2-Arylbenzimidazoles as Antiviral and Antiproliferative Agents-Part 1

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Abstract: Being involved in an anti-Flaviviridae Project, and because of the role played by benzimidazole derivatives as promising inhibitors of the HCV helicase and RNA polymerase, as well as of the Zn finger transcription factor, we synthesized a new series of 2-arylbenzimidazoles and evaluated them for antiviral activity, as well as for antiproliferative activity.

Compounds were tested in cell-based assays against viruses representative of: i) two of the three genera of the *Flaviviridae* family, i.e. *Flaviviruses* and *Pestiviruses*; ii) other RNA virus families, such as *Retroviridae, Picornaviridae, Paramyxoviridae, Rhabdoviridae and Reoviridae;* iii) two DNA virus families (*Herpesviridae and Poxviridae*). Compounds **15**, **28** and **29** resulted moderately active only against Yellow Fever Virus (a *Flavivirus*) (range 6-27 μM), whereas none of the title benzimidazoles showed any antiviral activity at concentrations not cytotoxic for the resting cell monolayers.

Compounds were also tested for antiproliferative activity against a panel of exponentially growing cell lines derived from human haematological and solid tumors. Several new benzimidazoles turned out active. Among them, compound **27** was the most potent against human haematologic and solid tumor cells and turned out to be as potent as Etoposide and more potent than 6-mercaptopurine (6-MP), used as reference antitumor agents.

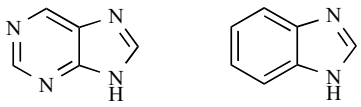
Key Words: Antiviral activity, flaviviridae, antiproliferative activity, arylbenzimidazoles.

INTRODUCTION

Benzimidazoles represent a class of heterocycles which are receiving much interest in the field of anti-infective therapies. Up to 1980 the most extensively marketed benzimidazoles spanned from pharmaceuticals (mainly human and veterinary antihelmintic, a few antineoplastic, antihistaminic, vasodilator and psychoactive agents), insecticides to fungicides [1]. After 1980 benzimidazole derivatives have emerged as a new class of commercially successful antiulcer agents. Among these, Omeprazole (AstraZenecka) is still on the top ten worldwide marketed drugs. A special attention was dedicated to benzimidazole derivatives as promising antiviral agents [2].

Benzimidazole (**1**) (Fig. **1**), in fact, may be considered a 1,3-dideazapurine and may replace this base in the process involving formation of nucleosides in viral replication.

Variously benzene ring substituted benzimidazoles, bearing a carbocyclic ring at position 1 instead of a normal sugar, have been considered as nucleoside analogues of purines. For example, compounds of structure **2** are reported to be active against hepatitis B virus at 0.74 μM [3], while compound **3** exhibited an *in vitro* inhibiting activity against both DNA and RNA virus [2].



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1,3-dideazapurine or benzimidazole

Fig. (1). Structure of purine and benzimidazole (**1**).

Successively, compounds **4** and **5** revealed to be 100-fold more potent [4] against the same strain of virus (Fig. **2**).

However, emerging strategies using benzimidazole derivatives as antiviral agents broke through after the discovery of hepatitis C virus (HCV) and identification of its genome that took advantage of enormous efforts spent in both disclosing and fighting the HIV replication phases. Since chronic infections with hepatitis C virus is a serious worldwide public health problem, the demand for effective HCV antiviral therapeutics is at present very high. Thus, a recent review has defined the status of the research in this field, where benzimidazole derivatives play an important role as promising anti-HCV agents [5]. Viropharma patented a series of compounds of formula **6-9** as inhibitors of NS3 helicase, with IC_{50} ranging from 0.7-10 μM [6] (Fig. **3**).

Two similar series of benzimidazole derivatives were patented independently by Japan Tobacco, Bristol-Meyers-Squibb and among these compounds **10** [7] and **11** [8] were reported as strong inhibitors of NS5B RNA polymerase. More recently, Japanese Authors described compound **12** [9]

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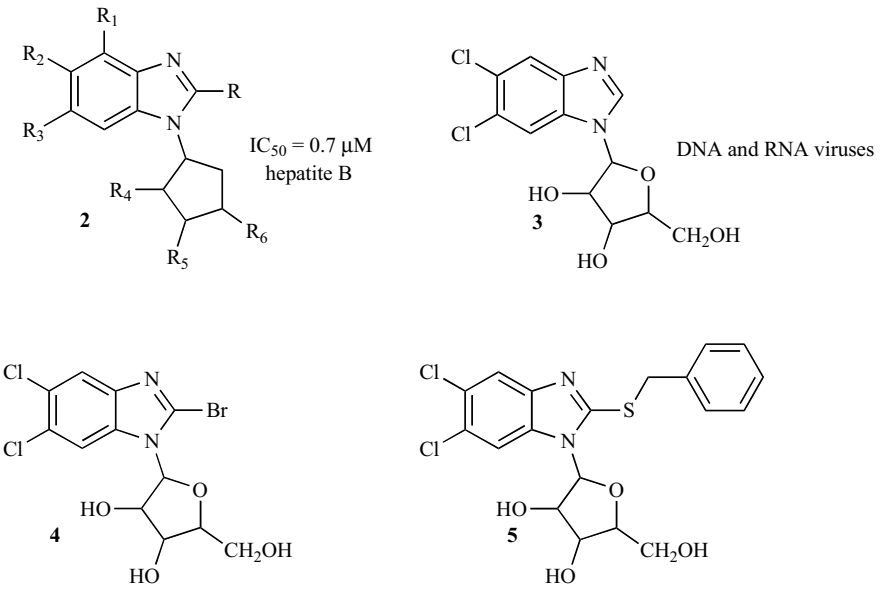


Fig. (2). Benzimidazoles active as antiviral agents.

as an improvement of this type of NS5B RNA polymerase inhibitors (Fig. **4**).

Our interest in the description of the biological and pharmacological properties of benzimidazoles started long time ago, when we reported analgesic-antinflammatory [10, 11] and choleretic [12,13] activities, as well as a chloropromazine-like activity in the conditioned avoidance response [14,15]. In the last five years several teams coordinated by one of us (P. La Colla) have been involved in a national MIUR program aimed at the development of novel anti-HCV agents. Within this project, two groups belonging to different Universities (Genova and Sassari, Italy) jointly afforded a program for the preparation of HCV non-nucleoside inhibitors based on the benzimidazole structure. Thus, taking into account the recent disclosure of their activity as inhibitors of

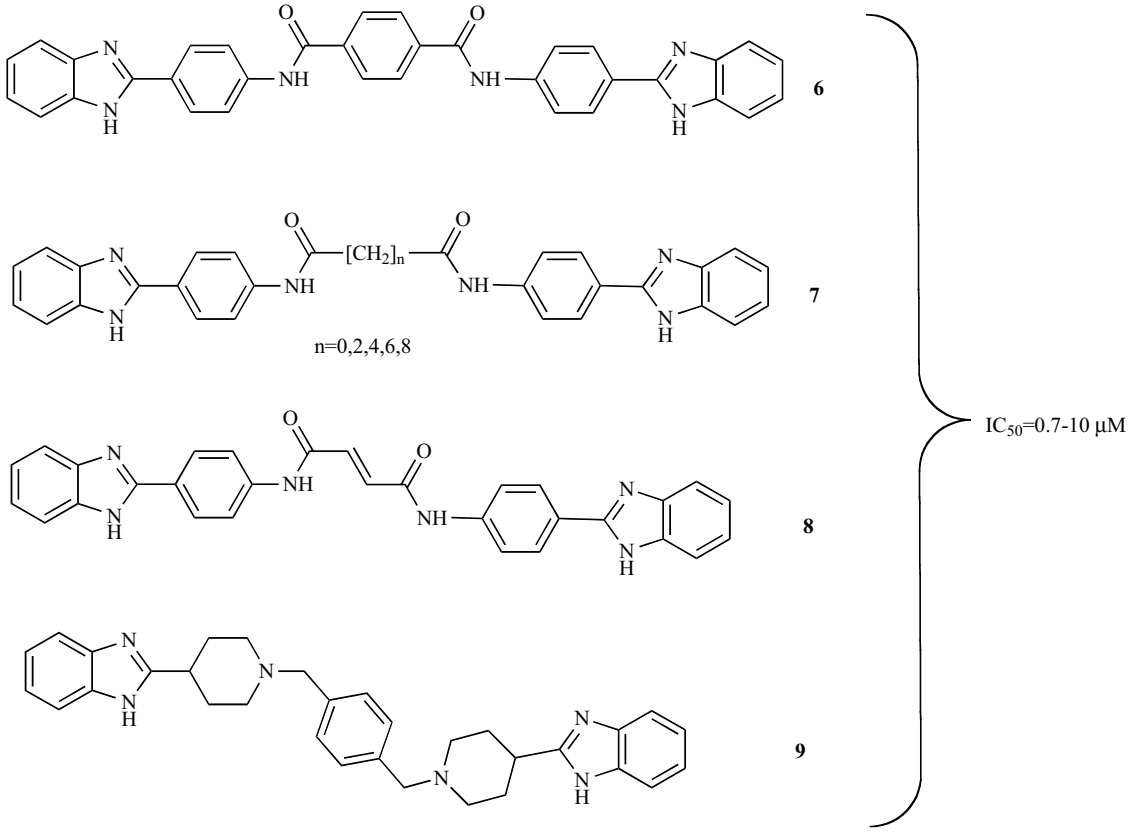
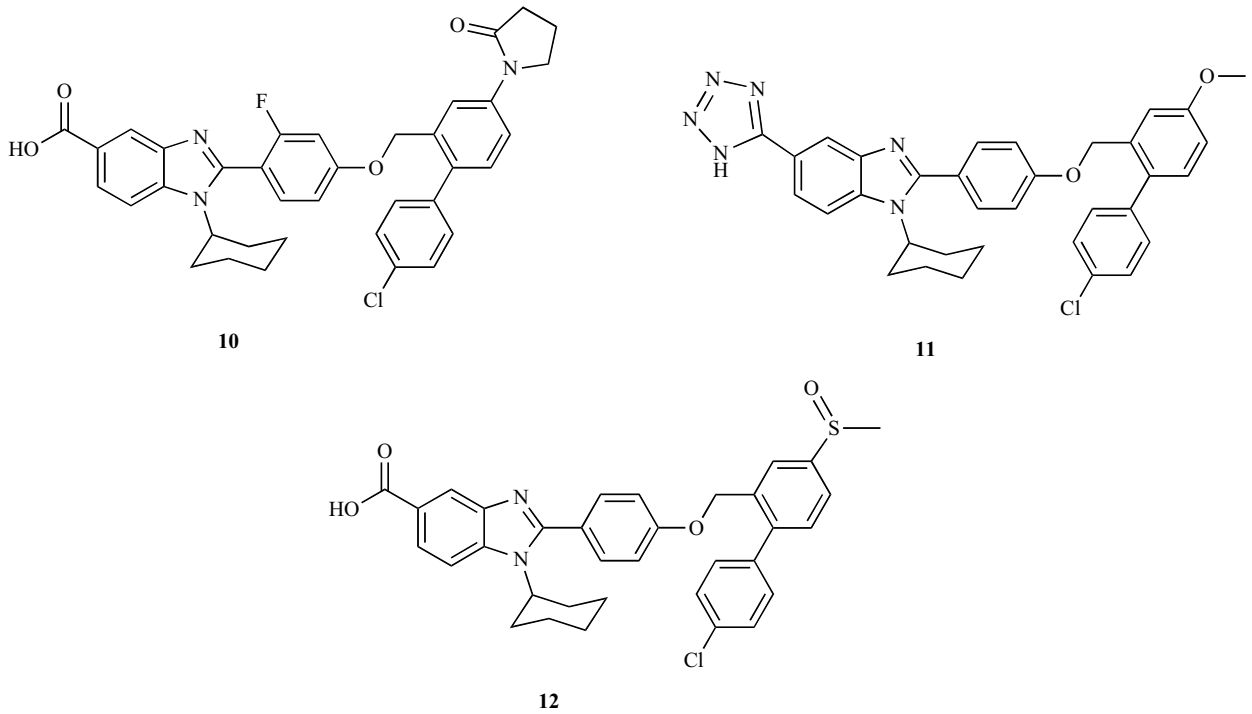


Fig. (3). Benzimidazoles patented as inhibitors of NS3 helicase.



10 **11** **12**

Fig. (4). Benzimidazoles patented as NS5B RNA polymerase inhibitors.

the HCV helicase and RNA polymerase [6-9], our group considered that part of this program could be oriented towards a further development of the benzimidazoles chemistry.

In this context, two convergent strategies were developed. On the one hand, we synthesized compounds of structure **A** (Fig. **5**), derived from a series previously described by Sparatore *et al*. [10,11,14,15] of which we designed homologues of 2-phenylbenzimidazoles by inserting, at position 2, "spacers" such as a 4'-substitued-diphenyle, a -styryl substituent or, simply, an α-naphtyl group. On the other hand, we extended the series of 5(6)-substitued–(1*H*) or 1-alky-2-benzyl and/or 2-phenylbenzimidazoles developed at the Genova University by Boido *et al*. [16], by synthesizing the compounds of structure B (Fig. **5**).

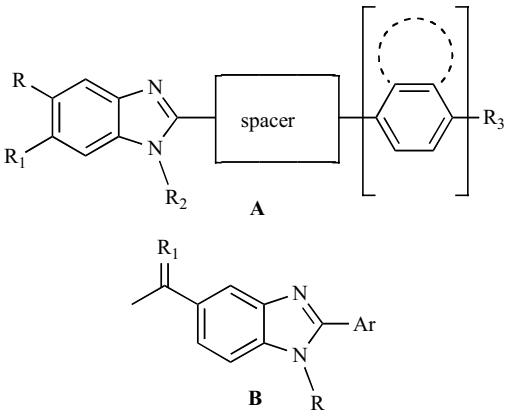


Fig. (5). General Structure of the designed drugs.

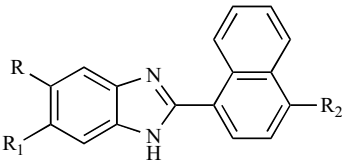
In this paper we report the preparation and the biological activity of compounds **13-36** of Fig. (**6**), as part of the above described program, in order to select novel potential leads against the cited targets and discover a possible antiproliferative activity. The naphtylbenzimidazoles are new compounds and represent the simplest case of homologation of 2-phenylbenzimidazoles. The effect of a bulkier substituent at position 2 of heteroring has not been so far considered in this class of compounds and the extra phenyl ring might result oriented preferentially towards or against the most prototropic stable imidazole form. Conformational analysis of compound **13**, using montecarlo method, confirms that three minima were obtained and the two rings are not coplanar. At the lowest energies the naphtyl group is almost planar as shown in Fig. (**7**).

It is worth noting that compound **13** has been recently described as an example of a small-molecule switch for zinc finger transcription factor and its inhibitory activity has been reported [17]. Because of the above indication, we deemed important to check whether any antiproliferative activity might be shown by derivatives with this particular structure.

CHEMISTRY

The preparation of 2-[naphtalen-1-yl]-1*H*-benzimidazoles (**13-36**) has been accomplished according to Scheme (**1**).

The diamines **a-f** were condensed with bisulphite compounds **g** and **h** according to the procedure described by Ridley *et al*. [18] to give the desired derivatives **13-24** in good yield (Table **1**). The nitro derivatives **19-24** were in turn converted into amines by reduction with hydrazine in the presence of palladium as catalyst. The primary amines **25-30** were then acetylated with acetic anhydride. The yields recorded in the last steps were from very high to moderate. Elucidation of the structures of prepared compounds were achieved by the whole of the elemental analyses and spectro-



13-36

Compd.	R	R_1	R_2
13	H	H	H
14	CH_3	H	H
15	CH_3	CH_3	H
16	Cl	H	H
17	Cl	Cl	H
18	CF_3	H	H
19	H	H	NO_2
20	CH_3	H	NO_2
21	CH_3	CH_3	NO_2
22	Cl	H	NO_2
23	Cl	Cl	NO_2
24	CF_3	H	NO_2
25	H	H	NH_2
26	CH_3	H	NH_2
27	CH_3	CH_3	NH_2
28	Cl	H	NH_2
29	Cl	Cl	NH_2
30	CF_3	H	NH_2
31	H	H	$NHCOCH_3$
32	CH_3	H	$NHCOCH_3$
33	CH_3	CH_3	$NHCOCH_3$
34	Cl	H	$NHCOCH_3$
35	Cl	Cl	$NHCOCH_3$
36	CF_3	H	$NHCOCH_3$

Fig. (6). General formula of the compounds synthesized.

scopic data (Table **1**). From the spectroscopic point of view it is interesting to note the differences existing in the UV spectra between the analogues 2-phenyl-1*H*-benzimidazoles described by some of us [13] and the 2-naphtyl derivatives of Table (**1**). 2-Phenylbenzimidazoles possess fine structure whereas the presence of a naphtyl ring at the same position causes the appearance of a broad intense band in the range of 309-289 nm for **13-18** and 327-306 nm for **30-36.** In the case of 2-[4-nitronaphtalen-1-yl]-*1H*-benzimidazoles (**19-24**) the maxima descend to a flat band at higher wavelength 358-323 nm because of conjugation with the nitro group. Compounds (**25**-**28**) exhibited three set of maxima around 332-325, 284-279 and 234-232 nm, while in compounds **29** and **30** the

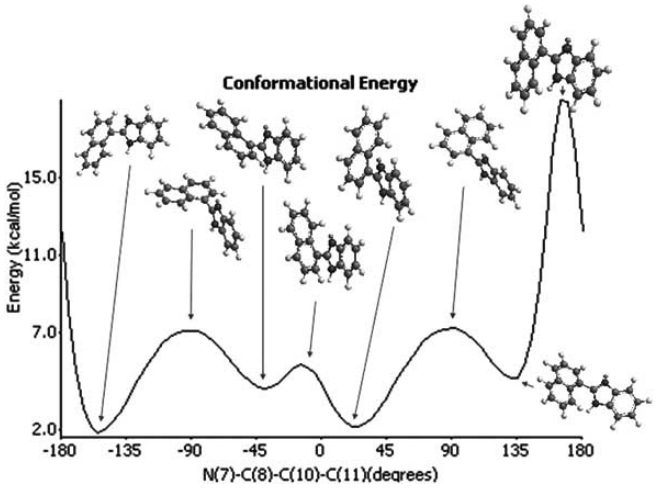


Fig. (7). Energy minimization profile of compound **13**.

band around 280 nm decreases and the most intense band is batochromically shifted at 342 nm.

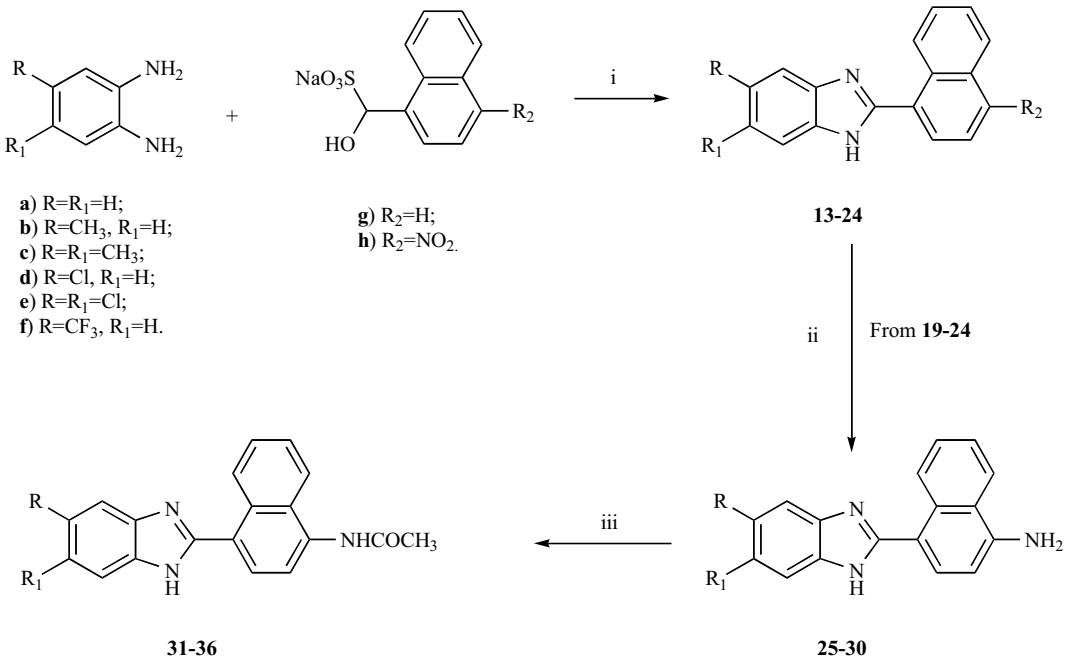
RESULTS AND DISCUSSION

As reported in Table (**2**), title compounds were evaluated *in vitro* against $ssRNA^+$ viruses representative of the *Flaviviridae* and *Picornaviridae* families. In particular, viruses representative of two of the three genera of the *Flaviviridae* family, [i.e., YFV (*Flaviviruses*) and BVDV (*Pestiviruses*), as HCV (*Hepacivirus*) can hardly be used in routine cell based assays], and of one genus of the *Picornaviridae* family [CVB-2 and Polio 1 (*Enteroviruses*)] were tested. Compounds were also tested against an additional $ssRNA^+$ virus [Human immunodeficiency virus (HIV-1)], and representatives of dsRNA viruses [*Reoviridae* (Reo-1)] and $ssRNA^-$ viruses, *Paramyxo* [respiratory syncytial virus (RSV)] and *Rhabdo* [vesicular stomatitis virus (VSV)]. Two representatives of DNA virus families were also included: HSV-1 (*Herpesviridae*) and VV (*Poxviridae*).

As far as the antiviral activity is concerned, compounds **15**, **28** and **29** resulted moderately active against the sole YFV (range 6-27 μM). In particular, against this virus **15** resulted only 6-fold less potent than the reference nucleoside analogue 2'-C-Me-guanosine (NM108). The other benzimidazole derivatives showed activity neither against HIV-1, nor against representatives of dsRNA (Reo) and $ssRNA^-$ viruses (RSV or VSV) (data not shown) at not cytotoxic concentrations.

The benzimidazole derivatives (**15, 16, 18, 21, 22, 24, 27, 28, 30, 33, 34, 36)** which showed some cytotoxicity for resting cell monolayers were evaluated against a panel of exponentially growing human cell lines derived from haematological (CCRF-CEM, WIL-2NS and CCRF-SB) (Table **3**) or solid tumours (SK-MEL-28, MCF7, SK-MES-1, HepG2 and DU145) (Table **4**). They were also evaluated against cell lines (MRC-5 and CRL7065) derived from normal human tissues (Table **5**). In these assays, etoposide and 6-mercaptopurine (6-MP) were used as reference drugs.

The data reported in Tables (**3-5**) show that compound **27** was the most active against both haematological and solid



Scheme (1). Synthesis of 5-mono- or 5,6-di- substituted -2-(4-R_2-naphtyl-1-yl)-1*H*-benzimidazoles (**13-36**).

tumour cells, resulting as potent as etoposide and more potent than 6-MP against all the exponentially growing cell lines tested.

In the series of compounds examined it seems evident that substituents in both benzimidazole and naphthalene rings affect cytotoxicity. In the set of compounds which are unsubstituted at the 4' position of the naphthalene moiety, the presence of two methyl electron-releasing groups at positions 5 and 6 of the heterocycle (compound **15**) strongly influence the activity (0.06 μM), which is equivalent to that of compound **27,** bearing an amino group at 4' position. The nitro-precursor **21** is 5-fold less potent, while its acetyl derivative (**32**) is 26-fold less potent. Among the other substituents at the benzimidazole ring, the dichloro and trifluoromethyl groups are the least cytotoxic. This behaviour was also observed in the antiproliferative activity (Tables **3** and **4**).

EXPERIMENTAL SECTION

General Remarks

Melting points were carried out with a Köfler hot stage or Digital Electrothermal melting point apparatus and are uncorrected. Infrared spectra were recorded as nujol mulls on NaCl plates with a Perkin-Elmer 781 IR spectrophotometer and are expressed in ν (cm^{-1}). UV spectra are qualitative and were recorded in nm for solutions in EtOH with a Perkin-Elmer Lamba 5 spectrophotometer. Nuclear magnetic resonance (^{1}H-NMR) spectra were determined in $CDCl_3$, DMSO-d_6, $CDCl_3$/DMSO-d_6 (in the ratio 1:3) and were recorded in a Varian XL-200 (200 MHz). Chemical shifts (δ scale) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard. Splitting patterns are designated, as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; br s, broad singlet; dd, double doublets.

The assignment of exchangeable protons (OH and NH) was confirmed by the addition of D_2O. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel F-254. Pure compounds showed a single spot in TLC. For flash chromatography, Merck silica gel 60 was used with a particle size 0.040-0.063 mm (230-400 mesh ASTM). Elemental analyses were performed on a Perkin-Elmer 2400 instrument at Laboratorio di Microanalisi, Dipartimento di Chimica, Università di Sassari, Italy, and results were within ±0.4% of theoretical values.

Chemistry

Intermediates

The diamines (**a-e)** were commercially available. The diamine (**f)** was prepared according to Walley [19] starting from the commercially available parent nitro compound (Aldrich). The bisulphite compounds (**h** and **g**) were obtained in high yields from the commercially available 1-naphtaldehyde and the purposely prepared 4-nitro-naphtalene-1-carbaldehyde following the procedure described by Katritzky and Xie [20].

General Procedure for Preparation of Compounds (13-24)

To a solution of 0.45 g (2.54-4.16 mmol) of the appropriate diamine (**a-f)** in ethanol (60 mL) was added in 1:1 molar ratio the sodium hydroxy(4-R_2-napht-1-yl)methanesulfonate (**h**,**g**). Then, the mixture was heated under reflux for 8 h. On cooling, a small amount of inorganic compound was filtered off and the ethanol mother liquors were evaporated to dryness in vacuo. The solid residues, coloured from white to

Table 1. Yields, Physical and Spectroscopic Data of Compounds of Scheme 1

Compd.	Yield %	M.p. °C	Analysis for	UV λ_{max}(EtOH) nm	IR ν_{max}(Nujol) cm^{-1}	^{1}H-NMR ($CDCl_3$-DMSO-d_6), *J* = Hz, δ:
13[22]	79	220-222	$C_{17}H_{12}N_2$	303, 224, 207	3344	8.79-8.75 (1H, m, H-8'), 8.04-7.94 (3H, m, arom.), 7.74 (5H, m, arom.), 7.34-7.28 (2H, m, arom.).
14	78	223-225	$C_{18}H_{14}N_2$	309, 222, 206	3346	8.83-8.78 (1H, m, H-8'), 8.01-7.91 (3H, m, arom.), 7.69-7.46 (5H, m, arom.), 7.11 (1H, d, *J* = 8.4; H-7), 2.51 (3H, s, 5-CH_3).
15	66	245-250	$C_{19}H_{16}N_2$	298, 205	3362	8.86-8.81 (1H, m, H-8'), 8.00-7.90 (3H, m, arom.), 7.78 (1H, s, H-4), 7.64-7.59 (3H, m, arom.), 7.45 (1H, s, H-7), 2,40 (6H, s, 5,6-CH_3).
16	81	138-140	$C_{17}H_{11}ClN_2$	(386, 377), 289, 248, 208	3351	8.75-8.72 (1H, m, H-8'), 8.00-7.89 (4H, m, arom.); 7.67-7.57 (4H, m, arom.); 7.24 (1H, dd, $J_{6,7}$ = 8,8, $J_{6,4}$ = 2,0, H-6), 6.63 (1H, m, arom.).
17	85	>300	$C_{17}H_{10}Cl_2N_2$	300, 208	3360	13.10 (1H, s, NH), 8.98-8.94 (1H, m, H-8'), 8.08 (1H, s, H-4), 8.04-7.92 (3H, m, arom.), 7.74 (1H, s, H-7), 7.69-7.50 (2H, m, arom.).
18	92	215-218	$C_{18}H_{11}F_3N_2$	290, 209	3361	8.88-8.84(1H, m, H-8'), 8.07-7.97 (4H, m, arom), 7.82-7.78 (1H, m, arom.), 7.68-7.51(4H, m, arom.).
19	76	289-291	$C_{17}H_{11}N_3O_2$	334, 222sh	1515	12.90 (1H, s, NH), 9.21-9.16 (1H, m, H-8'), 8.51-8.48 (1H, m, H-5'), 8.30 (1H, d, *J* = 7.9, H-2'), 8.08 (1H, d, J = 7.9, H-3'), 7.85-7.62 (4H, m, arom.), 7.33-7.29 (2H, m, arom.).
20	87	124-127	$C_{18}H_{13}N_3O_2$	349, 232sh	3319, 1506, 1329	8.21 (1H, d, *J* = 7.9, H-8'), 8.49 (1H, d, *J* = 7.9, H-5'), 8.29 (1H, d, *J* = 7.9, H-2'), 8.06 (1H, d, *J* = 8,0, H-3'), 7.83 (2H, m, H-6',7'), 6.51 (1H, d, *J* = 8.4, H-6), 7.49 (1H, s, H-4), 7.13 (1H, d, *J* = 8.2, H-7), 2.52 (3H, s, CH_3).
21	60	250-254	$C_{19}H_{15}N_3O_2$	358, 224sh	1516, 1337	9.03-8.99 (1H, m, H-8'), 8.53-8.49 (1H, m, H-5'), 8.19 (1H, d, *J* = 8.0, H-2'), 7,92 (1H, d, *J* = 8.0, H-3'), 7.78-7.42 (4H, m, arom.), 2.42 (6H, s, 5,6-CH_3).
22	73	122-125	$C_{17}H_{10}ClN_3O_2$	334, 224sh	1508, 1329	9.15-9.11 (1H, m, H-8'), 8.52-8.47 (1H, m, H-5'), 8.29 (1H, d, *J* = 8.2, H-2'), 8.07 (1H, d, *J* = 8.2, H-3'), 7.85-7.64 (4H, m, H-4,7,6',4'), 7.27 (1H, dd, $J_{6,7}$ = 8.6, $J_{6,4}$ = 2.0, H-6).
23	50	285-290	$C_{17}H_9Cl_2N_3O_2$	336, 232	3359, 1516, 1337	13.30 (1H, s, NH), 9.18-9.14 (1H, m, H-8'), 8.46-8.42 (1H, m, H-5'), 8.34 (1H, d, *J* = 7.6, H-2'), 8.13 (1H, s, H-4), 8.10 (1H, d, *J* = 7.6, H-3'), 8.02 (1H, s, H-7), 7.92-7.70 (2H, m, H-6'7').
24	66	121-123	$C_{18}H_{10}F_3N_3O_2$	330, 242sh, 205	1513, 1335	9.12-9.07 (1H, m, H-8'), 8.50 (1H, dd, $J_{6,7}$ = 8.6, $J_{4,6}$ = 1.6, H-6), 8.30 (1H, d, *J* = 8.6, H-2'), 8.09 (1H, d, *J* = 8.6, H-3'), 8.04 (1H, s, H-4), 7.85 (4H, m, arom.), 7.52 (1H, d, *J* = 8.4, H-7).

(Table 1. Contd….)

Compd.	Yield %	M.p. °C	Analysis for	UV λ_{max}(EtOH) nm	IR ν_{max}(Nujol) cm^{-1}	^{1}H-NMR ($CDCl_3$-DMSO-d_6), *J* = Hz, δ:
25	89	277-279	$C_{17}H_{13}N_3$	327, 280, 233	3443, 3366	8.98-8.94 (1H, m, H-8'), 8.10-8.03 (1H, m, H-5), 7.73-7.49 (5H, m, arom.), 7.30-7.20 (2H, m, arom.), 6.85-6.82 (1H, m, arom), 5.41 (2H, s, NH_2).
26	92	250-255	$C_{18}H_{15}N_3$	325, 280, 233	3453, 3343, 3160	8.62-8.56 (1H, m, H-8'), 7.66-7.62 (1H, m, H-5'), 7.50 (1H, d, *J* = 7,8, H-2'), 7.32-7.26 (4H, m, H-4,6,6',7'), 7.05 (1H, d, *J* = 7.9, H-3'), 6.49 (1H, d, *J* = 7.6, H-7), 4.57 (2H, s, NH_2), 2.45 (3H, s, CH_3).
27	83	224-226	$C_{19}H_{17}N_3$	327, 285, 235	3376	8.90-8.86 (1H, m, H-8'), 8.13-8.09 (1H, m, H-5'), 7.71 (1H, d, *J* = 8.0, H-2'), 7.60-7.44 (2H, m, arom.), 7.37 (2H, s, H-4,7), 6.83 (1H, d, *J* = 8,0, H-3'), 5.75 (2H, s, NH_2), 2.38 (6H, s, 5,6-CH_3).
28	83	222-225	$C_{17}H_{12}ClN_3$	332, 280, 233	3404, 3383, 3334	8.75-8.71 (1H, m, H-8'), 7.84-7.80 (1H, m, H-5'), 7.68-7.48 (3H, m, arom.), 7.30-7.18 (2H, m, arom.) 6.74-6.68 (2H, m, arom.), 4.41 (2H, s, NH_2).
29	90	295-300	$C_{17}H_{11}Cl_2N_3$	342, 234	3402, 3234	12.60 (1H, s, NH), 9.07 (1H, d, *J* = 8.4, H-8'), 8.13 (1H, d, *J* = 8.0, H-5'), 7.76 (1H, d, *J* = 8,0, H-2'), 7.63-7.43 (4H, m, arom.), 6.83 (1H, d, *J* = 8.0, H-3'), 5.92 (2H, s, NH_2).
30	90	228-230	$C_{18}H_{12}F_3N_3$	342, 248, 205	3460, 3374	8.75-8.71 (1H, m, H-8'), 7.84-7.80 (2H, m, arom.), 7.59-7.46 (6H, m, arom.), 6.70 (1H, d, *J* = 8.6, H-7), 4.44 (2H, s, NH_2).
31	75	>300	$C_{20}H_{14}N_2O$	307, 216	3263, 1660	10.04 (1H, s, NHCO), 9.09-9.04 (1H, m, H-8'), 8.27-8.22 (1H, m, H-5'), 8.00-7.95 (2H, m, arom.), 7.68-7.59 (4H, m, arom.), 7.27-7.22 (2H, m, arom.), 2.29 (3H, s, CH_3).
32	78	>300	$C_{21}H_{16}N_2O$	306, 214	3234, 1658, 1529	10.03 (1H, s, NHCO), 9.07-9.02 (1H, m, H-8'), 8.25-8.22 (1H, m, H-5'), 7.99-7.93 (2H, m, arom.), 7.62-7.52 (4H, m, arom.), 7.43 (1H, s, H-4), 7.07 (1H, d, *J* = 8.6, H-7), 2.50 (3H, s, CH_3), 2.29 (3H, s, CO-CH_3).
33	65	>300	$C_{22}H_{18}N_2O$	(388), 309, 211	3268, 1658, 1528	10.02 (1H, s, NHCO), 9.10-9.05 (1H, m, H-8'), 8.25-8.20 (1H, m, H-5'), 8.02 (1H, s, H-4), 7.92 (1H, s, H-7), 7.61-7.32 (4H, m, arom.), 2.39 (6H, s, 5,6-CH_3), 2.28 (3H, s, CO-CH_3).
34	70	>300	$C_{20}H_{13}ClN_2O$	(386), 308, 216	3239, 1660, 1529	10.02 (1H, s, NHCO), 9.10-9.02 (1H, m, H-8'), 8.28-8.20 (1H, m, H-5'), 7.99-7.95 (2H, m, arom.), 7.63-7.58 (3H, m, arom.), 7.26-7.18 (2H, m, arom.), 2.29 (3H, s, CH_3).
35	53	>300	$C_{20}H_{12}Cl_2N_2O$	328, 233, 209	1660, 1528	9.97 (1H, s, NHCO), 8.90-8.85 (1H, m, H-8'), 8.28-8.23 (1H, m, H-5'), 8.00 (1H, d, *J* = 8.2, H-2'), 7.93 (1H, d, *J* = 8,0, H-3'), 7.81 (2H, s, H-4,7), 7.64-7.59 (2H, m, H-6,7), 2.33 (3H, s, CH_3).
36	50	>300	$C_{21}H_{13}F_3N_2O$	326, 232, 205	1658, 1529	9.99 (1H, s, NHCO), 9.01-8.98 (1H, m, H-8'), 8.28-8.25 (1H, m, H-5'), 7.97-7.60 (6H, m, arom.), 7.50 (1H, d, *J* = 8.4, H-7), 2.32 (3H, s, CH_3).

dark brown, were purified by recrystallization from ethanol or methanol. Melting points, yields, analytical and spectroscopic data are reported in Table (**1**).

General Procedure for Preparation of Compounds (25-30)

To a solution of 0.36 g (1.0-1.24 mmol) of compounds (**19-24**) in ethanol (20 mL) was added an excess of hydrazine

Table 2. Antiviral Activity Against ssRNA$^+$ Viruses

Compd.	R	R_1	R_2	EC_{50} [μM]				
				[a]HIV-1	[b]BVDV	[c]YFV	[d]CVB-2	[d]Sb-1
13	H	H	H	>0.7	>4	>80	>100	>100
14	CH_3	H	H	>0.2	>0.4	>15	>60	>60
15	CH_3	CH_3	H	>0.06	>0.5	6	>2	>2
16	Cl	H	H	>0.3	>0.7	>13	>2	>2
17	Cl	Cl	H	>0.9	>2	>100	>100	>100
18	CF_3	H	H	>0.5	>1	>6	>75	>75
19	H	H	NO_2	>6	>20	>58	>100	>100
20	CH_3	H	NO_2	>6	>8	>18	>33	>33
21	CH_3	CH_3	NO_2	>0.3	>4	>9	>5	>4
22	Cl	H	NO_2	>1.7	>2.5	>14	>4	>4
23	Cl	Cl	NO_2	>5	>4	>6	>18	>18
24	CF_3	H	NO_2	>3	>6	>2.3	>22	>22
25	H	H	NH_2	>0.7	>2.4	>6	>20	>20
26	CH_3	H	NH_2	>0.2	>0.4	>26	>4	>4
27	CH_3	CH_3	NH_2	>0.06	>0.3	>3	>0.6	>0.6
28	Cl	H	NH_2	>0.3	>0.7	27	>1	>1
29	Cl	Cl	NH_2	>0.6	>0.7	16	>33	>33
30	CF_3	H	NH_2	>0.5	>0.8	>6	>20	>20
31	H	H	$NHCOCH_3$	>2	>100	>100	>100	>100
32	CH_3	H	$NHCOCH_3$	>2	>17	>53	>100	>100
33	CH_3	CH_3	$NHCOCH_3$	>1.6	>16	>21	>65	>65
34	Cl	H	$NHCOCH_3$	>1.8	>6	>17	>11	>11
35	Cl	Cl	$NHCOCH_3$	>2	>5	>54	>100	>100
36	CF_3	H	$NHCOCH_3$	>5	>7	>8	>40	>40
°AZT				0.01	§ND	ND	ND	ND
***NM108**				>100	4	1	30	35
^NM176				≥100	40	>100	25	23

[a]Compound concn. (μM) required to achieve 50% protection of MT-4 cells (CD4+ Human T-cells containing an integrated HTLV-1 genome) from the HIV-1-induced cytopathogenicity, as determined by the MTT method.

[b]Compound concn. (μM) required to achieve of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method.

[c]Compound concn. (μM) required to achieve 50% protection of BHK (Kidney fibroblast) cells from the YFV- and Reo-induced cytopathogenicity, as determined by the MTT method.

[d]Compound concn. (μM) required to reduce the plaque number of CVB-2 and Sb-1 by 50% in Vero 76 monolayers.

°AZT. 3'-azido-thymidine

§ND. Not Determined

*****2'-C-Me-guanosine

^2'-Ethynyl-cytidine

Variation among duplicate samples was less than 15%.

Table 3. Antiproliferative Activity Against Human Cells from Haemathologic Tumours

Compd.	[a]CC_{50} [μM]			
	MT-4	CCRF-CEM	WILS-2NS	CCRF-SB
15	0.06±0.04	0.6±0.3	1.2±0.2	0.7±0.05
16	0.3±0.01	0.5±0.1	0.05±0.7	0.7±0.05
18	0.5±0.1	1.2±0.1	1.8±0.7	1.0±0.01
21	0.3±0.02	2.4±0.05	2.8±0.4	2.0±0.1
22	1.7±0.2	2.7±0.4	2.4±0.2	2.2±0.05
24	3.0±0.1	4.7±0.7	5.9±0.6	3.9±0.3
27	0.06±0.05	0.2±0.05	0.4±0.03	0.2±0.05
28	0.3±0.01	0.5±0.1	0.9±0.2	0.6±0.1
30	0.5±0.1	0.9±0.05	0.8±0.05	0.8±0.05
33	1.6±0.4	4.8±1	8.7±2	5.0±0.9
34	1.8±0.6	3.7±0.3	7.0±0.3	3.9±0.3
36	5.0±0.1	15±1	15±0.2	12±0.5
Etoposide	0.09±0.01	0.09±0.01	0.2±0.05	0.08±0.03

[a]Compound concn. (μM) ±SD required to reduce the viable cell number of MT-4 cells (CD4+ Human T-cells containing an integrated HTLV-1 genome), CCRF-CEM (CD4+ human acute T-lymphoblastic leukaemia), WIL-2NS (human splenic B-lymphoblastoid cells) and CCRF-SB (human acute B-lymphoblastic leukaemia) by 50%, as determined by the MTT method.

hydrate (98%) (1.5 ml) in 1:1.25 molar ratio and the mixture was refluxed in the presence of 45 mg of 10% Pd charcoal for 1 h. The solution was then allowed to reach room temperature and the catalyst was removed by filtration through filter paper washed with ethanol. The mother liquors were evaporated in rotavapor under reduced pressure. Compounds **25-30** were obtained as coloured solids, which were purified by triturating with methanol.

Melting points, yields, analytical and spectroscopic data are reported in Table (**1**).

General Procedure for Preparation of Compounds (31-36)

A mixture formed by 0.1 g (3.00-3.9 mmol) of the amines (**25-30**) and acetic anhydride (3-4 mL) was kept under stirring at 80-100 °C for about 15 min. On cooling, the precipitate formed was collected and washed with diethyl ether. The acetyl derivatives (**31-36**) were obtained pure. Melting points, yields, analytical and spectroscopic data are reported in Table (**1**).

Biology

Compounds

Compounds were dissolved in DMSO at 100 mM and then diluted in cell culture medium so that the highest compound concentration used (100 μM) contains a DMSO concentration of 0.1% which, when used alone, is not cytotoxic for any of the cell lines used in this study.

Cells and Viruses

Cell lines were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell lines supporting the multiplication of RNA viruses were the following: $CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4); Madin-Darby Bovine Kidney (MDBK); Baby Hamster Kidney (BHK-21); Monkey kidney (Vero 76) cells.

Cytotoxic / Antiproliferative Assays

For cytotoxicity tests, run in parallel with antiviral assays, MDBK, BHK and Vero 76 cells were resuspended in 96 multiwell plates at an initial density of $6x10^5$, $1x10^6$ and $5x10^5$ cells/mL, respectively, in maintenance medium, without or with serial dilutions of test compounds. Cell viability was determined after 48-96 hrs at 37 °C in a humidified CO_2 (5%) atmosphere by the MTT method. The cytotoxicity of test compounds for Vero 76 monolayers was determined by staining with the crystal violet dye and, after extensive washing, comparing the amount of dye (recovered with a 0.1% SDS solution) to that of untreated monolayers.

It is worth noting that, due to the high cell density needed for virus growth, all the above cell monolayers contained resting cells.

For antiproliferative evaluations, exponentially growing cells derived from human haematological tumors [$CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-

Table 4. Antiproliferative Activity Against Human Cells from Solid Tumors

Compd.	[a]CC_{50} [μM]				
	SK-MEL-28	MCF7	SK-MES-1	HepG2	DU145
15	8.7±1	17±3	7.4±2	11±1	7.5±0.5
16	51±2	37±2	2.3±0.3	7.7±0.5	2.8±0.5
18	3.1±0.1	4.2±0.5	3.2±0.1	6.5±2	4.3±0.2
21	10±3	7.2±0.6	9.7±0.9	8.3±0.8	7.2±2
22	11±1	7.2±0.4	11±0.2	8.6±0.5	6.1±0.9
24	8.8±0.1	9.5±0.6	8.8±0.7	10±1	7.7±0.5
27	1.0±0.05	0.7±0.1	0.9±0.04	1.1±0.1	0.9±0.05
28	2.9±0.5	2.8±0.3	2.6±0.8	3.3±0.05	2.2±0.3
30	1.9±0.2	2.1±0.2	1.9±0.1	2.8±0.3	1.9±0.2
33	34±4	56±7	34±4	>100	33±2
34	33±5	10±0.01	7.5±0.3	23±1	2.8±0.1
36	11±0.1	6.0±0.8	4.6±0.9	25±1	3.5±0.2
6-MP	15±2	3.2±1.2	>100	8.0±2.6	2.0±0.2
Etoposide	1.2±0.5	1.0±0.7	0.3±0.2	0.7±0.1	0.4±0.2

[a]Compound concn. (μM) ±SD required to reduce the viable cell number of SK-MEL-28 (skin melanoma), MCF-7 (breast adenocarcinoma), SK-MES-1 (lung squamous carcinoma), HepG2 (hepatocellular carcinoma) and DU-145 (prostate carcinoma) by 50%, as determined by the MTT method.

4); $CD4^+$ human acute T-lymphoblastic leukaemia (CCRF-CEM); Human splenic B-lymphoblastoid cells (WIL-2NS); Human acute B-lymphoblastic leukemia (CCRF-SB)] were seeded at an initial density of $1x10^5$ cells/ml in 96 well plates in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 units/mL penicillin G and 100 μg/mL streptomycin. Human cell lines derived from solid tumours [skin melanoma (SK-MEL-28); breast adenocarcinoma (MCF-7); lung squamous carcinoma (SK-MES-1); hepatocellular carcinoma (HepG-2); prostate carcinoma (DU-145)] or normal tissues [lung fibroblasts (MRC-5); foreskin fibroblasts CRL-7065)] were also seeded at $1x10^5$ cells/ml in 96 well plates in the same specific media supplemented with 10% FCS and antibiotics as above. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. The extent of viable cell growth was determined after 96 hrs at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [21]. It is worth noting that the effect of compounds on these cell lines was determined under experimental conditions allowing cells to undergo exponential growth for at least three cell cycles.

In conclusion, we will refer to the effect of compounds on the various cell lines as "cytotoxic" or "antiproliferative", according to whether the cells are resting or proliferating, respectively.

Antiviral Assays

Activity of compounds against Human Immunodeficiency virus type-1 (HIV-1) was based on inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected with a multiplicity of infection (m.o.i.) of 0.01. Briefly, 50 μL of RPMI containing $1x10^4$ MT-4 were added to each well of flat-bottom microtitre trays containing 50 μL of RPMI, without or with serial dilutions of test compounds. Then, 20 μL of an HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4-day incubation, cell viability was determined by the MTT method.

Activity of compounds against Yellow fever virus (YFV), 17D vaccine strain and Reo virus type-1 (Reo-1) was based on inhibition of virus-induced cytopathogenicity in acutely infected BHK-21 cells. Activities against Bovine viral diarrhoea virus (BVDV), in infected MDBK cells were also based on inhibition of virus-induced cytopathogenicity.

BHK and MDBK cells were seeded in 96-well plates at a density of $5x10^4$ and $3x10^4$ cells/well, respectively, and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Cell monolayers were then infected with 50 μL of a proper virus dilution (in serum-free medium) to give an m.o.i = 0.01. One hr later, 50 μL of MEM Earle's medium, supplemented with inactivated foetal calf serum (FCS), 1% final concentration, without or with serial dilutions of test compounds, were added. After a 3 day incubation at 37 °C, cell viability was determined by the MTT method.

Activity of compounds against Coxsackie virus, B-2 strain (CVB-2), Polio virus type-1 (Sb-1), Sabin strain, Vesicular Stomatitis Virus (VSV) and against Respiratory syncytial virus (RSV), A-2 strain, in infected Vero 76 cells, was

Table 5. Antiproliferative Activity Against Human Cells from Normal Tissues

Compd.	[a]CC_{50}[μM]	
	MRC-5	CRL7065
15	9.0±3	30±0.5
16	40±6	56±9
18	5.8±1	6.0±0.2
21	10±1	93±7
22	10±0.7	40±6
24	8.6±0.7	15±4
27	1.1±0.1	44±6
28	2.6±0.4	>100
30	2.7±0.7	5.0±2
33	41±5	83±5
34	32±1	>100
36	21±3	>100
6-MP	>100	>100
Etoposide	>100	>100

[a]Compound concn. (μM) ±SD required to reduce the viable cell number of MRC-5 (lung fibroblast) and CRL7065 (foreskin fibroblast) by 50%, as determined by the MTT method.

determined by plaque reduction assays in Vero 76 cell monolayers. To this end, Vero 76 cells were seeded in 24-well plates at a density of $2.5x10^5$ cells/well and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Then, monolayers were infected with 250 μL of proper virus dilutions to give 50-100 PFU/well. Following removal of unadsorbed virus, 500 μL of Dulbecco's modified Eagle's medium supplemented with 1% inactivated FCS and 0.75% methyl cellulose, without or with serial dilutions of test compounds, were added. Cultures were incubated at 37 °C for 2 (Sb-1 and VSV), 3 (CVB-2) or 5 days (RSV) and then fixed with PBS containing 50% ethanol and 0.8% crystal violet, washed and air-dried. Plaques were then counted. 50% effective concentrations (EC_{50}) were calculated by linear regression technique.

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Linear Regression Analysis

Viral and cell growth at each drug concentration was expressed as percentage of untreated controls and concentrations resulting in 50% (EC_{50} and CC_{50}) growth inhibition were determined by linear regression analysis.

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