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Synthesis and Characterization of Methyl-2-hydroxy-5-{(1)-1-[2-(pyridin-4-ylcarbonyl) hydra zinylidene]butyl}benzoate, a New Isonicotinoylhydrazone Derivative of Methyl Salicylate

Glenn V. Alea[1], Faith Marie G. Lagua[1] and Margarita N.S. Caparas[1*]
[1] *Department of Chemistry, De La Salle University - Manila*
**Corresponding Author:* megnscaparas@yahoo.com

Abstract: One of the challenges faced by physicians in curing Tuberculosis (TB) is the presence of resistant strains of the bacteria towards the first-line of drugs (Isoniazid, Rifampicin, Pyrazinamide and Ethambutol) used to treat the disease. This has been a result of poor patient compliance to treatment regimens, enabling the bacteria to develop resistance towards these drugs. The derivatization of the first-line of drugs has been the subject of current researches as one of the strategies to combat the existence of resistant strains of the bacteria. Isonicotinoylhydrazones, are a class of isoniazid derivaties with potential anti-tuberculosis (TB) activity. This study deals with the synthesis and characterization of an Isonicotinoylhydrazone derivative, methyl 2-hydroxy-5-{(1)-1-[2-(pyridin-4-ylcarbonyl)hydrazinylidene]butyl}benzoate (1c). The synthesis involves the use Friedel-Crafts acylation of methyl salicylate with butanoyl chloride followed by the imine formation with Isoniazid. The target compound (1c) was generated as a dirty white powder in 75.98% yield. Characterization of the compound involved the use of mass spectrometry, infrared spectroscopy and its structure was confirmed through ^{1}H-NMR analysis. The synthesized compound may exhibit similar or higher anti-tubercular activity against susceptible and resistant strains of *Mycobacterium tuberculosis* towards INH. It may also be used as part of a structure activity relationship study on a set of previously synthesized Isonicotinoylhydrazones with varying alkyl chains.

Key Words: Isonicotinoylhydrazones, Isoniazid, Tuberculosis, Friedel-Crafts acylation, Methyl salicylate

1. INTRODUCTION

The number of people who fell ill with TB dropped to 8.8 million in 2010, including 1.1 million cases among people with HIV. The number has been falling since 2005 as reported in the Global Strategy for Containment of Antimicrobial Resistance 2010 of the World Health Organization. However, it is estimated that between 2002 and 2020, approximately 1 billion people would be newly infected with the disease, more than 150 million people would get sick, and 36 million would die of TB if new disease prevention and treatment measures are not developed. (Pavan et al., 2010)

Tuberculosis is a chronic bacterial infection caused by *Mycobacterium tuberculosis*. It is transmitted through the air and commonly targets the lungs. If the immune system cannot keep the tubercle bacilli under control, the bacilli would begin to multiply rapidly (TB disease) (Parumasivam et al., 2013). Anti-TB

chemotherapeautics lasts for at least 6 months and in some cases even longer. Regimens include a dosage form of combining at least two of the four first-line anti-TB drugs (Isoniazid, Rifampicin, Pyrazinamide and Ethambutol). The patient's inconsistent observance to drug administration and non-compliance to the prescribed drugs over a long treatment period (e.g.when the drug has been resumed after a gap of a few days to months.) are factors that influence the treatment outcome. This causes the emergence of multi-drug resistant tubercular bacilli (MDR-TB).

Isoniazid, being one of the most prominent and most inhibitory first-line drugs, has been the subject of studies trying to create anti tb agents derivatives. Recent studies have shown that coupling these first-line drug derivatives with potential molecules, such as methyl salicylate and salicylic acid may generate a more effective compound against susceptible and resistant strains of the bacilli.

In this study, an acylated derivative of methyl salicylate was coupled with Isoniazid. Methyl salicylate, commonly known as "oil of wintergreen" is a colorless liquid methyl ester of salicylic acid. Salicylic acid and its derivatives have been found to disrupt the stress sytem of the bacteria, by inhibiting its adhesion to tissues and production of toxins, causing reduced infection to host cell. A research also proved that the inhibition of energy production by salicylates when co-administered with first line of drugs may increase its activity in depleting the membrane energy of M.tuberculosis. (Austria and Lim, 2012; Bocanegra-García et al., 2011) The synthesized Isonicotinoylhydrazone derivative may exhibit a higher potency against susceptible and resistant strains of the mycobacteria towards Isoniazid.

2. RESULTS AND DISCUSSION

The strategy for the generation of the target compound (1c) which contains an isoniazid group and an acylated methyl salicylate group bridged via an imine functionality involved the use of Friedel Craft's acylation of methyl salicylate with butanoyl chloride followed by the imine formation with Isoniazid as shown in Figure 1.

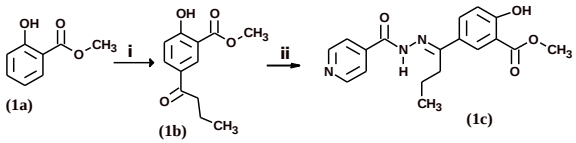

Fig 1. Synthetic pathway for compound (1c)
i. Butanoyl Chloride, $ZnCl_2$, CH_2Cl_2 ; ii. Isoniazid, Methanol

2.1 Synthesis of Methyl-5-butanoylsalicylate (1b)

The synthesis of the target compound (1c) involved the generation of compound (1b), which contains a 4-carbon acyl chain attached to a methyl salicylate moiety.

Compound (1b) was generated as white crystals with a yield of 30.86%. The TLC of the crude product gave a single spot with an R_f value of 0.36 (60% CH_2Cl_2:40% C_6H_{14}) which is more polar than methyl salicylate, 0.581 (60% CH_2Cl_2:40% C_6H_{14}). The melting point range of 69-71 ^oC indicates that the compound generated is pure. The functional groups present in the compound were identified using IR spectroscopy (KBr Disk). A peak at 1610 cm^{-1} found in the compound synthesized confirmed the attachment of ketone. A peak at 2960.34 cm^{-1} corresponds to the C-H stretch present at the compound synthesized. The mass spectrum of the compound showed a pseudomolecular ion peak at m/z 245.1 [M+Na], which is due to the mass of the target compound with potassium. This is consistent with the expected molar mass of the target compound (MM: 222.06, $C_{12}H_{14}O_4$).

2.2 Synthesis of Methyl 2-hydroxy-5-{(1)-1-[2-(pyridin-4-ylcarbonyl)hydrazinylidene] butyl}benzoate (1c)

The synthesis of compound (1c) involved the coupling of the synthesized acylated derivative of methyl salicylate (1b) with Isoniazid via imine formation.

The reaction produced dirty white powder with a yield of 75.98 %. The TLC of the crude product gave a single spot with an R_f value of 0.45 (95% EtOH: 5% CH_3OH) which is less polar than Isoniazid, 0.180 (95% EtOH: 5% CH_3OH) and Methyl-5-n-butanoylsalicylate 0.387, (95% EtOH:

5% CH_3OH). A higher R_f value for the product compared to the starting material, Methyl-5-n-butanoylchloride and Isoniazid indicates that it is a less polar compound, having a larger distance travelled on the TLC as the result of the attachment of methyl-5-n-butanoylsalicylate (1b).

The melting point range of 193-197 ^{O}C indicates that the compound generated is pure. The functional groups present in the compound were confirmed using IR spectroscopy (KBr Disk). The IR spectrum of the synthesized compound showed absorption bands at 3583 cm^{-1}, 3431 cm^{-1}, 1653 cm^{-1}, 1253 cm^{-1} corresponding to the aromatic amine, O-H functionality, aromatic ester and imine group respectively. The presence of the imine functionality confirms the attachment of the Isoniazid moiety.

The mass spectrum of the compound showed pseudomolecular ion peaks at m/z 342.14 [M+H], 364.12 [M+Na], which is due to the mass of the target compound with hydrogen and sodium respectively. This is consistent with the expected molar mass of the target compound (MM: 341.40, $C_{18}H_{19}N_3O_4$)

The structure of the synthesized compound (1c) was confirmed through ^{1}H-NMR analysis as shown in Figure 2. A strong singlet is found at 3.34 ppm corresponds to the hydrogen atom (H24) on the OH group attached to the acylated methyl salicylate aromatic ring. This is a strong signal due to the intramolecular H-bonding between the alcohol group and the carbonyl of the ester group. A singlet is found at 3.93 ppm corresponds to hydrogen atoms (H23) on the ester group attached to the acylated methyl salicylate aromatic ring. A triplet at 0.94 ppm (J value= 8 Hz) corresponds to the hydrogen atoms (H14) at the terminal carbon of the alkyl chain. This is due to the methylene protons adjacent to it. Another triplet at 2.88 ppm (J value= 8 Hz) corresponds to the hydrogen atoms (H12) along 4-Carbon alkyl chain.

Table 1. ^{1}H-NMR Spectra for methyl 2-hydroxy-5-{(1E)-1-[2-(pyridin-4-ylcarbonyl) hydrazinylidene]butyl}benzoate (1c)

Proton Chemical Shift	Integration	Multiplicity	Coupling Constant, J (Hz)	Assignment
0.94	3H	(t)	8	H14
1.48	2H	(m)	-	H13
2.88	2H	(t)	8	H12
3.34	1H	(s)	-	H24
3.93	3H	(s)	-	H23
11.42	1H	(s)	-	H9
8.76-8.78	2H	(d)	4	H2, H6
7.98-8.00	2H	(d)	4	H3, H5
7.06-7.08	1H	(d)	4	H19
7.74-7.69	1H	(d)	4	H20
8.26	1H	(s)	8	H16

A multiplet is found at 1.48 ppm which corresponds to the hydrogen atoms (H13) along the alkyl chain. A singlet appeared at 11.42 ppm that corresponds to the hydrogen atom (H9) next to the imine bond.

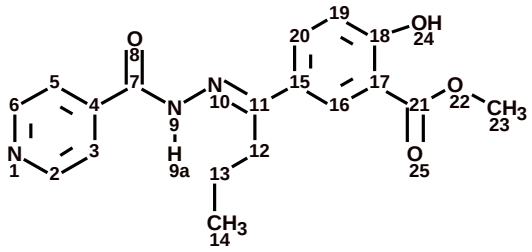

Fig 2. Structure of Methyl *2-hydroxy-5-{(1)-1-[2-(pyridin-4-ylcarbonyl)hydrazinylidene] butyl}benzoate (1c)*

For the peaks found that corresponds to the hydrogen atoms on the isoniazid ring, a doublet is found at δ7.98-8.00 (J value=4 Hz) for H3 and H5 and a doublet is found at 8.76-8.78 ppm (J value=4 Hz) that corresponds to H2 and H6. Lastly, for the peaks observed that corresponds to the hydrogen atoms on the phenyl ring, a singlet is found at 8.26 ppm corresponds to H16 (J value = 8 Hz). Another doublet found at 7.74-7.73 ppm corresponds to H20

(J value= 4 Hz) and a doublet appeared at 7.06-7.08 ppm corresponds to the hydrogen atom H19 (J value= 4 Hz) on the phenyl ring.

3. METHODOLOGY

The synthesis of methyl 2-hydroxy-5-{(1)-1-[-(pyridin-4-ylcarbonyl)hydrazinylidene]butyl} benzoate (1c) was done in two steps. For the first part, an acylated methyl salicylate precursor, methyl-5-butanoylsalicylate, was first synthesized via Friedel Craft's acylation which was followed by imine formation of isoniazid.

3.1 Synthesis of Methyl-5-butanoylsalicylate (1b)

A solution of zinc chloride (6.407 g, 47.00 mmol) dissolved in CH_2Cl_2 (6ml) was added to methyl salicylate (5.0 ml, 35.58 mmol) in a round bottom flask. This was followed by the drop-wise addition of butanoyl chloride (6.1 ml, 58.73 mmol) resulting into a cloudy white mixture. Syringe needles were placed on the rubber septum cover of the round bottom flask to release pressure. Leaving the reaction mixture stirring for 50 hours produced an emerald green mixture. The reaction mixture was extracted with CH_2Cl_2 (22 ml) and washed with distilled H_2O (15 ml, 3x). The color of the reaction mixture, while being shaken in the separatory funnel, went from emerald green to maroon orange. The organic layer was collected, dried with anhydrous $MgSO_4$, and filtered. A clear dark orange to mustard yellow liquid was observed. The solution was concentrated *in vacuo*. The resulting crude product was recrystallized with petroleum ether (5ml, 4x). The crystals that formed in the solution were isolated and washed with cold petroleum ether (3 ml, 4x) while being filtered using a Hirsch funnel. The product generated white crystals (2.503 g, 30.86%); R_f value: 0.36 (60% CH_2Cl_2:40% C_6H_{14}); MP: 69-71 ^{O}C; IR (KBr Disk): 3403 cm^{-1} Ar(OH), 1610 cm^{-1} (Ar-Ketone); MS/ESI(m/z): $[M+Na]^+$ 245.1; MF: $C_{12}H_{14}O_4$ [MM: 222.06]

3.2 Synthesis of methyl 2-hydroxy-5-{(1)-1-[2-(pyridin-4-ylcarbonyl) hydrazinylidene]butyl}benzoate (1c)

Methyl-5-n-butanoylsalicylate (1b) (0.104 g, 0.495 mmol) was placed in a round bottom flask together with isoniazid (0.112 g, 0.909 mmol) dissolved in methanol (3 ml). The contents were heated on a hot water bath while stirring for 3 hours with a maintaining temperature of 70-80 ^{O}C. A light yellow solution was observed. Stirring the solution for an additional 72 hours produced a creamy white liquid with sand-like sediments at the bottom and white solids at the sides of the flask. The contents in the round bottom flask were extracted with cold CH_2Cl_2 (5 ml, 3x) followed by washing with cold distilled H_2O (4 ml, 4x) while being filtered using a Hirsch funnel. The product generated dirty white powder (0.1284 g, 75.98 %); R_f value: 0.45 (95% EtOH:5% CH_3OH); MP: 193-197 ^{O}C; IR (KBr Disk): 3583 cm^{-1}(Amine group), 3431 cm^{-1}(O-H functionality), 1653 cm^{-1}(Ar-Ester), 1253 cm^{-1} (Ar-Amine); MS/ESI(m/z): 342.14 [M+H], 364.12 [M+Na]; MF: $C_{18}H_{19}N_3O_4$; [MM: 341.13]; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.42 (1H, s, , H9); 8.76-8.78 (2H, d, J value = 4Hz, H2 and H6); 8.26 (1H, s, J value = 8Hz, H16); 7.98-8.00 (2H, d, J value = 4Hz, H3 and H5); 7.74-7.69 (1H, d, J value = 4Hz, H20); 7.06-7.08 (1H, d, J value = 4Hz, H19); 3.93 (3H, s, H23); 3.34 (1H, s, H24); 2.88 (2H, t, J value = 8Hz, H12); 1.48 (2H, m, H13; 0.94 (3H, t, J value = 8Hz, H14)

4. CONCLUSIONS

The isonicotinoylhydrazone derivative of methyl salicylate (1c) was synthesized and characterized. The compound generated in 75.98% yield. The synthesis involved the preparation of the acylated methyl salicylate precursor (1b) followed by imine formation with isoniazid to generate (1c). This isonicotinoylhydrazone derivative may exhibit increased anti-mycobacterial activity against susceptible and resistant strains of *Mycobacterium tuberculosis.* It is then, recommended that the final products be subjected to anti-mycobacterial testing to determine the efficacy of the compounds against *Mycobacterium tuberculosis.*

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