Supplementary Table 1. Inhibition of trypanothione synthetase (TryS) by N^5-substituted, 3-chlorokenpaullones.

				% TryS inhibition at 30 µM[a] or *IC₅₀ value* (µM)[b,c]	
N^5-linker chain	**Compound**	**N^5-substitution**		***Tc*TryS**	***Tb*TryS**
none	**1**	H		NA	NA
	2	CH_2CH_2OH		NA	NA
CH_2CO-OR_1	**3**	R_1 = H		**~ 30**	NA
	4	R_1 = CH_3		37.4 ± 2.6	17.1 ± 4.0
	5	R_1 = CH_2CH_3		19.1 ± 55	29.8 ± 5.5
	6	R_1 = *tert*-butyl		10.3 ± 5.8	NA
CH_2CO-NR_1,R_2	**7**	R_1= H,	R_2 = H	5.2 ± 1.2	14.5 ± 5.5
	8	R_1 = H,	R_2 = NH_2	10.1 ± 3.1	8.3 ± 5.8
	9	R_1 = H,	R_2 = CH_3	22.8 ± 2.3	44.4 ± 3.6
	10	R_1 = CH_3,	R_2 = CH_3	14.9 ± 6.5	24.6 ± 3.8
	11	R_1 = H,	R_2 = CH_2CH_3	41.4 ± 8.3	NA
	12	R_1 = CH_2CH_3,	R_2 = CH_2CH_3	7.8 ± 2.6	***~ 30***
	13	R_1 = H,	R_2 = $(CH_2)_2OH$	NA	NA
	14	R_1 = H,	R_2 = C-$(CH_2OH)_3$	NA	12.6 ± 7.0
	15	R_1 = H,	R_2 = *tert*-butyl	5.8 ± 3.3	10.9 ± 6.8
	16	R_1 = H,	R_2 = 1,3,4-thiadiazol-2-yl	***~ 30***	18.8 ± 3.3
	17	R_1 = H,	R_2 = 4,5-dihydro-1,3-thiazol-2-yl	16 ± 2.2	41.3 ± 5.6
	18	R_1 = H,	R_2 = 1,3-oxazol-2-yl	6.9 ± 3.9	12.2 ± 6.5
	19	R_1 = H,	R_2 = phenyl	29.6 ± 2.5	***~ 30***
CH_2CO-R_1	**20**	R_1 = piperazin-1-yl, hydrochloride		17.1 ± 4.9	***~ 30***
	21	R_1 = 4-methylpiperazin-1-yl		NA	31.6 ± 6.0
	22	R_1 = 4-BOC-piperazin-1-yl		NA	18.7 ± 1.9
	23	R_1 = 4-(pyrimidin-2-yl)piperazin-1-yl		40.6 ± 3.7	27.3 ± 3.9
	24	R_1 = piperidin-1-yl		7.1 ± 4.5	19.9 ± 2.5
	25	R_1 = pyrrolidin-1-yl		NA	9.8 ± 2.8
	26	R_1 = morpholin-4-yl		NA	NA
CH_2CO-NH-CH_2-CH_2-NR_1,R_2	**Mol2008[d]**	R_1 = H,	R_2 = CH_3	40.5 ± 5.9	59.0 ± 6.0
	27	R_1 = H,	R_2 = H, hydrochloride	26.3 ± 3.6	27.3 ± 5.9
	28	R_1 = CH_2CH_3,	R_2 = CH_2CH_3	17.1 ± 1.1	16.0 ± 5.6
	29	R_1 = H,	R_2 = BOC	38.5 ± 4.5	28.3 ± 3.2
CH_2CO-NH-CH_2-CH_2-R_1	**30**	R_1 = morpholin-4-yl		11.3 ± 5.1	6.1 ± 4,9
	31	R_1 = piperidin-1-yl		21.4 ± 0.0	20.5 ± 3.2
	32	R_1 = piperazin-1-yl, dihydrochloride		7.9 ± 8.4	NA
	33	R_1 = 4-methylpiperazin-1-yl		16.8 ± 5.7	8.1 ± 6.2
	34	R_1 = 4-BOC-piperazin-1-yl		***20 ± 3***	24.8 ± 3.6
CH_2CO-NH$(CH_2)_4$-NH-R_1	**35**	R_1 = H, hydrochloride		6.7 ± 8.8	10.8 ± 2.2
	36	R_1 = BOC		***25 ± 3***	27.9 ± 4.6

[a] Enzyme inhibition is expressed as % TryS inhibition ± $2\sigma^{n-1}$. [b] IC_{50} values with their corresponding standard deviation (± $2\sigma^{n-1}$) are highlighted in bold italics. [C] For compounds inhibiting TryS by 50 ± 5% at 30 µM, an IC_{50} of ***~ 30*** µM is assumed. [d] Data published in [20]. NA, not active (enzyme activity at 30 µM is > 95%). BOC, refers to a *tert*-butyloxycarbonyl group.

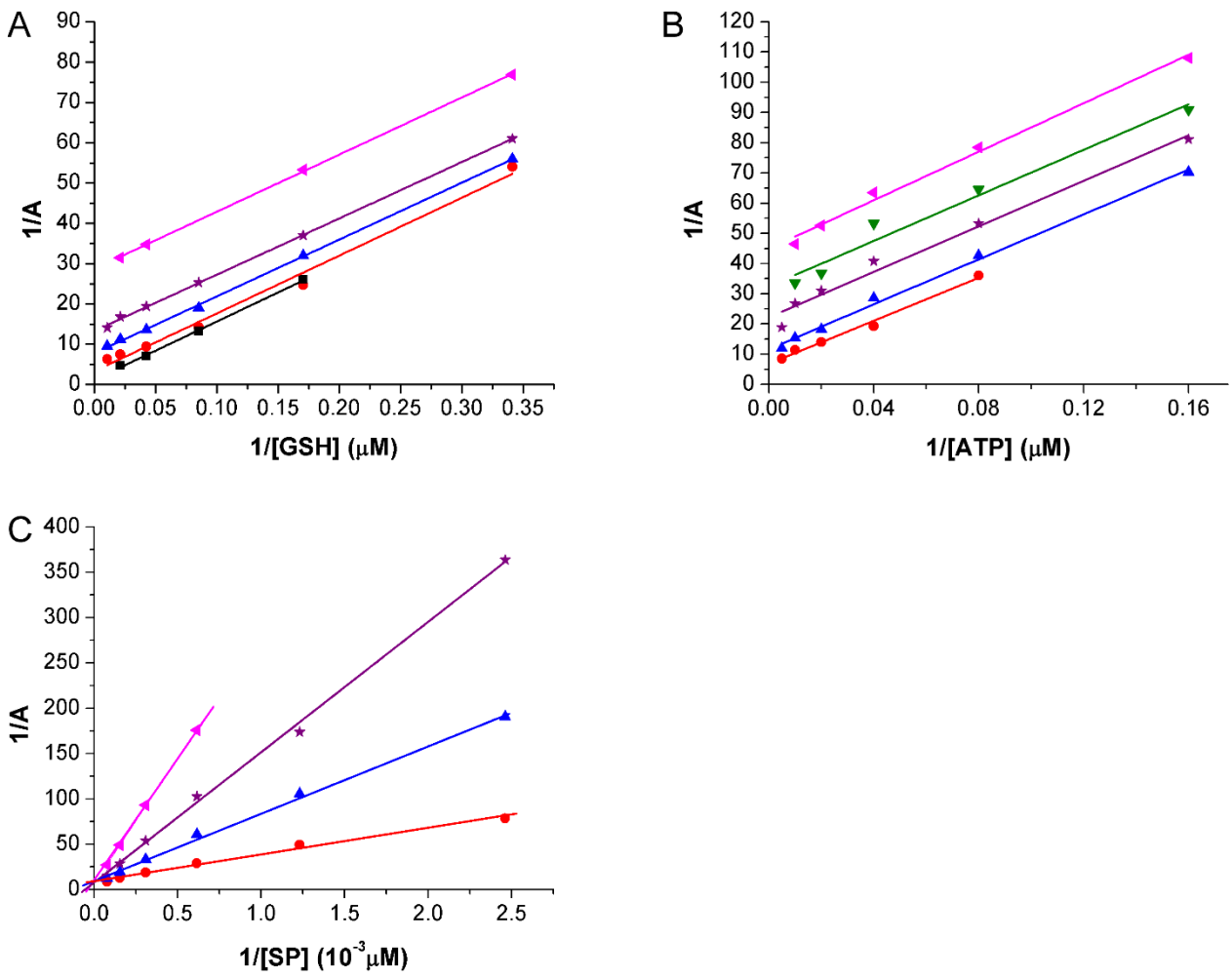

Figure S1. Mode of *Li*TryS inhibition by compound 20. Lineweaver-Burk reciprocal plots for the *Li*TryS activity measured at different inhibitor (black square: 0 μM, red circle: 0.31 μM, blue triangle: 0.775 μM, violet star: 1.55 μM, green inverted triangle: 2.3 μM and magenta inclined triangle: 3.1 μM) and varying substrate concentrations while maintaining fixed the concentration of the co-substrates. The maximum concentration of SP and ATP used in these studies that do not interfere with the colorimetric assay were 13 mM (~ 9.1-fold the K_M value) and 200 μM (~ 4.6-fold the K_M value), respectively. For GSH, the maximum concentration tested was 100 μM because at higher values there is a marked enzyme inhibition and loss of linearity. The enzyme velocities were measured using the end-point assay based on BIOMOL GREEN™ reagent (Enzo Life Sciencies) and the reciprocal plots are shown for the varying substrate: **A)** GSH, **B)** ATP, **C)** SP.