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Synthesis and antibacterial activity of *N*-(5-benzylthio-1,3,4-thiadiazol-2-yl) and *N*-(5-benzylsulfonyl-1,3,4-thiadiazol-2-yl)piperazinyl quinolone derivatives

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Abstract—A series of *N*-(5-benzylthio-1,3,4-thiadiazol-2-yl) and *N*-(5-benzylsulfonyl-1,3,4-thiadiazol-2-yl) derivatives of piperazinyl quinolones was synthesized and evaluated for antibacterial activity against Gram-positive and Gram-negative microorganisms. Some of these derivatives exhibit high activity against Gram-positive bacteria; *Staphylococcus aureus* and *Staphylococcus epidermidis*, comparable or more potent than their parent *N*-piperazinyl quinolones norfloxacin and ciprofloxacin as reference drugs. The SAR of this series indicates that both the structure of the benzyl unit and the S or SO_2 linker dramatically impact antibacterial activity.

Quinolones have become a major class of antibacterial agents, which are under extensive clinical development. They have an attraction because of their extremely potent activity, rapid bactericidal effects, and low incidence of resistance development.[1] The main disadvantage of the quinolones is their limited activity against Gram-positive pathogens (because of their intrinsically low activity against these species) and methicillin-resistant *Staphylococcus aureus* (MRSA) (because of the development of resistance).[2] In addition, quinolones can cause certain adverse effects, such as CNS effects, phototoxicity, tendonitis, hypoglycemia, and serious cardiac dysrhythmias.[3,4] Thus, despite many advances in the fluoroquinolone field, there exists continuous need for novel quinolones with better activity profile, pharmacokinetics, and tolerability, to overcome the limitations of existing drugs.

Most of the quinolone antibacterial research has been focused on the functionality at C-7 position since the introduction of norfloxacin **1** and ciprofloxacin **2** (Figs. 1 and 2). Moreover, C-7 substituent is the most adaptable site for chemical change and is an area that determines potency and target preference. This area also controls the pharmacokinetic properties of the drugs, with a basic nitrogen.[5–7] A five- or six-membered ring is the most commonly found substitution at position C-7, for example, gemifloxacin and trovafloxacin, have an aminopyrrolidine substituent at C-7.[8,9] Piperazine substitution at C-7 position has resulted in a wide range of clinically useful fluoroquinolone antibacterial agents namely norfloxacin, ciprofloxacin, perfloxacin, pefloxa-

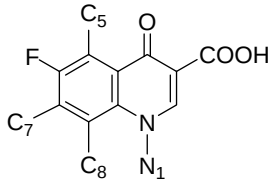

Figure 1. Quinolone core with main substitution sites.

Keywords: Quinolone; 1,3,4-Thiadiazole; Antibacterial activity.
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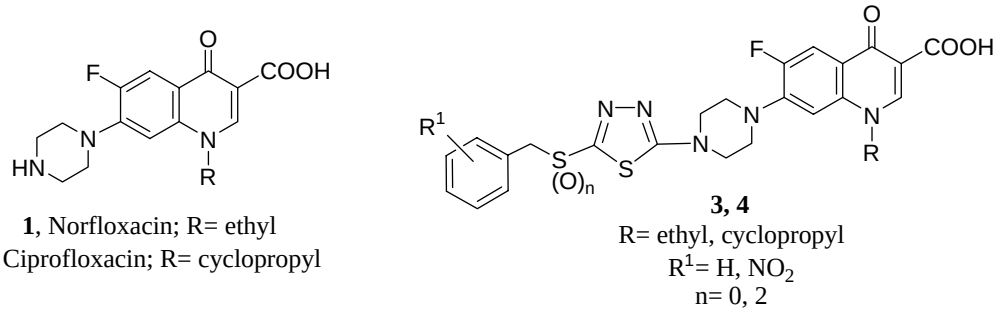


1, Norfloxacin; R= ethyl
2, Ciprofloxacin; R= cyclopropyl

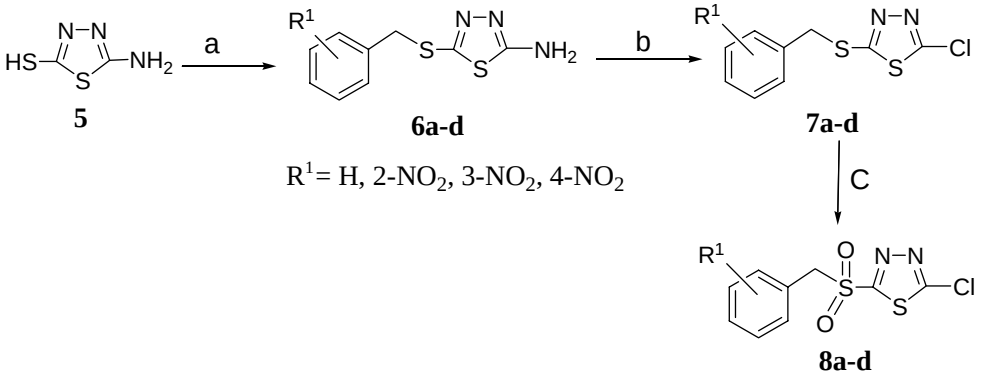
3, 4
R= ethyl, cyclopropyl
R^1= H, NO_2
n= 0, 2

Figure 2.

cin, ofloxacin, amifloxacin, fleroxacin, lomefloxacin, sparfloxacin, difloxacin, enoxacin, enrofloxacin, levofloxacin, marbofloxacin, and orbifloxacin.[8,9] Fluoroquinolones with 7-piperazinyl moiety have been reported to possess potent antibacterial activity.[10–17] According to the inhibition mechanisms of the quinolones, proposed by Shen et al.[7,18–20] the site near the C-7 substituent is regarded as the drug–enzyme interaction domain. In addition, Klopman et al. also concluded that the cell permeability is dominantly controlled by C-7 substituent.[21] These facts motivate our concern to C-7 substituent of quinolone. The piperazine moiety of 7-piperazinyl quinolones possess enough structural flexibility to allow product optimization. In the preceding papers, we described a number of *N*-substituted piperazinyl quinolones by introducing a specific substituents in the piperazine unit of 7-piperazinyl quinolones.[22–29]

As 2,5-disubstituted-1,3,4-thiadiazole derivatives are reported to show antibacterial activity,[30–33] in the present study we have aimed to achieve a better antimicrobial profile at lower concentration, by preparing *N*-substituted piperazinyl quinolones **3** and **4** carrying benzylthio- and benzylsulfonyl-1,3,4- thiadiazole derivatives (Fig. 2).

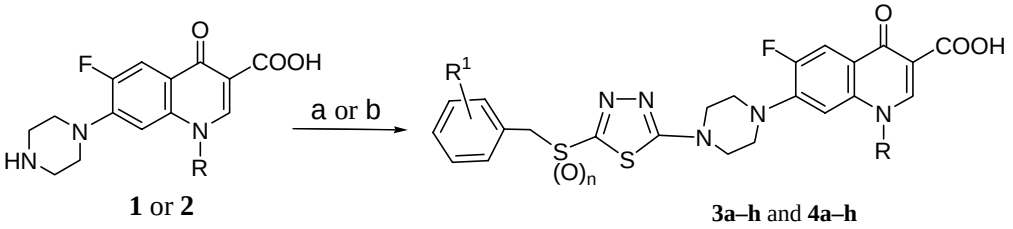
Our synthetic pathway to intermediates **7a**–**d** and **8a**–**d**, and target compounds **3a**–**h** and **4a**–**h** is presented in Schemes 1 and 2. The 2-amino-5-(benzylthio)-1,3,4-thiadiazole derivatives **6** was obtained from commercially available 5-amino-1,3,4-thiadiazol-2-thiol **5**. Thus, treatment of **5** with benzylbromide derivatives in presence of NaOH in 80% ethanol at room temperature afforded *S*-benzyl intermediates **6**.[34] Diazotization of amine **6** with $NaNO_2$ in hydrochloric acid in the presence of copper powder gave the chlorothiadiazoles **7**.[34,35] Sulfones **8** were prepared by usual procedure from sulfides **7**, using an excess of 30% H_2O_2 in CH_3COOH at 60 °C.[34] Reaction of compounds **7a**–**d** with **1** or **2** in DMF in the presence of $NaHCO_3$ at 130 °C gave compounds **3a**–**h**.[29]



5 **6a-d** **7a-d** **8a-d**

R^1= H, 2-NO_2, 3-NO_2, 4-NO_2

Scheme 1. Synthesis of intermediates **7** and **8**. Reagents and conditions: (a) appropriate benzyl halide, NaOH, EtOH 80%, rt, 12 h; (b) $NaNO_2$, HCl, Cu, 0 °C, 1 h, then rt, 2 h; (c) H_2O_2 30% (excess), AcOH, 60 °C, 30–40 min.



1 or **2** **3a–h** and **4a–h**

Scheme 2. Synthesis of compounds **3a**–**h** and **4a**–**h**. Reagents and conditions: (a) compounds **7a**–**d**, $NaHCO_3$, DMF, 130 °C; (b) compounds **8a**–**d**, DMF, $NaHCO_3$, DMF, 90 °C.

Similarly, the reaction of compounds **8a**–**d** with **1** or **2** at 90 °C gave the corresponding compounds **4a**–**h**.

Compounds **3a**–**h** and **4a**–**h** were tested in vitro by conventional agar-dilution method[36] against Gram-positive (*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228 and *Bacillus subtilis* PTCC 1023) and Gram-negative (*Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, and *Pseudomonas aeruginosa* ATCC 9027). The minimum inhibitory concentration (MIC) values were determined by comparison to parent quinolones, norfloxacin **1** and ciprofloxacin **2** as reference drugs.

The MIC values in Table 1 indicate that most compounds showed significant activity (MIC = 0.03–4 μg/mL) against Gram-positive bacteria and moderate to poor activity (MIC = 1–64 μg/mL) against Gram-negative pathogens.

Table 1 reveals that compounds **3g** and **3h** followed by **4g** are superior in inhibiting the growth of *S. aureus* (MIC = 0.5–1 μg/mL). However, compounds **3b**–**f** and **4f** are statistically equivalent in antibacterial activity and better than the remaining tested compounds. Antibacterial screening of compounds **3a**–**h** and **4a**–**h** against *S. epidermidis* reveals that compounds **3b**–**d**, **3f**–**h** and **4f**–**h** show a comparable or better activity (MIC = 0.03–0.5 μg/mL) with respect to the reference drugs (MIC = 0.25–0.5 μg/mL). Indeed, compounds **3h** and **4f** were the most active compounds against *S. epidermidis*, their activities were found to be 8- to 16-fold more than reference drugs. Most compounds had respectable in vitro activity against *B. subtilis*, but were less active than reference drugs.

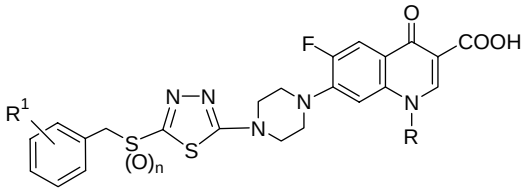
Compound **3h** was found to exhibit the most potent in vitro antibacterial activity against Gram-positive bacteria, with MIC of 0.5, 0.03, and 0.5 μg/mL against *S. aureus*, *S. epidermidis*, and *B. subtilis*, respectively.

Generally, most compounds showed moderate activity (MIC = 1–16 μg/mL) against Gram-negative bacteria, with an exception of antibacterial activity against *P. aeruginosa*. All compounds showed poor or no activity against *P. aeruginosa*. In fact, Compounds **3h** and **4f** were the most potent against all Gram-negative bacteria, with an MIC value of 1–32 μg/mL.

The following SAR trends have been observed. In general, benzylthio- or benzylsulfonyl-1,3,4-thiadiazole groups are well tolerated in terms of Gram-positive activity, as exemplified by the potency of ciprofloxacin analogs **3e**–**h** (MIC range of 0.5–2 and 0.03–1 μg/mL for *S. aureus* and *S. epidermidis*, respectively). In many cases, the compounds had superior activity to their parent quinolones. As is evident from the data, higher susceptibilities (lower MICs) were observed with Gram-positive and poorer susceptibilities, with Gram-negative bacteria. Thus, introduction of 1,3,4-thiadiazole carrying benzylthio derivatives at the N-4 position of piperazine ring changes the antibacterial potency profile of piperazinyl quinolones.

The effect of positional substitution was primarily investigated by preparing all three possible nitro regioisomers on benzyl moiety. It was seen that introduction of a nitro group in ciprofloxacin derivatives (R = cyclopropyl) improved the potency against all tested pathogens, but in norfloxacin derivatives (R = ethyl) improved the potency only against Staphylococci. In some cases, the

Table 1. Antibacterial activities of compounds **3a**–**h** and **4a**–**h** against selected strains (MICs in μg/mL)



Compound	R	R^1	*n*	*S. aureus*	*S. epidermidis*	*B. subtilis*	*K. pneumoniae*	*P. aeruginosa*	*E. coli*
3a	Ethyl	H	0	16	2	8	16	>64	16
3b	Ethyl	2-NO_2	0	4	0.5	8	32	>64	16
3c	Ethyl	3-NO_2	0	2	0.25	8	16	>64	16
3d	Ethyl	4-NO_2	0	4	0.5	2	64	>64	32
3e	Cyclopropyl	H	0	2	1	4	32	>64	16
3f	Cyclopropyl	2-NO_2	0	2	0.06	4	16	64	2
3g	Cyclopropyl	3-NO_2	0	0.5	0.06	4	2	64	2
3h	Cyclopropyl	4-NO_2	0	0.5	0.03	0.5	2	32	1
4a	Ethyl	H	2	64	16	8	>64	>64	>64
4b	Ethyl	2-NO_2	2	>64	>64	16	64	>64	32
4c	Ethyl	3-NO_2	2	>64	64	64	>64	>64	>64
4d	Ethyl	4-NO_2	2	64	8	32	>64	>64	>64
4e	Cyclopropyl	H	2	16	4	4	>64	>64	>64
4f	Cyclopropyl	2-NO_2	2	4	0.03	1	4	32	1
4g	Cyclopropyl	3-NO_2	2	1	0.05	8	16	64	8
4h	Cyclopropyl	4-NO_2	2	8	2	1	64	>64	>64
1	(Norfloxacin)			1	0.5	0.06	0.25	2	0.25
2	(Ciprofloxacin)			0.5	0.25	0.015	0.06	0.5	0.06

effect of nitro group was relatively similar for *para*, *meta*, and *ortho* position. Nevertheless, the effect of nitro-substitution and its position dependent on the other substituents seems to have different influence on the antibacterial activity against various bacterial strains. Comparison between MIC values of thio analogs **3a**–**h** and sulfones **4a**–**h** revealed that *S*,*S*-dioxidation of thio compounds caused a diminution in antibacterial activity against most bacterial species. Sulfonyl analogs of norfloxacin **4a**–**d** were at best only weakly active as antibacterial as measured by MICs.

The results of MIC tests against both Gram-positive and Gram-negative bacteria revealed that ciprofloxacin derivatives (R = cyclopropyl) were usually more potent than norfloxacin derivatives (R = ethyl) especially against Gram-positive pathogens.

In the nitrobenzylthio-1,3,4-thiadiazole series **3b**–**h** comparison with the corresponding nitrophenyl-1,3,4-thiadiazole derivatives[29] brought to the fore that incorporation of methylthio linkage between phenyl and thiadiazole in many cases exerted an excellent positive effect: for example, compound **3g** was more active than nitrophenyl-1,3,4-thiadiazole analog against all of the tested strains.

In light of the above structure–activity relationships, it can be suggested that 1,3,4-thiadiazole bearing a certain pendent group is well tolerated at N-4 position of piperazine ring. Methylthio linker is necessary for activity and nitro-substituent on benzyl moiety enhances antibacterial potency especially against Gram-positive bacteria. Moreover, the effects on antibacterial activity correlate with the inherent activity of the parent quinolone.

Previously, we reported that novel nitroaryl- and nitroheteroaryl-1,3,4-thiadiazolyl quinolones differing from ciprofloxacin or norfloxacin solely by the linkage of various nitroaryl- and nitroheteroaryl-1,3,4-thiadiazolyl groups to the piperazinyl residue at C-7 of the parent drug have particularly high in vitro activity against Gram-positive cocci such as *S. aureus*. Similarly, our new series of compounds exhibit high activity against Gram-positive and marginal activity against Gram-negative bacteria.

Although the nature of the C-7 substituent is known to influence quinolone activity in bacteria,[5–7] we identify addition of the 1,3,4-thiadiazole group as a particular chemical modification that allows manipulation of selectivity and potency. In exploring possible causes of this alteration of potency, it was noted that our title compounds **3** and **4** could be viewed as hybrid drugs incorporating a quinolone and a functionalized thiadiazole moiety that, in itself, shows some antibacterial activity.[30–33] Conversely, two general factors that contribute to antibacterial potency of quinolones are the kinetics of drug uptake and the ability to inhibit gyrase or topoisomerase IV.[37] The mechanism by which quinolones enter the bacterial cell is complicated and not entirely understood. Intracellular quinolone concentrations depend on the balance between drug influx through porin channels, drug transport across the cytoplasmic membrane, and efflux of drug out of the cytoplasm and the cell.[38] The physicochemical properties of quinolones (e.g., relative hydrophobicity, charge or molecular mass) are important for penetration into bacterial cell and have a different role in Gram-negative and Gram-positive bacteria. Increasing molecular mass and bulkiness of substituent at C-7 position hinder penetration of quinolones into Gram-negative organisms through the porin channels, although hydrophobic molecules appear to enter via the lipopolysaccharide or across the lipid bilayer.[39–41] Gram-positive bacteria do not possess an outer membrane, and so lack outer membrane proteins and lipopolysaccharide. Accumulation by Gram-positive bacteria (e.g., *S. aureus*) is thought to take place by simple diffusion across the cytoplasmic membrane.[42,43] Accordingly, we suggest that compounds like thiadiazolyl quinolones (**3** and **4**), which have high molecular mass and bulky substituent at C-7 position, are accumulated in the Gram-positive bacteria more favorably than ciprofloxacin and norfloxacin. Moreover, ciprofloxacin and norfloxacin are zwitterionic quinolones, but their structural modification to yield the corresponding thiadiazole derivatives produces compounds with different ionizable groups in the biological pH range. This charge difference is suggested to account for the better uptake into Gram-positive bacteria, for the thiadiazolyl quinolones **3** and **4**.

In conclusion, we have identified a series of *N*-substituted piperazinyl quinolones **3** and **4** in which the N-4 hydrogen of piperazinyl group of norfloxacin and ciprofloxacin replaced with various nitrobenzylthio- and nitrobenzylsulfonyl-1,3,4-thiadiazolyl moieties with in vitro microbiological activity against Gram-positive organisms comparable or higher than respective parent quinolones, ciprofloxacin, and norfloxacin. The SAR of this series indicates that both the structure of the benzyl unit and the S or SO_2 linker dramatically impact antibacterial activity.

Acknowledgment

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References and notes

1. Hooper, D. C.; Wifson, J. C. *Antimicrob. Agents Chemother.* **1985**, *28*, 716.
2. Ball, P. *Infection* **1994**, *22*, S140.
3. Ball, P.; Mandell, L.; Niki, Y.; Tilloston, G. *Drug Safety* **1999**, *21*, 407.
4. Ball, P. *J. Antimicrob. Chemother.* **2000**, *45*, 557.
5. Domagala, J. M.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Nichols, J. B.; Solomon, M.; Worth, D. F. *J. Med. Chem.* **1988**, *31*, 991.
6. Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nordeen, C. W.; Maleczka, R. E.; Pernet, A. G. *J. Med. Chem.* **1985**, *28*, 1558.

7. Shen, L. L.; Mitscher, L. A.; Sharma, P. N.; O'Donnell, T. J.; Chu, D. W. T.; Cooper, C. S.; Rosen, T.; Pernet, A. G. *Biochemistry* **1989**, *28*, 3886.
8. De Sarro, A.; De Sarro, G. *Curr. Med. Chem.* **2001**, *8*, 371.
9. Anderson, V. E.; Osheroff, N. *Curr. Pharm. Des.* **2001**, *7*, 339.
10. Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358.
11. Hagihara, M.; Kashiwase, H.; Katsube, T.; Kimura, T.; Komai, T.; Momata, K.; Ohmine, T.; Nishigaki, T.; Kimura, S.; Shimada, K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3063.
12. Domagala, J. M.; Hanna, L. D.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Solomon, M. *J. Med. Chem.* **1986**, *29*, 394.
13. Konda, H.; Sakamoto, F.; Kodera, Y.; Tsukamoto, G. *J. Med. Chem.* **1986**, *29*, 2020.
14. Fang, K.-C.; Chen, Y.-L.; Sheu, J.-Y.; Wang, T.-C.; Tzeng, C.-C. *J. Med. Chem.* **2000**, *43*, 3809.
15. Gootz, T. D.; McGuirk, P. R.; Moynihan, M. S.; Haskell, S. L. *Antimicrob. Agents Chemother.* **1994**, *38*, 130.
16. Pandeya, S. N.; Sriram, D.; Nath, G.; DeClercq, E. *Eur. J. Med. Chem.* **2000**, *35*, 249.
17. Chu, D. T. W.; Fernandes, P. B.; Maleczka, R. E.; Nordeen, C. W.; Pernet, A. G. *J. Med. Chem.* **1987**, *30*, 504.
18. Shen, L. L.; Pernet, A. G. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 307.
19. Shen, L. L.; Kohlbrenner, W. E.; Weigl, D.; Baranowski, J. *J. Biol. Chem.* **1989**, *264*, 2973.
20. Shen, L. L.; Baranowski, J.; Pernet, A. G. *Biochemistry* **1989**, *28*, 3879.
21. Klopman, G.; Macina, O. T.; Levinson, M. E.; Rosenkranz, H. S. *Antimicrob. Agents Chemother.* **1987**, *31*, 1831.
22. Foroumadi, A.; Emami, S.; Davood, A.; Moshafi, M. H.; Sharifian, A.; Tabatabaiee, M.; Tarhimi Farimani, H.; Sepehri, G.; Shafiee, A. *Pharm. Sci.* **1997**, *3*, 559.
23. Foroumadi, A.; Emami, S.; Haghighat, P.; Moshafi, M. H. *Pharm. Pharmacol. Commun.* **1999**, *5*, 591.
24. Foroumadi, A.; Davood, A.; Mirzaei, M.; Emami, S.; Moshafi, M. H. *Boll. Chim. Farm.* **2001**, *140*, 411.
25. Foroumadi, A.; Soltani, F.; Emami, S.; Davood, A. *Boll. Chim. Farm.* **2002**, *141*, 247.
26. Mirzaei, M.; Foroumadi, A. *Pharm. Pharmacol. Commun.* **2000**, *6*, 351.
27. Foroumadi, A.; Mansouri, S.; Kiani, Z.; Rahmani, A. *Eur. J. Med. Chem.* **2003**, *38*, 851.
28. Foroumadi, A.; Ashraf-Askari, R.; Moshafi, M. H.; Emami, S. *Pharmazie* **2003**, *58*, 432.
29. Foroumadi, A.; Soltani, F.; Moshafi, M. H.; Ashraf-Askari, R. *Il Farmaco* **2003**, *58*, 1023.
30. Russo, F.; Santagati, M. *Il Farmaco* **1976**, *31*, 41.
31. Tsotinis, A.; Varvaresou, A.; Calogeropoulou, T.; Siatra-Papastaikoudi, T.; Tiligada, A. *Arzneim.-Forsch. Drug Res.* **1997**, *47*, 307.
32. Dogan, H. N.; Duran, A.; Rollas, S.; Sener, G.; Uysal, M. K.; Gülen, D. *Bioorg. Med. Chem.* **2002**, *10*, 2893.
33. Varvaresou, A.; Siatra-Papastaikoudi, T.; Tsotinis, A.; Tsantili-Kakoulidou, A.; Vamvakides, A. *Il Farmaco* **1998**, *53*, 320.
34. Shafiee, A.; Foroumadi, A. *J. Heterocycl. Chem.* **1996**, *33*, 1611.
35. Foroumadi, A.; Daneshtalab, M.; Shafiee, A. *Arzneim.-Forsch. Drug Res.* **1999**, *49*, 1035.
36. Baron, E. J.; Finegold, S. M. *Bailey and Scott's Diagnostic Microbiology*, 8th ed.; C.V. Mosby: St. Louis, 1990; pp 184–188.
37. Hooper, D. C. *Clin. infect. Dis.* **2001**, *32*, S9.
38. Piddock, L. J. V. *J. Antimicrob. Chemother.* **1991**, *27*, 399.
39. Chapman, J. S.; Georgopapadakou, N. H. *Antimicrob. Agents Chemother.* **1988**, *32*, 438.
40. Hirai, K.; Aoyama, H.; Irikura, T.; Iyobe, S.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* **1986**, *29*, 535.
41. Dalhoff, A.; Schmitz, F.-J. *Eur. J. Clin. Microbiol. Infect. Dis.* **2003**, *22*, 203.
42. McCaffrey, C.; Bertasso, A.; Pace, J.; Georgopapadakou, N. H. *Antimicrob. Agents Chemother.* **1992**, *36*, 1601.
43. Piddock, L. J. V.; Hall, M. C.; Wise, R. *Antimicrob. Agents Chemother.* **1990**, *34*, 1088.