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SCIENCE DIRECT
Antiviral Research 71 (2006) 7–14
Antiviral Research
www.elsevier.com/locate/antiviral

Synthesis and the biological evaluation of 2-benzenesulfonylalkyl-5-substituted-sulfanyl-[1,3,4]-oxadiazoles as potential anti-hepatitis B virus agents

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Received 19 October 2005; accepted 15 February 2006

Abstract

Current treatments for chronic hepatitis B virus (HBV) infection include the use of interferon-α and of nucleoside analogs lamivudine, adefovir and entecavir. However, the use of interferon-α has many side effects while that of nucleosidic inhibitors can lead to the emergence of resistant viruses. Hence, new drugs for the treatment of HBV infection are still highly desired. Oxadiazoles have been observed to exhibit antiviral activities against RNA viruses. In this study, a facile synthesis of 2-benzenesulfonylalkyl-5-substituted-sulfanyl-[1,3,4]-oxadiazoles is reported. The compounds were then evaluated for their anti-HBV activity. 1-{2-[5-(1-Benzenesulfonyl-propyl)-[1,3,4]oxadiazol-2-yl-sulfanyl]-ethyl}-4-(2-methoxy-phenyl)-piperazine (**1i**) was able to inhibit the expression of the viral antigens, HBsAg and HBeAg in a concentration-dependent manner with no cytotoxic effects and without any effects on the expression of viral transcripts. Concentration- and time-dependent reductions in virion production were also observed. The inhibition of virion production was comparable to that of lamivudine and EC_{50} values of 1.63 and 2.96 μM were obtained for compound **1i** and lamivudine, respectively. Thus, in addition to the antiviral effects on RNA viruses, oxadiazoles also have anti-HBV activities.

Keywords: Hepatitis B virus; Hepatitis B virus-s antigen; Hepatitis B virus-e antigen; Antiviral activity; Oxadiazoles

1. Introduction

Hepatitis B virus (HBV) is the major cause of acute and chronic hepatitis which could lead to hepatocellular carcinoma. Chronic hepatitis B infection affects more than 400 million people worldwide and 1–2% of them die each year from virus related complications (Lin and Kirchner, 2004). Currently, interferon-α and nucleosidic inhibitors of HBV reverse transcriptase/polymerase, lamivudine (3TC), adefovir and entecavir are the only drugs approved for the treatment of chronic HBV infection (Ocama et al., 2005; Mailliard and Gollan, 2006). However, interferon-α produces many side effects and its efficacy is partial with less than 30% of the chronic carriers responding to treatment. In addition, approximately 50% of the patients who respond positively to interferon-α treatments are known to suffer a recurrence of the viremia after cessation of the treatment (Thomas, 1998; Fattovich et al., 1998). In contrast, 3TC has more universal applicability and is able to reduce the viral load very rapidly. However, this initial rapid response is often followed by a slow elimination of the residual virus. Emergence of drug resistance commonly occurs during this slower phase thus preventing long-term treatment (Fischer et al., 2001). Adefovir is effective against 3TC-resistant viruses but long-term monotherapy can also result in resistance leading to reduced response to adefovir (Angus et al., 2003; Villeneuve et al., 2003; Fung et al., 2005). Resistance to entecavir has also been reported in lamivudine-resistant patients (Tenney et al., 2004). The wide prevalence of chronic HBV infection together with the lack of an effective drug available for the treatment warrants the search for novel therapeutic agents against the virus.

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doi:10.1016/j.antiviral.2006.02.007

Oxadiazoles are useful targets in the search for antivirals as they have been associated with a wide variety of interesting properties. Members of this class of compound are known to possess tyrosine kinase inhibitory (Vu et al., 1999), anti-inflammatory (Nicolaides et al., 1998), monoamine oxidase inhibitory (Matsumoto et al., 1994) and antitumor activities (Chimirri et al., 1996). More recently, 2,5-disubstituted-[1,3,4]-oxadiazoles have also gained considerable attention because of their uses as organic light-emitting devices (Zhang et al., 2005), orally active COX-2 inhibitors (Song et al., 1999) and heterocyclic amide and ester isosteres (Luthman et al., 1999).

Earlier works have shown that oxadiazoles possess antiviral activities against RNA viruses and are able to inhibit HIV replication (El-Emam et al., 2004). Antiviral activity towards picornaviruses has been described and to date, the best characterized is pleconaril (Diana et al., 1994; Fendrick, 2003; Webster, 2005) which binds to the hydrophobic pockets in the base of canyons on the surface of picornaviruses (Badger et al., 1988). This inhibits virus attachment to cells (Pevear et al., 1989; Shepard et al., 1993) and also inhibits the uncoating of the viral capsid (Fox et al., 1986; Shepard et al., 1993). Herein, we describe the synthesis of a new class of 2,5-disubstituted-[1,3,4]-oxadiazoles, the 2-benzenesulfonylalkyl-5-substituted-sulfanyl-[1,3,4]-oxadiazoles, and the biological evaluation of their anti-HBV activity.

2. Materials and methods

2.1. Synthesis of oxadiazoles

All chemicals were obtained from commercial suppliers and used without purification. The three 1-(2-chloroethyl)-4-substituted-piperazines used for the preparation of **1f–1i** were prepared according to a modified reported procedure (Modica et al., 2001). Analytical TLC was carried out on pre-coated plates (Merck Silica Gel 60, F254) and visualized with UV light. Flash column chromatograph was performed with silica (Merck, 230–400 mesh). NMR spectra (^{1}H and ^{13}C) were recorded at 298 K on the Bruker DPX300 or AMX500 Fourier Transform spectrometer. Chemical shifts are expressed in δ (ppm), relative to the internal standard of tetramethylsilane (TMS). Mass spectra were performed on VG Micromass 7035 spectrometer under EI or ESI.

2.1.1. Preparation of α-sulfonylesters (*3*)

Sodium benzenesulfinate **2** (0.80 g, 5 mmol) was dissolved in DMF (30 mL) in which NBu_4I (0.18 g, 0.5 mmol), KI (1.0 g, 6 mmol) and the α-bromoester (6 mmol) were added. The mixture was stirred at room temperature for 4 h. DMF was then removed by freezing–drying and the residual yellow oil obtained was purified by flash column chromatography to give **3**. **3a**: Yield = 98%. IR: 1747, 1327, 1153 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.70 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 7.58 (t, 2H, J = 7.8 Hz, $2H_{arom}$), 7.69 (t, 1H, J = 7.8 Hz, H_{arom}), 7.94 (d, 2H, J = 7.8 Hz, $2H_{arom}$). ^{13}C NMR ($CDCl_3$): δ 52.9, 60.7, 128.4, 129.1, 134.2, 138.6, 162.7. **3b**: Yield = 97%. IR: 1741, 1323, 1149 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.56 (d, 3H, J = 7.2 Hz, CH_3-3), 3.68 (s, 3H, OCH_3), 4.06 (q, 1H, J = 7.2 Hz, CH), 7.58 (t, 2H, J = 7.8 Hz, $2H_{arom}$), 7.70 (t, 1H, J = 7.8 Hz, H_{arom}), 7.89 (d, 2H, J = 7.8 Hz, $2H_{arom}$). ^{13}C NMR ($CDCl_3$): δ 11.8, 52.9, 65.3, 128.9, 129.2, 134.2, 136.8, 166.6. **3c**: Yield = 94%. IR: 1737, 1326, 1149 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.97 (t, 3H, J = 7.2 Hz, CH_3-4), 1.16 (t, 3H, J = 7.2 Hz, OCH_2CH_3), 1.91–2.14 (m, 2H, CH_2-3), 3.86 (dd, 1H, J = 4.0 Hz, 11.2 Hz, CH-2), 4.12 (q, 2H, J = 7.2 Hz, OCH_2), 7.56 (t, 2H, J = 7.2 Hz, $2H_{arom}$), 7.66 (t, 1H, J = 7.2 Hz, H_{arom}), 7.88 (d, 2H, J = 7.2 Hz, $2H_{arom}$). ^{13}C NMR ($CDCl_3$): δ 11.3, 13.8, 20.4, 62.0, 72.2, 128.8, 129.2, 134.0, 137.2, 165.7.

2.1.2. Preparation of α-sulfonylcarboxylic hydrazides (*4*)

Hydrazine hydrate (1.2 g, 20 mmol) was added to a solution of α-sulfonylester (2 mmol) in ethanol (50 mL). The resulting solution was refluxed for 16 h. The solvent was then evaporated and the residue was purified by flash column chromatography (50% EtOAc in hexane) to give **4**. **4a**: Yield = 92%. ^{1}H NMR (DMSO-d_6): δ 4.25 (s, 2H, CH_2), 4.39 (b, 2H, NH_2), 7.69 (t, 2H, J = 7.2 Hz, $2H_{arom}$), 7.79 (t, 1H, J = 7.2 Hz, H_{arom}), 7.92 (d, 2H, J = 7.2 Hz, $2H_{arom}$), 9.34 (b, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 59.3, 127.9, 129.0, 133.8, 139.5, 160.0. HRMS (EI) calcd. for $C_8H_{10}N_2O_3S$, 214.0412; found, 214.0414. **4b**: Yield = 87%. ^{1}H NMR (DMSO-d_6): δ 1.29 (d, 3H, J = 7.2 Hz, CH_3), 4.03 (q, 1H, J = 7.2 Hz, CH), 4.35 (b, 2H, NH_2), 7.66 (t, 2H, J = 7.2 Hz, $2H_{arom}$), 7.75–7.83 (m, 3H, $3H_{arom}$), 9.33 (b, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 11.9, 62.9, 128.8, 129.0, 134.0, 136.8, 163.6. HRMS (EI) calcd. for $C_9H_{12}N_2O_3S$, 228.0569; found, 228.0570. **4c**: Yield = 90%. ^{1}H NMR (DMSO-d_6): δ 0.78 (t, 3H, J = 7.2 Hz, CH_3), 1.68–1.79 (m, 2H, CH_2), 3.34 (b, 2H, NH_2), 3.83 (dd, 1H, J = 6.0, 9.0 Hz, CH), 7.64 (t, 2H, J = 7.5 Hz, $2H_{arom}$), 7.74–7.81 (m, 3H, $2H_{arom}$), 9.40 (b, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 10.9, 19.9, 69.5, 128.8, 129.0, 134.0, 137.2, 162.9. HRMS (EI) calcd. for $C_{10}H_{14}N_2O_3S$, 242.0725; found, 242.0726.

2.1.3. Preparation of 5-(1-benzenesulfonyl-alkyl)-3H-[1,3,4]-oxadiazole-2-thione (*5*)

Carbon disulfide (0.38 g, 5 mmol) and potassium hydroxide (86 mg, 1 mmol) were added to a solution of **4** (1 mmol) in ethanol (10 mL) at 0 °C. The mixture was then refluxed for 12 h. The solvent was evaporated and the residue obtained was dissolved in water and acidified with dilute HCl. **5** precipitated from the solution was pure enough to be used in the next step of the reaction. **5a**: Yield = 86%. IR: 1610, 1338, 1229, 1150 cm^{-1}. ^{1}H NMR (CD_3OD): δ 4.82 (s, 2H, CH_2), 7.62–7.68 (m, 2H, $2H_{arom}$), 7.75–7.79 (m, 1H, H_{arom}), 7.90 (d, 2H, J = 7.8 Hz, $2H_{arom}$). ^{13}C NMR (DMSO-d_6): δ 51.6, 128.0, 129.5, 134.6, 137.6, 154.2, 178.0. HRMS (ESI, M + H) calcd. for $C_9H_9N_2O_3S_2$, 257.0055; found, 257.0054. **5b**: Yield = 80%. IR: 1606, 1363, 1241, 1165 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.75 (d, 3H, J = 7.2 Hz, CH_3), 4.41 (q, 1H, J = 7.2 Hz, CH), 7.59–7.64 (m, 2H, $2H_{arom}$), 7.71–7.76 (m, 1H, H_{arom}), 7.83 (d, 2H, J = 7.5 Hz, $2H_{arom}$). ^{13}C NMR (DMSO-d_6): δ 11.0, 57.0, 128.7, 129.5, 134.9, 135.7, 157.4, 177.8. HRMS (ESI, M + H) calcd. for $C_{10}H_{11}N_2O_3S_2$, 271.0211; found, 271.0219. **5c**: Yield = 84%. IR: 1604, 1332, 1202, 1153 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.01 (t, 3H, J = 7.2 Hz,