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Novel 2,3-disubstituted 1,4-naphthoquinone derivatives and their metal complexes – Synthesis and *in vitro* cytotoxic effect against mouse fibrosarcoma L929 cells

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ABSTRACT

Quinones have various pharmacological properties including antibacterial, antifungal, antiviral, anti-inflammatory, antipyretic and anticancer activity. Two novel 2,3-disubstituted 1,4-naphthoquinones and their metal complexes were synthesised, characterized and tested. The cytotoxic potential of the novel 2,3-disubstituted 1,4-naphthoquinones and their metal complexes was studied against L929 murine fibroblasts cells grown *in vitro*. The treatment resulted in a concentration-dependent cytotoxicity as indicated by MTT assay.

1. Introduction

Research in the field of cancer therapy has led to the development of many modern medicines and therapeutics with promising anticancer activity. Among the many natural and synthetic compounds explored for anticancer potential, compounds with quinone containing moieties form a major part. Quinones are widely distributed compounds in nature and they are reported to exhibit diverse pharmacological properties including anticancer activity [1,2]. One of the mechanisms by which quinones could induce cytotoxicity was induction of oxidative stress [3]. According to this proposed mechanism, quinones are first reduced to hydroquinones or semiquinone radicals by cellular reductases at the expense of NADPH. Then, both hydroquinones and semiquinone radicals undergo rapid autoxidation with the regeneration of the parent quinones and the concomitant formation of superoxide [3]. These quinones with the ability to induce oxidative stress are responsible for initiation of tissue damage selectively in tumour cells and this seems to be a promising approach for targeting cancer cells [4].

1,4-naphthoquinone pharmacophore is known to impart pronounced biological effects in 1,4-naphthoquinone derivatives, leading to antitumour [5,6], antiproliferative [7], antimycobacterial [8], antiplatelet, anti-inflammatory, antiallergic [9], antimalarial [10] and antileishmanial activities [11]. The incorporation of sulphur atom in 1,4-naphthoquinone derivatives has led to antifungal, antibacterial, antiviral, and anticancer activities [12–16].

It has been reported that menadione (an analogue of 1,4-naphthoquinone) could induce reactive oxygen species (ROS), which mediate DNA damage in many human cultured cells, suggesting that this activity might be important for its therapeutic effects of naphthoquinone derivatives [17,18].

The literature offers data related to the complex compounds formed by transition metals and ligands having conjugated double bonds [19–21]. The special interest about these complexes is due, among others, to the fact that the complex compounds readily participate in reversible electron-transfer reactions. Literature mentions the important biologic-active, antimalaric, antiviral and antitumoural properties of this type of ligands; the same properties are shown by the complexes that these ligands form with metal ions, which act in the biological structures as essential microelements [19–21]. The importance of these compounds can be exemplified by

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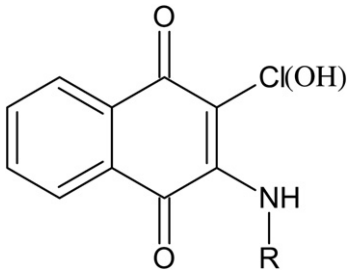

Fig. 1. 3-alkyl- or 3-alkylamino-substituted derivatives of 2-hydroxy-1,4-naphthoquinone or 2-chloro-1,4-naphthoquinones.

the interesting biological activities associated with many 3-alkyl- or 3-alkylamino-substituted derivatives of 2-hydroxy-1,4-naphthoquinone or 2-chloro-1,4-naphthoquinones (Fig. 1) [22].

Further need for a study of compounds of this type is illustrated by the observation that the ortho-amino quinoid unit is present in many antitumoural antibiotics such as actinomycins, mitomycin C, porfiromycin and streptonigrin [23].

In this study, in order to continue the existing investigations on 2,3-disubstituted 1,4-naphthoquinones [24], we introduced substituents groups containing N, S or O atoms, knowing the fact that in many natural structures, such as proteins or aminoacids, these groups are responsible for various biological activities. Moreover, the potential biological activity of the new synthesized compounds can be cumulated due to the 1,4-naphthoquinone properties. Also, because there are few literatures [25–27] regarding the complexes of the general [M-N2S2] type, especially metal complexes with naphthoquinonic ligands, we hope that the data provided by this paper will contribute to enrich the knowledge in this insufficiently studied field.

Cell lines are useful models for doing research since they provide large amounts of consistent cells for prolonged use and because most cellular characteristics are maintained, reliable experimental data can be compared among research reports, in which the same cell lines are used. L929 is a cell line popular in many experiment aspects such as material biocompatibility testing [28–30], drug cytotoxicity testing [31,32] and cell biology studies [33–35].

In order to develop new antitumoural drugs with less secondary effects, the final purpose of this study was to evaluate the potential biological activity of the novel synthesized 2,3-disubstituted 1,4-naphthoquinone compounds in terms of cytotoxicity by MTT assay [36], using L929 mouse fibroblasts.

2. Methods

2.1. Chemicals

The reagents and the solvents for the synthesis were used without further purification. 2-amino-3-chloro-1,4-naphthoquinone and 2,3-dichloro-1,4-naphthoquinone were purchased from Fluka (Steinheim, Germany), chloroacetic acid from Merck (Darmstadt, Germany), ethanol, methanol, dilituric acid, anhydrous nickel chloride and hexahydrate chloroplatinic acid from Sigma–Aldrich (Steinheim, Germany), metallic sodium from Aldrich (Steinheim, Germany), thiourea, NaOH and glacial acetic acid from UTCHIM (Ramnicu Valcea, ROMANIA) and Water Chromasolv from Riedel-de Haën (Seelze, Germany).

2.2. Instruments

Melting points (m.p.) of the ligands and metal complexes were determined by open capillary method using Sunsim electric melting point apparatus and were uncorrected. Elemental analysis of C, H, N, S and O was performed with a Thermo Finnigan Flash 2000 Automatic Elemental Analyzer. The metal content was determined using an Analytic Jena NOVAA 300 Absorption Atomic Spectrophotometer. The IR spectra were recorded in the region 4000–400 cm^{-1} on a FT-IR MAGNA 750 NICOLET in anhydrous KBr pellets. A Jasco V-670 UV–Vis spectrophotometer was used to perform the electronic spectra.

2.3. In vitro cytotoxicity assay

In order to determine the cytotoxicity of the compounds, the *in vitro* cytotoxicity MTT assay was performed using L929 mouse fibroblasts (kindly provided by Dr. Ronald Doyle, University of Louisville School of Medicine, Louisville, KY, USA).

The cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) (Sigma–Aldrich, Inc. St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) (BIOCHROM AG, Berlin, Germany) and 100 U/mL penicillin-streptomycin (Lonza, Verviers, Belgium) and incubated at 37 °C in a humidified atmosphere with 5% CO_2, in flat-bottom 96-well tissue culture plates (starting concentration: 6 × 105 cells/mL, 100 μL/well).

Test compounds were dissolved in dimethylsulphoxide (DMSO) (Merck, Darmstadt, Germany) at a final concentration of 1% in culture medium.

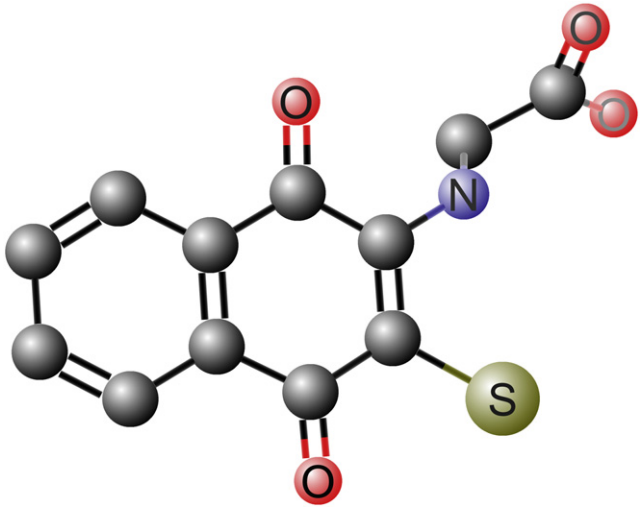

Fig. 2. 2-acetamino-3-mercapto-1,4-naphthoquinone.

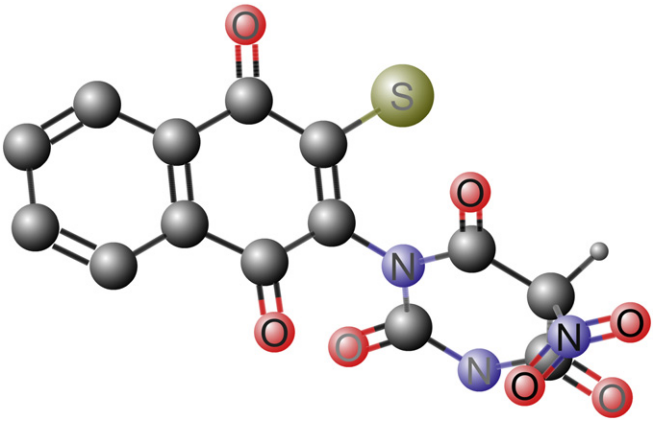

Fig. 3. 2-mercapto-3(5-nitrobarbituro)-1,4-naphthoquinone.