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Synthesis and antitumor activity of alkylated bile acids and oxazolines

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ABSTRACT

Bile acid derivatives with a free carboxylic group or an oxazoline ring in the side chain and with different lengths of alkyl chains on steroid skeleton were synthetized and their antitumor activity against six human cancer cell lines was investigated. Methyl, ethyl, butyl or octyl chains were introduced stereoselectively by Grignard reaction at C-7 of acid and oxazoline, and at C-12 of oxazoline. Carbonyl group at C-12 of acid compound gave addition product only with methyl Grignard reagent, and complex mixture of products with other used reagents. Due to enolization, the C-3 carbonyl group did not participate in the Grignard reaction. Steric reasons are a main cause of this chemical behavior. Compounds with a butyl chain at the C-7 position showed very good antitumor activity with $IC_{50} < 5$ μM.

1. Introduction

Perception of bile acids (BAs) as merely lipid emulsifying and solubilizing molecules was fundamentally changed after discovery of their hormonal roles. BAs are natural agonists for a number of nuclear and transmembrane receptors involved in a variety of metabolic functions, ranging from BA biosynthesis to energy homeostasis.[1] These findings considerably broadened therapeutic potential of BA and their derivatives.

Relation of hydrophobic/hydrophilic area of BA is important for their biological properties. Hydrophobic BAs, such as secondary BAs, deoxycholic acid (DCA) and lithocholic acid (LCA) which lack one hydroxyl group relative to corresponding primary BAs, can induce apoptosis and have been implicated as tumor promoters.[2,3] BAs with oxo groups have lower membranolytic activity then their hydroxyl analogues.[4,5] Also, obeticholic acid, semisynthetic BA, with ethyl group connected to steroid C-6 is a strong farnesoid X receptor (FXR) agonist, and in the final phases of clinical studies for treatment of some liver diseases.[6,7] In our previous study we have showed that introduction of an ethylidene group at the C-7 position on the steroid skeleton greatly influenced antitumor activity of these compounds.[8] Perception of BA steroid skeleton as a pharmacophore, and findings that introduction of hydrophobic groups on BA steroid skeleton can significantly influence biological activity prompted us to investigate the most common way to change hydrophobicity-introduction of alkyl groups. This work should give insights into specific chemical behavior of bile acid steroid skeleton in alkylation reactions with Grignard reagents and in vitro antitumor activity of alkyl derivatives.

2. Results and discussion

2.1. Chemistry

In order to investigate the possibilities of bile acid alkylation we decided to employ Grignard reactions as they are simple, cheap and fast. Also we decided to try Grignard reactions on bile acid derivatives with free carboxylic groups, as well as with protected carboxylic groups in form of oxazoline. For the starting compound we used cheap and commercially available cholic acid (CA). By known procedures we oxidized CA in corresponding 7-oxo (**1**), 12-oxo (**2**) and 3-oxo (**3**) derivatives.[9–11] 7,12-Diformyloxy-3-oxo compound **4** was obtained by known method.[12]

Protection of carboxylic group in the form of oxazoline can resolve low solubility of bile salt in ether solvents and potential

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problems of carboxylic carbon participation in Grignard reaction. The synthesis of bile oxazoline **5** is described in our previous publication.[8] By similar manner we have synthesized 12-oxo **6** and 3-oxo **7** oxazoline in good yields (Scheme 1.). The sequence included formylation (**8**), condensation of carboxylic group with 2-amino-2-methyl-1-propanol (**9**, **10**) using EEDQ as coupling agent followed by cyclization to oxazoline (**11**, **12**) ring by treatment with thionyl chloride and deformylation (**6** and **7**).

Bile acid derivatives, 7-oxo bile acid **1** and 7-oxo bile oxazoline **5**, were subjected to Grignard reaction with methyl, ethyl, butyl and octylmagnesium halides. Reaction condition, products and yields of reactions are shown in Scheme 2.

Both oxo derivatives, **1** and **5,** gave corresponding alkyl adducts in good yields. However, adducts with free carboxyl group are formed in better yields then oxazolines with similar reaction conditions. Reaction times were slightly shorter for **5** then for **1** probably due to better solubility of oxazolines in THF. Reactions with Grignard reagent were carried out in dry THF on temperature of −20 °C. When higher temperature was applied oxazoline ring was partially hydrolyzed into amides **21**, **22** and **23** (Fig. 1.), though alkylation of C-7 carbonyl group still occurs.

Yields of alkylated amides at −20 °C were less than 8%, while at reflux yields of byproduct were increased up to 26%. Alkylation reactions were stereoselective, and only one epimer was detected. Determination of configuration in steroid skeleton is always challenging, especially in the case when newly introduced substituent is alkyl group. NOE NMR experiments usually give ambiguous results due to considerable overlap of signals in NMR spectra, also bile acid derivatives with long hydrocarbon chains are hard to crystallize. Fortunately, we succeeded to crystallize carboxylic derivate with methyl group **13**, and determined configuration at C-7 by X-ray crystallography experiment. Structure of **13** shown in Fig. 2, shows that methyl group at C-7 is β orientated. This configuration was expected considering considerable steric hindrance of α side of steroid by A ring, and β methyl group has thermodynamically favorable equatorial orientation.

If a nucleophile approaches from the α side of the steroidal skeleton the maximal overlap of HOMO orbitals of nucleophile and LUMO orbitals of carbonyl group would result in 105°–107° angle of attack (Bürgi-Dunitz angle α_{BD}, Nu-C(carbonyl)-O(carbonyl)). This would make nucleophilic carbon of Grignard reagent in syn-axial orientation with C-4, C-9 and C-12 hydrogens. That means that there is no variance in Flippin-Lodge angle (α_{FL}) in α-azimuth plane, and that nucleophile attack from α side is excluded. While in attack from β side at $\alpha_{BD} \approx 107°$ angle Flippin-Lodge angle (β-azimuth plane) shows maximal variance, i. e. attack is not sterically inhibited (Appendix A – **Supplementary data**).

Example of **13** gave enough evidence that is safe to conclude, with considerable degree of certainty, that other 7-alkyl derivatives have also β-orientated alkyl groups.

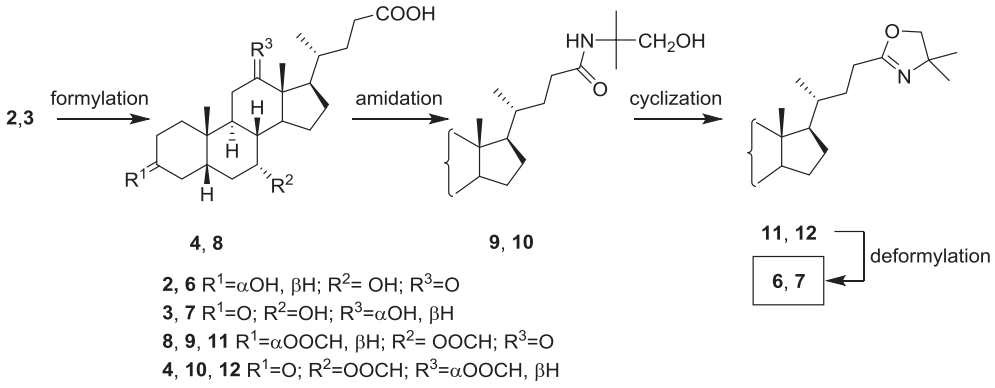
Somewhat different results were obtained in the reaction of 12-oxo BA **2** or 12-oxo oxazoline **6** with Grignard reagents. Reaction condition, products and yields of these reactions are shown in Scheme 3.

Alkylation of 12-oxo BA was only successful with MeMgI, and **24** was isolated in moderate yield. Reactions of **2** with EtMgBr and BuMgCl were carried out at three temperatures: at −20 °C after 2 h the reaction did not occur; at 0 °C or room temperature (RT) after 5 h complex mixture of products was obtained with CA as main product (10% with BuMgCl). In reaction with EtMgBr reaction mixture was not purified, and CA as main product was detected by TLC check.

Oxazoline **6** with MeMgI at 0 °C after 30 min gave only adduct **25** but with EtMgBr and BuMgCl, at −20 °C and then at 0 °C, beside alkylated products **26** i.e **28**, gave also reduced product **27**. Reduced product **27**, was created as a result of the reduction of the keto group with Grignard reagent, and it is related to steric hindrance of C-12 carbonyl group.[12] Reaction of **6** with OctMgBr was carried out at 0 °C for 3 h, and only octyl product **29** was isolated. These findings show that higher reaction temperatures (0 °C) disfavors reduction reaction, and only addition of alkyl group occurs.

Configuration at C-12 of **24** was determined by ROESY NMR experiment, and a strong cross-peak between methyl groups on C-12 and C-13 indicate β orientation of C-12 methyl group. Same stereochemistries at C-12 presumably have all C-12 alkyl derivatives, as Flippin-Lodge angle in α-azimuth plane tends to zero value (Appendix B **- Supplementary data**).

The difference in the chemical behavior between 7-oxo and 12-oxo BAs is attributed to a lower steric accessibility of C-12 carbonyl carbon. In this way it could be also explained different reactivity of these groups in Grignard reaction. The Grignard reagent reacted relatively quickly with the C-7 carbonyl group of **1**, to give the corresponding addition product, while in the case of **2** only methyl adducts were isolated in poor yield, and ethyl- and butyl-reagents gave complex mixture of products. Approach of the alkylmagnesium halide reagent to C-12 carbonyl of **2** is hampered by C-18 methyl group, therefore reaction occurs only at the higher temperatures (RT), and at higher temperatures side reactions also occur. Weakly reactive carboxylic carbon C-24 undergoes addition reactions on room temperature, which with the combination of C-12 adducts and reduction of C-12 carbonyl group leads to complex mixture of products. Smaller value of Flippin-Lodger angle in β-azimuth plane of C-12 carbonyl makes successful collision with Grignard reagent molecules only with molecules that approach



Scheme 1. Synthesis of oxazoline derivatives.