Environmental Photochemistry of Fenamate NSAIDs and their Radical Intermediates

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Electronic Supplementary Information

The electronic supplementary information contains 21 pages numbered S1-S21, 20 figures, 4 tables, and details on methods for determining, $\log D_{ow}$ values, determination of the bimoleular reaction rate constant with $^1O_2$, Fraction of D_2O in solutions for KSIE experiments, calculation of steady-state concentrations of singlet oxygen and hydroxyl radicals, calculation of light screening factor, water column model, and calculation of $^3PN^*$ quenching caffeic acid (Text S1-S7).

Table S1. $LogD_{ow}$ for Fenamates - Predicted with ACD/Labs software (www.chemspider.com).

Fenamate Drug	$LogD_{ow}$ (ionized, pH 7.4)
Diclofenac	1.69
Flufenamic acid	2.29
Meclofenamic acid	2.95
Mefenamic acid	2.04
Tolfenamic acid	2.47

Text S1. Methods for determining the bimolecular rate constant with singlet oxygen.

Rose Bengal and visible light (> 455 nm)

This is a steady-state experiment in open borosilicate test tubes containing 40 μM FFA, 3 μM Rose Bengal as a $^1O_2$ source and 5 μM of the test compound. Samples were irradiated with a Xenon lamp using a 455 nm longpass filter to inhibit any direct photolysis of the test compounds. Samples were taken at 0, 2.5, 5, 7.5, 10, 12.5, and 15 min for FFA quantification. Aliquots were taken at 0, 5, 10, 15, 20, 25, 30, and 40 min for the fenamate drugs. Dark controls of the samples kept in amber vials during the irradiation were also included. UV-vis absorbance measurements of samples at time 0 and 40 min were compared to determine the photobleaching of Rose Bengal during the experiment.

$^1O_2$ phosphorescence (laser spectroscopy)

Singlet oxygen phosphorescence was recorded at (1270 ± 5 nm) using a near-IR photo-multiplier tube detector. This configuration was built in our lab and modeled after the singlet oxygen phosphorescence detector described by Bilski et al[1] and Jiménez-Banzo et al[2]. Quartz cuvettes were filled with sensitizer in a solvent composition of 50:50, H_2O:ACN. For these experiments 100 μM perinaphthenone and 5 μM Rose Bengal were used as sensitizers. A pump beam of 360 nm and 550 nm were used to excite PN and RB, respectively and their corresponding power was 80 and 70 mW. Increasing concentrations of fenamates were present in solution, 100 – 500 μM for flufenamic, mefenamic and tolfenamic acid, and 500 μM – 3000 μM for diclofenac and meclofenamic acid. The various fenamate concentrations also contained different amount of solvent (acetonitrile), which can also affect the lifetime of $^1O_2$, so controls were done with the same solvent composition, which did not contain fenamates to correct for this difference. The decay portion of the $^1O_2$ signal was fitted and represented the lifetime of the $^1O_2$. The decay rate (k_{obs}, s^{-1}) corresponds to 1/lifetime. The decay rate for each amount of fenamates added were plotted against each other in a Stern-Volmer plot. The data was collected using a software called TimeHarp. Data analysis was performed using Origin 9.1

Non-photochemical generation of $^1O_2$: H_2O_2 decomposition with molydate catalyst

Non-photochemical generation of $^1O_2$ was done using a method adapted from one used by Boreen et al [3]. This experiment was carried out in amber sample vials that were covered to prevent photolysis. 50 μL of 1 mM H_2O_2 was added to 4.95 mL of a carbonate (pH 10.0, 10 mM) solution containing 10 μM fenamate, 40 μM FFA, and 1 mM MoO_4^{2-}. 375 μL aliquots were taken at 0, 10, 30, 60, 120, 180, and 360 min and added to 125 μL of sodium azide (507 mM) to quench the reaction. Samples

were analyzed with HPLC. Controls were done without MoO_4^{2-} and without H_2O_2 to check whether fenamates were reactive with the catalyst or hydrogen peroxide.

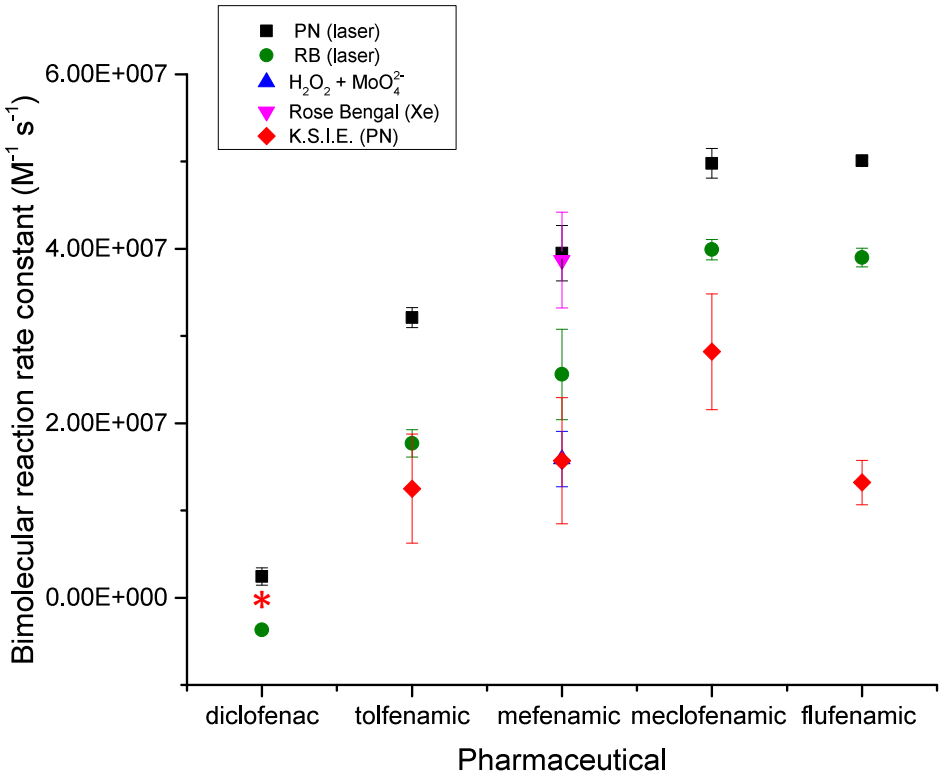

Figure S1. Comparison of $k_{rxn}(_{1O2,fen})$ determined via different experimental methods. Black squares (■), and green circles (●), represent $^1O_2$ phosphorescence using perinaphthenone (PN) and Rose Bengal (RB) as a $^1O_2$ source, respectively. Blue triangles (▲) used chemical generation of $^1O_2$ with H_2O_2 and MoO_4^{2-}. Pink triangles (▼), show photochemical generation of $^1O_2$ with RB and light > 455 nm. Red diamonds (◆) represent kinetic solvent isotope effect (KSIE) experiments using PN as a $^1O_2$ source.

* = no kinetic solvent isotope effect observed for diclofenac, so no rate constant could be determined.

Text S2. Composition of D_2O in KSIE experiments

The fraction of D_2O in solution can be calculated using the following equation:

$$k_{solv} = \chi H_2O \cdot k_{H2O} + \chi D_2O \cdot k_{D2O}$$

with k_{solv} being the observed reaction rate constant of the probe molecule furfuryl alcohol (FFA) in the solution tested, k_{H2O} being the observed reaction rate constant of FFA in 100% H_2O, and k_{D2O} being the reaction rate constant of FFA in 100% D_2O, as well as the unknown mole fractions of in H_2O and D_2O of the tested solution.

$$\chi H_2O = \frac{k_{solv} - k_{D2O}}{k_{H2O} - k_{D2O}}; \quad \chi D_2O = 1 - \chi H_2O$$

$k_{H2O} = 2.54 \times 10^5\ s^{-1}$; $k_{D2O} = 1.79 \times 10^4\ s^{-1}$, (based on lifetimes of $^1O_2$)[4]
The calculated fractions of H_2O and D_2O in the experiments for all test compounds are listed in
Table S2.

Table S2. Fraction of D_2O in solutions for KSIE experiments.

Fenamate	Mole Fraction H_2O	Mole Fraction D_2O
Diclofenac	9.8%	90.2%
Flufenamic acid	11.3%	88.7%
Meclofenamic acid	18.0%	82.0%
Mefenamic acid	13.8%	86.1%
Tolfenamic acid	21.6%	78.4%

Text S3. Calculation of steady-state concentration of singlet oxygen, [1O2]ss

$$[\,^1O_2]_{ss} = \frac{k_{obs}(FFA)}{k_{rxn,FFA}}$$

k_{obs} = observed degradation rate constant for FFA

$$\ln k_{rxn,FFA} = -\frac{(1.59 \pm 0.06)\times 10^3}{273.16 + T[\,^\circ C]} + (23.82 \pm 0.21)$$

T = temperature in degrees Celsius
see Appiani et al.[5]

118 **Text S4. Calculation of steady-state concentration of hydroxyl radical, [•OH]ss**

119

120 $$[\,^{\bullet}OH]_{ss} = \frac{\mathrm{d}[hTPA])/_{dt}}{k_{rxn,TPA} \cdot [TPA] \cdot Y}$$

121

122 d[hTPA]/dt = rate of change for hTPA (in M s^{-1})

123 [TPA] = initial concentration of TPA,

124 $k_{rxn,TPA}$ = 4.4 x 10^9 M^{-1} s^{-1}, Y = 35% (production yield)[6]

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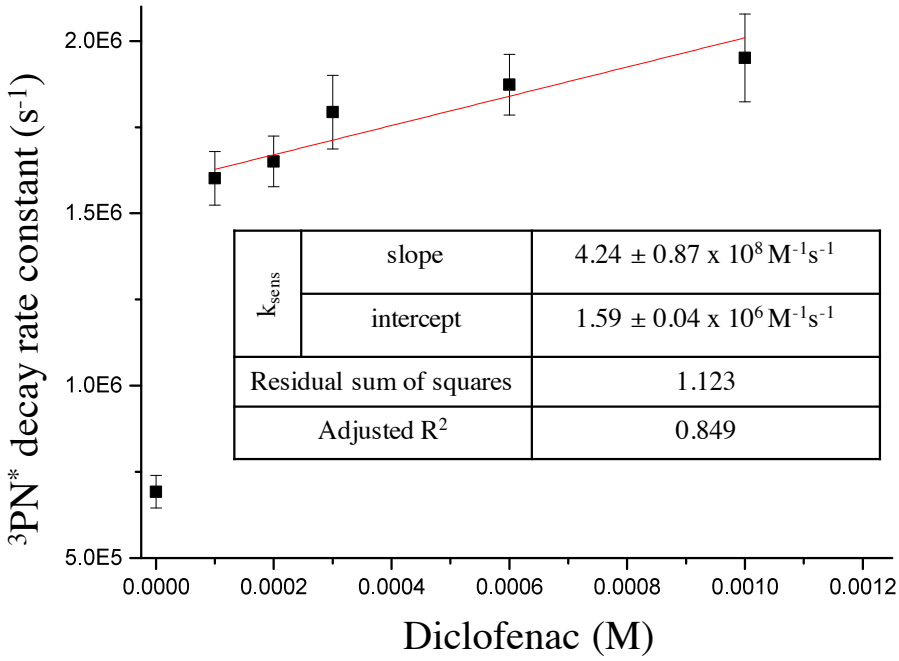
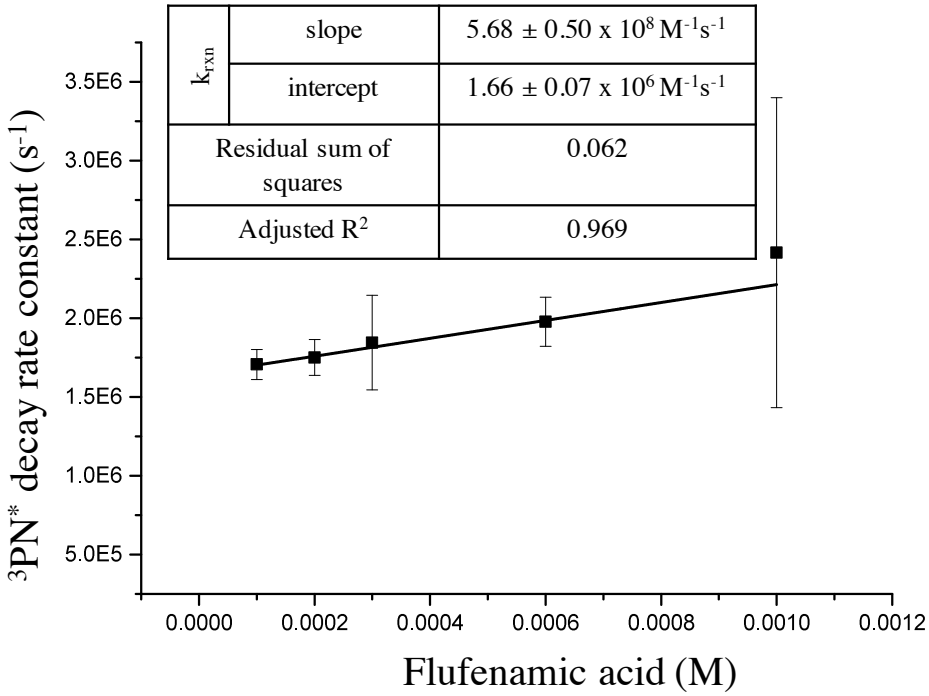
126

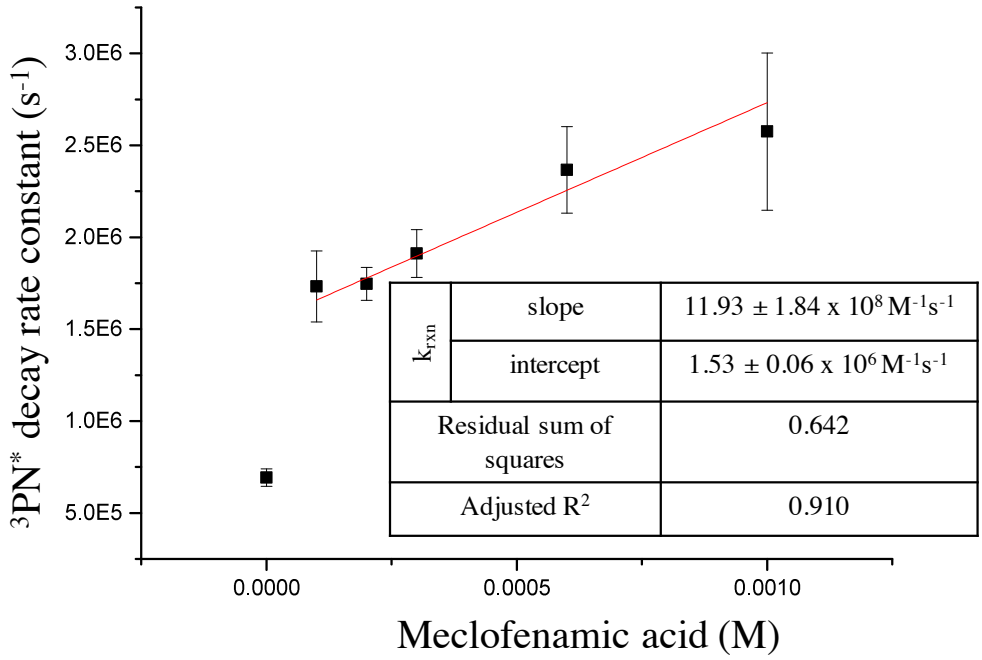
127 **Stern-Volmer Plots for Calculating Reaction Rate Constant with triplet**
128 **perinaphthenone - k_{rxn}($_{fen,3PN}$)**



129
130 **Figure S2. Stern-Volmer Plot for determination of bimolecular reaction rate**
131 **constant for reaction between triplet perinaphthenone and diclofenac.**



132
133 **Figure S3. Stern-Volmer Plot for determination of bimolecular reaction rate**
134 **constant for reaction between triplet perinaphthenone and flufenamic acid.**



135
136 **Figure S4. Stern-Volmer Plot for determination of bimolecular reaction rate**
137 **constant for reaction between triplet perinaphthenone and meclofenamic acid.**

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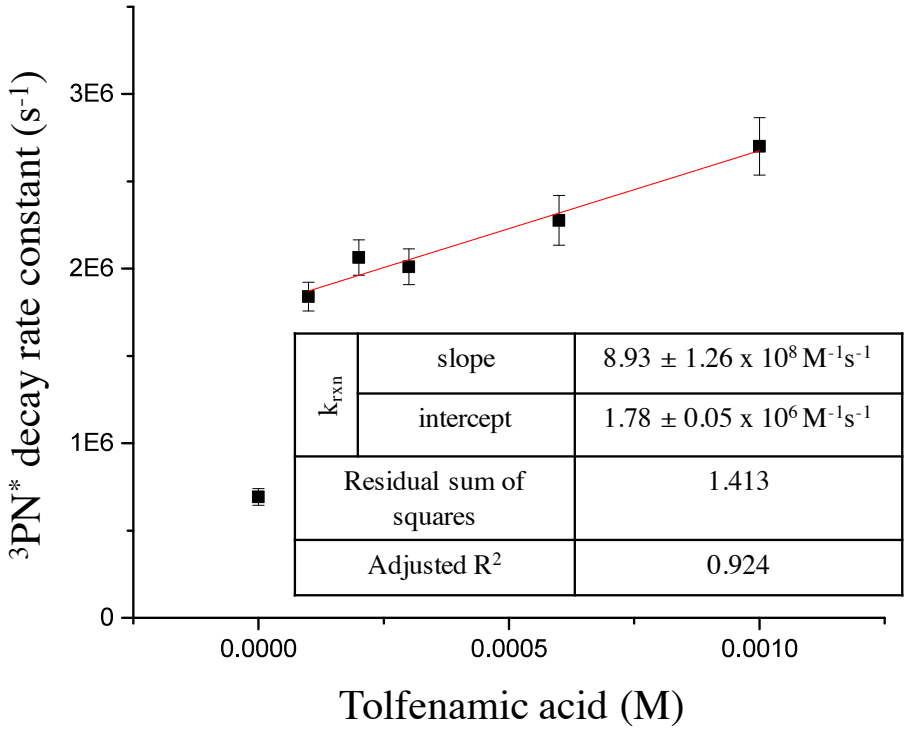

139
140 **Figure S5. Stern-Volmer Plot for determination of bimolecular reaction rate**
141 **constant for reaction between triplet perinaphthenone and tolfenamic acid.**

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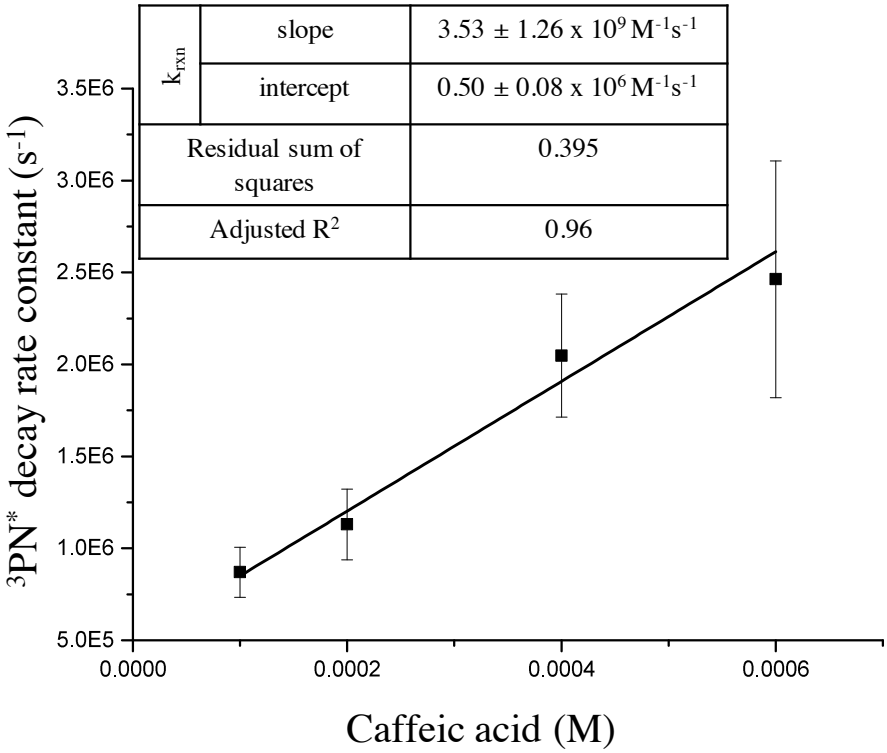
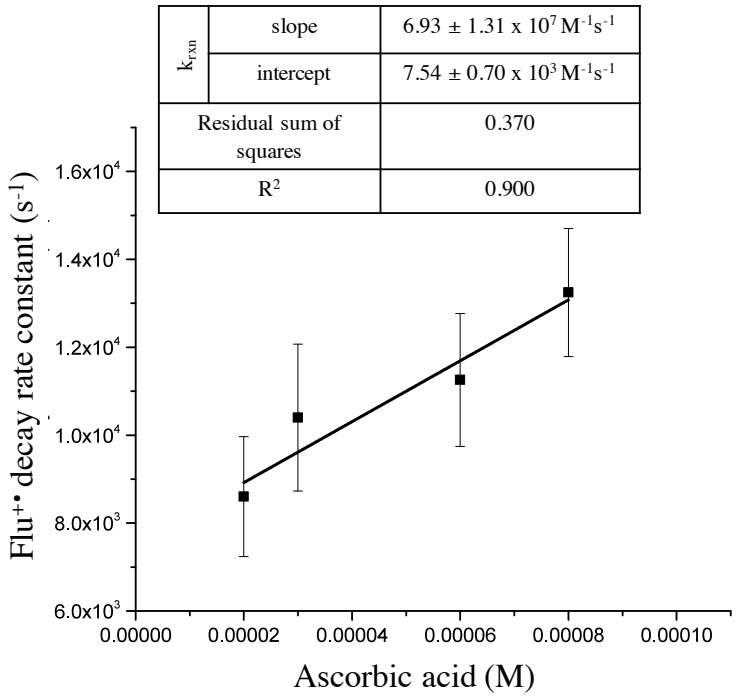


143
144 **Figure S6. Stern-Volmer Plot for determination of bimolecular reaction rate**
145 **constant for reaction between triplet perinaphthenone and caffeic acid.**

146 **Stern-Volmer Plots for Calculating Reaction Rate Constant with Antioxidant -**
147 **k_{rxn}($_{fen+.,ascorbic\ acid}$)**
148



149
150 **Figure S7. Stern-Volmer Plot for determination of bimolecular reaction rate**
151 **constant for reaction between flufenamic acid radical cation ($Flu^{+\bullet}$) and ascorbic**
152 **acid.**

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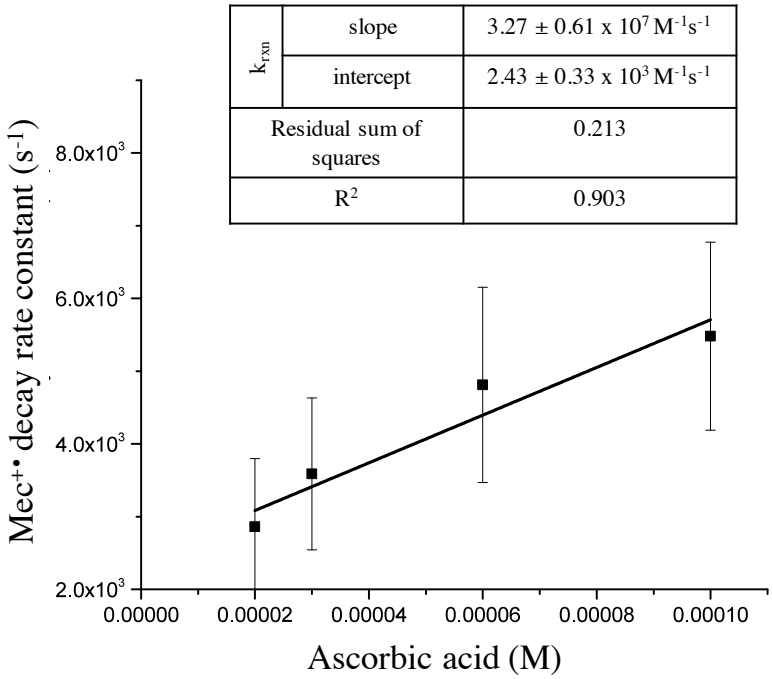
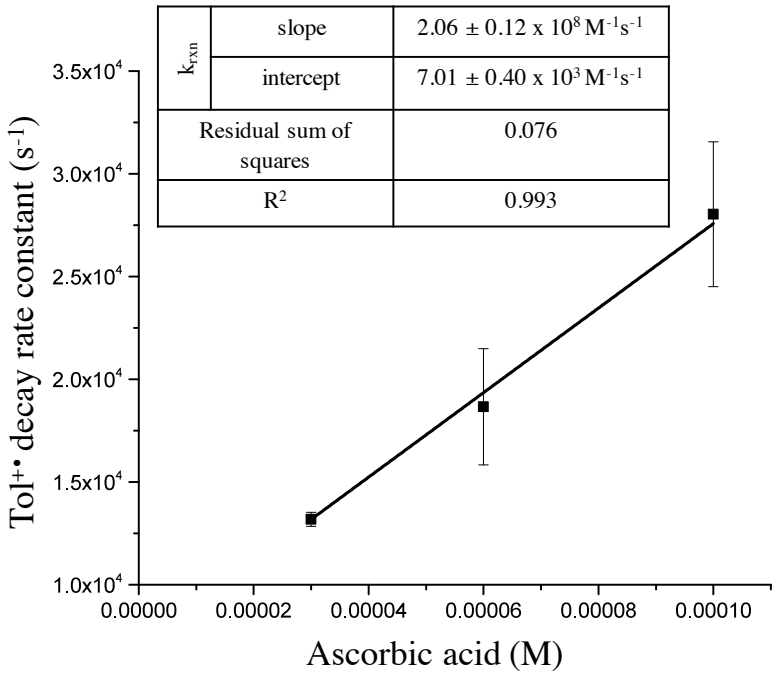


154
155 **Figure S8. Stern-Volmer Plot for determination of bimolecular reaction rate**
156 **constant for reaction between flufenamic acid radical cation ($Mec^{+\bullet}$) and**
157 **ascorbic acid.**



158

159 **Figure S9. Stern-Volmer Plot for determination of bimolecular reaction rate**
160 **constant for reaction between flufenamic acid radical cation ($Tol^{+\bullet}$) and ascorbic**
161 **acid.**

162
163

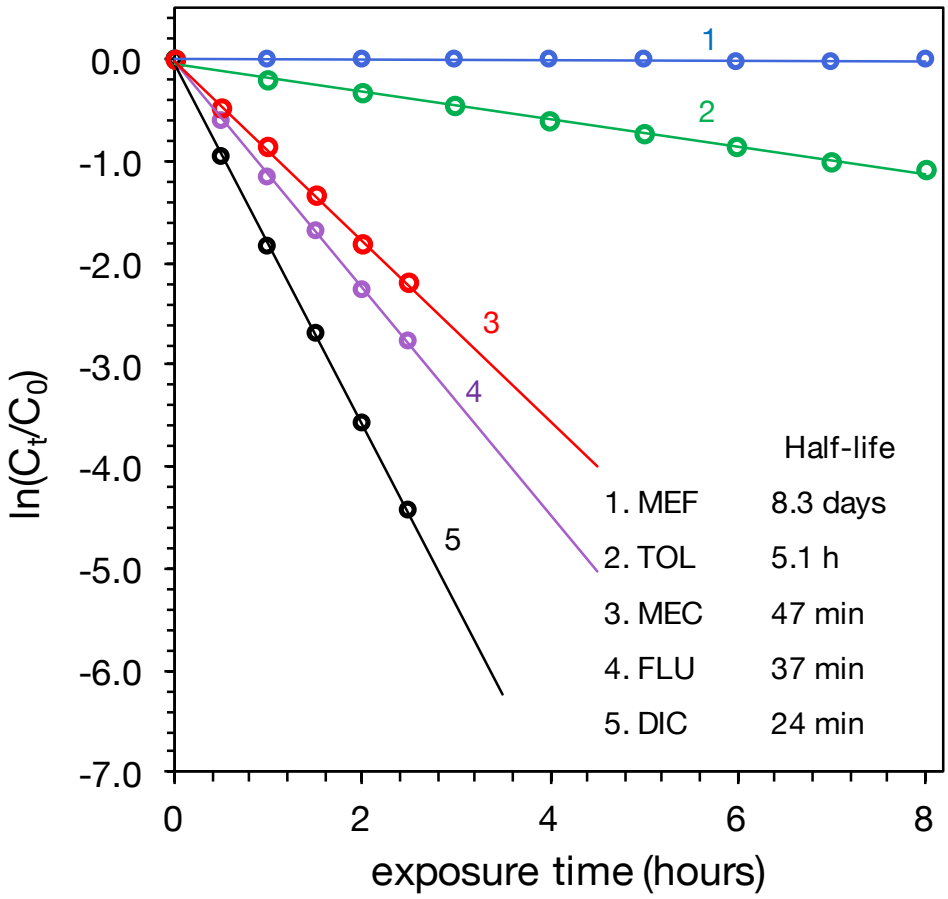
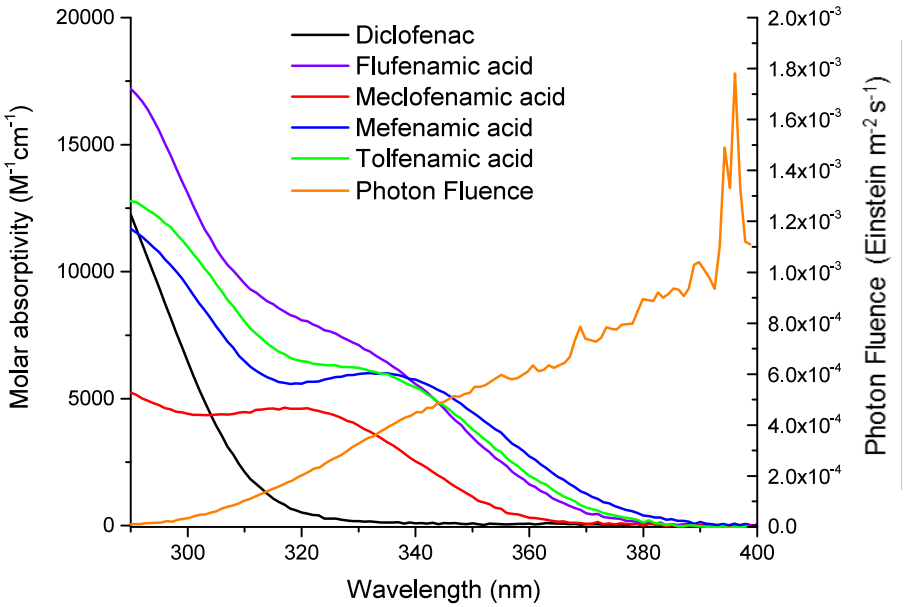


Figure S10. Pseudo-first order degradation plots of the fenamate drugs in the solar simulator controlled at pH 7.5 for mefenamic acid (1, blue), tolfenamic acid (2, green), meclofenamic acid (3, red), flufenamic acid (4, purple), and diclofenac (5, black). The table inset describes their half-lives under experimental conditions.



172
173 **Figure S11. Absorbance spectra for diclofenac and fenamates from 200 – 400 nm**
174 **(left axis). The photo fluence rate for the solar simulator output is plotted on the**
175 **right axis. Overlap of molar absorptivity peaks with solar simulator output peak**
176 **represents light that can be absorbed by the molecules.**

177

178

Text S3. Calculation of Light Screening Correction Factor.

Using Figure S11 above, decide the range of wavelengths where the light source and the test compound overlap. This range will be used to determine the screening correction factor based on method from Leifer et al.[7].

First, calculate the "S-factor".

$$S = \frac{1 - 10^{-az}}{2.303 \cdot az}$$

a = optical density at λ, dependent on the absorption of the sensitizer; DOM.
z = pathlength of light through test-tube

Next calculate the intensity of light absorbed by the system, $I_{t\lambda}$.

$$I_{t\lambda} = \frac{S \cdot I_{0\lambda}}{\sum I_{0\lambda}}$$

$I_{0\lambda}$ = incident light intensity of the light source, measured using a radiometer

Then, the amount of light that is transmitted (T) is calculated,

$$T = \frac{I_{t\lambda}}{I_{0\lambda}}$$

And finally from the transmission, it is possible to calculate the correction factor.

$$CF = \frac{1}{T}$$

CF = correction factor

Table S3. Light Screening Correction Factor for diclofenac and fenamates in 10 mg_c L^{-1} PLFA to account for light attenuation from DOM.

Fenamate Drug	Light Screening Correction Factor
Diclofenac	1.15
Flufenamic acid	1.09
Meclofenamic acid	1.10
Mefenamic acid	1.08
Tolfenamic acid	1.09

209 **Table S4. Summary of contribution to indirect photodegradation from various**
210 **photochemically produced reactive intermediates (PPRIs) under simulated**
211 **sunlight with 10 mg_C L^{-1} PLFA.**

Compound	Effect of DOM Δk_{obs} (%)	$^1O_2$ (%)	$^{\bullet}OH$ (%)	$^3CDOM^*$ (%)
Mefenamic acid	+95	24.0	1	71
Tolfenamic acid	+9	9.9	>1	0
Meclofenamic acid	-14	4.0	>1	n.a.[a]
Flufenamic acid	-2	1.4	>1	n.a.[a]
Diclofenac	+19	0.0	>1	19

212 [a]Not analyzed because of overall net quenching effect of DOM
213
214
215
216

Text S4. Water Column Model (Figure 2b)

To estimate the change of direct and indirect photochemical degradation in a water column, the absorption spectrum of the organic matter solution and the test compounds was recorded and the light intensity of solar irradiation were used.

First, the wavelength dependent change of light intensity as a function of water depth $I_{\lambda,z}$ (mE cm^{-2} s^{-1}), was calculated as

$$I_{\lambda,z} = I_{\lambda,0} \cdot 10^{-z \cdot a}$$

with $I_{\lambda,0}$ being incident light intensity at the water surface, a (cm) being the optical density at wavelength, λ, dependent on the absorption of the sensitizer; DOM, and z (cm) being the water pathlength of light representing the water depth.

Second, the depth and wavelength dependent rate of light absorbance, $k_{a,z}$ (mE cm^{-3} s^{-1}), by the sensitizer DOM and the test compound were estimated as

$$k_{a,z} = 2.303 \cdot a \cdot I_{\lambda,z}.$$

At each depth, the rates were summed across the wavelength spectrum where the DOM and test compounds absorb light within the solar spectrum, being 290-500 nm for DOM and 290-400 nm for diclofenac, mefenamic acid, tolfenamic acid, meclofenamic acid, and flufenamic acid.

The change in $k_{a,z}$ as a function of depth for DOM and test compounds is directly proportional to the relative decrease of indirect and direct photodegradation, respectively.

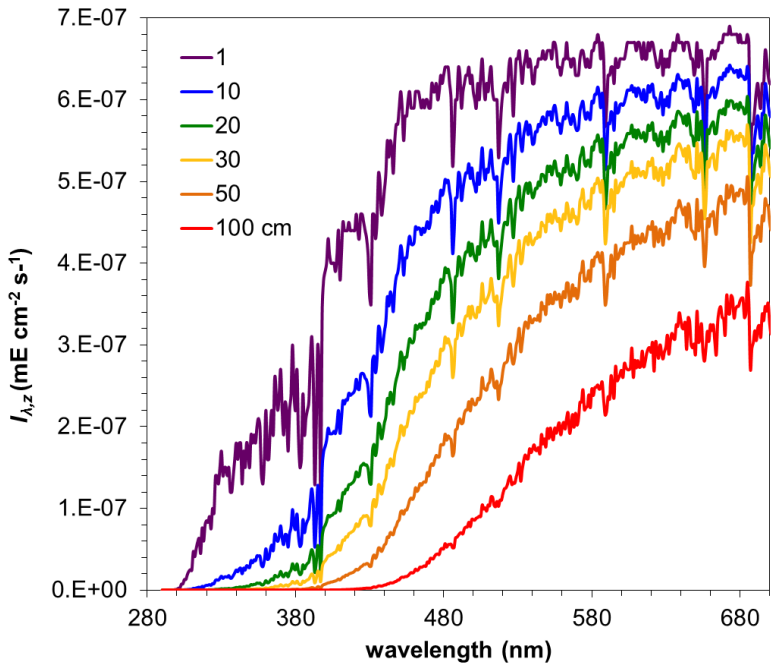
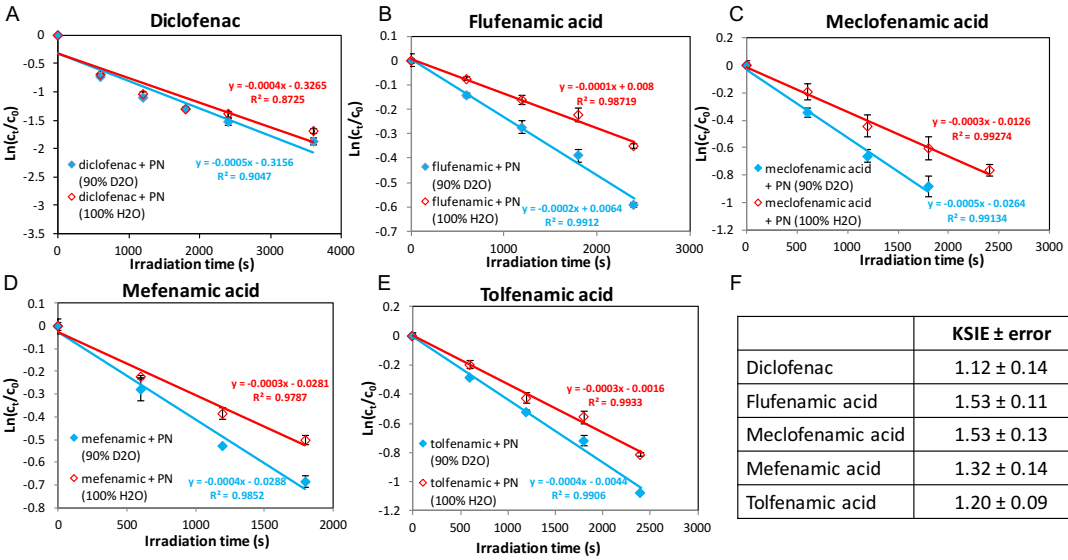


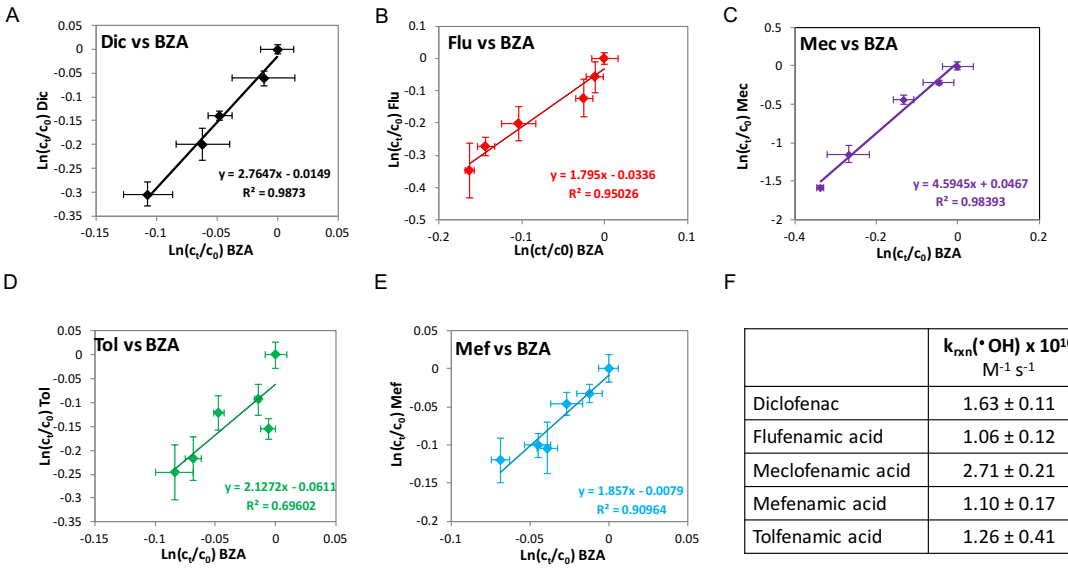
Figure S12. Light intensity of wavelengths 200-700 nm up to 1 m in the water column. Purple = 1 cm, blue = 10 cm, green = 20 cm, yellow = 30 cm, orange = 50 cm and red = 1 m depth.



F

	KSIE ± error
Diclofenac	1.12 ± 0.14
Flufenamic acid	1.53 ± 0.11
Meclofenamic acid	1.53 ± 0.13
Mefenamic acid	1.32 ± 0.14
Tolfenamic acid	1.20 ± 0.09

Figure S13. Pseudo-first order degradation of (A) diclofenac, (B) flufenamic acid, (C) meclofenamic acid, (D) mefenamic acid, (E) tolfenamic acid in 0.7 μM perinaphthenone. Blue diamonds represent degradation in 90% D_2O and red hollow diamonds represent degradation in 100% H_2O. Panel F shows the KSIE (ratio of k_{obs} (D_2O/H_2O)).



F

	k_{rxn}(•OH) x 10^{10} M^{-1} s^{-1}
Diclofenac	1.63 ± 0.11
Flufenamic acid	1.06 ± 0.12
Meclofenamic acid	2.71 ± 0.21
Mefenamic acid	1.10 ± 0.17
Tolfenamic acid	1.26 ± 0.41

Figure S14. Competition Plots vs. pseudo-first order degradation of benzoic acid (BZA) for (A) diclofenac, (B) flufenamic acid, (C) meclofenamic acid, (D) tolfenamic acid, and (E) mefenamic acid. Panel F shows the calculated bimolecular reaction rate constants for diclofenac and the fenamates.

Role of Triplet Sensitizing and Antioxidant moieties in DOM

Below are the remaining pseudo-first order degradation plots for flufenamic, meclofenamic, mefenamic, tolfenamic acid for the steady-state experiment with 0.77 μM perinaphthenone in air, argon sparged and with 10 μM caffeic acid.

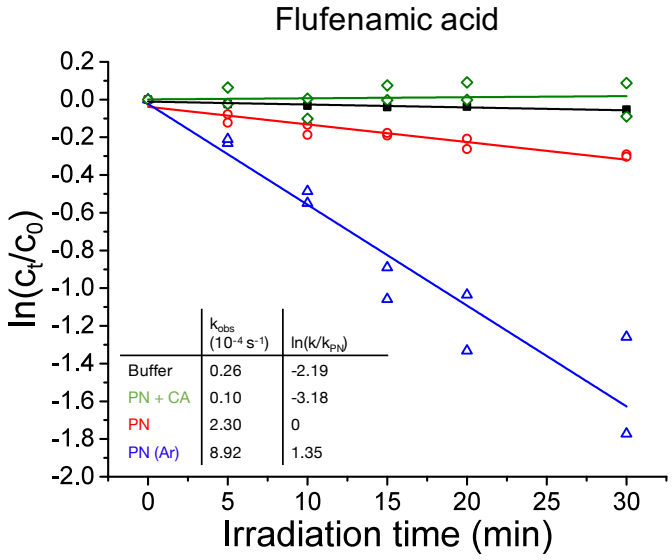


Figure S15. Pseudo-first order degradation of flufenamic acid (5 μM) in enhanced UVA light in phosphate buffer (pH 7.5) only: black squares (■), in the presence of the triplet sensitizer perinaphthenone (PN, 0.7 μM): red circles (○), with PN and argon sparged: blue triangles (△), and with PN and the antioxidant caffeic acid (CA, 10 μM): green diamonds (◇), and the inset shows the reaction rate constants, k_{obs} and the log-normalised ratio of k_{obs}, normalized to k_{obs} while sensitized with PN, $\ln(k/k_{PN})$.

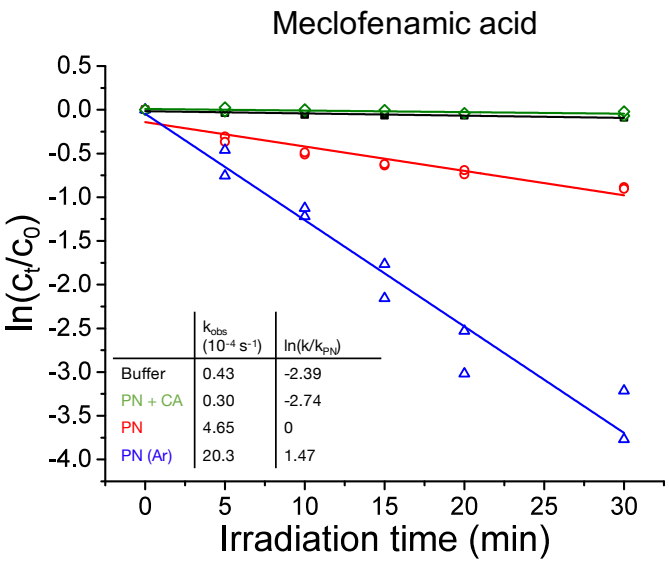


Figure S16. Pseudo-first order degradation of meclofenamic acid (5 μM) in enhanced UVA light in phosphate buffer (pH 7.5) only: black squares (■), in the presence of the triplet sensitizer perinaphthenone (PN, 0.7 μM): red circles (○), with PN and argon sparged: blue triangles (△), and with PN and the antioxidant caffeic acid (CA, 10 μM): green diamonds (◇), and the inset shows the reaction rate constants, k_{obs} and the log-normalised ratio of k_{obs}, normalized to k_{obs} while sensitized with PN, $\ln(k/k_{PN})$.

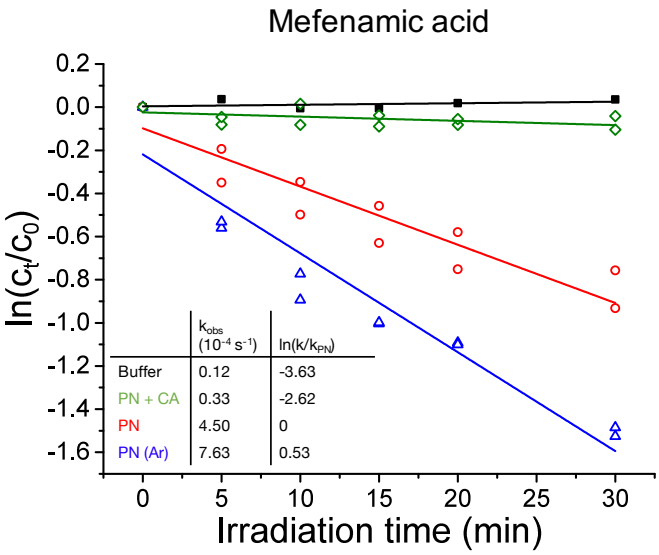


Figure S17. Pseudo-first order degradation of mefenamic acid (5 μM) in enhanced UVA light in phosphate buffer (pH 7.5) only: black squares (■), in the presence of the triplet sensitizer perinaphthenone (PN, 0.7 μM): red circles (○), with PN and argon sparged: blue triangles (△), and with PN and the antioxidant caffeic acid (CA, 10 μM): green diamonds (◇), and the inset shows the reaction rate constants, k_{obs} and the log-normalised ratio of k_{obs}, normalized to k_{obs} while sensitized with PN, $\ln(k/k_{PN})$.

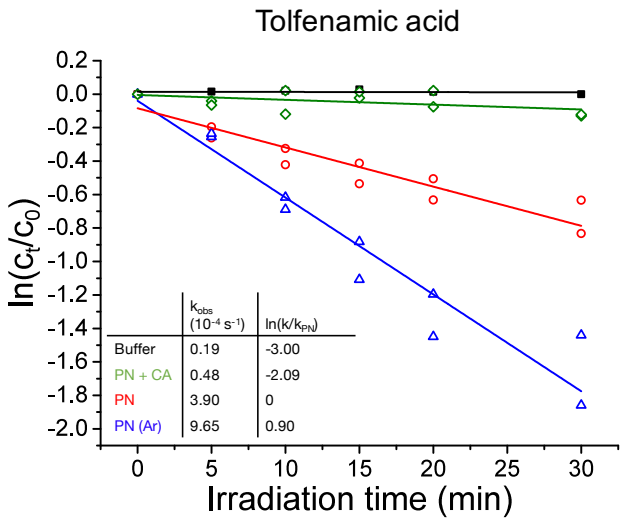


Figure S18. Pseudo-first order degradation of tolfenamic acid (5 μM) in enhanced UVA light in phosphate buffer (pH 7.5) only: black squares (■), in the presence of the triplet sensitizer perinaphthenone (PN, 0.7 μM): red circles (○), with PN and argon sparged: blue triangles (△), and with PN and the antioxidant caffeic acid (CA, 10 μM): green diamonds (◇), and the inset shows the reaction rate constants, k_{obs} and the log-normalised ratio of k_{obs}, normalized to k_{obs} while sensitized with PN, $\ln(k/k_{PN})$.

Text S5. Calculation of ^{3}PN* quenching by caffeic acid for steady-state experiments

The decay (k_d) of ^{3}PN* in air sparged solution was calculated by the inverse its lifetime ($\tau = 1.7\ \mu s$).

$$k_d = 5.8 \times 10^5\ s^{-1}$$

The amount of triplet decay due to caffeic acid (k_{CA}) can be calculated by multiplying the bimolecular reaction rate constant of k(^{3}PN*, Figure S6) with the caffeic acid concentration for the steady-state experiment.

$$k_{CA} = (3.5 \times 10^9 M^{-1} s^{-1}) \times (10\ \mu M)$$
$$= 3.5 \times 10^4\ s^{-1}$$

$$Additional\ decay = \frac{k_d + k_{CA}}{k_d}$$
$$= 6\%$$

Therefore, caffeic acid would contribute to 6% decay of ^{3}PN* in solution.

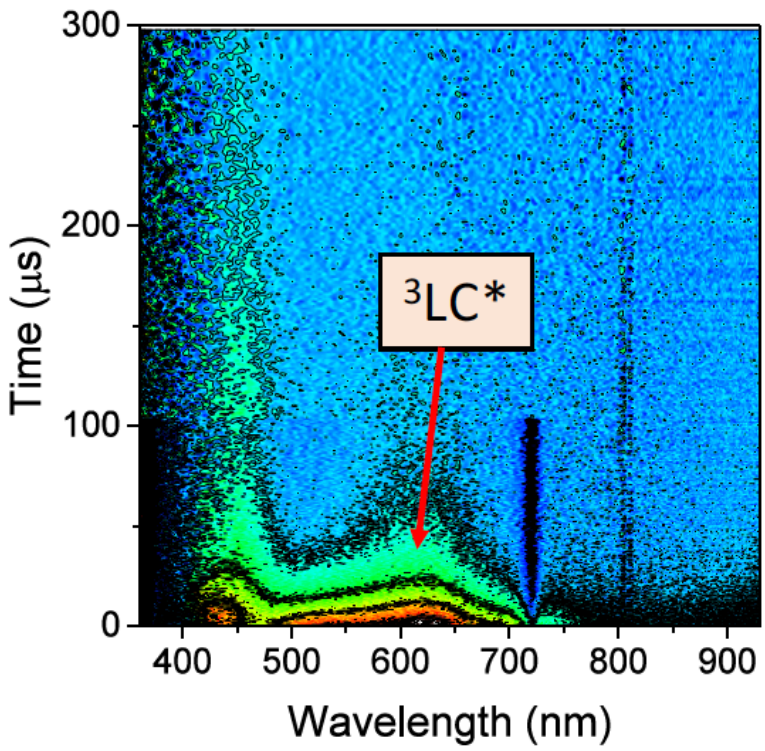


Figure S19. Blank for triplet Lumichrome (^{3}LC*). Pump beam = 360 nm. Sparged continuously with argon gas.

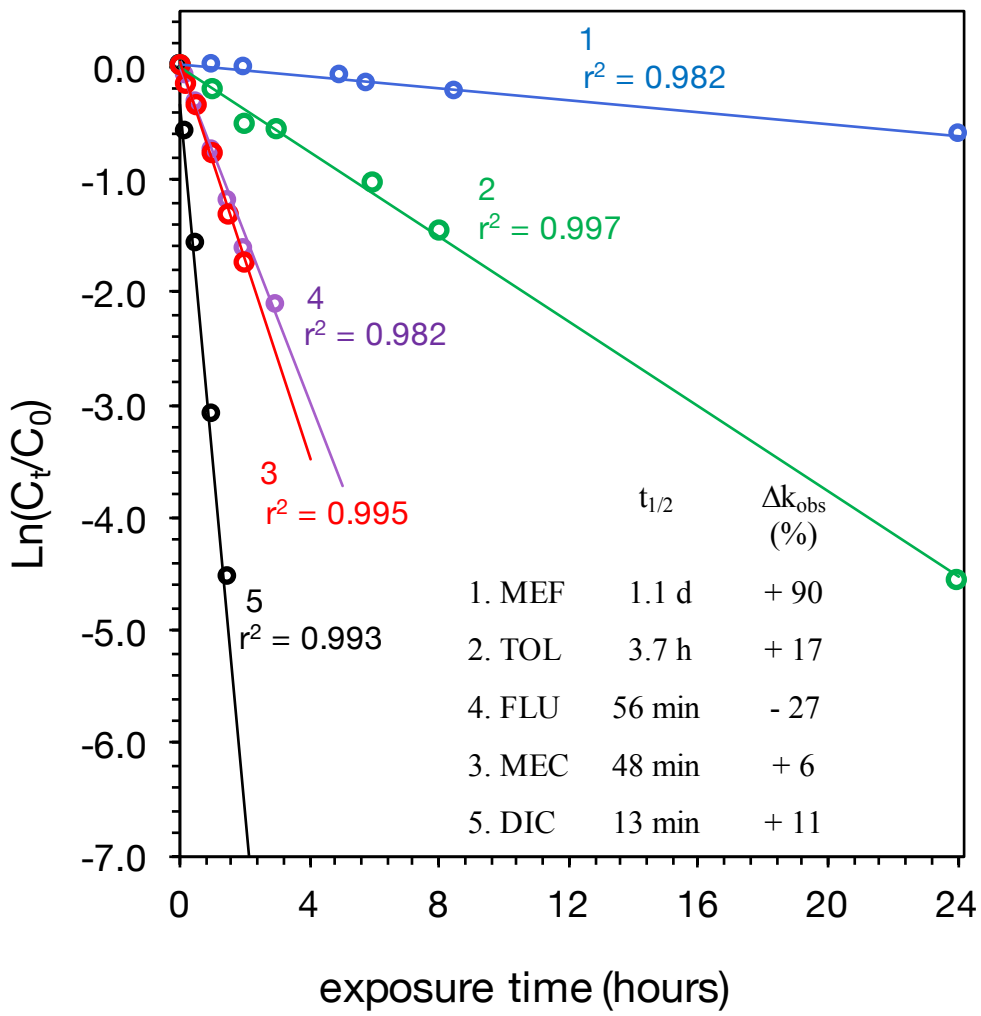


Figure S20. (A) Pseudo-first order degradation plots of the fenamate drugs in the solar simulator in solution with 10 mg_c L^{-1} Suwannee River Fulvic Acid, controlled at pH 7.5, for mefenamic acid (1, blue), tolfenamic acid (2, green), flufenamic acid (4, purple), meclofenamic acid (3, red), and diclofenac (5, black). The table inset shows the half-lives under experimental conditions and the net change in reaction rate (Δk_{obs}) due to the DOM.

References

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