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Nicotine alkaloids as antioxidant and potential protective agents against *in vitro* oxidative haemolysis

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ABSTRACT

The capacity of eleven nicotine alkaloids to reduce oxidative stress was investigated. In order to provide a structure-activity relationships analysis, new nicotine derivatives with a substituent introduced into the pyrrolidine ring were synthesized and investigated together with nicotine and its known analogs. All newly synthesized compounds were characterized by ^{1}H, ^{13}C NMR and EI-MS technique. The antioxidant properties of nicotine, its known analogs and newly produced derivatives, were evaluated by various antioxidant assays such 1,1-diphenyl-2-picryl-hydrazyl free radical ($DPPH^{\bullet}$) scavenging, ferrous ions (Fe^{2+}) chelating activity and total reducing ability determination by $Fe^{3+} \rightarrow Fe^{2+}$ transformation assay. The protective effects of all compounds tested against 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and *tert*-butyl hydroperoxide (*t*-BuOOH)-induced oxidative haemolysis and morphological injury of human erythrocytes, were estimated *in vitro*. The results showed that nicotine alkaloids exhibited various antiradical efficacy and antioxidant activity in a structure- and a dose-dependent manner. In addition, the capacity of nicotine alkaloids to protect erythrocytes from AAPH- and *t*-BuOOH-induced oxidative haemolysis, was dependent on its incubation time with cells. Our findings showed that chemical and biological investigations conducted simultaneously can provide comprehensive knowledge concerning the antioxidant potential of nicotine alkaloids. This knowledge can be helpful in better understanding the properties of nicotine alkaloids under oxidative stress conditions.

1. Introduction

Plant alkaloids represent one of the largest groups of natural products. A well-studied class of biologically active compounds includes (*S*)-(-)-nicotine (compound **1**), the major alkaloid present in *Nicotiana tabacum*. It is a major pharmacologically active substance in tobacco and is the main cause of physiological addiction in smoking. Nicotine together with anabasine and anabasamine constitutes a group of natural ligands of nicotinic acetylocholine receptors (nAChRs). Anabasine (also called neonicotine) is a minor tobacco alkaloid established to be a selective α7-nAChRs agonist in an animal model with low toxicity for the potential treatment of schizophrenia [1–3]. It blocks the function of nAChRs *via* desensitization with a mechanism similar to that of nicotine. Anabasamine was shown to inhibit the catalytic activity of the enzyme acetylcholinesterase [4] and exhibit anti-inflammatory activity [5]. The action of nicotine has been extensively investigated in humans, and animals as well as in a variety of cell systems. It plays an important role in the developments of lung cancer, and cardiovascular disease in smokers [6–8]. Smoking is also considered to induce oxidative stress that can result in the oxidation of lipids, inactivation of certain proteins, disruption of biological membranes, and induction of DNA single-strand breakage [9,10].

Although many studies have investigated the antioxidant properties of nicotine the results still remain controversial. Several *in vivo* experiments suggest that the beneficial/protective effects of nicotine in both Parkinson's disease, and Alzheimer's disease may be due to antioxidant mechanisms, while other studies have reported that nicotine induces oxidative stress in different tissues [11–13]. Recent studies showed that nicotine and cotinine analogs can be potential neuroprotective agents for Alzheimer disease [14].

In continuation of our interest in the chemistry and biology of natural-product-based compounds [15,16], a series of pyrrolidine-modified nicotine analogs was synthesized to elucidate the

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structure–antioxidant activity relationship. We applied three different antioxidant assays such 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH•) scavenging, ferrous ions (Fe^{2+}) chelating activity and a total reducing ability determination by $Fe^{3+} \rightarrow Fe^{2+}$ transformation assay to evaluate the antioxidant properties of newly synthesized compounds, as well as those of nicotine and its known analogs (compounds **1**–**11,** see Fig. 1). Ferulic acid (FA), butylated hydroxytoluene (BHT) and Trolox were used as the reference antioxidants.

Additionally, the capacity of nicotine alkaloids to protect isolated human erythrocytes from oxidative damage induced by two free radicals inductors, namely hydrophilic 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and hydrophobic *tert*-butyl hydroperoxide (*t*-BuOOH), was evaluated. Erythrocytes are the most abundant blood cells and are susceptible to oxidative damage because of their natural role as oxygen transporters. Under oxidative stress conditions the erythrocytes membrane cytoskeleton is damaged and as a consequence, specific cell shape transformations as well as haemolysis are observed [17,18]. The dual effects of nicotine in relation to oxidative damage and antioxidant as well as cytoprotection, including human erythrocytes, was reported [7,19], therefore performing a study with a series of nicotine alkaloids can shed some light on the relationship between the chemical structure and antioxidant properties of each compound.

In our previous paper we showed that nicotine noticeably decreases the cell membrane-perturbing potential of hydrophobic bile acids [15]. In this work we clarify the antioxidant potential of nicotine, its analogs and new derivatives using various antioxidant assays, including the cellular system. We aimed to evaluate the relationship between the chemical structure of each nicotine alkaloid and its effect on oxidative stress *in vitro*. This study represents the first estimation of eleven nicotine alkaloids, including those which are newly synthesized, as antioxidant and human erythrocyte protective agents *in vitro*.

2. Materials and methods

2.1. Chemicals and instruments

Starting materials and reagents used in reactions were obtained commercially from Aldrich and were used without purification. Proton nuclear magnetic resonance (^{1}H-NMR) and Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on a Varian 300/400 MHz spectrometer. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (TMS) for all recorded NMR spectra. EI-MS mass spectra were recorded on 320MS/450GC Bruker mass spectrometer. The absorbance (Abs) was measured at the appropriate wavelength by using spectrophotometer SEMCO, EPOLL 2000 ECO (methods 2.3–2.8).

2.2. Synthesis and characterization of nicotine derivatives and analogs

Synthesis of cotinine (2): Nicotine (162 mg, 1 mmol), mercury(II) acetate (478 mg, 1.5 mmol) and EDTA (438 mg, 1.5 mmol) were dissolved in 20 mL of water and refluxed for 18 h. Brown solution was separate from metallic mercury and refluxed with 30% H_3PO_3 (20 mL) for 2 h. Potassium hydroxide was added to pH = 7 and solution was extracted by CH_2Cl_2 until absence of alkaloids in organic phase (Dragendorff test). Combined organic phases was dried over $MgSO_4$ and solution was evaporated. Brown oil was obtained. Yield: 87.4%. Anal. Calcd. for $C_{10}H_{12}N_2O$:C, 68.18; H, 6.82; N, 15.91. Found: C, 68.20; H, 6.91; N, 15.78. ^{1}H NMR (300 MHz, $CDCl_3$, TMS, ppm): δ 8.56 (1H-6′, ArH), 8.51 (1H-2′, ArH), 7.76 (1H-4′, ArH), 7.51 (1H-5′, ArH), 4.68 (1H-5), 3.40 (*N*–CH_3), 2.45 (1H-4), 2.35 (1H-3), 2.31 (1H-3), 1.77 (1H-4). ^{13}C NMR (75 MHz, $CDCl_3$, TMS, ppm): δ 174.30, 149.12, 148.34, 136.94, 134.70, 123.99, 60.87, 29.52, 27.50, 27.50.

Cotinine thio- (**3**) and seleno- (**4**) analogs were obtained according to the literature [20,21].

Anabasamine (6) was obtained from anabasamine trichloride – Anabasamine x 3 HCl was dissolved in 2 N HCl (5 mL). The solution was alkalized with KOH and (after cooling) extracted with diethyl ether. The ether solution was dried with KOH pellets, evaporated under pressure and oily residue was crystallized from MeOH. White crystalline **6** was obtained (96%), m.p. 64–65 °C. Anal. Calcd. for $C_{16}H_{19}N_3$:C, 75.88; H, 7.51; N, 16.60. Found: C, 75.80; H, 7.48; N, 16.70. ^{1}H NMR (300 MHz, CD_3OD, TMS, ppm): δ 9.16 (1H-2″, ArH), 8.63 (1H-2′, ArH), 8.59 (1H-6″, ArH), 8.43 (1H-4″, ArH), 7.94 (1H-4′, ArH), 7.92 (1H-5′, ArH), 7.57 (1H-5″, ArH), 3.07 (1H-2), 3.02 (1H-6), 2.23 (1H-2), 2.05 (*N*–CH_3), 1.84 (1H-4), 1.77 (1H-5), 1.76 (2H-3), 1.74 (1H-4), 1.66 (1H-5).

***S*-benzylation reaction, general procedure:** nicotine thiolactam (96 mg, 0.5 mmol), benzyl alcohol (162 mg, 1.5 mmol) or 2-/4-methoxy benzyl alcohol (207 mg, 1.5 mmol) were dissolved in dichloromethane and BF_3 etherate (246 mg, 2 mmol) was added dropwise to the solution. The reaction mixture was stirred at room temperature for 72 h. The excess of dichloromethane was

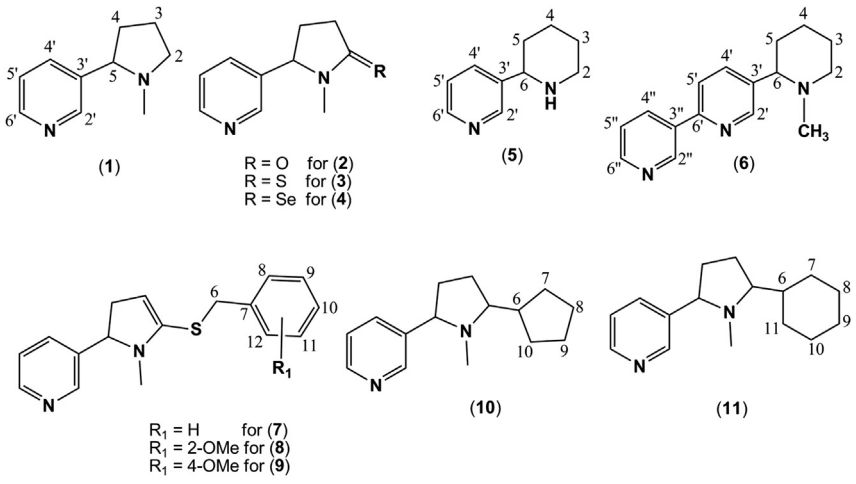

Fig. 1. Chemical structure of compounds investigated.