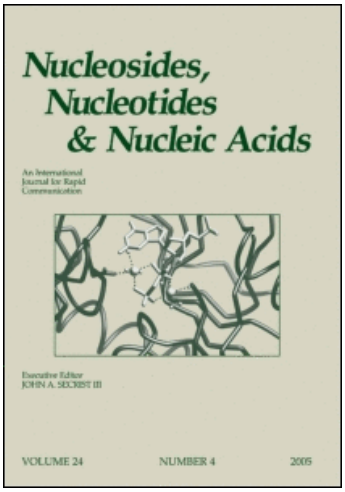


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

6-Hydroxy Derivative as New Desfluoroquinolone (DFQ): Synthesis and DNA-Binding Study

Oriana Tabarrini[a]; Claudia Sissi[b]; Arnaldo Fravolini[a]; Manlio Palumbo[b]
[a] Istituto di Chimica e Tecnologia del Farmaco, Università degli Studi di Perugia, Perugia, Italy [b] Dipartimento di Scienze Farmaceutiche, Università di Padova, Padova, Italy

To cite this Article Tabarrini, Oriana , Sissi, Claudia , Fravolini, Arnaldo and Palumbo, Manlio(2000) '6-Hydroxy Derivative as New Desfluoroquinolone (DFQ): Synthesis and DNA-Binding Study', Nucleosides, Nucleotides and Nucleic Acids, 19: 8, 1327 — 1336
To link to this Article: DOI: 10.1080/15257770008033055
URL: http://dx.doi.org/10.1080/15257770008033055

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(8), 1327–1336 (2000)

6-HYDROXY DERIVATIVE AS NEW DESFLUOROQUINOLONE (DFQ): SYNTHESIS AND DNA-BINDING STUDY [1]

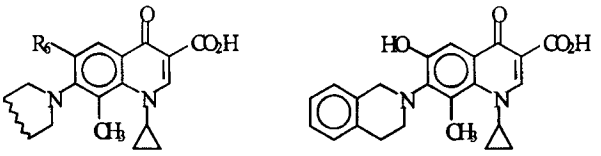
Oriana Tabarrini, [a] Claudia Sissi, [b] Arnaldo Fravolini, [a*] and Manlio Palumbo [b*]

[a] Istituto di Chimica e Tecnologia del Farmaco, Università degli Studi di Perugia, 06123 Perugia, Italy, [b] Dipartimento di Scienze Farmaceutiche, Università di Padova, 35131 Padova, Italy.

ABSTRACT. A new 6-desfluoroquinolone derivative, characterized by the presence of a 6-hydroxyl group instead of the usual fluorine atom at the C-6 position, was synthesized with the aim to better understand the mechanistic role of the C-6 substituent in the quinolone/DNA/DNA-gyrase interaction. The antibacterial activity unambiguously shows that the hydroxyl group is a good substitute for the C-6 fluorine atom, especially against Gram-positive bacteria. On the contrary, it is a very weak inhibitor of the target DNA gyrase, displaying the highest IC_{50} value observed for all the C-6 substituted analogues. This behaviour could be explained on the basis of its DNA binding properties.

Introduction. Quinolones are an important class of antibacterial agents. Although the earlier compounds were not fluorinated, it soon became apparent that the introduction of a fluorine atom at the C-6 position, increased the potency and amplified the activity spectrum, overcoming the pharmacokinetic gap of their predecessors. Starting with norfloxacin, the fluorine atom at C-6 represents a structural feature common to all quinolones in therapeutic and clinical development. We recently examined a large series of 6-desfluoroquinolones (DFQ), 6-amino[2-4] and 6-hydrogen[5] derivatives (FIG. 1) and showed that a high level of activity was still retained when the C-6 fluorine atom was replaced with an amino group or even with a hydrogen atom when an appropiate substituent set is coupled in the quinolone ring.

The 6-desfluoroquinolone DNA-binding properties were also investigated in the presence/absence of Mg^{++} ions.[6] Both in the 6-amino and 6-fluoro series, a correlation was found with gyrase inhibition activity, whereas the unsubstituted analogue exibited



6-desfluoroquinolones
$R_6 = NH_2$, H

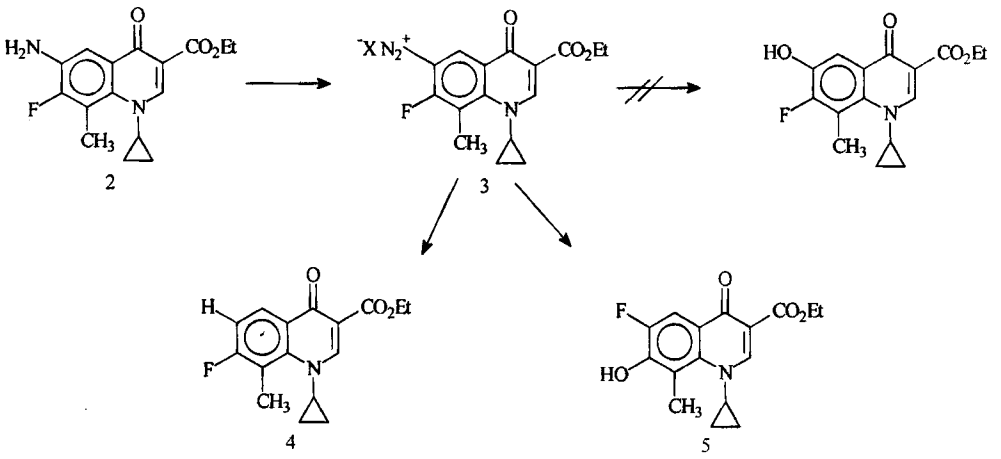
1

FIGURE. 1: Chemical structure of compound 1 and reference derivatives

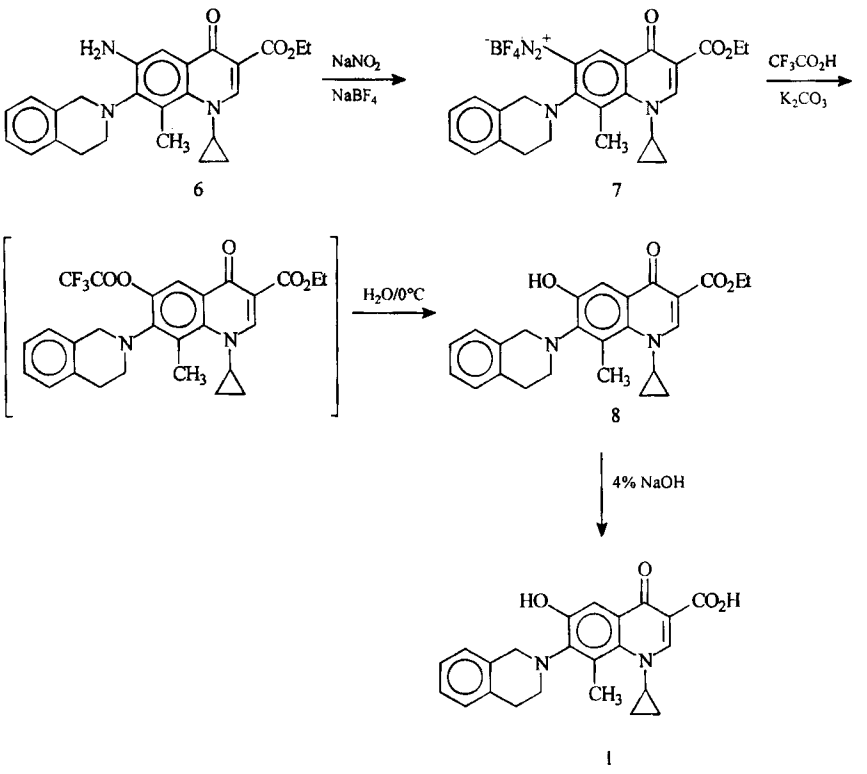
enzyme poisoning at higher drug concentrations. This observation supports the idea that, besides playing a pharmacokinetic role, the C-6 substituent is involved in the recognition of an enzyme pocket through polar interaction, perhaps a hydrogen bond.

To better understand the mechanistic role of C-6 substitution, we have introduced a hydroxyl group at that position obtaining the first 6-hydroxyquinolone (**1**) (FIG. 1). In addition, it bears those substituents which were previously shown to grant the highest activity: a methyl group at C-8, a cyclopropyl group at N-1 and a tetrahydroisoquinolinyl side chain at the C-7 position. In this paper, we report the synthesis, the DNA-binding properties and the biological activity of 1-cyclopropyl-6-hydroxy-8-methyl-4-oxo-7-(1,2,3,4-tetrahydro-2-isoquinolinyl)-1,4-dihydroquinoline-3-carboxylic acid (**1**), and compare the results with those previously reported for the corresponding 6-desfluoro (NH_2, ^3H [5]) as well as 6-fluoro analogues.[3]

Chemistry. Our initial strategy to prepare 6-hydroxyquinolone **1** was by hydroxy-de-diazoniation of 6-amino-7-fluoro derivative **2** obtained by catalytic reduction of previously synthesized ethyl 1-cyclopropyl-7-fluoro-8-methyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate analogue[3] (SCHEME 1). Thus amino derivative **2** was converted into its diazonium salt **3** (chloride or tetrafluoborate) and then decomposed under various experimental conditions. Unfortunately, all attempts to obtain the desired 6-hydroxy derivative failed, while two main by-products were identified as the 6-hydrogen derivative **4** and 6-fluoro-7-hydroxy derivative **5**. Therefore we had to carry out a total synthesis starting directly from 6-amino-7-tetrahydroisoquinolinyl derivative **6**, which was synthesized as previously reported[3] (SCHEME 2).



SCHEME 1



SCHEME 2

Among the many hydroxy-de-diazonation conditions used, (boiling aqueous H_2SO_4, boiling trifluoroacetic acid/ K_2CO_3,[7] or addition of Cu_2O to a diluted solution of diazonium salt in large excess of $Cu(NO_3)_2$ solution[8]), only the decomposition of diazonium tetrafluoborate **7** with a mixture of K_2CO_3 in trifluoroacetic acid, followed by water hydrolysis of trifluoroacetate intermediate, allowed the hydroxy derivative **8** to be obtained, even if in low yield.

Hydrolysis with 4 % NaOH gave the target 6-hydroxy carboxylic acid **1**.[9]

The logP [10] value calculated for the new derivative **1**, as well as for other structurally related quinolones, are reported in Table 1.

Metal ion interaction. All classical quinolones efficiently bind metal ions like Mg^{2+}, a process which also modulates DNA complex formation. Addition of Mg^{2+} to compound **1** induces a remarkable decrease of the drug fluorescence quantum yield, supporting the formation of a quinolone-metal ion complex. Titration experiments were performed to calculate the ion-quinolone binding constant. Assuming a 1:1 complex stoichiometry, as in previous cases[11], in 10 mM TRIS, 20 mM NaCl, pH 7.0, 25°C, we obtained a complex formation constant $K_{Mg} = 2200 \pm 110\ M^{-1}$.[12] A comparison with literature data, shows a very efficient interaction of the 6-hydroxy derivative to Mg^{2+}, with the K_{Mg} being the highest thus far observed for structurally related quinolones.[6] As previously pointed out, the presence of a tetrahydroisoquinolinyl side chain at the C-7 position makes the drug negatively charged at pH 7.0, at which classical quinolones are zwitterionic; this obviously favors complexation to the positively charged ion. In addition, the introduction of a hydroxyl group on the planar ring system tends to increase the electron density at the metal coordination site.

DNA binding. The DNA binding properties of **1** were investigated using both single- and double-stranded DNA in the presence/absence of Mg^{2+}.[13] In the absence of the metal ion no evidence for quinolone interaction was observed with either DNA. However, in the presence a near physiological concentration of Mg^{2+} (1 mM) a modest binding to dsDNA was observed with a complex formation constant $K_i \approx 200\ M^{-1}$ (data not shown). In the presence of ssDNA and Mg^{2+} (1 mM), the formation of two different complexes was monitored. The addition of ssDNA to a solution of **1** initially induces a decrease in

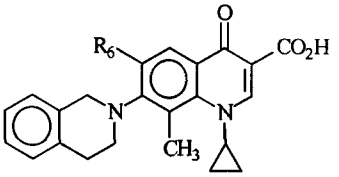


TABLE 1. Comparative In Vitro Antibacterial Activity, Inhibitory Effect (IC_{50}, μg/mL) on Gyrase Supercoiling Activity from *E. coli*, and logP for 6-Hydroxy-8-methyl-7-tetrahydroisoquinolinyl derivative (**1**), and Its Reference Agents.

		MICs, μg/mL				
organism[a]	R_6	**OH**	**H** [b]	**NH_2** [c]	**F** [b]	**CPX**[d]
Gram-positives						
S. au. ATCC 29213		≤0.016	≤0.016	≤0.016	≤0.016	0.5
S. au. MPR 5		≤0.016	0.03	≤0.016	≤0.016	0.25
S. au. M-R[e] POMM 6214		≤0.016	0.25	≤0.016	≤0.016	1
S. au. CPX-R[f] OBT 687		1	2	2	1	>16
S. py. OMNFI BI		0.16	0.25	≤0.016	0.03	0.5
S. pn. I 043		1	1	0.25	0.25	1
E. fe. LEP Br		0.25	1	0.25	0.5	1
E. fe. UCMC 39690		0.06	0.5	0.125	0.06	0.5
Geometric means		0.099	0.272	0.075	0.074	0.917
Gram-negatives						
E. co. ATCC 25922		0.125	1	0.125	0.125	≤0.016
E. co. 120		0.125	2	0.125	0.25	≤0.016
E. co CPX-R OBT 431		>16	16	>16	>16	8
E. cl. OMNFI 174		2	0.03	0.5	0.5	≤0.016
P. mi. OBT 505		2	>16	>16	4	0.06
P. vu. CNUR 6		16	>16	8	4	0.5
K. pn. ATCC 10031		0.06	0.125	0.03	0.06	≤0.016
P. ae. ATC1C 9027		1	>16	8	4	0.06
P. ae. OBT 307		>16	>16	>16	16	1
H. in.		≤0.016	0.06	≤0.016	≤0.016	≤0.016
geometric means		0.869	1.851	0.998	0.869	0.083
IC_{50}[g]		14.41	7.8	3.6	3.0	0.68
logP[h]		3.50	3.78	3.00	3.92	1.14

[a] Organisms selected are as follows: *S. au.*, *Staphylococcus aureus*; *S. py.*, *Streptococcus pyogenes*; *S. pn.*, *Streptococcus pneumoniae*; *E. fe.*, *Enterococcus faecalis*; *E. co.*, *Escherichia coli*; *E. cl.*, *Enterobacter cloacae*; *P. mi.*, *Proteus mirabilis*; *P. vu.*, *Proteus vulgaris*; *K. pn.*, *Klebsiella pneumoniae*; *P. ae.*, *Pseudomonas aeruginosa*; *H. in.*, *Haemophilus influenzae*. [b] See Reference 5. [c] See Reference 3. [d] CPX = ciprofloxacin. [e] M-R = methicillin-resistant. [f] CPX-R = ciprofloxacin-resistant. [g] Calculated by quantitative measurement of the supercoiled DNA peak in an agarose gel by densitometric assay.

the fluorescence quantum yield which is later reversed at high polynucleotide concentrations (FIG. 2). In the latter case, the maximum is shifted to a lower wavelength. For the second complex, it was not possible to reach saturation. It is noteworthy that increasing the Mg^{2+} concentration progressively suppresses the first binding mode. Like to other quinolones, it can be proposed that the complex at a low DNA-quinolone ratio corresponds to the models proposed in the literature with drug stacking onto DNA bases.[14] The second complex is probably related to a different geometry of interaction which involves the 6-hydroxy group and the metal ion. Since this is predominant at high Mg^{2+} concentrations, a double Mg-bridging could be proposed, coordinating quinolone to DNA phosphates.

BIOLOGICAL EVALUATION

Inhibition of DNA gyrase. The ability to inhibit DNA gyrase supercoiling activity was investigated by using a previously described protocol.[15] The results are summarized in TABLE 1, where data relative to quinolones bearing different substituents at the C-6 position, as well as to ciprofloxacin, are reported. The IC_{50} value for compound **1** is the highest observed for 8-methyl-7-tetrahydroisoquinolinyl derivatives. Clearly, its recognition of the *E. coli* DNA gyrase/DNA cleavable complex is rather poor. This can be related to the very weak interaction between the 6-hydroxy analogue and ssDNA. A inverse linear relationship between the extent of the Mg^{2+}-mediated quinolone/DNA interaction and the gyrase inhibitory concentration was previously observed.[6]

Antibacterial activity. The 6-hydroxyquinolone **1** was tested *in vitro* against an assortment of eight Gram-positive and ten Gram-negative organisms, including some clinical isolates and a ciprofloxacin-resistant *Escherichia coli* and *Staphylococcus aureus* (MRSA). The minimun inhibitory concentrations (MICs, μg, mL) determined by the microdilution technique using nutrient broth, according to NCCLS,[16] were reported in TABLE 1.

In the bacterial strains, the 6-hydroxy derivative exhibits an activity comparable to previously tested 8-methyl-derivatives bearing a fluorine or an amino group at C-6 position. As previously pointed out for other 6-desfluoroquinolones, the tested drug

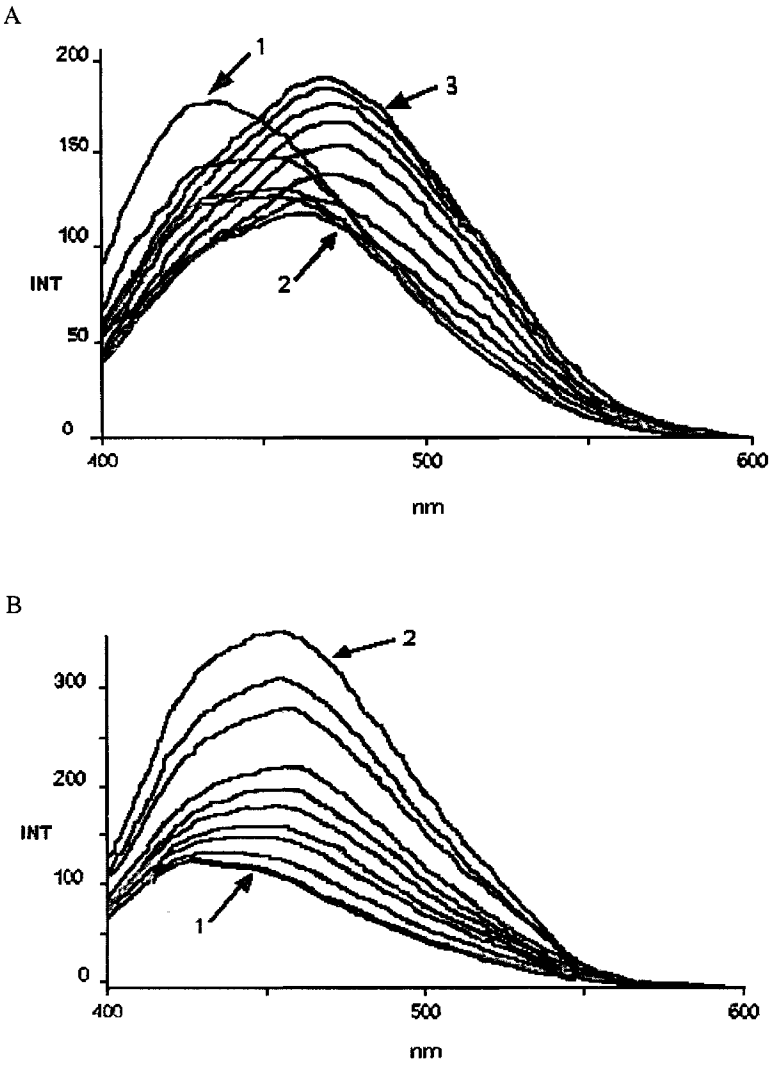


FIGURE. 2: Fluorometric titrations of compound **1** (2.6 μM) with ssDNA in 10 mM TRIS, 20 mM NaCl pH 7.00, 25°C: Panel A: in the presence of 1 mM Mg^{2+}. Arrows 1, 2 and 3 refer to [ssDNA] = 0, 0.77 and 3.95 mM respectively. Panel B: in the presence of 10 mM Mg^{2+}. Arrows 1 and 2 refer to [ssDNA] = 0 and 47 mM respectively.

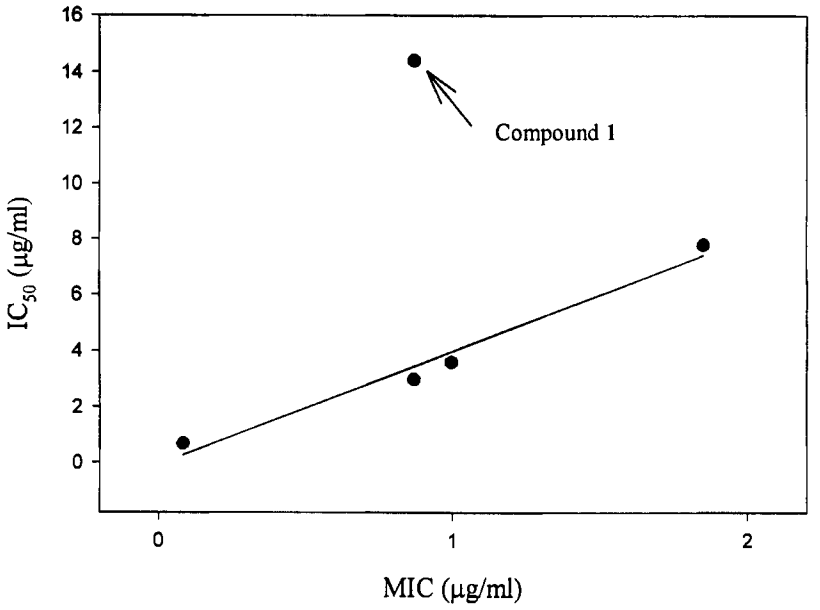


FIGURE. 3: Dependence of Gram-negative geometric mean MICs to DNA gyrase IC_{50} for test compounds

exhibits a higher activity in Gram-positive organisms than ciprofloxacin with MICs one order of magnitude lower.

CONCLUSIONS

The novel 6-hydroxy compound confirms the activity pattern found for the 6-fluoro and 6-amino analogues. In particular, it is remarkably active against Gram-positive bacteria, whereas on the average the latter performs ten times better against Gram-negative strains. Our data unambiguously show that the OH group can successfully replace F or NH_2 in terms of antibacterial response; clearly the activity inversion from Gram-negatives to Gram-positives is related to the structure of C-7 substituent (compare fluoro derivative and ciprofloxacin in TABLE 1).

As far as gyrase poisoning is concerned, the novel compound shows a remarkably reduced effect when compared to the congeners; it is even worse than the unsubstituted analogue. It was previously noted that the tetrahydroisoquinoline substitution at C-7

position increases IC_{50} value by a factor ≈ 5. In the case of derivative **1**, we observed a much higher (≈ 20) increment compared to ciprofloxacin. A plot of IC_{50} vs. Gram-negative MICs shows that derivative **1** can be considered an outlier with reference to its congeners (FIG. 3). This might be related to its dual DNA binding mode, only one of which is apparently adequate to be effective at the gyrase/DNA complex level. Nonetheless, the relatively high biological activity suggests another mode of bacterial cell killing by the 6-hydroxy derivative. It is very unlikely that the low MIC can be granted by a much improved uptake of compound **1**, which exhibits a logP value close to that of the parent drugs.

REFERENCES AND NOTES

1. Part of this work was previously presented: Cecchetti, V.; Fravolini, A.; Palumbo, M.; Sissi, C.; Tabarrini, O. First Italian-Swiss Meeting on Medicinal Chemistry, Torino, September **1997**, ML20.
2. Cecchetti, V.; Clementi, S.; Cruciani, G.; Fravolini, A.; Pagella, P. G.; Savino, A.; Tabarrini, O. *J. Med. Chem.* **1995**, *38*, 973-982.
3. Cecchetti, V.; Fravolini, A.; Lorenzini, M. C.; Tabarrini, O.; Terni, P.; Xin, T. *J. Med. Chem.* **1996**, *39* , 436-445.
4. Wise, R.; Pagella, P. G.; Cecchetti, V.; Fravolini, A.; Tabarrini, O. *Drugs* 1995, 49, 272-273.
5. Cecchetti, V.; Fravolini, A.; Palumbo, M.; Sissi, C.; Tabarrini, O.; Terni, P.; Xin, T. *J. Med. Chem.* **1996**, *39* , 4952-4957.
6. Sissi, C.; Andreolli, M.; Cecchetti, V.; Fravolini, A.; Gatto, B.; Palumbo, M. *Bioorg. Med Chem.* **1998**, *6*, 1555-1561.
7. Horning, D. E.; Ross, D. A.; Muchowsk, J. M. *Can. J. Chem.* **1977**, *42*, 2347-2348.
8. Cohn, T.; Dietz, A. G. Jr.; Miser, J. R. *J. Org. Chem.* **1977**, *42*, 2053-2058.
9. Compound **1**: mp 256-258 °C; ^{1}H NMR(DMSOd_6) δ 0.75-0.95 and 1.05-1-15 (each 2 H, m, cyclopropyl CH_2), 2.65 (3 H, s, CH_3), 2.85-3.10 and 3.30-3.60 (each 2 H, m, isoquinoline CH_2), 4.25-4.50 (3 H, m, isoquinoline CH_2, cyclopropyl CH), 5.10-5.50 (1 H, bs, OH), 7.00-7.25 (4 H, m, isoquinoline Ar-H), 7.35 (1 H, s, H-5), 8.60 (1 H, s, H-2). *Anal.* Calcd. for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.17.Found: C, 70.90; H, 5.87; N, 7.15.

10. Molecular Operating Environment (MOE 1998, 10), chemical Computing Group, Inc, 1255 University St., Suite 1600, Montreal, Qubec, Canada, H3B 3x3.
11. Palumbo, M.; Gatto, B.; Zagotto, G.; Palù. G. *Trends Microbiol.* **1993**, *1*, 232-235.
12. Stock solutions (1 mg/ml) were made in DMSO and diluted to the working concentration in the desired buffer Titrations were carried out in TRIS 10 mM, NaCl 20 mM, pH 7.0, 25 °C. Experimental procedure and data analysis were performed as previously reported.
13. DNA Gyrase and relaxed pBR 322 were from Lucent Ltd (Leicester, UK). Inhibition assays were performed according to the protocol provided by the supplier.
14. Palù, G.; Valisena,S.; Ciarrocchi, G.; Gatto, B.; Palumbo, M. *Proc. Nat. Acad. Sci. U. S. A.* **1992**, *89*, 9671-9675
15. Antonello, C.; Uriarte, E; Palumbo, M.; Valisena, S.; Parolin, C.; Palù, G. *Eur J. Med. Chem.* **1993**, *28*, 291-296.
16. Method for dilution: antimicrobial susceptibility tests for bacteria grown aerobically; approved standard M7-A3. National Committee for Clinical Laboratory Standards. 1993; NCCLS: Villanova, PA, 1993.