Abstract: The arbocyclic nucleosides aristeromycin and neplanocin have been studied as a source for new antiviral agents. A convenient synthesis of C-5′-truncated 3-deaza-1′,6′-isoneplanocin, which combines the features of antiviral candidates 5′-noraristeromycin and 3-deaza-1′,6′-isoneplanocin is reported from (−)-cyclopentenone to give the two C-4′ epimers of 5′-nor-3-deaza isoneplanocin. Antiviral assays showed activity against the JC virus (EC_{50} = 1.12 \mu M for (4′R)-8; EC_{50} = 59.14 \mu M for (4′S)-7) and inactivity of both compounds against several DNA and RNA viruses. Both compounds lacked cytotoxicity.

Keywords: antivirals; carbocyclic nucleosides; neplanocin; Ullmann reaction

1. Introduction

Emerging and reemerging viral infectious diseases are continuously posing huge threats to global public health and have had a substantial socioeconomic impact. For example, a total of 28,616 confirmed and suspected cases with 11,310 deaths were reported during the 2014–2016 Ebola outbreak [1]. At the end of 2019, a novel coronavirus, named SARS-CoV-2, emerged and has infected 12,970,605 people in 188 countries/regions with 570,220 deaths (as of 13 July 2020 [2]) and continues to increase.

In the search for antiviral countermeasures, repurposed or newly designed nucleosides and nucleotide analogues are serving as a resource for the frontline defense, especially in those urgent situations [3,4]. For instance, BCX 4430 (Galidesivir, a) and GS-5734 (Remdesivir, b) (Figure 1) were developed during the 2014–2016 Ebola outbreak [5]. Because of its activity towards SARS-CoV-2, Remdesivir is being repurposed for treatment in this current pandemic.

Figure 1. Examples of antivirals as nucleosides and nucleotides analogues: (a) BCX 4430 (Galidesivir); (b) GS-5734 (Remdesivir).

Galidesivir (a) and Remdesivir (b) are C-nucleosides with the glycosidic linkage replaced by a more stable C-C bond and, hence, are metabolically stable to hydrolytic and phosphorlytic breakdown,
a relevant feature for nucleoside-based therapeutic candidates [6]. A similar property is seen with carbocyclic nucleosides, such as the naturally occurring aristeromycin (1) and neplanocin A (2), (Figure 2) which possess antibacterial, -parasitic, -viral and -cancer properties [3,7], due, principally, to the non-selective inhibition of S-adenosylhomocysteine hydrolase (SAHase). The therapeutic of 1 and 2 is limited by their cytotoxicity as a result of biomolecular inference by their 5′-phosphate metabolites.

![Structures of carbocyclic nucleosides: 1. Aristeromycin; 2. Neplanocin; 3. 5′-noraristeromycin; 4. 4′-deoxymethylene neplanocin; 5. 5′-norneplanocin.](image1)

To address this undesirable feature, the C-4′ truncated variations (3 and 4) were prepared and found to be effective against a number of viruses and to be non-cytotoxic [8]. A similar modification on neplanocin A (that is, 5) is, however, unlikely due to its enolic structure (red structure in Figure 2).

Another carbocyclic nucleoside structural modification developed in our labs has been the 1′,6′-isoneplanocin series (herein designated as isoneplanocin and represented by the 3-deaza analogue, 6) that displays a broad-based, non-cytotoxic antiviral profile [9]. We have recently desired to combine the features of 3 with 6 and, thus, set 7 and 8 as targets. (Figure 3) These results are reported here.

![Isoneplanocin analogues and designed target compounds: 6. 3-deaza-isoneplanocin; 7. (4′S)-3-deaza-5′-norisoneplanocin; 8. (4′R)-3-deaza-5′-norisoneplanocin.](image2)

2. Results

Ullmann coupling of a vinyl iodide with an adenine moiety is well established in our lab as a powerful synthetic tool for the preparation of 1′,6′-isoneplanocin analogues [9]. For the purposes of this investigation, vinyl halide 11 was foreseen as the requisite building block. Its synthesis (Scheme 1) began with the iodination of protected (−)-cyclopentenone 9, available from ribose [10,11], to 10. Luche reduction of 10 to allylic alcohol 11, which, upon acid catalyzed isopropylidene rearrangement was expected [12] to provide 12 but resulted in an inseparable mixture with unreacted 11. As a consequence, this mixture was subjected to the Ullmann conditions with 3-deazaadenine [13], and a low yield of 13 (that is, its protected form, 8) occurred.
Our attention turned to employing the Ullmann coupling of 11 and 3-deazaadenine. This succeeded in giving 14 (Scheme 2) in a moderate yield in contrast to 12, suggesting a hydroxyl substituent adjacent to the vinyl coupling site was necessary for the Ullmann to succeed. Acid deprotection of 14 availed the desired (4′R)-8. In addition to NMR data, the structure of 8 was confirmed by X-ray crystallography (CCDC 2018731), which served to confirm the regiochemistry of the cyclopentenyl and the 3-deaza base of 8 (Supplementary Materials).

Scheme 2. Reagents and conditions: (a) 3-deazaadenine, K₂CO₃, dipivaloylmethane, CuI, 120 °C, overnight, 51%; (b) 2 M HCl/MeOH, rt., 1 h, 85%; (c) HCl, MeOH, 13, 42%; (56% recovered 14); rt., overnight. (d) Ph₃P, DIAD, benzoic acid, THF, rt., 12 h, 65%; (e) LiOH, THF-H₂O (1:1), rt., 6 h, 95%; (f) HCl, MeOH, rt., overnight, 90%.

To achieve epimer 7, acid catalyzed isopropylidene rearrangement of 14 to 13 was followed by a Mitsunobu C-4′ inversion to 15. Basic removal of the benzoate of 15 to 16 and subsequent acid deprotection yielded (4′S)-7.

3. Discussion

Compounds 7 and 8 were subjected to antiviral assays [14]. Compound 8 displayed potent activity (EC₅₀ = 1.12 μM) against the JC virus, a polyomavirus. Compound 7 had much lower activity (EC₅₀ = 59.14 μM) against the JC virus. Both epimers showed no cytotoxicity (CC₅₀ > 150 μM) towards the host COS7 cell-line. There was no activity for either compound against human cytomegalovirus, adenovirus, vaccinia virus, Epstein–Barr virus and human norovirus. No cytotoxicity was found as a result of these assays.

Further studies will consider variations of 8 for improving its JC antiviral potential, correlating its enzymatic effects (for example, towards SAHase) with the parent 6, and its usefulness for developing novel C-4′ hydroxyl-based analogues within the 3-deazaisoneplanocin series.
4. Materials and Methods

General Procedure of Ullmann Reaction

Vinyl iodide (1 mmol) was dissolved in DMSO (10 mL) under N₂. 3-Deazaadenine (1.25 mmol), K₂CO₃ (117 mg), dipivaloylmethane (DPM) (27 µL) and Cul (13 mg) were added in sequence. The reaction was heated to 120 °C in an oil bath overnight. The solvent was evaporated under vacuum and the residue was purified by column chromatography (EtOAc:hexanes = 1:1).

(1S,2R,3S)-4-(4-amino-1H-imidazo[4,5-c]pyridin-1-yl)cyclopent-4-ene-1,2,3-triol ((4′R)-7): ¹H NMR (500.3 MHz, D₂O) δ 8.42 (s, 1H), 7.61 (d, J = 7.0 Hz, 1H), 7.23 (d, J = 7.0 Hz, 1H), 6.26 (d, J = 2.0 Hz, 1H), 5.03 (m, 1H), 4.85 (m, 1H), 4.11 (t, J = 5.0 Hz, 1H); ¹³C NMR (125.8 MHz, D₂O) δ 151.3, 141.5, 140.8, 139.2, 138.3, 126.3, 121.2, 100.1, 71.8, 71.5, 70.5. Analogue was calculated for C₁₁H₁₅N₄O₃: C, 53.22; H, 4.87; N, 22.57. Found: C, 53.01; H, 4.94; N, 22.29.

(1R,2R,3S)-4-(4-amino-1H-imidazo[4,5-c]pyridin-1-yl)cyclopent-4-ene-1,2,3-triol ((4′S)-7): ¹H NMR (500.3 MHz, DMSO-δ₆) δ 8.34 (s, 1H), 7.76 (d, J = 6.0 Hz, 1H), 7.31 (d, J = 6.0 Hz, 1H), 6.27 (s, 2H), 6.15 (d, J = 2.0 Hz, 1H), 5.13 (d, J = 8.0 Hz, 1H), 4.84 (m, 2H), 4.54 (d, J = 7.5 Hz, 1H), 4.49 (m, 1H), 4.12 (m, 1H). ¹³C NMR (125.8 MHz, DMSO-δ₆) δ 152.6, 141.7, 139.8, 139.5, 137.0, 126.4, 118.6, 98.1, 71.7, 71.3, 70.0. HRMS (ESI) was calculated for C₁₁H₁₅N₄O₃: 249.0988. Found (M + H)⁺ 249.0987.

Supplementary Materials: The following are available online. Figure S1: ¹HNMR spectrum of 7, Figure S2: ¹³C NMR spectrum of 7, Figure S3: ¹H NMR spectrum of 8, Figure S4: ¹³C NMR spectrum of 8, Figure S5: X-ray crystallography of 8, crystallographic data (excluding structure factors) is available in the Cambridge Crystallographic Data Centre, CCDC 2018731.

Author Contributions: Investigation, Q.C., S.W.S., C.L., K.L.J., T.S.; Writing—review & editing Q.C., S.W.S.

We are grateful to Slippery Rock University and Auburn University for support of this research. We thank Phani Pokkuluri at Auburn University for the X-ray crystallography data. We are indebted to the NIAID in vitro assay team for the viral data presented herein: Don Smee, Utah State University; Brent Korba, Georgetown University; Mark Prichard, University of Alabama—Birmingham; Michael Murray, Southern Research Institute; and Mark Lewis, Bioqual, Inc.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Centers of Disease Control and Prevention. Available online: https://www.cdc.gov/vhf/ebola/index.html (accessed on 13 July 2020).
2. Johns Hopkins University: Coronavirus Resource Center. Available online: https://coronavirus.jhu.edu/us-map (accessed on 13 July 2020).
3. Yates, M.K.; Seley-Radtke, K.L. The evolution of nucleoside analogue antivirals: A review for chemists and non-chemists. Part I: Early structural modifications to the nucleoside scaffold. Antiviral Res. 2018, 154, 66–86.
4. Yates, M.K.; Seley-Radtke, K.L. The evolution of antiviral nucleoside analogues: A review for chemists and non-chemists. Part II: Complex modifications to the nucleoside scaffold. Antiviral Res. 2019, 162, 5–21. [CrossRef] [PubMed]
5. Liu, C.; Zhou, Q.; Li, Y.; Garner, L.V.; Watkins, S.P.; Carter, L.J.; Smoot, J.; Gregg, A.C.; Daniels, A.D.; Jervey, S.; et al. Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. ACS Cent. Sci. 2020, 6, 315–331. [CrossRef] [PubMed]
6. Clercq, E.D. New nucleoside analogues for the treatment of hemorrhagic fever virus infections. Chem. Asian J. 2019, 14, 3962–3968. [CrossRef] [PubMed]
7. Wolfe, M.S.; Borchardt, R.T. S-Adenosyl-L-homocysteine hydrolase as a target for antiviral chemotherapy. J. Med. Chem. 1991, 34, 1521–1530. [CrossRef] [PubMed]
8. Seley, K.L.; Schneller, S.W. Does the anti-hepatitis B virus activity of (+)-5′-noraristeromycin exist in its 4′-epimer and 4′-deoxygenated derivatives? J. Med. Chem. 1998, 41, 2168–2170. [CrossRef] [PubMed]
9. Liu, C.; Chen, Q.; Schenller, S.W. Enantiomeric 3-deaza-1′,6′-isoneplanocin and its 3-bromo analogues: Synthesis by the Ullmann reaction and their antiviral properties. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 928–930. [CrossRef]

10. Yang, M.; Ye, W.; Schneller, S.W. Preparation of carbocyclic S-adenosylazamethionine accompanied by a practical synthesis of (−)-aristeromycin. *J. Org. Chem.* **2004**, *69*, 3993–3996. [CrossRef]

11. Liu, C.; Chen, Q.; Schneller, S.W. Both enantiomers of 6′-Isoneplanocin. *Nucleos. Nucleot. Nucl.* **2017**, *36*, 631–636. [CrossRef] [PubMed]

12. Chen, Q.; Smith, A. L-Like 3-deazaneplanocin analogues: Synthesis and antiviral properties. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 1266. [CrossRef]

13. Chen, Q.; Davidson, A. Synthesis, conformational study and antiviral activity of L-Like neplanocin derivatives. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 4436–4439. [CrossRef] [PubMed]

14. Liu, C.; Coleman, R.; Archer, A.; Hussein, I.; Bowlin, T.L.; Chen, Q.; Schneller, S.W. Enantiomeric 4′-Truncated 3-deaza-1′, 6′-isoneplanocins: Synthesis and antiviral properties including Ebola. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 2480–2482. [CrossRef] [PubMed]

**Sample Availability:** Samples of the compounds are not available from the authors.