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S-Aryltriazole acyclonucleosides: Synthesis and biological evaluation against hepatitis C virus

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ABSTRACT

Novel *S*-aryltriazole acyclonucleosides were designed as structural analogs based on the previously identified antiviral aryltriazole acyclonucleosides in our laboratories. These *S*-aryltriazole nucleosides were synthesized in excellent yields via S_NAr-mediated S-arylation, followed by subsequent ammonolysis. X-ray structural analysis revealed special structural feature brought by the S-linkage, which may represent an unfavorable factor contributing to the lack of anti-HCV activity for this family of triazole nucleosides.

Synthetic nucleoside mimics with modified nucleobase and/or sugar moieties are of considerable importance in the search for new structural leads exhibiting biologically interesting activity.[1] Appending aromatic systems to a nucleobase has been shown to create nucleosides with unique properties and biological activities. The resulting enlarged aromatic system may confer stronger and more efficient binding properties to the corresponding nucleoside for interaction with biological targets, leading to novel mechanism of action. One successful example is HEPT (Fig. 1) an acyclonucleoside analog displaying the potent and selective anti-HIV activity.[2] Of particular importance is the appended phenylthio group which is attached on the pyrimidine nucleobase of HEPT at the 6-position. This aryl interacts with the amino acid residues Tyr188 and Leu100 of the HIV reverse transcriptase enzyme, leading to the conformational change and consequent inhibition of HIV replication. The mode of action for HEPT is therefore completely different from that of the conventional nucleoside analogs.[3]

Over the last five years we have been interested in developing structurally novel and diverse triazole nucleosides bearing aromatic systems anchored onto the triazole nucleobases.[4–12] By

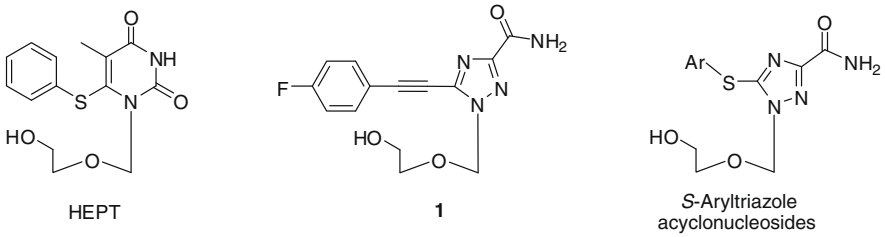

Figure 1. Chemical structure of HEPT, **1** and proposed *S*-aryltriazole acyclonucleoside compounds.

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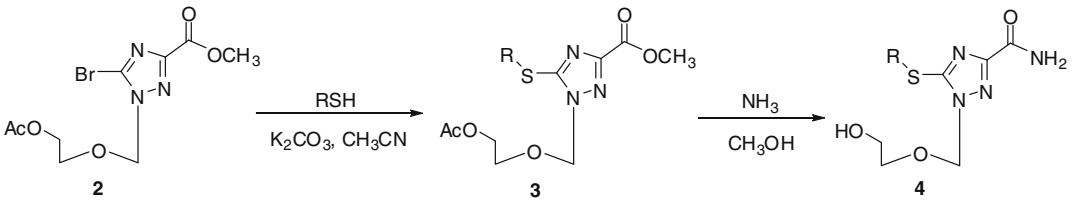
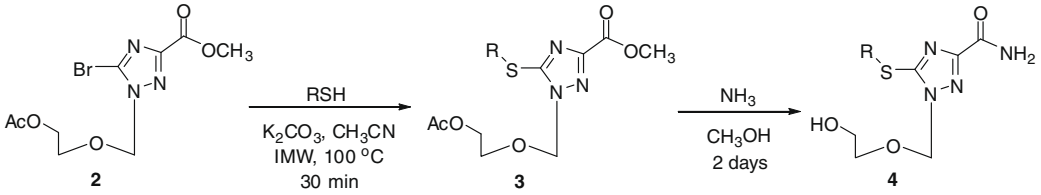
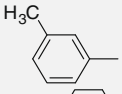
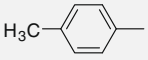
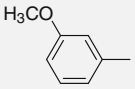
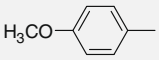
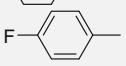
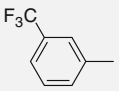
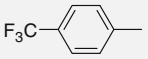
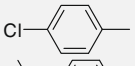
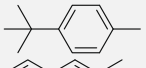
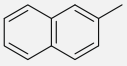
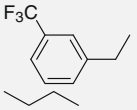


Scheme 1. Synthesis of *S*-aryltriazole acyclonucleosides **3** and **4** starting with **2** followed by subsequent ammonolysis.

Table 1
Synthesis of *S*-aryltriazole acyclonucleosides **3** and **4** via direct S-arylation on **2** followed by subsequent ammonolysis.



Entry	R	Products	Yields (%)	Products	Yields (%)
1		**3a**	99	**4a**	84
2		**3b**	91	**4b**	99
3		**3c**	98	**4c**	95
4		**3d**	97	**4d**	73
5		**3e**	98	**4e**	99
6		**3f**	93	**4f**	67
7		**3g**	99	**4g**	81
8		**3h**	99	**4h**	94
9		**3i**	96	**4i**	99
10		**3j**	92	**4j**	79
11		**3k**	98	**4k**	77
12		**3l**	96	**4l**	61
13		**3m**	92	**4m**	85
14		**3n**	79	**4n**	80
15		**3o**	92	**4o**	95
16		**3p**	93	**4p**	76
17		**3q**	93	**4q**	63
18		**3r**	96	**4r**	86

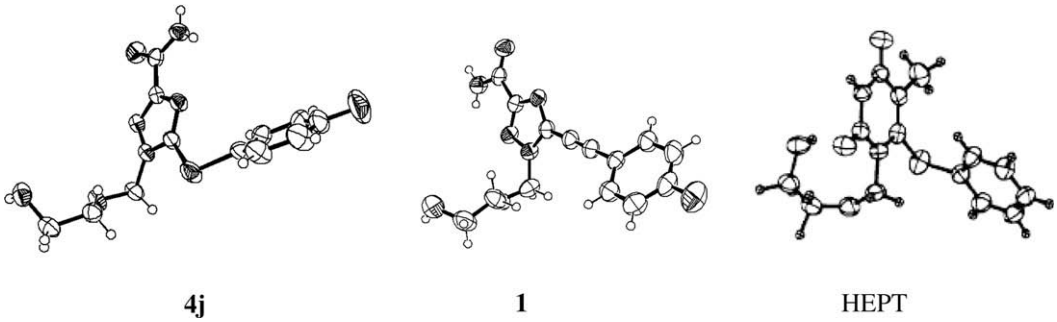


Figure 2. Crystal structure of **4j** in comparison with those of **1**[6] and HEPT.[2]

combining the intrinsic properties of the triazole ring[13] with the appended aromatic moieties in an enlarged aromatic system,[14] we aimed to identify novel triazole nucleoside analogs displaying advantageous binding properties with the corresponding biological targets via aromatic interactions and thus novel mechanism of action. Some of these compounds indeed show promising antiviral and anticancer activities.[4–8,12b] Of particular interest, acyclic nucleoside lead **1** (Fig. 1) elicits significant in vitro antiviral activity against the hepatitis C virus (HCV).[6]

Based on the identified anti-HCV lead compound **1**[6] and inspired by the structural features of HEPT,[3] we decided to synthesize *S*-aryltriazole acyclonucleosides (Fig. 1) in the view to undertake a study on structure–activity relationship and explore their anti-HCV activity as well. Whereas this family of compounds do share some features with **1**, that is, the presence of aryl appended triazole heterocycle as the nucleobase, there are some structural differences, that is, the aryl moiety is linked to the triazole ring via structurally flexible S-linkage rather than the rigid triple bond bridge in **1**. In this way, they may resemble HEPT which has an aryl moiety appended on the nucleobase via S-linkage. Here we report the synthesis and the anti-HCV activity of these *S*-aryltriazole acyclonucleosides.

The synthesis of the corresponding S-arylation nucleosides was performed via S_NAr-mediated S-arylation reaction between the bromotriazole nucleoside **2**[10] and various thiols to provide the nucleosides **3**,[15] which were then subjected to subsequent ammonolysis to furnish the corresponding deprotected nucleosides **4** (Scheme 1).[16]

We first optimized the S-arylation of **2** using 4-methylbenzenethiol as a thiol counterpart. We were pleased to find that the S-arylation proceeded readily, which was in line with the results observed by Liu and Robins for S-arylation on 6-halopurine nucleoside analogs.[17] By systematically varying the bases (K_2CO_3, Na_2CO_3, Li_2CO_3, triethylamine, etc.), solvents (CH_3CN, THF, toluene etc.), reaction temperature and microwave irradiation duration (data not shown), we determined the most suitable conditions for S-arylation, namely, treating **2** with thiol in the presence of two equivalent K_2CO_3 in CH_3CN under microwave irradiation at 100 °C for 30 min. Most S-arylation reactions proceeded in almost quantitative yields,[15] except for that with 4-chlorothiophenol (Table 1, entry 14). The relatively lower yield for **3n** was probably due to the incomplete transformation, for a small amount of **2** remained in the reaction mixture, as revealed by TLC analysis. It should be noted that the presence of the electron-donating (Table 1, entries 5–7) or electron-withdrawing groups (Table 1, entries 8–13) on thiophenol did not significantly affect the yield of S-arylation, and that no steric effect was observed (Table 1, entries 2, 5, and 11). Alkyl thiols also gave excellent yields (Table 1, entries 17 and 18). The overall high-yielding S-arylation was significantly different from the N-arylation on **2**.[12a] This difference can be mainly ascribed to the much stronger nucleophilicity of the thiol compared to the amine functionality.

Further treatment of **3** in NH_3/MeOH at room temperature resulted in the deprotection of the sugar moiety and amination of the carboxylester group, affording the corresponding deprotected nucleoside **4** in good to excellent yields (Table 1).[16] Some compounds were obtained in only moderate yields due to their relatively poor solubility leading to considerable loss during the purification process.

Finally, we obtained single crystals of **4j** and determined its structure by X-ray analysis (Fig. 2).[18] To our great surprise, the phenyl ring in **4j** adopted very different orientations compared to that in **1** and in HEPT. In addition, the carboxylamide group in **4j** also projected in an almost opposite direction with respect to that in **1**. All these structural deviations can be directly ascribed to the S-linkage introduced between the appended aryl group and triazole ring in **4j**.

All the synthesized *S*-aryltriazole acyclonucleosides were subjected to an antiviral assay against hepatitis C virus using a HCV subgenomic RNA replicon test in Huh-5-2 cells.[19,20] Unfortunately, none of the synthesized compounds elicited any notable antiviral activity. Based on earlier identified anti-HCV activity of lead **1** as well as on the different structural features revealed by the crystal structures of **4j**, **1** and HEPT, we can reasonably assume that the S-linkage introduced between the appended aromatic groups and triazole ring might represent an unfavorable factor contributing to the lack of anti-HCV activity.

In conclusion, we synthesized and characterized a novel family of S-arylated triazole acyclonucleosides. The aromatic groups were introduced directly at the 5-position on the triazole ring via a simple and efficient S_NAr-mediated S-arylation procedure, giving the corresponding products in excellent yields. Due to the special structure feature brought by the S-linkage, none of the synthesized S-arylated triazole nucleosides elicited any significant antiviral activity against HCV. However, the easy and efficient S-arylation reaction presented in this study will allow us to prepare structurally diverse *S*-aryltriazole nucleoside analogs in the view to undertake a detailed structure–activity relationship analysis in our future drug discovery program based on triazole nucleoside analogs.

Acknowledgments

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correction of manuscript. Dr. Yi Xia is supported by a post-doctoral fellowship from la Fondation pour la Recherche Médicale.

Supplementary data

^{1}H NMR and ^{13}C NMR spectra of all the new compounds described. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.115.

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15. *General procedure for preparing* ***3****:* Methyl 1-((2-acetoxyethoxy)methyl)-5-bromo-1H-1,2,4-triazole-3-carboxylate (32.2 mg, 0.1 mmol), the corresponding thiol (0.12 mmol), and K_2CO_3 (27.6 mg, 0.2 mmol) were suspended in 2 mL of fresh distilled CH_3CN under argon. The vessel was sealed and the reaction was carried out under microwave irradiation at 100 °C for 30 min, and then cooled to room temperature. The reaction mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 2:1). The purified material was dried in vacuo to afford the corresponding product **3** as colorless oil.
16. *General procedure for preparing* ***4****:* The corresponding starting material **3** was dissolved in 12 mL of a saturated NH_3/MeOH solution and stirred at room temperature for 2 days. Then the solvent was removed and the residue was washed three times with CH_2Cl_2. The washed residue was dried in vacuo to afford the corresponding product **4**.
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18. Single crystals of product **4j** suitable for X-ray crystallographic analysis were obtained via slow evaporation of CH_2Cl_2–CH_3OH solution. Crystallographic data of **4j**: $M_w = 312.32$, monoclinic, space group $P2(1)/n$, $a = 7.3609(9)$ Å, $b = 27.335(3)$ Å, $c = 7.7357(9)$ Å, $\alpha = 90.00°$, $\beta = 117.268(2)°$, $\gamma = 90.00°$, $V = 1383.5(3)$ Å^3, $Z = 4$, $D_{calcd} = 1.499$ g/cm^3, $\mu = 0.262$ mm^{-1}, $R_1 = 0.0330$ and $wR_2 = 0.0712$ ($I > 2\sigma(I)$), GOF = 1.005. CCDC 762236 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Center, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
19. *Anti-HCV assay in Huh-5-2 cells*: Huh-5-2 cells were seeded at a density of 5000 per well in a tissue culture-treated white 96-well view plate (Packard, Canberra, Canada) in complete Dulbecco's modified Eagle's medium (DMEM) supplemented with 250 μg/mL G418. After incubation for 24 h at 37 °C (5% CO_2), medium was removed and threefold serial dilutions in complete DMEM (without G418) of the test compounds were added in a total volume of 100 μL. After 4 days of incubation at 37 °C, cell culture medium was removed and luciferase activity was determined using the Steady-Glo luciferase assay system (Promega, Leiden, The Netherlands); the luciferase signal was measured using a Luminoskan ascent (Thermo, Vantaa, Finland).
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