

## Synthesis and Antimicrobial Study of Possible Mutual Prodrugs of Amoxicillin and Metronidazole by Direct and Indirect Coupling through Spacer

Ammar A. Ali Beg\* and Ahlam J. Qasir\*\*<sup>1</sup>

\*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Kufa, Najaf, Iraq.

\*\*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

### Abstract

Amoxicillin have been conjugated with metronidazole as possible mutual prodrug to get a wider spectrum of activity by acting on aerobic and anaerobic bacteria, have antifungal activity, to provide protection for beta lactam ring of amoxicillin and also to improve patient compliance as it given as a single dose therapy. The structures of the synthesized compound were confirmed and characterized using elemental microanalysis (CHN), IR and some physicochemical properties. Biological study was done by using disc diffusion method against different bacterial strains which are , *Staphylococcus aureus* , *Salmonella typhie* , *Pseudomonas aeruginosa* , *E. coli* , *Klebsiella pneumonia* and fungi ( *Candida albicans* ) . using nutrient agar medium and 5 mm diameter paper discs. The synthesized compounds showed maintenance or improvement of the antimicrobial activity especially compound (II) showed superior activity in comparison with compounds (I, III) .

**Key words:** Amoxicillin , Metronidazole , Mutual prodrug .

### تخليق ودراسة الفعالية المضادة للميكروبات كمقدمات دوائية تبادلية لعقاري الاموكسيسيلين والمترونيدازول بطريقة مباشرة وغير مباشرة بواسطة ذراع

عمار عبد العزيز علي بييج\* و احلام جميل قصير\*\*<sup>1</sup>

\* فرع الكيمياء الصيدلانية، كلية الصيدلة، جامعة الكوفة، النجف، العراق .

\*\* فرع الكيمياء الصيدلانية، كلية الصيدلة، جامعة بغداد، بغداد، العراق .

### الخلاصة

في هذا البحث نقوم بربط عقار الاموكسيسيلين مع عقار المترونيدازول كمقدمات دوائية لكي نحصل على فعالية ذات طيف أوسع من خلال عمله على البكتريا الهوائية و اللاهوائية , لديه فعالية ضد الفطريات , توفير حماية لحلقة البيتا لاكتام في الاموكسيسيلين و لتحسين استساغة المريض للعلاج . تم تشخيص المركبات المصنعة باستخدام التحليل الدقيق للعناصر المكونة لها و من خلال الأشعة تحت الحمراء و بعض الخواص الفيزيائية والكيميائية . دراسة الفعالية الحيوية تمت باستخدام طريقة الانبعاث القرصي ضد بعض سلالات البكتريا المختلفة و الفطريات و التي هي *Staphylococcus aureus* , *Salmonella typhie* , *Pseudomonas aeruginosa* , *E. coli* , *Klebsiella pneumonia* and *Candida albicans* . ٥ مل . لقد أظهرت المركبات المصنعة تحسنا أو حفاظا على الفعالية المضادة للميكروبات وخصوصا المركب (II) حيث اظهر فعالية عالية مقارنة بالمركبات (I,III) .

الكلمات المفتاحية : الاموكسيسيلين ، المترونيدازول ، مقدمات دوائية تبادلية.

### Introduction

Anaerobic infections characteristically are polymicrobial (mixed) and include both anaerobic and facultative organisms<sup>(1)</sup>. The organisms tend to be acquired endogenously. The particular mix of pathogens reflects the combined influence of the complex commensal flora at a specific body site and the unique micro biota of the underlying conditions<sup>(2)</sup>. Because these organisms are generally of low pathogenicity, anaerobic or mixed infections generally develop as a consequence of either structural alterations in the normal mucosal barrier or tissue ischemia with lowered oxidation - reduction potential<sup>(3)</sup>. Knowledge of the anatomic location of the primary source of infection and the underlying condition of the host, therefore, is essential in predicting the probable organisms causing

anaerobic and mixed infections associated with the indigenous micro flora<sup>(4)</sup>.

Microorganism in mixed infections may respond to antimicrobial agents differently than do those in monomicrobial infections, and it may not be necessary to eradicate every bacterial species in mixed infection to achieve cure<sup>(5)</sup>. In the treatment of mixed aerobic anaerobic infection we use antibiotics that act on both type of bacteria so here we used metronidazole with amoxicillin as there is synergistic effect between them as shown In 1989, by van Winkelhoff et al.<sup>(6)</sup> who showed that mechanical treatment followed by a regimen of metronidazole and amoxicillin for 7 days is effective in eliminating *A. actinomycetem-comitans* from the infected sites. Recently, this was confirmed in a large patient group, in which more than

<sup>1</sup>Corresponding author E-mail:ahlamqasir@yahoo.com

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An explanation for the in vivo efficacy may be the in vitro synergism against 95% eradication of the microorganism was reported <sup>(7)</sup>.

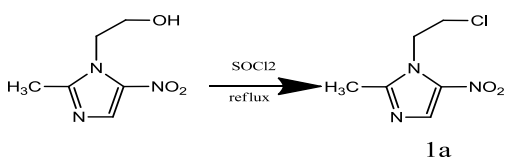
*A.actinomycetem - comitans* not only between metronidazole and amoxicillin but also between metronidazole and its hydroxymetabolite as well as between amoxicillin and the hydroxymetabolite of metronidazole <sup>(8)</sup>.

However, there are potential advantages in giving such coadmi -nistered drugs having comple -mentary pharmacological activities in the form of a single chemical entity. Such agents are named as mutual prodrugs which are designed with improved physicochemical property.

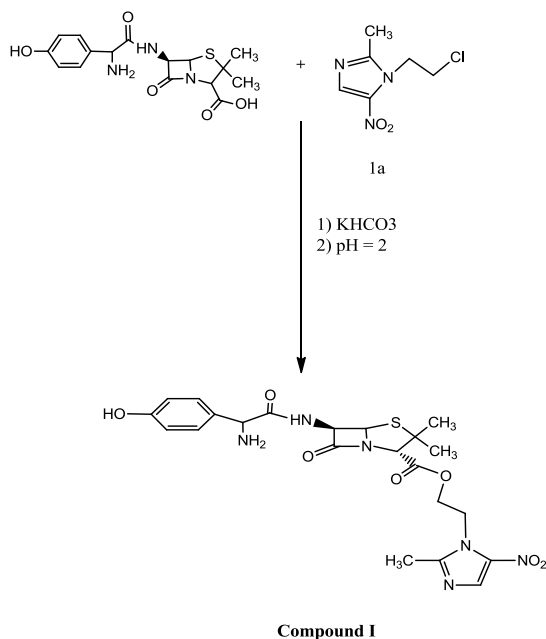
In the view of this background, the present study was conducted to the design, synthesis, and preliminary biological study of mutual prodrugs of amoxicillin with metronidazole to get antibiotic with broader spectrum of activity and given by single dose.

**Chemistry**

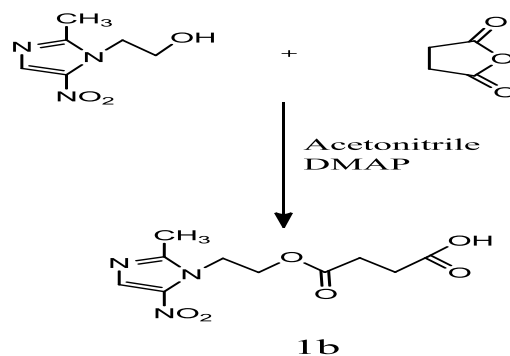
The synthetic pathways for the designed target compounds (1a- c and I, II and III) are illustrated in (schemes 1-6).



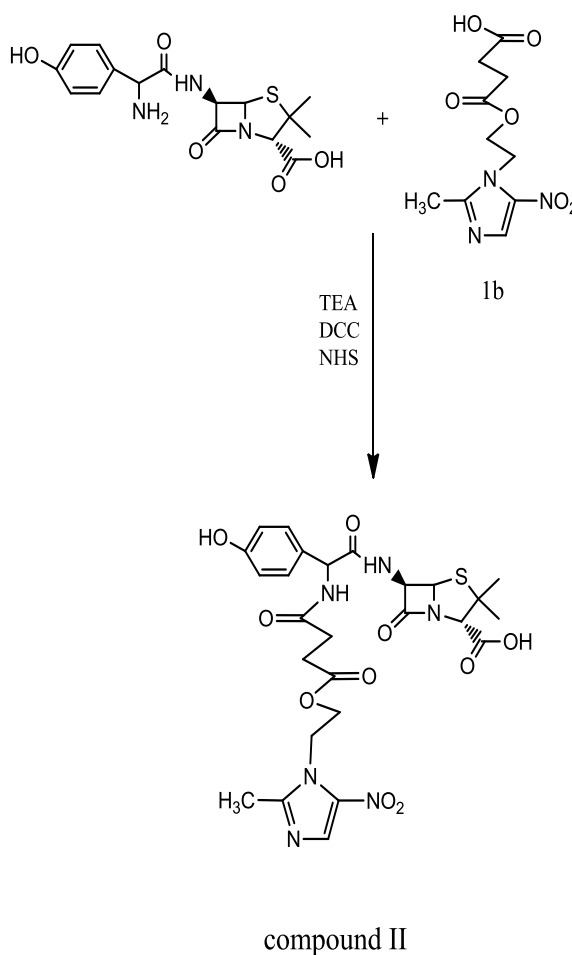
**Scheme (1) synthesis of MTZ-Cl**



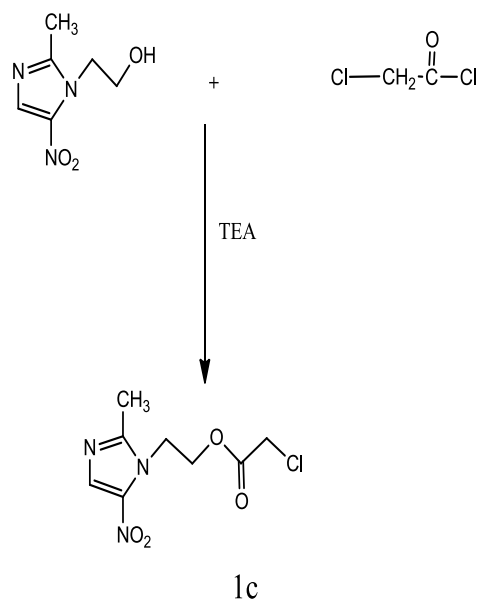
**Scheme (2) synthesis of compound I**



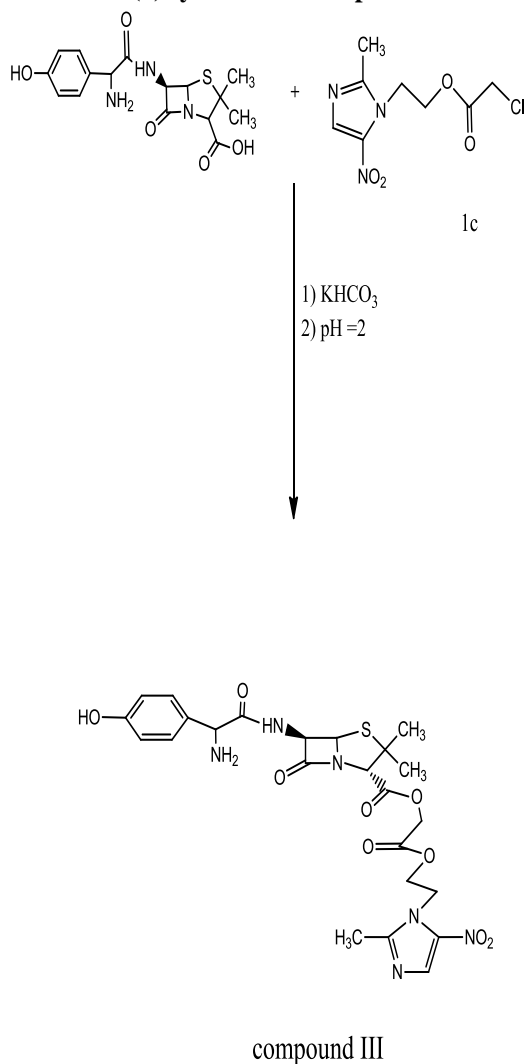
**Scheme(3)synthesis of compound 1B**



**Scheme (4) synthesis of compound II**



Scheme (5) synthesis of compound 1c



Scheme (6) synthesis of compound III

## Experimental

### A - Chemistry

All reagents and anhydrous solvents were of analytical grade and were used as received from the commercial suppliers (Merck-Germany, Riedel-Dehaen-Germany, BDH-England and Fluka-Switzerland). Amoxicillin and Metronidazole were purchased from SDI Company, Iraq. Thin layer chromatography (TLC) was run on Kieselgel GF254 (60), Merck (Germany), to check the purity of the products as well as monitoring the progress of reactions. FT-IR spectra were recorded at College of Pharmacy, Kufa University

by using Shimadzu - Japan spectro - photometer and the determination of the spectra were performed by using KBr discs. CHNO microanalysis has been done at the central laboratory of kufa uniuising euroEA Shimadzu-Japan elemental analyzer.

### Synthesis of 1-(2-chloroethyl)-5-methyl-2-nitro-1H-imidazole, (1a) <sup>(9)</sup>:

To a solution of metronidazole ( 5 mmol , 0.858 gm ) in THF ( 20 ml ) was added thionyl chloride (6.5 mmol , 0.75 ml) dropwise under stirring. then the reaction mixture was refluxed for 7 hrs. Excess thionyl chloride was removed azeotropically. The residue was taken into ethyl acetate (30 mL) and washed with water (10 mL x 3). Organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to provide a brown solid . The percent yield, physical appearance, melting point and R<sub>f</sub> value are listed in table (1).

### Synthesis of compound (I) <sup>(10)</sup>:

A suspension of amoxicillin trihydrate (1 mmol ,0.4195gm) in 5 ml DMF was prepared and (1 mmol ,0.01 gm) of potassium bicarbonate and [(0.203) mL (2 mmol)] benzaldehyde were added at 0 °C ( to provide protection for amine group0 . The mixture was stirred at 0 °C for 3.5 h. At the end of this time (1 mmol ,0.1 gm) of potassium bicarbonate and (1 mmol ) of compound (1a) were added and stirred at 0 °C for additional 6 hrs. The mixture was poured onto ice water and was extracted with (5 mL x 3) of ethyl -acetate. After washing the organic phase with 10% sodium chloride and drying over anhydrous sodium sulfate, it was evaporated to dryness in vacuo at 20 °C and a yellow oily residue was obtained. The residue was dissolved in 5 mL of acetonitrile. The pH of the solution was adjusted to 2 with 1N HCl and it was stirred for 30 min at 0-5 °C . In order to remove acetonitrile, 6 mL of water was added and the solution was evaporated in vacuo at 20 °C . After washing the aqueous solution with (6 mL x 3) of ethylacetate and saturating with

sodium chloride it was extracted with (6 mL x 3) of dichloro -methane. The organic phase was dried over anhydrous sodium sulfate and it was left overnight at 5 °C The residue was filtered and dried .

The percent yield, physical appearance, melting point and R<sub>f</sub> value are listed in table (1) .

**Synthesis of 4- (2- (5- methyl - 2 - nitro - 1H -imidazol - 1 -yl ) ethoxy)-4-oxo butanoic acid ( compound Ib )<sup>(11)</sup> :**

Metronidazole (3 mmol ,0.5135gm) was dissolved in 30 mL of acetonitrile and (3 mmol , 0.3 gm) of the Succinic anhydride followed by 4-dimethyl aminopyridine (0.3 mmol , 0.037 gm) were added. The mixture was left at ambient temperature for 48 hrs. until the reaction was completed as evidenced by TLC , the products was precipitated after solvent evaporation and addition of cold water. The percent yield, physical appearance, melting point and R<sub>f</sub> value are listed in table (1) .

**Synthesis of compound (II)<sup>(12)</sup> :**

Compound (Ib) (1.133 mmol , 0.303 g ) was dissolved in 10 ml of anhydrous DMSO and (1.699 mmol , 0.24 ml ) of TEA was added under stirring ; then ( 2.266 mmol , 0.260 gm) of NHS and then (2.266 mmol, 0.4675 gm) of DCC were added . The reaction was let under stirring overnight at room temperature in the dark . Dicyclohexylurea ( DCU ) was filtered out and the solution was dropped into 200 ml of diethyl ether. After 4 hrs. At 4 °C the activated ( Ib- NHS ) was recovered by filtration and washed with Diethyl ether and then dried under vacuum .

To ( 1 mmol , 0.4194 gm ) of Amoxicillin dissolved in 10 ml DMSO , ( 5 mmol ) of ( Ib-NHS ) dissolved in 6 ml of DMSO was added . To the mixture ( 0.02 ml ) of Et<sub>3</sub>N was added and the reaction was let to react over night under stirring at room temperature in the dark . Then 5 ml of water was added and the PH adjusted to 7.5 and 5ml of DMF was also added to help phase separation . the ( compound II ) was extracted from aqueous phase by ethyl acetate ( 50 ml x 4 ) . The organic layer was dried over anhydrous sodium sulphate and evaporate the ethyl acetate to get the compound (II). The percent yield, physical appearance, melting point and R<sub>f</sub> value are listed in table (1) . To an ice-cooled solution of

metro -nidazole (1.1 mmol , 0.1883 gm) and TEA (2.2 mmol , 0.3 ml) in dichloromethane (10 mL) , chloroacetyl chloride (2.2 mmol, 0.18 ml) was added under stirring. The reaction mixture was further stirred at 0-5 °C for 30 min.

**Synthesis of Chloro- acetic acid 2- ( 5- methyl-2-nitro - 1 H-imidazol - 1 - yl ) ethyl ester ( compound 1c )<sup>(9)</sup> :**

Dichloromethane was distilled off and the residue was taken in ethyl acetate (20 mL). The ethyl acetate layer was washed with water (10 mL x 3) and dried over anhydrous sodium sulfate. Sodium sulfate was filtered off and washed with ethyl acetate (5 mL x 2). The filtrate was concentrated to give a brown semisolid . The percent yield, physical appearance, melting point and R<sub>f</sub> value are listed in table (1) .

**Synthesis of compound (III)<sup>(10)</sup> :**

A suspension of amoxicillin trihydrate (1 mmol ,0.4195gm) in 5 ml DMF was prepared and (1 mmol ,0.01 gm) of potassium bicarbonate and [(0.203) mL (2 mmol)] benzaldehyde were added at 0 °C . The mixture was stirred at 0 °C for 3.5 h. At the end of this time (1 mmol ,0.1 gm) of potassium bicarbonate and (1 mmol ) of compound (1c) were added and stirred at 0 °C for additional 6 hrs. The mixture was poured onto ice water and was extracted with (5 mL x 3) of ethyl - acetate. After washing the organic phase with 10% sodium chloride and drying over anhydrous sodium sulfate, it was evaporated to dryness in vacuo at 20 °C and a yellow oily residue was obtained. The residue was dissolved in 5 mL of acetonitrile. The pH of the solution was adjusted to 2 with 1N HCl and it was stirred for 30 min at 0-5 °C . In order to remove acetonitrile, 6 mL of water was added and the solution was evaporated in vacuo at 20 °C . After washing the aqueous solution with (6 mL x 3) of ethylacetate and saturating with sodium chloride it was extracted with (6 mL x 3) of dichloro -methane. The organic phase was dried over anhydrous sodium sulfate and it was left overnight at 5 °C The residue was filtered and dried . The percent yield, physical appearance, melting point and R<sub>f</sub> value are listed in table (1) .

**Table (1) The percent yield, physical appearance, melting point and  $R_f$  of the intermediate and final products.**

Compounds and intermediates	Empirical formula	Molecular weight	Description	% yield	Melting point °C	$R_f$ value
1a	C <sub>6</sub> H <sub>8</sub> ClN <sub>3</sub> O <sub>2</sub>	189.6	yellow crystals	60.1	77-79	*A=0.33 B=0.42
1b	C <sub>10</sub> H <sub>13</sub> N <sub>3</sub> O <sub>6</sub>	271.23	White crystals	65.3	125-128	A=0.21 B=0.33
1c	C <sub>8</sub> H <sub>10</sub> ClN <sub>3</sub> O <sub>4</sub>	247.64	Semisolid brown	76.3		A=0.3 B=0.41
I	C <sub>22</sub> H <sub>26</sub> N <sub>6</sub> O <sub>7</sub> S	518.5	Faint yellow powder	59.4	189-191	A=0.29 B=0.41
II	C <sub>26</sub> H <sub>30</sub> N <sub>6</sub> O <sub>10</sub> S	618.6	White powder	66.9	203-205	A=0.25 B=0.37
III	C <sub>24</sub> H <sub>28</sub> N <sub>6</sub> O <sub>9</sub> S	576.58	Off white-yellow powder	55.3	197-199 d	A=0.27 B=0.38

A =(dichloromethane : Ethanol : ethylacetate) (6:1:3) Polarity index= 3.7

B=(dichloromethane : Ethanol : ethylacetate) (1:6:3) Polarity index= 4.75

**1-(2-chloroethyl)-5-methyl-2-nitro-1H-imidazole (1a) :**

IR (KBr): 2976 cm<sup>-1</sup> [C-H], 1532 cm<sup>-1</sup> [N-O], 1460 cm<sup>-1</sup> [C=N], 1365 cm<sup>-1</sup> [N-O], 756 cm<sup>-1</sup> [C-Cl].

**4-(2-(5-methyl-2-nitro-1H-imidazol-1-yl)ethoxy)-4-oxo butanoic acid (1b) :**

IR (KBr): 3181 cm<sup>-1</sup> [O-H], 2962 cm<sup>-1</sup> [C-H], 1732 cm<sup>-1</sup> [C=O] of ester, 1531 cm<sup>-1</sup> [N-O], 1476 cm<sup>-1</sup> [C=N], 1266 cm<sup>-1</sup> [C-O].

**Chloro-acetic acid 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl ester ( compound 1c) :**

IR (KBr): 3019 cm<sup>-1</sup> [C-H], 1747 cm<sup>-1</sup> [C-O], 1529 cm<sup>-1</sup> [N-O], 1471 cm<sup>-1</sup> [C=N], 1262 cm<sup>-1</sup> [C-N], 825 cm<sup>-1</sup> [C-Cl].

**Compound (I) :**

IR (KBr): 3506 cm<sup>-1</sup> asy. Str. [N-H] of amine, 3444 cm<sup>-1</sup> sy. Str. [N-H] of amine, 3151 cm<sup>-1</sup> [O-H] of phenol, 1780 cm<sup>-1</sup> [C=O] of lactam, 1738 cm<sup>-1</sup> [C=O] of ester, 1697 cm<sup>-1</sup> [C=O] of amide, 1550 cm<sup>-1</sup> [N-O] of nitro, 1261 & 1174 cm<sup>-1</sup> [C-O]. CHN Calculated: C, 50.96; H, 5.05; N, 16.21; O, 21.6; Found: C, 51.21; H, 5.13; N, 16.18; O, 21.27.

**Compound (II) :**

IR (KBr): 3334 cm<sup>-1</sup> [N-H] of amide, 3230 cm<sup>-1</sup> [O-H] of phenol, 2966 cm<sup>-1</sup> [C-H], 1778 cm<sup>-1</sup> [C=O] of lactam, 1742 cm<sup>-1</sup> [C=O] of ester, 1674 cm<sup>-1</sup> [C=O] of amide, 1638 cm<sup>-1</sup> [C=N], 1519 cm<sup>-1</sup> asy. Str. [N-O] of nitro, 1371 cm<sup>-1</sup> sy. Str. [N-O] of nitro, 1273 & 1174 cm<sup>-1</sup> [C-O]. CHN Calculated: C,

50.48; H, 4.89; N, 13.59; O, 25.86; Found: C, 50.67; H, 5.02; N, 13.87; O, 25.46.

**Compound (III) :**

IR (KBr): 3511 cm<sup>-1</sup> asy. Str. [N-H] of amine, 3441 cm<sup>-1</sup> sy. Str. [N-H] of amine, 3207 cm<sup>-1</sup> [O-H] of phenol, 1778 cm<sup>-1</sup> [C=O] of lactam, 1751 & 1740 cm<sup>-1</sup> [C=O] of ester, 1671 cm<sup>-1</sup> [C=O] of amide, 1553 cm<sup>-1</sup> [N-O] of nitro, 1275 & 1175 cm<sup>-1</sup> [C-O]. CHN Calculated: C, 49.99; H, 4.89; N, 14.58; O, 24.97; Found: C, 51.21; H, 5.21; N, 14.03; O, 24.42.

**B - Antimicrobial studies**

Antibacterial activity for the synthesized compounds was investigated by disc diffusion method against different bacterial strains and fungi such as, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *E.coli*, *Klebsiella pneumonia* and *Candida albicans*, using nutrient agar medium and 5 mm diameter paper discs (Whatman No. 1). The investigated compounds *i.e.* the final products, were dissolved in DMSO at a concentration of 5000 µg/ml. The filter paper disc were soaked in solutions of the test compounds, dried, and then placed in Petri plates previously seeded with the test organisms. The plates were incubated for 24 h at 37 °C and the inhibition zone in the region of each disc was measured.

The results obtained are tabulated in table (2).

## Results and Discussion

The synthesis of the designed compounds has been successfully achieved. Characterization and structural formulas of the synthesized compounds were confirmed by melting point determination,  $R_f$  values, FTIR spectroscopy and elemental micro-analysis. The antimicrobial study for the synthesized

compounds showed maintenance or improvement of the antibacterial activity against both gram negative and gram positive bacteria with activity against fungi. Especially compound II show superior activity in comparison with the other synthesized compounds.

**Table (2) Antimicrobial activities (a) of the compounds (I-III) .**

Compound	<i>S.aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginos</i>	<i>Klebsiella pneumoniae</i>	<i>Candida albicans</i>
Control (DMSO)	-	-	-	-	-
Amoxicillin	25	22	23	23	-
Metronidazole	6	-	14	-	17
I	31	23	32	21	19
II	29	25	31	29	20
III	28	24	30	28	19

(a) Growth inhibition diameter (mm)

## References

1. Yoonseon Park, Jun Young Choi, Dongeun Yong, Clinical Features and Prognostic Factors of Anaerobic Infections: A 7-Year Retrospective Study, The Korean Journal of Internal Medicine, 2009;24:1- 13.
2. Nagy E. Anaerobic infections: update on treatment considerations. Drugs. 2010;70 :841-58.
3. zianabos AO et al : Anaerobic infections: General concepts, in Principles and Practice of Infectious Diseases, 6th ed, GL Mandell et al (eds). Elsevier, 2005, pp 2810–2816
4. Anthony W. Chow, m.d., f.a.c.p, f.r.c.p.c. Anaerobic infections ACP Medicine, (chapter 141) 2006, pp 1604-1624.
5. Brook I: Beta-lactamase-producing bacteria in mixed infections. Clin. Microbiol Infect, 2004 ; 10 :777 - 784.
6. van Winkelhoff, A. J., J. P. Rodenburg, R. J. Goen6, F. Abbas, E. G. Winkel, and J. de Graaff. Metronidazole plus amoxicillin in the treatment of *A. actinomycetem - comitans* associated periodontitis. J. Clin. Periodontol. 1989 ; 16 :128-131.
7. van Winkelhoff, A. J., C. J. Tjihof, and J. de Graaff. Microbiological and clinical results of metronidazole plus amoxicillin therapy in *Actinobacillus actinomycetem-comitans*-associated periodontitis. J. Periodontol. 1992 ; 63 :52-57.
8. Pavicic M.J. , van Winkelhoff A.J.and J.de.Graaff, Synergistic effects between amoxicillin, metronidazole, and its hydroxyl metabolite against *Actinobacillus actinomycetem – comitans*. Aantimicrob. Agents Chemother. 1991; 35:961- 966.
9. Lalit Kumar,a Amit Sarswat,a Nandlal,a Vishnu L. Sharma,a, Imidazole derivatives as possible microbicides with dual protection, European Journal of Medicinal Chemistry, 2010; 45(2) :817-824.
10. Meltem Ceylan "UNL" Usoy, Nurten Altanlar, Rahmiye Ertan, Synthesis and Antimicrobial Activities of Some New Flavonyl Pro-drug Esters of Ampicillin, Turk J Chem 2005 ; 29 : 187 -191.
11. Nadia M. Mahfouz,Tarek Aboul-Fadl . Ahmed K. Diab, Metronidazole twin ester prodrugs: synthesis, physico -chemical properties, hydrolysis kinetics and anti-giardial activity, Eur: J. Med. Chem. 1998; 33: 675-683.
12. Pasut G, Canal F, Dalla Via L, Arpicco S, Veronese FM, Schiavon O. Antitumoral activity of PEG-gemcitabine prodrugs targeted by folic acid. J Control Release, 2008 ; 127( 3) : 239-248.