

Synthesis, Characterisation and *In Silico* Study Of Vanillyl Mandelic Acid

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ABSTRACT

The hormones epinephrine and norepinephrine are the catecholamines secreted by adrenal glands situated on each kidney. These hormones are released into the blood stream and prepare the body for physical stress. During this heart beat increases, lungs expand, liver fat cells are mobilised and release the stored energy. Break down of epinephrine into metaepinephrine and vanillyl mandelic acid (VMA), norepinephrine into meta norepinephrine and VMA take place and are excreted through urine. The increase in VMA can cause cancers of adrenal glands and nervous system along with neuroendocrine disorders. The title compound synthesised was checked confirmed the functional groups using FTIR, melting point and for its anticancer property. This was docked to the receptor protein with PDB ID 1UNG. Prior to docking, the pharmacophore features of the ligand was also analysed. The results of these works are being discussed in this paper.

Key words: epinephrene, norepinephrene, VMA, receptor protein

INTRODUCTION

Mandelic acid (MA) is an important metabolite of styrene. In humans, measurement of its concentration in urine provides an important assessment of the overall level of styrene exposure in workers of the reinforced plastic manufacturing industry¹. Recent literature about the biological monitoring of styrene-exposed workers is reviewed. Styrene primarily exhibits its toxicity on the central and peripheral nervous systems, although its mutagenicity and chromosome damaging ability also may be relevant. Uptake, transformation and excretion of styrene show that, beside the usual biological indicators such as urinary mandelic and phenylglyoxylic acids (main metabolites), other indicators also may be of interest².

During a program on clinical standards conducted at the National Bureau of Standards, a need for large scale preparation of pure DL-4-hydroxy-3-methoxymandelic acid (IV) was needed³. Denantiomorph of IV is one of the major metabolites of epinephrine and norepinephrine in normal human urine⁴. Also, abnormally high concentrations of IV are found in the urine of patients having pheochromocytoma⁵. Henceforth, mandelic acid serves to be a marker for the diagnosis of certain diseases. Urinary levels of mandelic acid (MA) and phenylglyoxylic acid (PGA) were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS)^{6,7}.

Vanillyl mandelic acid (VMA) is a chemical intermediate in the synthesis of artificial vanilla flavorings⁸. Bearing the importance of mandelic acid and its derivatives, an attempt has been made to synthesize VMA. The biological importance of mandelic acid is already well known. It has immense applications in the cosmetic arena in skin related issues. The potential of the synthesised product VMA, we have analysed its anti bacterial property using insilico approach. The ADMET analysis has also been done. Cancer is a deadly disease and faced as a threat globally. Difficulties are still experienced by scientists, working on this disease till date in finding a good drug for it. In an attempt to search for more drugs, alternative methods are also used. Here compounds from natural sources, synthetic compounds are the ones normally followed. The relation of anticancer activity to chemical structure help in determining the anticancer property of synthetic organic compounds⁹. We have already used insilico analysis on cancer inhibition¹⁰ (Pommagar) and other areas (collagen, antimicrobial metabolite)^{11,12}. The hydrogen bonding interactions in the compound shows the DNA binding which results in anticancer agents formation¹³. The ligands from the synthetic arena, we have come forward with a compound VMA synthesised in our laboratory.

MATERIALS AND METHODS

Synthesis and Characterisation of VMA

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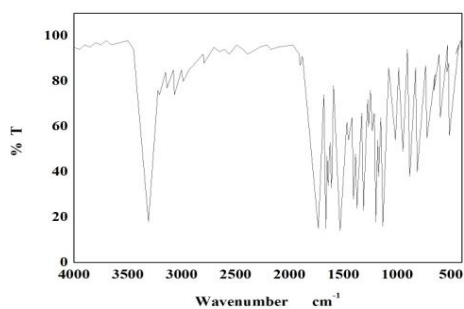


Figure 1: FTIR of VMA

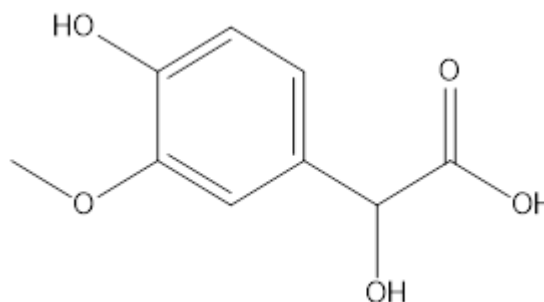


Figure 2: Structure of VMA



Figure 3: Structure of the drug target protein CDK5

1.52 g of Vanillin (10 mmol), 3 ml of chloroform (43 mmol), 2.6 g sodium hydroxide (65 mmol), 9 ml (500 mmol) of water, TBAB 0.16 g (0.46 mmol) are taken in a sterilised beaker, stirred well for 15 minutes and microwaved for 5 minutes at 50-55° C. The mixture is then cooled to room temperature and the pH of the mixture is adjusted to 3.0 - 3.5. The contents are then extracted in CHCl₃, evaporated to give a crystalline solid (yield 70%), homogenous on TLC, melted at 132°C.

FTIR (Figure 1) showed -OH hydrogen bonded stretching at 3160 cm⁻¹ and carbonyl stretching at 1680 cm⁻¹. Carbon NMR showed a peak at 172 ppm corresponding to carboxylic acid and proton NMR showed phenolic, aromatic and carboxylic protons at 5.3ppm, 6-8.5 ppm and 11 ppm respectively. The mass spectrum shows 198 g which corresponds to VMA. Structure of the compound is shown here in the Figure 2.

Retrieval of protein from protein database

The structure of the target protein CDK5 and its x-ray crystallographic structure, having a resolution of 2.3, bearing the PDB ID 1UNG was retrieved from the protein data bank (<http://www.rcsb.org/pdb/>).

Docking Analysis

Preparation of protein and Ligand

The target protein, 1UNG, after being retrieved from PDB was subjected to the removal of hetero atoms present in it. This was followed by applying the force field CharmM using Accelrys Discovery Studio 2.1¹⁴, followed by energy minimization by 1000 iterations. The synthesized compound was drawn using Chemscketch and was saved as a mol file. This ligand was also subjected to the above process as for the protein. The lipinski's rule was checked for the synthetic ligand and was found to satisfy the requirements.

Pharmacophore analysis of the ligand

The pharmacophore features of the ligand were also analyzed by choosing the best conformations which can be generated from the ligand. There are 10 best conformations generated for the ligand. Pharmacophores are generated based on the hip hop algorithm available in Accelrys Discovery Studio¹⁵

RESULTS AND DISCUSSION

This molecule which serves as a ligand is first analysed for the lipinkis rule¹⁶ which has to be satisfied. Upon examination, it was found the compound satisfies the criteria to be ligand. In this work, it is found that the ligand interacts with the protein. The cyclindependent kinases (CDK) CDK1, CDK2, CDK 4, and CDK6 are serine/threonine protein kinases targeted in cancer therapy due to their role in cell cycle progression¹⁷. ASP92, GLY199 and LYS88 are the residues found in the binding site of the target protein. The energy of the protein after minimisation is -17981.68931 K cal/mol and for the ligand it is -14.76513 Kcal/mol. The figure3 shows the structure of the drug target protein.

The pharmacophore feature of a compound signifies its biological importance as a drug (Figure 4). The pharmacophore analysis of the compound shows five hydrogen bond acceptors, 5 hydrogen bond donors and 5 hydrophobic groups. The interfeature distance maintained during the analysis was 2.7 Å. The molecule has a ALogP value of 0.358 and a molecular weight of 184.146 amu.

Docking is an *in silico* approach where the possibility of interaction between a target protein and a ligand is predicted. The non bonded interactions between the ligand (VMA) and the protein CDK5 along with the docking score serves as a measure of the feasibility for the complex formation. Using the ADME protocol of accelrys, the pharmacophore kinetic analysis of the ligand was analysed. Figure 5 shows the results of the analysis.

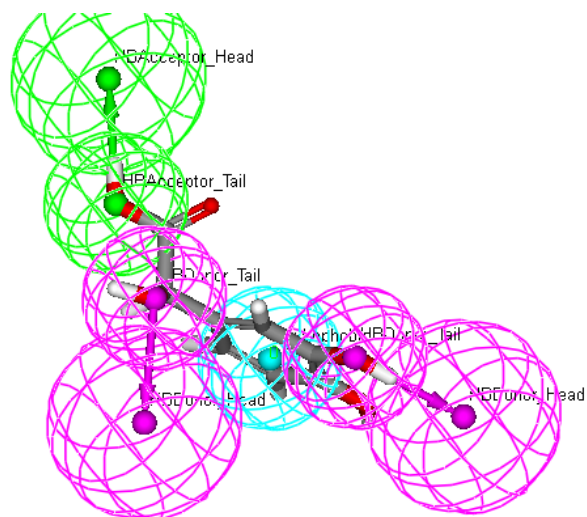


Figure 4. Pharmacophore features of the compound VMA

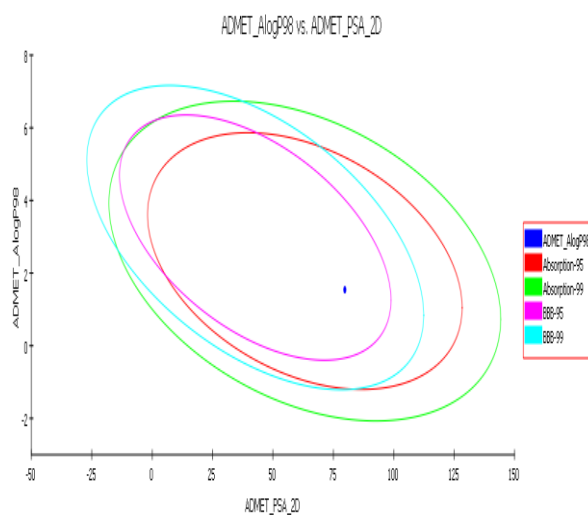


Figure 5. ADME analysis of the compound VMA

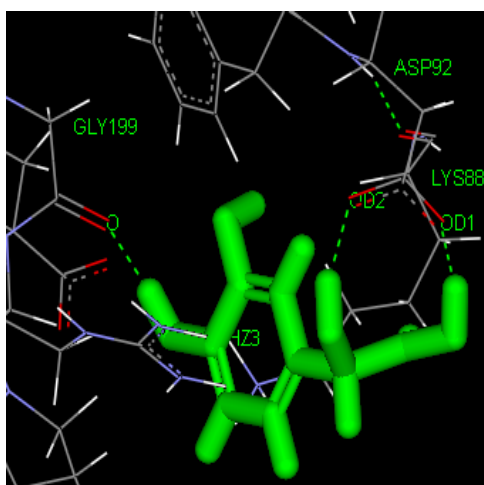


Figure. 6-A

Figure 6-A. Interaction between the target protein and the ligand.

The figure 6-B Surface area of the protein bound to the ligand.

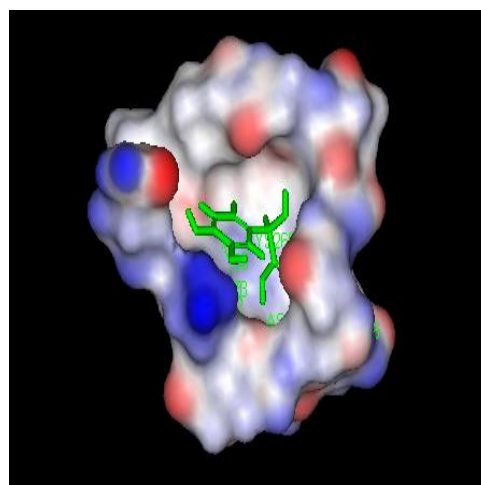


Figure. 6-B

It can be seen that the ligand satisfies all the parameters. On performing docking, seven binding sites were predicted for the target protein. In the present case, the results show the highest dock score of 48.287 at the binding site of the target protein. This site also holds 4 hydrogen bonding interactions. The interactions are found to be between O11 of the ligand and HZ3 of the residue lysine88 of the protein, H19, H16 and H14 of the ligand with O of Gly199, OD2 and OD1 of Asp 92 Figure 6-A and 6-B shows the interaction between the target protein and the ligand and the ligand positioned in the binding cavity of the protein.

CONCLUSION

The ligand has been synthesised with the substitution of alpha hydroxy acid in vanillin. The biological importance of the synthesised compound has been analysed by using computational approach. It has been found that the ligand could be a potential lead compound in the inhibition of

the CDK5 protein, which needs a verification from the other studies.

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