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Synthesis and antiplasmodial activity of purine-based C-nucleoside analogues†

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A series of homologous C-nucleoside mimics have been synthesized *via* an efficient and facile synthetic protocol involving the conjugate addition of purine to sugar derived olefinic ester in good yields. The synthesized compounds were evaluated for their antiplasmodial activity *in vitro* against both the CQ-sensitive and resistant strains of *P. falciparum*. Interestingly, all the synthesized nucleoside analogs exhibited an IC₅₀ of <5 μM, while compounds **22a**, **23a**, and **23b** showed promising antiplasmodial activity with an IC₅₀ of 1.61, 0.88, and 1.01 μM against the CQ-sensitive Pf3D7 strain and 1.14, 1.01, and 2.57 μM against the CQ-resistant PfK1 strain, respectively.

1. Introduction

Malaria, an infectious disease caused by the protozoan parasites *Plasmodium falciparum* (*P. falciparum*), *P. ovale*, *P. vivax* and *P. malariae*, is transmitted from the bite of an *Anopheles* mosquito. *P. falciparum* is mainly responsible for the most severe form of human malaria and millions of deaths globally. As per World Health Organization reports, malaria affected about 216 million people in 2016, resulting in 445 000 deaths, mostly in infants.¹ Resistance to chloroquine and other conventionally used antimalarial drugs such as pyrimethamine/sulfadoxine in *P. falciparum* is reported in many endemic areas.^{2,3} With the emergence of resistance to almost all the quinolones and antifolate drugs in *P. falciparum* malaria,⁴ artemisinin and its derivatives are the mainstay to treat malaria. Artemisinin-based combination therapies (ACTs) act as the frontline treatments against *P. falciparum* malaria.⁵ Resistance to artemisinins in several areas (WHO 2015) has led to the development of urgently needed novel drugs that could selectively target *P. falciparum*.⁶

Plasmodium parasites are purine auxotrophs as they are unable to perform *de novo* purine biosynthesis.⁷ The parasite

depends upon the salvage of purine from the host for its survival and proliferation.⁸ Several unique enzymes for the purine metabolism in parasites hold significant promise as drug targets. Purine-based nucleosides exhibit antiplasmodial activity by inhibiting these crucial enzymes involved in purine biosynthesis of parasites.^{9–13} Purine-based acyclic nucleosides are potent inhibitors of PfHGXPRT, an enzyme essential for the synthesis of nucleoside monophosphates.^{14–16} 5'-Methylthio coformycin (**1**) and its analogs are reported to selectively inhibit *P. falciparum* adenosine deaminase (PfADA) to the human adenosine deaminase (hADA).¹⁷ N⁶-(2,2-diphenylethyl)-5'-N-phenylcarboxamidoadenosine (**2**) exhibits activity against the CQ-resistant strains (IC₅₀ of 1.8 μM).¹⁸ Methylthio-immucillin-H (MT-immucillin-H, **3**) is reported to selectively inhibit the purine nucleoside phosphorylase of *P. falciparum* (PfPNP).¹⁹ The purine nucleoside MDL73811 (**4**) exhibited good antimalarial activity with an IC₅₀ of 2–3 μM against *P. falciparum* strains by inhibiting S-adenosylmethionine decarboxylase (AdoMetDC) against both CQ-sensitive and CQ-resistant strains.²⁰ Another

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Fig. 1 Purine nucleoside-based antimalarial nucleosides and general structures of synthesized nucleoside analogs.

nucleoside analog (6'*R*)-6'-*C*-methylneplanocin A (RMNPA, 5) effectively inhibits *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase resulting in significant antimalarial activity (IC₅₀ of 0.10 μM) (Fig. 1).^{21,22}

In view of the above, we thought to access few nucleoside analogs which could inhibit the crucial enzymes of *Plasmodium* parasites and exhibit antimalarial activity against both the CQ-sensitive (*Pf*3D7) and resistant (*Pf*K1) strains.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds was accomplished starting from 1',2':5',6'-di-*O*-isopropylidene- α -D-glucufuranose **7**²³ easily prepared from D-glucose. The latter was transformed to 3-*O*-benzyl and 3-*O*-(2-nitrophenyl)glycofuranosylenoate derivatives **15** and **16** in good yields (>95%) by the earlier reported methods of Horner-Wadsworth-Emmons olefination of the respective aldoses (**13** and **14**) with triethyl phosphonate (Scheme 1).²³

The glycofuranosylenoate (**15** and **16**) on DBU catalyzed conjugate addition of purine derivatives (**17–19**) in ethanol gave the diastereoisomeric mixture of the respective glycoconjugates of purines (**20a–23a** & **20b–23b**). The addition of purines to the olefinic ester was regioselective, and in each reaction, a diastereomeric mixture of two compounds was formed with only the N-9 attachment of purine

ring, and two diastereomers have the *S* (β -L-ido) and *R* (α -D-*gluco*) configuration at the C-5' in the sugar units (Scheme 1). The formation of diastereomers was evident from TLC and NMR spectral data. The diastereomers were isolated using column chromatography, and the structures were confirmed by various 1D (¹H, ¹³C) and 2D (COSY, NOESY, HSQC, and HMBC) NMR experiments and ESI-MS analysis. 2D-NMR spectroscopy revealed the structures of **20a** and **20b** and configuration at the C-5' of the sugar units. HMBC correlation of CH₂-7'' to C-3' revealed the connectivity of the benzyl moiety to the sugar ring. H-5' showed HMBC correlations with C-4 and C-8. H-8 showed HMBC correlation with C-5'. These essential HMBC correlations confirmed the N-9 connectivity of the adenine moiety to the sugar unit. HMBC correlations of **20a** and **20b** were similar and suggested that the adenine moiety was attached to the sugar moiety through N-9 in both the compounds. Few nOe correlations of the above compounds were found to be different. In compound **20b**, nOe correlations of H-5' to H-3' established that the adenine moiety is above the plane, *i.e.* the α -D-*gluco* (*R*) configuration at C-5' in the sugar units, whereas these nOe correlations are absent in compound **20a** and indicated that the adenine moiety in compound **20a** is below the plane at C-5' having the β -L-ido (*S*) configuration (Fig. 2).

The reduction of the above ethanoates/methanoates **20a–23a** & **20b–23b** with LiAlH₄ led to the formation of the

Scheme 1 Synthesis of glycosyl esters (**15** and **16**) and purine-based C-nucleoside analogues (**20a–23a** & **20b–23b**) and (**24–29**). Reagents and conditions: (a) NaH (1 equiv.), TBAB (20 mol%), THF, 0–25 °C, 12 h, 90–95%; (b) 70% AcOH aq., 25 °C, 8 h, 98%; (c) NaIO₄ aq. (1 equiv.), MeOH, 0–25 °C, 3 h, 90–92%; (d) TEPA (1 equiv.), LiOH (1 equiv.), THF, 25 °C, 8 h, 92–94%; (e) DBU (10 mol%), MeOH or EtOH, reflux, 16–18 h; (f) LiAlH₄ (1 equiv.), anh. THF, N₂ atm., 0–25 °C, 3 h, 88–94%.

Fig. 2 Important NOESY and HMBC correlation of 20a and 20b.

respective alcohol analogs (20–29) in excellent yields (Scheme 1).

For SAR activity, we have also synthesized the 3-*O*-(2-aminophenyl)enoate derivative (30) by Pd/C reduction of the nitro group in the nitrophenyl ring of 21a. The ester group in compound 30 was hydrolyzed to the respective acid derivative (31) with Et₃N in THF in good yield (Scheme 2).

In order to explore the role of the sugar unit and to check the activity of arylated purines, the *N*-9-arylated adenine derivative (33)²⁴ was also synthesized by the reaction of adenine (17) with 4-fluoroacetophenone (32) *via* SNAr reaction in DMSO in the presence of K₂CO₃ at 140 °C. The reduction of the ketone to the respective alcohol (34) was carried out using NaBH₄. The carboxyethoxy propyl derivative (36) was synthesized by the Michael addition of adenine (17) to the ethyl acrylate (35) in refluxing ethanol in the presence of NaOEt (Scheme 3).²⁵

2.2. Biology

***In vitro* antiparasmodial activity.** All of the above-synthesized compounds (20a–23a, 20b–23b, 24–29, and 30–36) were evaluated for their *in vitro* antiparasmodial activity against the CQ-sensitive (3D7) and CQ-resistant (K1) strains of *P. falciparum* with chloroquine as the reference drug (Table 1).

The nucleoside analogs having the 3-*O*-benzyl (20a and 20b) or 3-*O*-(2-nitrophenyl) (21a and 21b) substituents have shown activity (IC₅₀ = <5 μM) against both the strains.

Scheme 2 Synthesis of amino ester (30) and amino acid (31)-based C-nucleoside analogues. Reagent conditions: (a) Pd–C (10 mol%), H₂ atm., 2 h, 98%; (b) Et₃N (3 equiv.), 50% EtOH aq., 36 h, 88%.

Scheme 3 Synthesis of *N*-9-phenyl and alkyl-purine derivatives. Reagents and conditions: (a) K₂CO₃ (2.5 equiv.), DMSO, 140 °C, 36 h, 62%; (b) NaBH₄ (1 equiv.), MeOH, 25 °C, 2 h, 99%; (c) EtONa (1 equiv.), EtOH, reflux, 3 h, 98%.

6-Amino *N*-alkylated purine nucleoside analogs 22a (IC₅₀ of 1.61 μM and 1.14 μM) and 23a (IC₅₀ of 0.88 μM and 1.13 μM) displayed antiparasmodial activity only at the lower concentration with the IC₅₀ < 2 μM against both the CQ-sensitive and resistant strains. Compounds 22a (IC₅₀ of 1.61 μM & 1.14 μM) and 23a (IC₅₀ of 0.88 μM & 1.13 μM) with the β-*L*-ido (*S*) configuration showed better *in vitro* antiparasmodial activity as compared to compounds 22b (IC₅₀ of 3.60 μM & 2.27 μM) and 23b (IC₅₀ of 1.01 μM & 2.57 μM) with α-*D*-*gluco* (*R*) configuration against the CQ-sensitive and resistant strains, respectively. Comparing the antiparasmodial activity of nucleoside analogs (20a–23a & 20b–23b) with that of the ester and alcohol (24–29) groups, esters showed better activity as compared to alcohol analogs. However, between the nucleoside analogs with carboxyl and ester groups, the former (31) has IC₅₀ values of 4.82 and 3.72 μM while the latter (30) displayed antiparasmodial activity with IC₅₀ values of 4.47 and >5 μM against the CQ-sensitive and resistant strains, respectively. The adenine derivatives 33 and 34 with the aryl group rather than the sugar unit as the *N*-9 substituent did not display any significant activity (IC₅₀ > 5 μM) indicating the importance of sugar units in antiparasmodial response. Adenine derivative 36 with an *N*-9 propanoate substituent also did not display any significant activity against either of the strains *in vitro* (IC₅₀ > 5 μM). All the sixteen nucleoside analogs having an IC₅₀ of <5 μM against either of the strains were further evaluated for their cytotoxicity against VERO cell line and their CC₅₀ and selectivity index (SI) against both *Pf* strains are reported in Table 1. The results showed that all these compounds have no discernible cytotoxicity. The most active antiparasmodial compounds (22a and 23a) exhibited a higher CC₅₀ of 57.49 & 81.46 μM, respectively, and selectivity index (SI = 35.70 & 50.42 for 22a and 92.56 & 72.08 for 23a) as compared to compounds 22b and 23b with a CC₅₀ of 53.27 & 44.95 μM, respectively, and selectivity index (SI = 14.79 & 23.46 for 22b and 44.50 & 17.49 for 23b) for both the CQ-sensitive and resistant strains. Resistance index (RI) is a parameter, which describes the activity of a compound regardless of the susceptibility of parasite strain and any potent

Table 1 *In vitro* antiparasitodal activity^f and cytotoxicity of final compounds

Comp.	Structure	IC ₅₀ ^{a,b} (μM)		RI ^c (μM)	CC ₅₀ ^d (μM)	SI ^e (μM)	
		Pf3D7	PfK1			Pf3D7	PfK1
20a		4.44 ± 0.31	4.79 ± 0.25	1.07	68.43	15.41	14.28
20b		4.12 ± 0.22	4.09 ± 0.03	0.99	86.31	20.94	21.10
21a		3.56 ± 0.43	4.93 ± 0.15	1.38	96.31	27.05	19.53
21b		4.24 ± 0.06	4.25 ± 0.005	1.00	118.99	28.06	27.99
22a		1.61 ± 0.09	1.14 ± 0.14	0.70	57.49	35.70	50.42
22b		3.60 ± 0.01	2.27 ± 0.17	0.63	53.27	14.79	23.46

Table 1 (continued)

Comp.	Structure	IC ₅₀ ^{a,b} (μM)		RI ^c (μM)	CC ₅₀ ^d (μM)	SI ^e (μM)	
		Pf3D7	PfK1			Pf3D7	PfK1
23a		0.88 ± 0.01	1.13 ± 0.02	1.28	81.46	92.56	72.08
23b		1.01 ± 0.25	2.57 ± 0.86	2.54	44.95	44.50	17.49
24		4.31 ± 0.12	4.99 ± 0.2	1.15	97.14	22.53	19.46
25		3.62 ± 0.37	4.70 ± 0.19	1.11	148.07	37.11	33.34
26		3.99 ± 0.03	4.44 ± 0.21	0.75	61.24	19.56	25.83
27		4.52 ± 0.16	4.09 ± 0.05	1.29	148.07	40.90	31.50

Table 1 (continued)

Comp.	Structure	IC ₅₀ ^{a,b} (μM)		RI ^c (μM)	CC ₅₀ ^d (μM)	SI ^e (μM)	
		Pf3D7	PfK1			Pf3D7	PfK1
28		3.13 ± 0.10	2.37 ± 0.46	0.90	51.80	11.46	12.66
29		4.11 ± 0.04	4.99 ± 0.35	1.21	74.87	18.21	15.00
30		4.47 ± 0.09	>5	>1.11	89.68	20.06	17.93
31		4.82 ± 0.75	3.72		67.07	13.91	18.02
33		>5	ND		ND	NA	NA
34		>5	ND		ND	NA	NA
36		>5	ND		ND	NA	NA
	Chloroquine	0.0049 ± 0.0001	0.83 ± 0.01	169.3			

^a 50% inhibitory concentration against the chloroquine-sensitive (3D7) and resistant (K1) strains of *P. falciparum*. ^b Values are represented as the average of at least duplicate determinations. ^c Resistance index (RI) = IC₅₀ K1/IC₅₀ 3D7. ^d 50% cytotoxic concentrations; the monkey kidney cell line (VERO) was obtained from the tissue culture lab, CDRI. ^e Selectivity index (SI) = CC₅₀/IC₅₀. ND = not done. NA = not applicable. ^f We used human RBCs that were obtained from a blood bank under the approval CDRI/IEC/2017/A4 (the CDRI Human Ethics Committee clearance number for the *P. falciparum* culture in human RBCs).

drug should have a low RI value. Delightfully, the majority of the synthesized nucleoside analogs have RI values <1.5, whereas the RI of the reference drug chloroquine was found to be 169.3 (Table 1).

3. Conclusion

In summary, purine-based derivatives, *C*-nucleoside analogs were synthesized and evaluated for their *in vitro* antiparasmodial activity against chloroquine-sensitive and resistant strains of *P. falciparum*. All of the nucleoside analogs exhibited an IC₅₀ of <5 μM; however, the non-glycosylated purines did not show any significant activity against either of the CQ-sensitive or resistant strains. Among these, three nucleoside analogs exhibited an IC₅₀ of <2 μM against either of the strains. From the initial SAR study, it has been found that the compounds with a β-L-ido (S) configuration (22a & 23a) showed better activity than compounds with an α-D-glucopyranosyl (R) configuration (22b & 23b), and 6-amino alkylated purine nucleoside ester analogues (22a, 23a, and 23b) exhibited good antiparasmodial activity having an IC₅₀ of 0.88–2.57 μM.

Conflicts of interest

The authors declare no competing interest.

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