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INFLUENCE OF THE STRUCTURE OF THE SUGAR MOIETY ON THE CYTOTOXIC AND ANTIVIRAL PROPERTIES OF SUGAR ELECTROPHILES

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Blocked sugars bearing a 2-nitrovinyl, 1-cyanovinyl, 3-nitrochromen-2-yl or 4-cyanochromen-2-yl group have been prepared. Their cytotoxicity and chemotherapeutic properties depend on the structure of the sugar moiety. Some of these compounds active against *Polyoma virus* constitute leads for the development of new antiviral drugs.

KEYWORDS—*Polyoma virus*; antibacterial; α-methylideneurononitrile; α-methylidenealdononitrile; nitrochromene; cyanochromene

Michael acceptors constitute a class of soft organic electrophiles whose toxicity depends on their ability to react with soft biological nucleophiles such as mercapto groups. The general toxicity of this family of compounds has been converted to useful therapeutic activity in special cases such as ethacrinic acid[1] where a specific fixation on accessory sites of the receptor and a control of the biodistribution of the drug were operating.

We report herein more unexpected large differences in biological activities in a series of closely related blocked sugar electrophiles.

Sugar derivatives (**1a-b, 1d-e, 1g-k**) submitted to the Henry reaction following a described procedure[2] led to the corresponding nitroalcohols **2**,[3] which were dehydrated to the nitroenose derivatives **3** (**3f** being obtained from its unacetylated nitroalcohol precursor). Treating **3** with cyanide anion[4] led to the expected α-methylidenenitriles **4**. Compounds **5b** and **5c** were obtained from the reaction of the corresponding aldehydes with the conjugate base of acrylonitrile. Condensation of some of the nitroenoses **3** with aromatic *ortho*-hydroxyaldehydes, led to the nitrochromenes **6l, 7a, 7b, 7d, 7l, 7j, 7l** and **8l** obtained as a mixture of two epimers at the new asymetric carbon, which, in most instances, were not separated. Treatment of the 3-nitrochromenes **6l, 7a** and **7j** with cyanide anion easily gave the corresponding 4-cyanochromenes **9l, 10a** and **10j** respectively. The yields of reactions cited (Chart), fell in the range of 50-95%.

Representative examples of these sugar electrophiles were submitted to a limited panel of toxicity tests (see Table). The oncogenic *Polyoma virus* was chosen for its bearing both lytic and oncogenic activities and also for its low molecular weight, easily isolated DNA. Large variation in every kind of tested toxic activity were found in each family (bearing the same electrophilic group), at least one member of each showing some cytoxicity. Every possible type of toxicity profile was encountered :

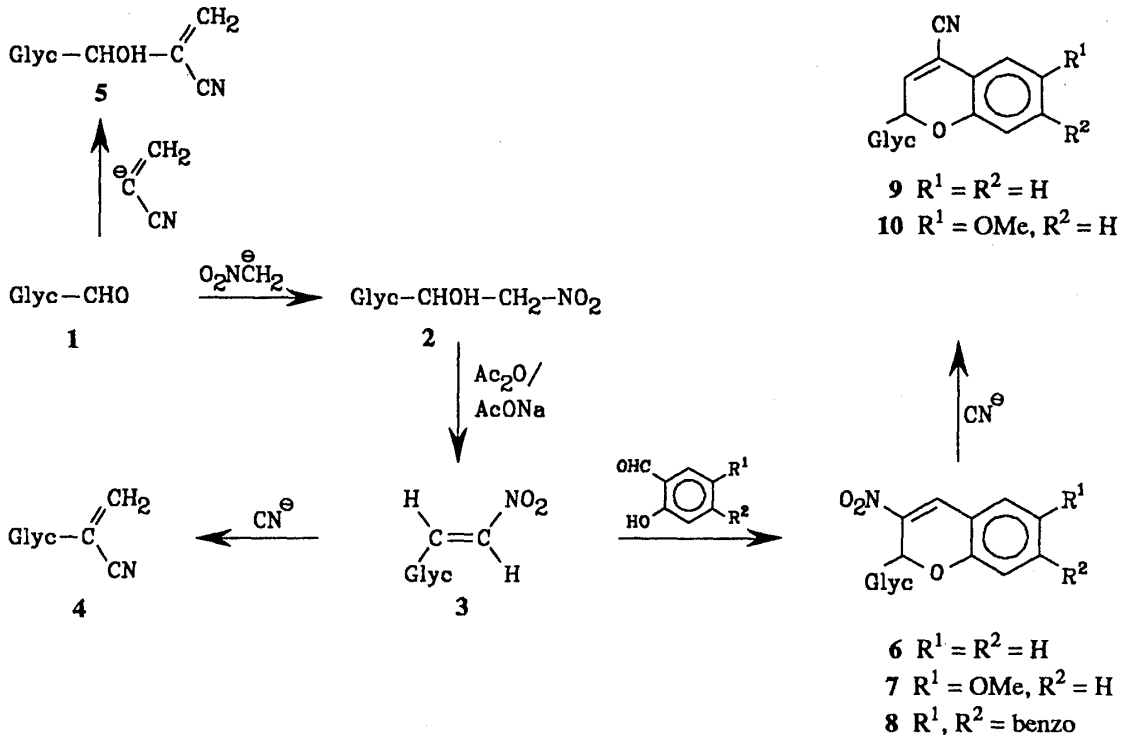
Table. Some Cytotoxic, Antiviral and Antibacterial Activities of Sugar Electrophiles

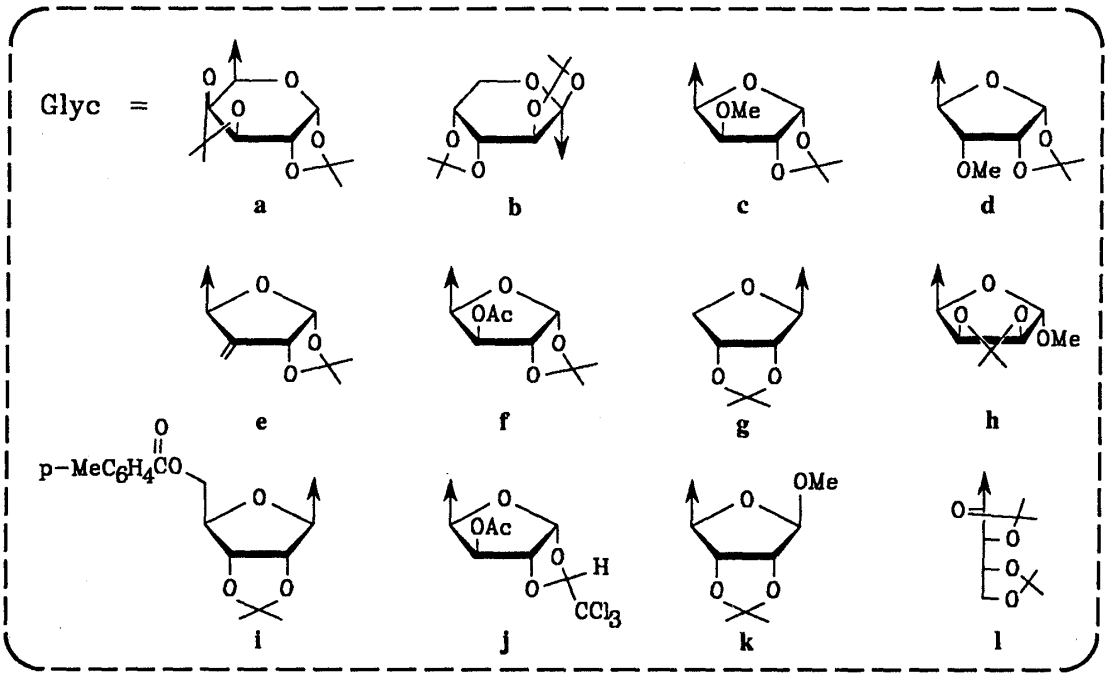
Compounds	Cytotoxic activity [a]	Activity against *Polyoma virus* [b]	Activity against *E. coli* MIC (μM)	Activity against *B. subtilis* MIC (μM)
3a	16.6	NA	83	40
3b	8.3	NA	83	41
3g	23.2	NA	116	56
3j	27.6	NA	>276[d]	69
3k	81.6	NA	102	51
3l	18.3	9.1 (PI)	91	11
4a	71.1	NA	89	44
4b	355.5	71.1 (PI)	>355[d]	53
4d	111	NA	>2220	>1776[d]
4e	48.3	4.8 (TI)	483	241
4g	128.1	64.0 (6h D)	640	640
4j	2[c]	----	>234	>234
4k	22.2	NA	444	444
4l	19.8	9.9 (PI)	>395	99
5b	128.5	64.2 (24h D)	80	19
5c	78.3	NA	98	98
7a	12[c]	----	>184[d]	>184
7b	229.7	NA	>230	57
7d	>263.6	NA	66	66
7i	5	2.5 (PI)	24	12
7j	40.3	20.1 (24h D)	>50[d]	25
7k	1.6[c]	----	211	>211
7l	245.5	NA	>123	>123[d]
8l	58.5	NA	117	58
10a	>120.4	NA	>241	>241
10j	5.2	NA	13	6

[a] Minimum cytotoxic concentration (μM) on 3T6 cells (morphological observation). [b] Cultivated on 3T6 cells: NA no activity, PI partial inhibition, TI total inhibition, D delay in the development relative to the untreated colony. [c] On COS cells. [d] At these concentrations, sporulation and/or a delay of more than 24 h was noted.

- atoxic compounds (**4d, 7b, 7d, 7l, 10a**),
- general toxic without antiviral selectivity (**10j**),
- general toxic with marginal antiviral selectivity (**7i**),
- compounds having some kind of selective toxicity with (**3l, 4l, 5b, 7j**) or without (**3b, 3j**) antiviral selectivity,
- compounds of low general toxicity with marginal (**4b, 4g**) or significant (**4e**) antiviral selectivity.

The only compound showing antipolyoma activity with an acceptable therapeutic index (**4e**) was submitted to a more detailed virological study following previously described procedures.[5] Growing mouse 3T6 cells were





Chart

exposed 48 h after seeding to different concentrations of **4e**, for either 1 or 24 h, and then labelled with ^{3}H-thymidine or ^{3}H-uridine. The presence of 3 μg/ml (14.4 μM) of **4e** during 1 h reduced ^{3}H-thymidine incorporation by about 60% and ^{3}H-uridine incorporation by 94%. With 10 μg/ml, the incorporation of both precursors was less than 3% of that of untreated control cultures. Treatment of the cultures during 24 h with 1 μg/ml had little inhibitory effect whereas with 3 μg/ml, incorporation of ^{3}H-thymidine and ^{3}H-uridine was less than 3% as compared to untreated cultures.

In another series of experiments, we tested the effects of compound **4e** on quiescent mouse kidney cells infected with *Polyoma virus*. In these cells, *Polyoma virus* induces a lytic infection, whereby mouse chromatin duplication parallels viral DNA replication.[6] By a selective extraction procedure, viral DNA can be separated from the high molecular weight mouse DNA and further purified by velocity sedimentation.[7] In one set of cultures, compound **4e** was present at a concentration of 1 μg/ml from 8 to 28 h after infection, and in another set at 3 μg/ml from 24 to 28 h after infection. The cultures were labelled with ^{3}H-thymidine from 28 to 30 h after infection. Radioactivity was determined in the supernatant fraction containing mostly viral DNA, as well as in the pellet fraction (mostly mouse DNA). While 1 μg/ml of **4e** had little effect on ^{3}H-thymidine incorporation, 3 μg/ml present for 4 h before the labelling period, reduced incorporation of ^{3}H-thymidine by about 70-75% in both the supernatant and the pellet fractions.[8] Velocity sedimentation of the supernatant fractions showed that radioactivity in the viral DNA peak was decreased by 97% when **4e** was present at 3 μg/ml and by 20% at a concentration of 1 μg/ml during 20 h, as compared to untreated infected cultures.

As compounds **4e**, **4l** and **7l** showed other antiviral properties and particularly anti-HSV-1 activity at concentrations slightly below the cytotoxicity threshold,[9] they constitute new leads to hopefully useful antiviral compounds. A molecular modeling program along this line is being undertaken.

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