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Synthesis and Characterization of a Pyrazinamide derivative of Imidazo[2,1-b][1,3,4]thiadiazole, a potential Anti-tuberculosis drug

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Abstract: Despite the availability of first- and second-line drugs for the treatment of tuberculosis, the increasing prevalence of multi-drug resistance and extensively drug-resistant strains of *Mycobacterium tuberculosis* still poses a major threat to global health. With this, researchers are motivated to design and synthesize new molecules that possess promising anti-tubercular properties. Imidazo[2,1-b][1,3,4]thiadiazole derivatives have diverse pharmacological properties, one of which is that derivatives of this core molecule have been proven to have anti-tuberculosis properties.

In this research, apyrazinamide derivative of Imidazo[2,1-b][1,3,4]thiadiazole (6) was synthesized and characterized. It was generated in four steps that involved the formation of athiadiazole derivative containing a propyl group followed by the formation of the imidazo[2,1-b][1,3,4]thiadiazole core incorporating the 4-chlorophenyl group. Finally, an aldehyde group on the 5th position generated via a Vilsmeier-Haack reaction enabled the attachment of the pyrazinamide moiety via imine bond formation with 2-pyrazinehydrazide.Compound 6 was generated as a yellow brownish solid in 66% yield. This molecule may serve as a potential anti-tuberculosis drug due to the coupling of the pyrazinamide drug moiety and an Imidazo[2,1-b][1,3,4]thiadiazole core. The identity and structure of all the precursor compound and the final compound were confirmed usingm.p., IR, GC-EI-MS, and ^{1}H-NMR methods.

Key Words: Imidazothiadiazole derivative; anti-tuberculosis; pyrazinamide

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1. INTRODUCTION

Tuberculosis more commonly known as TB, is a chronic disease and one of the world's leading causes of death. It is caused by the acid-fast bacteria *Mycobacterium tuberculosis* (Todar, 2012).This bacterium commonly attacks the lungs but can also spread to different parts of the body which include the kidney, brain, and even the liver. This bacterium is very deadly since it can remain dormant for a very long time and can reactivate given the proper conditions. The disease mainly spreads through the air, as a person can easily get infected when near an infected individual via coughing, sneezing, or talking (WHO, 2017).

Treatment of Tuberculosis comes usually with different types of drugs, which include the different first line drugs: Isoniazid, Rifampin, Ethambutol, Streptomycin, and Pyrazinamide (WHO, 2012 and CDC, 2012).

Bacteria however evolve in different ways, which leads to them being able to resist the drug treatments given; this is called antimicrobial drug resistance. Drug resistance can be caused by different factors, these include the enzymes that the bacterium carry which help in its survival via control of various important processes like replication. In the case of *Mycobacterium tuberculosis* these enzymes have the ability to modify its metabolism, and slow down its replication when it is in its dormant stage, which renders the different lines of drugs useless in the long run[8]. Due to the presence of these drug resistant bacteria the number of deaths caused by TB has drastically increased. A set of second line of drugs have been administered to counter the drug resistant bacterium, these include Kanamycin, Capreomycin, Amikacin, Fluoroquinones, Ethionamide, Prothionamide, Clofazimine, p-amino Salicylic Acid, and Cycloserine (CDC, 2012; Jnawali and Ryoo, 2013). Second line drugs are usually combined with first line drugs in an attempt to overcome the drug resistant properties of the bacterium.

Imidazo[2,1-b][1,3,4]thiadiazole is a heterocyclic compound that has previously been commonly studied in the field of pharmacology due to its wide range of pharmacological properties which include it being anti-cancer, anti-inflammatory, anti-fungal, anti-bacterial, anti-leishmanial, anti-tumor, anti-consulvant, analgesic, anthelmintic, and anti-tubercular. Previous researchers that have studied the different derivatives of Imidazo[2,1-b][1,3,4]thiadiazoles have shown effectivity of the moiety against *Mycobacterium tuberculosis*. (Alwan et. al, 2015 and Kolavi et. al, 2006)

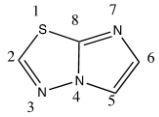
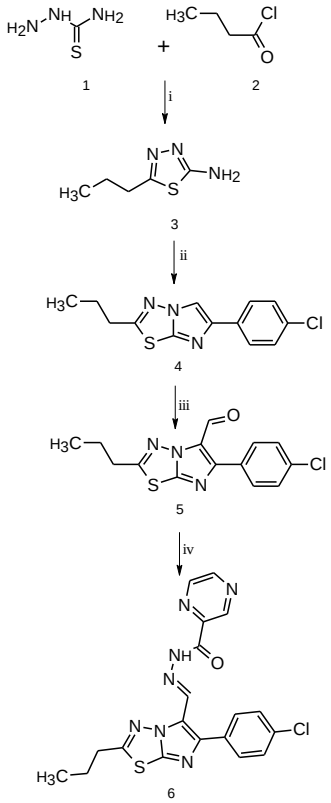


Figure 1. Imidazo[2,1-b][1,3,4]thiadiazole ring

The main objective of this research is to synthesize and characterize a potential anti-tubercular drug that may show more bactericidal properties against *Mycobacterium tuberculosis*. The synthesis of the pyrazinamide derivative of Imidazo[2,1-b][1,3,4]thiadiazole was carried out in four steps starting with the formation of the thiadiazole ring system. It is expected that this compound will show a potentially effective anti-tuberculosis drug since it fuses the pyrazinamide moiety and an imidazothiadiazole core which has shown a variety of biological applications which includes anti-tuberculosis properties as well.

2. METHODOLOGY

The synthesis of the pyrazinamide derivative of Imidazo[2,1-b][1,3,4]thiadiazole was done in 4 steps as shown in Scheme 1. The initial step was the generation of the thiadiazole ring, followed by the formation of the imidazothiadiazole core. Upon Vilsmeier-Haack reaction, an aldehyde moiety was introduced in the 5th position of the imidazothiadiazole core which enabled the attachment of the pyrazinamide group via imine formation.



Scheme 1. Synthesis of the Pyrazinamide derivative of Imidazo[2,1-b][1,3,4]thiadiazole (**6**).
i. $POCl_3$, Reflux (90-100°C), 4hrs; ii. 2-bromo-4'-chloroacetophenone, dry EtOH, Reflux (90-95°C), 14hrs; iii. $POCl_3$/DMF (0°C) – 30 mins, RT-2hrs, 60°C-2hrs, Na_2CO_3 sol'n - 90°C – 2hrs; iv. 2-pyrazinehydrazide, RT, 48 hrs – stirring.

3. RESULTS AND DISCUSSION

The synthesis of the target compound 6 was done in 4 steps. The generation of the thiadiazole ring with the propyl group (3) was achieved via nucleophilic acyl substitution using butyryl chloride (1) and thiosemicarbazide (2). This was followed by the formation of the imidazothiadiazole core using 2-bromo-4'-chloroacetophenone via a dehydrocyclization of the iminothiadiazole intermediate. It was envisioned that the coupling with the pyrazinamide group be situated at the 5thposition of the imidazothiadiazole core. This was achieved by the formation of an aldehyde functionality via a Vilsmeier-Haack reaction to generate compound 5 followed by the attachment of the pyrazinamide group thru an imine bond formation.

3.1 Synthesis of 5-propyl-1,3,4-thiadiazol-2-amine (3)

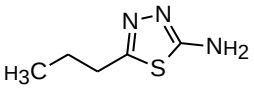


Figure 2

Compound 3 as shown in Figure 2 was generated as a solid brown powder in 7% yield (0.2008g, 7%). The sharp melting point range of 155-157°C and the single spot TLC profile generated from the compound also confirms its purity. The GC-EI-MS (m/z) generated for the compound showed a peak at 142.1 $[M+H]^+$ which was consistent with the expected calculated molar mass of 143.21 g/mol. *Solid brown powder. (0.2008g, 7%); Rf value: 0.767 (25EtOAc:1EtOH1CH$_3$COOH); MP: 155 -157°C; GC-EI-MS (m/z) 142.1 [M+H]$^+$; IR (KBr): 3104.48 cm^{-1} (N–H), 3270.59 cm^{-1} (N–H)*

3.2 Synthesis of 6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (4)

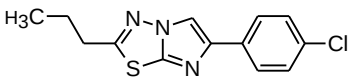


Figure 3

Compound 4 as shown in Figure 3 was generated as a yellow solid in 54% yield (0.561g, 54%). The single TLC spot with an Rf value of 0.384 and its sharp melting point of 147-149°C confirms the purity of the compound. Its identity was confirmed with the ^{1}H-NMR data of the compound which showed signals that were consistent with its structure.The propyl group gave signals at 3.01ppm (t, J=7.4 Hz, 2H), 1.77 ppm (m, 2H), and 0.98 ppm (t, J=7.4 Hz, 3H). The 4-chlorophenyl group was confirmed by the signals generated at 7.85 ppm (d, J=8.4 Hz, 2H) and 7.45 ppm (d, J=8.8 Hz, 2H). The

hydrogen at the 5th position of the imidazothiadiazole core was seen at *8.67 ppm (s, 1H). Yellow solid (0.561g, 54%); Rf value: 0.384 (C_6H_{14}:CH_2Cl_2); mp: 147 - 149°C; IR (KBr): 3132.69cm^{-1} (Ar-CH), 2961.88cm^{-1} (C_{sp^3}- H); ^{1}HNMR (400 MHz, DMSO-d_6) δ8.67 (s, 1H), 7.85 (d, J=8.4 Hz, 2H), 7.45 (d, J=8.8 Hz, 2H), 3.01 (t, J=7.4 Hz, 2H), 1.77 (m, 2H), 0.98 (t, J=7.4 Hz, 3H).*

3.3 Synthesis of the aldehyde precursor of the Pyrazinamide derivative of Imidazo[2,1-b][1,3,4]thiadiazole(5)

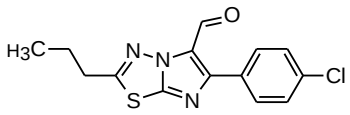


Figure 4

Compound 5 as shown in Figure 4 was generated as a dark brown liquid in 83% yield. Purity of the compound was confirmed with a single TLC spot at 0.525. The GC-EI-MS of the compound showed a molecular ion peak at 305.1[M+H]$^+$which was consistent with the molar mass of the compound that is equivalent to 305.81 g/mol. The ^{1}H-NMR also confirmed the structure of the compound with the loss of the chemical shift for the hydrogen at the 5th position and the occurrence of the peak at 9.98 ppm which corresponds to the hydrogen of the aldehyde group which is situated at a more deshielded region. This was also confirmed by the peak at 1672 cm^{-1} which corresponds to the presence of a carbonyl functionality in the molecule.*Dark brown liquid which later solidified. (0.521g, 83%); Rf value: 0.525 (1C_6H_{14}:1CH_2Cl_2); IR (KBr):3000cm^{-1}(Ar–CH), 1672.06cm^{-1} (C=O); GC-EI-MS ($C_{14}H_{12}ClN_3OS$) (305.78) (m/z): 305.1 [M+H]$^{+\ 81}$; ^{1}HNMR (400 MHz, DMSO-d_6) δ9.98 (s, 1H), 7.99 (d, J=8.4 Hz, 2H), 7.57 (d, J=8.8 Hz, 2H), 3.11 (t, J=7.4 Hz, 2H), 1.84-1.72 (m, 2H), 0.98 (t, J=7.4 Hz, 3H).*

3.4 Synthesis of the Pyrazinamide derivative of Imidazo[2,1-b][1,3,4]thiadiazole (6)

Compound 6 was generated as a yellow-brownish solid in 66% yield. It was TLC pure with an Rf value of 0.88 (9EtOAc:1EtOH) and a relatively short mp range of 215-220°C. The ^{1}H-NMR signals of the compound confirmed the expected structure as shown in Figure 5. The peaks corresponding to the pyrazinamide moiety appeared at 9.04 (s, J=1.5 Hz, 1H), 8.92 ppm (d, J=2.4, 1.5 Hz, 1H), and 8.78 ppm (d, J=2.5 Hz, 1H). The proximity of these proton signals which were exhibited by the similar values of the coupling constant signified that they belonged to the same electronic environment of the pyrazinamide ring and that confirms the presence of the pyrazinamide group. The attachment of the pyrazinamide group was then confirmed by the presence of the imine hydrogen signal seen at 9.26 ppm (s, 1H) and the amide hydrogen at 12.46 ppm (s, 1H) which were not observed in compound 5. This indicates that the pyrazinamide was successfully attached because of the formation of the imime bond and the loss of the aldehyde hydrogen signal. *Yellow-brownish solid (0.372g, 66%); Rf value: 0.88 (9$C_4H_8O_2$:1EtOH); IR (KBr): 1671.28 cm^{-1} (C=O), 3405.52 cm^{-1} (N–H); ^{1}HNMR (400 MHz, DMSO – d_6) δ12.46 (s, 1H), 9.26 (s, 1H), 9.04 (s, J=1.5 Hz, 1H), 8.92 (d, J=2.4, 1.5 Hz, 1H), 8.78 (d, J=2.5 Hz, 1H), 8.01 (d, J=8.6 Hz, 2H), 7.53 (d, J=8.6 Hz, 2H), 3.09 (t, J=7.4 Hz, 2H), 1.86-1.76 (m, 2H), 1.01 (t, J=7.3 Hz, 3H).*

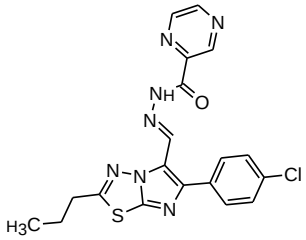


Figure 5

4. CONCLUSIONS

The pyrazinamide derivative of Imidazo[2,1-b][1,3,4]thiadiazole (6) was generated in 4 steps starting with the formation of the thiadiazole ring, followed by the imidazothiadiazole core. The formation of the aldehyde group at the 5th position was done to attach the pyrazinamide group via an imine bond formation. This generated the final compound (6) as a yellow-brownish solid in 66% yield. The signals obtained from the ^{1}H-NMR spectra of compound 6 confirmed the identity and structure of the compound.

Further characterizations have to be made on the precursors and the final compound to complete the confirmation of their identities. Also, the final compound has to be subjected to anti-microbial and anti-mycobacterial biological tests to check its potency as an anti-tuberculosis therapeutic molecule.

5. ACKNOWLEDGMENTS

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