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Original paper

Relationship between the structure and cytotoxic activity of new unsaturated ketonucleosides tested on eight cell lines

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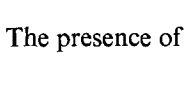
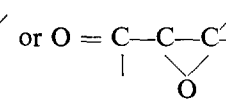
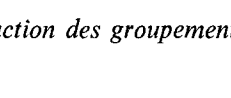
Summary — The synthesis and cytotoxic activity of 14 ketonucleosides on 8 normal, transformed and leukemic cell lines are described. Compounds **1**, **2**, **3**, **4**, **5** and **6** proved to be the most cytotoxic for transformed and leukemic cells; compound **7** presents an intermediate activity, whereas compounds **8**, **9**, **10**, **11**, **12**, **13** and **14** exhibited little or no cytotoxicity. The presence of the  or  system in the sugar moiety of the molecule was required for cytotoxic activity. Introduction of bromine into the sugar moiety of compound **1** made it highly toxic, whereas the introduction of an *O*-acetyl group into the sugar moiety of compound **5** seemed to moderate its cytotoxic activity. The degree of this activity was independent of the anomeric configuration, axial or equatorial position of the bases and the L or D configuration of the sugar.

Ketonucleosides **1**, **2**, **3** and **5** inhibited DNA synthesis in leukemic REH_6 cells more markedly than RNA and protein syntheses.

Résumé — **Relation structure—activité cytotoxique de nouveaux cétonucléosides insaturés essayés sur huit lignées cellulaires.** *Les auteurs décrivent la synthèse et l'activité cytotoxique d'une série de cétonucléosides, sur 8 lignées cellulaires: normales, transformées et leucémiques. Parmi les 14 composés testés, les cétonucléosides* ***1****,* ***2****,* ***3****,* ***4****,* ***5*** *et* ***6*** *ont une activité cytotoxique élevée vis-à-vis des cellules transformées ou leucémiques, le composé* ***7*** *a une activité intermédiaire, alors que les composés* ***8****,* ***9****,* ***10****,* ***11****,* ***12****,* ***13*** *et* ***14*** *sont inactifs. L'introduction des groupements*  *ou* *au sein de la partie sucrée de ces molécules est indispensable à cette activité. L'introduction du brome dans le* ***1*** *augmente la toxicité, tandis que la présence de l'*O*-acétyle au niveau du* ***5*** *modère celle-ci. Enfin, l'activité cytotoxique semble indépendante de la configuration anomérique, de la position axiale ou équatoriale de la base et de la configuration* L *ou* D *du sucre.*

Par ailleurs, les composés ***1****,* ***2****,* ***3*** *et* ***5*** *inhibent les synthèses de l'ADN, de l'ARN et des protéines. Cette inhibition est plus marquée sur l'ADN que sur l'ARN et les protéines.*

ketonucleosides / unsaturated ketonucleosides / cytotoxicity / macromolecular synthesis / structure—activity relationship

Introduction

The intensive efforts made in the synthesis of nucleosides including what are termed 'modified sugars' is largely due to the discovery of new oxidative procedures that readily permit the preparation of aldosuloses [1, 2]. Furthermore, since the anti-tumoral properties of arabinose—cytosine (Ara—C) and its homologs were discovered [3—5], nucleosides have proven to be effective drugs in the therapeutic stock, especially for the treatment of leukemia and certain solid tumors [2, 6]. Unfortunately, the first procedures of synthesis did not permit the preparation of the corresponding ketonucleosides postulated as key intermediates in many biosynthetic routes. Subsequently, most of the nucleosides obtained possess structures similar to those of the natural nucleosides [1] and some have the disadvantage of being rapidly inactivated *in vivo* by cleavage of the glycosidic bond or by deamination. These include, for example, the analogs of adenosine or cytosine, whose activities depend upon their deaminase/nucleotide kinase ratio [7, 8].

Recently, authors reported the synthesis of hexosulosyl purines and deoxyhexosulosyl purines, by direct oxidation of an assymetric carbon atom, and have shown that these oxonucleosides can be used as synthetic intermediates for

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many 'modified' nucleosides possessing biological interest [9—11].

The first reported biological study in the ketonucleoside series has given the most encouraging results. They inhibit the growth of viruses [12], and of cancerous KB cells [13, 14] and several of them display significant anti-tumor activity against L1210 leukemia cells in mice [14, 15]. Similarly, the first unsaturated ketonucleoside with a double bond in its sugar moiety conjugated to the carbonyl group [9] exhibited a high degree of cytotoxic activity. In all cases, the parent compounds were completely inactive [13, 15].

In the present study, we tested the cytotoxic activity of 14 new ketonucleosides (Fig. 1), on normal, leukemic or transformed lines of cells in order to detect the most active compounds, the most sensitive cells and any selective action on normal, transformed and leukemic cells. We also report the preliminary pharmacological effects of the active drugs on DNA, RNA and protein synthesis.

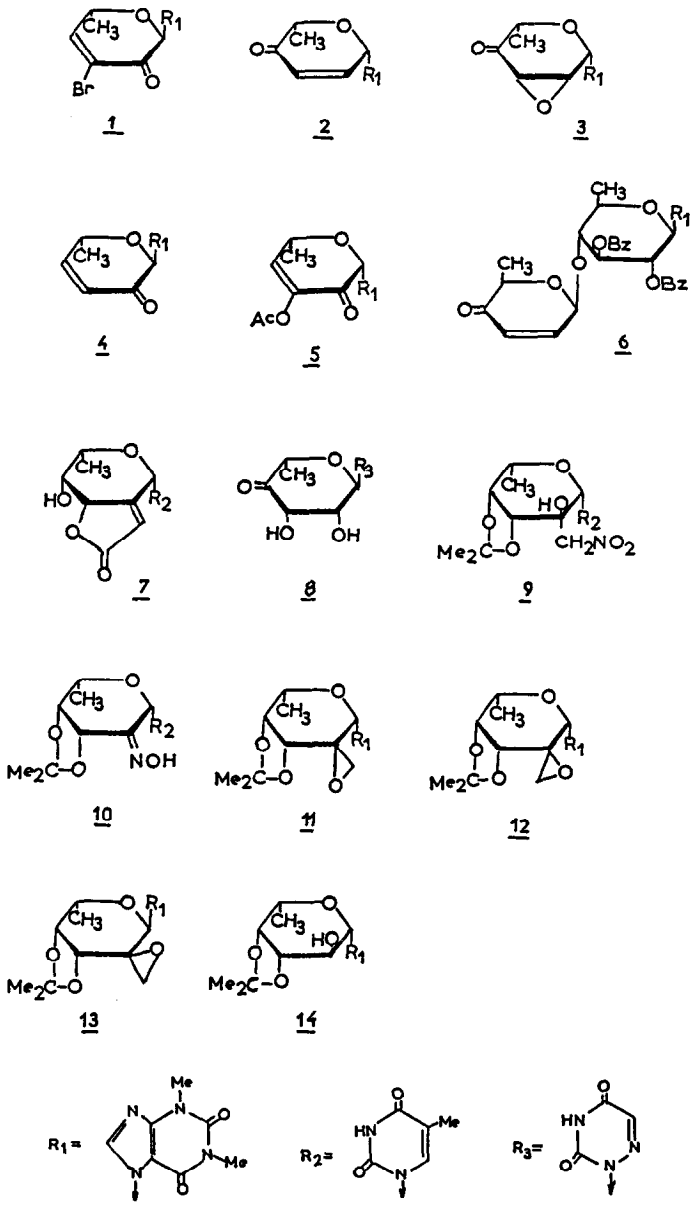

Fig. 1. Formulae of the ketonucleosides tested.

Chemistry

Several of the compounds examined were recently described [9, 10] (**1**—**7**, **11**—**14**). For the newest compounds, a detailed description of their synthesis is given under Experimental protocols.

Ketonucleosides such as compounds **8** and **3** were obtained by direct oxidation of an isolated hydroxyl group in the sugar moiety of two suitably protected parent hexosyl purines, respectively 6-aza-1-(2′,3′,*O*-isopropylidene-α-L-rhamnosyl)-uracil and 7-(2′,3′-anhydro-β-L-rhamnosyl)-theophylline.

The total synthesis of the newly obtained compound **8** was carried out as follows: condensation of bis-trimethylsilyl-aza-uracil with tetracetyl rhamnose under the conditions described by Vorbrüggen [16] gave high yields of the starting acetylated nucleoside **15**. It should be noted, however, that a mixture of the two isomers N-1 and N-3 was obtained when this reaction occurred at a temperature above 50°C.

Deacetylation using sodium methoxide gave compound **16**, which subsequently reacted with 2,2-dimethoxypropane in acetic acid to give the selectively protected nucleoside **17**. Ruthenium tetroxide oxidation [17] of **17** led to the formation of the corresponding ketonucleoside **18**. Cleavage of the protecting isopropylidene group with trifluoracetic acid/methanol [18] yielded **8**.

Unsaturated ketonucleosides **1**, **2**, **4**, **5** and **6** were obtained either by acylation of the corresponding ketonucleosides or by oxidation of partially acylated nucleosides [11, 19]. Of the various oxidative methods used, the most efficient proved to be the pyridinium chlorochromate or dichromate molecular sieve system [20]. The fused lactone nucleoside

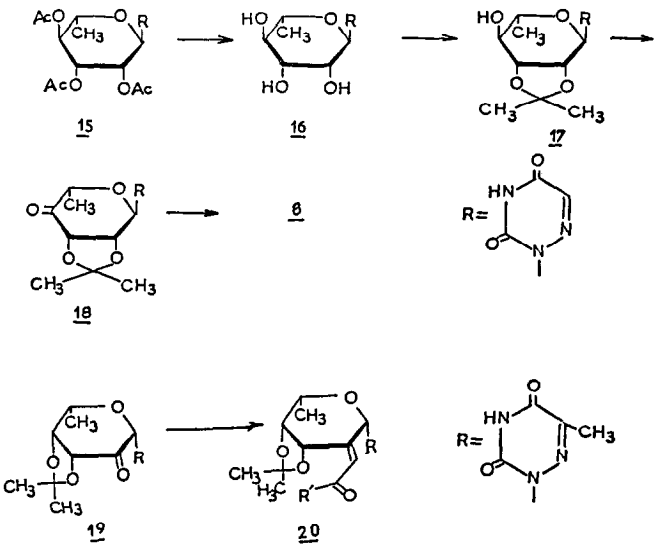

Fig. 2.

7, was recently obtained [21] by Wittig olefination of the parent 2′-ketonucleoside **19**.

The branched-chain sugar nucleosides **9** and **10** were obtained by nucleophilic addition of nitromethane and hydroxylamine, respectively, to ketonucleoside **19** [11]. These reactions were found to be highly stereospecific, since no isomer was isolated.

Nucleosides **11**, **12** and **13**, which constitute the first synthesized spiroepoxynucleosides, were obtained by the construction of the exocyclic ring from ketonucleoside precursors. Treatment of 2′-ketonucleosides with dimethyl sulfoxonium methylide and addition of the sulfurylid produced the β-L-talo- and α- and β-galacto-spironucleosides **11**, **13** and **12**, respectively [21].

The structures of the nucleosides examined were defined by analytical and spectroscopic methods, especially ^{1}H NMR spectroscopy.

Results and Discussion

Estimation of ketonucleoside cytotoxic activity in terms of ID_{50}, expressed in nmol/ml of medium, showed that compounds **1**, **2**, **3**, **4**, **5** and **6** displayed considerable cytotoxic activity in the transformed animal cell lines and human leukemic cell lines. The activity of compound **7** was intermediate and that of compounds **8**, **9**, **10**, **11**, **12**, **13** and **14** was very low (ID_{50} = 1000 μg/ml) or exhibited no dose—response-related activity at all (*e.g.*, compounds **8**, **14**) (Tables I, II).

To facilitate comparison and discussion, we used the logarithmic scale advocated by Venugopal & Luckery [29] (only for transformed and leukemic cells), with a '— log ID_{50}' value for each substance, indicated in this case in μmol/ml (Fig. 3).

When we considered the effect of each compound on pairs of cells and from each line tested, **2** was the most active in V79 cells, with an ID_{50} of 14 pmol/ml, followed in decreasing order, by **1**, **3**, **4**, **5**, **6** and **7** (Table I). This classification was also valid for rhabdomyosarcoma cells.

1 was the most cytotoxic in the Syrian hamster cells transformed by SV40 (ID_{50} = 5.40 pmol/ml), followed by **2**, **3**, **4**, **6**, **5** and **7**.

In the human leukemia cell lines, the most cytotoxic compound was **1** with an ID_{50} of 3.39 nmol/ml for the Daudi cell line and 1.08 nmol/ml for the REH_6 line; then came **2**, **3**, **6**, **4**, **5** and **7**. The REH_6 line turned out to be much more sensitive than the Daudi line.

When the chemical structure of the most cytotoxically active molecules is considered (Fig. 1), it should be noted that: 1) all of them have a ketonic group either in position 4′ of the sugar, as for **2**, **3** and **6** or in position 2′, as for **4**, **5** and **1**; 2) except for compound **3**, which has an epoxy function in position 2′, 3′ of the sugar, all these molecules have a double ethylenic bond, either in position 2′, 3′ of the sugar cycle, as for **2** and **6** or in position 3′, 4′ as for **5**, **1** and **4**; 3) all of them have a methyl group in position 5′ of the sugar; 4) the nitrogenous bases can be at position β for the L-conformation in the case of compounds **2**, **3** and **5** or at position α for compounds **1**, **4** and **6**.

In conclusion, the sugar moiety of all six molecules includes an O = C—C = C conjugate system which seems to be connected with their cytotoxic activity. This is confirmed by the structure of the almost inactive products,

Table I. Inhibiting dose 50 (ID_{50}) (in nmol/ml) for cell lines growing in monolayers.

Cells	Ketonucleosides						
	1	**2**	**3**	**4**	**5**	**6**	**7**
V79	0.27	0.014	3.14	6.10	30	20	227.4
CHN	1.57	0.69	2.06	6.86	60	7.21	284.6
RMC	0.14	0.069	0.36	1.66	8.22	1.58	278.9
REC	0.41	0.17	0.0065	1.10	40	1.72	57.24
SHSV40	0.0054	0.69	0.72	4.79	30	6.47	130
FH36	1.27	0.41	3.95	2.55	100	1.43	54.6

Exponentially growing cells were exposed throughout the incubation period to increasing concentrations of ketonucleosides. The percentage of survival of drug-treated cells was determined by the cloning efficiency method described under Experimental protocols. ID_{50} were estimated from the corresponding survival curves by linear regression analysis. V79: cell line established from Chinese hamster lung; CHN: Chinese hamster primary culture; RMC: rat rhabdomyosarcoma cells; REC: primary rat embryo cell culture; SHSV40: SV40 transformed hamster embryo cells; FH36: early secondary culture of hamster embryo cells.

Table II. Inhibiting dose 50 (in nmol/ml) for cells growing in suspension.

Cells	Ketonucleosides						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Daudi	3.39	11.97	9.31	23.97	44.14	10.45	427
REH_6	1.08	1.72	2.45	5	16.32	1.28	3263

Exponentially growing Daudi and REH_6 cells, seeded at 0.5 × 10^6 cells/ml, were grown in medium supplemented with various ketonucleoside concentrations. Cells excluding trypan blue dye were counted after 72 h of incubation. ID_{50} was determined from the resulting cytotoxicity curves.

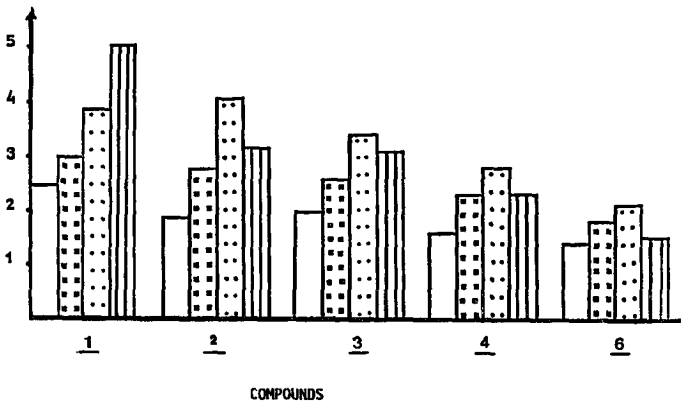

Fig. 3. Comparative cytotoxicity of ketonucleosides. Each bar represents the '— log ID_{50}' (in μmol/ml) for each ketonucleoside in: rhabdomyosarcoma cells (RMC), transformed SV40 hamster embryo cells (SHSV40), and human leukemia cell lines: Daudi and REH_6.

such as **7**, **8**, **9** and **10** which do not possess this conjugate system. Furthermore, compound **14**, the homolog of **5** before oxidation, did not display any cytotoxic activity.

The position of the O = C—C = C< system inside the pyranic cycle does not seem to be determinant (the carbonyl may be in positions 2′ or 4′ of the sugar). However, in some cases, the molecule may be cytotoxic when an epoxy function replaces the double ethylenic bond. Thus, the activity of compound **3**, which has an O = C—C—C< (O) system with an epoxy function in position 2′, 3′ of the cycle, did not change much compared to that of its homologs. The introduction of an electrophilic agent such as bromine at position 3′ of compound **1** increased its toxicity.

The introduction of an *O*-acetyl group into the sugar moiety of compound **5** seemed to moderate its cytotoxic activity. Compounds **5**, **1** and **2** possess the same basic structure with a bromine for **1** and an *O*-acetyl for **5**. Consequently, compound **1** displayed high cytotoxicity due to the presence of bromine. **4**, which is the equivalent of **1** without bromine or of **5** without an *O*-acetyl group, displayed intermediate cytotoxic activity, and **5** had a very low activity (Fig. 1).

The degree of cytotoxicity appears to be independent of the L or D configuration of the sugar, suggesting that the electrophilic O = C—C = C< or O = C—C—C< (O) system was in itself sufficient to endow these molecules with cytotoxic properties.

Finally, the role of the bases is still difficult to evaluate. Whatever the base, the molecules devoid of the O = C—C = C< system proved to be inactive. As already explained, the lower or null cytotoxic activity exhibited by compounds **8**, **9**, **10**, **11**, **12** and **13** is probably due to the fact that they lack this system. The presence of pyrimidine bases in some of them (thymine in compounds **7**, **9**, **10** and uracil in compound **8**), does not entitle us to conclude that these bases are responsible for the absence of cytotoxic activity in these compounds. However, other studies with only the one 'sugar structure' are necessary to validate this hypothesis. Numerous nucleosides, closely related to these compounds, analogs of pyrimidines, such as amicetin, gougerotin and blasticidin, have proven to be as active as purine analogs [1, 8, 17].

The position of the bases in relation to the cycle of the active compounds does not seem to be involved in their cytotoxic activity. Thus, the activity of compound **4**, an analog of compound **5** with theophylline is as strong as that of one of its homologs.

A slight difference between the sensitivity of tumoral or transformed cells and normal cells was observed in some cases, but this selective effect cannot be considered significant.

In conclusion, the study showed that: 1) the main structural requirement for significant cytotoxic activity is the presence of the O = C—C = C< or O = C—C—C< (O) system in the sugar moiety of the molecule; 2) the introduction of the electrophilic agent bromine leads to fairly high cytotoxicity; 3) the presence of an *O*-acetyl group moderates this activity; and 4) the degree of activity appears to be independent of the anomeric configuration of the sugar, and its L or D configuration.

To elucidate the cytotoxic mechanism of ketonucleosides, we also investigated their effects on macromolecular synthesis in REH_6 cells. Table III shows that incubation of these REH_6 cells with various concentrations of ketonucleosides **1**, **2**, **3** and **5** caused dose-dependent inhibition of the incorporation of all three precursors tested ([^{3}H]thymidine, [^{3}H]uridine and [^{14}C]leucine). However, DNA synthesis was preferentially inhibited in relation to RNA and protein synthesis.

Table III. Incorporation of [^{3}H]thymidine, [^{3}H]uridine and [^{14}C]leucine into DNA, RNA and proteins, respectively (% of control).

Compounds	Conc. (nmol/ml)	DNA	RNA	Proteins
1	0.5	55.0 ± 9.20	75.0 ± 7.75	80.0 ± 9.20
	1	23.4 ± 7.30	52.5 ± 7.85	85.0 ± 8.33
	2	10.7 ± 2.50	32.3 ± 9.35	55.5 ± 10.77
2	0.5	62.5 ± 10.13	70.0 ± 3.38	112.0 ± 15.35
	1	22.0 ± 3.35	90.0 ± 5.77	70.0 ± 7.82
	2	8.6 ± 1.62	65.0 ± 10.12	60.0 ± 6.32
3	1	68.0 ± 7.35	90.0 ± 7.70	98.0 ± 8.12
	2	40.0 ± 4.47	60.0 ± 9.35	80.0 ± 8.75
	4	22.5 ± 8.75	40.6 ± 11.00	52.0 ± 7.35
5	6	80.2 ± 2.35	102.0 ± 8.35	158.0 ± 25.10
	12	55.1 ± 11.10	87.0 ± 3.35	80.0 ± 7.35
	14	30.8 ± 10.77	67.0 ± 10.10	70.0 ± 8.22

[^{3}H]Thymidine, [^{3}H]uridine and [^{14}C]leucine were incorporated as described under Experimental protocols. Each value corresponds to the mean value of two separate experiments ± SEM.

At small doses, which did not alter the cell growth rate (0.5 nmol/ml for **1** and **2**, 1 nmol/ml for **3**, 6 nmol/ml for **5**), DNA synthesis was inhibited more than RNA and protein syntheses, suggesting that the cell replication pattern was altered. At higher cytotoxic doses, syntheses of all three were affected and maximum inhibition was again observed for DNA synthesis. The most active compound of all seemed to be **1**, for which the results resembled those for cytotoxicity.

Although the synthesis of DNA was more inhibited by ketonucleosides than those of RNA and protein, the similarity of these inhibitions makes it difficult to distinguish between primary and secondary effects. It does suggest, however, that these drugs function by a mechanism which is fairly non-specific for any particular macromolecular process. Several hypotheses have been reported in this connection: they include the direct inhibition of the DNA template, inactivation of DNA or RNA polymerase, deple-

tion of an essential precursor or action specifically at the level of DNA or other biochemical mechanism. The latter hypothesis includes drug interaction with the cell membrane. In this respect, a recent study showed that unsaturated ketonucleosides are highly reactive with sulfhydryl groups, particularly on the membrane surface of L1210 cells [30].

Experimental protocols

Chemistry

A Varian T60 spectrometer was used for ^{1}H NMR spectra (internal standard Me_4Si). Optical rotations were determined with a Rousset-Jouan, Quick polarimeter. UV spectra were recorded on a Varian 635 spectrophotometer. Reactions were monitored by TLC on Schleicher and Schüll silica gels (plastic sheets). Preparative TLC (1.5 mm layers) was carried out on silica gel PF 254 (Merck). Nucleosides were detected by UV light or by charring with sulfuric acid. HPLC was performed on a Dupont Zorbax—Sil column (0.75 × 50 cm), using a Dupont 850 chromatograph equipped with a UV absorbance detector and a Rheodyne 7000 sampling valve. Melting points were not corrected. Elemental analyses were obtained from the Laboratoire de Microanalyse du CNRS (Paris, France).

Solutions were concentrated at 40°C under reduced pressure. Dichloromethane was purified by storage for 1 week over a 4A molecular sieve. Solutions in organic solvents were dried on Whatman 1 PS hydrophobic filters.

6-Aza-1-(2′, 3′, 4′-O-acetyl-α-L-rhamnosyl)-uracil ***15***
A solution of tetraacetyl rhamnose (332 mg, 1 mmol) and tin (IV) chloride (0.16 ml, 1.4 eq) in dry 1,2-dichloroethane, was added to freshly prepared bis-trimethylsilyl-6-azauracil (1.1 mmol). The mixture was heated at 50°C for 2.5 h. After completion of the reaction ascertained by TLC (EtOAc), the reaction mixture was cooled, and washed with aqueous sodium hydrogen carbonate until neutral. After evaporation of the organic phase, compound **15** was crystallized from cyclohexane/ethanol (300 mg, 80%). mp 100°C, $[\alpha]_D$ = — 81° (c = 0.1; MeOH), UV λ_{max} 260 nm (ε = 5000), NMR (DMSO d_6) δ: 7.65 (s, 1H, H5); 6.00 (d, 1H, H1′, J_{1-2} 3 Hz); 5.60—5.50 (m, 2H, H2′, H3′); 5.00 (t, 1H, H4′, J_{3-4}, J_{4-5} 8 Hz); 4.00 (m, 1H, H5′); 2.00 (s, 9H, OAc); 1.16 (d, 3H, H6′, J_{5-6} 6 Hz); Anal. ($C_{14}H_{19}N_5O_4$) CHN.

6-Aza-1-(α-L-rhamnosyl)-uracil ***16***
15 (385 mg, 1 mmol) was dissolved in methanol (5 ml), 1 N sodium methoxide (1 ml, 1 eq) was added and the mixture was stirred at room temperature for 1 h. After neutralization with Amberlite resin (IR 120) and evaporation, the residue was crystallized from methanol (230 mg, 90%). mp 250°C, $[\alpha]_D$ = — 95° (c = 0.1; MeOH), UV λ_{max} 260 nm (ε = 5700), NMR (DMSO d_6) δ: 7.3 (s, 1H, H5); 5.7 (d, 1H, H1′, J_{1-2} 7 Hz); 4.0—3.5 (m, 3H, H2′, H3′, H4′); 3.16 (mn, 1H, H5′); 1.00 (d, 3H, H6′, J_{5-6} 6 Hz); Anal. ($C_9H_{13}N_3O_6$) CHN.

6-Aza-1-(2′, 3′-O-isopropylidene-α-L-rhamnosyl)-uracil ***17***
To a suspension of compound **16** (259 mg, 1 mmol) in acetic acid (10 ml), 2,2-dimethoxypropane (0.6 ml, 5 eq) was added. The mixture was heated at 90°C in the presence of 4A molecular sieve until dissolution occurred (1 h). It was then coevaporated, several times, with carbon tetrachloride to a white powder which did not crystallize (240 mg, 80%). $[\alpha]_D$ = — 62° (c = 0.1, MeOH), UV λ_{max} 260 nm (ε = 6700), NMR (DMSO d_6) δ: 7.6 (s, 1H, H5); 6.0 (d, 1H, H1′, J_{1-2} 2 Hz); 4.5—4.3 (m, 2H, H2′, H3′); 4.16 (t, 1H, H4′, J_{3-4}, J_{4-5} 6 Hz); 3.4 (m, 1H, H5′); 1.45 (s, 3H, CH_3); 1.3 (s, 3H, CH_3); 1.1 (d, 3H, H6′, J_{5-6} 6 Hz). Anal. ($C_{12}H_{17}N_3O_6$) CHN.

6-Aza-1-(2′, 3′-O-isopropylidene-4′-keto-α-L-rhamnopyranosyl)-uracil ***18***
To an aqueous solution of sodium *m*-periodate (428 mg, 2 mmol) and sodium hydrogen carbonate (168 mg, 2 mmol), ruthenium dioxide (133 mg, 1 mmol) was added and the suspension was stirred until dissolution. To the deep yellow solution of the ruthenium tetroxide thus obtained, an ethyl acetate solution of compound **17** (299 mg, 1 mmol) was added and the mixture was stirred for 1 h. Excess isopropanol was carefully added to reduce the tetroxide, and the solution was filtered on phase separating paper. After concentration of the organic phase, compound **18** was crystallized from ether (148 mg, 50%). mp 176°C, $[\alpha]_D$ = — 74° (c = 0.1, MeOH), UV λ_{max} 260 nm (ε = 5200), NMR (DMSO d_6) δ: 7.6 (s, 1H, H5); 6.0 (d, 1H, H1′, J_{1-2} 1 Hz); 4.87 (mn, 2H, H2′, H3′); 4.56 (t, 1H, H5′, J_{5-6} 6 Hz); 1.5 (s, 3H, CH_3); 1.4 (s, 3H, CH_3); 1.3 (d, 3H, H6′). Anal. ($C_{12}H_{15}N_3O_6$) CHN.

6-Aza-1-(4′-keto-α-L-rhamnopyranosyl)-uracil ***8***
Compound **18** (297 mg, 1 mmol) was dissolved in trifluoroacetic acid/methanol (90/10, 5 ml). After a few minutes, the solution was concentrated to dryness and washed free of acid with ether. Recrystallization from methanol/water gave compound **8** (230 mg, 90%). mp 249°C, $[\alpha]_D$ = — 98° (c = 0.1, MeOH), UV λ_{max} 260 nm (ε = 6400), NMR (DMSO d_6) δ: 7.6 (s, 1H, H5); 5.75 (d, 1H, H1′, J_{1-2} 1 Hz); 1.2 (d, 3H, H6′, J_{5-6} 6 Hz). Anal. ($C_9H_{11}N_3O_6$) CHN.

1-(6-Deoxy-3′, 4′-O-isopropylidene-2′-C-nitromethyl-α-L-galactopyranosyl)-thymine ***9***
To a solution of 1-(6′-deoxy-3′,4′, *O*-isopropyliden-α-L-hexopyranosylulose)-thymine **19** (1 g, 3.22 mmol) in nitromethane (10 ml), sodium methoxide (50.2 ml) was added and the mixture was stirred for 10 min. Resin Amberlite IR 120 was then added and the solution was filtered and concentrated. The residue was crystallized from ethanol to give **9** (1.012 g, 85%); mp 140—141°C, $[\alpha]_D$ = — 155° (c = 0.2, MeOH), UV λ_{max} (MeOH) 275 nm (ε = 11 507); NMR (CD_3 COOD): δ: 7.82 (s, 1H, H6); 6 (s, 1H, H1′); 5.6 (s, 2H, CH_2NO_2); 4.1—3.7 (m, 3H, H3′, H4′, H5′); 1.9 (s, 3H, Me—C5); 1.4 (d, 3H, J = 7 Hz, H6′). Anal. ($C_{15}H_{20}N_3O_8$) CHN.

1-(6′-Deoxy-3′, 4′-O-isopropylidene-α-L-lyxohexopyranosylulose oxime) thymine ***10***
Hydroxylamine hydrochloride (0.140 g, 2 mmol) was dissolved in a mixture of pyridine/water (1/1, 5 ml). 310 mg (1 mmol) of 1-(6′-deoxy-3′,4′-*O*-isopropylidene-L-lyxohexopyranosylulose)-thymine **19** was added and the mixture was kept at room temperature for 4 h. The solution was concentrated and dichloromethane was added and distilled twice from the residue. The pure **10** isolated as a semi-crystalline material (260 mg, 80%) had $[\alpha]_D$ = — 50° (c = 0.2, MeOH); UV λ_{max} (MeOH) 264 nm (ε = 10 562); NMR ($CDCl_3$) δ: 7.9 and 7.7 (s, 1H, H6); 6.5 and 6.7 (s, 1H, H1); 5.9 and 5 (d, 1H, J = 7 Hz, H3′); 4.3 (dd, 1H, J = 7 and 2 Hz, H4′); 3.7 (dq, 1H, 6.5 and 2 Hz, H5′); 1.8 (s, 3H, Me—C5); 1.57 and 1.4 (s, 2 × 3H, CH_3 isopropylidene); 1.25 (d, 3H, J = 7 Hz, H6′). Anal. ($C_{14}H_{19}O_6N_3$) CHN.

Pharmacological studies

Cells
Pairs of cells of the following lines were used. They included both normal and either transformed or leukemic cells: V79 Chinese hamster lung fibroblasts (V79) were obtained from the International Agency for Research on Cancer, Lyon, France, and primary cultures of Chinese hamster lung fibroblasts (CHN) were prepared from a 3 week old hamster, as previously described [22]. Rhabdomyosarcoma cells (RMC) were derived from a primary 9-4/0 tumor induced by intramuscular injection of metallic nickel powder into a male WAG rat [23], and primary cultures of WAG rat embryo cells (REC) were prepared by successive trypsinizations [22]. SV40 transformed Syrian hamster embryo cells (SHSV40) were obtained from Dr. I. Gresser (IRSC, Villejuif, France) and normal hamster embryo cells (FH36) were prepared from 13—14 day old Syrian hamster embryos.

V79, CHN, RMC, REC, SHSV40 and FH36 cell lines were grown as monolayers and cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (200 U/ml of penicillin and 50 μg/ml of streptomycin). Cells were incubated in a humidified atmosphere at 37°C with 10% CO_2.

Two human leukemia cell lines were also used: Daudi cells from a human peripheral lymphocyte cell line transformed by Epstein—Barr virus, and REH_6 cells, a human acute lymphocytic leukemia cell line, with no T or B cell characteristics [24].

These cells were obtained from Dr. Rosenfeld (IRSC, Villejuif, France) and grown as a monodispersed suspension culture in RPMI 1640 (Eurobio, Paris, France) with 10% heat-inactivated FCS and antibiotics.

Toxicity assays

Various concentrations of cells were incubated at 37°C for 24 h until they reached exponential growth. They were then exposed to increasing ketonucleoside concentrations ranging from 0.125 to 10 μg/ml of culture medium for compounds **1**, **2**, **3** and **4**, and from 10 to 80 μg/ml of culture medium for compounds **5**—**14**. Cytotoxicity was evaluated by measuring cloning efficiency for cells grown as monolayers and by trypan blue dye exclusion for cells grown in suspension.

The procedure used for testing cloning efficiency has already been described [22]. Briefly, when untreated colonies exceeded a median diameter of 0.25 mm, the plastic petri dishes (Falcon) were rinsed with Hank's balanced saline solution (HBSS), immediately fixed with ethanol, stained with 4% Giemsa and counted. Results are expressed in percentage of toxicity as follows:

$$100 - \frac{\text{cloning efficiency in treated cells}}{\text{cloning efficiency in control cells}} \times 100$$

Cloning efficiency is the number of visible colonies obtained relative to the number of cellular units plated.

Trypan blue dye was prepared as a 0.4% solution in HBSS at pH 7.2 according to Phillips [25], except that no methyl-*p*-hydroxybenzoate preservative was added. For staining, cells were suspended in HBSS at about 10^6 cells/ml and 0.1 ml of 0.4% stain was added per ml of cells. Cells were counted in a hemocytometer within a few minutes of staining. The percentage of viable cells is equal to non stained cells/(stained + non stained cells) × 100 and results are expressed as the percentage of toxicity, calculated as follows:

$$100 - \frac{\text{percentage viable treated cells}}{\text{percentage viable control cells}}$$

For each compound tested, two independent experiments were run.

Effect of ketonucleosides on precursor incorporation into DNA, RNA and protein

REH_6 cells (10^6 cells/ml) were exposed to compounds **1**, **2**, **3** and **5** for 24 h. Immediately after treatment, labeled precursors, which contained 0.5 μCi/ml of [^{3}H]methylthymidine (44 Ci/mM) and either 1 μCi/ml [^{14}C]leucine (46 mCi/mM) or 0.5 μCi/ml [^{3}H]uridine (27 Ci/mM) (CEA, Saclay, France), were added as a 30 min pulse.

At the end of the pulse, incorporation was stopped with 0.15 M NaCl, the preparation was centrifuged and radioactive precursor incorporation into DNA, RNA and protein was determined by the modified method described earlier [26]. Briefly, cells labeled with [^{3}H]-methylthymidine and [^{14}C]leucine were washed and precipitated with 10% perchloric acid (PCA). Warm 10% PCA was added to the precipitate and heated to 90°C for 30 min. The filtered supernatant was used for DNA estimation, by measurement of absorbance at 260 nm in a UV spectrophotometer (Varian) with calf thymus DNA as the standard, and for measurement of radioactivity incorporation into DNA. The precipitate was solubilized in 20°C ammonia and the filtrate was used to estimate protein incorporation of [^{14}C]leucine. Protein was measured by the Bradford technique [27].

Cells labeled with [^{3}H]uridine were treated with cold 0.6 N PCA. The pellet was washed with cold 0.2 N PCA suspended in 0.3 N KOH and incubated at 37°C for 18 h. After addition of 1.2 N PCA, the supernatant was used to estimate RNA, at 260 nm using yeast RNA as the standard, and radioactivity.

Radioactivity incorporation was expressed in μg for DNA and RNA and in mg for protein. Measurements were made on duplicate samples and results were expressed as the % of control values.

Statistical analysis

ID_{50} was determined by a linear regression curve [28].

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