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Original Article

Synthesis and characterization of N-Mannich based prodrugs of ciprofloxacin and norfloxacin: *In vitro* anthelmintic and cytotoxic evaluation

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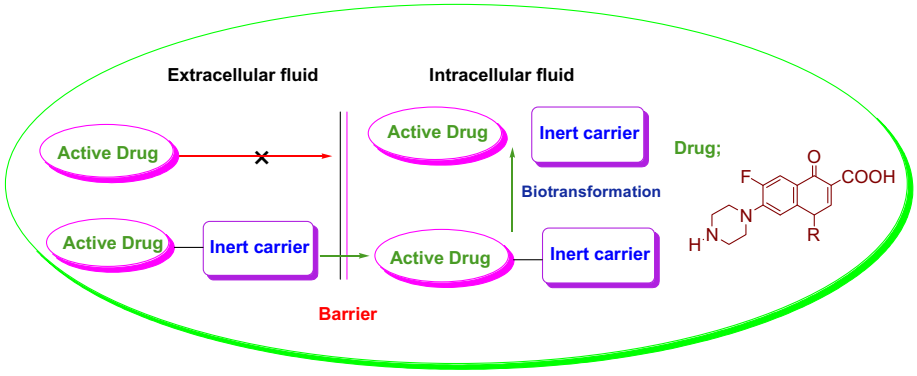
GRAPHICAL ABSTRACT



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ABSTRACT

Prodrugs, the inert derivatives of existing drugs have successfully contributed to the modification of their physicochemical properties. The improved antimicrobial potential due to enhanced lipophilicity of some of the synthesized prodrugs of antibacterial agents by various schemes has already been reported. In the current study, synthesis, characterization, and biological evaluation of some more lipid based prodrugs/-compounds of ciprofloxacin and norfloxacin has been carried out. The synthesized prodrugs/compounds have been screened for anthelmintic activity using Indian earthworms and cytotoxic activity against human lung cancer cell lines A-549 employing sulforhodamine B (SRB) assay method. The prodrugs FQF1, 6b, 6c, and 6k were found to possess promising anthelmintic activity due to improved partition coefficient. Growth of selected cells lines was found to decrease with increase in concentration of prodrugs as compared to parent drug. Prodrug, 6k having GI_{50} value 28.8, has been proved to be the most active among all the synthesized prodrugs. Results of present investigation reveal that some of the synthesized prodrugs/compounds were found to possess promising biological activity.

Introduction

The fluoroquinolone is a series of synthetic antibacterial agents depicting a broad spectrum of antimicrobial activity, relatively low incidence of adverse and toxic effects as well as an excellent safety

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profile [1]. They are being successfully utilized in the treatment of a variety of bacterial infections since their discovery, more than 3–4 decades ago. These agents work by inhibiting the two enzymes namely DNA gyrase and topoisomerase IV. These are vital bacterial enzymes essential for DNA replication as well as transcription leading to cell death [2,3].

In spite of a great and considerable advancements as well as huge requirement in the antibacterial therapy, these drugs have been found to possess various limitations such as poor hydrophilicity, less oral bioavailability, narrow spectrum of activity, short half life, poor systemic distribution, unpleasant taste and less lipophilicity [4–10]. To get rid of these problems associated with fluoroquinolone drugs, two main approaches can be adopted; the synthesis of some novel therapeutic substances or a more efficient utilization of already accessible pharmaceutical agents through various means. Now days, the second approach has also been widely utilized due to problems coupled with the preparation and refinement of novel active compounds [11]. Several methods have been acquired by various scientists to conquer the constraints related to these drugs. One of the preferred strategies to unravel these imperfections of existing drugs which ultimately results in improvement of the bioavailability is prodrug synthesis. Prodrugs can provide many advantages over parent drugs, such as increased solubility, enhanced stability, improved bioavailability, reduced side effects, and better selectivity. Synthesis of various N-Mannich based prodrugs of ciprofloxacin and norfloxacin by two schemes has already been reported and those synthesized prodrugs with enhanced lipophilicity were found to possess significant antimicrobial potential [10,12]. As per available report, synthesis of N-Mannich bases reduces the amines pK_a about 3 points resulting in enhancement of lipophilicity of parent drug and consequently, diffusion potential. Moreover, N-Mannich base hydrolyses at varied rate in buffers depending upon its pH to liberate the bioactive compound [13]. Hence, it may be anticipated that the synthesized prodrugs may undergo hydrolysis reaction to liberate the active drug molecule.

Cancer is the second leading source of fatality in the world after heart related diseases, but it may be the primary cause of death in the near future. Patients with cancer are more prone to various infectious diseases [14]. Keeping in view, the elevated requirement of anticancer drugs with high therapeutic efficiency and increasing severity of infectious diseases in the cancer patients, there is an urgent demand of novel agents/modified existing drugs, which can act as dual anticancer-anti-infective agents having modified biological potential in singular molecular framework. Besides being good antibacterial agents, fluoroquinolones have been found to possess remarkable anticancer activity and anthelmintic activity as reported in the previous articles [15,16]. Hence, encouraged by the promising antimicrobial activity results of synthesized prodrugs [10,12] and to harness the maximum therapeutic potential of these agents, the present investigation was undertaken. It was felt worthwhile to evaluate these prodrugs for anthelmintic activity against Indian earthworms (*Pheretima posthuma*) and check cytotoxicity against human cancer cell lines A-549 by SRB assay method.

Experimental

Materials and methods

The cell lines and media used in the studies comprising of human lung cancer cell lines A-549 and standard drug adriamycin (Adr) were procured and maintained at Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Kharghar, Mumbai during the experimental work. To carry out the anthelmintic activity, Indian earthworm (*Pheretima posthuma*) was procured from Department of Agriculture, Gurukul, Kurukshetra. Norfloxacin and ciprofloxacin were obtained as gift samples from Combitic Global Caplet Pvt. Ltd., (Sonepat, Haryana, India), and labetalol hydrochloride was obtained from Steadfast Medishield (Delhi, India). All other solvents were procured from SD Fines (Mumbai, India) and were of high grade. Standard drug albendazole was purchased from local suppliers (Kurukshetra, Haryana, India).

Chemistry

Various prodrugs of ciprofloxacin and norfloxacin were synthesized employing two schemes.

In Scheme 1 (Fig. 1) lipid based prodrugs were synthesized in five steps. In first step, fatty acid esters (II a/b) were synthesized from fatty acids (I a/b) by refluxing them with excess of methanol in the presence of concentrated sulphuric acid for 3–4 h. Fatty acid hydrazides (III a/b) were synthesized in second step by refluxing fatty acid esters (II a/b) obtained in first step with excess of 98% hydrazine hydrate (three times the moles of fatty acid esters) in ethanol as solvent for 3–4 h. For synthesis of 5-formyl salicylamide (V) in third step, the pH of solution of labetalol hydrochloride (IV) in water was adjusted upto 10. Equimolar quantity of sodium periodate in H_2O was added drop wise over 30 min to this basic solution and allowed to stir for an additional 30 min. The resulting mixture was acidified by adding concentrated HCl to obtain 5-formyl salicylamide (V). Mannich bases (VII a/b) of selected antibacterial drugs were synthesized in proceeding steps by refluxing the mixture of antibacterial drugs (VI a/b), 5-formyl salicylamide (V) and formalin in glacial acetic acid/methanol as solvent and in final step, prodrugs (VIII) were synthesized by reacting fatty acid hydrazides (III a/b) and mannich bases (VII a/b) obtained from second step and fourth step respectively. Four prodrugs (FQF1-FQF4) have been synthesized from Scheme 1. The solids thus obtained were filtered, washed with water and products were recrystallized from chloroform. Synthesis of prodrug FQF1 has already been reported [12]. The synthetic route for prodrugs/compounds, FQF1-FQF4 is outlined in Fig. 1. These prodrugs/ compounds were appropriately evaluated for spectral and physicochemical characterization.

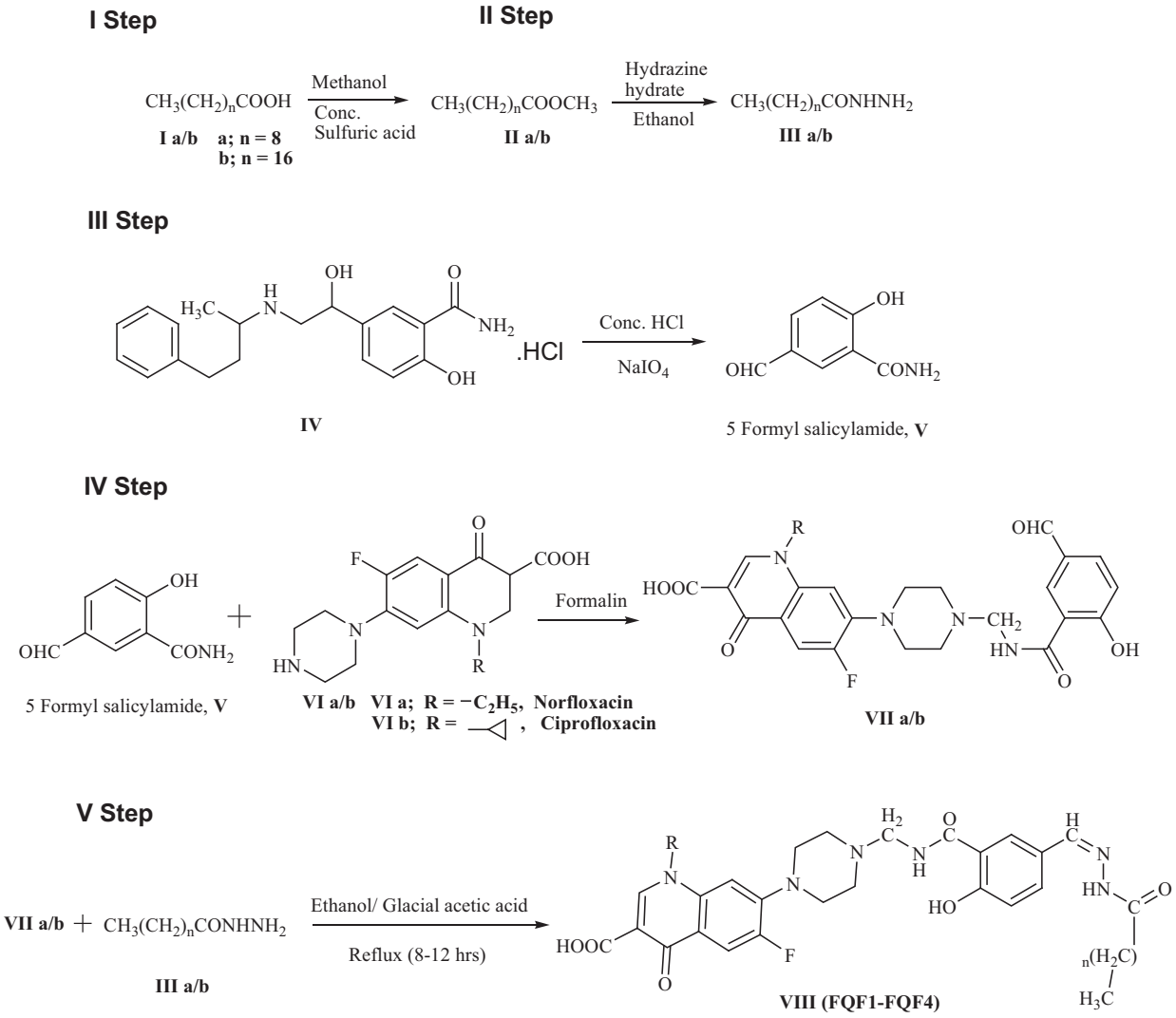
Determination of partition coefficient

Partition coefficient of synthesized prodrugs/compounds has also been determined by shake flask method, employing organic as well as aqueous solvents. The quantity of prodrugs in both phases was determined using standard curve in the respective phases [10,12].

(FQF2); Yield 62%; m.p.: 187–190 °C; IR (KBr cm^{-1}): 3152 (N—H), 2943 (C—H), 1627 (C=O), 1496 (C—N). ^1H NMR (DMSO-d_6) δ ppm: 1.21 (t, 3H, —CH_3 ethyl), 2.1 (quin, 2H, CH_2,), 3.18–3.54 (m, 8H, piperazne-H), 4.57 (d, 2H, N-CH_2-N, methylene), 6.9–7.35 (m, 5H, aromatic and H_5, H_8-quinolone), 15.3 (s, 1H, COOH) 7.7 (s, 1H, H_2-quinolone), MS: m/z = 776.9 (M^+).

(FQF3); Yield 65%; m.p.: 190–192 °C; IR (KBr cm^{-1}): 3209 (N—H), 2952 (C—H), 1632 (C=O), 1494 (C—N). ^1H NMR (DMSO-d_6) δ ppm: 1.28 {m, 2H, CH_2 (cyclopropyl)}, 2.98–3.51 (m, 8H, piperazine-H), 3.34 {quin, 1H, CH (cyclopropyl)}, 4.5 (d, 2H, N—CH_2—N, methylene), 6.53–7.49 {m, 5H, aromatic (H_5, H_8-quinolone)}, 15.2 (s, 1H, COOH) 8.12 (s, 1H, H_2-quinolone), MS: m/z = 676.3 (M^+).

(FQF4); Yield 68%; m.p.: 201–204 °C; IR (KBr cm^{-1}): 3164 (N—H), 2889 (C—H), 1652 (C=O), 1498 (C—N). ^1H NMR (DMSO-d_6) δ ppm: 1.34 {m, 2H, CH_2 (cyclopropyl)}, 4.36 (d, 2H, N—CH_2—N, methylene), 3.12–3.72 (m, 8H, piperazine-H), 6.92–7.82 {m, 6H, aromatic (H_5, H_8-quinolone)}, 3.51 {quin, 1H, CH (cyclopropyl)},



S. No.	Prodrug	n	R	S. No.	Prodrug	n	R
1.	FQF1	8	-C_2H_5	3.	FQF3	8	
2.	FQF2	16	-C_2H_5	4.	FQF4	16	

Fig. 1. Synthetic scheme 1 for preparation of prodrugs FQF1-FQF4.

14.5 (s, 1H, COOH) 7.91 (s, 1H, H_2-quinolone), MS: m/z = 788.9 (M^+).

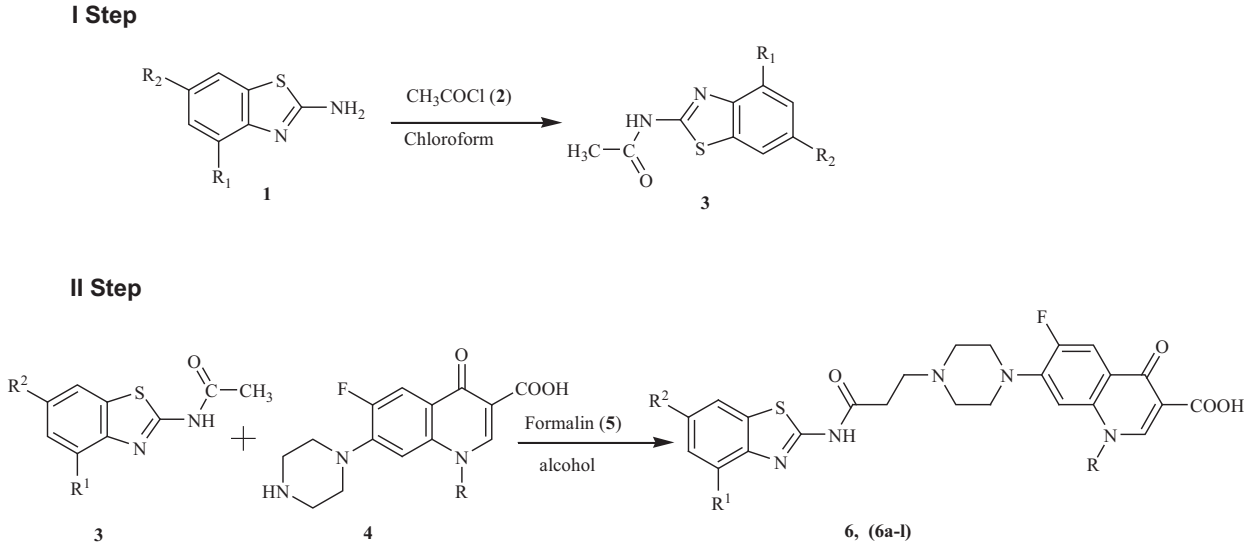
In another scheme, benzothiazoles clubbed prodrugs of selected antibacterial agents were synthesized employing N-Mannich base approach. The synthesis of prodrugs was carried out in two steps. In the first step, various benzothiazoles (1) were acetylated by refluxing them with acetyl chloride (2) in chloroform as solvent for 10–12 h, to produce acetylated benzothiazoles (3); followed by N-Mannich base synthesis in second step. N-Mannich bases of selected antibacterial agents (6) were synthesized by refluxing the mixture of these acetylated benzothiazoles (3), antibacterial agents (4), and 37% formalin (5) in methanol as solvent. Twelve prodrugs (6a-l) were synthesized from this scheme, reported elsewhere [10] and spectral as well as physicochemical characterization was carried out. The general scheme for synthesis of prodrugs (6a-l) has been represented in Scheme 2 (Fig. 2).

The ^{1}H NMR spectra of synthesized N-Mannich base prodrugs of ciprofloxacin and norfloxacin (6a-l) in DMSO-d_6 displayed —CH_2-—CH_2— ethylene bridge at 3.90–4.42 ppm as multiplet. The signals of NH proton and COOH proton were obtained as singlet at 4.71–4.91 ppm and broad singlet at 13.07–15.3 ppm, respectively. The IR spectrum of prodrugs showed characteristics absorption peaks approximately at 1600, 1700, 1250, 3000, and 3300 cm^{-1}. Mass spectra of all prodrugs were found to be in accordance with its proposed molecular formula.

Biological evaluation

Anthelmintic activity

The anthelmintic screening of the test compounds has been carried out to find their capability to eradicate the worms. The



Prodrug	R	R^1	R^2	Prodrug	R	R^1	R^2
6a	$-C_2H_5$	-H	$-OCH_3$	**6g**		-H	$-OCH_3$
6b	$-C_2H_5$	-H	$-OC_2H_5$	**6h**		-H	$-OC_2H_5$
6c	$-C_2H_5$	-H	$-CH_3$	**6i**		-H	$-CH_3$
6d	$-C_2H_5$	-H	-Cl	**6j**		-H	-Cl
6e	$-C_2H_5$	-H	$-NO_2$	**6k**		-H	$-NO_2$
6f	$-C_2H_5$	-Cl	-H	**6l**		-Cl	-H

Fig. 2. Synthetic scheme 2 for preparation of prodrugs 6a-l.

anthelmintic activity of reported synthesized prodrugs/ compounds has been carried out *in vitro* against Indian earthworm (*Pheretima posthuma*) procured from Department of Agriculture, Gurukul, Kurukshetra of nearly equal sizes (4–5 cm in length and 0.1–0.2 cm in width). The earthworm was washed with standard saline (0.9% *w/v*) to remove all the fecal material and soil particles. This earthworm was selected due to its analogous anatomical and physiological characteristics with intestinal roundworm, parasites of human beings.

Suspensions of synthesized prodrugs/compounds and standard drugs were dissolved in a minimum quantity of DMSO and the volume was adjusted to 40 mL with standard saline (0.9% *w/v*) to prepare the concentration 0.2% *w/v* of test compounds. The test solutions (0.2% *w/v*) were taken in petri dishes (2 inches). The standard drug albendazole was also used in the form of the suspension with the same concentration in the same way [17,18].

For the experimentation purpose, a group of six earthworms were placed into each of 40 mL of standard drug and the test

suspensions (0.2% *w/v*). Earthworms tested in standard saline (0.9% *w/v*) were taken as control group. The petri dishes containing the earthworms were kept under observation to note down their individual time of paralysis and death for up to 5 h of the test period. The mean paralysis time was recorded by shaking the worms and being assured that no movement could be observed except when the worms were shaken vigorously. Death time was recorded after ascertaining that earthworms neither moved when shaken vigorously nor when dipped in water having temperature upto 50 °C. The mean paralysing time and mean death time was calculated [19,20].

Cytotoxic activity

Cytotoxic studies of selected synthesized prodrugs/compounds have been carried out in human lung cancer cell lines A-549 employing sulforhodamine (SRB) protocol. The growth medium selected for cells was RPMI medium containing 10% fetal bovine serum and 2 mM glutamine. Cells were allowed to inoculate into 96-well microtiter plates at appropriate cellular density depending upon replication time of selected cell lines. The microtiter plates containing cells, after inoculation were allowed to incubate at temperature 37 °C, 95% air, 5% CO_2, and 100% RH for 24 h before addition of synthesized test prodrugs/compounds.

After solubilization into appropriate solvent (DMSO) in different concentrations *i.e.* 10 μg/mL, 20 μg/mL, 30 μg/mL, and 40 μg/mL, an aliquots of 10 μL of these solutions were added into microtiter plates and incubated for 48 h. The experiment was terminated by adding 30% of cold TCA and allowed to incubate for another 60 min at 4 °C. After washing with tap water several times, sulforhodamine B solution at 0.4% *w/v* in 1% acetic acid was added into the wells. The plates were incubated for 20 min at room temperature, washed with 1% acetic acid, and air dried. The bound stain was eluted with trizma base and absorbance was noted using ELISA reader at λ_{max} 540 nm with reference wavelength of 690 nm.

Percentage control growth was calculated for test compounds relative to control and expressed as ratio of average absorbance of test compounds containing wells to average absorbance of control wells multiplied by 100. Using absorbance at time zero (Tz), control growth (C) and test growth at four concentration levels (Ti), the percentage growth was calculated at all the selected drug concentrations. The percentage growth inhibition was calculated by the following formulae:

$$\text{Percentage growth inhibition} = \text{Ti}/\text{C} \times 100$$

Growth inhibition of 50% (GI_{50}) is the drug concentration at which half (50%) reduction of net protein increase takes place as measured by SRB staining [21]. It was determined by:

$$[(\text{Ti} - \text{Tz})/(\text{C} - \text{Tz})] \times 100 = 50.$$

Results and discussion

Development and characterization of ciprofloxacin and norfloxacin prodrugs (FQF2-FQF4). The synthesis and characterization of lipid based prodrugs/compounds of ciprofloxacin and norfloxacin was carried out as outlined in Fig. 1. N–Mannich bases of ciprofloxacin and norfloxacin were synthesized by refluxing these antibacterial agents with 5-formyl salicylamide in presence of formalin. These N-Mannich bases were further reacted with fatty acid hydrazides to prepare the prodrugs/compounds FQF2-FQF4. All the synthesized prodrugs/compounds were characterized by appropriate spectral methods *i.e.* IR, ^{1}H NMR spectroscopy, and mass spectrometry. The characteristic peaks at 4.36–4.57 ppm (d, 2H) due to —CH_2 methylene bridge in ^{1}H NMR spectra revealed the synthesis of N-Mannich bases of norfloxacin and ciprofloxacin, the intermediate compounds. The disappearance of peaks at 9.91 and 10.1 ppm (1H, s) of CHO group assured the synthesis of final prodrugs/compounds. Shake flask method was employed to find out the partition coefficient of synthesized prodrugs FQF2-FQF4. Prodrugs FQF2, FQF3, and FQF4 were found to possess 0.98, 0.43, and 0.39 partition coefficient, respectively, which was greater than their parent drugs, norfloxacin (0.46), and ciprofloxacin (0.28), respectively. Partition coefficient of prodrugs 6a-f was found to be greater (1.99–2.87) compared to norfloxacin and prodrugs. As well, 6g-l exhibited better partition coefficient (0.33–0.46) than that of ciprofloxacin. Being a significant factor in improving the penetrability of active pharmaceutical agent through biological membrane, modified partition coefficient would be assumed to be the contributory factor for superior biological potential of synthesized prodrugs.

These synthesized prodrugs were evaluated for antimicrobial activity and some of them were found to possess significant antimicrobial activity as reported elsewhere. These prodrugs were evaluated for some other activities. The results of those biological evaluations have been discussed as follows:

Anthelmintic activity

The anthelmintic activity of synthesized (FQF2-FQF4) and reported (FQF1, 6a-l) prodrugs was determined against Indian earthworm (*Pheretima posthuma*). They demonstrated promising anthelmintic activity at concentration of 200 mg/100 mL. The anthelmintic activity results of prodrugs obtained from two schemes has been discussed separately here. The outcomes of anthelmintic activity of prodrugs/compounds FQF1-FQF4 have been summarized in Table 1 and shown in Fig. 3.

Table 1
Anthelmintic activity of synthesized prodrugs (FQF1-FQF4).

Prodrug	Concentration of prodrug (mg/100 mL)	Mean paralysing time (min) ± S.D	Mean death time (min) ± S.D
FQF1	200	15.50 ± 1.29	51.25 ± 1.26
FQF2	200	21.00 ± 1.83	62.00 ± 1.41
FQF3	200	33.75 ± 1.50	75.50 ± 2.08
FQF4	200	43.25 ± 0.96	92.50 ± 1.29
NFX	200	55.75 ± 0.96	92.00 ± 2.16
CFX	200	62.75 ± 1.50	114.50 ± 1.29
*Standard	200	15.00 ± 0.82	55.25 ± 0.50
**Control	–	–	–

Each value is mean ± s.d. n = 6.
* Albendazole.
** DMSO, saline water.

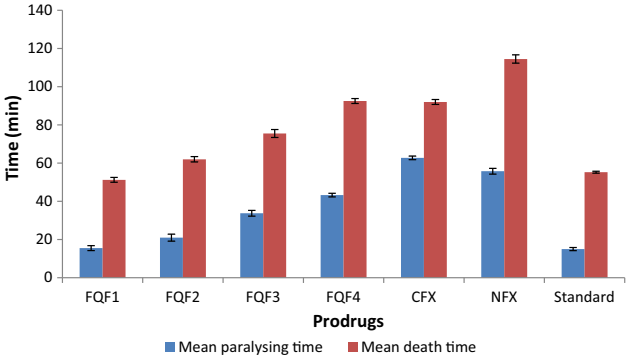


Fig. 3. Anthelmintic evaluation of FQF1-FQF4.

Due to improved lipophilicity, these prodrugs were found to exhibit reduced mean paralysing time (15.50 ± 1.29 to 43.25 ± 0.96 min) and death time (51.25 ± 1.26 to 92.50 ± 1.29 min) compared to parent drugs, norfloxacin (paralysing time 55.75 ± 0.96 min, death time 92.00 ± 2.16 min) and ciprofloxacin (paralysing time 62.75 ± 1.50 min, death time 114.5 ± 1.29 min).

Having highest partition coefficient (1.15) and consequently penetrability, prodrug FQF1 having mean paralysing time 15.50 ± 1.29 min and mean death time 51.25 ± 1.26 min exhibited comparable anthelmintic activity compared to standard drug, albendazole (mean paralysing time 15.00 ± 0.82 min and mean death time 55.25 ± 0.5 min). Synthesis of N-Mannich base and incorporation of fatty acids resulted in improvement of the partition coefficient of synthesized prodrugs and consequently their biological potential.

The mean paralysing time and mean death time for prodrugs 6a-l was calculated and summarized in Table 2 and depicted in Fig. 4. These prodrugs having improved partition coefficient showed better anthelmintic activity with mean paralysing time ranging from 8.75 ± 0.96 to 53.00 ± 1.83 min and death time in between 20.25 ± 0.96 to 86.25 ± 1.50 min compared to parent drugs, norfloxacin and ciprofloxacin. The prodrugs 6b, 6c, and 6k were found to possess remarkable activity having mean paralysing time 8.75 ± 0.96, 9.75 ± 0.96, and 13.00 ± 0.82 min and mean death time 20.25 ± 0.96, 36.00 ± 0.82, and 39.75 ± 1.71 min, respectively, compared to both parent as well as standard drug at the same concentration. Results of anthelmintic activity of prodrugs 6a-l

Table 2
Anthelmintic activity of synthesized prodrugs (6a-l).

Prodrug	Concentration of prodrug (mg/100 mL)	Mean paralysing time (min) ± S.D	Mean death time (min) ± S.D
6a	200	33.00 ± 1.41	73.05 ± 1.29
6b	200	08.75 ± 0.96	20.25 ± 0.96
6c	200	09.75 ± 0.96	36.00 ± 0.82
6d	200	20.50 ± 1.29	57.25 ± 1.71
6e	200	53.00 ± 1.83	63.00 ± 1.63
6f	200	35.75 ± 1.71	86.25 ± 1.50
6g	200	34.50 ± 1.30	49.00 ± 0.82
6h	200	37.75 ± 1.26	84.75 ± 1.26
6i	200	23.75 ± 1.71	51.75 ± 1.71
6j	200	25.00 ± 0.82	45.50 ± 1.73
6k	200	13.00 ± 0.82	39.75 ± 1.71
6l	200	28.75 ± 1.71	62.25 ± 0.96
NFX	200	55.75 ± 0.96	92.00 ± 2.16
CFX	200	62.75 ± 1.50	114.5 ± 1.29
*Standard	200	15.00 ± 0.82	55.25 ± 0.50
**Control	–	–	–

Each value is mean ± s.d. n = 6.
* Albendazole.
** DMSO, Saline water.

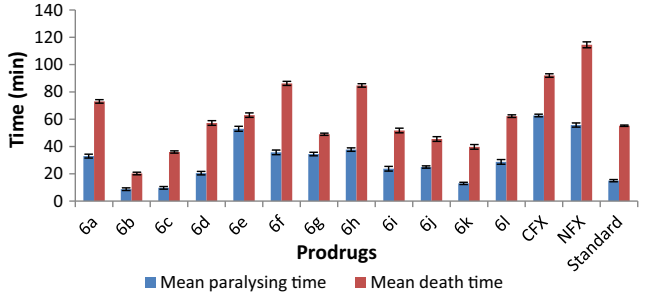


Fig. 4. Anthelmintic evaluation of prodrugs 6a-l.

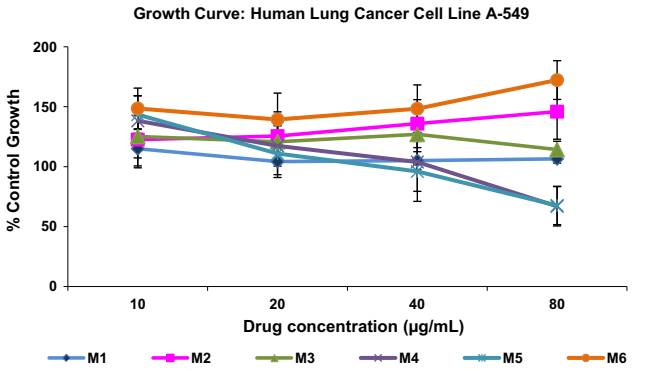


Fig. 5. Effect of different concentrations of synthesized prodrugs on percentage growth inhibition of Human lung cancer cell A-549.

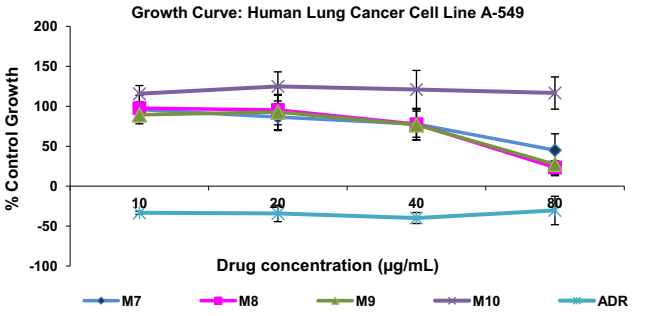


Fig. 6. Effect of different concentrations of synthesized prodrugs on percentage growth inhibition of human lung cancer cell A-549.

Table 3
GI_{50} and percentage control growth of synthesized prodrugs.

Prodrug	Samples ID	Human lung cancer cell line A-549 % Control growth Drug concentration in µg/mL (Average values)				GI_{50}
		10	20	30	40	
FQF1	M1	115.0 ± 16.0	104.3 ± 13.3	105.1 ± 7.4	106.5 ± 3.5	67.3
FQF2	M2	122.6 ± 22.0	125.5 ± 20.2	135.9 ± 20.0	145.9 ± 23.0	>80
FQF3	M3	125.2 ± 17.9	120.8 ± 18.1	126.9 ± 25.0	114.2 ± 6.95	>80
6a	M4	138.2 ± 21.0	117.3 ± 16.8	103.9 ± 24.5	66.9 ± 16.4	>80
6b	M5	143.4 ± 15.6	110.9 ± 17.6	96.0 ± 25.0	67.6 ± 16.0	>80
6f	M6	148.6 ± 17	139.4 ± 22	148.3 ± 19.8	172.2 ± 16.2	>80
6i	M7	95.9 ± 17.9	86.5 ± 16.8	77.8 ± 19.7	45.0 ± 20.6	44.5
6k	M8	97.6 ± 4.80	95.3 ± 18.5	77.7 ± 16.3	23.4 ± 8.50	28.8
6l	M9	89.3 ± 6.10	92.8 ± 22.1	76.8 ± 19.1	27.4 ± 14.3	40.3
Adriamycin	Standard	−33.4 ± 1.9	−34.1 ± 10.3	−39.8 ± 6.9	−30.4 ± 17.8	<10
Norfloxacin	M10	115.7 ± 10.3	124.9 ± 18.2	120.9 ± 24.0	116.6 ± 20.1	>80

Each value is mean ± s.d. n = 3.

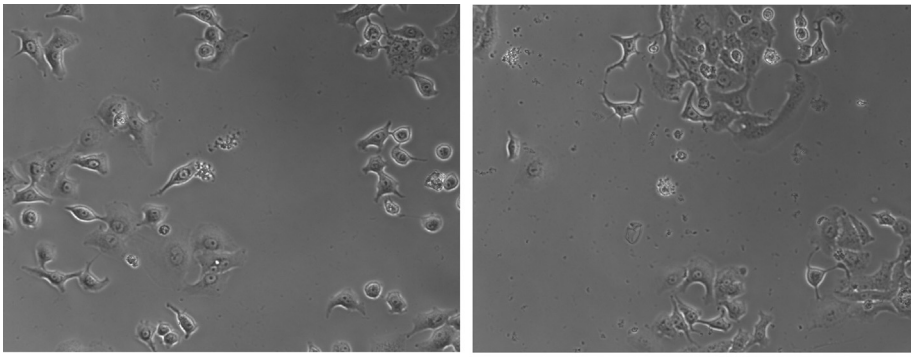


Fig. 7. Cytotoxic activity of prodrugs M8 (a) and M9 (b) against human lung cancer cell lines.

suggested that clubbing of fluoroquinolones with benzothiazoles (having substitutions of electron withdrawing and electron donating groups at 4th and 6th position) via synthesis of N-Mannich base in scheme 2 (Fig. 2) have positively increased the lipophilicity of the prodrugs/compounds and helped in improvement of anthelmintic activity of synthesized prodrugs.

Cytotoxic activity

The synthesized prodrugs/compounds were evaluated for cytotoxic activity to find out the effect of their improved partition coefficient. The synthesized prodrugs/compounds that possessed significant antibacterial activity were selected for cytotoxic activity. To determine the cytotoxic activity, sulforhodamine B (SRB) protocol was employed and human lung cancer cell lines A-549 were selected for this study. The results have been determined in the terms of GI_{50} and percentage control growth in Table 3 and shown in Figs. 5 and 6.

Percentage growth of selected cell lines was found to decrease with increase in concentration of prodrugs, FQF1, 6i, 6k, and 6l. On the other hand, the prodrugs FQF2 (M2) and 6f (M6) were found to exhibit growth promoting effect. Study of GI_{50} values on human lung cancer cell lines indicated that the lipid based prodrug FQF1 and other prodrugs such as 6i, 6k, and 6l having methyl (weak electron donating) and chloro, nitro (electron withdrawing groups), respectively substituted at 6th and 4th position, were found to exhibit good activity against human lung cancer cell line A-549 when compared with parent drug. However, no direct relationship could be established between functional groups and cytotoxicity possessed by various prodrugs. In the human lung cancer cell lines GI_{50} value of prodrugs FQF1 and 6i, 6k, 6l was found to be 67.3 μg/mL and 28.8–44.5 μg/mL, respectively, as compared to >80 μg/mL for parent drug.

Although, no cell death was detected with these prodrugs/compounds, the growth of the cells was reduce with increase in concentration, possibly due to improved lipophilicity and better penetration of synthesized prodrugs through cellular membrane when compared to parent drug. The prodrugs FQF1, FQF2, and FQF3 correspond to sample ID M1, M2, and M3. The extent of biological activities has not been quantified however; the improvement of biological activity *i.e.* antimicrobial, anthelmintic, and cytotoxic activities of synthesized prodrugs indicates their greater penetrability owing to improved partition coefficient than the pure drug itself.

Prodrugs 6a, 6b, 6f, 6i, 6k, and 6l corresponds to samples ID M4, M5, M6, M7, M8, and M9, respectively. The parent drug norfloxacin correspond to M10. The prodrugs M8 and M9 were found to exhibit promising GI_{50} and their cytotoxic effects on human lung cancer cell lines has been depicted in Fig. 7.

Conclusions

In current investigation, lipid based prodrugs has been synthesized and evaluated for physicochemical and spectral characterization. The effect of improved partition coefficient of synthesized prodrugs/compounds has also been studied on cell lines and Indian earthworms. The *in vitro* screening of synthesized prodrugs/compounds exhibited superior therapeutic efficiency as compared to parent drugs. Therefore, taking into account the data presented herein, it can be inferred that the improvement of absorption characteristics owing to increased lipophilicity of prodrugs can be useful in furtherance of endeavors towards reduction in doses.

Conflict of Interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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