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Antiproliferative Activities of Cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornithine] and its *in vitro* anti-VEGFR-2 Inhibition

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Research is continuing for synthesizing new molecules with potent pharmacological effects against cancer. In the current work we prepared a new tripeptide and investigated its *in vitro* as well as *in vivo* antiproliferative effects. Firstly, cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornithine] was prepared and then tested against 17 different cancer cell lines. Results showed that the prepared compound showed increased cytotoxic effects (in terms of decreased IC50 value), which reached about 20.3, 28.1, 18.3 and 72.3% against RKOP27, K561, GOTO, HT1080 cell lines, respectively, in comparison to standard positive controls. Furthermore, cancer cell viability was probably affected through VEGFR-2 kinase inhibition.

Keywords: Anticancer, Kinase inhibition, Macrocyclic, *in vivo*, amino acid ester, cyclic tripeptide.

Introduction

In nature, protein synthesis including a series of peptide coupling reactions (amide bond formation between two a-amino acids or peptides) is very complex. To solve this problem, the amino acid must be transformed to amino acid ester (as intermediates) *in vivo* by a selective activation process catalyzed by enzymes. This intermediate is then involved in a process mediated by the coordinated interplay of more than a hundred macromolecules, including mRNAs, tRNAs, activating enzymes and protein factors, in addition to ribosomes.[1] Amide bond formation between an acid and an amine are formally condensations, whereas, on mixing an amine with a carboxylic acid, an acid– base reaction occurs first to form a stable salt. The amide bond formation has to fight against adverse thermodynamics as the equilibrium shown in the following equation and lies on the side of hydrolysis rather than synthesis.[2]

Pharmacological studies have proved that many peptides, including those synthetic and isolated from plants, have a potential antitumor[3–5], antiviral[6], antimicrobial[7], and anticancer[8] activities. Additionally, in our previous work reported that heterocyclic candidates possess antimicrobial[9–12], analgesic and antiinflammatory[13], inhibitors of VEGFR-2 tyrosine kinase[14], and anti -breast cancer[15] activities. In continuation of our interest in the chemical and pharmacological properties of di-substituted pyridine and amino-acid derivatives, we report herein *in vitro* anti-VEGFR-2 Inhibition of cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornithine].

Materials and Methods

Chemistry

The tested compound **4** was elucidated previously by physical and spectroscopic data by Amr *et al*.[16]

Synthesis of cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornthene] (4)

Compound **4** was synthesized by reaction of 3, 5-pyridine dicarbony dichloride (**1**) with L- valine methyl ester followed by hydrolysis to corresponding diacid **2,** which was cyclized with L-ornithine methyl ester by using Mixed anhydride method according to reported procedure[1] to give macrocyclic tripeptide **3**. Treatment of **3** with methanolic sodium hydroxide gave the target compound **4**.

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Anticancer activity

The prepared compound was screened for its potential antiproliferative effects against different cell lines using standard MTT assay.[17,18] Cell lines tested were chosen to cover a wide range of cancerous diseases in different tissues and organs. They included cervical carcinoma (KB), ovarian carcinoma (SKOV-3), CNS cancer (SF-268), non-small lung cancer (NCI H460), colon adenocarcinoma (RKOP27), leukemia (HL60, U937 and K562), melanoma (G361 and SK-MEL-28), neuroblastoma (GOTO and NB-1), cervical carcinoma (HeLa), breast carcinoma (MCF-7), fibrosarcoma (HT1080), liver carcinoma (HepG2) and prostate carcinoma (PC-3). Cell propagation, maintenance and assay procedure were followed as per our previous work.[17,18] Additionally standard reference drugs were used throughout the work as positive controls.

***In vivo* evaluation of antiprostate cancer activity**

The *in vivo* evaluation was carried out according to Kinoyama *et al.*[19] using Male Wistar rat model. The protocol followed the ethical guidelines stated for animal care.

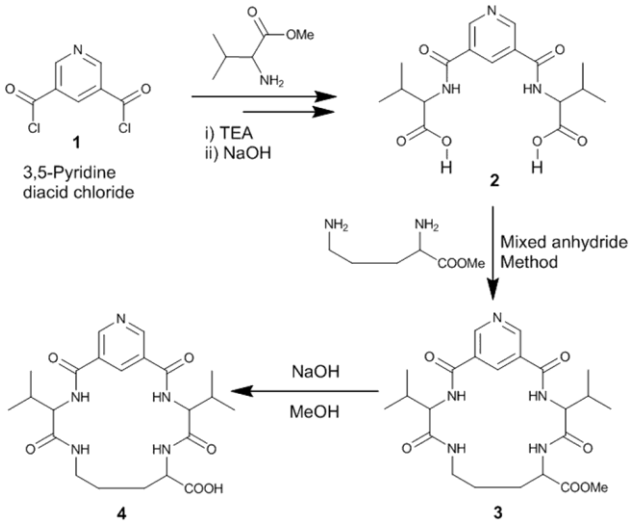
ELISA determination of kinase inhibition

The assay was performed according to our previously established protocol.[17] Briefly, wells previously coated with poly (Glu, Tyr) were seeded with ATP solution and different serial dilutions of the prepared derivative. Results were compared to Sorafenib. VEGFR-2 kinase was added to wells to start the experiment, and after 1 h at 37 °C, plates were washed. The 2nd antibody (Goat anti-mouse IgG horseradish peroxidase) was used to complete the reaction and the assay followed the standard.

Result and Discussion

Chemistry

Cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornithine] **4** was synthesized according to our previous work.[16] Treatment of acid chloride **1** with L-valine methyl ester in the presence of trimethylamine gave the corresponding bis-ester followed by hydrolyzed with sodium hydroxide to corresponding bis-acid **2**. The latter compound **2** was cyclized with L-ornithine methyl ester by using mixed anhydride method to give macrocyclic tripeptide ester **3**. Hydrolysis of **3** with methanolic sodium hydroxide afforded the corresponding title compound **4** (Scheme 1).



Scheme 1 — Chemical structure for synthesized compounds[16]

Antiproliverative screening

After chemical synthesis, the prepared compound was then subjected to detailed cytotoxic evaluation against different cancer cell lines, both *in vivo* and *in vitro*. Preliminary results (Fig. 1) revealed that the prepared compound was effective against all tested cell lines, and showed a varying degree of cell toxicity, which was cell type dependent. However, the most potential antiproliferative activities obtained were noticed against RKOP27, K561, GOTO, HT1080, HepG2 and PC3 cell lines. The obtained IC_{50} values for these cell lines recorded 3.45, 4.79, 3.57, 3.67, 3.45 and 8.81 nM, respectively. Furthermore, it can be seen that these obtained IC_{50} values were either similar or much less than those obtained by the corresponding control drugs; 4.33, 6.66, 4.37, 13.24, 3.44 and 8.22 nM for Capecitabine, Doxorubicin, Imatinib, Gemcitabine and Bicalutamide, respectively, for the same order of cell lines. From these data, it can be seen that the prepared compound showed an increase in its cytotoxic activities (in terms of IC_{50} values), which reached about 20.3, 28.1, 18.3 and 72.3% from the values obtained for the standard reference drugs. Accordingly, the potential of the prepared compound can be outlined as a base for preparing pharmaceutically drugs active against cancer cells.

***In vivo* antiprostate cancer activity**

Secondly, the *in vivo* cytotoxic effects of the synthesized derivative against prostate cancer developed in mice showed promising results, where the obtained ED_{50} value was significant

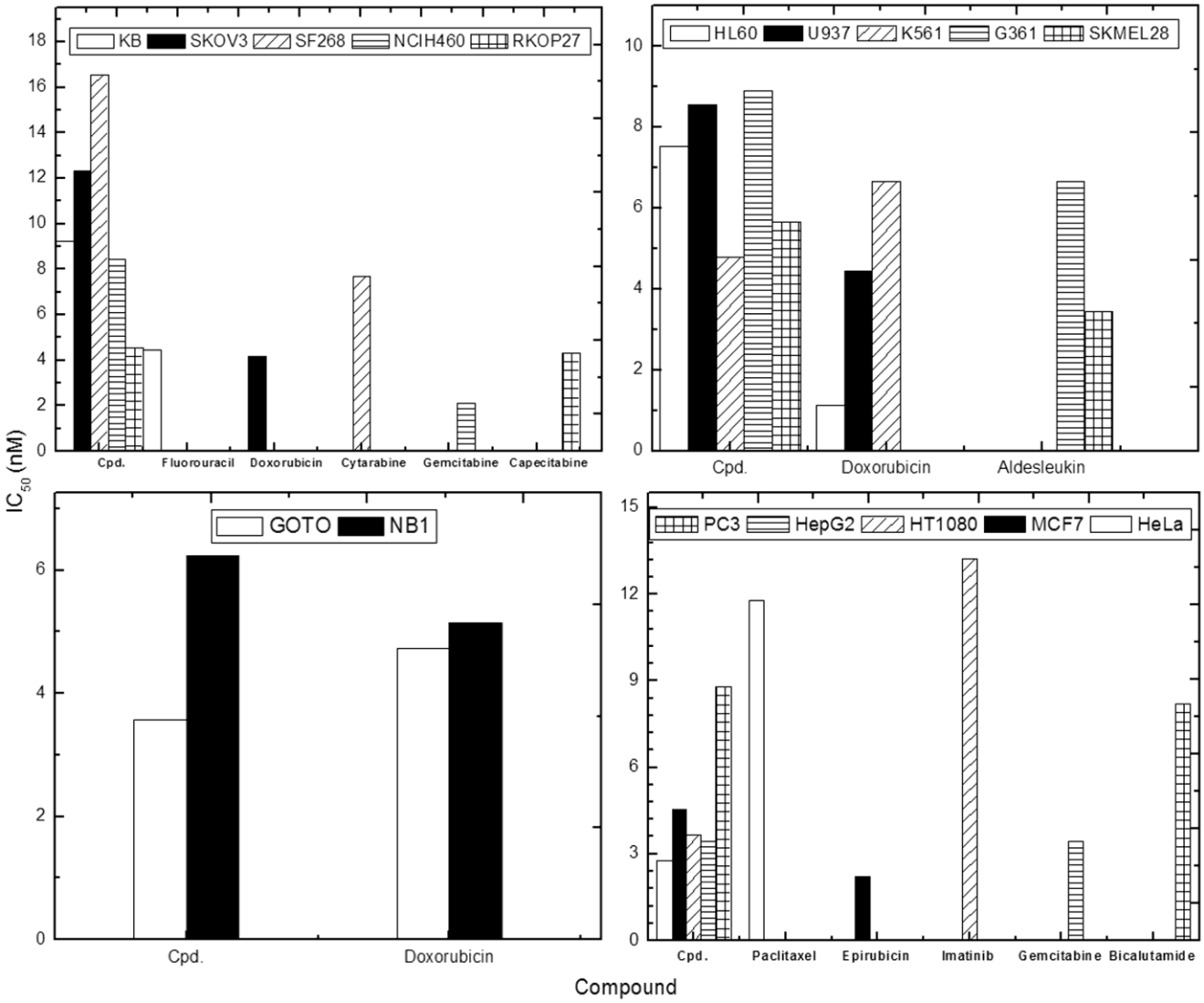


Fig. 1 — Antiproliferative activities of the cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornithine] towards different cancer cell lines

(4.65 ± 0.03 μM) when compared to that obtained for the reference drug investigated *in vivo* (11.60 ± 0.09 μM). It can be seen that our prepared compound showed about 2.5-folds increase in its effects against *in vivo* prostate cancer model.

Anti-VEGFR-2 inhibition

In order to gain an overview on the possible mode of action, by which the newly synthesized derivative may act on cancer cells, the inhibitory effect of the synthesized compound was tested against VEGFR-2 kinase enzyme. The compound exhibited a potential effect with an inhibitory IC_{50} value for VEGFR-2 kinase of 1.88 nM. Furthermore, comparing our derivative with the standard Sorafenib (IC_{50} 2.0 nM) showed that compound 4 has increased inhibitory effect (about 6% increase).

Conclusion

A tripeptide, namely cyclo (N^{α}-pyrido)-bis-[(L-valinyl)-L-ornithine] was prepared by the cyclization of the corresponding diacid with L-ornithine methyl ester. The prepared tripeptide revealed increased effects against RKOP27, K561, GOTO, HT1080 cell lines, where the obtained IC_{50} values for these cell lines were lower than their corresponding positive controls. Additionally, the compound showed 2.5-folds increase in its activity *in vivo* against PC3 carcinoma. Therefore, the new compound can serve as a promising molecule in drug discovery.

Abbreviations

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

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References

1 Christian A G N M & Falque V, Amide bond formation and peptide coupling, *Tetrahedron*, **61** (2005) 10827–10834.
2 Ulijn R V, Moore B D, Janssen A E & Halling P J, A single aqueous reference equilibrium constant for amide synthesis–hydrolysis, *J Chem Soc*, **2** (2002) 1024–1028.
3 Prabha M R & Ramachandramurty B, Sequence determination of a novel tri-peptide isolated from the young leaves of *Azadirachta indica* A. Juss., *Int J Peptides*, **2013** (2013) Article ID 629549.
4 Wele A, Zhang Y, Ndoye I, Brouard J P, Pousset J L & Bodo´B, A cytotoxic cyclic heptapeptide from the seeds of *Annona cherimola*, *J Nat Prod*, **67** (2004) 1577–1579.
5 Ung P & Winkler D A, Tripeptide motifs in biology: targets for peptidomimetic design, *J Med Chem*, **54** (2011) 1111–1125.
6 Hubatsch I, Lazorova L, Vahlne A & Artursson P. Orally active antiviral tri-peptide glycyl-prolyl-glycinamide is activated by CD26 (dipeptidyl peptidase IV) before transport across the intestinal epithelium, *Antimicrob Agents Chemother*, **49** (2005) 1087–1092.
7 Hong J, Chen T-T, Hu P & Wang S-Y, A novel antibacterial tri-peptide from Chinese leek seeds, *Eur Food Res Technol*, **240** (2015) 327–333.
8 Thundimadathil J, Cancer treatment using peptides: current therapies and future prospects, *J Amino Acids*, **2012** (2012), Article ID 967347.
9 Al-Harbi N O, Bahashwan S A, Fayed A A, Aboonq M S & Amr A E, Anti-parkinsonism, hypoglycemic and anti-microbial activities of new poly fused ring heterocyclic candidates, *Int J Biol Macromol*, **57** (2013) 165–173.
10 Ghozlan S A S, Al-Omar M A, Amr A E, Ali K A & El-Wahab A A, Synthesis and antimicrobial activity of some heterocyclic 2,6-bis(substituted)-1,3,4-thiadiazolo-, oxadiazolo-, and oxathiazolidino-pyridine derivatives from 2,6-pyridine dicarboxylic acid dihydrazide. *J Heterocyc Chem*, **48** (2011) 1103–1110.
11 Hossan A S M, Abu-Melha H M A, Al-Omar M A & Amr A E, Synthesis and antimicrobial activity of some new pyrimidinone and oxazinone derivatives fused with thiophene rings using 2-chloro-6-ethoxy-4-acetylpyridine as starting material, *Molecules*, **17** (2012) 13642–13655.
12 Mohamed S F, Youssef M M, Amr A E & Kotb E R, Antimicrobial activities of some synthesized pyridines, oxazines and thiazoles from 3-aryl-1-(2-naphthyl)prop-2-en-1-ones, *Sci Pharmac*, **76** (2008) 279–303.
13 Khalifa N M, Al-Omar M A, Amr A E, Baiuomy A R & Abdel-Rahman R F, Synthesis and biological evaluation of some novel fused thiazolo [3,2-a] pyrimidines as potential analgesic and anti-inflammatory agents, *Russ J Bioorg Chem*, **41** (2015) 192–200.
14 Xi L, Zhang J, Liu Z, Zhang J, Yan J, Jin Y & Lin J, Novel 5-anilinoquinazoline-8-nitro derivatives as inhibitors of VEGFR-2 tyrosine kinase: synthesis, biological evaluation and molecular docking, *Org Biomol Chem*, **11** (2013) 4367–4378.
15 Amr A E, El-Naggar M, Al-Omar M A, Elsayed E A & Abdalla M M, *In vitro* and *in vivo* anti-breast cancer activities of some synthesized pyrazolinyl-estran-17-one candidates, *Molecules*, **23** (2018) 1572.
16 Amr A E, Naglah A M, Sabry N M, Ibrahim A A, Elsayed E A & Attar A. Synthesis and investigation of 3,5-bis-linear and macrocyclic tripeptidopyridine candidates by using l-valine, N,N′-(3,5-pyridinediyldicarbonyl)bis-dimethyl ester as synthon, *Zeitschrift für Naturforsch* B, **74** (2019) 473–478.
17 Amr A E, Abo-Ghalia M H, Moustafa G O, Al-Omar MA, Nossir E & Elsayed EA, Design, synthesis and docking studies of novel macrocyclic pentapeptides as anticancer multi-targeted kinase inhibitors, *Molecules*, **23** (2018) 2416.
18 Amr A E, El-Naggar M, Al-Omar M A, Elsayed E A & Abdalla M M, *In vitro* and *in vivo* anti-breast cancer activities of some synthesized pyrazolinyl-estran-17-one candidates. *Molecules*, **23** (2018) 1572**.**
19 Kinoyama I, Taniguchi N, Kawaminami E, Nozawa E, Koutoku H, Furutani T, Kudoh M & Okada M, N-Arylpiperazine-1-carboxamide derivatives: a novel series of orally active non-steroidal androgen receptor antagonists, *Chem Pharm Bull*, **53 (**2005) 402–409.