

IN-SILICO ANALYSIS OF PHYTOCOMPOUNDS FOR INHIBITION OF NICOTINE ACETYL- COA RECEPTOR (NACHR) MEDIATED LUNG CANCER

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ABSTRACT

Objective: Lung cancer is mostly found in cigarette smokers. Nicotine Acetyl-CoA receptor (nAChR) is found to be responsible for this cancerous activity. When cigarette containing nicotine binds with this receptor of lung cells the carcinogenic activity is initiated. Hence, there is a possibility for the prevention of lung cancer formation when the nicotine is competitively inhibited by an interfering molecule preferably from natural products. This will add additional support in the management of lung cancer. This study was planned to identify the natural compounds which have more affinity to the nicotine Acetyl-CoA receptor (nAChR) than the nicotine by *in silico* evaluation using Auto dock software.

Methods: About six compounds were subjected and proved with preclinical studies were obtained from a literature survey and were computed for docking and characterization.

Results: Results showed that Taxol had minimum energy level of -11.54 kcal/mol to the nicotine Acetyl-CoA receptor (nAChR) compared to the nicotine energy level of -7.01. Other molecules are having the activity in the following order Combretastatins < Camptothecin < Liriodenine < Curcumin and Podophyllotoxin < Taxol.

Conclusion: It is concluded that these data may be useful for the preparation of different combinations and formulations for the management of lung cancer.

Keywords: Anti-cancer activity, Docking, Ligands, Lung cancer, Nicotine Acetyl-CoA receptor (nAChR) and Tubulin.

INTRODUCTION

Lung cancer is referred as bronchiogenic cancer or bronchiogenic carcinoma. There are two major forms of lung cancer, small cell lung cancer and non-small cell lung cancer. Both of these cancers arise from the epithelial cells that line the airways of the lungs.

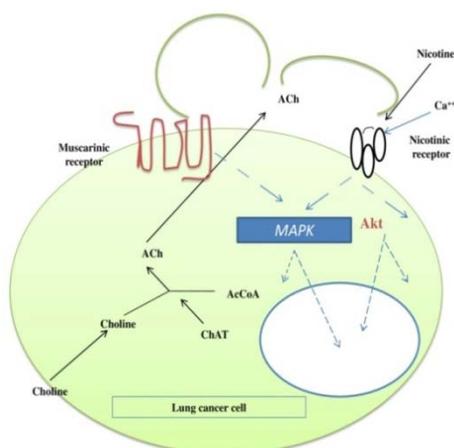


Fig. 1: Model of cholinergic signaling in SCC. Exogenous nicotine activates MAPK and an Akt proliferative pathway through nicotinic cholinergic receptors. Endogenous ACh similarly activates both MAPK and Akt pathways through both muscarinic and nicotinic receptors. Thus, muscarinic antagonists have potential to block proliferation induced both by exogenous nicotine and endogenous ACh as well as antagonizing the up-regulation in cholinergic signaling induced by nicotine (Pingfang et al. 2008).

Among these two types non-small lung cell cancer is mostly found in cigarette smokers [1, 2]. Based on this fact a study was planned with some plant based molecule like Combretastatins, Camptothecin, Liriodenine, Curcumin, Podophyllotoxin and Taxol for anticancer activity screening against nicotine Acetyl-CoA receptor (nAChR) responsible cancer induction [3,4]. The list of molecules selected for the study and their properties related to the aim of this study was chosen by literature review and listed here.

Combretastatin A-4 (CA-4) analogues are biaryls connected by an ethylene bridge and have excellent antitumoral and antivascular activities, hence it has attracted considerable interest among medicinal chemists [5]. The microtubule destabilising agents, combretastatin-A-4 (CA-4) led to microtubular array disorganization, arrest in mitosis and abnormal metaphases, accompanied by the presence of numerous centrosome-independent star-like structures containing tubulin and aggregates of pericentrosomal matrix components like γ -tubulin, pericentrin and ninein, whereas the structural integrity of centrioles was not affected by treatment [6].

Camptothecin is a cytotoxic alkaloid made up of a pentacyclic ring structure which contains a pyrrole (3, 4 β) quinoline moiety possessing strong anticancer activity and it is isolated mainly from the bark and stem of Chinese ornamental tree, *Camptotheca acuminata*. These analogs work by inhibiting DNA topoisomerase I which play a major role in various DNA functions like replication and transcription [7].

Liriodenine is an apomorphine alkaloid belongs to H-Benzo (G)-1,3-benzodioxolo (6,5,4-dequinolin-8-one) class of isoquinoline alkaloid of aporphine subgroup, it can intercalate into the neighboring base pairs of DNA double helix, to which its significant antitumor activity can be typically attributed. Therefore, it also catalytically inhibits topoisomerase to block DNA synthesis. Moreover, it also catalytically inhibits topoisomerase to block DNA synthesis and increase p53 and iNOS expression to induce cell cycle G1 arrest [8].

Curcumin (diferuloylmethane) is a polyphenolic compound isolated from the Indian plant spice, *Curcuma longa*. It finds its application as potent anti-cancer compounds. The anti-carcinogenic effects induced by curcumin in cancer cells are mediated via the modulation of multiple oncogenic signaling transduction elements [9]. Potential mechanisms of anti-carcinogenic effects induced by curcumin in cancer cells include the down-regulation of the epidermal growth factor receptor (EGFR) family members (EGFR/erbB1 and erbB2/HER2) insulin-like growth factor type-1 receptor (IGF-1R), sonic hedgehog (SHH/GLIs) and Wnt/b-catenin and their downstream signaling effectors [10].

Podophyllotoxin is derived from the roots of *Podophyllum* species, to be specific, *Podophyllum peltatum* Linnaeus and *Podophyllum emodi* Wallich. It is a naturally occurring lignan with important antineoplastic and antiviral properties [11]. A TOP-53 derivative of podophyllotoxin exhibited twice the inhibitory activity of eto-poside (VI-16) against topoisomerase II and induced DNA strand breaks but showed no inhibitory activity against tubulin polymerization. Taxol is a natural product with significant antitumor activity, has been approved for the treatment of breast, ovarian, and lung carcinomas. It exhibits marked anti-tumour activity against a broad range of rodent tumors [12, 13].

The major cellular target for Taxol is the tubulin / microtubule system of the cancer cell. Taxol has a specific binding site on the microtubule and incubation of cells with Taxol causes the formation of stable bundles of microtubules that disrupt the normal polymerization/depolymerization cycle of microtubules and suppresses microtubule dynamics [14].

Lung cancer is mostly found in cigarette smokers. Nicotine Acetyl-CoA receptor (nAChR) is found to be responsible for this cancerous activity. When cigarette containing nicotine binds with this receptor of lung cells the carcinogenic activity is initiated [15, 16, 17]. Hence, there is a possibility for the prevention of lung cancer formation when the nicotine is competitively inhibited by an interfering molecule preferably from natural products. Herbal medicines usually act on many targets because of their multiple interaction sites [Figure 1: Ping fang Song et al., 2008]. Bioinformatics-based methodologies offer alternative approaches and have the potential to cut costs in identifying drug targets [18, 19]. In this point of view, *In silico* screening of anticancer activity for the above identified molecules with nicotine Acetyl-CoA receptor (nAChR) was performed [20, 21].

MATERIALS AND METHODS

Materials

Plant derivatives and analogs were selected for the present study. Ligands selected for *In silico* docking studies are combretastatins, liriiodenine, camptothecin, podophyllotoxin, curcumin and Taxol. The sources used for data base collection include Drugbank, PubChem and RCSB protein data bank (PDB). The chemical structures from the collected data sources are drawn using chemsketch software (10.1) Fig. 2 (2a, 2b, 2c, 2d, 2e and 2f) and Auto Dock 4.2.5 is used for effective protein-ligand docking.

Protein preparation and optimization

The crystal structure of nicotine Acetyl-CoA receptor (nAChR) used in this study was retrieved from RCSB protein data bank (<http://www.rcsb.org/pdb>).

Ligand preparation and optimization

Using Chemskech Software the structures of the drugs and analogs were sketched and generated into MOL File which results in subsequent generation of their 3-D structures. These structures are viewed using web lab viewer tool which is a molecule format converter in PDB. After Ligands preparation optimization was carried out using Auto Dock 4.2.5 [10].

Docking stimulation

The docking analysis of receptor and their analogs with nicotine Acetyl-CoA receptor (nAChR) were carried out by Auto Dock docking software 4.2.5.

RESULT AND DISCUSSION

The docking of nicotine Acetyl-CoA receptor (nAChR) and the conventional drug Combretastatins, Camptothecin, Liriiodenine, Curcumin, Podophyllotoxin, Taxol and reference drug Nicotine were given in (Figure 3 (a,b,c,d,e and f) and 3A) and Table 1.

Table 1: Represents the binding site of the six different ligands with single protein.

Ligands	Binding site
combretastatins	Gln 208 N - H - O 2.7 A
Camptothecin	N - H - O Val 1023 = 3.4 A Arg 1031 NH2 - H - O = 3.0 A Arg 1031 NH1 - H - O = 2.4 A
Curcumin	Arg 977 NH2 - H - O = 2.7 A Arg 977 NE - H - O = 2.7 A
Liriiodenine	No Interactions
podophyllotoxins	Ser 424 O - H - O = 3.2 A Ser 424 CB - H - O = 3.3 A Thr 281 N - H - O = 2.9 A
Taxol	Asp 696 OD2 - H - H = 1.9 A

The Protein-ligand interaction plays a significant role in structure based drug designing. Structure of ligands were drawn by Chemskech and docked with the active site of receptor protein one by one. Docking scores obtained from Auto Dock 4.2.5 for nicotine Acetyl-CoA receptor (nAChR) and with various ligands (Combretastatins, Camptothecin, Liriiodenine, Curcumin, Podophyllotoxin and Taxol) selected from the *in vitro* experimental evidences indicating possibility for anti-cancer activity (Scores: ΔG kcal/mol) were -6.06, -9.85, -10.96 -8.78, -9.36 and -11.54 kcal/mol respectively. The reference drug Nicotine showed binding energy (ΔG) -7.01 kcal/mol Table 2.

Table 2: Represents energy value of all phytocompounds. The result positive and negative indicate the possibility for the anticancer activity of compounds. These are arrived based on the relativity observed from the free energy values between phytocompounds and reference drug nicotine.

Protein	Free energy value [kcal/mol]	Result
Nicotine	-7.01	Ref Drug
Taxol	-11.54	Positive
Liriiodenine	-10.96	Positive
Camptothecin	-9.85	Positive
Podophyllotoxin	-9.36	Positive
Curcumin	-8.78	Positive
Combretastatins	-6.06	Negative

Nicotine has the higher energy than Taxol. Hence, Taxol could block the activity of nicotine and prevent nicotine inducible cancer. Study shows that combretastatins is a ligand which has higher ΔG (-6.06 kcal/mol) than the nicotine as well as other ligands that denotes that it is not suitable therapeutically purpose.

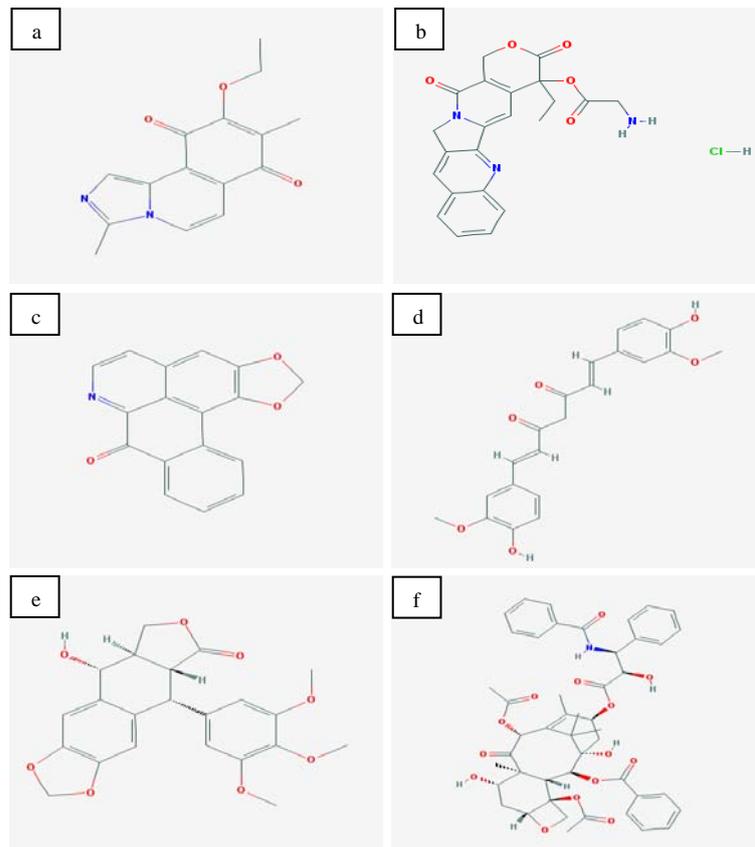


Fig. 2: (a) Combretastatins (b) Camptothecin (c) Liriodenine (d) Curcumin (e) Podophyllotoxin (f) Taxol

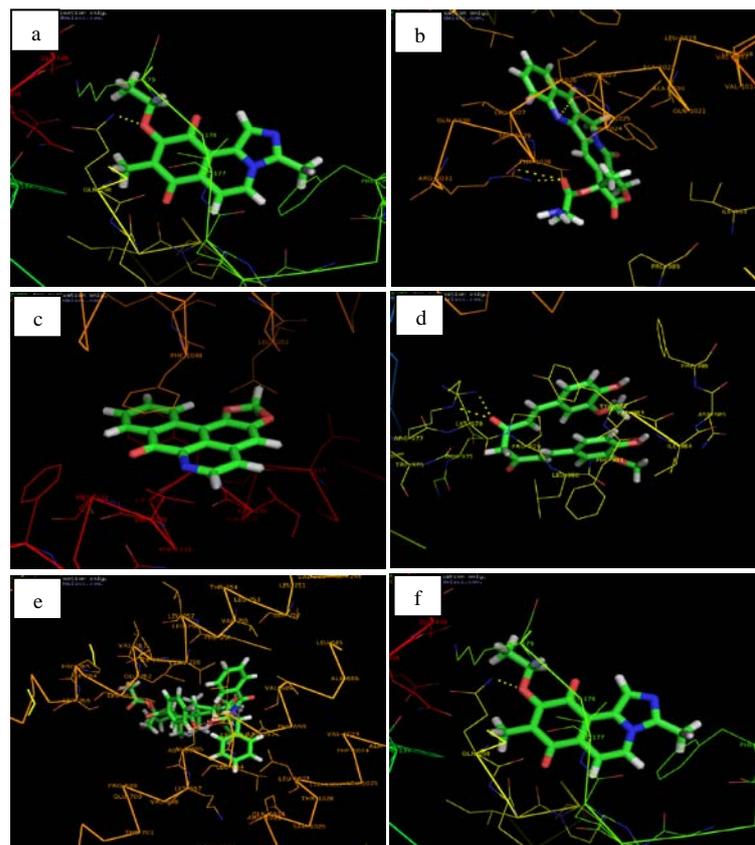


Fig. 3: (a) Combretastatins (b) Camptothecin (c) Liriodenine (d) Curcumin (e) Podophyllotoxin and (f) Taxol

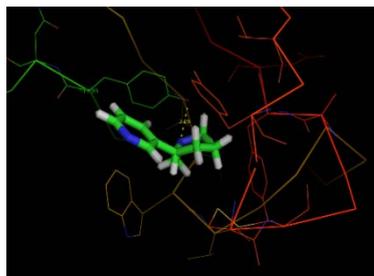


Fig. 3A: (Reference drug Nicotine)

CONCLUSION

This study concludes that Taxol has very low binding energy than the other ligands as well as with reference drug (Nicotine). Hence it may be a best choice for lung cancer treatment. Other drugs selected for this study having lesser binding energy than the Nicotine may also be considered based on other factors like solubility, toxicity, possibility for synthesis and so on.

CONFLICT OF INTERESTS

Declared None

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