antioxidants

MDPI

Supplementary Material

Dual carbonic anhydrase IX/XII inhibitors and Carbon Monoxide Releasing Molecules modulate LPS-mediated inflammation in mouse macrophages

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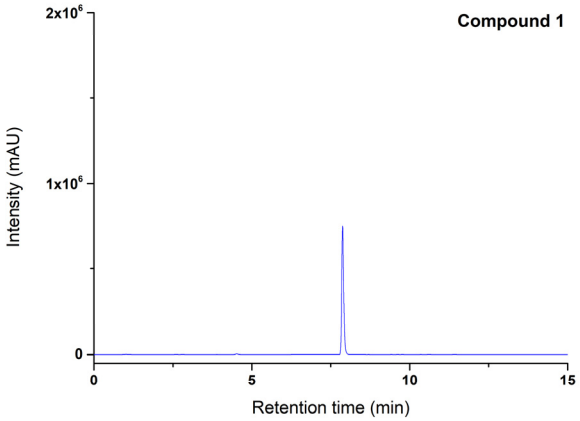

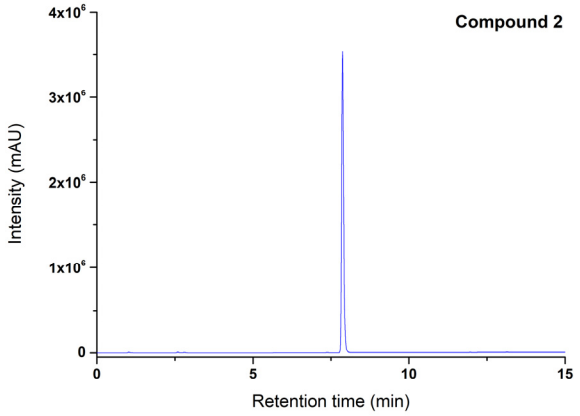

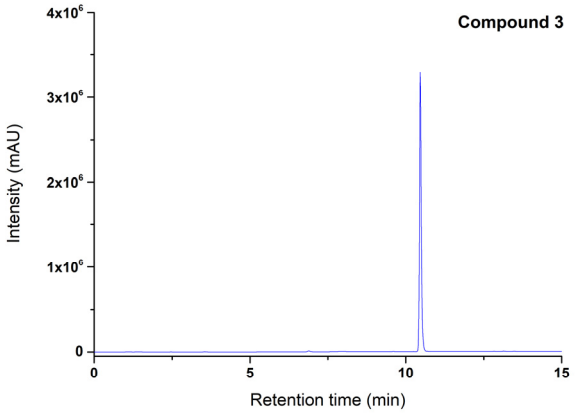
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3x10^6
2x10^6
1x10^6
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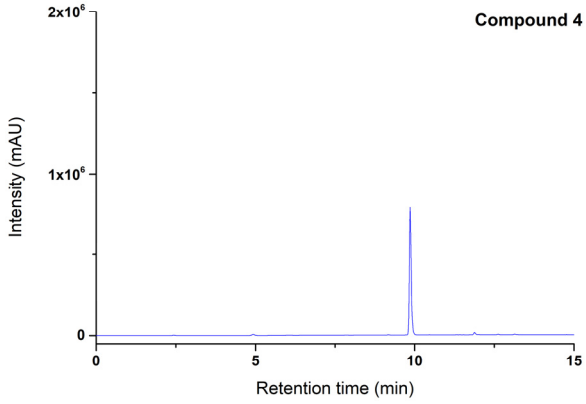
0
5
10
15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0
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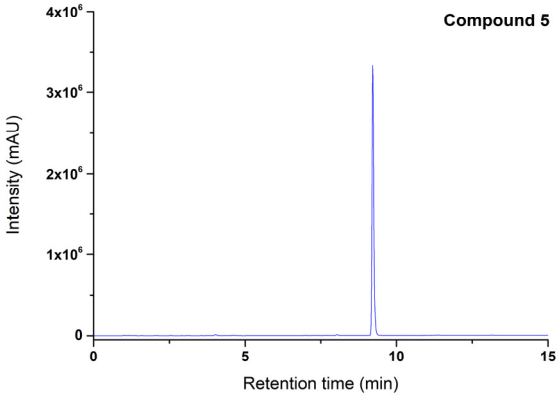
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Intensity (mAU)
Retention time (min)
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10
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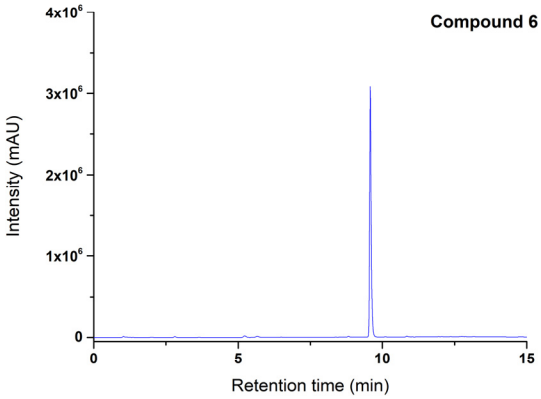
Compound 6
Intensity (mAU)
Retention time (min)
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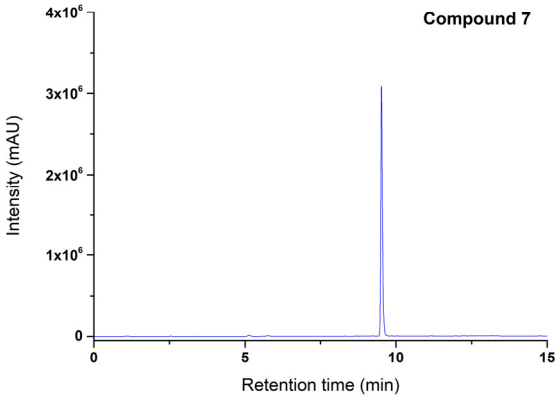
Compound 7
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Retention time (min)
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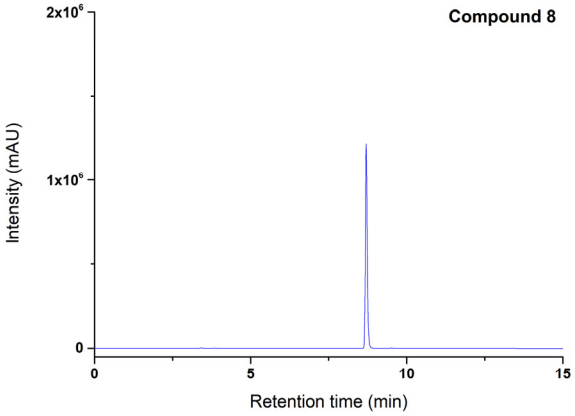
Compound 8
Intensity (mAU)
Retention time (min)
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1x10^6
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5
10
15

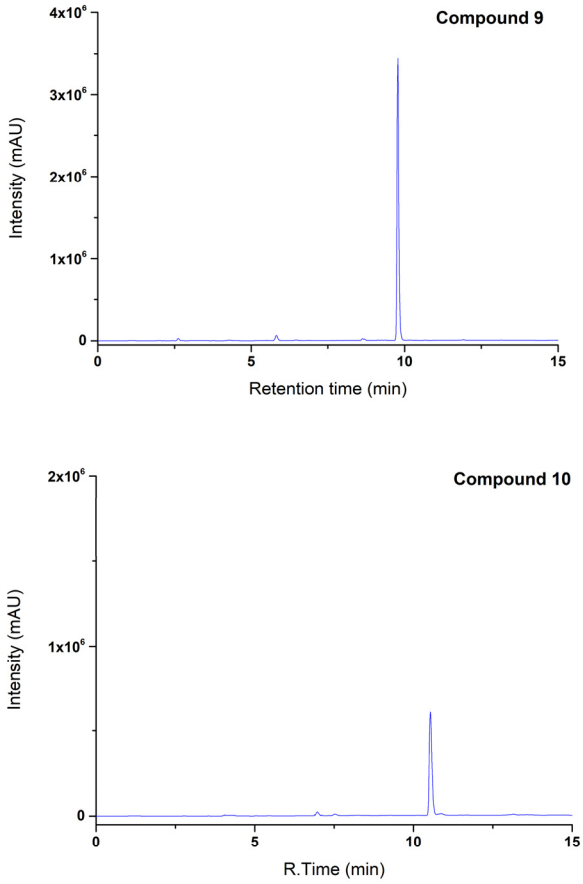

Figure S1. Chromatograms (HPLC) of compounds **1**-**10**.

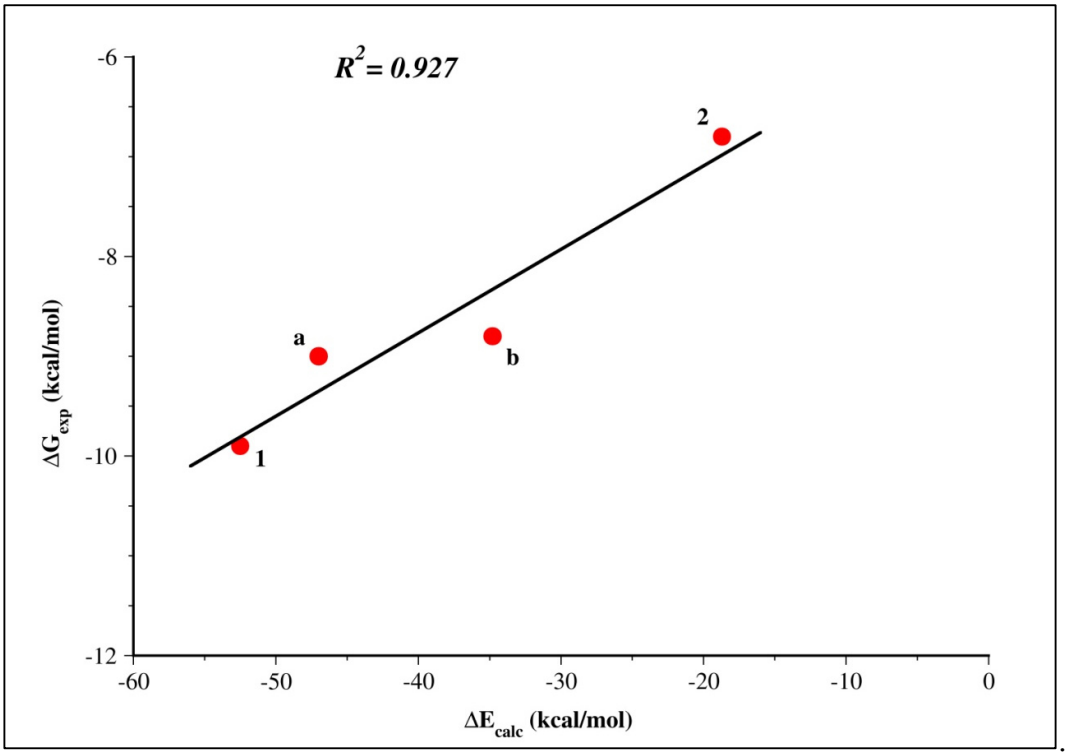

Figure S2. Correlation between ΔE_{calc} and ΔG_{exp} values, defined for complexes of hCA IX-mimic with **a**, **b**, **1** and **2**.

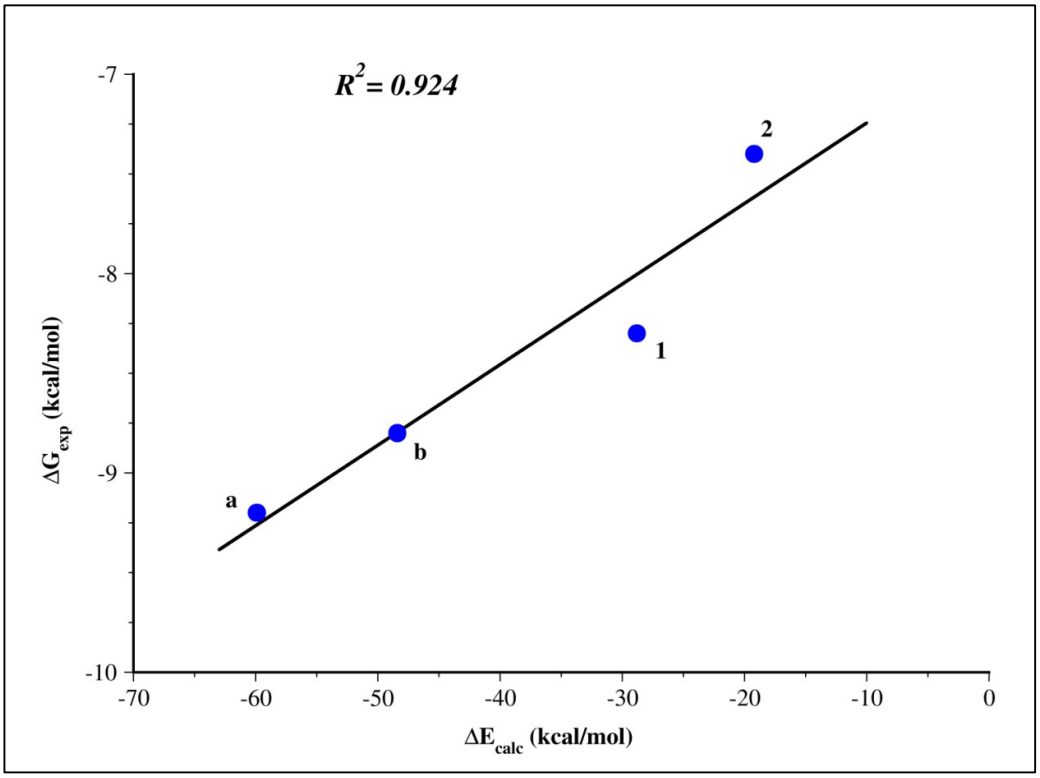

Figure S3. Correlation between ΔE_{calc} and ΔG_{exp} values, defined for complexes of hCA XII with **a**, **b**, **1** and **2**.

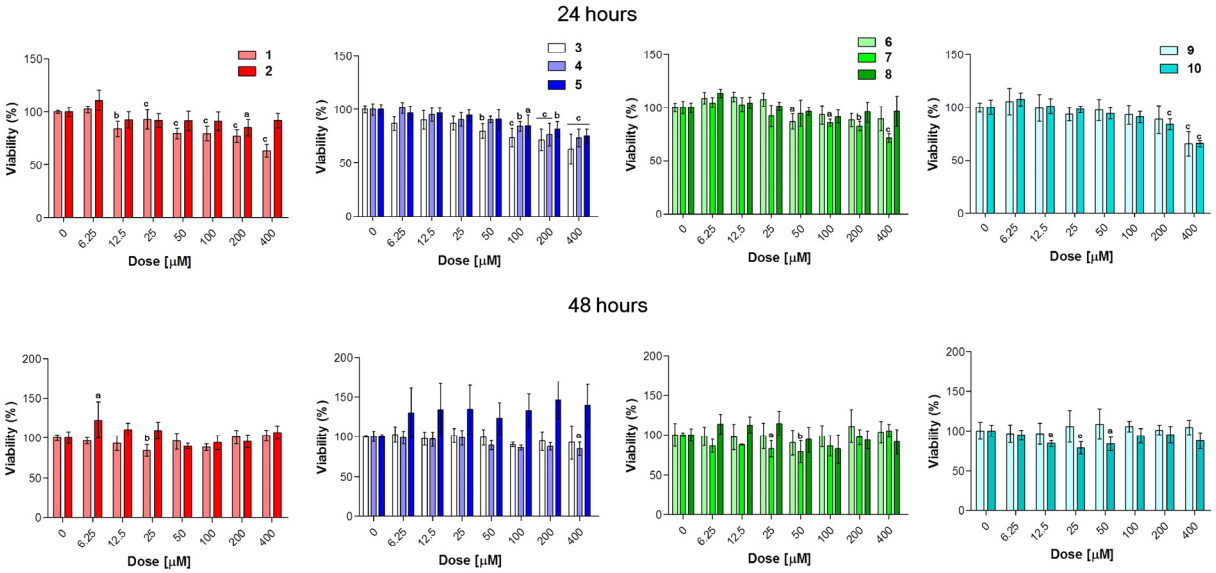

Figure S4. Cell metabolic activity of RAW 264.7 mouse macrophages in the presence of increasing concentrations (0-400 μM) of compounds **1-10**. Data shown are the means ± S.D. of six replicates and are expressed as percentages of untreated cultures (0 μM) set as 100% (a = p<0.05 between compounds and untreated cells; b = p<0.01 between compounds and untreated cells; c = p<0.001 between compounds and untreated cells).

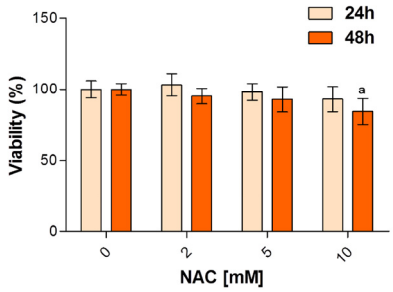

Figure S5. Cell metabolic activity of RAW 264.7 mouse macrophages in the presence of increasing concentrations (0-10 mM) of NAC. Data shown are the means ± S.D. of six replicates and are expressed as percentages of untreated cultures (0 mM) set as 100% (a = p<0.05 between NAC and untreated cells).

Table S1. Inhibition data against hCA I, hCA II, hCA IX and hCA XII of propargylated precursors and the standard sulfonamide inhibitor acetazolamide (**AAZ**) by a Stopped-Flow CO_2 hydrase assay.

	K_I (nM)[a]			
compound	**hCA I**	**hCA II**	**hCA IX**	**hCA XII**
a[b]	>100000	>100000	252.0	178.0
b	>100000	>100000	358.0	378.0
c	>100000	>100000	>100000	>100000
d	>100000	>100000	>100000	>100000
e	>100000	>100000	>100000	>100000
f	>100000	>100000	695.0	4920
g	>100000	>100000	315.5	353.0
h	>100000	>100000	>100000	>100000
i	>100000	>100000	>100000	>100000
l	>100000	>100000	>100000	>100000
AAZ	250	12.1	25.8	5.7

[a]Mean value from three independent assays (errors were in the range of ± 5-10%). [b]from ref. (De Monte, C; Carradori, S; Secci, D; D'Ascenzio, M; Vullo, D; Ceruso, M; Supuran, CT. Cyclic tertiary sulfamates: Selective inhibition of the tumor-associated carbonic anhydrases IX and XII by *N*- and *O*-substituted acesulfame derivatives. *Eur. J. Med. Chem.* 2014, *84*, 240-246).

Table S2. calculated binding energies in solution, ΔE_{calc}, and experimental free energies, ΔG_{exp}, for hCA IX-mimic and hCA XII, with **1** and **2** and their propargyl precursors, **a** and **b**. R^2 values are also provided.

Molecule	**hCA IX**			**hCA XII**		
	***ΔE_{calc}**	***ΔG_{exp}**	**[#]K_I**	***ΔE_{calc}**	***ΔG_{exp}**	**[#]K_I**
a	-47.0	-9.0	252	-59.9	-9.2	178
1	-52.5	-9.9	56.3	-28.8	-8.3	788.4
b	-34.8	-8.8	358	-48.4	-8.8	378
2	-18.7	-6.8	10000	-19.2	-7.4	3462
R^2	0.927			0.924		

*Energy values in kcal/mol; [#]K_I in nM.