Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 891–894

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Substituted Quinolines Induce Inhibition of Proliferation of HTLV-1 Infected Cells

Alain Fournet,[a,b] Renaud Mahieux,[c] Mohammed A. Fakhfakh,[a] Xavier Franck,[a] Reynald Hocquemiller[a] and Bruno Figadère[a,*]

[a]*Laboratoire de Pharmacognosie (associé au CNRS-BioCIS) Faculté de Pharmacie, Université de Paris-Sud, rue J. B. Clément, 92296 Châtenay-Malabry, France*

[b]*Institut de Recherche pour le Développement (IRD), 213 rue Lafayette, 75480 Paris, France*

[c]*Unité d'Epidemiologie et Physiopathologie des Virus Oncogènes, 28 rue du Dr. Roux, Institut Pasteur, 75724 Paris, France*

Received 25 October 2002; revised 9 December 2002; accepted 11 December 2002

Abstract—Several quinolines were synthesized and evaluated against HTLV-1 infected cells. Some of them were able to inhibit HTLV-1 cell-growth at 10 μM. Some structure–activity relationships were observed.
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Ten to twenty millions of individuals are infected by human T-lymphotropic virus type 1 (HTLV-1) worldwide, mainly in south Japan, intertropical Africa, the Caribbean region and some regions of South and Central America.[1,2] HTLV-1 is transmitted from mother-to-child through prolonged breast feeding, by sexual contacts and by transmission of infected lymphoid cells. While most of the HTLV-1 carriers will remain asymptomatic, one can estimate that 1–5% of the infected individuals will develop one of the two severe HTLV-I-associated diseases: for example, adult-T-cell leukemia/lymphoma (ATLL), a CD4+ lymphoproliferation of poor prognosis,[3] or a slowly progressive neurological disorder named tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM).[4] About 800 cases of ATLL are diagnosed each year in southern Japan, with a survival median of six months in the acute leukemic form. In the Martinique Island (French West Indies), nearly 300 cases of TSP/HAM are diagnosed for only 400,000 inhabitants.[5] Because HTLV-1 cells are resistant to most apoptosis-inducing agents,[6] treatment of adult T cell leukemia/lymphoma (ATLL) patients, using conventional chemotherapy, has very limited benefit. The combination of zidovudine (AZT) with interferon-α is a partially effective in vivo, but not in vitro, treatment against ATLL.[7] In vitro, retinoic acid or arsenic trioxide (As_2O_3), in combination with interferon-α, can also induce cell-death in HTLV-1 transformed cells, through mechanisms that involve the down-regulation of NF-κB in the latter case.[8]

We were recently interested in quinolines, especially 2-alkylquinolines and 2-arylquinolines, isolated from plants[9] or prepared by synthesis.[10a–c] These compounds, with low-molecular weight, exhibited a variety of biological properties such as antiprotozoal activity (e.g., against *Leihsmania* sp.,[11] *Plasmodium*,[12] *Trypanosoma cruzi*[13]), and were found to be potent inhibitors of the human immunodeficiency virus of type-1 (HIV-1) integrase.[14,15] A very few quinolines (imidazoquinolinamines) such as imiquimod have also been demonstrated to be potent inducers of IFN-α and cytosines both in in vitro and in vivo experiments.[16] The low efficiency of compounds used in the treatment of ATLL led us to study various 2-alkyl, 2-alkenyl, 2-arylquinolines, and two bisquinolines against HTLV-1 transformed cell lines (HUT-102).

Compounds **3**, **6**, **10**, **14** and **22** were isolated from *Galipea longiflora*[9] and other compounds (Fig. 1) were prepared using previously published methods.[10a–c] All the compounds were characterized by nuclear magnetic resonance (^1H and ^{13}C NMR) spectrometry, IR, elemental analyses and mass spectrometry. They were dissolved in dimethyl sulfoxide (DMSO, Sigma) and were

*Corresponding author. Tel.: +33-01-46-83-55-92; fax: +33-01-46-83-53-99; e-mail: bruno.figadere@cep.u-psud.fr

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doi:10.1016/S0960-894X(02)01085-5

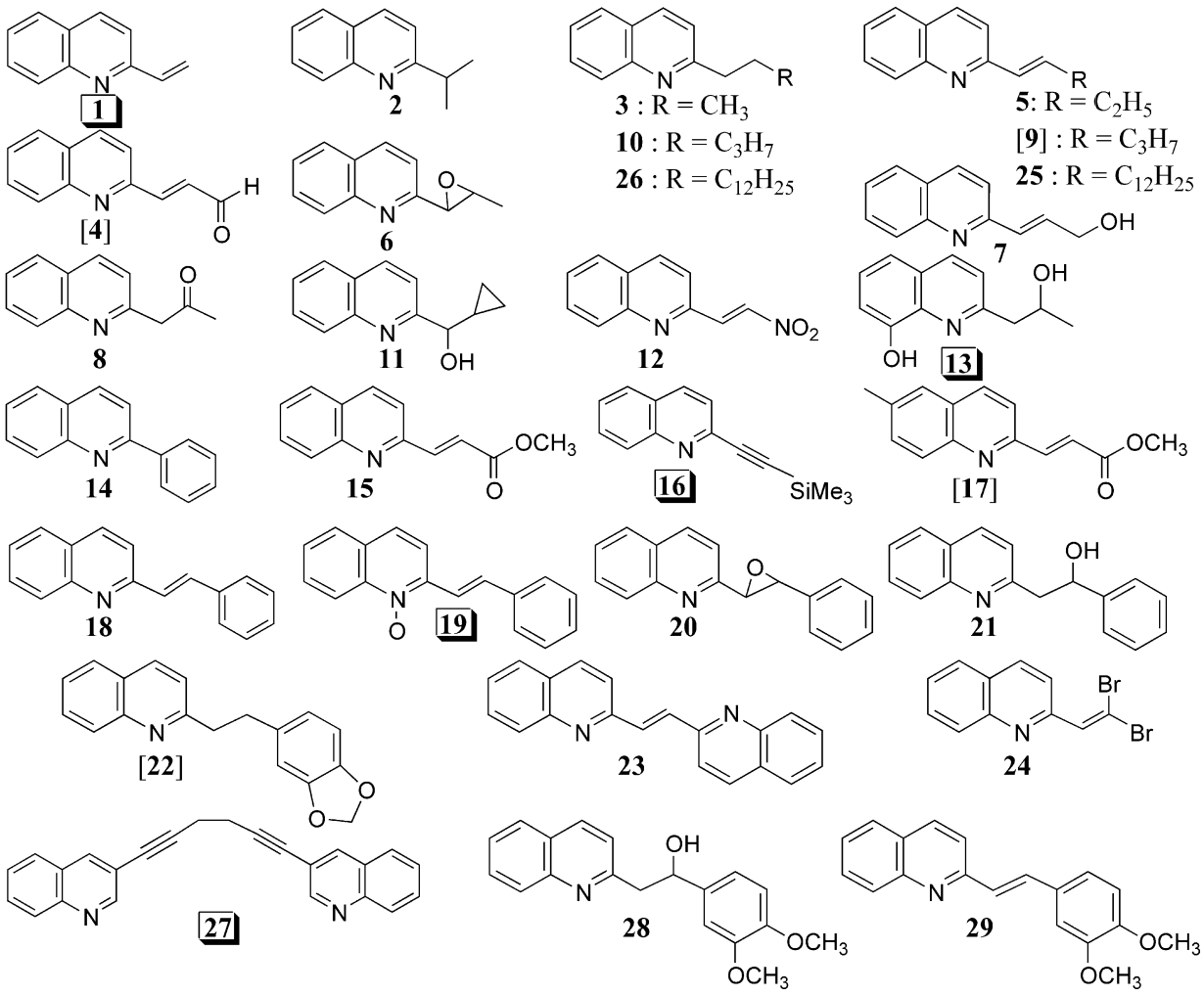

Figure 1. Compounds 1–29 tested against HTLV-1 transformed cells (most active ones in shadowed boxes, and medium ones in brackets).

used at concentrations of 50 and 10 μM. HUT-102, MT-2, C8166 and C91/PL are HTLV-1 transformed cell lines, and MOLT4 and Jurkat are leukemic cells that are not infected by HTLV-1. All these cell lines were grown in Rosewell Park Memorial Institute medium (RPMI-1640°; Life Technologies, Gaithesburg, MD, USA) supplemented with 10% fetal calf serum, glutamin, and penicillin-streptomycin. To measure cellular proliferation or viability, a cell proliferation-viability kit (XTT°; Roche Molecular Biochemicals) was used. In this assay, tetrazolium salt XTT is cleaved to form an orange formazan dye by metabolically active cells. This dye is directly quantified using an enzyme-linked immunosorbent assay reader at 492 nm.

Twenty-nine quinolines, 1–29, were evaluated against HTLV-1 transformed cells (HUT-102) (see Table 1). The cellular proliferation was quantified after 48 h of treatment. The compounds were then classified as inactive, or antiproliferative. Twenty compounds were found to have no or little effect either at 50 or 10 μM. These compounds are seven alkylquinolines **2**, **3**, **6**, **8**, **10**, **11**, and **26**, six 2-alkenylquinolines **5**, **7**, **12**, **15**, **25** and **29**, four 2-arylquinolines **14**, **20**, **21**, and **28**, two 2-styrylquinolines, **18** and **29**, and one 2,2′-bisquinoline **23**. Four compounds inhibited the cellular proliferation moderately: 2-alkenylquinoline **9** (containing a pent-2-en-1-yl chain), **17** (with a methylcarboxylate function), **22** (possessing an alkyl chain substituted by an aryl group), and **4** (possessing a conjugated formyl group to the ethenyl chain). The most active compounds were the simple 2-substituted quinoline **1** (with an ethenyl side chain), compound **13** (possessing one hydroxyl at the 8-position on the heteroaromatic ring, and another hydroxyl at the 2-position of the propyl chain), a 2-styrylquinoline **19** (containing a *N*-oxyquinoline ring), and compound **16** (with a trimethylsilylethynyl side chain). One 3-3′-bisquinoline, **27** (with a hex-1,5-diyn-1,1-diyl tether), showed also a remarkable activity.

From this comparative study, it is possible to draw some structure–activity relationships. Compound **9**, pent-2-en-1-yl-quinoline, shows 46% inhibition at 10 μM, whereas analogues with a shorter alkyl chain (compounds **1** and **16**) show a stronger activity whereas one with a longer chain (compound **25**) shows very low activity.

Interestingly, saturation of the alkenyl chain of **9**, leading to compound **10**, afforded an inactive compound. However, the presence of a hydroxyl on the quinoline ring and another hydroxyl group at the 2-position of the

Table 1. Antiproliferative activity of quinolines 1–29 against HTLV-1 infected HUT-102 cells

Compd	Inhibition (%)	Compd	Inhibition (%)
1	10 μM:87	16	10 μM:87
2	10 μM:0	17	10 μM:45
3	10 μM:0	18	10 μM:0
4	10 μM:34	19	10 μM:86
5	10 μM:0	20	10 μM:0
6	10 μM:0	21	10 μM:16
7	10 μM:0	22	10 μM:50
8	50 μM:0	23	50 μM:0
9	10 μM:46	24	10 μM:0
10	10 μM:0	25	10 μM:6
11	10 μM:0	26	10 μM:12
12	50 μM:0	27	10 μM:82
13	10 μM:85	28	10 μM:0
14	10 μM:0	29	10 μM:0
15	10 μM:4		

propyl chain in compound 13, increased the activity. Comparatively, compound 7, which possesses both a hydroxyl and a conjugated unsaturation, showed no activity. Compounds 11 and 6, having a hydroxyl and an epoxide function, respectively, did not show activity at the same concentration (even at 50 μM). The *N*-oxystyrylquinoline 19 showed an interesting activity (86% inhibition at 10 μM), whereas the simple styrylquinoline 18 was completely inactive. However, compound 21, possessing a 2-hydroxyl-2-phenylethyl chain, shows some activity (16% at 10 μM), but the 1,2-epoxystyrylquinoline 20 was again inactive. Finally, the 3,3′-bisquinoline 27 showed a surprising activity at 10 μM (82% inhibition) whereas the 2,2′-bisquinoline 23 was completely inactive. It is not sure wither activity is due to the alkynyl chain, or to the 3-substitution or to the bisquinoline unit (or a combination of several fac-

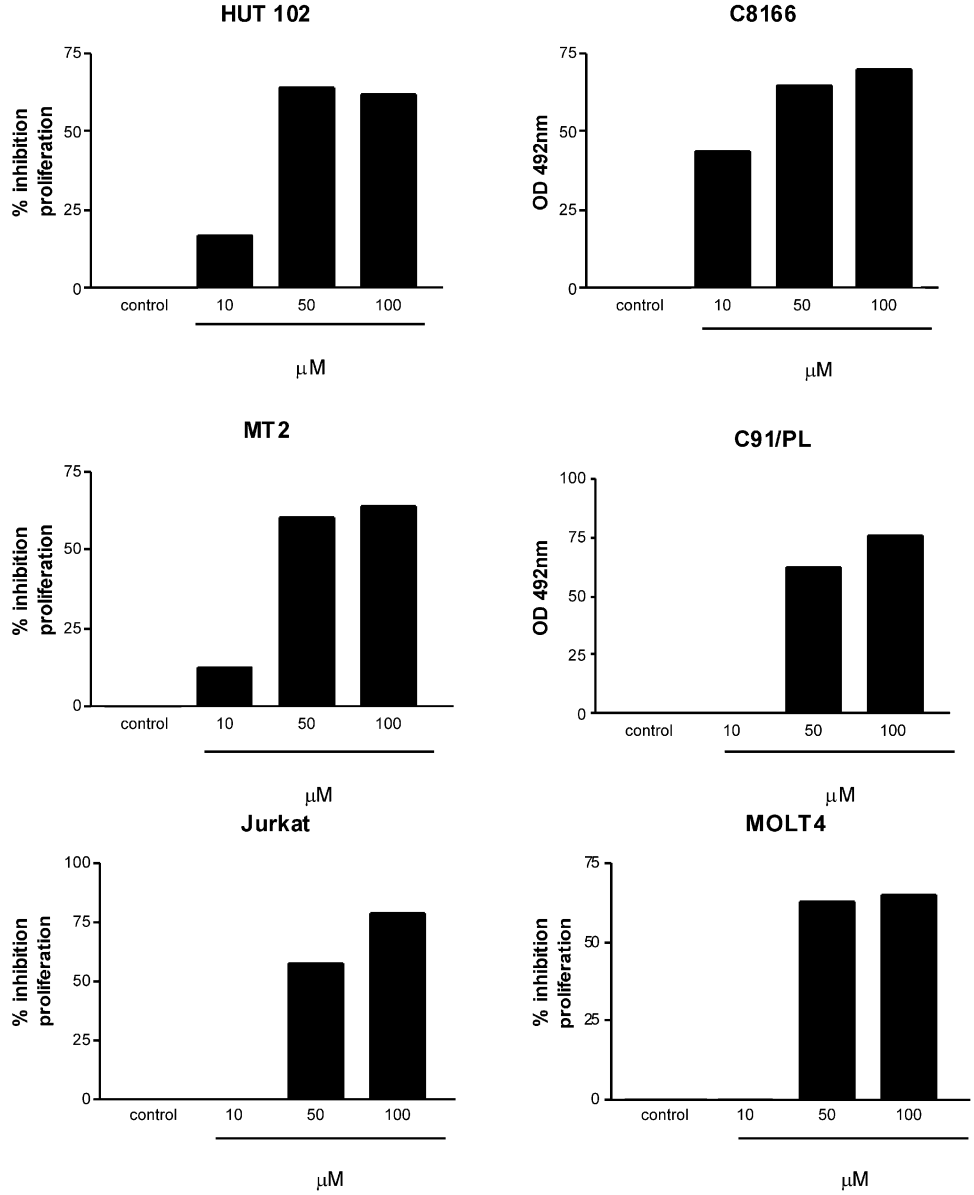

Figure 2. HTLV-1 cells from patients are sensitive to *E*-1-(2-quinolyl)-pentene 9, (A) HTLV-1 transformed cell lines (HUT-102, C8166, C91/PL, MT-2), (B) non HTLV control cell lines (MOLT-4, Jurkat).

tors). Compound **9** was then evaluated on a series of HTLV-1 transformed cells (HUT-102, C8166, MT2 and C91/PL) (Fig. 2) or non-infected control cells (Jurkat and MOLT-4). After 48 h treatment, the cell proliferation was measured. While control Jurkat and MOLT-4 cells were not affected by compound **9** at 10 μM, 3 out of 4HTLV-1 infected cell-lines, that is, HUT102, C8166, and MT2 were sensitive to the drug at 10 μM. Interestingly, the most sensitive cells were C8166. These cells express only the viral Tax protein. Several new quinolines have now been designed from these results and are planned to be synthesized on a large scale, in order to get more information on the SAR.

In conclusion, from this study several 2- or 3-substituted quinolines were found to exhibit significant activity against HTLV-1 transformed cells. Further studies are now to be performed to evaluate the efficiency of these compounds for the treatment of ATLL. Furthermore, new analogues will be designed, in order to obtain compounds with low cytotoxic effects.

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