Journal of Advances in Medical and Pharmaceutical Sciences

19(2): 1-13, 2018; Article no.JAMPS.45276
ISSN: 2394-1111

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

Toluwase Hezekiah Fatoki[1], Olusola Olalekan Elekofehinti[2], Afolabi Clement Akinmoladun[3*] and David Morakinyo Sanni[1*]

[1]Enzymology Unit, Department of Biochemistry, Federal University of Technology, P.M.B. 704, Akure, Nigeria.
[2]Bioinformatics and Molecular Biology Unit, Department of Biochemistry, Federal University of Technology, P.M.B. 704, Akure, Nigeria.
[3]Biochemical Pharmacology and Toxicology Unit, Department of Biochemistry, Federal University of Technology, P.M.B. 704 Akure, Nigeria.

Author's contribution

This work was carried out in collaboration between all authors. Author THF designed this study, analyzed and prepared the draft of the manuscript. Authors OOE, ACA and DMS revised the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2018/45276
Editor(s):
(1) Dr. Sadaf Jamal Gilani, Department of Pharmaceutical Chemistry, The Glocal University, Saharanpur, U.P, India.
Reviewers:
(1) Ho Tat Bang, Ho Chi Minh City University of Medicine and Pharmacy, Viet Nam.
(2) A. O. Sijuade, Ekiti State University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/28144

Original Research Article

Received 09 September 2018
Accepted 27 November 2018
Published 05 January 2019

ABSTRACT

Aim: To identify 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 computational methods which include target prediction, pharmacokinetic prediction and molecular docking.
Results: Bioactive components of *Lobelia inflata* exhibited therapeutic potential on the targets which include vesicular monoamine transporter 2 (VMAT2) and sodium dependent serotonin/ dopamine receptor, while current drugs (sulfamethoxazole and trimethoprim) targeted endothelin receptors (ET_AR and ET_BR) and macrophage colony-stimulating factor 1 receptor (MCSF1R) respectively, in addition to the well-known dihydrofolate reductase. The binding energies from the docking analyses showed that all the functional compounds of *Lobelia inflata* studied have strong

**Corresponding author: E-mail: acakinmoladun@futa.edu.ng, dmsanni@futa.edu.ng;*

affinity for MCSF1R followed by dihydrofolate reductase, ET_BR and VMAT2. However, ambrisentan 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 proposing possible potential targets, as well as possible utilization and optimization of functional compounds of *Lobelia inflata* as therapeutants.

Keywords: Chronic cough; Lobelia inflata, ambrisentan; macrophage colony-stimulating factor-1 receptor; vesicular monoamine transporter 2; endothelin receptors; dihydrofolate reductase.

1. INTRODUCTION

Cough is a reflex response to microbial invasion or chemical irritation of the throat, mediated by the receptors and ion-channels in the airway of the larynx, through signal transduction involving nerves and fibers to the receptors and ion-channels in the brainstem of the central nervous system. Cough is recognized as a defense reflex mechanism, with three phases: an inspiratory phase; a forced expiratory effort against a closed glottis; and opening of the glottis with subsequent rapid expiration which 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 extent of prevalence in the community has been 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). Cough may be the presenting symptom of patients with some rarer conditions such as eosinophilic bronchitis and idiopathic pulmonary fibrosis [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

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	[5]
Ammi visnaga	linalool, bornyl acetate, thymol, croweacin	[6]
	visnagin, visnadin, khellin, bisabolol, nerol, 2,2-dimethylbutanoic acid	[7]
Marrubium persicum	acetophenone, m-tolualdehyde, β-caryophyllene, germacrene D	[8]
Equisetum arvense	1,8-cineol, cis-geranyl acetone, trans-β-Ionone, caryophyllene oxide, Hexahydrofarnesyl acetone, trans-Phytol	[9]
Marrubium vulgare	γ-eudesmol, β-citronellol, citronellyl formate, ledene, geranyl tiglate	[10]
Abrus precatorius	abrusoside A,C,D, abrussic acid, abruquinone A-C,E, abrectorin, abrine	[11]
Adhatoda vasica	vasicine, vasicol, vasinone	[11]
Eucalyptus globules	1,8-cineol, α-phellandrene, limonene, terpinen-4-ol, aromadendrene, epiglobulol, piperitone, globulol	[11]
Lobelia inflata	lobeline, isolobinine, lobelanidine, lobinaline, chelidonic acid.	[11]
Verbascum thapsus	crocetin, hesperidin, ascorbic acid, coumarin, verbascoside	[11]
Acorus calamus	azulene, β-asarone, elemicin, eugenol, galangin	[11]

**These bioactive compounds were selected based on the previous report and molecular weight. Duplication of bioactive compounds from these selected medicinal plants has been avoided.*

brainstem medullary central cough generator through the intermediary relay neurons in the nucleus tractus solitarius (NTS) [1]. Purinergic receptors (such as P2X3, P2X2 and P2X7) are found mainly on peripheral sensory nerves, with some expression in the NTS of the brainstem. Studies have 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 terminals and are capable of initiating action potentials, with voltage-gated sodium channels (NaV) responsible for action potential transmission [3].

Centrally acting neuromodulators such as gabapentin, pregabalin, morphine, guaifenesin, codeine, dexbrompheniramine, dextromethorphan, amitriptyline and baclofen, have been found to heightened neural sensitisation that is involved in the pathogenesis of cough [2]. The present drugs in the market are limited in their efficacy in treatment of chronic cough due to significant side effect. Moreover, there is urgent need to discover and develop new functional compounds that will meet the challenge posed by a chronic cough in the twenty-first century. This can be achieved through discovery of potential and novel targets implicated 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 have shown various medicinal plants used in the treatment of cough (Table 1). Phytochemicals are bioactive chemical compounds naturally present in plants that provide human health benefits and have the potential of reducing the risk of major chronic diseases [12]. Dramatic improvements in drug discovery and development process have been recorded due to increasing knowledge of biomacromolecule and small molecular weight compounds coupled with computational tools [13]. In this study, novel potential targets involved in chronic cough were investigated from array of medicinal plants and standard reference drugs.

2. MATERIALS AND METHODS

2.1 *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 used as cough suppressant or antitussive agent from ethnomedicinal studies (Table 1), were adapted from the literatures (cross-cultural). The reference ligands used were sulfamethoxazole, trimethoprim, reserpine, citalopram, ambrisentan, methotrimeprazine and imatinib. The chemical structures in structure data file (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).

2.2 Pharmacokinetics Prediction

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

2.3 Protein Target Prediction

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

2.4 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 structures in PDB were modeled separately using Swiss Model (https://swissmodel.expasy.org) [17,18]. The modeled structure with best quality was then used. The VMAT2 (UniProt ID: Q05940) model was built on the template of solute carrier family 2 facilitated glucose transporter member 1 (PDB:

Table 2. Grid box parameters selected for the target proteins

Target Protein	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 (PDB: 1OHJ)	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, 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

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. The *3D LigandSite* (http://www.sbg.bio.ic.ac.uk/3dligandsite) [19], was used to predict the active site amino acid residues of the novel targets.

2.5 Ligands 3D 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].

2.6 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 to capture the entire catalytic site of each target protein and accommodate each of the ligand to move freely during docking. Other docking parameters were set to the software's default values. 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. Due to the complex structure of VMAT2 and endothelin B receptor (ET_BR), the docking studies of both proteins were done on two parts.

3. RESULTS 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 probability on target of 80-90% on dihydrofolate reductase, endothelin-1 receptor and endothelin B receptor (ET_BR) while trimethoprim showed probability on target of 80-90% 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 compound with high binding potential for the endothelin receptors (Table 3).

Studies have 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 one 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 with 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 (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. This study corroborate previous reports on the involvement of the

identified targets in the pathophysiology of cough [27-30], while the less explored and novel potential targets were further considered in this study.

As shown in Fig. 1, citalopram is a specific inhibitor of serotonin transport [27], methotrimeprazine is an inhibitors of muscarinic receptor and reserpine inhibits vesicular amine transport [31]. The antagonists of endothelin receptor include ambrisentan and bosentan [32], imatinib is an antagonist of MCSF1R while sulfamethoxazole and trimethoprim inhibit dihydrofolate reductase. 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 increasing negativity of the binding free energy (ΔG), defines better affinity and spontaneity of the interaction.

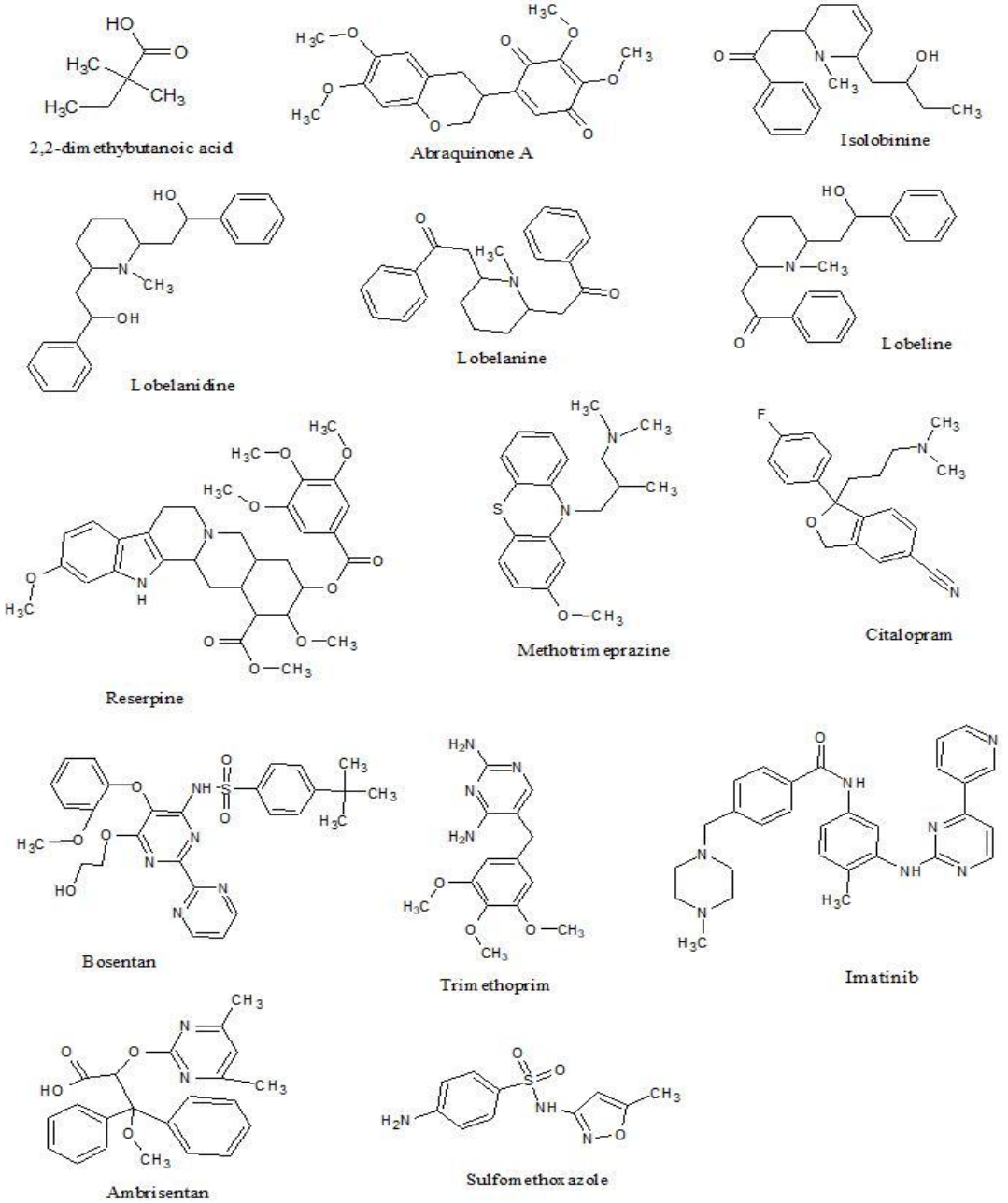

Fig. 1. Structure of potent bioactive compounds and reference drug compounds

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

Table 5. Predicted binding free energies (docking scores) observed between the ligands and the target proteins

Ligands	Novel potential targets with predicted binding energies (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

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 target proteins when compared to the bioactive compounds and other standard drug compounds used in this study (Table 5).

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 implicate cough reflex action. MCSF1R belongs to the type III protein tyrosine kinase receptor family and it has been implicated in the pathological development of inflammatory arthritis and cancer [33, 34]. The data shown in this study suggest that MCSF1R is a suitable target 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 defunctionalised, saturated analog of lobeline [40] and study has shown that chemical defunctionalisation 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 lead to vasodilation of the human pulmonary arteries, vascular smooth muscle and endothelia cells in the peripheral human lung with effect 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 depolarise 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].

4. 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 utilisation and optimisation of functional compounds of *Lobelia inflata* as therapeutants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chung KF, Pavord ID. Prevalence, pathogenesis and causes of chronic cough. Lancet. 2008;371:1364–74.
2. Gibson PG, Vertigan AE. Management of chronic refractory cough. BMJ. 2015;351:h5590.
   DOI: 10.1136/bmj.h5590
3. Smith JA, Woodcock A. Chronic cough. New England Journal of Medicine. 2016;375:1544-51.
4. Ford AP. In pursuit of P2X3 antagonists: Novel therapeutics for chronic pain and afferent sensitization. Purinergic Signalling. 2012;8(Suppl1):S3–S26.
   DOI: 10.1007/s11302-011-9271-6
5. Hussein HM, Hameed IH, Ibraheem OA. Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of Adiantum Capillus-Veneris using GC-MS and FTIR Spectroscopy. International Journal of Pharmacognosy and Phyto-chemical Research. 2016;8(3):369-385.
6. Khalfallah A, Labeb A, Sembra Z, Kaki A, Kabouche A, Touzani R, Kabouche Z. Antibacterial activity and chemical composition of the essential oil of *Ammi visnaga* L. (Apiaceae) from constantine, Algeria. International Journal of Medicinal and Aromatic Plants. 2011;1(3):302-305.
7. Hashim S, Jan A, Marwat KB, Khan MA. Phytochemistry and medicinal properties of *Ammi visnaga* (Apiacae). Pakistan Journal of Botany. 2014;46(3):861-867.
8. Hamedeyazdan S, Fathiazad F, Asnaashari S. Chemical composition of the essential oil from *Marrubium persicum* C. A. Mey. (Lamiaceae). Pharmaceutical Sciences. 2013;19(2):35-38.
9. Radulović N, Stojanović G, Palić R. Composition and antimicrobial activity of *Equisetum arvense* L. essential oil. Phytotherapy Research. 2006;20:85–88.
10. Kadri A, Zarai Z, Bekir A, Gharsallah N, Damak M, Gdoura R. Chemical composition and antioxidant activity of *Marrubium vulgare* L. essential oil from Tunisia. African Journal of Biotechnology. 2011;10(19):3908-3914.
11. Sultana S, Khan A, Safhi MM, Alhazmi HA. Cough suppressant herbal drugs: A review. International Journal of Pharmaceutical Science Invention. 2016; 5(5):15-28.
12. Liu RH. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. Journal of Nutrition. 2004;134:3479–3485.
13. Sanni DM, Fatoki TH. Computational evaluation of pharmacokinetics and potential protein targets of ginger (*Zingiber officinale*); 2017.
14. Antoine D, Michielin O, Zoete V. Swiss ADME: A free web tool to evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules. Scientific Reports. 2017;7: 42717.
    DOI: 10.1038/srep42717
15. Gfeller D, Michielin O, Zoete V. Shaping the interaction landscape of bioactive molecules. Bioinformatics. 2013;29:3073-3079.
16. Sanni DM, Fatoki TH, Kolawole AO, Akinmoladun AC. Xeronine structure and function: Comparative mastery of its mystery. In silico Pharmacology. 2017;5:8.
    DOI: 10.1007/s40203-017-0028-y
17. Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T, Kiefer F,

Cassarino TG, Bertoni M, Bordoli L, Schwede T. SWISS-MODEL: Modelling protein tertiary and quaternary structure using evolutionary information. Nucleic Acids Research. 2014;42:W252-W258.

18. Remmert M, Biegert A, Hauser A, Söding J. HHblits: lightning-fast iterative protein sequence searching by HMM-HMM alignment. Nature Methods. 2012;9:173-175.
19. Wass MN, Kelley LA, Sternberg MJ. 3D Ligand Site: Predicting ligand-binding sites using similar structures. Nucleic Acids Research. 2010;38:W469-73.
20. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open babel: An open chemical toolbox. Journal of Cheminformatics. 2001;3:33.
21. Gasteiger J, Marsili M. Iterative partial equalization of orbital electronegativity - a rapid access to atomic charges. Tetrahedron. 1980;36(22):3219–3228.
22. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. Auto Dock 4 and Auto Dock Tools 4: automated docking with selective receptor flexibility. Journal of Computational Chemistry. 2009;30(16):2785–2791.
23. Trott O, Olson AJ. Auto dock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry. 2010;31(2):455–461.
24. Lipinski CA, Lombardo F, Dominy V, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews. 2001;46:3–26.
25. Felpin F, Lebreton J. History, chemistry and biology of alkaloids from *Lobelia inflata*. Tetrahedron. 2004;60:10127-10153.
26. Dwoskin LP, Crooks PA. A novel mechanism of action and potential use for lobeline as a treatment for psychostimulant abuse. Biochemical Pharmacology. 2002; 63:89-98.
27. Pacholczyk T, Blakely RD, Amara SG. Expression cloning of a cocaine and antidepressant-sensitive human noradrenaline transporter. Nature. 1991; 350:350-353.
28. Costello RW, Jacoby DB, Fryer AD. Pulmonary neuronal M2 muscarinic receptor function in asthma and animal models of hyperreactivity. Thorax. 1998; 53:613–618.
29. Haga K, Kruse AC, Asada H, Yurugi-Kobayashi T, Shiroishi M, Zhang C, Weis WI, Okada T, Kobilka BK, Haga T, Kobayashi T. Structure of the human M2 muscarinic acetylcholine receptor bound to an antagonist. Nature. 2012;482(7386): 547–551.
30. Wess J, Eglen RM, Gautam D. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. Nature Review Drug Discovery. 2007;6: 721–733.
31. Schuldiner S, Liu Y, Edwards RH. Reserpine binding to a vesicular amine transporter expressed in Chinese Hamster ovary fibroblast. The Journal of Biological Chemistry. 1993;268(1):29-34.
32. Dupuis J, Hoeper MM. Endothelin receptor antagonists in pulmonary arterial hypertension. European Respiratory Journal. 2008;31:407-415.
33. Bischof RJ, Zafiropoulos D, Hamilton JA, Campbell IK. Exacerbation of acute inflammatory arthritis by the colony-stimulating factors CSF-1 and granulocyte macrophage (GM)-CSF: Evidence of macrophage infiltration and local proliferation. Clinical and Experimental Immunology. 2000;119(2):361–367.
34. Cannarile MA, Weisser M, Jacob W, Jegg A, Ries CH, Ruttinger D. Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. Journal for Immuno Therapy of Cancer. 2017;5:53.
DOI: 10.1186/s40425-017-0257-y
35. Dajani R, Ali EA, Dajani B, (2011). Macrophage colony stimulating factor and monocyte chemoattractant protein 2 are elevated in intrinsic asthmatics. Cytokine, 56: 641-647.
36. Peter D, Jimenez J, Liu Y, Kim J, Edward RH. The chromaffin granule and synaptic vesicle amine transporters differ in substrate recognition and sensitivity to inhibitors. The Journal of Biological Chemistry. 1994;269(10):7231-7237.
37. Eiden LE, Weihe E. VMAT2: A dynamic regulator of brain monoaminergic neuronal function interacting with drug of abuse. Annals of the New York Academic of Sciences. 2011;1216:86-98.
38. Erickson JD, Schafer MKH, Bonner TI, Eiden LE, Weihe E. Distinct pharmacological properties and distribution in

neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. Proceedings of National Academy of Science. 1996;93: 5166-5171.
39. Peter D, Liu Y, Sternini C, de Giorgio R, Brecha N, Edwards RH. Differential expression of two vesicular monoamine transporters. Journal of Neuroscience. 1995;15:6179-6188.
40. Zheng G, Dwoskin LP, Deaciuc AG, Crooks PA. Defunctionalized lobeline analogues: Structure-activity of novel ligands for the vesicular monoamine transporter. Journal of Medicinal Chemistry. 2005;48:5551-5560.
41. Nickell JR, Krishnamurthy S, Norrholm S, Deaciuc AG, Siripurapu KB, Zheng G, Crooks PA, Dwoskin LP. Lobelane inhibits methamphetamine evoked dopamine release via inhibition of the vesicular monoamine transporter-2. Journal of Pharmacology and Experimental Therapeutics. 2010;332:612- 621.
42. Sauvageau S, Thorin E, Caron A, Dupuis J. Evaluation of endothelin-1-induced pulmonary vasoconstriction following myocardial infarction. Experimental Biology and Medicine (Maywood). 2006;231:840–846.
43. Sauvageau S, Thorin E, Caron A, Dupuis J. Endothelin-1- induced pulmonary vasore activity is regulated by ET_A and ET_B receptor interactions. Journal of Vascular Research. 2007;44:375–381.
44. Knott PG, D'Aprile AC, Henry PJ, Hay DW, Goldie RG. Receptors for endothelin-1 in asthmatic human peripheral lung. British Journal of Pharmacology. 1995;114:1–3.
45. Goldie RG, D'Aprile AC, Self GJ, Rigby PJ, Henry PJ. The distribution and density of receptor subtypes for endothelin-1 in peripheral lung of the rat, guinea-pig and pig. British Journal of Pharmacology. 1996; 117:729–735.
46. Pollock DM, Keith TL, Highsmith RF. Endothelin receptors and calcium signaling. FASEB Journals. 1995;9:1196-1204.
47. Meyer-Lehnert H, Wanning C, Predel HC, Backer A, Stelkens H, Kramer HJ. Effects of endothelin on sodium transport mechanisms: Potential role in cellular calcium mobilization. Biochemical and Biophysical Research Communication. 1989;163:458-465.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/28144