Pyrimido[1,6-*a*]benzimidazoles: A New Class of DNA Gyrase Inhibitors[1]

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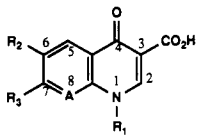
Substituted 4-quinolone- (1, A = CH) and 1,8-naphthyrid-4-one- (1, A = N) 3-carboxylic acids are currently the only classes of clinically useful antibacterial agents exerting their activity by inhibiting the subunit A of DNA gyrase. Pyrimido[1,6-*a*]benzimidazoles 11 have been found to be a new class of inhibitors of this enzyme. The design, synthesis, and biological activity of these compounds are reported.

Since the discovery of nalidixic acid (**1a**) in 1962,[2] extensive variations of the ring substituents and ring system (1: A = CH, N) have been carried out. This effort resulted in the identification of fluorinated quinolones, a group of broad-spectrum antibacterial agents which exert their activity by inhibiting the subunit A of DNA gyrase.[3,4]

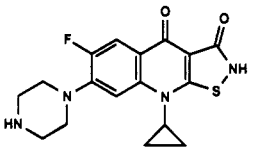


1a : R_1 = Et; R_2 = H; R_3 = Me; A = N

1b : R_1 = Et; R_2 = F; R_3 = 1-piperazinyl; A = CH

1c : R_1 = 2-fluoroethyl; R_2 = F; R_3 = 4-N-Me-piperazinyl, A = CF

Detailed structure–activity relationships within this class of compounds have been reviewed recently.[5] Clinically useful members of this class of antibiotics invariably contain a condensed N-substituted 4-pyridone-3-carboxylic acid moiety. As a matter of fact, with the exception of compounds of type 2,[6] the above mentioned structural



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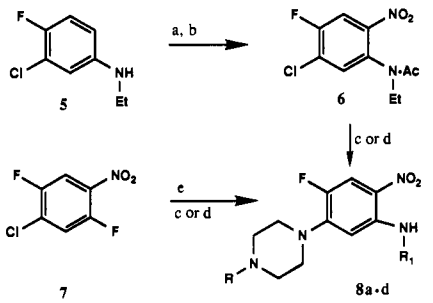
element is present in all potent DNA gyrase inhibitors. It was the aim of the present study to find inhibitors of DNA gyrase structurally different from this established class of compounds, in particular compounds lacking a β-keto acid group, which has been a hallmark of inhibitors of the subunit A of DNA gyrase. Substituted pyrimido[1,6-*a*]-benzimidazoles 11 (Scheme III) were found to be a new class of DNA gyrase inhibitors. Their design, synthesis, enzyme inhibitory, and antibacterial activities are reported herein.

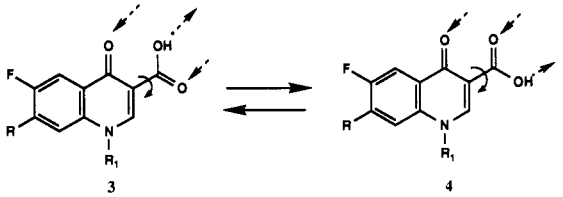
Scheme I[a]



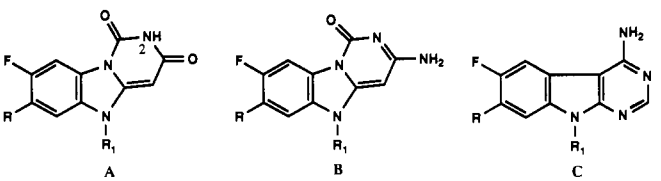
[a] (a) Ac_2O, AcOH; (b) KNO_3, H_2SO_4; (c) *N*-methylpiperazine; (d) *N*-acetylpiperazine, then KOH, MeOH, and optionally $(BOC)_2O$; (e) cyclopropylamine.

Design of DNA Gyrase Inhibitors

In accordance with the recently introduced model on the mechanism of inhibition of DNA gyrase by quinolones,[7] we hypothesized that the β-keto acid moiety of a quinolone might recognize structural elements of unpaired nucleic acids in the DNA–gyrase–inhibitor ternary complex. The two rotamers **3** and **4** of a quinolone carboxylic acid exhibit



two hydrogen-bonding patterns: acceptor–donor–acceptor and acceptor–acceptor–donor, respectively. Of course, we were aware that this statement is a gross oversimplification with respect to the situation under physiological conditions, where these molecules should exist as a zwitterion or, possibly, as the magnesium chelates. Nevertheless, we took the nonionized forms **3** and **4** and deduced that their β-keto acid moiety has the potential to recognize the Watson–Crick hydrogen-bond patterns of thymine and/or adenine and guanine (but not cytosine) in the cleavage site of the DNA–gyrase complex. Under this premise, structures of type A–C were designed by using molecular



A B C

(1) Part of the work was presented at the 11th International Symposium on Medicinal Chemistry, Jerusalem, September 3, 1990.

(2) Lesher, G.; Froelich, E.; Gruett, M.; Bailey, I.; Brundage, R. J. 1.8-Naphthyridine derivatives: A new class of chemotherapeutic agents. *J. Med. Pharm. Chem.* **1962**, *5*, 1063–1065.

(3) Crumplin, G. C.; Midgley, J. M.; Smith, J. T. Mechanism of action of nalidixic acid and its congeners. *Top. Antibiot. Chem.* **1979**, *3*, 9–38.

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(7) Shen, L. L.; Baranowski, J.; Pernet, A. G. Mechanism of inhibition of DNA gyrase by quinolone antibacterials: Specificity and cooperativity of drug binding to DNA. *Biochemistry* **1989**, *28*, 3879–3885. 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. Mechanism of inhibition of DNA gyrase by quinolone antibacterials: A cooperative drug–DNA binding model. *Biochemistry* **1989**, *28*, 3886–3894.

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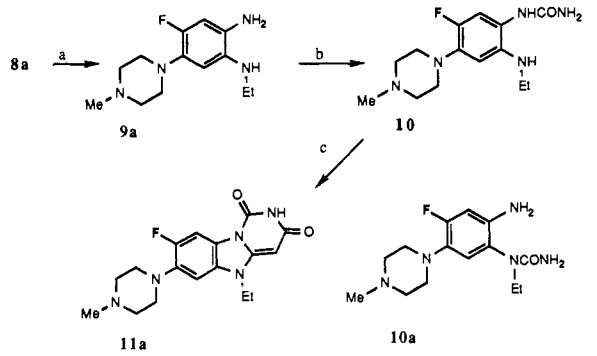
Scheme II[a]



[a] (a) Pd/C, H_2, EtOH; (b) NaOCN, HCl; (c) $CH_2(CO_2Et)_2$, MeONa, MeOH.

modeling. In these structures, the 4-pyridone-3-carboxylic acid moiety of a quinolone was replaced by structural elements of the complementary nucleic acids thymine, cytosine, and adenine, while the relative position of all other structural features of a quinolone (i.e. substituents in position 1, 6, and 7 in structure 1) was maintained. This approach is illustrated by an overlap of quinolone **3** and compound **11c**.

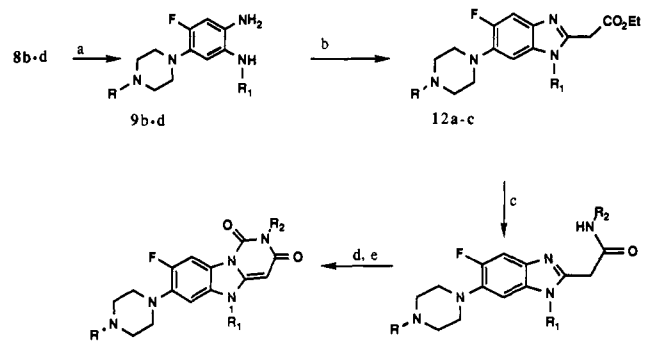
In the course of the work it has been shown that representative examples of structures B (**14c**) and C (**19**) were devoid of any appreciable DNA gyrase inhibitory or antibacterial activities. However, compounds **11a,c,d,f**, representatives of the generic structure A, were found to be a new class of DNA gyrase inhibitors. Therefore, additional compounds in this series, the *N*-2-hydroxy and *N*-2-amino derivatives **11h–v** (Table III) were prepared, even though some of these derivatives contain combinations of functional groups that conflict with our initial hypothesis of nucleotide complementarity.

Chemistry

Synthesis of Pyrimido[1,6-*a*]benzimidazoles 11 (Type A Compounds). The trisubstituted nitrobenzoles **8a–d** required for the preparation of compounds **11** (Schemes II and III) were obtained starting either from *N*-ethylaniline derivative **5**[8] or from nitrobenzole derivative **7**[9] (Scheme I, Table I). Two different synthetic pathways were used to build up pyrimidobenzimidazole structure **11**. The first (Scheme II), used for the synthesis of **11a**, suffered from a low regioselectivity. During conversion of amine **9a** to the desired urea **10**, the unwanted isomer **10a** was formed as well. So, compounds **11b–v** were prepared using an alternative route shown in Scheme III. Reaction of *o*-phenylenediamines **9b–d** with ethyl β-amino-β-ethoxyacrylate led to the formation of 2-benzimidazoleacetates **12a–c** (Table II). Further treatment of esters **12** with ammonia, *O*-benzylhydroxylamine, *tert*-butyl carbazate, *tert*-butyl 2-methylcarbazate, or hydrazine afforded compounds **13a–j** (Table II). The latter were cyclized in a base-catalyzed reaction with 1,1'-carbonyldiimidazole and, where appropriate, deprotected to give the target compounds **11a,c,d,f,h,k,m,p,r,t,v** (Table III).

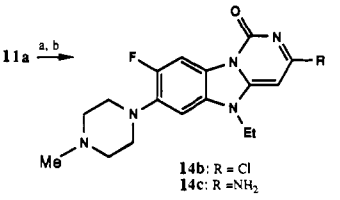
Synthesis of Pyrimido[1,6-*a*]benzimidazole 14c (Type B Compounds). Treatment of pyrimidobenzimidazoledione **11a** with phosphoryl chloride, followed by

Scheme III[a]



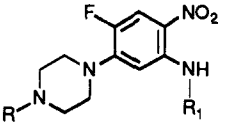
[a] (a) Pd/C, H_2, MeOH; (b) HN=C(OEt)CH_2CO_2Et, DMF; (c) NH_4OH, NH_4Cl, EtOH or NH_2OH·HCl, EtONa, EtOH followed by BzBr, DBU, THF or NH_2NHBOC or $NH_2NMeBOC$; (d) (imidazole)$_2CO$, DBU, THF; (e) Pd/C, H_2, EtOH and/or CF_3CO_2H.

Scheme IV[a]



[a] (a) $POCl_3$; (b) NH_3 in MeOH.

Table I. Yields and Physicochemical Properties of o-Nitroanilines 8



compd	R	R_1	% yield	mp, °C	elemental anal.
8a	Me	Et	87	149–150[a]	$C_{13}H_{19}FN_4O_2$
8b	Me	cyclopropyl	97	130	$C_{14}H_{19}FN_4O_2$
8c	BOC	Et	95	155–156	NA[c]
8d	BOC	cyclopropyl	84	159–160[b]	$C_{16}H_{25}FN_4O_4$

[a] Crystallized from EtOH/H_2O. [b] Crystallized from EtOH. [c] NA = not available.

reaction with ammonia in methanol, yielded **14c** (Scheme IV).

Synthesis of Pyrimidoindole 19 (Type C Compounds). 1,3-Difluoro-4,6-dinitrobenzene (**15a**) was partially reduced to nitroaniline **15b**. Condensation of the latter compound with cyanomalonamide followed by thermal ring closure yielded indole derivative **16**, which in turn was reacted with triethyl orthoformate to form pyrimidoindole **17a**. The piperazine substituent was built up after reduction of the nitro group and subsequent dialkylation with *N*-benzylbis(2-chloroethylamine). Reaction of **17c** with phosphoryl chloride, followed by selective N-ethylation with triethyl phosphate,[16] yielded **18b**, which was treated with benzylamine to give **18c**. Hydrogenolytic deprotection of **18c** over a mixture of palladium on charcoal and palladium oxide resulted in the target compound **19** (Scheme V).

Biological Results and Discussion

The results of the inhibition of the *Escherichia coli* DNA gyrase and the in vitro antibacterial activities of compounds **11**, **14**, and **19** against selected representative microorganisms are summarized in Table IV. For com-

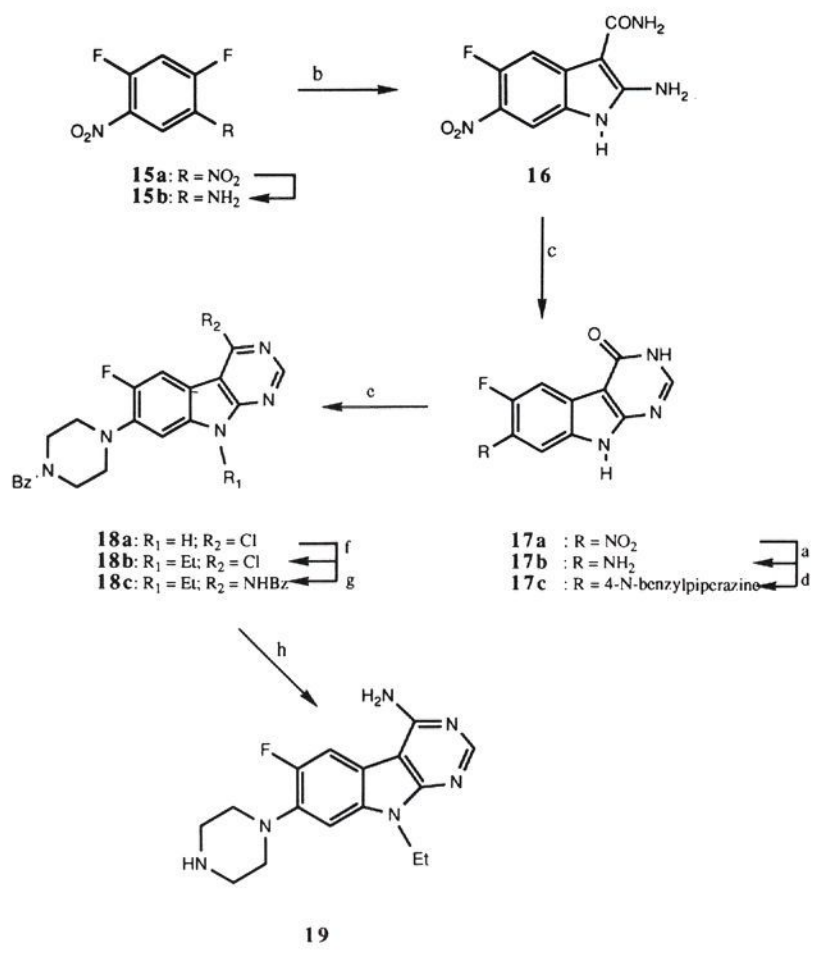
(8) Kim, C. U.; Luh, B. Y. Novel synthesis of quinolone-3-sulfonic acid derivatives. *Heterocycles* 1988, *27*, 1119–1122.
(9) Finger, C. G.; Oesterling, R. E. Aromatic fluorine compounds. VI. Displacement of aryl fluorine in diazonium salts. *J. Am. Chem. Soc.* 1956, *78*, 2593–2596.

Table II. Yields and Physicochemical Properties of Benzimidazoles **12** and **13**

compd	R	R_1	R_2	% yield	mp, °C	elemental anal.
12a	Me	cyclopropyl	COOEt	42	143–145	$C_{19}H_{25}FN_4O_2$
12b	BOC	Et	COOEt	67	131–133[a]	$C_{22}H_{31}FN_4O_4$
12c	BOC	cyclopropyl	COOEt	78	151–152[a]	$C_{23}H_{31}FN_4O_4$
13a	Me	cyclopropyl	$CONH_2$	92	254–258[b]	$C_{17}H_{22}FN_5O$
13b	BOC	Et	$CONH_2$	36	162–164[a]	$C_{20}H_{28}FN_5O_3$
13c	BOC	cyclopropyl	$CONH_2$	53	219[a]	$C_{21}H_{28}FN_5O_3$
13d	Me	Et	CONHOBz	44	165–168[a]	$C_{23}H_{28}FN_5O_2$
13e	Me	cyclopropyl	CONHOBz	41	158	$C_{24}H_{28}FN_5O_2$
13f	BOC	Et	CONHOBz	44	191–194[a]	$C_{27}H_{34}FN_5O_4$
13g	BOC	cyclopropyl	CONHOBz	36	187[c]	$C_{28}H_{34}FN_5O_4$
13h	BOC	cyclopropyl	CONHNHBOC	23	202–203[c]	$C_{26}H_{37}FN_6O_5$
13i	BOC	cyclopropyl	CONHNMeBOC	33	180–182[d]	$C_{27}H_{39}FN_6O_5$
13j	BOC	cyclopropyl	$CONHNH_2$	58	172–173[a]	$C_{21}H_{29}FN_6O_3$
13k	BOC	cyclopropyl	$CONHNMe_2$	70	179–181[c]	$C_{23}H_{33}FN_6O_3$

[a] Crystallized from AcOEt/*n*-hexane. [b] Crystallized from EtOH. [c] Crystallized from AcOEt/MeCN. [d] Crystallized from *n*-hexane.

Scheme V[a]



[a] (a) Pd/C, H_2, AcOEt, then $NaHSO_3$ in EtOH; (b) $CH_2(CN)C(=NH)NH_2$, *t*-BuOK, DMF; (c) $HC(OEt)_3$; (d) $BzN(CH_2CH_2Cl)_2$, KI, DMF; (e) $POCl_3$; (f) $PO(OEt)_3$, K_2CO_3; (g) $BzNH_2$; (h) Pd/C, PdO, H_2, in EtOH, 1 N HCl.

parison, the activities of norfloxacin (**1b**) and fleroxacin (**1c**) are also included.

At the enzyme level, all intermediates and the protected forms of the target compounds were inactive with MNEC values >10 μg/mL (individual data not shown).

Compounds **11a,c,d,f** (type A compounds), incorporating structural features of thymine in the place of the β-keto acid moiety of quinolones, showed enzyme inhibitory activities comparable with those of the reference compounds norfloxacin (**1b**) and fleroxacin (**1c**). By contrast, compound **19**, which contains the structural patterns of adenine (type C compound), and compound **14c**, a cytosine analogue (type B compound), were found to be virtually inactive both at the enzyme level and in vitro.

Within the group of compounds **11**, no clear trends in structure–activity relationships (SAR) emerged (Table IV).

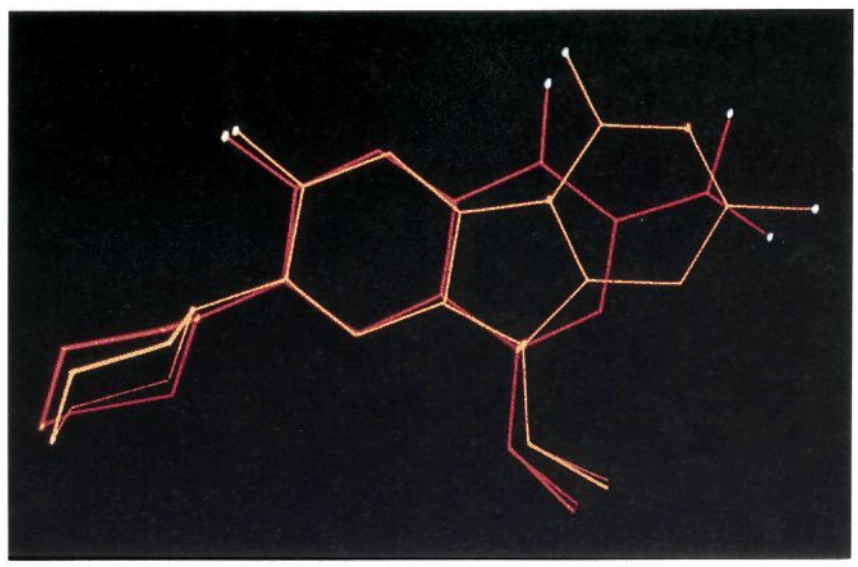


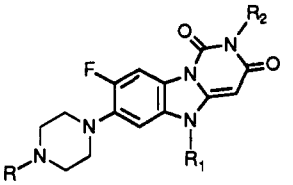
Figure 1. Superposition of structures **3** (red, R = 1-piperazinyl, R_1 = cyclopropyl) and **11a** (orange).

All target compounds showed MNEC values between 0.1 and 2 μg/mL. Within the scope of this work no major influence of either R or R_1 substituents on enzyme inhibitory activities was observed. A cyclopropyl group as R_1 substituent was slightly better than an ethyl group in the series where R_2 = H (i.e. **11f** and **11c**), with no difference in other substituent combinations (i.e. **11k** vs **11p** and **11h** vs **11m**). Compounds where R_1 = H were completely inactive (data not shown). Hence, the influence of the R_1 substituent, which according to Figure 1 can be well-matched with the N-1 substituent of quinolones, does not unambiguously follow the same SAR pattern as the latter within the group of quinolone antibacterials.[5]

The compounds **11h,k,m,p,r,t,v**, with R_2 substituents other than hydrogen, exhibited MNEC values comparable to those of the parent structures.

The MNEC values in the range of 0.1–0.5 μg/mL for the newly synthesized DNA gyrase inhibitors are within the range of enzyme inhibitory activities of clinically useful antibacterials with this mode of action. Their in vitro antibacterial activities are, however, lower than one would expect from their enzyme inhibition data in analogy to quinolones. One of the reasons for this discrepancy could be that the endpoint used in the enzyme assay may not be the true indication of the enzyme inhibition potency of the compounds discussed. As in the quinolone series, in vitro antibacterial activity of the compounds with R_1 = cyclopropyl is clearly superior to those having an ethyl group at this position (e.g. **11d** vs **11a**). On the other hand,

Table III. Yields and Physicochemical Properties of the Pyrimidobenzimidazoles 11



compd	R	R_1	R_2	% yield	mp, °C	elemental anal.
11a	Me	Et	H	17	>250[i]	$C_{17}H_{21}ClFN_5O_2$
11b	BOC	Et	H	67	270–273[a]	$C_{21}H_{26}FN_5O_4$
11c	H	Et	H	70	264–266[c]	$C_{16}H_{18}FN_5O_2$
11d	Me	cyclopropyl	H	23	>260[d]	$C_{16}H_{20}FN_5O_2$
11e	BOC	cyclopropyl	H	73	261	$C_{22}H_{28}FN_5O_4$
11f	H	cyclopropyl	H	60	>250[j]	$C_{19}H_{19}F_4N_5O_2$
11g	Me	Et	OBz	58	250–252[d]	$C_{24}H_{26}FN_5O_3$
11h	Me	Et	OH	53	262	$C_{17}H_{20}FN_5O_3$
11i	BOC	Et	OBz	79	205–207[b]	$C_{28}H_{32}FN_5O_5$
11j	BOC	Et	OH	90	243[g]	$C_{21}H_{26}FN_5O_5$
11k	H	Et	OH	40	>250[i]	$C_{16}H_{19}FClN_5O_3$
11l	Me	cyclopropyl	OBz	74	267–268[d,h]	$C_{25}H_{26}FN_5O_3$
11m	Me	cyclopropyl	OH	60	>250	$C_{16}H_{20}FN_5O_3$
11n	BOC	cyclopropyl	OBz	61	227[b]	$C_{29}H_{32}FN_5O_5$
11o	BOC	cyclopropyl	OH	72	NA[k]	$C_{22}H_{26}FN_5O_5$
11p	H	cyclopropyl	OH	58	>250[i]	$C_{17}H_{19}ClFN_5O_3$
11q	BOC	cyclopropyl	NHBOC	20	219	$C_{27}H_{35}FN_6O_6$
11r	H	cyclopropyl	NH_2	46	232[a]	$C_{17}H_{18}FN_6O_2$
11s	BOC	cyclopropyl	NMeBOC	59	224–225[b]	$C_{28}H_{37}FN_6O_6$
11t	H	cyclopropyl	NHMe	43	229[a]	$C_{16}H_{21}FN_6O_2$
11u	BOC	cyclopropyl	$N(Me)_2$	53	201–203[f]	$C_{24}H_{31}FN_6O_4$
11v	H	cyclopropyl	$N(Me)_2$	42	207–209[f]	$C_{19}H_{23}FN_6O_2$

[a] Crystallized from EtOH. [b] Crystallized from AcOEt/*n*-hexane. [c] Crystallized from MeOH/water. [d] Crystallized from MeOH. [e] Crystallized from water. [f] Crystallized from AcOEt. [g] 1:1 DMF adduct. [h] 1:1 MeOH adduct. [i] Hydrochloride. [j] Trifluoroacetate. [k] NA = not available.

Table IV. Inhibition of *E. coli* DNA Gyrase and Antibacterial Activity of Selected Target Compounds[a,b]

compd	MNEC,[c] μg/mL	minimal inhibitory concentration (MIC),[c] μg/mL									
		Ec(A)	Ec(B)	Ec(C)	Ko	Et	Pa	Sa	Sh	Bsu	Mlu
11a	2	16	2	32	>32	16	>32	>32	NA[d]	16	>32
11c	1	16	8	>64	>64	64	>64	>64	NA	64	>64
11d	1	2	0.25	2	8	4	32	16	>32	4	>32
11f	0.2	2	0.5	8	16	8	32	>32	NA	8	>32
11h	1	2	1	4	4	2	2	16	16	8	16
11k	1	8	8	32	16	8	4	>64	>64	32	32
11m	1	≤0.03	≤0.03	0.12	0.25	0.12	2	4	32	2	32
11p	1	≤0.25	≤0.25	2	1	1	2	>64	>64	4	8
11r	0.2	1	0.25	2	4	2	8	64	>64	4	>64
11t	0.1	1	0.5	8	8	4	32	16	128	8	128
11v	0.5	8	1	32	32	32	64	16	>128	4	>128
14c	>10	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
19	5	64	16	>128	>128	>128	>128	>128	>128	128	NA
1b	0.5	0.12	0.12	0.12	0.25	0.25	1	4	>64	1	32
1c	0.5	0.12	0.06	0.06	0.12	0.12	0.5	2	32	0.5	16

[a] Structures are shown in Table III. [b] Organisms selected for the Table are as follows: Ec(A), *E. coli* 1346; Ec(B), *E. coli* B; Ec(C), *E. coli* 1527E; Ko, *Klebsiella oxytoca* 1082E; Et, *Enterobacter cloacae* P99; Pa, *Pseudomonas aeruginosa* 799/61; Sa, *Staphylococcus aureus* 887; Sh, *Staphylococcus haemolyticus* 75 (quinolone-resistant); Bsu, *Bacillus subtilis* ATCC 585369; Mlu, *Micrococcus luteus* ATCC 8340. [c] See Experimental Section. [d] NA = not available.

unlike in the group of quinolone and naphthyridine-type antibacterials, the 4′-*N*-methyl substitution both enhanced and broadened the antibacterial spectrum when compared with the corresponding secondary amines (i.e. 11m and 11p). However, even the in vitro activity of 11d, the most active compound within the group of compounds with R_2 = H, is rather disappointing. The reason for this discrepancy may be the fact that the N-substituted 4-pyridone-3-carboxylic acid moiety of quinolone antibacterials, in addition to its hydrogen-bonding properties, possesses the capability of chelating bivalent cations. Magnesium-chelating properties may facilitate the penetration of the compounds into the bacterial cell and enhance their antibacterial activity.[10] The variation of the substituent R_2 in 11 offered the possibility to modulate the biological activity in this class of compounds by introducing functionalities which would facilitate the building of metal complexes. Such compounds as 11 for example (with R_2 = OH) were expected, according to our working hypothesis, to be less suited for specific recognition of unpaired nucleic base(s), which, in turn, should be reflected by increased MNEC values. On the other hand, the chelating properties of the compounds could enhance their antibacterial activity in vitro. The data showed that, al-

(10) Chapman, J. S.; Georgopapadakou, N. H. Routes of quinolone permeation in *Escherichia coli*. *Antimicrob. Agents Chemother.* 1988, *32*, 438–442.

though the introduction of OH, NH_2, NHMe, or NMe_2 group in R_2 did not affect the MNEC values significantly, the in vitro antibacterial activity of compounds where R_2 = OH has increased. This was in particular the case in the pair of compounds **11f** and **11p**, where the introduction of a hydroxyl group as R_2 reduced the enzyme inhibitory activity and increased the in vitro antibacterial activity considerably. The hydroxamic acid derivatives **11h,k,m,p** are, indeed, according to our preliminary data able to chelate magnesium (data not shown).

In conclusion, pyrimido[1,6-*a*]benzimidazoles **11** have been found to be a new class of potent DNA gyrase inhibitors. Their antibacterial activity is, however, inferior to the quinolone DNA gyrase inhibitors and antibacterial agents like norfloxacin or fleroxacin. Further studies directed toward the elucidation of the mechanism of action of this class of DNA gyrase inhibitors, which are expected to lead to a clearer view of SAR, are currently under way.

Experimental Section

Melting points were determined with a Büchi 510 melting point apparatus and are uncorrected. The ^1H NMR spectra were recorded with a Brucker AC 250 (250 MHz) spectrophotometer. Chemical shifts (δ) are in ppm relative to internal tetramethylsilane. The IR spectra were recorded on a Nicolet FTIR spectrometer as KBr pellets. Mass spectra were determined on a MS 9 spectrometer (updated with ZAB console VG Altrincham) with a SS 300 Finnigan MAT data system. The IR, NMR, and mass spectral data of all compounds were consistent with the assigned structures. Elemental analysis were obtained for all new compounds reported. Carbon, hydrogen, and nitrogen analysis were within 0.4% of the theoretical values. All organic phases were dried over anhydrous $MgSO_4$ and concentrated on a Büchi rotatory evaporator at aspiratory pressure. Chromatography was done using the medium-pressure flash method[11] and Merck silica gel 60 (230–400 mesh ASTM).

1. **Preparation of the Nitroanilines 8a–d (Table I). 1-[5-(Ethylamino)-2-fluoro-4-nitrophenyl]-4-methylpiperazine (8a).** A solution of **5**[8] (97.4 g, 0.56 mol) in acetic acid (225 mL) was cooled to 5 °C and treated dropwise with acetic anhydride (105 mL, 1.12 mol). After stirring for 1 h, the solution was poured onto ice (250 mL) and extracted with ethyl acetate (2 × 200 mL). The combined organic layers were sequentially washed with water (100 mL), 2 N NaOH (100 mL), saturated $NaHCO_3$ (100 mL), and water (100 mL), then dried, and evaporated. Recrystallization from *n*-hexane gave 102 g (84%) of 3′-chloro-*N*-ethyl-4′-fluoroacetanilide: NMR ($CDCl_3$) δ 1.11 (t, J = 7 Hz, 3 H), 1.84 (s, 3 H), 3.71 (q, J = 7 Hz, 2 H), 7.07 (m, 1 H), 7.25 (m, 2 H). Anal. ($C_{10}H_{11}ClFNO$) C, H, N, Cl, F.

A solution of 3′-chloro-*N*-ethyl-4′-fluoroacetanilide (101 g, 0.46 mol) in concentrated H_2SO_4 (300 mL) was cooled to 5 °C and treated dropwise with concentrated H_2SO_4 (220 mL) containing KNO_3 (57 g, 0.56 mol). The reaction mixture was stirred at this temperature overnight, then poured into ice/water (200 mL), and extracted with ethyl acetate (2 × 200 mL). The combined organic layers were sequentially washed with water (100 mL), saturated $NaHCO_3$ (100 mL), and brine, then treated with charcoal, dried, and evaporated. Recrystallization from ether/*n*-hexane gave 40.3 g (48%) of 5′-chloro-*N*-ethyl-4′-fluoro-2′-nitroacetanilide (**6**) as colorless crystals. The mother liquor was chromatographed (20% ethyl acetate/*n*-hexane) affording another 18 g (21%): mp 68 °C; NMR ($CDCl_3$, mixture of two rotamers) δ 1.09 and 1.28 (2 t, J = 7.5 Hz, 3 H), 1.86 and 2.27 (2 s, 3 H), 3.4 and 3.9 (2 m, 2 H), 7.35 and 7.46 (2 d, J = 6.7 Hz, 1 H), 7.87 (d, J = 6.7 Hz, 1 H). Anal. ($C_{10}H_{10}ClFN_2O_3$) C, H, N.

A mixture of **6** (74 g, 0.28 mol) and *N*-methylpiperazine (126 mL, 1.13 mol) was warmed to 60 °C for 2 h. The excess of reagent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (250 mL) and sequentially washed with water (3 × 100 mL) and 10% aqueous NaCl solution. The organic layer was dried and evaporated. Crystallization from ethyl acetate/*n*-hexane gave 73.5 g (78.5%) of *N*-ethyl-4′-fluoro-5′-(4-methyl-1-piperazinyl)-2′-nitroacetanilide as yellow crystals: mp 117–119 °C; NMR ($CDCl_3$, mixture of two rotamers) δ 1.07 and 1.23 (2 t, J = 7.5 Hz, 3 H), 1.83 and 2.27 (2 s, 3 H), 2.35 and 2.37 (2 s, 3 H), 2.59 (m, 4 H), 3.33 (m, 4 H), 3.4 and 3.95 (2 m, 2 H), 6.61 and 6.64 (2 d, J = 6.5 Hz, 1 H), 7.87 (d, J = 6.5 Hz, 1 H). Anal. ($C_{15}H_{21}ClFN_4O_3$) C, H, N.

A solution of *N*-ethyl-4′-fluoro-5′-(4-methyl-1-piperazinyl)-2′-nitroacetanilide (20 g, 0.61 mol) in MeOH (160 mL) was treated with KOH (34.6 g, 0.61 mol) and the mixture was warmed to 80 °C for 3 h. After cooling, the resulting crystals were collected by filtration, washed with water, and dried, affording 13.7 g of **8a**: NMR ($CDCl_3$) δ 1.37 (t, J = 7.5 Hz, 3 H), 2.36 (s, 3 H), 2.57 (m, 4 H), 3.31 (m, 6 H), 5.98 (d, J = 7 Hz, 1 H), 7.83 (d, J = 12 Hz, 1 H), 8.20 (br, 1 H).

***tert*-Butyl 4-[5-(Cyclopropylamino)-2-fluoro-4-nitrophenyl]-1-piperazinecarboxylate (8d).** A solution of **7**[9] (36.7 g, 0.189 mol) in a mixture of triethylamine (26.3 mL, 0.189 mol) and cyclopropylamine (14.55 mL, 0.208 mol) was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (500 mL) and extracted with water (2 × 200 mL). The organic layer was washed with 10% aqueous NaCl solution. After drying and evaporation, the residue was recrystallized from EtOH, affording 35 g (80%) of 5-chloro-*N*-cyclopropyl-2-nitro-4-fluoroaniline: mp 73–75 °C; NMR (DMSO-d_6) δ 0.65 and 0.89 (2 m, 2 × 2 H), 2.62 (m, 1 H), 7.52 (d, J = 6 Hz, 1 H), 8.01 (br, 1 H), 8.10 (d, J = 10 Hz, 1 H). Anal. ($C_9H_8ClFN_2O_2$) C, H, N.

The intermediate 5-chloro-*N*-cyclopropyl-2-nitro-4-fluoroaniline (3.5 g, 15.2 mmol) and *N*-acetylpiperazine (3.9 g, 30.4 mmol) were dissolved in triethylamine (3.1 mL, 22.8 mmol) and stirred at 60 °C overnight. The resulting suspension was partitioned between water (100 mL) and ethyl acetate (200 mL). The organic layer was sequentially washed with water and 10% aqueous NaCl solution, then dried, and evaporated. The residue was crystallized from EtOH, yielding 4.57 g (93%) of 1-acetyl-4-[5-(cyclopropylamino)-2-fluoro-4-nitrophenyl]piperazine as yellow crystals: mp 142–143 °C; NMR (90 MHz, DMSO-d_6) δ 0.6 (m, 2 H), 0.85 (m, 2 H), 2.05 (s, 3 H), 2.60 (m, 1 H), 3.30 (m, 4 H), 3.65 (m, 4 H), 6.62 (d, J = 7.5 Hz), 7.80 (d, J = 15 Hz, 1 H), 8.25 (br, 1 H). Anal. ($C_{15}H_{19}FN_4O_3$) C, H, N.

A solution of 1-acetyl-4-[5-(cyclopropylamino)-2-fluoro-4-nitrophenyl]piperazine (1 g, 3.1 mmol) in MeOH (25 mL) was treated with finely powdered KOH (0.87 g, 15.5 mmol) and water (2 mL). The solution was stirred at 80 °C for 3 h and then evaporated under reduced pressure. The residue, dissolved in ethyl acetate (50 mL), was sequentially washed with water (2 × 30 mL) and 10% aqueous NaCl solution. After drying and evaporation, the residue was chromatographed (10% MeOH/ethyl acetate), affording 0.75 g (85%) of 4-[5-(cyclopropylamino)-2-fluoro-4-nitrophenyl]piperazine: NMR (90 MHz, DMSO-d_6) δ 0.6–1.0 (m, 4 H), 2.60 (m, 1 H), 2.8 (m, 4 H), 3.20 (m, 4 H), 6.40 (d, J = 7.5 Hz, 1 H), 7.75 (d, J = 15 Hz, 1 H), 8.20 (s, 1 H).

A solution of 4-[5-(cyclopropylamino)-2-fluoro-4-nitrophenyl]piperazine (0.75 g, 2.7 mmol) in dioxane (5 mL) was treated with $(BOC)_2O$ (0.7 g, 3.2 mmol) and a solution of $NaHCO_3$ (268 mg, 3.2 mmol) in water (2.5 mL). The mixture was stirred at room temperature for 2 h then diluted with water (10 mL). The resulting crystals were collected by filtration and recrystallized from EtOH, affording 0.86 g of **8d** as yellow crystals: NMR (DMSO-d_6) δ 0.61 (m, 2 H), 0.90 (m, 2 H), 1.42 (s, 9 H), 2.65 (m, 1 H), 3.29 (m, 4 H), 3.50 (m, 4 H), 6.62 (d, J = 7.5 Hz, 1 H), 7.80 (d, J = 15 Hz, 1 H), 8.20 (br, 1 H).

***tert*-Butyl 4-(5-Ethyl-2-fluoro-4-nitrophenyl)-1-piperazinecarboxylate (8c).** The title compound was obtained from **6** and *N*-acetylpiperazine in analogy to the preparation of **8d**: NMR (DMSO-d_6) δ 1.24 (t, J = 7.5 Hz, 3 H), 1.42 (s, 9 H), 3.2–3.5 (m, 10 H), 6.20 (d, J = 7.5 Hz, 1 H), 7.76 (d, J = 15 Hz, 1 H), 8.20 (br, 1 H); IR 1691, 1630, 1581 cm^{-1}.

1-[5-(Cyclopropylamino)-2-fluoro-4-nitrophenyl]-4-methylpiperazine (8b). The title compound was obtained from **7** and *N*-methylpiperazine in analogy to the preparation of **8d**.

2. Preparation of Pyrimidobenzimidazole 11a. [2-(Ethylamino)-5-fluoro-4-(4-methyl-1-piperazinyl)phenyl]-urea (10) and 1-[2-Amino-4-fluoro-5-(4-methyl-1-piperazinyl)phenyl]-1-ethylurea (10a). A solution of **8a** (13.7

(11) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* 1978, *43*, 2923–2925.

g, 48.5 mmol) in MeOH (900 mL) was hydrogenated over 5% Pd/C. At the end of the reduction, the mixture was quickly filtered into a reaction flask containing NaOCN (3.78 g, 58.2 mmol). The reddish solution was kept under argon and the pH was adjusted to pH 5 by addition of 1 N HCl (about 25 mL) and stirred at room temperature for 6 h. The reaction mixture was evaporated to dryness. The residue was triturated with MeOH/ethyl acetate (4:1; 450 mL) and the solid was filtered off and discarded. The mother liquor was treated with charcoal, filtered, and evaporated under reduced pressure. The crystals formed upon cooling were filtered and yielded 2.8 g (21%) of **10a**: mp 246 °C (MeOH); NMR ($CDCl_3$) δ 0.96 (t, J = 7.5 Hz, 3 H), 2.19 (s, 3 H), 2.41 (m, 4 H), 2.83 (m, 4 H), 3.1 and 3.65 (2 m, 2 H), 4.85 (br, 2 H), 5.4 (br, 2 H), 6.51 (d, J = 8.3 Hz, 1 H), 6.55 (d, J = 4.7 Hz, 1 H). Anal. ($C_{14}H_{22}FN_5O$) C, H, N.

The mother liquor containing **10** was concentrated under reduced pressure and directly used in the next step (assumed yield 60%): NMR ($CDCl_3$) δ 1.25 (t, J = 7.5 Hz, 3 H), 2.36 (s, 3 H), 2.60 (m, 4 H), 3.11 (m, 4 H), 3.12 (q, J = 7.5 Hz, 2 H), 3.90 (br, 1 H), 4.8 (br, 2 H), 6.20 (br, 1 H), 6.25 (d, J = 8 Hz, 1 H), 6.87 (d, J = 12 Hz, 1 H).

5-Ethyl-8-fluoro-7-(4-methyl-1-piperazinyl)pyrimido[1,6-*a*]benzimidazole-1,3(2*H*,5*H*)-dione Hydrochloride (11a). A freshly prepared solution of $NaOCH_3$, obtained from sodium (2.38 g, 0.1 mol) and MeOH (100 mL), was treated with a mixture of **10** (~10 g, ~30 mmol) and diethyl malonate (10.5 mL, 69 mmol) in MeOH (100 mL). The solution was refluxed overnight, then poured into a mixture of ice (100 mL) and 2 N HCl (200 mL). The resulting crystals were filtered and recrystallized from water, affording 1.9 g of **11a**: NMR (DMSO-d_6) δ 1.21 (t, J = 7.5 Hz, 3 H), 2.24 (s, 3 H), 2.51 (m, 4 H), 3.05 (m, 4 H), 4.05 (q, J = 7.5 Hz, 2 H), 5.24 (s, 1 H), 7.15 (d, J = 6 Hz, 1 H), 7.74 (d, J = 10 Hz, 1 H), 11.1 (br, 1 H).

3. Preparation of Benzimidazoles 12a–c and 13a–k (Table II). *tert*-Butyl 4-[5-(Cyclopropylamino)-2-fluoro-4-aminophenyl]-1-piperazinecarboxylate (9d). A solution of **8d** (0.76 g, 2 mmol) in MeOH (100 mL) was hydrogenated over 5% Pd/C. At the end of the reduction, the catalyst was removed by filtration under argon and the filtrate was evaporated. The residue, dissolved in ethyl acetate/*n*-hexane (100 mL; 1:1), was treated with Fuller's earth and evaporated under reduced pressure, yielding 0.65 g (93%) of **9d** as an unstable pink oil: NMR (DMSO-d_6) δ 0.4–0.6 (m, 4 H), 1.35 (s, 9 H), 2.20 (m, 1 H), 2.8 (m, 4 H), 3.40 (m, 4 H), 4.50 (s, 2 H), 4.8 (s, 1 H), 6.35 (d, J = 15 Hz, 1 H), 6.55 (d, J = 9 Hz, 1 H).

Following the same procedure, compounds **9a**, **9b**, and **9c** were obtained in situ starting from **8a**, **8b** and **8c**, respectively.

Ethyl 6-[4-(*tert*-Butoxycarbonyl)-1-piperazinyl]-1-cyclopropyl-5-fluoro-2-benzimidazoleacetate (12c). A solution of **9d** (15.49 g, 44.2 mmol) in DMF (150 mL) was treated portionwise with ethyl β-amino-β-ethoxyacrylate hydrochloride (17.3 g, 88.4 mmol)[12] and the resulting solution was stirred at 50 °C for 1.5 h. The solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (500 mL) and sequentially washed with water (2 × 200 mL) and 10% aqueous NaCl solution. The organic layer was dried and evaporated, leaving reddish crystals which were purified by chromatography (ethyl acetate) and crystallization from ethyl acetate/*n*-hexane, affording 15.5 g of **12c**: NMR (DMSO-d_6) δ 1.0–1.2 (m, 4 H), 1.20 (t, J = 7.5 Hz, 3 H), 1.43 (s, 9 H), 2.97 (m, 4 H), 3.20 (m, 1 H), 3.52 (m, 4 H), 4.09 (s, 2 H), 4.13 (q, J = 7.5 Hz, 2 H), 7.15 (d, J = 7.5 Hz, 1 H), 7.36 (d, J = 15 Hz, 1 H).

2-Benzimidazoleacetates **12a** and **12b** were prepared in analogy to **12c**, starting from **9b** and **9c**, respectively.

***tert*-Butyl 4-[2-(Carbamoylmethyl)-1-cyclopropyl-5-fluoro-6-benzimidazolyl]-1-piperazinecarboxylate (13c)**. A solution of **12c** (4.46 g, 10 mmol) and NH_4Cl (5.35 g, 100 mmol) in EtOH (250 mL) was saturated with NH_3 for 1 h. The resulting suspension was stirred at room temperature for 24 h, then concentrated NH_4OH (50 mL, 50%) was added, and the suspension was further stirred at 50 °C for 24 h. The solvents were evaporated under reduced pressure. The residue was suspended in ethyl acetate (2 × 100 mL) and filtered off. The combined filtrates were concentrated to 50 mL and chromatographed (10% MeOH/ethyl acetate), affording 2.2 g of **13c** after crystallization from ethyl acetate/*n*-hexane: NMR (DMSO-d_6) δ 1.0 (m, 2 H), 1.15 (m, 2 H), 1.43 (s, 9 H), 2.96 (m, 4 H), 3.25 (m, 1 H), 3.52 (m, 4 H), 3.85 (s, 2 H), 7.14 (d, J = 7.5 Hz, 1 H), 7.33 (d, J = 15 Hz, 1 H), 7.10 and 7.70 (2 × br, 2 × 1 H).

2-Benzimidazoleacetamides **13a** and **13b** were prepared in analogy to **13c**, starting from **12a** and **12b**, respectively.

***tert*-Butyl 4-[2-[[(Benzyloxycarbonyl)carbamoyl]-methyl]-1-cyclopropyl-5-fluoro-6-benzimidazolyl]-1-piperazinecarboxylate (13g)**. A solution of **12c** (703 mg, 1.68 mmol) in water/EtOH (5 mL; 1:1) was reacted with *O*-benzylhydroxylamine (450 mg, 5 mmol) and stirred at 50 °C for 72 h. The solvents were evaporated, and the residue was triturated with water. The resulting crystals were filtered and recrystallized from ethyl acetate, yielding 317 mg of **13g** as colorless crystals: NMR ($CDCl_3$) δ 1.05 (m, 2 H), 1.20 (m, 2 H), 1.50 (s, 9 H), 3.05 (m, 4 H), 3.20 (m, 1 H), 3.65 (m, 4 H), 3.95 (s, 2 H), 4.95 (s, 2 H), 7.02 (d, J = 9 Hz, 1 H), 7.21 (d, J = 15 Hz, 1 H), 7.3–7.4 (m, 5 H), 10.95 (br, 1 H).

The *N*-alkoxy-2-benzimidazoleacetamide derivatives **13e** and **13f** were prepared in analogy to **13g**, starting from **12a** and **12b**, respectively.

***tert*-Butyl 3-[[6-[4-(*tert*-Butoxycarbonyl)-1-piperazinyl]-1-cyclopropyl-5-fluoro-2-benzimidazolyl]-acetyl]carbazate (13h)**. A solution of **12c** (1.8 g, 4 mmol) in pyridine (40 mL) was reacted with *tert*-butyl carbazate (2.1 g, 16 mmol) for 65 h at 115 °C. The solvent was evaporated under reduced pressure and the residue was triturated with ether. The crystals were collected by filtration and recrystallized from ethyl acetate, yielding 490 mg of **13h**: NMR (DMSO-d_6) δ 1.05 (m, 2 H), 1.20 (m, 2 H), 1.38 (s, 9 H), 1.43 (s, 9 H), 2.99 (m, 4 H), 3.20 (m, 1 H), 3.55 (m, 4 H), 3.90 (s, 2 H), 7.15 (d, J = 9 Hz, 1 H), 7.35 (d, J = 15 Hz, 1 H), 8.85 (br, 1 H), 9.95 (br, 1 H).

2-Benzimidazoleacetohydrazide derivatives **13i** and **13j** were prepared in analogy to **13h**, starting from **12c** using *tert*-butyl 2-methylcarbazate and hydrazine hydrate, respectively.

***tert*-Butyl 4-[1-Cyclopropyl-2-[(3,3-dimethylcarbazoyl)-methyl]-5-fluoro-6-benzimidazolyl]-1-piperazinecarboxylate (13k)**. A solution of **13j** (430 mg, 1 mmol) in MeOH (22 mL) was reacted with a 35% formaldehyde solution (102 mg, 1.2 mmol) at 50 °C for 20 min. The reaction was allowed to cool and reacted with $NaBH_4$ (46 mg, 1.2 mmol). The reaction was stirred for 20 min and the whole process was repeated three times. The reaction mixture was concentrated under reduced pressure and the residue was taken up in CH_2Cl_2 and washed with 10% aqueous NaCl solution. The organic layer was dried, evaporated, and chromatographed (eluent CH_2Cl_2, then CH_2Cl_2/MeOH 9:1). The relevant fractions were pooled, evaporated under reduced pressure, and crystallized from ethyl acetate, yielding 320 mg of **13k** as colorless crystals.

4. Preparation of Pyrimidobenzimidazoles 11b–v (Table III). *tert*-Butyl 4-[5-Cyclopropyl-8-fluoro-1,2,3,5-tetrahydro-1,3-dioxopyrimido[1,6-*a*]benzimidazol-7-yl]-1-piperazinecarboxylate (11e). A solution of **13c** (0.98 g, 2.35 mmol) in dry THF (10 mL) was treated with *N*,*N*'-carbonyldiimidazole (0.716 g, 4.69 mmol) and DBU (0.2 mL). The solution was warmed to 60 °C for 2 h, then cooled to 0 °C, and filtered. The resulting crystals were washed with THF and ether, giving 0.76 g of **11e** as colorless crystals: NMR (DMSO-d_6) δ 0.9–1.2 (m, 4 H), 1.42 (s, 9 H), 3.0 (m, 4 H), 3.40 (m, 1 H), 3.50 (m, 4 H), 5.15 (s, 1 H), 7.0 (d, J = 9 Hz, 1 H), 7.80 (d, J = 15 Hz, 1 H), 11.2 (s, 1 H).

The corresponding pyrimidobenzimidazole derivatives **11b**, **11g**, **11i**, **11l**, **11n**, **11q**, **11s**, and **11u** were prepared in an analogous way, starting from the benzimidazole derivatives **13b**, **13d**, **13f**, **13e**, **13g**, **13h**, **13i**, and **13k**, respectively.

***tert*-Butyl 4-[5-Cyclopropyl-8-fluoro-1,2,3,5-tetrahydro-2-hydroxy-1,3-dioxopyrimido[1,6-*a*]benzimidazol-7-yl]-1-piperazinecarboxylate (11o)**. A solution of **11n** (0.98 g, 1.87 mmol) in EtOH (250 mL) was hydrogenated over 10% Pd/C. At the end of the reduction, water (100 mL) was added and the mixture was refluxed, filtered, and evaporated to dryness, affording 0.256 g (31%) of **11o**. Another 0.35 g (41%) was obtained by

(12) Glickman, S. A.; Cope, A. C. Structure of β-amino derivatives of α,β-unsaturated lactones and esters. *J. Am. Chem. Soc.* 1945, *67*, 1017–1020.

treatment of the catalyst with hot DMF. NMR (DMSO-d_6) δ 0.96 (m, 2 H), 1.17 (m, 2 H), 1.42 (s, 9 H), 3.01 (m, 4 H), 3.05 (m, 1 H), 3.51 (m, 4 H), 5.32 (s, 1 H), 7.03 (d, J = 7.5 Hz, 1 H), 7.81 (d, J = 13 Hz, 1 H), 10.5 (s, 1 H).

In an analogous procedure, compounds **11j**, **11h**, and 11m were prepared starting from 11i, **11g**, and 11l, respectively.

5-Cyclopropyl-8-fluoro-7-(1-piperazinyl)pyrimido[1,6-*a*]benzimidazole-1,3(2*H*,5*H*)-dione Trifluoroacetate (11f). A solution of **11e** (443 mg, 1 mmol) in TFA (2 mL) was stirred at room temperature for 2 h. The excess reagent was removed under reduced pressure and the solid dried under high vacuum. The colorless powder was suspended in water (5 mL), treated with $NaHCO_3$ (200 mg), filtered, and then washed with water and water/EtOH (1:1). Recrystallization from water/EtOH (5:95) gave 208 mg of **11f** as colorless crystals: NMR (DMSO-d_6) δ 0.9–1.2 (m, 4 H), 3.05 (m, 1 H), 3.30 (m, 8 H), 5.16 (s, 1 H), 7.0 (d, J = 7 Hz, 1 H), 7.76 (d, J = 11 Hz, 1 H), 8.90 (s, 2 H), 11.2 (s, 1 H).

In an analogous way, the deprotected target compounds **11c**, **11k**, **11p**, **11r**, 11t, and 11v were prepared starting from **11b**, **11j**, **11o**, **11q**, **11s**, and **11u**, respectively.

5. Preparation of Aminopyrimidobenzimidazole 14c. 3-Chloro-5-ethyl-8-fluoro-7-(4-methyl-1-piperazinyl)pyrimido[1,6-*a*]benzimidazol-1(5*H*)-one (14b). A suspension of **11a** (465 mg, 1.35 mmol) in $POCl_3$ (10 mL) was stirred at 130 °C for 2 h. The resulting brown yellow solution was evaporated under reduced pressure. The oily residue was taken up in $CHCl_3$ (100 mL) and the solution was treated dropwise with saturated aqueous $NaHCO_3$ solution. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (2 × 100 mL). The combined organic layers were dried and evaporated, affording 293 mg (60%) of **14b** as a yellow powder: NMR (90 MHz, DMSO-d_6) δ 1.55 (t, J = 7.5 Hz, 3 H), 2.50 (s, 3 H), 2.82 (m, 4 H), 3.30 (m, 4 H), 4.22 (q, J = 7.5 Hz, 2 H), 6.32 (s, 1 H), 6.92 (d, J = 6 Hz, 1 H), 8.41 (d, J = 10 Hz, 1 H); MS m/e 363 (M^+).

3-Amino-5-ethyl-8-fluoro-7-(4-methyl-1-piperazinyl)pyrimido[1,6-*a*]benzimidazol-1(5*H*)-one (14c). A suspension of **14b** (200 mg, 0.55 mmol) in MeOH saturated with ammonia (50%, 50 mL) was heated in a stainless steel bomb at 100 °C for 12 h. At the end of the reaction, the volatiles were evaporated, and the residue was chromatographed (eluent ethyl acetate/MeOH 8:2). The relevant fractions were pooled, affording 78 mg (41%) of **14c** as colorless crystals: mp 278–280 °C (EtOH/ethyl acetate); NMR (DMSO-d_6) δ 1.25 (t, J = 7.5 Hz, 3 H), 2.24 (s, 3 H), 2.50 (m, 4 H), 3.06 (m, 4 H), 4.10 (q, J = 7.5 Hz, 2 H), 5.43 (s, 1 H), 6.72 (br, 2 H), 7.17 (d, J = 6 Hz, 1 H), 7.95 (d, J = 10 Hz, 1 H). Anal. ($C_{17}H_{21}FN_6O$) C, H, N.

6. Preparation of Pyrimidoindole 19. 2,4-Difluoro-5-nitroaniline (15b). A solution of **15a** (25 g, 122.5 mmol) in ethyl acetate (1200 mL) was partially hydrogenated (3.25 L of H_2) over 5% Pd/C (3.0 g). After dilution with EtOH (1.2 L), $NaHSO_3$ (500 mL, 10% in H_2O) was added and the mixture was vigorously stirred for 15 min. After separation, the aqueous layer was extracted with ethyl acetate (3 × 1 L). The combined organic layers were washed with brine (3 × 1 L), dried, concentrated, and chromatographed (20% ethyl acetate/*n*-hexane), affording 11.0 g (52%) of **15b**: mp 94–96 °C; NMR ($CDCl_3$) δ 3.90 (s, 2 H), 6.97 (dd, J = 10.3 Hz, 1 H), 7.52 (dd, J = 6.8 Hz, 1 H). Anal. ($C_6H_4F_2N_2O_2$) C, H, N.

2-Amino-5-fluoro-6-nitroindole-3-carboxamide (16). A solution of **15b** (10 g, 57 mmol) in DMF (100 mL) was rapidly added at room temperature to a solution of potassium *tert*-butoxide (12.9 g, 115 mmol) and 2-cyanacetamide (9.7 g, 115 mmol) in DMF (500 mL). After stirring at room temperature for 1.5 h the dark purple solution was poured into ice/water (500 mL) and the pH was immediately adjusted to 5–6 using 2 N HCl. After extraction with ethyl acetate (3 × 0.5 L), the combined organic layers were washed with brine (3 × 0.3 L), dried, and concentrated. The residue was dissolved in DMF (250 mL) and warmed to 55 °C for 2 h. After cooling, the reaction mixture was poured into ice/water. The resulting crystals were collected by filtration, affording, after drying, 8.5 g (59%) of **16**: mp >270 °C; NMR (DMSO-d_6) δ 6.87 (s, 2 H), 7.56 (d, J = 14.0 Hz, 1 H), 7.59 (s, 2 H), 7.88 (d, J = 6.9 Hz, 1 H), 11.0 (s, 1 H). Anal. ($C_9H_7FN_4O_3$) C, H, N.

8-Fluoro-2,5-dihydro-7-nitro-1*H*-pyrimido[4,5-*b*]indol-1-one (17a). A suspension of **16** (8.5 g, 36 mmol) in triethyl orthoformate (170 mL) was warmed to 120 °C for 1.5 h. After cooling, the reaction mixture was diluted with ethyl acetate (170 mL). The crystals were collected by filtration and washed with ethyl acetate, affording 7.2 g (82%) of **17a**: mp >250 °C; NMR (DMSO-d_6) δ 7.86 (d, J = 11.6 Hz, 1 H), 8.20 (d, J = 6.3 Hz, 1 H), 8.29 (s, 1 H), 12.7 (s, 2 H); IR (KBr) 1690 cm^{-1}; MS m/e 248 (M^+), 218 (M^+ – NO), 202 (M^+ – NO_2).

7-Amino-8-fluoro-2,5-dihydro-1*H*-pyrimido[4,5-*b*]indol-1-one (17b). A solution of **17a** (7.2 g, 29 mmol) in DMF (1.2 L) was hydrogenated over 5% Pd/C (2.4 g). After concentration and filtration, the solid was washed with EtOH, affording 6.2 g (98%) of **17b**: mp >250 °C; NMR (DMSO-d_6) δ 5.20 (s, 2 H), 6.82 (d, J = 6.3 Hz, 1 H), 7.43 (d, J = 11.7 Hz, 1 H), 7.96 (s, 1 H), 11.76 (s, 1 H), 12.07 (s, 1 H). Anal. ($C_{10}H_7FN_4O$) C, H, N.

7-(4-Benzyl-1-piperazinyl)-8-fluoro-2,5-dihydro-1*H*-pyrimido[4,5-*b*]indol-1-one (17c). A mixture of **17b** (6.2 g, 28 mmol), bis(β-chloroethyl)benzylamine hydrochloride (23 g, 86 mmol), KI (23.7 g, 143 mmol), and triethylamine (20 mL, 142 mmol) in DMF (300 mL) was warmed to 110 °C for 5 h. The reaction mixture was concentrated, treated with water (1 L), and filtered. The solid was triturated in ethyl acetate and filtered, affording 10 g of **17c**, directly used in the next step. An analytical sample was obtained after chromatography (10% MeOH in CH_2Cl_2): mp >220 °C (dec); NMR (DMSO-d_6) δ 2.58 (m, 4 H), 3.05 (m, 4 H), 3.55 (s, 2 H), 7.03 (d, J = 6.3 Hz, 1 H), 7.20–7.38 (m, 5 H), 7.58 (d, J = 11.7 Hz, 1 H), 8.06 (s, 1 H), 12.09 (s, 1 H), 12.19 (s, 1 H); IR 1666 cm^{-1}; MS m/e 377 (M^+), 286 (M^+ – Bz).

7-(4-Benzyl-1-piperazinyl)-1-chloro-8-fluoro-5*H*-pyrimido[4,5-*b*]indole (18a). A suspension of **17c** (10 g; ~26 mmol, crude material) in a mixture of $POCl_3$ (200 mL) and DMF (50 mL) was warmed to 75 °C for 2 h. After concentration, the reaction mixture was diluted with ethyl acetate (500 mL), poured into 1 N HCl (1 L), and warmed to 70 °C for 30 min. After cooling, the pH was adjusted to 6–7 with Na_2CO_3. After extraction with ethyl acetate (3 × 2 L), the combined organic layers were washed with brine, dried, and concentrated. The oily residue was crystallized (MeOH), yielding 1.66 g (15% from **17b**) of **18a**: mp 238–241 °C; NMR (DMSO-d_6) δ 2.62 (m, 4 H), 3.16 (m, 4 H), 3.57 (s, 2 H), 7.13 (d, J = 7.0 Hz, 1 H), 7.24–7.38 (m, 5 H), 7.90 (d, J = 12.6 Hz, 1 H), 8.71 (s, 1 H), 12.68 (s, 1 H). MS m/e 395 (M^+), 304 (M^+ – Bz). Anal. ($C_{21}H_{19}FClN_5$) C, H, N.

7-(4-Benzyl-1-piperazinyl)-1-chloro-5-ethyl-8-fluoro-5*H*-pyrimido[4,5-*b*]indole (18b). A suspension of **18a** (1.66 g, 4.19 mmol) and potassium carbonate (1.8 g, 13 mmol) in $PO(OEt)_3$[16] (30 mL) was warmed to 100 °C for 2.5 h. The solvent was evaporated under reduced pressure and the residue suspended in dry ether. HCl was bubbled into the solution for 10 min. The resulting colorless crystals were filtered, washed with dry ether, dissolved in saturated aqueous $NaHCO_3$, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated, yielding 1.54 g (86%) of **18b**, which was directly used in the next step: NMR (DMSO-d_6) δ 1.35 (t, J = 7.0 Hz, 3 H), 2.58 (m, 4 H), 3.20 (m, 4 H), 3.59 (s, 2 H), 4.54 (q, J = 7.0 Hz, 2 H), 7.24–7.46 (m, 6 H), 7.94 (d, J = 12 Hz, 1 H), 8.78 (s, 1 H); MS m/e 423 (M^+).

1-(Benzylamino)-7-(4-benzyl-1-piperazinyl)-5-ethyl-8-fluoro-5*H*-pyrimido[4,5-*b*]indole (18c). A solution of **18b** (0.464 g, ~1.1 mmol, crude) in benzylamine (6 mL) was warmed to 100 °C for 1 h. After concentration under reduced pressure, the residue was chromatographed (60% ethyl acetate/*n*-hexane), yielding after crystallization (MeOH) 0.275 g (44% from **18a**) of **18c**: mp 106–111 °C; NMR (DMSO-d_6) δ 1.30 (t, J = 7.0 Hz, 3

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H), 2.60 (m, 4 H), 3.16 (m, 4 H), 3.60 (s, 2 H), 4.41 (q, J = 7.0 Hz, 2 H), 4.84 (d, J = 6.0 Hz, 2 H), 7.17-7.40 (m, 11 H), 7.26 (t, J = 6.0 Hz, 1 H), 8.32 (s, 1 H), 8.34 (d, J = 13 Hz, 1 H). Anal. ($C_{30}H_{31}FN_6$) C, H, N.

1-Amino-5-ethyl-8-fluoro-7-(1-piperazinyl)-5*H*-pyrimido[4,5-*b*]indole (19). A solution of **18c** (0.258 g, 0.52 mmol) in a mixture of EtOH (100 mL) and 1 N HCl (10 mL) was hydrogenated over 5% Pd/C (0.1 g) and PdO (0.1 g) at 78 °C for 4 h. After filtration, the pH was adjusted to 9 with 1 N NaOH and the solution concentrated in vacuo. The colorless solid was triturated with water (50 mL) and filtered, yielding 50 mg (31%) of **19**: mp 219-221 °C; NMR (DMSO-d_6) δ 1.28 (t, J = 7.0 Hz, 3 H), 2.90 (m, 4 H), 3.00 (m, 4 H), 4.39 (q, J = 7.0 Hz, 2 H), 7.13 (s, 2 H), 7.20 (d, J = 7.0 Hz, 1 H), 8.21 (d, J = 13 Hz, 1 H), 8.24 (s, 1 H); MS m/e 314 (M^+).

7. Enzyme Inhibition. The compounds synthesized were evaluated in a DNA supercoiling assay.[13] Equimolar quantities of each DNA gyrase subunit[14,15] were reconstituted to the A_2B_2 complex by incubation for 30 min at 25 °C. Relaxed pUC18 DNA (7.5 nM) was incubated with DNA gyrase (3.85 nM) in 35 mM Tris-HCl (pH 7.5), 24 mM KCl, 4 mM $MgCl_2$, 1.8 mM spermidine, 9 μg/mL tRNA, 5 mM DTT, 1.4 mM ATP, 100 μg/mL BSA and the compound tested at concentrations between 0.1 and 100 μg/mL at 37 °C for 30 min. Samples were electrophoresed on a 0.8% agarose gel and the inhibiting activity of the compound tested was expressed as MNEC (maximal noneffective concentration), i.e. the highest inhibitor concentration at which all DNA is still completely supercoiled.

8. In Vitro Antibacterial Activity. The in vitro antibacterial activity of the compounds was determined in a side-by-side comparison with fleroxacin by standard agar dilution method on DST agar (Oxoid). The compounds were incorporated into a melted medium of 50 °C just prior to the pouring and use of the plates. The inoculum of approximately 10^4 colony-forming units (CFU) was prepared from appropriately diluted overnight cultures and applied to the agar surface with a multipoint inoculating device (Denley A400 multipoint inoculator). The lowest concentration of the drug that prevented the macroscopic growth of a culture after 18 h of incubation at 35 °C was recorded as minimal inhibitory concentration (MIC).

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Preparation and in Vitro and in Vivo Evaluation of Quinolones with Selective Activity against Gram-Positive Organisms

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A series of quinolones were prepared which contained oximes or substituted oximes as replacements for the amine substituents normally found on the pyrrolidine or piperidine fragments of quinolone antibacterial agents. These substituents led to compounds that had selective activity against Gram-positive organisms. These compounds showed in vivo activity against *Staphylococcus aureus*. Only compound **29** had in vivo activity against *Streptococcus pneumoniae*.

Introduction

The topoisomerases are a group of enzymes that control the linking number of double-stranded DNA molecules, and they are divided into three groups. The enzyme DNA gyrase is a member of the topoisomerase II group.[1] The inhibition of bacterial DNA gyrase has been the target of a worldwide research effort which began with the discovery of nalidixic acid (1) in the early 1960s.[2] Structure–activity relationships (SAR) of compounds based on nalidixic acid have led to a large group of synthetic antibacterial agents known collectively as the quinolones.[3] The compounds

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