Journal of Scientific & Industrial Research
Vol. 79, April 2020, pp. 292–296

Cyclo (N^{α}-dinicotinoyl)-bis-[(L-valinyl)-L-lysinyl acid hydrazide]: Assessment of its Role in Cancer and Kinase Activity Inhibition

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Received 9 June 2019; revised 17 December 2019; accepted 15 February 2020

Current research aimed at evaluating the *in vitro* as well as i*n vivo* anticancer activities of a newly synthesized peptide hydrazide; i.e. 4,14-diisopropyl-2,5,13,16-tetraoxo-3,6,12,15-tetraaza-1(3,5)-pyridinacyclohexadecaphane-7-carbohydrazide. The hydrazide was synthesized from methyl 4,14-diisopropyl-2,5,13,16-tetraoxo-3,6,12,15-tetraaza-1(3,5)-pyridinacyclohexadecaphane-7-carboxylate **2** by acting of hydrazine hydrate. It showed significant anticancer effects against different tested cell lines, where cervical, breast, liver, colon, prostate, brain, fibrosarcoma, leukemia and melanoma cell lines were the most affected cell types. The prepared derivative also inhibited VEGF-2 kinase enzyme significantly and exhibited an *in vivo* tumorigenic effects in mice model.

Keywords: Anticancer, Synthesis, Peptides, Hydrazide, Mechanism of action.

Introduction

Hydrazides have been known to be associated with Antibacterial[1], antifungal (against three species such as: *Candida albicans*, *Candida glabrata*, and *Candida tropicalis*[2]), anthelmintic[3], and anticonvulsant[4] activities. On the other hand, hydrazide and their derivatives "hydrazones" constitute a large class of organic candidates, which played very important role in medicinal chemistry due to the fact that they contain azomethine group (–NH–N=CH–) connected with carbonyl group, which is responsible for their different pharmaceutical applications and makes possible the synthesis of different heterocyclic scaffolds.[5] In recent years, a lot of biologically important heterocyclic and hydrazide derivatives with a number of functional groups have been synthesized[6–11] from many different carbonyl compounds. In addition, they were found to possess anticancer[12–15], anti-inflammatory[16], anticonvulsant[17], antiviral[18], and antiprotozoal[19] activities. The aim of the current work was to synthesize a new hydrazide tripeptide as a macrocyclic tripeptidopyridine and to evaluate its possible anticancer activities *in vitro* as well as *in vivo*.

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Materials and Methods

Reagents

3,5-Pyridinedicarbonylchloride, L-Valene, methanol, dichloromethane, hydrazine hydrate, and L-lysine methyl ester, 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. 1H 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).

Synthesis of 4,14-diisopropyl-2,5,13,16-tetraoxo-3,6,12,15-tetraaza-1(3,5)-pyridinacyclo-hexadecaphane-7-carbohydrazide3

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 **(2)** (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 4, 14-diisopropyl-2,5,13,16-tetraoxo-3,6,12,15-tetraaza-1(3,5)-pyridinacyclohexadecaphane-7 carbohydrazide**3**. The obtained compound **3** was compared with authentic sample previously prepared and characterized by Amr *et al*.[20]

Anticancer screening

Serial dilutions of the prepared hydrazide (in DMSO) were evaluated for their anticancer activities using standard MTT assay following the protocol previously developed in our laboratories.[21,22] The method is based on the reaction between the effector and the dehydrogenases within cellular mitochondria. Absorbance was measured using a microplate reader as 560 nm. Reference drugs were used as positive controls depending on tested cell lines.

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

This section was carried out using Male Wistar rats injected with androgensto develop prostate cancer *in vivo*. The assay and measurement protocols were adapted from our previous works.[23]

Mechanism of action studies

In order to evaluate the possible mechanistic pathway, by which the prepared hydrazide may affect cancer cells, we investigated the inhibition of vascular endothelial growth factor (VEGF receptors). The assay was done according to our previously published protcools.[24]

Result and Discussion

Chemistry

We have previously synthesized some macrocyclic tripeptidopyridine candidate**3**and they characterized by physical, chemical and spectroscopic evidences in advance according to our previous work.[20] Compound **2** was synthesized by treating ofdiacid chloride **1** with L-valine methyl ester to give the corresponding bis-ester followed by hydrolysis with methanolic sodium hydroxide to give the corresponding bis-acid derivative. The latter compound was cyclized with L-lysine methyl ester hydrochloride by using mixed anhydride method gave cyclized product **2**. Treatment of **2** with hydrazine hydrate in methanol gave the corresponding hydrazide **3** (Scheme 1).

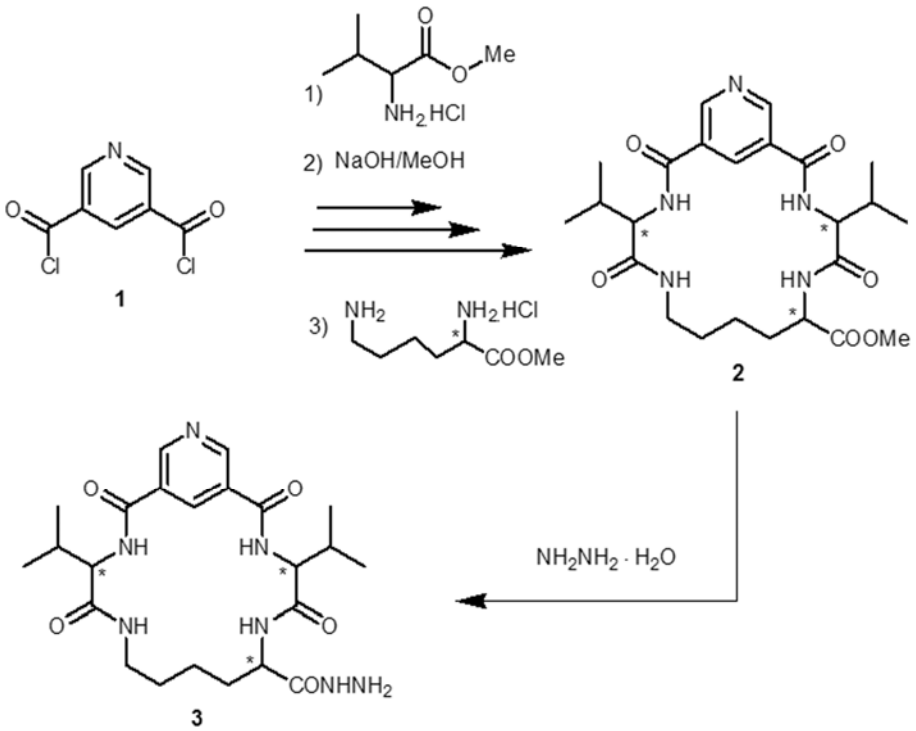
***In vitro* anticancer evaluation**

The present section aimed at investigation the possible anticancer effects of the synthesized derivative (compound **3**) against various cancer cell lines. Results in Fig. 1 showed clearly that the prepared compound have an anticancer effect against all tested cell lines and also in a dose-dependent relationship. However, the effect varied depending on the tested cell line. This is in great accordance with our previously published work on the evaluation of anticancer potential of synthesized molecules.[22,24,25] This effect can be attributed to the varied architecture of membrane structure of various cell types. Secondly, it can be seen that comparing the obtained anticancer effects with standard pharmaceutical drugs, which are usually used in clinical studies as well as scientific research, showed that the tested cell lines can be divided into two main categories, in terms of their response to compound 5. The first class, where the cells were affected, however, with a lower potential than the tested drugs, includes SKOV3 (ovarian), NCIH460 (non-small lung cancer), HL50 and U937 (leukemia), GOTO and NB1 (melanoma) cell lines. It can be seen that the reported IC50 values for were lower than the values obtained for positive drugs by about 1.1-, 1.25-, 4.8-, 1.5-, 1.58- and 1.28-folds for SKOV3, NCIH460, HL60, U937, GOTO and NB1 cells, respectively.

On the other hand, the remaining tested cell lines were affected with higher degrees of biological activities, where the obtained IC50 values (0.45, 4.56, 1.45, 2.67, 5.43, 1.24, 0.45, 2.0, 1.0, 2.0 and 5.99 nM) were higher by about 9.9-, 1.7-, 2.99-, 2.5-, 1.23-, 2.8-, 26.2-, 1.1-, 13.4-, 1.72- and 1.4-folds for KB (cervical), SF268 (CNS), RKOP27 (colon), K561 (leukemia), G361 (melanoma), SKMEL28 (melanoma), HeLa (cervical), MCF7 (breast), HT1080 (fibrosarcoma)), HepG2 (liver) and PC3 (prostate) cells, respectively. The values recorded for their corresponding positive drugs were 4.46, 7.68, 4.33, 6.66, 6.66, 3.45, 11.8, 2.22, 13.24, 3.44 and 8.22 nM for Florouracil, Cytarabine, Capecitabine, Doxorubicin, Aldesleukin, Aldesleukin, Paclitaxel, Epirubicin, Imatinib, Gemcitabine and Bicalutamide, respectively, for the same order of cell lines.

***In vivo* anticancer potential**

The second section was designed to investigate the potential of compound **3** to affect the growing tumor of prostate cancer *in vivo* in animal model. Results obtained showed that the synthesized derivative potentially decreased the *in vivo* prostate tumor growth by an ED_{50} value of 1.28 ± 0.032 μM. It can be seen that this value is significant when compared to that obtained for Flutamide as a positive drug



Scheme 1— Synthetic pathway for compound **3**

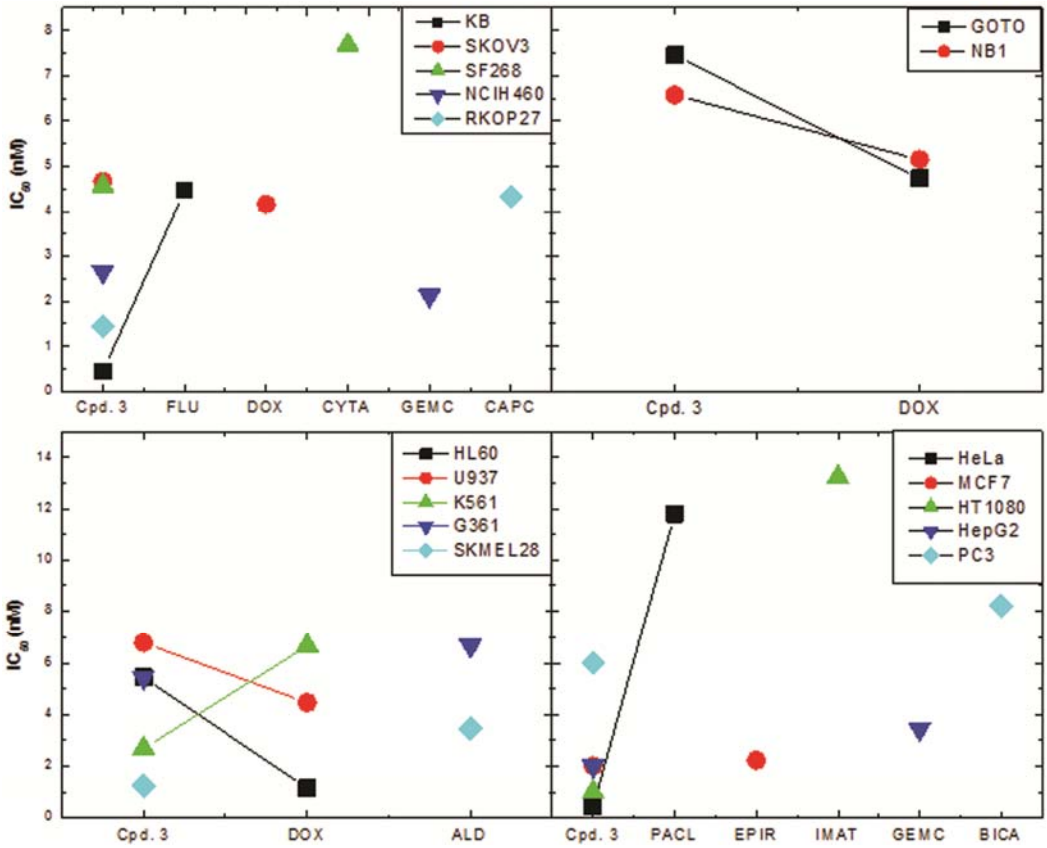


Fig. 1 — Effect of compound **3** on the viability of different tested cancer cell lines

(11.60 ± 0.09 μM), where the *in vivo* effect increased by about 8839% from the positive control.

Mechanism of action investigation

Finally, the inhibition of the receptors for vascular endothelial growth factor-2 was evaluated in response to different concentrations of the prepared derivative. Results showed that the kinase enzyme was significantly inhibited at an IC_{50} of 1.02 nM, when compared to the value obtained for reference drug inhibition (Sorafenib, IC_{50} = 2 nM). These results suggest that the prepared compound 5 may exhibit its anticancer activity via blocking the receptors for VEGF-2 kinase enzymatic pathway.

Conclusion

Within the framework of our continuing efforts to provide the pharmaceutical industry with biologically active molecules exhibiting potential anticancer activities against a wide range of tumorigenic cell lines, we prepared a Cyclo (N^{α}-dinicotinoyl)-bis-[(L-valinyl)-L-lysinyl acid hydrazide] via hydrazonolysis mechanism. The prepared acid hydrazide showed great potential against all tested cell lines. Furthermore, the effect was cell line-dependent. HeLa and HT1080 cells were affected by about 26.2- and 13.4-folds higher that the evaluated positive references. Additionally, results showed that the prepared hydrazide has a potential *in vivo* anticancer activity, and that these *in vitro* as well as *in vivo* biological effects may be related to the inhibition of cell receptors for VEGF-2 kinase enzyme.

Acknowledgments

Authors are grateful to King Saud University for funding the work through Researchers Supporting Project (Project No. RSP-2019/66).

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