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Synthesis and antiviral assays of some benzimidazole nucleosides and acyclonucleosides

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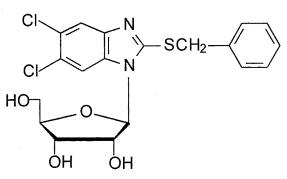
Abstract

Some benzimidazole nucleosides and acyclonucleosides were synthesized and tested in vitro as antiviral agents. None of them showed significant activity. Replacement of the benzenesulphonyl group at N-1 with the ribofuranosyl moiety or with the acyclovir side-chain was deleterious. © 2001 Elsevier Science S.A. All rights reserved.

Keywords: Benzimidazoles; Synthesis; Nucleosides; Acyclonucleosides; Antiviral assays

1. Introduction

Among the antiviral agents, benzimidazole nucleosides and acyclonucleosides have received much attention. The 5,6-dichloro-1-(β-D-ribofuranosyl)-benzimidazole (DRB) [1] was found to have multiple biological activities including activity against RNA and DNA viruses [2]. Earlier, Townsend et al. reported that certain ribosylated polyhalogenated benzimidazoles are potent and selective inhibitors of HCMV replication at noncytotoxic concentrations [3,5]. 2-Benzylthio derivative **1** is also active, even if more cytotoxic [6]. The mechanism of action of these nucleosides does not involve inhibition of DNA synthesis but does involve inhibition of DNA processing. Several benzimidazole acyclonucleosides have also been studied and tested for antiviral activity against HCMV and HSV-1 [7]. Only the 2-thiobenzyl analogues **2** showed any activity, confirming the favorable influence of a thioether linkage at the 2-position. Recently we reported the synthesis and antiviral activity of some *N*-benzenesulphonylbenzimidazoles [8,9]. Some of them, related to general structure **3**, showed good antiviral activity against two RNA viruses at micromolar concentration. In continuation of our studies in this field, we became interested in the preparation of other benzimidazole derivatives, in which the sulphonyl group has been replaced with the β-D-ribofuranosyl moiety or with the acyclovir side-chain. The aim is to evaluate if the base structure, a benzimidazole nucleus bound to a 2-pyridyl moiety by a bridge, is useful for antiviral activity even with a different mechanism of action. In fact, although the exact mechanism of action of the sulphonylated benzimidazoles remains unclear, they probably inhibit RNA replication and, specifically they selectively inhibit (+)-strand RNA synthesis. The mechanism of action of most nucleoside analogues is dependent, at least in part, upon their phosphorylation by viral cellular nucleoside kinases. This results in the formation of the triphosphate form of the nucleoside analogue, which is then responsible for inhibition of the viral nucleic acid poly-



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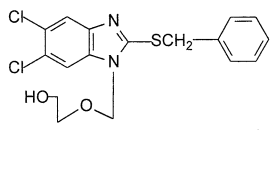

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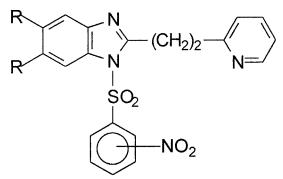

3 R=H, Cl

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merase leading to the disruption of viral nucleic acid replication [10]. We report herein the synthesis of some 2-(2-pyridylethyl and 2-pyridylmethylthio)-1-(β-D-ribofuranosyl and -1-[(2-hydroxyethoxy)methyl] benzimidazoles and their antiviral assays.

2. Chemistry

The derivatives **4**–**7** were reported in literature [9]. Silylation of **4**–**7** with bis(trimethylsilyl)-acetamide (BSA) in acetonitrile was followed by ribosylation with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of trimethylsilyltrifluoromethanesulfonate (TMSOTf). The compounds **8**–**11** were obtained in 40–50% yields. Glycosilation of **4**–**7** yielded the respective β-nucleosides as the major products; only a trace amount of the α-anomers was observed.

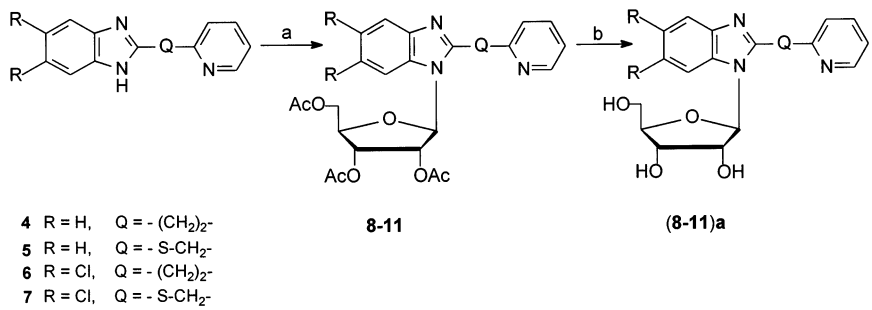
In fact the preferential formation of the β anomer was expected with a protected sugar bearing a participating group at the 2-position [4]. Deprotection of these was achieved with methanolic ammonia to yield (**8**–**11**)**a** (Scheme 1).

Sodium salts of **4**–**7** have been obtained by treatment of the free base with sodium hydride in acetonitrile. These salts were then condensed with (2-acetoxyethoxy)methylbromide [11] to give the blocked acyclic nucleosides **12**–**15**. Treatment of these compounds with sodium carbonate in aqueous ethanol (90%), at room temperature, afforded (**12**–**15**)**a** derivatives in 40–50% yield (Scheme 2).

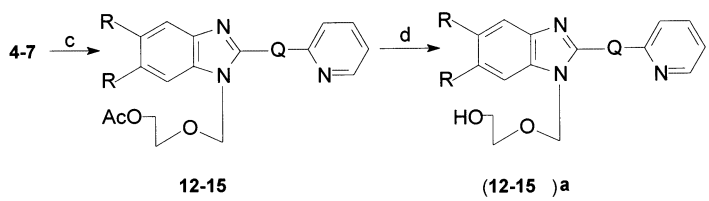
3. Results and discussion

All compounds were investigated with respect to inhibition of HSV 2, (CVM), Coxsackievirus B2 and Poliovirus 1 replication in cell cultures. In order to verify the antiviral effect of all compounds against HSV 2, CMV, Coxsackievirus B2 and Poliovirus 1, several experiments on cytotoxicity in infected and uninfected cell cultures, tested in parallel with inhibition of virus-induced cytopathic effect, were carried out. The minimum cytotoxic concentration, obtained by MTT method, was >100 μg/ml for the compounds (**8**, **9**, **10**, **12**, **13**, **14**)**a**; >50 μg/ml for the compound (**11**,**15**)**a** (>200 μg/ml for Ribavirin and >100 μg/ml for Ganciclovir). None of the analyzed derivatives had a significant effect on replication of the different virus strains included in this study with respect to the reference drugs.

The lack of antiviral activity of all compounds leads to some considerations. The importance of substituents at the 2-position of the benzimidazole ring is once more confirmed. In fact, in contrast to the potent activity of 2-chloro, 2-bromo and 2-benzylthio analogues [3], the presence of 2-(2-pyridinylmethylthio) and 2-(pyridinylethyl) moieties causes the loss of inhibitory effects. Moreover, considering the activity shown by 2-benzylthio derivatives in the nucleoside [6] and acyclonucleoside [7] series, it is evident that the replacement of the benzene ring by a pyridine is deleterious. With regard to the N-1 position, the substitution of the benzenesulphonyl group by the ribofuranosyl moiety and by the acyclovir side-chain completely abolishes the antiviral activity. It may be concluded that the in-



Scheme 1. (a) (1) Bis(trimethylsilyl) acetamide/CH_3CN. (2) 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, trimethylsilyl trifluoromethanesulfonate. (b) NH_3/MeOH.



Scheme 2. (c) NaH, (2-acetoxyethoxy)methyl bromide/CH_3CN; (d) Na_2CO_3/EtOH.

hibitory effect of structure **3** is associated with the presence of the benzensulphonyl moiety and of a nitro group.

4. Experimental

4.1. Chemistry

Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates) and visualized with iodine or aqueous potassium permanganate. ^{1}H NMR and ^{13}C NMR were determined in DMSO-δ_6 solutions with a Varian VXR 300 spectrometer, peaks positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard. The EI MS were recorded on a VG 7070E at 70 eV; the positive ion is reported as m/z. Melting points were determined on a Buchi–Tottoli instrument and are uncorrected. Chromatographies were performed with Merck 60-200 mesh silica gel. All products reported ^{1}H NMR spectra in agreement with the assigned structures. Elemental analyses were performed by the microanalytical laboratory of Dipartimento di Chimica, University of Ferrara, and agreed within 0.4% of the theoretical values.

4.1.1. General procedure for the synthesis of compounds 8–11

The appropriate benzimidazole (**4**–**7**) (3.2 mmol) was suspended in dry acetonitrile (15 ml), and BSA (1 ml, 3.8 mmol) was added to effect a clear solution. After 15 min 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (1.9 g, 3.2 mmol) and TMSOTf (1 ml, 5.1 mmol) were added. The mixture was heated to 50 °C in an oil bath and allowed to stir for 20 h. The acetonitrile was removed under reduced pressure and the protected nucleoside was separated on a silica gel column, eluting with $CHCl_3$: MeOH (95:5). All compounds appeared as a foam and were characterized without crystallization. **8**: 52%; ^{1}H NMR ($CDCl_3$): δ 2.01 (s, 3H), 2.17 (s, 3H), 2.21 (s, 3H), 3.46 (m, 4H), 4.3 (m, 1H), 4.5 (m, 2H), 5.5–5.7 (m, 2H), 6.2 (m, 1H), 7.1–7.4 (m, 4H), 7.6–7.8 (m, 3H), 8.6 (m, 1H). **9**: 54%; ^{1}H NMR ($CDCl_3$): δ 2 (s, 3H), 2.16 (s, 3H), 2.21 (s, 3H), 4.3–4.5 (m, 3H), 4.7–4.9 (m, 2H), 5.5–5.7 (m, 2H), 6.1–6.2 (m, 1H), 7.2–7.4 (m, 3H), 7.5–7.8 (m, 4H), 8.6 (m, 1H). **10**: 58%; ^{1}H NMR ($CDCl_3$): δ 2.04 (s, 3H), 2.20 (s, 3H), 2.32 (s, 3H), 3.45 (m, 4H), 4.1–4.4 (m, 2H), 4.6–4.7 (m, 1H), 5.5 (m, 2H) 6. 1 (m, 1H), 7.1–7.3 (m, 1H), 7.5–7.7 (m, 2H), 7.7–7.9 (m, 2H), 8.5 (m, 1H). **11**: 57%; ^{1}H NMR ($CDCl_3$): δ 1.99 (s, 3H), 2.17 (s, 3H), 2.30 (s, 3H), 4.3–4.5 (m, 3H), 4.7–4.9 (m, 2H), 5.3–5.5 (m, 2H), 6 (m, 1H), 7.2 (m, 1H), 7.5 (m, 1H), 7.6–7.8 (m, 3H), 8.6 (m, 1H).

4.1.2. General procedure for the synthesis of compounds (8–11)a

The protected nucleosides (**8**–**11**) (0.81 mmol) were treated with methanolic ammonia (methanol saturated with ammonia at 0 °C) at room temperature for 18 h. The mixture was concentrated to dryness under reduced pressure. The nucleosides (**8**–**11**)**a** were separated on a column using 10% MeOH–$CHCl_3$. The isolated compounds were crystallized from ethyl acetate–petroleum ether.

8a: 42%; m.p. 112 °C, ^{1}H NMR (DMSO-d_6): δ 3.7 (m, 4H), 3.9 (m, 2H), 4.1 (m, 2H), 4.4 (m, 1H), 5.1 (m, 1H, OH), 5.2 (m, 1H, OH), 5.4 (m, 1H, OH), 5.8 (m, 1H), 7.2 (m, 3H), 7.4 (m, 1H), 7.5 (m, 1H), 7.7 (m, 1H), 7.9 (m, 1H), 8.5 (m, 1H).

^{13}C NMR (DMSO-d_6): δ 26.86, 34.97, 61.39, 69.60, 71.56, 85.65, 88.14, 113.02, 118.58, 121.52, 121.62, 121.82, 123, 133.31, 136.54, 142.72, 148.96, 154.56, 160.12.

9a: 53%; m.p. 127 °C, ^{1}H NMR (DMSO-d_6): δ 3.6 (m, 2H), 3.9 (m, 1H), 4.1 (m, 1H), 4.4 (m, 1H), 4.7 (m, 2H), 5.1 (m, 1H, OH), 5.2 (m, 1H, OH), 5.4 (m, 1H, OH), 5.7 (m, 1H), 6.9–7.2 (m, 3H), 7.5–7.9 (m, 4H), 8.5 (m, 1H).

^{13}C NMR (DMSO-d_6): δ 38.04, 61.39, 69.74, 71.10, 85.67, 88.70, 112.36, 117.78, 121.75, 121.93, 122.47, 123.04, 134.22, 136.81, 143.37, 149.08, 151.43, 156.34.

10a: 61%; m.p. 195 °C, ^{1}H NMR (DMSO-d_6): δ 3.7 (m, 4H), 4 (m, 2H), 4.1 (m, 2H), 4.4 (m, 1H), 5.3 (m, 1H, OH), 5.4 (m, 1H, OH), 5.5 (m, 1H, OH), 5.8 (m, 1H), 7.2 (m, 1H), 7.4 (m, 1H), 7.5–7.7 (m, 1H), 7.9 (m, 1H), 8.3 (m, 1H), 8.5 (m, 1H).

^{13}C NMR (DMSO-d_6): δ 26.66, 34.53, 61.04, 69.55, 72.04, 86.03, 88.07, 114.67, 119.46, 121.35, 122.8, 124.17, 124.31, 132.50, 136.32, 142.20, 148.74, 157.08, 159.60.

11a: 58%; m.p. 203 °C, ^{1}H NMR (DMSO-d_6): δ 3.7 (m, 2H), 4 (m, 1H), 4.1 (m, 1H), 4.4 (m, 1H), 4.6–4.8 (m, 2H), 5.25 (m, 1H, OH), 5.33 (m, 1H, OH), 5.44 (m, 1H, OH), 5.7 (m, 1H), 7.3 (m, 1H), 7.5 (m, 1H), 7.7–7.9 (m, 2H), 8.3 (m, 1H), 8.5 (m, 1H).

^{13}C NMR (DMSO-d_6): δ 38.03, 61.15, 69.81, 71.60, 86.21, 88.79, 114.06, 118.73, 122.55, 123.07, 124.25, 124.73, 133.57, 136.85, 143, 149.15, 154.70, 155.96.

4.1.3. General procedure for the synthesis of compounds (12–15)

A stirred suspension of the appropriate benzimidazole (**4**–**7**) (1.79 mmol) in dry acetonitrile (60 ml), was added of NaH (0.062 g, 2.6 mmol) under a N_2 atmosphere. The mixture was stirred until a clear solution was obtained (30 min). 2-(Acetoxyethoxy)methyl bromide (0.423 g, 2.15 mmol) in CH_3CN (10 ml) was added dropwise. The reaction mixture was stirred for an additional 6 h, then concentrated under reduced pressure. The residue was diluted with H_2O (50 ml), extracted with EtOAc (3 × 50 ml). The organic phase

dried on Na_2SO_4 was concentrated in vacuo and the resulting oil was purified by column chromatography on silica gel eluting with $CHCl_3$:MeOH (95:5). **12**: 60%; ^{1}H NMR (DMSO-d_6): δ 1.9 (s, 3H), 3.33 (m, 4H), 3.6 (m, 2H), 4.1 (m, 2H), 5.68 (s, 2H), 7.1–7.4 (m, 4H), 7.5–7.8 (m, 3H), 8.5 (m, 1H). **13**: 59%; ^{1}H NMR (DMSO-d_6): δ 1.81 (s, 3H), 3.44 (s, 2H), 4.3 (m, 2H), 4.5 (m, 2H), 4.71 (s, 2H), 7.1–7.3 (m, 2H), 7.5–7.6 (m, 2H), 7.7–7.8 (m, 3H), 8.5 (m, 1H). **14**: 60%; ^{1}H NMR (DMSO-d_6): δ 1.98 (s, 3H), 3.35 (m, 4H), 3.6 (m, 2H), 4.1 (m, 2H), 5.6 (s, 2H), 7.1–7.4 (m, 2H), 7.6–7.8 (m, 3H), 8.5 (m, 1H). **15**: 57%; ^{1}H NMR (DMSO-d_6): δ 1.8 (s, 3H), 3.41 (s, 2H), 3.6 (m, 2H), 4.1 (m, 2H), 4.7 (s, 2H), 7.2–7.4 (m, 2H), 7.6–7.8 (m, 3H), 8.5 (m, 1H).

4.1.4. General procedure for the synthesis of compounds (12–15)a

A mixture of the appropriate benzimidazole (**12**–**15**) (1.2 mmol), Na_2CO_3 (0.3 g, 2.8 mmol), and EtOH (10% aqueous, 100 ml) was stirred at room temperature for 15 h. The ethanol was removed under reduced pressure to dryness. The residue was diluted with H_2O (50 ml), extracted with EtOAc (3 × 50 ml). The organic phase dried on Na_2SO_4 was concentrated in vacuo and the resulting oil was purified by column chromatography on silica gel eluting with $CHCl_3$:MeOH (93:7). Evaporation of the appropriate fractions gave a solid which was recrystallized from EtOAc. **12a**: 40%; m.p. 70–72 °C, ^{1}H NMR (DMSO-d_6): δ 3.33 (m, 4H), 3.6 (m, 2H), 4.2 (m, 2H), 5 (m, 1H, OH), 5.6 (s, 2H), 7.1–7.4 (m, 4H), 7.5–7.8 (m, 3H), 8.5 (m, 1H). **13a**: 32%; m.p. 56–58 °C, ^{1}H NMR (DMSO-d_6): δ 3.4 (s, 2H), 3.6 (m, 2H), 4.2 (m, 2H), 4.7 (s, 2H), 5 (m, 1H, OH), 7.1–7.3 (m, 2H), 7.4–7.6 (m, 2H), 7.7 (m, 3H), 8.5 (m, 1H). **14a**: 40%; m.p. 88 °C, ^{1}H NMR (DMSO-d_6): δ 3.41 (m, 4H), 3.6 (m, 2H), 4.3 (m, 2H), 5 (m, 1H, OH), 5.7 (s, 2H), 7.2–7.4 (m, 2H), 7.6–7.9 (m, 3H), 8.5 (m, 1H). **15a**: 20%; m.p. 62–64 °C, ^{1}H NMR (DMSO-d_6): δ 3.3 (s, 2H), 3.7 (m, 2H), 4.3 (m, 2H), 4.6 (s, 2H), 5.5 (m, 1H, OH), 7.2–7.4 (m, 2H), 7.5–7.8 (m, 3H), 8.5 (m, 1H).

4.2. Biology

4.2.1. Cells and viruses

HeLa and human embryonic lung (HEL) cells were used. Cells were propagated in Minimal Essential Medium (MEM) with 10% fetal calf serum (FCS), 1% L-glutamine, 100 U/ml of penicillin and 100 U/ ml of streptomycin. All experiments were performed using laboratory strains of Herpes Simplex Virus type 2 (HSV 2), cytomegalovirus (CMV), Coxsackievirus B2 and Poliovirus1.

4.2.2. Peroxidase staining for determination of virus titers

Cells monolayers in 96-well plates (Nunc Denmark) were overlaid with 100 μl of each virus dilution (dilutions from 10^{-1} to 10^{-9}). After adsorption for 1 h at 37 °C and 5% CO_2, the medium was removed and replaced with 100 μl of fresh complete MEM. After 24 h of incubation, peroxidase staining was performed [12]. After fixation of cells with methanol for 15 min at −20 °C, the monolayers were incubated for 15 min with PBS supplemented with 1% bovine serum albumin (PBS/BSA) at 37 °C. Pool of reference sera against HSV2, CMV, Coxsackievirus B2 and Poliovirus 1, respectively (dilution of 1:20 in PBS/BSA) were added. After 45 min of incubation at 37 °C the monolayers were washed three times with PBS and peroxidase-conjugated antibodies anti-human immunoglobulinG (Dako Denmark) were added. After 45 min of incubation at 37 °C the monolayers were washed three times with PBS and substrate (HRP color development reagent, Bio-Rad Laboratories) was added. Plaques were counted under a light microscope. The viral titers were 3×10^8 PFU/ml for HSV2, 1.8×10^6 PFU/ml for CMV, 2×10^6 PFU/ml for Coxsackievirus B2 and 3×10^5 PFU/ml for Poliovirus 1.

4.2.3. Cytotoxicity assays

Confluent cells monolayers grown in 96 well tissue culture plates were incubated with MEM supplemented with 2% of FCS, 1% L-glutamine in the presence of different concentration of compounds dissolved in dimethylsulphoxide (DMSO) in triplicate. Cell cultures were incubated at 37 °C, 5% CO_2 for 96 h. The viabilities of the cells were assessed by the MTT method (Boehringer Mannheim). The cytotoxicities of compounds against HeLa and HEL cells were evaluated in terms of the CC_{50} (the concentration required to reduce cell number by 50% relative to the number of cells in the untreated control cell cultures) [13].

4.2.4. Antiviral assays

Evaluation of anti-HSV2, anti-CMV, anti-Coxsackievirus B2 and anti-Poliovirus 1 activity was based on the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, a serial twofold dilution of compounds starting at 400 μg/ml (100 μl aliquots in DMSO diluted in MEM) was prepared in a 96-well tissue culture tray (Nunc, Denmark) with three wells used for each concentration. Ribavirin and Ganciclovir were used as reference drugs. To each well, 5×10^4 cells (in 50 μl of DMSO/MEM) were added. When the monolayers had become confluent, usually after 24 h, the medium was aspirated and viruses, at the M.O.I. of 10^{-3} PFU/cell, (in 50 μl of DMSO/MEM) were added. After 1 h adsorption at 37 °C the inoculum was removed and the culture medium or, in the case of the controls DMSO/MEM, was added to the compounds and to the reference drugs dissolved in DMSO. Each compound was assayed in triplicate. To determine the

median effective concentration (EC_{50}) of the compounds, 10 μl of MTT was added to each well. The mixture was incubated at 37 °C for 4 h, and reduced MTT (formazan) was extracted from the cells by the addition of 100 μl of solubilization mixture (Boehringer Mannheim). After an overnight incubation at 37 °C the absorbance of the formazan was measured by using a microplate reader (Labsystems Multiscan MS-DASIT) at two different wavelengths (540–690 nm). The EC_{50} of each compounds was defined as the concentration that achieved 50% protection of virus-infected cells from virus-induced destruction [14].

Acknowledgements

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