Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(19):424-432
(http://derpharmachemica.com/archive.html)

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

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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 Satphylococcus aureus, Bacillus subtilis Escherichia coli and proteus, also active against tested fungilike 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 naldixic 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). Spotswere visualized by using either UV-lamp at 254nm or iodine. Electro thermal melting point apparatus and open capillary tubes were used to determine the melting points and are uncorrected. Infrared spectra were recorded as KBr disc by using FT- IR spectrophotometer Shimadzu 8400s, and elemental microanalysis at 400 MHz Vario macro cube-the art of elemental analysis were performed in college of pharmacy, AL-Mustansiriyah University College of pharmacy. The ^{1}HNMR 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 (l5 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}):

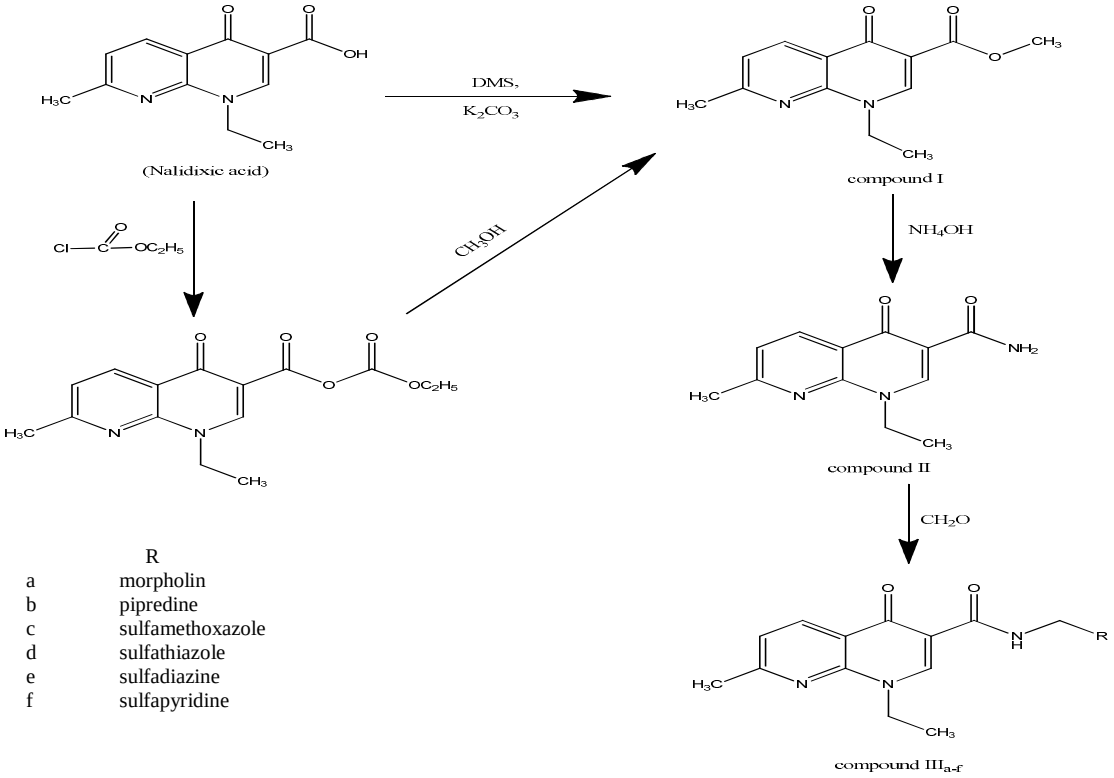
General Procedure for Synthesis of nalidixic acid mannich base derivatives from secondary amines ($III_{a,b}$) :

Secondary amine (a or b) (0.865 mmole) was added to an ethanolic solution (50 mL) of compound II (0.865 mmole, 0.2g) 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, 0.2g) that prepared by dissolving it in ethanol (20 mL). 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 1.



R	
a	morpholin
b	pipredine
c	sulfamethoxazole
d	sulfathiazole
e	sulfadiazine
f	sulfapyridine

Scheme 1. Shows the synthesis of target compounds (III_{a-f})

Methyl 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate(I)
$[C_{13}H_{14}N_2O_3]$: 90% yield; m.p. 154-156°C;IR (ƛ max cm^{-1}): 3088 & 3028 (Ar-H), 1691 (C=O ester), 1643 (C=O ketone); Analysis: Calcd C, 63.40; H, 5.73; N, 11.38; 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)
$[C_{12}H_{13}N_3O_2]$: 67% yield; m.p. 243-245°C; IR (ƛ max cm^{-1}): 3317& 3194 (NH), 3063 & 2995 (Ar-H), 1662 (C=O), 1606 (C=C Aromatic); ^{1}HNMR(δ ppm): 7.46 (NH_2), 1.39 (CH_3), 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(III_a)
$[C_{17}H_{22}N_4O_3]$:63% yield; m.p. 197-199°C; IR(ƛ max cm^{-1}): 3178 (NH), 3082 & 3045 (Ar-H), 2852 & 2816(CH_2 bridge), 1658 (C=O), 1114 (C-O); ^{1}HNMR(δ ppm):10.12 (NH), 4.22 (CH_2 bridge), 3.57 & 2.47 (CH_2 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-4-oxo-N-(piperidin-1-ylmethyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide (III_b)
$[C_{18}H_{24}N_4O_2]$: 74% yield; m.p. 218-220°C; IR (ƛ max cm^{-1}): 3188 (NH), 3032 &2972 (Ar-H), 2852& 2802 (CH_2 bridge), 1656 (C=O), 1217, 1166 &1033 (C-N pipredine); ^{1}HNMR (δ ppm): 10.06 (NH), 4.19 (CH_2 bridge), 1.39, 1.48 & 2.45 (CH_2pipredine); 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 (III_c)
$[C_{23}H_{24}N_6O_5S]$: 56% yield; m.p. 117°C; IR (ƛ max cm^{-1}): 3363 (NH sulfonamide), 3255 (NH amide), 2889 & 2831 (CH_2 bridge), 1665 (C=O), 1340 & 1161 (S=O); ^{1}HNMR (δ ppm): 10.00 (NH sulfonamide), 10.26 (NH amide), 7.48- 7.56 (Ar-H), 4.78 (CH_2 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(III_d)
[$C_{22}H_{22}N_6O_4S_2$]:71% yield; m.p. 171-173°C; IR (ƛ max cm^{-1}): 3380 (NH sulfonamide), 3255 (NH amide), 3101 (NH amine), 2877 (CH_2 bridge), 1662 (C=O), 1340 & 1145 (S=O); ^{1}HNMR (δ ppm): 11.14 (NH), 10.28 (NH amide), 4.82 (CH_2 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 (III_e)
[$C_{23}H_{23}N_7O_4S$]: 69% yield; m.p. 194-196°C; IR (ƛ max cm^{-1}):3201 (NH sulfonamide), 3086 (NH amide), 2872 (CH_2 bridge), 1656 (C=O), 1340 &1155 (S=O); ^{1}HNMR (δ ppm): 11.05 (NH sulfonamide), 10.40 (NH amide), 4.78 (CH_2 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(III_f)
[$C_{24}H_{24}N_6O_4S$]:80% yield; m.p. 251-253°C; IR (ƛ max cm^{-1}): 3383 (NH sulfonamide), 3196 (NH amide), 2823 (CH_2 bridge), 1658 (C=O), 1350 & 1155 (S=O); ^{1}HNMR (δ ppm):11.35 (NH), 10.40 (NH amide), 4.77 (CH_2 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.

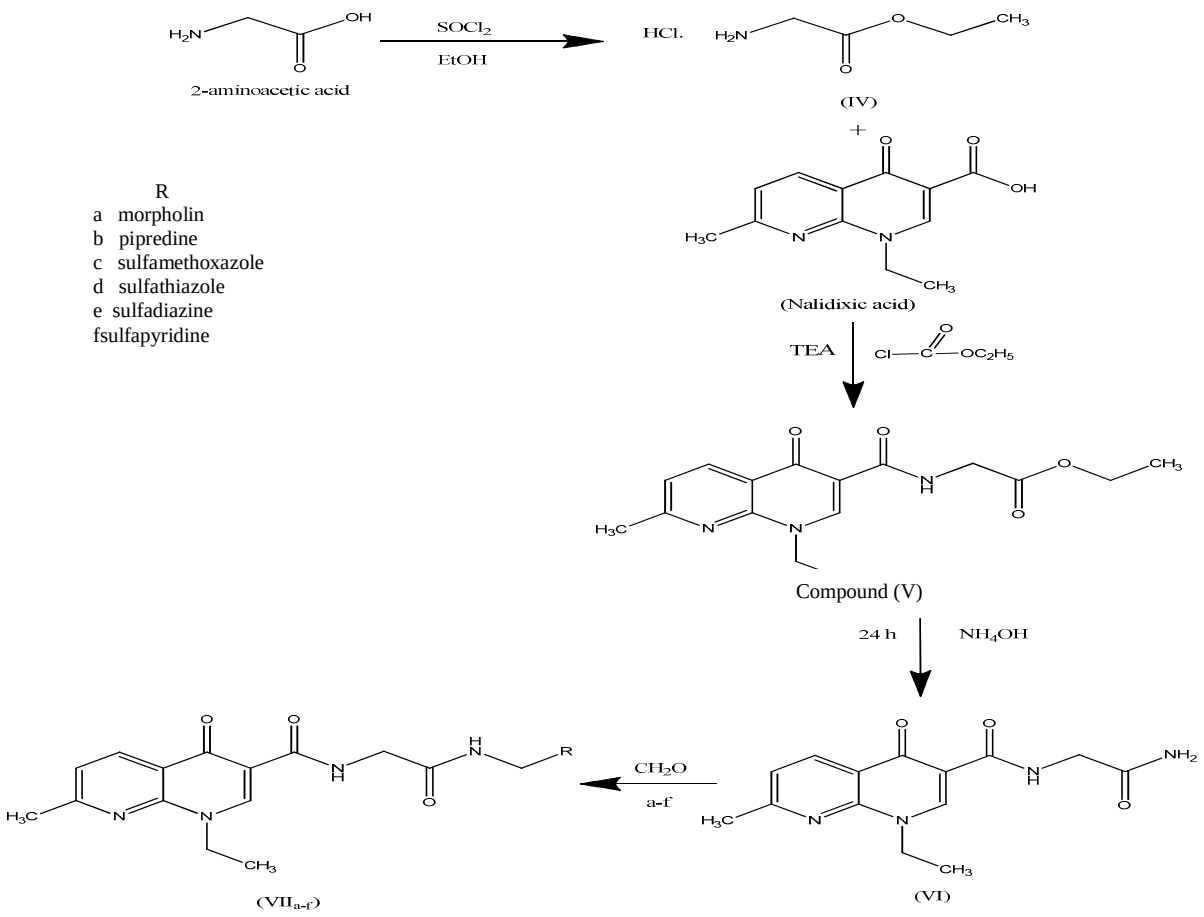
Synthesis of Ethyl-2-amino acetate hydrochloride (IV):
Thionyl chloride (11mmole, 0.8 mL) was added gradually to absolute ethanol (10 mL) cooled to (0°C). 2-Aminoacetic acid (10 mmole, 0.75g) was suspended in the reaction mixture and subjected to ultra-sonication at room temperature for (45 min.), on 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 $CHCl_3$ (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].

Synthesisof 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 mannich base derivatives (VII_{a-f}):
General Procedure for Synthesis of mannich base derivatives ($VII_{a,b}$):
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 mannich base derivatives from primary amines (VII_{c-f}):
Sulphonamide derivative (c-f) (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.



Scheme 2. Shows the synthesis of target compounds (VII_{a-f})

Ethyl amino acetate hydrochloride (IV)
[$C_4H_{10}NO_2Cl$]**:**92% yield; m.p. 143-145°C; IR (ƛ max cm^{-1}): 2978 (NH_2 amine), 2673& 2637 (CH_3), 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)
[$C_{16}H_{19}N_3O_4$]: 94% yield; m.p. 198-200°C; IR (ƛ 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)
[$C_{14}H_{16}N_4O_3$]: 66% yield; m.p. 313-315°C; IR (ƛ max cm^{-1}): 3416, 3279 & 3205 (NH amides), 3037 (Ar-H), 1668 (C=O), 1606 (S=C); ^{1}HNMR (δ ppm): 10.05 (NH amide), 7.10 (NH_2 amide), 3.96 (CH_2-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 (VII_a)
[$C_{19}H_{22}N_5O_4$]: 73% yield; m.p. 252-254°C; IR (ƛ max cm^{-1}): 3290 & 3228 (NH amide), 3081 & 3033 (Ar-H), 2891 & 2850 (CH_2 bridge), 1652 (C=O), 1114 (C-O morpholin); ^{1}HNMR (δ ppm): 10.10 (NH amide), 4.05 (CH_2 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 (VII_b)
[$C_{20}H_{27}N_5O_3$]: 65% yield; m.p. 211-213°C; IR (ƛ max cm^{-1}): 3292 & 3236 (NH amide), 3045 (Ar-H), 2852 & 2787 (CH_2 bridge), 1654 (C=O), 1228, 1165 & 1031 (C-N pipredine); ^{1}HNMR (δ ppm): 10.08 (NH amide), 4.02 (CH_2 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)

[$C_{25}H_{27}N_7O_6S$]: 70% yield; m.p. 288-290°C; IR (λ max cm^{-1}): 3420 & 3351 (NH sulfonamide), 3258 & 3100 (NH amide), 3073 (Ar-H), 2869 & 2803 (CH_2 bridge), 1651 (C=O), 1324 & 1147 (S=O); ^{1}HNMR (δ ppm): 11.04 (NH sulfonamide), 10.06 (NH amide), 4.80 (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)-1,4dihydro-1,8-naphthyridine-3-carboxamide (VII_d)

[$C_{24}H_{25}N_7O_5S_2$]: 78% yield; m.p. 253-255°C; IR (λ max cm^{-1}): 3365 (NH sulfonamide), 3203 & 3103 (NH amide), 3052 (Ar-H), 2852 (CH_2 bridge), 1660 (C=O), 1365 & 1138 (S=O); ^{1}HNMR (δ 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_e)

[$C_{25}H_{26}N_8O_5S$]: 80% yield; m.p. 280-281°C; IR (λ 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);^{1}HNMR (δ 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_f)

[$C_{26}H_{27}N_7O_5S$]: 82% yield; m.p. 272-274°C; IR (λ max cm^{-1}): 3362 (NH sulfonamide), 3205 (NH amide), 3049 (Ar-H), 2856 & 2823 (CH_2 bridge), 1662 (C=O), 1338 & 1134 (S=O);^{1}HNMR (δ 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, 5.83; 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

Sample Code and Standard	Concentration (µg/ml)	Zone of Inhibition (mm)					
		Gram Negative		Gram Positive		Fungal	
		E. coli	*Proteus*	*Bacillus*	*Staph. aureus*	*Candida albicana*	*Candida tropicalis*
II	250	15	17	20	15	19	16
	125	12	15	18	13	18	13
	62.5	11	13	12	11	16	10
	31.25	-	10	12	10	13	-
III_a	250	15	16	28	19	20	19
	125	15	11	22	17	18	16
	62.5	13	10	19	11	17	12
	31.25	11	-	17	-	16	11
III_b	250	16	17	19	15	21	19
	125	13	14	16	12	19	17
	62.5	11	12	13	11	18	14
	31.25	11	10	10	10	15	12
III_c	250	20	18	25	17	very high	20
	125	18	16	22	15	very high	18
	62.5	15	12	19	11	very high	17
	31.25	11	12	19	10	23	15
III_d	250	16	18	22	17	very high	17

	125	14	15	17	17	23	16
	62.5	12	12	16	15	21	12
	31.25	10	10	13	10	18	-
III_e	250	22	19	25	28	20	19
	125	18	16	21	21	18	18
	62.5	17	16	17	17	18	15
	31.25	13	11	14	13	16	14
III_f	250	18	24	18	16	18	20
	125	13	20	18	15	16	19
	62.5	11	18	15	12	16	19
	31.25	10	15	11	10	15	17
VI	250	14	17	16	20	16	16
	125	12	16	12	17	15	13
	62.5	-	12	9	16	14	11
	31.25	-	10	8	10	12	-
VII_a	250	20	15	20	22	22	19
	125	17	13	17	19	20	19
	62.5	12	12	13	16	18	17
	31.25	10	-	11	12	15	14
VII_b	250	17	16	20	15	20	21
	125	13	12	15	11	18	18
	62.5	12	10	12	10	18	17
	31.25	-	10	10	-	16	14
VII_c	250	22	19	24	17	very high	22
	125	19	16	21	15	very high	20
	62.5	16	15	19	11	very high	19
	31.25	14	11	19	8	very high	16
VII_d	250	19	20	24	17	24	19
	125	18	17	21	14	22	17
	62.5	15	16	18	11	22	16
	31.25	11	12	15	10	21	14
VII_e	250	18	21	22	19	22	21
	125	18	19	20	16	21	19
	62.5	16	18	18	15	18	17
	31.25	13	16	15	11	16	17
VII_f	250	19	18	19	20	19	22
	125	16	15	17	18	19	20
	62.5	16	11	16	18	17	19
	31.25	13	8	11	15	16	17
Nalidixic acid	250	16	15	13	14	12	9
	125	12	12	11	12	10	-
	62.5	10	9	10	8	-	-
	31.25	-	-	-	-	-	-
Sulfamethoxazole	250	17	16	17	12	Very high	16
	125	14	15	13	10	Very high	14
	62.5	11	12	12	-	Very high	14
	31.25	10	10	12	-	16	13
Sulfathiazole	250	15	14	17	13	24	-
	125	14	14	15	10	23	-
	62.5	12	12	14	-	22	-
	31.25	12	-	-	-	19	-
Sulfadiazine	250	18	16	19	14	17	16
	125	14	12	17	12	16	15
	62.5	13	9	15	10	13	14
	31.25	10	-	11	10	13	12
Sulfapyridine	250	16	19	19	17	16	17
	125	14	14	18	14	15	17
	62.5	13	11	16	10	14	16
	31.25	11	-	13	-	14	15
DMSO	pure	-	-	-	-	-	-

RESULTS AND DISCUSSION

Synthesis of the target compounds (III_{a-f}&VII_{a-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 δ 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 250µg/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_e) may regard the best one and (III_a) 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.

Acknowledgements
The authors are thankful to the laboratory for pharmaceutical Chemistry, Al-mustansiriyah University, Baghdad, Iraq, for providing the necessary facilities to conduct research work.

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