OPEN ACCESS

BBR BIOMEDICAL AND BIOPHARMACEUTICAL RESEARCH

Volume 22, Issue 2 (2025)
10.19277/bbr.22.2.371
Supplemental Material

INVESTIGAÇÃO BIOMÉDICA E BIOFARMACÊUTICA IBB

# Supporting Information / Informações Suplementares

Ruiz-Soriano, A., et al. (2025)
Dehydroabietic Acid vs. Azo Derivatives: Structural Integrity as a Key to Preserving Antimicrobial and Antibiofilm Potency.
*Biomedical and Biopharmaceutical Research*, 22(2), 1-17.
https://doi.org/10.19277/bbr.22.2.371

## Table of Contents

1. General Information.....S2

2. Synthesis and Characterization.....S3

3. NMR Spectra.....S8

4. UV-Vis Spectra.....S11

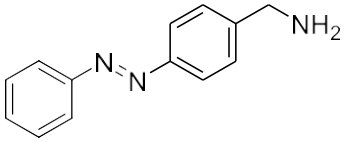
5. Antimicrobial activity: MIC and MBC values of the tested samples.....S12

# 1. General Information

All solvents and reagents used were purchased from commercial suppliers and used without further purification. TLC analysis was carried out on aluminum sheets coated with silica gel and visualized using UV light. $^{1}$H-NMR spectra were obtained at room temperature on a Bruker 400 MHz spectrometer. $^{13}$C-NMR spectra were obtained at 100 MHz. All NMR spectra were processed using MestReNova NMR software. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are reported in Hz. Splitting patterns are reported as follows: singlet (s), doublet (d), triplet (t), quadruplet (q), quintuplet (quint), doublet of doublets (dd), doublet of doublets of doublets (ddd), multiplet (m), etc. NMR signals were assigned using the appropriate 2D NMR experiments (*i.e.* HSQC and HMBC when necessary). High-resolution mass spectrometry (HRMS) and EI-MS were performed by Unitat de Cromatografia de Gasos-Espectrometria de Masses Aplicada, Centres Científics i Tecnològics de la Univeristat de Barcelona (CCiTUB). All manipulations between irradiations and analysis by UV-Vis were carried out in a dark room. Irradiation with blue light was performed using a 4 W LED bulb. Irradiation with UV light (365 nm) was performed with a TLC visualization lamp. Irradiation under sunlight was performed exposing solutions of compounds to sunlight outdoors. UV-Vis spectra were recorded after irradiation at different wavelengths (356, 380, 405, 420, 455, 470, 530 and 650 nm) of 50 μM solutions in DMSO in a 96-well plate.

# 2. Synthesis and Characterization

**(*E*)-(4-(phenyldiazenyl)phenyl)methanamine (S1).**

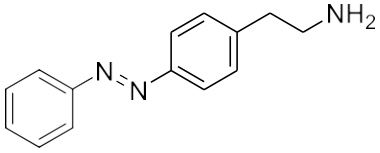


To a stirred solution of (*E*)-2,2,2-trifluoro-*N*-(4-(phenyldiazenyl)benzyl)acetamide[1] (2.10 g, 6.83 mmol) in THF (3.6 mL) was added an aqueous solution of NaOH 10% (13.6 mL). After 6 h the reaction was checked by TLC (EtOAc/MeOH, 9:1) to confirm full conversion. The reaction mixture was diluted with $H_2O$ (volume?), and the aqueous phase was extracted with $CH_2Cl_2$ (3 x 15 mL). The organic fractions were combined, washed with brine (volume?), dried with anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The title product was obtained as an orange solid (1.40 g, quant.). $^1$H-NMR matches reported spectra.[2]

**$^1$H-NMR** (400 Hz, $CDCl_3$): δ 7.94 (m, $C_{Ar}H$, 4 H), 7.50 (m, $C_{Ar}H$, 5 H), 3.96 (s, $CH_2$, 2 H) ppm. $NH_2$ not observed.

**$^{13}$C-NMR** (100 Hz, $CDCl_3$): δ 152.7 ($C_{Ar}$), 151.7 ($C_{Ar}$), 146.6 ($C_{Ar}$), 130.9 ($C_{Ar}$H), 129.1 ($C_{Ar}$H), 127.7 ($C_{Ar}$H), 123.2 ($C_{Ar}$H), 122.8 ($C_{Ar}$H), 46.2 ($CH_2$) ppm.

**(*E*)-2-(4-(phenyldiazenyl)phenyl)ethan-1-amine (S2).**



To a stirred solution of (*E*)-2,2,2-trifluoro-*N*-(4-(phenyldiazenyl)phenethyl)acetamide[1] (1.70 g, 5.29 mmol) in THF (2.8 mL) was added an aqueous solution of NaOH 10% (10.6 mL). After 6 h the reaction was checked by TLC (EtOAc/MeOH, 9:1) to confirm full conversion. The reaction mixture was diluted with $H_2O$, and the aqueous phase was extracted with DCM (3 x 15 mL). The organic fractions were combined, washed with brine (volume?), dried with anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The title product was obtained as an orange solid (1.21 g, quant.). $^1$H-NMR matches reported spectra.[3]

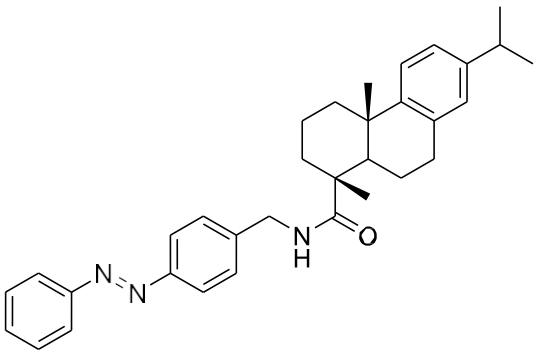
**$^1$H-NMR** (400 Hz, $CDCl_3$): δ 7.94-7.84 (m, $C_{Ar}H$, 4 H), 7.55-7.42 (m, $C_{Ar}H$, 3 H), 7.34 (d, $J$ = 8.6 Hz, $C_{Ar}H$, 2 H), 3.01 (t, $J$ = 6.8 Hz, $CH_2$, 2 H), 2.82 (t, $J$ = 6.8 Hz, $CH_2$, 2 H) ppm. $NH_2$ not observed.

**$^{13}$C-NMR** (100 Hz, $CDCl_3$): δ 152.8 ($C_{Ar}$), 151.4 ($C_{Ar}$), 143.4 ($C_{Ar}$), 130.9 ($C_{Ar}$H), 129.6 ($C_{Ar}$H), 129.1 ($C_{Ar}$H), 123.1 ($C_{Ar}$H), 122.9 ($C_{Ar}$H), 43.5 ($CH_2$), 40.1 ($CH_2$) ppm.

[1] Ruiz-Soriano, A.; Lamelza, L.; Pizzamiglio, E.; Just-Baringo, X. *J. Org. Chem.* **2024**, *89*, 17141-17146.
[2] Shimogaki, T.; Oshita, S.; Matsumoto, A. *Macromol. Chem. Phys.* **2011**, 212, 1767–1777.
[3] Gu, L.; Liu, X.; Dong, S.; Chen, Z.; Han, R.; He, C.; Wang, D.; Zheng, Y. *Polym. Chem.* **2020**, 11, 1871-1876.

**$^1$H-NMR** (400 Hz, $CDCl_3$): δ 7.86-7.79 (m, $C_{Ar}H$, 4 H), 7.51-7.45 (m, $C_{Ar}H$, 2 H), 7.40 (tt, *J* = 7.2, 1.2 Hz) 7.34 (dt, *J* = 8.8, 2.8 Hz, $C_{Ar}H$, 2 H), 4.05 (brs, $NH_2$, 2H) ppm.

**(1*R*,4a*S*)-7-isopropyl-1,4a-dimethyl-*N*-(4-((*E*)-phenyldiazenyl)benzyl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (DHA_Azo1).**



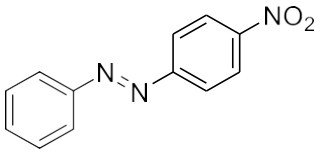
To a thick-wall glass reaction tube charged with dehydroabietic acid (50 mg, 0.17 mmol) dissolved in $CH_2Cl_2$ (1.3 mL) was added thionyl chloride (24 µL, 0.33 mmol). The tube was sealed and the reaction mixture was stirred at 65 °C. After 4 h, the mixture was allowed to cool to room temperature and volatiles were co-evaporated with the aid of toluene. The acid chloride of the dehydroabietic acid thus obtained was purged with Argon, dissolved in anhydrous $CH_2Cl_2$ (0.8 mL) and added dropwise to a stirring solution of amine **S1** (35.1 mg, 0.17 mmol) and $NEt_3$ (32 µL, 0.23 mmol) in anhydrous $CH_2Cl_2$ (0.8 mL). After stirring at room temperature for 18 h, the reaction mixture was diluted with $CH_2Cl_2$ (10 mL) and $H_2O$ (10 mL). The organic phase was washed with 1 N HCl (3 × 10 mL), 2 M NaOH (3 × 10 mL) and brine (1 × 10 mL). Then, the organic phase was dried with anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc, 97.5:2.5 + 1% $NEt_3$ to 80:20 + 1% $NEt_3$), affording the title compound as an orange oil (55 mg, 68%).

**$^1$H-NMR** (400 Hz, $CDCl_3$): δ 7.96-7.87 (m, $C_{Ar}H$ Azo, 4 H), 7.56-7.45 (m, $C_{Ar}H$ Azo, 3 H), 7.41 (d, *J* = 8.4 Hz, $C_{Ar}H$ Azo, 2 H), 7.17 (d, *J* = 8.0 Hz, $C_{Ar}H$, 1 H), 7.00 (dd, *J* = 8.0, 2.0 Hz, $C_{Ar}H$, 1 H), 6.88 (d, *J* = 2.0 Hz, $C_{Ar}H$, 1 H), 6.26 (t, *J* = 5.6 Hz, CON*H*, 1 H), 4.54 (dd, *J* = 14.8, 5.6 Hz, $CH_2$NHCO, 1 H), 4.52 (dd, *J* = 14.8, 5.6 Hz, $CH_2$NHCO, 1 H), 2.91-2.78 (m, 3 H), 2.32 (d, *J* = 13.2 Hz, $CH_2$, 1 H), 2.18 (dd, *J* = 12.4, 2.0 Hz, C*H*, 1 H), 1.89-1.48 (m, 7 H), 1.31 (s, $CH_3$, 3 H), 1.25-1.20 (m, $CH_3$ + $CH(CH_3)_2$, 9 H) ppm.

**$^{13}$C-NMR** (100 Hz, $CDCl_3$): δ 178.6 (*C*ONH), 152.8 ($C_{Ar}$N), 151.6 ($C_{Ar}$N), 147.1 ($C_{Ar}$), 145.8 ($C_{Ar}$), 142.6 ($C_{Ar}$), 134.8 ($C_{Ar}$), 131.1 ($C_{Ar}$H Azo), 129.7 ($C_{Ar}$H Azo), 129.2 ($C_{Ar}$H Azo), 127.0 ($C_{Ar}$H DHHA), 123.3 ($C_{Ar}$H Azo), 122.9 ($C_{Ar}$H Azo), 124.1 ($C_{Ar}$H DHHA), 124.0 ($C_{Ar}$H DHHA), 47.4 (*C*), 45.5 (*C*H), 40.8 ($CH_2$), 38.0 ($CH_2$), 37.4 (*C*), 37.2 ($CH_2$), 35.8 ($CH_2$), 33.6 (*C*H), 30.1 ($CH_2$), 25.3 ($CH_3$), 24.1 ($CH_3$), 21.2 ($CH_2$), 18.9 ($CH_2$), 16.6 ($CH_3$) ppm.

**HRMS** calcd for $C_{33}H_{40}ON_3$ $[M+H]^+$ :494.3166, found 494.3175.

**(*E*)-1-(4-nitrophenyl)-2-phenyldiazene (S3).**

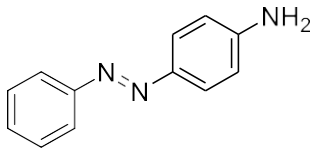


A solution of *p*-nitroaniline (2.97 g, 21.5 mmol) in $CH_2Cl_2$ (75 mL, 0.3 M) was added to a round bottom flask containing a solution of Oxone® (13.2 g, 21.5 mmol) in $H_2O$ (75 mL, 0.3 M). The mixture was stirred vigorously with an oval stirring bar at room temperature. The progress of the reaction was checked by TLC ($CH_2Cl_2$/MeOH, 90:10). The solution gradually turned green as the desired nitroso compound formed. Once the TLC showed full conversion, the layers were separated, and the aqueous layer was extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic layers were washed with 1 N aqueous HCl (40 mL), saturated aqueous $NaHCO_3$ (40 mL), $H_2O$ (40 mL) and brine (40 mL). Then, the organic phase was dried with anhydrous $Na_2SO_4$ and concentrated under reduced pressure to around 15 mL. The crude thus obtained was checked by $^1$H-NMR to confirm clean full conversion into the nitroso intermediate and was used without purification.

To a three-neck round bottom flask loaded with a stirring solution of the crude nitroso compound in acetic acid (14 mL) at room temperature under $N_2$, aniline (1.97 mL, 21.5 mmol) was added dropwise. After 18 h stirring at room temperature, the solvent was removed under reduced pressure. The residue obtained was dissolved in EtOAc (75 mL) and the resulting solution was washed with 2 M NaOH (3 × 30 mL), saturated aqueous $NaHCO_3$ (1 × 30 mL) and brine (1 × 30 mL). Then, the organic phase was dried with anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The crude was purified by silica gel column chromatography (hexane/EtOAc, 98:2), affording the title compound as an orange solid (2.53 g, 52%). $^1$H-NMR matches reported spectra.[4]

**$^1$H-NMR** (400 Hz, $CDCl_3$): δ 8.39 (d, *J* = 8.8 Hz, $C_{Ar}H$, 2 H), 8.04 (d, *J* = 8.8 Hz, $C_{Ar}H$, 2 H), 8.0-7.95 (m, $C_{Ar}H$, 2 H), 7.60-7.53 (m, $C_{Ar}H$, 3 H) ppm.

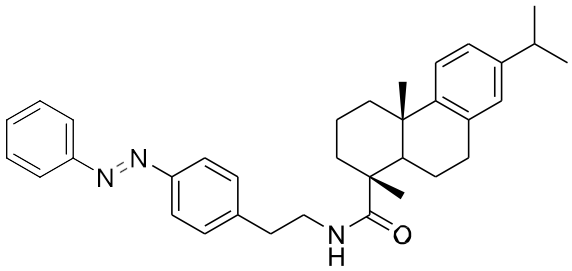
**(*E*)-4-(phenyldiazenyl)aniline (S4).**



To a stirred solution of **S3** (2.53 g, 11.1 mmol) in THF:$H_2O$ (3:1, 145 mL) was added $Na_2S$ (8.12 g, , 32% purity 33.3 mmol) and stirred at reflux. After 3 h, a second portion of $Na_2S$ (8.12 g, , 32% purity 33.3 mmol) was added and the mixture and stirred for 2 more hours. After TLC showed full conversion, the reaction was cooled to room temperature and concentrated under reduced pressure. We added 150 mL of EtOAc and washed with 1 M NaOH (3 × 50 mL) and brine (50 mL). Then, the organic phase was dried with anhydrous $Na_2SO_4$ and concentrated under reduced. The crude was purified by silica gel column chromatography ($CH_2Cl_2$/MeOH, 100:0 to 97.5:2.5), affording the title compound as an orange solid (1.76 g, 80%). $^1$H-NMR matches reported spectra.[3]

[4] Schönberg, M.; Althaus, M.; Fronius, M.; Clauss, W.; Trauner, D. *Nature Chemistry* **2014**, 6, 712–719.

**(1*R*,4a*S*)-7-isopropyl-1,4a-dimethyl-*N*-(4-((*E*)-phenyldiazenyl)phenethyl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (DHA_Azo2).**



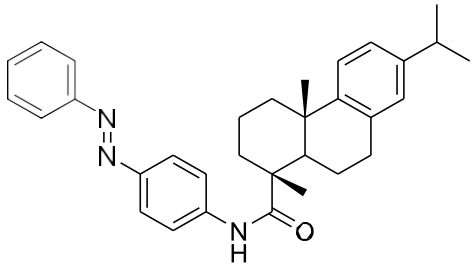
To a thick-wall glass reaction tube charged with dehydroabietic acid (50 mg, 0.17 mmol) dissolved in $CH_2Cl_2$ (1.3 mL) was added thionyl chloride (24 µL, 0.33 mmol). The tube was sealed and the reaction mixture was stirred at 65 °C. After 4 h, the mixture was allowed to cool to room temperature and volatiles were co-evaporated with toluene. The acid chloride of the dehydroabietic acid thus obtained was purged with Argon, dissolved in anhydrous $CH_2Cl_2$ (0.8 mL) and added dropwise to a stirring solution of amine **S2** (37.4 mg, 0.17 mmol) and $NEt_3$ (32 µL, 0.23 mmol) in anhydrous $CH_2Cl_2$ (0.8 mL). After stirring at room temperature for 18 h, the reaction mixture was diluted with $CH_2Cl_2$ (10 mL) and $H_2O$ (10 mL). The organic phase was washed with 1 N HCl (3 × 10 mL), 2 M NaOH (3 × 10 mL) and brine (1 × 10 mL). Then, the organic phase was dried with anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc, 97.5:2.5 + 1% $NEt_3$ to 80:20 + 1% $NEt_3$), affording the title compound as an orange oil (34 mg, 39%).

**$^1$H-NMR** (400 Hz, $CDCl_3$): δ 7.92 (d, *J* = 6.8 Hz, $C_{Ar}H$ Azo, 2 H), 7.88 (d, *J* = 8.4 Hz, $C_{Ar}H$ Azo, 2 H), 7.56-7.44 (m, $C_{Ar}H$ Azo, 3 H), 7.35 (d, *J* = 8.4 Hz, $C_{Ar}H$ Azo, 2 H), 7.14 (d, *J* = 8.0 Hz, $C_{Ar}H$, 1 H), 6.98 (dd, *J* = 8.0, 2.0 Hz, $C_{Ar}H$, 1 H), 6.83 (d, *J* = 2.0 Hz, $C_{Ar}H$, 1 H), 5.83 (t, *J* = 6.0 Hz, CONH, 1 H), 3.60 (q, *J* = 6.4 Hz, $CH_2$NHCO, 2 H), 2.92 (t, *J* = 6.8 Hz, $CH_2CH_2$NHCO, 2 H), 2.80 (m, $CH_2$ + CH, 3 H), 2.28 (d, *J* = 12.8 Hz, $CH_2$, 1 H), 2.08 (dd, *J* = 12.4, 2.4 Hz, CH, 1 H), 1.80-1.32 (m, 7 H), 1.21 (s, $CH_3$, 3 H), 1.20-1.18 (m, $CH_3$ + $CH(CH_3)_2$, 9 H) ppm.

**$^{13}$C-NMR** (100 Hz, $CDCl_3$): δ 178.6 (*C*ONH), 152.8 ($C_{Ar}$N), 151.6 ($C_{Ar}$N), 147.1 ($C_{Ar}$), 145.8 ($C_{Ar}$), 142.6 ($C_{Ar}$), 134.8 ($C_{Ar}$), 131.1 ($C_{Ar}$H Azo), 129.7 ($C_{Ar}$H Azo), 129.2 ($C_{Ar}$H Azo), 127.0 ($C_{Ar}$H DHA), 123.3 ($C_{Ar}$H Azo), 122.9 ($C_{Ar}$H Azo), 124.1 ($C_{Ar}$H DHA), 124.0 ($C_{Ar}$H DHA), 47.4 (*C*), 45.5 (*C*H), 40.8 ($CH_2$), 38.0 ($CH_2$), 37.4 (*C*), 37.2 ($CH_2$), 35.8 ($CH_2$), 33.6 (*C*H), 30.1 ($CH_2$), 25.3 ($CH_3$), 24.1 ($CH_3$ ×2), 21.2 ($CH_2$), 18.9 ($CH_2$), 16.6 ($CH_3$) ppm.

**HRMS** calcd for $C_{34}H_{42}ON_3$ $[M+H]^+$ :508.3322, found 508.3326.

**(1*R*,4a*S*)-7-isopropyl-1,4a-dimethyl-*N*-(4-((*E*)-phenyldiazenyl)phenyl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (DHA_Azo0).**



To a thick-wall glass reaction tube charged with dehydroabietic acid (50 mg, 0.17 mmol) dissolved in $CH_2Cl_2$ (1.3 mL) was added thionyl chloride (24 µL, 0.33 mmol). The tube was sealed and the reaction mixture was stirred at 65 °C. After 4 h, the mixture was allowed to cool to room temperature and volatiles were co-evaporated with toluene. The acid chloride of the dehydroabietic acid thus obtained was purged with Argon, dissolved in anhydrous $CH_2Cl_2$ (0.8 mL) and added dropwise to a stirring solution of amine **S4** (32.7 mg, 0.17 mmol) and $NEt_3$ (32 µL, 0.23 mmol) in anhydrous $CH_2Cl_2$ (0.8 mL). After stirring at room temperature for 18 h, the reaction mixture was diluted with $CH_2Cl_2$ (10 mL) and $H_2O$ (10 mL). The organic phase was washed with 1 N HCl (3 × 10 mL), 2 M NaOH (3 × 10 mL) and brine (1 × 10 mL). The crude was purified by column chromatography (Hexane:EtOAc, 97.5:2.5 + 1% $NEt_3$ to 8:2 + 1% $NEt_3$), affording product the title compound as an orange oil (32 mg, 40%).

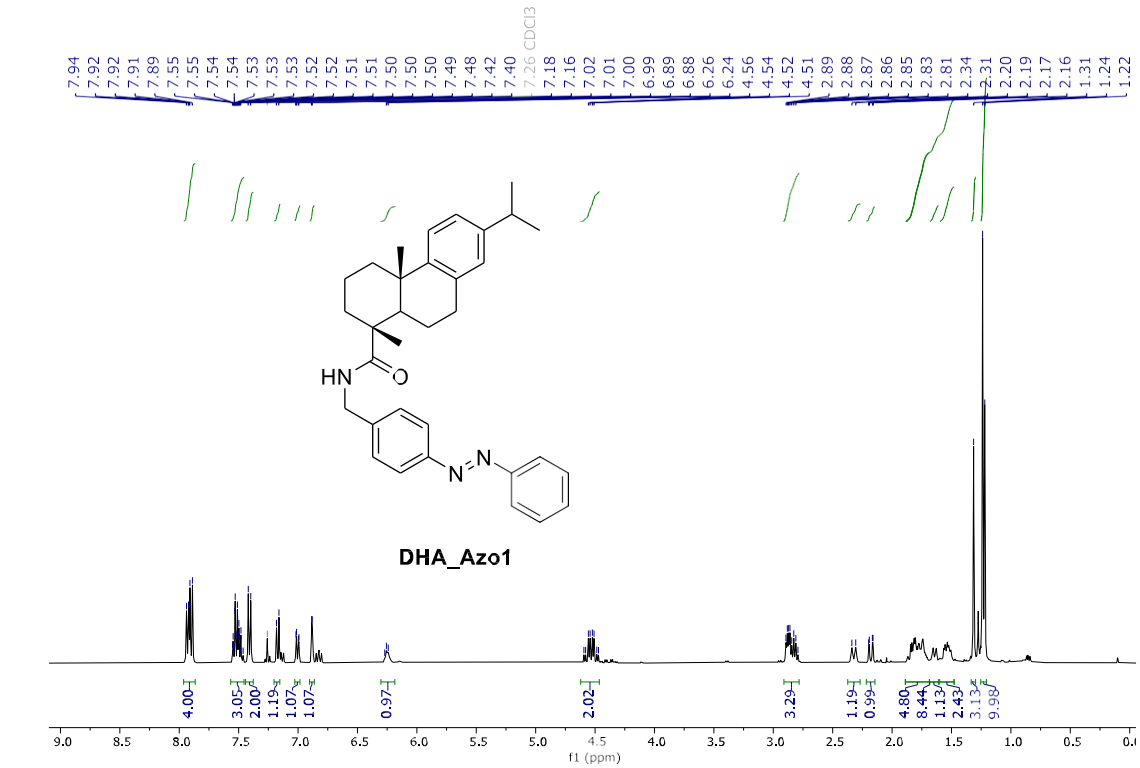
**$^1$H-NMR** (400 Hz, $CDCl_3$): δ 7.97-7.88 (m, $C_{Ar}H$ Azo, 4 H), 7.71 (d, *J* = 8.8 Hz, $C_{Ar}H$ Azo, 2 H), 7.67 (s, CON*H*, 1 H), 7.55-7.41 (m, $C_{Ar}H$ Azo, 3 H), 7.20 (d, *J* = 8.4 Hz, $C_{Ar}H$, 1 H), 7.03 (dd, *J* = 8.4, 2.0 Hz, $C_{Ar}H$, 1 H), 6.89 (d, *J* = 2.0 Hz, $C_{Ar}H$, 1 H), 2.96-2.88 (m, $CH_2$, 2 H), 2.84 (sept, *J* = 7.0 Hz, $CH(CH_3)_2$, 1 H), 2.38 (d, *J* = 13.2 Hz, C*H*, 1 H), 2.23 (dd, *J* = 12.4, 2.4 Hz, $CH_2$, 1 H ), 1.96-1.54 (m, 7 H), 1.44 (s, $CH_3$, 3 H), 1.28 (s, $CH_3$, 3 H), 1.24 (d, *J* = 7.0 Hz, $CH(CH_3)_2$, 6 H) ppm.

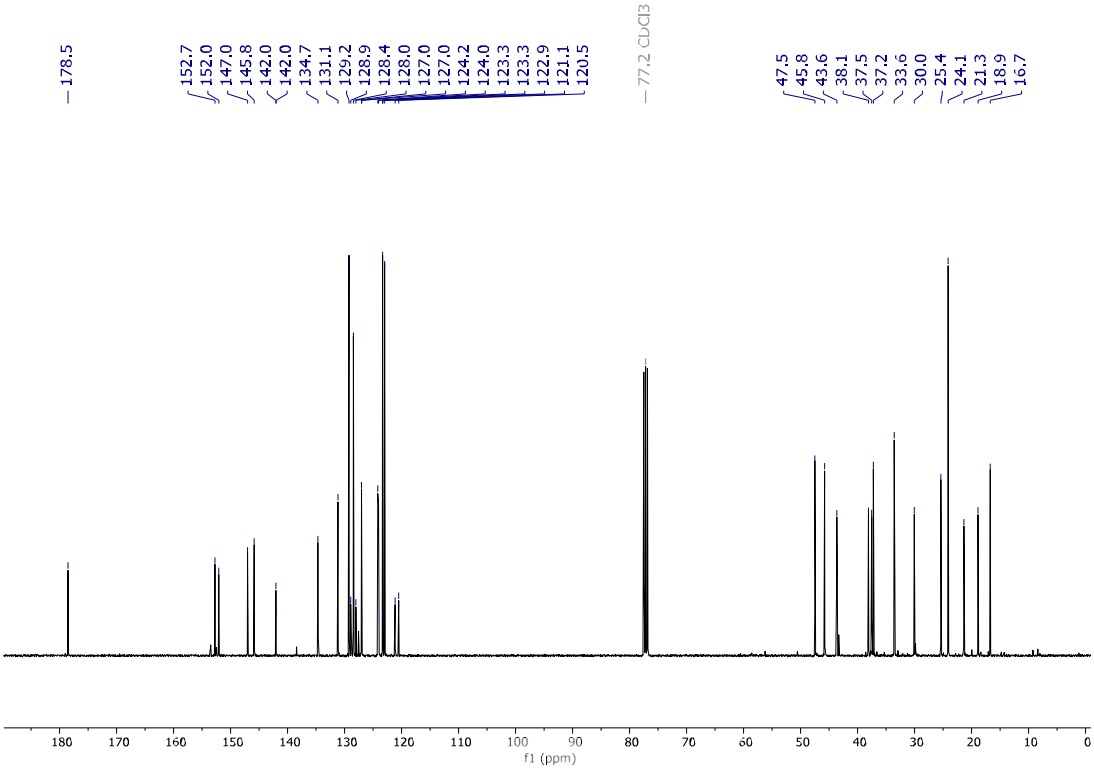
**$^{13}$C-NMR** (100 Hz, $CDCl_3$): δ 176.9 (*C*ONH), 152.8 ($C_{Ar}$N), 149.1 ($C_{Ar}$N), 146.9 ($C_{Ar}$), 146.0 ($C_{Ar}$), 140.8 ($C_{Ar}$), 134.6 ($C_{Ar}$), 130.9 ($C_{Ar}$H Azo), 129.2 ($C_{Ar}$H Azo), 127.1 ($C_{Ar}$H DHA), 124.2 ($C_{Ar}$H DHA), 124.1 ($C_{Ar}$H DHA), 124.1 ($C_{Ar}$H Azo), 122.9 ($C_{Ar}$H Azo), 120.2 ($C_{Ar}$H Azo), 48.6 (*C*), 46.0 (CH), 38.1 ($CH_2$), 37.5 (C), 37.3 ($CH_2$), 33.6 ($CH(CH_3)_2$), 30.0 ($CH_2$), 25.3 ($CH_3$), 24.1 ($CH(CH_3)_2$), 24.1 ($CH(CH_3)_2$), 21.4 ($CH_2$), 18.9 ($CH_2$), 16.8 ($CH_3$) ppm.

**HRMS** calcd for $C_{32}H_{38}ON_3$ $[M+H]^+$ :480.3009, found 480.3020.

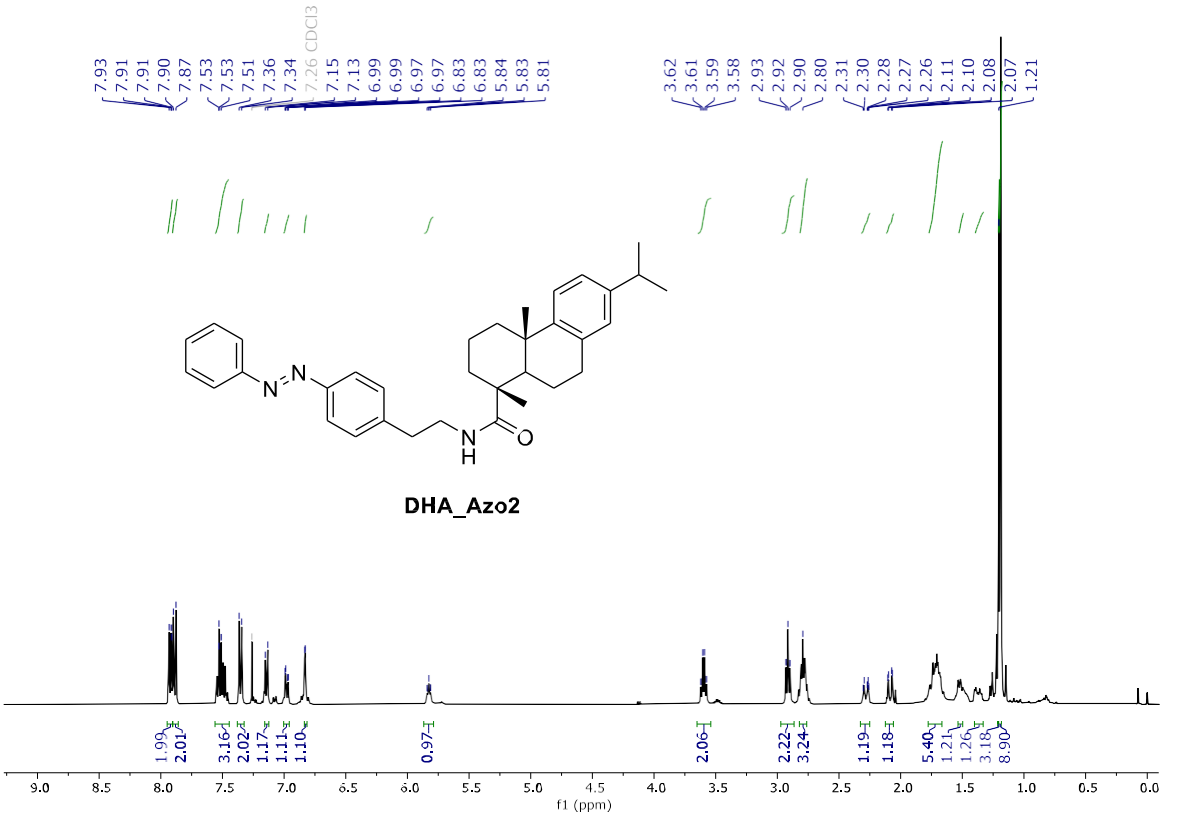
# 3. NMR Spectra

**(1*R*,4a*S*)-7-isopropyl-1,4a-dimethyl-*N*-(4-((*E*)-phenyldiazenyl)benzyl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (DHA_Azo1).**

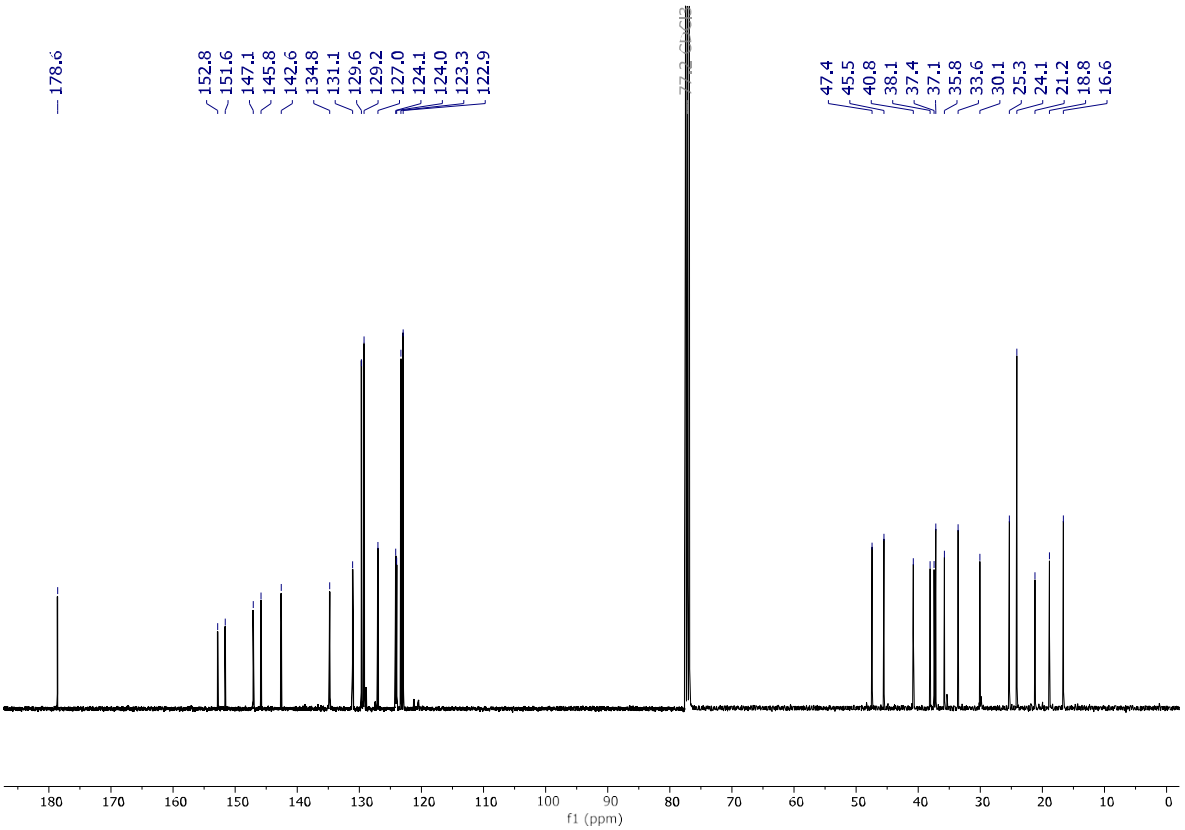


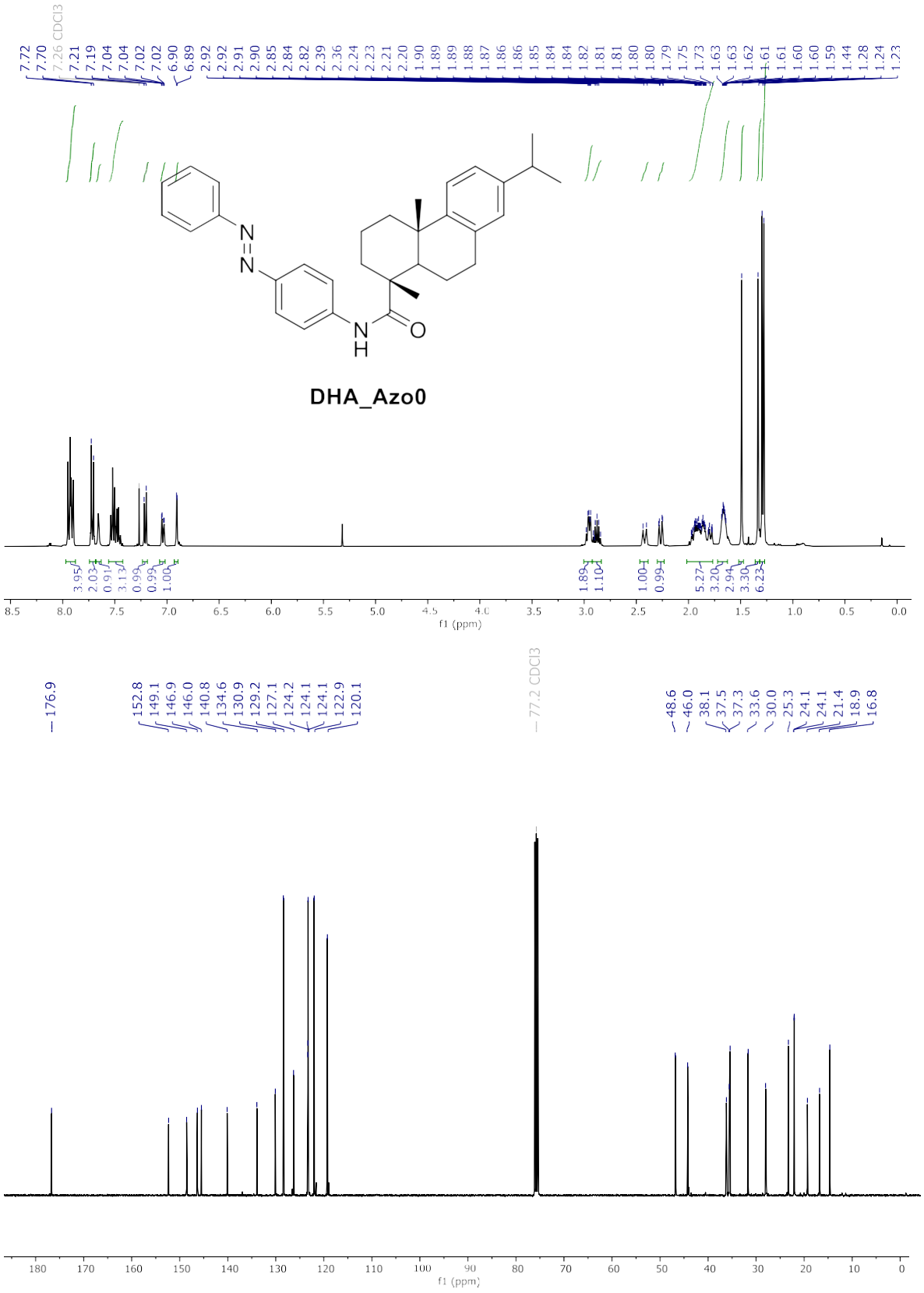
**(1*R*,4a*S*)-7-isopropyl-1,4a-dimethyl-*N*-(4-((*E*)-phenyldiazenyl)phenethyl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (DHA_Azo2).**



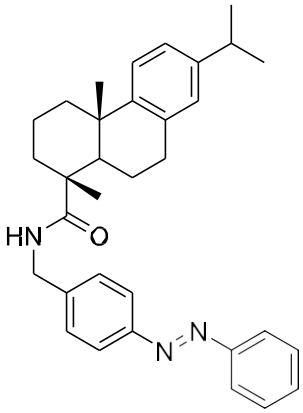

**DHA_Azo2**



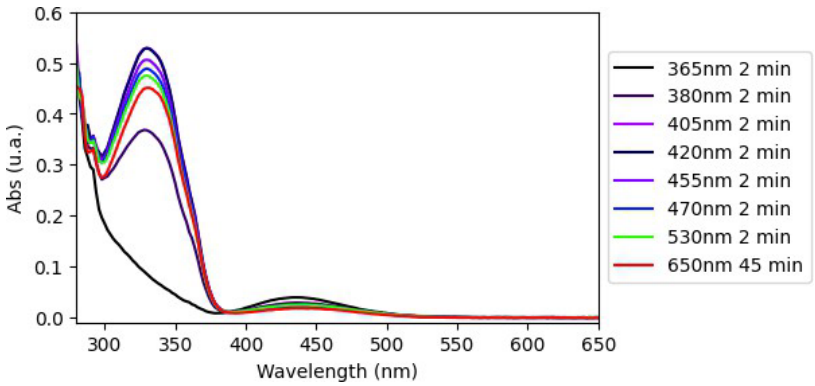
**(1*R*,4a*S*)-7-isopropyl-1,4a-dimethyl-*N*-(4-((*E*)-phenyldiazenyl)phenyl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (DHA_Azo0).**

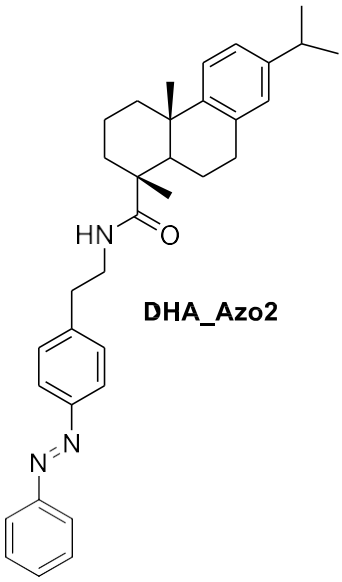


# 4. UV-Vis Spectra

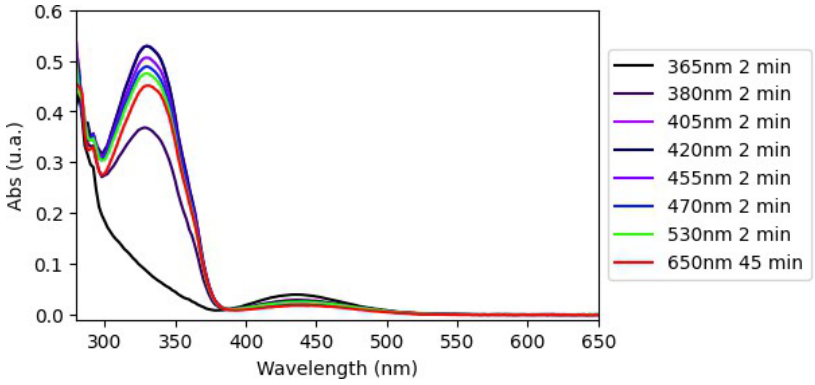


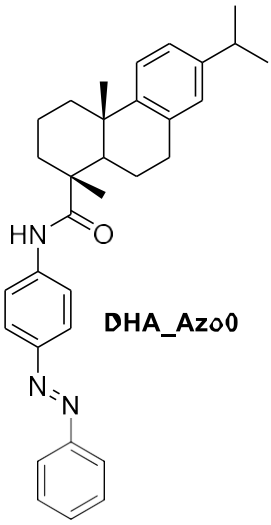

**DHA_Azo1**



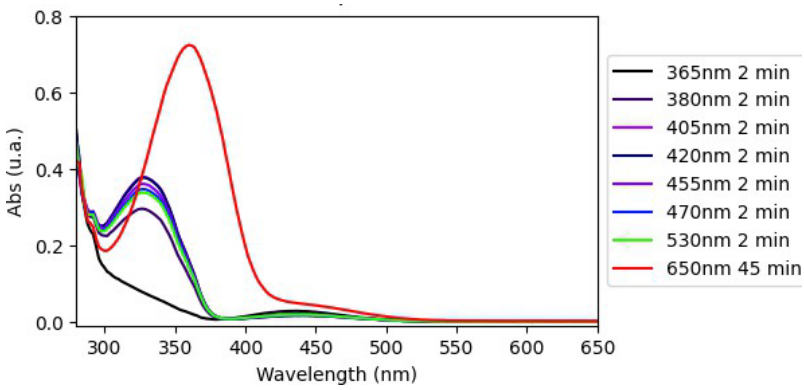




**DHA_Azo2**




**DHA_Azo0**



## 5. Antimicrobial activity: MIC and MBC values of the tested samples

**Table S1:** MIC and MBC values of the tested samples in µg/mL. Data represent the median values of at least three replicates.

| | ***S. aureus 25923*** | | ***S. aureus 43866*** | | ***E. faecalis*** | | ***E. coli*** | | ***P. aeruginosa*** | | ***S. cerevisiae*** | | ***C. albicans*** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** |
| **DHA_Azo1_Blue** | 125 | 500 | 31.25 | 500 | 62.5 | 500 | 62.5 | 250 | 62.5 | 500 | 62.5 | 125 | 62.5 | 125 |
| **DHA_Azo1_UV** | 125 | 500 | 62.5 | 500 | 62.5 | 500 | 62.5 | 500 | 125 | 500 | 62.5 | 62.5 | 31.25 | 125 |
| **DHA_Azo1_Sun** | 125 | 500 | 31.25 | 500 | 125 | 500 | 125 | 500 | 125 | 500 | 31.25 | 31.25 | 31.25 | 62.5 |
| **DHA_Azo2_Blue** | 125 | 500 | 62.5 | 500 | 62.5 | 500 | 62.5 | 250 | 62.5 | 500 | 62.5 | 125 | 62.5 | 125 |
| **DHA_Azo2_UV** | 125 | 500 | 62.5 | 500 | 62.5 | 500 | 62.5 | 500 | 125 | 500 | 62.5 | 125 | 31.25 | 62.5 |
| **DHA_Azo2_Sun** | 125 | 500 | 31.25 | <125 | 125 | <500 | 125 | <500 | 125 | 500 | 31.25 | 31.25 | 31.25 | 125 |
| **DHA_Azo0_Blue** | 125 | <500 | 62.5 | 500 | 31.25 | <500 | 62.5 | 500 | 125 | 500 | 62.5 | 250 | 62.5 | 62.5 |
| **DHA_Azo0_UV** | 125 | 500 | 62.5 | 500 | 62.5 | 500 | 125 | 500 | 125 | 500 | 62.5 | 125 | 62.5 | 125 |
| **DHA_Azo0_Sun** | 125 | 500 | 62.5 | <250 | 125 | <500 | 125 | 500 | 125 | 500 | 31.25 | 31.25 | 31.25 | 125 |
| **DHA_Blue** | 3.91 | <31.25 | 0.98 | 3.91 | 0.98 | 7.81 | 62.5 | 250 | 62.5 | 500 | 62.5 | 125 | 31.25 | 62.5 |
| **DHA_UV** | 15.63 | 125 | 0.98 | 7.81 | 1.95 | 15.63 | 62.5 | 500 | 62.5 | 250 | 62.5 | 62.5 | 31.25 | 125 |
| **DHA_Sun** | 3.91 | nt | 0.49 | <7.81 | 1.95 | <15.63 | 62.5 | 500 | 125 | 500 | 31.25 | 31.25 | 15.63 | 62.5 |
| **Positive control** | 0.98 | 500 | 0.49 | nt | >0.49 | <500 | 0.49 | 500 | 0.98 | 500 | 7.8 | 500 | 1.95 | 62.5 |
| | VAN | | VAN | | VAN | | NOR | | NOR | | NYS | | NYS | |

nt, not tested; VAN – Vancomycin; NYS – Nystatin; NOR – Norfloxacin