6-Aminoquinolones as New Potential Anti-HIV Agents

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Received July 1, 1999

A series of 6-aminoquinolone compounds were evaluated for their in vitro activity against human immunodeficiency virus type 1 (HIV-1). Compound **12a**, bearing a methyl substituent at the N-1 position and a 4-(2-pyridyl)-1-piperazine moiety at the C-7 position, was the most active in inhibiting HIV-1 replication on de novo infected C8166 human lymphoblastoid cell lines. The **12a** EC_{50} value was 0.1 μM, a 7−20-fold lower concentration relative to that for compounds **8a** and **7a** containing a cyclopropyl and *tert*-butyl substituent at the N-1 position, respectively. When the C-6 amino group was replaced with a fluorine atom, a decreased antiviral effect was observed. The observed effects are selective, since potency is substantially reduced when testing the compounds against the herpes simplex virus type 1 (HSV-1). Active quinolone derivatives very efficiently interact with TAR RNA, which suggests a nucleic acid-targeted mechanism of action.

Introduction

The search for new anti-HIV compounds continues to be a major challenge in antiviral chemotherapy. Despite the recent success of highly active antiretroviral therapy (HAART) in prolonging survival of AIDS patients and in reducing morbidity due to opportunistic infections,[1] long-term toxicity of combinations of antireverse transcriptase and antiprotease drugs and selection of drug-resistant viral mutants are still a major concern. Efforts are therefore devoted to the discovery of compounds with diverse mechanisms of action.

It has been recently found that antibacterial fluoroquinolones can exhibit antiviral activity per se. Two analogues,[2−4] bearing a 3,4-didehydro-4-phenyl-1-piperidinyl moiety at the C-7 position, exhibited EC_{50} < 50 nM in chronically infected cells. They were suggested to act, at least in part, through inhibition of cellular factors working or cooperating with Tat.[4]

Preliminary tests suggest that also the new family of 6-aminoquinolones, originally synthesized as potential antibacterials,[5] could exhibit anti-HIV and anti-HSV activities, a possible lead being represented by the 1-*tert*-butyl-7-[4-(2-pyridyl)-1-piperazinyl] derivative **7a**.[6] We report here the synthesis and antiviral activities of additional 6-aminoquinolones (**7c**−**f**, **8a**, **9**, **12a**−**14a**, and **16a**) and a preliminary characterization on their mechanism of action. Several comparative 6-fluoro analogues (**20a**−**22a**) were also prepared and tested.

Chemistry

The cycloaracylation procedure was employed to build the quinolone framework. Thus, starting with suitable ethyl α-aroyl-*β*-(dimethylamino)acrylate derivatives by sequential reaction with appropriate amines and cyclization, the desired N-substituted ethyl 7-chloro-6-nitro(or fluoro)-4-oxo-1,4-dihydroquinoline-3-carboxylate intermediates were obtained. The synthons, ethyl 7-chloro-1-*tert*-butyl(or cyclopropyl)-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylates, were elaborated to the 6-amino-1-*tert*-butyl and 6-amino-1-cyclopropyl target acids **7a**−**f** and **8a** respectively, as well as ethyl 7-fluoro-1,8-dimethyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate to the 6-amino-1,8-dimethyl acid **16a**, through a three-step sequence: nucleophilic displacement of the C-7 halogen with appropriate heterocyclic bases, catalytic reduction of the C-6 nitro group, and basic or acidic hydrolysis.

Similarly, 6-amino-1-methyl acid **12a** was obtained from ethyl 7-chloro-1-methyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate via intermediate **10a** (ethyl 1-methyl-6-nitro-7-[4-(2-pyridyl)-1-piperazinyl]-4-oxo-1,4-dihydroquinoline-3-carboxylate) and its reduced 6-amino analogue **11a**. The intermediate **10a** was exploited to obtain amide derivative **13a**; thus, it was hydrolyzed to the corresponding nitro acid, which was coupled with 1-(2-pyridyl)piperazine utilizing 1,1′-carbonyldiimidazole (CDI) and then reduced. On the other hand, intermediate **11a** was elaborated into 6-methylamino derivative **14a** via the trifluoroacetamide derivative.

The target acid derivative **9**, bearing a 4-(2-pyridyl)-1-piperazinyl at the N-1 position, was obtained from 7-chloro-1-[4-(2-pyridyl)-1-piperazinyl]-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate by catalytic reduction and subsequent acid hydrolysis. Finally, the C-6 fluoro acid derivatives **20a**−**22a** were prepared from the corresponding ethyl 7-chloro-1-*tert*-butyl(or cyclopropyl or methyl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate**,** respectively, through nucleophilic and acidic hydrolysis steps.

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10.1021/jm9903390 CCC: $19.00
Published on Web 09/19/2000

Biological Evaluation

Antiviral Activity. Anti-HIV-1 activity and toxicity data, reported in Table 1, show that the most potent drug is 7-[4-(2-pyridyl)-1-piperazinyl] derivative **12a**, which bears a methyl group at the N-1 position. It exhibits an EC_{50} in the submicromolar range, followed by compounds **8a** and **7a** containing a *tert*-butyl and cyclopropyl at the N-1 position, respectively. The corresponding 6-fluoro congeners were 5−10-fold less potent (compare **7a**, **8a**, and **12a** vs **20a, 21a**, and **22a**, respectively). However, the N-1 substituent effect on their relative activity was in the same order as that found for 6-aminoquinolones: **22a** (methyl) > **21a** (cyclopropyl) > **20a** (*tert*-butyl).

Alkylation of the C-6 amino group, as in the 6-methylamino derivative **14a**, drastically decreased antiviral response, pointing to the importance of an unsubstituted amine at C-6. Comparison of compounds **12a** and **16a** shows that the C-8 substituent is also crucial in terms of interaction with the molecular target. Esterification of the carboxyl group at the C-3 position dramatically reduced antiviral activity and cytotoxic potential, thus confirming the key role generally played by this portion of the molecule in the biological activity of quinolones.

The test compounds appear to be selective for HIV, since they were substantially less potent (the TC_{50} increases by almost 2 orders of magnitude) against an HSV-1 wild-type strain, not withstanding their low level of toxicity for Vero cells (Table 1).

Interaction with Viral Nucleic Acid Sequences. Fluorometric titration experiments were carried out with compounds **11a**, **12a,** and ciprofloxacin in the presence of RNA and DNA sequences. The key results are reported in Figure 1. Clearly, addition of TAR RNA largely reduces the fluorescence quantum yield of **12a** (panel A), indicating the formation of a stable complex. Conversely, derivative **11a** was practically unaffected by addition of the viral nucleic acid (panel B). The same was true for a classical fluoroquinolone like ciprofloxacin (not shown). Control experiments showed much lower changes in fluorescence emission of **12a** in the presence of a TAR-unrelated t-RNA sequence or calf thymus DNA, for both of which a slight increase, rather than a decrease, in fluorescence emission is observed, even in the presence of a 1000-fold excess of the nucleic acid (panel C). Hence, specific recognition of the viral ribonucleic acid depends on the structure of the test quinolone and reflects HIV-1 inhibition properties. In addition, complex formation between TAR and **12a** is quite effective, since we observed saturation of fluorescence emission at a nucleic acid concentration in the submicromolar range.

Discussion and Conclusions

The data presented here show that 6-aminoquinolones represent useful novel leads for the development of new and effective drugs for AIDS treatment. Indeed, 6-amino derivatives appear to be more potent than the corresponding fluorinated analogues as anti-HIV-1 agents. Quite remarkably, **12a** is the most potent compound of the quinolone structural class so far described and also the one with the most favorable selectivity index in C8166 cells.

We have identified three important features that contribute to the enhancement of antiviral activity: (a) the presence of the carboxylic acid at the C-3 position; (b) the presence of a small polar group at the C-6 position and bulky substituents at the C-7 position, and (c) the presence of small substituents at the N-1 position of the quinolone moiety. As experimentally demonstrated, there are some structural limitations at the N-1 and C-8 positions: bulky substituents are not well tolerated at either N-1 and/or C-8, whereas no steric restrictions are present around the C-7 position. RNA-binding data confirm that the carboxylic function of quinolones has to be free for efficient interaction. In addition, to ensure a specific contact to TAR, appropriate substituents must be located at positions 6 and 7 of the quinolone moiety, as indicated by the lack of response exhibited by **11a** and ciprofloxacin. It is worth observing that the latter two compounds are very poorly active as anti-HIV-1 agents (Table 1), which supports the hypothesis that RNA−quinolone interaction is relevant to antiviral activity. Indeed, improved affinity for the target seems to represent the basis for the improved selectivity profile observed for **12a**. In keeping with this, preliminary data suggest that compounds having a poorer SI (and activity), like **7a**, also exhibit reduced binding.

Since active 6-aminoquinolones are cytotoxic and since the highest selectivity index obtained is 85, one could assume that this class of molecules perturbs physiological cellular functions or signals acting through a nucleic acid intermediate as well. To address the issue of antiviral versus cytotoxic activity, it will be now crucial to further elucidate the mechanism of action of the new quinolones at the molecular and biochemical levels. In particular, the interactions of 6-aminoquinolones with other genomic viral sequences should be investigated to assess their interference with the main events of HIV-1 replication.

Experimental Section

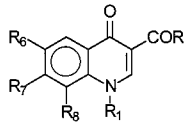
Antiviral and Cytotoxicity Assays. All compounds were dissolved in dimethyl sulfoxide at 25 mM or higher concentration to exclude any cytotoxic effect of dimethyl sulfoxide after dilution in the appropriate media. The anti-HIV-1 activities and toxicities of compounds were assessed in C8166 with HIV-1IIIB as described before.[7] The anti-HSV-1 activity was assayed in Vero cells by a plaque reduction method as previously described.[7] The inhibitory activity against HIV-1 was determined by examining cell cultures for syncytium formation and by measuring reverse trancriptase (RT) activity of culture supernatants.[8] The antiviral activity was also evaluated by measuring antigen gp120 production with an ELISA method and by using a cytopathic effect reduction assay as reported by Weinslow et al.[9] The results shown in Table 1 represent the mean of at least three different experiments performed in triplicate.

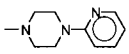
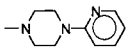
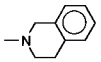
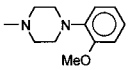
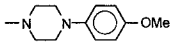
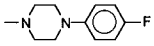
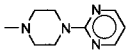
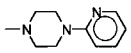
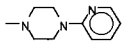
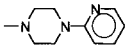
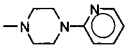
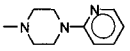
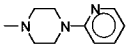
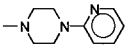
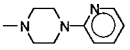
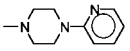
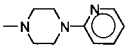
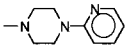
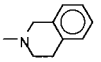
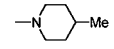
Nucleic Acids: TAR RNA. T7 polymerase was used for in vitro transcription of pGEM7 zf(+) containing HIV-1 sequences (HXBc2 strain) from nucleotide +454 to nucleotide +558, where +1 represents the first nucleotide of the 5′-LTR.[10] RNA was precipitated by adding 1/10 volume of 3 M sodium acetate and 2 volumes of ethanol at 0 °C.

t-RNA (from Wheat germ) and highly polymerized calf-thymus DNA were purchased from Sigma Chemical Co. and used following phenol extraction.

Spectroscopic Titrations with RNA and DNA. Fluorometric titrations were performed exploiting the drugs high fluorescence yield upon excitation at 350 nm. Fluorescence

Table 1. Structural, Anti-HIV-1, and Anti-HSV-1 Properties of the Tested 6-Aminoquinolones



Compd.	R_1	R_6	R_7	R_8	R	Biological activity [a]		
						EC_{50} [b]	TC_{50} [c]	SI [d]
5a	*t*-Bu	NH_2		H	OEt	51.2	77.9	1.5
						>300 [e]	>300 [e]	-
7a	*t*-Bu	NH_2		H	OH	2.0	8.1	4
						82 [e]	290 [e]	3.5 [e]
7b	*t*-Bu	NH_2		H	OH	40	80	2
7c	*t*-Bu	NH_2		H	OH	3.2	20	6
						33 [e]	300 [e]	9 [e]
7d	*t*-Bu	NH_2		H	OH	9.5	13.8	1.4
7e	*t*-Bu	NH_2		H	OH	8.44	10.7	1.2
7f	*t*-Bu	NH_2		H	OH	9.48	42.6	4.0
8a	*c*-Pr	NH_2		H	OH	0.7	9	13
						38 [e]	95 [e]	2.5 [e]
9		NH_2	Cl	H	OH	500	>1000	>2
11a	Me	NH_2		H	OEt	37	57	1.5
						>300 [e]	>300 [e]	-
12a	Me	NH_2		H	OH	0.1	7	70
						30 [e]	270 [e]	9 [e]
13a	Me	NH_2		H		*f*	*f*	-
14a	Me	NHMe		H	OH	3.5	19.5	5
16a	Me	NH_2		Me	OH	7.5	46.2	6
20a	*t*-Bu	F		H	OH	7.5	40	5
21a	*c*-Pr	F		H	OH	5	65	13
22a	Me	F		H	OH	1.3	7	5
23	*c*-Pr	NH_2		Me	OH	80	400	5
24	*c*-Pr	NH_2		Me	OH	20	80	4
CPX [g]						100	160	1.6
RFX [h]						200	400	2
AZT						0.02	500	25,000

[a] Antiviral activity against human immunodeficiency virus type 1 in C8166 cells. [b] EC_{50} represents the concentration of drug (μM) which reduced reverse trancriptase (RT) activity of C8166 culture supernatants by 50%. [c] TC_{50} represents the concentration of drug (μM) which reduced cell growth by 50%. [d] SI represents the selectivity index (ratio of TC_{50} to EC_{50}). [e] Antiviral activity against herpes simplex virus type 1 in Vero cells. [f] Not tested due to its low solubility. [g] CPX = ciprofloxacin. [h] RFX = rufloxacin.

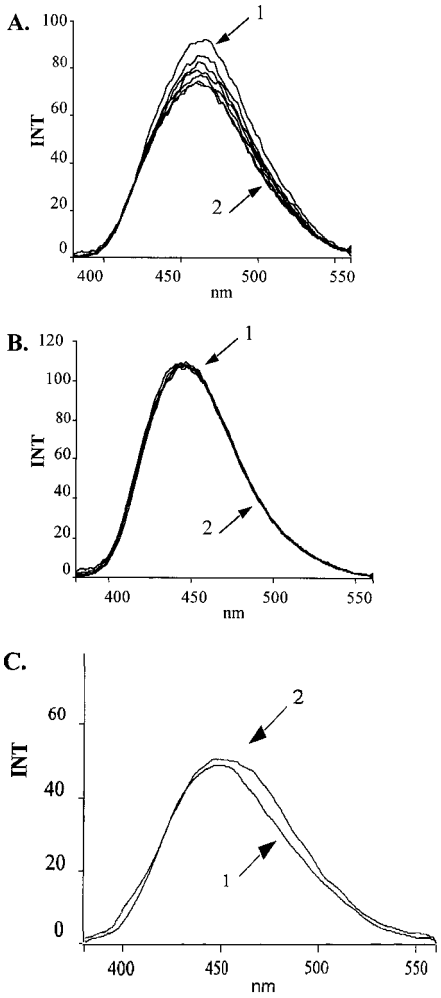


Figure 1. Aminoquinolone **12a** exhibits high-affinity binding to TAR RNA. Panel A: Changes in emission spectrum of **12a** (2 μM, spectrum 1) upon addition of increasing amounts of TAR RNA up to 0.2 μM (spectrum 2). Panel B: Same measurements for **11a** (6 μM, spectrum 1) up to 0.2 μM TAR RNA (spectrum 2). Panel C: Changes in emission spectrum of **12a** (1 μM, spectrum 1) upon addition of large excess (1 mM) of calf thymus DNA (spectrum 2). Excitation wavelength was 350 nm, and every spectrum was recorded at 25 °C after allowing 5 min of equilibration in 10 mM Tris, pH 7.0, 20 mM NaCl, and 1 mM $Mg(ClO_4)_2$.

spectra were recorded at 25 °C on a luminescence spectrophotometer LS50B (Perkin-Elmer). Titrations were performed in Tris HCl (10 mM, pH 7.0), NaCl (20 mM) and $Mg(ClO_4)_2$ (1 mM) by adding increasing amounts of concentrated nucleic acid to diluted quinolones solutions and recording the emission spectra 5 min after mixing to ensure equilibrium. Quinolone concentration was evaluated by absorption spectroscopy. Each solution was filtered through a 5-μm filter before use to eliminate any particulate material that would interfere with the fluorescence response.

Acknowledgment. We acknowledge financial support through AIDS Grant No. 407-76002 to G.P., MURST Grants 60% and 40% to G.P. and M.P., Grant No. 770/97 from Regione Veneto to M.P., and a grant from the University of Perugia to A.F.

Supporting Information Available: Reaction schemes and detailed information on the synthesis and characterization of individual compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM9903390