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Original Research Article

Functional Compounds of *Lobelia inflata* Revealed Novel and Potential Targets for Chronic Cough Therapy

ABSTRACT

Aim: To identify the potential targets involved in chronic cough pathophysiology toward discovery and development of new therapeutants.

Methodology: This work was carried out on bioactive compounds of selected medicinal plants with reference to known therapeutic agents using combination of computational methods which include target prediction, pharmacokinetics prediction and molecular docking.

Results: It was observed that bioactive components of *Lobelia inflata* provides therapeutic potential on the targets which include vesicular monoamine transporter 2 (VMAT2), sodium dependent serotonin/dopamine receptor, while sulfamethoxazole and trimethoprim targeted endothelin receptors (ET_AR and ET_BR) and macrophage colony-stimulating factor 1 receptor (MCSF1R) respectively in addition to well-known dihydrofolate reductase. The binding energies from the docking analysis showed that all the functional compounds of *Lobelia inflata* studied possibly serves as antagonists of MCSF1R followed by dihydrofolate reductase, ET_BR and VMAT2. However, ambrisentan which is an antagonist of endothelin receptors was found to be the most effective against all the targets used in this study with minimum predicted binding energy of -11.6 kcal/mol.

Conclusion: This *in silico* study provides an insight to the mechanism of chronic cough pathophysiology and therapy by implication of potential targets, as well as possible utilization and optimization of functional compounds of *Lobelia inflata* as therapeutants.

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Keywords: chronic cough, *Lobelia inflata*, lobelanine, lobeline, ambrisentan, macrophage colony-stimulating factor-1 receptor, vesicular monoamine transporter 2, endothelin receptors, dihydrofolate reductase.

INTRODUCTION

Cough is a reflex response to microbial invasion or chemical irritants, which is mediated by sensory neurons in the airways which transmit through neurons present in the brainstem of the respiratory tract. Cough is recognized as a defense reflex mechanism, with three phases which include: an inspiratory phase; a forced expiratory effort against a closed glottis; and opening of the glottis with subsequent rapid expiration, that generates a characteristic cough sound [1]. Cough has been identified as one of the most frequent reasons for consultation with a general or respiratory physician, and the estimation of the community prevalence of cough was reported to be 2.3-18% of the adult population [2].

Cough has been divided into acute self-limiting cough which lasts for less than 3 weeks, and chronic persistent cough, which usually lasts for more than 8 weeks. Some types of cough can last for an intermediate period of 3–8 weeks, which is called subacute cough [1]. The reported percentage of global occurrence of cough are; Europe (12.7%), Oceania (18.1%), and America (11.0%) than in Asia (4.4%) and Africa (2.3%) [2]. Chronic cough is a feature of many common respiratory diseases (e.g., chronic obstructive pulmonary disease, asthma, and bronchiectasis) and of some common non-respiratory conditions (e.g., gastroesophageal reflux and rhinosinusitis), and it may be the presenting symptom of patients with some rarer conditions (e.g., idiopathic pulmonary fibrosis and eosinophilic bronchitis) [3].

Pharmacologically, Laryngeal and pulmonary receptors, such as rapidly adapting receptors (RARs), C-fibres, and slowly adapting fibres (SAR), and cough receptors provide input to the brainstem medullary central cough generator through the intermediary relay neurons in the nucleus tractus solitarius (NTS) [1]. P2X3 purinergic receptors are found mainly on peripheral sensory nerves, with some expression in the NTS of the brainstem. Studies has also revealed P2X3 receptor as a target for the management of chronic pain associated with central nervous system purinergic signaling, which include chronic cough [4]. Transient receptor potential ankyrin 1 (TRPA1) and transient receptor potential vanilloid 1 (TRPV1) are found on nerve

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terminals and are capable of initiating action potentials, and voltage-gated sodium channels (NaV) are responsible for action potential transmission [3].

Neuromodulators that acts centrally which include gabapentin, pregabalin, morphine, guaifenesin, codeine, dexbrompheniramine, dextromethorphan, amitriptyline, and baclofen, has been found to act on the heightened neural sensitization that is involved in the pathogenesis of cough [2]. The present drugs in the market are limited in their efficacy or limited in treatment of chronic cough due to significant side effect. Moreover, there is urgent need to discover and development new functional compounds that will meet the challenge posed by chronic cough in the twenty-first century. This can be possible by identify novel and potential targets that involved in pathophysiological mechanism of cough. Thus, the aim of this study was to identify the novel and potential targets involved in chronic cough pathophysiology toward discovery and development of therapeutants.

Ethnomedicinal studies has showed various medicinal plants that are used in the treatment of cough as shown in Table 1. Phytochemicals are bioactive chemical compounds naturally present in plants that provides human health benefits and have the potential of reducing the risk of major chronic diseases [5]. Dramatic improvement in drug discovery and development process has been recorded due to increasing knowledge of biomacromolecule and small molecules coupled with computational tools [6]. In this study, novel and potential targets involved in chronic cough were investigated using selected medicinal plants and standard reference drugs.

Table 1: Bioactive components of selected cough suppressant and antitussive medicinal plants.

Medicinal Plant	**Selected Functional Compounds**	**References**
Adiantum capillus-veneris	D-Carvone, Estragole, 3-Allyl-6- methoxyphenol, Tributyl acetylcitrate, 10,13-Dioxatricyclo-[7. 3.1.0(4,9)]-tridecan-5-ol-2-carboxylic acid	[7]
Ammi visnaga	Linalool, Bornyl acetate, thymol, Croweacin	[8]
	Visnagin, Visnadin, Khellin, bisabolol, nerol, 2,2-dimethylbutanoic acid	[9]
Marrubium persicum	Acetophenone, m-Tolualdehyde, β-Caryophyllene, Germacrene D	[10]
Equisetum arvense	1,8-Cineol, cis-Geranyl acetone, trans-β-Ionone, Caryophyllene oxide, Hexahydrofarnesyl acetone, trans-	[11]

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	Phytol	
Marrubium vulgare	γ-eudesmol, β-citronellol, citronellyl formate, Ledene, Geranyl tiglate	[12]
Abrus precatorius	Abrusoside A,C,D, abrussic acid, abruquinone A-C,E, abrectorin, abrine	[13]
Adhatoda vasica	vasicine, vasicol, vasinone	
Eucalyptus globules	1,8-Cineol, α-phellandrene, limonene, terpinen-4-ol, aromadendrene, epiglobulol, piperitone, globulol	
Lobelia inflata	Lobeline, isolobinine, lobelanidine, lobinaline, chelidonic acid.	
Verbascum Thapsus	Crocetin, hesperidin, ascorbic acid, coumarin, verbascoside	
Acorus calamus	Azulene, β-asarone, elemicin, eugenol, galangin	

MATERIALS AND METHODS

In silico preparation of ligands

Experimental bioactive compounds (ligands) were obtained from Gas Chromatography Mass Spectrometry or Liquid Chromatography Mass Spectrometry (GC-MS/LC-MS) data of the available medicinal plants which are known as cough suppressant or antitussive agent from ethnomedicinal studies (Table 1), were adapted from the literatures (cross-cultural). The chemical structures in sdf and canonical SMILES (Simplified Molecular Input Line Entry Specification) format were obtained from the PubChem Compound Database (http://www.ncbi.nlm.nih.gov/pccompound) of the National Center for Biotechnology Information (NCBI).

Pharmacokinetics Prediction

At this stage, library of ligands was then subjected to *in silico* ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity). To this end, the designed ligands were loaded into the *SwissADME* server (http://www.swissadme.ch) and ADME screening was performed at default parameters [14].

Protein Target Prediction

The identification of potential protein targets for the prepared ligands were carried out using the *SwissTargetPrediction* server (http://www.swisstargetprediction.ch). *Homo sapiens* was selected as target organism [15,16] and the results were compared with the list of known coughs target proteins. The target proteins that are novel were compiled for further analysis.

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Molecular Docking Studies

Protein preparation and identification of binding site residues

The available crystal structure of the identified novel targets was retrieved from the RCSB Protein Data Bank (PDB) (www.rcsb.org/pdb/home/home.do). All water molecules and hetero atoms were removed from the crystal structures by using PyMOL molecular graphic system, version 2.0.7 (www.pymol.org), while other targets with unavailable 3D structure were modeled separately using *SwissModel* (https://swissmodel.expasy.org) [17, 18]. The modeled structure with best quality was then used. The *3DLigandSite* (http://www.sbg.bio.ic.ac.uk/3dligandsite) [19], was used to predict the active site amino acid residues of the novel targets.

Ligands Preparation

Ligands with therapeutic potential for the novel targets were used for docking with reference to selected drug compounds in the market. The pose and binding energy of each ligand on novel targets for cough therapy were evaluated. All file conversions required for the docking study were performed using the open source chemical toolbox Open Babel version 2.4.1 [20]. All rotatable bonds present on the ligands were treated as non-rotatable to perform the rigid docking. The Gasteiger charge calculation method was used and partial charges were added to the ligand atoms prior to docking [21].

Grid box preparation and docking

Blind docking parameters (Table 2) were set using AutoDock Tools (ADT), a free graphic user interface of MGL software packages (version 1.5.6) [22]. The grid box was generated that was enough to capture the entire protein's catalytic site and accommodate all above mentioned ligands to move freely during docking. Other docking parameters were set to the software's default values.

121 Table 2: Grid box parameters selected for the target proteins.
122

Protein Targets	Coverage	Center grid box (points)	Size (points)	Spacing (Å)	Predicted binding site residues
VMAT2 Q05940 (PDB: Model)	A	585.725 x -47.120 x 201.492	120 x 120 x 120	0.375	Ile25, Val26, Phe27, Leu28, Ala29, Leu28, Ala29, Leu30, Leu31, Leu32, Tyr158, Pro159, Ile160, Pro161, Ile162, Phe163, Ala164, Gly165, Phe166, Cys167, Met169, Phe170, Leu255, Ala256, Leu258, Val259, Leu260, Asp262, Gly263, Gln266, Val269
	B	584.917 x -21.771 x 202.968	120 x 120 x 120	0.375	
Solute carrier family 22 member 6 Q4U2R8 (PDB: Model)	All	26.991 x -21.151 x 128.854	120 x 120 x 120	0.375	Cys341, Met344, Leu345, Ala348, Gly393, Arg394, Arg395, Pro396, Ala397, Gln398, Met399, Ala400, Ala401, Leu402, Leu403, Leu404, Ala405, Leu434, Phe442, Pro494, Val495, Ala496, Ser498, Ala499, Val502, Leu504, Pro505, Glu506
Endothelin B receptor P24530 (PDB: 5GLI)	A	-4.327 x -46.157 x -6.677	110 x 110 x 110	0.375	Ile85, Ser86, Pro87, Pro8, Thr244, Met245, Asp246, Tyr247, Lys248, Gly249, Tyr251
	B	-12.568 x -9.675 x -12.702	120 x 120 x 120	0.375	
Histone deacetylase 3 O15379 (PDB: 4A69)	All	42.007 x 54.565 x 23.700	120 x 120 x 120	0.375	Asp169, Ile170, His171, Asp258, Gly295, Tyr297
Dihydrofolate reductase P00374	All	14.948 x 24.077 x 16.919	110 x 110 x 110	0.375	Val8, Ala9, Ile16, Gly17, Lys18, Gly20, Asp21, Leu22, Trp24, Gly53, Lys54, Lys55, Ser59, Leu75, Ser76, Arg77, Glu78, Arg91,

(PDB: 1OHJ)					Ser92, Leu93, Val115, Gly116, Gly117, Ser118, Val119, Val120, Tyr121, Glu123, Thr146
MCSF1R Tyrosine kinase P07333 (PDB: 2I0V)	All	15.483 x 28.561 x -0.714	110 x 110 x 110	0.375	Ala614, Glu664, Tyr665, Cys666, Leu785, Asp796, Phe797, Gly798, Leu799, Ala800, Arg801

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The molecular docking program AutoDock Vina (version 1.1.2) [23], was employed to perform the docking experiment from the command line. After docking, the ligands were ranked according to their binding energy and close interactions of binding of the target with the ligands were analyzed through AutoDock Tools.

RESULT AND DISCUSSION

Sixty-five bioactive compounds were screened computationally and those that showed high probability of therapeutic potential toward targets relevant to chronic cough were selected as shown in Table 3. The most effective bioactive compounds which include isolobine, lobelanidine, lobelanine, and lobeline, were obtainable from *Lobelia inflata*. Moreover, analysis of sulfamethoxazole, a common drug used in the treatment of cough that originated from microbial infection, showed 80-90% probability on dihydrofolate reductase, endothelin-1 receptor and endothelin B receptor (ET_BR) while trimethoprim showed 80-90% probability on dihydrofolate reductase and 60-70% on macrophage colony-stimulating factor 1 receptor (MCSF1R). Thus, dihydrofolate reductase, endothelin B receptor (ET_BR) and macrophage colony-stimulating factor 1 receptor (MCSF1R) were included in the docking analysis to serve as reference for the phytochemicals. The abruquinone A from the plant *Abrus precatorius*, was the only bioactive that showed probability of target for endothelin receptors as shown in Table 3.

Study has shown the parameter of a drug-like compound to be $5 \leq$ lipophilicity $\geq 0 \leq$ hydrophilicity ≥ -5 [16] and may possibly violates not more than 1 of the Lipinski's rule [24]. The blood brain barrier permeability, lipophilicity (consensus LogP), gastrointestinal absorption and solubility reported in the Table 4, showed that cough therapeutants must have excellent ADME and central nervous system (CNS) properties. *Lobelia inflata*, a biannual or annual flowering plant grown primarily in Eastern North America, has lobeine as the principal alkaloid with higher percent found in the seed [25]. The extract of *Lobelia* has been used as respiratory stimulant for the treatment of asthma, expectorant, emetic, anti-spamodic, diuretic and muscle relaxant [26].

As shown in Table 3, the implicated targets for chronic cough therapy with at least 80-90% predictions were sodium-dependent noradrenaline transporter, sodium-dependent serotonin transporter, sodium-dependent dopamine transporter, muscarinic acetylcholine receptor M1-M5, synaptic vesicular amine transporter {SVAT) (also known as vesicular monoamine transporter

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(VMAT)),, chromaffin granule amine transporter (CGAT), sigma non-opioid intracellular receptor 1, gamma-aminobutyric acid receptor (complex), CHRNA7-FAM7A fusion protein, and neuronal acetylcholine receptor subunit alpha-7. Neurodegenerative disorder can occur from most of these targets due to mutation while in terms of the chronic cough, mutation is not the case but microbial infection and accumulation of inhaled toxic compounds. There are previous reports on possible involvement of most of the targets identified in this study in the pathophysiology of cough [27-30], which excluded from further exploration for novelty in this work.

As shown in Figure 1, the potent antagonists of noradrenaline transport are desipramine and nortriptyline, while specific inhibitor of serotonin transport include citalopram and paroxetine [27]. The antagonist of sigma non-opioid intracellular receptor is rimcazole while the inhibitors of muscarinic receptor include scopolamine, methotrimeprazine, and ethopropazine. The drug reserpine and tetrabenazine are the two principal agents that inhibit vesicular amine transport [31], while antagonists of endothelin receptor include ambrisentan, bosentan, and macitentan [32]. Also, the antagonist of MCSF1R are imatinib and sunitinib, while sulfamethoxazole and trimethoprim inhibit dihydrofolate reductase.

The VMAT2 (UniProt ID: Q05940) model was built on the template of Solute carrier family 2 facilitated glucose transporter member 1 (PDB: 4pyp.1.A) with sequence identity, sequence similarity, coverage, and global model quality estimation (GMQE) of 16.01%, 0.27, 0.74 and 0.40 respectively, while the model of Solute carrier family 22 member 6 (UniProt ID: Q4U2R8) used template of Solute carrier family 2, facilitated glucose transporter member 5 (PDB: 4ybq.1.A), with 26.41%, 0.33, 0.69 and 0.41 respectively. The best GMQE and coverage equals to 1. Due to the complex structure of VMAT2 and endothelin B receptor (ET_BR), the docking studies was done on two parts, of which both showed possible binding of the ligands (Table 2 and 5). The data in Table 5 showed that all the potent bioactive component of *Lobelia inflata*, have highest affinities for MCSF1R followed by dihydrofolate reductase, ET_BR and VMAT2. The more the negative is the binding free energy (ΔG), the better the affinity and spontaneous of the interaction.

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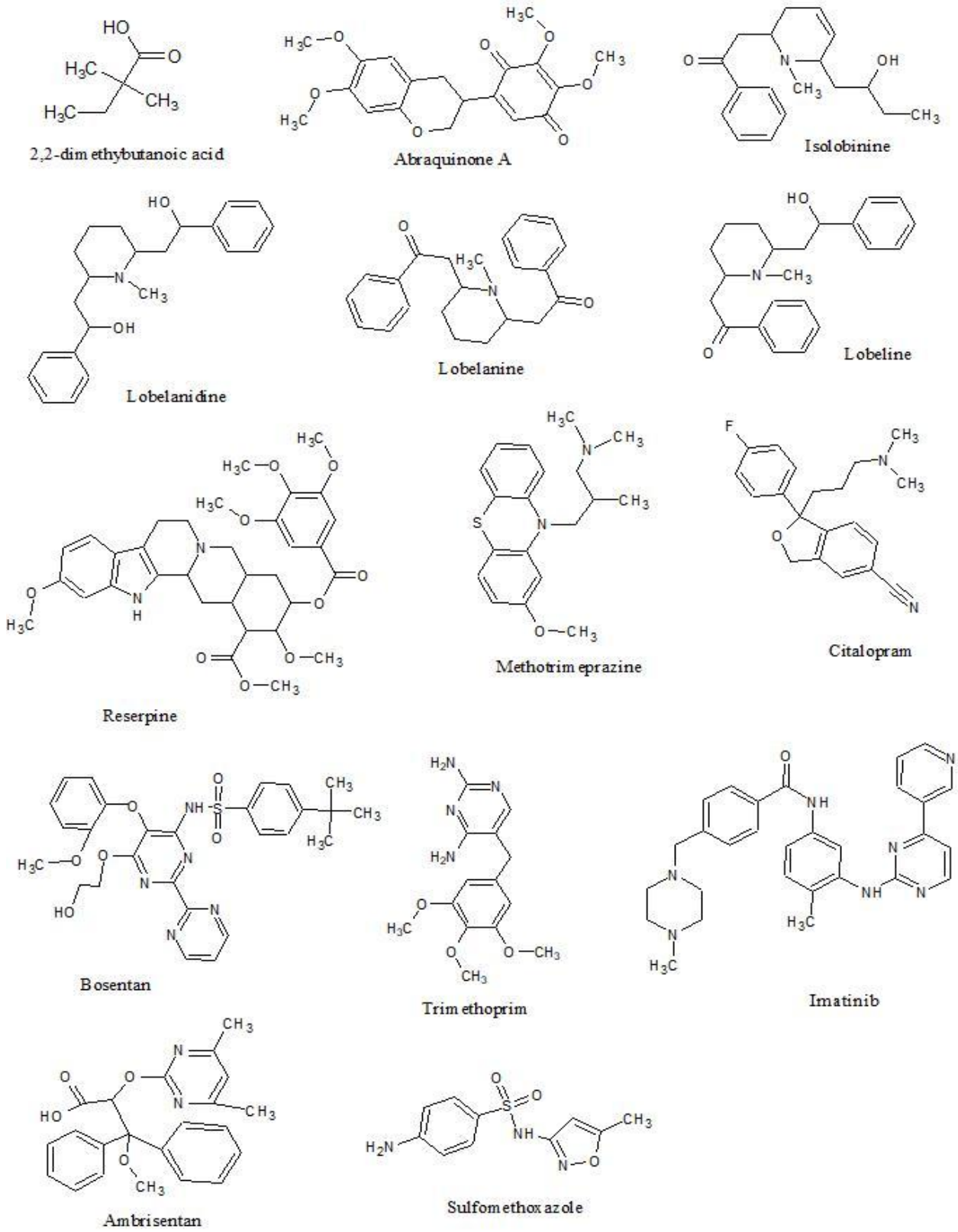

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184 Figure 1: Structure of potent bioactive and reference drug compounds

185 Table 3: Target prediction for selected bioactive compounds for chronic cough therapy.

TARGETS	UniProt ID	BIOACTIVE COMPOUNDS										
		1	2	3	4	5	6	7	8	9	10	11
Androgen receptor	P10275	****		***								
Cytochrome P450 19A1 (Aromatase)	P11511	****		****				**				
Sodium-dependent noradrenaline transporter	P23975	***	*	***	**	*	*****	**	****	****		
Sodium-dependent serotonin transporter	P31645	***		***	**	*	*****		****	****		
Sodium- and chloride-dependent glycine transporter 1, 2	P48067, Q9Y345	***		***	**				***			
Sodium-dependent dopamine transporter	Q01959	***	*	***	**	*	*****	**	****	****		
Sodium- and chloride-dependent neutral and basic amino acid transporter B(0+)	Q9UN76	***		***	**				***			
Muscarinic acetylcholine receptor M1, M2, M3, M4, M5	P11229, P08172, P20309, P08173, P08912	***	*	***			*****	**				
Steroid 17-alpha-hydroxylase/17,20 lyase	P05093			***				**				
Synaptic vesicular amine transporter	Q05940				***		*****			*****		
Chromaffin granule amine transporter	P54219				***		*****			*****		
Opioid receptor Mu-type, Delta-type, Kappa-type	P35372, P41143, P41145				**							
Sigma non-opioid intracellular receptor 1	Q99720				**		*****		***			
Sodium-dependent proline transporter	Q99884				**				***			
D(2) dopamine receptor	P14416				**				***	****		
Prostaglandin G/H synthase 1, 2	P23219, P35354					***						
Gamma-aminobutyric acid receptor (Complex)	P47870/P18507/P14867					**			***	*****		
CHRNA7-FAM7A fusion protein	Q494W8						****		***	*****		
Neuronal acetylcholine receptor subunit alpha-7	P36544						****		***	*****		

5-hydroxytryptamine receptor 1B, 2A, 2C	P28222, P28223, P28335								***	****		
FAD-linked sulfhydryl oxidase ALR	P55789								***			
Histamine H1 receptor	P35367									****		
Endothelin-1 receptor and Endothelin B receptor	P25101, P24530										*	
Cannabinoid receptor 1, 2	P21554, P34972										*	
Solute carrier family 22 member 6, 9, 10, 24, 25	Q4U2R8, Q8IVM8, Q63ZE4, Q8N4F4, Q6T423											***
Histone deacetylase 1, 2, 3	Q13547, Q92769, O15379											**

1: Bisabolol; 2: D-Limonene; 3: Gamma-Eudesmol; 4: Isolobinine; 5: Thymol; 6: Lobelanidine; 7: Terpinen-4-ol; 8: Lobelanine; 9: Lobeline, 10: Abruquinone A. 11: 2,2-Dimethylbutanoic acid * (40-50%), ** (50-60%), *** (60-70%), **** (70-80%), ***** (80-90%) Probability on Target. Probabilities have been computed based on a cross-validation. They may therefore not represent the actual probability of success for any new molecule.

Table 4: Pharmacokinetics prediction for selected bioactive compounds for chronic cough therapy.

Pharmacokinetics Parameter	**BIOACTIVE COMPOUNDS**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Molecular Weight	222.37	136.23	222.37	287.4	150.22	339.47	154.25	335.44	337.46	360.36	116.16
Molar Refractivity	72.36	47.12	70.46	90.09	48.01	106.18	48.80	104.70	105.44	91.54	32.47
Total Polar Surface Area	20.23	0	20.23	40.54	20.23	43.70	20.23	37.38	40.54	80.29	37.3
Consensus LogP	3.76	3.37	3.75	2.86	2.80	3.41	2.60	3.65	3.51	1.99	1.34

ESOL Class	Soluble	Soluble	Soluble	Soluble	Soluble	Moderately Soluble	Soluble	Moderately Soluble	Moderately Soluble	Soluble	Very Soluble
Gastrointestinal Absorption	High	Low	High	High	High	High	High	High	High	High	High
Blood Brain Barrier Permeant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Pgp substrate	No	No	No	No	No	Yes	No	No	No	No	No
Lipinski #violations	0	0	0	0	0	0	0	0	0	0	0
Bioavailability Score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.56	0.56
Synthetic Accessibility	3.95	3.46	3.88	3.94	1.0	3.36	3.28	2.86	3.23	3.94	1.0

1: Bisabolol; 2: D-Limonene; 3: Gamma-Eudesmol; 4: Isolobinine; 5: Thymol; 6: Lobelanidine; 7: Terpinen-4-ol; 8: Lobelanine; 9: Lobeline, 10: Abruquinone A. 11: 2,2-Dimethylbutanoic acid

195 Table 5: Predicted binding free energies (docking scores) and detailed interactions observed between the ligands and the target
196 enzyme.

Ligands	**Novel targets with predicted binding energy (kcal/mol)**							
	VMAT2 Q05940 (PDB: Model)		Solute carrier family 22 member 6 Q4U2R8 (PDB: Model)	Endothelin B receptor P24530 (PDB: 5GLI)		Histone deacetylase 3 O15379 (PDB: 4A69)	Dihydrofolate reductase P00374 (PDB: 1OHJ)	MCSF1R Tyrosine kinase domain P07333 (PDB: 2I0V)
	A	B	All	A	B	All	All	All
Isolobinine	-7.9	-7.3	-7.6	-8.0	-8.0	-7.8	-8.0	-9.2
Lobelanidine	-8.8	-8.9	-8.4	-9.3	-8.5	-8.1	-9.8	-10.0
Lobelanine	-9.2	-9.2	-8.7	-9.4	-9.4	-8.8	-9.9	-10.2
Lobeline	-9.2	-9.3	-8.7	-9.4	-9.5	-8.1	-9.7	-10.2
Abruquinone A	-8.8	-8.3	-7.9	-8.4	-8.2	-6.6	-7.3	-7.6
2,2-Dimethylbutanoic acid	-5.0	-4.8	-4.7	-5.5	-4.9	-5.2	-4.9	-5.3
Sulfamethoxazole	-7.9	-7.2	-9.6	-8.0	-8.4	-8.4	-8.1	-9.4
Trimethoprim	-7.2	-6.6	-6.4	-6.5	-7.2	-6.6	-5.9	-7.9
Reserpine	-10.0	-9.4	-10.2	-8.5	-8.4	-8.1	-8.4	-11.1
Citalopram	-9.7	-9.8	-9.0	-9.6	-9.5	-9.4	-9.7	-12.3
Ambrisentan	-12.9	-12.9	-12.7	-12.1	-11.8	-11.6	-13.0	-14.9
Methotrimeprazine	-8.9	-9.5	-7.9	-7.9	-8.5	-7.5	-7.7	-10.4
Imatinib	-11.0	-11.0	-10.8	-10.6	-9.6	-10.1	-9.0	-11.6

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The compound, 2,2-dimethylbutanoic acid that previously showed high probability for solute carrier family 22, has least binding energy for the same, and high affinity for ET_BR. Moreover, sulfamethoxazole and trimethoprim that has been acclaimed as inhibitor of dihydrofolate reductase, both showed better affinity for solute carrier family 22 member 6, histone deacetylase, MCSF receptor-tyrosine kinase and VMAT2 respectively. Also, reserpine and citalopram showed better affinity for MCSF1R than VMAT2 and dihydrofolate reductase, while ambrisentan, methotrimeprazine and imatinib showed highest affinity for MCSF1R, followed by VMAT2. Surprisingly, ambrisentan which is a high affinity endothelin-A receptor (ET_AR) antagonist with a high selectivity for ET_AR than ET_BR, showed highest binding energy for all the targets compared to the bioactive of medicinal plant and known drug compounds.

All the evidences of the study favored MCSF1R, followed by VMAT2 and endothelin receptors as possible targets in chronic cough therapy. These targets were involved in the signal transduction that may probably implicated cough reflex action. MCSF1R belongs to the type III protein tyrosine kinase receptor family, which has been implicated in the pathogenesis of inflammatory arthritis and cancer [33, 34]. The data shown in this study suggest that MCSF1R is suitable targets for treating chronic cough or other macrophage-induced inflammatory diseases. This corroborate the report by Dajani *et al.* [35], that MCSF was found elevated in sera of intrinsic asthmatics compared to normal control female human subjects.

A direct comparison showed that SVAT (VMAT) has a higher affinity than CGAT for monoamine substrates, particularly for histamine, and four classes of vesicular neurotransmitter transport activity were reported to include monoamines, acetylcholine, glutamate and γ-aminobutyric acid and glycine [36]. The VMAT exists in humans in two isoforms, VMAT1 and VMAT2, encoded by separate genes SLC18A1 and SLC18A2, respectively [37]. VMAT1 is located primarily in endocrine cells found in adrenal medulla chromaffin cells and absent in adult neuronal cells. Studies have shown that VMAT2 is located in neuronal cells of the CNS as well as in sympathetic adrenal chromaffin cells and neurons in the intestine and stomach [38, 39]. VMAT2 represents a vital protein in regulating all neuronal monoamine transmission because it is responsible for monoamine packaging into small synaptic vesicles. Lobeline interacts with VMAT2 to redistribute dopamine from vesicles, increasing cytosolic dopamine concentrations. Lobelane is a defunctionalized, saturated analog of lobeline [40] and study has shown that

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chemical defunctionalization of lobelane resulted in decreased water solubility compared to lobeline, but lobelane showed increased potency to inhibit dopamine uptake at VMAT2 compared to lobeline [41].

Endothelin receptors (ETRs) are part of the G-protein coupled receptor (GPCR) family possessing seven transmembrane domains [32]. The biology and pharmacology of ETRs are quite complex and remain incompletely understood, which include processes such as heart development, respiratory gaseous exchange, penile erection, blood pressure, smooth muscle contraction, response to hypoxia, calcium-mediated signaling etc. There is also evidence that the ET_AR and ET_BR can form functional heterodimers which may be of some pharmacological importance [42]. Endothelin is a potent pulmonary vasoconstrictor, and its binding to ET_AR and ET_BR cause vasoconstriction and vasodilation respectively. The combined blockade of both the ET_AR and the ET_BR leads to vasodilation of the human pulmonary arteries, vascular smooth muscle, and endothelia cells in the peripheral human lung with impart on influx of extracellular calcium [42-46].

The results of this study evidently set forth possible association of chronic cough and low libido or erectile dysfunction, due to reduction of testosterone by aromatase to form estrogen. The high level of estrogens in men contribute to the occurrence of prostate cancer, heart disease and gynecomastia. Bisabolol and gamma-eudesmol function as inhibitors of aromatase activity and bind to androgen receptor causing activation that lead to spermatogenesis and positive regulation of cellular proliferation and differentiation in target tissues. More importantly, the results suggested high ingestion of sodium and calcium during the period of cough may reduce the level of chronicity. Meyer-Lehnert et al [47] earlier reported that endothelin was responsible for modulating Na^+ transport mechanisms and that Na^+ fluxes into and out of the cell could contribute to Ca^{2+} homeostasis in vascular smooth muscle. The endothelin-induced Ca^{2+} influx has been found possible based on the dependence of the L-type Ca^{2+} channels opening on the initial influx of Na^+ which depolarize the membrane [46]. However, gabapentin and pregabalin which are existing therapeutants for cough, have been found as modulators of calcium channels in central and peripheral pathways [3].

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CONCLUSION

Knowledge of the molecular interactions of protein targets with array of functional compounds is practically useful for the understanding of mechanism of action relevant to pharmacological applications. This *in silico* study provides insight into the mechanism of chronic cough pathophysiology and therapy by implication of potential targets, as well as possible utilization and optimization of functional compounds of *Lobelia inflata* as therapeutants. Does MCSF1R fitted best as a target for cancer therapy or cough therapy? This will be explored by further research to guide key decision in drug discovery and development.

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