

**MINISTRY OF EDUCATION  
AND TRAINING**

**VIETNAM ACADEMY OF  
SCIENCE AND TECHNOLOGY**

**GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY**



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**STUDY ON EXTRACTION, DETERMINATION OF  
CHEMICAL STRUCTURE AND EVALUATION OF  
THE RECOMBINANT ClpC1 PROTEIN INHIBITION  
OF THE COMPOUNDS FROM SOME VIETNAMESE  
ACTINOMYCETE SPECIES**

**SUMMARY OF DISSERTATION ON ORGANIC CHEMISTRY**

**Code: 9440114**

**Ha Noi - 2024**

The dissertation is completed at: Graduate University of Science and Technology, Vietnam Academy Science and Technology

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## LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

**1. Huynh Thi Ngoc Ni**, Pham Thi Ninh, Tran Van Chien, Nguyen Thi Dung, Dinh Thi Ngoc Mai, Nguyen Thi Van, Nguyen Hong Minh, Ngo Van Hieu, Ho Ngoc Anh, Jinhua Cheng, Joo-Won Suh, Tran Van Sung, Nguyen Kim Nu Thao, Tran Thi Phuong Thao, *Screening for antimycobacterial activity of actinomycetes collected in Vietnam - Isolation and activity of metabolites from Streptomyces alboniger (A121)*, Natural Product Communications, 2024, 19(1), 1-13. DOI: 10.1177/1934578X231224994. (SCIE)

**2. Huynh Thi Ngoc Ni**, Pham Thi Ninh, Nguyen Thi Dung, Tran Van Chien, Nguyen Quynh Uyen, Ho Ngoc Anh, Joo-Won Suh, Jinhua Cheng, Nguyen Kim Nu Thao, Nguyen Minh Duc, Tran Thi Phuong Thao, *ClpC1 protein inhibition, antimycobacterial, and anti-inflammatory properties of the metabolite from Streptomyces wuyuanensis collected in Nghe An province, Vietnam*, Vietnam Journal of Chemistry, 2024, 62(1), 85-91. Doi: 10.1002/vjch.202300345. (Q3)

**3. Huynh Thi Ngoc Ni**, Pham Thi Ninh, Ho Ngoc Anh, Jinhua Cheng, Joo-Won Suh, Tran Van Sung, Nguyen Kim Nu Thao, Le Thi Hong Nhung, Nguyen Quynh Uyen, Nguyen Minh Duc, Tran Thi Phuong Thao, *Secondary metabolites from Actinoplanes missouriensis and inhibitory activity of Mycobacterium tuberculosis ClpC1 protein*, Journal of Science and Technology, Hanoi University of Industry, 2023, 58 (6A), 116-120.

**4. Tran Thi Phuong Thao, Huynh Thi Ngoc Ni**, Pham Thi Ninh, Nguyen Thi Dung, Tran Van Chien, Nguyen Quynh Uyen, Ho Ngoc Anh, Joo-Won Suh, Jinhua Cheng, Nguyen Kim Nu Thao, Tran Van Sung and Nguyen Minh Duc, *Metabolites from Streptomyces aureus (VTCC43181) and their inhibition of Mycobacterium tuberculosis ClpC1 protein*, Molecules, 2024, 29(3), 720-730. Doi: 10.3390/molecules29030720. (Q1)

## INTRODUCTION

Tuberculosis (TB) is an infection caused by *Mycobacterium tuberculosis*, most commonly found in the lungs but can also affect the central nervous system (tuberculous meningitis), lymphatic system, circulatory system (miliary tuberculosis), genitourinary system, bones and joints. Currently, tuberculosis is one of the main and most common infectious diseases, affecting 2 billion people in the world, with 9 million new cases each year and causing 2 million deaths. This disease is common in developing countries. Almost 90% of the people infected with *M. tuberculosis* do not develop the active disease and are asymptomatic. Around 10% of latent infections progress to active disease which, if left untreated, kill about half of those affected. Tuberculosis is still one of the world's deadliest infectious diseases after HIV. Negligence in TB control programs has led to a resurgence of TB. Furthermore, multidrug-resistant (MDR) tuberculosis is one of the biggest challenges for scientists. According to a World Health Organization (WHO) report, totally Drug-Resistant TB emerged in India which accounted for the highest number of tuberculosis (TB) cases in the world. The 1st, 2nd, and 3rd generation antibiotic have been researched and extensively used to treat TB disease. Nevertheless, the number of TB drug resistances is still growing fast. Hence, finding new antibiotics and more effective drugs are urgently needed to treat TB.

Recent studies showed that the trend of exploiting active compounds from microbial sources: bacteria, actinomycetes... was increasingly receiving attention from scientists. Among them, actinomycetes are known to be the largest group of microorganisms for production of antibiotics

Vietnam has a great diversity of natural ecosystem including tropical ever-green forest, mangrove forest, coastal island, high mountains, beach, etc. which are the potential resources to discover actinomycetes

diversity and natural antibiotics. However, up to now, there are only few reports on the antituberculosis metabolites from actinomycetes in Vietnam. Therefore, the topic “*Study on extraction, determination of chemical structure and evaluation of the recombinant ClpC1 protein inhibition of the compounds from some Vietnamese actinomycete species*” is necessary and of practical significance, contributing to finding new safe and effective natural drugs to treat tuberculosis.

### **Objectives of the thesis:**

Searching for compounds have affected recombinant protein ClpC1 from some actinomycete strains in Vietnam.

With the above objectives, the thesis sets out the following research contents:

- Collect actinomycete samples in different regions, receive and process actinomycete biomass samples from the Institute of Microbiology and Biotechnology, Hanoi National University.
- Evaluate antimycobacterial activity against *M. smegmatis* (strain similar to *M. tuberculosis*) of actinomycete strains.
- Create extracts from cultures of actinomycete strains using different solvents.
- Separate and purify pure compounds from culture solutions of actinomycete strains.
- Determine the chemical structure of the isolated pure compounds.
- Evaluate the effect of isolated pure compounds on the target recombinant protein ClpC1.

### **New contributions of the thesis:**

- For the first time, antimycobacterial activity against *M. smegmatis* (strain similar to *M. tuberculosis*) of eight actinomycete strains was evaluated. Strains include *Streptomyces spiroverticillatus* VH19-A067, *Streptomyces wuyanensis* VH19-A079, *Streptomyces alboniger* VH19-A105B, *Streptomyces alboniger* VH19-A121, *Streptomyces aureus*

VTCC43181, *Streptomyces cyaneus* VTCC43860, *Streptomyces sp.* VTCC43168 và *Actinoplanes missouriensis* VTCC40900.

- For the first time, 14 compounds were isolated from the above eight actinomycete strains. These compounds include: obscurolide B<sub>2β</sub> (**AT.01**), chartreusin (**AT.02**), indole-3-carboxylic acid (**AT.03**), nocardamin (**AT.04**), pleurone (**AT.05**), halolitoralin A (**AT.06**), (6Z)-15-methyl-6-hexadecenoic acid (**AT.07**), cardoltriene (**AT.08**), cardoltriene M (**AT.09**), 7-deoxyauramycinone (**AT.11**), 7-acetyl-3,6-dihydroxy-8-methyl tetralone (**AT.12**), valin (**AT.13**), flufuran (**AT.14**), trehalose (**AT.15**). In which, there was 1 new compound named **cardoltriene M (AT.09)**.

For the first time, antimycobacterial activity against *M. smegmatis* of compound chartreusin (**AT.02**) was evaluated.

- For the first time, compounds isolated from actinomycetes were evaluated for ATPase hydrolysis activity of the recombinant ClpC1 protein, an important regulatory protein of *M. tuberculosis*.

## CHAPTER 1. OVERVIEW

### **1.1. Tuberculosis situation in Vietnam and the world - Drug resistance situation in tuberculosis bacteria**

#### *1.1.1. Tuberculosis situation in Vietnam and the world*

#### *1.1.2. Drug resistance in tuberculosis bacteria in Vietnam and the world*

### **1.2. Anti-tuberculosis compounds from actinomycetes in the world and in Vietnam**

#### *1.2.1. Introduction to actinomycetes*

#### *1.2.2. Anti-tuberculosis compounds from actinomycetes in the world*

##### *1.2.2.1. Aminoglycoside*

##### *1.2.2.2. Nitroimidazole*

##### *1.2.2.3. Macrolide*

##### *1.2.2.4. Cyclopeptide*

##### *1.2.2.5. Diaza-anthracene*

##### *1.2.2.6. Polyketide*

#### *1.2.3. Anti-tuberculosis compounds from actinomycetes in Vietnam*

### **1.3. Overview about some actinomycete species that are research subjects**

#### *1.3.1. Streptomyces alboniger*

##### *1.3.1.1. Morphological characteristics of Streptomyces alboniger*

##### *1.3.1.2. Bioactive compounds isolated from Streptomyces alboniger*

#### *1.3.2. Streptomyces wuyuanensis*

#### *1.3.3. Streptomyces aureus*

##### *1.3.3.1. Morphological characteristics of Streptomyces aureus*

##### *1.3.3.2. Bioactive compounds isolated from Streptomyces aureus*

#### *1.3.4. Streptomyces spiroverticillatus*

##### *1.3.4.1. Morphological characteristics of Streptomyces spiroverticillatus*

##### *1.3.4.2. Bioactive compounds isolated from Streptomyces spiroverticillatus*

#### *1.3.5. Streptomyces cyaneus*

##### *1.3.5.1 Morphological characteristics of Streptomyces cyaneus*

*1.3.5.2. Bioactive compounds isolated from Streptomyces cyaneus*

### ***1.3.6. Actinoplanes missouriensis***

*1.3.6.1. Morphological characteristics of Actinoplanes missouriensis*

*1.3.6.2 Bioactive compounds isolated from Actinoplanes missouriensis*

## **1.4. Overview about ClpC1 protein**

The method of screening for anti-tuberculosis compounds targeting ClpC1 is a new and modern method being used by reputable research groups in the world to screen anti-tuberculosis metabolites from actinomycetes. This method is non-toxic and not dangerous because it does not directly use tuberculosis bacteria in the screening process. This method allows for mass screening of active metabolites from actinomycetes as well as from other natural sources. During the process of building a screening model, the mechanism of action of compounds on ClpC1 target of tuberculosis bacteria will also be clarified, helping to save costs and time compared to conventional testing methods.

Thus, the secondary metabolites with antituberculosis activity are mostly isolated from actinomycetes. Actinomycetes are the most abundant and abundant source of anti-tuberculosis compounds, and have become potential research objects for scientists to discover new anti-tuberculosis drugs. Therefore, the PhD thesis topic "*Study on extraction, determination of chemical structure and evaluation of the recombinant ClpC1 protein inhibition of the compounds from some Vietnamese actinomycete species*" is necessary, scientific significance, contributing to the search for anti-tuberculosis compounds from actinomycetes in Vietnam and orienting future research in medicine and pharmacy.



## CHAPTER 2. SUBJECTS, METHODOLOGY AND EXPERIENCE

### 2.1. Research subjects

#### 2.1.1. Actinomycetes belong to the genus *Streptomyces*

Table 2.1. the strains isolated from different areas along the Northern to Central Vietnam

No	Strain codes	Natural source	Locations for actinomycetes collection	Latitude
1	<i>Streptomyces spiroverticillatus</i> VH19-A067	Soil	The confluence of Day river and Vac river, Thuong Kiem commune, Kim Son district, Ninh Binh province	N20°3'0,8''E106°6'52,8''
2	<i>Streptomyces wuyuanensis</i> VH19-A079	Soil	Quynh Luong Mangrove Forest, Quynh Luong commune, Quynh Luu district, Nghe An province	N19°8'42,1''E105°41'43,1''
3	<i>Streptomyces alboniger</i> VH19-A105B	Soil	On the way to the top of May Bac mountain, Cuc Phuong National Park, Nho Quan district, Ninh Binh province	N20°21'0,8''E105°36'15,7''
4	<i>Streptomyces alboniger</i> VH19-A121	Soil	On the top of May Bac mountain, Cuc Phuong National Park, Nho Quan district, Ninh Binh province (648 m above sea level)	N21°21'21,9''E105°36'36,2''

*Streptomyces cyaneus* VTCC43860, *Streptomyces* sp VTCC43168, *Streptomyces aureus* VTCC43181 was cultured and provided by the National Microbial Gene Resource Center, Institute of Microbiology and Biotechnology, Hanoi National University.

#### 2.1.2. Rare actinomycetes belong to the genus *Actinoplanes*

*A. missouriensis* VTCC40900 was cultured and provided by the

National Microbial Gene Resource Center, Institute of Microbiology and Biotechnology, Hanoi National University.

## **2.2. Chemical, research equipment**

### **2.2.1. Chemical**

### **2.2.2. Research equipment**

## **2.3. Method**

### **2.3.1. Collection and isolation of actinomycetes**

#### *2.3.1.1. Collection and isolation of actinomycetes*

#### *2.3.1.2. Isolation of actinomycetes*

### **2.3.2. Extraction**

### **2.3.3. Isolation of compounds from extracts**

### **2.3.4. Determination structures of isolated compounds**

### **2.3.5. Assessment of biology activity**

#### *2.3.8.1. Evaluation of antimycobacterial activity against *Mycobacterium smegmatis**

#### *2.3.8.2. Evaluation of ATPase activity of recombinant protein ClpC1*

## **2.4. Extraction and isolation of compounds from the culture of actinomycetes**

### **2.4.1. Extraction and isolation of compounds from the culture of *Streptomyces alboniger* VH19-A121**

**AT.01 (Obscuroside B<sub>2β</sub>):** Yellow powder; C<sub>15</sub>H<sub>17</sub>NO<sub>4</sub>;  $[\alpha]_D^{27} = +20,4$  (c 0,1, CH<sub>2</sub>Cl<sub>2</sub>), HR-ESI-MS (m/z): 276.1221 (calculated for C<sub>15</sub>H<sub>18</sub>NO<sub>4</sub> 276,1236 [M+H]<sup>+</sup>), 298.1045 (calculated for C<sub>15</sub>H<sub>17</sub>NO<sub>4</sub>Na 298.1055 [M+Na]<sup>+</sup>); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>), δ<sub>H</sub> (ppm): 9.77 (1H, s, CHO), 7.73 (1H, d, *J* = 8.5, H-3', H-5'), 6.67 (1H, d, *J* = 8.5, H-2', H-6'), 5.88 (1H, dd, *J* = 15.0, 7.0, H-6), 5.64 (1H, dqd, *J* = 15.0, 7.0, 2.0, H-7), 4.60 (1H, brs, C5-OH), 4.41 (1H, m, H-3), 4.35 (1H, dd, *J* = 7.2, 3.0, H-4), 4.32 (1H, m, H-5), 3.18 (1H, dd, *J* = 18.0, 7.5, H-2a), 2.44 (1H, dd, *J* = 18.0, 3.5, H-2b), 1.74 (3H, dd, *J* = 6.5, 1.5 Hz, H-8); <sup>13</sup>C NMR (125 MHz,

CDCl<sub>3</sub>),  $\delta_c$  (ppm): 190.3 (C-7'), 174.9 (C-1), 150.9 (C-1'), 132.3 (C-3', C-5'), 131.4 (C-6), 128.2 (C-7), 128.1 (C-4'), 112.6 (C-2', C-6'), 87.3 (C-4), 73.1 (C-5), 51.0 (C-3), 36.3 (C-2), 17.8 (C-8).

**AT.02 (Chartreusin):** Greenish-yellow powder; C<sub>32</sub>H<sub>32</sub>O<sub>14</sub>; HR-ESI-MS ( $m/z$ ): 641.1876 (calculated for C<sub>32</sub>H<sub>33</sub>O<sub>14</sub> 641.1870 [M+H]<sup>+</sup>). The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra data: see Table 3.3

#### 2.4.2. Extraction and isolation of compound from the culture of *Streptomyces wuyuanensis* VH19-A079

**AT.03 (Indole-3-carboxylic acid):** Light yellow powder; C<sub>9</sub>H<sub>7</sub>NO<sub>2</sub>; ESI-MS ( $m/z$ ): 178.5 [M-H+H<sub>2</sub>O]<sup>-</sup>; The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra data: see Table 3.4.

#### 2.4.3. Extraction and isolation of compounds from the culture of *Streptomyces aureus* VTCC43181

**AT.04 (Nocardamin):** Colorless powder; C<sub>27</sub>H<sub>48</sub>N<sub>6</sub>O<sub>9</sub>; HR-ESI-MS ( $m/z$ ): 601.3527 (calculated for C<sub>27</sub>H<sub>49</sub>N<sub>6</sub>O<sub>9</sub> 601.3561 [M+H]<sup>+</sup>), 623.3353 (calculated for C<sub>27</sub>H<sub>48</sub>N<sub>6</sub>O<sub>9</sub>Na 623.3380 [M+Na]<sup>+</sup>). The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra data: see Table 3.5.

**AT.05 (Pleurone):** White, powder, C<sub>4</sub>H<sub>2</sub>O<sub>4</sub>; ESI-MS ( $m/z$ ): 119 [M+Na-H<sub>2</sub>O]<sup>+</sup>. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra data: see Table 3.6.

**AT.06 (Halolitoralin A):** White soil; C<sub>27</sub>H<sub>48</sub>N<sub>6</sub>O<sub>6</sub>; HR-ESI-MS ( $m/z$ ): 553.3730 (calculated for C<sub>27</sub>H<sub>49</sub>N<sub>6</sub>O<sub>6</sub> 553.3714 [M+H]<sup>+</sup>), 575.3548 (calculated for C<sub>27</sub>H<sub>48</sub>N<sub>6</sub>O<sub>6</sub>Na 575.3533 [M+Na]<sup>+</sup>). <sup>1</sup>H-NMR ( $\delta_H$ ,  $J$ , 500 MHz, CD<sub>3</sub>OD): 3.57-3.54 (2H, m,  $\alpha$ -H of Leu<sup>1</sup> & Leu<sup>2</sup>), 3.50 (1H, d,  $J$  = 3.5 Hz,  $\alpha$ -H of Ile), 3.43 (3H, d,  $J$  = 4.5 Hz,  $\alpha$ -H of Ala<sup>1</sup>, Ala<sup>2</sup> & Ala<sup>3</sup>), 1.97-1.95 (1H, m,  $\beta$ -H của Ile), 1.83-1.77 (2H, m,  $\gamma$ -H của Leu<sup>1</sup> & Leu<sup>2</sup>), 1.83-1.59 (4H, m,  $\beta$ -H of Leu<sup>1</sup> & Leu<sup>2</sup>), 1.66-1.59 (2H, m,  $\gamma$ -H of Ile), 1.08-1.07 (3H, d,  $J$  = 7.0 Hz,  $\beta$ -H of Ala<sup>3</sup>), 1.05-1.01 (9H, m,  $\beta$ -H of Ala<sup>1</sup> & Ala<sup>2</sup>,  $\beta'$ -H of Ile), 1.00-0.96 (15H, m,  $\delta$ -H of Leu<sup>1</sup> & Leu<sup>2</sup>,  $\delta$ -H of Ile); <sup>13</sup>C-NMR ( $\delta_c$ ,  $J$ , 125 MHz, CD<sub>3</sub>OD): 175.2 (C=O, Leu<sup>1</sup> & Leu<sup>2</sup>), 174.5 (C=O, Ala), 174.0 (C=O, Ile), 61.8 ( $\alpha$ -C, Ala<sup>1</sup>, Ala<sup>2</sup> & Ala<sup>3</sup>), 60.9 ( $\alpha$ -C, Ile), 54.7

( $\alpha$ -C, Leu<sup>1</sup> & Leu<sup>2</sup>), 41.8 ( $\beta$ -C, Leu<sup>1</sup> & Leu<sup>2</sup>), 37.7 ( $\beta$ -C, Ile), 25.9 ( $\gamma$ -C, Ile), 25.8 ( $\gamma$ -C, Leu<sup>1</sup> & Leu<sup>2</sup>), 23.2 ( $\delta$ -C, Leu<sup>2</sup>), 22.0 ( $\delta$ -C, Leu<sup>1</sup>), 19.1 ( $\beta$ -C, Ala<sup>3</sup>), 17.7 ( $\beta$ -C, Ala<sup>1</sup> & Ala<sup>2</sup>), 15.6 ( $\beta'$ -C, Ile), 12.2 ( $\delta$ -C, Ile).

**2.4.4. Extraction and isolation of compound from the culture of *Streptomyces spiroverticillatus* VH19-A067**

**AT.07 ((6Z)-15-methyl-6-hexadecenoic acid):** Pale pink solid; C<sub>17</sub>H<sub>32</sub>O<sub>2</sub>; HR-ESI-MS ( $m/z$ ): 267.2335 (calculated for C<sub>17</sub>H<sub>31</sub>O<sub>2</sub> 267.2324 [M-H]<sup>-</sup>). <sup>1</sup>H-NMR ( $\delta$ <sub>H</sub>,  $J$ , 600 MHz, CD<sub>3</sub>OD): 5.36 (2H, m, H-6, H-7), 2.30 (2H, m, H-2), 2.06 (4H, m, H-5, H-8), 1.61 (2H, m, H-3), 1.54 (1H, m, H-15), 1.34 (2H, m, H-4), 1.33 – 1.31 (10H, m, H-9, H-10, H-11, H-12, H-13), 1.20 (2H, m, H-14), 0.91 (6H, m, H-16, H-17); <sup>13</sup>C-NMR ( $\delta$ <sub>C</sub>,  $J$ , 125 MHz, CD<sub>3</sub>OD): 177.8 (C-1), 130.8 (C-6, C-7), 40.2 (C-14), 35.0 (C-2), 33.0 (C-9, C-12), 30.7 (C-4), 28.5 (C-10, C-11), 29.1 (C-15), 28.1 (C-5), 26.1 (C-3), 23.7 (C-13), 23.0 (C-16, C-17).

**2.4.5. Extraction and isolation of compounds from the culture of *Streptomyces alboniger* VH19-A105B**

**AT.08 (Cardoltriene):** Brown oil; C<sub>21</sub>H<sub>30</sub>O<sub>2</sub>; HR-ESI-MS ( $m/z$ ): 313,2196 (calculated for C<sub>21</sub>H<sub>29</sub>O<sub>2</sub> 313,2168 [M-H]<sup>-</sup>). The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra data: see Table 3.7.

**AT.09 (Cardoltriene M):** Brown oil; C<sub>36</sub>H<sub>47</sub>NO<sub>5</sub>; HR-ESI-MS ( $m/z$ ):  $m/z$  596.3325 (calculated for C<sub>36</sub>H<sub>47</sub>NO<sub>5</sub>Na 596.3352 [M+Na]<sup>+</sup>), 572.3406 (calculated for C<sub>36</sub>H<sub>46</sub>NO<sub>5</sub> 572.3376 [M-H]<sup>-</sup>). The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra data: see Table 3.7.

**(Chartreusin):** The NMR spectra data of **SH.03** coincided with **SA.02**.

**AT.11 (7-deoxyauramycinone):** Orange soil; C<sub>21</sub>H<sub>18</sub>O<sub>7</sub>; HR-ESI-MS ( $m/z$ ): 382.1053 (calculated for C<sub>21</sub>H<sub>18</sub>O<sub>7</sub> 382.1059 [M]). The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra data: see Table 3.8.

**2.4.6. Extraction and isolation of compound from the culture of *Streptomyces cyaneus* VTCC43860**

**AT.12 (7-acetyl-3,6-dihydroxy-8-methyl tetralone):** White powder;  $C_{13}H_{15}O_4$ ; HR-ESI-MS ( $m/z$ ): 235.0960 (calculated for  $C_{13}H_{15}O_4$  235.0970  $[M+H]^+$ ), 257.0783 (calculated for  $C_{36}H_{47}NO_5Na$  257.0790  $[M+Na]^+$ ), 233.0827 (calculated for  $C_{36}H_{46}NO_5$  233,0814  $[M-H]^-$ ). The  $^1H$ -NMR and  $^{13}C$ -NMR spectra data: see Table 3.9.

**2.4.7. Extraction and isolation of compound from the culture of *Streptomyces sp. VTCC43168***

**AT.13 (Valin):** White soil;  $C_5H_{11}NO_2$ ; ESI-MS ( $m/z$ ): 115.8  $[M-H]^-$ .  $^1H$ -NMR ( $CD_3OD$ , 500 MHz),  $\delta_H$  (ppm),  $J$  (Hz): 3.32-3.40 (1H, m, H-2), 2.27 (1H, m, H-3), 1.08 (3H, d,  $J = 7.0$  Hz, H-4), 1.03 (3H, d,  $J = 7.0$  Hz, H-5);  $^{13}C$ -NMR ( $\delta_C$ ,  $J$ , 125 MHz,  $CD_3OD$ ): 180.3 (C-1), 61.8 (C-2), 31.0 (C-3), 19.3 (C-4), 17.7 (C-5).

**2.4.8. Extraction and isolation of compounds from the culture of *Actinoplanes missouriensis VTCC40900***

**AT.14 (Flufuran):** Colorless, needle-shaped crystals;  $C_6H_6O_4$ , ESI-MS:  $m/z = 140.7$   $[M-H]^-$ .  $^1H$ -NMR ( $CD_3OD$ , 500 MHz)  $\delta_H$ : 7.95 (1H, s, H-2), 6.51 (1H, s, H-4), 4.42 (2H, s, H-7);  $^{13}C$ -NMR ( $CD_3OD$ , 125 MHz)  $\delta_C$ : 177.2 (C-6), 170.2 (C-5), 147.4 (C-3), 141.0 (C-2), 110.6 (C-4), 61.2 (C-7).

**AT.15 (Trehalose):** White soil;  $C_{12}H_{22}O_{11}$ , ESI-MS:  $m/z = 364.9$   $[M+Na]^+$ .  $^1H$ -NMR ( $CD_3OD$ , 500 MHz)  $\delta_H$  5.14 (2H, d,  $J = 3.5$  Hz), 3.84 (6H, m), 3.70 (2H, m), 3.49 (2H, dd,  $J = 10.0, 3.5$  Hz), 3.33 (2H, m);  $^{13}C$ -NMR ( $CD_3OD$ , 125 MHz)  $\delta_C$  95.0 (C-1), 74.6 (C-3), 73.8 (C-2), 73.2 (C-5), 71.9 (C-4), 62.6 (C-6).

## CHAPTER 3. RESULTS AND DISCUSSION

### 3.1. Results of isolation and identification of actinomycetes

#### 3.1.1. Isolation of actinomycetes

181 different actinomycete strains were isolated from 26 soil and sediment samples collected from various regions along the North to Central Vietnam based on their differences in colony characteristics such as color, colony size, filamentous structure, mycelium growth, and pigment secretion... Preliminary assessment of actinomycete morphology showed that of 181 total strains there were 135 strains belonging to the genus *Streptomyces* (74.6%), while the rest one (46 strains) were rare actinomycetes (25.4%).

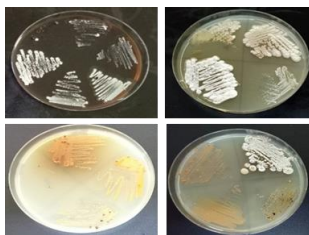


Figure 3.1. Colony characteristics of isolated some actinomycetes

#### 3.1.2. Antimycobacterial actinomycetes Against *Mycobacterium Smegmatis*

Agar plate assay was used for the screening of actinomycete strains against *M. smegmatis*. The evaluation result showed that of 181 actinomycete strains, 14 strains (7.7%) were able to inhibit *M. smegmatis* (PL1).

The strains VH19-A002, VH19-A067 and VH19-A079 showed an equivalent activity with an inhibition zone of 9 mm diameter, while the strains VH19-A105B was active against *M. smegmatis* with 8 mm of inhibited zone. The strain VH19-A121 was the most active one with an inhibition zone of 11 mm in diameter.

#### 3.1.3. Classification of actinomycete strains

The most active strains (VH19-A002, VH19-A067, VH19-A079, VH19-A105B, VH19-A121) against *M. smegmatis* were classified based on their cultural characteristic, the color of the substrate mycelia, the colony morphology, the ability to produce pigment, the morphology of the spore chain and 16S rDNA sequences and conducted by Master Vu Ha Phuong, Department of Biology, Life Sciences Research Center, University of Natural Sciences - Hanoi National University. By analysis of the above data, the actinomycetes VH19-A002, VH19-A067, VH19-A079, VH19-A105B and VH19-A121 were identified as *Streptomyces avidinii* VH19-A002, *Streptomyces spiroverticillatus* VH19-A067, *Streptomyces wuyanensis* VH19-A079, *Streptomyces alboniger* VH19-A105B và *Streptomyces alboniger* VH19-A121, respectively.

### 3.2. Results of determining the structure of compounds isolated from the culture of actinomycetes

#### 3.2.1. Structural elucidation of the compounds isolated from *Streptomyces alboniger* VH19-A121

##### 3.2.1.1. AT.01

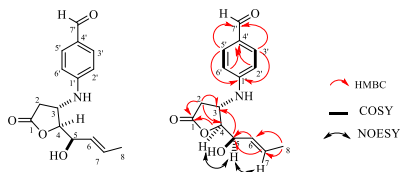


Figure 3.4. The structure, HMBC, COSY and NOESY correlations of compound AT.01

Compound **AT.01** was obtained as a yellow powder. The spectral data of **AT.01** are consistent with the spectral data of obscurolide B<sub>2β</sub>. Therefore, compound **AT.01** was identified as obscurolide B<sub>2β</sub>. This is the first time this compound has been isolated from *S. alboniger*.

##### 3.2.1.2. AT.02

Compound **AT.02** was obtained as a greenish-yellow powder. Compound **AT.02** was identified as chartreusin, a benzonaphthopyranone

glycoside isolated previously from *S. chartreusis*. This is the first time chartreusin has been isolated from *S. alboniger*.

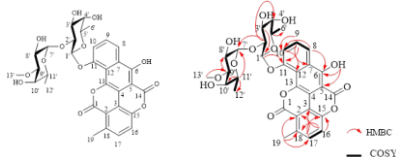


Figure 3.7. The structure, HMBC, COSY and NOESY correlations of compound **AT.02**

### 3.2.2. Structural elucidation of the compound isolated from *Streptomyces wuyuanensis* VH19-A079

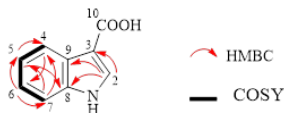


Figure 3.15. The structure of compound **AT.03**

Compound **AT.03** was obtained as a light yellow powder. Combination of NMR and MS data and comparing with the reference allowed to determine compound **AT.03** as indole-3-carboxylic acid.

### 3.2.3. Structural elucidation of the compounds isolated from *Streptomyces aureus* VTCC43181

#### 3.2.3.1. **AT.04**

Compound **AT.04** was obtained as a colorless powder. Combination of NMR and MS data and comparing with the reference allowed to determine compound **AT.04** as nocardamin, a cyclic trimer of N-hydroxy-N'-succinylcadaverine.

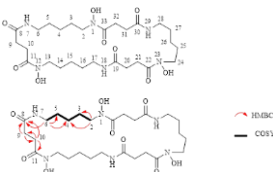
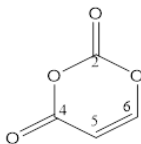


Figure 3.16. The structure, HMBC and COSY correlations of compound **AT.04**

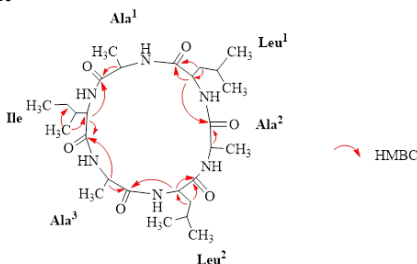
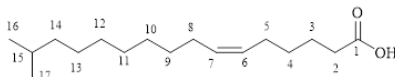


3.2.3.2. **AT.05**Figure 3.18. The structure of compound **AT.05**

Compound **AT.05** was obtained as a white powder. Combination of the spectral data and comparing with the reference allowed to determine compound **AT.05** as pleurone (4*H*-1,3-dioxine-2,4-dione) with the molecular formula of  $C_4H_2O_4$ .

3.2.3.3. **AT.06**

Compound **AT.06** was obtained as a white solid. By analysis of the spectral data and comparing it with the published data, compound **AT.06** was determined as halolitoralin A, a hexapeptide containing three Ala, two Leu, and one Ile unit

Figure 3.19. The structure and HMBC correlations of compound **AT.06**3.2.4. **Structural elucidation of the compounds isolated from *Streptomyces spiroverticillatus* VH19-A067**Figure 3.20. The structure of compound **AT.07**

Compound **AT.07** was obtained as pale pink solid. By analysis of the spectral data and comparing it with the published data, compound **AT.07** was determined as (6*Z*)-15-methyl-6-hexadecenoic acid.

### 3.2.5. Structural elucidation of the compounds isolated from *Streptomyces alboniger* VH19-A105B

#### 3.2.5.1. AT.08

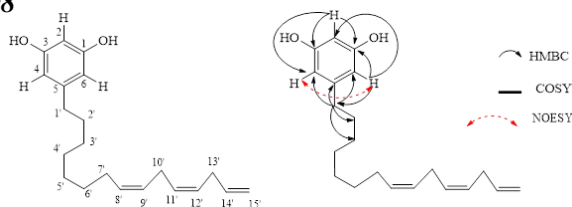


Figure 3.22. The structure, HMBC, COSY and NOESY correlations of compound AT.08

Compound AT.08 was obtained as brown oil. Combination of NMR and HR-ESI-MS data and comparing with the reference allowed to determine compound AT.08 as cardoltriene.

#### 3.2.5.2. AT.09

Compound AT.09 was obtained as brown oil. The HRESI-MS spectrum of AT.09 showed molecular ion peaks at  $m/z$  596.3325 (calculated for  $C_{36}H_{47}NO_5Na$  596,3352  $[M+Na]^+$ ), 572.3406 (calculated for  $C_{36}H_{46}NO_5$  572.3376  $[M-H]^-$ ) allowed to determine the molecular formula of compound AT.09 as  $C_{36}H_{47}NO_5$ .

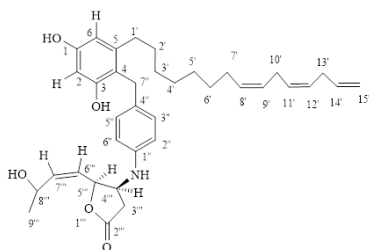


Figure 3.28. The structure of compound AT.09

The  $^1H$ -NMR spectrum of compound 1 appeared six aromatic protons at  $\delta_H$  6.98 (2H, d,  $J = 8.5$ , H-3'', H-5''), 6.47 (2H, dd,  $J = 8.5, 3.0$ , H-2'', H-6''), 6.29 (1H, m, H-6), 6.22 (1H, *br s*, H-2), nine olefin protons at  $\delta_H$  5.88 (1H, m, H-7'''), 5.83 (1H, m, H-14'), 5.73 (1H, m, H-6'''), 5.42

(1H, m, H-12'), 5.41 (1H, m, H-11'), 5.36 (1H, m, H-8'), 5.34 (1H, m, H-9'), 5.04 (2H, m, H-15') and eleven methylene groups at  $\delta_{\text{H}}$  3.86 (2H, s, H-7''), 2.96 (1H, m, H-3'''), 2.40 (1H, d,  $J = 3.5$ , H-3'''), 2.82 (2H, m, H-13'), 2.77 (2H, m, H-10'), 2.50 (2H, t,  $J = 7.5$ , H-1'), 2.02 (2H, m, H-7'), 1.48 (2H, m, H-2'), 1.27 (8H, m, H-3', H-4', H-5', H-6'). Furthermore, two methine groups at  $\delta_{\text{H}}$  4.79 (1H, m, H-5'''), 3.97 (1H, m, H-4''') and one methyl group at  $\delta_{\text{H}}$  1.25 (3H, m, H-9''') were observed.

The  $^{13}\text{C}$ -NMR spectrum indicated twelve aromatic carbons at  $\delta_{\text{C}}$  155.5 (C-3), 154.8 (C-1), 144.1 (C-5), 143.8 (C-1''), 130.3 (C-4''), 129.1 (C-3'', C-5''), 117.1 (C-4), 114.1 (C-2'', C-6''), 108.8 (C-6), 101.0 (C-2), 8 olefin carbons at  $\delta_{\text{C}}$  138.5 (C-7'''), 136.9 (C-14'), 130.9 (C-8'), 129.3 (C-12'), 127.6 (C-9'), 126.9 (C-11'), 124.6 (C-6'''), 114.7 (C-15'), 11 anky groups at  $\delta_{\text{C}}$  33.5 (C-1'), 31.0 (C-2', C-13'), 30.0 (C-3', C-4', C-5', C-6'), 27.2 (C-7'), 25.6 (C-10'), 23.2 (C-9'''). Furthermore, one methylene group at  $\delta_{\text{C}}$  35.3 (C-3'''), 2 methine groups at  $\delta_{\text{C}}$  84.7 (C-5'''), 55.5 (C-4''') and 1 lacton carbonyl group at  $\delta_{\text{C}}$  174.9 (C-2'''). Comparison of the  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectral data of **AT.09** with data of **AT.08** showed that the structures of compounds **T.08** và **AT.09** were similar.

The assignment of all protons and carbons in compound **2** was further corroborated by 2D NMR (COSY, HSQC, HMBC and NOESY) spectral data. The COSY correlations of H-2'' ( $\delta_{\text{H}}$  6.47)/H-3'' ( $\delta_{\text{H}}$  6.98); H-6'' ( $\delta_{\text{H}}$  6.47)/H-5'' ( $\delta_{\text{H}}$  6.98) confirmed the location of the aromatic protons in the aromatic ring and correlations of protons in the allyl groups between H-15' ( $\delta_{\text{H}}$  5.04)/H-14' ( $\delta_{\text{H}}$  5.83); H-12' ( $\delta_{\text{H}}$  5.42)/H-13' ( $\delta_{\text{H}}$  2.82); H-13' ( $\delta_{\text{H}}$  2.82)/ H-14' ( $\delta_{\text{H}}$  5.83); H-11' ( $\delta_{\text{H}}$  5.41)/H-10' ( $\delta_{\text{H}}$  2.77); H-9' ( $\delta_{\text{H}}$  5.34)/H-10' ( $\delta_{\text{H}}$  2.77); H-7' ( $\delta_{\text{H}}$  2.02)/H-8' ( $\delta_{\text{H}}$  5.36), H-6' ( $\delta_{\text{H}}$  1.27); H-1' ( $\delta_{\text{H}}$  2.50)/H-2' ( $\delta_{\text{H}}$  1.48); H-2' ( $\delta_{\text{H}}$  1.48)/H-3' ( $\delta_{\text{H}}$  1.27); H-7''' ( $\delta_{\text{H}}$  5.88)/H-8''' ( $\delta_{\text{H}}$  4.34), H-6''' ( $\delta_{\text{H}}$  5.73); H-8''' ( $\delta_{\text{H}}$  4.34)/H-9''' ( $\delta_{\text{H}}$  1.25); H-3''' ( $\delta_{\text{H}}$  2.96)/H-4''' ( $\delta_{\text{H}}$  3.97); H-4''' ( $\delta_{\text{H}}$  3.97)/H-5''' ( $\delta_{\text{H}}$  4.79); H-5''' ( $\delta_{\text{H}}$  4.79)/H-6''' ( $\delta_{\text{H}}$  5.73).

The allyl group was attached to the aromatic ring at C-5, approved by the HMBC correlation between H-1' ( $\delta_{\text{H}}$  2.50) to C-4 ( $\delta_{\text{C}}$  117.1), C-5 ( $\delta_{\text{C}}$  144.1), C-6 ( $\delta_{\text{C}}$  108.8); H-2 ( $\delta_{\text{H}}$  6.22) to C-4 ( $\delta_{\text{C}}$  117.1); H-6 ( $\delta_{\text{H}}$  6.29) to C-2 ( $\delta_{\text{C}}$  101.0), C-4 ( $\delta_{\text{C}}$  117.1), C-1' ( $\delta_{\text{C}}$  33.5). The phenyl group was attached to the phenolic ring at C-4, approved by the HMBC correlation between H-7'' ( $\delta_{\text{H}}$  3.86) to C-3 ( $\delta_{\text{C}}$  155.3), C-5 ( $\delta_{\text{C}}$  144.1); H-5'' ( $\delta_{\text{H}}$  6.98) to C-1'' ( $\delta_{\text{C}}$  143.8), C-3'' (129.1), C-7'' (29.6); H-3'' ( $\delta_{\text{H}}$  6.98) với C-1'' ( $\delta_{\text{C}}$  143.8), C-5'' (129.1); H-2'' ( $\delta_{\text{H}}$  6.47) với C-6'' ( $\delta_{\text{C}}$  114.1). The NOESY correlation between H-2'' ( $\delta_{\text{H}}$  6.47)/H-4''' ( $\delta_{\text{H}}$  3.97), H-5''' ( $\delta_{\text{H}}$  4.79); H-4''' ( $\delta_{\text{H}}$  3.97)/H-5''' ( $\delta_{\text{H}}$  4.79); H-5''' ( $\delta_{\text{H}}$  4.79)/H-6''' ( $\delta_{\text{H}}$  5.73), H-7''' ( $\delta_{\text{H}}$  5.88); H-6''' ( $\delta_{\text{H}}$  5.73)/H-7''' ( $\delta_{\text{H}}$  5.88); H-8' ( $\delta_{\text{H}}$  5.36)/ H-9' ( $\delta_{\text{H}}$  5.34)'; H-11' ( $\delta_{\text{H}}$  5.41)/ H-12' ( $\delta_{\text{H}}$  5.42)' allowed to determine the relative configuration of **AT.09** as Figure 3.39.

The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra of compound **AT.09** were provided in Table 3.7. From the spectral data presented above, new compound **AT.09** was named cardoltriene M.

Table 3.7. The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra of compounds **AT.08** và **AT.09**

C	AT.08		AT.09		Cardoltriene	
	$\delta_{\text{H}}$ (ppm) <sup>a</sup>	$\delta_{\text{C}}$ (ppm) <sup>b</sup>	$\delta_{\text{H}}$ (ppm) <sup>c</sup>	$\delta_{\text{C}}$ (ppm) <sup>d</sup>	$\delta_{\text{H}}$ (ppm) <sup>e</sup>	$\delta_{\text{C}}$ (ppm) <sup>f</sup>
1		159.3		154.8		156.4
2	6.09 (1H, m)	100.9	6.22 (1H, br s)	101.0	6.17 (1H, br s)	100.2
3		159.3		155.3		156.4
4	6.14 (1H, d, $J = 2.5$ Hz)	107.9		117.1	6.24 (1H, d, $J = 1.8$ Hz)	108.1
5		146.3		144.1		146.2
6	6.14 (1H, d, $J = 2.5$ Hz)	107.9	6.29 (1H, m)	108.8	6.24 (1H, d, $J = 1.8$ Hz)	108.1
1'	2.45 (2H, t, $J =$	36.9	2.50 (2H, t, $J =$	33.5	2.44 (2H, t, $J =$	35.8

	7.5 Hz)		7.5 Hz)		7.7 Hz)	
2'	1.56 (2H, m)	32.4	1.48 (2H, m)	31.0	1.53 (2H, m)	31.0
3'	1.30 (2H, m)	30.4	1.27 (2H, m)	30.0	1.28 (2H, m)	29.6
4'	1.30 (2H, m)	30.8	1.27 (2H, m)	30.0	1.28 (2H, m)	29.4
5'	0.93 (2H, m)	23.0	1.27 (2H, m)	30.0	1.28 (2H, m)	29.3
6'	1.32 (2H, m)	23.7	1.27 (2H, m)	30.0	1.28 (2H, m)	29.2
7'	2.08 (2H, m)	28.1	2.02 (2H, m)	27.2	2.03 (2H, q, $J = 6.6$ Hz)	27.2
8'	5.35 (1H, m)	130.2	5.36 (1H, m)	130.9	5.37 (1H, m)	130.4
9'	5.34 (1H, m)	130.2	5.34 (1H, m)	127.6	5.37 (1H, m)	129.3
10'	2.81 (2H, m)	26.4	2.77 (2H, m)	25.6	2.80 (2H, m)	25.6
11'	5.40 (1H, m)	127.8	5.41 (1H, m)	126.9	5.37 (1H, m)	127.6
12'	5.42 (1H, m)	128.7	5.42 (1H, m)	129.3	5.37 (1H, m)	126.8
13'	2.83 (2H, m)	32.3	2.82 (2H, m)	31.0	2.80 (2H, m)	31.5
14'	5.83 (1H, m)	137.9	5.83 (1H, m)	136.9	5.82 (1H, ddt, $J = 17.1, 10.3, 6.2$ Hz)	136.8
15'	5.02 (1H, m) 4.96 (1H, m)	115.0	5.04 (1H, m)	114.7	5.05 (1H, dq, $J = 17.1, 1.6$ Hz) 4.98 (1H, dq, $J = 10.3, , 1.6$ Hz)	114.7
1''				143.8		
2''			6.47 (1H, dd, $J = 8.5, 3.0$ Hz)	114.1		
3''			6.98 (1H, d, $J = 8.5$ Hz)	129.1		
4''				130.3		
5''			6.98 (1H, d, $J = 8.5$ Hz)	129.1		
6''			6.47 (1H, dd, $J$	114.1		

			= 8.5, 3.0 Hz)			
7''			3.86 (2H, s)	29.6		
1'''						
2'''				174.9		
3'''			2.96 (1H, m) 2.40 (1H, d, <i>J</i> = 3.5 Hz)	35.2		
4'''			3.97 (1H, m)	55.5		
5'''			4.79 (1H, m)	84.7		
6'''			5.73 (1H, m)	124.6		
7'''			5.88 (1H, m)	138.5		
8'''			4.34 (1H, m)	67.5		
9'''			1.25 (3H, m)	23.2		

<sup>a</sup>500 MHz, CD<sub>3</sub>OD; <sup>b</sup>125 MHz, CD<sub>3</sub>OD; <sup>c</sup>500 MHz, CDCl<sub>3</sub>; <sup>d</sup>125 MHz, CDCl<sub>3</sub>; <sup>e</sup>300 MHz, CDCl<sub>3</sub>; <sup>f</sup>100 MHz CDCl<sub>3</sub>

### 3.2.5.3. AT.10

Compound **AT.10** was isolated as a yellow-green powder. Based on the NMR spectrum, it showed that the structure of compound **AT.10** coincided with compound **AT.02** (chartresin).

### 3.2.5.4. AT.11

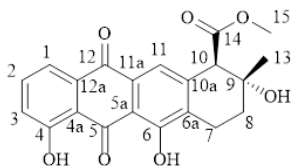


Figure 3.40. The structure of compound **AT.11**

Compound **AT.09** was obtained as orange soil. Combination of NMR, MS data and comparing with the reference allowed to determine compound **AT.11** as 7-deoxyauramycinone (9-epi-7-deoxynogalamycinone).

### 3.2.6. Structural elucidation of the compound isolated from *Streptomyces cyaneus* VTCC43860

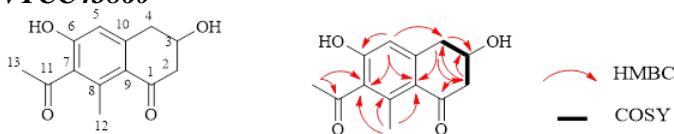


Figure 3.42. The structure, HMBC and COSY correlations of compound **AT.12**

Compound **AT.12** was obtained as white powder. Combination of NMR, MS data and comparing with the reference allowed to determine compound **AT.12** as 7-acetyl-3,6-dihydroxy-8-methyl tetralone.

### 3.2.7. Structural elucidation of the compound isolated from *Streptomyces* sp. VTCC43168

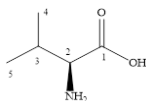


Figure 3.43. The structure of compound **AT.13**

Compound **AT.13** was obtained as white soil. By analysis of the spectral data, compound **AT.13** was determined as valin (Acid 2-amino-3-methylbutanoic).

### 3.2.8. Structural elucidation of the compounds isolated from *Actinoplanes missouriensis* VTCC40900

#### 3.2.8.1. **AT.14**

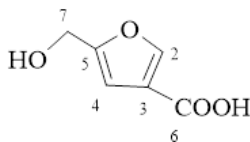


Figure 3.44. The structure of compound **AT.14**

Compound **AT.14** was obtained as colorless, needle-shaped crystals. The NMR spectrum of **AT.14** completely matched of 5-hydroxymethylfuran-3-carboxylic acid hay flufuran.

### 3.2.8.2. *Chất AT.15*

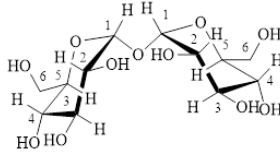


Figure 3.47. The structure of compound **AT.15**

Compound **AT.15** was obtained as white soil. Combination of NMR, MS data and comparing with the reference allowed to determine compound **AT.15** as trehalose.

## 3.3. Results of evaluating the activity of isolated pure compounds

### 3.3.2. Antimycobacterial activity against *Mycobacterium smegmatis*

Compounds **AT.01** and **AT.02** were evaluated for their antimycobacterial activity against *M. smegmatis*. The results showed that compound **AT.02** displayed strong activity against *M. smegmatis* with 15 mm of inhibition zone diameter, while compound **AT.01** was inactive. Chartreusin was reported to have potential antimycobacterial activity against *M. tuberculosis* 607 and *My. tuberculosis* H37Rv. Our study reported for the first time the production of chartreusin from *Streptomyces alboniger* and its antimycobacterial activity against *M. smegmatis*.

### 3.3.2. Evaluation of ATPase activity of recombinant protein ClpC1

The result showed that ClpC1 proteins were located in the cell membrane and only collected after breaking the cell membrane with ultrasonic waves. The recombinant ClpC1 protein was obtained with an approximate molecular weight of 93.5 kDa.

ATPase activity of ClpC1 was determined by the phosphate content released. The result showed that ATP hydrolysis activity via ClpC1 was recorded at 10  $\mu$ M, following the gradual increase of the ATP concentration in the reaction. The stability of ATPase activity in ClpC1 protein was also examined. As a result, the ATPase activity remained at its original properties for seven days but was largely changed and unstable by



day 8.

Ecumicin and rufomycin were used as positive controls in this study. Different concentrations of compounds **AT.01**, **AT.02**, **AT.03**, **AT.04**, **AT.05**, **AT.06**, **AT.07**, **AT.08**, **AT.09**, **AT.11**, **AT.12**, **AT.14** (0.1  $\mu$ M, 1.0  $\mu$ M and 10.0  $\mu$ M) were evaluated and optimized for the ATPase activity of the ClpC1 protein. The results showed that ATPase activity increased gradually with the concentration of compounds **AT.03**, **AT.06** and **AT.14**. Therefore, these compounds were effective for the ATP hydrolysis of ClpC1 protein, similarly to ecumicin, but with lower ATP hydrolysis affection. ATPase activity decreased gradually with the concentration of compounds **AT.08** (Cardoltriene), **AT.09** (Cardoltriene M), thus, two these compounds were effective for the ATP hydrolysis of ClpC1 protein, similarly to rufomycin, but with higher ATP hydrolysis affection. With the effect of reducing the ATP hydrolysis activity by nearly 50% at 10 $\mu$ M of compounds **AT.08** and **AT.09**, it showed that these compounds are considered to have high anti-tuberculosis potential through the mechanism of targeting the regulatory protein ClpC1 in the direction of reducing ATPase activity while compounds **AT.03**, **AT.06** and **AT.14** increase ATPase activity of ClpC1 protein. However, Further research is needed to study the mechanism and active sites of these compounds on *M. tuberculosis* ClpC1 protein.

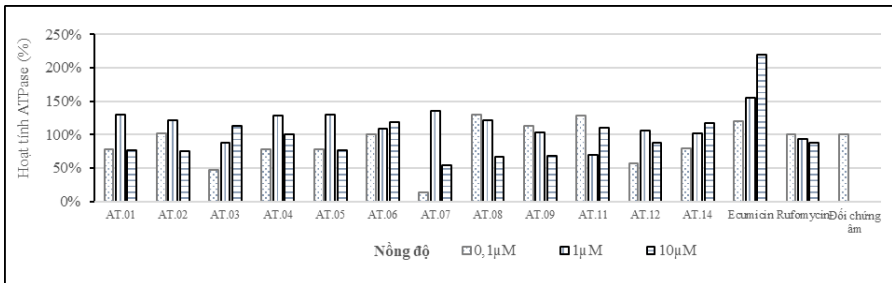


Figure 3.52. Affection of compounds to ATPase activity

## CONCLUSIONS AND RECOMMENDATIONS

### ➤ Conclusion

1. 181 different actinomycete strains were isolated from 26 soil and sediment samples collected from various regions along the North to Central Vietnam and were screened for their antimycobacterial activity against *M. smegmatis* which is a strain similarity to *M. tuberculosis*. The results showed that the five most active strains were *S. avidinii* VH19-A002 (Mangrove forest in Phu Long commune), *S. spiroverticillatus* VH19-A067 (The confluence of Day river and Vac river), *S. wuyanensis* VH19-A079 (Quynh Luong mangrove forest), *S. alboniger* VH19-A105B (on the way to the top of May Bac mountain) and *S. alboniger* VH19-A121 (the top of May Bac mountain).

2. 14 compounds from the biomass of actinomycete strains were isolated and determined their structures using modern spectroscopic methods such as HR-ESI-MS, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, HSQC, HMBC, COSY, NOESY, as following:

+ 2 compounds from *S. alboniger* VH19-A121 are obscurolide B2β (**AT.01**) belongs to obscurolide framework, chartreusin (**AT.02**) belongs to the benzonaphthopyranone glycoside framework.

+ 1 compound from *S. wuyanensis* VH19-A079 is indole-3-carboxylic acid (**AT.03**) belongs to indole carboxylic acid framework.

+ 3 compounds from *S. aureus* VTCC43181 are nocardamine (**AT.04**) belongs to cyclopeptide framework, pleurone (**AT.05**) belongs to lactone framework, halolitoralin A (**AT.06**) belongs to cyclopeptide framework.

+ 1 compound from *S. spiroverticillatus* VH19-A067 is (6Z)-15-methyl-6-hexadecenoic acid (**AT.07**) belongs to carboxylic acid framework.

+ 3 compounds from *S. alboniger* VH19-A105B are cardoltriene (**AT.08**) belongs to polyphenol compound, cardoltriene M (**AT.09**, new) belongs to polyphenol compound, 7-deoxyauramycinone (**AT.11**) belongs to anthraquinone framework. Among them, there is a new compound named cardoltriene M (**AT.09**).

+ 1 compound from *S. cyaneus* VTCC43860 is 7-acetyl-3,6-dihydroxy-8-methyl tetralone (**AT.12**) belongs to tetralone framework.

+ 1 compound from *Streptomyces* sp. VTCC43168 is valine

(**AT.13**) belongs to amino acid compound.

+ 2 compounds from *A. missouriensis* VTCC40900 are flufuran (**AT.14**) belongs to furan framework, trehalose (**AT.15**) belongs to the glycoside framework.

3. Compounds **AT.01** and **AT.02** were evaluated for their antimycobacterial activity against *M. smegmatis*. The results showed that compound **AT.02** displayed strong activity against *M. smegmatis* with 15 mm of inhibition zone diameter, while compound **AT.01** was inactive.

4. Twelve compounds (**AT.01**, **AT.02**, **AT.03**, **AT.04**, **AT.05**, **AT.06**, **AT.07**, **AT.08**, **AT.09**, **AT.11**, **AT.12**, **AT.14**) were evaluated for their ability to affect the ATPase activity of recombinant ClpC1 protein. This is the first time these compounds have been evaluated for their effect on ATPase activity of the recombinant ClpC1 protein, a regulatory protein of *M. tuberculosis*. The results showed that there were 5 compounds including indole-3-carboxylic acid (**AT.03**), halolitoralin A (**AT.06**), flufuran (**AT.14**), cardoltriene (**AT.08**) and cardoltriene M (**AT.09**) have the effect of inhibition ClpC1 protein through ATP hydrolysis of ClpC1 protein. Research results showed that the compounds isolated from the studied actinomycetes affected the ClpC1 protein of tuberculosis bacteria. Among them, cardoltriene (**AT.08**) and cardoltriene M (**AT.09**) are considered to have high anti-tuberculosis potential through the mechanism of targeting the regulatory protein ClpC1.

#### ➤ **Recommendations**

- Continue to research the isolated actinomycete strains as well as the culture conditions for actinomycete strains to search for new compounds with antimicrobial and antituberculosis activities.

- There are further, more in-depth studies on the mechanism and active sites of compounds such as indole-3-carboxylic acid (**AT.03**), halolitoralin A (**AT.06**), flufuran (**AT.14**), cardoltriene (**AT.08**) and cardoltriene M (**AT.09**) to recombinant protein ClpC1.

- Research on the process of isolating and cultivating large quantities of actinomycete strains (*S. spiroverticillatus* VH19-A067, *S. wuyanensis* VH19-A079, *S. alboniger* VH19-A105B and *S. alboniger* VH19-A121) to search for new anti-tuberculosis compounds for use in medicine and pharmacy.