Supporting Information
For

NAD(P)H: quinone oxidoreductase 1 (NQO1) enzyme responsive nanocarrier based on mesoporous silica nanoparticles for tumor targeted drug delivery *in vitro* and *in vivo*

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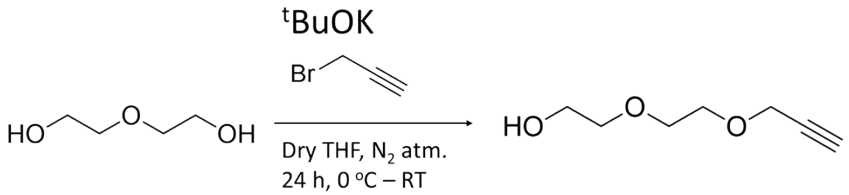
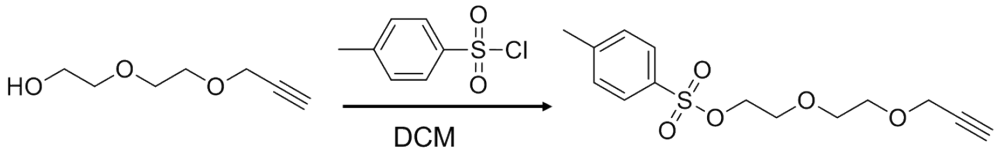
Contents

1. Synthesis of 2-(2-(prop-2-yn-1-yloxy)ethoxy)ethanol (1a) [S1]



2,2'-Oxydiethanol (4.775 g, 45 mmol) was added to the suspension of ^{t}BuOK (2.55 g, 22.75 mmol) in dry THF (75 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 30 min; then propargyl bromide (3.345 g, 22.5 mmol) in dry THF (15 mL) was added dropwise to the mixture for a period of 30 min. The resulting mixture was stirred at room temperature for 24 h. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc) to give the product as a pale yellow liquid (2.23 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.06 (d, J = 2.4 Hz, 2H), 3.54 (br, 6H), 3.44 (br, 2H), 2.45 (t, J = 2.3 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 79.42, 74.87, 72.50, 69.95, 68.93, 61.3, 58.19.

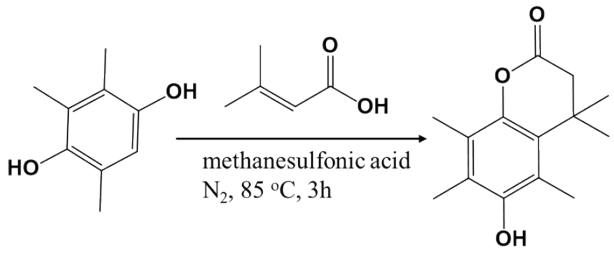
2. Synthesis of 2-(2-(prop-2-yn-1-yloxy)ethoxy)ethyl 4-methylbenzenesulfonate (1)[S2]



To a stirred solution of 2-(2-(prop-2-yn-1-yloxy)ethoxy)ethanol (**1a**) (2.0 g, 13.87 mmol) in CH_2Cl_2 (25 mL), Et_3N (6 mL, 43 mmol) and DMAP (17 mg, 0.14 mmol) were added at 0 °C and stirred for 30 min at the same temperature. Then, to the reaction mixture at 0 °C, a solution of 4-methylbenzene-1-sulfonyl chloride (2.9 g, 15.3 mmol) in CH_2Cl_2 (25 mL) was added dropwise.

The mixture was allowed to stir at room temperature for 24 h. After the addition of saturated $NaHCO_3$ solution, the reaction mixture was extracted with EtOAc. The combined organic layers were washed with brine solution, H_2O and dried over $MgSO_4$ and solvent was removed under reduced pressure. The resultant residue was purified by silica gel column chromatography (hexane/EtOAc = 4/1) to afford as a pale brown color oil (3.8 g, 91%). ^{1}H NMR(300 MHz, $CDCl_3$): δ = 7.78 (d, J= 7.9 Hz, 2 H), 7.34 (d, J= 7.9 Hz, 2 H), 4.16–4.13 (m, 4 H), 3.67 (m, 2 H), 3.61 (m, 4 H), 2.44 (s, 1 H,), 2.43(s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 144.8, 133.0, 129.8, 128.0, 79.5, 74.7, 70.5, 69.2, 69.0, 68.7, 58.4, 21.6; MS (ESI): m/z = 299.2 ($[M+H]^+$), 321.2 ($[M+Na]^+$).

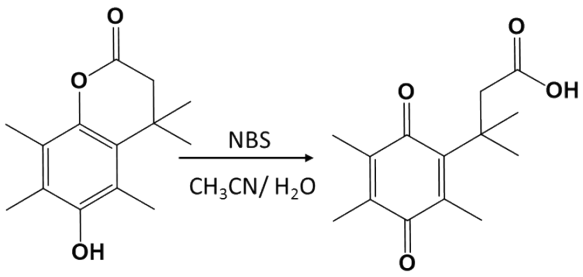
3. Synthesis of 6-hydroxy-4,4,5,7,8-pentamethylchroman-2-one (2a)[S3]



2,3,5-Trimethylhydroquinone (1 g, 6.5 mmol) and 3,3-dimethylacrylic acid (0.72 g, 7.2 mmol) were added in 10 mL methanesulfonic acid. The mixture was stirred at 85 ° C under nitrogen for 3 h and then cooled to room temperature. 100 g of ice was added was added to the mixture, with stirring. The precipitate was extracted with ethyl acetate (50 mL) for four times. The combined organic layer was washed with saturated $NaHCO_3$ (250 mL) and water (50 mL) twice and dried over $MgSO_4$ and solvent was removed under reduced pressure. The obtained residue was recrystallized from hexane and ethyl acetate (2:1, v/v) to give of the desired product as a white solid (1.44 g, 84 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.69 (s, 1H), 2.56 (s, 2H), 2.37 (s, 3H), 2.23

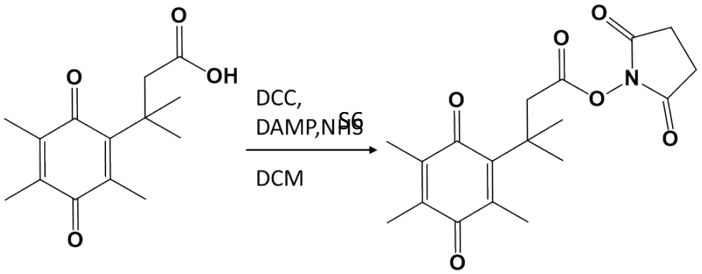
(s, 3H), 2.9 (s, 3H), 1.46 (s, 6H). MS (EI): m/z (%) = 234.1 (100), 219.1 (27), 192.2 (39), 177.1 (31), 160.1 (13), 149.1 (10).

4. **Synthesis of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl) butanoic acid (2b)**[S3]



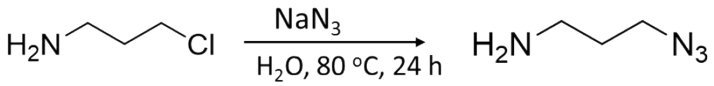
To a solution of 3,4-dihydro-6-hydroxy-4,4,5,7,8-pentamethylchromen-2-one (1.58 g, 6.74 mmol) in a mixture of acetonitrile (15 mL) and water (3 mL) was added *N*-bromosuccinimide (1.26 g, 7.08 mmol) in portions with stirring at room temperature. After 1 hour, the organic solvents were evaporated under reduced pressure, and the remaining solution was extracted with CH_2Cl_2 (30 mL) two times. The combined organic layer was dried over $MgSO_4$, and the solvent was removed to give 1.65 g (98 %) of a yellow oily product (3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoic acid), which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.04 (s, 2H), 2.15 (s, 3H), 1.96 (m, 3H), 1.94 (m, 3H), 1.45 (s, 6H). MS (EI): m/z (%) = 250.1 (40), 235.1 (40), 204.1 (34), 189.1 (100), 175.1 (37), 163.1 (62), 135.1 (35).

5. **Synthesis of 2,5-dioxopyrrolidin-1-yl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoate (2c)**[S3]



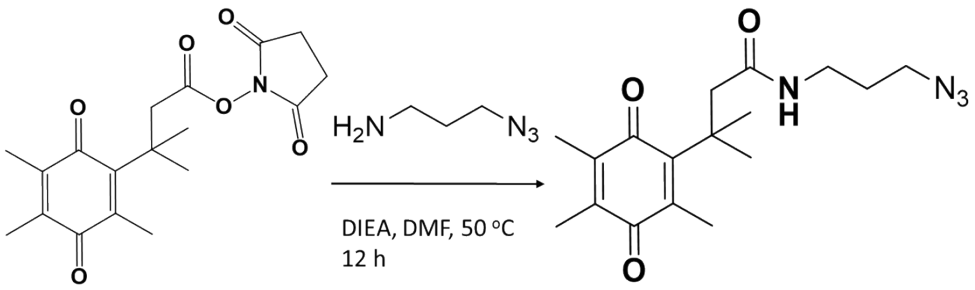
To a solution of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl) butanoic acid (650 mg, 2.6 mmol) and *N*-hydroxysuccinimide (304 mg, 2.7 mmol) in CH_2Cl_2 (40 mL) was added 1,3-dicyclohexylcarbodiimide (DCC, 540 mg, 2.6 mmol) portionwise, followed by a catalytic amount of *N,N*-(dimethylamino)- pyridine (DMAP). The reaction mixture was stirred for 1 h. The white precipitate was filtered, and the filtrate was concentrated. The residue was redissolved in cold ethyl acetate (15 mL), and insoluble impurities were filtered. Solvent was removed and recrystalized in EA/HX (1/5, v/v). The final products (3-Methyl-3-(2,4,5-trimethyl- 3,6-dioxocyclohexa-1,4-dienyl)bu-tanoic Acid, N-Hydroxysuccinimidyl Ester were obtained 419 mg (90 %) of a yellow, foamy solid product. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.27 (s, 2H), 2.77 (s, 4H), 2.15 (s, 3H), 1.94 (s, 6H), 1.51 (s, 6H). MS (EI): m/z (%) = 347.2 (20), 331.2 (100), 316.2 (21), 288.2 (92), 249.2 (24), 205.2 (24), 191.2 (30), 163.2 (19).

6. Synthesis of 3-azidopropan-1-amine (2d)[S4]



3-Azidopropan-1-amine was synthesized based on literature procedure.[S4] Sodium azide was added (1.5 g, 21.4 mmol) to a stirred solution of 3-chloropropylamine hydrochloride (1 g, 7.7 mmol) dissolved in water (10 ml), and the mixture heated to 80 ºC. After 15 h, KOH in pellets were added to basify the solution, followed by extraction with diethyl ether (3 × 10 mL). The combined organic phases were dried over $MgSO_4$ and concentrated to give the desired amine as a colorless volatile oil (510 mg, 68% yield); ^{1}H NMR (300 MHz, $CDCl_3$), δ:3.27 (t, *J* = 6.8 Hz, 2H), 2.70 (t, *J* = 6.8 Hz, 2H) 1.64 (qn, *J* = 6.8 Hz, 2H), 1.45 (br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 49.2, 39.2, 32.3; MS (ESI): m/z (%) = 101 ([M+H] +, 68%), 76 (32), 58 (100).

7. Synthesis of N-(3-azidopropyl)-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanamide (2)[S3]



To a solution of 2c (200 mg, 0.58 mmol) in DMF (3 mL), diisopropylethylamine (DIEA, 240 μL, 1.38 mmol) and 3-azidopropylamine (70 mg, 0.7 mmol) were added. The reaction mixture was stirred overnight at 50 °C, diluted with ethyl acetate (15 mL), washed with NH_4Cl and brine, and dried over MgSO4. Solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (hex/EtOAc, 2:1) to give 140 mg (73%) of product as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.32 (t, *J* = 6.6 Hz, 2H), 3.22 (q, *J* = 6.6 Hz, 2H), 2.81 (s, 2H), 2.12 (s, 3H), 1.97 (m, 3H), 1.95 (m, 3H), 1.73 (quint, *J* = 6.6 Hz, 2H), 1.41 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 191.3, 187.56, 172.02, 153.34, 143.48, 138.03, 137.67, 49.29, 49.18, 38.25, 36.87, 28.89,28.7914.11, 12.71,12.15; MS (ESI): m/z (%)= 333.0 ($[M + 1]^+$).

Figures.

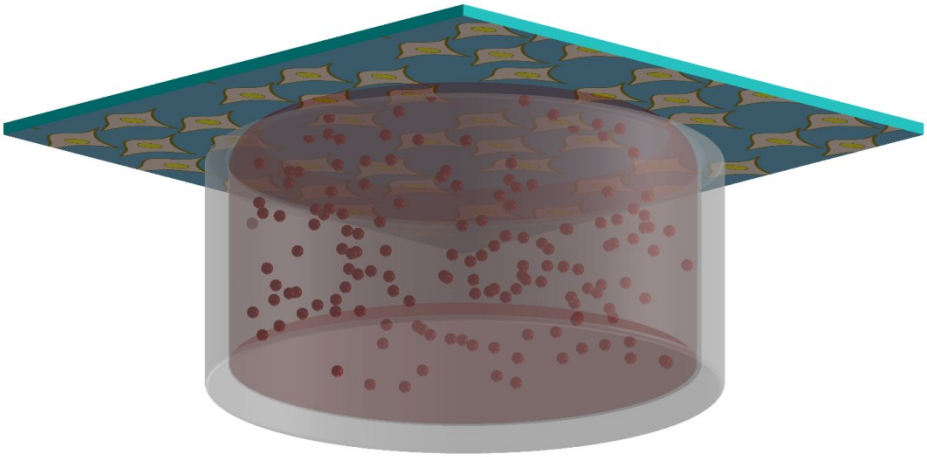


Figure S1. Schematic diagram of the cells treated with DOX loaded MSNP-NQO1 nanoparticles in an inverted configuration.

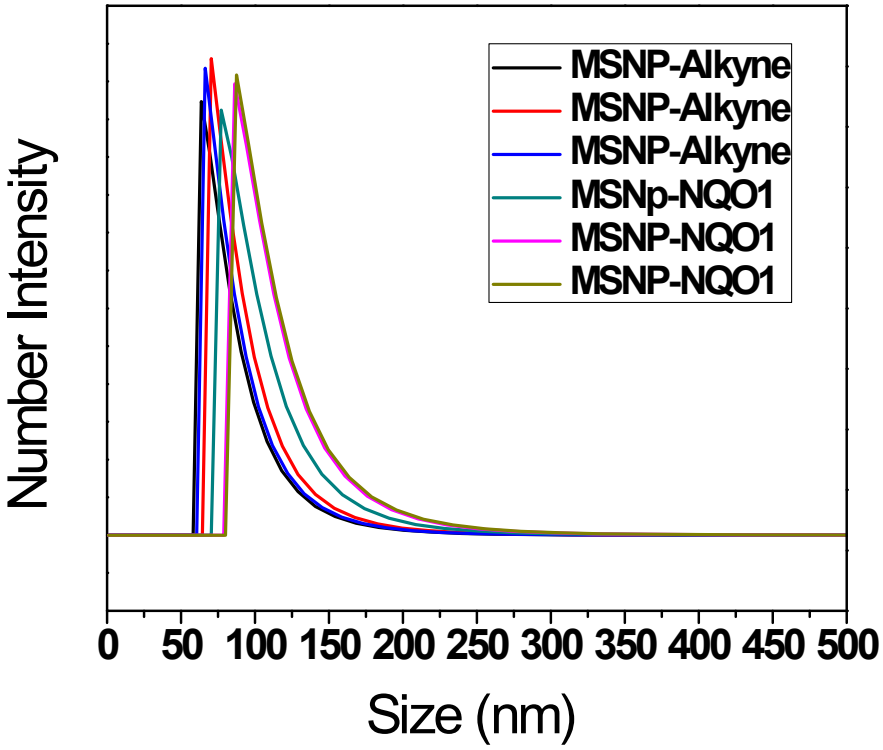


Figure S2. DLS particle size analysis of MSNP-Alkyne and MSNP-NQO1.

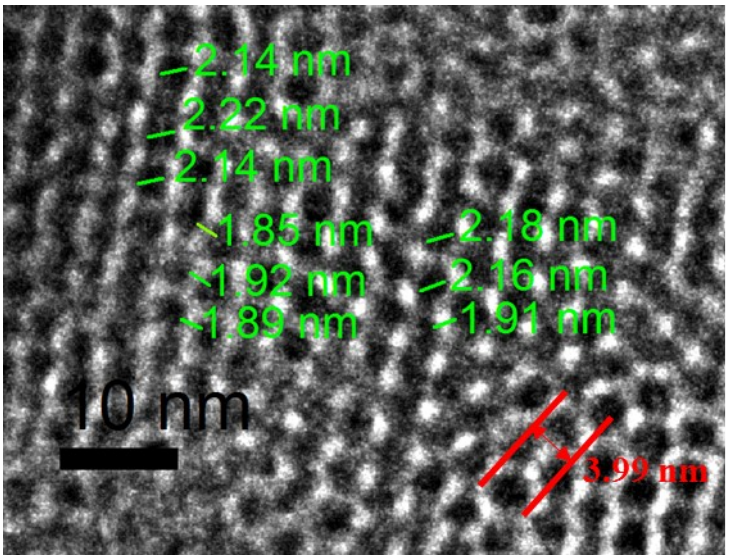


Figure S3. Enlarged HR-TEM images of **MSNP-Alkyne** for calculating the interplanar distance of d_{100} planes and thickness of the mesopore wall.

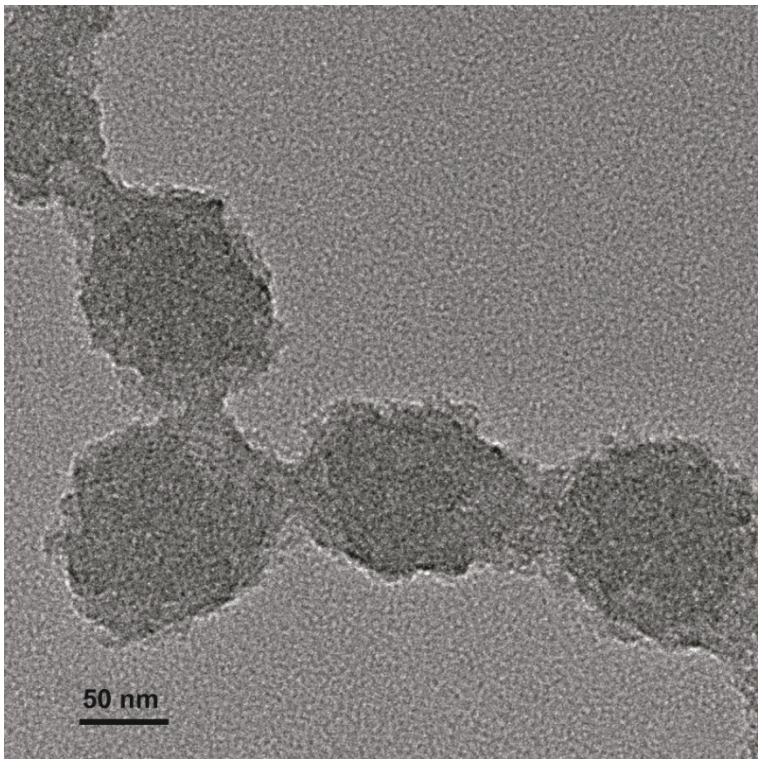


Figure S4. (a) HR-TEM images of MSPN-NQO1.

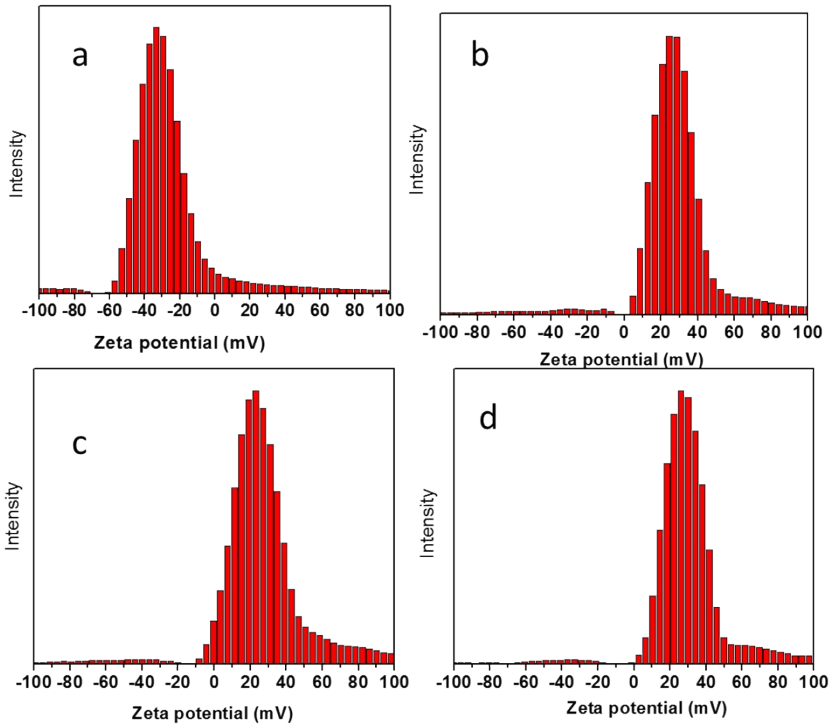


Figure S5. Zeta potential values of different mesoporous silica nanoparticles during the functionalization (a) MSNP, (b) MSNP-NH_2, (c) MSNP-Alkyne and (d) MSNP-NQO1

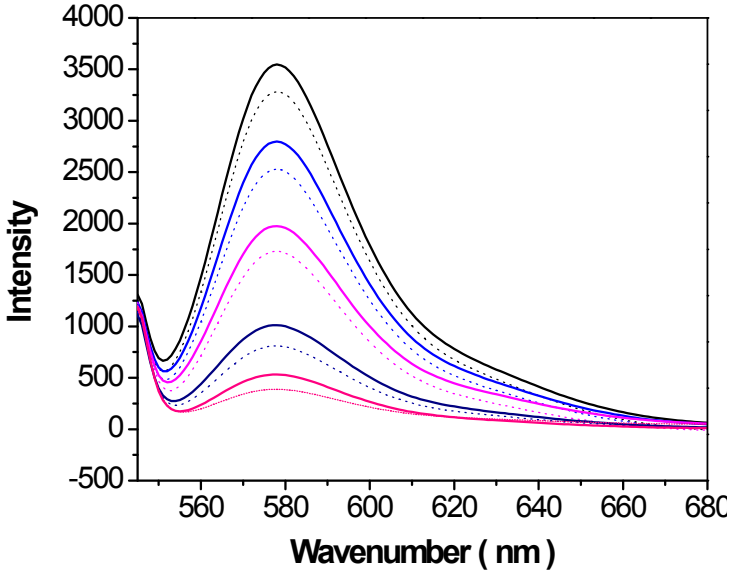


Figure S6. Fluorescence spectra of rhodamine B (——) before loading and (- - -) after loading under different nanoparticle-to-rhodamine b loading weight ratios. Weight ratio of Rh B_{mg}/$MSNP_{mg}$ is from 0.5:10 to 5:10

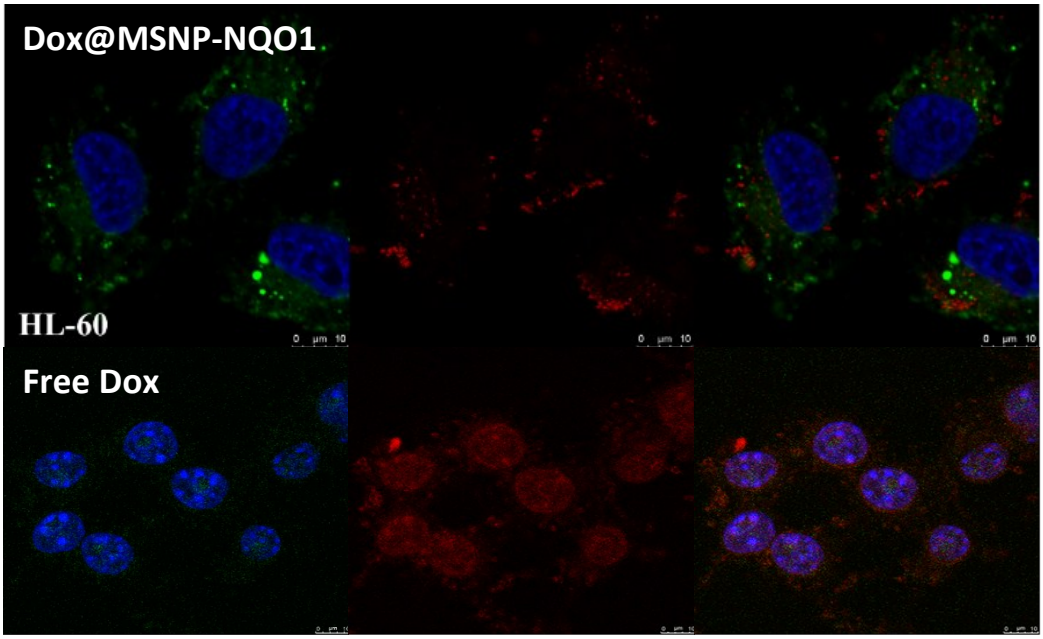


Figure S7. Confocal microscopy images of HL-60 cells incubated with Dox loaded MSNP-NQO1 and free Dox for 2 h. Blue signal is given by nuclei staining of DAPI, green signal is given by plasma membrane marker DiO, and red signal is given by Dox.

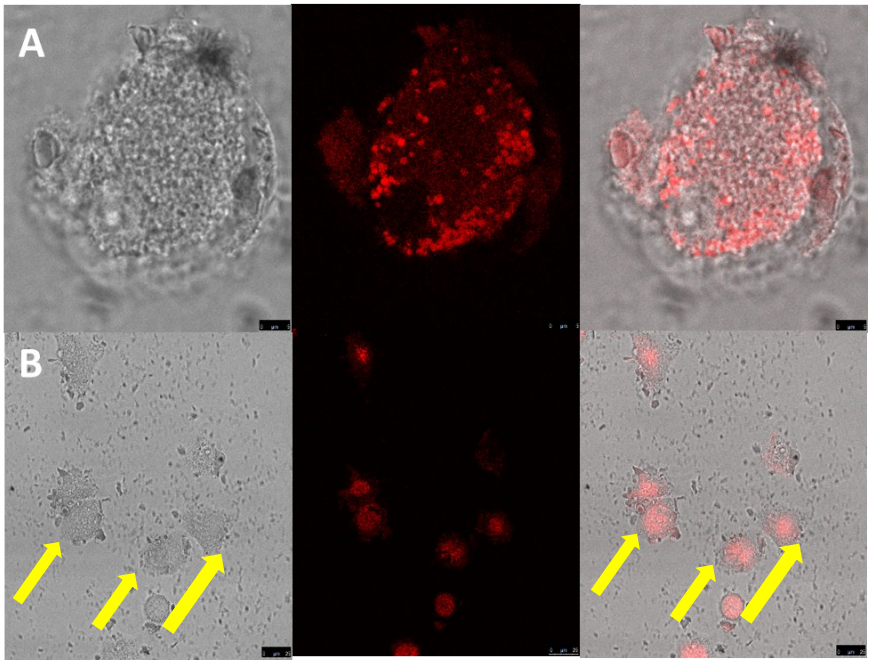


Figure S8. Fluorescence microscopic images of A549 cells after being treated with 50 µg/mL Dox-loaded MSNP-NQO1 after 6 h at 37 ^{0}C. Yellow colored arrows shows apoptosis of A549 cells induced by Dox of Dox-loaded MSNP-NQO1. (A) After 6h at 37^{o}C (B) after 6 h at 37^{o}C without zooming factor. Yellow colored arrow shows apoptosis of A549 cells induced by Dox.

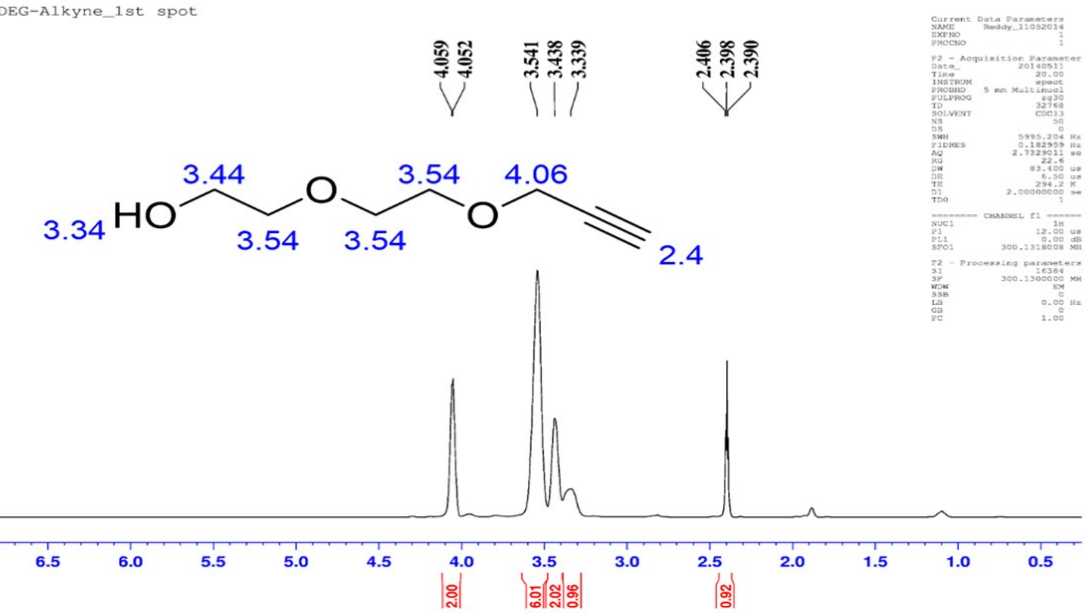


Figure S9. [1]HNMR spectrum of 2-(2-(prop-2-yn-1-yloxy)ethoxy)ethanol

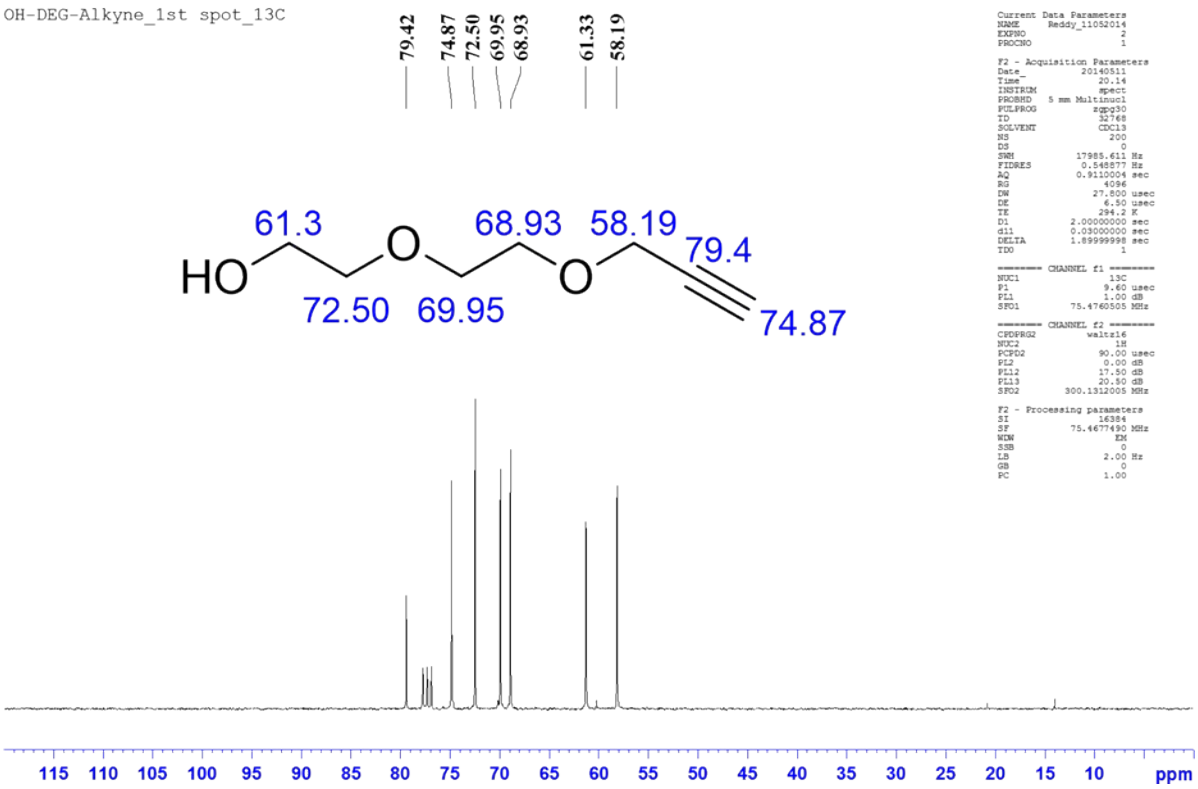


Figure S10. ^{13}CNMR spectrum of 2-(2-(prop-2-yn-1-yloxy)ethoxy)ethanol

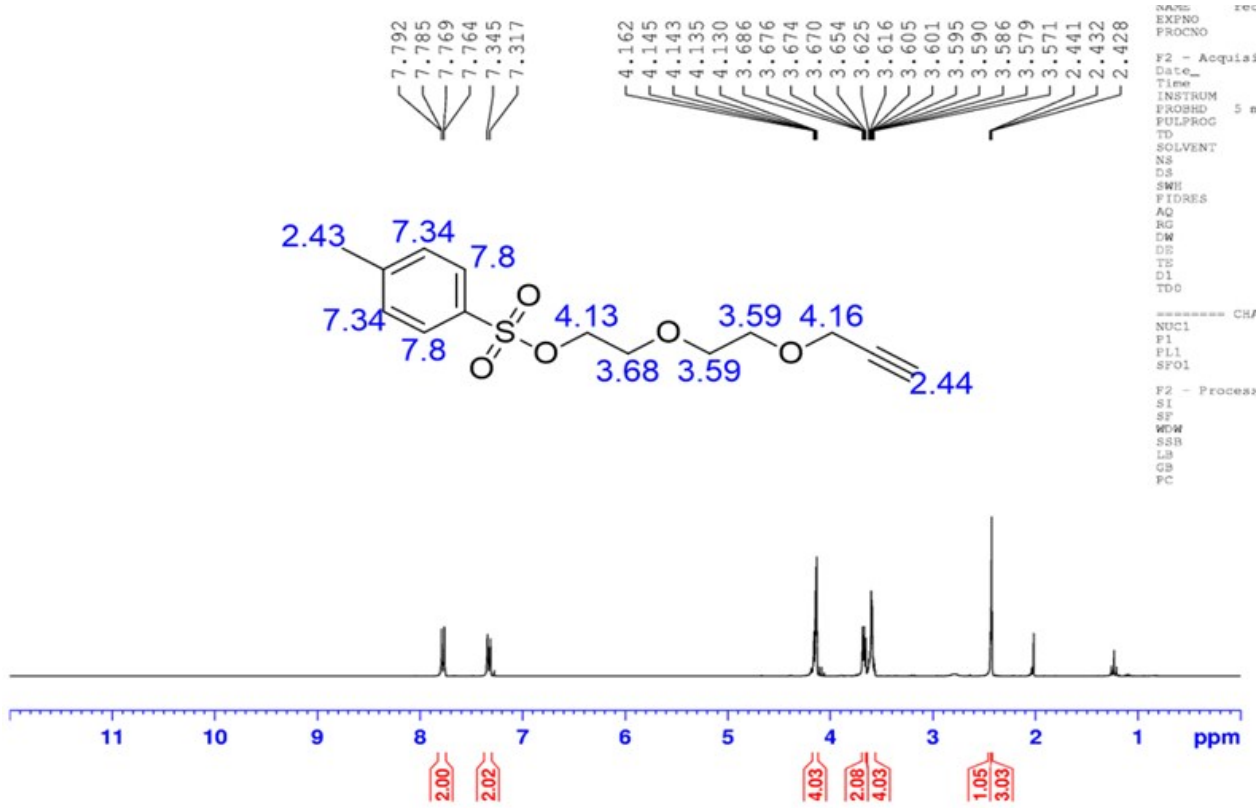


Figure S11. [1]HNMR spectrum of 2-(2-(prop-2-yn-1-yloxy)ethoxy)ethyl 4-methylbenzenesulfonate

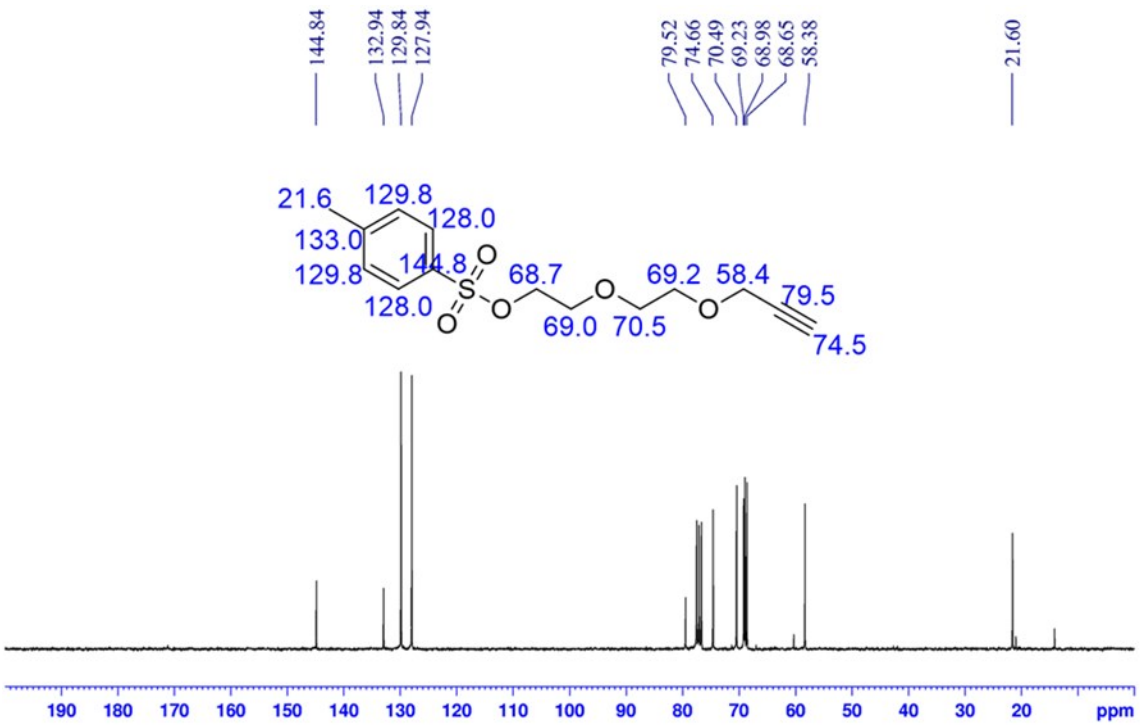


Figure S12. ^{13}CNMR spectrum of 2-(2-(prop-2-yn-1-yloxy)ethoxy)ethyl 4-methylbenzenesulfonate

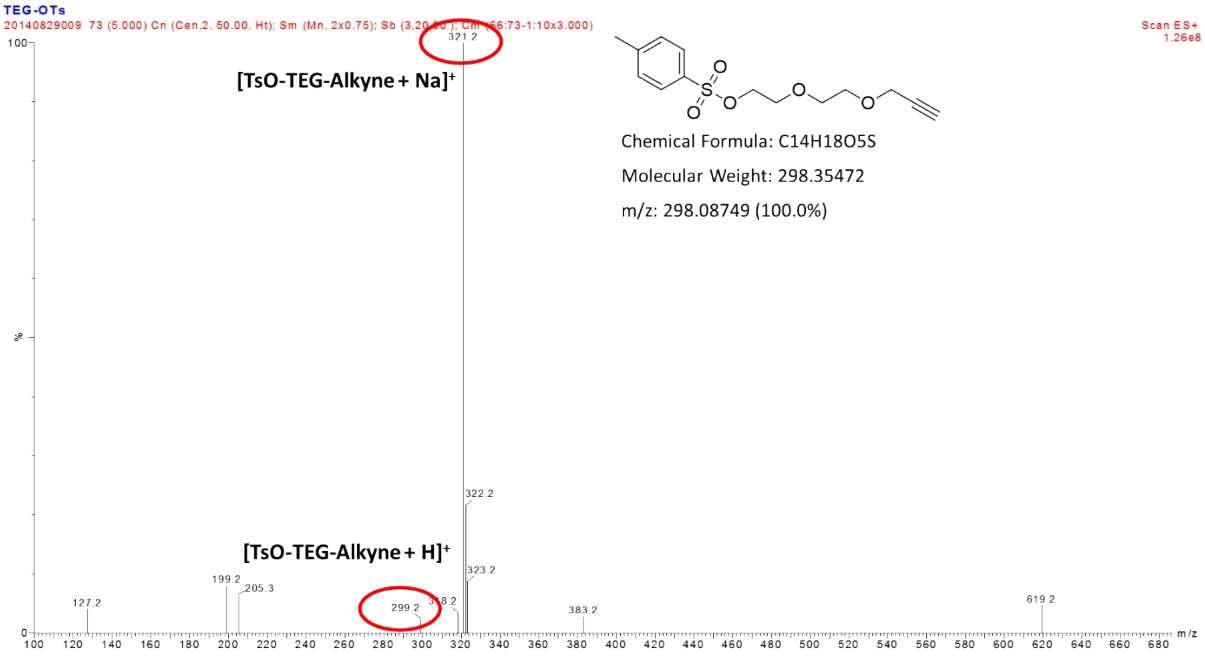


Figure S13. EI-MS spectrum of 2-(2-(prop-2-yn-1-yloxy)ethoxy)ethyl 4-methylbenzenesulfonate

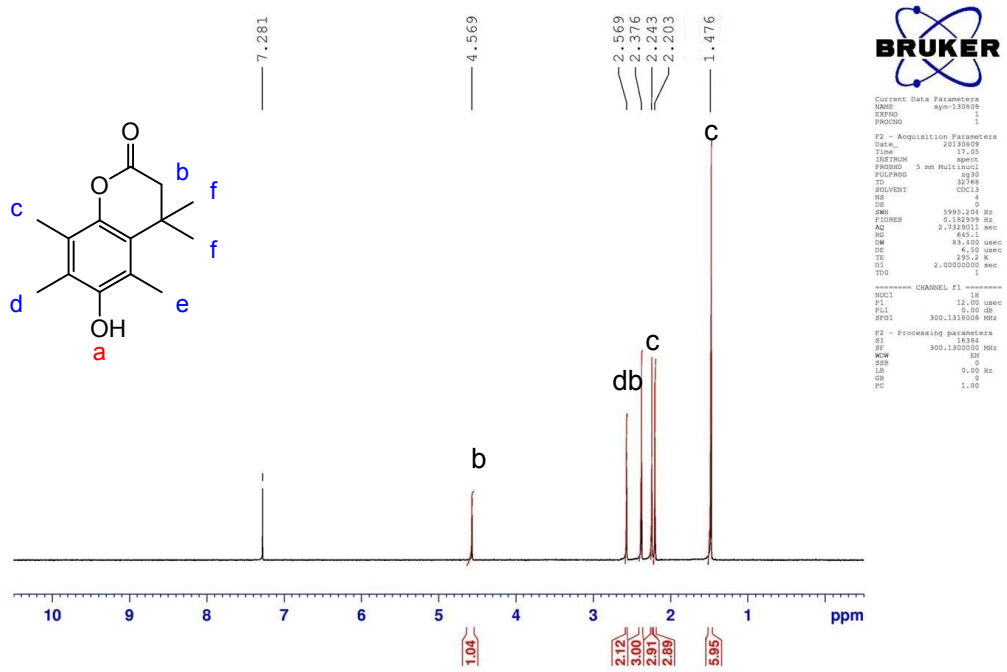


Figure. S14. [1]HNMR spectrum of 3,4-dihydro-6-hydroxy-4,4,5,7,8-pentamethyl- chromen-2-one.

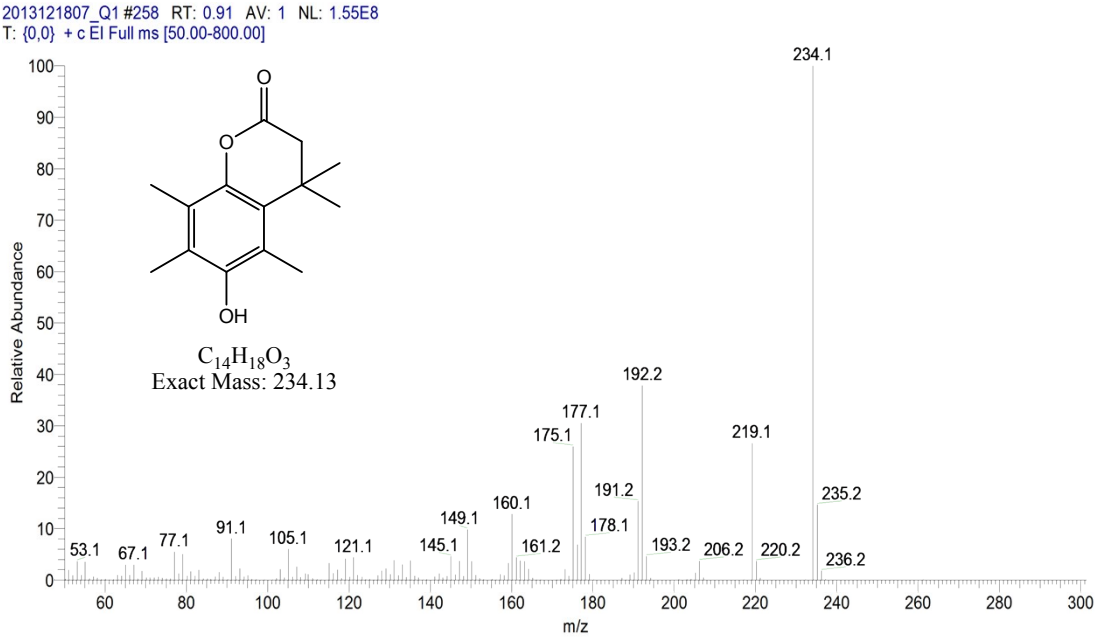


Figure S15. EI-MS spectrum of 3,4-dihydro-6-hydroxy-4,4,5,7,8-pentamethyl- chromen-2-one.

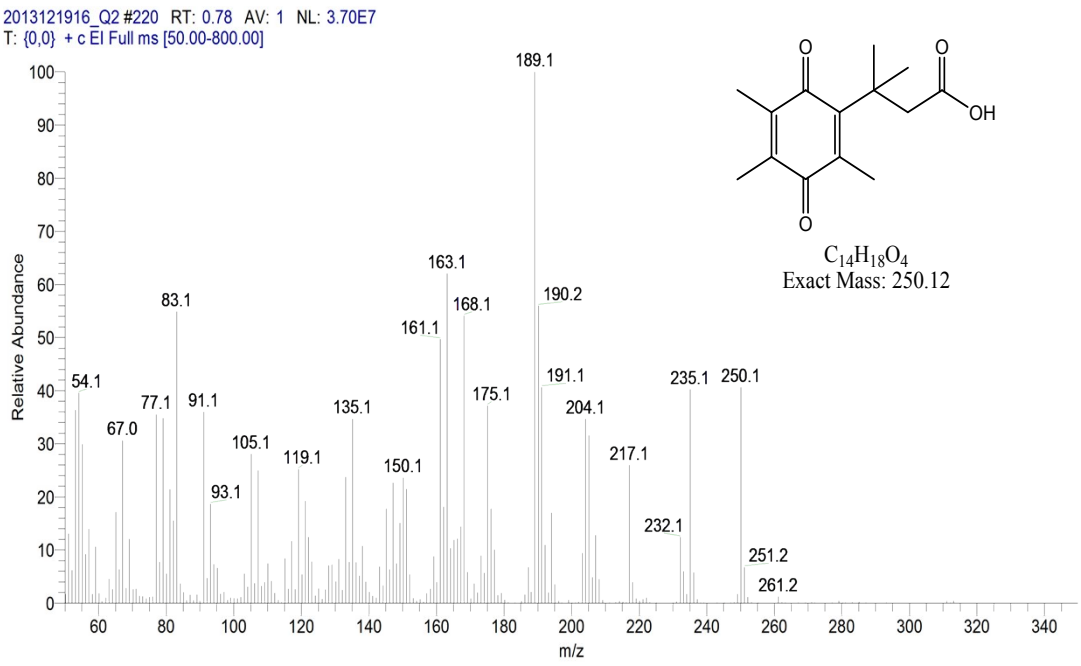


Figure S16. [1]HNMR spectrum of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa- 1,4-dienyl)butanoic acid.

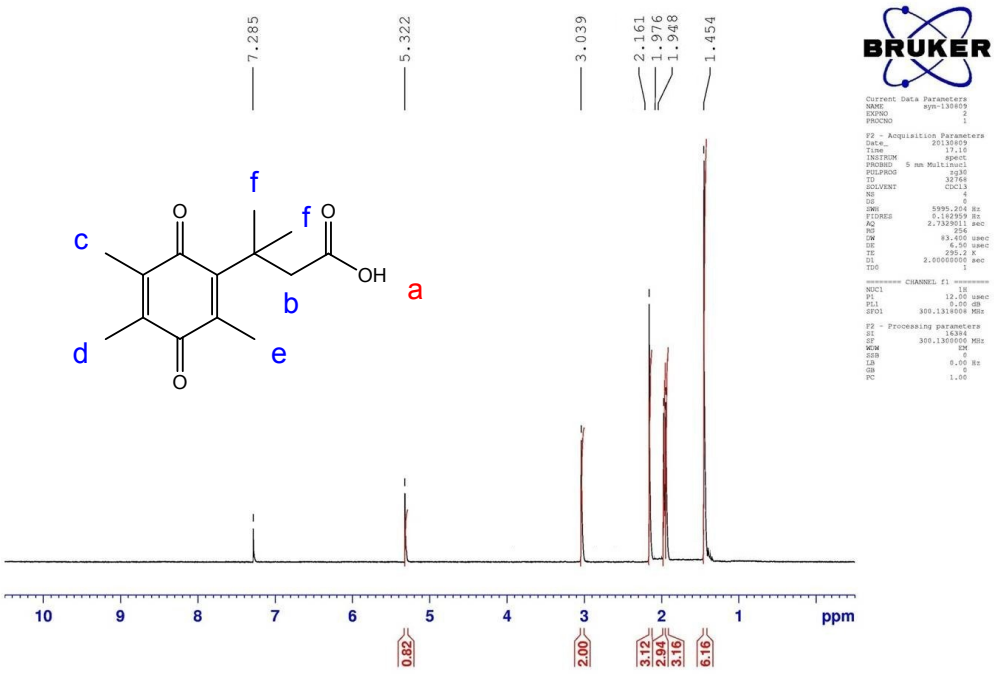


Figure S17. EI-MS spectrum of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa- 1,4-dienyl)butanoic acid.

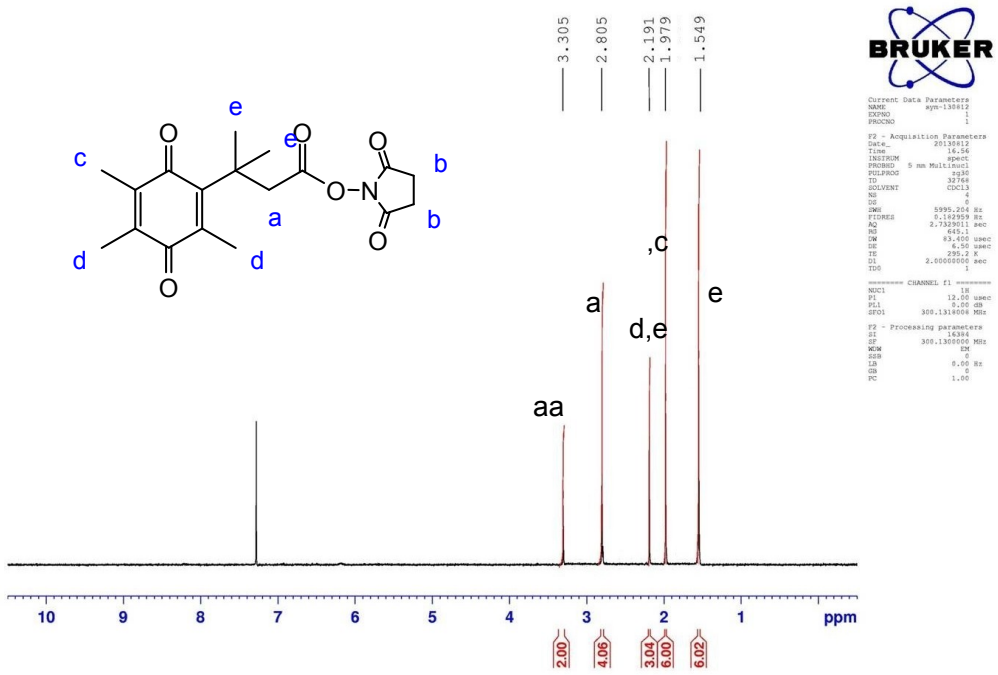


Figure S18. [1]HNMR spectrum of 2,5-dioxopyrrolidin-1-yl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoate

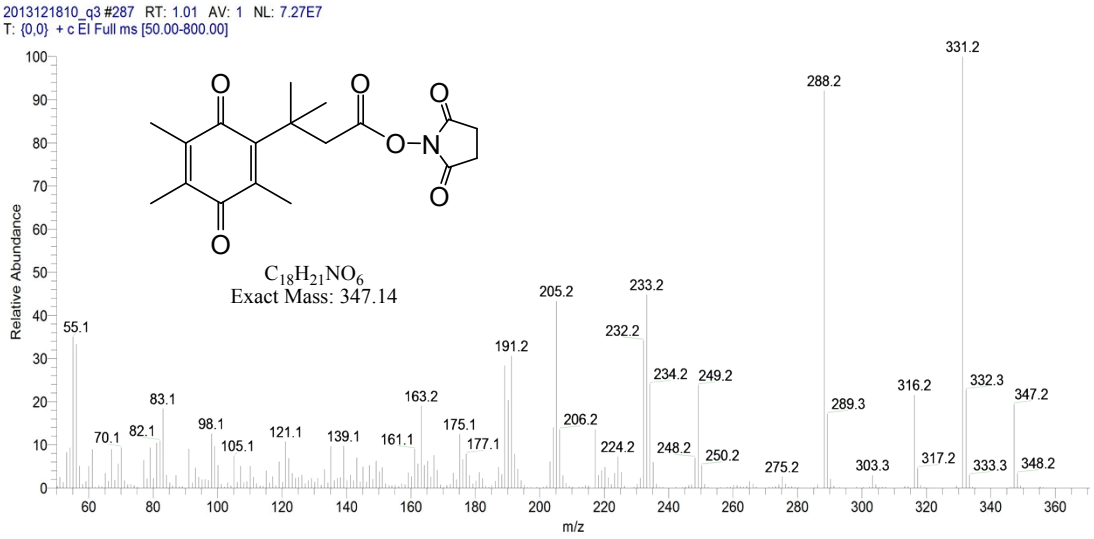


Figure S19. EI-MS spectrum of 2,5-dioxopyrrolidin-1-yl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoate

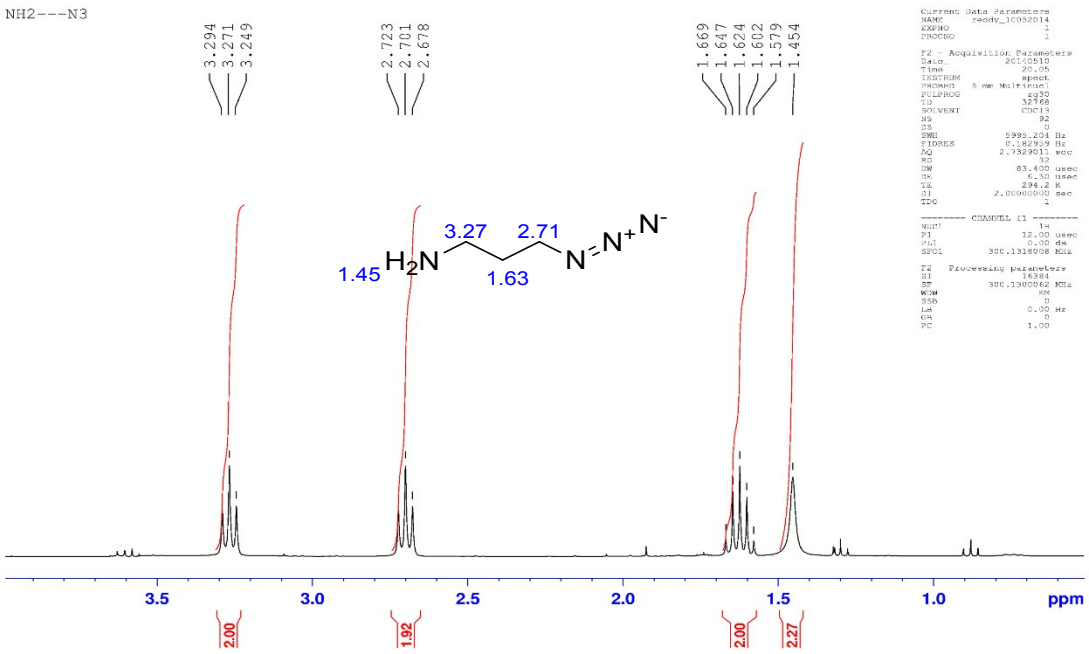


Figure S20. [1]HNMR spectrum of 3-azidopropan-1-amine

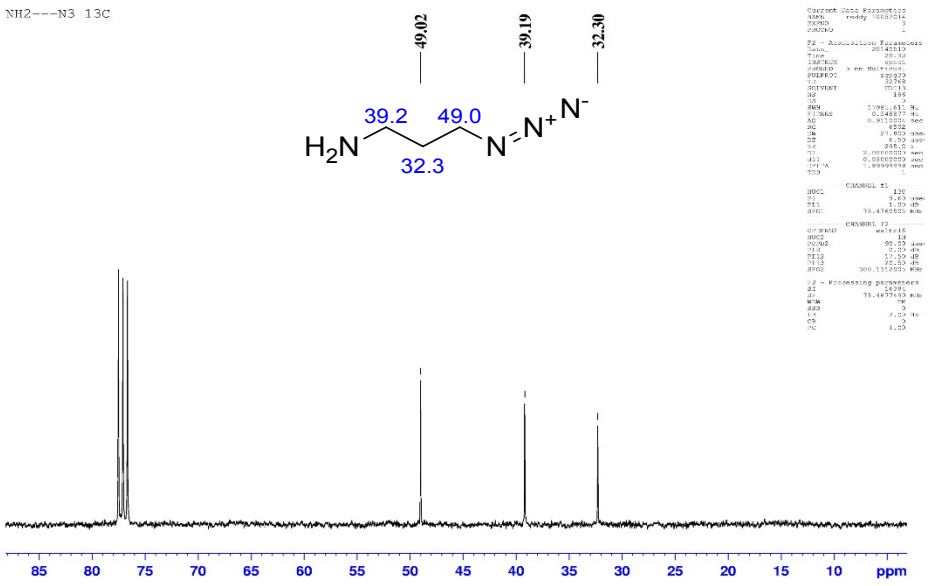


Figure S21. [13]CNMR spectrum of 3-azidopropan-1-amine

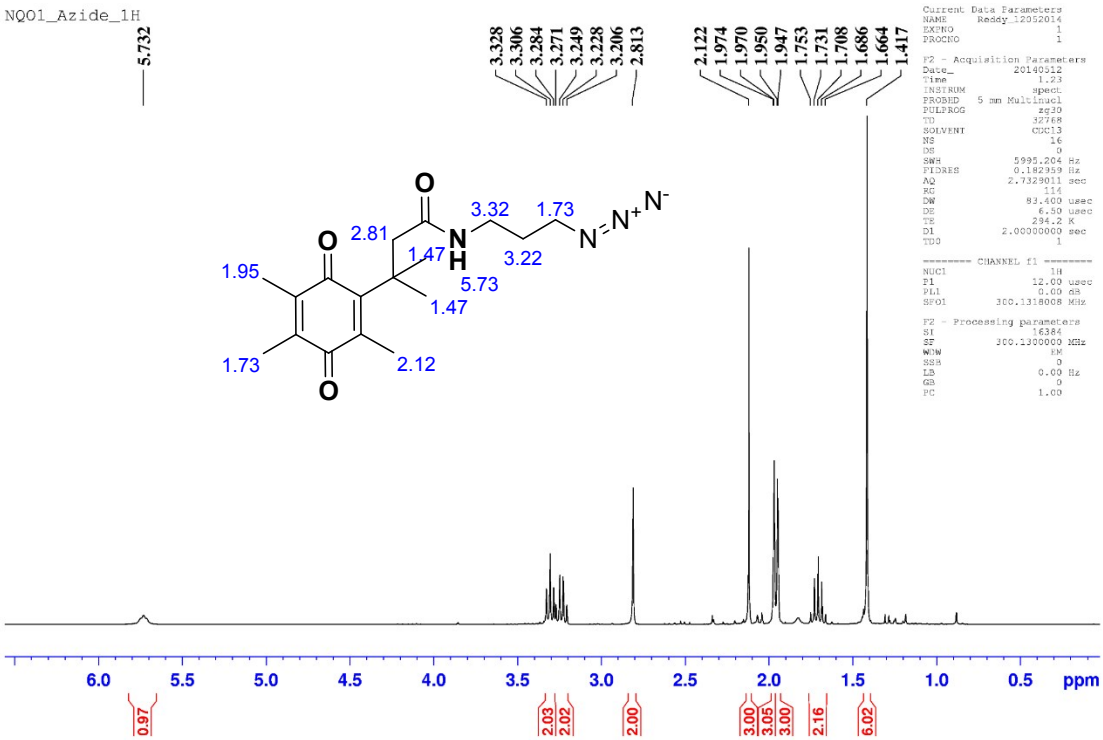


Figure S22. [1]HNMR spectrum of N-(3-azidopropyl)-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanamide

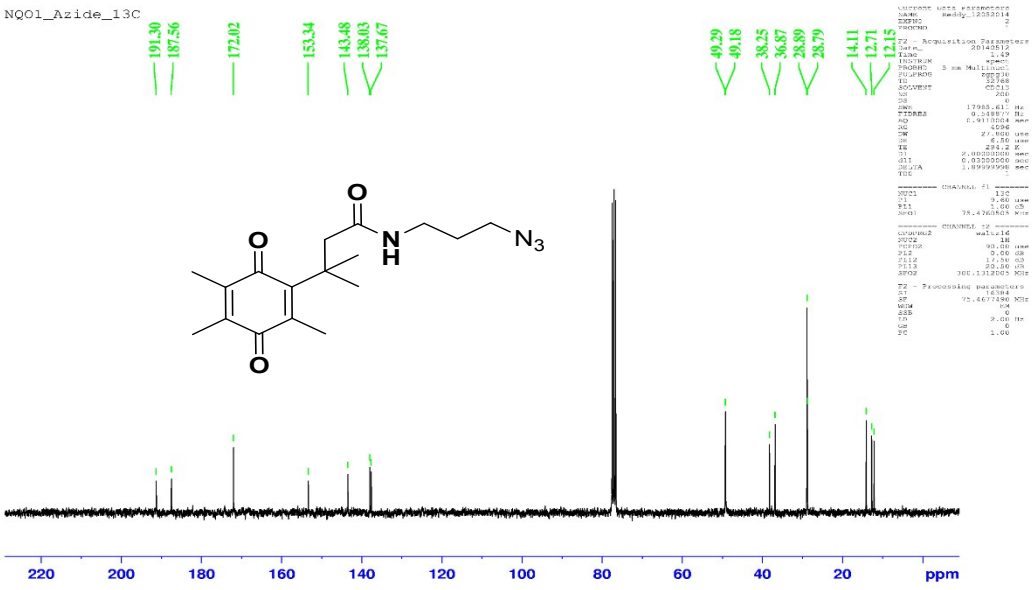


Figure S23. [13]CNMR spectrum of N-(3-azidopropyl)-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanamide

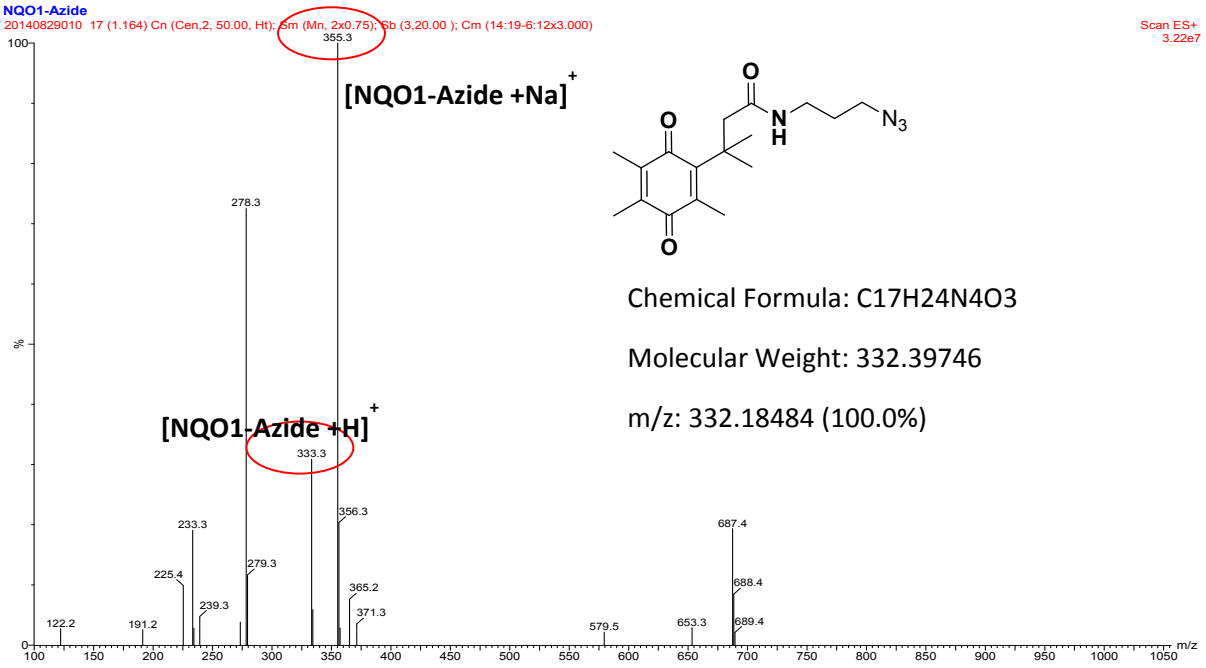


Figure S24. ESI-MS spectrum of N-(3-azidopropyl)-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanamide

Reference:

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