Discovery of Thalicthuberine as a Novel Antimitotic Agent from Nature that Disrupts Microtubule Dynamics and Induces Apoptosis in Prostate Cancer Cells

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Supplementary Information

Supplementary Figures S1, S2, S3, and figures legends Supplementary Table S1–S2 Supplementary Videos S1–S4 and legends

SNORD46	5.57	7.40	
SNORD71	3.05	3.54	
TP53I3	2.32	1.92	3.0
PGF	2.29 1.61		
EGR1	2.11	1.93	
MDM2	2.09	1.87	
GPR87	1.93	1.90	
FOS	1.89	1.62	
LRRFIP1	1.88	2.47	
CCNB1	1.86 2.14		
CDC20	1.83	2.26	
HMMR	1.83	1.97	
CCL20	1.81	1.92	
LHX3	1.80	1.47	
ITLN2	1.75	1.75	
KIF18A	1.73	1.94	
DLGAP5	1.71	1.89	2.5
CDKN1A	1.71	1.41	
CCNB2	1.70	1.84	
TNFAIP8L1	1.69	1.75	
NEK2	1.68	1.78	
CKS2	1.68	1.94	
EBLN2	1.67	2.07	
GPSM2	1.67	2.03	
GAS2L3	1.66	1.57	
AURKA	1.66	2.10	
PRR11	1.65	1.79	
DDIT3	1.64	1.53	
SNORA58	1.60	1.91	
PSRC1	1.58	1.84	
BUB1	1.57	1.70	2.0
SCLT1	1.56	1.70	
NUF2	1.52	1.65	
BIRC5	1.52	1.75	
COX8C	1.52	1.41	
CENPF	1.52	1.80	
DEPDC1	1.52	1.59	
CENPE	1.51	1.67	
AMY2B	1.51	1.43	
TNFAIP3	1.51	1.45	
GTSE1	1.50	1.77	
ASCC3	1.50	1.46	
KIF20A	1.48	1.79	
FAM209B	1.47	1.55	
TPX2	1.44	1.73	- 1.5
IER3	1.43	1.55	
CCDC83	1.42	1.58	
	тн	Vinb	

Supplementary Figure S1: TH and vinblastine caused similar transcriptional changes in LNCaP cells

Heatmap depicting the fold changes of genes differentially expressed (*P* value ≤ 0.1 , foldchange of ≥ 1.4) in LNCaP cells after 24 h treatment with TH (1×IC₅₀) or vinblastine (Vinb, 1×IC₅₀). Red indicates up-regulation and blue down-regulation. The darker the shade of color, the higher the fold-change of expression.



Supplementary Figure S2: TH does not interact with DNA nor induces DNA damage through double-strand breaks.

(A) Analysis of DNA melting temperature revealed that TH did not induce a shift in temperature and did not displace of SYBR Green, suggesting that TH does not directly interact

with DNA. The temperature-dependent dissociation of SYBR Green-stained double-stranded DNA in the presence of TH (1–1000 μ M) was monitored using a Real-Time PCR instrument. H₂O, DMSO and DAPI (0.12–1 μ M) were used as controls (triplicate, mean ± SD).

(B) Immunofluorescence microscopy coupled with automated image analysis (CellProfiler) was used to quantify the number and the mean intensity of γ H2AX foci in LNCaP cells after 24 h of treatment (upper panel, n = 2, mean ± SD). Doxorubicin (Dox) was used as positive control. TH (batch A) did not induce a significant increase in γ H2AX foci (number and mean intensity). Representative images are shown (bottom panel). Scale bar = 20 µm.



Supplementary Figure S3: Batches A and B of TH induced a mitotic arrest and disrupted the mitotic spindle organization.

LNCaP cells were treated with both batches A and B of TH, and vinblastine for 24 h, and cells were subjected to fluorescence microscopy of DAPI-stained DNA. Representative images of mitotic cells displaying condensed chromatin. Scale bar = $50 \mu m$.

Supplementary Table S1: List of selected mitosis-related genes regulated by TH as identified in the microarray

Gene symbol	Gene description	Fold change
CDC20	cell division cycle 20	1.83
HMMR	hyaluronan-mediated motility receptor (RHAMM)	1.83
AURKA	aurora kinase A	1.66
CENPE	centromere protein E	1.51
CCNB1	cyclin B1	1.86
KIF18A	kinesin family member 18A	1.73
CKS2	CDC28 protein kinase regulatory subunit 2	1.68
KIF20A	kinesin family member 20A	1.48
CCNB2	cyclin B2	1.70
NUF2	NDC80 kinetochore complex component	1.52
TPX2	TPX2, microtubule-associated	1.44
NEK2	NIMA (never in mitosis gene a)-related kinase 2	1.68
BUB1	budding uninhibited by benzimidazoles 1 homolog	1.57
BIRC5	baculoviral IAP repeat containing 5 (survivin)	1.52
CENPF	centromere protein F	1.52
DNAH12	dynein, axonemal, heavy chain 12	1.65
TUBA3C	tubulin, alpha 3c	-1.44
TUBA3D	tubulin, alpha 3d	-1.57
TUBA3E	tubulin, alpha 3e	-1.57

For genes with multiple probes, the 'differentially expressed reference probe with the highest fold change' was used to represent the gene for functional analysis.

Supplementary Table S2: Statistical data for Figure 3A

Compound	Sub G ₀ -G ₁	G_0 - G_1	S	G ₂ -M
TH (1.25 μM)	ns	ns	ns	ns
TH (2.5 µM)	***	ns	ns	****
TH (5 μM)	***	****	ns	****
Vinb (10 nM)	****	****	ns	****

For each phase of the cell cycle, one-way ANOVA with Dunnett's multiple comparisons test was used (comparison to DMSO, ns=non-significant, *** P < 0.001, **** P < 0.0001).

Supplementary Videos

Supplementary Video S1. Time-lapse microscopy of HeLa-H2B-GFP cells treated with DMSO or vinblastine (20 nM). HeLa-H2B-GFP cells were imaged using an Olympus IX81 (1 image/5 min for 24 h). Top panels: brightfield, bottom panels: H2B-GFP. DMSO-treated cells normally progressed through mitosis, while cells treated with vinblastine (Vinb, 20 nM) showed delayed mitosis, asymmetric divisions, and cell death. Scale bar = $20 \,\mu$ m.

Supplementary Video S2. Time-lapse microscopy of HeLa-H2B-GFP cells treated with TH (1.25 and 5 μ M). HeLa-H2B-GFP cells were imaged using an Olympus IX81 (1 image/5 min for 24 h). Top panels: brightfield, bottom panels: GFP channel. TH increased in a concentration-dependent manner mitotic duration, leading to cell death or asymmetric division. Scale bar = 20 μ m.

Supplementary Video S3. Time-lapse microscopy of HeLa-H2B-GFP cells treated with TH (2.5 μ M), BI2536 (50 nM), and the combination of TH and BI2536. HeLa-H2B-GFP cells were imaged using an Olympus IX81 (1 image/5 min for 24 h). Top panels: brightfield, bottom panels: GFP channel. TH and BI increased mitotic duration in a concentration-dependent manner. TH led to both cell death and asymmetric division, while BI2536 alone or in combination with TH exclusively induced cell death. Scale bar = 20 μ m.

Supplementary Video S4. Interphase microtubule dynamics in HeLa-EB1-GFP treated with DMSO, TH (5 μ M), or vinblastine (20 nM). EB1-GFP was imaged by spinning disk microscopy (1 image/s for 1 min). In DMSO-treated cells, EB1 comets were clearly discernible and followed regular trajectories. In TH- and vinblastine-treated cells. Scale bar = 10 μ m.