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Synthesis of new compounds with promising antiviral properties against group A and B Human Rhinoviruses

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ABSTRACT

The human common cold, which is a benign disease caused by the Rhinoviruses, generally receives palliative symptomatic treatments, since no specific therapy against any of these viruses currently exists. In this work, some original synthetic compounds were produced and tested, in order to find non-toxic substances with an improved protection index (PI) for infected cells, as compared to reference drugs such as Pirodavir. We designed a series of novel molecules with a double oxygen in the central hydrocarbon chain and some modifications of the lateral methylisoxazole and propoxybenzoate moieties of lead compound 6602 (ethyl 4-{3-[2-(3-methyl-1,2-isoxazol-5-yl)ethoxy]propoxy}benzoate). It was found that most of these substances were actually less toxic than Pirodavir; in addition, the new molecule indicated as **8c** was more than 30 times less toxic than Pirodavir, about twice as active on the group A strain of Rhinovirus HRV14, and even four times more effective on the group B strain HRV39, as compared to Pirodavir's PI.

1. Introduction

Several groups of viruses that cause an extraordinarily wide range of illnesses belong to the Picornaviridae family.[1,2] The Rhinovirus genus contains the common cold agents and is formed from a great number of serotypes, making it extremely difficult to prepare an effective vaccine.[3] Due to the lack of specific antiviral therapy, most Rhinovirus illnesses are managed symptomatically. Although effective antiviral therapy is not yet clinically available, some promising anti-picornavirus drugs are able to reduce the duration of illness in adults. Capsid-binding compounds such as Disoxaril and Pirodavir have proved to be partially effective in the intranasal treatment of patients with a common cold caused by some strains of Human Rhinoviruses.[4–10]

The Rhinovirus capsid consists of 4 polypeptides VP 1, 2, 3, and 4, which all derive from the cleavage of a larger protein. The capsid proteins determine host and tissue tropism by recognition of cell-specific cell-membrane receptors and the virus uses these membrane receptors to enter the target cells.[11–13] Most human Rhinovirus strains, for example, bind to the intracellular adhesion molecule 1 (ICAM-1), an immunoglobulin-like molecule, and consequently, capsid proteins are a potential target for the action of specific anti-Rhinovirus drugs.[14–19]

In a previous study[20] we tested several novel anti-Rhinovirus compounds and demonstrated that some modifications of the central hydrocarbon molecule chain could lead to new interesting drugs with improved activity for certain Rhinovirus strains and decreased cytotoxicity on cell cultures. Thus, considering that the VP1 viral canyons of different species of Picornaviruses have a peculiar structure in terms of size, depth and shape, we designed novel SAR studies targeted at modifying the central chain and lateral moieties of lead compound 6602 (**1a**), in order to find original molecules whose antiviral and cytotoxic properties could prove more effective against group A and B Rhinovirus strains.

In the present work we describe some original molecules with very promising properties, one of which presents improved cytotoxic and anti-viral activity, as compared to lead drug 6602 and also to the reference drug Pirodavir.

2. Drug design

The molecules described in this study were derived from lead compound 6602 (**1a**) described by Laconi et al.[20] and represented in Figure 1. Pirodavir (**1b**) was also used as a reference compound.

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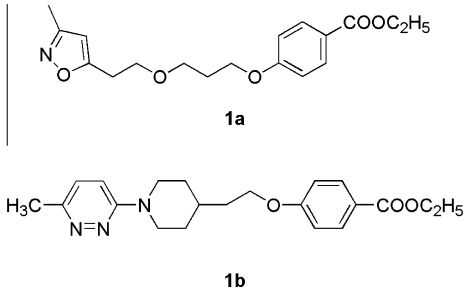


Figure 1. Compound **1a**: The chemical structure of lead compound 6602 (ethyl 4-{3-[2-(3-methyl-1,2-isoxazol-5-yl)ethoxy]propoxy}benzoate). Compound **1b**: The general reference compound Pirodavir.

Since compounds **1a** and **1b** undergo easy in vivo hydrolysis of the ester functionality to the corresponding acid derivatives which are almost devoid of anti-HRV activity, we disclosed some new compounds lacking such a function in the aryloxy group.

With this aim, we substituted the above-mentioned group with either a 1,3-benzodioxol-5-yl (**6b**, **7b**, **8b**), a 4-acetylphenyl (**6c**, **8c**, **9c**, **10c**), a 4-cyanophenyl (**8a**), or a 4-fluorophenyl group (**9d**) (Fig. 2). In some derivatives the methylisoxazolyl moiety was substituted by either a 2-ethoxy-1,3-benzoxazol-6-yl (**8a–c**) or a 1,3-benzodioxol-5-yl group (**9c–d**, **10c**). The central aliphatic chain was shortened in molecules **7b**, **8a–c**, **9c–d** and **10c**.

3. Results and discussion

3.1. Chemistry

The general synthetic pathways for the preparation of the reported compounds are shown in Scheme 1. Compounds **6–10** were obtained through nucleophilic substitution between derivatives **3–5** and the aryloxypropyl bromides **2a–d**, obtained from the corresponding phenols by treatment with excess of 1, 3-dibromopropane. The primary alcohol **3** [2-(3-methyl-1,2-isoxazol-5-yl)ethanol, $n = 2$] was prepared following previously described methods.[21] Compound **4** was prepared by the reaction of 4-aminobenzene-1,3-diol with an excess of tetraethyl orthocarbonate. Compounds **3** ($n = 0$) and **5** are commercially available.

3.2. Antiviral activity

The cytotoxicity and the anti-Rhinovirus activity of 9 novel original compounds derived from lead 6602 are indicated in Table 1.

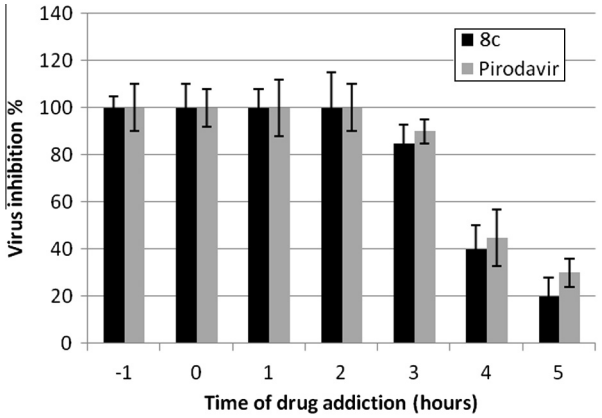
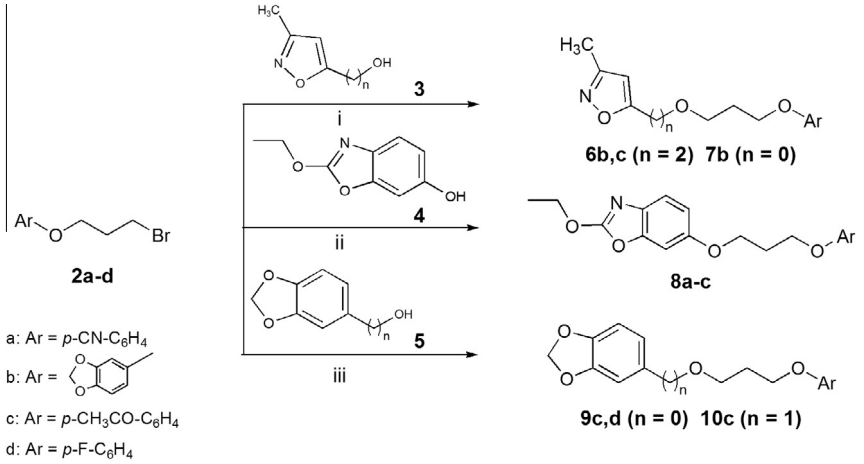


Figure 2. Study of compound **8c** antiviral activity when added to the cells at different times after infection. Compound **8c** and Pirodavir were used at a concentration of 5 μg/mL. Both compounds were 100% effective when added to the cells from h −1 until the 2nd h post infection; after this their activity decreased rapidly.

Two different strains of Rhinoviruses were used in these tests, representing the 2 major groups of this genus, namely groups A and B. Each compound was tested for the maximum non-toxic dose (CD_{50}), the inhibitory dose (ID_{50}) and the protection index (PI = CD/ID).

3.2.1. Chemical modification and structure–activity relationship (SAR)

All the new compounds tested showed a CD equal or even lower than 6602, but all of them resulted far less toxic than Pirodavir on HeLa cells; moreover, the insertion of an ethoxybenzoxazol-6-yl group coupled with either a benzodioxolyl or a 4-acetylphenyl group produced two compounds (**8b** and **8c**) that were more than 60 times less toxic than pirodavir and 4 times less toxic than 6602. The introduction of the 1,3-benzodioxol-5-yl group in **6b**, **7b**, **8b**, **9c**, **9d**, **10c** led to substances with poor antiviral activity. Although their cytotoxicity was low, the PIs were far less significant than those of 6602 and Pirodavir. The insertion of a cyano substituent into the aryloxy group produced a compound (**8a**) with a PI of 16 and 33, respectively, still worse than 6602. It should also be noted that shortening the central aliphatic chain as in **7b** till **10c** did not seem to have a relevant effect on either the cytotoxicity or the antiviral effect. Finally, when the aryloxy group was replaced by a 4-acetylphenyl group as in **6c** and **8c**, the results were extremely



Scheme 1. Synthesis of compounds **6–10**. Reagents and conditions: (i) $n = 2$, NaH, THF, −10 °C; $n = 0$, K_2CO_3, CH_3CN, 80 °C; (ii) K_2CO_3, CH_3CN, 80 °C; (iii) $n = 1$, NaH, THF, −10 °C; $n = 0$, K_2CO_3, CH_3CN, 80 °C.

Table 1
Screening of cytotoxic activity and anti-Rhinovirus properties of the novel compounds and reference molecules 6602 and Pirodavir

Compounds	Cytotoxic activity	Anti-Rhinovirus activity			
	CD_{50}[a] (μg/mL)	HRV14 ID_{50}[b] (μg/mL)	PI[c]	HRV39 ID_{50} (μg/mL)	PI
6602	50	0.07	714	0.07	714
6b	50	50	1	50	1
6c	50	25	2	6	8
7b	50	25	2	12	4
8a	50	25	2	1.5	33
8b	200	25	8	12	16
8c	200	0.5	400	0.1	2000
9c	25	25	1	25	1
9d	25	25	1	25	1
10c	50	6	8	6	8
Pirodavir	3.1	0.012	258	0.006	516

[a] CD: Cytotoxic dose on HeLa cells.
[b] ID: Inhibitory dose.
[c] PI: Protection index (CD/ID).

different depending on the concomitant modification of the isoxazolyl moiety. **6c** showed a modest PI of 8, whilst **8c**, where the methylisoxazolyl group was substituted by a 2-ethoxy-1,3-benzoxazolyl group, presented a dramatic improvement of its activity and cytotoxicity on cultured cells. Its PI for the group A Rhinovirus was 400 (about half of that shown by 6602, but almost twice that of Pirodavir = 258), but for group B it was 2000, far better than 6602 (714) and Pirodavir (516). Although the overall specific antiviral activity of **8c** was lower than that of 6602 and Pirodavir, it is important to consider that in all cases, compound **8c** was less toxic on cell cultures than all the other lead or reference compounds tested, so that in most cases its PI was highly improved with respect to both 6602 and pirodavir.

3.2.2. Action mechanism of the new compounds

Figure 2 shows the antiviral activity of compound **8c** on HRV14 when it is added to the infected cell culture at different times after infection. Pirodavir was used as the reference molecule. **8c** exerts its inhibition effect on the Rhinovirus when added to the cells within the 3rd h after infection. From the 4th h onwards the activity rapidly decreases to non-significant levels. This behaviour is quite similar to that expressed by Pirodavir, and means that **8c** is an antiviral molecule which exerts its activity in the early phases of viral replication, such as during virus-to-cell adhesion and virus entry into the cells. In this context, compound **8c** seems to behave exactly like the reference Pirodavir, which alters virion adhesion to the cells by blocking the interaction between the Rhinovirus capsid canyon and the cell receptor ICAM 1. Moreover, it is important to point out that substance **8c**, just like Pirodavir, has a reversible effect on Rhinovirus inhibition and does not show any kind of direct virucidal activity.

4. Conclusions

The aim of this study was to induce some modifications on lead compound 6602 in order to obtain a number of derivatives with improved cytotoxic and anti-Rhinovirus activities. In fact, by substituting the isoxazolyl moiety with a benzoxazolyl group, and the ethyl ester with a 4-acetylphenyl group, we obtained a new compound, indicated by the label **8c**, which was 4 times less toxic than molecule 6602 and more than 60 times less toxic than the reference compound Pirodavir. Furthermore, anti-Rhinovirus activity resulted highly improved, since the protection index of **8c** was about 3 times superior to that of compound 6602 on HRV39, about twice as high as that of Pirodavir on HRV14 and up to 4 times better that that shown by Pirodavir on HRV39. We conclude that the novel original molecule named **8c** has acquired some properties that render it less toxic and more active on Rhinoviruses than the reference compounds. Since Pirodavir, the most studied reference compound, has already shown some sort of anti-common cold activity at least as an in vivo topical treatment, but has yet to be launched onto the drug market because of its toxicity, we believe that substance **8c** has shown a significant improvement in terms of cell toxicity and anti-viral activity and merits possible development for in vivo studies and for preclinical testing.

5. Experimental

5.1. Synthesis and characterization

5.1.1. General

NMR spectra were recorded in $CDCl_3$ on 500 Varian spectrometer at room temperature and TMS as internal standard. Chemical shifts are reported as follows: chemical shifts, multiplicity and coupling constants. Mass spectra were recorded on Agilent 5973 N (Cpsil 32 m) and Nermag R10-10 (quartz-Cpsil 5.25 m). (E.I. 70 eV) Microanalysis was performed with a Perkin Elmer 2400 series 2. Infrared spectra were obtained using a Bruker-FT-IR.

Analytical thin layer chromatography was performed using 0.25 mm silica gel 60-F plates. Flash chromatography was performed using 200–400 mesh silica gel (Merk KGaA). Unless otherwise stated, yields refer to chromatography and spectroscopically pure materials. THF was freshly distilled from sodium/benzophenone. Reactions requiring anhydrous conditions were performed in oven dried glassware under Argon atmosphere.

5.1.2. Preparation of 4-(3-bromopropoxy)benzonitrile (2a)

A solution of 1,3-dibromopropane (4.24 g, 21.2 mmol, 2.13 mL) in CH_3CN (8 mL), was added dropwise to a stirred mixture of 4-hydroxybenzonitrile (0.50 g, 4.2 mmol) and K_2CO_3 (0.87 g, 6.3 mmol) in CH_3CN (10 mL) at room temperature and then stirred at 80 °C for 7 h. The reaction mixture was cooled, the solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (30 mL), washed with 10% NaOH (2 × 20 mL), and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography with petroleum ether (40–60)/diethyl ether (4:1) as eluent to give **2a** (0.68 g, 68%).

Colourless oil; IR (neat): 2226 cm^{-1} (CN). ^1H NMR ($CDCl_3$, 500 MHz): δ 2.29 (m, CH_2CH_2Br), 3.55 (t, CH_2Br, J = 6.5 Hz), 4.12 (t, OCH_2, J = 6.0 Hz), 6.92 (d, 2 Ar*H*, J = 9.0 Hz), 7.53 (d, 2 Ar*H*, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$, 125,8 MHz): δ 29.6 (CH_2), 31.9 (CH_2), 65.7 (CH_2), 104.1 (*C*), 115.2 (2*C*H), 119.1 (*C*N), 133.9 (2*C*H), 161.9 (*C*); MS (EI): *m/z* (%) 241 (29), 239 (27), 160 (4), 119 (100), 102

(12), 64 (10), 41 (55). Anal. Calcd for $C_{10}H_{10}BrNO$: C, 50.02; H, 4.20; N, 5.83. Found: 50.16; H, 4.12; N, 5.76.

5-(3-Bromopropoxy)-1,3-benzodioxole (**2b**)[22] (0.73 g, 68%), **1-[4-(3-bromopropoxy)phenyl]ethanone** (**2c**)[23] (0.74 g, 69%) and **1-(3-bromopro-poxy)-4-fluorobenzene** (**2d**)[24] (0.69 g, 70%) were prepared in a manner similar to that described above for **2a**, using 1,3-benzodioxol-5-ol (0.58 g, 4.2 mmol),or 1-(4-hydroxy-phenyl)ethanone (0.57 g, 4,2 mmol), or 4-fluorophenol (0.47 g, 4.2 mmol), in place of 4-hydroxybenzonitrile, respectively, and the ^{1}H NMR, ^{13}C NMR and MS data were in accordance with literature values.

5.1.3. Preparation of 6b and 6c

A solution of 2-(3-methyl-1,2-isoxazol-5-yl)ethanol (0.20 g, 1.6 mmol)[21] in anhydrous THF (1 mL) was added at room temperature to a stirred suspension of pentane-washed sodium hydride (60% w/v in mineral oil, 0.09 g, 2,3 mmol) in THF (5 mL) in an argon atmosphere. After 1 h the appropriate halides **2b** (0,41 g, 1.6 mmol) or **2c** (0,41 g, 1.6 mmol) in THF (5 mL) were added at -10 °C and the resulting mixture was stirred and warmed to a room temperature over 18 h and then treated with brine (20 mL). The organic layer was extracted with diethyl ether (3 × 20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with petroleum ether (40–60)/diethyl ether (4:1) as eluent to give the final products.

5.1.3.1. 5-{2-[3-(1,3-Benzodioxol-5-yloxy)propoxy]ethyl}-3-methyl-1,2-isoxazole (6b). (0.19 g, 40%), colourless oil; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.01 (qnt, $OCH_2CH_2CH_2O$, J = 6.0 Hz), 2.23 (s, CH_3), 2.98 (t, Isoxazole-CH_2, J = 6.5 Hz), 3.63 (t, $OCH_2CH_2CH_2O$, J = 6.0 Hz), 3.73 (t, Isoxazole-CH_2CH_2O, J = 6.5 Hz), 3.96 (t, CH_2O-Benzodioxole, J = 6.0 Hz), 5.88 (s, Isoxazole-*H*), 5.92 (s, OCH_2O), 6.32 (dd, Ar-*H*, J = 2.5 Hz, J = 8,0 Hz), 6.50 (dd, Ar-*H*, J = 2.5 Hz, J = 6.0 Hz), 6.71 (dd, Ar-*H*, J = 3.0 Hz); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 11.3 (CH_3), 27.5 (CH_2), 29.3 (CH_2), 65.5 (CH_2), 67.4 (CH_2), 67.9 (CH_2), 98.0 (*C*H), 101.1 (OCH_2O), 102.4(*C*H, Isoxazole), 105.7 (*C*H), 107.9 (*C*H), 141.6 (*C*), 149.9 (*C*), 159.6 (*C*, Isoxazole), 159.8 (*C*), 170.4 (*C*, Isoxazole); MS (EI): *m*/*z* (%) 305 (51), 138 (100), 110 (37), 68 (85), 41 (55). Anal. Calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: 62.86; H, 6.12; N, 4.76.

5.1.3.2. 1-(4-{3-[2-(3-Methyl-1,2-isoxazol-5-yl)ethoxy]propoxy} phe-nyl)ethanone (6c). (0.23 g, 47%), colourless oil; IR (neat): 1669 cm^{-1} (C═O). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.03 (qnt, $OCH_2CH_2CH_2O$, J = 6.0 Hz), 2.18 (s, CH_3), 2.52 (s, CH_3CO), 2.95 (t, Isoxazole-CH_2, J = 6.5 Hz), 3.60 (t, $OCH_2CH_2CH_2O$, J = 6.0 Hz), 3.70 (t, Isoxazole-CH_2CH_2O, J = 6.0 Hz), 4.06 (t, CH_2OAr, J = 6.0 Hz), 5.83 (s, 1H, Isoxazole-*H*), 6.90 (d, 2 Ar-*H*, 8.5 Hz), 7.90 (d, 2 Ar-*H*, 9 Hz); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 11.3 (CH_3), 26.3 (CH_3CO), 27.5 (CH_2), 29.4 (CH_2), 64.9 (CH_2), 67.1 (CH_2), 67.9 (CH_2), 102.4 (*C*H, Isoxazole), 114.1 (2*C*H), 130.4 (*C*), 130.6 (2*C*H), 159.8 (*C*, Isoxazole), 162.9 (*C*), 170.3 (*C*, Isoxazole), 196.7 (*C*═O); MS (EI): *m*/*z* (%) 303 (22), 288 (16), 161 (25), 149 (22), 121 (45), 110 (41), 68 (87), 43 (100). Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: 67.46; H, 6.72; N, 4.74.

5.1.4. Preparation of 7b

5-(3-Bromopropoxy)-1,3-benzodioxole (**2b**) (0.41 g, 1.6 mmol) in CH_3CN (2 mL), was added dropwise at room temperature to a stirred solution of 3-methylisoxazol-5-(4H)-one morpholine salt (0.30 g 1.6 mmol), K_2CO_3 (0.69 g, 5.0 mmol) in CH_3CN (10 mL), and then was stirred at 80 °C for 7 h. The reaction mixture was cooled, the solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (30 mL), washed with 10% NaOH (2 × 20 mL), and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography with petroleum ether (40–60)/diethyl ether (4:1) as eluent to give the final product.

5.1.4.1. 5-{[3-(1,3-Benzodioxol-5-yloxy)propoxy]-3-methyl-1,2-iso-xazole (7b). (0.17 g, 41%), yellow crystals; mp: 100–102 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.16 (s, CH_3), 2.23 (qnt, $OCH_2CH_2CH_2O$, J = 6.0 Hz), 4.04 (t, Isoxazole-OCH_2, J = 6.0 Hz), 4.33 (t, CH_2OAr, J = 6.0 Hz), 5.06 (s, Isoxazole-*H*), 5.91 (s, OCH_2O), 6.31 (dd, Ar-*H*, J = 2.0 Hz, J = 8.5 Hz), 6.48 (d, Ar*H*, J = 2.0 Hz), 6.69 (d, Ar-*H*, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 125,8 MHz): δ 12.3 (CH_3), 28.9 (CH_2), 64.5 (CH_2), 68.7 (CH_2), 86.7 (*C*H), 98.1 (*C*H, Isoxazole), 101.1 (OCH_2O), 105.7 (*C*H), 107.9 (*C*H), 141.8 (*C*), 148.3 (*C*), 154.1 (C, Isoxazole), 162.2 (*C*), 173.2 (*C*, Isoxazole); MS (EI): *m*/*z* (%) 277 (42), 149 (52), 140 (100), 138 (46), 82 (56). Anal. Calcd for $C_{14}H_{15}NO_5$: C, 60.64; H, 5.45; N, 5.05. Found: 60.48; H, 5.52; N, 4.94.

5.1.5. 2-Ethoxy-1,3-benzoxazol-6-ol (4)[25]

To a mixture of of 4-aminobenzene-1,3-diol hydrochloride (1.00 g, 6.2 mmol) and anhydrous sodium acetate (0.52 g, 6.2 mmol) in anhydrous $CHCl_3$ (30 mL), a slight excess of tetraethyl orthocarbonate (2.50 g, 13.0 mmol) was added and then stirred for 16 h at 60 °C. The reaction was worked up by adding saturated NaCl solution (20 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The organic extracts were dried ($NaSO_4$) and evaporated under reduced pressure, and the residue purified by silica gel column chromatography with petroleum ether (40–60)/ethyl acetate (3:1) as eluent to give **4** (0.78 g, 70%).

The ^{1}H-NMR and ^{13}C NMR data were in agreement with the literature values. MS (EI): *m*/*z* (%) 179 (34), 151 (100), 122 (39), 107 (12), 95 (88), 80 (16), 67 (30), 52 (31), 39 (41). Anal. Calcd for $C_9H_9NO_3$: C, 60.33; H, 5.06; N, 7.82; O, 26.79. Found: 60.28; H, 5.11; N, 7.76; O, 26.85.

5.1.6. 4-{3-[(2-Ethoxy-1,3-benzoxazol-6-yl)oxy]propoxy} benzonitrile (8a)

A solution of 2-ethoxy-1,3-benzoxazol-6-ol **4** (0.30 g, 1.7 mmol) in CH_3CN (2 mL), was added dropwise to a stirred mixture of 4-(3-bromopropoxy)benzonitrile **2a** (0.40 g, 1.7 mmol) and K_2CO_3 (0.35 g, 2.5 mmol) in CH_3CN (10 mL) at room temperature and then stirred at 80 °C for 7 h. The reaction mixture was cooled, the solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (30 mL), washed with 10% NaOH (2 × 20 mL), and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography with petroleum ether (40–60)/diethyl ether) (4:1) as eluent to give **8a** (0.51 g, 89%).

White solid; mp: 122 °C; IR (nujol): 2224 cm^{-1} (CN). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.51 (t, CH_3, J = 7.0 Hz), 2.31 (qnt, OCH_2CH_2-CH_2O, J = 6.0 Hz), 4.17 (t, OCH_2, J = 6.0 Hz), 4.23 (t, CH_2O, J = 6.0 Hz), 4.59 (q, CH_3CH_2, J = 7.0 Hz), 6.83 (dd, Benzoxazole-*H*, J = 2.0 Hz, J = 8.5 Hz), 6.95 (d, Benzoxazole-*H*, J = 2.0 Hz), 6.97 (d, 2 Ar-*H*, J = 9.0 Hz), 7.35 (d, Benzoxazole-*H*, J = 8.5 Hz), 7.59 (d, 2 Ar-*H*, J = 8.5 Hz). ^{13}C NMR ($CDCl_3$, 125,8 MHz): δ 14.3 (CH_3), 29.1 (CH_2), 64.8 (CH_2), 65.0 (CH_2), 68.1 (CH_2), 96.8 (*C*H), 104.1 (*C*), 111.6 (*C*H), 115.2 (2*C*H), 117.8 (*C*H), 119.1 (*C*N), 134.0 (2*C*H), 134.9 (C), 148.8 (*C*), 155.3 (*C*), 162.1 (*C*, Oxazole), 163.0 (*C*); MS (EI): m/z (%) 338 (44), 151 (100), 132 (52), 102 (42), 90 (12), 77 (25), 41 (39). Anal. Calcd for $C_{19}H_{18}N_2O_4$: C, 67.44; H, 5.36; N, 8.28. Found: 67.36; H, 5.22; N, 8.38.

8b and **8c** were prepared using a similar synthetic method to that described above for **8a**, using 5-(3-bromopropoxy)-1, 3-benzodioxole **2b** (0.44 g, 1.7 mmol) or 1-[4-(3-bromopropoxy)phenyl]ethanone **2c** (0.43 g, 1.7 mmol) in place of 4-(3-bromopropoxy)benzonitrile, respectively.

5.1.6.1. 6-[3-(1,3-Benzodioxol-5-yloxy)propoxy]-2-ethoxy-1,3-ben-zoxazole (8b). (0.42 g, 69%), white solid; mp: 104–106 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.51 (t, CH_3, J = 7.0 Hz), 2.24

(qnt, $OCH_2CH_2CH_2O$, J = 6.0 Hz), 4.10 (t, CH_2O J = 6.0 Hz), 4.16 (t, OCH_2, J = 6.0 Hz), 4.59 (q, CH_3CH_2, J = 7.0 Hz), 5.92 (s, OCH_2O), 6.35 (dd, Benzodioxole-*H*, J = 2.5 Hz, J = 8.5 Hz), 6.52 (d, Benzodioxole-*H*, J = 2.5 Hz), 6.71 (d, Benzodioxole-*H*, J = 8.5 Hz), 6.84 (dd, Benzoxazole-*H*, J = 2.0 Hz, J = 8.5 Hz), 6.96 (d, Benzoxazole-*H*, J = 2.0 Hz), 7.35 (d, Benzoxazole-*H*, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 125,8 MHz): δ 14.3 (CH_3), 29.4 (CH_2), 65.37 (CH_2), 65.42 (CH_2), 68.0 (CH_2), 96.8 (*C*H), 98.1 (*C*H), 101.1 (OCH_2O), 105.7 (*C*H), 107.9 (*C*H), 111.6 (*C*H), 117.7 (*C*H), 134.7 (*C*), 141.7 (*C*), 148.2 (*C*), 148.8 (*C*), 154.4 (*C*), 155.5 (*C*), 162.9 (*C*, Oxazole); MS (EI): *m*/*z* (%) 357 (70), 192 (61), 178 (32), 164 (23), 149 (100), 137 (62), 121 (41), 107 (32), 93 (22), 79 (24), 65 (34). Anal. Calcd for $C_{19}H_{19}NO_6$: C, 63.86; H, 5.36; N, 3.92. Found: 63.76; H, 5.32; N, 3.78.

5.1.6.2. 1-(4-{3-[(2-Ethoxy-1,3-benzoxazol-6-yl)oxy]propoxy} phe-nyl)ethanone (8c). (0.30 g, 49%), white solid; mp: 112–114 °C; IR (nujol) 1668 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.52 (t, CH_3CH_2, J = 7.0 Hz), 2.32 (qnt, $OCH_2CH_2CH_2O$, J = 6.0 Hz), 2.57 (s, CH_3CO), 4.19 (t, CH_2O, J = 6.0 Hz), 4.26 (t, OCH_2, J = 6.0 Hz), 4.60 (q, CH_3CH_2, J = 7.0 Hz), 6.85 (dd, Benzoxazole-*H*, J = 2.5 Hz, J = 8.5 Hz), 6.96 (d, Benzoxazole-*H*, J = 2.5 Hz), 6.97 (d, 2 Ar-*H*, J = 9.0 Hz), 7.36 (d, Benzoxazole-*H*, J = 8.5 Hz), 7.95 (d, 2 Ar-*H*, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$, 125,8 MHz): δ 14.3 (CH_3), 26.3 ($COCH_3$), 29.2 (CH_2), 64.7 (CH_2), 65.1 (CH_2), 68.1 (CH_2), 96.8 (*C*H), 111.6 (*C*H), 114.2 (2*C*H), 117.7 (*C*H), 130.4 (*C*), 130.6 (2*C*H), 134.8 (*C*), 148.8 (*C*), 155.4 (*C*), 162.8 (*C*, Oxazole), 163.0 (*C*), 196.7 (*C*=O); MS (EI): *m*/*z* (%) 355 (86), 177 (22), 162 (26), 151 (59), 135 (15), 121 (22), 106 (23), 91 (24), 77 (25), 43 (100). Anal. Calcd for $C_{20}H_{21}NO_5$: C, 67.59; H, 5.96; N, 3.94. Found: 67.44; H, 5.82; N, 3.78.

5.1.7. Preparation of 9c and 9d

A solution of 1,3-benzodioxol-5-ol (0.50 g, 3.6 mmol) in CH_3CN (5 mL), was added dropwise to a stirred mixture of appropriate halide **2c** (0.92 g, 3.6 mmol) or **2d** (0.83 g, 3.6 mmol) and K_2CO_3 (0.74 g, 5.4 mmol) in CH_3CN (20 mL) at room temperature and then stirred at 80 °C for 7 h. The reaction mixture was cooled, the solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (30 mL), washed with 10% NaOH (2 × 20 mL), and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography with petroleum ether (40–60)/diethyl ether (2:1) as eluent to give the final products.

5.1.7.1. 1-{4-[3-(1,3-Benzodioxol-5-yloxy)propoxy]phenyl}ethanone (9c). (1.11 g, 98%),white solid; mp: 135–137 °C; IR (nujol): 1669 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.27 (qnt, $OCH_2CH_2CH_2O$, J = 6.0 Hz), 2.57 (s, CH_3CO), 4.10 (t, OCH_2, J = 6.0 Hz), 4.23 (t, CH_2O, J = 6.0 Hz), 5.92 (s, OCH_2O), 6.35 (dd, Benzodioxole-*H*, J = 2.5 Hz, J = 8.5 Hz), 6.51 (d, Benzodioxole-*H*, J = 2.5 Hz), 6.71 (d, Benzodioxole-*H*, J = 8.5 Hz), 6.95 (d, 2 Ar-*H*, J = 8.5 Hz), 7.94 (d, 2 Ar-*H*, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 26.3 (CH_3), 29.2 (CH_2), 64.7 (CH_2), 65.1 (CH_2), 98.1 (*C*H), 101.1 (OCH_2O), 105.7 (*C*H), 107.9 (*C*H), 114.2 (2*C*H), 130.4 (*C*), 130.6 (2*C*H), 141.7 (*C*), 148.3 (*C*), 154.3 (*C*), 162.8 (*C*), 196.7 (*C*=O); MS (EI): *m*/*z* (%) 314 (84), 149 (23), 138 (100), 121 (12), 107 (14), 91 (12), 43 (44). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: 68.64; H, 5.72.

5.1.7.2. 5-[3-(4-Fluorophenoxy)propoxy]-1,3-benzodioxole (9d). (0.71 g, 68%), white solid; mp: 96–98 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.24 (qnt, $OCH_2CH_2CH_2O$, J = 6.0 Hz), 4.09 (t, OCH_2, J = 6.0 Hz), 4.13 (t, CH_2O, J = 6.0 Hz), 5.92 (s, OCH_2O), 6.35 (dd, Benzodioxole-*H*, J = 2.5 Hz, J = 8.5 Hz), 6.51 (d, Benzodioxole-*H*, J = 2.5 Hz), 6.71 (d, Benzodioxole-*H*, J = 8.5 Hz), 6.86 (dd, 2 Ar-*H*, J = 9.0 Hz, J_{HF} = 4.5 Hz), 6.98 (t, 2 Ar-*H*, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 29.3 (CH_2), 65.0 (2CH_2), 98.1 (*C*H), 101.1 (OCH_2O), 105.7 (*C*H), 106.9 (*C*), 107.9 (*C*H), 115.5 (2*C*H, *d*, J_{CF} = 7.9 - Hz), 115.8 (2*C*H, *d*, J_{CF} = 23.0 Hz), 122.3 (*C*), 154.9 (*C*), 155.0 (*C*), 157.3 (*C*F, *d*, J_{CF} = 238.1 Hz); MS (EI): *m*/*z* (%) 290 (55), 138 (100), 125 (45), 107 (14), 95 (39). Anal. Calcd for $C_{16}15_{15}FO_4$: C, 66.20; H, 5.21. Found: 66.32; H, 5.30.

5.1.8. 1-{4-[3-(1,3-Benzodioxol-5-ylmethoxy)propoxy]phenyl}-ethanone (10c)

A solution of 1,3-benzodioxol-5-ylmethanol (0.50 g, 3.3 mmol) in anhydrous THF (5 mL) was added at room temperature to a stirred suspension of pentane-washed sodium hydride (60% w/v in mineral oil, 0.17 g, 4.2 mmol) in THF (20 mL) in an argon atmosphere. After 1 h the halide **2c** (0.84 g, 3.3 mmol) in THF (5 mL) was added at room temperature and the resulting mixture was stirred at 60 °C for 16 h and then treated with brine (20 mL). The organic layer was extracted with diethyl ether (3 × 20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with petroleum ether (40–60)/diethyl ether (1:1) as eluent to give **10c** (0.52 g, 48%).

Yellow solid; mp 42–44 °C; IR (nujol): 1669 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.09 (qnt, CH_2, J = 6.0 Hz), 2.55 (s, CH_3 CO), 3.63 (t, 2H, OCH_2, J = 6.0 Hz), 4.13 (t, $ArOCH_2$, J = 6.0 Hz), 4.56 (s, Benzodioxole-CH_2O), 5.94 (s, OCH_2O), 6.76 (d, Benzodioxole-*H*, J = 6.5 Hz), 6.79 (d, Benzodioxole-*H*, J = 6.5 Hz), 6.86 (s, Benzodioxole-*H*), 6.91 (d, 2 Ar-*H*, J = 9.0 Hz), 7.92 (d, 2 Ar-*H*, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$, 125,8 MHz): δ 26.3 (CH_3), 29.6 (CH_2), 65.1 (CH_2), 66.2 (CH_2), 72.9 (CH_2), 101.0 (OCH_2O), 108.0 (*C*H), 108.4 (*C*H), 114.2 (2*C*H), 121.2 (*C*H), 130.4 (*C*), 130.6 (2*C*H), 134.9 (*C*), 147.1 (*C*), 147.8 (*C*), 163.0 (*C*), 196.9 (*C*=O); MS (EI): *m*/*z* (%) 328 (25), 135 (100), 121 (12), 105 (10), 77 (28), 43 (19). Anal. Calcd for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14. Found: 69.42; H, 6.08.

5.2. Biological assay

5.2.1. Cells and viruses

The cytotoxic and antiviral activity of the compounds was studied on both HeLa cells (Ohio strain) and Hep-2 cells grown in DMEM with 1% non-essential amino acids, 200 μg/mL streptomycin, 200 units/mL penicillin G and 10% fetal calf serum (GIBCO Laboratories INC). Cell lines were kept at 37 °C in a humidified atmosphere with 5% CO_2. Rhinoviruses HRV14 (group A) and HRV39 (group B), were purchased from the American Type Culture Collection (ATCC). For all the above mentioned viruses, working stocks were prepared as cellular lysates using DMEM with 2% heat inactivated fetal calf serum.

5.2.2. Cytotoxic activity

The cytotoxicity of the test compounds was evaluated by measuring the effect produced on cell morphology and cell growth in vitro. Cell monolayers were prepared in 24-well tissue culture plates and exposed to various concentrations of the compounds. Plates were checked by light microscopy after 24, 48 and 72 h. Cytotoxicity was scored as morphological alterations (e.g., rounding up, shrinking, detachment). The viability of the cells was determined by a tetrazolium-based colorimetric method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bro-mide (MTT), as previously described.[24,26] The 50% cytotoxic dose (CD_{50}) is the concentration of the compound that reduced the absorbance of the control sample by 50%.

5.2.3. Inhibition of virus multiplication

The Rhinovirus inhibition assay was evaluated by a one-step viral infection of cell monolayers, followed by virus yield titration in an agar-plaque assay. HeLa cell monolayers were prepared in

24 multiwell plates and were infected by the Rhinoviruses at a MOI of 1. Next, serial dilutions of the test compounds were added and after 24–36 h of incubation at 33 °C and 3% CO_2, when the cytopathic effect in the control cells was almost total, the monolayers were frozen and thawed and the viruses in the supernatant were titrated by the plaque assay method. The antiviral activity assay on the Rhinoviruses was carried out by the 50% plaque reduction assay in Hep2 cells as previously described.[20] The compound concentration required to inhibit virus plaque formation by 50% is expressed as the 50% inhibitory concentration (ID_{50}), and calculated by dose–response curves and linear regression.

5.2.4. Time-drug addiction assay

Time-reduction studies were performed on cell monolayers grown in 24 well plates, as indicated by Laconi et al.[20] The compounds were added to the cells from h −1 to h +6 after viral infection. The cells were incubated in a CO_2 atmosphere for 24–36 h, washed twice with HBSS, after which the virus yield was detected by a plaque assay.

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Supplementary data

Supplementary data (^1H NMR, ^{13}C NMR and MS spectra) for new compounds **6**–**10** associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.05.066. These data include MOL files and InChiKeys of the most important compounds described in this article.

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