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Synthesis and anti-HIV properties of new hydroxyquinoline–polyamine conjugates on cells infected by HIV-1 LAV and HIV-1 BaL viral strains

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Abstract—To find new derivatives that block different virus strains entry in cells bearing specific surface receptors represent an interesting challenge for medicinal chemists. Here, we report the synthesis and the anti-HIV properties of a new series of analogues based on the introduction of quinoline moiety on various polyamine backbones, including polyazamacrocycles. Three compounds **7**, **8**, and **10** of this series were found active on PBMCs cells infected by HIV-1 LAV or by HIV-1 BaL, in contrast the well-known reference compound **1a** (AMD 3100) was found only active on HIV-1 LAV strain.

Viruses that are broadly resistant to currently available anti-retroviral medications represent a growing challenge for HIV therapy. Unlike many existing HIV drugs that target the virus after it has infected a healthy cell, it had been shown that compounds like AMD3100 **1a**, its analogue **1b** (Fig. 1) and other related analogues block the virus from entering a healthy cell preventing the replication process. These compounds block cell surface G-protein coupled receptors, such as CCR5 or CXCR4.[1–3] AMD 3100 has reached phase II clinical trials and is presently considered as a prototype of this class of compounds. A recent review on these analogues has been published by Hatse et al.[4] Nevertheless, the existence of two azamacrocycles is not a prerequisite for anti-HIV activity.

In order to enter and infect cells, HIV must bind to either CXCR4 or CCR5 chemokine receptors. Different HIV strains prefer one receptor to the other. Blocking CXCR4 and CCR5 receptors can prevent the relevant HIV strains from entering and infecting the target cells. An infected individual cell may harbor different levels of both CXCR4 and CCR5. Research on HIV disease suggests that approximately one-half of patients are infected with HIV strains that use CXCR4 as an entry receptor as well as strains that use CCR5.

AMD3100 **1a** is extremely specific in its affinity for the CXCR4 receptor, and this property depends, at least in part, on an electrostatic interaction between the basic (positively charged) nitrogens of the cyclam moieties and the acid (negatively charged) carboxylates of the aspartic acid residues located at positions 171, 182, 193, and 262 of the CXCR4 receptor. In particular,

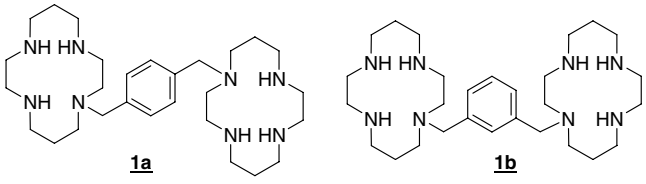

Figure 1. AMD 3100 structure **1a** and a related analogue **1b**.

Keywords: Polyazamacrocycles; 8-Hydroxyquinoline; HIV-1 LAV; HIV-1 Bal; CXCR4 receptor; CCR5 receptor.
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the aspartate residues 171 and 262, located at the junction of the transmembraneous segments with the extracellular loops of the CXCR4, have proven to be crucial in the binding of **1a** with its receptors.[5] Analogue **1a** affinity for CXCR4 can be enhanced by its complexation with Cu^{2+}, Zn^{2+} or Ni^{2+} metal ions[6,7] and this enhanced affinity is explained through a better interaction with the aspartate in position 262 (For additional details on the anti-HIV activities of polyazamacrocycle metal complexes, see references Hatse et al.[4] and Princen et al.[8]). These observations led us to investigate the possibility to design new anti-HIV drugs incorporating in their structure specific ligands with transition metal chelating properties, which could block virus entry in healthy cells mediated either by CXCR4 or CCR5 receptors. As far as, 8-hydroxy-quinolines were known to form tetradentate complexes with transition metals such as copper and zinc with good binding affinity values[9] (log *K*s values being around 10) they were selected as suitable ligands for the design of new chemokine receptor antagonists. As far as clioquinol **2** (5-chloro-7-iodo-8-hydroxy-quinoline), a drug in clinical trials for Alzheimer's disease treatment, being known to form zinc and copper complexes.[10] We attempt to introduce 8-hydroxy-quinoline moieties on different polyamine backbones, to give rise to mono- or di-quinoline analogues of general structures represented in Figure 2. Since length and chemical structure of the linker joining the two polyazamacrocycles in AMD3100 were determinative for the anti-HIV activity, it was of interest to use different diamines, polyamines or related conjugates as clioquinol linkers. Finally, we report the synthesis of new polyamine-conjugate and their anti-HIV properties. Polyamine can be either: (a) a monocyclam or bicyclam AMD 3100 or (b) the diamines: 1,3-diamino propane, 1,4-diaminomethylbenzene, and piperazine.

The new analogues were synthesized according to methodologies summarized in Schemes 1 and 2.

The whole new analogues were obtained starting from a common intermediate, chloromethyl quinoline **3** which was synthesized from chloromethylation of commercially available quinoline, according to a method described by Zheng.[11]

Several diamino linkers 1,3-diamino propane, 1,4-diamino xylene, piperazine, 1,4,8-tri-terbutoxy carbonyl 1,4,8,11-tetraazacyclotetradecane, 1,8,12-tri-terbutoxy carbonyl 1,4,8,12-tetraazacyclopentadecane, and penta Boc-protected bis-polyazamacrocycles **10d** (The synthetic scheme for **10d** starting from cyclam is shown on Scheme 2) were condensed on intermediate **3**, in acetonitrile as solvent in the presence of K_2CO_3 as base. Final compounds (**4**, **5**, and **6**), were obtained, respectively, in 55%, 60%, and 50% yields, while the Boc-protected analogues **7a** and **8a** were obtained in 45% and 55% yields. After Boc-deprotection in acidic media, the final corresponding analogues (**7** and **8**) were isolated in quantitative yields (Scheme 1).

The synthesis of bis-polyazamacrocycle–quinoline conjugate **10** required a specific synthetic route summarized in Scheme 2. Final compound **10** was obtained through the synthesis of the key intermediate 1,11-diBoc-polyazamacrocycle **10b**. This later was synthesized starting from cyclam (three equivalents) by addition of one equivalent of di terbutyldicarbonate reagent. After a

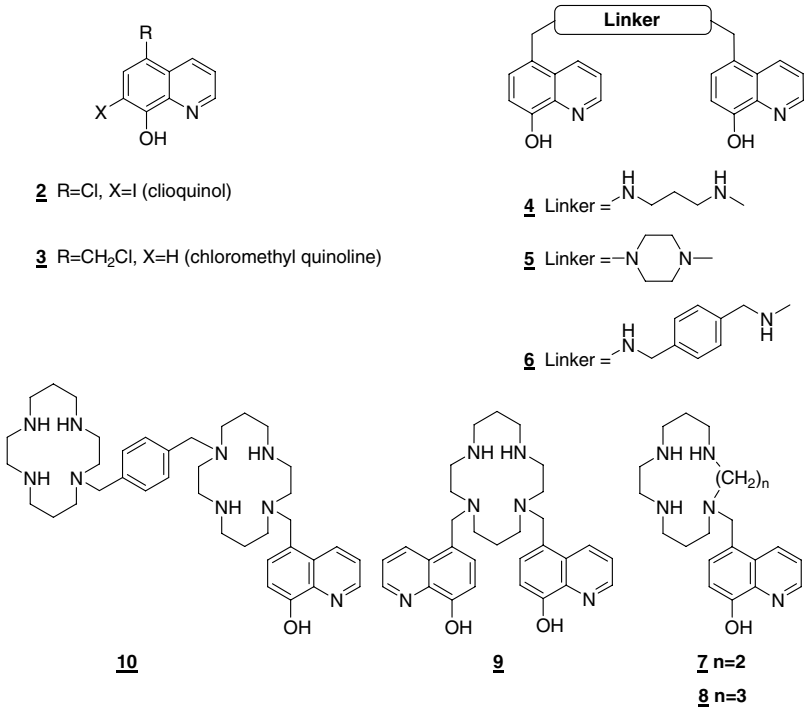
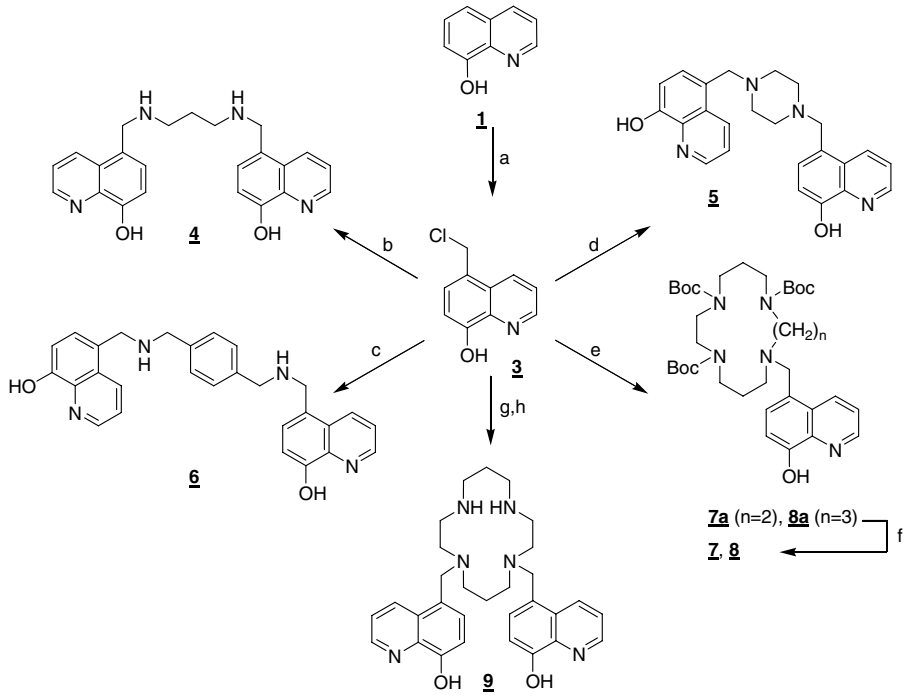
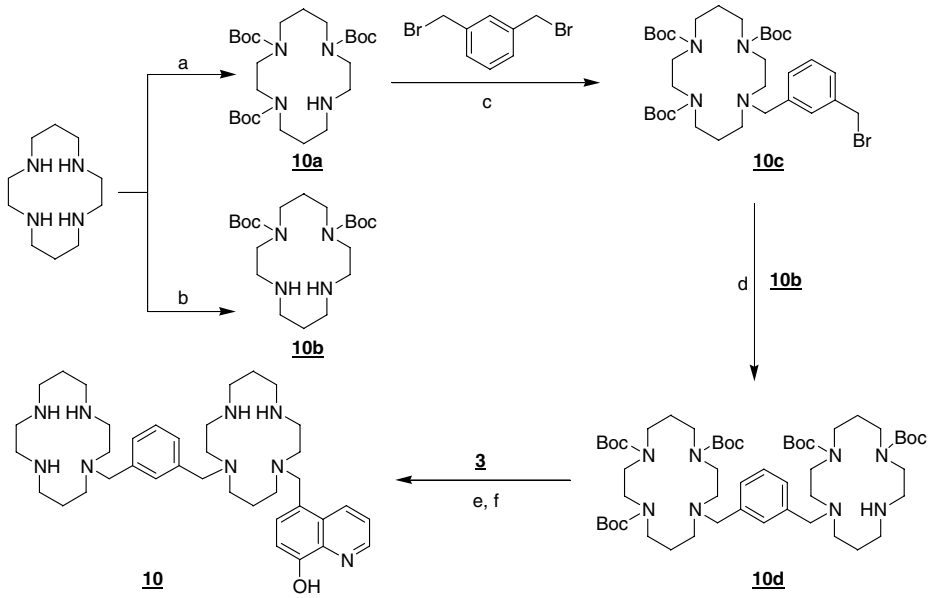


Figure 2. General structures of the new synthesized polyamine–quinoline conjugates.



Scheme 1. Reagents and conditions: (a) HCHO, HCl, 0 °C, overnight; (b) diamino propane–K_2CO_3, CH_3CN, rt, overnight; (c) α,α′-diamino xylene, CH_3CN, rt, 3 days; (d) piperazine, K_2CO_3, CH_3CN, rt, 3 days; (e) 1,4,8-tri-terbutoxy carbonyl-1,4,8,11-tetraazacyclotetradecane or 1,8,12-tri-terbutoxy carbonyl-1,4,8,12-tetraazacyclopentadecane, DIEA, CH_2Cl_2, overnight, rt; (f) HCl, ether, rt; (g) analogue **10b** (Scheme 2), DIEA, CH_2Cl_2, overnight, rt; (h) HCl, ether, rt, overnight.



Scheme 2. Reagents and conditions: (a), (b) Boc_2O, CH_2Cl_2, rt, overnight; (c) K_2CO_3, DMF, 3 days, rt; (d) **10b**, K_2CO_3, CH_3CN; (e) **3**, K_2CO_3, CH_3CN, rt; (f) HCl, ether, rt.

tedious column chromatography, 1,11-diBoc-protected **10b** was isolated in 20% overall yield and its structure was unambiguously assigned through NMR studies consistently with NMR published data.[12] The other possible expected analogue (1,8-diBoc-polyazamacrocycle) which differs from analogue **10b** (1,11-diBoc-polyazamacrocycle), only by the position of the Boc protecting groups on the polyazamacrocyclic core, was isolated in very low yield (less than 5%) (not shown in Scheme 2). Condensation of one equivalent of the triprotected tetraazamacrocycle **10a** already described in the literature[13] with one equivalent of α,α′-dibromoxylene led to intermediate **10c**, which after condensation on the diBoc-protected polyaza macrocycle **10b** gave intermediate

Table 1. Anti-HIV activities of the new conjugates on BaL and LAV HIV strains

Compound	HIV-1 LAV EC_{50}(μM)	HIV-1 BaL EC_{50}(μM)	CC_{50}[a] (μM)
1a	0.47	Inactive	>10
1b	0.05	Inactive	>10
2	Inactive	Inactive	2
3	5.10	Inactive	>10
2,3,2,3-Polyazamacrocycle	Inactive	Inactive	>10
2,3,3,3-Polyazamacrocycle	Inactive	Inactive	>10
4	Inactive	Inactive	>10
5	Inactive	Inactive	>10
6	Inactive	Inactive	>10
7	4.10	4.35	>10
8	1.50	1.25	>10
9	4.10	toxic	8
10	0.70	3.50	>10

EC_{50}: concentration in μM required to inhibit HIV RT activity by 50%.
[a] CC_{50}: concentration in μM to cause 50% death of uninfected cells. The highest tested concentration being 10 μM.

10d (overall yield 40%). Condensation of this later on chloromethyl quinoline **3** led after Boc-standard deprotection to the desired analogue **10** (overall yield 60%). The structures of the whole final analogues were clearly identified by spectroscopic methods: ^{1}H NMR, ^{13}C NMR, and MS (data available on request).

The anti-HIV activities of the whole series of compounds were assayed according to previously described methods.[14,15] PBMCs were infected with two virus strains: HIV-1 LAV[15] and HIV-1 BaL.[16] It was of interest to assay the new analogues against these two strains which use two different chemokines (GPCRs, CXCR4, and CCR5) to enter the cells. Results are expressed as means of 50% effective concentrations (EC_{50}) and cytotoxic concentrations (CC_{50}) represent concentrations required to cause 50% death of uninfected PBMCs. The results are presented in Table 1. Clioquinol **2**, 5-chloromethyl-8-hydroxy-quinoline **3**, and bis-polyazamacrocycle **1a** were used as benchmarking compounds for comparative anti-HIV activity with that of the new analogue antiviral activity.

As it can be observed in Table 1, compounds **7**, **8**, and **10** show anti-HIV activities on both strains HIV-1 LAV and HIV-1 BaL with EC_{50} values ranging from 0.5 to 5 μM (LAV strain) and from 1.2 to 5 μM (BaL strain). The case of compound **9** should be dissociated from the other active derivatives since its EC_{50} value is very close to its CC_{50} value, it cannot be considered as an active compound. In contrast, Biscyclam **1a** or AMD 3100 or its analogue **1b** was found totally inactive on HIV-1 BaL strain. This result was quite interesting since these bis-polyazamacrocycles are reported specific CXCR4 antagonist prototypes. They do not interact with any other CXCR or CCR receptors, they block X4 HIV-1 replication only through CXCR4 antagonization.[17]

It can be also observed that the structure of diamino spacers on which the quinoline moiety is linked appears to be determinative for the HIV-activities on both viral strains. Compounds **4**, **5**, and **6**, including 1,3-diaminopropane, piperazine or 1,4-diaminomethylbenzene as respective spacers, were totally denied of any anti-HIV activity. Moreover monopolyazamacrocycles such as cyclam (2,3,2,3) or its larger analogue (2,3,3,3) were also ineffective on both HIV virus strains. Clioquinol **2** it self was found inactive.

Since it is known that in specific conditions, CD_4+ cells express both CXCR4 and CCR5 mRNA co-receptors,[18] it could be possible that PBMCs used in this screening express both co-receptors, in this hypothesis the structure of new analogues allows interactions with both receptors, which results in both cases to a blockade of virus entry. BaL strains use CCR5 receptors to penetrate specific cells while HIV-1 LAV strains use more specifically CXCR4 receptors to enter in MT4 cells (classification of different HIV strains for the cells expressing different kind of receptors has been given by Princen et al.[19]). At present, we have no evidence that introduction of quinoline moiety on the bis-polyazamacrocycle backbone allows interactions with both CXCR4 and CCR5. It could be hypothesized that the bis-polyazamacycle moiety interacts with CXCR4 receptors, while quinoline moiety allows interactions with CCR5 co-receptors. Nevertheless, this hypothesis of inhibitors endowed with a dual activity on both types of receptors (CXCR4 and CCR5) could be a new concept of interest, if it can be confirmed by additional studies. Moreover such HIV drugs could find therapeutic application in the case of AIDS-related dementia complex (ADC) very often caused by HIV-1 BaL strain infections. Indeed it is known that productive HIV infection in the CNS is limited to macrophages and microglial cells, which contribute to the production of a number of putative neurotoxins including quinolinic acid.[20] Elevated levels of quinolinic acid are observed in vivo in cerebrospinal fluid of patients with AIDS-related dementia complex (ADC).[21] In this feature, such new analogues after optimization could be of potential value for ADC treatment.

Nevertheless, other action mechanisms for these new drugs cannot be ruled out since the new analogues can also interact with other targets within the viral replication cycle.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2006.08.114.

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