

Structure-activity relationships study of psoralen derivatives as inhibitors of *Mycobacterium tuberculosis* proteasome

Kaja Rožman ^{1,2}, Evan M. Alexander ², Eva Ogorevc ¹, Krištof Bozovičar ¹, Izidor Sosič ¹, Courtney C. Aldrich ² and Stanislav Gobec ^{1,*}

Supplementary data

Contents

1. Protein expression and isolation	2
2. Enzymatic assay	2
3. Determination of K_i and K_m values	11
a. Bortezomib.....	11
b. PR-957.....	12
c. Compound 8.....	14
d. Compound 11.....	16
e. Compound 13.....	17
f. Compound 15.....	19
g. Determination of K_m value for Suc-LLVY-AMC.....	20
4. Dilution assay	21
a. Bortezomib.....	21
b. PR-957.....	22
c. Compound 8.....	23
d. Compound 11.....	24
e. Compound 13.....	25
f. Compound 15.....	26
5. Analytic and synthetic data for compounds.....	Error! Bookmark not defined.

1. Protein expression and isolation

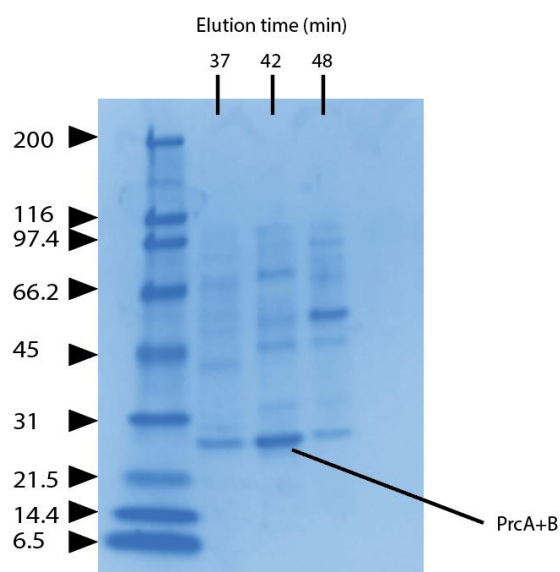
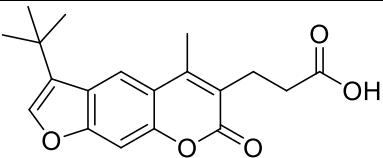
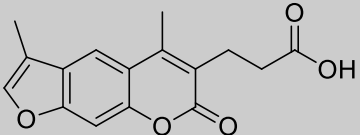
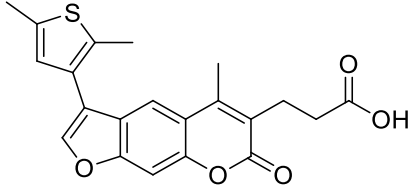
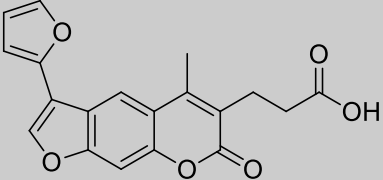
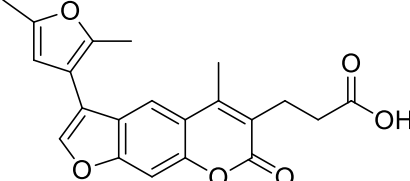
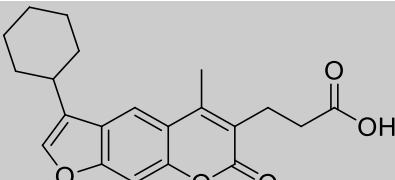
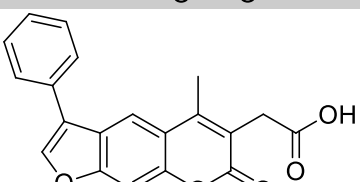
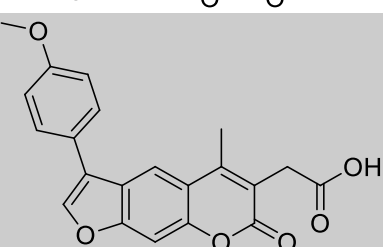
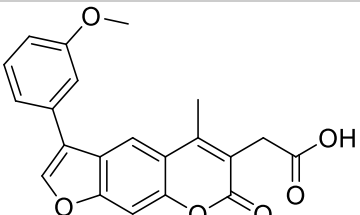


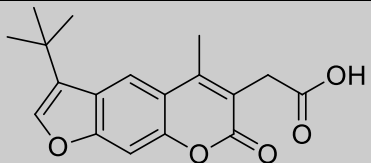
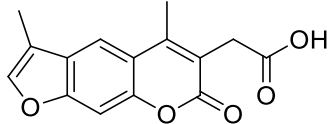
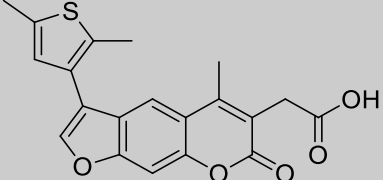
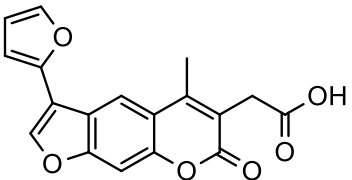
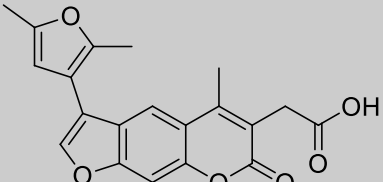
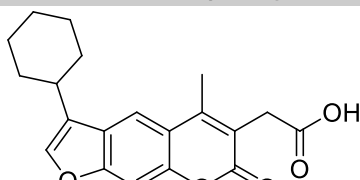
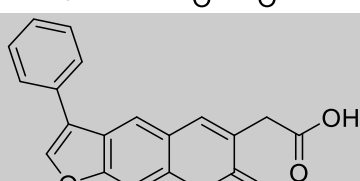
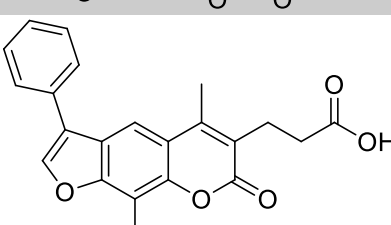
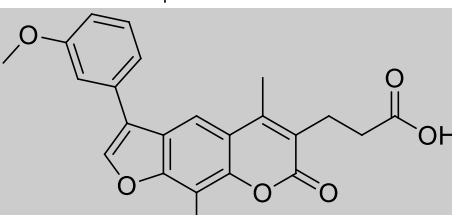
Figure S1. Gel image of purified Mtb proteasome with a clear band at 26-27 kDa representing processed α and β subunits.

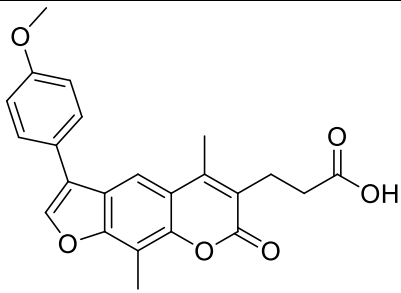
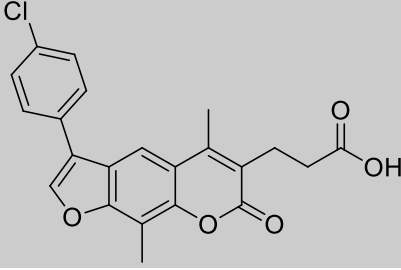
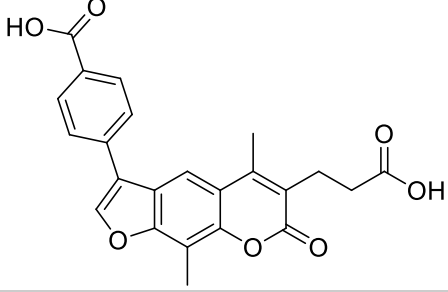
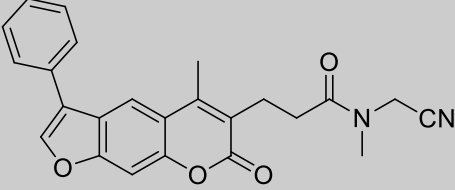
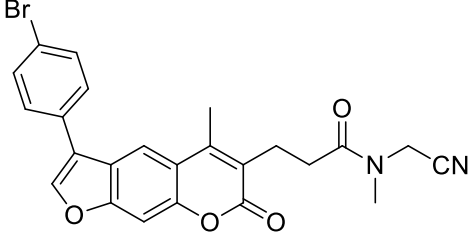
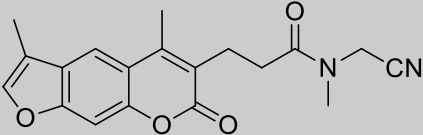
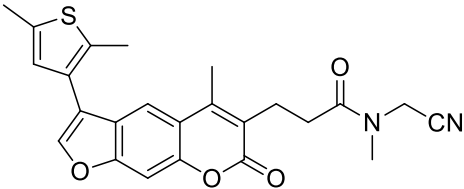
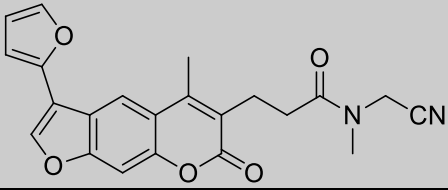
2. Enzymatic assay

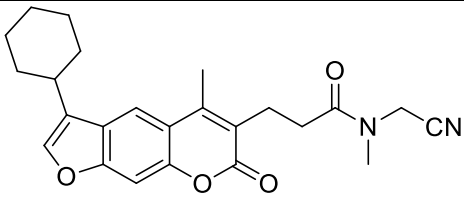
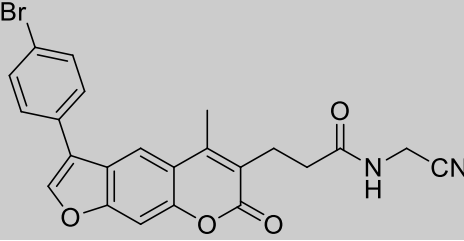
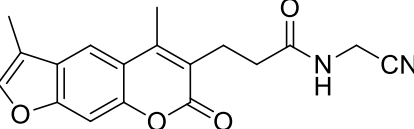
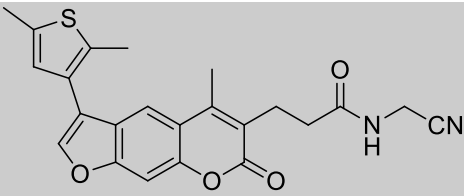
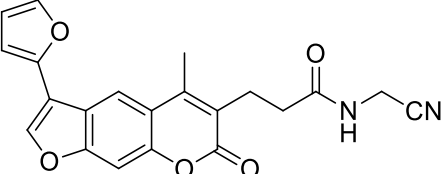
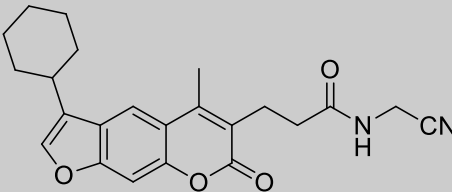
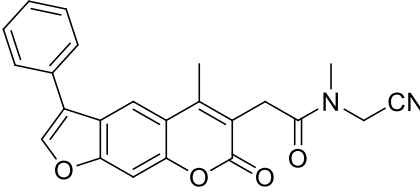
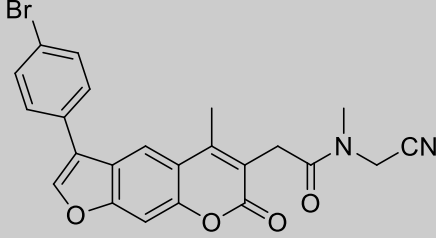
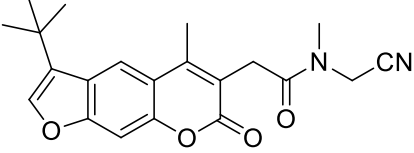
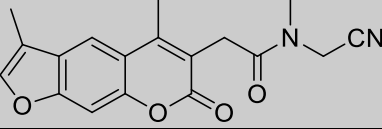
Table S1. Chemical structures, molecular weights and residual activities of inactive psoralens.

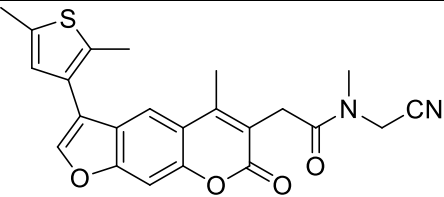
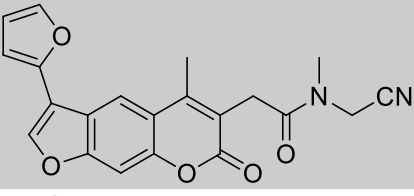
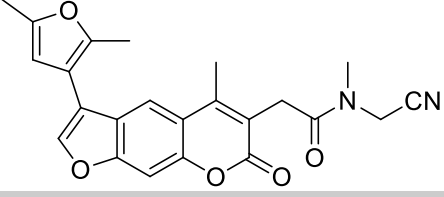
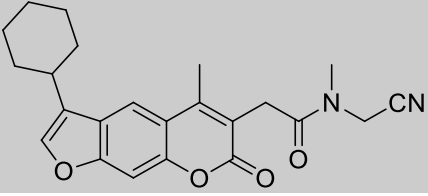
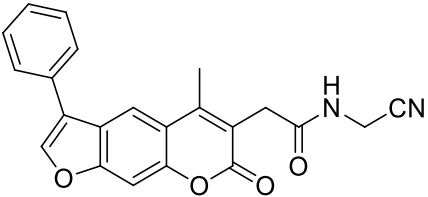
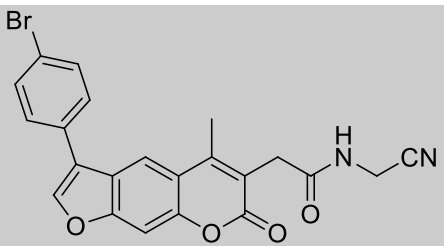
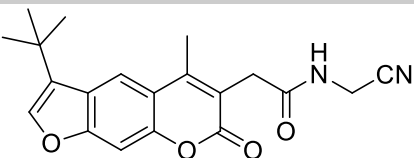
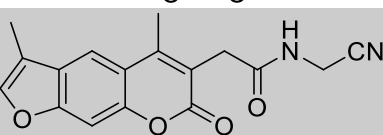
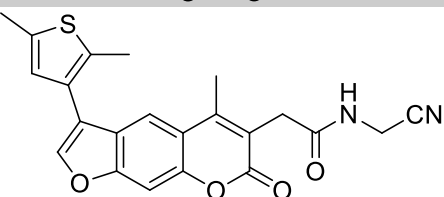
Compound	Chemical structure	M (g/mol)	RA ^a at 100 μ M against <i>Mtb</i> (%)	RA ^a at 10 μ M (%) or K_i or IC_{50} (μ M) against $\beta 5i$ of IP ^b
16		348.35	72%	$K_i = 1.6 \pm 0.7 \mu$ M
17		378.37	100%	84%
18		378.37	100%	$K_i = 29.6 \pm 3.9 \mu$ M

19		328.36	95%	83%
20		286.28	93%	94%
21		382.43	99%	$K_i = 52.3 \pm 18 \mu\text{M}$
22		338.31	55%	$K_i = 116 \pm 27 \mu\text{M}$
23		366.36	76%	100%
24		354.40	71%	102%
25		334.32	96%	$K_i = 2.4 \pm 0.4 \mu\text{M}$
26		364.35	50%	$K_i = 8.2 \pm 0.5 \mu\text{M}$
27		364.35	98%	$K_i = 7.0 \pm 0.2$

28		314.34	97%	85%
29		272.26	61%	88%
30		368.40	93%	$K_i = 36.5 \pm 7.1 \mu\text{M}$
31		324.28	79%	$K_i = 89.3 \pm 2.0 \mu\text{M}$
32		352.34	100%	$K_i = 351 \pm 48 \mu\text{M}$
33		340.37	90%	$K_i = 198 \pm 21 \mu\text{M}$
34		320.30	54%	$K_i = 3.8 \pm 1.1 \mu\text{M}$
35		362.38	100%	$K_i = 43.1 \pm 15 \mu\text{M}$
36		392.40	87%	72%

37		392.40	87%	87%
38		396.82	47%	89%
39		406.38	98%	ND
40		400.43	100%	92%
41		479.32	91%	107%
42		338.36	99%	93%
43		434.51	55%	107%
44		390.39	82%	86%

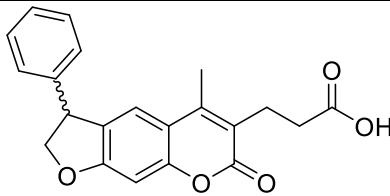
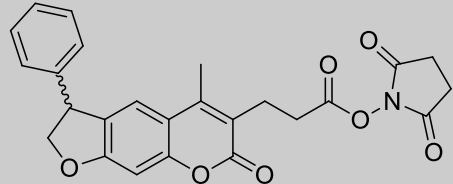
45		406.47	92%	111%
46		465.30	82%	107%
47		324.33	95%	105%
48		420.48	100%	109%
49		376.36	100%	97%
50		392.45	96%	102%
51		386.40	93%	97%
52		465.30	70%	86%
53		366.41	97%	96%
54		324.34	95%	99%

55		420.48	88%	103%
56		376.36	53%	103%
57		404.42	100%	96%
58		392.45	100%	101%
59		372.37	92%	101%
60		451.27	87%	105%
61		352.38	92%	91%
62		310.31	60%	ND
63		406.45	95%	101%

64		362.34	91%	98%
65		378.42	91%	99%
66		445.42	66%	$IC_{50} = 0.009 \pm 0.002 \mu M$
67		524.32	96%	$IC_{50} = 1.9 \pm 0.9 \mu M$
68		475.45	93%	$IC_{50} = 0.64 \pm 0.13 \mu M$
69		475.45	72%	$IC_{50} = 0.93 \pm 0.57 \mu M$
70		425.43	70%	41%
71		383.35	97%	66%
72		479.50	95%	$IC_{50} = 1.8 \pm 0.8 \mu M$

73		431.29	79%	$IC_{50} = 6.36 \pm 0.61 \mu M$
74		510.29	61%	101%
75		411.40	88%	53%
76		369.33	53%	101%
77		421.36	56%	44%
78		437.44	98%	62%
79		459.45	100%	$IC_{50} = 3.26 \pm 1.49 \mu M$
80		305.33	55%	ND
81		372.38	100%	101%

82		359.37	68%	90%
83		373.40	52%	116%
84		291.31	100%	88%
85		358.35	93%	115%
86		345.35	95%	$IC_{50} = 20.51 \pm 1.36 \mu M$
87		359.37	93%	102%
88		377.37	84%	$IC_{50} = 0.013 \pm 0.004 \mu M$
89		391.40	65%	$IC_{50} = 0.12 \pm 0.05 \mu M$
90		405.42	69%	$IC_{50} = 0.20 \pm 0.04 \mu M$

91		350.36	95%	94%
92		447.44	95%	36%

^a The data were calculated as residual activities (RAs) in the presence of 10 μM (for $\beta 5\text{i}$) or 100 μM (for *Mtb* proteasome) of each compound (standard errors for RAs were <15%). ^b Published previously in: Sosič, I.; Gobec, M.; Brus, B.; Knez, D.; Živec, M.; Konc, J.; Lešnik, S.; Ogrizek, M.; Obreza, A.; Žigon, D.; Janežič, D.; Mlinarič-Raščan, I.; Gobec, S., Nonpeptidic selective inhibitors of the chymotrypsin-like ($\beta 5\text{i}$) subunit of the immunoproteasome. *Angew Chem Int Ed Engl* **2016**, *55* (19), 5745-8; Shannon Schiffrer, E.; Sosič, I.; Šterman, A.; Mravljak, J.; Mlinarič-Raščan, I.; Gobec, S.; Gobec, M., A focused structure–activity relationship study of psoralen-based immunoproteasome inhibitors. *Med Chem Commun* **2019**, *10* (11), 1958-65. ND, not determined.

3. Determination of K_i and K_m values

K_m for Suc-LLVY-AMC of $60 \pm 15 \mu\text{M}$ was determined (See *Material and Methods* for details).

Michaelis-Menten kinetics – plot of reaction rate (v) *vs* substrate concentration [S]

Dixon plot – plot of the reciprocal of the reaction rate ($1/v$) *vs* substrate concentrations [S]

Lineweaver – Burk plot – plot of the reciprocal of the reaction rate ($1/v$) *vs* the reciprocal of the substrate concentration [S]

a. Bortezomib

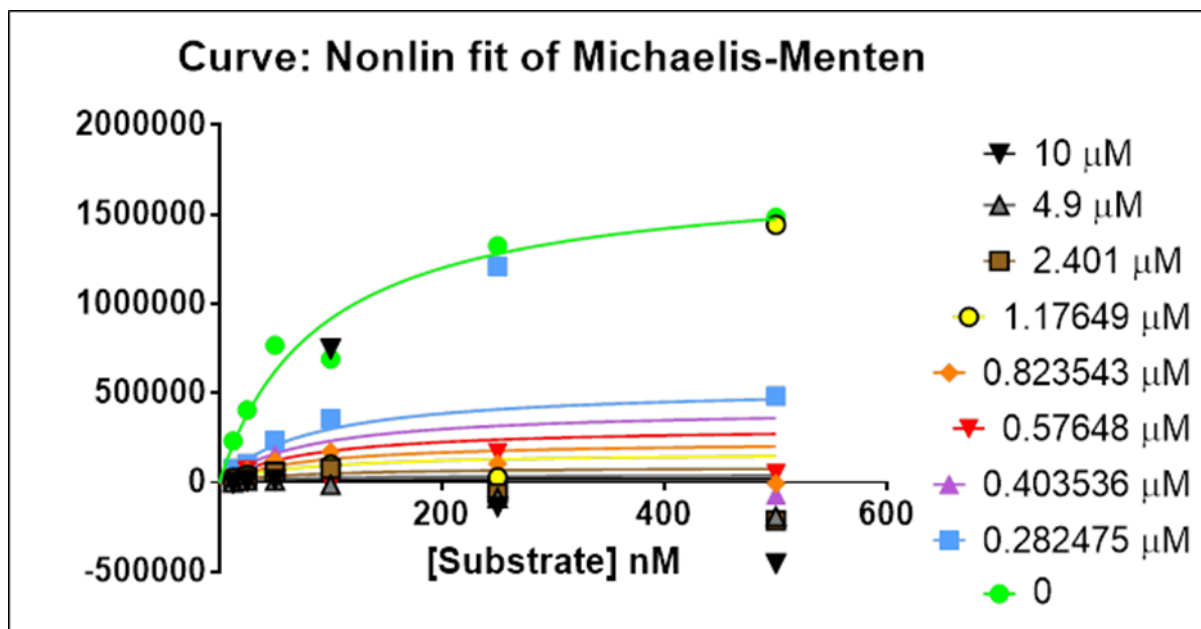


Figure S2. Michaelis-Menten plot for bortezomib based on enzyme activity (10 nM) at various concentrations of the compound (written on the right) and various concentrations of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

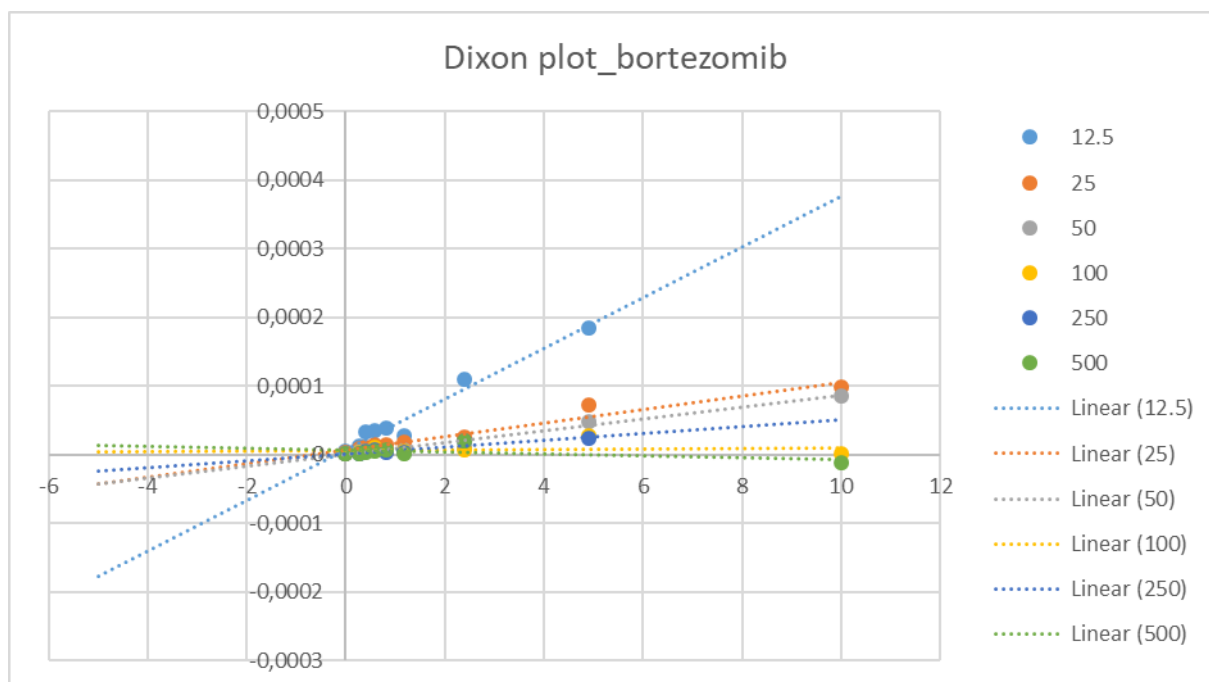


Figure S3. Dixon plot for bortezomib based on enzyme activity (10 nM) at various concentration of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

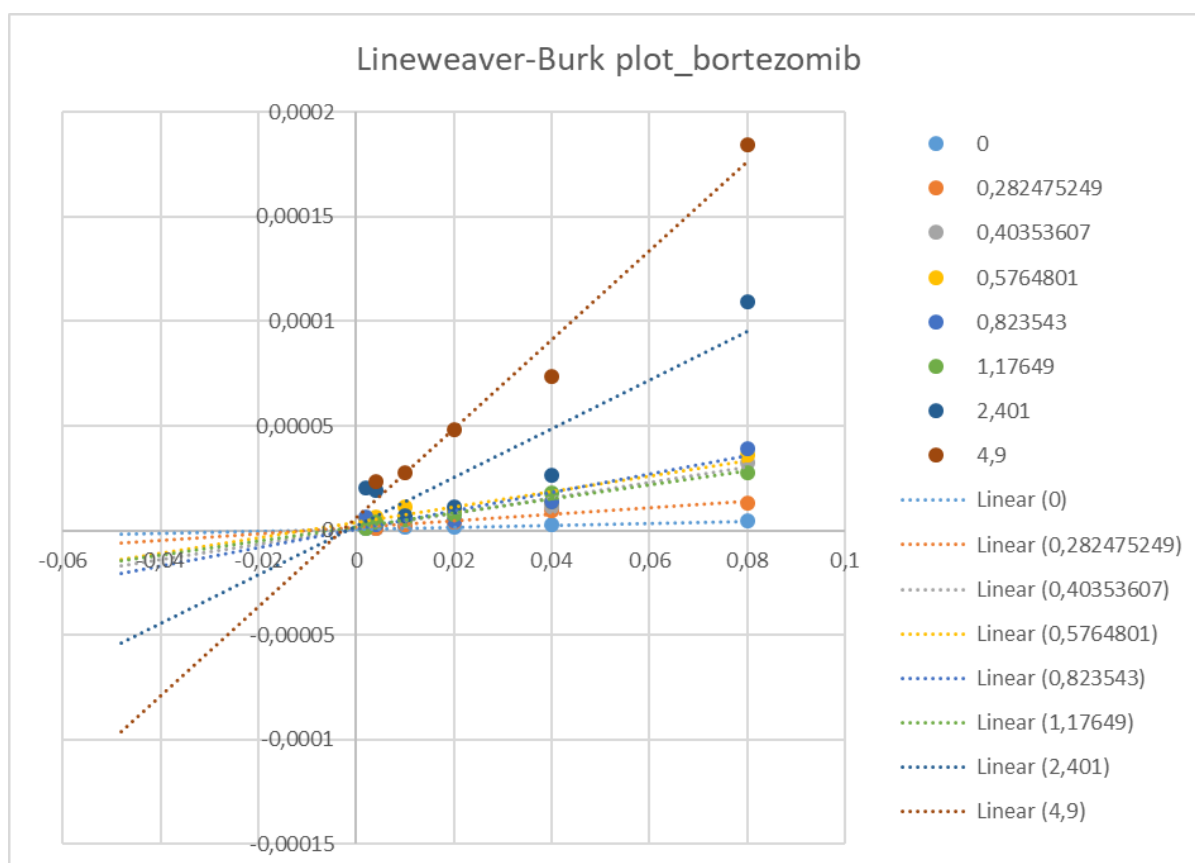


Figure S4. Lineweaver-Burk plot for bortezomib based on enzyme activity (10 nM) at various concentrations of the compound (written on the right).

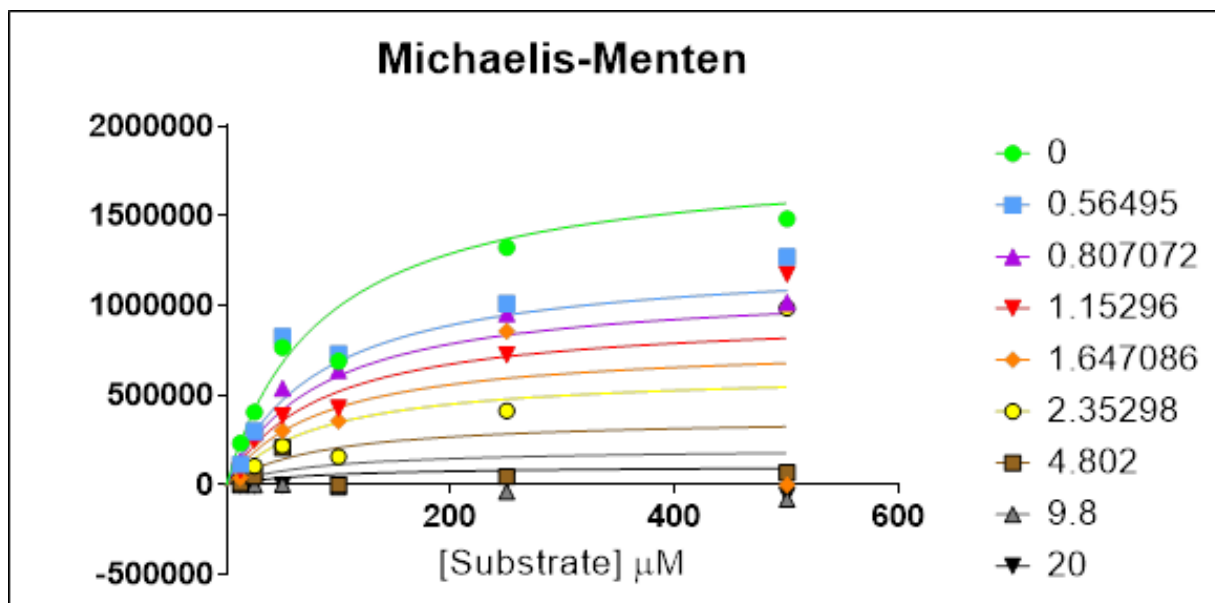


Figure S5. Michaelis-Menten plot for compound **PR-957** based on enzyme activity (10 nM) at various concentrations of the compound (written on the right) and various concentrations of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

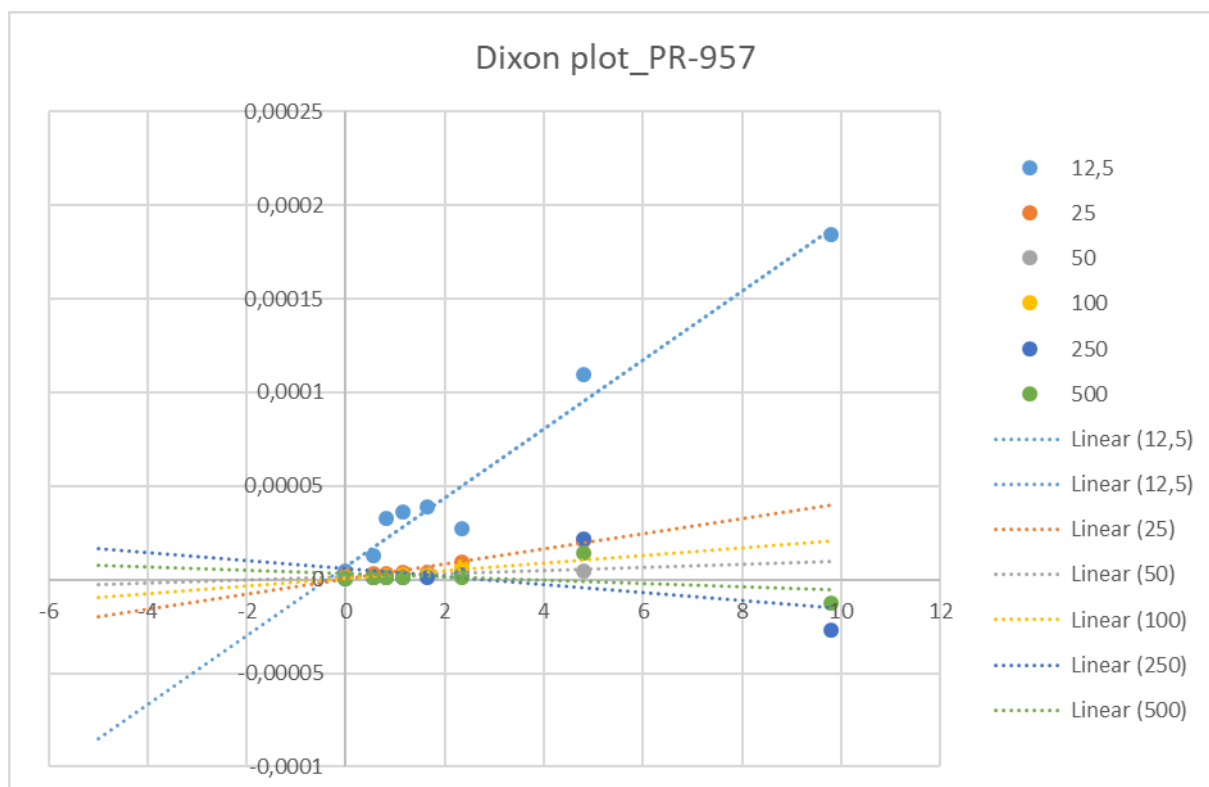


Figure S6. Dixon plot for compound **PR-957** based on enzyme activity (10 nM) at various concentration of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

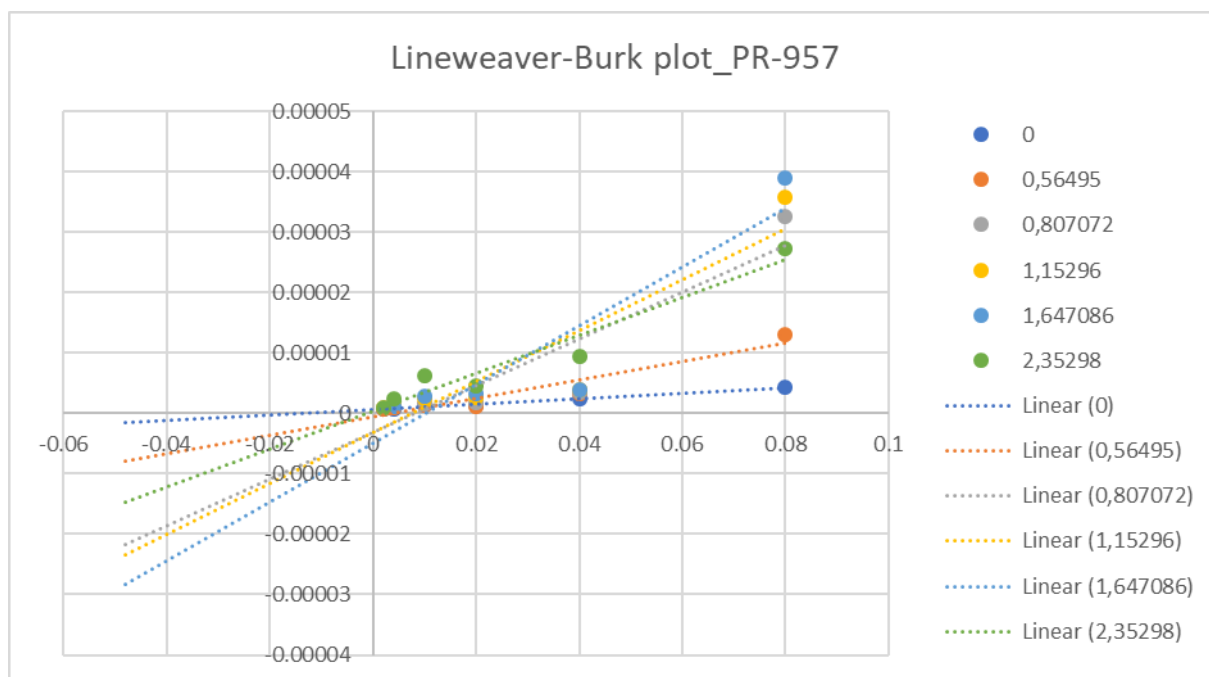


Figure S7. Lineweaver-Burk plot for compound **PR-957** based on enzyme activity (10 nM) at various concentrations of the compound (written on the right).

c. Compound 8

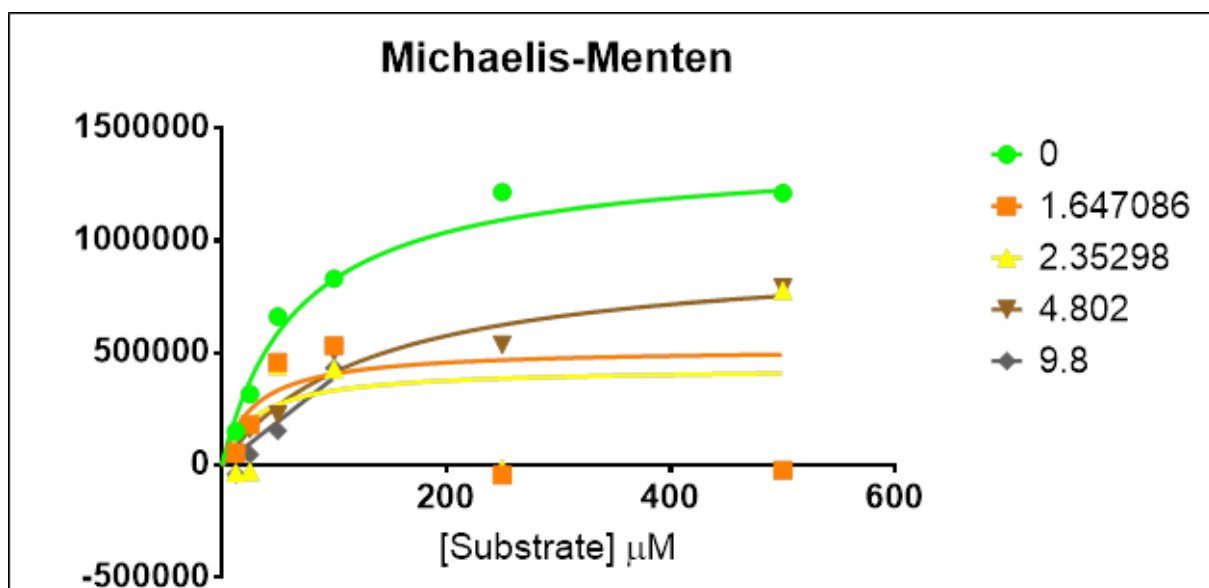


Figure S8. Michaelis-Menten plot for compound **8** based on enzyme activity (10 nM) at various concentrations of the compound (written on the right) and various concentrations of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

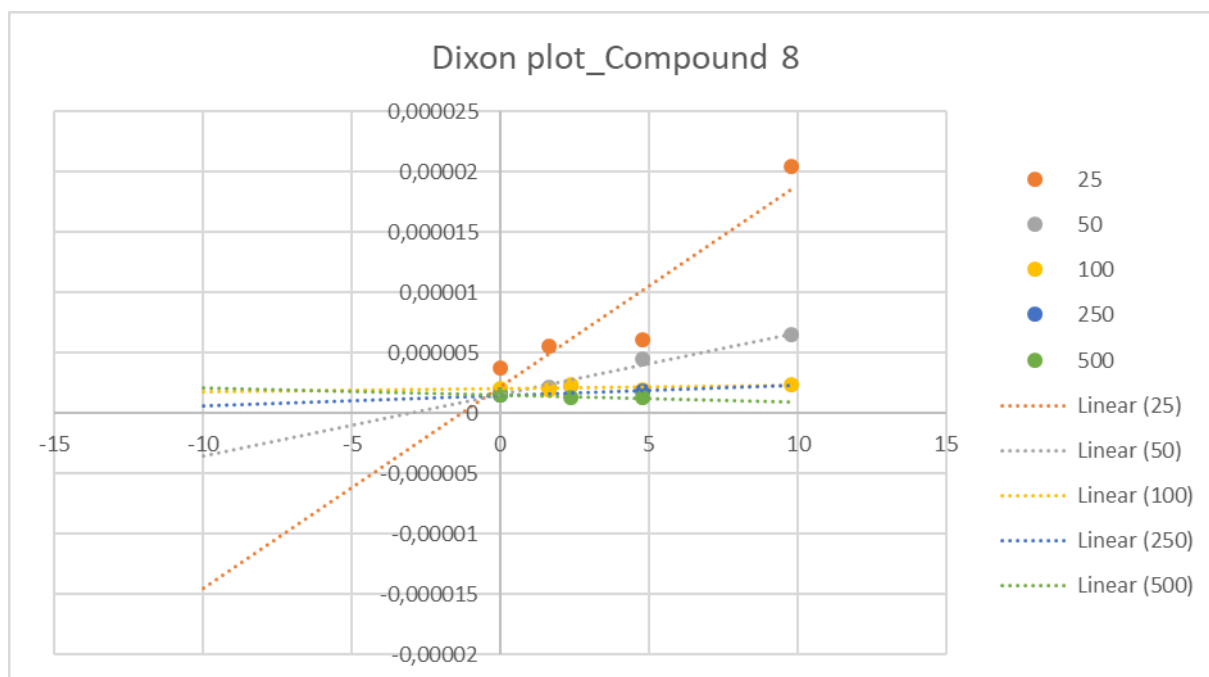


Figure S9. Dixon plot for compound 8 based on enzyme activity (10 nM) at various concentration of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

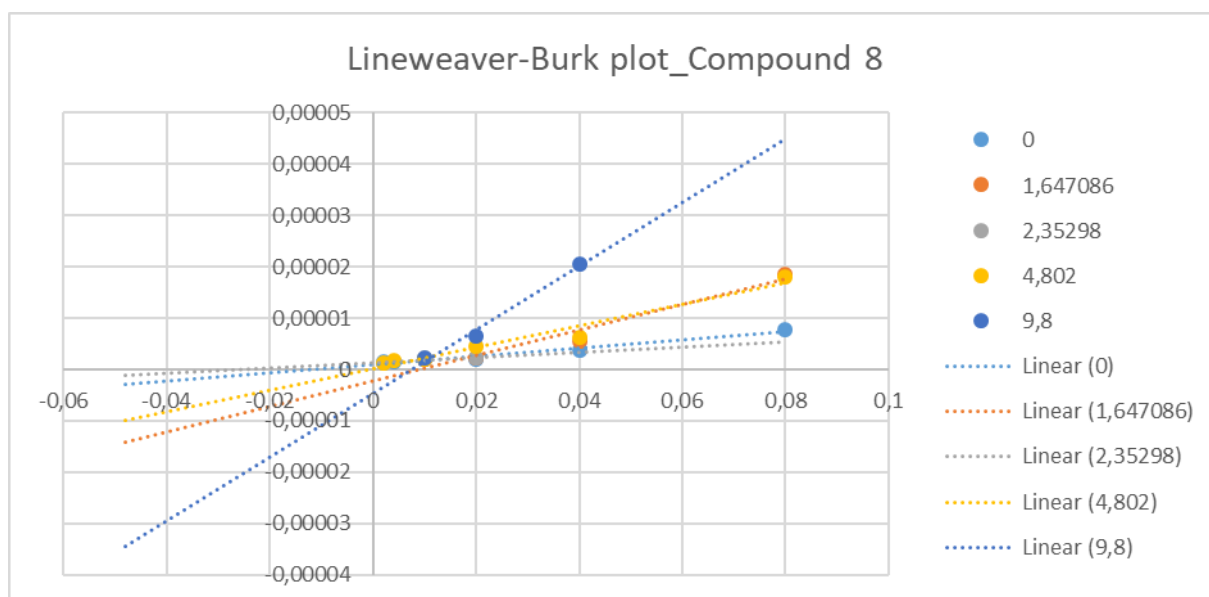


Figure S10. Lineweaver-Burk plot for compound 8 based on enzyme activity (10 nM) at various concentrations of the compound (written on the right).

d. Compound 11

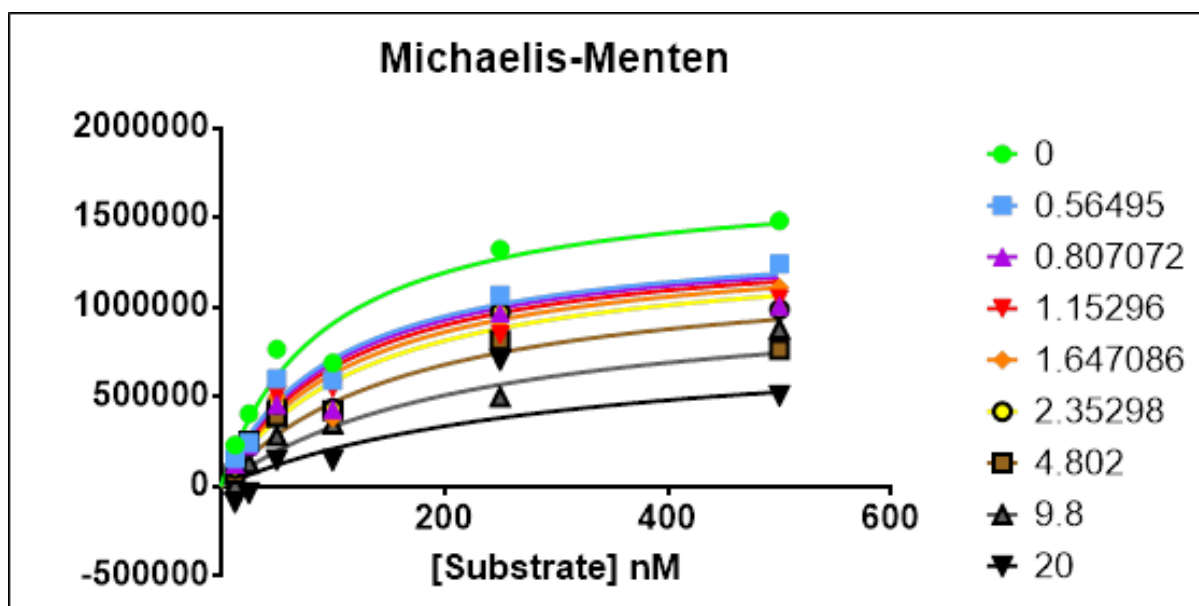


Figure S11. Michaelis-Menten plot for compound 11 based on enzyme activity (10 nM) at various concentrations of the compound (written on the right) and various concentrations of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

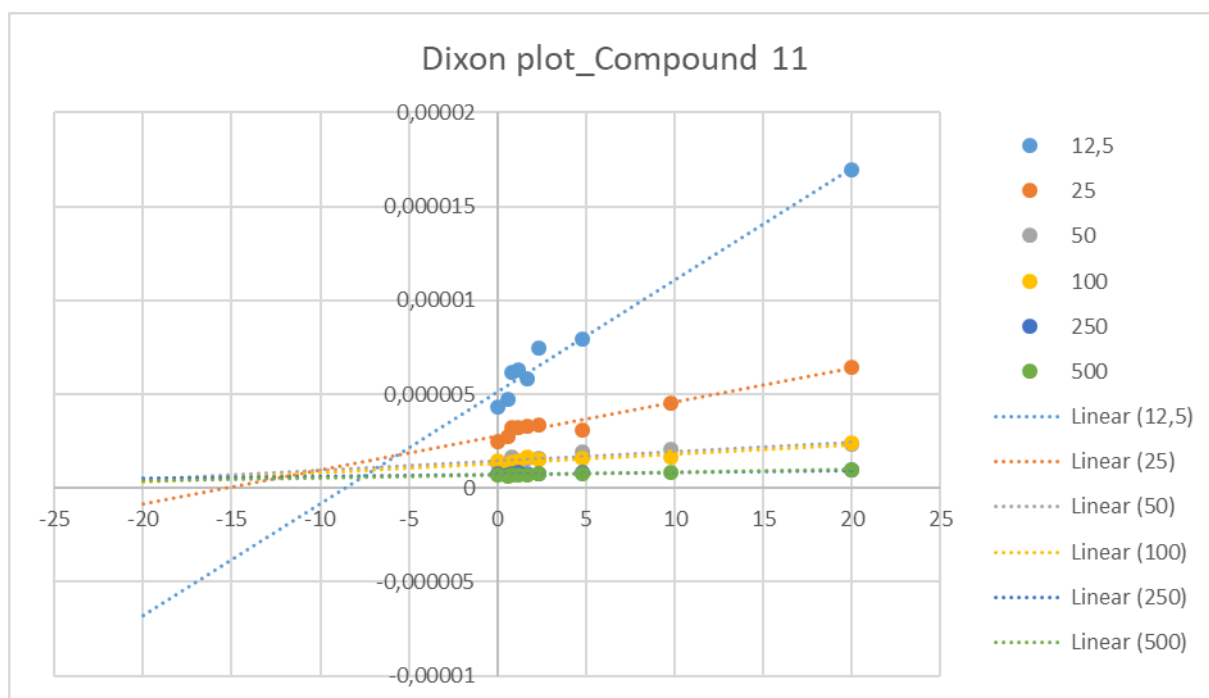


Figure S12. Dixon plot for compound 11 at various concentration of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

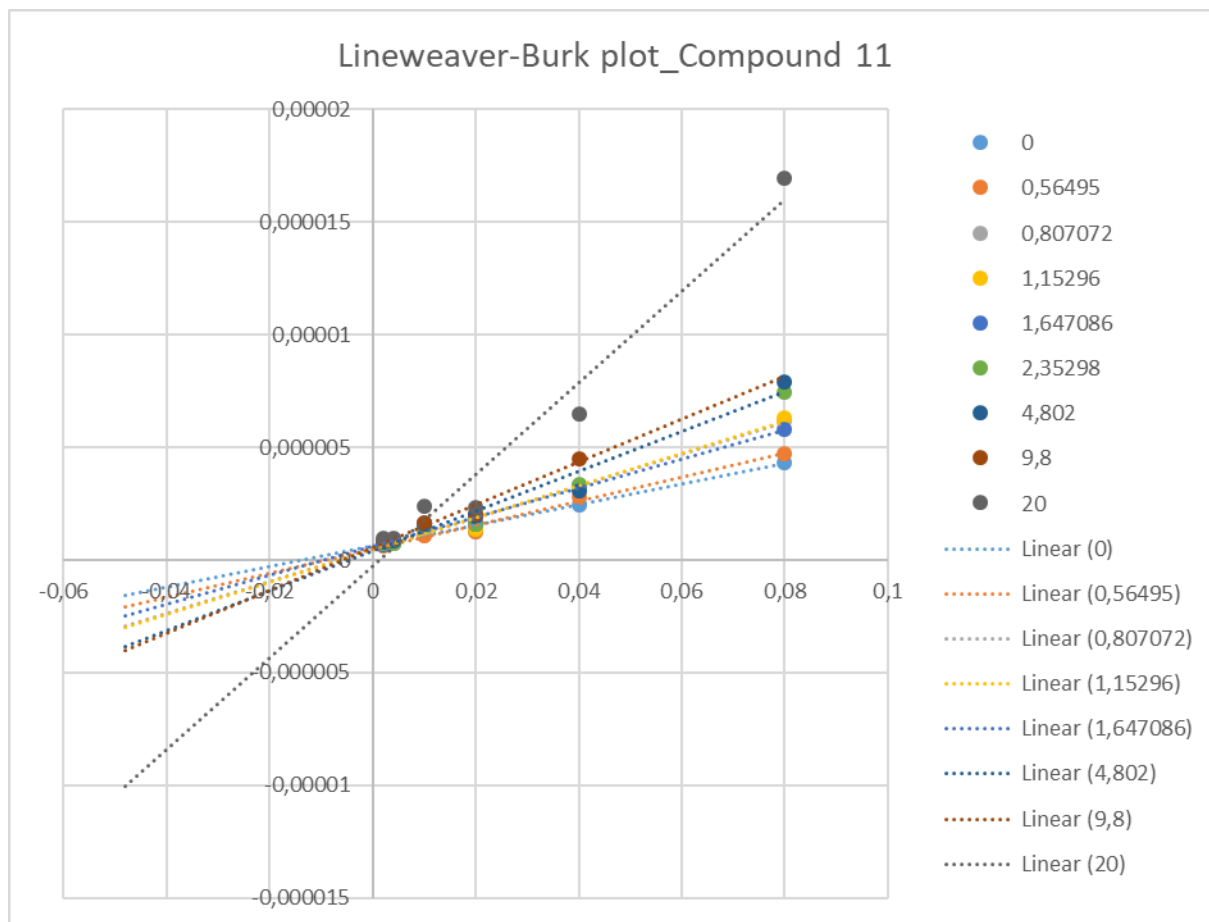


Figure S13. Lineweaver-Burk plot for compound **11** at various concentrations of the compound (written on the right).

e. Compound 13

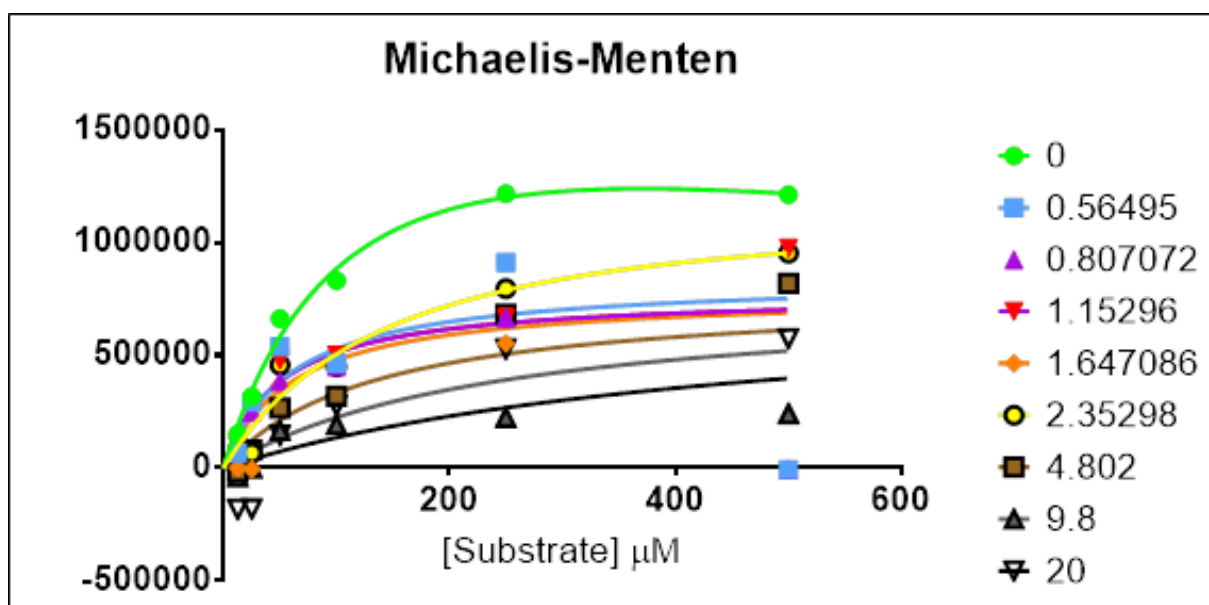


Figure S14. Michaelis-Menten plot for compound **13** based on enzyme activity (10 nM) at various concentrations of the compound (written on the right) and various concentrations of the substrate Suc-LLVY-AMC AMC (12.5, 25, 50, 100, 250 and 500 μM).

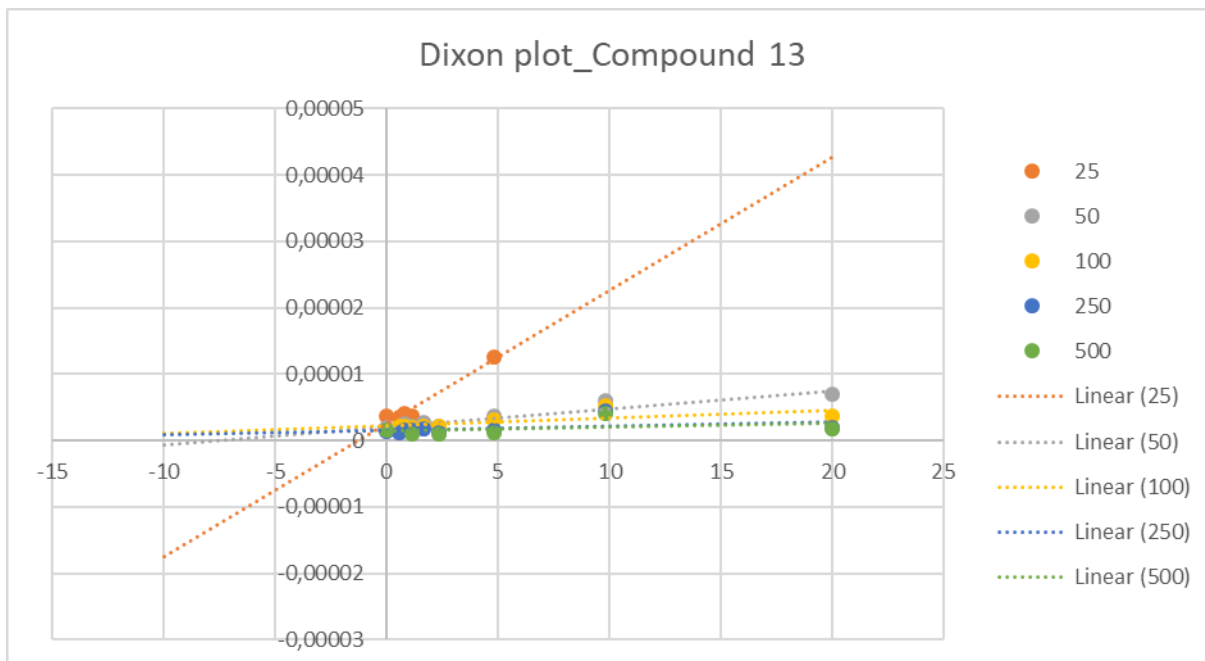


Figure S15. Dixon plot for compound 13 at various concentration of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

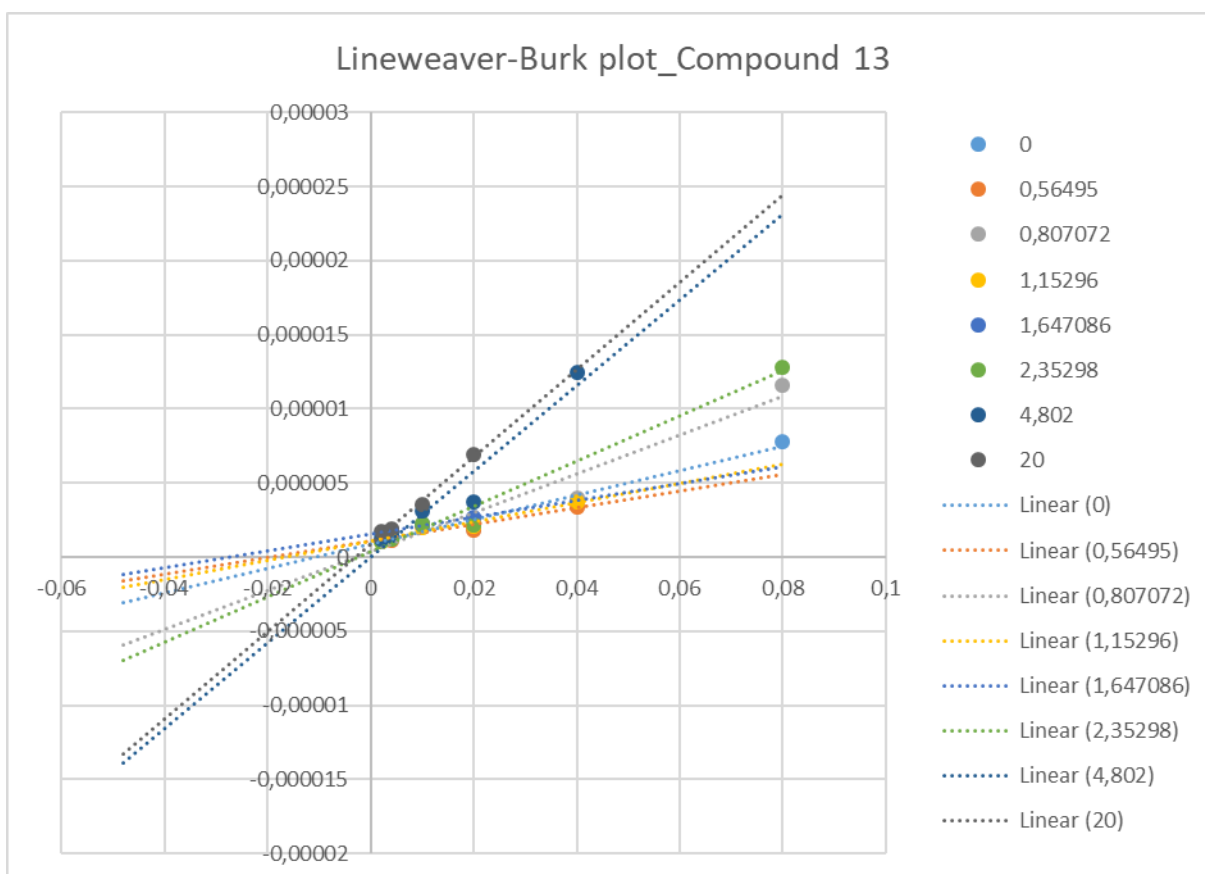


Figure S16. Lineweaver-Burk plot for compound 13 at various concentrations of the compound (written on the right).

f. Compound 15

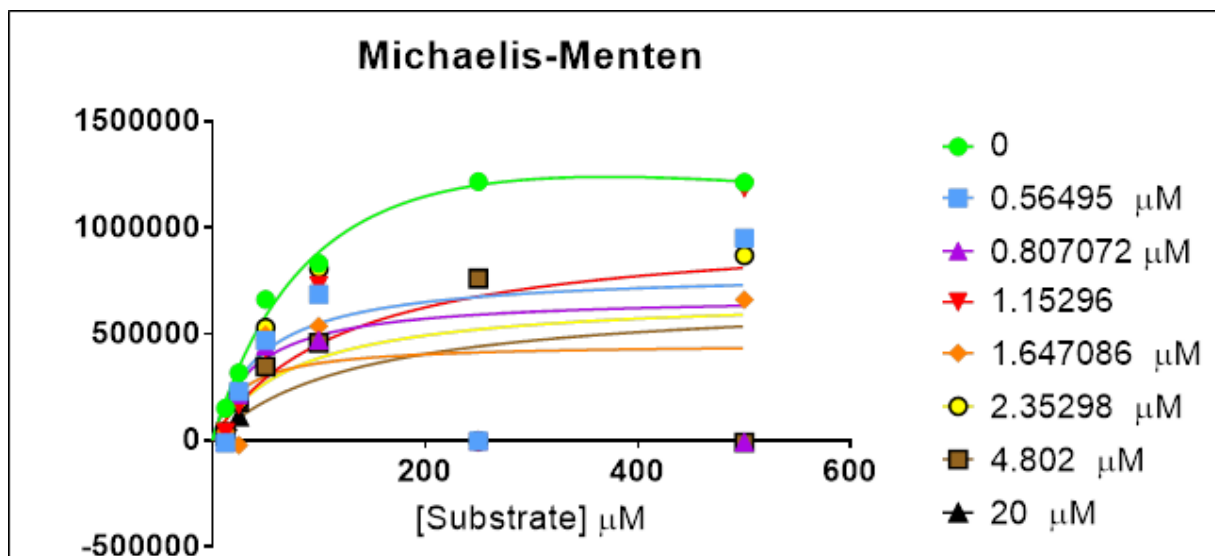


Figure S17. Michaelis-Menten plot for compound **15** based on enzyme activity (10 nM) at various concentrations of the compound (written on the right) and various concentrations of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

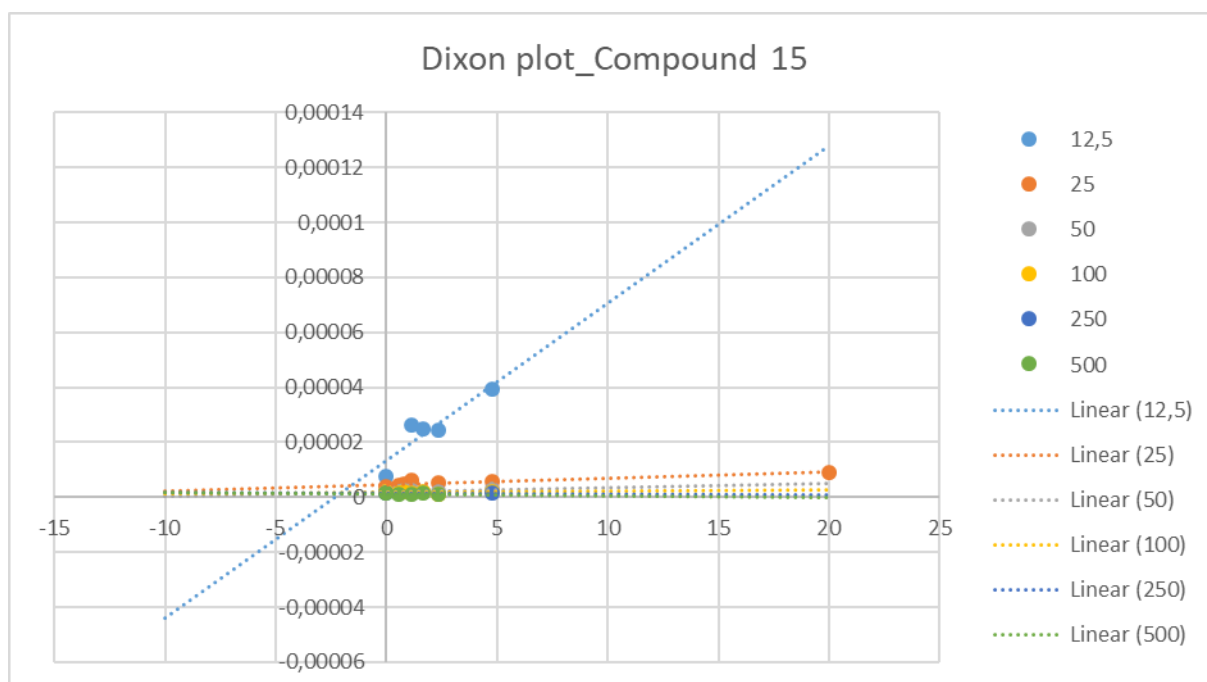


Figure S18. Dixon plot for compound **15** at various concentration of the substrate Suc-LLVY-AMC (12.5, 25, 50, 100, 250 and 500 μM).

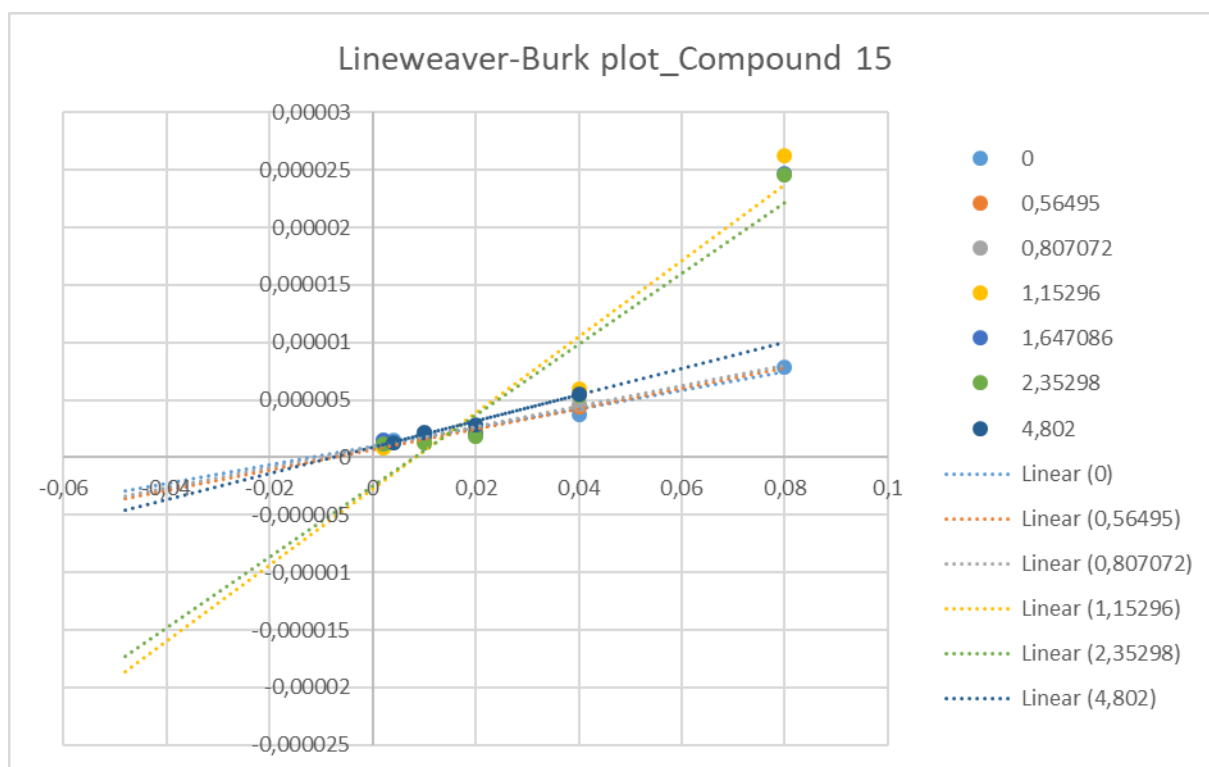


Figure S19. Lineweaver-Burk plot for compound 15 at various concentrations of the compound (written on the right).

g. Determination of K_m value for Suc-LLVY-AMC

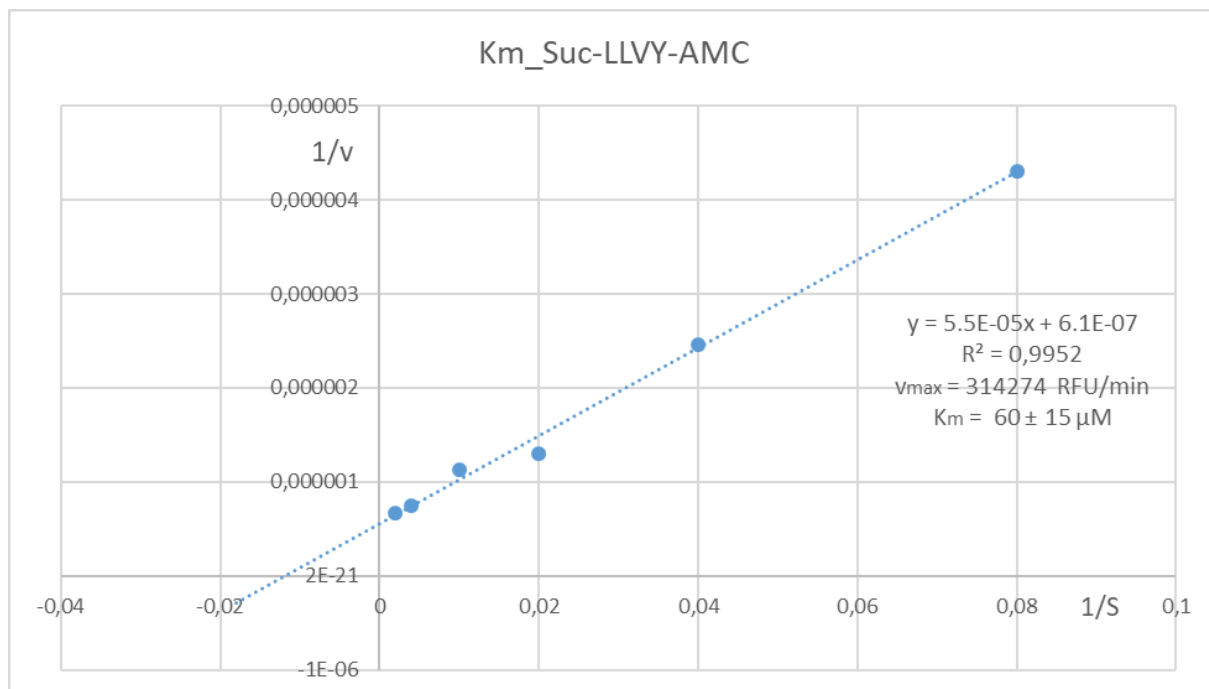


Figure S20. Lineweaver-Burk plot showing the increase in initial velocity for Mtb proteasome catalytic reaction in the presence of increasing concentration of the substrate Suc-LLVY-AMC.

4. Dilution assay

a. Bortezomib

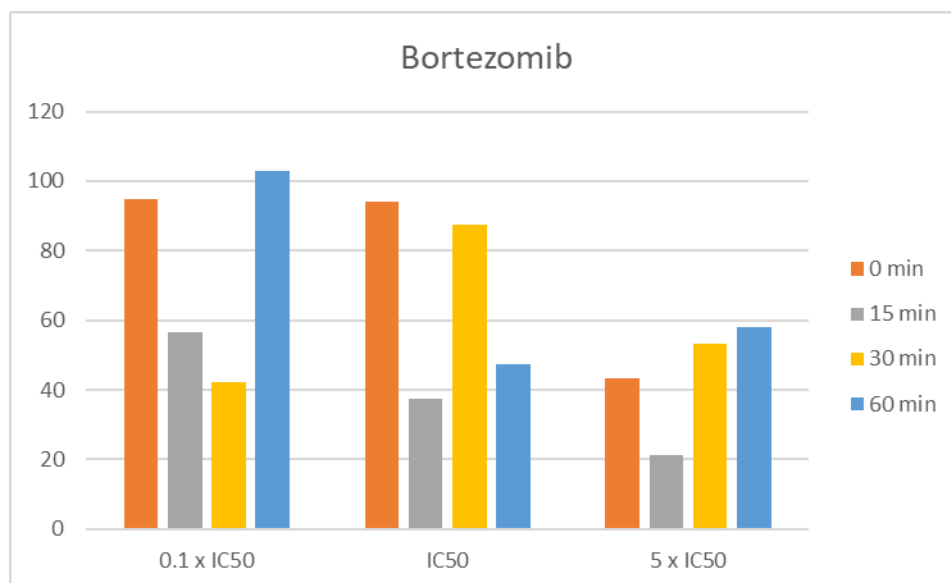


Figure S21. Restored activity (%) of Mtb proteasome after preincubation of enzyme with bortezomib at various concentrations (0.1 x IC₅₀, IC₅₀ and 5 x IC₅₀) for various amounts of time (0, 15, 30 and 60 min).

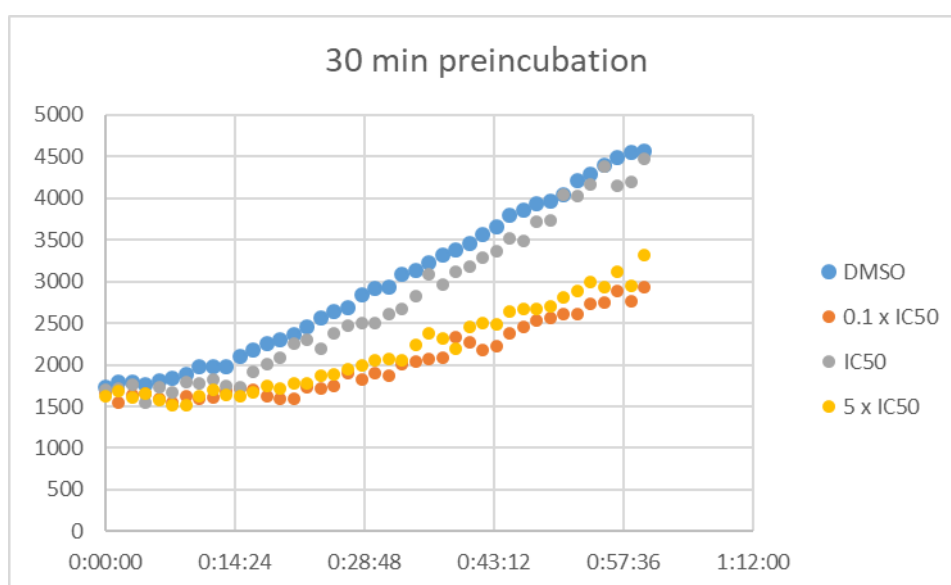


Figure S22. Restored activity (RFU) of Mtb proteasome after 30-min preincubation of enzyme with bortezomib at various concentrations (0.1 x IC₅₀, IC₅₀ and 5 x IC₅₀).

b. PR-957

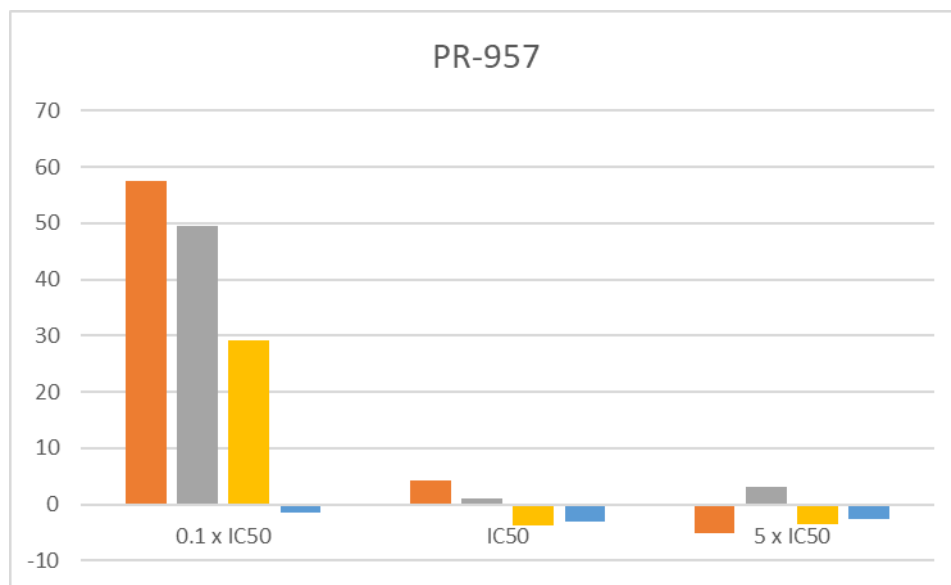


Figure S23. Restored activity (%) of Mtb proteasome after preincubation of enzyme with **PR-957** at various concentrations (0.1 x IC₅₀, IC₅₀ and 5 x IC₅₀) for various amounts of time (0, 15, 30 and 60 min).

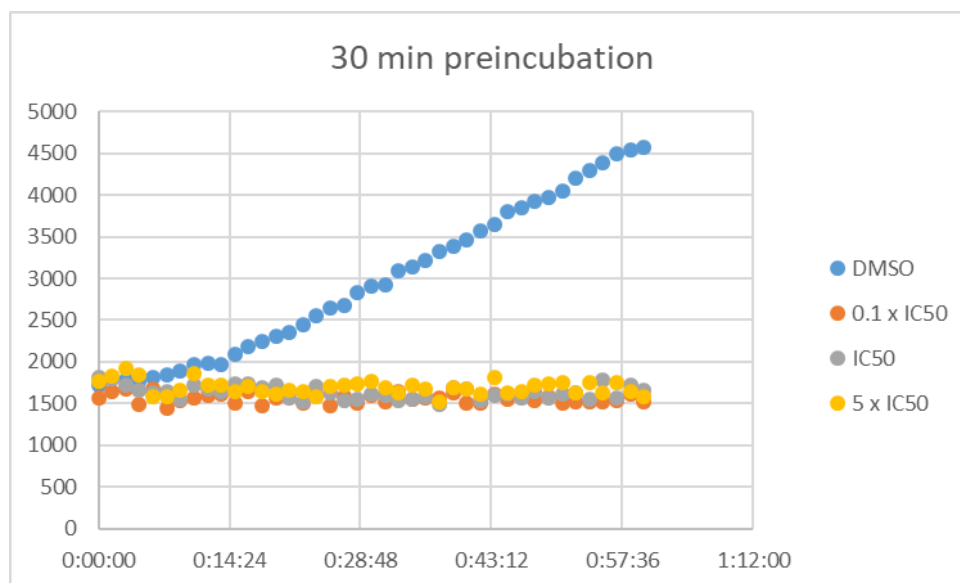


Figure S24. Restored activity (RFU) of Mtb proteasome after 30-min preincubation of enzyme with **PR-957** at various concentrations (0.1 x IC₅₀, IC₅₀ and 5 x IC₅₀).

c. Compound 8

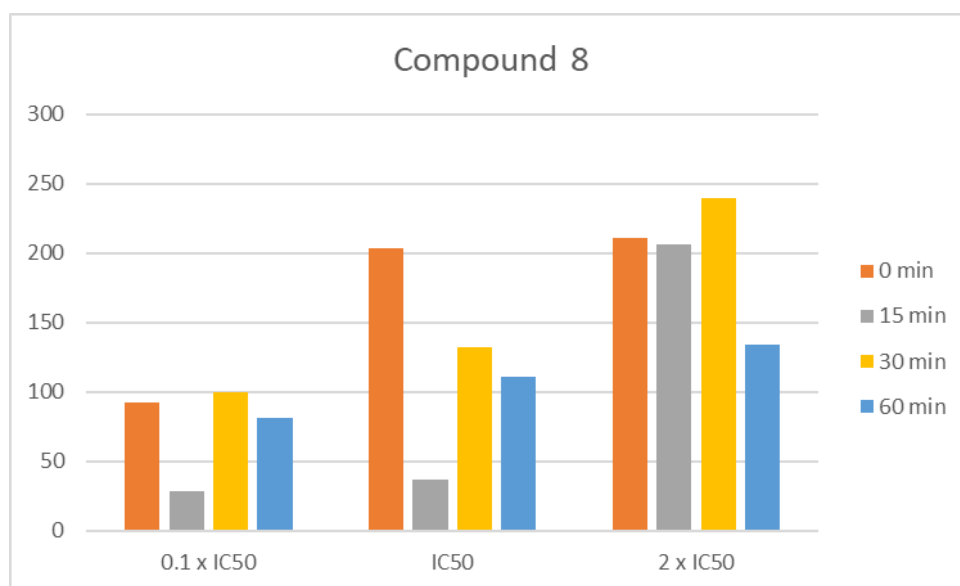


Figure S25. Restored activity (%) of Mtb proteasome after preincubation of enzyme with compound 8 at various concentrations (0.1 x IC₅₀, IC₅₀ and 2 x IC₅₀) for various amounts of time (0, 15, 30 and 60 min).

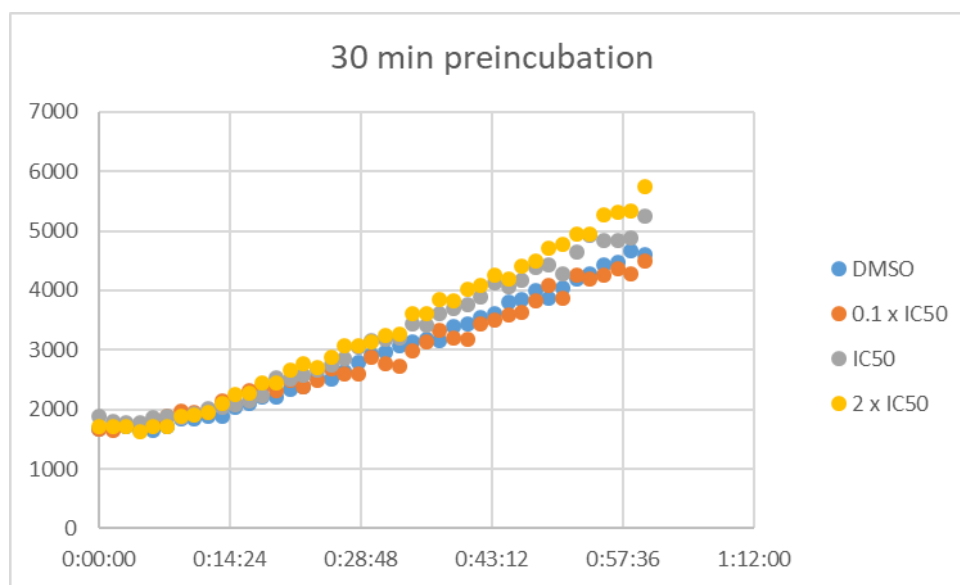


Figure S26. Restored activity (RFU) of Mtb proteasome after 30-min preincubation of enzyme with compound 8 at various concentrations (0.1 x IC₅₀, IC₅₀ and 2 x IC₅₀).

d. Compound 11

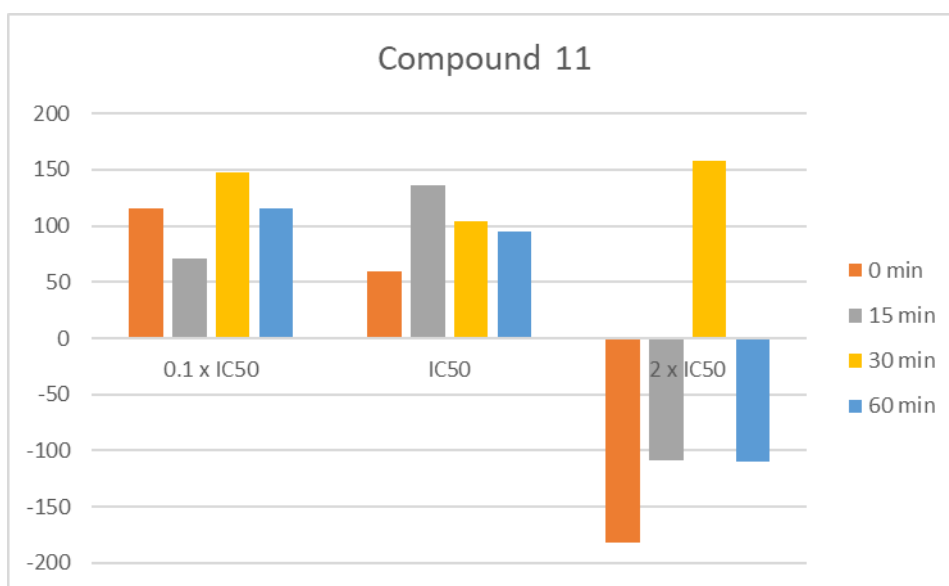


Figure S27. Restored activity (%) of Mtb proteasome after preincubation of enzyme with compound **11** at various concentrations (0.1 x IC₅₀, IC₅₀ and 2 x IC₅₀) for various amounts of time (0, 15, 30 and 60 min).

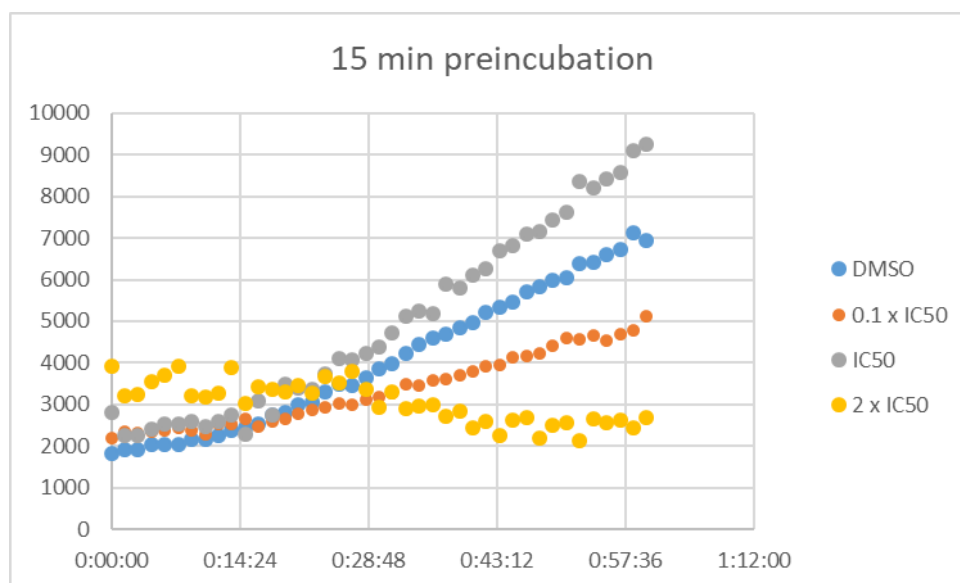


Figure S28. Restored activity (RFU) of Mtb proteasome after 15-min preincubation of enzyme with compound **11** at various concentrations (0.1 x IC₅₀, IC₅₀ and 2 x IC₅₀).

e. Compound 13

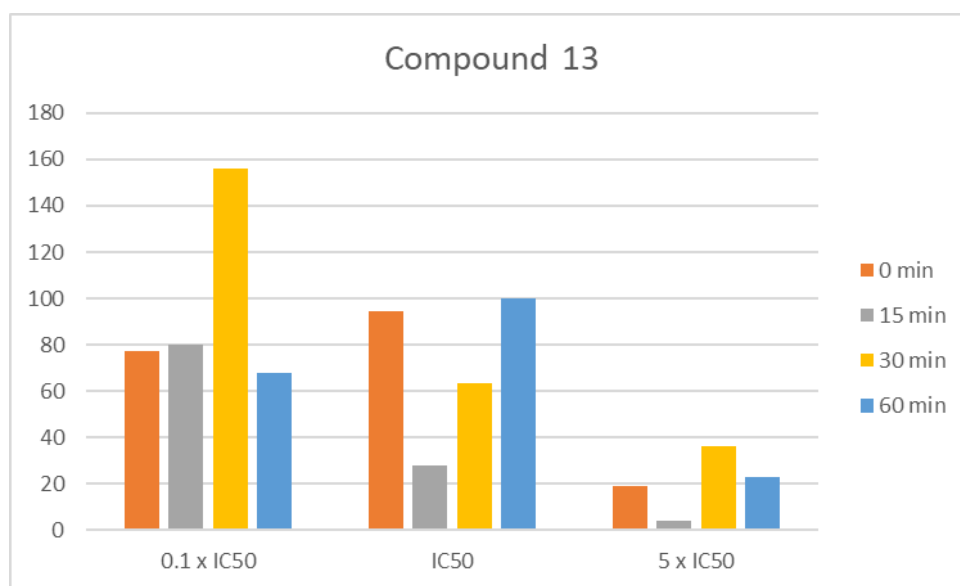


Figure S29. Restored activity (%) of Mtb proteasome after preincubation of enzyme with compound 13 at various concentrations (0.1 x IC₅₀, IC₅₀ and 5 x IC₅₀) for various amounts of time (0, 15, 30 and 60 min).

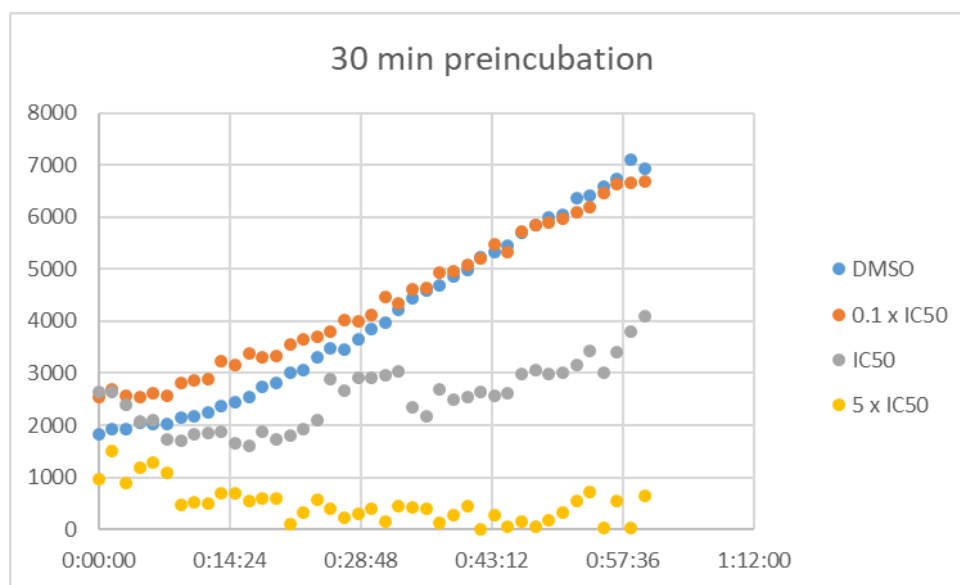


Figure S30. Restored activity (RFU) of Mtb proteasome after 30-min preincubation of enzyme with compound 13 at various concentrations (0.1 x IC₅₀, IC₅₀ and 5 x IC₅₀).

f. Compound 15

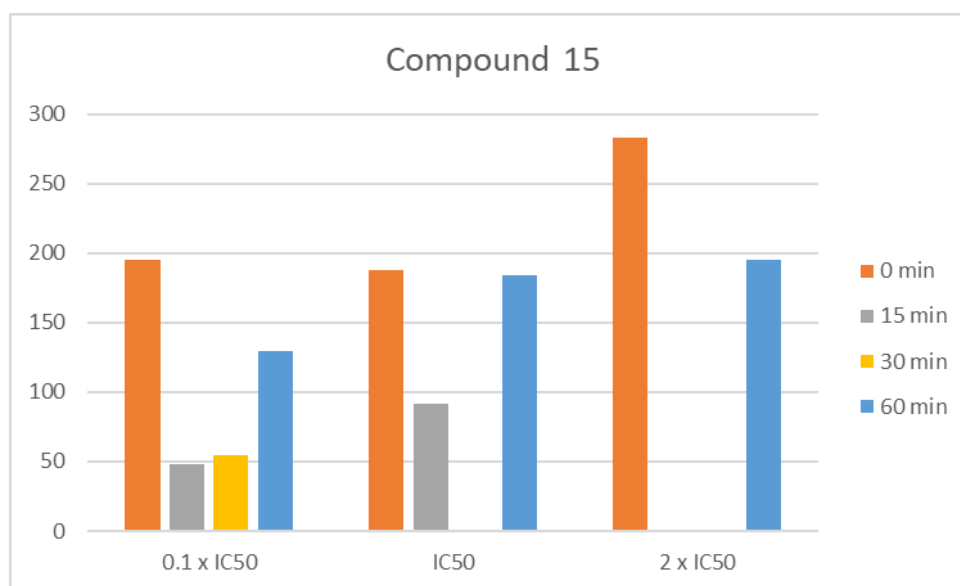


Figure S31. Restored activity (%) of Mtb proteasome after preincubation of enzyme with compound 15 at various concentrations (0.1 x IC₅₀, IC₅₀ and 2 x IC₅₀) for various amounts of time (0, 15, 30 and 60 min).

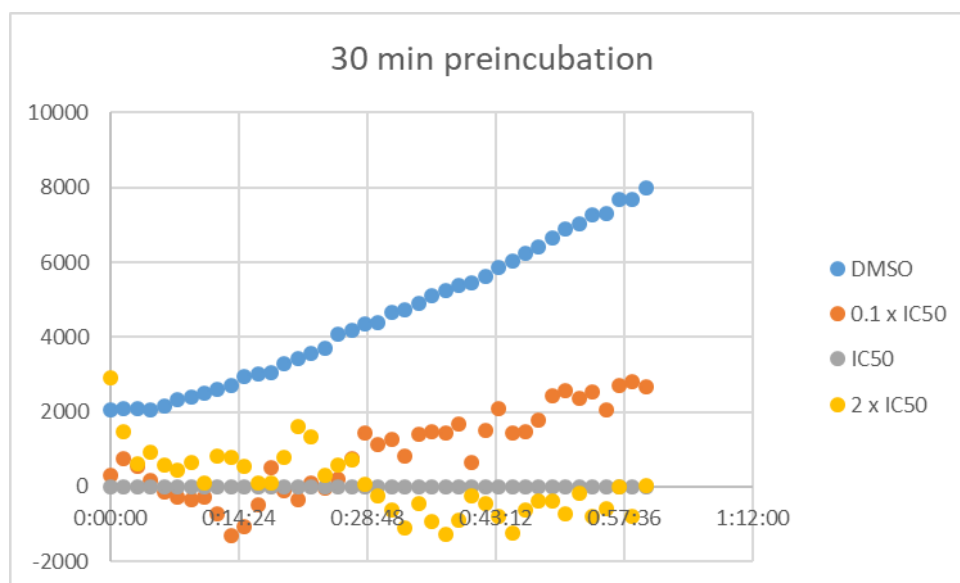


Figure S32. Restored activity (RFU) of Mtb proteasome after 30-min preincubation of enzyme with compound 15 at various concentrations (0.1 x IC₅₀, IC₅₀ and 2 x IC₅₀).

5. Analytic and synthetic data for compounds

Determination of k_{inact}/K_i values for PR-957

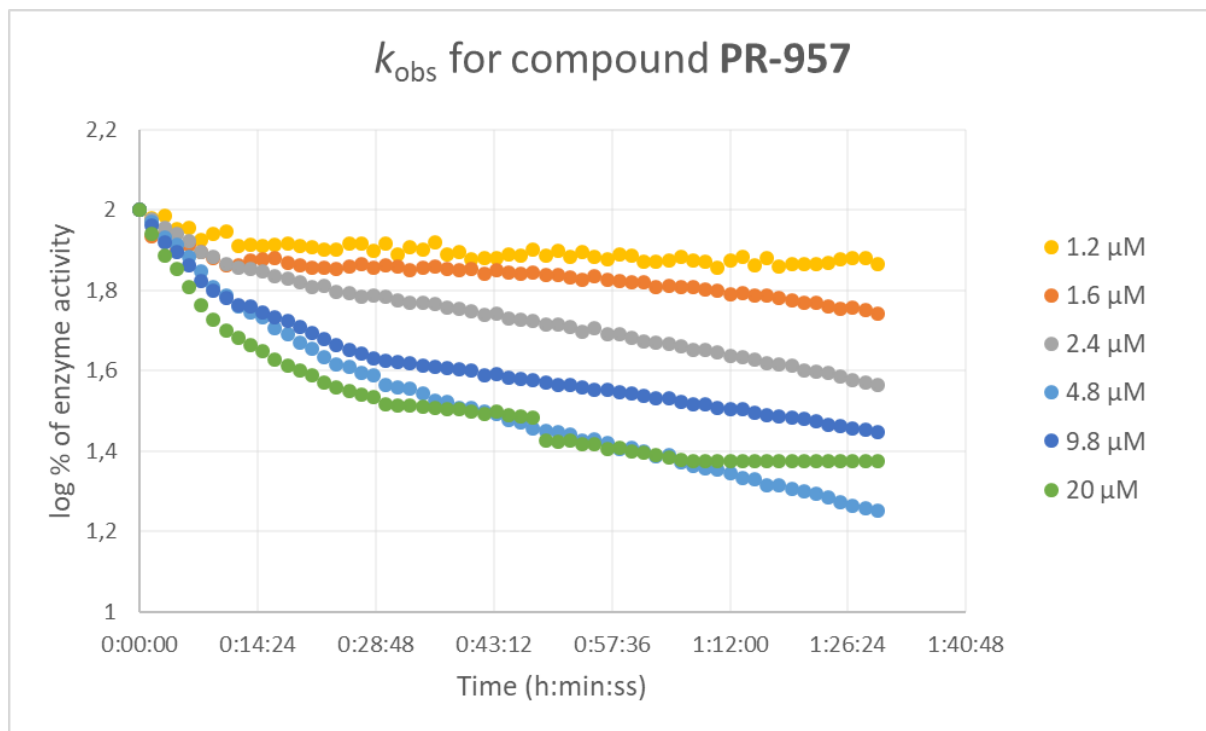


Figure S33. Plot of logarithm of % of enzyme activity vs time for compound PR-957 during the 90-min reaction.

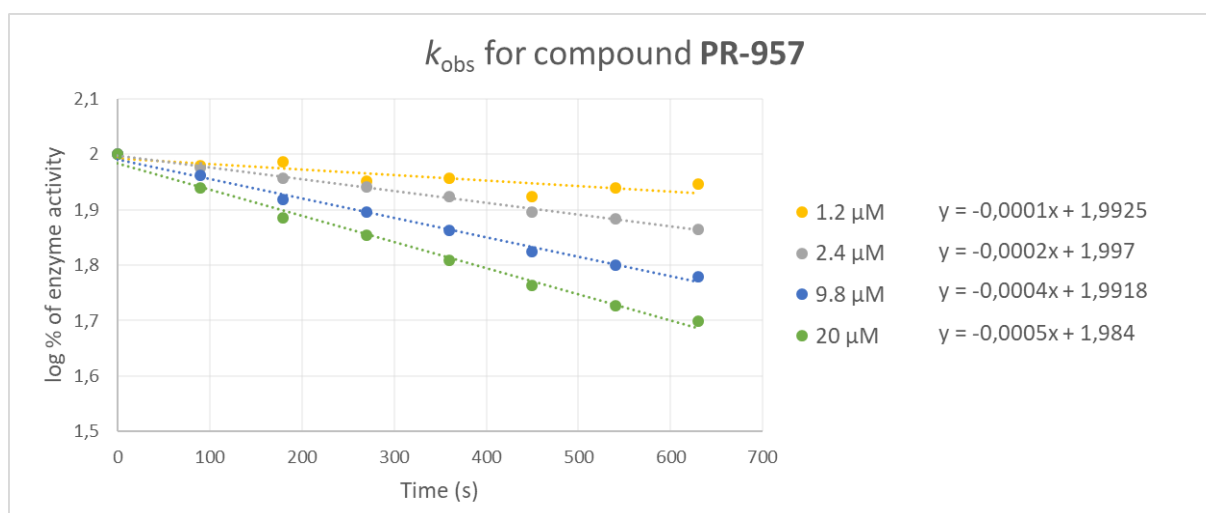


Figure S34. Determination of initial rate of the reaction, k_{obs} , by plotting logarithm of % of enzyme activity vs time (first 10 min of the reaction).

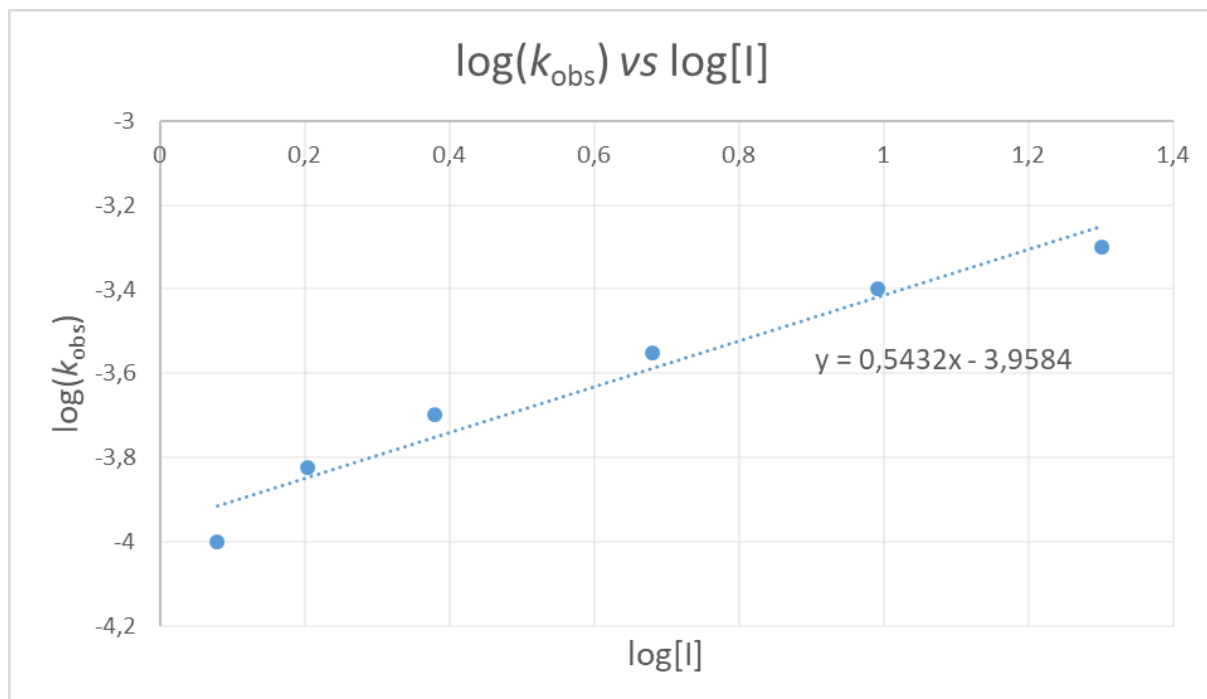


Figure S35. Plot of logarithm of k_{obs} vs logarithm of inhibitor concentration for compound PR-957.

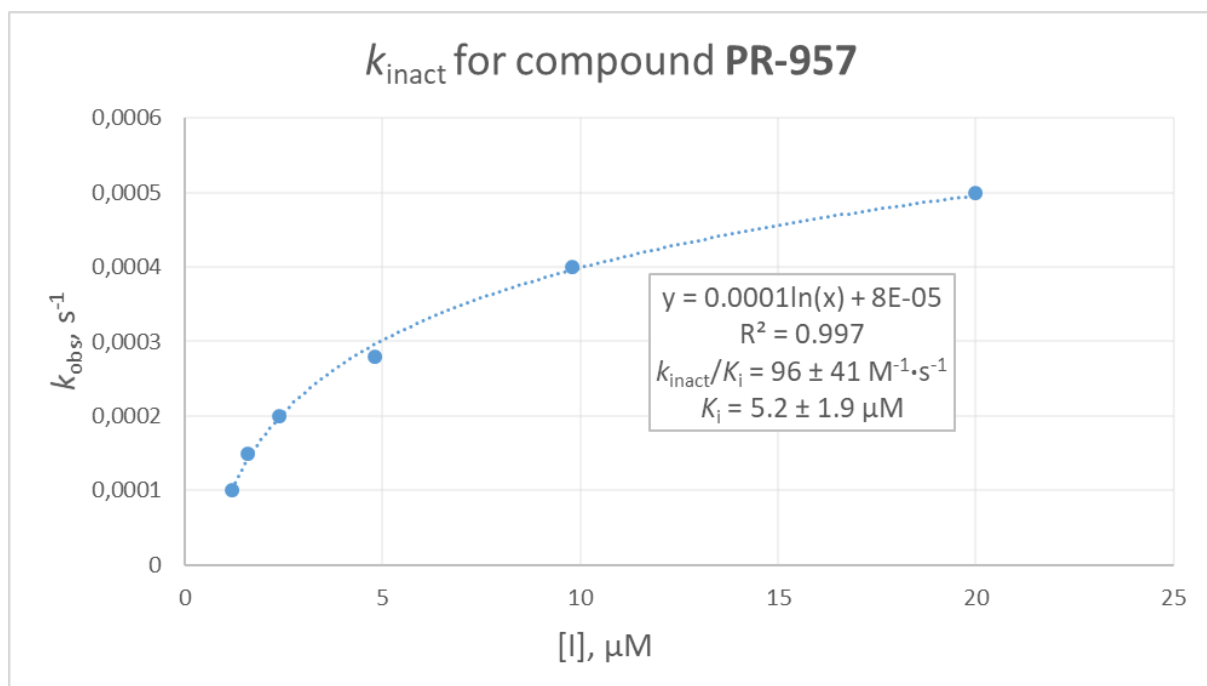


Figure S36. Determination of constant of inactivation, k_{inact} , from plot of k_{obs} vs inhibitor concentration, [I].