SYNTHESIS AND ANTIBIOTIC, ANTIFUNGAL ACTIVITIES OF SOME 3-(5'-CHLOROSALICYLAMIDO) RHODANINE AND 3-(5'-CHLOROSALICYLAMIDO) THIORHODANINE DERIVATIVES

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ABSTRACT

Background: *Thiazolidines were synthesised more on the world but the derivatives of 3-salicylamidothiazolidine weren't synthesised. By combining thiazolidine ring with acid salicylic we hope that will obtain some products that have both antibiotic and antifugi activies.* ***Objectives:*** *to synthesize and determine the structure of 3-(5'-chlorosalicylamido) rhodanine and it's derivatives; to test the antibiotic and antifugi activities of the products.* ***Results:*** *Obtained 3-(5□-chlorosalicylamido) rhodanine, it's four 5-arylidene -3-(5'-chlorosalicylamido)rhodanine derivatives, and converse carbonyl group (-C=O) to thion group (-C=S) gain 5-chlorosalicylamido-4-mercaptothiazole-2(3H)-thione. All of derivatives have able against to MSSA and MRSA.****Conlusion:*** *from salicylic acid by many steps of reaction obtain 5-chlorosalicylamidorhodanine and it's four derivatives. Heating 5-chlorosalicylamidorhodanine with P2S5 in dry dioxane to gain 5-chlorosalicylamido-4-mercaptothiazole-2(3H)-thione. All of them were examined by model spectrometries and had structure as expected. All of them have activity against bacteria MSSA and MRSA, just 5-chlorosalicylamido-4-mercaptothiazole-2(3H)-thione can against Candida albicans.*

Keywords: *rhodanine, chlorosalicylamidorhodanine, antibiotic, fungistatic, NMR, mass spectrometry.*

I. INTRODUCTION

Thiazolidine is a heterocyclic nucleus which appears in many antibiotics such as penicillines. According to many studies, a group of thiazolidine derivatives which has antibiotic and fungistatic actions are salicylamidorhodanines [2], [3], [4], [5]. In this study, we focused on chlorosalicylamidorhodanines and changed from the C=O to C=S form chlorosalicylamidothiorhodanines, in the hope that antibiotic and antifungal activities may be enhanced.

II. MATERIALS AND METHOD

2.1. Materials for synthesis

5-chlorosalicylic acid, hydrochloric acid, sulphuric acid, ethanol, methanol, hydrazine hydrate, carbon disulfide, monochloroacetic acid, P2S5 are from China.

2-nitrobenzaldehyde, 2-furaldehyde, salicylaldehyde, 4-methoxybenzaldehyde, 4-nitrobenzaldehyde, 4-chlorobenzaldehyde, paradimethylamino-benzaldehyde are the pure chemicals for synthesis from Acros.

2.2. Method

UV spectrometry: 2010 – Hitachi.

IR spectrometry: FTIR 8101 – Shimadzu.

Thin layer chromatogrphy: Silicagel thin layer GF, 254 – Merck.

1H - NMR : Brucker 500 MHz.

13C – NMR: Brucker AV 125MHz

Solvents:

A: Chloroform : acetic acid (9 :1)

B: Chloroform : ethyl acetate (3:7)

C: Chloroform : ethyl acetate : methanol (20:20:2)

D: Toluen: ethyl acetate: methanol (31:8:1)

E: Toluen: ethyl acetate: methanol (15:4:1)

F: Toluen: ethyl acetate: methanol (15:4:2,5)

G: n-Hexane: ethyl acetate (1:1)

Melting point: Gallenkamp.

Antibiotic and antifugal actions are determined by dilution test (agar method) using trypticase soy and sabouraud media

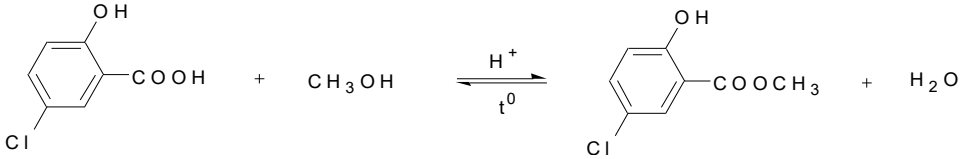
The bacteria: Streptococcus faecalis ATCC 29212
Escherichia coli ATCC25922
Staphycoccus aureus ATCC29213
MRSA

The fugi: Candida albicans ATCC 10231

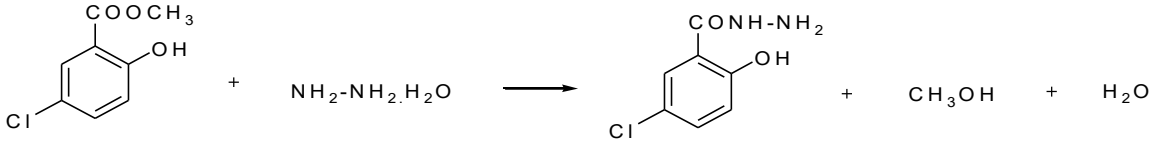
III. RESULTS

3.1. To synthesize 3-(5'-chlorosalicylamido) rhodanine and it's derivatives

From 5-chlorosalicylic acid we gained 5-chlorosalicylate methyl by esterazation with methanol (catalysis by H_2SO_4).

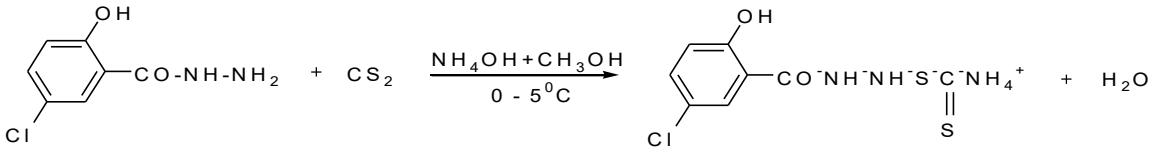


Then by carried out the reaction between ester anh hydrazin we obtained 5-chlorosalicylhydrazide.



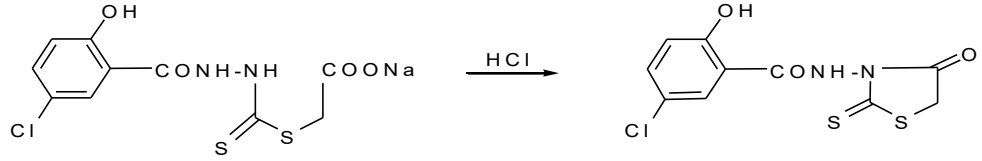
At last, through three steps we got 3-(5′-chlorosalicylamido) rhodanine.

a. To synthesize amonium dithiocarbamate (step 1)

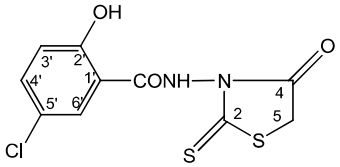


b. To synthesize sodium 5-chlorosalicylamido dithiocarbaminyl glycolate (step 2)

*c. To synthesize 3-(5′-chlorosalicylamido)rhodanine (**1**) (step 3)*



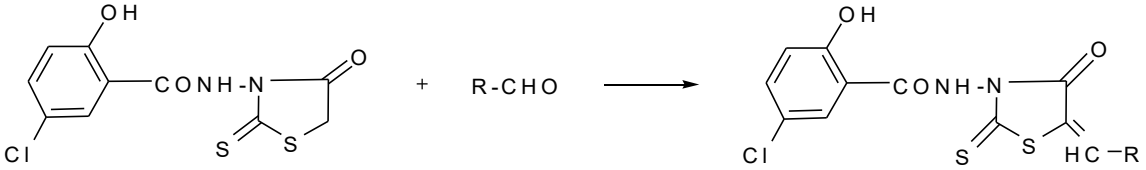
***(1): 3-(5′-chlorosalicylamido)rhodanine**:*



$C_{10}H_7ClN_2O_3S_2$; MW:302.76; Yield 88%; Yellow crystals; Mp (^oC): 114^0C. TLC, Rf (solvents): 0.56 (B), 0.4 (E), 0.34 (G). UV (λ_{max}, nm): 294; 210. IR (ν, cm^{-1}): 3282, (C-N amide); 1768 (-C=O); 1654 (C=O amide); 825 (C-Cl). ^{1}H-NMR (500MHz, DMSO-d_6, δ ppm): 7. 6(*d*, *J*=8.5, 1H, ArCH=); 7.52 (*d*, *J*=8.5, 1H, ArCH=); 7.61 (*s*,1H, ArCH=); 4.54 (*s*, 2H,-CH_2). ^{13}C-NMR (125MHz, DMSO-d_6, δ ppm): 199.7(C_2); 170.1(C_4); 163.4(NH-CO); 156.2(C_2'); 134.0(C_4'); 129.1(C_6'); 123.1(C_5'); 119.1(C_1'); 117.1(C_3'); 33.3(C_5).

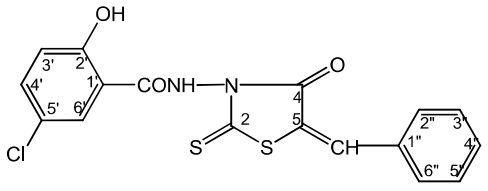
ESI- MS : $[M]^+$ (m/z): 303

3.2. To synthesize derivatives of 5-arylidene -3-(5′-chlorosalicylamido)rhodanine



The 5-arylidene -3-(5′-chlorosalicylamido)rhodanine derivatives were synthesised by Knoevenagel reaction between 3-(5′-chlorosalicylamido)rhodanine and the aromatic aldehydes. The reaction is carried out in acetic acid glacial with catalysis of dimethylamine. We get five derivatives (**1a – 1e**)

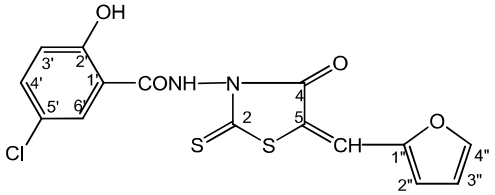
(1a): 5-benzylidene-3-(5′-chlorosalicylamido)rhodanine



$C_{17}H_{11}ClN_2O_3S_2$; MW: 390.87; Yield 65%; Yellow crystal; Mp (^oC): 218 ^oC. TLC, Rf (solvents): 0.62 (B), 0.59 (E), 0.50 (G). UV (λ_{max}, nm): 373; 273; 210. IR (νcm^{-1}) : 1714.6 ($\nu_{C=O}$; rhodanine); 1660.6 ($\nu_{C=O}$; amide); 1593.1; 1571.9; 1510.8 ($\nu_{C=C}$; aromatic); 1029.9 ($\nu_{C=S}$; rhodanine). ^{1}H-NMR (500 MHz, DMSO-*d*$_6$, δ ppm): 11.70 (*s*; 1H; -OH); 11.25 (*s*; 1H; -CONH); 7.98 (*s* ; 1H; =CH-); 7.87 (*s*; 1H; $H_{6'}$); 7.72 (d; *J* = *7*; 2H; $H_{2''}$, $H_{6''}$); 7.58 (*m*; 4H; $H_{4'}$, $H_{3''}$, $H_{5''}$, $H_{4''}$); 7.09 (*d*; *J* = *9*; 1H; $H_{3'}$). ^{13}C-NMR (125 MHz, DMSO-*d*$_6$, δ ppm): 190.2 (C_2); 163.4 (-CONH-); 163.2 (C_4); 156.2 ($C_{2'}$); 134.7 ($C_{1''}$); 134.1 (=CH-); 132. 6 ($C_{4'}$); 131.4 ($C_{2''}$, $C_{6''}$); 130.8 ($C_{3''}$, $C_{5''}$); 129.6($C_{6'}$); 129.31 ($C_{5'}$); 123.3 ($C_{4''}$); 119.4 ($C_{1'}$); 119.1 ($C_{3'}$); 117.2 (C_5). DEPT (125 MHz, DMSO-*d*$_6$, δ ppm): 134.1 (=CH-); 132. 6 ($C_{4'}$); 131.4 ($C_{2''}$; $C_{6''}$); 130.8 ($C_{3''}$; $C_{5''}$); 129.6 ($C_{6'}$); 123.3 ($C_{4''}$); 119.1 ($C_{3'}$).

ESI- MS : $[M+H]^+$ (m/z): 391.90.

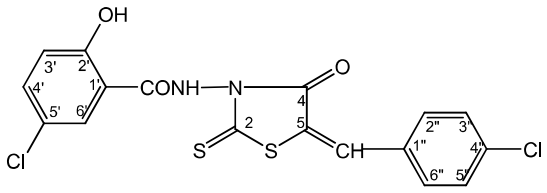
(1b): 5-furylidene-3-(5′-chlorosalicylamido)rhodanine



$C_{15}H_9ClN_2O_4S_2$; MW: 380.83; Yield 67%. Yellow crystal. Mp (^oC): 251 ^oC. TLC, Rf (solvents): 0,61 (B), 0,57 (E), 0,45 (G). UV (λ_{max}, nm): 394, 288, 207. IR (νcm^{-1}) :1706.9 ($\nu_{C=O}$; rhodanin); 1654.8 ($\nu_{C=O}$; amide); 1604.7; 1539.1; 1517.9 ($\nu_{C=C}$; aromatic); 1014.5 ($\nu_{C=S}$; rhodanine). ^{1}H-NMR (500 MHz, DMSO-*d*$_6$, δ ppm): 1.,63 (*s*; 1H; -OH); 11.14 (*s*; 1H; -CONH); 8.20 (*s*; 1H; $H_{6'}$); 7.85 (*d*; 1H; $H_{4''}$); 7.81 (*s*; 1H; =CH-); 7.54 (*d*; *J* = *9*; 1H; $H_{4'}$); 7.31 (*d*; *J* = *3,5*; 1H; $H_{2''}$); 7.08 (*d*; *J* = *8.5*; 1H; $H_{3'}$); 6.83 (*d*; *J* = *3.5*; 1H; $H_{3''}$). ^{13}C-NMR (125 MHz, DMSO-*d*$_6$, δ ppm): 191.2 (C_2); 163.3 (-CONH-); 163.0 (C_4); 156.2 ($C_{2'}$); 149.4 ($C_{1''}$); 149.1 ($C_{4''}$); 134.1 (=CH-); 129.3 ($C_{4'}$); 123.2 ($C_{6'}$); 121.5 ($C_{5'}$); 120.4 ($C_{3''}$); 119.1 ($C_{1'}$); 117.2 ($C_{3'}$); 115.9 (C_5); 114.2 ($C_{2''}$). DEPT (125 MHz, DMSO-*d*$_6$, δ ppm): 149.1 ($C_{4''}$); 134.1 (=CH-); 129.3 ($C_{4'}$); 123.2 ($C_{6'}$); 120.4 ($C_{3''}$); 117.2 ($C_{3'}$); 114.2 ($C_{2''}$).

ESI- MS : $[M]^+$ (m/z): 380.80

(1c): 5-(4″-chlorobenzyliden)-3-(5′-chlorosalicylamido)rhodanine



$C_{15}H_9ClN_2O_4S_2$; MW: 380.83; Yield 70%. Yellow crystal. Mp (^oC): 220 ^oC. TLC, Rf (solvents): 0,64 (B), 0,57 (E), 0,61 (G). UV (λ_{max}, nm): 207, 276, 375.

IR (νcm^{-1}) : 1728.1 ($\nu_{C=O}$; rhodanine); 1637.5 ($\nu_{C=O}$; amide); 1600.8; 1585.4; 1508.2 ($\nu_{C=C}$; aromatic); 1056.9 ($\nu_{C=S}$; rhodanine).

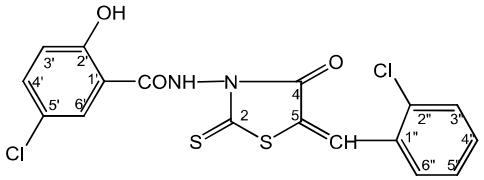
^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 11.45 (*s*; 2H; -OH; -CONH); 7.98 (*s*; 1H;=CH-); 7.85 (*s*; 1H; $H_{6'}$); 7.74 (*d* ; *J = 8.5*; 2H; $H_{2''}$, $H_{6''}$), 7.65 (*d*; *J = 8.5*; 2H; $H_{3''}$, $H_{5''}$); 7.55 (*d*; *J = 8.5*; 1H; $H_{4'}$); 7.08 (*d*; *J =9*; 1H; $H_{3'}$).

^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 189.8 (C_2); 163.2 (-CONH-); 163.1 (C_4); 156.3 ($C_{2'}$); 136.0 ($C_{4''}$); 134.1 (=CH-); 133.4 ($C_{4'}$); 132.5 ($C_{2''}$, $C_{6''}$); 131.6 ($C_{1''}$); 129.6 ($C_{6'}$); 129.4 ($C_{3''}$, $C_{5''}$); 123.2 ($C_{5'}$); 120.1 ($C_{1'}$); 119.2 ($C_{3'}$); 117.3 (C_5). (PL 10.5)

DEPT (125 MHz, DMSO-d_6, δ ppm): 134.1 (=CH-); 133.4 ($C_{4'}$); 132.5 ($C_{2''}$, $C_{6''}$); 129.6 ($C_{6'}$); 129.4 ($C_{3''}$, $C_{5''}$); 119.2 ($C_{3'}$).

ESI- MS : $[M+H]^+$ (m/z) 426.50

(1d): 5-(2′′-chlorobenzyliden)-3-(5′-chlorosalicylamido)rhodanine

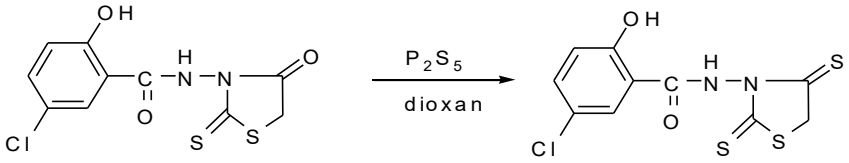


$C_{17}H_{10}Cl_2N_2O_3S_2$; MW: 425.31. Light yellow crystal. Yiel 65%. Mp (^{o}C): 122 ^{o}C. TLC, Rf (solvents): 0.65 (B), 0.59 (E), 0.50 (G). UV (λ_{max}, nm): 209; 272; 364.

IR (νcm^{-1}) : 1732.0 ($\nu_{C=O}$; rhodanine); 1639.4 ($\nu_{C=O}$; amide); 1607; 1593.1; 1511.0 ($\nu_{C=C}$; aromatic); 1047.3 ($\nu_{C=S}$; rhodanine). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 11.65 (*s*; 1H; -OH); 11.2 (*s*; 1H; -CONH); 8.08 (*s*; 1H; =CH-); 7,87 (*s*; 1H; $H_{6'}$); 7,67 (*m*; 2H; $H_{4'}$, $H_{3''}$); 7,56 (*m*; 3H, $H_{6''}$, $H_{5''}$, $H_{4''}$); 7,09 (*d*; 1H; $H_{3'}$).)^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 189.9 (C_2); 163.2 (-CONH-); 162.9 (C_4); 156.3 ($C_{2'}$); 134.9 (=CH-); 134.1 ($C_{4'}$); 132.6 ($C_{1''}$); 130.5 ($C_{2''}$); 129.6 ($C_{3''}$); 129.5 ($C_{4''}$); 129.4 ($C_{6'}$); 128.3 ($C_{6''}$); 123.2 ($C_{5'}$); 123.0 ($C_{5''}$); 119.2 ($C_{1'}$); 117.2 ($C_{3'}$); 113.0 (C_5). DEPT (125 MHz, DMSO-d_6, δ ppm): 134.9 (=CH-); 134.1 ($C_{4'}$); 129.6 ($C_{3''}$); 129.5 ($C_{4''}$); 129.4 ($C_{6'}$); 128.3 ($C_{6''}$); 123.0 ($C_{5''}$); 117.2 ($C_{3'}$).

ESI- MS : $[M]^+$ (m/z): 425.60

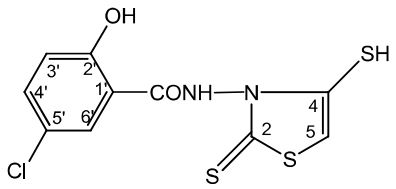
3.3. To synthesize of *3-(5'-chlorosalicylamido)-4-mercaptothiazole-2(3H)-thione* (2)



There are some method to converse -C=O to -C=S, in this study we use dry dioxane as the solvent and P_2S_5 as the catalyst of reaction [1].

Product has red color.

***(2): 3-(5'-chlorosalicylamido)-4-mercaptothiazole-2(3H)-thione*:**



$C_{10}H_7ClN_2O_2S_3$, MW: 318.,88. Yield 50%. Red power. Mp (^oC): 198^{0}C. . TLC, Rf (solvents): 0.62 (B), 0.40 (E), 0.18 (G). UV (λ_{max} nm): 214; 249; 315; 415; 435.

IR (νcm^{-1}) : 1652.9 ($\nu_{C=O}$; amide); 1602.7; 1593.,1; 1539,1 ($\nu_{C=C}$; aromatic); 1070.4 ($\nu_{C=S}$; rhodanine). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 11.94 (*s*; 1H; -CONH); 7.98 (*s*; 1H; $H_{6'}$); 7.52 (*d*; *J=8.5*; 1H; $H_{4'}$); 7.07 (*d*; *J=8.5*; 1H; $H_{3'}$); 7.01 (*s*; 1H; H_5). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 174.6 (C_2); 161.7 (-CONH-); 155.6 ($C_{2'}$); 136.7 (C_4); 133.6 ($C_{4'}$); 126.5 ($C_{6'}$); 123.6 ($C_{5'}$); 118.9 ($C_{3'}$); 116.48 ($C_{1'}$); 96.4 (C_5). DEPT (125 MHz, DMSO-d_6, δ ppm): 133.6 ($C_{4'}$); 126.5 ($C_{6'}$); 118.9 ($C_{3'}$); 96.4 (C_5).

ESI- MS : $[M]^+$ (m/z): 319,00

3.4. Antibiotic, antifungal activities of the products

The results of antibiotic and antifugi are presented in tab 1

Table 1. MIC of antibiotic and fungistatic agents

Products	MIC (µg/ml)				
	E. Coli	**MSSA**	***Strep.***	**MRSA**	***C. albicans***
1a	256	64	128	8	>512
1b	256	64	128	32	>512
1c	256	16	16	8	>512
1d	256	16	64	16	>512
2	256	4	256	4	256

IV. DISCUSSION

4.1. Synthesis and determine structure

***3-(5′-chlorosalicylamido)rhodanine* (1)**

The product (**1**) has resonant signals of ^{1}H-NMR in the range 6 – 8 ppm which are chemical shift of aromatic protons. The number of proton is five that is fixed with the hydrogen atoms in structure.

The signal of carbons are ten that is suitable with the number of carbon atoms in structure. The ^{13}C-NMR has a signal at 33.3 ppm that is the signal of -CH_2 in thiazolidine ring. The mass spectrometry result is 303 equivelent with the molecular weigh (302.76).

So the product is 3-(5′-chlorosalicylamido)rhodanine.

***5-arylidene -3-(5′-chlorosalicylamido)rhodanine derivatives* (1a-1d)**

All products have the number of protons fixed with the hydrogen atoms in their structures. The chemicel shift of peak of carbons are suitable, the number of peaks are fixed with number of carbons in structures. On DEPT135, there are no negative peaks that mean the -CH_2 at 5th position in thiazolidines ring were reacted with aromatic aldehydes and the products are 5-arylidene -3-(5′-chlorosalicylamido)rhodanine derivatives

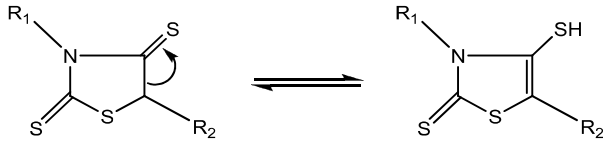
All the products are pure because there are no unwanted peaks.

***3-(5'-chlorosalicylamido)-4-mercaptothiazole-2(3H)-thione* (2)**

In synthesis thiorhodanine we choose P_2S_5 because it's cheap and we can synthesize in lab, further more when using the dry dioxane and P_2S_5 the yiel was higher than other materials.

On IR spectrum there was no waver at 1750-1740 cm^{-1} of group >C=O in thiazolidine ring so that the thionization is appeared, but ^{13}C-NMR spectrum lost one peak in range 160 – 180 ppm of quaternary carbon(-C=S) instead of we see one more peak at 136 ppm of aromatic carbon. We also don't see a peak at 33 ppm of -CH_2 at 5th position in thiazolidin ring but there is new peak at 96 ppm. The mass spectrometry is 319.0 that is equivalent with molecular weigh of expected product.

By combination the results of IR spectrum, ^{1}H-NMR, ^{13}C-NMR and MS we realize that thionization reaction has occurred but by the resonant of free electron pair on nitrogen and sulfur atoms so there is a interconversion between two carbon at position 4 and position 5 on structure, hence thiorhodanine can occur in thione or thienol form.



Thione Thienol

Our product (**2**) exists in thienol form and it's named 3-(5'-chlorosalicylamido)-4-mercaptothiazole-2(3H)-thione

4.2. The antibiotic and antifugi activities

Almost 3-(5-clorosalicylamido)rhodanine′s derivatives have strong antibiotic activitive, especially against MRSA. 3-(5'-chlorosalicylamido)-4-mercaptothiazole-2(3H)-thione (**1**) have strongest activitive on MRSA which MIC is 4 (µg/ml). Only (**1**) can against *C. albicans*

IV. CONCLUSIONS

We obtain six products include:

5-chlorosalicylamindo rhodanine (**1**).

5-benzylidene-3-(5′-chlorosalicylamido) rhodanine (**1a**).

5-furylidene-3-(5′-chlorosalicylamido) rhodanine (**1b**).

5-(4′′-chlorobenzyliden)-3-(5′-chlorosalicylamido) rhodanine (**1c**).

5-(2′′-chlorobenzyliden)-3-(5′-chlorosalicylamido) rhodanine (**1d**).

3-(5'-chlorosalicylamido)-4-mercaptothiazole-2(3H)-thione (**2**).

All of them have have structure as expected. We haven't found them in the documents.

The results obtained on the test of antibiotic and antifulgal activities determined that all have effect on tested bacteria Gram (+). The replacement of –C=O group by –C=S leaded to enhance antibacterial activity.

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(Received: 09/11/2018 - Accepted: 11/01/2019)

PHYTOCHEMICAL INVESTIGATION OF THE LICHEN PARMOTREMA SANCTI-ANGELII (LYNGE) HALE (PARMELIACEAE)

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ABSTRACT

Background: *Lichens are fungal and algal/cyanobacterial symbioses resulting in the production of specific metabolites with a great variety of effects such as antibiotic, antimycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic and antiproliferative. So far, lichens have been used in folk medicine in many countries. Objectives: As apart of searching bioactive compounds from lichens, a phytochemical investigation was conducted on a foliose lichen, Parmotrema sancti-angelii (Lynge) Hale, collected in Lam Dong province, Vietnam.* ***Methods:*** *Open column silica gel chromatography and preparative TLC techniques were employed to isolate compounds. Their structures were elucidated by HRMS, NMR analysis and compared with those in references.* ***Results:*** *Our present study led to the isolation of four compounds, atranol (1), methyl haematomate (2), orsellinic acid (3) and lecanoric acid (4).* ***Conclusion:*** *From 300 g of lichen P. sancti-angelii, four compounds were isolated and identified by spectroscopic methods and compared with literature data. This study contributed to investigate chemical constituents of lichens in Vietnam which have been ignored.*

Keywords: *Parmeliaceae, Parmotrema, atranol, orsellinic acid.*

I. INTRODUCTION

Lichens are symbiotic organisms, usually composed of a fungal partner (the mycobiont) and one or more photosynthetic partners (the photobiont) which is most often