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Synthesis and biological evaluation of novel neamine–nucleoside conjugates potentially targeting to RNAs

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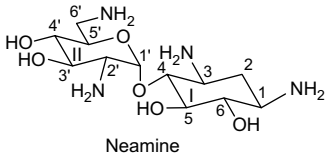
ABSTRACT

Eighteen novel neamine–nucleoside conjugates with ethylenediamine–lysine or ethylenediamine–arginine as the linker were synthesized and their potential binding to A site of 16S RNA and TAR RNA was evaluated using SPR (surface plasmon resonance). Compared with neamine, compounds **10i** and **10q** show 6.3 and 4.8 times potential in binding to A site of 16S RNA and eight and six times potential in binding to TAR RNA, respectively. According to the data of SPR, it indicates that amino acid residue and nucleobase moieties of the designed neamine–nucleosides conjugates exhibit the important contributions for the binding to A site of 16S RNA and TAR RNA. The molecular docking study on the interaction between the ligands and A site of 16S RNA is in agreement with the experimental data. The novel type of modification may provide a promising way for the development of neamine derivatives effectively targeting to RNAs.

1. Introduction

With the research advances in the clearance of RNA structure and its important function in the life cycle of pathogenic microorganisms and the processing of diseases, RNA has been viewed as an important small molecular drug target for finding potential drugs.[1,2] Aminoglycoside antibiotic family including neomycin, kanamycin, paramomycin, and so on has been known to bind potentially to many important RNAs such as bacterial A site of 16S rRNA,[3,4] HIV-1 transactivation response element (TAR) RNA,[5] HIV-1 Rev responsive element (RRE) RNA,[6] group I introns,[7] and the hammerhead ribozyme.[8] But the high toxicity, which mainly resulted from the nonspecific electrostatic interactions limited their clinic use at a large degree,[9] and also, aminoglycosides are prone to lead to drug resistance because of their own structural instability and the modification of aminoglycoside-modifying enzymes.[10] The detailed study has shown that neamine (Fig. 1), as the common part of neomycin-class aminoglycosides, is the minimum motif that can specifically bind to the A site of 16S RNA and affect the translation procedure in protein synthesis.[4] It seems a promising way to keep the neamine core and develop novel potential neamine derivatives. Up to now, many efforts have been made to optimize the core structure neamine, among which neamine–peptide or nucleobase conjugates have been proved to be a convenient and effective strategy to improve the binding to RNAs. The neamine conjugates with arginine/lysine,[11,12] arginine peptide,[13] nucleobase,[14] aromatic rings,[15] peptide nucleic acid,[16] and alkylamines[17] can enhance its binding affinity and some of them have been showed certain binding selectivity. Until now there are no general rules for the design of the specific as well as potential RNA-binding small molecules, and all of the reported aminoglycoside conjugates were designed by connecting neamine with peptide or nucleobase directly to one of amino groups on the aminosugar moiety.



Neamine

Figure 1. Structure of neamine.

Previously, two types of neamine–nucleoside conjugates were synthesized from our group by the condensation of azidodisaccharide and nucleoside using ethylenediamine as a linker. Results from the data of SPR evaluation suggested that the nucleobase played a significant role to bind to A site of 16S RNA and an ethylenediamine

Abbreviations: Ac, acetyl; Arg, argnine; BAIB, [bis(acetoxy)-iodo]benzene; BOC, *tert*-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; DMF, *N*,*N*-dimethyl formamide; DMSO, dimethyl sulfoxide; Eda, ethylenediamine; Et, ethyl; Fmoc, 9-fluorenylmethyloxycarbonyl; HOBT, 1-hydroxybenzotriazole; Lys, lysine; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; Py, pyridine; TEMPO, 2,2,4,6-tetramethyl-1-piperidinyloxyl; rt, room tempreture; THF, tetrahydrofuranyl; Tf_2O, trifluoromethanesulfonic anhydride.

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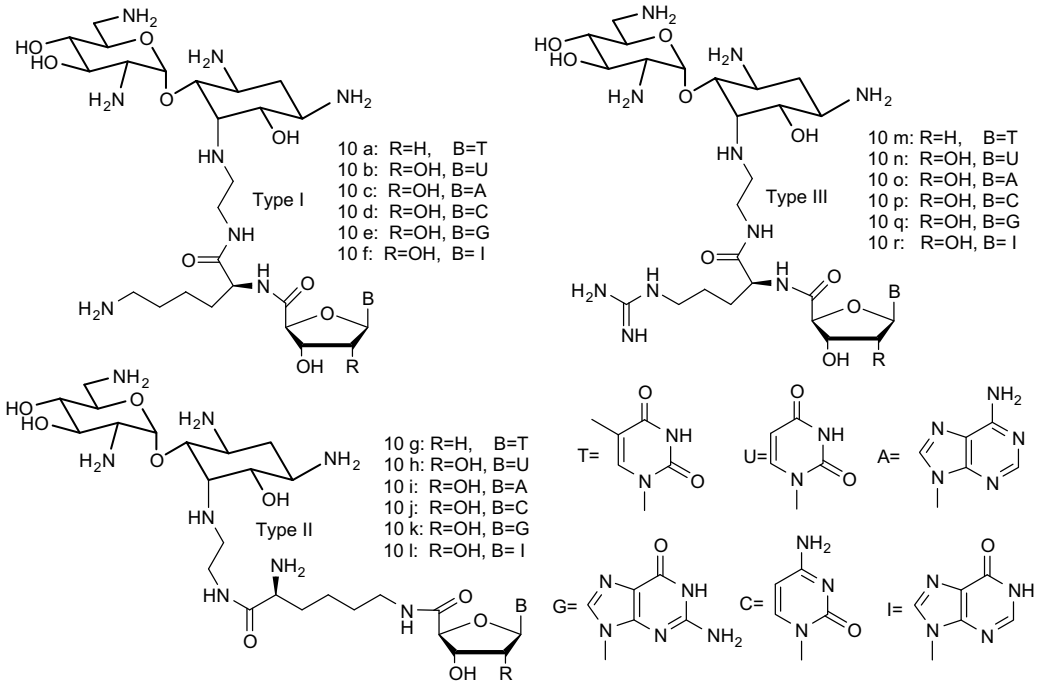


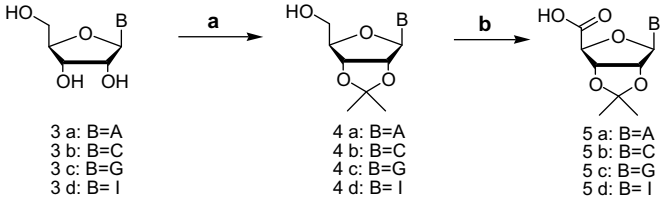
Figure 2. Structures of neamine–nucleoside conjugates.

linker between the neamine and nucleoside was favorable for this binding.[18] In this report we prepared a new class of neamine–nucleoside conjugates (Fig. 2), in which an ethylenediamine–amino acid was used as linker for the modification of the 5-hydroxyl group of neamine by nucleosides. The amino groups on neamine play an important role in their binding to the target RNAs. Keeping the amino group free in the neamine molecule and modification of other positions of neamine may lead the derivatives contributing a various interaction to RNA. Compounds **10a**–**r** were designed using a flexible side chain to connect the neamine and nucleosides, which were expected to recognize the specific site of RNA. Moreover, lysines and arginines are often present in the RNA-binding proteins (for example, HIV-1 transactiviting (Tat) proteins and regulator of expression of virion (Rev) proteins),[19,20] neamine–arginine and neamine–lysine conjugates both enhance the inhibition of HIV-1 TAR-Tat interaction compared with their mother compound neamine.[11] Based on these backgrounds, three types of neamine–nucleoside conjugates were synthesized: *type I* (compounds **10a**–**f**) consists in neamine derivatives with ethylenediamine–lysine as the linker and nucleosides connecting at the α-NH_2 group of lysine; *type II* (compounds **10g**–**l**) is the similar structure as type II but the nucleosides are condensed with the ε-NH_2 group of lysine; *type III* (compounds **10m**–**r**) is formed by a number of neamine derivatives with ethylenediamine–arginine as the linker and nucleoside connecting at the α-NH_2 group of arginine. The interactions between compounds **10a**–**r** with the A site of 16S RNA and TAR RNA were evaluated by dissociation constants (K_D values in μM) using SPR method. The dissociation constants clearly indicated that three types of neamine–nucleoside conjugates showed the better binding properties to 16S RNA and TAR RNA than the neamine derivatives reported previously.[18]

2. Results and discussion

2.1. Synthesis of neamine–nucleoside conjugates 10a–r

Six protected 5′-carboxylic acid-nucleosides **2a**, **b**, **5a**–**d** were synthesized (Scheme 1, Fig. 3), among which **2a** and **2b** were



Scheme 1. Synthesis of protected 5′-carboxylic acid-nucleosides **5a**, **5b**, **5c**, and **5d**. Reagents and conditions: (a) CH_3COCH_3, 70% $HClO_4$, rt; (b) BAIB, TEMPO, CH_3CN, H_2O, rt.

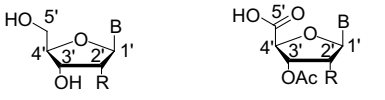


Figure 3. Structures of protected 5′-carboxylic acid-nucleosides **2a** and **2b**.

prepared from compounds **1a** and **1b** according to the published procedures.[18] Compounds **5a**, **5b**, **5c**, and **5d** were synthesized from the nucleosides **3a**–**d**. After the protection of 2′- and 3′-hydroxyl group by isopropylidene group, the nucleoside intermediates **4a**, **4b**, **4c**, and **4d** were obtained in good yield. Compounds **4a**–**d** were oxidized by BAIB and TEMPO in mild conditions to afford the desired 5′-carboxylic acid-nucleosides **5a**, **5b**, **5c**, and **5d** in 75–82% yields.

For the synthesis of compounds of types I–III (Schemes 2 and 3), compound **6** was prepared by the reported procedure and used as the starting material.[18] Compounds **8a** and **8b** were synthesized by the condensation reaction of compound **6** with Boc-Lys(Fmoc)-OH to give compound **7a** first. For compound **8a**, the Boc-protection group on α-NH_2 group of lysine was selectively removed by CF_3COOH in CH_2Cl_2 (1:4) and compound **8b** was obtained by the removal of Fmoc-protection group on ε-NH_2 group of lysine moiety using Et_2NH in DMF (1:9). Compound **6** was condensed with Fmoc-Arg(Pbf)-OH to yield **7b**, after the removal of the Fmoc group on the α-NH_2 group of arginine using the same procedure as the