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Evaluation of *in vitro* and *in vivo* Cytotoxic Activities and Kinase Inhibition of Newly Synthesized Cyclo (N^{α}-Dinicotinoyl)-Bis-[(L-Valinyl)-L-Lysine Methyl Ester]

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Cancer is a major risk disease affecting human survival. The pharmaceutical companies are continuing searching for new drug candidates with promising anticancer activities, and reduced side effects. The current work aimed at synthesized a new tripeptide with potential pharmacological properties. L-Valine methyl ester was used to prepare cyclo (N^{α}-dinicotinoyl)-bis-[(L-valinyl)-L-lysine methyl ester. The new compound revealed promising *in vitro* cytotoxic activities against different neuroblastoma, cervical carcinoma, fibrosarcoma as well as hepatocellular carcinomas. Furthermore, we also found that the obtained IC_{50} of the compound decreased by about 50% during its *in vivo* anti-prostate cancer evaluation. Furthermore, the mechanism of action studies proposes that the new prepared derivative affects cancer cells trough the inhibition of VEGFR-2 kinase enzyme.

Keywords: Macrocyclic tripeptidopyridine ester, i*n vitro* cytotoxicity, i*n vivo* antiprostate, VEGFR-2 kinase activity

Introduction

Cancer is one of the most powerful mankind threatening disease, due to its high mortality rate as well as difficult treating procedures and harmful side effects[1,2]. The pharmaceutical industry encourages continuous discovery of novel organic derivatives possessing potential anticancer activities, with the aim to combat cancer[3]. Heterocyclic ester derivatives have been identified as biologically significant components of medicinal chemistry[1], such as: antiviral, anti-inflammatory, antioxidant, anti-HIV, and anticancer agents[4,5]. On the other hand, peptide candidates have been recognized as interesting exhibiting a broad spectrum of biological and pharmacological activities[6-8]. In view of these observations and in continuation of our previous work[9-17] in heterocyclic and peptide chemistry, herein we reported the*in vitro* and *in vivo* cytotoxic activities and kinase inhibition of newly synthesized cyclo (N^{α}-dinicotinoyl)-bis-[(L-valinyl)-L-lysine methyl ester] derivative.

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Materials and Methods

Synthesis of cyclo (N^{α}-dinicotinoyl)-bis-[(L-valinyl)-L-lysine methyl ester] (3)

Compound **3** was synthesized by reaction of 3, 5-pyridine dicarbonyl dichloride (**1**) with L-valine methyl ester followed by hydrolysis to corresponding diacid **2,** which wascyclized with L-lysine methyl ester by using Mixed anhydride method according to reported procedure. The tested compound **3** was previously characterized by physical and spectroscopic data which were reported by Amr *et al.*[15].

Biological Activities

In vitro cytotoxicity screening

Various cancer cell lines (Table 1) were evaluated. Cells were cultivated and propagated using standard medium and conditions using standard cell culture protocols developed by our group[18-21]. After inoculation, plates were incubated at standard conditions for 24 h. After that, two plates/cell line, were fixed using TCA. Control references were dissolved in DMSO at comparable concentration doses. Plates were then processed as previously mentioned in the protocols. Absorbance was read at 515 nm and the IC_{50}values were calculated from the regression analysis of the data.

In vivo cytotoxic activity

During this section, we used male Wistar rats obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. Experiments were performed according to standard protocol[22].

VEGFR-2 kinase activity assays by ELISA

This work was carried out according to standard protocol[23] using Sorafenibas a positive control. The absorbance was measured at 492 nmand IC_{50} values were calculated from the inhibition rate percentage equation[23].

Results and Discussion

Chemistry

Macrocyclic tripeptidopyridine candidate **3** was synthesized (Figure 1) and characterized by physical, chemical and spectroscopic evidences in advance according to our previous work[19]. In the current study, we report the evaluation and activities of these compounds as possible anticancer agents.

Biological Screening

Screening of in vitro cytotoxic activities

The cytotoxicity of the tested compounds was determined on seventeen different human cancer cell lines (Figure 2). Screening results showed that the prepared compound exhibited variable potentials against difference tested cancer cells. This is in accordance with our previous results obtained showing that different cells respond differently towards investigated drugs[15,17-19]. This is due to differences between different cell types in terms of membrane structure and cell organization. Furthermore, comparing obtained results (in terms of IC_{50} values) with those recorded for standard pharmaceutical drugs showed that comparable IC_{50}

Table 1 — Different cell lines and reference drugs used throughout the work

Cell line	Type	Cell line	Type	Cell line	Type
KB	Cervical carcinoma	U937	Leukemia	HeLa	Cervical carcinoma
SKOV-3	Ovarian carcinoma	K562	Leukemia	MCF-7	Breast carcinoma
SF-268	CNS cancer	G361	Melanoma	HT1080	Fibrosarcoma
NCI H460	Non-small lung cancer	SK-MEL-28	Melanoma	HepG2	Liver carcinoma
RKOP27	Colon adenocarcinoma	GOTO	Neuroblastoma	PC-3	Prostate carcinoma
HL60	Leukemia	NB-1	Neuroblastoma		
Reference drugs					
Sorafenib		Fluorouracil		Doxorubicin	Cytarabine
Gemcitabine		Capecitabine		Aldesleukin	Paclitaxel
Epirubicin		Imatinib		Bicalutamide	Flutamide

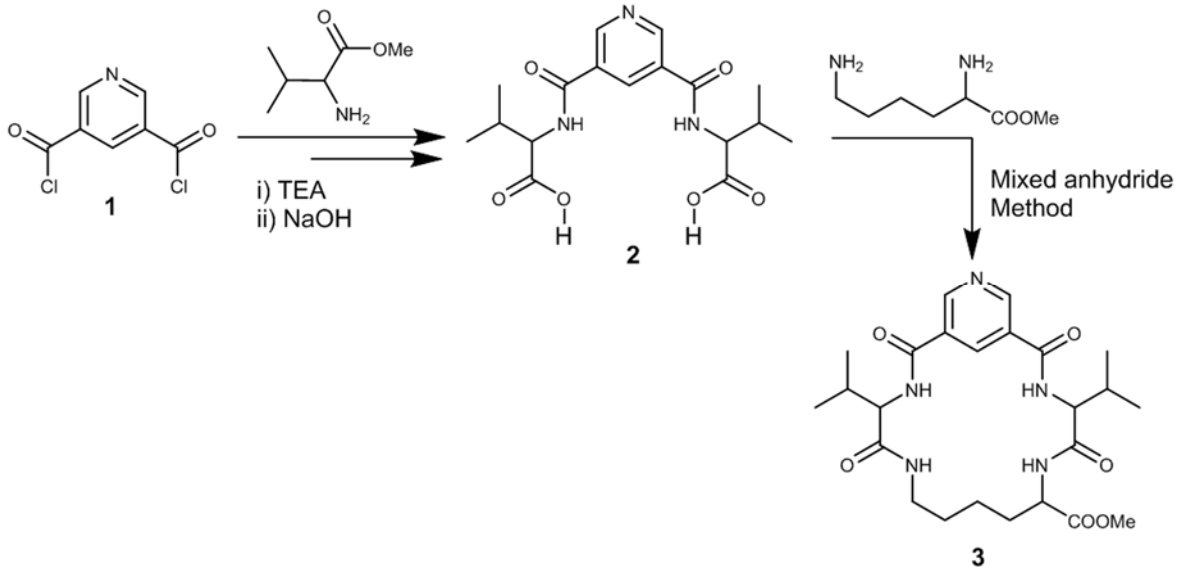

Fig. 1 — Synthetic route for cyclo (N^{α}-dinicotinoyl)-bis-[(L-valinyl)-L-lysine methyl ester] **3**.

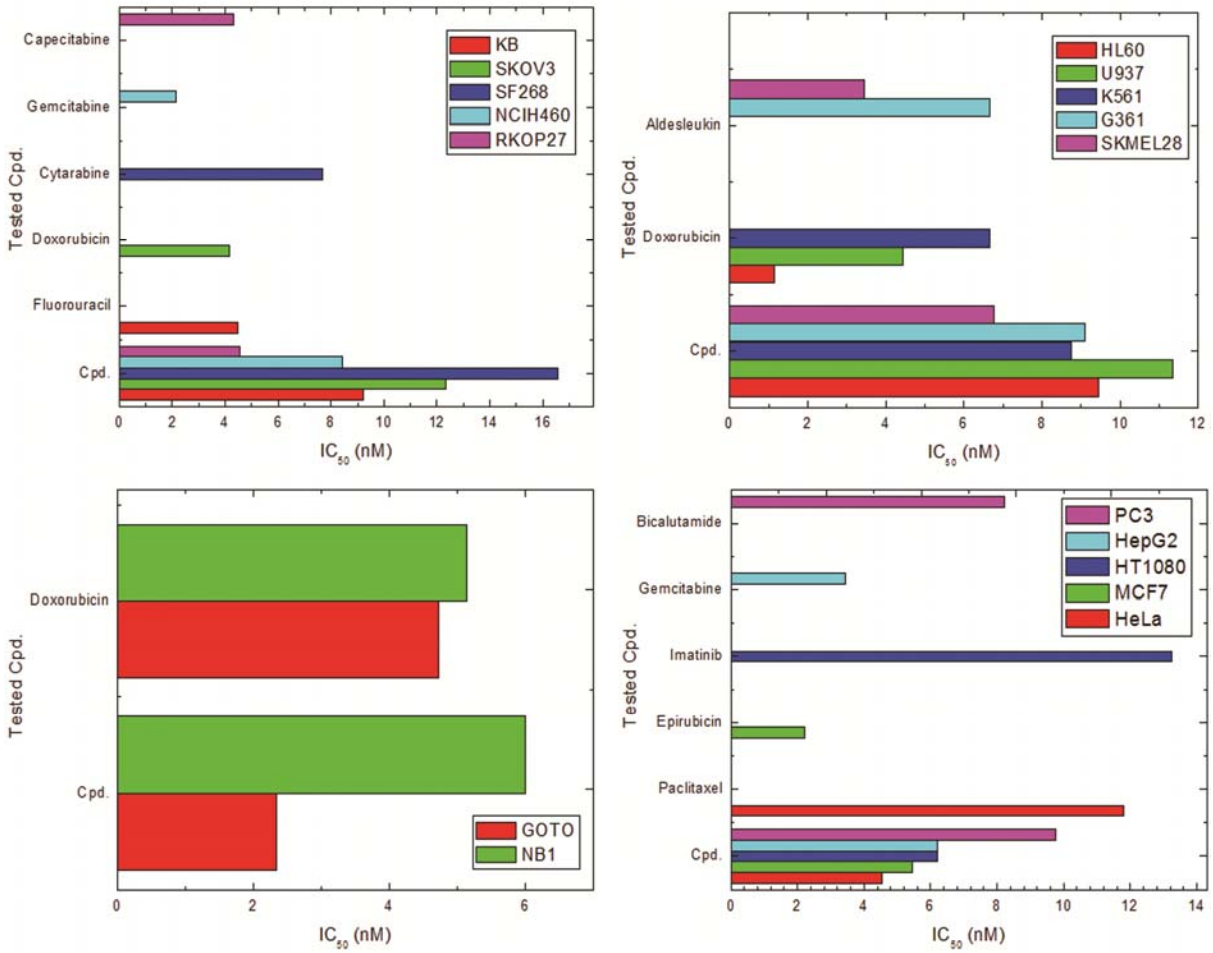

Fig. 2 — Cytotoxic activities of Cpd. 3 against different evaluated cell lines

values were obtained for colon adenocarcinoma (4.56 and 4.33 nM for compound**3** and Capecitabine, respectively), for the neuroblastoma NB-1 (6.0 and 5.15 nM for compound **3** and Doxorubicin, respectively), as well as the prostate cancer PC-3 cell line (9.76 and 8.22 nM for compound **3** and Bicalutamide, respectively). On the other hand, our prepared compound showed higher potential cytotoxic activities, where it showed almost 2-folds activity against the neuroblastoma GOTO cells (IC_{50}: 2.34 and 4.37 nM for compound **3** and Doxorubicin, respectively), 2.6-folds activity against HeLa cells (IC_{50}: 4.55 and 11.8nM for compound **3** and Paclitaxel, respectively), 21-folds activity against the fibrosarcoma HT1080 (IC_{50}: 6.2 and 130.24 nM for compound **3** and Imatinib, respectively) and 5.5-folds activity against the HepG2 cells (IC_{50}: 6.2 and 34.4 nM for compound **3** and Gemcitabine, respectively).

***In vivo* activity**

In vivo anti-prostate data showed potential anti-prostate activity for compound **3** in experimental animal model. The obtained significant ED_{50} value observed for compound (**5**) (5.26 ± 0.034 μM) was 2.2-folds higher than that obtained for the Flutamide reference drug (11.6 ± 0.09 μM).

VEGFR-2 inhibition

Anti-VEGFR-2 results revealed that the synthesized compound (**3)** exhibited potent VEGFR-2 inhibitory activity, where the obtained IC_{50} value (1.91nM) showed about 5% activity higher than the Sorafenib reference drug (2 nM).

Conclusion

Within the framework of the current investigation, we were able to synthesize a new cyclo (N^{α}-dinicotinoyl)-bis-[(L-valinyl)-L-lysine methyl ester from the reaction of dicarbonyl dichloride and L-valine methyl ester. The new derivative exhibited potent *in vitro* cytotoxic activities against neuroblastoma, cervical carcinoma, fibrosarcoma as well as hepatocellular carcinomas in comparison to standard

reference drugs. Furthermore, the new derivative showed 2.2-folds increased *in vivo* anti-prostate cancer activity, compared to control drug. These results support the potential use of the new derived tripeptide as a promising pharmaceutical molecule with potential *in vitro* and *in vivo* anticancer properties.

Abbreviations

IC_{50} Half maximal inhibitory concentration
ED_{50} Median effective dose

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