New procedure for the synthesis of indolo[3,2-b]quinoline derivatives with DNA G-quadruplex stabilization capacity

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Introduction

Cancer is one of the leading causes of death worldwide and the occurrence of resistance to common anticancer drugs demands new and innovative drug design approaches. G-quadruplexes (G4s) DNA structures in oncogenic promoter regions (of *c-MYC* and *KRAS* oncogenes, for example) and telomeres are potential targets for cancer therapy. Small molecules could serve as DNA G4 stabilizers and down-regulate the targeted gene expression leading to induction of programmed cell death by apoptosis [1][2].

Indolo[3,2-b]quinoline and indolo[3,2-c]quinoline derivatives were previously reported as stabilizers of G4 DNA structures (**Figure 1**) and promising selective anticancer leads, as these compounds are able to preferentially target the G4 motifs in the *KRAS* promoter and inhibit the transcription and translation of this oncogene, inducing apoptosis of KRAS-dependent colon cancer cells by up-regulating the expression of the pro-apoptotic transcription factor p53 (**Figure 2**) [3][4]. Activation of p53 by small molecules is also expected to be a valuable approach in fighting cancer. In this area of research, we have previously identified other promising indole-based compounds with activity as p53 gene expression or p53 function activators [5][6]. Thus, the improvement of the synthetic procedures of these compounds are highly relevant. Herein we describe an alternative experimental procedure for the synthesis of 7-carboxylate indolo[3,2-b]quinoline tri-alkylamine derivatives **1a** and **2a**.

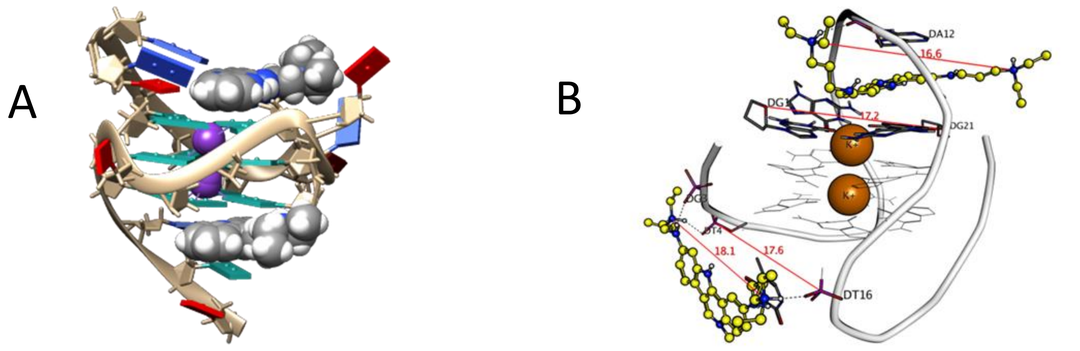


Figure 1 - A) Structure of 2:1 complex of an indolo[3,2-b]quinoline derivative with *c-MYC* promoter G4 (PDB ID: 2L7V). B) Molecular modelling 2:1 complex of an indolo[3,2-c]quinoline derivative with antiparallel human telemore G4 (PDB ID: 143D).[7]

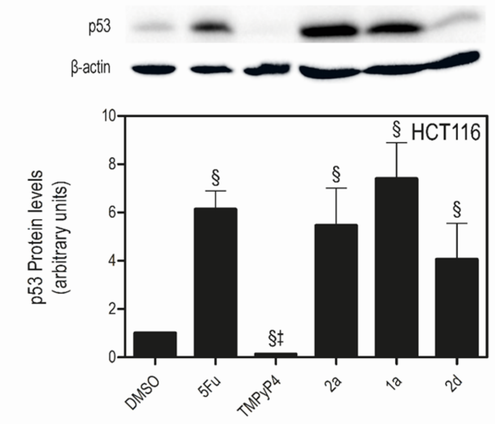
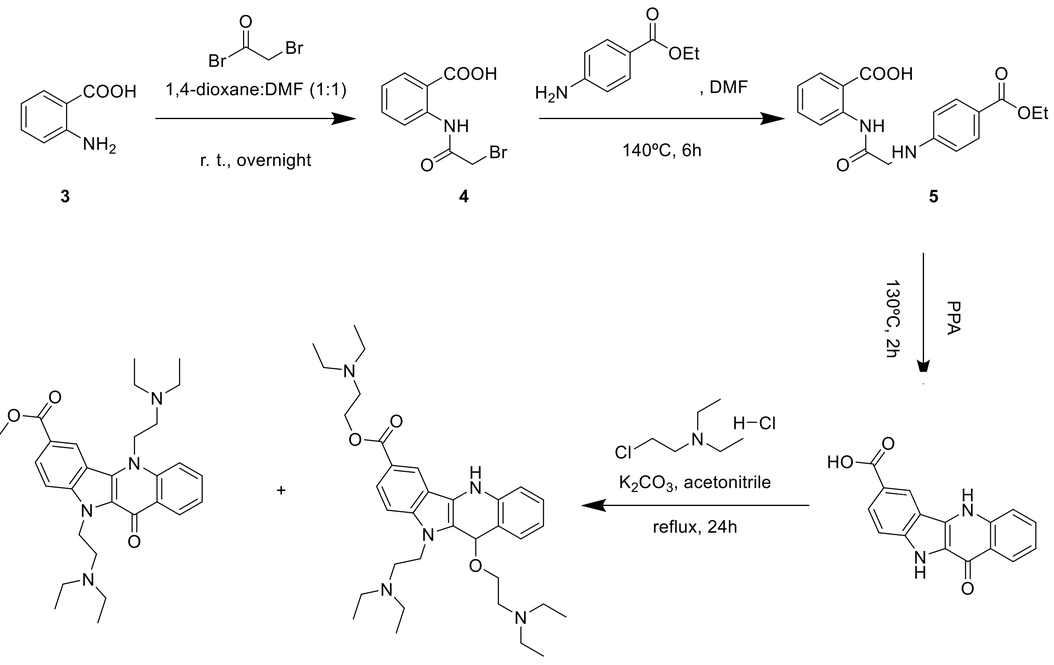


Figure 2 - Exposure to indolo[3,2-b]quinoline derivatives **1a** and **2a** increases p53 steady-state protein expression in HCT116 cells. (Figure from [3])

Synthetic Procedures



Compounds **1a** and **2a** were synthesized as previously described [3], with the following modifications:

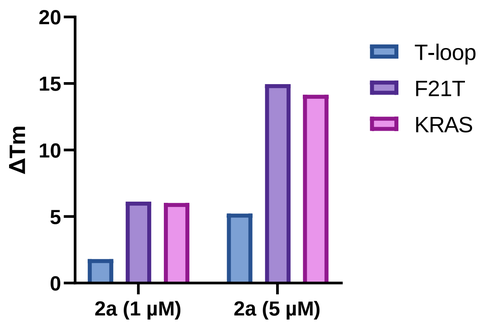
•**Synthesis of intermediate 5:**

Previous Procedure and yield	Present Procedure and yield
A mixture of compound 4 and 4-ethylaminobenzoate in DMF was placed in a microwave apparatus, at 300 W, 140ºC, for 4 hours. Yield: 62 %.	A mixture of compound 4 and 4-ethylaminobenzoate in DMF was heated at 140ºC in a pressure tube for 6 hours. Yield: 40-60 %.

•**Work-up procedure for intermediate 6:**

Previous Procedure and yield	Present Procedure and yield
The reaction mixture is basified to pH 4 with KOH and the product is isolated by liquid-liquid extraction with ethyl acetate. Yield: 20 %.	The reaction mixture is basified to pH 4 with KOH and the dark green sticky precipitate that is formed is filtered and then dissolved in metanol. The solubilized product is separated from unsoluble by-products by filtration. Yield: 66 %.

G-quadruplex stabilization by FRET melting assay



Indolo[3,2-b]quinoline 2a preferentially binds and stabilize DNA G4 than ds-DNA. Melting temperature variations (ΔTm) of labeled G4s present in promoters of *k-RAS* (KRAS), human telomere (F21T) and hairpin loop sequence (T-loop) at 0.2 μM, stabilized by compound **2a**. ΔTm values are averages from a triplicate experiment; std errors < 0.25 °C.

Spectra

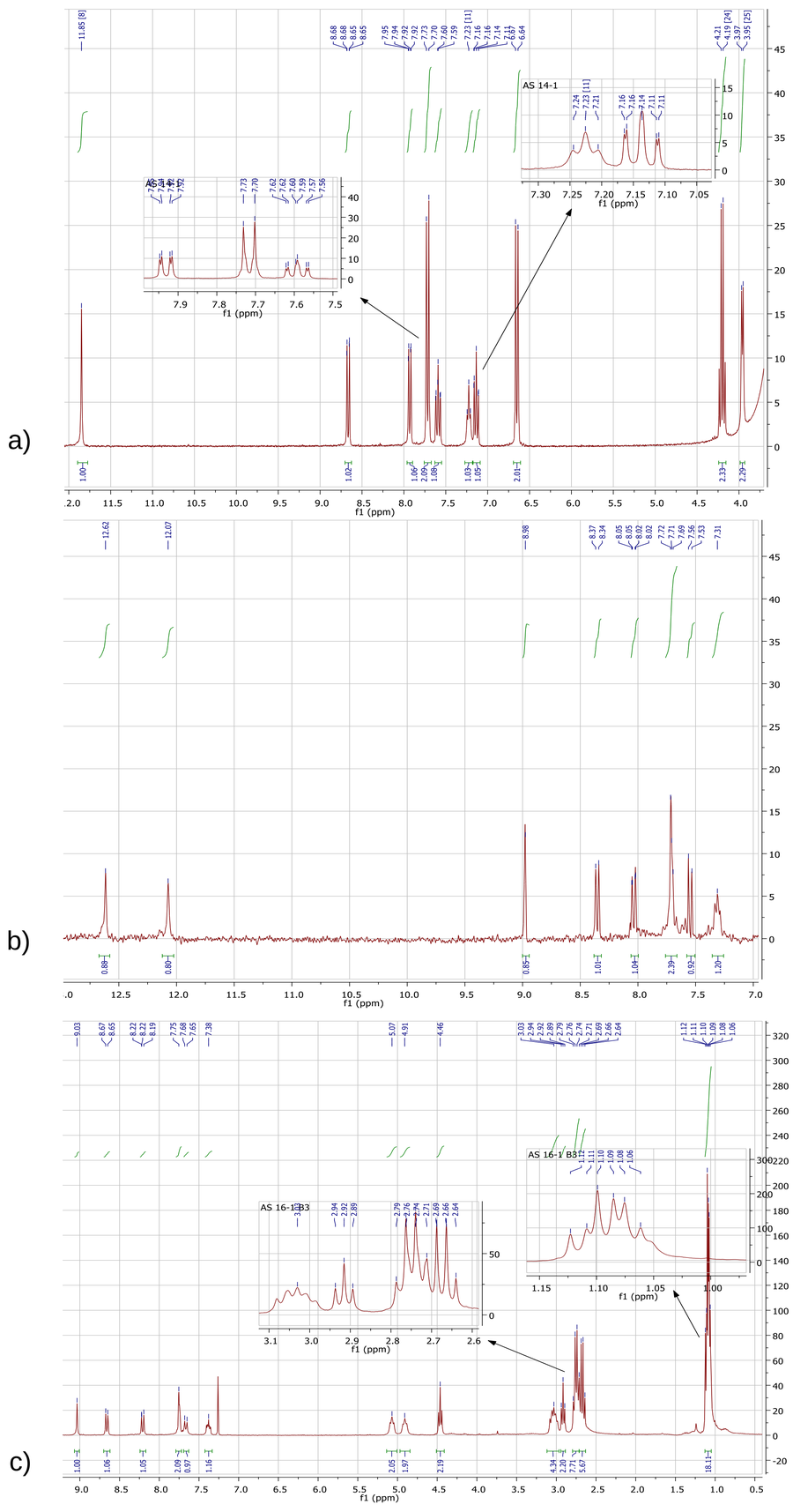


Figure 3- Proton NMR of compounds **5** (a)), **6** (b)) and **2a** (c))

Conclusions

- With the herein reported **alternative method for the synthesis of intermediate 5** the microwave apparatus can be replaced by a pressure tube, a much more economic lab equipment.
- An **improved work-up procedure to obtain intermediate 6** is also here reported. With this procedure the yield increases from 20% to 66%.

References

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Acknowledgments

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