

Design, Synthesis, and Antibacterial Study of New Gatifloxacin-Antioxidants as Mutual Prodrugs

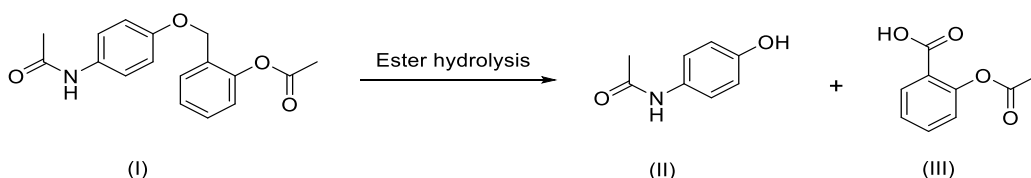
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Abstract

Objective: The objective of this search is to design, and synthesize new mutual prodrugs in order to obtain synergistic antibacterial activity. **Methods:** The hydroxyl group of three antioxidants (menthol, thymol, and vanillin), was linked to chloroacetyl chloride through nucleophilic substitution reaction, to give intermediates (Ia-Ic), which were reacted with a carboxyl group of Gatifloxacin to form three final compounds (I-III) having ester linkage. **Results:** The Antibacterial activity effect of the target compounds (I-III) has been tested for *in vitro* inhibitory activity against Gram-positive bacteria: *Staphylococcus aureus* and Gram-negative bacteria: *Escherichia coli* by using spots diffusion method. All the tested compounds show a remarkable antibacterial activity against tested bacteria. **Conclusion:** The synthesized prodrugs were characterized and identified through FT-IR spectroscopy, ¹H-NMR spectrum, and various physicochemical parameters. The antibacterial study of the compounds showed various activities toward the two types of pathogenic bacteria which are *Staphylococcus aureus* and *Escherichia coli*. Compounds [I, II, and III] revealed that *Staphylococcus aureus* was sensitive to synthesized compounds but *Escherichia coli* showed a reverse activity with some resistance for antibacterial drugs.

Key words: Gatifloxacin, Antioxidants, Mutual Prodrugs, Antibacterial activity



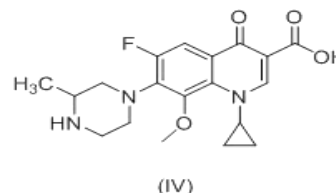
Gatifloxacin (IV) is a fourth-generation fluoroquinolone antibacterial agent (Sharma et al., 2009), which is a synthetic compound that acts through suppression of bacterial DNA synthesis by inhibition of gyrase or topoisomerase II enzyme in gram-negative bacteria, and topoisomerase IV enzyme in gram-positive bacteria (Dougherty et al., 2014; Hawkey, 2003). Fluoroquinolone possesses a broad antibacterial spectrum due to

Introduction

Prodrugs are biologically inactive compounds that are activated either chemically or enzymatically to give the active parent components (Ratnadeep V. Ghadage, 2013). They are classified to different classes according to the type of moieties (Zawilka et al., 2013). The carrier linked prodrugs in which the drug molecule (active moiety) was linked with carrier molecule (inactive moiety), while the mutual prodrugs in the two active drug molecules were linked to each other, either directly or through specific linker reduce the steric factors between the two moieties (Abu-jaiash et al., 2014). Different bonds were used in the synthesis of prodrug molecules as ester, amide...etc.), which hydrolyzed by esterase and amidase enzymes respectively (Naser, 2018).

There are numerous objectives for prodrug synthesis such as increasing water solubility, increasing oral bioavailability, increasing chemical stability, reducing pain at injection site, reducing side effects, increasing organs selectivity (Datar and Shendge, 2015), and synergistic effect which is one of the important objectives of mutual prodrugs like Benorilate (I) in which the paracetamol (II) linked through ester linkage to aspirin (III) in order to give synergistic analgesic effect, and reduce gastric ulceration (Zhi-Z and Jiang, 2012).

its effect against gram-positive bacteria like *Staphylococci*, gram-negative bacteria like *Escherichia coli*, *Klebsiella*, *Serratia*, and *Pseudomonas aeruginosa*, anaerobic *Chlamydia*, *Mycoplasma*, *Legionella*, *Brucella*, and *Mycobacterium* (Xia et al. 2013; Raul et al., 2015).

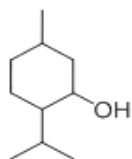


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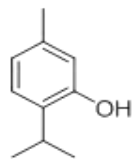
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In order to increase its antibacterial activity, it was linked with different anti-oxidants as menthol (V) (Leyva-López et al., 2017), thymol (VI) (Waliwitiya et al., 2010), and vanillin (VII) (Kumar

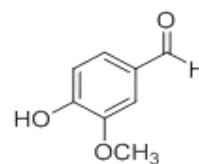
et al., 2012), as they possess strong antibacterial activity, in addition to their anti-oxidant, anticancer, and anti-inflammatory properties (Chahal et al., 2017).



(V)



(VI)



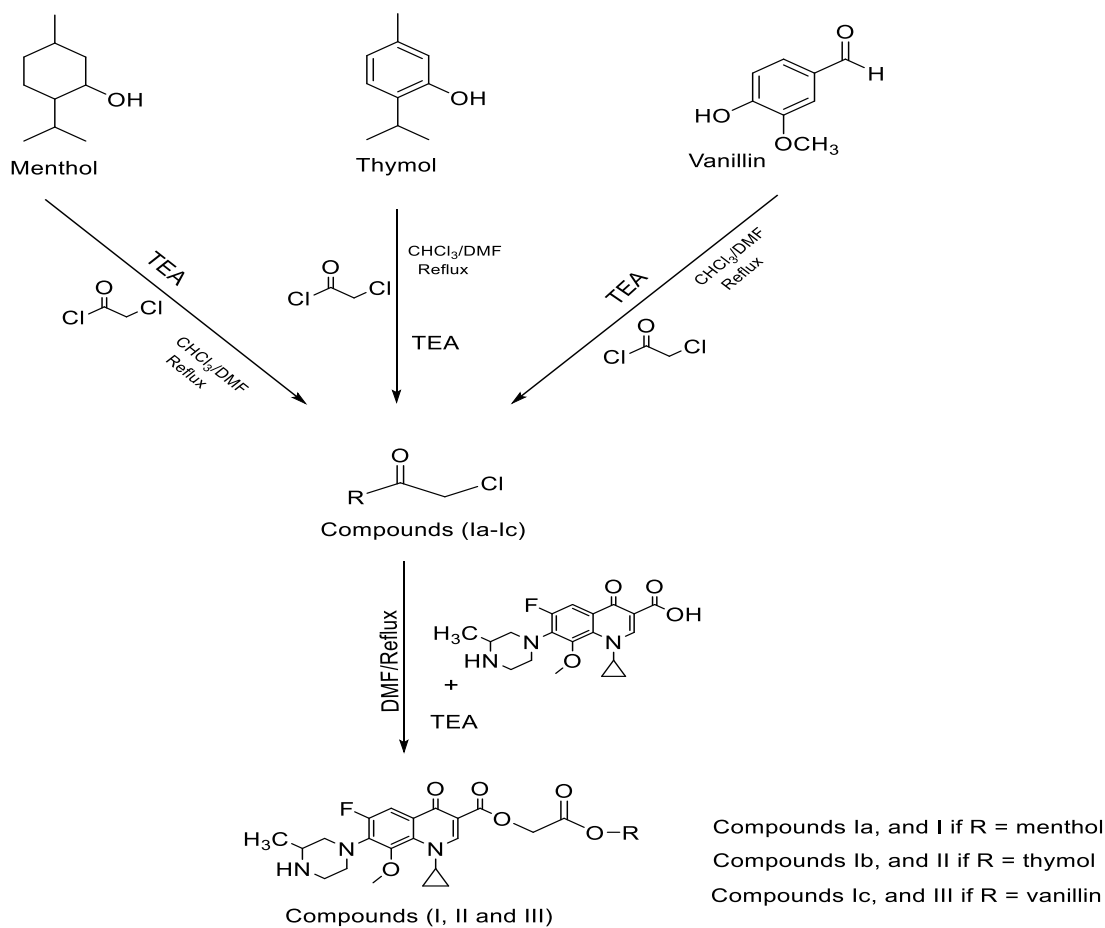
(VII)

Materials and Methods

Experimental

The reagents and anhydrous solvents were of analytical grade and supplied from (Reidal Dehean Germany; Sigma-Aldrich Germany; BDH England). Melting points (uncorrected) were determined by the capillary tube method by Thomas hover apparatus (England). R_f values were determined through using ascending thin layer chromatography, on DC-Kartan SI Alumina 0.2 mm to ensure the purity and progress of the reaction, using methanol: benzene (50:50) as a mobile phase (Ahmed et al.,

2016). Determination of FT-IR spectra was done by using FT-IR spectrophotometer and KBr discs, at the faculty of pharmacy, Kufa University. Proton nuclear magnetic resonance (¹H-NMR) spectra were recorded using NMR ultra shield spectrophotometer 500 MHz, Bruker Avance III (Switzerland), at the college of Tehran, Iran. Steps of synthesis of all compounds are presented in scheme 1. Antioxidants (menthol, thymol, and vanillin) were coupled with chloroacetylchloride in the presence of TEA, to give intermediates Ia, Ib, and Ic. Then the coupling reaction of Gatifloxacin with intermediates Ia, Ib, and Ic result in the synthesis of compounds I, II, and III respectively.



Scheme 1: Synthesis of the compounds I, II, and III.

*Chemistry:**Coupling reaction of antioxidants with chloroacetylchloride:*

Antioxidant (19.1 mmol), was dissolved in DMF: CHCl₃ (25:75) mixture (40 ml), then TEA (2.66 ml, 19.1 mmol) was added. The reaction mixture was stirred on ice bath; chloroacetylchloride (1.5 ml, 19.1 mmol in 10 ml CHCl₃) was added in dropwise with continuous stirring over a period of one hour, followed by refluxing of the mixture for three hours. Then excess cold water was added, and the precipitated compound was filtered and crystallized from ethanol, to give intermediate Ia, Ib, and Ic (Noor et al., 2018). The percent yield, physical appearance, and R_f values were given in Table 1.

Spectral Analysis:

2-isopropyl-5-methylcyclohexyl 2-chloroacetate (Ia); FT-IR (cm⁻¹): 2,972 (C-H) of alkane, 1,747 (C=O) of ester, and 1,226 (C-O) of ester.

2-isopropyl-5-methylcyclohexyl 2-chloroacetate (Ib); FT-IR (cm⁻¹): 2,970 and 2,939 (C-H) of alkane, 1,741 (C=O) of ester, and 1,581 and 1,479 (C=C) of aromatic.

4-formyl-2-methoxyphenyl 2-chloroacetate (Ic); FT-IR (cm⁻¹): 2,978 and 2,738 (C-H) of alkane, 1,789 (C=O) of aldehyde, and 1,685 (C=O) of ester.

Coupling reaction of Gatifloxacin with intermediates Ia, Ib, and Ic.

A mixture of intermediated Ia, Ib or Ic (17.5 mmol), and Gatifloxacin (17.5 mmol), were dissolved in DMF (25 ml), then TEA (2.5 ml, 17.5 mmol) was added. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated; the residue was triturated with acetone and crystallized from methanol (Noor et al., 2018). The percent yield, physical appearance, and R_f values were given in Table 1.

Spectral Analysis:

2-((2-isopropyl-5-methylcyclohexyl)oxy)-2-oxoethyl-1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (I); FT-IR (cm⁻¹): 3,048 (C-H) of aromatic, 2,978 and 2,850 (C-H) of alkane, 1,782 (C=O) of ketone, and 1,743 (C=O) of ester. ¹H-NMR (DMSO-d₆) δ(ppm): 8.7 (s,1H,CH of alkene), 7.7 (s,1H,CH-Ar), 5.2 (s,2H,-OCH₂), 4.5 (m,1H,C-H), 4.1 (m, 1H, C-H of cyclopropane),3.8 (s,3H,OCH₃), 3.5-2.6 (3m,7H, CH and CH₂ of piperazine ring), 1.8-1.3 (m,13H,CH of alkane), 1.11 (d,3H,CH₃), 1.08 (m,1H,NH), 0.88 (high intensity doublet, 9H,3CH₃).

2-(2-isopropyl-5-methylphenoxy)-2-oxoethyl 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (II); FT-IR (cm⁻¹): 3,038 (C-H) of aromatic, 2,974 and 2,939 (C-H) of alkane, 1,732 (C=O) of

ketone, and 1,620 (C=O) of ester. ¹H-NMR (DMSO-d₆) δ(ppm): 8.7 (s,1H,CH of alkene), 7.7 (s,1H,CH-Ar), 7.2-7 (m,3H,CH-Ar), 5.2 (s,2H,-OCH₂), 4.1 (m, 1H, C-H of cyclopropane),3.8 (s,3H,OCH₃), 3.5-2.6 (3m,7H, CH and CH₂ of Piperazine ring), 3 (m,1H,CH), 2.35 (s,3H,CH₃-Ar), 1.3 (m,4H,2CH₂ of cyclopropane), 1.2 (high intensity doublet,6H,2CH₃), 1.11 (d,3H,CH₃), 1.08 (m,1H,NH).

2-(4-formyl-2-methoxyphenoxy)-2-oxoethyl 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (III); FT-IR (cm⁻¹): 3,012 (C-H) of aromatic, 2,931 and 2,904 (C-H) of alkane, 1,732 (C=O) of aldehyde, and 1,643 (C=O) of ester. ¹H-NMR (DMSO-d₆) δ(ppm): 9.6 (s,1H,CH of aldehyde), 8.7 (s,1H,CH of alkene), 7.7-7.3 (4d,4H,CH-Ar), 5.2 (s,2H,-OCH₂), 4.1 (m, 1H, C-H of cyclopropane),3.8 (s,6H,2 -OCH₃), 3.5-2.6 (3m,7H, CH and CH₂ of Piperazine ring), 1.3 (m,4H,2CH₂ of cyclopropane), 1.11 (d,3H,CH₃), 1.08 (m,1H,NH).

Antibacterial Study

The *in vitro* antibacterial activity of the synthesized compounds was investigated against several pathogenic representative Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative bacteria like *Escherichia coli* by agar well diffusion method, using Muell-Hinton agar as a medium (Ullah and Ali, 2017). All bacteria used were obtained from the microbiology laboratory in Middle Euphrates Hospital. 1ml of the spore suspension of each bacterium was spread all over the surface of the cold solid media placed in the petri-dish. The tested compounds were dissolved in DMSO. An amount (0.1ml) of the solutions was added accurately in spots on the surface of the injected solid media.

The Petri-dishes were incubated at 37° C for 24 hours. The inhibition zone formed by the compounds against the two types from tested bacterial determined the antibacterial activities of the synthetic compounds. The mean value obtained for two individual replicates was used to calculate the zone of growth inhibition of each compound.

Results and Discussion*Chemistry:*

Mutual ester prodrugs of Gatifloxacin with antioxidants (menthol, thymol, and vanillin) were synthesized according to the scheme that was shown above. Their physicochemical characters were represented in Table 1, and their structures were confirmed by the FT-IR spectroscopy and ¹H-NMR spectra. Anti-oxidants (menthol, thymol, and vanillin) underwent nucleophilic substitution reaction (S_N²) in presence of equimolar of chloroacetyl chloride when the hydroxyl group in their structures attacked the electrophilic carbonyl carbon in chloroacetyl chloride leading to the displacement of the chlorine atom. The reaction occurred in the presence of equimolar of triethylamine which acted as a base to neutralize the hydrogen chloride formed. This reaction led to the formation of compounds Ia, Ib, and Ic.

The rate of an SN² reaction follows second order kinetics, just like how the rate of limiting step depends on the nucleophile concentration as well as the concentration of the substrate (Smith and March, 2007). This mechanism depends on the solvent, temperature, and concentration of the nucleophile and that of the leaving group. It is generally favored in primary or secondary alkyl halides with an aprotic solvent like (DMF) (Marye Anne FOX and James K. Whitesell, 2004). The synthesized compounds undergo another nucleophilic substitution reaction with Gatifloxacin, in which the secondary amine in the later compound act as a nucleophile to attack the electrophile and cause displacement of the chlorine atom from compounds Ia, Ib, and Ic. In aliphatic heterocyclic compounds, the nitrogen atom is a part of a saturated heterocyclic ring and the lone pair of electrons is available for reaction with protons (e.g. Piperazine). In the base strength compounds of this type are similar to their open-chain aliphatic counterparts with typical pKa values of 8-9 (Donald, 2008).

Table 1: Physicochemical Properties of the Synthesized Compounds.

Compounds	Empirical Formula	Molecular weight	Description	% Yield	Melting point °C	R _f values
Ia	C ₁₂ H ₂₁ ClO ₂	232.75	Deep brown crystals	60.5	200-201	0.63
Ib	C ₁₂ H ₁₅ ClO ₂	226.7	Pale yellow powder	80	251-252	0.79
Ic	C ₁₀ H ₉ ClO ₄	228.63	Pale Brown crystals	84	202 d	0.8
I	C ₃₁ H ₄₂ FN ₃ O ₆	571.69	Brown Powder	76	198-200	0.72
II	C ₃₁ H ₃₆ FN ₃ O ₆	565.64	Pale yellow Powder	62	289 d	0.62
III	C ₂₉ H ₃₀ FN ₃ O ₈	567.57	White crystals	63	184-186	0.84

Antibacterial activity:

The Antibacterial activity effect of the compounds has been tested for *in vitro* growth inhibitory activity against Gram-positive bacteria: *Staphylococcus aureus* and Gram-negative bacteria: *Escherichia coli* by using spots diffusion method (Sonmez et al., 2019; Sharma et al., 2019). All the tested compounds show a remarkable antibacterial activity against tested bacteria. The results were listed in Table 2, and their statistical results were shown in Figure 1. Compounds [I, II, and III] revealed that *Staphylococcus aureus* was sensitive to compounds but *Escherichia coli* showed a reverse activity with some resistance to antibacterial drugs and this can be discussed under four points:- 1-inhibition of cell membrane function, 2-inhibition of cell wall 3-inhibition of nucleic acid and 4-inhibition of protein synthesis (Ullah and Ali, 2017).

Table 2: Antibacterial activity data (zone of inhibition in nm) of the compounds I, II, and III.

Compound	Bacteria	
	G(+Ve)	G(-Ve)
	<i>S.aureus</i>	<i>E.coli</i>
S	15	10
I	23	16
II	21	17
III	20	11

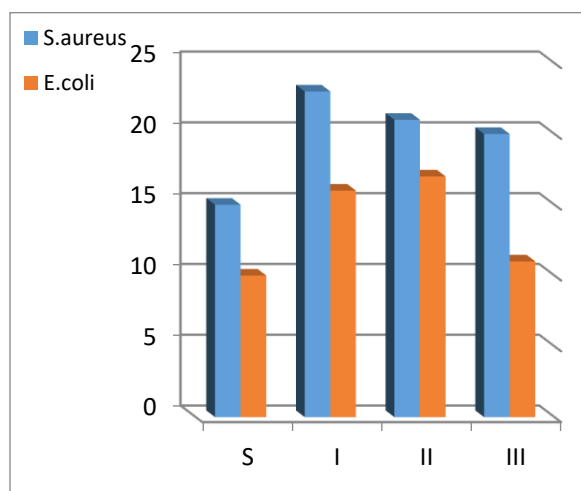


Figure 1: Antibacterial activity data (Zone of inhibition in nm) of the compounds.

Conclusion:

The designed compounds have been synthesized successfully as shown in scheme 1 and their structures were confirmed, using Fourier Transform Infrared Spectroscopy (FT-IR spectra), Proton Nuclear Magnetic Resonance Spectroscopy (¹H-NMR), and their purity was confirmed by their physical data (melting points and R_f values).

All the tested compounds show a remarkable antibacterial activity against tested bacteria. Compounds [I, II and III] revealed that *Staphylococcus aureus* was sensitive to synthesized compounds but *Escherichia coli* showed a reverse activity with some resistance to antibacterial drugs.

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