The medicinal and chemical aspects of naphthoquinones isolated from *Euclea natalensis* A. DC. on *Mycobacterium tuberculosis*

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Abstract

The isolation and antimycobacterial activity of several naphthoquinones from *Euclea natalensis* were previously reported and initiated this study into the occurrence, chemistry and biological activity of this class of compounds. The structure activity relationship of the isolated naphthoquinones, and commercially available derivatives were also studied.

Several plant species were investigated to establish a possible link between their traditional use for chest related symptoms (including tuberculosis infection) and the occurrence of 7-methyljuglone in these plants. The plants were extracted and tested qualitatively with the use of three analytical tools for the presence of 7-methyljuglone or related naphthoquinones.

Due to its commercial unavailability, the chemical synthesis of two of these naphthoquinones, 7-methyljuglone and diospyrin, was attempted with varying degrees of success. The Friedel-Crafts acylation method was used to synthesise 7-methyljuglone from *m*-cresol and maleic anhydride as starting material. The optimisation of the synthesis was also investigated. Through a two-step pathway of epoxidation and steam distillation, diospyrin was

successfully synthesised albeit in small quantities. During the attempts to synthesise diospyrin, two other related compounds were also synthesised. These compounds, neodiospyrin and mamegakinone, are structural isomers of diospyrin.

The stability of some of the naphthoquinones was tested in various carriers in an attempt to explain the influence this will have on the obtained antituberculosis and toxicity data. The BACTEC vial solution, which is widely used to determine potency against *Mycobacterium tuberculosis*, was analysed with HPLC to determine the stability of these compounds in it. In addition the stability in organic solvents especially DMSO, was also tested as this is the solvent of choice for hydrophobic compounds in almost all bioassays.

The antituberculosis activity and/or toxicity of 7-methyljuglone was investigated with three bioassays, to broaden our knowledge on the mechanism of action of naphthoquinones. Vero cells were employed to determine the inhibitory concentration (IC50) of most of the naphthoquinones. Mice experiments were carried out to determine the toxicity of 7-methyljuglone and diospyrin *in vivo*. In addition the lead compound, 7-methyljuglone, was tested on *Musca domestica* (house fly) to establish its toxicity on this organism.

In order to find the pharmacophore of this class of compounds, a preliminary structure-activity relationship was conducted. During this study the active site in the compounds which confers potency and toxicity was partly established.

The mode of action of some of the naphthoquinones was investigated and it was established that the compounds might interfere with the mycobacterial electron transport chain. A fluorinated 7-methyljuglone stops the production of menaquinone which transports electrons from the NADH dehydrogenase complex to the cytochrome bc complex and effectively kills the mycobacterium.

Keywords: diospyrin, electron transport chain, 7-methyljuglone, *Mycobacterium tuberculosis*, structure-activity relationship.

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