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Design, Synthesis, And Antibacterial Study Of New Gatifloxacin Conjugated With Guaiacol And Paracetamol Analogues As Mutual Prodrug

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Abstract: :

Objective: The objective of the work to design, and synthesize new mutual prodrugs of gatifloxacin to improve the therapeutic action as anti-microbial mainly as anti-bacterial to overcome the emergent anti-bacterial resistance of the gatifloxacin in first approach and to broaden the anti-bacterial spectrum secondly .In our study we choose the ligand (quaiacol and paracrtamol) to link via an ester bond with gatifloxacin by spacer arm chloroacetyl chloride. The quaiacol is a natural anti-oxidants with mark able anti-bacterial action ,while paracetamol is a centrally non - steroidal anti-inflammatory drugs (NSAIDs) ,the up-to-date studies of paracetamol referred to it has a good anti-bacterial action. Design of the new analogous of gatifloxacin with quiaicol&paracetamol respectively with complementary anti-bacterial action to synergize the therapeutic action and impair the microbial mechanism of resistance that is the major challenges of the most anti-bacterial antibiotic.

Methods: The hydroxyl functional groupe of (quaiacol ,parcetamol), was conjugated to chloroacetyl chloride via a nucleophilic substitution profile reaction respectively, to produce an intermediates (IA-IB), which were further reacted with a carboxyl functional group of Gatifloxacin to produce the designed compounds (I-II) having an ester linkage as mutual prodrug.

Results: The Antibacterial activity effect of the designed compounds (I-II) has been investigated for in vitro inhibitory anti-bacterial action against Gram-(+) bacteria like Staphylococcus aureus and Escherichia coli as Gram-negative bacteria; using the diffusion of the spots procedure. All the tested compounds show a remarkable antibacterial activity toward the tested bacteria.

Conclusion: The synthesized designated mutual prodrugs were synthesized ,characterized and identified by using FT-IR spectroscopic analysis, 1H-NMR spectroscopic analysis, and important physicochemical properties. The antibacterial assay of the designated compounds report different activities toward the two types of pathogenic bacteria which are Staphylococcus aureus and Escherichia coli. Compounds [I, II] revealed that Staphylococcus aureus was sensitive to synthesized designated compounds but Escherichia coli report a reverse activity with some resistance for antibacterial drugs .

Key words: Gatifloxacin, NSAID, Quaiacolpenolic antioxidants Mutual Prodrugs, Antibacterial actionassay.

Introduction:

The emergent bacterial resistance to most anti-bacterial anti-biotics is the highly global problem today. Many researches among the world to attempt to discover anew anti biotics to challenge the new strains of bacteria that resistant to most anti biotic today.Prodrug approach is the one of successful approaches to enhance the therapeutic criteria of drugs generally .The bacterial resistance mode examples as enzymatic degrading resistance , target disposition resistance , over expression of efflux pumps uptake down up resistance . The bacterial resistance is the most important mortality causation according to the World Health Organization (WHO) .(1) A prodrug strategy is the solution for the drug that lacking the appropriate pharmacokinetic or elevated host toxicity profile . A special advancing in application of the prodrug strategy in the area of bacterial –antibiotic resistance of the target that is resist by bacteria by designing of the prodrugs that needed the bacterium-specific system of enzymes to cleavage the active drug. so, the prodrug strategy in producing chemo therapies that are active against anti biotic - resistant bacteria.(2)The prodrug strategy is growing nucleus of the research area , and it is not only modify the pharmacokinetic of drug but is also for the molecular and cellular factors modification level. Example as the expression/distribution of cellular proteins ,the influx/efflux membrane transporters and drug targeting and enhancing the delivery system to achieve the maximum therapeutic successful treatment. (3,4,5,6)

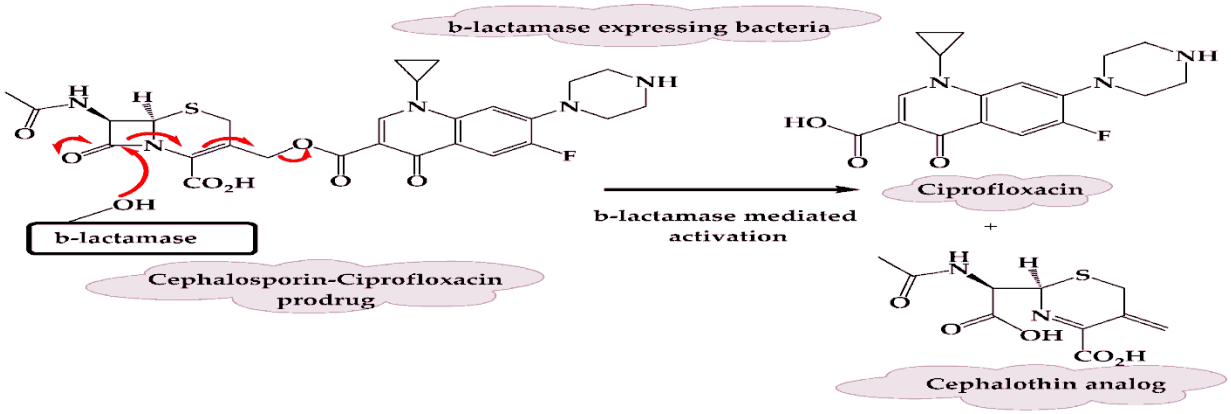
There are abroad-spectrum of antibiotics, such as ciprofloxacin, that are extensively prescribe to treat (UTI) urinary tract infections caused by Escherichia coli (7) as β-lactam-resistant bacteria species. The use of broad-spectrum antibiotics associated commonly with secondary infection due to antibiotic-resistant bacteria because of their action on normal flora in G.I.T. (8,9)

The developing of the cephalosporin conjugated fluoroquinoloneprodrug was done (10). The objective of design this prodrug was deliver the ciprofloxacin selectively to the bacteria only that express β-lactamase enzyme system , while having a minor action on bacteria that are not express the same mode of the resistance (the β-lactamase enzymes).

by this prodrug strategy, the prodrug capable substrate turnover rather than inhibit the β-lactamase enzyme system.

A cephalosporin core was choosing corner for the β-lactam substrate , the cephalosporins are β-lactams sufficiently highly hydrolyzed by β-lactamases.

In general, cephalosporins are considering as excellent prodrug carrier due to their allowing to the thebioreversible alteration of other active drugs by the linkage of one of their functional groups at the 3'_position of the cephem ring structure .



Gatifloxacin: is advanced fluoroquinolone with approval for treatment of infections of the respiratory and genitourinary tracts, such as S. pneumoniae and S. aureus and other gram positive bacteria.

Gatifloxacin involves a methoxy substituent at position (8) of the quinolone ring system that improving it is activity among bacteria with enhanced the antibacterial activity (static and cidal) activity as well as reduced choosing of resistant mutants (12).

Although gatifloxacin has been assayed in antibacterial activity against S. pneumoniae (13) and Escherichia coli(, also there are anew researches of gatifloxacin resistance profile in S. aureus .

Quinolones antibiotics essentially produce their action by interact with two essential bacterial enzymes system firstly DNA gyrase and secondly topoisomerase IV, in initiating their bactericidal activity.

Interpretation of these interactions has based on studies of genetically manipulated drug-resistant mutants to determine the primary and secondary drug targets and to evaluate the pattern of the resistance mutations that may influence drug activity. Over use of quinolones for treating of infections by gram-positive bacteria and with appear quinolone resistance in gram-positive species, particularly methicillin-resistant strains of Staphylococcus aureus (11) (14).

Acetaminophen, anti-inflammatory activities also have direct and indirect antimicrobial effects (15) some studies revel to that acetaminophen at normal therapeutic plasma levels can induce β-lactamase activity enzymatic system (16). paracetamol at low plasma therapeutic levels also decreases the capability of S. marcescens to kanamycin and cefotaxime (16).

The study of Candida spp shows that during use of ibuprofen(NSAID) or ASA aspirin in combination with amphotericin B and azoles antifungal producing the synergistic effects (17).
The most important study about the synergistic action of ibuprofen and flucanazol indicate decreasing up to 128-fold in MIC of candidiaspp.via reversing the over expression of the efflux pumps, also ibuprofen can minimize the candida resistance.

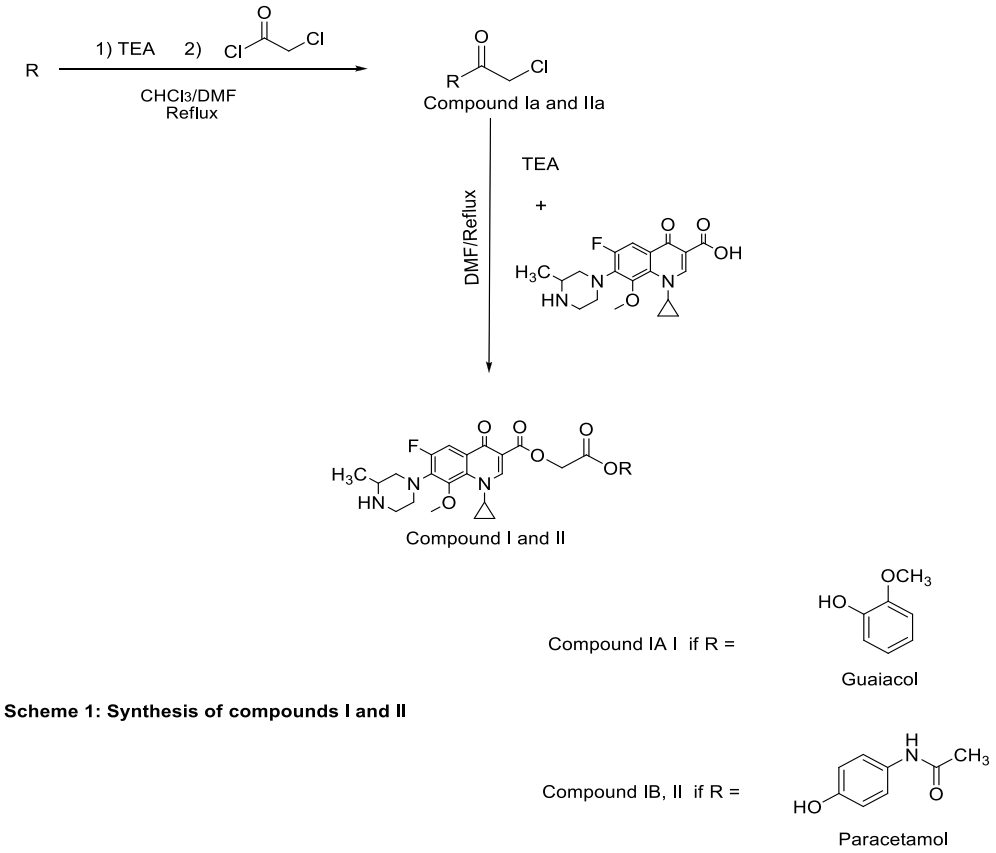
azithromycin and moxifloxacin their transportation and release by polymorphonuclear leukocytes (PMNL) have been reduced by very low concentration of paracetamol and ibuprofen. (19). Further practice particularly revel the action of NSAIDs on anti biotic susceptibility pathogenic microorganism are mediated by either changes in penetrations via cell membrane of bacteria or increasing or decreasing in efflux through bacterial membrane. (20,21)
quaiacol is a natural phenolic anti-oxidant and has a goodanti-microbial activity against wide range of microorganism (22).Especially when given as a combination simultaneously (23) .The designated compounds (I,II) having the commentary anti bacterial activity to the gatiflixacin nucleus own antibacterial activity as well as diminish the an emergent bacterial resistance to gatifloxacin.

Materials and Methods:

The reactants and solvents supplied from (ReidalDehean Germany; Sigma-Aldrich company Germany; BDH company England).Analytical grade .

Melting points by Advanced Melting Point Apparatus, (SMP30), Stuart by using capillary tube method .

On DC-Kartan SI Alumina 0.2 mm to proven progress of the reaction and the purity and, the solven .Rf ratio were done by thin layer chromatography, use methanol: benzene in a ratio (50:50) as a mobile phase (Ahmed et al., 2016). Determination of FT-IR spectra chart was measured by spectrophotometer FT-IR shimadzo and KBr discs, in the faculty of pharmacy, Kufa University central lab. (1H-NMR)Proton nuclear magnetic resonance spectra were recorded using NMR ultra shield spectrophotometer (Switzerland)500 MHz, BrukerAvance III, in the college of Tehran, Iran. Steps to synthesis of the target compounds are shown in scheme 1. (Quaiacol, Paracetamol) were linked with chloroacetylchloride moiety as spacer arm in the presence of triethylamine, to produce compounds IA, IB, . Then the conjugation of Gatifloxacin with intermediates IA, IB, and form in the synthesis of final designated compounds I, II respectively.



Scheme 1: Synthesis of compounds I and II

Chemistry:

reaction of guaiacol or paracetamol with chloroacetylchrolide:

(Guaiacol 5mmol , 0.62 gm),or (paracetamol 5 mmol,0755gm) was dissolved in a co solvent system containing DMF:$CHCl_3$ in ratio(50:50) mixture to form (40 ml), then TEA (0,696 ml, 5mmol) was added. Then, reaction was stirred on ice bath, then, the chloroacetylchloride (0.4 ml, 5mmol was dissolved in 20 ml CHCl3) was added slowly with acontinuous stirring at zero Celsius over of 1.5 hour, complete the reaction by refluxing of the reaction mixture for two hours.
Then excess of cold water was added, to precipitate the product that has obtained was filtered, and recrystallize from ethanol, to produce compounds IA and IB.

The physical characteristic, percent yield, and Rfratio were shown in table(1).

reaction ofGatifloxacin with compounds Ia and IIa.to form the target compound 1and II.

A mixture containing compound IA or IB (5mmol.0933 gm of Ia or 1.068 gm of Ia), &Gatifloxacin(5mmol,1.877 gm), were dissolved in DMF (30 ml), then, TEA triethylamine (0,696ml,

5mmol), were added. The reaction mixture was reacted by stirring at 25celsius for covering the night time . The solvent was evaporated under reduced pressure ; the resulting product was triturated with acetone and, then methanol recrystallization to ensure more purity. The physical appearance ,percent yield and Rfratio were given in table 1.

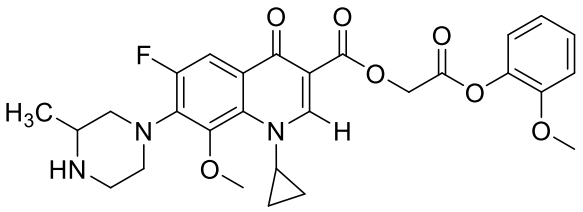
TABLE 1

Compound	l Formula	Molecular weight	Characteristic	% Yield	M.P melting point oC	Rfratio
COMPOUND I	**C28H30FN3O7**	**539.55**	**Yellow crystal**	**58%**	**187-191**	**0.7.**
COMPOUND II	**C29H31FN4O7**	**566.58**	**White powder**	**64%**	**234-238**	**0.59**

RESULTS:

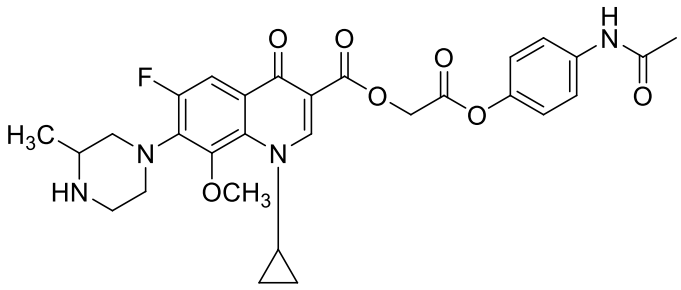
Mutual prodrugs of Gatifloxacin with (quaiacol or paracetamol) were done according to the procedure and schematic presentation illustrated above . The physico-chemical characterization, were measured and recorded in Table 1, and the structures of the target compounds were evidenced by the FT-IR spectroscopy data , and were illustrated by Table 2. F.T.IR spectra .

Compound I(Gatifloxacin-gauciol):



FT-IR (cm−1): 3,001 (CH) of aromatic, 2,941 and 2,890 (C-H) of alkane, and 1,752 (C=O) of ketone. 1H-NMR (DMSO-d6)δ(ppm): (C28H30FN3O7); 8.67 (s,1H,CH of alkene), 7.7-6.92 (1s and 1m,5H,CH-Ar),5.33 (s,2H,-OCH2-C=O), 4.14 (m, 1H, C-H of cyclopropane),3.84 (s,6H,2 -OCH3), 3.2-2.7(3m,7H, CH and CH2 of Piperazine ring), 1.5 (m,4H,2CH2 of cyclopropane), 1.1(d,3H,CH3), 1.06 (s,1H,NH).

Compound II (Gatifloxacin-paracetamol):



FT-IR (cm−1): 3,030 (CH) of aromatic, 2,950 and 2,893 (C-H) of alkane, and 1,682 (C=O) of amide. 1H-NMR (DMSO-d6)δ(ppm): (C29H31FN4O7); 9.86 (s, 1H, NH-C=O), 8.95 (s,1H,CH of alkene), 7.7-7.1 (1s and 2d,5H,CH-Ar),5.31 (s,2H,-OCH2-C=O), 4.13 (m, 1H, C-H of cyclopropane),3.83 (s,3H, Ar-OCH3), 3.4-2.8(3m,7H, CH and CH2 of Piperazine ring), 2.0 (s,3H, NH-O-CH3), 1.6 (m,4H,2CH2 of cyclopropane), 1.1(d,3H,CH3), 1.05 (s,1H,NH).

Antibacterial activity:

The Antibacterial activity effect of the drugs has been checked for in vitro inhibitory activity of growth : against Staphylococcus aureus as Gram-(+) bacteria and Escherichia colit that selected as Gram(-) bacteria:by diffusional spots procedure (24). the tested compounds show significant antibacterial activity toward the investigated bacteria. The results in Table 2, and their statistical values were listed in Figure 1. Compounds [**I**, **II**] revealed that Staphylococcus aureus was sensitive to compounds but Escherichia coli showed a reverse activity with some resistance to antibacterial compounds and the interpretations of the results can be analysis in four proposed mechanisms :- 1- destruction of cell wall, 2-alteration and dye function of cell membrane 3-block of nucleic acid synthesis and 4- modified of protein synthesis (25).

Table 2: Antibacterial activity results inhibition zone was measured in nm of the target comp. I and II.

Compound	**Bacteria**	
	G(+Ve)	G(-Ve)
	S.aureus	**E.coli**
S	**15**	**10**
I	**16**	**11**

II	**18**	**13**

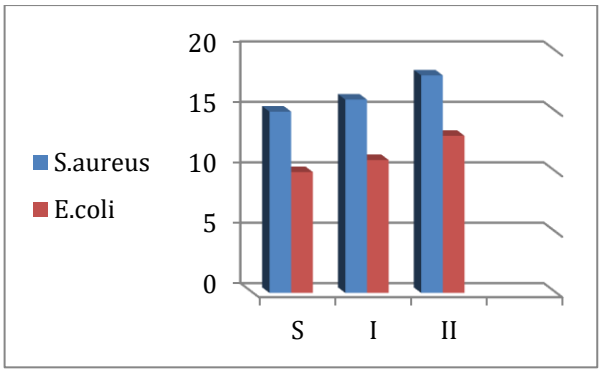


Figure 1: Antibacterial activity data of the compounds.

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