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Use of Pd-catalyzed Suzuki–Miyaura coupling reaction in the rapid synthesis of 5-aryl-6-(phosphonomethoxy)uracils and evaluation of their inhibitory effect towards human thymidine phosphorylase

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ABSTRACT

A number of new 5-aryl substituted pyrimidine acyclic nucleoside phosphonates were synthesized and tested for their ability to inhibit human TP. Their rapid synthesis using Pd-catalyzed Suzuki–Miyaura coupling reactions of various arylboronic acids with 5-bromo-4-(phosphonomethoxy)-2,6-dibutoxypyrimidine was successfully applied. For a series of 5-aryl-6-phosphonomethoxyuracils, an increased inhibitory effect was determined. This effect is supported by the results found for 4-fluorophenyl (K_i^{dThd}=4.89±0.62) and 3-nitrophenyl (K_i^{dThd}=3.98±0.46) substituents.

1. Introduction

Acyclic nucleoside phosphonates (ANPs) are known as compounds possessing various kinds of biological activities.[1] Currently, the biological effects for pyrimidine ANP derivatives could be also extended in context of their potential inhibitory potency towards thymidine phosphorylase (TP).[2,3] This enzyme catalyzes reversible phosphorolysis of 2′-deoxythymidine (dThd) to thymine and 2-deoxy-D-ribose 1-phosphate,[4] which is dephosphorylated to 2-deoxy-D-ribose. Regarding the identical principle of TP to platelet-derived endothelial cell growth factor (PD-ECGF), a potential inhibition of this enzyme may be important in tumour angiogenesis.[5] For ANPs designed as 'multisubstrate inhibitors' of TP with various interfered pyrimidine bases and phosphonoalkyl groups at thymine and phosphate-binding sites[2] there are several advantages compared to most single-substrate inhibitors. These compounds are catabolically stable and flexible in structure and there could be more points for interactions with enzyme through their functionalized acyclic linkage. Therefore, ANP inhibitors may find utility as efficient suppressors of tumour growth[5c] in future.

In our study, we have focused on the development of synthetic methods for the rapid preparation of novel ANPs, which were further tested for their ability to inhibit human TP. Recently, we published a series of ANPs, such as 1-[2-(phosphonomethoxy)ethyl]thymine (PMET), 1-[3-hydroxy-2-(phosphonomethoxy)propyl]thymine (HPMPT) and 1-[3-fluoro-2-(phosphonomethoxy)propyl]thymine (FPMPT) and many others,[6–9] which possess a considerable inhibitory effect newly tested on TP isolated from SD-lymphoma.[6] Unfortunately, these compounds showed a marginal activity on human enzymes expressed in V79 hamster cells as well as the enzyme isolated from human placenta. It seems the differences in recognition of active sites of both enzymes are probably appreciable and these findings may therefore exclude the utilization of TP from SD-lymphoma as a model enzyme.

In contrast, it is known that various 5-alkyl and 5-aryl-6-halogeno substituted uracils[10] as single-substrate inhibitors demonstrate a considerable impact on the inhibitory activity towards human TP probably due to their potential hydrophobic interaction of alkyl and aryl substituents directed by the halogen electron-withdrawing effect (Fig. 1a).

On the other hand, [5-chloro-6-(2-iminopyrrolidin-1-yl)methyl-2,4-(1*H*,3*H*)-pyrimidine] (TPI)[11] represents the most efficient inhibitor towards required TP (Fig. 1b). However, its interaction with active site of enzyme is probably energetically favoured through the

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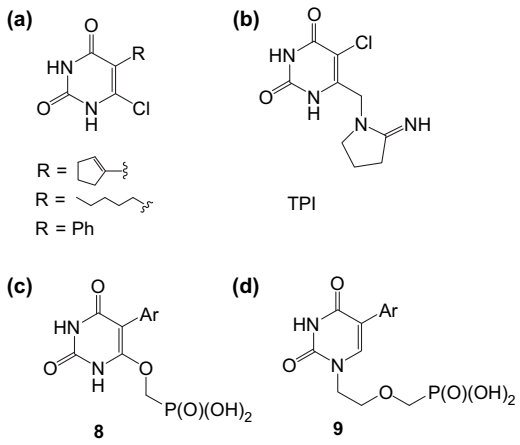
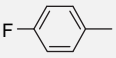
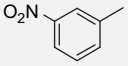
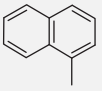
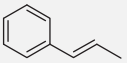


Figure 1. Pyrimidine derivatives as investigated inhibitors of human TP.

solvatation of its positively charged imino group with phosphate and/or H_2O located near the phosphate binding site. Thus activated TPI may be similar to the thymidine cleavage.[12] From this point of view, a mechanism of inhibitor binding with TP may be explained by several ways depending on their structure.[2,12] Based on this proposal, our strategy was to mimic a potential solvatation of TPI in conjunction with hydrophobic effect expected in those of 5-alkyl and 5-aryl-6-chlorouracils. That means we tried to introduce a catabolically stable phosphonomethoxy group to C-6-position and various aryl groups bearing electron-withdrawing substituents, heteroatoms or other conjugated moieties to the position C-5 of pyrimidine base such as phenyl, naphth-1-yl, (*E*)-2-(phenyl)vinyl, 4-fluorophenyl, 3-nitrophenyl, 3-furyl, 3-thienyl, pyridin-3-yl and -4-yl. Thus we have started the synthesis of 5-aryl-6-(phosphonomethoxy)uracils **8a–i** as a new class of ANPs aimed at positive influence of inhibitory effect towards human TP (Fig. 1c; Table 1).

Table 1
Suzuki-coupling reaction and deprotection for 5-aryl-C-6-subtituted uracil ANPs

Entry	R (Ar)[b]	Solvent	Reaction time (h)	Yield[a] of **7** (%)	Yield of **8** (%)
a		Toluene	23	45	33
b		Toluene (DMF)	12 (7)	—	—
c		DMF	6	77	23
d		DMF	6	72	53
e		DMF	4	66	68
f		DMF	4.5	32	61
g		DMF	4	78	29
h		DMF	5.5	43	57
i		DMF	6	25	—
j	H	—	—	—	58

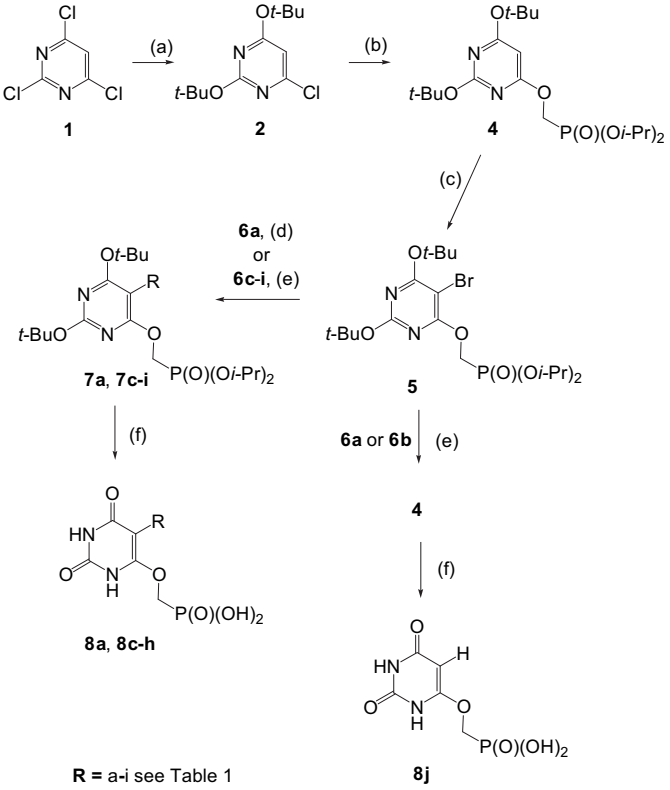
[a] Conditions: **5** (1.0 equiv), **6** (2 equiv), Pd catalyst (0.1 equiv), base (3.3–4.7 equiv of Na_2CO_3), 130 °C; solvent/H_2O 1:6 (entries **a** and **b**) and 8:1 (entries **c–i**).

[b] Aryl subtituents of ANPs **9a–i** correspond to entries **a–i**. The yields of these compounds are mentioned in Ref. 15.

2. Results and discussion

For the efficient arylation method of uracil moiety in some pyrimidine ANPs we utilized Suzuki–Miyaura cross-coupling,[13,14] which have generally been used widely due to their low-toxicity. As the arylation of pyrimidine ANPs has not been studied in detail via this process[14] we decided to introduce various aryl and heteroaryl groups to pyrimidine ANP derivatives using this synthetic method.

As indicated in our preliminary communication,[15] we have successfully employed the Suzuki–Miyaura cross-coupling for the preparation of N^1-substituted 5-arylpyrimidine ANPs **9a–i** (Fig. 1d; Table 1). We have extended this work by studying the arylation of a 5-bromo uracil derivative **5** (Scheme 1). In addition, we have introduced various functionalized aryl groups.



Scheme 1. Reagents and conditions: (a) *t*-BuONa (2 equiv), THF, 0 °C→reflux, 45%; (b) NaH, $HOCH_2P(O)(Oi\text{-}Pr)_2$ (**3**), THF, 0 °C→rt, 39%; (c) NBS, AIBN, THF, 60 °C, 99%; (d) $Pd(PPh_3)_4$ (0.10 equiv), Na_2CO_3, toluene, H_2O, 130 °C, 45%; (e) $Pd(PPh_3)_4$ (0.10 equiv), Na_2CO_3, DMF, H_2O, 130 °C, 43–78%; (f) $(CH_3)_3SiBr$, CH_3CN, rt, 0 °C, 23–68%.

First we prepared the 5-bromo derivative **5** by a three-step synthesis from commercially available 2,4,6-trichloropyrimidine **1**. In this case, we selectively protected **1** by reaction with 2 equiv of sodium *tert*-butoxide to give chloro derivative **2**. In a further reaction step, we chose hydroxymethylphosphonate **3** as a simple phosphonate building block with the assumption that the final structure of **8** could largely mimic a leading structure of TPI in interaction with additional phosphate or water, respectively.[12] The reaction of chloro derivative **2** with **3** converted quantitatively in the presence of sodium hydride into phosphonate **4** (monitoring by TLC). In contrast to a previous method of bromination,[15] we left a deprotection of methoxy groups in uracil moiety regarding to possible difficulty with unfavourable cleavage of phosphonomethoxy substituents. Instead,