RESEARCH ARTICLE

Synthesis of New Antibiotic Agent Based on Mannich Reaction

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ABSTRACT

Three new anti-bacterial compounds were designed and synthesized via the Mannich reaction and evaluated in vitro against different species of bacteria. The compounds have been characterized based on Fourier transform infrared (FT-IR), ^{1}HNMR, and Carbon-13 Nuclear magnetic resonance (^{13}C NMR), A significant part of the compounds obtained, showed anti-bacterial activity towards Gram-positive and Gram-negative species.

Keywords: Mannich reaction, Antibacterial, Ciprofloxacin, Amoxicillin, Ampicillin, and Cefalexin

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INTRODUCTION

One of the most significant and fundamental chemical synthesis reactions is the Mannich reaction. It's a three-component condensation between molecules having active hydrogen, *i.e.,* one acidic hydrogen atom, an aldehyde, and a primary or secondary amine.[1] The process involves the removal of a water molecule and produces a Mannich base.[2,3] Mannich reactions exhibit a variety of biological effects, including carbonic cytotoxicity,[4-7] anhydrase inhibition,[8-10] anti-inflammatory,[11] and anticonvulsant.[12,13] Quinolones constitute an important class of synthetic broad-spectrum anti-bacterial agents; they have been the center of considerable scientific and clinical interest since their discovery. Nalidixic acid I, showed moderate activity against Gram-negative bacteria and low absorption. It is being marketed for the treatment of UTIs.[14]

MATERIAL AND METHODS

Instrument

The 400 MHz NMR (^{1}H and ^{13}C) analysis was used (Iran). The FTIR was recorded at Al-Zahrawi University College (IRAQ).

Synthesis of Compounds (1-3)

In three distinct reaction containers, 1-mmol of amoxicillin, ampicillin, and cefalexin were mixed in 50 mL of DMSO, then 37% formaldehyde (0.2 mL) was added to each container and gently agitated for 15 minutes. One mmol of ciprofloxacin was added to a solution of amoxicillin, penicillin, and cefalexin with formaldehyde and agitated overnight, after which distilled water was added and the precipitate formed was filtered and washed multiple times with distilled water. Compounds 1, 2, and 3 could be obtained.[15,16]

Antimicrobial Activity:

The disc diffusion assay was conducted for the anti-bacterial test of the synthesized compounds 1-3. Compounds 1-3 were dissolved in DMSO with a range of concentrations from 12.5 to 50 mg/mL. Filter papers with 1.6 mm were saturated with the solution of sample and negative control DMSO and placed on the surface of the agar. The plates were incubated for 48 hours at 37°C. The diameter of zone inhibitions was measured in millimeters (mm) to evaluate the effect of new compounds against the microbial used.[17]

Statistical Analysis

One-way ANOVA was utilized to compare anti-bacterial activities, followed by Tukey's post hoc analysis and mean SEM to express the data with p 0.05. SPSS software update version was used for the analysis, and GraphPad Prism program v8.0.2 was used to create the graphs.

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Table 1: Inhibitory zones of novel synthesis compounds at different percentage concentrations vs. stander references (*). (statistics analysis by one ANOVA Mean ± Std. Error)

Bacteria/concentration	*Compounds*	*Mean ± Std. Error*	*95% Confidence interval for mean*		*p value*
			Lower bound	*Upper bound*	
Staphylococcus aureus/ (12.5%)	Ciprofloxacin-amoxicillin	21.25 ± 0.52	19.01	23.49	.000
	Ciprofloxacin-Ampicillin	28.17 ± 1.13	23.31	33.02	
	Ciprofloxacin-cephalexin	19.30 ± 1.08	14.66	23.94	
	Ciprofloxacin*	0.27 ± 0.18	-0.49	1.03	
	Amoxicillin*	31.97 ± 1.07	27.37	36.56	
S. aureus/(25%)	Ciprofloxacin-amoxicillin	28.10 ± 1.30	22.51	33.69	.000
	Ciprofloxacin-ampicillin	33.70 ± 1.19	28.57	38.83	
	Ciprofloxacin-cephalexin	26.27 ± 1.22	21.02	31.51	
	Ciprofloxacin*	0.23 ± 0.12	-0.28	0.75	
	Amoxicillin*	31.43 ± 2.72	19.74	43.12	
S. aureus/(50%)	Ciprofloxacin-Amoxicillin	34.23 ± 0.62	31.58	36.89	.000
	Ciprofloxacin-Ampicillin	37.97 ± 0.84	34.36	41.57	
	Ciprofloxacin-cephalexin	31.50 ± 0.98	27.26	35.74	
	Ciprofloxacin*	0.33 ± 0.33	-1.10	1.77	
	Amoxicillin*	40.23 ± 1.13	35.36	45.10	
Klebsiella pneumoniae/ (12.5 mm)	Ciprofloxacin-Amoxicillin	17.93 ± 0.92	13.96	21.91	.000
	Ciprofloxacin-ampicillin	19.87 ± 1.10	15.14	24.60	
	Ciprofloxacin-cephalexin	22.10 ± 0.84	18.49	25.71	
	Ciprofloxacin*	28.17 ± 1.07	23.56	32.78	
	Amoxicillin*	0.33 ± 0.33	-1.10	1.77	
K. pneumoniae/(25 mm)	Ciprofloxacin-Amoxicillin	25.03 ± 0.78	21.68	28.39	.000
	Ciprofloxacin-ampicillin	24.30 ± 1.08	19.66	28.94	
	Ciprofloxacin-cephalexin	26.03 ± 0.90	22.18	29.88	
	Ciprofloxacin*	31.47 ± 9.55	-18.63	63.56	
	Amoxicillin*	0.50 ± 0.29	-0.74	1.74	
K. pneumoniae/(50 mm)	Ciprofloxacin-Amoxicillin	28.00 ± 0.75	24.77	31.23	.009
	Ciprofloxacin-ampicillin	28.23 ± 1.07	23.61	32.86	
	Ciprofloxacin-cephalexin	30.27 ± 0.76	26.99	33.55	
	Ciprofloxacin*	36.57 ± 0.30	35.29	37.84	
	Amoxicillin*	0.43 ± 0.30	-0.84	1.71	

RESULTS AND DISCUSSION

Chemistry Results

Three different compounds were synthesized utilizing the Mannich process, as illustrated in Scheme 1. In a DMSO solution, the ciprofloxacin and formaldehyde (37%) were agitated at room temperature overnight. The yields of the reactions ranged from 74 to 82 percent. $^{1}H–C^{13}$ NMR IR and were used to determine the structures of the new compounds 1, 2, and 3.

Compound (1) (Ciprofloxacin-Amoxicillin); 1H NMR (DMSO-d6): 0.45-0.75 (m, 6H, CH_3), 1.10-1.44 (m, 4H, cyclopropyl), 3.25-3.75 (m, 8H, Diethylenediamine) 4.15-5.48 (m, 3H, 3,3-dimethyl-7-oxo-4-thia-1 azabicycloheptane-2-carboxylic acid), 5.18 (s, 2H, CH2 of linker), 6.98-9.11 (Ar-H), 9.80 and 10.01 (s, 2H, NH), 12.16 (1H, OH) 13.62-14.54 (2H, COOH). C13 NMR (DMSO-d6): 192, 185, 182, 180 and 176 (5 carbonyl groups), 118-151 (14 sp2 carbons), 73 CH2 of linker, 27-30 (2CH3 groups). FTIR 1640 and 1720 cm^{-1}, (carbonyl groups). 3200 and 3230 cm^{-1} (NH groups), 3420 cm^{-1} (OH groups)

Compound (2) (Ciprofloxacin-Ampicillin); 1H NMR (DMSO-d6): 0.25 and 0.52 (m, 6H, CH_3), 1.75-2.01 (m, 4H, cyclopropyl), 3.28 and 3.92 (m, 8H, Diethylenediamine) 4.51-5.55 (m, 3H, 3,3-dimethyl-7-oxo-4-thia-1-azabicycloheptane-2-carboxylic acid), 4.96 (s, 2H, CH_2 of linker), 6.45-9.62 (Ar-H), 10.01 and 10.52 (s, 2H, NH), 13.23 and 14.01 (2H, COOH). FTIR 1620 and 1740 cm^{-1}, (carbonyl groups). 3240 and 3280 cm^{-1} (NH groups), 3500 cm^{-1} (OH groups)

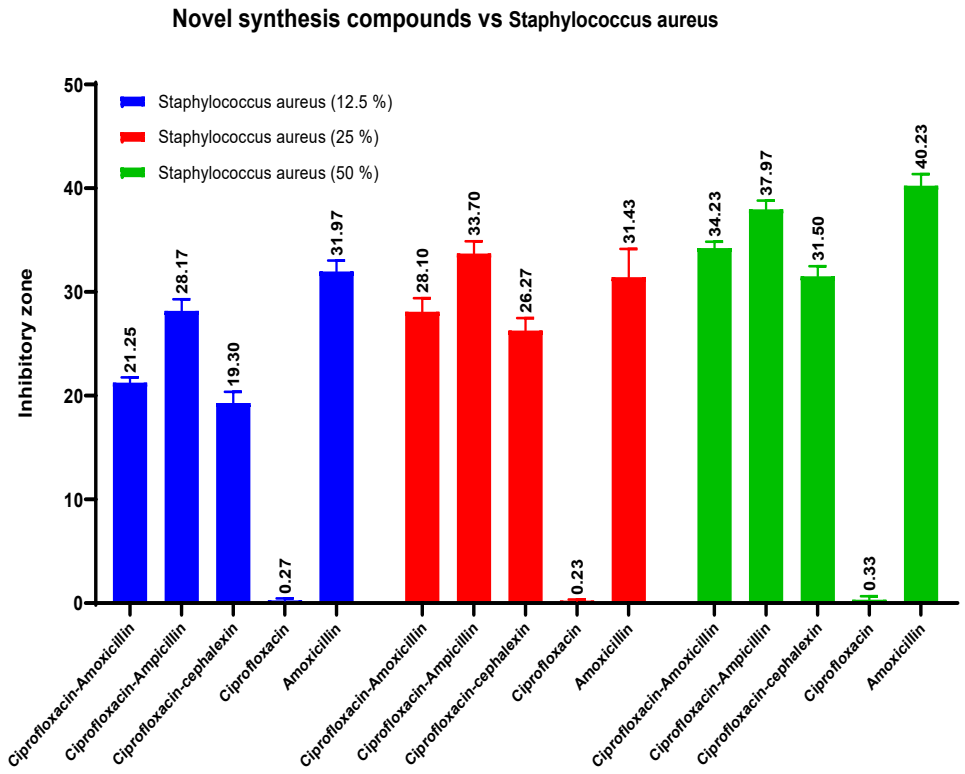

Figure 1: Anti-bacterial activities of novel compounds against *S. aureus* vs. standers.

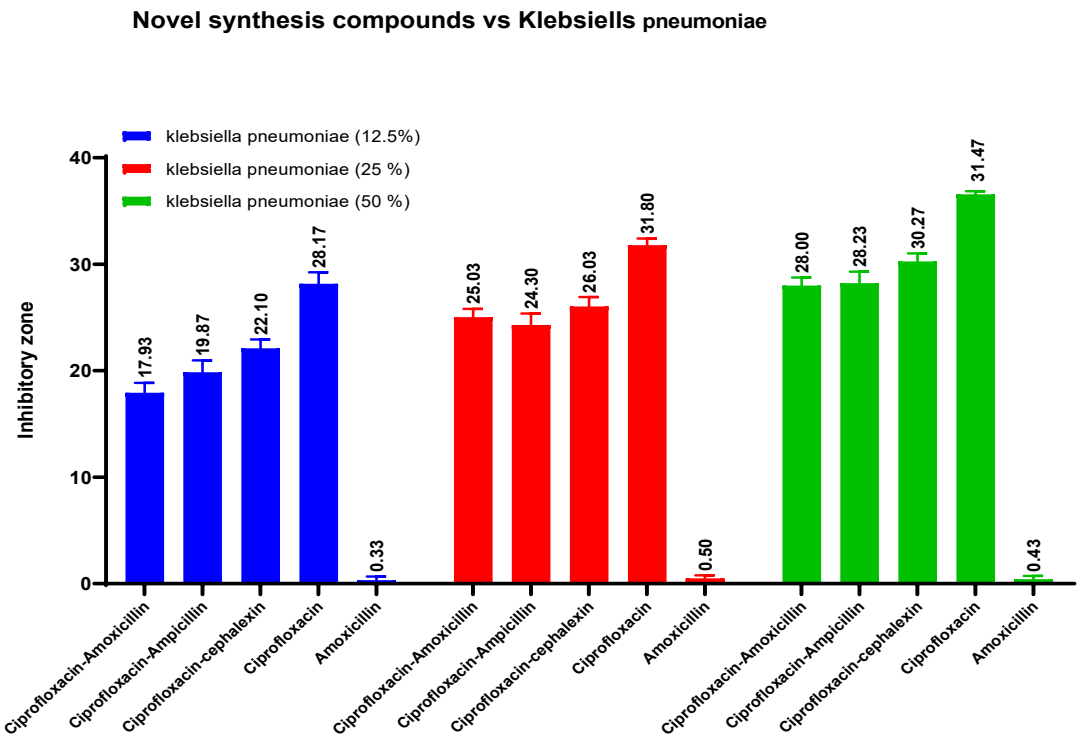

Figure 2: Inhibitory zone of novel synthesis compounds vs. *K. pneumonia.*

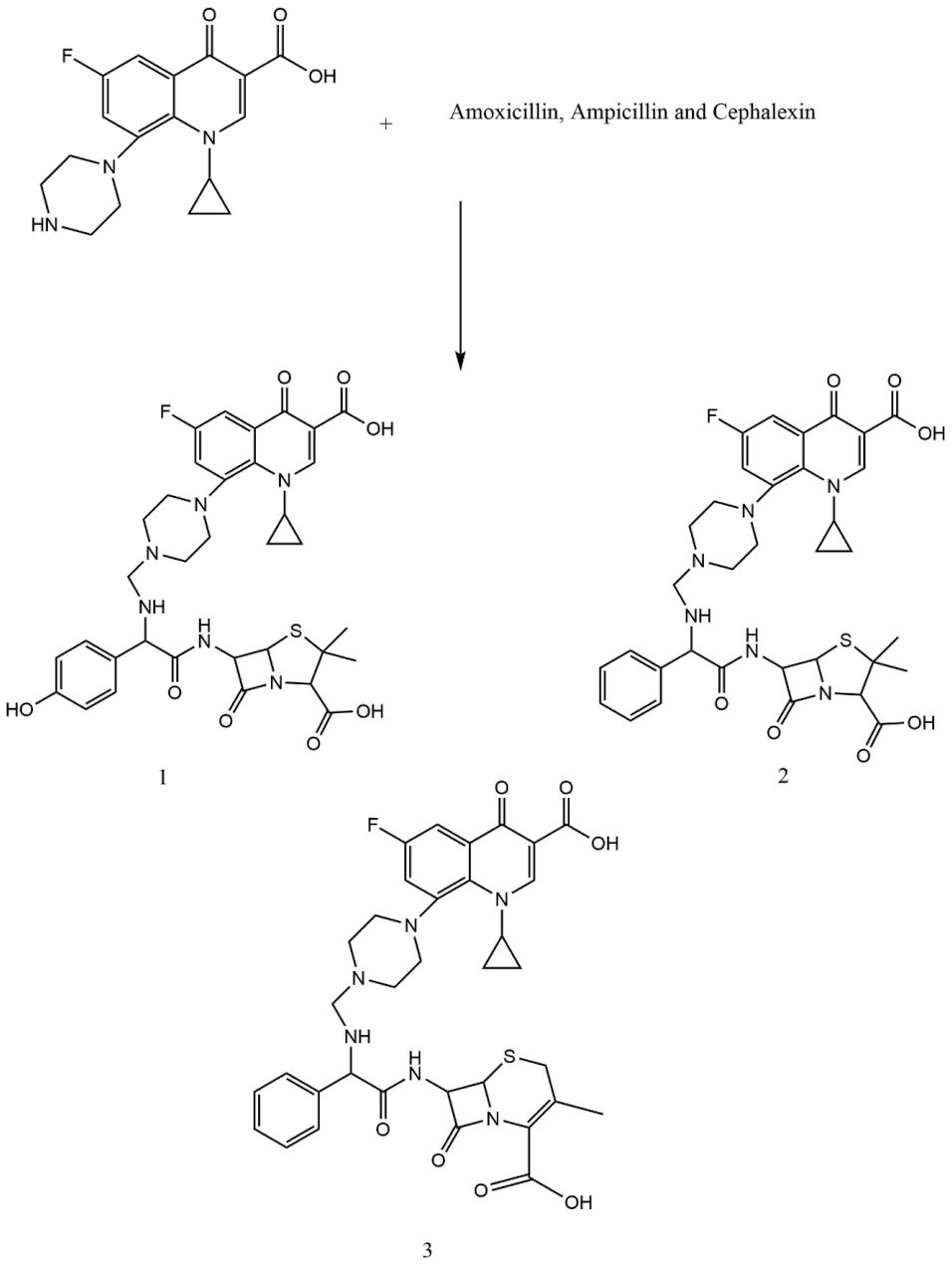

Figure 3: Synthesis compound 1-3

Compound (3) (Ciprofloxacin-Cephalexin); ^{1}H NMR (DMSO-d6): 2.02 (s, 3H, CH_3), 1.00-1.61 (m, 4H, cyclopropyl), 3.12 and 3.62 (m, 8H, Diethylenediamine) 4.22-5.55 (m, 4H, 3-methyl-8-oxo-5-thia-1-azabicyclooct-2-ene-2-carboxylic acid), 5.22 (s, 2H, CH_2 of linker), 6.68-9.22 (Ar-H), 13.00-13.52 (2H, COOH). FTIR 1616 and 1680 cm^{-1}, (carbonyl groups). 3180 and 3260 cm^{-1} (NH groups), 3340 cm^{-1} (OH groups)

Anti-bacterial Results

We compared or tested the anti-bacterial activities between the new compounds and our standards by measuring the inhibitory zone at different percentage concentrations. We found that the newly synthesized compounds have to vary anti-bacterial activities.

Novel Compounds Against *S. Aureus*

Our results showed that amoxicillin has the highest inhibitory zone compared to other novel synthesis compounds. However, the ciprofloxacin showed the lowest or nearly null inhibitory zone. There is an insignificant difference ($p > 0.05$) between Ciprofloxacin-Amoxicillin and Ciprofloxacin-cephalexin compounds; Ciprofloxacin-Ampicillin and amoxicillin inhibit bacterial growth; meanwhile, there is a significant difference ($p < 0.05$) between ciprofloxacin-ampicillin and

both Ciprofloxacin-Amoxicillin and Ciprofloxacin-cephalexin compounds (Table 1 and Figure 1).

Novel Compounds Against *K. Pneumonia*

The anti-bacterial activities of the new compounds were differed against klebsiella pneumonia as compared with *s. aureus.* The inhibitory zone of ciprofloxacin was higher as compared with other novel synthesis compounds. However, amoxicillin showed the lowest or nearly null inhibitory zone.

At a low percent (12.5%) concentration, there is an insignificant difference ($p > 0.05$) between Ciprofloxacin-Amoxicillin with both Ciprofloxacin-cephalexin compounds and Ciprofloxacin-Ampicillin to inhibit bacterial growth; meanwhile, there is a significant difference ($p < 0.05$) between ciprofloxacin all newly synthesis compounds.

At 25% concentration, there is an insignificant difference ($p > 0.05$) between ciprofloxacin and new synthesis compounds, and within these groups, we notice the insignificant difference. But as an increased percentage of concentration to 50%, we found a significant difference ($p < 0.05$) between ciprofloxacin all newly synthesized compounds but still no significant difference ($p < 0.05$) between them (Figure 2 and 3).

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