Supporting Information

The Essential Medicinal Chemistry of Curcumin

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Supplemental Table 1. Prototypical examples of assays reporting curcumin bioactivity.

Target	Potency	Selectivity	Assay descriptions	Comments	Ref(s)
p300	IC_{50} = ~25 μM	G9a-GST, HDAC1	[^{3}H]acetyl-CoA HAT assays; *in vitro* transcription assay; cell-based HAT assays	1. No detergent in assays 2. Incubation times allow for compound degradation 3. Micromolar compound concentrations 4. Reactive mechanism(s) not excluded	[1]
HDAC8	IC_{50} = 115 μM	None	Molecular modeling (docking); fluorometric HDAC assay with HeLa nuclear extract	1. No detergent in assays 2. Enzyme source nuclear extract containing other HDACs and HATs 3. No counter-screens for fluorescence interference performed 4. No evidence of direct target engagement 5. Heavy reliance on docking	[2]
GSK-3β	IC_{50} = 66.3 nM	None	Docking; tau phosphoELISA assay; liver glycogen in Balb/c mice;	1. No detergent or thiol-scavenging agents in assays. 2. Heavy reliance on docking 3. No evidence of direct target engagement 4. Long incubation times for *in vitro* assay; no confirmation of compound stability 5. Non-specific *in vivo* readout	[3]
Tau fibril formation	IC_{50} = 3.5 μM	None	ThT fluorescence assay (primary, AID 1460); FP mP (secondary, AID 1468); FP total (counter-screen, AID 1463)	1. Long incubation time (6 h) in primary assay 2. Fluorescent readouts 3. Inactive in FP mP secondary assay 4. Active in FP total counter-screen (IC_{50} = 13 uM) 5. Not selected for follow-up (AIDs 1558, 1559, 1712)	PubChem AID 1475
Amyloid fibril formation	IC_{50} = 1–64 μM	None	ThT fluorescence assay on Tg2576 mouse brain sections; aggregation ELISA (6E10)	1. Fluorescent readouts 2. Long incubation times (> 1 h) allow for compound degradation 3. Concentrations tested above aggregation threshold 4. Follow-up work showed no affect of curcumin on Aβ aggregation states	[4-5]
CFTR	appK_i = 5–15 μM (against SERCA)	None	Nasal epithelium potential changes in ΔF508 mice	1. No evidence of target engagement 2. No confirmation of compound presence *in vivo* 3. Results irreproducible by separate lab	[6]
CB1	K_i = 5.9 nM	446-fold over CB2 (K_i > 2 μM)	Incubation with membrane fraction of CHO cells stably expressing CB1 or CB2	1. Incubation time sufficient for compound degradation 2. No orthogonal confirmation of target engagement 3. Work retracted when results were irreproducible	[7-8]
TrxR	Rat IC_{50} = 3.6 μM HeLa cells IC_{50} = 15 μM	None	DTNB reduction assay; *in vitro* and with HeLa cells	1. Concentrations tested above aggregation threshold 2. No detergent in assays 3. No target engagement for cellular assay 4. Long incubation time (2–6 h) sufficient for degradation	[9]
IRAK	IC_{50} ~ 10 μM	None	Incubation with whole cells; immunoprecipitation/ Western blot detection by anti-IRAK antibodies	1. Results do not exclude membrane disruption 2. No evidence of target engagement 3. Concentrations tested above chemical aggregation threshold	[10]
ErbB2	Only tested at 50 μM	None	Whole cell incubations; radioassay with immunoprecipitated protein	1. Results do not exclude membrane disruption 2. Incubation time (1–4 h) sufficient for compound degradation 3. No confirmation of target engagement 4. Concentration tested above chemical aggregation threshold	[11]

Supplemental Table 2. Reported half-lives of curcumin at a variety of conditions.[12-13] Note: RPMI 1640 contains glutathione but no other proteins, lipids, or growth factors.

pH	Temperature	Buffer system	$t_{1/2}$ (min)
3.0	37 °C	0.1 M citrate-phosphate	118.63
5.0	23 °C	0.1 M citrate-phosphate	> 20.0
5.0	37 °C	0.1 M citrate-phosphate	199.08
6.0	23 °C	0.1 M citrate-phosphate	> 20.0
6.0	37 °C	0.1 M phosphate	195.69
6.5	37 °C	0.1 M phosphate	153.02
6.8	37 °C	0.1 M phosphate	39.75
7.0	23 °C	0.1 M citrate-phosphate	> 20.0
7.0	37 °C	RPMI 1640	~ 20.0
7.0	37 °C	RPMI 1640 + fetal bovine serum	360–480
7.2	37 °C	0.1 M phosphate	9.40
7.2	37 °C	0.5 M phosphate	9.54
7.2	37 °C	0.025 M phosphate	9.47
7.40	37 °C	Human blood	360–480
7.5	23 °C	0.1 M citrate-phosphate	20.0
8.0	23 °C	0.1 M citrate-phosphate	~ 4.0
8.0	37 °C	0.1 M phosphate	1.05
8.5	23 °C	0.1 M phosphate	~ 7.5
9.0	23 °C	0.1 M phosphate	~ 11
10.0	37 °C	0.1 M carbonate	14.05

Supplemental Table 3. Reported activities of curcumin that are potential toxic side effects. Assay values reported as IC_{50} values unless otherwise indicated. AMMC: 3-[2-(N,N-diethyl-N-methylammonium)ethyl]-7-methoxy-4-methylcoumarin; BFC: 7-benzyloxy-4-(trifluoromethyl)-courmarin; BQ: 7-benzyloxyquinoline; CEC: 3-cyano-7-ethoxycoumarin; CDNB: 1-chloro-2,4-dinitrobenzene; DBF: dibenzylfluorescein; EROD: ethoxyresorufin deethylation; K_f = formation constant; MFC: 7-methoxy-4-(trifluoromethyl)-courmarin; βNF: β-napthoflavone; PB: phenobarbital; PROD: pentoxyresorufin depentylation;

Curcumin Reactivity	Evidence
hERG (KCNH2 potassium voltage-gated channel, subfamily H (EAG-related), member	5.55 μM, (whole cell patch-clamp HEK293; dose-dependent inhibition)[14] 4.4 ± 1.4 μM, (thallium influx); 22 μM (patch clamp)[15]
Cellular toxicity	31 μM, (murine macrophage cells, J774.1); 15.2 μM, (kidney cells, HEK 293T)[16]
CYP450 inhibition	**1A1/1A2:** K_i 0.14 μM, IC_{50} 2 μM; (competitive inhibition of EROD activity in βNF-induced rat liver microsomes) **2B1/2B2:** K_i 76.02 μM, IC_{50} 14 μM, (competitive inhibition of PROD activity in PB-induced microsomes)[17] **3A4:** 14.9 ± 1.4 μM (BFC); 54.4 ± 18.3 μM (BQ); 44.1 ± 4.2 μM (DBF) **2C9**: 6.0 ± 1.4 μM (MFC) **2D6:** 175 ± 47.0 μM (AMMC) **1A2:** 104.6 ± 22.1 μM (CEC)[18]
Glutathinone S-transferase activity	K_i 2.29 μM, inhibition of GST activity toward CDNB[17, 19-20]
Protein reactivity	See Table 1
Iron chelation	$K_f = 10^{22}$ Fe(III)-curcumin complex. Curcumin induced a phenotype of iron deficiency in mice fed a low-iron diet (5 mg iron/kg)[21] and significantly reduced iron stores in mice fed a Western-type diet.[22]

Examples of covalent modification of proteins by curcumin

TrxR (thioredoxin reductase). Curcumin has been reported to covalently modify both Cys^{496} and Sec^{497} in the C-terminal active site of the cytosolic TrxR1.[9] This ubiquitous enzyme expressed in all living cells is an essential mammalian selenocysteine (Sec)-containing flavoenzyme. It catalyzes the NADPH-dependent reduction of the redox-active disulfide in thioredoxin (Trx), an enzyme that is involved in cellular redox control. Covalent modification leads to an induction of NADPH oxidase activity that leads to an increased production of reactive oxygen species (ROS). The authors of this work speculated that this increase in the production of ROS might be beneficial as there is an overexpression of TrxRs in a variety of tumor types.

IRAK (interleukin-1 (IL-1) receptor-associated kinase). Another reported covalent target of curcumin is IRAK.[10] This kinase features five vicinal cysteines, four of which are located near arginine or histidine residues, making them particularly sensitive to reaction with electrophiles. Recruitment of IRAK to the IL-1 receptor is an early event in inflammatory signaling cascades. Alkylation of IRAK thiols by curcumin was demonstrated in a murine T-cell line stably overexpressing IKAK ($EL\text{-}4^{IRAK}$).

ErbB2. Finally, curcumin has been shown to covalently modify ErbB2 (Her2/neu), a transmembrane tyrosine kinase that acts as a coreceptor for other epithelial growth factor receptors. This covalent modification initiates the CHIP-dependent ErbB2 ubiquinination that leads to ErbB2 depletion.[11] ErbB2 was immunoprecipitated from cell lysates and [^{3}H]-curcumin binding was evaluated. The radioactivity signal increased from protein incubated with [^{3}H]-curcumin was competed away with cold curcumin.

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