A tuned affinity-based staurosporine probe for \textit{in situ} profiling of protein kinases

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1. General Information

All chemicals were purchased from commercial vendors and used without further purification, unless indicated otherwise. All reaction requiring anhydrous conditions were carried out under argon or nitrogen atmosphere using oven-dried glassware. HPLC-grade solvents were used for all reactions. Reaction progress was monitored by TLC on pre-coated silica plates (Merck 60 F254, 0.25 μm) and spots were visualized by UV or iodine stain. Flash column chromatography was carried out using Merck 60 F254, 0.040-0.063 μm silica gel. All NMR spectra (1H-NMR, 13C-NMR) were recorded on a Bruker NMR spectrometer (300MHz for 1H and 75 MHz for 13C). Chemical shifts are reported in parts per million referenced with respect to appropriate internal standards or residual solvent peaks (CDCl3 = 7.26 ppm, CD3OD = 3.31 ppm, DMSO-d6 = 2.50 ppm). The following abbreviations were used in reporting spectra, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, br = broad. All analytical HPLC were carried out on Shimadzu LCMS (IT-TOF) system or Shimadzu LCMS-2010EV system equipped with an auto-sampler using reverse-phase Phenomenex Luna 5 μm C18(2) 100 Å 50 × 3.0 mm columns. Water with 0.1% TFA and acetonitrile with 0.1% TFA were used as eluents and the flow rate was 0.6 mL/min. For enzyme inhibition and IC50 measurements, Tecan microplate reader (Multimode Reader, Infinite® 200) in luminescence mode with i-controlTM software was used. Fluorescence scanning of the SDS-PAGE gels was carried out with Typhoon 9410 fluorescence gel scanner (Amersham Biosciences). Imaging was done with the Leica TCS SP5X confocal microscope system equipped with Leica HCX PL APO 63×/1.20 W CORR CS, 405 nm diode laser, white laser (470−670 nm, with 1 nm increments, with eight channels AOTF for simultaneous control of eight laser lines, each excitation wavelength provides 1.5 mV), and a photomultiplier tube (PMT) detector ranging from 410 to 700 nm for steady state fluorescence. Images were processed with Leica Application Suite Advanced Fluorescence (LAS AF). All enzymes used were expressed in \textit{E. coli} strain BL21-DE3 and purified as described previously.1 Staurosporine (98%) was purchased from Invitrogen. Tris(2-carboxyethyl) phosphine (TCEP), and the click chemistry ligand, tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) were purchased from Sigma-Aldrich. Antibodies against c-Src (B-12) (sc-8056) and CDK1 (ab133327) were purchased from Santa Cruz Biotechnology and Abcam, respectively.

![Fig. S1. Structures of the two different azide reporters used in the present study.](image-url)
2. Synthesis of probe STS-C1

(9H-Fluoren-9-y1)methyl (3-(tert-butoxy)-1-oxo-1-(prop-2-yn-1-ylamino)propan-2-yl)carbamate (2). To the solution of Fmoc-O-tert-Butyl-L-serine (3.83 g, 10 mmol), HBTU (5.7 g, 15 mmol) and HOBut (2.7 g, 20 mmol) in dry DMF (150 mL), after 10 min of stirring, was added propargyl amine (0.96 mL, 15 mmol) and DIEA (3.5 mL, 20 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 h. Solvent was removed in vacuo to give the residue which was purified by column chromatography to give a white solid. (4.0 g, 95%). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.77 (d, $J = 7.5$ Hz, 1H), 7.60 (d, $J = 7.3$ Hz, 1H), 7.41 (t, $J = 7.2$ Hz, 1H), 7.32 (t, $J = 7.4$ Hz, 1H), 6.89 (s, 1H), 5.72 (s, 1H), 4.42 (d, $J = 6.8$ Hz, 1H), 4.05 (dd, $J = 5.1$, 2.6 Hz, 1H), 3.81 (s, 1H), 3.37 (t, $J = 8.4$ Hz, 1H), 2.24 (t, $J = 2.5$ Hz, 1H), 1.22 (s, 5H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 172.74, 79.46, 73.36, 71.19, 63.57, 54.88, 28.60, 27.25.

2-Amino-3-(tert-butoxy)-N-(prop-2-yn-1-yl)propanamide (3). The solution of compound 2 (2 g, 4.8 mmol) in piperidine-DCM (1:4, 50 mL) was stirred at room temperature for 30 min. The reaction was concentrated in vacuo to give a white solid which was purified by column chromatography to give compound 3 as a colorless oil. (0.94 g, quantitative). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.66 (s, 1H), 4.29 – 3.79 (m, 2H), 3.66 – 3.20 (m, 3H), 2.21 (t, $J = 2.5$ Hz, 1H), 1.14 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 172.74, 79.46, 73.36, 71.19, 63.57, 54.88, 28.60, 27.25.

3-(tert-Butoxy)-2-(2-chloroacetamido)-N-(prop-2-yn-1-yl)propanamide (4). To the solution of 2-chloroacetyl chloride (1.13 g, 10 mmol) and DIEA (2.6 mL, 15 mmol) in dry DCM (10 mL) was added a solution of compound 3 (0.94 g, 5 mmol) in dry DCM (5 mL) at 0°C. After 30 min of stirring at 0°C, the reaction was allowed to warm to room temperature. The reaction mixture was next concentrated in vacuo and the resulting residue was redissolved in DCM (10 mL), washed with aqueous saturated sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulphate and concentrated. The resulting residue was purified by column chromatography to give a white solid. (1.0 g, 72%). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.49 (d, $J = 6.5$ Hz, 1H), 7.12 (s, 1H), 4.40 (td, $J = 7.4$, 4.4 Hz, 1H), 4.12 – 3.84 (m, 4H), 3.72 (dd, $J = 8.7$, 4.2 Hz, 1H), 3.35 (t, $J = 8.3$ Hz, 1H), 2.21 (t, $J = 2.2$ Hz, 1H), 1.14 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 169.43, 166.24, 78.97, 74.26, 71.01, 63.57, 54.88, 28.60, 27.16.

2-(2-Chloroacetamido)-3-hydroxy-N-(prop-2-yn-1-yl)propanamide (5). Compound 4 (0.8 g, 2.9 mmol) at room temperature was added TFA (15 mL). After 45 min of stirring, the reaction was concentrated. The resulting residue was redissolved in ethyl acetate (10 mL) and washed with aqueous saturated sodium bicarbonate. The aqueous layer was extracted with ethyl acetate (10 mL X 3). The combined organic layers were dried over anhydrous sodium sulphate, concentrated and further purified by column chromatography to give the desired product as a
white solid (0.25 g, 39%). $^1$H NMR (500 MHz, MeOD) $\delta$ 4.44 (t, $J = 5.1$ Hz, 1H), 4.24 – 4.08 (m, 2H), 4.08 – 3.92 (m, 2H), 3.80 (qd, $J = 11.2$, 5.2 Hz, 2H), 2.58 (t, $J = 2.5$ Hz, 1H); $^{13}$C NMR (75 MHz, MeOD) $\delta$ 171.57, 169.28, 80.29, 72.34, 62.85, 56.89, 43.20, 29.61.

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Electronic Supplementary Material (ESI) for Chemical Communications

3. Anti-proliferation

Cell viability was determined using the XTT colorimetric cell proliferation kit (Roche) following manufacturer’s guidelines, as well as previous published protocols. $^2$ Briefly, cells were grown to 20-30% confluence (since they will reach ~90% confluence within 48 to 72 h in the absence of drugs) in 96-well plates under the conditions described above. The medium was aspirated, and then washed with PBS, and treated in duplicate, with 0.1 mL of the medium containing different concentrations of probes (10, 100 nM) or drug (10, 100 nM, as a positive control). Probes were applied from DMSO stocks whereby DMSO never exceeded 1% in the final solution. The same volume of DMSO was prepared according to reference 2 $(5$ mg, 8.8 pmol; prepared according to reference 2) in DMSO (0.5 mL) was added compound 5 (3.9 mg, 17.8 pmol), EDC (15.4 mg, 80 pmol) and DMAP (2.4 mg, 20 pmol) at 0 °C. Then the reaction was allowed to warm to r.t. and stirred at r.t. overnight until no more product formed monitored by LC-MS. Upon further purification by reverse-phase semi-preparative HPLC (10-95% ACN/H$_2$O), the desired product was obtained (0.5 mg, 7.4%). $^1$H NMR (300 MHz, DMSO) $\delta$ 9.28 (d, $J = 8.2$ Hz, 1H), 8.67 (t, $J = 5.1$ Hz, 1H), 8.60 (s, 1H), 8.55 (d, $J = 8.1$ Hz, 1H), 8.05 (d, $J = 7.9$ Hz, 1H), 7.99 (d, $J = 8.6$ Hz, 1H), 7.67 (d, $J = 8.5$ Hz, 1H), 7.49 (t, $J = 7.9$ Hz, 2H), 7.41 – 7.33 (m, 1H), 7.29 (t, $J = 7.4$ Hz, 1H), 7.03 (t, $J = 6.9$ Hz, 1H), 5.00 (s, 3H), 4.64 – 4.57 (m, $J = 14.1$, 5.9 Hz, 2H), 4.33 – 4.15 (m, 5H), 3.92 (s, 2H), 2.82 (s, 3H), 2.78 (s, 3H), 2.73 (s, 1H), 2.68 (s, 1H), 2.54 (s, 1H), 2.33 (s, 3H), 2.21 (m, 1H); LCMS m/z calcd. for C$_{40}$H$_{35}$ClN$_{6}$O$_{8}$ [M+H]$^+$ 767.2596, found 767.244; C$_{40}$H$_{35}$ClN$_{6}$O$_{8}$ [M+Na]$^{2+}$ 789.2416, found 789.224 (IT-TOF).

3. Anti-proliferation

Cell viability was determined using the XTT colorimetric cell proliferation kit (Roche) following manufacturer’s guidelines, as well as previous published protocols. $^2$ Briefly, cells were grown to 20-30% confluence (since they will reach ~90% confluence within 48 to 72 h in the absence of drugs) in 96-well plates under the conditions described above. The medium was aspirated, and then washed with PBS, and treated in duplicate, with 0.1 mL of the medium containing different concentrations of probes (10, 100 nM) or drug (10, 100 nM, as a positive control). Probes were applied from DMSO stocks whereby DMSO never exceeded 1% in the final solution. The same volume of DMSO was used as a negative control. Fresh medium, along with two probes and one corresponding drug, were added every 24 h. After a total treatment time of 72 h, proliferations were assayed using the XTT colorimetric cell proliferation kit (Roche) following manufacturer’s guidelines (read at 450 nm). Data represent the average (s.d. for two trials).

4. Pure enzyme labeling

To investigate the specificity of our probes, labelling with recombinantly purified proteins was performed. Briefly, different proteins (at a final concentration of 100 nM) were incubated with STS-C1 (200 nM) and a previously published probe, STS-2 (1000 nM), in above reaction buffer for 2 h at r.t. followed by click-chemistry with Rhodamine azide (Rh-PEG-N3). After 2 h of click reaction, 6xSDS loading dye was added and the mixture was heated to 95 °C for 10 min. The resulting proteins were resolved by SDS-PAGE, followed by in-gel fluorescence scanning (FL) and silver staining.
Fig. S2. In vitro labelling of recombinantly purified proteins including BSA and several kinases with STS-C1 (200 nM) and STS-2 (1 µM). Part of above results are reproduced as Figure 3C in the main text.

5. Pure enzyme activity assay

Concentration-dependent experiments were performed to determine the inhibition potency and the binding affinity of the probes towards the catalytic domain of the kinases. The inhibition assay was performed with Kinase-Glo® Plus Luminescent Kinase assay kit from Promega following the manufactures instructions. Recombinant kinase, ATP and the probe were mixed in the HEPES buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM MgCl₂) at a volume of 27.5 µL in a flat-bottom solid white 384-well plate. The incubation was allowed to continue for 20 min at 37 °C and the reaction was subsequently quenched by the addition of an equal volume of the Kinase-Glo reagent. After 5 min of incubation, the luminescence readouts from the wells were measured using Tecan microplate reader with i-control software. The ATP and substrate peptide concentrations used in the assay were 100 µM and 50 µM, respectively. The IC₅₀ values of the probes were calculated from the percentage activity vs. log [concentration of probe] curves generated using GraphPad Prism software.

6. Bacterial lysate labeling

Bacterial lysates were prepared using modified procedures based on previous reports. The cultures of LB (100 mL) with a single colony containing c-Src were grown at 37 °C with shaking to reach OD₆₀₀ = 1.2. Subsequently, IPTG (0.2 mM) was added to induce protein expression and incubated for further 18 h at 18 °C with shaking (230 rpm). 5 mL LB was taken out before and after inducing, respectively, and harvested by centrifugation at 4000 rpm for 10 min at 4 °C. The resulting pellets were resuspended in the lysis buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM MgCl₂, 50 µM PMSF) and sonicated (to complete lysis, 10 rounds of 3 s on and 3 s off, at 28 % amplitude) followed by centrifugation for 15 min (13,000 rpm at 4 °C). The total protein concentrations of these lysates were then quantified by Bio-Rad protein assay (Bio-Rad USA), and stored in -20 °C, and used for all subsequent labeling experiments. For probe concentration-dependent in vitro labeling, 20 µg of above bacterial lysates was mixed with the probe in different concentrations and the reactions were incubated for 2 h at r.t., followed by click-chemistry with Rhodamine azide (Rh-PEG-N₃). After 2 h of click reaction, 6 x SDS loading dye (4 µL) was added and the mixture was heated to 95 °C for 10 min. The resulting proteins were resolved by SDS-PAGE. In-gel fluorescence scanning was used to visualize the labelled protein bands. Both in-gel fluorescence scanning (FL) and coomassie Brilliant Blue staining (CBB) were always carried out on the gels upon SDS-PAGE separation of labeled samples. For lysate concentration-dependent in vitro labelling, to different amounts of above bacterial lysates, the probe (200 nM) was added and the reactions were incubated for 2 h at r. t., followed by the same procedure as the previous labelling experiment.
For competitive labelling experiments, to 20 µg of above bacterial lysates, after different concentrations of Staurosporine (STS) was incubated at r.t. for 30 min, the probe (200 nM) was added and the reactions were incubated for 2 h at r.t., followed by the same procedure as the previous labelling experiment.

**Fig. S3.** (A) *in vitro* labelling of bacterial lysate over-expressing c-Src (20 µg) with STS-C1 (0-1000 nM); (B) Dose-dependent labelling of bacterial lysates over-expressing c-Src (0-20 µg); (C) Competitive labelling experiment in which 20 mg of the c-Src over-expressing bacterial lysate was labelled with STS-C1 (200 nM) in the presence of different amounts of STS (0-2 µM). Parts of above results are reproduced as Figure 3D in the main text.

7. **In vitro and in situ labeling over cancer cell lines**

For *in vitro* proteome labelling, the probe was added to 100 µg fresh HepG2 cell lysates (prepared as previously described) in 100 µL of HEPES buffer at a desired concentration. Samples incubated with probe were incubated for 2 h at room temperature. Four microliters of a freshly premixed click chemistry reaction cocktail (100 µM Rh-PEG-N3 from 10 mM stock solution in DMSO, 100 µM TBTA from 10 mM freshly prepared stock solution in deionized water, and 1 mM CuSO4 from 100 mM freshly prepared stock solution in deionized water) was added. The reaction was further incubated for 2 h with gentle mixing, before being terminated by addition of pre-chilled acetone (0.4 mL; 30 min incubation at -20 °C). Precipitated proteins were subsequently collected by centrifugation (13000 rpm X 10 min at 4 °C). The supernatant was discarded and the pellet was washed with 200 µL of pre-chilled methanol. The air-dried pellet was added 2 X loading buffer and heated for 10 min at 95 °C. Around 20 µg (per gel lane) of proteins were separated by SDS-PAGE (10% gel) and then visualized by in-gel fluorescence scanning. For *in situ* labelling, HepG2 cells were grown to 80~90% confluency in 24-well plates under conditions described above. The medium was aspirated, and the cells were trypsinized and pelleted by centrifugation. Eventually, the cell pellets were re-suspended in HEPES buffer (0.5% NP-40, 100 µL), homogenized by sonication, and diluted to 1 mg/mL with HEPES buffer. All subsequent procedures were similar to those from *in vitro* experiments. The protein pellets were then re-suspended in 20 µL of 1 X SDS- loading buffer and heated for 10 min at 95 °C. Around 20 µg (per gel lane) of proteins were separated by SDS-PAGE (10% gel) and then visualized by in-gel fluorescence scanning.

8. **Pull down experiment and validation**

To identify potential cellular targets of the probes, pull-down (PD) experiments were carried out, and followed by Western blotting (WB) and LC-MS/MS, where applicable. The general pull-down procedure was based on previously reported procedures, with the following optimizations. Fresh cell lysates were prepared and their protein concentrations determined, as described earlier. For *in vitro* pull-down experiment, cellular lysates...
(5 mg) were supplemented with 200 µL 5× HEPES buffer (125 mM HEPES, pH 7.5; 750 mM NaCl; 10 mM MgCl₂), the reaction volume was adjusted to 1 mL with milli-Q water. Subsequently, a solution of probe (final 1μM) was added, and equilibration was carried out for 2 h at r.t. Subsequently, the reaction was reacted by click chemistry with Rh-biotin-N₃ under the conditions described before, acetone precipitated, and resolubilized in 1% SDS in PBS with brief sonication. This re-suspended sample was then incubated with avidin–agarose beads (100 µL/mg protein) at r.t. overnight. After centrifugation, supernatant was removed and the beads were washed with 0.1% SDS once and PBS for 1 times, then washed with buffer A (8M Urea 200 mM NaCl 2% SDS 100 mM Tris pH 8), buffer B (8M Urea 1.2 M NaCl 0.2% SDS 100 mM Tris 10% Ethanol 10% Isopropanol pH 8), buffer C (8M Urea 100 mM Tris pH 8) and PBS. After washing, the beads were boiled in 1× SDS loading buffer (200 mM Tris pH 6.8, 400 mM DTT, 8% SDS) for 15 min. Control PD using DMSO was carried out concurrently. For in situ PD, the probe (1μM) was directly added to live cells, followed by incubation for 2 h. DMSO should never exceed 1% in the final solution. After 2 h of incubation at 37 °C/5% CO₂, the medium was aspirated, and cells were washed twice gently with PBS to remove the excessive probe. The cells were then trypsinized and pelleted by centrifugation. Eventually, the cell pellets were resuspended in PBS (50 μL), homogenized by sonication, and diluted to 1 mg/mL with PBS. The labeled lysates were then subjected to click reaction with Rh-biotin-N₃, and all subsequent experiments were carried out as above described. Control PD using DMSO was carried out concurrently with live cells. WB experiments were carried out as previously described using the corresponding antibodies.

After SDS-PAGE, digestion process was introduced as the followings: the collected gel cut into small particles was washed twice with ~400 µl of 25 mM ammonium bicarbonate/50% acetonitrile and vortex for 10 min; Gel pieces was then washed with 400 μl of 100 mM ammonium bicarbonate at pH= 8 for 10 min while vortexing, and dehydrated with ~400 µl of 100% acetonitrile. After repeating rehydration and dehydration and Removing the solution, gel particles was incubated with 300 µL of 0.05 mg/mL trypsin solution for 16 h at 37 °C; the solution was combined with two additional extractions using 2 vol of 5% FA/50% acetonitrile and concentrated in vacuo. The peptides were separated and analyzed on a Shimadzu UFLC system (Shimadzu, Japan) coupled to an LTQ-FT Ultra (Thermo Electron, Germany). Mobile phase A (0.1% formic acid in H₂O) and mobile phase B (0.1% formic acid in acetonitrile) were used to establish the 60 min gradient comprising 0−35% B, 8 min of 35−50% B, and 2 min of 80% B, followed by re-equilibrating at 5% B for 5 min. Peptides were then analyzed on LTQ-FT with an Advance Captive Spray Source (Michrom Bio Resources) at an electrospray potential of 1.5 kV. A gas flow of 2 L/min, ion transfer tube temperature of 180 °C, and collision gas pressure of 0.85 m Torr were used. The LTQ-FT was set to perform data acquisition in the positive-ion mode, except that the m/z range of 350−1600 was used in the full MS scan. The raw data were converted to mgf format. The database search was performed with an in-house Mascot server (version 2.2.07, Matrix Science) with MS tolerance of 10 ppm and MS/MS tolerance of 0.8 Da. Two missed cleavage sites of trypsin were allowed. Carbamidomethylation(C) was set as a fixed modification, and oxidation (M) and phosphorylation (S, T, and Y) were set as variable modifications. “False” hits that appeared in negative control pull-down/LCMS experiments were further eliminated. All proteins were identified by a minimum score of 40 and at least one unique peptides. Based on these criteria, a list of the hits, which was potential targets of STS-C1, was generated in Table S1& S2, for in vitro and in situ PD, respectively. Some “sticky” or contaminated proteins such as keratin, actin, and tubulin have been deleted. “False” hits that appeared in negative control pull-down/LCMS experiments have been further eliminated. The kinases were extracted into Table S3.

For Western blotting analysis, the PVDF membrane was blocked with 3% BSA-TBST (Tweens-20, 0.1%) at 4 °C overnight after the proteins on gel was transferred. Then PVDF membrane was incubated with primary antibody in 3% BSA-TBST (tween-20, 0.1%) at r.t. for 1 h followed by washing with TBST (Tweens-20, 0.1%) for 10 min × 4. The PVDF membrane was further incubated with corresponding secondary antibody at r.t. for 1 h followed by washing with TBST (tween-20, 0.1%) for 10 min × 4. SuperSignal West Dura Kit (Thermo Scientific) was used to develop the blot.

Fig. S4. Western blot validation of large-scale pull-down experiment of HepG2 with STS-C1. CDK1 was used as a representative example.

9. Molecular Modeling

Molecular docking studies were carried out using AutodockVina software.² The Src structure was obtained from the PDB databank (PDB ID:3F6X). Explicit hydrogen atoms were added, all water molecules were then deleted. The peptide ligand was removed and the protein structure was processed using AutoDock Tools. Compound STS-C1 was prepared for docking using AutoDock Tools to assign AD4atom types, calculate Gasteiger charges, and set all rotatable bonds as active torsions. The ligand was docked into the protein using
AutoDockVina (version 1.11, The Scripps Research Institute). The exhaustiveness parameter was set to 100 (default = 8, linear scale); all other default settings were used. The macromolecule molecular surface and secondary structure were displayed by PyMol (version 0.99, DeLano Scientific LLC).

10. **Live-cell imaging in HepG2 Cells.**

HepG2 cells were seeded in glass bottom dishes (CELLview™, Cat. No. 627861) and grown till ~ 60% confluence. The cells were incubated with 0, 200 nM, 500 nM and 1 µM of STS-C1 in fresh growth medium (200 µL). The cells were further incubated for 2 h at 37°C/CO₂. Then the cells were washed with PBS three times. Subsequently, cells were fixed with 3.7% formaldehyde in PBS for 20 min at 37°C/CO₂, washed twice again and permeabilized with 0.1% Triton X-100 in PBS for 15 min at 37°C/CO₂ washed twice again. Cell were then blocked with 2% BSA, 0.05% Tween-20 in PBS for 30 min at room temperature, washed twice with PBS and then subsequently treated with a freshly premixed click chemistry reaction in a 200 µL (10 µM Rh-PEG-N₃, 200 µM TBTA, 2 mM TCEP, 2 mM CuSO₄) for 1 h at room temperature with gentle shaking. Cells were washed with 2x PBS, several times PBS containing 1% Tween-20 and 0.5 mM EDTA (until there was no small crystal under microscopy), 2x PBS, 2x methanol, 2x PBS. The cells were incubated with nucleus stain (Hoechst, 0.2 µg/mL final concentration) for 10 min and washed twice with PBS. Finally, the cells were washed imaged.

![Fig. S5. Fluorescence imaging of HepG2 cells. Cells were treated with STS-C1 for 2 h, then fixed and clicked with Rh-PEG-N₃ (red); After washing, the cells were incubated with Hoechst (blue) for 10 min. Parts of above results are reproduced as Figure 4D in the main text.](image)

11. **References**


