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Synthesis of pyrrolo[2,3-*d*]pyrimidine nucleoside derivatives as potential anti-HCV agents

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Abstract

Several Toyocamycin (**4**) analogues were examined for their ability to inhibit HCV RNA in a replicon assay. Among the compounds examined 4-methylthio (**18**) and 5-carboxamide oxime derivatives (**23** and **27**) of Toyocamycin were found to have good activity and selectivity.
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Keywords: HCV NS5B inhibitors; Toyocamycin; Pyrrolo[2,3-*d*]pyrimidine nucleosides

1. Introduction

Hepatitis C virus (HCV) infection is highly persistent and lacks an effective and well tolerated antiviral therapy. This has spurred intensive efforts to discover novel anti-HCV agents. The RNA-dependent RNA polymerase (RdRp), NS5B of HCV, constitutes a valid target for drug discovery as it is the centerpiece for viral replication. Compared to the host RNA and DNA polymerases, NS5B RdRp has distinct sub cellular localization at the interface of the endoplasmic reticulum membrane and cytoplasm, a novel catalytic

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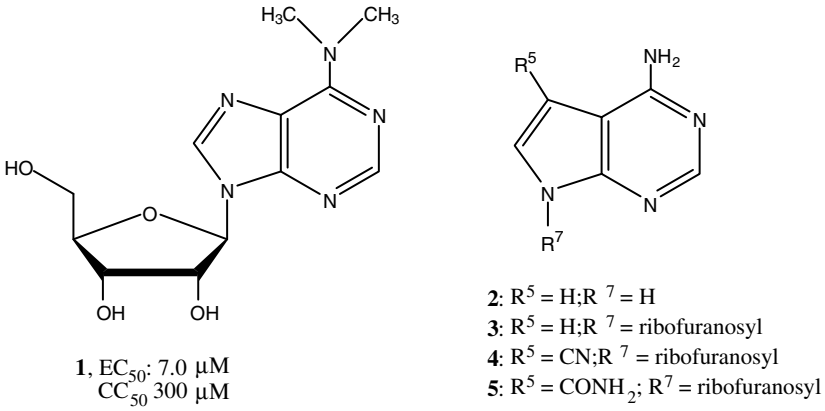


Fig. 1. Adenosine lead derivative (**1**) and Toyocamycin (**4**) analogues.

mechanism and many unique structural features, all of which make it an attractive target for developing effective anti-HCV therapeutics [1].

Currently, combinations of pegylated interferons and ribavirin are the leading therapy [2] for hepatitis C virus. However, even these combinations have limited efficacy. Many classes of nucleoside and non-nucleoside inhibitors of NS5B RdRp have been identified and appeared in literature and patent applications in pursuit of a better treatment for HCV.

Our continued interest [3] to develop a promising treatment for HCV infection has led us to screen our nucleoside library against HCV polymerase in a replicon assay. This effort identified N^6,N^6-dimethyl adenosine (**1**, Fig. 1) as the lead compound (EC_{50}: 7 μM, CC_{50}: 300 μM). Whereas Toyocamycin [4] (**4**) has long been, along with its congener nucleosides Tubercidin (**3**) and Sangivamycin (**5**), studied for its antiviral (human cytomegalovirus, [HCMV]) and antitumor potencies. Accordingly, Toyocamycin analogues comprising nucleoside [5] and the related heterocycle (**2**), pyrrolo[2,3-*d*]pyrimidine [6], modifications were examined against HCMV. The nucleoside modifications included both of sugar and aglycone moieties while pyrrolo[2,3-*d*]pyrimidine **2** heterocycle modifications involved C5 and N7 positions. As part of SAR studies in order to improve the activity of lead adenosine derivative **1** several pyrrolo[2,3-*d*]pyrimidine nucleoside analogues related to Toyocamycin **4** were synthesized and screened for their potential in the inhibition of HCV polymerase in replicon assay [7]. In this report, we disclose results of our study.

2. Materials and methods

2.1. General

^{1}H NMR spectra were obtained using Varian Gemini 300 MHz instrument. Majority of reagents were procured from Aldrich Chemical Company, MI, USA and used as received. All anhydrous reactions were carried out under Argon atmosphere. The reactions were monitored either by thin layer chromatography (TLC), using TLC sheets coated with silica gel 60 F_{254} (Merck) and UV light visualization, or by LC-MS (waters) Micromass). The products were purified by flash chromatography on Merck silica gel 60 (230–400 mesh ASTM). The replicon assay, in presence of the inhibitors, was carried out as described in the literature [7].

2.2. 3,4-Dihydro-4-oxo-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**6**)

To a solution of Toyocamycin **4** (7.275 g, 25 mmol) in a mixture of distilled water (200 ml) and glacial acetic acid (50 ml) solid $NaNO_2$ (17.25 g, 250 mmol) was slowly added at 50 °C. The reaction mixture was heated at 70 °C for 1 h. The volatiles were evaporated under reduced pressure to dryness and the residue was extracted with hot acetone (4× 100 ml). The combined acetone extracts were evaporated and the residue was purified over flash silica gel chromatography to obtain the title product (6.13 g, 84%). ^{1}H NMR (CD_3OD): δ 8.18 (s, 1H), 8.00 (s, 1H), 6.14 (d, $J = 5.4$ Hz, 1H), 4.43 (t, $J = 5.4$ Hz, 1H), 4.27 (t, $J = 3.9$, 5.4 Hz, 1H), 4.10 (dd, $J = 3.0$, 6.6 Hz, 1H), 3.80 (ddd, $J = 2.7$, 12.0 and 32 Hz, 2H). ES-MS $(m/z+1)^+$: 293.

2.3. 3,4-Dihydro-4-oxo-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**7**)

To a solution of 3,4-dihydro-4-oxo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **6** (5.84 g, 20 mmol) in pyridine (50 ml) acetic anhydride (8.46 ml, 90 mmol) was added and stirred at room temperature for 16 h. The volatiles evaporated and the residue was dissolved in a mixture of CH_2Cl_2 (200 ml) and water (200 ml). The organic layer was separated and washed with water (200 ml), aq. HCl (1 N, 2× 200 ml), and water (200 ml), dried ($MgSO_4$) and evaporated over a rotary evaporator. The residue was purified over flash silica gel chromatography (CH_2Cl_2/MeOH using as eluants with increasing polarity of the mixture) to obtain the titled product as a colorless solid (6.94 g, 83%). ^{1}H NMR ($CDCl_3$): δ 12.69 (br s, D_2O exchangeable, 1H), 8.13 (s, 1H), 7.69 (s, 1H), 6.31 (d, $J = 5.4$ Hz, 1H), 5.62 (t, $J = 5.4$ Hz, 1H), 5.47 (dd, $J = 4.2$, 5.4 Hz, 1H), 4.45 (m, 1H), 4.40 (d, $J = 4.2$ Hz, 2H), 2.18 (s, 3H), 2.15 (s, 3H), 2.09 (s, 3H). ES-MS $(m/z+1)^+$: 419.

2.4. 4-Chloro-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**8**)

3,4-Dihydro-4-oxo-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile, **7** (4.18 g, 10 mmol) was dissolved in phosphorous oxychloride (25 ml) and refluxed for 1 h. The reaction mixture was cooled to room temperature and the volatiles evaporated. The residue was suspended in ice cold water and stirred vigorously. The solids separated were filtered and dried (P_2O_5) under reduced pressure to obtain the titled crude product in a quantitative yield. The hygroscopic solid was used in the next reactions without further purification. ES-MS $(m/z+1)^+$: 437.

2.5. The following procedure for the synthesis of 4-methylamino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine-5-carbonitrile **10** exemplifies the general method for the synthesis of mono N^4-alkyl Toyocamycin derivatives **10–15**. The rest of the compounds in the series were characterized by LC-MS analysis

4-Chloro-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **8** (0.3 mmol) was treated with a solution of methylamine (3 ml, 2 M) in THF was added and the reaction flask was closed tightly. The reaction mixture stirred at room temperature for 16 h. The volatiles were evaporated and the residue was purified over flash silica gel

chromatography using CH_2Cl_2 and MeOH as eluants with increasing polarity to obtain 4-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **10** (63%). ^{1}H NMR (CD_3OD): δ 8.13 (s, 1H), 8.02 (s, 1H), 6.00 (d, $J = 6.3$ Hz, 1H), 4.62 (dd, $J = 5.4$, 6.3 Hz, 1H), 4.27 (dd, $J = 3.0$, 5.4 Hz, 1H), 4.13 (dd, $J = 3.0$, 6.0 Hz, 1H), 3.80 (ddd, $J = 2.4$, 12.3 and 33.2 Hz, 2H), 3.05 (s, 3H). ES-MS $(m/z+1)^+$: 306.

2.6. 4-Chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**9**)

4-Chloro-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **8** (1.9 g, 4.36 mmol) was treated with a cold (−20 °C) solution of saturated methanolic ammonia and the reaction mixture kept at 5 °C for 2.5 h with occasional shaking. The volatiles evaporated and the residue was purified over flash silica gel chromatography using CH_2Cl_2 and MeOH mixture with increasing polarity to obtain the title product (1.1 g, 81.5%). ^{1}H NMR (CD_3OD): δ 8.16 (s, 1H), 8.01 (s, 1H), 6.12 (d, $J = 5.4$ Hz, 1H), 4.40 (t, $J = 5.4$ Hz, 1H), 4.27 (t, $J = 3.9$, 5.4 Hz, 1H), 4.09 (dd, $J = 3.0$, 6.6 Hz, 1H), 3.80 (ddd, $J = 2.7$, 12.0 and 32 Hz, 2H). ES-MS $(m/z+1)^+$: 312.

2.7. 4-Dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**16**)

To a solution of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **9** (0.3 g, 0.96 mmol) in EtOH (10 ml) a solution of dimethylamine (10 ml, 2 M) in THF was added and the reaction mixture was refluxed for 1 h. The volatiles evaporated and the residue was purified by flash silica gel chromatography using CH_2Cl_2/MeOH mixture with increasing polarity to obtain the titled product (0.2 g, 65.15%). ^{1}H NMR (CD_3OD): δ 8.34 (s, 1H), 8.24 (s, 1H), 6.13 (d, $J = 5.1$ Hz, 1H), 4.52 (t, $J = 5.1$ Hz, 1H), 4.27 (dd, $J = 3.9$, 5.1 Hz, 1H), 4.12 (dd, $J = 2.7$, 6.6 Hz, 1H), 3.82 (ddd, $J = 2.4$, 12.3 and 37.5 Hz, 2H), 3.39 (s, 6H). ES-MS $(m/z+1)^+$: 320.

2.8. 4-O-Methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**17**)

A solution of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **9** (0.310 g, 1 mmol) and sodium methoxide (0.054 g, 5 mmol) in methyl alcohol (10 ml) was refluxed for 16 h. The reaction mixture was cooled to room temperature and volatiles evaporated. The residue was purified over flash silica gel chromatography (CH_2Cl_2/MeOH mixture as eluant) to obtain the titled product (0.16 g, 52%) as a white solid. ^{1}H NMR (CD_3OD): δ 8.77 (s, 1H), 8.58 (s, 1H), 6.20 (d, $J = 5.4$ Hz, 1H), 4.53 (t, $J = 5.4$ Hz, 1H), 4.22 (dd, $J = 3.9$, 4.8 Hz, 1H), 4.13 (dd, $J = 3.0$, 7.2 Hz, 1H), 3.80 (ddd, $J = 2.7$, 12.3 and 34.2 Hz, 2H), 4.02 (s, 3H). ES-MS $(m/z+1)^+$: 307.

2.9. 4-Methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**18**)

A suspension of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **9** (0.310 g, 1 mmol) and sodium methanethiolate (0.105 g, 1.5 mmol) in isopropyl alcohol (10 ml) was refluxed for 1 h. The reaction mixture cooled to room temperature and volatiles evaporated. The residue was purified over flash silica gel chromatography (CH_2Cl_2/MeOH mixture as eluant) to obtain the titled product (0.23 g, 72%) as a white solid. ^{1}H NMR (CD_3OD): δ 8.67 (s, 1H), 8.48 (s, 1H), 6.23 (d, $J = 5.4$ Hz, 1H), 4.51 (t,

$J = 5.4$ Hz, 1H), 4.29 (dd, $J = 3.9$, 4.8 Hz, 1H), 4.12 (dd, $J = 3.0$, 7.2 Hz, 1H), 3.82 (ddd, $J = 2.7$, 12.3 and 34.2 Hz, 2H), 2.72 (s, 3H). ES-MS $(m/z+1)^+$: 322.

2.10. 4-Methylamino-7-(β-D-ribofuranosyl) pyrrolo[2,3-d]pyrimidine-5-carboxamidoxime (23)

To a solution of 4-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **10** (0.305 g, 1 mmol) in EtOH (10 ml) an aqueous solution of hydroxylamine (0.5 ml, 50% w/w) was added and the reaction mixture refluxed for 16 h. The volatiles were evaporated and the residue was recrystallized from minimum amount of methanol to obtain the title product (0.21 g, 62%) as a white solid. ^{1}H NMR (CD_3OD) δ: 8.07 (s, 1H), 7.68 (s, 1H), 5.97 (d, $J = 6.6$ Hz, 1H), 4.61 (dd, $J = 5.4$, 6.6 Hz, 1H), 4.26 (dd, $J = 2.7$, 5.1 Hz, 1H), 4.10 (dd, $J = 3.0$, 5.7 Hz, 1H), 3.79 (ddd, $J = 34.8$, 12.3 and 2.7 Hz, 2H), 3.02 (s, 3H). ES-MS $(m/z+1)^+$: 339.

2.11. 4-Methylamino-7-(β-D-ribofuranosyl) pyrrolo[2,3-d]pyrimidine-5-carboxamidine (24)

To a solution of 4-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamidoxime **23** (0.14 g, 0.41 mmol) in a mixture of ethanol and water (10 ml, 1:1) ammonium chloride (0.022 g, 0.41 mmol) and thoroughly washed Raney nickel (0.5 ml) in water was successively added. The reaction mixture was shaken well under hydrogen atmosphere (50 psi) at room temperature for 16 h, filtered over celite and the filtrate was evaporated to obtain the titled product (0.1 g, 67%) as an hydrochloride salt. ^{1}H NMR (CD_3OD) δ: 8.28 (s, 1H), 8.14 (s, 1H), 6.10 (d, $J = 5.7$ Hz, 1H), 4.59 (t, $J = 5.4$ Hz, 1H), 4.29 (dd, $J = 4.8$, 3.3 Hz, 1H), 4.14 (dd, $J = 6.3$, 3.0 Hz, 1H), 3.79 (ddd, $J = 32.4$, 12.3 and 2.4 Hz, 2H), 3.10 (s, 3H). ES-MS $(m/z+1)^+$: 323.

2.12. 4-Dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxamidoxime (25)

To a solution of 4-dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile, **16** (0.1 g, 0.31 mmol) in EtOH (10 ml) an aqueous solution of hydroxylamine (0.5 ml, 50% w/w) was added and the reaction mixture refluxed for 16 h. The volatiles were evaporated and the residue was purified over flash silica gel chromatography (CH_2Cl_2/MeOH mixture as eluant) to obtain the title product (0.06 g, 54.5%) as a white solid. ^{1}H NMR (CD_3OD) δ: 8.14 (s, 1H), 7.57 (s, 1H), 6.06 (d, $J = 6.0$ Hz, 1H), 4.58 (t, $J = 5.7$ Hz, 1H), 4.27 (dd, $J = 5.4$, 3.0 Hz, 1H), 4.09 (dd, $J = 6.0$, 2.7 Hz, 1H), 3.77 (ddd, $J = 33.3$, 12.3 and 2.7 Hz, 2H), 3.17 (s, 6H). ES-MS $(m/z+1)^+$: 353.

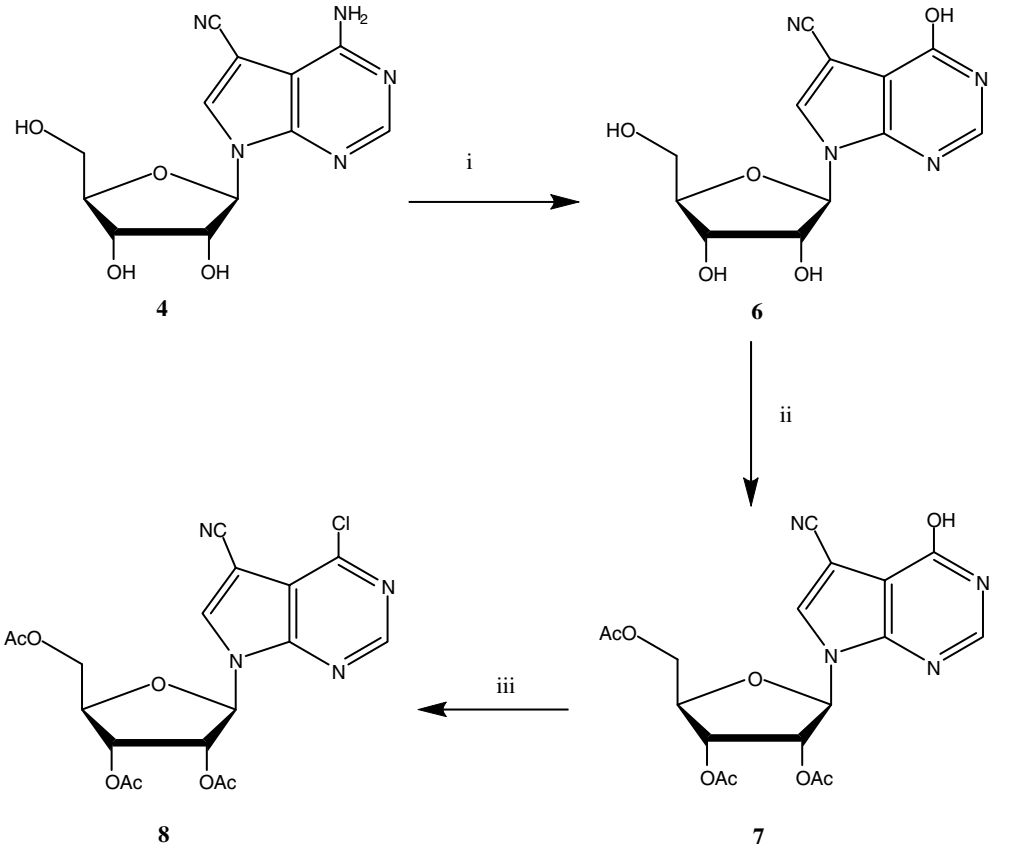
2.13. 4-Dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxamidine (26)

To a solution of 4-dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamidoxime **25** (0.1 g, 0.28 mmol) in a mixture of ethanol and water (5 ml, 1:1) ammonium chloride (0.015 g, 0.28 mmol) and thoroughly washed Raney nickel (0.5 ml) in water was successively added. The reaction mixture was shaken well under hydrogen atmosphere (50 psi) at room temperature for 16 h. The reaction mixture was filtered over

celite and the filtrate evaporated to obtain the titled product (0.05 g, 49.5%) as an hydrochloride salt. ^{1}H NMR (CD_3OD) δ: 8.29 (s, 1H), 8.23 (s, 1H), 6.16 (d, $J = 6.0$ Hz, 1H), 4.58 (t, $J = 5.4$ Hz, 1H), 4.30 (dd, $J = 4.5$, 3.9 Hz, 1H), 4.13 (br d, $J = 2.7$ Hz, 1H), 3.82 (ddd, $J = 29.1$, 12.6 and 2.7 Hz, 2H), 3.14 (s, 6H). ES-MS $(m/z+1)^+$: 337.

2.14. 4-Methythio-7-(β-D-ribofuranosyl)pyrrolo[2,3- d]pyrimidine-5-carboxamidoxime, (*27*)

To a solution of 4-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile, **18** (0.2 g, 0.62 mmol) in EtOH (10 ml) an aqueous solution of hydroxylamine (0.5 ml, 50% w/w) was added and the reaction mixture refluxed for 16 h. The volatiles were evaporated and the residue was purified over flash silica gel chromatography (CH_2Cl_2/MeOH mixture as eluant) to obtain the product (0.21 g, 72.7%) as a white solid. ^{1}H NMR (CD_3OD) δ: 8.57 (s, 1H), 7.81(s, 1H), 6.21 (d, $J = 5.4$ Hz, 1H), 4.56 (t, $J = 6.0$ Hz, 1H), 4.29 (br m, 1H), 4.10 (br m,1H), 3.79 (br m, 2H), 2.62 (s, 3H). ES-MS $(m/z+1)^+$: 356.



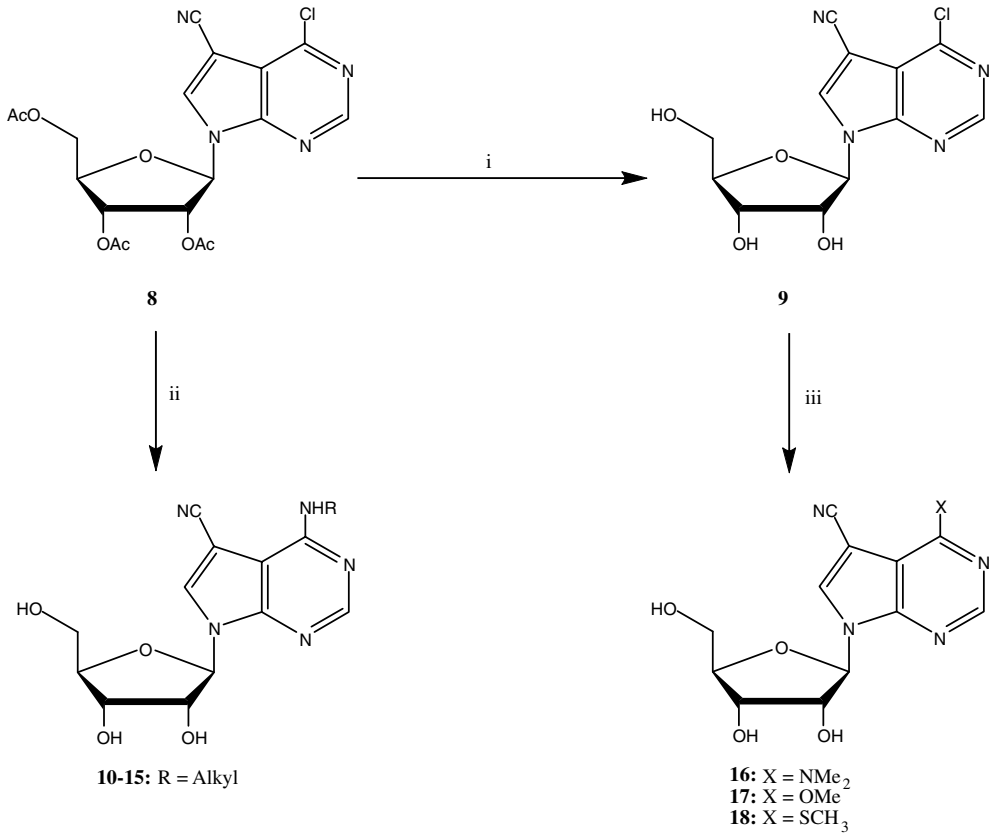
4 **6** **8** **7**

Scheme 1. Reagents: (i) $NaNO_2$, water, glacial AcOH; (ii) Ac_2O, Py.; (iii) $POCl_3$.

3. Results and discussion

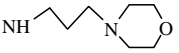
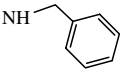
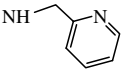
The present study involved modifications of exocyclic amino group and cyano group, at C4 and C5 positions, respectively, of Toyocamycin (**4**). To explore the various substitutions tolerated on exocyclic amino group several N^4-alkyl derivatives of **4** were synthesized (Scheme 2). The desired intermediate chloro derivative (**8**) was synthesized as shown in Scheme 1. Thus, Toyocamycin **4** was diazotized by a modified procedure [8] to obtain desaminotoyocamycin (**6**) which was protected as the corresponding triacetate (**7**) in high yield. Hydroxy derivative **7** was then refluxed [9] in $POCl_3$ for 16 h to obtain the crucial intermediate **8** in quantitative yield.

The treatment of intermediate **8** with saturated methanolic ammonia at 5 °C for 2.5 h furnished 4-chloro-pyrrolo[2,3-*d*]pyrimidine nucleoside (**9**). The derivatives (**10**–**18**) modified at C4 position of Toyocamycin **4** were obtained from either of the intermediates **8** or **9**. Thus mono N^4-alkyl derivatives **10**–**15** (Table 1) were then generated in a small library fashion by exposing **8** to respective amines. Although the larger size of alkyl group has improved the activity more than two fold (**10** vs. **13**) it also increased the cytotoxicity multiple times. Apart from mono N^4-alkyl derivatives (**10**–**15**), the corresponding dialkyl



Scheme 2. Reagents: (i) Saturated methanolic ammonia; (ii) RNH_2, THF; (iii) $NHMe_2$, THF (or) NaOMe, MeOH (or) NaSMe, isopropyl alcohol.

Table 1
SAR of the substitution at C4 position of Toyocamycin

Compound	R	EC_{50}(μM)	CC_{50}(μM)
10	NHMe	11	300
11		8	100
12		7	50
13		4.5	40
14		5	75
15		5	100
16	NMe_2	3	250
17	OCH_3	0.4	40
18	SCH_3	0.4	250

(N^4,N^4-dimethyl) derivative [9] (**16**) was also synthesized to understand the influence of substituting both protons of exocyclic amino group at C4 of Toyocamycin **4**.

Thus, chloro derivative **9** upon exposure to dimethyl amine in refluxing THF afforded the N^4,N^4-dimethyl derivative **16**. Derivative **16** was found to show better activity than that of mono N^4-alkyl derivatives **10**–**15**. This indicated that the two protons on the exocyclic amino group at C4 in Toyocamycin **4** were not essential for the activity. This was further validated by the altogether replacement of exocyclic amino group by different hetero-atom groups as in the case of methoxy (**17**) and methylthio (**18**) derivatives [9]. These derivatives (**17** and **18**) were obtained from **9** by the reaction with NaOMe or NaSMe. Between the derivatives **17** and **18**, the latter was found to be less toxic than the former. The excellent activity of derivatives **17** and **18** over that of **10**–**15** might suggest that the hydrogen bond acceptors were better tolerated than the hydrogen bond donors at C4 position in the absence of any modification of cyano group at C5 position of Toyocamycin **4**.

After establishing the modifications allowed at C4 position of Toyocamycin **4**, to improve the activity of original lead adenosine derivative **1**, we then focused our attention on modifications involving both C4 and C5 positions (Table 2). Initially, the C5 cyano group in desaminotoyocamycin **6** was modified. The target derivatives carboxamide

Table 2
SAR of the substitution at C4 and C5 positions of Toyocamycin

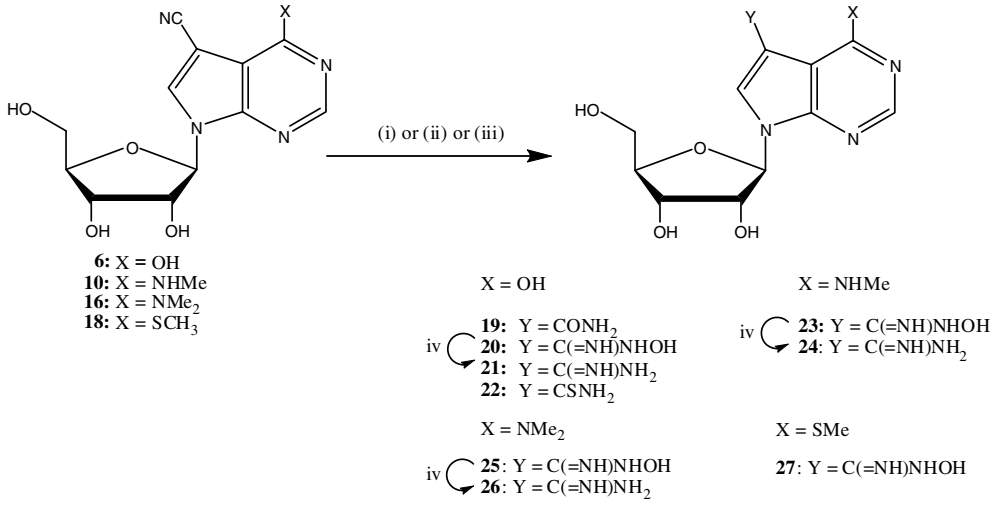
Compound	EC_{50}(μM)	CC_{50}(μM)
19	4	33
20	33	300
21	100	300
22	25	250
23	0.6	200
24	3.7	300
25	9	300
26	2.5	300
27	1	200

(**19**), carboxamido oxime (**20**), carboxamidine (**21**) and thioamide (**22**) were prepared (Scheme 3) as described previously in literature [8]. Of these 5-carboxamido derivative **19** had shown comparable activity as that of compounds **10**–**18** derived from single modification. Although carboxamide **19** exhibited good activity but it's also found to be relatively toxic (CC_{50}: 33 μM), whereas derivatives **20**–**22** were weakly active but they were not toxic.

This prompted us to explore carboxamide oxime and carboxamidine modifications of cyano group in N^4-methyl **10** and N^4,N^4-dimethyl **16** and methylthio **18** derivatives. Accordingly, derivatives **10**, **16** and **18** were converted into the corresponding carboxamide oximes (**23**, **25** and **27**), respectively, upon reaction with aqueous hydroxylamine in refluxing ethanol. The corresponding carboxamidines (**24** and **26**) of the carboxamide oximes **23** and **25** were obtained under catalytic hydrogenation conditions.

Among the carboxamide oximes **20**, **23**, **25** and **27** of this study only the carboxamide oxime **23** derived from nitrile **10** was found to have highest activity. A closer look of oxime **23** revealed that it also has good selectivity [selectivity index (SI): 333] only next to that of nitrile derivative **18** (SI: 625). Furthermore, the carboxamidines **24** and **26** were also found to have good activity and selectivity over that of **21**. This confirms to the fact that absence of hydrogen bond donors, such as substituted amino groups, at C4 of Toyocamycin **4** enhances the activity even when cyano group at C5 is modified. The thioamides (not shown) derived from compounds **10**, **16** and **18** were also found to be inactive as that of **22**. Thus, simultaneous modification of C4 and C5 positions of Toyocamycin **4** did not enhance the activity to a large extent over modifications at C4 alone.

In conclusion, we have synthesized and screened a variety of Toyocamycin **4** analogues (**10**–**27**) for their ability to inhibit HCV RNA replication in replicon cells. The derivatives **17** and **18** from single modifications at C4 and analogues **23** and **27** from combined modifications of C4 and C5 positions of Toyocamycin **4** showed good activity over the rest of the compounds examined in this study. However derivatives **18**, **23** and **27** were found to have good selectivity in decreasing order.



Scheme 3. Reagents: (i) H_2O_2, NH_4OH; (ii) aq NH_2OH, EtOH; (iii) H_2S, NEt_3, py.; (iv) Raney Ni, H_2.

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