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DIGITALIS-LIKE COMPOUNDS: SYNTHESIS AND BIOLOGICAL EVALUATION OF 3β-(AMINOALKYLTHIO) DERIVATIVES

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Abstract: The synthesis and the binding affinities to the digitalis receptor on the Na^+,K^+-ATPase of a series of 3β-(aminoalkylthio) digitalis derivatives are described. In most cases these derivatives showed a higher binding affinity than that of the corresponding 3β-hydroxy and 3β-mercapto parent compounds.

Digitalis cardiac glycosides are well known drugs for treatment of congestive heart failure;[1] their action is mainly due to inhibition of Na^+,K^+-ATPase.[2] The most potent inhibitors of Na^+,K^+-ATPase are natural products such as ouabain, digoxin, digitoxin, and digitoxigenin (**1**, **Scheme 1**); some of them or their derivatives are still widely used today, although their toxicity makes their use not completely safe.

Recently the search for endogenous digitalis-like factors that may be responsible for essential hypertension[3] has opened a new field in the study of compounds acting on the Na^+,K^+-ATPase. The H_1-H_2 and H_3-H_4 domains of the α-subunit of Na^+,K^+-ATPase are believed to be the binding site of digitalis steroids. These domains contain six free carboxylic groups that would give several binding possibilities for basic ligands.[4] A single example of a digitalis-like derivative bearing a nitrogen-containing side chain at 3β position has been described, namely the *N*-(4'-amino-*n*-butyl)-3-aminoacetyl derivative of strophanthidin,[5] compound that showed a better affinity compared with the parent genin.

With the aim to study in depth this hypothesis we designed, as a first approach, the synthesis of 3β-(aminoalkyloxy) derivatives of digitoxigenin, but these compounds were found very difficult to obtain owing to the presence of the α,β-unsaturated lactone in 17β position, that was unstable in all the reaction conditions used e.g. those of Williamson reaction. Yields were in any case lower than 20%.

We then turned our attention to the synthesis of 3β-(aminoalkylthio) derivatives of digitoxigenin **1** by alkylation of the previous unknown 3β-mercapto-14β-hydroxy-5β-card-20(22)-enolide **5**, assuming that this time the reaction conditions would be compatible with the presence of an α,β-unsaturated lactone. Digitoxigenin **1** was oxidised to digitoxigenone **2**[6] (89% yield) with TPAP/NMO in CH_2Cl_2 at room temperature;[7] the reduction of **2** with $NaBH_4$ in dioxane/water at room temperature gave the equatorial 3α-hydroxy derivative (3-epi-digitoxigenin) **3**[6] in 92% yield. The thioacetyl derivative **4** was obtained from **3** by a Mitsunobu[8] reaction with thioacetic acid in the presence of DIAD (64% yield on the reacted starting material; 50% of it was recovered unreacted)[9] or in two steps through the methansulfonate ester of **3** and potassium acetate in DMF (62% overall yield), as described by Abramson.[10]

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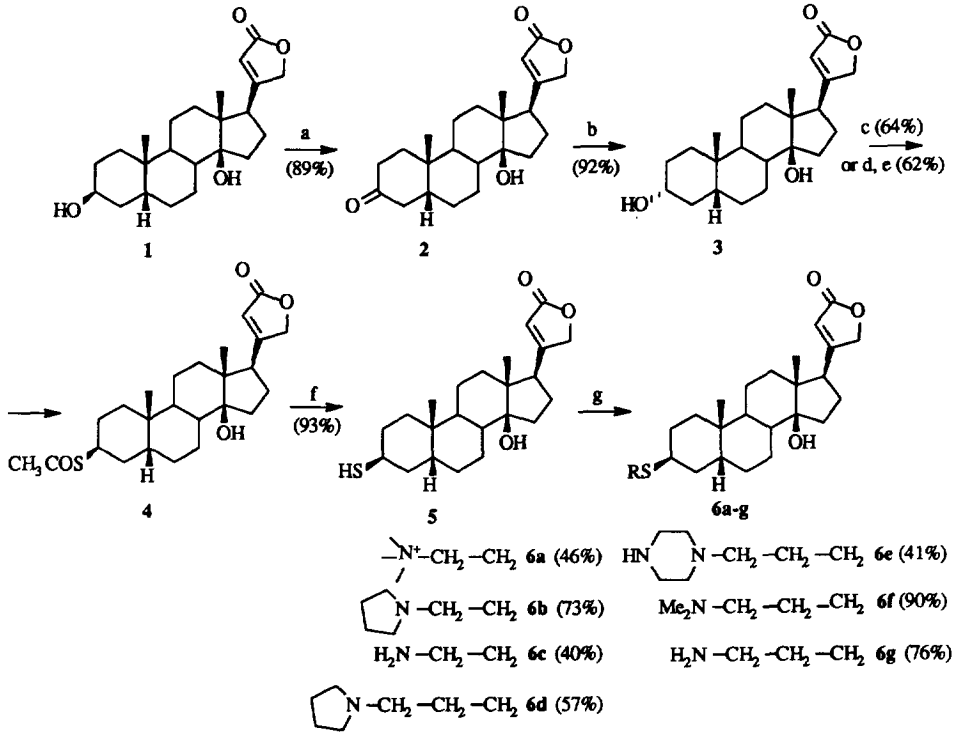
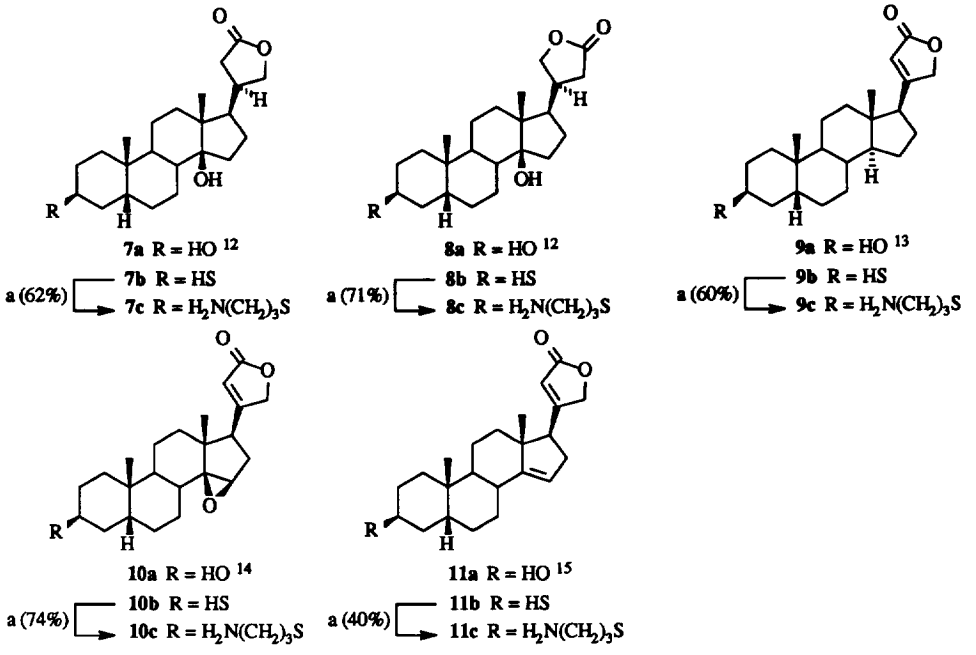
Scheme 1



Reagents and conditions: a: TPAP, NMO, CH_2Cl_2, rt; b: $NaBH_4$, dioxane/water, rt; c: thioacetic acid, DIAD, triphenylphosphine, DMF, rt to 60 °C; d: methanesulfonyl chloride, pyridine, 0 °C; e: potassium thioacetate, DMF, 40 °C; f: NH_3 gas., MeOH/THF, rt; g: R-Cl, NaH, DMF, 0 °C to rt.

The presence of the α,β-unsaturated lactone did not permit to obtain the thiol derivative **5** by normal cleavage conditions of thioacetyl group e.g. aqueous NaOH or KOH or non aqueous bases such as MeONa, EtONa or BuLi, or the use of reducing agents such as $LiAlH_4$. The presence of a labile tertiary hydroxy group in position 14β prevented also the use of aqueous acids such as HCl, HBr. The thiol derivative **5** could be obtained in 93% yield, by bubbling anhydrous ammonia into a MeOH/THF solution of compound **4**, from which oxygen had been accurately removed with a nitrogen stream.[11] The thiol derivative **5** could be easily alkylated with various aminoalkyl chlorides to the desired 3β-(aminoalkylthio) derivatives **6a-g** in 40-90% yields after chromatography: the reactions were conducted in carefully deoxygenated DMF, in the presence of sodium hydride (1.0 equivalent) and the specific aminoalkyl chloride (2.0 equivalents). The different digitalis aglicones 3β-mercapto-14β-hydroxy-5β-20(R)-cardanolide **7b**, 3β-mercapto-14β-hydroxy-5β-20(S)-cardanolide **8b**, 3β-mercapto-5β,14α-card-20(22)-enolide **9b** and 3β-mercapto-14β,15β-epoxy-5β-card-20(22)-enolide **10b** reported in **Figure 1** were synthesized in a similar way as described in **Scheme 1**, starting from the corresponding 3β-hydroxy or 3-oxo parent compounds. The 3β-mercapto-5β-carda-14,20(22)-dienolide **11b** was synthesized starting from compound **4** by dehydration with thionyl chloride (85% yield) and subsequent ammonolysis.

Figure 1



Reagents and conditions: a: 3-aminopropyl chloride, NaH, DMF, 0 °C to rt.

From the 3β-mercapto aglicones **7b-11b** the 3β-(aminopropylthio) derivatives **7c-11c** were prepared as described above;[16] the aminopropyl chain was chosen as the standard derivative for verify the effect of this substitution on the binding affinity of different digitalis steroids. The binding affinities for the digitalis receptor site of the Na^+,K^+-ATPase of the 3β-(aminoalkylthio) derivatives as well as of the corresponding 3β-hydroxy and 3β-mercapto parent compounds are listed in **Table 1**.

Table 1 Binding affinity on Na^+,K^+-ATPase

Compound	Binding[a] -log IC_{50}	Compound	Binding[a] -log IC_{50}
1	7.2	8a	5.8
5	6.0	8b	5.5
6a	7.0	8c	6.5
6b	6.7	9a	5.2
6c	7.5	9b	NA[b]
6d	6.8	9c	5.8
6e	6.5	10a	6.6
6f	7.0	10b	5.6
6g	7.1	10c	6.6
7a	5.6	11a	5.5
7b	5.0	11b	NT[c]
7c	6.5	11c	5.8

[a] Average of three values. The affinity for the receptor site of Na^+,K^+-ATPase was evaluated by the displacement of the specific [^{3}H]-ouabain binding from Na^+,K^+-ATPase receptor[17] isolated from dog kidney and purified according to Jørghensen.[18] [b] NA: compound that did not reach the half-maximal displacement at the maximal concentration tested (10^{-5} M). [c] NT: this compound showed an insufficient stability in solution.

All the aminoalkylthio derivatives of digitoxigenin had a higher affinity than the thiol derivative **5**. The better results were obtained with primary aminoalkylthio derivatives **6c** (twice the affinity of the parent compound) and **6g** (about the same affinity of the parent compound); the tertiary amino derivatives had also good affinities, with the dimethylamino **6f** showing the best one and the quaternary ammonium compound **6a** with approximately the same affinity as the tertiary amino derivatives; a second amino function in the piperazinyl derivative **6e** sensitively reduced the affinity. The aminopropylthio derivatives of the other compounds of **Figure 1** showed a common feature i.e. an affinity higher than the corresponding parent 3β-hydroxy (except for **10a**) and 3β-mercapto derivatives. As the 17β-unsaturated lactone and the 14β-hydroxy group are two of the peculiar characteristic of digitalis steroids[19] it is noteworthy that digitalis derivatives with a saturated lactone substituent **7c** and **8c**, had binding affinities only 5 times lower than digitoxigenin and that the affinities of compounds without the 14β-hydroxy group **9c** and **11c** were close to micromolar.

In summary we have synthesized a series of 3β-(aminoalkylthio) digitalis derivatives that in most cases showed a higher binding affinity than that of the corresponding 3β-hydroxy and 3β-mercapto parent compounds; these results are in accordance with the hypothesis that the presence of six free carboxylic groups in the H_1-H_2 and H_3-H_4 domains of the α-subunit of Na^+,K^+-ATPase, that are believed to be the binding site of digitalis steroids, give several binding possibilities for basic ligands.

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