Supplementary Materials

for

Design, synthesis and pharmacological evaluation of new quinoline-based Panx-1 channel blockers

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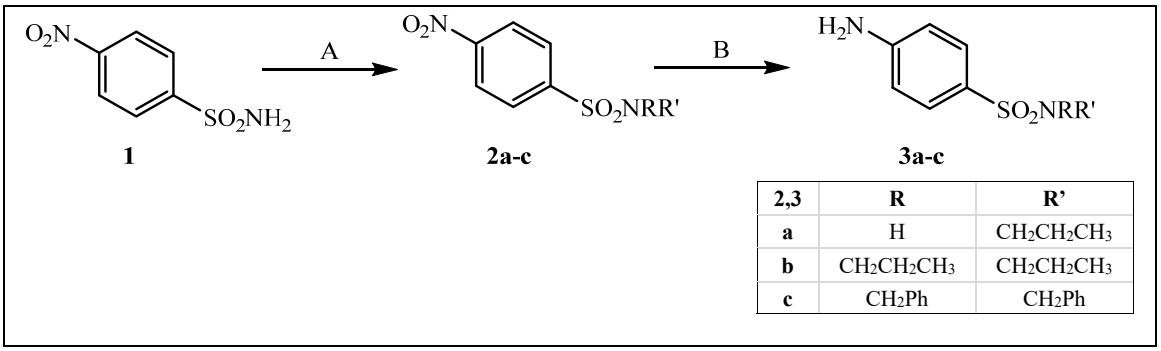
Table of Contents:

5. ADMET assessment (**Tables S6–S11**).
6. Elemental analysis (**Table S12**).

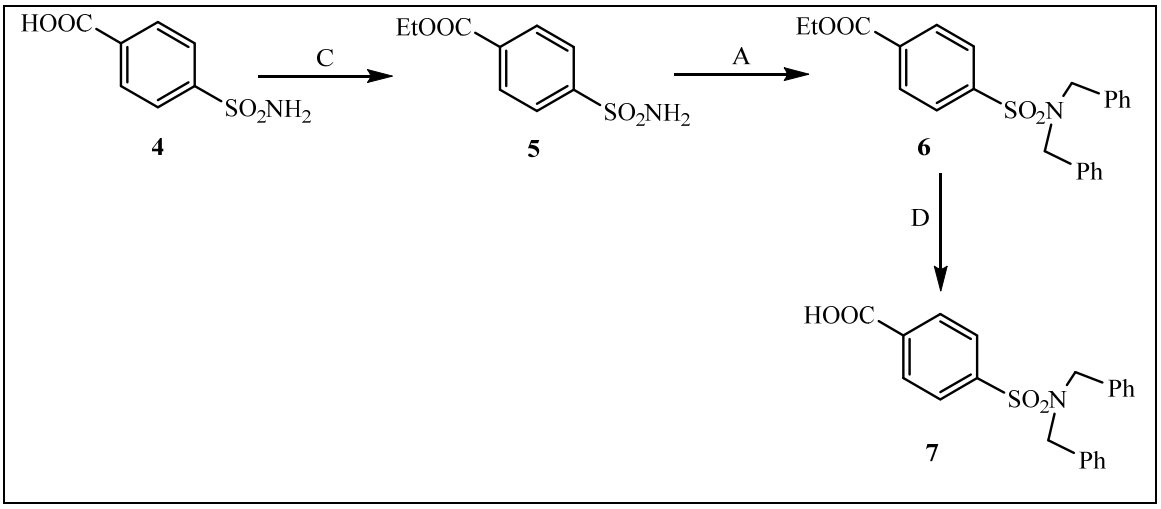
1. Synthesis of reaction intermediates

The scheme describes the procedures used for the synthesis of some reagents (others are commercially available) to obtain the final compounds. For reagents **3a-c**, the synthetic process involves alkylation of commercial 4-nitrobenzenesulfonamide (**1**) with the appropriate halide derivative and K_2CO_3 in dry DMF. Specifically, 1-bromopropane is used to obtain the monoalkylated and bialkylated products **3a,b** (Carmellino, Caccialanza, & Borgogna, 1983) and benzyl bromide is used to obtain the bialkylated product **3c** (Hui Po & Sheu Ju, 1987). Finally, for compound **7**, the synthetic process involved three steps: esterification of commercial 4-sulfamoylbenzoic acid (**4**) resulting in the corresponding ethyl ester 5 (Turkes, et al., 2019); alkylation of the sulfonamide with benzyl bromide (using the same procedure reported above for reagents 3a-c) leading to the dibenzylated intermediate **6** (Caturla, et al., 2004); and finally, hydrolysis of the ester to acid **7** (Hanke, et al., 2013).



1 **2a-c** **3a-c**

2,3	R	R'
a	H	$CH_2CH_2CH_3$
b	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$
c	CH_2Ph	CH_2Ph



Reagent and conditions:

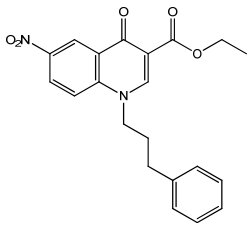
A: K_2CO_3, dry DMF, r.t., 30', then appropriate halide derivative, 80°C, 3h.

B: H_2, Pd/C, EtOH abs, 30 psi, r.t., 1h 30'.

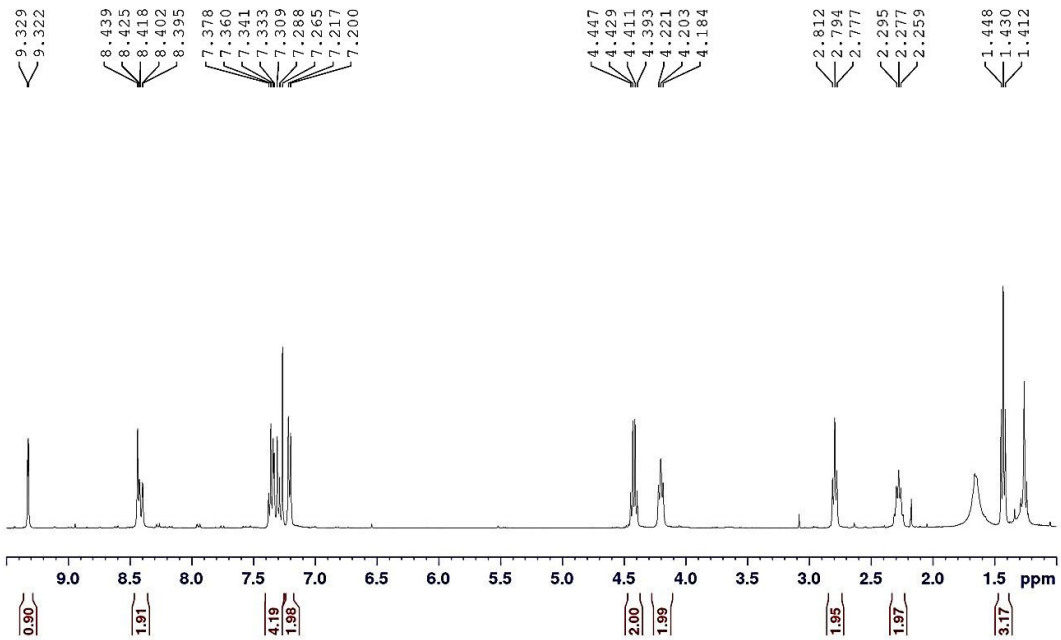
C: EtOH abs, H_2SO_4 conc., reflux, 5h.

D: EtOH 96%, NaOH 40%, reflux, 2h.

2. NMR spectra of some reaction intermediates and representative final compounds



1H NMR compound 4c
CDCl3



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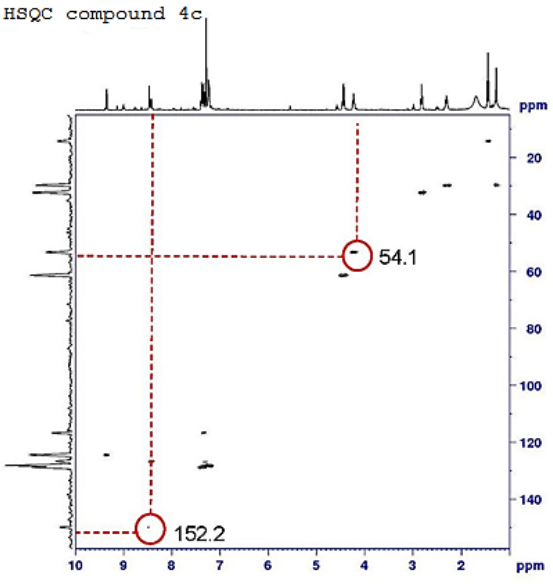

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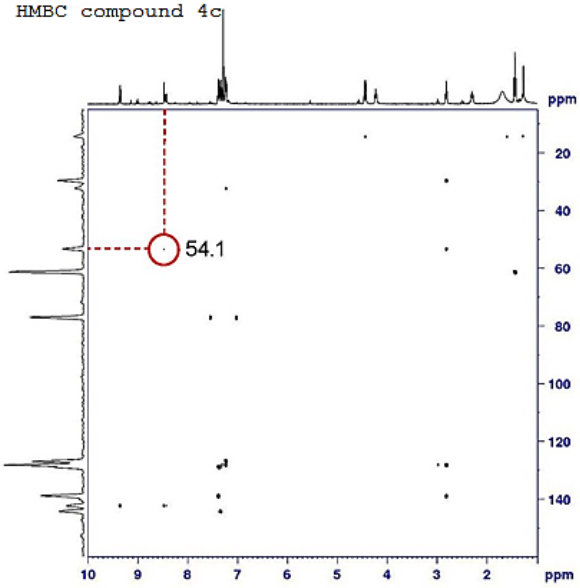

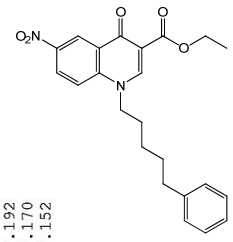

1H NMR compound 4e
CDCl3

9.306
9.299
8.475
8.458
8.451
7.564
7.541
7.313
7.295
7.283
7.277
7.229
7.210
7.205
7.192
7.170
7.152
4.449
4.431
4.413
4.395
4.245
4.226
4.207
2.675
2.656
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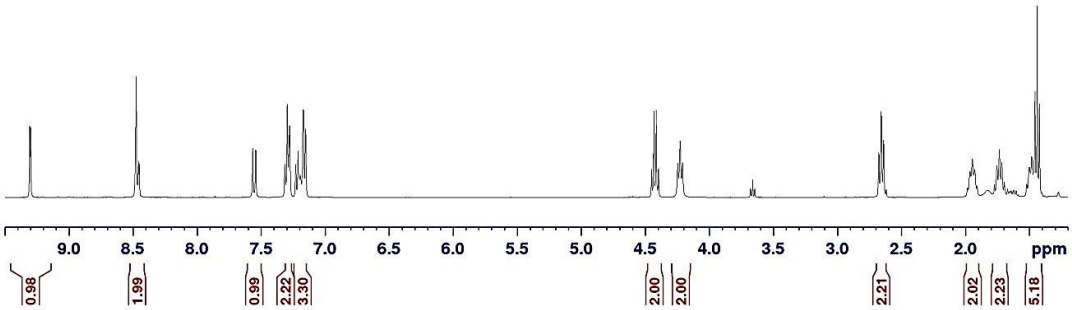
1.733
1.715



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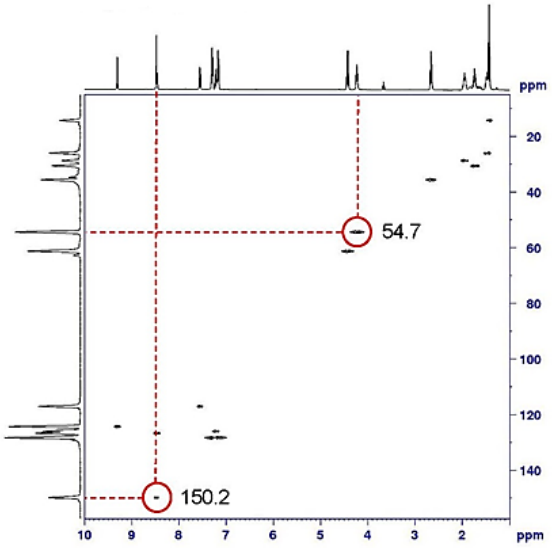

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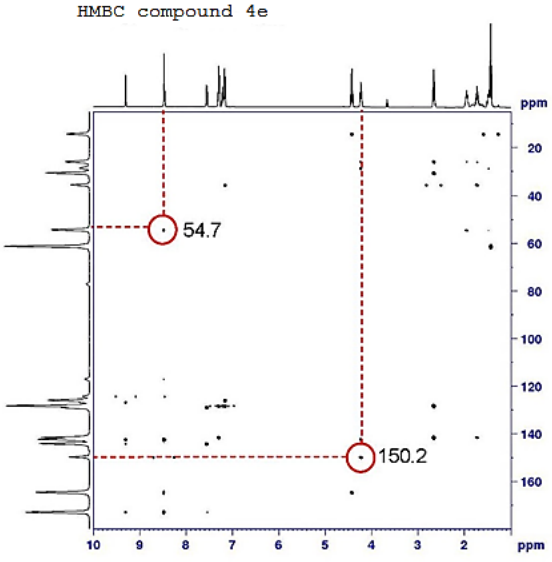
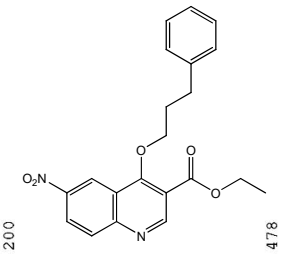
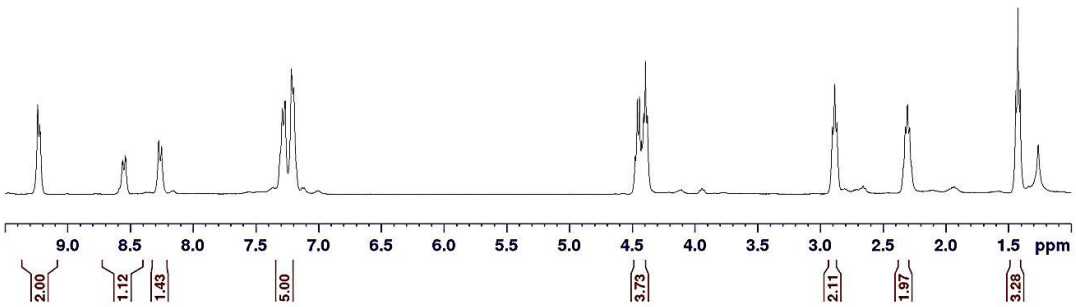


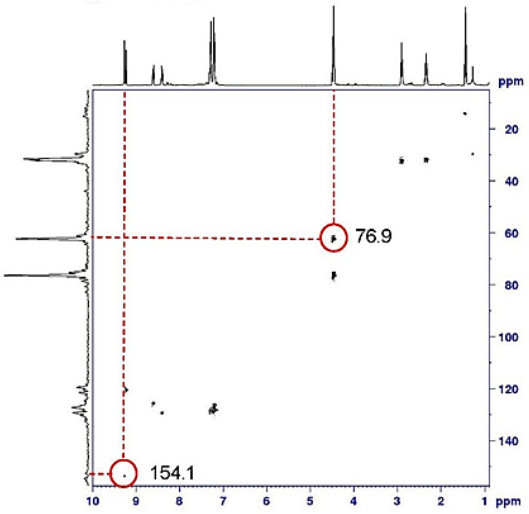
1H NMR compound 5c
CDCl3



9.240 9.225 9.219 8.566 8.560 8.544 8.538 8.275 8.253 7.290 7.272 7.266 7.217 7.200 4.478 4.460 4.442 4.424 4.410 4.394 4.378 2.904 2.886 2.867 2.324 2.305 2.288 1.442 1.424 1.407



HSQC compound 5c



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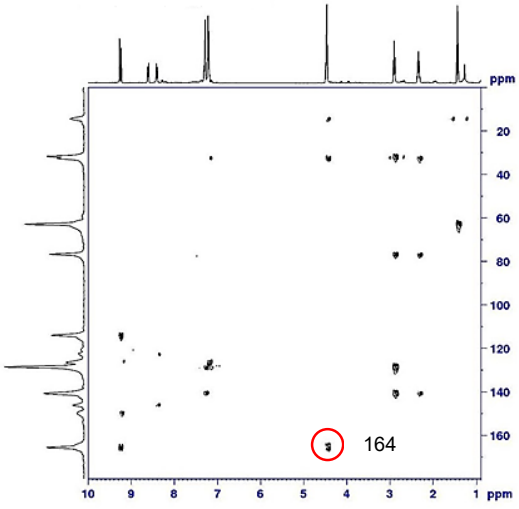

1H NMR compound 5e
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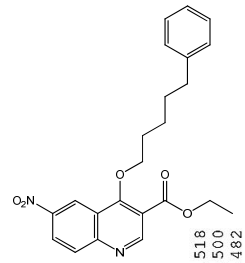
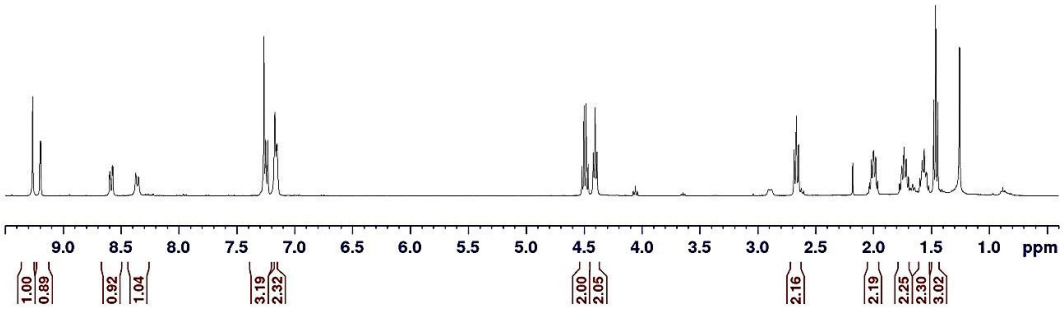
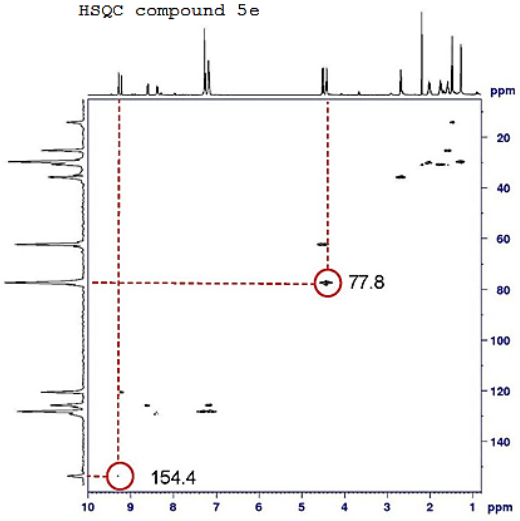


9.264 9.200 9.194 8.599 8.593 8.576 8.570 8.373 8.350
7.296 7.266 7.251 7.233 7.171 7.158 7.152
4.518 4.500 4.482 4.464 4.421 4.405 4.388
2.685 2.666 2.647 2.623 2.034 2.018 1.999 1.980 1.964 1.774 1.755 1.736 1.717 1.698 1.599 1.579 1.570 1.561 1.548 1.480



HSQC compound 5e



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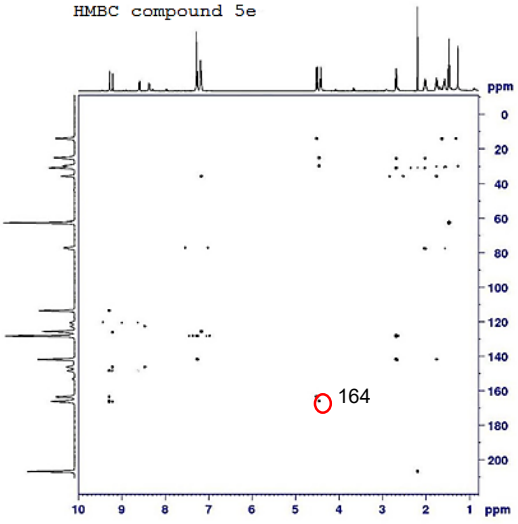
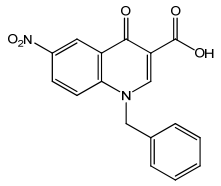
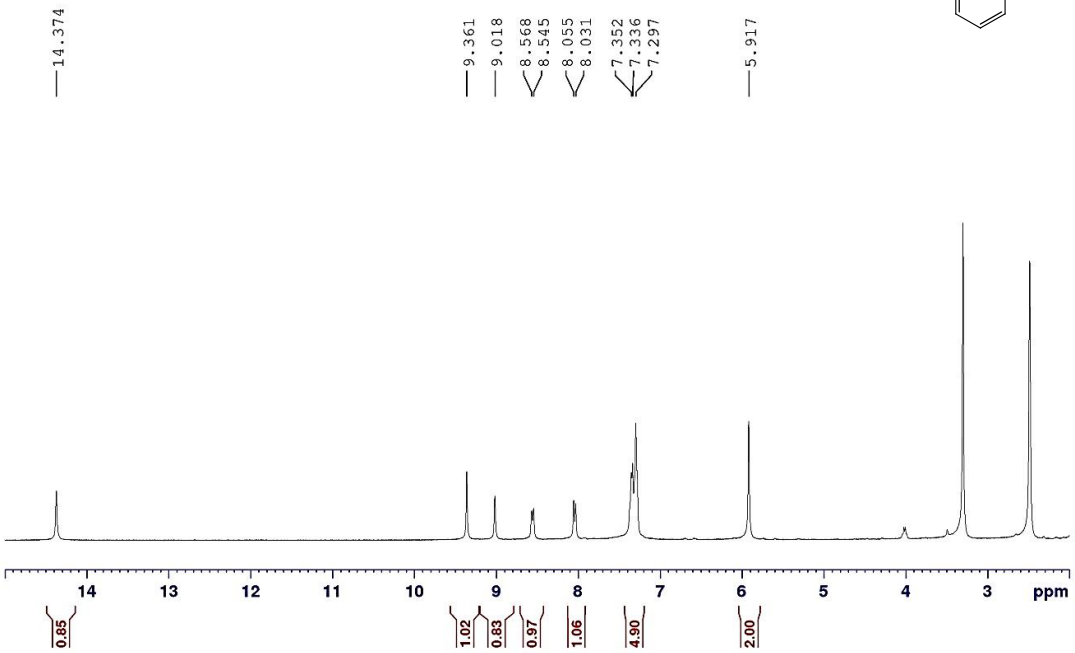
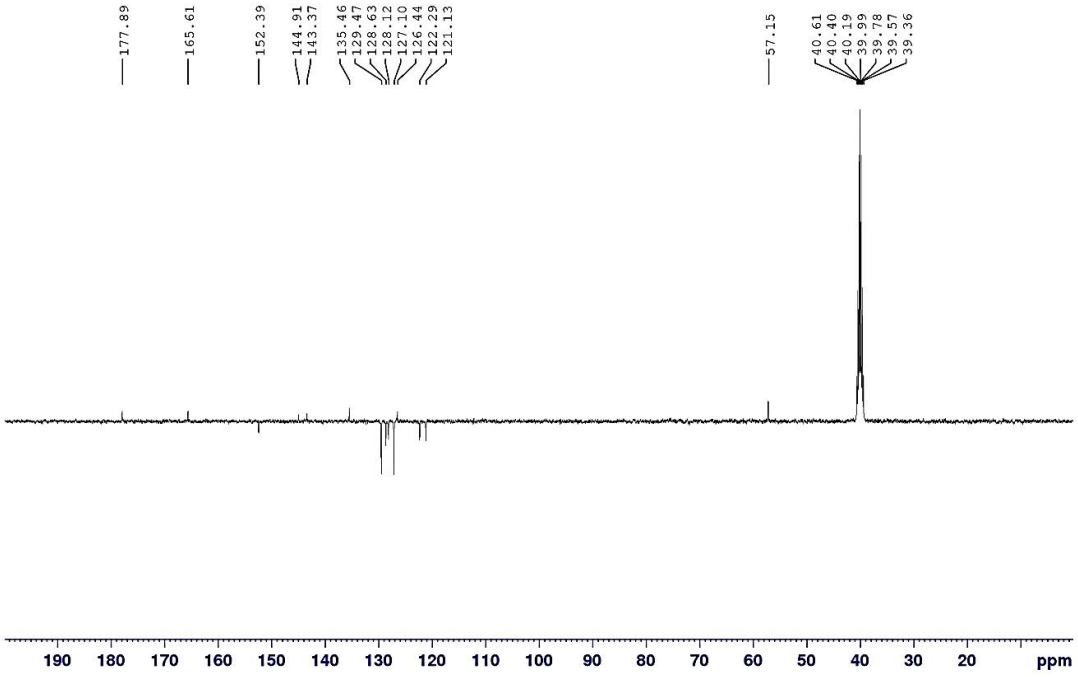


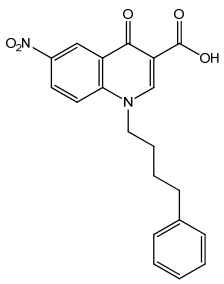


1H NMR compound 6a
DMSO



13C NMR compound 6a
DMSO





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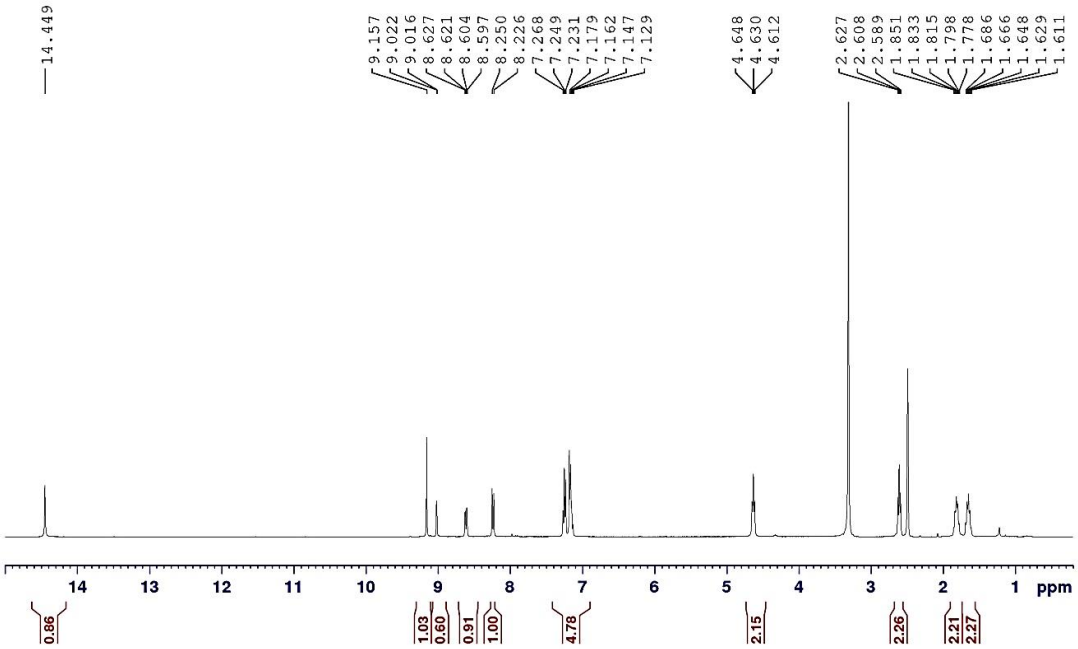
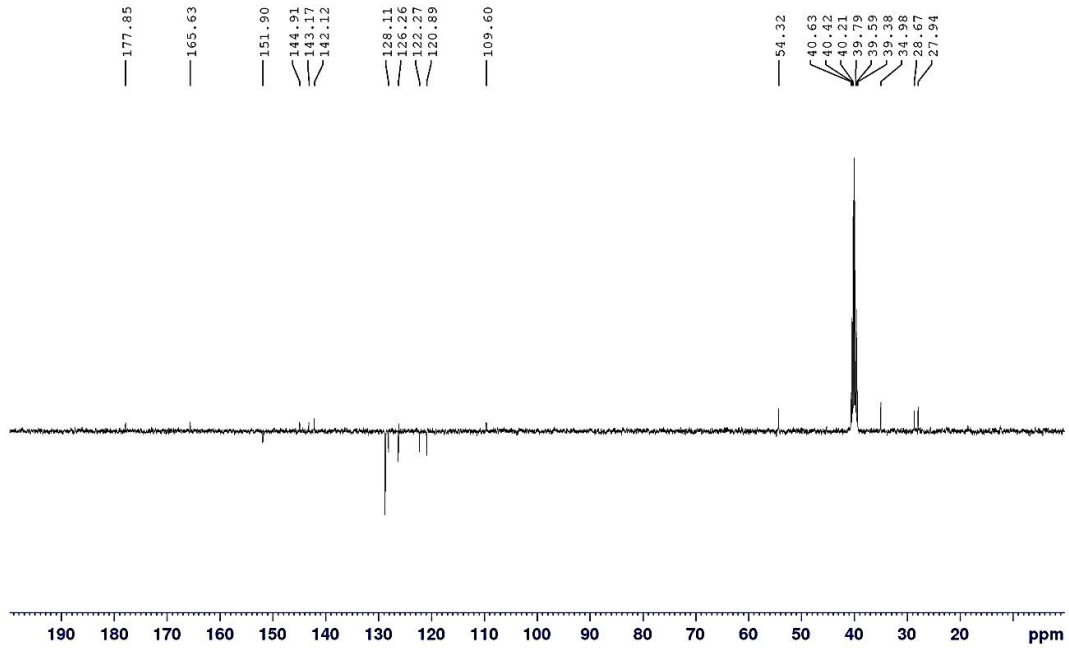
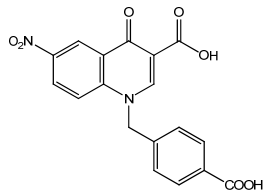


13C NMR compound 6d
DMSO





1H NMR compound 6g
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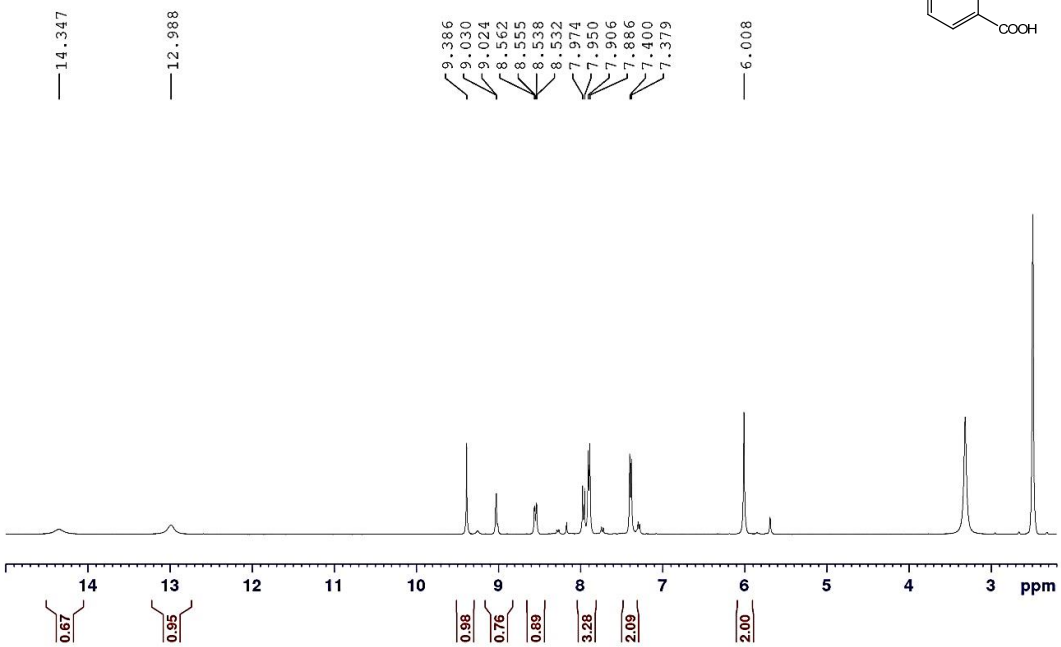
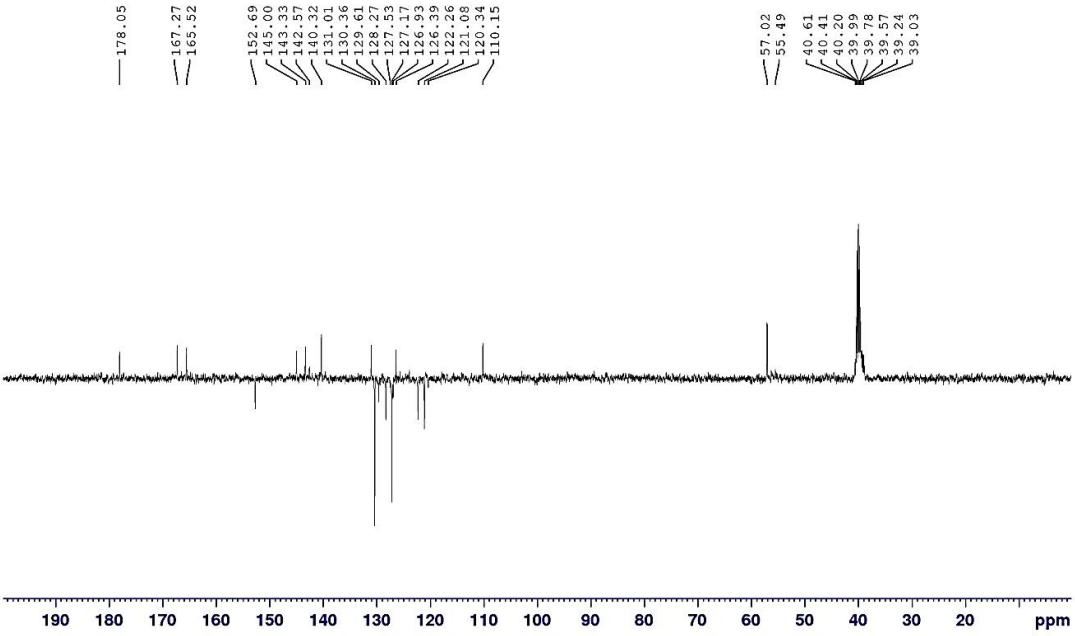
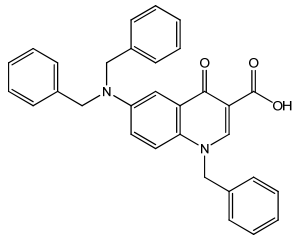
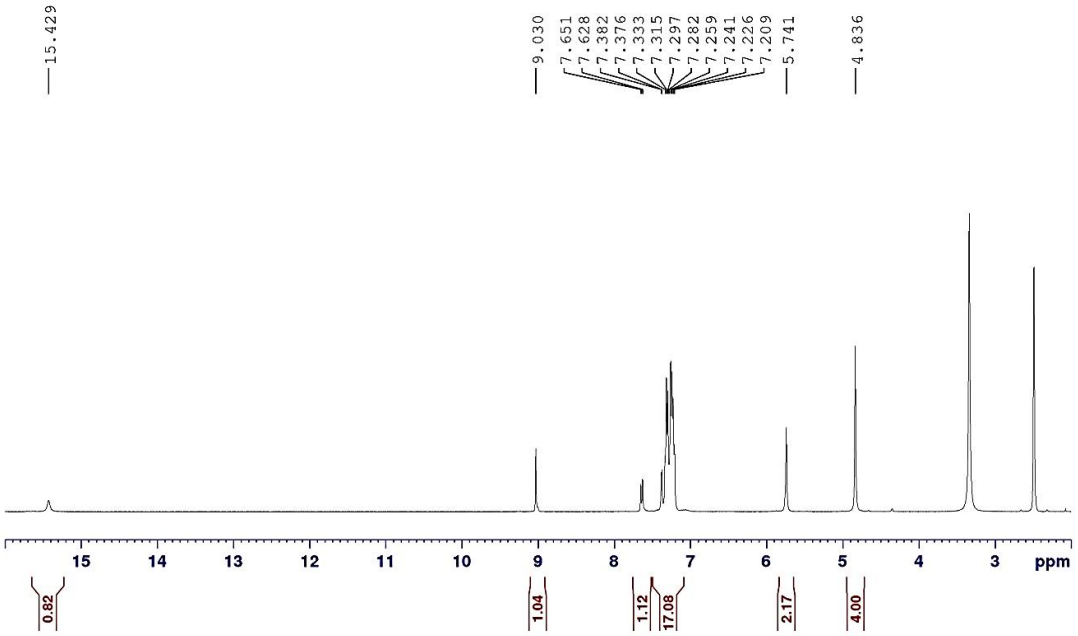


13C NMR compound 6g
DMSO





1H NMR compound 10
DMSO



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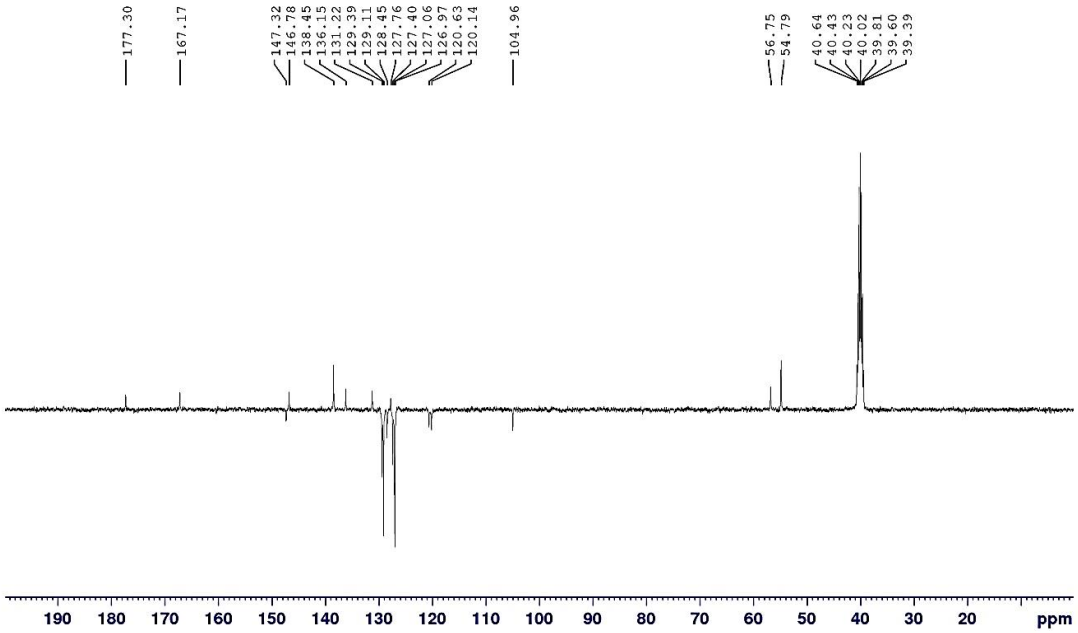

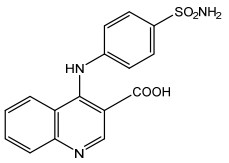

1H NMR compound **13c**
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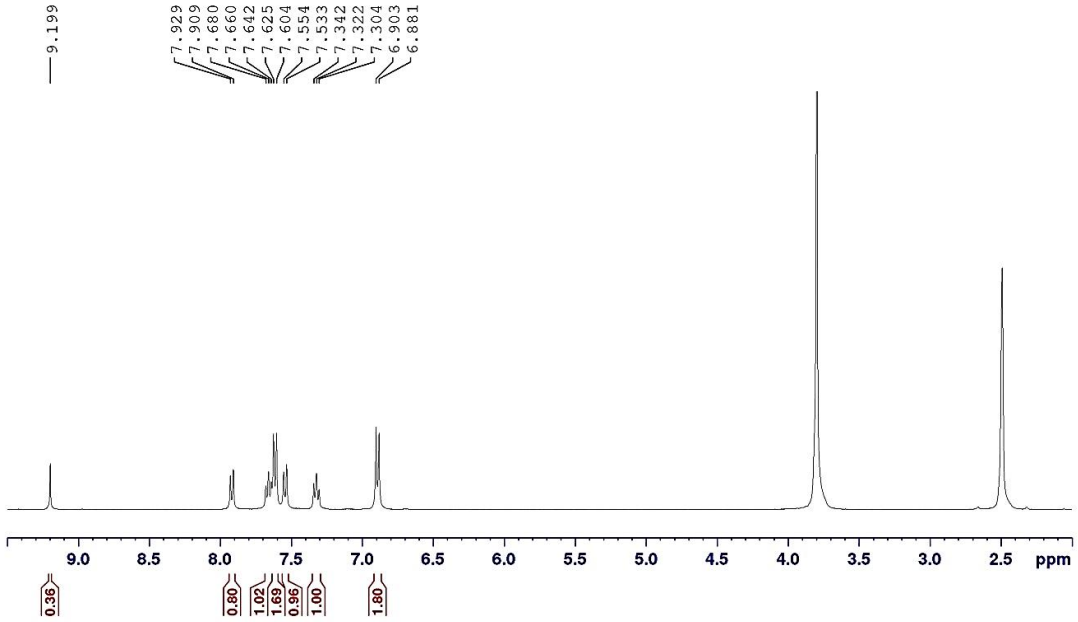

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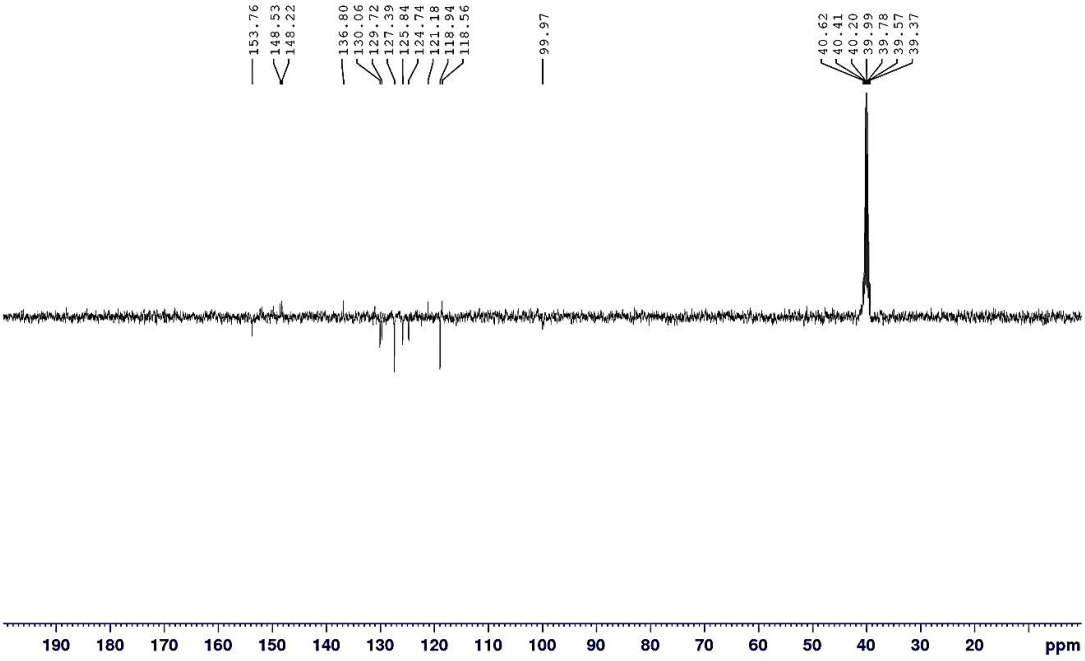

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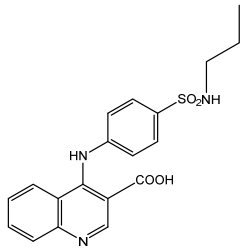
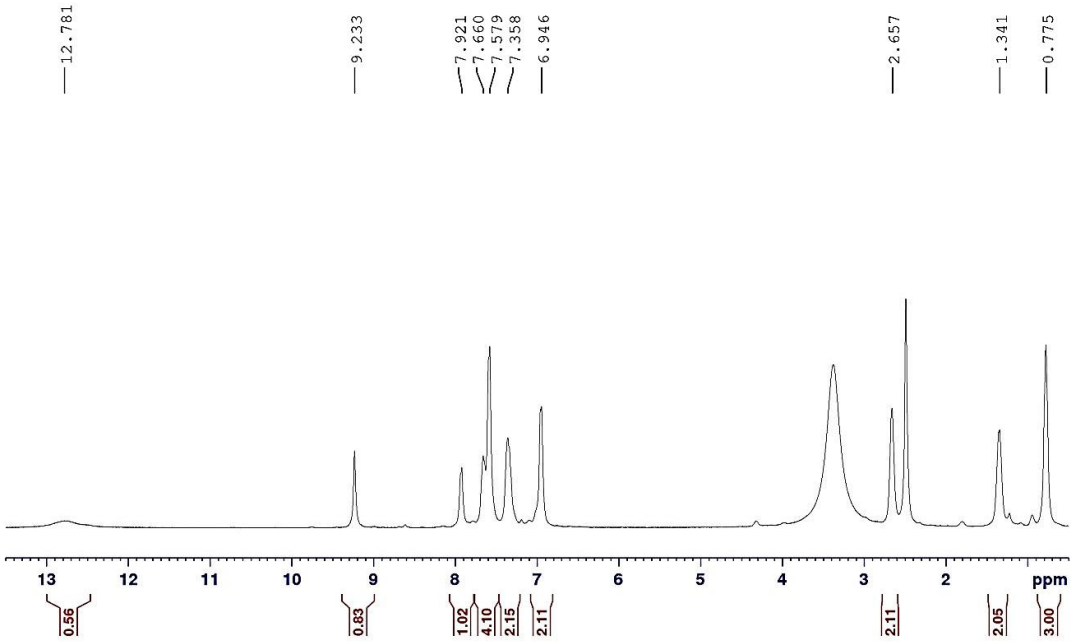
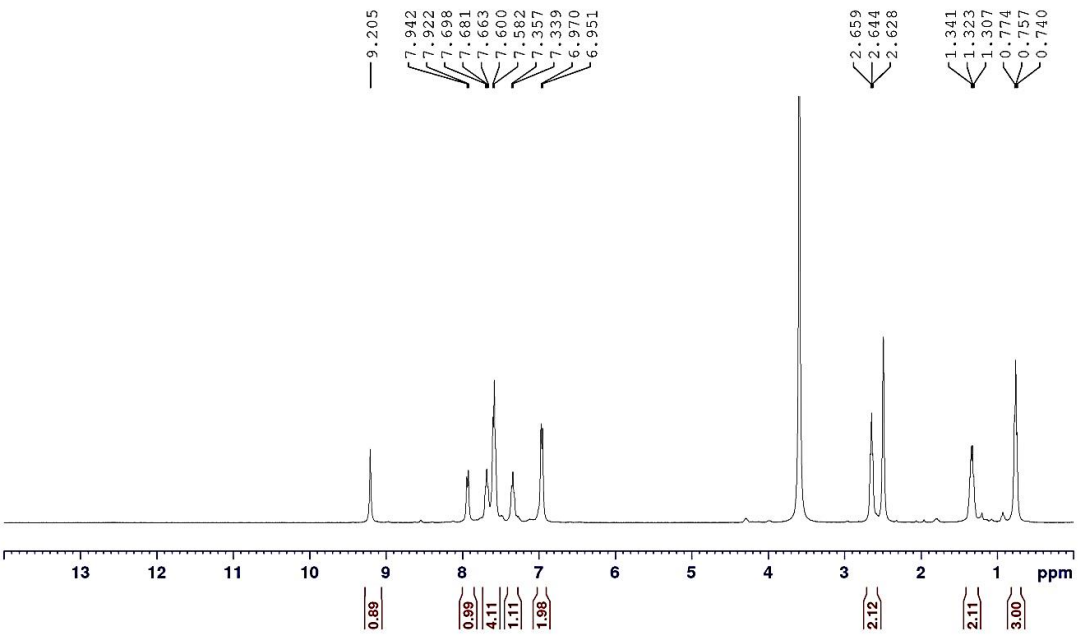
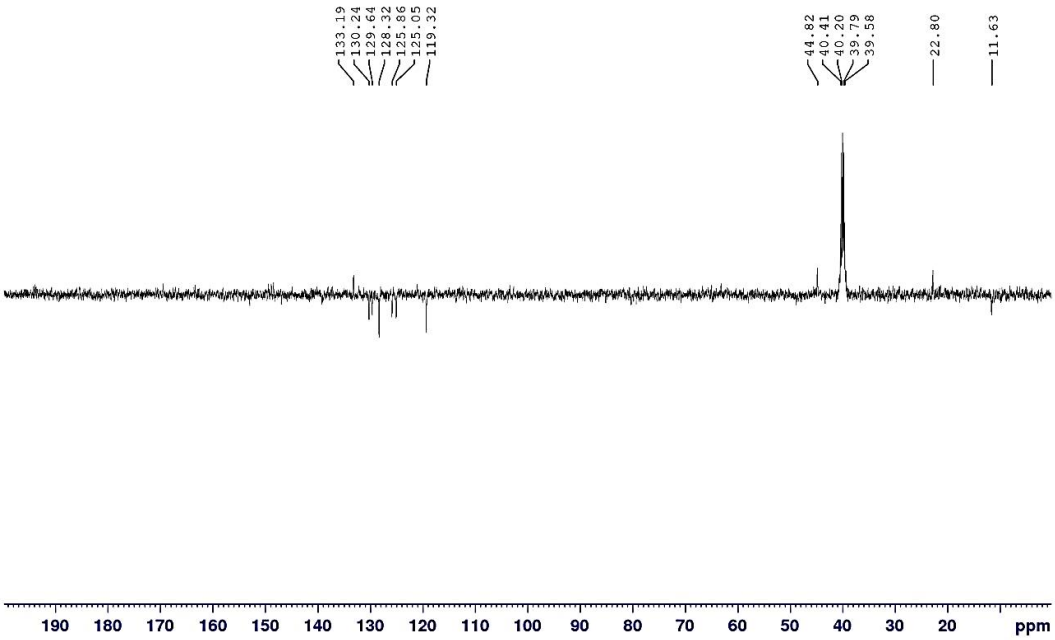


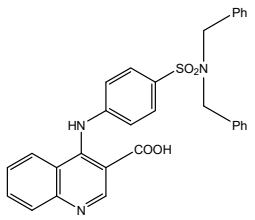


1H NMR compound **13d**
DMSO+D2O

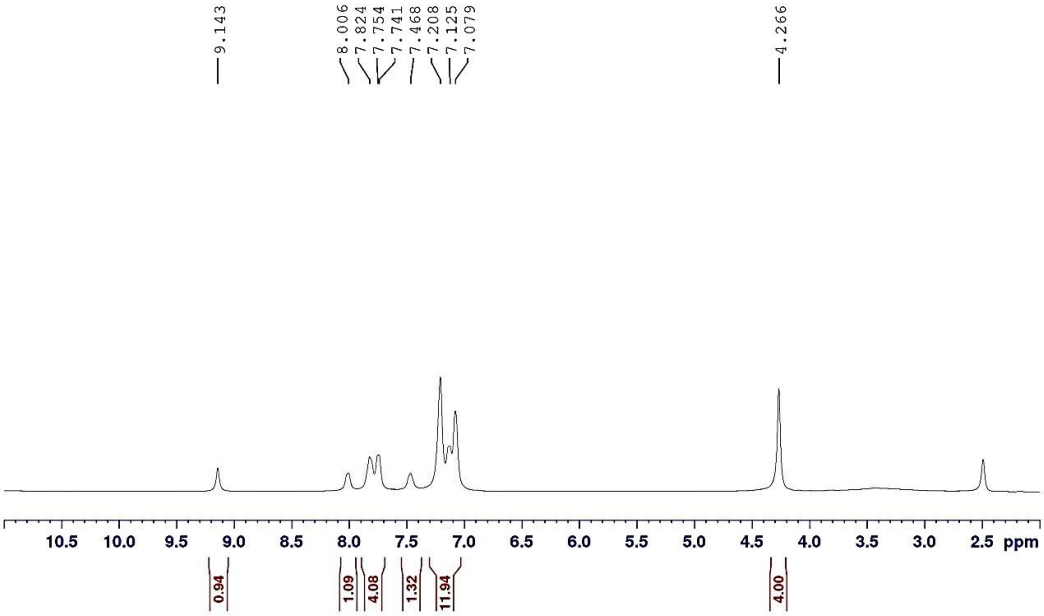


13C NMR compound **13d**
DMSO

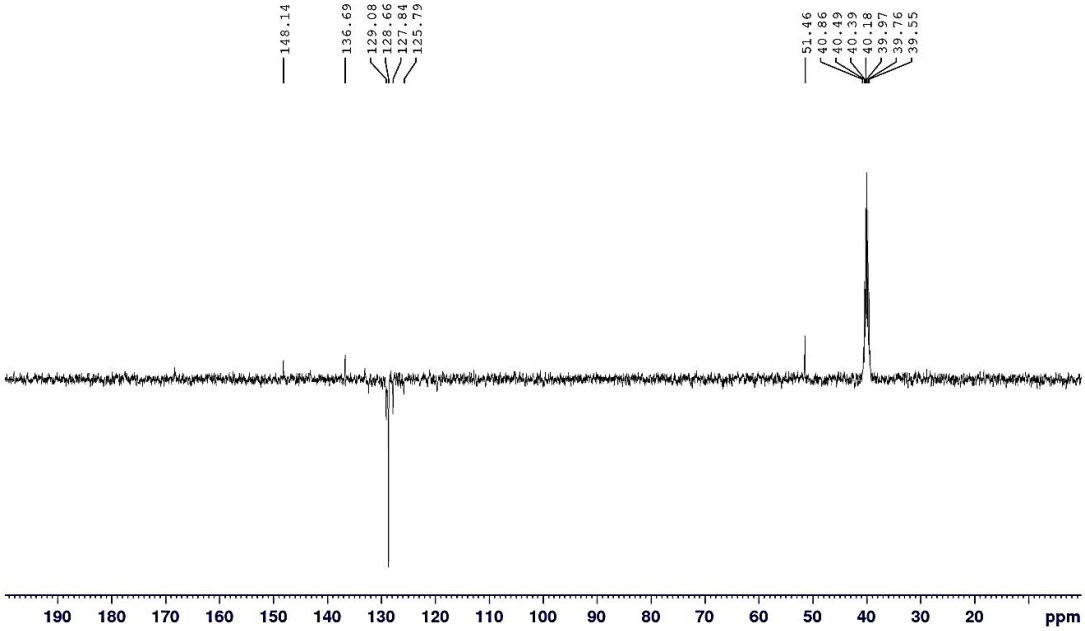


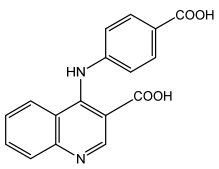


1H NMR compound **13f**
DMSO

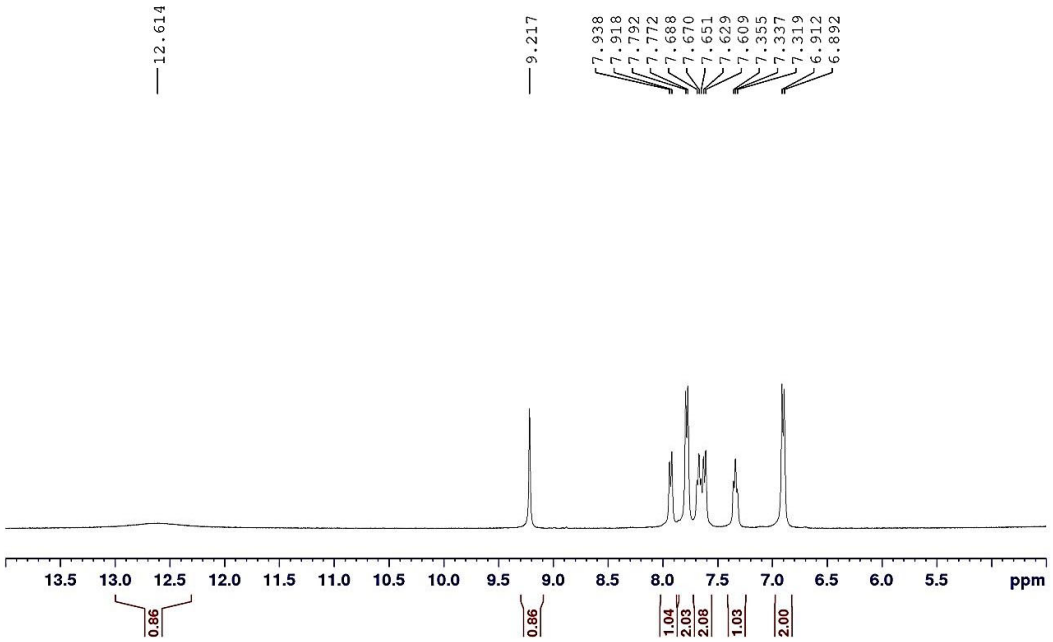


13C NMR compound **13f**
DMSO





1H NMR compound : **13g**
DMSO



13C NMR compound **13g**
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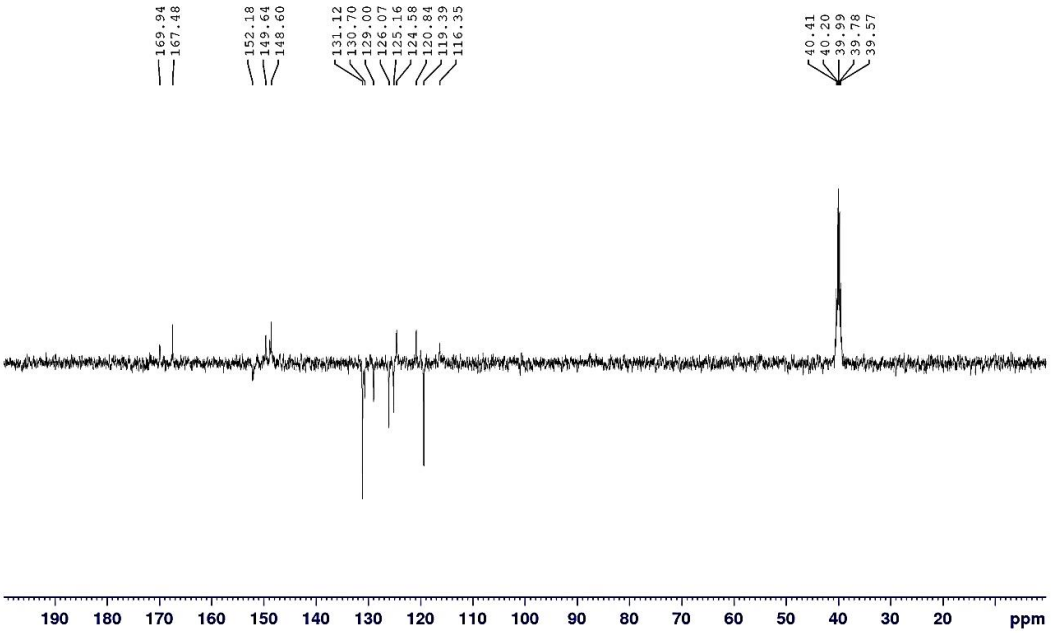
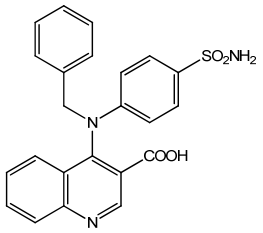
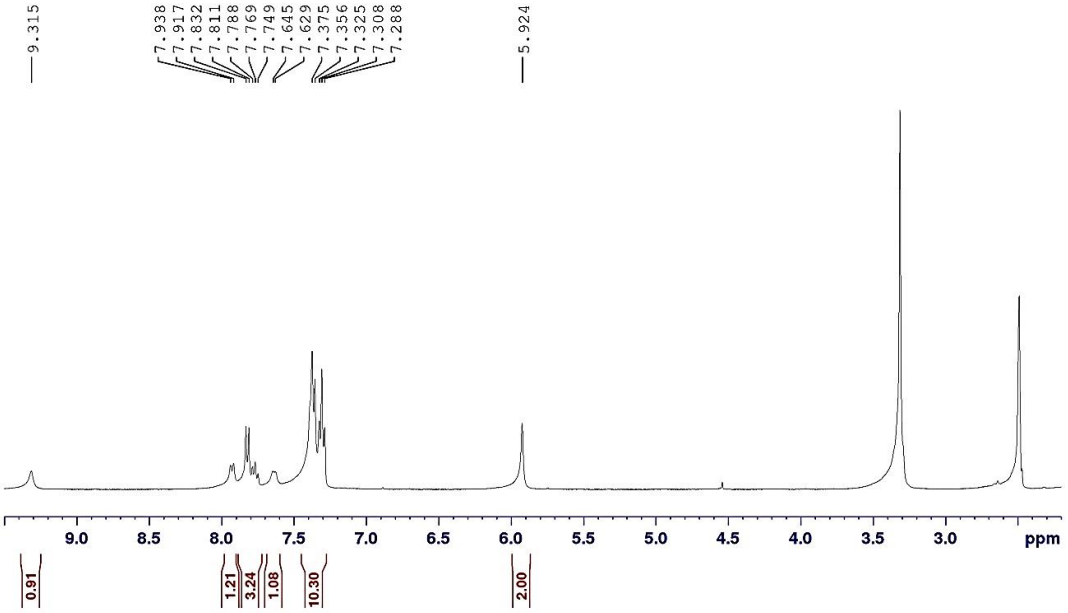
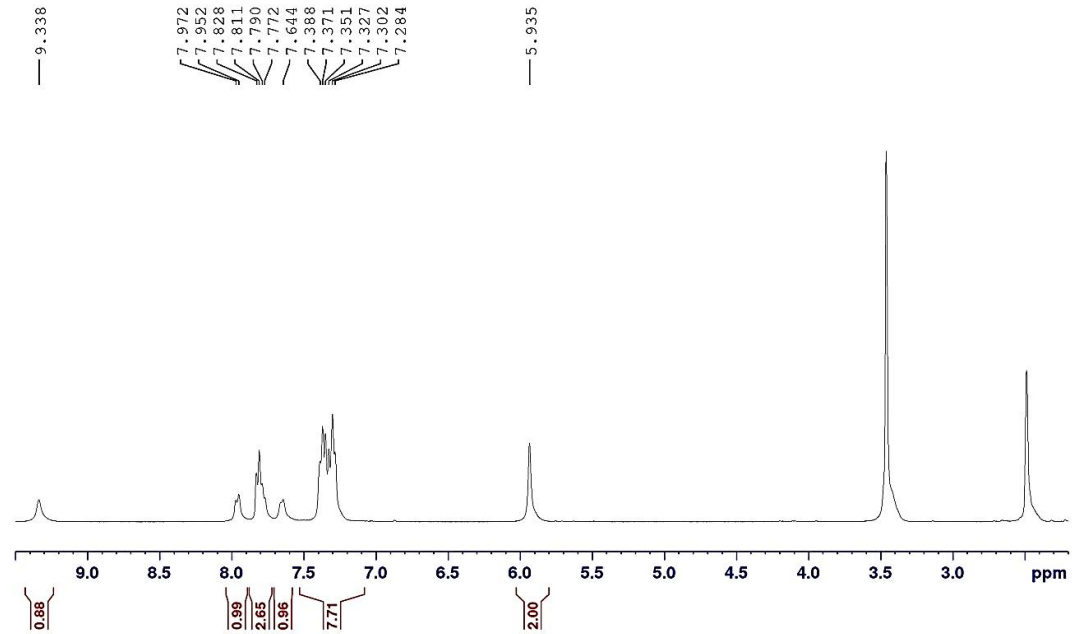




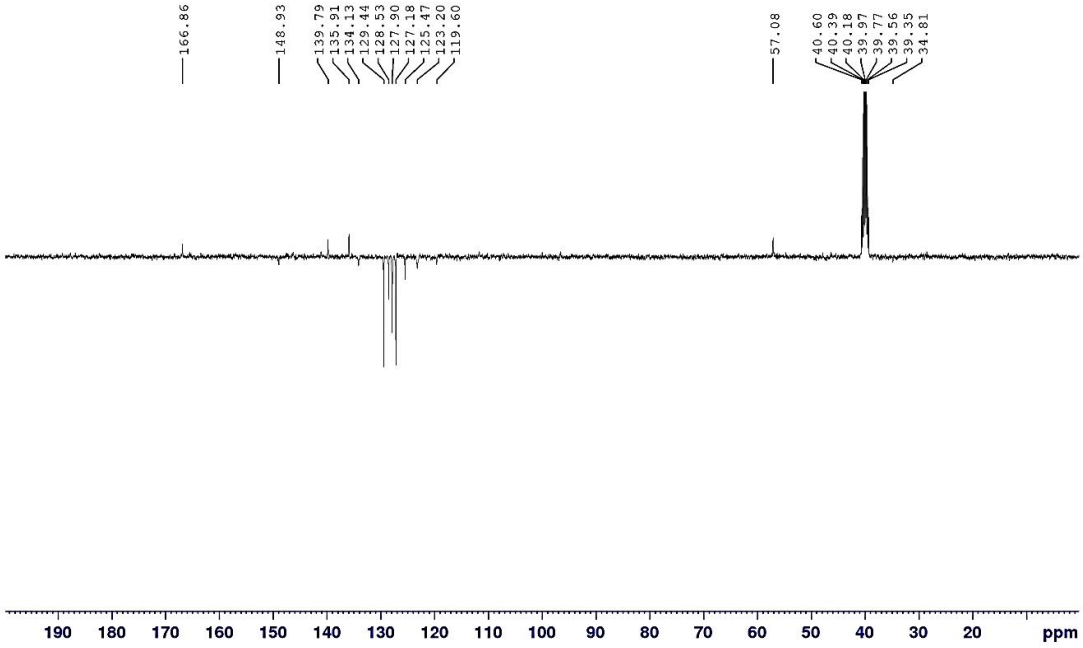
1H NMR compound 15
DMSO



1H NMR compound 15
DMSO+D2O



13C NMR compound 15
DMSO



3. Chemical stability tests

Table S1: Stability of compounds and KEE in PBS and plasma

Comp.	PBS (min)	H-pl (min)
4a	>120	>120
4b	>120	>120
4d	>120	>120
4e	>120	>120
4f	>120	>120
KEE Control	-	119

Table S2: Elution gradient of mobile phase used for LC-MS/MS analyses

Time (min)	A (%)	B (%)	Flow (mL min^{-1})
0.00	60	40	0.25
2.50	10	90	0.25
4.50	10	90	0.25
4.51	90	10	0.25
6.50	90	10	0.25

solvent A: 5 mM of ammonium formate and 10mM of formic 875 acid in mQ water: acetonitrile 90:10 (v/v) solution;
solvent B: 5 mM of ammonium for-876 mate and 10mM of formic acid in mQ water: acetonitrile 10:90 (v/v) solution.

Table S3: MRM parameters

Compounds	Precursor ion (m/z)	Quantitation ion (m/z) [CE (V)]	Qualification ion (m/z) [CE (V)]
IS	455	165 [30]	-
4a	353	91 [15]	307 [15]
4b	367	105 [15]	321 [15]
4d	395	91 [15]	349 [20]
4e	409	217 [30]	363 [20]
4f	432	170 [25]	386 [15]

KEE	283	209 [15]	105 [30]

Table S4: Linear regressions data, linearity coefficients, LOD and LOQ values for each analyte

Compounds	Slope (PAR/ng ml^{-1})	Intercept (PAR)	R^2	y-SD LOD (ng mL^{-1})	y-SD LOQ (ng mL^{-1})
4a	0.102	0.151	0.998	2.5	7.5
4b	0.112	0.142	0.997	3.4	10.1
4d	0.115	0.191	0.997	3.4	10.2
4e	0.112	0.260	0.996	4.3	12.8
4f	0.029	0.031	0.999	2.0	6.0

Solution stability profiles

The solution stability profiles in PBS and human plasma were obtained by monitoring the variation of analyte concentration at different incubation times (0, 30, 60 e 120 min. at 37°C). They are reported in **Figures S1-S5**.

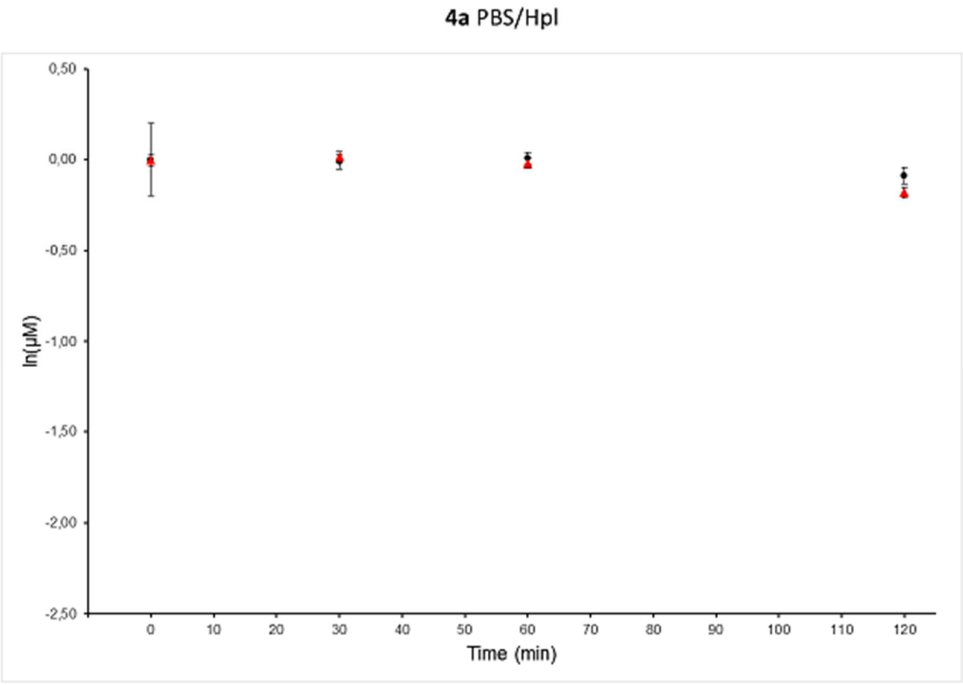


Figure S1. Degradation plots of compound **4a** in PBS (black spot) and human plasma (red triangle).

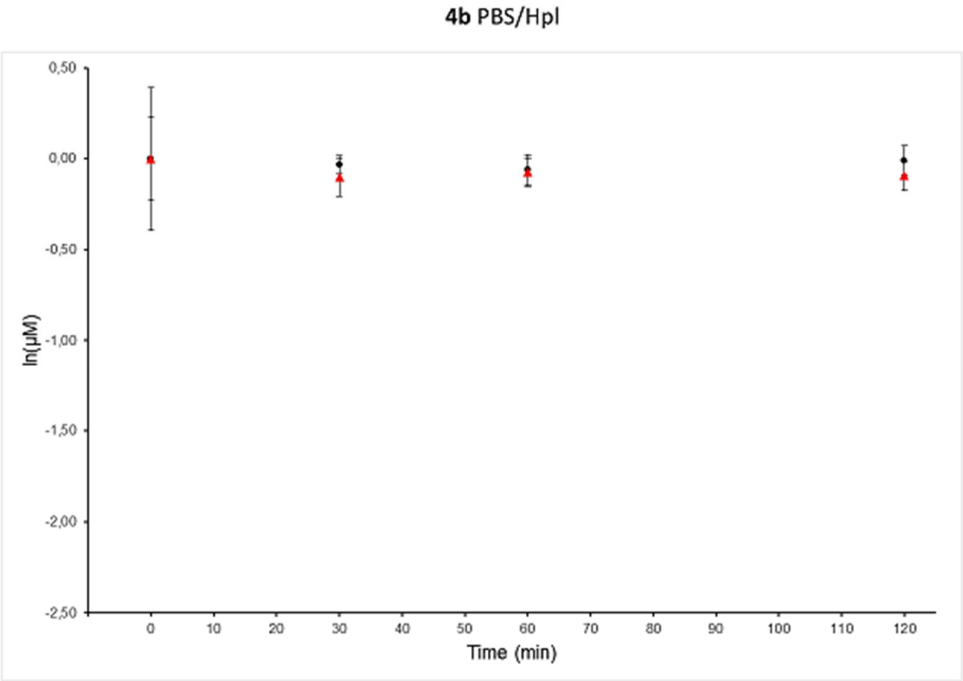


Figure S2. Degradation plots of compound **4b** in PBS (black spot) and human plasma (red triangle).

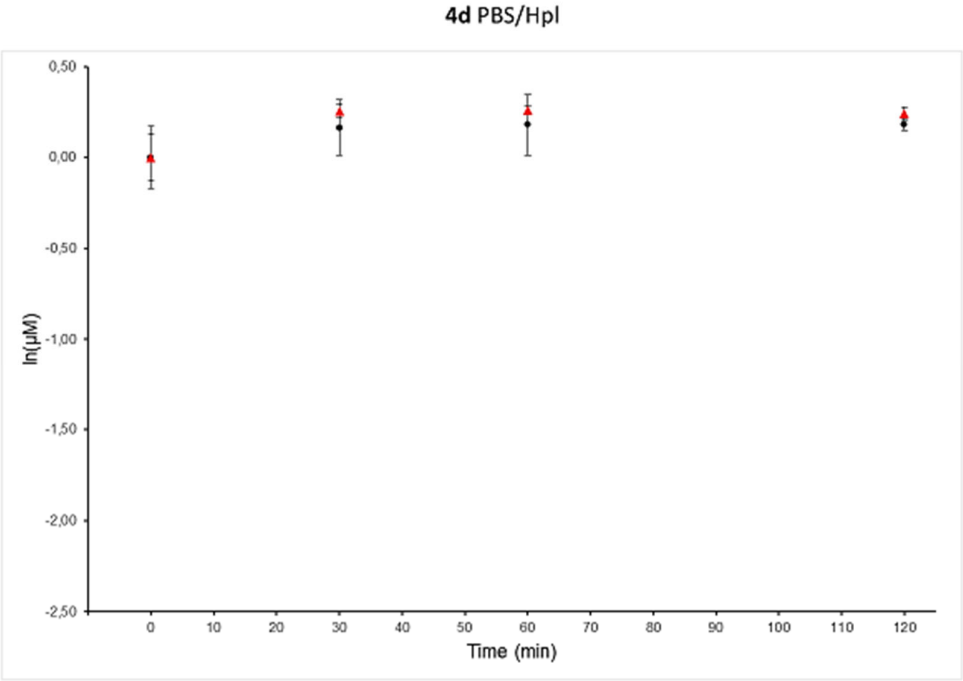


Figure S3. Degradation plots of compound **4d** in PBS (black spot) and human plasma (red triangle).

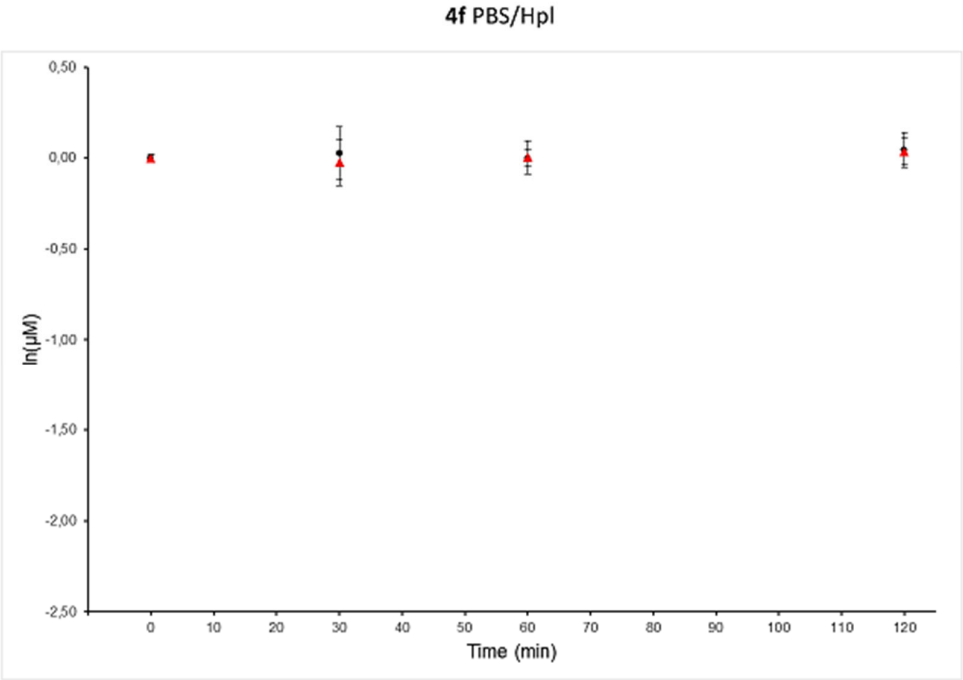


Figure S4. Degradation plots of compound **4f** in PBS (black spot) and human plasma (red triangle).

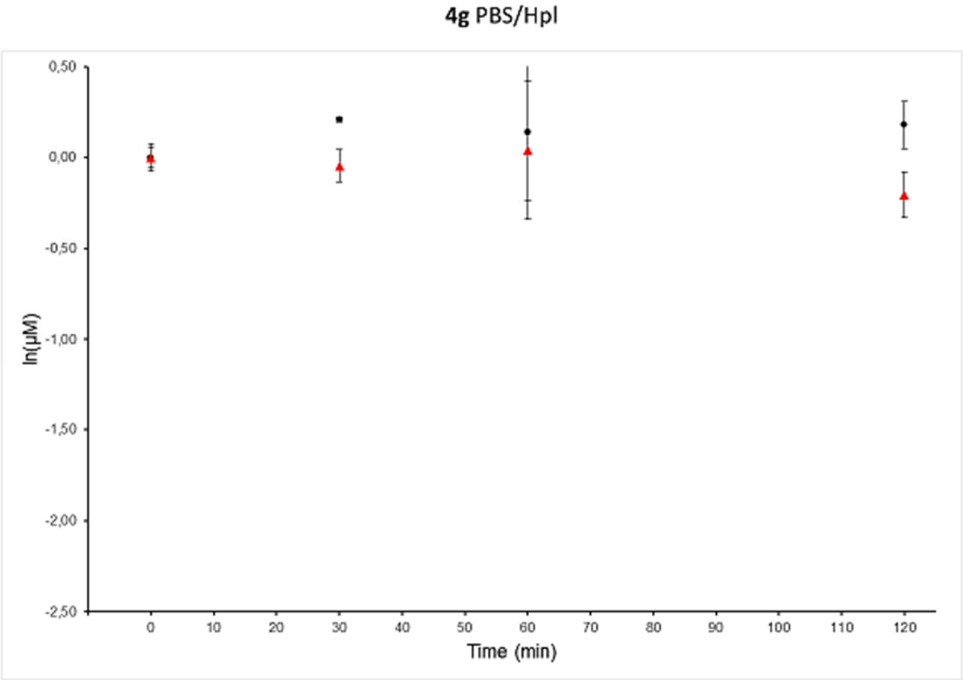


Figure S5. Degradation plots of compound **4g** in PBS (black spot) and human plasma (red triangle).

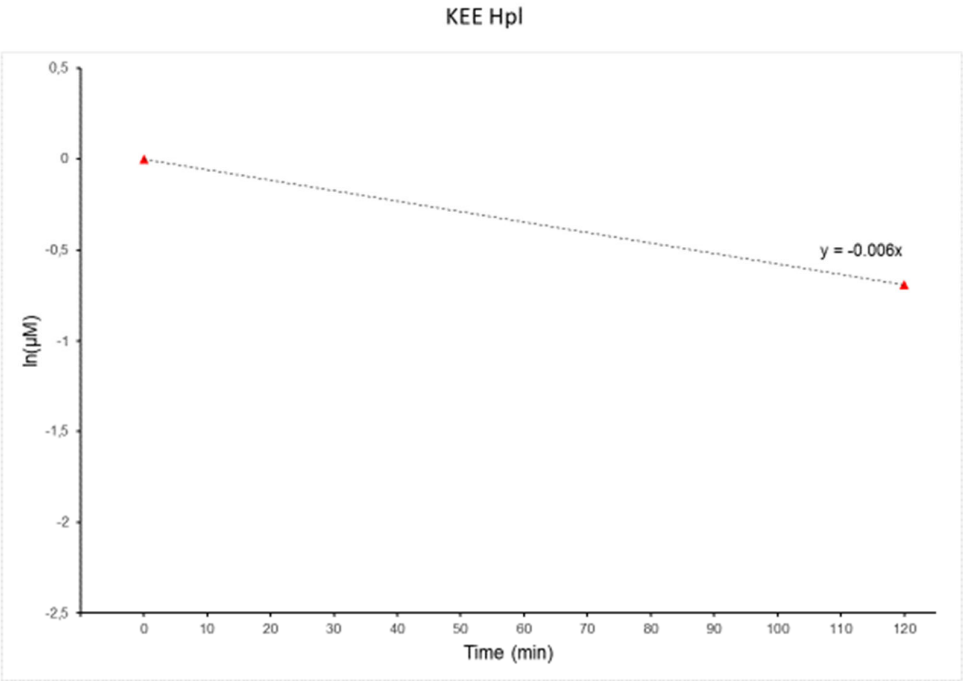


Figure S6. Degradation plot of Ketoprofen Ethyl Ester (KEE), reference compound, in human plasma.

4. Single-Crystal X-ray Diffraction

Table S5. Crystallographic data and refinement parameters for **4d**.

	4d
empirical formula	$C_{22}H_{22}N_2O_5$
formula weight	394.42
T (K)	100
Crystal system, space group	Triclinic, P-1
λ (Å)	1.54178
Unit cell dimensions (Å, °)	a = 10.451(2), α = 81.071(8) b = 13.852(2), β = 71.945(8) c = 15.030(2), γ = 69.602(9)
***V* (Å^3)**	1936.2(5)
Z, dcalc(g/cm^3)	4, 1.353
μ (mm^{-1})	0.797
F(000)	832
Reflections collected/unique	26025 / 6301
Data/paramenters	6301 / 523
Final R indices [I>2σ(I)]	R1 = 0.0946, wR2 = 0.2538
R indices all data	R1 = 0.1355, wR2 = 0.3205
GoF	1.168

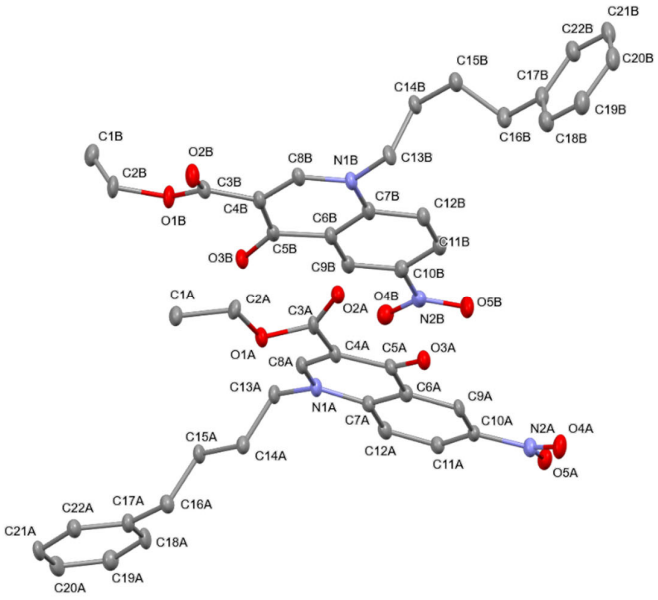


Figure S7. Ortep-3 view of the asymmetric unit of **4d**. Hydrogen atoms have been omitted for sake of clarity.

5. ADME assessment (Tables S6-S11)

Table S6. SwissADME calculated physiochemical properties.

Mol	Molecular Weight	LogP	TPSA
6f	403.372	1.3036	173.66 Å²
6g	368.301	2.3544	142.42 Å²
13e	427.526	4.4873	107.98 Å²
13g	308.293	3.3748	99.52 Å²

Table S7. SwissADME calculated lipophilicities parameters.

Mol	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT Log P	Consensus Log P
6f	0.42	1.87	2.91	-0.38	-0.77	0.81
6g	0.86	2.83	2.88	0.69	0.54	1.56
13e	3.14	4.57	5.57	0.91	2.67	3.41
13g	1.39	3.34	3.37	0.64	2.02	2.15

Table S8. SwissADME calculated water solubility parameters.

Mol	ESOL Log S	ESOL Solubility (mg/ml)	ESOL Solubility (mol/l)	ESOL Class	Ali Log S	Ali Solubility (mg/ml)	Ali Solubility (mol/l)	Ali Class
6f	-3.61	9.86E-02	2.44E-05	Soluble	-5.14	2.93E-03	7.27E-06	Moderately soluble
6g	-4.01	3.56E-02	9.66E-05	Soluble	-5.48	1.22E-03	3.32E-06	Moderately soluble
13e	-5.28	2.22E-03	5.20E-06	Moderately soluble	-6.75	7.64E-05	1.79E-07	Poorly soluble
13g	-4.11	2.41E-02	7.83E-05	Moderately soluble	-5.11	2.41E-03	7.82E-06	Moderately soluble

Table S9. SwissADME calculated pharmacokinetics parameters.

Mol	GI absorp.	BBB perm.	Pgp sub.	CYP2D6 substrate[a]	CYP3A4 substrate[a]	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
6f	Low	No	No	No	No	No	No	Yes	No	No	-7.43
6g	Low	No	No	No	No	No	No	Yes	No	No	-6.54

13e	Low	No	No	No	No	No	Yes	Yes	No	Yes	-5.54
13g	High	No	No	No	No	Yes	No	No	No	No	-5.81

[a] pkCSM (http://biosig.unimelb.edu.au).

Table S10. Swiss ADME calculated drug likeness parameters.

Mol	**Lipinski #violations**	**Ghose #violations**	**Veber #violations**	**Egan #violations**	**Muegge #violations**
6f	0	0	1, TPSA>140	1, TPSA>131.6	1, TPSA>150
6g	0	0	1, TPSA>140	1, TPSA>131.6	0
13e	0	0	0	0	0
13g	0	0	0	0	0

Table S11. pkCSM calculated excretion properties.

Mol	**Total Clearance Log(ml/min/kg)**	**Renal OCT2 substrate**
6f	0.89	No
6g	0.73	No
13e	0.70	No
13g	0.34	No

6. Table S12. Elemental analysis

Comp.	Formula (MW)	Anal. Calcd.				Anal. Found		
		C	H	N		C	H	N
4b	$C_{20}H_{18}N_2O_5$ (366.37)	65.57	4.95	7.65		65.83	4.97	7.68
4c	$C_{21}H_{20}N_2O_5$ (380.39)	66.31	5.30	7.36		66.57	5.32	7.39
4d	$C_{22}H_{22}N_2O_5$ (394.42)	66.99	5.62	7.10		66.72	5.59	7.07
4e	$C_{23}H_{24}N_2O_5$ (408.45)	67.63	5.92	6.86		67.36	5.90	6.83
4f	$C_{19}H_{17}N_3O_7S$ (431.42)	52.90	3.97	9.74		53.11	3.98	9.78
4g	$C_{20}H_{16}N_2O_7$ (396.35)	60.61	4.07	7.07		60.85	4.09	7.10
5c	$C_{21}H_{20}N_2O_5$ (380.39)	66.31	5.30	7.36		66.57	5.32	7.39
5e	$C_{23}H_{24}N_2O_5$ (408.45)	67.63	5.92	6.86		67.36	5.90	6.83
6a	$C_{17}H_{12}N_2O_5$ (324.29)	62.96	3.73	8.64		62.71	3.71	8.60
6b	$C_{18}H_{14}N_2O_5$ (338.31)	63.90	4.17	8.28		63.64	4.15	8.25
6c	$C_{19}H_{16}N_2O_5$ (352.34)	64.77	4.58	7.95		64.51	4.56	7.92
6d	$C_{20}H_{18}N_2O_5$ (366.37)	65.57	4.95	7.65		65.83	4.97	7.68
6e	$C_{21}H_{20}N_2O_5$ (380.39)	66.31	5.30	7.36		66.57	5.32	7.39
6f	$C_{17}H_{13}N_3O_7S$ (403.37)	50.62	3.25	10.42		50.42	3.24	10.38
6g	$C_{18}H_{12}N_2O_7$ (368.30)	58.70	3.28	7.61		58.46	3.27	7.58
8	$C_{17}H_{14}N_2O_3$ (294.30)	69.38	4.79	9.52		69.66	4.81	9.56
9	$C_{33}H_{30}N_2O_3$ (502.60)	78.86	6.02	5.57		78.54	5.99	5.54
10	$C_{31}H_{26}N_2O_3$ (474.55)	78.46	5.52	5.90		78.77	5.54	5.92
12a	$C_{15}H_{13}N_3O_2S$ (299.35)	60.18	4.38	14.04		60.42	4.40	14.09
12b	$C_{16}H_{12}N_2O_2$ (264.28)	72.72	4.58	10.60		72.43	4.56	10.55
12c	$C_{18}H_{17}N_3O_4S$ (371.41)	58.21	4.61	11.31		58.44	4.63	11.35
12d	$C_{21}H_{23}N_3O_4S$ (413.49)	61.00	5.61	10.16		61.24	5.63	10.20
12e	$C_{24}H_{29}N_3O_4S$ (455.57)	63.28	6.42	9.22		63.53	6.44	9.25
12f	$C_{32}H_{29}N_3O_4S$ (551.66)	69.67	5.30	7.62		69.39	5.38	7.59
12g	$C_{19}H_{16}N_2O_4$ (366.35)	67.85	4.80	8.33		67.58	4.82	8.36
13c	$C_{16}H_{13}N_3O_4S$ (343.36)	55.97	3.82	12.24		55.75	3.80	12.19
13d	$C_{19}H_{19}N_3O_4S$ (385.44)	59.21	4.97	10.90		59.45	4.99	10.94
13e	$C_{22}H_{25}N_3O_4S$ (427.52)	61.81	5.89	9.83		61.56	5.87	9.79
13f	$C_{30}H_{25}N_3O_4S$ (523.61)	68.82	4.81	8.03		68.54	4.79	8.00
13g	$C_{17}H_{12}N_2O_4$ (308.29)	66.23	3.92	9.09		66.49	3.93	9.12
14	$C_{25}H_{23}N_3O_4S$ (461.53)	65.06	5.02	9.10		65.32	5.04	9.13

15	$C_{23}H_{19}N_3O_4S$ (433.48)	63.73	4.42	9.69		63.47	4.40	9.65