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New insights into 3-(aminomethyl)naphthoquinones: Evaluation of cytotoxicity, electrochemical behavior and search for structure–activity correlation

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ABSTRACT

Herein we describe the structure–activity relationship of a large library of Mannich bases (**MBs**) synthesized from 2-hydroxy-1,4-naphthoquinone. In general, the compounds have shown high to moderate activity against the HL-60 (promyelocytic leukaemia) cell line with IC_{50} = 1.1–19.2 μM. Our results suggest that the nature of the aryl moiety introduced in the structure of **MBs** by the aldehyde component is crucial to the cytotoxicity, and although the group originated from the primary amine has a lesser influence, aromatic ones were found to suppress the activity. Thus, **MBs** derived from salicylaldehydes or 2-pyridinecarboxaldehyde and aliphatic amines are the most active compounds. A satisfactory correlation of the E_{pIIc} versus IC_{50} (μM) in dimethylsulfoxide was observed. The most cytotoxic **MBs** (**Series a–c**, derived from salicylaldehydes) showed the least negative E_{pIIc} values. Noteworthy, however, **Series d** (derived from 2-pyridinecarboxaldehyde) did not follow this correlation. They exhibited both the lowest IC_{50} and the most negative E_{pIIc} values, thus suggesting that other factors also influence the cytotoxicity of the **MBs**, such as lipophilicity, electronic distribution and hydrogen bonding.

Quinones are known for their wide range of pharmacological properties.[1] Naphthoquinones and anthraquinones, for instance, are active against parasites,[2] bacteria and fungi,[3] and their antitumor properties[4,5] have attracted increasing research interest. These compounds have been shown to act via oxidative stress, topoisomerases inhibition and DNA alkylation or intercalation, eventually causing strand breaks.[5,6]

3-(Aminomethyl)-2-hydroxy-1,4-naphthoquinones known as Mannich bases (**MBs**) constitute an interesting class of naphthoquinones that were first synthesized in the 40s by Leffer and coworkers who also described their antimalarial activity.[7] They are obtained by the condensation reaction of 2-hydroxy-1,4-naphthoquinone (lawsone), a non-enolizable aldehyde and a primary or secondary amine.[7,8] Methodologies for the synthesis of **MBs** derived from anilines and heterocyclic amines have also been reported using metal ions as catalysts.[9,10]

We have reported the bactericidal[11] and antiviral[12,13] profiles of novel **MBs**, which have shown very good activities. Furthermore, **MBs** derived from 2-pyridinecarboxaldehyde exhibited moderate to high activities against some tumor cell lines.[14] Their respective platinum(II) and platinum(IV) complexes were also evaluated and in some cases they were more active than cisplatin, one of the most commonly used drugs in chemotherapy.[14,15] It was also found that the **MBs** were more active than their respective platinum(II) complexes against most cell lines, even though the latter were found to interact strongly with DNA and induce DNA strand breaks in vitro.[16] The higher cytotoxicity of the **MBs** has been proposed to be due to deamination with formation of an *ortho*-quinone methide, which may cause DNA strand breaks and cell death.[16]

Continuing our efforts to obtain other **MB** derivatives with improved cytotoxicities, we have synthesized a series of novel 3-(*R*-amino-(*R*′)-methyl)-2-hydroxy-1,4-naphthoquinones

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and compared their cytotoxic activity against melanoma (MDA-MB-435), promyelocytic leukaemia (HL-60), colorectal adenocarcinoma (HCT-8) and glioblastoma (SF-295) with those of analogous compounds reported previously.[14] To look for possible correlations between their redox properties and cytotoxic activities, the electrochemical behavior of these molecules was evaluated in DMSO, a non-aqueous aprotic solvent, which has been suggested to mimic the cell membrane environment.[17]

The synthesis of the **MBs** was carried out following a procedure described previously and illustrated in Figure 1A.[11] Except for **MBs 4a**, **5a**, **8a**, **4b**, **5b**, **4c**, **5c**, **4d**–**8d**, **9e**, **5g**, **4h** and **5h** the compounds are novel. The **MBs** were isolated as analytically pure orange powders with yields ranging from 33% to 94%. They are all stable in the solid state, but undergo slow decomposition in solution (MeOH, EtOH, $CHCl_3$ and DMSO) when left for long periods of time. This process is probably related to the deamination of the **MBs**, extensively discussed in the literature.[18] The compounds were formulated based on elemental analysis and their structures, confirmed by spectroscopic data (see Experimental, Supplementary data).

The ^{1}H NMR spectra show similar profiles. Hydrogens *H5*–*H8* are observed in the δ 8.0–7.0 range as dd or ddd for *H5* and *H8*, and as td for *H6* and *H7*. A singlet attributed to *H11* is also observed around δ 6.0–5.5, except for compounds of **Series i** where this singlet is around δ 4.0. The aromatic hydrogens (*H13*–*H17*) are observed in the expected region and were attributed to the respective hydrogens of the aryl or pyridyl groups based on coupling constant values (*J*) and ^{1}H–^{1}H COSY experiments.[11] The aliphatic peaks are the most shielded signals at δ 4.0–1.0. The spectra of the **MBs** containing *Boc*-protected amines exhibit a singlet attributed to the three methyl groups, at δ 1.41–1.48. The ^{13}C (APT) NMR spectra show all the expected peaks (see Supplementary data).

Similarly the IR spectra exhibit the expected bands for the synthesized **MBs**[19] (see Supplementary data). The UV–Vis spectra show the same pattern for all compounds, with three bands, λ_1 = 270–280 nm, λ_2 = 330–340 nm and λ_3 = 460–470 nm, attributed, respectively, to π–π^* transitions of the benzene and naphthoquinone rings, naphthoquinone π–π^* transition and carbonyl groups n–π^* transition.[20–22]

The cytotoxicity of the **MBs** was investigated and compared to the data described previously for compounds **4d**–**8d**.[14] Aiming to improve their solubility in water, some **MBs** (**4a**, **5a**, **8a**, **4b**, **5b**, **4c**, **5c**, **9e**, **5g**, **4h** and **5h**)[11,23] were converted into their hydrochloride forms upon treatment with acetyl chloride. The **MBs·HCl** were obtained in quantitative yields and formulated based on elemental analysis data. Nevertheless they were not soluble enough in water and therefore the cytotoxicity assays of all compounds were undertaken in 1% DMSO (DMSO concentration did not exceed 1% in the highest drug concentration solution, see Experimental, Supplementary data).

All **MBs** described in Figure 1 have been tested, but only those that have shown cytotoxic activity (IC_{50} < 40 μmol L^{-1}) against the tested tumor cell lines are shown in Table 1, which also contains compounds **4d**–**8d**[14] for comparison. The results show that the activity of the **MBs** is mostly dependent on the nature of the substituent on C11. Thus, the **MBs** derived from benzaldehyde (**Series e**), 3-hydroxybenzaldehyde (**Series f**), 2,4-dichlorobenzaldehyde (**Series g**), 4-nitrobenzaldehyde (**Series h**) and formaldehyde (**Series i**) are inactive.

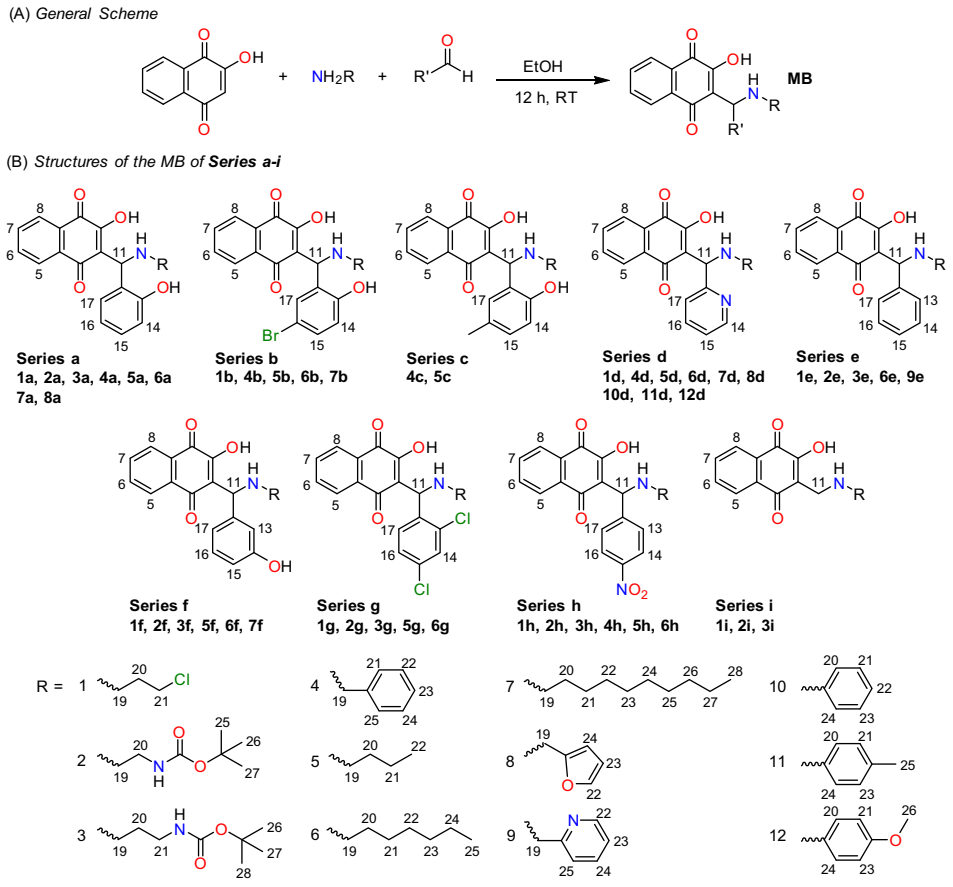

Figure 1. (A) General procedure for the preparation of 3-(*R*-amino-(*R*′)-methyl)-2-hydroxy-1,4-naphthoquinones and (B) structures of the synthesized **MBs** (**Series a**–**i**).