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# New $N$-4 piperazinyl derivatives of norfloxacin: design, synthesis, and correlation of calculated physicochemical parameters with antibacterial activity

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**Abstract:** A group of $N-4$ piperazinyl derivatives of norfloxacin was synthesized and identified by different spectroscopic techniques. The $N-4$ piperazinyl substituent in target compounds **2a**–**2k, 3a**–**3c**, and **4a** and **4b** was designed to have different electronic, steric, and physicochemical properties. The antibacterial activity of the newly synthesized compounds was evaluated against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* strains using norfloxacin as a reference. Results showed that most of the tested compounds had higher activity against *E. coli* and *K. pneumoniae* than norfloxacin, whereas only five derivatives were more active against *P. aeruginosa*. On the other hand, all derivatives were less active than norfloxacin against *S. aureus*. The biological activity of the target compounds, expressed in log MIC, is correlated with lipophilicity, polarizability, and topology parameters. Results showed that none of the calculated parameters could determine the biological activity. Consequently, the total volume of the molecule, bulkiness at C-7, electronic factors, and lipophilicity are important factors that should be considered in the design of new fluoroquinolones.

**Key words:** Norfloxacin, fluoroquinolones, antibacterial, quantitative structure activity relationship, lipophilicity, polarizability, thermodynamic parameters

## 1. Introduction

Fluoroquinolones represent an important class of synthetic antibiotics during the last decades.[1] They possess a broad antibacterial effect against a wide variety of gram-positive and gram-negative microorganisms.[2] Fluoroquinolones act by targeting two isozymes: DNA-gyrase and topoisomerase IV.[3] The two enzymes, bound to fluoroquinolones, are transformed into toxic enzymes that target bacterial chromosomes. The 1,4-dihydro-3-carboxylic-4-one skeleton is considered an essential pharmacophore for binding fluoroquinolones with DNA-gyrase. Meanwhile, it is believed that the 6-fluoro and 7-piperazinyl groups are responsible for the broad-spectrum and anti-*Pseudomonas* activities of fluoroquinolones.[2] The physicochemical parameters of fluoroquinolones are believed to be essential for the cell permeability of these antibiotics and, consequently, their antibacterial activity.[4] Chemical modifications at C-7 of the fluoroquinolone skeleton enable the control of pharmacokinetic properties and thus might cause a change in the cell permeability, potency, and spectrum of

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activity.[2] Although fluoroquinolones are broad-spectrum antibiotics, bacterial resistance to them has made the search for newer antibiotics a continuous challenge.

Moreover, a recent quantitative structure activity relationship (QSAR) study used quantum chemistry and chemometric methods to prove the importance of molecular properties for the anti-P. aeruginosa activity for selected fluoroquinolones.[5] In addition, QSAR studies showed a linear correlation between the antibacterial activity of benzene sulfonamide fluoroquinolone derivatives with electronic parameters along with steric parameters. On the other hand, hydrophobic properties showed a minor role in the activity.[6] Additionally, a moderate correlation was found among 15 developed quinolones between the minimum inhibitory concentrations (MICs) and hydrophobicity (r = 0.61). On the other hand, analysis of QSAR among 40 fluoroquinolones revealed that the MIC increment ratio was significantly correlated to the bulkiness of the C-7 substituent.[7] The thermodynamic parameters for the association of some quinolones with double-strand and single-strand DNA showed that the change in Gibbs free energy is negative, indicating that the complex formation is spontaneous. This observation suggests that the association of the quinolone with double-strand as well as single-strand DNA is an energetically favorable (exothermic) process, whereas the entropy change is unfavorable. The effect of the substituents at the quinolone ring is less prominent in the single-strand DNA case compared to double-strand DNA due to the steric effect.[8] Meanwhile, another study showed the importance of electronic and acid-base properties of quinolones for biological activity. Deprotonation of the piperazinyl–NH group greatly affects the charge distribution in the studied compounds.[9]

Collectively, optimizing the overall molecular configurations enhances the number of intracellular targets for antimicrobial action and impedes the efficiency of efflux proteins that diminish intracellular penetration.[10]

Based on the above findings and in continuation of our research to explore the effect of N-4 piperazinyl substitution on antibacterial activity,[11] a number of different N-4 piperazinyl substituents are designed. The target substituents have variable physicochemical, volume, and electronic properties. Moreover, this research aims at investigating the correlation between biological activity expressed in log MIC with different lipophilicity, topological, electronic, and thermal parameters theoretically calculated.

## 2. Results and discussion

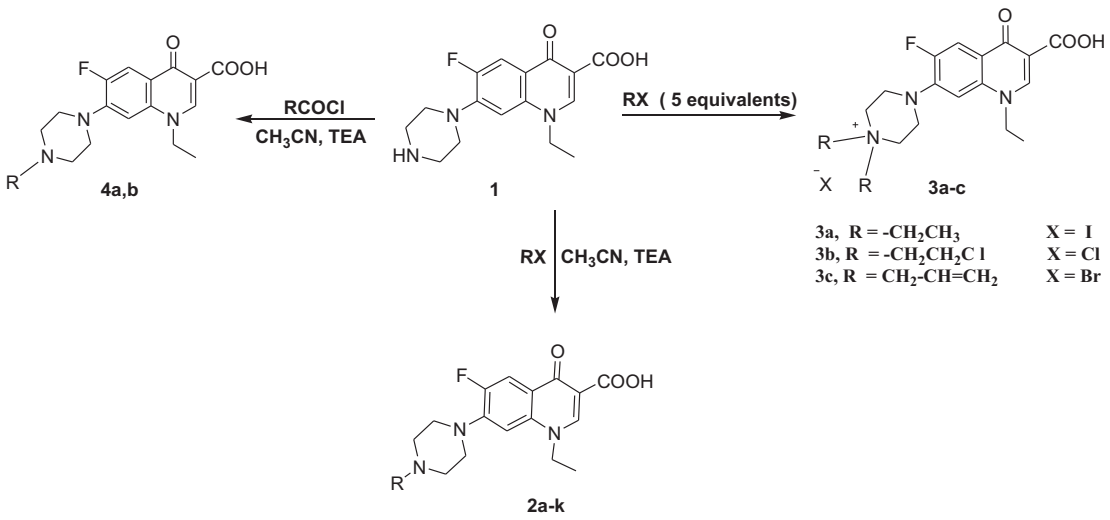
### 2.1. Synthesis of norfloxacin analogs

Synthesis of the target compounds is outlined in the Scheme. Alkylated norfloxacin derivatives **2a**–**2k** were prepared by refluxing equimolar ratios of norfloxacin **1** and the respective alkyl halide in acetonitrile using triethylamine as a base (Scheme). Acylated intermediates of PABA (p-aminobenzoic acid, Figure A), adamantine-1-amine (Figure 1B), 4-*p*-tolylthiazol-2-amine (Figure C), and 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine (Figure D) were prepared through the reaction of the respective amine with bromoacetyl bromide or chloroacetyl chloride in dichloromethane using potassium carbonate as a base.

Quaternary ammonium derivatives of norfloxacin **3a**–**3c** were prepared using a procedure reported for a similar reaction[12] by heating norfloxacin **1** in acetonitrile with five equivalents of the respective alkyl halide. $^1$H NMR spectra of compounds **3a**–**3c** showed a downfield shift of the piperazine protons to $\delta$ 3.0–4.0 ppm.

Similarly, acylated norfloxacin derivatives **4a** and **4b** were synthesized by heating equimolar ratios of **1** with the respective acyl halide in acetonitrile using triethylamine as a base.

All the synthesized compounds were confirmed using NMR spectroscopy; the definite pattern of norfloxacin (two doublets and a singlet at $\delta$ 7.2–8.9 ppm) supported the success of the coupling processes at the




**Scheme.** Synthesis of the target compounds **2a**–**2k**, **3a**–**3c**, and **4a** and **4b**.

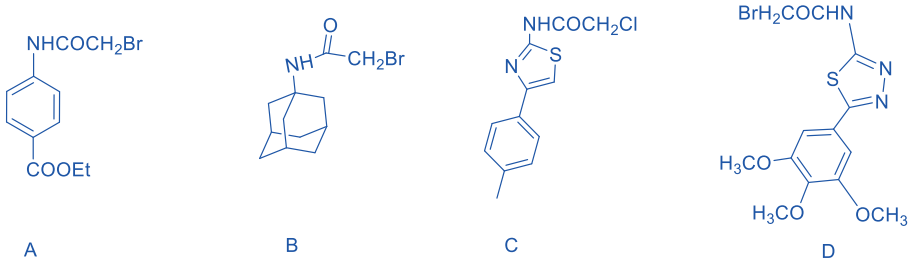



**Figure.** Structure of acylated intermediates used in the synthesis of the designed compounds. A- Acylated derivative of PABA (p-aminobenzoic acid); B- adamantine-1-amine; **C-** 4-*p*-tolylthiazol-2-amine; **D-** 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine.

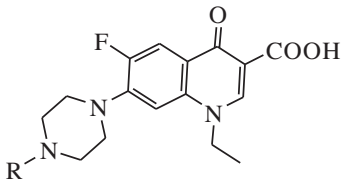
norfloxacin nucleus. Aliphatic protons appeared as expected. For example, compound **2d** showed characteristic allylic protons at $\delta$ 5.13, 5.19, and 5.8 ppm. A singlet at $\delta$ 3.7 ppm accounting for the spacer $CH_2$ at **2j** and the pattern at $\delta$ 1.6–2.9 ppm of the adamantane motif for compound **2f** also confirm the success of the coupling processes, and so on. Elemental analyses also confirmed the formation of the designed compounds.

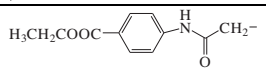
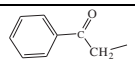
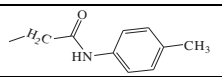
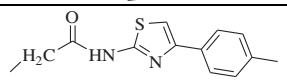
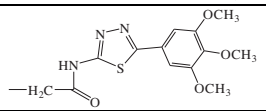
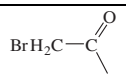
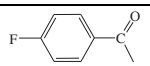
### 2.2. Antibacterial activity

Antibacterial activity of the prepared compounds **2a**–**2k, 3a**–**3c**, and **4a** and **4b** in addition to the parent norfloxacin was determined against four standard strains of *S. aureus* (ATCC 6538), *K. pneumoniae* (ATCC 10031), *P. aeruginosa* (ATCC 10145), and *E. coli* (ATCC 8739). Results are expressed as minimum concentration required to inhibit bacterial growth (MIC, $\mu$g/mL) and are listed in Table 1. Results were judged according to Clinical and Laboratory Standards Institute (CLSI) guidelines as follows; if the MIC of the tested compound is $\leq 4\mu$g/mL, the microorganism is considered sensitive; at $\geq$16 the microorganism is resistant. If the MIC is around 8 $\mu$g/mL, the compound is regarded as intermediately active (Table 2).

Results showed that compounds **2a**, **2h**, **2j**, and **4b** had comparable or higher activity than the reference norfloxacin. They had MICs of 1.10–4.70 $\mu$g/mL against gram-positive bacteria while compounds **2a**, **2b**, **2d**, **2g**, **2k**, **3a**, and **4a** showed the best activity against *E. coli* (MIC values were 1.1–2.52 $\mu$g/mL vs. 1.85–1.95

**Table 1**. Different substitutions and minimum inhibitory concentrations (MICs) ($\mu$g/mL) of the tested compounds **2a**–**2k**, **3a**–**3c**, **4a**, **4b**, and norfloxacin **1** against *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.



| Comp. No. | R | Minimum inhibitory concentration (MIC, $\mu$g/mL) | | | |
|---|---|---|---|---|---|
| | | Staph. aureus ATCC 6538* | E. coli ATCC 8739 | Klebsiella pneumonia ATCC 10031 | Ps. aeruginosa ATCC 10145 |
| 1 | H | 1.85 | 1.95 | 2.48 | 27.69 |
| 2a | Ethyl | 1.40 | 1.10 | 3.437 | 6.17 |
| 2b | Isopropyl | 13.19 | 1.41 | 3.61 | 5.30 |
| 2c | $-CH_2COOH$ | 120.22 | 27.63 | 5.24 | 349.86 |
| 2d | $-CH_2-CH=CH_2$ | 149.06 | 1.40 | 2.33 | 193.13 |
| 2e |  | 208.70 | 4.10 | 30.57 | 52.80 |
| 2f |  | 304.40 | 157.8 | 115.44 | 233.32 |
| 2g |  | 20.27 | 1.84 | 1.90 | 15.50 |
| 2h |  | 4.70 | 82.99 | 22.60 | 4.22 |
| 2i |  | NA | 1.69 | 1.87 | NA |
| 2j |  | 1.88 | 15.24 | 20.09 | 498.09 |
| 2k |  | 38.48 | 2.39 | 1.88 | 96.82 |
| 3a | Bis-(ethyl)- | 88.90 | 2.50 | 1.10 | 151.8 |
| 3b | Bis-(2-chloroethyl)- | 113.50 | 1.20 | 93.5 | 19.02 |
| 3c | Bis-(allyl)- | 166.88 | 21.7 | 378.05 | 295.70 |
| 4a |  | 55.92 | 2.52 | 12.97 | NA |
| 4b |  | 1.70 | 7.33' | 57.90 | NA |

NA: Not active.

**Table 2**. MIC of norfloxacin against the tested organisms according to CLSI.

| Microorganism | MIC ($\mu$g/mL) | | |
|---|---|---|---|
| | S | I | R |
| *S. aureus* | $\leq 4$ | 8 | $\geq 16$ |
| *E. coli* | $\leq 4$ | 8 | $\geq 16$ |
| *K. pneumoniae* | $\leq 4$ | 8 | $\geq 16$ |
| *P. aeruginosa* | $\leq 4$ | 8 | $\geq 16$ |

|S= Sensitive, I = intermediate, R= resistant.

$\mu$g/mL for norfloxacin). The same compounds appeared to be less active against the rest of the gram-negative bacteria; they showed MICs of 2.50 to >100 $\mu$g/mL against different gram-negative bacteria.

On the other hand, introducing a bulky $N$4-adamantane carbamoyl group in compound **2f** did not show any significant activity against all the tested microorganisms (MICs = 115–304 $\mu$g/mL, Table 1). Meanwhile, compound **3b** having a bis-(2-chloroethyl) group and **3a** with a bis-ethyl group showed moderate activity against the gram-positive *S. aureus* (MIC = 88.90 $\mu$g/mL) but higher activity against *E. coli* and *K. pneumoniae* (MIC = 2.50 and 1.10 $\mu$g/mL, respectively, Table 1). Consequently, the $N$-isopropyl compounds **2b** and **2g** showed moderate activity against *S. aureus*, while compounds **2a**, **2b**, **2d**, **2g**, **2i**, **3b**, and **4b** showed better activity than the reference norfloxacin against *E. coli* with MIC values that ranged between 1.00 and 1.84 $\mu$g/mL. In addition, compounds **2e** and **3a** showed good activity, but less so than norfloxacin. Compounds **2b**, **2d**, **2g**, **2i**, **2k**, and **3a** showed comparable activity to norfloxacin against *Klebsiella pneumoniae*, while **2a**, **2b**, **2g**, and **2h** showed better activity against *P. aeruginosa*.

Overall, most compounds showed considerable activity against *S. aureus* and *E. coli* and lower activity against *K. pneumoniae* but low or no activity against *P. aeruginosa*.

### 2.3. Calculation of lipophilicity, polarizability, and topology parameters

Calculation of lipophilicity, topological, electronic, and thermal parameters of the target compounds and norfloxacin were carried out using the ChemBio 3D Ultra 2014 program. Calculated parameters include the Balaban index as a topological parameter. Diameter, electronic energy, molecular mass, and Wiener index were calculated as steric parameters. Some electronic parameters were also calculated including dipole moment and *N-4* piperazinyl charge density. Moreover, the heat of formation, molar refractivity, Gibbs free energy, and log p, as thermodynamic parameters, were measured. It is expected that these aforementioned parameters have an effect on the permeability of fluoroquinolones to bacterial cells. They may also affect binding with the target site, DNA-gyrase or its isozyme topoisomerase IV. The Wiener index is a measure of the compactness of molecules.[13] It was also reported to be connected with the molecular Van der Waals areas. The Wiener index provides a rough measure of the molecular surface, though it cannot be considered as either a measure of molecular volume or of the volume-to-surface ratio.[14] Moreover, some reports showed the importance of the steric effect and Gibbs free energy or heat of formation on the binding of quinolones with DNA.[9] Additionally, partitioning of quinolones in 1-octanol/water was found to be an entropy-driving process with absorbing heat and water solvating drug molecules more easily than 1-octanol. The enthalpy changes for quinolone molecules partitioning in 1-octanol/water are all positive, and the degrees of the system decrease.[15] As several reports focused on the importance of these parameters for the activity of fluoroquinolones, lipophilicity, polarizability, and topology parameters for the prepared compounds are calculated and outlined in Table 3.

**Table 3**. Calculated parameters for the target compounds including molecular mass, Balaban index, Wiener index, diameter, molar refractivity, log P, heat of formation, Gibbs energy, $N-4$ charge, and steric energy.

| Compound # | Molecular mass | Balaban index | Molecular topology: Wiener index | Topological diameter bond(s) | Molar refractivity, $cm^3/mol$ | Log P | Heat of formation [kJ/mol] | Gibbs energy [kJ/mol] energy | $N$-4 charge | Steric energy [kcal/mol] |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 319.13 | 317490 | 1116 | 11 | 86.98 | 1.37 | –462.39 | –51.63 | –0.205 | 0.9175 |
| **2a** | 347.16 | 487665 | 1462 | 13 | 95.52 | 2.08 | –467.79 | 0.38 | –0.113 | 8.0456 |
| **2b** | 361.18 | 593330 | 1649 | 13 | 99.9371 | 2.40 | –493.71 | 6.36 | –0.121 | 9.9998 |
| **2c** | 377.14 | 728512 | 1884 | 14 | 96.855 | 1.01 | –818.06 | –343.53 | –0.108 | 4.4280 |
| **2d** | 359.16 | 601011 | 1672 | 14 | 99.9336 | 2.43 | –363.64 | 96.64 | –0.108 | 5.6741 |
| **2e** | 419.19 | 1229616 | 2592 | 16 | 110.866 | 2.10 | –852.27 | –287.17 | –0.115 | 8.7039 |
| **2f** | 510.26 | 2137506 | 4856 | 18 | 139.19 | 2.42 | –579.26 | 182.45 | 0.124 | 10.3550 |
| **2g** | 524.21 | 3481397 | 5649 | 22 | 139.028 | 2.42 | –857.98 | –190.51 | 0.107 | 5.1816 |
| **2h** | 437.18 | 1420037 | 3205 | 17 | 120.673 | 2.66 | –467.68 | 34.39 | –0.109 | 5.8818 |
| **2i** | 466.20 | 1965249 | 3950 | 19 | 128.393 | 2.74 | –499.42 | 103.02 | –0.115 | 3.0377 |
| **2j** | 549.18 | 3336946 | 6031 | 22 | 149.815 | 4.56 | –309.85 | 330.11 | –0.100 | 12.891 |
| **2k** | 626.20 | 5891793 | 8442 | 23 | 165.225 | 3.41 | –717.30 | 81.32 | –0.103 | 15.8520 |
| **3a** | 503.11 | 704817 | 1820 | 13 | – | – | – | – | 0.741 | –22.3132 |
| **3b** | 479.09 | 1006351 | 2264 | 14 | – | – | – | – | 0.733 | –25.3265 |
| **3c** | 479.12 | 1006351 | 2264 | 14 | – | – | – | – | 0.766 | –37.4125 |
| **4a** | 439.05 | 720419 | 1862 | 14 | 102.749 | 1.66 | –554.04 | –114.22 | –0.033 | 1.8876 |
| **4b** | 441.15 | 1390532 | 3138 | 17 | 115.431 | 3.07 | –654.62 | –178.47 | –0.012 | 0.9140 |

Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations ($P < 0.05$). All statistical analyses were performed using SPSS 17 (SPSS Inc., Chicago, IL, USA). The Pearson correlation is +1 in the case of a perfect direct (increasing) linear relationship (correlation), –1 in the case of a perfect decreasing (inverse) linear relationship (anticorrelation),[16] and some value in the open interval (–1, 1) in all other cases, indicating the degree of linear dependence between the variables. As it approaches zero, there is a decreased relationship (closer to uncorrelated). The closer the coefficient is to either –1 or 1, the stronger the correlation is between the variables. If the variables are independent, Pearson's correlation coefficient is 0, but the converse is not true because the correlation coefficient detects only linear dependencies between two variables.

Correlation results between the antibacterial activity expressed in log MIC and the calculated parameters of the tested compounds and the reference norfloxacin are outlined in Table 4. It was clear that the $N-4$ substituent has an important impact on log P, $N$-4 charge density, and steric energy among other parameters. The $N$-4 charge ranged from 0.7 with a bis alkyl substitution to –0.12 with large bulky adamantyl group. Additionally, the $N$-4 charge had an impact on antibacterial activity, especially against gram-positive bacteria (correlation coefficient = 0.34), while changing the $N$-4 charge did not cause any significant change in anti-*E. coli* activity (correlation coefficient = 0.01, Table 4)

**Table 4**. Correlation coefficient between log MIC of the tested strains and the calculated parameters of the target compounds and the reference norfloxacin.

| | Correlation cooffiecient | | | |
|---|---|---|---|---|
| | *Staph. aureus* ATCC 6538 | *E. coli* ATCC 8739 | *Klebsiella pneumoniae* ATCC 10031 | *P. aeruginosa* ATCC 10145 |
| Molecular mass | 0.211600 | 0.205800 | –0.131400 | 0.280200 |
| Balaban index | –0.001138 | 0.081990 | –0.244200 | 0.106100 |
| Wiener index | 0.14130 | 0.19880 | –0.19520 | 0.15530 |
| Topological diameter bond(s) | 0.01722 | 0.19910 | –0.19260 | 0.19470 |
| Molar refractivity, $cm^{-1}$/mol | 0.13680 | 0.27010 | 0.15590– | 0.20250 |
| Log P | –0.23160 | 0.10250 | 0.01530 | 0.17250 |
| Heat of formation [kJ/mol] | –0.319500 | –0.040480 | 0.132300 | –0.004659 |
| Gibbs free energy [kJ/mol] | –0.08832 | 0.13680 | –0.05435 | 0.06598 |
| $N$4-charge | 0.34210 | 0.01965 | 0.20450 | 0.05620 |
| Steric energy [kcal/mol] | –0.28180 | 0.04281 | –0.21380 | 0.09526 |

Moreover, molecular mass, diameter, and steric energy also had an impact on the antibacterial activity of the tested norfloxacin derivatives. Increasing molecular mass had a small negative impact on the activity against *K. pneumoniae* (correlation coefficient = –0.13) and a higher positive impact on the other microorganisms (correlation coefficient = 0.21–0.28). These data are consistent with the published data in the literature.[17] The diameter of the molecules had a weak impact on the anti-*E. coli* and *P. aeruginosa* activity (correlation coefficient = 0.19) and increasing diameter negatively affected activity against *K. pneumoniae* (correlation coefficient = –0.19).

Additionally, a negative to weak correlation was shown between the Balaban index, the Wiener index, and the tested activities. Log P had a moderate to weak correlation with the antibacterial activity in most of the tested strains, which means that hydrophobicity of the molecule is not the only factor affecting the entrance of the molecule into the bacterial cell. The highest impact for hydrophobicity was observed against *S. aureus* strains, where higher log P values decreased the observed antibacterial activity (correlation coefficient = –0.23, Table 4). A previous correlation study reported that passive diffusion is not the defining mechanism for fluoroquinolone entry to bacterial cells minimizing the role of lipophilicity as observed in the current study.[18] Similarly, heat of formation and Gibbs energy showed weak correlations with the activity against *S. aureus*, whereas they showed weak or no impact on the activity against different gram-negative strains. Change in Gibbs free energy is usually negative, indicating that the complex formation between a compound and target enzyme is spontaneous. The association is driven by favorable $\Delta H$ (negative enthalpies) and therefore is exothermic. Gibbs free energy did not show a significant correlation with the antibacterial activity. Additionally, the increase in steric energy moderately decreased both anti-*Staphylococcus* and anti-*Klebsiella* activities, while no similar effect was observed on the rest of the tested strains.

Moreover, data in Table 5 show that there is a highly significant correlation between molar mass and Weiner index (r = 0.965) and a strong negative correlation between steric energy and *N-4* charge (r = –0.948). It is obvious that the increase of *N*-4 charge density has a deleterious effect on log P (r = –0.940). As expected, there was a medium correlation between Gibbs energy and the heat of formation (r = 0.314). It was obvious that there was a weak to moderate effect of the topological parameters on log P that may affect the permeability of quinolones. On the other hand, there was no significant correlation between log P and the Weiner index. In summary, there was no calculated factor that could determine the biological activity. However, the total volume of the molecule, bulkiness at C-7, electronic factors, and lipophilicity can determine the activity. It is obvious that when designing newer fluoroquinolones, the total volume of the molecule, bulkiness at C-7, electronic factors, and lipophilicity factors should be considered. Abuo-Rahma et al. previously studied correlations of other fluoroquinolone properties with antimicrobial activity and their results supported the fact that no single parameter is most effective in determining the activity of fluoroquinolones where biological activity is a factor of a group of lipophilicity, molecular mass, and electronic factors.[12] Similar results were found in the literature as the antibacterial activity of fluoroquinolones depended on certain electronic effects and steric effects of bulky groups positioned on the piperazine ring where electronic properties of $OCH_3$ and Cl groups significantly enhanced the antibacterial activity of a series of sulfonamide-substituted fluoroquinolones while the $CH_3$ steric effect decreased the activity.[19]

### 2.4. Conclusion

Fifteen norfloxacin derivatives containing different substituents at the *N*4-piperazinyl moiety have been synthesized. These derivatives are designed to have different lipophilicity, polarizability, and topology properties. Some of the prepared compounds showed reasonable antibacterial activity parallel to or greater than the reference norfloxacin. Isopropyl derivative **2b** and *p*−aminobenzoic acid derivative **2g** showed broad-spectrum antibacterial activity against all the tested strains. Generally, test compounds have higher activity against *S. aureus* and *E. coli* among the examined microorganisms. Correlation between antibacterial activities, expressed in log MIC, and lipophilicity, polarizability, and topology parameters showed that none of the studied parameters could exclusively affect the antibacterial activity. Indeed, the total volume of the molecules, bulkiness, electronic factors, and lipophilicity can determine the antibacterial activity. For example, though some

**Table 5**. Correlation coefficient between all tested parameters of the new compounds and the reference norfloxacin.

| | Molecular mass | Balaban index | Wiener index | Topological diameter bond(s) | Molar refractivity $cm^{-1}$/mol | Log P | Heat of formation [kJ/mol] | Gibs free energy Gibbs free energy [kJ/mol] | *N*4-charge | Steric energy [kcal/mol] |
|---|---|---|---|---|---|---|---|---|---|---|
| Molecular mass | | 0.974323 | 0.965624 | 0.909384 | 0.936761 | 0.103195 | –0.58673 | 0.45710 | –0.27782 | 0.527731 |
| Balaban index | | | 0.969158 | 0.97951 | 0.97777 | 0.28444 | –0.42964 | 0.64539 | –0.47708 | 0.682676 |
| Wiener index | | | | 0.92457 | 0.98582 | 0.073290 | –0.60270 | 0.54360 | –0.31828 | 0.51235 |
| Topological diameter bond(s) | | | | | 0.96683 | 0.44737 | –0.25877 | 0.78560 | –0.64054 | 0.80082 |
| Molar refractivity, $cm^{-1}$/mol | | | | | | 0.22063 | –0.46759 | 0.67662 | –0.46845 | 0.63129 |
| Log P | | | | | | | 0.742911 | 0.79667 | –0.94043 | 0.89379 |
| Heat of formation [kJ/mol] | | | | | | | | 0.313555 | –0.56107 | 0.366455 |
| Gibbs free energy [kJ/mol] | | | | | | | | | –0.95347 | 0.922978 |
| *N*4-charge | | | | | | | | | | –0.94803 |
| | | | | | | | | | | |

parameters like the charge on $N-4$ affect activity against *S. aureus*, others such as log P showed almost no effect on activity on *K pneumoniae*. Thus, all these parameters should be considered in the design of new fluoroquinolones.

## 3. Experimental

### 3.1. Materials

All reagents and solvents were of commercially available reagent grade quality and were used without further purification. The IR spectra were recorded on a Nicolet iS5 FT-IR spectrometer (Fisher Scientific, USA). The $^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Avance III (USA) at 400 MHz or 600 MHz. Chemical shifts are reported in $\delta$ ppm using TMS as an internal standard and coupling constants ($J$) are expressed in Hz. Abbreviations indicating multiplicity were used as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. Melting points were measured on a Stuart SMP10 melting point apparatus (Germany). Elemental analyses were carried out at the Regional Center

of Mycology and Biotechnology, Al-Azhar University, Cairo. LC/MS/MS was carried out using an Agilent UPLC/LC/MS/MS 1260 Infinity II (USA) with 6420 Triple Quad LC/MS detector at the Faculty of Pharmacy, Minia University. Norfloxacin was purchased from Medical Union Pharmaceutical (MUP) Abu-Sultan, Ismailia, Egypt. Compounds **2a**,[20] **2b**,[21] **2d**,[1] 2h,[22] and **3a**[12] were prepared as reported.

#### 3.1.1. *N*-((3s,5s,7s)-Adamantan-1-yl)-2-bromoacetamide[23] B

$^1$H NMR (400 MHz, DMSO-$d_6$) 1.59–1.60 (6H, m, adamantane 3C$\underline{H}_2$), 1.89–1.90 (3H, m, 3 C$\underline{H}$ ($CH_2$)), 1.96–1.98 (6H, m, adamantane 3NC$\underline{H}_2$), 4.02 (1H, s, COC$\underline{H}_2$), 7.23 (1H, s, N$H$).

#### 3.1.2. 2-Chloro-*N*-(4-(*p*-tolyl)thiazol-2-yl)acetamide C[24]

Pale yellow crystals, mp: 162–164 °C (reported: 165–167 °C); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.43 (3H, s, C$\underline{H}_3$), 4.43 (2H, s, COC$\underline{H}_2$), 7.29–7.30 (3H, m, thiazole C5 & 2 Ar H), 7.78 (2H, d, $J$ = 7.50, Ar H), 10.90 (1H, s, NH).

#### 3.1.3. 2-Bromo-*N*-(5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl) acetamide D

Yellow powder; 3.10 g, 84.00% yield; mp: 274–276 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.74 (3H, s, OC$\underline{H}_3$), 3.89 (6H, s, OC$\underline{H}_3$), 4.22 (2H, s, -COC$\underline{H}_2$), 7.21 (2H, s, Ar-$H$), 12.29 (1H, s, -N$H$).

#### 3.1.4. 7-(4-Allylpiperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 2d

Pale yellow powder; yield = 65%, mp = 237–238 °C, $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$1.35 (3H, t, $J$= 6.9 Hz, $NCH_2C\underline{H}_3$), 2.95–3.05 (4H, m, piperazinyl-$H$), 2.99 (2H, d, $J$= 6.2 Hz, $NC\underline{H}_2CH$), 3.34–3.36 (4H, m, piperazinyl-4$H$), 4.54 (2H, q, $J$ = 6.9 Hz, $C\underline{H}_2CH_3$), 5.13 (H, d, $J_{cis}$ = 10.3 Hz, $N$-$CH_2$-CH=C$\underline{H}_2$), 5.19 (H, d, $J_{trans}$= 17.2 Hz, $NC\underline{H}_2CH$), 5.79–5.82 (1H, m, N-$CH_2$-C$\underline{H}$=$CH_2$), 7.14 (1H, d, $J$ = 6.8 Hz, $H-8$), 7.88 (1H, d, $J$ = 13 Hz, $H$-5), 8.91 (1H, s, $H$-2), 15.32 (1H, brs, COO$H$); $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 14.32, 49.08, 49.50, 52.21, 60.67, 106.10, 107.44, 111.57, 118.10, 120.48, 137.09, 144.60, 148.50, 166.32, 176.40. Anal. Calcd. for $C_{19}H_{22}FN_3O_3$ (359.16): C, 63.50; H, 6.17; N, 11.69. Found: C, 63.64; H, 6.21; N, 11.84.

#### 3.1.5. 7-(4-(1-Ethoxy-1-oxopropan-2-yl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 2e

Pale yellow powder; yield = 74%, mp = 209–211 °C, $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$1.17 (3H, d, $J$ = 7.00 Hz, $CH_3$CH-), 1.18 (3H, t, $J$ = 6.8 Hz, -$OCH_2C\underline{H}_3$), 1.37 (3H, t, $J$= 6.9 Hz, $NCH_2C\underline{H}_3$), 2.72 (1H, d, $J$= 7.0 Hz, $\underline{CH}CH_3$), 3.35–3.95 (8 H, m, piperazinyl-8$H$), 4.08 (2H, q, $J$ = 6.8 Hz, $OC\underline{H}_2CH_3$), 4.55 (2H, q, $J$= 6.9 Hz, -N-$C\underline{H}_2CH_3$), 7.14 (1H, d, $J$ = 6.9 Hz, $H-8$), 7.88 (1H, d, $J$ = 14 Hz, $H$-5), 8.91 (1H, s, $H$-2); $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 14.46, 14.57, 40.28, 48.57, 49.50, 50.07, 60.06, 61.52, 106.10, 107.31, 111.30, 119.48, 137.47, 146.05, 148.82, 157.50, 166.44, 172.36, 175.50. Anal. Calcd. for $C_{21}H_{26}FN_3O_5$ (419.19): C, 60.13; H, 6.25; N, 10.01. Found: C, 60.41; H, 6.39 1; N, 10.43.

### 3.1.6. 7-(4-(2-((3s,5s,7s)-Adamantan-1-ylamino)-2-oxoethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 2f

Pale yellow powder; yield = 62%, mp >300 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$1.40 (3H, t, $J$ = 6.8 $NCH_2C\underline{H}_3$), 1.60–1.65 (6H, m, adamantane $3C\underline{H}_2$), 1.93–2.06 (9H, m, adamantane $3NC\underline{H}_2$ and 3 $C\underline{H}$(CH2)), 2.63–3.65 (4H, m, piperazinyl-$H$), 3.32–3.34 (6H, m, piperazinyl-4$H$ and $COCH_2N$), 4.58 (2H, q, $J$ = 6.8 Hz, $NC\underline{H}_2CH_3$), 7.11 (1H, s, N$H$), 7.17 (1H, d, $J$ = 7.2 Hz, $H-8$), 7.89 (1H, d, $J$ = 13Hz, $H$-5), 8.95 (1H, s, $H$-2), 15.10 (1H, brs, COO$H$). Anal. Calcd. for $C_{28}H_{35}FN_4O_4$ (510.26): Calcd. C, 65.86; H, 6.91; N, 10.97. Found: C, 65.98; H, 6.98; N, 11.09.

### 3.1.7. 7-(4-(2-((4-(Ethoxycarbonyl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 2g

Pale yellow powder; yield = 66%, mp = 270–273 °C, $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$1.26 (3H, t, $J$ = 6.8 $OCH_2C\underline{H}_3$), 1.36 (3H, t, $J$ = 7.6 Hz, $NCH_2C\underline{H}_3$), 2.73–2.85 (4H, m, piperazinyl-$H$), 3.36 (2H, s, -$COC\underline{H}_2NH$), 3.47–3.49 (4H, m, piperazinyl-4$H$), 4.24 (2H, q, $J$ = 6.8 Hz, $OC\underline{H}_2CH_3$), 4.58 (2H, q, $J$ = 7.6 Hz, $NC\underline{H}_2CH_3$), 7.22 (1H, d, $J$ = 6.9 Hz, $H-8$), 7.75 (2H, d, $J$ = 6.8 Hz, Ar$-\underline{H}$), 7.85 (2H, d, $J$ = 6.8 Hz, Ar$-\underline{H}$), 7.89 (1H, d, $J$ = 12Hz, $H$-5), 8.93 (1H, s, $H$-2), 10.10 (1H, brs, N$H$), 15.10 (1H, brs, COO$H$); $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 14.20, 14.44, 42.72, 46.59, 49.13, 52.26, 60.48, 106.80, 107.14, 107.28, 111.60, 118.88, 130.25, 137.21, 137.31, 144.00, 148.87, 152.70, 153.10, 158.80, 165.44, 166.14, 176.31. Anal. Calcd. for $C_{27}H_{29}FN_4O_6$ (524.21): C, 61.82; H, 5.57; N, 10.68. Found: C, 61.94; H, 5.54; N, 10.84.

### 3.1.8. 7-(4-((*p*-Tolylcarbamoyl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid 2i

Pale yellow powder; yield = 72%, mp = 294–296 °C, $^1$H NMR (400 MHz, DMSO-$d_6$) 1.43 (3H, t, $J$ = 7.2 Hz, $NCH_2C\underline{H}_3$), 2.26 (3H, s, *p-tolyl*-$C\underline{H}_3$), 3.16–3.89 (8H, m, piperazinyl-$H$), 4.33 (2H, s, -$CH_2$-CO), 4.63 (2H, q, $J$ = 7.6 Hz, $NC\underline{H}_2CH_3$), 7.15 (2H, d, $J$ = 8.00 Hz, Ar$-\underline{H}$), 7.24 (1H, d, $J$ = 6.9 Hz, $H-8$), 7.54 (2H, d, $J$ = 8.00 Hz, Ar$-\underline{H}$), 7.94 (1H, d, $J$ = 12.8 Hz, $H$-5), 8.94 (1H, s, $H$-2), 10.98(1H, brs, N$H$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 14.98, 20.94, 42.39, 46.81, 49.63, 51.78, 57.03, 106.97, 107.66, 111.79, 112.02, 119.97, 120.40, 129.76, 133.71, 135.93, 137.59, 144.32, 144.42, 149.15, 151.89, 154.37, 162.91, 166.50, 176.61. Anal. Calcd. for $C_{25}H_{27}FN_4O_4$ (466.2): LC/MS/MS data: Calculated (466.2), Found: 466.2

### 3.1.9. 1-Ethyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-((4-(p-tolyl)thiazol-2-yl)amino)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid 2j

Pale yellow powder; yield = 49%, mp = 294–296 °C, $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$1.38 (3H, t, $J$ = 6.8 Hz, $NCH_2C\underline{H}_3$), 2.29 (3H, s, $C\underline{H}_3$), 2.45–2.46 (4H, m, piperazinyl-$H$), 3.51–3.55 (6H, m, piperazinyl-4$H$ *and* $COC\underline{H}_2$), 4.58 (2H, q, $J$ = 6.8 Hz, $C\underline{H}_2CH_3$), 7.21 (3H, d, $J$ = 8 Hz, $H-8$ and 2 Ar$H$), 7.61 (1H, s, Ar$H$), 7.75 (2H, d, $J$ = 8 Hz, Ar$H$), 7.93 (1H, d, $J$ = 12.4 Hz, $H$-5), 8.94 (1H, s, $H$-2), 15.32 (1H, brs, COO$H$); $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 14.79, 21.12, 39.34, 40.34, 49.47, 100.49, 107.58, 111.35, 114.5, 122.50, 126.07, 129.77, 131.90, 135.10, 137.74, 146.50, 149.10, 151.20, 152.50, 159.80, 163.40, 166.53, 176.10. Anal. Calcd. for $C_{28}H_{28}FN_5O_4S$ (549.18): C, 61.19; H, 5.13; N, 12.74. Found: C, 61.04; H, 5.23; N, 12.90.

### 3.1.10. 7-(4-((5-(3,4,5-Trimethoxyphenyl)-1,3,4-thiadiazol-2-ylcarbamoyl)methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 2k

Pale yellow powder; yield = 74%, mp = 278–280 °C, $^1$H NMR (400 MHz, DMSO-$d_6$) 1.42 (3H, t, $J$= 7.2 Hz, $NCH_2C\underline{H}_3$), 2.80 (4H, m, piperazinyl-$H$), 3.55 (4H, m, piperazinyl-$H$), 3.73 (2H, s, -$CH_2$-CO), 3.84 (3H, s, -$OCH_3$), 3.88 (6H, s, 2-$OCH_3$), 4.59 (2H, q, $J$= 7.6 Hz, $NC\underline{H}_2CH_3$), 7.20 (2H, s, 2Ar H), 7.18 (2H, d, $J$ = 7.2 Hz, H-8), 7.94 (1H, d, $J$ = 12.8 Hz, $H$-5), 8.94 (1H, s, $H$-2), 15.15 (1H, brs, -COOH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 14.81, 49.54, 49.96, 52.48, 56.59, 60.22, 60.64, 104.72, 106.34, 107.54, 111.53, 111.76, 119.97, 126.03, 137.67, 139.94, 142.00, 145.97, 148.94, 153.87, 154.02, 158.47, 162.33, 166.59, 169.19, 176.64. Anal. Calcd. for $C_{29}H_{31}FN_6O7S$ (626.20): LC/MS/MS data: Calculated: (626.2), Found: (626.2).

### 3.1.11. 4-(3-Carboxy-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolin-7-yl)-1,1-diethylpiperazin-1-ium iodide 3a

Pale yellow powder; yield = 68%, mp >300 °C, $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$1.22 (6H, t, $J$ = 6.8 Hz, $NCH_2C\underline{H}_3$), 1.38 (3H, t, $J$ = 6.8 Hz, $NCH_2C\underline{H}_3$), 3.20–3.32 (4H, m, piperazinyl-$H$), 3.48–3.85 (4H, m, piperazinyl-$H$), 4.57 (2H, t, $J$ = 6.8 Hz, $NC\underline{H}_2CH_3$), 4.00 (4H, q, $J$ = 6.8 Hz, $NC\underline{H}_2CH_3$), 4.57 (2 H, q, $J$ = 6.8 Hz, $NC\underline{H}_2CH_3$), 7.20 (1H, d, $J$ = 7 Hz, $H-8$), 7.90 (1H, d, $J$ = 12 Hz, $H$-5), 8.91 (1H, s, $H$-2); $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 9.10, 14.45, 42.75, 46.56, 49.17, 50.22, 50.89, 106.65, 107.23, 111.55, 120.01, 137.16, 148.78, 151.77, 153.76, 166.11, 176.26. Anal. Calcd. for $C_{20}H_{27}FIN_3O_3$ (503.11): C, 47.72; H, 5.41; N, 8.35. Found: C, 47.89; H, 5.49; N, 8.51.

### 3.1.12. 4-(3-Carboxy-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolin-7-yl)-1,1-bis(2-chloroethyl)piperazin-1-ium chloride 3b

Pale yellow powder; yield = 44%, mp = 237–239 °C, $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$1.37 (3H, t, $J$ = 7.5 Hz, $NCH_2C\underline{H}_3$), 2.58 (4H, t, $J$ = 6.8 Hz, $ClCH_2CH_2N$-), 3.26–3.46 (4H, m, piperazinyl-$H$), 3.46–3.51 (4H, m, piperazinyl-4$H$), 4.07 (4H, q, $J$ = 7.0 Hz, $CH_2C\underline{H}_2Cl$), 4.57 (4H, t, $J$ = 6.8 Hz, -$NC\underline{H}_2CH_3$), 7.22 (1H, d, $J$ = 6.8 Hz, $H-8$), 7.93 (1H, d, $J$ = 13 Hz, $H$-5), 8.94 (1H, s, $H$-2); $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 14.61, 42.75, 46.66, 46.71, 49.29, 51.75, 60.12, 69.24, 106.10, 107.44, 111.57, 120.28, 137.39, 144.63, 149.04, 152.40, 166.32, 176.48. Anal. Calcd. for $C_{20}H_{25}Cl_3FN_3O_3$ (479.09): C, 49.96; H, 5.24; N, 8.74. Found: C, 50.03; H, 5.22; N, 8.89.

### 3.1.13. 1,1-Diallyl-4-(3-carboxy-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-ium bromide 3c

Pale yellow powder; yield = 63%, mp = 229–230 °C, $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$1.43 (3H, t, $J$ = 8 Hz, $NCH_2C\underline{H}_3$), 2.52–2.59 (4H, m, piperazinyl-$H$), 3.05 (4H, d, $J$ = 6.2 Hz, $2NC\underline{H}_2CH$), 3.32–3.34 (4H, m, piperazinyl-$H$), 4.60 (2H, q, $J$ = 8 Hz, $C\underline{H}_2CH_3$), 5.18 (2H, d, $J_{cis}$= 4 Hz, 2N-$CH_2$-CH=$C\underline{H}_2$), 5.24 (2H, d, $J_{trans}$ = 12 Hz, $2NC\underline{H}_2CH$), 5.85–5.87 (2H, m, 2N-$CH_2$-$C\underline{H}$=$CH_2$), 7.19 (1H, d, $J$ = 8 Hz, $H-8$), 7.92 (1H, d, $J$ = 12 Hz, $H$-5), 8.96 (1H, s, $H$-2), 15.37 (1H, brs, $COOH$). Anal. Calcd. for $C_{22}H_{27}BrFN_3O_3$ (479.12): C, 55.01; H, 5.67; N, 8.75. Found: C, 55.17; H, 5.71; N, 8.89.

#### 3.1.14. 7-(4-(2-Bromoacetyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid[25] 4a

White powder; yield: 0.370 g (84%); mp: 247–249 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): 1.45 (3H, t, $J$ = 7.6 Hz, NCH$_2$C$\underline{H}_3$), 3.38–3.42 (4H, m, piperazinyl-$H$), 3.70–3.74 (4H, m, piperazinyl-$H$), 4.20 (2H, s, BrC$\underline{H}_2$), 4.59 (2H, q, $J$ = 7.6 Hz, NC$\underline{H}_2$CH$_3$), 7.22 (1H, d, $J$H-F = 7.6 Hz, $H$8), 7.96 (1H, d, $J$H-F = 13.6 Hz, $H$5), 8.94 (1H, s, $H$2), 15.22 (1H, s, COO$\underline{H}$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 14.76, 28.29, 41.61, 46.11, 49.56, 106.86, 107.58, 111.73 (d, $J$ = 23 Hz), 120.06 (d, $J$ = 8 Hz), 137.62, 145.56 (d, $J$= 11 Hz), 149.14, 153.29 (d, $J$= 247 Hz), 165.49, 166.57, and 176.67; Anal. Calcd for C$_{18}$H$_{19}$BrFN$_3$O$_4$: C, 49.11; H, 4.35; N, 9.54. Found: C, 49.34; H, 4.31; N, 9.78.

### 3.2. Antibacterial activity

#### 3.2.1. Bacterial strains

Standard strains of *S. aureus* (ATCC 6538), *K. pneumoniae* (ATCC 10031), *P. aeruginosa* (ATCC 10145), and *E. coli* (ATCC 8739) were obtained from the Microbiological Resource Centre (MIRCIN), Agriculture Faculty, Ain Shams University.

#### 3.2.2. Determination of minimum inhibitory concentrations[26]

Microorganisms (0.5 mL) of 1 × 10$^6$ CFU/mL (0.5 McFarland turbidity) were plated in sterile petri dishes and then 20 mL of sterile, molten, and cooled (45 °C) Muller Hinton agar medium was added to all petri dishes. The plates then were slowly rotated to ensure the uniform distribution of the microorganisms, allowing the solidification on a flat surface. After solidification, four equidistant and circular wells of 10 mm in diameter were carefully punched using a sterile cork bore.

Twofold serial dilutions were performed on the tested compounds and the reference norfloxacin. Equal volumes of the tested compounds and the reference were well applied separately to each one in the three replicates using a micropipette. All plates were incubated overnight at 37 °C and then collected. Zones of inhibition that developed were measured. The average of the zones of inhibition was calculated. The MIC was calculated by plotting the natural logarithm of the concentration of extract against the square of the zones of inhibition. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of the concentration axis gave the MIC value.

### 3.3. Statistical analysis

Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations ($P < 0.05$). All statistical analyses were performed using SPSS 17 (SPSS Inc., Chicago, IL, USA).

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