Synthesis, Characterization and Antimicrobial Study of New Nalidixic Acid Mannich Base Derivatives

Monther Faisal Mahdi1, Ayad Mohammed Rasheed Rauf1 and Shahbaa Shafeeq Rzoqi2

1Department of Pharmaceutical Chemistry, College of Pharmacy, University of Al-Mustansiriyha, Baghdad, Iraq
2Ministry of Health, Dyala Health Directorate, Baquba General Hospital

ABSTRACT

The present research paper has been focused on synthesis of nalidixic acid mannich base derivatives by adopting appropriate synthetic steps with expected broader spectrum of activity. Purification of intermediates and final titled compounds has been done by recrystallization. Characterization of all the synthesized mannich derivatives including intermediates by physical and spectral data like IR, Proton NMR and elemental microanalysis. Biological evaluation of the newly synthesized compounds for their pharmacological activity has been studied and found that the synthesized compounds are active against tested Gram positive and Gram negative bacteria like Staphylococcus aureus, Bacillus subtilis Escherichia coli and proteus, also active against tested fungid like Candida albicana and Candida tropicalis by adopting standard protocols.

Keywords: Nalidixic acid, Mannich base derivatives, Characterization, Antimicrobial activities.

INTRODUCTION

Infection can be defined as the invasion of a host organism's bodily tissues by disease causing organisms. Infections are caused by microorganisms such as viruses, prions, bacteria, and viroids, and larger organisms like parasites and fungi [1]. Urinary tract infection is one of the most common infectious diseases among men and women [2]. Antimicrobial drugs or chemicals are the substances used to kill or slow down the growth of microorganisms. They include antibiotics, antiviral, antifungal and anti-parasitic agents [3]. The first clinically useful quinolone was nalidixic acid, discovered by Lesher and co-workers in 1962, which was generated from chloroquine, an antimalarial agent [4]. Unfortunately, bacteria could develop a rapid resistance to this agent [5, 6]. Also among the antibacterial agents known, sulfonamide drugs were used for the cure and prevention of bacterial infection in human beings [7]. Sulfonamides also play a very important role as key constituent in number of biologically active molecules. Almost all of the major categories of antibiotics in the clinical application showed resistance to microorganism specially β- lactam, macrolides, vancomycin and quinolones derived bacterial drug’s resistance is a source of concern for healthcare officials [8-10]. So, there is a real need to discover new compounds with high efficiency towards pathogens and less toxicity, which may be different from available resistant drugs [11]. Numbers of reports are available for the synthesis of Mannich base using a liphatic, aromatic, substituted aromatic and hetero aldehyde [12-14]. Mannich bases are known to play a vital role in the development of synthetic pharmaceutical chemistry. The literature studies revealed that mannich bases are very reactive and can be easily converted to other compounds, for example, reduced to form physiologically active amino alcohols [15]. It’s also known to possess potent activities like antibacterial, antifungal [16-18], anthelmintic [19], antimalarial [20], antiviral [21],
antitubercular, anti-HIV [22, 23], anti-inflammatory [24, 25], anticancer [26, 27], anticonvulsant [28], analgesic [29], and antipsychotic activity [30].

The mutual prodrug is an efficient approach for drug optimization, the term mutual prodrug refers to two or more therapeutic compounds bonded via a covalent chemical linkage. Regardless of being similar to prodrug it differs in having inactive group replacement by active group, which are coupled directly or indirectly by a cleavable spacer [31].

MATERIALS AND METHODS

Chemicals and instrumentation
Nalidixic acid was purchased from Almansour Company, while other reagents such as sulfonamides were purchased from BDH, formaldehyde from Sigma-Aldrich, ethylchloroformate and glycine from Fluka, morpholin and pipredine from Himedia and used as it is. All of the solvents and materials used were of analar type and used without further purification. Precoated silica gel plates, 60G F254, obtained from Merck, Darmstadt(Germany) and were used for thin layer chromatography (TLC). Spots were visualized by using either UV-lamp at 254nm or iodine. The materials and methods are performed in college of pharmacy, AL-Mustansiriya University College of pharmacy. The $^1$H NMR spectra was performed by Bruker Avance (III) 400 MHz at the university of Ain shams, college of pharmacy.

Synthesis of 3-methyl 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (I):
Ethyl chloroformate (11.55 mmole, 1.1 mL) was added dropwise to a stirred suspension of nalidixic acid (10.00 mmole, 2.32 g) and triethylamine (2 mL) in CHCl$_3$ (20 mL). The temperature was maintained at 5-10°C throughout the addition and stirred for further 30 min. Methanol (15 mL) was added and the mixture was stirred for further 4 hours at room temperature. The solution was then evaporated to dryness under reduced pressure and the residue was recrystallized from ethyl acetate/light petroleum [32].

Synthesis of 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (II):
Methyl nalidixate (0.869 mmole, 0.20 g) was added to saturated aqueous ammonium hydroxide solution (24 mL) and stirred at room temperature for 24 hours. Water (30 mL) was added and the mixture was extracted with dichloromethane (3×30 mL). The combined organic solvent was evaporated under reduced pressure to afford compound (II) [33].

Synthesis of nalidixic acid mannich base derivatives (III$_{a-f}$):
General Procedure for Synthesis of nalidixic acid mannich base derivatives from secondary amines (III$_{a,b}$):
Secondary amine (a or b) (0.865 mmole) was added to an ethanolic solution (50 mL) of compound II (0.865 mmole, 0.2 g) in flat bottom flask. One half of (2.2 ml) of formaldehyde solution 37 % was added slowly with constant stirring. The reaction mixture was stirred at 70-75 °C on a magnetic stirrer for 5.5 and 8.5 hours, depending upon the secondary amine taken. The remaining portion of formaldehyde solution was added in two installments at an interval of one hour.

The reaction mixture was kept overnight in the refrigerator. Evaporation of the solvent under reduced pressure and recrystallization of the product with dry ethanol [34].

General Procedure for Synthesis of nalidixic acid mannich base derivatives from primary amines (III$_{c-f}$):
Sulphonamide derivative (c-f) (0.865 mmole) was added slowly to a solution of compound II (0.865 mmole, 0.2 g) that prepared by dissolving it in ethanol (20 mL). The pH of the mixture was adjusted to 3.5 by adding HCl (0.5 ml), and then formaldehyde solution 37 % (2.2 mL) was added slowly with constant stirring. The mixture was kept at efficient ice cooling for half an hour, and then refluxed on water bath. Reflux time varied according to the sulphonamide used. The refluxed mixture was kept at 0°C for four days when crystalline product was obtained. The product was recrystallized from dry ethanol and dioxane-water (1:1)[34] as shown in Scheme 1.
Scheme 1. Shows the synthesis of target compounds (IIIₐ–f).

Methyl 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate(I)
[C₆H₇NO₂]: 90% yield; m.p. 154-156°C; IR (λ max cm⁻¹): 3088 & 3028 (Ar-H), 1691 (C=O ester), 1643 (C=O ketone); Analysis: Calcd C, 63.40; H, 5.73; N, 11.32; Found: C, 62.89; H, 5.75; N, 11.27.

1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide(II)
[C₆H₇NO₂]: 67% yield; m.p. 243-245°C; IR (λ max cm⁻¹): 3151 & 3194 (NH), 3063 & 2995 (Ar-H), 1662 (C=O), 1606 (C=C Aromatic); ¹H NMR (δ ppm): 7.46 (NH₂), 1.39 (CH₃), 7.46, 8.56 & 8.98 (naphthalin); Analysis: Calcd C, 62.33; H, 5.67; N, 18.17; Found: C, 62.09; H, 5.87; N, 18.02.

1-ethyl-7-methyl-N (morpholinomethyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide(IIIₐ)
[C₆H₇N₂O₂]: 63% yield; m.p. 197-199°C; IR (λ max cm⁻¹): 3175 & 3151 (NH), 3082 & 3045 (Ar-H), 2852 & 2816(CH₂ bridge), 1658 (C=O), 1114 (C-O); ¹H NMR (δ ppm): 10.12 (NH₂), 4.22 (CH₂ bridge), 3.57 & 2.47 (CH₂ morpholin); Analysis: Calcd C, 61.80; H, 6.71; N, 16.96; Found: C, 61.29; H, 6.43; N, 17.27.

1-ethyl-7-methyl-N-(piperidin-1-ylmethyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide(IIIₐ)
[C₆H₇N₂O₂]: 74% yield; m.p. 218-220°C; IR (λ max cm⁻¹): 3188 (NH), 3032 & 2972 (Ar-H), 2852 & 2802 (CH₂ bridge), 1656 (C=O), 1217, 1166 & 1033 (C-N piperidine); ¹H NMR (δ ppm): 10.06 (NH₂), 4.19 (CH₂ bridge), 1.39, 1.48 & 2.45 (CH₂piperidine); Analysis: Calcd C, 65.83; H, 7.37; N, 17.06; Found: C, 66.16; H, 7.55; N, 16.89.

1-ethyl-7-methyl-N-((4-(5-methylisoxazol-3-yl)sulfonyl)phenyl)amino)methyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (IIIₐ)
[C₆H₇N₂O₂S]: 56% yield; m.p. 117°C; IR (λ max cm⁻¹): 3363 (NH sulfonamide), 3255 (NH amide), 2889 & 2831 (CH₂ bridge), 1665 (C=O), 1340 & 1161 (S=O); ¹H NMR (δ ppm): 10.00 (NH sulfonamide), 10.26 (NH amide), 7.48–7.56 (Ar-H), 4.78 (CH₂ bridge); Analysis: Calcd C, 55.63; H, 4.87; N, 16.93; S, 6.46; Found: C, 56.29; H, 4.95; N, 17.42; S, 6.27.
1-ethyl-7-methyl-4-oxo-N-(((4-(thiazol-2-ylsulfonyl)phenyl)amino)methyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide (IIIa)

[C22H22N6O4S2]: 71% yield; m.p. 171-173°C; IR (ν max cm⁻¹): 3380 (NH sulfonamide), 3255 (NH amide), 3101 (NH amine), 2877 (CH₂ bridge), 1662 (C=O), 1340 & 1145 (S=O); ¹HNMR (δ ppm): 11.14 (NH), 10.28 (NH amide), 4.82 (CH₂ bridge); Analysis: Calcd C, 53.00; H, 4.45; N, 16.86; S, 12.86; Found: C, 53.48; H, 4.31; N, 17.39; S, 13.38.

1-ethyl-7-methyl-4-oxo-N-(((4-(pyrimidin-2-ylsulfonyl)phenyl)amino)methyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide (IIIb)

[C23H23N7O4S]: 69% yield; m.p. 194-196°C; IR (ν max cm⁻¹): 3201 (NH sulfonamide), 3086 (NH amide), 2872 (CH₂ bridge), 1656 (C=O), 1340 & 1155 (S=O); ¹HNMR (δ ppm): 11.05 (NH sulfonamide), 10.40 (NH amide), 4.78 (CH₂ bridge); Analysis: Calcd C, 55.97; H, 4.70; N, 19.87; S, 6.50; Found: C, 55.47; H, 4.87; N, 19.44; S, 6.38.

1-ethyl-7-methyl-4-oxo-N-(((4-(pyridin-2-ylsulfonyl)phenyl)amino)methyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide (IIIc)

[C24H24N6O4S]: 80% yield; m.p. 251-253°C; IR (ν max cm⁻¹): 3383 (NH sulfonamide), 3196 (NH amide), 2823 (CH₂ bridge), 1658 (C=O), 1350 & 1155 (S=O); ¹HNMR (δ ppm): 11.35 (NH), 10.40 (NH amide), 4.77 (CH₂ bridge); Analysis: Calcd C, 58.52; H, 4.91; N, 17.06; S, 6.51; Found: C, 59.10; H, 5.01; N, 17.51; S, 6.22.

Synthesis of Ethyl-2-amino acetate hydrochloride (IV):
Thionyl chloride (11mmole, 0.8 mL) was added gradually to absolute ethanol (10 mL) cooled to (0°C). 2-Aminoacetic acid (10 mmole, 0.75g) was suspended in the reaction mixture and subjected to ultra-sonication at room temperature for (45 min.), on completion of the reaction, the solvent was removed under reduced pressure and the residue was purified by recrystallization from methanol: diethyl ether to give compound (IV) [35].

Synthesis of ethyl 2-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamido)acetate (V):
Ethyl chloroformate (11.55 mmole, 1.1 mL) was added dropwise to an ice cooled stirred suspension of nalidixic acid (10 mmole, 2.32 g) and triethylamine (2 mL) in dry CHCl₃ (20 mL). The temperature was maintained at 5-10°C throughout the addition and for further 30 min. Compound IV (1.395g, 10 mmole) was then added together with an equivalent amount of triethylamine (10 mmole, 1.39mL) and the mixture was stirred for further 4 hours at room temperature. The solution was then evaporated to dryness under reduced pressure and the residue was crystallized from aqueous ethanol to offer compound (V) [36].

Synthesis of N-(2-amino-2-oxoethyl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (VI):
Compound V (0.63 mmole, 0.2 g) was added to saturated aqueous ammonium hydroxide solution (24 mL) and stirred at room temperature for 24 hours. Then filtration of the solution will produce compound (VI) [33].

Synthesis of nalidixic acid manich base derivatives (VIIa,b):
General Procedure for Synthesis of manich base derivatives (VIIa,b):
Dissolved compound VI (0.868 mmole, 0.25g) in a solvent system (50 mL) of ethanol: dimethyl sulphoxide (1:1) and then secondary amine (a or b) (0. 868 mmole) was added to it. One half of (2.2 mL) of formaldehyde solution 37% was added slowly with constant stirring. The reaction mixture was stirred at 70-75 °C on a magnetic stirrer for 5.5 and 8.5 hours, depending upon the secondary amine taken. The remaining portion of formaldehyde solution was added in two installments at an interval of one hour. The reaction mixture was kept overnight in the refrigerator. Evaporation of the solvent under reduced pressure and recrystallization of the product with dry ethanol [34].

General Procedure for Synthesis of nalidixic acid manich base derivatives from primary amines (VIIc,d):
Sulphonamide derivative (c-l) (0.868 mmole) was added slowly to a solution of compound VI (0.868 mmole, 0.2g) that prepared by dissolving it in a solvent system of (50 mL) ethanol: dimethyl sulphoxide (1:1). The pH of the mixture was adjusted to 3.5 by adding HCl (0.5ml), and then formaldehyde solution 37% (2.2 mL) was added slowly with constant stirring. The mixture was kept at efficient ice cooling for half an hour, and then refluxed on water bath. Reflux time varied according to the sulphonamide used. The refluxed mixture was kept at 0°C for four days when crystalline product was obtained. The product was recrystallized from dry ethanol and dioxane-water (1:1) [34] as shown in Scheme 2.
Monther Faisal Mahdi et al. *Der Pharma Chemica, 2016, 8 (19): 424-432*

**Scheme 2. Shows the synthesis of target compounds (VIIa, b)**

**Ethyl amino acetate hydrochloride (IV)**
[C₆H₅NO₂Cl]: 92% yield; m.p. 143-145°C; IR (ν, max cm⁻¹): 2978 (NH₂ amine), 2673 & 2637 (CH₃), 1748 (C=O ester), 1250 (C-O-C ester), 1136 (C-N).

**Ethyl 2-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamido)acetate (V)**
[C₆H₅NO₂]: 94% yield; m.p. 198-200°C; IR (ν, max cm⁻¹): 3186 (NH amide), 3086 & 3045 (Ar-H), 1737 (C=O ester), 1653 (C=O amide); Analysis: Calcd C, 60.56; H, 6.03; N, 13.24; Found: C, 60.00; H, 5.77; N, 13.10.

**N-(2-amino-2-oxoethyl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (VI)**
[C₆H₅NO₂]: 66% yield; m.p. 313-315°C; IR (ν, max cm⁻¹): 3416, 3279 & 3205 (NH amides), 3037 (Ar-H), 1668 (C=O), 1606 (S=C); ¹HNMR (δ ppm): 10.05 (NH amide), 7.10 (NH₂ amide), 3.96 (CH₂-NH); Analysis: Calcd C, 58.32; H, 5.59; N, 19.43; Found: C, 58.87; H, 5.38; N, 18.87.

**1-ethyl-7-methyl-N-(2-morpholino-2-oxoethyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (VIIa)**
[C₆H₂NO₂]: 73% yield; m.p. 252-254°C; IR (ν, max cm⁻¹): 3290 & 3228 (NH amide), 3081 & 3033 (Ar-H), 2891 & 2850 (CH₃ bridge), 1652 (C=O), 1114 (C-O morpholin); ¹HNMR (δ ppm): 10.10 (NH amide), 4.05 (CH₂ bridge); Analysis: Calcd C, 58.90; H, 6.50; N, 18.08; Found: C, 58.47; H, 6.36; N, 18.50.

**1-ethyl-7-methyl-4-oxo-N-(2-oxo-2-(piperidin-1-yl)ethyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide (VIIb)**
[C₆H₂NO₂]: 65% yield; m.p. 211-213°C; IR (ν, max cm⁻¹): 3292 & 3236 (NH amide), 3045 (Ar-H), 2852 & 2787 (CH₂ bridge), 1654 (C=O), 1228, 1165 & 1031 (C-N piperidine); ¹HNMR (δ ppm): 10.08 (NH amide), 4.02 (CH₂ bridge); Analysis: Calcd C, 62.32; H, 7.06; N, 18.17; Found: C, 61.85; H, 7.21; N, 17.68.
1-ethyl-7-methyl-N-(2-((4-((5-methylisoxazol-3-yl)sulfonyl)phenyl)amino)-2-oxoethyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (VII)

\[C_{25}H_{27}N_{7}O_{6}S\]: 70% yield; m.p. 288-290°C; IR (\(\lambda_{\text{max}}\) cm\(^{-1}\)): 3420 & 3351 (NH sulfonamide), 3258 & 3100 (NH amide), 3073 (Ar-H), 2869 & 2803 (CH\(_2\) bridge); Analysis: Calcd C, 54.24; H, 4.92; N, 17.71; S, 5.79; Found: C, 54.89; H, 4.73; N, 17.77; S, 5.21.

1-ethyl-7-methyl-4-oxo-N-(2-oxo-2-((4-(thiazol-2-ylsulfonyl)phenyl)amino)ethyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide (VII)

\[C_{24}H_{25}N_{7}O_{5}S_2\]: 78% yield; m.p. 253-255°C; IR (\(\lambda_{\text{max}}\) cm\(^{-1}\)): 3365 (NH sulfonamide), 3203 & 3103 (NH amide), 3052 (Ar-H), 2852 (CH\(_2\) bridge), 1660 (C=O), 1365 & 1138 (S=O); 1HNMR (\(\delta_{\text{ppm}}\)): 11.29 (NH sulfonamide), 10.06 (NH amide), 4.14 (CH\(_2\) bridge); Analysis: Calcd C, 51.88; H, 4.54; N, 17.65; S, 11.54; Found: C, 52.45; H, 4.66; N, 17.11; S, 12.10.

1-ethyl-7-methyl-4-oxo-N-(2-oxo-2-((4-(pyrimidin-2-ylsulfonyl)phenyl)amino)ethyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide (VII)

\[C_{25}H_{26}N_{8}O_{5}S\]: 80% yield; m.p. 280-281°C; IR (\(\lambda_{\text{max}}\) cm\(^{-1}\)): 3362 (NH sulfonamide), 3221 & 3103 (NH amide & amine), 3039 (Ar-H), 2868 & 2814 (CH\(_2\) bridge), 1656 (C=O), 1327 & 1153 (S=O); 1HNMR (\(\delta_{\text{ppm}}\)): 11.28 (NH), 10.06 (NH amide), 4.69 (CH\(_2\) bridge); Analysis: Calcd C, 54.54; H, 4.76; N, 20.35; S, 5.82; Found: C, 54.99; H, 4.55; N, 20.74; S, 6.01.

1-ethyl-7-methyl-4-oxo-N-(2-oxo-2-((4-(pyridin-2-ylsulfonyl)phenyl)amino)ethyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide (VII)

\[C_{26}H_{27}N_{7}O_{5}S\]: 82% yield; m.p. 272-274°C; IR (\(\lambda_{\text{max}}\) cm\(^{-1}\)): 3362 (NH sulfonamide), 3205 (NH amide), 3049 (Ar-H), 2856 & 2823 (CH\(_2\) bridge), 1662 (C=O), 1338 & 1134 (S=O); 1HNMR (\(\delta_{\text{ppm}}\)): 11.22 (NH), 10.07 (NH amide), 4.40 (CH\(_2\) bridge); Analysis: Calcd C, 56.82; H, 4.95; N, 17.84; S, 5.66; Found: C, 56.45; H, 4.75; N, 18.42; S, 5.66.

Antimicrobial Activity:

The antibacterial activity of the target compounds was done in college of Pharmacy / university of Al-mustansiriyah. A preliminary antibacterial activity has been carried out according to Well Diffusion Method. The tested compounds have been studied for their antibacterial activity in vitro against four tested bacteria (Staphylococcus aureus, Bacillus Subtalus, as gram positive bacteria and Proteus vulgaris, Escherichia coli, as gram negative bacteria) were clinically activated and maintained on nutrient agar medium for testing antibacterial. (Nalidixic acid, Sulfamethoxazole, Sulfathiazole, Sulfadiazine, Sulfapyridine) were used as a reference drugs for antibacterial activity. While the antifungal activity of the tested compounds was done in college of education for pure science Ibn-Alhaitham / university of Baghdad, they were tested against two types of fungi (Candida albicana & Candida tropicalis). The results were shown in Table 1.

Table 1: Antimicrobial activity of synthesized compounds

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RESULTS AND DISCUSSION

Synthesis of the target compounds (\( \text{III}_a-\text{f} \) & \( \text{VII}_a-\text{f} \)) were characterized by spectral technique, sharp absorption band in the range 1668- 1650 cm\(^{-1}\) indicating the formation of amide bond (CONH), also two major absorption bands
appeared in the range 2895-2750 cm\(^{-1}\) where clearly attributed to the presence of CH\(_2\) bridge indicating the formation of mannich base.

The major proton NMR spectral peak appeared at \(\delta\) 3.96-4.82 was assigned to CH\(_2\) bridge of mannich base.

The synthesized compound were also screened for their antimicrobial activity against tested microorganisms Proteus, Escherichia coli, Bacillus , Staphylococcus aureus, Candida albicana and Candida tropicalis at concentrations of (31.25, 62.5, 125 and 250 \(\mu\)g/mL) except the control which used pure.

Table 1 illustrates the inhibition zone in (mm.) for each concentration of all tested compounds.

These tested compounds exert significant antimicrobial activity in comparison to DMSO as control group, and these obtained results are compatible with many studies showed some quinolones have good antimicrobial action especially ciprofloxacin [37]. In comparison to standard compound (Sulfonamide drugs), tested compound exert higher activity against tested microbial.In comparison the antimicrobial results among the tested compounds (III) may regard the best one and (III) the lower one which may lead to the conclusion that sulfonamide derivatives have higher antimicrobial activity than secondary amines derivatives.

CONCLUSION

The antimicrobial activity of the synthesized compounds showed significant activity against tested gram positive, gram negative bacteria and fungus comparable to nalidixic acid. The use of glycine spacer in series 2 gave them a higher antimicrobial activity than series 1. The use of primary amines (sulfonamides) showed higher antimicrobial activity against most of the organism used in the test compared to the use of secondary amines.

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REFERENCES