SYNTHESIS AND INVESTIGATION OF THE ANTITUMORAL
ACTIVITY OF SHORTENED ANALOGS OF LULIBERIN

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With the aim of obtaining antitumoral drugs possessing a binary action mechanism, we have synthesized a series of shortened analogs of luliberin, including some containing a 1-carboxymethyl-5-fluorouracil residue. Their antitumoral and hormonal activities have been investigated.

Synthetic luliberin analogs are widely used in oncology for the treatment of hormone-dependent tumors [1]. Here, two mechanisms of the antitumoral action of the compounds are possible — mediated, connected with a fall in the level of steroid sex hormones, and direct, through the action of the peptide on the tumor cells (it has been shown that, in a number of cases, cancer cells have receptors for luliberin-like peptides [2]).

In addition, because of the heterogeneity of tumor tissues they always contain cells insensitive to hormone treatment and, during treatment, a tumor is always transformed into the hormone-independent type. A high therapeutic effect is given by the use of various chemotherapeutic drugs, but a common defect of them is low selectivity and, as a consequence, high toxicity.

The addition of a cytotoxic grouping to the peptide chain will permit a simultaneous rise in the efficacy of a peptide drug and decrease in such an effect of the chemotherapeutic agent. Then aimed transport of compounds with a cytotoxic action directly to target cells having the appropriate receptors is possible. The use of such an approach in the synthesis of luliberin analogs has been described in [3].

With the aim of a more detailed study of the prospects of this direction, we have synthesized luliberin analogs covalently bound with 5-fluorouracil (5-FU). In this case great importance is attached to the choice of spacer grouping, which must, in the first place, ensure stability of the bond between the 5-FU and the peptide under the conditions of the conjugation reaction and subsequent purification, while, in the second place, the modification must not lead to an appreciable fall in biological activity of either component.

According to the literature, 5-FU derivatives of the carbamoyl type possess improved pharmacological properties as compared with the unmodified drug. Initially, therefore, in order to include the cytotoxic grouping in the peptide sequence we used 5-FU carbamoyl chloride [4]. In this way we obtained the tert-butyl ester of N\textsuperscript{a}-(5-fluorouracil-1-yl)carbonyl]-N\textsuperscript{a}-Z-lysine,

\[ \text{O=C-[5-FU]COCl} \rightarrow \text{O=C-[5-FU]COO} \]

and then the corresponding p-nitrophenyl ester. However, in the selection of the conditions for conjugation with the peptide we came up against certain difficulties. Thus, it is possible to perform the reaction only in DMFA in the absence of contact...
with moisture; the use of a base is excluded; and, because of the instability of the compound in aqueous and alcoholic media, the subsequent purification and isolation of the product is difficult. In subsequent work, therefore, for the modification of the peptide we selected a considerably more stable alkyl derivative of 5-FU — 1-carboxymethyl-5-fluorouracil (CMFU):

\[
\begin{align*}
\text{HN} & \quad \text{N} \quad \text{F} \\
\text{CH}_2 \text{COOH} &
\end{align*}
\]

It is known that this compound, when added to a polymer by an ester or amide bond, has a fairly high cytotoxic activity.

CMFU was obtained by condensing 5-FU in an aqueous solution of alkali with an excess of chloroacetic acid and was isolated by treatment with the ion-exchange resin Dowex 2×8. The p-nitrophenyl ester of CMFU (CMFU—ONp) can be used in peptide synthesis without serious limitations [6].

For modification by the cytotoxic agent we selected luliberin analogs with shortened amino acid sequences. Investigations that we performed previously had shown that such compounds possess a considerable biological activity and, at the same time, are far more accessible synthetically than the releasing hormone itself and its superagonists and antagonists [7, 8].

The following analogs were synthesized in the course of this work:

\[
\begin{align*}
\text{H-Ser-Tyr-D-Asp-Leu-Arg-Pro-NHEt} & \\
\text{CMFU-Ser-Tyr-D-Asp-Leu-Arg-Pro-NHEt} & \\
\text{H-Pro-Ser-Tyr-D-Asp-Leu-Arg-Pro-NHEt} & \\
\text{CMFU-Pro-Ser-Tyr-D-Asp-Leu-Arg-Pro-NHEt} &
\end{align*}
\]

The hexapeptide (1), the starting point for all the other compounds, was synthesized by the fragment condensation method, using a (2+1)+3 scheme (Fig. 1).

In the preparation of the C-terminal fragment, Z-Arg(NO_2)-Pro-NHEt, the deblocking of Z-Pro-NHEt by catalytic hydrogenolysis led to the formation of by-products, and the benzoxycarbonyl protection was therefore eliminated by the action of HBr in acetic acid. The condensation of the hydrobromide so obtained with Z-Arg(NO_2)-OPcp in the presence of triethylamine took place slowly [7], apparently because of the high basicity of the imino group. To eliminate HBr from the reaction medium, the amino component was treated with Dowex 2×8 in methanol, which permitted a substantial increase in yield.

For the introduction of a D-Asp residue in position 6 of the natural sequence of the hormone we used a combination of the azide and the diphenyl phosphorazidate methods. This expedient is convenient in working with amino acids requiring a laborious multistage preparation of derivatives or imposing methodological limitations on the further course of synthesis.

After elimination of the protective groups by hydrogenolysis and purification on ion-exchange Sephadex, the initial hexapeptide (1) was used for obtaining the other analogs by the activated-ester method. In this process, the guanidine group of Arg was not protected.