SUPPORTING INFORMATION

Selective Inhibition of Extracellular Thioredoxin by Asymmetric Disulfides

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SUPPLEMENTAL FIGURES

Figure S1:

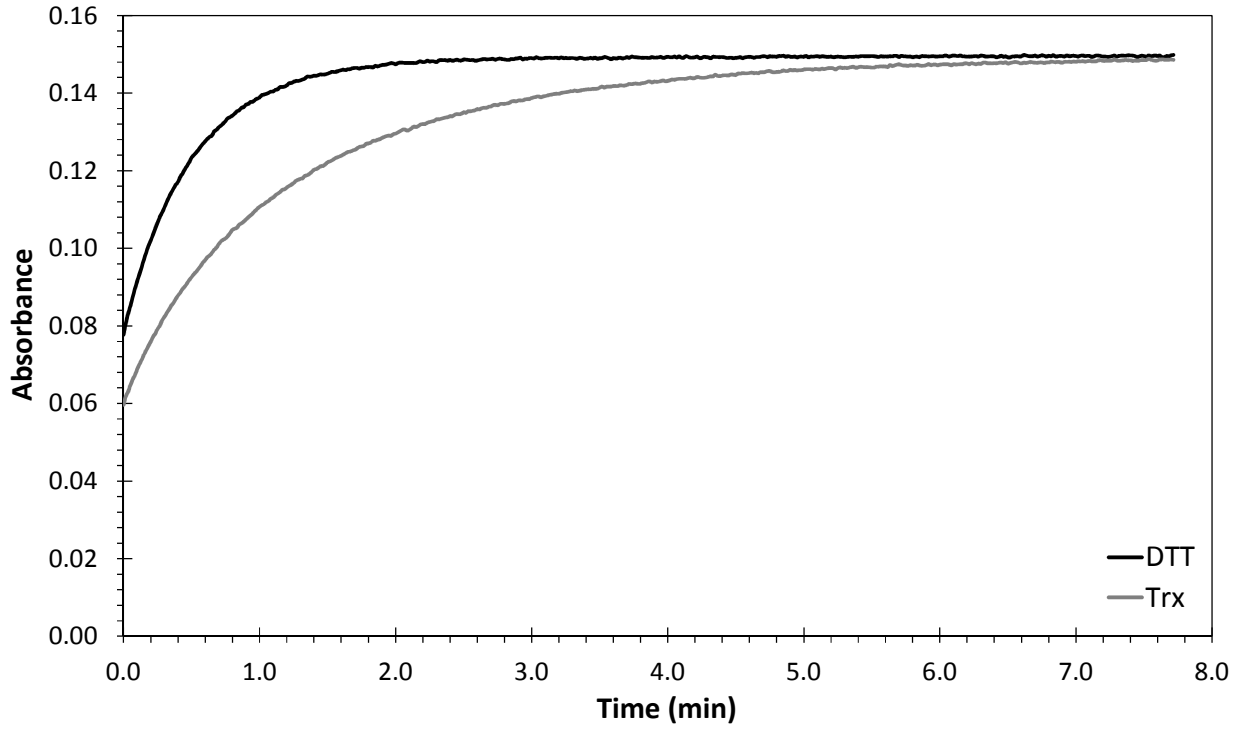


Figure S1: Time course of the reaction between 10 μM PX-12 and either 250 μM DTT or 10μM Trx, measured at 252 nm. Reactions were performed in 0.1 M citrate-phosphate buffer at pH 6.

Figure S2:

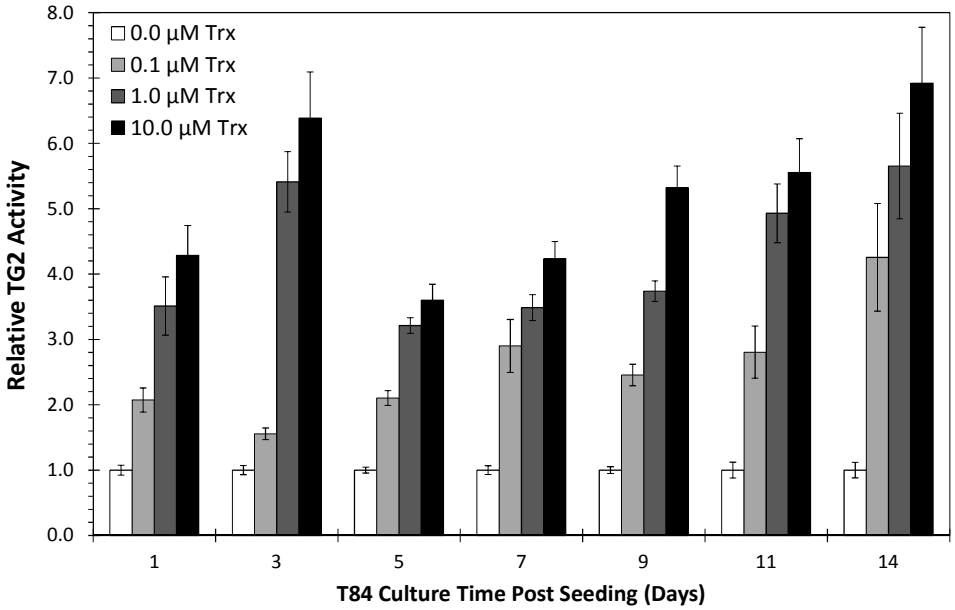


Figure S2: Effect of maturity of T84 monolayers on Trx-mediated TG2 activation. T84 cells were grown for 1-14 days post-seeding, and treated with 0-10 µM recombinant human Trx and 200 µM 5BP for 3 h. Following removal of all well contents, cells were washed, fixed, washed, and blocked as described in the Experimental Methods. Horseradish peroxidase conjugated streptavidin was used to quantify the amount of extracellular 5BP incorporation. TG2 activity is normalized to the activity observed in the absence of any Trx at the corresponding time point. Experiments were performed in triplicate Values reported are averages +/- standard errors.

Figure S3:

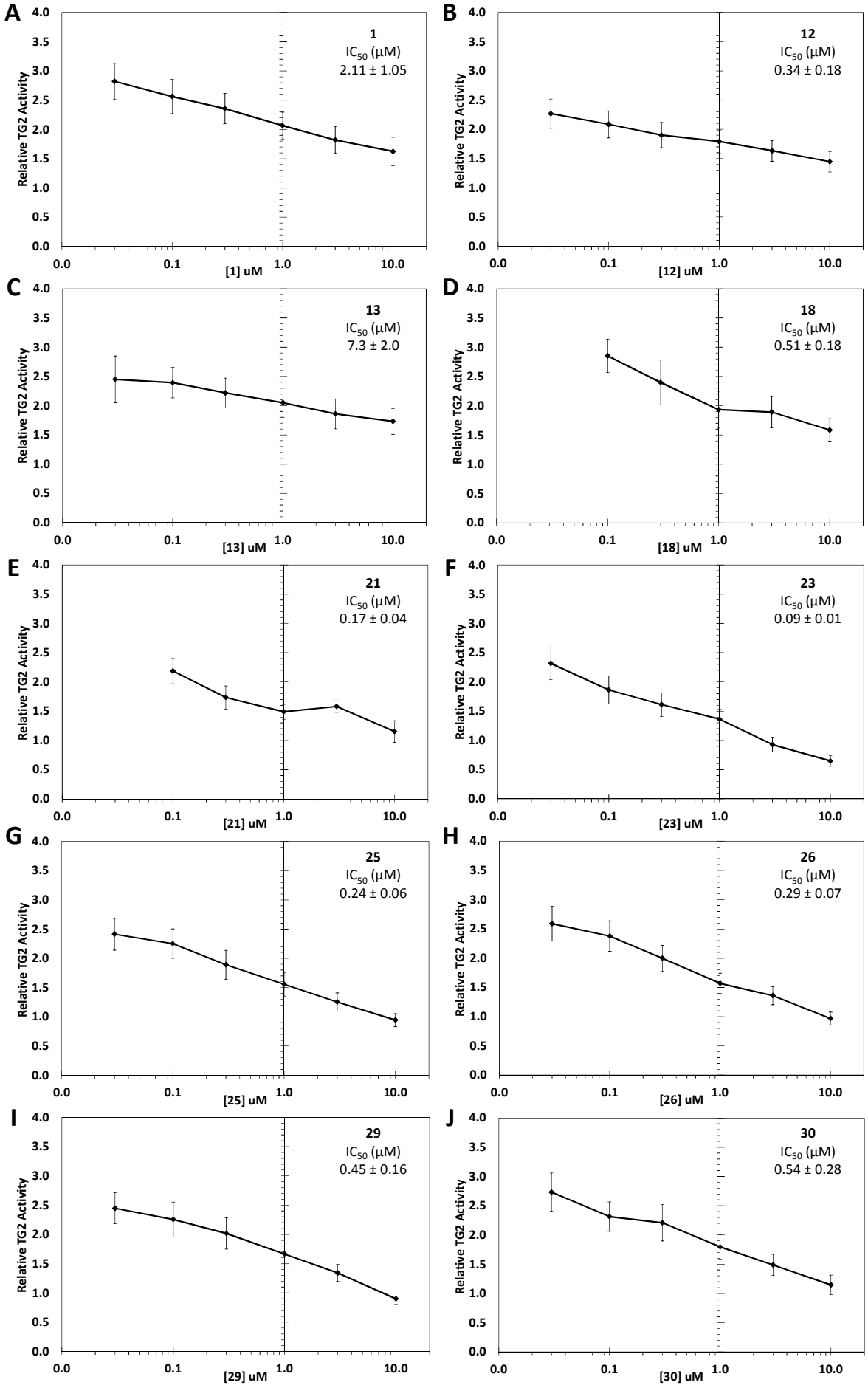
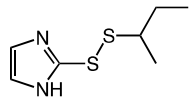
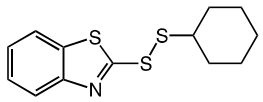
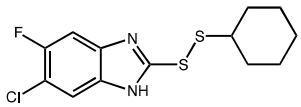
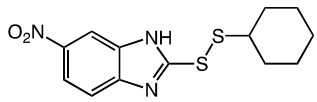
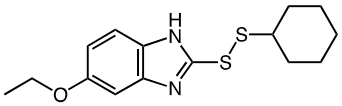
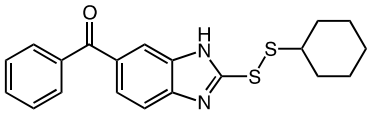
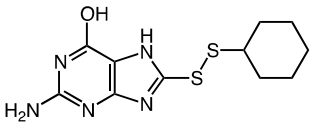
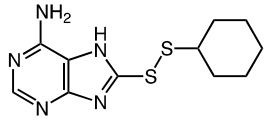
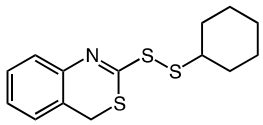
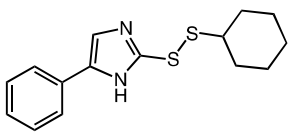
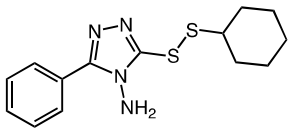

A
1
IC50 (μM)
2.11 ± 1.05
B
12
IC50 (μM)
0.34 ± 0.18
C
13
IC50 (μM)
7.3 ± 2.0
D
18
IC50 (μM)
0.51 ± 0.18
E
21
IC50 (μM)
0.17 ± 0.04
F
23
IC50 (μM)
0.09 ± 0.01
G
25
IC50 (μM)
0.24 ± 0.06
H
26
IC50 (μM)
0.29 ± 0.07
I
29
IC50 (μM)
0.45 ± 0.16
J
30
IC50 (μM)
0.54 ± 0.28
Relative TG2 Activity
[1] uM
[12] uM
[13] uM
[18] uM
[21] uM
[23] uM
[25] uM
[26] uM
[29] uM
[30] uM

Figure S3: Inhibition of Trx mediated TG2 activation in T84 enterocytes via asymmetric disulfides. Recombinant human Trx was added to T84 cells, as described in the experimental methods section. T84 cells were treated with 0.03 μM Trx, 200 μM 5BP, and 0-10 μM compounds (A) **1**, (B) **12**, (C) **13**, (D) **18**, (E) **21**, (F) **23**, (G) **25**, (H) **26**, (I) **29**, and (J) **30** for 3 h. Following removal of all well contents, cells were washed, fixed, washed, and blocked as described in the Experimental Methods. Horseradish peroxidase conjugated streptavidin was used to probe the amount of extracellular 5BP incorporation. TG2 activity is normalized to the activity observed in the absence of any exogenous Trx. IC_{50} values reported were calculated using linear regressions of TG2 activity versus logarithmic inhibitor concentration. Experiments were performed in triplicate. Values reported are averages +/- standard errors.

SUPPLEMENTAL TABLE

Table S1: Comparison of the reactivity (k_{inh}/K_i) of selected inhibitors against Trx versus bimolecular rate constant against DTT, glutathione, and cysteine.

Inhibitor Structure	Compound	Trx k_{inh}/K_i ($\mu M^{-1}min^{-1}$)	Trx/ DTT	Trx/ Glutathione	Trx/ Cysteine
	**1**	0.076	7	6	4
	**18**	0.031	52	150	51
	**21**	0.14	200	120	79
	**23**	0.34	170	180	95
	**24**	0.27	110	64	45
	**25**	0.076	150	210	130
	**26**	0.072	120	60	40
	**27**	0.089	74	37	21
	**28**	0.16	65	52	52
	**29**	0.087	73	36	29
	**30**	0.18	42	33	23