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Design, synthesis, antibacterial activity and physicochemical parameters of novel *N*-4-piperazinyl derivatives of norfloxacin

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ABSTRACT

We report herein the synthesis of some *N*-Mannich bases in addition to different N-4 substituents of norfloxacin. The antibacterial activities of the newly synthesized compounds were evaluated and correlated with their physicochemical properties. Results revealed that some of the tested compounds exhibited better inhibitory activities than the reference antibiotic norfloxacin against *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia and Staphylococcus aureus* strains. Correlation results showed that there is no single physicochemical parameter that can determine the effect of *N*-4 piperazinyl group on the activity of these fluoroquinolones, where lipophilicity, molecular mass and electronic factors may influence the activity.

1. Introduction

Fluoroquinolone antibacterial agents represent a fast growing group of antibiotics; various derivatives were synthesized and tested for their antimicrobial activities. The new generations of fluoroquinolones achieved significant improvement in potency, spectrum and physicochemical properties.[1] The SAR studies revealed that, the fluorine atom and the 1-alkyl, 1,4-dihydro-4-oxo-quinoline-3-carboxylic acid skeleton of fluoroquinolones is responsible for potency represented in binding with type-II topoisomerase enzymes, DNA gyrase and topoisomerase IV.[2] In addition, it is believed that the 6-fluoro and 7-piperazinyl groups are responsible for the broad spectrum and antipseudomonal activities of fluoroquinolones.[2] Moreover, it is clear that chemical modifications at C-7 is suitable for controlling of the pharmacokinetic properties and hence changes in the cell permeability of these antibiotics.[3] The third generation fluoroquinolones as lomefloxacin and fleroxacin exhibited longer duration of action, while sparfloxacin and tosufloxacin revealed higher potency against Gram-positive *cocci* and anaerobic bacteria than the earlier members[4] The more recently released fluoroquinolone, levofloxacin, possesses a broad-spectrum antibacterial activity similar to that of earlier quinolones. However, it has a greater activity against Gram-positive and atypical organisms.[5]

Several *N*-4 piperazinyl derivatives of fluoroquinolones were introduced and demonstrated various biological activities like benzenesulfonamide fluoroquinolones as broad spectrum antibacterials[6] and 7-{4-[2-(hydroxyiminoethyl]-1-piperazinyl derivatives which demonstrated excellent activities against renal cancer.[7] Also, isatine Mannich bases of gatifloxacin were emerged as potent anticancer agents.[8] In addition, research groups introduced recently *N*-4 piperazinyl aralkyl,[9] Mannich bases[10] or cephalosporin[11] fluoroquinolone derivatives effective as potential antimycobacterial agents. Other norfloxacin Mannich bases were synthesized and showed anti-HIV, antifungal in addition to antibacterial activities.[12]

N-Mannich bases have been used as potentially useful prodrug candidates for imides, amides, amines, hydantoin and urea derivatives.[13] It is believed that the *N*-Mannich base functional group can increase the lipophilicity of the parent amines at physiological pH values by decreasing their protonation resulting in enhancement of absorption through bio-membranes.[14] The lipophilicity of fluoroquinolones can influence their ability to cross bacterial membranes. Consequently, partition coefficient could be an important parameter affecting the biological activity.[15] Furthermore, it is clear that the neutral species of fluoroquinolones are more lipophilic than zwitterionic form. So, factors that can affect N-4 protonation, like steric and electronic effects or charge density, can also affect lipophilicity.[16] Moreover, it was proposed that lipophilicity is a very important factor in the quinolones intestinal absorption[17] that is responsible for the poor relationship between in vivo and in vitro activity of fluoroquinolones.

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Herein, we report the synthesis of two series of norfloxacin derivatives. The first series describes synthesis of bis(2-hydroxyethyl)piperazin-1-ium bromide derivative with expected full positive charge at pH 7; 5,6-diphenyl 1,2,4-triazinyl and cyclopentyl *N*-4 piperazinyl substituents in addition to the *N*-formyl derivatives, the known active metabolite[18] of norfloxacin. The second series describes synthesis of various *N*-4 piperazinyl Mannich bases as prodrugs of norfloxacin. The designed *N*-4 piperazinyl substituents were chosen with expected variable lipophilicities, steric, electronic and molecular masses. Also, partitioning behavior of these derivatives between octanol and buffer solutions (pH 7.4) is a matter of interest to be correlated with their antibacterial activities. It seems reasonable to calculate the N-4 charge density, due to its importance in determining the N-4 protonation, consequently, correlation between partition coefficient, molecular mass and N-4 charge density with log MIC was carried out. The methods of synthesis of the designed compounds are summarized in Schemes 1 and 2.

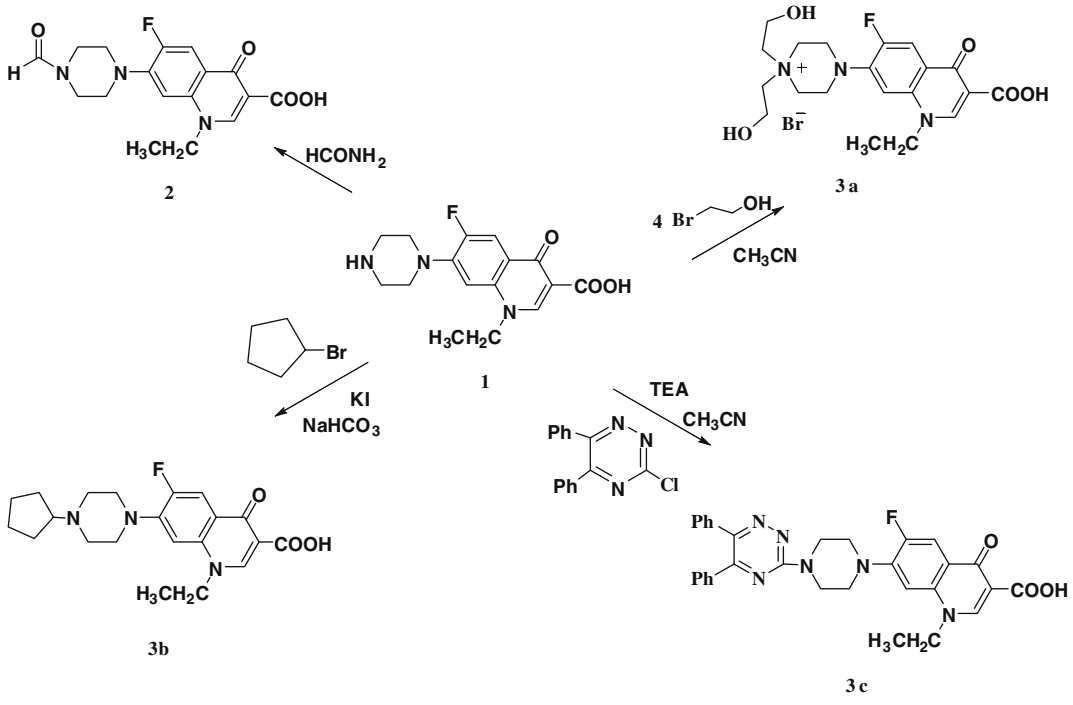
2. Results and discussion

2.1. Synthesis

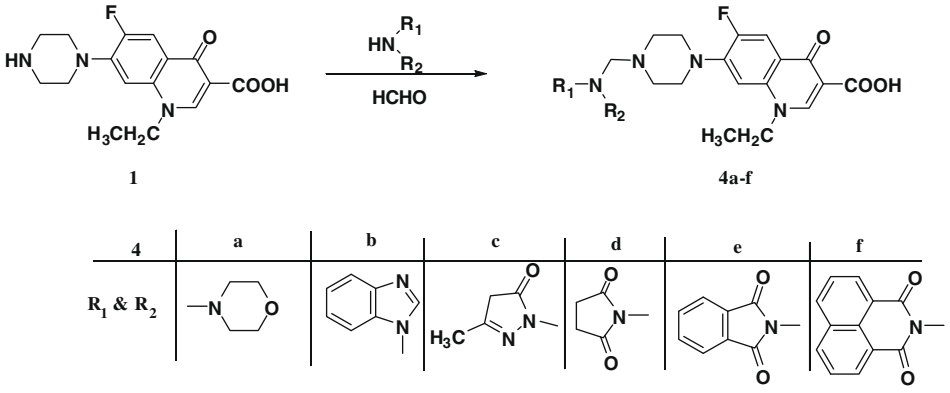
Although the *N*-formyl derivative **2** was prepared previously[1] in 50% yield using formic acid without spectroscopic specifications, it was also recently prepared[19] in DMF using an independent reaction without spectroscopic data. It is prepared in this investigation using a procedure reported for synthesis of other *N*-formyl[20] by refluxing norfloxacin **1** and formamide in glacial acetic acid in boiling steam bath. It was difficult to react the formed *N*-formyl group with amines or hydrazides. Heating at reflux of norfloxacin **1** with four equivalents of bromoethanol in acetonitril afforded *N-4* bis(2-hydroxyethyl)piperazin-1-ium bromide derivative **3a** in high yield. The isolated compound exhibited a melting point over 300 °C. Hence, the possibility of identification of the product as the tertiary analogue, the *N*-4-hydroxyethyl derivative, is excluded where it is reported in the literature with a variable melting point due to difference in melting point of this compound with that reported in literature (229–230 °C).[1] Also, the ^{1}H NMR spectrum of compound **3a** in DMSO-d_6 showed the two –CH_2– groups of the two hydroxyethyl moieties and the piperazine moiety appeared as a multiplet from 3.0 to 4.1 ppm. The 2 OH absorption appeared at δ 5.3 ppm. Elemental analysis data confirmed the N-quaternary derivative **3a**. Heating at reflux of norfloxacin **1** with 3-chloro-5,6-diphenyl-1,2,4-triazine using triethylamine as a base in acetonitrile afforded the derivative **3c**. On the other hand, preparation of the cyclopentyl derivative **3b** required stirring of norfloxacin **1** with cyclopentyl bromide using sodium bicarbonate as a base and potassium iodide as a catalyst in DMF at room temperature.

Mannich bases of norfloxacin **4a–f** were prepared by heating at reflux of norfloxacin **1** and the respective secondary amine, imide with excess formaldehyde in ethanol. The yield of the formed Mannich bases range from 49.5% to 76%. The purity of the synthesized compounds was checked by thin layer chromatography and elemental analysis. The structural formulae of the products were assigned by spectral data. The ^{1}H NMR spectra were identified by their chemical shifts, multiplicities and coupling constants. In general, ^{1}H NMR spectra showed the characteristic chemical shifts for the fluoroquinolone nucleus. It showed the absorption characteristic for the –*N*–CH_2CH_3 as triplet–quartet; the piperazine 8 protons appeared either as a multiplet or two multiplets in the range of 2.9–4.3 ppm, the doublet signal at a range of 6.8–7.25 ppm with J_{H-F} of about 6.6–7.5 Hz is characteristic for H-8; the doublet signal at a range of 7.8–8.1 ppm with J_{H-F} of about 12.0–14 Hz is characteristic for H-5 and the singlet signal characteristic for H-2 at about 8.63–8.95 ppm. The ^{1}H NMR spectrum of the *N*-formyl derivative **2** showed the characteristic signal for –*N*–C*H*O group as a singlet at 8.1 ppm. The ^{1}H NMR of the Mannich bases **4a–f** showed the characteristic singlet (2H) for the –N–CH_2–N– at 4.7–4.8 ppm in $CDCl_3$ or 5.2–5.3 in DMSO-d_6.

It is noteworthy noting that the ^{1}H NMR spectrum of the Mannich base **4c** in $CDCl_3$ showed absorptions corresponding to two tautomeric forms in the 3-methyl-pyrazole-5(4*H*)-one moiety. This result is consistent with a previous finding about tautomerism in

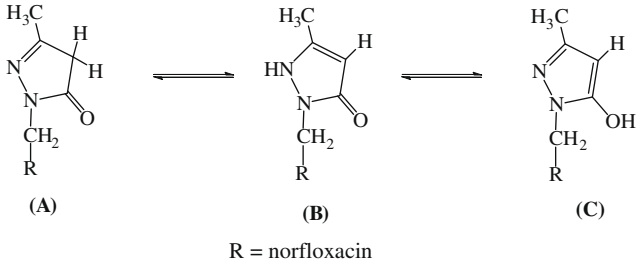


Scheme 1. Synthesis of compounds **2** and **3a–c**.



Scheme 2. Synthesis of the Mannich bases **4a–f**.

pyrazolones.[21] This may be attributed to the presence of both the CH (**A**) and the NH-(**B**) isomers. The –N–CH_2–N– group appeared as two absorptions at δ 4.69 and 4.36 ppm owing to the different chemical environment corresponding to the two isomers. Other absorption corresponding to the 4-CH_2 moiety in pyrazolone tautomer (**A**) multiplet at 3.38–3.50 ppm. In addition, a singlet characteristic for the =CH group for the isomer (**B**) appears at 4.2 ppm. The prevalence of the two tautomers is in the ratio of about 40% of the tautomer (**A**) and 60% of tautomer (**B**). The possibility of the presence of the –OH tautomer (**C**) is excluded due to the absence of both a downfield aromatic proton absorption corresponding to the =CH and the enolic OH.



2.2. Antibacterial screening

The synthesized compounds **2**, **3a–c**, **4a–f** in addition to the reference norfloxacin were tested for their in vitro antibacterial activity against *P. aeruginosa*, *Escherichia coli, Klebsiella pneumonia and Staphylococcus aureus* strains. The diameter of the zones of inhibition (mm) are given in Table 1, their minimum inhibitory concentrations (MICs) are given in Table 2. From the obtained data, it is obvious that, the pyrazolone Mannich base **4c** exhibited good inhibition activity against all the tested Gram-positive and Gram-negative organisms which is more pronounced than that exhibited by the reference, norfloxacin. The succinimide Mannich base **4d** and the *N*-formyl analogue **2** revealed a better activity than norfloxacin in all the tested strains except *K. pneumonia*, where the inhibition is weak. Other compounds showed variable inhibition activities against the used strains. In comparison, the *N*-formyl **2**, the pyrazolone **4c** and succinimide Mannich base **4d** gave antipseudomonal activities comparable to norfloxacin. The *N*-formyl **2** and the quaternary derivative **3a**, in addition to the Mannich bases **4c** and **4d** showed better activity against *E. coli* than norfloxacin that is excellent in case of the Mannich bases **4c** and **4d** (MICs < 1). None of the newly synthesized compounds exhibited better activity against *K. pneumonia* than norfloxacin, but compounds **3a**, **4c** and **4e** showed good comparable activities. The *N*-formyl **2** and the Mannich base **4a** showed excellent inhibition against *S. aureus* (MICs < 1). It is noteworthy noting that the triazinyl derivative **3c** and the Mannich base **4f** characterized by their relatively higher molecular weight (550.28 and 528.53, respectively) exhibited no pronounced activities against all the tested strains.

2.3. Partition coefficient

The experimental partition coefficient between octanol and phosphate buffer pH 7.4 expressed in (log *D*) of the newly synthesized fluoroquinolones, **2**, **3a**, **3c**, **4a–d, 4f** in addition to the parent norfloxacin ranged from 0.158 to −0.90 (Table 3). The results of the calculated partition coefficient values (log *P*) are listed along with the experimental partition coefficients for comparison in Table 3. To determine the relationship between the calculated and experimental partition coefficient values, a simple regression was performed. There was found a weak correlation between the calculated and the experimental values (r^2 = 0.29) Figure 1. This weak correlation may be attributed to the zwitterionic nature of the fluoroquinolones at the physiological pH which is close to the isoelectric point (pH 7.0) that is not considered in the calculated log *P*. It is assumed that the partitioning of quinolones was generally consistent with the knowledge that the neutral species of like compounds is more lipophilic than the zwitterionic species. The ratio of the zwitterions to the neutral species is a very important feature to describe the lipophilicity of fluoroquinolones[22] (Scheme 3).

2.4. Correlation between partition coefficient and log MIC

The parabolic relationship between the antibacterial activities (log MIC) and both log *D* or the calculated log *P* has been fitted. The following general regression equation was therefore applied.

$$Y = a \log X^2 + b \log X + C$$

where *Y* = log MIC; log *X* = log *D* or calcd log *P*; *a*, *b* and *c* are constants.

The parabolic curve-fittings between log MIC and both the calculated log *P* or the experimental log *D* values in all the tested microorganisms are described. The correlation between calculated log *P* and log MIC in case of *P. aeruginosa* showed strong positive correlation (r^2 = 0.94). (Fig. 2). On the other hand, this correlation is weaker in case of *K. pneumonia, E. coli* and *S. aureus* (r^2 = 0.85, 0.67, 0.62, respectively), Table 4.

The correlation between log *D* and log MIC is weaker than in case of calculated log *P* where r^2 = 0.54, 0.43, 0.38 and 0.25 for *E.*

Table 1
The diameter of the zone of inhibition (mm)

Compd	Organisms	Diameter of inhibition zone (mm)				
		4 (μg/mL)	**16** (μg/mL)	**64** (μg/mL)	**128** (μg/mL)	**512** (μg/mL)
Norf.	*Pseudomonas aeruginosa*	0	2	5	15	20
	Escherichia coli	0	0	4	6	19
	Klebsiella pneumonia	0	0	5	8	12
	Staphylococcus aureus	0	0	0	3	20
2	*Pseudomonas aeruginosa*	0	2	7	13	15
	Escherichia coli	2	2	2	4	13
	Klebsiella pneumonia	0	0	0	0	8
	Staphylococcus aureus	7	8	8	11	12
3a	*Pseudomonas aeruginosa*	0	0	0	0	0
	Escherichia coli	0	4	7	9	12
	Klebsiella pneumonia	0	0	4	5	11
	Staphylococcus aureus	0	0	0	0	14
3b	*Pseudomonas aeruginosa*	0	0	0	0	0
	Escherichia coli	0	0	0	3	8
	Klebsiella pneumonia	0	0	2	3	8
	Staphylococcus aureus	0	0	4	7	15
3c	*Pseudomonas aeruginosa*	0	0	0	0	0
	Escherichia coli	0	0	0	0	0
	Klebsiella pneumonia	0	0	0	0	0
4a	*Pseudomonas aeruginosa*	0	0	3	3	15
	Escherichia coli	0	0	0	0	30
	Klebsiella pneumonia	0	0	0	0	20
	Staphylococcus aureus	5	7	7	8	10
4b	*Pseudomonas aeruginosa*	0	0	0	0	15
	Escherichia coli	0	3	6	7	10
	Klebsiella pneumonia	0	0	3	8	20
	Staphylococcus aureus	0	0	0	0	3
4c	*Pseudomonas aeruginosa*	0	3	3	7	18
	Escherichia coli	8	8	10	10	10
	Klebsiella pneumonia	0	2	3	6	11
	Staphylococcus aureus	0	0	1	3	3
4d	*Pseudomonas aeruginosa*	0	9	13	15	27
	Escherichia coli	0	0	0	0	5
	Klebsiella pneumonia	8	15	20	22	31
	Staphylococcus aureus	0	4	12	13	22
4e	*Pseudomonas aeruginosa*	0	0	0	10	12
	Escherichia coli	0	0	0	0	9
	Klebsiella pneumonia	0	0	4	8	10
	Staphylococcus aureus	0	0	0	0	0
4f	*Pseudomonas aeruginosa*	0	0	0	0	10
	Escherichia coli	0	0	0	0	13
	Klebsiella pneumonia	0	0	0	8	9
	Staphylococcus aureus	0	0	0	0	15

coli, K. pneumonia, S. aureus and P. aeruginosa strains, respectively, Table 4. These non-significant correlation results may be explained by the literature results[22] which revealed that lipophilicity of the molecule and hence penetration into bacterial cell is not the only factor which can affect its activity. Indeed, other factors like the affinity of the compounds for their target DNA Gyrase and Topoisomerase IV can affect their MIC.[23] Furthermore, the heterogeneous nature and the difference in the steric effect of the tested *N*-4-piperazinyl substituent may play an important role in the accommodation of these molecules on the active site of the enzyme.[23] The weaker correlation between log MIC and log *D* may be due to the zwitterionic nature of fluoroquinolone analogues at the physiological pH. Hence, protonation on the *N*-4 piperazinyl can influence their lipophilicity. It is reported that the electronic factors can play a pronounced effect on the protonation of *N*-4 piperazinyl group.[23] Consequently, this may affect the state of the molecule to be either in the neutral or zwitterionic form (Scheme 3). Furthermore, lipophilicity and so permeability into bacterial cells is a function of the electronic effects. According to these findings, it is important to calculate the residual charge on the piperazinyl N-4 of the synthesized compounds and norfloxacin. The calculated charges for the tested compounds are listed in Table 3. The parabolic curve-fittings between the charge density and log *D* or log *P* are described. A weak correlation between log *D* or log *P* and charge density (r^2 = 0.28 and 0.34, respectively) was found. These results revealed that the N-4 charge density is not the only factor determining the N-4 protonation and hence partition coefficient. Other factors, like steric effect could possibly affect N-4 protonation. These findings are consistent with a previous study about the hydrophobicity of quinolones that revealed no good correlation between MIC with hydrophobicity[24] Similarly, another study revealed weak correlation between MIC and cell accumulation.[15,25]

2.5. Correlation between molecular mass and log MIC

It is reported[15,26] that the molecular mass and bulkiness of the substituent at C-7 position hinder penetration of quinolones into Gram-negative organisms through the porine channel, although hydrophobic molecules appear to enter via the lipopolysaccharide or across the lipid bilayer. Conversely, Gram-positive bacteria do

Table 2
Minimum inhibitory concentration (*MIC*) of the tested active compounds and the reference norfloxacin

Compd	Minimum inhibitory concentration (μg/mL)			
	P. aeruginosa	*E. coli*	*K. pneumonia*	*S. aureus*
Norfl.	9	14	8	64
2	6	11	128	<1
3a	>512	4	10	128
3b	>512	64	13	11
3c	>512	>512	>512	64
4a	16	128	128	<1
4b	128	4	14	128
4c	9	<1	8	10
4d	5	<1	128	4
4e	64	128	9	>512
4f	128	128	64	128

not possess an outer membrane, and so lack outer membrane proteins and lipopolysaccharide. Hence accumulation of Gram-positive bacteria is thought to take place by simple diffusion across the cytoplasmic membranes and not affected by increasing the bulkiness of the N-7 substituents.[27] Parabolic curve-fitting between molecular mass and log MIC for the tested compounds (Table 4) resulted in a weak correlation (r^2 = 0.19, 0.53 and 0.62) for the Gram-negative organisms *K. pneumonia, P. aeruginosa* and *E. coli*, respectively. Conversely, this correlation is stronger in case of the Gram-positive *S. aureus* (r^2 = 0.67) Table 4. These results are consistent with a previous QSAR study about quinolones showing that absorption is increased by higher lipophilicity up to a limit due to steric hindrance.[16]

3. Conclusion

A group of *N*-Mannich bases, as prodrugs of norfloxacin in addition to their related N-4-substituents; *N*-formyl, bis(2-hydroxyethyl)piperazin-1-ium bromide, *N*-triazinyl and *N*-cyclopentyl derivatives were prepared and identified by spectroscopic and elemental methods. Some of the prepared compounds exhibited better inhibitory activities than the reference antibiotic norfloxacin against the tested microorganisms. The *N*-formyl **2** and the Mannich base **4d** showed better activity than norfloxacin against *P. aeurginosa*. The Mannich bases **4c** and **4d** exhibited a superior activity against *E. coli* (MICs < 1 μg/mL), while the *N*-formyl **2** and the Mannich base **4a** exhibited a superior activity against *S. aureus* (MICs < 1 μg/mL). In addition, the Mannich base **4c** exhibited a pronounced broad spectrum activity against all the tested Gram-positive and Gram-negative microorganisms. Results revealed that there is no significant correlation between log MIC and log *P*, log *D* and molecular mass. Hence, assuming the similarity in affinity of these molecules to enzymes, there is no single physicochemical parameter that can definitely be identified as an affluent on the biological effect.

4. Experimental

4.1. Chemistry

All the chemicals and solvents used were purchased from commercial sources and were of the highest pure form. Melting points (uncorrected) were determined on a Stuart electrothermal melting point apparatus. ^{1}H NMR spectra were run on GEMINI-200 NMR spectrometer (200 MHz) and on MERCURY-300BB NMR spectrometer (300 MHz) and also on Varian Em-360L NMR spectrometer (60 MHz) using tetramethylsilan (TMS) as internal standard. Chemical shifts are expressed in δ ppm, the coupling constants *J* are expressed in hertz. Mass spectra were performed on JEOL JMS600 or HP mass spectrometers. Elemental microanalyses were performed on a Perkin Elmer 2400 CHN Elemental analyzer or on a Hereaus Vario EL apparatus. The reactions follow up and checking the purity of the compounds were made by TLC (Kieselgel 60 F254 precoated plates, E. Merck, Dermastadt, Germany), the spots were visualized by exposure to UV lamp at λ_{max} 254 nm. Yields are of purified product and were not optimized. Norfloxacin was purchased from the (Medical Union Pharmaceuticals, Abu-sultan, Ismailia, Egypt). 3-Chloro-5,6-diphenyl-1,2,4-triazine was prepared as reported.[28] 3-Methyl-1*H*-pyrazol-5(4*H*)-one was prepared as reported.[29]

4.1.1. 1-Ethyl-6-fluoro-7-(4-formylpiperazin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (2)

Norfloxacin (3.19 g, 0.01 mol) was heated with formamide (50 mL) over a steam bath for 2 h. While standing in the cold overnight, needle crystals were formed, which were filtered off. Recrystallization with methanol afforded the pure product **2** as pale yellow crystals, mp 292–293 °C in 73% yield; IR (KBr) cm^{-1}: 3435, 3035, 2925, 1691, 1665, 1608, 1250, 1190, 802, 745; ^{1}H NMR spectrum (DMSO-d_6) 400 MHz 1.39 (t, 3H, *J* = 7 Hz, –CH_2CH_3); 3.20–3.60 (m, 8H, piperazine 8H); 4.53 (q, 2H, *J* = 7 Hz, –CH_2CH_3); 7.14 (d, 1H, J_{H-F} = 6.9 Hz, H-8); 7.80 (d, 1H, J_{H-F} = 12.9 Hz, H-5); 8.10 (s, 1H, –CHO); 8.88 (s, 1H, H-2). FAB-MS *m*/*z* MH$^+$ 348.19 (4.00%). Anal. Calcd for $C_{17}H_{18}FN_3O_4$ (347.13): Calcd: C, 58.78; H, 5.22; N, 12.10. Found: C, 58.59; H, 5.21; N, 12.14.

4.1.2. 4-(3-Carboxy-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolin-7-yl)-1,1-bis(2- hydroxyethyl)piperazin-1-ium bromide (3a)

A mixture of norfloxacin (0.79 g, 0.0025 mol) and bromoethanol (1.24 g, 0.01 mol) in acetonitril (50 mL) was heated at reflux overnight. The precipitated solid was filtered off, washed with hot

Table 3
Log *p*, log *D*, molecular mass, N-4 charge and log MIC for compounds **1**, **2**, **3a–c** and **4a–f**

No.	Correlation parameters				Log MIC			
	Log *P*	Log *D*	Mol. mass	N-4 Charge	*P. aeruginosa*	*E. Coli*	*K. pneumonia*	*S. aureus*
1	1.49	−0.41	319.13	−0.209	0.95	1.45	0.00	1.81
2	0.55	0.087	347.13	0.417	0.78	1.04	2.11	0.00
3a	1.90	0.158	487.11	0.739	2.71	0.60	1.00	2.11
3b	3.99	ND	387.20	−0.143	2.71	1.81	1.11	1.04
3c	3.11	−0.072	550.21	0.188	2.71	2.71	2.71	1.81
4a	2.31	−0.043	418.20	−0.163	1.20	2.11	2.11	0.00
4b	3.74	−0.043	449.19	−0.127	2.11	0.60	1.15	2.11
4c	1.16	−0.85	429.18	−0.152	0.95	0.00	0.90	1.00
4d	0.43	−0.80	430.17	−0.149	0.70	0.00	2.11	0.60
4e	2.60	ND	478.17	−0.149	1.81	2.11	0.95	2.71
4f	5.09	−0.90	528.18	−0.145	2.11	2.11	1.81	2.11

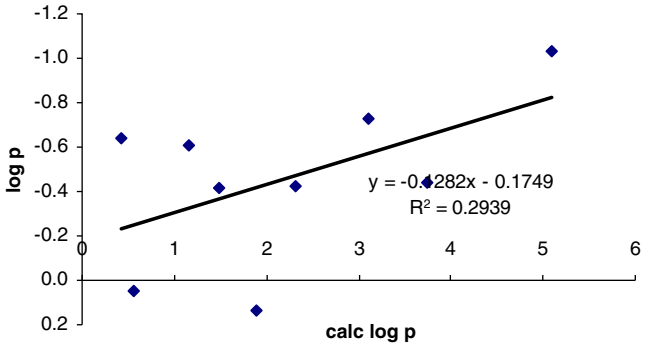


Figure 1. Straight-line correlation between calculated log *P* and log *D*.

methanol and dried to afford 0.97 g of brownish yellow crystals, mp > 300 °C, % yield = 79.88; IR (KBr) cm^{-1}: 3385, 3000, 2925, 1690, 1615, 1263, 1194, 797, 739; ^1H NMR spectrum (DMSO-d_6) 200 MHz 1.42 (t, 3H, J = 7 Hz, –CH_2CH_3); 3.0–4.1 (m, 16H, piperazine 8H and –N–CH_2CH_2OH 8H); 4.60 (q, 2H, J = 7 Hz, –CH_2CH_3); 5.31 (br s, 2H, O*H*); 7.24 (d, 1H, J_{H-F} = 7.2 Hz, -H-8); 7.89 (d, 1H, J_{H-F} = 14.2 Hz, -H-5); 8.92 (s, 2H, H-2), 15.23 (br s, 1H, -COO*H*). EI-MS *m*/*z*: M−HBr 406.00 (3.00%), 364.30 (78%), 320.20 (100%). Anal. Calcd for $C_{20}H_{27}BrFN_3O_5$ (487.11): Calcd: C, 49.19; H, 5.57; N, 8.60. Found: C, 49.41; H, 5.41; N; 9.46.

4.1.3. 7-(4-Cyclopentylpiperazin-1-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (3b)

A mixture of norfloxacin (0.5 g, 1.57 mmol), sodium bicarbonate (0.13 g, 1.65 mmol), potassium iodide (0.05 g, 0.5 mmol) and cyclopentyl bromide (0.278 g, 1.86 mmol) in 40 mL DMF was stirred at room temperature for 10 h. The reaction mixture was poured into 20 mL water, extracted with chloroform (2 × 75) mL. The chloroform extract was collected, dried over anhydrous sodium sulfate. The dried solution was evaporated to dryness, crystallized from ethanol/chloroform affording 0.42 g white powder, mp: 260–262 °C, 70% yield; IR (KBr) cm^{-1}: 3445, 3025, 2845, 1724, 1610, 1247, 1208, 798, 746; ^1H NMR 200 MHz (DMSO-d_6): δ 1.34 (t, 3H, J = 7 Hz, –CH_2CH_3); 2.42–2.66 (m, 8H, -cyclopentyl 8H); 3.1–3.27 (m, 8H, piperazine 8H); 4.05 (m, cyclopentyl H-1); 4.47 (q, 2H, J = 7.4 Hz, N–CH_2CH_3); 7.05 (d, 1H, J_{H-F} = 6.6 Hz, H-8); 7.80 (d, 1H, J_{H-F} = 12.0 Hz, H-5); 8.85 (s, 1H, H-2); 15.25 (s, 1H, COO*H*). EI-MS *m*/*z* $M^{\cdot+}$ 387.00 (28.30%). Anal. Calcd for $C_{21}H_{26}FN_3O_3 \cdot 0.25H_2O$ (387.20): C, 64.35; H, 6.81; N, 10.72. Found: C, 64.37; H, 6.66; N, 10.57.

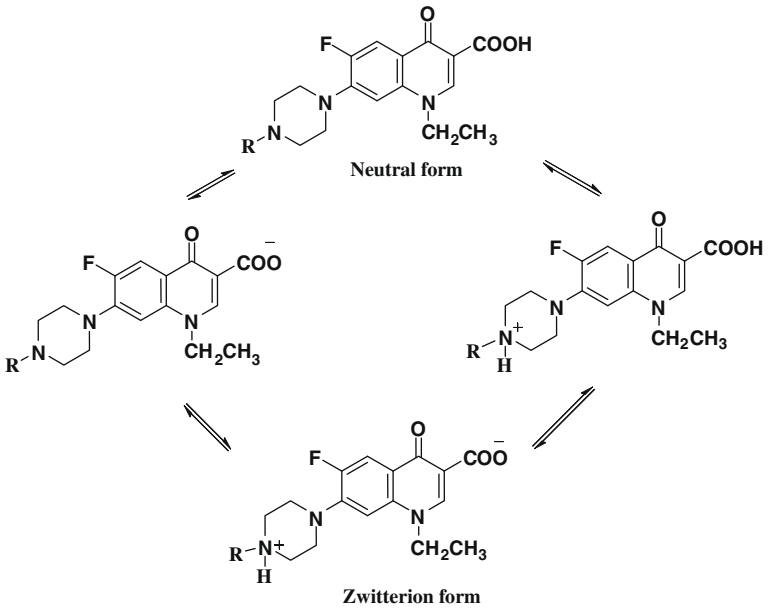
4.1.4. 1-Ethyl-6-fluoro-1,4-dihydro-7-(4-(5,6-diphenyl-1,2,4-triazin-3-yl)piperazin-1-yl)-4-oxoquinoline-3-carboxylic acid (3c)

A mixture of norfloxacin (3.19 g, 0.01 mol), 3-chloro-5,6-diphenyl-1,2,4-triazine (2.5 g, 0.01 mol) and TEA (1.2 g, 0.02 mol) in acetonitril (50 mL) was heated at reflux overnight. The reaction mixture was evaporated to dryness. To the residue was added 30 mL water, the precipitated solid was filtered off, washed with ethanol. Crystallization from methanol/chloroform afforded 3.0 g of yellow powder, mp > 300 °C; 54.55% yield; IR (KBr) cm^{-1}: 3405, 3030, 2905, 1720, 1611, 1240, 1194, 796, 744; ^1H NMR 200 MHz ($CDCl_3$): δ 1.63 (t, 3H, J = 7 Hz, –CH_2CH_3); 3.51 (br s, 4H, piperazine 4H); 4.20–4.40 (m, 6H, piperazine 4H + N–CH_2CH_3); 6.93 (d, 1H, J_{H-F} = 6.6 Hz, -H-8); 7.29–7.57 (m, 10H, Aromatic 10H); 8.10 (d, 1H, J_{H-F} = 12.8 Hz, H-5); 8.70 (s, 1H, H-2); 15.09 (br s, 1H, –COO*H*). FAB-MS *m*/*z* MH^+ 551.02 (4.30%) Anal. Calcd for $C_{31}H_{27}FN_6O_3 \cdot 0.5H_2O$ (550.21): C, 66.54; H, 5.04; N, 15.02. Found: C, 66.55; H, 5.23; N, 15.08.

4.1.5. General procedure for preparation of the Mannich bases (4a–g)

To an equimolar mixture of norfloxacin and the respective amine or imide in ethanol, 1 mL of formaline (37%) was added. The reaction mixture was heated at reflux overnight, cooled. The precipitated solid was filtered off under vacuum. Crystallization from the respective solvent afforded the Mannich bases **4a–f** in 40–76% yields, respectively.

4.1.5.1. 1-Ethyl-6-fluoro-1,4-dihydro-7-(4-(morpholinomethyl)-piperazin-1-yl)-4-oxoquinoline-3-carboxylic acid (4a). Compound **4a** was prepared using the above general procedure in 63% yield, crystallization solvent: DMF; mp = 287–288 °C; IR (KBr) cm^{-1}: 3410, 3075, 2910, 1712, 1615, 1251, 1194, 818, 744; ^1H NMR 300 MHz (DMSO-d_6) δ 1.42 (t, 3H, J = 6.9 Hz, –CH_2CH_3); 3.13–3.53 (m, 16H, piperazine 8H and morpholine 8H); 4.63(q, 2H, J = 6.9 Hz,



Scheme 3. The possible protonation states of norfloxacin derivatives.

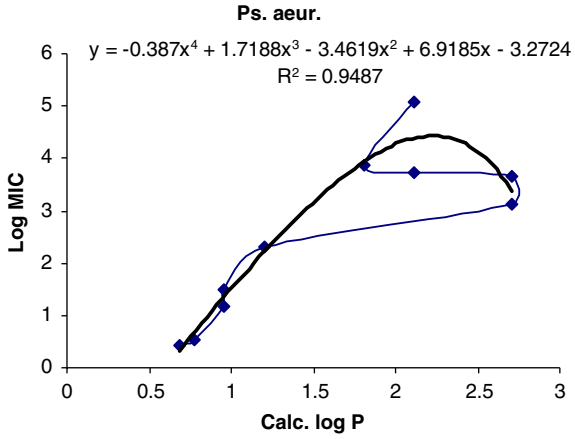


Figure 2. Correlation between calc. log *P* and log MIC.

Table 4
Correlation coefficient (r^2) between log MIC of the tested strains and log *P*, log *D*, molecular mass and N-4-charge

Microorganism	Correlation parameters			
	Log *P*	Log *D*	Molecular mass	N-4-Charge
P. aeurginosa	0.948	0.246	0.529	0.580
E. coli	0.67	0.539	0.626	0.155
K. pneumonia	0.842	0.42	0.197	0.582
S. aureus	0.616	0.378	0.677	0.249

N–C*H*$_2$CH$_3$); 5.31 (br s, 2H, –N–C*H*$_2$N–); 7.25 (d, 1H, J_{H-F} = 7.5 Hz, H-8); 7.94 (d, 1H, J_{H-F} = 13.2 Hz, H-5); 8.95 (s, 1H, H-2). 15.23 (br s, 1H, COO*H*). Anal. Calcd for $C_{21}H_{27}FN_5O_4$ (418.20): C, 60.27; H, 6.50; N, 13.39. Found: C, 60.20; H, 6.09; N, 13.48.

4.1.5.2. 7-{4-[(1*H*-Benzo[*d*]imidazol-1-yl)methyl]piperazin-1-yl}-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4b). Compound **4b** was prepared using the above general procedure in 76% yield; crystallization solvent: methanol/chloroform; mp = 262–264 °C (dec.); IR (KBr) cm^{-1}: 3395, 3030, 2880, 1725, 1609, 1249, 1197, 796, 744; ^{1}H NMR 200 MHz ($CDCl_3$) δ 1.58 (t, 3H, *J* = 7.0 Hz, –CH$_2$C*H*$_3$); 2.90 (m, 4H, piperazine H); 3.34 (m, 4H, piperazine H) 4.34 (q, 2H, *J* = 7.0 Hz, –N–C*H*$_2$CH$_3$); 4.74 (s, 2H, –N–C*H*$_2$N–); 6.83 (d, 1H, J_{H-F} = 6.6 Hz, H-8); 7.57–8.00 (m, 6H, benzimidazol 5H and H-5); 8.65 (s, 1H, H-2), 15.10 (br s, 1H, COO*H*). Anal. Calcd for $C_{24}H_{24}FN_5O_3$ (449.19): C, 64.13; H, 5.38; N, 15.58. Found: C, 64.22; H, 5.22; N, 15.88.

4.1.5.3. 1-Ethyl-6-fluoro-1,4-dihydro-7-{4-[(4,5-dihydro-3-methy-5-oxopyrazol-1-yl)methyl]piperazin-1-yl}-4-oxoquinoline-3-carboxylic acid (4c). Compound **4c** was prepared using the above general procedure in 59% yield; crystallization solvent: methanol/chloroform; mp = 255–257 (dec.); IR (KBr) cm^{-1}: 3445, 3030, 2995, 1785, 1714, 1610, 1250, 1207, 800, 757; ^{1}H NMR spectrum ($CDCl_3$) 300 MHz, δ 1.59 (t, 3H, *J* = 7.2 Hz, –CH$_2$C*H*$_3$); 2.80 (s, 3H, –CH$_3$ of the pyrazolone); 2.92 (m, 4H, piperazine H); 3.35 (m, 4H, piperazine H) 3.43 (m, 0.8H, –CH$_2$ of the pyrazolone); 4.2 (s, 1.2H, =CH of the pyrazolone); 4.34 (q, 2H, *J* = 7.2 Hz, –N–C*H*$_2$CH$_3$); 4.37 (s, 1.2H, –N–C*H*$_2$N–); 4.70 (0.8H, –N–C*H*$_2$N–); 7.18 (d, 1H, J_{H-F} = 7.0 Hz, -H-8); 7.88 (d, 1H, J_{H-F} = 13.6 Hz, -H-5); 8.94 (s, 1H, H-2). 15.37 (br s, 1H, –COO*H*). FAB-MS *m*/*z* MH$^+$+Na 454.30 (2.60%) Anal. Calcd for $C_{21}H_{24}FN_5O_4$ (429.18): C, 58.73; H, 5.63; N, 16.31. Found: C, 58.70; H, 5.68; N, 16.32.

4.1.5.4. 7-{4-[(2,5-Dioxopyrrolidin-1-yl)methyl]piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (4d). Compound **4d** was prepared using the above general procedure in 49.5% yield; crystallization solvent: methanol/chloroform; mp = 266–267 °C (dec.); IR (KBr) cm^{-1}: 3430, 3025, 2840, 1756, 1689, 1616, 1251, 1206, 797, 742; ^{1}H NMR spectrum ($CDCl_3$) 200 MHz, 1.60 (t, 3H, *J* = 6.9 Hz, –N–CH$_2$C*H*$_3$); 2.85 (s, 4H, –pyrrolidin H); 2.9 (4H, piperazine H); 3.35 (m, 4H, – piperazine H); 4.35 (q, 2H, *J* = 7.20 Hz, N–C*H*$_2$CH$_3$); 4.65 (s, 2H, –N–C*H*$_2$N–); 6.80 (d, 1H, J_{H-F} = 7.20 Hz, -H-8); 8.05 (d, 1H, J_{H-F} = 13.8 Hz, H-5); 8.65 (s, 1H, H-2); 15.10 (br s, 1H, –COO*H*). FAB-MS *m*/*z* MH$^+$ 431.25 (3.00%) Anal. Calcd for $C_{21}H_{23}FN_4O_5$ (430.17): C, 58.60; H, 5.39; N, 13.02. Found: C, 58.60; H, 5.40; N, 12.98.

4.1.5.5. 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-{4-[(1,3-dioxoisoindolin-2-yl)methyl]piperazin-1-yl}quinoline-3-carboxylic acid (4e). Compound **4e** was prepared as white powder using the above general procedure in 52.70% yield; crystallization solvent: methanol/chloroform; mp = 256–257 °C (dec.); IR (KBr) cm^{-1}: 3450, 3030, 2881, 1761, 1702, 1611, 1245, 1203, 797, 732; ^{1}H NMR spectrum ($CDCl_3$) 200 MHz, 1.57 (t, 3H, *J* = 6.9 Hz, –CH$_2$C*H*$_3$); 2.93 (br s, 4H, -piperazine H); 3.35 (br s, 4H, -piperazine H); 4.31 (q, 2H, *J* = 7.2 Hz, N–C*H*$_2$CH$_3$); 4.71 (s, 2H, –N–C*H*$_2$N–); 6.81(d, 1H, J_{H-F} = 6.6 Hz, -H-8); 7.75–7.9 (m, 4H, benzene H); 7.97 (d, 1H, J_{H-F} = 12.9 Hz, H-5); 8.63 (s, 1H, H-2). EI-MS *m*/*z* M−H$^+$ 477.00 (1.40%). Anal. Calcd for $C_{25}H_{23}FN_4O_5$ (478.17): C, 62.76; H, 4.85; N, 11.71. Found: C, 62.34; H, 4.84; N, 11.70.

4.1.5.6. Preparation of 7-[4-(1,3-dioxo-2,3-dihydro-1H-2-phenalenyl) piperazino]-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (4f). Compound **4f** was prepared as white powder using the above general procedure in 40% yield; crystallization solvent: DMF; mp = 287–288 °C (dec.); IR (KBr) cm^{-1}: 3420, 3030, 2819, 1797, 1688, 1616, 1240, 1194, 796, 770, 691; ^{1}H NMR spectrum (DMSO-d_6) 60 MHz, 1.60 (t, 3H, *J* = Hz, –CH$_2$C*H*$_3$); 2.9–4.0 (m, 8H, -piperazine H); 4.9 (q, 2H, *J* = Hz, N–C*H*$_2$CH$_3$); 5.3 (s, 2H, –N–C*H*$_2$N–); 8.3–9.5 (m, 8H, 6H of benzene; H-5- and H-2 of the fluoroquinolone ring); 13.2 (br s, 1H, –COO*H*, exchangeable with D_2O). EI-MS *m*/*z* M−H$^+$ 527.00 (2.60%). Anal. Calcd for $C_{29}H_{25}FN_4O_5$ (528.18): C, 65.90; H, 4.77; N, 10.60. Found: C, 65.65; H, 4.77; N, 11.00.

4.2. Antimicrobial screening[30,31]

Compounds **2, 3a–c** and **4a–f** were screened for their in vitro antibacterial activity against *P. aeruginosa*, *E. coli*, *K. pneumonia* (as Gram-negative bacteria) *and S. aureus* (as Gram-positive organism).

Bacterial strains were supplied from department of Microbiology, Faculty of Pharmacy, El-Minia University. Suspension of each microorganism was prepared to 10^6 colony forming units (CFU/mL). The tested compounds, as well as norfloxacin (Sigma, USA), were dissolved in DMF to an initial concentration of 10 mg/mL and dilutions of the test compounds were prepared at concentrations 512, 128, 64, 16, 4 μg/mL.

4.2.1. Preparation of the inoculated media[32]

All strains were cultured on Muller-Hinton agar medium which was supplied from Oxoid Chemical Co. UK, and prepared according to the instructions of the manufacturers. The media were molten on a water bath, inoculated with 0.5 mL of the culture of the specific microorganism and poured into sterile Petri dishes to form a layer of about 3–4 mm thickness. The layer was allowed to cool and harden. With the aid of cork-porer, cups of about 10 mm diameter were done.

4.2.2. Agar diffusion technique[31]

Different concentrations of the tested compounds in DMF were placed separately in cups in the agar medium. All plates were

incubated at 37 °C overnight. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined to be the intercept of the grave of logarithm concentrations versus diameter of the inhibition zones.

4.3. Determination of partition coefficient[23,33]

The experimental partition coefficient (D) between n-octanol and phosphate buffer was determined by a slight modification of the method earlier described by Vazquez et al.[23] Briefly, 100 μL of a stock solution (0.2 mg/mL) was diluted with 1.9 mL of appropriate phosphate buffer solution (pH 7.4) and mixed with 2 mL of octanol (the organic and aqueous phase was mutually saturated), the vials were protected from light by wrapping in aluminum foil. The two phases were vortexed for 3 min and agitated for 5 h in a shaking water bath at 25 + 0.1 °C. After equilibration, the octanol phase was removed with a Pasteur pipette and both phases were assayed spectrophotometrically (λ = 270–280 nm) to determine drug concentration. The partition coefficient was calculated as the ratio between molar concentration in n-octanol and aqueous phase. The total concentration in both phases was measured by spectrophotometry and the experimental partition coefficients (D) were determined from:

$$D = C_i - C_w/C_w \cdot V_w/V_o$$

where C_i and C_w represent the solute concentration in the aqueous phase before and after distribution, respectively; V_w represents the volume of the aqueous and V_o the volume of the organic phase. All partition coefficient determinations were made in triplicate. Spectronic Genesys, connected to an IBM computer loaded with the Winspec application software (Milton Roy, USA) and Jenway 6505 (Jenway LTD., UK) ultraviolet–visible spectrophotometers with matched 1 cm quartz cells were used throughout this study for all measurements. The calcd log P was calculated by ACD/Labs (TM) Software for MS Windows (TM). Copyright (C) 1993–1997 Advanced Chemistry Development, Inc. The calculation of charge density was carried out using CHEM 3D pro 10 program.

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