Development of a Pharmacophore Model for Inhibition of PP2A

Christopher Wirth, James Gordon IV, Christopher Dietz, Christopher Stoj PhD, Ronny Priefer PhD, Lawrence Mielnicki PhD, Mary McCourt PhD
Niagara University, Department of Biochemistry, Chemistry and Physics

Cantharidin is a naturally occurring compound in the Spanish Fly and Chinese Blister Beetle and it also happens to be a very effective anti-cancer agent. Unfortunately, it is a bit too effective. With a lethal limit of 1mg/kg, it will most likely get rid of the cancer when administered to a patient, but will also most likely be fatal to them in the process.[1] Given the potential of cantharidin, the present research effort seeks to devise a potent analog based on the cantharidin structure that will be just as effective against cancer, but at the same time have a greatly decreased toxicity level. The approach to developing the optimal analog is based on an integrated analysis of synthesis, biochemical testing and molecular modeling techniques. A systematic analysis of the structure of cantharidins and the requirements for binding to its target protein led to the design of over 100 analogs. This was done by altering the original cantharidin molecule in certain places, such as the bridgehead position and double bond, which were believed to change the efficacy and/or toxicity of the analogs. Previous studies showed an oxygen atom at the bridgehead position is directly related to the inhibition.[2] Each new analog was designed, model built and then optimized using the AM1 semi-empirical program.[3] Charges were calculated and the optimized structures were analyzed. Of the original 100 designed, 9 were synthesized and their efficacy was tested by administering them to Human HepG2 hepatocarcinoma cell lines. Analog activity was assessed by measuring cell growth. Of the nine synthesized and tested cantharidin analogs, only one possessed similar effects to that of the parent compound. Within the cell, cantharidin inhibits protein phosphatase 2A (PP2A), a major regulator of phosphorylation associated with tumor initiation and growth. The analogs were modeled into the active site of the PP2A to identify potential structure activity relationships (SARs).[4] This process showed that there is no clear-cut relationship between analog efficacy and the bridgehead oxygen. Relationships were analyzed between bridgehead atom, bridgehead charge, open/closed ring formations and the presence of a double bond and none alone were responsible for their efficacy. There was some association of the bridgehead structure and the ring structure with PP2A inhibition and cell growth effects respectively. Future work suggests SAR models, based on the relative charges and positions of the bridgehead atom and the double bond.

References:

1 – Matsuzawa, M., Graziano, M. J. & Casida, J. E. “Endothal and Cantharidin Analogues: Relation of Structure to Herbicidal Activity and Mammalian Toxicity” (1987) *J. Agric. Food Chem*. 35: 823-829.
2 – McCluskey, A., Walkom, C., Bowyer, M., Ackland, S., Gardiner, E., Sakoff, J. “Cantharimides: A New Class of Modified Cantharidin Analogues Inhibiting Protein Phosphatases 1 and 2A.” (2001) *Bioorganic & Medicinal Chemistry Letters*. 11: 2941-2946.
3 – Tripos. “SYBYL”. St. Louis, MO.
4 – PDB ID: 2NYM. Xu, Y., Xing, Y., Chen, Y., Chao, Y., Lin, Z., Fan, E., Yu, J.W., Strack, S., Jeffrey, P.D., Shi, Y. “Structure of the protein phosphatase 2A holoenzyme.” (2006) *Cell* 127: 1239-1251.