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SYNTHESIS AND BIOLOGICAL ACTIVITY OF INTERCALATOR SUBSTITUTED BILE STEROIDS

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Abstract. Cholic acid has been functionalised at the 3 and 24 positions with quinoline and quinoxaline chromophores. The 24-quinoline monoester shows significant activity against L1210 mouse leukaemia cells compared to the 3,24-disubstituted quinoline and quinoxaline esters.

The design and synthesis of DNA bisintercalators has attracted much interest due to the potential of these systems to increase binding selectivity and affinity for DNA.[1,2] Studies carried out on synthetic bisintercalators have shown that ligand rigidity plays a major role in determining sequence specificity, the mode of interaction with DNA, and antitumor activity.[2] From a design perspective, the linker in a DNA bisintercalator should be sufficiently rigid to prevent aggregation and formation of intermolecular complexes, and ideally should contain DNA binding groups, which may increase the binding constant of the drug-DNA complex (although this is not a necessary condition), and provide a mechanism whereby sequence selectivity may be achieved. The vast majority of synthetic bisintercalators reported[2] incorporate flexible linkers in their structures that serve only the role of separating the chromophores.

This paper reports the synthesis of potential DNA binding compounds based on bile steroids. Intercalators were attached to derivatives of cholic acid as (i) steroids provide a curved, rigid backbone for attachment of intercalators in a defined geometry (ii) physical association *in vitro* between DNA and certain classes of steroids has been reported,[3] and (iii) interaction with DNA has been implicated in the mutagenic action of certain bile steroids.[4] Intercalators have been combined previously with DNA-active functionalities such as alkylating agents,[5] platinum complexes[6] and monointercalators.[7]

Functionalisation of cholic acid was carried out in two ways to give steroids **4**, **5**, and **6** which have the same intercalators at positions 3 and 24, and steroid **7**, which contains different intercalators at positions 3 and 24. Thus, reduction of cholic acid (BH_3/THF or $LiAlH_4$) afforded the tetrahydroxy steroid **1** in 85% yield. Treatment of **1** with 4-5 equivalents of 2-quinoxaloyl, 2-quinolinoyl and 3-quinolinoyl chlorides (THF/NEt_3) afforded the corresponding bisesters **4**, **5** and **6** respectively, which were purified by chromatography (silica, acetone/light petroleum 1:4). However, the products were obtained in poor yields (20-30%). The preferred route to these compounds involved conversion of **1** to the trifluoroacetate tetraester (triflouroacetic anhydride/ether), followed by selective removal of the trifluoroacetyl groups at positions 3 and 24 ($MeOH/H_2O/NaHCO_3$) to give the 3,24-diol **2** (Scheme 1). This compound has improved solubility compared to the parent compound **1**. Treatment of **2** with 2 equivalents of the appropriate acid chloride (NEt_3/THF) afforded the corresponding bisesters. Selective removal of the trifluoroacetyl groups (NH_3/H_2O/THF) afforded **4**, **5** and **6** in >95% yield.[8] A similar route was used to prepare the 24-ester **3**.

The dihydroxy steroid **2** also allows entry into systems with different intercalators at positions 3 and 24. Thus, reaction of **2** with 1 equivalent of 2-quinolinecarboxylic acid chloride in the presence of NEt_3 afforded the 24-quinolyl ester. Reaction with a further equivalent of 2-quinoxalinecarbonyl chloride followed by removal of the trifluoroacetyl groups afforded compound **7**.[8] In principle, steroids such as **7** are also accessible directly from the tetrahydroxy steroid **1** by successive reaction with 1 equivalent of two different carboxylic acid chlorides. However, the poor solubility of the tetrahydroxy steroid **1** in solvents suitable for the reaction led to experimental difficulties and only poor yields of products.

Preliminary biological testing of steroids **3-7** against mouse leukaemia L1210 cells has been carried out. For the bisintercalator steroids **4-7**, the IC_{50} values for continuous 48 hours drug exposure in a growth inhibition assay were all in the range 20 to > 50 μM. However, steroid **3**, in which only one intercalator is attached to position 24, showed significant activity with an IC_{50} value of 4.8 μM. For comparison, cholic acid and quinoline gave IC_{50} values of > 50 μM. These results are somewhat surprising, but suggest that selective modification of the 24-position of these steroids by attachment of an intercalator may lead to a potential new class of antitumor agents. Studies are now focused on related 24-substituted steroids in order to elucidate their mechanism of action, as well as extension of the biological testing to include breast and ovarian cell lines.

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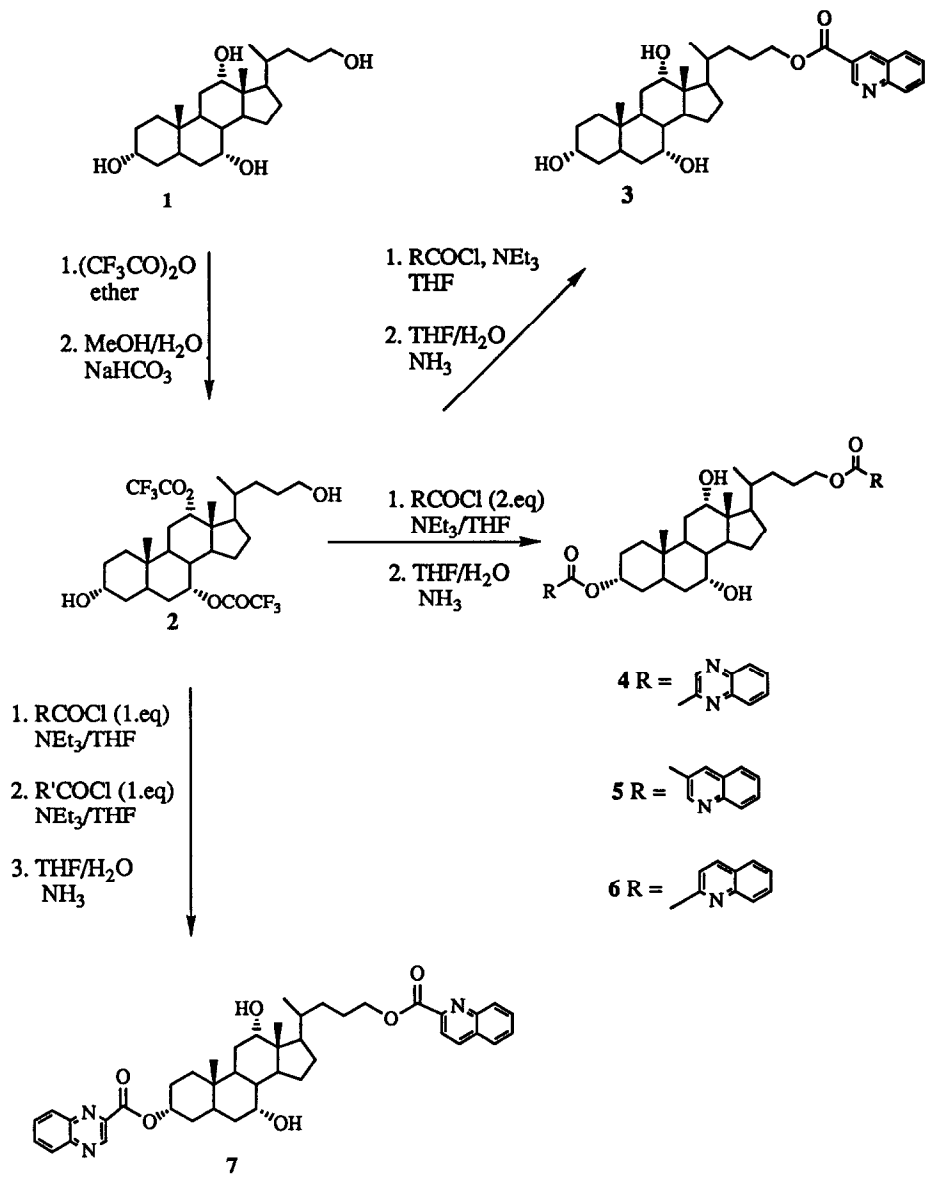
1
3
1.(CF3CO)2O
ether
2. MeOH/H2O
NaHCO3
1. RCOCl, NEt3
THF
2. THF/H2O
NH3
2
1. RCOCl (2.eq)
NEt3/THF
2. THF/H2O
NH3
4 R =
5 R =
6 R =
1. RCOCl (1.eq)
NEt3/THF
2. R'COCl (1.eq)
NEt3/THF
3. THF/H2O
NH3
7

SCHEME 1

References and Notes

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8. Selected 600 MHz ^{1}H NMR data ($CDCl_3$) and mass spectral data (FAB or ES); full details and assignments will be reported elsewhere.

3 δ 0.64 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.99 (d, *J* 6.6 Hz, 21-CH_3), 3.35-3.42 (m, 3-H), 3.79 (s, 7-H), 3.94 (s, 12-H), 4.30-4.39 (m, 24-H); FAB-MS 550.3 $(M + H)^+$.

4 mp 175-8°C; δ 0.73 (s, 18-CH_3), 0.97 (s, 19-CH_3), 1.07 (d, *J* 6.6 Hz, 21-CH_3), 2.32 (td, *J* 12 Hz, 4.3 Hz), 2.66 (app.q, *J* 12.7 Hz), 3.89 (q, 7-H), 4.04 (t, 12-H), 4.47-4.55 (m, 24-H), 5.03 (tt, *J* 11.4 Hz, 4.4 Hz, 3-H); FAB-MS 708.7 $(M + 2\ H)^+$.

5 mp 142-5°C; δ 0.75 (s, 18-CH_3), 0.97 (s, 19-CH_3), 1.07 (d, *J* 6.6 Hz, 21-CH_3), 2.23 (td, *J* 12.2 Hz, 4.6 Hz), 2.58 (app.q, *J* 12.7 Hz), 3.90 (q, 7-H), 4.06 (t, 12-H), 4.37 - 4.45 (m, 24-H), 4.94 (tt, *J* 11.3 Hz, 4.4 Hz, 3-H); FAB-MS 705.5 $(M+ H)^+$.

6 δ 0.72 (s, 18-CH_3), 0.97 (s, 19-CH_3), 1.05 (d, *J* 6.6 Hz, 21-CH_3), 2.32 (td, *J* 12.2 Hz, 4.6 Hz), 2.64 (app.q, *J* 12.6 Hz), 3.88 (q, 7-H), 4.03 (t, 12-H), 4.40-4.50 (m, 24-H), 4.99 (tt, *J* 11.4 Hz, 4.4 Hz, 3-H); FAB-MS 705.5 $(M + H)^+$.

7 δ 0.732 (s, 18-CH_3), 0.96 (s, 19-CH_3), 1.05 (d, *J* 6.6 Hz, 21-CH_3), 2.32 (td, *J* 12 Hz, 4.8 Hz), 2.64 (app.q, *J* 12.7 Hz), 3.88 (q, 7-H), 4.03 (t, 12-H), 4.42 - 4.52 (m, 24-H), 5.03 (tt, *J* 11.6 Hz, 4.5 Hz, 3-H); ES-MS 706.3 $(M + H)^+$.

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