Supplementary Information

# Serendipitous fragment-based drug discovery: Ketogenic diet metabolites and statins effectively inhibit carbonic anhydrases I - XIV

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***Supplementaryt Table 1.*** Inhibition constants of isozymes hCA II, IV, VA and IX with several carboxylates, for the $CO_2$ hydration reaction, at 20 °C.[1,2]

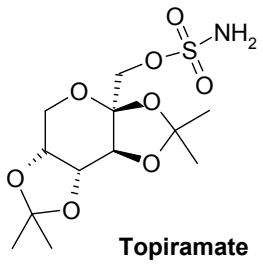
| Inhibitor | $K_I$ (mM) | | | |
|---|---|---|---|---|
| | hCA II | hCA IV | hCA VA | hCA IX |
| formate | 24.0 | 1.25 | 9.97 | 1.22 |
| acetate | 0.13 | 0.55 | 25.9 | >150 |
| malonate | 1.62 | 0.028 | 6.00 | 1.27 |
| pyruvate | 2.11 | 0.014 | 5.50 | 1.12 |
| L-lactate | 3.22 | 1.53 | 3.37 | >150 |
| L-malate | 1.87 | 0.054 | 2.49 | 7.42 |
| citrate | 2.16 | $9.9 \times 10^{-5}$ | 1.67 | 4.93 |
| benzoate | 0.03 | 0.075 | 7.01 | >150 |

## CA inhibition assay

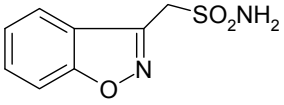
An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed $CO_2$ hydration activity.[3] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM $Na_2SO_4$ (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed $CO_2$ hydration reaction for a period of 10-100 s.[32] The $CO_2$ concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5-10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in the assay buffer and dilutions up to 0.01 nM were done thereafter with the same buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares

methods using PRISM 3, as reported earlier,[4] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.[4,5]

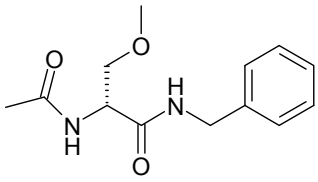
**Structure of sulfonamide/sulfamate CA inhibitors and of lacosamide**




**Topiramate**




**Zonisamide**




**Lacosamide**

**References**

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