MINISTRY OF EDUCATION AND TRAINING
VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

HUYNH THI NGOC NI

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

Hanoi - 2024

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

Supervisors:

1. Supervisor1: Assoc. Prof. Dr. Tran Thi Phuong Thao, Institute of Chemistry, Vietnam Academy Science and Technology
2. Supervisor 2: Prof. Dr. Tran Van Sung, Institute of Chemistry, Vietnam Academy Science and Technology

Referee 1: Assoc. Prof. Dr. Vu Quoc Trung
Referee 2: Assoc. Prof. Dr. Vu Dinh Hoang
Referee 3: Assoc. Prof. Dr. Ngo Dinh Binh

The dissertation will be examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at 14-hour 00 minute, on June 06th, 2024

The dissertation can be found at:
1. Graduate University of Science and Technology Library
2. National Library of Vietnam

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 *Mycobacterium 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. These 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, include: obscurolide $B_{2\beta}$ (**AT.01**), chartreusin (**AT.02**), indole-3-carboxylic acid (**AT.03),** nocardamin (**AT.04**), pleurone (**AT.05**), halolitoralin A (**AT.06**), (6*Z*)-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 compounds obscurolide $B_{2\beta}$ (**AT.01**) and chartreusin (**AT.02**) were 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*. Five compounds indole-3-carboxylic acid (**AT.03**), halolitoralin A (**AT.06**), flufuran (**AT.14**), cardoltriene (**AT.08**) and cardoltriene M (**AT.09**) were ClpC1 protein inhibition, through the affection of ATPase hydrolysis

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. 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	*Streptomyces spiroverticillatus* VH19-A067	Soil	The confluence of Day river and Vac river, Thuong Kiem commune, Kim Son district, Ninh Binh province	N20^{0}3'0,8''E106^{0}6'52,8''
2	*Streptomyces wuyuanensis* VH19-A079	Soil	Quynh Luong Mangrove Forest, Quynh Luong commune, Quynh Luu district, Nghe An province	N19^{0}8'42,1''E105^{0}41'43,1''
3	*Streptomyces alboniger* VH19-A105B	Soil	On the way to the top of May Bac mountain, Cuc Phuong National Park, Nho Quan district, Ninh Binh province	N20^{0}21'0,8''E105^{0}36'15,7''
4	*Streptomyces alboniger* 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^{0}21'21,9''E105^{0}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 solation 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 a*ntimycobacterial 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 (Obscurolide $B_{2\beta}$): Yellow powder; $C_{15}H_{17}NO_4$; $[\alpha]_D^{27} = +20{,}4$ (c 0,1, CH_2Cl_2), HR-ESI-MS (m/z): 276.1221 (calculated for $C_{15}H_{18}NO_4$ 276,1236 $[M+H]^+$), 298.1045 (calculated for $C_{15}H_{17}NO_4Na$ 298.1055 $[M+Na]^+$); ^{1}H-NMR (500 MHz, $CDCl_3$), δ_H (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); ^{13}C NMR (125 MHz,

$CDCl_3$), δ_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; $C_{32}H_{32}O_{14}$; HR-ESI-MS (*m/z)*: 641.1876 (calculated for $C_{32}H_{33}O_{14}$ 641.1870 $[M+H]^+$). The ^{1}H-NMR and ^{13}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; $C_9H_7NO_2$; ESI-MS (*m/z*): 178.5 $[M-H+H_2O]^-$; The ^{1}H-NMR and ^{13}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; $C_{27}H_{48}N_6O_9$; HR-ESI-MS (*m/z)*: 601.3527 (calculated for $C_{27}H_{49}N_6O_9$ 601.3561 $[M+H]^+$), 623.3353 (calculated for $C_{27}H_{48}N_6O_9Na$ 623.3380 $[M+Na]^+$). The ^{1}H-NMR and ^{13}C-NMR spectra data: see Table 3.5.

AT.05 (Pleurone): White, powder, $C_4H_2O_4$; ESI-MS (*m/z*): 119 $[M+Na-H_2O]^+$. The ^{1}H-NMR and ^{13}C-NMR spectra data: see Table 3.6.

AT.06 (Halolitoralin A): White soil; $C_{27}H_{48}N_6O_6$; HR-ESI-MS (*m/z*): 553.3730 (calculated for $C_{27}H_{49}N_6O_6$ 553.3714 $[M+H]^+$), 575.3548 (calculated for $C_{27}H_{48}N_6O_6Na$ 575.3533 $[M+Na]^+$). ^{1}H-NMR (δ_H, *J*, 500 MHz, CD_3OD): 3.57-3.54 (2H, m, α-H of Leu^1 & Leu^2), 3.50 (1H, d, *J* = 3.5 Hz, α-H of Ile), 3.43 (3H, d, *J* = 4.5 Hz, α-H of Ala^1, Ala^2 & Ala^3), 1.97-1.95 (1H, m, β-H của Ile), 1.83-1.77 (2H, m, γ-H của Leu^1 & Leu^2), 1.83-1.59 (4H, m, β-H of Leu^1 & Leu^2), 1.66-1.59 (2H, m, γ-H of Ile), 1.08-1.07 (3H, d, *J* = 7.0 Hz, β-H of Ala^3), 1.05-1.01 (9H, m, β-H of Ala^1 & Ala^2, β'-H of Ile), 1.00-0.96 (15H, m, δ-H of Leu^1 & Leu^2, δ-H of Ile); ^{13}C-NMR (δ_C, *J*, 125 MHz, CD_3OD): 175.2 (C=O, Leu^1 & Leu^2), 174.5 (C=O, Ala), 174.0 (C=O, Ile), 61.8 (α-C, Ala^1, Ala^2 & Ala^3), 60.9 (α-C, Ile), 54.7

(α-C, Leu[1] & Leu[2]), 41.8 (β-C, Leu[1] & Leu[2]), 37.7 (β-C, Ile), 25.9 (γ-C, Ile), 25.8 (γ-C, Leu[1] & Leu[2]), 23.2 (δ-C, Leu[2]), 22.0 (δ-C, Leu[1]), 19.1 (β-C, Ala[3]), 17.7 (β-C, Ala[1] & Ala[2]), 15.6 (β'-C, Ile), 12.2 (δ-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; $C_{17}H_{32}O_2$; HR-ESI-MS (*m/z):* 267.2335 (calculated for $C_{17}H_{31}O_2$ 267.2324 $[M-H]^-$). ^{1}H-NMR (δ_H, *J*, 600 MHz, CD_3OD): 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); ^{13}C-NMR (δ_C, *J*, 125 MHz, CD_3OD): 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; $C_{21}H_{30}O_2$; HR-ESI-MS (*m/z)*: 313,2196 (calculated for $C_{21}H_{29}O_2$ 313,2168 $[M-H]^-$). The ^{1}H-NMR and ^{13}C-NMR spectra data: see Table 3.7.

AT.09 (Cardoltriene M): Brown oil; $C_{36}H_{47}NO_5$; HR-ESI-MS (*m/z)*: *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 $[M-H]^-$). The ^{1}H-NMR and ^{13}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; $C_{21}H_{18}O_7$; HR-ESI-MS (*m/z):* 382.1053 (calculated for $C_{21}H_{18}O_7$ 382.1059 [M]). The ^{1}H-NMR and ^{13}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 ^{1}H-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 $[M-H]^-$. ^{1}H-NMR (CD_3OD, 500 MHz), δ_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 $[M-H]^-$. ^{1}H-NMR (CD_3OD, 500 MHz) δ_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) δ_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 $[M+Na]^+$. ^{1}H-NMR (CD_3OD, 500 MHz) δ_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) δ_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%).

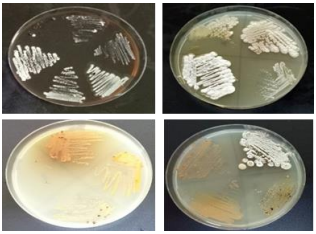

Figure 3.1. Growth of some actinomycetes isolated Vietnam

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 morphological, physiological, biochemical characteristics according to the ISP 1966 lassification, 16S rDNA sequences and was performed 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***

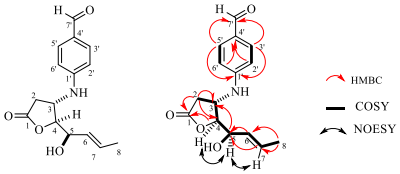

Figure 3.4. The structure, HMBC, COSY and NOESY correlations of compound ***AT.01***

Compound **AT.01** was obtained as a yellow powder. The spectral data of **AT.01** are consistent with the spectral data of obscurolide $B_{2\beta}$. Therefore, compound **AT.01** was identified as obscurolide $B_{2\beta}$. 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*.

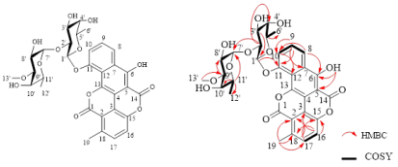

*Figure 3.7. The structure, HMBC, COSY and NOESY correlations of compound **AT.02***

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

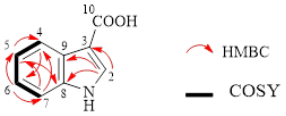

*Figure 3.15. The structure of compound **AT.03***

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.

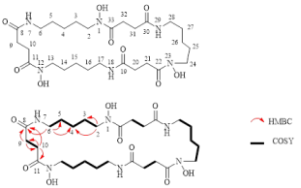

*Figure 3.16. The structure, HMBC and COSY correlations of compound **AT.04***

3.2.3.2. ***AT.05***

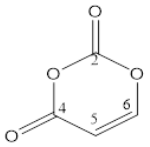

Figure 3.18. The structure of compound ***AT.05***

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 (*4H*-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

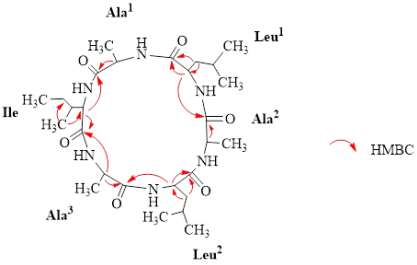

Figure 3.19. The structure and HMBC correlations of compound ***AT.06***

3.2.4. Structural elucidation of the compounds isolated from Streptomyces spiroverticillatus VH19-A067

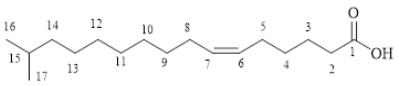

Figure 3.20. The structure of compound ***AT.07***

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***

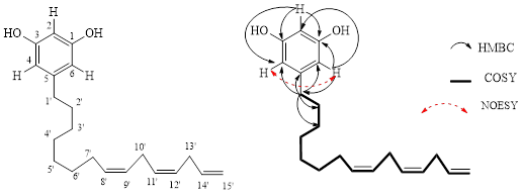

Figure 3.22. The structure, HMBC, COSY and NOESY correlations of compound ***AT.08***

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 $C_{36}H_{47}NO_5$.

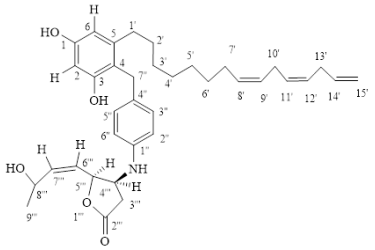

Figure 3.28. The structure of compound ***AT.09***

The ^{1}H-NMR spectrum of compound **1** appeared six aromatic protons at δ_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 δ_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 δ_H 3.86 (2H, s, H-7''), 2.96 (1H, m, H-3'''a), 2.40 (1H, d, *J* = 3.5, H-3'''b), 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 δ_H 4.79 (1H, m, H-5'''), 3.97 (1H, m, H-4''') and one methyl group at δ_H 1.25 (3H, m, H-9''') were observed.

The ^{13}C-NMR spectrum indicated twelve aromatic carbons at δ_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 δ_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 ankyl groups at δ_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 δ_C 35.3 (C-3'''), 2 nmethine groups at δ_C 84.7 (C-5'''), 55.5 (C-4''') and 1 lacton carbonyl group at δ_C 174.9 (C-2'''). Comparison of the ^{1}H and ^{13}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'' (δ_H 6.47)/H-3'' (δ_H 6.98); H-6'' (δ_H 6.47)/H-5'' (δ_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' (δ_H 5.04)/H-14' (δ_H 5.83); H-12' (δ_H 5.42)/H-13' (δ_H 2.82); H-13' (δ_H 2.82)/ H-14' (δ_H 5.83); H-11' (δ_H 5.41)/H-10' (δ_H 2.77); H-9' (δ_H 5.34)/H-10' (δ_H 2.77); H-7' (δ_H 2.02)/H-8' (δ_H 5.36), H-6' (δ_H 1.27); H-1' (δ_H 2.50)/H-2' (δ_H 1.48); H-2' (δ_H 1.48)/H-3' (δ_H 1.27); H-7''' (δ_H 5.88)/H-8''' (δ_H 4.34), H-6''' (δ_H 5.73); H-8''' (δ_H 4,34)/H-9''' (δ_H 1.25); H-3''' (δ_H 2.96)/H-4''' (δ_H 3.97); H-4''' (δ_H 3.97)/H-5''' (δ_H 4.79); H-5''' (δ_H 4.79)/H-6''' (δ_H 5.73).

The allyl group was attached to the aromatic ring at C-5, approved by the HMBC correlation between H-1’ (δ_H 2.50) to C-4 (δ_C 117.1), C-5 (δ_C 144.1), C-6 (δ_C 108.8); H-2 (δ_H 6.22) to C-4 (δ_C 117.1); H-6 (δ_H 6.29) to C-2 (δ_C 101.0), C-4 (δ_C 117.1), C-1’ (δ_C 33.5). The phenyl group was attached to the phenolic ring at C-4, approved by the HMBC correlation between H-7’’ (δ_H 3.86) to C-3 (δ_C 155.3), C-5 (δ_C 144.1); H-5’’ (δ_H 6.98) to C-1’’ (δ_C 143.8), C-3’’ (129.1), C-7’’ (29.6); H-3’’ (δ_H 6.98) với C-1’’ (δ_C 143.8), C-5’’ (129.1); H-2’’ (δ_H 6.47) với C-6’’ (δ_C 114.1). The NOESY correlation between H-2’’ (δ_H 6.47)/H-4’’’ (δ_H 3.97), H-5’’’ (δ_H 4.79); H-4’’’ (δ_H 3.97)/H-5’’’ (δ_H 4.79); H-5’’’ (δ_H 4.79)/H-6’’’ (δ_H 5.73), H-7’’’ (δ_H 5.88); H-6’’’ (δ_H 5.73)/H-7’’’ (δ_H 5.88); H-8’ (δ_H 5.36)’/ H-9’ (δ_H 5.34)’; H-11’ (δ_H 5.41)’/ H-12’ (δ_H 5.42)’ allowed to determine the relative configuration of **AT.09** as Figure 3.28**.**

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

C	**AT.08**		**AT.09**		**Cardoltriene**	
	δ_H (ppm)[a]	δ_C (ppm)[b]	δ_H (ppm)[c]	δ_C (ppm)[d]	δ_H (ppm)[e]	δ_C (ppm)[f]
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, *J* = 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		

[a]500 MHz, CD_3OD; [b]125 MHz, CD_3OD; [c]500 MHz, $CDCl_3$; [d]125 MHz, $CDCl_3$, [e]300 MHz, $CDCl_3$; [f]100 MHz $CDCl_3$

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** (chartreusin).

3.2.5.4. **AT.11**

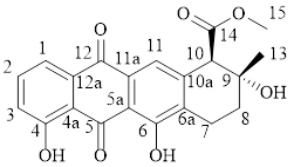

*Figure 3.40. The structure of compound **AT.11***

Compound **AT.11** 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-deoxy-nogalamycinone).

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

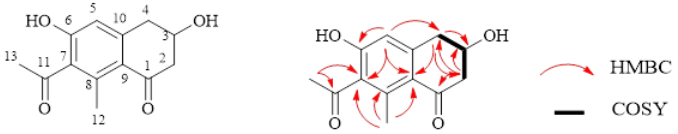

*Figure 3.42. The structure, HMBC and COSY correlations of compound **AT.12***

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

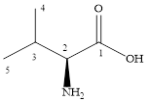

*Figure 3.43. The structure of compound **AT.13***

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**

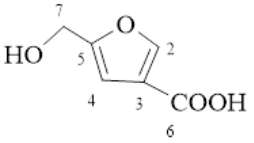

*Figure 3.44. The structure of compound **AT.14***

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. **AT.15**

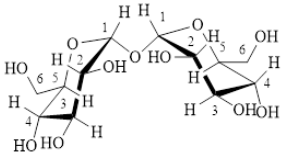

*Figure 3.47. The structure of compound **AT.15***

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* (strain similar to *M. tuberculosis*). 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 wasrecorded at 10 µM, following the gradual increase of the ATP concentration in the reaction. The stability of ATPase activity in ClpC1 proteinwas 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 µM, 1.0 µM and 10.0 µM) 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 10µM 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.

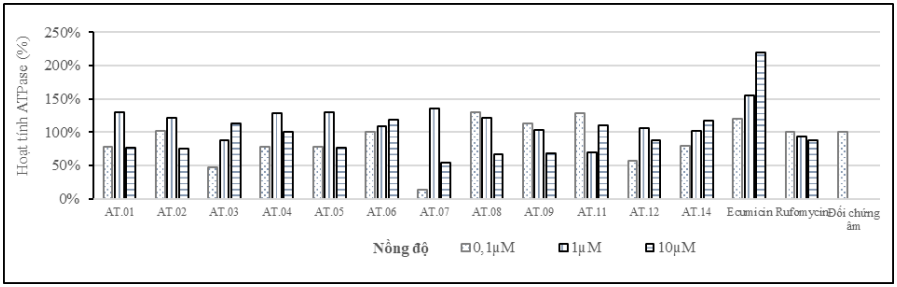

Figure 3.49. Affection of compounds to ATPase activity

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 *Mycobacterium smegmatis* which is a strain similarity to *Mycobacterium tuberculosis*. The results showed that the five potential active strains were *Streptomyces avidinii* VH19-A002 (Mangrove forest in Phu Long commune), *Streptomyces spiroverticillatus* VH19-A067 (The confluence of Day river and Vac river), *Streptomyces wuyanensis* VH19-A079 (Quynh Luong mangrove forest), *Streptomyces alboniger* VH19-A105B (on the way to the top of May Bac mountain) and *Streptomyces alboniger* VH19-A121 (the top of May Bac mountain).

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

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

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

+ 3 compounds were isolated from *S. aureus* VTCC43181 including two cyclopeptide compounds nocardamine (**AT.04**) and halolitoralin A (**AT.06**), one lactone compound pleurone (**AT.05**).

+ 1 carboxylic acid compound isolated from *S. spiroverticillatus* VH19-A067 was (6Z)-15-methyl-6-hexadecenoic acid (**AT.07**).

+ 3 compounds were isolated from *S. alboniger* VH19-A105B including two phenolic compounds cardoltriene (**AT.08**) and cardoltriene M (**AT.09**, new), one anthraquinone compound 7-deoxyauramycinone

(**AT.11**). There is a new compound named cardoltriene M (**AT.09**).

+ 1 phenolic compound isolated from *S. cyaneus* VTCC43860 was 7-acetyl-3,6-dihydroxy-8-methyl tetralone (**AT.12**).

+ 1 amino acid compound isolated from *Streptomyces* sp. VTCC43168 is valine (**AT.13**).

+ 2 compounds isolated from *A. missouriensis* VTCC40900 were one furan compound flufuran (**AT.14**) and one disacaride compound trehalose (**AT.15**).

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. Among them, three compounds indole-3-carboxylic acid (**AT.03**), halolitoralin A (**AT.06**) and flufuran (**AT.14**) were effective for the ATP hydrolysis of ClpC1 protein, similarly to ecumicin. Cardoltriene (**AT.08**) and cardoltriene M (**AT.09**) were effective for the ATP hydrolysis of ClpC1 protein, similarly to rufomycin.

- **Recommendations**

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.

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. (Scopus)

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)