Supporting information for

Preventive oral treatment with resveratrol pro-prodrugs drastically reduce colon inflammation in rodents

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Figure S1. In vitro anti-inflammatory activity of resveratrol (**1**) and glucosyl resveratrol derivatives (**2-5**). (**A**) and resveratrol (**1**) and 6-acyl glucosyl resveratrol derivatives (**6-10**) (**B**) in CCD18-Co colon cells. Activity was measured as the capability to prevent prostaglandin E_2 (PGE_2) production by human colon CCD18-Co cells upon stimulation with 1 ng/mL of IL-1β for 18h. Bars with the same letter are not significantly different at $P < 0.01$.

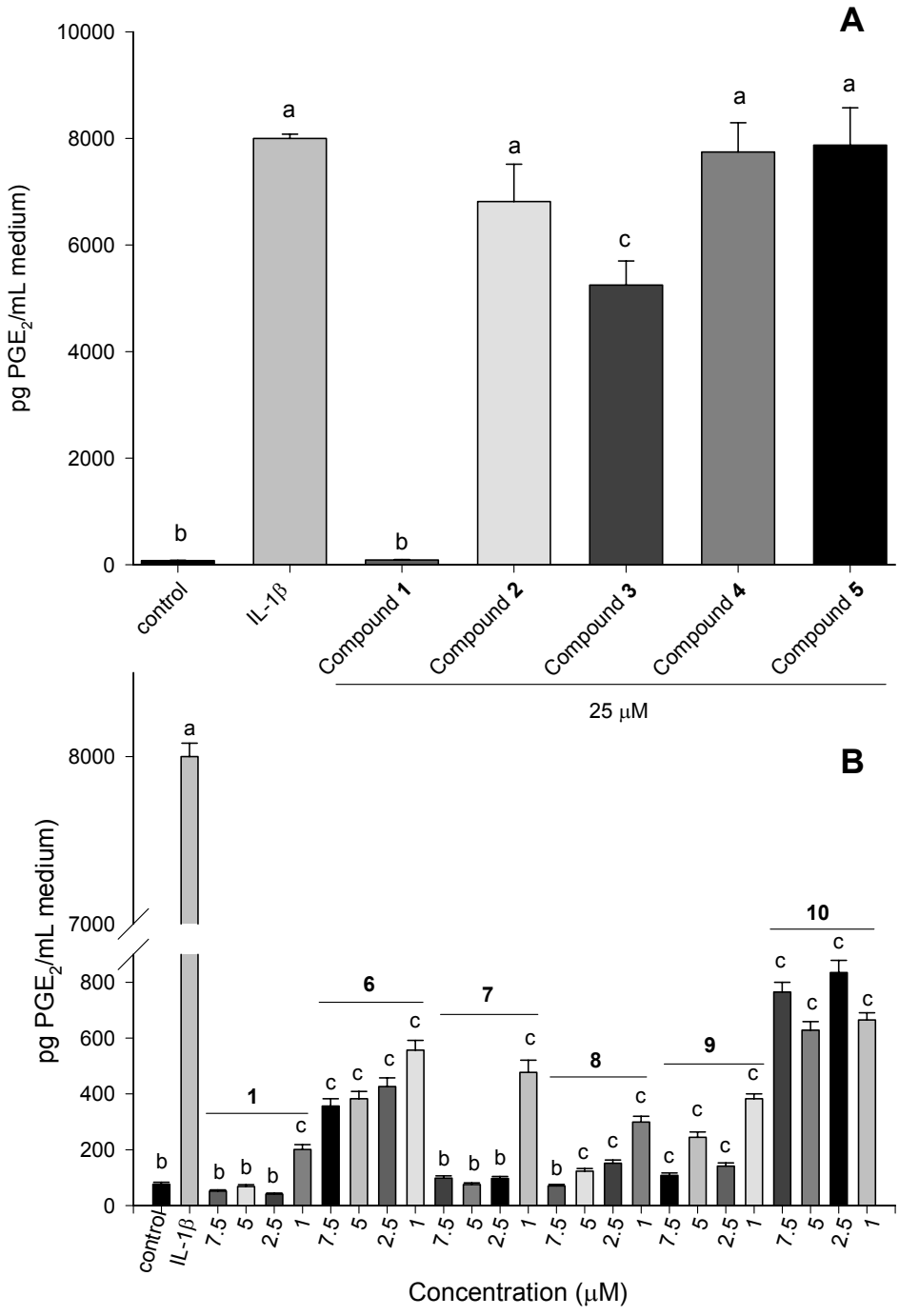


Figure S2. Chromatographic profiles of cell media samples after the incubation of colon Caco-2 cells with glucosyl- resveratrol derivatives for 6 h. (**A**) Compound **1**, (**B**), Compound **2**, (**C**) Compound **3**, (**D**) Compound **4**, (**E**) Compound **5**. a, *trans*-Resveratrol glucuronide; b, *cis*-resveratrol glucuronide; c, *cis*-resveratrol; d, *cis*-piceid; e, *cis*-resveratrol-3,5-diglucoside.

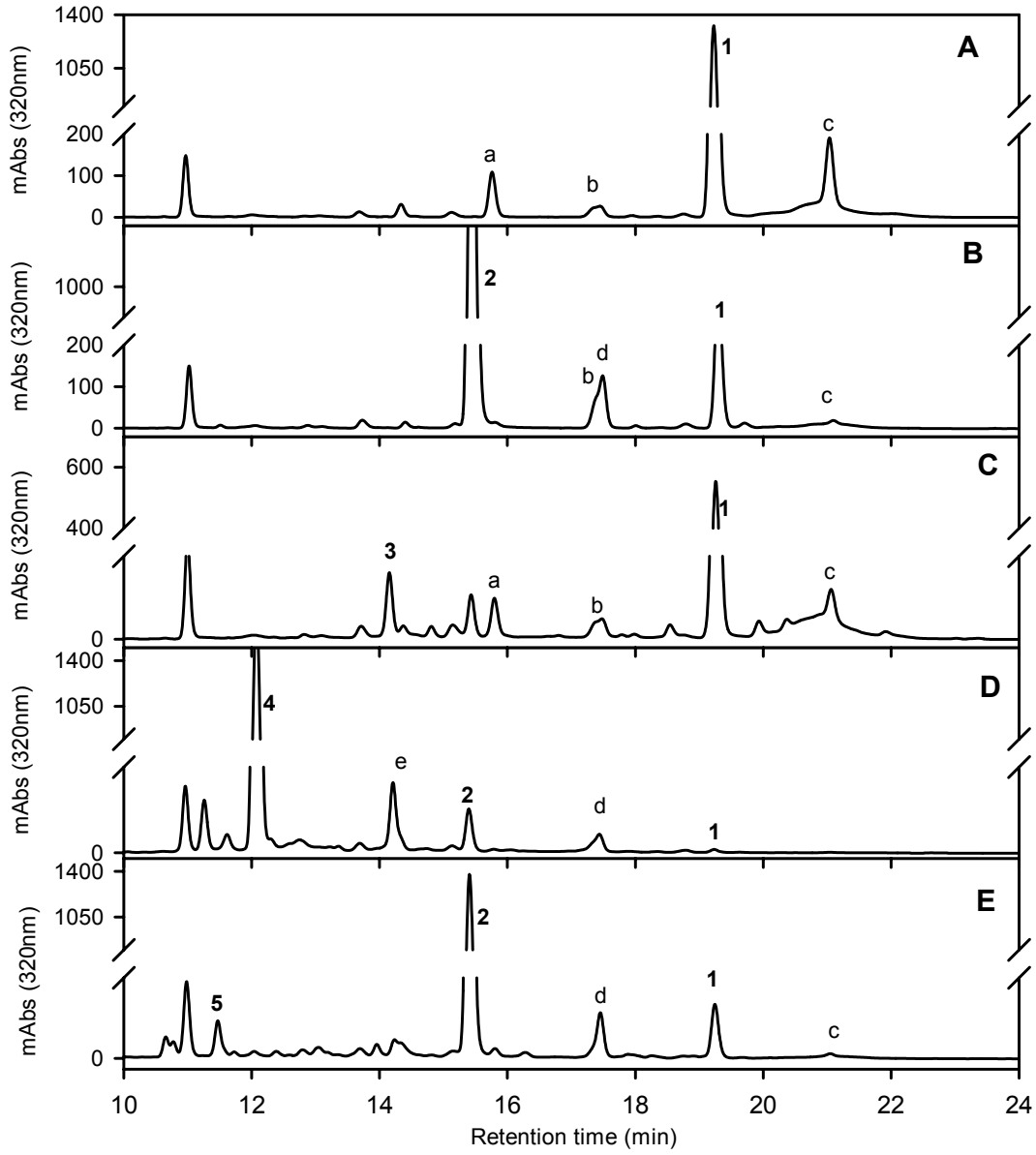
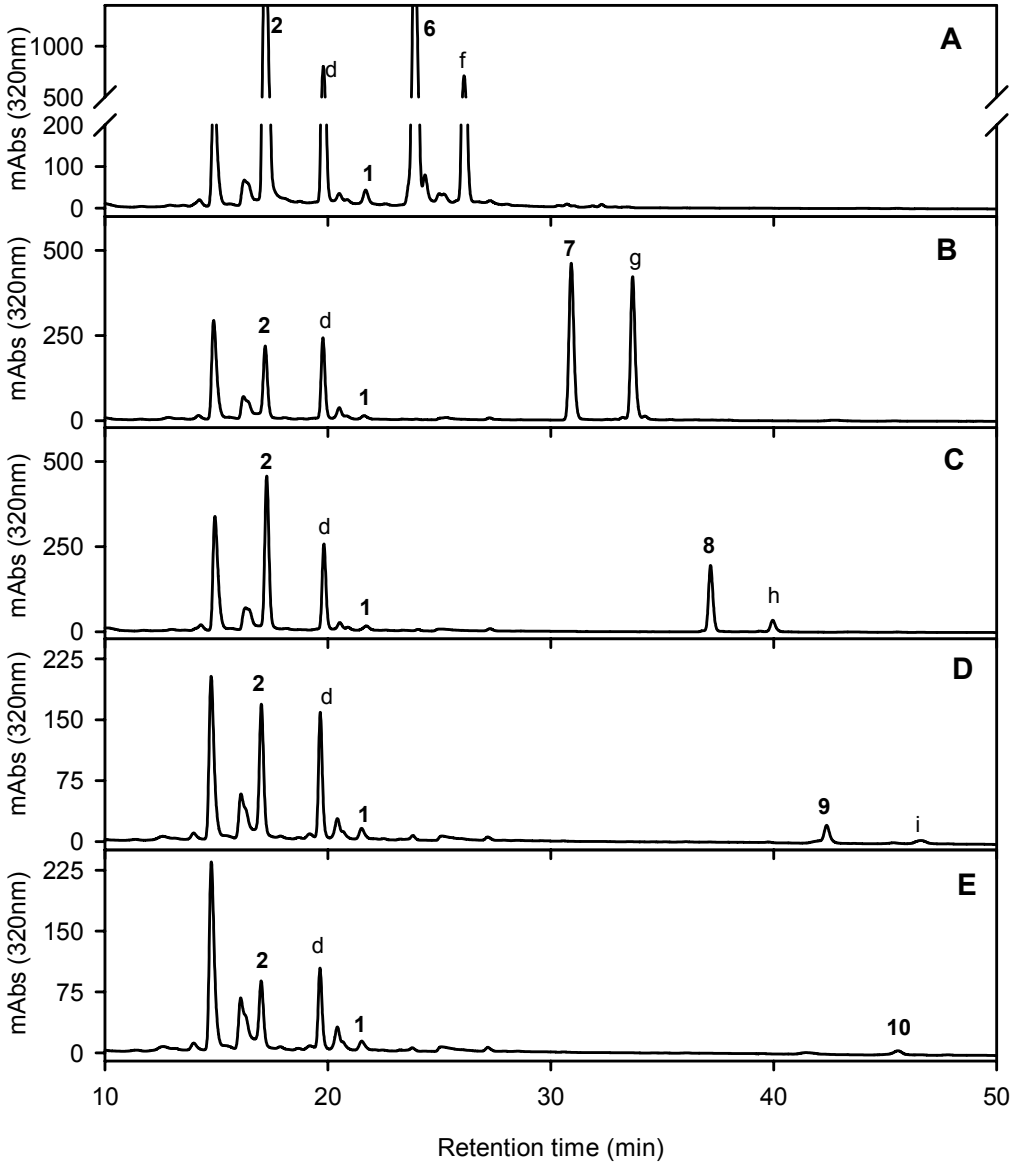


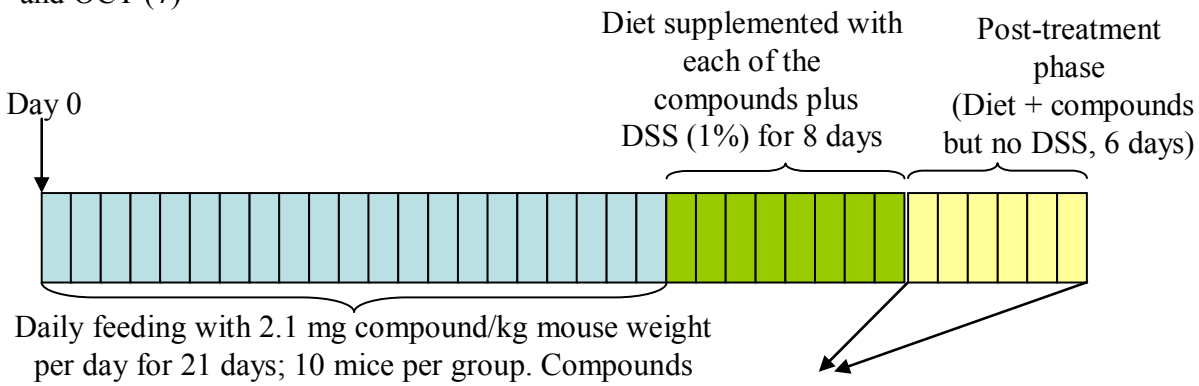
Figure S3. Chromatographic profiles of cell media samples after the incubation of colon Caco-2 cells with glucosyl-acyl resveratrol derivatives for 6 h. (**A**) *trans*-resveratrol-3-*O*-(6'-*O*-butanoyl)-β-D-glucopyranoside (**6**) (**B**), *trans*-resveratrol-3-*O*-(6'-*O*-octanoyl)-β-D-glucopyranoside (**7**), (**C**) *trans*-resveratrol-3-*O*-(6'-*O*-lauroyl)-β-D-glucopyranoside (**8**), (**D**) *trans*-resveratrol-3-*O*-(6'-*O*-palmitoyl)-β-D-glucopyranoside (**9**), (**E**) *trans*-resveratrol-3-*O*-(6'-*O*-stearoyl)-β-D-glucopyranoside (**10**). d, *cis*-piceid; f, *cis*-resveratrol-3-*O*-(6'-*O*-butanoyl)-β-D-glucopyranoside; g, *cis*-resveratrol-3-*O*-(6'-*O*-octanoyl)-β-D-glucopyranoside; h, *cis*-resveratrol-3-*O*-(6'-*O*-lauroyl)-β-D-glucopyranoside and i, *cis*-resveratrol-3-*O*-(6'-*O*-palmitoyl)-β-D-glucopyranoside.



Scheme S1. Experimental inflammation assays approached in the present study.

(A) *Inflammation assay-1: 'Pre-feeding' with the compounds before DSS administration*

Seven groups: control, DSS, RES (**1**), PIC (**2**), DIGLUC (**4**), BUT (**6**), and OCT (**7**)



Evaluations:

-Overall asppearance of mice (days 29 and 35)
-DAI (weight, stool blood and stool form) (days 29 and 35)
-Fecal microbiota (at 0, 21 and 29 days)
-Antibody array in colon mucosa (29^{th} d)
-Haptoglobin and fibrinogen serum levels (29^{th} d)
-Histological analyses of colon (29^{th} d)
-Prostaglandins in colon mucosa (29^{th} d)
-Myeloperoxidase in colon mucosa (29^{th} d)
-Colon lenght (29^{th} d)

(B) *Inflammation assay-2: 'Co-administration' of compounds with DSS from day 0 (no pre-feeding)*

Seven groups: control, DSS, RES (**1**), PIC (**2**), DIGLUC (**4**), BUT (**6**), and OCT (**7**)

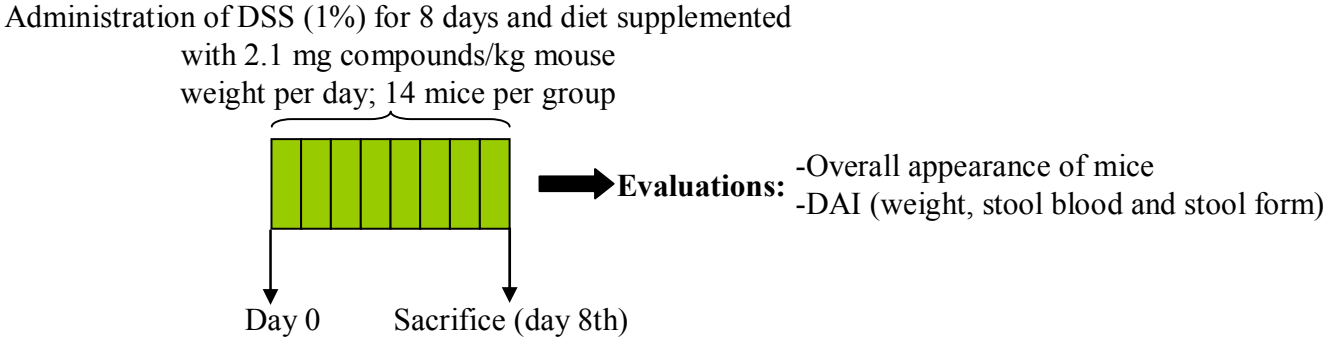


Figure S4. Evolution of mice weight from the different groups along the study.
Bars have not been depicted in the Figure to improve the clarity. The bar SD indicates the highest standard deviation obtained. The letters a and b indicate the presence of two significantly different clusters at $P < 0.05$: a, CONTROL, BUT and OCT, and b, DSS, RES, PIC, and DIGLUC.

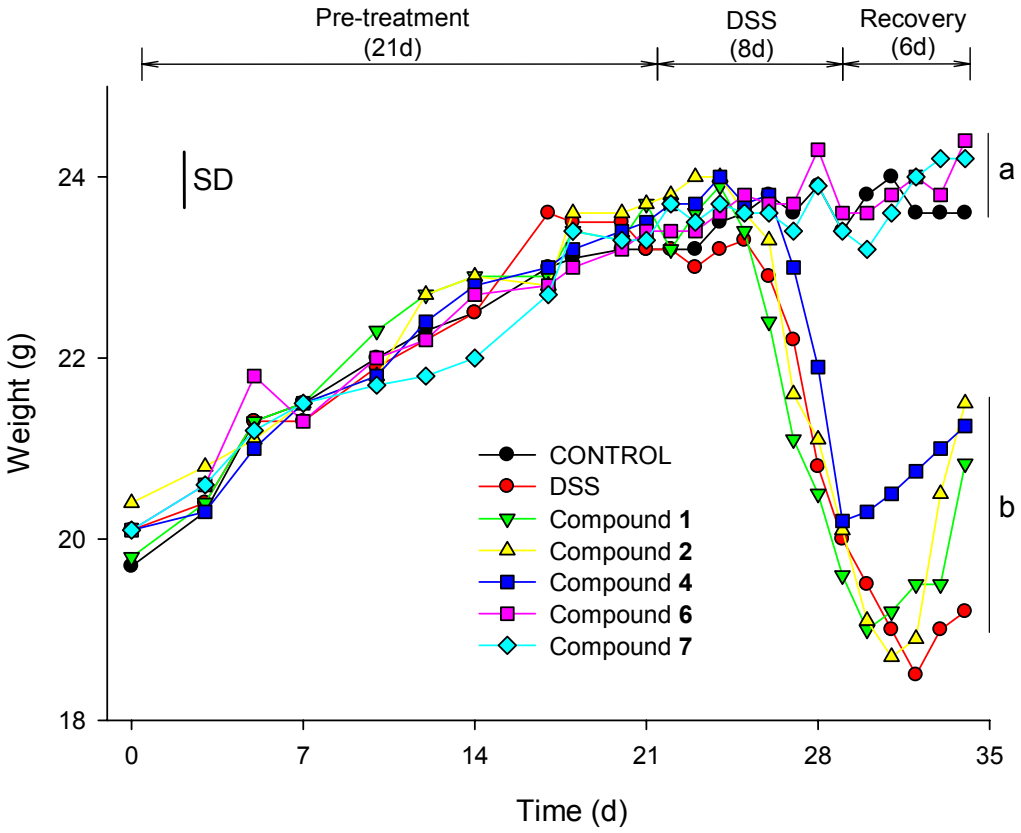


Figure S5. Representative pictures of C57Bl/6 mice used in the present study after DSS treatment in the absence (DSS group) or presence of the compounds **1** (RES group), **6** (BUT group) or **7** (OCT group). DSS, RES, BUT and OCT groups are shown. PIC and DIGLUG groups (not shown) presented a similar appearance to RES group. The pictures were taken the last day after DSS administration (See also Figure 2, Figure S4 or Scheme S1 for details of the experimental design). The arrows designate rectal bleeding.

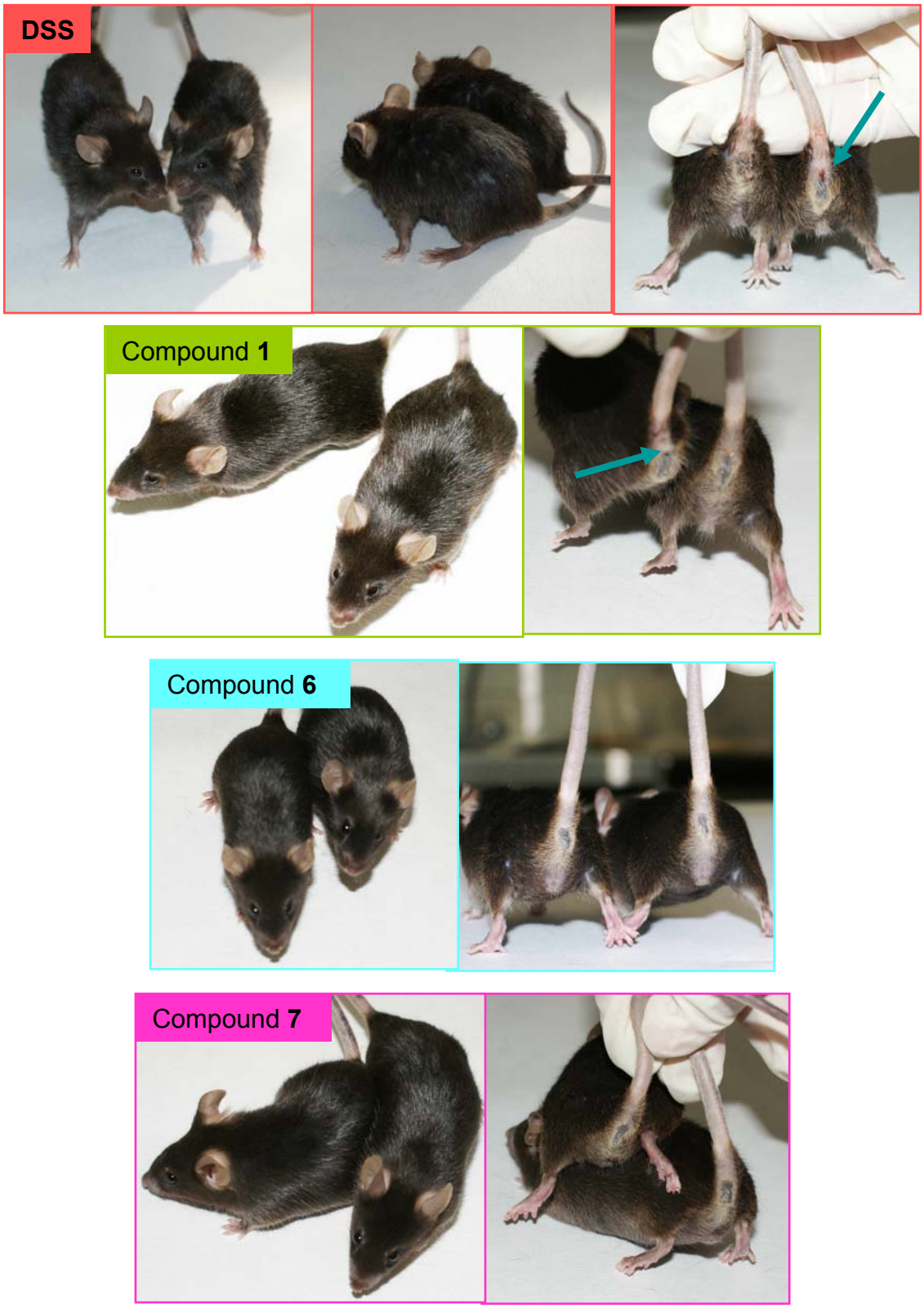


Figure S6. Evolution of DAI values (see Supplementary Methods) upon co-administration of 1% DSS and each of the compounds tested for 8 days without pre-treatment with the compounds for 21 days.

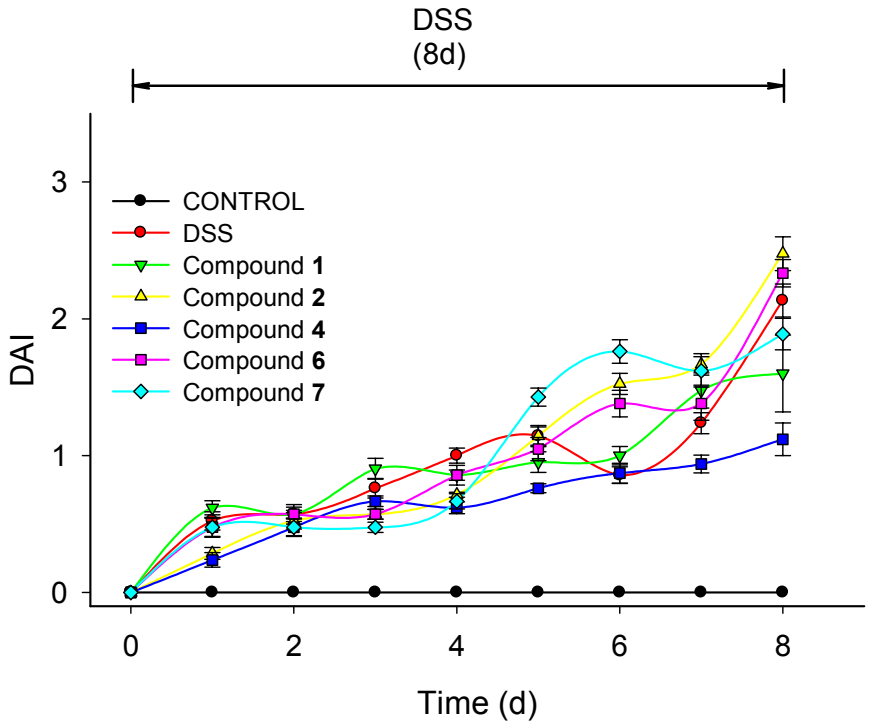


Table S1. Histological scores for evaluating colonic tissue damage.

Parameter evaluated	**Control**	**DSS**	Compound **1**	Compound **2**	Compound **4**	Compound **6**	Compound **7**
Loss of epithelium	0	2.6 ± 0.3	1.2 ± 0.9	0.8 ± 0.3[a]	0.4 ± 0.2[a]	0.1 ± 0.0[a]*	0.2 ± 0.2[a]
Crypt damage	0	3.2 ± 0.5	2.0 ± 0.7	2.6 ± 0.4	1.8 ± 0.3	0.8 ± 0.3[a,b]	0.5 ± 0.2[a,b]
Infiltration of inflammatory cells	0	3.5 ± 0.3	3.0 ± 0.7	3.6 ± 0.2	2.8 ± 0.2	2.6 ± 0.5	1.5 ± 0.2[a,b,c]
Total	0	9.3 ± 1.1	6.2 ± 1.8	7.0 ± 0.8	5.0 ± 0.7[a]	3.4 ± 0.8[a,b]	2.2 ± 0.4[a,b,c]

[a]Significantly different compared with DSS group, [a]$P < 0.05$; [a*]$P < 0.005$
[b]Significantly different compared with PIC group, $P < 0.05$
[c]Significantly different compared with DIGLUC group, $P < 0.05$

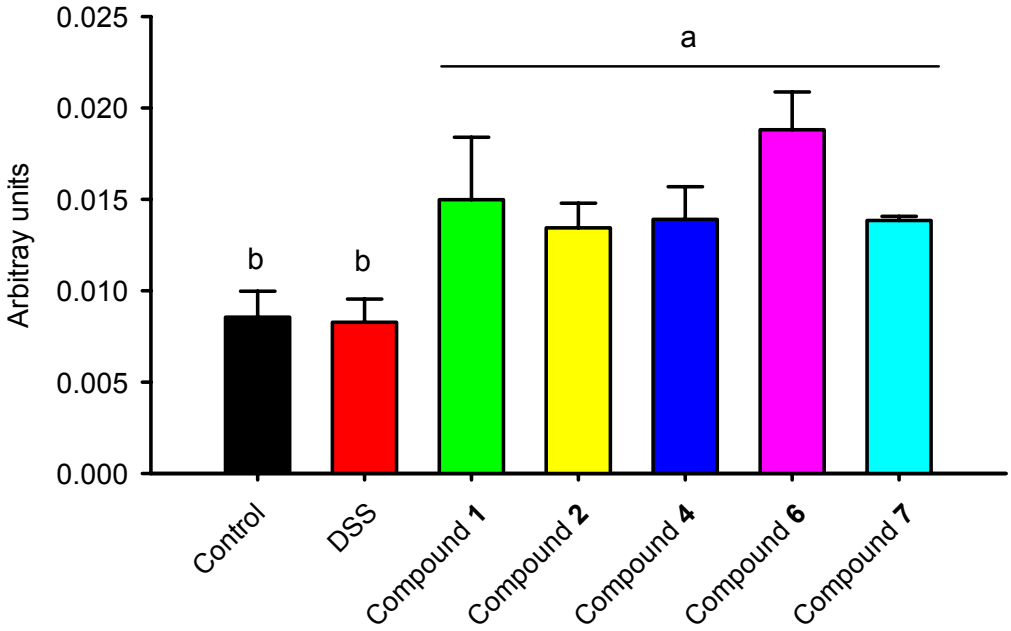
Figure S7. Schematic representation of RayBio™ Mouse inflammation antibody array used in the present study. See also the Experimental section for protocol used as well as Fig. 4 and Fig. S8 for quantitative changes observed.

POS	POS	NEG	NEG	Blank	BLC	CD30L	EOTAX	EOTAX-2	F-L	FRACTAL	GCSF
GM-CSF	IFNγ	IL-1α	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-9	IL-10	IL-12p40/p70	IL-12p70
IL-13	IL-17	I-TAC	KC	LEP	LIX	LPTN	MCP-1	MCSF	MIG	MIP-1α	MIP-1γ
RANTES	SDF-1	TCA-3	TECK	TIMP-1	TIMP-2	TNFα	sTNF RI	sTNF RII	Blank	Blank	POS

Original antibody array membrane contains each cytokine in duplicate. Down-regulated protein levels in green. Up-regulated protein levels in red.

Legend: POS, positive control; **NEG**, negative control; **Blank**, background signal; **BLC**, B-lymphocyte chemoattractant (CXCL13) protein; **CD30L**, CD30 ligand (belongs to the TNF ligand family); **EOTAX**, Eotaxin (eosinophil chemoattractant protein type 1); **EOTAX-2**, Eotaxin-2 (eosinophil chemoattractant protein type 2); **F-L**, Fas-Ligand; **FRACTAL**, Fractalkine (CX3CL1, chemokine ligand 1**)**; **GCSF**, Granulocyte colony-stimulating factor**; GM-CSF**, Granulocyte macrophage colony-stimulating factor**; IFNγ**, Interferon-γ; **Il-1α**, Interleukin-1α, **IL-1β**, Interleukin-1β; **IL-2**; Interleukin-2; **IL-3**, Interleukin-3; **IL-4**, Interleukin-4; **IL-6**, Interleukin-6; **IL-9**, Interleukin-9; **IL-12p40/p70**, Ratio between the IL-12p40 subunit and the IL12-p70 heterodimer; **IL-12p70**, IL-12 heterodimer consisting of two disulfide-bonded subunits, IL-12p35 and IL-12p40; **IL-13**, Interleukin-13; **IL-17**, Interleukin-17; **I-TAC**, Interferon-inducible T-cell alpha chemoattractant (CXCL11) protein; **KC**, Keratinocyte-derived cytokine; **LEP**, Leptin; **LIX**, Lipopolysaccharide-induced CXC chemokine (CXCL5); **LPTN**, Lymphotactin (Lptn/XCL1); **MCP-1**, Monocyte chemotactic protein-1; **MCSF,** Macrophage colony-stimulating factor**; MIG**, IFNγ-inducible T cell chemoattractant monokine; **MIP-1α**, Macrophage inflammatory protein-1-alpha (CCL3); **MIP-1γ**, Macrophage inflammatory protein-1-gamma (CCL9); **RANTES**, Regulated on Activation, Normal T Expressed and Secreted (belongs to IL-8 supefamily, CCL5); **SDF-1**, Stromal cell-derived factor 1; **TCA-3**, T-cell activation-3 chemokine (CCL1, murine homolog of human I-309); **TECK**, Thymus expressed chemokine (CCL25); **TIMP-1**, Tissue inhibitor of metalloproteinases-1; **TIMP-2**, Tissue inhibitor of metalloproteinases-2; **TNFα**, Tumor necrosis factor-alpha; **sTNF RI**, p55 subunit of the TNFα receptor (TNFRp55, CD120a); **sTNF RII**, p75 subunit of the TNFα receptor (TNFRp75, CD120b).

Figure S8. Levels of interleukin-3 (IL-3) in mice colon mucosa samples. Samples were taken on day 29 of the experimental procedure (8 days after DSS administration). The letters a and b indicate the presence of two significantly different clusters at $P < 0.05$.



Scheme S2. Experimental designs for metabolism assays.

(A) ***Metabolism assay-1: Time-course evolution of parent compounds 1, 2, 4, 6 and 7 and appearance of derived metabolites in blood and gastrointestinal tract in healthy mice.***

Single intragastric administration of 84 mg of each compound/kg mouse weight.
5 groups (**1**, **2**, **4**, **6**, **7**)

Nine time-points (0, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h) after compounds administration.
3 mice per time-point in each group

Plasma
Stomach content
Small intestine content
Colon content

HPLC-MS-MS

(B) ***Metabolism assay-2: Evaluation of resveratrol content in mouse colon after 4 h of intragastric administration of 1, 6 and 7 in mice with DSS-induced colitis.***

Mice fed with 2.1 mg of compound/kg mouse weight per day for 21 days followed by 8 days of treatment DSS (1%)

Three groups: RES (**1**), BUT (**6**) and OCT (**7**)

After 8 days of DSS administration (day 29)

Single intragastric administration of 84 mg of compound/kg mouse weight.
Three groups (RES, BUT, OCT); 4 mice in each group. One time-point (4h) analyzed after intragastric administration.

Colon content

HPLC-MS-MS

Figure S9. Kinetics of the glucosyl- and acyl-glucosyl-resveratrol parent compounds (**a**), of the derived intermediate piceid (**b**) and of free resveratrol (**c**) in the stomach after intragastric administration of compounds **1**, **2**, **4**, **6** and **7** (84 mg/kg body weight). AUC and C_{max} values are shown in Table S2.

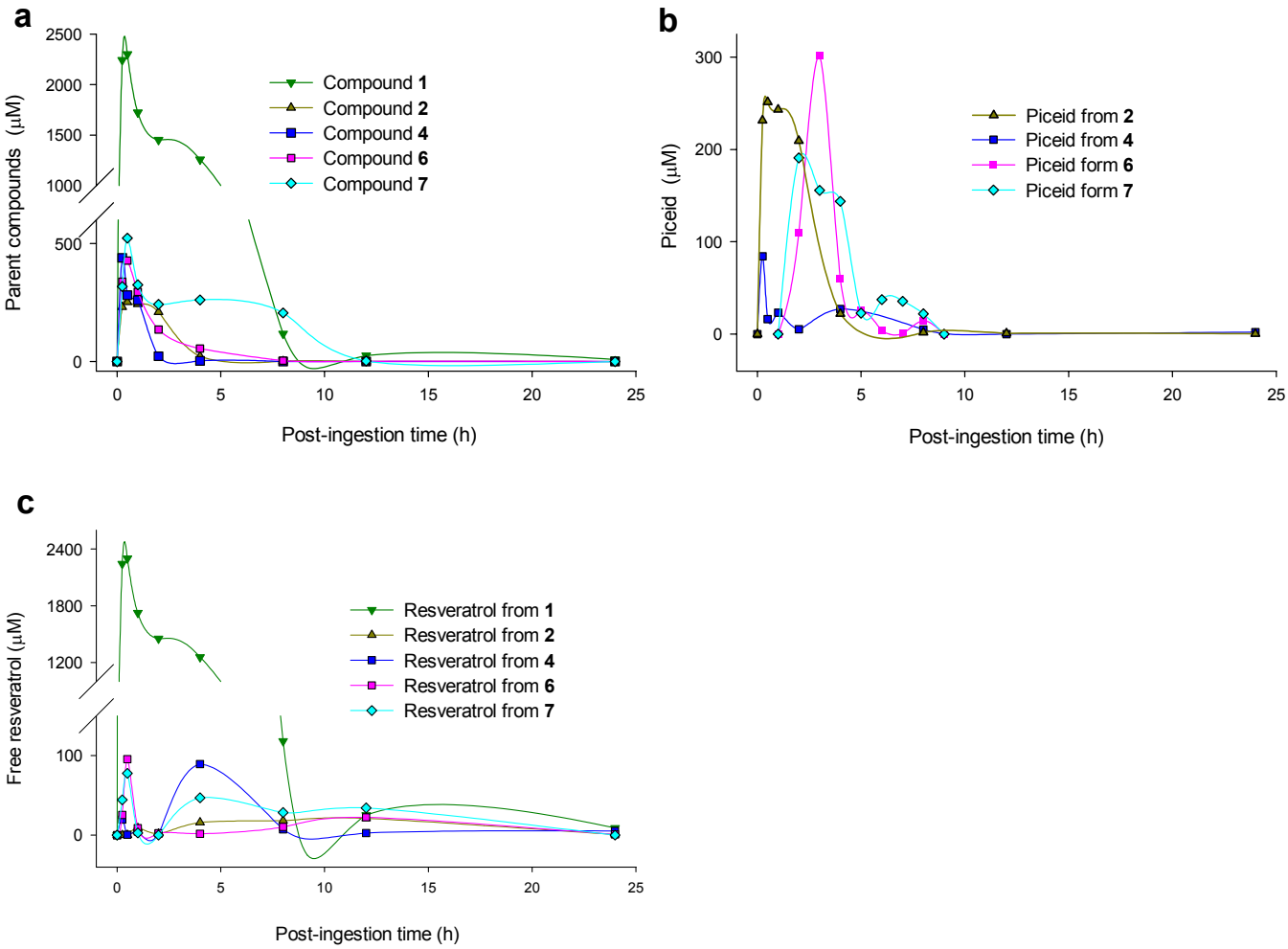


Figure S10. Kinetics of free resveratrol in the small intestine upon intragastric administration of compounds **1**, **2**, **4** , **6** and **7** (84 mg/kg body weight). AUC and C_{max} values are indicated in Table S2.

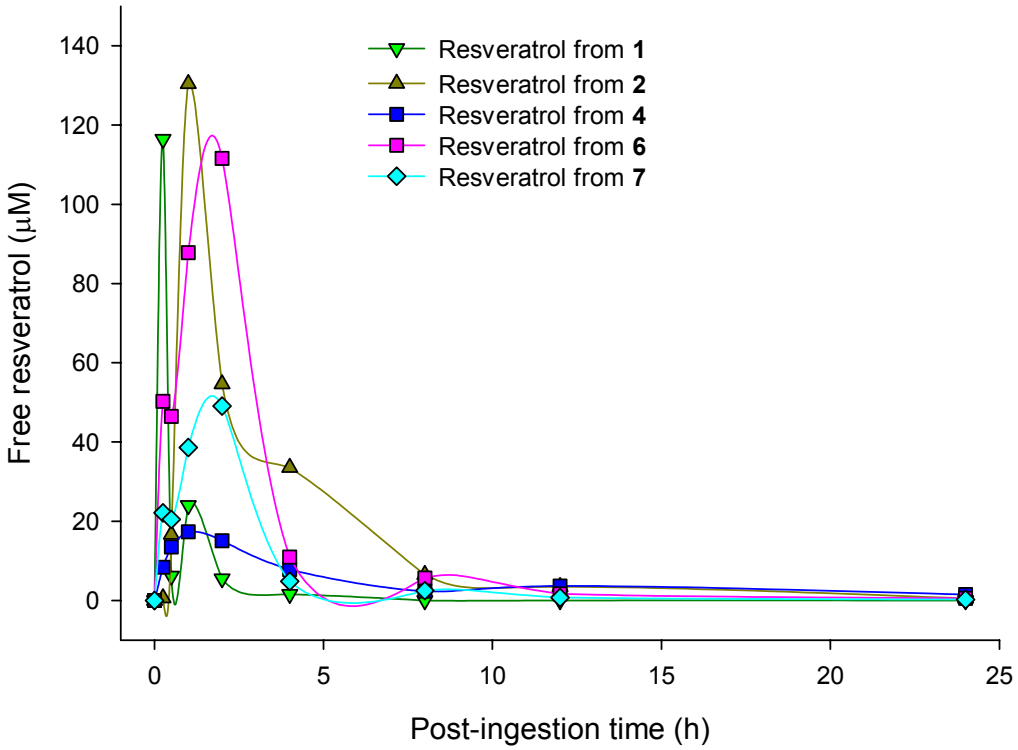


Figure S11. Kinetics of resveratrol glucuronide in plasma upon intragastric administration of compounds **1**, **2**, **4**, **6** and **7** (84 mg/kg body weight). AUC and C_{max} values are shown in Table S2.

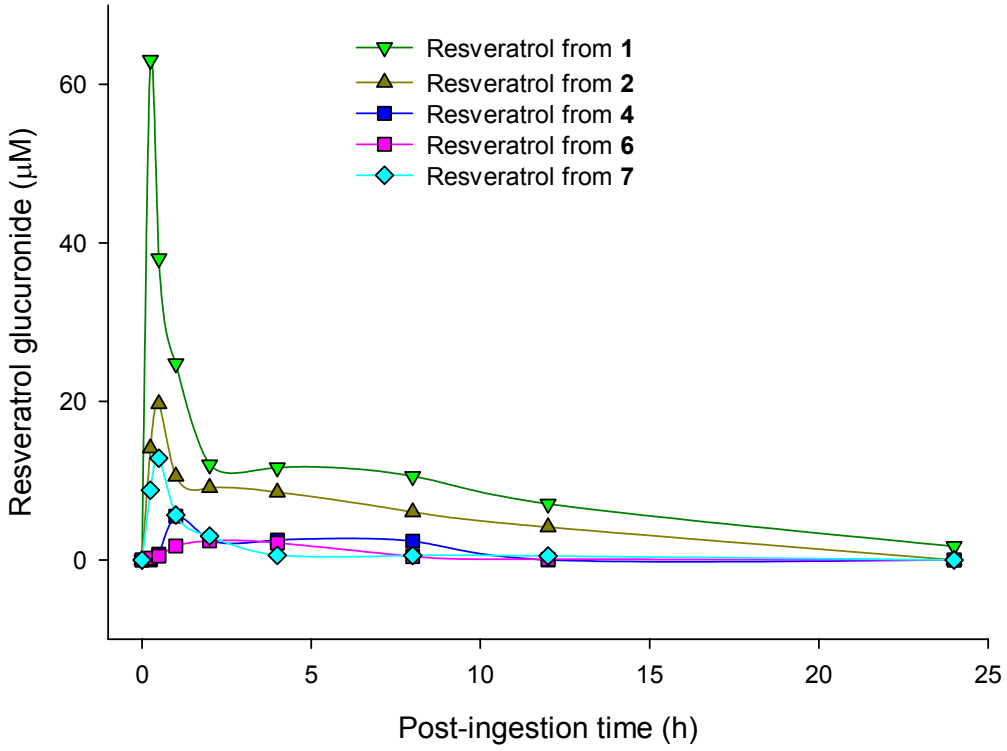


Table S2. Gastro-intestinal transit and plasma kinetics (24 h) of resveratrol and resveratrol derivatives in healthy mice after single ingestion of 84 mg/kg of compounds **1**, **2**, **4**, **6** or **7**. AUC values are expressed as μg h/mL and C_{max} values as μM.

	COMPOUNDS QUANTIFIED					
COMPOUNDS INGESTED	**STOMACH** Parent compounds	**STOMACH** *trans*-Piceid	**STOMACH** Free resveratrol	**PLASMA** Resveratrol glucuronide	**SMALL INTESTINE** Free resveratrol	**COLON** Free resveratrol
Compound **1**						
AUC:	1,071.37	-	1,071.37	47.98	142.44	90.17
C_{max}:	1,150.80	-	1,150.80	63.05	116.42	52.50
Compound **2**						
AUC:	166.66	166.66	69.42	5.06	78.54	148.37
C_{max}:	251.26	251.26	21.12	19.71	130.44	110.74
Compound **4**						
AUC:	103.28	37.91	82.81	5.74	26.00	79.73
C_{max}:	438.89	84.21	89.20	5.53	17.40	75.31
Compound **6**						
AUC:	195.81	80.98	64.71	3.18	76.53	191.22
C_{max}:	427.16	301.57	95.47	2.42	111.60	166.18
Compound **7**						
AUC:	571.81	154.66	130.19	5.37	33.51	139.65
C_{max}:	522.29	190.82	77.71	12.85	49.10	129.46

Areas under the curve (AUC) and C_{max} values were calculated from data shown in Figure 7 (colon) and in Figures S10 (stomach), S11 (small intestine) and S12 (plasma) by using the WinNonlin v. 5.2.1 program (Pharmasight Corporation, Mountain View, CA, USA). Resveratrol glucuronide and resveratrol sulphate were also detected in the colon from RES and PIC groups (fed with **1** and **2**, respectively). No initially administered prodrug was detected in the colon.

SUPPLEMENTARY METHODS

In Vitro Anti-Inflammatory Activity. The human normal colon fibroblast cell line CCD18-Co was obtained from the American Type Culture Collection (ATCC number CLR-1459) (Rockville, USA) and was grown in Minimal Essential Medium (MEM) supplemented with 10% v/v fetal bovine serum (FBS), 2 mM glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 units/mL penicillin and 100 μg/mL streptomycin. Cells were maintained at 37 °C under a 5% CO_2 and 95% air atmosphere at constant humidity. Stocks of compounds were dissolved in DMSO and were added to the culture media at the specified concentration. DMSO did not exceed 0.5% as final concentration. CCD18-Co cells were seeded and allowed to reach confluence, subsequently medium was replaced with fresh medium containing 0.1% FBS for 24 h previous to the inflammatory stimulus. Cells were then treated with compounds **1** (at 1, 2.5, 5, 7.5 and 25 μM), **2**, **3**, **4** and **5** (at 25 μM) and **6**, **7**, **8**, **9** and **10** (at 1, 2.5, 5 and 7.5 μM) and co-stimulated with 1 ng/mL of IL-1β for 18 h. CCD-18Co cells were used from population doubling level (PDL) 28 to 35 for all the experiments.

Cell Uptake and Metabolism. The human colon cancer cell line Caco-2 was obtained from the American Type Culture Collection (ATCC) (Rockville, MD, USA) and the cells grown as previously reported (ref. 36, main manuscript). Cells were seeded at 10^4 cells cm^{-2} on 6-well plates (Nunc, Roskilde, Denmark), allowed to adhere for 48 h. To examine the ability of Caco-2 cells to form and excrete derived metabolites, cells were incubated for 6, 24 and 48 h with the following compounds: i) 25 μM of **1**, **2**, **3**, **4** and **5** and ii) 7.5 μM of **6**, **7**, **8**, **9** and **10**. All the compounds were dissolved in DMSO (<0.5 % in the culture medium) and filtered (0.2 μm) prior to addition to the culture media. Control cells were treated with DMSO. Experiments were all done in triplicate. At the end of each treatment, samples of media (100 μL) were analyzed by LC-MS/MS.

Cell Viability. Cells were seeded in 96 well plates and treated as above. In the in vitro inflammatory and metabolism assays, after 18h and 48h, respectively, of co-incubation with the different compounds mentioned above, cells were washed three times with PBS and the MTT assay was performed according to Morgan et al.[1] .

Animals and Experimental Designs. Mice were housed in a temperature-controlled environment (22 ± 2 ºC) with 55 ± 10% relative humidity and controlled lighting (12 h light/dark cycle). They were fed with mouse standard chow (Panlab, Barcelona, Spain) containing 60% carbohydrates, 16% proteins, 12% humidity, 5% minerals and ashes, 4% fibers and 3% fat (2,900 kcal/kg). DSS solution (dextran sulphate sodium salt, average molecular weight 36,000-50,000) (MP Biomedicals, Illkirch, France) was freshly prepared and both food and water intake were measured daily. The selected compounds (**1**, **2**, **4**, **6** and **7**) were homogenously mixed with ground standard chow and further re-pelleted. Compounds-enriched diets were protected from light. Compounds content in the diet was routinely checked by HPLC along the study. The compound **1** (*trans*-resveratrol, >99% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and the compound **2** (*trans*-piceid, >98% purity) was obtained from Seebio Biotech. Inc. (Shanghai, China). The animal dose used in the inflammation assays for each compound was 2.1 mg/kg mouse weight per day which was equivalent to 0.143 mg/kg day in humans (10 mg of compound for a 70 kg-person) according to the human equivalent dose formula (HED), which takes into account the body surface area instead of the direct animal weight-human weight extrapolation: HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{0.33}$ (ref. 31, main manuscript). In the inflammation assays, after day 8 of colitis induction, mice were anaesthetized with a mixture (1:1 v/v; 1 mL/kg body weight) of xylazine (Xilagesic 2%) (Calier Laboratories, Barcelona, Spain) and ketamine (Imalgene 1000) (Merial Laboratories, Barcelona, Spain) and sacrificed by exanguination by cardiac puncture. For the metabolic studies, mice were sacrificed at each time-point studied as described in the scheme S2.

Disease Activity Index and Animal Appearance. Disease activity index (DAI) was determined by combining scores of body weight loss, stool consistency and stool blood. Each score was determined as follows:

Change in body weight: 0 < 1%; 1: 1-5%; 2: 6-10%; 3: 11-15%; 4 > 15%.

Stool consistency: Normal: 0; slightly soft: 1; soft: 2; very soft: 3; diarrhea: 4.

Stool blood was evaluated by test slides based on the peroxidase activity of hemoglobin from red blood cells (Siemens, HealthCare Diagnosis Inc, Tarrytown, NY, USA). The fecal samples were diluted 1:50 with saline solution and tested directly for reactivity on the slides. After 60 s, the color of the reagent strip was checked

against the manufacturer's color chart. Reactions were scored from 0 for a negative result to 4 for the most intensive color change.

Sampling Procedure. Animals were anesthetized with an intraperitoneal injection of 0.1 mL/100 g body weight of ketamine/xylazine (150 mg/kg and 10 mg/kg, respectively) and blood samples (approximately 1 mL) were obtained immediately by cardiac puncture. The collected blood was immediately separated in plasma by centrifugation at 3000*g* for 10 min at 4 ºC in a Sigma 1-13 microcentrifuge (Braun Biotech. International, Melsungen, Germany) and immediately frozen at -80 ºC for further analyses.

Colons were removed and rinsed with PBS, blotted dried and the length was measured. Samples of freeze-dried feces (0.5 g) were processed as reported elsewhere (ref. 38, main manuscript). An aliquot of 50 µL was diluted with 50 µL water, filtered through a Millex-HV13 0.45 µm membrane filter (Millipore Corp., Bedford, MA, USA) and injected (6 µL) in the LC-MS-MS equipment.

Haptoglobin and Fibrinogen. Serum concentrations of haptoglobin were quantified by a spectrophotometric method using a Phase Range Haptoglobin assay (Tridelta Development, Ireland). These assays were performed in a Cobas Mira Plus analyzer (ABX Diagnostic, Montpellier, France) following the manufacturer's instructions. Fibrinogen was measured according to the method of Clauss et al.[2]

Prostaglandin Assay. PGE_2 levels were measured in distal colon mucosa homogenates using an EIA immunoenzymatic method (Cayman Chemicals, San Diego, CA,). Samples of distal colon mucosa (10 mg) were homogenized in 1 mL of 0.1 M sodium phosphate pH 7.4 containing 1 mM EDTA and 10 µM indomethacin and centrifuged at 9,000×*g* for 20 min. A dilution of sample supernatant 1:50 was used.

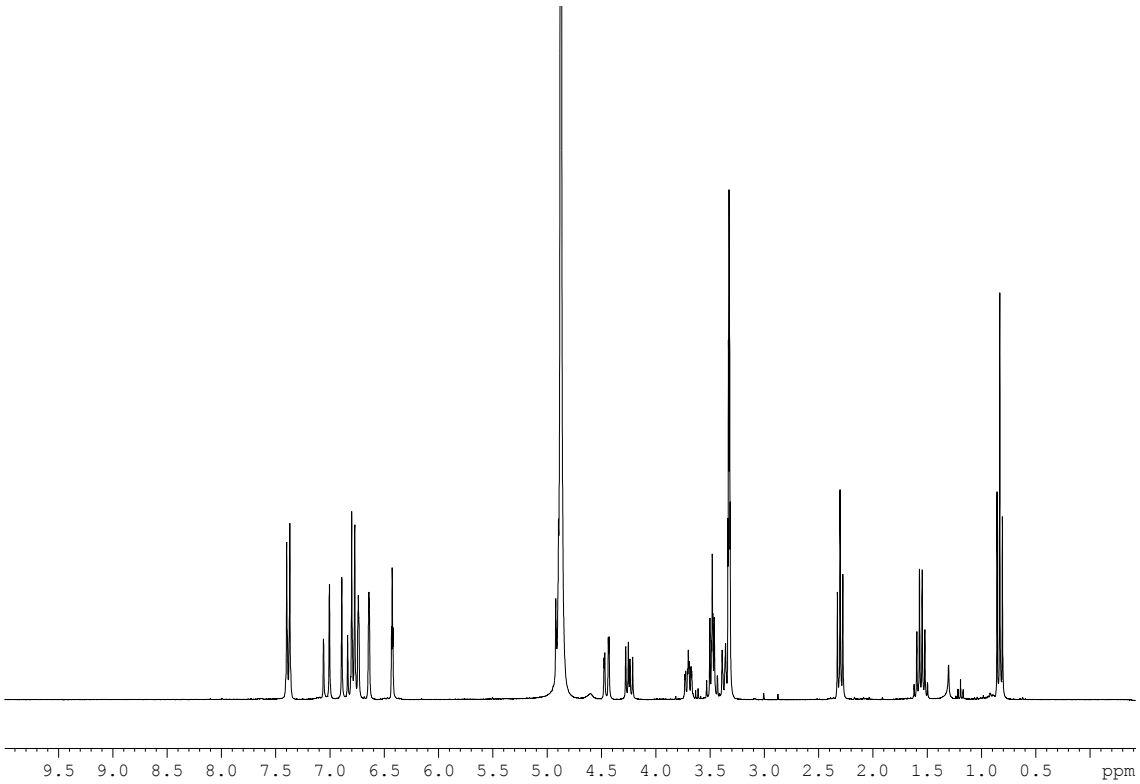
Histological Analysis of Colon Samples. Tissue samples from the distal colon were fixed in 10% neutral buffered formalin, dehydrated in graded ethanol series and embedded in paraffin. For histological evaluation, 5 µm-thick tissue sections were stained with haematoxylin-eosin and observed under a Leica DMRB light microscope (Leica Ultracut, Wetzlar, Germany) at x20 magnification. Photographs taken from the colon samples were digitalized using a camera DC-500 (Leica) with TWAIN driver software.

Fecal Microbiota Analysis. Fresh fecal samples were collected on days 0, 21 and on the day of sacrifice after starting the supplementation with the different compounds. Fecal samples were homogenized in buffered peptone water (100 mg/mL) (AES Laboratoire, Combourg, France) (1:10 dilution) using filter stomacher bags (Seward Limited, London, UK) and a stomacher (IUL Instrument, Barcelona, Spain) for 90 s. Ten-fold serial dilutions were made in the same medium and aliquots of 0.1 mL of the appropriate dilution were spread onto different agar media. *Lactobacillus* and *Bifidobacterium* were enumerated on Man Rogosa Sharpe (MRS) agar and on MRS agar supplemented with 0.5 mg/L dicloxacilin, 3 g/L LiCl and 0.5 g/L L-cysteine hydrochloride, respectively. Enterobacteria were obtained by inoculating decimal dilutions in violet red bile glucose agar. *Escherichia coli* were enumerated using chromocult coliform agar. *Clostridium* spp. were enumerated on reinforced clostridial media containing 20 µg/mL of polymixine sulphadiacine. Culture plates were incubated at 37 °C for 24–48 h in an anaerobic chamber (CO_2:H_2:N_2, 5:15:80). All media were obtained from Oxoid (Basingstoke, UK). Antibiotics and other supplements were obtained from Sigma. Microbial counts were expressed as log CFU g^{-1}. The mean and standard error per group were calculated from the log values of the CFU g^{-1}.

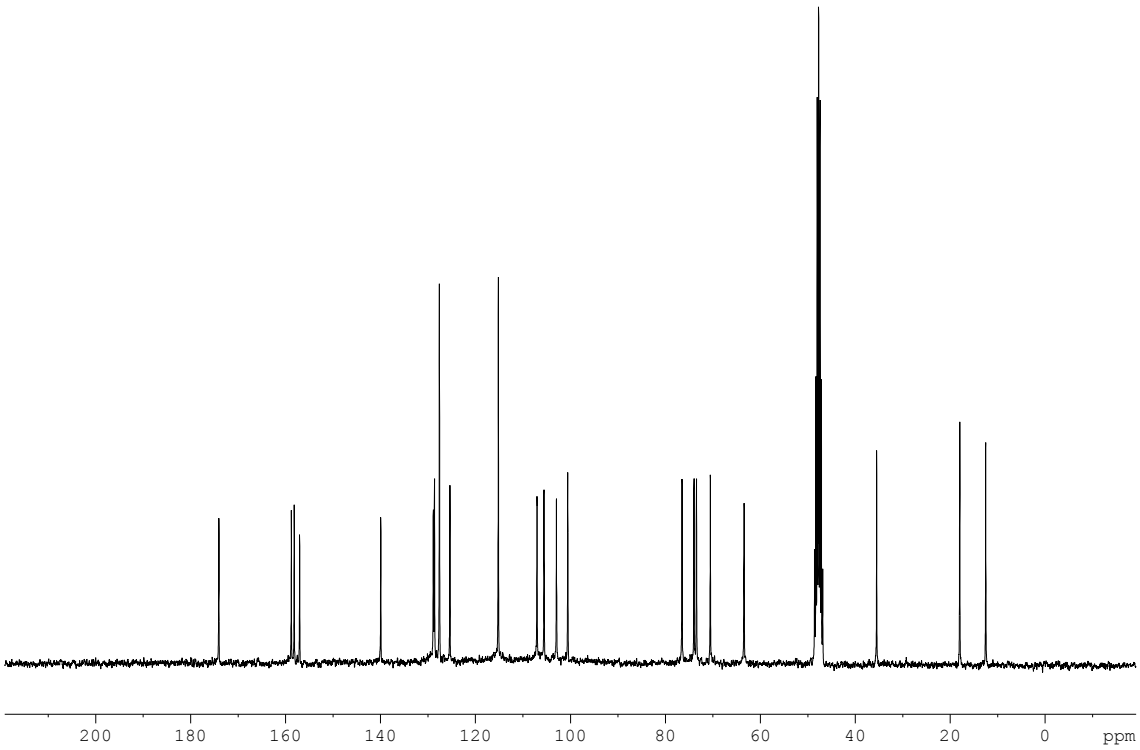
HPLC-MS-MS Analyses. Analyses were carried out in a 1200 series HPLC-DAD system (Agilent Technologies, Waldbronn, Germany), equipped with an HTC Ultra mass detector in series (Bruker Daltonics, Bremen, Germany). The mass detector was an ion-trap mass spectrometer equipped with an electrospray ionisation (ESI, capillary voltage, 4 kV; nebuliser 15 psi; dry gas 5 L/min; dry temperature, 350 °C) system. Mass scan (MS) and MS/MS daughter spectra were measured from *m/z* 100 to 1200 using the Ultra scan mode (26,000 *m/z* per second). Collision-induced fragmentation experiments were performed in the ion trap using helium as collision gas, and the collision energy was set at 50%. Mass spectrometry data were acquired in the negative ionization mode. Chromatographic separations of cell media, cell extracts, blood and stomach, small intestine and colon content samples were carried out on a 150 x 0.5 mm i.d., 5 µm, reverse phase SB C18 Zorbax column (Agilent, Waldbronn, Germany) using water/formic acid (99:1, v/v) (A) and acetonitrile (B) as the mobile phases at a flow rate of 10 µL/min. The gradient started with 1% B in A to reach 60% B at 30 min, 90% B at 30 min for 5 min and returning to the initial conditions (1% B). UV chromatograms were recorded at 280 and 320 nm. Identification of compounds **1-10** was carried out by

chromatographic comparisons (UV and MS) with the corresponding commercially available standards (**1** and **2**) as well as the synthesized compounds in the present study (**3-10**). Resveratrol derivatives (glucuronides, sulfates, etc.) were quantified as resveratrol **1** at 320 nm. Resveratrol metabolites were identified according to their UV and MS spectra as well as MS/MS fragments.

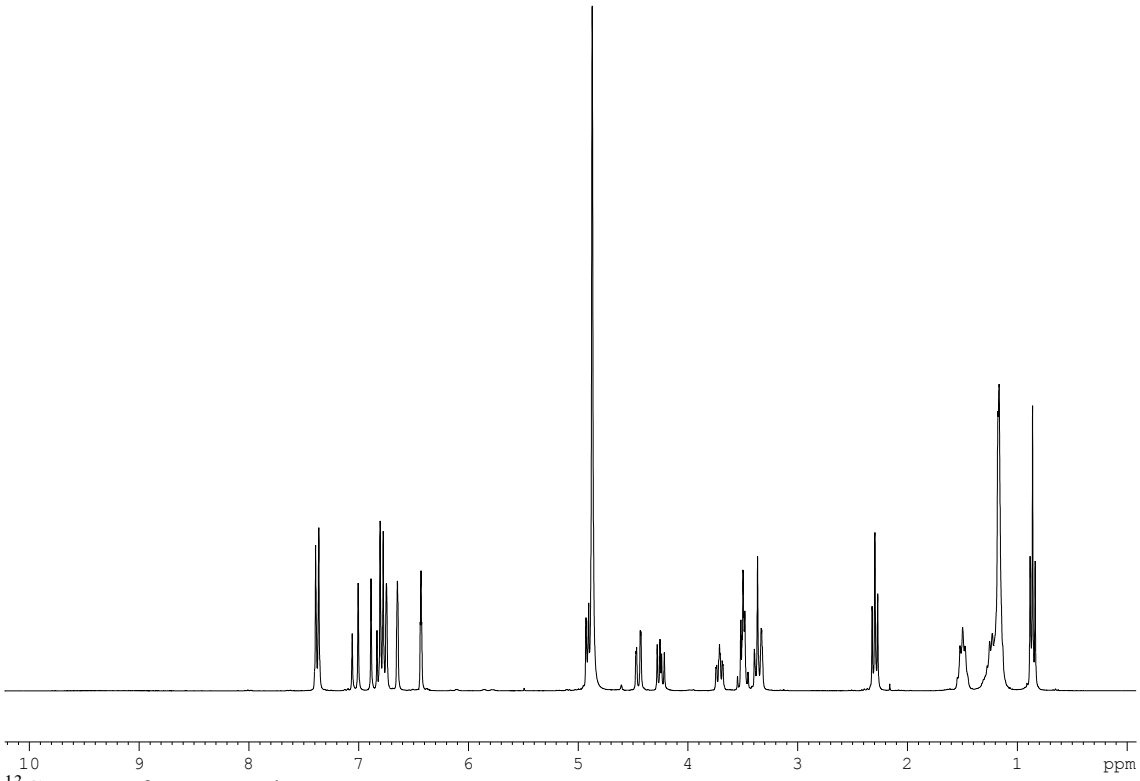
^{1}H NMR of compound **6**:



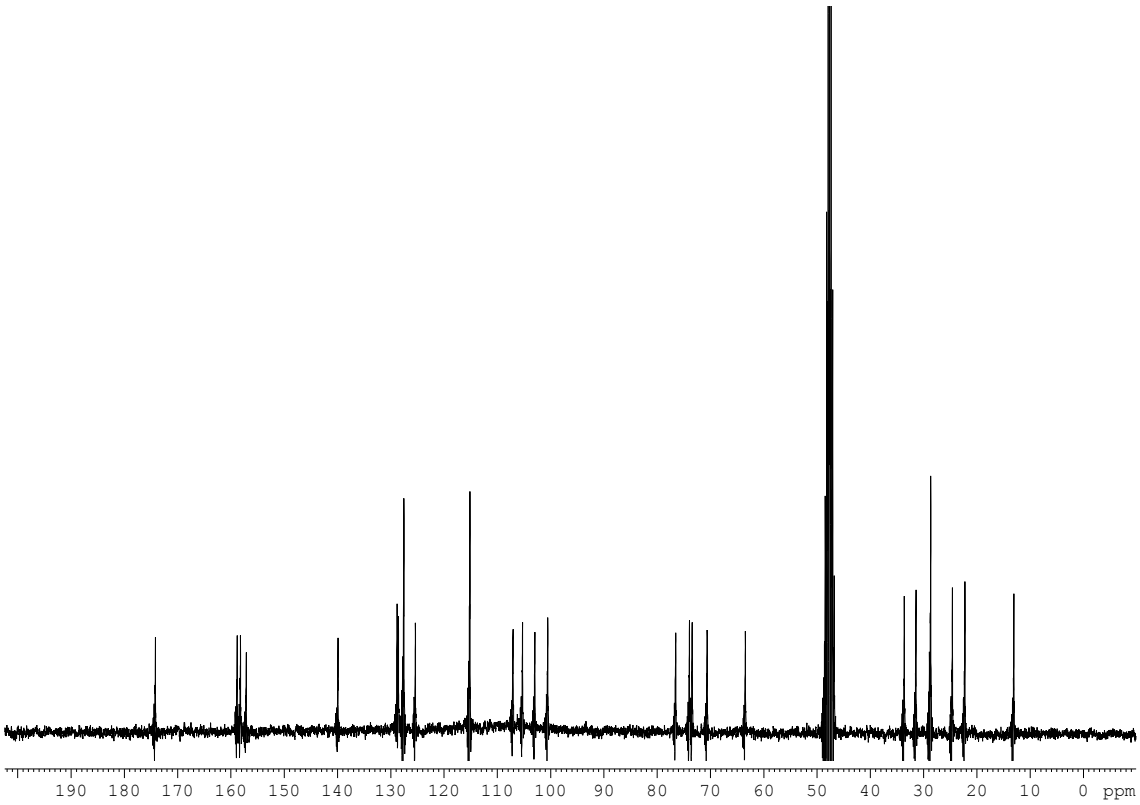
^{13}C NMR of compound **6**:



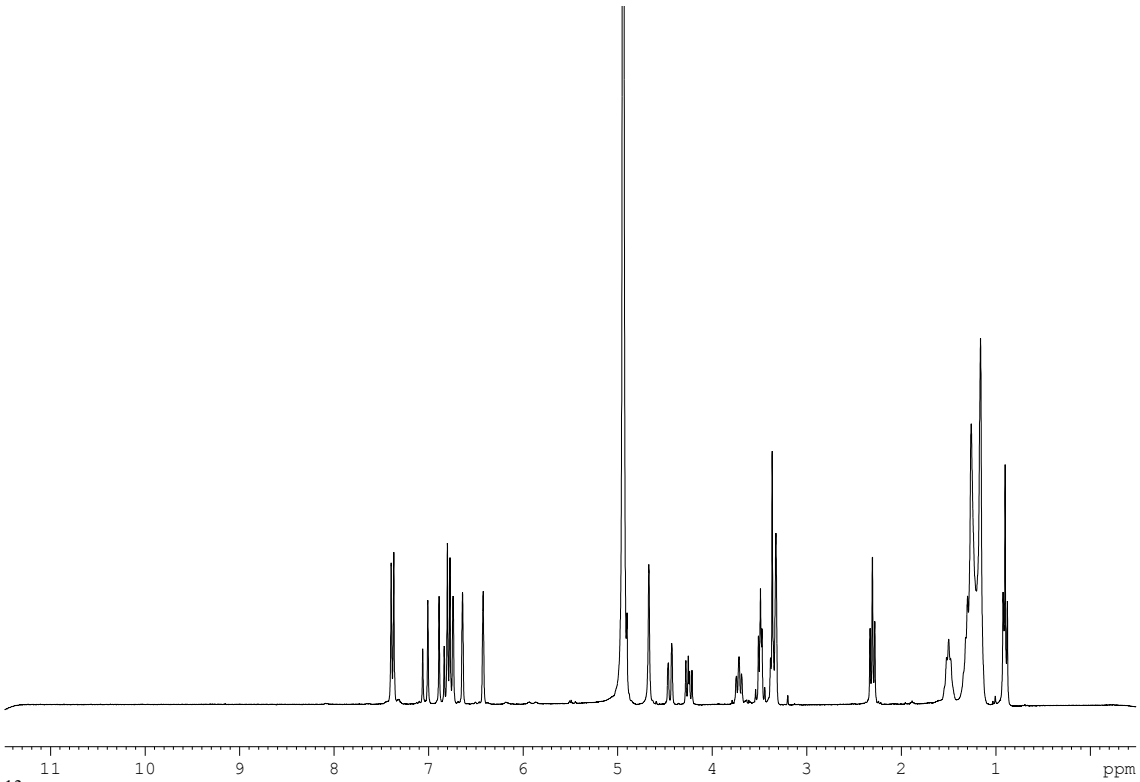
^{1}H NMR of compound **7**:



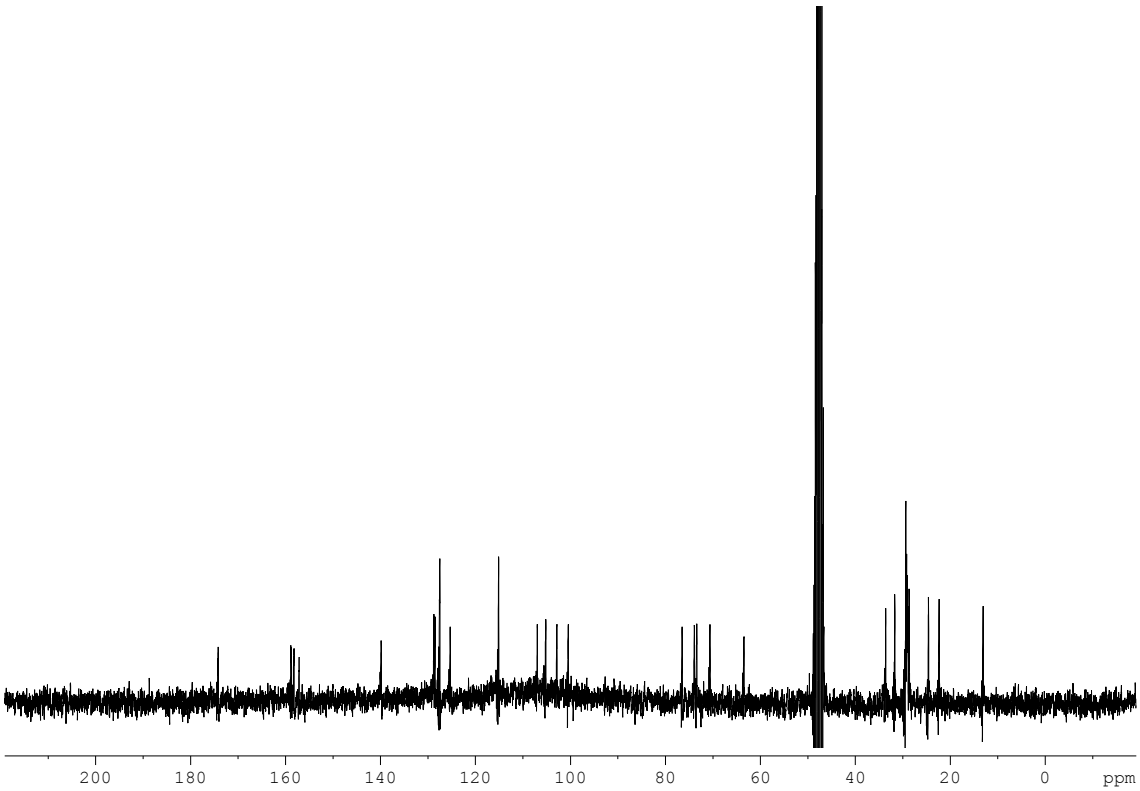
^{13}C NMR of compound **7**:



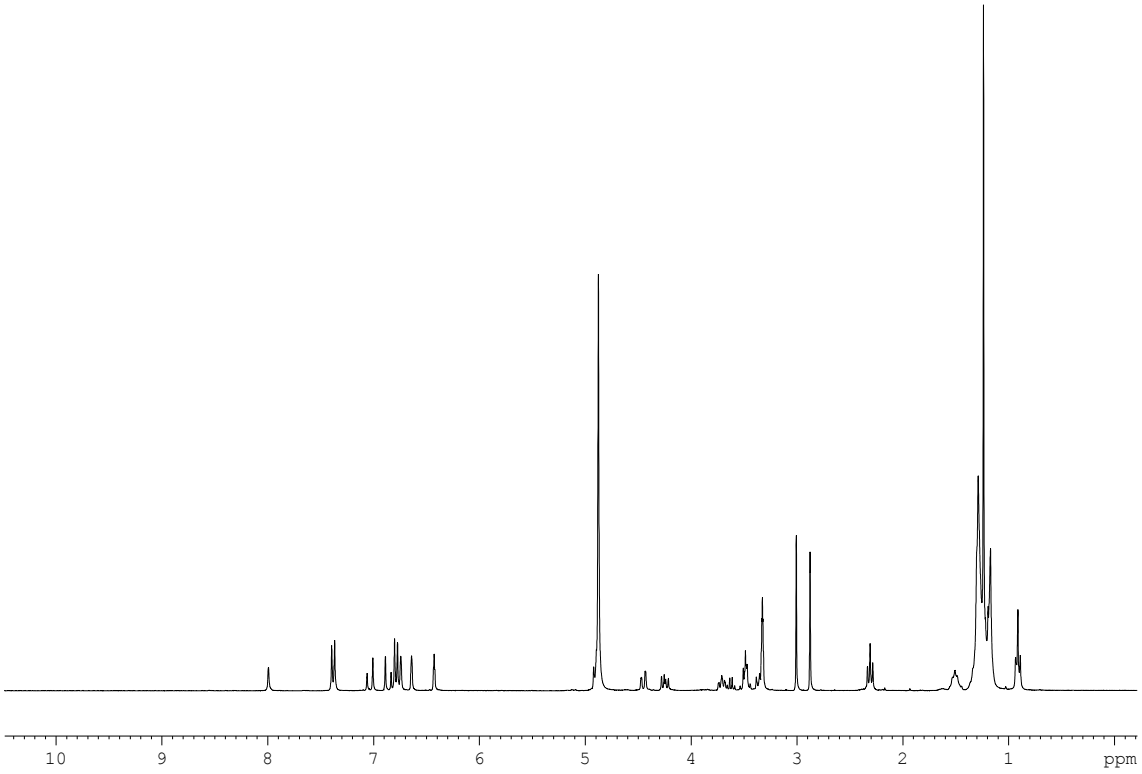
^{1}H NMR of compound **8**:



^{13}C NMR of compound **8**:



^{1}H NMR of compound **9**:



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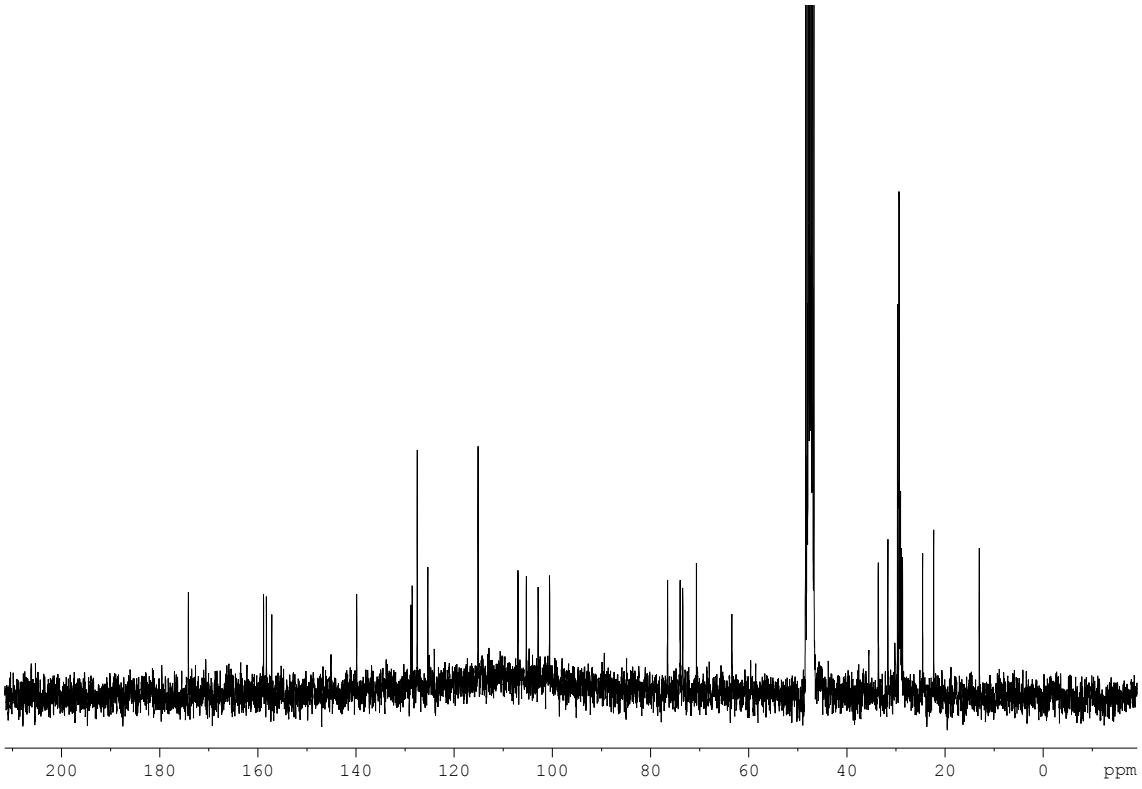

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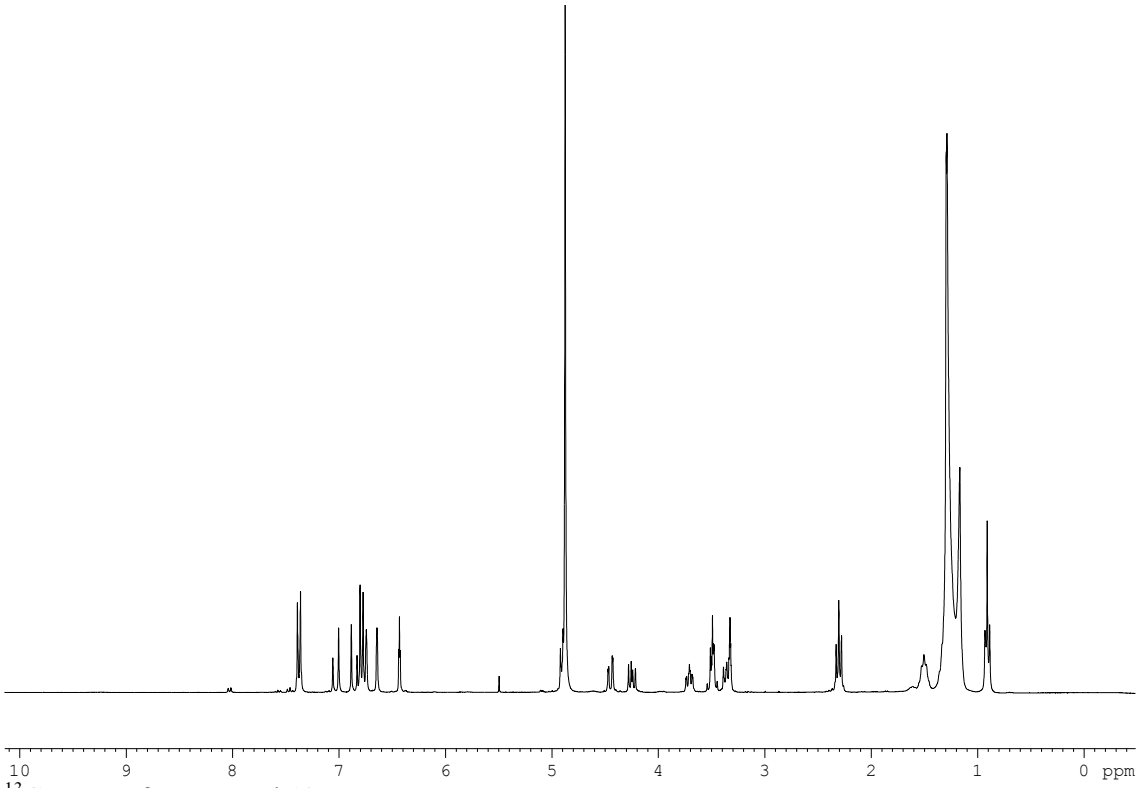

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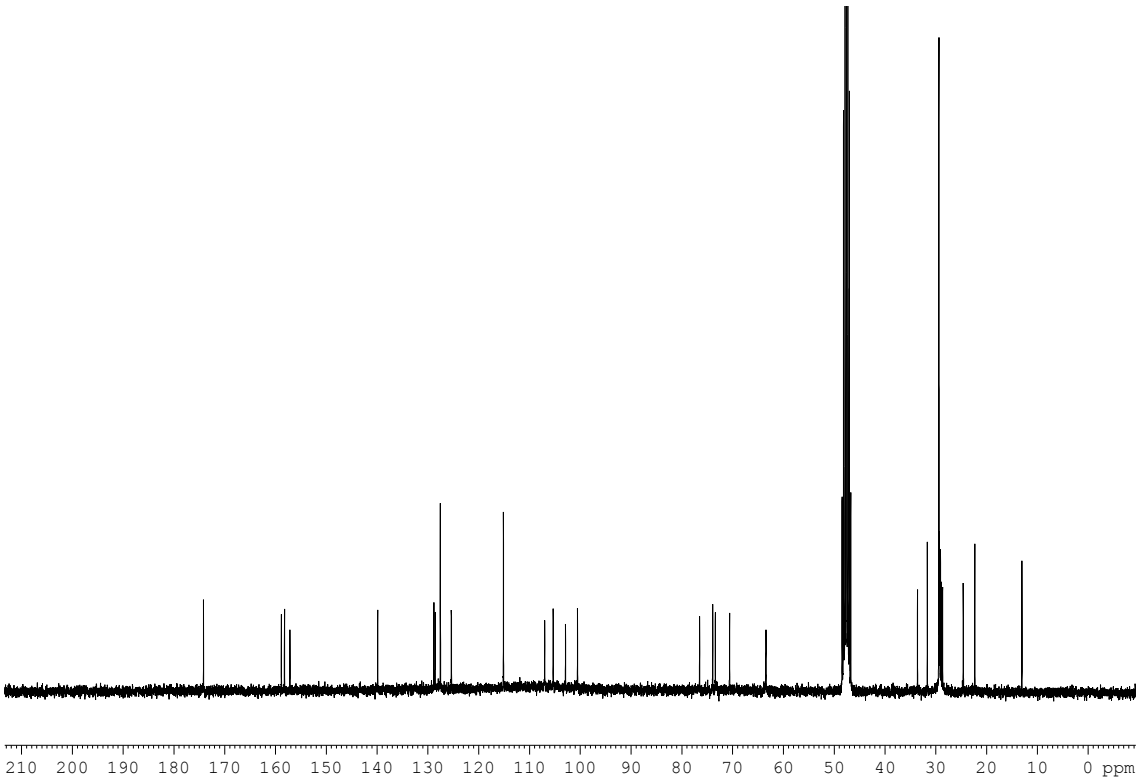

References

1. Morgan, D. M. Tetrazolium (MTT) assay for cellular viability and activity. *Methods Mol. Biol.* **1998**, *79*, 179-183.
2. Giffen, P. S; Turton, J.; Andrews, C. M.; Barrett, P.; Clarke, C. J.; Fung, K. W.; Munday, M. R.; Roman, I. F.; Smyth, R.; Walshe, K.; York, M. J. Markers of experimental acute inflammation in the Wistar Han rat with particular reference to haptoglobin and C-reactive protein. *Arch. Toxicol.* **2003**, *77*, 392–402.