Human glutamate carboxypeptidase II (GCPII) is a zinc-dependent metallopeptidase implicated in diverse pathologies. GCPII-specific ligands can be used in diagnostic and therapeutic applications in prostate cancer and various neurological disorders [1]. Such ligands typically consist of a glutamate moiety linked to a zinc-binding group to ensure high specificity and affinity, respectively [2]. Hydroxamate functionality is one of the prominent zinc-binding functions used in the field, however, there are no structural data describing interactions between hydroxamates and GCPII. Here we report X-ray structures of six complexes between GCPII and hydroxamate-based inhibitors. Our structures reveal unexpected positioning of hydroxamates in the internal GCPII pocket that differs markedly from binding modes of matching prototypical GCPII inhibitors featuring different zinc-binding groups. They provide mechanistic explanation for prior structure-activity relationship studies and can be exploited for the structure-assisted design of novel GCPII-specific compounds.


**Keywords:** Prostate-specific membrane antigen, glutamate carboxypeptidase II, hydroxamate-based inhibitors