Available online on www.ijddt.com
International Journal of Drug Delivery Technology 2017; 7(2); 89-95
doi: 10.25258/ijddt.v7i2.8922
ISSN: 0975 4415

Research Article

Design, Development and Evaluation of N-Mannich Base Prodrugs of Norfloxacin For *In Vitro* Release Behavior and Antimicrobial Properties

Sharma Prabodh Chander*, Piplani Mona

Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119, India.

Received: 6th Dec, 16; Revised 27st March, 17, Accepted: 12th June, 7; Available Online:25th June, 2017

ABSTRACT

In this research work, prodrugs of norfloxacin with various benzothiazoles were synthesized and studied for hydrolytic studies at various physiological pH. The results indicated that all of the prodrugs exhibited more and faster hydrolysis mainly in phosphate buffer (pH 7.4) rather than in HCl buffer (pH 1.2). These prodrugs were characterized by FTIR, ^{1}H NMR, mass spectroscopy and physical analysis. The synthesized prodrugs showed better partition coefficient as compared to parent compound, norfloxacin. All of the prodrugs were tested for antimicrobial activity against selected microbial strains. Among the synthesized prodrugs, **M1** was found to exhibit significant antibacterial efficacy having MIC 6.25 µg/ml against *S. aureus* MTCC 96 and prodrug **M6** depicted good antibacterial activity (MIC 6.25 µg/ml) against *E. coli* MTCC 443 when compared with norfloxacin (MIC 10 µg/ml). Prodrugs **M2** and **M4** showed comparable activity against *E. coli* MTCC 443 and *P. aeruginosa* MTCC 1688 respectively to standard drug norfloxacin. The antibacterial activity of prodrug **M4** (MIC 25 µg/ml) was found to be better than ciprofloxacin (MIC 50 µg/ml) against *S. pyogenus* MTCC 442. Moreover, prodrugs **M4** and **M6** possessed better antifungal activities (MIC 250, 75 µg/ml respectively) against *C. albicans* MTCC 227 while **M2** showed significant potency against *A. niger* MTCC 282 and *A. clavatus* MTCC 1323 (MIC 50 µg/ml) compared to standard drug nystatin (MIC 100 µg/ml).

Keywords: Prodrugs, lipophilicity, antibacterial activity, benzothiazoles, dissolution.

INTRODUCTION

Norfloxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7(piperazin-1-yl)quinoline-3-carboxylic acid] primarily prescribed fluoroquinolone at clinical level is a potent chemotherapeutic oral antimicrobial agent[1]. This drug with wide bacterial spectrum has been utilized in number of infectious diseases caused by many pathogenic microorganisms[2]. As lipophilicity is an important parameter for absorption of the drug into the cell, norfloxacin gets slowly absorbed through the cellular membrane owing to less lipophilicity[3]. Moreover, norfloxacin possesses less bioavailability (30-40%) and shortest plasma $t_{1/2}$ of 3.5-4.5 h among fluoroquinolones due to less ability to bind with plasma proteins[4]. Hence, norfloxacin gets significantly excreted through renal pathway from the body and mainly used for curing urinary tract infections despite of having good potential against many Gram positive and Gram negative bacteria[5]. Therefore, main attention of scientific researchers is to retain this antibacterial agent into the body and enhance its penetration into the cellular membrane.

This undesired feature of less lipophilicity of norfloxacin can be conquered by adopting prodrug approach. Prodrug is conventional model in which the parent drug is chemically modified for changing its pharmacokinetic and pharmaceutical characteristics and hence, improving its therapeutic index[6]. This bioreversible inert drug precursor will convert to pharmacologically active compound on biotransformation. To overcome the limitations associated with fluoroquinolone drugs, several prodrugs of these drugs have been developed and evaluated by number of scientists across the world. Various prodrugs of norfloxacin have been reported in the form of esters, amides, polyesters, *etc*[5,7,8]. Although immense research in this area for development of prodrug of norfloxacin has been carried out to improve its bioavailability still there is scope for development of novel prodrugs with improved pharmacokinetic features. N-Mannich bases of amine and NH group containing compounds have been synthesized and proved to be potentially useful prodrugs, since N-Mannich bases hydrolyzes in physiological buffers depending upon its pH and release the parent compound[9,10]. They are found to possess various potent biological activities in association with improving the lipophilicity of parent compounds by depressing their protonation[10,11].

Benzothiazole, a versatile, heterocyclic compound exhibiting significant biological potential is of great scientific interest now a days[12,13]. They have been found to exhibit several pharmacological activities like: antimicrobial, antihelmintic, anticancer, antidiabetic activities, *etc*[14,15]. Hence, as an ongoing part of our

*Author for Correspondence: sharma_prabodh@rediffmail.com

research on fluoroquinolones and benzothiazoles[16-19], the present study contemplates the synthesis and evaluation of N-Mannich bases of norfloxacin with benzothiazoles as prodrugs to improve the pharmacokinetic parameter of norfloxacin in association with utilizing therapeutic efficacy of active benzothiazoles, which would be helpful in exhibiting promising pharmacological potential.

Experimental

General

Norfloxacin was obtained as a gift sample from Combitic Global Caplet Private Limited, Sonepat, Haryana, India. Benzothiazoles were procured from Sigma Aldrich (Banglore, India). All other chemicals and solvents were obtained from SD Fines (Mumbai, India), Rankem Lab (New Delhi, India) and were of analytical grade. Uncorrected melting point was measured by capillary method on MR-VIS Labindia, melting visual range apparatus. IR spectra were obtained on Perkin Elmer IR spectrophotometer (KBr pellets). ^{1}H NMR spectrum was recorded on Bruker Avance II 400 NMR spectrometer. The absorbance of sample solution was noted on Systronic double beam UV, spectrophotometer.

Chemistry

General procedure for synthesis of acetylated benzothiazoles (2a-f)

The reaction mixture of equimolar quantity of benzothiazole (0.01 M), (1a-f) and acetyl chloride (0.01 M) in chloroform were taken in round bottom flask and allowed to reflux in presence of K_2CO_3 for 10-12 hrs (~80-85 ℃). Reaction mixture was concentrated to approximately half of its initial volume. The concentrated product was allowed to wash with 5% $NaHCO_3$ (w/v) and subsequently with water. The dried product was collected and purified by the process of recrystallization using methanol[20]. Six different acetylated benzothiazoles (2a-f) were synthesized following this procedure. Purity of the synthesized compounds was checked by TLC.

General procedure for synthesis of N-Mannich base of fluoroquinolone (M1-M6)

To a solution of norfloxacin (0.01 M) in glacial acetic acid was added acetylated benzothiazole (0.01 M) (2a-f) and 37% formalin (0.5 ml). The reaction mixture was allowed to reflux on heating mantle for 2-3 hrs. After reflux, the resulting mixture was reduced to half of its original volume and precipitates were obtained. Further purification was carried out by recrystallization using DMF and water. The precipitates were collected and dried[21]. Employing the above described procedure six N-Mannich base prodrugs (**M1-M6**) have been synthesized. Physical and analytical data of synthesized prodrugs is given in Table 1.

7-(4-(2-(6-Methoxybenzo[d]thiazol-2-ylcarbamoyl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid **M1.** Yield 71%, m.p. 249-251 ℃. IR (KBr, cm^{-1}) 3380 (NH str), 3054-2837 (C-H str), 1718 (C=O str), 1665 (CONH str), 1637 (C=O str), 1258 (C-N str); ^{1}H NMR (DMSO-*d6*) δ ppm: 1.37 (t, 3H, -CH_3 ethyl), 2.32-2.42 (m, 2H, -CH_2 ethyl), 3.36-3.60 (m, 8H, piperazine-H), 3.69 (3H, s, methoxy), 4.1 (m, 4H, -CH_2-CH_2- ethylene bridge), 4.81 (s, 1H, -NH), 6.89-7.88 {m, 5H, aromatic (H_5, H_8-quinolone and $H_{5'}$, $H_{7'}$, $H_{8'}$- benzothiazole)}, 8.09 (s, 1H, H_2-quinolone), 15.12 (s br, 1H, -COOH); MS: m/z = 553.6 (M^+).

7-(4-(2-(6-Ethoxybenzo[d]thiazol-2-ylcarbamoyl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid **M2.** Yield 69%, m.p. 255-257 ℃. IR (KBr, cm^{-1}) 3350 (NH str), 3072-2842 (C-H str), 1716 (C=O str), 1672 (CONH str), 1632 (C=O str), 1251 (C-N str); ^{1}H NMR (DMSO-*d6*) δ ppm: 1.29 (t, 3H, -CH_3 ethyl), 1.33 (3H, t, CH_3 ethoxy), 2.29-2.38 (m, 2H, -CH_2 ethyl), 3.41-3.79 (m, 8H, piperazine-H), 3.79 (2H, q, CH_2 ethoxy), 4.09 (m, 4H, -CH_2-CH_2- ethylene bridge), 4.72 (s, 1H, -NH), 6.76-7.74 {m, 5H, aromatic (H_5, H_8-quinolone and $H_{5'}$, $H_{7'}$, $H_{8'}$-benzothiazole)}, 8.12 (s, 1H, H_2-quinolone), 14.8 (s br, 1H, -COOH); MS: m/z = 567.6 (M^+).

7-(4-(2-(6-Methylbenzo[d]thiazol-2-ylcarbamoyl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid **M3.** Yield 73%, m.p. 278-281 ℃. IR (KBr, cm^{-1}) 3370 (NH str), 3067-2843 (C-H str), 1712 (C=O str), 1670 (CONH str), 1629 (C=O str), 1253 (C-N str); ^{1}H NMR (DMSO-*d6*) δ ppm: 1.19 (t, 3H, -CH_3 ethyl), 2.31-2.43 (m, 2H, -CH_2 ethyl and 3H, -CH_3, benzothiazole), 3.31-3.58 (m, 8H, piperazine-H), 4.28 (m, 4H, -CH_2-CH_2- ethylene bridge), 4.85 (s, 1H, -NH), 6.79-7.85 {m, 5H, aromatic (H_5, H_8-quinolone and $H_{5'}$, $H_{7'}$, $H_{8'}$- benzothiazole)}, 7.92 (s, 1H, H_2-quinolone), 15.3 (s br, 1H, -COOH); MS: m/z = 537.6 (M^+).

7-(4-(2-(6-Chlorobenzo[d]thiazol-2-ylcarbamoyl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid **M4.** Yield 65%, m.p. 262-265 ℃. IR (KBr, cm^{-1}) 3350 (NH str), 3063-2852 (C-H str), 1715 (C=O str), 1662 (CONH str), 1626 (C=O str), 1257 (C-N str); ^{1}H NMR (DMSO-*d6*) δ ppm: 1.27 (t, 3H, -CH_3 ethyl), 2.26-2.35 (m, 2H, -CH_2 ethyl), 3.42-3.53 (m, 8H, piperazine-H), 4.16 (m, 4H, -CH_2-CH_2- ethylene bridge), 4.75 (s, 1H, -NH), 6.85-7.93 {m, 5H, aromatic (H_5, H_8-quinolone and $H_{5'}$, $H_{7'}$, $H_{8'}$-benzothiazole)}, 8.42 (s, 1H, H_2-quinolone), 14.4 (s br, 1H, -COOH); MS: m/z = 558 (M^+).

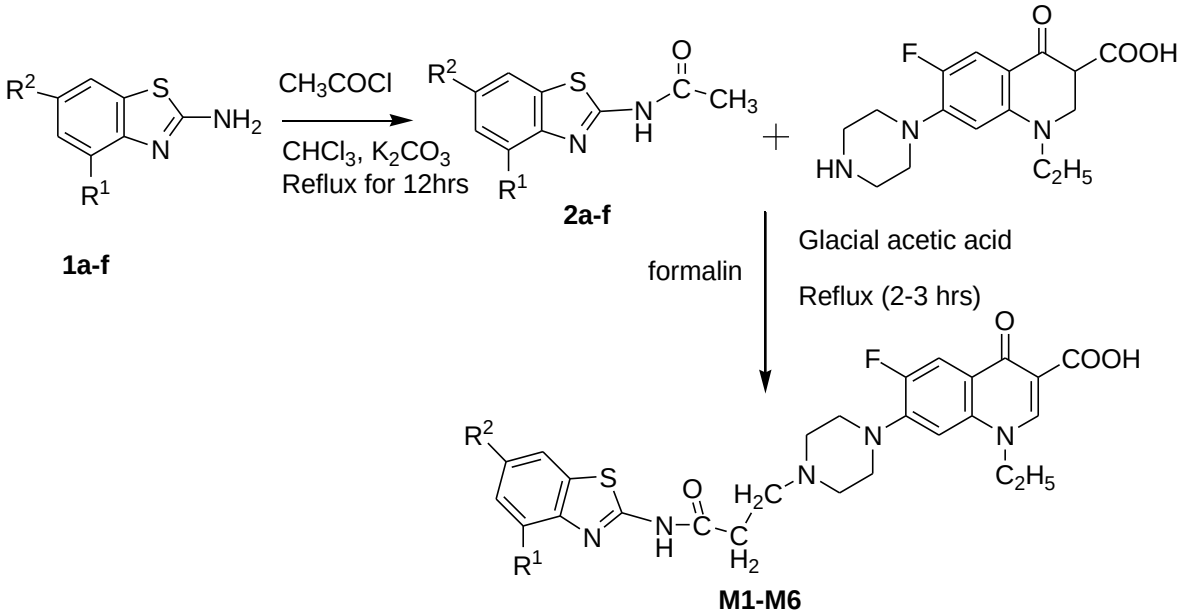
7-(4-(2-(6-Nitrobenzo[d]thiazol-2-ylcarbamoyl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid **M5.** Yield 61%, m.p. 251-253 ℃. IR (KBr, cm^{-1}) 3360 (NH str), 3039-2867 (C-H str), 1719 (C=O str), 1676 (CONH str), 1630 (C=O str), 1258 (C-N str); ^{1}H NMR (DMSO-*d6*) δ ppm: 1.37 (t, 3H, -CH_3 ethyl), 2.34-2.44 (m, 2H, -CH_2 ethyl), 3.28-3.67 (m, 8H, piperazine-H), 4.34 (m, 4H, -CH_2-CH_2- ethylene bridge), 4.87 (s, 1H, -NH), 6.92-7.96 {m, 5H, aromatic (H_5, H_8-quinolone and $H_{5'}$, $H_{7'}$, $H_{8'}$-benzothiazole)}, 8.19 (s, 1H, H_2-quinolone), 14.91 (s br, 1H, -COOH); MS: m/z = 568.6 (M^+).

7-(4-(2-(4-Chlorobenzo[d]thiazol-2-ylcarbamoyl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid **M6.** Yield 59%, m.p. 247-249 ℃. IR (KBr, cm^{-1}) 3370 (NH str), 3049-2839 (C-H str), 1715 (C=O str), 1668 (CONH str), 1634 (C=O str), 1249 (C-N str); ^{1}H NMR (DMSO-*d6*) δ ppm: 1.21 (t, 3H, -CH_3 ethyl), 2.20-2.39 (m, 2H, -CH_2

Table 1: Physicochemical characterization of synthesized prodrugs.

Prodrug	R^1	R^2	Molecular Formula*	Mol. Wt.*	Melting Point (°C)*	Yield (%)	R_f
M1	-H	$-OCH_3$	$C_{27}H_{28}FN_5O_5S$	553.61	249-251	71	0.47
M2	-H	$-OC_2H_5$	$C_{28}H_{30}FN_5O_5S$	567.63	255-257	69	0.51
M3	-H	$-CH_3$	$C_{27}H_{28}FN_5O_4S$	537.61	278-281	73	0.67
M4	-H	-Cl	$C_{26}H_{25}ClFN_5O_4S$	558.02	262-265	65	0.59
M5	-H	$-NO_2$	$C_{26}H_{25}FN_6O_6S$	568.58	251-253	61	0.65
M6	-Cl	-H	$C_{26}H_{25}ClFN_5O_4S$	558.02	247-249	59	0.62

*Calculated values



Scheme 1

Table 2: Partition coefficient and drug contents of synthesized prodrugs.

Prodrug	Partition coefficient	Drug content (%)
M1	2.87	89
M2	2.32	85
M3	1.99	82
M4	2.25	88
M5	2.36	74
M6	2.38	78

ethyl), 2.96-3.67 (m, 8H, piperazine-H), 3.9 (m, 4H, $-CH_2-CH_2-$ ethylene bridge), 4.80 (s, 1H, -NH), 7.12-8.27 {m, 5H, aromatic (H_5, H_8-quinolone and $H_{5'}$, $H_{6'}$, $H_{7'}$-benzothiazole)}, 8.23 (s, 1H, H_2-quinolone), 14.56 (s br, 1H, -COOH); MS: m/z = 558 (M^+).

In vitro hydrolysis studies

In vitro hydrolysis studies of synthesized prodrugs were carried out in phosphate buffer of 7.4 pH and HCl buffer of 1.2 pH. The hydrolysis studies were performed using 100 ml of HCl buffer (pH 1.2) in 500 ml of beaker at 37 ± 0.1 °C. The prodrug (10 mg) was taken in suspension form in dialysis membrane tied at both ends, hanged in the buffer contained in beaker and kept on magnetic stirrer rotated at 100 rpm. The samples (2 ml) were withdrawn after predetermined time intervals (30, 60, 90, 120, 150, 180, 210, 240, 270, 300 and 360 min) and filtered through 0.45 µm membrane. Sink condition was maintained after every withdrawal of sample by replacing with equal quantity of buffer. The samples were diluted to suitable concentration with buffer and analyzed for release of norfloxacin against HCl buffer taken as blank and λ_{max} 278 nm on UV/VIS double beam spectrophotometer. The same procedure was followed for dissolution studies in phosphate buffer (pH 7.4)[5].

Partition coefficient

Shake flask method was adopted for determination of partition coefficient of synthesized prodrugs. Chloroform pre-saturated with phosphate buffer (10 ml, pH 7.0) and phosphate buffer pre-saturated with chloroform (10 ml) were taken in separating funnel. After addition of prodrug sample (10 mg), the separating funnel was shaken occasionally for 30 minutes. Complete separation of the phases was carried out by keeping the separating funnel stationary for few minutes. The amount of prodrug sample in respective phases was calculated by employing standard curve of the same sample in both phases[22,23]. Partition coefficient was determined by the following equation:

$PC = C_o/C_w$

C_o = the concentration of the prodrug in organic phase,

C_w = the concentration of the prodrug in aqueous phase.

Drug content

The drug content of prodrug was determined following alkaline hydrolysis method. The sample prodrug (10 mg) was added in 1N NaOH solution and allowed to get

Table 3: Minimum inhibitory concentration in µg/ml against bacterial strains.

Prodrug	Antibacterial Activity (Minimum inhibitory concentration in µg/ml)			
	Gram Positive Bacteria		Gram Negative Bacteria	
	S. Aureus (MTCC 96)	*S. pyogenus* (MTCC 442)	*P. aeruginosa* (MTCC 1688)	*E. coli* (MTCC 443)
M1	6.25	62.5	25	500
M2	250	250	12.5	12.5
M3	500	500	200	100
M4	500	25	12.5	100
M5	250	250	100	125
M6	250	250	200	6.25
Norfloxacin	10	10	10	10
Ciprofloxacin	50	50	25	25

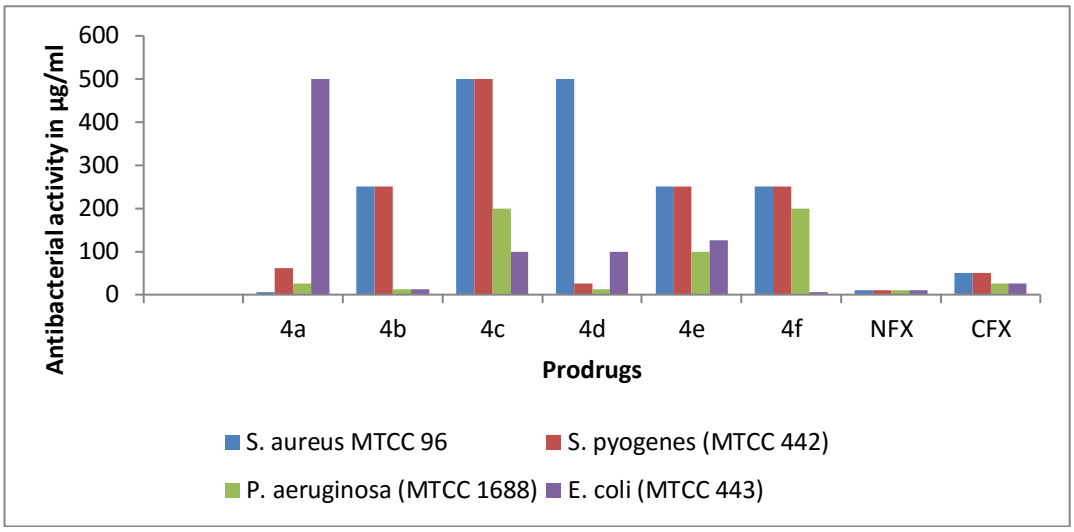


Figure 1: Antibacterial evaluation of synthesized prodrugs (M1-M6).

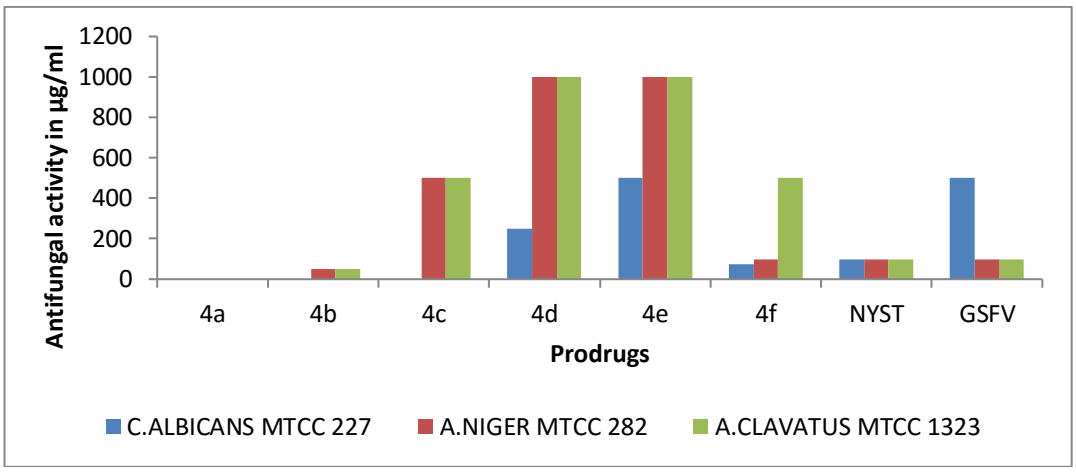


Figure 2: Antifungal evaluation of synthesized prodrugs (M1-M6).

hydrolyze for 24 hrs. The amount of norfloxacin attached to benzothiazoles was determined by UV/VIS spectrophotometer[24,25].

Antimicrobial screening

The antibacterial evaluation of synthesized prodrugs in terms of minimum inhibitory concentration (MIC) were carried out using tube dilution method against four bacterial strains *i.e.* Gram positive bacteria: *Staphylococcus aureus* MTCC 96, *Streptococcus pyogenus* MTCC 442, Gram negative bacteria: *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688 and compared with standard drugs[26,27]. The prodrugs were also screened for antifungal activities employing same method against three fungal strains: *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323 and compared with standard drugs greseofulvin and nystatin.

RESULTS AND DISCUSSION

Table 4: Minimum inhibitory concentration in µg/ml against fungal strains.

Prodrug	Antifungal Activity (Minimum inhibitory concentration in µg/ml)		
	C. albicans (MTCC 227)	*A. niger* (MTCC 282)	*A. clavatus* (MTCC 1323)
M1	>1000	>1000	>1000
M2	>1000	50	50
M3	>1000	500	500
M4	250	1000	1000
M5	500	1000	1000
M6	75	100	500
Greseoful vin	500	100	100
Nystatin	100	100	100

Synthesis and characterization of prodrugs (M1-M6). The synthetic pathway to obtain the target prodrugs in the present study is given in Scheme 1. Initially acetylated benzothiazoles were prepared in good yield by refluxing benzothiazoles with acetyl chloride in chloroform as solvent containing pinch of anhydrous potassium carbonate. Finally, prodrugs **M1-M6** were synthesized by reacting acetylated benzothiazoles, norfloxacin and formalin in presence of glacial acetic acid. Products were purified by recrystallization using mixture of DMF and water. The newly synthesized prodrugs were characterized by FTIR, ^{1}H NMR and mass spectroscopy. ^{1}H NMR spectra illustrated characteristic peaks due to proton of ethylene bridge appeared as multiplet at 3.9-4.5 ppm, NH proton as well as C-2 proton of quinolone ring as a singlet at 4.72-4.87 and 7.92-8.42 ppm respectively and a broad singlet at 14.6-15.3 ppm due to COOH proton. The appearance of peak at δ (3.9-4.5 ppm, 2H) confirms the synthesis of N-Mannich bases between norfloxacin and benzothiazoles. *In vitro dissolution studies of prodrugs in HCl buffer (1.2 pH). In vitro* dissolution studies of synthesized prodrugs were carried out in HCl buffer (pH 1.2) and phosphate buffer (pH 7.4). Percentage release of norfloxacin from prodrugs (**M1-M6**) versus time in minutes in HCl buffer (pH 1.2) has been shown in Figure **1**. *In vitro dissolution* studies revealed that for prodrugs **M5** and **M6**, no hydrolysis was observed for first half an hour while **M1** and **M4** started releasing norfloxacin after 1 h in HCl buffer (1.2 pH). They were found to exhibit more stability in HCl buffer (pH 1.2) as compared to other prodrugs. Moreover, the synthesized prodrugs **M1-M6** were found to release only 6.26-15.3% of parent drug in 6 h as shown in Figure 1. Each experiment was carried out in triplicate.

In Vitro dissolution studies of prodrugs in phosphate buffer (7.4 pH). While in phosphate buffer (pH 7.4), hydrolysis of these prodrugs started in first half an hour and were found to release 14.03%-51.21% of parent drug in 6 h as shown in Figure 2. **M1, M2** and **M4** released approximately 5-7% of drug in alkaline environment as compared to 1-2% of drug release in acidic environment in first 2 h. It was observed that N-Mannich bases hydrolyzed more in phosphate buffer (at pH 7.4) as compared to HCl buffer (1.2 pH).

Partition coefficient and drug content determination of prodrugs. Partition coefficient of all prodrugs was determined experimentally in chloroform:phosphate buffer (pH 7.0) using shake flask method. The concentration of prodrugs in each phase was determined by UV/VIS spectrophotometer utilizing standard curve of the prodrugs in both phases at their respective $\boldsymbol{\lambda}_{max}$ and is given in Table 2. The results indicated that lipophilicity of the synthesized prodrugs was found to be increased (1.99-2.87) when compared with parent drug, norfloxacin (1.94). This enhanced lipophilicity and stability of prodrugs in HCl buffer might be helpful in improving the passage of prodrugs through the bacterial membrane positively, as it has been considered a significant factor for the biological prospective of norfloxacin.

Drug content of each prodrug was calculated after complete hydrolysis of prodrug in 1N NaOH for 24 h. The amount of norfloxacin linked to various benzothiazoles as evaluated by UV/VIS spectrophotometer and employing standard calibration curve of norfloxacin was found to be 74-89 % and is summarized in Table 2.

Antibacterial evaluation of prodrugs. The synthesized prodrugs (**M1-M6**) were evaluated for antimicrobial activity against Gram positive strains: *Staphylococcus aureus* MTCC 96, *Streptococcus pyogenus* MTCC 442, Gram negative bacteria: *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688 and fungal strains: *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323. The results of antibacterial activity of prodrugs and their standard drugs have been summarized in Table 3 and depicted in Figure. The results of antibacterial activities of prodrugs revealed that prodrug **M1** having methoxy group at 6 position of benzothiazole showed significant activity against *S. aureus* MTCC 96 (MIC value of 6.25 µg/ml) when compared with norfloxcain (MIC value of 10 µg/ml). Prodrugs **M2** (having ethoxy group at 6 position of benzothiazole) and **M4** (having chloro group at 6 position of benzothiazole) exhibited better antibacterial activities with MIC 12.5 µg/ml against *P. aeruginosa* MTCC 1688 than that of standard drug ciprofloxacin (MIC value of 25 µg/ml). Moreover, prodrugs **M6** (having chloro group at 4th position) and **M2** (MIC value of 6.25 µg/ml and 12.5 µg/ml respectively) were found to possess good antibacterial activities against *E. coli* MTCC 443 as compared to standard drugs. The antibacterial activity against *S. pyogenus* MTCC 442 indicated that prodrug **M4** (MIC value of 25 µg/ml) exhibited superior antibacterial activity when compared with standard drug ciprofloxacin (MIC value of 50 µg/ml).

Determination of Antifungal activity. The antifungal potency of the synthesized prodrugs was compared with standard drugs greseofulvin as well as nystatin and the individual minimum inhibitory concentration (MIC in µg/ml) values are listed in Table 4 and presented in Figure. The study of MIC values indicates that prodrug **M2** (6-ethoxy) exhibited strong inhibition against *A. niger* MTCC 282 (MIC value 50 µg/ml) and *A. clavatus* MTCC 1323

(MIC value 50 µg/ml) compared to both standard drugs greseofulvin and nystatin having MIC value 100 µg/ml. On comparing the antifungal activity of prodrug **M6** (4-chloro) with standard drugs it was found that prodrug **M6** showed superior activity (MIC value of 75 µg/ml) against *C. albicans* MTCC 227 and comparable activity (MIC value of 100 µg/ml) against *A. niger* MTCC 282. **M4** also exhibited good potency (MIC value 250 µg/ml) against *C. albicans* MTCC 227.

CONCLUSION

In the present study, an efficient method for the synthesis of N-Mannich bases of norfloxacin with various substituted benzothiazoles is described. The prodrugs are characterized by spectral and physicochemical analysis and found to possess improved pharmacokinetic properties and bioavailability. Partition coefficient of the synthesized prodrugs determined by shake flask method confirms that synthesis of N-Mannich bases of amine group containing drugs results in improved lipophilicity. The synthesized prodrugs have been screened for antimicrobial activities and some of them showed significant activities against selected microbial strains. *In vitro* dissolution studies indicate that the prodrugs exhibited good stability in HCl buffer (pH 1.2) as compared to phosphate buffer (pH 1.2). Therefore, there are possibilities for the intact prodrugs to be absorbed in more concentration at gastric pH due to improved partition coefficient and may have potential for better utilization in future.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ACKNOWLEDGEMENTS

One of authors Ms. Mona Piplani is grateful to Indian Council of Medical Research, New Delhi, India, for awarding Senior Research Fellowship vide Letter no. 45/07/2013/PHA/BMS. We express our sincere thanks to Micro-care laboratory, Surat for providing antimicrobial activity data and SAIF, Chandigarh for ^{1}H NMR spectra.

REFERENCES

1. H. Kondo, F. Sakamoto, T. Uno, Y. Kawahata and G. Tsukamoto. Studies on prodrugs. 11. Synthesis and antimicrobial activity of N-[(4-methyl-5-methylene-2-oxo-1,3dioxolan4-yl) oxy]norfloxacin. *Journal of Medicinal Chem*istry, 1989, 32(3): 671-674.
2. J. Alexander, R. A. Fromtling, J. A. Bland, B. A. Pelak and E. C. Gilfillan. (Acy1oxy) alkyl carbamate prodrugs of norfloxacin. *Journal of Medicinal Chem*istry, 1991, 34(1): 7-81.
3. V. Coessens, E. H. Schacht and D. Domurado. Synthesis and *in vitro* stability of macromolecular prodrugs of norfloxacin. *Journal of Controlled Release*, 1997, 47(3): 283-291.
4. A. Kumar, S. Singh, G. Sharma, S. Sharma, A. Verma, R. Kaur and U. K. Jain. Formulation, optimization and evaluation of gastro-retentive floating microsphere of norfloxacin. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 2013, 3(23): 12-17.
5. S. Dhaneshwar, K. Tewari, S. Joshi, D. Godbole and P. Ghosh. Diglyceride prodrug strategy for enhancing the bioavailability of norfloxacin. *Chemistry of Physics and Lipids*, 2011, 164(4): 307-313.
6. A. Verma, B. Verma, S. K. Prajapati and K. Tripathi. Prodrug as a chemical delivery system: a review. *Asian Journal of Research in Chemistry*, 2009, 2(2): 100-103.
7. S. A. Khan, A. Ahmad and A. Husain. Synthesis, characterization and evaluation of amide based prodrugs of norfloxacin. *World Journal of Pharmacy*, 2015, 3(8): 1607-1611.
8. F. Dubar, R. Wintjens, É. S. Martins-Duarte, R. C. Vommaro, W. Desouza and D. Dive, *et al*. Ester prodrugs of ciprofloxacin as DNA-gyrase inhibitors: synthesis, antiparasitic evaluation and docking studies. *Medicinal Chemistry Communication*, 2011, 2(5): 430-435.
9. H. Bundgaard and M. Johansen. Prodrugs as drug delivery systems IV: N-Mannich bases as potential novel prodrugs for amides, ureides, amines, and other NH-acidic compounds. *Journal of Pharmaceutical Sciences*, 1980, 69(1): 44-46.
10. H. Bundgaard, U. Klixbüll and E. Falch. Prodrugs as drug delivery systems. O-acyloxymethyl salicylamide N-Mannich bases as double prodrugs forms for amines. *International Journal of Pharmaceutics*, 1986, 29(1): 19-28.
11. S. Bala, N. Sharma, A. Kajal, S. Kamboj and V. Saini. Mannich bases: an important pharmacophore in present scenario. *International Journal of Medicinal Chemistry*, 2014, 1-15.
12. P. S. Yadav, D. Prakash and G. P. Senthilkumar. Benzothiazoles: different methods of synthesis and diverse biological activities. *International Journal of Pharmaceutical Sciences and Research*, 2011, 3(1): 01-07.
13. R. Ali and N. Siddiqui. Biological aspects of emerging benzothiazoles: a short review. *Journal of chemistry*, 2013, 1-12.
14. G. Achaiah, N. S. Goud, K. P. Kumar and P. Mayuri. Review on 2-substituted benzothiazoles: diversity of synthetic methods and biological activities. *International Journal of Pharmaceutical Sciences and Research*, 2016, 7(4): 1375-1382.
15. G. N. Raju, B. S. Karumudi, N. R. Rao. Benzothiazole: versatile heterocyclic nucleus in medicinal chemistry. *International journal of pharmaceutical chemistry*, 2015, 5(4): 104-114.
16. P. C. Sharma, A. Jain, M. S. Yar, R. Pahwa, J. Singh and S. Goel. Synthesis and antibacterial evaluation of novel analogs of fluoroquinolones annulated with 6-substituted-2-aminobenzothiazoles. *Arabian Journal of Chemistry*, 2015, 8(5): 671-677.
17. P. C. Sharma, R. Kumar, M. Chaudhary, A. Sharma and H. Rajak. Synthesis and biological evaluation of novel benzothiazole clubbed fluoroquinolone derivatives. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2013, 28(1): 1-10.
18. P. C. Sharma, A. Jain, M. S. Yar, R. Pahwa, J. Singh, P. Chanalia. Novel fluoroquinolone derivatives bearing

N-thiomide linkage with 6-substituted-2-aminobenzothiazoles: synthesis and antibacterial evaluation. *Arabian Journal of Chemistry*, 2013, Article in press.

19. P. C. Sharma, A. Jain and S. Jain. Fluoroquinolone antibacterials: a review on chemistry, microbiology and therapeutic prospects. *Acta Poloniae Pharmaceutica-Drug Research*, 2009, 66(6): 587-604.
20. H. Kaur, H. Kaur, A. Chawla, U. S. Baghel and R. K. Dhawan. A review: synthesis schemes of antimicrobial and anticancer thiazole derivatives. *Journal of Global Trends in Pharmaceutical sciences*, 2014, 5(2): 1684-1691.
21. S. N. Pandeya, D, Sriram, G. Nath, E. D. Clercq. Synthesis, antibacterial, antifungal and anti-HIV activities of norfloxacin mannich bases. *European Journal of Medicinal Chemistry*, 2000, 35(2): 249-255.
22. S. Dubey, V. Jain and G. B. Preethi. Evaluation of lipophilicity, antimicrobial activity and mutagenicity of some novel ester prodrugs of metronidazole. *Indian Journal of Chemistry*, 2009, 48B: 571-1576.
23. A. J. Qasir and A. D. Jassim. Synthesis of mutual prodrug of p-amino salicylic acid (PAS) and isoniazid (INH) with possible enhancement physicochemical properties. *Journal of Al-Nahrain University*, 2010, 13(3): 7-13.
24. N. Agrawal, M. J. N. Chandrasekar, U. V. S. Sara and A. Rohini. Poly (HEMA- Zidovudine) conjugate: a macromolecular prodrug for improvement in biopharmaceutical properties of the drug. *Drug Delivery*, 2011, 18(4): 272-280.
25. C. Mura, D. Valenti, C. Floris, R. Sanna, M. A. De Luca, A. M. Fadda and G. Loy. Metronidazole prodrugs: synthesis, physicochemical properties, stability and *ex vivo* release studies. *European Journal of Medicinal Chemistry*, 2011, 46(9): 4142-4150.
26. N. B. Patel and F. M. Shaikh. Synthesis and antimicrobial activity of new 4-thiazolidinone derivatives containing 2-amino 6-methoxybenzothiazole. *Saudi Pharmaceutical Journal*, 2010, 18(3): 129-136.
27. M. Piplani, A.C. Rana, P.C. Sharma. Synthesis, characterization and evaluation of produgs of ciprofloxacin clubbed with benzothiazoles through N-Mannich base approach. *Chemical Biology Letters*, 2016, 3(2): 52-57.