Supplementary Material

Oxime esters on 4-nitrobenzaldehyde and 9,10-anthraquinone-2-carboxaldehyde templates: DNA- and albumin-binding and photocleavage studies

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Table of Contents

A. NMR Spectra

4-Nitrobenzaldehyde *O*-(4-bromobenzoyl) oxime (5a)

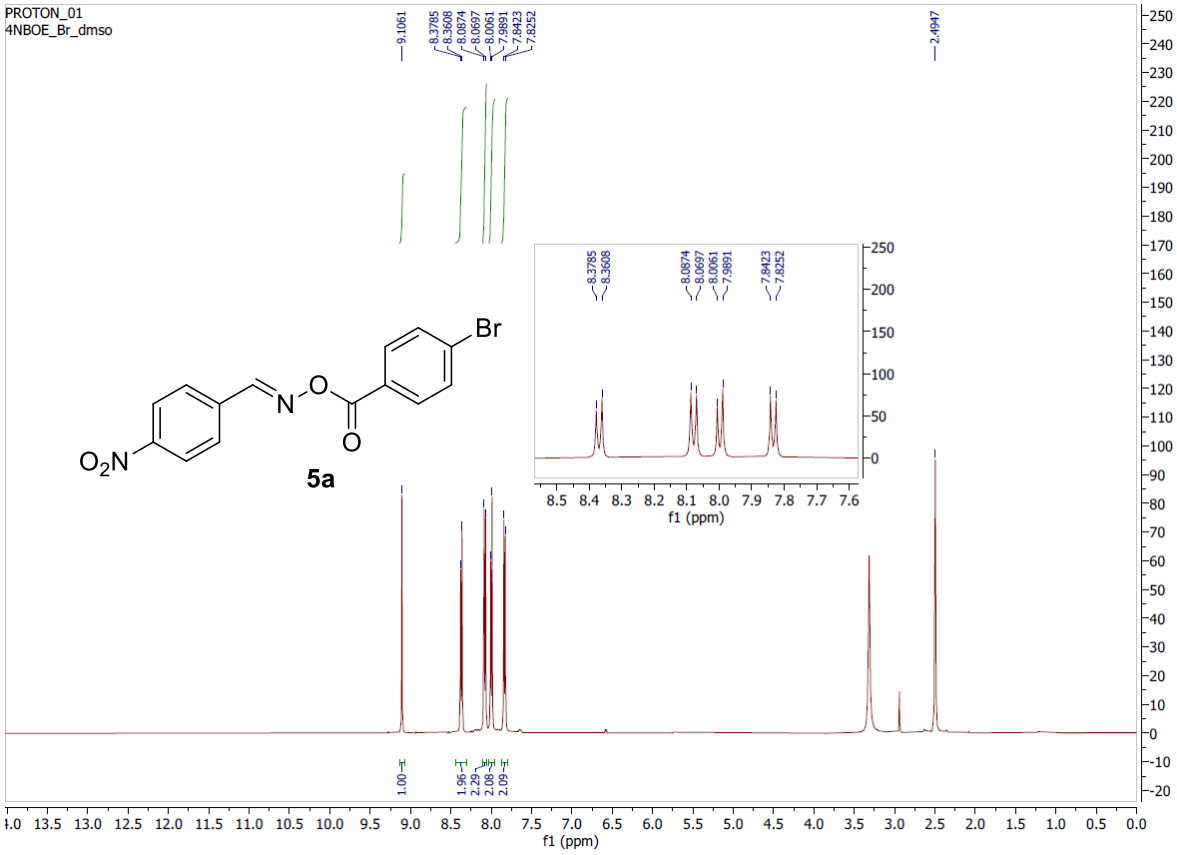


Figure S1. ^{1}H NMR spectra of 4-nitrobenzaldehyde *O*-(4-bromobenzoyl) oxime.

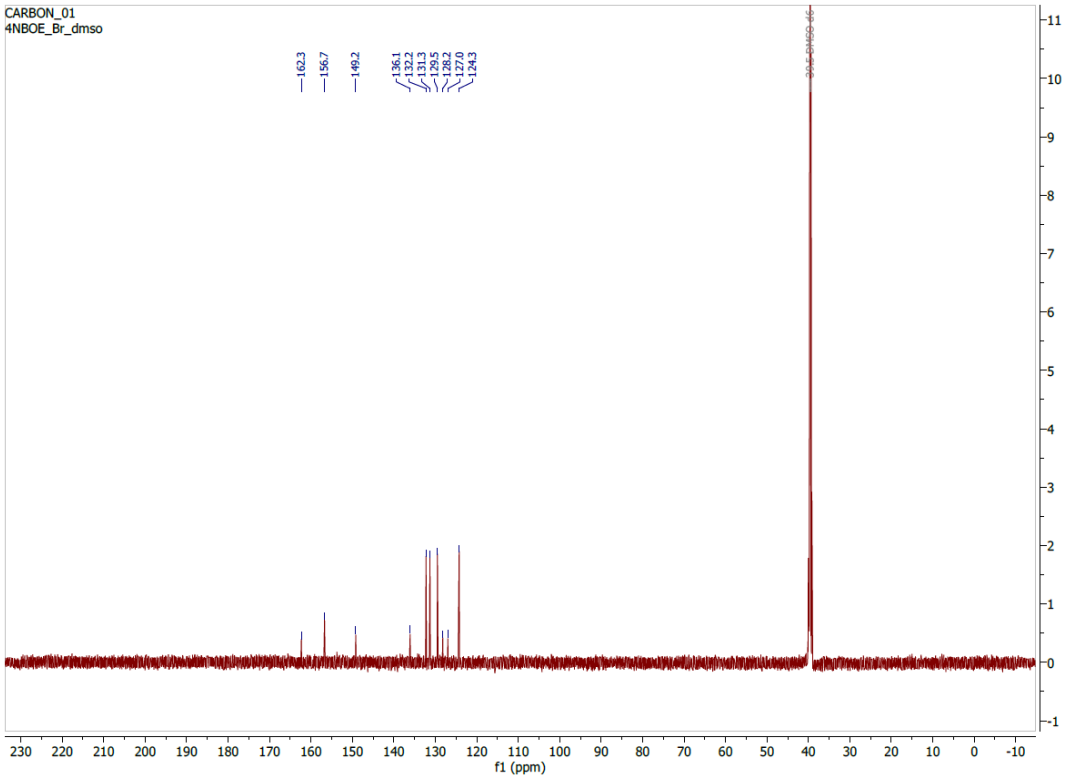


Figure S2. ^{13}C NMR spectra of 4-nitrobenzaldehyde *O*-(4-bromobenzoyl) oxime.

4-Nitrobenzaldehyde *O*-(4-methoxybenzoyl) oxime (5b)

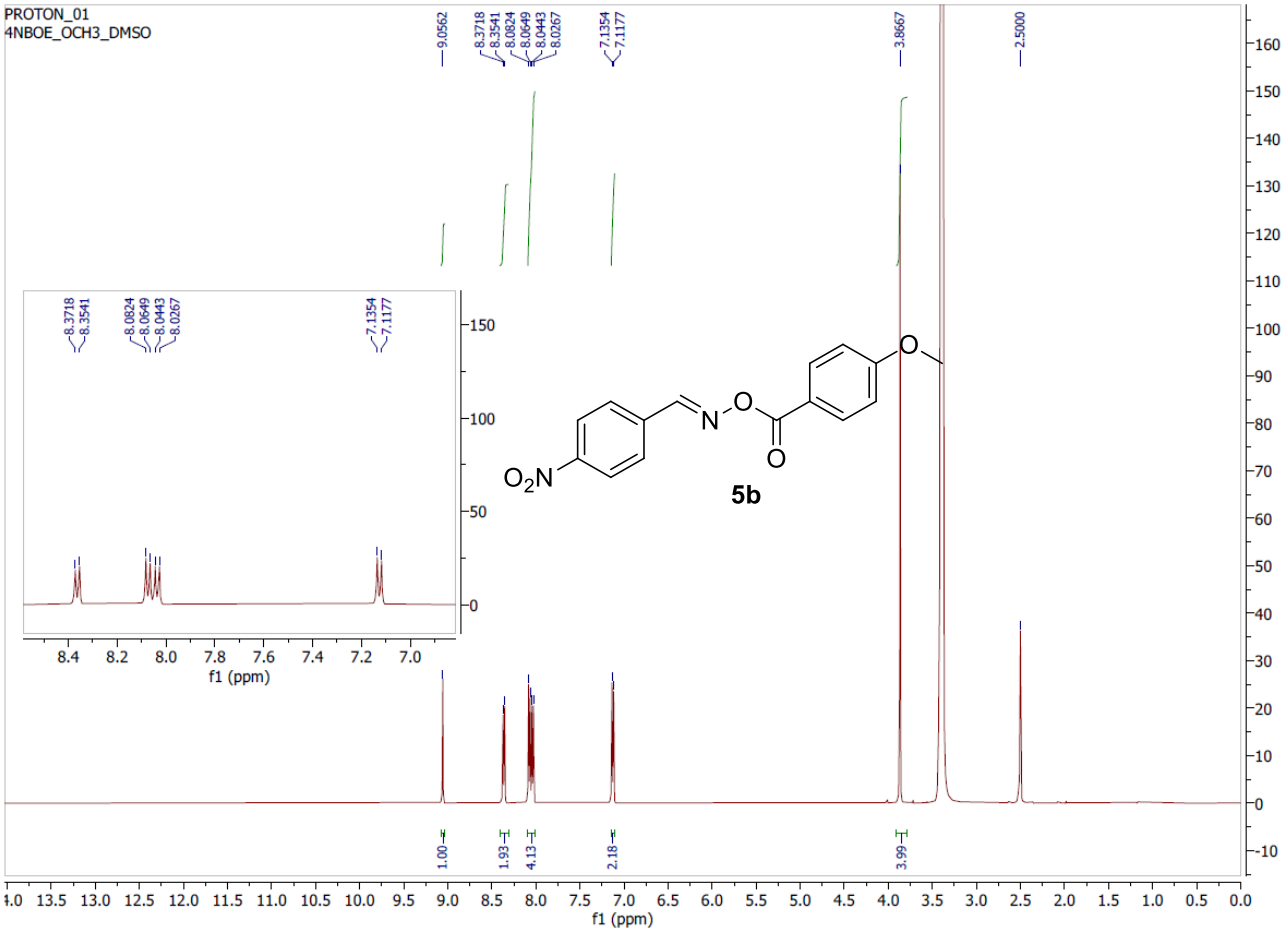


Figure S3. ^{1}H NMR spectra of 4-nitrobenzaldehyde *O*-(4-methoxybenzoyl) oxime.

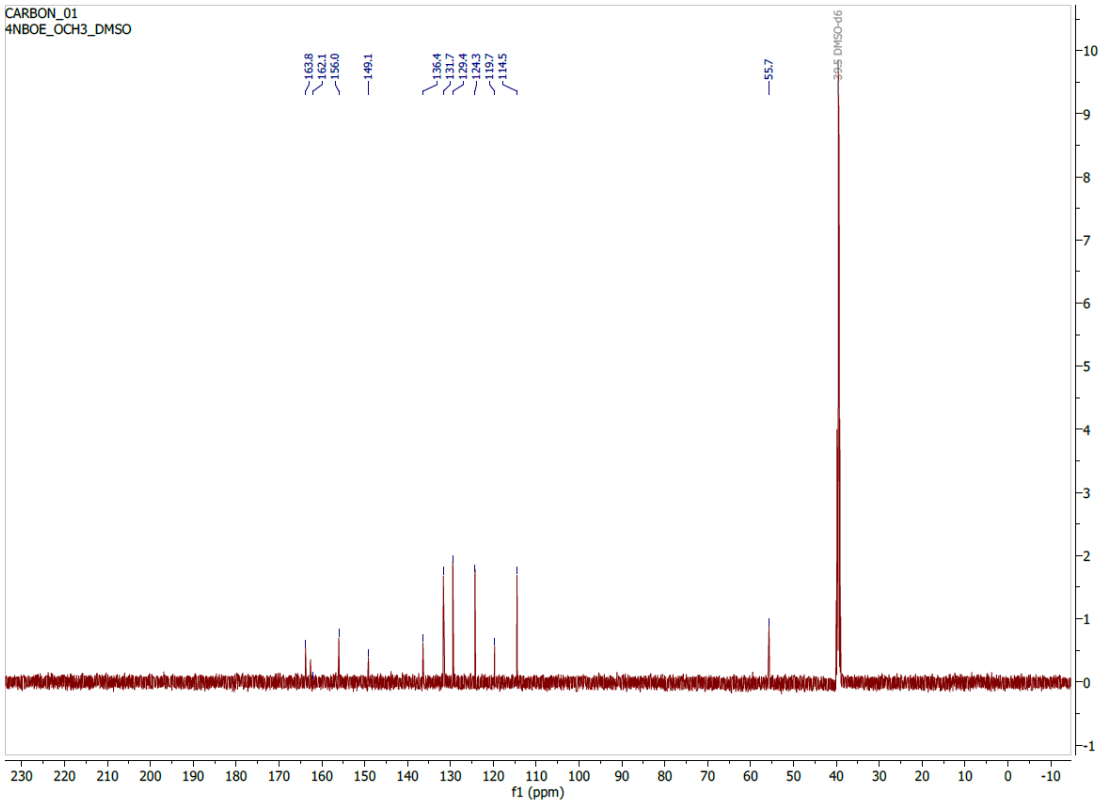


Figure S4. ^{13}C NMR spectra of 4-nitrobenzaldehyde *O*-(4-methoxybenzoyl) oxime.

4-Nitrobenzaldehyde *O*-isobutanoyl oxime (5c)

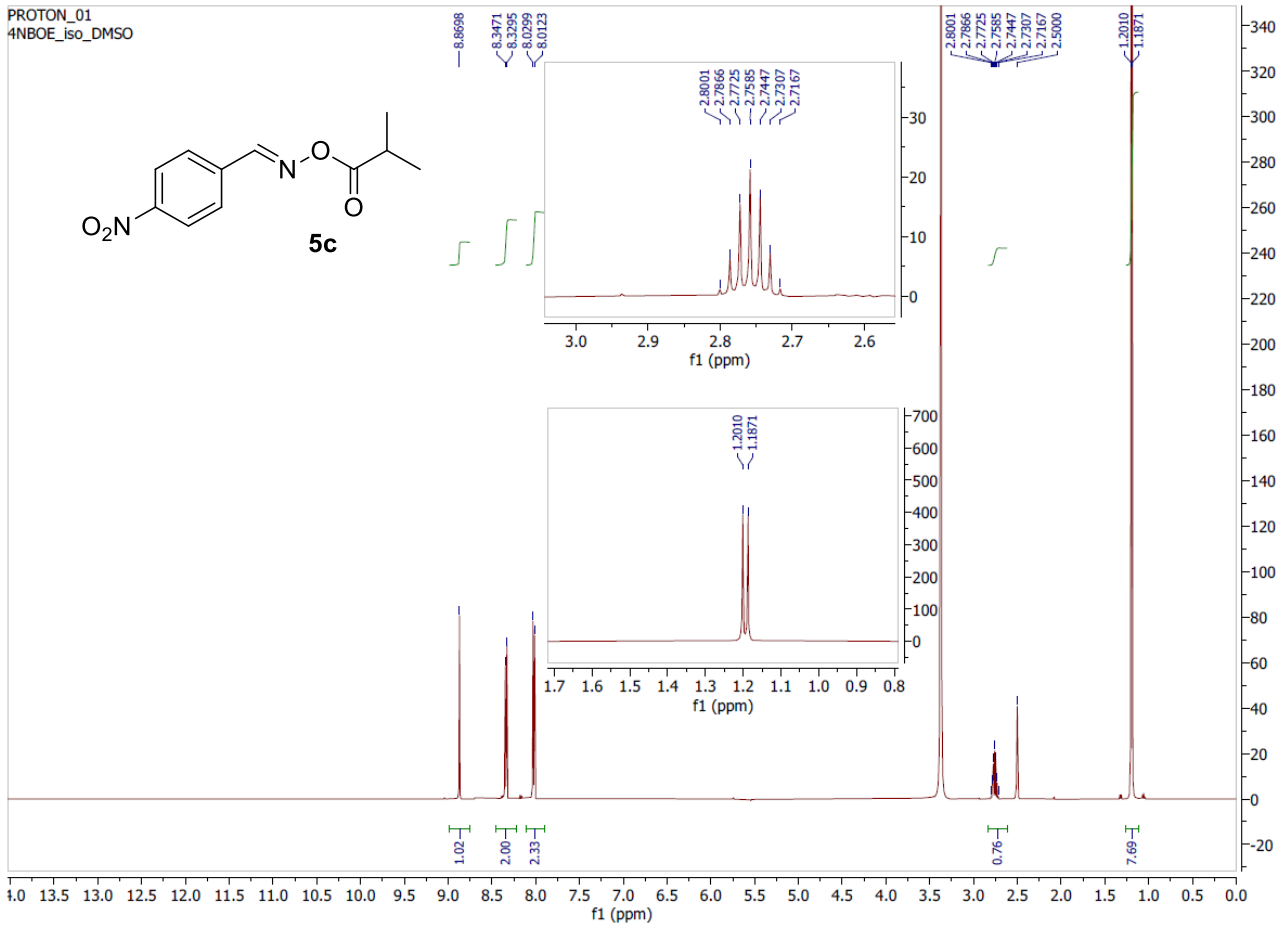


Figure S5. ^{1}H NMR spectra of 4-nitrobenzaldehyde *O*-isobutanoyl oxime.

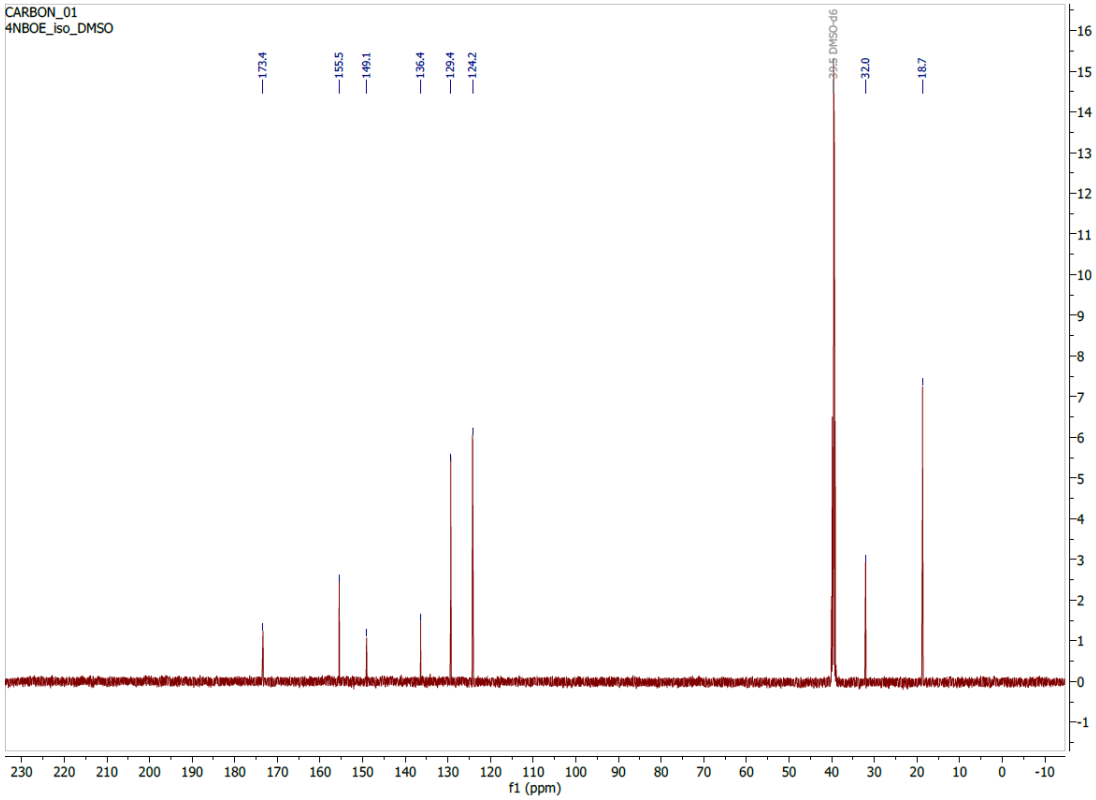


Figure S6. ^{13}C NMR spectra of 4-nitrobenzaldehyde *O*-isobutanoyl oxime.

4-Nitrobenzaldehyde *O*-(2-phenylpropanoyl) oxime (5d)

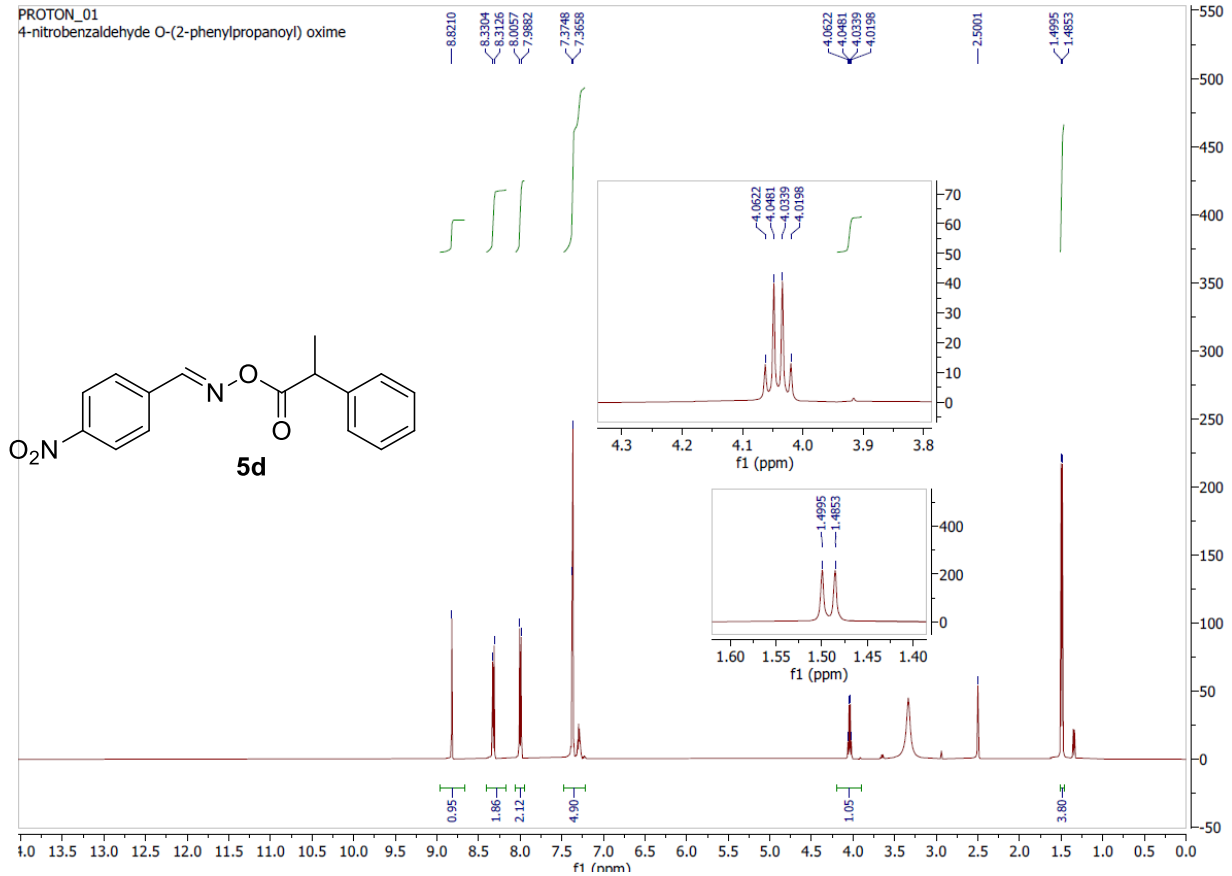


Figure S7. ^{1}H NMR spectra of 4-nitrobenzaldehyde *O*-(2-phenylpropanoyl) oxime.

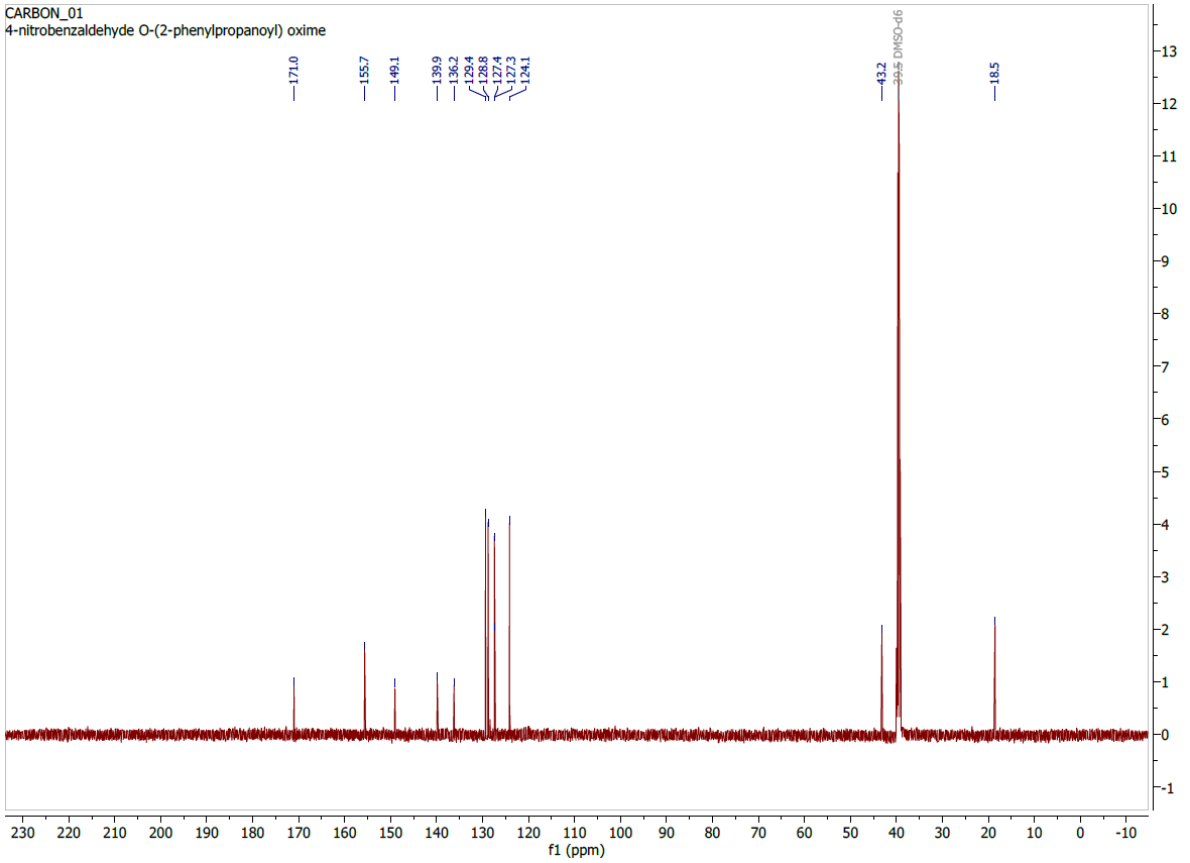


Figure S8. ^{13}C NMR spectra of 4-nitrobenzaldehyde *O*-(2-phenylpropanoyl) oxime.

9,10-Dioxo-9,10-dihydroanthracene-2-carbaldehyde (7)

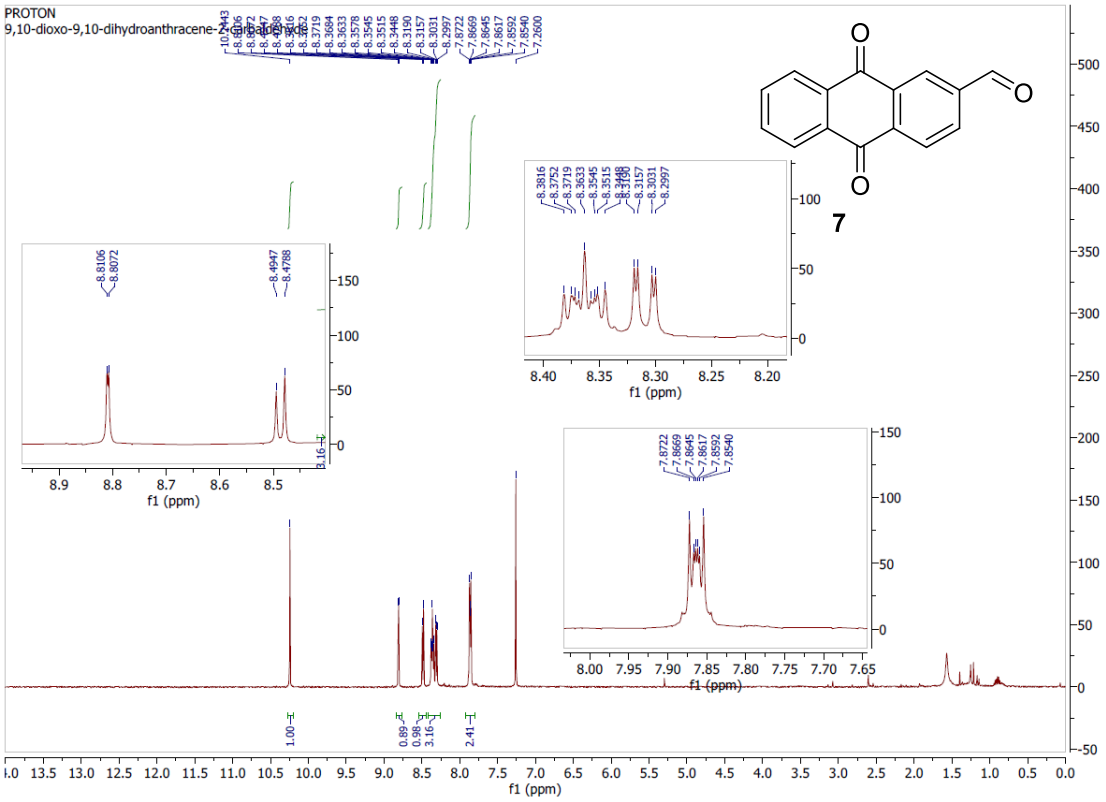


Figure S9. ^{1}H NMR spectra of 9,10-dioxo-9,10-dihydroanthracene-2-carbaldehyde.

9,10-Dioxo-9,10-dihydroanthracene-2-carbaldehyde oxime (8)

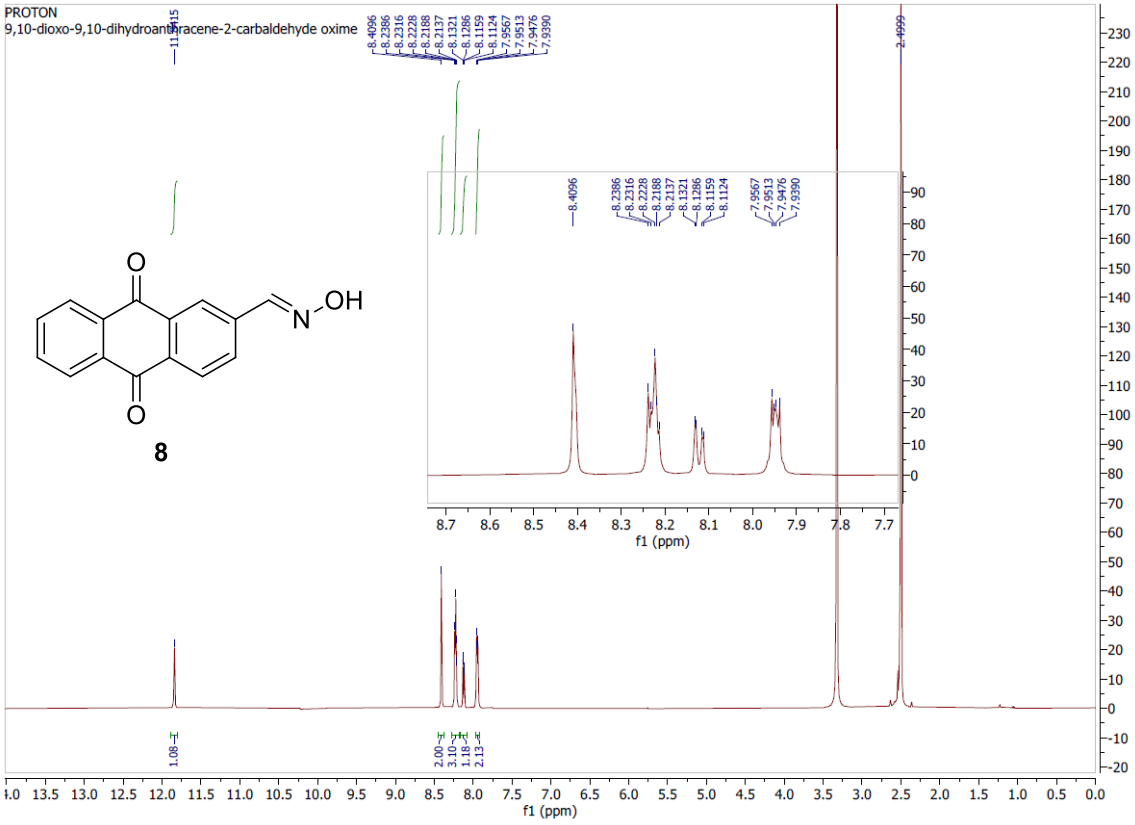


Figure S10. ^{1}H NMR spectra of 9,10-dioxo-9,10-dihydroanthracene-2-carbaldehyde oxime.

9,10-Dioxo-9,10-dihydroanthracene-2-carbaldehyde *O*-(4-bromobenzoyl) oxime (9a)

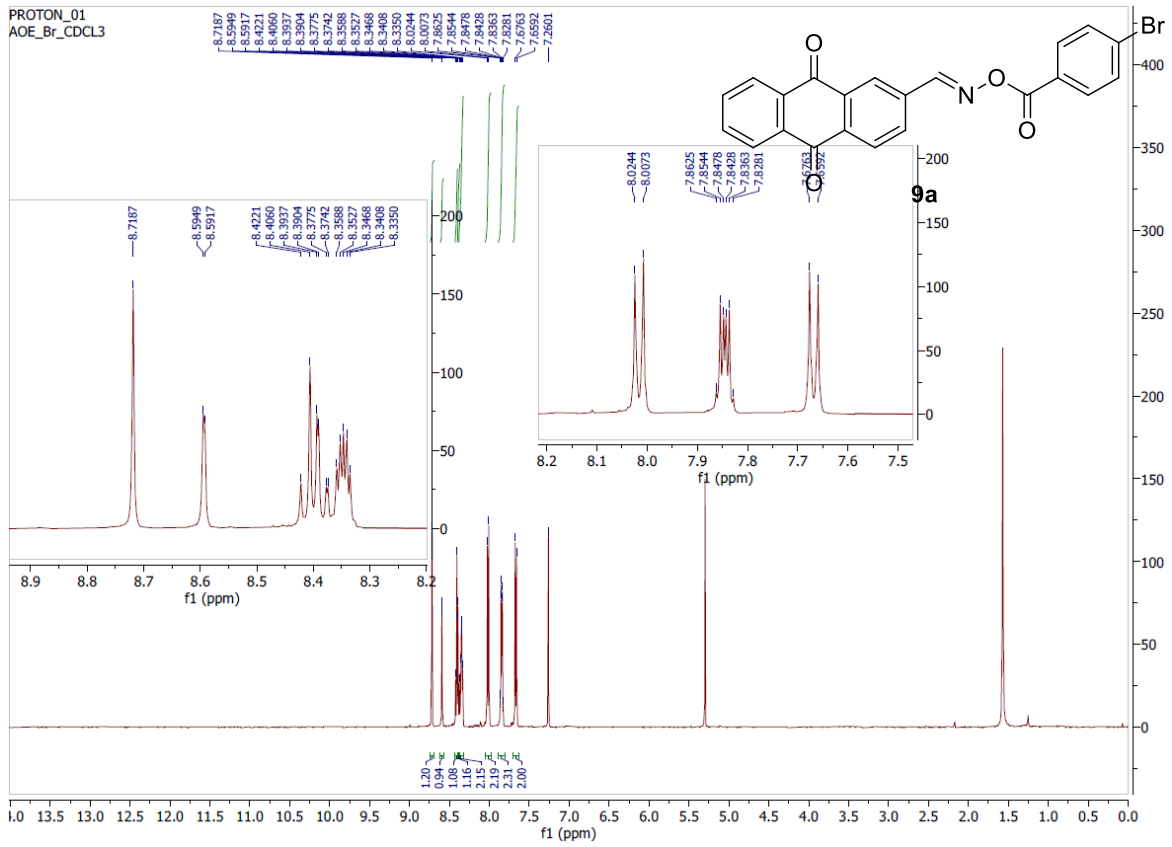


Figure S11. ^{1}H NMR spectra of 9,10-dioxo-9,10-dihydroanthracene-2-carbaldehyde *O*-(4-bromobenzoyl) oxime.

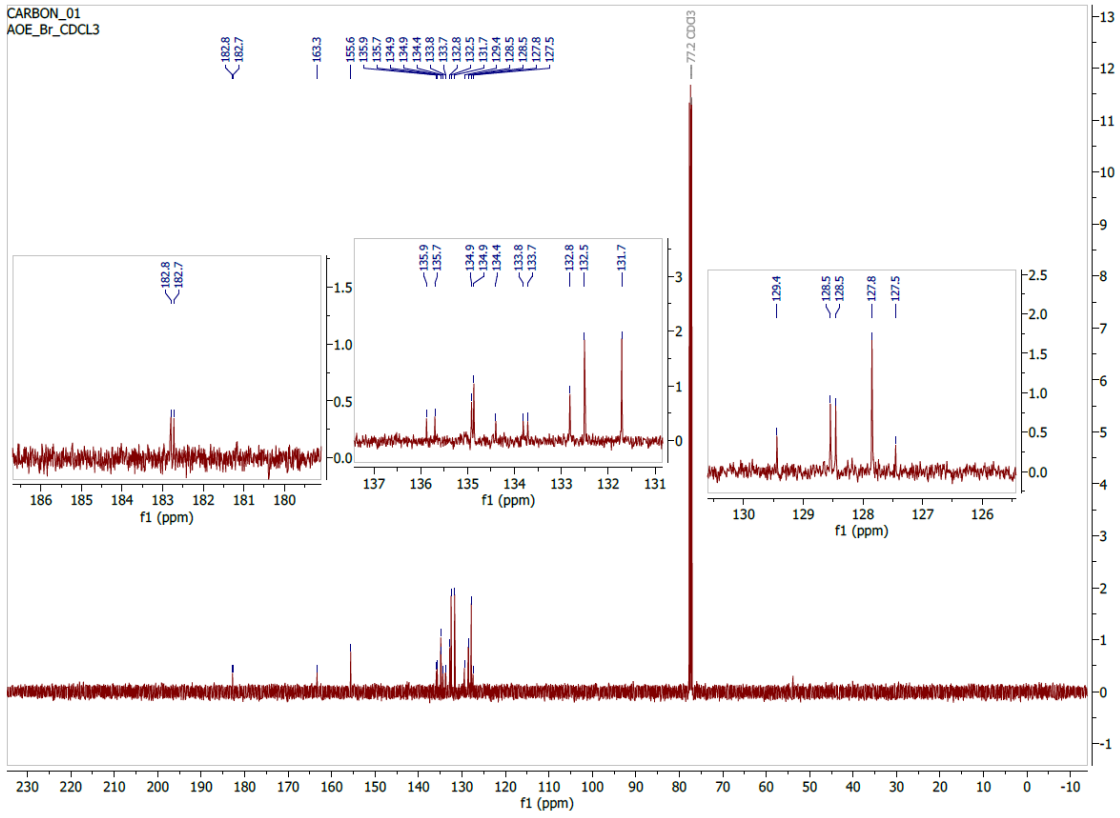


Figure S12. ^{13}C NMR spectra of 9,10-dioxo-9,10-dihydroanthracene-2-carbaldehyde *O*-(4-bromobenzoyl) oxime.

B. Binding Assays with Calf Thymus DNA

The synthesized compounds were dissolved in DMSO (1 mM). These solutions were further mixed along with DNA buffer solutions (containing 150 mM NaCl and 15 mM trisodium citrate at pH 7.0) in which case no more than 5% (v/v) DMSO was used due to low solubility of the compounds. The interaction of the compounds with CT DNA was studied with UV–vis spectroscopy, cyclic voltammetry and viscosity measurements and via competitive studies with EB by fluorescence emission spectroscopy. It should be noted that the DMSO effect was taken into account and appropriate corrections were performed.

1. UV-Vis spectroscopy

UV-vis spectroscopy was employed to investigate the interaction between the compounds and CT DNA. The UV-vis spectra of each compound recorded in the presence of CT DNA in various mixing ratios are presented in Figure S13.

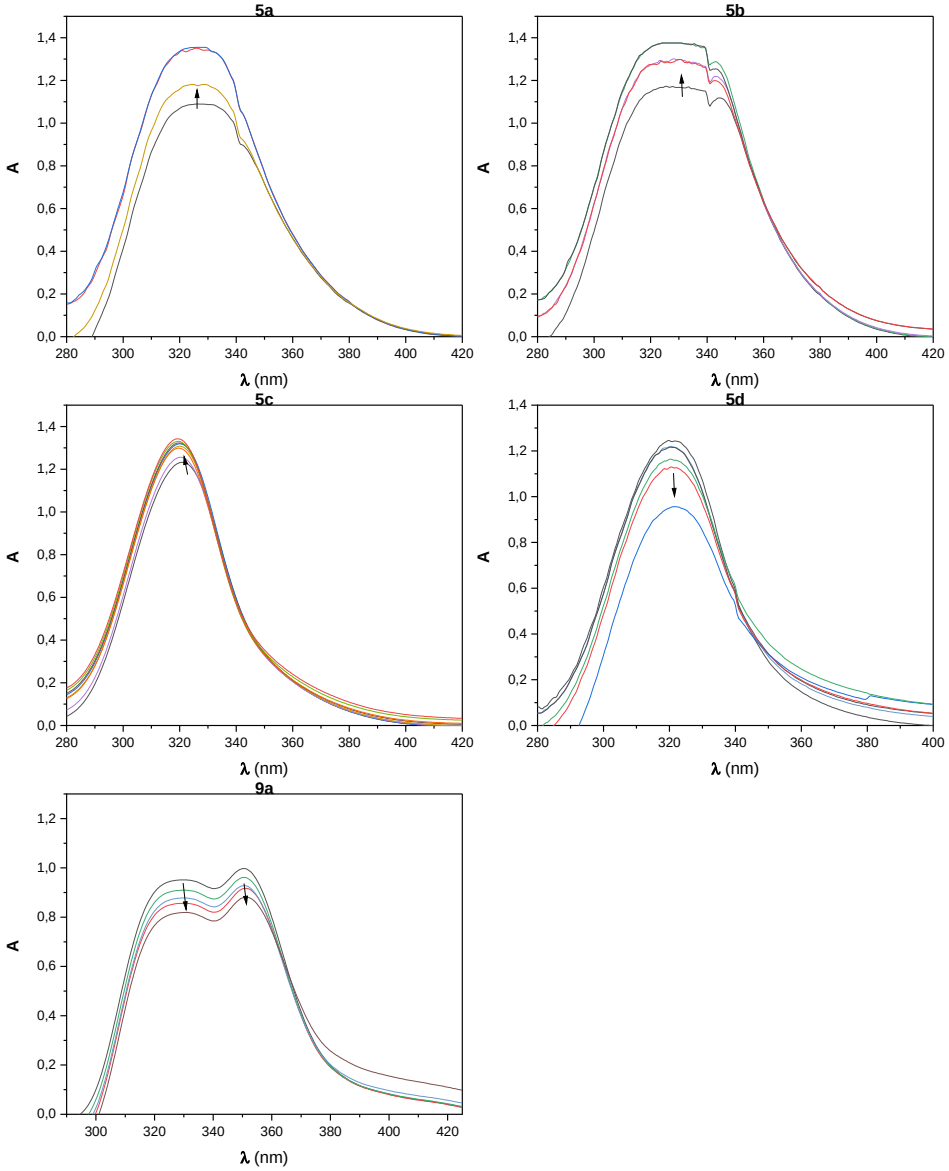


Figure S13. UV−vis spectra of a DMSO solution of compounds **5a-d** and **9a** in the presence of increasing amounts of CT DNA. The arrows show the changes upon increasing amounts of CT DNA.

From the plots [DNA]/(ε_A-ε_F) versus [DNA] (Figure S14), the K_b constant can be calculated according to the Wolfe-Shimer equation[1]:

$$\frac{[DNA]}{\varepsilon_A-\varepsilon_f} = \frac{[DNA]}{\varepsilon_b-\varepsilon_f} + \frac{1}{Kb(\varepsilon_b-\varepsilon_f)} \qquad \text{(Equation S1)}$$

where [DNA] is the concentration of DNA in base pairs, ε_A=A/[compound], ε_f the extinction coefficient for the free compound while ε_b the corresponding coefficient for the compound in the fully bound form. K_b is determined by the ratio of slope to the y intercept in plots [DNA]/(ε_A-ε_f) versus [DNA].

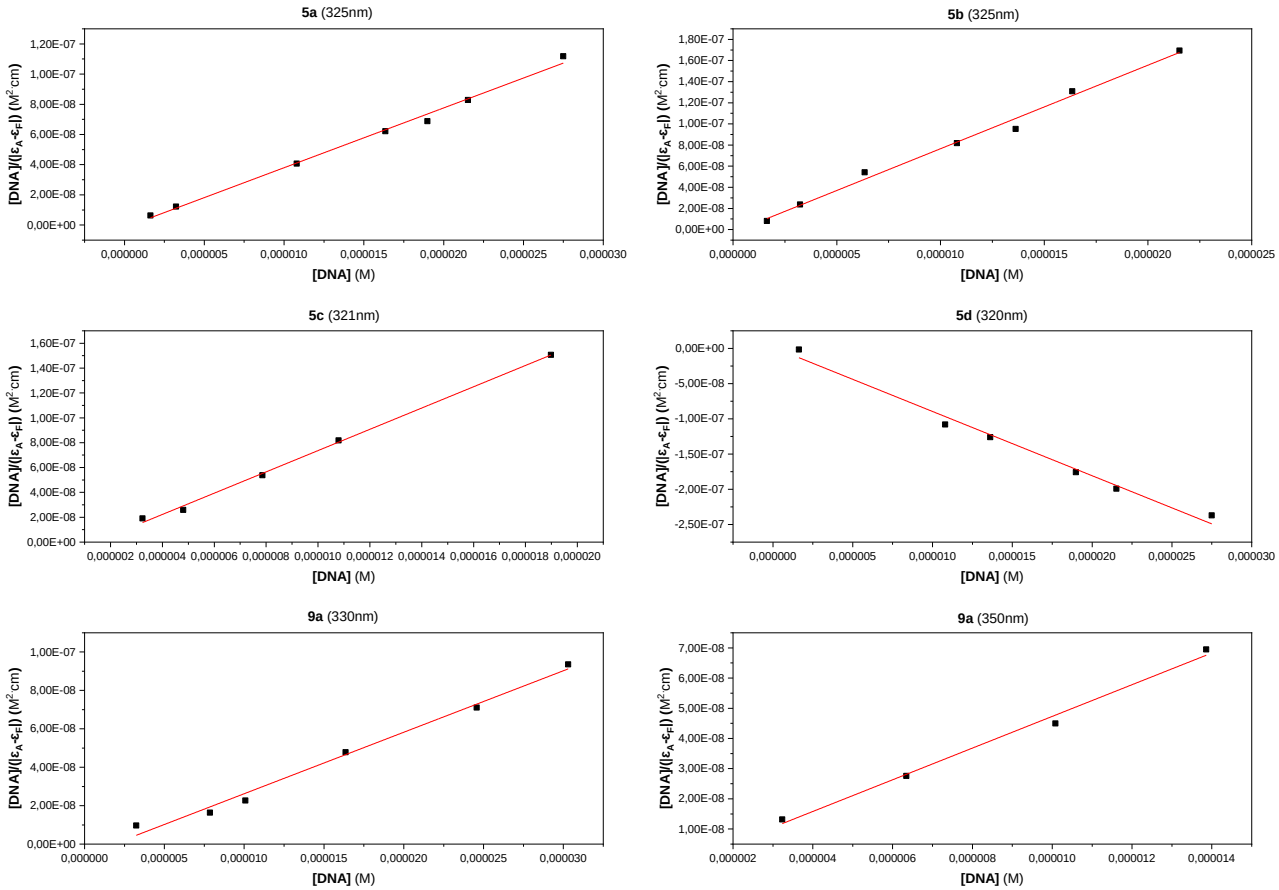


Figure S14. From left to right and top to bottom [DNA]/(e_A-e_F) vs. [DNA] plots of ester **5a-d**, **9a**.

2. Viscosity measurements

The interaction mechanism between DNA and each compound was examined by measuring the viscosity of the CT DNA buffer solution (where the concentration of DNA was 0.1 mM) in the presence of increasing amounts of the compounds up to value r = [compound]/[DNA] = 0.27. All measurements were performed at room temperature and the obtained data are presented in the main article as $(\eta/\eta_0)^{1/3}$ *versus* r, where η is the viscosity of DNA in the presence of the compound and η_0 is the viscosity of DNA alone in buffer solution.

3. EB competitive studies

The competitive studies of the compounds with EB for the DNA-intercalating sites (by displacing it from its EB-DNA adduct) were investigated with fluorescence emission spectroscopy. The EB-DNA adduct was formed by treating 40 μM EB and 45 μM CT DNA in buffer solution. The interaction was studied by adding increasing amounts of each compound and the fluorescence emission spectra of the EB-DNA solution were recorded (Figure S15).

Moreover, using the I_0/I *versus* [compound] plots (Figure S16), the Stern-Volmer constant (K_{SV}) was calculated along with the quenching constant k_q from the following equation[2]:

$$4. \quad \frac{I_0}{I} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q] \qquad \text{(Equation S2)}$$

where I_0 and I correspond to the emission intensities of the EB-DNA solution in the absence and the presence of the compound, respectively, τ_0 is the average lifetime of the emitting system (EB-DNA) without the quencher (23 ns)[3] and k_q the quenching constant. The quenching constant (k_q, in $M^{-1}s^{-1}$) of the compound was calculated according to equation S3:

$$5. \quad K_{SV} = k_q \tau_0 \qquad \text{(Equation S3)}$$

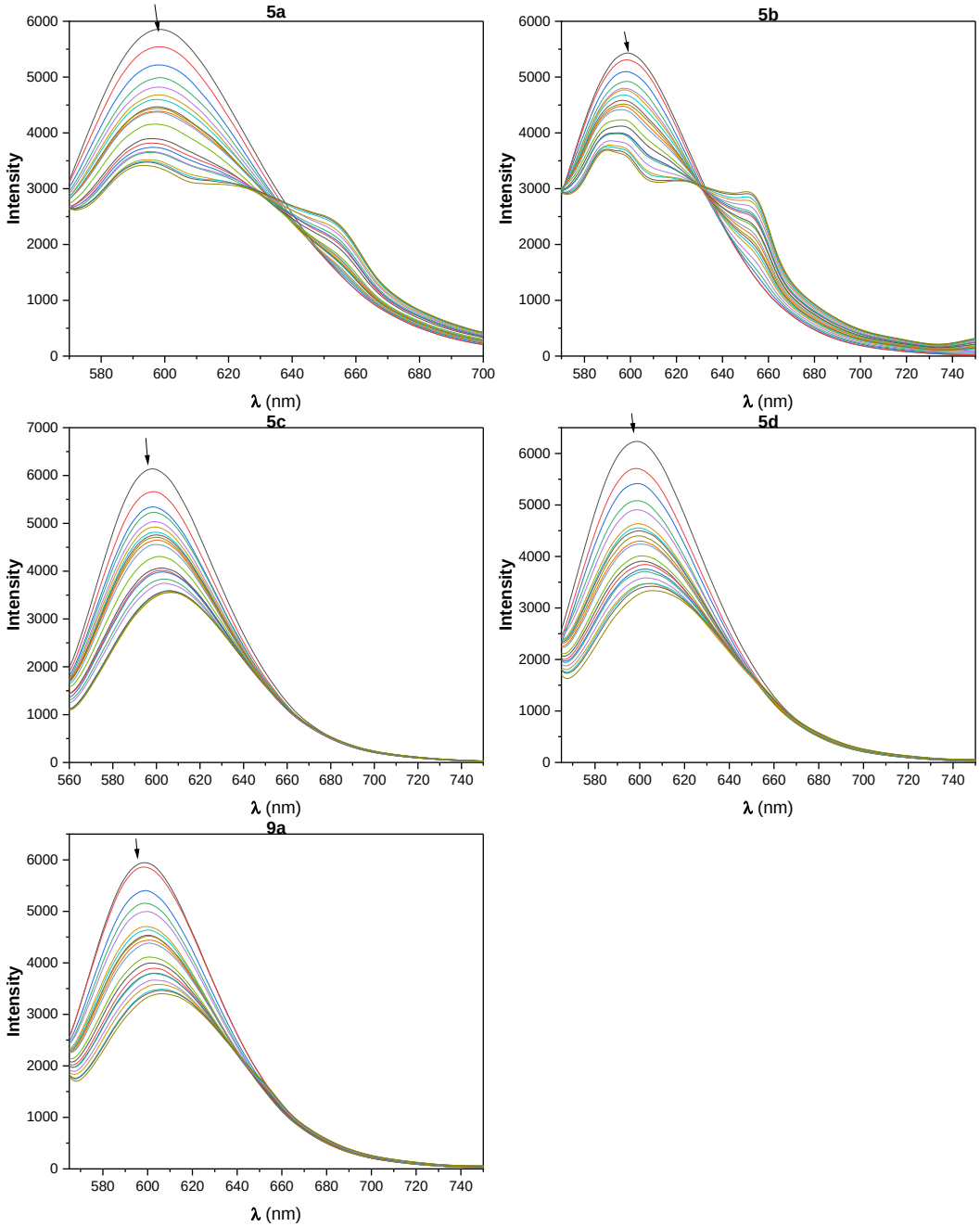


Figure S15. Fluorescence emission spectra ($\lambda_{excitation}$ = 540 nm) for EB-DNA ([EB] = 40 µM, [DNA] = 45 µM,) in buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0) in the absence and presence of increasing amounts of compounds **5a-d** and **9a**. The arrow shows the changes of intensity upon increasing amounts of the compound.

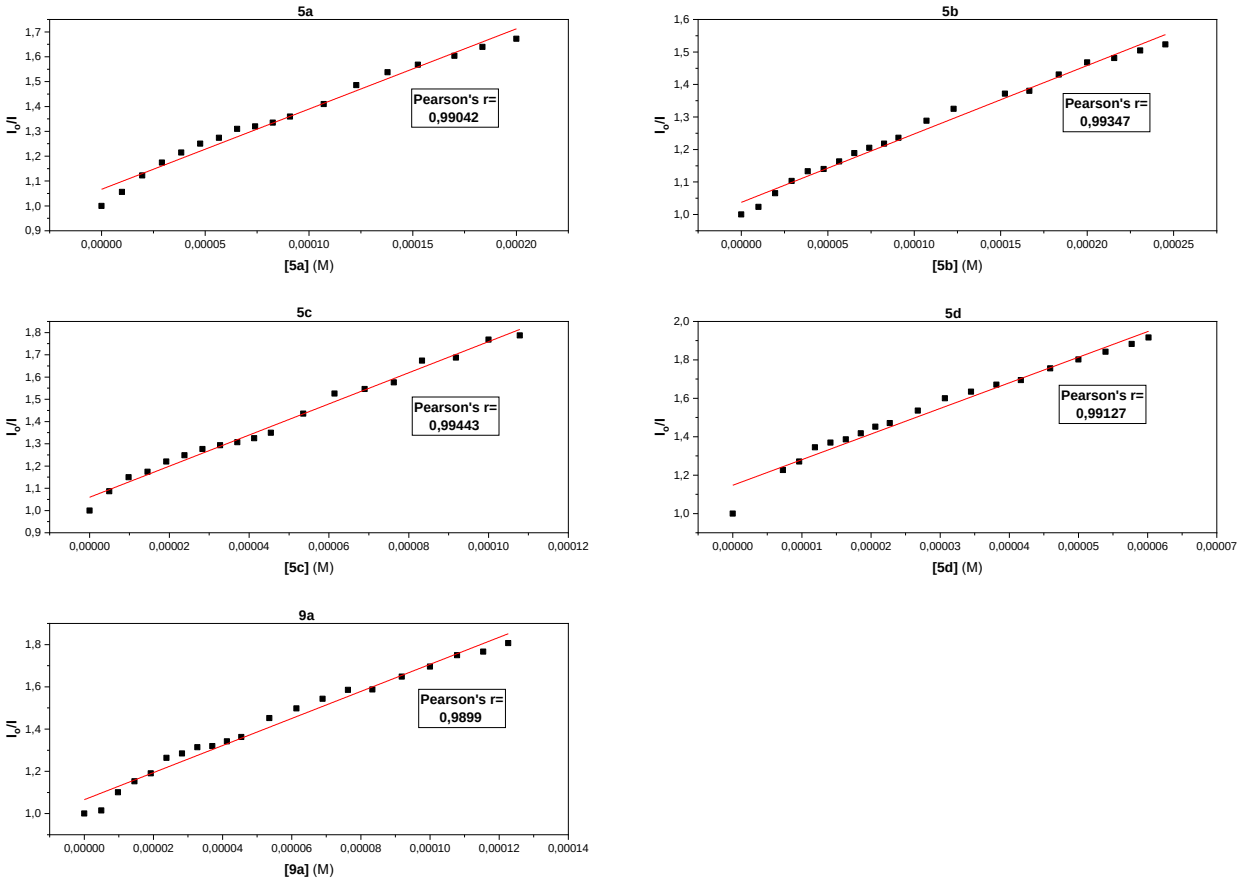


Figure S16. From left to right Stern−Volmer plots of the EB−DNA quenching experiments upon addition of compounds **5a-d** and **9a**.

C. Albumin Assays

The albumin-binding study was conducted to examine whether the synthesized compounds can bind to a carrier protein like the albumins BSA and HSA. This study was performed with tryptophan fluorescence quenching experiments using BSA (3 μM) or HSA (3 μM) in buffer (containing 15 mM trisodium citrate and 150 mM NaCl at pH 7.0). The fluorescence emission spectra were recorded with an excitation wavelength of 295 nm. The quenching of the emission intensity of tryptophan residues of BSA at 345 nm or HSA at 340 nm was monitored using fluorescence emission spectroscopy (Figures S17 and S20) and by subtracting the spectra of the free albumin from that in the presence of the compound.

The influence of the inner−filter effect on the measurements was evaluated by equation S4:

$$I_{corr} = I_{meas} \times 10^{\frac{\varepsilon(\lambda_{exc})cd}{2}} \times 10^{\frac{\varepsilon(\lambda_{em})cd}{2}} \qquad \text{(Equation S4)}$$

where I_{corr} = corrected intensity, I_{meas} = the measured intensity, c = the concentration of the quencher, d = the cuvette (1 cm), $\varepsilon_{(\lambda exc)}$ and $\varepsilon_{(\lambda em)}$ = the ε of the quencher at the excitation and the emission wavelength, respectively, as calculated from the UV−vis spectra of the compound.[4]

The Stern−Volmer and Scatchard graphs were used to study the interaction of the compound with the albumins. According to Stern−Volmer quenching equation (equation S2), where Io = initial tryptophan fluorescence intensity of the albumin, I = tryptophan fluorescence intensity of the albumin after the addition of the quencher, k_q = quenching constant, K_{SV} = Stern−Volmer constant, τ_o = average lifetime of the albumin without the quencher, K_{SV} (in M^{-1}) was obtained by the slope of the diagram Io/I *versus* [compound] (Stern−Volmer plots) (Figures S18 and S21). Taking as fluorescence lifetime (τ_o) of tryptophan in the albumin at around 10^{-8} s,[2] the k_q (in $M^{-1}s^{-1}$) was calculated with equation S3.

From the Scatchard equation (equation S5):

$$\frac{\Delta I/_{\mathrm{Io}}}{[Q]} = \mathrm{nK} - K\frac{\Delta \mathrm{I}}{\mathrm{Io}} \qquad \text{(Equation S5)}$$

where n is the number of binding sites per albumin and K is the albumin−binding constant (K, in M^{-1}) was calculated from the slope in plots (ΔI/Io)/[compound] *versus* (ΔI/Io) (Figures S19 and S22) and n was given by the ratio of y intercept to the slope.[5]

1. BSA Fluorescence emission spectroscopy

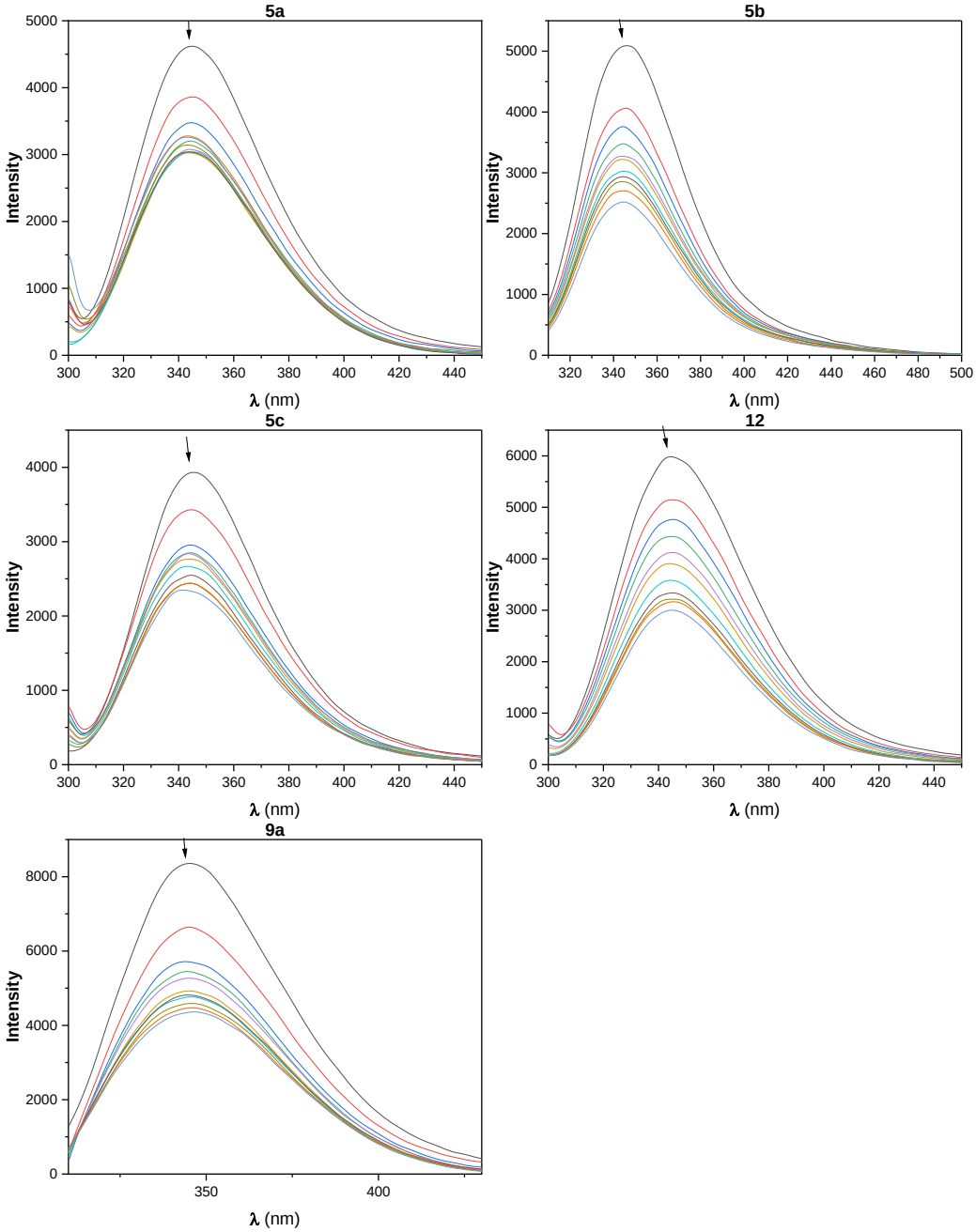


Figure S17. Fluorescence emission spectra ($\lambda_{excitation}$ = 295 nm) of a buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0) of BSA (3 µM) in the presence of increasing amounts of compounds **5a-d** and **9a**. The arrow shows the changes of intensity upon increasing amounts of the compound.

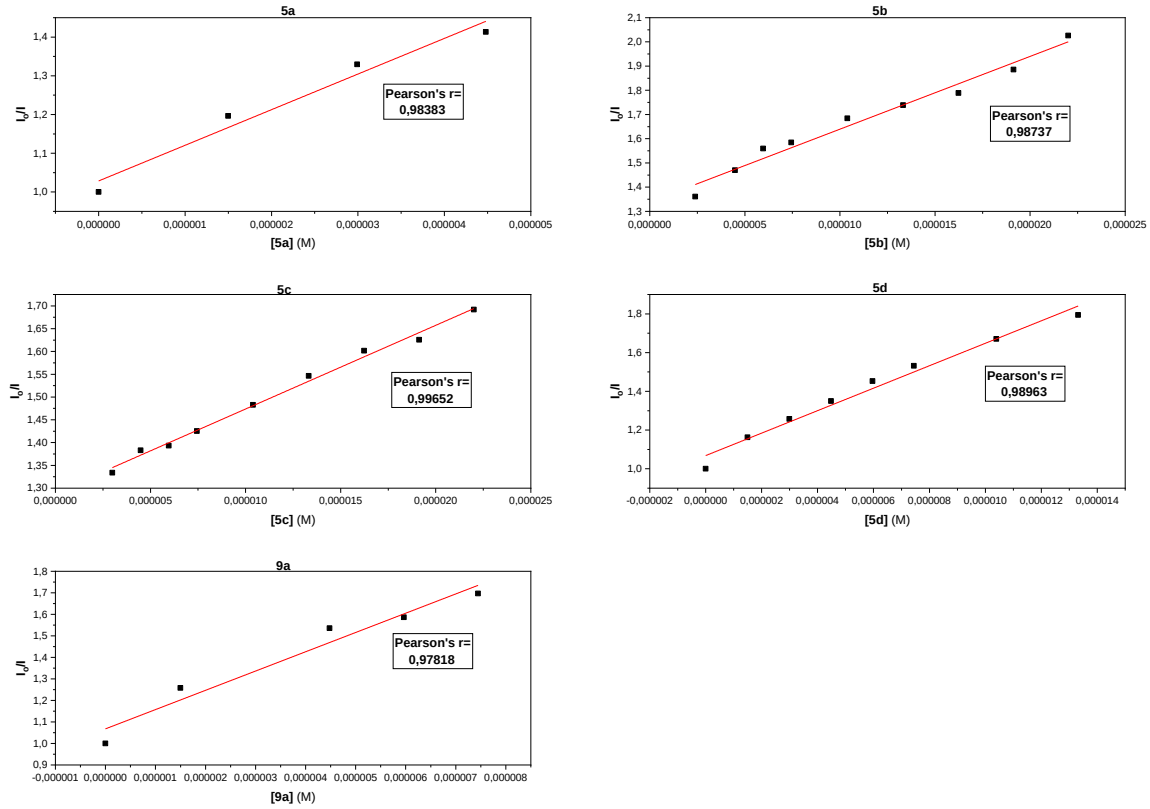


Figure S18. From left to right Stern−Volmer plots of the BSA quenching experiments upon addition of compounds **5a-d** and **9a**.

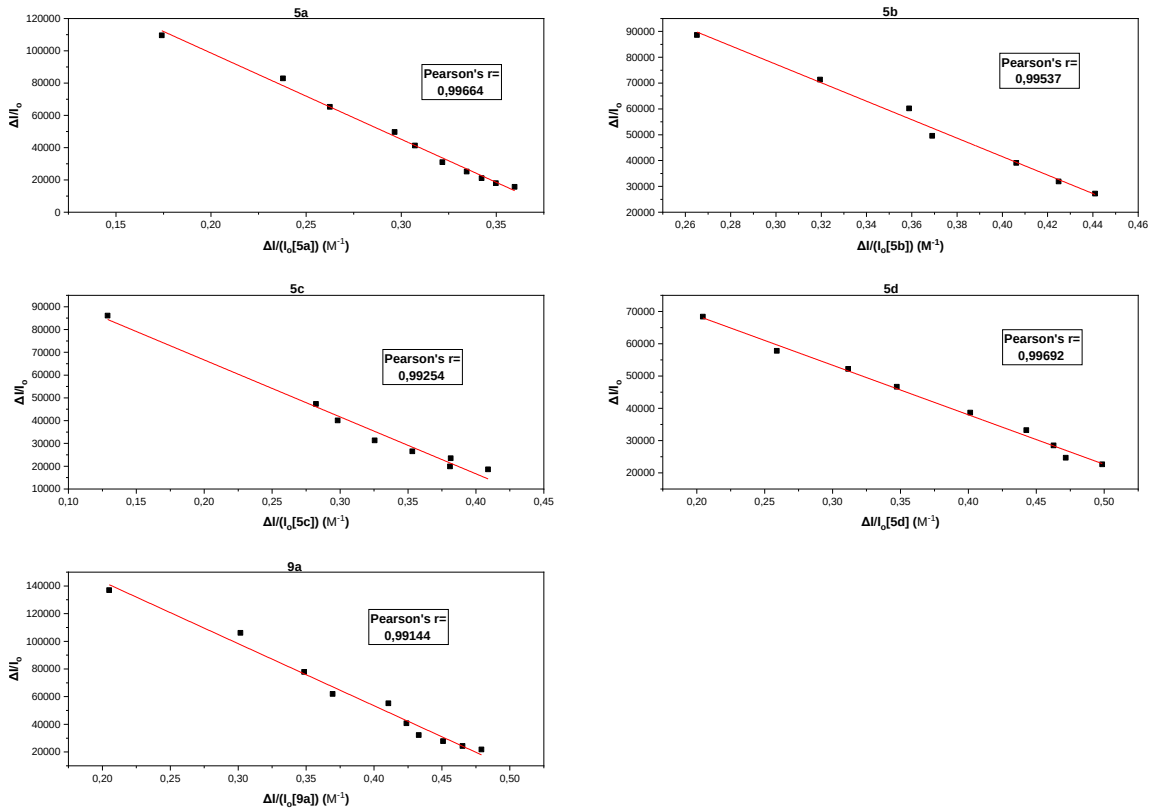


Figure S19. From left to right Scatchard plots of the BSA quenching experiments upon addition of compounds **5a-d** and **9a**.

2. HSA Fluorescence emission spectroscopy

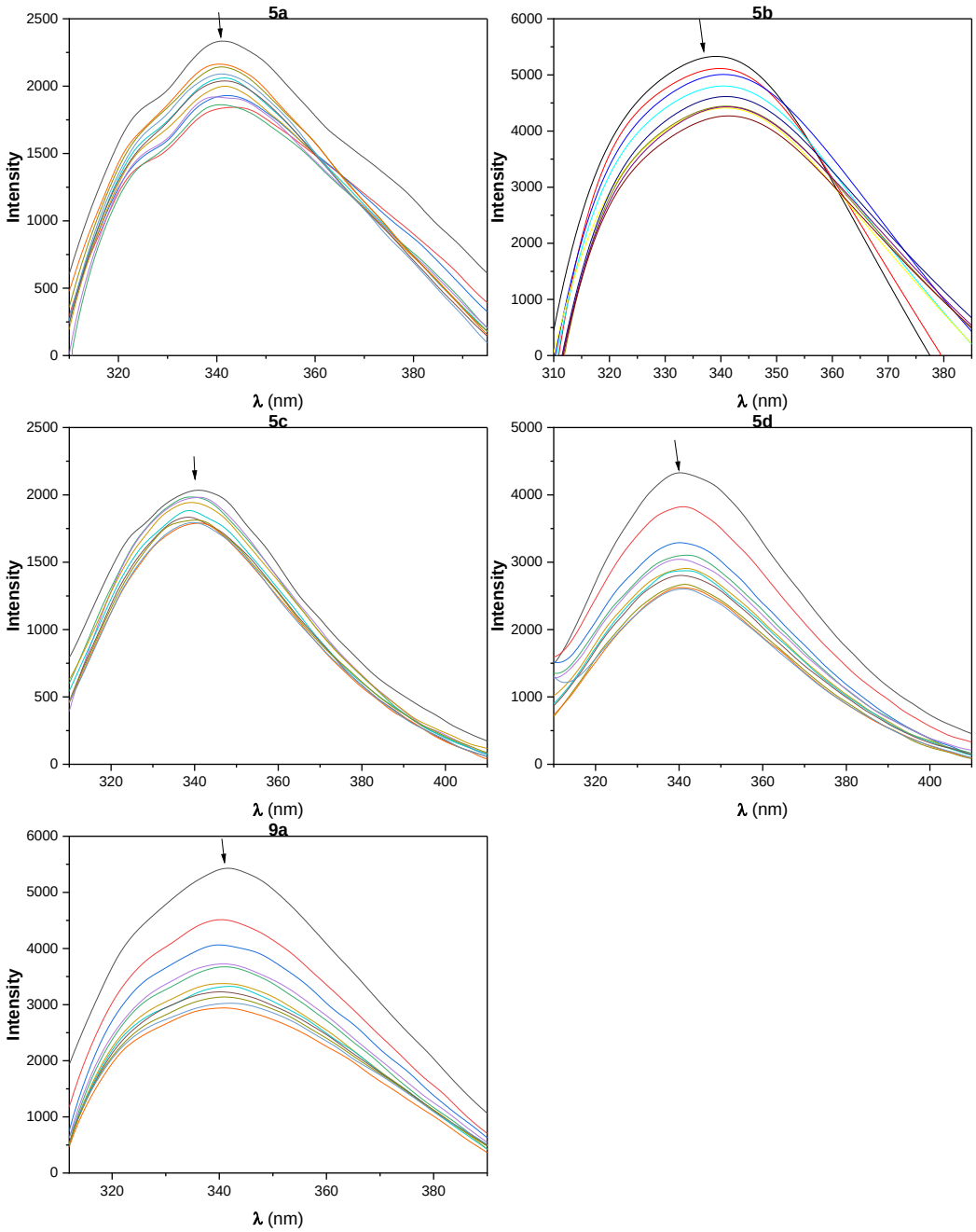


Figure S20. Fluorescence emission spectra ($\lambda_{excitation}$ = 295 nm) of a buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0) of HSA (3 µM) in the presence of increasing amounts of compounds **5a-d** and **9a**. The arrow shows the changes of intensity upon increasing amounts of the compound.

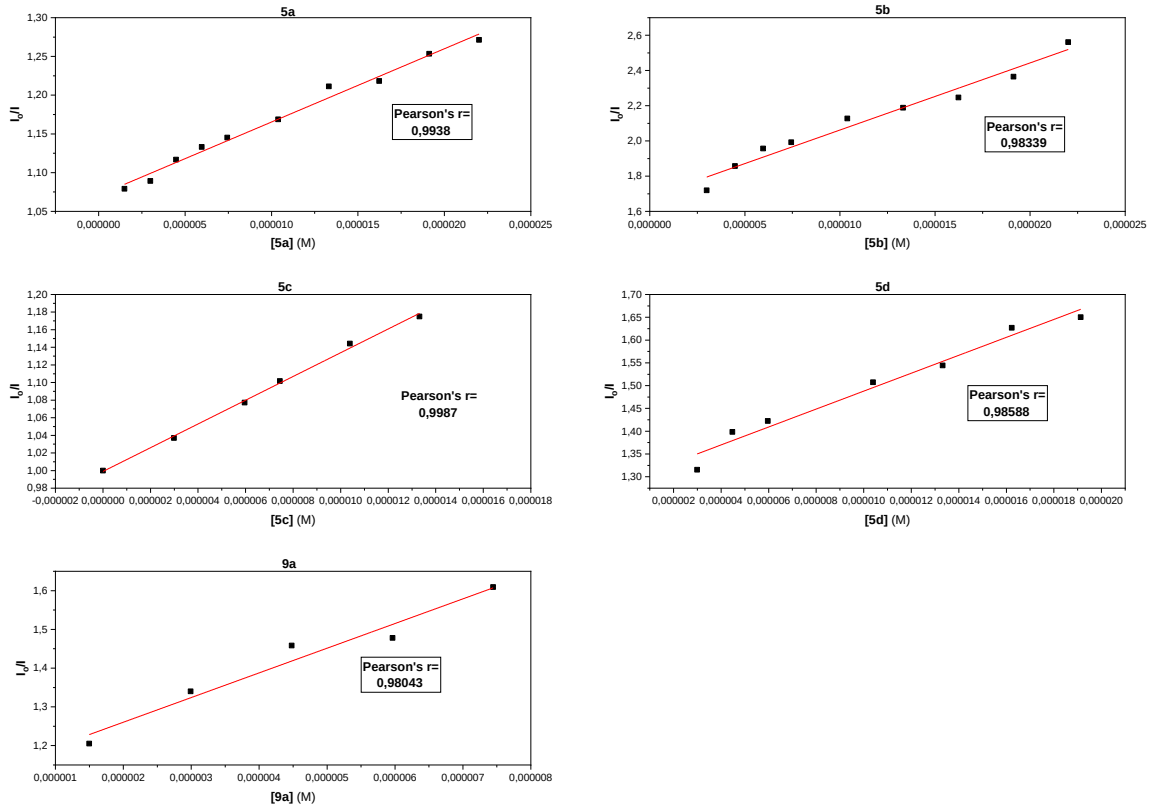


Figure S21. From left to right Stern–Volmer plots of the HSA quenching experiments upon addition of compounds **5a-d** and **9a**.

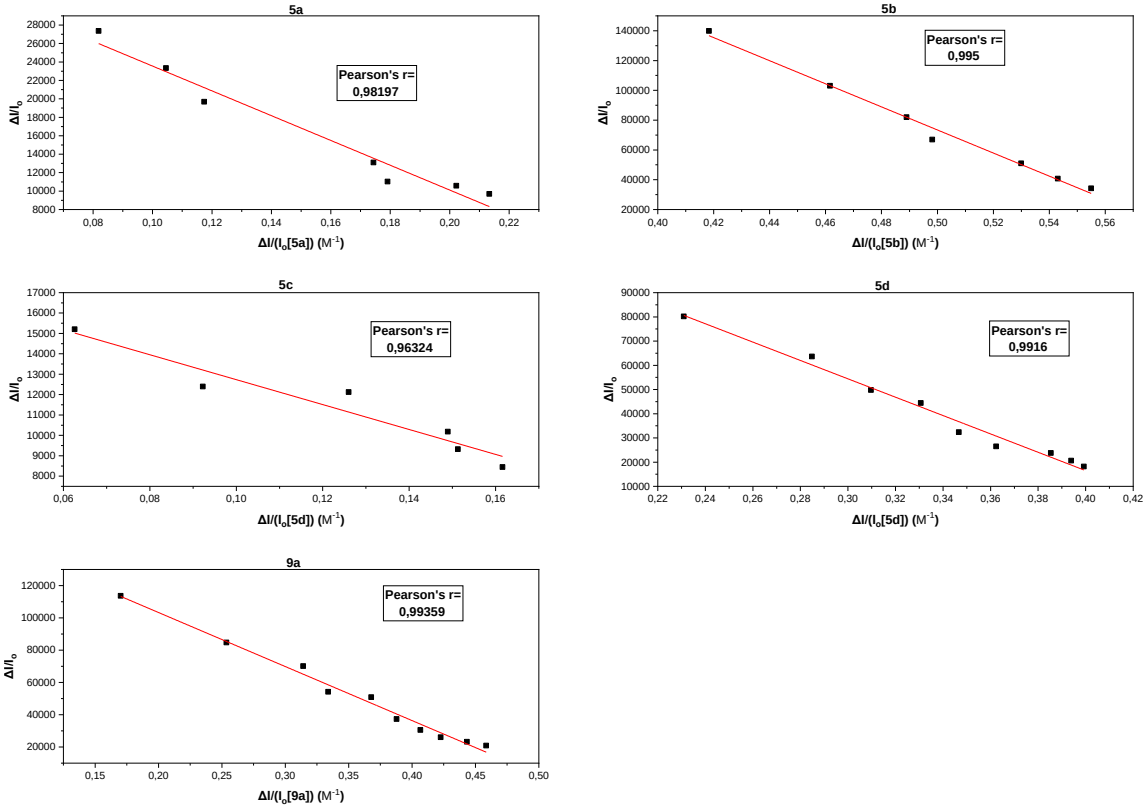


Figure S22. From left to right Scatchard plots of the HSA quenching experiments upon addition of compounds **5a-d** and **9a**.

D. DNA Irradiation Protocol

For the preparation of all the solutions and the experimental procedures, deionized water was used which will be referred from now on described as water or H_2O, unless stated otherwise.

Preparation and electrophoresis of samples

For a 20 μL reaction, 1 μL pBluescript KS II DNA, 19 μL Tris-HCl 0.05M for reference sample and 1 μL DNA, Tris-HCl and oxime diluted in DMSO (amounts depending on the desired concentration to test each oxime) for our oximes were transferred in Eppendorf. The contents are centrifuged for 1', incubated at 37 ^oC for 30', centrifuged again for 1' and irradiated for 2 hours under UV-A lamp at 10 cm distance.

The samples were centrifuged for 1', added commercially available dye 6x (gel loading dye purple 6x B7024S from Biolabs) and were loaded at the wells starting from left with the ladder and moving to the right with the reference sample and following the oximes. The gel run for 40' under 90V current.

Gel analysis was achieved through image capture under ultraviolet light using MiniBIS Pro from DNR Bio Imaging Systems.

E. BSA and HSA Irradiation Protocol

For the preparation of all the solutions and the experimental procedures, deionized water was used which will be referred from now on as water or H_2O, unless stated otherwise.

Aliquoting is the procedure of splitting a solution into Eppendorf containers and used to lessen the risk of cross contamination and due to the fact that very small quantities are used in each experiment.

For the duration of the albumin irradiation experiments, master mix solution was used which refers to a premixed solution that consists of the same elements that the sample solution has apart from the oxime. The advantages include the quick preparation of samples, reduction of analytical errors and possible cross contamination due to less pipetting and last but not least, greater producibility of results.

Preparation and electrophoresis of samples

In every experiment performed two reference samples were made, one that contains master mix and water and is irradiated along with the rest of the samples and one consisting of master and DMSO (being the solvent of our samples) that is not irradiated.

For a 20 μL reaction, 18 μL master mix (containing trisodium citrate 0.015 M, NaCl 0.15 M, $Co(NH_3)_6Cl_3$ 0.001 M and either BSA or HSA 1 μg/μL) and 2 μL of oxime and water in amounts depending on the desired concentration of oxime are mixed together in Eppendorf container. The contents are centrifuged for 1', irradiated for a 2- or 4-hour period using UV-A lamp at 10 cm distance and centrifuged again for 1'. Laemmli buffer 5x (consisted of Tris-HCl (pH=6.8) 0.1 M, glycerol 20.01%, DTT, SDS and bromophenol blue) was added to the samples, centrifuged for 1' and vortexed, incubated at 85 ^oC for 5', centrifuged and vortexed again and lastly, they were added on the gel. The two first leftmost positions were occupied by the reference samples and then the rest of the samples were added. The gel run for 2 hours under 120V current.

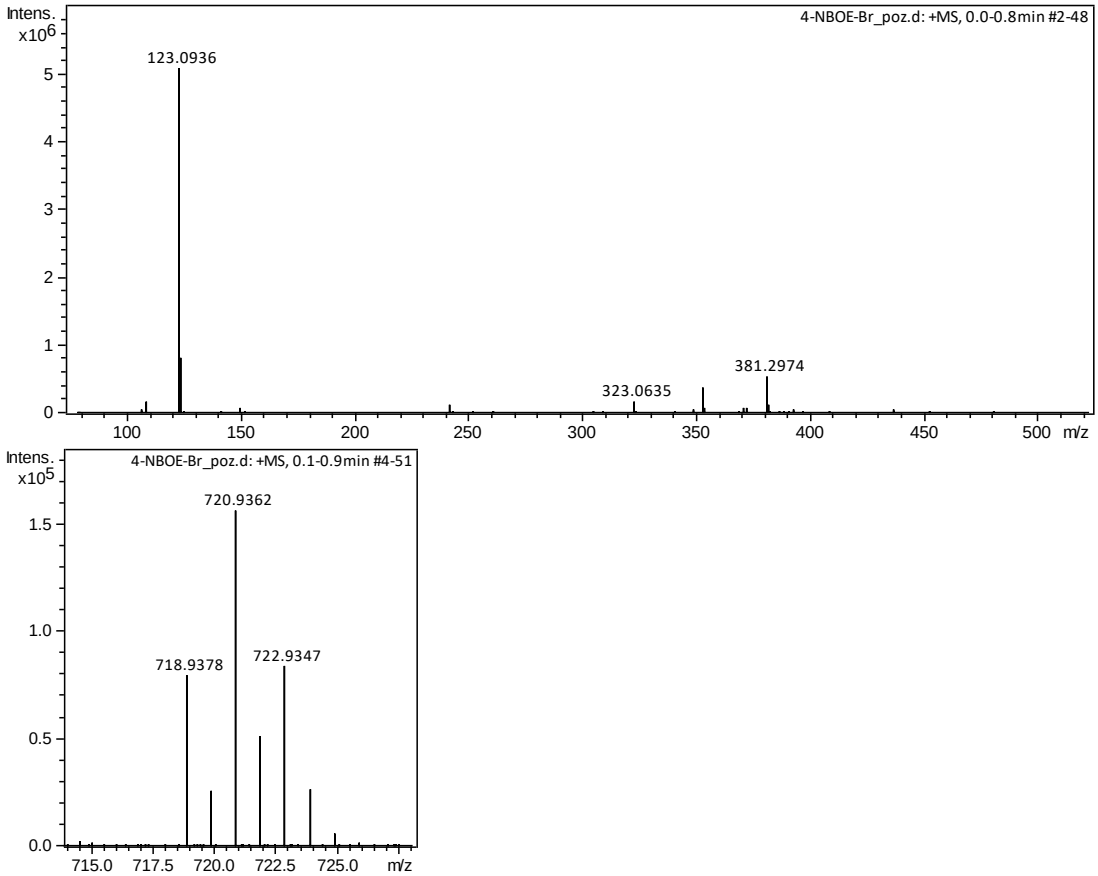
Gel analysis was achieved through image capture under visible light using MiniBIS Pro from DNR Bio Imaging Systems.

F. References

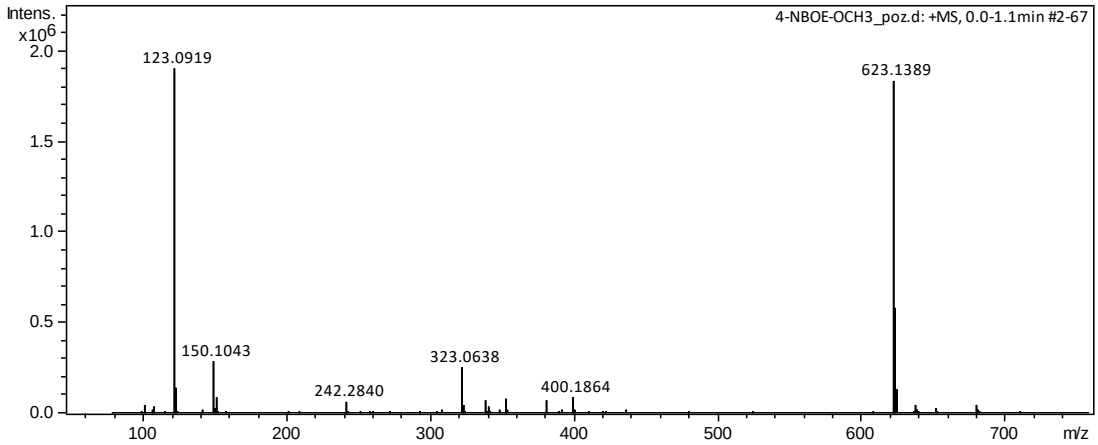
1. Wolfe, A.; Shimer, G. H. J.; Meehan, T. *Biochemistry* **1987**, *26*, 6392. DOI:10.1021/bi00394a013.
2. Lakowicz, J. R. *Principles of fluorescence spectroscopy, 3rd Principles of fluorescence spectroscopy, Springer, New York, USA, 3rd edn, 2006.*; **2006**. DOI:10.1007/978-0-387-46312-4.
3. Heller, D. P.; Greenstock, C. L. *Biophys. Chem.* **1994**, *50*, 305. DOI:10.1016/0301-4622(93)e0101-a.
4. Stella, L.; Capodilupo, A. L.; Bietti, M. *Chem. Commun.* **2008**, 4744. DOI:10.1039/b808357f.
5. Wang, Y.-Q.; Zhang, H.-M.; Zhang, G.-C.; Tao, W.-H.; Tang, S.-H. *J. Lumin.* **2007**, *126*, 211. DOI:https://doi.org/10.1016/j.jlumin.2006.06.013.

G. HRMS of compounds 5a-c and 9a

5a



5b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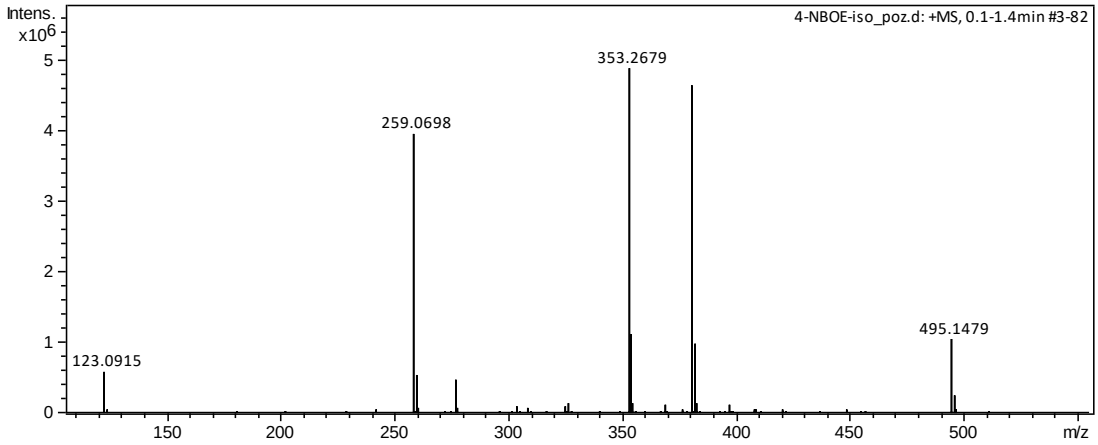
5c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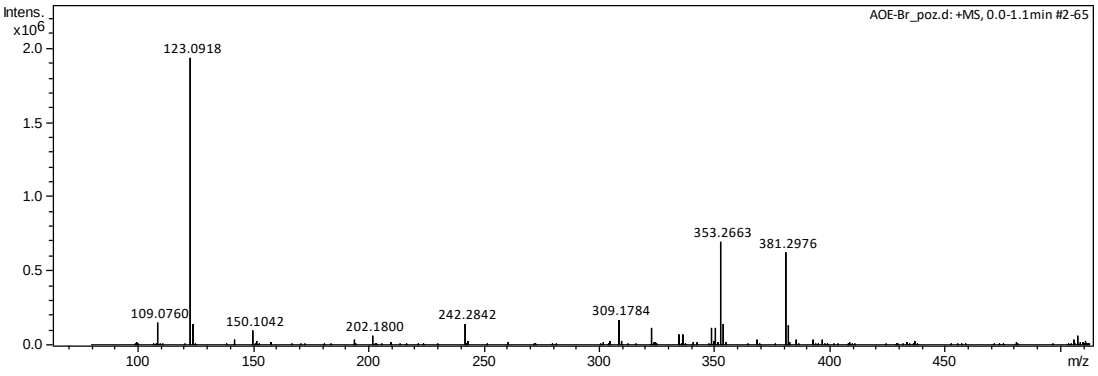
9a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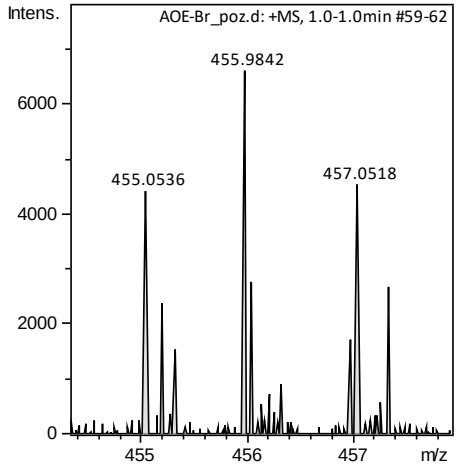
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