ФАРМАЦИЯ
PHARMACIA

Том/Volume LVIII **2011** **Книжка/Number 1-4**

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С Ъ Д Ъ Р Ж А Н И Е

Оригинални статии

C O N T E N T S

Original articles

ФАРМАЦИЯ 1-4/2011

ISSN 0428-0296 УДК 615

Организационен секретар *Св. Цветанова*
Стилова редакция *Св. Цветанова и д-р Б. Станчева (на англ. ез.)*
Корекция *Св. Цветанова*
Терминологичен и семантичен контрол *д-р Б. Станчева*
Форматиране *О. Маркова*

Подписана за печат на 09.01.2011 г.
Печатни коли 15, формат 60 x 90/8

Централна медицинска библиотека
1431 София, ул. „Св. Г. Софийски" № 1, тел. 952-16-45, Fax: 851 82 65
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SYNTHESIS AND PHYSICOCHEMICAL CHARACTERIZATION OF NEW SALICYLALDEHYDE BENZOYL HYDRAZONE DERIVATIVE WITH HIGH CYTOTOXIC ACTIVITY

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Summary. Aroylhydrazones derived from pyridoxal and salicylaldehyde are compounds with interesting biological properties including a high anticancer activity. Salicylaldehyde benzoylhydrazone (SBH) is an unusually potent inhibitor of DNA synthesis and cell growth in a variety of cultured human and rodent cells. The current study reports synthesis, physicochemical characterization and pharmacological investigations of a new derivative – 3-methoxy-salicylaldehyde isonicotinoylhydrazone (mSIH). The hydrazone was prepared by the Schiff base condensation between isonicotinoyl hydrazide and 3-methoxy-salicylaldehyde in ethanol. The structure of the compound was confirmed by elemental and thermo-gravimetric analyses, IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The cytotoxic effect of the new compound was examined on some human tumor cell lines using the MTT-dye reduction assay. The obtained IC_{50} values revealed that 3-methoxy-salicylaldehyde isonicotinoylhydrazone proved to be equipotent or more active than cisplatin, and far more active than another utilized anticancer drug melphalan.

Key words: 3-methoxy-salicylaldehyde isonicotinoylhydrazone, characterization anti-cancer activity

СИНТЕЗ НА ФИЗИКОХИМИЧНА ХАРКАТЕРИСТИКА НА НОВ ДЕРИВАТ НА САЛИЦИЛАЛДЕХИТ БЕНЗОЛ ХИДРАЗОНА С ВИСОКА ЦИТОТОКСИЧНА АКТИВНОСТ

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Резюме. Ароилхидразоните, получени от пиридоксал и салициладехид, са съединения с интересни биологични свойства, включително висока антиканцерогенна активност. Салицилалдехид бензоилхидразонът (SBH) е необикновено мощен инхибитор на ДНК синтеза и растежа на клетките в множество култивирани човешки клетки и клетки на гризачи. Представеното проучване разглежда синтеза, физикохимичната характеристика и фармакологичните изследвания на нов дериват 3-метокси-салицилалдехид изоникотиноил хидразон (mSIH). Хидразонът е получен чрез Schiff base кондензация на изоникотиноил хидразид и 3-метокси-салицилалдехид в етанол. Структурата на съединението е потвърдено с елементен и термогравиметричен анализ, IR, ^{1}H-NMR и ^{13}C-NMR спектроскопия. Цитотоксичното действие на новото съединение е изпробвано върху някои линии на човешки туморни клетки с използване на MTT-dye редукционен анализ. Получените IC_{50} стойности показват, че 3-метокси-салицилалдехид изоникотиноил хидразонът е еднакво силен или по-активен от цисплатината и далеч по-активен от другото използвано средство срещу рак – мелфалан.

Ключови думи: 3-метокси-салицилалдехид изоникотиноил хидразон характеристика, антиканцерогенен ефект

Introduction

Interest in the study of hydrazones has been growing in the last 30 years because of their antimicrobial, antituberculosis, and antitumour activity [1]. Aroylhydrazones derived from condensation of salicylaldehyde and different acid hydrazides are very effective anti-proliferative agents. The compound salicylaldehyde benzoylhydrazone (SBH) is an unusually potent inhibitor of DNA synthesis and cell growth in a variety of cultured human and rodent cells. Various derivatives of salicylaldehyde benzoylhydrazone have been synthesized in order to discover new more effective antiproliferative agents [2-6]. These promising results with salicylaldehyde benzoylhydrazone prompted us to design a new SBH analogue. In order to receive a compound with high cytotoxic activity we used 3-methoxy-salicylaldehyde and the biologically active isonicotinic acid hydrazide.

In this work we report the synthesis and pharmacological characterization of new potential therapeutic agent 3-methoxy-salicylaldehyde isonicotinoylhydrazone (3mSIH) (fig. 1).

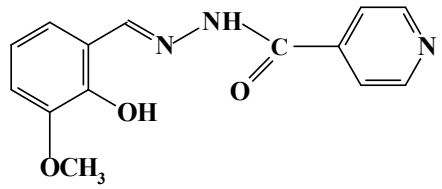

Fig. 1. *3-methoxy-salicylaldehyde isonicotinoylhydrazone (3mSIH)*

Materials and methods

Synthesis of 3mSIH

A solution of 3-methoxy-salicylaldehyde (0,01 mol) in 96% ethanol (10 ml) was added to the isonicotinic acid hydrazide (0,01 mol) in 50 % aqueous ethanol (40 ml) at constant stirring and heating until the resulting solid phase was dissolved. The solution was allowed to cool and stand at room temperature for 24 hours. During this time, crystals of the product were obtained, then filtered. The solid hydrazone was dried for 2 days in a vacuum desiccator.

Computational details

Density functional theory (DFT) calculations for the molecule were carried out with Becke's 3-parameter hybrid functional, combined with the Lee-Yang-Parr correlation functional - B3LYP with 6-31+G (d,p) basis set using the Gaussian 03 program suite.

Cytotoxicity assessment

The cytotoxic effect of the newly synthesized salicylaldehyde benzoyl hydrazone derivative against the panel of human leukemic and tumor cell lines were determined using the standard MTT-dye reduction assay for cell viability following a 72 h continuous exposure.

Data processing and statistics

The cell survival data were normalized as percentage of the untreated control (set as 100% viability). The statistical processing of biological data included the Student's t-test whereby values of $p \leq 0.05$ were considered statistically significant. In addition, IC_{50} values were derived from the concentration-response curves using non-linear regression analysis.

Results and discussion

Chemistry

Elemental Analysis

The results of the elemental analysis of the new compound are summarized in Table 1.

Infrared spectra

The important infrared frequencies with their possible assignments from the spectra of the new hydrazone are listed in Table 2.

NMR spectra

The ^{1}H- and ^{13}C-NMR spectra were determined in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) are reported in part per million (ppm) and the important peaks are given in Table 3. The numbers of protons obtained by integration of the peaks are in good agreement with the expected structure.

Table 1. *Characterization data of the compound*

Molecule	Molecular formula	Yield (%)	Colour	m.p. (°C)	Found/Calculated (%)			
					C	H	N	O
3mSIH	$C_{14}H_{13}O_3N_3$	89	brightly yellow	236°-237°	62.10/61.99	4.94/4.83	15.67/15.49	17.29/17.69

***Table 2**. Infrared frequencies (cm-1) from spectra of 3mSIH in the solid state (KBr)*

Assignment	3mSIH
ν(O – H)	3350
ν(N – H)	3203
ν(C = O)	1691
ν(C = N)	1604
ν(C – NH)	1566
δ(O – H)	1289
ν(C – O)	1247

Computational study

In order to predict the molecular structure of the new compound, quantum-chemical calculations were carried out. The structural parameters of the new hydrazone were computed at B3LYP level of theory using a 6-31+G (d,p) basis set. The calculated bond lengths and angles were compared with the experimental crystallographic data for SBH. The optimized geometry of the compound is shown in Fig. 2.

Pharmacology

It is evident from the IC_{50} values that the hydrazone proved to be potent antiproliferative agent causing concentration-dependent cytotoxic effect at low micromolar concentrations.

Among the cell lines under evaluation, the human acute myeloid leukaemia HL-60 proved to be the most sensitive to mSIH treatment, actually the IC_{50} value in these cells is the lowest (Table 4).

Throughout the panel of tested cell lines, the novel compound 3mSIH proved to be equipotent or more active than cisplatin, and far more active as compared to the other reference antineoplastic agent melphalan (Fig. 3).

Conclusion

In conclusion, the new hydrazone, derivative of salicylaldehyde benzoyl hydrazone was synthesized. The antiproliferative activity of the novel compound was investigated in a wide spectrum of cell lines, representative for some important types of human cancer.

Based on the results of the MTT-dye reduction assay, the compound mSIH deserves more detailed toxicological and pharmacological investigation for the development of new anticancer drugs.

***Table 3.** Magnetic resonance peaks (ppm) and their assignments for mSIH*

1H-NMR						13C-NMR		
OCH3	Aryl ring (from 3-methoxy-salicylaldehyde)	Aryl ring (from benzhydrazide)	CH=N	NH	OH	CH3	CH=N	C=O
3.82	6.85-7.22	7.84-8.81	8.72	10.72	12.23	55.82	150.34	161.29

***Table 4** Cytotoxic activity of the tested hydrazone in a panel of human tumor cell lines after 72 h continuous exposure (MTT-assay)*

Compound	IC_{50} (μmol/l)						
	HL-60	KE-37	K-562	BV-173	SaOS-2	MDA-MB-231	MCF-7
mSIH	2.7 ± 0.9	3.3 ± 1.2	8.7 ± 2.0	3.7 ± 1.1	11.9 ± 3.2	16.4 ± 2.0	11.7 ± 2.4
Cisplatin	4.7 ± 3.4	5.2 ± 3.5	12.5 ± 4.2	4.2 ± 2.1	21.3 ± 6.6	17.9 ± 3.9	19.9 ± 7.4
Melphalan	18.5 ± 2.1	21.4 ± 3.9	28.2 ± 7.1	31.3 ± 3.9	31.4 ± 5.4	42.2 ± 3.7	33.7 ± 4.2

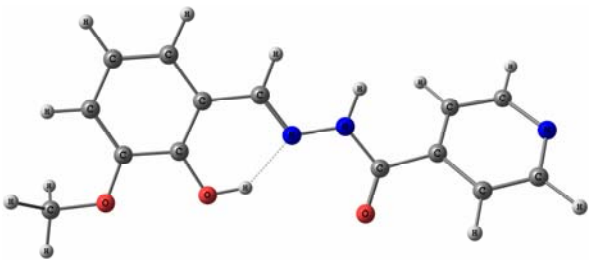

***Fig. 2.** B3LYP/6-31+G (d,p) optimized structure of 3mSIH*

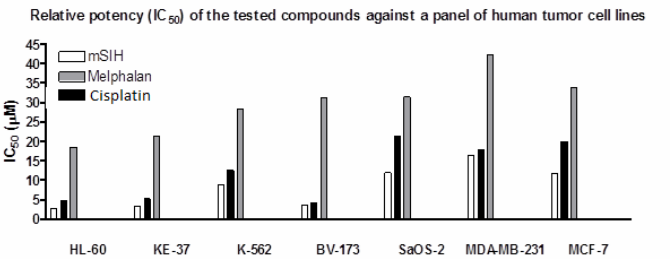

***Fig 3.** Relative potency (IC50) of the tested compounds against a panel of human tumor cell lines*

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