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Synthesis and Spectroscopic Analyses of Metronidazole-Naphthol Derivative

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Abstract

The synthesis of metronidazole-naphthol derivative (Sample ***5****) was achieved by the reduction of metronidazole (Sample* ***1****) with zinc in aqueous solution of hydrochloric acid and diazotization of the reduced product (Sample* ***2****) with aqueous solutions of $NaNO_2$ and HCl at a temperature range of 0 – 5 ^{0}C. Diazo-coupling of 2- Naphthol unto the diazonium salt of metronidazole gave rise to metronidazole-naphthol derivative. The synthesis produced a yield of 28.2 and 36.4% for Sample* ***2*** *(1-(2-Hydroxyethyl)-2-methyl-5-aminoimidazole) and Sample* ***5*** *respectively. The purity of samples 1, 2 and 5 were verified from their melting points of 158 – 160 ^{0}C, 154 – 156 ^{0}C, 145 – 146 ^{0}C respectively. The* ***colour*** *variation of Samples 1, 2 and 5 were cream, light- brown and red respectively. The chemical structures and bonding of the samples were verified with the use of Ultraviolet / Visible spectroscopic analysis, results obtained shows a maximum wavelength of 217 – 236 nm, 514 – 571 nm and 500 – 545 nm for Samples 1, 2 and 5 respectively. Fourier transform infrared spectroscopic analyses (FTIR) were employed to confirm the functional groups in the compounds. The appearance of vibrational peaks at wavelengths of 3733.72 - 3627.33 cm^{-1}, 3218.04 cm^{-1}, 1464.71 cm^{-1}, 1362.24 – 1213.85 cm^{-1} for amino (-N-H), hydroxyl (-OH), stretching (-CH2) and cyano (C-N) groups respectively signifies the presence of the proposed amino product of metronidazole (Sample* ***2****) while the peaks at 3381.24 cm^{-1} (-OH), 3099.11 (C-H), 1617.00 cm^{-1} (C=C), 1533.85 (-N=N-), 1474.79 (-CH2) and 1366.51 - 1239.15cm^{-1}(-C-N) confirms the structure of metronidazole-naphthol derivative (Sample 5).*

Keywords: Functional group, vibration, melting point, wavelength, peaks.

INTRODUCTION

The compound metronidazole is known as 2-(2-methyl-5-nitro-1H-imidazole) ethanol based on international union of pure and applied chemist (IUPAC) nomenclature; it is commercially called flagyl around the globe especially in Nigeria. Metronidazole is an unsaturated organic heterocyclic belonging to the imidazole family with antimicrobial properties (Patel *et al.,* 2021) The different functional groups in the chemical structure of metronidazole have a lot of impact on the molecules' activity. A molecule of metronidazole consists of an imidazole ring as the nucleus with 2-hydroxylethyl substituted at C_1, it also possesses a methyl functional group at C_2 with a nitro group at C_5 (Mirzael *et al.,* 2008). A typical structure of metronidazole is shown in figure 1.

The history of heterocyclic compounds dates to the 18th century and some of the notable developments such as the isolation of alloxan from uric acid by Luigi Brugnatelli in 1818,

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production of furfural by J. W. Dobereiner in 1832 and dry distillation of bones to obtain pyrrole in 1834 by F.F Runge have been reported (Arora *et al.,* 2012). Metronidazole **1** as a heterocycle has an imidazole ring made up of heteroatoms such as carbon and nitrogen. Therefore, according to classification of heterocycles by Eicher et al., (2003), it can be classified as a five-membered two heteroatom heterocyclic compound. Examples of other compounds with different heteroatoms are Stibole, Arsinane, Bismolane, Borinane, Pyrrole, Furan **7**, etc (Celebioglu and Uyar, 2019).

These compounds (**2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13, 14 and 15**) as shown in figure 2 contains antimony, arsenic, bismuth, boron, nitrogen, oxygen, phosphorus, selenium, silicon, sulphur, tellurium and tin respectively. Other examples of elements that can be found in heterocyclic compounds include germanium in 4-benzyl-2,2,6,6- tetramethylperhydro-1,4,2,6-oxazadigermine **14** , lead in 10,10- dimethylphenoxaplumbin **15** and mercury in 5H,13H-12-oxa-5- aza-7-mercura-benzo[4,5]cyclohepta[1,2-a]naphthalene-6-one **16** (Yaragatti and Kulkarni, 2010).

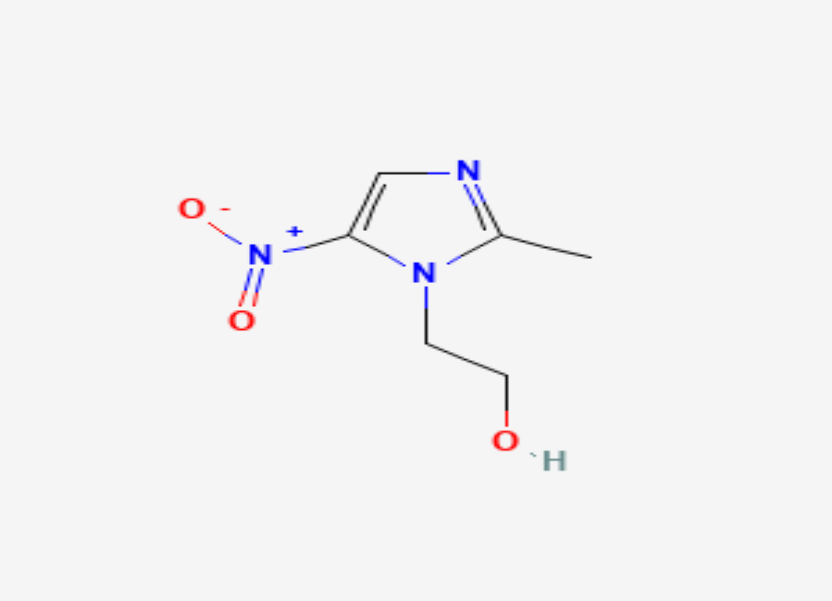


Figure 1: Chemical structure of metronidazole 1

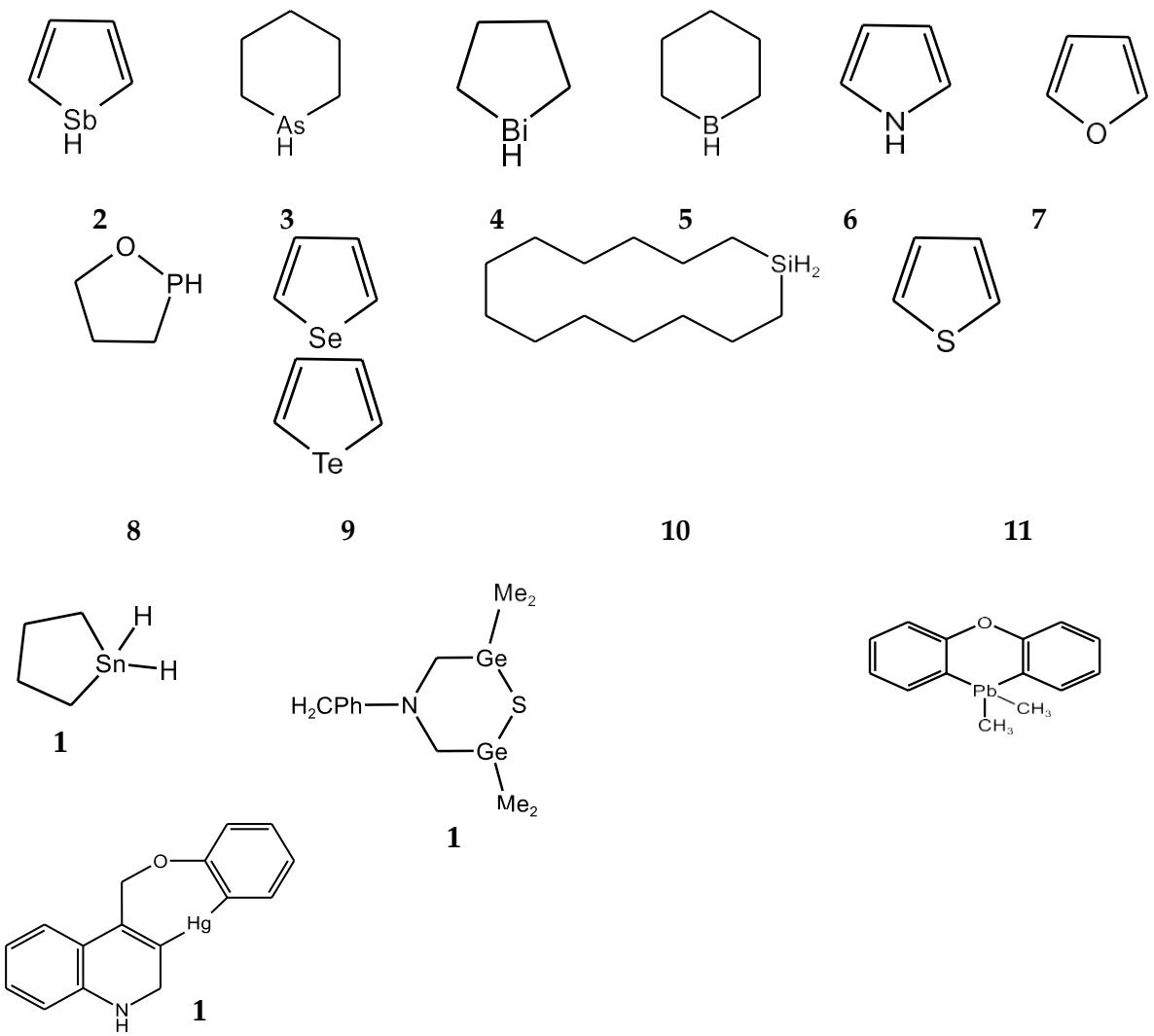
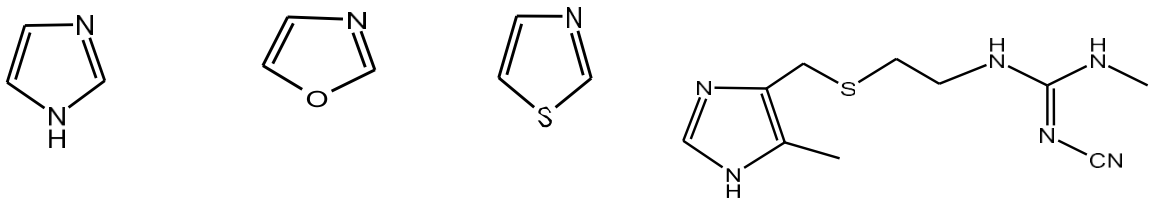


Figure 2: Examples of organic heterocycles with different heteroatom

Imidazole **17** is the heterocyclic nucleus of metronidazole, it is a member the class of the 1,3-azoles, other members of this class are oxazole **18** and thiazole **19.** Among the1,3-azoles, imidazole is the most researched unsaturated heterocycle due to its pharmacological, biological and catalytic effects. Some simple derivatives of imidazole have been associated with antimicrobial and fungicidal activities and majority are seen to possess simple structural features with different substitution patterns, Its ring structure can also be recognized in synthetic drugs such as cimetidine **20** and metronidazole **2** (Al-Masri *et al.*, 2011). The structures of imidazoles and other 1,3 azoles are shown in figure 3

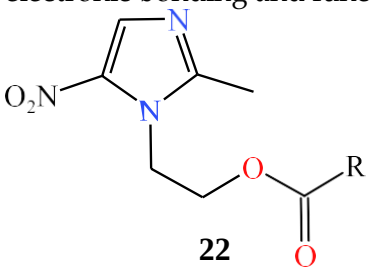


17 **18** **19** **20**

Figure 3: Structures of Imidazole, thiazole, oxazole and cimetidine

Most parasite and bacterial stocks have the potential to develop metronidazole resistance hence the need to synthesize a wide range of metronidazole derivatives. Microorganisms such as *Trichomonas vaginalis, Gardia lambia, Entamoeba Histolytica, Clostridium difficile etc* are resistant to metronidazole. Numerous research teams have produced a wide range of synthetic derivatives of metronidazole (which do not lose their nitro group) by substituting different structural counterparts, such as flavones, chalcones, hydrazides, acids, aliphatic and aromatic amines, alcohols and nitrogen heterocycles for example quinolone, indole, imidazole, thiazole, pyrazole, triazole oxadiazole and isoquinoline), or the *N*-alkyl linker and Methyl group of metronidazole, or both of the groups on metronidazole having different structural counterparts (Patel *et al.*, 2021; Mustapha *et al.*, 2008). Some substituted derivatives of metronidazole are aminomethylbenzoate esters of metronidazole 22, metronidazole phosphate ester 23, metronidazole benzoate 24, acetic acid of metronidazole 25 etc. as shown in figure 4.

The aim of this study is to synthesize a metronidazole naphthol derivative which is anticipated to increase the antibacterial and antiparasitic proficiency of metronidazole. The study is also aimed at carrying out ultraviolet and infrared spectroscopic analyses on the produced metronidazole derivative to ascertain its active ingredients in terms of the electronic bonding and functional groups in the compound.



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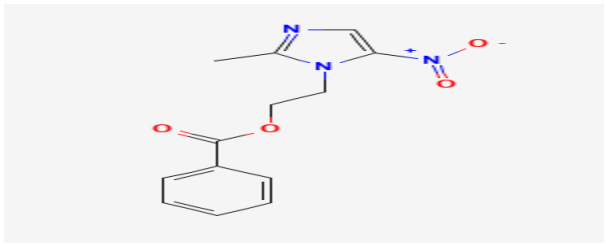

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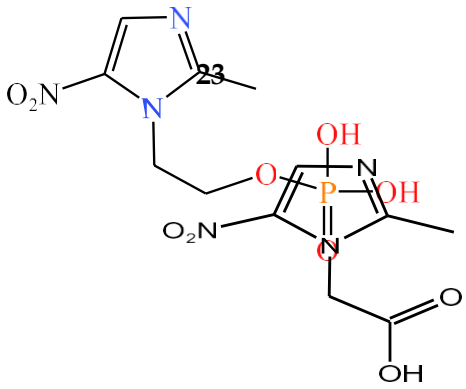

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Figure 4: Metronidazole Derivatives

Materials and Methods

Sample Collection and Preparation

All the chemicals / reagents used in this study were obtained from the analytical laboratory of the department of pure and industrial chemistry, university of Port Harcourt, Nigeria.

Melting point Determination of Reactants and Products

The melting points of the starting materials (Metronidazole and 2-Naphthol) as well as that of the products (during synthesis) were determined to ascertain their respective purities. A capillary tube was heated at one end for 2-3 minutes while continuously rotating it closing the other end. The open end of the capillary tube was dipped into the finely powdered sample to 1-2 cm of the tube with the closed end of the tube held between the finger and the

thumb. The capillary tube was attached to a thermometer with the help of a thread and placed in the groove of an aluminium block, ensuring that the capillary tube holding the sample was at the middle of the groove. The aluminium block was placed on a tripod stand above a kerosene burner and heated continuously while watching the temperature. The temperature at which the sample started melting was noted as well as the temperature at which it melted completely. The average of both readings was recorded as the melting point of the sample (Dingsdag *et al.,* 2017)

Synthesis of 1-(2-Hydroxyethyl)-2-methyl-5-aminoimidazole (Sample 2)

A 15 g (0.084 moles) metronidazole was accurately weighed and transferred into a 250mL Erlenmeyer flask together with 70ml of 1.0M hydrochloric acid (HCl) solution and stirred for 10 minutes. A 50 g (0.0765 moles) zinc dust was measured and added gently into the stirring solution of metronidazole and HCl. Gradually, as the zinc dust was being added, the colour of the resulting mixture changed from yellowish white to reddish brown. The entire mixture was stirred adequately for a period of two hours and the temperature of the reaction increased slightly from 40- 60 ^{o}C. When the time elapsed, the mixture was allowed to cool to room temperature, filtered using a Whatman filter paper and the filtrate collected and washed. The reaction was monitored using the colour changes that appeared during the reaction process. The organic product formed in the mixture was extracted three times with 30mL of chloroform and 30mL of ethanol. The organic and inorganic layers were separated using a 500mL separating funnel. The organic layer collected was dried with enough anhydrous Na_2SO_4, filtered and was allowed to evaporate at room temperature. On completion of evaporation, reddish brown crystals of 3.5g were obtained with a yield of 28.2%. (Tolomeu and Fraga, 2023) The reaction for this process is shown in equation 1:

Diazotization of Sample 2 and coupling with 2-Naphthol.

A 2.5 g of sample **2** was dissolved in 10 mL of 1M HCl solution in a 100 mL beaker marked **A** and cooled in an ice water bath maintained at a temperature of 0 – 5 ^{0}C for 20 minutes. In a test tube marked **B**, 2g of $NaNO_2$ was mixed with 10 mL of distilled water and cooled in the same ice water bath for another 20 minutes. A 2g of 2-Naphthol was accurately weighed and dissolved in 10 mL of 1M NaOH solution in a separate 100 mL beaker marked **D**. The resulting solution was placed in the ice water bath for 20 minutes. After cooling, the content of test tube **B** was poured gradually into the 100 mL beaker marked **A** containing sample **2** and hydrochloric acid solution with constant stirring in the ice water bath resulting in sample **3** (metronidazole diazonium ion) as shown in equation 2. The temperature of each solution in beaker **A** and **D** was constantly monitored and maintained in the range of 0 – 5^{o}C. A solution of sulfamic acid was added into the mixture to remove excess or any unreacted sodium nitrite.

The content in beaker **A** was transferred gently into the beaker **D** containing the 2-Naphthol solution with occasional stirring of the solution. After stirring, a red precipitate appeared. The mixture was filtered, the residue dried and recrystallized with ethanol. The recrystallized solution was filtered, allowed to evaporate at room temperature and air dried. A reddish compound, weighing 0.91g with a yield of 36.4% was obtained and this is sample **5** which is the metronidazole naphthol derivative as shown in equation 3 (Wang *et al.,* 2013).

Analyses of Samples using Ultraviolet-Visible Spectrophotometer (UV-Vis)

An ultraviolet visible spectroscopic analysis was carried out on samples 1, 2 and 5 respectively using a Jenway 6850 UV/Vis spectrophotometer. The cuvette was filled with

the sample and placed in the already calibrated spectrophotometer in the appropriate direction. The cuvette containing the sample was covered to prevent ambient light from penetrating. The start button of the processing unit connected to the spectrophotometer was initiated, the absorbance spectrum of the sample was downloaded by allowing the instrument to scan through different wavelengths. The wavelength corresponding to the maximum absorbance was obtained from the absorbance spectrum as shown in figures 5, 6 and 7 respectively (Saheed *et al.,* 2020).

Analyses of samples using Fourier Transform Infrared Spectrophotometer (FTIR)

Potassium bromide (KBr) equivalent to 100 mg was properly mixed with the sample. The KBr-sample mixture was added to the sample compartment and further mixed for transparent disc formation and then introduced to the equipment for analyses. A beam of infrared light was passed through an interferometer which splits into two separate beams. Each of the beams passes through the sample and reference respectively. The distribution of the infrared light that passes through the interferometer was altered using a moving mirror placed inside the apparatus. The signal directly recorded, called an "interferogram", represents light output as a function of mirror position. The beams pass through a splitter and then reflected back towards a detector. The splitter alternates the beams that enter the detector. A data-processing technique called Fourier transform turns this raw data into the desired result which is the sample's spectrum. The functional groups present in the sample was interpreted from the spectrum (Adegoke and Umoh, 2009).

Results

Table 1: Melting Points of Metronidazole (Sample 1), 1-(2-Hydroxyethyl)-2-methyl-5-aminoimidazole (Sample 2) and Metronidazole naphthol derivative (Sample 5)

Samples	Melting points (^{o}C)	Colour
Sample **1**	158 – 160	Cream
Sample **2**	154 – 156	Light brown
Sample **5**	145 – 146	Red

Table 2: UV/Vis and FTIR Characteristics of Samples 1, 2 and 5

Samples	UV/Vis, λ max (nm)	FT-IR (cm^{-1})
Sample **1**	217 – 236	3212.39 (-OH), 3099.41 (C-H aromatic), 1558.81 – 1506.78 (-NO_2) 1486.93 – 1424.38 (-CH_2) 1366.94 – 1263.99 (C–N aromatic)
Sample **2**	514 - 571	3733.72 - 3627.33 (-N-H), 3218.04 (-OH), 1464.71 (-CH_2), 1362.24 – 1213.85 (C–N aromatic)
Sample **5**	500 - 545	3381.24 (-OH), 3099.11 (C-H aromatic), 1617 (C=C aromatic), 1533.85 (-N=N-) 1474.79 (-CH_2) 1366.51 - 1239.15 (C-N aromatic)

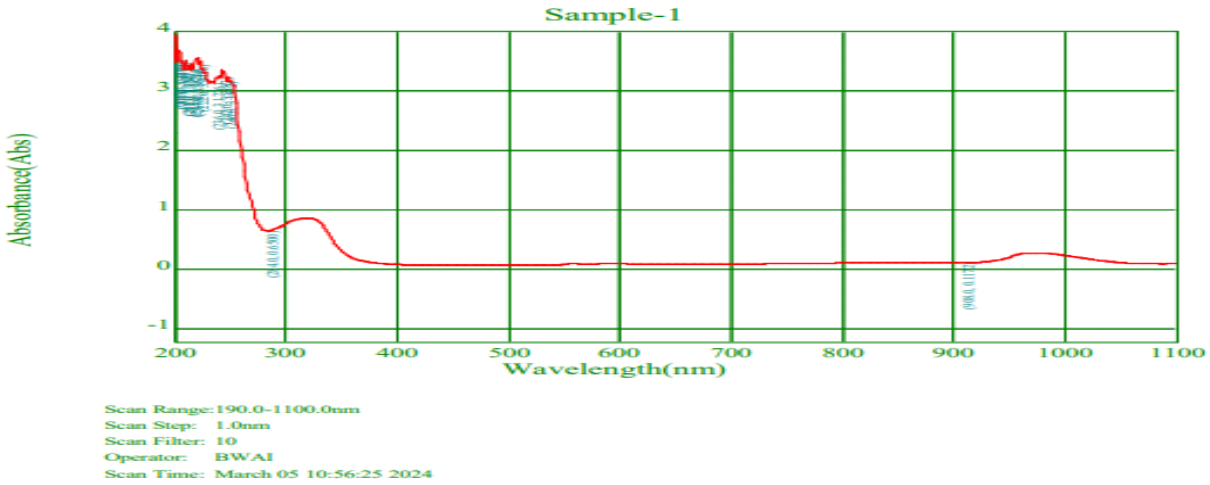


Figure: 5: UV/Vis spectrum of Sample 1 (metronidazole)

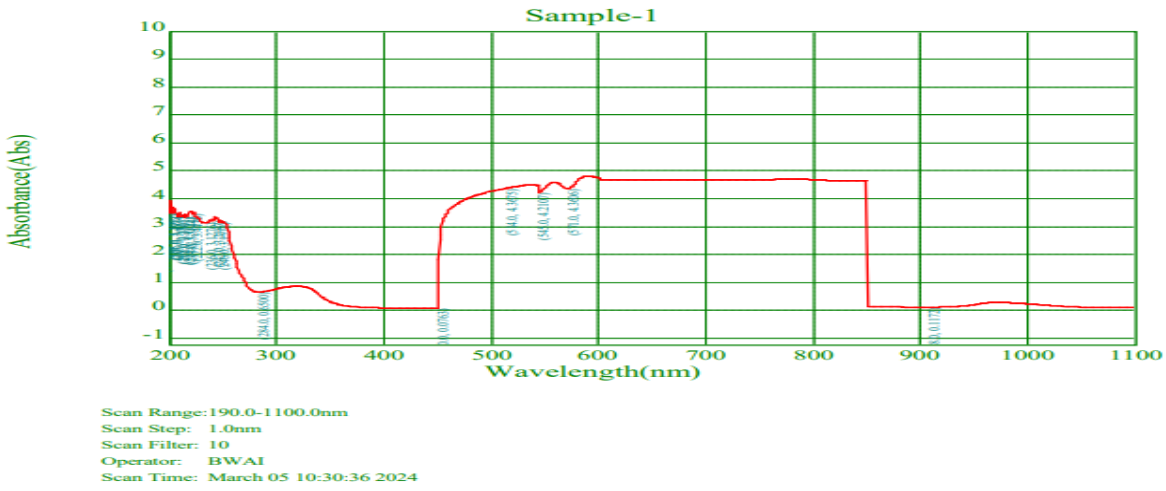


Figure 6: UV/Vis spectrum of Sample 2 (1-(2-Hydroxyethyl)-2-methyl-5-aminoimidazole)

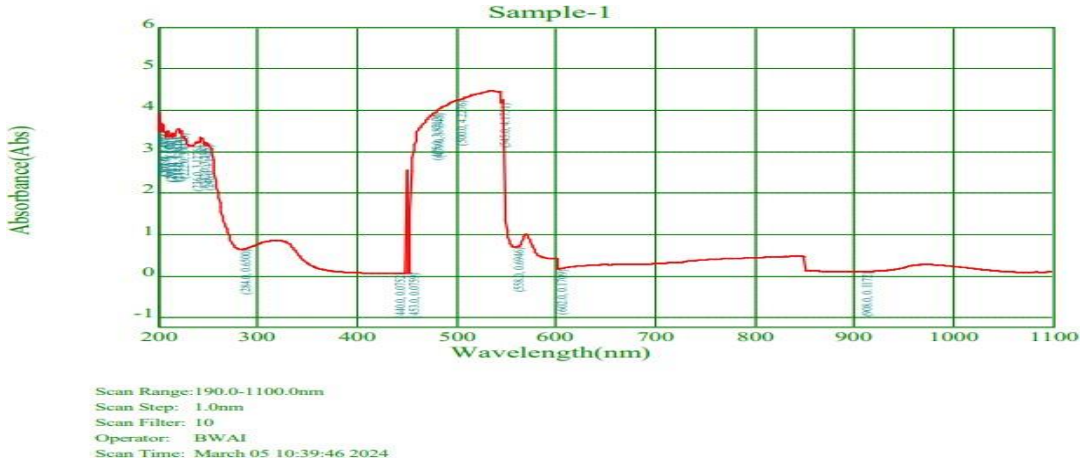


Figure 7: UV/Vis spectrum of Sample 5 (metronidazole - naphthol derivative)

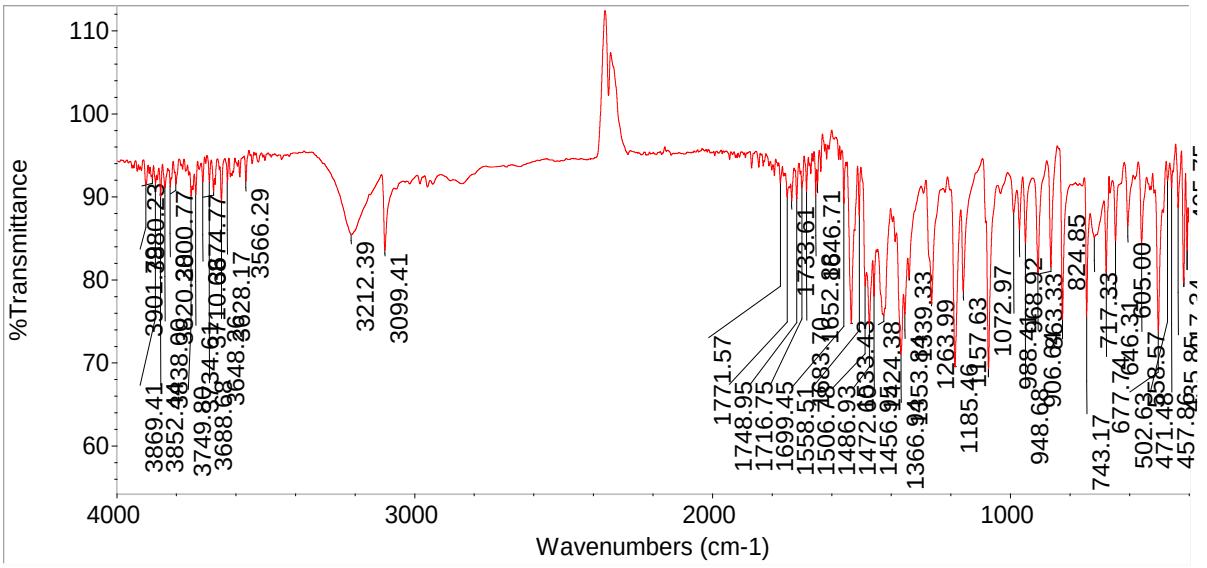


Figure 8: FT-IR spectra of sample 1 (metronidazole).

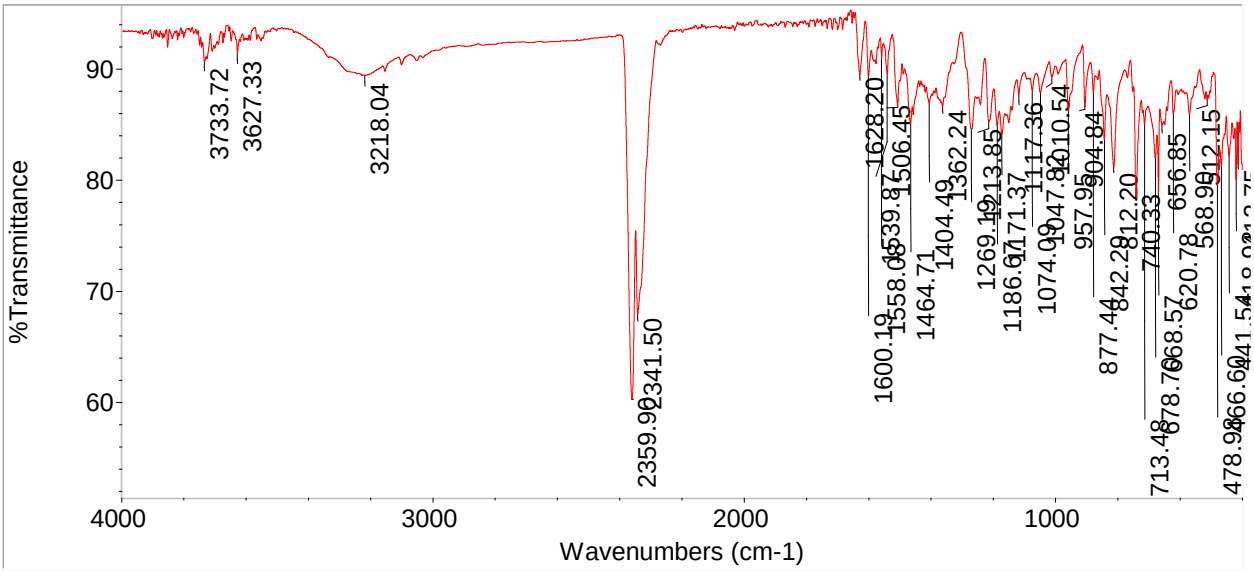


Figure 9: FT-IR spectra of sample 2 ((1-(2-Hydroxyethyl)-2-methyl-5-aminoimidazole

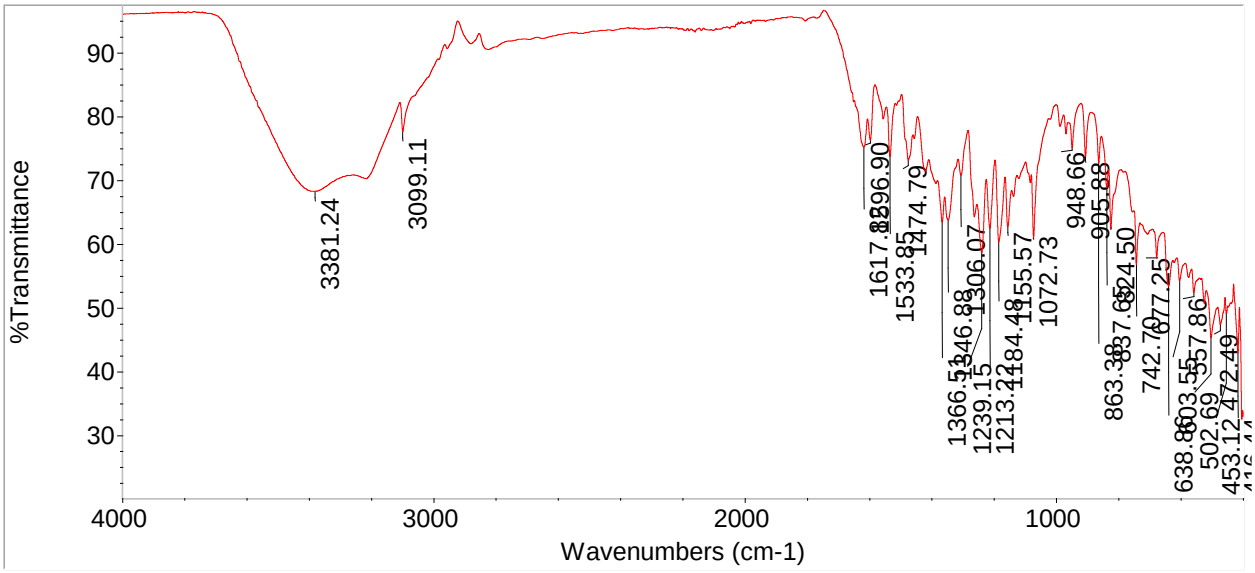
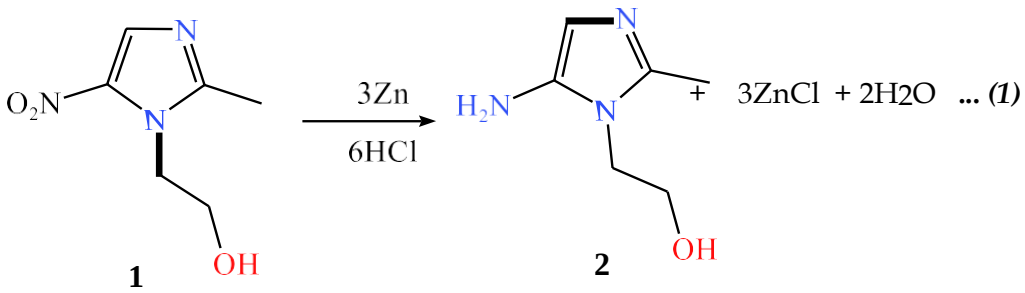
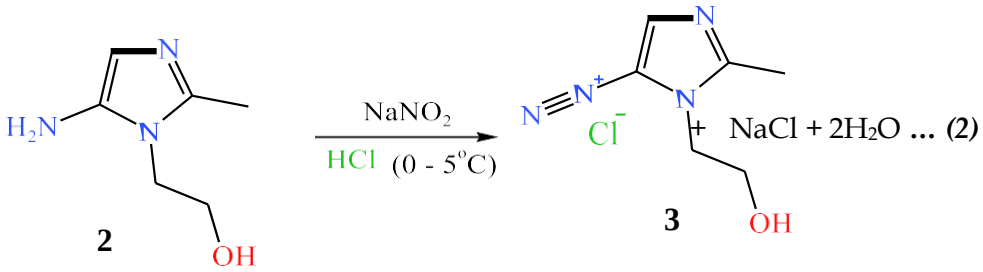


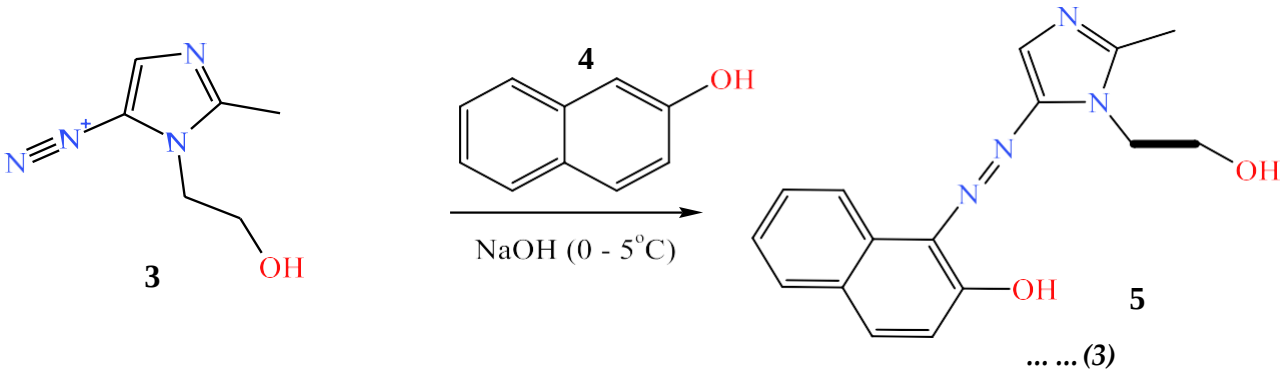
Figure 10: FT-IR spectra of sample 5 (metronidazole - naphthol derivative)

Synthesis of 1-(2-Hydroxyethyl)-2-methyl-5-aminoimidazole (Sample 2)



Diazotization of compound 2 and coupling with 2-Naphthol.





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Discussion

Melting point is one of the critical physical properties that indicates the level of purity of organic compounds. Metronidazole (Sample **1**) which is the starting material was converted to a light brown crystal after the reduction process to obtain 1-(2-Hydroxyethyl)-2-methyl-5-aminoimidazole (Sample **2**) with a percentage yield of 28.2% with a slightly reduced melting point from the starting material metronidazole (Sample **1**) as shown in Table 1. The diazotization process of Sample **2** using solutions of $NaNO_2$ and HCl as shown in equation 2 was carried out at very low temperatures below 5 ^{0}C in an ice water bath with a thermometer inserted for monitoring the temperature, this is because at elevated temperatures, diazonium salts become unstable resulting in the release of nitrogen gas from the diazonium ion, which creates unwanted substituents that interferes with the reaction process (Alonzo *et al.,* 2019). The diazotization of Sample **2** led to the formation of metronidazole diazonium ion. The 2-naphthol in equation 3 was first dissolved in NaOH in situ to produce naphthoxide ions which is more reactive than 2-naphthol to facilitate the coupling process unto Sample 3. The product of the coupling reaction is a reddish diazo product of metronidazole naphthol derivative with a percentage yield of 36.4 % and a melting point of 145-146 ^{0}C which is Sample **5**. From Table 1 it can be deduced that there is a reduction in melting point from the metronidazole starting material to the metronidazole naphthol derivative product. The absorption of the active ingredients of pharmaceutical compounds such as metronidazole increases with decrease in their melting point. The fraction absorbed for drugs with high melting points is limited to a greater degree compared to drugs with low melting points of the same dose (Ang *et al.,* 2017). Results obtained therefore shows that the efficacy of metronidazole naphthol derivative is more promising than metronidazole judging from their melting points.

The UV/Vis spectrum of samples 1, 2 and 5 in aqueous solutions were analyzed. The wavelength of their respective maximum absorbances were determined as shown in Table 2. Results obtained corresponds with the chemical bonding of the chemical structure of metronidazole, 1-(2-Hydroxyethyl)-2-methyl-5-aminoimidazole and metronidazole naphthol derivative as shown in figures 5, 6 and 7 respectively. Absorbances are represented by absorption peaks as shown in the spectrum, the wavelength at which the highest peak elutes is the maximum wavelength. Results obtained confirms that metronidazole (Sample **1**) absorbs energy radiation in the ultraviolet range (200-400nm) of the electromagnetic spectrum while 1-(2-Hydroxyethyl)-2-methyl-5-aminoimidazole (Sample **2**) and metronidazole naphthol derivative (Sample **5**) absorbs within the visible range of the electromagnetic spectrum. Absorption either ultraviolet or visible could result

in electronic transitions of molecules, that is molecular electrons moving from a lower energy to a higher energy molecular orbital (Saheed *et al.,* 2020).

Table 2 also shows the functional groups of samples 1, 2 and 5 as obtained from the infrared spectroscopic analyses performed on the samples. The infrared spectrum of Sample **1** as shown in Appendix **1** affirms the presence of -OH and -NO_2 functional groups at wavelengths of 3212.39 and 1558.1 – 1506.73 cm^{-1} respectively. The spectrum of Sample **2** in Appendix **2** shows the disappearance of -NO_2 group and the presence of -NH_2 with vibrational peaks at 3733.72 – 3627 cm^{-1} wavelengths representing -N-H stretching vibrations due to the reduction of the nitro group to form the amino group as shown in equation 1. The product obtained after diazotization and coupling of Sample **2** with 2-Naphthol was confirmed to be sample **5** with the appearance of a broad peak at 3381.24 cm^{-1}, accompanied by a slightly visible band at 3099.11 cm^{-1} indicating the presence of -OH group and C-H stretching respectively. The azo linkage (-N=N-) was confirmed with a single band in 1533.85 cm^{-1} while the -C=C aromatic peak was confirmed with a peak at 1617 cm^{-1} wavelength. The -C-N aromatic peak at 1366.94 – 1263.99 cm^{-1} and the -CH_2 symmetric stretching at 1464.71 cm^{-1} (located as a substituent) were found in Samples **1, 2** and **5** serving as an additional confirmation for the structures of all three samples (Saffaj *et al.,* 2004). The infrared spectrum of Sample **5** is shown in Appendix **3.**

Conclusion

The synthesis of metronidazole-naphthol derivative involves the reduction of metronidazole with zinc dust in aqueous solution and diazotization of the reduced compound in cold solutions of nitrite and hydrochloric acid, producing a diazonium salt which undergoes subsequent coupling with 2-Naphthol. The reaction process was monitored using the color changes that were observed from the addition of zinc dust to the coupling of 2-naphthol at 0 – 5 ^{o}C. The purity of the starting material (metronidazole), the products 1-(2-Hydroxyethyl)-2-methyl-5-aminoimidazole and metronidazole naphthol derivative were confirmed from their melting points which followed a downward trend affirming a higher activity of metronidazole naphthol derivative as opposed to metronidazole. The chemical bonding and structures of the synthesized compounds were affirmed using a UV/Vis spectrometer while the functional groups were confirmed through infrared spectroscopic analyses. Metronidazole naphthol derivatives can be applied in the production of medicines, pigments, fungicides, insecticides, dyes, and perfumes; Previously employed as an anthelmintic, they are also utilized as antioxidants for rubber, fats, and oils, as well as an antiseptic and lubricant for electric motors, steam turbines, hydraulic equipment, and instruments.

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