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Investigation of Growth Inhibitory Effects of cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornthenyl acid hydrazide] on Various Cancer Cells as well as *in vitro* VEGFR-2 Kinase Inhibition

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During the current work, we synthesized a new peptide derivative; 4,13-diisopropyl-2,5,12,15-tetraoxo-3,6,11,14-tetraaza-1(3,5)-pyridinacyclopentadecaphane-7-carbohydrazide. The prepared hydrazide was investigated for its *in vivo* as well as *in vitro* anticancer effects. Results revealed that this derivative has a great potential against 6 cancerous cell lines. Furthermore, the highest effect was obtained against HT1080 and HeLa cells, where the compound showed 7.4- and 15.1-folds increased activity against them, respectively. Additionally, the compound seems to exert its potential anticancer effect by affecting the kinase enzyme VEGFR-2. Finally, the compound showed promising results when tested in *in vivo* against prostate cancer developed animal models.

Keywords: Azide method**,** Macrocyclic tripeptidopyridine, Cell lines, *In vivo, In vitro*, Kinase activity.

Introduction

From thirty years ago, peptide magainin was discovered and used as anticancer agent[1,2], and thus, attention was directed towards the anticancer activities of a wide range of host defense peptides of the innate immune system.[3–5] In our previous work[6–13], we reported that certain of newly synthesized compounds incorporated heterocyclic and amino acid moieties exhibited antimicrobial, [6–9]antiinflammatory[10], anticancer[12], and Monoamino oxidase inhibitors[13] activities. On the other hand, carbohydrazide derivative were synthesized and evaluated as potential antitumor activity.[14] In addition, some of substituted macrocyclic peptide derivatives were evaluated for Topoisomerase I and II inhibitory activity[15], cytotoxicity against several human cancer cell lines[16,17] and kinase inhibitory activities.[18] In this study, we reported the *in vitro* anti-VEGFR-2 kinase activity, *in vitro* cytotoxicity as well as *in vivo* antiprostate effects of the newly synthesized macrocyclic tripeptidopyridine candidate.

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Materials and methods

Reagents

3,5-Pyridinedicarbonylchloride was prepared and elucidated according to literature procedures.[11] L-Valene, methanol, dichloromethane, hydrazine hydrate, and L-lornithine methyl estere, were all purchased from Sigma-Aldrich (Switzerland). Melting point was determined in Electro Thermal Digital melting point apparatus IA9100. IR (KBr) spectra were recorded on a Nexus 670 FTIR Fourier Transform infrared spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a JEOL 500 MHz instrument (Tokyo, Japan). Mass spectra were run on a MAT Finnigan SSQ 7000 spectrometer (Shimadzu, Kyoto, Japan; Model: QP2010 ultra), using the electron impact technique (EI). The compound **5** was previously characterized by physical and spectroscopic data which were reported by Amr *et al.*[11].

Synthesis of 4,13-diisopropyl-2,5,12,15-tetraoxo-3,6,11,14-tetraaza-1(3,5)-pyridinacyclopentadecaphane-7-carbohydrazide 5

To a solution of methyl 4,13-diisopropyl-2,5,12,15-tetraoxo-3,6,11,14-tetraaza-1(3,5)-pyridin-acyclopentadecaphane-7-carboxylate (**4**) (0.475 g,

1 mmol) in absolute methanol (25 mL), hydrazine hydrate (0.8 mL, 16 mmol) was added with stirring. The reaction mixture was heated under reflux for 6 h, and then evaporated to dryness under vacuum. The formed residue was washed with diethyether several times, filtered off, and crystallized from dioxane-water to afford the corresponding cyclo hydrazide derivative **5**. The obtained compound **5** was compared with authentic sample previously prepared and characterized by Amr *et al*.[11]

Biological Activities

Effect of the prepared hydrazide on cancer cell viability

The prepared hydrazide was tested for its effect on viability of 17 cancerous cell lines covering almost all important cancer types.[21,22] The assay was performed according to our previously developed protocol using standard MTT assay method.

Effect of compound 5 on kinase inhibition

The method used throughout the work was adopted from our previously optimized and published protocol depending on ELISA.[22]

Antiprostate cancer animal model investiagtion

In our previous work[22], we used male Wistar rats to initiate and evaluate prostate cancer as affected by our synthesized hydrazide. Our experimental protocol was strictly followed for evaluating the degree of affecting prostate cancer in animal models.

Result and Discussion

Chemistry

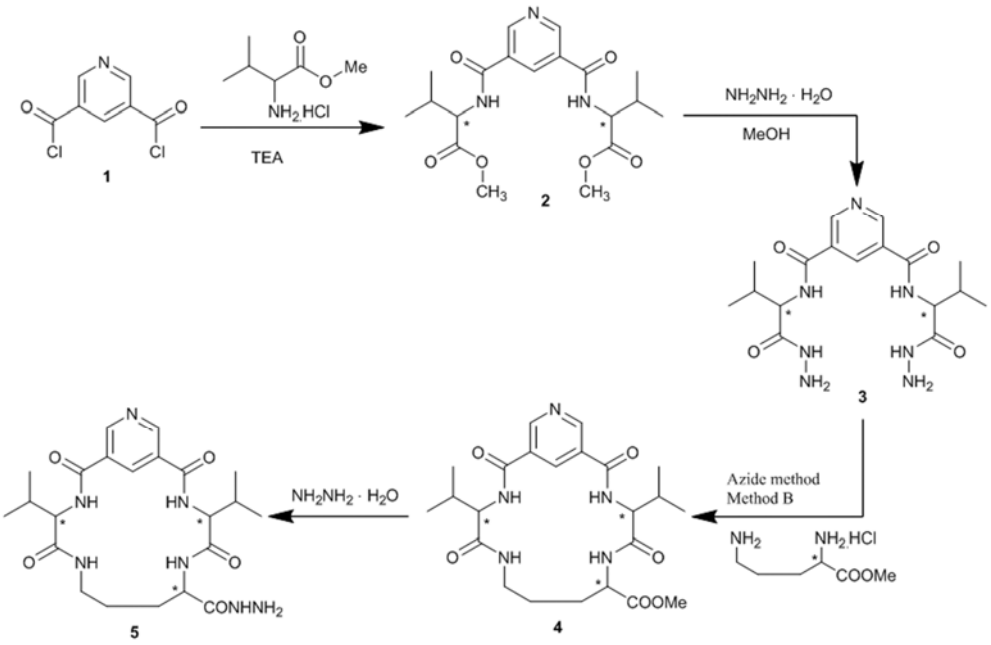
We have previously synthesized some macrocyclic tripeptidopyridine candidate **5** and they characterized by physical, chemical and spectroscopic evidences in advance according to our previous work.[11] Macrocyclic tripeptide **5** was synthesized by using methyl 4,13-diisopropyl-2,5,12,15-tetraoxo-3,6,11,14-tetraaza-1(3,5)-pyridinacyclopentadecaphane-7-carboxylate **(4)** as starting materials, which was synthesized from 3,5-pyridine dicarbonyl dichloride **1**, according to a reported procedure.[19,20] Reaction of dipeptide **3** with L-ornithine methyl ester by using azide method afforded cyclic derivative**4**, which was treated with $NH_2NH_2.H_2O$ to give cyclic tripeptide hydrazide **5** (Scheme 1). In the current study, we report the evaluation and activities of this compound as possible anticancer agents.

Biological Screening

Effect of compound 5 on the viability of different cancer cell lines

The second section of the current work was designed to evaluate the possible effects of the prepared cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornthenyl acid hydrazide] on the viability of different cancer cell lines. The results were obtained with positive drug controls. The obtained results, presented in Fig. 1, showed that the effect of compound **5** on cancer cell lines can be classified into three different categories. Firstly, compound **5** exhibited more or less similar effects in comparison to positive controls against cervical carcinoma (KB) and the melanoma G361 cell lines. The obtained IC_{50} values recorded 4.45 and 6.78 nM for KB and G361 cells, respectively, while their corresponding positive controls showed IC_{50} values of 4.46 and 6.66 nM for Fluorouracil and Aldesleukin, respectively. On the other hand, compound **5** showed potential anticancer activities against prostate cancer cell line (PC-3), liver carcinoma (HepG2), leukemia (K561), colon adenocarcinoma (RKOP27), fibrosarcoma (HT1080) and the cervical carcinoma (HeLa) cell lines. However, comparing the obtained results with their corresponding positive controls, revealed that cells were differently affected with the tested compound. The obtained IC_{50} values against PC3 and HepG2 cells increased by about 15.8 and 19.2% from their corresponding positive controls (6.92 and 2.78 nM for PC3 and HepG2 cells, respectively, and 8.22 and 3.44 nM for Bicalutamide and Gemcitabine, respectively. For, K561 and RKOP27 cells, the obtained IC_{50} (3.35 and 1.54 nM, respectively) increased by about 49.7 and 64.4%, respectively, from their positive controls (6.66 and 4.33 nM for Doxorubicin and Capecitabine, respectively. The most prominent effect was obtained against HT1080 and HeLa cells, where there was an increased cytotoxic effect of about 7.4- and 15.1-folds from their positive controls. Regarding ovarian carcinoma (SKOV-3), CNS cancer (SF-268), non-small lung cancer (NCI H460), leukemia (HL60 and U937), melanoma (SK-MEL-28), neuroblastoma (GOTO and NB-1), breast carcinoma (MCF-7), it can be seen that although these cell lines were also affected by the prepared compound (**5**), however, the obtained IC_{50} values for them were much lower than their corresponding positive controls as shown in Fig. 1.

Generally, different cells react differently towards affecting compounds of natural extracts due to their



Scheme 1 — Synthetic pathway for compound **5**

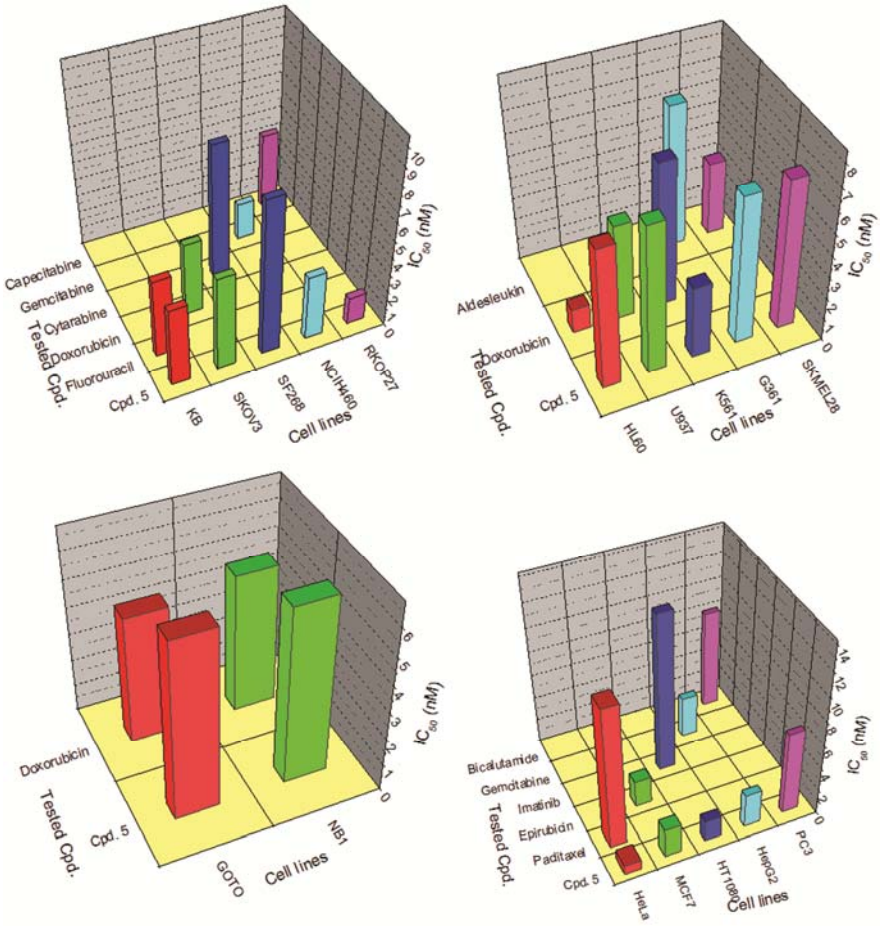


Fig. 1 — Anti-VEGFR-2 activity of the synthesized compound **5**

built-in differences in membrane structure and organization.[23,24]

In vitro anti-VEGFR-2 screening

The prepared compound (Cpd. **5**) was evaluated for its inhibitory effect of VEGFR-2 kinase activity in order to have an estimated idea about its possible mechanism of action. Results showed that the prepared cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornthenyl acid hydrazide] showed increased inhibitory effect against VEGFR-2 kinase enzyme, where the inhibitory effect obtained (IC_{50} = 1.33 nM) was about 33.5% higher than the effect obtained by the positive control (Sorafenib, IC_{50} = 2 nM).

***In vivo* antiprostate cancer activity**

Finally, the potential of the prepared cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornthenyl acid hydrazide] to exhibit antitumorigenic effects in animal model was evaluated against *in vivo* antiprostate cancer developed in mice. In terms of ED_{50} value, Cpd. 5 showed a potential antitumorigenic activity (ED_{50} = 1.65 ± 0.021 μM), which was about 7-folds higher than the obtained results for Flutamide (ED_{50} = 11.60 ± 0.09 μM).

Conclusion

We reported the potential *in vitro* and *in vivo* anticancer activities of a newly synthesized cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornthenyl acid hydrazide]. Results showed that the prepared compound can adversely affect the viability of all tested cancerous cell lines with variable extents. Additionally, the newly prepared hydrazide may affect the viability of cancerousss cells by interfering with the VEGFR-2 kinase activity. Also, it showed a great potential against *in vivo* developed prostate cancer model. The present study shows the importance of synthesizing chemical starting molecules which may have an impact on the pharmaceutical drug industry in its fight against cancer diseases.

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