Structure−Activity Relationship Study on Anti-HIV 6-Desfluoroquinolones

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On the basis of our recent findings that 6-aminoquinolones inhibit the HIV Tat-mediated transactivation, we have designed a broad series of derivatives identifying novel potent agents such as the 6-desfluoroquinolones **24** (**HM12**) and **27** (**HM13**), which showed pronounced anti-HIV activity in acutely, chronically, and latently HIV-1 infected cell cultures. We demonstrate here that highly potent molecules can be obtained by optimizing the substituent in the various positions of the quinolone nucleus.

Introduction

The HIV pandemic remains one of the most serious threats to public health. While the currently available drugs effectively reduce the levels of the rapidly replicating virus, the benefit is only short-term due to the emergence of resistant strains[1] and the inability of the current anti-HIV chemotherapy to completely eradicate viral infection[2] caused by the occurrence of stable latent reservoirs mainly represented by latently infected resting $CD4^+$ lymphocytes[3] and monocyte/macrophages (M/M[a]).[4] Therefore, there is an urgent need to develop anti-HIV agents with a new mechanism of action able to overcome these problems.

An innovative intervention site could be the transcription from the 5′ LTR (long terminal repeat) promoter, a crucial step in the HIV replication cycle. Inhibitors of this process will be able to suppress HIV replication not only in acutely but also in chronically infected cells.[5] Various attempts have been made to discover suitable selective inhibitors of HIV-1 gene expression and transcription,[6,7] but to date, none of the compounds have led to a successful clinical development due to low bioavailability and/or high toxicity.

In this scenario, the 6-aminoquinolones[8,9] have emerged as a promising new class of HIV expression inhibitors, interfering with the Tat-mediated transactivation of the HIV-1 LTR promoter.[10] The advent of quinolones as antiviral agents is particularly attractive because quinolones are small extremely versatile molecules, easily synthesized at low cost on a large scale and endowed with well-known biochemical properties that make them very suitable pharmacophore structures. Therefore, it is extremely important to explore this class of compounds in an effort to design even more selective anti-HIV inhibitors. In fact, the first quinolone-based structure (GS-9137/JTK-303)[11] with very strong antiretroviral properties is currently in advanced clinical trials. Interestingly, in contrast to 6-aminoquinolones, this compound owes its anti-HIV activity exclusively to the inhibition of the viral enzyme integrase.[12]

Starting from **1** (**WM5**),[8] the prototype of the 6-aminoquinolone series, and in order to determine the optimal substitution pattern, we have designed new derivatives challenging all the features thought to be essential for the biological activity (Chart 1). The carboxylic function was replaced by a primary amide (**2**), secondary amides (**3** and **4**), a keto group (**5**), or oxime moieties (**6** and **7**). In a more drastic structural modification, the C-3 carboxylic function was banned, synthesizing the C-3 decarboxylate quinolinone derivatives **8**−**10**. Concerning the N-1 position, earlier structure−activity relationship (SAR) studies showed that the highest antiretroviral activity was achieved with a small substituent, with a methyl group being the most optimal. In this study, we synthesized derivatives **11**−**14** to explore the effect of the absence of a substituent at this position. We also turned our attention to the C-7 position, modifying the 4-(2-pyridinyl)-1-piperazinyl moiety characterizing the lead **1**, in both the piperazine and N-4 aryl rings, synthesizing derivatives **15**−**22**. In a previous study, we showed that the elimination of the 6-amino group in **1** resulted in the 6-hydrogen analogue **23**[9] (Chart 1), which had a weak antiviral activity in MT-4 cells but a markedly lower cytotoxicity (about 100 times less toxic than **1**). In this study, we aimed at maintaining low cytotoxicity while increasing antiviral potency, and therefore we have prepared new 6-hydrogenquinolones, **24**−**28** (Chart 1), by introducing those 4-arylpiperazines which conferred high potency on previous 6-aminoquinolone series at the C-7 position.

Chemistry

The C-3 amide derivatives **2**−**4** were prepared in good yield by reacting the ethyl 6-amino-1-methyl-4-oxo-7-[4-(2-pyridinyl)-1-piperazinyl]-1,4-dihydro-3-quinolinecarboxylate[8] with a saturated ammonia solution in EtOH in a closed vessel or with the selected benzylamines in neat. The synthesis of C-3 keto (**5**) and oxime derivatives (**6** and **7**) (Scheme 1) was accomplished by starting from 3-chloro-4-nitroaniline, which was elaborated to synthone **31** by reaction with methyl 2-acetyl-3-ethoxy-2-propenoate[13] to give the acrylate **29**, which was successively thermically cyclized to derivative **30** and finally N-1 methylated. The nucleophilic reaction with 1-(2-pyridinyl)piperazine followed by catalytic reduction, gave the 6-amino-3-acetyl derivative **5**, which was further elaborated to oxime derivatives **6** and **7** by reaction with hydroxylamine and methoxylamine, respec-

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[a] Abbreviations: 6-DFQs, 6-desfluoroquinolones; GFP, green fluorescent protein; HCMV, human cytomegalovirus; HIV, human immunodeficiency virus; LTR, long terminal repeat; M/M, human primary monocyte/macrophages; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBMCs, peripheral blood mononuclear cells; SAR, structure−activity relationship.

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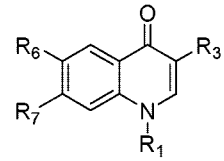
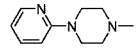
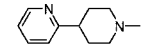
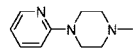
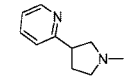
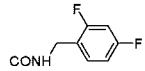
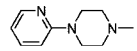
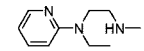
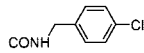
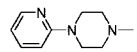
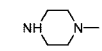
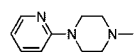
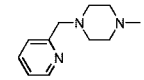
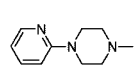
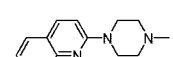
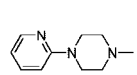
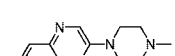
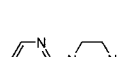
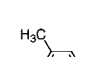
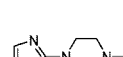
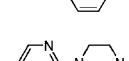
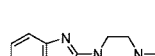
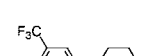
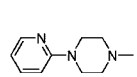
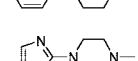
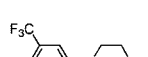
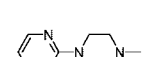
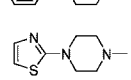
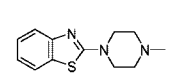
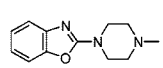
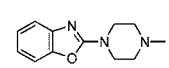
Chart 1. Structure of the 6-DFQs Synthesized in this Study and Reference Compounds



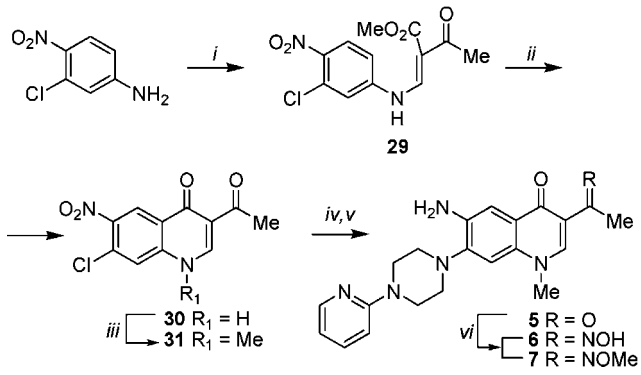
compd	R_1	R_3	R_6	R_7	compd	R_1	R_3	R_6	R_7
1[8]	Me	CO_2H	NH_2		**15**	Me	CO_2H	NH_2	
2	Me	$CONH_2$	NH_2		**16**	Me	CO_2H	NH_2	
3	Me		NH_2		**17**	Me	CO_2H	NH_2	
4	Me		NH_2		**18**	Me	CO_2H	NH_2	
5	Me	COMe	NH_2		**19**	Me	CO_2H	NH_2	
6	Me	C(NOH)Me	NH_2		**20**	Me	CO_2H	NH_2	
7	Me	C(NOMe)Me	NH_2		**21**	Me	CO_2H	NH_2	
8	Me	H	NH_2		**22**	Me	CO_2H	NH_2	
9	Me	H	NH_2		**23**[9]	Me	CO_2H	H	
10	Me	H	NH_2		**24**	Me	CO_2H	H	
11	H	CO_2H	NH_2		**25**	Me	CO_2H	H	
12	H	CO_2H	NH_2		**26**	Me	CO_2H	H	
13	H	CO_2H	NH_2		**27**	Me	CO_2H	H	
14	H	CO_2H	NH_2		**28**	Me	CO_2H	H	

tively. To obtain the C-3 decarboxylate quinolones **8**−**10** (Scheme 2), carboxylic acid **32**[14] was decarboxylated, even if in very low yield, to quinolinone **33** by using Ph_2O at reflux for 15 h. The successive nucleophilic reaction with selected 4-arylpiperazines gave the nitro derivatives **34**−**36**, which were then catalytically reduced to the 6-amino target derivatives. The route followed to synthesize 4-hydroxyquinolinyl derivatives **11**−**14** (Scheme 3) parallels the cycloaracylation procedure used to synthesize similar compounds,[8,9] which starts with acrylate **37**[15] and proceeds through quinolone **38**[16] and C-7 functionalized intermediates **39**−**42**. The 6-aminoquinolones **15**−**22** were prepared by conventional route (Scheme 4) starting from synthone **43**,[8] which was reacted with various side chains to give intermediates **44**−**51** and in turn hydrogenated and finally hydrolyzed. The 6-hydrogen derivatives **24**−**28** were prepared as previously reported for **23**[9] by nucleophilic reaction of 7-chloro-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid borine complex[14] with selected 4-arylpiperazines in DMSO and Et_3N.

Results and Discussion

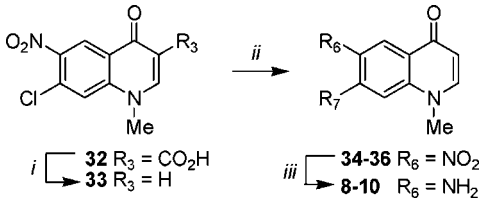
All the synthesized compounds were initially evaluated for anti-HIV-1 (III_B) and anti-HIV-2 (ROD) activity in MT-4 cells. The cytotoxicity of the compounds was determined in parallel. The results of the active compounds are shown in Table 1, together with reference compounds **1**[8] and **23**,[9] for comparative purposes (the results of inactive compounds are reported in Table S1 of Supporting Information). The anti-HIV activity of some selected compounds was also evaluated in the human lympho-

Scheme 1[a]



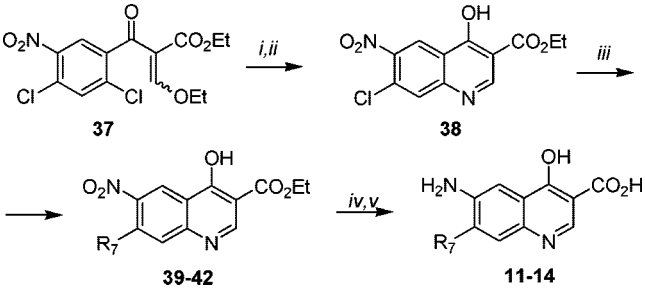
[a] Reagents: (i) EtOCH=C(COMe)CO_2Me, 120 °C; (ii) Dowtherm A, 200 °C; (iii) MeI, K_2CO_3, DMF, 90 °C; (iv) 1-(2-pyridinyl)piperazine, DMF, 80 °C; (v) H_2, Raney Ni, DMF; (vi) $H_2NOR \cdot HCl$, pyridine, 65 °C.

Scheme 2[a]



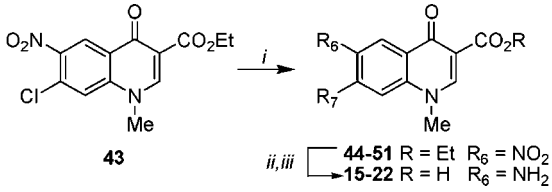
[a] Reagents: (i) Ph_2O, reflux; (ii) R_7H, DMF, 90 °C; (iii) H_2, Raney Ni, DMF or HCO_2NH_4, 10% Pd/C, DMF.

Scheme 3[a]



[a] Reagents: (i) NH_3/EtOH/Et_2O; (ii) K_2CO_3, DMF, 100 °C; (iii) R_7H, DMF, 80-110 °C; (iv) H_2, Raney Ni, DMF or HCO_2NH_4, 10% Pd/C, DMF; (v) 6N HCl/EtOH, reflux, or 1N NaOH, reflux.

Scheme 4[a]



[a] Reagents: (i) R_7H, DMF, 70-110 °C or K_2CO_3, CH_3CN, 90 °C; (ii) H_2, DMF, Raney Ni or 5% Pd/C; (iii) 6N HCl/EtOH, reflux or 1N NaOH, reflux.

cytic CEM cell line and PBMCs (see Table S2 in Supporting Information).

SAR studies confirmed that the C-3 carboxylic moiety is indispensable for the biological activity. In fact, its replacement with an amide function, as well as with keton or oxime groups, resulted in derivatives **2**−**7** that were completely devoid of cytotoxicity and antiviral properties. The same was also observed with **8**−**10**, which lacked the C-3 carboxylic moiety; this is in agreement with what has been previously observed by Baba and co-workers for anti-HIV fluoroquinolones.[17] For the C-7 position, the piperazine ring was confirmed as the best linker between quinolone and pyridine rings; it cannot be replaced by either its piperidine bioisoster (**15**) or ring constrained pyrrolidine (**16**) without a loss of antiviral activity. The opening of the piperazine ring was also detrimental for activity in that compound **17** was not active at subtoxic concentrations. When we eliminated the aromatic portion on the N-4 position of the piperazine ring (**18**) or introduced one methylenic unit in the aromatic portion (**19**), the antiviral activity disappeared. On the contrary, the 7-quinolinylpiperazinyl derivative **20** and 7-quinoxalinylpiperazinyl derivative **21** showed good anti-HIV activity. Both compounds also showed high anti-HIV-1 and HIV-2 activity in CEM cells (**20**, $EC_{50} = 0.036$ and 0.075 μg/mL; **21**, $EC_{50} = 0.05$ and 0.28 μg/mL), while they are less toxic resulting in higher SI values (115 and 55 on HIV-1 and HIV-2, respectively, for **20**).

The most interesting results came from the SAR investigations on the N-1 and C-6 positions. In particular, the absence of a substituent at the N-1 position, did not cause a loss of activity in contrast with the well-established SAR for both antibacterial[18] and antiviral quinolone agents.[19] In fact, derivatives **11**−**14**, generally maintain good antiviral activity in both MT-4 and CEM cell cultures, and more importantly they were also active in PBMCs with EC_{50} values ranging from 0.094 to 0.21 μg/mL. It is worth noting that the absence of the substituent at the N-1 position gives rise to the keto/enol tautomers, and the relative equilibrium, under physiological or assay conditions, is not easily predictable.[20] Considering that the 4-keto-3-carboxylic arrangement was previously reported as indispensable for target recognition, through a Mg^{2+} bridge, in the antibacterial quinolones[21] as well as for the anti-HIV 6-aminoquinolones,[8] fluorimetric titration experiments were carried out for the derivative **11**[22] to check its ability to form complexes with this divalent ion. The experiments showed that it was still able to complex Mg^{2+} ions even if with a lower constant ($K_{Mg} = 2530$ M^{-1}) compared to its N-1 methyl counterpart **1** ($K_{Mg} = 6650$ M^{-1})[23] but superior to that of the antibacterial ciprofloxacin ($K_{Mg} = 790$ M^{-1}).[23]

The synthesis of the new quinolones lacking the C-6 substituent resulted in very interesting compounds. In particular, at concentrations lower than 125 μg/mL in MT-4 and 100 μg/mL in CEM cells, the 6-hydrogenquinolones **25**, **26**, and **28** were not toxic but the anti-HIV activity was compromised. In contrast, a balanced activity was displayed by the *m*-(trifluoromethyl)phenylpiperazinyl derivative **24** (**HM12**), which in MT-4 cells showed $EC_{50} = 0.07$ and 0.13 μg/mL and $CC_{50} = 2.61$ μg/mL. Very good anti-HIV activity was also observed with the benzothiazolpiperazinyl derivative **27** (**HM13**), which was the most interesting compound when assayed in acutely infected CEM cell cultures because it displayed the same anti-HIV-1 and HIV-2 activity as observed in MT-4 but was 10 times less toxic, resulting in SI values of 281 and 104, respectively. Compounds **24** and **27** were also tested in PBMCs, where they maintained good anti-HIV-1 activity with $EC_{50} = 0.28$ and 0.15 μg/mL, respectively.

Whereas other quinolones also blocked the in vitro replication of HCMV (human cytomegalovirus), compounds **24** and **27** have an antiviral activity that is limited to HIV-1 and -2.

To study the molecular mechanism of action of the new 6-desfluoroquinolones (6-DFQs), time-of-addition experiments were carried out for derivative **27**, including reference compounds with a known mode of action. This experiment determines how long the addition of an HIV replication inhibitor

Table 1. Anti-HIV-1 and -HIV-2 Activity and Cytotoxicity of Selected Quinolones in MT-4 cells.

compd	HIV-1 (III_B) EC_{50} (μg/mL)[a,c]	HIV-2 (ROD) EC_{50} (μg/mL)[a,c]	CC_{50} (μg/mL)[b,c]	SI[d] (III_B)	SI[d] (ROD)
1[8]	0.058 ± 0.021	0.097 ± 0.053	0.84 ± 0.40	15	10
11	0.45 ± 0.27	0.16 ± 0.02	2.16 ± 0.18	5	14
12	0.38 ± 0.04	0.46 ± 0.08	1.64 ± 0.24	4	4
13	1.21 ± 0.55	1.92 ± 0.17	7.61 ± 2.07	7	5
14	0.23 ± 0.09	0.28 ± 0.04	1.95 ± 0.45	11	7
20	0.035 ± 0.004	0.022 ± 0.017	0.21 ± 0.10	6	10
21	0.18 ± 0.04	0.19 ± 0.10	3.20 ± 1.02	18	17
23[9]	19.20 ± 3.53	>65.53	65.53 ± 26.39	3.4	<1
24	0.07 ± 0.01	0.13 ± 0.02	2.61 ± 0.66	38	21
25	10.03 ± 1.80	12.70 ± 0.57	>125	>12	>10
26	58.25 ± 31.32	16.35 ± 0.49	>125	>2	8
27	0.03 ± 0.01	0.04 ± 0.03	0.44 ± 0.12	14.7	5.5
28	5.76 ± 4.84	11.86 ± 6.99	>125	>22	>11

[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cells from HIV induced cytopathogenicity, as determined by the MTT method. [b] CC_{50}: concentration of compound that reduces the viability of mock-infected cells by 50%, as determined by the MTT method. [c] All data represent mean values ± standard deviations for at least two separate experiments. [d] SI: ratio of CC_{50}/EC_{50}.

can be postponed before losing its antiviral activity. Compound **27** kept its antiviral activity when added at least up to 10 h postinfection, suggesting that it inhibits the viral replication at a late stage (details and Figure S1 in Supporting Information). To study if these compounds act on a postintegrational target of the HIV replication, compounds **24** and **27** were tested for their effect on virus expression from persistently infected human T lymphoma cell line $HuT78_{IIIB}$. These cells contain integrated provirus and constitutively release virus in the supernatant. In this assay, compounds **24** and **27** efficiently prevent virus production at nontoxic concentrations, suggesting a postintegrational mechanism of action (details and Figure S2 in Supporting Information). Subsequently, the effect on Tat-mediated gene expression was investigated using a Tat-assay in Jurkat cells. In this assay, compounds **27** and **28** (selected due to its nontoxic profile) inhibited the Tat-mediated green fluorescent protein (GFP) gene expression from the HIV-1 LTR promoter in a dose-dependent manner, suggesting a target of interaction related to HIV-1 gene expression (details and Figure S3 in Supporting Information).

In conclusion, this study provided new insights in the SAR of anti-HIV quinolones and led to the identification of new potent and selective derivatives. While the presence of a carboxylic function and the 4-arylpiperazine ring at the C-3 and C-7 positions, respectively, turned out to be crucial to preserve antiviral activity, the expansion of the heteroaryl system at the N-4 piperazine side chain increased the activity as shown by derivatives **20** and **21**. The real novelty of this study, however, emerged from the elimination of any substituent at the N-1 or C-6 positions of the quinolone nucleus. The preserved anti-HIV activity of the N-1 unsubstituted derivatives **11**−**14** challenges the well-established quinolone SAR. This unexpected structure−activity feature should be taken into account in the design of additional selective anti-HIV quinolone analogues. The most significant result was definitely achieved with the synthesis of a variety of new 6-hydrogenquinolones. In fact, when the appropriate 4-arylpiperazine was introduced at the C-7 position, very active and selective derivatives were obtained such as the 6-hydrogenquinolones **24** and **27**. These compounds are endowed with very strong activity on HIV-1 acutely infected MT-4, CEM, and PBMCs cells as well as on chronically infected HuT78. A potent antiviral activity was also observed in latently HIV-1 infected M/M cells at drug concentrations as low as 40 ng/mL.[24] This activity was further confirmed in an in vivo model for HIV-1 latency, which provides encouraging evidence for the use of quinolones in the control of HIV-1 infection in vivo.[24] The mechanism of action studies clearly demonstrated that the new 6-DFQs interfere with a postintegrational target of the HIV-1 replication cycle, principally a target involving HIV-1 gene expression.

Experimental Section

General Procedure for Coupling Reaction (Method A). A mixture of the appropriate synthone (1 equiv) and selected side chains (3−5 equiv) in dry DMF was heated at 70−110 °C until no starting material could be detected by TLC (1−36 h). The reaction mixture was worked up and purified as reported in the description of the single compounds.

General Procedure for Reduction of 6-Nitro Group (Method B). A stirred solution of the selected 6-nitro derivative in DMF was hydrogenated over a catalytic amount of Raney nickel at room temperature and atmospheric pressure until no starting material could be detected by TLC (1−30 h). The mixture was then filtered over celite, and the filtrate was evaporated to dryness to afford a residue which, by treatment with EtOH/EtOAc, gave a solid that was filtered and dried to give the corresponding 6-amino derivative.

General Procedure for Acid Hydrolysis (Method C). A mixture of the selected ester (1 mequiv) in EtOH (6 mL) and 6N HCl (6 mL) was refluxed until no starting material could be detected by TLC (2−72 h). It was worked up and purified as reported in the description of the single compounds.

6-Amino-4-hydroxy-7-[4-(2-pyridinyl)-1-piperazinyl]-3-quinolinecarboxylic acid (11). The title compound was prepared starting from synthone **38**[16] through general coupling procedure (method A), using 1-(2-pyridinyl)piperazine (110 °C, 30 h); after cooling, the precipitate solid was filtered, washed with EtOH, and dried to give compound **39**. It was then reduced by general reduction procedure (method B) (3 h) and hydrolyzed by general hydrolysis procedure (method C) (30 h): after cooling, the mixture was filtered and the filtrate was neutralized by adding 10% NaOH. The solid obtained was collected by filtration, washed with water and EtOH and finally recrystallized by EtOH/DMF to give acid **11** in 15% overall yield.

1-Methyl-4-oxo-7-{4-[3-(trifluoromethyl)phenyl]-1-piperazinyl}-1,4-dihydro-3-quinolinecarboxylic acid (24). A mixture of 7-chloro-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid BF_2 chelate[9] (0.80 g, 2.80 mmol), 1-[3-(trifluoromethyl)phenyl]piperazine (3.22 g, 14 mmol), and Et_3N (1.4 g, 14 mmol) in dry DMSO (10 mL) was heated at 90 °C for 30 min. After cooling, the precipitate was filtered, washed with ice/water, dried, and then recrystallized from DMSO to give **24** (0.30 g, 25%).

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Supporting Information Available: Experimental details corresponding to the synthesis and analytical data of compounds described in this paper. A table containing elemental analysis data for target compounds **2**−**22** and **24**−**28**. Complete descriptions of biological protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hirsch, M. S.; Conway, B.; D'Aquila, R. T.; Johnson, V. A.; Brun-Vezinet, F.; Clotet, B.; Demeter, L. M.; Hammer, S. M.; Jacobsen, D. M.; Richman, D. D. Antiretroviral Drug Resistance Testing in Adult HIV-infection. *J. Am. Med. Assoc.* **2000**, *279*, 1984–1991.

(2) Marcello, A. Latency: the Hidden HIV-1 Challenge. *Retrovirology* **2006**, *3*, 7.

(3) Chun, T.-W.; Justement, J. S.; Lempicki, R. A.; Yang, J.; Dennis, G. D.; Hallahan, C. W.; Sanford, C.; Pandya, P.; Liu, S.; McLaughlin, M.; Ehler, L. A.; Moir, S.; Fauci, A. S. Gene Expression and Viral Production in Latently Infected, Resting $CD4^+$ T cells in Viremic versus Aviremic HIV-infected Individuals. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 1908–1913.

(4) McElrath, M. J.; Pruett, J. E.; Cohn, Z. A. Mononuclear Phagocytes of Blood and Bone Marrow: Comparative Roles as Viral Reservoirs in Human Immunodeficiency Virus Type 1 Infections. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 675–679.

(5) Daelemans, D.; Vandamme, A. M.; De Clercq, E. Human Immunodeficiency Virus Gene Regulation as Target for Antiviral Chemotherapy. *Antivir. Chem. Chemother.* **1999**, *10*, 1–10.

(6) For recent review see:(a) Baba, M. Recent Status of HIV-1 Gene Expression Inhibitors. *Antivir. Res.* **2006**, *71*, 301–306.

(7) Stevens, M.; De Clercq, E.; Balzarini, J. The Regulation of HIV-1 Transcription: Molecular Targets for Chemotherapeutic Intervention. *Med. Res. Rev.* **2006**, *5*, 595–625.

(8) Cecchetti, V.; Parolin, C.; Moro, S.; Pecere, T.; Filipponi, E.; Calistri, A.; Tabarrini, O.; Gatto, B.; Palumbo, M.; Fravolini, A.; Palù, G. 6-Aminoquinolones as New Potential Anti-HIV Agents. *J. Med. Chem.* **2000**, *3*, 3799–3802.

(9) Tabarrini, O.; Stevens, M.; Cecchetti, V.; Sabatini, S.; Dell'Uomo, M.; Manfroni, G.; Palumbo, M.; Pannecouque, C.; De Clercq, E.; Fravolini, A. Structure Modifications of 6-Aminoquinolones with Potent anti-HIV Activity. *J. Med. Chem.* **2004**, *47*, 5567–5578.

(10) Stevens, M.; Balzarini, J.; Tabarrini, O.; Andrei, G.; Snoeck, R.; Cecchetti, V.; Fravolini, A.; De Clercq, E.; Pannecouque, C. Cell-Dependent Interference of a Series of New 6-Aminoquinolone Derivatives with Viral (HIV/CMV) Transactivation. *J. Antimicrob. Chemother.* **2005**, *56*, 847–855.

(11) Sato, M.; Motomura, T.; Aramaki, H.; Matsuda, T.; Yamashita, M.; Ito, Y.; Kawakami, H.; Matsuzaki, Y.; Watanabe, W.; Yamataka, K.; Ikeda, S.; Kodama, E.; Matsuoka, M.; Shinkai, H. Novel HIV-1 Integrase Inhibitors Derived from Quinolone Antibiotics. *J. Med. Chem.* **2006**, *49*, 1506–1508.

(12) Daelemans, D.; Lu, R.; De Clercq, E.; Engelman, A. Characterization of a Replication-Competent, Integrase-defective Human Immunodeficiency Virus (HIV)/Simian Virus 40 Chimera as a Powerful Tool for the Discovery and Validation of HIV Integrase Inhibitors. *J. Virol.* **2007**, *81*, 4381–4385.

(13) Schenone, P.; Sansebastiano, L; Mosti, L. Reaction of 2-Dimethylaminomethylene-1,3-diones with Dinucleophiles. VIII Synthesis of Ethyl and Methyl 2,4-disubstituited 5-pyrimidinecarboxylates-[1]. *J. Heterocycl. Chem.* **1990**, *27*, 295–305.

(14) Barton, N.; Crowther, A. F.; Hepworth, W.; Richardson, D. N.; Driver, G. W. Quinolones and Therapeutic Compositions Containing Them. UK Patent GB830832, 1960.

(15) Petersen, U.; Grohe, K.; Kuck, K. H. Quinolonecarboxylic Acid Derivatives and Their Use as Bactericidal Agents. German Patent DE 3308908, 1985.

(16) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. Structure−Activity Relationships of Antibacterial 6,7- and 7,8-Disubstituted 1-Alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acids. *J. Med. Chem.* **1980**, *23*, 1358–1363.

(17) Baba, M.; Okamoto, M.; Kawamura, M.; Makino, M.; Higashida, T.; Takashi, T.; Kimura, Y.; Ikeuchi, T.; Tetsuka, T.; Okamoto, T. Inhibition of Human Immunodeficiency Virus Type 1 Replication and Cytokine Production by Fluoroquinolone Derivatives. *Mol. Pharmacol.* **1998**, *53*, 1097–1103.

(18) Mitscher, L. A.; Devasthale, P.; Zavod, R. Structure−Activity Relationships In *Quinolone Antimicrobial Agents*, 2nd ed.; Hooper, D. C., Wolfson, J. S., Eds.; American Society for Microbiology: Washington, D.C., 1998; pp3−51.

(19) Richter, S.; Parolin, C.; Palumbo, M.; Palù, G. Antiviral Properties of Quinolone-based Drugs. *Curr. Drug Targets−Infect. Disord.* **2004**, *4*, 111–116.

(20) (a) For a discussion of the tautomer equilibrium in the 4-hydroxyquinoline heterocycle, see:De la Cruz, A.; Elguero, J.; Goya, P.; Martínez, A. Tautomerism and Acidity in 4-Quinolone-3-Carboxylic Acid Derivatives. *Tetrahedron* **1992**, *48*, 6135–6150.

(21) Palumbo, M.; Gatto, B.; Zagotto, G.; Palù, G. On the Mechanism action of Quinolone Drugs. *Trends Microbiol.* **1993**, *1*, 232–235.

(22) Fluorimetric tritation experiments for **11** were performed in similar conditions to that reported for compound **1** and ciprofloxacin in ref 23.

(23) Richter, S.; Gatto, B.; Tabarrini, O.; Fravolini, A.; Palumbo, M. Antiviral 6-Aminoquinolones: Molecular Basis for Potency and Selectivity. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4247–4251.

(24) Stevens, M.; Pollicita, M.; Pannecouque, C.; Verbeken, E.; Tabarrini, O.; Cecchetti, V.; Acquaro, S.; Perno, C. F.; Fravolini, A.; Schols, D.; De Clercq, E; Balzarini, J. A Novel in Vivo Model for the Study of HIV-1 Transcription Inhibitors: Evaluation of New 6-Desfluoroquinolone Derivatives (6-DFQs). *Antimicrob. Agents Chemother.* **2007**, *4*, 1407–1413.

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