Supplementary materials and methods

Discovery of novel sulfonated small molecules that inhibit vascular tube formation

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Materials

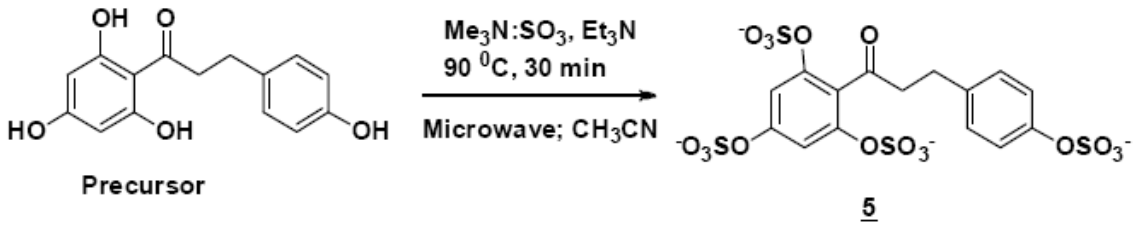
Bovine lung microvascular endothelial cells (BLMVEC) of low passage were kindly provided by Dr. Randall Dull of the University of Utah. MCDB-131 complete media was purchased from Vec Technologies (Rensselaer, NY). Reduced growth factor basement membrane matrix (RGF-BME), calcein, and sulforaphane were purchased from Trevigen Inc. (Gaithersburg, MD). Tryp LE Express was purchased from Invitrogen Inc. (Carlsbad, CA). All other reagents were purchased from Aldrich Chemical (Milwaukee, WI) and used without further purification. Polyphenolic precursors used in the synthesis of sulfonated molecules were either synthesized in the laboratory as described earlier[1] or purchased from Indofine (Somerville, NJ) and Sigma (St. Louis, MO).

In vitro matrigel tube formation assay

BLMVEC were cultured in MCDB-131 media in a humidified 37 ºC incubator. Cells were split 24 hrs prior to conducting tube formation assays in order to keep them in the log phase of growth. RGF-BME was thawed overnight at 4 ºC in a frost free refrigerator. Fifty µl of RGF-BME was then plated out in wells of a chilled 96 well plate using chilled pipette tips. The RGF-BME was allowed to solidify to form the matrigel matrix in the humidified incubator at 37 ºC. Concurrently, BLMVEC were suspended by incubation with Tryp LE Express and counted using a hemacytometer. After the matrigel had formed, a premixed solution of 1 x 10^5 cells, heparan sulfate mimetic inhibitors, and MCDB-131 media were then added to wells in the 96 well plates in duplicate. The plates were then incubated in the humidified incubator for 16 hrs prior to Calcein staining and imaging. Cells were first observed under a light microscope to observe their morphology. Subsequently, media was removed from each well containing cells by gentle dabbing with a paper towel. The wells were then washed twice with chilled PBS and 100 µl of 2 µM Calcein AM was added to each well. After incubating cells for 30 minutes in the incubator, the cells were washed twice with chilled PBS. They were immediately imaged with an Olympus IX81 microscope attached to a color CCD Filter and a GFP emission filter using 485 nm excitation/520 nm emission.

Synthesis of sulfonated molecules

Procedure for microwave-assisted sulfonation: To a stirred solution of the polyphenol or polyalcohol in MeCN (1 – 5 mL) at RT, Et_3N (10 eqv per –OH group) and $Me_3N{:}SO_3$ complex (6 eqv per –OH) were added. The reaction vessel was sealed and micro-waved (CEM Discover synthesizer, Cary, NC) for 30 min at 90 ^{0}C. Several reaction tubes were pooled for isolation of the product. The MeCN layer was decanted and pooled, while the residue was washed with MeCN (5 mL) and centrifuged. The combined MeCN layers were concentrated in vacuo. Water (5 mL) was added to the residue and stirred for 10 min. The water layer was concentrated to approximately 2 mL, loaded onto a Sephadex G10 column (160 cm) and chromatographed using water as eluent. Fractions were combined based on capillary electrophoresis profiles, concentrated and re-loaded onto a SP Sephadex C25 column for sodium exchange. Appropriate fractions were pooled, concentrated in vacuo, and lyophilized to obtain a white powder.



Scheme 1. A general reaction scheme for synthesizing sulfonated small molecular scaffolds.

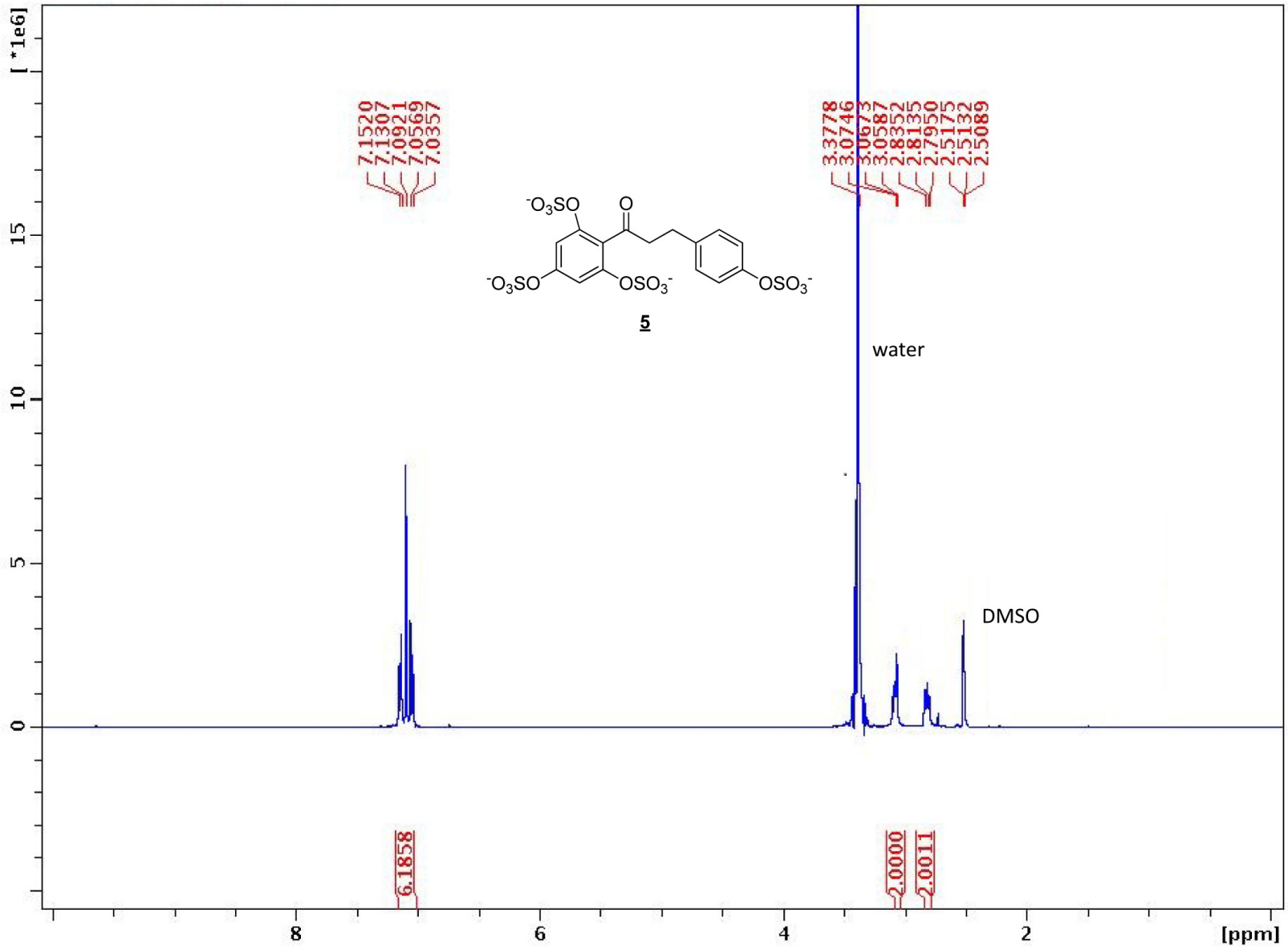
Spectral characteristics of the sulfonated compounds not reported earlier are as follows:

5: ^{1}H NMR (DMSO, 400 MHz): 7.09 (m, 6 H), 3.06 (t, 2 H, *J*= 2.04Hz), 2.81 (t, 2 H, *J*=2.04Hz). ^{13}C NMR (DMSO, 100 MHz): 153.5, 151.3, 149.6, 136.13, 128.38, 120.38, 109.33, 39.74, 35.23. ESI-MS (-ve) m/z Calcd for $C_{15}H_{10}Na_4O_{17}S_4$: 681.84; found, 659.02 $(M-Na)^+$.

6: ^{1}H NMR (DMSO, 400 MHz): 8.18 (m, 1 H), 7.85 (m, 1 H), 7.6 (m, 6 H). ^{13}C NMR (DMSO, 100 MHz): 178.22, 157.34, 154.92, 151.63, 136.1, 133.7, 131.22, 130.71, 125.08, 124.74, 123.88, 122.35, 121.77, 120.01, 118.46. ESI–MS (+ve) m/z Calcd for $C_{15}H_8Na_2O_{10}S_2$: 458.33; found, 481.08 $(M+Na)^+$.

8: ^{1}H NMR (DMSO, 400 MHz): 7.97(m, 2 H), 7.83 (m, 1 H), 7.55 (m, 1 H), 7.41 (m, 2 H), 7.24 (m, 1 H). ^{13}C NMR (DMSO, 100 MHz): 172.84, 158.09, 155.24, 153.19, 135.17, 131.82, 128.49, 125.99, 123.92, 122.53, 120.38, 118.46, 106.79. ESI-MS (-ve) m/z Calcd for $C_{15}H_7Na_3O_{14}S_3$: 576.33; found, 530.14 $(M-2Na)^+$.

10: ^{1}H NMR (DMSO, 400MHz): 7.91 (m, 1 H), 7.61 (d, 1 H, *J*=3.2 Hz), 7.55 (m, 2 H), 7.35 (d, 1 H, *J*=2.1 Hz), 7.05 (m, 1 H). ^{13}C NMR (DMSO, 100 MHz): 175.4, 155.5, 151.04, 135.6, 131.12, 127.18, 119.1, 117.08, 114.47, 113.51. ESI-MS (-ve) m/z Calcd for $C_{15}H_6Na_4O_{18}S_4$: 693.80; found, 670.99 $(M-Na)^+$.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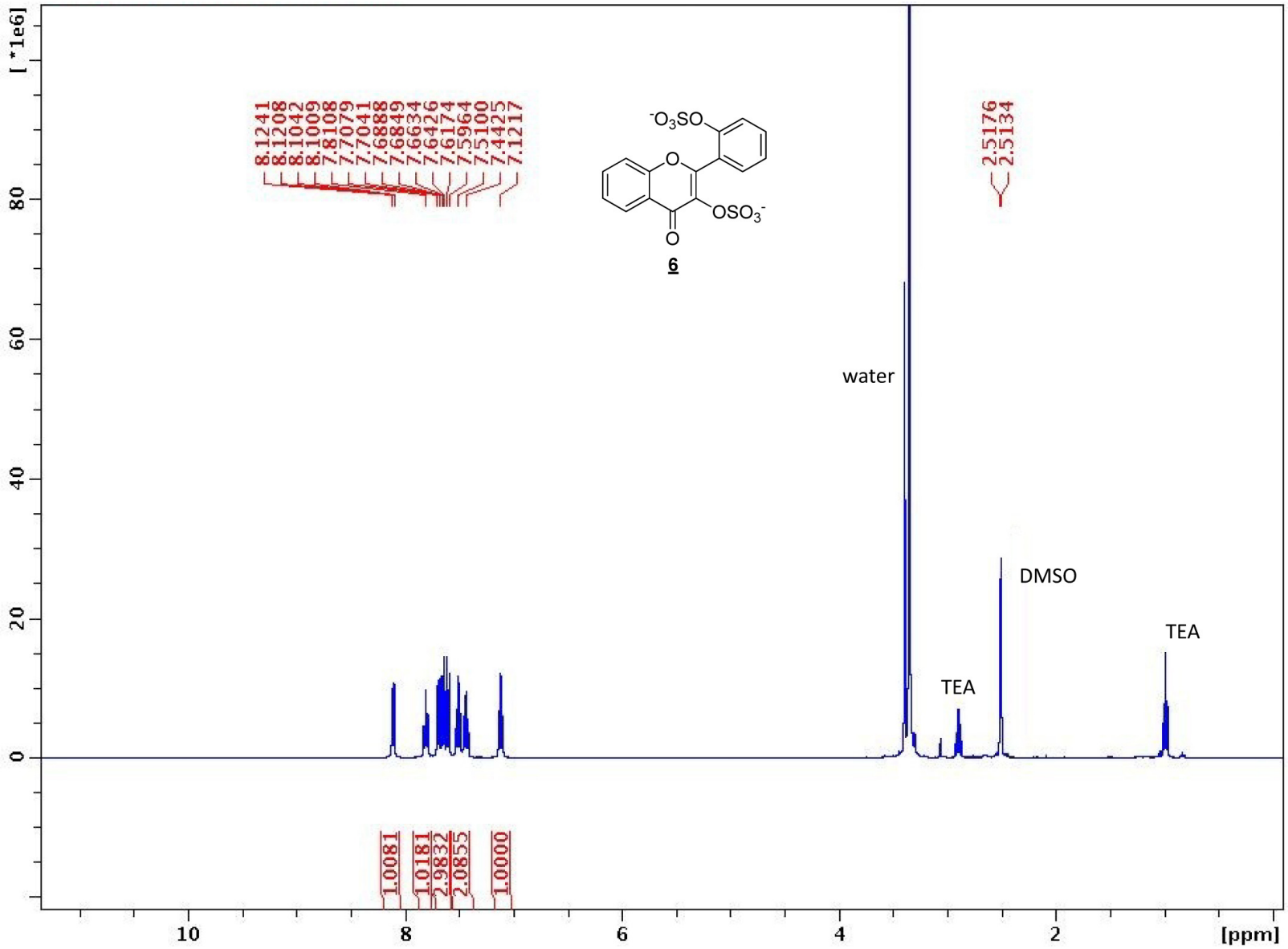
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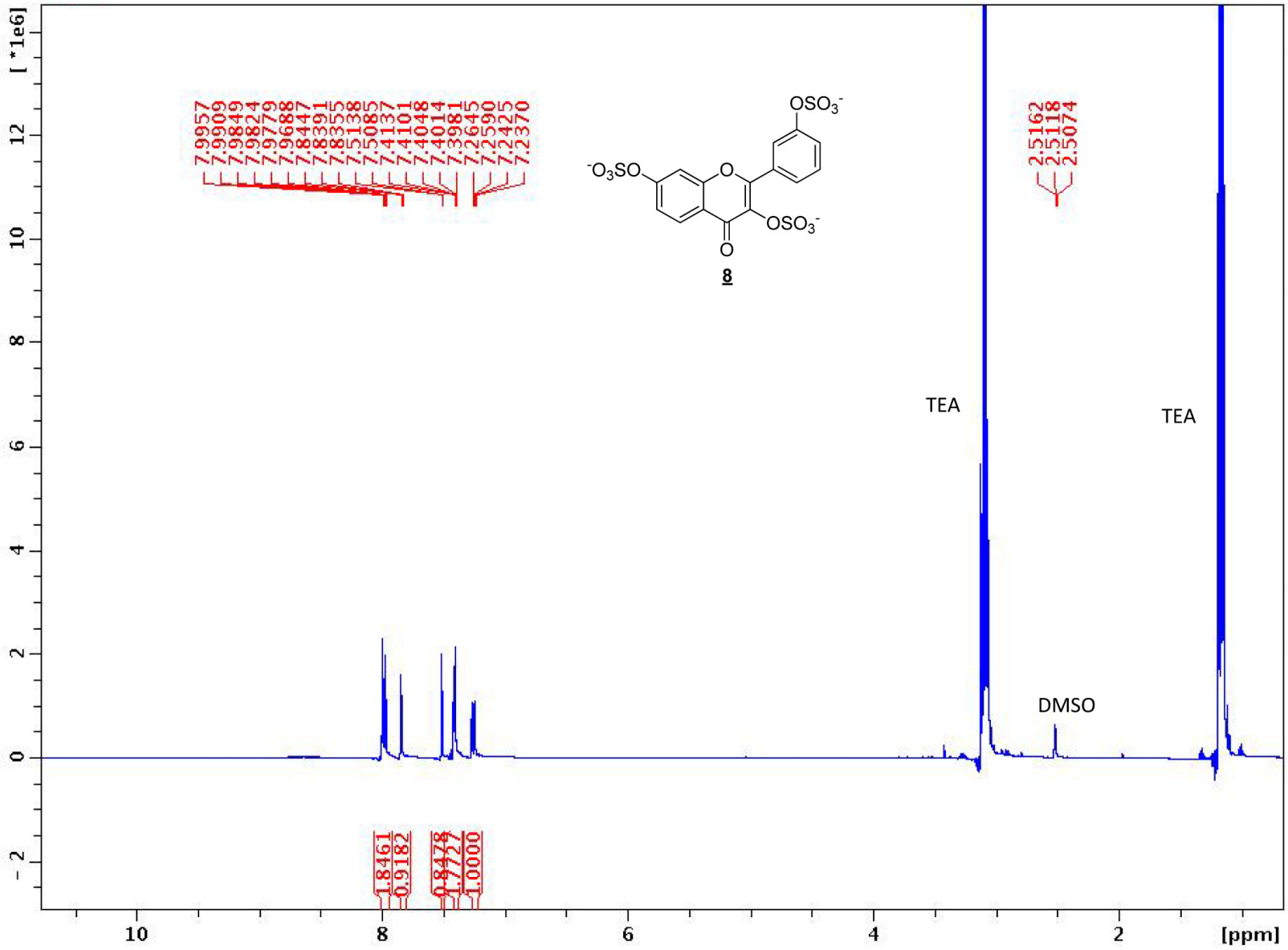
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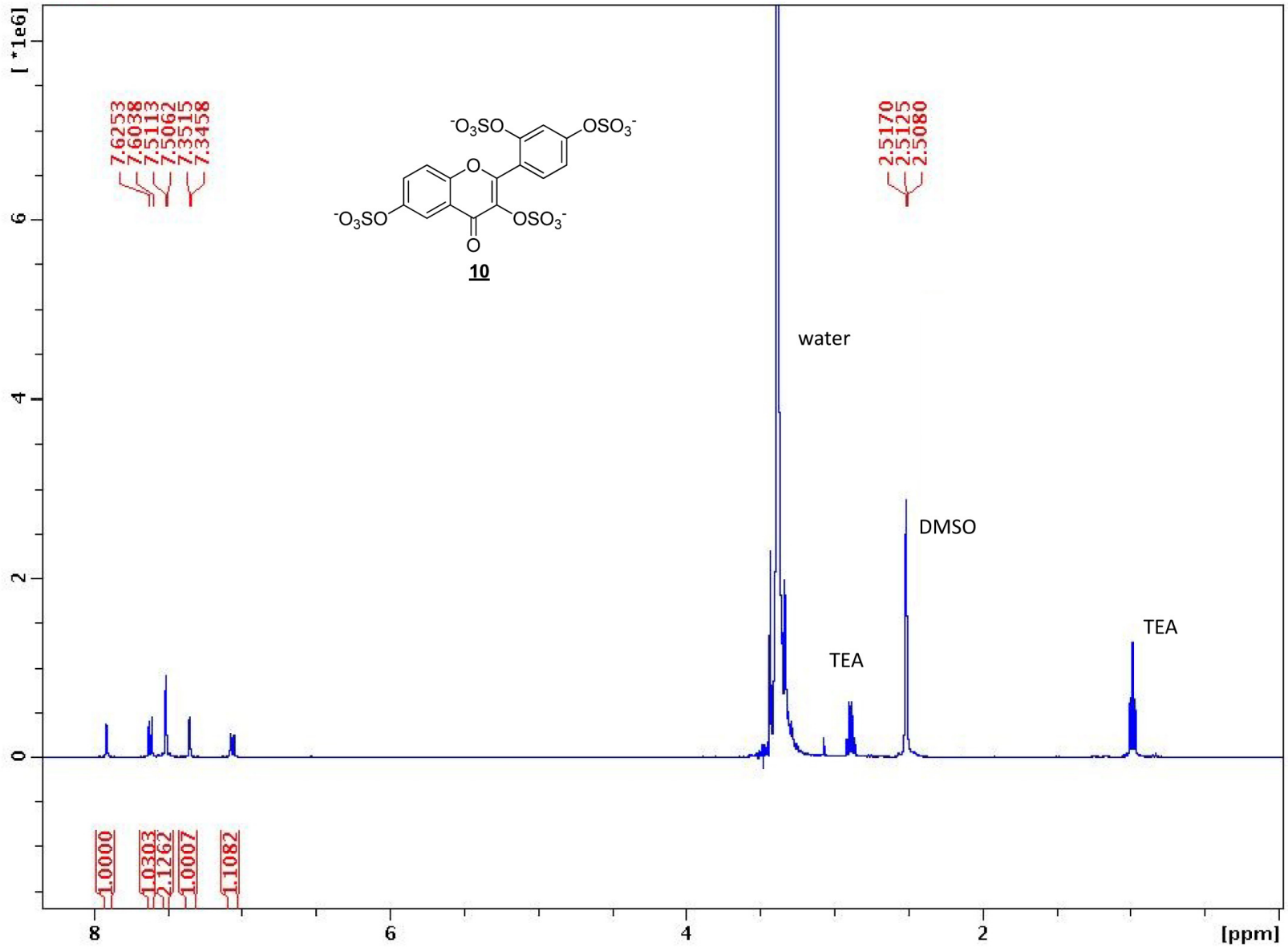
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References

[1] Verghese, J.; Liang, A.; Sidhu, P. P.; Hindle, M.; Zhou, Q.; Desai, U. R. Bioorg Med Chem Lett. 2009, 19, 4126.