Antiviral Research 87 (2010) 338–344

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Quinoline-based compounds as modulators of HIV transcription through NF-κB and Sp1 inhibition

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ARTICLE INFO

Article history:
Received 9 February 2010
Received in revised form 6 May 2010
Accepted 14 June 2010

Keywords:
Quinoline derivatives
HIV
AIDS
Antiviral

ABSTRACT

18 quinoline-based compounds were tested for antiviral properties against human immunodeficiency syndrome (HIV). The compounds tested here contain quinoline or tetrahydroquinoline rings and can be divided into two main groups: group 1 includes 4-(2-oxopyrrolidinyl-1)-1,2,3,4-tetrahydroquinolines with 2-(3-nitrophenyl) substituent (N-series) or 2-(3-aminophenyl) moiety (H-series), and group 2 includes 2-(3-nitrophenyl)- or 2-(3-aminophenyl)-substituted quinolines (S-series). Two different antiviral assays were performed in order to test the anti-HIV activity of compounds: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and recombinant virus assay (RVA). Results showed that the most active compounds were 2-aryl quinolines, particularly those containing methoxy substituents or no substituents in the quinoline skeleton. HIV transcription inhibition appears to be their target in both resting and phorbol myristate acetate (PMA) activated primary lymphocytes, and nuclear factor-κB (NF-κB) and specificity protein-1 (SP1) seems to be the most important transcription factors involved in their action.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) due to infection with the human immunodeficiency virus (HIV) continues to be a worldwide epidemic. Existing therapies are targeted at the virus-associated reverse transcriptase, protease, gp41 or fusion peptide and recently CCR5 and integrase antagonists have been included as a strategy to inhibit viral replication (Warnke et al., 2007; Battegay et al., 2008; Menéndez-Arias and Tözsér, 2008). The emergence of drug-resistant virus strains and the occurrence of side effects are major disadvantages of the current therapies for the treatment of AIDS and make currently available drugs insufficient to maintain a safe therapeutic arsenal against HIV (Bagasra, 2006; Williams and Greene, 2007; Hirsch et al., 2008). Although effective synergy has been found when using combinations of drugs directed against different targets, therapeutic failure is relatively frequent, especially from a long-term perspective. In view of these considerations, there is a definitive need for new classes of drugs suitable for use that could replace, or partially substitute existing drugs, and preferably act on new targets engaging novel molecular mechanisms (Greene et al., 2008; Hughes et al., 2008; Coiras et al., 2009). Therefore, the development of new anti-HIV agents is focusing on novel heterocyclic structures.

Heterocyclic systems with quinoline nucleus represent privileged moieties in medicinal chemistry, and are ubiquitous sub-structures associated with biologically active natural products. Quinoline derivatives have been shown to display a wide spectrum of biological activities such as antibacterial (Hoemann et al., 2002; Martínez-Grueiro et al., 2005; Lilienkampf et al., 2009), antifungal (Vargas et al., 2003; Meléndez Gómez et al., 2008), anti-parasitic (Kouznetsov et al., 2004, 2007), antiviral (Grande et al., 2008; Chen et al., 2009), cytotoxic and antineoplastic (Jacquemond-Collet et al., 2002) and anti-inflammatory and immunosuppressive behaviours (Dorey et al., 2000; Liu et al., 2009). Due to their broad biological activity, quinoline compounds have been considered as good starting materials for the search of novel anti-HIV agents. In this study, we focused on the HIV inhibitory activity of 18 quinoline-based compounds and we have shown that some of them are potent inhibitors of HIV replication. We also present evidence of the exact mechanism of action of these compounds, as well as discussing some of the unique structure–activity relationships associated with this series of quinolinic molecules.

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doi:10.1016/j.antiviral.2010.06.006

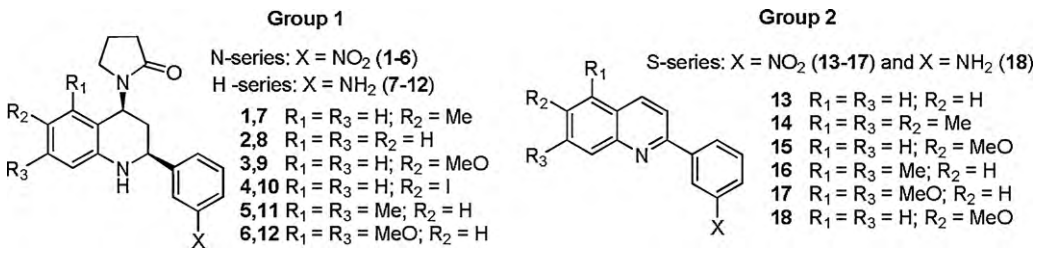

Fig. 1. Chemical structure of synthesized quinolines.

2. Materials and methods

2.1. Compounds

The compounds evaluated in this research were selected as part of a preliminary systematic screening, and were prepared using chemical procedures already reported by us (Kouznetsov et al., 2006). The synthesized quinolines have been divided into two groups according their chemical structure, based mainly on the nature of the quinoline moiety (Fig. 1). Group 1 (N-series and H-series) includes 2-(3-nitrophenyl) or 2-(3-aminophenyl)-4-(2-oxopyrrolidinyl-1)-1,2,3,4-tetrahydroquinolines and group 2 (S-series) includes 2-(3-nitrophenyl)- or 2-(3-aminophenyl)-substituted quinolines. Compounds were dissolved in DMSO and assayed at concentrations ranging from 0 to 500 μg/ml.

2.2. Cells

MT-2 cells (American Type Culture Collection, Rockville, MD) (Harada et al., 1985) were cultured in RPMI 1640 medium containing 10% (v/v) fetal bovine serum, 2 mM L-glutamine, penicillin (50 IU/ml) and streptomycin (50 μg/ml) (all Whittaker M.A. Bio-Products, Walkerville, MD). MT-2 cells were cultured at 37 °C in a 5% CO_2 humidified atmosphere and split twice a week. 293T cells (Dubridge et al., 1987; Pear et al., 1993) were cultured in DMEM medium containing 10% (v/v) fetal bovine serum, 2 mM L-glutamine, penicillin (50 IU/ml) and streptomycin (50 μg/ml) (all Whittaker M.A. Bio-Products, Walkerville, MD). 293T cells were cultured at 37 °C in a 5% CO_2 humidified atmosphere and split twice a week.

2.3. Mononuclear cell preparation

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy blood donors by centrifugation through a Ficoll-Hypaque gradient (Pharmacia Corporation, North Peapack, NJ) and were resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum (PAN Biotech GmbH, Aidenbach, Germany), 2 mM L-glutamine and antibiotics (100 μg/ml streptomycin and 100 IU/ml penicillin) before culture at a concentration of 2×10^6 cells/ml (Sancho et al., 2004). Phorbol myristate acetate (PMA) was obtained from Sigma–Aldrich, St. Louis, MO (USA) and used at 0.1 μM.

2.4. Plasmids

The vector pNL4.3-Luc was generated by cloning the luciferase gene in the HIV-1 proviral clone pNL4.3 (Adachi et al., 1986). Plasmid pNL4.3 Renilla was generated by cloning the renilla gene in the *nef* site of pNL4.3 (Garcia-Perez et al., 2007) and pJR Renilla plasmid was generated by cloning the *env* gene of HIV-1 JR_{CSF} in the pNL4.3 Renilla plasmid (Connor et al., 1995). The 3-enh-κB-ConA-luc plasmid (Arenzana-Seisdedos et al., 1993) carries a luciferase gene under the control of three synthetic copies of the κB consensus of the immunoglobulin κ-chain promoter cloned into the BamHI site located upstream from the conalbumin transcription start site. The Sp1-luc plasmid (a kind gift from Dr. Solís-Herruzo, Hospital 12 de Octubre, Madrid, Spain) contains two consensus sequences for SP1 cloned into the p19LUC vector. DNA for the vesicular stomatitis virus (VSV) G glycoprotein was cloned in the pcDNA3.1 plasmid (pcDNA-VSV).

2.5. Anti-HIV activity evaluation

Anti-HIV activity of compounds was evaluated with the classic 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and recombinant virus assays (RVA). Classic antiviral assays were performed by either infecting or not MT-2 cells with HIV (NL4.3 strain) in the presence of different concentrations of the compounds and maintained at 37 °C 5% CO_2. After 7 days, the MTT reagent was added and left in culture for 2 h. Afterwards, the cell culture was lysed and cell viability determined by UV absorption (Pauwels et al., 1988). The RVA was performed as follows: infectious supernatants were obtained from calcium phosphate transfection on 293T cells of plasmids pNL4.3-Luc or co-transfection of the pNL4.3-Luc-R^-E^- (AIDS Research and Reference Reagent Program, NSAID, National Institutes of Health), full-length HIV-DNA that do not express HIV envelope, and pcDNA3-VSV (Oberlin et al., 1996), that express G protein of VSV. These supernatants were used to infect cells in the presence or absence of the compounds to evaluate. Anti-HIV activity quantification was performed 48 h post-infection. Briefly, cells were lysed with 100 μl of buffer provided by "Luciferase Assay System Kit with Reporter Lisis Buffer" (Promega, Madison, WI). Relative luminescence units (RLUs) were obtained in a luminometer (Berthold Detection Systems, Pforzheim, Germany) after the addition of substrate to cell extracts. Viability was performed in parallel treated cells with the same concentrations of compound as in the classic or RVA. After 48 h, cell viability was evaluated with the CellTiter Glo (Promega) assay system following the Manufacturer's specifications. Inhibitory concentrations 50 (IC_{50}) and cytotoxic concentrations 50% (CC_{50}) were calculated using GraphPad Prism Software (sigmoidal dose–response).

2.6. Retrotranscriptase assay

PBMCs were seeded in 24 well plates and infected with a wild type NL4.3 obtained from MT-2 infected cells in the presence or absence of the compounds on trial. After 18 h, genomic DNA was extracted from cell cultures with QUIA DNA blood mini kit (QUIAGEN). Genomic DNA was subjected to a real time PCR in an Applied biosystems PCR with the following primers: R/U5 (forward), 5′-GGC TAA CTA GGG AAC CCA CTG-3′; LTR/gag (reverse), 5′-CCT GCC TCG AGA GAG CTG CTC TGG-3′. DNA quantification was performed as compared to a non-infected control and a non-treated but infected control (NL4.3). Actin primers were used as standard genomic DNA. DNA expression was calculated as Ct (Cycle threshold, number of cycles needed to reach a significant fluorescence signal) and expressed as percentage of relative expression as compared to an untreated control (100%).

Table 1
Antiviral activity evaluation of compounds.

	MTT				RVA				PVA	
	IC_{50} μM	SE	CC_{50} μM	SE	IC_{50} μM NL4.3-Luc	SE	CC_{50} μM	SE	IC_{50} μM Delta-VSV-Luc	SE
1	>50	ND	>50	ND	>142	ND	53.47	3.62	ND	ND
2	>50	ND	>50	ND	>148	ND	51.58	3.92	ND	ND
3	>50	ND	>50	ND	>136	ND	47.33	3.20	ND	ND
4	>50	ND	>50	ND	>108	ND	60.85	2.75	ND	ND
5	>50	ND	>50	ND	53.77	4.91	>137	ND	ND	ND
6	>50	ND	>50	ND	53.73	4.33	>126	ND	ND	ND
7	>50	ND	>50	ND	74.64	4.60	>156	ND	ND	ND
8	>50	ND	>50	ND	>163	ND	>163	ND	ND	ND
9	69.07	18.75	103.35	49.25	31.30	6.69	63.36	4.41	25.97	5.32
10	>50	ND	>50	ND	54.42	3.15	>115	ND	ND	ND
11	88.86	7.88	>50	ND	>149	ND	>149	ND	ND	ND
12	116.27	6.34	>50	ND	113.73	4.46	75.39	3.82	ND	ND
13	**63.67**	**11.67**	**135.77**	**24.80**	**19.66**	**4.77**	**76.68**	**5.98**	**24.63**	**7.66**
14	>50	ND	>50	ND	>189	>8	>189	ND	ND	ND
15	**87.86**	**13.66**	**122.46**	**3.43**	**8.97**	**4.73**	**>178**	**ND**	**18.57**	**6.18**
16	>50	ND	>50	ND	110.27	5.21	>180	ND	ND	ND
17	**18.48**	**8.04**	**102.89**	**6.68**	**16.74**	**4.74**	**>161**	**ND**	**18.86**	**3.98**
18	72.71	15.05	107.60	46.81	9.67	6.71	109.43	5.61	156.56	41.5

MTT classic assay results are shown in the columns on the left, recombinant virus assay (RVA) results in the columns in the middle and pseudotyped virus assay (PVA) in the columns on the right. MTT assay was performed by infecting MT-2 cells with 2 ng/well of NL4.3 viral clone in the presence or absence of different concentrations of compounds. Cell culture was maintained for 7 days and HIV-infected or mock-infected cell viability evaluated by MTT addition and absorbance measurement. RVA or PVA was performed by infecting MT-2 cell with NL4.3-Luc (20 ng/well) or NL4.3-Δ-env-VSV-Luc (20 ng/well) in the presence or absence of different concentrations of compounds. After 48 h, cell culture was lysed and RLUs measured in a luminometer. Toxicity in RVA and PVA was performed in mock-infected MT-2 cells treated in parallel with the same concentrations of compounds and cell viability was measured by CellTiter Glo assay. Inhibitory concentration 50 (IC_{50}) and cytotoxic concentration 50 (CC_{50}) were determined using sigmoidal dose–response curves (GraphPad software).

2.7. Transfection assays

Freshly isolated PBMCs were maintained in culture without stimuli for 1 day prior to the transfection assay. Afterwards, PBMCs were resuspended in 350 μl of RPMI without serum and antibiotics and electroporated using an Easyject plus Electroporator (Equibio, Middlesex, UK). PBMCs were transfected at 320 V, 1500 μF, and maximum resistance with the plasmids at a concentration of 0.5 μg/10^6 cells. After transfection, cells were incubated in RPMI with 10% fetal calf serum at 37 °C, activated or not with different stimuli and harvested 48 h later. Luciferase activity (RLUs) was measured in a luminometer.

2.8. Isolation of nuclear extracts and western blot

MT-2 cells (15×10^6/well) were pretreated with the active quinolines (20 μM) or PMA 0.1 μM for 30 min. After 2 h of stimulation, cells were washed twice with cold PBS and nuclear proteins were isolated by standard procedures. Protein concentration was determined by the Bradford method (Bio-Rad, Richmond, CA) and 40 mg of proteins were boiled in Laemmli buffer and electrophoresed in 7.5% SDS/polyacrylamide gels. Separated proteins were transferred to nitrocellulose membranes (0.5 A at 100 V; 4–8 °C) for 1 h. Blots were blocked in TBS solution containing 0.1% Tween 20 and 5% non-fat dry milk overnight at 4–8 °C, and immunodetection of specific proteins was carried out with primary antibodies for p65, IκB and Sp1 (Santa Cruz, CA) using an ECL system (GE Healthcare, Uppsala, Sweden).

3. Results

Anti-HIV activity of compounds was evaluated with the classic MTT and RVA. Both assays enabled us to identify those compounds with antiviral activity, although RVA is more sensitive, since it is able to identify compounds just 48 h after infection (7 days for the MTT assay) and it allowed us to perform assays in PBMCs, since MTT assay is not sensitive enough to test viability of primary lymphocytes. Briefly, cells were treated with different concentrations of compounds and left in culture for 2 h. Afterwards, cells were infected with HIV (NL4.3 clone) and left in culture for 7 days. Such a long treatment was able to detect compounds with high potency, but those with low stability are not easily detected. To resolve this, RVA was also performed. This assay is performed by infecting PBMCs with recombinant virus carrying luciferase reporter genes. Thus, viral replication is directly evaluated leading us to test compounds in just 48 h.

As shown in Table 1, compounds of S-series (13–18) were more potent than all compounds of N-series (1–6) or H-series (7–12) (Fig. 1). Among N-series, no activity was found in any of the compounds tested with MTT assay, although RVA was able to identify two tetrahydroquinolines with moderate activity (5 and 6) against HIV infection. Among H-series, three tetrahydroquinoline molecules were active in both assays (9, 11 and 12), with IC_{50} values ranging from 50 to 100 μM, although toxicity suggests a non-specific mechanism of action, especially for compounds 9 and 12. Interestingly, just one molecule of this series (9) was also active against VSV-pseudotyped HIV (NL-Δ-env-VSV-Luc) infections.

The most potent series, S-series, showed three quinoline molecules (13, 15 and 17) with the highest potency in both assays. Compound 17 was the most potent in MTT assay (IC_{50} of 18.48 μM), although IC_{50} values in RVA suggest similar potency for the three compounds (range 10–20 μM with CC_{50}s above 70 μM). On the other hand, the activity of compounds 13, 15 and 17 had similar IC_{50} values against both virus (HIV and VSV-pseudotyped), suggesting a target which is not related to HIV envelope, and thus, to viral entry.

To further study the potential action of these compounds, a retrotranscriptional assay was performed. In this assay, viral DNA is directly quantified by real time PCR. In the case that the compounds inhibit reverse transcriptase or a related target, lower amounts of DNA would be detected, as compared to a positive control and zidovudine, as a control of retrotranscription inhibition. No inhibition was detected for any of the active compounds in this assay (Table 2), although zidovudine was able to effectively decrease the total DNA amount. Therefore, the total DNA amount in cell infections is not reduced by treatment with active compounds. This fact suggests that a later step would be the target of these compounds.

Table 2
Viral DNA quantification real time PCR after treatment with active quinolines.

	Relative expression
Not treated	100%
Zidovudine 1 μM	25%
9	188%
13	117%
15	175%
17	168%
18	381%

Results are shown as relative expression as compared to a not treated control (100%). Zidovudine at 1 μM was used as inhibition control. Quinoline derivative treatment was performed at a single concentration of 50 μM.

To further analyze the target of active compounds, viral transcription was studied in resting or stimulated primary lymphocytes. To this end, MT-2 cells or resting PBMCs obtained from healthy donors by standard procedures, were transfected with a luciferase construct under the control of the whole genome of HIV (NL4.3-Luc) and treated with PMA (0.1 μM) and compounds at 20 μM. The culture was maintained at 37 °C in 5% CO_2 for 48 h and cells were harvested and lysed following the manufacturer's instructions (Luciferase assay system, Promega). Luciferase activity was measured in cell lysates in a luminometer.

As shown in Table 3, the most potent compounds in antiviral assays are also the most active in the transfection experiments in both PMA-activated PBMCs and MT-2 cells. Compounds 13, 15 and 17 inhibit HIV transcription in MT-2 cells and transcription induced by PMA in resting PBMCs.

Since HIV transcription is a complex process in which a lot of viral and cellular factors are involved, the three most potent compounds were further studied as inhibitors of transcription. To this end, resting PBMCs or MT-2 cells were transfected with luciferase constructs under the control of nuclear factor-κB (NF-κB) or specificity protein-1 (Sp1) transcription factors.

As shown in Table 4, the three most potent compounds are able to decrease HIV transcription through NF-κB and Sp1 inhibition. NF-AT induced transcription treated with PMA 0.1 μM, ionomycine and quinolines were not affected (data not shown).

Since viral latency has always been a cause of concern, effect of quinolines derivatives on viral transcription was further studied in resting PBMCs without treatment with PMA. To this end, resting PBMCs were again transfected with luciferase reporter constructs under the control of complete HIV genome (NL4.3-Luc). PBMCs were not treated with PMA, and remained resting, and were treated with quinoline derivatives. After 48 h, cells were lysed and luciferase activity (RLUs) measured in a luminometer.

As shown in Table 5, transactivation of resting PBMCs was effectively achieved by PMA. On the other hand, quinolines were also able to inhibit HIV replication in resting PBMCs, although the transcription signal was at least 10 times lower. Curiously, compound 17 was more potent in non-PMA-treated cells than with PMA-induced transcription, since the percentage of transcription inhibition is lower.

Table 3
Effect of quinoline compounds on HIV transcription.

Compound	Transcription PBMCs		Transcription MT-2	
	NL4.3-Luc PMA 0.1 μM + c. 20 μM		NL4.3-Luc + c. 20 μM	
	% RLUs	SD	% RLUs	SD
1	268%	32%	152%	8%
2	272%	33%	129%	1%
3	193%	23%	153%	9%
4	148%	18%	105%	18%
5	176%	21%	134%	2%
6	127%	15%	129%	6%
7	173%	21%	92%	6%
8	245%	29%	121%	2%
9	269%	32%	70%	4%
10	222%	27%	141%	4%
11	296%	36%	132%	6%
12	226%	27%	142%	5%
13	40%	5%	18%	1%
14	197%	24%	119%	6%
15	41%	15%	9%	1%
16	112%	13%	101%	8%
17	27%	3%	8%	0%
18	259%	31%	98%	6%

Transcription PBMCs: resting PBMCs were transfected with a luciferase expression vector under the control of complete HIV provirus (NL4.3) and treated with compounds (20 μM) and PMA (0.1 μM). 48 h later, cells were lysed and luciferase expression measured in a luminometer. Results are expressed as percentages compared to a PMA-treated culture (100%). Results are the mean and standard deviation of at least three independent experiments.
Transcription MT-2: MT-2 cells were transfected with a luciferase expression vector under the control of complete HIV provirus (NL4.3) and treated with compounds (20 μM). 18 h later, cells were lysed and luciferase expression measured in a luminometer. Results are expressed as percentages compared to a non-treated culture (100%). Results are the mean and standard deviation of at least three independent experiments.

To further study the action of active quinolines in transcription, the expression of p65 and IκB proteins was measured by western blot in nuclear extracts of MT-2 cells. As shown in Fig. 2, the three compounds, but particularly 17, diminish the expression of p65. This suggests that quinolines act through the blockade of the nuclear translocation of p65, and thus inhibit the action of NF-κB. On the other hand, the inhibitor of NF-κB, IκB, is less expressed in quinoline-treated cell nuclear extracts. Since p65 is able to stimulate the synthesis of its inhibitor, the blockade of the action of NF-κB also blocks the re-synthesis of IκB, and it is thus not present in nuclear extracts when treated with quinolines as compared to a non-treated cell control.

4. Discussion

Since the 1980s, AIDS has become a disastrous epidemic which greatly threatens the health of mankind and brings crises and chal-

Table 4
Effect of compounds on HIV transcription.

	Transcription PBMCs				Transcription MT-2			
	3-enh-KB-Luc + c. 20 μM	SD	Sp1-Luc + c. 20 μM	SD	3-enh-KB-Luc + c. 20 μM	SD	Sp1-Luc + c. 20 μM	SD
13	**24%**	6%	**26%**	6%	**10%**	1%	**8%**	0%
15	**21%**	4%	**25%**	11%	**5%**	2%	**4%**	1%
17	**77%**	16%	**47%**	7%	**49%**	6%	**45%**	7%

Transcription PBMCs: resting PBMCs were transfected with a luciferase expression vector under the control of NF-κB (3-enh-κB-Luc) or Sp1 (Sp1-Luc) and treated with compounds (20 μM) and PMA (0.1 μM). 48 h later, cells were lysed and luciferase expression measured in a luminometer. Results are expressed as percentages compared to a PMA-treated culture (100%). Results are the mean and standard deviation of at least three independent experiments.
Transcription MT-2: MT-2 cells were transfected with a luciferase expression vector under the control of NF-κB (3-enh-κB-Luc) or Sp1 (Sp1-Luc) and treated with compounds (20 μM). 18 h later, cells were lysed and luciferase expression measured in a luminometer. Results are expressed as percentages compared to a non-treated culture (100%). Results are the mean and standard deviation of at least three independent experiments.

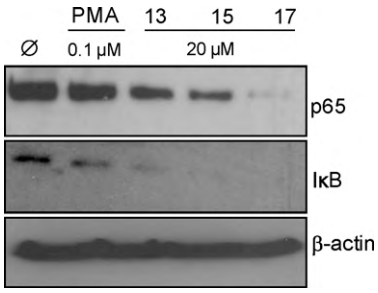

Fig. 2. Expression of p65 and IκB proteins in nuclear extracts of MT-2 cells treated with the active quinolines at 20 μM. MT-2 cells were treated with 20 μM quinolines (13, 15 and 17) or PMA at 0.1 μM for 2 h. Nuclear extracts were obtained and expression of p65 and IκB were detected with specific antibodies.

lenges to the healthcare system, with more than 38 million people infected worldwide (Wakabi, 2007; El Amari and Hirschel, 2009). Today, there are more than 20 drugs available in the clinic for the treatment of HIV infection. In spite of a wide variety of potent HIV inhibitors, a number of severe problems are still associated with HIV infection. Two major concerns are the emergence of resistant or even multi-resistant viruses following combination therapy, and the noxious side effects associated with some inhibitors. In consequence, as long as it remains impossible to eradicate the virus and until a protective vaccine is available, there is an urgent need for effective inhibitors capable of controlling resistant virus variants with a more favorable tolerance profile.

Antiviral drug development has substantially increased. The pharmaceutical industry has shown a continued interest in further exploiting existing drug targets, obtaining proof of concept for new ones and initiating new drug development programs focusing on novel structures. Considerable attention has been paid to the synthesis of quinoline derivatives, as this structural framework is often found in synthetic pharmaceuticals with unique biological activities. Moreover, 4-quinolones, oxo-derived quinolines, have previously shown anti-HIV activity through transcription inhibition (Stevens et al., 2005; Tabarrini et al., 2008).

The compounds tested here contain quinoline or tetrahydroquinoline rings and can be divided into two main groups: group 1 includes 4-(2-oxopyrrolidinyl-1)-1,2,3,4-tetrahydroquinolines with 2-(3-nitrophenyl) substituent (N-series) or 2-(3-aminophenyl) moiety (H-series), and group 2 includes 2-(3-nitrophenyl)- or 2-(3-aminophenyl)-substituted quinolines (S-series). Two different antiviral assays were performed in order to test the anti-HIV activity of the compounds: MTT and RVA. Compounds of the S-series were more potent than the H-series, although there were five compounds with moderate activity in the MTT assay (5, 6, 9, 11 and 12). Interestingly, in RVA, the active compounds were tetrahydroquinoline molecules 5, 6, 7, 9, 10 and 12, although these differences could be related to the low potency of most of this class of compounds. Moreover, most of them were active only against HIV recombinant virus, and not against VSV-pseudotyped infection, suggesting an HIV envelope-related mechanism of action, except for compound 9, which was also active against VSV-pseudotyped infections. However, the most interesting molecules were 13, 15 and 17 with the highest potency in both assays, although compound 17 was more potent in MTT assay. Similar IC_{50} values were obtained for the recombinant virus assay against both viruses, suggesting a target not related to the HIV envelope, and thus, to viral entry.

Table 5
Effect of quinoline compounds on HIV transcription.

Transcription PBMCs		
	NL4.3-Luc + c. 20 μM	SD
PMA 0.1 μM	503%	136%
13	14%	7%
15	23%	9%
17	8%	11%

Resting PBMCs were transfected with a full-length HIV-DNA carrying a luciferase reporter gene in the position of *nef* (NL4.3-Luc) and treated with compounds (20 μM). 48 h later, cells were lysed and luciferase expression measured in a luminometer. Results are expressed as percentages compared to a non-treated culture (100%). Results are the mean and standard deviation of three independent experiments.

Although IC_{50} values do not suggest a strong anti-HIV activity, the chemically related antibacterial fluoroquinolones showed minimum inhibitory concentrations (MICs) in the same range, around 4 μg/ml (anti-HIV quinolines around 10–90 μM which would be 5–25 μg/ml) with typical doses of between 200 and 400 mg per day (data obtained from FDA). Therefore, if the pharmacokinetics are comparable, anti-HIV quinolines could be administered at similar doses to reach active drug serum concentrations.

To further study the potential action of these compounds, a real time PCR-based retrotranscriptional assay was performed. However, the total viral DNA amount in cell infections is not reduced by treatment with active compounds, suggesting that a later step is probably the target of these molecules. Since HIV transcription is a complex process involving many different factors, the three most potent compounds were further studied as inhibitors of viral transcription. The results showed that the three compounds were able to decrease HIV transcription induced by PMA through NF-κB and Sp1 inhibition.

Quinolines diminish the expression of NF-κB and its inhibitor IκB in nuclear extracts, showing that inhibition of HIV replication is related to the inhibitory effect of these compounds on NF-κB activity. In fact, low nuclear levels of IκB also reflect decreased NF-κB activity as IκB gene transcription and nuclear location of the protein are dependent on continuous re-synthesis driven by NF-κB (Coiras et al., 2008).

Moreover, transcription inhibition was also effectively achieved in non-PMA-stimulated PBMCs, suggesting that viral reservoirs, which are thought to be mainly a small pool of CD4+ T cells (Siliciano et al., 2003; Coiras et al., 2009), are also a target of the quinoline-based compounds. The stronger potency observed against the transcription in resting cells could be due to the level of HIV transcription. In PMA-activated PBMCs transcription was 10 times higher than in resting cells and this is related with the induction of transcription factors involved in HIV transcription. The inhibitory effect observed in activated PBMCs is related with the blockade of NF-κB and Sp1 by the compounds tested. Low transactivation levels are found in resting lymphocytes as has been described by our group (Alcamí et al., 1995) and other authors. We have shown that this low-level activity in resting cells is related to the translocation of low amounts of NF-κB in resting cells (Coiras et al., 2007, 2008). In consequence an inhibitory effect is found in resting PBMCs, although lower levels of transcription factor are present, and thus higher potency of compounds is observed. The effect found for the active quinoline compounds could therefore be related with the inhibition of this activation pathway in resting lymphocytes.

Some relationships can be extracted from the analysis of the structures and the activities displayed. The 2-aryl-4-(2-oxopyrrolidinyl-1)-1,2,3,4-tetrahydroquinoline moiety is not by itself sufficient for antiviral activity, as this is clearly suggested by the lack of activity of many compounds of the N-series and H-series. However, moderate activity has been shown with those with substituents on the quinoline skeleton in the C-5 and C-7 position (compounds 5 and 6 from N-series and compounds 11 and 12 from H-series), with a target probably related to the HIV envelope. The substitution with a methoxy group in the C-6 position of the quinoline skeleton is only favourable in compounds bearing a $-NH_2$

group in the aryl substituent at C-2 position (compound 9). Interestingly, this compound was also active against VSV-pseudotyped virus infections, suggesting a target which is not dependent on the HIV envelope.

Within the S-series, the quinoline skeleton without any substituents (compound 13) or with methoxy substituents (compounds 15 and 17) was the most active. In this series, the structure–activity relationship around the substituents on the quinoline moiety did not seem to have as dramatic an effect as in the aryl ring, but some trends were evident. Substitutions with methyl at the 6-position and/or at the 5- and 7-position were detrimental to antiviral activity, as is apparent from the lack of activity of compounds 14 and 16. In addition, the methoxy substitution in the quinoline skeleton was detrimental in compound 18 bearing a NH_2 group in the aryl substituent at C-2 position.

5. Conclusion

In this work, 18 quinoline-based compounds were tested for antiviral properties against HIV. The most active quinoline molecules (13, 15 and 17) were further studied in order to obtain an overview of their exact mechanism of action. Results showed that the three compounds are able to decrease HIV transcription in MT-2 cells and resting or PMA-treated PBMCs through NF-κB and Sp1 inhibition. From the analysis of the structures and the activities displayed, some relationships could be extracted. Results showed that the most active compounds were 2-aryl quinolines, particularly those containing methoxy substituents or no substituents in the quinoline skeleton. These studies provide more insight into the structure–activity relationships of quinoline derivatives such as HIV inhibitors, and should help in designing new quinoline derivatives, which could be useful for the development of antiviral agents.

Acknowledgements

Research in our laboratory is supported by grants from the European Union (EUROPRISE and CHAARM), the Instituto de Salud Carlos III (RETIC RD06/0006 and the Intrasalud Programme), the Comunidad de Madrid (VIRHOST Network) and Fundación para la Investigación y Prevención del Sida en España (FIPSE). Luis Astudillo is grateful to project PBCT Anillo ACT-38 for financial support. The technical assistance of Ms. Brooke-Turner is gratefully acknowledged.

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