Synthesis and properties of antitumor poly-functional derivatives of heteroareneanthraquinones

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Relevance of the topic. Anthraquinone derivatives (anthracene-9,10-dione) have a high practical value due to their unique spectral and redox properties, photochromism, as well as a wide range of biological activity. Of particular importance is the antitumor effect of compounds based on anthraquinone (doxorubicin, mitoxantrone, etc.) used for the treatment of cancer. However, an emergence of drug resistance and the trends aimed at the creation of targeted drugs contribute to the search and study of new anthraquinone derivatives. Annelation of anthraquinone with various heterocycles is one of prior directions in the development of antitumor substances. Linear heteroarene-fused anthraquinones with one heteroatom have a high antiproliferative activity, affecting a number of intracellular targets. Anthrafuran-3-carboxamide LCTA-2034 developed at the Gause Institute of New Antibiotics (Moscow, Russia) was recommended for clinical trials as a medication for hematological malignancies and solid tumors. Another example – 4,11-di((2-guanidinoethyl)amino)anthra[2,3-b]thiophene-5,10-dione LCTA-1581, which is a high-affinity ligand of G-quadruplex structures (G4) of nucleic acids, suppresses the growth of tumor cells.

Despite advances in the preparation and study of the properties of heterocyclic anthraquinone derivatives, there are a number of gaps in the understanding of structure-activity relationship. The available synthetic approaches do not allow them to be filled. Systematic development of the methodology for the synthesis and chemical modification of poly-functional heteroarenanthraquinones, and above all, derivatives of carboxylic acids, as well as the study of their antitumor activity can contribute to the creation of new chemotherapeutic drugs. Therefore, a focused analysis of the patterns between the structure of compounds in the series of poly-functional derivatives of heteroarene anthraquinones and their antitumor properties in highly demand. For example, it seems important to evaluate the role of the heterocycle, the position of the carboxamide fragment and other substituents in the heterocyclic ring, the structure of the cyclic diamine in the amide group, as well as substituents in the peri-positions of the anthraquinone

The purpose of research work is the development a methodology for the synthesis of poly-functional derivatives of heteroarene-fused anthraquinones to obtain substances with promising antitumor properties.

To achieve this goal, the following main issues were addressed:

1) development of schemes for the synthesis of poly-functional

heteroarenanthraquinones, including derivatives of anthra[2,3-b]furan, anthra[2,3-b]thiophene, naphtho[2,3-f]indole, naphtho[2,3-g]quinoline and naphtho[2,3-g]chromene;

2) search and optimization of ways for chemical modification of substituents in the heterocyclic fragment, primarily to synthesize the carboxylic acid derivatives of heteroarene-fused anthraquinones;

3) development of methods for transformation of substituents and functional groups in the peri-positions of the quinone ring of heteroarene anthraquinone derivatives;

4) target-oriented synthesis of a library of poly-functional heteroarenanthraquinones bearing the pharmacophore groups in the heterocyclic core and peri-positions of the quinone core to obtain antitumor substances;

5) structure-activity relationship of heteroarene anthraquinones, as well as identification of the role of individual structural elements in the observed biological effects.

Scientific novelty. Schemes for the synthesis of anthra[2,3-b]furan, anthra[2,3-b]thiophene and naphtho[2,3-f]indole derivatives containing an ester group in positions 2 and 3 of the heterocycle, as well as esters of naphtho[2,3-g]quinoline-3- and naphtho[2,3-g]chromene-3-carboxylic acids have been developed. A method for the preparation of anthra[2,3-b]furans and naphtho[2,3-f]indoles containing various substituents at position 2, based on a Pd(0)-catalyzed cross-coupling/heterocyclization reaction has been proposed. Effective ways of chemical modification of substituents of the heterocyclic ring of heteroarene anthraquinone derivatives discovered, in particular: hydrolysis of ester groups in positions 2 and 3; alcoholysis of the 2-nitrile group of anthrathiophenes; reduction of the carboxyl group in position 3 of anthrafurans to a formyl group and carbinol; oxidation of the formyl group in position 3 of anthrathiophenes to a carboxyl group; decarboethoxylation of anthrafurans, transformation of carboxyl groups into amide groups, etc. New methods for modifying substituents in the peri-positions of heteroarene-fused anthraquinones, such as dealkylation of alkoxy groups and alkylamino groups, have been developed. A method for introducing substituents on the nitrogen atom of naphtho[2,3-f]indole-3-carboxylic acid derivatives and a method for N-, S-, O-functionalization of the 2-position of 2-oxonaphtho[2,3-g]quinoline-3-esters carboxylic acids has been found. A series of 4,11-dihydroxy derivatives of heteroarene anthraquinones containing a carboxamide fragment based on cyclic diamines in various positions of the heterocycle was obtained. 4,11-Diaminoderivatives of heteroarene anthraquinones were synthesized, having an additional side chain with a terminal amino group connected to the heterocycle via a carboxamide spacer. The antiproliferative activity of new carboxylic acid derivatives of heteroarene anthraquinones was screened and the role of individual functional groups and substituents in cytotoxic properties was analyzed. The relationship between the structure of compounds and their effect on such intracellular targets as duplex and G4 DNA, topoisomerase 1, as well as the ability to induce ROS and cause apoptosis

of tumor cells has been studied. The cytotoxic properties of heteroarene-fused anthraquinones may be associated with the inhibition of tumor-associated NADH oxidase (tNOX) and NAD-dependent deacetylase Sirtuin 1. An influence of the heterocycle and the diamine residue in the amide group of 4,11-dihydroxyheteroarene anthraquinone carboxamides on the ability to overcome Pgp-mediated mechanism for the removal of xenobiotics from tumor cells was postulated. An increase in the affinity and selectivity of G4 ligands upon the introduction of a carboxamide group with a side chain with a terminal guanidino group into position 2 of heteroarene anthraquinones was experimentally demonstrated, validating the calculated model of interaction with the target. Using anthra[2,3-b]furan-2-carboxamide derivatives conjugated with biotin as an example, the interaction of ligands with G4 under low abundance cellular concentrations is demonstrated.

The theoretical and practical significance of the work is argued by the development of new methods for annelation of various heterocycles to anthraquinone, which can be used for the preparative production of poly-functional heteroareneanthraquinones. Effective ways of transformation of substituents and functional groups of the heterocyclic ring and anthraquinone fragment have been found for the functionally oriented design and synthesis of compounds with a given structure. A number of new highly active compounds have been obtained that suppress the growth of tumor cells, including those with activated mechanisms of resistance to chemotherapeutic drugs. The use of computer-aided design combined with synthetic chemistry techniques has led to the development of selective G4 ligands based on heteroarene anthraquinones. The discovery of the ability of compounds to simultaneously act on several promising targets for antitumor therapy contributes to further optimization of the structure and development of new multi-targeted drugs. The applied value of the work is supported by the identification of candidate compounds that have demonstrated reliable antitumor effects in vivo and are promising for in-depth preclinical study.

Provisions submitted for defense:

- 1,4-dihydroxyanthraquinone and its 2-methyl analogue are universal starting compounds for annelation of the majority of heterocycles to anthraquinone, including poly-functional derivatives of heteroareneanthraquinones;
- the conversion of 4,11-dihydroxy derivatives of heteroarenanthraquinone-3-carboxylic acids into the corresponding amides is more efficiently realize through intermediate acid chlorides, while a similar reaction of 2-isomeric acids easily occurs using peptide synthesis reagents;
- oxidative dealkylation of alkylamino groups makes it possible to obtain heteroarene anthraquinones containing primary amino groups in positions 4 and 11, while the structure of the heterocyclic core significantly affects the reactivity;
- the peptide coupling reagents promote N-, S-, O-functionalization of 2-oxonaphtho[2,3-

g]quinoline-3-carboxylic acid esters under mild conditions, but the method has limitations;
- the majority of 4,11-dihydroxyheteroarenanthraquinone carboxamides overcome Pgp-mediated efflux from tumor cells, and this ability depends on the structure of the heterocycle and the structure of the carboxamide group;
- the introduction of an additional side chain into position 2 of 4,11-diamino derivatives of heteroarene-fused anthraquinones increases the affinity for telomeric G4 and selectivity of binding over DNA duplex;
- heteroarenanthraquinone derivatives have multi-targeted antitumor effects, including binding to DNA duplex and quadruplexes, suppression of topoisomerase 1, inhibition of tNOX and Sirtuin 1, as well as induction of ROS.

Publications. The main content of the dissertation work is presented in 24 articles in scientific journals from the list of Higher Attestation Commissions, indexed by the international databases Web of Science/Scopus, more than 50 abstracts of reports have been published at international and all-Russian scientific conferences, and 4 Russian patents for inventions have been received.

Structure of the dissertation. The dissertation includes an introduction part, a literature review, a discussion of the results, an experimental part, conclusions and a bibliography of 362 titles. The work is presented on 380 pages, including 154 figures, 12 tables and 1 appendix.