Chapter 4

Results and Discussion

4.1 NMR Spectrometry characterization of ACA analogs

NMR spectroscopy was used to determine the structure of ACA analogs. All of the ^{1}H, ^{13}C NMR spectra were recorded on a Bruker-Ultra Shield (400 and 100 MHz for ^{1}H and ^{13}C, respectively), using $CDCl_3$ as a solvent with trace of $CHCl_3$ as an internal standard.

4.1.1 NMR Characterization of 1-(4-bromophenyl)but-3-en-1-ol 109

The ^{1}H NMR spectroscopic data appeared the characteristic resonances for aromatic ring in 2,6-positions and 3,5-positionas at 7.24 (J = 7.6 Hz) and 7.43 (J = 7.6 Hz) ppm as a pair of doublet. The differences of chemical shift of aromatic protons were affected by inductive effect of bromide atom *para*- positon on aromatic ring. The resonances for a proton α to the alcohol showed at 4.71 ppm as triplet (J = 7.2 Hz) coupling to CH_2-2' at 2.48 ppm as double triplet (J = 14, 8 Hz) and appeared OH at 2.02 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 121.14 - 142.82 ppm. The α-carbon and β-carbon to OH group appeared at 72.63 and 43.56 ppm, respectively.

4.1.2 NMR Characterization of 1-(4-bromophenyl)nonan-1-ol 110

The ^{1}H NMR spectroscopic data showed the characteristic resonances for aromatic proton (CHs) 2,6-positions and 3,5-position as a pair of doublet at 7.13 (J = 8 Hz) and 7.40 ppm (J = 8.4), respectively. The differences of chemical shift of aromatic protons were affected by inductive effect of bromide atom *para*- positon on aromatic ring. The resonances for a proton α to the alcohol showed at 4.54 ppm as triplet with a coupling constant of 13.2 Hz coupling to CH_2-2' at 1.61 ppm as multiplet and appeared OH at 1.88 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 121.14 - 143.93 ppm**.** The carbon to OH group appeared at 73.99 ppm. The carbon of side chains appeared in the region of 14.09 - 38.87 ppm**.**

4.1.3 NMR Characterization of 1-(4-bromophenyl)tridecan-1-ol 111

The ^{1}H NMR spectroscopic data appeared the characteristic resonances for aromatic ring in 2,6-positions and 3,5-positionas at 7.15 (J = 8.4 Hz) and 7.39 (J = 8.4 Hz) ppm as a pair of doublet. The differences of chemical shift of aromatic protons were affected by inductive effect of bromide atom *para*- positon on aromatic ring. The resonances for a proton α to the alcohol showed at 4.56 ppm (J = 13.2) as triplet coupling to CH_2-2' at 1.61 ppm as multiplet and appeared OH at 0.81 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 121.25 - 145.64 ppm**.** The carbon to OH group appeared at 76.73 ppm. The carbon side chains of all methyl groups appeared in the region of 19.79 - 42.93 ppm**.**

4.1.4 NMR Characterization of 1-(4-bromophenyl)-2-phenylethanol 112

The ^{1}H NMR spectroscopic data appeared the characteristic resonances for two aromatic rings in 4'-8' and 2,6-positions and 3,5-positionas at 7.19 and 7.39 ppm as multiplet and doublet (J = 8.4 Hz) respectively. The differences of chemical shift of aromatic protons were affected by inductive effect of bromide atom *para*- positon on aromatic ring. The resonances for a proton α to the alcohol showed at 4.79 ppm as triplet (J = 8 Hz) coupling to CH_2-2' at 2.89 ppm as multiplet and appeared OH at 1.18 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of two aromatic rings in the region of 117.39 - 132.75 ppm**.** The α-carbon and β-carbon to OH group appeared at 74.70 and 44.06 ppm respectively

4.1.5 NMR Characterization of 1-(4-chlorophenyl)but-3-en-1-ol 113

The ^{1}H NMR spectroscopic data showed the characteristic resonances for aromatic proton (CHs) 2,6-positions and 3,5-positions as a doublet at 7.24 (J = 7.6 Hz) and 7.43 (J = 7.6 Hz) ppm respectively as a pair of doublet. The differences of chemical shift of aromatic protons were affected by inductive effect of chloride atom *para*- positon on aromatic ring**.** The resonances for a proton α to the alcohol showed at 4.71 ppm as triplet (J = 7.2 Hz) coupling to CH_2-2' at 2.49 ppm as double triplet (J = 14, 8 Hz) and appeared OH at 2.02 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 124.57 - 131.95 ppm. The α-carbon and β-carbon to OH group appeared at 72.62 and 43.56 ppm respectively.

4.1.6 NMR Characterization of 1-(4-chlorophenyl)tridecan-1-ol 114

The ^{1}H NMR spectroscopic data appeared the characteristic resonances for aromatic ring in 2,6-positions and 3,5-positionas at 7.30 (J = 8 Hz) and 7.32 (J = 8.4 Hz) ppm as a pair of doublet. The differences of chemical shift of aromatic protons were affected by inductive effect of chloride atom *para*- positon on aromatic ring. The resonances for a proton α to the alcohol showed at 4.67 ppm (J =13.2) as triplet coupling to CH_2-2' at 2.89 ppm as multiplet and appeared OH at 0.90 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 127.07 - 128.79 ppm**.** The carbon to OH group appeared at 74.03 ppm. The carbon side chains of all methyl groups appeared in the region of 14.11 - 39.99 ppm**.**

4.1.7 NMR Characterization of (4-chlorophenyl)(cyclopentyl)methanol 115

The ^{1}H NMR spectroscopic data showed the characteristic resonances for aromatic proton (CHs) 2,6-positions and 3,5-positions at 7.20 (J = 8.4 Hz) and 7.23 (J = 8.4 Hz) ppm respectively as a pair of doublet. The differences of chemical shift of aromatic protons were affected by inductive effect of chloride atom at *para*-positon on aromatic ring. The resonances for a proton α to the alcohol showed at 4.40 ppm as doublet with a coupling constant of 8.0 Hz coupling to CH-2' at 2.17 ppm as sextet (J = 8.4) and appeared OH at 1.35 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 121.14 - 143.93 ppm**.** The carbon to OH group appeared at 73.99 ppm. The carbon side chains of all methyl groups appeared in the region of 25.56 - 37.75 ppm**.**

4.1.8 NMR Characterization of 1-(2-chlorophenyl)but-3-en-1-ol 116

The ^{1}H NMR spectroscopic data appeared the characteristic resonances for aromatic ring in 5-position, 4,6-positions and 3-position at 7.21 (J = 13.6 Hz) as triplet, 7.28 as multiplet and 7.57 ppm (J = 7.6 Hz) as doublet. The differences of chemical shift of aromatic protons were affected by inductive effect of bromide atom at *ortho*- positon on aromatic ring. Character of H-2' showed individual Ha and Hb with coupling constant to each other at 8-8.8 Hz. The resonances for a proton α to the alcohol showed at 4.12 ppm as double doublet (J = 14, 7.2 Hz) coupling to CH-2'a

and CH-2'b (this affected by rigid rotation of C1-C2 bond which influenced by *m*-chlorophenyl group) and appeared OH at 1.92 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 118.65 - 141.20 ppm**.** The α-carbon to OH group appeared at 69.64 ppm.

4.1.9 NMR Characterization of 1-(2-chlorophenyl)-2-phenylethanol 117

The ^{1}H NMR spectroscopic data appeared the characteristic resonances for aromatic ring in 5-position, 4,6-positions and 3-position as at 7.06 (J = 12.1 Hz), 7.13 (J = 17.1, 7.8 Hz) and 7.15 ppm (J = 7.2 Hz), as triplet, double triplet and doublet respectively. The differences of chemical shift of aromatic protons were affected by inductive effect of chloride atom at *ortho*- positon on aromatic ring. The resonances for a proton α to the alcohol showed at 5.33 ppm as double doublet (J = 6, 9.2 Hz) and appeared OH at 1.89 ppm as broad singlet. Character of H-2' showed individual Ha and Hb with coupling constant to each other at 2.38 and 2.64 Hz as a pair of double triplet (this affected by rigid rotation of C1-C2 bond which influenced by *o*-chloro phenyl group). The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 126.58 -142.40 ppm**.** The carbon to OH group appeared at 72.85 ppm.

4.1.10 NMR Characterization of 1-(3-chlorophenyl)but-3-en-1-ol 118

The ^{1}H NMR spectroscopic data appeared the characteristic resonances for aromatic ring in 2-position, 6-position and 4,5-positions as at 7.13 (J = 10.2 Hz), 7.17 (J = 12.6, 6.4 Hz) and 7.20 ppm, as doublet, double doublet and multiplet respectively. The differences of chemical shift of aromatic protons were affected by inductive effect of chloride atom at *meta*- positon on aromatic ring. The resonances for a proton α to the alcohol showed at 4.65 ppm as triplet (J = 8 Hz) and appeared OH at 1.77 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 123.98 -133.91 ppm**.** The α-carbon to OH group appeared at 69.64 ppm.

4.1.11 NMR Characterization of 1-(3-chlorophenyl)-2-phenylethanol 119

The ^{1}H NMR spectroscopic data appeared the characteristic resonances for aromatic ring in 6-position, 5-positions and 2,4-positions as at 7.07 (J = 8 Hz), 7.15 (J = 11.2 Hz) and 7.19 ppm (J = 17.2, 8 Hz), as doublet, triplet and doublet triplet respectively. Another aromatic ring appeared the characteristic resonances in Ar'H-4-position at 7.11 ppm (J = 11 Hz), Ar'H-2,6-positions at 7.13 ppm (J = 7.2 Hz) and Ar'H-3,5-positions 7.23 (J = 7.2 Hz) as triplet, doublet and doublet, respectively. The differences of chemical shift of aromatic protons were affected by inductive effect of atom at *meta*- positon on aromatic ring. The resonances for a proton α to the alcohol showed at 4.81 ppm as triplet with a coupling constant of 8 Hz and appeared OH at 2.04 ppm as broad singlet.

4.1.12 NMR Characterization of 4-(1-hydroxybut-3-enyl)phenol 120

The ^{1}H NMR spectroscopic data appeared the characteristic resonances for aromatic ring in 2,6-positions and 3,5-positionas at 6.82 (J = 8 Hz) and 7.15 (J = 7.2 Hz) ppm as a pair of doublet. The differences of chemical shift of aromatic protons were affected by inductive effect of hydroxyl group at *para*- positon on aromatic ring. The resonances for a proton α to the alcohol showed at 4.12 ppm as triplet (J = 7.4 Hz) and appeared OH at 2.06 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 115.64 - 155.62 ppm**.** The α-carbon to OH group appeared at 83.39 ppm.

4.1.13 NMR Characterization of (*E*)-1-phenylhexa-1,5-dien-3-ol 121

The ^{1}H NMR spectroscopic data appeared the characteristic resonances for aromatic ring in 4-position, 3,5-positions and 2,6-positionas at 7.17 (J = 14.4 Hz), 7.24 (J = 14 Hz) and 7.30 (J = 7.2 Hz) ppm as triplet, triplet and doublet. The resonances for a proton α to the alcohol showed at 5.04 ppm as double triplet (J = 16.4, 10 Hz) which coupled to H-4' at 2.44 ppm as double doublet (J =10.4, 7.2 Hz) and H-2' at 6.08 ppm as double doublet (J = 16, 8.8), respectively and appeared OH at 2.02 ppm as broad singlet. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 125.41 - 133.27 ppm. The α-carbon and β-carbon to OH group appeared at 130.58 and 124.79 ppm respectively.

4.1.14 NMR Characterization of 1-(thiophen-2-yl)but-3-en-1-ol 122

The ^{1}H NMR spectroscopic data of the thiophene showed the characteristic resonance for the 4,5-position and 3-position at 6.89 (J = 8 Hz) and 7.18 ppm (J = 6 Hz) respectively as a pair of doublet. The resonances for a proton α to the alcohol at 4.92 ppm as triplet (J = 12.8 Hz) and appeared OH at 2.1 ppm as broad singlet. This also confirmed by ^{13}C NMR showed the carbon of thiophene ring in the region of 124.48-138.33 ppm. The α-carbon to OH group appeared at 69.42 ppm coupling to CH_2-2' at 2.54 ppm as multiplet.

4.1.15 NMR Characterization of 1-(4-bromophenyl)nonyl acetate 123

The ^{1}H NMR spectroscopic data showed the characteristic resonances for methyl group at 2.19 ppm (J = 7 Hz) as triplet which influenced by one of CH_2 on carbon long chain. A proton at 1'-position shifted to lower field region at 5.73 ppm as triplet (J = 11.4 Hz) when compared with 4.54 ppm of 1-(4-bromophenyl)nonan-1-ol **110**. This is because of the effect of electron withdrawing group (OAc) at 1'-position.

4.1.16 NMR Characterization of 1-(4-bromophenyl)tridecyl acetate 124

The ^{1}H NMR spectroscopic data showed the characteristic resonances for protons of methyl group at 0.88 ppm (J = 12.8 Hz) as triplet which influenced by one of CH_2 on carbon long chain. A proton at 1'-position shifted to lower field region at 5.70 ppm as triplet (J = 14 Hz) when compared with 4.56 ppm of 1-(4-bromophenyl) tridecan-1-ol **111**. This is because of the effect of electron withdrawing group (OAc) at 1'-position. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 121.91 - 139.24 ppm. The α-carbon and β-carbon to OAc group appeared at 76.72 and 50.26 ppm respectively. The carbon of alkoxy group showed OCH_3 and COO at 23.35 and 139.95 ppm, respectively.

4.1.17 NMR Characterization of 1-(4-bromophenyl)-2-phenylethyl acetate 125

The ^{1}H NMR spectroscopic data showed the characteristic resonances for two aromatic rings in the region of 7.06-7.48 ppm and observed methyl group at 2.12 ppm as singlet. A proton at 1'-position appeared at 5.89 ppm as triplet that shifted to lower field region when compared with a proton at 1'-position of 1-(4-bromophenyl)-2-phenylethanol **112** at 4.79 ppm. This is because of the effect of electron withdrawing group (OAc).

4.1.18 NMR Characterization of 1-(4-chlorophenyl)tridecyl acetate 126

The ^{1}H NMR spectroscopic data showed the characteristic resonances for methyl group at 2.26 ppm as triplet (J = 7 Hz). A proton at 1'-position shifted to lower field region at 4.56 ppm as triplet (J = 12.8 Hz) when compared with 1-(4-chloro phenyl)tridecan-1-ol **114.** This is because of the effect of electron withdrawing group (OAc) at 1'-position. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 127.95 – 143.93 ppm. The α-carbon and β-carbon to OAc group appeared at 75.45 and 36.24 ppm respectively. The carbon of alkoxy group showed OCH_3 and COO at 18.20 and 165.57 ppm, respectively.

4.1.19 NMR Characterization of (4-chlorophenyl)(cyclopentyl)methyl acetate 127

The ^{1}H NMR spectroscopic data showed the characteristic resonances for protons of methyl group at 2.14 ppm as a singlet. A proton at 1'-position shifted to lower field region at 5.44 ppm as triplet (J = 8 Hz) when compared with 4.40 ppm of (4-chlorophenyl)(cyclopentyl)methanol **115**. This is because of the effect of electron withdrawing group (OAc) at 1'-position. The ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 128.59 - 139.23 ppm. The α-carbon and β-carbon to OAc group appeared at 80.61 and 45.31 ppm respectively. The carbon of alkoxy group showed OCH_3 and COO at 25.20 and 166.34 ppm, respectively**.**

4.1.20 NMR Characterization of 1-(2-chlorophenyl)but-3-enyl acetate 128

^{1}H NMR spectroscopic data was observed by the appearance of protons of methyl group at 2.19 ppm (J = 10 Hz) as triplet. A proton at 1'-position shifted to lower field region at 6.22 ppm as triplet (J = 12 Hz) when compared with 4.12 ppm of 1-(2-chlorophenyl)but-3-en-1-ol **116** because the effect of electron withdrawing group (OAc) at 1'-position. ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 118.32-132.72 ppm. The α-carbon and β-carbon to OAc group appeared at 72.49 and 39.51 ppm respectively. The carbon of alkoxy group showed OCH_3 and COO at 20.07 and 168.00 ppm, respectively.

4.1.21 NMR Characterization of 1-(3-chlorophenyl)but-3-enyl acetate 129

^{1}H NMR spectroscopic data was observed by the appearance of protons of methyl group at 1.77 ppm as a singlet. A proton at 1'-position shifted to lower field region at 5.56 ppm as triplet (J = 7.6 Hz) when compared with 4.65 ppm of 1-(3-chlorophenyl)but-3-en-1-ol **118** because the effect of electron withdrawing group (OAc) at 1'-position. ^{13}C NMR spectra showed the carbon of aromatic ring in the region of 118.79-141.80 ppm. The α-carbon and β-carbon to OAc group appeared at 75.60 and 40.53 ppm respectively. The carbon of alkoxy group showed OCH_3 and COO at 21.24 and 166.19 ppm, respectively.

4.1.22 NMR Characterization of 1-(4-acetoxyphenyl)but-3-enyl acetate 130

^{1}H NMR spectroscopic data was observed by the appearance of protons of 2 methyl groups at 2.38 and 3.22 ppm each as singlets. A proton at 1'-position shifted to lower field region at 4.17 ppm (J = 7.3 Hz) as triplet because the effect of electron withdrawing group (OAc) compared to 4.12 ppm of 4-(1-hydroxybut-3-enyl)phenol **120**. ^{13}C NMR spectra showed the carbon of aromatic ring at 121.45 – 150.06 ppm. The α-carbon and β-carbon to OAc group appeared at 83.11 and 56.72 ppm respectively. The carbon of both alkoxy groups showed OCH_3 and COO at 21.12 (OCH_3-1'), 42.50 (OCH_3-4) and 169.40 (2 × COO) ppm, respectively.

4.1.23 NMR Characterization of (*E*)-1-phenylhexa-1,5-dien-3-yl acetate 131

^{1}H NMR spectroscopic data was observed by the appearance of protons of methyl group at 2.10 ppm as singlet. Compared with (*E*)-1-phenylhexa-1,5-dien-3-ol **121**, a proton at 3'-position shifted from 5.04 ppm to lower field region at 5.65 ppm as multiplet because the effect of electron withdrawing group (OAc). ^{13}C NMR spectra showed the carbon of aromatic ring at 127.15 – 133.14 ppm**.** The α-carbon to OAc group appeared at 73.78 ppm. The carbon of alkoxy group showed OCH_3 and COO at 21.24 and 165.20 ppm, respectively.

4.1.24 NMR Characterization of 1-(thiophen-2-yl)but-3-enyl acetate 132

^{1}H NMR spectroscopic data was observed by the appearance of protons of methyl group at 2.09 ppm as singlet. A proton at 1'-position shifted to lower field region at 6.98 ppm when compared to 4.92 ppm of 1-(thiophen-2-yl)but-3-en-1-ol **122**. This is because the effect of electron withdrawing group (OAc). The thiophene showed the characteristic resonance for the 5-position, 4-position and 3-position at 6.97, 7.06 and 7.29 ppm respectively as doublet. ^{13}C NMR spectra showed the carbon of thiophene ring in the region of 125.65-142.92 ppm. The carbon of alkoxy group appeared OCH_3 and COO at 18.29 and 170.11 ppm, respectively.

4.2 Mass spectrometry characterization of ACA analogs

Mass spectroscopy (MS) was used to determine the molecular weight and possibly identifying components of ACA analogs. Mass spectra of some ACA analogs were recorded with a Thermo Finnigan LCQ Advantage ion trap mass spectrometer.

4.2.1 MS characterization of 1-(4-bromophenyl)but-3-en-1-ol 109

1-(4-Bromophenyl)but-3-en-1-ol **109** which has a molecular weight of 228. MS appeared molecular ion peak (M+1) at *m/z* = 229.3. Fragmentation via loss of 17 (-OH) gives a common fragment seen for alkyl benzenes at m/z = 212. Loss of 27 (-$CH{=}CH_2$) gives a common fragment seen for alkyl benzenes at m/z = 201. Loss of 56 (-$CH_2{=}CH{-}C_2H_5$) from the molecular ion gives 157 corresponding to the phenyl cation.

4.2.2 MS characterization of 1-(4-bromophenyl)nonan-1-ol 110

1-(4-Bromophenyl)nonan-1-ol **110** which has a molecular weight of 298. MS appeared molecular ion peak (M+1) at *m/z* = 299. Fragmentation via loss of 86 ($-C_6H_{14}$) gives a common fragment seen for alkyl benzenes at *m/z* = 213.

4.2.3 MS characterization of 1-(4-bromophenyl)-2-phenyl ethanol 112

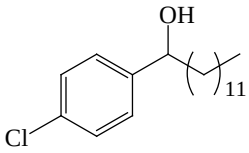
1-(4-Bromophenyl)-2-phenyl ethanol **112** which has a molecular weight of 276. MS appeared molecular ion peak (M+1) at *m/z* = 277. Fragmentation via loss of 121 ($-C_7H_7$-CH(OH)) gives a common fragment at *m/z* = 156**.**

4.2.4 MS characterization of 1-(2-chlorophenyl)but-3-en-1-ol

1-(2-Chlorophenyl)but-3-en-1-ol which has a molecular weight of 182. MS appeared molecular ion peak (M+1) at *m/z* = 185. Fragmentation via loss of 41 ($-CH_2$-CH=CH_2) from the molecular ion gives *m/z* = 144.

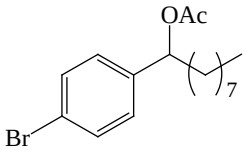
4.3 Chemistry

We synthesized ACA analogs through Grignard reaction and acetylation reaction as showed in **Scheme 11**. ACA analogs **110 – 132 (Table 3)** were successfully prepared from aromatic aldehydes, including *p*-bromobenzadehyde **102**, *p*-chlorobenzadehyde **103**, *o*-chlorobenzadehyde **104**, *m*-chlorobenzadehyde **105**, *p*-hydroxybenzadehyde **106**, cinnamaldehyde **107** and thiophene-2-carbaldehyde **108** by Grignard reaction in 9-59 yield%**.** We used allylmagnesium bromide, allylmagnesium chloride, octylmagnesium bromide, dodecylmagnesium bromide, benzylmagnesium bromide and cyclopentylmagnesium bromide as a Grignard reagent. The alcohol analogs were then converted into ester analogs by acetylation reaction in 13-54 %yield. Importantly, this report found novel compounds **(Figure 34)** which no previous reports of the successful preparation.



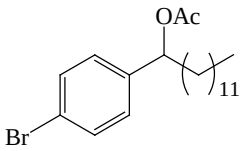
1-(4-chlorophenyl)tridecan-1-ol

114



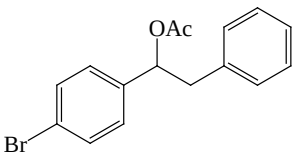
1-(4-bromophenyl)nonyl acetate

123



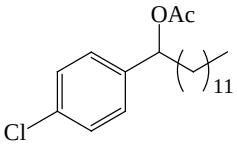
1-(4-bromophenyl)tridecyl acetate

124



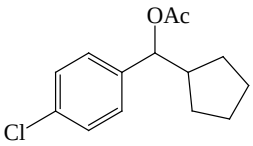
1-(4-bromophenyl)-2-phenylethyl acetate

125



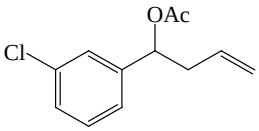
1-(4-chlorophenyl)tridecyl acetate

126



(4-chlorophenyl)(cyclopentyl) methyl acetate

127

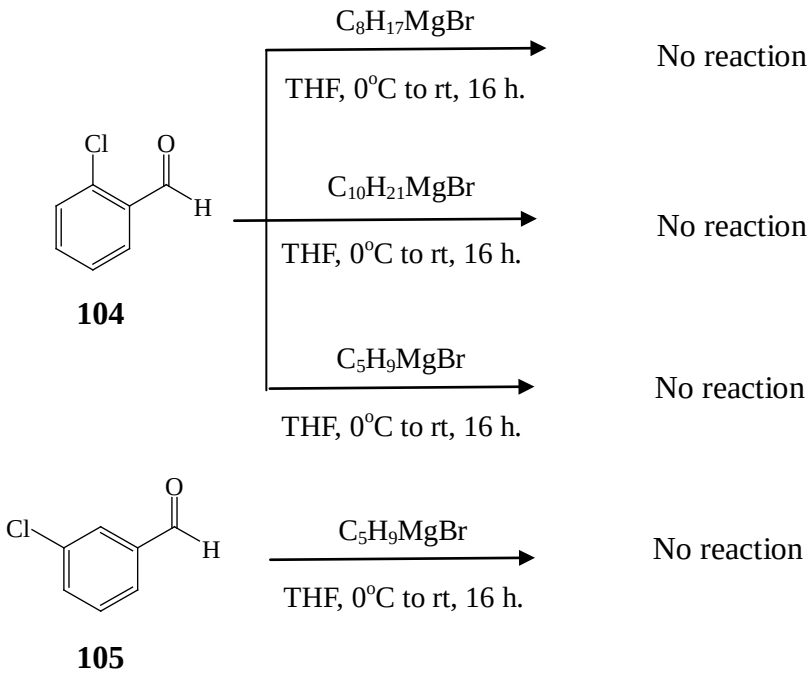


1-(3-chlorophenyl)but-3-enyl acetate

129

Figure 34. Novel compounds

We could not synthesize the reactions of *o*-chlorobenzadehyde **104** with octylmagnesium bromide ($C_8H_{17}MgBr$), dodecylmagnesium bromide ($C_{10}H_{21}MgBr$) and cyclopentyl magnesium bromide (C_5H_9MgBr). *m*-Chlorobenzadehyde **105** could not be reacted with cyclopentylmagnesium bromide (C_5H_9MgBr) **(Scheme 36)**. 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.



Scheme 36. Pathway of non-reacted compounds

We studied quantitative analysis by reporting %yields as showed in **Table 3**. Firstly, we studied the effect of substituents on benzene ring. Compound **120-TBDMS** with siloxyl group at *para*- position of benzene ring was obtained high %yield (86 %yield starting from **139**). Compounds with bromide atom **110-112** at *para*- position of benzene ring were afforded in 34-51 %yield. This also caused by size and steric hinder of the Grignard reagents (to be discussed later in this chapter). Compounds with chloride atom at *para*- **114**, *ortho*- **116** or *meta*- **118** and **119** position of benzene ring achieved in 23-30 %yield. With an exception of compounds **115** (*p*-Cl) and **117 (***o*-Cl) were obtained in low %yield (13 and 9 %yield, respectively). These results also caused by size and steric hinder of the Grignard reagents and the effect of position of chloride atom on benzene ring. (to be discussed later in this chapter). Consideration of compounds **111** and **114** with dodecyl group substitution at position 1´ of chavicol showed that compound **111** with bromide atom at *para*- position of benzene ring was obtained in higher %yield than compound **114** with chloride atom at *para*- position of benzene ring. We compared compounds **121**, **126** and **120-TBDMS** with allylmagnesium bromide as a Grignard reagent. It showed that compound **120-**

TBDMS (*p*-OTBDMS) was achieved in higher %yield than compound **121** (*p*-Br) and compound **126** (*p*-Cl), respectively. This may be due to the inductive effect of substitutents group on *para*- position of benzene ring. Siloxyl group of **120-TBDMS**, bromide atom **121** and chloride atom **126** at *para*- position of benzene ring were electronegatively induced electron density in the aromatic system toward themselves creating bond polarity. Siloxyl group of **120-TBDMS** achieved higher %yield than bromide atom **121** and chloride atom **126** at *para*- position of benzene ring, respectively. This is because of an inductive effect of substituent at *para*- position of benzene ring increased the electrophilicity of aldehyde. The aldehyde acted as good electrophilic substrates to react with nucleophile of Grignard reagent. Compound **121** with non-substituted benzene ring had low %yield (with 12.1 %yield). However, compound **122** with thiophene ring gave good %yield (59 %yield). This is because 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

Secondly, we tempted to compare the effect of chlorine atom at different positions of benzene ring (*para*-, *meta*- or *ortho*- position). Consideration of compounds with allyl substitution at position 1´ of chavicol analogs **126**, **116** and **118** showed that compound **126** (*p*-Cl) was obtained higher %yield than compounds **116** (*o*-Cl) and **118** (*m*-Cl). Compounds **116** (*o*-Cl) and **118** (*m*-Cl) were not different on %yield in the case of allylmagnesium bromide as a Grignard reagent. Compounds **117** (*o*-Cl) and **119** (*m*-Cl) were reacted with benzylmagnesium bromide as a Grignard reagent. Compound **119** with chloride atom at *meta*- position of benzene ring was obtained in higher yield than compound **117** with chloride atom at *ortho*- position of benzene which had shown a steric effect on the reaction. These results indicated the important of chloride atom presence on benzene ring. Chloride atom at *para*- position of benzene ring caused less steric hindered to the reagent than chloride atom at *meta*- and *ortho*- position of benzene ring, respectively. The yields were not different between compounds **116** (*o*-Cl) and **118** (*m*-Cl) because allylmagnesium bromide is a small Grignard reagent, therefore the presence of chlorine atom on benzene ring showed similar steric effect on the reaction.

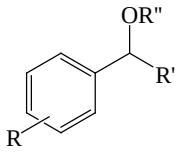
Thirdly, we studied the effect of R´ position on C-1´ of chavicol analogs. Regarding to compounds containing bromide atom at *para*- position of benzene ring,

compounds **110** (49.7 %yield) and **111** (50.6 %yield) with aliphatic side chain (8 and 12 carbons, respectively) at R´ position gave better %yield than compound **112** (33.5 %yield) with benzyl substitution. Acetylation of compound **123** (35.29 %yield) and **124** (48.8 %yield) with aliphatic side chain (8 and 12 carbons, respectively) at R´ position showed better %yield than compound **125** (23.22 %yield) with benzyl substitution as well. When compounds contain chloride atom at *para*- position of benzene ring, we found that compound **114** (22.7 %yield) with dodecyl group showed better %yield than compound **115** (13.2 %yield) with cyclopentyl group. Acetylation of compound **126** (47.2 %yield) with dodecyl group gave in higher %yield than compound **127** (13.2 %yield) with cyclopentyl group. Moreover, when contain chloride atom at *ortho*- position of benzene ring, we could obtain compound **116** (26.5 %yield) with allyl group in higher %yield than compound **117** (9.2 %yield) with benzyl substitution. This may be due to the size and steric hinder of the Grignard reagents. More bulky and hindrance substituted compounds, such as cyclopentyl magnesium bromide and benzylmagnesium bromide, gave lower %yields than less bulky or non-hindrance Grignard reagent, such as octylmagnesium bromide and dodecylmagnesium bromide. Therefore, using Grignard reaction, compound with aliphatic side chain substitution at R´ position gave higher %yield than bulky substitution (cyclopentyl group and benzyl group) at R´ position as expected.

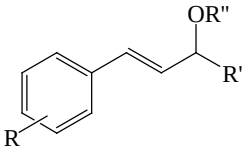
Lastly, we determined the effect of protecting group for hydroxyl group at position 1´ of chavicol analogs. Compound **130** with siloxyl group at *para*- position of benzene ring and allyl substitution at position 1´ of chavicol analogs was achieved in high yield (90 %yield). Consideration of compounds with halide atom (bromide and chloride atom) at *para*- position of benzene ring **123-127**, compounds **123**, **124** and **126** with aliphatic side chain (octyl and dodecyl grop) at position 1´ of chavicol analogs were obtained in better %yield than compounds **125** and **127** with bulky group (benzyl and cyclopentyl) at position 1´ of chavicol analogs. These results indicated that better product yields could be achieved by increasing the nucleophilic strength of the alkoxides of ACA analogs. However, these results also caused by size and steric hinder of the Grignard reagents.

TABLE 3.

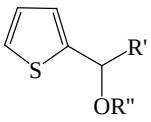
THE ANTI-TUBERCULOSIS ACTIVITY OF 1´-ACETOXYCHAVICOL ACETATE ANALOGS AGAINST MYCOBACTERIUM TUBERCULOSIS H37Ra



110-120, 123-130



121, 131



122, 132

Cpds.	R	R´	R´´	%Yield	[a] MIC		Activity	[b] %Inhibition at 50 µg/ml
					µg/ml	µM		
110	*p*-Br	C_8H_{17}	H	49.7	50	167.08	Active	n.d.
111	*p*-Br	$C_{12}H_{25}$	H	50.6	-	-	Inactive	-166.04
112	*p*-Br	C_7H_7	H	33.5	-	-	Inactive	n.d.
114	*p*-Cl	$C_{12}H_{25}$	H	22.7	-	-	Inactive	-31.38
115	*p*-Cl	C_5H_9	H	13.2	-	-	Inactive	-69.14
116	*o*-Cl	C_3H_5	H	26.5	-	-	Inactive	-200.21
117	*o*-Cl	C_7H_7	H	9.2	50	202.65	Active	95.88
118	*m*-Cl	C_3H_5	H	26.4	-	-	Inactive	-65.27
119	*m*-Cl	C_7H_7	H	29.6	-	-	Inactive	20.36
120	*p*-OH	C_3H_5	H	60	-	-	Inactive	80
121	H	C_3H_5	H	12.1	50	286.96	Active	99.36
122	-	C_3H_5	H	59.0	-	-	Inactive	n.d.
123	*p*-Br	C_8H_{17}	Ac	35.29	12.50	36.63	Active	101.49
124	*p*-Br	$C_{12}H_{25}$	Ac	48.8	-	-	Inactive	-105.47
125	*p*-Br	C_7H_7	Ac	23.22	-	-	Inactive	-15.45
126	*p*-Cl	$C_{12}H_{25}$	Ac	47.2	-	-	Inactive	-33.92
127	*p*-Cl	C_5H_9	Ac	13.2	-	-	Inactive	-6.58
128	*o*-Cl	C_3H_5	Ac	54.5	-	-	Inactive	-260.99
129	*m*-Cl	C_3H_5	Ac	31.1	-	-	Inactive	-10.84
130	*p*-OH	C_3H_5	Ac	90	-	-	Inactive	50
131	H	C_3H_5	Ac	26.3	-	-	Inactive	28.59
132	-	C_3H_5	Ac	50.1	-	-	Inactive	-170.38

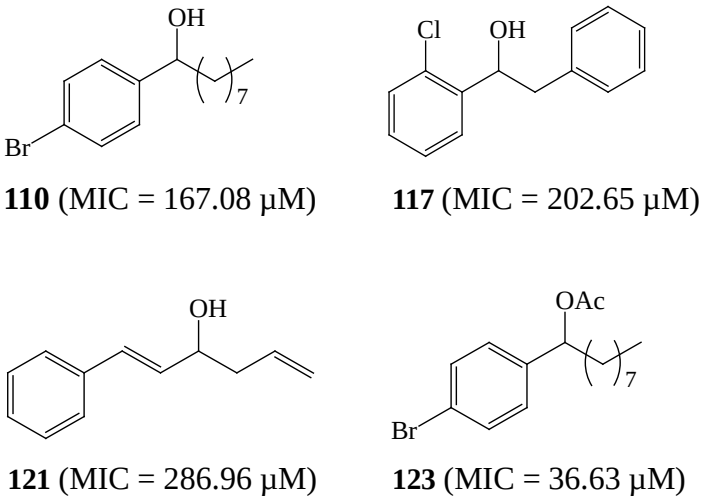
MIC of positive control: Rifampicin = 0.003–0.012 µg/ml , Ofloxacin = 0.391-0.781 µg/ml
Streptomycin = 0.156–0.313 µg/ml, Isoniazid = 0.023-0.046 µg/ml

[a] Minimum Inhibitory Concentration at ≥ 90% inhibition (active and MIC included)

[b] Interpretation at < 90 % inhibition (inactive)

4.4 Antimicrobial activity

All synthesized compounds were tested for their activity against M. *tuberculosis* H37Ra at National Science and Technology Development Agency (NSTDA), using Anti-*Mycobacterium tuberculosis* H37Ra Assay and Green fluorescent Protein (GFP)-based fluorescent detection method. MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after incubation. The maximum concentrations of all test samples were 50 μg/ml. The minimum inhibitory concentration (MIC) was measured when the compounds have greater than or equal to 90% inhibition. Compounds having less than 90% inhibition were defined as inactive compound. We used %inhibition to define a tendency of inactive compounds to anti-TB activity. Compounds with high %inhibition could show better tendency against *Mycobacterium tuberculosis* than compounds with less %inhibition. The results of in *vitro* antimycobacterial activities are summarized in **Table 3.** In our previous study, inductive effects of substituents (chlorine or bromine atom) on a benzene ring have significantly enhanced the anti-TB activity (Singkhonrat, Bunthitsakda, Kedpokasiri, & Nuampipat, 2010). Therefore, we synthesized and investigated the high electronegativity of chlorine or bromine atom on aromatic benzene ring for the structure activity relationship of ACA analogs. There were four compounds **(110**, **117**, **121** and **123)** showed activity against M. *tuberculosis* H37Ra with MIC of 167.08, 202.65, 286.96 and 36.63 μM, respectively **(Figure 35).** The other compounds in this series failed to inhibit the growth of M. *tuberculosis* H37Ra. As we mentioned above, they were defined as the inactive compounds because they exhibited less than 90 %inhibition. We used %inhibition to define a tendency of inactive compounds to inhibit M. *tuberculosis* H37Ra.



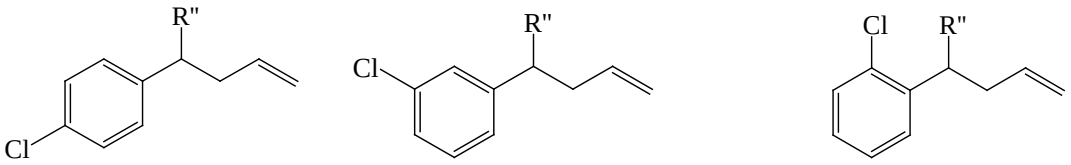
110 (MIC = 167.08 µM) **117** (MIC = 202.65 µM)

121 (MIC = 286.96 µM) **123** (MIC = 36.63 µM)

Figure 35. The structure of active ACA analogs of this series

Firstly, we tempted to compare the effect of chlorine atom at different positions of benzene ring (*para*-, *meta*- or *ortho*- position) on anti-tuberculosis activity. Regarding to compounds with allyl substitution at position 1´ of chavicol analogs, compounds **116**, **118, 128** and **129** showed no inhibitory activity. We used %inhibition to define a tendency of inactive compounds to anti-TB activity. Compounds with more negative %inhibiton decreased the potential ability to inhibit *Mycobacterium tuberculosis.* According to capability of inhibitory showed by %inhibition at 50 µg/ml, these data could show the tendency of the required LEAD structures which could suggest for further study. According to compounds **116** (*o*-Cl) and **118** (*m*-Cl) with allyl substitution at C1´ of chavicol analogs, compound **118** with chloride atom at *meta*- position on benzene ring tended to inhibit growth of bacteria better than compound **116** with chloride atom at *ortho*- position on benzene ring (-65.27 and -200.21 %inhibition, respectively). This showed the opposite tendency when acetylation of compound **116** which showed that *ortho*- position on benzene ring **128** would offer a better inhibitory activity than *meta*- position on benzene ring **129 (Figure 36)**. Our previous study (Singkhonrat, et al., 2010) showed that 1-(4-chlorophenyl)but-3-en-1-ol **PCB-OH**, and 1-(4-chlorophenyl)but-3-enyl acetate **PCB-OAc**, contained a chloride atom at *para*- position of benzene ring, appeared activity against M. *tuberculosis* H37Ra (with MIC of 17.11 and 55.64 µM, respectively). Compounds **120** and **130** with hydroxyl group at *para*- position on

benzene ring loss anti-TB activity (50 and 80 %inhibition, respectively). However, we found that compounds **120** and **130** with hydroxyl group at *para*- position on benzene ring tended to achieve inhibitory activity more than compounds **116** and **128** with chloride atom at *ortho*- position on benzene ring and compounds **118** and **129** with chloride atom at *meta*- position on benzene ring. These results showed that compound with halogen atom at *para*- positon of benzene ring achieved biological activity similar to the previous report (Singkhonrat, et al., 2010) **(Figure 36)**



R´´ = OH (MIC 17.11 µM) **PCB-OH** R´´ = OH (-65.27 %inhibition) **118** R´´ = OH (-200.21 %inhibition) **116**
R´´ = OAc (MIC 55.64 µM) **PCB-OAc** R´´ = OAc (-260.99 %inhibition) **129** R´´ = OAc (-10.84 %inhibition) **128**

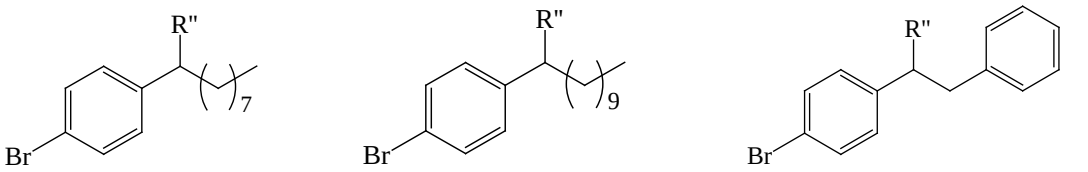
Active **Inactive**

PCB-OH > **PCB-OAc** >>>>>>>>>>>>> **128**>**118**>>> **116**>**129**

Figure 36. Summary of the effect of chloride atom at different positions of benzene ring

Secondly, we studied the effect of alkyl substitution at position 1´ of chavicol analogs **(Figure 37)**. Regarding to alcohol analogs **110-112** with bromide atom at *para*- position of benzene ring, compound **110** (R´ = C_8H_{17}) found to give inhibitory activity with MIC 167.08 µM, while compounds **111** (R´ = $C_{12}H_{25}$) and **112** (R´ = C_7H_7) showed no activity. Ester analogs **123-125** with bromide atom at *para*-position of benzene ring, compound **123** (R´ = C_8H_{17}) showed activity against *M. tuberculosis* H37Ra (MIC 36.63 µM), while compounds **124** (R´ = $C_{12}H_{25}$), **125** (R´ = C_7H_7) showed no activity as well. It indicated that compounds **110** and **123** with octyl substitution at C1´ of chavicol analogs showed anti-TB activity and the structure required to be acetylated for better activity. The length of R´ side chain at C1´ of chavicol analogs was also an importance for activity. Compounds **111** and **124** with

longer side chain with more than 8 carbons lost activity. However, compounds **111** tended to achieve inhibitory activity more than compounds **112** with benzyl substitution at position 1´ of chavicol analogs. This showed the opposite tendency when acetylation of **111** and **112** which showed that the bulky substituent of benzyl group of **125** preferred to offer a better inhibitory activity than aliphatic substituent of **124**. According to compounds with chloride atom at *para*- position of benzene ring, both alcohol analogs **114** and **115** and ester analogs **126** and **127** with dodecyl or cyclopentyl at C1´ of chavicol analogs showed no activity. The results of inhibitory activity revealed preferably the acetylated compounds with less carbon atoms than 8 carbons, even the substituent was cyclopentyl group. Moreover, the electronegativity effect of chloride atom **114** and **126** at *para*- position showed better inhibitory activity than compounds with bromide atom **111** and **124**.



R´´ = OH (MIC 167.08 µM) **110** R´´ = OH (-166.04 %inhibition) **111** R´´ = OH Inactive **112**
R´´ = OAc (MIC 36.63 µM) **123** R´´ = OAc (-105.47 %inhibition) **124** R´´ = OAc (-15.45 %inhibition) **125**

R´´ = OH (-31.38 %inhibition) **114** R´´ = OH (-69.14 %inhibition) **115**
R´´ = OAc (-33.92 %inhibition) **126** R´´ = OAc (-6.58 %inhibition) **127**

	Active	**Inactive**
p-Br:	**123**>**110**>>>>>>>>>>	>**125**>>**124**>**111**
p-Cl:		**127**>>>>>> >**126** > **114**>**115**

Figure 37. Summary of the effect of alkyl substitution at position 1´ of chavicol analogs

In our previous work (Singkhonrat, et al., 2010) found that compounds with allyl substituent at C1′ of chavicol analogs and R position contained bromide or chloride at *para*- position of benzene ring showed good activity (with MIC of 13.78 µM and 17.11 µM, respectively). In this study, compound **121** with allyl substituent at C1′ of chavicol analogs and addition of carbon atom with conjugation to benzene ring from C1′ without substituent on benzene ring could maintained the activity. (with MIC of 286.96 µM). Whereas, compound **116** (R = *o*-Cl, R′ = C_3H_5) and **118** (R = *m*-Cl, R′ = C_3H_5) caused the loss of anti-TB activity. This may caused by the effect of substituent position on benzene ring at *ortho*- and *meta*- position interferring the mode of action. We found that aliphatic side chain at R′ position should contain less than 8 carbons. Compounds **111**, **114**, **124** and **126** which consisted more than 8 carbons aliphatic side chain (dodecyl substitution) at C1′of chavicol analogs, led to the significant loss of activity. Compounds **115, 125** and **127** with bulky substituent (cyclopentyl or benzyl substitution) were led to loss of activity as well. In this series, the structure with *ortho*- positon on benzene ring was possible only for compound **117** (R = *o*-Cl, R′ = C_7H_7) with MIC of 202.65 µM. These results indicated that the size of the alkyl chain could be responsible for the loss of biological activity. However, if bulky group was necessary, the structure must consist of with high electronegative group at *ortho*-position of benzene ring. 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. Regarding to compounds **110** and **123** with bromide atom at *para*- position of benzene ring and octyl substitution at C1′ of chavicol analogs. Compound **123** with acetyl protecting group appeared better activity than compound **110** with free hydroxyl group (36.63 µM and 167.08 µM, respectively). Contrarily, compound **121** and **131** with allyl substitution at position 1′ of chavicol analogs offered anti-TB activity of compound **121** with free hydroxyl group (286.96 µM), while compound **131** with protecting group of free hydroxyl group failed to inhibit M. *tuberculosis*. Compounds **111** (R = *p*-Br, R′ = $C_{12}H_{25}$, R′′ = OH), **114** (R = *p*-Cl, R′ = $C_{12}H_{25}$, R′′ = OH), **115** (R = *p*-Cl, R′ = C_5H_9, R′′ = OH), **116** (R = *o*-Cl, R′ = C_3H_5, R′′ = OH), **118** (R = *m*-Cl, R′ = C_3H_5, R′′ = OH), **120** (R = *p*-OH, R′ = C_3H_5, R′′ = OH), **124** (R = *p*-Br, R′ = $C_{12}H_{25}$, R′′ = OAc), **126** (R = *p*-Cl, R′ = $C_{12}H_{25}$, R′′ = OAc), **127** (R = *p*-Cl, R′ =

C_5H_9, R´´ = OAc), **128** (R = *o*-Cl, R´ = C_3H_5, R´´ = OAc), **129** (R = *m*-Cl, R´ = C_3H_5, R´´ = OH) and **130** (R = *p*-OH, R´ = C_3H_5, R´´ = OAc) showed no activity. We found that compounds **124**, **127** and **129** with acetoxy group at C1´ of chavicol analogs tended to inhibit growth of bacteria better than compounds **111**, **115** and **118** with free hydroxyl group at C1´ of chavicol analogs. On the other hand, compounds **114**, **116** and **120** with free hydroxyl group at position 1´ of chavicol analogs tended to achieve better activity than compounds **126, 128** and **130** with acetoxy group at position 1´ of chavicol analogs. It indicated that the presence of protecting group for hydroxyl group at C1´ of chavicol analogs was not major effect for anti-TB activity.

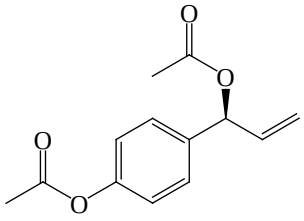
Finally, we studied the effect of different aromatic ring on activity against M. *tuberculosis* H37Ra. Compounds containing allyl substituent at R´ position showed that compound **121** with conjugated system with benzene ring offered anti-TB activity (286.96 µM), while compound **122** with thiophene ring led to a loss of activity. It indicated the important of aromaticity to biological activity. Phenyl group was attached to carbon atom possessing high electronegative group at *para*- position of benzene ring such as chloride or bromide atom could appropriate physical properties to access the target cell more than thiophene ring.

In this series, compound 1-(4-bromophenyl)nonyl acetate **123** with bromine atom at *para*- position of benzene ring, consisting octyl group and acetoxy group at position 1´ of chavicol, was found to be the most active compound against M. *tuberculosis* H37Ra with MIC 36.63 µM in these series.

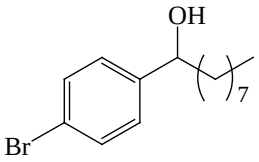
This study showed that the key structure of ACA analogs that important on anti-TB activity should have the following structural requirements 1) the *para*-substitution of halide atom at the benzene ring was essential; 2) the presence of aliphatic side chain at C1´ of chavicol should be less than 8 carbons; 3) the presence of acetyl group as a protecting group for hydroxyl group at C1´ of active compound (with 8 carbons aliphatic side chain) was an importance for improve biological activity; 4) phenyl group was attached to carbon atom possessing high electronegative group at *para*- position of benzene ring such as chloride or bromide atom could has appropriate physical properties to access the target cell more than other aromatics.

TABLE 4.

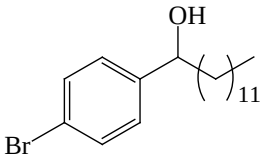
THE CYTOTOXICITY OF 1´*S*-1´-ACETOXYCHAVICOL ACETATE (*S*-ACA) AND ANALOGS



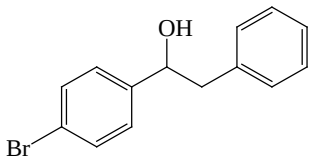
S-ACA



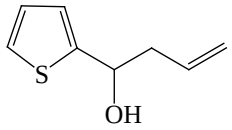
110



111



112



122

Cpds.	**R**	**R´**	**R´´**	**Cytotoxicity Activity**	[a] **IC_{50} (µg/ml)**
***S*-ACA**	AcO	C_2H_3	OAc	Cytotoxic	2.00 (Saradee, 2009)
110	*p*-Br	C_8H_{17}	OH	Cytotoxic	15.68
111	*p*-Br	$C_{12}H_{25}$	OH	Non-Cytotoxic	-
112	*p*-Br	C_7H_7	OH	Cytotoxic	8.58
122	-	C_3H_5	OH	Non-Cytotoxic	-

[a] The half maximal inhibitory concentration

Some compounds were further examined for toxicity (IC_{50}) against primate cell. The cytotoxicity activity test of ACA analogs was performed by National Science and Technology Development Agency (NSTDA).

The cytotoxicity activity of ACA analogs are shown in Table 4. Compound **110** appeared anti-TB activity, and also appeared cytotoxic (15.68 µg/ml). Compound **112** failed to inhibit M. *tuberculosis* H37Ra, while it appeared toxicity at 8.58 µg/ml. Compounds **111** and **122** did not showed anti-TB activity, neither did cytotoxicity. The results indicated that these compounds may not have appropriate physico-

chemical properties to access the target cell. The development of any new compounds for TB would depend on an excellent safety profile since it would need to be used for several months in combination with other antitubercular agents.