IDENTIFICATION OF NEW CANTHARIDIN DERIVATIVES AS NOVEL POTENTIAL INHIBITORS OF PROTEIN PHOSPHATASES 2 AND 5 (PP2A, PP5)

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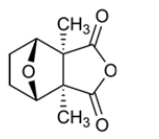
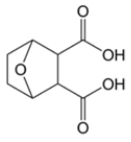
Cantharidin, a natural compound occurring in medicinal insect blister beetle (*Mylabris phalerata* Pallas), and its water-soluble demethylated synthetic analog norcantharidin (better known as endothall), historically used in the purification of phosphorylated proteins, were identified as inhibitors of serine-threonine protein phosphatases (PP). Cantharidin (CID 5944, hydrolyzing into cathartic acid - CID 2544) is a potent selective inhibitor of PP2A and PP5 [PMID: 1334551], while norcantharidin (CID 93004) is a medium-strength inhibitor of PP2A only [PMID:12003183, 23809227]. These compounds have extensively been studied as promising leads in the development of more effective therapeutics for the treatment of cancer, neurodegenerative disorders, and type 1 diabetes mellitus. As no highly PP5 selective inhibitors have been reported, the design and evaluation of novel biologically active derivatives of known inhibitors are of extreme importance.

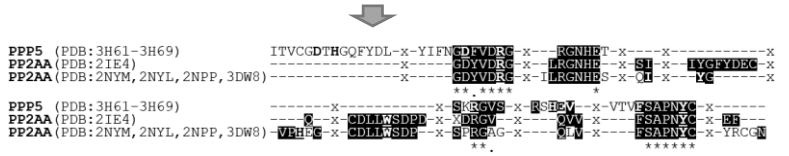


MW – 196.2, ALogP – 0.64
RotBonds – 0, PSA – 52.6
HBA – 4, HBD – 0
AromRings – 0, HAC – 14



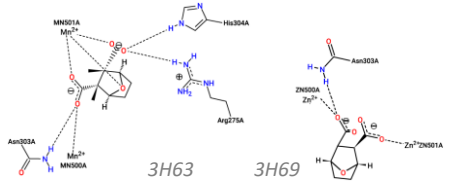
MW – 186.2, ALogP – 0.05
RotBonds – 2, PSA – 83.83
HBA – 3, HBD – 2
AromRings – 0, HAC – 13

Inhibitor	PDB	PP type	UniProtKB	Water molecules	Binding sites
Cantharidin	3H61, 3H68, 3H63, 3H67, 3H62	PPP5	P53041	5	3
Norcantharidin	3H69, 3H64	PPP5	P53041	5	1

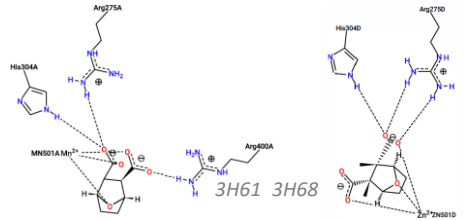


Binding site profiles

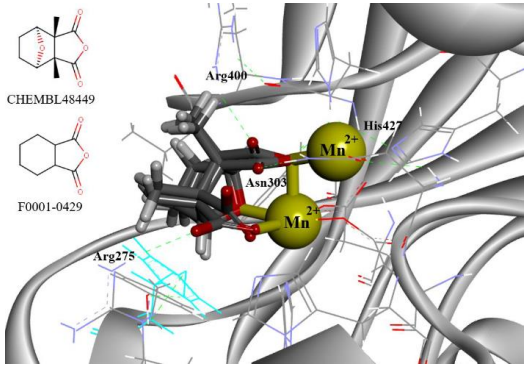
Based on the template structures of human PP5 (PDB IDs: 3H68, 3H63, 3H67, 3H62, 3H61, 3H69, 3H64) in complex with cantharidin and endothall and PP2A (UniprotKB: Q07099), we reconstructed its full 3D model in Schrödinger. The ligands for docking input were prepared using CCDC Hermes. Cantharidin and endothall binding sites in PP5 and PP2A showed high similarity for protein phosphatases of different origin.



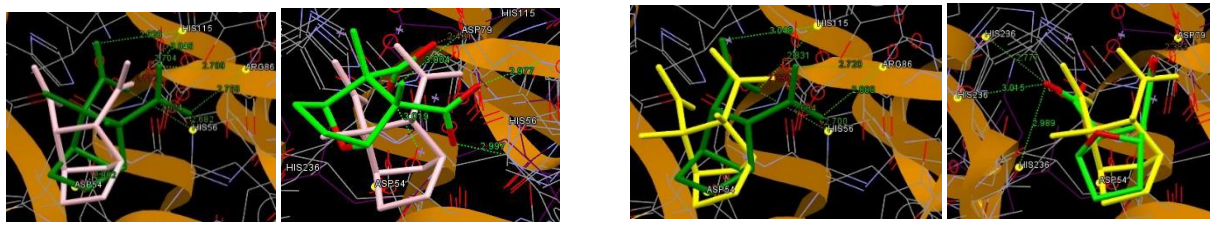
The key residues forming the binding pocket are shown in two-dimensional diagrams



The docking results showed that three amino acid residues, Arg275, Asn303, His304, and Arg275, His304, Arg400 (PP5 and PP2A, respectively) were the most important for the ligand binding. In total, nine residues were demonstrated to be involved in the binding of the inhibitors. The completeness of the amino acid sequence and the profile of the site interaction were determined by pairwise and profile alignment with the original sequence (with ClustalX). The binding site for PP5 was specified within a 15 Å radius from the atom -NE2 (His125).

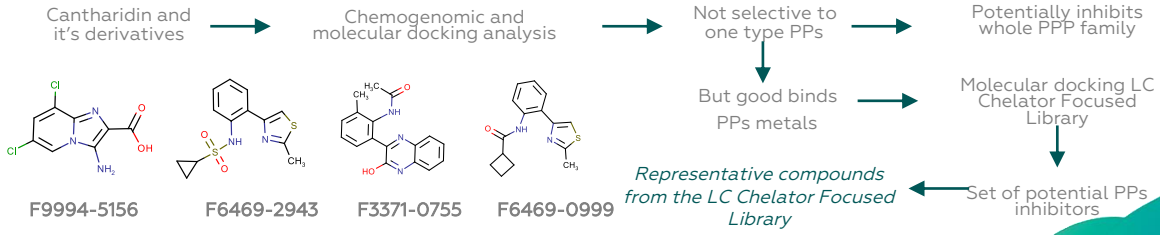


PP5 with cantharidin, LC analog and Mn ion molecules



A zoomed view of the substrate-binding pocket of norcantharidin with protein phosphatases from Human and Arabidopsis

With the aid of chemoinformatic analysis, we selected over 50 most promising derivatives of cantharidin from the PubChem Database and the Life Chemicals Chelator Focused Library. Protein-ligand complexes of these derivatives were predicted by flexible docking and evaluated both with CCDC GOLD scoring functions and molecular dynamics simulation in GROMACS. As a result, we have identified 11 novel potential PP5 and PP2A inhibitors, previously not described in the literature.



Representative compounds from the LC Chelator Focused Library

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