

Assessment of the Inhibition Mechanism of Phoxymethylketones Against Cruzipain by Hybrid QM/MM-MD Simulations [†]

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Abstract: Cruzipain (CZP) is the main cysteine protease of *T. cruzi*, the causative agent of Chagas disease, and, thus, a therapeutic target of interest for rational drug design. In this work, the inhibition mechanism of the most potent reported phoxymethylketone (PMK) derivative was evaluated by hybrid quantum mechanics/molecular mechanics molecular dynamics (QM/MM-MD) studies. Three feasible inhibition pathways were explored against both monomeric and homodimeric CZP. QM/MM-MD simulations were performed on the Tupac supercomputer (Argentina), employing the Amber20 package. The *SCC-DFTB* semiempirical method and the *ff14SB* and *TIP3P* force fields were applied for the QM and classic regions, respectively.

A two-step inhibition pathway was found to be the most energetically favorable, involving the formation of a tetrahedral thiohemiacetal intermediate, followed by a rearrangement yielding the final thioether adduct with tetrafluorophenol displacement. An alternative stepwise mechanism via a protonated thiohemiacetal intermediate and a concerted one showed less or non-feasibility of occurrence. Additionally, lower activation energies were observed for the reactions entailing the homodimer, suggesting potential homodimeric CZP activity. Overall, these findings contribute to the understanding of the molecular mechanism of PMK derivatives against CZP, paving the way for the design of new inhibitors for the effective treatment of Chagas disease.

Keywords: Cruzipain; inhibition mechanism; molecular dynamics; QM/MM.

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Conflicts of Interest

The authors declare no conflict of interest.