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Click chemistry approach to characterize curcumin-protein interactions *in vitro* and *in vivo*

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Abbreviations: Keap1, Kelch-like ECH-associated protein 1; **Nrf2**, nuclear factor erythroid 2-related factor 2; **NF-κB**, nuclear factor kappa-light-chain-enhancer of activated B cells; **mono-Cur**, mono-propargyl curcumin; **di-Cur**, di-propargyl curcumin; **DMEM**, Dulbecco's modified Eagle's medium; **FBS**, fetal bovine serum; **MTT**, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **THPTA**, tris-hydroxypropyltriazolylmethylamine.

Abstract

Curcumin, a bis-α, β-unsaturated β-diketon dietary compound from turmeric, is among the most promising dietary compounds for preventing chronic diseases. Previous research has shown that curcumin is highly reactive toward protein thiols to form curcumin-protein adducts, however, the interactions of curcumin with proteins are under-studied. Here we report the design and synthesis of "click" chemistry probes of curcumin, mono-propargyl curcumin (mono-Cur) and di-propargyl curcumin (di-Cur), and use the click probes to study curcumin-proteins interactions *in vitro* and *in vivo*. We find that compared with di-Cur, the mono-Cur probe has more potent biological effects and enhanced effects to label proteins in cultured cells, suggesting that mono-Cur is a better click probe to study the biological actions of curcumin. Furthermore, using the mono-Cur probe, we find that oral administration of this probe in mice leads to formation of curcumin-protein adducts in colon and liver tissues of C57BL/6 mice, suggesting that curcumin could covalently modify cellular proteins *in vivo*. Together, these results could help us to better understand protein-curcumin interactions. These results could in part explain the poor pharmacokinetics of curcumin; in addition, formation of these protein adducts could contribute to the health-promoting effects of curcumin.

Keywords: curcumin; click chemistry probe synthesis; protein adducts; distribution

Introduction

Curcumin, a dietary compound from turmeric, is among the most promising dietary compounds for preventing chronic diseases such as inflammation and cancer [1, 2]. Notably, two Phase II human clinical trials have shown that curcumin reduced the risks of colorectal cancer [3] and pancreatic cancer [4]. Currently the biological effects of curcumin are being evaluated in over 100 human clinical trials [5]. A better understanding of its biological effects and action mechanisms could help to develop novel anti-cancer agents.

Previous research to study the pharmacokinetics and/or tissue distribution of curcumin has focused on analyzing the tissue concentrations of free-form curcumin or its metabolites such as curcumin glucuronide and curcumin sulfate [6-8]. However, curcumin has a bis-α,β-unsaturated β-diketone moiety, and therefore it is highly reactive toward protein thiols to form curcumin-protein adducts via Michael reaction [9]. It is important to study the formation of curcumin-protein adducts, since substantial research has shown that some protein thiols contribute to cell signaling, and covalent modification of these thiol groups could have important biological significance. For example, previous research has shown that covalent modification of thiol groups of Kelch-like ECH-associated protein 1 (Keap1) protein leads to disruption of Keap1-nuclear factor erythroid 2-related factor 2 (Nrf2) complex and induction of phase II detoxification enzymes [10], and covalent modification of thiol groups of p65 subunit of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) protein complex impairs NF-κB-DNA binding and suppresses NF-κB-mediated inflammatory responses [11]. However, to date, the curcumin-protein interactions are under-studied. In particular, it mains unknown whether curcumin could covalently modify cellular proteins *in vivo* after oral ingestion.

Alkene and azide [3+2] cycloaddition click chemistry-based imaging is a novel and powerful method for analysis of the interactions of proteins with small molecules [12]. In this study, we report the chemical synthesis and biological activity of "click" probes of curcumin, and use the click chemistry approach to study the interactions of curcumin with proteins *in vitro* and *in vivo*.

Material and Methods

Chemical synthesis of mono-propargyl curcumin (mono-Cur)

Chemical synthesis of 3-Methoxy-4-(prop-2-yn-1-yloxy) benzaldehyde: Vanillin (1 g, 6.57 mmol, Sigma-Aldrich, St. Louis, MO) and potassium carbonate (4.54 g, 33 mmol) were mixed in 50 mL methanol, followed by addition of 80 wt% propargyl bromide solution dissolved in toluene (4.91 g, 33 mmol). The reaction mixture was stirred under nitrogen at room temperature overnight. The reaction product was extracted with ethyl acetate and the organic layers were combined, washed with water, dried with anhydrous magnesium sulfate, and evaporated under vacuum using a rotatory evaporator to offer 3-Methoxy-4-(prop-2-yn-1-yloxy) benzaldehyde. The product was used for reaction without purification. All the regents are purchased from Thermo Fisher Scientific (Waltham, MA) except specially provided.

Chemical synthesis of 3, 5-Hexadien-2-one, 4-hydroxy-6-(4-hydroxy-3-methoxyphenyl)-:
Boric anhydride (0.69 g, 10 mmol, Sigma-Aldrich) was added to 50 mL anhydrous ethyl acetate, followed by addition of acetylacetone (4.12 mL, 40 mmol). The mixture was stirred at 50 °C for 30 min. Subsequently, vanillin (1.52 g, 10 mmol) and tributyl borate (5.38 mL, 20 mmol) were added and the mixture was stirred at 50 °C for another 30 min. Then n-butylamine (0.73 mL, 10 mmol) dissolved in 15 mL anhydrous ethyl acetate was added dropwise. The reaction mixture was stirred

under nitrogen at 80 °C for 4 h and then at room temperature overnight. After 30 mL hydrochloric acid solution was added, the mixture was stirred for 30 min to quench the reaction. The reaction product was extracted with ethyl acetate. The crude product was purified by column chromatography using 50% ethyl acetate in hexane as the mobile phase and silica gel as the stationary phase to afford 3,5-Hexadien-2-one, 4-hydroxy-6-(4-hydroxy-3-methoxyphenyl)-.

Chemical synthesis of mono-Cur: Boric anhydride (0.60 g, 0.85 mmol) was added to 30 mL anhydrous ethyl acetate, followed by addition of furoylacetone (0.40 g, 1.71 mmol). The mixture was stirred at 50 °C for 30 min. 3-Methoxy-4-(prop-2-yn-1-yloxy) benzaldehyde (0.32 g, 1.71 mmol) and tributyl borate (460 µL, 1.71 mmol) were added and the reaction mixture was stirred at 50 °C for another 30 min. Then n-butylamine (169 µL) dissolved in 10 mL anhydrous ethyl acetate was added dropwise. The reaction mixture was stirred under nitrogen at 80 °C for 4 h and at room temperature overnight. After 30 mL hydrochloric acid was added, the mixture was stirred for 30 min to quench the reaction. The reaction product was extracted with ethyl acetate, the organic layers were combined, washed with water, dried with anhydrous magnesium sulfate, and evaporated to dryness under vacuum using a rotary evaporator. The crude product was purified by a silica gel column chromatography eluted with a gradient of ethyl acetate-hexane gradient (40:60 to 50:50) to afford mono-Cur probe.

mono-Cur probe was obtained as orange plate. High resolution ESI-MS showed [M-H^+] at 405.1342 (calcd. for 405.1344). The purity of the synthesized compound was also checked by ^{1}H NMR and HPLC analysis. For the HPLC analysis, it was conducted on Agilent 1100 HPLC (Agilent, Santa Clara, CA, USA) using Kromasil 100-5-C18 column (4.6 x 250 mm, 5 µm), eluted with a mobile phase of 20% water with 0.1% acetic acid (phase A) and 80% methanol with 0.1% acetic acid (phase B), at a flow rate of 1.0 mL/min and detection wavelengths at 254 nm and 420 nm.

Chemical synthesis of di-propargyl curcumin (di-Cur)

Boric anhydride (0.35 g, 5 mmol) was added to 50 mL anhydrous ethyl acetate, followed by addition of acetylacetone (1.03 mL, 10 mmol). The mixture was stirred at 50 °C for 30 min. 3-Methoxy-4-(prop-2-yn-1-yloxy) benzaldehyde (3.8 g, 20 mmol) and tributyl borate (10.8 mL, 40 mmol) were added and the mixture was stirred at 50 °C for another 30 min. Then n-butylamine (0.4 mL, 5 mmol) dissolved in 15 mL anhydrous ethyl acetate was added dropwise. The reaction mixture was stirred under nitrogen at 80 °C for 4 h and at room temperature overnight. After 30 mL hydrochloric acid was added, the mixture was stirred for 30 min to quench the reaction. The reaction product was extracted with ethyl acetate, the organic layers were combined, washed with water, dried with anhydrous magnesium sulfate, and evaporated to dryness under vacuum using a rotary evaporator. The crude product was purified after recrystallization from methanol. Di-Cur probe was obtained as orange powder. High resolution ESI-MS showed [M-H^+] at 443.1494 (calcd. for 443.1500). The purity of the compound was validated by HPLC analysis, as described above.

Cell culture

MC38 colon cancer cells (a kind gift from Prof. Ajit Varki at the University of California San Diego) were cultured in Dulbecco's modified Eagle's medium (DMEM, Lonza, Allendale, NJ) supplemented with 10% fetal bovine serum (FBS, Corning, NY) in a 37 °C incubator under an atmosphere with 5% CO_2.

Cell proliferation assay

MC38 cells were seeded in 96-well plates at a density of 8,000 cells per well in 100 μL DMEM complete medium and were allowed to attach overnight. The cells were treated with test compounds

or DMSO vehicle in complete medium for 24 h. Cell proliferation was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO) assay.

Click chemistry-based fluorescence SDS-PAGE

MC38 cells were treated with curcumin, mono-Cur, di-Cur, or DMSO vehicle in complete medium for 2-4 h. After aspirating the medium, the cells were washed with PBS, centrifuged at 1,000 rpm at 4 °C for 5 min. Cellular proteins were then extracted using RIPA lysis buffer (Boston BioProducts, Ashland, MA) with protease inhibitor cocktail (100 ×, Boston BioProducts) for 30 min at 4 °C. After centrifugation at 12,000 rpm at 4 °C for 20 min, the supernatants were collected and the protein concentrations were determined using a BCA protein assay kit (Thermo Fisher Scientific).

The supernatant (containing 60 μg protein) was incubated with $CuSO_4$ (50 μM, Thermo Fisher Scientific), ascorbic acid (50 μM, Thermo Fisher Scientific), tris-hydroxypropyltriazolylmethylamine (THPTA, 50 μM, Lumiprobe, Hunt Valley, MD), and CruzFluor sm^{TM} 8 azide (50 μM, Santa cruz, Dallas, TX) for 1 h at room temperature in dark. After the reaction, the solution was 1:1 mixed with SDS-PAGE loading buffer (Amresco, Solon, OH), heated at 95 ºC for 10 min, and resolved on 15% SDS-PAGE gels. To visualize “click” labelled proteins, in-gel fluorescence imaging was performed using Odyssey imaging system (LI-COR Biosciences) at 800 nm. The total protein levels in the gels were determined by coomassie blue staining.

Click chemistry-based proteomics

MC38 cells were treated with curcumin, mono-Cur, or DMSO vehicle in complete medium for 2-4 h, then cell lysis was performed as described above. To facilitate protein purifications, we used a click reaction to label the target proteins with biotin. Briefly, the supernatant from the lysed cell was

incubated with $CuSO_4$ (50 μM, Thermo Fisher Scientific), ascorbic acid (50 μM, Thermo Fisher Scientific), THPTA (50 μM, Lumiprobe), and biotin azide (PEG4 carboxamide-6-azidohexanyl biotin, 50 μM, Thermo Fisher Scientific) for 1 h at room temperature in dark. The biotinylated proteins were purified using SoftLink™ soft release avidin resin (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, ~1.5 mL resin was poured into a polypropylene chromatography column (BioRad, Hercules, CA). To generate the resin, the resin was saturated with 5 mM biotin (Thermo Fisher Scientific) and then eluted with Tris buffer (50 mM Tris, 150 mM NaCl, pH 7.9) until the column was equilibrated. The reaction mixture was loaded into the column, washed with Tris buffer, and eluted with 5 mM biotin in Tris buffer to obtain the biotinylated proteins which were then concentrated and de-salted by Pierce concentrator (10K MWCO, Thermo Fisher Scientific). For LC-MS/MS analysis, the isolated protein was 1:1 mixed with SDS-PAGE loading buffer (Amresco), heated at 95 ºC for 10 min, and resolved on 15% SDS-PAGE gels. The gels were stained by coomassie blue, and the gels containing proteins were cut for LC-MS/MS at the Mass Spectrometry Facility at the University of Massachusetts Worcester.

Animal experiment

The animal experiment was conducted in accordance with the protocols (No. 2017-0019) approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Amherst. C57BL/6 male mice (age = 6 weeks) were purchased from Charles River (Wilmington, MA). After acclimation in the animal facility, the mice were divided into 3 groups, and treated with: (1) curcumin (dose = 100 mg/kg/day) dissolved in 200 μL polyethylene glycol 400 (PEG 400, EMD Millipore, Billerica, MA), (2) mono-Cur (dose = 100 mg/kg/day) dissolved in PEG 400, and (3) PEG 400 vehicle, by oral gavage. After 4 h, the mice were sacrificed, and tissues were collected for analysis.

Click chemistry-based fluorescence SDS-PAGE for animal tissues

The animal tissues (colon, small intestine, etc) were grounded after frozen by liquid nitrogen. The proteins from the homogenized animal tissues were extracted with RIPA lysis buffer (Boston BioProducts) with a protease inhibitor cocktail (100 ×, Boston BioProducts). The click chemistry reaction and SDS-PAGE fluorescence imaging were performed as described above.

Statistical analysis

Data are expressed as means ± SEM. Statistical comparison of two groups was performed using either Student's *t* test, and comparison of three groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The statistical analyses were performed using Prism 6 (GraphPad Software Inc., San Diego, CA) and $P < 0.05$ was considered statistically significant.

Results

Effects of mono-Cur and di-Cur probes on cell proliferation in MC38 colon cancer cells

We designed and synthesized two click chemistry probes of curcumin, mono-Cur and di-Cur (see chemical synthesis in **Fig. 1**). The purities and structures of the synthesized compounds were validated by HPLC, NMR, and MS analysis (HPLC data see supplemental Fig. S1 and S2, NMR data see Table S1 and S2).

We studied the biological effects of curcumin and its click probes in MC38 colon cancer cells **(Fig. 2)**. Compared with curcumin, mono-Cur had similar potency to suppress MC38 cell proliferation, while di-Cur probe had weaker effects. Notably, at a concentration of 50 μM, treatment with curcumin and mono-Cur for 24 h caused a ~80% inhibition of MC38 cell proliferation, while di-Cur probe only inhibited ~40% of cell proliferation.

Effects of mono-Cur and di-Cur on protein labeling in MC38 colon cancer cells

We tested the effects of curcumin click probes on protein labeling in MC38 colon cancer cells. We treated MC38 cells with vehicle (DMSO), curcumin, mono-Cur and di-Cur (50 μM) for 1-4 h, then performed click chemistry-based in-gel fluorescence to image labelled proteins (**Fig. 3A**). Both click probes (mono-Cur and di-Cur) were capable of labeling multiple cellular proteins in MC38 cells (**Fig. 3B**). In comparison, mono-Cur had more potent effect to label proteins than di-Cur. As expected, treatment with vehicle (DMSO) or curcumin showed little fluorescence signals.

Next, we studied the dose-dependent effects of curcumin click probes on protein labeling in MC38 cells. We treated MC38 cells with vehicle (DMSO), curcumin, mono-Cur and di-Cur (20-100 μM) for 4 h, then performed in-gel fluorescence imaging (**Fig. 3C**). In agreement with results above (**Fig. 3B**), both curcumin click probes were capable of labeling proteins *in vitro*, and mono-Cur was more effective to label cellular proteins in MC38 cells (**Fig. 3C**).

We used a LC-MS/MS-based proteomics to identify the labelled proteins. We treated MC38 cells with vehicle (DMSO), curcumin, or mono-Cur for 24 h, then performed biotin-based click chemistry for protein isolation and subsequent LC-MS/MS analysis. LC-MS/MS results showed that 12 proteins were only detected in mono-propargyl curcumin-treated cells, but not in vehicle- or curcumin-treated cells (see **supplemental information Table S3**), supporting that these proteins could be binding proteins of mono-Cur.

Effects of mono-Cur on protein labeling *in vivo*

We tested whether curcumin could label cellular proteins after oral ingestion in mice. The mice were treated with vehicle (PEG 400), curcumin, or mono-Cur via oral gavage. After 4 h, we harvested

different organs and performed click chemistry-based in-gel fluorescence to image labelled proteins in tissues (**Fig. 4A**). We found that liver (**Fig. 4B**) and colon (**Fig. 4C**) had clear fluorescence signals, illustrating the presence of labelled proteins in these tissues. In contrast, other organs, such as intestine (**Fig. S3**), had little fluorescence imaging signals, suggesting little presence of labelled proteins in these tissues.

Discussion

In this study, we have designed and synthesized two "click" probes of curcumin (mono-Cur and di-Cur), and used these probes to characterize curcumin-protein interactions *in vitro* and *in vivo*. Our results support that both of the two click probes mono-Cur and di-Cur can be detected using the fluorescence with the protein combination. Compared with di-Cur, the mono-Cur probe is a better probe to study the biological actions of curcumin. Indeed, mono-Cur has similar biological action compared with curcumin, while di-Cur has weaker effect. Consistent with this result, we further found that compared with di-Cur, mono-Cur has more potent effect to label cellular proteins *in vitro*. Together, these results support that mono-Cur probe is a better click chemistry probe to study the biological actions of curcumin. There could be several reasons for the different actions of mono-Cur and di-Cur. For example, di-Cur has chemical modifications on both hydroxyl (-OH) groups in curcumin whereas mono-Cur only changed one -OH group. More deletion of -OH group in di-Cur could have reduced polarity and attenuated permeability across cell membrane. These results also suggest that the hydroxyl (-OH) groups contribute to the biological actions of curcumin.

A central finding of our research is that curcumin could covalently modify cellular proteins *in vitro* and *in vivo*. Previous research has shown that protein thiols contribute to cell signaling, therefore, covalent modification of protein thiols could have important biological significance. For example,

covalent modification of cysteine residues of Keap1 protein leads to disruption of Keap1-Nrf2 complex and activation of Nrf2-mediated phase II detoxification signaling pathway [10, 13, 14], and covalent modification of Cys38 residue of p65 protein (a component of NF-κB protein complex) blocks NF-κB-DNA binding and suppresses NF-κB-mediated inflammatory responses. Therefore, it is likely that formation of curcumin-protein complex could contribute to the health-promoting effects of curcumin. A recent click chemistry-based proteomics study showed that curcumin covalently modifies casein kinase I gamma, and this protein is critical for the bioactivities of curcumin [9]. Here our LC-MS/MS-based proteomics did not detect this protein, and this difference could be because different cell lines were used in the experiments. Further elucidation of the direct cellular targets of curcumin could help to better understand the action mechanisms of curcumin, facilitating the development of novel compounds for disease prevention.

Using the click chemistry probe of curcumin (mono-Cur), we studied whether curcumin could covalently label proteins *in vivo* after oral ingestion. Our results showed that 4 h after oral consumption of mono-Cur, click chemistry-based in-gel fluorescence imaging showed the formation of labelled proteins in the colon and liver tissues, which are the two important metabolic organs of curcumin. Multiple studies showed that curcumin can be used as a chemopreventive agent for colorectal cancer through many molecular pathways, such as NF-κB and activator protein 1 (AP-1) [15, 16], which may be explained by our results. This result suggests that after oral administration of curcumin, curcumin could enter intracellular space, covalently modify the proteins, and get trapped in the intracellular space. This may contribute to the poor pharmacokinetics of curcumin. Indeed, previous studies have shown that after oral intake of curcumin, the concentrations of free-form curcumin in plasma and tissues are extremely low [17-20]. Notably, after a single oral intake of 10-12 g of curcumin, the free form of curcumin was barely detected in human plasma [6]. This leads to the hypothesis that the biological effects of curcumin could be mediated by its metabolites. However,

the major metabolites of curcumin detected in the circulation are curcumin sulfate and curcumin glucuronide, which have been shown to be less-active or inactive [6-8, 21]. Here, our results showed that curcumin forms the complexes with proteins in specific tissues in mice, such as liver and colon, supporting that these protein complexes may contribute to the beneficial effects of curcumin. However, it is hard to compare the detected fluorensece proteins *in vivo* and *in vitro* because of the different systems. Moreover, further studies are needed to clarify the protein targets of curcumin *in vivo* and the roles of curcumin-protein complexes in the biological actions of curcumin. Meanwhile, it is important to look for the approaches to improve the structural stability of curcumin as well. For example, nanoparticle curcumin with increased water solubility is developed and found that it increased the bioavailability in human subjects [22]. Other developing approaches such as liposomes, micelles and phospholipid complexes of curcumin were also widely reported to enhance the bioavailability of curcumin and reviewed by Mirzaei et al. (2017) [23].

Together, using click chemistry-based in-gel fluorescence imaging, our results support that curcumin can covalently modify cellular proteins *in vitro* and *in vivo*. These results could in part explain the poor pharmacokinetics of curcumin observed in previous studies. In addition, formation of these protein adducts could contribute to the health-promoting effects of curcumin.

Author contributions

H.Y., E.S. and G.Z. designed the experiments. H.Y., E.S., Z.D., W.W., M.A., Y.N., and J.Z. performed the experiments. H.Y, E.S., and G.Z analyzed the data and wrote the manuscript. All the authors read and approved the final manuscript.

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The authors have declared no conflict of interest.

Supporting information

Figure S1. HPLC analysis of mono-Cur.

Figure S2. HPLC analysis of di-Cur.

Figure S3. Click chemistry imaging of colon tissues. (A) fluorescence imaging; (B) protein coomassie blue staining. n = 4 per group.

Table S1. ^{1}H NMR chemical shifts and J-coupling constants for mono-Cur (400 MHz, chloroform-d)

Table S2. ^{1}H NMR chemical shifts and J-coupling constants for di-Cur (400 MHz, chloroform-d)

Table S3. List of proteins binding with the mono-Cur probe in MC 38 cells identified by LC-MS/MS.

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Figure legends

Figure 1. Chemical synthesis strategy to prepare the click chemistry probes of curcumin (di-Cur and mono-cur).

Figure 2. Effects of curcumin and its click probes on cell proliferation. The results are expressed as mean ± SEM, n = 6/group.

Figure 3. Click chemistry-based in-gel SDS-PAGE fluorescence imaging in MC38 colon cancer cells. (A) Scheme of the experiment. (B) Time-course effects of curcumin click probes to label cellular proteins in MC38 cells. MC38 cells were treated with 50 μM curcumin, mono-Cur, or di-Cur, or DMSO vehicle for 1-4 h. Left panel: fluorescence imaging; Right panel: coomassie blue staining. (C) Dose-dependent effects of curcumin click probes to label cellular proteins in MC38 cells. MC38 cells were treated with varied doses of curcumin, mono-Cur, or di-Cur, or DMSO vehicle for 4 h. Left panel: fluorescence imaging; Right panel: coomassie blue staining.

Figure 4. Click chemistry-based in-gel SDS-PAGE fluorescence imaging in mice. (A) Scheme of the experiment. (B) Click chemistry imaging of liver tissues. Left panel: fluorescence imaging; Right panel: protein coomassie blue staining. (C) Click chemistry imaging of colon tissues. Left panel: fluorescence imaging; Right panel: protein coomassie blue staining. n = 4 per group.

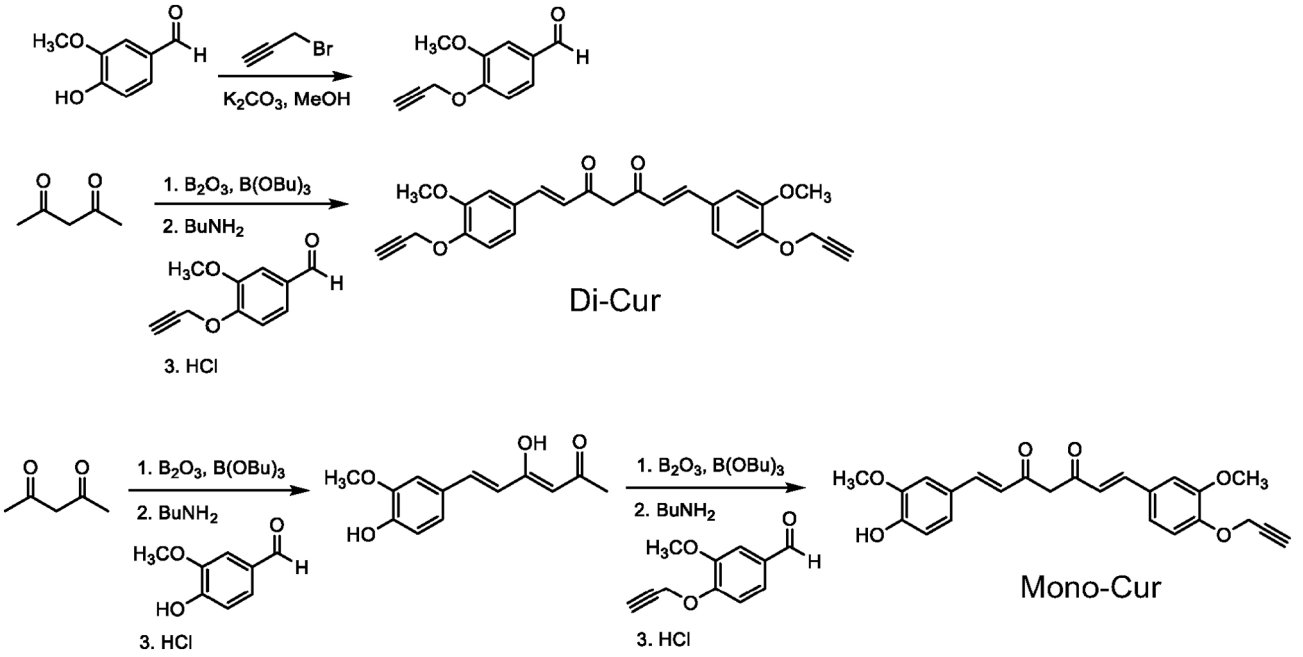
H3CO
O
H
HO
Br
K2CO3, MeOH
1. B2O3, B(OBu)3
2. BuNH2
3. HCl
OCH3
Di-Cur
OH
Mono-Cur

Figure 1

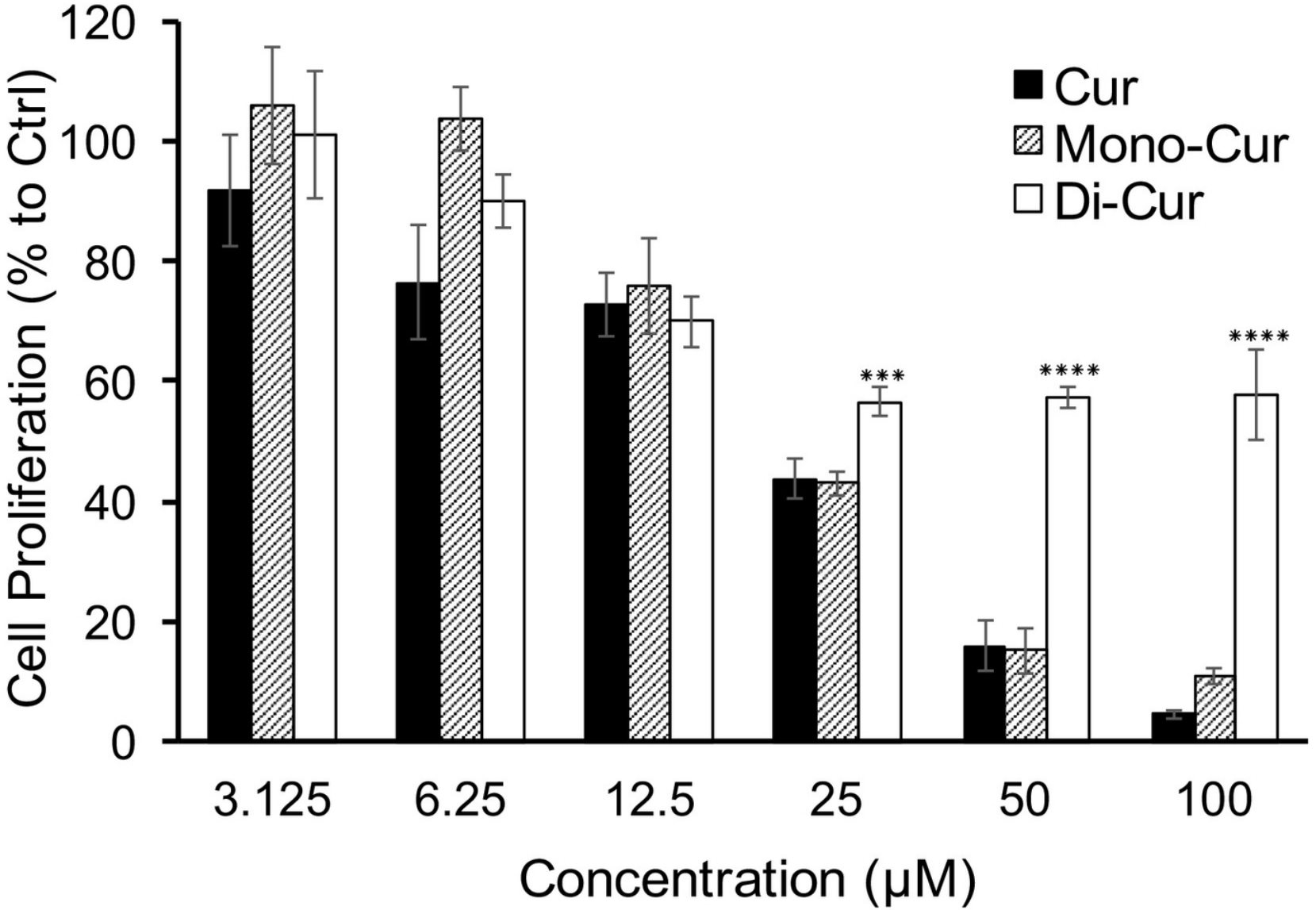
120
100
80
60
40
20
0
Cell Proliferation (% to Ctrl)
Cur
Mono-Cur
Di-Cur
***
****
****
3.125
6.25
12.5
25
50
100
Concentration (μM)

Figure 2

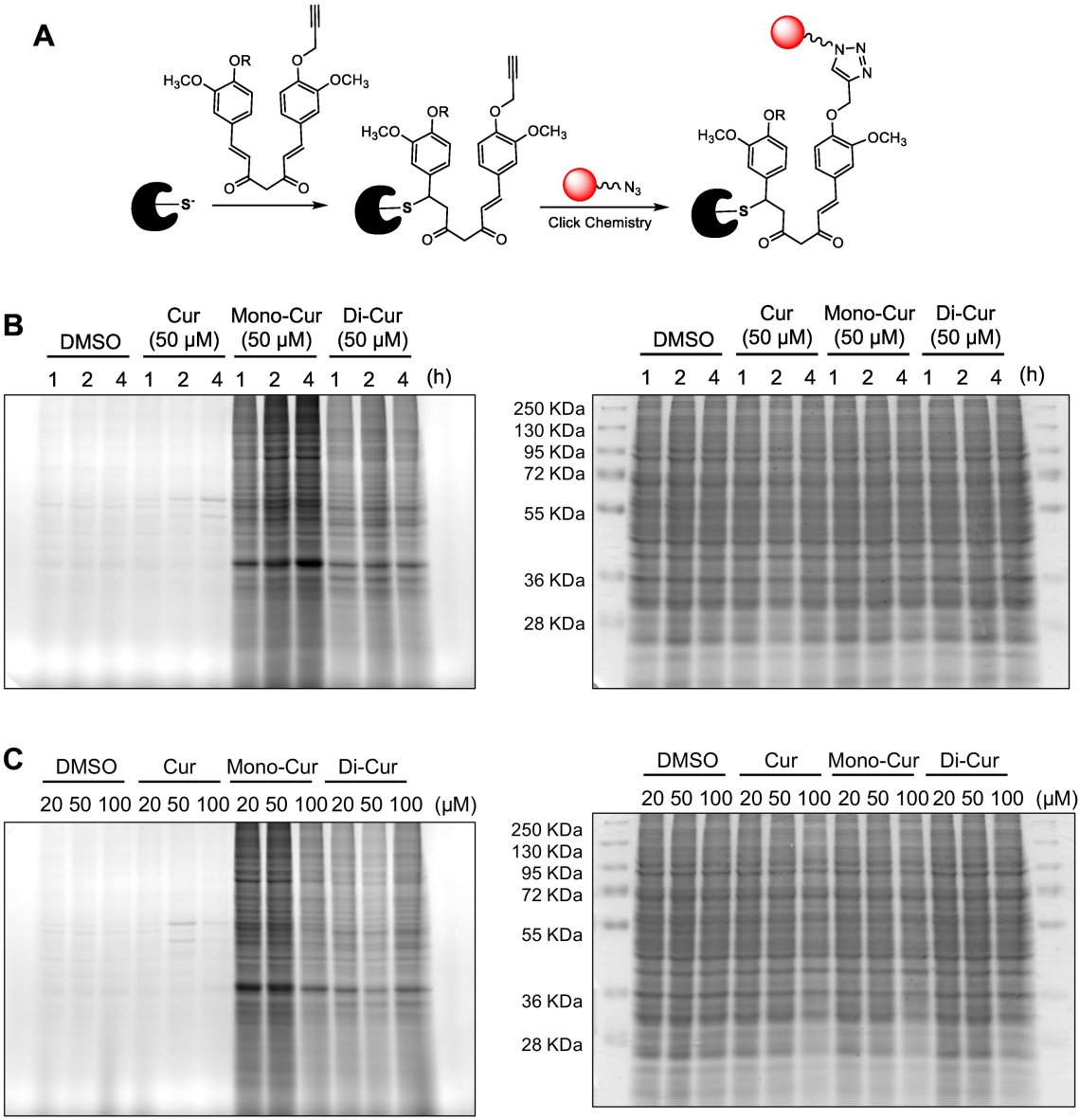
A
Click Chemistry
B
DMSO
Cur (50 μM)
Mono-Cur (50 μM)
Di-Cur (50 μM)
1 2 4 1 2 4 1 2 4 1 2 4 (h)
250 KDa
130 KDa
95 KDa
72 KDa
55 KDa
36 KDa
28 KDa
C
DMSO
Cur
Mono-Cur
Di-Cur
20 50 100 20 50 100 20 50 100 20 50 100 (μM)

Figure 3

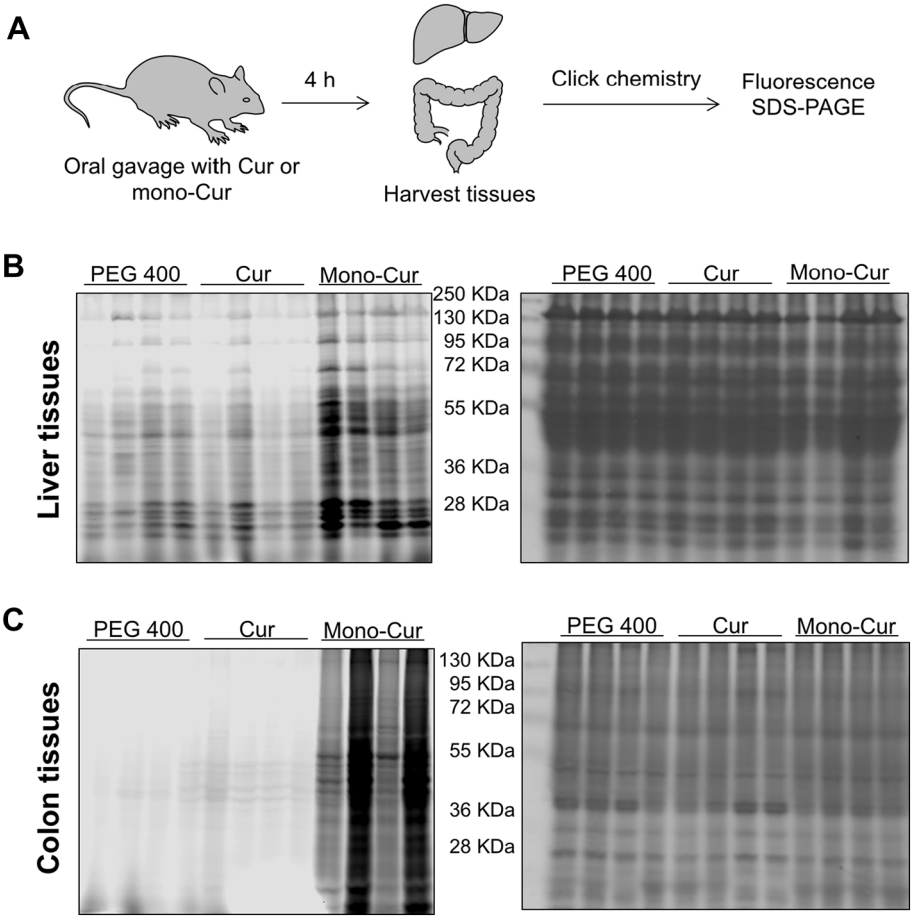
A
4 h
Oral gavage with Cur or mono-Cur
Harvest tissues
Click chemistry
Fluorescence SDS-PAGE
B
Liver tissues
PEG 400
Cur
Mono-Cur
250 KDa
130 KDa
95 KDa
72 KDa
55 KDa
36 KDa
28 KDa
PEG 400
Cur
Mono-Cur
C
Colon tissues
PEG 400
Cur
Mono-Cur
130 KDa
95 KDa
72 KDa
55 KDa
36 KDa
28 KDa
PEG 400
Cur
Mono-Cur

Figure 4