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Bioorganic & Medicinal Chemistry Letters 17 (2007) 4934–4937
Bioorganic & Medicinal Chemistry Letters

Interaction of *O*-(undec-10-en)-yl-D-glucose derivatives with the *Plasmodium falciparum* hexose transporter (PfHT)

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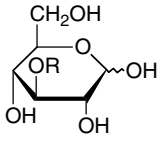
Received 30 April 2007; revised 6 June 2007; accepted 7 June 2007
Available online 10 June 2007

Abstract—All-*O*-undec-en-10-yl derivatives of D-glucose have been prepared and their affinities for the *Plasmodium falciparum* hexose transporter (PfHT) determined; the *O*-2 derivative displays a good apparent affinity for PfHT (K_I = 2 μM) with no significant interaction with the mammalian transporter GLUT1. This selectivity points to position −2 of glucose as an appropriate substitution site for the development of inhibitors of *P. falciparum* glucose uptake.

There is an urgent need for new antimalarial agents because of the emergence of multidrug resistant *Plasmodium falciparum* infections. PfHT (*P. falciparum* hexose transporter) is a member of the Major Facilitator Superfamily of transport proteins and has been identified as a protein that delivers hexoses including the main energy source, D-glucose, to the parasite.[1–3] PfHT is a product of a single copy gene and is the major route of entry of glucose into the intracellular parasite. It is essential for parasite survival because *P. falciparum* needs a continuous supply of glucose to provide for its energy requirements. Glucose deprivation causes an immediate drop in intracellular pH of the parasite[4] and prolonged deprivation kills parasites. Hence, inhibitors of PfHT should block supply of glucose and kill parasites rapidly and safely, provided such compounds do not also interfere with mammalian hexose transporters and incur undue toxicity.

To gain knowledge about sites of interactions of glucose with PfHT, deoxyglucose analogues that lack hydroxyl groups at carbon positions of D-glucose mapped the *O*-3 position to be important for hydrogen bonding with the parasite transporter and mammalian GLUT1.[2] More detailed studies confirmed and elucidated differences between parasite and mammalian transporter by the use of *O*-methyl-glucose derivatives.[2,5] These compounds, in which one of the hydroxyl groups of D-glucose has been replaced by a methoxy group, were also systematically evaluated. Comparison of affinities for PfHT of this series of derivatives showed that 3-*O*-methyl-D-glucose (3-OMG) interacted preferentially with PfHT,[2,5] and led to the preparation of a series of O-3-substituted analogues[6] as well as establishing that 3-*O*-(undec-10-en)yl-D-glucose (CM3361-structure **1**) inhibits PfHT significantly (K_I = 53 ± 19 μM). Moreover, this inhibition is selective as interaction with GLUT1, the ubiquitous mammalian hexose transporter, is of relatively low apparent affinity (K_I for GLUT1 = 3.3 ± 0.3 mM).[5] Inhibitory effects of this compound on PfHT orthologues in other plasmodial species have also been observed, confirming broad spec-



3-OMG : R = CH_3,
1 : R = -$(CH_2)_9$-CH=CH_2

Keywords: Glucose; Carbohydrate transporters; PfHT; Inhibitors; Malaria.
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doi:10.1016/j.bmcl.2007.06.021

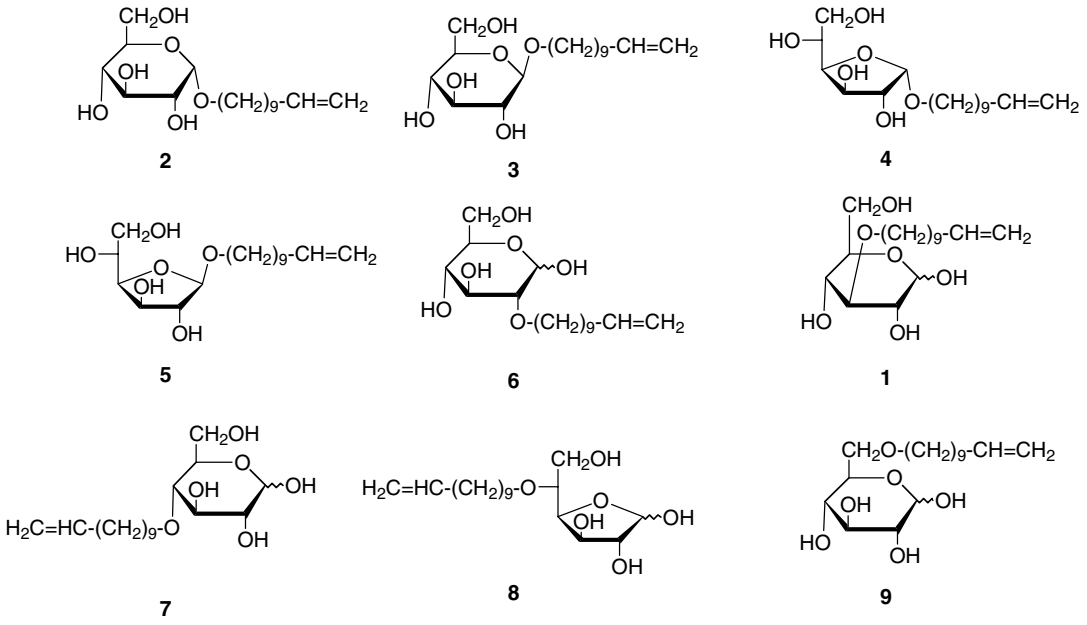
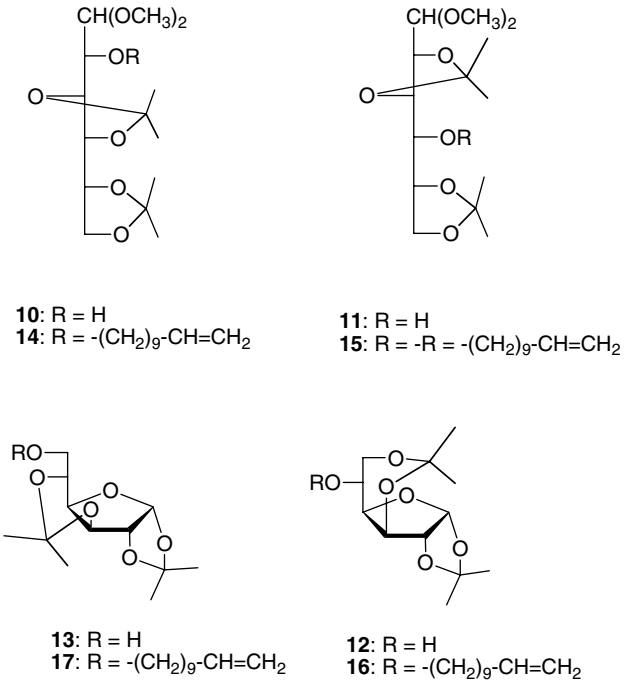


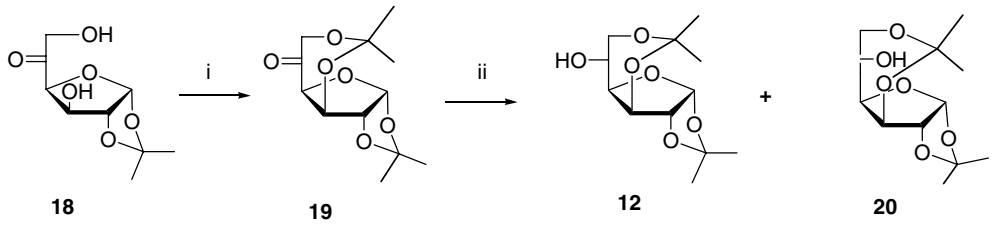
Figure 1. Structures of the *O*-(undec-10-en)-l-D-glucose derivatives.

ificity of the compound for the *Plasmodium* genus of the Apicomplexan family.[5] Furthermore, **1** was shown to kill *P. falciparum* and drug-resistant forms of parasites in culture at concentrations that do not disrupt host red cells. This parasitocidal effect is antagonised by increasing concentrations of D-glucose and D-fructose in cultures of *P. falciparum*.[5] In an in vivo murine model of *Plasmodium berghei* almost 40% suppression in parasitaemia was observed with this compound in a standardized 4-day suppression test, validating hexose transporters of plasmodial species as targets of **1**. Direct effects of this compound were also observed on isolated parasites, highlighting PfHT as being the target for inhibition, rather than other unrelated pathways such as parasite hexokinase.[4] Addition of inhibitor produced a drop in ATP levels and consequential loss of intracellular pH control[4] consistent with previous findings.[7] More recently a series of *O*-3 derivatives were also tested and confirmed that a chain length between C8–C13 is necessary to render hexose-base derivatives more inhibitory.[6]

Although the choice of *O*-3 as a site for substitution has arisen from a comparison of O-methylated glucose derivatives,[5] the large difference in the observed affinities against PfHT of **1** ($K_I = 53 \pm 19$ μM) and of 3-OMG ($K_I = 1.3 \pm 0.3$ mM) raises the possibility that inhibitory properties are brought about by the undecenyl substituent itself. Therefore a comparison of all *O*-(undec-10-en)-yl derivatives of glucose was attempted to possibly unravel a better substitution site for further modifications of D-glucose.

To address this question, glycosides **2**–**5** and ethers **6**–**9**, that is, derivatives of glucose in which the undec-10-enyl chain has been introduced successively on every hydroxyl group of D-glucose (see Fig. 1), have been prepared. With regard to glycosides, the α-pyranoside **2** can be obtained conventionally[8] and the β anomer, **3**, was derivatized from acetobromoglucose,[9] whereas to obtain **4** and **5**, the pre-complexation of glucose with barium chloride before a ferric chloride-catalysed glycosidation was used.[10] To obtain ethers **6**–**9**, glucose derivatives **10**, **11**,[11] **12** and **13**[12] were used as in these compounds all hydroxyl groups but one are protected as acetals; thus, after Williamson-type alkylation of the free hydroxyl groups with undecen-10-yl bromide (to get **14**–**17**, respectively),[13] acidic hydrolysis of the acetals afforded the desired products **6**–**9**.[14] The preparation of **12** (see Scheme 1) deserves





Scheme 1. (i) 2-methoxy-propene (2.5 equiv.), TsOH (cat.), DMF, 15 h (63%); (ii) $NaBH_4$,CH_3OH (quant.).

comments; although acetalation with acetone or 2,2-dimethoxy-propane under acid catalysis of **18**[15] failed, it proceeded cleanly with the use of 2-methoxy-propene under kinetic conditions;[16] the carbonyl group of **19** was next reduced to give a ca. 3:1 mixture of two epimeric alcohols; to assign their relative structures (**12** vs **20**) the mixture was submitted to acidic cleavage, which gives D-glucose and L-idose; ^{13}C NMR spectroscopy analysis[17] shows that D-glucose is the major component and therefore **12** is the major reduction product; interestingly it turned out that it is not necessary to separate **12** from **20** before the alkylation step as only **12** was found to react[18] (to yield **16**).

Derivatives were primarily screened at 0.5 mM to identify analogues exhibiting potent inhibitory effects on glucose transport activity mediated by PfHT and GLUT1 expressed in *Xenopus laevis* oocytes (data not shown and Table 1). Those derivatives (**2**–**5**) that did not inhibit by 50% in the primary screen were excluded from further evaluation, except for **8** and **9** that demonstrated ~50% inhibition of total transport activity.

It can be seen that most derivatives do not exert significant interaction with PfHT or with mammalian GLUT1 (see Table 1). With regard to *O*-1 substituted derivatives (**2**–**5**), affinity is lost irrespective of the anomeric configurations in pyranoside and furanoside forms; this is in agreement with the finding that the 1-OH is important for interaction with both PfHT and GLUT1.[2] Introduction of the lipophilic chain at *O*-5 and *O*-6 (**8** and **9**, respectively) also decreases the affinity for PfHT, showing K_I's greater than 0.5 mM; note that GLUT1 is not inhibited by **8** but that some inhibition is observed with **9**, suggesting a free hydroxymethyl group (OH-6) is important for GLUT1 interaction. When the undecenyl substituent is located at positions neighbouring that of **1** (the parent compound) the affinities are good: **7** (the *O*-4 substituted derivative) generates a K_I half of **1** but selectivity for PfHT (0.026 ± 0.011 mM) is reduced; however, inhibition of GLUT1 (K_I = ~0.15 mM, average of two determinations) is also apparent. In contrast **6** (the *O*-2 substituted derivative) interacts with PfHT with affinity in the low μM range ($K_I = 0.002 \pm 0.00033$ mM), which is ⩾25-fold more potent compared with that of **1**; moreover no inhibition of GLUT1 mediated D-glucose uptake can be observed when tested over this concentration (data not shown), a result that underscores selectivity for PfHT.

Table 1. K_I (mean ± SEM where appropriate) for D-glucose uptake of *O*-(undecen-10-yl)-D-glucose derivatives against PfHT and GLUT1

Compound	Oxygen substituted	PfHT K_I(mM)	GLUT1 K_I (mM)
1	3[a]	0.053 ± 0.019	3.30 ± 0.21
2	1α[b]	>0.5[d]	NI[g]
3	1β[b]	>0.5[d]	>0.5[d]
4	1α[c]	>0.5[d]	NI[g]
5	1β[c]	>0.5[d]	NI[g]
6	2	0.0020 ±0.0003	NI[g]
7	4	0.026 ± 0.011	0.098, 0.200[f]
8	5	1.5[e]	NI[g]
9	6	2[e]	0.4[e]

[a] Reported in Ref. 5.
[b] Pyranoside form.
[c] Furanoside form.
[d] When tested in a primary screen at 0.5 mM as denoted by >0.5, 50–60% inhibition of D-glucose uptake was observed suggesting higher concentrations of these inhibitors would be necessary to achieve 50% inhibition of total transport activity.
[e] A single determinant as insufficient material to carry out repeats.
[f] Two experiments (i.e., two independent values).
[g] NI: no inhibition.

IC_{50} values were also obtained for those inhibitors demonstrating inhibition of PfHT based on the oocyte data. Only **6** and **7** were screened at various concentrations on asynchronous cultures constituting 1% parasite infection of blood cultured over 48 h (Fig. 2); the remaining derivatives were excluded from this analysis due to the lack of potency in inhibiting the malarial transporter expressed in oocytes. Interestingly, **6** appeared to be the most potent of all the glucose-base derivatives tested to date

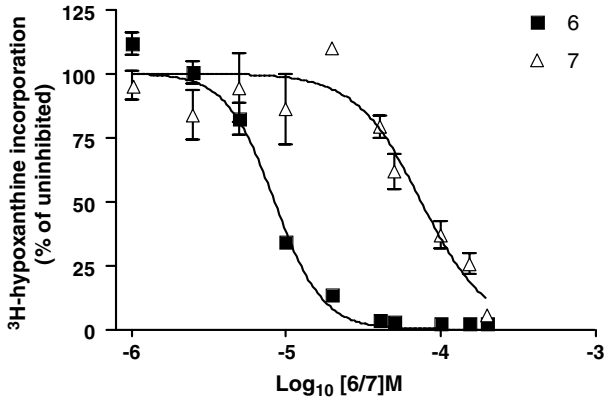


Figure 2. IC_{50} curves of compounds **6** and **7** in cultures of *P. falciparum* (3D7) grown in 4 mM glucose-containing media. Curves are of a single experiment that was repeated 4 times for **6** and 3 times for **7**. The IC_{50}'s for this experiment are 0.0084 and 0.075 mM, respectively. Table 2 presents mean IC_{50}'s of all experiments.

Table 2. IC_{50} data for compounds **6** and **7** were obtained from cultured *P. falciparum*

Compound	IC_{50} (mM)
1	0.0157 ± 0.0017[a]
6	0.00820 ± 0.0018[b]
7	0.123 ± 0.029[b]

[a] Reported in Ref. 4.
[b] ±SEM; for **6**: four determinations and for **7**: three determinations.

with an IC_{50} twofold more effective than **1** (Table 2). In contrast, for **7** the IC_{50} value for killing parasites is high.

Previous structure/affinity studies using *O*-3 derivatives explored chain length, hydrophilicity/lipophilicity balance and their inhibitory effects on PfHT-mediated D-glucose uptake. These established an alkyl/alkenyl C8–C13 chain as being necessary to retain selective affinity for the malarial transporter.[6] In the present study, introduction of the same undecenyl chain on each *O*-substitution site within glucose was carried out. **6** was found to be a much better inhibitor than the previous lead compound (**1**), with an excellent selectivity for PfHT as no inhibition of mammalian GLUT1 is observed at concentrations effective against the malarial transporter. Moreover this inhibitor in culture demonstrated almost a twofold higher potency than the parent inhibitor 3361. This points out to position −2 of D-glucose[19] for further modifications, with 2-*O*-(undec-10-en)-yl-D-glucopyranose[20] becoming a new lead.

Acknowledgments

M.I. is grateful to the LEDSS for financial support and A.P.P. is grateful to the MRC for a studentship.

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12. **13** was obtained from the readily available corresponding bromide (Hodosi, G.; Podanyi, B.; Kuszmann, J. *Carbohydr. Res.* **1992**, *230*, 327), by reaction with potassium nitrite (Raduchel. B., *Synthesis* **1980**, 292).
13. Alkylation procedure: To a stirred solution of alcohols **10**–**13** in dry DMF (1–2 mL/mmol) at 4 °C was added sodium hydride (1.1 equiv—60% suspension in mineral oil) and the resulting mixture stirred for 30–60 min at the same temperature; then undec-10-enyl bromide (1.1 equiv). was added and the cooling bath was removed. After overnight stirring, quenching with water was followed by extraction with 1:1 *n*-pentane/diethyl ether and **14**–**17** were obtained (20–50% yield) after column chromatography on silica gel.
14. Acetal cleavage procedure: To a solution of **14**–**17** in dioxane (4 mL/mmol) stirred at 60 °C was added dropwise 2 N hydrochloric acid (4 mL/mmol) and the mixture was stirred overnight. After cooling, water was added and the pH set to 7.0 (1 M NaOH). The resulting solution was concentrated to half volume, the pH was checked and if necessary set to 7.0 (0.1 N NaOH) and the solution concentrated to half volume, before being extracted with diethyl ether. Column chromatography on silica gel using dichloromethane with increasing amounts of ethanol afforded **6**–**9** (20–60% yield).
15. Obtained in three steps from D-glucose; see (a) Tsuda, Y.; Hanajima, M.; Matsuhira, N.; Okuno, Y.; Kanemitsu, K. *Chem. Pharm. Bull.* **1989**, *37*, 2344; (b) Baxter, E. W.; Reitz, A. B. *J. Org. Chem* **1994**, *59*, 3175.
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17. D-Glucose and L-idose can be easily differentiated by the chemical shifts of their anomeric carbons.
18. The low reactivity of the alkoxide derived from **20** stems presumably from steric congestion and/or low nucleophilicity, possibly due to its interactions with neighbouring oxygens.
19. The affinity for PfHT is retained with 2-deoxy-D-glucose, which shows that the 2-OH group is not critical (See Ref. 2).
20. Only the β-pyranose anomer of **6** was detected by NMR spectroscopy.