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Review Article

Simplified Digitalis-like Compounds acting on Na^+, K^+-ATPase

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Digitalis compounds are used in the treatment of congestive heart failure as positive inotropic agents; their action is mainly due to the inhibition of Na^+,K^+-ATPase. A well-known drawback is their arrhythmogenic potential. Attempts to find safer digitalis-like compounds by means of molecular simplifications of the typical 5β,14β-steroidal skeleton, which appeared in the medicinal chemistry literature from 1990 until 2002, are briefly reviewed. Several novel achievements were obtained in order to better understand the requisites of the digitalis binding site on Na^+, K^+-ATPase. Only minor simplification, such as cleavage of the D ring of the digitalis skeleton, could preserve the desired inotropic activity, while highly simplified digitalis-like compounds failed to give sufficiently high inotropic potency, even in the presence of a powerful pharmacophore, such as the *O*-aminoalkyloxime group.

Keywords: Simplified Digitalis-like compounds; Na^+, K^+-ATPase; Congestive heart failure; Inotropic

INTRODUCTION

Digitalis compounds are used in the treatment of congestive heart failure as positive inotropic agents. Heart failure is the pathophysiological state in which the heart is unable to pump blood at a rate adequate for the requirements of metabolism. When the term congestive is added, it means that there is congestion of the systemic and pulmonary venous circulation due to ventricular failure. Patients with heart failure display symptoms of fatigue, breathlessness, and peripheral oedema which severely limit their normal activities.

Congestive heart failure affects 1–2% of the population and even 10% of the very elderly.[1] The primary defect is a loss of cardiac contractile function. Therapy is addressed at improving the pumping performance of the heart and reducing cardiac load by reducing vasoconstriction. Different drugs[2] are available to counteract the decline of the pump efficiency: inotropic agents, such as digitalis glycosides, to improve cardiac output; diuretics and salt restriction to reduce systemic congestion; vasodilators to reduce vascular resistance and hence the afterload.

DIGITALIS INOTROPIC AGENTS

Digitalis compounds are used to improve the pumping performance of the heart. Their action is mainly due to inhibition of Na^+,K^+-ATPase, a plasma membrane bound enzyme which promotes the outward transport of Na^+ and the inward transport of K^+. When it is inhibited, Na^+ concentration inside the cell is increased and, as a consequence, Ca^{++} is introduced by exchange with Na^+, through the Na^+, Ca^{++} exchanger. The final result is a higher concentration of Ca^{++} available to activate contraction.

Among digitalis compounds, digoxin (Figure 1) is the most prescribed drug. Digoxin can improve the symptoms, the exercise tolerance, and reduce the hospitalization, while it has a neutral effect on mortality.[3] Although the incidence and severity of digitalis intoxication are decreasing, vigilance is

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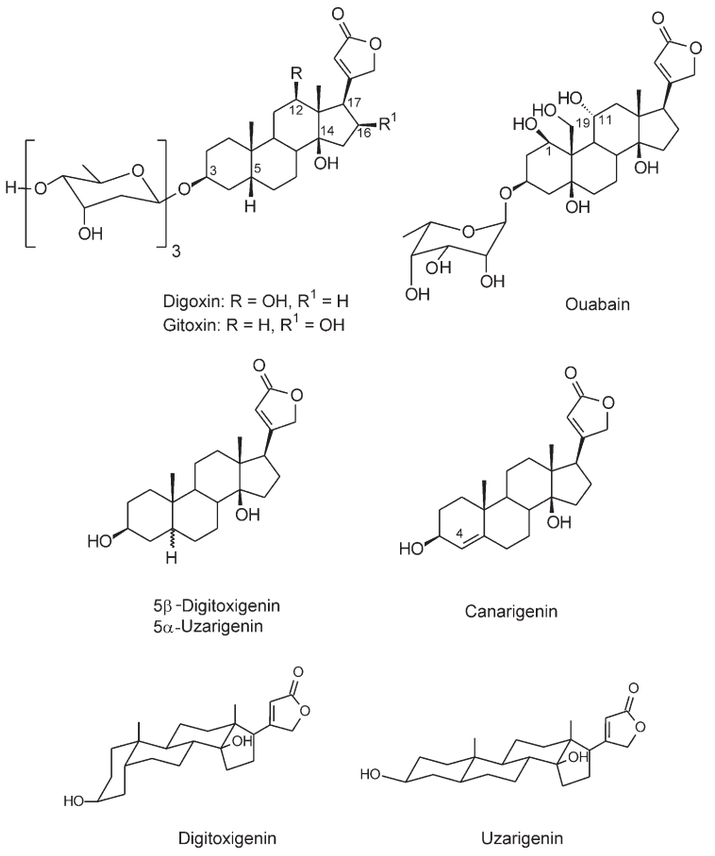


FIGURE 1 Structures of natural occurring digitalis compounds and differences between the steroidal skeleton's shape for digitoxigenin (5β-H, AB cis junction) and uzarigenin (5α-H, AB trans junction).

necessary to avoid disturbances of conduction and cardiac arrhythmias.

Digitalis compounds and synthetic digitalis-like ones, act as competitive inhibitors of the enzyme; when tested on isolated enzyme preparations, in the determination of their affinity, they are used to displace the specific binding of tritiated ouabain. It is worth noting that ouabain or a close related isomer is believed to be the endogenous inhibitor of the Na^+,K^+-ATPase,[4] affecting the balance of ions across the plasma membrane.

Digitalis compounds[5] are characterized by the presence of a cis/trans/cis steroidal skeleton with an α,β-unsaturated lactone in the 17β-position, a 14β-hydroxy group and a 3β-hydroxy group, usually linking one or more sugar rings. The first three moieties are essential for the inotropic activity, while the glycosides are responsible for the pharmacokinetics of the compounds.

The search for safer inotropic agents started some decades ago.[5] The unsaturated lactone was replaced with other α,β-unsaturated substituents or heterocycles (e.g. 3-furyl, 3-pyridyl, 4-pyridazinyl) leading always to less potent compounds. The 14β-hydroxy group can be replaced only by a 14β,15β-epoxide, while other hydroxy groups can be present in positions 1, 5, 12, 16, and 19 always on the β side (digoxin, gitoxin, digitoxigenin, and ouabain; Figure 1) and only in position 11 on the α side (ouabain). Attempts to change the junctions of the rings were partially successful only on the A/B fusion, as 5α (uzarigenin; Figure 1) and Δ^4 (canarigenin; Figure 1) derivatives maintain some of the original activity.

SIMPLIFIED DIGITALIS-LIKE COMPOUNDS

In an attempt to better understand the requisites of the digitalis binding site of the Na^+,K^+-ATPase and possibly overcome the toxicity of digitalis compounds, molecular simplifications of the digitalis skeleton were under taken. In this review we will deal with those digitalis-like compounds where there was a molecular simplification of the typical 5β,14β-steroidal skeleton that appeared in the medicinal chemistry literature from 1990 until 2002; modifications or substitutions of the α,β-unsaturated lactone ring in position 17β will not be dealt with. To the best of our knowledge, only one other group from the University of Salamanca, beside ours, have worked in this field during the aforementioned period.

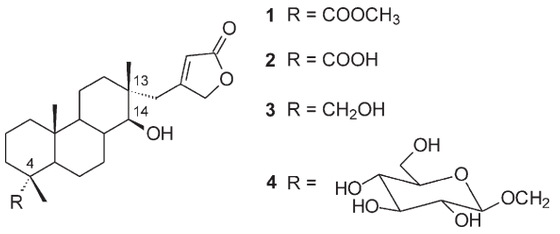


FIGURE 2 Structures of diterpenic butenolide derivatives.

San Feliciano's group (University of Salamanca, Spain) reported examples of simplified digitalis-like compounds, namely: diterpenic butenolides[6] (Figure 2), cyclohexylmethyl butenolides[7,8] (Figure 3), and hydroindene amidinohydrazones[9–11] (Figure 4).

The diterpenic cardenolide analogues (Figure 2) lack the D ring of the steroidal system and have substituents at position 4. In this series, compound **1** did not modify the inotropic response, while compounds **2**–**4** produced a negative inotropic effect. The authors hypothesized that the lack of positive inotropic activity could be because either the compounds interact at the digitalis binding site of Na^+,K^+-ATPase itself as antagonists or because they bind to a different receptor, since they have a different conformation due to lack of the D ring.

The cyclohexane derivatives **5**–**9** (Figure 3) introduce a greater simplification, since they are equivalent to the C ring of the steroid skeleton; again, these compounds were either inactive or even negative inotropic agents.

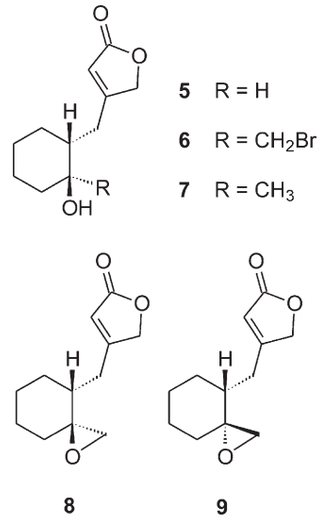


FIGURE 3 Structures of cyclohexylmethyl butenolide derivatives.

The hydroindene derivatives **10**–**13** (Figure 4) resemble the C and D rings of the digitalis steroidal skeleton, although they do not have the typical cis junction or carry the peculiar 14β-hydroxy substituent. They are substituted with the amidinohydrazone moiety at position 5 and/or an α,β-unsaturated system at position 1; both of which are known pharmacophoric groups at the digitalis binding site.[5]

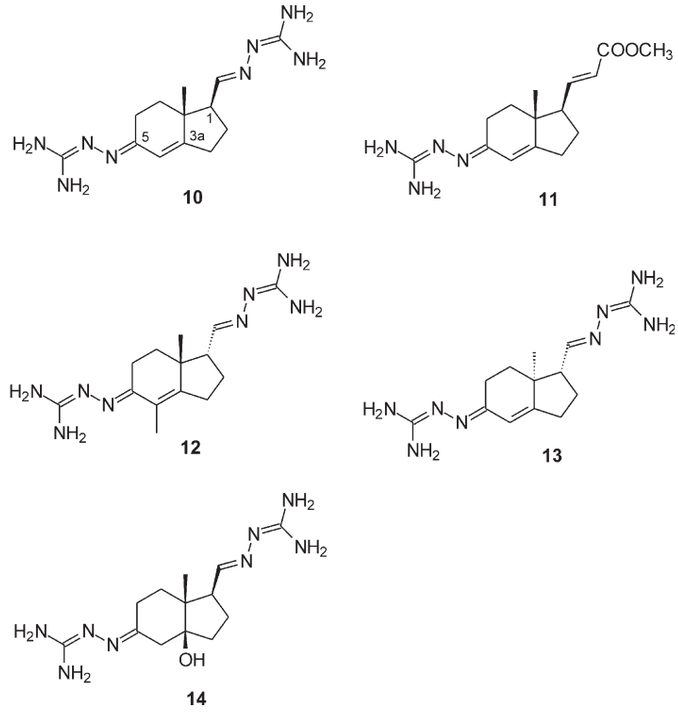


FIGURE 4 Structures of hydroindene amidinohydrazones.

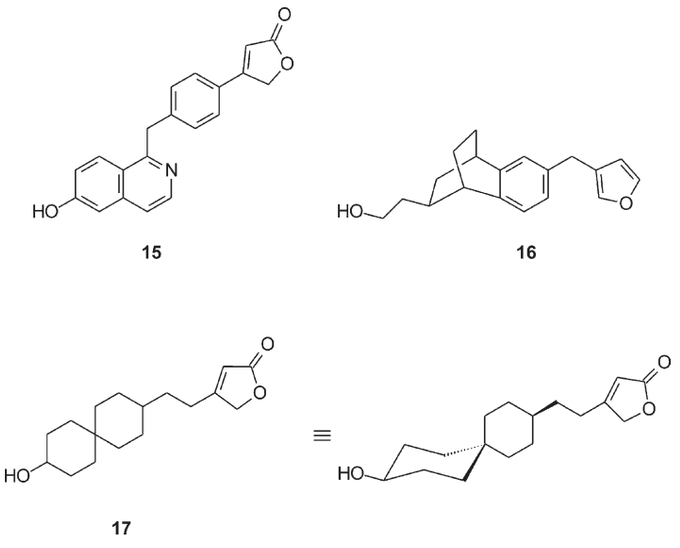


FIGURE 5 Structures of 1-benzyl-6-hydroxyisoquinoline (**15**), *endo*-1′,2′,3′,4′-tetrahydro-1′,4′-ethano-2′-naphthylethanol (**16**), and spiro[5.5]undecane (**17**) derivatives.

Among these compounds, **10**, **12**, and **13** showed positive inotropic effects, even though at high concentrations in *in vitro* tests (0.3 mM), notwithstanding the α- or β-stereochemistry at position 1 (**10** vs. **12**) or even when the opposite enantiomer was tested (**13** vs. **10**). Also in this series, some compounds showed negative inotropic effects (**11** and **14**), even when the cyclic system is in the same configuration as that of the C and D rings of digitalis compounds, and the 3aβ-hydroxy (equivalent to 14β-hydroxy in digitalis compounds) substituent is present (**14**).

Our research on digitalis-like compounds at Prassis started about ten years ago. In the initial studies, we examined some classes of simplified digitalis compounds (Figure 5), namely, 1-benzyl-6-hydroxyisoquinolines[12] (**15**), *endo*-1′,2′,3′,4′-tetrahydro-1′,4′-ethano-2′-naphthylethanol derivatives[13] (**16**), and spiro[5.5]undecane derivatives[14] (**17**). The spiroundecane and the tetrahydronaphthylethanol derivatives could resemble the bent steroidal skeleton of the cardiac glycosides, while the hydroxyisoquinolines mimic deoxybenzoin derivatives reported by Prigent as inhibitors of Na^+, K^+-ATPase and positive inotropic agents.[15] These compounds showed inhibitory activities on Na^+, K^+-ATPase in the range of 0.1 mM and none of them showed appreciable inotropic activity.

All of these attempts, from both our group and the other workers, suffer from poor or weak activity at the Na^+, K^+-ATPase digitalis binding site. The explanation could be that simplified digitalis-like compounds lack the appropriate interactions with the binding site due to the absence of the complete steroidal skeleton. The hypothesis was advanced that successful compounds would be derived from these classes only if the reduced hydrophobic interactions could be augmented by a novel stronger pharmacophore, different from those previously reported.

Minor simplifications of the digitalis skeleton led to new insights into the minimum requirements for the binding to the Na^+, K^+-ATPase.

Cleavage of the D-ring of the digitalis skeleton gave compounds with affinities of the same order of magnitude as digitoxigenin (binding IC_{50} 0.063 μM), showing that the D ring is not essential for recognition by the digitalis binding site of Na^+, K^+-ATPase (Figure 6).[16] Some requirements were maintained, such as the 14β-hydroxy group

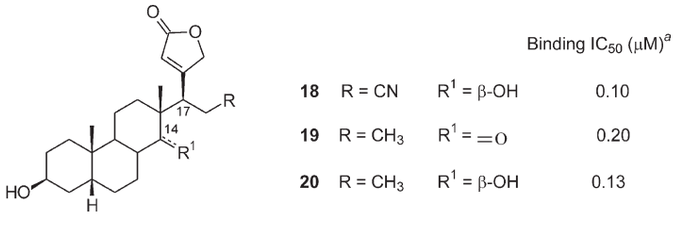


FIGURE 6 Structures of 14,15-seco derivatives. [a]Means of values determined in two to three separate experiments in duplicate. The affinity for the digitalis binding site of Na^+,K^+-ATPase was evaluated by the displacement of the specific [^{3}H]-ouabain binding from Na^+, K^+-ATPase isolated from dog kidney.

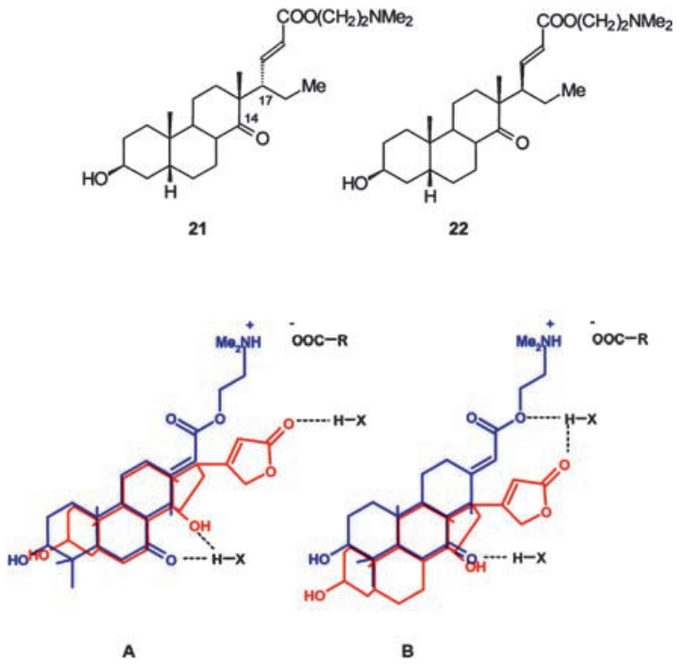


FIGURE 7 Structures of 2-dimethylaminoethyl 14,15-seco-17-acrylates and a model of the superposition between cassaine (blue) and digitoxigenin (red). Model A is the traditional superposition, model B is the novel superposition proposed by our group.

(**18**, and **20**), but the 14-keto (**19**) was also accepted without a great loss of affinity.

On the basis of the structural and stereochemical similarities among digitoxigenin, 14,15-secodigitoxigenin analogues, and cassaine (an *Erythrophleum* alkaloid with digitalis-like behavior), a new model was constructed for the relative alignment of cassaine and digitalis derivatives: instead of overlapping the A, B, and C rings, as in the traditional model,[17,18] the C ring and 14β-OH of digitalis

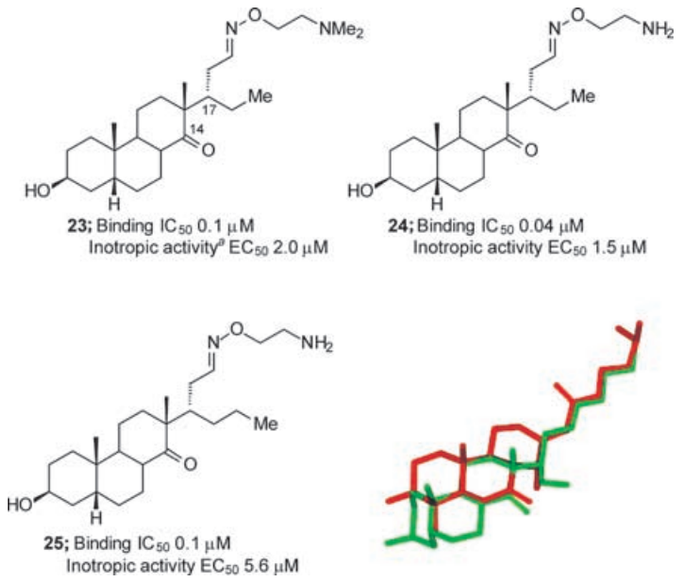


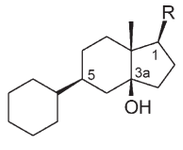
FIGURE 8 Structures of 14,15-seco-17α-(*O*-aminoalkyloxime) derivatives and model of superposition between cassaine (red) and compound **24** (green). [a]Concentrations producing 50% of the maximal increase in force of contraction were calculated from concentration-response curves. The experiments were performed on electrically driven guinea pig left atrium.

compounds were superimposed on the B ring and 7-keto group of cassaine, respectively; greater importance was given to the 17α space of digitalis derivatives. As a consequence, high binding affinities were predicted and demonstrated for the two epimeric 2-dimethylaminoethyl 14,15-seco-17α-acrylate (**21**; binding $IC_{50} = 0.12\,\mu M$) and 17β-acrylate (**22**; binding $IC_{50} = 0.25\,\mu M$) (Figure 7).[19]

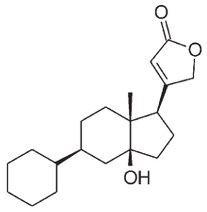
The discovery of the *O*-aminoalkyloximes as substituents capable of conferring a very potent inhibition of Na^+, K^+-ATPase,[20] led us to apply this finding to classes of compounds in which the typical digitalis skeleton was simplified or replaced, with the aim of obtaining different interactions with the binding site or with the Na^+, K^+-ATPase isoforms.

Putting together the powerful *O*-aminoalkyloxime substituent and the observations on the seco-D derivatives, new 17α-(*O*-aminoalkyloxime) derivatives with the seco-D digitalis skeleton were synthesized: high binding affinities and high inotropic activities were found (Figure 8),[21] in spite of the previous reports on the need of a 17β stereochemistry for digitalis glycosides.

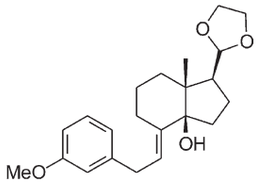
Different seco derivatives were also considered, particularly perhydroindene derivatives, formally derived from the cleavage of the B ring of the digitalis skeleton. Again, the *O*-aminoalkyloxime chain provided good binding affinity and inotropic activity (**26**–**30**),[22] in contrast with the very low result for the corresponding butenolide derivative **31**,[23] or the 4-ene compound **32**[24] (Figure 9). In the series of



	Binding IC_{50} μM	Inotropic Activity EC_{50} μM
26 R = (*E*) CH=NO$(CH_2)_2N(CH_3)_2$	1.6	12
27 R = (*E*) CH=NO$(CH_2)_2NH_2$	1.0	nd
28 R = (*E*) CH=NO$(CH_2)_3NH_2$	0.8	nd
29 R = (*E,E*) CH=CHCH=NO$(CH_2)_2N(CH_3)_2$	1.0	20
30 R = (*E,E*) CH=CHCH=NO$(CH_2)_2NH_2$	1.0	15

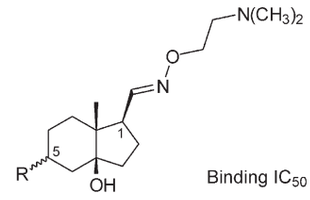


31; Binding IC_{50} > 0.1 mM



32; Binding IC_{50} > 0.1 mM

FIGURE 9 Structures of perhydroindene derivatives. nd: not determined.



	Binding IC_{50} μM
33 R = α–$CH_2C_6H_5$	> 100
34 R = α–$CH_2C_6H_{11}$	100
35 R = α–C_6H_5	> 100
36 R = β–$CH_2C_6H_5$	3.2
37 R = β–$CH_2C_6H_{11}$	4.0
38 R = β–C_6H_5	3.2

FIGURE 10 Structures of 5α- and 5β-substituted perhydroindene derivatives.

perhydroindene *O*-aminoalkyloximes[22] it is worth mentioning the importance of the conformation of the skeleton to the binding affinity. 5α-Substituted compounds (**33**–**35**) were 25–30 times less potent than the corresponding 5β derivatives (**36**–**38**) (Figure 10). The reason can be found in the different conformations of the two epimeric series. As shown in Figure 11, all perhydroindene derivatives have their minimum conformational energy with the substituents at C5 in the equatorial position and this results in different conformations for the two epimeric hydrindane derivatives. The 5α-substituted perhydroindenes present a non-digitalis-like conformation of the CD rings, while the 5β substituted compounds are in the digitalis-like conformation, which is most probably the reason for their higher affinity to the receptor. Although good binding affinities were reached in the series of 5β-substituted 1β-(*O*-aminoalkyloxime) perhydroindenes, they were about 4 times less potent than uzarigenin (the corresponding digitalis-like compound with the same conformation of the skeleton), the inotropic activity of these compounds was low (EC_{50} in the range of 12–20 μM, see Figure 9).

CONCLUSIONS

Attempts to find new simplified digitalis-like compounds failed to give sufficiently high inotropic potency when the structure of the new derivatives was too different from the classical digitalis skeleton. Not even a powerful pharmacophore, such as the *O*-aminoalkyloxime group, could strengthen the interaction with Na^+,K^+-ATPase. Only minor simplification, such as cleavage of the D ring of the digitalis skeleton, could preserve the desired

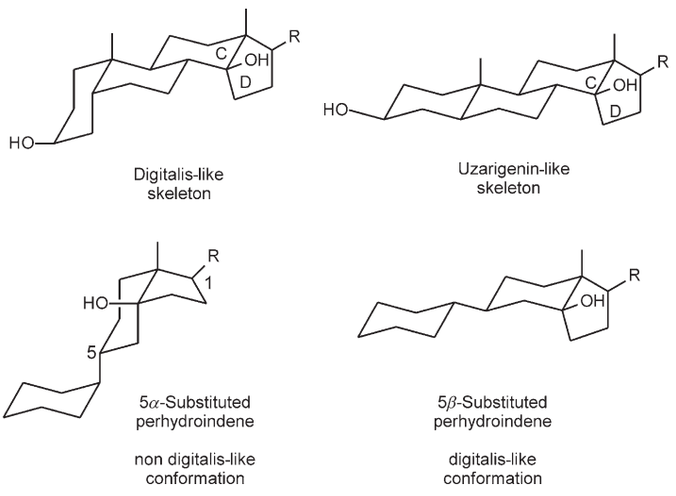


FIGURE 11 Conformations of 5α- and 5β-substituted perhydroindene derivatives in comparison with digitalis-like and uzarigenin-like conformations.

inotropic activity. This could mean that a wide surface capable of binding to the digitalis binding site through strong Van der Waals interactions, such as the steroidal skeleton, is of great importance for a high affinity to Na^+,K^+-ATPase.

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