DRUG DESIGN OF MEDICINAL PRODUCTS ON THE BASIS OF NATURAL CARDIOSTEROIDS

Iu. Gubin, O. Romelashvili, T. Zborovska

1. Introduction

An important trend in the development of modern pharmacy is the increase in the number of drugs based on chemically transformed natural substances. New biologically active substances that are derivatives of cardiosteroids are synthesized. This work is a continuation of works initiated by prof. Makarevich I. F. with co-authors [1]. The original optimized method for the synthesis of aldimes was first applied. Synthesis is based on the interaction of cardenolides with organic amines with the formation of imines.

The lack of biological activity in bis-cardenolides, obtained in the form of azometin C19, despite the fact that they are easily hydrolyzed to the high-level monocardenolides in the body, have not been found for a long time [2].

Only the use of modern methods of QSAR analysis allowed to make an assumptions about such a phenomenon.

In addition, it was necessary to study the specific physicochemical properties of bis-ketoimines of C3-cardenolides, which consist in their resistance to hydrolysis [3, 4].

Drug design of new cardiotonic drugs remains relevant because cardiovascular diseases and their mortality have been at the forefront of the world for a long time. The aim of the present study was to elucidate the relationship between pharmacological activity and the structure of a number of cardiosteroids, with the aim of creating new drugs that affect the cardiovascular system. Significant toxicity and low therapeutic index of known cardiosteroids greatly limits their use.

2. Planning (methodology) of research

Previous research in this field has been directed above all to create new molecules with significant cardi-activity, which have led to an increase in their toxicity. Thus, it is believed that in some cardiosteroids, it is not possible to create effective drugs with a satisfactory benefit / risk ratio.

The data accumulated by the authors did not always reflect the existing views on the relationship between structure and activity, and were usually discarded.

This paper proposes the synthesis of previously unknown substances but not existing in nature, and the analysis of new and previously obtained results using modern computational methods using QSAR analysis.

3. Materials and methods

The accumulated positive experience with chemically or biochemically modified cardiosteroids allows us to speak about the expediency of further work on the transformation of these biologically active substances. Modern methods of calculating and studying the active formation of imines.

Висновки. Сформульовано гіпотезу існування явища неінгібуючого зв’язування з Na+,K+-АТФазою, при якому, речовина діє як антидот по відношенню до кардіостероїдів.

Ключові слова: кардіостероїди, карденолід, драгомін, жорсткий бар’єр і стабільна конфігурація в альдимінах карденолідів.

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molecule at different levels of the hierarchy of the structure (atomic, molecular and supramolecular) make it possible to monitor and predict the relationship between the structure of matter and its biological effect.

Today, the strategy of obtaining new biologically active substances includes:

- QSAR analysis – Establishing of the quantitative relationship "structure – biological action". Descriptive modeling – a forecast of biological activity based on the analysis of pharmacophores.
- Virtual screening (in silico) – calculation and theoretical study of the interaction of target receptors with biologically active molecules [5].

To find out the dependence of the structure-action of the cardenolides, we used the Field Align computer program version 2.1.1. Cresset Bio-Molecular Discovery Limited.

As the reference (basic) molecule 3-acetyl-19-aldoxim strophanthidine was used – one of the most active substance in a cardiosteroids series. As the reference pharmacophores, negative fields were used in the region of the C21-C23 atoms, and positive fields in the region of the C14 atom.

Synthesis methods are given in the publication [2].

4. Results
Previous studies of transformed cardiosteroids have shown that aldimines of cardenolides have been shown to be low-toxic cardiotonics (e.g., adamantyl-iminostrophantidine) [3]. In this regard, it seemed advisable to continue these studies, having received a wider set of new substances with subsequent pharmacological screening and the detection of the most promising in practical terms, physiologically active derivatives.

For chemical transformations, strophanthidin (cardenolide), as well as erysimin and cymarin (cardenolide glycosides), which contain an angular aldehyde group in their structures, were taken as the source of natural cardiosteroids. Synthesis of aldimines of cardenolides was carried out by two methods:

1. Natural aldehydes were directly interacting with organic compounds containing a free primary amino group. It has been established that the formation of imines occurs most promptly and fully at the maximum possible concentrations of the interacting substances, when the reaction mixture resembles the molten mass at the boiling point of the corresponding solvent with azeotrope distillation of water.

2. The second method is because the primary amines are more stable in the form of salts. In these cases, the reaction with aldehydes group of cardiosteroids was carried out in a boiling solution in the presence of sodium acetate.

The structure of the received substances is confirmed by the data of element analysis, IR and PMR spectra. Infrared spectra is characterized by the absence of an absorption band of the aldehyde group and the presence of the absorption band C = N-group (1650 cm⁻¹).

5. Discussion
In the synthesis of aldimines of cardenolides, we had that comparatively exceptional case when two types of isomerism – conformational (isomers) and geometrical.
Such an explanation does not contradict the fact that in the PMR spectrum of strophanthidin oxime, a three-proton signal of an angular methyl group of 18-CH₃ appears in the region of 0.74 ppm, that is, it is shifted to a strong field, which is obviously due to the spatial shielding effect of proton C-19-H to this group. The spatial proximity of proton C-19-N and 18-CH₃ is clearly visible on 3D models (Fig. 3).

Nor does it contradict the fact that this substance forms with a salt of metals a stable complex soluble in organic solvents. The formation of such a complex is quite possible due to the chelating fragment of the molecule, which is formed by OH groups in the C-3 and C-5 and the oxime group.

For example, the formation of aldimes strophanthidin complexes with copper ions, soluble in organic solvents (green (Fig. 4) [3].

The formation of the complex involves the nitrogen atom through a pair of electrons and OH groups in C3 and C5. In the PMR spectra of aldimes, the signal of the angular methyl group of 18-CH3 is manifested in the 0.66–0.74 ppm region, that is, it is shifted to a strong field due to the influence of 19C-H proton on this group.

This orientation of the 19C-H proton directly affects geometric isomerism due to the spatial arrangement of functional groups around the double C = N-bond.

Of the geometric isomers, the most likely are anti (E) isomers. It is the E-isomer that can easily form complexes with copper ions, which is observed in this case.

Son (Z) – an isomer cannot form such complexes.

Computer modelling of stereo structures confirms that the anti (E) –isomers are the most acceptable.

Based on the foregoing, it is reaffirmed that aldoxime strophanthidin has a structure of the Z-isomer (Fig. 5, 7, 8) and not the E-isomer (Fig. 6) (aldoximes group circled in red).

We believe that it is chelate groups in the aldoximes of aglycones that cause such a significant increase in biological activity. Apparently, such structures allow cardenolide to interact more strongly with the functional groups of receptors in the body compared with natural aglycones. The above explanation does not exclude others. In particular, aldoximes of aglycones, due to the presence of chelate groups, may be selective carriers of calcium ions through membranes of myofibrillar cells and thereby increase biological activity, as it is known [9, 10] that calcium ions increase the contraction of myofibrils. It is also possible that both of these effects take place in a living organism.
Fig. 6. E-isomer aldoxime strophanthinidin

Fig. 7. Distribution of charges in Z-isomer aldoxime strophanthinidin
Some decrease in biological activity in aldoxime glycosides, in comparison with initial glycosides, can partly be explained by a violation of the chelate structure. It should be borne in mind that the angular aldehyde group increases the biological activity. "Closing" it, without creating a chelate structure, in principle should lead to a decrease in biological activity.

Regarding cimarin-19-aldoxim, it can be noted that the decrease of its biological activity in comparison with digitoxigenin is natural, since it does not contain the 3b-ON group, which is important for the cardiotonic action of aglycones, and there is no chelate structure, which was already discussed [11].

Ketogenic cardenolides until recently remained a poorly understood part of the chemistry of steroid cardiotonics [4]. This is mainly because, in nature, keto-cardenolides are unknown, in contrast to the widespread natural aldehyde cardenolides. Available keto-cardenolides became after the development of methods of oxidation in natural substances of secondary OH groups is very convenient for labile substances by oxidizing agent – Saretta reagent. This reagent is a complex of chromic anhydride – pyridine [12]. In modified views, it is described in Collins's work and was used by us for synthesis.

From the literature, only two synthesized close-to-structure structures of the N-derivatives of the digitoxigenone are known: guanidylhydrazone – and S-methyliso-thiosemicarbazone-digitoxigenone.

Derived natural substances for the synthesis of derivatives served as digoxygenin and cardigenin, which were obtained from the corresponding natural glycosides. Digitoxigenin is obtained by hydrolysis from digitoxin, and cardigenin (14,16-diangiogrigidoxygenin) is from the gytoxin. Synthesis was carried out in two stages. The first one is the oxidation of digitoxigenin and cardigenin with the release of the corresponding 3-keto-derivatives. The second stage is the interaction of the obtained ketone with the primary amines by boiling in the corresponding solvents with azeotropic effluent of water released during the reaction.

Taking into account the worked out methodological methods of synthesis of these substances, a new group of substances – bis-kardenolids – was obtained. Primary diamines of the following structure were used as reagents: $\text{H}_2\text{N}(\text{CH}_2)_n\text{nNH}_2$, where $n=3$ and above; $\text{H}_2\text{N}-\text{CH}_2-S-S-\text{CH}_2-\text{NH}_2$; $\text{H}_2\text{N}- (\text{C}_6\text{H}_5) – (\text{C}_6\text{H}_5) -\text{NH}_2$.

In obtaining ammonium salts of strophanthinid acid, in addition, mixed primary, secondary and tertiary diamines are used. From cardenolides in the reaction was taken strophanthinid, dihytoxigenone and strophanthinid acid.

Synthesis of bis-cardenolides by reaction with diamines is carried out mainly in the same way as aldimines of cardenolides [3, 4]. Bis-ketoimines in each case have two isomers, clearly visible on the chromatograms of TLC. The isomers were obtained in an individual state by column chromatography on aluminium oxide. Attention is drawn to the abnormality of the physico-chemical properties of bis-ketoimin C3. Unexpectedly, for us, they were highly polar in comparison with the original digitoxigenone, and, secondly, both substances are difficult to hydrolyse in acidic media, which is completely unusual for imines. Conventional imines are known to hydrolyse even with water, especially when heated.

In addition, dinitrostrophantidol, synthesized by I.F. Makarevich, showed an unexpected combination of structure – activity. Therefore, 17a-nitrostrophantidine (Figure 9) has an expected low cardiotonic activity, whereas 17β-nitrostrophantidine (Figure 10), instead of higher activity, was inactive. Such anomalies are encouraging in the fact that cardenolides is still potentially effective cardiotonics, which has recently been questioned by some researchers.

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Fig. 8. Huckel analysis

Fig. 9. 17a-nitrostrophantidine
**Limitations of the research.** The research have shown the potential capabilities for non-inhibitory binding of Na+, K+ -ATPhase, but do not show studies in animals or isolated animal tissue.

**Prospects for further research.** Substances I, II and III (Fig. 11) are believed to be antidotes for cardiac glycosides.

A further experiment should involve the administration of these substances to animals (pigeons, cats, frogs, or studies on isolated tissue) before cardiac glycosides are used.

Then inject active cardiac glycosides. For example, strophanthidin or digoxin in doses – LD100 or twice as much, that for strophanthidin or digoxin are 100–200 mcg / kg.

The introduction of digoxin or strophanthidin, after the use of substances I, II or III should cause less inotropic effect than the same administration of digoxin or strophanthidin, without preliminary treatment of the animal or isolated tissue with the proposed substances.

**Substances I**

**Substances II**

**Substances III**

**Fig. 11. Substances I-III**

**6. Conclusions**

Summing up, we pay attention to abnormal facts discovered in the course of this work and works carried out earlier. The use of these anomalies can lead to the creation of new types of drugs.

An unexpected result was the fact that bis-strophanthidine benzidinazomethin was cardiotonically inactive, despite the fact that it is relatively easy to hydrolyse in water to the original strophanthidine – a very active cardiotonic (strophanthidin). Based on the results we have formulated the hypothesis of the existence of a phenomenon of non-inducing binding to Na+, K+ -ATPhase, in which the substance acts as an antidote for cardioteroids. It can be used to create drugs without side effects of cardioteroids.

The results will be used to search for the synergy of the biological effects of natural and synthetic substances in the direction of certain physiological effects in order to develop a new series of drugs. In addition, the findings are used to find new approaches to optimize the composition of new drugs.

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Iurii Gubin, PhD, Senior Researcher, Department of Quality Management, National University of Pharmacy
Pushkinska str., 53, Kharkiv, Ukraine, 61002
E-mail: gubin990756@gmail.com

Olena Romelashvili, PhD, Associate Professor, Department of Quality Management, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002
E-mail: osromelashvili@gmail.com

Tatiana Zborovska, PhD, Associate Professor, Department of Quality Management, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002
E-mail: t.v.zborovska@gmail.com