# Acyclic Nucleoside Analogues as Inhibitors of *Plasmodium falciparum* dUTPase

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We report the discovery of novel uracil-based acyclic compounds as inhibitors of deoxyuridine 5′-triphosphate nucleotidohydrolase (dUTPase), an enzyme involved in nucleotide metabolism that has been identified as a promising target for the development of antimalarial drugs. Compounds were assayed against both *P. falciparum* dUTPase and intact parasites. A good correlation was observed between enzyme inhibition and cellular assays. Acyclic uracil derivatives were identified that showed greater or similar potency and in general increased selectivity compared to previously reported inhibitors. The most active compound reported here against the *P. falciparum* enzyme had a $K_i$ of 0.2 $\mu$M. Molecular modeling studies provided a good rationale for the observed activities. Preliminary ADME studies indicated that some of the lead compounds are drug-like molecules. These compounds are useful tools for further investigating *P. falciparum* dUTPase for the development of much-needed novel antimalarial drugs.

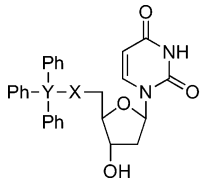
## Introduction

Malaria is a major health problem, especially in the tropical regions of the world. Annually there are 300 million cases of malaria, and at least a million people, mainly children in sub-Saharan Africa, die from the disease.[1] The parasite (*Plasmodium falciparum)* that causes the most acute form of malaria is quick to become resistant to chemotherapeutic agents. For example, in many places there is a major problem of resistance to chloroquine, a 4-aminoquinoline that was for many years the mainstay of treatment for malaria. Hence, there is urgent need for the development of new drugs against new molecular targets, where cross-resistance to existing agents is not likely to be a problem. MMV[a] estimates a new drug is required for combating malaria every 5 years.[2]

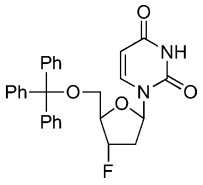
We have recently discovered that some 5′-tritylated nucleosides (**1a**−**d**, Table 1) are selective inhibitors of the *P. falciparum* enzyme deoxyuridine nucleotidohydrolyase (dUTPase).[3] This enzyme, which has not been exploited as a drug target, is involved in nucleotide metabolism. It is responsible for the hydrolysis of dUTP to dUMP and in doing so fulfils two roles. First, it provides a source of dUMP, the substrate for thymidylate synthase involved in the only route that is available for biosynthesis of dTMP (which is subsequently converted into dTTP). Second, it helps maintain a low dUTP/dTTP ratio, reducing erroneous incorporation of uracil into DNA. dUTPase is found in almost all organisms, and it has been shown to be essential for viability where this has been investigated.[4−6] The *Plasmodium* and human dUTPase enzymes both belong to the trimeric subclass of dUTPases and share many kinetic and structural characteristics. They are very selective for dUTP over other deoxyribose nucleoside triphosphates and also ribose nucleoside triphosphates (e.g. UTP).[7] Both enzymes share a common overall topology and show the five conserved motifs characteristic of trimeric dUTPases.[7,8] Interestingly there is an insertion of approximately 23 amino acids in the *Plasmodium* dUTPase.[9] Despite the overall similarities, there are subtle structural differences between the *Plasmodium* and human dUTPases, which allow for selective inhibition of the *P. falciparum* enzyme.[3,9]

Previously reported inhibitors of dUTPases include non-hydrolyzable isosteres of nucleoside triphosphates, where the

**Table 1.** Previously Reported Data for Inhibition of *P. falciparum* DUTPase and Growth Inhibition of *P. falciparum* Cultured in Red Blood Cells by 5′-Tritylated Nucleosides[3]



**1a**, X = O, Y = C
**1b**, X = NH, Y = C
**1c**, X = O, Y = Si



**1d**

| | dUTPase | | in vitro | |
|---|---|---|---|---|
| compound | *P. falciparum* $K_i$ ($\mu$M) | human $K_i$ ($\mu$M) | *P. falciparum* $IC_{50}$ ($\mu$M) | L6-cells $IC_{50}$ ($\mu$M) |
| **1a** | 1.8 | 18 | 6 | 192 |
| **1b** | 0.2 | 46 | 4.5 | 44 |
| **1c** | 2.8 | 909 | 1.1 | nd |
| **1d** | 5.0 | 457 | 2.0 | nd |

[a] nd: not determined. The $IC_{50}$ values are the means of two independent assays performed in duplicate.

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[a] Abbreviations: dUTPase, deoxyuridine nucleotidohydrolase; MMV, Medicine for Malaria Venture, d4U, 2′,3′-deoxy-2′,3′-didehydrouridine, ADME, adsorption, distribution, metabolism, excretion; PS-$PPh_3$, polymer-supported triphenylphosphine; TPSO, triphenylsilyloxy; DTT, dithiothreitol; IPTG, isopropyl $\beta$-D-thiogalactopyranoside; PMSF, α-toluenesulfonyl fluoride, FBS: fetal bovine serum; BSA,bovine serum albumin.

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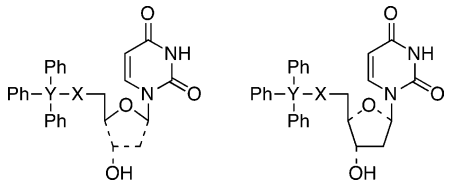


**Figure 1.** Analogy between acyclic and deoxyuridine derivatives.

α,β bridging oxygen atom is replaced by a methylene or an amino group.[10,11] These have been excellent tools for investigating the kinetics of the dUTPase enzyme and for generating crystal structures of the enzyme from various species. However, such compounds are unlikely to be drug candidates owing to chemical and enzymatic instability, poor bioavailability, and membrane permeability. On the contrary, the 5′-tritylated deoxyuridine analogues that we have reported and shown to possess selective antiplasmodial activity are more drug-like, having improved stability, reasonable logP values and low molecular weight.[3]

In this paper we discuss further modifications of the 5′-tritylated deoxyuridine derivatives, in particular generation of acyclic analogues (Figures 1 and 2). These may offer advantages in terms of optimizing the distance between the base (uracil) and trityl group and allow for further alterations and optimization of drug structure. Acyclic nucleoside derivatives are successful antiviral agents; e.g. aciclovir for the treatment of herpes infections. Initially we prepared an acyclic derivative in which there was an oxygen atom in the chain (Figure 2, compound **2a**). However, this particular analogue proved to be metabolically unstable, poorly selective, and fairly cumbersome to purify. Therefore we prepared a series of compounds with no oxygen in the chain, using simple and versatile methodologies (Figure 2, Series **3** to **6**). In addition, to investigate the effect of adding rigidity to the side-chain, compounds in which there was both an *E* and *Z* double bond in the acyclic chain (compounds **7a** and **8a**) were synthesized. Previous studies have shown that d4U analogues[3] were potent inhibitors of the *Plasmodium* dUTPase, possibly due to favorable π interactions with a tyrosine residue (Tyr112) within the enzyme active site. Finally, some branched acyclic derivatives were prepared: γ-branched analogues of the penciclovir type form Series **9**, while Series **10** encompasses the β-branched analogues. Such branched structures were designed to partially mimic the backbone of 2′-deoxyuridine and possibly increase affinity for the enzyme active site.

## Synthesis

**Series 2.** 1-[2-(Benzoyloxyethoxy)methyl]uracil **2g** was synthesized by condensation of bis-silylated uracil (prepared in situ by reaction of uracil with *N*,*O*-bis[trimethylsilyl]-acetamide) with the commercially available 2-(chloromethoxy)-ethyl benzoate, in the presence of the Lewis acid catalyst trimethylsilyl triflate. Purification proved cumbersome (possibly due to impurities present in chloromethoxyethyl benzoate), and **2g** was obtained in 30% yield, although with small traces of impurities. Removal of the benzoate ester by treatment with potassium carbonate in methanol afforded the alcohol 1-[2-(hydroxyethoxy)methyl]uracil (**11**, 49%), which was further derivatized with trityl chloride and *tert*-butyldimethylsilyl chloride (TDMSCl), to produce the trityl ether (**2a**, 20%) and TBDMS ether (**2f**, 56%), respectively.

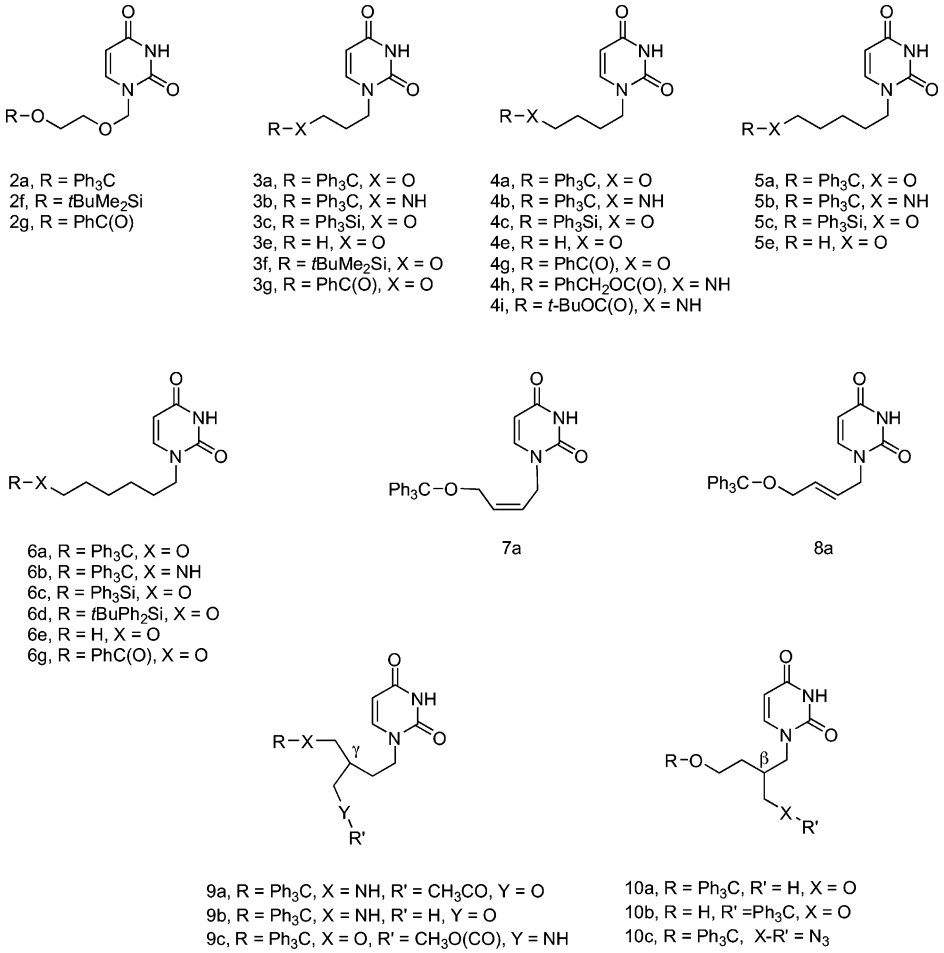
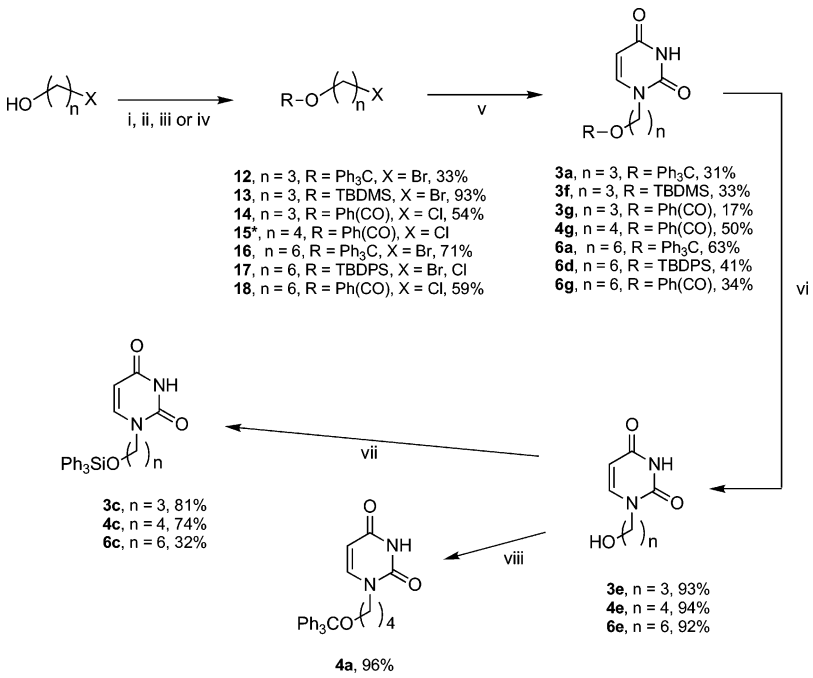


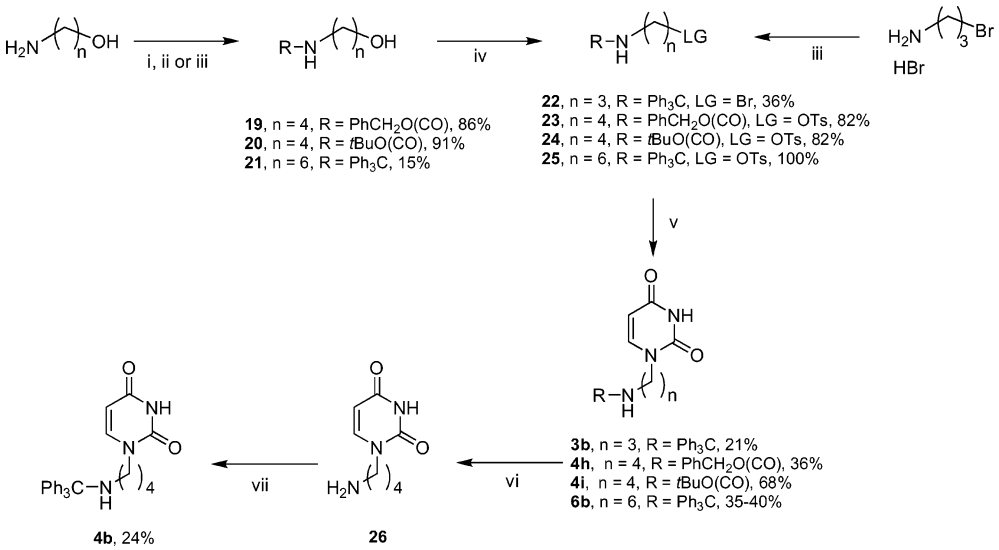

**Figure 2.** Structures of compounds assayed.

**Scheme 1**[a]




[a] (i) TrCl, $Et_3N$, DMAP, DCM, rt; (ii) TBDMSCl, imidazole, DMF, rt; (iii) PhCOCl, pyridine, 0 °C−rt; (iv) TBDPSCl, imidazole, DMF, 0 °C−rt; (v) uracil, $Cs_2CO_3$, DMF, 40−90 °C; (vi) From **3g**, **4g**, **6g**; MeONa, MeOH, rt; (vii) $Ph_3SiCl$, pyridine, 0 °C; (viii) TrCl, pyridine, 50 °C. *Compound **15** was purchased from Acros.

**Scheme 2**[a]



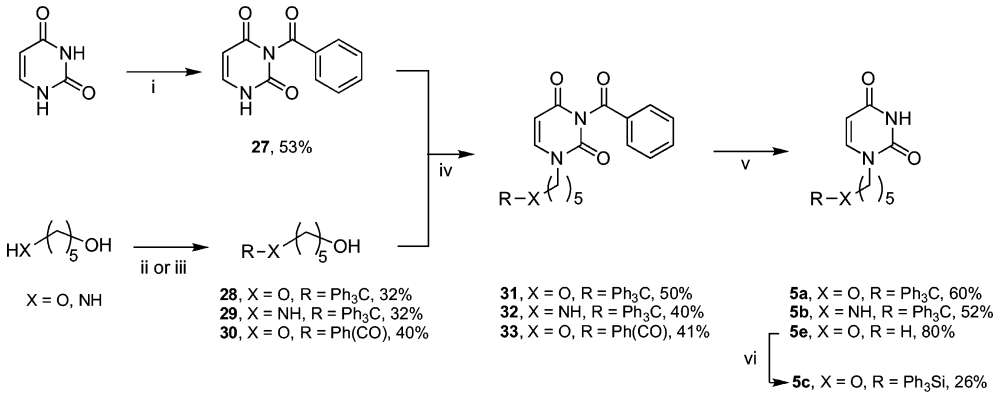

[a] (i) $Boc_2O$, $Et_3N$, DCM, rt; (ii) $PhCH_2OCOCl$, $Na_2CO_3$, $H_2O$, 0 °C-rt; (iii) TrCl, $Et_3N$, DCM, 0 °C; (iv) TsCl, pyridine, 0 °C or TsCl, $Et_3N$, DMAP, 0 °C; (v) uracil, $Cs_2CO_3$, DMF, 40 °C; (vi) From **4h**, $H_2$, 5% Pd/C, MeOH, rt; (vii) TrCl, pyridine, 50 °C.

**Series 3, 4, and 6.** The preparation of these compounds is summarized in Schemes 1 and 2. The key step was the direct coupling of uracil with the relevant halogenated aliphatic side-chain, in the presence of cesium carbonate in DMF under moderate heating (40 °C). The chemoselectivity of the alkylation was verified by two-dimensional NOESY NMR (showing the coupling through space between 1′-H of the side-chain and 6-H of the uracil, see Supporting Information).

As depicted in Scheme 1, the alcohol functionality of the linear side-chain fragment was protected as either a benzoate ester, silyl ether, or trityl ether. In the case of the C4 analogues, an ether protection was not suitable due to the ease of intramolecular cyclization of the side-chain moiety (formation of a five-membered ring). Therefore, the trityl ether and triphenylsilyl ether analogues were not prepared directly but via etherification of 1-(4-hydroxybutyl)uracil (**4e**). Hydroxy uracil analogues **3e**, **4e**, and **6e** were prepared very neatly in high yields by treatment of the benzoate ester derivatives **3g**, **4g**, and **6g** with sodium methoxide in methanol. Furthermore, as the triphenylsilyl (TPS) group was expected not to be completely stable under the reaction conditions required for the coupling step, its introduction was left until last. Analogues **3c**, **4c**, and **6c** were obtained by reaction of the corresponding alcohols with TPSCl in pyridine at 0 °C, in 81, 74, and 32% yields, respectively.

Derivatives bearing a terminal amino group in their side-chain (Scheme 2) were prepared following a methodology similar to their oxygen analogues. Tritylaminopropyl and tritylaminohexyl

**Scheme 3**[a]




[a] (i) 1. PhCOCl, pyridine, $CH_3CN$, rt; 2. $K_2CO_3$, dioxane; 30 min; (ii) TrCl, $Et_3N$, DCM, 0 °C−rt; (iii) PhCOCl, $Me_2SnCl_2$, $K_2CO_3$, THF, rt; (iv) DIAD, PS-$PPh_3$, THF, rt; (v) NaOH, dioxane, rt, or MeONa, MeOH, rt; (vi) $Ph_3SiCl$, pyridine, rt.

analogues **3b** and **6b** could be obtained by direct coupling of uracil with the suitably activated aliphatic fragment (**22** and **25**, respectively). For the C4 analogue, the side-chain was N-protected as a carbamate (**23** or **24**) prior to coupling to the uracil to avoid intramolecular cyclization of the side-chain. Removal of the benzyloxycarbonyl group by hydrogenation in the presence of 5% Pd/C yielded the amino analogue **26**, which was finally tritylated to give the target **4b**.

**Series 5, Compounds 7a and 8a.** The synthesis of compounds from Series 5 is described in Scheme 3. As with their C4 analogues, the C5 side-chain precursors can be prone to intramolecular cyclization, in this case due to the ease of formation of six-membered rings. A way to overcome this problem is to use the Mitsunobu reaction in the key coupling step: in the presence of diisopropyl azadicarboxylate (DIAD) and triphenylphosphine ($PPh_3$), uracil can react with a variety of aliphatic chains bearing an alcohol without need for it to be activated. A convenient procedure developed by Hernández et al.[12] employs polymer-supported $PPh_3$ (PS-$PPh_3$) which facilitates the otherwise potentially tricky removal of the triphenylphosphine oxide byproduct and excess $PPh_3$ reagent. To avoid $N^3$ alkylation, uracil was protected prior to coupling. This was achieved in a two-step procedure consisting of $N^1$,$N^3$ double protection with benzoyl chloride in pyridine, followed by selective $N^1$ deprotection carried out using potassium carbonate in acetonitrile.[13] Pentane-1,5-diol and 5-aminopentan-1-ol were tritylated using trityl chloride and triethylamine in DCM, with an excess of diol or amino alcohol to minimize ditritylation. The diol was also monobenzoylated using benzoyl chloride in the presence of dimethyl tin dichloride as the catalyst inducing monoprotection.[14] Alcohols **28**−**30** thus obtained were successfully coupled to $N^3$-benzoyluracil using the Mitsunobu methodology to give the $N^3$-benzoyl precursors **31**−**33** in 40−50% yield (Scheme 3). Cleavage of the benzoyl group was performed either with sodium methoxide in methanol or with NaOH in dioxane to yield the target analogues **5a**, **5b**, and **5e** in 52−80% yield. Finally, the C5 triphenylsilyl ether **5c** was obtained from the hydroxyuracil derivative **5e,** as previously described for the corresponding C3, C4, and C6 analogues.

The same general strategy was applied to the synthesis of unsaturated derivatives **7a** and **8a**, using (*Z*)-4-trityloxybut-2-en-1-ol (**34**) and the *E* isomer (**36**), as the respective side-chain units in the Mitsunobu coupling step. Intermediates **34** and **36** were prepared as described by Hernandez et al.[12] and McDonald et al.[15] (see Supporting Information).

**Series 9 and 10.** The branched acyclic analogues (Series 9 and 10) were synthesized through a combination of standard protections/deprotections of orthogonal protecting groups, from the branched precursors **39**, **42** (Schemes 4 and 5), and **47** (1-[2-azidomethyl-4-*tert*-butyldimethylsilyloxybutyl]uracil). For a first screen, no attempts were made at producing enantiomerically pure samples, and the compounds were tested as enantiomeric mixtures. Noteworthy is the successful use of microwave irradiation to produce the tritylamino analogue **9c**, where various attempts using conventional heating failed. Finally, compound **10c** was prepared in two straightforward steps from the TBDMSO branched analogue **47**: removal of the TBDMS group with TBAF in THF to free the hydroxyl group (intermediate **48**) followed by tritylation.

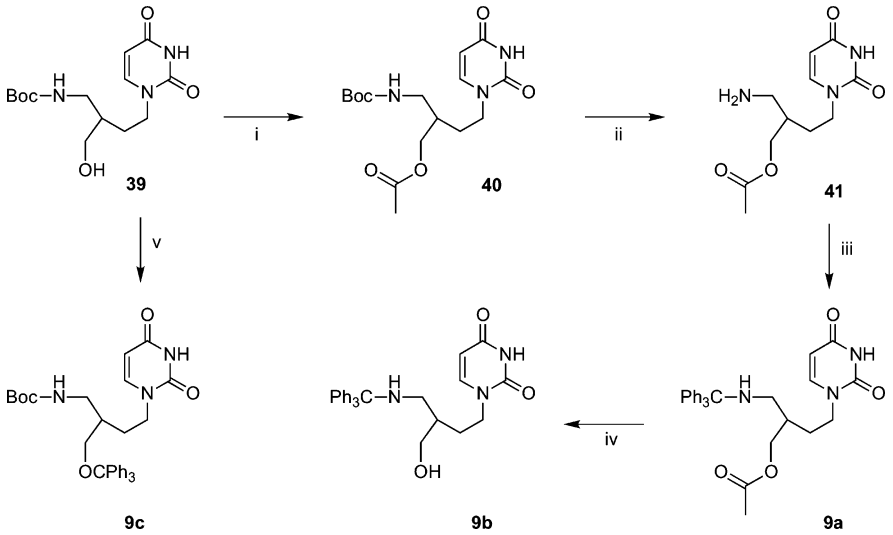
## Biology

**Enzyme Assays.** Compounds were screened against the recombinant *P. falciparum* dUTPase and recombinant human dUTPase, to evaluate inhibition and selectivity (SI, selectivity index). The results from these assays are shown in Tables 2 and 3.

Compound **2a** was the closest single chain acyclic analogue of 5′-trityloxy-2′-deoxyuridine **1a**; it retained similar or even slightly improved (2-fold) enzyme activity and selectivity (**2a**, $K_i = 0.7$ $\mu$M, SI = 25; **1a**, $K_i = 1.8$ $\mu$M, SI = 10). Replacing the trityl (Tr) ether by a *tert*-butyldimethylsilyl (TBDMS) ether, a benzoate ester or an adamantyl amide group resulted in loss of activity, suggesting that lipophilicity and steric bulk alone did not account for enzyme inhibition. This is in agreement with conclusions previously reported for deoxyuridine derivatives[3] and is discussed later in this paper for other series of compounds assayed.

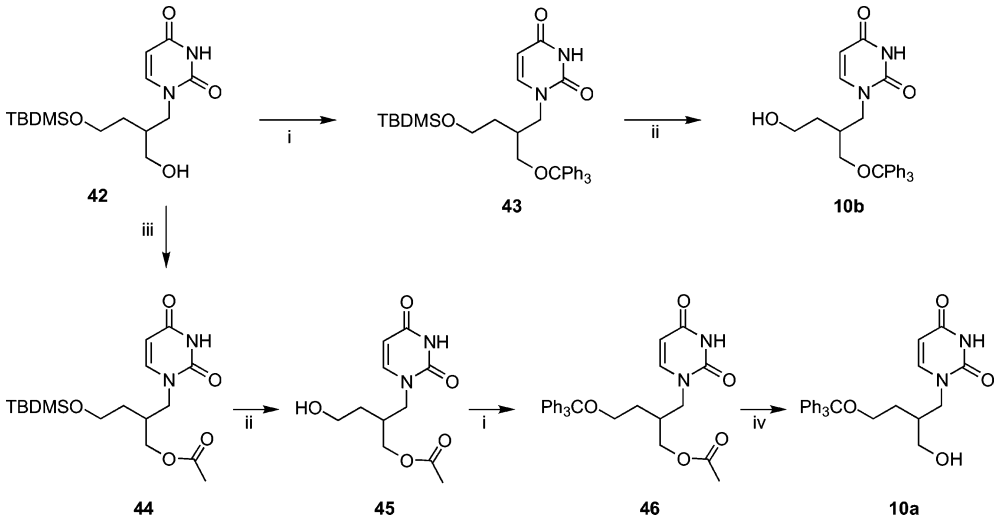
Compound **4a** gave rise to a similar inhibition of the *P. falciparum* enzyme compared to compound **2a** (**2a**, $K_i = 0.7$ $\mu$M; **4a**, $K_i = 1.6$ $\mu$M), which demonstrated that while having an oxygen in the side-chain probably is a closer mimic of the nucleoside, this is not a key feature for activity. Furthermore, **4a** showed a much lower affinity for the human enzyme than **2a** (Table 2; **4a**, $K_i > 1000$ $\mu$M; **2a**, $K_i = 17$ $\mu$M) and consequently was at least 25 times more selective (selectivity index = [$K_i$ human]/[$K_i$ *P. falciparum*)]; Table 3). Hence, it can be postulated that while the oxygen does not play a significant role in *P. falciparum* dUTPase inhibition, it undergoes interactions that are more important within the human enzyme active site. In addition, the 2-(trityloxyethoxy)methyl acyclic derivative **2a** was found (i) to be prone to rapid degradation by human liver microsomes (see Discussion, ADME tests) and (ii) to require a cumbersome purification (see Synthesis). Therefore, Series **2** was not progressed further.

**Scheme 4**[a]




[a] (i) $Ac_2O$, pyridine, rt, 88%; (ii) TFA, DCM, rt; (iii) TrCl, $Et_3N$, DCM, rt, 70%; (iv) MeONa, MeOH, rt, 99%; (v) TrCl, pyridine, microwave irradiation (three times 160 °C for 5 min), 79%.

**Scheme 5**[a]




[a] (i) TrCl, pyridine, 70 °C; **42**, 76%; **45**, 90%; (ii) TBAF, THF, rt; **10b**, 47%; **44**, 78%; (iii) $Ac_2O$, pyridine, rt, 67%; (iv) MeONa, MeOH, rt, 57%.

Instead, using the simple and versatile synthetic methodology used to prepare compound **4a**, more analogues with varying side-chain length ($n$ = 3 to 6) in combination with a selection of functional groups at the end of their aliphatic chain were assayed (Table 3). Functional moieties considered (Figure 2, Table 3) included trityl ether, tritylamine, various silyl ethers, alcohol, benzoate ester, and carbamates (Cbz and Boc) for varying steric bulk, lipophilicity and electronic characteristics.

**Effect of Side-Chain Length**. No general correlation between activity and side-chain length was revealed. Within the trityloxy Series **a**, the lowest $K_i$ values were obtained for **4a** and **5a**. Within the tritylamino Series **b**, compound **3b** was the most active, although the least selective as well. Within the triphenylsilyloxy Series **c**, activity was retained with no significant change in $K_i$ when the chain length was varied from three to six carbons. However, the chain length of three carbon atoms correlated with a lower selectivity for the parasite over the human enzyme. Possibly there is also a slight reduction in selectivity for the chain length of six carbon atoms. Compound **4b** (acyclic analogue of lead **1b**) appeared as the best overall inhibitor ($K_i$ = 0.9 $\mu$M, SI > 1111).

In addition, when focusing on the effect of the O/NH switch at the end of the side-chain within a series (series **3** to **6** considered individually), few variations are observed as regards activity, with the clear exception of the C3 analogues; indeed while the trityl ether **3a** did not show significant inhibition of *P. falciparum* dUTPase ($K_i$ = 87 $\mu$M), the corresponding tritylamine **3b** showed a greatly improved activity (over 400-fold) with a $K_i$ = 0.2 $\mu$M. A 5-fold increase in activity along with a 10-fold improvement in selectivity can also be noted when replacing the trityloxy by a tritylamino group in the C6 series (Table 2; **6a**, $K_i$ = 9.6 $\mu$M, SI = 50; and **6b**, $K_i$ = 1.8 $\mu$M, SI > 543). Very similar observations can be made when considering a C/Si switch within the trityl group itself (that is when replacing the trityloxy by a triphenylsilyloxy group). Indeed, (i) the silyl ether **3c** was much more active than its analogue **3a** ($K_i$ = 1.6 and 87 $\mu$M, respectively); (ii) within Series **6**, the silyl ether **6c** showed both increased activity and greater selectivity compared to **6a** (Table 2; **6c**, $K_i$ = 1.7 $\mu$M,

**Table 2.** Inhibition of the *P. falciparum* and Human dUTPases, and Growth Inhibition of *P. falciparum* and Mammalian Cell Line (rat L6-cells)

| | dUTPase | | in vitro | |
|---|---|---|---|---|
| compound | *P. falciparum* $K_i$ ($\mu$M) | Human $K_i$ ($\mu$M) | *P. falciparum*[a] $IC_{50}$ ($\mu$M) | L6-cells[b] $IC_{50}$ ($\mu$M) |
| **2a** | 0.7 | 17 | 0.9 | 39 |
| **2f** | 44 | 63 | 62 | >299 |
| **2g** | 98 | 311 | 127 | 202 |
| **3a** | 87 | 313 | 7.5 | 34 |
| **3b** | 0.2 | 1.4 | 4.4 | 107 |
| **3c** | 1.6 | 25 | 0.58 | 70 |
| **3e** | 368 | >1000 | >29 | nd |
| **3f** | 73 | 73 | >18 | 192 |
| **3g** | 9.2 | 898 | >18 | nd |
| **4a** | 1.6 | >1000 | 4.9 | 42 |
| **4b** | 0.9 | >1000 | 3.8 | 33 |
| **4c** | 2.2 | >1000 | 0.36 | nd |
| **4e** | >1000 | >1000 | 235 | nd |
| **4g** | 224 | 496 | 38 | nd |
| **4h** | 17 | >1000 | 15 | nd |
| **4i** | 112 | >1000 | 17 | nd |
| **5a** | 2.0 | >1000 | 1.1 | 24 |
| **5b** | 4.3 | >1000 | 2.2 | 39 |
| **5c** | 3.8 | >1000 | 0.38 | 47 |
| **5e** | >1000 | >1000 | 20 | >454 |
| **6a** | 2.3 | 476 | 2.3 | 44 |
| **6b** | 1.8 | >1000 | 1.1 | 40 |
| **6c** | 1.7 | 383 | 0.46 | 50 |
| **6d** | 8.5 | >1000 | 3.9 | 18 |
| **6e** | >1000 | 636 | 19 | nd |
| **6g** | 32 | >1000 | 3.4 | nd |
| **7a** | 1.2 | 5.2 | 3.5 | 39 |
| **8a** | 0.6 | >1000 | 3.7 | 34 |
| **9a** | 1.3 | 74 | 2.8 | 37 |
| **9b** | 0.24 | 5.7 | 3.2 | 55 |
| **9c** | 2.5 | >1000 | 0.9 | 15 |
| **10a** | 2.2 | 260 | 1.2 | 33 |
| **10b** | 0.9 | 21 | 1.9 | 57 |
| **10c** | 23 | >1000 | 0.64 | 18 |
| $Ph_3SiOH$ | >1000 | >1000 | 0.65 | 138 |
| $Ph_3COH$ | >1000 | >1000 | 12 | nd |
| TDBMSOH | nd | nd | >37 | >680 |

[a] *Plasmodium falciparum*; [b] Measure of cytotoxicity; nd: not determined. Controls: for *P. falciparum*, chloroquine, $IC_{50} = 0.1$ $\mu$M; for cytotoxicity, podophyllotoxin, $IC_{50} = 0.012$ $\mu$M. The $IC_{50}$ values are the means of four values of two independent assays done in duplicate.

SI = 226; and **6a**, $K_i = 9.6$ $\mu$M, SI = 50); and (iii) little variation was noticeable within Series **4** and **5**.

**Unsaturated Acyclic Analogues 7a and 8a**. The two isomers **7a** and **8a** showed similar levels of inhibition of the *Plasmodium* dUTPase, also comparable to that of their saturated analogue **4a**, with the *E* isomer **8a** being possibly slightly more active (Table 2; **4a**, $K_i = 1.6$ $\mu$M; **7a**, $K_i = 1.2$ $\mu$M; **8a**, $K_i = 0.6$ $\mu$M). On the other hand, a great difference in activity against the human dUTPase was observed: **7a** gave rise to some inhibition of the enzyme ($K_i = 5.2$ $\mu$M) whereas **8a** was totally inactive ($K_i > 1$ mM). Consequently, while **7a** showed very poor selectivity (SI = 4), its *E* isomer **8a** was very selective toward the *P. falciparum* enzyme (SI > 1666). This striking observation suggests that introduction of rigidity through a double bond in position 2,3 of the side-chain can be beneficial (Table 2, compare **4a** [$K_i = 1.6$ $\mu$M; SI > 617] and **8a** [$K_i = 0.6$ $\mu$M; SI > 1666]); however, the choice of conformation of the double bond is crucial if selectivity is to be retained.

**Branched Acyclic Analogues (Series 9 and 10)**. One of the key SAR features established so far for both acyclic and cyclic[3] analogues is the requirement for a group bearing two or even three phenyl rings, trityl and triphenylsilyl being the groups of choice. Therefore, the branched acyclic derivatives prepared to further our investigations all contained one such group (either TrO or TrNH), as well as another functional group in the branched chain. Furthermore, it was decided that one chain would be four carbons long as a mimic of the nucleoside analogues.

The $\gamma$-branched derivative **9b** represented the closest analogue to lead **1b** and was found to be as potent an inhibitor of *P. falciparum* dUTPase, with a $K_i$ value of 0.2 $\mu$M. However this result was partially contrasted by the 10-fold loss in selectivity observed for **9b** (Table 2). This selectivity loss was a direct consequence of an increased inhibition of the human dUTPase (Table 2 and Figure 1; **1b**, $K_i = 46$ $\mu$M; **9b**, $K_i = 5.7$ $\mu$M). Thus, increasing the flexibility of the inhibitor seemed to allow enhanced interactions unfortunately only within the human dUTPase.

Blocking the free hydroxyl in **9b** as an acetate ester in **9a** resulted in a noticeable 7-fold loss of activity against the *P. falciparum* dUTPase. However, a slight increase in selectivity was also observed. This suggests that the loss of the OH hydrogen bond donor has a greater effect on interactions within the human dUTPase than with the *P. falciparum* enzyme.

Compound **9c**, that possesses a trityl ether at the end of one C4 chain and a Boc carbamate at the end of the other C4 chain, gave very similar results to the unbranched analogue **4a**.

In series **10**, there is branching at the $\beta$-position. Similar inhibition of the *Plasmodium* dUTPase was seen for the analogue with a $\beta$-hydroxymethyl compared to the unbranched analogue **4a**, although with a slight loss of selectivity. Replacing the hydroxyl group with an azide group (**10c**) led to a 10-fold loss in activity.

Compound **10b** has a three carbon chain with a $\beta$-hydroxyethyl branch. Comparison of **10b** with its unbranched C3 trityloxy analogue **3a**, shows that the introduction of a hydroxyethyl $\beta$-branch enhanced antiplasmodial activity (40-fold) as well as selectivity (6-fold). However, the latter remains in the low range (SI = 23).

## In Vitro Assays

Compounds were screened against *P. falciparum* cultured in red blood cells to evaluate antiplasmodial activity and against a mammalian cell line (L-6 rat cells) as a test for cytotoxicity (Tables 2 and 4).

In general, compounds with a trityloxy (Series **a**), tritylamino (Series **b**), or triphenylsilyloxy (Series **c**) substituent inhibited the growth of *P. falciparum*, with $IC_{50}$ values in the order of 0.4 to 8 $\mu$M (Table 2). The most potent inhibitors were the TPSO analogues **4c**, **5c**, and **6c** ($IC_{50} = 0.36$, 0.38, and 0.43 $\mu$M, respectively), with $IC_{50}$ values up to 10-fold lower than compounds from the TrO and TrNH series. Compound **6d**, in which there is a diphenyl substituent, also gave good inhibition of parasite growth ($IC_{50} = 3.9$ $\mu$M). Furthermore, these compounds were selective for the parasite over mammalian (L-6) cells, the degree of selectivity varying from about 5-fold to over 100-fold.

Compounds lacking a triphenyl or diphenyl substituent showed much weaker inhibition of *P. falciparum* growth, with only compound **6g** giving an $IC_{50}$ value under 10 $\mu$M (Table 2). This mirrors the trends observed for the nucleoside analogues.[3] Furthermore, acyclic inhibitors show similar or more potent parasite growth inhibition than their deoxyuridine counterparts (e.g. **4a**, $IC_{50} = 4.9$ $\mu$M and **1a**, $IC_{50} = 6$ $\mu$M; **4b**, $IC_{50} = 3.8$ $\mu$M and **1b**, $IC_{50} = 4.5$ $\mu$M; **4c**, $IC_{50} = 0.36$ $\mu$M and **1c**, $IC_{50} = 1.1$ $\mu$M). The side-chain length did not appear

**Table 3.** Overview of Enzyme Inhibition for Compounds from Series **2** to **6**[a]

| no. | R | **2**, $R(CH_2)_2OCH_2U$ | **3**, $R(CH_2)_3U$ | **4**, $R(CH_2)_4U$ | **5**, $R(CH_2)_5U$ | **6**, $R(CH_2)_6U$ |
|---|---|---|---|---|---|---|
| **a** | $Ph_3CO$ | 0.7 (25) | 87 (4) | 1.6 (>617) | 2.0 (>500) | 9.6 (50) |
| **b** | $Ph_3CNH$ | | 0.2 (7) | 0.9 (>1111) | 4.3 (>233) | 1.8 (>543) |
| **c** | $Ph_3SiO$ | | 1.6 (16) | 2.2 (>446) | 3.8 (>265) | 1.7 (227) |
| **d** | *t*-$BuPh_2SiO$ | | | | | 8.5 (>118) |
| **e** | HO | | 368 (>2) | >1 mM | >1 mM | >1 mM |
| **f** | *t*-$BuMe_2SiO$ | 44 (1.4) | 73 (1) | | | |
| **g** | PhC(O) | 98 (3) | 9.2 (>97) | 224 (2) | | 32 (>31) |
| **h** | $PhCH_2OC(O)NH$ | | | 17 (60) | | |
| **i** | *t*-BuOC(O)NH | | | 112 (>8) | | |

[a] $K_i$ values against *P. falciparum* dUTPase are given in $\mu$M. The selectivity index defined as ($K_i$ human/$K_i$ *P. falciparum*) is given in parentheses. U = uracil.

**Table 4.** Overview of *P. falciparum* Growth Inhibition for Compounds from Series **2** to **6**[a]

| no. | R | **2**, $R(CH_2)_2OCH_2U$ | **3**, $R(CH_2)_3U$ | **4**, $R(CH_2)_4U$ | **5**, $R(CH_2)_5U$ | **6**, $R(CH_2)_6U$ |
|---|---|---|---|---|---|---|
| **a** | $Ph_3CO$ | 0.9 (78) | 7.5 (5) | 4.9 (8) | 1.1 (21) | 2.3 (19) |
| **b** | $Ph_3CNH$ | | 4.4 (24) | 3.8 (8.8) | 2.2 (18) | 1.1 (37) |
| **c** | $Ph_3SiO$ | | 0.58 (121) | 0.36 (na) | 0.38 (123) | 0.46 (109) |
| **d** | *t*-$BuPh_2SiO$ | | | | | 3.9 (5) |
| **e** | HO | | >29 (na) | 235 (na) | 20 (>23) | 19 (na) |
| **f** | *t*-$BuMe_2SiO$ | 62 (>4) | >18 (<11) | | | |
| **g** | PhC(O) | 127 (1.6) | >18 (na) | 38 (na) | | 3.4 (na) |
| **h** | $PhCH_2OC(O)NH$ | | | 14.5 (na) | | |
| **i** | *t*-BuOC(O)NH | | | 17 (na) | | |

[a] $IC_{50}$ values are given in $\mu$M. The selectivity index defined as ($IC_{50}$ *P. falciparum*/$IC_{50}$ L6-cells) is given in parentheses. U = uracil. na: not available.

to have a clear influence on antiplasmodial activity in vitro, as seen in the case of enzyme inhibition.

Evidence from the literature suggests that bonds between silicon and oxygen could be labile in cellular conditions.[16,17] Therefore, the commercially available triphenylsilanol (TPSOH) and *tert*-butyldimethylsilanol (TBDMSOH) were also investigated for their antiparasitic activity (Table 2). TBDMSOH was inactive against *P. falciparum* ($IC_{50}$ > 37 $\mu$M). On the other hand, TPSOH was found to be a potent inhibitor of *P. falciparum* growth, with a level of activity ($IC_{50}$ = 0.3 $\mu$M) similar to that of the TPSO analogues (Series **c**). It may be possible that compounds from Series **c** owe some or all of their antiplasmodial activity to TPSOH potentially released as a result of hydrolysis under cellular assay conditions. However, it was shown previously[3] that the *P. falciparum* dUTPase was inhibited by the TPSO derivatives but not by TPSOH ($K_i$ > 1000 $\mu$M) nor by the corresponding alcohols (Series **e**), which demonstrates that the TPSO analogues, as such, were responsible for enzyme inhibition. Therefore, if TPSOH is inhibiting parasite growth then it is acting on a molecular target other than dUTPase (and not yet identified).

Both unsaturated analogues **7a** and **8a** were good inhibitors of *P. falciparum* growth with virtually identical $IC_{50}$ values (3.5 and 3.7 $\mu$M, respectively), but with relatively low selectivity compared to mammalian cells growth ($IC_{50}$ = 39 and 34 $\mu$M, respectively).

Branched analogues from Series **9** and **10** all showed strong inhibition of *P. falciparum* growth in vitro, with $IC_{50}$ values ranging from 0.6 to 3.2 $\mu$M. None of the compounds were found to be very cytotoxic (Table 2). Furthermore, compared to their nucleoside and single chain analogues, the branched derivatives exhibited similar or slightly better antiplasmodial activity (Table 2). In particular, compare the $IC_{50}$ values for $\gamma$-branched **9b** (3.2 $\mu$M) and its nucleoside analogue **1b** (4.5 $\mu$M); for the $\gamma$-branched **9a** (2.8 $\mu$M, hydroxymethyl branch) and **9b** (3.2 $\mu$M, acetoxymethyl branch) and their single chain analogue **4b** (3.8 $\mu$M); for **9c** (0.9 $\mu$M) and the straight chain **4a** (4.9 $\mu$M). A hydroxymethyl (**10a**) or azidomethyl (**10c**) $\beta$-branch added onto **4a** increased antiplasmodial activity (four times in the case of **10a** and a more significant eight times in the case of **10c**). Similarly, compared to acyclic **3a** ($IC_{50}$ = 7.5 $\mu$M), its hydroxyethyl $\beta$-branched analogue **10b** ($IC_{50}$ = 1.9 $\mu$M) was slightly more active in vitro.

## Molecular Modeling

In an attempt to rationalize the results of the enzyme inhibition, the inhibitors were docked into the active site of the *P. falciparum* enzyme. Previously we have reported the crystal structure of *P. falciparum* dUTPase complexed with **1d**.[9] This was used as a starting point for the modeling. FlexX[18] as implemented in the Sybyl software[19] was used for the docking. To validate the modeling, compound **1d** was docked in to the P. *falciparum* dUTPase active site, in an attempt to reproduce the binding conformation found in the crystal structure. There was an RMSD of 1.14 Å between the docked and crystallographically determined conformations of the **1d** (Figure 3a), suggesting that the docking was reliable.

Hence, all the inhibitors were docked into the active site. In general, it may be observed that the inhibitors bind in a manner similar to which **1d** was found to bind crystallographically (Figure 3b and 3c).[9] All the inhibitors have the uracil moiety positioned in the bottom of the active site forming hydrogen bonds with the side chain of the residue Asn103 and the chain of residue Ile117. Also the inhibitors have the trityl moiety positioned in a hydrophobic part of the active site, interacting with the side chain of residues Phe46 (forming a $\pi-\pi$ interaction) and Ile117 (Figure 3d). Furthermore, it is possible that Lys96 might also interact with the triphenyl group forming a cation$-\pi$ interaction. Interestingly, Phe46 is not present in the human structure, but is replaced by Val42, which may partially account for the selectivity of the compounds for the parasite.

The aliphatic chain that links the uracil group and the trityl moiety interacts with Tyr112 by van der Waals interaction. The flexibility of these acyclic nucleoside derivatives allows them to adopt a folded conformation which still retains the uracil and trityl compounds in their binding pockets.

A complete table of docking scores obtained with FlexX is reported and included in the Supporting Information of this

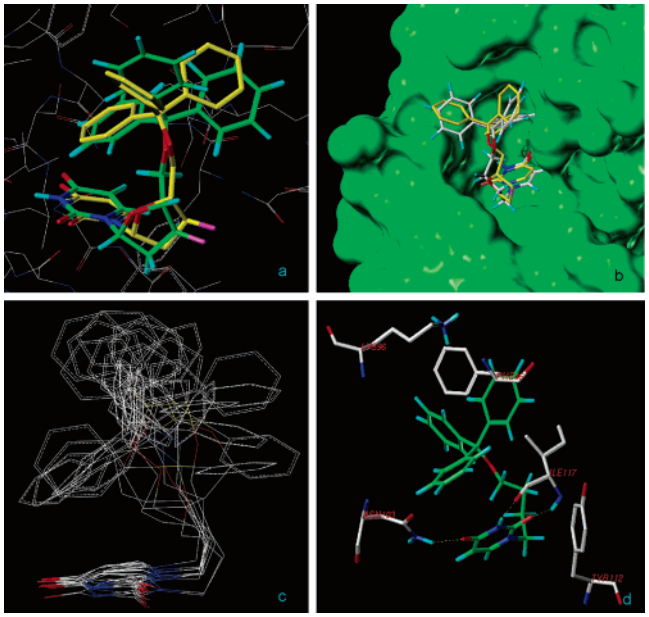


**Figure 3.** (a) Superimposition of the binding conformation of compound **1d** obtained crystallographically (yellow) and by modeling (green). (b) As Figure 3a, with the protein shown as a surface. (c) Superimposition of the docked conformations of compounds of Series **2**−**6** (except **4h**). (d) Interactions of **4a** with key residues in the active site of the *P. falciparum* dUTPase.

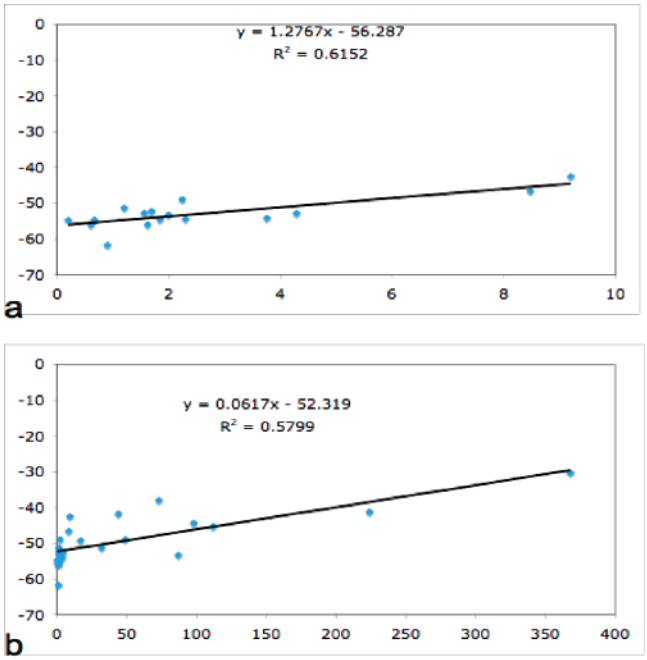



**Figure 4.** (a) Correlation between $K_i$ (*x*-axis) and FlexX score (*y*-axis) for compounds with a $K_i < 10$ $\mu$M against *P. falciparum* dUTPase. (b) Correlation between $K_i$ (*x*-axis) and FlexX score (*y*-axis) for compounds with a $K_i < 400$ $\mu$M.

article. The computed energy scores obtained from FlexX suggest a favorable interaction between *P. falciparum* dUTPase and most of the ligands with low constant of inhibition; this is evident from the high negative binding score values. Compounds with very poor experimental binding affinity ($K_i > 500$ $\mu$M) also showed poor scores with FlexX, which is consistent with the model possibly showing predictive potency. There appears to be a correlation between observed $K_i$ and docking score, which gave a linear correlation with an $R^2$ of 0.61 (Figure 4) {this correlation excludes the branched chain compounds **9** and **10**}.

## Discussion

**Correlation Enzyme/in Vitro Assays.** In general, a good correlation between enzyme and cellular assays results was observed. Potent inhibitors of the enzyme contained a triphenyl substituent (or in one case a diphenyl substituent), and all of these gave good inhibition of parasite growth. Compounds lacking the triphenyl moiety failed to give inhibition of the enzyme and also gave much weaker inhibition of parasite growth. There was not an obvious correlation between chain length and enzyme inhibition or growth inhibition. The docking calculations suggest that the chains can fold to accommodate the molecules so that the uracil and triphenyl groups bind in their respective pockets. Slight differences were observed regarding the extent of inhibition in the enzyme and cellular assays. This may be due to differences in cellular permeability of compounds. Additionally, some compounds may be metabolized; for example an acetoxy group may be hydrolyzed in cellular conditions to produce a more active analogue. Thus, whereas the hydroxyl derivative **9a** was 5−10 times more active against *P. falciparum* dUTPase than analogues **9b** (acetoxy derivative) and **9c** (carbamate derivative), it showed similar or even possibly slightly less activity in the cellular assay.

However, in four instances, assay results at the enzyme and parasite levels did not correlate. The acyclic derivative **3g** (with a benzoyloxypropyl side-chain) inhibited the enzyme with a $K_i$ of 9.2 $\mu$M, but showed no *P. falciparum* growth inhibition ($IC_{50} > 18$ $\mu$M). This may be explained by hydrolysis of the benzoate occurring in cell culture, with **3g** being converted into the inactive hydroxyl analogue **3e**. On the other hand, the trityl ether **3a**, the benzoate acyclic **6g** and the $\gamma$-branched derivative **10c** inhibited *P. falciparum* growth but not the target dUTPase. Here, the lack of correlation could be due to the compounds acting on a molecular target other than *P. falciparum* dUTPase.

In the case of the unsaturated analogues **7a** and **8a**, although there was a good correlation between inhibition of the target dUTPase and inhibition of *P. falciparum* growth, the striking difference of affinity for the human enzyme was not reflected in the cytotoxicity cellular assay. Indeed, although compound **7a** inhibited the human dUTPase ($K_i = 5.4$ $\mu$M) whereas its geometrical isomer **8a** did not ($K_i > 1$ mM), both analogues showed similar cytotoxicity on mammalian cells ($IC_{50}$ around 35 $\mu$M), although only one mammalian cell type has been investigated.

**Preliminary ADME Studies.** Preliminary ADME tests showed that compounds **1b**, **2a**, and **1d** have reasonable drug-like properties,[20] with the acyclic analogue **2a,** however, lacking metabolic stability as evidenced by the high clearance rate by human liver microsomes (Table 5). Tests on additional acyclic analogues focused on stability. These included (i) incubation with human liver microsomes (HLM), which assesses the overall response to all liver metabolising enzymes and therefore provides information regarding metabolic stability and also gives early guidance relating to drug half-life; and (ii) stability in simulated gastric, which is particularly relevant, as oral administration is a key requirement for the antimalarial drugs sought. Key results are shown in Table 5.

HLM intrinsic clearance for **2a** and **4a** indicated that having an oxygen in the side-chain facilitated degradation by liver enzymes. The Si/C replacement ($Ph_3Si$ in place of $Ph_3C$) did not seem to have any effect on metabolic stability (compare HLM intrinsic clearance for **4a** and **4c**). On the other hand,

**Table 5.** Preliminary ADME tests carried out

| compound | HLM intrinsic clearance[a] $\mu$L/min/mg | HLM intrinsic clearance relative to control | incubation in simulated gastric fluid amount remaining/incubation time | log $D^b$ |
|---|---|---|---|---|
| **2a** | 96 (55) | 1.7 | Na | 4.93 |
| **4a** | 68 (29) | 2.3 | 2%/5 min | 5.48 |
| **4c** | 69 (29) | 2.4 | <2%/0.2 min | 6.28 |
| **6c** | 35 (29) | 1.2 | <2%/0.2 min | 7.04 |
| **4b** | 64 (52) | 1.2 | 94%/150 min | 4.42 |

[a] Value for control (MV026048) is given in parentheses. [b] Estimated using ACDLabs 7.0; na: not available.

introducing a tritylamino group in place of a trityloxy group (NH/O replacement) appeared to enhance stability (compare **4a** and **4b**). Finally, results suggested that side-chain length may be important for metabolic stability. Indeed, compound **6c** (side-chain of six carbons) was found to be much more stable toward liver enzymes than its analogue with a side-chain of four carbons (**4c**).

Looking at stability in simulated gastric fluid, results obtained for compounds **4a**, **4b**, and **4c** showed that the tritylamino group provided stability whereas the trityl ether and triphenylsilyl ether were predicted to undergo significant degradation in the stomach, especially so in the case of the silyl ether **4c**, almost instantly hydrolyzed.

That inhibition of *P. falciparum* dUTPase was found to be closely associated with the presence of trityl or triphenylsilyl groups raises the issue of high lipophilicity. According to Lipinski investigations, good bioavailability or permeability is more likely for drugs with a logP below 5.[21] LogP values can only be predicted for uncharged substances, and for compounds that can exist in different ionic forms (particularly relevant for tritylamines at physiological pH), logD values represent better predictions. Clearly, predicted logD values were high due to the three phenyl groups (Table 5). However, the presence of the ionizable amine moiety lowered lipophilicity (by 1 logD unit; compare **4b** and **4a**), while the introduction of a silicon atom had the opposite effect (compare **4a** and **4c**).

Overall, these preliminary results suggest that for further lead development (especially in view of an orally administered drug), a tritylamino unit is a better option than trityloxy and triphenylsilyloxy groups from a lipophilicity (logD) point of view, as well as for stability, both in simulated gastric fluid simulation and toward liver enzymes.

## Conclusion

We have described a cost-effective synthesis of a selection of acyclic analogues of deoxyuridine. Potent and selective nontoxic inhibitors were identified. The compounds prepared showed equal or better antiplasmodial properties (inhibition of *P. falciparum* dUTPase and parasite growth, nontoxicity) compared to cyclic analogues previously reported,[3] especially with regards to selectivity. Structure−activity relationships were found to be in good agreement with previous observations, emphasizing the importance of a trityl or triphenylsilyl group for activity. Furthermore, potential issues related to stability and lipophilicity associated with such groups were considered for some of the most promising inhibitors. Finally, we have tested a simple docking model which reproduced well experimental data and may have some potential as a qualitative screening tool. Globally, the once neglected *P. falciparum* dUTPase is now much better understood and stands as a very promising target for the development of novel antimalarial drugs.

## Experimental Section

**Chemistry.** Chemicals were purchased from Aldrich, Sigma, Lancaster, Acros, Avocado, or Fluka and were used without further purification. Compounds **39**, **42**, and **47** were provided by Medivir AB. Dry solvents were generally purchased from Fluka in sure-seal bottles and stored over molecular sieves. Reactions were performed in predried apparatus under an atmosphere of nitrogen unless otherwise stated. Reactions using microwave irradiation were carried in a Biotage Initiator microwave. Thin-layer chromatography (TLC) was performed on Merck silica gel $60F_{254}$ plates. Column chromatography was carried out using either Fisons matrix silica 60 (35−70 micron) or prepacked ISOLUTE SI columns purchased from Argonaut. $^1$H, $^{13}$C, and $^{19}$F NMR were recorded on a Bruker Avance DPX 300 spectrometer or on a Bruker Avance DPX 500 with tetramethylsilane as the internal standard. Deuterated solvents were purchased from Goss unless stated otherwise. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrometer. Melting points (mp) were measured on a Gallenkamp melting point apparatus and were uncorrected. Low-resolution mass spectra were recorded on a Fisons VG Platform II spectrometer or on a Brucker MicroTof spectrometer. High-resolution mass spectra were determined by the EPSRC Mass Spectrometry Centre, Swansea, UK. Microanalyses were carried out by MEDAC Ltd.

**General Procedure A. Coupling of Uracil with Protected Side-Chain Activated via a Bromo, Chloro. or Tosylate.** Uracil (1−1.5 equiv) and cesium carbonate (1 equiv) in dry DMF were stirred at room temperature under nitrogen for 30 min. The suitably protected and activated side-chain fragment (1 equiv) in dry DMF was added dropwise via a syringe. The reaction mixture was stirred at 30−60 °C for 4−64 h. Water was added and the solution was extracted with EtOAc. The organic layers were washed with water, dried over $Na_2SO_4$, and concentrated in vacuo. The desired compound was obtained after purification by flash column chromatography.

**General Procedure B. Ester Hydrolysis with Sodium Methoxide.** The benzoate or acetate ester (1 equiv) was dissolved in a 0.2 M solution of sodium methoxide (10 equiv) in $CH_3OH$. The reaction was stirred at room temperature until the disappearance of the starting esters was observed by TLC. The solution was neutralized with Dowex 50WX8−200 ion-exchange resin ($H^+$ form). The resin was filtered and washed with $CH_3OH$. The filtrate was concentrated in vacuo, and the residue was coevaporated with ethanol several times to give the desired alcohol. Alternatively (when specified) the residue was chromatographed.

**General Procedure C. Tritylation.** The alcohol or amine precursor (1 equiv), trityl chloride (1.2 equiv), and, when stated, DMAP (catalytic amount) were stirred in dry pyridine at 50 °C for up to 64 h. The reaction mixture was partitioned between cold water and $CH_2Cl_2$. The organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was purified by flash column chromatography to give the desired product.

**General Procedure D. Silylation of Alcohols with Triphenylsilyl Chloride.** A solution of triphenylsilyl chloride (TPSCl, 1.4 equiv) in dry pyridine was added dropwise to a solution of the alcohol precursor (1 equiv) in dry pyridine previously cooled in an ice-salt bath. The reaction mixture was stirred at 0 °C under nitrogen for 2−4 h. If necessary (presence of unreacted alcohol detected by TLC), more TPSCl in dry pyridine was added. Once the reaction had reached completion, it was quenched with $CH_3OH$. The solvent was then removed in vacuo, and the crude residue was purified by flash column chromatography (ISOLUTE SI column eluted with 0 → 5% $CH_3OH$ in $CHCl_3$ unless otherwise stated) to give the desired triphenylsilyl ether.

**1-[2-(Trityloxy)ethoxymethyl]uracil (2a).** Compound **2a** was prepared following general procedure C, from the alcohol **11** (0.50 g, 2.69 mmol) and trityl chloride (0.82 g, 2.96 mmol) in dry pyridine (20 mL). White solid (0.23 g, 20%). $R_f$ = 0.71 (10% $CH_3OH$/$CHCl_3$); mp 145−148 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 3.32 (2H, m, 2′-H), 3.78 (2H, m, 3′-H), 5.29 (2H, s, 1′-H), 5.82 (1H, d, $J$ = 7.9 Hz, 5-H), 7.29−7.51 (16H, m, Ph-H and 6-H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 63.3 (3′-$CH_2$), 69.7 (2′-$CH_2$), 77.4 (1′-$CH_2$), 87.2 ($Ph_3C$), 103.6 (5-CH), 127.5 (Ph-CH), 127.7 (Ph-CH), 128.3 (Ph-CH), 129.1 (Ph-CH), 143.5 (6-CH), 144.2 (Ph-C), 151.4 (2-C), 163.8 (4-C); IR (KBr) 3021, 1702, 1673, 760, 1452, 1087, 703 $cm^{-1}$; LRMS ($ES^+$) $m/z$ 451 ([M + Na]$^+$, 100%); 473 (6%), 452 (13%), 243 (7%); HRMS ($ES^+$) calcd for $C_{26}H_{24}N_2NaO_4$ (M + Na)$^+$ 451.1634, found 451.1626.

**1-(3-Trityloxypropyl)uracil (3a).** Compound **3a** was prepared following general procedure A, from 3-bromo-1-*O*-tritylpropanol **12** (0.40 g, 1.05 mmol), $Cs_2CO_3$ (0.38 g, 1.05 mmol), and uracil (0.13 g, 1.15 mmol) in DMF (10 mL). White solid (134 mg, 31%). Flash column chromatography was performed eluting the column (ISOLUTE SI) with 0 → 30% EtOAc/hexane. Mp 161−164 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 2.14−2.02 (2H, m, 2′-H), 3.22 (2H, t, $J$ = 5.67 Hz, 3′-H), 4.02 (2H, t, $J$ = 6.58 Hz, 1′-H), 5.47 (1H, dd, $J$ = 2.4, 7.9 Hz, 5-H), 6.95 (1H, d, $J$ = 7.9 Hz, 6-H), 7.50−7.22 (15H, m, Ph-H), 8.10 (1H, bs, 3-NH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 26.5 (2′-$CH_2$), 48.0 (1′-$CH_2$), 67.0 (3′-$CH_2$), 97.2 ($Ph_3C$ C-10), 101.7 (5-CH), 128.9 (Ph-CH), 128.4 (Ph-CH), 127.7 (Ph-CH), 137.6 (Ph-C), 148.7 (6-CH), 150.7 (2-C), 160.0 (4-C); Anal. ($C_{26}H_{24}N_2O_3$) C, H, N.

**1-(3-Tritylaminopropyl)uracil (3b).** Compound **3b** was prepared following general procedure A from uracil (0.23 g, 2.05 mmol), and $Cs_2CO_3$ (0.46 g, 1.42 mmol) in DMF (8 mL), reacted with 3-bromo-1-tritylaminopropane **22** (0.51 g, 1.33 mmol) in DMF (3 mL). Chromatography was carried out using 20 → 50% EtOAc in hexane; a second column (0 → 10% $CH_3OH$ in $CHCl_3$) was necessary to remove traces of DMF. Compound **3b** was isolated as a white solid (0.116 g, 21%). Mp 228−230 °C (dec); $^1$H NMR (300 MHz, $CDCl_3$ + $CD_3OD$) $\delta$ 1.87 (2H, m, 2′-H), 2.20 (2H, t, $J$ = 6.5 Hz, 3′-H), 3.85 (2H, t, $J$ = 7.2 Hz, 1′-H), 5.61 (1H, d, $J$ = 7.9 Hz, 5-H), 7.09 (1H, d, $J$ = 7.9 Hz, 6-H), 7.15−7.35 (9H, m, Ph-H), 7.47 (6H, m, Ph-H); $^{13}$C NMR (75 MHz, $CDCl_3$ + $CD_3OD$) $\delta$ 30.3 (2′-$CH_2$), 40.4 (3′-$CH_2$), 47.2 (1′-$CH_2$), 71.2 ($Ph_3C$), 102.4 (5-CH), 126.8 (Ph-CH), 128.3 (Ph-CH), 129.0 (Ph-CH), 144.9 (6-CH), 146.1 (Ph-C), 151.4 (2-C), 164.8 (4-C); LRMS ($ES^+$) $m/z$ 845 ([2M + Na]$^+$, 3%), 434 ([M + Na]$^+$, 22%), 412 ([M + H]$^+$, 10%), 243 ($Ph_3C^+$, 42%), 87 (100%); HRMS ($ES^+$) calcd for $C_{26}H_{26}N_3O_2$ (M + H)$^+$ 412.2020, found 412.2018; Anal. ($C_{26}H_{25}N_3O_2$, 0.19 HBr) C, H, N; Calcd Br, 3.56; found Br, 4.91.

**1-(3-Triphenylsilyloxypropyl)uracil (3c).** Compound **3c** was prepared following general procedure D, from a solution of 1-(3-hydroxypropyl)uracil **3e** (0.19 g, 1.13 mmol) in dry pyridine (4 mL) stirred at 0 °C with TPSCl (0.43 g, 1.46 mmol) in dry pyridine (3 mL) for 4 h 30 min, and for 15 min after further addition of TPSCl (0.204 g, 0.692 mmol) in dry pyridine (1 mL). White solid (0.392 g, 81%). $R_f$ = 0.52 (10% $CH_3OH$/$CHCl_3$); mp 152−153 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 2.01 (2H, m, 2′-H), 3.94 (4H, m, 1′-H and 3′-H), 5.54 (1H, d, $J$ = 7.9 Hz, 5-H), 7.04 (1H, d, $J$ = 7.9 Hz, 6-H), 7.51 (9H, m, Ph-H), 7.68 (6H, m, Ph-H), 9.49 (1H, bs, 3-NH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 31.5 (2′-$CH_2$), 46.4 (1′-$CH_2$), 60.3 (3′-$CH_2$), 102.1 (5-CH), 128.3 (Ph-CH), 130.8 (Ph-CH), 134.0 (Ph-C), 135.8 (Ph-CH), 145.6 (6-CH), 151.3 (2-C), 164.3 (4-C); LRMS ($ES^+$) $m/z$ 879 ([2M + Na]$^+$, 8%), 451 ([M + Na]$^+$, 38%), HRMS ($ES^+$) calcd for $C_{25}H_{28}N_3O_3Si$ (M + $NH_4$)$^+$ 446.1894, found 446.1887; Anal. ($C_{25}H_{24}N_2O_3Si$) C, H, N.

**1-(3-*tert*-Butyldimethylsilyloxypropyl)uracil (3f).** Compound **3f** was prepared following general procedure A, from 3-bromo-1-*tert*-butyldimethylsilylpropanol **13** (0.64 g, 2.68 mmol), uracil (0.30 g, 2.68 mmol), and $Cs_2CO_3$ (0.87 g, 2.68 mmol) in DMF (10 mL). Colorless oil (250 mg, 33%). Flash column chromatography was performed eluting the column (ISOLUTE SI) with 0 → 30% EtOAc/hexane. Mp 120−122 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.00 (6H, s, [$CH_3$]$_2$Si), 0.95 (9H, s, C[$CH_3$]$_3$), 1.94 (2H, quintet, $J$ = 6.6 Hz, 2′-H), 3.70 (2H, t, $J$ = 6.6 Hz, 1′-H), 3.91 (2H, t, $J$ = 6.6 Hz, 3′-H), 5.72 (1H, d, $J$ = 7.9 Hz, 5-H), 7.30 (1H, d, $J$ = 7.9 Hz, 6-H), 9.15 (1H, bs, 3-N); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ −5.0 ([$CH_3$]$_2$Si), 18.6 (C[$CH_3$]$_3$), 26.3 (C[$CH_3$]$_3$), 31.6 (2′-$CH_2$), 46.6 (1′-$CH_2$), 59.4 (3′-$CH_2$), 102.0 (5-CH), 145.8 (6-CH), 151.2 (2-C), 164.3 (4-C); LRMS ($ES^+$) $m/z$ 307 ([M + Na]$^+$, 100%).

**1-(5-Trityloxypentyl)uracil (5a).** The $N^3$-benzoyluracil **31** (231 mg; 0.42 mmol) was stirred for 48 h at room temperature with a mixture of 1 M NaOH and dioxane (v/v 1:1, 8 mL). After 48 h, brine (8 mL) was added to the solution which was then extracted with EtOAc (3 × 8 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. The crude residue was purified by flash column chromatography eluting the silica gel column with 10% $CH_3OH$ in $CH_2Cl_2$. The colorless oil isolated was treated with a mixture of water/ethanol (v/v, 1:1) to give compound **5a** as a white solid (112 mg; 60%). $R_f$ = 0.64 (10% $CH_3OH$/$CH_2Cl_2$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.50−1.60 (2H, m, 3′-H), 1.74−1.84 (4H, m, 2′-H and 4′-H), 3.22 (2H, t, $J$ = 6.2 Hz, 5′-H), 3.83 (2H, t, $J$ = 7.3 Hz, 1′-H), 7.34−7.46 (9H, m, Ph-H), 5.80 (1H, d, $J$ = 7.9 Hz, 5-H), 7.21 (1H, d, $J$ = 7.9 Hz, H-6), 7.56 (6H, m, Ph-H), 9.13 (1H, bs, 3-NH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 23.6 (3′-$CH_2$), 29.2 (2′-$CH_2$), 29.9 (4′-$CH_2$), 49.2 (1′-$CH_2$), 63.5 (5′-$CH_2$), 86.9 ($Ph_3C$), 102.5 (5-CH), 127.4 (Ph-CH), 128.4 (Ph-CH), 129.1 (Ph-CH), 144.8 (Ph-C), 144.9 (6-CH), 151.4 (2-C), 164.6 (4-C); LRMS ($ES^+$) $m/z$ 463 ([M + Na]$^+$, 100%); HRMS ($ES^+$) calcd for $C_{28}H_{32}N_3O_3$ (M + $NH_4$)$^+$ 458.2438, found 458.2439.

**1-(5-Tritylaminopentyl)uracil (5b).** Compound **5b** was prepared following general procedure B, from the $N^3$-benzoyluracil derivative **32** (0.19 g; 0.35 mmol) treated with 0.2 M $CH_3ONa$ in $CH_3OH$ (17 mL, 3.4 mmol). After removal of the Dowex resin and concentration of the filtrate in vacuo, the residue was purified by flash column chromatography, eluting the silica gel column with 10% $CH_3OH$ in $CH_2Cl_2$. White foam (240 mg; 52%). $R_f$ = 0.50 (10% $CH_3OH$/$CH_2Cl_2$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.36−1.49 (2H, m, 3′-H), 1.63−1.75 (4H, m, 2′-H and 4′-H), 2.18 (2H, t, $J$ = 7.2 Hz, 5′-H), 3.74 (2H, t, $J$ = 7.3 Hz, 1′-H), 5.73 (1H, d, $J$ = 7.9 Hz, 5-H), 7.14 (1H, d, $J$ = 7.9 Hz, 6-H), 7.22−7.36 (9H, m, Ph-H), 7.52 (6H, d, $J$ = 9.9 Hz, Ph-H), 8.70 (1H, bs, 3-NH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 24.6 (3′-$CH_2$), 29.5 (4′-$CH_2$), 30.8 (2′-$CH_2$), 43.7 (5′-$CH_2$), 49.2 (1′-$CH_2$), 71.3 ($Ph_3C$), 102.5 (5-CH), 126.7 (Ph-CH), 128.2 (Ph-CH), 129.0 (Ph-CH), 144.8 (6-CH), 146.6 (Ph-C), 151.0 (2-C),163.8 (4-C); LRMS ($ES^+$) $m/z$ 440 [M$^+$ , 50%], 243 ($Ph_3C^+$, 100%); HRMS ($ES^+$) calcd for $C_{28}H_{30}N_3O_2$ (M + H)$^+$ 440.2341, found 440.2333.

**1-(5-Triphenylsilyloxypentyl)uracil (5c).** Compound **5c** was prepared following general procedure D, from a solution of 1-(5-hydroxypentyl)uracil **5e** (0.05 g, 0.25 mmol) in pyridine (1 mL) treated with TPSCl (0.08 g, 0.27 mmol) in pyridine (1 mL) at room temperature for 24 h. Flash column chromatography was performed on a silica gel column eluted with 1% $CH_3OH$ in $CH_2Cl_2$. Light orange syrup (30 mg, 26%). $R_f$ = 0.69 (5% $CH_3OH$/$CH_2Cl_2$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.43−1.51 (2H, m, 3′-H), 1.66−1.76 (4H, m, 2′-H and 4′-H), 3.72 (2H, t, $J$ = 7.3 Hz, 1′-H), 3.87 (2H, t, $J$ = 6.1 Hz, 5′-H), 5.69 (1H, d, $J$ = 7.9 Hz, 5-H), 7.08 (1H, d, $J$ = 7.9 Hz, 6-H), 7.43−7.54 (9H, m, Ph-H), 7.67 (6H, m, Ph-H), 9.26 (1H, s, 3-NH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 23.2 (3′-$CH_2$), 29.0 (2′-$CH_2$), 32.2 (4′-$CH_2$) 49.2 (1′-$CH_2$), 63.8 (5′-$CH_2$), 102.4 (5-CH), 128.7 (Ph-C), 130.5 (Ph-CH), 134.4 (Ph-CH) 136.4 (Ph-CH), 144.9 (6-CH), 151.2 (2-C), 164.2 (4-C); LRMS ($ES^+$): $m/z$ 479 ([M + Na]$^+$, 100%); HRMS ($ES^+$) calcd for $C_{27}H_{28}N_2NaSiO_3$ (M + Na)$^+$ 479.1761, found 479.1780; Anal. ($C_{27}H_{28}N_2SiO_3$, 0.55 $H_2O$) C, H; Calcd N, 6.00; found N, 5.45.

**1-[(*Z*)-4-Trityloxy-2-butenyl]uracil (7a).** Compound **7a** was prepared following general procedure B, from the $N^3$-benzoyluracil **37** (0.05 g, 0.09 mmol) treated with 0.2 M $CH_3ONa$ in $CH_3OH$ (5 mL, 1.0 mmol). After removal of the Dowex resin and concentration of the filtrate in vacuo, the residue was purified by flash column chromatography, eluting the silica gel column with 10% $CH_3OH$ in $CH_2Cl_2$. White solid (38 mg, 90%). $R_f$ = 0.66 (10% $CH_3OH$/$CH_2Cl_2$); mp 65−68 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 3.79 (2H,

d, $J$ = 6.2 Hz, 4′-H), 4.28 (2H, d, $J$ = 7.4 Hz, 1′-H), 5.69−5.98 (2H, m, 5-H and 3′-H), 6.01−6.09 (1H, m, 2′-H), 7.16 (1H, d, $J$ = 8.0 Hz, 6-H), 7.29−7.41 (9H, m, Ph-H), 7.49−7.52 (6H,m, Ph-H), 8.21 (1H, bs, 3-NH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 44.9 (1′-$CH_2$), 59.9 (4′-$CH_2$), 87.8 ($Ph_3C$), 102.1 (5-CH), 126.1 (3′-CH), 127.7 (Ph-CH), 128.4 (Ph-CH), 129.0 (Ph-CH), 132.5 (2′-CH), 144.1 (Ph-C), 144.2 (6-CH), 151.2 (2-C), 164.0 (4-C); LRMS ($ES^+$) $m/z$ 447 ([M + Na]$^+$, 100%); HRMS ($ES^+$) calcd for $C_{27}H_{24}N_2O_3Na$ (M + Na)$^+$ 447.1679, found 447.1680; Anal. ($C_{27}H_{24}N_2O_3$, 0.4 $H_2O$) C, H, N.

**1-[(*E*)-4-trityloxy-2-butenyl]uracil (8a).** Compound **8a** was prepared following general procedure B, from the $N^3$-benzoyluracil **38** (0.05 mg, 0.09 mmol) treated with 0.2 M $CH_3ONa$ in $CH_3OH$ (5 mL, 1.0 mmol). After removal of the Dowex resin and concentration of the filtrate in vacuo, the residue was purified by flash column chromatography, eluting the silica gel column with 0→5% $CH_3OH$ in $CHCl_3$. White solid (38 mg, 90%). $R_f$ = 0.51 (10% $CH_3OH/CHCl_3$); mp 70−72 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 3.74 (2H, d, $J$ = 3.9 Hz, 1′-H), 4.42 (2H, d, $J$ = 5.6 Hz, 4′-H), 5.77 (1H, d, $J$ = 7.9 Hz, 5-H), 5.83−5.98- (2H, m, 2′-H and 3′-H), 7.19 (1H, d, $J$ = 7.9 Hz, 6-H), 7.27−7.39 (9H, m, Ph-H), 7.49 (6H, d, $J$ = 7.8 Hz, Ph-H), 9.04 (1H, bs, 3-NH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 49.6 (1′-$CH_2$), 64.0 (4′-$CH_2$),87.5 ($Ph_3C$), 102.9 (5-CH), 124.1 (3′-CH), 124.1 (Ph-CH), 128.3 (Ph-CH), 129.0 (Ph-CH), 133.3 (2′-CH), 144.1 (6-CH), 144.3 (Ph-C), 151.1 (2-C), 163.9 (4-C); LRMS ($ES^+$) $m/z$ 447 ([M + Na]$^+$, 100%); HRMS ($ES^+$) calcd for $C_{27}H_{28}N_3O_3$ (M + $NH_4$)$^+$ 442.2125, found 442.2128; Anal. ($C_{27}H_{24}N_2O_3$, 0.2 $H_2O$) C, H, N.

**1-[4-Acetyloxy-3-(tritylaminomethyl)butyl]uracil (9a).** Trityl chloride (0.13 g, 0.46 mmol) was added to a solution of compound **41** (0.10 g, 0.43 mmol) and triethylamine (0.64 mL, 0.46 mmol) in dry $CH_2Cl_2$ (5 mL). The reaction mixture was stirred at room temperature for 6 h. Further trityl chloride (0.06 g, 0.23 mmol) and triethylamine (0.32 mL, 0.23 mmol.) were added, and the reaction was left stirring overnight. Water (5 mL) was added. The organic layer was extracted and washed with 0.1M HCl (5 mL), dried over $MgSO_4$, concentrated in vacuo, and purified by flash column chromatography eluting with 0 → 5% $CH_3OH$ in $CH_2Cl_2$. The title compound was obtained as a white foam (150 mg, 70%). $R_f$ = 0.60 (10% $CH_3OH/CHCl_3$); mp 68−72 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.65−1.83 (3H, m, 2′-H and 3′-H), 2.03 (3H, s, C$H_3$CO), 2.17 (2H, d, $J$ = 6.0 Hz, C$H_2$NHTr), 3.68 (2H, m, 1′-H), 4.27 (2H, d, $J$ = 5.0 Hz, 4′-H), 5.66 (1H, d, $J$ = 7.9 Hz, 5-H), 7.02 (1H, d, $J$ = 7.9 Hz, 6-H), 7.19−7.30 (9H, m, Ph-H), 7.46 (6H, d, $J$ = 7.3 Hz, Ph-H), 9.07 (1H, bs, 3-NH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 21.3 ($CH_3$CO), 29.7 (2′-$CH_2$), 37.0 (3′-CH), 44.6 ($CH_2$-NHTr), 47.4 (1′-$CH_2$), 65.3 ($CH_3CO_2CH_2$ or 4′-$CH_2$), 71.3 ($Ph_3C$), 102.7 (5-CH), 126.8 (Ph-CH), 128.3 (Ph-CH), 129.0 (Ph-CH), 144.7 (6-CH), 146.2 (Ph-C), 151.1 (2-C), 164.0 (4-C), 171.5 ($CH_3CO$); LRMS (CI) $m/z$ 498.3 ([M + H]$^+$, 100%); HRMS ($ES^+$) calcd for $C_{30}H_{32}N_3O_4$ (M + H)$^+$ 498.2387, found 498.2384; Anal. ($C_{30}H_{31}$-$N_3O_4$,1.5 $H_2O$) C, H, N.

**1-[4-Hydroxy-3-(tritylaminomethyl)butyl]uracil (9b).** The intermediate **9a** (0.11 g, 0.22 mmol) was deacetylated according to general procedure B using 0.2 M $CH_3ONa$ in $CH_3OH$ (5 mL, 1.0 mmol). Compound **9b** was obtained as a white foam (100 mg, 99%). $R_f$ = 0.66 (10% $CH_3OH/CHCl_3$); mp 75−80 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.47−1.68 (2H, m, 2′-H), 1.84 (1H, bs, 3′-H), 2.40−2.48 (2H, m, C$H_2$NHTr), 3.57−3.82 (3H, m, 1′-H and 4′-C$H$H), 3.90−3.95 (1H, m, 4′-CH$H$), 5.26 (2H, bs, $CH_2OH$ and $Ph_3$-CN$H$), 5.65 (1H, d, $J$ = 7.9 Hz, 5-H), 7.03 (1H, d, $J$ = 7.9 Hz, 6-H), 7.35−7.23 (9H, m, Ph-H), 7.41−7.48 (6H, m, Ph-H), 8.16 (1H, bs, 3-NH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 29.4 (2′-$CH_2$), 38.3 (3′-CH), 47.5 (1′-$CH_2$), 48.1 ($CH_2$NHTr), 67.0 (4′-$CH_2$), 97.8 ($Ph_3C$), 102.7 (5-CH), 127.2 (Ph-CH), 128.5 (Ph-CH), 128.9 (Ph-CH), 144.7 (6-CH), 145.1 (Ph-C), 150.9 (2-C), 163.7 (4-C); LRMS ($ES^+$) $m/z$ 456 ([M + H]$^+$, 100%); HRMS ($ES^+$) calcd for $C_{28}H_{30}N_3O_3$ (M + H)$^+$ 456.2282, found 456.2283; Anal. ($C_{28}H_{29}$-$N_3O_3$, 0.5 $H_2O$, 1.0 HCl) C, H, N.

**1-[4-(*tert*-Butoxycarbonylamino)-3-(trityloxymethyl)butyl]uracil (9c).** 1-[4-(*tert*-Butoxycarbonylamino)-3-(hydroxymethyl)butyl]uracil **39** (0.05 g, 0.15 mmol), trityl chloride (0.05 mg, 0.19 mmol), and dry pyridine (2 mL) were irradiated with microwaves to reach the temperature of 160 °C for 5 min. The irradiating cycle was repeated three times. The solvent was removed in vacuo, and the crude product was purified by flash column chromatography using 40% hexane in EtOAc. Compound **9c** was obtained as a white foam (70 mg, 79%). $R_f$ = 0.27 (40% hexane/EtOAc); mp 86−88 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.47 (9H, s, C[C$H_3$]$_3$), 1.53−1.62 (1H, m, 3′-H), 1.67−1.85 (2H, m, 2′-H), 3.05−3.10 (1H, m, 4′-C$H$H), 3.23−3.31 (3H, m, 1′-H and 4′-CH$H$), 3.76−3.84 (2H, m, 3′-H), 4.58 (1H, t, $J$ = 6.1 Hz, N$H$Boc), 5.67 (1H, dd, $J$ = 2.4, 7.9 Hz, 5-H), 7.27−7.40 (10H, m, 6-H and Ph-H), 7.48 (6H, d, $J$ = 7.0 Hz, Ph-H), 8.08 (1H, bs, 3-NH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 28.4 (C[$CH_3$]$_3$), 29.7 (2′-$CH_2$), 36.9 (3′-$CH_2$), 40.8 (4′-$CH_2$), 46.8 (1′-$CH_2$), 64.3 ($CH_2$OTr), 79.2 (C[$CH_3$]$_3$), 86.8 ($Ph_3C$), 101.9 (5-CH), 126.9 (Ph-CH), 127.8 (Ph-CH), 128.6 (Ph-CH), 143.7 (Ph-C), 145.0 (6-CH), 150.8 (2-C), 156.5 (NH$CO_2$), 163.8 (4-C); HRMS ($ES^+$) calcd for $C_{33}H_{38}N_3O_5$ (M + H)$^+$ 498.2387, found 498.2384; Anal. ($C_{33}H_{37}N_3O_5$, 0.30 $H_2O$) C, H; Calcd N, 7.49; found N, 6.98.

**1-[2-(Hydroxymethyl)-4-(trityloxy)butyl]uracil (10a).** The intermediate **46** (0.14 g, 0.28 mmol) was deacetylated according to general procedure B using 0.2 M $CH_3ONa$ in $CH_3OH$ (5 mL, 1.0 mmol). Compound **10a** was obtained as a white solid (73 mg, 57%). $R_f$ = 0.51 (10% $CH_3OH/CH_2Cl_2$); mp 83−85 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.64−1.77 (2H, m, 3′-H), 3.21−3.28 (2H, m, 4′-H), 2.03−2.10 (1H, m, 2′-H), 3.44 (1H, dd, $J$ = 4.4, 12.0 Hz, C$H$HOH), 3.49 (1H, dd, $J$ = 3.4 Hz, 12.0 Hz, CH$H$OH), 3.63 (1H, dd, J = 4.9, 14.0 Hz, 1′-C$H$H), 3.78 (1H, dd, J = 9.0, 14.0 Hz, 1′-CH$H$), 5.69 (1H, d, $J$ = 7.9 Hz, 5-H), 7.12 (1H, d, $J$ = 7.9 Hz, 6- H), 7.27 (3H, m, Ph-H), 7.33 (6H, d, $J$ = 7.5 Hz, Ph-H), 7.45 (6H, d, $J$ = 7.4 Hz, Ph-H), 9.67 (1H, bs, 3-NH); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 29.1 (3′-$CH_2$), 38.3 (2′-CH), 49.0 (1′-$CH_2$), 61.0 ($CH_2$OH), 61.1 (4′-$CH_2$), 87.0 ($Ph_3C$), 102.4 (5-CH), 127.3 (Ph-CH), 127.9 (Ph-CH), 128.7 (Ph-CH), 143.9 (Ph-C), 145.6 (6-CH), 151.9 (2-C), 163.8 (4-C); LRMS ($ES^+$) $m/z$ 479 ([M + Na]$^+$, 100%); HRMS ($ES^+$) calcd for $C_{28}H_{28}N_2NaO_4$ (M + Na)$^+$ 479.1941, found 479.1946; Anal. ($C_{28}H_{28}N_2O_4$, 1.20 $H_2O$) C, H, N.

**1-[4-Hydroxy-2-(trityloxymethyl)butyl]uracil (10b).** The TBDMS protected intermediate **43** (0.13 g, 0.23 mmol) was dissolved in THF (3 mL). Tetrabutylammonium fluoride (0.07 g, 0.27 mmol) was added, and the reaction mixture was stirred at room temperature until complete disappearance of the starting material was observed by TLC. The solvent was removed in vacuo, and the crude product was purified by flash column chromatography eluting with 5 → 10% $CH_3OH$ in $CHCl_3$. The title compound was obtained as a white solid (49 mg, 47%). $R_f$ = 0.57 (10% $CH_3OH/CHCl_3$); mp 85−88 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.69−1.77 (2H, m, 3′-H), 2.14 (1H, bs, 4′-OH), 2.21 (1H, m, 2′-H), 3.14−3.19 (2H, m, 4′-H), 3.71−3.76 (2H, m, 1′-C$H$H and CH$H$OTr), 3.79−3.84 (1H, m, C$H$HOTr), 3.99 (1H, dd, $J$ = 4.1, 13.8 Hz, 1′-CH$H$), 5.32 (1H, d, $J$ = 7.9 Hz, 5-H), 6.69 (1H, d, $J$ = 7.9 Hz, 6-H), 7.27−7.30 (6H, m, Ph-H), 7.34 (3H, t, $J$ = 7.5 Hz, Ph-H), 7.43 (6H, d, $J$ = 7.6 Hz, Ph-H), 8.23 (1H, bs, 3-NH); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 32.3 (3′-$CH_2$), 36.1 (2′-CH), 49.4 (1′-$CH_2$), 60.2 ($CH_2$OTr), 62.5 (4′-$CH_2$), 86.7 ($Ph_3C$), 101.5 (5-CH), 127.3 (Ph-CH), 128.0 (Ph-CH), 128.6 (Ph-CH), 143.4 (Ph-C), 145.3 (6-CH), 151.1 (2-C), 163.7 (4-C); LRMS ($ES^+$) $m/z$ 479 ([M + Na]$^+$, 100%); HRMS ($ES^+$) calcd for $C_{28}H_{28}N_2O_4Na$ (M + Na)$^+$ 479.1941, found 479.1935; Anal. ($C_{28}H_{28}N_2O_4$, 1.40 $H_2O$) C, H, N.

**1-[2-(Azidomethyl)-4-(trityloxy)butyl]uracil (10c).** Compound **10c** was prepared by tritylation of 1-[2-(azidomethyl)-4-(hydroxy)-butyl]uracil **48** (0.10 g, 0.42 mmol) with trityl chloride (0.16 g, 0.58 mmol) in dry pyridine (5 mL), according to general procedure C. White solid (163 mg, 82%). $R_f$ = 0.57 (10% $CH_3OH/CHCl_3$); mp 75−80 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.42−1.51 (2H, m, 3′-H), 2.03−2.11 (1H, m, 2′-H), 2.96−3.14 (4H, m, 1′-H and 4′-H), 3.42−3.46 (1H, m, C$H$H$N_3$), 3.50 (1H, dd, $J$ =6.1, 13.9 Hz, CH$H$$N_3$), 5.49 (1H, dd, $J$ = 2.0, 7.9 Hz, 5-H), 6.90 (1H, d, $J$ = 7.9 Hz, 6-H), 7.06−7.09 (3H, m, Ph-H), 7.14 (6H, t, $J$ = 7.6 Hz, Ph-H), 7.25 (6H, d, $J$ = 7.3 Hz, Ph-H), 8.52 (1H, bs, 3-NH); $^{13}$C NMR

(125 MHz, $CDCl_3$) $\delta$ 29.6 (3′-$CH_2$), 36.5 (2′-CH), 50.5 (1′-$CH_2$), 51.9 ($CH_2N_3$), 60.5 (4′-$CH_2$), 102.5 (5-CH), 127.1 (Ph-CH), 127.9 (Ph-CH), 128.6 (Ph-CH), 143.9 (Ph-C), 144.8 (6-CH), 150.8 (2-C), 163.3 (4-C); IR (KBr) 3061, 2102, 1593, 1449 $cm^{-1}$; LRMS ($ES^+$) *m*/*z* 504 ($[M + Na]^+$, 100%); LRMS ($ES^-$) *m*/*z* 482 ($M^-$, 100%); HRMS ($ES^+$) calcd for $C_{28}H_{24}N_5O_2Na$ $(M + Na)^+$ 504.2006, found 504.1997; Anal. ($C_{28}H_{24}N_5O_2$) C, H; Calcd N, 14.54; found N, 13.79.

**Biological Assays. Enzyme Purification and Inhibition Assays.** Both recombinant *P. falciparum* and human dUTPases were expressed in *E. coli* BL21 (DE3) cells which had been transformed with the pET11*Pfdut*[9] and pET3*Hudut* (kindly provided by P. O. Nyman, Lund University, Sweden) expression vectors, respectively. For dUTPase purification, the same procedure was used for both the human and the *Plasmodium* enzymes. Cell pellets from a 2-L IPTG-induced culture were resuspended in 40 mL of buffer A (20 mM sodium acetate (pH 5.5), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT) and 20 $\mu$M PMSF. The cells were lysed by sonication, and the cell extract was cleared by centrifugation at 15 000 rpm for 45 min. The supernatant was loaded onto a 50 mL phosphocellulose (Whatman P-11) column at 4 °C and was eluted with a 50 mM to 2 M NaCl gradient in buffer A. The enzyme was then dialyzed against buffer A, prior to gel filtration chromatography on a Superdex 200 HA 10/30 column at 4 °C. Purified fractions contained dUTPase of approximately 96% purity.

Nucleotide hydrolysis was monitored by mixing enzyme and substrate with a rapid kinetic accessory (Hi-Tech Scientific) attached to a spectrophotometer (Cary 50) and connected to a computer for data acquisition and storage as described previously.[22] Protons, released through the hydrolysis of nucleotides, were neutralized by a pH indicator in weak buffered medium with similar p$K_a$ and monitored spectrophotometrically at the absorbance peak of the basic form of the indicator. The ratio between the indicator and the buffer concentration was 50:2000 ($\mu$M), and the absorbance changes were kept within 0.1 units. The indicator/buffer pair used was red cresol/bicine (pH 7.5−8.5, 573 nm). Assay mixes contained 30 nM of *Pf*dUTPase, 50 $\mu$M dUTP, 5 mM $MgCl_2$, 2.5 mM DTT, 1.25 mg/mL BSA, and 100 mM KCl. $V_{max}$ and $K_{Mapp}$ were calculated by fitting the resulting data to the integrated Michaelis−Menten equation. The apparent $K_M$ values were plotted against inhibitor concentration, and $K_i$ values (Table 2) were obtained according to eq 1.

$$K_{Mapp} = \frac{K_M}{K_i}[I] + K_M \qquad (I)$$

**In Vitro Assays.** All in vitro assays were carried out twice independently in duplicate.

***Plasmodium falciparum.*** In vitro activity against the erythrocytic stages of *P. falciparum* was determined by using a $^3$H-hypoxanthine incorporation assay[23,24] using the chloroquine and pyrimethamine resistant K1 strain and the standard drugs chloroquine (Sigma C6628) and artemisinin (Arteannuin, Qinghaosu; Sigma 36,159-3). Compounds were dissolved in DMSO at 10 mg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100U/mL), Albumax (5 g/L), and washed human red cells $A^+$ at 2.5% haematocrit (0.3% parasitaemia). Serial doubling dilutions of each drug were prepared in 96-well microtiter plates and incubated in a humidified atmosphere at 37 °C; 4% $CO_2$, 3% $O_2$, 93% $N_2$.

After 48 h, 50 $\mu$L of $^3$H-hypoxanthine (= 0.5 $\mu$Ci) was added to each well of the plate. The plates were incubated for an additional 24 h under the same conditions. The plates were then harvested with a Betaplate cell harvester (Wallac, Zurich, Switzerland), and the red blood cells were transferred onto a glass fiber filter and then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid and were counted in a Betaplate liquid scintillation counter (Wallac, Zurich, Switzerland). $IC_{50}$ values were calculated from sigmoidal inhibition curves using Microsoft Excel.

**Cytotoxicity.** Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine at a density of $4 \times 10^4$ cells/mL. After 24 h, the medium was removed and replaced by fresh medium containing a serial drug dilution, and the plate was incubated at 37 °C under a 5% $CO_2$ atmosphere for 72 h. Alamar Blue (10 $\mu$L) (Trinova, Giessen, Germany) was then added to each well and incubation continued for a further 2−4 h. The plates were read in a microplate fluorescence scanner (Spectramax Gemini XS by Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. From the sigmoidal inhibition curve, $IC_{50}$ values were calculated.

**Molecular Modeling. Molecular Docking Studies.** The ligands under study were built employing the SKETCH module of SYBYL6.9 and minimized using Tripos Force Field. Docking studies were carried out using the FlexX program[19] interfaced with SYBYL 6.9. FlexX is a fast-automated program based on incremental construction procedure. In this method the flexibility of the ligands is considered by including several conformations of ligands while maintaining a rigid structure for the biomolecule. All the molecules were docked into the active sites of the *P. falciparum* dUTPase. The 3D coordinates of the active sites were taken from the X-ray crystal structures of the *P. falciparum* dUTPase reported as complexes with the inhibitor **1d**, deposited in the Brookhaven Protein Databank (PDB codes: 1VYQ). Molecules of water were deleted. While creating the receptor description file (rdf), the active site was defined as the area within 6.5 Å around the cocrystallized ligand. Formal charges were assigned to all the molecules and FlexX run was submitted.

For most compounds the top rank-score was selected as a result of the docking. For a few compounds (**2g**, **3a**, **4a**,**g**,**i**, **5b**, **6b**, and **7a**) a lower rank-score was chosen, as this represented a better mimic the conformation of the crystal structure in these cases.

**Preliminary ADME Studies. Metabolic Stability in Human Liver Microsomes. Materials.** NADPH was purchased from Sigma Aldrich, Sweden AB. Pooled human liver microsomes were purchased from In Vitro Technologies, Inc..

**Metabolic Incubation.** Incubation mixture consisted of liver microsomes (0.5 mg microsomal protein/ml) substrate, (2 $\mu$M), and NADPH (1 mM) in a total volume of 0.3 mL of phosphate buffer (100 mM, pH 7.4). The reaction was initiated by the addition of NADPH, and the sample was incubated at 37 °C. At time points 0, 10, 20, and 30 min, aliquots (50 $\mu$L) were removed and added to 150 $\mu$L of acetonitrile in order terminate the reaction. Following mixing and centrifugation the supernatant was analyzed by LC-MS.

**Calculation of in Intrinsic Clearance, Clint.** The natural logarithm (residual concentration) versus time (min) is plotted. The slope of the line gives the elimination rate constant = *k*. The elimination half-life $T_{1/2} = -0.693/k$. Clint = $(0.693/T_{1/2}) \times$ mL incubation/mg microsomes.

**Stability in Simulated Gastric Fluid. Materials**. The Simulated Gastric Fluid consisted of 0.2% sodium chloride, 0.32% pepsin, and 0.7% hydrochloric acid. The pH of the final solution was 1.2 (USP 23).

**Incubation.** The compound was incubated in 0.5 mL of the gastric fluid at a final concentration of 10 $\mu$M. At time points; 0, 0.2, 5, 15, 30, 60, 150 min, aliquots (50 $\mu$l) were removed and mixed with 50 $\mu$L of phosphate buffer, (pH 7.4) and 0.2 mL of acetonitrile. The sample was centrifuged and the supernatant analyzed by LC−UV (254 nm).

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**Supporting Information Available:** Experimental procedures and analytical data for all the compounds not included in the Experimental Section, a table of elemental analysis data obtained for selected compounds, a table with the docking scores of FlexX and a NOESY spectrum of compound **4b**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) http://www.who.int/topics/malaria/en/.
(2) http://www.who.int/tdr/publications/tdrnews/news60/mmv.htm.
(3) Nguyen, C.; Kanisathan, G.; Leal-Cortijo, I.; Musso-Buendia, A.; Kaiser, M.; Brun, R.; Ruiz-Pérez, M.; Johansson, N.-G.; González-Pacanowska, D.; Gilbert, I. H. Deoxyuridine Triphosphate Nucleotidohydrolase as a Potential Antiparasitic Drug Target. *J. Med. Chem.* **2005**, *48*, 5942−5954.
(4) El-Hajj, H. H.; Zhang, H.; Weis, B. Lethality of a dut (deoxyuridine triphosphatase) Mutation in *Escherichia Coli*. *J. Bacteriol*. **1988**, *170*, 1069−1075.
(5) Gadsden, M. H.; McIntosh, E. M.; Game, J. C.; Wilson, P. J.; Haynes, R. H. dUTP Pyrophosphatase is an Essential Enzyme in *Saccharomyces cerevisiae*. *EMBO J.* **1993**, *12*, 4425−4431.
(6) Hidalgo-Zarco, F.; González-Pacanowska, D. Trypanosomatid dUTPases as Potential Targets for Drug Design. *Curr. Protein Pept. Sci*. **2001**, *2*, 389−397.
(7) Mol, C. D.; Harris, J. M.; McIntosh, E. M.; Tainer, J. A. Humand dUTP pyrophosphatase: uracil recognition by a $\beta$ hairpin and active sites formed by three separate subunits. *Structure* **1996**, *4*, 1077−1092.
(8) Persson, R.; Cedergren-Zeppezauer, E. S.; Wilson, K. S. Homotrimeric dUTPases; Structural solutions for specific recognition and hydrolysis of dUTP. *Curr. Protein Pept. Sci.* **2001**, *2*, 287−300.
(9) Whittingham, J. L.; Leal, I.; Kasinathan, G.; Nguyen, C.; Bell, E.; Berry, C.; Benito, A.; Turkenburg, J.; Dodson, E. J.; Ruiz-Pérez, L. M.; Wilkinson, A. J.; Johansson, N. G.; Brun, R.; Gilbert, I. H.; González-Pacanowska, D.; Wilson, K. Novel Inhibitors of *Plasmodium falciparum* dUTPase Provide a Platform for Anti-Malarial Drug Design. *Structure* **2005**, *13*, 329−338.
(10) Zalud, P.; Wachs, W. O.; Nyman, P. O.; Zeppezauer, M. Inhibition of the Proliferation of Human Cancer Cells in vitro by Substrate Analogues Inhibitors of dUTPase. *Adv. Exp. Med. Biol.* **1995**, *370*, 135−138.
(11) Persson, T.; Larsson, G.; Nyman, P. O. Synthesis of 2′-Deoxyuridine 5′($\alpha$,$\beta$-imido)triphosphate, a Substrate Analogue and Potent Inhibitor of dUTPase. *Bioorg. Med. Biochem.* **1996**, *4*, 553−556.
(12) Hernández, A.-I.; Balzarini, J.; Karlsson, A.; Camarasa, M.-J.; Pérez-Pérez, M.-J. Acyclic Nucleoside Analogues as Novel Inhibitors of Human Mitochondrial Thymidine Kinase. *J. Med. Chem.* **2002**, *45*, 4254−4263.
(13) Frieden, M.; Giraud, M.; Reese, C. B.; Song, Q. Synthesis of 1-[cis-3-(Hydroxymethyl)cyclobutyl]-uracil, -Thymine and -Cytosine. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2827−2832.
(14) Maki, T.; Iwasaki, F.; Matsumura, Y. A new convenient method for selective monobenzoylation of diols. *Tetrahedron Lett.* **1998**, *39*, 5601−5604.
(15) McDonald, W. S.; Verbicky, C. A.; Zercher, C. K. Two-Directional Synthesis of Polycyclopropanes. An Approach to the Quinquecyclopropane Fragment of U-106305. *J. Org. Chem.* **1997**, *12*, 11215−1222.
(16) Showell, G. A.; Mills, J. S. Chemistry Challenges in Lead Optimization: Silicon Isosteres in Drug Discovery. *Drug Discov. Today*. **2003**, *8*, 551−556.
(17) Bains, W.; Tacke, R. Silicon Chemistry as a Novel Source of Chemical Diversity in Drug Design. *Curr. Opin. Drug Discovery Dev.* **2003**, *6*, 526−543.
(18) Rarey, M.; Krame, B.; Lengauer, Y.; Klebe, G. A Fast Flexible Docking Method using an Incremental Construction Algorithm. *J. Mol. Biol*. **1996**, *261*, 470−489.
(19) Sybyl 6.9, Tripos Inc., 1699 South Hanley Road, St Louis, MO 63144.
(20) Properties considered included cellular permeability (Caco-2 cells test), metabolic stability (incubation with human liver microsomes), and possible effect of CYP3A4 (substrate properties and inhibition of this important cytochrome P450 enzyme gives an early warning of metabolism, toxicity, and potential interactions with coadministered drugs). N. G. Johansson (Medivir AB), personal communication.
(21) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3−25.
(22) Hidalgo-Zarco, F.; Camacho, A. G.; Bernier-Villamor, V.; Nord, J.; Ruiz-Perez, L. M.; Gonzalez-Pacanowska, D. Kinetic properties and inhibition of the dimeric dUTPase-dUDPase from Leishmania major. *Protein Sci*. **2001**, *10*, 1426−1433.
(23) Desjardins, R. E.; Canfield, C. J.; Haynes, D.; Chulay, J. D. Quantitative Assessment of Antimalarial Activity in Vitro by a Semiautomated Microdilution Technique. *Antimicrob. Agents Chemother*. **1979**, *16*, 710−718.
(24) Matile, H. Pink, J. R. L. *Plasmodium falciparum* Malaria Parasite Cultures and their Use in Immunology. In *Immunological Methods*; Academic Press: San Diego, 1990; pp 221−234.

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