

Supplementary Information

Polypharmacology of sulfonamides: Pazopanib, a multitargeted receptor tyrosine kinase inhibitor in clinical use, potently inhibits several mammalian carbonic anhydrases

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CA inhibition assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO₂ hydration activity.¹ 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 Na₂SO₄ (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO₂ hydration reaction for a period of 10-100 s. The CO₂ 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 distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay 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,² and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.^{3,4}

References

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