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Facile Synthesis of Ciprofloxacin Prodrug Analogues to Improve its Water Solubility and Antibacterial Activity

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Ciprofloxacin is a broad spectrum fluoroquinolone antibiotic that acts by the inhibition of bacterial topoisomerase type II (DNA gyrase) and have a potent activity against Gram positive and negative bacteria. However, due to its rigid and solid-state structure it suffers from limited water solubility. Improving its pharmacokinetic properties especially its water solubility is the concern of this work through the synthesis of various derivatives of ciprofloxacin with ethylene glycols through prodrug strategy without reducing its antibacterial activity. Shake-flask method was used to measure the aqueous solubility in phosphate buffer. The ciprofloxacin analogues were adequately stable at acidic and physiological pH. Synthesized derivatives were subjected to esterase-mediated hydrolysis reaction for the release of the drug from its prodrug form and showed a total hydrolysis after 25 min. Also antibacterial activity was studied against *Staphylococcus aureus* and *Escherichia coli*. Compared to ciprofloxacin, the solubility was increased for the three derivatives, and the antimicrobial activity was enhanced up to 40%.

Introduction

The physiochemical characteristics of drug substances, including solubility and permeability, can generally affect the pharmacokinetic (PK) and thereby the pharmacodynamic (PD) behavior of these drugs. An important PK parameter is the oral bioavailability, which is the fraction of the total oral dose that can access the systemic blood circulation. Besides intestinal permeability, oral bioavailability is greatly dependent on the extent of drug absorption and thereof on the rate of dissolution of that drug in aqueous medium, which makes it challenging for poorly soluble compounds. In addition, the stability of the formulations itself is influenced by drug solubility.[1] On the other hand, the intestinal permeability of a certain drug is dependent on the extent of drug's lipid solubility. In spite of this paradoxical relationship between drug's solubility and intestinal permeability, a dynamic balance must exist in order to have an effective absorption.[2]

Unfortunately, such a balance doesn't exist for many valuable drugs like ciprofloxacin, which is a broad spectrum second generation fluoroquinolone antibiotic, structurally related to nalidixic acid, effective against most Gram-negative bacteria and many Gram-positive bacteria,[3] and works by the inhibition of bacterial DNA gyrase.[4] Ciprofloxacin has low solubility and low permeability, and therefore considered as a class 4 compound according to the biopharmaceutics classification system (BCS).[5]

Ciprofloxacin is small, rigid, and flat molecule having a crystal lattice that lowers its aqueous solubility.[5] This is due to the strong intermolecular bonds (van der Waals and hydrogen bonding interactions) that allow the molecules to pack densely in the crystal. Various studies have been made on ciprofloxacin either to increase its antimicrobial activity or to improve its solubility and permeability, but the results were not satisfactory enough.[6] The aim of this study is to develop a facile synthesis of ciprofloxacin analogues using prodrug strategy by introducing various derivatives of ethylene glycols (EGs). The synthesized analogues have more flexible molecular structures, which disrupt the crystal lattice of ciprofloxacin. Therefore, they will increase the solubility of ciprofloxacin in the aqueous environments to allow a faster and complete solvation of the drug. Finally, the antibacterial activity of the different ciprofloxacin derivatives was tested and compared with the ciprofloxacin on Gram positive bacteria (Staphylococcus aureus) and Gram negative bacteria (Escherichia coli).

Results and Discussion

One of the interesting approaches that is used to improve the pharmacokinetic profile of a drug is the prodrug strategy.[7] Prodrug term is simply defined as the masked form of an active drug that is designed to be activated after enzymatic or chem-

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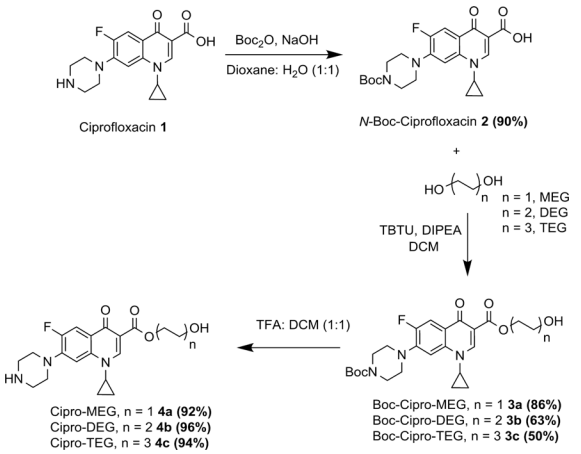
ical reaction once they have been administered into the body.[8] Therefore, prodrugs are normally considered as inactive molecule or have less activity than the released drug. In this work, we decided to utilize this strategy in order to improve the water solubility of ciprofloxacin. The synthesized prodrugs are based on the formation of ester bond that can be easily hydrolyzed by esterase enzyme in the body to the active ciprofloxacin.[9] The esterification reaction is conducted with three derivatives of ethylene glycols that showed a total safety profile in human and used in many food and pharmaceutical industries.[10] Three ciprofloxacin analogues (Scheme 1, **4a-c**) were



Scheme 1. Synthetic scheme of ciprofloxacin derivatives.

synthesized by esterification of ciprofloxacin. The ciprofloxacin base was initially protected from the piperazine ring by reacting it with Boc_2O in basic condition giving 90% yield. Then the *N*-Boc-protected ciprofloxacin was forced to react with monoethylene glycol (MEG, 86% yield), diethylene glycol (DEG, 63% yield) and triethylene glycol (TEG, 50 % yield) using TBTU as coupling agent and DIPEA as Hünig's base. Followed by deprotection reaction to all the products by triflouroacetic acid (TFA) resulting in Cipro-MEG (92% yield), Cipro-DEG (96% yield) and Cipro-TEG (94% yield) as shown in Scheme 1.

The basic chemical structures of the synthesized derivatives were confirmed by measuring NMR spectra. Representative spectra of ^{1}H NMR of the final three ciprofloxacin derivatives (Cipro-MEG, Cipro-DEG, Cipro-TEG) are shown in Figure 1. The ^{1}H NMR spectra of the all derivative contain all expected resonance peaks characteristic for aromatic ring of the ciprofloxacin between 7.5-9.0 ppm, CH_2OCO around 4.5 ppm. The multiples between 3.0-4.0 ppm matching the piperazine ring and the remaining chain of MEG, DEG or TEG. The last doublet peak around 1.2 ppm correspond to cyclopropyl.

Solubility of ciprofloxacin and its three analogues was measured using Shake-Flask method at 37°C. The solubility was determined by direct calibration method using UV spectrophotometer to measure the absorbance at λ_{max} of the products determined from UV-Vis spectrum scan between 200-400 nm. The

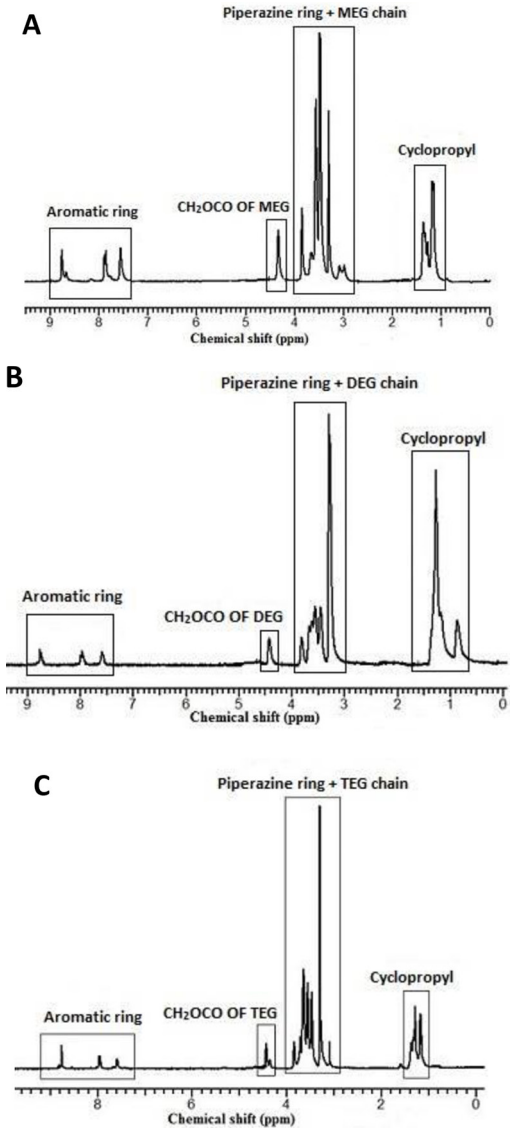


Figure 1. ^{1}H NMR spectra of A) ciprofloxacin-MEG B) ciprofloxacin-DEG C) ciprofloxacin TEG.

readings were taken after 24, 48, and 72 hr until reaching the equilibrium solubility.

Higher solubility was obtained for all three derivatives of ciprofloxacin as compared with that of ciprofloxacin base, in a sodium phosphate buffer at pH 7.4 as shown in Table 1.

Table 1. Solubility and dose number of ciprofloxacin and its three derivatives in phosphate buffer at pH 7.4 (37° C).

Ciprofloxacin analogue	Solubility (mg\ml)	Folds	Dose number (Do)
Ciprofloxacin base	0.1	-	30
Ciprofloxacin-MEG	7.4	73	0.4
Ciprofloxacin-DEG	26.3	262	0.1
Ciprofloxacin-TEG	38.7	386	0.07

Ciprofloxacin-TEG scored the highest solubility (38.7 mg/ml) followed by ciprofloxacin-DEG (26.3 mg/ml) then ciprofloxacin-MEG (7.4 mg/ml).

Once the solubility has been determined, it is used to calculate the dose number (Do). A dose number equal or lower than 1 indicated high-solubility, and Do >1 signified a low-solubility compound.[11] Therefore, all our derivatives are considered high soluble molecules as shown in table 1. This can be explained due to the addition of EG's gave the structures increased level of hydrophilicity and hence higher aqueous solubility. Furthermore, the introduction of large flexible groups disrupted the rigid crystal lattice that contributed to ciprofloxacin solid-state limited solubility. Moreover, these esterification reactions eliminated the zwitterionic nature of ciprofloxacin and resulted in basic derivatives with a single pKa of ~ 8.4 and reduced hydrogen bond capacity as calculated by Chem 3D® program (CambridgeSoft Corporation). Moreover, the introduction of ethylene glycol derivatives dramatically increased the flexibility of ciprofloxacin by disrupting the rigid crystal lattice and reducing the intermolecular forces between ciprofloxacin molecules.

The stability of ciprofloxacin analogues was investigated using spectrophotometry. The chemical stability for the three ciprofloxacin analogues was determined by evaluating the hydrolysis rates in 100 mM isotonic buffers (pHs 1.2 and 7.4). There were neither color change nor precipitation at either pH values. In addition, the absorbance data showed a great stability at both conditions as shown in Table 2.

Table 2. Stability of ciprofloxacin analogues at different pH values over one week

Ciprofloxacin analogue	% of remaining prodrug pH 1.2	pH 7.4
Ciprofloxacin-MEG	> 95%	> 90%
Ciprofloxacin-DEG	> 98%	> 92%
Ciprofloxacin-TEG	> 98%	> 95%

Esterification of ciprofloxacin transformed it into a prodrug. Hence, we studied the transformation of the inactive prodrug into the parent compound. The derivatives were subjected to a hydrolysis reaction catalyzed by the esterase enzyme at 37°C. We used 10 U mL^{-1} of the esterase, where this enzyme activity would be comparable to levels found in mouse serum.[12]

In order to determine the release profile of the prodrugs, we have constructed calibration curves of ciprofloxacin and its three derivatives as shown previously in Figures 2. As λ_{max} of each derivative is different from the parent drug (ciprofloxacin), it is can be easily determined the release hydrolysis profile of each analogue.

The Conversion of ciprofloxacin-EGs to ciprofloxacin was rapid, evident by UV-Vis spectroscopy illustrated by decrease in the prodrugs maximum absorbance peak (275 nm for Cipro-MEG, 280 nm for Cipro-DEG, 285 nm for Cipro-TEG) with concomitant peak increase for ciprofloxacin (260 nm). Thus esterase spontaneously cleaved the ester linkage between ciprofloxacin carboxyl group and EGs and each derivative regenerated the free carboxylic acid group of ciprofloxacin. The concentration of released drug was determined every 5 min using UV spectrophotometer as shown in Figure 2. As can be observed a rapid and total hydrolysis of the parent drug after 25 minutes of the incubation with the enzyme.

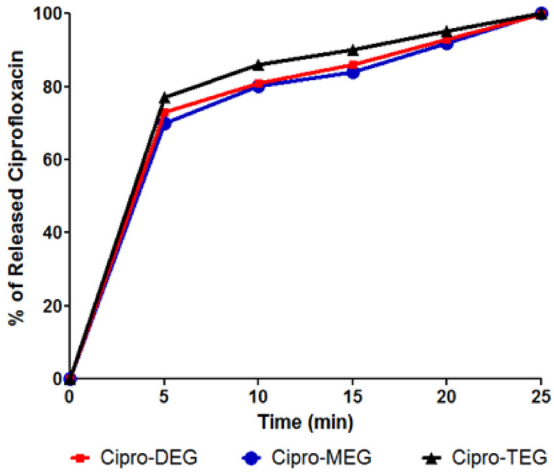


Figure 2. Percentage of released ciprofloxacin over time during esterase mediated hydrolysis.

In order to confirm that the analogues can be totally hydrolyzed and can give the same activity of ciprofloxacin, antibacterial activity of ciprofloxacin and its three synthesized analogues was tested against Gram negative bacteria *Escherichia coli (E. coli)* and Gram positive bacteria *Staphylococcus aureus (S. aureus)* in the presence and absence of esterase enzyme. Ciprofloxacin analogues showed weak antibacterial activity in the absence of esterase enzyme (Table 3). On the other hand, when esterase enzyme (10 U/ml) was added to the medium to release the products from their prodrug states, there was an enhancement in the activity of ciprofloxacin analogues by up to 40 % and 29 % against *E. coli* and *S. aureus*, respectively (Table 3 and 4).

Table 3. MIC ±SD of ciprofloxacin and the synthesized analogues tested against E. coli and S. aureus with/without esterase enzyme.

	with esterase		without esterase	
	MIC(μM) ± SD E. coli	MIC(μM) ± SD S. aureus	MIC(μM) ± SD E. coli	MIC(μM) ± SD S. aureus
Cipro	0.05 ± 0.007	1.51 ± 0.003	0.05 ± 0.007	1.51 ± 0.003
Cipro-MEG	0.04 ± 0.016	1.33 ± 0.013	0.67 ± 0.001	21.32 ± 0.001
Cipro-DEG	0.04 ± 0.005	1.19 ± 0.003	0.6 ± 0.001	19.08 ± 0.001
Cipro-TEG	0.03 ± 0.003	1.08 ± 0.003	0.54 ± 0.001	17.27 ± 0.001

Eze and co-authors[6a] have tried to form complexes of ciprofloxacin with Iron (III); solubility and permeability have been

Table 4. Percentage of enhanced antibacterial activity for the synthesized analogues against E. coli and S. aureus in the presence of esterase enzyme.

Ciprofloxacin analogue	% enhanced activity	
	E. coli	S. aureus
Cipro-MEG	20%	12%
Cipro-DEG	20%	21%
Cipro-TEG	40%	29%

raised, but a decrease in the antimicrobial activity has been noticed. Formation of crystalline and amorphous salts with different stiochiometries between ciprofloxacin and succinic acid has been accomplished by Paluch et al.[6b] and resulted in diverse solubility characteristics. Reddy and colleague have engineered three novel salts of ciprofloxacin with carboxylic acid; improved solubility with no change in permeability was obtained.[6c]

Conclusions

The synthesis of three different derivatives of ciprofloxacin was successful. The molecular structures of these derivatives were confirmed by measuring their corresponding NMR spectra. The solubility of the three analogues was increased compared to that of ciprofloxacin base, with triethylene glycol having the highest solubility and dose number. Also, the analogues were adequately stable at pH 1.2 and 7.4. The release of ciprofloxacin by an esterase-mediated hydrolysis reaction was fast, and the antimicrobial activity of the products were improved. We recommend studying the detailed pharmacokinetic properties of the three derivatives and measure the *in vivo* behavior of the synthesized products.

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Keywords: Ciprofloxacin · Prodrug strategy · Solubility · Antibacterial activity

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