Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1833–1838

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Potent and Selective Inhibition of Parainfluenza 1 (Sendai) Virus by New 6-Oxiranyl-, 6-Methyloxiranyluracils, and 4(3H)-Pyrimidinone Derivatives.

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Received 6 April 1998; accepted 12 June 1998

Abstract: Several new 6-oxiranyl-, 6-methyloxiranyluracils, and pyrimidinone derivatives, synthesized by the lithiation-alkylation sequence of 1,3,6-trimethyluracil, 1,3-dimethyl-6-chloromethyluracil, and 2-alkoxy-6-methyl-4(3H)-pyrimidinones, showed a potent and selective antiviral activity against the parainfluenza 1(Sendai) virus replication.

The parainfluenza viruses (enveloped viruses with nonsegmented negative-strand RNA genome) include important respiratory tract pathogens of infants and children, as well as important viruses of mammals and birds.[1] They were initially isolated from infants and children with lower respiratory tract diseases and were shown to be a major cause of croup as well as pneumonia and bronchiolitis.[2] As a group, parainfluenza viruses 1, 2, and 3 are second only to respiratory syncytial viruses as a cause of serious respiratory tract diseases in infants and children. Sendai virus (SV) was classified as the murine subtype of Parainfluenza virus 1. It has been used extensively in studies that have defined many of the basic biochemical and molecular biological properties of the paramyxoviruses. Moreover it has been employed as in *vivo* experimental model of parainfluenza virus infection, or for in *vitro* studies of new antiviral agents.[3]

To date, different substances such as: synthetic peptides,[4] protease inhibitors[5] or natural proteases,[6] that are capable of interfering with the different types of membrane fusion events[7] have been found to have an inhibitory effect on SV infections. Besides low-molecular weight compounds, the effects against SV of thapsigargin (a specific inhibitor of Ca^{2+}-ATPase),[8] ambazone (1,4-benzoquinone-guanylhydrazone-thiosemicarbazone),[9] dihydroheptaprenol,[10] and methylprednisolone acetate,[11] have also been investigated. However, the different products studied show varying degree of success as well as limitations due to a low selectivity and a specific antiviral treatment is not available at the moment.

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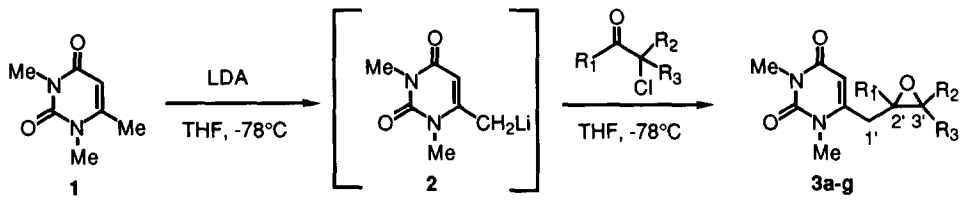
0960-894X/98/$19.00
PII: S0960-894X(98)00314-X

During the last few years there has been a growing interest in the biological activity of substituted pyrimidines,[12] and some uracil and pyrimidinone derivatives substituted either at C-5 and C-6 position have emerged in the field of antiviral chemotherapy.[13] Being involved in the synthesis and biological evaluation of new 6-substituted uracil and pyrimidinone derivatives,[14] and within a part of a project aimed to obtain selective modifications of nucleic acids and their components,[15] we have recently reported the synthesis of 5,6-disubstituted-5,6-dihydrouracil derivatives characterized by selective inhibitory activity against SV.[16] The present paper reports the synthesis of 6-oxiranyl- and 6-methyloxiranyluracil and pyrimidinone derivatives **3a-g**, **6a-c**, and **9a-d**, selective and potent inhibitors of SV replication in cultured cells as measured by decreased haemagglutinin units (HAU)[17] in the supernatant of the infected cells. To the best of our knowledge this is the first reported example of 6-substituted uracil and pyrimidinone derivatives strongly active against SV.

Chemistry

Our previous work on the synthesis of bipyrimidones and bipyrimidinylmethane derivatives showed that the metalation of 6-methyl-4(3H)-pyrimidinones takes place at the methyl in the 6-position in an essentially regiospecific manner.[18] This metalation-alkylation sequence was used for the preparation of several types of 6-substituted uracils.[19] In order to fully exploit the synthetic potential of this procedure we started to investigate the reaction of the lithium derivative of 1,3,6-trimethyluracil **1** with several acyclic and cyclic α-halogenoketones as electrophiles. The reaction of **2**, prepared from **1**[20] with 1.2 equiv. of lithiumdiisopropylamide (LDA) in THF at -78°C, with chloroacetone, 2-chloroacetophenone, 1-chloropinacolone, 3-chloro-2-butanone, 2-chlorocyclopentanone, and 2-chlorocyclohexanone afforded 6-methyloxiranyl uracil derivatives **3a-g** in acceptable yields (Scheme 1, Table).



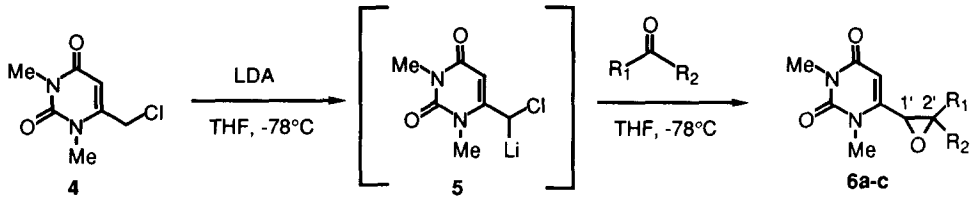
3a: R_1=Me, R_2=R_3=H. **3b**: R_1=Ph, R_2=R_3=H. **3c**: R_1=t-Bu, R_2=R_3=H. **3d**: R_1=R_3=Me, R_2=H. **3e**: R_1=R_2=Me, R_3=H. **3f**: R_1=R_2=-$CH_2CH_2CH_2$-, R_3=H. **3g**: R_1=R_2=-$CH_2CH_2CH_2CH_2$-, R_3=H.

Scheme 1

In the case of 3-chloro-2-butanone two possible diastereoisomers, compounds **3d** and **3e**, were obtained after chromatographic purification (ratio value **3d**:**3e**=1:5), and their stereochemistry was determined by a series of 1D-NMR NOE measurements. In particular, the proximity of the Me-2' and Me-3' protons in **3e** was

revealed by their mutual NOE effect (8.1%), suggesting the E-stereochemistry. In compound **3d** the Z-stereochemistry was confirmed by the lack of any detectable NOE effect between Me-2' and Me-3' protons.

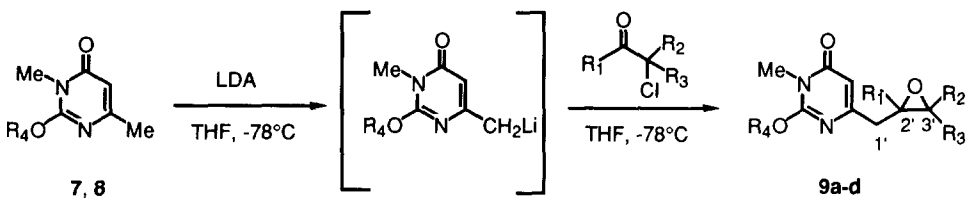
With the aim to apply the metalation-alkylation sequence for the synthesis of 6-oxiranyluracil derivatives, we studied the reaction of the lithium derivative of 1,3-dimethyl-6-chloromethyluracil **4** with carbonyl compounds as electrophiles. The reaction of **5**, prepared from **4**[21] with 1.2 equiv. of lithiumdiisopropylamide (LDA) in THF at -78°C, with acetophenone and benzaldehyde afforded compounds **6a-c** in acceptable yields (Scheme 2, Table). In compound **6a**, obtained as the only recovered product, the proximity of the Phe-2' and H-1' protons was revealed by their mutual NOE effect (7.5%) suggesting the E-stereochemistry. In the case of diastereoisomers **6b** and **6c** (ratio value **6b**:**6c**=1:1.2) the Z- and E-stereochemistry was assigned on the basis of the value of the coupling constant between vicinal H-1' and H-2' protons (8.3 Hz, and 1.9 Hz, respectively).



6a: R_1=Me, R_2=Ph. **6b**: R_1=H, R_2=Ph. **6c**: R_1=Ph, R_2=H.

Scheme 2

Finally, the metalation-alkylation sequence was applied to 2-*n*-propyloxy-3,6-dimethyl-4(3H)-pyrimidinone **7** and 2-cyclohexyloxy-3,6-dimethyl-4(3H)-pyrimidinone **8**,[22] in the presence of 2-chlorocyclohexanone, chloroacetophenone, and 3-chloro-2-butanone, to afford the 6-methyloxiranyl-4(3H)-pyrimidinone derivatives **9a-d** in yields ranging from 40% to 73% (Scheme 3, Table).



7: R_4=n-Pr. **8**: R_4=Cyclohexyl. **9a**: R_1= R_2=-$CH_2CH_2CH_2CH_2$-, R_3 =H, R_4=n-Pr. **9b**: R_1=Ph, R_2= R_3=H, R_4=n-Pr. **9c**: R_1=Ph, R_2= R_3=H, R_4=Cyclohexyl. **9d**: R_1=R_3=Me, R_2=H, R_4=Cyclohexyl.

Scheme 3

In compound **9d** the proximity of the Me-2' and H-3' protons was revealed by their mutual NOE effect (9%). It is interesting to note that the reported metalation-alkylation sequence was very selective. In fact, no

bipyrimidinylmethane derivatives were detected, showing that the possible addition/elimination reaction on the C-2 position of the 4(3H)-pyrimidinone ring,[19] was an uncompetitive reaction under these experimental conditions.

Biology

All new products[23] have been assayed for antiviral activity on parainfluenza 1 (Sendai) virus replication in Madin Darby canine kidney cells (MDCK cells) by the measure of the haemagglutinin units (HAU) in the supernatant of the infected cells, as described by Garaci et al.[17] Cytotoxicity tests were based on microscopic evaluation of cell morphology and viability (confluent MDCK cells) and on viability of proliferating mouse myeloma cells (NSO cells from American Tissue Culture Collection) and normal human lymphocytes stimulated with PHA (5 μg/ml). Viability was measured by using tritiated thymidine incorporation tests. Statistical evaluation of the results allowed us to calculate CC_{50} for both proliferating mouse myeloma cells and normal human lymphocytes. Most of compounds analysed showed interesting inhibitory activity, in particular, **3a-c**, **3f**, and **6a-b**, presented an ED_{50} lower than micromolar while that of **9a-d** was two order of magnitude less potent. Some of the compounds tested showed an associated toxic effect that, in the case of **3c**, **3f**, **6a**, and **6b**, was not microscopically detectable at the concentration range in which compounds have been found active.

Table: Activity against parainfluenza 1 (Sendai) virus and cytotoxicity of compounds **3a-g**, **6a-c**, and **9a-d**

compd.	ED_{50}, μM[a]	MTC, μM[b]	CC_{50}, μM[c]	Yield, %[d]
3a	0.24	0.48	4.0	48
3b	0.18	0.37	3.7	65
3c	0.59	>0.79	7.9	67
3d	>2.0	>2.0	89	20
3e	>2.0	>2.0	89	47
3f	0.42	>0.84	8.4	68
3g	>2.0	>2.0	40	83
6a	0.47	>0.81	7.3	56
6b	0.38	>0.80	7.7	43
6c	193	380	>380	35
9a	176	>352	>352	40
9b	16.6	330	>330	65
9c	147	294	>294	69
9d	171	342	>342	73

[a]Inhibitory concentration required to reduce virus-induced cytopathicity by 50%.

[b]Minimum toxic concentration required to cause a microscopically detectable alteration of normal cell morphology. The results listed are the mean values of three independent determinations.

[c]Concentration required to inhibit cell proliferation by 50%. [d] Yields referred to the synthesis of analyzed products.

On the basis of the above data the following structure-activity relationships can be tentatively reported: i) The N,N-dimethyluracil scaffold, very unusual for antiviral compounds, along with the C-6 substitution on the uracil ring, seems to be an important feature for active compounds. ii) The position, the substitution pattern, and the stereochemistry of the oxirane ring play an important role in modulating both the activity and the toxic effect of the products. In particular, 6-oxiranyl derivatives **6a**, and **6b**, are more active than the corresponding 6-methyloxiranyl derivatives. In the latter case the substitution at the terminal carbon, usually but not always, gives rise to less interesting compounds. The inactivity of **3d**, **3e**, and **3g**, in the range of concentration of the biological tests, are quite difficult to be rationalised in terms of stucture-activity relationships but some more work is in progress in our laboratory for a complete study of this new interesting class of parainfluenza 1 (Sendai) virus inhibitors.

Acknowledgement: Financial support from Italian MURST is acknowledged. One of us (M. Botta) wishes to thank for a grant from the Merck Research Laboratories (Academic Development Program in Chemistry).

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20. Compound **1** was prepared by reaction of commercially available 6-methyluracil with CH_2N_2 in MeOH at 25°C.
21. Compound **4** was prepared by reaction of commercially available 6-chloromethyluracil with CH_2N_2 in MeOH at 25°C.
22. Compounds **7** and **8** were prepared by trans-alkoxylation reactions starting from 2-methoxy-6-methyl-4(3H)-pyrimidinone and appropriate sodium alkoxylates as reported in: Botta, M.; De Angelis, F.; Finizia, G.; Gambacorta, A.; Nicoletti, R. *Synthetic Comm.* **1985**, 15, 27.
23. The structure of new compounds were determined by EI-MS, ^1H- and ^{13}C-NMR spectroscopy. All new compounds gave satisfactory (0.4% of the theoretical values) elemental analyses.