

Synthesis, X-Ray Crystal structure and Evaluation of Biological Activities of 1-(Acetoxyethyl)-2-Methyl-5-Nitroimidazol

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ABSTRACT

1-(Acetoxyethyl)-2-methyl-5-nitroimidazol **1** has been synthesized from 2-(2'-methyl-5'-nitro-1H-imidazol-1'-yl) ethanol known as metronidazole. X-Ray Crystal structure of the compound **1** is now described and shows a stereopair in the asymmetric unit, also a crystal packing of these two molecules on each other in attachment by Van der Waals contact. The Crystal system of this compound is monoclinic with a Space group P21/c and a R-value of 4.34%. Moreover, the characterization of the compound **1** has been confirmed by using of the proton, carbon-13 and DEPT-135 Nuclear Magnetic Resonance data. The evaluation of its *in vitro* antibacterial activities shows that the compound **1** is most active than its analogue 2-(2'-methyl-5'-nitro-1H-imidazol-1'-yl) ethanol taken as reference. Against the anaerobic bacteria *Eutrobacterium species* and the aerobic strong bacteria *Pseudomonas aeruginosa* ATCC, 1-(Acetoxyethyl)-2-methyl-5-nitroimidazol **1** presents a Minimal Inhibition Concentration (MIC) respectively around 62.50 mg/l and 1000 mg/l, whereas the MIC of metronidazole amount around 125 mg/l against the bacteria *Eutrobacterium species*. Against the aerobic bacteria *Pseudomonas aeruginosa* ATCC, metronidazole doesn't show any antibacterial activity. This confirms the accuracy of the biological results, because metronidazole, according to the mechanism of action, is only cytotoxic to anaerobic bacteria cells. An antibacterial activity of the derivative **1** against the aerobic strong bacteria *Pseudomonas aeruginosa* ATCC allows us to think that this derivative can be used to some extent against the mixed infections.

Keywords: 1-(Acetoxyethyl)-2-methyl-5-nitroimidazol, monoclinic Crystal system, Crystal packing of a stereopair in the asymmetric unit, higher antibacterial activity in mixed infections

INTRODUCTION

Known as metronidazole and marketed by Sanofi-Aventis under the trade name Flagyl, 2-(2'-methyl-5'-nitro-1H-imidazol-1'-yl) ethanol is an antibiotic and antiprotozoal medication that can be used against anaerobic bacteria and protozoa^{1,2}.

Anaerobic bacteria are revealed responsible for some infections; among them intra-abdominal infections including peritonitis, intra-abdominal abscess³, gynecological infections⁴.... Protozoa cause for instance amoeba from which the symptoms appear of progressive form after the ingestion of the mature cysts⁵.

In order to fight anaerobic bacteria such as *Eutrobacterium species* and protozoal parasites like *Entamoeba histolytica*, metronidazole goes on to be administered; but the presence, in lots of cases, of mixed infections of both aerobic and anaerobic bacteria on the one hand, the regulation without control of antibiotics like metronidazole on the other hand, and therefore its misuses have favored the emergence of antibacterial drug resistant pathogens⁶. Nowadays, for the effective treatment against all attack stages of some bacteria and protozoal parasites, metronidazole can be used only in combination with other antibiotics such as norfloxacin⁷. The combination of metronidazole and norfloxacin is marketed under the trade name Normet or Normegyl.

According to the mechanism of action, metronidazole inhibits nucleic acid synthesis by disrupting the DNA of microbial cells. This function occurs only when metronidazole is partially reduced; and because this reduction usually happens only in anaerobic cells, it has relatively little effect upon human cells or aerobic bacteria⁷. DI. Edwards adds that metronidazole is cytotoxic to facultative anaerobic bacteria, but the mechanism of this action is not well

understood⁸.

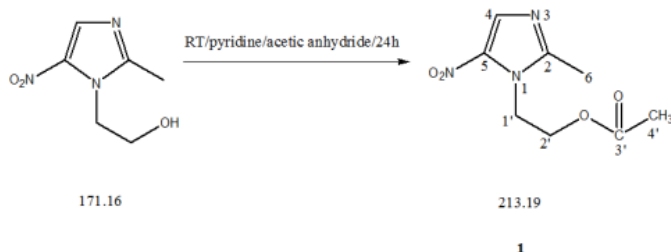
Owing to the presence, in lots of cases, of mixed infections, the increase of the resistance to metronidazole and the ambiguous mechanism of action of this drug, we have thought that we can favor the penetration of the drug in the cells for improving the antibacterial activity. For this reason, we have proposed to transform the polar hydroxyl group of metronidazole in less polar ester group by acetylation because of an apolar character of membrane cells. The derivative obtained by structural modification and metronidazole(taken as reference) will be subjected to *in vitro* antibacterial experiments above cell culture assays.

The design of the synthesis of 1-(Acetoxyethyl)-2-methyl-5-nitroimidazol, the determination of its Crystal structure by X-Ray diffraction accompanied of the *in vitro* evaluation of its antibacterial activities in covering to the anaerobic bacteria such as *Eutrobacterium species* and the aerobic strong bacteria *Pseudomonas aeruginosa* ATCC expressed by the Minimal Inhibition Concentration (MIC) make up important contributions of this work.

The absence of the antibacterial activity of the metronidazole against the aerobic bacteria *Pseudomonas aeruginosa* ATCC confirms the accuracy of the biological results, because metronidazole, according to the mechanism of action, is only cytotoxic to anaerobic bacteria cells. An antibacterial activity of the derivative **1** against the aerobic strong bacteria *Pseudomonas aeruginosa* ATCC allows us to think that this derivative can be used to some extent against the mixed infections.

Synthesis of 1-(acetoxyethyl)-2-methyl-5-nitroimidazol (1)

Compounds **1** has been synthesized according to Scheme 1. The most acetylation reactions of a hydroxyl group are carried out by using a catalyst which eases the reaction^{9,10}. On the basis of the availability of the reagents, we have designed and synthesized 1-(Acetoxyethyl)-2-methyl-5-nitroimidazol(**1**) by using pyridine as a base for the deprotonation of the hydroxyl group of metronidazole and as solvent for the reaction. A solution of copper sulfate was used as indicator for the presence of pyridine that must be removed. It was observed that a reaction time of 24h at room temperature with acetic anhydride was necessary for getting the derivative **1**, after extraction of this product from the reaction mixture with ethyl acetate and evaporation of this solvent, as light brown crystals in good yield of 60%.



Scheme 1. The synthesis of 1-(Acetoxyethyl)-2-methyl-5-nitroimidazol(**1**)

EXPERIMENTAL PART

Materials. The chemicals for the synthesis were procured from commercial sources, and a structural confirmation of metronidazole was carried out by NMR analyses.

TLC: aluminium sheets silica gel (60 F254 Merck).

1-(Acetoxyethyl)-2-methyl-5-nitroimidazol (1**).** To the powder of 2-(2'-methyl-5'-nitro-1H-imidazol-1'-yl) ethanol (2.5g, 14.6mmol) in a 100ml round-bottom flask was added pyridine (25ml, 309.1mmol). The resulting solution was stirred during 25min at room temperature. Acetic anhydride (25ml, 265mmol) was added dropwise, and the reaction mixture was further stirred for 24h. Then, the reaction mixture was poured in 150ml water, and the product was extracted with ethyl acetate (3x50ml). The combined organic extracts were washed with water (a solution of CuSO₄ was used for the detection of pyridine), dried over MgSO₄ and evaporated to dryness under reduce pressure to give light brown crystals. Yield 60%.

The structure of the synthesized compound was characterized by X-Ray diffraction and confirmed by ¹H-NMR, ¹³C-NMR and ¹³C-DEPT-135.

The Crystal structure of the derivative **1** was carried out with the collection of X-ray intensities at 100K on a SMART 6000 diffractometer equipped with CCD detector using CuK α radiation ($\lambda=1.54178\text{\AA}$), using ϕ and ω scans. The images were interpreted

and integrated with the program SAINT from Bruker¹¹. The structure was solved by direct methods and refined by full-matrix least-squares on F² using the SHELXTL program package¹². During the refining procedure, non-hydrogen atoms were refined anisotropically and the hydrogen atoms in the riding mode and isotropic temperature factors at 1.2 times U(eq) of the parent atoms (1.5 times for methyl groups). Details of crystal data and parameters of data collection are given in Table 1.

¹H-, ¹³C-NMR and ¹³C-DEPT-135 spectra were recorded at room temperature on a Bruker 600 instrument operating at a frequency of 600 MHz for ¹H-NMR and 150 MHz for ¹³C-NMR and ¹³C-DEPT-135; tetramethylsilane and CDCl₃ with respect to TMS as internal reference. For ¹H-NMR, δ in ppm and J in Hz, chemical shift multiplicities are reported as s = singlet, t = triplet; for ¹³C-NMR, δ in ppm, fragments with =C=, -CH=, -CH₂-, -CH₃ were designed respectively with s, d, t and q. ¹H-, ¹³C-NMR and ¹³C-DEPT Nuclear Magnetic Resonance data are given respectively in Table 2, Table 3 and Table 4.

Biological testing

1.Method¹⁴. The used method for the *in vitro* antibacterial tests was the agar disc-diffusion method. This method consists in moistening of sterile filter paper discs (8 mm diameter) with different aqueous solutions of metronidazole and its ester derivative. These filterpaper discs were carefully placed on the agar culture plates that had been previously inoculated separately with the bacteria. After the incubation of plates at 37°C, the diameters of the growth inhibition zones were measured after 24 hours.

2. Determination of minimal Inhibition Concentration (MIC)¹⁵. The MIC values were determined by serial two-fold dilution method and as the lowest concentration that completely inhibited visible growth of the microorganism as detected by unaided eye.

RESULTS AND DISCUSSION

1.Chemical results

The progress of the reaction for obtaining the metronidazole derivative **1** has been monitored by thin layer chromatography. Metronidazole shows a R_f value of 0.18, whereas its acetylated derivative **1** has a R_f value of 0.37 with ethyl acetate as eluent. The increase of the nucleophilie of the oxygen of the hydroxyl group by the deprotonation with pyridine as base eases the substitution reaction. This led up to say that there has been effectively a reaction which has given a product whose the structure is fully supported by X-ray data, ¹H-NMR, ¹³C-NMR and ¹³C-DEPT-135 Nuclear Magnetic Resonance data.

1.1.Structural elucidation by the X-ray analysis

The X-ray structural parameters of the metronidazole derivative **1** are given in Table 1.

Table 1. Crystallographic Data of the metronidazole derivative **1**

Crystal dimensions	0.4x0.2x0.2mm	No.mol./cell	2
Formula	C ₈ H ₁₁ N ₃ O ₄	d _{calc} [g/cm ³]	1.390
Formula weight(g.mol ⁻¹)	213.19	μ (CuK α)[mm ⁻¹]	0.967
Crystal system	monoclinic	F(000)	896.0
Space group	P 21/c	Temperature[K]	100
Hall group	-P2ybc	θ max[°]	70.490
a[Å]	21.6293(11)	Radiation[Å]	CuK α ($\lambda=1.5418$)
b[Å]	8.7981(4)	Scan mode	ω/ϕ

c[Å]	10.7624(6)	Collected intensities(h,k,lmax)	26, 10, 13
α [°]	90.0	No. of reflections measured	3845
β [°]	95.989(2)	No. of reflections used in Analysis I>2 σ (I)	3443
γ [°]	90.0	Data completeness	0.987
V[Å ³]	2036.87(18)	R	0.0434
Z	8	WR2	0.1064(3845)

X-Ray structure of the compound **1** shows a stereopair in the asymmetric unit, also a crystal packing of these two molecules on each other in attachment. Quite interestingly, the two imidazol rings of the derivative **1** show different ring conformations in the solid-state structure. The crystal packing in the asymmetric unit cell is apparently determined by Van der Waals contact, and no short intermolecular distances are observed (Fig.1).

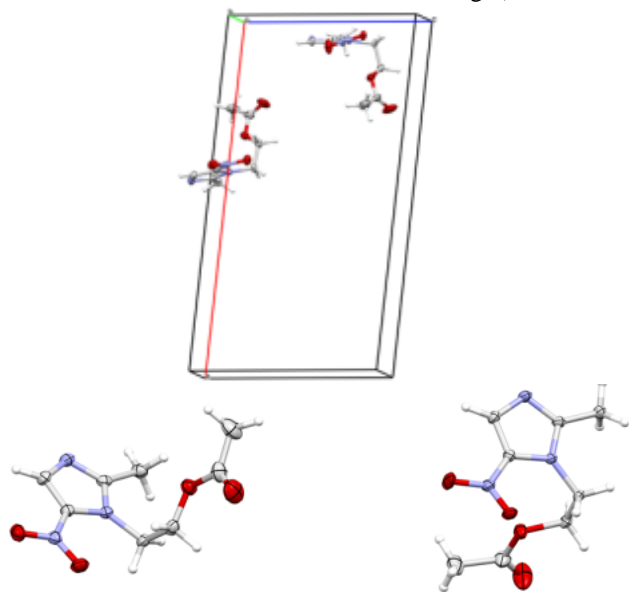


Fig.1. Stereopair of the derivative **1** in the asymmetric unit

The bond lengths and angles show usual features. The N-atoms in both imidazol rings approach planarity. The two imidazol are parallel and overlap at Van der Waals separation. This is expected to stabilize the structure¹³ (Fig.2 and 3)

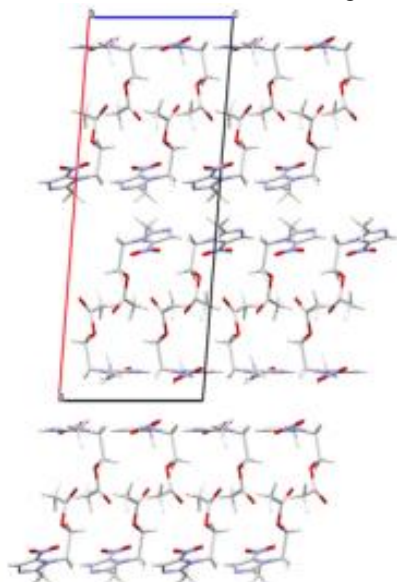


Fig.2. Projection along the crystallographic b axis showing the crystal packing of the metronidazole derivative **1**

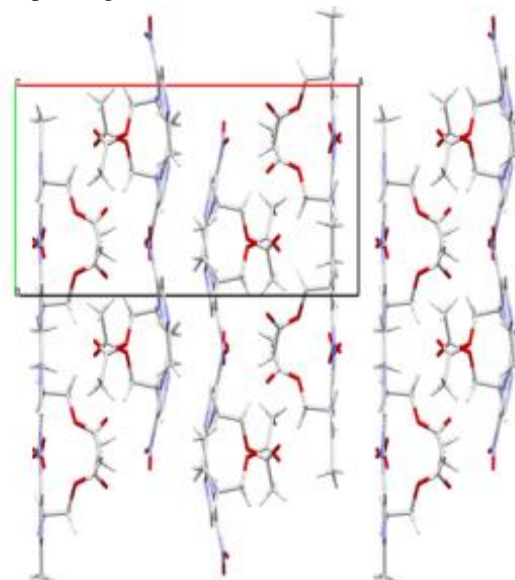


Fig.3. Projection along the crystallographic c axis showing the crystal packing of the metronidazole derivative **1**

1.2. Structural elucidation by NMR analyses

The correlation of X-ray structural and spectral parameters of the chemical compounds is an important goal in chemistry.

Table 2. ¹H-NMR Data (CDCl₃) of the metronidazole derivative **1** at 600 MHz; δ in ppm and J in Hz, internal standard TMS

H-C(4)	7.80	s
H-C(2')	4.47	t
H-C(1')	4.27	t
H-C(6)	2.40	s
H-C(4')	1.90	s

J(1',2')	5.0
J(2',1')	5.0

Table 3. ¹³C-NMR Data (CDCl₃) of the metronidazole derivative **1** at 150 MHz; δ in ppm, internal standard TMS

C(3')	170.2	s
C(5)	151.1	s
C(2)	138.5	s
C(4)	133.0	d
C(2')	62.5	t
C(1')	45.0	t
C(4')	20.5	q
C(6)	14.2	q

Table 4. ¹³C-DEPT-135 Data (CDCl₃) of the metronidazole derivative **1** at 150 MHz; δ in ppm, internal standard TMS

C(3')	-
C(5)	-
C(2)	-
C(4)	Oriented positively
C(2')	Oriented negatively
C(1')	Oriented negatively
C(4')	Oriented positively
C(6)	Oriented positively

In comparing the ^1H -NMR spectra of the metronidazole derivative **1** and metronidazole, we observe the apparition of the signal of a second methyl group (4') as a singlet at 1.90 ppm in the spectrum of the derivative **1**. In ^{13}C -NMR, the changes are also perceived through the presence of the carbon (3') of the carbonyl group at 170.2 ppm and the carbon (4') of the methyl group at 20.5 ppm in the spectrum of the derivative **1**. The presence of a second methyl group and a carbonyl group, and their chemical shifts have been proved by the structure analysis ^{13}C -DEPT-135: The carbon of the

methyl group is oriented positively, whereas the carbonyl group one doesn't appear in this spectrum.

All this leads up to confirm that there has been an acetylation reaction by substitution up to the oxygen of the hydroxyl group of metronidazole, and that the formed product is then 1-(acetoxyethyl)-2-methyl-5-nitroimidazol.

After the determination of the structure of the synthesized product, the *in vitro* antibacterial activities of the derivative **1** have been evaluated in relation to that one of metronidazole taken as reference.

2. Biological results

The *in vitro* antibacterial activities of the metronidazole and its acetylated derivative **1** against the anaerobic bacteria *Eutrobacterium species* and the aerobic strong bacteria *Pseudomonas aeruginosa ATCC* are summarized in Tables 5-8.

Table 5. Antibacterial activity of metronidazole against *Eutrobacterium species*

Concentration(mg/l)	250	125	62.50	31.25	15.63	7.81
Diameter of growth inhibition zone(mm)*	9.98±0.18	6.95±0.15	-	-	-	-

*(-): Inactive

Table 6. Antibacterial activity of the derivative **1** against *Eutrobacterium species*

Concentration(mg/l)	250	125	62.50	31.25	15.63	7.81
Diameter of growth inhibition zone(mm)*	16.90±0.20	13.05±0.15	9.98±0.18	-	-	-

*(-): Inactive

Table 7. Antibacterial activity of metronidazole against *Pseudomonas aeruginosa ATCC*

Concentration(mg/l)	1000	500	250	125	62.50	31.25	15.63	7.81
Diameter of growth inhibition zone(mm)*	-	-	-	-	-	-	-	-

* (-): Inactive

Table 8. Antibacterial activity of the derivative **1** against *Pseudomonas aeruginosa ATCC*

Concentration(mg/l)	1000	500	250	125	62.50	31.25	15.63	7.81
Diameter of growth inhibition zone(mm)*	5.75±0.15	-	-	-	-	-	-	-

*(-): Inactive

From these results, metronidazole and its acetylated derivative **1** exhibit varying degrees of inhibition against the tested bacteria. Strong activity was displayed by the compound **1**, which produced a diameter growth inhibition zone of 16.90 mm against *Eutrobacterium species* and a diameter growth inhibition zone of 5.75 mm against *Pseudomonas aeruginosa ATCC*. Thus, the MICs of metronidazole and its derivative **1** against the bacteria *Eutrobacterium species* amount around 125 mg/l and 62.50 mg/l respectively. Referring to the aerobic strong bacteria *Pseudomonas aeruginosa ATCC*, the derivative **1** acts on these at the concentration of 1000 mg/l with a diameter growth inhibition zone of 5.75 mm, whereas metronidazole doesn't exhibit any antibacterial activity. This lead us up to confirm the accuracy of these biological results, because metronidazole, according to the mechanism of action, is only cytotoxic to the anaerobic bacteria cells. An antibacterial activity of the derivative **1** against the aerobic strong bacteria *Pseudomonas aeruginosa ATCC*, on the other hand, shows that this derivative can be used to some extent against the mixed infections.

The structure-activity relationship was analyzed to understand the

influence of structures of metronidazole and its derivative **1** on their antibacterial activities. This difference in their activities can only come from their structures and not from the mechanism of action, because the both compounds contain the nitro group, that is partially reduced during this mechanism of action. Metronidazole is more polar than its acetylated derivative because of the polar character of the hydroxyl group. The acetylated derivative contains the ester group, which is less polar. Therefore, we can think that this weak polarity of the derivative **1** eases the penetration of this drug through the lipophile(apolar) membrane cells according to the principle "like dissolves like".

CONCLUSION

The design of the synthesis of a new agent for antibacterial uses by acetylation reaction, the description of its monoclinic X-ray crystal structure with a stereopair on each other in attachment by Van der Waals contact in the asymmetric unit cell and a space group P21/c accompanied of the biological tests make up the important aspects of this work.

1-(Acetoxyethyl)-2-methyl-5-nitroimidazol exhibits *in vitro* a

higher antibacterial activity than metronidazole against the anaerobic bacteria *Eutrobacterium species* and the aerobic strong bacteria *Pseudomonas aeruginosa ATCC*. This can be explained by the decrease of the polar character in the derivative **1**, that eases the penetration of this compound in the apolar membrane cells. The inactivity of the metronidazole against the aerobic bacteria *Pseudomonas aeruginosa ATCC* proves the accuracy of the biological tests, because metronidazole, according to the mechanism of action, is only cytotoxic to anaerobic bacteria cells. An antibacterial activity of the derivative **1** against the aerobic strong bacteria *Pseudomonas aeruginosa ATCC* shows that this derivative can be used to some extent against the mixed infections.

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