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Full Length Research Paper

Preparation and identification of two new phthalocyanines and study of their anti-cancer activity and anti-bacterial properties

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Nickel phthalocyanines chloride and nickel phthalocyanines nitrate abbreviated as NiPcCl and NiPc (NO_3) respectively were synthesized and characterized. These nickel phthalocyanines are prepared by reaction of phetalic anhydride with urea, ammonium chloride and ammonium heptamolybdate, nickel related salt. Characterization of them was made by microanalyses, Fourier transform infrared spectroscopy (FT-IR) and Ultra violet (UV) visible spectroscopies. The biological properties such as antitumor and antibacterial activities of these compounds were studied. These new compounds showed excellent anti-tumor activity against colon cancer. The anti-bacterial properties of these compounds were studied on Gram-negative and Gram-positive bacterizes, such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella, Staphylococcus aureus, Bacillus subtilis*.

Key words: Nickel phthalocyanines, synthesis, characterization, biological properties, anticancer, anti-bacterial.

INTRODUCTION

Phthalocyanines (Pcs) are traditionally used as dyes and pigments. Their characteristic blue-green color and robustness account for their traditional use as industrial pigments. Their use has recently extended too many high technological processes. They can be used in many applications with appropriate substitution in the peripheral position of the macrocycle, such as in optical recording materials, optical limiters, field-effect transistors, Langmuir-Blodgett films, thin films (or solar cells), gas sensors and liquid crystals (Cook, 1994). Furthermore, they are used as catalysts for photo or oxidative degradation of pollutants and as photosensitizers for photodynamic therapy (Oleinick et al., 1993). The synthetic methodologies for this class of compounds are relatively well established (Costa, 2009; Nemykin and Lukyanets, 2010). The most comprehensive early study of phthalocyanines was conducted by Linstead and co-workers in 1930s (Berezin, 1981). For the first thirty years after their discovery, phthalocyanines were widely used as blue and green light-resistant pigments and dyes in the paper and textile industries because of their high thermal, chemical, and photochemical stabilities (Herbst and Hunter, 2004). Phthalocyanine is an intensely blue-green coloured macrocyclic compound that is widely used in dyeing (Kandel and Schuster, 1977; Johanson and Balster, 1978; Kadish, 2003). Phthalocyanines form coordination complexes with most elements of the periodic table. These complexes are intensely colored and are also used as dyes or pigments. Almost all early phthalocyanine complexes were unsubstituted on the periphery and had a low solubility in most known solvents even in such high-boiling aromatic solvents as 1-chloro- or 1-bromonaphthalene and quinoline, with sulfuric acid found to be the best solvent for them (Nemykin and Lukyanets, 2010). Change of the central atom and/or its axial coordination, change of the *meso*-atoms in the phthalocyanine macrocycle, and its peripheral

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modification (Sharman and van Lier, 2003) are the major ways of modifying the phthalocyanine structure, the last one of which turned out to be the most fruitful. Because the scope and limitations of the first two modification approaches are rather well explored, here we will discuss only the peripheral modification of the phthalocyanine core. Metallo phthalocyanines are multipurpose synthetic electroactive materials with particular electronic and structural characteristics which show semiconducting and nonlinear optical properties (Costa, 2009). Phthalocyanines can also be polymerized in one- or two-dimensional arrays. This architectural flexibility facilitates the tailoring of their properties over a very broad range (Nemykin and Lukyanets, 2010). The preparation properties and applications of phthalocyanines have been recently reviewed (Lezno and Lever, 1989). On the other hand, the wide range of condensed phases that they can show, such as monocrystals, mesophases, Langmuir-Blodgettlms, etc., has contributed to the development of electronic and electrooptic devices based on these compounds (Cook, 1994; Costa, 2009). Therefore, phthalocyanines have a great technological potential in areas related to intrinsic semiconductors and conducting polymers, nonlinear optics, chemical sensors, electrochromic display devices, laser recording materials, information storage systems and liquid-crystal colour display applications, among other and potentially biological properties. (Hanack et al, 1996; Nyokong, 2007; De La Torre et al., 2004). In this paper we prepared and identified nickel phthalocyanines and studied their biological properties.

EXPERIMENTAL

Chemicals and reagents

Compounds purchased from Aldrich and the Merck Company (Analytical Grade) were used without purification. All solvents were used without purification. Infrared spectra were recorded as KBr disks on a Bruker Tensor model 27 spectrophotometer. The UV–Vis spectra were recorded with a Perkin Elmer 25 Lambda spectrophotometer in DMF as solvent.

Preparation of phthalocyanines: General method

Phetalic anhydride (1.56 g), urea (2.33 g), nickel salt, ammonium Chloride (0.29 g) and ammonium heptamolybdate (0.02 g) was dissolved. The mixture was put inside the microwave for 10 to 30 min at 120 to 150°C to give a blue or green powder. At the end of the reaction the resulting solids was mixed at HCl (1 N, 30 ml) and the resulting phthalocyanine filtered.

NiPcCl analytical data

Mp = 360ºC; Yield = 83%; Elemental analysis of NiPcCl Found (%): C: 67.18, H: 2.82, N: 19.77; Calculated (%): C: 67.28, H: 2.95, N: 19.85%; FTIR (KBr pellet, cm^{-1}): 3209.66 (s, O-H), 8271.43 (m, CH_2), 1606.06 (m, C-C), 1765.98 (m, C=N), 1345.40 (s, C-N), 532.79 (w, Ni-N). UV–Vis, λmax (nm)/ε ($M^{-1}cm^{-1}$); 325, 579 and 665 (Figures 1 and 2).

The structure of the general phthalocyanines part is as shown in Figure 3.

NiPc (NO_3) analytical data

Mp = 365ºC; Yield = 75%. Elemental analysis of NiPc (NO_3) Found (%): C: 67.88, H: 2.82, N: 19.79; Calculated (%): C: 67.95, H: 2.93, N: 19.85%; FTIR (KBr pellet, cm^{-1}): 3254.35 (s, O-H), 907.91 (m, CH_2), 1627.28 (s, C-C), 1309.52 (s, C-N), 531.196 (w, Ni-N). UV–Vis, λmax (nm)/ε ($M^{-1}cm^{-1}$); 320, 596 and 660 (Figures 4 and 5).

In vitro activities

Cell culture

NiPcCl and NiPc (NO_3) compounds were assayed for cytotoxicity in vitro against colon cancer. The cell line was provided by the Pasteur Institute Laboratory of natural and Biomimetic in Iran. The H29: human colon adenocarcinoma cell line, used for treatment with the drugs, was provided. H29 cells were grown at 37°C in an atmosphere containing 5% CO_2, with RPMI-1640 MEDIUM HEPES Modification with L-glutamine and 25 mM HEPES (SIGMA-ALDRICH CHEMIE GmbH) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco), 2.7% sodium bicarbonate and 500 mg/L ampicillin. Percentage of live cells was given in Tables 1 and 2.

Bacterial cultures and test of antibacterial activities

Films with antimicrobial activity could help control the growth of pathogenic and spoilage microorganisms. An antimicrobial film is particularly desirable due to its acceptable structural integrity and barrier properties imparted by the compounds matrix, and the antimicrobial properties contributed by the natural antimicrobial agents impregnated within (Lou and Stutzenberger, 2008). Five types of bacteria *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella, Staphylococcus aureus, Bacillus subtilis* were used as model bacteria in this study. Luria broth (LB) and agar were used as sources for culturing *E. coli* at 37°C on a rotary platform in an incubator. *In vitro* activity test was carried out using the growth inhibitory zone (well method). The potency of components was determined against the Gram-positive bacteria and also against the Gram-negative bacteria. Microorganisms (obtained from enrichment culture of the microorganisms in 1 mL Muller-Hinton broth incubated at 37°C for 3 h were cultured on Muller-Hinton agar medium. The inhibitory activity was compared with that of standard antibiotics, such as gentamicin (10 µg). After drilling wells on the medium using a 6 mm cork borer, 100 µL of solution from different compounds were poured into each well. The plates were incubated at 37°C overnight. The diameter of the inhibition zone was measured as precisely as possible. Each test was carried out in triplicate, and the average was calculated for inhibition zone diameters. A blank containing only DMSO showed no inhibition in a preliminary test. The macro dilution broth susceptibility assay was used for the evaluation of minimal inhibitory concentration (MIC). The use of 12 test tubes is required by the macro dilution method. By including 1 mL Muller-Hinton broth in each test and then adding 1 mL extract with 100 mg/mL concentration in the first tube, we made a serial

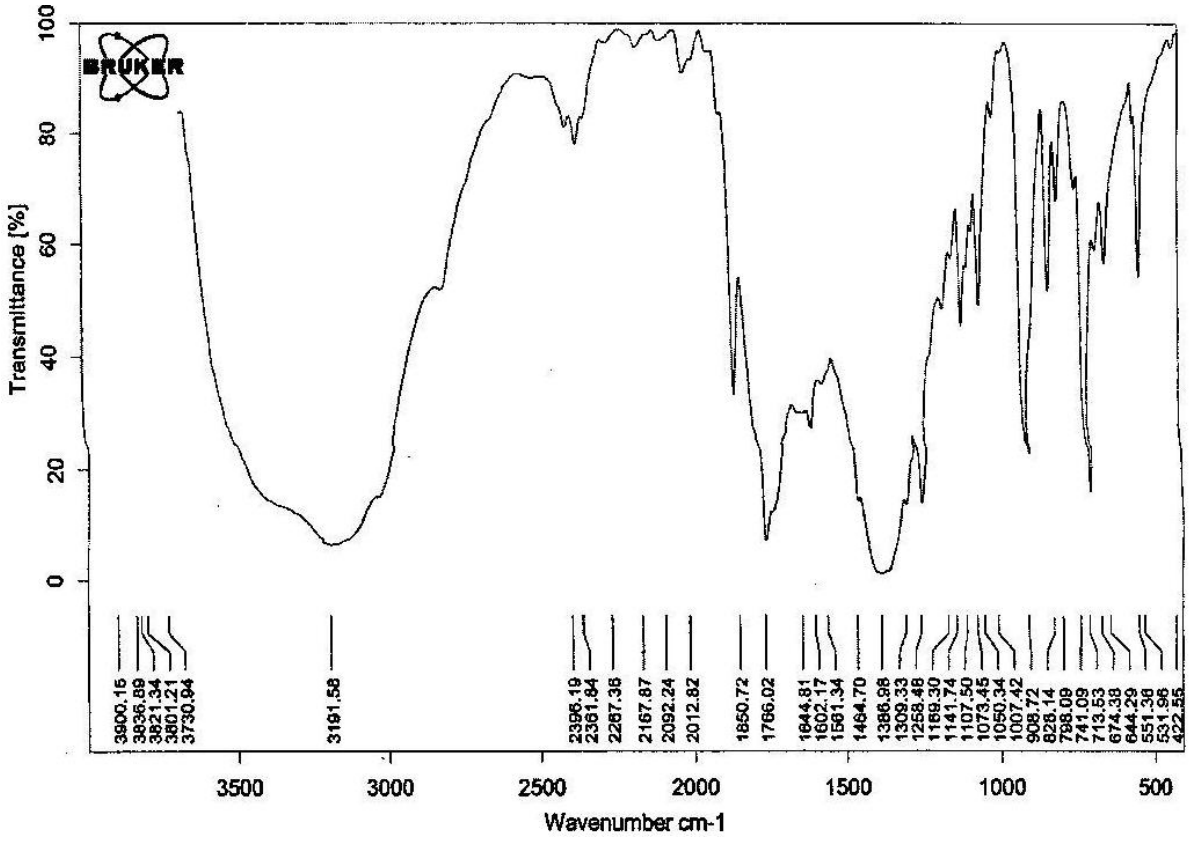

Figure 1. FTIR spectrum of NiPcCl (KBr Disk).

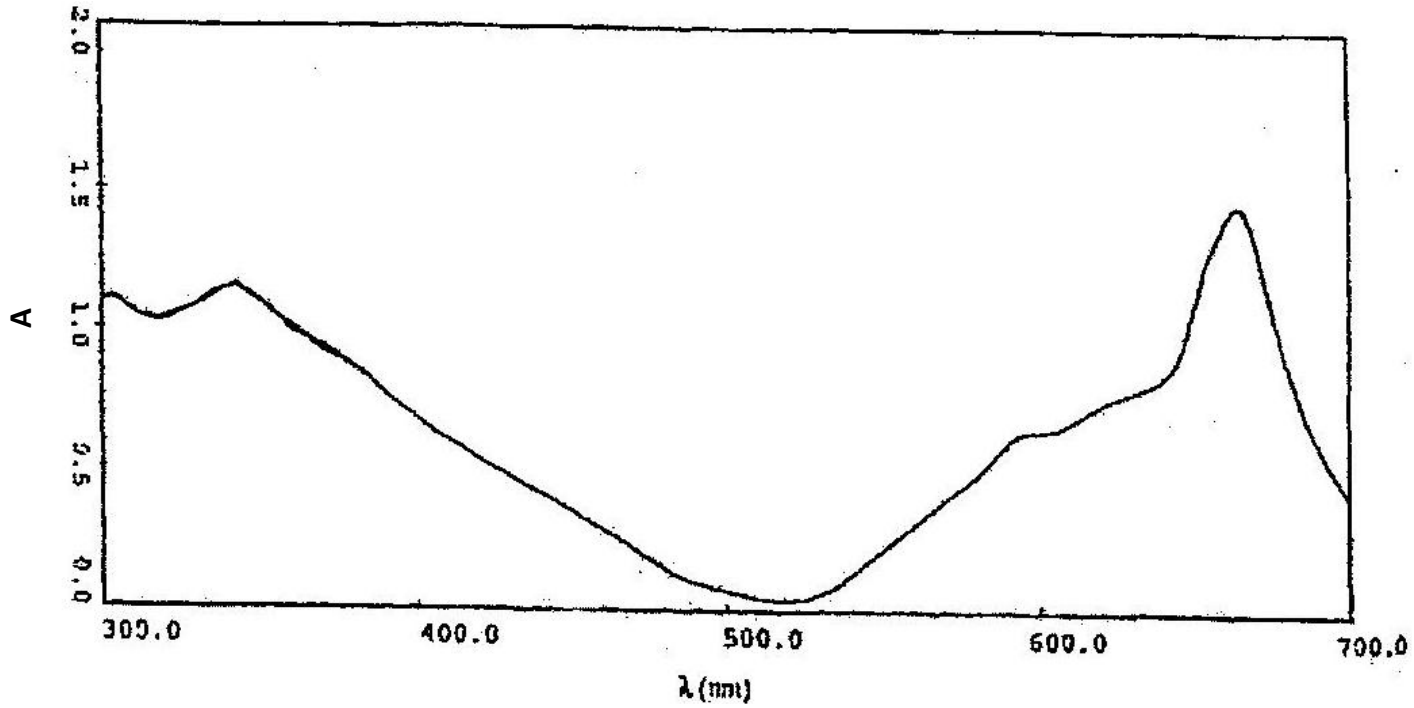

Figure 2. UV/Vis spectrum of NiPcCl (in DMF, C= 10^{-3}M).

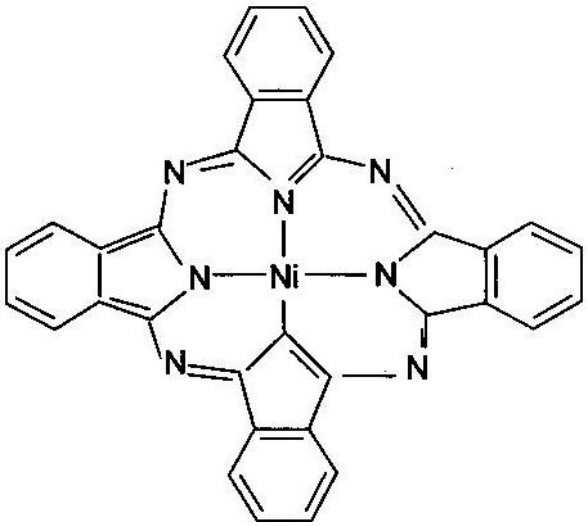

Figure 3. The structure of NiPc.

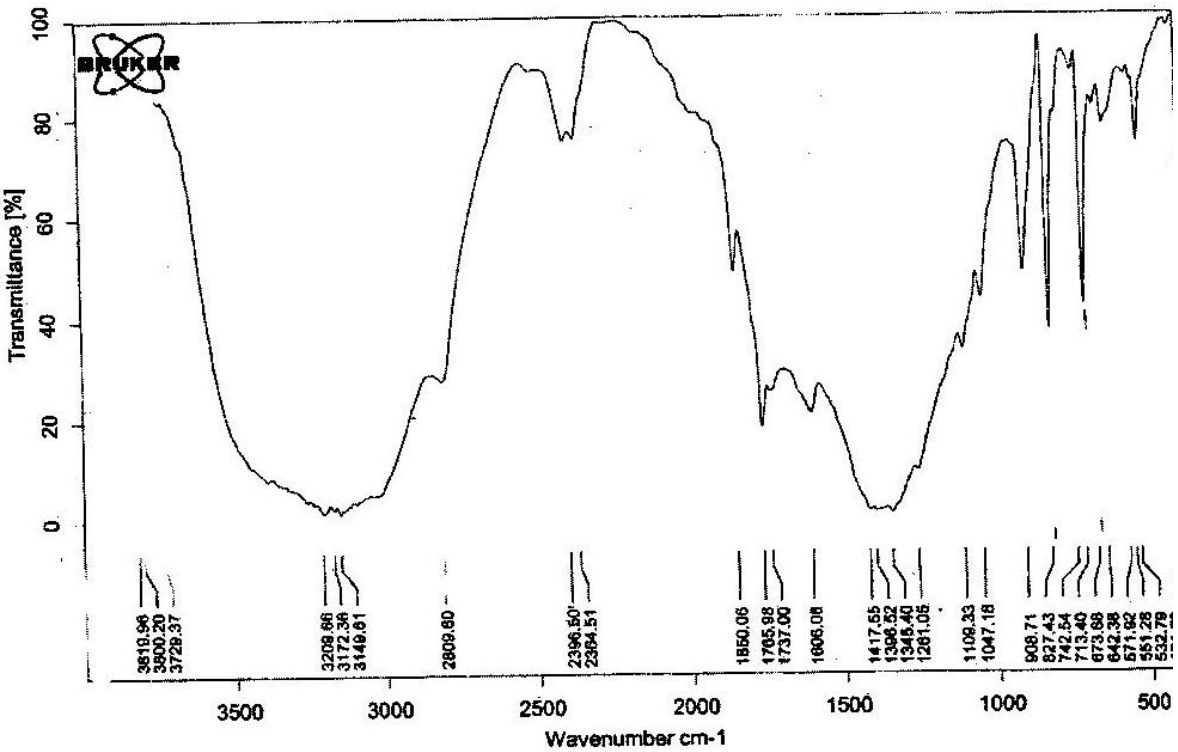

Figure 4. FTIR spectrum of NiPc (NO_3) (KBr Disk).

dilution of this extract from the first tube to the last tube. Bacterial suspensions were prepared to match the turbidity of 0.5 McFarland turbidity standards. Matching this turbidity provided bacterial inoculums concentration of 1.5×10^8 cfu/mL. Then 1 mL of bacterial suspension was added to each test tube. After incubation at 37°C for 24 h, the last tube was determined as the minimal inhibitory concentration (MIC) without turbidity.

RESULTS

The Phthalocyanine (Pc) macromolecule is able to coordinate various metal cations (Fe, Ni, Co, etc) in its center with the four central nitrogens belonging to the pyrrolic subunits. With Nickel, this is called Nickel

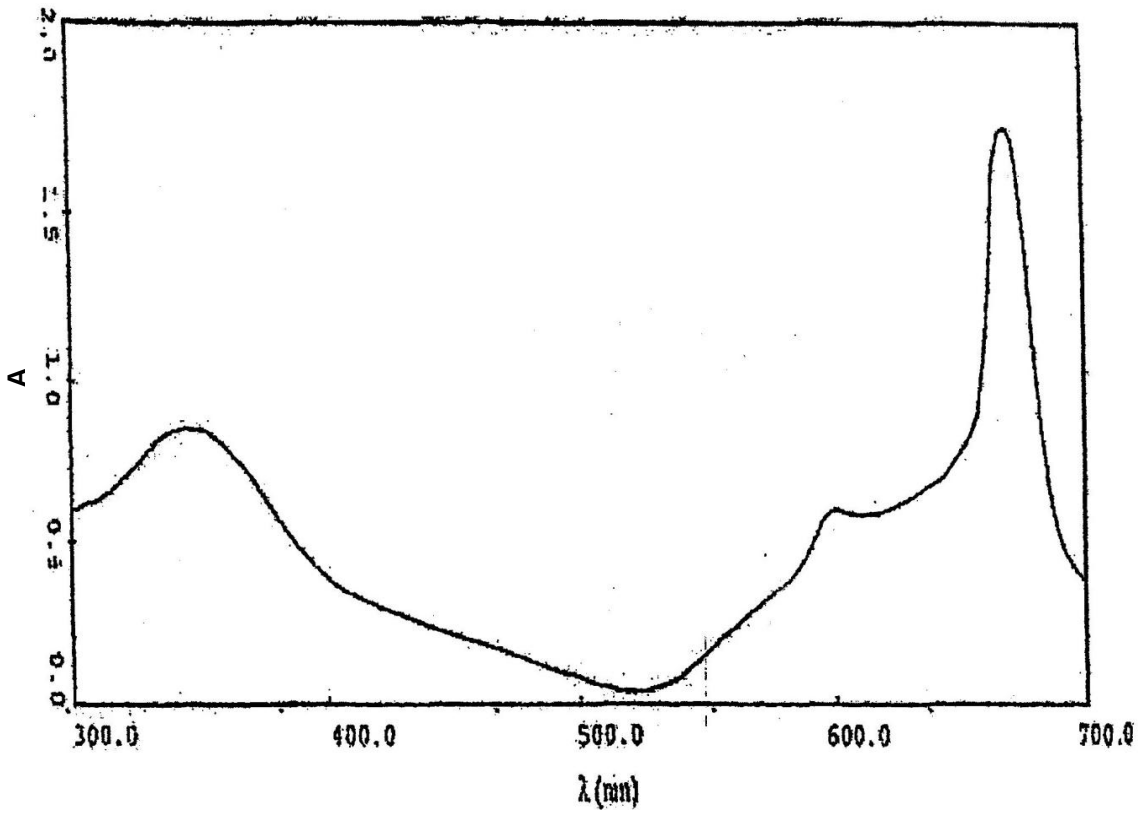

Figure 5. UV/Vis spectrum of NiPc (NO_3) (in DMF, C= 10^{-3}M).

Table.1. The life percentage of cell of NiPcCl.

NiPcCl concentration	Blank concentration	Life percentage of cell
0.025	0.7267	0
0.125	0.7267	0.83
0.25	0.7267	1.31

Table 2. The life percentage of cell of NiPc (NO_3).

NiPc (NO_3) concentration	Blank concentration	Life percentage of cell
0.025	0.7267	2.5
0.125	0.7267	2.98
0.25	0.7267	1.31

phthalocyanine ($NiC_{32}H_{16}N_8$, NiPc), which is useful in carbon nanotube synthesis. In this paper these Nickel phthalocyanine compounds were obtained in relatively high yield, 83 and 75% respectively. These title compounds are soluble in solvents such as ethanol, dimethylformamide, acetonitrile and acetone, less soluble in methanol and insoluble in water and ether. The strong bands at 3209.66 and 1345.40 cm^{-1} were assigned to the O-H and C-N in NiPcCl. The strong bands at 3254.35, 1627.28, 1560.36 and 1309.52 cm^{-1} were assigned to the O-H, C-C, C=C and C-N in NiPc (NO_3). Our procedure for producing compounds has some advantages. For example, there are no side products in preparing NiPcCl and NiPc (NO_3) compounds in our method, the reactions are quite fast and do not require any severe conditions such as high pressure or high temperature, and it is not sensitive to air. Tables 3 and 4 show the antibacterial activity of NiPcCl and NiPc (NO_3) compounds.

In vitro anti-cancer activities

The compounds were assayed for cytotoxicity *in vitro* against H29: human colon adenocarcinoma cell line. The cell lines were provided by the Pastour Institute Laboratory of Natural and Biomimetics in Iran. The procedure for cytotoxicity studies was similar to that reported earlier (Wahab et al., 2004). Briefly, in order to calculate the concentration of each drug, 190 mL of cell suspension (5×104 cell/mL) were exposed to various concentrations of compound dissolved in sterile DMSO.

Table 3. Antibacterial test of this NiPcCl.

NiPcCl	Bacteria
Low Antibacterial activity	*Staphylococcus aureus*
Antibacterial activity	*Escherichia coli*
Low Antibacterial activity	*Salmonella*
Antibacterial activity	*Pseudomonas aeruginosa*
Antibacterial activity	*Bacillus subtilis*

Table 4. Antibacterial test of this NiPc (NO_3).

NiPc (NO_3)	Bacteria
Antibacterial activity	*Staphylococcus aureus*
Antibacterial activity	*Escherichia coli*
Low antibacterial activity	*Salmonella*
Antibacterial activity	*Pseudomonas aeruginosa*
Low antibacterial activity	*Bacillus subtilis*

The final concentration of DMSO in the growth medium was 2% (v/v) or lower, concentrations without effect on cell replication (Zhao et al., 1998; Ishida et al., 1998). After incubation periods 72 h for all cell lines, the cell concentrations were determined both in control and in drug-treated cultures. All experiments were carried out six times.

DISCUSSION

In this study we have reported the synthesis of two new Phthalocyanine derivatives. The chemical characterizations of synthesized compounds were made by using the elemental analysis, IR and UV spectral techniques. Phthalocyanine reacts with Nickel chloride and Nickel nitrate in 2:1(L/M) molar ratio in solvent to afford complex. These compounds are stable at room temperature. The formation of the compounds was also confirmed by UV–vis spectra. The absorption spectra of the compounds were recorded as 10^{-3} M DMF using a quartz cuvette of 1 cm path length. The spectrum of the NiPcCl compound in DMF solutions shows that absorption bands were observed at 325, 579 and 665 nm, however, in NiPc (NO_3) compound in DMF solutions absorption bands were observed at 320, 596 and 660 nm. The results of antitumor activity show that these compounds exhibit anti-tumor properties. The mechanism by which these compounds act as antitumor agents is apoptosis. It has also been proposed that concentration plays a vital role in increasing the degree of inhabitation.

Cytotoxicity studies

The Nickel compounds have been tested against one H29: human colon adenocarcinoma cell line. The general method used for testing anti-tumor properties of these compounds is the standard testing method that has been previously described in greater detail in some papers (Vicente et al., 2008) and abbreviated in some others.

After preincubation lasting 24 h at 37°C in a 5% CO_2 atmosphere and 100% humidity, the tested compounds in the concentration range 0.1 to 28 µM of complexes were added. The incubation lasted 72 h and at the end of this period the dead cells and live cells was measured by Trypan blue. The compounds were first dissolved in DMSO and then filtrated. After the incubation periods (72h for all cell lines), the cell concentrations were determined both in control and in drug-treated cultures. All experiments were done six times.

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