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Original article

Neamine-heterocycle conjugates as potential anti-HIV agents

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ABSTRACT

A series of neamine-heterocycle conjugates were designed and synthesized. All new compounds displayed more potent inhibitory effect on HIV replication than neamine, among them two compounds displayed stronger anti-HIV activity than neomycin B. The results suggested that it might be a promising approach to modify neamine for the discovery of new anti-HIV agents.

1. Introduction

Aminoglycosides, usually binding to a variety of RNAs, are a large family of clinically important antibiotics effective against a broad range of bacteria [1]. They are multiply charged compounds of high flexibility. The positive charges are attracted to the negatively charged RNA backbone. The flexibility of aminoglycosides facilitates accommodation into a binding pocket for making specific contacts. Among them, neomycin B (Fig. 1) has been proved to display anti-HIV activity [2]. Further investigations suggested that neamine, a component of neomycin B, is the key moiety in the interaction with HIV target RNAs [3]. Neamine is a hydrophilic *pseudo*-disaccharide possessing several amino functionalities that are mostly protonated under physiological conditions and have good cellular uptake ability [4]. By means of electrostatic and H-bond effects, it can bind to RNAs in a sequence-specific fashion [5]. So far many neamine derivatives have been prepared, and their affinities to target RNAs were evaluated by fluorescence titration, surface plasmid resonance (SPR), or other methods. Considerable new compounds exhibited stronger affinities to target RNAs of HIV than neamine [6]. However, previous research on antibacterial evaluation revealed that there is no good correlation between affinity and bioactivity [7], and corresponding data on anti-HIV potency have been less reported, therefore whether these modifications benefit the actual bioactivity remains unknown. Herein we report the design and synthesis of some new neamine-heterocycle conjugates and their bioassay on anti-HIV. In order to avoid the specious results in bioactivity assessment, we observed the potency of these compounds to inhibit the replication of HIV on cell level.

2. Experimental

Non-aqueous reactions were performed under nitrogen atmosphere at room temperature, unless otherwise noted. All commercial reagents were purchased from Aldrich or Acros and were used without further purification. Anhydrous pyridine and dichloromethane were obtained by distilling commercial ones over CaH_2 and anhydrous DMF by distilling over P_2O_5 under reduced pressure. Routine ^1H and ^{13}C nuclear magnetic resonance spectra were recorded. Samples were dissolved in deuterated chloroform ($CDCl_3$) or deuterium oxide (D_2O) and tetramethylsilane (TMS) was used as reference. Elemental analyses were performed for key compounds. Elemental analyses were performed in PE-2400C analyzer. Mass spectra were recorded on PE SCLEX QSTAR mass spectrometer (for low resolution ESI-MS) or Bruker APEX IV mass spectrometer (for high resolution ESI-MS). Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254}. Compounds were visualized by UV light (254 nm) and/or by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL) (for intermediates) or ninhydrin solution in ethyl acetate (5%) followed by heating (for final compounds).

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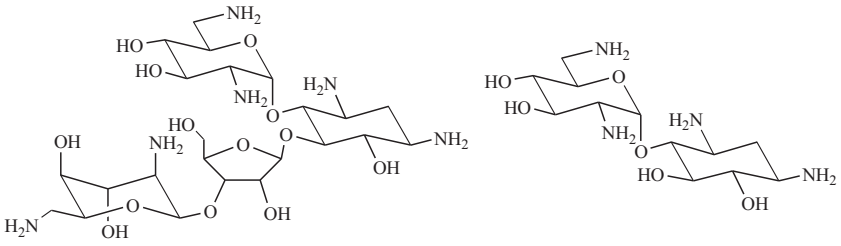


Neomycin B, **1** Neamine, **2**

Fig. 1. The structures of neomycin B and neamine.

*2.1. epi-5-(2-Aminoethyl)-amino-1,3,2′,6′-tetraazido-6,3′,4′-tri-O-acetyl neamine (**14**)*

To a stirring mixture of **13** (300 mg, 0.54 mmol), pyridine (0.2 mL, 2.48 mmol) and dichloromethane (10 mL) was added triflic anhydride (0.18 mL, 1.08 mmol) solution in dichloromethane (1 mL) dropwise by a syringe in a period of 10 min at room temperature. After stirring for 3.5 h, TLC assay showed that compound **13** was consumed. After removal of the solvent of the reaction mixture, the residue was dissolved in 20 mL of ethyl acetate and the resulting solution was washed with brine (2× 60 mL). The organic layer was collected, dried over anhydrous sodium sulfate, and concentrated to foam in vacuo. Then it was dissolved in 5 mL of acetonitrile and to the solution was added ethylene diamine (1 mL) in one portion while stirring. Half an hour later, this reaction mixture was poured into cold water (8 mL) and extracted with dichloromethane (3× 30 mL), and the combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated in vacuum. The residue was purified by column chromatography on silica gel (chloroform/methanol = 30/1) to provide a white foam (200 mg, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.48 (t, 1H, J = 10.0 Hz), 5.10–5.03 (m, 2H), 4.70 (dd, 1H, J_1 = 2.5 Hz, J_2 = 10.0 Hz), 4.32–4.19 (m, 2H), 4.03–3.94 (m, 1H), 3.70–3.61 (m, 2H), 3.47–3.41 (m, 2H), 3.30 (dd, 1H, J_1 = 5.0 Hz, J_2 = 13.5 Hz), 2.98–2.83 (m, 3H), 2.67–2.64 (m, 1H), 2.36 (ddd, 1H, J_1 = 13.5 Hz, J_2 = J_3 = 5.0 Hz), 2.15 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.35 (ddd, 1H, J_1 = J_2 = J_3 = 13.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9, 169.7, 94.3, 77.9, 76.0, 70.9, 69.5, 68.9, 61.3, 57.4, 56.1, 50.6, 50.5, 41.6, 32.2, 20.9, 20.6. HRESI-MS calcd. for $C_{20}H_{30}N_{14}O_8$ $[M+H]^+$: 595.2443; found: 595.2431.

*2.2. epi-5-[2-(6-Hydroxynicotinyl)aminoethyl]-amino-1,3,2′,6′-tetraazido-6,3′,4′-tri-O-acetyl neamine (**15a**)*

Compound **14** (100 mg, 0.17 mmol), 6-hydroxyl-nicotinic acid (35 mg, 0.26 mmol), HBTU (115 mg, 0.39 mmol), DIPEA (68 mg, 0.5 mmol) and DMF (15 mL) were mixed in one portion. After stirring for 12 h, TLC assay showed the reaction was completed. The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel with chloroform/methanol (30/1) as eluent to afford the desired product (92 mg, 76% yield) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.53 (d, 1H, J = 2.1 Hz), 7.90 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.4 Hz), 6.52 (d, 1H, J = 8.4 Hz), 6.44 (br, 1H), 5.48 (t, 1H, J = 9.9 Hz), 5.09–5.03 (m, 2H), 4.77 (s, 2H), 4.72 (dd, 1H, J_1 = 2.5 Hz, J_2 = 10.1 Hz), 4.32–4.26 (m, 1H), 4.12–4.03 (m, 1H), 3.93–3.84 (m, 1H), 3.70–3.61 (m, 3H), 3.48–3.37 (m, 3H), 3.30 (dd, 1H, J_1 = 5.1 Hz, J_2 = 13.5 Hz), 2.98–2.91 (m, 2H), 2.37 (ddd, 1H, J_1 = 13.2 Hz, J_2 = J_3 = 5.0 Hz), 2.13 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.39 (ddd, 1H, J_1 = J_2 = J_3 = 12.5 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.0, 169.9, 169.7, 160.3, 147.5, 137.0, 120.2, 107.9, 94.4, 77.8, 75.8, 70.9, 69.6, 69.0, 61.3, 57.4, 56.2, 55.9, 50.6, 49.5, 40.4, 32.0, 20.9, 20.6. Anal. calcd. for $C_{26}H_{33}N_{15}O_{10}$: C 43.64, H 4.65, N 29.36; found: C 43.56, H 4.87, N 29.19. HRESI-MS calcd. for $C_{26}H_{33}N_{15}O_{10}$ $[M+H]^+$: 716.2608; found: 716.2617.

*2.3. epi-5-[2-(6-Hydroxynicotinyl)aminoethyl)-amino neamine (**5**)*

To a solution of **15a** (78 mg, 0.11 mmol) in methanol (10 mL) was added sodium methoxide solution in methanol (30%, 0.1 mL) while stirring. When TLC showed the reaction was completed, the solvent was removed. After the residue was purified by a short column chromatography on silica gel (eluent: chloroform/methanol = 10/1), the resulting product was dissolved in a mixture of pyridine (3 mL), triethylamine (2 mL), and water (1 mL). Then the mixture was bubbled with H_2S until it turned deep green. After stirring overnight, the solvent was removed in vacuum. The residue was suspended in 1.5 mL of methanol and the resulting mixture was absorbed in a proper quantity of silica gel. After removal of the volatile component, the mixture was transferred onto a short silica gel column. The column was washed with eluents as following: petroleum/ethyl acetate (25 mL/25 mL), ethyl acetate (50 mL), ethyl acetate/methanol (25 mL/25 mL), methanol (50 mL), and methanol/concentrated aqua ammonia (100 mL/10 mL). Finally the desired product was obtained as a white powder (42 mg, 0.09 mmol) in 79% yield over two steps.

*2.4. epi-5-Amino-1,3,2′,6′-tetraazido-6,3′,4′-tri-O-benzyl neamine (**17**)*

Compound **16** (300 mg, 0.43 mmol), pyridine (1.2 mL), and dichloromethane (15 mL) were mixed in one flask. To the solution was added triflic anhydride (1.2 mL, 7.15 mmol) solution in dichloromethane (10 mL) dropwise in a period of 2 h while stirring at room temperature. After completion of the addition of triflic anhydride, the reaction was stirred for another 4 h. Then the reaction mixture was poured into the mixture of ice-water (40 mL) and was stirred for 20 min. The organic layer was separated out, dried over anhydrous sodium sulfate, and then concentrated to residue. After running column chromatography on silica gel with petroleum/ethyl acetate (10/1) as eluent, the crude product was dissolved in acetone (15 mL). Then the solution was cooled down with an ice bath and bubbled with ammonia for 1 h. After stirring for another 1 h, TLC assay showed that all of the triflate was consumed. Then the reaction mixture was concentrated and the resulting residue was subjected to column chromatography on silica gel with petroleum/ethyl acetate (8/1) as eluent. Finally the desired product was obtained (155 mg, 52% yield from **16**) as a white semisolid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39–7.25 (m, 15H), 4.95 (d, 1H, J = 4.0 Hz), 4.90–4.88 (m, 3H), 4.70–4.61 (m, 3H), 4.12–4.02 (m, 4H), 3.80 (t, 1H, J = 3.0 Hz), 3.63–3.50 (m, 3H), 3.41 (dd, 1H, J_1 = 3.5 Hz, J_2 = 10.0 Hz), 3.36 (dd, 1H, J_1 = 4.5 Hz, J_2 = 13.5 Hz), 3.27 (dd, 1H, J_1 = 3.5 Hz, J_2 = 9.5 Hz), 2.23 (ddd, 1H, J_1 = 13.5 Hz, J_2 = J_3 = 5.0 Hz), 1.22 (ddd, 1H, J_1 = J_2 = J_3 = 12.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 137.6, 137.5, 137.3, 128.6, 128.5 (2 C),

128.1, 128.0, 127.9, 127.7, 93.9, 81.5, 80.4, 78.6, 78.1, 75.7, 75.0, 72.2, 71.3, 63.5, 57.6, 57.3, 50.9, 47.9, 32.6. Anal. calcd. for $C_{33}H_{37}N_{13}O_5$: C 56.97, H 5.36, N 26.17; found: C 57.20, H 5.13, N 25.89. ESI-MS calcd. for $C_{33}H_{37}N_{13}O_5$ $[M+H]^+$: 696; found: 696.

2.5. epi-5-[(2-Amino-6-benzyloxy-purine-9-yl) acetyl]-amino-1,3,2′,6′-tetraazido-6,3′,4′-tri-O-benzyl neamine (**18a**)

Compound 17 (52 mg, 0.075 mmol), (2-amino-6-(benzyloxy)-purine-9-yl) acetic acid (27 mg, 0.090 mmol), TBTU (35 mg, 0.108 mmol), triethylamine (45 mg, 0.45 mmol) and DMF (8 mL) were mixed in one flask. After stirring for 12 h, another portion of (2-amino-6-(benzyloxy)-purine-9-yl) acetic acid (14 mg, 0.045 mmol) and TBTU (18 mg, 0.054 mmol) was added to the reaction mixture. The reaction was stirred for another 24 h. After removal of the solvent followed by purification with gradient column chromatography on silica gel (eluent: petroleum/ethyl acetate 2/1 to 1.5/1), the desired product was obtained as white solid (36 mg, 49% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.60 (s, 1H), 7.55 (d, 1H, J = 5.0 Hz), 7.46 (d, 2H, J = 6.0 Hz), 7.36–7.22 (m, 20H), 5.59 (d, 1H, J = 12.5 Hz), 5.55 (d, 1H, J = 12.0 Hz), 5.24 (d, 1H, J = 4.0 Hz), 5.10 (m, 1H), 4.90 (s, 2H), 4.86 (d, 1H, J = 11.0 Hz), 4.80–4.69 (m, 4H), 4.59 (t, 1H, J = 11.5 Hz), 4.41 (d, 1H, J = 11.0 Hz), 3.93 (dd, 1H, J_1 = 9.0 Hz, J_2 = 10.0 Hz), 3.88 (m, 1H), 3.58–3.34 (m, 7H), 3.29 (dd, 1H, J_1 = 5.5 Hz, J_2 = 13.0 Hz), 2.14 (ddd, 1H, J_1 = 13.5 Hz, J_2 = J_3 = 4.5 Hz), 1.23 (ddd, 1H, J_1 = J_2 = J_3 = 13.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.5, 161.5, 159.2, 153.7, 139.8, 137.5, 136.6, 136.0, 128.5 (2C), 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 115.6, 93.6, 79.9, 78.7, 78.4, 75.5, 75.0, 74.6, 71.7, 71.4, 68.7, 63.0, 58.3, 57.9, 51.0, 47.8, 44.9, 31.9. Anal. calcd. for $C_{47}H_{48}N_{18}O_7$: C 57.78, H 4.95, N 25.81; found: C 57.83, H 5.17, N 25.63. ESI-MS calcd. for $C_{47}H_{48}N_{18}O_7$ $[M+H]^+$: 977; found: 977.

2.6. epi-5-(2-Aminoethyl)-amino-1,3,2′,6′-tetraazido-6,3′,4′-tri-O-benzyl neamine (**19a**)

The synthesis of 5-*O*-triflyl-1,3,2′,6′-tetraazido- 6,3′,4′-tri-*O*-benzyl neamine was the same as described in synthesis of **17**. To the triflate (600 mg, 0.72 mmol) solution in acetonitrile (20 mL) was added ethylene diamine (1 mL) while stirring. When TLC showed that all of the triflate was consumed, the solvent was removed and the residue was purified by column chromatography on silica gel. The final product was obtained as a yellow gel (471 mg, 67% overall yield from **16**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38–7.26 (m, 15H), 5.28 (d, 1H, J = 12.0 Hz), 5.02 (d, 1H, J = 3.6 Hz), 4.89 (d, 1H, J = 13.2 Hz), 4.86 (s, 2H), 4.71–4.60 (m, 3H), 4.11–3.98 (m, 4H), 3.59–3.45 (m, 5H), 3.34 (dd, 1H, J_1 = 4.5 Hz, J_2 = 13.5 Hz), 3.26 (dd, 1H, J_1 = 2.4 Hz, J_2 = 9.9 Hz), 2.96–2.89 (m, 1H), 2.82–2.72 (m, 3H), 2.25 (ddd, 1H, J_1 = J_2 = 5.1 Hz, J_3 = 13.2 Hz), 1.19 (ddd, 1H, J_1 = J_2 = J_3 = 13.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.6, 137.5, 137.3, 128.5, 128.1, 128.0, 127.9, 127.7, 93.8, 82.8, 80.2, 78.5, 77.7, 77.2, 75.6, 75.0, 72.4, 71.3, 63.5, 58.0, 54.2, 52.3, 50.9, 42.3, 32.9. HRESI-MS calcd. for $C_{35}H_{42}N_{14}O_5$ $[M+H]^+$: 739.3535; found: 739.3543.

2.7. epi-5-[(Guanine-9-yl)acetyl]-amino neamine (**9**)

Compound **18a** (100 mg) was dissolved in the mixture of pyridine (3 mL), triethylamine (2 mL) and water (0.5 mL). Then, H_2S gas was passed into the mixture until it turned deep green. The reaction mixture was stirred for 10 h at room temperature. The mixture was concentrated and passed through a short silica column with eluents as following: petroleum/ethyl acetate (25 mL/25 mL), ethyl acetate (50 mL), ethyl acetate/methanol (25 mL/25 mL), methanol (50 mL), and methanol/concentrated aqua ammonia (100 mL/10 mL). The crude product was then dissolved in 5 mL of methanol and pH of the resulting solution was adjusted to 3–4 with hydrochloric acid (1 mol/L). Then catalytic amount of Pd/C was added. The mixture was stirred for 5 days under H_2 atmosphere. After TLC showed that the reaction was completed, the solvent was evaporated to give the product as white amorphous powder in 67% yield.

2.8. epi-5-(2-Aminoethyl)-amino neamine (**3**)

A mixture of **19a** (21 mg, 0.028 mmol), triphenylphosphine (60 mg, 0.228 mmol), THF (10 mL) and water (0.5 mL) was heated under reflux for 24 h. Then the solvent was removed and the residue was subjected to a short column chromatography on silica gel with eluents as following: EtOAc (40 mL), EtOAc/MeOH (1/1, 60 mL), MeOH/NH_3(aq) (40/1, 80 mL). The fraction containing amine was collected and concentrated to residue. The residue was dissolved in the mixture of THF (5.6 mL), acetic acid (1.4 mL) and water (1.4 mL). Then catalytic amount of Pd/C was added. The mixture was stirred under H_2 atmosphere for 72 h. The mixture was filtered, and the filtrate was concentrated to afford the desired product in the form of acetate as yellowish amorphous powder (18 mg, 95% yield).

3. Results and discussion

It is known that neamine is a weak antibiotic and can bind to RRE RNA with a low affinity [6c], we wonder whether the introduction of an additional heterocyclic moiety can enhance its activity. So a series of new neamine derivatives, which are characterized by the neamine moiety conjugated with a heterocycle, typically a nucleobase or its mimics, by a linker, were designed (Fig. 2). Except for the inherent electrostatic and H-bond effects of neamine, we anticipated the additional base or its mimics can exert the base–base-like interaction with target RNA, just like it is observed in classic nucleic acids, thus enhancing the anti-HIV activities.

To introduce heterocycles onto neamine, two synthetic routes were employed. The first route used perazidotriacetyl neamine derivative **14** as the key intermediate, as depicted in Scheme 1. Perazidotriacetyl neamine (**13**) [8] was prepared from neamine by an improved diazotransfer procedure [9]. Treatment of compound **13** with triflic anhydride in the presence of pyridine in dichloromethane gave its triflate, which reacted with ethylene diamine to yield **14**. Then heterocyclic carboxylic acids were coupled with **14** to form the corresponding amides **15a**–**d**. Deacetylation of compounds **15a**–**d** which was followed by reduction of azido groups with H_2S provided the target compounds **5**–**8** [10].

For the synthesis of neamine-heterocycle conjugates **9**–**12**, the second approach was used, as outlined in Scheme 2. The benzylated neamine derivative **17** served as the intermediate. Firstly, 3′,4′,6-tribenzylperazido neamine (**16**) was prepared according to the literature procedure [6b]. The triflation of 5-hydroxyl in **16** which was followed by nucleophilic substitution with ammonia afforded intermediate **17**, which was coupled with various heterocyclic carboxylic acids [8,11], yielding the neamine-heterocycle conjugates **18a**–**d**. The target compounds **9**–**12** [12] were accomplished successfully by reduction of azido groups with H_2S which was followed by removal of benzyl groups with palladium-catalyzed hydrogenolysis. By a similar way, two simple neamine derivatives, epi-5-(2-aminoethyl)-amino neamine (**3**) and epi-5-(2-hydroxyethyl)-amino neamine (**4**) were also obtained [13].

In order to test the effects of these compounds on the HIV replication, different compounds were added to each HIV *pseudo*-type virus (HIVpp) infected cells, respectively. HIV *pseudo*-type

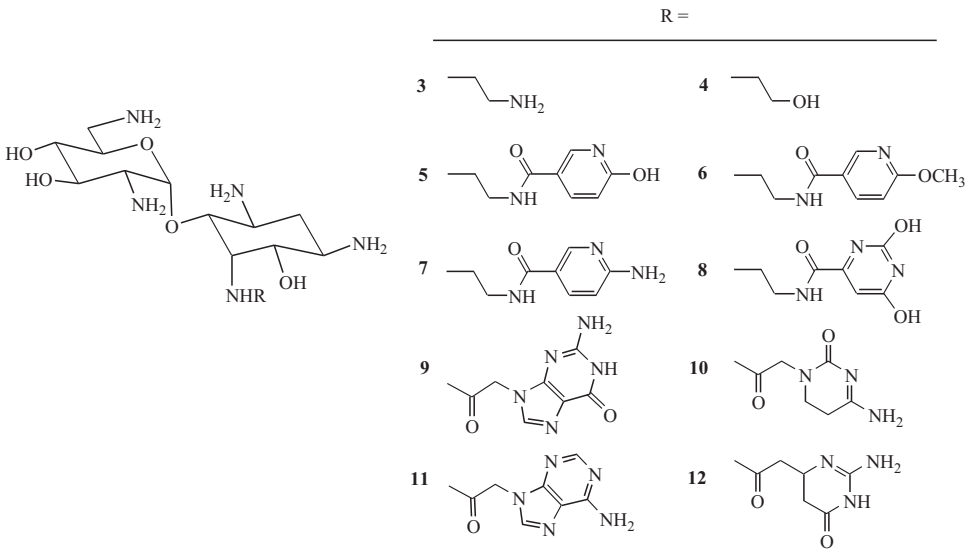
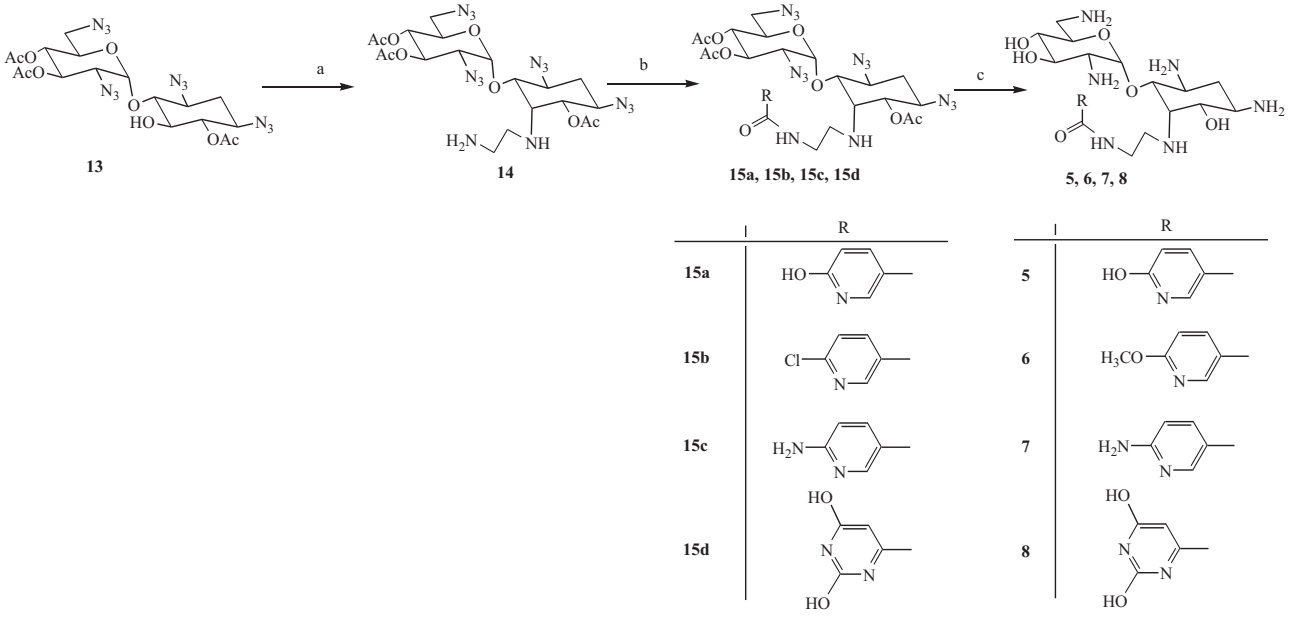


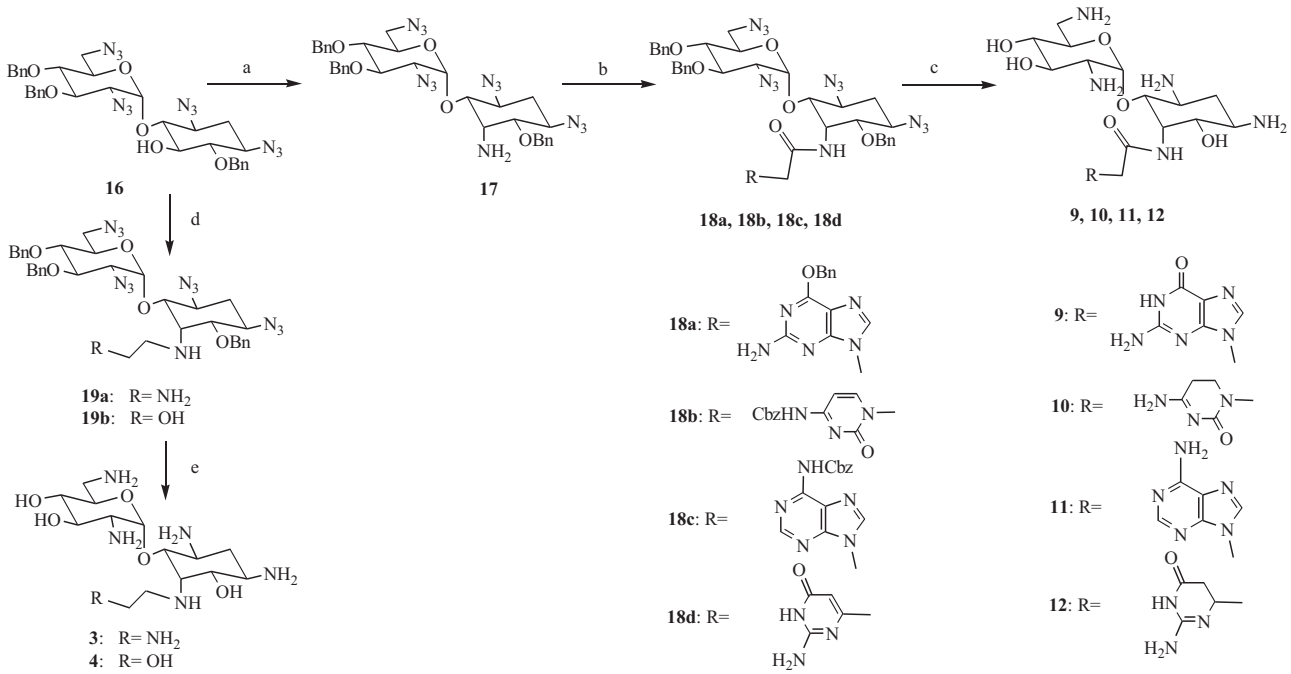
Fig. 2. Structures of the designed neamine derivatives.

virus was produced by co-transfection of HEK 293T cells with a plasmid phRL-null vector (Promega) and a pNL4-3.luc.R$^-$E$^-$ (NIH) containing the env deficient HIV proviral genome and an intact luciferase gene. The phRL-null vector [hRluc/SV40] (Promega) was used as the internal control Renilla luciferase reporter. The replication of the HIVpp in 293T cells was measured through normalizing the transfection efficacy with Luciferase and Renilla luciferase activities. The relative luciferase activities represented the viral replication efficacy of HIVpp. For better understanding the potency of these compounds on anti-HIV, AZT, a well-known anti-HIV drug, was used as a positive control during evaluation.

As shown in Fig. 3, all of the tested compounds displayed inhibitory effects on the HIV replication when comparing with the control group without drugs. Joyfully, all new compounds exhibited stronger potency than neamine, the parent compound. Compounds **9**–**12** with heterocycle-acetyl moieties showed relatively weak inhibitory activities. To our surprise, compounds **3** and **4**, two simply-modified derivatives of neamine, showed a remarkable increase in anti-HIV potency, even beyond some compounds containing heterocycle moiety. Compounds **5**–**7** contain the nicotine moiety substituted by the hydroxyl, methoxy, and amino groups, respectively. Among these three compounds, compound **6** with the methoxy substituent exhibited the strongest potency. Compound **8** with the aminoethyl linkage also showed potent anti-HIV activity. At the same concentration, the suppression effects of **6** and **8** exceeded that of neomycin B. In fact, compound **6** showed the best inhibitory effect on anti-HIV among all compounds with nucleobase-like heterocycles. Generally, our experiments demonstrated that for neamine-heterocycle conjugates, compounds with the aminoethyl linkage showed better anti-HIV activities than the compounds with heterocycle-acetyl moieties.



Scheme 1. Synthesis of neamine-heterocycle conjugates **5**, **6**, **7**, and **8**. Reagents and conditions: (a) (i) Tf_2O, Py, CH_2Cl_2; (ii) ethylene diamine and CH_3CN, 63%; (b) heterocyclic acids, HOBT/HBTU or DCC, DMF, 76% for **15a**, 75% for **15b**, 43% for **15c**, 33% for **15d**; (c) (i) NaOMe, MeOH; (ii) H_2S, pyridine/Et_3N/H_2O, 79% for **5**, 81% for **6**, 85% for **7**, 68% for **8**.



Scheme 2. Synthesis of neamine-heterocycle conjugates **9**, **10**, **11**, **12** and compounds **3**, **4**. Reagents and conditions: (a) (i) Tf_2O, pyridine, CH_2Cl_2; (ii) NH_3, acetone, 52%; (b) heterocyclic acids, HBTU, DIPEA, DMF, 49% for **18a**, 80% for **18b**, 50% for **18c**, 67% for **18d**; (c) (i) H_2S, pyridine/Et_3N/H_2O; (ii) H_2, Pd/C, 67% for **9**, 72% for **10**, 77% for **11**, 61% for **12**; (d) (i) Tf_2O, pyridine, CH_2Cl_2; (ii) 2-amino-ethanol or ethylene diamine, CH_3CN, 67% for **19a**, 63% for **19b**; (e) (i) Ph_3P, THF, H_2O; (ii) H_2, Pd/C, THF/HOAc/H_2O, 95% for **3**, 89% for **4**.

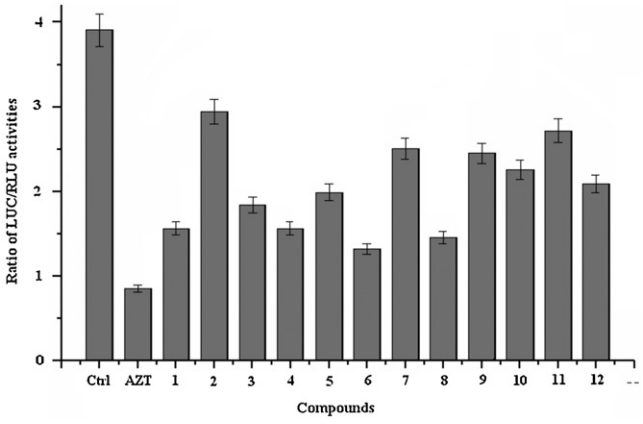


Fig. 3. Anti-HIV activities of the synthetic neamine derivatives. The control (Ctrl) refers to no drugs or compounds added. 2×10^5 293T cells of each well were transfected with 0.8 μg of pNL4-3.luc.R^-E^- and 0.1 μg of phRL-null vector and incubated with 0.2 μmol/L different compounds for 48 h. The experiments were repeated three times. The error bars represent the standard errors of the means. AZT concentration was 65 μmol/L.

4. Conclusion

In conclusion, modifications on neamine resulted in a distinct increase in anti-HIV activities. Among no more than ten synthetic derivatives, two compounds displayed stronger effect than neomycin B. These findings may hold the potential to discover the neamine-modified derivatives with more potent anti-HIV activities.

Acknowledgments

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[10] Compound **5**: ^{1}H NMR (500 MHz, D_2O, in the hydrochloride form): δ 8.35 (d, 1H, J = 2.0 Hz), 8.20 (dd, 1H, J_1 = 2.0 Hz, J_2 = 9.5 Hz), 7.10 (d, 1H, J = 9.5 Hz), 5.73 (d, 1H, J = 3.5 Hz), 4.50 (dd, 1H, J_1 = 3.5 Hz, J_2 = 11.0 Hz), 4.38 (t, 1H, J = 4.0 Hz), 4.34 (dd, 1H, J_1 = 4.0 Hz, J_2 = 11.0 Hz), 4.12 (dd, 1H, J_1 = 9.0 Hz, J_2 = 10.0 Hz), 4.03 (m, 1H), 3.89 (ddd, 1H, J_1 = J_2 = 4.5 Hz, J_3 = 12.0 Hz), 3.84–3.73 (m, 4H), 3.67–3.61 (m, 3H), 3.52 (dd, 1H, J_1 = 3.5 Hz, J_2 = 13.5 Hz), 3.41 (dd, 1H, J_1 = 7.0 Hz, J_2 = 13.5 Hz), 2.66 (ddd, 1H, J_1 = 12.5 Hz, J_2 = J_3 = 4.5 Hz), 2.02 (ddd, 1H, J_1 = J_2 = J_3 = 12.5 Hz). ^{13}C-NMR (125 MHz, D_2O, in the hydrochloride form): δ 168.0, 155.6, 142.2, 137.6, 119.1, 114.7, 92.7, 72.0, 71.3, 70.5, 68.9, 68.4, 58.9, 53.6, 51.8, 48.9, 47.7, 40.4, 38.7, 28.7. HRESI-MS calcd. for $C_{20}H_{35}N_7O_7$ $[M-e]^+$: 485.2593; found: 485.2598. Compound **6**: ^{1}H NMR (300 MHz, D_2O): δ 8.34 (d, 1H, J = 2.1 Hz), 7.91 (dd, 1H, J_1 = 2.4 Hz, J_2 = 9.0 Hz), 6.79 (d, 1H, J = 9.0 Hz), 4.91 (d, 1H, J = 3.6 Hz), 3.80 (s, 1H), 3.56–3.12 (m, 9H), 2.97–2.82 (m, 4H), 2.64 (dd, 1H, J_1 = 6.6 Hz, J_2 = 13.5 Hz), 2.55 (dd, 1H, J_1 = 3.6 Hz, J_2 = 10.5 Hz), 1.84 (ddd, 1H, J_1 = 12.9 Hz, J_2 = J_3 = 4.5 Hz), 0.90 (ddd, 1H, J_1 = J_2 = J_3 = 12.3 Hz). ^{13}C NMR (75 MHz, D_2O): δ 171.2, 168.6, 149.1, 141.4, 126.1, 113.0, 98.0, 81.6, 78.6, 76.2, 75.0, 73.9, 60.9, 57.7, 57.1, 52.7, 50.6, 49.7, 44.1, 42.7, 38.5. HRESI-MS calcd. for $C_{21}H_{37}N_7O_7$ $[M+H]^+$: 500.2827; found: 500.2829. Compound **7**: ^{1}H NMR (500 MHz, D_2O, in the hydrochloride form): δ 8.14 (d, 1H, J = 2.5 Hz), 7.99 (dd, 1H, J_1 = 2.5 Hz, J_2 = 9.5 Hz), 6.67 (d, 1H, J = 9.5 Hz), 5.73 (d, 1H, J = 3.5 Hz), 4.51 (dd, 1H, J_1 = 3.5 Hz, J_2 = 11.0 Hz), 4.44 (t, 1H, J = 4.0 Hz), 4.37 (dd, 1H, J_1 = 4.5 Hz, J_2 = 11.0 Hz), 4.11 (dd, 1H, J_1 = 8.5 Hz, J_2 = 10.0 Hz), 4.04 (m, 1H), 3.91–3.59 (m, 8H), 3.52 (dd, 1H, J_1 = 3.5 Hz, J_2 = 14.0 Hz), 3.41 (dd, 1H, J_1 = 7.0 Hz, J_2 = 14.0 Hz), 2.67 (ddd, 1H, J_1 = 13.0 Hz, J_2 = J_3 = 4.5 Hz), 2.04 (ddd, 1H, J_1 = J_2 = J_3 = 13.0 Hz). ^{13}C NMR (125 MHz, D_2O, in the hydrochloride form): δ 169.6, 165.8, 141.3, 138.6, 119.8, 114.9, 92.7, 71.6, 71.3, 70.4, 69.0, 68.1, 58.8, 53.6, 52.4, 48.9, 47.6, 40.4, 38.6, 28.6. HRESI-MS calcd. for $C_{20}H_{37}N_8O_6$ $[M+H]^+$: 485.2831; found: 485.2820. Compound **8**: ^{1}H NMR (300 MHz, D_2O): δ 6.05 (s, 1H), 4.95 (d, 1H, J = 3.6 Hz), 3.67 (m, 1H), 3.47–2.90 (m, 10H), 2.95–2.85 (m, 3H), 2.60 (dd, 1H, J_1 = 3.6 Hz, J_2 = 10.2 Hz), 1.96 (ddd, 1H, J_1 = 12.6 Hz, J_2 = J_3 = 4.5 Hz), 1.07 (ddd, 1H, J_1 = J_2 = J_3 = 12.3 Hz). ^{13}C NMR (75 MHz, D_2O): δ 170.1, 167.3, 161.4, 159.4, 98.3, 95.6, 78.2, 74.5, 73.7, 71.9, 70.7, 58.1, 55.4, 50.0, 48.9, 47.2, 41.5, 40.3, 34.2. HRESI-MS calcd. for $C_{19}H_{35}N_8O_8$ $[M+H]^+$: 503.2572; found: 503.2575.

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[12] Compound **9**: ^{1}H NMR (500 MHz, D_2O): δ 8.40 (s, 1H), 5.53 (d, 1H, J = 3.0 Hz), 5.26 (d, 1H, J = 17.5 Hz), 5.18 (d, 1H, J = 17.5 Hz), 5.08 (br, 1H), 4.26 (dd, 1H, J_1 = 3.0 Hz, J_2 = 11.0 Hz), 4.09 (dd, 1H, J_1 = 4.0 Hz, J_2 = 11.0 Hz), 4.03 (t, 1H, J = 11.0 Hz), 3.91–3.87 (m, 1H), 3.79 (ddd, 1H, J_1 = 12.0 Hz, J_2 = J_3 = 4.0 Hz), 3.66 (ddd, 1H, J_1 = 12.0 Hz, J_2 = J_3 = 4.5 Hz), 3.53–3.49 (m, 2H), 3.45 (dd, 1H, J_1 = 3.5 Hz, J_2 = 11.0 Hz), 3.32 (dd, 1H, J_1 = 6.0 Hz, J_2 = 14.0 Hz), 2.63 (ddd, 1H, J_1 = 13.0 Hz, J_2 = J_3 = 4.5 Hz), 1.94 (ddd, 1H, J_1 = J_2 = J_3 = 12.5 Hz). ^{13}C NMR (125 MHz, D_2O): δ 171.3, 158.0, 155.5, 140.3, 112.8, 92.6, 72.8, 71.1, 70.1, 69.1, 69.0, 53.7, 50.1, 49.3, 47.9, 46.8, 40.6, 29.0. HRESI-MS calcd. for $C_{19}H_{32}N_{10}O_7$ $[M+H]^+$: 513.2528; found: 513.2521. Compound **10**: ^{1}H NMR (500 MHz, D_2O): δ 5.48 (d, 1H, J = 3.5 Hz), 5.12 (t, 1H, J = 4.0 Hz), 4.54 (d, 1H, J = 18.0 Hz), 4.28 (dd, 1H, J_1 = 4.0 Hz, J_2 = 11.0 Hz), 4.09–4.02 (m, 3H), 3.94–3.90 (m, 1H), 3.78 (ddd, 1H, J_1 = 12.5 Hz, J_2 = J_3 = 3.0 Hz), 3.72–3.67 (m, 1H), 3.62–3.49 (m, 5H), 3.32 (dd, 1H, J_1 = 2.0 Hz, J_2 = 14.0 Hz), 2.87–2.76 (m, 2H), 2.60 (ddd, 1H, J_1 = 13.0 Hz, J_2 = J_3 = 4.5 Hz), 1.95 (ddd, 1H, J_1 = J_2 = J_3 = 13.0 Hz). ^{13}C NMR (125 MHz, D_2O): δ 174.9, 173.7, 155.6, 92.0, 72.0, 71.2, 69.9, 69.1, 69.0, 53.9, 51.6, 49.3, 49.1, 48.1, 44.8, 40.8, 30.8, 28.9. HRESI-MS calcd. for $C_{18}H_{34}N_8O_7$ $[M+H]^+$: 475.2623; found: 475.2622. Compound **11**: ^{1}H NMR (500 MHz, D_2O): δ 8.48 (s, 1H), 8.35 (s, 1H), 5.52 (d, 1H, J = 3.5 Hz), 5.42 (d, 1H, J = 17.5 Hz), 5.38 (d, 1H, J = 17.5 Hz), 5.09 (t, 1H, J = 4.0 Hz), 4.28 (dd, 1H, J_1 = 3.0 Hz, J_2 = 11.0 Hz), 4.11–4.03 (m, 2H), 3.92–3.88 (m, 1H), 3.81 (ddd, 1H, J_1 = 12.0 Hz, J_2 = J_3 = 4.5 Hz), 3.54–3.45 (m, 3H), 3.32 (dd, 1H, J_1 = 7.0 Hz, J_2 = 14.0 Hz), 2.63 (ddd, 1H, J_1 = 13.0 Hz, J_2 = J_3 = 4.5 Hz), 1.94 (ddd, 1H, J_1 = J_2 = J_3 = 12.5 Hz). ^{13}C NMR (125 MHz, D_2O): δ 171.3, 150.7, 149.8, 146.1, 145.6, 118.8, 92.6, 72.7, 71.2, 70.1, 69.1, 68.9, 53.8, 50.1, 49.3, 48.0, 47.0, 40.7, 29.0. HRESI-MS calcd. for $C_{19}H_{32}N_{10}O_6$ $[M+H]^+$: 497.2579; found: 497.2583. Compound **12**: ^{1}H NMR (500 MHz, D_2O): δ 5.51 (d, 1H, J = 4.0 Hz), 5.50 (d, 1H, J = 3.5 Hz), 5.06 (t, 1H, J = 3.5 Hz), 5.04 (t, 1H, J = 3.5 Hz), 4.36–4.24 (m, 4H), 4.22–4.02 (m, 4H), 3.93–3.89 (m, 2H), 3.82–3.74 (m, 2H), 3.62–3.44 (m, 8H), 3.34–3.28 (m, 2H), 3.04–2.71 (m, 10H), 2.62–2.54 (m, 2H), 2.02–1.88 (m, 2H). ^{13}C NMR (125 MHz, D_2O): δ 174.6, 170.4, 154.2, 92.5, 92.3, 72.6, 72.4, 71.4, 70.1, 70.0, 53.8, 49.5, 49.3, 47.9, 46.2, 45.9, 40.8, 40.1, 39.8, 35.0, 34.8, 28.9. HRESI-MS calcd. for $C_{18}H_{34}N_8O_7$ $[M+H]^+$: 475.2623; found: 475.2631.

[13] Compound **3**: ^{1}H NMR (500 MHz, D_2O): δ 5.32 (d, 1H, J = 3.5 Hz), 3.94–3.90 (m, 1H), 3.87–3.79 (m, 3H), 3.75–3.71 (m, 1H), 3.66–3.58 (m, 3H), 3.47–3.41 (m, 3H), 3.22 (dd, 1H, J_1 = 3.0 Hz, J_2 = 13.5 Hz), 3.17 (dd, 1H, J_1 = 3.5 Hz, J_2 = 10.5 Hz), 3.09–3.05 (m, 1H), 2.94–2.89 (m, 1H), 2.35 (ddd, 1H, J_1 = 12.5 Hz, J_2 = J_3 = 4.5 Hz), 1.90 (s, 15H), 1.55 (ddd, 1H, J_1 = J_2 = J_3 = 12.5 Hz). ^{13}C NMR (75 MHz, D_2O): δ 184.2, 95.5, 77.8, 74.6, 73.7, 73.4, 71.5, 63.0, 59.1, 56.6, 54.6, 51.3, 49.4, 43.0, 33.3, 25.9. HRESI-MS calcd. for $C_{14}H_{32}N_6O_5$ $[M]^+$: 364.2429; found: 364.2413. Compound **4**: ^{1}H NMR (500 MHz, D_2O, in the form of acetate): δ 5.27 (d, 1H, J = 3.5 Hz), 3.90–3.73 (m, 4H), 3.67–3.60 (m, 1H), 3.56 (s, 1H), 3.49–3.38 (m, 3H), 3.23–2.99 (m, 6H), 2.34 (ddd, 1H, J_1 = 12.5 Hz, J_2 = J_3 = 4.5 Hz), 1.89 (s, 15H), 1.54 (ddd, 1H, J_1 = J_2 = J_3 = 12.5 Hz). ^{13}C NMR (75 MHz, D_2O, in the form of acetate): δ 182.0, 93.8, 75.2, 72.1, 71.8, 71.6, 69.4, 57.2, 54.6, 49.0, 47.3, 40.8, 40.2, 30.7, 23.8. HRESI-MS calcd. for $C_{14}H_{31}N_5O_6$ $[M+H]^+$: 366.2347; found: 366.2346.