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Synthesis and molecular docking studies of 1-phenyl-4-glycosyl-dihydropyridines as potent antileishmanial agents

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ABSTRACT

A series of 1-phenyl-4-glycosyl-dihydropyridines (**4**–**17** and **19**–**21**) were prepared by the one pot multicomponent reaction of glcosyl aldehyde, β-keto compounds and aniline or substituted aniline in the presence of TBAHS as catalyst. The compounds were screened *in vitro* and *in vivo* for their antileishmanial activities. Most of the compounds exhibited moderate to good activity against amastigotes and promastigotes of *Leishmania donovani*. The compounds **4**, **11**, **12**, **13**, and **17** exhibited potent *in vivo* activity with selectivity index (SI) values 7.43–18.93. Molecular docking studies with these compounds revealed *L. donovani* PTR1 as the possible target to show antileishmanial activities.

1. Introduction

Leishmaniasis, a disease caused by protozoan parasite belonging to the genus *Leishmania* is transmitted by the bite of mainly two genera of sandflies – *Lutzomyia* in the New World and *Phlebotomus* in the Old World [1]. The disease affects almost 2 million people across the 88 countries throughout the world (www.who.int/tdr/disease/leish).

The parasites have a digenetic life cycle, first residing in the gut of phlebotomine sand fly where they replicate as procyclic promastigotes. After a blood meal, the parasites are transmitted and engulfed by vertebrate mononuclear phagocytic system cells and are transformed into the amastigote stage. These amastigotes divide within the acidified phagolysosomes [2]. Human infection is caused by about 21–30 species of *Leishmania* parasites and the two forms of this disease cutaneous leishmaniasis (CL) and visceral leishmanias (VL) are very important for health and socioeconomic point of view. The VL is severe form of leishmaniasis as the parasites migrate to the vital organs of the body such as liver, spleen, bone marrow and other lymphoid tissues and sometimes it is fatal. No effective vaccines are available [3] and the treatment of leishmaniasis relies mainly on chemotherapy [4] with few drugs, which are quite toxic. Chemotherapy is inadequate and drug resistance against the known antileishmanials warrants the introduction of newer and safer chemotherapeutic agents. A diverse group of chemical structures have been reported as antileishmanial agents. These include mostly the nitrogen heterocycles; quinolines [5], acridines [6], phenothiazines [7], pyrimidines [8,9], purines [10], and many other class of compounds including anilines [11], flavonoids [12], quinines [13,14], amino acid esters and amides [15,16], amino alcohols [17], alkyl phospholipids [18], and certain Pt complexes [19].

However, rational drug design approach involves identification of selective drug targets and development of inhibitors of enzymes present in the parasite but absent from their mammalian host. The pteridine reductase 1 (PTR1, EC 1.5.1.33) of *Leishmania* is an excellent drug target due to the unusual salvage of pterin from the host while the host synthesizes pterin derivatives *de novo* from GTP and lack PTR1 activity [20]. Biochemical studies indicate that this enzyme is a NADPH dependent pterin reductase and exhibit its enzymatic activity as a tetramer [21–23]. PTR1 reduces biopterin to

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H2-biopterin and H4-biopterin; it is also capable of reducing folate to 7,8-dihydrofolate and tetrahydrofolate. Recently we have shown that dihydropyrimidinones and 1-phenyl-4-glycosyl-dihydropyridines inhibit PTR1 of leishmania parasite and possess potent *in vitro* activities also [24–26]. At least two of the dihydropyridine class of compounds one bearing the phenyl and the other sugar residue at the 4th-position of the dihydropyridine ring were studied in great detail in our group for their possible mode of antilieishmanial action [26,27]. In order to optimize this molecule we have synthesized a series of 1-phenyl-4-glycosyl-dihydropyridines and evaluated there *in vitro* and *in vivo* activities against *Leishmania donovani*. Docking studies with 3D structure of PTR1 were also performed in order to find out essential features of this skeleton responsible for binding with Leishmanial PTR1 and eliciting the biological response.

2. Results and discussion

2.1. Chemistry

The starting xylofuranosyl dialdoses, 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuran-1,5-dialdose (**1a**), 1,2-*O*-isopropylidene-α-D-xylofuran-1,5-dialdose (**1b**) and 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuran-1,5-dialdose (**1c**) were prepared from D-glucose according to the procedure reported in literature [28–30]. Thus, reaction of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuran-1,5-dialdose (**1a**) with methylacetoacetate and 4-methoxy aniline in the presence of tetrabutylammonium hydrogensulphate (TBAHS) in diethylene glycol as our earlier reported method [31] resulted in 2,6-dimethyl-4-(3′-*O*-benzyl-1′,2′-*O*-isopropylidene-β-L-*threo*-pentofuranos-4′-yl)-1-(4-methoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**4**) in good yield. It was characterized on the basis of its spectroscopic data and analyses. The IR, MS and NMR (^{1}H and ^{13}C) spectral data are consistent with the proposed structure **4**.

To see the scope of this reaction with different susbstrates, condensation of the xylofuranosyl dialdoses **1a**, **1b** and **1c** with different anilines (**2a**–**2h**) and methyl acetoacetate (**3a**) or ethyl acetoacetate (**3b**) were carried out under the above mentioned condition give the respective 1-phenyl-4-(β-L-*threo*-pentofuranos-4′-yl)dihydropyridines (**5**–**17**) (Scheme 1, Table 1) in good yields. The structures of these compounds were also established on the basis of their spectroscopic data and microanalysis.

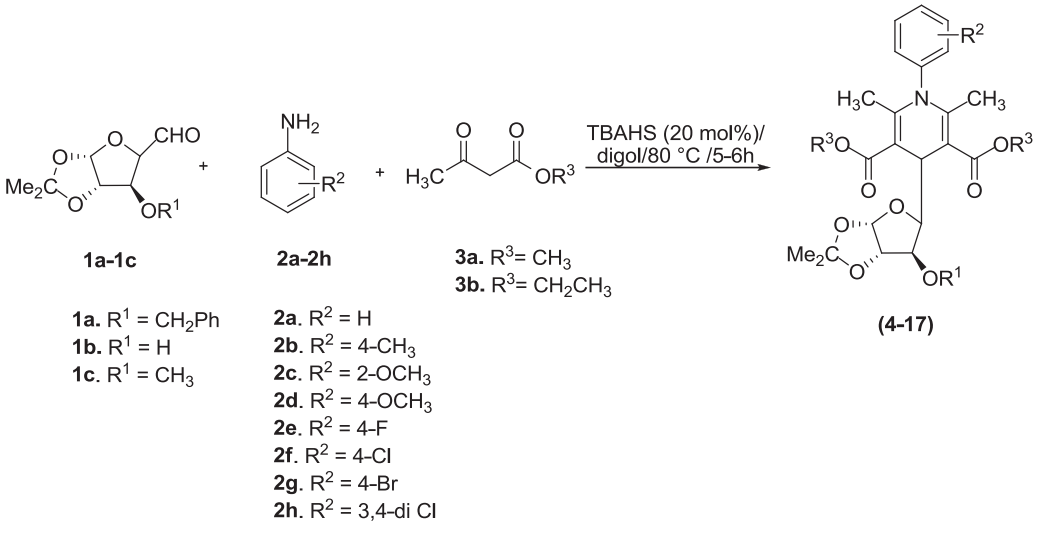
Table 1
Synthesis of dihydropyridines (**4**–**17** and **19**–**21**) with different glycosyl aldoses, anilines, and β-keto esters.

Entry	Glycosyl aldoses	R^2	β-keto esters	Product	Time (h)	Isolated yield (%)
1	**1a**	4-OMe	**3a**	**4**	7	67
2	**1a**	4-Cl	**3a**	**5**	8	74
3	**1a**	4-CH_3	**3a**	**6**	6	78
4	**1a**	4-F	**3a**	**7**	9	70
5	**1a**	4-F	**3b**	**8**	10	76
6	**1b**	3,4-diCl	**3a**	**9**	8	76
7	**1b**	H	**3a**	**10**	7	68
8	**1b**	4-Cl	**3a**	**11**	8	77
9	**1c**	4-CH_3	**3a**	**12**	6	78
10	**1c**	4-Br	**3a**	**13**	7	64
11	**1c**	4-OMe	**3a**	**14**	7	70
12	**1c**	4-Cl	**3a**	**15**	8	73
13	**1c**	4-CH_3	**3b**	**16**	6	69
14	**1c**	2-OMe	**3b**	**17**	7	71
15	**18**	4-Cl	**3a**	**19**	7	76
16	**18**	4-OMe	**3a**	**20**	7	78
17	**18**	NH_2	**3a**	**21**	8	66

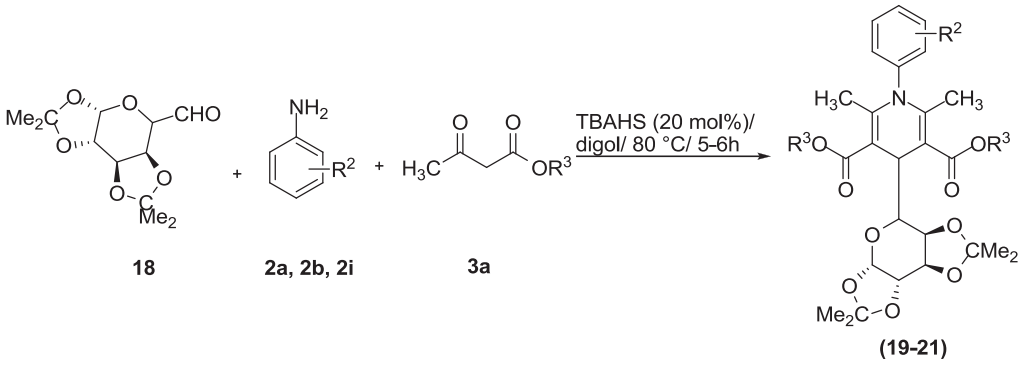
In order to see the effect of pyranosyl sugar in the above reaction, similarly condensation of galactopyranosyl dialdose **18** with methyl acetoacetate and three different anilines, 4-amino-, 4-methoxy- and 4-chloroanilines were carried out separately as above to give good yields of 4-(1′,2′:3′,4′-di-*O*-isopropylidene-α-L-arabinopyranos-5′-yl)-1,4-dihydropyridines (**19**–**21**) (Scheme 2, Table 1).

2.2. Biology

The above synthesized compounds were evaluated *in vitro* against transgenic *L. donovani* promastigotes and intracellular amastigotes at 40 μg/ml concentrations to identify the potent compounds. Compounds showing >80% inhibition at this dose against both the stages of *L. donovani* were further screened at seven different concentrations to determine the IC_{50} values. The compound with *in vitro* $IC_{50} > 15$ μg/ml was considered to be inactive and not pursued further for evaluation. Out of all the active compounds with IC_{50} values ≤15 μg/ml and better safety index (SI) than the standard drugs Pentamidine and Miltefosine, only five



Scheme 1. Synthesis of 1-phenyl-4-glycosyl-dihydropyridines (**4**–**17**).



Scheme 2. Synthesis of 1-phenyl-4-glycosyl-dihydropyridines (**19**–**21**).

compounds were further evaluated for *in vivo* activity via intraperitoneal route at 50 mg/kg × 5 i.p dose against *L. donovani*/Hamster model (*Mesocricetus auretus*).

The IC_{50} and SI values of 1-phenyl-4-glycosyl-dihydropyridines against promastigotes and intracellular amastigotes of *L. donovani* are depicted in Table 2. As evident from Table 2 most of the 1-phenyl-4-glycosyl-dihydropyridines displayed potent inhibition of both the promastigotes and amastigotes of *L. donovani*. Among all the compounds, only compounds **4**, **11**, **12**, **13**, and **17** have shown IC_{50} in the range of 0.75–6.18 μg /ml with SI (selectivity index) values of 18.93, 9.21, 7.43, 13.58, and 8.04, respectively, against intracellular amastigotes. Further, it is also evident that SI values of these glycosyl dihydropyrdines are several folds better than the standard drugs Pentamidine and Miltefosine. The above five compounds (**4**, **11**, **12**, **13**, and **17**) were further screened for their *in vivo* activity against *L. donovani* in Hamster model intracellular. Compound **12** have shown better inhibition (58.79%) as compared to compounds **11**, **13** and **17** which exhibited 44.9%, 43.9% and 49.73% inhibition, respectively. The compound **4** has only a marginal activity (26.78%).

As evident from antileishmanial activity of the compounds, in general 3,5-dimethyl ester of dihydropyridines are more active than their counterparts the ethyl ester derivatives except compound **17**. Further, it is clear that compounds having 3′-*O*-methyl substituent are more potent than those with 3′-*O*-benzyl substituent. The glycosyl dihydropyridines **4**, **11**, **12**, **13** and **17** have better SI as compared to the standard drugs. Although no definite conclusion of structure activity relationship could be drawn from the above but these glycosyl dihydropyridines open the door to synthesize better analogs as the possibility of optimization with sugar as one of the components is highly enhanced.

2.3. Docking results of synthesized compounds

To explore their probable binding modes within the active site *of L. donovani* PTR1, molecular docking of all the compounds was performed by means of LigandFit program. Since no experimental three-dimensional structure for *L. donovani* PTR1 is known till date, all the compounds were docked into the active site of previously reported *L. donovani* PTR1 homology model [25] using the same protocol.

Molecular docking studies of dihydropyridine derivatives with *L. donovani* PTR1 binding site revealed very clear preference for most of the compounds. All the highly active dihydropyridine derivatives occupy similar spatial position as shown for highly active compound **11** in the *L. donovani* PTR1 binding site in Fig. 1. The docking scores of **11** matches well with the biological activity and compound **11** was among the top ranking compounds in docking scored (Table 3). The docking results indicated that the phenyl substituted dihydropyridine ring could fit well in a hydrophobic pocket formed by residues *Ala* 230, *Tyr* 191, *Tyr* 194, *Phe* 113, *Pro* 224, *Leu* 18. Because the active site of *L. donovani* PTR1 was

Table 2
In vitro and *in vivo* antileishmanial activity of compounds (**4**–**9**, **11**–**17**, and **19**–**21**).

S. No.	Compound No.	*In vitro* assessment IC_{50} (μg/ml)		Cytotoxicity CC_{50} (μg/ml)	Selective index (SI) CC_{50}/IC_{50}	*In vivo* activity (dose-50 mg/kg × 5, i.p.) percent inhibition
		Anti-promastigote activity	Anti-amastigote activity.			
1	**4**	1.94	0.75	14.14	18.93	26.78 ± 24.2
2	**5**	1.86	3.80	4.22	1.109	ND
3	**6**	3.62	4.89	7.66	3.394	ND
4	**7**	3.67	Inactive	ND	ND	ND
5	**8**	1.65	8.08	59.93	7.42	ND
6	**9**	3.72	10.13	29.94	2.96	ND
7	**11**	0.86	3.85	35.49	9.21	44.90 ± 9.8
8	**12**	1.74	4.45	33.07	7.43	58.79 ± 23.5
9	**13**	0.88	3.57	48.49	13.58	43.90 ± 10.3
10	**14**	12.41	4.42	18.61	4.21	ND
11	**15**	1.94	19.05	82.43	4.32	ND
12	**16**	Inactive	Inactive	ND	ND	ND
13	**17**	0.22	6.18	49.73	8.04	49.73 ± 12.0
14	**19**	4.17	14.35	15.23	1.06	ND
15	**20**	Inactive	Inactive	ND	ND	ND
16	**21**	Inactive	Inactive	ND	ND	ND
17	Pentamidine	0.643	12.11	31.31	2.58	92 ± 2.8 (40 × 5, i.p.)
18	Miltefosine	3.23	3.59	3.91	1.08	95.0 ± 3.7 (30 × 5, p.o.)

ND = Not done.

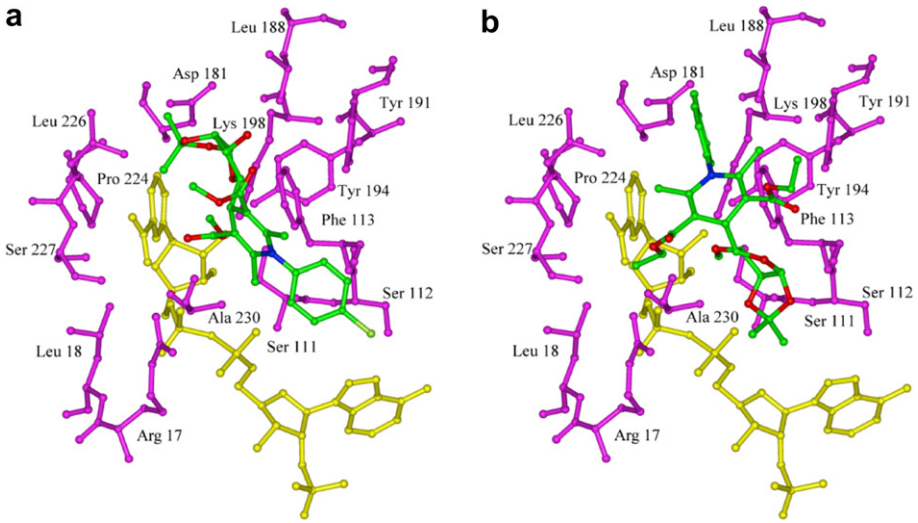


Fig. 1. Docked conformation (a) of compound **11** (atom color green) and (b) of compound **16** (atom color green), along with NADPH (yellow color), in the active site of *Leishmania donovani* PTR1 (magenta).

mostly surrounded by hydrophobic side chains, hydrogen bonding interactions with compound **11** were not prominent. However, dihydropyridine core of these compounds was held in the pocket by combination of van der Waals, hydrophobic, and edge to face π-stacking interactions with the protein. Major hydrophobic contacts occurred between the dihydropyridine core and substituted phenyl rings with the side chain of *Phe* 113, *Tyr* 194 and *Ala* 230. As seen from our docking studies, the *Phe* 113 is involved in edge to face π-stacking interaction with the pyridine core.

This edge to face or T shaped aromatic stacking interaction seems very important for *Leishmania donovani* PTR1 inhibition and may be responsible for the stability of the protein ligand complex (Fig. 1). The docking scores for inactive compound **16** were also in coherence with the biological activity, where it was among the low ranking compounds (Table 3). The binding mode for compound **16** in *L. donovani* PTR1 binding site revealed the reason for inferior activity of compound **16**, where it binds in opposite orientation to that of highly active compound **11**. The possible reason for this type of bound conformation may be the substitution of *O*-ethyl group at 3 and 5 position of the dihydropyridine ring which faces steric clashes with the cofactor NADPH. Therefore, to avoid steric clashes with the cofactor NADPH compound **16** prefers opposite orientation which lead to decrease in the hydrophobic and π-stacking interactions with the protein and hence lower the activity of the compound (Fig. 1). Our docking studies were however unable to explain the reason behind the high activity of compound **17** which was also among the low ranking compound.

3. Conclusion

We have developed an efficient synthesis of glycosyl dihydropyridines involving three component reactions of sugar aldehydes, β-keto esters and anilines. The compounds were evaluated against *L. donovani* both *in vitro* and *in vivo* and few of the compounds displayed potent activities. These compounds with antileishmanial activities comparable to standard drugs and better SI values have led to a new lead for further exploration and development of safe and effective antileishmanial drugs.

4. Experimental

4.1. Chemistry

Commercially available reagent grade chemicals were used as received. All reactions were followed by TLC on E. Merck Kieselgel 60 F_{254}, with detection by UV light, spraying a 20% $KMnO_4$ aq. solution and/or spraying a 4% H_2SO_4 ethanolic solution. Column chromatography was performed on silica gel (100–200 mesh E. Merck). IR spectra were recorded as thin films or on KBr pellets with a Perkin Elmer Spectrum RX-1 (4000–450 cm^{-1}) spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-300/200 in $CDCl_3$ and $CDCl_3 + CCl_4$. Chemical shift values are reported in ppm relative to TMS (tetramethylsilane) as internal reference, unless otherwise stated; s (singlet), d (doublet), t (triplet), m (multiplet); *J* in hertz. ESI mass spectra were performed

Table 3
LigandFit Docking scores of the compounds **4**–**6** and **10**–**17**.

Compound	DOCK Score	Ligscore1	Ligscore 2	PLP1	PLP2	JAIN	PMF	LUDI
4	−11.89	−1.07	−2.01	23.22	32.21	2.78	73.01	621
5	24.90	1.13	2.32	52.04	48.42	0.06	124.52	476
6	51.21	2.86	4.47	86.78	68.45	0.26	138.89	479
10	42.31	2.55	4.39	71.18	54.47	−0.4	98.21	392
11	48.56	3.19	4.58	80.38	65.73	1.08	122.29	507
12	32.49	1.88	2.89	67.04	55.31	−0.49	106.94	441
13	30.24	1.71	2.94	59.86	48.33	−0.31	91.35	388
14	20.38	2.31	3.59	61.03	45.78	−0.67	58.27	343
15	27.68	2.16	4.60	71.96	52.58	−2.05	83.89	290
16	−0.66	0.17	0.84	8.2	18.53	−0.83	74	280
17	−5.01	−0.51	−0.5	48	44.31	0.81	124.51	455

using Quattro II (Micromass). Elemental analyses were performed on a Perkin–Elmer 2400 II elemental analyzer. Optical rotations were measured in a 1.0 dm tube with a Rudolph Autopol III polarimeter in $CHCl_3$.

4.1.1. General procedure for the synthesis of 1-phenyl-4-glycosyl-dihydropyrdine (**4**–**17** and **19**–**21**)

A mixture of the selected dialdose (**1a** or **1b** or **1c** or **18**, 14.5 mmol), methyl/ethylacetoacetate (30.0 mmol) and selected aniline (14.5 mmol) in diethylene glycol in the presence of catalytic amount of tetrabutylammonium hydrogensulphate (20 mol%) was magenetically stirred at 80–100 °C till the disappearance of starting material. After completion of reaction (TLC), the reaction mixture was poured over crushed ice and precipitated solid was filtered. The crude solid mass was purified by column chromatography (SiO_2, 100–200 mesh) using appropriate eluant to give the respective dihydropyrdine derivative.

4.1.2. 2,6-Dimethyl-4-(3′-O-benzyl-1′,2′-O-isopropylidene-β-L-threo-pentofuranos-4′-yl)-1-(4-methoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**4**)

It was obtained as yellow solid in 67% yield; mp 95–98 °C; $R_f = 0.5$ (7:3 hexane–EtOAc); $[\alpha]_D = 13.36$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr): υ_{max} in cm^{-1} 2943, 1696, 1639, 1438, 1211, 1084; ^1H NMR (200 MHz, $CDCl_3$): $\delta = 7.40–7.24$ (m, 5H, ArH), 7.08 (d, $J = 8.76$ Hz, 2H, ArH), 6.91 (d, $J = 8.76$ Hz, 2H, ArH), 5.86 (d, $J = 3.94$ Hz, 1H, H-1′), 4.67–4.54 (m, 3H, OCH_2Ph, H-4), 4.48 (d, $J = 3.88$ Hz, 1H, H-2′), 4.01 (dd, $J_1 = 3.24$ Hz, $J_2 = 7.78$ Hz, 1H, H-4′), 3.83–3.81 (m, 4H, ArOCH_3, H-3′), 3.66, 3.46 (each s, 6H, 2×$COOCH_3$), 2.07, 2.02 (each s, 6H, 2×CH_3), 1.46, 1.27 (each s, 6H, $(CH_3)_2C$); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 169.0, 168.8, 159.6, 148.6, 148.2, 138.5, 133.3, 131.6, 128.6, 127.7, 127.2, 114.6, 111.2, 105.0, 103.6, 102.5, 83.8, 82.7, 82.1, 71.4, 55.7, 51.5, 32.6, 27.2, 26.7, 18.4, 18.1$. ESMS: $m/z = 580$ $[M + H]^+$. Anal. For $C_{32}H_{37}NO_9$: C, 66.31; H, 6.43; N, 2.42; Found C, 66.28; H, 6.45; N, 2.40.

4.1.3. 2,4-Dimethyl-4-(3′-O-benzyl-1′,2′-O-isopropylidene-β-L-threo-pentofuranos-4′-yl)-1-(4-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**5**)

It was obtained as yellow solid in 74% yield; mp 82–86 °C; $R_f = 0.5$ (7:3 hexane–EtOAc); $[\alpha]_D = -7.80$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 2947, 1697, 1641, 1212, 1084; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.42–7.20$ (m, 7H, ArH), 7.15 (d, $J = 8.54$ Hz, 2H, ArH), 5.85 (d, $J = 3.96$ Hz, 1H, H-1′), 4.65–4.53 (m, 3H, OCH_2Ph, H-4), 4.47 (d, $J = 3.82$ Hz, 1H, H-2′), 3.99 (dd, $J_1 = 3.28$ Hz, $J_2 = 7.18$ Hz, 1H, H-4′), 3.84 (d, $J = 3.26$ Hz, 1H, H-3′), 3.63, 3.48 (each s, 6H, 2×$COOCH_3$), 2.05, 2.02 (each s, 6H, 2×CH_3), 1.45, 1.27 (each s, 6H, $(CH_3)_2C$); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 168.7, 168.6, 147.9, 147.2, 139.3, 138.4, 134.9, 132.1, 129.8, 128.6, 127.8, 127.2, 111.3, 105.0, 104.3, 103.2, 83.9, 82.8, 82.1, 71.5, 51.5, 32.7, 32.0, 27.2, 26.6, 23.0, 18.4, 18.2, 14.5$. ESMS: m/z = 584 $[M + H]^+$. Anal. For $C_{31}H_{34}NClO_8$: C, 63.75; H, 5.87; N, 2.40; Found: C, 63.65; H, 5.90; N, 2.37.

4.1.4. 2,6-Dimethyl-4-(3′-O-benzyl-1′,2′-O-isopropylidene-β-L-threo-pentofuranos-4′-yl)-1-(4-methylphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**6**)

It was obtained as yellow solid in 78 % yield; mp 78–82 °C; $R_f = 0.5$ (7:3 hexane–EtOAc); $[\alpha]_D = 6.58$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 2986, 2946, 1696, 1211, 1058; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.32–7.24$ (m, 5H, ArH), 7.18 (d, $J = 8.08$ Hz, 2H, ArH), 7.05 (d, $J = 8.22$ Hz, 2H, ArH), 5.86 (d, $J = 3.96$ Hz, 1H, H-1′), 4.68–4.53 (m, 3H, OCH_2Ph, H-4), 4.48 (d, $J = 3.92$ Hz, 1H, H-2′), 4.02 (dd, $J_1 = 3.24$ Hz, $J_2 = 7.98$ Hz, 1H, H-4′), 3.83 (d, $J = 3.22$ Hz, 1H, H-2′) 3.66, 3.43 (each s, 6H, 2×$COOCH_3$), 2.40 (s, 3H, ArCH_3), 2.07, 2.02 (each s, 6H, 2×CH_3), 1.46, 1.27 (each s, 6H, $(CH_3)_2C$); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 169.0, 168.7, 148.2, 148.0, 138.7, 138.5, 138.1, 130.4, 130.2, 28.6, 127.7, 127.1, 111.2, 105.0, 103.7, 102.6, 83.8, 82.7, 82.1, 71.4, 51.5, 51.4, 32.6, 27.2, 26.7, 21.5, 18.4, 18.1$. ESMS: $m/z = 564$ $[M + H]^+$. Anal. For $C_{32}H_{37}NO_8$: C, 68.19; H, 6.62; N, 2.49; Found C, 68.12; H, 6.67; N, 2.45.

4.1.5. 2,6-Dimethyl-4-(3′-O-benzyl-1′,2′-O-isopropylidene-β-L-threo-pentofuranos-4′-yl)-1-(4-flurophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**7**)

It was obtained as yellow solid in 70% yield; mp 78–81 °C; $R_f = 0.5$ (7:3 hexane–EtOAc); $[\alpha]_D = -4.63$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 3020, 2926, 2361, 1691, 1509, 1215; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$), $\delta = 7.38–7.20$ (m, 5H, ArH), 7.20–7.10 (m, 4H, ArH), 5.84 (d, $J = 3.81$ Hz, 1H, H-1′), 4.63–4.51 (m, 3H, OCH_2Ph and H-4), 4.47 (d, $J = 3.87$ Hz, 1H, H-2′), 3.98 (dd, $J_1 = 3.18$ Hz, $J_2 = 6.72$ Hz, 1H, H-4′), 3.84 (d, $J = 3.15$ Hz, 1H, H-3′), 3.64 (s, 3H, $COOCH_3$), 3.51 (s, 3H, $COOCH_3$), 2.07 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 1.46, 1.28, (each s, 6H, $(CH_3)_2C$); ^{13}C NMR (75 MHz, $CDCl_3 + CCl_4$), $\delta = 168.1, 147.1, 147.1, 138.0, 132.1, 132.0, 128.2, 127.3, 126.8, 116.3, 116.0, 110.8, 104.5, 103.8, 102.7, 83.5, 82.5, 81.8, 71.0, 51.0, 32.3, 32.3, 26.8, 26.3, 17.9, 17.8$; ESI MS: 568.2 $[M + H]^+$, 590 $[M + Na]^+$, HRMS (JOEL MS Route) m/z calc. for $C_{31}H_{35}FNO_8$ $[M + H]^+$ 568.23467, found 568.23490.

4.1.6. 2,6-Dimethyl-4-(3′-O-benzyl-1′,2′-O-isopropylidene-β-L-threo-pentofuranos-4′-yl)-1-(4-flurophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid diethyl ester (**8**)

It was obtained as yellow solid in 76% yield; m.p. = 132–135 °C; $R_f = 0.5$ (7:3 hexane–EtOAc); $[\alpha]_D = 6.3$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 3021, 2930, 1684, 1639, 1508, 1215; ^1H NMR (300 MHz, $CDCl_3 + CCl_4$), $\delta = 7.38–7.26$ (m, 5H, ArH), 7.21–7.09 (m, 4H, ArH), 5.88 (d, $J = 3.90$ Hz, 1H, H-1′), 4.69–4.52 (m, 3H, OCH_2Ph and H-4), 4.50 (d, $J = 3.90$ Hz, 1H, H-2′), 4.14–4.00 (m, 5H, OCH_2CH$_3$ and H-4′), 3.88–3.84 (m, 1H, H-3′), 2.07 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.29–1.21 (m, 9H, OCH_2CH_3 and $(CH_3)_2C$), 1.16–1.11 (t, $J = 7.10$ Hz, OCH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3 + CCl_4$), $\delta = 168.1, 167.9, 146.9, 146.6, 138.1, 132.1, 132.0, 128.1, 127.3, 126.7, 116.2, 115.9, 110.9, 104.6, 104.0, 103.1, 83.5, 82.4, 81.8, 70.8, 59.8, 59.6, 32.6, 26.8, 26.3, 17.9, 17.7$; ESI MS: 596.2 $[M + H]^+$, 619.3 $[M + Na]^+$, HRMS (JOEL MS Route) m/z calc. for $C_{33}H_{39}FNO_8$ $[M + H]^+$ 596.26597, found 596.26815.

4.1.7. 2,6-Dimethyl-4-(1′,2′-O-isopropylidene-β-L-threo-pentofuranos-4′-yl)-1-(3,4-dichlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**9**)

It was obtained as light yellow solid in 76% yield; mp 137–141 °C; $R_f = 0.5$ (8:2 hexane–EtOAc); $[\alpha]_D = -15.06$ (c 0.1, $CHCl_3$, at 25 °C), IR (KBr) υ_{max} in cm^{-1}, 3020, 1684, 1216, 1076; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.50–7.45$ (m, 1H, ArH), 7.26–7.20 (m, 1H, ArH), 6.99–6.95 (m, 1H, ArH), 5.95 (d, $J = 3.08$ Hz, 1H, H-1′), 4.54 (dd, $J_1 = 3.54$ Hz, $J_2 = 6.42$ Hz, 1H, H-2′), 4.22 (s, 1H, H-4), 4.00 (s, 1H, H-4′), 3.73 (s, 1H, OH), 3.68, 3.66 (each s, 6H, 2×$COOCH_3$), 3.18 (d, $J = 2.74$ Hz, 1H, H-3′), 2.10, 1.51 (each s, 6H, 2×CH_3), 1.29, 1.14 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 171.4, 167.8, 156.0, 140.1, 133.4, 131.9, 130.1, 129.3, 112.3, 106.2, 96.5, 84.4, 83.7, 77.9, 77.8, 76.7, 74.3, 72.9, 72.6, 52.1, 51.4, 42.4, 33.0, 30.1, 27.1, 26.5, 26.0, 19.5$. ESMS: $m/z = 528$ $[M + H]^+$ Anal. For $C_{24}H_{27}NCl_2O_8$: C, 54.55; H, 5.15; N, 2.65; Found C, 54.52; H, 5.25; N, 2.67.

4.1.8. 2,6-Dimethyl-4-(1′,2′-O-isopropylidene-β-L-threo-pentofuranos-4′-yl)-1-phenyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**10**)

It was obtained as light yellow solid in 68% yield; mp 85–89 °C; $R_f = 0.5$ (8:2, hexane–EtOAc); $[\alpha]_D = -22.97$ (c 0.1, $CHCl_3$ at 25 °C); IR (KBr) υ_{max} in cm^{-1} 3020, 1676, 1216, 1089; ^1H NMR (300 MHz,

$CDCl_3$) $\delta = 7.48–7.31$ (m, 4H, ArH), 7.07–7.03 (m, 1H, ArH), 5.98 (d, $J = 3.66$ Hz, 1H, H-1′), 4.52 (d, $J = 3.60$ Hz, 1H, H-4), 4.27 (t, $J = 3.03$ Hz, 1H, H-2′), 4.05 (d, $J = 1.80$ Hz, 1H, H-4′), 3.77 (s, 1H, OH), 3.69, 3.68 (each s, 6H, 2×$COOCH_3$), 3.20 (d, $J = 2.76$ Hz, 1H, H-3′), 2.10, 1.54 (each s, 6H, 2×CH_3), 1.30, 1.27 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 171.4, 156.6, 139.2, 134.5, 133.1, 131.8, 129.6, 111.9, 106.2, 94.1, 84.2, 83.7, 74.3, 52.1, 51.3, 42.5, 33.0, 27.1, 26.5, 25.9, 19.5$ ESMS: $m/z = 460$ $[M + H]^+$; Anal. For $C_{24}H_{29}NO_8$: C, 62.73; H, 6.36; N, 3.05; Found: C, 62.53; H, 6.51; N, 3.10.

4.1.9. 2,6-Dimethyl-4-(1′,2′-O-isopropylidene-β-L-threo-pentofuranos-4′-yl)-1-(4-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**11**)

It was obtained as light yellow solid in 77% yield; mp 88–92 °C; $R_f = 0.5$ (7:3 hexane–EtOAc); $[\alpha]_D = -9.13$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 3020, 1698, 1597, 1216, 1043; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.40–7.34$ (m, 2H, ArH), 7.06–6.93 (m, 2H, ArH), 5.73 (d, $J = 3.42$ Hz, 1H, H-1′), 4.57 (d, $J = 3.66$ Hz, 1H, H-4), 4.19–4.15 (m, 2H, H-2′ and H-4′), 3.94 (s, 1H, OH), 3.69 (s, 6H, 2×$COOCH_3$), 2.56 (d, $J = 2.86$ Hz, 1H, H-3′), 2.08, 1.49 (each s, 6H, 2×CH_3), 1.30 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 170.3, 167.6, 155.2, 138.8, 134.6, 133.4, 131.9, 130.1, 129.3, 112.3, 106.2, 97.0, 84.4, 84.1, 76.9, 74.7, 51.9, 51.3, 43.2, 33.1, 27.4, 26.7, 25.4, 19.5$ ESMS: $m/z = 494$ $[M + H]^+$. Anal. For $C_{24}H_{28}NClO_8$: C, 58.36; H, 5.71; N, 2.84; Found C, 58.26; H, 5.78; N, 2.85.

4.1.10. 2,6-Dimethyl-4-(1′,2′-O-isopropylidene-3′-O-methyl-β-L-threo-pentofuranos-4′-yl)-1-(4-methylphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**12**)

It was obtained as light yellow solid in 78% yield; mp 185–188 °C; $R_f = 0.5$ (7:3 hexane–EtOAc); $[\alpha]_D = -59.69$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 3020, 1689, 1216, 1039; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.20$ (d, $J = 8.14$ Hz, 2H, ArH), 7.03 (d, $J = 8.24$ Hz, 2H, ArH), 5.86 (d, $J = 3.98$ Hz, 1H, H-1′), 4.47–4.44 (m, 2H, H-4, H-2′), 3.95 (dd, $J_1 = 3.18$ Hz, $J_2 = 7.30$ Hz, 1H, H-4′), 3.73, 3.72 (each s, 6H, 2×$COOCH_3$), 3.55 (d, $J = 3.20$ Hz, 1H, H-3′), 3.29 (s, 3H, OCH_3), 2.38 (s, 3H, ArCH_3), 2.05, 1.98 (each s, 6H, 2×CH_3), 1.45, 1.28 (each s, 6H, $(CH_3)_2C$); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 169.0, 168.8, 148.0, 147.9, 138.6, 138.1, 130.4, 130.1, 111.1, 105.1, 103.4, 102.0, 84.6, 83.6, 81.2, 57.2, 51.5, 33.1, 27.2, 26.6, 21.54, 18.3, 18.1$; ESMS: $m/z = 488$ $[M + H]^+$; Anal. For $C_{26}H_{33}NO_8$: C, 64.05; H, 6.82; N, 2.87; Found: C, 64.10; H, 6.89; N, 2.79.

4.1.11. 2,6-Dimethyl-4-(1′,2′-O-isopropylidene-3′-O-methyl-β-L-threo-pentofuranos-4′-yl)-1-(4-bromophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**13**)

It was obtained as light yellow solid in 64% yield; mp 209–212 °C; $R_f = 0.5$ (6:4 hexane–EtOAc); $[\alpha]_D = -33.56$ (c 0.1, $CHCl_3$, at 25 °C). IR (KBr) υ_{max} in cm^{-1} 3020, 1697, 1215, 1082; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.55$ (d, $J = 6.64$ Hz, 2H, ArH), 7.09 (d, $J = 6.62$ Hz, 2H, ArH), 5.86 (d, $J = 4.00$ Hz, 1H, H-1′), 4.47–4.42 (m, 2H, H-4, H-2′), 3.92 (dd, $J_1 = 3.24$ Hz, $J_2 = 6.22$ Hz, 1H, H-4′), 3.74, 3.73 (each s, 6H, 2×$COOCH_3$), 3.56 (d, $J = 3.22$ Hz, 1H, H-3′), 3.28 (s, 3H, OCH_3), 2.04, 2.00 (each s, 6H, 2×CH_3), 1.44, 1.25 (each s, 6H, $(CH_3)_2C$) ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 168.7, 147.8, 146.9, 139.9, 132.8, 132.5, 122.9, 111.2, 105.1, 104.1, 102.5, 84.9, 83.6, 81.2, 77.9, 77.3, 76.7, 57.2, 55.7, 51.6, 33.3, 27.2, 26.6, 18.3, 18.2$, ESMS: $m/z = 552$ $[M + H]^+$, 574 $[M + Na]^+$ Anal. For $C_{25}H_{30}NBrO_9$: C, 54.36; H, 5.47; N, 2.54; Found: C, 54.43; H, 5.44; N, 2.57.

4.1.12. 2,6-Dimethyl-4-(1′,2′-O-isopropylidene-3′-O-methyl-β-L-threo-pentofuranos-4′-yl)-1-(4-methoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**14**)

It was obtained as light yellow solid in 70% yield; mp 214–217 °C; $R_f = 0.5$ (6:4 hexane–EtOAc); IR (KBr) υ_{max} in cm^{-1} 3419, 2950, 1705, 1654, 1212, 1090; $[\alpha]_D = -38.05$ (c 0.1, $CHCl_3$, at 25 °C); ^1H NMR (300 MHz, $CDCl_3$) $\delta = 7.09$ (d, $J = 8.85$ Hz, 2H, ArH), 6.91 (d, $J = 8.85$ Hz, 2H, ArH), 5.90 (d, $J = 3.96$ Hz, 1H, H-1′), 4.51–4.48 (m, 2H, H-4, H-2′), 3.97 (dd, $J_1 = 3.18$ Hz, $J_2 = 7.08$ Hz, 1H, H-4′), 3.85 (s, 3H, $ArOCH_3$), 3.76, 3.75 (each s, 6H, 2×$COOCH_3$), 3.58 (d, $J = 2.12$ Hz, 1H, H-3′), 3.32 (s, 3H, OCH_3), 2.08, 2.02 (each s, 6H, 2×CH_3), 1.48, 1.27 (each s, 6H, $(CH_3)_2C$); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 169.0, 168.8, 159.6, 148.4, 148.1, 133.3, 131.6, 114.6, 111.2, 105.1, 103.4, 101.9, 84.7, 83.6, 81.2, 78.0, 77.3, 76.7, 57.2, 55.7, 51.5, 33.2, 27.2, 26.6, 18.3, 18.1$; ESMS: $m/z = 504$ $[M + H]^+$ Anal. For $C_{26}H_{33}NO_9$: C, 62.02; H, 6.61; N, 2.78; Found: C, 62.03; H, 6.59; N, 2.59.

4.1.13. 2,6-Dimethyl-4-(1′,2′-O-isopropylidene-3′-O-methyl-β-L-threo-pentofuranos-4′-yl)-1-(4-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**15**)

It was obtained light yellow solid in 73%; mp 190–194 °C; $R_f = 0.5$ (7: 3 hexane–EtOAc); $[\alpha]_D = -48.47$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 3020, 1692, 1652, 1316, 1216, 1046; ^1H NMR (300 MHz, $CDCl_3$) $\delta = 7.40$ (d, $J = 6.69$ Hz, 2H, ArH), 7.15 (d, $J = 6.81$ Hz, 2H, ArH), 5.87 (d, $J = 3.96$ Hz, 1H, H-1′), 4.49–4.46 (m, 2H, H-4, H-2′), 3.94 (dd, $J_1 = 3.18$ Hz, $J_2 = 6.33$ Hz, 1H, H-4′), 3.75, 3.74 (each s, 6H, 2×$COOCH_3$), 3.57 (d, $J = 3.18$ Hz, 1H, H-3′), 3.29 (s, 3H, OCH_3), 2.38 (s, 3H, $ArCH_3$), 2.05, 2.00 (each s, 6H, 2×CH_3), 1.45, 1.30 (each s, 6H, $(CH_3)_2C$); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 168.4, 168.4, 147.5, 146.6, 138.9, 134.5, 131.7, 129.4, 110.8, 104.7, 103.7, 102.0, 84.4, 83.2, 80.8, 56.8, 51.2, 51.1, 32.9, 26.7, 26.1, 17.9, 17.7$ ESMS: $m/z = 508$ $[M + H]^+$ Anal. For $C_{25}H_{28}NClO_8$: C, 59.11; H, 5.95; N, 2.76; Found: C, 59.10; H, 5.97; N, 2.80.

4.1.14. 2,6-Dimethyl-4-(1′,2′-O-isopropylidene-3′-O-methyl-β-L-threo-pentofuranos-4′-yl)-1-(4-methylphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid diethyl ester (**16**)

It was obtained as light yellow solid in 70% yield; mp 180–183 °C; $R_f = 0.5$ (6:4 hexane–EtOAc); $[\alpha]_D = -36.48$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 3020, 1687, 1215, 1085, 1034; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.19$ (d, $J = 8.18$ Hz, 2H, ArH), 7.03 (d, $J = 8.20$ Hz, 2H, ArH), 5.88 (d, $J = 4.00$ Hz, 1H, H-1′), 4.55–4.47 (m, 2H, H-4, H-2′), 4.26–4.12 (m, 4H, 2×$COOCH_2CH_3$), 3.99 (dd, $J_1 = 3.22$ Hz, $J_2 = 7.12$ Hz, 1H, H-4′), 3.57(d, $J = 3.20$ Hz, 1H, H-3′), 3.29 (s, 3H, OCH_3), 2.38 (s, 3H, $ArCH_3$), 2.04, 1.98 (each s, 6H, 2×CH_3), 1.46 (s, 3H, $(CH_3)_2C$), 1.34–1.25 (m, 9H, $(CH_3)_2C$ and 2×$COOCH_2CH_3$) ^{13}C NMR (50 MHz $CDCl_3$): $\delta = 168.8, 168.5, 147.7, 147.5, 138.6, 138.2, 130.5, 130.1, 111.1, 105.1, 103.7, 102.3, 84.7, 83.6, 81.3, 60.1, 60.0, 57.1, 33.2, 27.1, 26.6, 21.5, 18.4, 18.1, 14.8, 14.7$ ESMS: $m/z = 516$ $[M + H]^+$; Anal. For $C_{28}H_{37}NO_8$: C, 65.23; H, 7.23; N, 2.72; Found: C, 65.18; H, 7.30; N, 2.80.

4.1.15. 2,6-Dimethyl-4-(1′,2′-O-isopropylidene-3′-O-methyl-β-L-threo-pentofuranos-4′-yl)-1-(2-methoxyphenyl)1,4-dihydropyridine-3,5-dicarboxylic acid diethyl ester (**17**)

It was obtained as light yellow solid in 71% yield; mp 160–163 °C; $R_f = 0.5$ (6:4 hexane–EtOAc); $[\alpha]_D = -26.43$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 3020, 1682, 1216, 1042; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.40–7.31$ (m, 1H, ArH), 7.08–6.91 (m, 3H, ArH), 5.88 (d, $J = 3.94$ Hz, 1H, H-1′), 4.58–4.47 (m, 2H, H-4, H-2′), 4.25–4.15 (m, 4H, 2×$COOCH_2CH_3$), 3.98 (dd, $J_1 = 3.18$ Hz, $J_2 = 6.98$ Hz, 1H, H-4′), 3.82 (s, 3H, $ArOCH_3$), 3.56 (d, $J = 4.00$ Hz, 1H, H-3′), 3.28 (s, 3H, OCH_3), 2.02, 1.96 (each s, 6H, 2×CH_3), 1.46 (s, 6H, $(CH_3)_2C$), 1.34–1.24 (m, 9H, $(CH_3)_2C$ and 2×$COOCH_2CH_3$), ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 168.9, 168.7, 157.0, 148.6, 148.2, 131.2, 130.5, 29.8, 121.7, 111.7, 111.1, 105.2, 102.8, 101.3, 84.8, 83.4, 81.3, 60.0, 59.9, 57.1, 55.5, 33.2, 27.2, 26.6, 16.5, 16.2, 14.8, 14.8$ ESMS: $m/z = 532$ $[M + H]^+$, 554 $[M + Na]^+$ Anal. For $C_{28}H_{37}NO_9$: C, 63.26; H, 7.02; N, 2.63; Found : C, 63.30; H, 7.17; N, 2.59.

*4.1.16. 2,6-Dimethyl-4-(1′,2′,3′,4′-di-O-isopropylidene-α-^{l}-arabino-pyranos-5′-yl)-1-(4-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**19**)*

It was obtained as yellow solid in 76% yield; mp 190–194 °C; $R_f = 0.5$ (7:3 hexane–EtOAc); $[\alpha]_D = -73.83$(c 0.1, $CHCl_3$, at 25 °C), IR (KBr) υ_{max} in cm^{-1} 3020, 1689, 1216, 1068; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.40$ (dd, $J_1 = 2.00$ Hz, $J_2 = 6.58$ Hz, 2H, ArH), 7.19 (dd, $J_1 = 2.08$ Hz, $J_2 = 6.64$ Hz, 2H, ArH); 5.48 (d, $J = 4.86$ Hz, 1H, H-1′), 4.51 (dd, $J_1 = 2.16$ Hz, $J_2 = 8.03$ Hz, 1H, H-3), 4.40 (d, $J = 2.12$ Hz, 1H, H-4), 4.27 (dd, $J_1 = 1.38$ Hz, $J_2 = 7.90$ Hz, 1H, H-4′), 4.20 (dd, $J_1 = 2.16$ Hz, $J_2 = 4.88$ Hz, 1H, H-2′), 3.71, 3.68 (each s, 6H, 2×$COOCH_3$), 3.60 (dd, $J_1 = 1.28$ Hz, $J_2 = 3.76$ Hz, 1H, H-5′), 2.00 (s, 6H, 2×CH_3), 1.46, 1.39, 1.27, 1.25 (each s, 12H, 2×$(CH_3)_2C$); ^{13}C NMR (50 MHz $CDCl_3$): $\delta = 168.9$, 168.8, 149.7, 147.3, 139.9, 134.6, 132.1, 129.7, 125.2, 109.9, 109.4, 108.6, 103.1, 101.2, 97.2, 74.7, 73.3, 71.7, 71.5, 71.2, 51.5, 51.2, 35.6, 26.2, 25.5, 25.2, 18.5, 18.3 ESMS: $m/z = 564$ $[M+H]^+$ Anal. For $C_{28}H_{34}ClNO_9$: C, 59.63; H, 6.08; N, 2.48; Found : C, 59.61; H, 6.12; N,2.50.

*4.1.17. 2,6-Dimethyl-4-(1′,2′,3′,4′-di-O-isopropylidene-α-L-arabinopyranos-5′-yl)-1-(4-methoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**20**)*

It was obtained as yellow solid in 78% yield; mp 190–193 °C; $R_f = 0.5$ (7:3 hexane–EtOAc); $[\alpha]_D = -73.94$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 2984, 2934, 1697, 1637, 1206, 1073; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.10$ (dd, $J_1 = 2.72$ Hz, $J_2 = 6.72$ Hz, 2H, ArH), 6.88 (dd, $J_1 = 2.14$ Hz, $J_2 = 6.86$ Hz, 2H, ArH), 5.47 (d, $J = 2.94$ Hz, 1H, H-1′), 4.48 (dd, $J_1 = 2.12$ Hz, $J_2 = 7.90$ Hz, 1H, H-3′), 4.43 (d, $J = 9.28$ Hz, 1H, H-4), 4.24 (dd, $J_1 = 1.24$ Hz, $J_2 = 7.84$ Hz, 1H, H-4′), 4.17 (dd, $J_1 = 2.16$ Hz, $J_2 = 4.96$ Hz, 1H, H-2′), 3.81 (s, 3H, $ArOCH_3$), 3.69, 3.65 (each s, 6H, 2×$COOCH_3$), 3.57 (dd, $J_1 = 1.18$ Hz, $J_2 = 4.16$ Hz, 1H, H-2′), 2.00, 1.98 (each s, 6H, 2×CH_3), 1.46, 1.41, 1.26, 1.24 (each s, 12H, 2×$(CH_3)_2C$); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 168.8$, 168.7, 167.7, 159.4, 153.1, 150.1, 148.0, 145.7, 134.0, 132.6, 131.5, 126.2, 116.6, 115.0, 114.5, 110.3, 109.7, 109.3, 109.1, 109.0, 108.4, 108.2, 102.5, 100.8, 74.7, 73.3, 71.7, 71.5, 71.2, 51.5, 51.2, 35.6, 26.2, 25.5, 25.2, 18.5, 18.3 ESMS: $m/z = 560$ $[M+H]^+$ 582 $[M+Na]^+$; Anal. For $C_{29}H_{37}NO_{10}$: C, 62.24; H, 6.66; N, 2.50; Found : C, 62.30; H, 6.70; N, 2.47.

*4.1.18. 2,6-Dimethyl-4-(1′,2′,3′,4′-di-O-isopropylidene-α-L-arabinopyranos-5′-yl)-1-(4-aminophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**21**)*

It was obtained as yellow solid in 66% yield; mp 189–192 °C; $R_f = 0.5$ (7: 3 hexane–EtOAc); $[\alpha]_D = -73.94$ (c 0.1, $CHCl_3$, at 25 °C); IR (KBr) υ_{max} in cm^{-1} 3021, 2929, 1683, 1587, 1216; ^1H NMR (200 MHz, $CDCl_3$) $\delta = 6.94$ (d, $J = 8.12$ Hz, 2H, ArH), 6.67 (d, $J = 8.18$ Hz, 2H, ArH); 5.51 (d, $J = 2.94$ Hz, 1H, H-1′), 4.51–4.44 (m, 2H, H-3′ and H-4), 4.26–4.22 (m, 2H, H-4′ and H-2′), 3.70–3.61 (m, 9H, 2×$COOCH_3$, H-5′, NH_2), 2.05, 2.02 (each s, 6H, 2×CH_3), 1.39, 1.38, 1.27, 1.25 (each s, 12H, 2×$(CH_3)_2C$); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 169.5$, 150.2, 148.7, 146.6, 131.6, 131.3, 115.4, 109.5, 108.6, 102.2, 100.7, 72.8, 71.7, 71.6, 71.4, 51.4, 51.2, 35.5, 30.1, 29.7, 26.3, 25.5, 25.2, 18.4, 18.3; ESMS: $m/z = 567$ $[M+Na]^+$ Anal. For $C_{28}H_{36}N_2O_9$: C, 61.75; H, 6.66; N, 5.14; Found: C, 61.77; H, 6.62; N, 5.13.

4.2. Biology

4.2.1. Anti-promastigote assay

The *L. donovani* promastigotes (MHOM IN/Dd_8; originally obtained from Imperial college, London) were transfected with fire fly luciferase gene, and the transfectants were maintained in medium 199 (Sigma chemical Co., USA) supplemented with 10% fetal calf serum (GIBCO) and gentamycin (4μg/ml) solution (Sigma) under pressure of G 418 (Sigma) [26].

The *in vitro* effect of the compounds on the growth of promastigotes was assessed by monitoring the luciferase activity of viable cells after treatment. The transgenic promastigotes of late log phase were seeded at 5×10^5/100 μl medium 199 well in 96-well flat-bottomed microtiter (MT) plates (CELLSTAR) and incubated for 72 h in medium alone or in the presence of serial dilutions of drugs (1–40 μg/ml) in DMSO. Parallel dilutions of DMSO were used as controls. After incubation, an aliquot (50 μl) of promastigote suspension was aspirated from each well of a 96-well plate and mixed with an equal volume of Steady Glo® reagent (Promega) and luminescence was measured by a luminometer. The values were expressed as relative luminescence unit (RLU) [32]. The inhibition of parasitic growth is determined by comparison of the luciferase activity of drug treated parasites with that of untreated controls by the general formula:

$$\%\text{Inhibition} = (N - n \times 100)/N$$

where N is average relative luminescence unit (RLU) of control wells; and n is average RLU of treated wells.

4.2.2. Anti-amastigote assay

For assessing the activity of compounds against the amastigote stage of the parasite, mouse macrophage cell line (J-774A.1) infected with promastigotes expressing luciferase firefly reporter gene was used. Cells were seeded in a 96-well plate (4×10^4cell/100 μl/well) in RPMI-1640 containing 10% fetal calf serum and the plates were incubated at 37 °C in a CO_2 incubator. After 24 h, the medium was replaced with fresh medium containing stationary phase promastigotes (4×10^5/100 μl/well). Promastigotes invade the macrophages and are transformed into amastigotes. The test compounds are added at two fold dilutions up to 7 points in complete medium starting from 40 μg/ml conc. after replacing the previous medium and the plates were incubated at 37 °C in a CO_2 incubator for 72 h. After incubation, the drug containing medium was decanted and 50 μl PBS was added in each well and mixed with an equal volume of steady Glo reagent. After gentle shaking for 1–2 min, the reading was taken in a luminometer as repoeted earlier [33,34]. The values are expressed as relative luminescence units (RLU). Data are transformed into a graphic program (Excel). IC_{50} of antileishmanial activity was calculated by nonlinear regression analysis of the concentration–response curve using the four parameter Hill equations.

4.2.3. Cytotoxicity assay

The cell viability was determined using the MTT assay developed by Mossman et al. [35] Exponentially growing cells (J-774A.1) (1×10^5cells /100 μl/well) were incubated with different drug concentrations for 72 h and were incubated at 37 °C in a humidified mixture of CO_2 and 95% air in an incubator. Stock solutions of compounds prepared in DMSO were further diluted with fresh complete medium. After incubation, 25 μl of MTT reagent (5 mg/ml) in PBS medium, followed by syringe filtration were added to each well and incubated at 37 °C for 2 h. At the end of the incubation period, the supernatant was removed by tilting plate completely without disturbing cell layer and 150 μl of pure DMSO are added to each well. After 15 min of shaking the readings were recorded as absorbance at 544 nm on a micro plate reader. The cytotoxic effect were expressed as 50% lethal dose, i.e., as the concentration of a compound which provoked a 50% reduction in cell viability compared to cell in culture medium alone. IC_{50} values were estimated as described by Huber and Koella [36].

4.2.4. In vivo assay

The method of Beveridge [37], as modified by Bhatnagar et al. [38] and Gupta et al. [39] was used for *in vivo* screening. Golden hamsters (inbred strain) of either sex weighing 40–45 g were infected intracardially with 1×10^7 amastigotes per animal. The infection is well adapted to the hamster model and establishes itself in 15–20 days. Meanwhile, hamsters gain weight (85–95 g) and can be subjected to repeated spleen biopsies. Pre-treatment spleen biopsy in all the animals is carried out to assess the degree of infection. The animals with +1 infection (5–15 amastigotes/100 spleen cell nuclei) are included in the chemotherapeutic trials. The infected animals are randomized into several groups on the basis of their parasitic burdens. Five to six animals are used for each test sample. Drug treatment by intraperitoneal (i.p.) route is initiated after 2 days of biopsy and continued for 5 consecutive days. Post-treatment biopsies are done on day 7 of the last drug administration and amastigote counts are assessed by Giemsa staining. Intensity of infection in both, treated and untreated animals, as also the initial count in treated animals is compared and the efficacy is expressed in terms of % inhibition of amastigotes multiplication using the following formula:

$$\%\text{Inhibition} = 100 - [\text{ANAT} \times 100/(\text{INAT} \times \text{TIUC})]$$

where ANAT is Actual Number of Amastigotes in Treated animals, INAT is Initial Number of Amastigotes in Treated animals and TIUC is Times Increase of parasites in Untreated Control animals.

4.3. Molecular docking (materials and methods)

Molecular docking of the compounds in the active site of *L. donovani* PTR1 homology model was carried out using LigandFit program available with Cerius2 Version 4.10 [40]. This algorithm makes use of a shape comparison filter in combination with a Monte Carlo conformational search for generating ligand poses consistent with the active site shape. Candidate poses are minimized in the context of the active site using a grid-based method for evaluating protein–ligand interaction energies. The docked conformations predicted by LigandFit were ranked with built-in scoring functions in Cerius2. As each scoring function may rank binding poses differently, a consensus scoring approach was used with six different scoring functions: (i) Dock score, (ii) Ligscore, (iii) PLP1, (iv) PLP2, (v) potential mean force (PMF), and (vi) LUDI. The conformer with best consensus score was taken as the best docked conformation. The LigandFit scores for the compounds are presented in Table 3.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2010.02.018.

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