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Research Article

Phytochemical and in silico study on Lupinus subcarnosus Hook, its effect on neuronal $\alpha_4\beta_2$ nicotinic acetylcholine receptors (nAChRs) and the major alkaloids

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ABSTRACT

Neuronal nicotinic acetylcholine receptors, including α_4 and β_2 subunits, are the amplest nicotinic acetylcholine receptors present in the brain. Nicotine is a component of tobacco smoke that exerts its psychoactive impacts *via* binding to nicotinic acetylcholine receptors (nAChRs). Thus, targeting $\alpha_4\beta_2$ of the nAChRs is emerging as a promising tool implemented for smoking cessation. We isolated twelve (**1**-**12**) alkaloids from 75% EtOH extract of L. subcarnosus herb for the first time. The structures of the isolated alkaloids were established by spectroscopic methods, by comparis on with authentic samples. Based on the structural similarities between the smoking cessation compounds cytisine and varenicline, the isolated alkaloids (**1**-**12**) were docked into the $\alpha_4\beta_2$ nAChR active site. Compounds **9** and **11** revealed reasonable binding energy and interacted with the key amino acids Cys199, Cys200, and Trp156 within $\alpha_4\beta_2$ nAChR active site. The *in silico* ADME prediction toxicity and mutagenicity of lupin alkaloids were studied as well.

1. Introduction

Nicotine is the main addictive element in tobacco smoke; it exerts its activity through the interaction with nicotinic acetylcholine receptors (nAChRs) [1-3]. To a large extent, the reinforcing effects of nicotine result from the direct activation of neuronal $\alpha_4\beta_2$ nAChRs, which triggers downstream events such as increased dopamine release in the mesolimbic system. Therefore, the $\alpha_4\beta_2$ nAChR subtype has become a key target for developing therapeutic agents for smoking cessation [4, 5]. High-affinity partial agonists for $\alpha_4\beta_2$ nAChRs, such as cytisine, and varenicline, are molecules of interest because they exhibit the unique property of acting as a mixed agonist/antagonist [6]. High affinity varenicline binding competes with nicotine at the $\alpha_4\beta_2$ nAChR and, thereby, antagonizes the reward sensation of smoking. However, varenicline activates α4β2 nAChRs with low efficacy and desensitizes, leaving channels unopened even at high binding occupancy, thereby minimizing withdrawal symptoms and increasing the success rate of smoking cessation attempts [7, 8]. Lupinus subcarnosus Hook, family Leguminosae (Fabaceae), is native to Texas state (subcarnosus, texensis, perennis, concinnus, plattensis and havardii). Collectively, bluebonnets and the Texas legislature are considered the six species flowers as official state ornamental flowers. In the present study, twelve lupin-type quinolizidine alkaloids were isolated from the plant's herb for the first time (**Fig. 1**). The structural similarities of the isolated alkaloids with cytisine and varenicline prompted us to evaluate the in silico binding properties within α4β2 nAChRs active site. The in silico pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules was studied as a fast-predictive tool in drug discovery which reduces the fraction of pharmacokinetics-related failure in the clinical phases drastically [9].

2. Results and discussion

2.1. The phytochemical study

From the 75% EtOH extract of *L. subcarnosus* herb, twelve (**1-12**) were isolated for the first time from the plant. The alkaloids were isolated by repeated silica gel column chromatography and identified by spectroscopic methods (IR, ^{13}CNMR, DEPT, ^{1}HNMR, and mass spectroscopy), co-chromatography with authentic samples, mp, and optical activities (details in the supplementary data). Compound (**1**) colorless oil $[\alpha]_D^{25}$ + 82°, it showed $[M]^+$ at m/z 248. Compound (**2**) showed $[\alpha]_D^{25}$ +19.2°. The mass spectrum of **2** exhibited the molecular ion peak at m/z 248. Compound (**3**) was obtained as yellowish needles $[\alpha]_{D^{25}}$ 138°. The mass spectrum showed the molecular ion peak at m/z 262. Compound (**4**) was isolated as a colorless oil $[\alpha]_{D^{25}}$ +38° and exhibited the molecular ion peak at m/z 246. Compound (**5**) was obtained as yellowish oil $[\alpha]_{D^{25}}$ -56.0°. The MS showed the molecular ion peak at m/z 246. Compound (**6**) was isolated as yellow oil, $[\alpha]_D^{25}$ +52°, and the molecular ion peak at m/z 248. Compound (**7**), oil, $[\alpha]_D^{25}$ -291°. The EIMS showed the M^+ at m/z 246 and the base peak at 134. Compound (**8**), $[\alpha]_D^{25}$ 44° (C= 0.1, MeOH) and the M^+ at m/z 264.

Compound (**9**), $[\alpha]_D^{25}$ -94.4° (c= 0.015, CH_2C1_2), EIMS **m**/z (rel. int. %) showed the M^+ at m/z 244 and it is also the base peak. Compound (**10**)

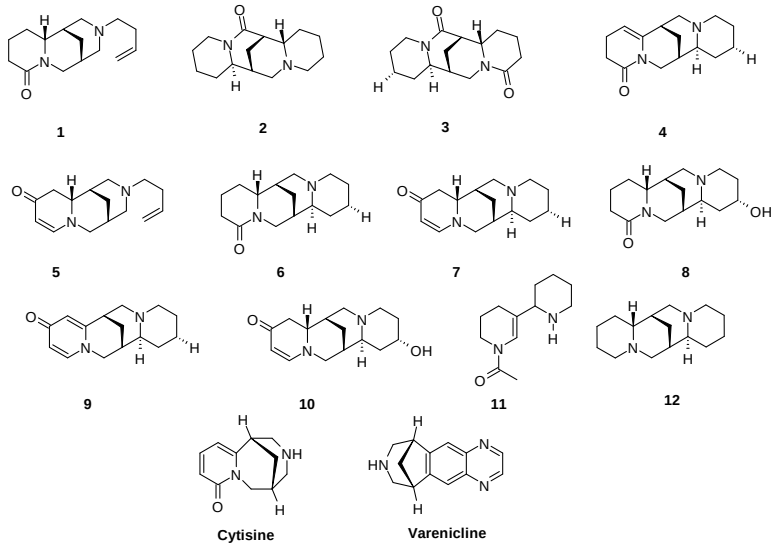


Fig. 1: Chemical Structures of varenicline, cytisine and alkaloids of the total basic fraction of the 75% EtOH extracts of *Lupinus subcarnosus* Hook.

$[\alpha]_D^{25}$ -331° (C=0.1, MeOH) The MS showed the M^+ at 262 the base peak at m/z 150. The peaks at 245 (M^+- OH) and 244 (M^+- H_2O) further confirmed the presence of a hydroxyl group which was indicated by IR peak at 3350 cm^{-1}. Compound **11** $[\alpha]_D^{25}$ +7.1° (C= 0.08, MeOH), The mass fragmentation showed the M^+ at 208. Compound **12** colorless oil, 142 mg, $[\alpha_D^{25}$ - 17° (C= 0.1, MeOH). EIMS m/z (rel. int. %) showed the molecular ion peak at 234 which proposed that **12** is (-)-sparteine**.**

2.2. Molecular Docking

Molecular docking simulation of compounds **1-12, cytisine** and **varenicline**, into the α4β2 nAChR active site was performed. They got stabilized at the α4β2 nAChR -binding site by variable several electrostatic interactions. Interestingly, all compounds showed nearly binding interactions like varenicline (**Table 1**, **Fig. 2, 3, S1**). The order of strength of binding was as follows: **11**>**5**>**1**>varenicline>**9**>cytisine>**3**>**8**>**6**>**4**>**7**>**2**>**10**, in which compounds **11**, **5**, and **1** were higher in the binding energy score than varenicline and cytisine. Confidentially, compound **11** showed the same interactions as the native ligand varenicline within $\alpha_4\beta_2$ nAChR binding site and showed three hydrogen bonds with the key residues Cys199, Cys200, and Trp156 (Interaction energy = -6.39 Kcal/mole) [10]. Despite the good energy scores of **5** and **1**, they did not reveal promising interactions within the active site, whereas **9** showed a reasonable interaction energy (-5.78 Kcal/mole) and better interactions with the abovementioned key amino acids. The superimposition of **9** and **11** with varenicline revealed that both compounds were oriented the same as the native ligand varenicline.

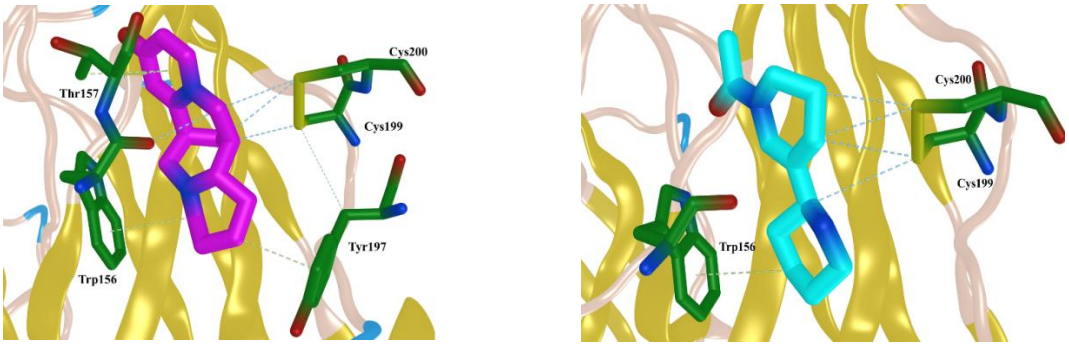
2.3 ADME and Toxicity Prediction

Studying the physicochemical properties and ADME of compounds **1-12**, cytisine, and

varenicline revealed that all compounds have a TPSA (23.5-43.8 Å²) as the reasonable TPSA is between 20 and 130 Å² (**Fig. S2**). Besides, the compounds are predicted to be highly absorbed by the GIT except compound **12**, which is predicted to have low GIT absorption. Only compounds **8**, **10**, and cytisine are predicted to be of low permeability to blood brain barrier (BBB), while the other compounds are expected to have good permeability to BBB. Noteworthy, all compounds showed no PAINS (pan assay interference compounds) and no violation against Lipinski role of five. Interestingly, the compounds are predicted to be better than varenicline as they did not inhibit P-gp substrate and CYP2D6. All test compounds, cytisine, and varenicline are supposed not to inhibit CYP1A2, CYP2C19, CYP2C9, and CYP3A4. Also, all compounds were studied for mutagenicity in *Salmonella typhimurium* and carcinogenicity in mice and rats using LAZAR toxicity prediction online tool https://lazar.in-silico.ch/predict and were found to be non-carcinogenic and non-mutagenic.

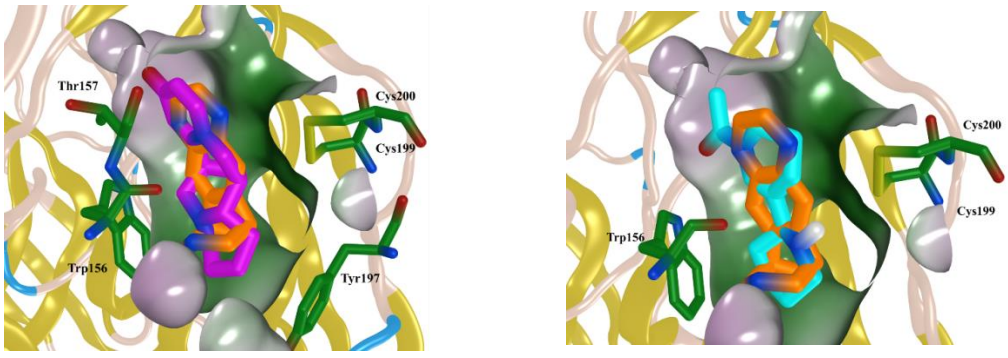
3. Conclusion

This study reveals the isolation of twelve alkaloids for the first time from *L. subcarnosus* herb. It contains tetracyclic quinolizidine type alkaloids with sparteine, lupanine type that were isolated as major compounds. Also,



a **b**

Fig. 2: 3D Docking poses of compounds **9 (a)**, and **11 (b)** into the varenicline binding site in the $\alpha_4\beta_2$ nicotinic acetylcholine receptor (nAChR)



a **b**

Fig. 3: (a) Superimposition of **9**, and varenicline; (**b**) Superimposition of **11**, and varenicline into the varenicline binding site in the $\alpha_4\beta_2$ nicotinic acetylcholine receptor (nAChR).

multiflorine and its derivative were also detected and isolated. The quinolizidine type alkaloids with the α-pyridone skeleton as anagyrine and the bicyclic lupinine type were not detected. *In silico* exploration was conducted for some of *Lupinus subcarnosus*

Hook phytocompounds that could mimic the action of varenicline by desensitizing α4β2 nAChRs. These phytocompounds could serve as potential candidates for the discovery of smoking cessation drugs.

Table 1: Receptor interaction of compounds **1**-**12**, cytisine and varenicline into the varenicline binding site in the α4β2 nicotinic acetylcholine receptor (nAChR)

Compound	dG_kcal/mole	Receptor
		Amino acid/Type of bonding
1	-6.11	Trp156/H-b
		Tyr204/H-π
2	-3.81	Cys199/H-b
		Cys200/H-b
3	-5.23	Cys199/H-b
		Trp156/H-b
		Tyr197/H-π
4	-4.47	Trp156/H-π
5	-6.16	Cys199/H-b
		Trp156/H-π
		Tyr100/H- π
		Tyr204/H-π
6	-4.48	Cys200/H-b
		Trp156/H-b
7	-4.02	Cys199/H-b
		Cys200/H-b
		Trp156/H-b
8	-4.77	Cys200/H-b
		Trp156/H-b
		Trp579/H-π
		Tyr100/ H- π
9	-5.78	Cys199/H-b
		Cys200/H-b
		Trp156/H-b
10	-3.52	Cys199/H-b
		Cys200/H-b
		Trp156/H-b
		Thr157/H-b
11	-6.39	Cys199/H-b
		Cys200/H-b
		Trp156/H-b
12	-4.8747	Tyr197/H-π
		Trp156/H-π
Varenicline	-5.90	Cys199/H-b
		Cys200/H-b
		Trp156/H-b
		Leu121/ H- π
Cytisine	-5.44	Trp156/H-b
		Ser155/H-b
		Thr157/ H- π
		Tyr204/ H- π

Supplementary Materials: The following are available online at https://odr.journals.ekb.eg/article_257288.html

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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