Selective targeting of lysosomal cysteine proteases with radiolabeled electrophilic substrate analogs
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Synthesis of diethyl (2R,3S)-1-bromo-3-hydroxy-butanedioate (I). A 30% solution of HBr in AcOH (35 ml) was added to diethyl-tartarate (7.2 g, 6 ml, 35 mmol) and stirred for 1 h at 0°C. The solution was allowed to warm up to room temperature and stirred overnight. Then it was poured into ice-water, and extracted with ether (3×). The combined ether layers were washed with water and brine, dried (Na2SO4), and concentrated. The remaining oil was dissolved in EOH (35 ml). A 30% solution of HBr in AcOH (21 ml) was added, and the resulting solution was refluxed for 4 h, and then concentrated in vacuo, removing most of the acetic acid by co-evaporation with toluene. The yellow oil was dissolved in ETOAc and washed with water, sat. NaHCO3, brine and dried (MgSO4). Silica column chromatography (16–25% ETOAc in hexanes) yielded I. (5.67 g, 21.1 mmol, 60%). Diethyl (2R,3S)-1-bromo-3-hydroxy-butanedioate: 1H NMR (300 MHz, CDCl3) δ 1.35 (t, 3H), 2.77 (m, 2H), 3.39 (d, 1H), 3.48 (m, 2H), 3.64 (d, 1H), 4.27 (m, 2H), 6.33 (t, 1H), 6.81 (d, 2H), 6.99 (d, 2H).

Synthesis of N-[(trans-3-carboxyoxirane-2-yl)-carbonyl]-3-methyl-butaneamide (VII). Compounds VI and VII were synthesized by removal of the ethyl ester of compounds V and IV, respectively. Ester hydrolysis was achieved by dissolving each compound in a minimal volume of ethanol followed by addition of 1 equivalent of sodium hydroxide as a 1 N aqueous solution. The resulting reaction was stirred for 2 h at room temperature at which time 1 equivalent of acetic acid was added and water added to the mixture. The water solution was washed with 3 volumes of ethyl acetate. The organic layers were combined, dried and concentrated by rotary evaporation. The crude colorless oils VI and VII were used without further purification.

Synthesis of N-[(trans-3-carboxyoxirane-2-yl)-carbonyl]-hydratide (VI) and N-[(trans-3-carboxyoxirane-2-yl)carbonyl]-3-methyl-butaneamide (VII). Compounds VI and VII were synthesized by dissolving each compound in a minimal volume of ethanol followed by addition of 1 equivalent of sodium hydroxide as a 1 N aqueous solution. The resulting reaction was stirred for 2 h at room temperature at which time 1 equivalent of acetic acid was added and water added to the mixture. The water solution was washed with 3 volumes of ethyl acetate. The organic layers were combined, dried and concentrated by rotary evaporation. The crude colorless oils VI and VII were used without further purification.

Synthesis of NH2-isoleucine–proline-OMe (IP-OMe). Boc-isoleucine (1.37 g, 13.2 mmol) and hydrobenzotriazole (HOBt; 1.95 g, 14.4 mmol) were dissolved in a minimal amount of a 1:1 (v:v) solution of DMF:CH2Cl2. Dicyclohexylcarbodiimide (DCC; 2.72 g, 13.2 mmol) was added as a solid in one portion to the stirring reaction mixture. The resulting reaction was stirred for 30 min at room temperature, the solid removed by filtration and the supernatant added to a flask containing proline methyl ester hydrochloride salt (2.0 g, 12 mmol). Disopropylethylamine (DIEA, 4.25 ml, 24 mmol) was added and the reaction stirred at room temperature for 1 h. Solvent was removed by rotary evaporation resulting in a yellow oil, which was dissolved in ethyl acetate, washed with 3× volumes of 0.1 N hydrochloric acid, 3× volumes saturated sodium carbonate, dried and re-concentrated by rotary evaporation. The crude oil was purified by flash chromatography (1:3, v:v ethyl acetate:hexanes) yielding Boc-IP-OMe as a colorless oil (3.6 g, 10.3 mmol, 84%). Boc-IP-OMe: 1H NMR (300 MHz, CDCl3) δ 0.82 (t, 3H), 1.04 (d, 3H), 1.24 (m, 2H), 1.44 (m, 9H), 1.76 (m, 1H), 2.08 (m, 2H), 2.36 (q, 2H), 2.67 (m, 2H), 2.65 (s, 3H), 3.65 (s, 3H), 4.35 (t, 1H), 4.55 (m, 1H), 5.24 (d, 1H).

Synthesis of NH2-isoleucine–proline-OMe (IP-OMe). The Boc group was removed by dissolving IP-OMe in a minimal amount of methylene chloride and adding an equal volume of trifluoroacetic acid (TFA). The reaction was stirred for 30 min at room temperature, a 2× volume of toluene added and the product concentrated by rotary evaporation. The resulting crude oil was used without further purification.

Synthesis of MB-074-OMe. Epoxy acid (71 mg, 308 µmol) was dissolved in a minimal volume of a 1:1 (v:v) mixture of methylene chloride and methanol. The reaction was quenched by dilution into diethyl ether followed by extraction with 3× volumes of brine and 0.1 N HCl. The resulting aqueous phase was dried and concentrated by rotary evaporation. The resulting crude oil was used without further purification.

Synthesis of N-[(trans-3-ethoxycarbonyloxirane-2-yl)-tyramide (V). Compound V was synthesized exactly as described for compound IV except tyramine was used instead of i-butyramine. N-[(trans-3-ethoxycarbonyloxirane-2-yl)-tyramide amide (V): 1H NMR (300 MHz, CDCl3) δ 1.35 (t, 3H), 2.77 (m, 2H), 3.39 (d, 1H), 3.48 (m, 2H), 3.64 (d, 1H), 4.27 (m, 2H), 6.33 (t, 1H), 6.81 (d, 2H), 6.99 (d, 2H).

Synthesis of N-[(trans-3-carboxyoxirane-2-yl)-carbonyl]-3-methyl-butaneamide (VII). Compounds VI and VII were synthesized by removal of the ethyl ester of compounds V and IV, respectively. Ester hydrolysis was achieved by dissolving each compound in a minimal volume of ethanol followed by addition of 1 equivalent of sodium hydroxide as a 1 N aqueous solution. The resulting reaction was stirred for 2 h at room temperature at which time 1 equivalent of acetic acid was added and water added to the mixture. The water solution was washed with 3 volumes of ethyl acetate. The organic layers were combined, dried and concentrated by rotary evaporation. The crude colorless oils VI and VII were used without further purification.

Synthesis of Boc-isoleucine-proline methyl ester (Boc-IP-OMe). Boc-isoleucine (1.37 g, 13.2 mmol) and hydrobenzotriazole (HOBt; 1.95 g, 14.4 mmol) were dissolved in a minimal amount of a 1:1 (v:v) solution of DMF:CH2Cl2. Dicyclohexylcarbodiimide (DCC; 2.72 g, 13.2 mmol) was added as a solid in one portion to the stirring reaction mixture. The resulting reaction was stirred for 30 min at room temperature, the solid removed by filtration and the supernatant added to a flask containing proline methyl ester hydrochloride salt (2.0 g, 12 mmol). Disopropylethylamine (DIEA, 4.25 ml, 24 mmol) was added and the reaction stirred at room temperature for 1 h. Solvent was removed by rotary evaporation resulting in a yellow oil, which was dissolved in ethyl acetate, washed with 3× volumes of 0.1 N hydrochloric acid, 3× volumes saturated sodium carbonate, dried and re-concentrated by rotary evaporation. The crude oil was purified by flash chromatography (1:3, v:v ethyl acetate:hexanes) yielding Boc-IP-OMe as a colorless oil (3.6 g, 10.3 mmol, 84%). Boc-IP-OMe: 1H NMR (300 MHz, CDCl3) δ 0.82 (t, 3H), 1.04 (d, 3H), 1.24 (m, 2H), 1.44 (m, 9H), 1.76 (m, 1H), 2.08 (m, 2H), 2.36 (q, 2H), 2.67 (m, 2H), 2.65 (s, 3H), 3.65 (s, 3H), 4.35 (t, 1H), 4.55 (m, 1H), 5.24 (d, 1H).

Synthesis of NH2-isoleucine–proline-OMe (IP-OMe). The Boc group was removed by dissolving IP-OMe in a minimal amount of methylene chloride and adding an equal volume of trifluoroacetic acid (TFA). The reaction was stirred for 30 min at room temperature, a 2× volume of toluene added and the product concentrated by rotary evaporation. The resulting crude oil was used without further purification.
Synthesis of CA-074b-OMe. CA-074b-OMe was synthesized exactly as described for MB-074-OMe except epoxy acid VII was used instead of epoxy acid VI. CA-074b-OMe 1H NMR (300 MHz, CDCl₃/CD₃OD) δ 0.75 (m, 6H), 1.12 (m, 3H), 1.19 (m, 2H), 1.58-1.70 (dm, 1H), 1.85 (m, 1H), 2.16 (m, 2H), 2.34 (m, 2H), 2.82-2.95 (dd, 2H), 3.16 (m, 2H), 3.38 (s, 1H), 3.58 (s, 1H), 3.63-3.92 (dm, 2H), 3.76 (s, 3H). 4.42 (m, 1H), 4.62 (t, 1H).

Synthesis of MB-074 and CA-074b. MB-074 and CA-074b were synthesized from MB-074-OMe and CA-074b by hydrolysis of the corresponding ester. Ester hydrolysis was achieved by dissolving each compound in a minimal volume of ethanol followed by addition of 1 equivalent of sodium hydroxide as a 1N aqueous solution. The resulting reaction was stirred for 2 h at room temperature or until the reaction was judged complete by TLC analysis. The reaction mixture was concentrated to dryness by rotary evaporation and the residue re-dissolved in a minimal amount of methanol. Ice cold diethyl ether was added and the product sodium salt was collected by centrifugation and washed with two portions of diethyl ether. The inhibitors were deemed pure by HPLC analysis and used directly for all further experiments. MB-074: 1H NMR (300 MHz, CDCl₃) δ 0.81 (t, 3H), 1.09 (d, 3H), 1.18 (m, 2H), 1.56 (m, 2H), 1.92 (m, 2H), 2.05 (m, 2H), 2.31 (m, 2H), 2.75-2.92 (dm, 2H), 2.83 (s, 2H), 3.19 (s, 1H), 3.42 (m, 1H), 3.58 (m, 1H), 3.77 (m, 2H), 4.32 (t, 1H), 4.42 (m, 1H), 6.36 (t, 1H), 6.79 (d, 2H), 6.99 (d, 2H). CA-074b 1H NMR (300 MHz, CDCl₃/CD₃OD) δ 0.75 (m, 6H), 1.12 (m, 3H), 1.19 (m, 2H), 1.58-1.70 (dm, 1H), 1.85 (m, 1H), 2.16 (m, 2H), 2.34 (m, 2H), 2.82-2.95 (dd, 2H), 3.16 (m, 2H), 3.38 (s, 1H), 3.58 (s, 1H), 3.63-3.92 (dm, 2H), 4.42 (m, 1H), 4.62 (t, 1H).

Figure S1