Supplementary material

Synthesis and evaluation of 1,4-naphthoquinone ether derivatives as *Sm*TGR inhibitors and new antischistosomal drugs

Authors' names

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Running title

Specific thioredoxin-glutathione reductase inhibitors

Fig. S1. Changes of the Absorption Spectra of the difluoromethylmenadione upon glutathione addition (Panels A-D).

(A) Absorption spectrophotometric variation and (B) Absorbance at 440 nm measured in the time course of the glutathionylation of the difluoromenadione. Solvent: 200µL MeCN + 265 µL NH_4OH buffer at pH 7.5; [difluoromenadione] = 2 x 10^{-4} M (10 µL stock at 10 mM); [GSH] = 10^{-3} M (25 µL stock at 20 mM); *T* = 25°C. (1) t = 0 s; (2) t = 600 s.

(C) Electronic absorption spectra measured for the difluoromenadione and its glutathione adducts.

(D) Distribution diagram of the difluoromenadione and its products as a function of time during the glutathionylation reaction. Solvent: 200µL MeCN + 265 µL NH_4OH buffer at pH 7.5; [difluoromenadione] = 2 x 10^{-4} M (10 µL stock at 10 mM); [GSH] = 10^{-3} M (25 µL stock at 20 mM); T = 25 °C.

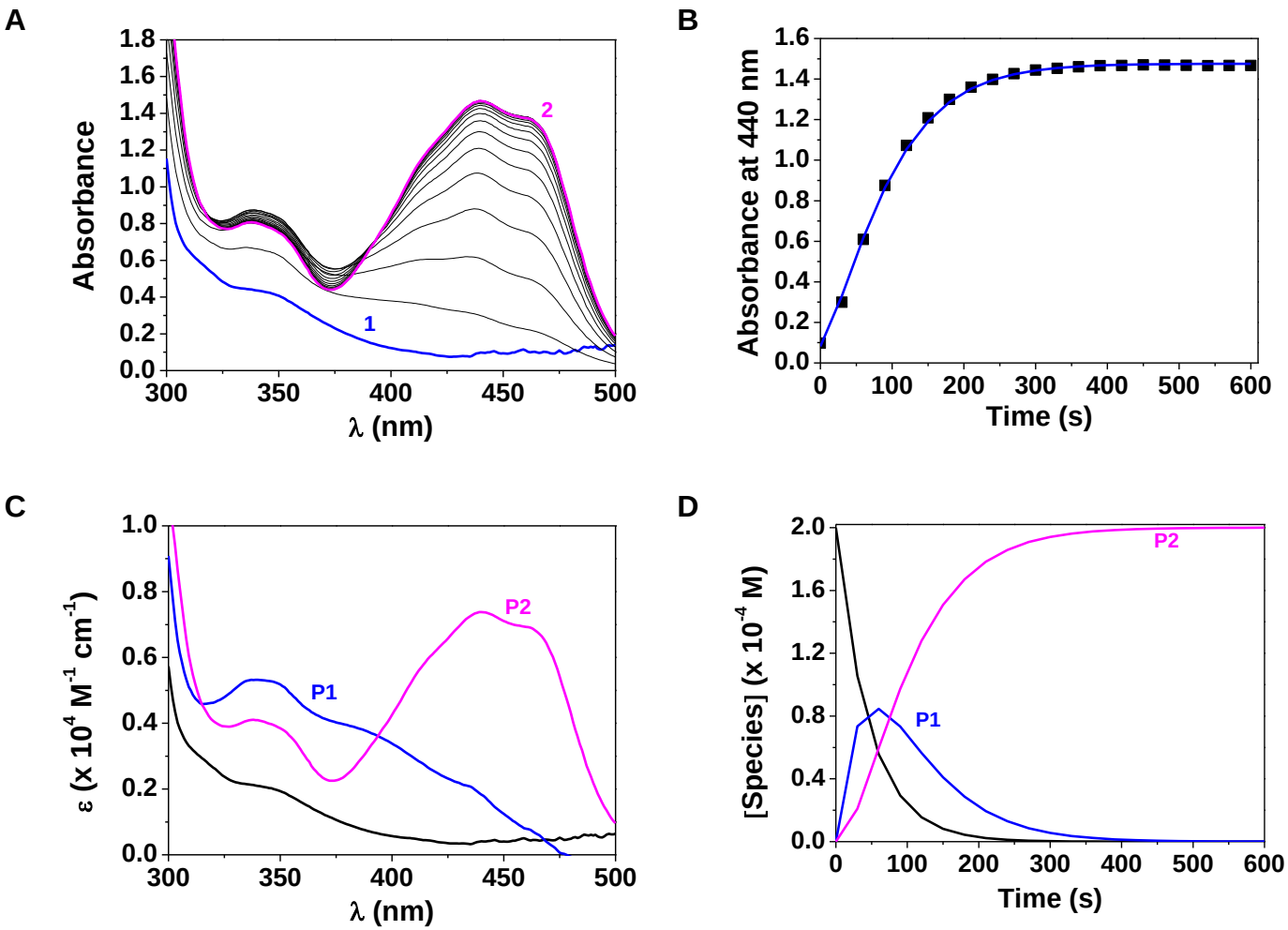
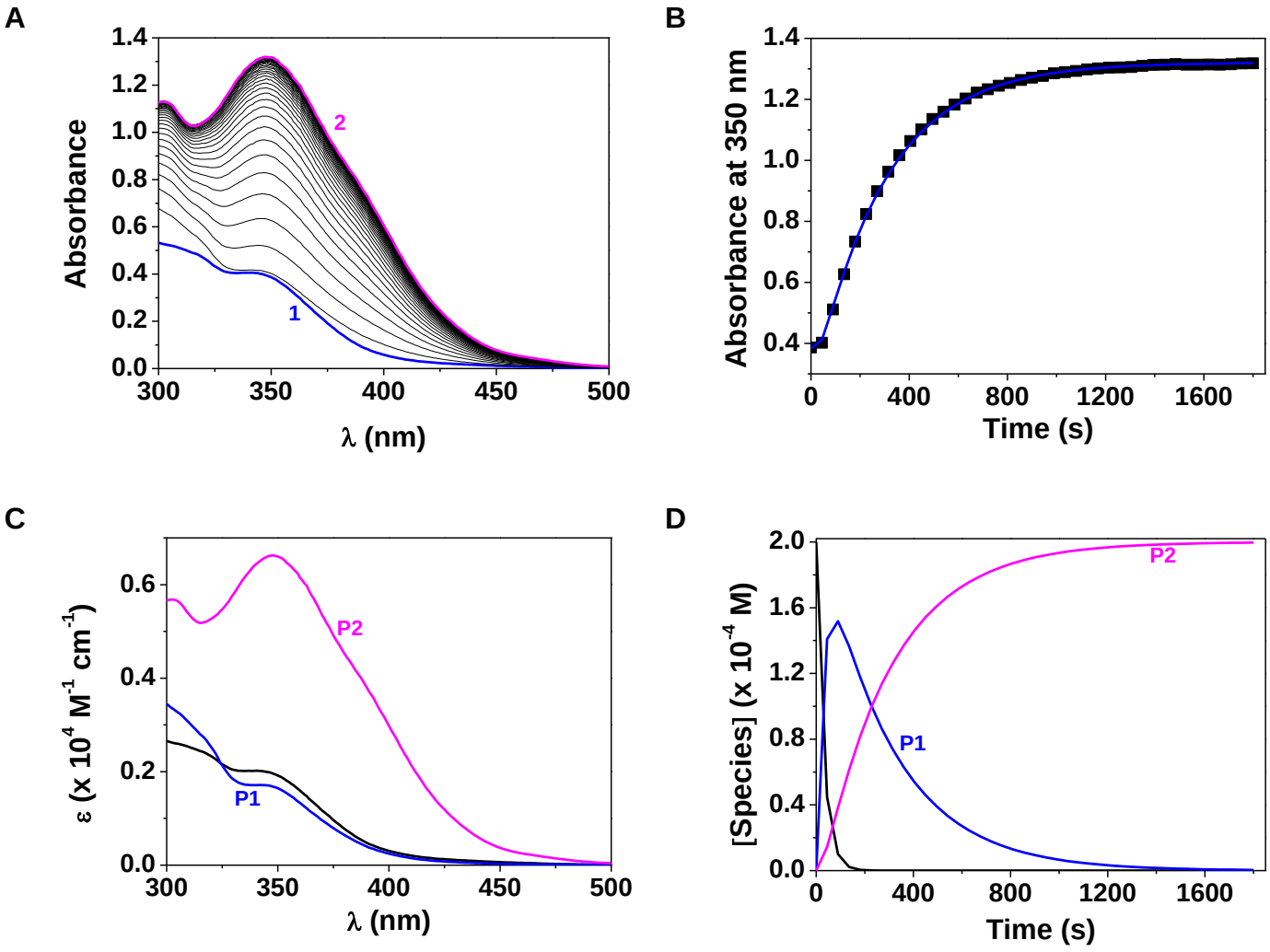


Fig. S2. Changes of the Absorption Spectra of the difluoromethyl derivative **8** upon glutathione addition (Panels A-D).

(A) Absorption spectrophotometric variation and (B) Absorbance at 350 nm measured in the time course of the glutathionylation of compound **8**. Solvent: 200µL MeCN + 265 µL NH_4OH buffer at pH 7.5; [**8**] = 2 x 10^{-4} M (10 µL stock at 10 mM); [GSH] = 10^{-3} M (25 µL stock at 20 mM); *T* = 25°C. (1) t = 0 s; (2) t = 1800 s.

(C) Electronic absorption spectra measured for compound **8** and its glutathione adducts and (D) Distribution diagram of compound **8** and its products as a function of time during the glutathionylation reaction. Solvent: 200µL MeCN + 265 µL NH_4OH buffer at pH 7.5; [**8**] = 2 x 10^{-4} M (10 µL stock at 10 mM); [GSH] = 10^{-3} M (25 µL stock at 20 mM); *T* = 25°C.



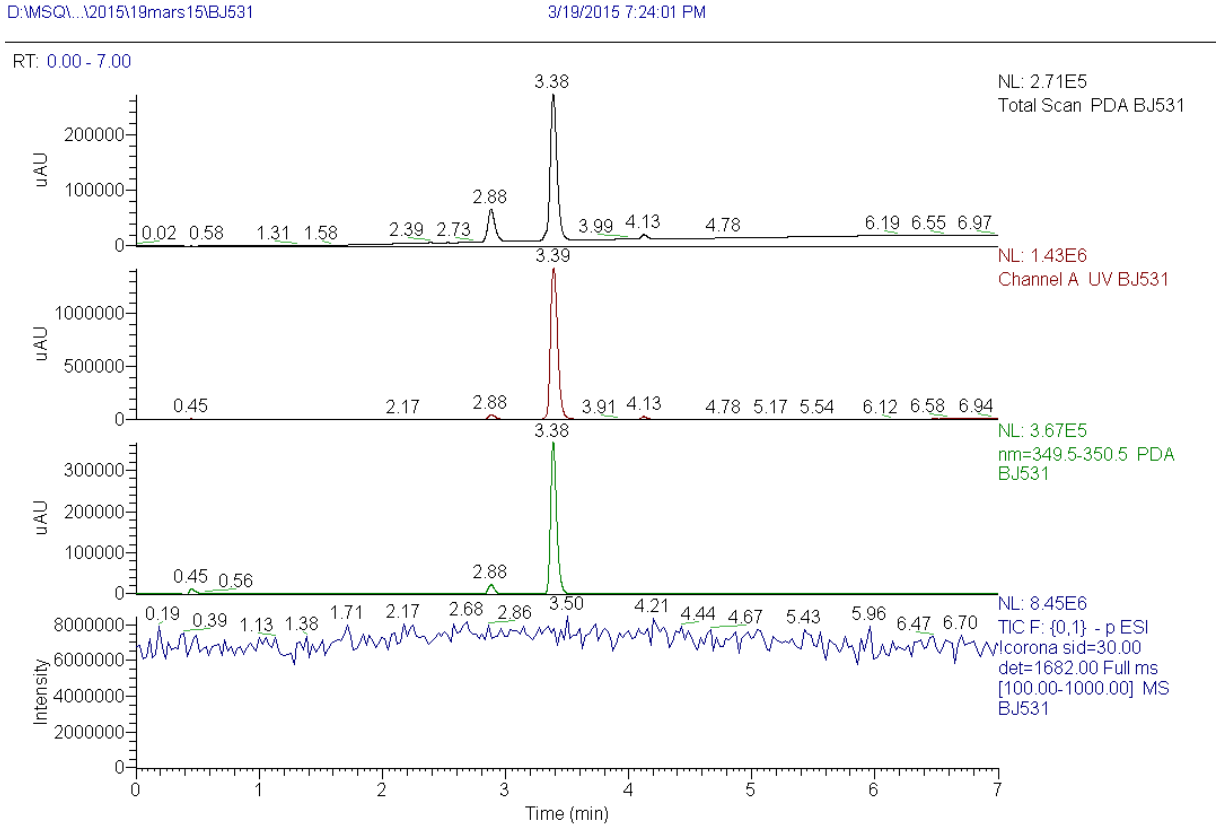
LC–MS analysis of the difluoromethylmenadiones–glutathione conjugates

After the sample was dissolved in the matrice solution (200 µl MeCN, 10µl of naphtoquinone, 265 µl of buffer NH_4OH and the addition of 25 µl GSH and to stop the reaction 10 µl of formic acid solution 50%), 5 µl was directly injected onto a Thermo Scientific Hypersil GOLD column (50×2.1 mm, 1.9 µm), in an Accela 600 liquid chromatography apparatus. The chromatogram was starting at a flow of 0.5 ml/min with a starting point (i) at 5% of buffer B (MeCN with 0.2% formic acid) and 95% of buffer A (water with 0.2% formic acid). From here (ii) a gradient was developed from 0 min to 5 min at 95% of buffer B and 5% of buffer A), (iii) from 5 to 8 min with an elution phase at the same condition, i.e. 95% of buffer B and 5% of buffer A, followed by a return to equilibration at the starting conditions on 6 min at 5% of buffer B and 95% of buffer A. A 50:50 % split was applied dividing the flow in two parts, one for the mass detector and the other in the photodiode array. The liquid chromatograph was coupled to a Thermo MSQ mass spectrometer (Thermo scientific) operated under a switching positive ionization mode and negative ionization mode with the following source settings: electro-spray source at 350 °C under N_2 nebulisation at 5 bar. The cone voltage applied 75 V in positive mode with a scan time of 0.5 sec and -30 V in negative mode with a scan time of 0.5 sec. Data acquisition was performed in full scan mode monitoring form 100 Dalton to 1000 Dalton. The photodiode array is scanning from 200 to 600 nm. Wavelengths extractions were studied at 254 nm and 350 nm. Xcalibur software (Thermo version 2.1) was used for data registration.

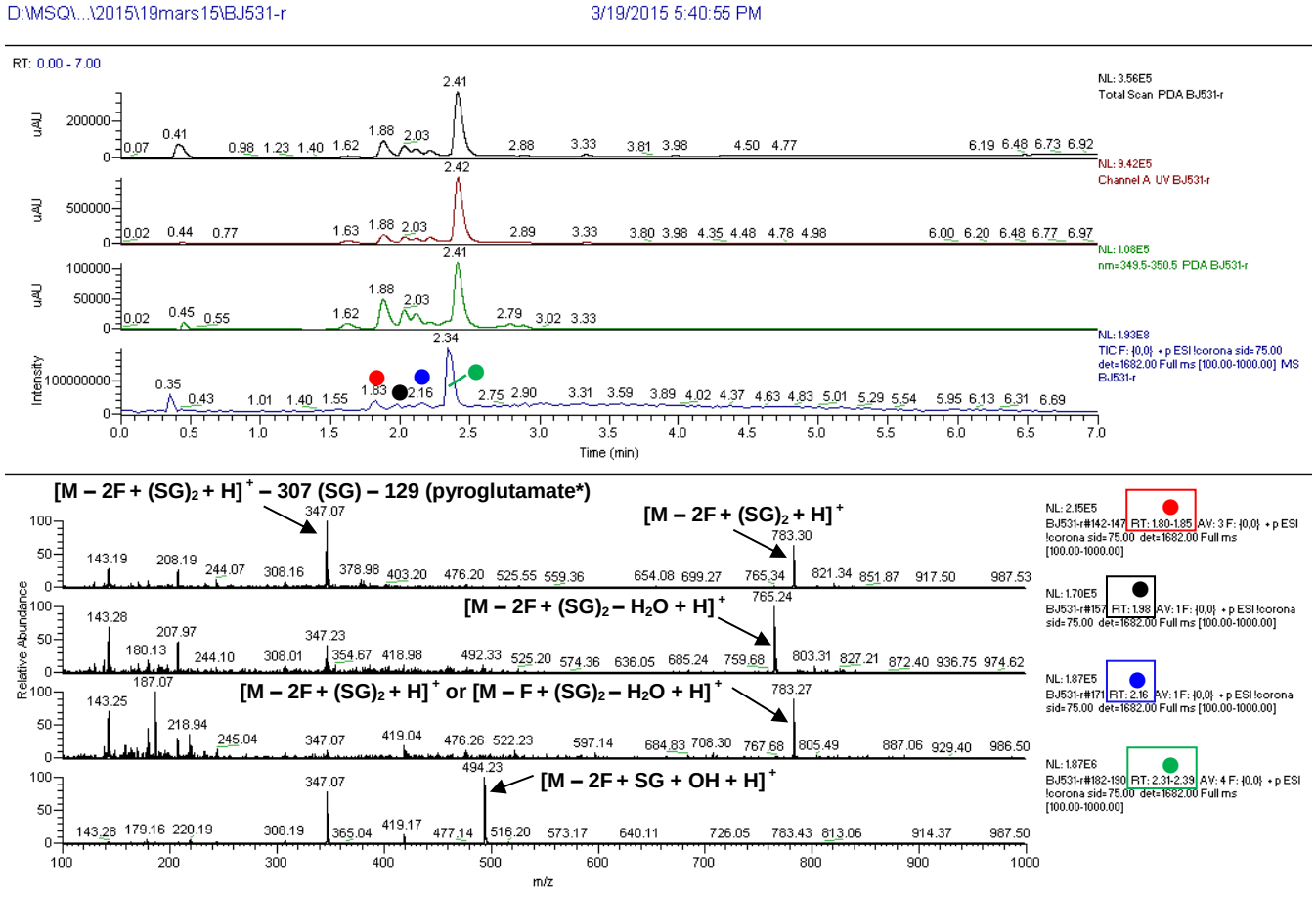
Figure S3. UV-Vis chromatograms and LC–MS traces After analysis of the glutathionylation reaction mixtures for difluoromethylmenadiones–GSH conjugate formation.

The data presented in the following section show 4 chromatograms (upper part): photodiode array (PDA) detection from 200 to 600 nm; UV trace at 254 nm; UV trace at 350 nm; and then TIC ESI (Total Ion Count in Electrospray at the specified tension) in a full scan from 100 to 1000 Dalton. An extracted view of each chromatogram is presented with a time range of 0 to 7 min. Noteworthy is to mention the time shift of 0.1 min between the UV detection and the mass spectra detection caused by the split system. The mass spectra of the compounds are shown in the lower part (except for the pure difluoromethylmenadione alone for which we could not detect the *m/z* signal at the compound concentration used). Both negative and positive ESI spectra were recorded using the following parameters: “-p ESI Corona sid = 30” meaning for negative ESI at the tension of 30V; “+p ESI Corona sid = 75” meaning for positive ESI at the tension of 75 V. Only one spectrum was shown according to the experiment.

difluoromethylmenadione alone

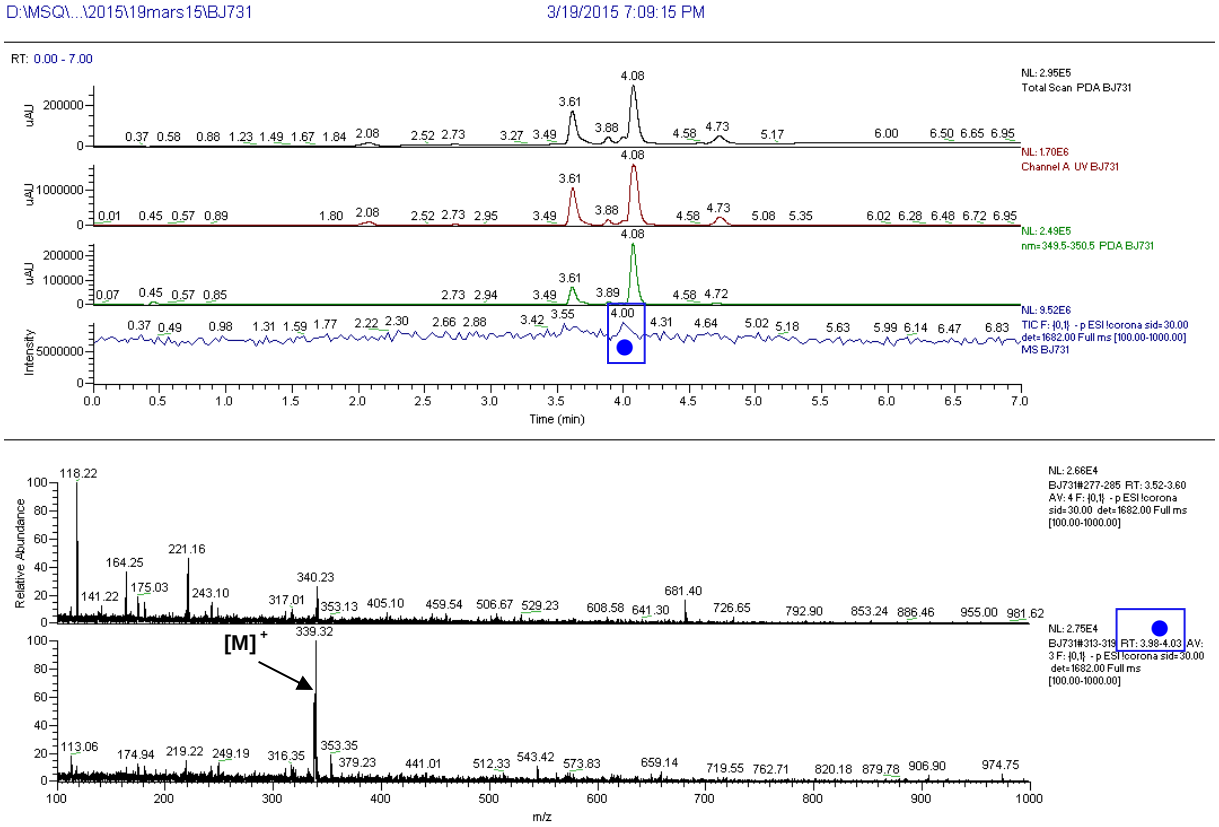


difluoromethylmenadione glutathionylation

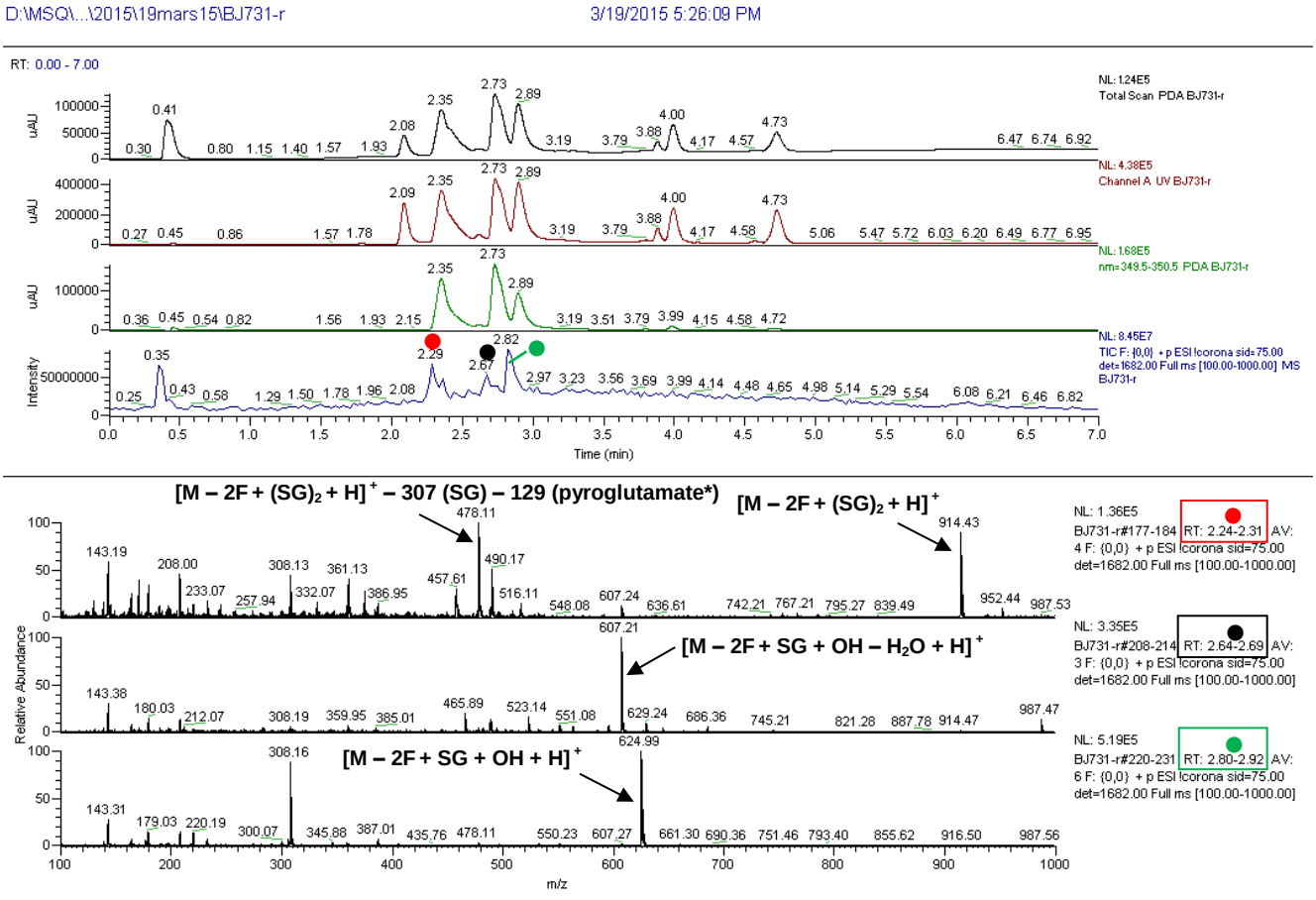


* the dissociation of the molecular ions of GSH adducts yielded fragment ions involving the loss of pyroglutamate (129 Da), which are typical of many GSH conjugates.

Compound **8** alone

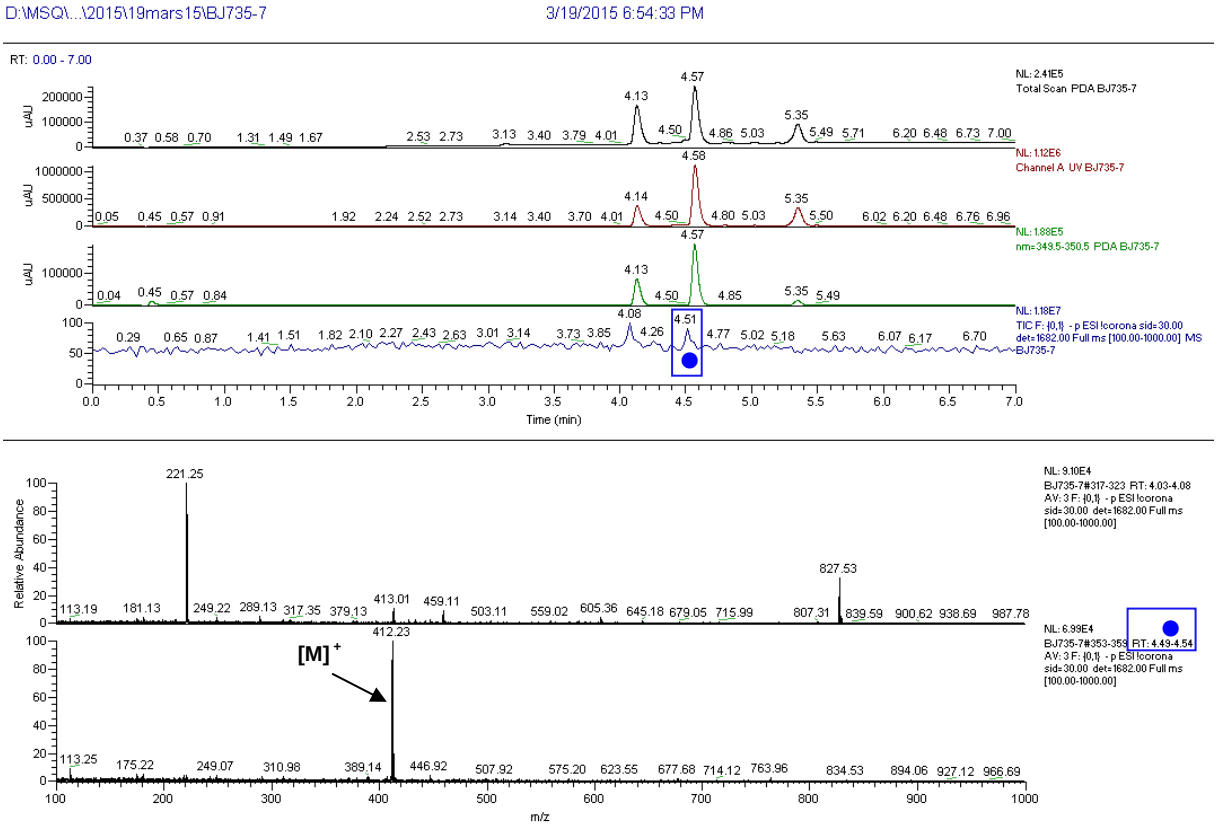


Compound **8** glutathionylation

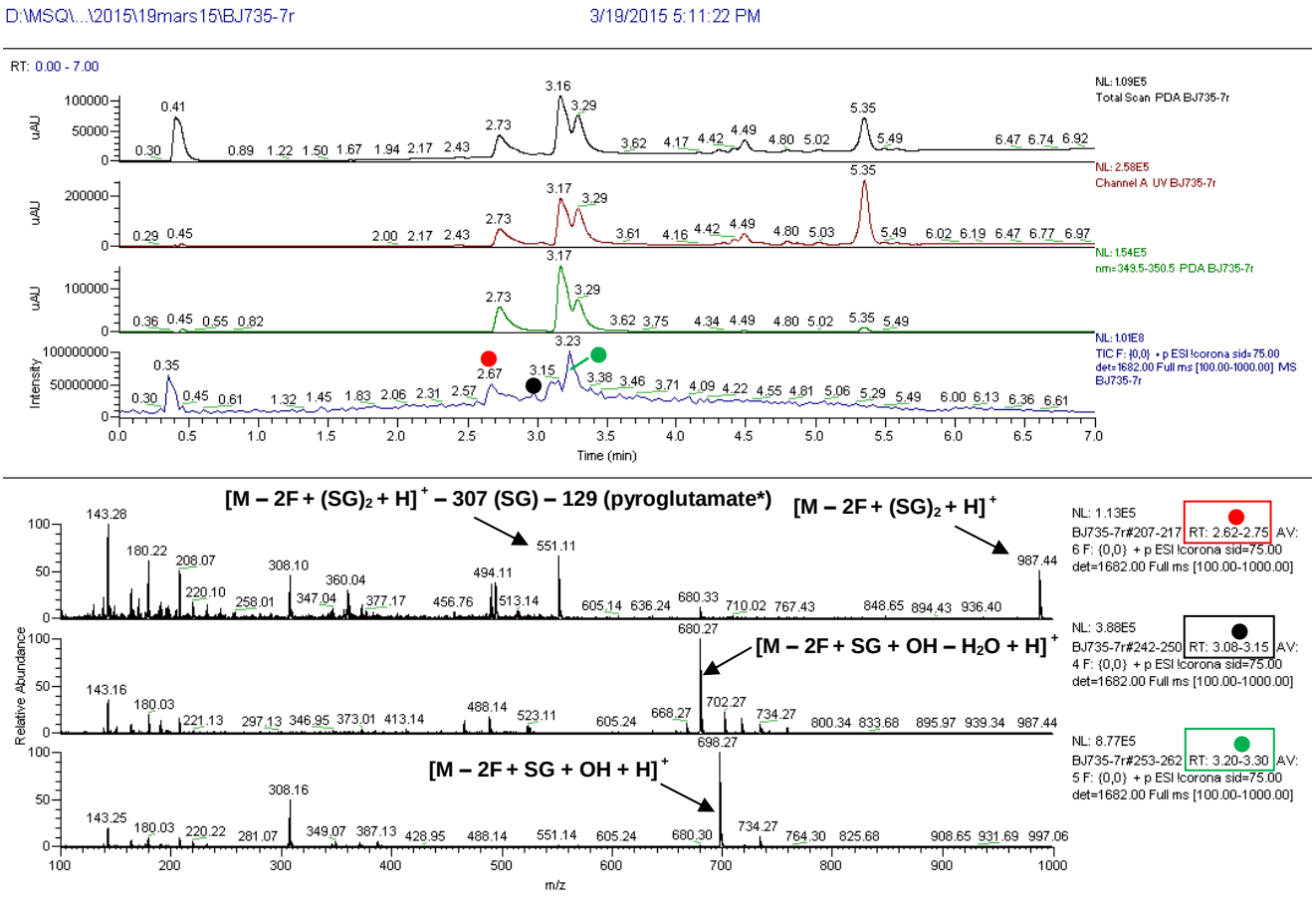


* the dissociation of the molecular ions of GSH adducts yielded fragment ions involving the loss of pyroglutamate (129 Da), which are typical of many GSH conjugates.

Compound **9** alone

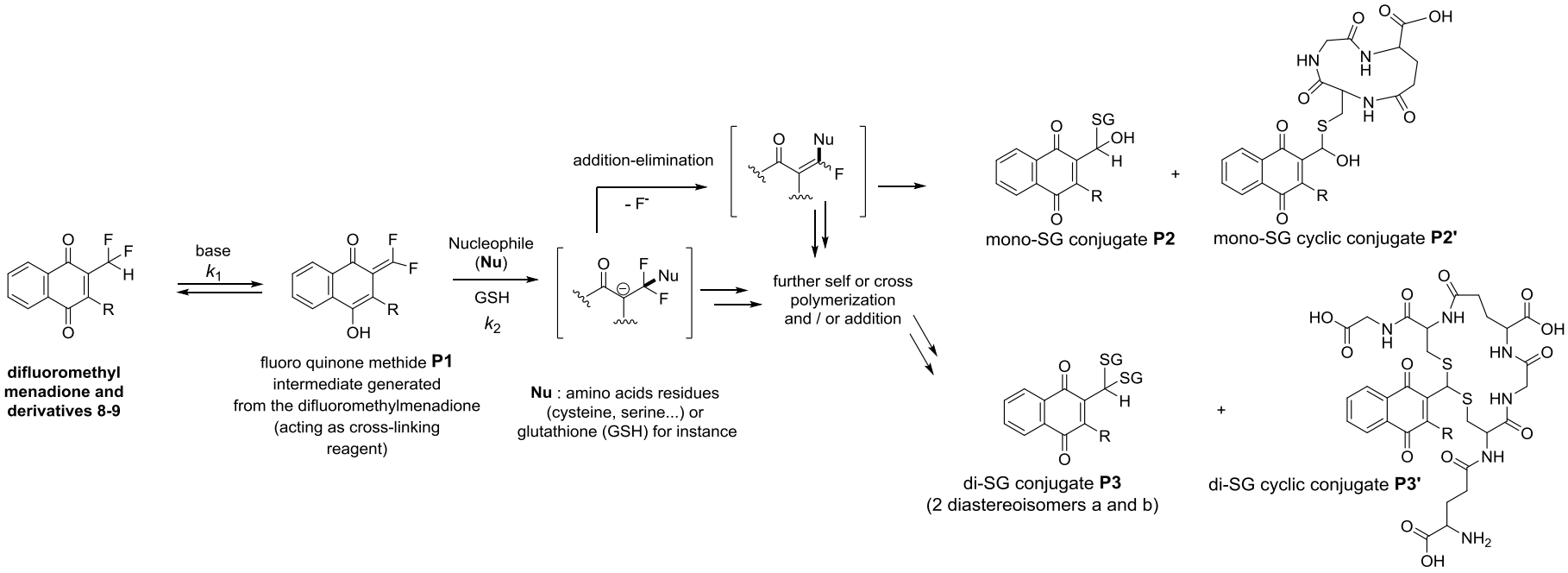


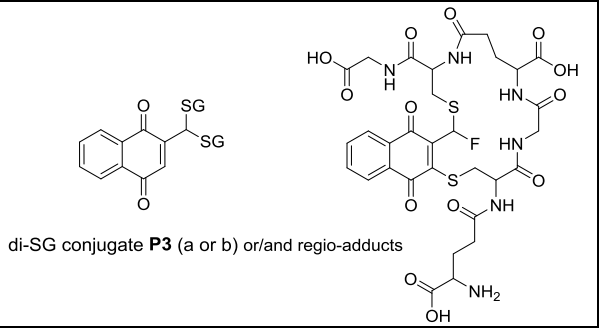
Compound **9** glutathionylation

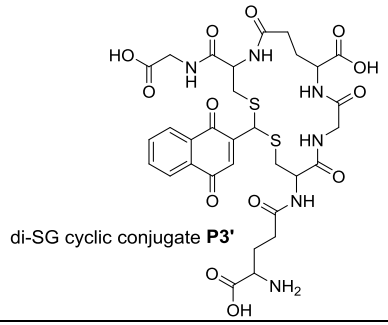
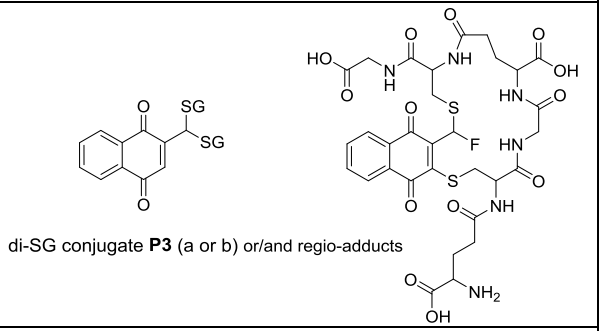
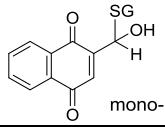


* the dissociation of the molecular ions of GSH adducts yielded fragment ions involving the loss of pyroglutamate (129 Da), which are typical of many GSH conjugates.

Table S1. Proposed chemical structures of difluoromethylmenadiones–glutathione adducts analyzed from the glutathionylation reaction mixtures by LC-MS analysis.



2-difluoromethyl-3-H or 3-phenoxymethyl menadione	LC-MS trace (retention time in min)	observed *m/z* $[M + H]^+$ (mass fragments)	predicted molecular chemical formula	proposed structure
difluoromethylmenadione	● (1.83)	783.3 (347.1)	$C_{31}H_{38}N_6O_{14}S_2$ or $C_{31}H_{35}FN_6O_{13}S_2$	 di-SG conjugate **P3** (a or b) or/and regio-adducts

	● (1.98)	765.2 (347.2)	$C_{31}H_{36}N_6O_{13}S_2$	 di-SG cyclic conjugate **P3'**
	● (2.16)	783.3 (187.1)	$C_{31}H_{38}N_6O_{14}S_2$ or $C_{31}H_{35}FN_6O_{13}S_2$	 di-SG conjugate **P3** (a or b) or/and regio-adducts
	● (2.35) major	494.2 (347.1)	$C_{21}H_{23}N_3O_9S$	 mono-SG conjugate **P2**

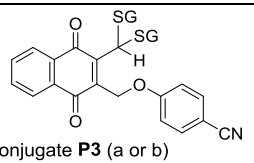
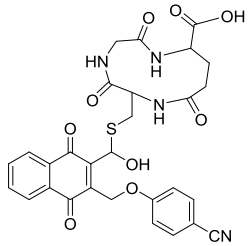
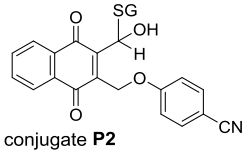
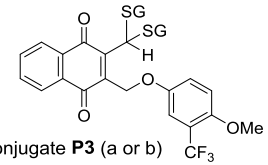
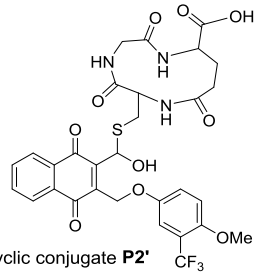
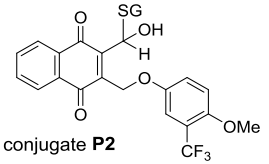
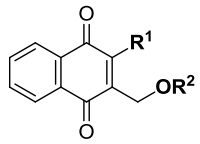
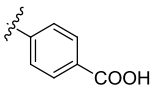
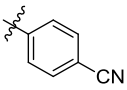
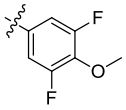
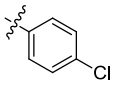
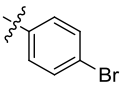
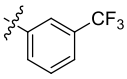
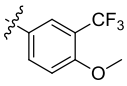
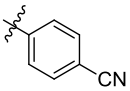
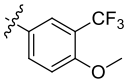
Compound **8**	● (2.28)	914.4 (607.2 ; 516.1 ; 490.2 ; 478.1)	$C_{39}H_{43}N_7O_{15}S_2$	 di-SG conjugate **P3** (a or b)
	● (2.62)	607.2 (523.1 ; 465.9)	$C_{29}H_{26}N_4O_9S$	 mono-SG cyclic conjugate **P2'**
	● (2.88) major	625.0 (308.2)	$C_{29}H_{28}FN_4O_{10}S$	 mono-SG conjugate **P2**
Compound **9**	● (2.69)	987.4 (511.1; 494.1)	$C_{40}H_{45}F_3N_6O_{16}S_2$	 di-SG conjugate **P3** (a or b)
	● (3.12)	680.3(488.1; 523.1)	$C_{30}H_{28}F_3N_3O_{10}S$	 mono-SG cyclic conjugate **P2'**
	● (3.25) major	698.3 (308.2)	$C_{30}H_{30}F_3N_3O_{11}S$	 mono-SG conjugate **P2**

Table S2. IC_{50} values for M_5 derivatives IC_{50} Values of 3-phenoxymethyl menadiones and their difluoromethymenadione derivatives as cytotoxic agents against human lung fibroblasts MRC-5 *in vitro*.

Compound			**IC_{50} (μM) in MRC-5 assay**
	R^1	**R^2**	
1	– Me		6.6
2	– Me		31.1
3	– Me		25.6
4	– Me		29.8
5	– Me		30.6
6	– Me		21.7
7	– Me		26.6
8	– CHF_2		6.7
9	– CHF_2		11.2