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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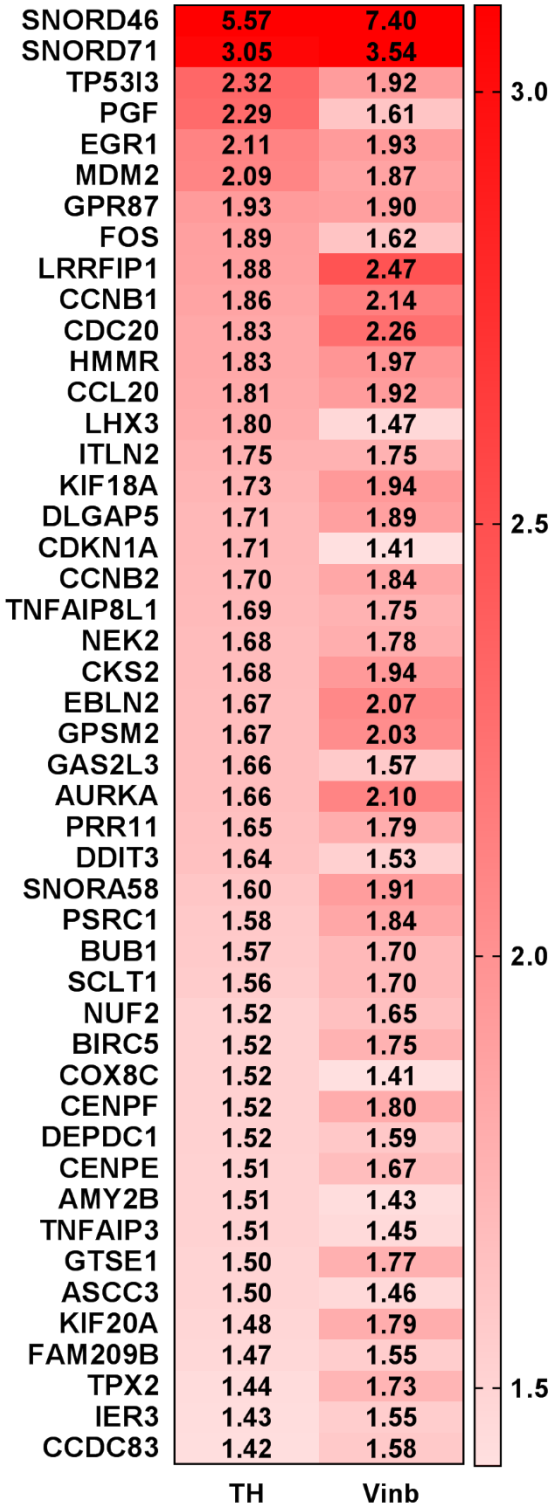
Supplementary Information

Supplementary Figures S1, S2, S3, and figures legends

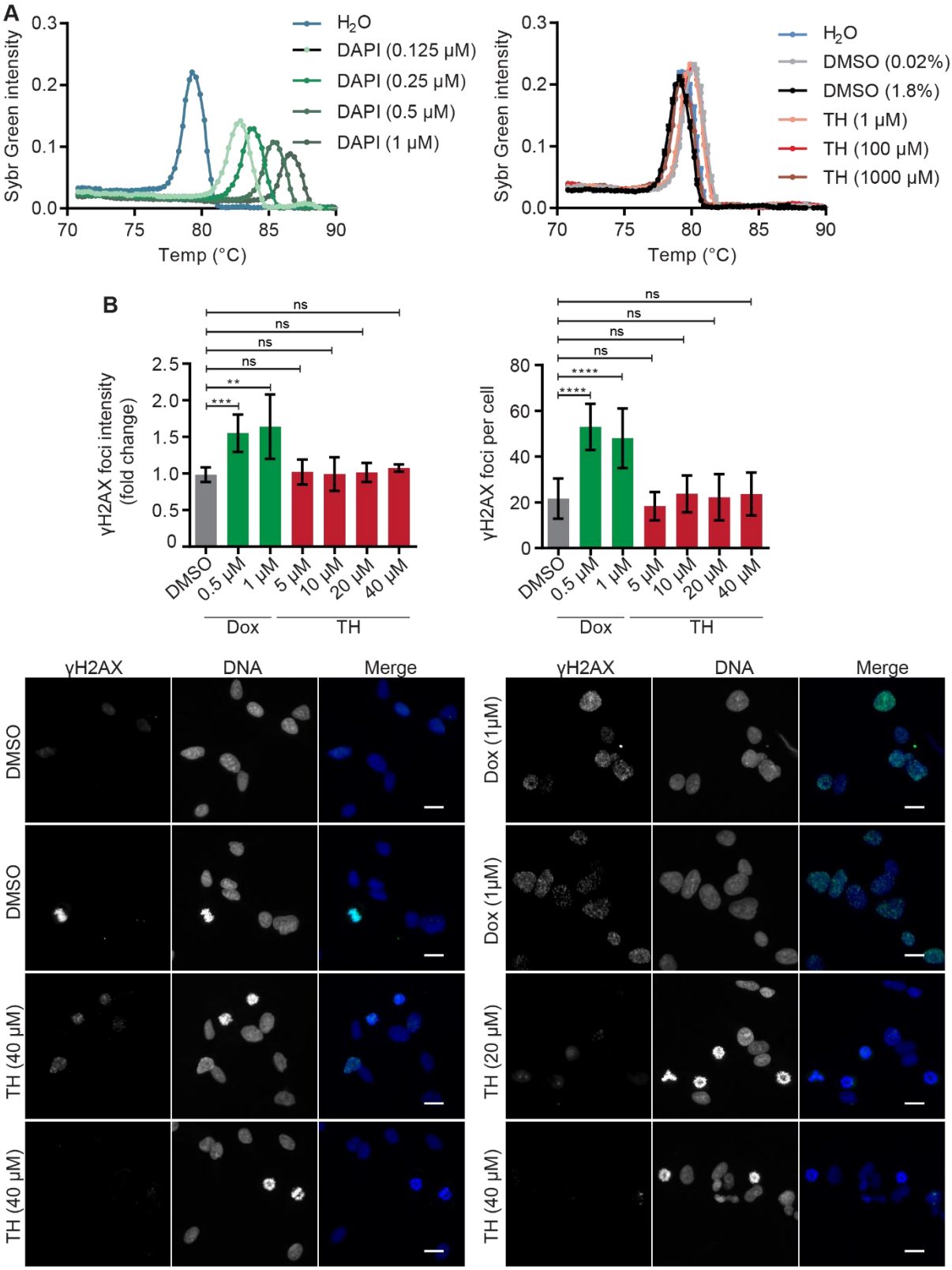
Supplementary Table S1−S2

Supplementary Videos S1−S4 and legends



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, fold-change of ≥1.4) in LNCaP cells after 24 h treatment with TH (1×IC_{50}) or vinblastine (Vinb, 1×IC_{50}). Red indicates up-regulation and blue down-regulation. The darker the shade of color, the higher the fold-change of expression.

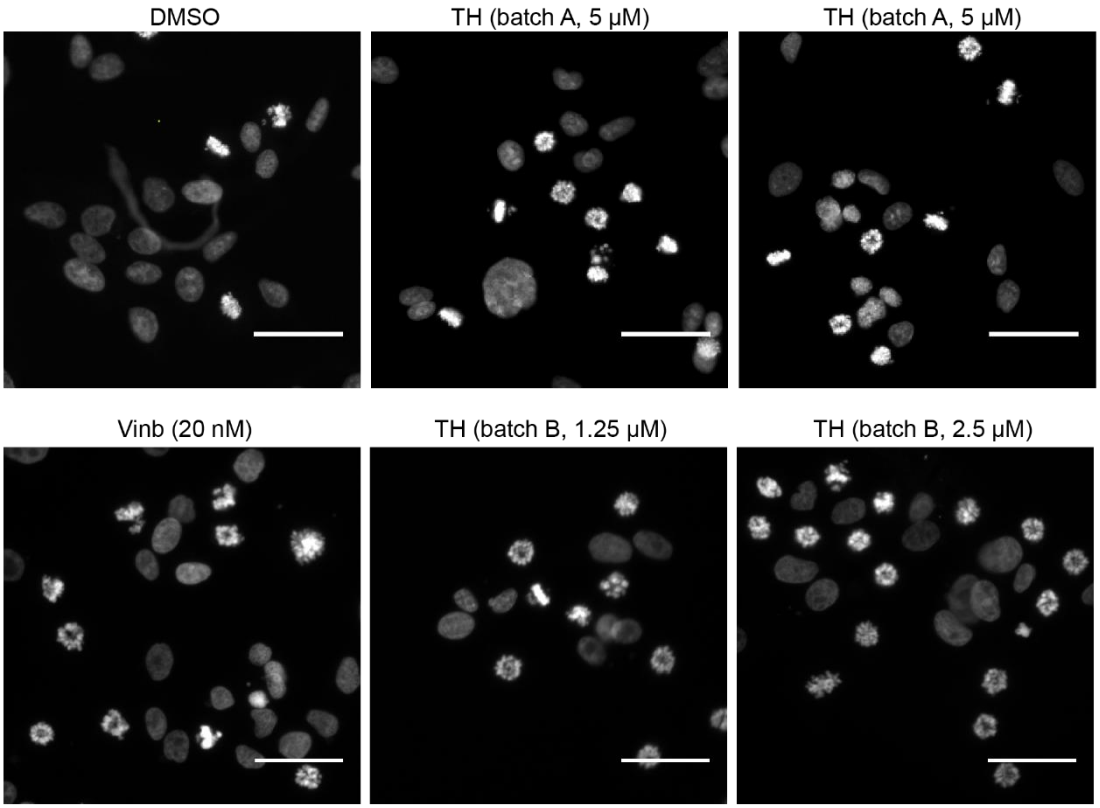


Supplementary Figure S2: TH does not interact with DNA nor induces DNA damage through double-strand breaks.

(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 µM) was monitored using a Real-Time PCR instrument. H_2O, DMSO and DAPI (0.12–1 µM) 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 γH2AX 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 γH2AX 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 µm.

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 µm.

Supplementary Video S2. **Time-lapse microscopy of HeLa-H2B-GFP cells treated with TH (1.25 and 5 µM).** 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 µm.

Supplementary Video S3. **Time-lapse microscopy of HeLa-H2B-GFP cells treated with TH (2.5 µM), 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 µm.

Supplementary Video S4. Interphase microtubule dynamics in HeLa-EB1-GFP treated with DMSO, TH (5 µM), 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 µm.