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Synthesis of Analogues of Natural Product 'Antheminone A' and Evaluation of Their *Anti*-Cancer Activity

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Abstract

Synthesis of two novel compounds (**1a** and **1b**) derived from analogue of antheminone A (**1c**) and their evaluation of *anti*-cancer activities are hereby described. This synthesis involved a multi-step reaction sequence involving the use of natural product (-)-quinic acid (**1d**) and unsymmetrical dicoumarol (**1e**) as precursors (Figure 1). Unsymmetrical dicoumarol (**1e**), a potent inhibitor of NQO1 was synthesized by coupling 4-hydroxyl coumarin and an appropriate benzaldehyde. Thus, in order to facilitate drug penetration through the barriers of cell membrane to NQO1 location, compound (**1e**) was re-modified by coupling with an analogue of antheminone A (**1c**). Spectral analyses of the products were carried out in order to confirm the identity of the compounds. Interestingly, the compounds which were obtained in good to moderate yield (51-68%) exhibited toxicity against the non-small cancer cell line, A549.

Keywords: synthesis, dicoumarol, antheminone A, enzyme assay, toxicity

1. Introduction

Compounds screened as potential *anti*-cancer agents can be either from natural or synthetic origin. Natural compounds have provided many guides and their structural identification have given abundant compounds with high-quality pharmacological activity and therapeutic potentials. The search for novel bioactive compounds has therefore improved over recent years. This effort has led to the discovery of several natural compounds and their synthetic analogues which have been identified as having a wide range of significant *anti*-cancer properties, such as inhibition of cell growth and proliferation (Zhang, X., *et al*. 2012).

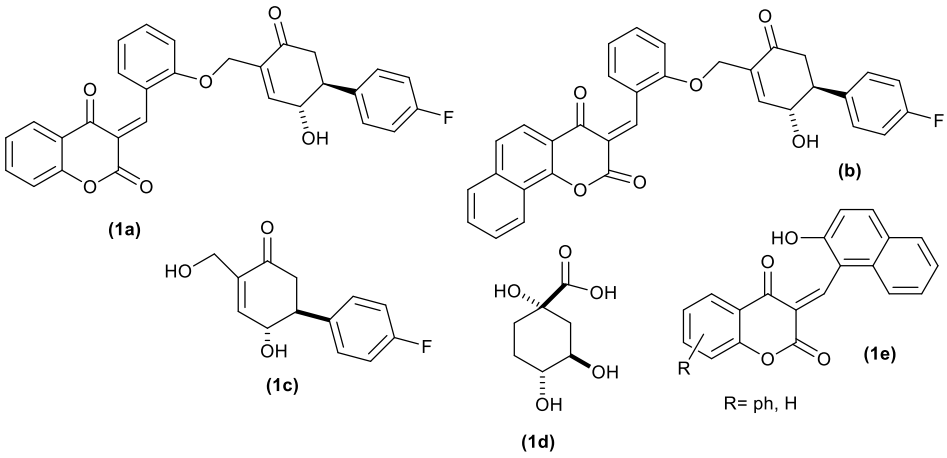


Figure 1. Structures of novel *anti*-cancer drugs (1a and 1b)

One outcome of this research interest has been the discovery of the two *anti*-cancer compounds: crotonyloxymethyl-(*4R,5R,6R*)-4,5,6-trihydroxyclohex-2-enone (COTC, (**2a**), (Takeuchi, *et al*. 1975) and anthemionone A (**2b**) (Collu, *et al*. 2008) (Figure 2).

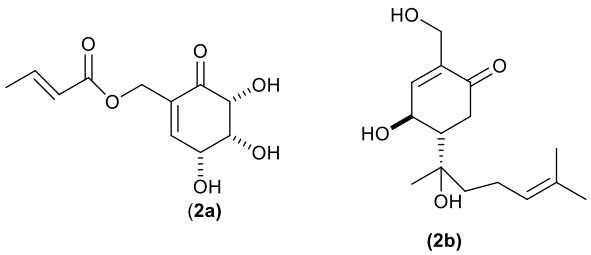
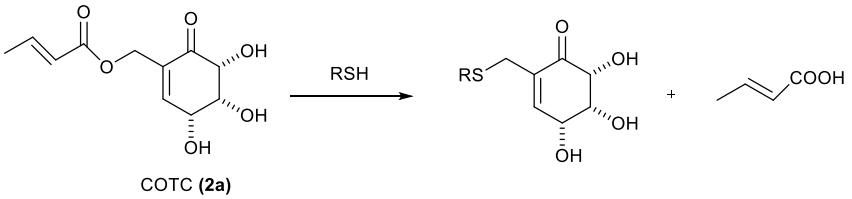


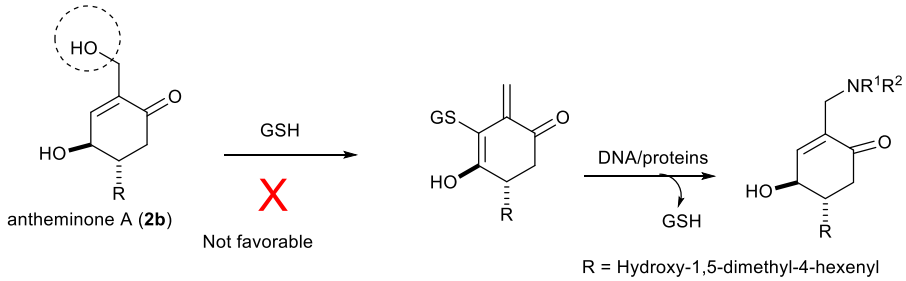
Figure 2. Structure of COTC (2a) and Antheminone A (2b)

According to research conducted by Takeuchi and his team, COTC was found to inhibit glyoxalase 1 enzyme obtained from the rat liver and yeast. Glyoxalase enzymes have been reported to support cell growth and regulation by controlling the level of toxic methylglyoxal produced by normal body metabolism (Szent-Gyorgi, A., 1968). Such inhibition will result in a carcinostatic effect by preventing the metabolism of methylglyoxal in tumor cells and therefore leading to cell apoptosis (French, F. A. and Freedlander B. L., 1958). The *anti*-cancer activity of COTC was a result of the nucleophilic substitution of the crotonate group by an intracellular sulfhydryl (-SH) group, such as that present in glutathione (GSH) (Scheme 1).



Scheme 1. Nucleophilic displacement of the carbonyl moiety of COTC (2a) by sulfhydryl containing compounds

Similar to COTC, the antheminone A (**2b**) also contain the *–α,β*-cyclohex-2-enone moiety. The research conducted by Collu and co-workers revealed that antheminone A showed a remarkable toxicity against all cancer cell lines. It also displayed substantial *anti*-growth activity toward cells related to immune system, such as HL-60, U-937 and Jurkat T, (Arthurs, C. L., 2010). The mode of action of antheminone A is still unknown, although it has a structural relationship with COTC. This assumption is chemically unlikely because the hydroxyl (OH) group is a poor leaving group in comparison to a crotonate group and thus will be less readily displaced by glutathionyl (SG) group (Scheme 2).

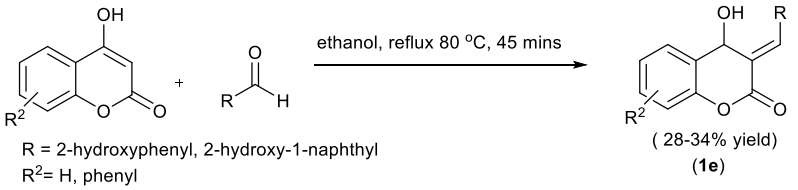


Scheme 2. The assumed mechanism of action of Antheminone A (2b). The reaction is not chemically favorable because OH is a relatively poor leaving group and thus not readily displaced by glutathione (GSH)

2. Result and Discussion

2.1 Synthesis of Unsymmetrical Dicoumarol (1e)

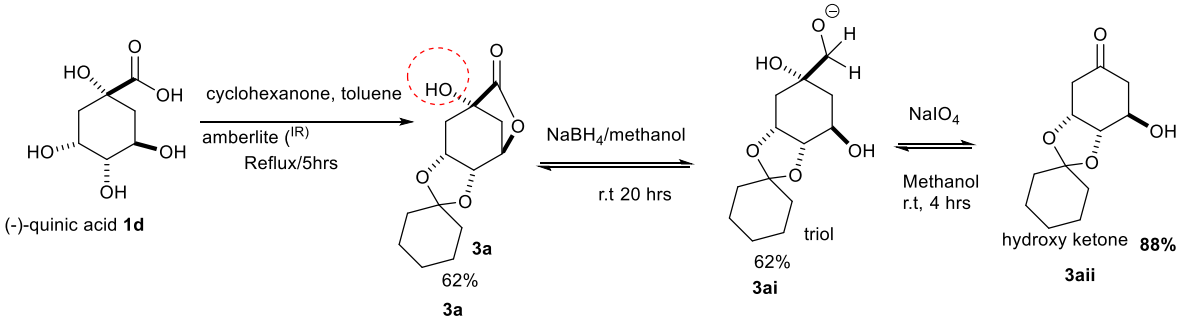
The reactions were carried out by reacting 1 equivalent of an appropriate 4-hydroxycoumarin and 1 equivalent of 2-hydroxyl benzaldehyde under thermal condition (Scheme 3). The yields were generally poor as a result of competitive dimer formation.



Scheme 3. Synthesis of compound (1e)

2.2 Synthesis of Cyclohexylidene Ketal (3a)

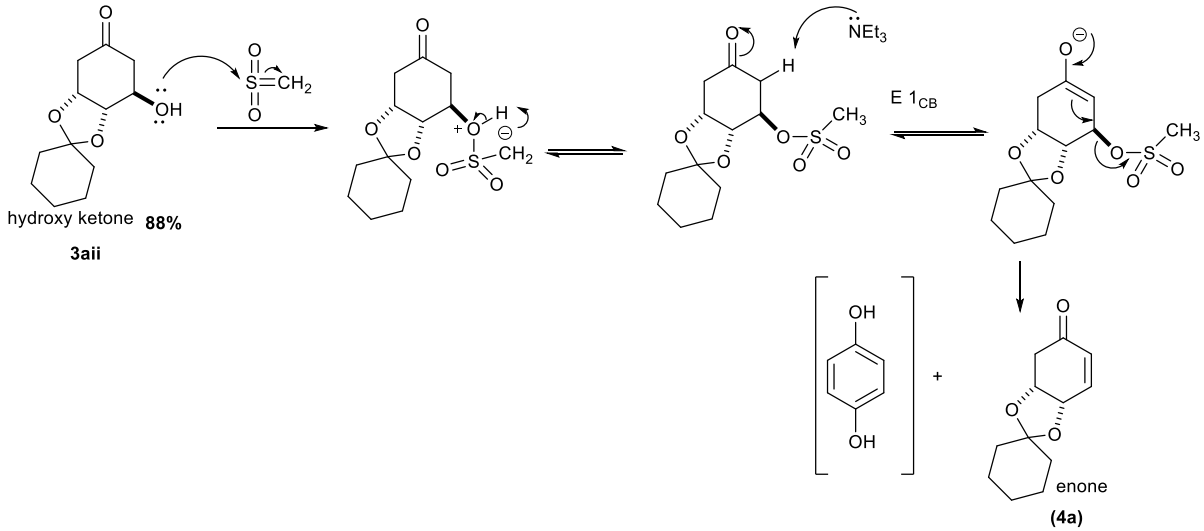
The ϒ-lactone (**3a**) had previously been synthesized by Gero by boiling a mixture of (-)-quninic acid, cyclohexanone and acid catalyst (Amberlite^{TM} IR (120 H) resin in a mixture of benzene and DMF (Gero, D., *et al*. 1971). In this reaction, toluene was used as a reaction medium owing to the health hazard use of benzene (Scheme 4).



Scheme 4. Synthesis of cyclohexylidene ketal (3a)

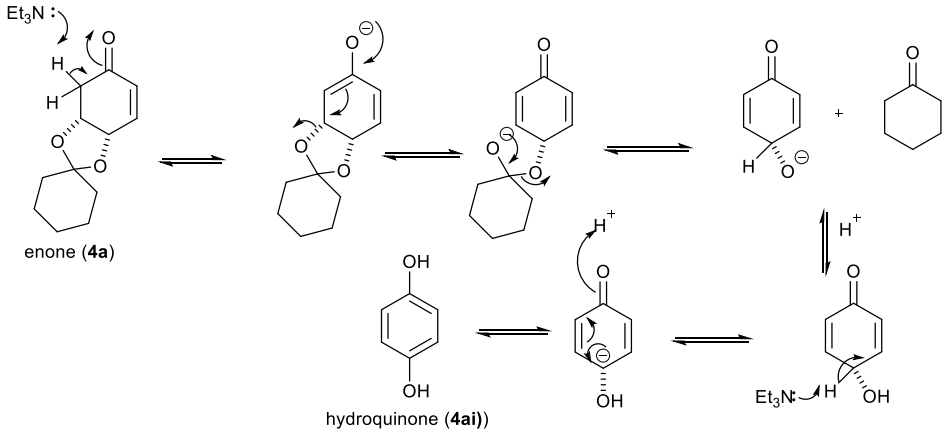
The reductive ring opening of cyclohexylidene ketal (**3a**) was achieved by using the procedure described by Schulz and co-workers (Schulz, J., *et al*. 2000). The cis-1,2-diol group of (-)-quinic acid was selectively protected with cyclohexanone in the presence of an acid catalyst (Amberlite^{TM} IR 120 (H) resin). The reductive ring opening was carried out using mild reducing agent such as $NaBH_4$ in methanol solvent to give triol (**3ai**). The reduction was successful due to the presence of the electron withdrawing hydroxyl group adjacent to the carbonyl which increases electrophilic nature and reactivity towards nucleophilic attack by the hydride ion.

The oxidative cleavage of triol was performed using free flowing silica gel-supported sodium periodate in methanol to give hydroxyl ketone (**3aii).** Elimination of *β*- hydroxyl was carried out using a procedure described by Danishefsky and co-workers (Danishefsky, S. J., *et al*. 1989). Hydroxyl group (OH) is a strong base compared to anion from a strong acid and thus it is a poor leaving group. To change it into a better leaving group, it was reacted with methanedulfonyl chloride in the presence of triethylamine to generate enone (**4a**) as depicted in the mechanism (Scheme 5).



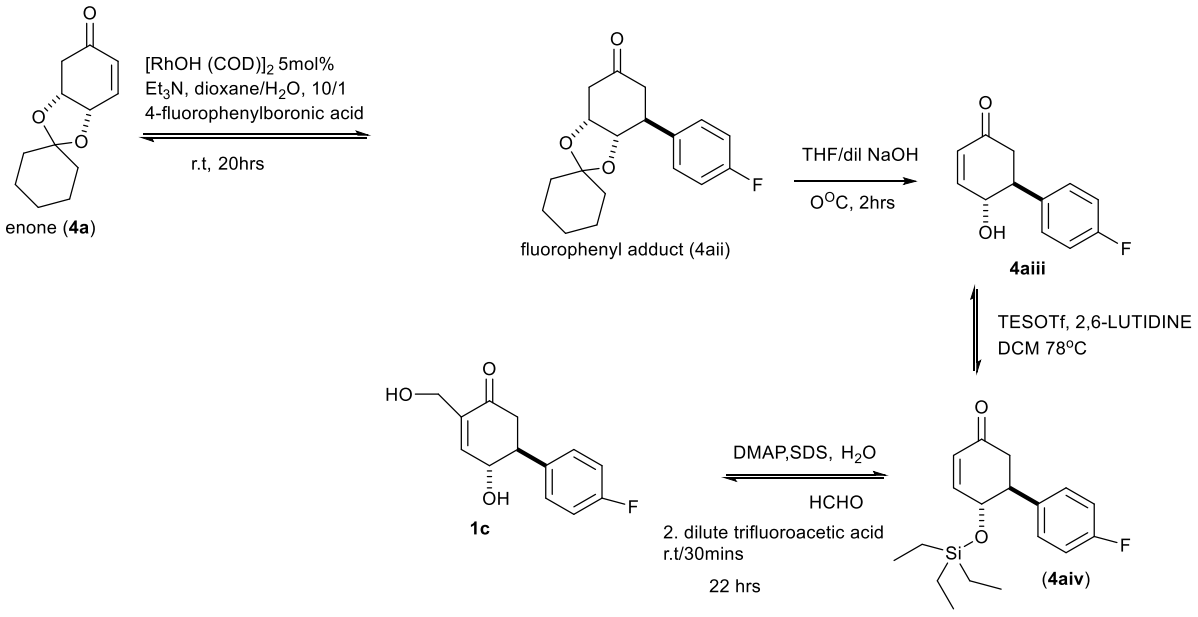
Scheme 5. Mechanism of formation of enone (4a)

A major difficulty was encountered at some stage in the synthesis of enone (**4a**) which was a consequence of the unstable nature of the compound. Thus, to avoid this, both the crude and pure enone were stored in the freezer to slow down any reaction with the residual triethylamine which could lead to elimination of cyclohexanone resulting in aromatization to give hydroquinone (**4ai**) as an impurity (Scheme 6).



Scheme 6. Mechanism of the formation of hydroquinone (4ai)

Also, 1,4-conjugate addition to enone was successfully carried out in the presence of triethylamine and dioxane/H_2O (10:1) to give fluorophenyl adduct (**4aii**) with 74% yield. The hydroxyl group at carbon 4 of (4aiii) was protected using trifluoromethanesulfonate (TESOTf) and 2,6-lutidine in DCM to give silylether (**4aiii**). The application of Morita-Baylis-Hillman reaction was used for the conversion of compound (**4aiv**) to an analogue of antheminone A (**4v**) as described in (Scheme 7). During this reaction, the protected compound (**4aiv**) was converted in a slightly longer period of 22 hours in the presence of SDS, DMAP and water at room temperature.

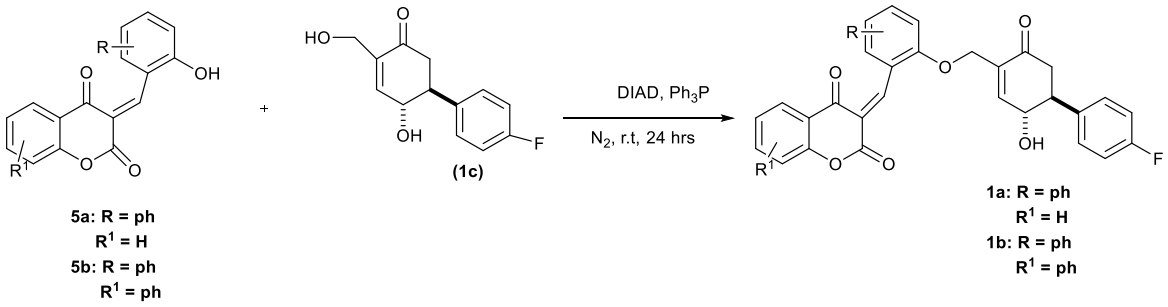


Scheme 7. Synthesis of compound (1c) using Morita-Baylis-Hillman reaction (Williams, C.M., *et al*. 2009)

2.3 Synthesis of Novel Anti-Cancer Drugs

The unsymmetrical dicoumarol (**5a** and **5b**) were previously reported by (Obi, J. C., 2019) as potential inhibitors of NQO1 with IC_{50} : (**5a**= 20 ± 6 nM) and (**5b** = 23 ± 5 nM). To gain access to their site of action, drugs must cross one or more barriers especially the plasma and the intracellular membranes of the cells. But there might be possibility of drug barrier to the target site of these inhibitors due to the phospholipid nature of the cell membrane, causing poor penetration of these inhibitors and hence low toxicity to the cancer cells. In view of this, the analogue of antheminone A (**4v**) were utilized for

the preparation of *anti*-cancer drugs (**1a** and **1b**) using Mitsunobu reaction as depicted in (Scheme 8).



Scheme 8. Synthesis of *anti*-cancer drugs (1a and 1b)

The Mitsunobu reaction which was discovered in 1967, has been used for the conversion of primary and secondary alcohols to esters, ethers, thoethers, etc (Mitsunobu, O. Y., *et al*. 1967). For this reaction to occur, one of the reacting species (the nucleophile) must be sufficiently acidic to enable protonation of the DIAD/DEAD. This will prevent side reactions from occurring.

3. MTT Cell Viability Assay

The IC_{50} value, in terms of enzyme assay, represents the concentration of a drug that is required for 50% inhibition in vitro, whereas, in terms of cytototoxicity, it represents the concentration of a drug required to inhibit the growth of cells by 50%. Thus, for the MTT cell viability assay, it represents the concentration at which half of the cells seeded remain viable at the end of the analysis. These novel drugs (**1a, 1b and 1c**) displayed good potency towards A549 cell line which has up-regulated level of NADH: oxidoreductase quinine 1 (NQO1) activity as shown in (Figure 3).

Finally, the cytotoxicity properties of these novel prodrugs though, are yet to be understood. Since the A549 cell is up-regulated in NQO1 activity, it could be hypothesized that NADH: oxidoreductase quinine 1 (NQO1) played a major role in the toxicity of the drugs.

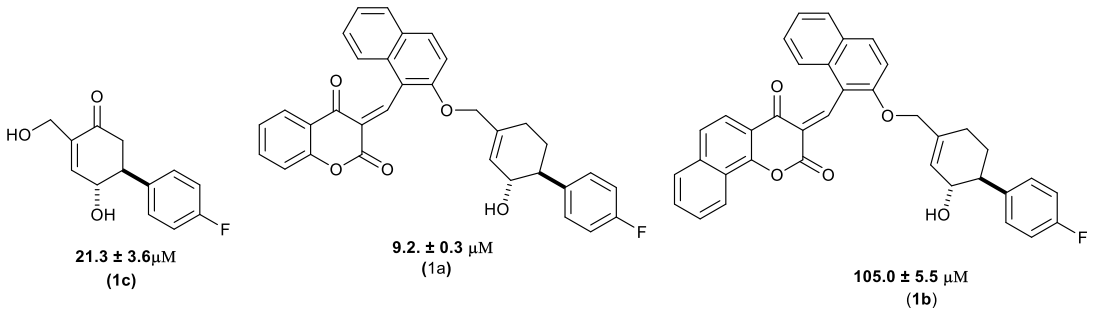


Figure 3. Cytotoxicity values of the prodrugs (1a, 1b, and 1c)

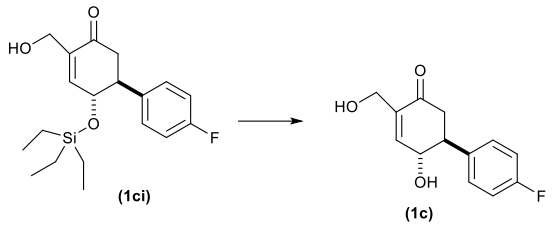
4. Conclusion

The unsymmetrical dicoumarol (**5a** and **5b**) was previously reported by the author as inhibitors of NADH:oxidoreductase quinine 1 (NQO1) were re-modified as prodrugs (**1a** and **1b**) by using a delivery agent related to natural prodruct antheminone A. This re-modification was very important in order to improve drug penetration through the barriers of cell membrane. Remarkably, these produgs exhibited toxicity towards the A549 cancer cell line.

Experimental

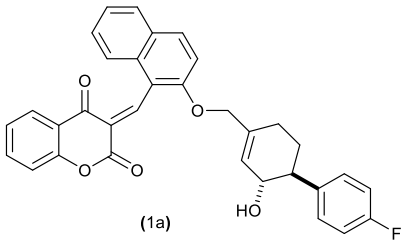
All the reagents used were obtained from commercial sources (Sigma-Aldrich Co., Alfa Aesar and Fisher Scientic). Melting point was measured using a Sanyo Gallenkamp MPD. 350 variable heater instrument and are uncorrected. IR spectra were recorded in solid state using a Bruker Alpha PFT-IR instrument. ^{13}C and ^{1}H NMR were recorded using Bruker Avance 400 spectrometers. Chemical shifts are given in ppm to the nearest 0.01 ppm and referenced to the solvent residual peak. Proton assignments were assisted by DEPT. HCOSY and ^{1}HMQC.

Synthesis of compound (1c).



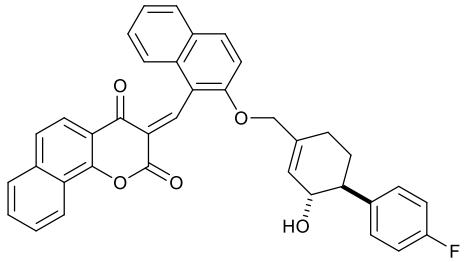
To a suspension of compound ((**1ci**) (49 mg, 0.14 mmol) in TFA: H_2O (7:1. 1 mL) was stirred for 30 mins at room temperature. The solvent were concentrated in *vacuo* to give a crude brown oil which was purified by flash silica chromatography (ethyl acetate : petroleum (2:1) to give compound (1c) as a colorless film (22 mg, 67%): V_{max} (film/cm^{-1} 3347 (br, O-H), 1668 (s, C=O); δ_H (400 MHz; $CDCl_3$) 2.65-2.76 (2H, M), 3.24 (1H, ~q, *J* 9.2), 4.30-4.40 (2H. m), 4.67 (1H, dd, *J* 9.2, 1.5), 6.95 (1H, ~ d, *J* 1.5), 7.06-7.12 (2H, m), 7.26 – 7.29 (2H, m); δ_C (100 MHz; $CDCl_3$) 43.4, 49.9, 61.0, 71.9, 116.0, 129.2, 135.0, 147.9, 198.4; δ_F (376 MHz; $CDCl_3$) -114.2 (s); *m/z* (+ES) 259 ([M + Na]$^+$, 100%; Found 259.0759, $C_{13}H_{13}Na]^+$), requires 259.0746).

Spectral identification of compound (1a).



Compound (1**a**) was isolated as a yellow powder (20.0 mg, 68%), R_f 0.5, [α]D^{29} -65.4 (c 0.5 in CH_2Cl_2): V_{max} (film/cm^{-1} 1726 (C=O), 1676, (1560); δ_H (400 MHz; $CDCl_3$) 2.18 (1H, dd), 2.38 (1H, dd), 2.78 (1H, ddd), 3.92 (1H, br, d), 4.65 (1H, ddd), 4.75 (1H,ddd), 6.72 (1H, d), 6.81-6.84 (2H, m), 696-703 (3H, m), 7.16 (1H, ddd), 7.52-7.59 (2H, m), 7.65 (1H,ddd), 7.78 (2H, ddd), 7.65 (1H, ddd), 7.78 (2H, ddd), 7.95 (1H, d), 8.09 (1H, d), 8.40 (1H, d), 9.02 (1H, s); δ_C (100 MHz; $CDCl_3$) 43.3, 49.4, 56.1, 64.4, 71.6, 104.7, 105.4, 113.1, 115.7, 115.9, 116.8, 121.8, 124.7, 126.6, 129.1, 129.9, 130.2, 131.9, 134.0, 135.8, 142.6, 148.7, 156.5, 158.1, 158.3, 160.6, 190.1, 196.4; δ_F (471 MHz; $CDCl_3$) -114.4 (s); *m/z* (+ES) 535.2 ([M + H]$^+$, 100%; Found 557.1391; $C_{33}H_{23}OFNa$ ([M+Na]$^+$), requires 557.1376).

Spectral identification of compound (1b).



Compound (1b) was isolated as a yellow solid (15.0 mg, 51%), R_f 0.4, [α]D^{29} -72.9 (c 0.45 in CH_2Cl_2): V_{max} (film/cm^{-1} 3426 (C-H), 2923, 1708 (C=O), 1668, 1647; δ_H (400 MHz; $CDCl_3$) 1.88 (1H, dd), 2.22 (1H, dd), 2.61 (1H, ddd), 3,77 (1H, dd), 4.60 (1H, ddd), 4.62 (1H,ddd), 6.81 (2H, dd), 7.01 (2H, t), 7.56-7.59 (2H, m), 7.76-7.87 (3H, t), 7.47 (1H, m), 7.87 (3H, m), 7.94 (1H, d), 8.00 (1H, d), 8.12 (1H, d), 8.31 (1H, d), 8.91 (1H, s); δ_C (100 MHz; $CDCl_3$) 42.7, 49.0, 71.4, 72.4, 113.1, 115.7, 115.9, 117.2, 121.8, 123.1, 125.3, 125.5, 126.8, 127.7, 128.0, 128.3, 128.8, 128.9, 129.1, 129.3, 129.6, 134.1, 134.9, 137.3, 139.2, 148.2, 155.1, 155.8, 158.6, 162.1, 166.4, 191.5, 196.0; δ_F (376 MHz; $CDCl_3$) -114.5 (s); *m/z* (+ES) 607.2 ([M + Na]$^+$, 100%; Found 607.1542, $C_{37}H_{25}NaF$([M+Na]$^+$), requires 607.1533).

5. Conclusion

The unsymmetrical dicoumrol (5a and 5b) which was previously reported by the author as inhibitors of NADH: oxidoreductase quinine 1 (NQO1) were re-modified as prodrugs (1a and 1b) by using a delivery agent related to natural product antheminone A. The re-modification was very important in order to improve drug penetration through the barriers of cell membrane. Amazingly, these prodrugs exhibited toxicity towards the A549 cancer cell line.

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