Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Epimeric methylsulfinyladenosine derivatives from the marine ascidian Herdmania momus

Jian Lin Li, Eun La Kim, Haibo Wang, Jongki Hong, Sook Shin, Chong-Kyo Lee, Jee H. Jung

College of Pharmacy, Pusan National University, Busan 609-735, South Korea
College of Pharmacy, Kyung Hee University, Seoul 130-701, South Korea
Department of Life Science, Sahmyook University, Seoul 139-742, South Korea
Korea Research Institute of Chemical Technology, Daejeon 305-343, South Korea
School of Medicine, Nantong University, Nantong 226-001, China

A R T I C L E   I N F O
Article history:
Received 12 April 2013
Revised 28 May 2013
Accepted 30 May 2013
Available online 7 June 2013

Keywords:
Nucleoside derivatives
Sulfinyl epimers
Ascidian
Herdmania momus

A B S T R A C T
Investigation of the secondary metabolites of the ascidian Herdmania momus led to the isolation and characterization of four new nucleoside derivatives (1–4). Structural studies showed that these derivatives represent a series of rare methylsulfinyladenosine derivatives of interconvertible transesterification isomers and/or sulfinyl epimers. The antiviral activities of these rare nucleosides were evaluated against a series of human pathogenic viruses.

Marine ascidians are a rich source of chemically diverse secondary metabolites, with remarkable biological activities.1,2 Such as amino acid derivatives and complex alkaloids.3–9 In our previous study on bioactive compounds from the solitary tunicate Herdmania momus, a series of amino acid derivatives (herdmanines A–M) were isolated.3,4 As a part of continuing study on the metabolites of the same tunicate, four new complex nucleosides (1–4) containing the rare methylsulfinyladenosine were isolated. Details of the isolation, structure elucidation, and of the results of the antiviral screening of these metabolites are described herein.

The combined MeOH and CH2Cl2 extracts were sequentially partitioned between CH2Cl2 and H2O, and n-BuOH and H2O. The n-BuOH-soluble fraction was subjected to chromatographic separation and purification to yield a series of nucleoside derived metabolites (1–4).10 Compounds 1 and 2 were found to be interconvertible, and on standing at room temperature after HPLC separation, each compound afforded the same equilibrium ratio of compound 1–2 (56:44). Compounds 3 and 4 were also interconvertible, and on standing at room temperature produced an identical equilibrium mixture (56:44) as that of 1 and 2. Spectral analysis of these interconvertible compounds was performed on equilibrium mixtures.

The equilibrium mixture of compounds 1 and 2 was a white powder. The (+)-FABMS spectrum of this mixture showed isotopic peaks of [M+2H]+ and [M+3H]+ at m/z 554/552 and 555/553, respectively, with an isotopic peak ratio of 1:1. This pseudomolecular ionic configuration is shared with various other nucleosides.
and amines.\(^{11}\) (+)-HRFABMS analysis of the mix of compounds 1 and 2 yielded the molecular formula C\(_{20}\)H\(_{13}\)BrN\(_{4}\)O\(_{3}\)S, with 14 degrees of unsaturation. The \(^1\)H and \(^{13}\)C NMR spectra of this equilibrium mixture revealed isomeric pairs of signals corresponding to two different molecular structures. Each set of signals can be delineated based on COSY, HMOC, and HMBC data. \(^1\)H and \(^{13}\)C NMR spectra indicated the presence of a nucleoside moiety. More specifically, characteristic chemical shifts of C-2 (\(\delta_c\) 145.8), C-4 (\(\delta_c\) 149.4), C-5 (\(\delta_c\) 125.5), C-6 (\(\delta_c\) 157.7), and C-8 (\(\delta_c\) 140.2) for one set of \(^{13}\)C NMR signals (compound 1) suggested that the purine base was adenine, and this was further confirmed by HMBC correlations between H-2 and C-4 and C-8 (Fig. 1). Based on the above, compound 1 was characterized as \(2S,3S,4R,5R\)-5-(6-amino-9H-purin-9-yl)-4-hydroxy-2-[(methylsulfinyl)methyl]tetrahydrofuran-3-yl-(6-bromo-5-hydroxy-1H-indole)-3-carboxylate and given the trivial name momusina A.

The \(^1\)H and \(^{13}\)C NMR spectra of another component (compound 2) were different from those of 1 only in splitting patterns and chemical shifts of some of the ribose ring proton signals. The signal for H-3 (\(\delta_1\) 4.84) was shifted upfield, whilst that of H-2 (\(\delta_1\) 5.94) was shifted downfield as compared to those of compound 1 (H-3, \(\delta_1\) 5.63; H-2, \(\delta_1\) 5.21), indicating an acyl substitution at C-2, and thereby, deshielding induced by the carbonyl group. This was further confirmed by the HMBC correlation between H-2' and C-8' (\(\delta_c\) 164.5) in compound 2.

Compounds 1 and 2 were interconvertible at room temperature affording an equilibrium mixture of 56:44 ratio. Likewise, a previous study reported that 3'-O-acetyl-ADP-ribose is an intramolecular transesterification product of 2'-O-acetyl-ADP-ribose produced by the Sir2 family of histone/protein deacetylases and the mixture of 3'- and 2'-acetate exist in equilibrium (55:45).\(^{17}\) Other examples of non-enzymatic acyl migration under mild conditions have also been reported.\(^{18–21}\) Furthermore, several research groups have studied the stability and/or acyl migration of many O-acyl glucuronides, and in all cases, acyl migration occurred when the hydroxyl groups of the glucuronic acid were essentially on the same face of the ring.

The equilibrium mixture of compounds 3 and 4 was also a white powder. The molecular formulas of compounds 3 and 4 were determined to be C\(_{20}\)H\(_{13}\)BrN\(_{4}\)O\(_{3}\)S based on NMR and MS data. The \(^1\)H and \(^{13}\)C NMR signals of each set of components were delineated based on COSY, HMOC, and HMBC. One set (compound 3) of \(^1\)H NMR signals was almost identical to those of compound 1, with the exception of a slight chemical shift difference at H-5' (Table 1). In addition, the carbon signals of C-5' (\(\delta_c\) 55.0) and C-7' (\(\delta_c\) 37.3) of compound 3 were shifted slightly upfield versus compound 1 (C-5', \(\delta_c\) 57.5; C-7', \(\delta_c\) 38.0) (Table 2). According to the above evidence, compounds 1 and 3 were deduced to be epimers as has been previously described for 5-deoxy-5-methylsulphinyladenosine\(^ {16}\) and the sulfoxide derivatives,\(^ {22}\) which only differ in their configuration with respect to the position of the sulfoxide group.

Comparisons of the \(^1\)H and \(^{13}\)C NMR data of 4 with those of 1-3 suggested that 4 was a 2'-3' transesterification derivative of compound 3, and a sulfoxide epimer of compound 2. Consequently, compounds 1, 3, and 2, 4 were deduced to be two pairs of epimers (Fig. 2A), and this was confirmed by oxidizing the sulfanyl group in mixtures with H\(_2\)O\(_2\) and subsequent HPLC analysis.\(^ {23}\) The mixtures of 1/2 (Fig. 2B) and of 3/4 (Fig. 2C) were converted to sulfonyleadenosine derivatives, and each mixture yielded identical

### Table 1

| Position | 1     | 2     | 3     | 4     |
|----------|-------|-------|-------|-------|
| 2        | 8.11 (s) | 8.08 (s) | 8.11 (s) | 8.08 (s) |
| 8        | 8.29 (s) | 8.26 (s) | 8.25 (s) | 8.23 (s) |
| 1'       | 6.16 (d, 5.5) | 6.34 (d, 4.0) | 6.16 (d, 6.0) | 6.32 (d, 4.0) |
| 2'       | 5.21 (t, 5.5) | 5.94 (dd, 5.5, 4.0) | 5.20 (t, 5.5) | 5.92 (t, 5.5) |
| 3'       | 5.63 (dd, 5.5, 4.5) | 4.84 (m) | 5.67 (dd, 5.5, 4.0) | 4.82 (m) |
| 4'       | 4.75 (m) | 4.55 (dd, 9.5, 6.5, 2.5) | 4.78 (m) | 4.63 (dd, 10.5, 7.5, 4.0) |
| 5a'      | 3.58 (dd, 13.0, 10.5) | 3.46 (dd, 13.0, 10.5) | 3.53 (dd, 14.0, 7.0) | 3.46 (dd, 13.5, 9.0) |
| 5b'      | 3.32 (m) | 3.32 (m) | 3.48 (dd, 13.5, 5.0) | 3.34 (m) |
| 7a'      | 2.72 (s) | 2.73 (s) | 2.70 (s) | 2.71 (s) |
| 7b'      | 8.08 (s) | 8.04 (s) | 8.08 (s) | 8.04 (s) |
| 4'       | 7.65 (s) | 7.59 (s) | 7.66 (s) | 7.59 (s) |
| 7'       | 7.59 (s) | 7.57 (s) | 7.58 (s) | 7.56 (s) |

\* Chemical shifts were assigned using COSY and HMBC spectral data.
at room temperature. The 13C NMR (see Tables 1–100) have been isolated from onion extracts. Usually the sulfoxide configuration of natural products is described for epimers differentiated by sulfoxide-asymmetry. In two-component (1/2) chromatogram of 1/2 (tR 25.39 min/24.57 min); (C) chromatogram of 3/4 (tR 25.54 min/24.54 min); (D) chromatogram of the oxidation products of 3/4 [5/6, tR 16.86/14.76 min]; (E) chromatogram of the oxidation products of 3/4 [5/6, tR 16.86/14.76 min]. RP HPLC condition for B–E was as follows; YMC-packed C-18 column, linear gradient elution system from 5% MeOH (0.1% TFA) to 90% MeOH (0.1% TFA) in 60 min, flow rate 0.5 mL/min, UV, 220 nm.

two-component (5 and 6) mixtures (Fig. 2D and E, respectively). This finding indicated that compounds 1, 3 and 2, 4 are epimeric at the sulfoxide group. 5′-Deoxy-5′-methylsulfinyladenosine epimers have been isolated from *Ganoderma lucidum*, and reported in onion extracts. Distinct biological activities have also been described for epimers differentiated by sulfoxide-asymmetry. Usually the sulfoxide configuration of natural products is (S). In the present study, we tried to determine the absolute stereochemistry of the sulfoxide moiety in compounds 1–4 by using MsrA (methylion sulfoxide reductase A, Protein Data Bank code: 1NWA), which selectively reduces S-configured sulfoxides. However, neither mixture 1/2 nor mixture 3/4 was reduced by MsrA, suggesting that these new nucleosides are not good substrates for MsrA and cannot bind properly to the active enzyme site. Rare sponge-derived nucleosides, such as, spongolide and spongeoridine served as antiviral leads and culminated in antiviral drugs like acyclovir and zanamivir. In some antiviral 5′-deoxyribonucleosides, substitution of a methylsulfonyl group at the 5′-position increased antiviral activity while securing low cytotoxicity. Moreover, 5-hydroxy-6-bromomido-3-carboxylic acid derivatives display antiviral activities against a number of viruses including influenza A/B viruses, avian coronavirus, infectious bronchitis virus, Marek’s disease virus, and hepatitis B/C viruses. Therefore, in an expectation of antiviral activity of compounds 1–4, we evaluated them for antiviral activity against human pathogenic viruses. However, none of the isolomers showed antiviral activity against human rhinoviruses (HRV14, HRV17, or HEV71, EC50 >100 μg/mL, CC50 >100 μg/mL), coxsackieviruses (CoxB1 or CoxB3, EC50 >100 μg/mL, CC50 >100 μg/mL), or poliovirus (PV3, EC50 >100 μg/mL, CC50 >100 μg/mL). Considering the chemical structure of those nucleoside derivatives, compounds 1–4 may exhibit antibacterial, analgesic, sedative, and cardiac depressant activities, however, further biological evaluations could not be performed due to paucity of materials.

Acknowledgment

This study was supported by the National Research Foundation of Korea (Grant No. 20090083538).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.05.097.

References and notes

1. Verbiest, J. F. J. Pharm. Belg. 1995, 50, 98.
2. Watters, D. J.; van den Brink, A. L. Toxicon 1993, 31, 1349.
3. Li, J. L.; Han, S. C.; Yoo, E. S.; Shin, S.; Hong, J.; Cui, Z.; Li, H.; Jung, J. H. J. Nat. Prod. 2011, 74, 1792.
4. Li, J. L.; Xiao, B.; Park, M.; Yoo, E. S.; Shin, S.; Hong, J.; Chung, H. Y.; Kim, H. S.; Jung, J. H. J. Nat. Prod. 2012, 75, 2082.
5. Ochi, M.; Kataoka, K.; Ariki, S.; Iwatsuki, C.; Kodama, M.; Fukuyama, Y. J. Nat. Prod. 1998, 61, 1043.
6. Santalová, E. A.; Denisenko, V. A.; Berdyshev, D. V.; Aminin, D. L.; Sanamyan, K. E. Nat. Prod. Commun. 2008, 3, 1617.
7. García, A.; Vázquez, M. J.; Quitiño, E.; Riguela, R.; Debitus, C. J. Nat. Prod. 1996, 59, 782.
8. Carroll, A. R.; Avery, V. M. J. Nat. Prod. 2009, 72, 696.
9. Davidson, B. S. Chem. Rev. 1993, 93, 1771.
10. Extraction and isolation: Frozen ascidians (0.4 kg) were chopped into small pieces and extracted with MeOH and CHCl3 at room temperature. The combined extract was partitioned between CHCl3 and H2O. The H2O fraction was further extracted with n-BuOH, and the BuOH fraction (6.0 g) was subjected to reverse-phase MPLC column chromatography (YMC gel ODS-A, 60 Å, 230 mesh) using a step gradient solvent system from 30% to 100% MeOH in 21 fractions. Fraction 8 (136.5 mg) was first purified on a SHodex-packed NH-5E column using 85% MeOH as eluent and further purified on a YMC-packed C-8 column (250 × 10 mm, 5 μm, 12 nm) using 35% MeOH as eluent to afford four components (1–4). However, 1 and 2 were interconvertible and 1.6 mg of an equilibrium mixture was obtained. Likewise, 3 and 4 were interconvertible and 2.0 mg of an equilibrium mixture was obtained.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.05.097.

| Table 2  |
|-----------|
| **13C** (100 MHz, CD3OD) NMR data for compounds 1–4** |
| Position | 1 | 2 | 3 | 4 |
| 1 | 145.8 | 145.8 | 145.8 | 145.8 |
| 2 | 149.4 | 149.4 | 149.3 | 149.3 |
| 3b | 125.5 | 125.5 | 125.5 | 125.5 |
| 4 | 157.7 | 157.7 | 157.7 | 157.7 |
| 5 | 140.2 | 140.3 | 140.2 | 140.2 |
| 6 | 90.2 | 88.6 | 90.2 | 88.6 |
| 7 | 72.6 | 74.9 | 72.4 | 74.5 |
| 8 | 74.6 | 72.9 | 74.7 | 72.6 |
| 9 | 76.9 | 78.3 | 77.0 | 78.2 |
| 10 | 57.5 | 57.4 | 55.0 | 54.5 |
| 11 | 38.0 | 38.1 | 37.3 | 37.3 |
| 12 | 133.8 | 133.8 | 133.8 | 133.8 |
| 13 | 106.8 | 106.8 | 106.8 | 106.8 |
| 14 | 126.7 | 126.7 | 126.7 | 126.7 |
| 15 | 106.0 | 106.0 | 106.0 | 106.0 |
| 16 | 149.4 | 149.4 | 149.3 | 149.3 |
| 17 | 105.0 | 105.0 | 105.4 | 105.0 |
| 18 | 115.8 | 115.8 | 115.8 | 115.8 |
| 19 | 131.9 | 131.9 | 131.8 | 131.8 |
| 20 | 164.5 | 164.5 | 164.5 | 164.5 |

* a Chemical shifts were assigned based on gHSQC and gHMBC spectral data.
  * b No signal was observed in the 13C spectrum.

Two-component (5 and 6) mixtures (Fig. 2D and E, respectively). This finding indicated that compounds 1, 3 and 2, 4 are epimeric at the sulfoxide group. 5′-Deoxy-5′-methylsulfinyladenosine epimers have been isolated from *Ganoderma lucidum*, and reported in onion extracts. Distinct biological activities have also been described for epimers differentiated by sulfoxide-asymmetry. Usually the sulfoxide configuration of natural products is (S). In the present study, we tried to determine the absolute stereochemistry...
Antiviral activity: 

50% cell culture inhibitory dose (log 10 McCID50) was determined for the compounds. Antiviral assays were performed at the Korea Research Institute of Chemical Technology using the virus-induced CPE (cytopathic effect) inhibition assay. 50% cytotoxic concentration (CC50) was defined as the concentration of a compound that protected 50% of the buffer used for all NMR experiments was 50 mM sodium phosphate in D2O (pD 7.4). In each case, reactions with MsrA and compounds were performed in an NMR tube for 2 days at 37 °C before 1H NMR spectroscopy. Reaction mixes were as follows: Compounds 1/2 (mixture) (0.50 mg, 0.91 mmol), DTT (1.0 mg, 4.7 mmol), MsrA (30 µg, in a solution of 50 mM Tris containing 150 mM NaCl), and NMR buffer (400 µL); and Compounds 3/4 (mixture) (0.50 mg, 0.91 mmol), DTT (0.9 mg, 4.2 mmol), MsrA (30 µg, in a solution of 50 mM Tris containing 150 mM NaCl) and NMR buffer (400 µL).