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INTERACTION BETWEEN B-CARBOLINE ALKALOIDS AND DNA USING SPECTROSCOPIC TECHNIQUES

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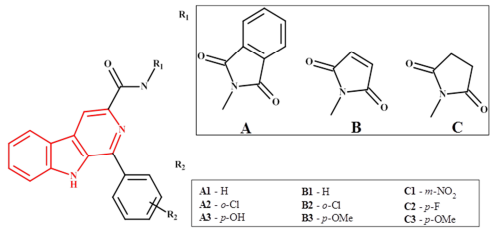
The β-carboline compounds represent a wide class of natural and synthetic indole alkaloids. Due to their biological and pharmacological properties, these alkaloids have been studied as antitumor agents [1]. In this context, the mapping of the interactions processes between small molecules and DNA is an important parameter to understand the molecular mechanisms of drug action, contributing to the development of new drugs DNA-target [2]. Thus, the purpose of this work was to evaluate the interaction between β-carboline alkaloids (Scheme 1.I) and ctDNA (*Calf thymus*), in order to comprise the binding mode of the synthetic alkaloids employing molecular fluorescence spectroscopy. The titrations were conducted by fixing alkaloids concentration (10 μM) with increasing additions of ctDNA. The experimental conditions was adjusted using Tris.HCl buffer (50 mM, pH = 7.4) with NaCl (100 mM). It was observed that addition of ctDNA in the alkaloid solution carried out the reduction of analytical signal, indicating the formation of non-fluorescent complexes between alkaloid and the quencher (DNA). The binding constants (K_b) were calculated, as well as the binding sites number (n) and the thermodynamic parameters (ΔG^0). The K_b values varied from 0.0011 to 18.4×10^5 M L^{-1} and it was observed that the values relative to the radical R_1, generally followed the order: B>C>A. The number of binding sites was approximately equal the unity, and ΔG^0 values varied from -17.4 to -35.7 kJ mol^{-1}, indicating that the process was spontaneous. To evaluate the DNA-ligand binding mode, assays with potassium iodide (KI) and ethidium bromide (EB) were performed. In this evaluation, it was suggested that the preferred interaction process occurs by intercalation, since the K_{SV} values (Scheme 1-II) were systematically lower in the presence of ctDNA, indicating that iodide cannot access the alkaloid. Additionally, by competition studies with EB, it was observed that the emission intensity of EB-DNA decreased after the alkaloid concentration increased. These results indicate that the binding mode is similar to the EB, a well-known DNA intercalator, corroborating with the KI assay, reinforcing the proposal that the interaction occurs by intercalation. The K_b values were correlated with the IC_{50} (μM) of the antiproliferative activity of nine tumor cells cultures. Only for the ovarian, resistant ovarian, breast and lung cell lines the determination coefficient (r^2) was satisfactory (at 95% of agreement), varying from 0.7110 to 0.8257. Therefore, for these cell tumor lines, the preferential mechanism of action might be associated with DNA interaction.

I



II

Comp.	Binding parameters K_b (10^5 L M^{-1})	K_{sv} (M^{-1}L) KI	K_{sv} (M^{-1}L) KI + ctDNA
A1	2.2 ± 0.01	14.2 ± 0.5	12.3 ± 0.5
A2	0.98 ± 0.01	23.9 ± 0.5	19.2 ± 0.4
A3	11.9 ± 0.01	16.8 ± 0.7	15.7 ± 0.5
B1	1.35 ± 0.01	13.3 ± 0.3	13.8 ± 0.2
B2	15.3 ± 0.01	45.9 ± 1.9	41.4 ± 1.7
B3	18.4 ± 0.01	33.2 ± 1.2	30.0 ± 1.6
C1	0.011 ± 0.001	17.5 ± 0.9	13.2 ± 0.7
C2	4.90 ± 0.01	29.5 ± 1.2	27.8 ± 0.9
C3	4.12 ± 0.01	82.7 ± 5.8	70.6 ± 4.2

Scheme 1. *I) Chemical structures of β-carboline alkaloids evaluated. II) K_b values and K_{SV} of β-carboline alkaloids quenching by KI in the absence and presence of ctDNA.*

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