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Supplementary information

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Methylarginine targeting chimeras for lysosomal degradation of intracellular proteins

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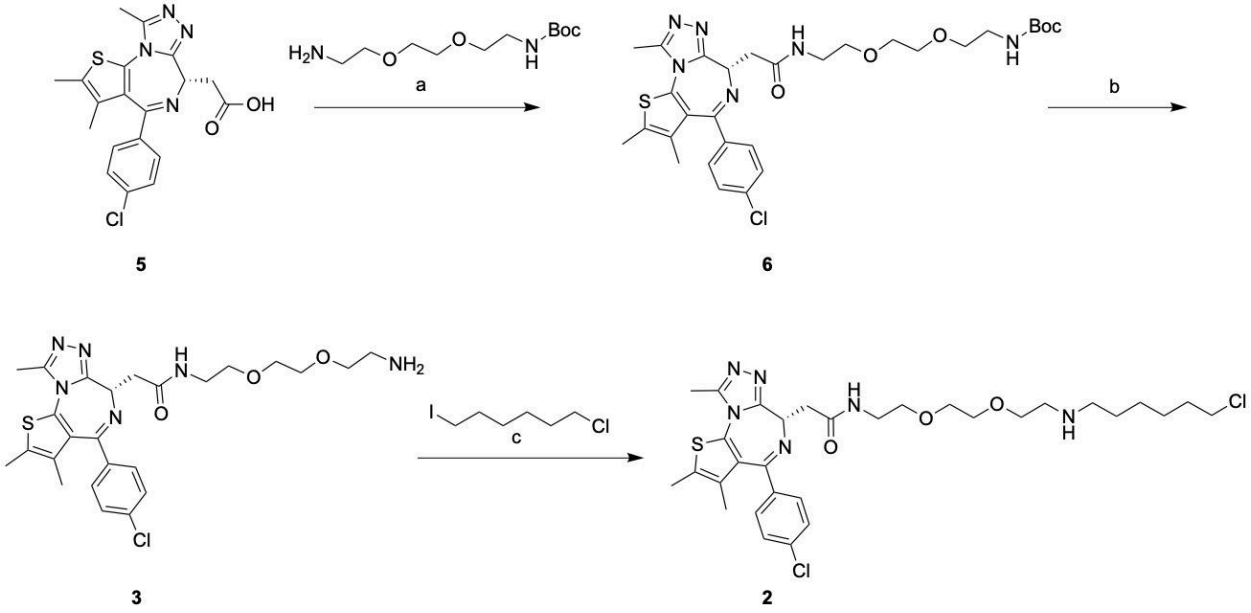
Content

Supplementary Note 1

Synthesis of MrTAC^{JQ1} (2)



Scheme 1: Reagents and Conditions: a) JQ1-COOH, PEG2, HOBT.H_2O, EDC, DIPEA, THF, rt, 12 h; **b)** TFA:DCM (95%:5%), rt, 1 h; **c)** 1-Chloro-6-Iodohexane, DIPEA, THF, rt, 12 h.

All anhydrous solvents were used under nitrogen. Purification was performed on Agilent 1260 Infinity II Analytical HPLC reverse phase with scalar (c18) column. The reactions were monitored by thin layer chromatography (TLC) and/or LCMS on Agilent 1260 Infinity II system with Single Quadrupole LC/MS System.
(S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (JQ1-COOH) was purchased from BLDpharm, 1-Chloro-6-iodohexane was purchased from Thermo Fisher Scientific. Tert-Butyl (2-(2-(2-aminoethoxy)ethoxy)ethoyl)carbamate was purchased from AmBeed.

Step a: To a stirred solution of (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (**5**, JQ1-COOH) (1 eq), HOBT.H_2O (1.5 eq.), and EDC (1.5 eq.) in THF, DIPEA (6 eq.) was added and left to stir for 15 minutes before addition of the corresponding BOC protected PEG linker. After linker addition, reaction mixture was stirred at room temperature for 12 h. Upon completion of reaction (TLC monitoring and LCMS), the reaction mixture was poured into a saturated $NaHCO_3$ solution and extracted with ethyl acetate. Organic layers washed with water and brine, dried over anhydrous sodium sulfate and solvent removed under vacuum. The crude product (**6**) was moved forward without purification.

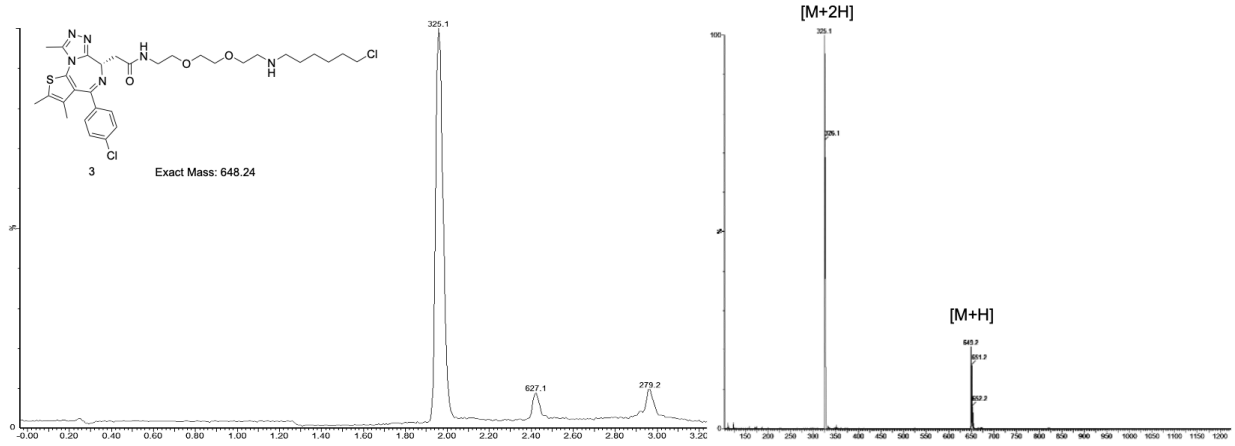
Step b: To a solution of **6** in DCM, 95% TFA was added. The reaction mixture was stirred at rt for 1 hr. After reaction completion (TLC monitoring), the reaction mixture was poured into saturated $NaHCO_3$

solution, and extracted with DCM 3x. The organic layers were washed with water and brine, dried over anhydrous sodium sulfate and the solvent removed under vacuum. Crude product (**3**) was subjected to the next step without further purification.

Step c: To a solution of **3** (1 eq) in THF, 1-Chloro-6-iodohexane (3 eq.) and DIPEA (6 eq.) was added. The reaction mixture was allowed to stir at rt for 12 hr. Upon completion of the reaction (TLC monitoring), the reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with ethyl acetate. Organic layers washed with water and brine, dried over anhydrous sodium sulfate and solvent removed under vacuum. The crude product (**2**) was purified by HPLC with a scalar (c18) column.

Characterization (HR-MS) of new chemical entity:

HRMS of MrTAC^{JQ1} (2)



HRMS: Theoretical $[M+H]^+$ = 649.2495, Acceptable: +/- 3.0 mDa; Observed $[M+H]^+$ = 649.2488, -0.7 mDa.