References

1 P. Valenti, P. Montanari, P. Da Re, G. Soldani and A. Bertelli, Arch. Pharm. (Weinheim) *313*, 280 (1980).
2 P. Giusti, M. Trevisan, L. Cima, P. Valenti and P. Montanari, Acta Anaesthesiol. Ital. *30*, 49 (1979).
3 G. Soldani, P. Valenti, P. Montanari and M. Recanatini, Acta Anaesthesiol. Ital. *30*, 77 (1979).
4 P. Valenti, P. Montanari, M.R. Gaion and P. Da Re, Communication presented at Fourteenth National Congress of S.C.I., Catania (Italy) 1981.
5 G.A. Alles and C.H. Ellis, J. Pharmacol. Exp. Ther. *104*, 253 (1952).
6 E. Bülbring and J. Wajda, J. Pharmacol. Exp. Ther. *85*, 78 (1945).
7 J.W. Lawson, J. Pharmacol. Exp. Ther. *160*, 22 (1968).
8 J.T. Litchfield and F. Wilcoxon, J. Pharmacol. Exp. Ther. *96*, 99 (1949).

[Ph 528]

Arch. Pharm. (Weinheim) *315*, 1007–1013 (1982)

Studies on Potential Antiviral Compounds, XXII***)

Synthesis and in Vitro Antiviral Activity of 1-(Hydroxyalkyl)-1*H*-benzimidazoles**

Laura Garuti+, Anna Ferranti+, Giuseppe Giovanninetti*)+, Giancarlo Scapini+, Loredano Franchi++ and Maria P. Landini++

+ Institut für Pharmazeutische Chemie der Universität, Via Belmeloro 6, I-40126 Bologna;
++ Institut für Mikrobiologie der Universität, Via Massarenti 9, I-40126 Bologna

Eingegangen am 20. November 1981

A series of 1-(hydroxyalkyl)-1*H*-benzimidazoles has been prepared and screened in vitro for activity against herpes simplex virus, type 2 (DNA) and poliovirus type 1 (RNA). 5,6-Dichloro-1-[2-(2-hydroxyethoxy)ethyl]-1*H*-benzimidazole (**9**, Table 1) was the most significant compound.

Potentiell antivirale Verbindungen, 22. Mitt.: Synthese und antivirale In-vitro-Aktivität von 1-(Hydroxyalkyl)-1*H*-benzimidazolen

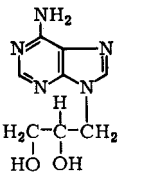
Einige (1-Hydroxyalkyl)-1*H*-benzimidazole wurden hergestellt und in vitro gegen Herpes simplex Typ 2 (DNA) und Poliovirus Typ 1 (RNA) geprüft. Als wirksamste Verbindung zeigte sich 5,6-Dichlor-1-[2-(2-hydroxyethoxy)ethyl]-1*H*-benzimidazol (**9**, Tab. 1).

** Dem italienischen Consiglio Nazionale delle Ricerche danken wir für die Unterstützung unserer Arbeit.

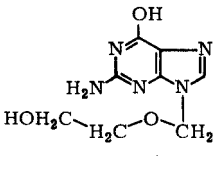
0365-6233/82/1212-1007 $ 02.50/0

(*S*)-9-(2,3-dihydroxypropyl)-adenine **(1)** [(*S*)-DHPA[1]] and 9-(2-hydroxyethoxymethyl)-guanine **(2)** [acycloguanosine[2,3]], two nucleoside analogues in which the cyclic carbohydrate moiety was replaced by an acyclic side chain, have recently been reported to possess marked antiviral activity in both cell culture systems and animal models. In cell cultures, (*S*)-DHPA inhibited the replication of several DNA and RNA viruses, but the mechanism of antiviral action has not yet been elucidated[4,5], whereas acycloguanosine showed a selective activity against herpes simplex virus (HSV-2), as a result of inhibition of virion-associated enzymes[3-6]. The biological properties exhibited by the above compounds encouraged further investigation on the acyclic nucleosides[7-12], which might also be interesting antitumoral agents[13,14]. In extensive studies on (*S*)-DHPA analogues, the broad-spectrum antiviral activity was dependent on the nature of the aliphatic side chain as well as the adenine base[8].

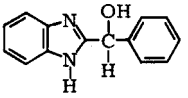
These findings and the fact that benzimidazoles and the purines have the same bicyclic skeleton with respect to size and a common imidazole ring[15] prompted us to synthesize the 1-(hydroxyalkyl)-1*H*-benzimidazoles (compounds **5–16**, table 1), described in this report, for evaluation as antivirals. Moreover, it is well known that interesting antiviral agents contain a benzimidazole moiety[15,16], though no clinical trial has been reported owing to a decreased in vivo activity or a limited discrimination between viral and host cell functions[15,17]. According to the procedure for the design of drugs incorporating two biological activities in one entity by chemical hybridization[18-22], the combination of benzimidazoles which are essentially RNA virus inhibiting with a hydroxyalkyl chain might afford nucleoside analogues possessing broad-spectrum antiviral properties and lower cell toxicity than reference compounds **1, 3, 4** (Fig. 1).



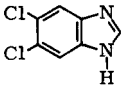
1



2



3



4

1-(Hydroxyalkyl)-1*H*-benzimidazoles (**5–16**, Table 1) were prepared by treating the appropriate benzimidazole in refluxing DMF with the hydroxyalkyl halide in the presence of sodium hydride. The reaction did not proceed satisfactorily when carried out in other solvents and in the presence of other bases (NH_3, NaOH, KOH). The procedure involving the benzimidazole sodium salt[23] in various solvents at different temperatures was also unsatisfactory.

The proposed structures and the N-1 alkylation are confirmed by ^{1}H-NMR spectra of the main compounds in the series, also in comparison with the spectra of the starting benzimidazole A and of its derivatives **3, 4** (see Experimental, Table 2).

Discussion

Nine out of twelve synthesized compounds inhibited HSV-2 replication in vitro (Table 1). This antiherpes effect, which has not been previously found in benzimidazole derivatives[16], is fairly significant for compounds **7** and **9** (1,30 $\log_{10}$ units: 95 % inhibition). On the contrary, a decreased activity in poliovirus growth inhibition by benzimidazoles[15] was observed, whereas that of 2-(α-hydroxybenzyl)-benzimidazole was enhanced by N-1 alkyl substitution in the benzimidazole moiety[16].

For the short series of benzimidazoles examined there was no preferential activity against either of the two viruses tested (Table 1). 5,6-Dichloro-1-[2-(2-hydroxyethoxy)-ethyl]-1*H*-benzimidazole **(9)** was the most significant compound, with 95 % inhibition of herpes virus replication associated with 86 % poliovirus growth inhibition at 50 μg/ml.

The greatest anti-poliovirus activity (1,30 $\log_{10}$ units = 95 % inhibition at 50 μg/ml) was displayed by 1-(3-hydroxypropyl)-2-(α-hydroxybenzyl)-benzimidazole **(14)**, which however, proved ineffective in inhibiting HSV-2 growth.

Experimental

Elemental analyses: Laboratory for Microanalysis of the Faculty of Pharmacy of the University of Pisa, Italy. *Mp's*: not corr.: Electrothermal Melting Point Apparatus. *Thin layer chromatography*: Baker-Flex Silica Gel IB2-F, ethyl acetate 95 % ethanol 9:1 v/v.

IR spectra: Perkin-Elmer 177 spectrophotometer (nujol mull technique). Spectra agreed with the proposed structures. *^{1}H-NMR spectra* (see Table 2): Varian EM-390 NMR spectrometer.

Microbiology. – The antiviral activities of the benzimidazoles in Table 1 were tested against a deoxyribovirus (herpes simplex-virus type 2) and a ribovirus (polio-virus type 1) using the cell system of human KB tumor cells.

The cell monolayers were infected with a virus suspension at a multiplicity ranging from 0,01 to 0,001 plaque-forming units (PFU) per cell. The cultures were incubated for 24 h in Eagle's maintenance medium, without calf serum, containing the compounds to be tested at the concentration reported in Table 1.

The infected cultures were frozen and thawed three times, then centrifuged. The supernatant was serially diluted and the viral titers were assayed on the cell monolayers by an ordinary plaque technique.

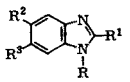
The activity of each compound was tested in triplicate cell cultures; infected cell cultures, incubated for 24 h in Eagle's maintenance medium without serum, were used as control.

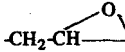
1-(Hydroxyalkyl)-1H-benzimidazoles **5–16**, *(Table 1)*

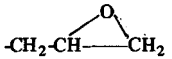
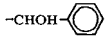
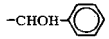
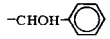
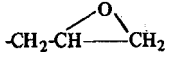
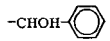
0.60 g (25 mmol) of NaH was added to a solution of 6.4 mmol of 1*H*-benzimidazole or one of its derivatives (compounds* **3** and **4**; fig. 1) in 20 ml anhydrous DMF. After the mixture was stirred at room temp. for 1 h, the appropriate hydroxyalkyl halide* (bromide or chloride, 84 mmol) was added and the mixture was heated with refluxing for the times listed in Table 1. After being cooled to room temp. the mixture was filtered and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column using ethyl acetate 95 % ethanol for elution.

* commercial products

Table 1: *1-(Hydroxyalkyl)-1H-benzimidazoles*



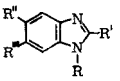
Comp.	R	R^1	R^2	R^3	Formula	Calc.	Found.	MP°	*** crystn solvent	yield %	reflux time: h	Growth Inhibition (log_{10} units) **** conc µg/ml	VHS-2	Poliovirus type-1
5*	$-CH_2-CH_2-O-CH_2-CH_2OH$	H	H	H	$C_{11}H_{14}N_2O_2$	C 64,1 H 6,84 N 13,6	64,1 6,66 13,1			34	6	50	0,90	0,15
6**	$-CH_2-CH_2-CH_2OH$	H	H	H	$C_{10}H_{12}N_2O$	C 68,2 H 6,86 N 15,9	67,8 6,63 15,8	54	A	27	10	50	0,42	0,15
7	$-CH_2-CHOH-CH_2OH$	H	H	H	$C_{10}H_{12}N_2O_2$	C 62,5 H 6,29 N 14,6	62,0 6,47 14,1	60	A	57	14	50	1,30	0,37
8		H	H	H	$C_{10}H_{10}N_2O$	C 68,9 H 5,78 N 16,1	68,5 6,13 15,8	52	B	33	10	50	0,60	0,15
9	$-CH_2-CH_2-O-CH_2-CH_2OH$	H	Cl	Cl	$C_{11}H_{12}Cl_2N_2O_2$	C 48,0 H 4,39 N 10,2	48,4 4,73 9,8	70–72	A	50	8	10	1,30	0,84
10	$-CH_2-CH_2-CH_2-OH$	H	Cl	Cl	$C_{10}H_{10}Cl_2N_2O$	C 49,0 H 4,11 N 11,4	49,6 4,18 11,0	98–100	A	54	12	5	0,30	0,30

11	$-CH_2-CHOH-CH_2OH$	H	Cl	Cl	$C_{10}H_{10}Cl_2N_2O_2$	C 46,0 H 3,86 N 10,7	45,8 4,29 10,3	156–157	B	60	9	25	0,35	0,30
12		H	Cl	Cl	$C_{10}H_8Cl_2N_2O$	C 49,4 H 3,31 N 11,5	49,7 3,07 11,2	126	A	57	10	25	0,60	0,82
13	$-CH_2-CH_2-O-CH_2-CH_2-OH$		H	H	$C_{18}H_{20}N_2O_3$	C 69,2 H 6,45 N 9,0	69,6 6,75 9,1			32	15	50	0,35	0
14	$-CH_2-CH_2-CH_2-OH$		H	H	$C_{17}H_{18}N_2O_2$	C 72,3 H 6,42 N 9,9	72,6 6,83 10,1	143–145	B	40	10	50	0	1,30
15	$-CH_2-CHOH-CH_2OH$		H	H	$C_{17}H_{18}N_2O_3$	C 68,4 H 6,08 N 9,4	68,9 6,44 8,8	86	B	51	14	25	0	0,40
16			H	H	$C_{17}H_{16}N_2O_2$	C 72,1 H 5,75 N 10,1	71,9 6,04 9,9	124–125	B	47	15	25	0	0,35

* amorphous, ** literature: liquid, BP = 186–190/0,8 Torr[24], *** A = 95 % ethanol-diethyl ether;
B = 95 % ethanol-ethyl acetate, **** maximum subtoxic concentrations

Solvents of crystallization and melting points of the compounds are listed in Table 1. The ^{1}H-NMR data of the most active products and the starting benzimidazoles **A**, **3** and **4** in CD_3OD are reported in Table 2.

Table 2: – (*) *^{1}H-NMR data in CD_3OD*



Comp..	R	R'	R''	R'''	$\delta_{R'}$	δ arom.	other signals
A	H	H	H	H	8.15s	7.1–7.8m(4H)	– – – – – – – – – – –
5	-$(CH_2CH_2O)_2$H	H	H	H	8.16s	7.1–7.8m(4H)	3.74(t,CH_2OH); 4.32 (t,N-CH_2); 3.3–3.7(m, CH_2-O-CH_2).
7	-CH_2CH(CH_2OH)OH	H	H	H	8.10s	7.0–7.8m(4H)	3.54(d,CH_2OH); 3.8–4.6(m,N-CH_2-CH).
4	H	H	Cl	Cl	8.19s	7.71s(2H)	– – – – – – – – –
9	-$(CH_2CH_2O)_2$H	H	Cl	Cl	8.13s	7.74s(1H); 7.80s(1H)	3.81(t,CH_2OH); 4.39 (t,N-CH_2); 3.4–3.7(m, CH_2-O-CH_2).
3	H	-CH(OH)-C_6H_5	H	H	– –	7.0–7.6m(9H)	5.98(s, CH-Ph)
14	-$(CH_2)_3$OH	-CH(OH)-C_6H_5	H	H	– –	7.1–7.8m(9H)	3.47(t,CH_2OH); 4.26 (t,N-CH_2); 6.25(s, CH-Ph); 1.4–2.1(m, C-CH_2-C).

(*) The OH of all the compounds exchanges with H_2O of the solvent. Chemical shifts are in ppm (δ; ± 0.01) from TMS as int. stand.

References

*** XXI: L. Garuti, A. Ferranti, G. Giovanninetti, M. Baserga and A. Mannini Palenzona, Farmaco Ed. Sci. *36*, 862 (1981).

1 E. De Clerq, J. Descamps, P. De Somer and A. Holy, Science *200*, 563 (1978).

2 H.J. Schaeffer, L. Beauchamp, P. de Miranda, G.B. Elion, D.J. Bauer and P. Collins, Nature (London) *272*, 583 (1978).

3 P.J. Roberts, Drugs Fut. *5*, 573 (1980).

4 E. De Clerq and P.F. Torrence, J. Carbohydr. Nucleosides Nucleotides *5*, 187 (1978).

5 W.E.G. Muller, Antibiot. Chemother, (Washington D.C.) *27*, 139 (1980).

6 D.I. Edwards in Antimicrobial Drug Action, Macmillan Press LTD, London 1980.

7 A. Holy, Collect. Czech. Chem. Commun. *43*, 3103 (1978).

8 E. De Clerq and A. Holy, J. Med. Chem. *22*, 510 (1979).
9 K.K. Ogilvie and M.F. Gillen, Tetrahedron Lett. *21*, 327 (1980).
10 R. Busson, L. Colla and H. Vanderhaeghe, Abstrs. VII Int. Symposium on Med. Chem., September 2–5, Torremolinos (Malaga), 1980.
11 J.L. Kelley, M.P. Krochmal and H.J. Schaeffer, J. Med. Chem. *24*, 472 (1981).
12 J.L. Kelley, J.E. Kelsey, W.R. Hall, M.P. Krochmal and H.J. Schaeffer, J. Med. Chem. *24*, 753 (1981).
13 D. Horton and R.A. Markovs, Carbohydr. Res. *80*, 263 (1980).
14 D. Horton and S.S. Kokrady, Carbohydr. Res. *80*, 364 (1980).
15 P.B. Sehgal and I. Tamm, Antibiot. Chemother. (Washington D.C.) *27*, 93 (1980).
16 I. Tamm and L.A. Caliguiri in International Encyclopedia of Pharmacology and Therapeutics, p. 115, D.J. Bauer Ed., Pergamon Press, Oxford 1972.
17 D.L. Swallow, Prog. Drug Res. *22*, 281 (1978).
18 J.J. Baldwin and coworkers, J. Med. Chem. *22*, 1285 (1979).
19 J.C. Jamoulle, C.L. Lapiere and J. Bourdais, Ann. Pharm. Fr. *37*, 331 (1979).
20 F. Sparatore, F. Savelli and G. Cordella, Farmaco Ed. Sci. *35*, 735 (1980).
21 G. Metz and M. Specker, Arzneim. Forsch. *30*, 1034 (1980).
22 R.G. Smith, R.A. Lucas and J.W.F. Wasley, J. Med. Chem. *23*, 952 (1980).
23 F. Benigni and L. Trevisan, Farmaco Ed. Sci. *29*, 936 (1974).
24 O. Ehrmann, F. Zimmermann and L. Friedrich, Ger. Offen. 2,300,018 (cl. C07d, A 61 K), 04 Jul. 1974, Appl. P 2300018.2, 02 Jan. 1973; C. A. *81*, 120632b (1974).

[Ph529]

Arch. Pharm. (Weinheim) *315*, 1013–1019 (1982)

Elektronenspektren – Halochromie – kationischer „push-pull"-Molekülsysteme im Vergleich zu den UV/VIS-Absorptionen überbrückter [10]-Annulenyl-1.3-benzodithiolyl-Kationen

Richard Neidlein*)[1] und Hartmut Zeiner[2]

Pharmazeutisch-Chemisches Institut der Universitäten Karlsruhe (TH) und Heidelberg, Im Neuenheimer Feld 364, D-6900 Heidelberg
Eingegangen am 23. November 1981

Es werden die UV/VIS-Spektren verschiedener kationischer „push-pull"-Molekülsysteme mit den UV/VIS-Absorptionen neuer überbrückter [10]-Annulenyl-1.3-benzodithiolyliumkationen verglichen und diskutiert.

Electron Spectra and Halochromy of Cationic "Push-Pull" Systems in Comparison with the U.V./VIS Absorptions of Bridged [10]Annulenyl-1,3-benzodithiolium Cations

UV/VIS spectra of cationic "push-pull" systems are discussed and compared with the absorptions of new bridged [10]annulenyl-1,3-dithiolylium cations.

0365-6233/82/1212-1013 $ 02.50/0