Supplementary Information

Discovery of small molecule inhibitors of the TLR1-TLR2 complex

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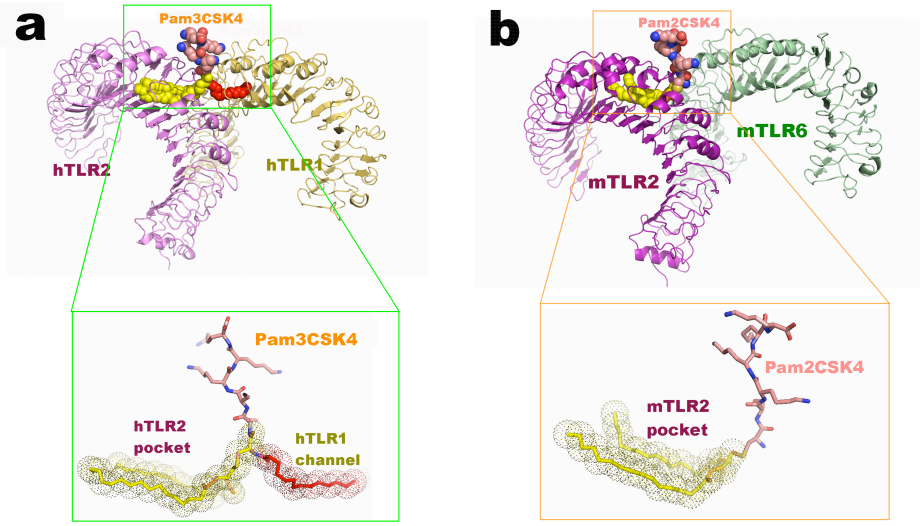
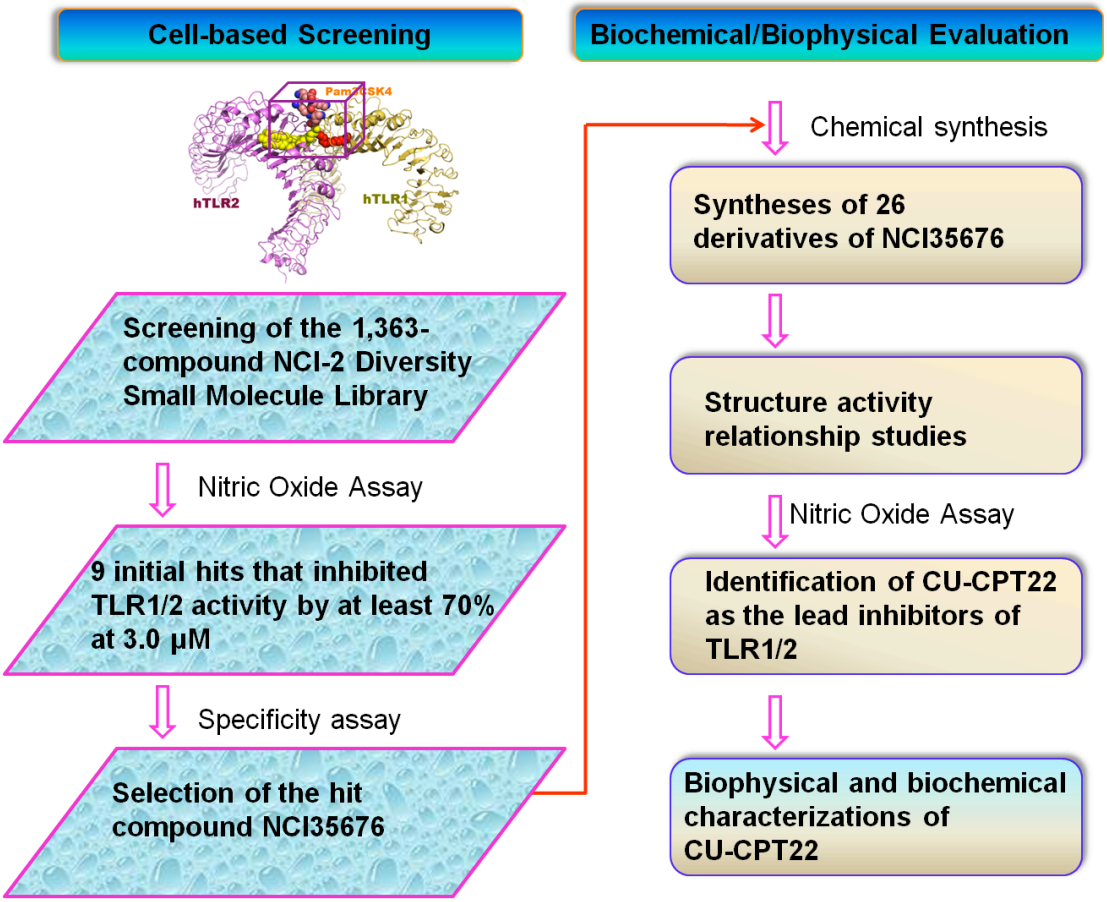
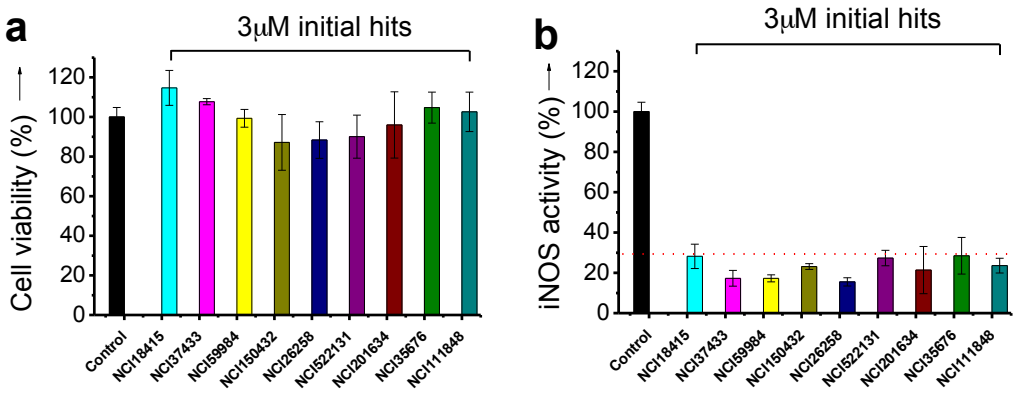


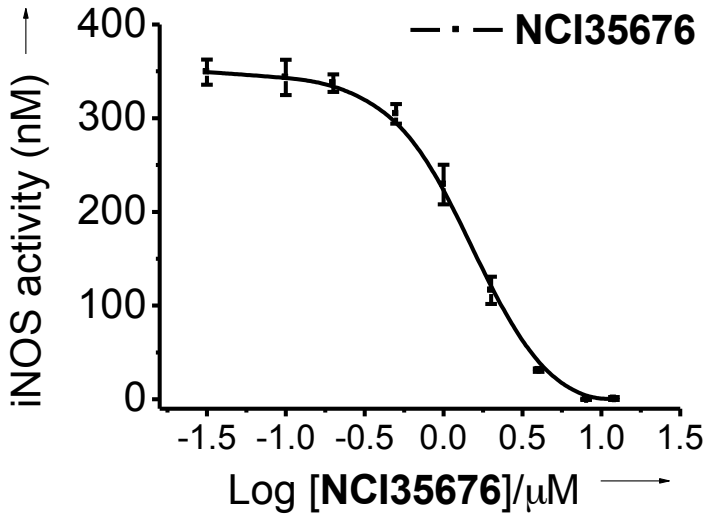
Supplementary Figure S1: Structural comparison of the different binding modes of ligands recognized the TLR1/TLR2 or TLR2/TLR6 complexes. (**a**) Crystal structure of TLR1/TLR2/Pam_3CSK_4 (Protein Data Bank (PDB) ID 2Z7X).[1] The Pam_3CSK_4-binding pocket in the hTLR1-TLR2 complex was shown in the close-up box. (**b**) Crystal structure of TLR2/TLR6/Pam_2CSK_4 (Protein Data Bank (PDB) ID 3A79).[2] The Pam_3CSK_4 or Pam_2CSK_4 binding pockets are represented in dots. TLR1, TLR2 and TLR6 are colored in yellow, purple, and green, respectively.



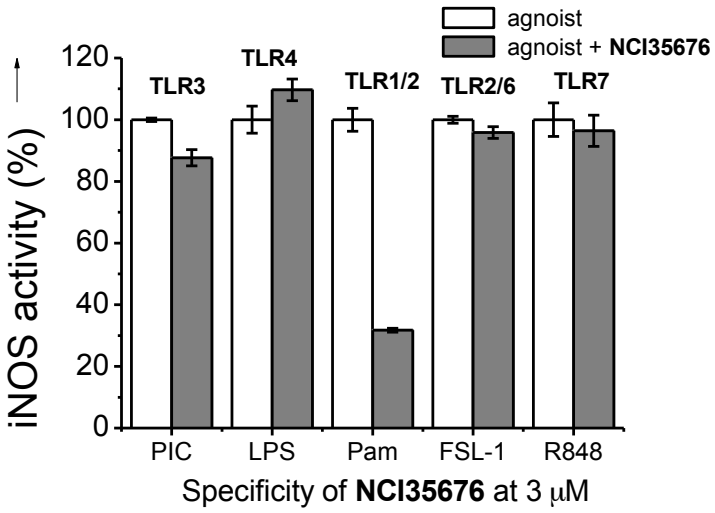
Supplementary Figure S2: Flow chart illustrating the development of **CU-CPT22** as a lead TLR1/2 inhibitor.



Supplementary Figure S3: Viability and inhibitory activity of the nine compounds selected from the library screening. (a) MTT assays showed that the identified 9 hits did not affect cell viability at 3 μM; while (b) showed >70% TLR1/2-mediated NO production inhibition in RAW 264.7 macrophage cells.



Supplementary Figure S4: Dose-dependent inhibitory activity of NO production by **NCI35676**. The IC_{50} is determined as 2.45 ± 0.25 μM.



Supplementary Figure S5: TLR specificity test for **NCI35676** (3 μM) with TLR-specific agonists in RAW 264.7 macrophage cells: (1) TLR3: 15 μg/mL Poly (I:C), (2) TLR4: 10 ng/mL LPS, (3) TLR1/2: 200 ng/mL Pam_3CSK_4, (4) TLR2/6: 10 ng/mL FSL-1, and (5) TLR7: 100 nM R848 were used to selectively activate respective TLRs.

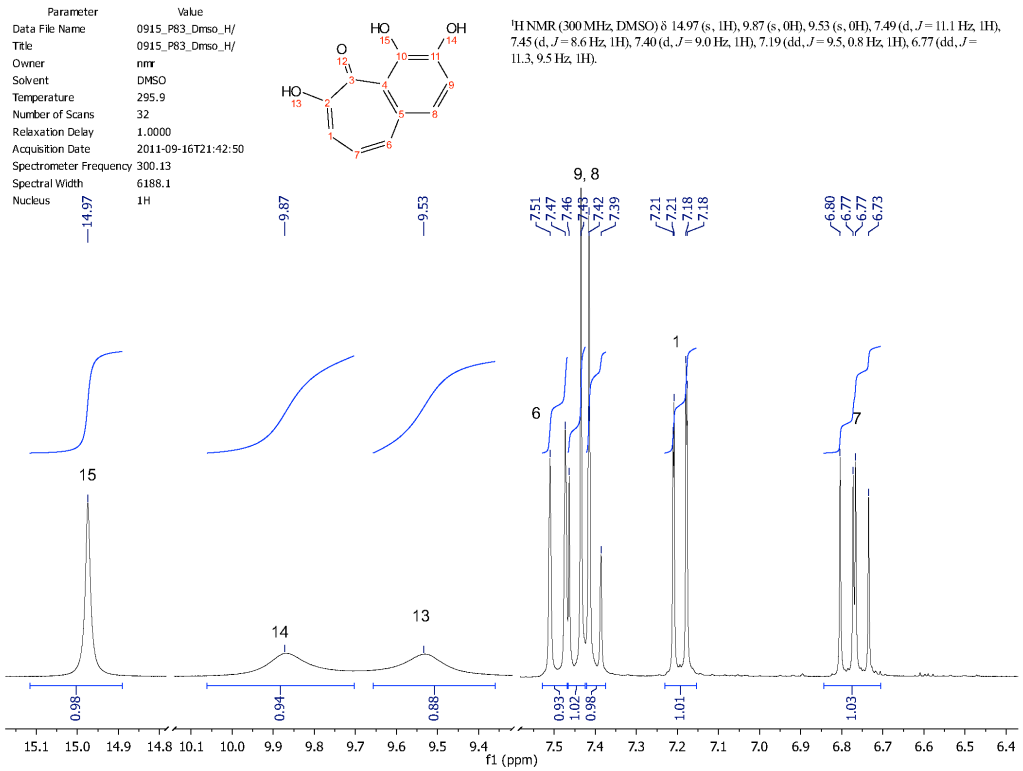


Figure S6 (a)

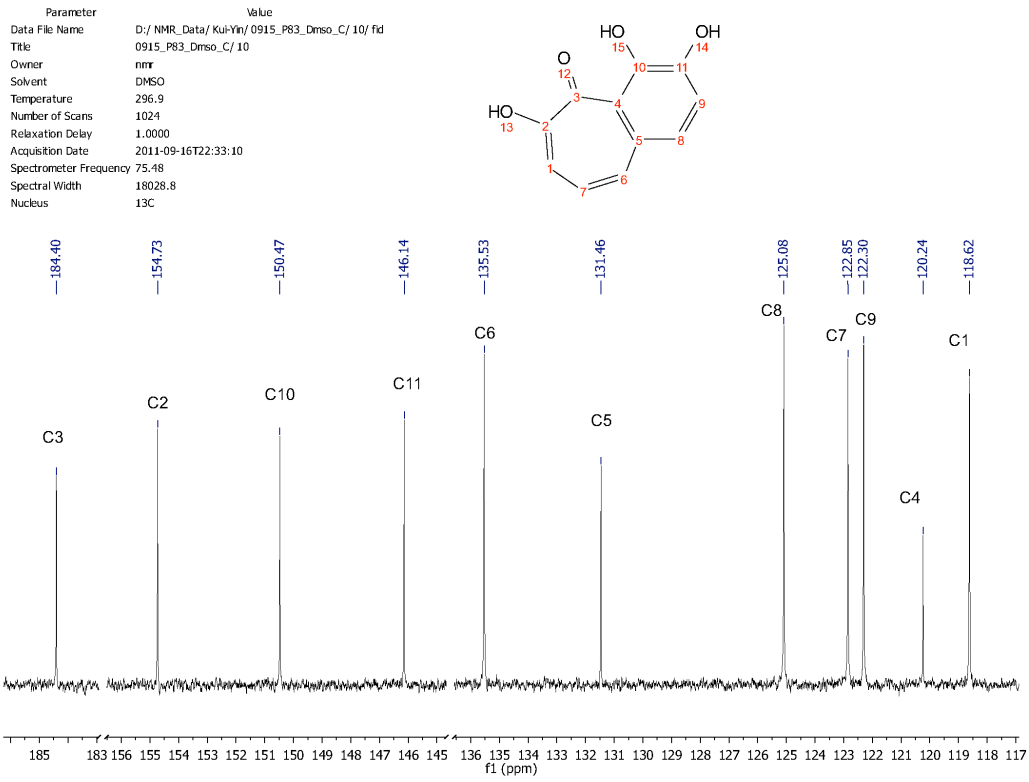


Figure S6 (b)

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Spectrometer Frequency	(300.13, 75.47)
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Nucleus	(1H, 13C)
Acquired Size	(512, 384)
Spectral Size	(1024, 2048)

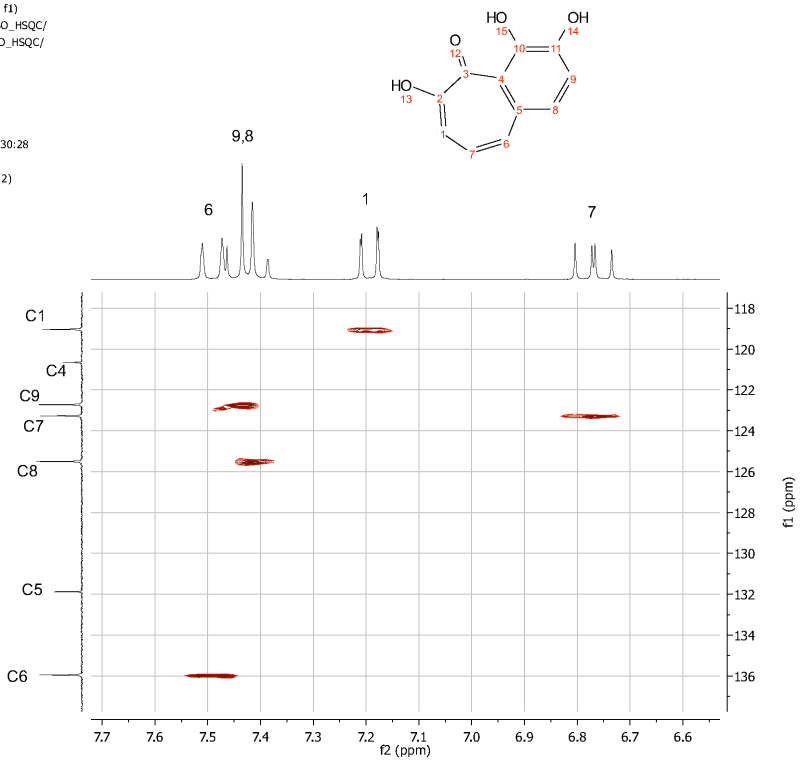

Figure S6 (c)

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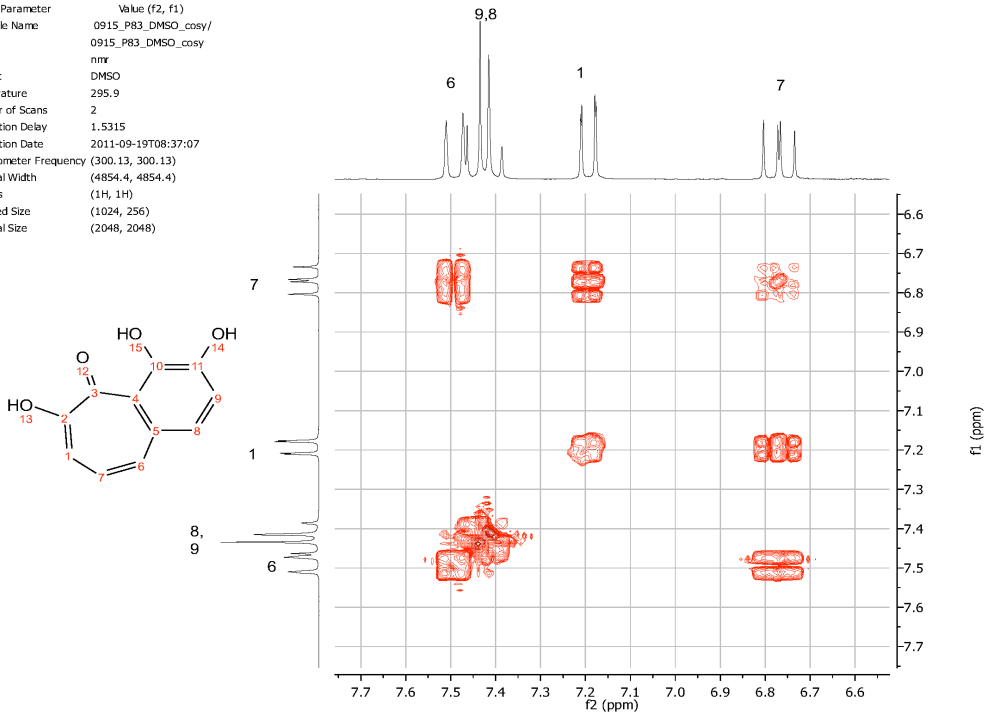
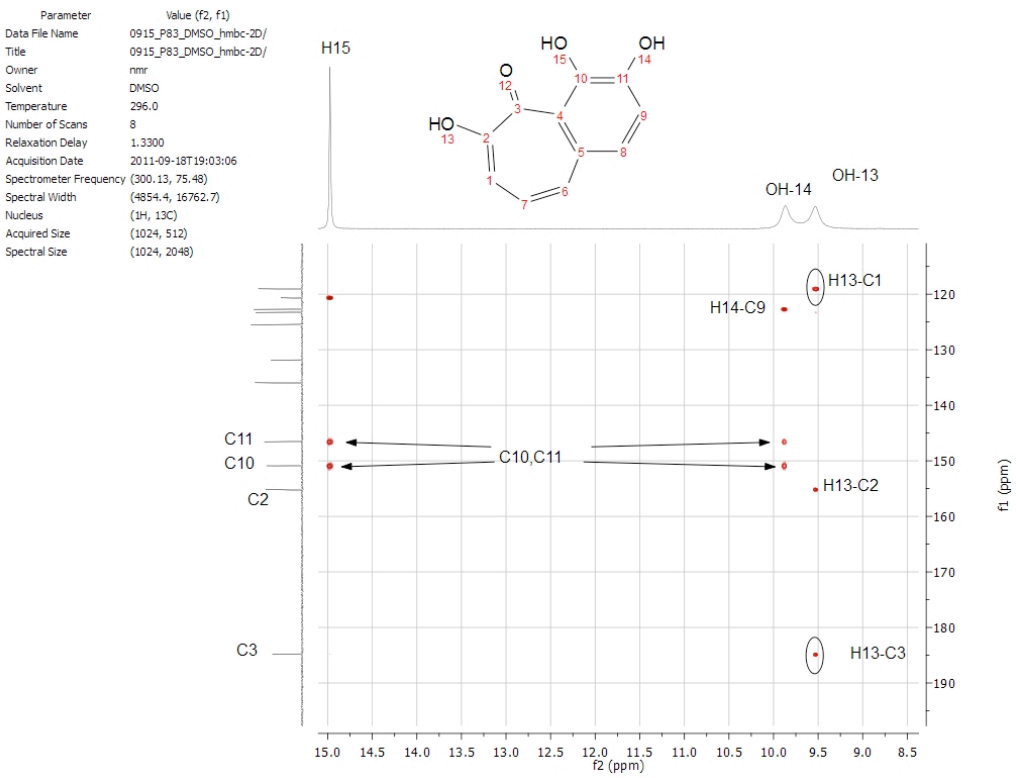


Figure S6 (d)



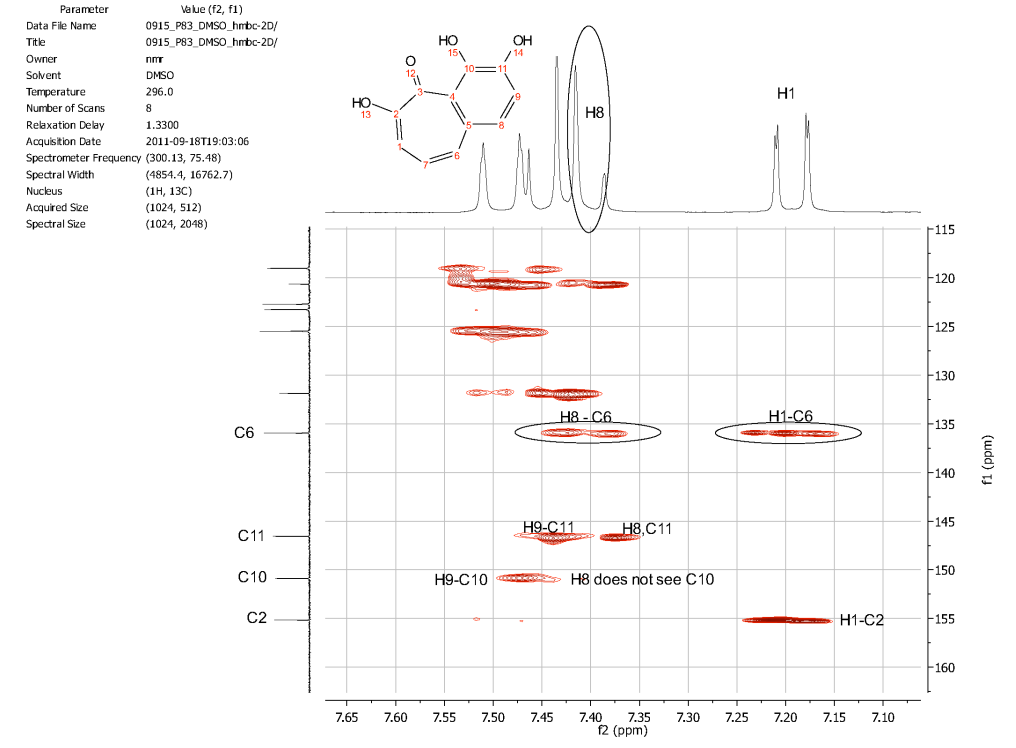
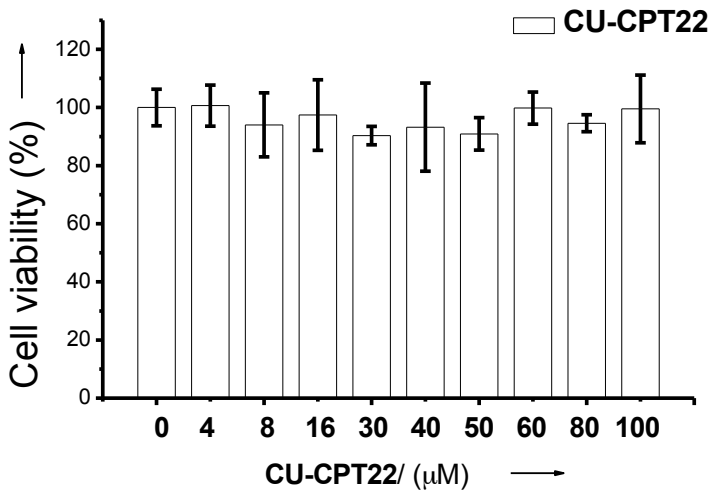
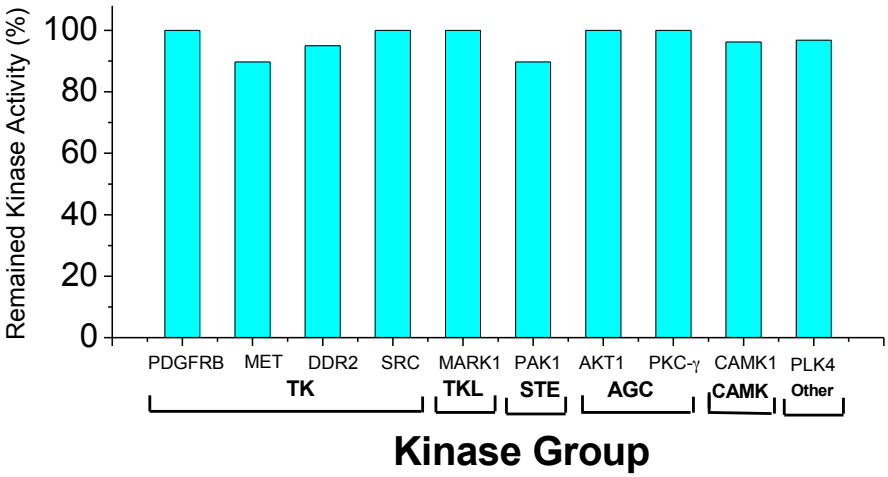


Figure S6 (**e**)

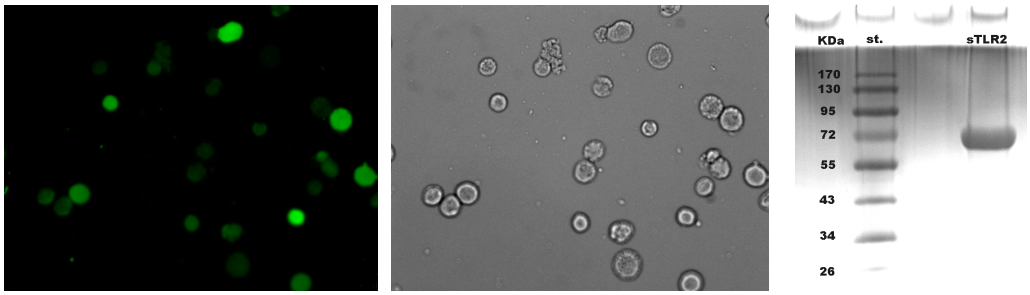
Supplementary Figure S6: NMR spectra of **2** monitoring (a) Chemical shifts of ^{1}H and (**b**) ^{13}C, the corresponding shifts was obtained using (**c**) HSQC, (**d**) COSY and (**e**) HMBC.



Supplementary Figure S7: MTT toxicity assay for **CU-CPT22**.

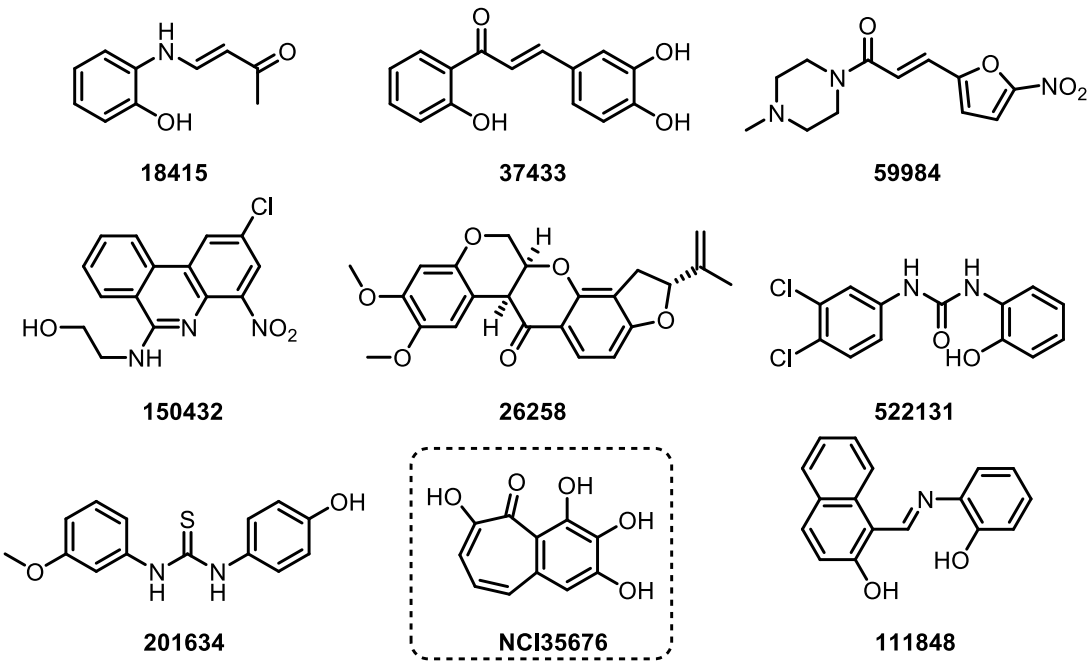


Supplementary Figure S8: Kinase selectivity screen for **CU-CPT22** at 5 μM. **CU-CPT22** was profiled against a panel of 10 kinases using the KinaseSeeker assay, showing minimal non-specific inhibitory effects of these kinases.

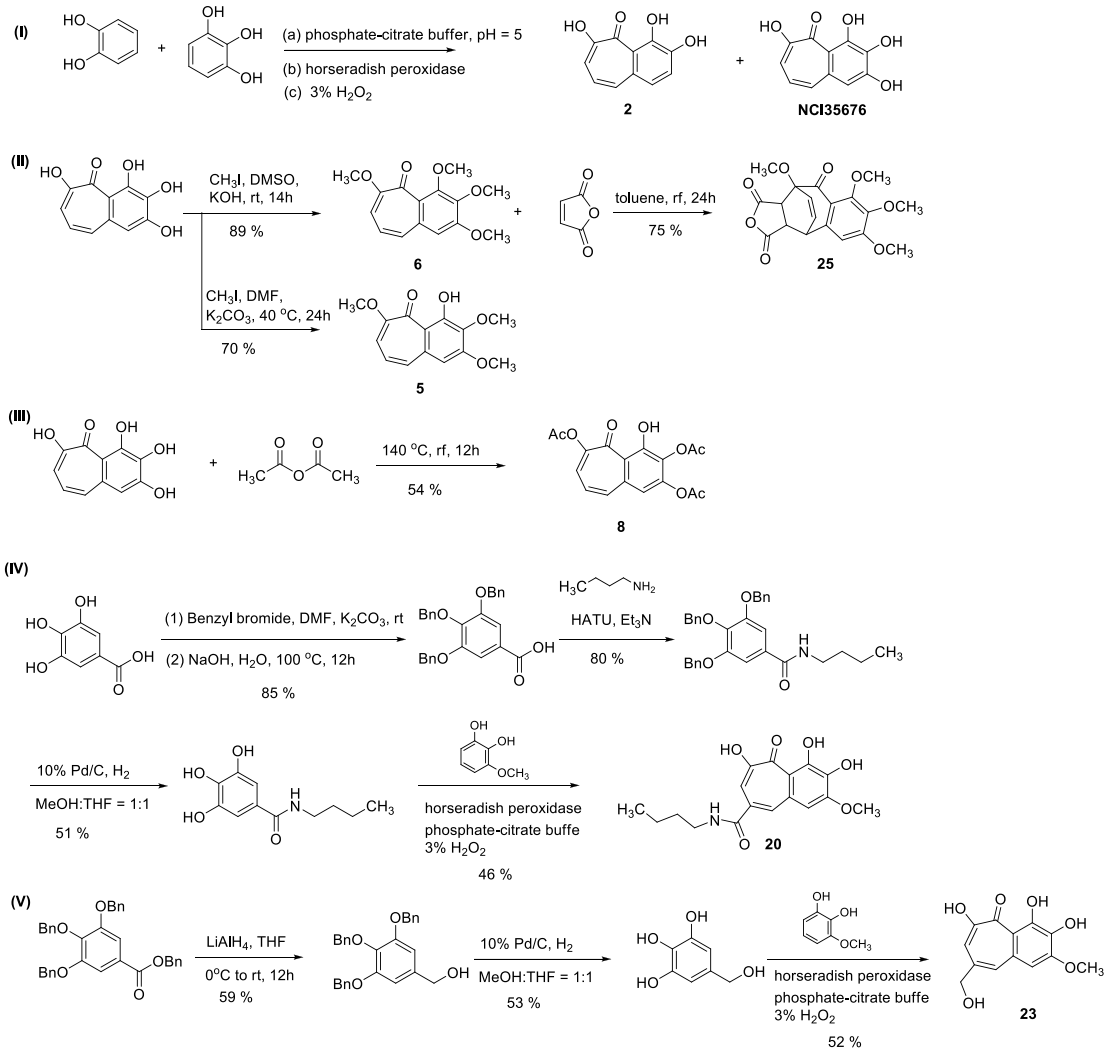


(a) (b) (c)

Supplementary Figure S9: Characterization of prepared human TLR2 (His tagged). (a) Fluorescent image of Hi 5 cells. The cells were imaged by excitation of 470/30 nm and emission of 565/55 nm after 3 days incubation with recombinant viruses. (b) Bright field image of Hi 5 cells. (c) TLR2 protein after purification as observed by Coomassie brilliant blue stain.



Supplementary *Scheme 1.* Structures of the nine top hits from the cell-based screening which can inhibit TLR1/2 at least 70% at 3.0 μM. **NCI35676** was selected for further investigation due to its high specificity.



Supplementary *Scheme 2*. General synthetic methods for the **NCI35676** derivatives.

Methods

General Methods.

NMR spectra were acquired on Bruker 300 or 400 spectrometers, running at 300 MHz (or 400 MHz) for ^{1}H and 75 (or 101 MHz) for ^{13}C, respectively. ^{1}H NMR spectra were recorded at 300 MHz in $CDCl_3$, $(CD_3)_2CO$, $(CD_3)_2SO$ or CD_3CN using residual $CHCl_3$ (7.28 ppm), $(CH_3)_2CO$ (2.05 ppm), $(CH_3)_2SO$ (2.50 ppm) and CH_3CN (1.94 ppm) as the internal standard. ^{13}C NMR spectra were recorded at 75 MHz in $CDCl_3$, $(CD_3)_2CO$ or $(CD_3)_2SO$ using residual $CHCl_3$ (77.16 ppm), $(CH_3)_2CO$ (29.84 and 206.26 ppm), $(CH_3)_2SO$ (39.97 ppm) and CH_3CN (1.32 and 118.26 ppm), as internal reference. Thin layer chromatography was performed on Merck Kieselgel 60 Å F254 or Silicycle 60Å F254 plates eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid. Compounds were purified using flash chromatography (FC) (Silica gel 60, 200-400 mesh, Sorbent Tech.) or recrystalization. Mass spectrometry was performed at the mass spectrometry facility of the Department of Chemistry at University of Colorado at Boulder on a double focusing high resolution mass spectrometer.

In Vitro TLR1/2 Inhibition Assay.

RAW 264.7 (Mouse leukaemic monocyte macrophage cell line) cells were grown in RPMI 1640 medium, supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 mg/mL). RAW cells were then plated in 96-well plates at 80,000 cells per well and grown for 24 h in the media descried previously at 37°C in a 5% CO_2 humidified incubator. After 24 h, non-adherent cells and media were removed and replaced with fresh unsupplemented RPMI 1640 medium. The adherent macrophages were treated with Pam_3CSK_4
(N-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-[R]-cysteinyl-[S]-seryl-[S]-lysyl-[S]-lysyl-[S]-lysyl-[S]-l
ysine.3HCl) (200 ng/mL) (Invivogen), an agonist of TLR1/2, and different concentrations of potential inhibitor. We prepared all of the compounds in stock solution of 20 mM (in DMSO), and then diluted it into the desired concentration with PBS. We have DMSO control in all of the experiment. Two rows were only treated with Pam_3CSK_4 as control. Plates were then incubated for an additional 24 h. Following incubation 100 µL of media was removed and added to flat black 96-well microfluor plates (Thermo Scientific, MA, USA). To each well, 10 µL of 2, 3-diaminonaphthalene (0.05 mg/mL in 0.62 M aqueous HCl solution) was added and incubated for 15 min in the dark. The reaction was quenched by addition of 5 µL of a 3 M aqueous NaOH solution and the plate was read on Beckman Coulter DTX880 reader (Beckman Coulter, CA, USA) with excitation at 365 nm and emission at 450 nm. The nitrite (a stable metabolite of nitric oxide) concentration was determined from a nitrite standard curve.[3] The inhibition rate (%) of NO release was determined using the following formula: Inhibition (%) = [$Pam_3CSK_4(OD_{450})$ – Compounds (OD_{450})]/[Pam_3CSK_4 (OD_{450})-Control (OD_{450})]×100. The IC_{50} values for both inhibition and cytotoxicity were determined graphically using software Origin v7.5.

Cytokine-specific ELISA.

RAW 264.7 cells were planted in 6-well plates at 1,000,000 cells per well with 3 mL of medium (RPMI 1640 medium, supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 mg/mL)) and grown for 24 h at 37°C in a 5% CO_2 humidified incubator. After 24 h, non-adherent cells and media were removed and replaced with fresh RPMI 1640 medium (3 mL/well). Two wells of adherent macrophages were treated with Pam_3CSK_4 (Invivogen, 300 ng/mL) as the positive control, two wells was treated with 8 µM compound **CU-CPT22**, and the other two wells ware treated with 8 µM compound DMSO. Another 6

well plate was treated with Pam_3CSK_4 (Invivogen, 300 ng/mL) and different concentration of **CU-CPT22**. Plates were then incubated for an additional 24 h. The medium was removed, the cells were washed with PBS (3 x 1 mL), the 6 well plates were put on ice, then 500 µL of lysis buffer was added to each well (Lysis Buffer: 120 µL 0.5M EDTA; 12 mL Mammalian Protein Extraction Reagent, 100 µL cocktail, 0.36 mL NaCl (5 M, aqueous)). After 5 min, the mixture was transferred into a corresponding 1.5 mL tube, spun for 20 min at 13.2 K rpm in a cold room. Approximately 400 µL of supernatant were collected into new tubes, frozen at -80 °C until ready for cytokine measurement. The production of the cytokine interleukin-1β (IL-1β) and TNF-α was quantified with enzyme-linked immunosorbent assays (ELISA) using cytokine-specific capture antibodies, biotinylated monoclonal detection antibodies, and recombinant human cytokine standards according to commercially available ELISA kits from R&D Systems. The cytokine level in each sample was determined in duplicate.

RAW 264.7 Cells Nitric Oxide TLR Selectivity Assay.

This assay was run in a similar manor as the "In Vitro TLR1/2 Inhibition Assay". High molecule weight Poly (I:C), LPS (lipopolysaccharide), FSL-1 ((S,R)-(2,3-bispalmitoyloxypropyl)-Cys-Gly-Asp-Pro-Lys-His-Pro-Lys-Ser-Phe), and R848 (4-amino-2-(ethoxymethyl)-α) were used to selectively activate TLR3, TLR4, TLR2/6 and TLR7 in place of Pam_3CSK_4, respectively.

TLR2 Protein Expression and Purification.

The TLR2 protein was expressed in the baculovirus insect cell expression system using the methods described by Kuroki et al.[4] Monolayers of Spodopera frugiperda (Sf-9) cells were cotransfected with Bright Baculovirus DNA (BD BaculoGold™) and the pVL1393 plasmid vector containing cDNA for TLR2. Viral titers were amplified to ~5-10 $\times 10^7$/mL virus particles. The recombinant viruses were used to infect suspension high 5 insect cells in serum-free medium (Insect-XPRESS™ Protein-free Insect Cell Medium with L-glutamine, Lonza) at 27 ^oC, 130 rpm. After incubation of high 5 insect cells with recombinant viruses for 3 days, the cells changed to green (Supplementary Figure S9a, S9b), the medium was collected after low-speed centrifugation and dialyzed (Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, Pierce) against 0.1 M Tris buffer (pH 8.0) containing 0.3 M NaCl. The dialyzed medium was filtered and purified by a column of nickelnitrilotriacetic acid beads (Qiagen, Santa Clarita, CA) according to the manufacturer's instruction. The purified protein was finally dialyzed against 5 mM Tris buffer (pH 7.4) containing 0.15 M NaCl and condensed by centrifugal concentrator (Millipore 10,000 MWCO). Electrophoretic analysis revealed that TLR2 exhibited a single band with a molecular mass of ~75 kDa (Supplementary Figure S9c). Approximately 100 μg of TLR2 protein was obtained from 500 mL of medium.

Fluorescence Anisotropy Assay.

In 500 µL Tris buffer (pH 7.2) add 10 µL (1 µg/mL) rhodamine-labeled Pam_3CSK_4 (Invivogen), test the fluorescence anisotropy at excitation of 549 nm and emission of 566 nm (Horiba Fluorolog 3). And 5 µL (20 µM) TLR1 (R &D) and 5 µL (20 µM) TLR2 was added into the above buffer, then retest the anisotropy. Following, **CU-CPT22** or compound **6** was added in the buffer from the concentration of 0 to 6 µM, and the fluorescence anisotropy was tested in the corresponding concentration.

Fluorescence polarization experiments were performed at 25 °C using a Horiba Fluorolog-3 fluorometer. For direct binding measurements, serial dilutions of TLR1 (R&D, MN) and TLR2 were made in Tris buffer

(pH 7.2), and an aliquot (10 μL) of 1 μg/mL rhodamine labeled Pam_3CSK_4 (Invivogen, CA), was added to a total volume of 500 μL. The competition binding solution was incubated for 30 minutes at 25 °C. Serial dilutions of **CU-CPT22** or compound **6** were incubated with 20 μM TLR1, 20 μM TLR2 and rhodamine-labeled Pam_3CSK_4 for 30 min at room temperature.

Regression analysis was carried out using Origin 7.5 (OriginLab) ligand binding macro module. Experimental data were fitted into equation (1) to determine the IC_{50} values, which in turn can be related to the known affinity of the Pam_3CSK_4 (K_d = 1.2 nM)[5] to acquire the inhibitory constant K_i using equation (2).

Equation (1): $y= min+ (max-min)/(1+10^{x-logIC50})$ (y= total binding, x= log concentration of and rhodamine-labeled Pam_3CSK_4, min= nonspecific binding, max= maximum binding in absence of ligand).

Equation (2): $K_i= IC_{50}/(1+[L]/K_d)$ ([L]= concentration of rhodamine-labeled Pam_3CSK_4).

***In Vitro* Cytotoxicity Assay.**

In a 96-well plate 10,000 cells in 200 µL media (RPMI 1640 medium, supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 mg/mL)) per well. Eight wells were left empty for blank controls. The plates were incubated (37 ^oC, 5% CO_2) overnight to allow the cells to attach to the wells. Add 20 µL (5 mg/mL) MTT solution to each well. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the MTT into the media. Incubate (37 ^oC, 5% CO_2) for 4 hours to allow the MTT to be metabolized. Dump off the media. Dry plate on paper towels to remove residue. Resuspend formazan (MTT metabolic product) in 200 µL DMSO. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the formazan into the solvent. When a clear difference could be seen by naked eye, results were read by spectrophotometer at 560 nm. Optical density should be directly correlated with cell quantity. Cytotoxicity (%) was determined using the following formula: Cytotoxicity (%) = (1 – [Compounds (OD_{560}) – Background (OD_{560})]/[Control (OD_{560})–Background (OD_{560})])×100.

Kinase Profiling.

The kinase profiling was performed by Luceome Biotechnologies, Tucson, AZ. Compound **CU-CPT22** was dissolved in DMSO at 10 mM and tested at concentrations of 5 µM. Each compound was first evaluated for false positive against split luciferase. If they did not inhibit luciferase control, then they were profiled in duplicate against the following kinases: PDGFRB, MET, DDR2, SRC, MAPK1, PAK1, AKT1, PKC-γ, CAMK1, and PLK4 using the protocol described by Ghosh and co-workers.[6] The percent inhibition and percent activity remaining was calculated using the following equations:

% inhibition = $[(ALU_{control} – ALU_{sample})/ ALU_{control}$ X 100

% activity remaining = 100 - % inhibtion

***In Silico* Docking**.

CU-CPT22 was docked into the TLR1/TLR2 binding domain (PDB: 2Z7X[1]) using Glide 5.6. The molecule was created, as appropriate, with multiple protonation and tautomeric states. The TLR1/2 conformations were prepared using standard Glide protocols. This includes addition of hydrogens, restrained energy-minimizations of the protein structure with the Optimized Potentials for Liquid Simulations-All Atom (OPLS-AA) force field, and finally setting up the Glide grids using the Protein and Ligand Preparation Module[7].

Synthesis method

Method I

A: Catechol (110 mg, 1.00 mmol) and pyrogallol (126 mg, 1.00 mmol) were dissolved in a mixture of acetone-pH 5.0 phosphate-citrate (1:1=0.2 M Na_2HPO_4: 0.1 M citrate) buffer (1:5 v/v, 5 mL), which contained 0.1 mg horseradish peroxidase (cas 9003-99-0, 5KU, Fisher). Four aliquots of 3% H_2O_2 (2 mL each) were added every 10 min over 40 min while stirring.[8] After 2-3 h, the resulting orange precipitate is filtered off, washed with water (3 x 6 mL) and dried under high vacuum condition to give a mixture of **2** and **NCI35676** (purpurogallin). The mixture was purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:4) as eluent, to give **2** as an orange solid (23 mg, 11 %).

B: Using 3-methoxycatechol (140 mg, 1.00 mmol) instead of catechol and gallic acid (170 mg, 1.00 mmol) instead of the pyrogallol, gave the single orange solid product **10** (160 mg, 57 %).

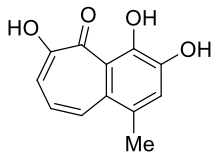
Method II

To a suspension of purpurogallin (44 mg, 0.200 mmol) and KOH (134 mg, 2.40 mmol) in anhydrous DMSO (8 mL), maintained at 0 ^{o}C under nitrogen, was added dropwise iodomethane (99 µL, 1.6 mmol), and the mixture was stirred overnight at room temperature.[8] After this time, the reaction was quenched with water (10 mL) and the mixture stirred for 20 min. After addition of EtOAc (20 mL), the organic phase was washed with water (3 × 20 mL), and the combined aqueous phases were extracted with EtOAc (20 mL). The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:5) as eluent, gave **6** as white solid (49 mg, 89 %).

A solution of **6** (28 mg, 0.101 mmol) and maleic anhydride (36.0 mg, 0.200 mmol) in toluene (2 mL) was heated to reflux in a sealed schlenk tube for 24 h.[9] The mixture was then evaporated to dryness under reduced pressure, and the resulting crude solid was purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:6) as eluent, to give **25** as white solid (28 mg, 75 %).

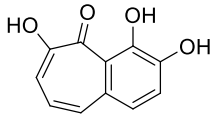
Method III

Acetic anhydride (56.7 μL, 0.600 mmol) was added slowly over a period of 30 min to a stirred solution of purpurogallin (44 mg, 0.200 mmol) in 2 mL of pyridine at 110 ^{o}C.[10] After an additional 12 h, 10 mL 1M HCl was added, and then extracted with dichloromethane (3 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated to give a yellow solid. This was purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:5) as eluent, to give **8** as a yellow powder (43 mg, 54 %).

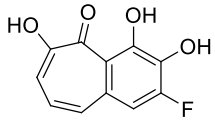


3,4,6-trihydroxy-1-methyl-5H-benzo[7]annulen-5-one (**1**). Following the general method IA, using 4-methylbenzene-1,2-diol (124 mg, 1.00 mmol) instead of catechol, gave an orange solid. The solid mixture was purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:4) as eluent, to give **1** as an orange solid (23 mg, 10 %): ^{1}H NMR (300 MHz, DMSO) δ 14.91 (s, 1H), 9.64 (s, 2H), 7.60 (d, *J* = 11.8 Hz, 1H), 7.40 (s, 1H), 7.19 (d, *J* = 9.3 Hz, 1H), 6.83 (dd, *J* = 11.8, 9.5 Hz, 1H), 3.39 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 185.01, 155.37, 149.33, 146.02, 130.38, 129.55, 129.42, 125.25, 123.14,

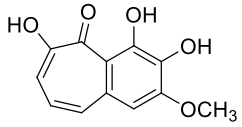
121.71, 118.64, 22.17; LRMS (ESI): calcd for: $C_{12}H_{10}O_4$ [M+Na]$^+$= 241.2, obsd [M+Na]$^+$= 241.0.



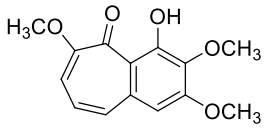
3,4,6-trihydroxy-5H-benzo[7]annulen-5-one (**2**). Following the general method IA, gave **2** as an orange solid (23 mg, 11 %): ^{1}H NMR (300 MHz, DMSO) δ 14.97 (s, 1H), 9.86 (s, 1H), 9.53 (s, 1H), 7.45 (td, *J* = 14.4, 10.1 Hz, 3H), 7.19 (dd, *J* = 9.5, 0.8 Hz, 1H), 6.77 (dd, *J* = 11.3, 9.5 Hz, 1H); ^{13}C NMR (75 MHz, DMSO) δ 184.82, 155.15, 150.89, 146.56, 135.95, 131.88, 125.50, 123.27, 122.72, 120.66, 119.04; LRMS (ESI): calcd for: $C_{11}H_8O_4$ [M+Na]$^+$= 227.1, obsd [M+Na]$^+$= 227.0.



2-fluoro-3,4,6-trihydroxy-5H-benzo[7]annulen-5-one (**3**). Following the general method IA, using 3-fluorocatechol (128 mg, 1.00 mmol) instead of catechol, gave an orange solid. The mixture was purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:4) as eluent, to give **3** as an orange solid (20 mg, 9 %): ^{1}H NMR (300 MHz, DMSO) δ 15.45 (s, 1H), 9.81 (s, 2H), 7.53–7.37 (m, 2H), 7.19 (dd, *J* = 9.6, 0.7 Hz, 1H), 6.86 (dd, *J* = 11.4, 9.6 Hz, 1H); ^{13}C NMR (75 MHz, DMSO) δ 184.20, 155.77, 154.17, 152.58, 134.70, 132.41, 124.98, 118.61, 118.17, 110.76, 110.50; HRMS (ESI): calcd for: $C_{11}H_7FO_4$ [M-H]$^-$ = 221.0255, obsd [M-H]$^-$ = 221.0255.

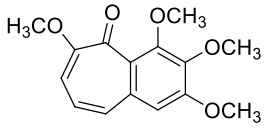


3,4,6-trihydroxy-2-methoxy-5H-benzo[7]annulen-5-one (**4**). Following the general method IA, using 3-methoxycatechol (140.1 mg, 1 mmol) instead of catechol, get an orange solid mixture. The mixture was purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:4) as eluent, to give **4** as an orange solid (26 mg, 11 %): ^{1}H NMR (300 MHz, DMSO) δ 15.14 (s, 1H), 9.50 (s, 1H), 9.38 (s, 1H), 7.52 (d, *J* = 11.2 Hz, 1H), 7.21–7.11 (m, 2H), 6.83 (dd, *J* = 11.3, 9.5 Hz, 1H), 3.97 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.00, 155.57, 152.74, 151.27, 135.85, 135.13, 133.26, 124.36, 117.53, 116.51, 106.97, 56.37; LRMS (ESI): calcd for: $C_{12}H_{10}O_5$ [M-H]$^-$ = 233.1, obsd [M-H]$^-$ = 233.0.

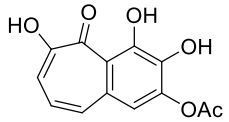


4-hydroxy-2,3,6-trimethoxy-5H-benzo[7]annulen-5-one (**5**). Following the general method II, to a suspension of purpurogallin (44 mg, 0.200 mmol) and anhydrous K_2CO_3 (249 mg, 1.80 mmol) in anhydrous DMF (8 mL), maintained at 0 °C under nitrogen, was added dropwise iodomethane (62 µL, 1.00 mmol), and the mixture was stirred overnight at room temperature. Then, the reaction was quenched with water (10 mL) and the mixture was stirred for an additional 20 min. After addition of EtOAc (20 mL), the organic phase was washed with water (3 x 20 mL), and the combined aqueous phases were

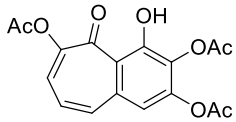
extracted with EtOAc (20 mL). The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting solid was purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:6) as eluent, give **5** as white solid (36 mg, 70%): ^{1}H NMR (300 MHz, DMSO) δ 15.27 (s, 1H), 7.40 (d, *J* = 10.9 Hz, 1H), 7.08 (s, 1H), 6.85 (dt, *J* = 11.1, 9.3 Hz, 2H), 3.96 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 185.62, 158.58, 157.95, 157.02, 136.66, 136.30, 134.41, 124.56, 117.50, 114.00, 106.64, 60.11, 56.69, 56.46; HRMS (ESI): calcd for: $C_{14}H_{14}O_5$ $[M+H]^+$ = 263.0914, obsd $[M+H]^+$ = 263.0914.



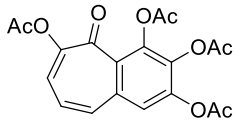
2,3,4,6-tetramethoxy-5H-benzo[7]annulen-5-one (**6**). Following the general method II, gave **6** as white solid (49 mg, 89 %): ^{1}H NMR (300 MHz, DMSO) δ 7.07 (s, 1H), 7.02 (d, *J* = 11.7 Hz, 1H), 6.56 (dd, *J* = 11.6, 8.6 Hz, 1H), 6.29 (d, *J* = 8.7 Hz, 1H), 3.91 (s, 1H), 3.84 (s, 1H), 3.79 (s, 1H), 3.72, (s, 1H); ^{13}C NMR (75 MHz, DMSO) δ 184.58, 158.40, 155.27, 152.06, 143.30, 132.57, 128.73, 125.16, 123.95, 107.74, 105.23, 62.63, 61.02, 56.44, 56.18; HRMS (ESI): calcd for: $C_{15}H_{16}O_5$ $[M+H]^+$ = 277.1066, obsd $[M+H]^+$ = 277.1070.



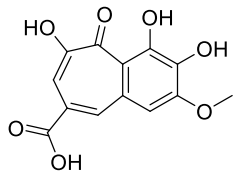
3,4,6-trihydroxy-5-oxo-5H-benzo[7]annulen-2-yl acetate (**7**). Following the general method III, using fewer equivalence of acetic anhydride (18.9 μL, 0.200 mmol) in the reaction, gave an orange solid mixture. The resulting solid was purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:5) as eluent, to give **7** as an orange powder (12 mg, 23 %): ^{1}H NMR (300 MHz, DMSO) δ 15.53 (s, 1H), 11.23 (s, 1H), 9.71 (s, 1H), 7.38 (t, *J* = 13.5 Hz, 1H), 7.07 (d, *J* = 8.9 Hz, 1H), 6.87 (dd, *J* = 10.9, 9.0 Hz, 2H), 2.32 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.57, 168.48, 158.20, 156.36, 155.72, 138.93, 134.08, 127.02, 126.98, 116.78, 115.03, 110.07, 20.79; HRMS (ESI): calcd for: $C_{13}H_{10}O_6$ $[M-H]^-$ = 261.0397, obsd $[M-H]^-$ = 261.0404.



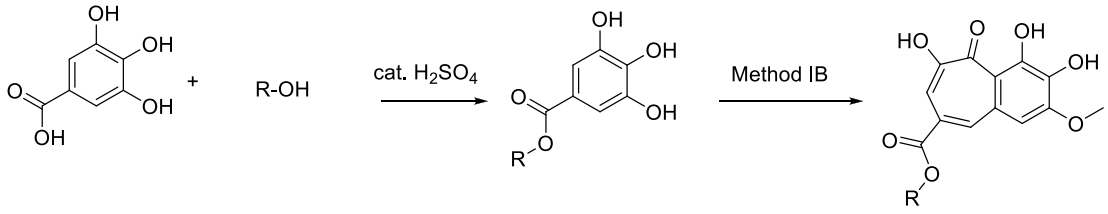
4-hydroxy-5-oxo-5H-benzo[7]annulene-2,3,6-triyl triacetate (**8**). Following the general method III, gave **8** as a yellow powder (43 mg, 54 %): ^{1}H NMR (300 MHz, DMSO) δ 14.96, δ 7.75–7.68 (m, 1H), 7.57–7.50 (m, 2H), 6.95 (dd, *J* = 11.5, 9.1 Hz, 1H), 2.38 (s, 1H), 2.37 (s, 1H), 2.32 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.96, 168.84, 167.39, 167.36, 159.10, 150.57, 147.22, 140.21, 136.21, 132.11, 129.37, 123.20, 120.62, 117.88, 20.76, 20.55, 20.29; HRMS (ESI): calcd for: $C_{17}H_{14}O_8$ $[M-H]^-$ = 345.1, obsd $[M-H]^-$ = 345.1.

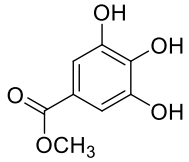


5-oxo-5H-benzo[7]annulene-2,3,4,6-tetrayl tetraacetate (**9**). Following the general method III, using more equivalence of acetic anhydride (18.9 μL, 0.200 mmol) in the reaction, get a light yellow solid. This was purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:5) as eluent, to give **9** as a light yellow powder (43.7 mg, 56 %): ^{1}H NMR (300 MHz, DMSO) δ 7.84 (s, 1H), 7.47 (dd, *J* = 12.3, 0.7 Hz, 1H), 7.11 (dd, *J* = 8.7, 0.7 Hz, 1H), 6.81 (dd, *J* = 11.6, 8.7 Hz, 1H), 2.39–2.32 (m, 6H), 2.27 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 180.87, 168.43, 168.17, 168.13, 167.64, 149.55, 145.48, 143.63, 137.23, 135.06, 134.26, 128.21, 124.70, 124.18, 123.79, 20.86, 20.59, 20.31; HRMS (ESI): calcd for: $C_{19}H_{16}O_9$ $[M+H]^+$ = 389.0857, obsd $[M+H]^+$ = 389.0867.

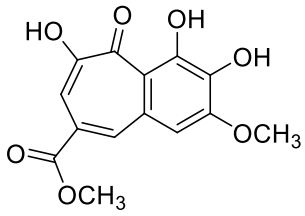


3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxylic acid (**10**). Following method IB, gave the orange solid product **10** (160 mg, 57 %): ^{1}H NMR (300 MHz, DMSO) δ 14.99 (s, 1H), 13.41 (s, 1H), 9.83 (s, 1H), 9.65 (s, 1H), 8.65–8.18 (m, 1H), 7.63 (d, *J* = 1.5 Hz, 1H), 7.45 (s, 1H), 4.00 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.52, 168.14, 154.00, 152.49, 151.84, 138.49, 137.96, 130.51, 124.99, 116.74, 115.12, 110.19, 56.66; LRMS (ESI): calcd for: $C_{13}H_{10}O_7$ $[M-H]^-$ = 277.1, obsd $[M-H]^-$ = 277.0.

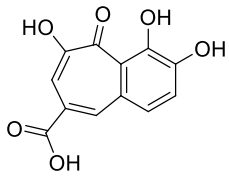




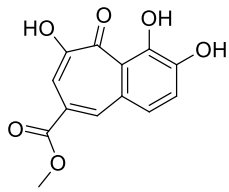
Methyl 3,4,5-trihydroxybenzoate. Gallic acid (850.6 mg, 5 mmol) and dissolved in MeOH (10 mL). Then, conc. H_2SO_4 (272.7 μL, 11 mmol) was added to the solution and was stirred at reflux for 12h (monitored by TLC).[11] The solvent was concentrated under reduced pressure. After extraction with EtOAc (20 mL), the solution was washed with distilled water (3 x 10 mL) and saturated $NaHCO_3$ (20 mL), and dried over Na_2SO_4. The solution was evaporated and purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:2) as eluent, to give methyl 3,4,5-trihydroxybenzoate as a white powder (782.6 mg, 85 %): ^{1}H NMR (300 MHz, MeOD) δ 7.06 (s, 2H), 4.98 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 166.76, 146.09, 146.02, 138.84, 119.73, 108.94, 52.04.



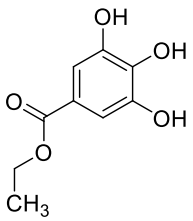
Methyl 3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxylate (**11**). Following the general method IB, using methyl 3,4,5-trihydroxybenzoate (185 mg, 1.00 mmol) instead of gallic acid, gave the orange precipitate. The resulting orange precipitate was filtered off, washed with water (3 x 6 mL) and dried under high vacuum to give the orange solid product **11** (172 mg, 59 %): ^{1}H NMR (300 MHz, DMSO) δ 14.94 (s, 1H), 9.90 (s, 1H), 9.73 (s, 1H), 8.39 (d, *J* = 1.2 Hz, 1H), 7.58 (d, *J* = 1.6 Hz, 1H), 7.47 (s, 1H), 4.01 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.60, 167.11, 154.19, 152.49, 151.91, 138.52, 138.24, 130.23, 123.91, 116.69, 114.49, 110.49, 56.71, 53.42; LRMS (ESI): calcd for: $C_{14}H_{12}O_7$ $[M+H]^+$= 293.2, obsd $[M+H]^+$= 293.1.



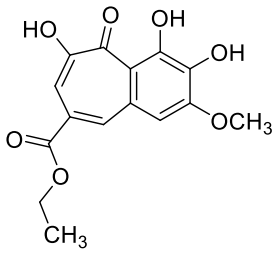
3,4,6-trihydroxy-5-oxo-5H-benzo[7]annulene-8-carboxylic acid (**12**). Following the general method IB, using 1,2-benzenediol (101 mg, 1.00 mmol) instead of 3-methoxycatechol, gave **12** as an orange precipitate (121 mg, 49 %): ^{1}H NMR (300 MHz, DMSO) δ 14.82 (s, 1H), 10.41 (s, 1H), 9.68 (s, 1H), 8.32 (d, *J* = 0.9 Hz, 1H), 7.65 (dd, *J* = 10.3, 5.3 Hz, 2H), 7.48 (d, *J* = 8.6 Hz, 1H); ^{13}C NMR (75 MHz, DMSO) δ 185.38, 168.07, 153.55, 151.65, 149.04, 139.18, 129.19, 128.64, 123.98, 122.43, 120.79, 116.42; LRMS (ESI): calcd for: $C_{12}H_8O_6$ $[M-H]^-$ = 247.1, obsd $[M-H]^-$ = 247.0.



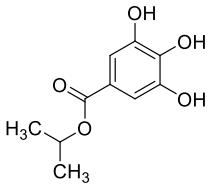
Methyl 3,4,6-trihydroxy-5-oxo-5H-benzo[7]annulene-8-carboxylate (**13**). Following **12** synthetic method, using 3,4,5-trihydroxybenzoate (185 mg, 1.00 mmol) instead of gallic acid, gave **13** as an orange precipitate (141 mg, 54 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.67 (s, 1H), 8.45 (d, *J* = 1.6 Hz, 1H), 8.19 (s, 1H), 8.01 (d, *J* = 1.6 Hz, 1H), 7.55 (s, 2H), 6.54 (s, 1H), 3.99 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 185.43, 167.00, 153.68, 151.74, 149.36, 139.24, 128.91, 128.87, 122.81, 122.40, 120.69, 115.68, 53.39; LRMS (ESI): calcd for: $C_{13}H_{10}O_6$ $[M-H]^-$ = 261.0, obsd $[M-H]^-$ = 261.0.



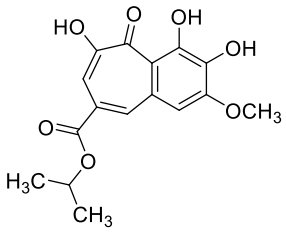
Ethyl 3,4,5-trihydroxybenzoate. Following methyl 3,4,5-trihydroxybenzoate synthetic method, using EtOH (10 mL) instead of MeOH, gave ethyl 3,4,5-trihydroxybenzoate as a white solid (903 mg, 91 %): ^{1}H NMR (300 MHz, DMSO) δ 9.26 (s, 2H), 8.93 (s, 1H), 6.94 (s, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO) δ 166.26, 145.99, 138.77, 120.01, 108.89, 60.44, 14.71.



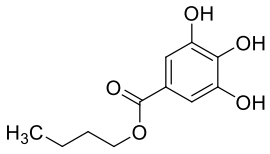
Ethyl 3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxylate (**14**). Following general method IB, using ethyl 3,4,5-trihydroxybenzoate (198 mg, 1.00 mmol) instead of gallic acid, gave **14** as an orange precipitate (158.6 mg, 51.8 %): ^{1}H NMR (300 MHz, DMSO) δ 14.95 (s, 1H), 9.89 (s, 1H), 9.72 (s, 1H), 8.37 (d, *J* = 1.6, 1H), 7.59 (d, *J* = 1.6 Hz, 1H), 7.46 (s, 1H), 4.35 (tt, *J* = 7.1, 3.5 Hz, 2H), 4.01 (s, 3H), 1.37 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.56, 166.57, 154.15, 152.48, 151.89, 138.44, 138.20, 130.24, 124.16, 116.68, 114.50, 110.44, 62.21, 56.71, 14.62; HRMS (ESI): calcd for: $C_{15}H_{14}O_7$ $[M-H]^-$ = 305.0668, obsd $[M-H]^-$ = 305.0666.



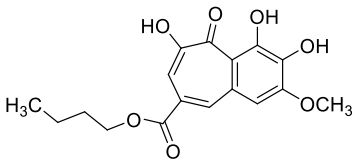
Isopropyl 3,4,5-trihydroxybenzoate. Following the method used for methyl 3,4,5-trihydroxybenzoate, using isopropyl alcohol (10 mL) instead of MeOH, gave isopropyl 3,4,5-trihydroxybenzoate as a white solid (941 mg, 89 %): ^{1}H NMR (300 MHz, DMSO) δ 9.18 (m, 3H), 6.93 (s, 2H), 5.42–4.82 (m, 1H), 1.26 (m, 6H); ^{13}C NMR (75 MHz, DMSO) δ 165.75, 145.95, 138.68, 120.39, 108.88, 67.57, 22.20.



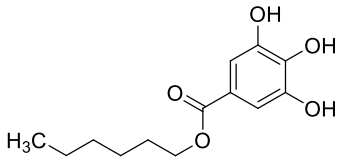
Isopropyl 3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxylate (**15**). Following general method IB, using isopropyl 3,4,5-trihydroxybenzoate (198 mg, 1.00 mmol) instead of gallic acid, gave **15** as an orange precipitate (157 mg, 49 %): ^{1}H NMR (300 MHz, DMSO) δ 14.96 (s, 1H), 9.88 (s, 1H), 9.72 (s, 1H), 8.53–8.28 (m, 1H), 7.58 (d, *J* = 1.5 Hz, 1H), 7.45 (s, 1H), 5.30–5.02 (m, 1H), 4.02 (s, 3H), 1.37 (d, 6H); ^{13}C NMR (75 MHz, DMSO) δ 183.55, 166.06, 154.13, 152.49, 151.88, 138.38, 138.17, 130.27, 124.51, 116.70, 114.54, 110.42, 69.83, 56.73, 22.08; HRMS (ESI): calcd for: $C_{16}H_{16}O_7$ $[M-H]^-$ = 319.0812, obsd $[M-H]^-$ = 319.0813.



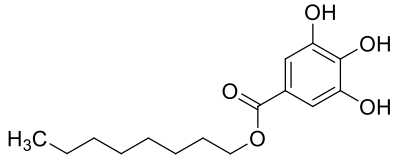
Butyl 3,4,5-trihydroxybenzoate. Following the method used for methyl 3,4,5-trihydroxybenzoate, using 1-butanol (10 mL) instead of MeOH, gave butyl 3,4,5-trihydroxybenzoate as a white solid (644 mg, 57 %). ^{1}H NMR (300 MHz, DMSO) δ 9.25-8.95 (m, 3H), 6.95 (s, 2H), 4.16 (t, *J* = 6.5 Hz, 2H), 1.69–1.56 (m, 2H), 1.48–1.30 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO) δ 166.30, 145.99, 138.78, 120.01, 108.89, 64.12, 30.80, 19.25, 14.06.



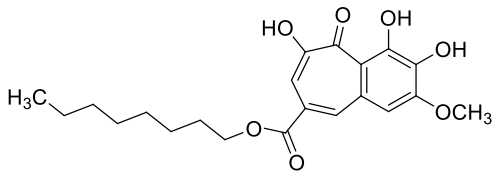
Butyl 3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxylate (**16**). Following general method IB, using butyl 3,4,5-trihydroxybenzoate (226 mg, 1.00 mmol) instead of gallic acid, gave **16** as an orange precipitate (171 mg, 51 %): ^{1}H NMR (300 MHz, DMSO) δ 14.95 (s, 1H), 9.89 (s, 1H), 9.73 (s, 1H), 8.36 (d, *J* = 1.1 Hz, 1H), 7.58 (d, *J* = 1.5 Hz, 1H), 7.44 (s, 1H), 4.31 (t, *J* = 6.6 Hz, 2H), 4.01 (s, 3H), 1.82–1.64 (m, 2H), 1.53–1.32 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.57, 166.61, 154.17, 152.49, 151.90, 138.47, 138.22, 130.24, 124.15, 116.70, 114.45, 110.47, 65.89, 56.72, 30.68, 19.17, 14.10; HRMS (ESI): calcd for: $C_{17}H_{18}O_7$ $[M-H]^-$ = 333.0978, obsd $[M-H]^-$ = 333.0979.



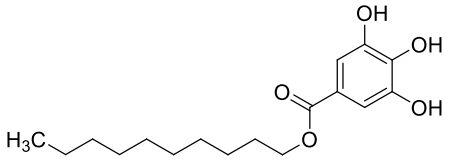
Hexyl 3,4,5-trihydroxybenzoate. Following methyl 3,4,5-trihydroxybenzoate synthetic method, using 1-hexanol (10 mL) instead of MeOH, gave hexyl 3,4,5-trihydroxybenzoate as a white solid (549 mg, 43 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (s, 2H), 5.99 (s, 3H), 4.29 (t, *J* = 6.6 Hz, 2H), 1.85–1.68 (m, 2H), 1.52–1.24 (m, 6H), 0.92 (dd, *J* = 8.6, 4.3 Hz, 3H); ^{13}C NMR (75 MHz, Acetone) δ 165.83, 145.08, 137.67, 121.21, 108.81, 64.10, 31.32, 28.60, 25.57, 22.33, 13.38.



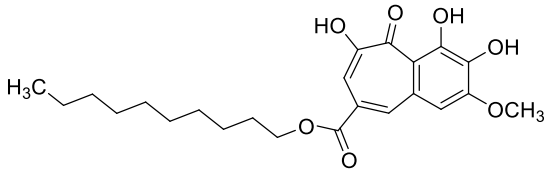
Octyl 3,4,5-trihydroxybenzoate. Following methyl 3,4,5-trihydroxybenzoate synthetic method, using 1-octanol (10 mL) instead of MeOH, gave octyl 3,4,5-trihydroxybenzoate as a white solid (643.7 mg, 46 %): ^{1}H NMR (300 MHz, DMSO) δ 9.15 (s, 3H), 6.94 (s, 2H), 4.15 (t, *J* = 6.5 Hz, 2H), 1.64 (dd, *J* = 14.2, 6.7 Hz, 2H), 1.45–1.21 (m, 10H), 0.86 (t, 3H); ^{13}C NMR (75 MHz, DMSO) δ 166.29, 145.99, 138.79, 119.99, 108.88, 64.40, 31.68, 29.11, 29.10, 28.74, 26.00, 22.53, 14.41.



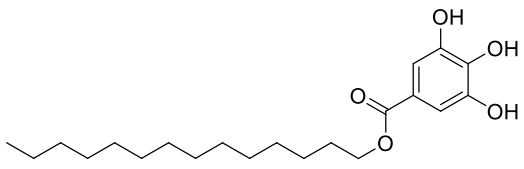
Octyl 3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxylate (**17**). Following general method IB, using octyl 3,4,5-trihydroxybenzoate (254 mg, 1.00 mmol) instead of gallic acid, gave **17** as an orange precipitate (205.7 mg, 52.7 %): ^{1}H NMR (300 MHz, DMSO) δ 14.94 (s, 1H), 9.88 (s, 1H), 9.71 (s, 1H), 8.34 (d, *J* = 1.2 Hz, 1H), 7.57 (d, *J* = 1.5 Hz, 1H), 7.41 (s, 1H), 4.29 (t, *J* = 6.7 Hz, 2H), 4.01 (s, 3H), 1.81–1.64 (m, 2H), 1.45–1.17 (m, 10H), 0.85 (dd, *J* = 8.8, 4.8 Hz, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.53, 166.57, 154.15, 152.46, 151.89, 138.46, 138.22, 130.21, 124.13, 116.67, 114.42, 110.42, 66.17, 56.68, 31.67, 29.10, 29.05, 28.56, 25.87, 22.53, 14.40; HRMS (ESI): calcd for: $C_{21}H_{26}O_7$ $[M\text{-}H]^-$ = 389.1606, obsd $[M\text{-}H]^-$ = 389.1605.



Decyl 3,4,5-trihydroxybenzoate. Following the method used for methyl 3,4,5-trihydroxybenzoate, using 1-decanol (10 mL) instead of MeOH, gave decyl 3,4,5-trihydroxybenzoate as a white solid (743 mg, 48 %): ^{1}H NMR (400 MHz, DMSO) δ 9.12 (s, 3H), 6.95 (s, 2H), 4.15 (t, *J* = 6.5 Hz, 2H), 1.64 (dd, *J* = 13.9, 6.8 Hz, 2H), 1.40–1.14 (m, 14H), 0.85 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 166.32, 146.00, 138.80, 120.03, 108.99, 108.92, 64.40, 31.76, 29.45, 29.42, 29.32, 29.16, 28.75, 25.99, 22.56, 14.40.

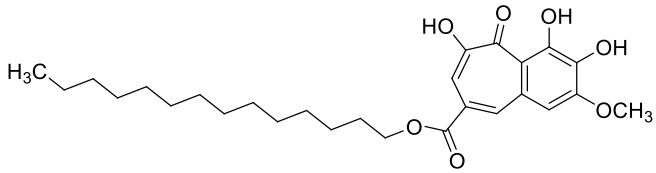


Decyl 3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxylate (**18**). Following general method IB, using decyl 3,4,5-trihydroxybenzoate (310 mg, 1.00 mmol) instead of gallic acid, gave **18** as an orange precipitate (222 mg, 53 %): ^{1}H NMR (300 MHz, DMSO) δ 14.96 (s, 1H), 9.76 (s, 2H), 8.34 (s, 1H), 7.57 (s, 1H), 7.42 (s, 1H), 4.29 (t, *J* = 6.6 Hz, 2H), 4.01 (s, 3H), 1.82–1.64 (m, 2H), 1.47–1.14 (m, 14H), 0.83 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.54, 166.58, 154.16, 152.47, 151.90, 138.47, 138.23, 130.22, 124.13, 116.68, 114.43, 110.43, 66.17, 56.68, 31.75, 29.38, 29.14, 29.10, 28.54, 25.85, 22.54, 14.39; HRMS (ESI): calcd for: $C_{23}H_{30}O_7$ $[M\text{-}H]^-$ = 417.1914, obsd $[M\text{-}H]^-$ = 417.1918.

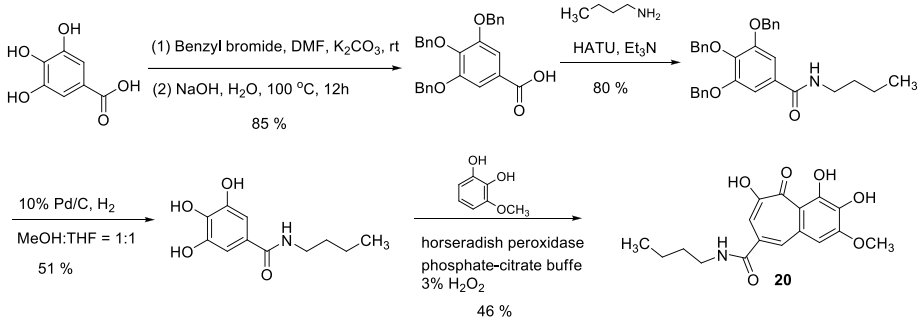


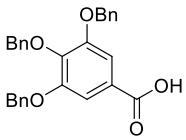
Tetradecyl 3,4,5-trihydroxybenzoate. Following the method used for methyl 3,4,5-trihydroxybenzoate, using 1-tetradecanol (10 g) instead of MeOH, to give tetradecyl 3,4,5-trihydroxybenzoate as a white solid

(754.9 mg, 41.2 %): ^{1}H NMR (300 MHz, Acetone) δ 7.13 (s, 2H), 4.22 (t, *J* = 6.6 Hz, 2H), 1.80–1.65 (m, 2H), 1.54–1.21 (m, 22H), 0.88 (m, 3H); ^{13}C NMR (75 MHz, Acetone) δ 165.83, 145.10, 137.69, 121.21, 108.83, 99.98, 64.10, 31.73, 25.90, 22.42, 13.45.



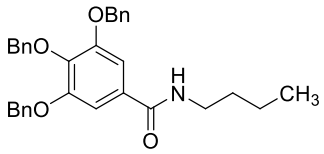
Tetradecyl 3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxylate (**19**). Following general method IB, using tetradecyl 3,4,5-trihydroxybenzoate (366.5 mg, 1 mmol) instead of gallic acid, gave **19** as an orange precipitate (195 mg, 41 %): ^{1}H NMR (300 MHz, DMSO) δ 14.94 (s, 1H), 9.81 (d, 2H), 8.36 (d, *J* = 1.2 Hz, 1H), 7.58 (d, *J* = 1.5 Hz, 1H), 7.43 (s, 1H), 4.30 (t, *J* = 6.6 Hz, 2H), 4.01 (s, 3H), 1.73 (ddd, *J* = 8.3, 6.5, 4.4 Hz, 2H), 1.31-1.15 (m, 22H), 0.84 (dd, *J* = 8.8, 4.7 Hz, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.56, 166.61, 154.14, 152.49, 151.89, 138.46, 138.36, 130.17, 124.10, 116.69, 114.46, 110.44, 66.15, 56.68, 31.74, 29.49, 29.45, 29.36, 29.31, 29.15, 29.03, 28.52, 25.82, 22.54, 14.40; HRMS (ESI): calcd for: $C_{27}H_{38}O_7$ [M-H]$^-$ = 473.2547, obsd [M-H]$^-$ = 473.2544.





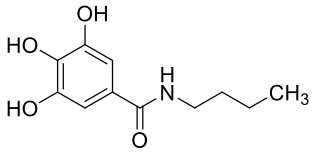
3,4,5-tris(benzyloxy)benzoic acid.

Gallic acid (1.70 g, 10.0 mmol) and anhydrous K_2CO_3 (11.3 g, 82.0 mmol) in DMF (80 mL) was stirred at room temperature for 1 h. BnBr (14.3 mL, 120 mmol) was added dropwise into the solution over 30 min at 40 °C under nitrogen. The reaction mixture was stirred for 12 h at 40 °C, then additional H_2O (40 mL) and EtOAc (100 mL) were added in the flask. The organic layer was washed with H_2O (3 x 100 mL), dried over anhydrous Na_2SO_4, filtered and was evaporated to dryness in vacuo. The residue was dissolved in aqueous ethanol (50%, 100 mL) containing 5 M NaOH and refluxed for 12 h.[12] The solution was diluted with H_2O (50 mL), adjusted to pH 2 with concentrated HCl and stirred for 30 min at rt. The precipitate was collected and recrystallized from methanol to give colorless needle crystal of 3,4,5-tris(benzyloxy)benzoic acid (3.75 g, 85 %): ^{1}H NMR (300 MHz, DMSO) δ 12.95 (s, 1H), 7.51–7.26 (m, 17H), 5.19 (s, 4H), 5.05 (s, 2H); ^{13}C NMR (75 MHz, DMSO) δ 167.29, 152.45, 141.41, 137.83, 137.30, 128.88, 128.65, 128.54, 128.48, 128.35, 128.24, 128.02, 127.06, 126.86, 126.45, 108.64, 74.68, 70.65.



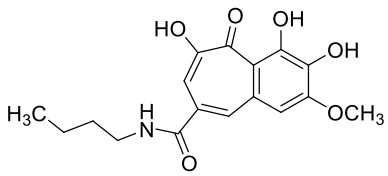
3,4,5-tris(benzyloxy)-*N*-butylbenzamide.

To a solution of 3,4,5-tris(benzyloxy)benzoic acid (440 mg, 1 mmol) in dry dichloromethane (5 mL) was added HATU (342 mg, 0.900 mmol), DIPEA (0.35 mL, 2.00 mmol) and 1-butanamine (98 µL, 1.00 mmol). The reaction mixture was stirred at room temperature overnight and the solution was evaporated and purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:6) as eluent, gave 3,4,5-tris(benzyloxy)-*N*-butylbenzamide as a white solid (398 mg, 80 %): ^{1}H NMR (300 MHz, Acetone) δ 7.82–7.68 (m, 1H), 7.59–7.19 (m, 17H), 5.18 (s, 4H), 5.09 (s, 2H), 3.38 (td, *J* = 7.1, 5.9 Hz, 2H), 1.67–1.50 (m, 2H), 1.46–1.26 (m, 6H), 0.90 (dd, *J* = 8.3, 5.4 Hz, 3H); ^{13}C NMR (75 MHz, Acetone) δ 165.64, 152.60, 140.48, 138.08, 137.28, 130.65, 128.39, 128.24, 127.99, 127.82, 127.64, 106.63, 74.56, 70.77, 39.62, 31.45, 29.59, 7.82, 26.55, 22.39, 13.43.



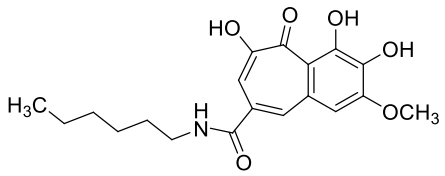
***N*-butyl-3,4,5-trihydroxybenzamide**.

To a solution of 3,4,5-tris(benzyloxy)-*N*-butylbenzamide (198 mg, 0.400 mmol) in THF/MeOH (15 mL, 1:1 v/v) was added palladium hydroxide on carbon (palladium 10 wt % on carbon). The suspension was stirred for 13 h at room temperature under a H_2 atmosphere.[13] The mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure, using ethyl acetate/hexane (1:4) as eluent, give *N*-butyl-3,4,5-trihydroxybenzamide as a white solid (46 mg, 51 %): ^{1}H NMR (300 MHz, Acetone) δ 8.15 (s, 2H), 7.62 (s, 1H), 7.06 (s, 2H), 3.46–3.25 (m, 2H), 1.57 (dq, *J* = 7.5, 6.6 Hz, 2H), 1.43–1.25 (m, 2H), 0.91 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, Acetone) δ 167.10, 145.24, 135.96, 125.83, 106.81, 39.35, 31.61, 19.90, 13.24.

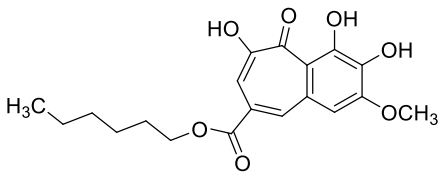


***N*-butyl-3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxamide** (**20**).

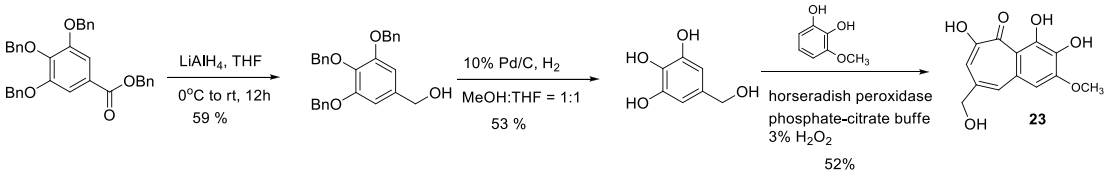
Following general method IB, using *N*-butyl-3,4,5-trihydroxybenzamide (113 mg, 0.500 mmol) instead of gallic acid to give **20** as an orange precipitate (78 mg, 46 %): ^{1}H NMR (300 MHz, DMSO) δ 14.98 (s, 1H), 9.74–9.54 (m, 2H), 8.60 (dd, *J* = 6.6, 2.9 Hz, 1H), 8.02 (s, 1H), 7.47 (s, 1H), 7.29 (s, 1H), 4.00 (s, 3H), 3.26 (dt, *J* = 12.8, 3.5 Hz, 2H), 1.63–1.46 (m, 2H), 1.45–1.05 (m, 78), 0.86 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.10, 167.66, 154.32, 152.60, 151.51, 137.05, 134.78, 131.27, 130.21, 116.56, 115.59, 108.93, 56.50, 31.48, 29.41, 26.64, 22.52, 14.39; HRMS (ESI): calcd for: $C_{19}H_{23}NO_6$ $[M-H]^-$ = 360.1454, obsd $[M-H]^-$ = 360.1452.

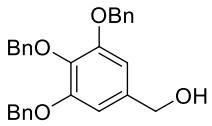


N-hexyl-3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxamide (**21**). Following the general method of **20**, we get **21** as an orange solid (76 mg, 42 %): ^{1}H NMR (300 MHz, DMSO) δ 14.98 (s, 1H), 9.74–9.54 (m, 2H), 8.60 (dd, *J* = 6.6, 2.9 Hz, 1H), 8.02 (s, 1H), 7.47 (s, 1H), 7.29 (s, 1H), 4.00 (s, 3H), 3.26 (m, 2H), 1.63–1.46 (m, 2H), 1.45–1.05 (m, 78), 0.86 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.10, 167.66, 154.32, 152.60, 151.51, 137.05, 134.78, 131.27, 130.21, 116.56, 115.59, 108.93, 56.50, 31.48, 29.41, 26.64, 22.52, 14.39; HRMS (ESI): calcd for: $C_{19}H_{23}NO_6$ [M-H]$^-$ = 360.1454, obsd [M-H]$^-$ = 360.1452.



Hexyl 3,4,6-trihydroxy-2-methoxy-5-oxo-5H-benzo[7]annulene-8-carboxylate (**22**, **CU-CPT22**). Following general method IB, using hexyl 3,4,5-trihydroxybenzoate (254 mg, 1.00 mmol) instead of gallic acid to give **CU-CPT22** as an orange precipitate (195 mg, 54 %): ^{1}H NMR (300 MHz, DMSO) δ 14.93 (s, 1H), 9.73 (s, 2H), 8.32 (d, *J* = 1.2 Hz, 1H), 7.56 (d, *J* = 1.5 Hz, 1H), 7.40 (s, 1H), 4.28 (dd, *J* = 8.6, 4.8 Hz, 2H), 4.01 (s, 3H), 1.72 (dd, *J* = 14.5, 6.8 Hz, 2H), 1.52–1.18 (m, 6H), 0.97–0.77 (m, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.51, 166.55, 154.13, 152.45, 151.89, 138.45, 138.23, 130.19, 124.11, 116.66, 114.41, 110.40, 66.16, 56.67, 31.34, 28.53, 25.52, 22.45, 14.33; HRMS (ESI): calcd for: $C_{19}H_{22}O_7$ [M-H]$^-$ = 361.1286, obsd [M-H]$^-$ = 361.1292.

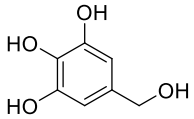




3,4,5-tris(benzyloxy)phenyl)methanol.

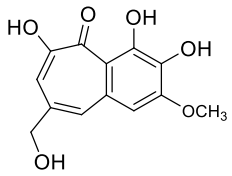
A solution of benzyl 3,4,5-tris(benzyloxy)benzoate (1.06 g, 2.00 mmol) in THF (10 mL) was added to a stirred suspension of lithium aluminium hydride (379 mg, 8.00 mmol) in THF (15 mL) at 0 °C over 1 hr. The mixture was then stirred at room temperature for 12 h and subsequently cooled to 0 °C before slowing addition of water (40 mL).[14] The mixture was extracted with EtOAc (3 x 20 mL). The organic extracts were dried with Na_2SO_4 and concentrated under reduced pressure. Purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:2) as eluent, gave 3,4,5-tris(benzyloxy)phenyl)methanol as a colorless oil (505 mg, 59 %): ^{1}H NMR (300 MHz, Acetonitrile)

δ 7.55–7.20 (m, 15H), 6.73 (s, 2H), 5.10 (s, 4H), 4.97 (s, 2H), 4.48 (d, *J* = 5.8 Hz, 2H), 3.19 (t, *J* = 5.9 Hz, 1H); ^{13}C NMR (75 MHz, Acetonitrile) δ 153.88, 139.41, 139.37, 138.70, 129.73, 129.63, 129.39, 129.17, 129.04, 106.77, 75.85, 71.83, 64.93.



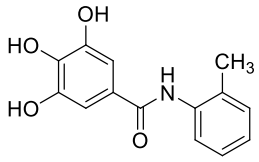
5-(hydroxymethyl)benzene-1,2,3-triol.

To a solution of 3,4,5-tris(benzyloxy)phenyl)methanol (171 mg, 0.401 mmol) in THF/MeOH (15 mL, 1:1 v/v) was added palladium hydroxide on carbon (palladium 10 wt % on carbon). The suspension was stirred for 13 h at room temperature under a H_2 atmosphere. The mixture was filtered through Celite, and the filtrate was evaporated and purified by flash chromatography on silica gel, using ethyl acetate/hexane (1:2) as eluent, give 5-(hydroxymethyl)benzene-1,2,3-triol as a colorless oil (33 mg, 53 %): ^{1}H NMR (300 MHz, Acetone) δ 7.58 (s, 2H), 6.42 (s, 2H), 4.42 (s, 2H); ^{13}C NMR (75 MHz, Acetone) δ 145.50, 133.52, 131.60, 106.29, 106.24, 105.86, 63.94.



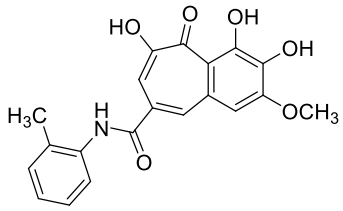
3,4,6-trihydroxy-8-(hydroxymethyl)-2-methoxy-5H-benzo[7]annulen-5-one (**23**).

Following general method IB, using 5-(hydroxymethyl)benzene-1,2,3-triol (78 mg, 0.500 mmol) instead of gallic acid, gave **23** as an orange precipitate (68 mg, 52 %): ^{1}H NMR (300 MHz, DMSO) δ 15.05 (s, 1H), 9.47 (s, 1H), 9.29 (s, 1H), 7.54 (s, 1H), 7.18–6.98 (m, 2H), 5.53 (dd, *J* = 7.4, 4.0 Hz, 1H), 4.43 (d, *J* = 5.0 Hz, 2H), 3.96 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 182.50, 154.53, 152.95, 151.15, 137.75, 135.25, 133.02, 131.39, 117.59, 116.16, 106.91, 66.10, 56.37; HRMS (ESI): calcd for: $C_{13}H_{12}O_6$ $[M\text{-}H]^-$ = 263.0558, obsd $[M\text{-}H]^-$ = 263.0561.



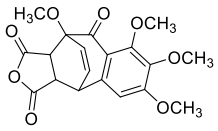
3,4,5-trihydroxy-*N*-*o*-tolylbenzamide.

Following general synthesize method of ***N*-butyl-3,4,5-trihydroxybenzamide**, using *o*-toluidine (160 µL, 1.00 mmol) instead of 1-butanamine, gave an oil mixture. This was purified by flash chromatography on silica gel. Using ethyl acetate/hexane (1:4) as eluent, gave 3,4,5-trihydroxy-*N*-*o*-tolylbenzamide as a colorless oil (203.77 mg, 78.6 %): ^{1}H NMR (300 MHz, Acetone) δ 8.77 (s, 1H), 8.18 (s, 2H), 7.66–7.56 (m, 1H), 7.28–7.02 (m, 5H), 2.33 (s, 3H); ^{13}C NMR (75 MHz, Acetone) δ 165.28, 145.31, 136.90, 136.36, 132.12, 130.23, 125.99, 125.92, 125.20, 125.17, 125.05, 124.93, 107.07, 17.31.



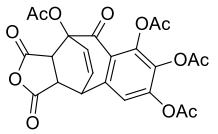
3,4,6-trihydroxy-2-methoxy-5-oxo-*N*-*o*-tolyl-5H-benzo[7]annulene-8-carboxamide (**24**).

Following general method IB, using 3,4,5-trihydroxy-*N*-o-tolylbenzamide (128 mg, 0.500 mmol) instead of gallic acid to give **24** as an orange precipitate (85 mg, 45 %): ^{1}H NMR (300 MHz, DMSO) δ 15.00 (s, 1H), 10.07 (s, 1H), 9.73 (d, 2H), 8.20 (d, *J* = 1.1 Hz, 1H), 7.54 (d, *J* = 1.4 Hz, 1H), 7.42–7.10 (m, 5H), 4.01 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 183.28, 167.13, 154.44, 152.62, 151.62, 137.27, 136.88, 135.43, 134.06, 131.15, 130.85, 130.16, 126.76, 126.51, 116.63, 115.61, 109.23, 56.55, 18.46; HRMS (ESI): calcd for: $C_{2O}H_{17}NO_6$ $[M-H]^-$ = 366.0984, obsd $[M-H]^-$ = 366.0982.



25

Following general method II, gave **25** as a white solid (28 mg, 75 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.65–6.56 (m, 2H), 6.16 (d, *J* = 9.2 Hz, 1H), 4.16 (dd, *J* = 7.2, 2.6 Hz, 1H), 3.98 (d, *J* = 0.8 Hz, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 3.84 (s, 3H), 3.74 (dd, *J* = 9.3, 2.4 Hz, 1H), 3.66 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.17, 171.15, 166.70, 157.30, 156.93, 143.10, 139.31, 133.82, 132.62, 119.40, 106.62, 85.23, 61.98, 60.95, 56.22, 54.20, 48.24, 44.61, 44.56; HRMS (ESI): calcd for: $C_{19}H_{18}O_8$ $[M+H]^+$= 375.1066, obsd $[M+H]^+$ = 375.1075.



26

Following general method II, using **9** (39 mg, 0.100 mmol) instead of **6** to give **26** as an white solid (25 mg, 52 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (s, 1H), 6.81–6.70 (m, 1H), 6.21–6.09 (m, 1H), 4.92–4.71 (m, 1H), 4.40–4.23 (m, 1H), 3.86 (dd, *J* = 6.0, 2.0, Hz, 1H), 2.313-2.29 (m, 12H); ^{13}C NMR (101 MHz, DMSO) δ 171.67, 169.16, 168.10, 167.91, 167.64, 167.28, 146.85, 146.09, 141.54, 136.36, 132.30, 122.26, 121.96, 121.85, 83.30, 48.51, 43.75, 42.55, 21.26, 20.79, 20.53, 20.19; HRMS (ESI): calcd for: $C_{23}H_{18}O_{12}$ $[M+Na]^+$= 509.0678, obsd $[M+Na]^+$ = 509.0691.

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