Chapter 5

Conclusions

Tuberculosis is a contagious disease with high mortality worldwide. The statistics indicated that 3 million people throughout the world die annually from tuberculosis and there are an estimated 8 million new cases each year, 95% of which occur in developing countries. In addition, about a third of the world's population harbors a dormant Mycobacterium tuberculosis infection, representing a significant reservoir of disease for the future. (Jaso, Zarranz, Aldana, & Monge, 2003, pp. 791-800)

Current frontline therapy consists of administering one of three drugs (isoniazid, rifampin or pyrazinamide) for 2 months followed by 4 months of follow-up therapy with isoniazid and rifampin. Thus, the problem arising due to MDR-TB requires the development of new therapeutic agents that have a unique mechanism of action, different from the currently used antitubercular drugs, in order to treat drug-resistant forms of the disease.

The 1'*S*-1'-acetoxylchavicol acetate (*S*-ACA) isolated from the rhizomes and the seeds of the *Zingiberaceae* plant showed very interesting biological properties such as antitumor, anti-inflammatory, antifungal, antioxidation, anti-HIV and xanthine oxidase inhibitory activity. Furthermore, it has been reported gastroprotective, antiallergic constituents and an inhibitory effect of nitric oxide in mouse peritoneal macrophages. The biological effects of *S*-ACA have been widely investigated. The activity against *M. tuberculosis* H37Ra and H37Rv has been reported with MIC values of 0.1-0.5 μg/ml and 0.6-1.6 μg/ml, respectively. However, its side effect has also been reported (2 μg/ml) (Saradee, 2009).

We report herein the synthesis of 1'-acetoxylchavicol acetate analogs and evaluation of their antimycobacterial activities against M. *tuberculosis* H37Ra. We have chosen to start from several aromatic aldehydes includind *p*-bromobenzadehyde, *p*-chlorobenzadehyde, *m*-chlorobenzadehyde, *o*-chlorobenzadehyde, cinnamaldehyde, thiophene-2-carbaldehyde and *p*-hydroxybenzadehyde reacted with Grignard reagents such as allylmagnesium bromide, octylmagnesium bromide, dodecylmagnesium

bromide, benzylmagnesium bromide and cyclopentylmagnesium bromide through Grignard reaction. *o*-Chlorobenzadehyde **104** non-reacted with octylmagnesium bromide, dodecylmagnesium bromide and cyclopentyl magnesium bromide. *m*-Chlorobenzadehyde **105** non-react with cyclopentylmagnesium bromide. These results caused by size and steric hinder of the Grignard reagents and the effect of position of chloride atom on benzene ring. Aromatic aldehydes with chloride atom at *ortho*- or *meta*- position of benzene ring were sterically hinder to reacted with bulky Grignard reagent. We found that seven compounds including 1-(4-chlorophenyl) tridecan-1-ol **114**, 1-(4-bromophenyl)nonyl acetate **123**, 1-(4-bromophenyl)tridecyl acetate **124**, 1-(4-bromophenyl)-2-phenylethyl acetate **125**, 1-(4-chlorophenyl)tridecyl acetate **126**, (4-chlorophenyl)(cyclopentyl) methyl acetate **127** and 1-(3-chlorophenyl) but-3-enyl acetate **129** had no previous reports of the successful preparation.

We studied quantitative analysis by reporting %yield. Compounds **110-122** were successfully synthesized through Grignard reaction with 9-60 %yields. Acetylation reaction of compounds **110-122** afforded compounds **123-132** in 13-90 %yields. The results showed that the inductive effect of the substituents on benzene ring was an importance. Low electronegativity of the substituent on benzene ring gave better %yield than high electronegativity (strength of electronegativity: chloride atom > bromide atom > siloxyl group). Compound **120-TBDMS** with *p*-OTBDMS on benzene ring achieved higher %yield than compound **109** with *p*-Br on benzene ring and compound **113** with *p*-Cl on benzene ring, respectively. Compound **122** with non-substituted benzene ring decreased %yield. However, compound **121** with thiophene ring gave good %yield due to the aromaticity of thiophene. The electron pairs on sulfur are significantly delocalized in the pi electron system which increased the ability strength of the molecular posed as a good electrophile. We found that compound with aliphatic side chain substitution at R´ position gave high %yield more than bulky substitution (cyclopentyl group and benzyl group) at R´ position. Acetylation reaction could be achieved better yields by increasing the nucleophilic strength of the alkoxides of ACA analogs without the effect of steric hindered group.

The synthesized compounds **110-130** were evaluated for *in vitro* anti-mycobacterial activity against M. *tuberculosis* H37Rv. The results were appeared in **Table 3**. Some of these compounds **110**, **117**, **121** and **123** showed inhibitory

properties with MIC of 167.08, 202.65, 286.96 and 36.63 µM, respectively. The other compounds in this series failed to inhibit the growth of M. *tuberculosis* H37Ra. Inactive compounds were defined when they exhibited less than 90 %inhibition. However, we used %inhibition to define a tendency of inactive compounds to inhibit M. *tuberculosis* H37Ra. Our work found the importance for enhanced the anti-TB activity of ACA analogs.

Firstly, we tempted to compare the effect of chlorine atom at different positions of benzene ring (R position). The results showed that the position of chloride atom on benzene ring was an important for enhancing anti-TB activity. Compound with halogen atom at *para*- positon of benzene ring achieved biological activity similar to the previous report (Singkhonrat, Bunthitsakda, Kedpokasiri, & Nuampipat, 2010). In order to emphasize the results, compounds **116** (*m*-Cl) and **118** (*o*-Cl) found to be failed activity.

Secondly, we studied the effect of alkyl substitution at position 1´ of chavicol analogs (R´ position). Compounds with bulky or more than 8 carbons aliphatic side chain at C1´ of chavicol were failed biological activity. We found that aliphatic side chain at C1´ of chavicol should less than 8 carbons. These may be the results of ability to access the target cell due to inappropriate physicochemical properties

Thirdly, we considered the effect of protecting group for hydroxyl group by acetylation. It indicated that the presence of acetyl group as a protecting group for hydroxyl group at C1´ of compound **123** (with 8 carbons aliphatic side chain) was an importance for improve biological activity (with nearly 5-fold times of **110**). However, the presence of protecting group for hydroxyl group at C1´ of chavicol analogs was not major effect for anti-TB activity.

Finally, we studied the effect of different aromatic system. It indicated that phenyl group was an importance for at-TB activity. Phenyl group was attached to carbon atom with high electronegative group (chloride or bromide) at *para*- position could offer appropriate physical properties to access the target cell more than thiophene ring and conjugated benzene ring.

Our data indicated that the ACA analogs offered a promising new lead for further development. For further study on ACA analogs for antituberculosis activity,

the study of the chirality effect at position 1´ of chavicol analogs is under investigation. We expect to achieve higher activity than their racemic compounds.