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Supporting Information

Chemiluminescent Duplex Analysis by Phenoxy-1,2-Dioxetane Luminophores with Color Modulation

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General methods

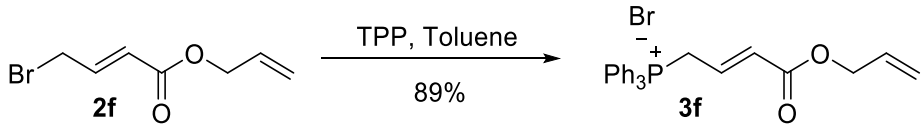
All reactions requiring anhydrous conditions were performed under an Argon atmosphere. All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were either A.R. grade or purified by standard techniques. Thin-layer chromatography (TLC): silica gel plates Merck 60 F254: compounds were visualized by irradiation with UV light. Column chromatography (FC): silica gel Merck 60 (particle size 0.040-0.063 mm), eluent given in parentheses. Reverse-phase high-pressure liquid chromatography (RP-HPLC): C18 5u, 250x4.6mm, eluent given in parentheses. Preparative RP-HPLC: C18 5u, 250x21mm, eluent given in parentheses. ^{1}H-NMR spectra were measured using Bruker Avance operated at 400MHz. ^{13}C-NMR spectra were measured using Bruker Avance operated at 100 MHz. Chemical shifts were reported in ppm on the δ scale relative to a residual solvent ($CDCl_3$: δ = 7.26 for ^{1}H-NMR and 77.16 for ^{13}C-NMR, DMSO-d_6: δ = 2.50 for ^{1}H-NMR and 39.52 for ^{13}C-NMR). Mass spectra were measured on Waters Xevo TQD. Chemiluminescence was recorded on Molecular Devices Spectramax i3x. Fluorescence was recorded on Tecan Infinite 200 Pro. All general reagents, including salts and solvents, were purchased from Sigma-Aldrich. Light irradiation for photochemical reactions: LED PAR38 lamp (19W, 3000K).

Abbreviations

ACN- Acetonitrile, **$CHCl_3$**- Chloroform, **DCM**- dichloromethane, **DIPEA**- N, N-Diisopropylethylamine, **DMF**- N, N' –Dimethylformamide, **EtOAc**- Ethyl acetate, **Hex**- Hexanes, **iPrOH**- Isopropyl alcohol, **K_2CO_3**- Potassium carbonate, **LiOH**- Lithium hydroxide, **MB**- Methylene blue, **MeOH**- Methanol, **NH_4Cl**- ammonium chloride, **$NaHCO_3^-$**- Sodium bicarbonate, **$Na_2S_2O_3^-$**- Sodium Thiosulfate, **$Na_2SO_4^-$**- Sodium sulfate, **$NaBH_4$**- Sodium borohydride, **NaI**- Sodium iodide, **THF**- Tetrahydrofuran, **TMS-Cl** - Trimethylsilyl chloride, **TPP** – triphenylphosphine.

Synthesis and characterization of phosphonium salts (3f-i, 3k, 3o) and phosphonates (3n):

Compound 3f

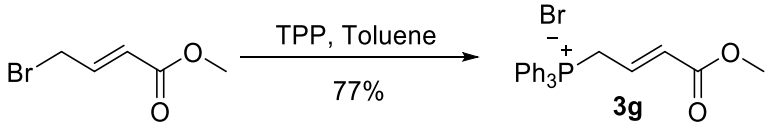


To a solution of compound **2f**[1] (1 gr, 4.90 mmol) in toluene (5 mL) was added triphenylphosphine (1.5 eq). The reaction was stirred at room temperature overnight upon which a white solid precipitated and TLC (Hex: EtOAc; 70:30) indicated the disappearance of starting material. The precipitate was collected and washed with toluene (20 mL) and hexanes (20 ml). The product was dried under reduced pressure to give the phosphonium salt **3f** as a white solid. (2.01 gr, 89% yield).

^{1}H-NMR (400 MHz, DMSO) δ 7.95 – 7.87 (m, 3H), 7.85 – 7.73 (m, 12H), 6.65 (dt, J = 14.1, 7.0 Hz, 1H), 6.16 (dd, J = 15.4, 4.9 Hz, 1H), 5.87 (ddd, J = 22.1, 10.7, 5.3 Hz, 1H), 5.20 (d, J = 6.2 Hz, 1H), 5.17 (s, 1H), 4.92 (dd, J = 17.4, 7.6 Hz, 1H), 4.55 (d, J = 5.4 Hz, 2H).

^{13}C-NMR (101 MHz, DMSO) δ 164.40, 135.91, 135.25, 135.16, 134.60, 134.39, 134.29, 132.93, 131.03, 130.91, 129.45, 129.32, 118.67, 118.46, 117.81, 65.35, 55.54.

Compound 3g

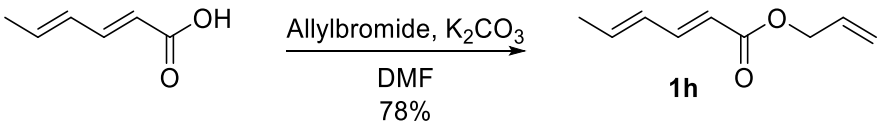


To a solution of commercially available methyl-4-bromocrotonate (1 gr, 5.56 mmol) in toluene (5 mL) was added triphenylphosphine (1.5 eq). The reaction was stirred at room temperature overnight upon which a white solid precipitated and TLC (Hex: EtOAc; 70:30) indicated the disappearance of starting material. The precipitate was collected and washed with toluene (20 mL) and hexanes (20 ml). The product was dried under reduced pressure to give the phosphonium salt **3g**[2] as a white solid. (1.72 gr, 77% yield). MS (ES+): m/z calc. for C23H22BrO2P: 440.0; found: 441.1 $[M+H]^{+}$.

^{1}H-NMR (400 MHz, DMSO) δ 7.94 – 7.89 (m, 3H), 7.78 (qt, *J* = 7.5, 5.7 Hz, 12H), 6.68 – 6.57 (m, 1H), 6.17 (dd, *J* = 15.4, 5.0 Hz, 1H), 4.98 (dd, *J* = 17.9, 7.6 Hz, 2H), 3.60 (s, 3H).

^{13}C-NMR (100 MHz, DMSO) δ 165.25, 135.90, 134.96, 134.86, 134.61, 134.40, 134.30, 131.03, 130.91, 129.45, 129.33, 118.70, 117.84, 52.40.

Compound 1h

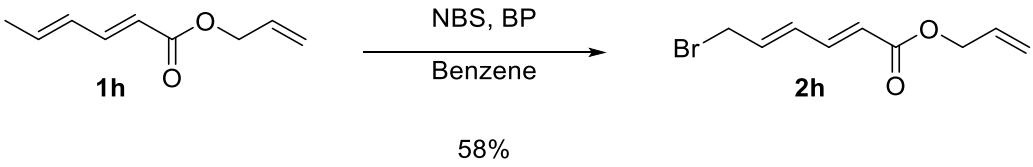


To a solution of commercially available sorbic acid (1 gr, 8.91 mmol) in DMF was added allyl bromide (1.2 eq) and K_2CO_3 (2 eq). The reaction was stirred for 2 hours at 40°C and monitored by TLC (Hex: EtOAc; 50:50). Upon completion, the reaction mixture was diluted with EtOAc and washed with brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by silica column chromatography (EtOAc: Hex; 20:80). The allyl ester **1h** was obtained as a pale-yellow oil (1.07 gr, 78% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.20 (dd, *J* = 15.4, 10.0 Hz, 1H), 6.16 – 6.00 (m, 2H), 5.87 (ddt, *J* = 17.1, 10.5, 5.7 Hz, 1H), 5.72 (d, *J* = 15.7 Hz, 1H), 5.20 (ddq, *J* = 35.5, 10.4, 1.4 Hz, 2H), 4.56 (dt, *J* = 5.7, 1.4 Hz, 2H), 1.77 (d, *J* = 5.9 Hz, 3H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 166.86, 145.38, 139.56, 132.53, 129.86, 118.71, 117.96, 64.90, 18.68.

Compound 2h

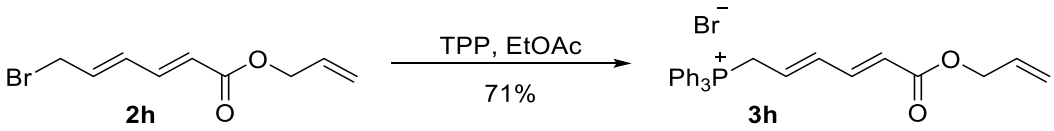


A mixture of allyl ester **1h** (0.95 gr, 6.25 mmol), benzoyl peroxide (0.2 eq), and N-bromosuccinimide (1.3 eq) in benzene (20 mL) was heated to reflux overnight. The reaction was monitored by TLC (EtOAc: Hex; 30:70). Upon completion, the reaction mixture was diluted with EtOAc and washed with brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by silica column chromatography (EtOAc: Hex; 20:80). The bromide **2h** was obtained as a yellow oil (0.83 gr, 58% yield).

^{1}H-NMR (400 MHz, CDCl3) δ 7.33 (dd, J = 15.4, 10.8 Hz, 1H), 6.41 (dd, J = 15.1, 10.9 Hz, 1H), 6.29 (dd, J = 15.0, 7.4 Hz, 1H), 5.93 (d, J = 15.4 Hz, 1H), 4.03 (d, J = 7.5 Hz, 2H).

^{13}C-NMR (101 MHz, CDCl3) δ 166.20, 143.09, 136.98, 132.29, 131.96, 122.96, 118.41, 65.31, 31.35.

Compound 3h

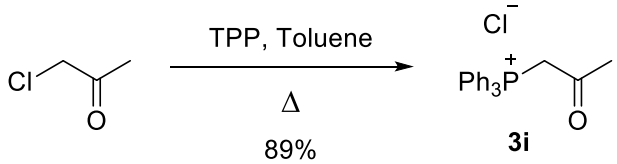


To a solution of bromide **2h** (0.83 gr, 3.62 mmol) in EtOAc (5 mL) was added triphenylphosphine (1.5 eq). The reaction was stirred at room temperature overnight, upon which yellow oil formed and TLC (Hex: EtOAc; 70:30) indicated the disappearance of starting material. The oil was triturated and washed with EtOAc (20 mL) and hexanes (20 ml). The product was dried under reduced pressure to give the phosphonium salt **3h** as a yellow oil. (1.26 gr, 71% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.67 (m, 10H), 7.60 (td, *J* = 7.5, 3.4 Hz, 5H), 6.99 (dd, *J* = 15.3, 11.0 Hz, 1H), 6.70 – 6.55 (m, 1H), 5.89 – 5.69 (m, 3H), 5.22 – 5.09 (m, 2H), 4.97 (dd, *J* = 16.3, 7.4 Hz, 2H), 4.50 (d, *J* = 5.7 Hz, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 166.22, 142.61, 142.56, 137.72, 137.58, 135.34, 134.12, 134.02, 132.31, 132.21, 132.12, 130.61, 130.48, 128.90, 128.78, 126.46, 126.35, 123.16, 118.40, 118.17, 117.32, 65.25, 31.62, 28.67, 28.18, 22.69, 14.19.

Compound 3i

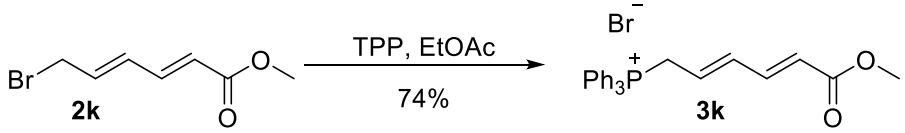


Phosphonium salt **3i** was synthesized according to a known procedure from chloroacetone.[3]

^{1}H-NMR (400 MHz, DMSO) δ 7.76 (ddt, J = 15.2, 7.7, 5.5 Hz, 15H), 5.93 (d, J = 12.2 Hz, 2H), 2.35 (d, J = 2.3 Hz, 3H).

^{13}C-NMR (100 MHz, DMSO) δ 201.93, 135.28, 134.29, 134.18, 130.66, 130.53, 120.02, 119.14, 32.29.

Compound 3k

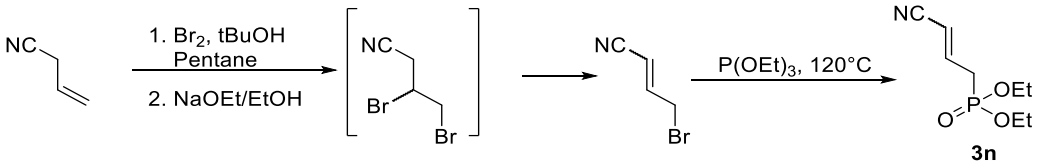


To a solution of compound **2k**[3] (1 gr, 4.90 mmol) in EtOAc (5 mL) was added triphenylphosphine (1.5 eq). The reaction was stirred at room temperature overnight, upon which a yellow solid precipitated, and TLC (Hex: EtOAc; 70:30) indicated the disappearance of starting material. The precipitate was collected and washed with EtOAc (20 mL) and hexanes (20 ml). The product was dried under reduced pressure to give the phosphonium salt **3k** as a yellow solid. (1.69 gr, 74% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.78 (ddd, *J* = 16.1, 10.2, 6.7 Hz, 10H), 7.64 (td, *J* = 7.6, 3.4 Hz, 5H), 7.03 (dd, *J* = 15.2, 11.3 Hz, 1H), 6.71 – 6.59 (m, 1H), 5.87 (dt, *J* = 22.1, 7.3 Hz, 1H), 5.78 (dd, *J* = 15.4, 2.4 Hz, 1H), 5.05 (dd, *J* = 16.3, 7.5 Hz, 2H), 3.65 (s, 3H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.16, 142.53, 137.80, 137.66, 135.34, 134.17, 134.07, 133.51, 132.69, 132.58, 130.62, 130.50, 129.27, 129.14, 126.37, 126.26, 123.12, 118.26, 117.41, 51.80, 28.74, 28.25.

Compound 3n

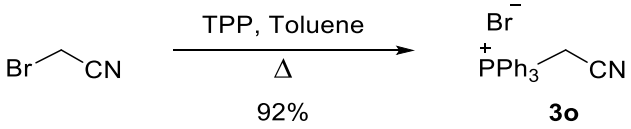


Phosphonate **3n** (E/Z-ratio of 1.2:1) was synthesized according to a known procedure from allyl cyanide.[4]

^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.69 – 6.45 (m, 1H), 5.48 (dddd, J = 7.5, 5.2, 2.8, 1.4 Hz, 1H), 4.17 – 4.04 (m, 4H), 2.96 (ddd, J = 23.3, 8.1, 1.3 Hz, 1H), 2.73 (ddd, J = 23.1, 7.8, 1.5 Hz, 1H), 1.31 (ddd, J = 7.0, 5.3, 3.5 Hz, 6H).

^{31}P-NMR (162 MHz, $CDCl_3$) δ 22.53, 22.38.

Compound 3o



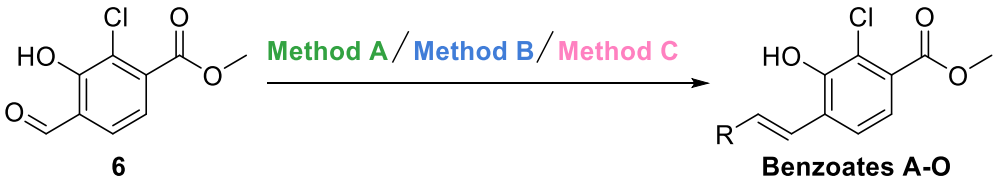
Phosphonium salt **3o** was synthesized according to a known procedure from 2-bromoacetonitrile.[5]

^{1}H-NMR (400 MHz, DMSO) δ 8.01 – 7.95 (m, 3H), 7.90 – 7.80 (m, 12H), 5.97 (d, J = 15.9 Hz, 2H).

^{31}P NMR (162 MHz, DMSO) δ 21.24.

Synthesis and characterization of Benzoates A-O

Benzoates **A-D** and Benzoates **O, L, and M** were synthesized according to a known procedure.[6–8]



Method A

To a solution of phosphonium salt or phosphonate **3** (1.1 eq) in DCM (1 mmol/ml) was added triethylamine (2 eq) and compound **6**[9] (1 eq). The reaction was stirred for 1 hour and monitored by TLC (Hex: EtOAc; 70:30). Upon completion, the reaction mixture was diluted with DCM and washed with 1M HCl followed by an additional wash with brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by silica column chromatography.

Method B

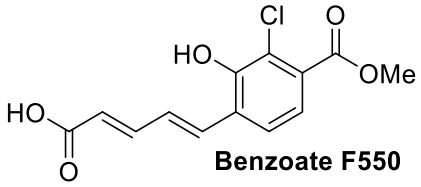
To a solution of phosphonium salt or phosphonate **3** (1.1 eq) in DCM (1 mmol/ml) was added triethylamine (2 eq) and compound **6**[9] (1 eq). The reaction was stirred for 1 hour and monitored by TLC (Hex: EtOAc; 70:30). Upon completion, the reaction mixture was diluted with DCM and washed with 1M HCl followed by an additional wash with brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude was further used without purification. The residue was dissolved in 1 ml of DCM, followed by the addition of DMBA (2 eq for each allyl group) and

tetrakis(triphenylphosphine)palladium (0.1 eq). The reaction was stirred at room temperature and monitored by TLC (Hex: EtOAc; 70:30). Upon completion, the solvent was removed by reduced pressure, and the product was purified by RP-HPLC.

Method C

Compound **6**[9] (1 eq), piperidine (2 eq), and acceptor (1.2 eq) were dissolved in 3 mL of ACN. The reaction mixture was stirred at reflux for 1 h and monitored by RP-HPLC (30-100 ACN in water with 0.1% TFA). Upon completion, the reaction mixture was evaporated under reduced pressure. The product was purified by RP-HPLC (gradient of ACN in water).

.66, 151.35, 133.91, 133.02, 127.35, 126.54, 125.90, 122.74, 121.81, 120.75, 119.53, 52.84.

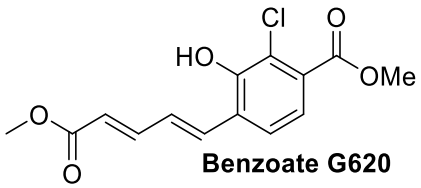


Benzoate F550

Benzoate F550 was synthesized from **3f** according to **Method B** (71% yield). MS (ES+): m/z calc. for C13H11ClO5: 282.1; found: 281.2 $[M-H]^-$.

^{1}H-NMR (400 MHz, DMSO) δ 7.61 (d, *J* = 8.2 Hz, 1H), 7.40 – 7.10 (m, 4H), 6.04 (d, *J* = 15.1 Hz, 1H), 3.82 (s, 3H).

^{13}C-NMR (101 MHz, DMSO) δ 167.99, 166.09, 151.88, 144.84, 133.89, 131.28, 130.13, 129.21, 125.49, 123.94, 122.02, 121.14, 53.10.

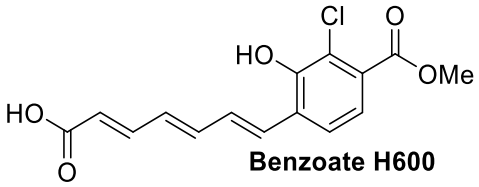


Benzoate G620

Benzoate G620 was synthesized from **3g** according to **Method A** (81% yield). MS (ES+): m/z calc. for C14H13ClO5: 296.1; found: 295.1 $[M-H]^-$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.38 (m, 3H), 7.19 (d, *J* = 15.8 Hz, 1H), 7.04 (dd, *J* = 15.7, 11.0 Hz, 1H), 6.43 (s, 1H), 6.04 (d, *J* = 15.3 Hz, 1H), 3.92 (s, 3H), 3.77 (s, 3H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.41, 165.42, 150.19, 144.80, 133.74, 130.06, 129.13, 127.56, 125.20, 123.16, 122.42, 120.64, 52.68, 51.82.

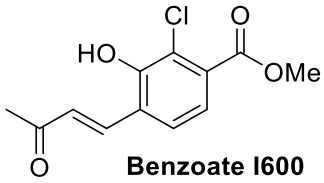


Benzoate H600

Benzoate H600 was synthesized from **3h** according to **Method B** (83% yield). MS (ES+): m/z calc. for C15H13ClO5: 308.0; found: 307.2 $[M-H]^-$.

^{1}H-NMR (400 MHz, DMSO) δ 9.95 (s, 1H), 7.55 – 7.52 (m, 1H), 7.32 – 7.23 (m, 2H), 7.17 – 7.03 (m, 2H), 6.93 (dd, *J* = 14.8, 10.1 Hz, 1H), 6.60 (dd, *J* = 14.4, 11.7 Hz, 1H), 5.94 (d, *J* = 15.2 Hz, 1H), 3.82 (s, 3H).

^{13}C-NMR (101 MHz, DMSO) δ 168.12, 166.10, 151.55, 144.51, 141.38, 132.63, 132.03, 131.96, 130.51, 130.21, 129.94, 129.30, 124.96, 122.97, 121.20, 53.05.

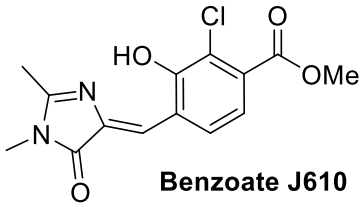


Benzoate I600

Benzoate I600 was synthesized from **3i** according to **Method A** (85% yield). MS (ES+): m/z calc. for C12H11ClO4: 254.1; found: 253.1$[M-H]^-$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 16.5 Hz, 1H), 7.48 (s, 2H), 6.85 (d, *J* = 16.5 Hz, 1H), 3.94 (s, 3H), 2.41 (s, 3H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 198.71, 165.29, 151.05, 136.68, 130.49, 126.28, 123.12, 52.80, 27.65.

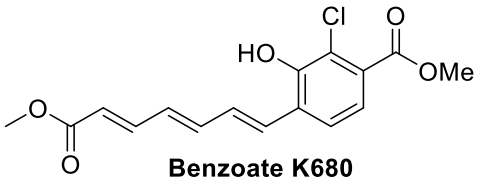


Benzoate J610

Benzoate J610 was synthesized from **3j** according to **Method C** (61% yield). MS (ES+): m/z calc. for C14H13ClN2O4: 308.1; found: 309.2$[M+H]^+$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.21 (d, *J* = 8.2 Hz, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 7.02 (s, 1H), 3.90 (s, 3H), 3.18 (s, 3H), 2.34 (d, 3H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.69, 166.52, 159.85, 154.58, 134.83, 134.54, 133.82, 127.66, 122.99, 120.26, 52.75, 26.99, 15.27.

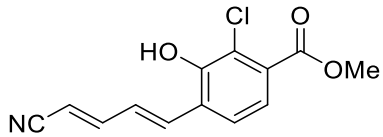


Benzoate K680

Benzoate K680 was synthesized from **3k** according to **Method A** (81% yield). MS (ES+): m/z calc. for C16H15ClO5: 322.1; found: 321.2[M-H]$^-$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40 (ddd, *J* = 22.4, 20.4, 9.8 Hz, 3H), 7.03 (d, *J* = 8.3 Hz, 2H), 6.75 (dd, *J* = 13.7, 8.5 Hz, 1H), 6.49 (dd, *J* = 14.8, 11.4 Hz, 1H), 6.35 (s, 1H), 5.95 (d, *J* = 15.3 Hz, 1H), 3.92 (s, 3H), 3.76 (s, 3H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.56, 165.48, 149.80, 144.38, 140.86, 131.93, 131.87, 129.90, 128.31, 124.72, 123.26, 121.47, 52.62, 51.76.

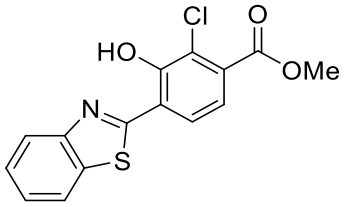


Benzoate N585

Benzoate K585 (E/Z ratio 1.3:1) was synthesized from **3n** according to **Method A** (81% yield). MS (ES+): m/z calc. for C13H10ClNO3: 263.1; found: 262.1[M-H]$^-$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.31 (m, 2H), 7.24 – 7.12 (m, 1H), 7.09 – 6.95 (m, 1H), 6.41 (d, *J* = 5.3 Hz, 1H), 5.54 – 5.27 (m, 1H), 3.96 – 3.92 (m, 3H).

^{13}C-NMR (101 MHz, CDCl3) δ 150.37, 134.72, 129.33, 128.92, 127.99, 125.39, 123.22, 100.02, 52.68, 30.73, 30.15, 29.82.



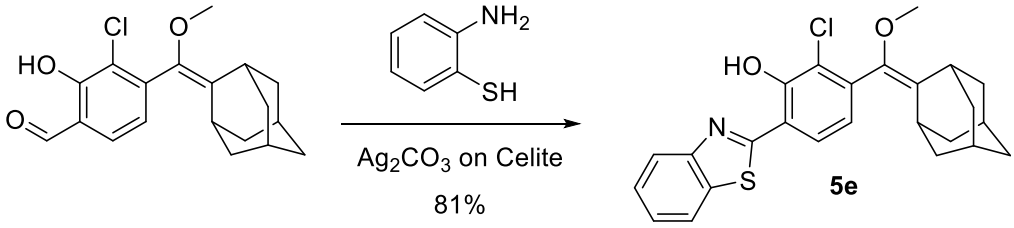
Benzoate E490

To a mixture of compound **6**[9] (50 mg, 0.15 mmol), commercially available 2-amino thiophenol (1.1 eq) in ethanol (2-3 ml) was added Ag_2CO_3/Celite (0.25 eq). The resulting mixture was stirred at 70 °C for 3 hours. Upon completion, the reaction mixture was filtered, diluted with EtOAc, and washed with 1M HCl and water. The organic layer was separated, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude residue was purified by silica column chromatography (Hex: EtOAc; 80:20) to obtain a brown solid (76% yield). MS (ES+): m/z calc. for C15H10ClNO3S: 319.0; found: 320.1 $[M+H]^+$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.2 Hz, 1H), 7.95 (d, *J* = 7.5 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.56 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H), 7.51 – 7.45 (m, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 3.98 (s, 1H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.78, 166.12, 154.

Synthesis and characterization of Enol Ether 5e:

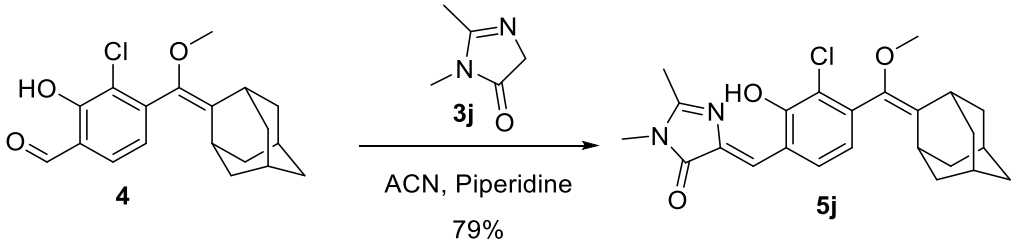


To a mixture of compound **4**[9] (50 mg, 0.15 mmol), commercially available 2-amino thiophenol (1.1 eq) in ethanol (2-3 ml) was added Ag_2CO_3/Celite (0.25 eq). The resulting mixture was stirred at 70 °C for 3 hours. Upon completion, the reaction mixture was filtered, diluted with EtOAc, and washed with 1M HCl and water. The organic layer was separated, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude residue was purified by silica column chromatography (Hex: EtOAc; 80:20) to obtain a brown solid (53 mg, 81%). MS (ES+): m/z calc. for C25H24ClNO2S: 437.1; found:436.4 $[M-H]^-$.

^{1}H-NMR (400 MHz, CDCl3) δ 7.97 (dd, J = 33.4, 8.3 Hz, 2H), 7.60 (d, J = 8.0 Hz, 1H), 7.54 (ddd, J = 8.3, 7.3, 1.2 Hz, 1H), 7.45 (td, J = 7.7, 1.1 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 3.38 (s, 3H), 3.31 (s, 1H), 2.17 (s, 1H), 1.99 – 1.66 (m, 14H).

^{13}C-NMR (101 MHz, CDCl3) δ 154.26, 151.56, 139.59, 139.18, 132.79, 132.36, 127.13, 126.10, 125.85, 122.49, 121.76, 117.04, 57.41, 39.40, 39.21, 38.76, 37.24, 33.10, 29.86, 28.55, 28.38.

Synthesis and characterization of Enol Ether 5j:

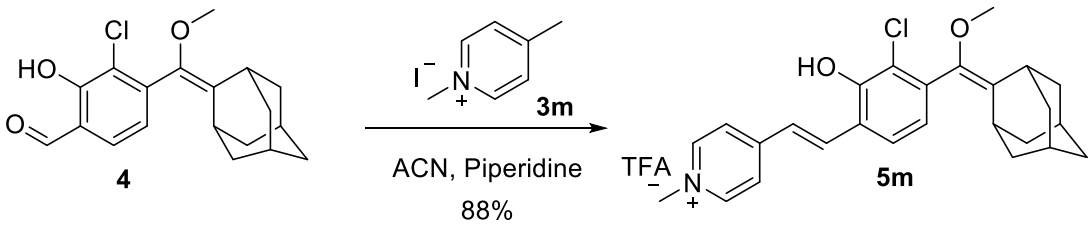


Compound **4**[9] (50 mg, 0.15 mmol), piperidine (2 eq), and compound **3j**[10] (1.2 eq) were dissolved in 3 mL of ACN. The reaction mixture was stirred at reflux for 1 hour and monitored by RP-HPLC (70-100 ACN in water with 0.1% TFA). Upon completion, the reaction mixture was diluted with EtOAc (100 mL) and washed with 1M HCl (100 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The product was purified by preparative RP-HPLC (gradient of ACN in water) and obtained as a yellow solid (51 mg, 79% yield). MS (ES+): m/z calc. for C24H27ClN2O3: 426.1. found:427.4 $[M+H]^+$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.18 (d, J = 8.1 Hz, 1H), 7.13 (s, 1H), 6.78 (d, J = 8.0 Hz, 1H), 3.34 (s, 3H), 3.26 (s, 1H), 3.24 (s, 3H), 2.41 (s, 3H), 2.14 (s, 1H), 1.98 – 1.68 (m, 13H).

^{13}C-NMR (101 MHz, $CDCl_3$) 167.75, 158.31, 154.55, 140.28, 139.96, 133.76, 133.22, 131.94, 129.14, 124.77, 122.45, 120.36, 57.38, 39.35, 39.18, 38.72, 37.25, 33.06, 29.78, 28.54, 28.38, 27.02, 15.24.

Synthesis and characterization of Enol Ether 5m:

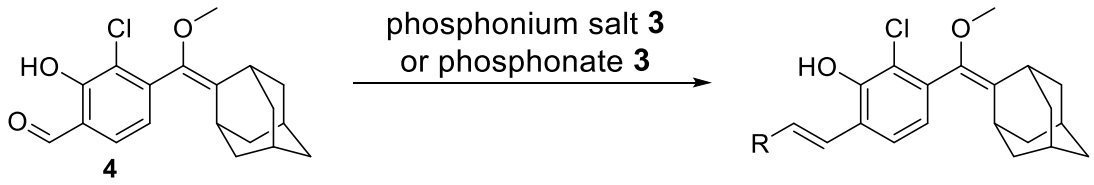


Compound **4**[9] (50 mg, 0.15 mmol), piperidine (2 eq), and compound **3m**[11] (1.2 eq) were dissolved in 3 mL of ACN. The reaction mixture was stirred at reflux for 1 h and monitored by RP-HPLC (70-100 ACN in water 0.1% TFA). Upon completion, the product was purified by preparative RP-HPLC (gradient of ACN in water) and obtained as a yellow solid (56 mg, 88% yield). MS (ES+): m/z calc. for C26H29ClNO2: 422.1. found: 422.4 $[M+H]^+$

^{1}H-NMR (400 MHz, DMSO) δ 8.84 (d, J = 6.8 Hz, 2H), 8.20 (d, J = 6.9 Hz, 2H), 8.12 (d, J = 16.3 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.50 (d, J = 16.3 Hz, 1H), 6.87 (d, J = 8.0 Hz, 1H), 4.25 (s, 3H), 3.21 (s, 3H), 3.16 (S, 1H), 2.00 (s, 1H), 1.94 – 1.58 (m, 14H).

^{13}C-NMR (100 MHz, DMSO) δ 153.07, 152.65, 145.77, 140.46, 137.23, 135.54, 130.23, 126.18, 124.95, 124.21, 123.53, 123.25, 56.98, 47.49, 39.06, 38.76, 38.67, 37.08, 33.04, 29.60, 28.32, 28.19.

Synthesis and characterization of Enol Ethers (5g, 5i, 5k,5n):

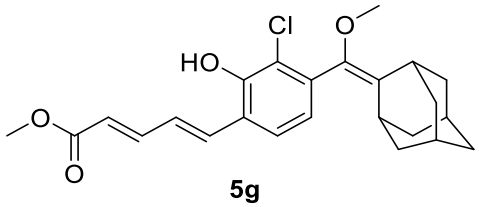


Method A

To a solution of phosphonium salt, **3** (1.1 eq) in DCM (1 mmol/ml) was added triethylamine (2 eq) and compound **4**[9] (1 eq). The reaction was stirred for 1 hour and monitored by TLC (Hex: EtOAc; 70:30). Upon completion, the reaction mixture was diluted with DCM and washed with 1M HCl followed by an additional wash with brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by silica column chromatography.

Method B

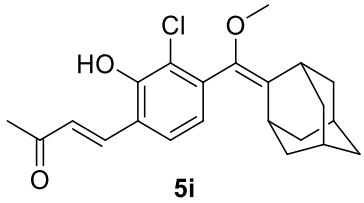
Phosphonate **3** (1.1 eq) was added dropwise to a suspension of NaH (2 eq, 60% in mineral oil) in dry THF (1 mmol/mL) at 0°C under an N_2 atmosphere. The mixture was stirred for 10 min, then compound **4**[9] (1 eq) was added dropwise for 5 min. The system was allowed to warm up to room temperature and stirred for 2.5 hours. The reaction was monitored by TLC and upon completion was diluted with EtOAc (50 mL) and washed with 1M HCl. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by silica column chromatography.



Enol Ether 5g was synthesized from **3g** according to **method A** (81% yield). MS (ES+): m/z calc. for C24H27ClO4: 414.1; found: 413.4 $[M-H]^-$.

^{1}H-NMR (400 MHz, CDCl3) δ 7.49 (dd, J = 15.1, 10.9 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.19 (d, J = 15.7 Hz, 1H), 7.01 (dd, J = 15.7, 11.1 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.16 (s, 1H), 6.00 (d, J = 15.3 Hz, 1H), 3.77 (s, 3H), 3.30 (s, 3H), 3.26 (s, 1H), 2.12 (s, 1H), 1.97 – 1.68 (m, 14H).

^{13}C-NMR (100 MHz, CDCl3) δ 167.56, 149.71, 145.35, 139.65, 135.29, 134.63, 132.60, 128.16, 125.32, 123.70, 121.45, 121.16, 57.27, 51.66, 39.25, 39.14, 38.91, 38.80, 37.16, 33.01, 29.81, 28.39.

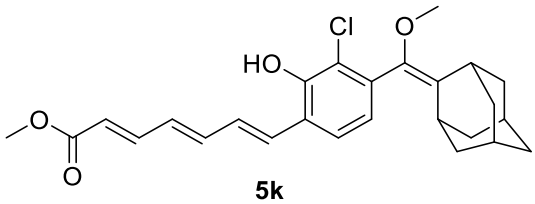


5i

Enol Ether 5i was synthesized from **3i** according to **method A** (83% yield). MS (ES+): m/z calc. for C22H25ClO3: 372.2; found: 371.3 $[M-H]^-$.

^{1}H-NMR (400 MHz, CDCl3) δ 7.82 (d, J = 16.5 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.82 (d, J = 16.5 Hz, 1H), 6.21 (s, 1H), 3.31 (s, 3H), 3.27 (s, 1H), 2.41 (s, 3H), 2.12 (s, 1H), 2.01 – 1.68 (m, 14H).

^{13}C-NMR (100 MHz, CDCl3) δ 167.68, 149.34, 144.73, 141.50, 139.76, 134.59, 132.55, 130.80, 130.66, 129.99, 124.88, 124.35, 123.75, 121.38, 120.68, 57.31, 51.71, 39.19, 37.20, 33.02, 29.84, 28.42.

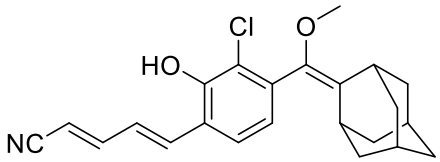


5k

Enol Ether 5k was synthesized from **3k** according to **method A** (72% yield). MS (ES+): m/z calc. for C26H29ClO4: 440.2; found: 439.4 $[M-H]^-$.

^{1}H-NMR (400 MHz, CDCl3) δ 7.41 – 7.33 (m, 2H), 6.99 (d, J = 8.8 Hz, 2H), 6.85 – 6.71 (m, 2H), 6.45 (dd, J = 14.7, 11.4 Hz, 1H), 6.08 (s, 1H), 5.92 (d, J = 15.2 Hz, 1H), 3.76 (s, 3H), 3.31 (s, 3H), 3.27 (s, 1H), 2.13 (s, 1H), 1.99 – 1.70 (m, 14H).

^{13}C-NMR (100 MHz, CDCl3) δ 167.68, 149.34, 144.73, 141.50, 139.76, 134.59, 132.55, 130.80, 130.66, 129.99, 124.88, 124.35, 123.75, 121.38, 120.68, 57.31, 51.71, 39.19, 38.92, 37.20, 33.02, 29.84, 28.42.



5n

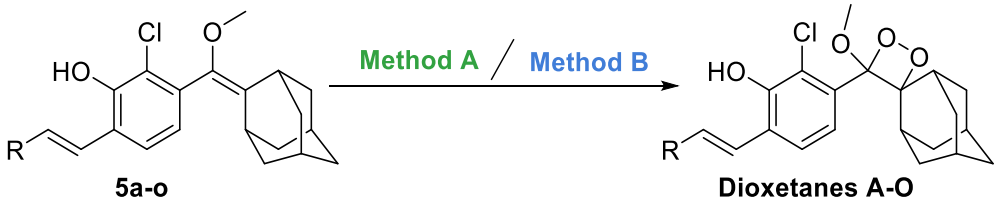
Enol Ether 5n (E/Z-ratio of 1.2:1) was synthesized from **3n** according to **method B** (65% yield). MS (ES+): m/z calc. for C23H24ClNO2: 381.2; found: 380.3 $[M-H]^-$.

^{1}H-NMR (400 MHz, CDCl3) δ 7.48 – 7.19 (m, 2H), 7.19 – 7.11 (m, 1H), 7.04 – 6.92 (m, 1H), 6.85 (dd, J = 7.9, 6.6 Hz, 1H), 6.29 (s, 1H), 5.35 (dd, J = 73.3, 13.5 Hz, 1H), 3.30 (s, 3H), 3.26 (s, 1H), 2.11 (s, 1H), 1.99 – 1.63 (m, 15H).

^{13}C-NMR (400 MHz, CDCl3) δ 151.00, 150.11, 149.81, 139.55, 136.07, 135.98, 135.71, 135.64, 132.97, 131.08, 127.48, 125.85, 125.69, 125.46, 123.89, 123.81, 123.58, 123.09, 123.02, 121.63, 121.51, 118.51, 116.84, 114.21, 98.54, 96.83, 57.41, 39.41, 39.17, 38.92, 37.16, 33.04, 31.77, 31.15, 30.45, 29.85, 28.40, 14.26.

Synthesis and characterization of Dioxetanes (A-O):

Dioxetane luminophores **A-D** and luminophores **O, and L** were synthesized according to a known procedure.[7,8]



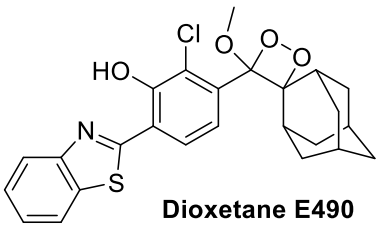
5a-o **Dioxetanes A-O**

Method A

Enol-ether **5** (0.1 mmol) was dissolved in THF: H_2O (4:1), followed by the addition of NaOH (5 eq). The reaction was stirred at room temperature and monitored by TLC (Hex: EtOAc 60:40) or HPLC (ACN gradient in water). Upon hydrolysis of esters, the reaction mixture was diluted with EtOAc and washed with 1M HCl. The organic phase was separated and concentrated under reduced pressure. The crude product was used for the next step without further purification. The residue was dissolved in 10 mL of DCM followed by the addition of a catalytic amount of methylene blue. Then, oxygen was bubbled through the solution while irradiating with yellow light. The reaction was monitored by RP-HPLC. Upon completion (about 7 min), the solvent was concentrated under reduced pressure and the product was purified by preparative RP-HPLC.

Method B

Enol ether **5** (0.1 mmol) and a catalytic amount of methylene blue were dissolved in 10 mL of DCM. Then, oxygen was bubbled through the solution while irradiating with yellow light. The reaction was monitored by RP-HPLC. Upon completion (7 min), the solvent was concentrated under reduced pressure and the product was purified by preparative RP-HPLC.

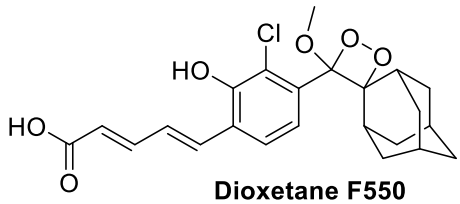


Dioxetane E490

Dioxetane E490 was synthesized from **Enol Ether 5e** according to **Method B** (85% yield). MS (ES+): m/z calc. for C25H24ClNO4S: 469.1; found:468.4 $[M-H]^-$.

^{1}H-NMR (400 MHz, DMSO) δ 8.23 (d, J = 7.4 Hz, 1H), 8.17 (d, J= 9.2 Hz, 1H), 8.15 (d, J = 9.0 Hz, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.61 (td, J = 7.2, 1.2 Hz, 1H), 7.53 (td, J = 7.8, 1.1 Hz, 1H), 3.15 (s, 3hH), 2.90 (s, 1H), 2.27 (d, J = 12.2 Hz, 1H), 1.99 (s, 1H), 1.84 – 1.48 (m, 12H).

^{13}C-NMR (100 MHz, DMSO) δ 166.50, 153.71, 151.51, 135.50, 134.14, 127.76, 127.31, 126.80, 124.04, 123.11, 120.80, 120.63, 111.93, 96.09, 50.03, 36.48, 33.90, 33.65, 32.56, 32.27, 31.70, 31.49, 26.13, 25.80.

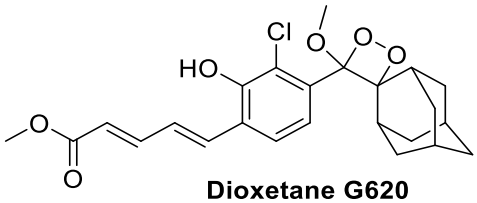


Dioxetane F550

Dioxetane F550 was synthesized from **Enol Ether 5g** according to **Method A** (65% yield overall). MS (ES+): m/z calc. for C23H25ClO6: 432.2; found: 431.3 $[M-H]^-$.

^{1}H-NMR (400 MHz, DMSO) δ 12.31 (s, 1H), 9.85 (s, 1H), 7.69 (d, *J* = 8.3 Hz, 1H), 7.47 (d, *J* = 8.3 Hz, 1H), 7.37 (dd, *J* = 15.1, 10.8 Hz, 1H), 7.29 (d, *J* = 15.7 Hz, 1H), 7.17 (dd, *J* = 15.5, 10.8 Hz, 1H), 6.04 (d, *J* = 15.1 Hz, 1H), 3.11 (s, 3H), 2.87 (s, 1H), 2.25 (d, *J* = 11.4 Hz, 1H), 1.96 (s, 1H), 1.82 – 1.29 (m, 12H).

^{13}C-NMR (100 MHz, CDCl3) δ 168.07, 152.16, 144.98, 134.25, 132.47, 129.53, 127.82, 125.40, 124.14, 123.59, 120.54, 112.02, 95.96, 49.84, 39.06, 36.51, 33.89, 33.57, 32.48, 32.31, 31.70, 31.43, 26.13, 25.82.

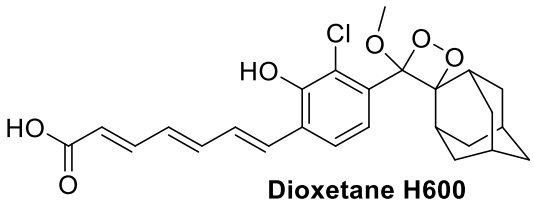


Dioxetane G620

Dioxetane G620 was synthesized from **Enol Ether 5g** according to **Method B** (90% yield). MS (ES+): m/z calc. for C24H27ClO6: 446.2; found: 445.3 $[M-H]^-$.

^{1}H-NMR (400 MHz, CDCl3) δ 9.87 (s, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.47-7.41 (m, 2H), 7.32 (d, J = 15.7 Hz, 1H), 7.18 (dd, J = 15.6, 10.9 Hz, 1H), 6.12 (d, J = 15.2 Hz, 1H), 3.67 (s, 3H), 3.09 (s, 3H), 2.85 (s, 1H), 2.22 (d, J = 12.1 Hz, 1H), 1.94 (s, 1H), 1.75 – 1.11 (m, 11H).

^{13}C-NMR (100 MHz, CDCl3) δ 167.14, 152.24, 145.62, 135.04, 132.62, 129.33, 127.70, 125.46, 124.16, 121.96, 120.55, 112.01, 95.96, 51.98, 49.83, 36.50, 33.88, 33.57, 32.48, 32.30, 31.69, 31.43, 26.12, 25.82.

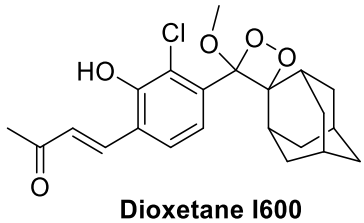


Dioxetane H600

Dioxetane H600 was synthesized from **Enol Ether 5k** according to **Method A** (54% yield overall). MS (ES+): m/z calc. for C25H27ClO6: 458.2; found: 457.3 $[M-H]^-$.

^{1}H-NMR (400 MHz, DMSO) δ 9.74 (s, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.44 (d, J = 8.3 Hz, 1H), 7.27 (dd, J = 15.0, 11.3 Hz, 1H), 7.14 – 7.04 (m, 2H), 6.92 (ddd, J = 14.0, 9.6, 4.5 Hz, 1H), 6.65 – 6.54 (m, 1H), 5.94 (d, J = 15.2 Hz, 1H), 3.09 (s, 3H), 2.85 (s, 1H), 2.27 – 2.15 (m, 1H), 1.95 (s, 1H), 1.64 (ddd, J = 38.9, 28.5, 16.9 Hz, 12H).

^{13}C-NMR (100 MHz, DMSO) δ 168.15, 151.76, 144.57, 141.51, 131.78, 131.32, 130.52, 128.42, 124.90, 124.19, 122.76, 120.49, 112.04, 95.95, 49.82, 38.16, 36.52, 33.90, 33.57, 32.49, 32.31, 31.71, 31.43, 29.47, 26.13, 25.83.

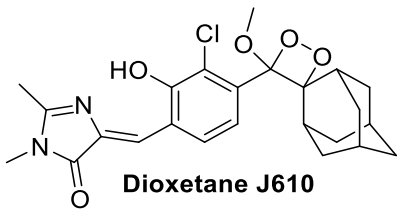


Dioxetane I600

Dioxetane I600 was synthesized from **Enol Ether 5i** according to **Method B** (92%). MS (ES+): m/z calc. for C22H25ClO5: 404.2; found: 403.3 $[M-H]^-$.

^{1}H-NMR (400 MHz, DMSO) δ 10.15 (s, 1H), 7.81 (d, J = 16.4 Hz, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.49 (d, J = 8.3 Hz, 1H), 6.87 (d, J = 16.4 Hz, 1H), 3.10 (s, 3H), 2.86 (s, 1H), 2.33 (s, 3H), 2.23 (d, J = 12.3 Hz, 1H), 1.93 (s, 1H), 1.72 – 1.28 (m, 11H).

^{13}C-NMR (100 MHz, DMSO) δ 198.65, 153.22, 137.36, 134.13, 129.70, 126.53, 126.03, 124.17, 120.75, 111.94, 95.97, 49.88, 36.49, 33.88, 33.59, 32.48, 32.27, 31.68, 31.43, 28.21, 26.12, 25.81.

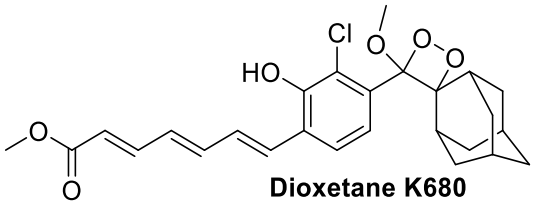


Dioxetane J610

Dioxetane J610 was synthesized from **Enol Ether 5j** according to **Method B** (83%). MS (ES+): m/z calc. for C24H27ClN2O5: 458.2; found: 459.5 $[M+H]^+$.

^{1}H-NMR (400 MHz, DMSO) δ 7.88 (d, J = 8.5 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 3.5 Hz, 1H), 3.13 (d, J = 2.1 Hz, 3H), 3.11 (s, 3H), 2.86 (s, 1H), 2.42 (s, 3H), 2.26 (d, J = 11.6 Hz, 1H), 1.94 – 1.50 (m, 13H).

^{13}C-NMR (100 MHz, DMSO) δ 168.08, 163.65, 154.38, 136.38, 135.58, 133.84, 133.49, 123.92, 123.59, 123.11, 120.26, 112.00, 96.06, 53.24, 49.93, 46.93, 36.53, 36.17, 33.83, 32.50, 31.70, 29.35, 27.44, 27.20, 26.14, 25.82, 15.78.

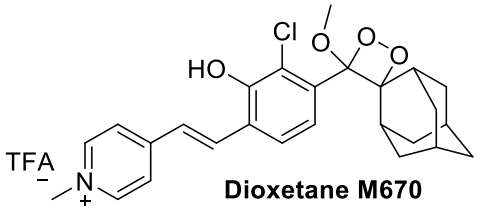


Dioxetane K680

Dioxetane K680 was synthesized from **Enol Ether 5k** according to **Method B** (85%). MS (ES+): m/z calc. for C26H29ClO6: 472.2; found: 471.4 $[M-H]^-$.

^{1}H-NMR (400 MHz, DMSO) δ 9.76 (s, 1H), 7.65 (t, J = 10.2 Hz, 1H), 7.44 (d, J = 8.3 Hz, 1H), 7.35 (dd, J = 15.2, 11.4 Hz, 1H), 7.13 – 7.05 (m, 2H), 6.98 (dd, J = 14.6, 9.5 Hz, 1H), 6.66 – 6.55 (m, 1H), 6.04 (d, J = 15.2 Hz, 1H), 3.66 (s, 3H), 3.09 (s, 3H), 2.85 (s, 1H), 2.23 (d, J = 12.2 Hz, 1H), 1.95 (s, 1H), 1.74 – 1.26 (m, 11H).

^{13}C-NMR (100 MHz, DMSO) δ 167.23, 151.81, 145.16, 142.26, 131.88, 131.68, 131.23, 130.94, 128.36, 124.95, 124.19, 121.15, 120.50, 112.04, 95.95, 51.93, 49.82, 36.52, 33.89, 33.57, 32.49, 32.31, 31.70, 31.43, 26.13, 25.83.

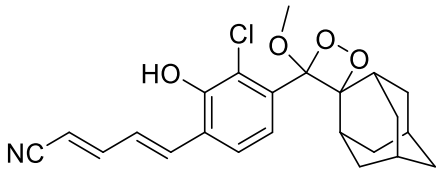


Dioxetane M670

Dioxetane M670 was synthesized from **Enol Ether 5m** according to **Method B** (76%). MS (ES+): m/z calc. for C26H29ClNO4: 454.18; found: 454.4 $[M+H]^+$.

^{1}H-NMR (400 MHz, DMSO) δ 10.40 (s, 1H), 8.87 (d, J = 6.8 Hz, 2H), 8.23 (d, J = 6.8 Hz, 2H), 8.11 (d, J = 16.4 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.59 – 7.51 (m, 2H), 4.26 (s, 3H), 3.12 (s, 3H), 2.87 (s, 1H), 2.24 (d, J = 12.1 Hz, 1H), 1.96 (s, 1H), 1.74 – 1.22 (m, 11H).

^{13}C-NMR (100 MHz, DMSO) δ 153.22, 152.82, 145.88, 135.06, 134.01, 126.81, 126.20, 124.43, 124.20, 120.92, 112.00, 96.03, 49.90, 47.59, 36.50, 33.88, 33.62, 32.51, 32.32, 31.68, 31.44, 26.13, 25.82.z



Dioxetane N585

Dioxetane N585 was synthesized from **Enol Ether 5n** according to **Method B** (55%). The compound was obtained as a diastereomeric mixture (E and Z) in a 3:2 ratio respectively. MS (ES+): m/z calc. for C23H24ClNO4: 413.1; found: 412.3 $[M-H]^{-}$.

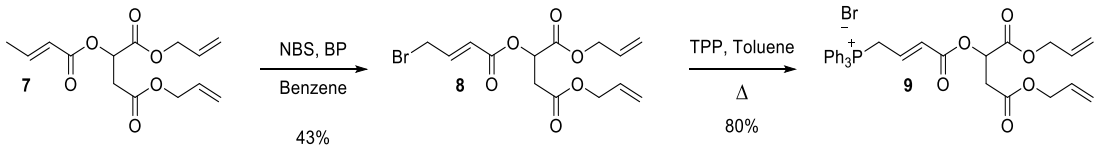
^{1}H-NMR (400 MHz, DMSO, diastereomer ratio 3:2): major diastereomer: δ 9.91 (s, 1H), 7.67 (dd, J = 14.0, 8.4 Hz, 1H), 7.52 – 7.45 (m, 1H), 7.44 (d, J = 10.8 Hz, 1H), 7.35 – 7.26 (m, 1H), 7.15 (dd, J = 15.6, 10.7 Hz, 1H), 5.88 (d, J = 15.8 Hz, 1H), 3.08 (s, 3H), 2.85 (s, 1H), 2.22 (d, J = 12.1 Hz, 1H), 1.94 (s, 1H), 1.75 – 1.41 (m, 11H).

^{1}H-NMR (400 MHz, DMSO, diastereomer ratio 3:2): minor diastereomer: δ 10.01 (s, 1H), 7.67 (dd, J = 14.0, 8.4 Hz, 1H), 7.52 – 7.45 (m, 1H), 7.35 – 7.26 (m, 3H), 5.67 (d, J = 12.0 Hz, 1H), 3.09 (s, 3H), 2.85 (s, 1H), 2.22 (d, J = 12.1 Hz, 1H), 1.94 (s, 2H), 1.75 – 1.41 (m, 11H).

^{1}H-NMR (400 MHz, DMSO, diastereomer ratio 3:2) δ 10.01 (s, 1H), 9.91 (s, 1H), 7.67 (dd, J = 14.0, 8.4 Hz, 2H), 7.52 – 7.45 (m, 2H), 7.44 (d, J = 10.8 Hz, 1H), 7.35 – 7.26 (m, 3H), 7.15 (dd, J = 15.6, 10.7 Hz, 1H), 5.88 (d, J = 15.8 Hz, 1H), 5.67 (d, J = 12.0 Hz, 1H), 3.09 (s, 3H), 3.08 (s, 3H), 2.85 (s, 2H), 2.22 (d, J = 12.1 Hz, 2H), 1.94 (s, 2H), 1.75 – 1.41 (m, 22H).

^{13}C-NMR (100 MHz, DMSO, diastereomer ratio 3:2) δ 152.67, 152.46, 151.67, 150.55, 136.64, 135.30, 133.13, 132.95, 129.12, 127.33, 127.11, 126.98, 126.55, 125.50, 124.31, 124.22, 120.59, 120.50, 119.40, 117.59, 111.97, 99.85, 98.19, 95.96, 49.84, 36.50, 33.88, 33.57, 32.49, 32.29, 31.68, 31.43, 26.13, 25.82.

Synthesis and characterization of DPLX-1, DPLX-2



Compound 8

A mixture of **7**[12] (1 gr, 3.55 mmol), benzoyl peroxide (0.2 eq), and N-bromosuccinimide (1.3 eq) in benzene (20 mL) was heated to reflux overnight. The reaction was monitored by TLC (EtOAc: Hex; 30:70). Upon completion, the reaction mixture was diluted with EtOAc and washed with brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by silica column chromatography (EtOAc: Hex; 20:80). The bromide **8** was obtained as a yellow oil (0.55 gr, 1.52 mmol, 43% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.04 (dt, *J* = 15.3, 7.3 Hz, 1H), 6.10 – 6.03 (m, 1H), 5.86 (ddtd, *J* = 17.2, 10.5, 5.7, 2.1 Hz, 2H), 5.55 (t, *J* = 6.1 Hz, 1H), 5.34 – 5.18 (m, 4H), 4.62 (ddd, *J* = 16.5, 8.9, 3.5 Hz, 4H), 3.99 (dd, *J* = 7.3, 1.3 Hz, 2H), 2.94 (d, *J* = 6.2 Hz, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 168.75, 168.41, 164.35, 143.64, 131.74, 131.36, 123.42, 119.10, 118.87, 68.65, 66.45, 65.91, 36.24, 28.99.

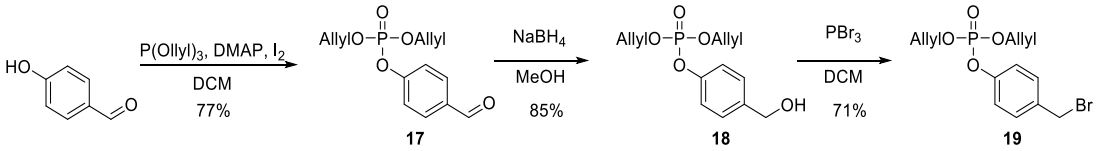
Compound 9

To a solution of bromide **8** (0.55 gr, 1.52 mmol) in toluene (5 mL) was added triphenylphosphine (1.5 eq). The reaction was stirred at room temperature overnight, upon which yellow oil formed and TLC (Hex: EtOAc; 70:30) indicated the disappearance of starting material. The oil was triturated and washed with ethyl acetate (20 mL) and hexane (20 ml). The product was dried under reduced pressure to give the phosphonium salt **9** as a yellow oil (0.76 gr, 1.22 mmol, 80% yield).

^{1}H-NMR (400 MHz, DMSO) δ 7.91 (dd, *J* = 9.6, 4.4 Hz, 11H), 7.83 – 7.69 (m, 4H), 7.65 – 7.49 (m, 2H), 7.39 – 7.31 (m, 2H), 7.22 (dd, *J* = 9.1, 5.3 Hz, 2H), 6.71 (dt, *J* = 14.0, 7.4 Hz, 1H), 6.21 (dd, *J* = 15.4, 4.8 Hz, 1H), 5.82 (ddd, *J* = 16.1, 10.4, 5.2 Hz, 2H), 5.41 (t, *J* = 5.8 Hz, 1H), 5.19 (ddd, *J* = 13.8, 13.0, 1.8 Hz, 4H), 5.00 (dd, *J* = 17.4, 7.5 Hz, 2H), 4.54 (dd, *J* = 29.0, 5.1 Hz, 4H).

^{13}C-NMR (101 MHz, DMSO) δ 172.22, 169.13, 168.35, 135.95, 134.50, 134.41, 134.31, 133.95, 133.76, 132.77, 132.41, 131.02, 130.90, 129.59, 129.41, 129.34, 118.60, 118.53, 118.44, 117.67, 68.94, 66.17, 65.51.

Synthesis of ALP trigger (compound 19):



Compound 17

4-hydroxy benzaldehyde (200 mg, 1.63 mmol) was dissolved in 2 ml of DCM, and 4-DMAP (2 eq) was added. The solution was stirred at room temperature. Triallyl phosphite (2.2 eq) was dissolved in 2 ml of DCM and cooled to 0°C. Iodine (2 eq) was added, and the reaction was stirred to homogeneity. The iodine solution was pipetted to the phenol solution and the reaction was stirred for 30 minutes and monitored by TLC. Upon completion, the reaction mixture was diluted with DCM (100 ml) and washed with brine (100 ml). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography (Hex: EtOAc; 70:30) afforded compound **17** as a yellow oil (350 mg, 77% yield). MS (ES+): m/z calc. for C13H15O5P: 282.1; found: 305.3 $[M+Na]^+$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.94 (s, 1H), 7.86 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 5.92 (dtd, J = 16.2, 10.9, 5.7 Hz, 2H), 5.39 – 5.23 (m, 4H), 4.67 – 4.59 (m, 4H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 190.83, 155.41, 133.49, 132.59, 131.94, 131.78, 120.76, 119.18, 118.71, 69.29, 69.23, 68.30, 68.24.

^{31}P NMR (162 MHz, CDCl3) δ -6.88.

Compound 18

Compound **17** (350 mg, 1.25 mmol) was dissolved in MeOH. $NaBH_4$ (2 eq) was added slowly. The reaction was monitored by TLC (EtOAc: Hex; 60:40). Upon completion, the reaction mixture was diluted with EtOAc, and washed with brine. The organic layer was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to afford compound **18** as an off-white powder (300 mg, 85% yield). MS (ES+): m/z calc. for C13H17O5P: 284.1; found: 307.3 $[M+Na]^+$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.26 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 7.8 Hz, 2H), 5.89 (ddd, J = 22.4, 10.8, 5.6 Hz, 2H), 5.28 (ddd, J = 13.8, 11.5, 1.3 Hz, 4H), 4.63 – 4.46 (m, 6H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 149.81, 149.74, 138.51, 132.14, 132.08, 128.37, 120.04, 119.99, 118.85, 69.03, 68.97, 64.22, 29.79.

^{31}P NMR (162 MHz, CDCl3) δ -6.30.

Compound 19

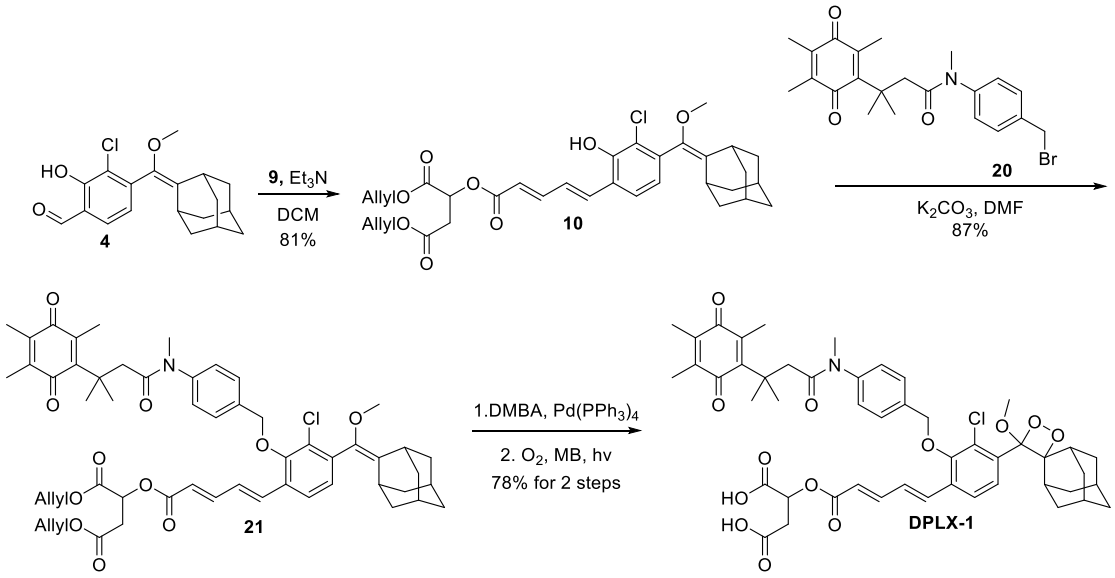
Compound **18** (300 mg, 1.03 mmol) was dissolved in 2 ml of DCM, and PBr_3 (1.1 eq) was added dropwise. Upon completion, the reaction mixture was diluted with DCM and washed with saturated $NaHCO_3$ solution. The organic layer was separated and dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure to afford compound **19** (307 mg, 71% yield) as a yellow oil. MS (ES+): m/z calc. for C13H16BrO4P: 346.0; found: 369.2 $[M+Na]^+$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.36 (d, J = 8.5 Hz, 2H), 7.18 (dd, J = 8.7, 1.0 Hz, 2H), 5.99 – 5.86 (m, 2H), 5.31 (ddd, J = 13.8, 11.6, 1.3 Hz, 4H), 4.63 (ddd, J = 8.5, 5.6, 1.4 Hz, 4H), 4.46 (s, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 150.66, 134.83, 132.15, 132.09, 130.67, 120.54, 120.49, 118.93, 69.09, 69.03, 32.72, 29.82.

^{31}P NMR (162 MHz, CDCl3) δ -6.32.

Synthesis of DPLX-1:



Compound 10

To a solution of phosphonium salt **9** (206 mg, 1.1 eq) in DCM (1 mmol/ml) was added triethylamine (2 eq) and compound **4**[9] (100 mg, 0.30 mmol, 1 eq). The reaction was stirred for 1 hour and monitored by TLC (Hex: EtOAc; 70:30). Upon completion, the reaction mixture was diluted with DCM and washed with 1M HCl followed by an additional wash with brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by silica column chromatography to give enol ether

10 as a white solid (143 mg, 0.24 mmol, 81% yield). MS (ES+): m/z calc. for C49H54ClNO13: 596.2; found: 595.5 $[M-H]^-$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.52 (dd, *J* = 15.2, 11.1 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 15.7 Hz, 1H), 6.99 (dd, *J* = 15.6, 11.1 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.27 (s, 1H), 6.03 (d, *J* = 15.3 Hz, 1H), 5.94 – 5.83 (m, 2H), 5.61 (t, *J* = 6.2 Hz, 1H), 5.36 – 5.20 (m, 4H), 4.71 – 4.58 (m, 4H), 3.29 (s, 3H), 3.25 (s, 1H), 3.00 – 2.96 (m, 2H), 2.11 (s, 1H), 1.96 – 1.67 (m, 14H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 168.96, 168.85, 165.85, 149.92, 146.88, 139.67, 135.55, 132.65, 131.81, 131.46, 127.95, 125.42, 123.73, 123.64, 121.54, 119.97, 119.33, 119.01, 118.89, 118.81, 118.67, 68.41, 67.44, 66.66, 66.40, 65.90, 65.78, 57.32, 39.29, 39.19, 39.16, 38.92, 38.87, 38.79, 37.19, 36.44, 33.03, 29.84, 28.42.

Compound 21

Enol ether **10** (100 mg, 0.16 mmol, 1eq) was dissolved in 1 ml dry DMF. K_2CO_3 (1.2eq) was added and the solution was stirred for 10 minutes before compound **20**[13](1eq) was added. The reaction mixture was stirred for 30 minutes at room temperature and monitored by TLC (Hex: EtOAc; 50:50). After completion, the reaction mixture was diluted with EtOAc (100 ml) and washed with saturated NH_4Cl (100 ml). The organic layer was separated, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (Hex: EtOAc; 50:50) and obtained as a white solid (195 mg, 0.20 mmol, 87% yield). MS (ES+): m/z calc. for C55H62ClNO11: 947.4; found: 970.8 $[M+Na]^+$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 7.9 Hz, 2H), 7.52 – 7.44 (m, 2H), 7.23 (dd, J = 19.7, 13.6 Hz, 3H), 7.09 (d, J = 8.0 Hz, 1H), 6.94 (dd, J = 15.4, 11.0 Hz, 1H), 6.06 (d, J = 15.3 Hz, 1H), 5.95 – 5.84 (m, 2H), 5.61 (t, J = 6.1 Hz, 1H), 5.36 – 5.21 (m, 4H), 5.00 (d, J = 1.4 Hz, 2H), 4.70 – 4.58 (m, 4H), 3.35 (d, J = 9.2 Hz, 3H), 3.28 (s, 1H), 3.17 (s, 3H), 2.99 (d, J = 6.2 Hz, 2H), 2.81 – 2.69 (m, 2H), 2.10 (d, J = 5.1 Hz, 3H), 1.99 – 1.61 (m, 22H), 1.31 (s, 3H), 1.25 (s, 6H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 191.36, 187.85, 172.22, 168.91, 168.74, 165.75, 154.91, 152.98, 146.20, 144.21, 143.74, 139.59, 137.90, 137.29, 136.37, 134.87, 132.60, 131.77, 131.41, 131.02, 129.78, 128.43, 128.15, 127.80, 124.53, 124.08, 123.56, 120.81, 119.09, 118.82, 115.95, 114.20, 75.09, 68.47, 67.42, 66.67, 65.91, 57.37, 47.79, 39.35, 38.77, 37.19, 36.37, 33.95, 33.12, 32.05, 31.76, 30.45, 29.82, 29.64, 29.28, 28.60, 22.82, 14.21, 12.89.

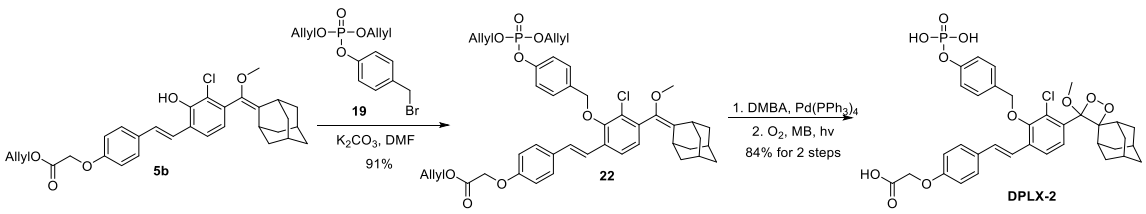
DPLX-1

A mixture of compound **21** (100 mg, 1 eq), DMBA (10 eq), and tetrakis(triphenylphosphine)palladium (0.1 eq) in DCM (2 mL) was stirred at room temperature for 3 hours and monitored by RP-HPLC (90-100% ACN in water, 20 min). Upon full consumption of starting material, a catalytic amount of methylene blue (~1 mg) was added. Oxygen was bubbled through the solution while irradiating with yellow light for 10 minutes. The reaction was monitored by RP-HPLC (70-100% ACN in water, 20 min). Upon completion, the reaction mixture was concentrated under reduced pressure, and the crude product was purified by preparative RP-HPLC (50-100% ACN in water, 20 min) to afford DPLX-1 (60 mg, 0.077 mmol, 78% yield) as a white solid. MS (ES+): m/z calc. for C49H54ClNO13: 899.3; found: 900.8 $[M+H]^+$.

^{1}H-NMR (400 MHz, DMSO) δ 7.81 (dt, J = 8.5, 6.4 Hz, 2H), 7.64 – 7.57 (m, 2H), 7.50 (dd, J = 15.3, 9.6 Hz, 1H), 7.33 – 7.19 (m, 4H), 6.19 (d, J = 15.2 Hz, 1H), 5.29 (dd, J = 8.5, 3.9 Hz, 1H), 4.97 (s, 2H), 3.13 (s, 3H), 3.04 (s, 1H), 2.90 – 2.82 (m, 2H), 2.76 (dd, J = 16.7, 8.7 Hz, 1H), 2.64 (s, 2H), 2.24 (d, J = 11.9 Hz, 1H), 1.99 (d, J = 12.8 Hz, 3H), 1.89 (d, J = 7.1 Hz, 3H), 1.75 – 1.30 (m, 11H), 1.22 (s, 9H).

^{13}C-NMR (101 MHz, DMSO) δ 190.94, 187.47, 171.28, 170.77, 165.75, 155.47, 153.58, 146.26, 144.34, 143.93, 137.34, 135.83, 134.19, 133.65, 130.59, 130.25, 129.05, 127.96, 127.43, 125.57, 122.33, 118.31, 111.86, 100.73, 96.04, 75.62, 69.18, 50.00, 47.50, 36.39, 35.71, 33.89, 33.66, 32.30, 31.69, 31.47, 29.62, 29.42, 29.29, 29.17, 28.70, 27.15, 26.11, 25.80, 22.69, 14.55, 14.32, 13.20, 12.34.

Synthesis of DPLX-2:



Compound 22

Enol ether **5b**[9] (150 mg, 0.28 mmol, 1eq) was dissolved in 1 mL dry DMF. K_2CO_3 (1.2eq) was added and the solution was stirred for 10 minutes before compound **19** (1eq) was added. The reaction mixture was stirred for 30 minutes at room temperature and monitored by TLC (Hex: EtOAc; 80:20). After completion, the reaction mixture was diluted with EtOAc (100 ml) and

washed with saturated NH_4Cl (100 ml). The organic layer was separated, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (Hex: EtOAc; 80:20) and obtained as a white solid (200 mg, 0.25 mmol, 91% yield). MS (ES+): m/z calc. for C44H48ClO9P: 786.3; found: 809.6 $[M+Na]^+$.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.48 (t, J = 5.7 Hz, 2H), 7.38 (d, J = 8.7 Hz, 2H), 7.23 (ddd, J = 18.0, 6.8, 4.3 Hz, 4H), 7.07 (dd, J = 13.7, 8.9 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.00 – 5.87 (m, 3H), 5.41 – 5.22 (m, 6H), 4.96 (d, J = 2.9 Hz, 2H), 4.67 (dddd, J = 12.3, 5.3, 2.9, 1.5 Hz, 10H), 3.33 (s, 3H), 3.28 (s, 1H), 2.11 (s, 1H), 1.82 (ddd, J = 34.7, 31.9, 13.4 Hz, 12H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 168.60, 157.89, 152.35, 150.75, 139.93, 134.98, 133.85, 132.68, 132.24, 131.81, 131.54, 130.49, 130.05, 129.87, 129.52, 129.24, 128.13, 127.89, 123.82, 121.01, 120.26, 120.12, 119.32, 118.86, 117.41, 115.13, 114.56, 74.85, 71.49, 71.29, 69.05, 68.99, 66.05, 65.47, 57.18, 39.35, 39.21, 38.81, 37.27, 33.07, 31.78, 30.45, 29.83, 28.56.

DPLX-2

A mixture of compound **22** (60 mg, 1 eq), DMBA (10 eq), and tetrakis(triphenylphosphine)palladium (0.1 eq) in DCM (2 mL) was stirred at room temperature for 3 hours and monitored by RP-HPLC (70-100% ACN in water, 20 min). Upon full consumption of starting material, a catalytic amount of methylene blue (~1 mg) was added. Oxygen was bubbled through the solution while irradiating with yellow light for 10 minutes. The reaction was monitored by RP-HPLC (70-100% ACN in water, 20 min). Upon completion, the reaction mixture was concentrated under reduced pressure, and the crude product was purified by preparative RP-HPLC (50-100% ACN in water, 20 min) to afford DPLX-2 (50 mg, 0.066 mmol, 84% yield) as a white solid. MS (ES+): m/z calc. for C35H36ClO11P: 698.3; found: 697.5 $[M-H]^-$.

^{1}H-NMR (400 MHz, DMSO) δ 7.82 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.29 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 17.1 Hz, 1H), 7.20 (d, J = 9.1 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 16.5 Hz, 1H), 6.85 (d, J = 8.7 Hz, 2H), 4.86 (s, 2H), 4.44 (s, 2H), 3.13 (s, 3H), 2.88 (s, 1H), 2.27 (d, J = 12.0 Hz, 1H), 1.95 (s, 1H), 1.78 – 1.17 (m, 11H).

^{13}C-NMR (101 MHz, DMSO) δ 171.60, 159.19, 155.01, 154.04, 134.91, 132.05, 131.17, 130.04, 129.97, 129.66, 128.61, 128.42, 127.17, 124.30, 120.30, 120.27, 115.39, 111.99, 96.00, 76.35, 66.79, 49.93, 36.53, 33.92, 33.67, 32.39, 32.34, 31.72, 31.46, 26.14, 25.82.

Bacterial Experiments

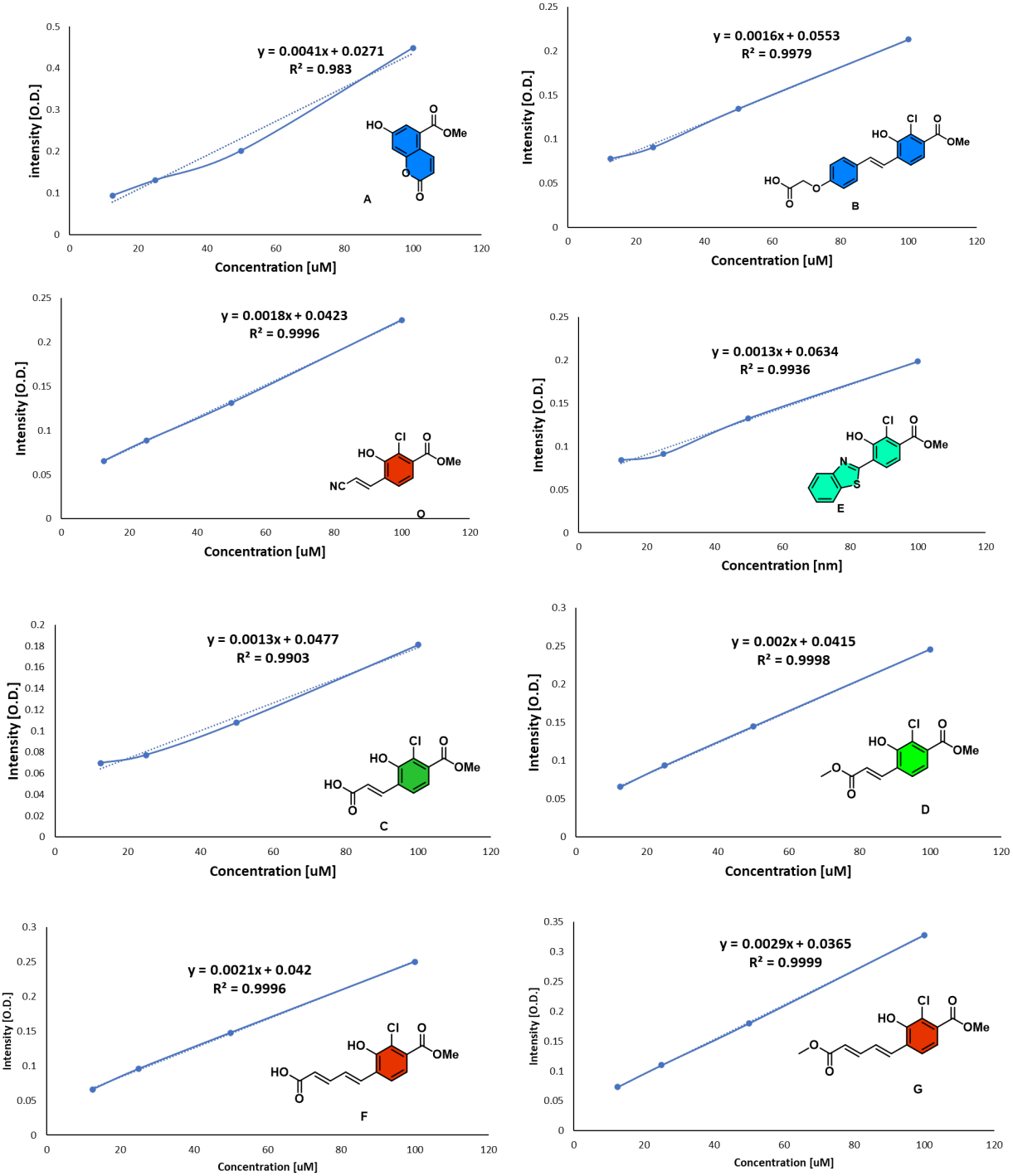
Detection of enzymes activity in a live bacterial assay

All bacterial strains used in this study were purchased from the American Type Culture Collection (ATCC): *S. aureus* (ATCC 35556) and *S. mutans* (ATCC 35668), or clinical isolate: *S. pyogenes* (glossy) was provided by Prof. Doron Steinberg (The Hebrew University of Jerusalem). Starter cultures were grown overnight with shaking at 37°C with shaking in a brain heart infusion (BHI) medium. Then cells were diluted 1:100 with fresh BHI medium and were grown for 2 hours with shaking at 37°C. Cultures were then centrifuged, washed with PBS buffer, and resuspended in PBS buffer (pH 7.4) to obtain OD600 of 0.75. 10µL sample of the tested probe in DMSO was added to a 90µL aliquot of bacterial cell sample to obtain a final concentration of 100µM (10% DMSO). Next, the total light emission and the chemiluminescence emission spectra of DPLX-1 and DPLX-2 were measured at 37°C.

Supplementary Figures

Measurements of extinction coefficient for Benzoates A-O

The extinction coefficients of all benzoates described in this paper were calculated from the correlation between intensity [O.D.] and concentration.



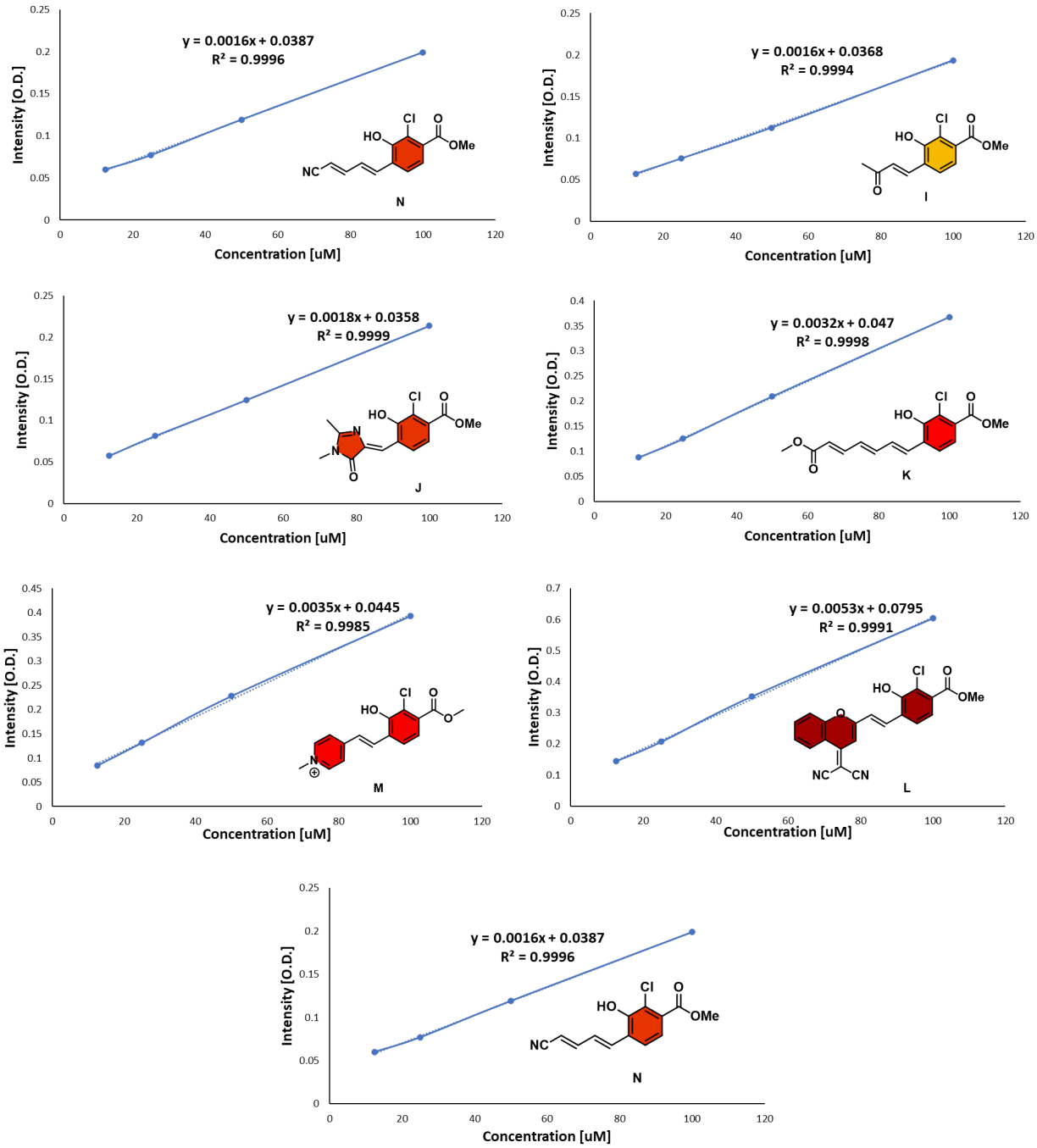


Figure S1. Absorbance [O.D.] versus concentration curve of **Benzoates A-O** [100, 50, 25, 12.5 uM], 10% DMSO in PBS 7.4 at 25°C.

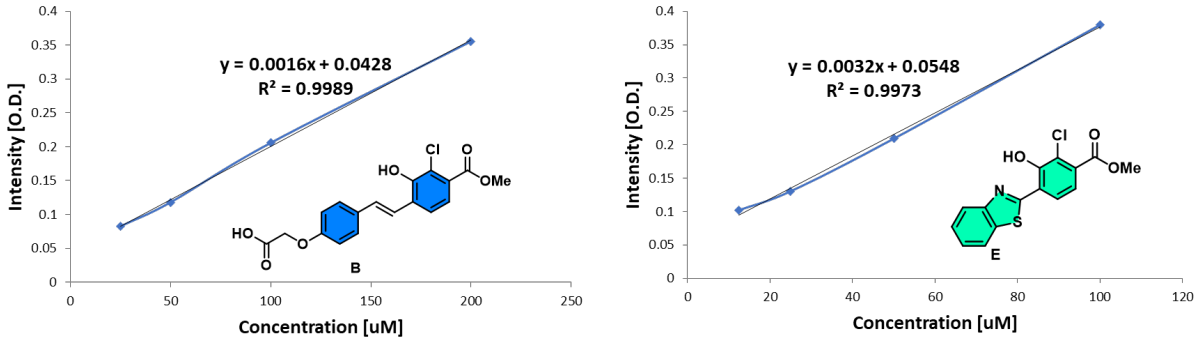
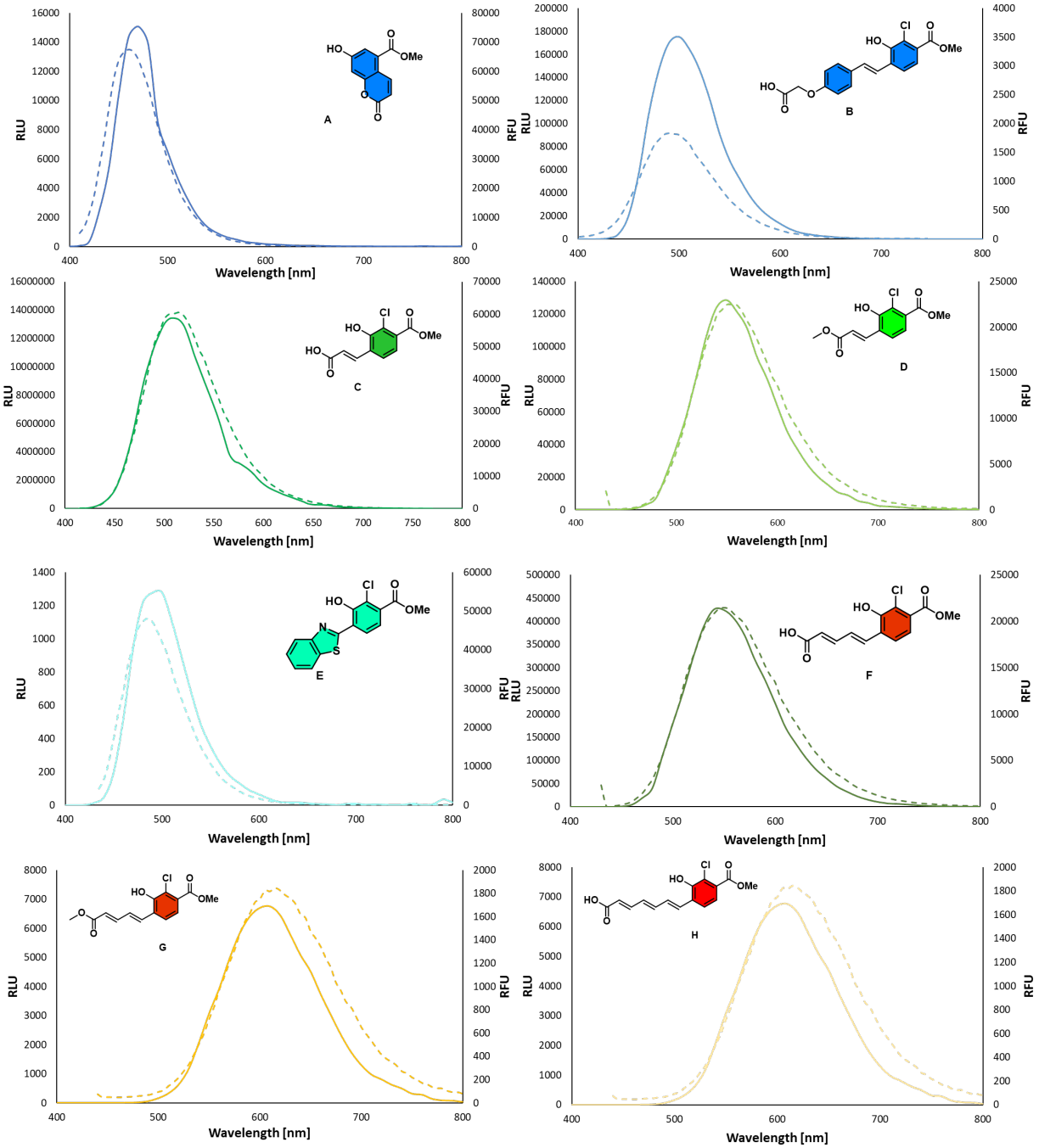


Figure S2. Absorbance versus concentration curve of **Benzoates B and E** [200, 100, 50, 25 uM] and [100, 50, 25, 12.5 uM] respectively, 10% DMSO in carbonate buffer pH 11 at 25°C.

Comparison of chemiluminescence and fluorescence emission spectra of benzoates A-O

Expectedly, the chemiluminescent and fluorescent emission spectra of all benzoates described in this paper were similar.



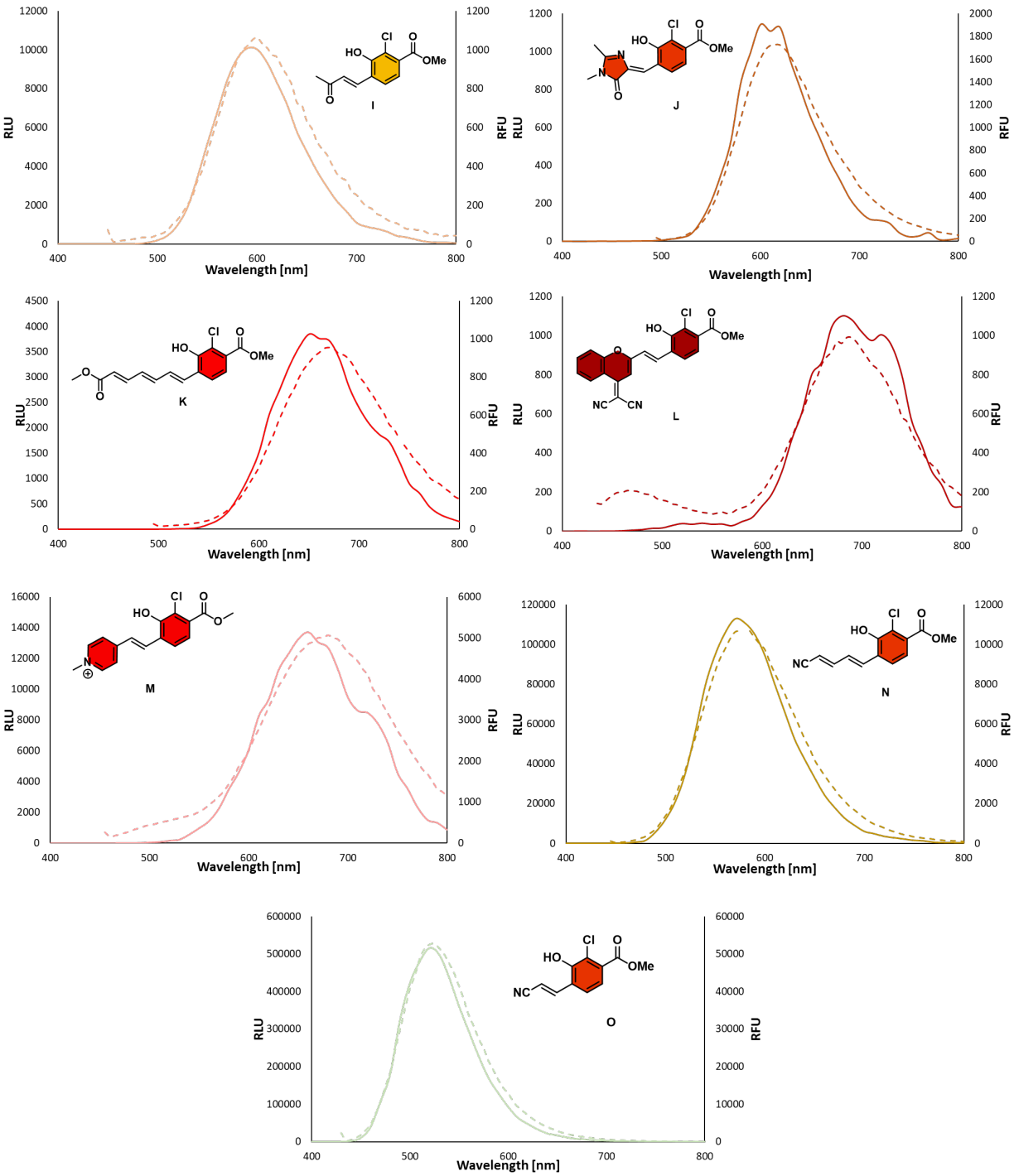
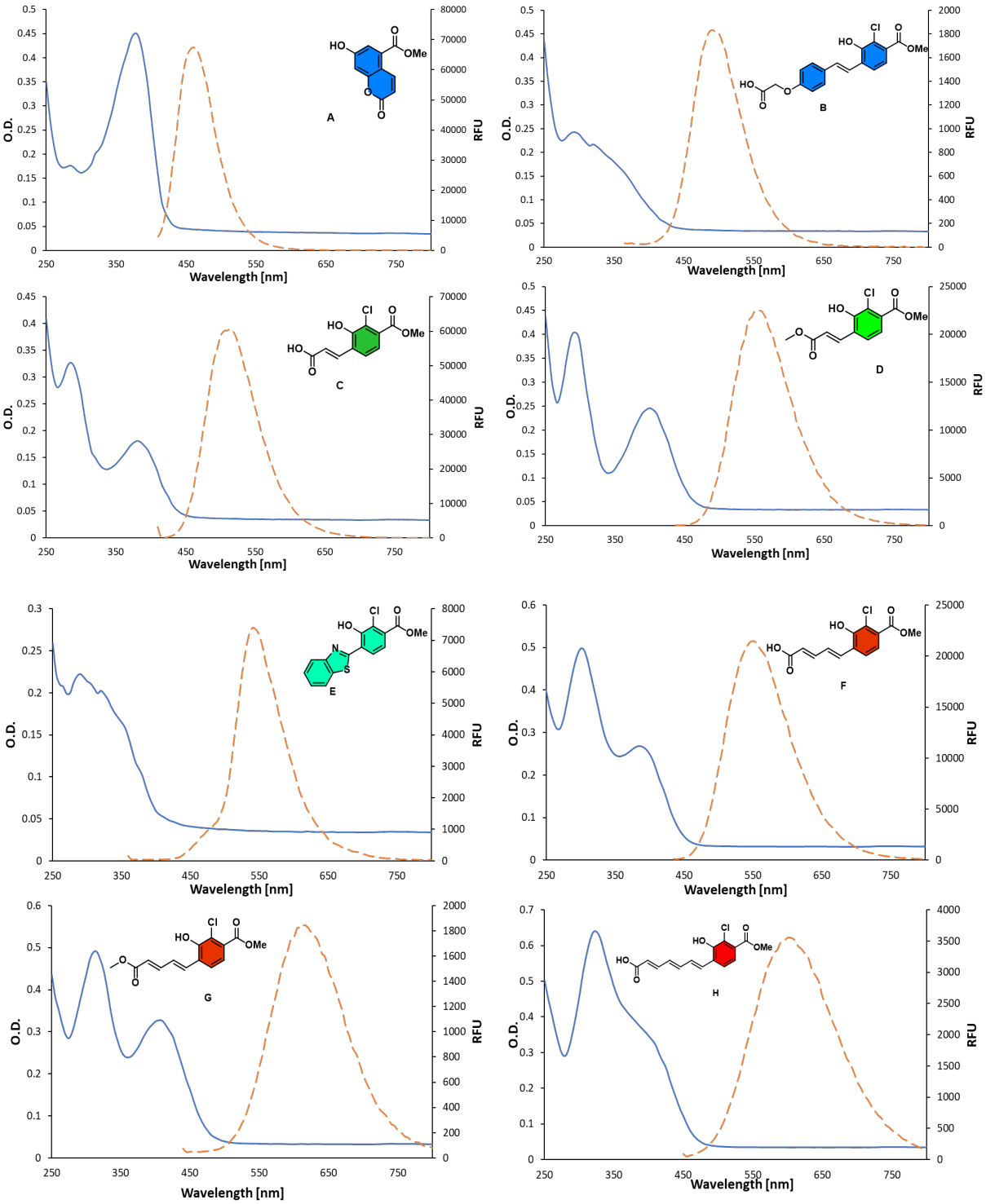


Figure S3. Fluorescence emission spectra (dashed line) and chemiluminescent emission spectra (solid line) of **Benzoates A-O** [100 uM] and **Dioxetanes A-O** [10 uM], 10% DMSO in PBS pH 7.4 at 25°C.


A
B
C
D
E
F
G
H
O.D.
RFU
Wavelength [nm]

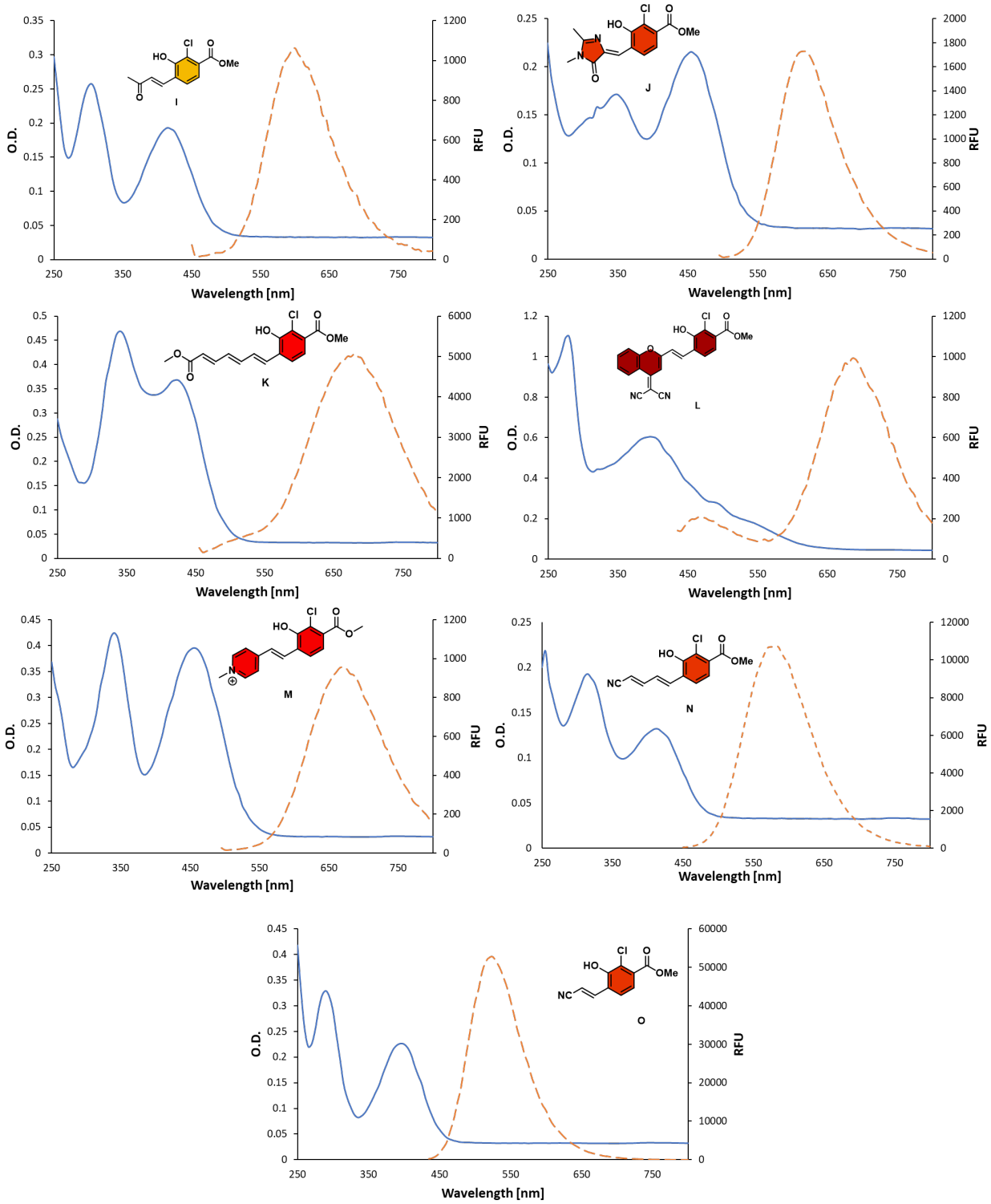


Figure S4. Absorbance (solid line) and fluorescence emission spectra (dashed line) of **Benzoates A-O** [100 uM], 10% DMSO in PBS pH 7.4 at 25°C.

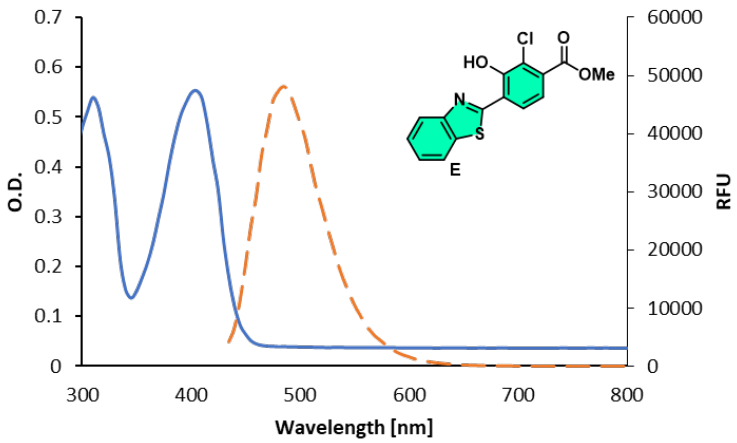
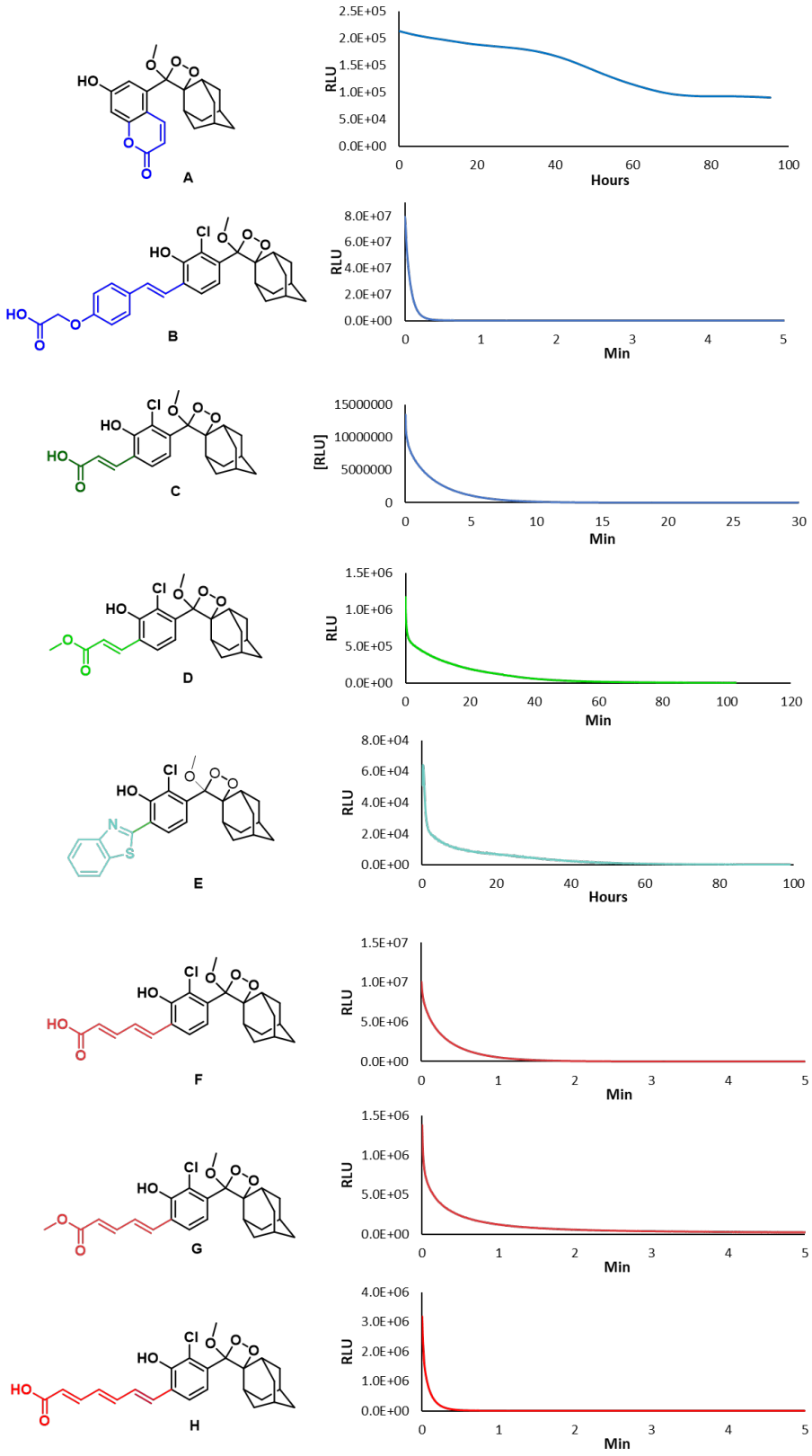


Figure S5. Absorbance (solid line) and fluorescence emission spectra (dashed line) of **Benzoate E** [100 uM], 10% DMSO in carbonate buffer pH 11 at 25°C.

Chemiluminescence kinetic profiles of Dioxetanes A-O



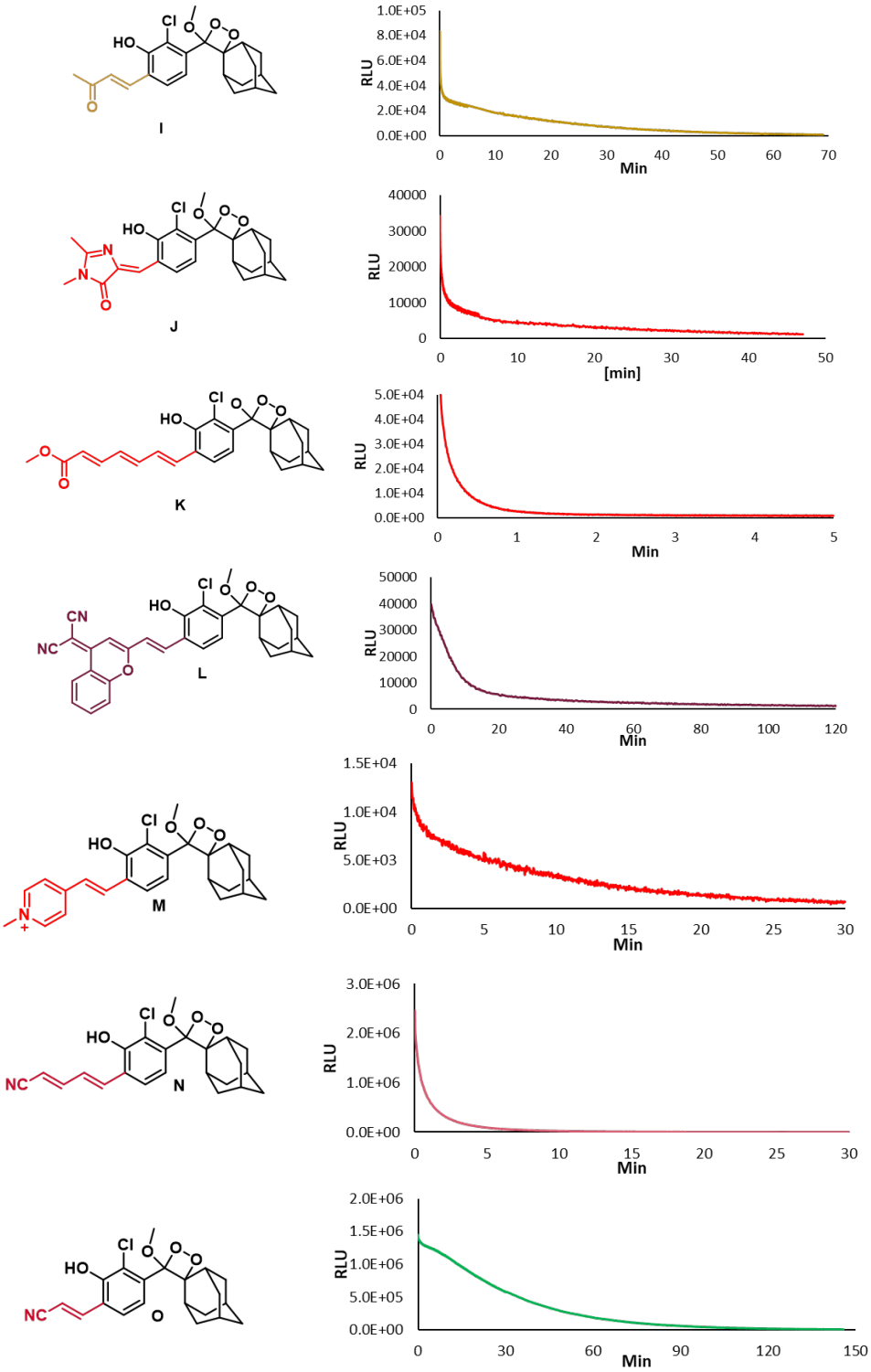


Figure S6. Chemiluminescence kinetic profiles of **Dioxetanes A-O** [1 uM], 10% DMSO in PBS pH 7.4 at 25°C.

Dioxetane E chemiluminescence kinetic profile in different pH values

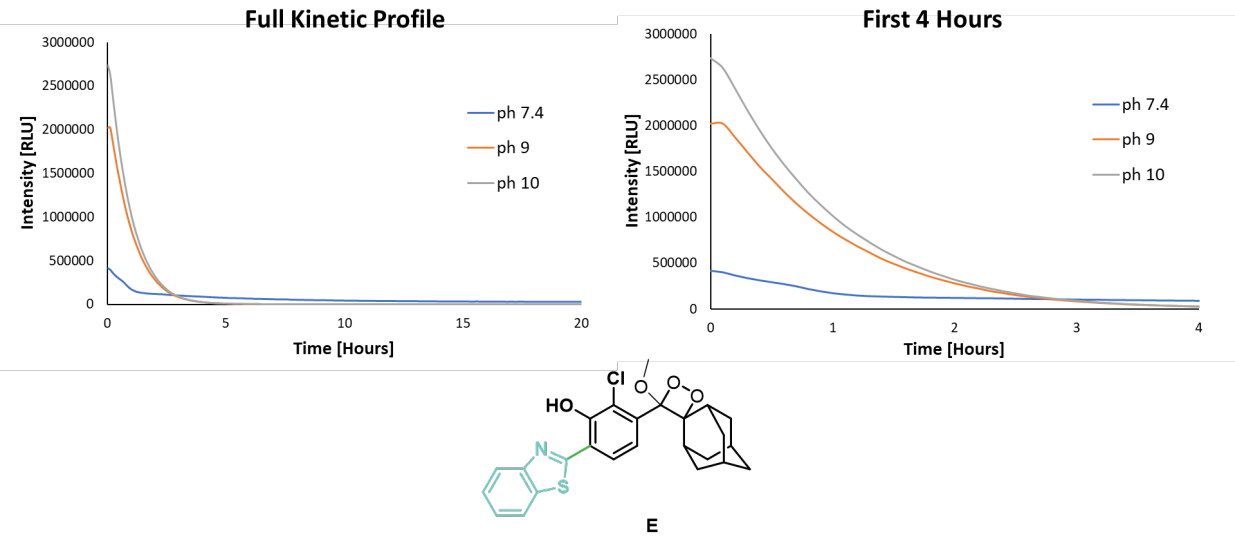


Figure S7. Chemiluminescence kinetic profile of **Dioxetane E** [1 uM], 10% DMSO in PBS 7.4, carbonate buffer pH 9, and pH 10 at 25°C.

Stability check of Dioxetanes A-O stock solutions

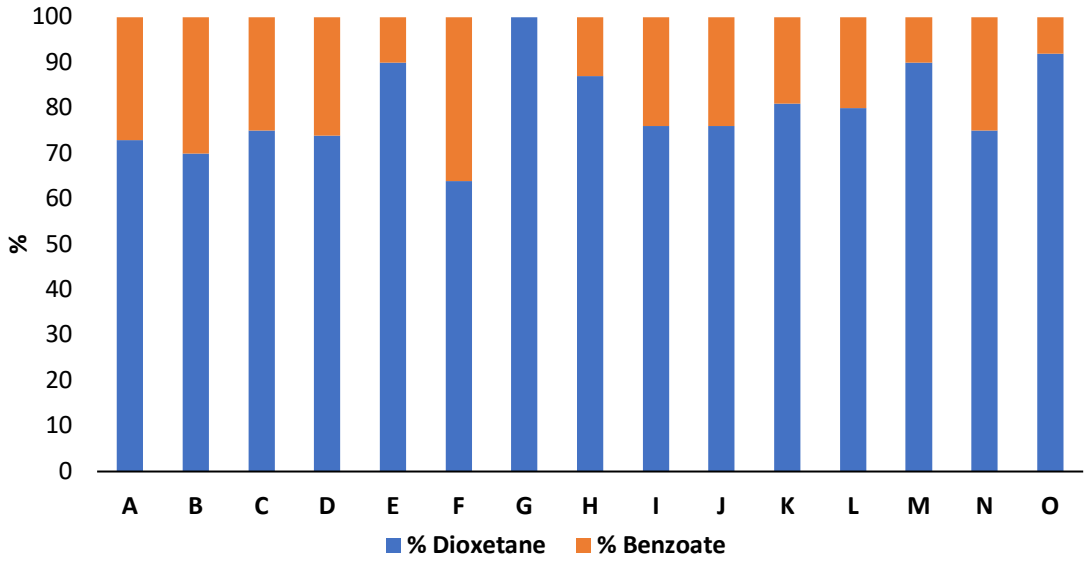
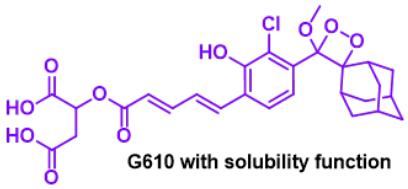


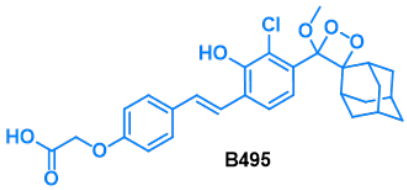
Figure S8. Stability of **Dioxetanes A-O** as stock solutions [10 mM] in DMSO. The solutions were stored at -20°C for a minimum period of one year and their stability was assessed as the percentage of benzoate formed during that period by RP-HPLC. Orange represents the benzoate percentage, and blue represents the dioxetane percentage. Amongst the stock solutions most were found to have at least 70% dioxetane which translates to great shelf stability.

Chemiluminescence properties of Dioxetanes G610 (with solubility function) and B495

	λ_{max} [nm]	$t_{1/2}$ [sec]	Φ_{CL} [%]
G610*	610	12.7	0.54
B495	500	3.6	0.55



G610 with solubility function



B495

Table S9. Chemiluminescence spectral properties of **G610*** and **B495** [1 uM] as measured in PBS 7.4, 10% DMSO at 37°C. *G610 methyl ester moiety was replaced by malic acid solubility function.

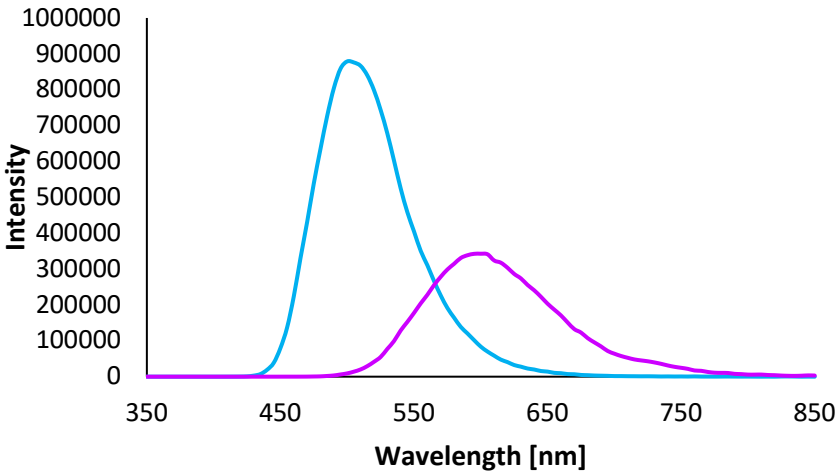


Figure S10. Chemiluminescence emission spectra of **G610*** and **B495** [1 uM], 10% DMSO in PBS pH 7.4 at 37°C.

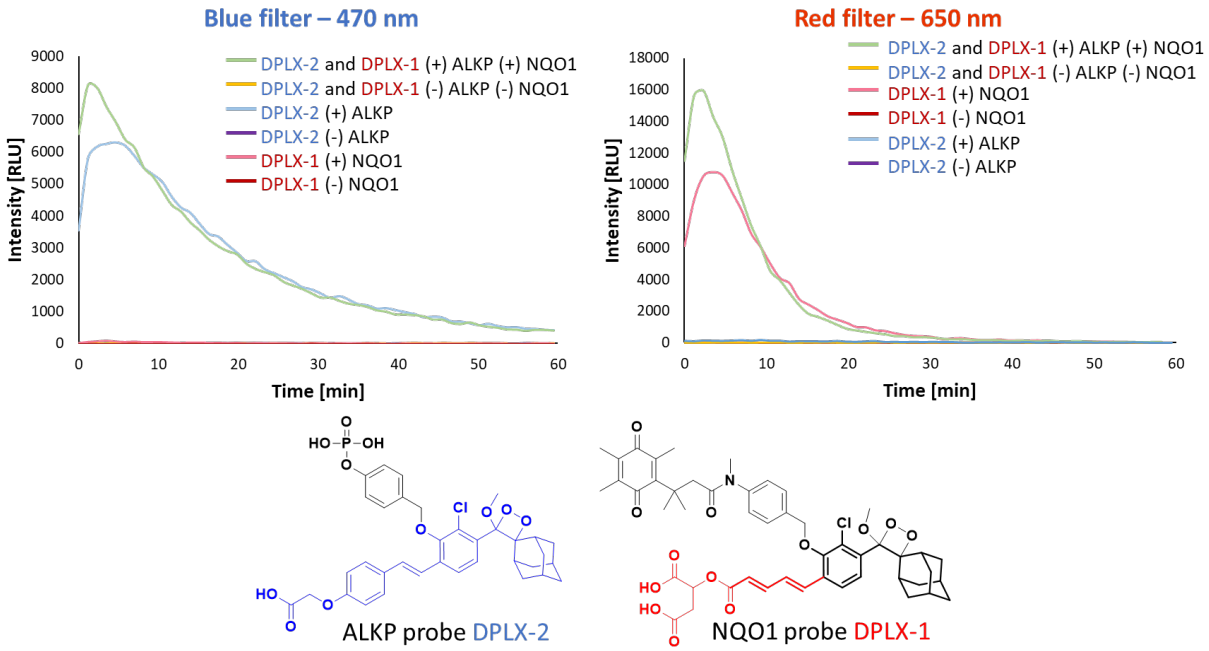


Figure S11. Chemiluminescence kinetic profile of DPLX-1 and DPLX-2 [10uM] with and without NQO1 [20 µgr/ml] and ALP [12.5 U/ml] in PBS 7.4, 10% DMSO at 25°C.The green curve represents the light captured when both probes were activated at the same time in the same well at 470 nm and 650 nm (slit 15 nm).

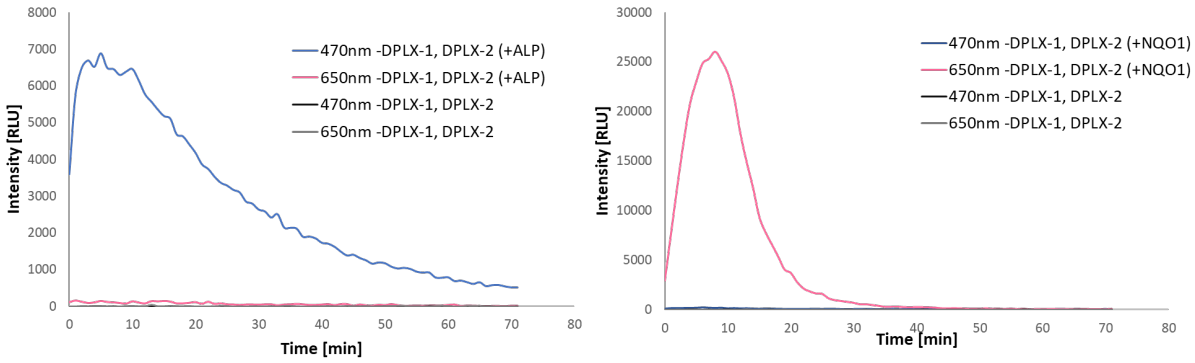


Figure S12. Control measurement of chemiluminescence kinetic profile of DPLX-1 and DPLX-2 [10uM] in the same well with and without NQO1 [20 µgr/ml] or ALP [12.5 U/ml] in PBS 7.4, 10% DMSO at 25°C.

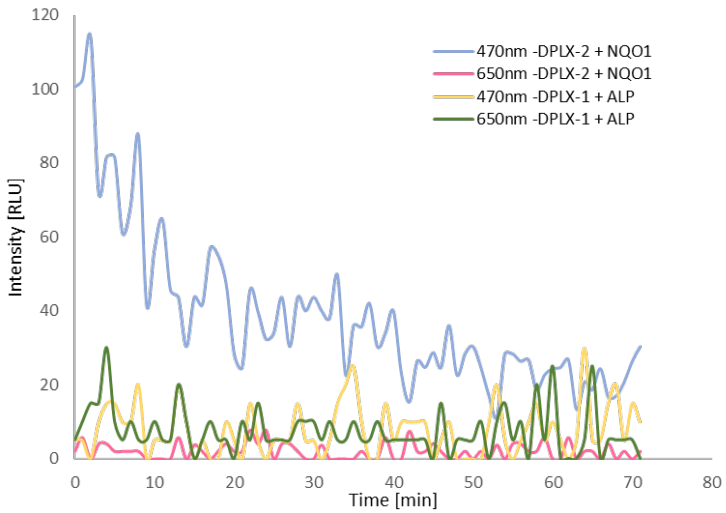


Figure S13. Control measurement of chemiluminescence kinetic profile of DPLX-1 activation with ALP [12.5 U/ml] and DPLX-2 [10uM] activation with NQO1 [20 µgr/ml] in PBS 7.4, NADH [100uM], 10% DMSO at 25°C.

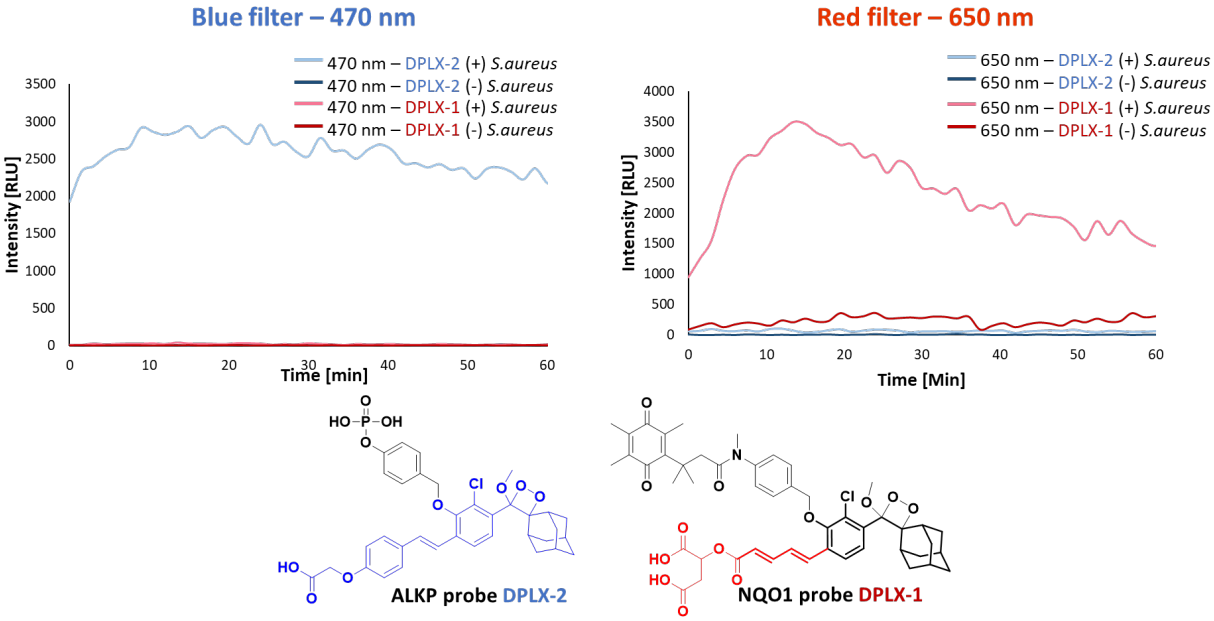


Figure S14. Chemiluminescence kinetic profiles of DPLX-1 and DPLX-2 [100uM] with and without *S.aureus* in PBS 7.4, 10% DMSO at 37°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm).

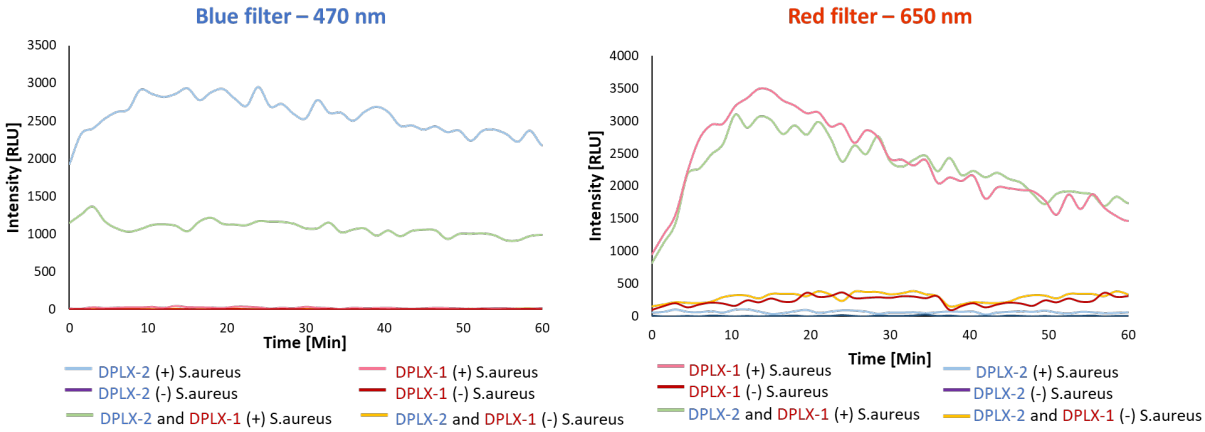


Figure S15. Chemiluminescence kinetic profile of DPLX-2 and DPLX-1 [100uM] with and without *S.aureus* in PBS 7.4, 10% DMSO at 37°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm). The green curve represents the light captured when both probes were activated simultaneously in the same well.

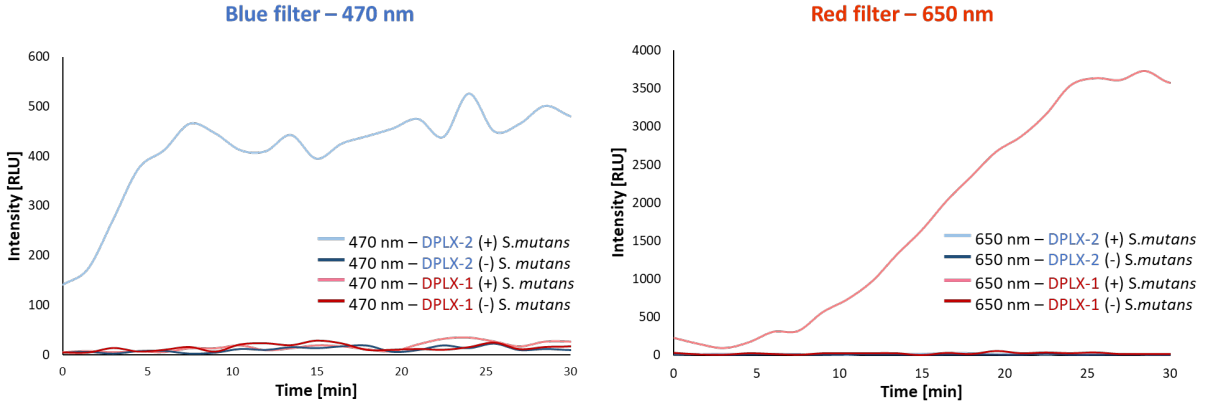


Figure S16. Chemiluminescence kinetic profiles of DPLX-1 and DPLX-2 [100uM] with and without *S. mutans* in PBS 7.4, 10% DMSO at 37°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm).

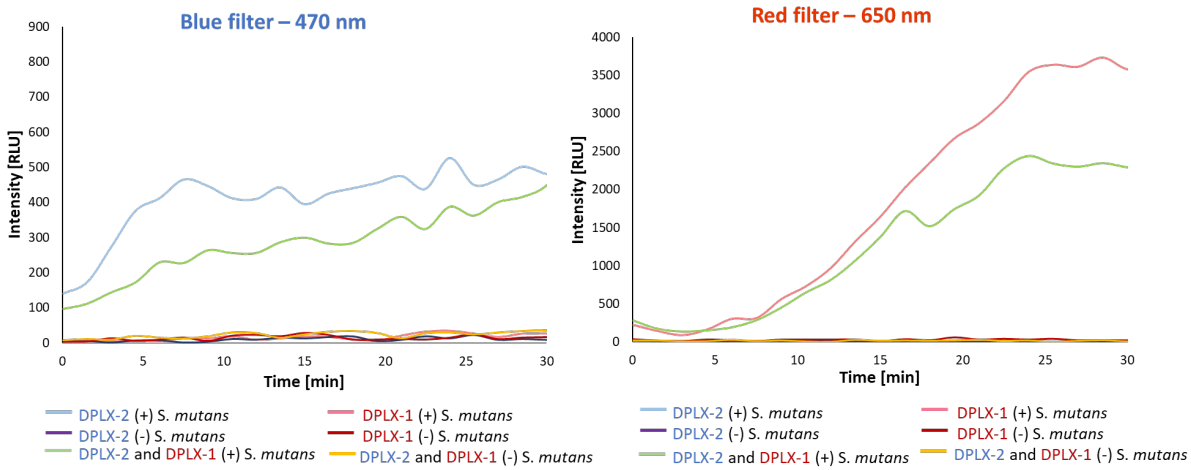


Figure S17. Chemiluminescence kinetic profile of DPLX-2 and DPLX-1 [100uM] with and without *S.mutans* in PBS 7.4, 10% DMSO at 37°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm). The green curve represents the light captured when both probes were activated simultaneously in the same well.

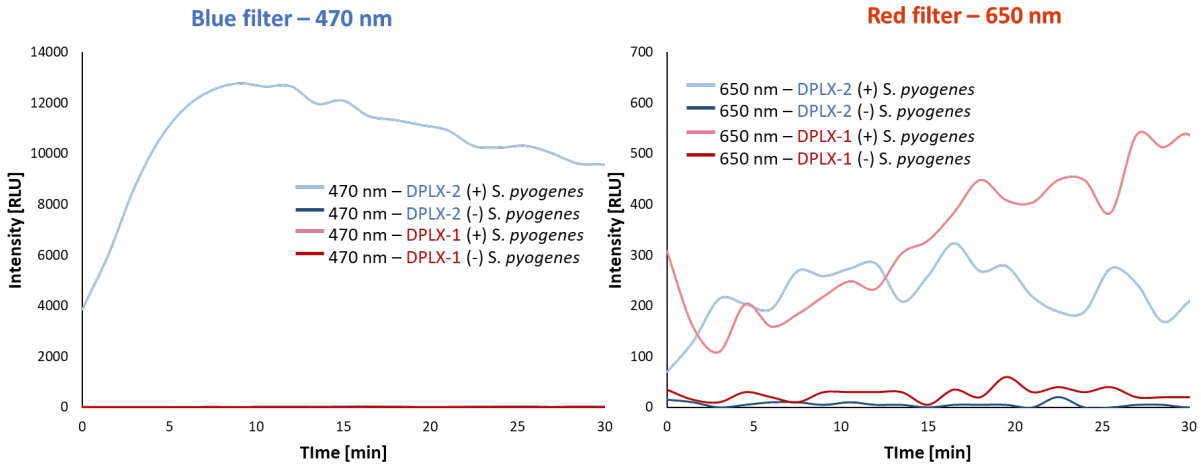


Figure S18. Chemiluminescence kinetic profiles of DPLX-1 and DPLX-2 [100uM] with and without *S. pyogenes* in PBS 7.4, 10% DMSO at 37°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm).

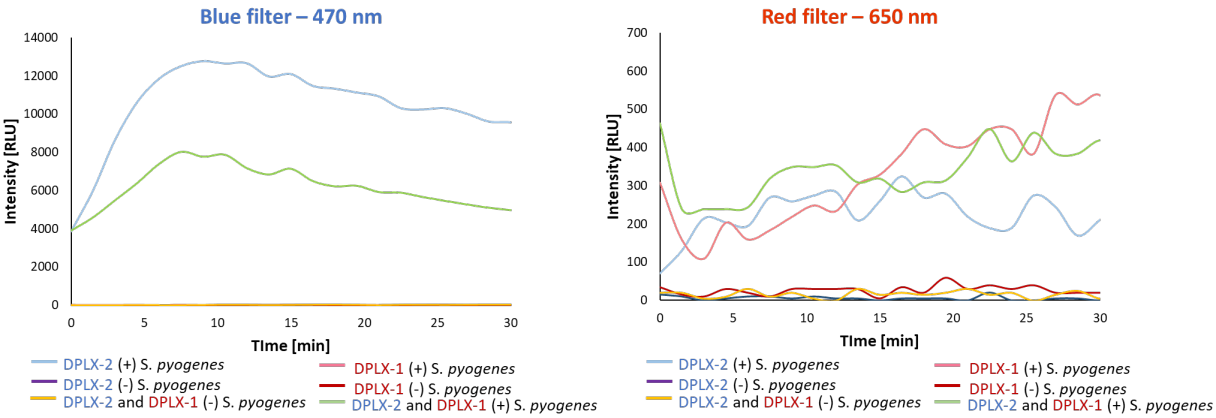


Figure S19. Chemiluminescence kinetic profile of DPLX-2 and DPLX-1 [100uM] with and without *S.pyogenes* in PBS 7.4, 10% DMSO at 37°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm). The green curve represents the light captured when both probes were activated simultaneously in the same well.

DPLX-1 and DPLX-2 inhibition assays with enzymes and bacteria

the alkaline phosphatase inhibitor cocktail according to our knowledge is a mixture of phosphate inhibitors directed towards Ser/Thr phosphatases, it contains, according to Sigma Aldrich, Calyculin A, Cantharidin, and (-)-p-Bromotetram- isole, in concentrations that are kept as a commercial secret. Please see Phosphatase Inhibitor Cocktail 3 (Cat. No. P0044) on the website:
https://www.sigmaaldrich.com/IL/en/technical-documents/technical-article/protein-biology/protein-lysis-and-extraction/phosphatase-inhibitor.

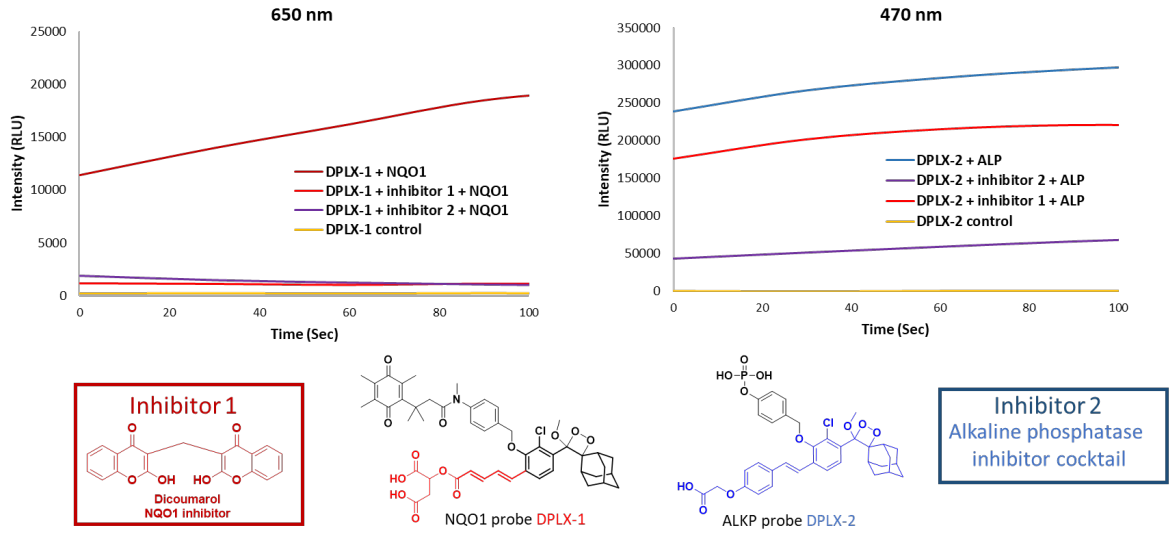


Figure S20. Chemiluminescence kinetic profile of DPLX-1 and DPLX-2 [1 uM] with and without NQO1 [10 µgr/ml] and ALP [1 U/ml]. Followed by 10 minutes of incubation of enzymes with dicoumarol (5 uM) and ALP inhibitor cocktail (1uL), the chemiluminescent probes were added

and the signal was measured during 5 minutes in PBS 7.4, 1% DMSO at 25°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm).

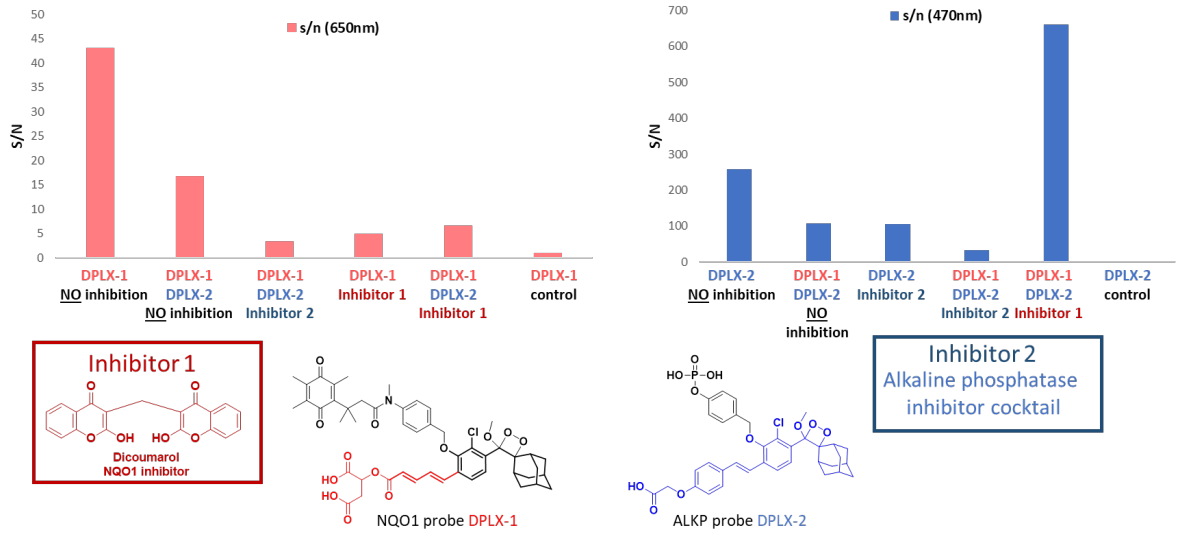


Figure S21. Chemiluminescence signal-to-noise ratio of DPLX-1 and DPLX-2 [100uM] incubated with *S. aureus*. Following 30 minutes of incubation of bacteria with or without dicoumarol (500uM) and ALP inhibitor (1uL), the chemiluminescent probes were added and the chemiluminescence signal was measured during 60 minutes at 37°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm).

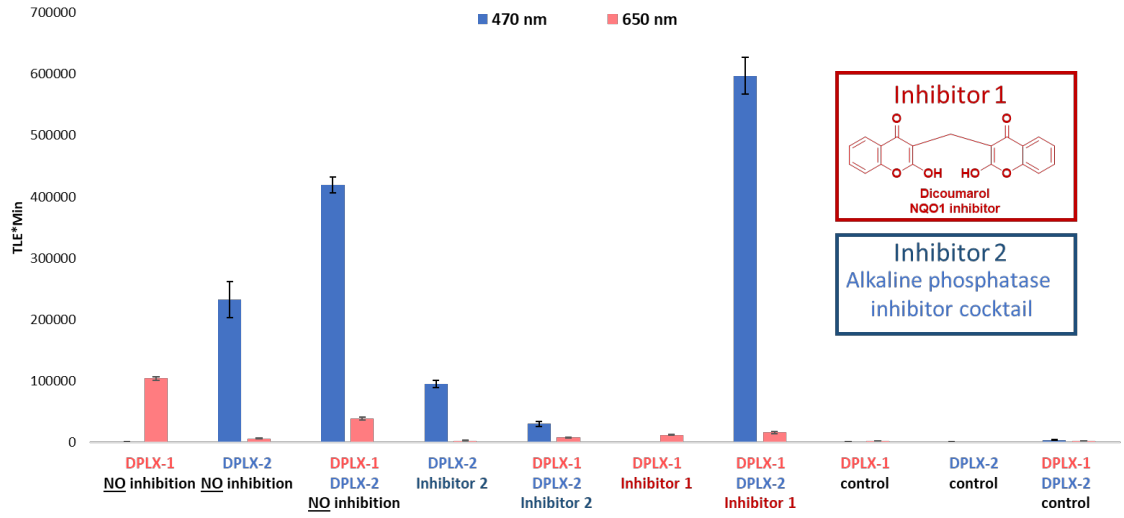


Figure S22. Chemiluminescence total light emission of DPLX-1 and DPLX-2 [100uM] incubated with S. aureus. Following 30 minutes of incubation of bacteria with or without dicoumarol (500uM) and ALP inhibitor (1uL), the chemiluminescent probes were added and the

chemiluminescence signal was measured during 60 minutes at 37°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm).

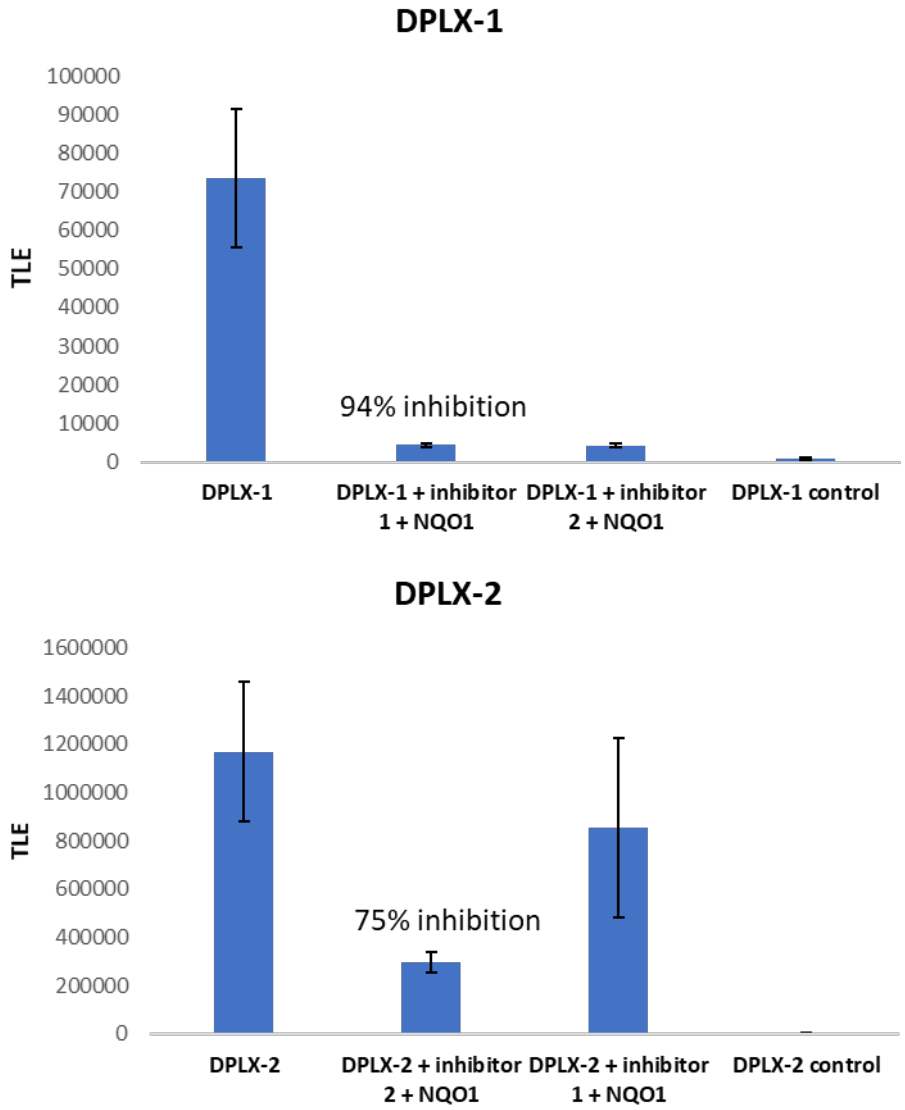
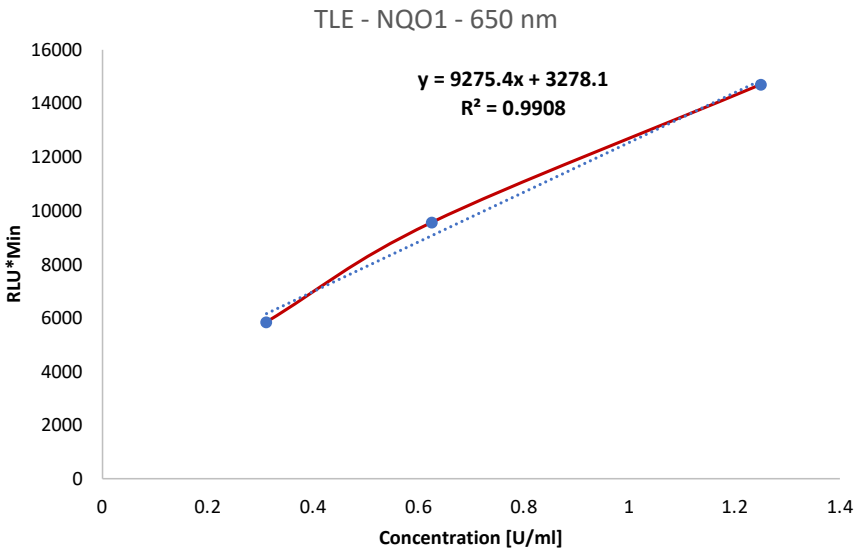
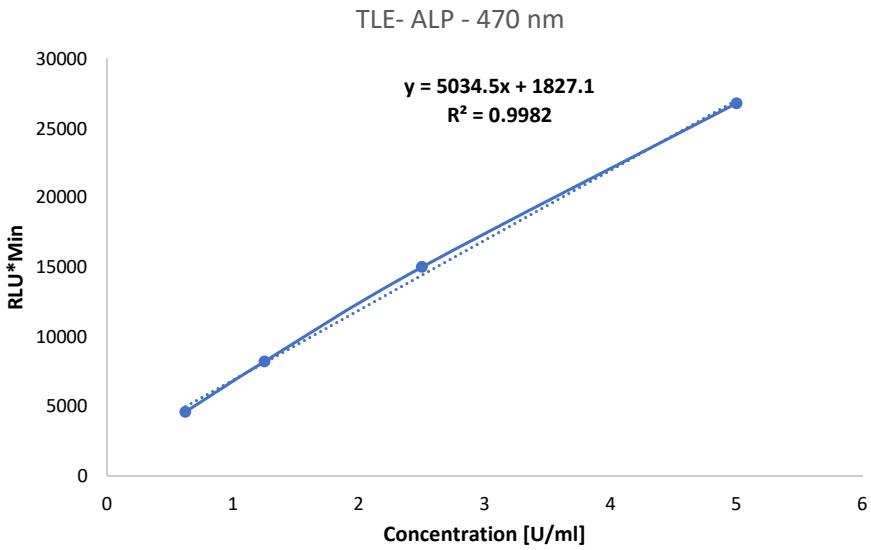


Figure S23. Total light emission of DPLX-1 and DPLX-2 [1 uM] with and without NQO1 [10 µgr/ml] and ALP [1 U/ml]. Followed by 10 minutes of incubation of enzymes with dicoumarol (5 uM) and ALP inhibitor cocktail (1uL), the chemiluminescent probes were added and the signal was measured during 5 minutes in PBS 7.4, 1% DMSO at 25°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm).



ALP	a	b	y (bacteria)	x
eq	5034.5	1827.1	10484.88333	1.71969
	ALP conc	1.72 U/ml		



NQO1	a	b	y (bacteria)	x
eq	9275.4	3278.1	16825.74	2.303268
	NQO1 conc	2.3 Ugr/ml		

Figure S24. Chemiluminescence total light emission versus different enzyme concentrations. From the calibration curve, we were able to calculate the estimated enzyme concentration in the bacterial measurements in *S. aureus*. NQO1 [1.25, 0.625, 0.312 ugr/ml] (upper) and ALP [5, 2.5, 1.25, 0.625 U/ml] (lower) enzyme concentrations measured with DPLX-1 and DPLX-2 [10uM], 10% DMSO in PBS 7.4. Chemiluminescence was measured after 8 minutes at room temperature. The measurements were conducted at 470 nm and 650 nm (slit 15 nm).
The estimated enzyme concentrations are shown in the table.

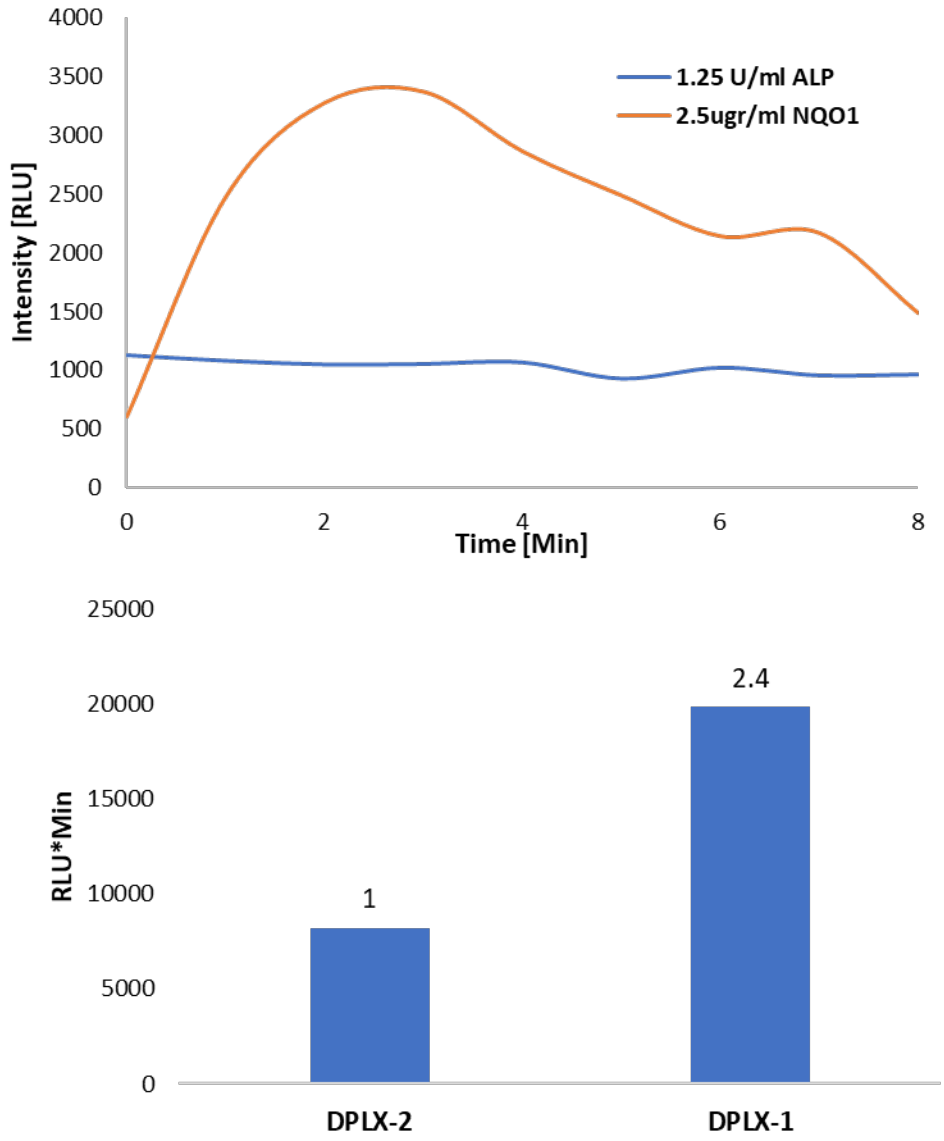


Figure S25. Chemiluminescence kinetic profile (upper) and total light emission (lower) of DPLX-1 and DPLX-2 [10uM], 10% DMSO in PBS 7.4 with and without ALP [1.5 U/ml] and NQO1 [2.5 ugr/ml]. Chemiluminescence was measured after 8 minutes at room temperature. The measurements were conducted at 470 nm and 650 nm (slit 15 nm).
This measurement correlates with the results obtained with S. aureus in Figure 5A.

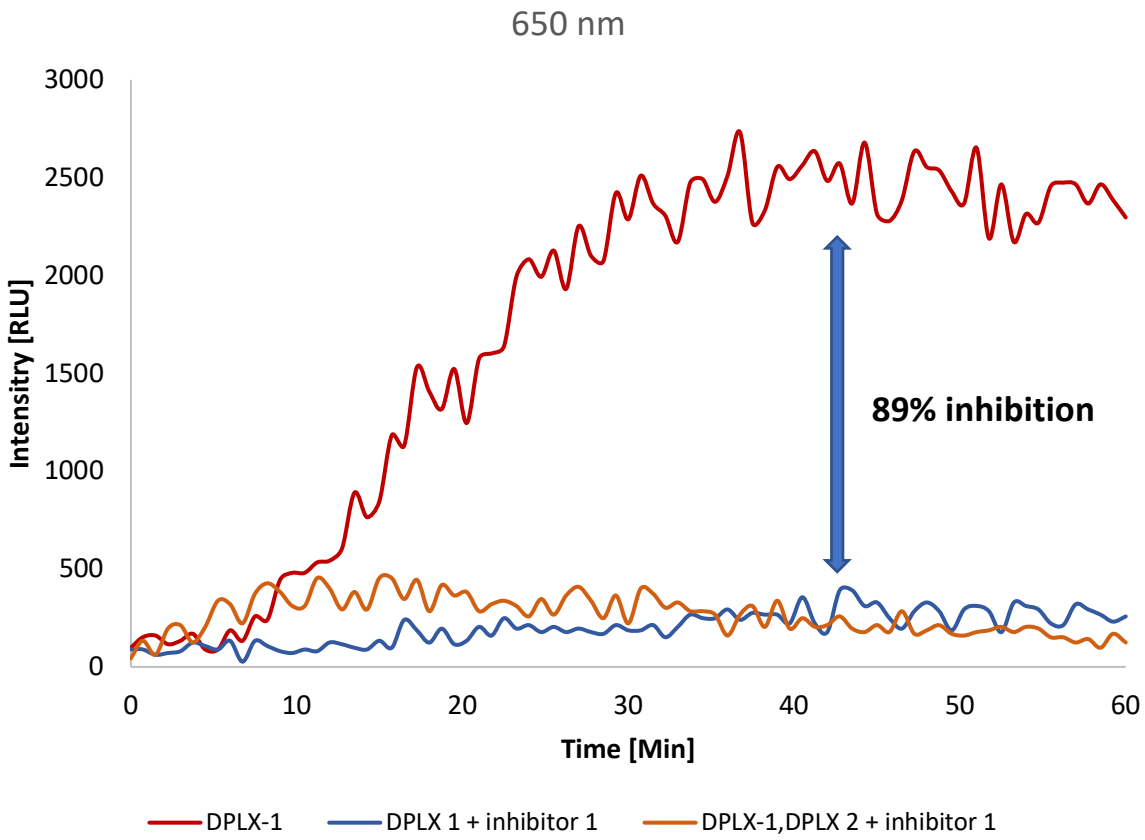


Figure S26. Chemiluminescence kinetic profile of DPLX-1 [100uM] incubated with S. aureus. Following 30 minutes incubation with or without dicoumarol and ALKP inhibitor, chemiluminescence was measured after 60 minutes at 37°C. The measurements were conducted at 470 nm and 650 nm (slit 15 nm).

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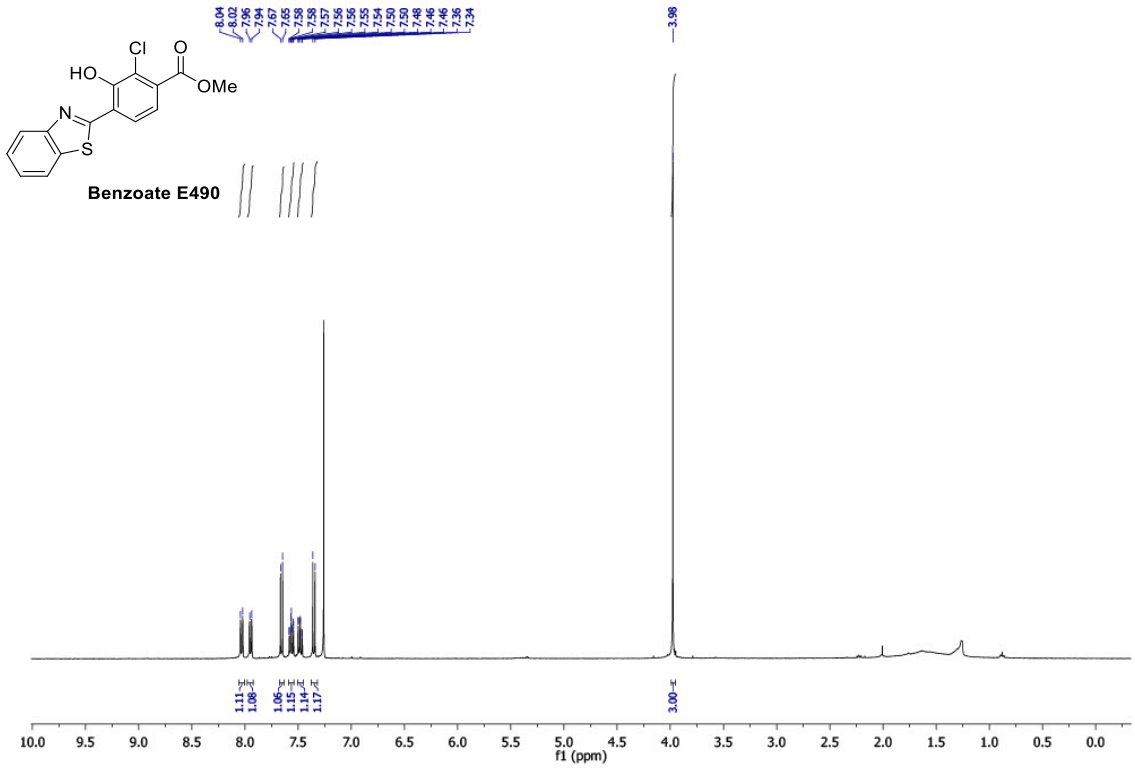
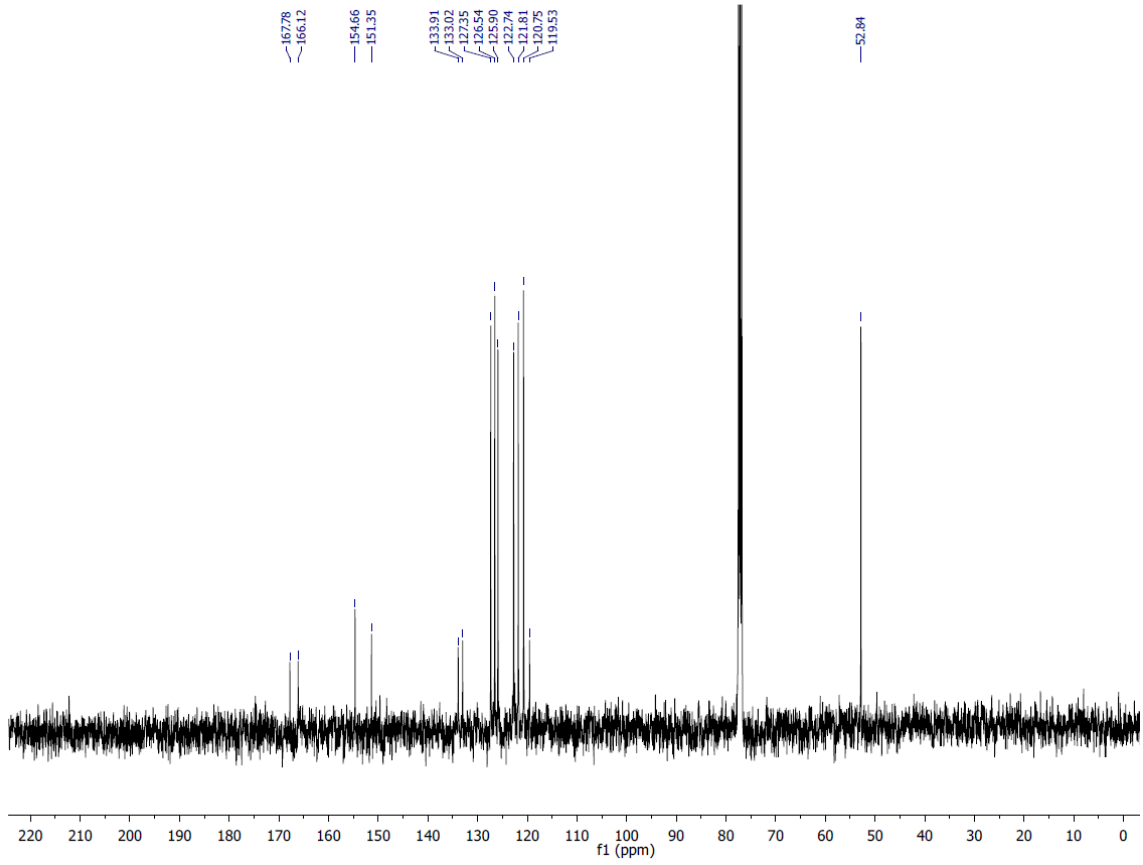
NMR spectra

^{1}H-NMR of Benzoate E490



^{13}C-NMR of Benzoate E490



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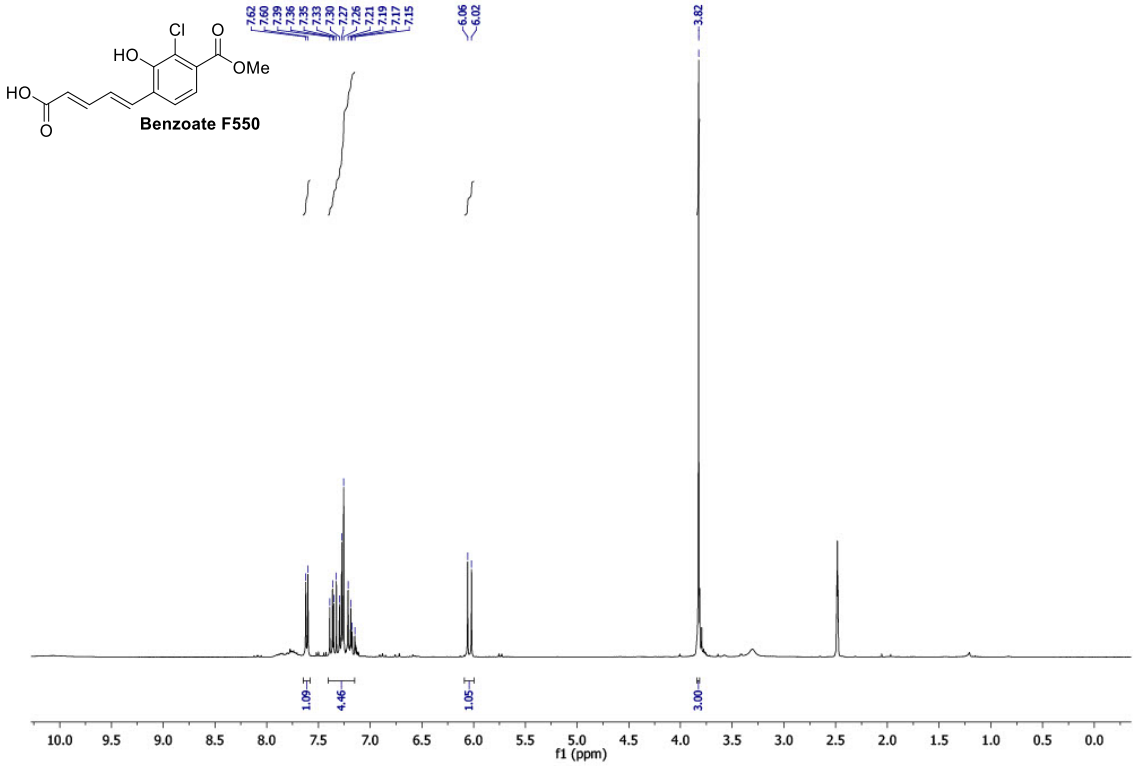

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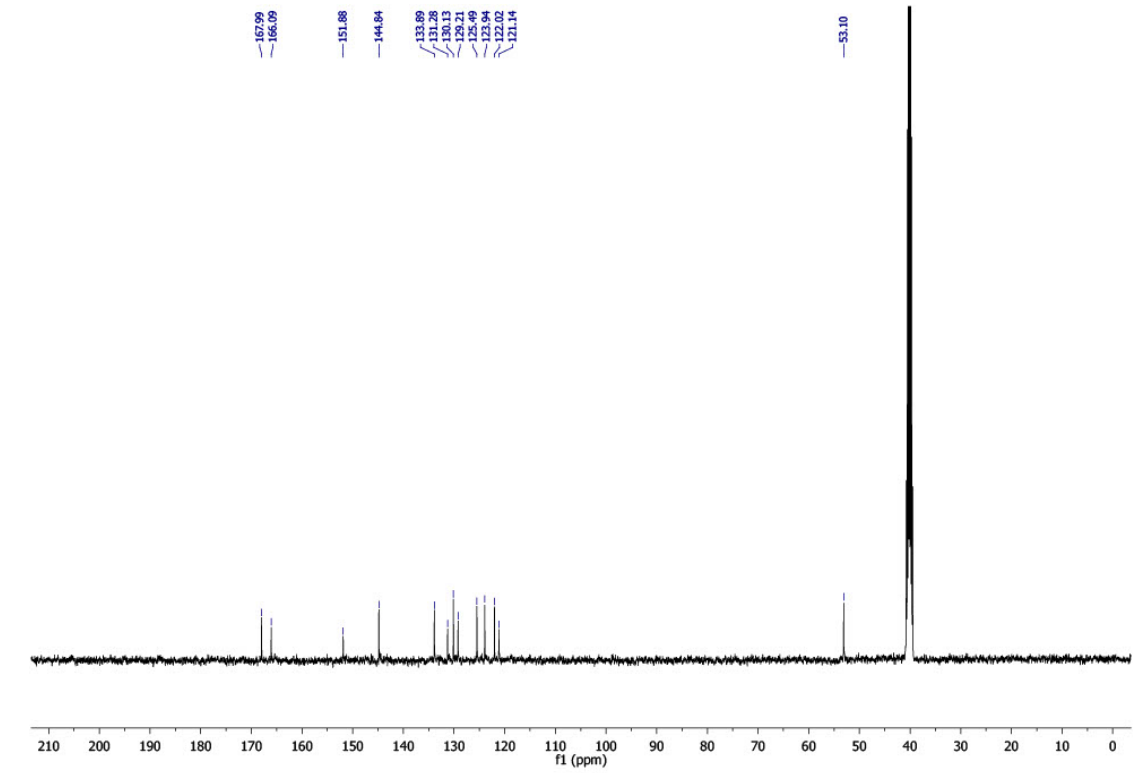

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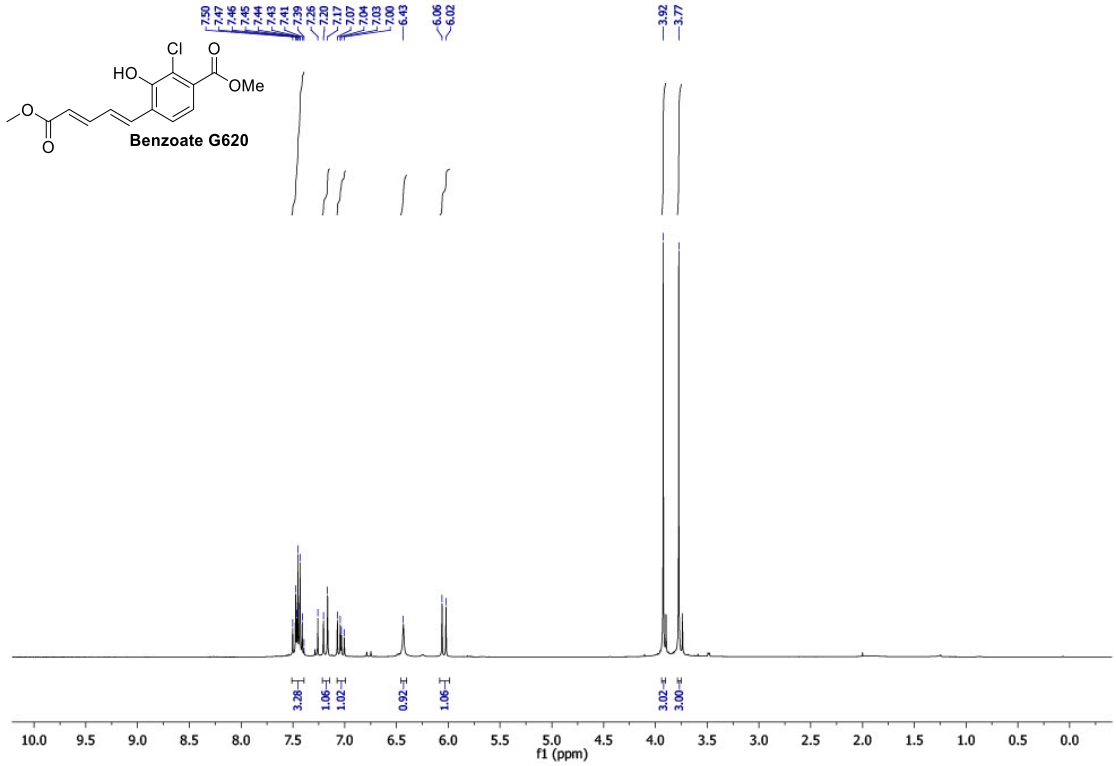

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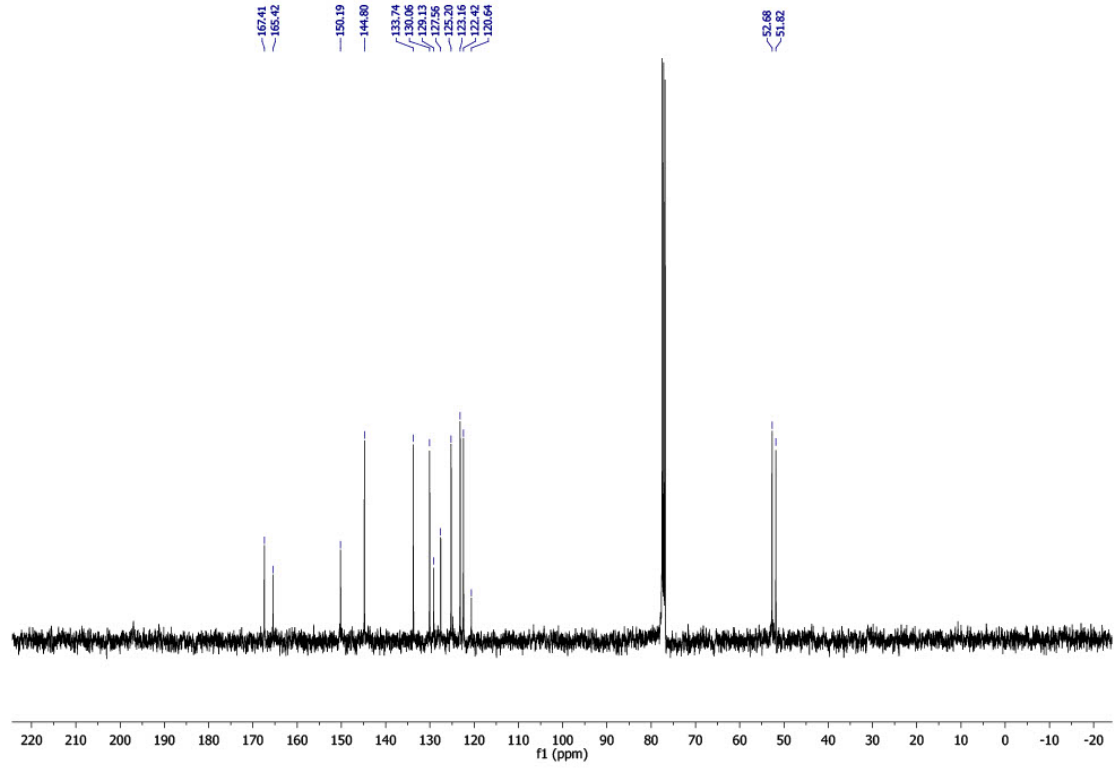

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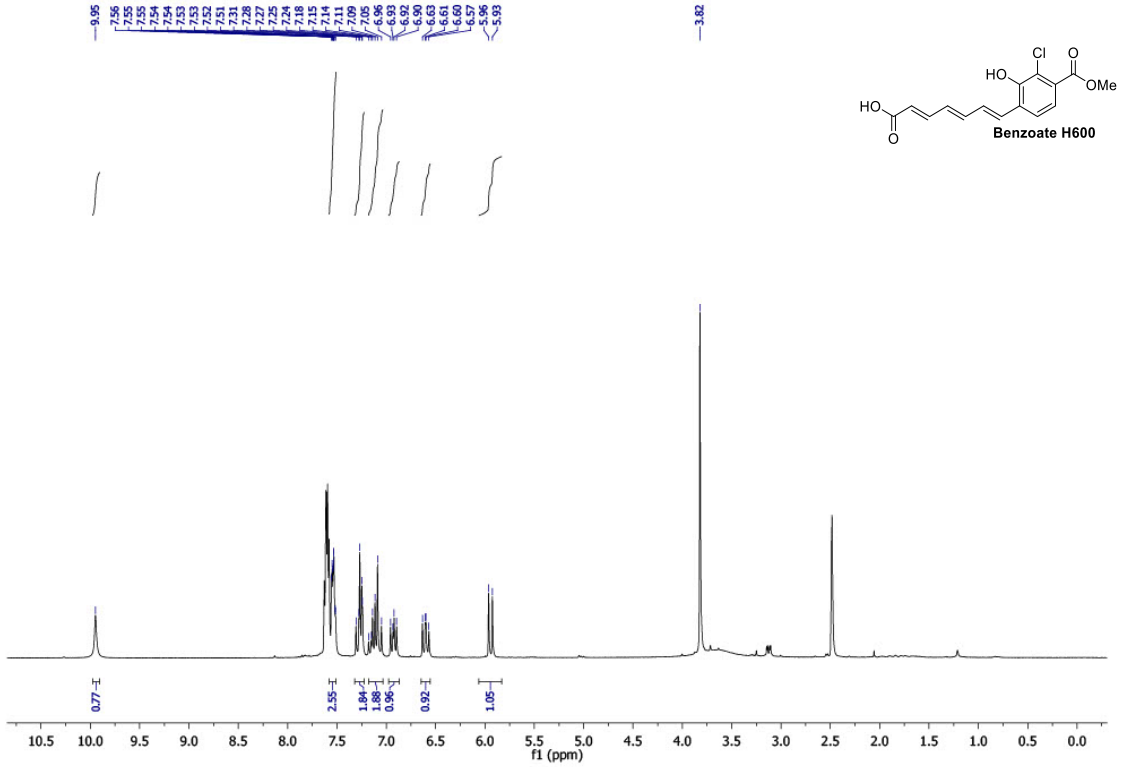

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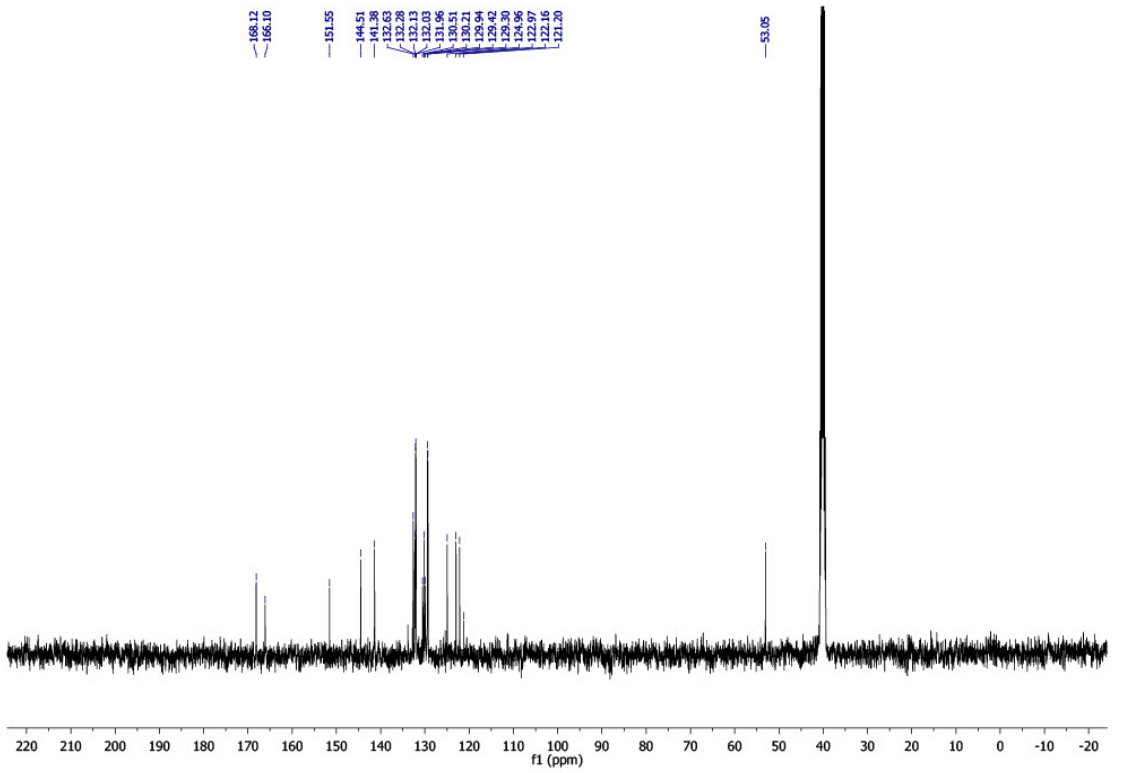

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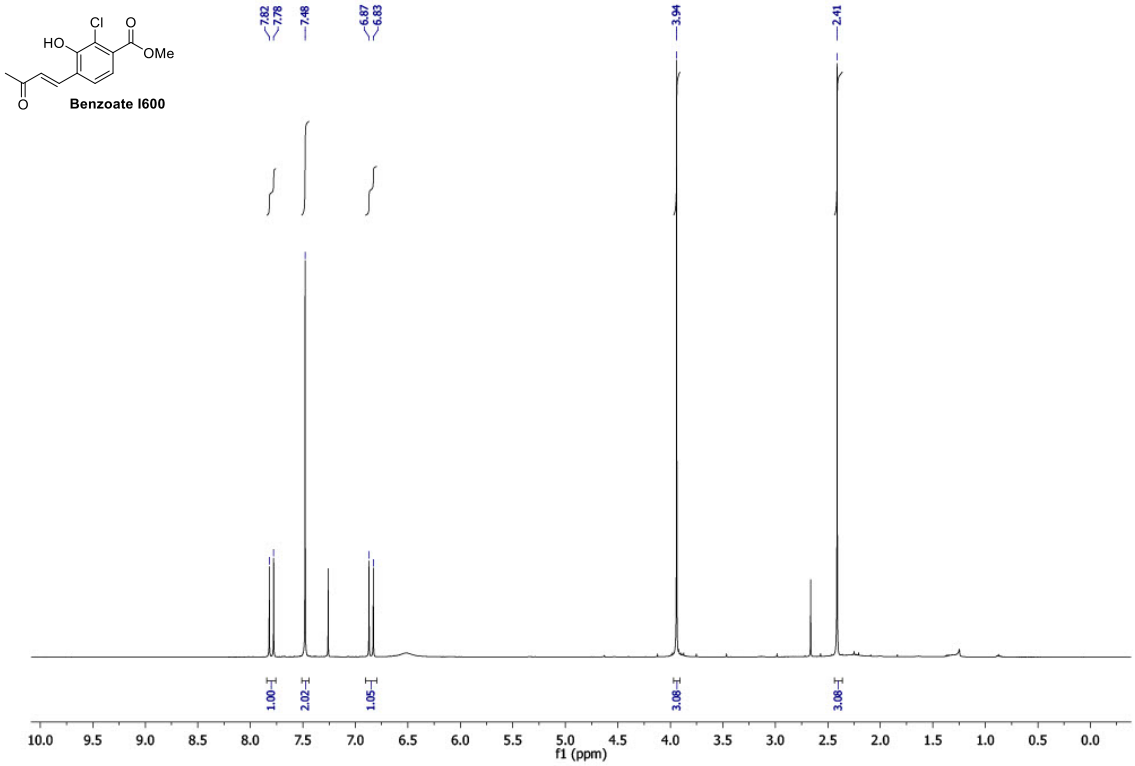

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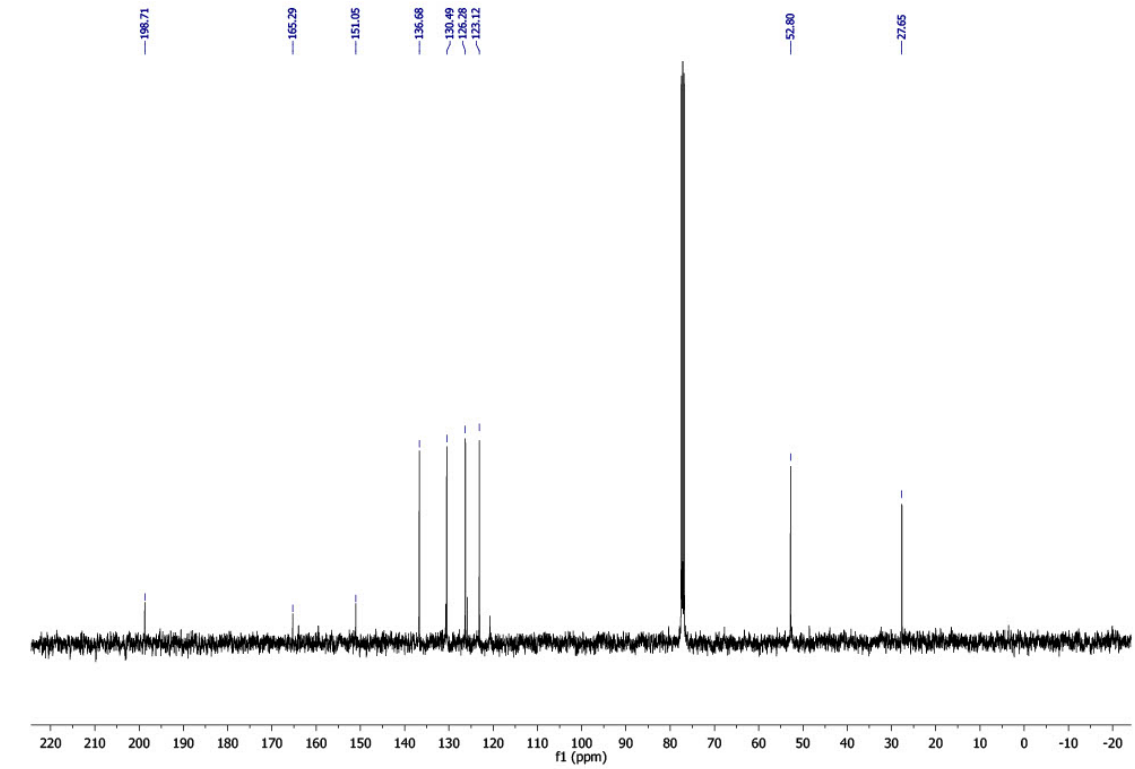
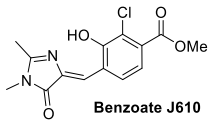
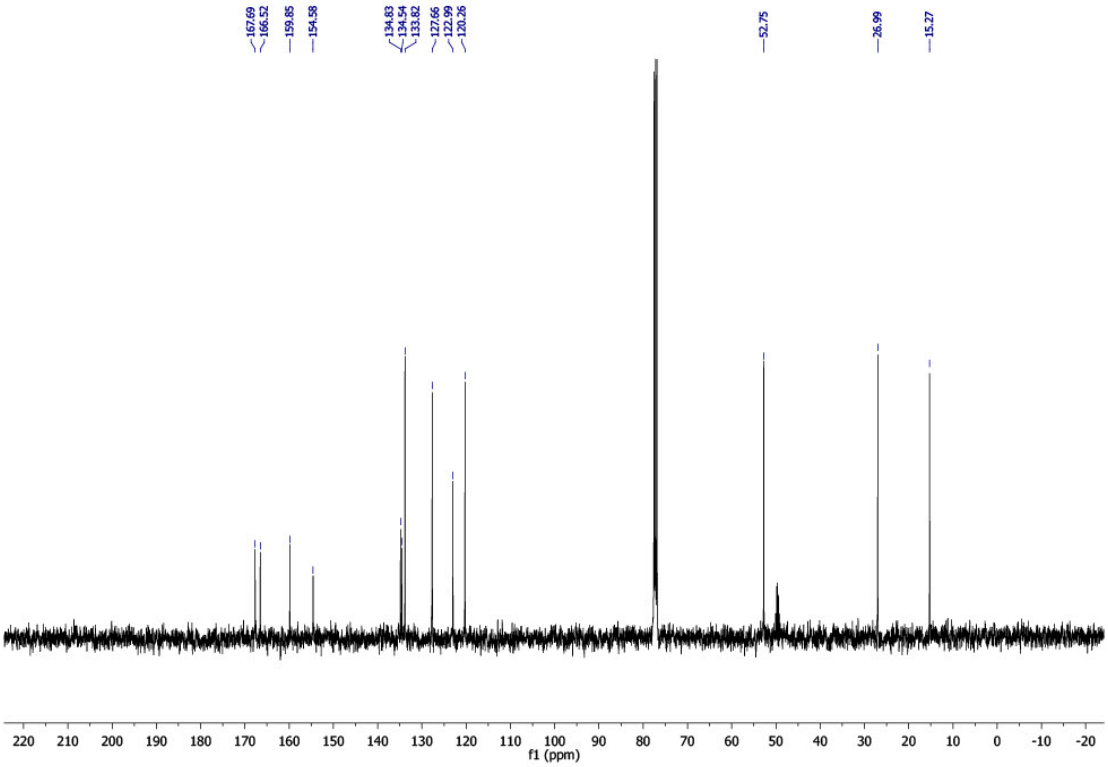


[1]H-NMR of Benzoate J610



Benzoate J610

[13]C-NMR of Benzoate J610



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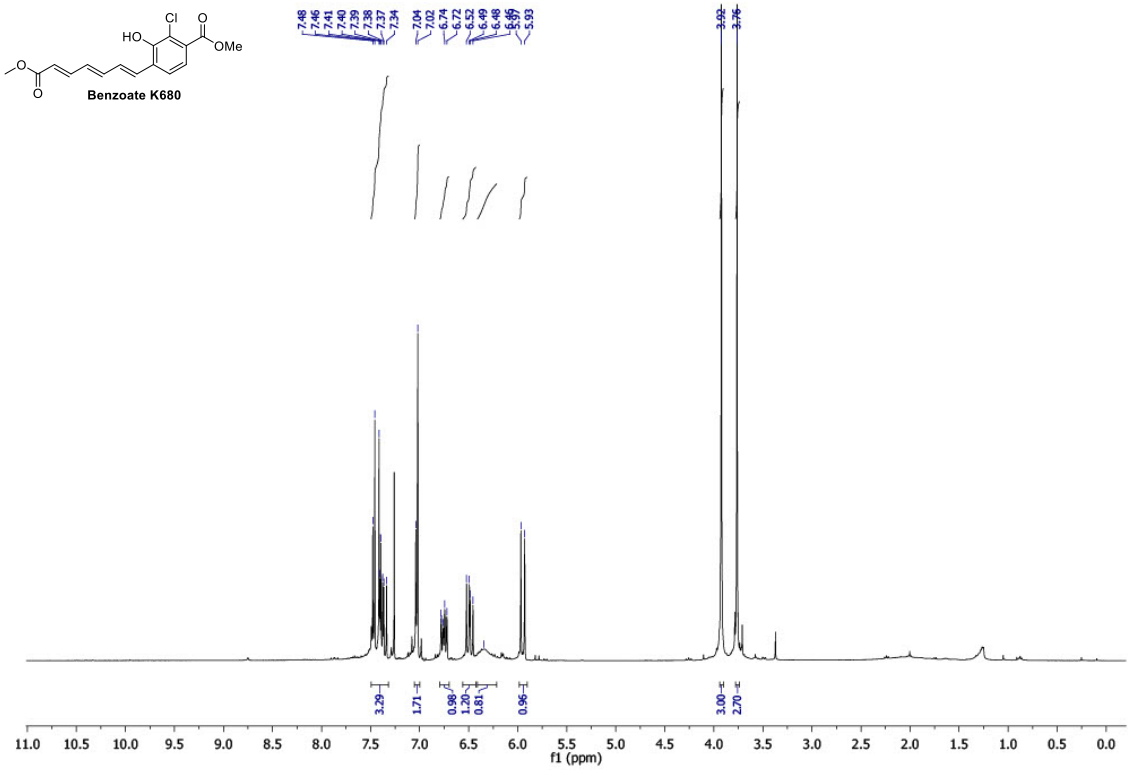

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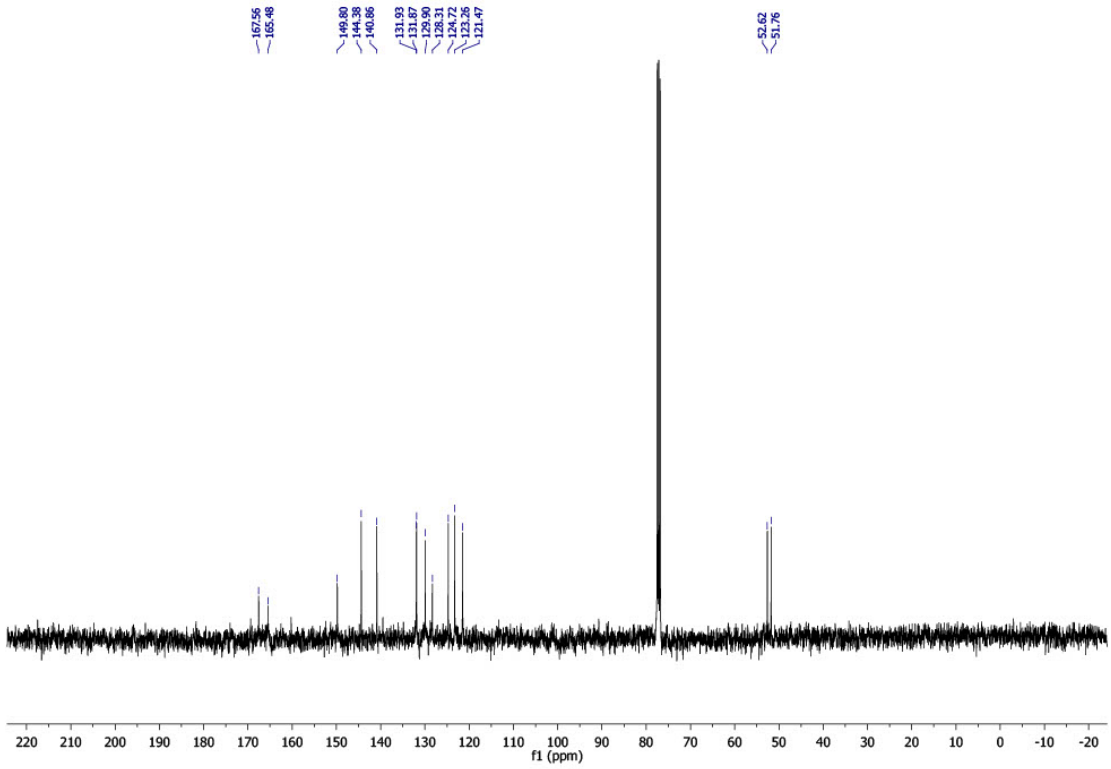

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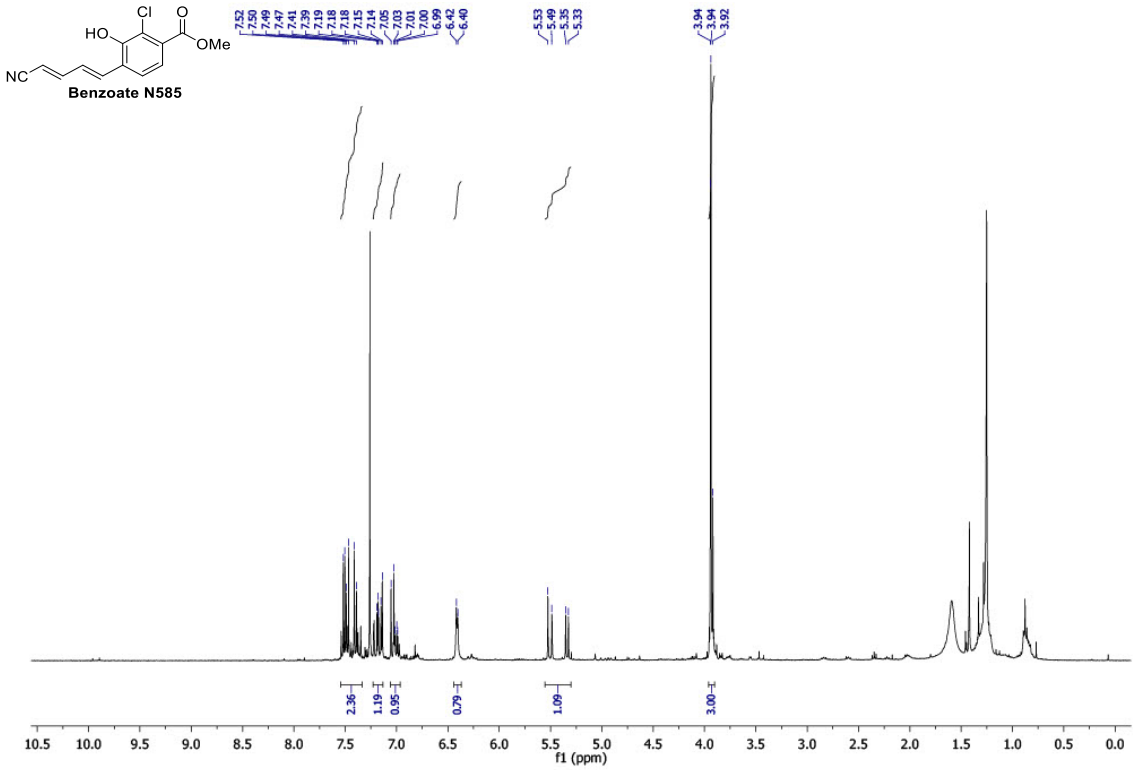

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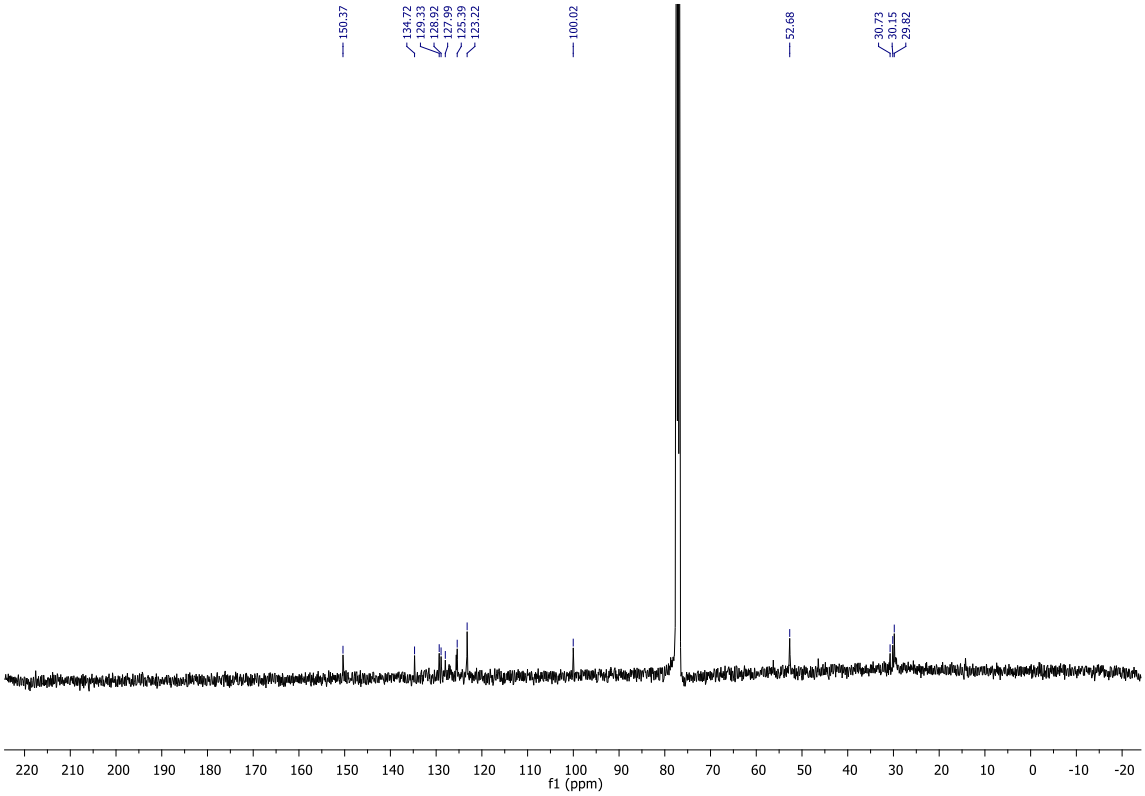
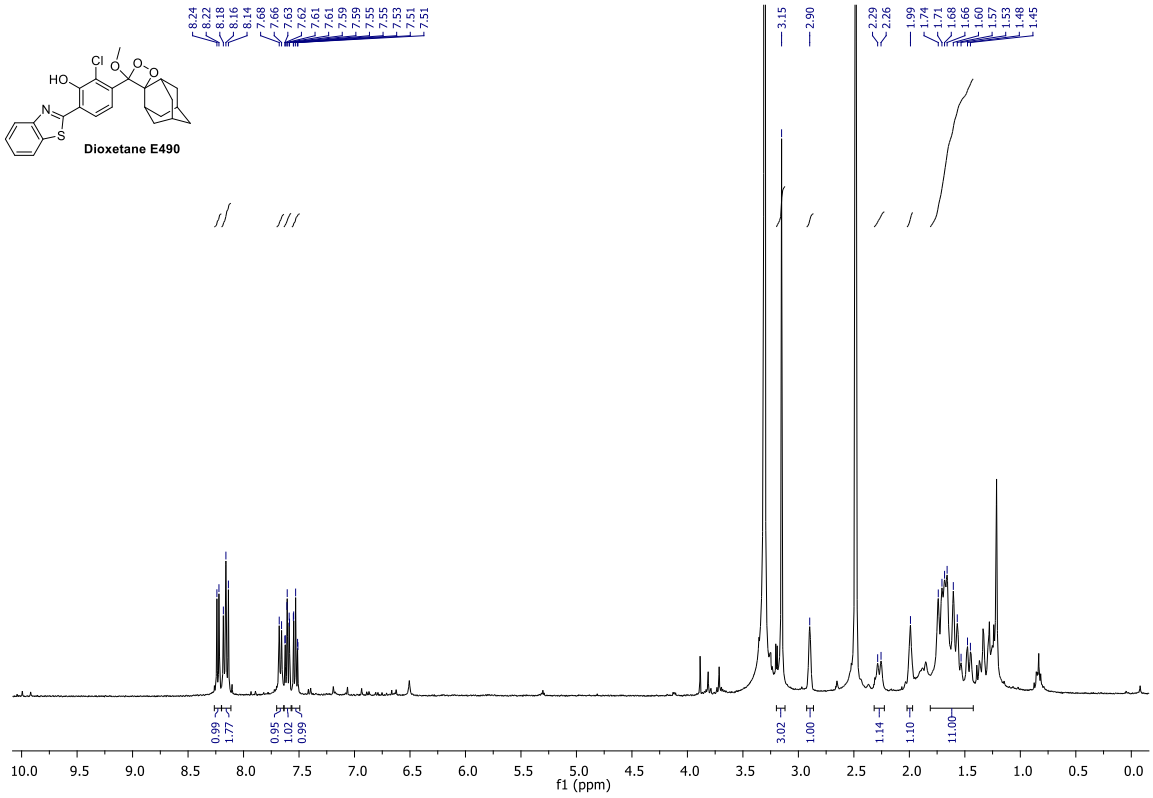


^{1}H-NMR of Dioxetane E490



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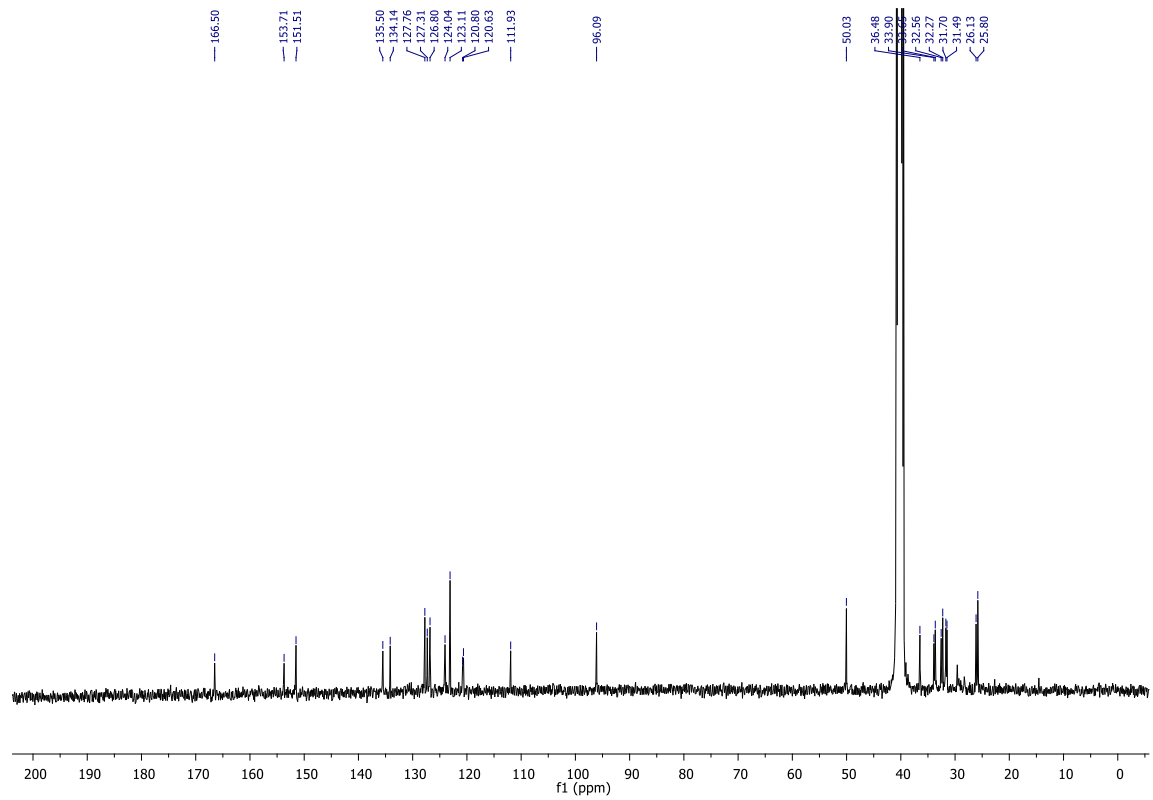

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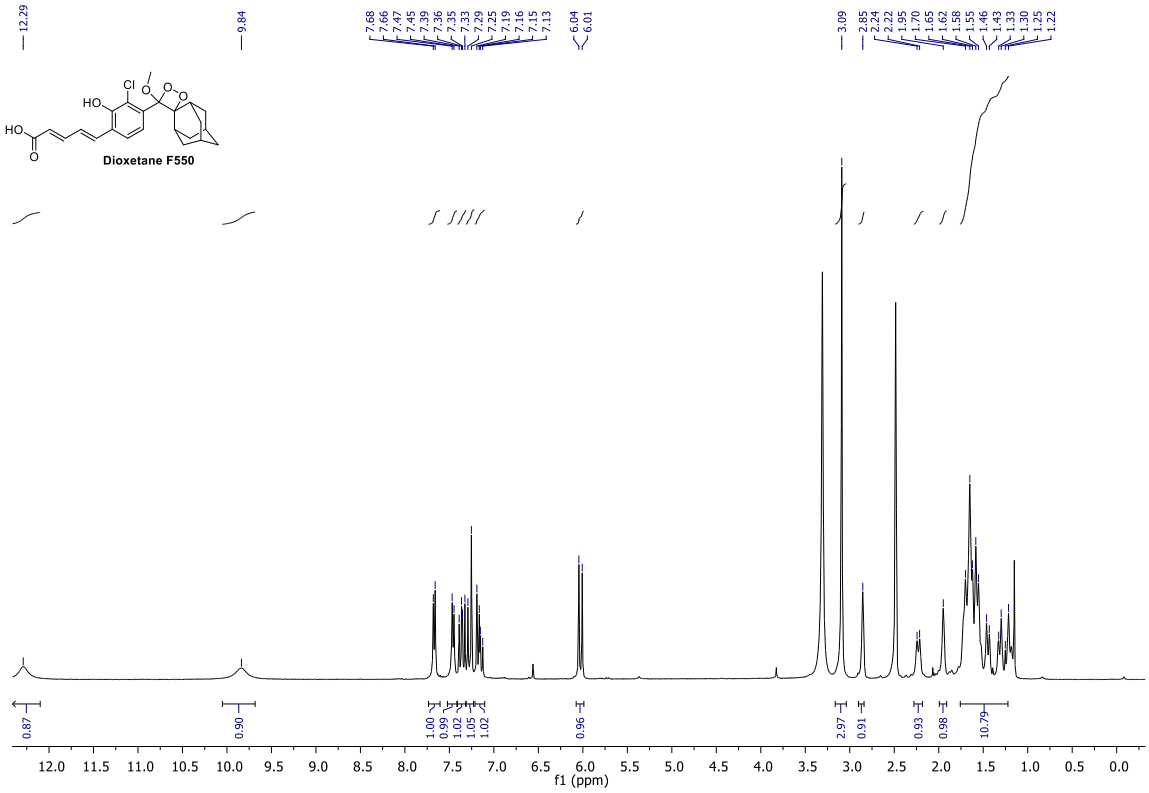

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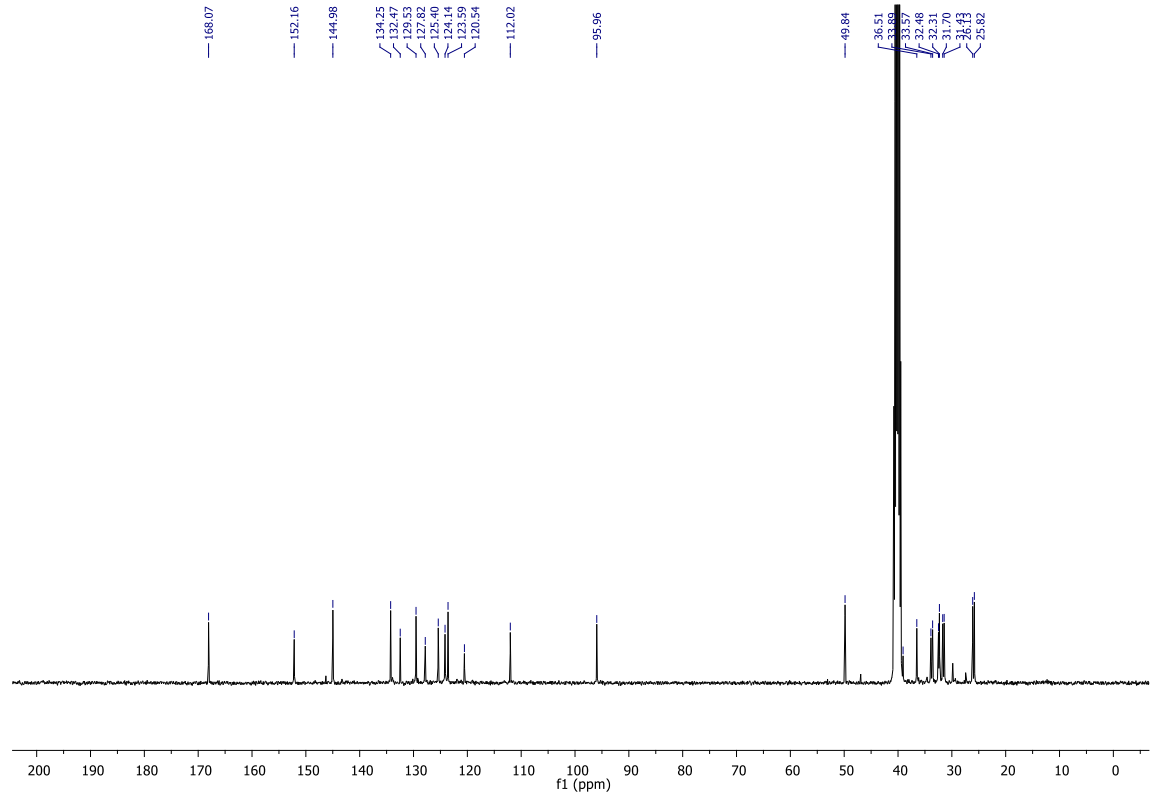

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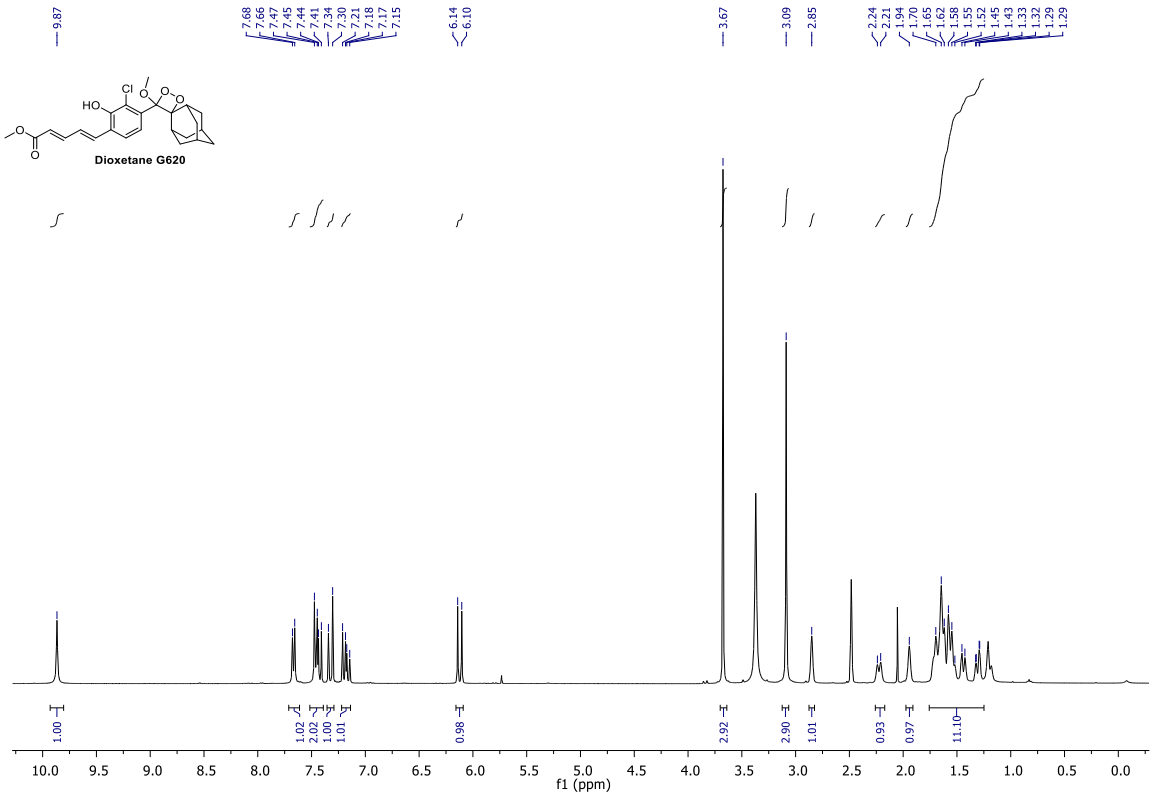

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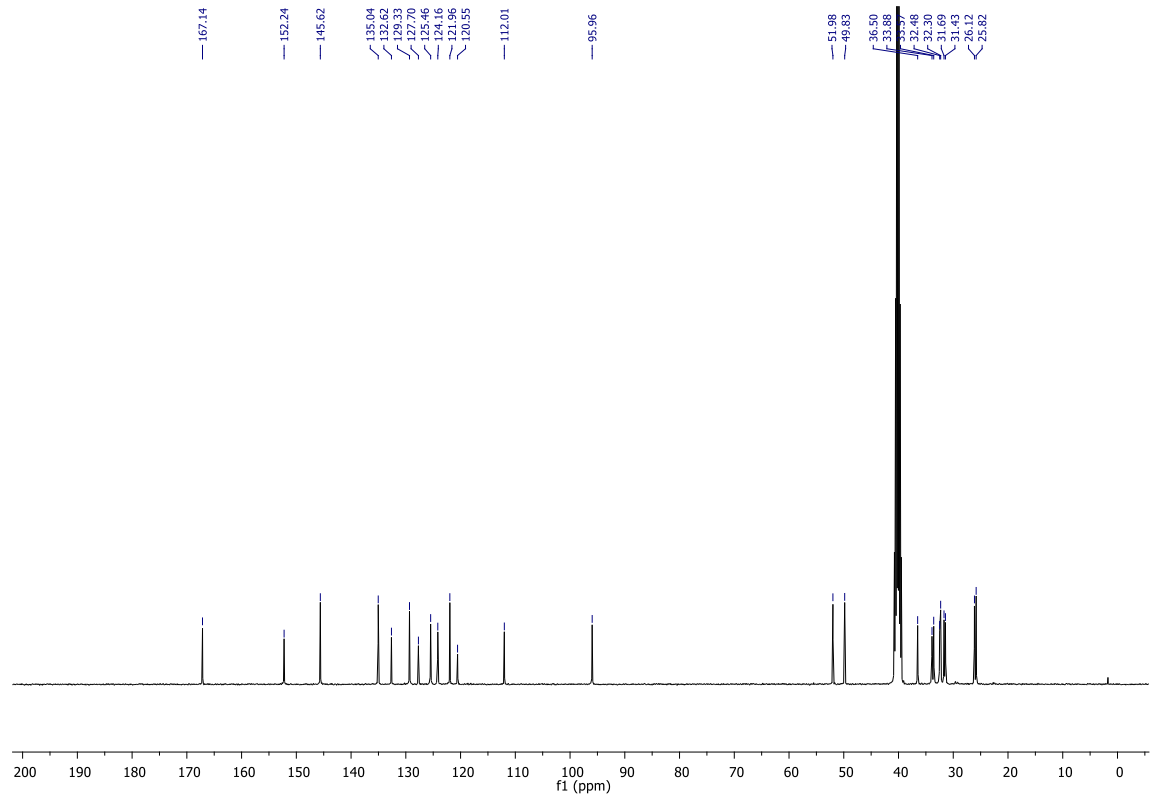

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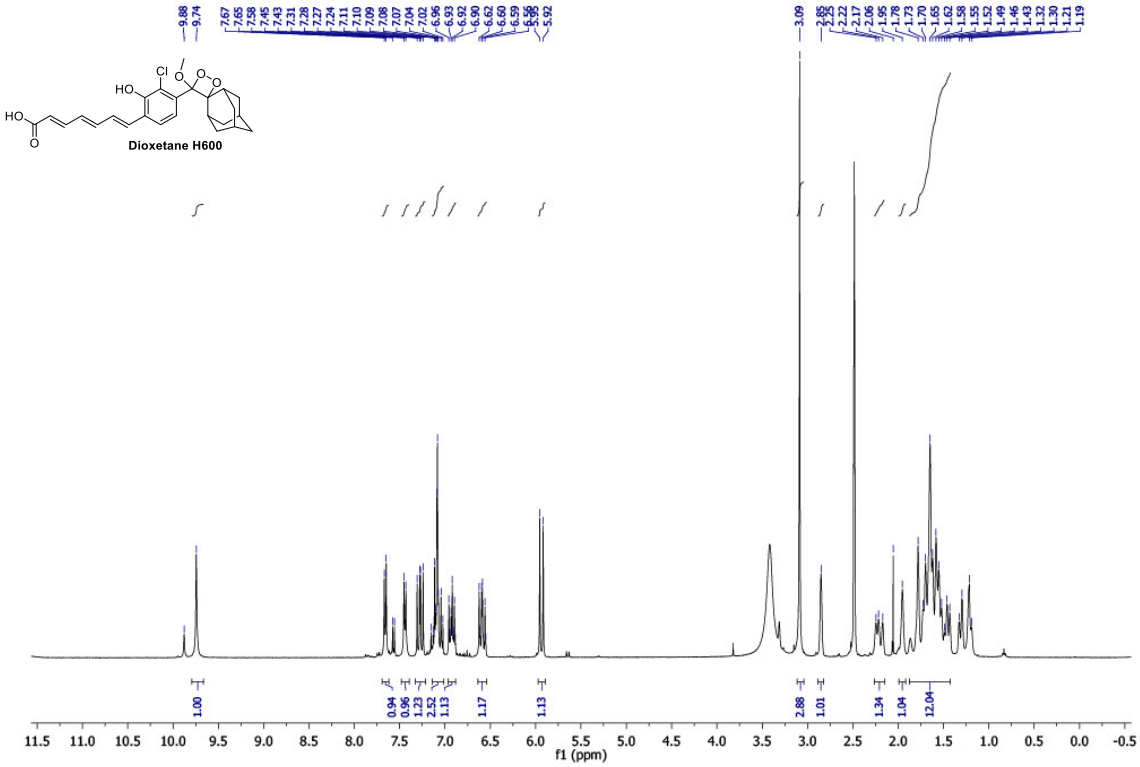

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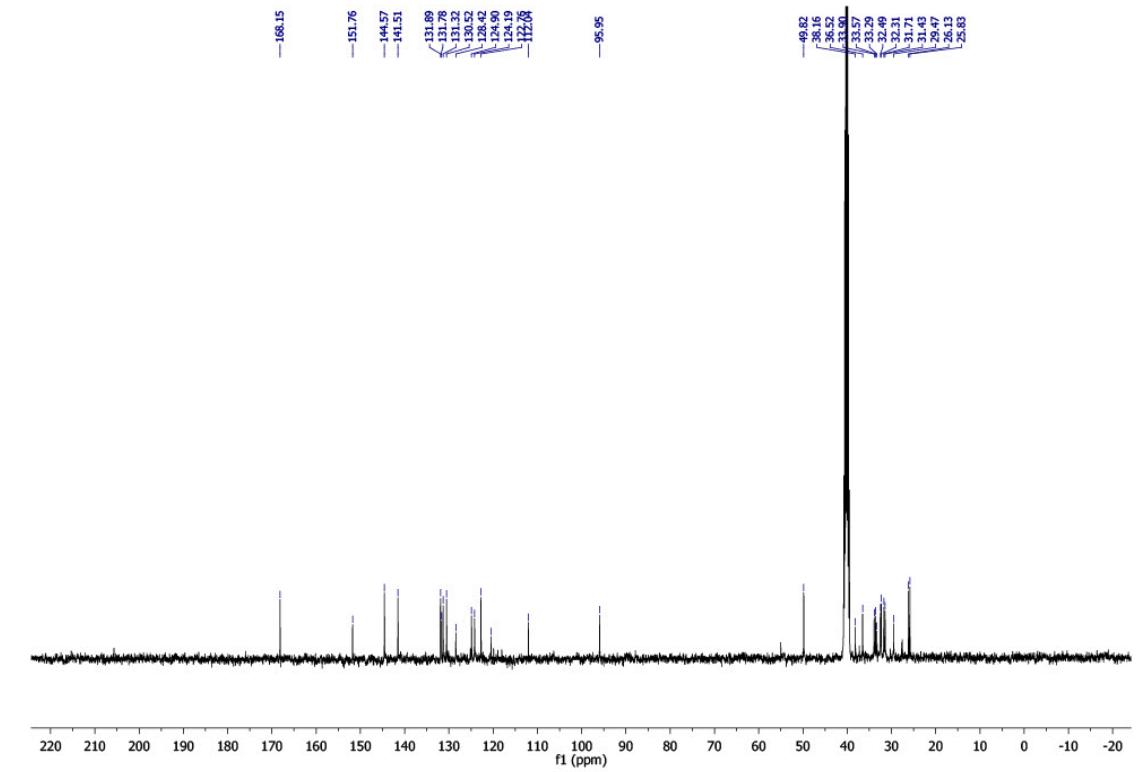

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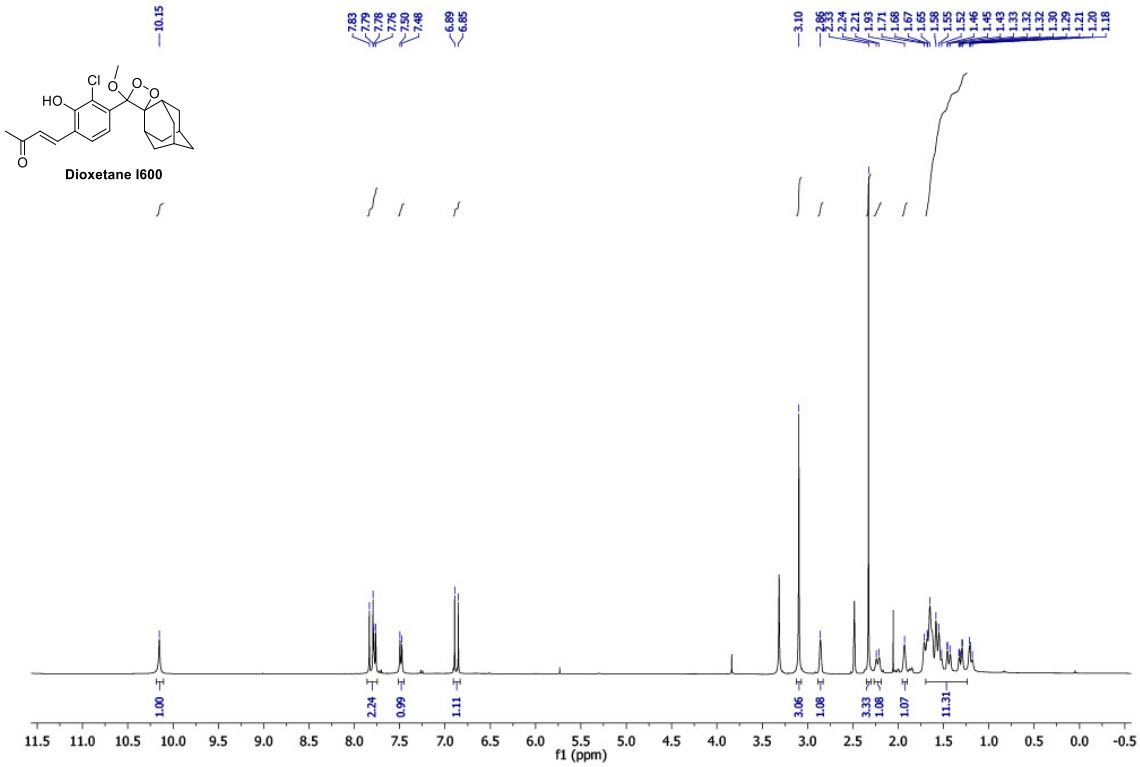

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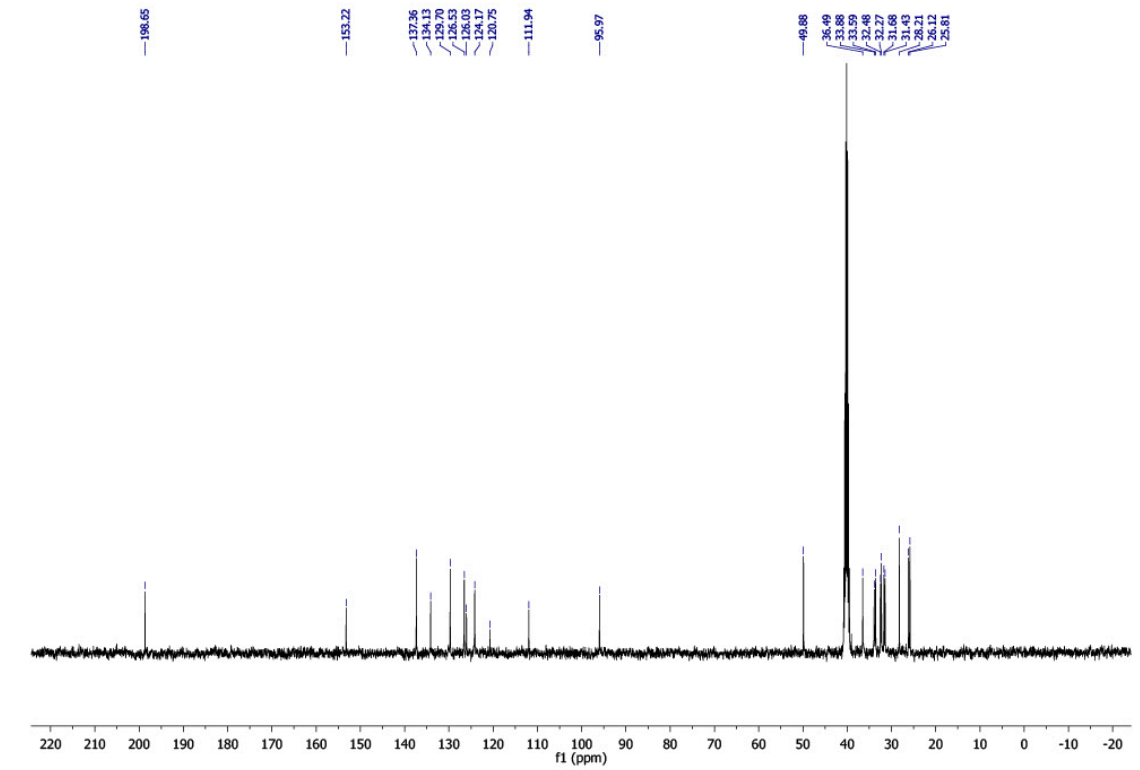

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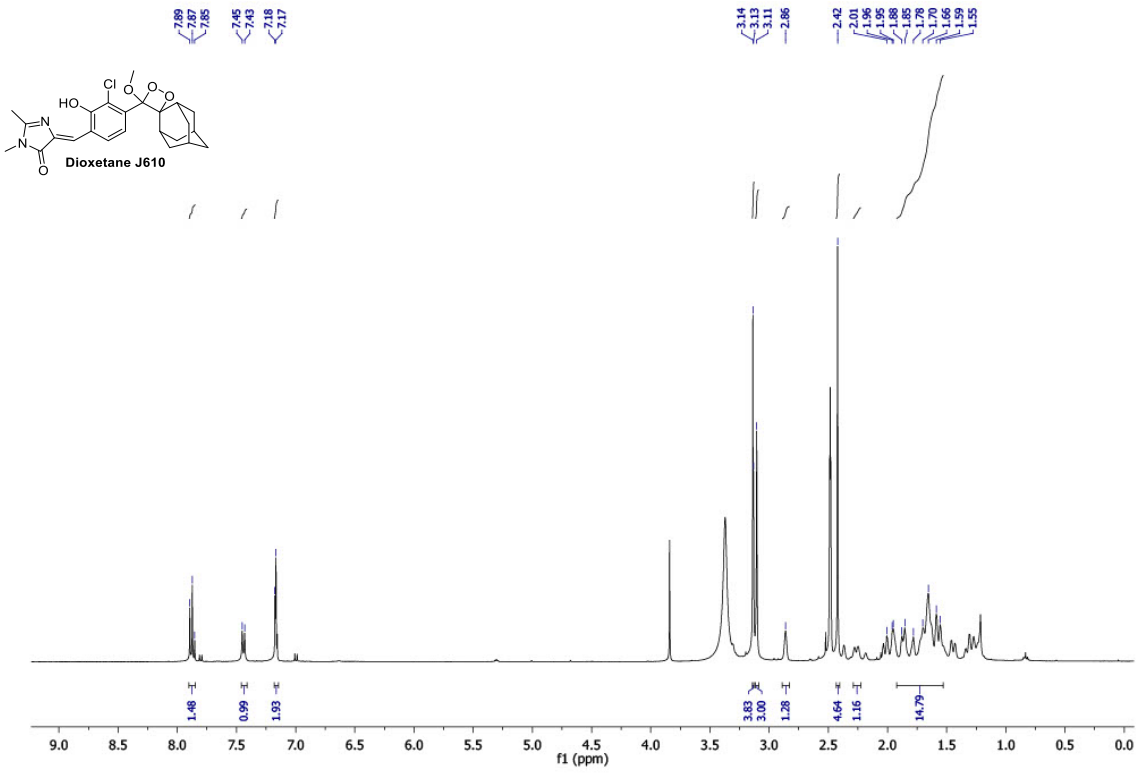

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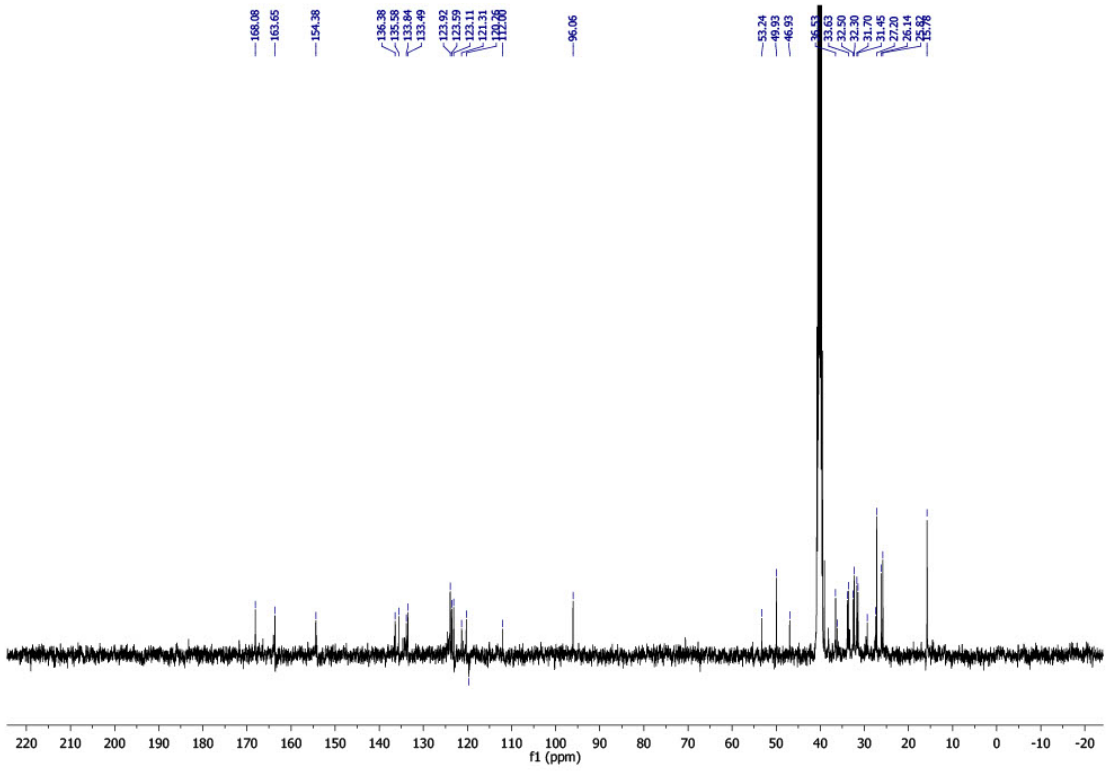

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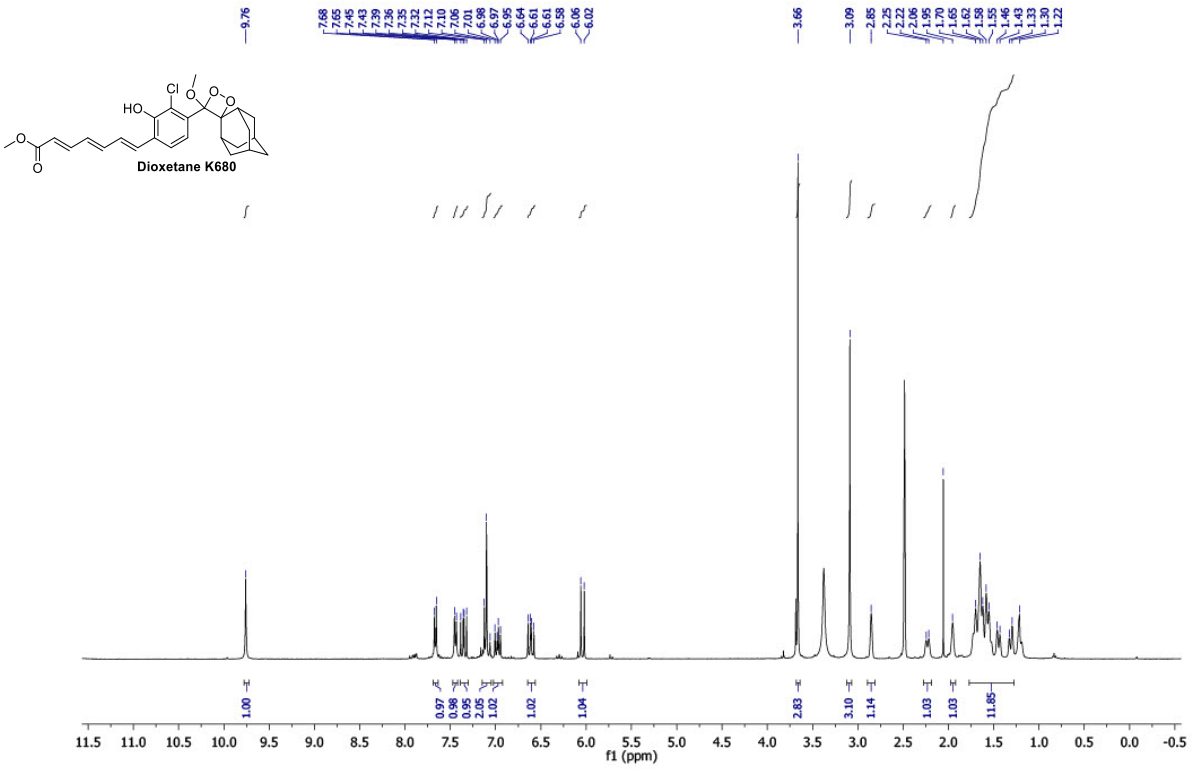

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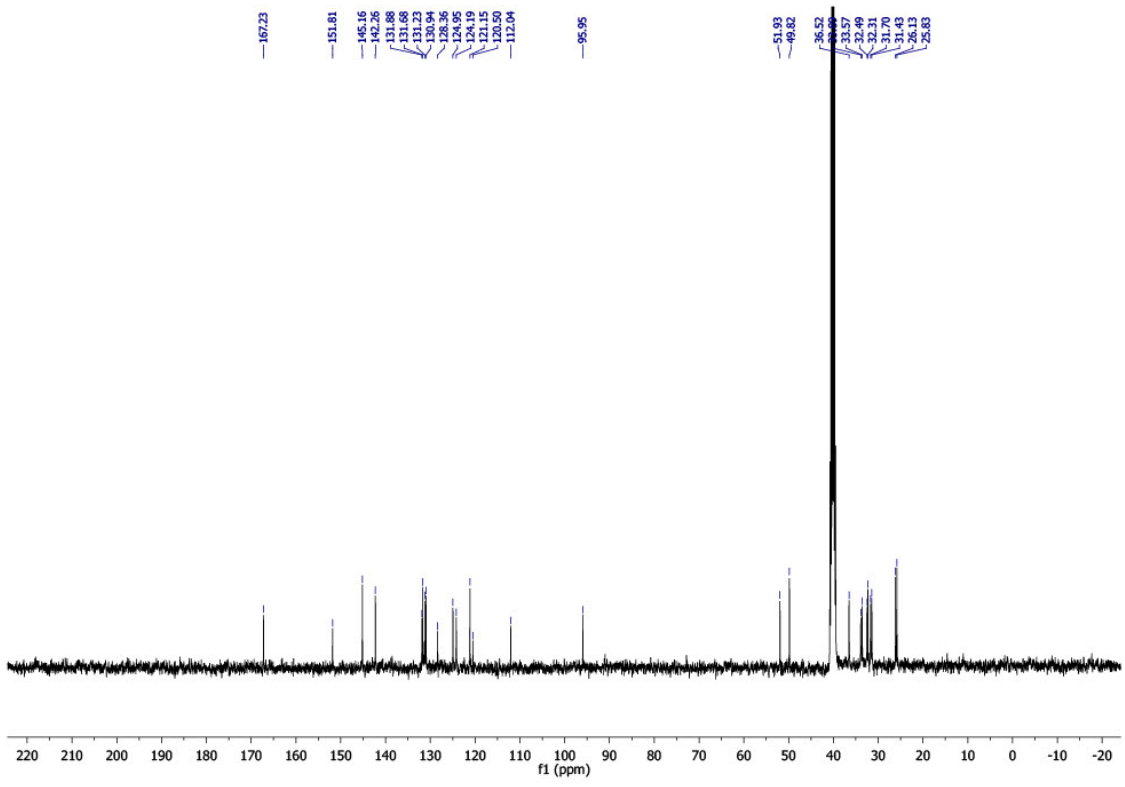

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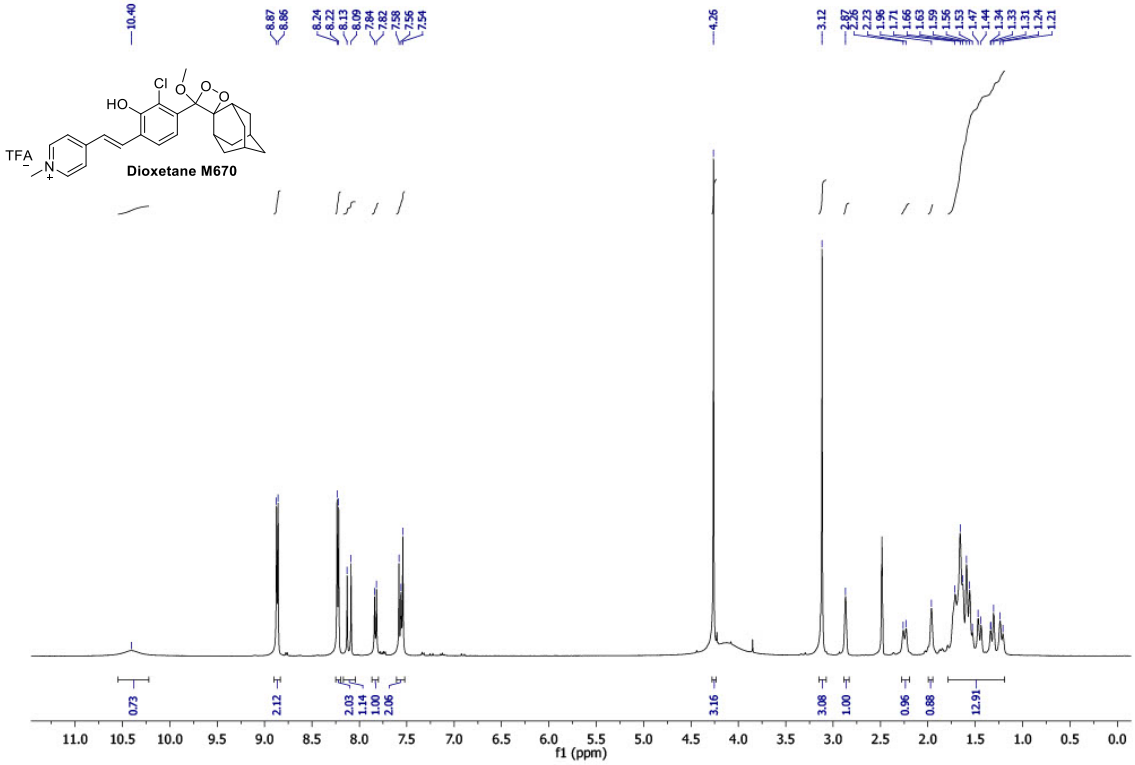

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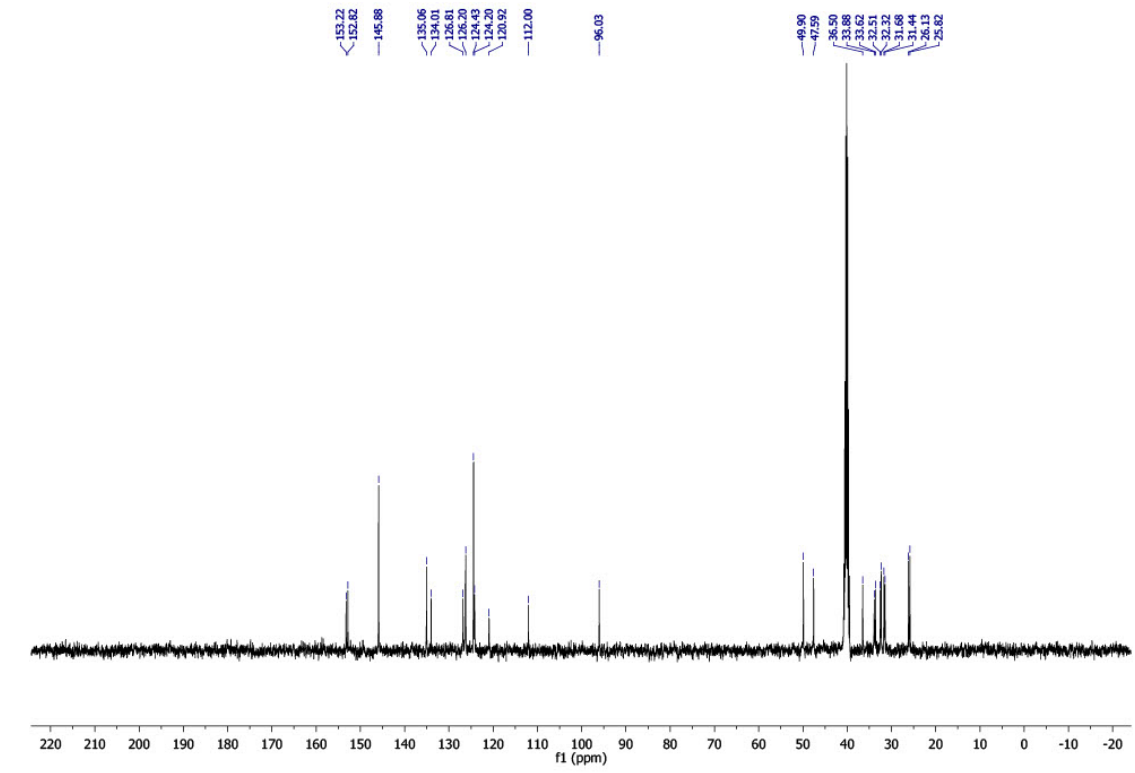

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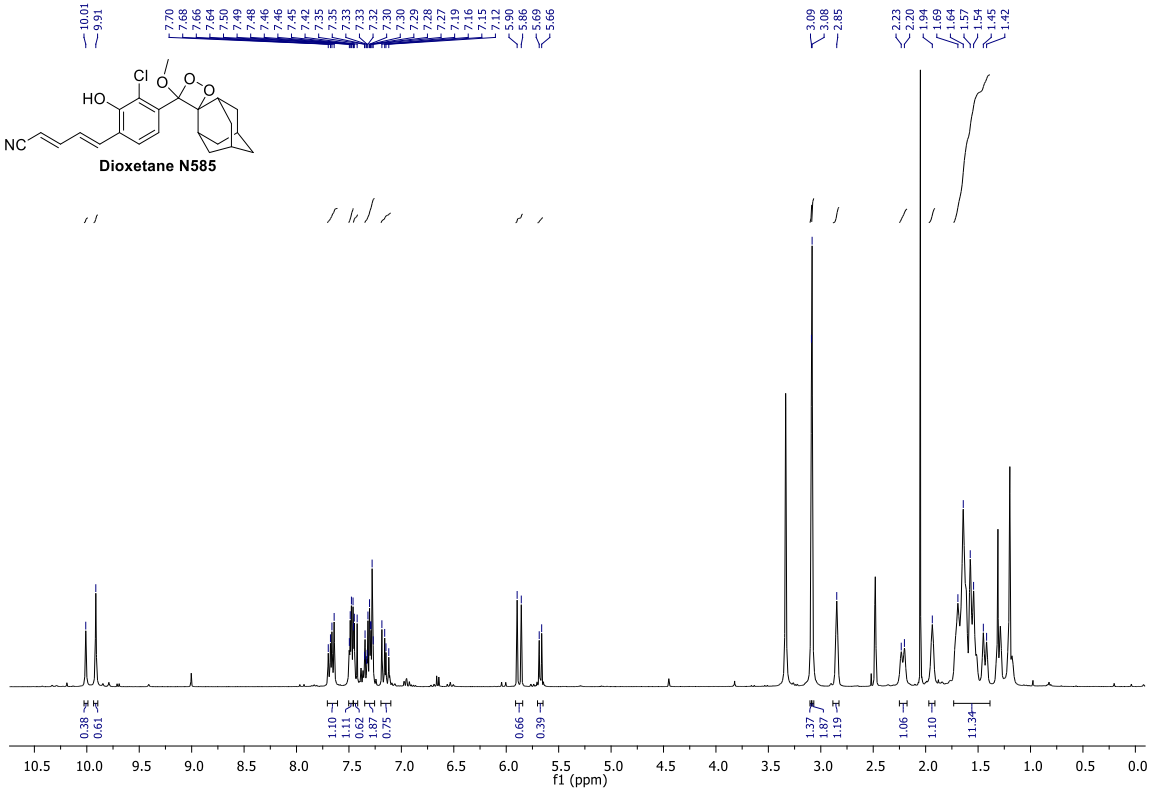

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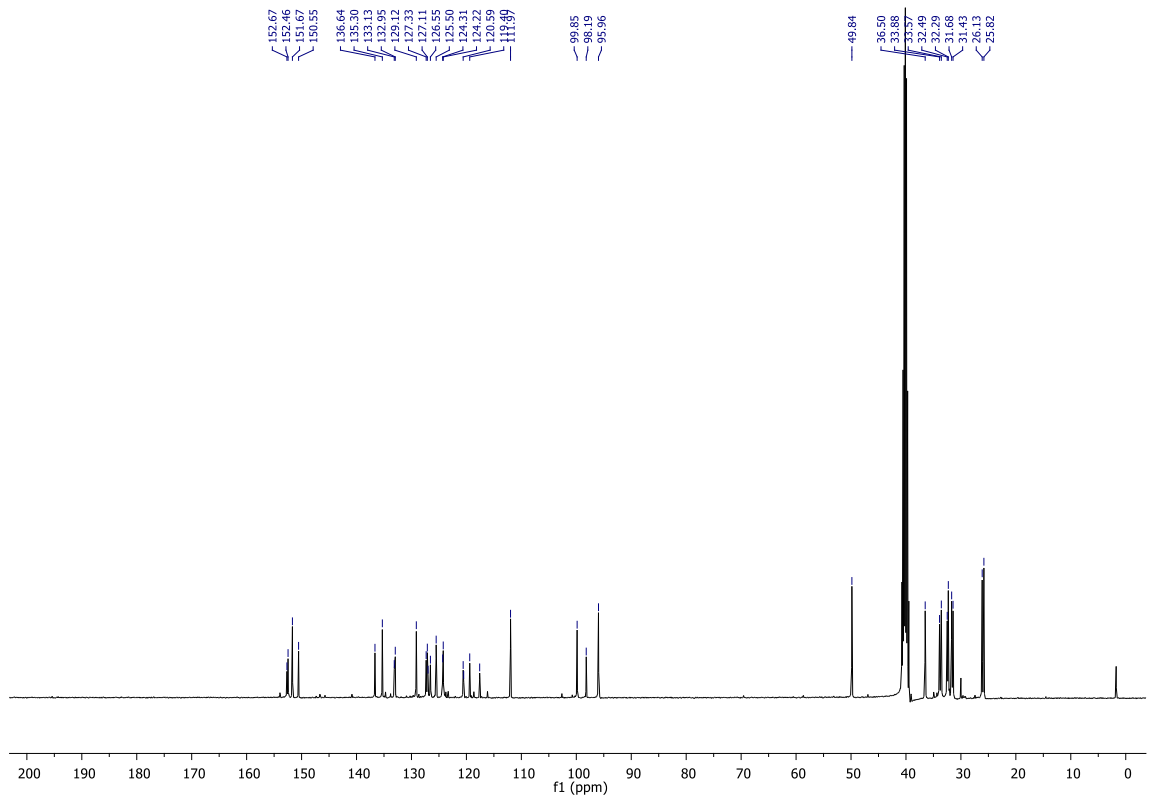

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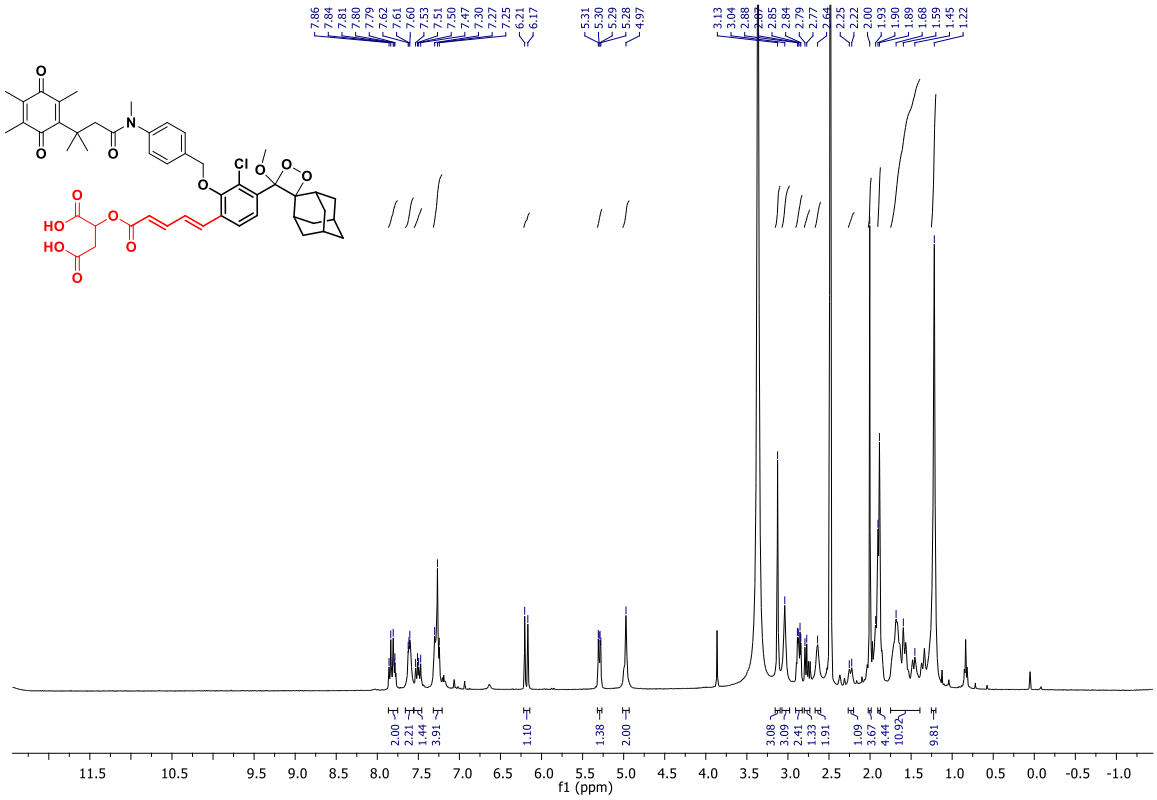

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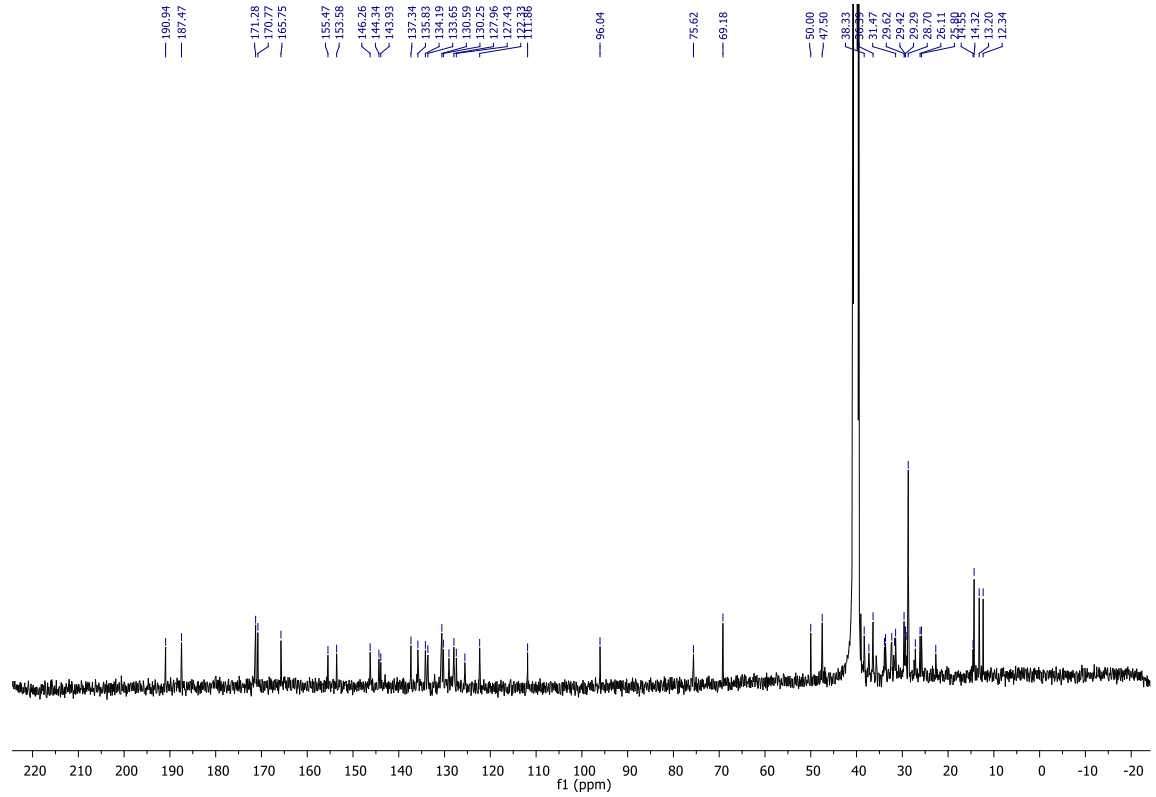

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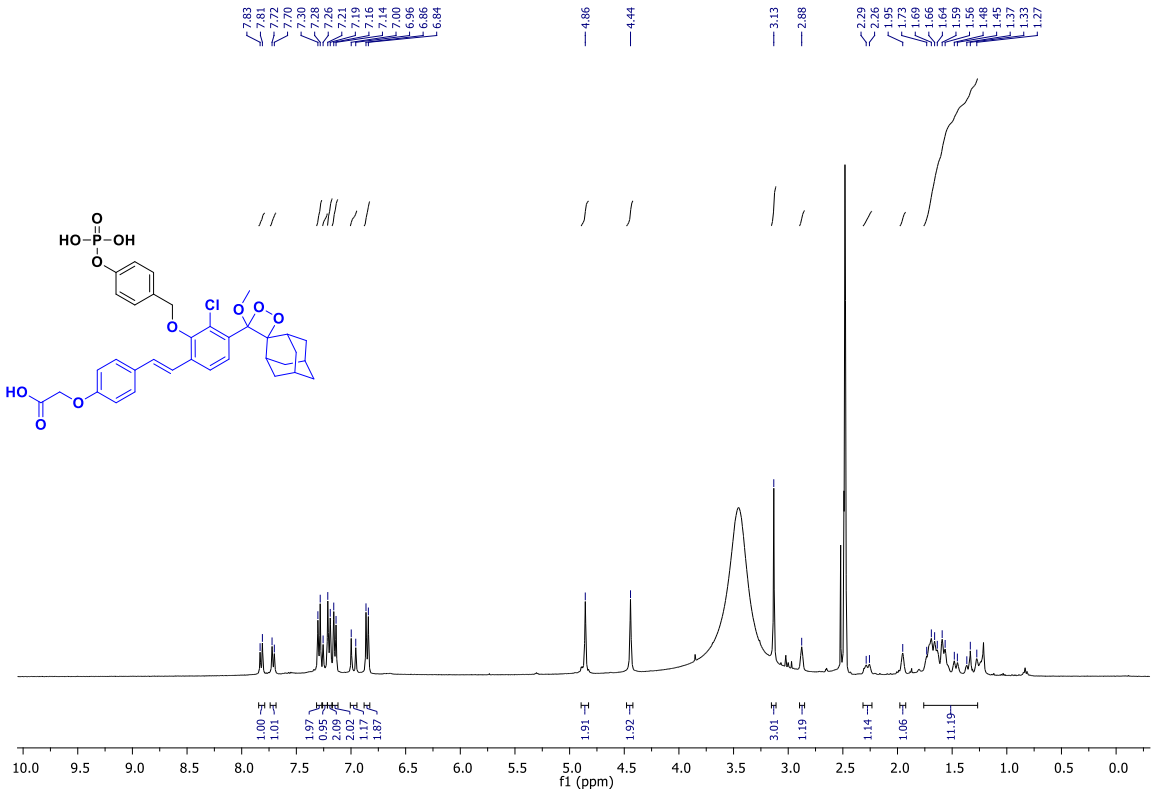

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