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Biological test against breast adenocarcinoma cells (MCF-7) of acylated products of 3-methyl-4-thiorhodanine

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ABSTRACT

Rhodanine and its derivatives are not only valued for their interesting chemical properties but also present a wide range of bioactivities and their chemical and pharmacological applications have been investigated. From 3-methyl-4-thiorhodanine we synthesized S-acylated and C-acylated

products using (some) carboxylic acid chlorides and the obtained products were studied the anti-proliferative activity by a SRB (sulforhodamine B) assay against a human breast cancer cell line. The results showed that C-acylated products are better inhibitors than S-acylated products.

Key words: 3-methyl-4-thiorhodanine, 2-thioxo-1,3-thiazolane-4-one, C-acylated, S-acylated, breast cancer cell line (MCF-7).

INTRODUCTION

Rhodanine derivatives have been proven to be attractive compounds due to their outstanding biological activities and have undergone rapid development as anticonvulsant, antibacterial, antiviral, and antidiabetic agents. At the same time, these have also been reported as Hepatitis C virus (HCV) protease inhibitors and used as inhibitors of uridine diphospho-*N*-acetylmuramate/L-alanine ligase. Recently, substituted rhodanines were investigated for the aggregation inhibitor properties. Rhodanines are classified as nonmutagenic and a long-term study

on the clinical effects of the rhodanine-based Epalrestat as an anti-diabetic, demonstrated that it is well tolerated. Additionally, rhodanines have been designed as inhibitors of various enzymes such as bacterial β -lactamase and Mur ligases. Rhodanine derivatives were found to have marked mildew-proofing activity. It is interesting to note that the new mildew-proofing agents contain the structure $\text{—}\overset{\text{N}}{\underset{\text{O}}{\text{C}}}\text{—}\text{S—}$ present in many plant fungicides (tetramethylthiuram disulfide and the salts of dithiocarbamic acid). Due to various possibilities of chemical

derivatization of the rhodanine ring, rhodanine-based compounds will probably remain a privileged scaffold in drug discovery. Therefore, the synthesis of these compounds is of considerable interest. Recent studies have revealed that 5-benzylidene-3-ethyl rhodanine induced cytotoxicity in a time- and concentration-dependent manner on leukemic cell line, CEM. [1, 2]

Cancer is one of the difficult diseases to be cured, and very few effective drugs are available. The development of novel, efficient, and less toxic anticancer agents remains an important and challenging goal in medicinal chemistry. For specific types of cancer occurring only in women, breast cancer is the second most frequent type of cancer in the world (1.05 million cases) and is by far the most common malignant disease in women (22 % of all new cancer cases) [3]. The MCF-7 human breast cancer cell line has been used as an excellent experimental model to improve the efficacy of different therapies before their use in patients [4].

The SRB (sulforhodamine B) method, as originally developed by Skehan et al. [5] is simple, accurate and yields reproducible results. The cells are briefly washed, fixed and stained with this dye (SRB). The incorporated dye is then liberated from the cells in a Tris base solution. An increase or decrease in the number of cells (total biomass) results in a concomitant change in the amount of dye incorporated by the cells in the

culture. This indicates the degree of cytotoxicity caused by the test material.

In this paper we report the chemical structures of novel derivatives of 3-methyl-4-thiorhodanine which are different in substituents and structures of carbon side chain. Furthermore, *in vitro* anticancer activity as well as the structure - activity relationships are described. The drastic influence of different substituents and side chains on the inhibition is shown in detail and could contribute to a deeper understanding of the anticancer properties of these interesting classes of compounds.

EXPERIMENTAL

Chemistry

The acylation of 3-methyl-4-thiorhodanine (3-methyl-1,3-thiazolane-2,4-dithione) with ten aromatic acid chlorides were performed at two different positions: carbon in position 5 and sulfur in position 4, respectively, to afford two different isomers [6]. By using pyridine as base, pure *S*-acylated products were exclusively obtained for all substituents, on the contrary, with sodium hydroxide as base, the obtained products were pure *C*-acylated ones for all substituents (see Figure 1). *S*-acylated products of 5 aliphatic acid chlorides (XCOC₂H₅ where X is methyl, ethyl, *iso*-propyl, *tert*-butyl and benzyl) with 3-methyl-4-thiorhodanine were also synthesized by the same method. The structures of these compounds were confirmed by their ¹H and ¹³C-NMR spectra.

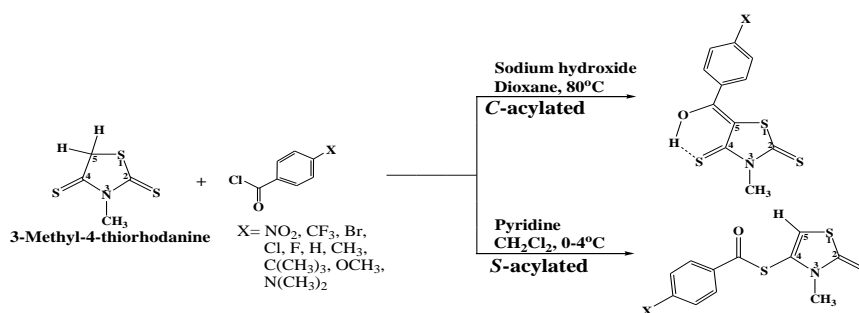


Figure 1. Chemical structures of the *C*- and *S*-acylated products of 3-methyl-4-thiorhodanine by ten aromatic carboxylic acid chlorides

Cytotoxicity

The cytotoxic activity of the products was screened by using the sulforhodamine B colorimetric assay method in the MCF-7 cell line. All the assays were done in Molecular Biological Laboratory- Genetic Department-University of Science- VNU-HCMC.

Cell culture

MCF-7 cells were cultured in E'MEM (Eagle's minimal essential medium) media which was supplemented with 10 % fetal bovine serum and the cells were maintained at 37 °C in a 5 % CO₂ atmosphere with 95 % humidity [7].

Protocol

All adherent cell lines were detached from the culture flasks by addition of 1mL of 0.05 % trypsin-EDTA to make single cell suspensions and viable cells were counted by trypan blue exclusion in a hemacytometer and diluted with medium to give the final concentration. Since the investigated compounds were insoluble in water, they were initially dissolved in DMSO and further diluted with culture medium for analysis. The concentration of DMSO was less than 0.5 % which was found to be non-toxic to the cells. Cells were inoculated at densities 10,000 cells per well and were preincubated for 24hrs at 37 °C to allow stabilization prior to addition of complexes. Subsequently, the different

complexes were incubated for 48 h in an atmosphere of 5 % CO₂ and 95 % relative humidity. The end point of the procedure was determined by the sulforhodamine B (SRB) method, as described below.

Briefly, adherent cell cultures were fixed *in situ* by adding 50 µL of cold 50 % (w/v) trichloroacetic acid (TCA) and incubated during for 60 minutes at 4 °C. The supernatant was then discarded, and the plates were washed five times with deionized water and dried. 100 µL of SRB solution (0.4 % w/v in 1 % acetic acid) was added to each well and incubated for 10 min at room temperature. The plates were air-dried and the bound stain was solubilized with Tris buffer and the optical densities were read on an automated spectrophotometric plate reader at 492 nm and 620 nm. All of the experiments were carried out at least three times [8].

Plate design

The components in each well of the control is the same as in each well of sample but positive control is camptothecin 0.01 µg/mL replaced for sample, negative control is complete medium, solvent control has no sample, blank control has no cell.

The growth inhibition was determined by using the following equation

$$\% I = \left(1 - \frac{OD_s}{OD_c}\right) \times 100$$

where

$$OD' = OD_{492} - OD_{620}$$

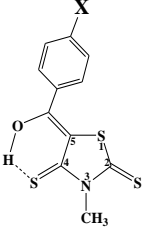
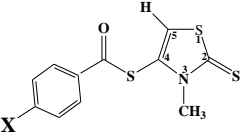
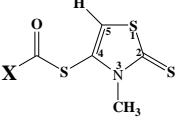
$$OD = OD'_{cell} - OD'_{blank}$$

$$OD_c: OD \text{ of } 0.25\% \text{ DMSO}$$

$$OD_s: OD \text{ of sample}$$

RESULT AND DISCUSSION

Table 1. *In vitro* cytotoxicity of obtained products (100 µg/mL) against MCF-7 cells

Structure of samples	Substituents (X)	Code of sample	Cell growth inhibition (I %)
			Mean ± SD
 Aromatic C-acylated products	NO ₂	C1	69.18 ± 3.099
	CF ₃	C2	67.14 ± 4.923
	Br	C3	68.22 ± 4.205
	Cl	C4	72.94 ± 2.407
	F	C5	85.31 ± 2.784
	H	C6	77.78 ± 1.700
	CH ₃	C7	71.88 ± 3.237
	C(CH ₃) ₃	C8	70.61 ± 1.444
	OCH ₃	C9	83.26 ± 2.138
	N(CH ₃) ₂	C10	28.90 ± 4.075
 Aromatic S-acylated products	NO ₂	S1	19.41 ± 5.068
	CF ₃	S2	Not determined *
	Br	S3	27.83 ± 5.589
	Cl	S4	13.82 ± 3.082
	F	S5	8.91 ± 3.025
	H	S6	5.67 ± 4.201
	CH ₃	S7	6.10 ± 5.795
	C(CH ₃) ₃	S8	12.98 ± 4.754
	OCH ₃	S9	59.73 ± 2.647
	N(CH ₃) ₂	S10	18.38 ± 2.255
 Aliphatic S-acylated products	CH ₃	S11	6.37 ± 11.298
	C ₂ H ₅	S12	-3.85 ± 4.713
	CH(CH ₃) ₂	S13	0.43 ± 4.849
	C(CH ₃) ₃	S14	-2.82 ± 7.435
	CH ₂ C ₆ H ₅	S15	7.70 ± 5.479
Camptothecin (Positive control)			38.60 ± 2.551

(*): lack of sample

The cytotoxic activity of the products against the MCF-7 cell line, screened by the SBR assay, expressed as a percentage of cell growth inhibition (I %), are presented in Table 1.

In general, tested compounds with a percentage of inhibition higher than 50% may be potential agents for cancer chemoprevention.

In Table 1, most of the C-acylated aromatic products (except the substituent N(CH₃)₂) at 100 µg/ml possess inhibitive percentages higher than 50 % (67-85 %) indicating a high cytotoxic activity against breast cancer cells.

On the contrary, almost all S-acylated aromatic products (except the substituent OCH₃) did not show any significant cytotoxicity against MCF-7, and especially, the S-acylated aliphatic products performed a very weak inhibitive

ability. The observation showed that the disappearance of benzene ring resulted in a reduction of cytotoxicity.

Among the *S*-acylated products, the one with substituent OCH_3 is the most active (59 %). Besides, the *C*-acylated derivative with substituent OCH_3 was also one of the products performing high cytotoxicity (83 %). The presence of a methoxy group on the benzene ring seems to be necessary to produce high cytotoxicity. That result is also in agreement with the comments in some papers [9].

The presence of the dimethylamino group in both *C*- and *S*-acylated products induced loss of cytotoxicity (%I was 28.90 % and 18.38 %, respectively).

In summary, *C*-acylated products were always more active than *S*-acylated products.

This result is in agreement with the observation by Matsumoto [10] and some other authors [9] that the presence of a methoxyl group in the molecule has an important role in the cytotoxic ability.

CONCLUSION

In the acylation reaction of 3-methyl-4-thiorhodanine, *C*- or *S*-acylated products can be obtained depending on the reaction conditions. We found out the best way to obtain either fully *S*-acylated or fully *C*-acylated products. *C*-acylated products showed strong inhibitory ability and may be potent inhibitors of MCF-7 cell proliferation used for cancer chemoprevention. Therefore the relevance of these results needs to be investigated further.

Khảo sát hoạt tính gây độc tế bào ung thư vú (MCF-7) của các dẫn xuất acyl hóa của 3-methyl-4-thiorhodanine

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TÓM TẮT

Phản ứng acyl hóa 3-methyl-4-thiorhodanin (2-thioxo-1,3-thiazolan-4-on) bởi các chlorur acid có thể xảy ra ở 2 vị trí: C số 5 hay S ở vị trí số 4 để tạo ra sản phẩm là dẫn xuất C-acyl hóa, S-acyl hóa tùy thuộc vào việc sử dụng loại xúc tác base nào.

Trong báo cáo này chúng tôi thử hoạt tính gây độc đối với tế bào ung thư vú (MCF-7) áp dụng trên các dẫn xuất S-acyl hóa và C-acyl hóa đã tổng hợp được và kết quả cho thấy các dẫn xuất C-acyl hóa có hoạt tính

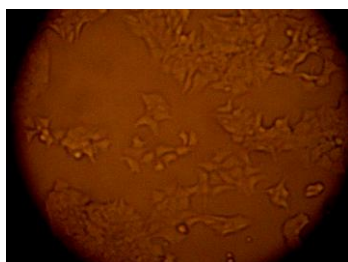
gây độc tế bào cao hơn các dẫn xuất S-acyl hóa.

Từ khóa: 3-Methyl-4-thiorhodanine, 2-thioxo-1,3-thiazolan-4-on, S-acyl hóa, C-acyl hóa, tế bào ung thư vú, MCF-7.

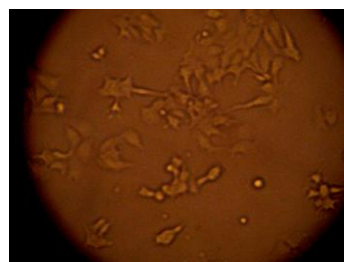
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APPENDIX

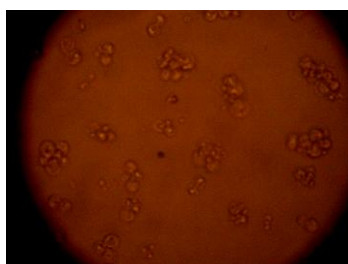


DMSO 0.25%-Negative control (0.00%)

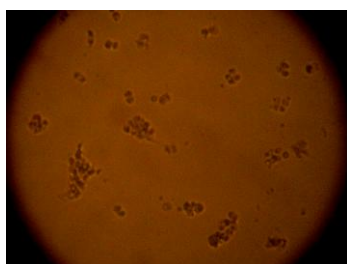


Camptothecin - Positive control (38.60%)

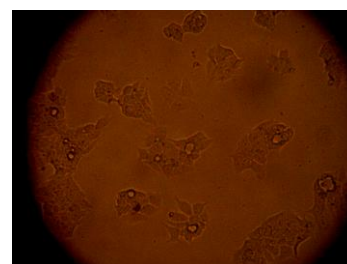
Appendix 1. Morphological observation of breast cancer cells (MCF-7) of negative and positive control after administration and the percentage of cell growth inhibition



C5 (85.31 %)

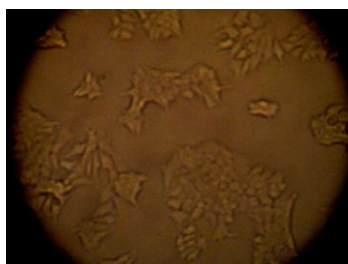


C9 (83.26 %)



C10 (28.90 %)

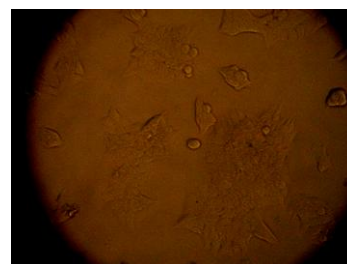
Appendix 2. Morphological observation of breast cancer cells (MCF-7) after administration of some *C*-acylated products and the percentages of cell growth inhibition



S6 (5.67 %)



S9 (59.73 %)



S12 (-3.85 %)

Appendix 3. Morphological observation of breast cancer cells (MCF-7) after administration of some *S*-acylated products and the percentages of cell growth inhibition