ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Identification of Human NMPrtase Inhibitors from *Adenocarpus mannii*; An *In-Silico* Approach

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Abstract:

Adenocarpus. mannii species belongs to family Fabaceae, well represented in south west region and west region of cameroon. Since, the cytotxic results of extracts and isolated compounds of *A. mannii* on human colon cancer cell lines are promising, it is decided to study their *in silico* binding studies towards the target protein involved. The present study focuses the *in-silico* docking studies of four flavonoids and one triterpene isolated from *Adenocarpus mannii* using Discovery Studio 4.1 Client. All the compounds exhibited a good interaction within the targeted domain compared to standards drugs. The compounds Isovitixin and chrysin 7-O-β-D-glucopyranoside show good docking properties with the highest activation energy of -1.14034 kcal/mol and -1.91612 kcal/mol respectively. Their physicochemical properties are also in accepatable range. These results suggest that the identified compounds can act as potential 'leads" to inhibit colon cancer cells.

Keywords: Color rectal cancer, docking, *A. mannii,* flavonoids, terpenoids

INTRODUCTION

Colorectal cancer or colon cancer is a malignant tumor arising from the inner wall of the large intestine. It is the third leading cause of cancer in males and fourth in females worldwide. Colorectal cancers are frequently encountered in hospital practice in Cameroon, where they represent 32% of digestive cancers [1]. Natural products are the most productive source for the discovery of new and novel compounds [2]. Despite the evolution of science, with the discovery of the Vinblastin is one of the alkaloids found in *Catharanthus roseus* and taxol isolated from *Taxus brevifolia* which exhibits cytotoxicity against several cancer cells [3] this disease remains uncontrolled. In developing countries, the cost of modern drug therapy is prohibitive and as such, many patients resort to traditional herbal medicine for the treatment since the medicinal plants have been used in the treatment of many diseases, such as cancer Thus researchers are embarking on the search for new biologically active molecules from medicinal plants.

Adenocarpus. mannii species belongs to family Fabaceae, well represented in the tropical regions of North Africa. Its native range includes Angola, Mozambique, Malawi, Guinea, South Africa, Tanzania, Burundi. In Cameroon, it is encountered in South West Region and West Region. *A. mannii* is a shrub of 5 to 6 m of height approximately, its leaves and branches are covered with soft hairs, the leaves are trifoliate and its flowers are yellow in the shape of a butterfly. The fruits of this plant are in the form of pods [4]. The Adenocarpus plants are used in traditional medicine for the treatment of several diseases: such as microbial infections, leprosy, scabies, acne, malaria and other plants species are used in agriculture as insecticidal properties. Phytochemical screening of this plant revealed the presence of starch, sugars, alkaloids, steroids, triterpenoniods and flavonoids [5]. The authors have investigated the cytotoxic effect of extracts of *A. mannii* on human colon cancer cell lines and the results are promising. The authors have also isolated a flavanoid namely 4',7-dihydroxy-5-methoxy isoflavone (**1**) from this plant and the work done by Ndjateu *et al*., 2014 have led to the isolation of other three flavonoids namely genistein (**2**), chrysin 7-O-β-D-glucopyranoside (**3**), isovitexin (**4**) and one triterpene oleanolic acid (**5**).

Figure 1. Aspect of leaf and branches of *A. mannii*

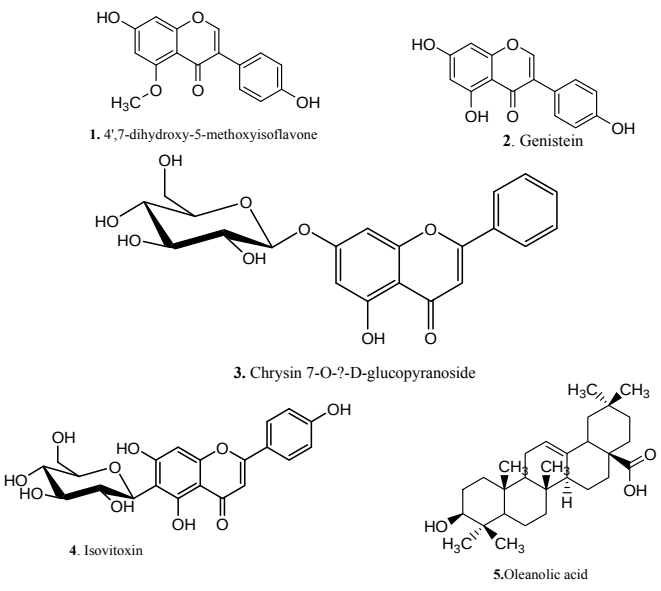


Figure 2. Selected compounds for *in-silico* docking

Nicotinamide Phosphor Ribosyl Transferase (NMPRTase) has a crucial role in the salvage pathway of NAD^+ biosynthesis. The potent inhibitors of NMPRTase can reduce cellular NAD^+ levels and induce apoptosis in tumors [6]. With the aim of further exploration of these plant and isolated compounds for anticancer potential, the present study is aimed to carry out *in-silico* docking of four flavonoids and one triterpene against human NMPRTase the protein that cause the colon cancer using Discovery Studio 4.1 Client.

MATERIALS AND METHODS

Preparation of Protein

The three dimensional crystal structure of Human NMPRTase (PDB id: 2E5D) in complex with the ligand nicotinamide was retrieved from the Protein Data Bank [7]. This protein has 475 residues. The complexes bound to the receptor molecule, all the hetero atoms and the non-essential water molecules were removed. Finally hydrogen atoms were merged to the target receptor molecule using Discovery Studio 4.1 Client. One missing segment found from SEQRES data: Missing segments containing non-standard residues or more than 20 residues and missing residues at N and C terminal are ignored. Loop1:10 residues missing at 2E5D: A: Lsy42, missing loop sequence: KTENSKLRKV. Loops added with insert amino Acid chain A: Lys43-A: Val52 (http://www.bmsc.washington.edu/CrystaLinks/man/pdb/part_35.html).

Preparation of Ligands

Totally 4 flavonoids and one triterpenoid which were isolated from the ethanol extract of *A. mannii* were identified for the study. The compounds were converted into their three dimensional structures and further used for docking studies.

Active site identification of human NMPRTase complexe

Human NMPRTase complexed with the selected compounds (2E5D) catalytic site predictions were analysed using Discovery Studio 4.1 Client. The following 67 residues were found to present in the ligand binding site. Leu172, Gly185, Leu186, Glu187, Tyr188, Lys189, Leu190, His191, Asp192, Phe192, Gly194, Try195, Arg196, His211, Leu212, Phe215, Lys215, Gly217, Thr218, Asp219, Thr220, Val221, Leu224, Gly239, Trr240,Ser241, Val242,Pro243, Ala244, Ala245, Glu246, Ile265, Phe269, Val272, Pro273, Val274, Ser275, Val276, Val277, Ser278, Arg302, Gln304, Ala306, Pro307, Leu308, Ile309, Ile310, Arg311, Pro312, Aps313, Arg349, Val350, Ile310, Arg311, Pro312, Asp313, Arg349, Val350, Ile351, Gln352, Gly353, Asp354, Val356, Leu361, Glu376, Asn377, Ile378, Ala379, Phe 380, Gly381, Ser382,Gly383, Leu386 shown in Figure 4.

Docking studies using Discovery Studio 4.1 Client

The docking analysis of Human NMPRTase complexed with the compounds was carried out by Discovery Studio 4.1 Client docking software. All the parameters used in Discovery Studio 4.1 Client docking were selected by default. Calculation type was set to dock mode and flexible mode was selected for the ligand. Grid resolution was set to 0.40 Å. Least energy indicated the easy binding character of ligand and receptor [8-9].

RESULTS AND DISCUSSION

Structure of the target protein

Human NMPRTase complexed (2E5D) has been exploited as a main therapeutic target for Colon Cancer. The three dimensional structure of Human NMPRTase complexed retrieved from the Protein Data Bank with PDB ID: 2E5D determined by X-Ray crystallography at a resolution of 2.0 (Å) was visualized in Discovery Studio 4.1 Client. 2E5D contains 475 acids and has been shown in the Figure 3.

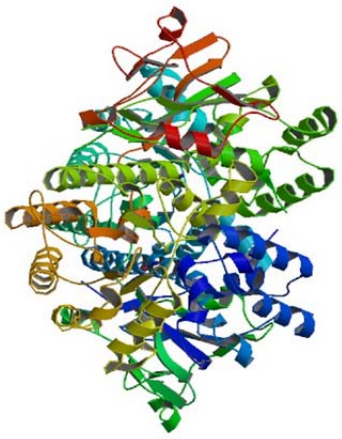


Figure 3: Three Dimensional Structure of Human NMPRTase complex (2E5D)

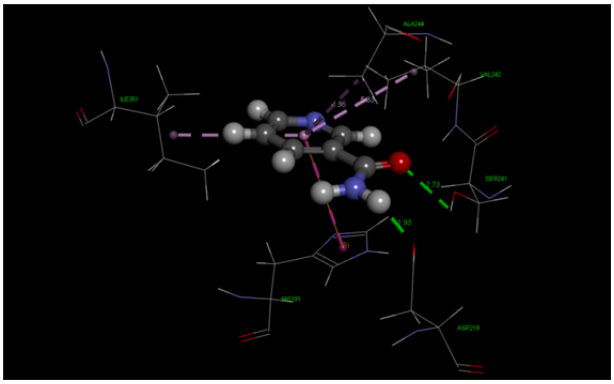


Figure 4. Active Binding Pocket using 67 residues of Human NMPRTase complex.
Predicted using Discovery Studio 4.1 Client

Docking analysis

The predicted 67 active residues were used as the catalytic sites for the five compounds used for docking studies. The results of the interaction between the active site residues of target Human NMPRTase complex and given in **Table 1**. By analyzing the docking interactions, Isovitoxin and chrysin 7-O-β-D-glucopyranoside were found to have the highest activation energy of -1.14034 kcal/mol (Figure 3) and -1.91612 kcal/mol (Figure 4) when compared with the standard drugs Capecitabine and 5-Fluorouracil which have respectively activation energy of -14.8538 kcal/mol (**Figure 8**) and 9.48069 kcal/mol (**Figure 7**). On the other hand Genistein and 4',7-dihydroxy-5-methoxyisoflavone had shown a weak activation energy in relation to Capecitabine (Table1), who shown wicked interaction with the site of the Human NMPRTase complexe . Chrysin 7-O-β-D-glucopyranoside having the high libdock

score (120.437) comparing with the standard drug Capecitabine (104.898) , 5-Fluorouracil (54.7846) and cocrystal (53.636). No refined poses found for ligand oléanolic acid. Fnally Isovitoxin and chrysin 7-O-β-D-glucopyranoside exhibits the best binding interaction with the Human NMPRTase complexed and futher it could be useful for identification and development of new preventive and therapeutic drug against colon cancer.

Table1. Docking results of Human NMPRTase complexed (2E5D)with 4 flavonoids and one triterpene isolated from *A.mannii*

Compound Name		LIBDOCK					CDOCKER	
	Libdock Score	Bond Type		Distance	Absolute Energy	Releative energy	CDOCKER_ Energy	CDOCKER Interaction Energy
Oléanolic acid	73.0463	-	-	-	-	-	No refined poses found for ligand	No refined poses found for ligand
4',7-dihydroxy-5-methoxyisoflavone	84.0086	(H…O) (H…O)	TRP156 -O27 GLN154 -O27	2,59669 2,90411	43,622	0	13.17	27.5664
Genistein	84.6061	(H…O) (H…O) (H…O)	H28 - SER199 H30 -GLN154 PRO158 -O20	2,65961 2,18278 2,50314	36,2669	0	17.6016	25.6413
Chrysin 7-O-β-D-glucopyranoside	120.437	(H…O)	H50 -GLN154	2,87068 2,92021	50,4692	2,37646	-1.91612	29.6198
Isovitoxin	78.1857	(H…O)	SER199- O11	2,52241	12,6846	34,0423	-1.14034	21.8577
Capecitabine	104.898	(H…O) (H…O) (H…O) (H…O)	TRP156- O5 H27 -SER199 PRO158- O7 H44 - GLY61	2,4682 2,60811 2,1185 2,99525	49,7196	6,34924	-14.8538	17.48
5-Fluorouracil	54.7846	(H…O)	GLN154 - O2	2,78634	6,94126	0	9.48069	16.9837
Nicotinamide	53.636	(H…O) (H…O)	SER241 - O8 NH15 - ASP219	2,73499 1,93276	22,3706	0,373029	13.1892	16.4567

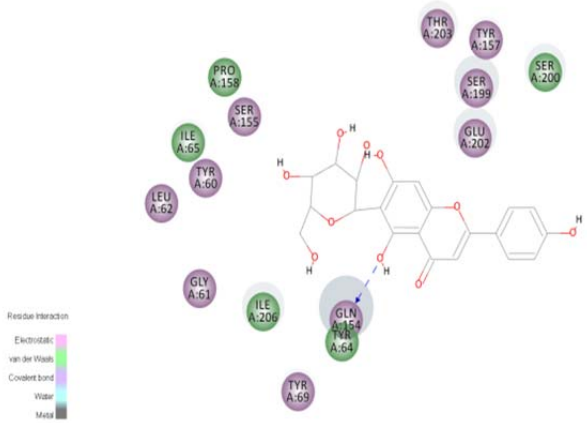


Figure 3: Docking 3D and 2D of Isovitoxin with Human NMPRTase complexed (2E5D)

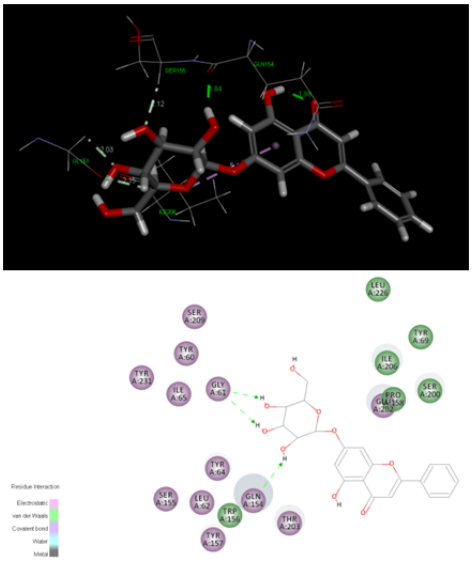


Figure 4: Docking 3D and 2D of chrysin 7-O-β-D-glucopyranoside with Human NMPRTase complexed (2E5D)

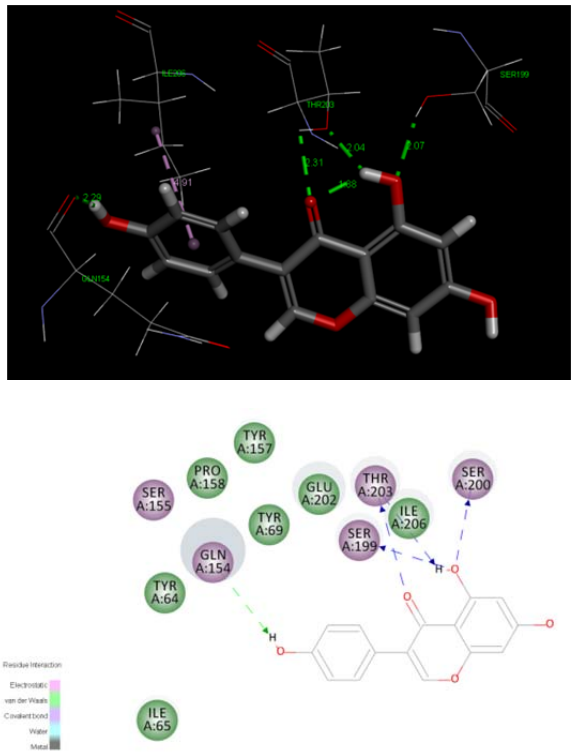


Figure 5: Docking 3D and 2D of Genistein with Human NMPRTase complexed (2E5D)

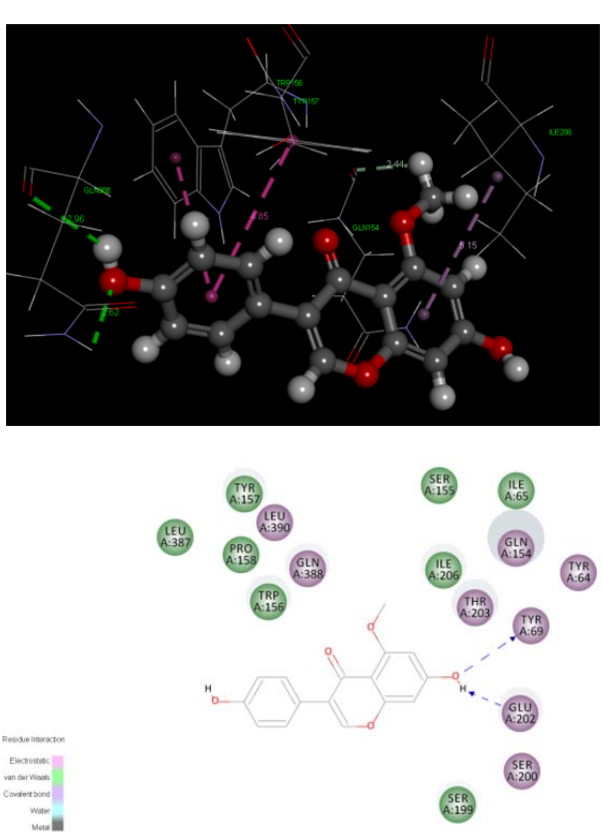
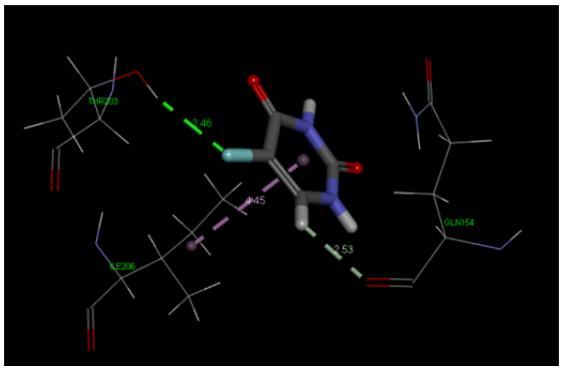


Figure 6: Docking of 4',7-dihydroxy-5-methoxyisoflavone with Human NMPRTase complexed (2E5D)



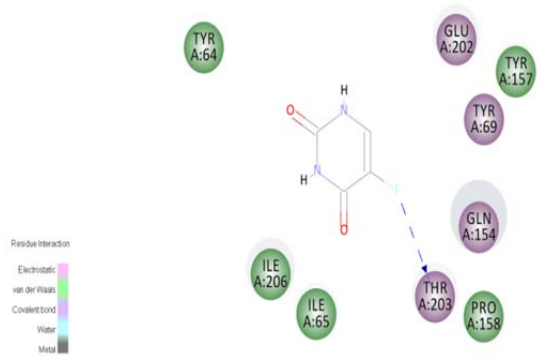
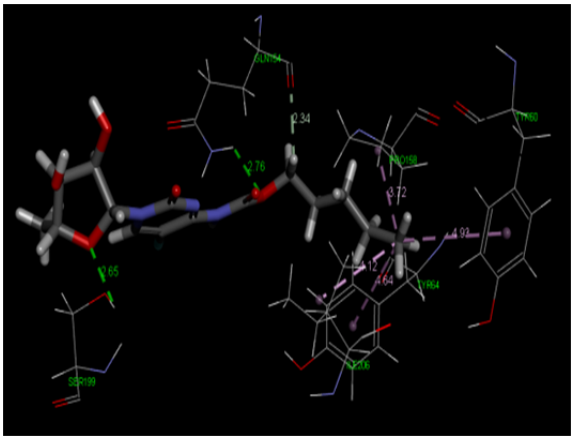


Figure 7: Docking of 5-Fluorouracil with Human NMPRTase complexed (2E5D)



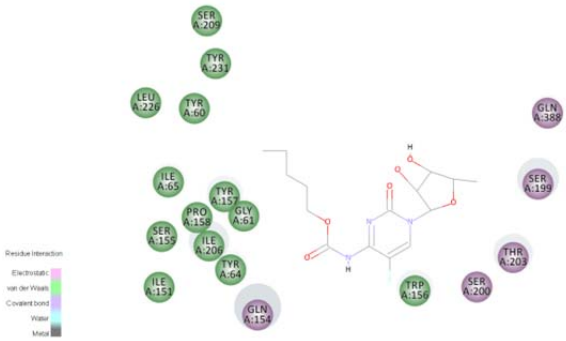


Figure 8: Docking of Capecitabine with Human NMPRTase complexed (2E5D)

Evaluation of drug likeness

Further the compounds were checked for Lipinski's Rule of five using Discovery Studio 4.1 Client. By evaluating the drug likeness we concluded that all the four anti colorectal cancers compounds have possessed through the Lipinski rule's of five criteria. Finally two compounds chrysin 7-O-β-D-glucopyranoside and Isovitoxin satisfy all the properties of pharmacological or biological properties with a best result when compared with known standards Capecitabine and 5-Fluorouracil.

ACKNOWLEDGMENTS

The authors are greatful to the University of Dschang, Cameroon, JSS College of Pharmacy, Ooty for learning and the research. One of the authors (BLAISE K. NGANOU) acknowledge the financial support by CICS, Chennai, India for providing INSA TATA JRD fellowship to carry out his research activities

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