Carbohydrate Research 345 (2010) 761–767

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Synthesis and antitubercular evaluation of new fluoroquinolone derivatives coupled with carbohydrates

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ARTICLE INFO

Article history:
Received 24 November 2009
Received in revised form 21 January 2010
Accepted 26 January 2010
Available online 1 February 2010

Keywords:
Fluoroquinolones
Carbohydrate
Tuberculosis
Microwave activation

ABSTRACT

We describe in this work the synthesis of nine new fluoroquinolone derivatives based on modifications at the C-7 position of the known fluoroquinolones cipro-, gati-, and moxifloxacin, as well as their antitubercular evaluation. The synthesis of these new analogues was improved using microwave irradiation, providing several advantages such as better yields and shorter reaction times, in comparison with classical reaction conditions. Derivatives **4**, **5,** and **7** exhibited promising antitubercular activities.

1. Introduction

Fluoroquinolones are nowadays an important class of antibacterial agents, possessing a broad spectrum of activity against Gram-positive, Gram-negative, and mycobacterial organisms, as well as against anaerobes, including resistant strains.[1] This class, which belongs to the family of gyrase inhibitors, has several advantages such as solubility, antimicrobial activity, prolonged serum half-life, fewer adverse side effects, and both oral and parenteral routes of administration.[2] In the area of modern antibacterial chemotherapy, these compounds, such as cipro-, gati-, and moxifloxacin (Scheme 1), have several applications: they can be used to treat complicated urinary tract infections, bacterial diarrhea, respiratory infections, sexually transmitted diseases, osteomyelitis, and some cases of wound infection. This class of compounds is also used in veterinary diseases (poultry, bovine, porcine, rabbits, deer, and fish).[1] In this context, another recent and important application of fluoroquinolones is in the treatment of tuberculosis (TB), a global health problem responsible for 1.7 million deaths each year. This contagious disease, caused by the bacterium *Mycobacterium tuberculosis* has increased around the world due to AIDS, poor socioeconomic conditions, immigration, the lack of new drugs on the market, the appearance of multi-drug resistant (MDR) bacteria, and more recently the emergence of extensively drug-resistant (XDR) bacteria, commonly defined as strains resistant to all the current first-line, as well as some second-line drugs.[3,4] Currently, fluoroquinolones are approved by WHO as second-line agents to treat TB in patients with resistance or intolerance to first-line anti-tuberculosis therapy.[5] However, the potential of fluoroquinolones as first-line agents is still under investigation. Two fluoroquinolones, gatifloxacin and moxifloxacin (Scheme 1), are under phase III clinical trials and are the most advanced compounds in clinical development as new anti-TB drugs.[6]

Due to the promising perspective of fluoroquinolones for TB treatment, as well as of other diseases, and in the context of our interest in this field,[7–11] the aim of this work was to synthesize new analogues of cipro-, gati-, and moxifloxacin having carbohydrates coupled either to the free amine group of these compounds or, starting from compound **1**,[12] via a diamine linker (Scheme 1). The reason for using carbohydrates is due to their importance in the cell wall of *M. tuberculosis*.[13,14] The carbohydrates used in this work are protected due to the composition of the cell wall of *M. tuberculosis*, which over 60% of its dry weight is occupied by lipids being important for the survival of this bacteria. For example, these lipids are able to help to fight against hydrophobic drugs and dehydration, and also allow this bacteria be more effective in the host's immune system by growing inside macrophages. In this context, protected carbohydrates could facilitate the penetration of these new fluoroquinolones through the cell wall. Diamines were chosen as linkers due to the fact that they are pharmacophore groups found in many antitubercular drugs including at the C-7 position

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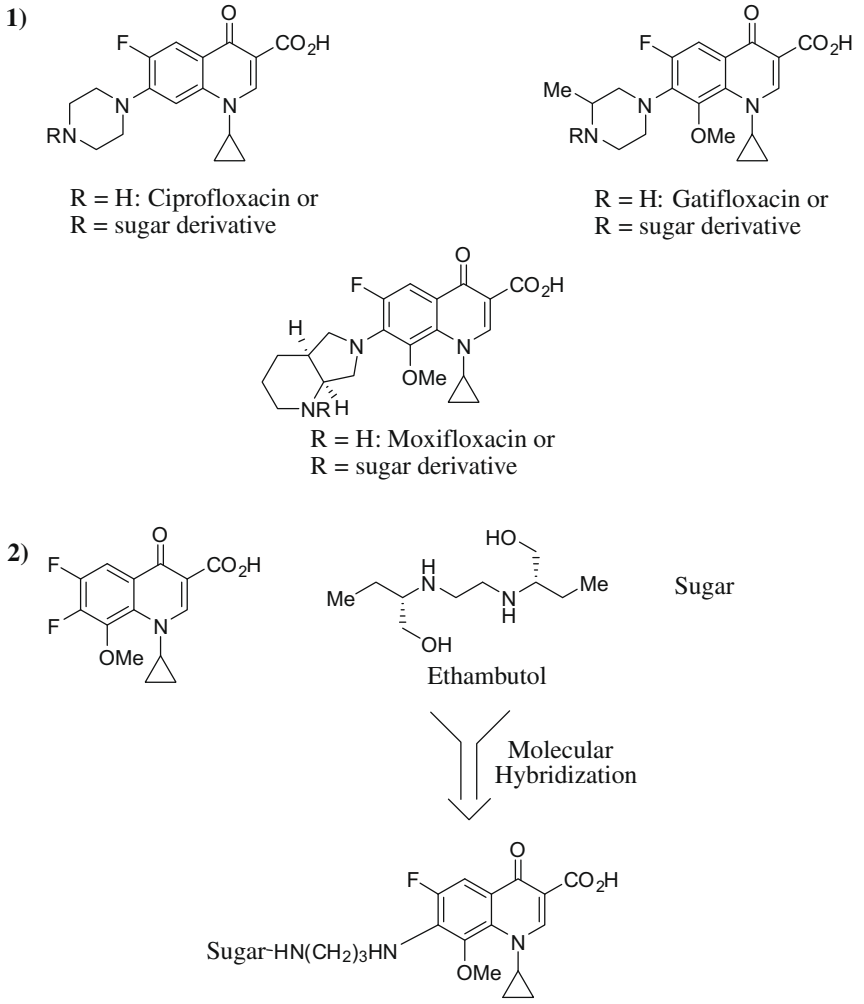
doi:10.1016/j.carres.2010.01.016



Scheme 1. Fluoroquinolone structures and their modification at the C-7 position.

of cipro-, gati-, and moxifloxacin and in ethambutol,[15,16] an important first-line drug used in TB treatment (Scheme 1).

2. Results and discussion

2.1. Chemistry

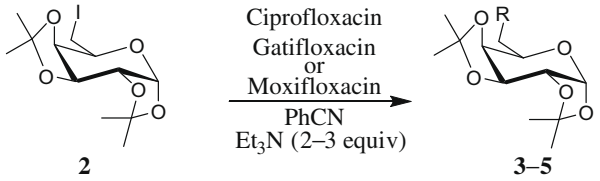
The synthesis of the new fluoroquinolones **3**–**5** was based on the coupling of 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**)[17] with cipro-, gati-, and moxifloxacin. The reaction, conducted under high temperature (140 °C) in the presence of base (triethylamine 2–3 equiv) and for extended times (24–48 h), led to the desired products with low yields in the range of 30–40%. To solve this problem, one important improvement in this synthesis was the utilization of microwave irradiation, furnishing the fluoroquinolone analogues in better yields (60–80%) and shorter time reaction times (20–45 min) (Scheme 2).

Another example of the importance of microwave irradiation in the synthesis of C-7-modified fluoroquinolones is demonstrated in Scheme 3. In this case, the use of microwave irradiation was critical for the success of the coupling reaction. Thus, reaction between gatifloxacin and methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-α-D-glucopyranoside (**6**)[18,19] did not occur under classical reaction conditions, even after eight days of reaction at 140 °C in the presence of a strong base (2 equiv of NaH). However, using microwave irradiation, the product of condensation was observed after 30 min, but in the case of gatifloxacin, the methyl group from the quinolone nucleus was lost as confirmed by HMBC experiments.

In addition to the synthesis of the new fluoroquinolones **3–5** and **7–9**, analogues linked to the sugars by diamines were also prepared. This was accomplished by an aromatic nucleophilic substitution at the C-7 position of fluoroquinolone **1**[12] by the sugar derivatives **10–12**, all previously prepared following literature procedures (Scheme 4).[20,21] In this case the use of microwave irradiation was not necessary, and the desired compounds were obtained in satisfactory yields in the range of 47–56%.

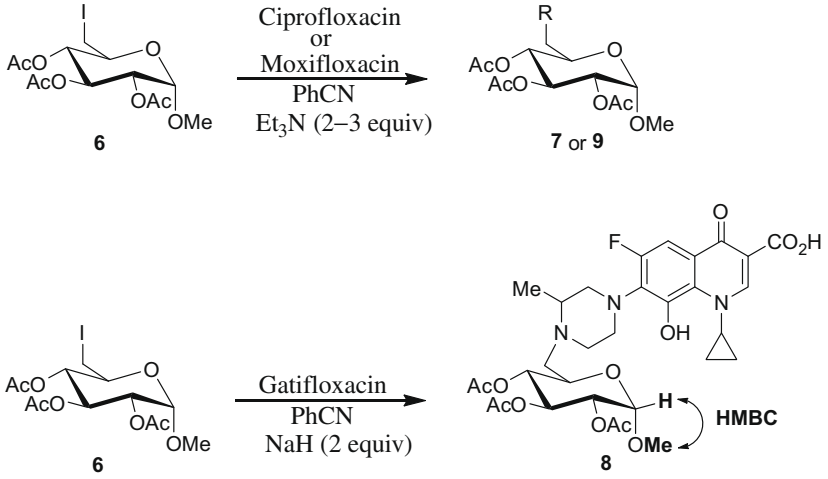
2.2. Antimycobacterial activity

The antimycobacterial evaluation of derivatives **3–5**, **7**, **8,** and **12–15** against *M. tuberculosis* ATTC 27294 was performed[22] using the micro plate Alamar Blue assay (MABA).[23] This methodology is nontoxic, uses thermally stable reagents and shows good correlation with proportional and BACTEC radiometric methods.[24,25] These tests were performed using the (NIH/NIAID) protocol,[26] and the results are shown in Table 1. Derivatives **4**, **5,** and **7** exhibited a promising inhibitory activity when compared with drugs used as the positive control such as ciprofloxacin, gatifloxacin, and moxifloxacin. In order to rationalize the observed activity, theoretical calculations were investigated for ciprofloxacin, gatifloxacin, moxifloxacin, and compounds **3** and **7**.



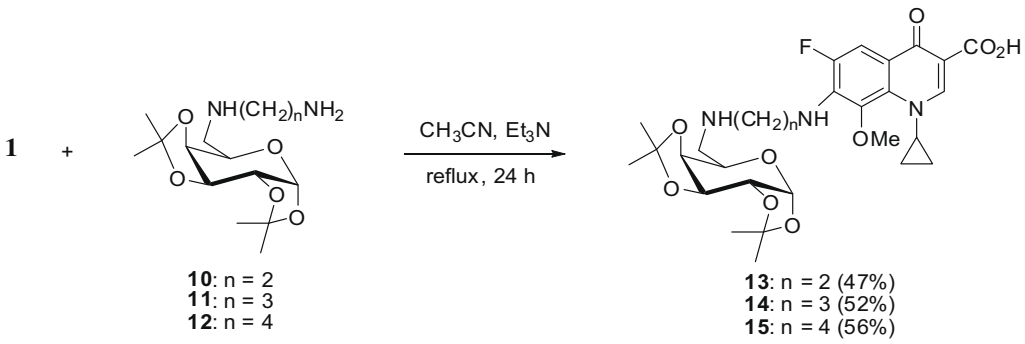
R	Compound	Reaction (140 °C) Time (h) and Yield (%)	MW Time (min) and Yield (%)
Ciprofloxacin	**3**	24 and 40	45 and 80
Gatifloxacin	**4**	48 and 32	20 and 60
Moxifloxacin	**5**	24 and 30	20 and 75

Scheme 2. Coupling of cipro-, gati-, and moxifloxacin with sugar derivative **2**.



R	Compound	Base and equiv	Reaction time (140 °C) (d) and yield (%)	MW; time (h) and yield (%)
Ciprofloxacin	**7**	Et_3N (3)	8 and 30	3.5 and 63
Gatifloxacin	**8**	NaH (2)	8 and no reaction	0.5 and 30
Moxifloxacin	**9**	Et_3N (2)	1 and 15	0.5 and 30

Scheme 3. Coupling of cipro-, gati-, and moxifloxacin with sugar derivative **6**.



Scheme 4. Coupling of fluoroquinolone (**1**) with sugar derivatives **10**–**12**.

Table 1
Antimycobacterial activity of fluoroquinolone derivatives

Compound no.	MIC (μg/mL)	IC_{50} (μm)	IC_{90} (μm)	% Growth inhibition
3	10	8.4	8.96	42.80
4	2.5	2.46	2.78	40.08
5	2.5	2.28	2.60	62.42
7	2.5	1.94	1.97	67.69
8	>10	N/A	N/A	−12.73
9	N/A	N/A	N/A	N/A
13	>10	N/A	N/A	−12.99
14	>10	N/A	N/A	−17.61
15	>10	N/A	N/A	−10.89
Ciprofloxacin	0.6	—	—	—
Gatifloxacin	0.1	—	—	—
Moxifloxacin	0.1	—	—	—

2.3. Computational procedure

In this computational study, 1000 conformations of each structure were generated by a random search Monte Carlo method[27] and optimized by PRCG molecular mechanics minimization[28] using the MACROMODEL (version 5.5) program[29] with the MM2* force field.[30] From these conformational searches, the most stable conformations were considered (Fig. 1). Ab initio gradient optimization of these structures using the B3LYP/631G basis set[31–34] have been performed with GAUSSIAN 03 program.[35] Mulliken atomic charges were analyzed to gain insight about their role in modulating the activity (Table 2).

In the present communication, the differences between our studied compounds are the substitutions on the C-7 and C-8 carbon atoms. Earlier investigations[36] demonstrated that the most versatile position for substitution of quinolones has been C-7, and that a cyclic system containing a secondary or tertiary amine moiety is usually the best. The beneficial effects are usually believed to be enhanced potency and favorable pharmacokinetics. An *O*-methyl group at C-8 often increases potency. Due to the presence of an *O*-methyl group at C-8, the Mulliken atomic charges on the C-8a carbon atom are greater for gatifloxacin and moxifloxacin (+0.37) than for ciprofloxacin, and compounds **3** and **7** (+0.34).

Moreover, it is noteworthy that the Mulliken atomic charges on the C-7 carbon atom is smaller for gatifloxacin, moxifloxacin, and compound **7** (+0.29 to +0.30) than for ciprofloxacin and compound **3** (+0.33), and that the Mulliken atomic charges on NA nitrogen atom is smaller for compound **7** (−0.60) than for ciprofloxacin and compound **3** (−0.62).

Further studies are underway to extend this work to a greater number and diversity of fluoroquinolones, which will serve to further improve the above-mentioned observations.

Table 2
Mulliken atomic charges on atoms in e

	Gatifloxacin	Moxifloxacin	Ciprofloxacin	Compound **3**	Compound **7**
MIC	0.1	0.5	1	10	2.5
N1	−0.72	−0.72	−0.71	−0.71	−0.71
C2	0.11	0.11	0.11	0.11	0.11
H2	0.22	0.22	0.21	0.21	0.21
C3	0.03	0.03	0.02	0.02	0.02
C4	0.25	0.25	0.26	0.26	0.26
O1	−0.43	−0.43	−0.43	−0.43	−0.43
C4a	0.003	0.0004	0.02	0.02	0.02
C8a	0.37	0.37	0.34	0.34	0.34
C5	−0.18	−0.18	−0.19	−0.19	−0.19
H5	0.19	0.20	0.19	0.19	0.19
C6	0.26	0.26	0.26	0.26	0.27
F	−0.33	−0.33	−0.33	−0.33	−0.33
C7	0.29	0.29	0.33	0.33	0.30
C8	0.19	0.20	−0.17	−0.17	−0.17
O8	−0.55	−0.55			
H8			0.15	0.15	0.15
NA[a]	−0.61	−0.64	−0.62	−0.62	−0.60
NB[b]	−0.58	−0.56	−0.57	−0.49	−0.48
HB	0.27	0.29	0.29		

[a] NA = *N*-fluoroquinolone.
[b] NB = *N*-sugar.

3. Experimental

All reagents and solvents were reagent grade unless otherwise stated and were used without prior purification. The optical rotation measurements were conducted on a JASCO P-1010 polarimeter. Melting points were measured in capillary tubes with a Buchi B-540 apparatus and are uncorrected. The IR spectra were acquired

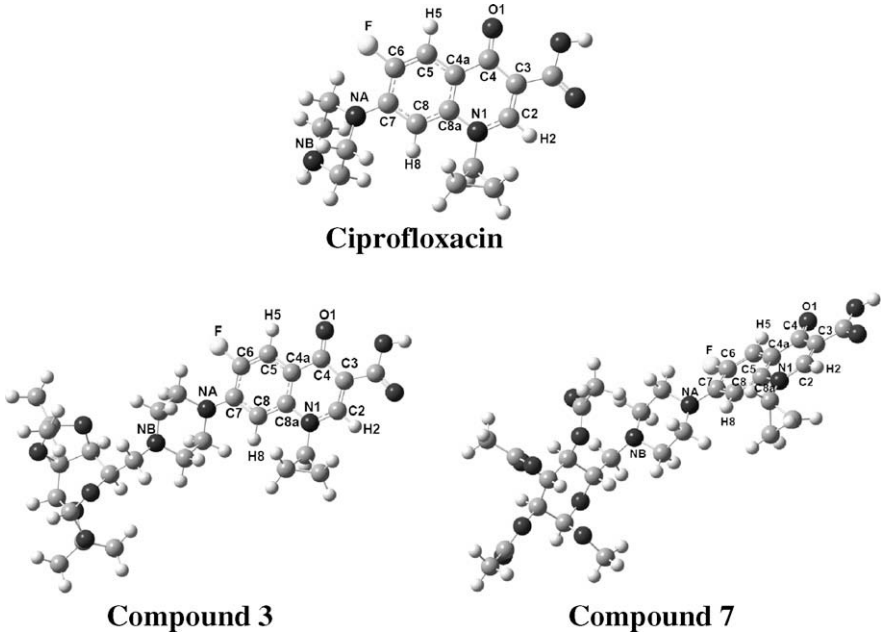


Figure 1. The most stable conformations for ciprofloxacin and compounds **3** and **7**.

on a Perkin–Elmer BX FTIR spectrophotometer as liquid films (neat) and only significant peaks were recorded. ^{1}H NMR (300 or 500 MHz) and ^{13}C NMR (75 or 125 MHz) spectra were recorded on solutions in $CDCl_3$ on a Bruker spectrometer. The high-resolution mass spectra were recorded on a Micromass LCT spectrometer, with electrospray ionization, at the Institut de Chimie des Substances Naturelles at Gif-sur-Yvette, France. The experiments were performed using a domestic microwave oven (Newtech® MO1180, 2450 MHz) specially modified for organic synthesis.

3.1. General procedure for the synthesis of compounds 3–9

The fluoroquinolones gati-, moxi-, and ciprofloxacin (0.5 mmol) in 8.0 mL of PhCN were treated with Et_3N or 60% NaH (2.0 mmol for gatifloxacin and moxifloxacin and 3.0 mmol for ciprofloxacin), and the mixtures were solubilized by sonication and heating. The iodo deoxysugar derivatives 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**, 1.0 mmol) and methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-α-D-glucopyranoside (**6**, 1.3 mmol) were added to the solution. The reaction mixture was then microwaved, and the reaction time was optimized for only one microwave pulse of 30 min, except for the ciprofloxacin derivative **7**, which required seven 30-min microwave pulses. The compounds were purified by column chromatography (CH_2Cl_2–MeOH), except for compound **9** which required purification in the dark by thin-layer chromatography (CH_2Cl_2–MeOH). The diastereomers of gatifloxacin derivative **8** were separated by UPLC–MS (for MS characterization) using an HSS C18 column (1.8 μm, 2.1 × 50 mm). The compounds were eluted isocratically with water (HCO_2H, 0.1%, v/v)/acetonitrile (HCO_2H, 0.1%, v/v), (70:30 v/v). The flow rate was fixed at 0.6 mL/min. Compounds **3**–**9** were obtained in 30–80% yields.

3.1.1. Physicochemical data for 1-cyclopropyl-7-[4-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)-1-piperazinyl]-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (3)

Mp 172–174 °C; $[\alpha]_D^{20}$ −45.5 (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 1725, 1626, 1466, 1256, 1067. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18 (m, 2H, $H_{c\text{-}Pr}$); 1.36 (m, 6H, CH_3); 1.38 (m, 2H, $H_{c\text{-}Pr}$); 1.46 (s, 3H, CH_3); 1.55 (s, 3H, CH_3); 2.62 (d, 1H, *J* = 13.5, $H_{6'}$); 2.76 (m, 4H, C*H*$_2$N); 2.85 (dd, 1H, *J* = 13.5 and 8.3, $H_{6''}$) 3.37 (m, 4H, C*H*$_2$N); 3.54 (tt, 1H, $H_{c\text{-}Pr}$, *J* = 7.2; 3.6); 4.01 (m, 1H, $H_{5'}$); 4.22 (dd, *J* = 8.0 and 1.4, $H_{4'}$); 4.33 (dd, *J* = 4.9 and 2.5, $H_{2'}$); 4.62 (dd, *J* = 7.7 and 2.2, $H_{3'}$); 5.59 (d, *J* = 4.9, $H_{1'}$); 7.33 (d, *J* = 7.1, H_8); 7.94 (d, *J* = 13, H_5); 8.71 (s, 1H, H_2); 15.02 (COOH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 8.4 (2 × $CH_{2c\text{-}Pr}$); 24.6, 25.0, 26.1, 26.2 (4 × $CH_{3i\text{-}Pr}$); 35.4 ($CH_{c\text{-}Pr}$); 49.8 (2 × CH_2N); 53.0 (2 × CH_2N); 58.0 (C-6′); 65.3 (C-5′); 70.5 (C-2′); 71.0 (C-3′); 72.7 (CH-4′); 96.8 (C-1′); 104.8 (d, $J_{8\text{-}F}$ 3.3, C-8); 108.2 ($C_{i\text{-}Pr}$); 108.6 (C-3); 109.5($C_{i\text{-}Pr}$); 112.4 (d, $J_{5\text{-}F}$ 24.1, C-5); 119.7 (d, $J_{7\text{-}F}$ 8.4, C-7); 139.2 (C-8a); 146.1 (C-4a); 147.5 (C-2); 153.8 (d, $Jc_{6\text{-}F}$ 249.5, C-6); 167.2 (COOH); 177.2 (C-4); HRESIMS: *m*/*z* calculated for $C_{29}H_{37}FN_3O_8$ $(M+H)^+$ 574.2565, found 574.2560.

3.1.2. Physicochemical data for (±)-1-cyclopropyl-7-[4-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)-3-methyl-1-piperazinyl]-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acid (4)

Mp 195–198 °C; IR (neat, cm^{-1}): 1728, 1617, 1441, 1242, 1066 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.00 (m, 2H, $H_{c\text{-}Pr}$); 1.15–1.21 (b, 5H, CHC*H*$_3$; 2xH, $H_{c\text{-}Pr}$); 1.34 (s, 3H, $CH_{3i\text{-}Pr}$); 1.35 (s, 3H, $CH_{3i\text{-}Pr}$); 1.47 (s, 3H, $CH_{3i\text{-}Pr}$); 1.54 (s, 3H, $CH_{3i\text{-}Pr}$); 2.67–2.77 (b, 3H, C*H*$_2$N, $CH_{piperazinyl}$); 3.04–3.13 (b, 3H, C*H*$_2$N, $H_{6'\ or\ 6''}$); 3.43 (b, 3H, C*H*$_2$N, $H_{6'\ or\ 6''}$); 3.75 (s, 1.5H, OMe); 3.77 (s, 1.5H, OMe) 3.99- 4.03 (m, 2H, $H_{5'}$, $CH_{c\text{-}Pr}$); 4.26–4.33 (b, 2H, $H_{4'}$, $H_{2'}$); 4.62 (m, 1H, $H_{3'}$); 5.55 (d, 0.5H, *J* = 5.0, $H_{1'}$); 5.57 (d, 0.5H, *J* = 5.0, $H_{1'}$) 7.85 (d, 1H, *J* = 12.0, H_5); 8.79 (s, 1H, H_2); 14.80 (COOH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.6–9.9 ($CH_{2c\text{-}Pr}$); 9.8 ($CH_{2c\text{-}Pr}$); 24.7–26.3 (4 × $CH_{3i\text{-}Pr}$); 40.7 ($CH_{c\text{-}Pr}$); 51.2–53.6 (3xCH_2N); 55.6 ($CH_{piperazinyl}$); 57.3 (C-6′); 64.8 (C-5′); 70.7–71.0 (C-2′, C-3′); 72.8 (C-4′); 96.9 (C-1′); 107.7–109.4 ($C_{i\text{-}Pr}$, C-5, C-3, $C_{i\text{-}Pr}$); 121.7 (C-7); 134.2 (C-8a); 140.0 (C-4a) 150.0 (C-2); 156.2 (C-6); 166.9 (COOH); 177.2 (C-4)); HRESIMS: *m*/*z* calculated for $C_{31}H_{41}FN_3O_9$ $(M+H)^+$ 618.2827, found 618.2823.

3.1.3. Physicochemical data for 1-cyclopropyl-7-[(4a*S*,7a*S*)-1-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)-octahydro-6*H*-pyrrolo[3,4-*b*]-pyridin-6-yl]-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acid (5)

Mp 196.5–198 °C; $[\alpha]_D^{20}$ −129.6 (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 1726, 1619, 1434, 1253, 1067; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (s, 3H, $CH_{3i\text{-}Pr}$); 1.11 (m, 2H, $H_{c\text{-}Pr}$); 1.26 (m, 2H, $H_{c\text{-}Pr}$); 1.32 (s, 3H, $CH_{3i\text{-}Pr}$); 1.33 (s, 3H, $CH_{3i\text{-}Pr}$); 1.52 (s, 3H, $CH_{3i\text{-}Pr}$); 1.58–184 (m, 4H, $H_{4'}$, $H_{5'}$); 2.28 (m, 1H, $H_{3'}$); 2.36–2.46 (m, 2H, $H_{6''\ or\ 6'''}$, $H_{4'a}$); 2.84–2.91 (m, 2H, $H_{6''\ or\ 6'''}$, $H_{3'}$); 2.98 (m, 1H, $H_{7'a}$); 3.33 (m, 1H, $H_{1'}$); 3.55 (s, 3H, OMe); 3.75–3.82 (m, 1H, $H_{6'}$); 3.89–3.95 (m, 2H, $H_{5''}$, $H_{1'}$); 3.97–4.03 (m, 2H, $CH_{c\text{-}Pr}$, $H_{6'}$); 4.16 (dd, 1H, *J*8.0 and 1.4, $H_{4''}$); 4.27 (dd, 1H, *J* = 4.9 and 2.5, $H_{2''}$); 4.48 (dd, 1H, *J* = 7.9 and 2.3, $H_{3''}$); 5.50 (d, 1H, *J* = 5.1, $H_{1''}$); 7.78 (d, *J* = 13.9, H_5); 8.75 (s, 1H, H_2); 15.13 (COOH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 8.9, 10.4 (2 × $CH_{2c\text{-}Pr}$); 22.1 (C-5′); 23.5 (C-4′); 24.2, 25.1, 26.0, 26.3 (4 × $CH_{3i\text{-}Pr}$); 37.8 (C-4′a); 40.7 ($CH_{c\text{-}Pr}$); 52.6 (C-3′); 53.5 (C-1′); 54.9 (C-6′); 55.0 (C-6″); 60.9 (OMe); 63.0 (C-7′a); 65.1 (C-5″); 70.7 (C-2″, C-3″); 71.7 (C-4″); 96.7(C-1″); 107.8 (d, $J_{5\text{-}F}$ 22.0, C-5); 108.6 (C-3, $C_{i\text{-}Pr}$); 109.0 ($C_{i\text{-}Pr}$); 117.5 (d, $J_{7\text{-}F}$ 8.6, C-7); 134.7 (C-8a); 137.8 (C-4a); 140.0 (C-8); 149.6 (C-2); 153.6 (d, $J_{6\text{-}F}$ 250.7, C-6); 167.4 (COOH); 176.7 (C-4)); HRESIMS: *m*/*z* calculated for $C_{33}H_{43}FN_3O_9$ $(M+H)^+$ 644.2983, found 644.2980.

3.1.4. Physicochemical data for 1-cyclopropyl-6-fluoro-1,4-dihydro-7-[4-(methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranosid-6-yl)-1-piperazinyl]-4-oxo-3-quinolinecarboxylic acid (7)

Mp 115–118 °C; $[\alpha]_D^{20}$ +71.7 (*c* 0.5, $CHCl_3$); IR (neat, cm^{-1}): 1746, 1626, 1454, 1222, 1034; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.19 (m, 2H, $CH_{2c\text{-}Pr}$); 1.39 ($CH_{2c\text{-}Pr}$); 2.00, 2.04, 2.07 (3 × CH_{3Ac}); 2.57 (m, 1H, $H_{6'}$); 2.62 (m, 1H, $H_{6'}$); 2.67 (m, 2H, C*H*$_2$N-Gluc); 2.82 (m, 2H, C*H*$_2$N-Gluc); 3.34 (m, 4H, 2 × C*H*$_2$N-FQ); 3.43 (s, 3H, OMe); 3.54 (m, 1H, $CH_{c\text{-}Pr}$); 3.99 (m, 1H, H-5′); 4.87 (dd, $J_{2''\text{-}1''}$ 3.2, $J_{2''\text{-}3''}$ 10.0, $H_{2'}$); 4.92 (m, 1H, $H_{1'}$); 5.10 (m, 1H, $H_{4'}$); 5.47 (m, 1H, $H_{3'}$); 7.33 (d, 1H, $J_{8\text{-}F}$ 6.0, H-8); 7.93 (d, 1H, *J* = 12.8, H_5); 8.71 (m, 1H, H_2); 15.00 (COOH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 8.4 (2 × $CH_{2c\text{-}Pr}$); 20.8, 20.9, 21.0 (3 × CH_{3Ac}); 35.5 ($CH_{c\text{-}Pr}$); 40.9, 50.0 (2 × CH_2N); 54.0 (2 × CH_2N); 55.7 (OMe); 58.3 (C-6′); 67.9 (C-5′); 70.4 (C-3′); 70.7 (C-4′); 71.1 (C-2′); 96.9 (C-1′); 104.9 (C-8); 108.2 (C-3); 112.4 (d, $J_{5\text{-}F}$ 23.6, C-5); 119.8 (d, $J_{7\text{-}F}$ 7.6, C-7); 139.2 (C-8a); 146.0 (d, $J_{4a\text{-}F}$ 10.3, C-4a); 147.5 (C-2); 153.8 (d, $J_{6\text{-}F}$ 252,0, C-6); 167.2 (COOH); 169.9, 170.3, 170.4 (3 × O*C*OCH$_3$); 177.2 (C-4); HRESIMS: *m*/*z* calculated for $C_{34}H_{43}FN_3O_{12}$ $(M+H)^+$ 656.2232, found 656.2233.

3.1.5. Physicochemical data for (±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-hydroxy-7-[3-methyl-4-(methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranosid-6-yl)-1-piperazinyl]-4-oxo-3-quinolinecarboxylic acid (8)

IR (neat, cm^{-1}): 1746, 1605, 1454, 1224, 1041; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.17 (m, 2H, $CH_{2c\text{-}Pr}$); 1.25 (s, 3H, CHC*H*$_3$); 1.28 (m, $CH_{2c\text{-}Pr}$); 2.02, 2.07, 2.09 (3 × CH_{3Ac}); 2.63 (br s, 4H, C*H*$_2$N, H-6′); 2.85 (br s, 3H, C*H*$_2$N, $CH_{piperazinyl}$); .3.15 (br s, 2H, $H_{6'}$); 3.47 (s, 3H, OMe); 4.02 (m, 1H, H-5′); 4.20 (br s, 1H, $CH_{c\text{-}Pr}$); 4.87 (m, 1H, $H_{2'}$); 4.92 (m, 1H, $H_{1'}$); 5.00 (m, 1H, $H_{4'}$); 5.49 (m, 1H, $H_{3'}$); 7.68 (d, 1H, *J* = 11.5, H_5); 8.80 (m, 1H, H_2); 14.66 (COOH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 10.0 (2 × $CH_{2c\text{-}Pr}$); 20.9, 21.0 (3 × CH_{3Ac}); 41.0 ($CH_{c\text{-}Pr}$); 51.3 (2 × CH_2N); 53.6 (2 × CH_2N); 55.9 (OMe); 57.9 (C-6′); 67.2 (C-5′); 70.2–71.2 (C-3′, C-4′, C-2′); 96.9 (C-1′); 103.1 (d, $J_{5\text{-}F}$ 22.4, C-5); 107.3 (C-3); 127.0 (d, $J_{7\text{-}F}$ 9.2, C-7); 147.1 (d, $J_{4a\text{-}F}$ 7.1, C-4a); 149.0 (C-2); 159.2 (d, $J_{6\text{-}F}$ 254.3,

C-6); 166.9 (COOH); 169.9, 170.3, 170.2 (3 × $OCOCH_3$); 177.2 (C-4); HRESIMS: *m*/*z* calculated for $C_{31}H_{39}FN_3O_{12}$ $(M+H)^+$ 664.2494, found 664.2493.

3.1.6. Physicochemical data for 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-1-(methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranosid-6-yl)-octahydro-6*H*-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid (9)

Mp 124.5–127 °C; $[\alpha]_D^{20}$ +29.6 (*c* 0.52, $CHCl_3$); IR (neat, cm^{-1}): 1747, 1619, 1445, 1227, 1047; ^1H NMR (500 MHz, $CDCl_3$): δ 1.01 (m, 1H, $CH_{2c\text{-}Pr}$); 1.17 (m, 1H, $CH_{2c\text{-}Pr}$); 1.60–1.80 (m, 4H, $H_{4'}$, $H_{5'}$), 1.98, 2.00, 2.05 (3s, 3 × CH_{3Ac}); 2.37 (m, 1H, $H_{4'a}$); 2.48–2.52 (m, 1H, $H_{3'}$); 2.61 (m, 2H, $H_{6''}$); 2.84 (m, 1H, $H_{3'}$); 3.20 (br s, 1H, $H_{7'a}$); 3.38 (s, 3H, OMe); 3.58 (br s, 5H, OMe; $H_{1'}$; $H_{3'}$); 3.71 (br s, 1H, $H_{1'}$) 3.79 (m, 1H, $H_{1'}$); 3.87 (m, 1H, $H_{5''}$); 4,01 (m, 1H, $CH_{c\text{-}Pr}$), 4.76 (dd, 1H, *J* = 3.4 and 10.0, $H_{2''}$); 4.83 (m, 1H, $H_{1''}$); 4.89 (m, 1H, $H_{4''}$); 5.39 (m, 1H, $H_{3''}$); 7.81 (d, 1H, $J_{5\text{-}F}$ 13.8, H_5); 8.78 (s, 1H, H_2); 15.11 (COOH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.52, 9.93 (2 × $CH_{2c\text{-}Pr}$); 20.9 (3 × CH_{3Ac}); 23.0 (C-5′); 23.9 (C-4′); 37.6 (C-4′a); 40.7 ($CH_{c\text{-}Pr}$); 51.5 (C-6′); 52.4 (C-1′); 54.3 (C-3′); 55.6 (OMe); 56.2 (C-6″); 61.1 (OMe); 62.9 (C-4′a); 69.0 (C-5″); 70.4 (C-3″); 71.0 (C-2″,C-4″); 96.7 (C-1″); 107.9 (d, $J_{5\text{-}F}$ 23.6, C-5); 108.1 (C-3); 117.5 (C-7); 134.7 (C-8a); 137.7 (d, $J_{4a\text{-}F}$ 12.0, C-4a); 140.6 (C-8); 149.8 (C-2); 153.7 (d, $J_{6\text{-}F}$ 250.4, C-6); 167.4 (COOH); 169.9, 170.3, 170.4, (3 × $OCOCH_3$); 176.9 (C-4); HRESIMS: *m*/*z* calculated for $C_{34}H_{43}FN_3O_{12}$ $(M+H)^+$ 704.2831, found 704.2830.

3.2. General procedure for the preparation of alkyl galactopyranose fluoroquinolone derivatives 13–15

A solution of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acid (**1**, 0.5 mmol), 6-(2-aminoethylamino, 3-aminopropylamino or 4-aminobutylamino)-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (0.5 mmol) and Et_3N (0.5 mmol) in 10 mL of CH_3CN was refluxed for 72 h. The solvent was removed to give a residue that was chromatographed on silica gel (CH_2Cl_2–MeOH) to furnish the desired compounds **13**, **14,** or **15** in 47%, 52%, and 56% yield, respectively.

3.2.1. Physicochemical data for 1-cyclopropyl-7-[*N*-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl-aminoethylamino)]-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acid (13)

Mp 80–83 °C; $[\alpha]_D^{20}$ −19.4 (*c* 1.26, $CHCl_3$); IR (neat, cm^{-1}): 1725, 1620, 1442, 1254, 1067; ^1H NMR (300 MHz, $CDCl_3$): δ 1.02 (m, 2H, $CH_{2c\text{-}Pr}$); 1.20 (m, 2H, $CH_{2c\text{-}Pr}$); 1.33 (m, 6H, $CH_{3i\text{-}Pr}$); 1.44 (s, 3H, $CH_{3i\text{-}Pr}$); 1.53 (s, 3H, $CH_{3i\text{-}Pr}$); 2.85 (dd, 1H, *J* = 12.7 and 3.6, $H_{6'}$); 2.96–3.03 (m, 3H, $H_{6'}$, CH_2N); 3.66 (m, 2H, CH_2N); 3.74 (s, 1H, OMe); 3.97 (m, 2H, $H_{5'}$, $CH_{c\text{-}Pr}$); 4.20 (dd, 1H, *J*8.0 and 1.9, $H_{4'}$); 4.33 (dd, 1H, *J*5.0 and 2.2, $H_{2'}$); 4.61 (dd, 1H, *J*7.9 and 2.3, $H_{3'}$); 5.47 (m, 1H, NH); 5.53 (d, *J* = 5.0, $H_{1'}$); 7.84 (d, 1H, *J* = 12.5); 8.75 (s, 1H, H_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.7, 9.8 (2 × $CH_{2c\text{-}Pr}$); 24.5, 25.1, 26.1, 26.2 (4 × $CH_{3i\text{-}Pr}$); 39.8 ($CH_{c\text{-}Pr}$); 44.2 (CH_2N); 48.9 (CH_2N); 49.0 (C-6′); 61.6 (OMe); 66.7 (C-5′); 70.7 (C-2′); 71.0 (C-3′); 72.0 (C-4′); 96.6 (C-1′); 107.6 (C-3); 108.5 (d, $J_{5\text{-}F}$ 22.5 Hz, C-5); 108.8 ($C_{i\text{-}Pr}$); 109.6 ($C_{i\text{-}Pr}$); 117.2 (d, $J_{7\text{-}F}$ 7.5, C-7); 133.4 (C-8a); 137.8 (C-4a); 149.6 (C-2); 151.3 (d, $J_{6\text{-}F}$ 247.0, C-6); 167.0 (COOH); 177.0 (C-4); HRESIMS: *m*/*z* calculated for $C_{28}H_{37}FN_3O_9$ $(M+H)^+$ 578.2514, found 578.2519.

3.2.2. Physicochemical data for 1-cyclopropyl-7-[*N*-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl-aminopropylamino)]-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acid (14)

Mp 104–107 °C; $[\alpha]_D^{20}$ −10.3 (*c* 0.93, $CHCl_3$); IR (neat, cm^{-1}): 1725, 1620, 1443, 1255, 1068; ^1H NMR (500 MHz, $CDCl_3$): δ 1.02 (m, 2H, $CH_{2c\text{-}Pr}$); 1.21 (m, 2H, $CH_{2c\text{-}Pr}$); 1.32 (s, 3H, $CH_{3i\text{-}Pr}$); 1.33 (s, 3H, $CH_{3i\text{-}Pr}$); 1.44 (s, 3H, $CH_{3i\text{-}Pr}$); 1.58 (s, 3H, $CH_{3i\text{-}Pr}$); 2.04 (m, 2H, CH_2CH_2N); 3.10 (m, 4H, $2H_{6'}$ and CH_2N); 3.70 (m, 2H, CH_2N); 3.75 (s, 3H, OMe); 3.98 (m, 1H, $CH_{c\text{-}Pr}$); 4.15 (m, 1H, $H_{5'}$), 4.23 (dd, 1H, *J*7.8, $H_{4'}$); 4.34 (dd, 1H, *J* = 4.8 and 1.9, $H_{2'}$); 4.63 (dd, 1H, *J* = 7.7 and 2.0, $H_{3'}$); 5.68 (m, 1H, NH); 5.49 (d, *J* = 4.6, $H_{1'}$); 7.82 (d, 1H, *J*12.2); 8.74 (s, 1H, H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.8 (2 × $CH_{2c\text{-}Pr}$); 24.4, 25.0, 26.1, 26.2 (4 × $CH_{3i\text{-}Pr}$); 28.7 ($CH_2CH_2CH_2N$); 39.8 ($CH_{c\text{-}Pr}$); 43.6 (CH_2N); 46.9 (CH_2N); 48.9 (C-6′); 61.8 (OMe); 65.4 (C-5′); 70.5 (C-2′); 70.9 (C-3′); 71.8 (C-4′); 96.4 (C-1′); 107.5 (C-3); 108.5 (d, *J*22.9, C-5); 109.2 ($C_{i\text{-}Pr}$); 109.8 ($C_{i\text{-}Pr}$); 117.2 (d, $J_{7\text{-}F}$ 7.5, C-7); 133.4 (C-8a); 137.6 (C-4a); 149.6 (C-2); 151.2 (d, $J_{6\text{-}F}$ 247.0, C-6); 167.3 (COOH); 177.0 (C-4); HRESIMS: *m*/*z* calculated for $C_{29}H_{39}FN_3O_9$ $(M+H)^+$ 592.2670, found 592.2668.

3.2.3. Physicochemical data for 1-cyclopropyl7-[*N*-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl-aminobutylamino)]-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acid (15)

Mp 108–109 °C; $[\alpha]_D^{20}$ −15.9 (*c* 0.98, $CHCl_3$); IR (neat, cm^{-1}): 1725, 1620, 1443, 1253, 1068; ^1H NMR (500 MHz, $CDCl_3$): δ 1.02 (m, 2H, $CH_{2c\text{-}Pr}$); 1.21 (m, 2H, $CH_{2c\text{-}Pr}$); 1.32 (s, 3H, $CH_{3i\text{-}Pr}$); 1.33 (s, 3H, $CH_{3i\text{-}Pr}$); 1.44 (s, 3H, $CH_{3i\text{-}Pr}$); 1.54 (s, 3H, $CH_{3i\text{-}Pr}$); 1.73 (m, 4H, $CH_2CH_2CH_2N$); 2.83 (CH_2N); 2.92 (m, 1H, $H_{6'}$); 2.92 (m, 1H, $H_{6'}$); 3.0 (m, 1H, $H_{6'}$); 3.56 (m, 2H, CH_2N); 3.72 (s, 3H, OMe); 3.97 (m, 1H, $CH_{c\text{-}Pr}$); 4.00 (m, 1H, $H_{5'}$); 4.21 (d, 1H, *J* = 7.9, $H_{4'}$); 4.33 (dd, 1H, *J* = 5.0 and 2.2, $H_{2'}$); 4.61 (dd, 1H, *J* = 7.5 and 1.8, $H_{3'}$); 4.86 (m, 1H, NH); 5.53 (d, 1H, *J* = 5.0, $H_{1'}$); 7.84 (d, 1H, *J* = 12.5); 8.75 (s, 1H, H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.8 (2 × $CH_{2c\text{-}Pr}$); 24.5, 25.1, 26.1, 26.2 (4 × $CH_{3i\text{-}Pr}$); 26.4 and 28.6 ($CH_2CH_2CH_2N$); 39.8 ($CH_{c\text{-}Pr}$); 45.3 (CH_2N); 48.9 (CH_2N); 49.3 (C-6′); 61.5 (OMe); 66.1 (C-5′); 70.7 (C-2′); 71.0 (C-3′); 72.0 (C-4′); 96.5 (C-1′); 107.6 (C-3); 108.6 (d, $J_{5\text{-}F}$ 22.1 Hz, C-5); 109.0 ($C_{i\text{-}Pr}$); 109.6 ($C_{i\text{-}Pr}$); 117.2 (d, $J_{7\text{-}F}$ 8.3, C-7); 133.4 (C-8a); 137.6 (C-4a); 149.6 (C-2); 151.1 (d, $J_{6\text{-}F}$ 246.2, C-6); 167.3 (COOH); 177.1 (C-4); HRESIMS: *m*/*z* calculated for $C_{30}H_{41}FN_3O_9$ $(M+H)^+$ 606.2827 found 606.2822.

4. Conclusions

In this work we have reported the synthesis of nine new analogues of cipro-, gati-, and moxifloxacin condensed with sugar derivatives, together with their antitubercular evaluation showing MICs between 2.5 and 10 μg/mL. The preparation of compounds **3–5** and **7–9** was improved using microwave irradiation. This method showed advantages in comparison with classical reaction conditions, such as better yields and shorter reaction times. Derivatives **4**, **5,** and **7** exhibited a promising inhibitory activity when compared with drugs used as positive controls.

Acknowledgments

The authors gratefully acknowledge CAPES and CNPq for fellowships and Dr. Robert H. Dodd for the revision of this work. This research was supported by FAPEMIG and partially funded by NIAD contract number N01-AI-60011.

References

1. De Souza, M. V. N.; De Almeida, M. V.; Couri, M. R. C.; Da Silva, A. D. *Curr. Med. Chem.* **2003**, *10*, 21–40.
2. De Souza, M. V. N. *Mini-Rev. Med. Chem.* **2005**, *5*, 1009–1018.
3. De Souza, M. V. N. *Curr. Opin. Pulmon. Med.* **2006**, *12*, 167–171.
4. De Almeida, C. G.; Diniz, C. G.; Silva, V. L.; Saraiva, M. F.; Le Hyaric, M.; De Almeida, M. V. *Med. Chem.* **2009**, *5*, 419–421.
5. De Souza, M. V. N.; Vasconcelos, T. R. A.; De Almeida, M. V.; Cardoso, S. H. *Curr. Med. Chem.* **2006**, *13*, 455–463.
6. Lugo, M. T. G.; Bewley, C. A. *J. Med. Chem.* **2008**, *51*, 2606–2612.

7. De Almeida, M. V.; Saraiva, M. F.; De Souza, M. V. N.; Da Costa, C. F.; Vicente, F. R. C.; Lourenco, M. C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5661–5664.
8. Faria, A. F.; De Souza, M. V. N.; De Oliveira, M. A. L. *J. Braz. Chem. Soc.* **2008**, *19*, 389–396.
9. Lourenco, M. C. S.; Neves Junior, I.; De Souza, M. V. N. *Med. Malad. Infect.* **2007**, *37*, 295–298.
10. De Oliveira, M. A. L.; Faria, A. F.; De Almeida, M. V.; De Souza, M. V. N. *Anal. Chim. Acta* **2006**, *579*, 185–192.
11. Boechat, N.; Kretlli, A.; De Souza, M. V. N.; Vasconcellos, T. A. *Int. J. Antimicrob. Agents* **2006**, *28*, 270–271.
12. Iwata, M.; Kimura, T.; Fujiwara, Y.; Katsube, T. EP 241206 A2 19871014, 1987.
13. De Souza, M. V. N.; Saraiva, M. F.; Pinheiro, A. C.; De Almeida, M. V.; Valle, M. S. *The Scientific World J.* **2008**, *8*, 720–751.
14. Taveira, A. F.; Le Hyaric, M.; Reis, E. F. C.; Araujo, D. P.; Ferreira, A. P.; De Souza, M. A.; Alves, L. L.; Lourenço, M. C. S.; Vicente, F. R. C.; De Almeida, M. V. *Bioorg. Med. Chem.* **2007**, *15*, 7789–7794.
15. Junior, C. O. R.; Le Hyaric, M.; Da Costa, C. F.; Correa, T. A.; Taveira, A. F.; Araujo, D. P.; Reis, E. F. C.; Lourenço, M. C. S.; Vicente, F. R. C.; De Almeida, M. V. *Mem. Inst. Oswaldo Cruz* **2009**, 703–705.
16. Deng, L.; Mikusova, K.; Robuck, K. G.; Scherman, M.; Brennan, P. J.; McNeil, M. R. *Antimicrob. Agents Chemother.* **1995**, *39*, 694–701.
17. Raymond, A. L.; Schroeder, E. F. *J. Am. Chem. Soc.* **1948**, *70*, 2785–2791.
18. Zief, M.; Hockett, R. C. *J. Am. Chem. Soc.* **1945**, *67*, 1267–1272.
19. Lin, T. S.; Harmon, R. E. *Org. Prep. Proced. Int.* **1975**, *7*, 11–18.
20. De Almeida, M. V.; Cesar, E. T.; Fontes, A. P. S.; Felicio, E. C. A. *J. Carbohydr. Chem.* **2000**, *19*, 323–329.
21. De Almeida, M. V.; Le Hyaric, M.; Amarante, G. W.; Lourenco, M. C. S.; Brandão, M. L. L. *Eur. J. Med. Chem.* **2007**, *42*, 1076–1083.
22. Canetti, G.; Rist, N.; Grosset, J. *Rev. Tuberc. Pneumol.* **1963**, *27*, 217–272.
23. Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. *J. Clin. Microbiol.* **1998**, *36*, 362–366.
24. Vanitha, J. D.; Paramasivan, C. N. *Diagn. Microbiol. Infect. Dis.* **2004**, *49*, 179–182.
25. Reis, R. S.; Neves, I., Jr.; Lourenço, S. L. S.; Fonseca, L. S.; Lourenço, M. C. S. *J. Clin. Microbiol.* **2004**, *42*, 2247–2248.
26. http://www3.niaid.nih.gov/LabsAndResources/resources/toolkit/protocol/.
27. Chang, G.; Guida, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379–4386.
28. Polak, E.; Ribiere, G. *Rev. Française Inf. Rech. Oper.* **1969**, *16-R1*, 35–43.
29. Mohamadi, F.; Richards, N. J. G.; Guida, W. C.; Liskamp, R.; Lipton, M. C.; Caufield, M.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.
30. Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134.
31. Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta* **1973**, *28*, 213–222.
32. Petersson, G. A.; Bennett, A.; Tensfeldt, T. G.; Al-Laham, M. A.; Shirley, W. A.; Mantzaris, J. *J. Chem. Phys.* **1988**, *89*, 2193–2218.
33. Petersson, G. A.; Al-Laham, M. A. *J. Chem. Phys.* **1991**, *94*, 6081–6090.
34. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
35. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski. V. G.; Montgomery, J. A.; Vreven, T., Jr.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T. A.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. GAUSSIAN 03, GAUSSIAN, Inc.: Wallingford CT, 2004.
36. Mitsher, L. A. *Chem. Rev.* **2005**, *105*, 559–592.