Synthesis and characterization of bioconjugates containing peptide moiety with potential anticancer activity

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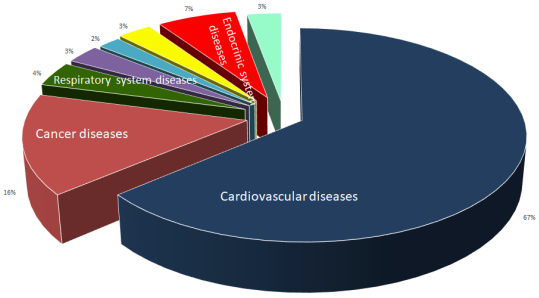
INTRODUCTION

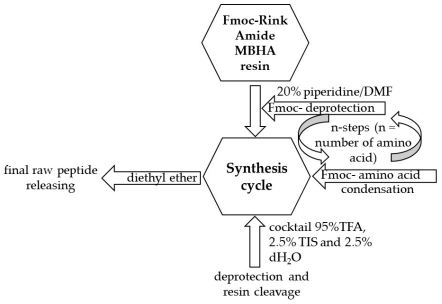
According to the world database Globocan cases of cancer diseases worldwide in 2022 are close to 20 million. A quarter of this number is distributed in Europe. Cancers have long reached pandemic proportions, and their treatment has proven to depend both on their early diagnosis and the discovery of new selective chemotherapeutics. Somatostatin (SST) and the derivatives lanreotid, vapreotide, etc., are group of peptides successfully used for treatment of different type of cancers in the medicinal practice. BIM-23052 is a linear C-terminal amid analog of SST which show very good in vitro inhibitory activity against Growth hormone in nanomolar range of concentration, which make this molecule a possible candidate as a new anticancer drug. In addition, naphthalimide derivatives are attracting much attention due to their proven antitumor activity on various tumor models and excellent stability [1]. Taking into account our previous experience in the creation of bioconjugates in a searching of a synergic effect between both part of the molecule as well as a targeting of the newly synthesized molecules the purpose of this work is to create new bioconjugates between monofluorinated analog of BIM-23052 and naphthalimide derivatives with potential synergic effect for treatment of cancers.



Data from the global database Globocan. estimated number of new cancer cases since 2020 until 2040
10 millions more, in total - close to 30 millions.

SYNTHESIS OF NEW BIOCONJUGATES

The 3 new bioconjugates of monofluorinated BIM-23052 with a general formula X-D-Phe-Phe(4-F)-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2, where X is A, B or C presented in Figure 1 were synthesized by using standard SPPS by means of Fmoc/O*t*Bu strategy (**Scheme 1**). Fmoc-Rink-Amide MBHA was used as a solid-phase carrier and DIC with additive of 1-hydroxysuccineimide (1-HOSu) served as coupling agent in a molar ratio Amino acid/DIC/1-HOSu/resin 3/3/3/1, respectively. The cleavage of the peptides from the resin was performed in acidic condition using a cocktail of . All deprotection and coupling reactions were monitored by standard Kaiser test.



Scheme 1. SPPS synthesis of targeted bioconjugates

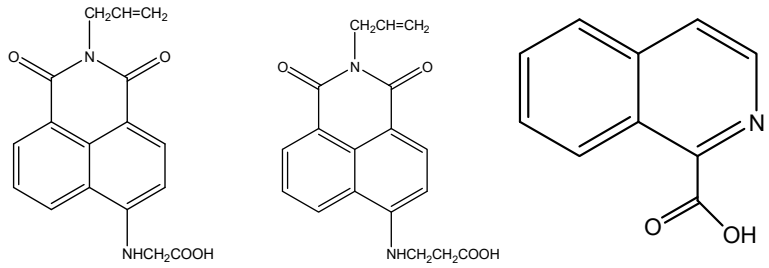


Figure. 1. A. N-allyl-4-(N-carboxymethyl)amino-1,8-naphtalimide (Nphtallyl-Gly); **B.** N-allyl-4-(N-carboxyethyl)amino-1,8-naphtalimide (Nphtallyl-β-Ala); **C.** Isoquinoline-1-carboxylic acid (IsoQui)

Code	Structure	Molecular formula	Mm exact, g/mol	$[M+nH]^+$ observed	t_R min	Chrom. purity, %
NIP1	Nphtallyl-Gly-D-Phe-Phe(4-F)-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2	$C_{78}H_{86}FN_{13}O_{13}$	1431.65	1432.75	6.680	99
NIP2	Nphtallyl-β-Ala-D-Phe-Phe(4-F)-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2	$C_{79}H_{88}FN_{13}O_{13}$	1445.66	1447.10	8.167	99
YT1	IsoQui-D-Phe-Phe(4-F)-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2	$C_{71}H_{79}FN_{12}O_{11}$	1294.60	1296.10	5.124	99

RESULTS AND DISCUSSION

Target structures with general formula X-D-Phe-Phe(4-F)-Phe-D-Trp-Lys-Thr-Phe-Thr-NH2, where X is a second pharmacophore A, B or C presented in Fig. 1 were synthesized. L-glycine or β-alanine in the fourth position of the naphthalimide ring are used as a linker between both parts of the molecule. The compounds were obtained by initially introduction of allylic group into the anhydride moiety and further the residue of the corresponding amino acid was introduced by nucleophilic substitution of a bromine atom. The second pharmacophore is also fluorescently active. Thus, the obtained bioconjugates could be also used as fluorescent markers in cancer diagnostics.

CHARACTERIZATION OF TARGETED BIOCONJUGATES

Purity of newly synthesized products was monitored by HPLC and their structures were proven by LC/MS/MS using Shimadzu Nexera X2, model 8045 apparatus with a linear binary gradient of phase A: H_2O (10 % AcCN; 0.1% HCOOH) and phase B: AcCN (5 % H_2O; 0,1 % HCOOH) and the following conditions:

time (min)	0.01	10.00	15.00	15.50	22.00
m.ph. A (%)	80	5	5	80	80
m.ph. B (%)	20	95	95	20	20

The other parameters of the chromatographic system are: Agilent Poroshell 120, 100 mm x 4.6 mm column; elution flow: 0.30 mL/min; temperature of column: 40ºC;
The structure of newly synthesized peptides is proven by ESI+ MS in SCAN mode in the following conditions:

Nebulizing gas flow	3 L/min
Heating gas flow	10 L/min
Interface temperature	350 ºC
DL temperature	200 ºC
Heat block temperature	400 ºC
Drying gas flow	10 L/min

CONCLUSIONS

Three new bioconjugates of monofluorinated BIM-23052 with a general formula X-D-Phe-Phe(4-F)-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2, where X is A, B or C presented in Fig. 1 were synthesized by using standard SPPS by means of Fmoc/O*t*Bu strategy and their structures and purity were proven with an HPLC/MS/MS. The anticancer activity is in a progress.

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