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Discovery of Potent Drug Candidates of *Adhatoda vasica* Against Target Proteins IL- 4 and IL- 13 of Asthma – An *in Silico*

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Abstract

Ayurveda mentioned in various Samhita that *Adhatoda vasica* is used in the treatment of Asthma. However, its role at molecular level is yet unknown. In this study, attempt has been made on *in-Silico* molecular studies for discovery of potent drug candidates among compounds of *Adhatoda vasica* against the Target Proteins IL- 13 & IL- 4 of Asthma.Target Proteins were identified by a study on Asthma Pathway derived from KEGG Pathway Database and cross validated using Potential Drug Target Database. The 3D Structure files of identified target proteins were retrieved from RCSB-PDB Server. 12 Compounds of *Adhatoda vasica* have been retrieved from Knapsack Family Data Base and toxicity assessment using OSIRIS Property Explorer. 8 Out of 12 Compounds viz. Deoxyvasicinone, Peganine, Vasicinol, Vasicinone, Vasicinolone, Vasicol, Vasicoline,Vasicolinone passed the virtual screening phase. Finally molecular docking was carried out between Identified Target Proteins and 8 Screened ligands using Autodock Vina showed *vasicinol* binds with IL-4 at minimum binding affinity of -6.1 kcal/mol and *Vasicolinone* with IL-13 at minimum binding affinity -6.5kcal/mol can be considered as the potent drug candidates for Bronchial Asthma.

Keywords: Bronchial Asthma; *Adhatoda vasica*; In-Silico; IL- 13; IL- 4

Introduction

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that varies over time and in intensity, together with variable expiratory airflow limitation [1]. Bronchial Asthma is the 14th most important disorder in the world in terms of the extent and duration of disability. The most recent revised global estimate of asthma suggests that as many as 334 million people have asthma. 14% of the world's children experience asthma symptoms, 8.6% of young adults (aged 18-45) experience asthma symptoms [2].

Ayurveda the world's most ancient science of natural medicine mentioned the use of *Adhatoda vasica* in the treatment of Asthma. However, the role of *Adhatoda vasica* functions at molecular level is yet unknown. In this study, an *in-silico* molecular approach was followed to discover the potent drug candidates from the compounds of Adhatoda vasica against the target proteins IL-4 & IL-13 involve in Asthma. IL- 13 resembles IL-4 to the locus and at primary, secondary and tertiary structural levels [3-6]. Both are the major cytokine of the asthma phenotype (airway hyperreactivity, goblet cell hyperplasia, eosinophilic inflammation). IL-4 controls the development of T lymphocytes, especially Th2 cells, IL-13 functions during the effector phase of immunity, mediating the physiological response of the target organ to Th2- induced inflammation [7-9]. Interleukin-4 (IL-4) functions in asthma including induction of the IgE isotype switch, expression of vascular cell adhesion molecule-1 (VCAM-1), promotion of eosinophil transmigration across endothelium, mucus secretion, and differentiation of T helper type 2 lymphocytes leading to cytokine release [10]. It's a key cytokine in the development of allergic inflammation and associated with induction of the ε isotype switch and secretion of IgE by B lymphocytes [11]. IgE-mediated immune responses are further enhanced by IL-4 through its ability to upregulate IgE receptors on the cell surface: the low-affinity IgE receptor (FcεRII; CD23) on B lymphocytes and mononuclear phagocytic cells and the high-affinity IgE receptor (FcεRI) on mast cells and basophils [12]. IgE-dependent mast cell activation induced by IL-4 has a pivotal role in the development of immediate allergic reactions. It contributes to airway obstruction in asthma is through the induction of mucin gene expression and the hypersecretion of mucus [13]. IL-4 increases the expression of eotaxin and other inflammatory cytokines from fibro- blasts that might contribute to inflammation and lung remodelling in chronic asthma [14].

Materials and Methods

The materials used were databases and software. The databases used for the study includes KEGG for pathway analysis and identifying drug targets, KNApSAcK Family Database for use to search for metabolites based on an accurate mass, molecular formula, metabolite name or mass spectra in several ionization modes, RCSB-Research Collaboratory of Structural Bioinformatics (RCSB) for retrieving pdb format of identified drug targets [15-17]. The Software used for the study includes Marvin Sketch to visualized the 3D structure of the compound, converted .mol formats to .smiles,.sdf and .pdb format to visualize the compounds in OSIRIS property explorer, OSIRIS for Toxicity Risk Assessment, cLogP Prediction, Solubility Prediction, Molecular Weight, Drug-Likeness Prediction, Overall Drug-Score [18,19]. Finally docking studies were made by Auto Dock Vina [20].

To understand the molecular basis of occurrence of asthma, we have performed pathway study from data base KEGG to identify potent drug targets IL- 13 & IL- 4.

The Target Proteins of Asthma IL- 13 & IL- 4 were identified, cross validated using Potential Drug Target Database, and the 3D Structure files of identified target protein were retrieved from RCSB Server in the form of .pdb File (text) format (Figure 1). On the other hand Compounds of Adhatoda vasica- Anisotine, Deoxyvasicinone, Peganine, Vasicinol, Vasicinone, Adhatodine, Vasicinolone, Vasicoline, Vasicol, Vasicolinone, 4,2'-dihydroxychalcone 4-glucoside and Peganidine have been retrieved from Knapsack Family Data Base in the form of .mol format, they are converted into .smiles and .pdb format using Marvin Sketch. Toxicity assessment of compounds was done using OSIRIS Property Explorer.

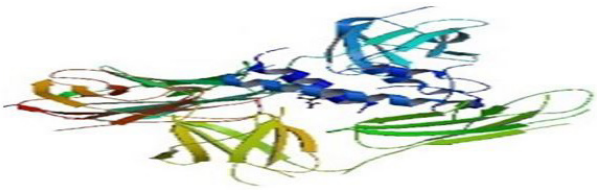
	
Protein Name:IL-4(PDBID:1BBN) [22] **Classification**: Cytokine **Structure Weight**: 15391.70 **Length**: 133 **Method**: SOLUTION NMR **Resolution[Å]:** > 1.8 **Molecular Function:** cytokine activity, cytokine receptor binding, interleukin-4 receptor binding, protein binding, growth factor activity	**Protein Name:**IL13(PDBID:3BPO) [23] **Classification:** Cytokine/cytokine Receptor **Structure Weight:** 74614.61 **Length:** 127 **Method:** X-RAY DIFFRACTION **Resolution[Å]:** 3.00 **Molecular Function:** cytokine receptor binding, cytokine receptor activity

Figure 1: Target Proteins of Asthma

Finally proteins were optimized for proteins ligands interaction studies by deleting all water molecules and optimized by minimization of energy by using AutoDock Vina. The grid parameter was set and the obtained structure was saved as .pdbqt. Ligands obtained from Knapsack Family Data Base in the form of .mol format converted into .pdb format using Marvin Sketch was optimized by using AutoDock Vina and saved in .pdbqt format.

To study the molecular descriptors of screened components of *Adhatoda vasica* were uploaded into online server, OSIRIS property explorer. This prediction process depends on comparison between pre-computed set of structural moieties whose properties are already known and the structural moieties of loaded molecules. Molecular descriptors like clogP, solubility, and drug score and side effects such as mutagenicity, tumorocity, irritant and reproductive effective were determined. To calculate the overall drug score, OSIRIS combined c logP, solubility, molecular weight, drug-likeness, drug score and toxicity risks into a single number to predict the molecule's over all drug potential. 8 out of 12 compounds are selected for molecular docking depending upon their drug score, toxicity risks, have high drug score and on the basis of drug-relevant properties – Lipinski's rule of 5.

In Docking Studies the 8 compounds selected based on experimental and insilico studies i.e. Vasicolinone, Vasicoline, Vasicol, Vasicinolone, Vasicinone, Vasicinol, Peganine, Deoxyvasicinone were docked with target proteins IL-4 and IL-13 using a genetic algorithm and simulated annealing approach to explore wide range of ligand conformational flexibility and rotational flexibility of Auto Dock. The protein ligand complex was analysed based on minimum binding affinity. Finally molecular docking was performed between Identified Target Proteins and the Screened ligands using Autodock Vina. The docked complexes were visualized in AutoDock Vina showing how the ligands interact with IL-4 and IL-13. We extended our study by going through an *In Silico* study on possible molecular level interaction of the best drug compound and target proteins IL-4 & IL-13 of Asthma [21].

Results of *In Silico* Studies

Phytochemicals of *Adhatoda vasica*: (Note:CN=Compound Name, CID=Compound Identification Number, MF = Molecular Formula, MW=Molecular Weight)

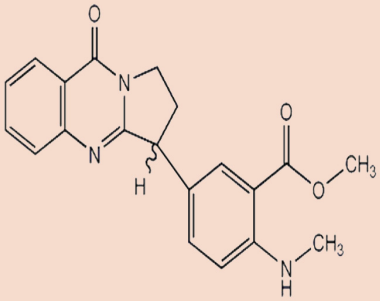
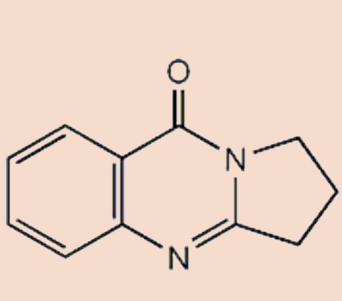
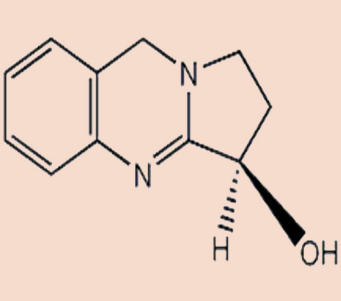
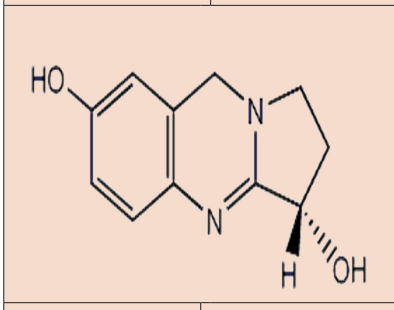
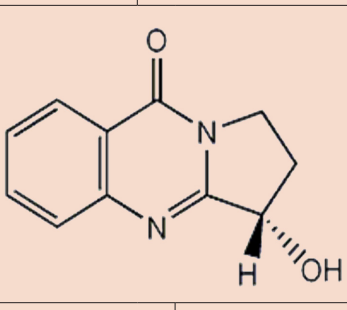
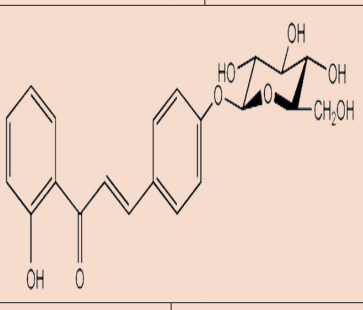
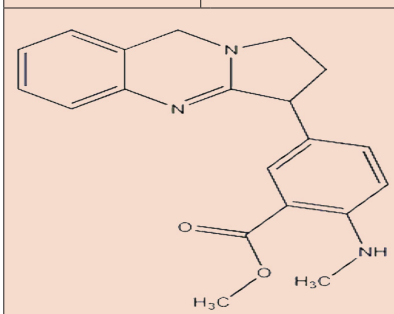
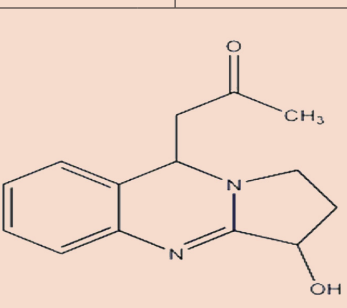
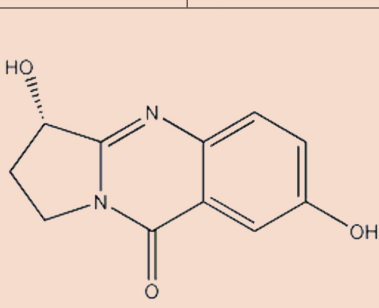
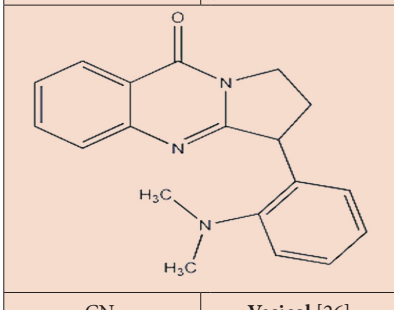
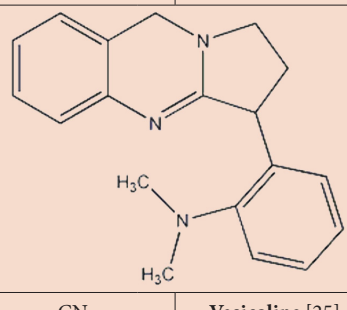
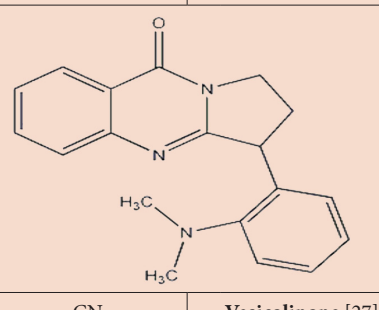
					
CN: CID: MF: MW:	**Anisotine** [24] C00002134 C20H19N3O3 349.1426415	CN: CID: MF: MW:	**Deoxyvasicinone** [24] CID:C00002150 C11H10N20 186.07931296	CN: CID: MF: MW:	**Peganine** [24] C00002191 C11H12N2O 188.09496302
					
CN: CID: MF: MW:	**Vasicinol** [24] C00002201 C11H12N202 204.08987764	CN: CID: MF: MW:	**Vasicinone** [24] C00002202 C11H10N202 202.07422758	CN: CID: MF: MW:	**4,2'dihydroxychalcone 4-glucoside** [28] C00007183 C21H22O8 402.13146768
					
CN: CID: MF: MW:	**Adhatodine** [25] C00026236 C20H21N3O2 335.16337694	CN: CID: MF: MW:	**Peganidine** [29] C00026249 C14H16N2O2 244.12117777	CN: CID: MF: MW:	**Vasicinolone** [25] C000026253 C11H10N203 218.0691422
					
CN: CID: MF: MW:	**Vasicol** [26] C000026254 C11H14N202 206.10552771	CN: CID: MF: MW:	**Vasicoline** [25] C000026255 C19H21N3 291.17354769	CN: CID: MF: MW:	**Vasicolinone** [27] C000026256 C19H19N30 305.15281225

Table 1: Phytochemicals of *Adhatoda vasica*

SL NO	LIGAND	MUTA-GENIC	TUMERO-GENIC	IRRITANT	REPRODUCTIVE EFFECT	ClogP	SOLU-BILITY	MOLECULAR WEIGHT	TPSA	DRUG LIKENESS	DRUG SCORE
1	**ANISOTINE**	No	Yes	No	No	2.55	-3.49	349.0	71.0	0.6	0.53
2	**DEOXY-VASICINONE**	No	No	No	No	1.25	-2.06	186.0	32.67	3.85	0.94
3	**PEGANINE**	No	No	No	No	0.22	-1.53	188.0	35.83	3.95	0.96
4	**VASICINOL**	No	No	No	No	-0.12	-1.24	204.0	56.06	3.41	0.96
5	**VASICINONE**	No	No	No	No	0.39	-1.66	202.0	52.9	4.61	0.96
6	**4,2'-DIHYDRO-XYCHALCONE 4-GLUCOSIDE**	No	No	No	No	0.62	-3.13	402.0	136.6	-4.95	0.41
7	**ADHATODINE**	No	Yes	No	No	2.38	-3.36	335.0	53.93	-0.02	0.5
8	**PEGANIDINE**	No	No	Yes	No	0.91	-1.79	244.0	52.9	2.51	0.55
9	**VASICINOLONE**	No	No	No	No	0.05	-1.36	218.00	73.13	4.39	0.96
10	**VASICOL**	No	No	No	No	-0.02	-1.48	206.0	66.56	2.57	0.93
11	**VASICOLINE**	No	No	No	No	2.72	-3.2	291.0	18.84	4.4	0.85
12	**VASICOLINONE**	No	No	No	No	2.89	-3.33	305.0	35.91	4.92	0.83

Note: The 4 ligands were screened out in the virtual screening phase ie. Anisotine for its tumerogenic effect; Paganidine for its irritant effect and 4, 2'-Dihydroxychalconoe 4-glucoside for its partial drug likeness and drug score

Table 2: Toxicity Assesment of Phytochemicals of Adhatoda Vasica

Sl No.	Ligands	Target Protein IL 4 with Binding Site	Binding Affinity (Kcal/mol)	Target Protein IL-13 with Binding Site	Binding Affinity (Kcal/mol)
1	DEOXYVASICINONE	A:ARG57:HH11 1	-4.7	B:LYSG7:HZ3 1	-5.1
2	PEGANINE	A:ARG57:HH22 1	-6.0	B:ASN137:HD22 1	-5.6
3	VASICINOL	A:ARG57:HH22 1	-6.1	B:TRP179:HE 1 1	-5.9
4	VASICINONE	A:ARG79:HH11 1, A:GLN82:HE22 1	-5.6	UNKO:H1	-6.1
5	VASICINOLONE	A:CYS7:HN1, A:ILE9:HN1	-6.0	B:GLN73:HN 1	-5.4
6	VASICOL	A:GLN12:HE21 1, A:LYS16:HZ21 1	-5.5	B:ARG175:HH21 1	-5.4
7	VASICOLINE	NILL	NILL	NILL	NILL
8	VASICOLINONE	UNKO:N 1	-6.3	A:ARG178:HE 1	-6.5

Table 3: Summarized table of the best binding affinity in the docked complex of target proteins and ligands

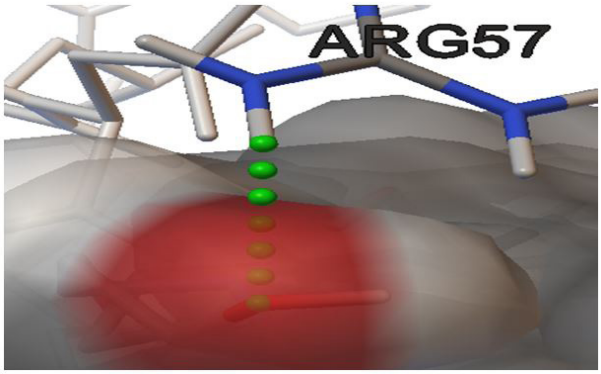


Figure 2(a): Interaction between ARG57 residue of IL-4 with Vasicinol (A:ARG57:HH22 1)

Figure 2(b): Interaction between ARG178 residue of IL-13 with Vasicolinone (A:ARG178:HE 1)

Figure 2: Visualization of potent ligand interaction with IL-4 & IL-13 in the docked complex

Discussion

Summarizing the entire study on molecular docking study all the 8 screened ligands out of 12 compounds of *Adhatoda vasica* (Table 1 and 2). i.e. Deoxyvasicinone, Peganine, Vasicinol, Vasicinone, Vasicinolone, Vasicol, Vasicoline, Vasicolinone have shown good binding affinity with the target proteins IL-4 & IL-13 of Asthma (Table 3). Based on binding energy and hydrogen bond formation, docking results were analyzed by using AutoDock tools and visualized through Auto docked. Among all the results the best binding affinity with its binding site in the docked complex of target proteins and ligands were selected to rule out the potent drug candidates. In this observation it is found that Vasicinol binds with IL-4 with ARG57 residue (A: ARG57: HH22 1) has minimum binding affinity of ΔG = -6.1 kcal/mol and Vasicolinone binds with IL-13 with ARG178 residue (A: ARG178: HE 1) has minimum binding affinity of ΔG -6.5 kcal/mol (Figure 2a and b). In this study Vasicolinone interact with IL-4 but the site of interaction is unknown with binding affinity of ΔG = -6.3 kcal/mol, so the ligand was not considered for best candidate amongst the compounds of *Adhatoda vasica*. Hence, Vasicinol and Vasicolinone are the potent drug candidates of *Adhatoda vasica* against target proteins IL-4 and IL-13 of Asthma.

The pathway study of cascade of Asthma and the present interaction study show the importance of *Adhatoda vasica* in molecular level with its probability mode of action againt Asthma. This study showed good results against target proteins IL-4 & IL-13 of Asthma. It might help in the further study to prevent induction of the IgE isotype switch and secretion of IgE by B lymphocytes as IgE leads to the mast cell activation and development of immediate allergic reactions, also might help in the prevention of airway obstruction, hypersecretion of mucus and lung remodelling in chronic asthma. This class of drug may represent the next generation of asthma therapy which need further scientific research.

Conclusion

The In Silico methods adopted in the present study helps to identify the ligands with target proteins which can be considered as the potent drug candidates for Bronchial Asthma for the further study in '*vitro*' and '*vivo*' reducing the time, cost in laboratory and subsequently before it enters the clinical trials.

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