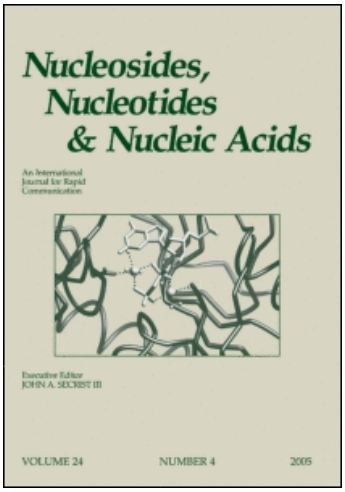

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Colombeau, Ludovic , Teste, Karine , Hadj-Bouazza, Amel , Chaleix, Vincent , Zerrouki, Rachida , Kraemer, Michel and Catherine, Odile Sainte(2008) 'Synthesis and Biological Activity of Chloroethyl Pyrimidine Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 27: 2, 110 — 120
To link to this Article: DOI: 10.1080/15257770701795813
URL: http://dx.doi.org/10.1080/15257770701795813

Nucleosides, Nucleotides, and Nucleic Acids, 27:110–120, 2008

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701795813

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF CHLOROETHYL PYRIMIDINE NUCLEOSIDES

Ludovic Colombeau,[1] Karine Teste,[1] Amel Hadj-Bouazza,[1] Vincent Chaleix,[1] Rachida Zerrouki,[1] Michel Kraemer,[2] and Odile Sainte Catherine[2]

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□ *The synthesis and biological activity of chloroethyl pyrimidine nucleosides is presented. One of these new nucleosides analogues significantly inhibited cell proliferation, migration and invasion as tested in vitro on the A431 vulvar epidermal carcinoma cell line.*

Keywords Nucleoside analogue; microwave activation; chloroethylnucleoside; anticancer; anti-migration; anti-invasion

INTRODUCTION

Several nucleoside analogues present interesting biological activities and can be used as antiviral or anticancer agents.[1] However, their limited uptake or quick metabolization, or the rapid emergence of resistant viral strain or multidrug resistant tumors, frequently impair their action or participate in their clinical toxicities.[2] These and other drawbacks strongly drive the search for new and diversified classes of nucleoside analogues.

As part of our research program on the synthesis of such compounds,[3] we became interested in the synthesis and biological evaluation of new nucleoside analogue drugs in which a chloroethyl chain is attached to the pyrimidine base (Figure 1).

Received 24 April, 2007; accepted 30 July, 2007.
Financial support from the "Conseil Régional du Limousin" is gratefully acknowledged. The authors are grateful to Dr. Michel Guilloton for useful comments on the manuscript.
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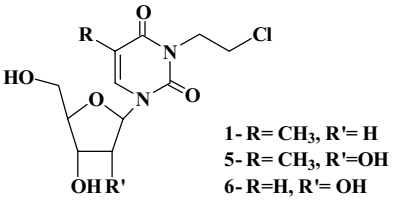


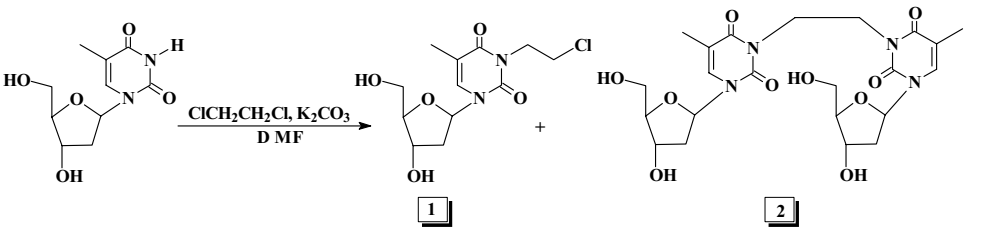
FIGURE 1 Chloroethylnucleosides.

RESULTS AND DISCUSSION

The first analogue synthesized was N-chloroethylthymidine **1**. Alkylation of thymidine with 1,2-dichloroethane (10 eq.) carried out under standard conditions in the presence of potassium carbonate (5 eq.) in N,N-dimethylformamide gave, after 2 hours at 80°C, a mixture of two products (Scheme 1).

Structural elucidation of these compounds indicated that one of them is the expected product (40%); the second one (compound **2**) results from reaction between *N*-chloroethylthymidine and thymidine (52.5%). We tried to apply microwave irradiation in order to improve these results. Microwave activation presents some advantages, such as a remarkable decrease in reaction times and, in a few cases, cleaner reactions and a good selectivity.[4] Selected results of alkylation of thymidine are summarized in Table 1.

Microwave activation (100 W, 8 minutes, 80°C), yielded 51% of the desired product and 35.5% of the second one. If, in these conditions, the proportion of N-chloroethylthymidine increased and the reaction time was reduced, the yield remained moderate. By the use of 1-bromo-2-chloroethane, N-chloroethylthymidine was isolated in 78% yield and dimer proportion dropped down to 18%. The best result was obtained with a large excess of 1,2-bromochloroethane (20 eq.) and in these conditions we obtained, after only 3 minutes, 90% of chloroethylthymidine and only 6% of 1,2-di-(thymidin-3-yl)ethane. Structures were established by NMR spectroscopy and were confirmed by mass spectrometry. The spectrum showed the presence of the protonated species $(M+H)^+$ m/z 305 and m/z



SCHEME 1 Alkylation of thymidine.

TABLE 1 Selected results of alkylation of thymidine

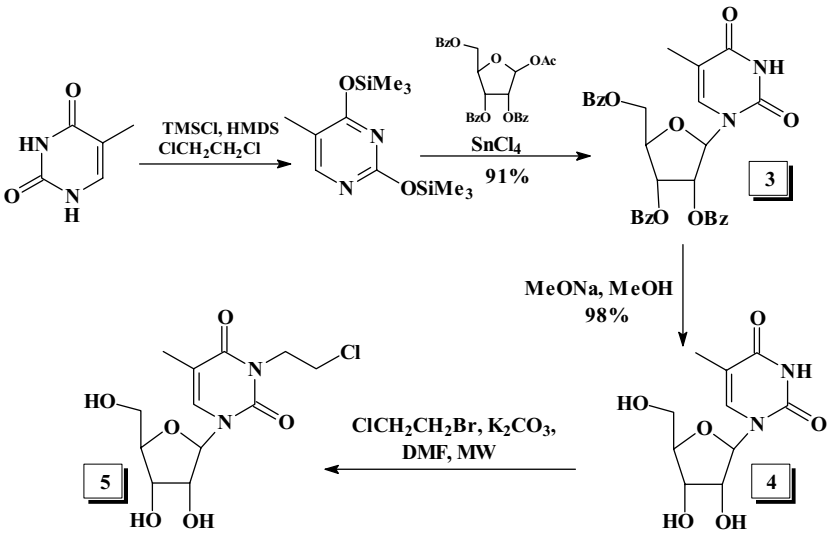
Entry	Activation	XCH_2CH_2X (eq.)	Reaction time	Chloroethylthymidine	dimer
1	Classical heating	$Cl(CH_2)_2Cl$ (10)	2 h	40%	52.5%
3	Microwave	$Cl(CH_2)_2Cl$ (10)	8 min	51%	35.5%
5	Microwave	$Cl(CH_2)_2Br$ (10)	4 min	78%	18%
6	Microwave	$Cl(CH_2)_2Br$ (20)	3 min	90%	6%

307, consequence of the two chlorine isotopes and in addition $(M+NH_4)^+$ m/z 322 and m/z 324.

The synthesis of the second nucleoside analogue, chloroethylhydroxythymidine **5**, consists of three steps (Scheme 2).

The starting material, 1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)thymine **3**, was prepared by the classical method of Vorbrüggen.[5] Thymine was silylated in dry 1,2-dichloroethane with TMSCl and HMDS, then addition of a solution of 1-*O*-acetyl-2,3,5-tribenzoylribofuranose and a Lewis acid in 1,2-dichloroethane to silylated thymine gave the expected protected nucleoside in 91% yield.[6] The next step was the total deprotection of the sugar moiety to give compound **4**. The removal of benzoyl groups proceeded readily with sodium methoxide in 98% yield.[6] Alkylation with 1-bromo,2-chloroethane using potassium carbonate, DMF and microwave activation (3 minutes) gave *N*-3(2-chloroethyl)-2′-hydroxythymidine (**5**) in 92% yield after purification.

Chemical ionization mass spectrometry analysis allowed identification of the molecular peaks MH^+ m/z 321 and m/z 323 consequence of the two chlorine isotopes, and in addition MNa^+ m/z 343 and m/z 345, MK^+ m/z 359 and m/z 361, MNa^+M m/z 663 and m/z 665.



SCHEME 2 Synthesis of 3-chloroethyl-2-hydroxythymidine.

The third analogue **6**, which resulted from alkylation of uridine, was realized in the same conditions using microwave activation (3 minutes), and gave after purification chloroethyluridine in 90% yield. In this case mass spectrometry showed the presence of the protonated species $(M+H)^+$ m/z 307 and m/z 309 and $(M+NH_4)^+$ m/z 324 and m/z 326.

BIOLOGICAL EVALUATION

First, we explored in vitro if chloroethyl pyrimidine nucleosides **1, 5**, and **6** could inhibit A431 cell proliferation. A431 human squamous carcinoma cells represent a good model of an aggressive, highly angiogenic and metastatic tumor.[7] A431 cells display an increase of epidermal growth factor receptors (EGFR) and produce large amounts of vascular endothelial growth factor (VEGF)[8] promoting neovascularization and angiogenesis.[9] Increased EGFR expression renders A431 cells less dependent on an exogenous source of epidermal growth factor (EGF) and enhances the EGF-induced mitogenic responses of squamous cell carcinoma cell lines compared with human epidermal keratinocytes, contributing to the invasiveness of malignant cells.[10]

In Vitro Effects of Chloroethyl Pyrimidine Nucleosides on Proliferation of A431 Tumor Cell Line

To investigate the effects of chloroethyl pyrimidine nucleosides on the cell proliferation, tumor cells were treated with increasing doses of chloroethyl pyrimidine nucleosides ranging from 15 μM to 1 mM (Figure 2).

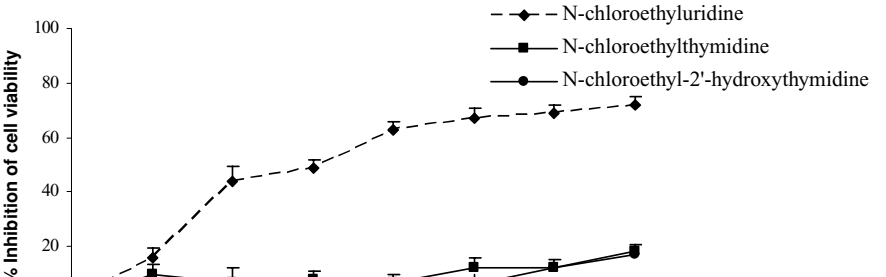


FIGURE 2 Dose-dependent effects of 3-chloroethylnucleosides **1**, **5**, **6** on A431 cell viability. A431 cells were treated with increasing concentrations (15 μM to 1 mM) of 3-chloroethylnucleosides for 72 hours. Results are mean ± SEM of three independent experiments.

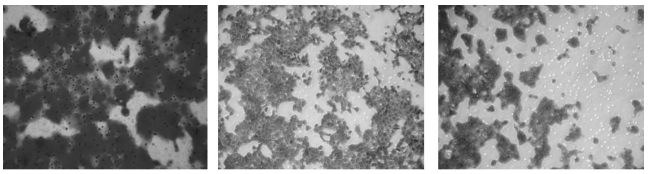


FIGURE 3 Effects of 3-chloroethyluridine (**6**) on the migration of A431 cells seeded on a fibronectin matrix in the upper chamber, with FCS 10% added to the lower chamber. Fewer cells migrated to the lower chamber in the presence of 3-chloroethyluridine. The migration of A431 cells was inhibited by 60% and 66%, respectively, by 3-chloroethyluridine at 50 μM and 100 μM. Original magnification x 200.

3-Chloroethylthymidine (**1**) and 3-chloroethyl-2′-hydroxythymidine (**5**) inhibited cell proliferation by less than 20% at 1 mM as compared to untreated control cells. 3-Chloroethyluridine (**6**) inhibited tumor cell proliferation in a dose-dependent manner. The concentration inducing 50% of maximal inhibition (IC_{50}) was 60 μM.

Then, as most tumors are able to disseminate via blood or lymphatic vessels,[11] we assessed the effects of 3-chloroethyluridine (**6**) on tumor cell migration and invasion. Invasion of tumor cells in the extracellular matrix is assumed by proteases as metalloproteases. We tested the effect of 3-chloroethyluridine (**6**) on cell migration, then, invasion, and ultimately on the expression of metalloproteases.

Cell Migration Assay

In the presence of a chemotactic stimulus as fetal calf serum (FCS) in the lower part of Boyden migration chambers, A431 cells migrated through the pores to the lower surface of the membrane. 3-Chloroethyluridine (**6**) significantly reduced cell migration. Compared with untreated control cells, migration of A431 significantly decreased by 60% ($p < 0.05$) and 66% ($p < 0.05$) in presence of 50 μM and 100 μM of 3-chloroethyluridine respectively (Figure 3).

Cell Invasion Assay

A Matrigel invasion assay was performed to study the effect of 3-chloroethyluridine **6** (50 μM and 100 μM) on the invasive ability of A431 cells. Compared with untreated control cells, invasion of A431 significantly decreased by 19% ($p < 0.05$) and 34% ($p < 0.05$) in presence of 50 μM and 100 μM of 3-chloroethyluridine respectively (Figure 4).

Chloroethyluridine Reduces Expression of Metalloproteases

Cell migration that takes place during angiogenesis requires a degradation of the extracellular matrix by proteases such as matrix metallopro-

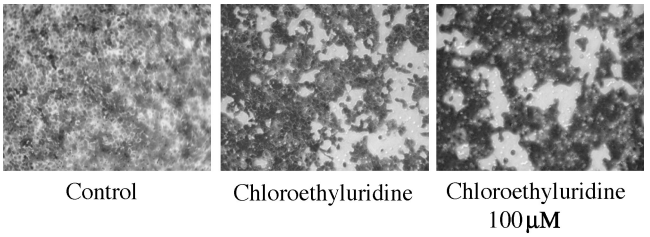


FIGURE 4 Effects of 3-chloroethyluridine (**6**) on the invasion of A431 cells seeded on a Matrigel matrix in the upper chamber, with FCS 10% added to the lower chamber. Fewer cells invaded to the lower chamber in the presence of 3-chloroethyluridine. The invasion of A431 cells was inhibited by 19% and 34% by 3-chloroethyluridine at 50 μM and 100 μM, respectively. Original magnification x 200.

teases (MMP).[12] Since 3-chloroethyluridine (**6**) inhibited migration and invasion of A431 cells, we then examined by zymography whether this compound could affect the secretion of MMP9 and MMP2 gelatinases by A431 cells. Analysis by quantitative zymography indicated that the amount of ProMMP9, MMP9, and ProMMP2, secreted into the medium and normalized to cell number after 24 hours or 48 hours of treatment with 15 μM of 3-chloroethyluridine, was not significantly different as compared to control cells. After 72 hours of treatment with 15 μM of 3-chloroethyluridine, the expression of MMP9 was totally abolished, whereas the expression of ProMMP9 decreased by 30%. ProMMP2 expression was not affected after 72 hours of treatment by 3-chloroethyluridine as compared to control cells (Figure 5).

The anti-migrative and anti-proliferative effects of 3-chloroethyluridine (**6**) can therefore been explained, as least in part, by an inhibition of MMP9 expression.

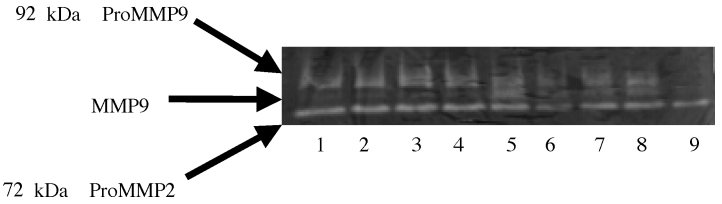


FIGURE 5 Effects of 3-chloroethyluridine (**6**) on ProMMP9, MMP9 and ProMMP2 expression by A431 cells. Conditioned media were collected after 24, 48, or 72 hours of incubation with 3-chloroethyluridine at 15 μM, normalized to cell number and subjected to gelatin zymography. Conditioned media from a positive control (HT 1080 cells, lane 1), pool of conditioned media from untreated A431 cells during 24, 48, and 72 hours (lane 2), pool of conditioned media from treated A431 cells during 24, 48, and 72 hours (lane 3), untreated cells for 24 hours (lane 4), A431 cells treated during 24 hours with 3-chloroethyluridine at 15 μM (lane 5), untreated cells for 48 hours (lane 6), A431 cells treated during 48 hours with 3-chloroethyluridine at 15 μM (lane 7), untreated cells for 72 hours (lane 8), A431 cells treated during 72 hours with 3-chloroethyluridine at 15 μM (lane 9).

CONCLUSION

In the present work, we reported the synthesis of new chloroethylpyrimidine nucleosides and our results on preliminary biological evaluation against highly invasive A431 tumor cells suggest that 3-chloroethyluridine could be efficient in inhibiting tumor growth and metastasis. Further investigation to demonstrates that this compound can act as an anticancer drug are actually in progress in our laboratory and additional data will be published elsewhere.

EXPERIMENTAL SECTION

Chemistry

All the solvents and chemicals were commercially available and, unless otherwise stated, and were used as received. DMF and $ClCH_2CH_2Cl$ were distilled twice over P_2O_5 and over CaH_2 just before use. Microwave irradiations were performed by the means of a monomode reactor (Synthewave 402, Prolabo) with focused waves. Reactions were monitored by thin-layer chromatography (TLC) on precoated 0.2 mm silica gel 60 F_{254} (Merck, Germany) plates and visualized in several ways: with an ultraviolet light source at 254 nm, by spraying with sulphuric acid (6 N) and heating at 200°C. Silica gel (Merck Kieselgel 60, 15–40 μm) was used for flash chromatography. ^{1}H NMR spectra were recorded at 400.13 MHz with a Brüker DPX spectrometer. Chemical shifts (δ) are expressed in ppm with Me_4Si as internal standard ($\delta = 0$). Data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; q, quintet; m, multiplet and br, broad), coupling constants (Hz) and assignment. Melting points (mp) were determined with a Kofler block and are uncorrected. IR spectra were recorded on a Perkin Elmer 1310 grating spectrophotometer and are reported in wave number (cm^{-1}). Chemical-impact mass spectra (CI) were recorded with a Kratos MS 580 mass spectrometer at the Laboratoire de Chimie Organique Structurale at the Université Pierre et Marie Curie (Paris VI).

General Procedure of Alkylation: In a typical procedure, a solution of nucleoside (1 mmol) with potassium carbonate (691 mg, 5 eq.) and 1-Bromo,2-Chloroethane (1.665 mL, 20 eq.) in DMF (8 mL) was irradiated (80°C, 100 W). After evaporation, the crude product was purified and gave the corresponding 3-chloroethylnucleoside.

***3-(2-Chloroethyl)thymidine* 1** (oil); *N*-3-(2-chloroethyl)thymidine was prepared starting from thymidine (242 mg, 1 mmol). Yield 90% (275mg); $R_f = 0.64$ (CH_2Cl_2/EtOH, 80/20, V/V); ^{1}H NMR (CD_3OD) δ: 7.85(q, 1H, J = 0.8Hz, H-6), 6.29 (t, 1H, J = 6.7Hz, H-1′), 4.39 (dt, 1H, J = 6.7Hz, J = 3.5Hz, H-3′), 4.25 (dt, 2H, J = 6.9Hz, J = 1.0Hz, H-α'), 3.91 (dd, 1H, J =

3.5Hz, J = 6.7Hz, H-4′), 3.80 (dd, 1H, J = 12.2Hz, J = 3.5Hz, H-5′a), 3.73 (dd, 1H, J = 12.2Hz, J = 3.5Hz, H-5′b), 3.71 (t, 2H, J = 6.9Hz, H-β'), 2.28 (ddd, 1H, J = 13.5Hz, J = 6.7Hz, J = 3.7Hz, H-2′a), 2.20 (ddt, 1H, J = 13.5Hz, J = 6.7Hz, J = 3.7Hz, H-2′b), 1.91 (d, 3H, J = 0.8 Hz, CH_3-thymine).

***1,2-di-(Thymidin-3-yl)ethane* 2** (oil): Yield 6% (16 mg), R_f = 0.16 (CH_2Cl_2/EtOH, 80/20, V/V); ^{1}H NMR (CD_3OD) δ: 7.78 (q, 2H, J = 1.1Hz, H-6), 6.17 (t, 2H, J = 6.5Hz, H-1′), 4.37 (dt, 2H, J = 6.5Hz, J = 3.8Hz, H-3′), 4.32 (m, 2H, H-α'), 4.19 (t, 2H, J = 6.9Hz, H-β'), 3.89 (q, 2H, J = 3.8Hz, H-4′), 3.80 (dd, 2H, J = 12.1Hz, J = 3.8Hz, H-5′a), 3.71 (dd, 2H, J = 12.1Hz, J = 3.8Hz, H-5′b), 2.26 (ddd, 2H, J = 13.5Hz, J = 6.5Hz, J = 3.8Hz, H-2′a), 2.20 (ddd, 2H, J = 13.5Hz, J = 6.5Hz, J = 3.8Hz, H-2′b), 1.83 (d, 6H, J = 1.1 Hz, CH_3-thymine).

***3-(2-Chloroethyl)-2-hydroxythymidine* 5** (white solid) was prepared starting from 2-hydroxythymidine (258 mg, 1 mmol). Yield 92% (295 mg); Tf = 92°C; R_f = 0.47 (CH_2Cl_2/EtOH, 90/10, V/V); ^{1}H NMR (CD_3OD) δ: 7.90 (q, 1H, J = 1Hz, H-6), 5.92 (m, 1H, H-1′), 4.26 (dt, 2H, J = 6.8Hz, J = 0.5Hz, CH_2-α'), 4.16 (m, 1H, H-2′), 4.16 (m, 1H, H-3′), 4.00 (m, 1H, H-4′), 3.86 (dd, 1H, J = 12.2Hz, J = 2.6Hz, H-5′a), 3.74 (dd, 1H, J = 12.2Hz, J = 2.6Hz, H-5′b), 3.71 (t, 2H, J = 6.8Hz, CH_2-β'), 1.90 (d, 3H, J = 1Hz, CH_3-thymine).

***3-(2-Chloroethyl)uridine* 6** (oil) was prepared starting from uridine (244 mg, 1 mmol). Yield 90% (275 mg) R_f = 0.55 (CH_2Cl_2/EtOH, 80/20, V/V); ^{1}H NMR (CD_3OD) δ: 8.05 (d, 1H, J = 8.2Hz, H-6), 5.91 (d, 1H, J = 5.1Hz, H-1′), 5.77 (d, 1H, J = 8.2Hz, H-5), 4.25 (dt, 2H, J = 6.8Hz, J = 0.7Hz, H-α'), 4.16 (t, 2H, J = 5.1Hz, H-2′), 4.15 (t, 1H, J = 5.1Hz, H-3′), 4.01 (dt, 1H, J = 5.1Hz, J = 2.8Hz, H-4′), 3.86 (dd, 1H, J = 12.2Hz, J = 2.8Hz, H-5′a), 3.74 (dd, 1H, J = 12.2Hz, J = 2.8Hz, H-5′b), 3.72 (t, 2H, J = 6.8Hz, H-β').

Biological Tests

A431 Cell Culture

A431 cells were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 U.mL^{-1} streptomycin (all obtained from Life Technologies, Inc.), at 37°C in a 5% CO_2 humidified atmosphere.

Cell Viability Experiments

Cell viability was evaluated using the MTT microculture tetrazolium assay,[13] which is based on the ability of mitochondrial enzymes to reduce 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) (Sigma, USA) into purple formazan crystals. Cells were seeded at a density of 5 × 10^3 cells in 96-well flat-bottom plates (Falcon, France) and incubated in complete culture medium for 24 hours. Then, the medium was removed

and replaced by 2% FCS-medium containing increasing concentrations of 3-chloroethyluridine varying from 1 mM to 15 μM. After 72 hours incubation, the cells were washed with phosphate buffered saline (PBS, Life Technologies) and incubated with 0.1 mL MTT (2 mg/mL, Sigma-Aldrich) for an additional 4 hours at 37°C. The insoluble product was then dissolved by addition of 200 μL DMSO (Sigma-Aldrich). The absorbance corresponding to solubilized formazan pellet (which reflects the relative viable cell number) was measured at 570 nm using a Labsystems Multiskan MS microplate reader.

Concentration-response curves were constructed and IC_{50} values (concentration of the compound inhibiting 50% of cell proliferation) were determined.

Cell Migration Assay

The influence of 3-chloroethyluridine (**6**) on migration of A431 cells was investigated as described previously[13] using Boyden invasion chambers with 8 μm pore size filters coated with 100 μL of fibronectin (100 μg/mL, Santa Cruz Biotechnology, USA) and were allowed to stand overnight at 4°C. 10^5 A431 untreated or 3-chloroethyluridine (100 μM or 50 μM during 4 hours)-pretreated cells were added to each insert (upper chamber). A strong chemoattractant (10% FCS) for A431 cells was added to the lower chamber. After 24 hours incubation at 37°C in a 5% CO_2-incubator, nonmigrated cells were removed by scraping and migrated cells were fixed in methanol and stained with haematoxylin. Cells migrating on the lower surface of the filter were counted in 10 fields using a Zeiss microscope. Results were expressed as a percentage, relative to controls normalized to 100%. Experiments were performed in triplicate.

Cell Invasion Assay

Cell invasion experiments were performed with Boyden chambers as described above. The inserts were coated with Matrigel membrane matrix (Falcon, Becton Dickinson Labware, USA). A431 cells (10^5) were seeded in the upper well of the Boyden chamber and 10% FCS was added to the lower chamber.

The cells were seeded in the upper chamber and 3-chloroethyluridine was added at 100 μM or 50 μM for 24 hours. After 24 hours at 37°C in a 5% CO_2-incubator, noninvaded cells in the upper chamber were wiped with a cotton swab and the filters were fixed, stained, and counted. Results were expressed as a percentage, relative to controls normalized to 100%. Experiments were performed in triplicate.

Zymography

A431 cells were seeded at a density of 50×10^4/well into 6-well tissue culture plates in DMEM-10% FCS. Cells were allowed to adhere for 24 hours.

Conditioned media were collected 24, 48, or 72 hours after treatment with 3-chloroethyluridine **6** (15 μM), normalized to cell number, mixed with non reducing Laemmli sample buffer, and subjected to 10% SDS-PAGE containing 0.1% (w/v) gelatin. The gel was washed 3 times at room temperature in a solution containing 2.5% (v/v) Triton X-100 in H_2O and incubated at 37°C for 24 hours in 50 mM Tris/HCL, pH 7.4, 0.2 M NaCl, 5 mM $CaCl_2$, and 0.05% Brij 35. The gel was stained for 60 min with 0.5% (w/v) R-250 Coomassie blue in 30% methanol (v/v)/10% acetic acid (v/v). ProMMP9, MMP9 and ProMMP2 were visualized as white zones on the gels indicating the gelatinolytic activity of proteinases. Gelatinase activity was quantified using a NIH image program.

REFERENCES

1. a) Hamamoto, Y.; Yamamoto, N.; Matsui, T.; Matsuda, A.; Ueda, T.; Yamamoto, N. Inhibitory effect of 2′,3′-didehydro-2′,3′-dideoxynucleosides on infectivity, cytopathic effects and replication of human immunodeficiency virus. *Antimicrob. Agents Chemother.* **1987**, 31, 907–910; b) Lin, T.-S.; Schinazi, R.F.; Prusoff, W.H. Potent and selective in vitro activity of 3′-deoxyadenosine, deoxythymidine-2′-ene (3′-deoxy-2′,3′-didehydrothymidine) against human immunodeficiency virus in vitro. *Biochem. Pharmacol.* **1987**, 36, 2713–2718; c) Gosselin, G.; Schinazi, R.F.; Sommadossi, J.-P.; Mathe, C.; Bergogne, M.C.; Aubertin, A.-M.; Kirn, A.; Imbach, J.-L. Anti-human immunodeficiency virus activities of the β-L-enantiomer of 2′,3′-dideoxycytidine and its 5-fluoro derivative in vitro. *Antimicrob. Agents Chemother.* **1994**, 38, 1292–1297; d) Lin, T.-S.; Luo, M.Z.; Liu, M.C.; Zhu, Y.L.; Gullen, E.; Dutchman, G.E.; Cheng, Y.-C. Design and synthesis of 2′,3′-dideoxy-2′,3′-didehydro-β-L-cytidine (β-L-d4C) and 2′,3′-dideoxy-2′,3′-didehydro-β-L-5-fluorocytidine (β-L-Fd4C), two exceptionally potent inhibitors of hepatitis B virus (HBV) and potent inhibitors of human immunodeficiency virus (HIV) in vitro. *J. Med. Chem.* **1996**, 39, 1757–1759; e) Daluge, S.M.; Good, S.S.; Faletto, M.B.; Miller, W.H.; St. Clair, M.H.; Boone, L.R.; Tisdale, M.; Parry, N.R.; Reardon, J.E.; Dornsife, R.E.; Averet, D.R.; Krenitsky, T.A. 1592U89, A novel carbocyclic nucleoside analogue with potent, selective anti-human immunodeficiency virus activity. *Antimicrob. Agents Chemother.* **1997**, 41, 1082–1093.
2. a) Richman, D.; Fischl, M.A.; Grieco, M.H.; Gottlieb, M.S.; Volberding, P.A.; Laskin, O.L.; Leedom, J.M.; Groopman, J.E.; Mildvan, D.; Hirsch, M.S. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N. Engl. J. Med.* **1987**, 317, 192–197; b) Yarchoan, R.; Pluda, J.M.; Thomas, R.V.; Mitsuya, H.; Brouwers, P.; Wyvill, K.M.; Hartman, N.; Johns, D.G.; Broder, S. Long-term toxicity/activity profile of 2′,3′-dideoxyinosine in AIDS or AIDS-related complex. *Lancet* **1990**, 336, 526–529; c) Larder, B.A. Viral resistance and the selection of antiretroviral combinations. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **1995**, 10 (Suppl. 1), S28–S33; d) Petersen, E.A.; Ramirez-Ronda, C.H.; Hardy, W.D.; Schwartz, R.; Sacks, H.S.; Follansbee, S.; Peterson, D.M.; Cross, A.; Anderson, R.E.; Dunkle, L, M. Dose-related activity of stavudine in patients infected with human immunodeficiency virus. *J. Infect. Dis.* **1995**, 171 (Suppl. 2), S131–S139.
3. a) Roy, V.; Zerrouki, R.; Krausz, P.; Schmidt, S.; Aubertin, A.M. Synthesis and antiviral evaluation of D4T analogues with a spacer arm between glucidic and base moieties. *Nucleosides, Nucleotides Nucleic Acids* **2004**, 23, 1625–1637; b) Zerrouki, R.; Billard, L.; Granet, R.; Krausz, P. Glycosylation Reaction of unprotected sugars with hydroxyalkylthymine. *Nucleosides, Nucleotides Nucleic Acids* **2004**, 23, 749–754; c) Davis, J.; Benhaddou, R.; Fedoryak, O.; Granet, R.; Krausz, P.; Bliard, C.; De Monte, M.; Aubertin, A.M. Potential antiviral agents. Part II. Synthesis and antiviral evaluation of pyrazinones substituted with acyclic chains. *Nucleosides Nucleotides* **1998**, 17, 1489–1504; d) Benjahad, A.; Benhaddou, R.; Granet, R.; Kaouadji, M.; Krausz, P.; Piekarski, S.; Bosgiraud, C.; Delebassee, S. Synthesis and antiretroviral evaluation of 3-alkyl-2-piperazinone nucleoside analogs. *Tetrahedron Lett.* **1994**, 35, 9545–9548.

4. Perreux, L.; Loupy, A. A tentative rationalization of microwave effects in organic synthesis according to the reaction medium, and mechanistic considerations. *Tetrahedron* **2001**, 57(45), 9199–9223.
5. Vorbrüggen, H.; Bennua, B. Nucleoside syntheses. XXV. A new simplified nucleoside synthesis. *Chem. Ber.* **1981**, 114(4), 1279–1286.
6. Hadj-Bouazza, A.; Zerrouki, R.; Krausz, P.; Laumond, G.; Aubertin, A.M.; Champavier, Y. New acyclonucleosides : synthesis and anti-HIV activity. *Nucleosides, Nucleotides Nucleic Acids* **2005**, 24, 1249–1263.
7. a) Di Benedetto, M.; Starzec, A.; Vassy, R.; Perret, G.Y.; Crepin, M.; Kraemer, M. Inhibition of epidermoid carcinoma A431 cell growth and angiogenesis in nude mice by early and late treatment with a novel dextran derivative. *Br. J. Cancer* **2003**, 88, 1987–1994; b) Guenin, E.; Ledoux, D.; Oudar, O.; Lecouvey, M.; Kraemer, M. Structure-activity relationships of a new class of aromatic bisphosphonates that inhibit tumor cell proliferation in vitro. A*nticancer Res.* **2005**, 25, 1139–1145; c) Hamma-Kourbali, Y.; Di Benedetto, M.; Ledoux, D.; Oudar, O.; Leroux, Y.; Lecouvey, M.; Kraemer, M. A novel non-containing-nitrogen bisphosphonate inhibits both in vitro and in vivo angiogenesis. *Biochem. Biophys. Res. Commun.* **2003**, 310, 816–23.
8. Myoken, Y.; Kayada, Y.; Okamoto, T.; Kan, M.; Sato, G.H.; Sato, J.D. Vascular endothelial cell growth factor (VEGF) produced by A-431 human epidermoid carcinoma cells and identification of VEGF membrane binding sites. *Proc. Natl. Acad. Sci. USA* **1991**, 88, 5819–5823.
9. Melnyk, O.; Shuman, M.A.; Kim, K.J. Vascular endothelial growth factor promotes tumor dissemination by a mechanism distinct from its effect on primary tumor growth. *Cancer Res.* **1996**, 56, 921–924.
10. Malliri, A.; Symons, M.; Hennigan, R.F.; Hurlstone, A.F.; Lamb, R.F.; Wheeler, T.; Ozanne, B.W. The transcription factor AP-1 is required for EGF-induced activation of rho-like GTPases, cytoskeletal rearrangements, motility, and in vitro invasion of A431 cells. *J. Cell Biol.* **1998**, 143, 1087–1099.
11. Mori, A.; Shigeki, A.; Furutani, M.; Hanaki, K.; Tadeka, Y.; Moriga, T.; Kondo, Y.; Gorin Rivas, M.J.; Imamura, M. Vascular endothelial growth factor-induced tumor angiogenesis and tumorigenicity in relation to metastasis in a HT1080 human fibrosarcoma cell model. *Int. J. Cancer* **1999**, 80, 738–743.
12. Heissig, B.; Hattori, K.; Friedrich, M.; Rafii, S.; Werb, Z. Argiogenesis: vascular remodeling of the extracellular matrix involves metalloproteinases. *Curr. Opin. Hematol.* **2003**, 10, 136–141.
13. Neaud, V.; Faouzi, S.; Guirouilh, J.; Le Bail, B.; Balabaud, C.; Bioulac-Sage, P. Rosenbaum, Human hepatic myofibroblasts increase invasiveness of hepatocellular carcinoma cells: evidence for a role of hepatocyte growth factor. *Hepatology* **1997**, 26, 1458–1466.