UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

DOCORATE SCHOOL

PhD THESIS

SUMMARY

FUNCTIONALIZED LIPOSOMES : NEW SYSTEMS FOR VECTORIZATION

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Key words: active principles, liposomes, nanotechnology, hydroxyapatite, affinity

SUMMARY

In the medical research field, liposomes represents current information. At base of their formulation stands nanotechnology. The necessity of liposomes formulation appears following the desire of achieving active principles targeting at the action place in body.

The PhD thesis follows synthesis of a chemical group, which shall us serve functionalizing liposomes, after this making affinity tests towards bone hydroxyapatite. The thesis is divided in two parts:

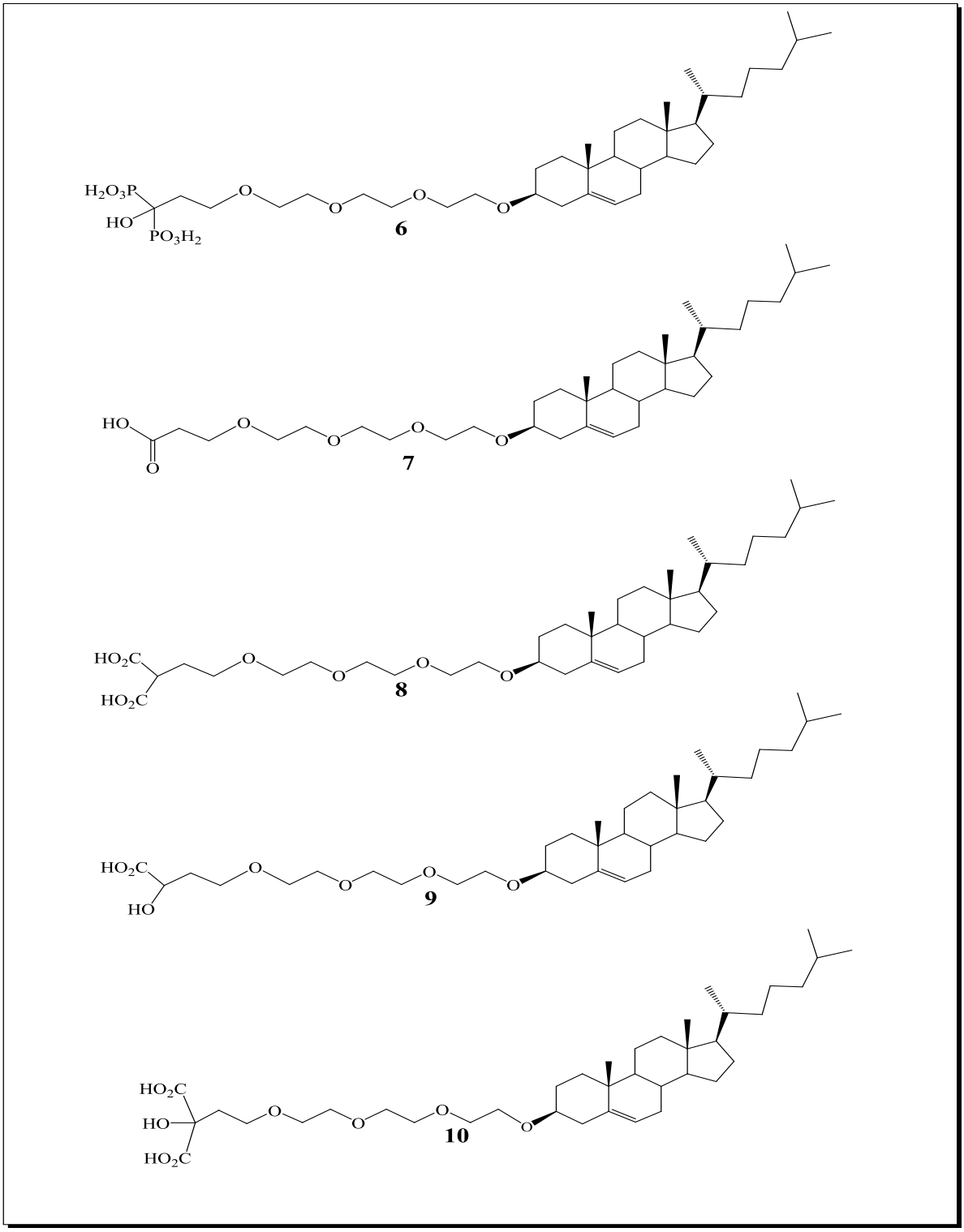
1. **The first part** contains information about the **knowledge current status** and is structured in two chapters:

Chapter I, entitled,,**Introductive notions: pharmaceutical forms with vectorization role'',** includes three subchapters. Within there are submitted general information regarding active principles insisting on their history and role along the time. Also contains notions about vectorization process of active principles to target organs describing active and passive transport. Pharmaceutical forms with vectorization role which we emphasis are the liposomes. The information which brings the largest chapter contribution refers to liposomes.

Chapter II entitled: **„ New osteotropic systems. Synthesis and comparisons.''**In the chapter beginning there is presented information about PhD thesis scope. The scope is multiple. We follow both the synthesis of chemical structures which could have osteotropic properties and the synthesis of liposomes functionalized with the obtained chemical structures, and in the end to make affinity

determination tests by incubate with hydroxyapatite. Are presented information about skeletal system and osteotropic systems reported along the time in specialty literature.

The chemical structures which synthesis is one of thesis scope and are presented in next table:



2. **The second part** describes the thesis personal contributions, containing the experimental part and three results and discussions chapters.

   The experimental part represents thesis' chapter III, the synthesis described here being one of the thesis' scopes.

   There are presented the general methods used in compound obtaining, analysis and purification but also the ones used for liposomes synthesis, analysis and testing.

   The result and discussion part starts with the presentation of preliminary tests, realized for researching the compounds affinity towards hydroxyapatite.

   There was investigated the charge distribution and electrostatic potential from the compounds polar heads level. These polar heads are responsible for the bone hydroxyapatite affinity, thus giving liposomes, eventually, osteotrope proprieties.

Chapter IV describes compound synthesis: hydroxybisphosphonic acid (6).

After bibliography study, the chosen method is that which assumes preparation of acidhydroxy fragment , followed by cholesterol binding. The group represented by acid hydroxybisphosphonic was introduced in final stage. Also, in this chapter, is presented the synthesis of acid 13-cholesteryloxy-4,7,10-trioxadodecanoic acid (7), compound which seems to have structure related to the others compounds with osteotropic properties.

ChapterV presents the results and discussions coming from acid 1-hydroxy-(12-cholesteryloxy-4,7,10-trioxa)dodecan-1,1-biscarboxylic (10) compound synthesis. Also in this case, the tartonic acid group introduction it is realized in the end. In this chapter there are described Synthesis of acid (13-cholesteryloxy-5,8,11-trioxa)-2-hydroxytridecanoic (9) compound synthesis, respectively . Synthesis of acid 12-cholesteryloxy-(4,7,10-trioxadodecan)-1,1-biscarboxylic (8). Compound 9 it is obtained in the last synthesis stage, next to compound 10, being its decarboxylated analogue. There are presented the multitude of purification methods, tested until obtaining compound 9 in pure state. Another thesis scope was obtaining compound 9 in pure state.

Compound 8 synthesis immerged as an opportunity, its structure being a totaly new one, which has not been reported yet, being confirmed by the interpretation of the spectrums, presented also in this chapter.

Chapter VI emphasise the liposomes synthesis, analysis and testing.

The liposomes have been obtained through agitation dispersion method. In order to analyze the liposomes, from all characteristic parameters, we have chosen dimension and zeta potential determination.

Liposome testing refers to hydroxyapatite affinity determination.

There were tested acid 1-hydroxy-(12-cholesteryloxy-4,7,10-trioxa)dodecan-1,1-bisphosfonic functionalized liposomes.

It has been done two tests:

1. The first consists in the affinity determination of the same hydroxyapatite concentration liposomes. The hydroxyapatite concentration is gradually increased from 0 to 10mg/ml.
2. The second fallows the liposomal solutions affinity determination with increasing quantities, towards constant concentration hydroxyapatite (10mg/ml).

Affinity is being calculated using the formula

A%=(x-y)X100/x, where:

A= affinity

x= initial fluorescence

y= final fluorescence

The obtained affinity values demonstrate synthesized molecules osteotropism capacity and have the order of measure with the ones reported by other authors in the literature.

Perspectives:

The Phd thesis has been focusing on:

- the synthesis of possible osteotropic proprieties chemical groups.

- the liposomes synthesis, later being functionalized with these chemical groups.

- realization of in vitro tests, in order to determine functionalized liposomes affinity towards bone hydroxyapatite.

During the Phd tesis liposomes functionazing and testing has been done only with one obtained chemical structure. Thus, one of the perspectives is represented by the functionalized liposomes synthesis with the remaining molecules, followed by in vitro tests. The obtained affinity tests had variations over the hydroxyapatite concentration and liposomal solution quantity. In the future there can be realized tests by varying the incorporated ligands percentages.

Due to positive results obtained during testing, meaning that the tested compound presents in vitro hydroxyapatite affinity, there can be also realized in vivo tests.

A last perspective and with major medical implications could be a drug, for example doxorubicin, in liposomes and testing in order to study the possible effect in stopping the evolution of cancerous cells.