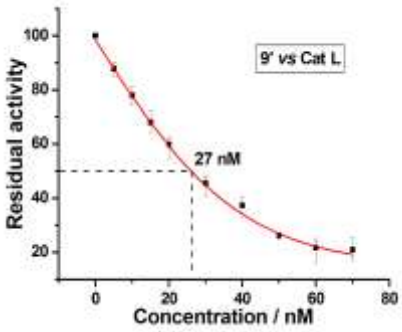


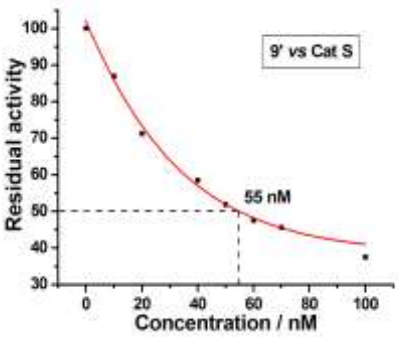
2.4 IC_{50} of Compound 7′ against Cathepsin K, L, S and B

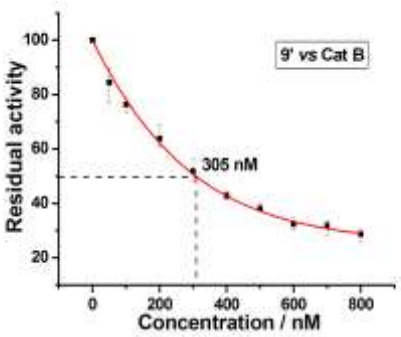


2.5 IC_{50} of Compound 9′ against Cathepsin K, L, S and B

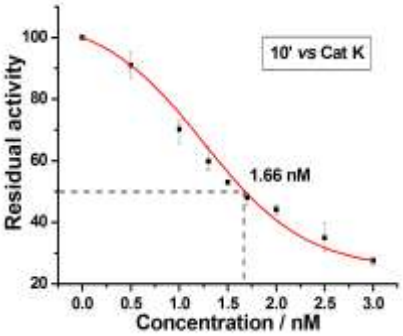


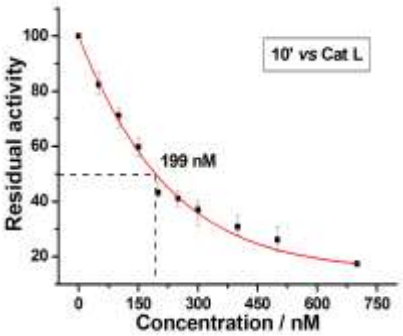


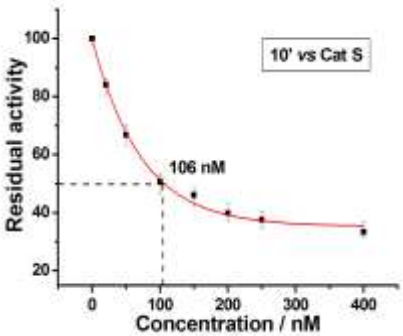


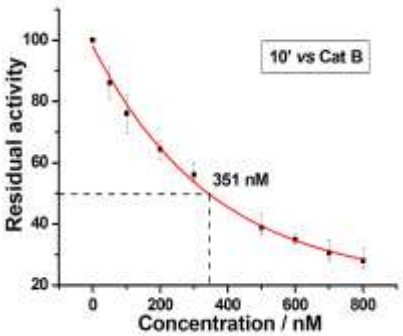


2.6 IC_{50} of Compound 10′ against Cathepsin K, L, S and B

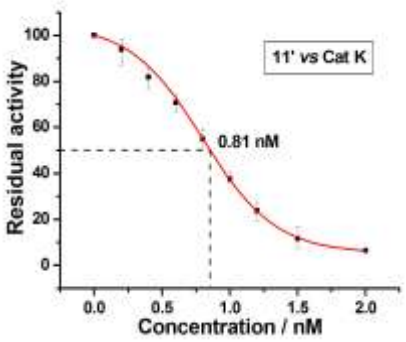


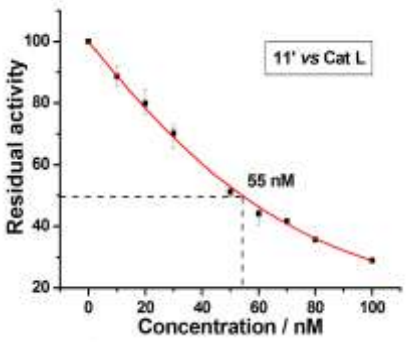


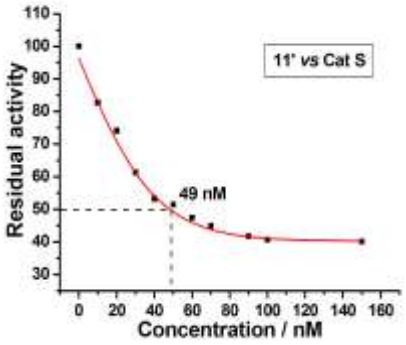


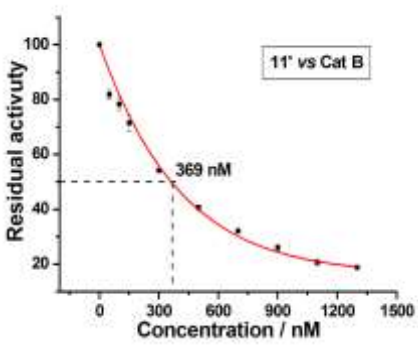


2.7 IC_{50} of Compound 11′ against Cathepsin K, L, S and B

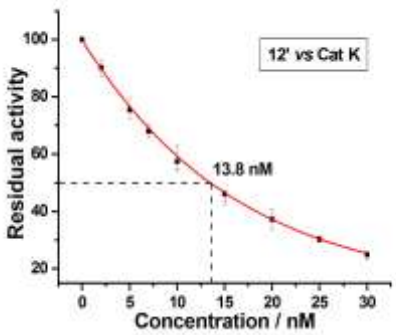


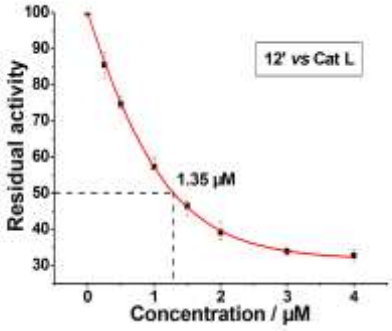


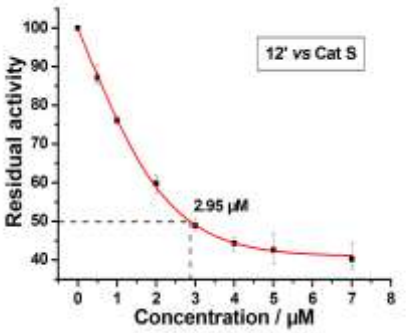


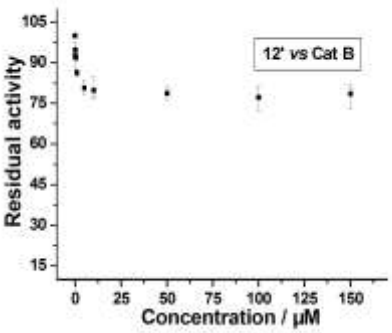


2.8 IC_{50} of Compound 12′ against Cathepsin K, L, S and B









2.9 IC_{50} of Compound 13′ against Cathepsin K, L, S and B

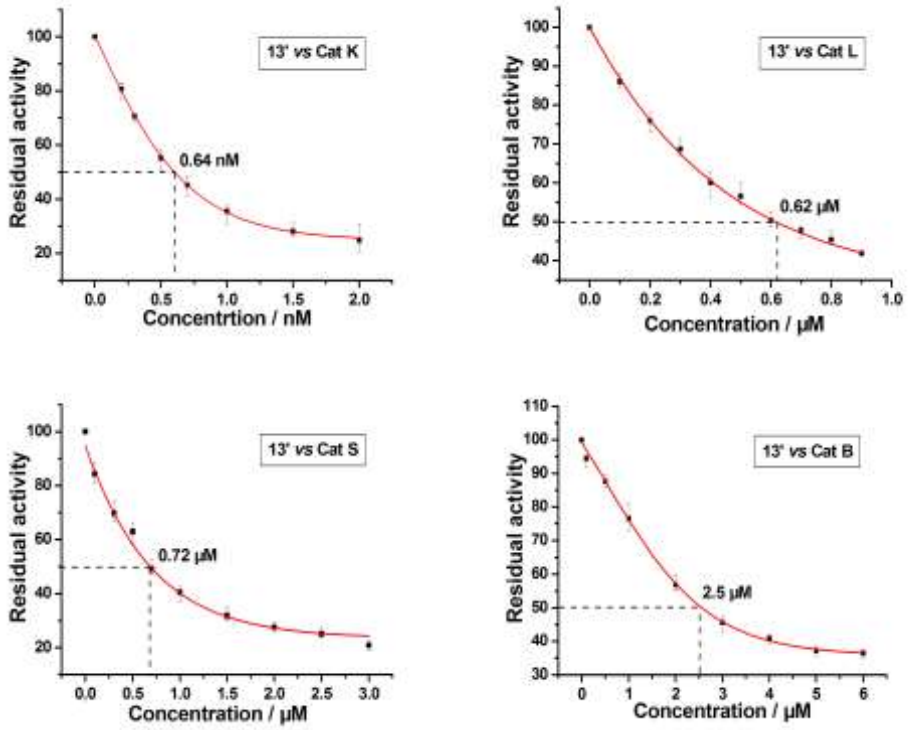


Table S1. Cathepsin inhibition (IC_{50}) by azadipeptide dipeptide nitriles

No. of Compounds	IC_{50} (nM)			
	Cat K	Cat L	Cat S	Cat B
5'	1.66	820	1060	1410
6'	2.17	2180	1130	740
7'	4.06	1980	880	1120
9'	0.67	27	55	305
10'	1.66	199	106	351
11'	0.81	55	49	369
12'	13.8	1350	2950	>0.15 mM
13'	0.64	620	720	2500

In our last paper, the values of K_m for Cathepsin K, L, S and B had been determined as 18.06 ± 0.22 μM, 3.525 ± 0.405 μM, 102.2 ± 1.52 μM and 157.5 ± 2.5 μM;[1] The condition of enzyme activity assay was similar in the two parts of this sequential studies, so the repeated work would not been exhibited here. The K_i values (Table S2) were calculated from the corresponding IC_{50} and K_m values and the

concentration of substrate, [S], by using the equation $K_i = IC_{50}/(1 + [S]/K_m)$.

Table S2. Cathepsin inhibition (K_i) by azadipeptide dipeptide nitriles

No. of Compounds	K_i (nM)			
	Cat K	Cat L	Cat S	Cat B
5'	0.79	123	762	1401
6'	1.03	327	812	735
7'	1.93	297	633	1113
9'	0.32	4.1	40	303
10'	0.79	30	76	349
11'	0.38	8.3	35	367
12'	6.54	202	2120	>0.149 mM
13'	0.29	93	518	2484

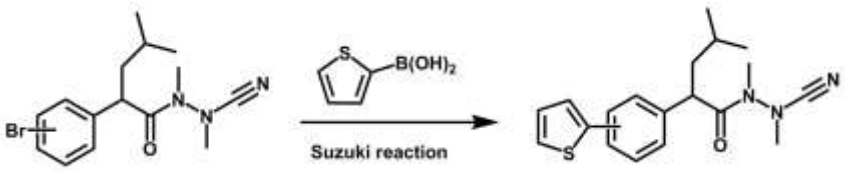
3. Preparation of Compounds

3.1. General Methods and Materials

Thin layer chromatography was performed on Branch of Qingdao Haiyang Chemical Co., Ltd aluminum sheets. Preparative column chromatography was performed on silica gel 60, 54-74μm. ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded on a Bruker Avance 500 spectrometer. ESI-MS spectra were recorded on a Bruker HCT mass spectrometer.

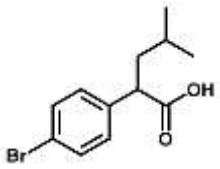
2-（3-bromophenyl）acetic acid, 2-（4-bromophenyl）acetic acid, were bought from Alfa Aesar China (Tianjin Co., Ltd.). LDA was obtained from Shanghai Darui Finechemical Co., Ltd.. 1-iodo-2-methylpropane was bought from J&K Scientific Ltd.. 1,2-dimethylhydrazine dihydrochloride was obtained from TCI Co., Ltd.. Cyanogen bromide was bought from ACROS Co., Ltd.. 2-thienylboronic acid, pyridin-4-ylboronic acid, phenylboronic acid and biphenyl-4-ylboronic acid were obtained from RED Chemical.

3.2. Schemes of synthesis



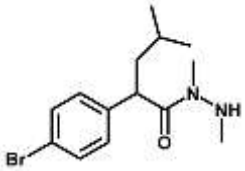
Scheme S1. Synthesis routes for compounds 5′-7′, and 9′-13′.

3.3. 4-methyl-2-phenylpentanoic acid:



2.15 g 2-(4-bromophenyl)acetic acid (10 mmol) was added to a 50 mL round-bottomed flask equipped with a magnetic stir bar, 20 mL tetrahydrofuran was then added. The mixture was degassed using five cycles of vacuum/nitrogen back-fill, then cooled to –15 °C. 7.5 mL LDA was injected into the round-bottomed flask under condition of stirring, and another 2 h at room temperature.[2] Next the reaction mixture was cooled to –15 °C in an ice bath again, and injected by 2.3 mL 1-iodo-2-methylpropane subsequently. The system was allowed to room temperature and stirred for additional 2 h. THF was removed in a rotary evaporator. The pH of the mixture was adjusted to 1-2, inducing a white solid precipitates. Then ethyl acetate was added to dissolve the precipitates, the organic layer was separated and the aqueous layer was extracted with ethyl acetate (15 mL×3). The combined organic phases were washed with brine twice (2×15 mL), then dried with Na_2SO_4 and evaporated to obtain the colorless oil. It was purified by column chromatography on silica gel with ethyl acetate / petroleum ether / formic acid (1:2:0.01) as eluent to obtain 4-methyl-2-phenylpentanoic acid as a colorless oil, which was then slowly solidified at room temperature.

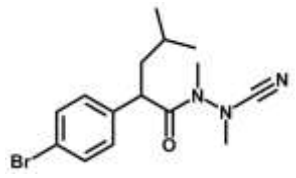
3.4. 2-(4-bromophenyl)-N,N',4-trimethylpentanehydrazide:



3.13 g 4-methyl-2-phenylpentanoic acid (10 mmol) was dissolved in THF (15 mL) and cooled to –25 °C. To the stirred solution, 1.11 mL N-methylmorpholine (10 mmol) and 1.32 mL isobutylchloroformate (10 mmol) were added consecutively. 2.66 g

1,2-dimethylhydrazine dihydrochloride (20 mmol) was dissolved in 5 mL H_2O, and 5 M NaOH (8 mL) was added under ice-cooling. When the precipitation of N-methylmorpholine hydrochloride occurred this solution was added to the reaction mixture. It was allowed to warm to room temperature within 30 min and stirred for additional 90 min. After evaporation of the solvent, the resulting aqueous residue was extracted with ethyl acetate (1×40 mL, 3×10 mL). The combined organic layers were washed with H_2O (1×15 mL), sat. $NaHCO_3$ twice (2×15 mL), H_2O (1×15 mL) and brine (1×15 mL) in turn. The solvent was dried with Na_2SO_4 and evaporated to obtain a colorless oil. It was purified by column chromatography on silica gel with ethyl acetate / petroleum ether (1:4) as eluent to obtain 2-(4-bromophenyl)-N,N',4-trimethylpentanehydrazide as a colorless oil, which was then slowly solidified at room temperature.

3.5. 2-(4-bromophenyl)-N'-cyano-N,N',4-trimethylpentanehydrazide (1′):



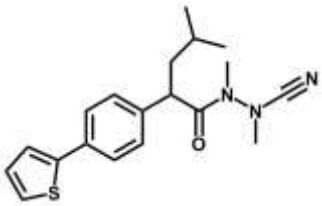
1.64 g sodium acetate (20 mmol) and 2.12 g cyanogen bromide (20 mmol) were added to a MeOH solution (35 mL) containing 3.38 g (10 mmol) 2-(4-bromophenyl)-N,N',4-trimethylpentanehydrazide. The mixture was stirred at room temperature for 5 h and then the solvent was removed under reduced pressure. The residue was suspended in 15 mL H_2O, a pH of 1-2 was adjusted by using 10% $KHSO_4$, and then it was extracted with ethyl acetate for three times (3×25 mL). The combined organic layers were washed with H_2O (1×20 mL), sat. $NaHCO_3$ (2×20 mL) and brine (1×20 mL) in turn. The solvent was dried by Na_2SO_4 and removed in vacuo. The oily residue was purified by column chromatography on silica gel using dichloromethane / petroleum ether (1:2) as eluent. The obtained oil was dried in a desiccator at room temperature for several days to obtain 2-(4-bromophenyl)-N' -cyano-N,N',4-trimethylpentanehydrazide as a colorless solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48 (d, *J* = 8.2 Hz, 2H), 7.18 (d, *J* = 8.1 Hz, 2H), 4.14 (t, *J* = 7.3 Hz, 1H), 3.19 (s, 3H), 3.14 (s, 3H), 2.04 (td, *J* = 13.3, 6.6 Hz, 1H), 1.65 – 1.54 (m, 1H), 1.46 (td, *J* = 13.3, 6.7 Hz, 1H), 0.93 (dd, *J* = 12.3, 6.4 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.29 (s), 138.66 (s), 132.05 (s), 129.64 (s), 121.14 (s), 114.34 (s), 45.62 (s), 43.46 (s), 35.37 (s), 30.59 (s), 25.55 (s), 22.67 (s), 22.31 (s).

MS (ESI) m/z: $[M + H]^+$ 338.0908.

3.6. N'-cyano-N,N',4-trimethyl-2-(4-(thiophen-2-yl)phenyl)pentanehydrazide (5′)



100 mL, round-bottomed Schlenk flask equipped with a magnetic stir bar was charged with 0.34 g maternal compound **1′** (1 mmol), 0.26 g 2-thienylboronic acid (2 mmol), 0.058 g tetrakis(triphenylphosphine)palladium (0.05 mmol), 2 mL 1 M aqueous solution of Na_2CO_3, and 30 mL of THF.[3] The mixture was degassed using five cycles of vacuum/nitrogen back-fill, and then was heated to 70 °C and kept there for 2.5 h with vigorous stirring. The mixture was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was then suspended in H_2O (10 mL) and ethyl acetate (30 mL), the organic layer was separated while the aqueous layer was extracted with ethyl acetate (3×15 mL). The combined organic phases were washed with brine (2×15 mL), then dried with Na_2SO_4 and evaporated to obtain the crude product of N'-cyano-N,N',4-trimethyl-2-(4-(thiophen-2-yl)phenyl)pentanehydrazide, which was purified by column chromatography on silica gel using Tetrahydrofuran / petroleum ether (1:4) as eluent.

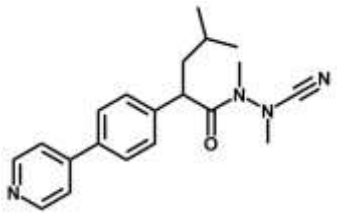
^{1}H NMR (500 MHz, DMSO): δ 7.64 (d, *J* = 7.7 Hz, 2H), 7.52 (dd, *J* = 19.7, 4.1 Hz, 2H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.14 (dd, *J* = 5.0, 3.7 Hz, 1H), δ 4.23 (t, *J* = 7.4 Hz,

1H)，3.20 (s,4H), 2.58 (s, 2H)，δ 2.10 (m, 1H), 1.55 (ddd, *J* = 20.1, 17.8, 11.1 Hz, 2H), 1.01 – 0.96 (m, 60

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.57 (s), 143.65 (s), 138.85 (s), 133.42 (s), 128.46 (s), 128.10 (s), 126.32 (s), 124.97 (s), 123.17 (s), 114.01 (s)， 45.97 (s), 43.57 (s), 40.46 (s), 30.64 (s)， δ 25.65 (s), 22.77 (s), 22.45 (s).

MS (ESI) m/z: $[M + H]^+$ 342.1654.

3.7. N'-cyano-N,N',4-trimethyl-2-(4-(pyridin-4-yl)phenyl)pentanehydrazide (6′)



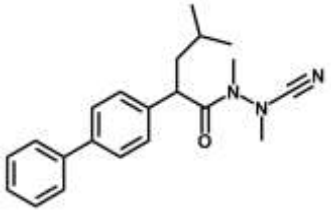
This compound was produced by using similar procedure as for compound **5′** (**Scheme S1**), but using 4-（4,4,5,5-tetramethyl-1,3-dioxolan-2-yl）pyridine [4] instead of 2-thienylboronic acid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.67 (d, *J* = 4.3 Hz, 2H).7.64 (d, *J* = 8.1 Hz, 2H), 7.53 – 7.40 (m, 4H), δ 4.23 (t, *J* = 7.4 Hz, 1H)， 3.20 (s,4H), 2.58 (s, 2H)， δ 2.10 (m, 1H), 1.55 (ddd, *J* = 20.1, 17.8, 11.1 Hz, 2H), 1.01 – 0.96 (m, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.45 (s), 149.92 (d, *J* = 21.7 Hz), 147.78 (s), 140.85 (s), 128.77 (s), 127.47 (s), 121.49 (s), 113.88 (s), 45.74 (s), 43.57 (s), 40.56 (s), 30.64 (s), 25.66 (s), 22.71 (s), 22.38 (s).

MS (ESI) m/z: $[M + H]^+$ 337.2013.

3.8. 2-(biphenyl-4-yl)-N'-cyano-N,N',4-trimethylpentanehydrazide (7′)



This compound was produced by using similar procedure as for compound **5′**

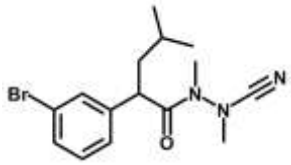
(**Scheme S1**), but using phenylboronic acid instead of 2-thienylboronic acid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.86 (d, *J* = 5.6 Hz, 2H), 7.64 (dt, *J* = 8.6, 6.1 Hz, 4H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 1H), 5.34 (td, *J* = 10.1, 3.7 Hz, 1H), 3.36 (s, 3H), 3.24 (s, 3H), 1.82 (dd, *J* = 12.1, 5.4 Hz, 1H), 1.77 – 1.63 (m, 2H), 1.12 – 1.00 (m, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.70 (s), 140.33 (s), 140.03 (s), 138.73 (s), 128.83 (d, *J* = 13.7 Hz), 128.42 (s), 127.86 – 126.76 (m), 114.08 (s), 45.86 (s), 43.61 (s), 40.41 (s), 30.59 (s), 25.67 (s), 22.56 (t, *J* = 23.3 Hz).

MS (ESI) m/z: $[M + H]^+$ 336.2082.

3.9. 2-(3-bromophenyl)-N'-cyano-N,N',4-trimethylpentanehydrazide (8′):



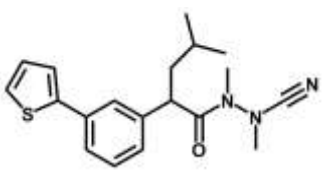
This compound was produced by using the similar procedures as for compound **1′** (**Scheme 1**), but staring with 2-(3-bromophenyl)acetic acid instead of 2-(4-bromophenyl)acetic acid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.45 (s, 1H), 7.41 (d, *J* = 6.6 Hz, 2H), 7.22 – 7.18 (m, 1H), 4.13 (t, *J* = 7.4 Hz, 1H), 3.18 (s, 3H), 3.13 (s, 3H), 2.04 – 1.95 (m, 1H), 1.59 (dt, *J* = 13.7, 6.9 Hz, 1H), δ 1.47 (dt, *J* = 13.2, 6.6 Hz, 1H), 1.00 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) : δ 174.79 (s), 131.32 (s), 130.90 (s), 130.46 (s), 126.60 (s), 122.92 (s), 114.34 (s), δ 45.87 (s), 43.47 (s), 40.44 (d, *J* = 34.7 Hz), 30.61 (s), 25.62 (s), 22.50 (d, *J* = 27.7 Hz).

MS (ESI) m/z: $[M + H]^+$ 338.0910.

3.10.N'-cyano-N,N',4-trimethyl-2-(3-(thiophen-2-yl)phenyl)pentanehydrazide (9′)



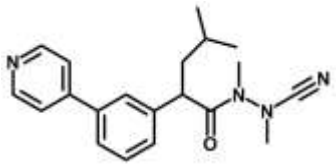
This compound was produced by using the similar procedures as for compound **5′** (**Scheme S1**), but it using compound **8′** instead of **1′** as maternal compound.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56 (d, *J* = 19.1 Hz, 2H), 7.39 (m, 2H), 7.35 (d, *J* = 4.9 Hz, 1H), 7.25 (d, *J* = 7.6 Hz, 1H), 7.16 – 7.10 (m, 1H), 4.22 (t, *J* = 7.3 Hz, 1H), 3.21 (s, 4H), 2.58 (s, 2H), 2.10 – 2.03 (m, 1H), 1.69 (dt, *J* = 13.7, 6.9 Hz, 1H), 1.56 (dt, *J* = 13.3, 6.6 Hz, 1H) , 1.01 (d, *J* = 6.5 Hz,3H), 0.98 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.48 (s), 143.71 (s), 140.44 (s), 135.06 (s), 129.52 (s), 128.14 (s), 126.96 (s), 125.21 (d, *J* = 5.3 Hz), 124.79 (s), 123.46 (s), 114.03 (s), 46.31 (s), 43.44 (s), 40.39 (d, *J* = 19.4 Hz), 30.63 (s), 25.64 (s), 22.72 (s), 22.46 (s).

MS (ESI) m/z: $[M + H]^+$ 342.1552.

3.11.N'-cyano-N,N',4-trimethyl-2-(3-(pyridin-4-yl)phenyl)pentanehydrazide (10′)



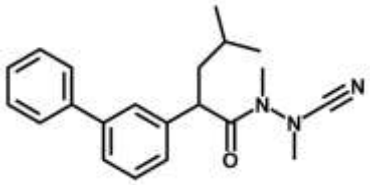
This compound was produced by using the similar procedures as for compound **5′** (**Scheme S1**), but it using compound **8′** instead of **1′** as maternal compound, and using pyridin-4-ylboronic acid instead of 2-thienylboronic acid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.67 (d, *J* = 4.4 Hz, 2H), 7.58 (s, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.51 (d, *J* = 5.0 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.38 (d, *J* = 7.4 Hz, 1H), 4.27 (t, *J* = 7.3 Hz, 1H), 3.17 (s, 4H), 2.57 (s, 2H), 2.07 – 2.02 (m, 1H), 1.67 (dt, *J* = 13.6, 8.8 Hz, 1H), 1.51 (dt, *J* = 12.9, 6.5 Hz, 1H), 0.97 (d, *J* = 6.2 Hz, 3H), 0.94 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.18 (s), 150.07 (d, *J* = 17.8 Hz), 147.40 (s), 140.62 (s), 138.50 (s), 129.56 (s), 128.60 (d, *J* = 15.2 Hz), 126.36 – 125.48 (m), 121.52 (d, *J* = 27.0 Hz), 113.80 (s), 45.80 (s), 43.56 (s), 40.38 (s), 30.38 (s), 25.57 (s), 22.49 (s), 22.35 (s).

MS (ESI) m/z: $[M + H]^+$ 337.2013.

3.12. 2-(biphenyl-3-yl)-N'-cyano-N,N',4-trimethylpentanehydrazide (11′)



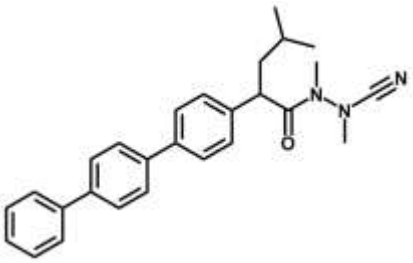
This compound was produced by using the similar procedures as for compound **5′** (**Scheme S1**), but it using compound **8′** instead of **1′** as maternal compound, and using phenylboronic acid instead of 2-thienylboronic acid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.58 (d, *J* = 7.5 Hz, 2H), 7.49 (d, *J* = 7.8 Hz, 2H), 7.44 (dd, *J* = 8.8 Hz, 3H), 7.37 (dd, *J* = 7.8 Hz, 2H), 4.20 (t, *J* = 7.3 Hz, 1H), 3.15 (s, 4H), 2.49 (s, 2H), 2.02 (dd, *J* =7.2 Hz, 1H), 1.66 (dt, *J* = 6.9 Hz, 1H), 1.50 (dd, *J* = 6.6 Hz, 1H), 0.96 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.5 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.67 – 174.65 (m), 141.88 (s), 140.52 (s), 140.27 (s), 129.35 (s), 128.89 (s), 127.64 (s), 127.35 (s), 127.10 (s), 126.84 (s), 126.53 (s), 125.96 (s), 114.04 (s), 46.46 (s), 43.61 (s), 40.39 (s), 30.65 (s), 25.63 (s), 22.74 (s), 22.47 (s).

MS (ESI) m/z: $[M + H]^+$ 336.1952.

3.13.N'-cyano-N,N',4-trimethyl-2-(4-(biphenyl-4-yl)phenyl)pentanehydrazide(12′)



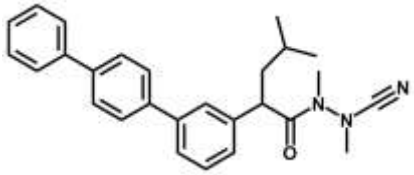
This compound was produced by using similar procedure as for compound **5′**(**Scheme S1**), but using biphenyl-4-ylboronic acid instead of 2-thienylboronic acid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.70 (d, *J* = 1.9 Hz, 1H), 7.69 (d, *J* = 2.4 Hz, 1H), 7.69 – 7.63 (m, 6H), 7.49 (t, *J* = 7.6 Hz, 2H), 7.39 (d, *J* = 3.4 Hz, 3H)，δ 4.23 (t, *J* = 7.4 Hz, 1H)，3.20 (s,4H), 2.58 (s, 2H)，δ 2.10 (m, 1H), 1.55 (ddd, *J* = 20.1, 17.8, 11.1 Hz, 2H), 1.01 – 0.96 (m, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.74 (s), 140.56 (s), 140.33 (s), 139.54 (s), 139.21 (s), 138.80 (s)，δ 128.86 (s), 128.44 (s), 127.43 (dd, *J* = 21.0, 15.7 Hz), 127.03 (s)，δ 114.05 (s)，δ 114.05 (s), 45.97 (s), 43.57 (s), 40.46 (s), 30.64 (s)，δ 25.65 (s), 22.77 (s), 22.45 (s).

MS (ESI) m/z: $[M + H]^+$ 412.2422.

3.14.N'-cyano-N,N',4-trimethyl-2-(3-(biphenyl-4-yl)phenyl)pentanehydrazide (13′)



This compound was produced by using the similar procedures as for compound **5′**(**Scheme S1**), but it using compound **8′** instead of **1′** as maternal compound, and using biphenyl-4-ylboronic acid instead of 2-thienylboronic acid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.70 (d, *J* = 3.5 Hz, 3H), 7.67 (d, *J* = 7.4 Hz, 3H), 7.57 (d, *J* = 7.7 Hz, 2H), 7.50 (t, *J* = 7.4 Hz, 2H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.30 (s, 1H)，δ 4.23 (t, *J* = 7.4 Hz, 1H)，3.20 (s,4H), 2.58 (s, 2H)，δ 2.10 (m, 1H), 1.55 (ddd, *J* = 20.1, 17.8, 11.1 Hz, 2H), 1.01 – 0.96 (m, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.67 (s), 141.35 (s), 140.56 (s), 140.36 (s), 139.37 (s), 129.43 (s), 128.88 (s), 127.62 (s), 127.48 (d, *J* = 5.3 Hz), 127.02 (d, *J* = 14.8 Hz), 126.38 (s), 125.84 (s), 114.05 (s), 46.48 (s), 43.65 (s), 40.42 (s), 30.67 (s), 25.67 (s), 22.63 (d, *J* = 33.6 Hz).

MS (ESI) m/z: $[M + H]^+$ 412.2418.

Reference

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2. T. Strzalko, L. Wartski, J. Corset, M. Castellà-Ventura, F. Froment, *J. Org. Chem.*, 2012, **77**, 6431−6442.
3. G. Kaupp, M. R. Naimi-Jamal, V. Stepanenko, *Chem. Eur. J.*, 2003, **9**, 1156-1160.