Synthesis, modeling and evaluation of 3′-(1-aryl-1*H*-tetrazol-5-ylamino)-substituted 3′-deoxythymidine derivatives as potent and selective human mitochondrial thymidine kinase inhibitors†

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Received 17th August 2010, Accepted 14th October 2010
DOI: 10.1039/c0ob00591f

Based on the presumed binding mode of an earlier identified inhibitor, we herein report new 3′-modified nucleosides as potent and selective inhibitors of mitochondrial thymidine kinase (TK2). A series of thirteen 3′-amino-, 3′-guanidino- and 3′-tetrazole-containing nucleosides were synthesized and evaluated for their TK2 inhibitory activity. Within the tetrazole series, compounds with nanomolar inhibitory activity were identified. A homology model of TK2 allowed to elucidate the observed activities. Introduction of a 2-bromovinyl group on C-5 of the pyrimidine base of the most promising 3′-derivative further improved the inhibitory activity, and caused a significant increase in the selectivity for TK2 *versus* TK1. Interestingly, for the current series of analogues, a strong correlation was observed between TK2 and *Drosophila melanogaster* dNK inhibition, further substantiating the phylogenetic relationship between these two nucleoside kinases.

Introduction

Deoxyribonucleoside kinases (dNKs) catalyze the first phosphoryl transfer from a suitable donor (in general ATP) to the 5′-OH of a 2′-deoxynucleoside. Mammalian cells contain four different dNKs: thymidine (dThd) kinase 1 (TK1), thymidine kinase 2 (TK2), deoxycytidine kinase (dCK) and deoxyguanosine kinase (dGK).[1] In resting cells TK2 is the principal, or maybe the only, active thymidine phosphorylating enzyme, and it has been shown that it plays a pivotal role in the mitochondrial salvage pathway in which nucleotides are provided for mtDNA synthesis.[1,2,3] Since long-term treatment with antiviral nucleoside analogues such as AZT and FIAU is known to induce severe mitochondrial toxicity, it is assumed that TK2 is involved in the toxicity of these nucleoside analogues.[4,5,6,7] In this respect, potent and selective inhibitors of TK2 could constitute valuable tools to unravel a variety of metabolic processes, including the role of TK2 in the maintenance of the mitochondrial dNTP pools, and they may also help to clarify the contribution of TK2 activity/inhibition to the mitochondrial toxicity of certain antiviral agents.

In an effort to optimize the TK2 inhibitory potency of a 3′-arylthiourea derivative of thymidine (**1**, Chart 1),[8] we recently found that a 1,4-disubstituted 1,2,3-triazole motif constitutes an attractive bioisostere for the thiourea linker as demonstrated by the high inhibitory activity of a series of 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine analogues (typified by **2**, Chart 1).[9]

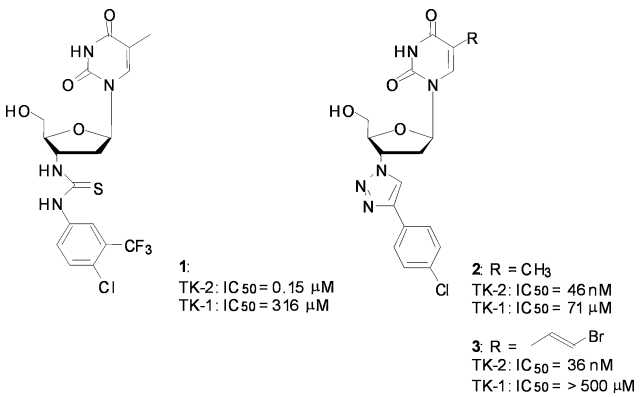

Chart 1 Known 3′-modified substrate-based inhibitors of TK-2.

Introduction of a 2-bromovinyl group (compound **3**, Chart 1), known to be a privileged substituent on C-5 of the pyrimidine base part for TK2 binding, only slightly improved the inhibitory activity, but caused a significant increase in the selectivity for TK2 *versus* TK1 (Chart 1).

In the present study, we further investigate the structure–activity relationships for TK2 inhibition through the synthesis of a series of novel thymidine and BVDU analogues containing a substituted amine or guanidine group instead of a thiourea at the 3′ position or a 1,5-disubstituted tetrazole linker. Modeling studies on the binding of **1** to human TK2 suggested that the nitrogen atoms of the thiourea urea group of **1** could interact favorably with the oxygens of the γ-phosphate of the co-substrate ATP[8] whereas for **2** a binding mode involving a water-mediated interaction with the side- chain of the central glutamate in the EEE lid region of the enzyme was proposed.[9] This novel array of hydrogen-bonding

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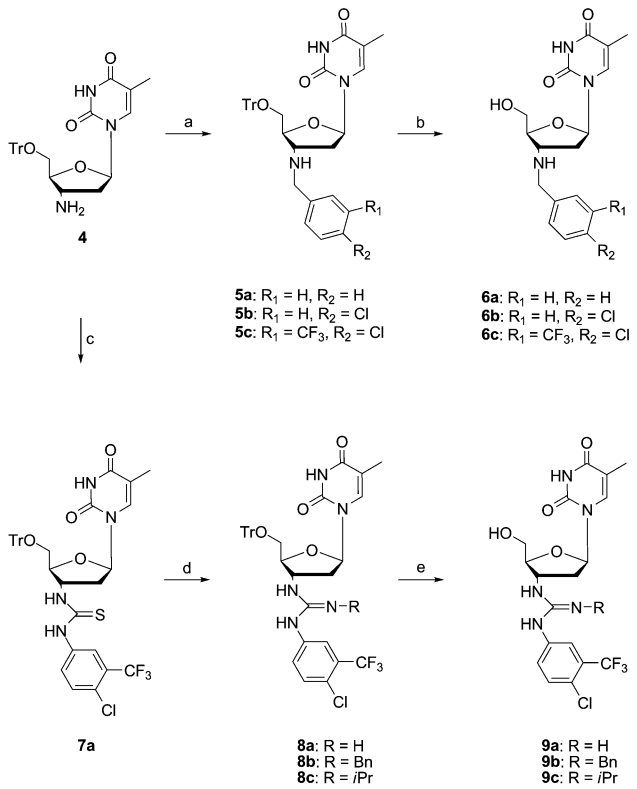
† Electronic supplementary information (ESI) available: Multiple sequence alignment and numbering of human TK-2 and related kinases Dm-dNK and HSV-1 TK, selected ^{1}H and ^{13}C NMR spectra and proof of purity (HPLC traces) of the final compounds. See DOI: 10.1039/c0ob00591f

functionalities at the 3′ position has helped us to identify those that are optimal for TK2 inhibition.

Results and Discussion

Chemistry

A small series of 3′-benzylamino analogues of thymidine (**6a–c**) was synthesized by treatment of amine **4**[10] with different aldehydes followed by *in situ* reduction of the resulting imines with $NaBH_4$ and final detritylation (Scheme 1).



Scheme 1 Synthesis of 3′-benzylamino and 3′-guanidine analogues of thymidine. *Reagents and conditions*: a) i) suitable benzaldehyde, dry MeOH, rt, 24 h; ii) $NaBH_4$, rt, 30 min, 16-88%; b) $ZnBr_2$, CH_2Cl_2/^{i}PrOH 85 : 15, rt, overnight, 34–58%; c) 4-chloro-3-(trifluoromethyl)-phenylisothiocyanate, DMF, 0 °C → rt, 3 h, 98%; d) suitable amine or 7 N NH_3, Et_3N, $HgCl_2$, DMF, 0 °C → rt, 20 h, 56–57%; e) HCOOH/Et_2O 7 : 3, rt, 1 h, 64–71%.

The guanidine analogues **9a–c** were synthesized by an $HgCl_2$-promoted guanylation[11] of the 3′-thiourea precursor **7a**,[12] which was prepared from the 3′-amino analogue **4**, followed by final detritylation (Scheme 1).

Interestingly, upon replacement of the amine by sodium azide in the mercury(II)-promoted guanylation reaction, thiourea **7a** was converted to the 5-aminotetrazole **10a** by spontaneous electrocyclization of the intermediate guanyl azide (Scheme 2).[11] The observed selectivity of the electrocyclization step is in accordance with earlier results, leading to the regioisomer in which the tetrazole is conjugated with the phenyl ring.[13]

Two typical methods were explored for the removal of the trityl protecting group of **10a**. Surprisingly, treatment with $ZnBr_2$ resulted in a 9 : 1-mixture of both tetrazole isomers **12a** and **13a**, which were separated using preparative HPLC. In our view, $ZnBr_2$ may promote the observed isomerization by first catalyzing opening of the tetrazole ring and subsequently acting as a catalyst in a non-specific ring closure.

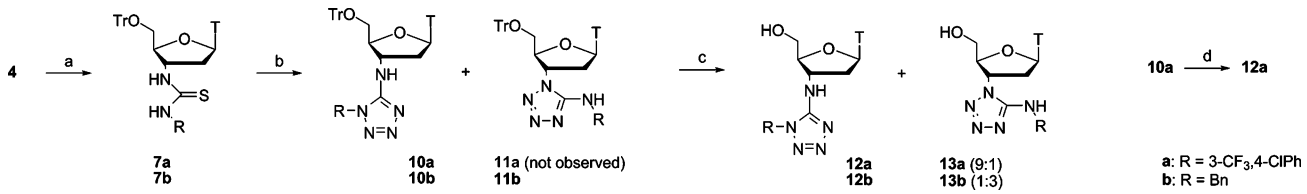
This isomerization could be prevented by performing the deprotection with formic acid in ether. In the case of the benzylthiourea analogue **7b**, competitive cyclization of the guanyl azide afforded a 1 : 2.2-mixture of the tetrazoles **10b** and **11b**. This isomer ratio was only slightly affected after further trityl deprotection with $ZnBr_2$ (1 : 2.6-mixture of isomers **12b** and **13b**). Identification of each isomer was based on the multiplicity of 3′-NH and 5″-NH (*e.g.*, **12b**: 3′-NH, d, J = 6.9 Hz; **13b**: 5″-NH, t, J = 5.7 Hz).

Application of an identical reaction sequence used for **7a** on the BVDU thiourea analogue **16** afforded **18** and **19** (3 : 1) in moderate yields (Scheme 3).

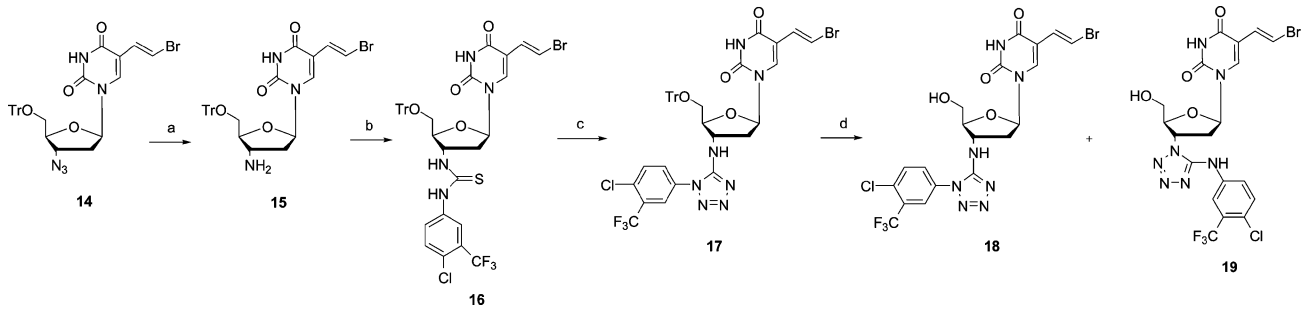
Biological Evaluation

All compounds were evaluated for their ability to inhibit dThd phosphorylation by recombinant purified human cytosolic TK1, human mitochondrial TK2, herpes simplex virus type 1 (HSV-1) TK, varicella-zoster virus (VZV) TK, and *Drosophila melanogaster* Dm-dNK (Table 1). The 3′-benzylamino analogues demonstrated modest inhibitory activity on TK2. This activity was influenced by the nature of the substituents at the phenyl ring. Derivatives containing either the unsubstituted (**6a**) or a 4-chloro-substituted (**6b**) phenyl gave micromolar inhibition, while the analogue possessing a 3-CF_3-4-Cl-substituted phenyl (**6c**) displayed significantly improved TK2 inhibitory activity. In addition, all the 3′-aminobenzyl derivatives showed poor inhibitory activity against TK1 (IC_{50} values, 442 to ≥ 500 μM).

dThd phosphorylation by TK2 was also inhibited by the substituted guanidine analogues (compounds **9a–c**), which yielded IC_{50} values ranging from 0.43 to 1.3 μM. The anti-TK2 activity was influenced by the nature of the third substituent on the guanidine moiety. The "unsubstituted" analogue **9a** and the ^{i}Pr-substituted analogue **9c** afforded high potency and excellent selectivity over TK1 (Table 1).



Scheme 2 Conversion of 3′-thiourea group to aminotetrazole moiety. *Reagents and conditions*: a) appropriate isothiocyanate, DMF, 0 °C → rt, 3 h, 98–99%; b) NaN_3, DMF, Et_3N, $HgCl_2$, 0 °C → rt, 6 h, 31% (**10a**), 62% (**10b/11b**); c) $ZnBr_2$, CH_2Cl_2/^{i}PrOH (85 : 15), rt, overnight, 32% (**12a/13a**), 54% (**12b/13b**); d) HCOOH/Et_2O 7 : 3, rt, 1 h, 45%.



Scheme 3 Synthesis of 3′-modified BVDU derivatives. *Reagents and conditions*: a) PPh_3, THF, H_2O, rt, overnight, 71%; b) 4-chloro-3-(trifluoromethyl)-phenylisothiocyanate, DMF, 0 °C → rt, 3 h, 82%; c) NaN_3, DMF, Et_3N, $HgCl_2$, 0 °C → rt, 6 h, 75%; d) $ZnBr_2$, CH_2Cl_2/^{i}PrOH (85 : 15), rt, overnight, 33%.

Table 1 Inhibitory effects of thymidine and BVDU analogues on 1 μM [CH_3-^{3}H]dThd phosphorylation by deoxyribonucleoside kinases from different origins

	IC_{50}[a]/μM				
Compd	TK1	TK2	HSV-1 TK	VZV TK	Dm-dNK
6a	> 500	4.6 ± 0.3	> 500	99 ± 74	48 ± 3
6b	≥ 500	3.3 ± 0.2	> 500	35 ± 5	41 ±4
6c	442 ± 37	0.33 ± 0.04	492 ± 12	28 ± 1	4.8 ± 0.2
9a	355 ± 69	0.43 ± 0.02	> 500	41 ± 3	11 ± 6
9b	468 ± 12	1.3 ± 0.5	372 ±39	41 ± 1	7.7 ± 4.1
9c	≥ 500	0.58 ± 0.29	> 500	277 ± 35	12 ± 4
12a	382 ± 52	**0.035 ± 0.007**	316 ± 39	63 ± 26	1.2 ± 0.0
12b	> 500	0.90 ±0.01	> 500	144 ± 42	10 ± 6
13a	29 ±1	0.59 ± 0.19	40 ± 0	5.5 ± 2.3	3.6 ± 0.7
13b	222 ± 112	2.6 ± 1.2	39 ± 1	3.2 ± 0.5	31 ± 4
18	> 500	**0.014 ± 0.001**	34 ± 3	26 ± 3	0.41 ± 0.04
19	> 500	0.40 ± 0.04	26 ± 4	6.1 ± 1.6	3.6 ± 0.2

[a] IC_{50} is the 50% inhibitory concentration of the test compounds, which was measured as the concentration required to inhibit 1 μM [CH_3-^{3}H]dThd phosphorylation by 50%. Data are the mean of at least 2 to 3 independent experiments (± S.D.).

In the 1,5-aminotetrazole series, the anti-TK2 activity was clearly influenced by a) the type of isomer, b) the substituent at position N-1 of the tetrazole ring, and c) the substituent on position 5 of the base. In all cases, the isomer in which the tetrazole ring is attached to C-3′ *via* a NH bridge (**12a**, **12b** and **18**), showed the best inhibitory activity against TK2 (IC_{50}s in the 0.014–0.90 μM range). The benzyl substituted analogue **12b** gave submicromolar inhibition, while introduction of an electron-withdrawing 3-CF_3-4-Cl phenyl substituent significantly improved the inhibitory activity (**12a**), which matches with that of the earlier described 3′-(4-(3,4-dichlorophenyl)-1,2,3-triazol-1-yl) analogue. Combination of this favorable 3′-modification with a 5′-*O*-trityl substituent led, as expected,[9] to an analogue (**10a**) that was completely devoid of TK2 inhibitory activity (IC_{50}s > 500 μM for all kinases tested). On the other hand, introduction of an additional 5-(2-bromovinyl) group (**18**), known to be a privileged substituent for TK2 recognition, proved to be compatible with the 3′-modification (IC_{50} TK2: 0.014 μM). This 5-(2-bromovinyl) substituent improved the TK2 inhibitory activity by 2- to 3-fold, and it further caused an increase in the selectivity for TK2 *versus* TK1, resulting in the complete lack of inhibitory effect against TK1-catalyzed dThd phosphorylation. A similar effect had previously been observed in the triazole series.[9] To the best of our knowledge, compound **18** is the most potent and selective inhibitor of TK2 reported to date. The isomeric aminotetrazole analogues in which the similar substituted tetrazole group is connected directly to the sugar ring were markedly less inhibitory to TK2 activity: **19** showed an IC_{50} value of 0.40 μM, that is, at least 25-fold higher than the IC_{50} value observed for **18** (Table 1). Interestingly, a close relationship for the inhibitory concentration of the test compounds was observed between TK2 and Dm-dNK (r = 0.951) but not between TK2 and HSV-1 TK (r = –0.229) (Table 1, Fig. 1).

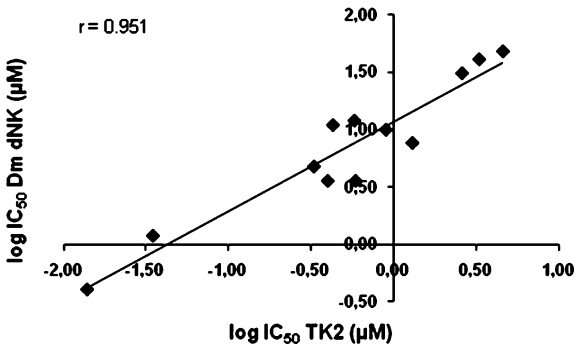


Fig. 1 Correlation between the IC_{50} values of the inhibitors shown in Table 1 against TK2 and Dm-dNK.

A prediction of the phylogenetic relation between the nucleoside kinases suggested a closer relationship between TK2 and Dm-dNK, than between TK2 and HSV-1 TK.[14] An amino acid sequence alignment of TK2 with Dm-dNK and HSV-1 TK showed that TK2 and Dm-dNK have around 55% sequence homology, which is only around 20% for the TK2 and HSV-1 TK (Fig. S1). In fact, TK2 is of similar length as Dm-dNK, whereas HSV-1 TK is longer due to an extended loop structure as also noticed by Johansson *et al.*[14] The ATP binding glycine-rich loop located in the N-terminal domain of the enzymes (25 EGNIAGGKTT 34) is highly conserved in TK2 and the other enzymes, although the amino acid composition of this site is closer between TK2 and Dm-dNK than between TK2 and HSV-1 TK.

Molecular Modeling

Our previously reported homology-built model of ATP-bound TK2 and the same methodology[8,9] were used to provide a

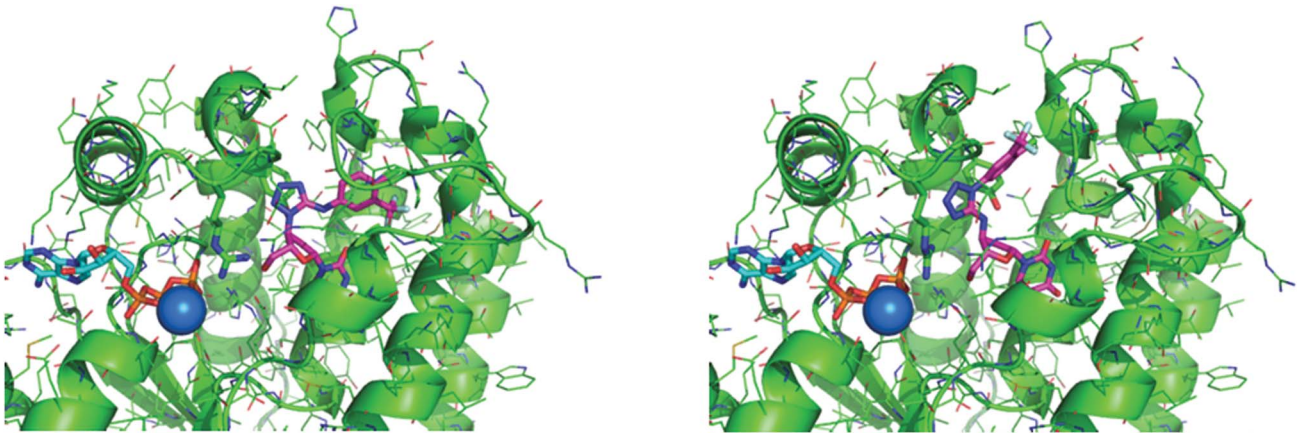


Fig. 2 Detail of the substrate binding site in the refined complexes of **12a** (left) and **13a** (right) with ATP-bound human TK2. Inhibitor, protein and ATP carbon atoms are colored magenta, green and cyan, respectively. The blue sphere represents the catalytic Mg^{2+} ion. The side chains of Arg167 and Tyr177 are shown as sticks. Amino acid numbering according to the alignments depicted in Fig. 1S (ESI†).

rationale for the present findings in atomic detail. The closed loop conformation of the enzyme is better stabilized by those inhibitors having the amino group bridging the tetrazole ring to the sugar moiety (*i.e.* **12**, **18**) relative to those having the amino group linking the tetrazole to the substituted phenyl ring (*i.e.* **6**). In the case of **12a**, the tetrazole moiety is proposed to establish a non-water-mediated interaction with the guanidinium of Arg167 whereas the amino group can establish a hydrogen bond with the hydroxyl group of Tyr177 (Fig. 2). Interestingly, whereas Arg167 in TK2 is positionally equivalent to Arg169 in Dm dNK and Arg222 in HSV-1 TK, the structural equivalent of Tyr177 in TK2 is also a tyrosine (Tyr179) in Dm dNK but a methionine (Met231) in HSV-1 TK. This latter amino acid cannot establish a hydrogen bond with the amino group of **12a** and this may contribute to the fact that these compounds are much better inhibitors against TK2 and Dm dNK than against HSV-1 TK.

Conclusions

Our ongoing efforts to identify potent and selective inhibitors of mitochondrial thymidine kinase 2 (TK2), led us to synthesize new 3′-modified thymidines related to the recently discovered 3′-[4-aryl-(1,2,3-triazol-1-yl)]-3′-deoxythymidine analogues. Replacement of the triazole linkage by a basic amine or guanidine, as possible interaction partners of the γ-phosphate group of the ATP co-substrate, generally led to a drop in inhibitory activity.

Interestingly, treatment of a 3′-arylthiourea-substituted 5′-*O*-tritylated thymidine precursor with $HgCl_2$ and sodium azide exclusively afforded the *N*-(3′-deoxythymidin-3′-yl)-1-substituted-1*H*-tetrazol-5-arylamine with an amino group between C-3′ of the deoxyribose and the tetrazole, while the same reaction performed on a 3′-benzylthiourea congener mainly gave rise to the regioisomer with the tetrazole ring directly connected to the sugar.

In the resulting 1,5-aminotetrazole series, the isomers in which the tetrazole ring is attached to C-3′ *via* a NH bridge exhibit the best anti-TK2 activity, with a 3-CF_3-4-Cl phenyl substituent at position N-1 of the tetrazole ring affording the best TK2-inhibition profile. Further introduction of a 5-(2-bromovinyl) substituent gave derivative **18**, which emerged as the most potent and selective TK2 inhibitor reported so far.

A TK2 homology model allowed to assess the binding mode of both tetrazole isomers. In the case of regioisomer **12a**, modeling studies indicated a contributing role of the tetrazole ring and the amino bridge to the overall affinity for TK2.

Experimental

All reagents were from standard commercial sources and of analytic grade. Precoated Merck silica gel F254 plates were used for TLC, spots were examined under ultraviolet light at 254 nm and further visualized by sulfuric acid-anisaldehyde spray. Column chromatography was performed on silica gel (63–200 μm, 60 Å, Biosolve, Valkenswaard, The Netherlands). NMR spectra were determined using a Varian Mercury 300 MHz and a Bruker 700 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to the residual solvent peak: in the case of DMSO-d_6, it is 2.54 ppm for ^{1}H and 40.5 ppm for ^{13}C; in the case of $CDCl_3$, it is 7.26 ppm for ^{1}H and 77.4 ppm for ^{13}C. Structural assignment was confirmed with COSY and DEPT. All signals assigned to hydroxyl groups were exchangeable with D_2O. Exact mass measurements were performed on a Waters LCT Premier XETM Time of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a CH_3CN–water (1 : 1) mixture at 10 μL min^{-1}.

General procedure for the synthesis of substituted 3′-benzylamino-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidine analogues 5a–c

To a suspension of amine **4** (1.0 equiv.) in dry MeOH was added a suitable benzaldehyde (1.05 equiv.). After 24 h stirring at room temperature, $NaBH_4$ (1.5 equiv.) was added and 30 min later the mixture was quenched with 1 M NaOH. The reaction mixture was extracted with CH_2Cl_2 and the organic layer washed with brine and dried over $MgSO_4$, filtered and evaporated to dryness. The residue was purified by column chromatography affording 3′-benzylamino analogues **5a–c** in moderate yield.

3′-Benzylamino-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidine 5a

Reaction of compound **4** (88 mg, 0.18 mmol), benzaldehyde (17 μL, 0.17 mmol) in 0.7 mL MeOH was performed as described in the general synthesis of 3′-benzylamino-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidine analogues affording **5a** as a colourless solid (17 mg, 16%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (3H, d, J = 1.2 Hz, 5-CH_3), 2.29 (2H, app t, J = 6.0 Hz, H-2′a and H-2′b), 3.33–3.47 (2H, m, H-5′a and H-5′b), 3.57 (1H, app dd, J = 6.3 Hz, J = 12.3 Hz, H-3′), 3.74 (2H, app q, J = 13.2 Hz, CH_2Ph), 3.91–3.93 (1H, m, H-4′), 6.26 (1H, app t, J = 6.0 Hz, H-1′), 7.20–7.40 (20H, m, Tr and CH_2Ph), 7.52 (1H, d, J = 0.9 Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.10 (5-CH_3), 39.60 (C-2′), 52.29 (CH_2Ph), 57.53 (C-3′), 63.80 (C-5′), 84.68 (C-1′), 85.11 (C-4′), 87.45 (Tr), 110.96 (C-5), 127.42, 127.51, 128.15, 128.71, 128.81 (CH_2Ph and Tr), 135.70 (C-6), 139.60 (CH_2Ph), 143.50 (Tr), 150.29 (C-2), 163.77 (C-4). HRMS (ESI-MS) for $C_{36}H_{36}N_3O_4$ $[M+H]^+$ found, 574.2770; calcd, 574.2700.

3′-(4-Chlorobenzylamino)-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidine 5b

Reaction of compound **4** (82 mg, 0.17 mmol), 4-chlorobenzaldehyde (23 mg, 0.16 mmol) in 0.6 mL MeOH was performed as described in the general synthesis of 3′-benzylamino-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidine analogues affording **5b** as a colourless solid (91 mg, 88%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.43 (3H, d, J = 0.9 Hz, 5-CH_3), 2.13–2.27 (2H, m, H-2′a and H-2′b), 3.16-3.24 (2H, m, H-5′a and H-5′b), 3.39 (1H, app dt, J = 5.7 Hz, H-3′), 3.67 (2H, app q, J = 14.1 Hz, CH_2Ph), 3.87–3.91 (1H, m, H-4′), 6.19 (1H, app t, J = 6.3 Hz, H-1′), 7.24–7.37 (19H, m, Tr and CH_2Ph), 7.51 (1H, d, J = 0.9 Hz, H-6), 11.32 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.75 (5-CH_3), 37.44 (C-2′), 49.98 (CH_2Ph), 56.83 (C-3′), 64.04 (C-5′), 83.26 (C-1′), 83.91 (C-4′), 86.30 (Tr), 109.38 (C-5), 127.14, 127.93, 128.01, 128.24, 129.70, 131.06 (Tr and CH_2Ph), 135.66 (C-6), 139.60 (CH_2Ph), 143.48 (Tr), 150.34 (C-2), 163.67 (C-4). HRMS (ESI-MS) for $C_{36}H_{35}ClN_3O_4$ $[M+H]^+$ found, 608.2357; calcd, 608.2311.

3′-[(3-Trifluoromethyl-4-chloro)-benzylamino]-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidine 5c

Reaction of compound **4** (108 mg, 0.22 mmol), 4-chloro-3-(trifluoromethyl)benzaldehyde (51 mg, 0.24 mmol) in 0.8 mL MeOH was performed as described in the general synthesis of 3′-benzylamino-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidine analogues affording **5c** as a colourless solid (103 mg, 68%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.44 (3H, d, J = 0.9 Hz, 5-CH_3), 2.18-2.26 (2H, m, H-2′a and H-2′b), 3.19 (2H, d, J = 3.6 Hz, H-5′a and H-5′b), 3.30–3.38 (1H, m, H-3′), 3.74 (2H, app q, J = 14.1 Hz, CH_2Ph), 3.88–3.89 (1H, m, H-4′), 6.18 (1H, app t, J = 6.6 Hz, H-1′), 7.24–7.34 (15H, m, Tr), 7.50 (1H, d, J = 1.5 Hz, H-6), 7.55–7.63 (2H, m, CH_2Ph), 7.76 (1H, d, J = 1.5 Hz, CH_2Ph), 11.31 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.79 (5-CH_3), 37.29 (C-2′), 49.49 (CH_2Ph), 57.09 (C-3′), 59.74 (C-5′), 83.22 (C-1′), 83.87 (C-4′), 86.28 (Tr), 109.38 (C-5), 121.15, 124.77, 126.93–128.22, 128.63 (Tr and CH_2Ph), 131.28 (CH_2Ph), 133.47 (CH_2Ph), 135.70 (C-6), 140.99 (CH_2Ph), 143.49 (Tr), 150.34 (C-2), 163.67 (C-4). HRMS (ESI-MS) for $C_{37}H_{34}ClF_3N_3O_4$ $[M+H]^+$ found, 676.2194; calcd, 676.2184.

3′-Benzylamino-3′-deoxy-β-D-thymidine 6a

Compound **5a** (94 mg, 0.16 mmol) was dissolved in a mixture of $ZnBr_2$ (592 mg, 2.63 mmol) in CH_2Cl_2/^{i}PrOH (2.6 mL, 85 : 15) and stirred overnight at room temperature. The reaction was quenched with water and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to dryness. The resulting residue was purified by column chromatography (CH_2Cl_2–MeOH 95 : 5), to yield **6a** (18.2 mg, 34%) as a colourless oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.77 (3H, m, 5-CH_3), 1.99–2.14 (2H, m, H-2′a and H-2′b), 3.27–3.33 (1H, m, H-3′), 3.53–3.71 (4H, m, H-5′a, H-5′b and CH_2Ph), 3.76–3.80 (1H, m, H-4′), 4.10 (1H, app br s, 3′-NH), 5.01 (1H, br s, 5′-OH), 6.15 (1H, app t, J = 6.3 Hz, H-1′), 7.19–7.32 (5H, m, CH_2Ph), 7.74 (1H, d, J = 1.2 Hz, H-6), 11.25 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.27 (5-CH_3), 37.55 (C-2′), 51.10 (CH_2Ph), 57.39 (C-3′), 61.75 (C-5′), 83.96 (C-1′), 85.32 (C-4′), 109.14 (C-5), 126.56, 127.96 and 128.09 (CH_2Ph), 136.25 (C-6), 140.78 (CH_2Ph), 150.42 (C-2), 163.77 (C-4). HRMS (ESI-MS) for $C_{17}H_{22}N_3O_4$ $[M+H]^+$ found, 332.1613; calcd, 332.1605.

3′-(4-Chlorobenzylamino)-3′-deoxy-β-D-thymidine 6b

Compound **6b** (14 mg, 56%, colourless solid) was synthesized from **5b** (40 mg, 0.066 mmol) using the same procedure as was described for the synthesis of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.76 (3H, d, J = 1.0 Hz, 5-CH_3), 2.04-2.10 (2H, m, H-2′a and H-2′b), 3.24–3.40 (1H, m, H-3′), 3.51–3.66 (2H, m, H-5′a and H-5′b), 3.69 (2H, d, J = 3.3 Hz, CH_2Ph), 3.75–3.79 (1H, m, H-4′), 5.02 (1H, br s, 5′-OH), 6.14 (1H, app t, J = 6.5 Hz, H-1′), 7.37 (4H, app s, CH_2Ph), 7.73 (1H, d, J = 1.3 Hz, H-6), 11.25 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.35 (5-CH_3), 37.58 (C-2′), 50.30 (CH_2Ph), 57.33 (C-3′), 61.77 (C-5′), 84.03 (C-1′), 85.35 (C-4′), 109.27 (C-5), 128.12, 129.88 and 131.12 (CH_2Ph), 136.35 (C-6), 139.95 (CH_2Ph), 150.51 (C-2), 163.90 (C-4). HRMS (ESI-MS) for $C_{17}H_{21}ClN_3O_4$ $[M+H]^+$ found, 366.1215; calcd, 366.1215.

3′-[(3-Trifluoromethyl-4-chloro)-benzylamino]-3′-deoxy-β-D-thymidine 6c

Compound **6c** (80 mg, 58%, colourless solid) was synthesized from **5c** (97 mg, 0.14 mmol) using the same procedure as was described for the synthesis of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.77 (3H, d, J = 1.2 Hz, 5-CH_3), 2.05–2.12 (2H, m, H-2′a and H-2′b), 3.25–3.33 (1H, m, H-3′), 3.52–3.67 (2H, m, H-5′a, H-5′b), 3.73–3.84 (3H, m, H-4′ and CH_2Ph), 4.07 (1H, app br s, 3′-NH), 5.01 (1H, br s, 5′-OH), 6.15 (1H, app t, J = 6.3 Hz, H-1′), 7.67 (2H, app s, CH_2Ph), 7.73 (1H, d, J = 0.9 Hz, H-6), 7.84 (1H, app s, CH_2Ph), 11.25 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.25 (5-CH_3), 37.43 (C-2′), 49.63 (CH_2Ph), 57.24 (C-3′), 61.62 (C-5′), 83.88 (C-1′), 85.22 (C-4′), 109.15 (C-5), 124.83, 126.06–128.57, 131.30 and 133.59 (CF_3 and CH_2Ph), 136.21 (C-6), 141.23 (CH_2Ph), 150.41 (C-2), 163.76 (C-4). HRMS (ESI-MS) for $C_{18}H_{20}ClF_3N_3O_4$ $[M+H]^+$ found, 434.1114; calcd, 434.1089.

N-(3′-Deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′-yl)-*N*′-(4-chloro-3-trifluoromethylphenyl)-thiourea 7a

A solution of 4-chloro-3-(trifluoromethyl)-phenylisothiocyanate (1.0 mL; 6.38 mmol) in 58 mL DMF was added to a suspension of compound **4** (2.36 g, 4.88 mmol) in 44 mL DMF at 0 °C under N_2 atmosphere. The reaction mixture was stirred at room temperature. After 3 h, solvents were evaporated to dryness and the residue was purified by column chromatography (CH_2Cl_2–MeOH 97 : 3) affording **7a** (3.44 g) in 98% yield as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.46 (3H, d, J = 0.9 Hz, 5-CH_3), 2.26-2.34 (1H, m, H-2′a), 2.48–2.58 (1H, m, H-2′b), 3.18 (2H, d, J = 4.8 Hz, H-5′a and H-5′b), 4.07–4.12 (1H, m, H-4′), 5.21 (1H, app br s, H-3′), 6.28 (1H, app t, J = 6.6 Hz, H-1′), 7.24–7.44 (15H, m, Tr), 7.60–7.71 (3H, m, subs Ph), 8.03 (1H, app s, H-6), 8.62 (1H, d, J = 6.3 Hz, 3′-NH), 9.84 (1H, s, N′H), 11.37 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.76 (5-CH_3), 35.77 (C-2′), 48.60 (C-3′), 64.00 (C-5′), 82.99 (C-1′), 83.63 (C-4′), 86.52 (Tr), 109.67 (C-5), 121.50, 124.50, 124.71, 126.09, 126.50, 126.91, 127.17, 127.97, 128.12, 128.31, 131.68, 135.55 (CF_3, subs Ph and Tr), 139.03 (C-6), 143.44 (Tr), 150.38 (C-2), 162.29 (C-4), 180.63 (C═S). HRMS (ESI-MS) for $C_{37}H_{32}ClF_3N_4NaO_4S$ $[M+Na]^+$ found, 743.1675; calcd, 743.1677.

General procedure for the synthesis of N-(3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′-yl)-*N*′-(4-chloro-3-trifluoromethylphenyl)-guanidine analogues 8a–c

To a solution of thiourea **7a** (1.0 equiv.) in dry DMF were added the appropriate amine (1.0 equiv.), Et_3N (2.0 equiv.) and $HgCl_2$ (1.0 equiv.) at 0 °C under N_2 atmosphere. The resulting black reaction mixture was stirred for several hours at room temperature or until TLC indicated complete consumption of starting material. The suspension was filtered through a pad of Celite, washing with CH_2Cl_2. The filtrate was diluted with water and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH_2Cl_2–MeOH 90 : 10 or 80 : 20) to give the appropriate guanidine in moderate yield.

N-(3′-Deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′-yl)-*N*′-(4-chloro-3-trifluoromethylphenyl)-guanidine 8a

Reaction of compound **7a** (232 mg, 0.32 mmol) with 10 mL 7 N NH_3 yielded compound **8a** (128 mg, 57%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.50 (3H, app s, 5-CH_3), 2.29-2.34 (1H, m, H-2′a), 2.49-2.52 (1H, m, H-2′b), 3.26-3.39 (2H, m, H-5′a and H-5′b), 4.00-4.02 (1H, m, H-4′), 4.64-4.66 (1H, m, H-3′), 6.21 (1H, app t, J = 5.7 Hz, H-1′), 7.23-7.61 (19H, m, Tr, subs Ph and H-6), 11.34 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.87 (5-CH_3), 37.24 (C-2′), 50.90 (C-3′), 62.94 (C-5′), 82.65 (C-1′), 83.35 (C-4′), 86.39 (Tr), 109.50 (C-5), 122.39, 124.59, 125.95, 126.73, 127.05, 127.16, 127.90, 127.97, 128.29, 132.21 (CF_3, subs Ph and Tr), 135.79 (C-6), 143.46 (Tr), 150.37 (C-2), 153.18 (C═N), 163.74 (C-4). HRMS (ESI-MS) for $C_{37}H_{34}ClF_3N_5O_4$ $[M+H]^+$ found, 704.2241; calcd, 704.2246.

N-(3′-Deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′-yl)-*N*′-(4-chloro-3-trifluoromethylphenyl)-benzylguanidine 8b

Reaction of compound **7a** (87 mg, 0.12 mmol) with benzylamine (13 µL, 0.12 mmol) afforded compound **8b** (54 mg, 56%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.43 (3H, d, J = 0.9 Hz, 5-CH_3), 2.20-2.28 (1H, m, H-2′a), 2.31-2.41 (1H, m, H-2′b), 3.23-3.32 (2H, m, H-5′a and H-5′b), 3.92-3.95 (1H, m, H-4′), 4.26 (2H, d, J = 5.7 Hz, CH_2Ph), 4.60–4.63 (1H, m, H-3′), 6.13–6.26 (3H, m, H-1′ and CH_2Ph), 6.73 (1H, d, J = 8.4 Hz, 3′-NH), 6.91 (1H, s, N′H), 7.19–7.41 (21H, m, Tr, CH_2Ph, subs Ph), 7.54 (1H, d, J = 0.9 Hz, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.81 (5-CH_3), 37.32 (C-2′), 44.48 (CH_2Ph), 54.91 (C-3′), 63.42 (C-5′), 83.46 (C-1′), 86.26 (C-4′ and Tr), 109.31 (C-5), 119.81, 126.63, 127.02, 127.06, 127.89, 128.11, 128.26, 131.62 (CF_3, subs Ph, Tr and CH_2Ph), 135.73 (C-6), 140.03 (CH_2Ph), 143.56 (Tr), 151.33 (C-2), 151.49 (C═N), 163.70 (C-4). HRMS (ESI-MS) for $C_{44}H_{40}ClF_3N_5O_4$ $[M+H]^+$ found, 794.2727; calcd, 794.2715.

N-(3′-Deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′-yl)-*N*′-(4-chloro-3-trifluoromethylphenyl)-isopropylguanidine 8c

Reaction of compound **7a** (232 mg, 0.32 mmol) with isopropylamine (27 µL, 0.32 mmol) afforded compound **8c** (128 mg, 57%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97–1.06 (6H, m, ^{i}Pr), 1.42 (3H, app s, 5-CH_3), 2.26–2.35 (2H, m, H-2′a and H-2′b), 3.24–3.35 (2H, m, H-5′a and H-5′b), 3.66 (1H, app sex, J = 6.9 Hz, ^{i}Pr), 3.95–3.96 (1H, m, H-4′), 4.64 (1H, app d, J = 5.4 Hz, H-3′), 5.29 (1H, d, J = 5.4 Hz, 3′-NH), 6.08 (1H, br s, N′H), 6.20 (1H, app t, J = 6.3 Hz, H-1′), 6.76 (1H, d, J = 8.7 Hz, subs Ph), 6.94 (1H, s, subs Ph), 7.21–7.42 (16H, m, Tr and subs Ph), 7.56 (1H, d, J = 1.2 Hz, H-6), 11.32 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.74 (5-CH_3), 22.65 (^{i}Pr), 37.20 (C-2′), 42.73 (^{i}Pr), 50.48 (C-3′), 63.51 (C-5′), 83.48 (C-1′), 83.75 (C-4′), 86.27 (Tr), 109.34 (C-5), 119.34, 121.30, 124.92, 126.42, 126.81, 127.07, 127.42, 127.87, 128.27, 128.54, 131.63 (CF_3, subs Ph and Tr), 135.72 (C-6), 143.54 (Tr), 150.33 (C-2), 151.32 (C═N), 163.68 (C-4). HRMS (ESI-MS) for $C_{40}H_{40}ClF_3N_5O_4$ $[M+H]^+$ found, 746.2717; calcd, 746.2715.

N-(3′-Deoxy-β-D-thymidin-3′-yl)-*N*′-(4-chloro-3-trifluoromethylphenyl)-guanidine 9a

A solution of compound **8a** (108 mg, 0.15 mmol) in 3.0 mL HCOOH/Et_2O 7 : 3 (v:v) reacted for 45 min at room temperature. The reaction was quenched with EtOAc and water, washed with sat. $NaHCO_3$ and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over $MgSO_4$ and the solvent evaporated. The residue was purified by column chromatography (CH_2Cl_2–MeOH 80 : 20) to yield compound **9a** (50 mg, 71%) as a yellow-brown solid. Treatment with a 4 N HCl solution in 1,4-dioxane gave the appropriate salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.78 (3H, d, J = 0.9 Hz, 5-CH_3), 2.16–2.36 (2H, m, H-2′a and H-2′b), 3.59–3.72 (2H, m, H-5′a and H-5′b), 3.81-3.84 (1H, m, H-4′), 4.29 (1H, app dd, J = 6.0 Hz, J = 12.9 Hz, H-3′), 5.76 (1H, br s, 5′-OH), 6.16 (1H, app t, J = 6.3 Hz, H-1′), 7.12 (1H, d, J = 7.5 Hz, subs Ph), 7.25 (1H, s, subs Ph), 7.50 (1H, d, J = 8.4 Hz, subs Ph), 7.75 (1H, d, J = 1.2 Hz, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.29 (5-CH_3), 37.33 (C-2′), 50.93 (C-3′), 61.17 (C-5′), 83.34 (C-1′), 85.37 (C-4′), 109.26 (C-5), 126.65, 127.06, 128.26 and 131.97 (subs Ph),

136.08 (C-6), 150.42 (C-2), 163.76 (C-4). HRMS (ESI-MS) for $C_{18}H_{20}ClF_3N_5O_4$ $[M+H]^+$ found, 462.1132; calcd, 462.1150.

N-(3′-Deoxy-β-D-thymidin-3′-yl)-*N*′-(4-chloro-3-trifluoromethylphenyl)-benzylguanidine 9b

Compound **9b** (15 mg, 68%) was synthesized from compound **8b** (33 mg, 0.041 mmol) using the same procedure as described for the synthesis of **9a**. Treatment with a 4 N HCl solution in 1,4-dioxane gave the appropriate salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.76 (3H, d, J = 0.6 Hz, 5-CH_3), 2.23 (2H, app t, J = 6.9 Hz, H-2′a and H-2′b), 3.51-3.3.57 (1H, m, H-5′a), 3.62–3.67 (1H, m, H-5′b), 3.77-3.81 (1H, m, H-4′), 4.27–4.32 (3H, m, H-3′ and CH_2Ph), 6.14 (1H, app t, J = 6.3 Hz, H-1′), 6.35 (1H, br s, 5′-OH), 6.93 (1H, app dd, J = 2.4 Hz, J = 8.7 Hz, subs Ph), 7.02–7.03 (1H, m, subs Ph), 7.20–7.35 (5H, m, CH_2Ph), 7.41 (1H, d, J = 8.7 Hz, subs Ph), 7.73 (1H, d, J = 1.2 Hz, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.27 (5-CH_3), 37.32 (C-2′), 44.59 (CH_2Ph), 51.01 (C-3′), 60.98 (C-5′), 83.32 (C-1′), 85.17 (C-4′), 109.18 (C-5), 120.07, 121.28, 121.52, 121.60, 124.90, 126.56, 126.70, 126.96, 127.13, 127.86, 128.18, 131.82, (CF_3, subs Ph and CH_2Ph), 136.18 (C-6), 140.01 (CH_2Ph), 150.40 (C-2), 152.17 (C═N), 163.77 (C-4). HRMS (ESI-MS) for $C_{25}H_{26}ClF_3N_5O_4$ $[M+H]^+$ found, 552.1620; calcd, 552.1620.

N-(3′-deoxy-β-D-thymidin-3′-yl)-*N*′-(4-chloro-3-trifluoromethylphenyl)-isopropylguanidine 9c

Compound **9c** (54 mg, 0.11 mmol, 64%) was synthesized from compound **8c** (123.7 mg, 0.17 mmol) using the same procedure as described for the synthesis of **9a**. Treatment with a 4 N HCl solution in 1,4-dioxane gave the appropriate salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.07-1.09 (6H, m, ^{i}Pr), 1.77 (3H, d, J = 1.2 Hz, 5-CH_3), 2.28 (2H, app t, J = 7.2 Hz, H-2′a and H-2′b), 3.56-3.84 (4H, m, H-4′, H-5′a, H-5′b and ^{i}Pr), 4.32 (1H, app dd, J = 6.6 Hz, J = 12.3 Hz, H-3′), 5.90 (1H, br s, 5′-OH), 6.18 (1H, app t, J = 6.6 Hz, H-1′), 7.06 (1H, app dd, J = 2.4 Hz, J = 8.7 Hz, subs Ph), 7.19 (1H, d, J = 2.1 Hz, subs Ph), 7.47 (1H, d, J = 8.7 Hz, subs Ph), 7.71 (1H, d, J = 1.2 Hz, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.25 (5-CH_3), 22.59 (^{i}Pr), 36.96 (C-2′), 43.23 (^{i}Pr), 51.64 (C-3′), 61.14 (C-5′), 83.30 (C-1′), 85.08 (C-4′), 109.36 (C-5), 120.86, 121.21, 121.41, 124.83, 126.64, 127.05, 127.57, 131.97 (CF_3 and subs Ph), 136.13 (C-6), 150.42 (C-2), 152.10 (C═N), 163.74 (C-4). HRMS (ESI-MS) for $C_{21}H_{26}ClF_3N_5O_4$ $[M+H]^+$ found, 504.1644; calcd, 504.1620.

General procedure for the synthesis of substituted 1,5-aminotetrazole analogues

To a suspension of a suitable thiourea (1.0 equiv.), sodium azide (3.0 equiv.) and mercuric chloride (1.1 equiv.) in dry DMF was added triethylamine (3.0 equiv.) under N_2 atmosphere. The resulting suspension was stirred for several hours at room temperature or until TLC indicated complete consumption of starting material. The suspension was filtered through a pad of Celite, washing with CH_2Cl_2. The filtrate was diluted with water and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (EtOAc–hexane 95 : 5 or CH_2Cl_2–MeOH 98 : 2) to give the appropriate tetrazole.

5-(3′-Amino-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′*N*-yl)-1-(4-chloro-3-trifluoromethylphenyl)-tetrazole 10

Reaction of compound **7a** (189 mg, 0.26 mmol), NaN_3 (51 mg, 0.79 mmol), $HgCl_2$ (78 mg, 0.29 mmol) and Et_3N (105 µL, 0.76 mmol) yielded compound **10a** (58 mg, 31%) as a yellow-brown solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.51 (3H, app s, 5-CH_3), 2.33-2.42 (1H, m, H-2′a), 2.46–2.53 (1H, m, H-2′b), 3.27–3.33 (1H, m, H-5′a), 3.41–3.46 (1H, m, H-5′b), 4.09–4.13 (1H, m, H-4′), 4.55–4.60 (1H, m, H-3′), 6.31 (1H, app t, J = 6.6 Hz, H-1′), 7.23–7.42 (15H, m, Tr), 7.50 (1H, d, J = 7.2 Hz, 3′-NH), 7.60 (1H, d, J = 1.2 Hz, H-6), 7.90–8.05 (3H, subs Ph), 11.36 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.82 (5-CH_3), 36.68 (C-2′), 59.74 (C-3′), 64.09 (C-5′), 82.60 (C-1′), 83.70 (C-4′), 86.44 (Tr), 109.73 (C-5), 120.36, 123.99, 124.96, 125.03, 127.17–127.67, 127.95–128.27, 128.51, 130.86, 131.90, 132.21, 133.21 (CF_3, subs Ph and Tr), 135.83 (C-6), 143.49 (Tr), 150.39 (C-2), 154.54 (C═N), 163.66 (C-4). HRMS (ESI-MS) for $C_{37}H_{31}ClF_3N_7O_4Na$ $[M+Na]^+$ found, 752.006; calcd, 752.1970.

5-(3′-Amino-3′-deoxy-β-D-thymidin-3′*N*-yl)-1-(4-chloro-3-trifluoromethylphenyl)-tetrazole 12a

A solution of compound **10a** (34 mg, 0.047 mmol) in 1.0 mL HCOOH/Et_2O 7 : 3 (v:v) reacted for 45 min. EtOAc and water were added to the mixture which was then washed with saturated $NaHCO_3$ and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over $MgSO_4$ and the solvent evaporated. The residue was purified by column chromatography (CH_2Cl_2–MeOH 95 : 5) to yield compound **12a** (10.0 mg, 0.021 mmol, 45%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.80 (3H, app s, 5-CH_3), 2.29–2.34 (2H, m, H-2′a and H-2′b), 3.65–3.73 (2H, m, H-5′a and H-5′b), 3.97–4.01 (1H, m, H-4′), 4.35–4.41 (1H, m, H-3′), 5.19 (1H, br s, 5′-OH), 6.25 (1H, app t, J = 6.9 Hz, H-1′), 7.81 (1H, d, J = 0.9 Hz, H-6), 7.96–8.03 (2H, m, subs Ph), 8.17 (1H, s, subs Ph). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.30 (5-CH_3), 37.05 (C-2′), 54.46 (C-3′), 61.51 (C-5′), 83.63 (C-1′), 84.66 (C-4′), 109.45 (C-5), 124.06-133.14 (subs Ph), 136.18 (C-6), 150.49 (C-2), 154.84 (C═N), 163.79 (C-4). HRMS (ESI-MS) for $C_{18}H_{18}ClF_3N_7O_4$ $[M+H]^+$ found, 488.1070; calcd, 488.1055.

5-(3′-Amino-3′-deoxy-β-D-thymidin-3′*N*-yl)-1-(4-chloro-3-trifluoromethylphenyl)-tetrazole 12a and 5-[amino-(4-chloro-3-trifluoromethylphenyl)]-1-(3′-deoxy-β-D-thymidin-3′*N*-yl)-tetrazole 13a

Compound **10a** (80 mg, 0.11 mmol) was dissolved in a mixture of $ZnBr_2$ (394 mg, 1.75 mmol) in CH_2Cl_2/^{i}PrOH (1.8 mL, 85 : 15) and stirred overnight at room temperature. The reaction was quenched with water and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to dryness. The resulting residue was purified using RP-HPLC (Phenomenex Luna C-18, 250 × 21.10 mm 5 µm, H_2O–CH_3CN, 90 : 10 → 0 : 100 in 29 min, flow: 17.5 mL min^{-1}), affording isomers **12a** and **13a** as white powders (17 mg, 32%, 9 : 1). **13a**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.83 (3H, app s, 5-CH_3), 2.50–2.2.62 (2H, m, H-2′a and H-2′b), 3.65–3.76 (3H, m, H-4′, H-5′a and H-5′b), 4.25 (1H, app s, H-3′), 5.43 (1H, br s, 5′-OH), 6.46 (1H, app t, J = 6.6 Hz, H-1′), 7.55–7.58 (1H, d, J = 8.1 Hz, subs Ph), 7.80–7.96 (2H, m, subs Ph), 8.15 (1H, app s,

H-6). ^{13}C NMR (175 MHz, DMSO-d_6): δ 12.73 (5-CH_3), 61.68 (C-5′), 84.32 and 84.54 (C-1′ and C-4′), 110.20 (C-5), 127.97–134.08 (subs Ph), 136.59 (C-6), 150.94 (C-2), 164.19 (C-4). HRMS (ESI-MS) for $C_{18}H_{18}ClF_3N_7O_4$ [M+H]$^+$ found, 488.1063; calcd, 488.1055.

N-(3′-Deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′-yl)-*N*′-benzylthiourea 7b

Reaction of compound **4** (364 mg, 0.75 mmol) with benzylisothiocyanate (114 mg, 0.75 mmol) was performed as described for the synthesis of compound **7a** affording compound **7b** in a 99% yield (471 mg, white foam). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.42 (3H, app s, 5-CH_3), 2.18-2.26 (1H, m, H-2′a), 2.45–2.55 (1H, m, H-2′b), 3.18–3.22 (1H, m, H-5′a), 3.38–3.43 (1H, m, H-5′b), 4.03 (1H, app s, H-4′), 4.68 (2H, app s, CH_2Ph), 5.10 (1H, app br s, H-3′), 6.21 (1H, app t, J = 6.6 Hz, H-1′), 7.21–7.42 (20H, m, Tr and CH_2Ph), 7.55 (1H, app s, 6-H), 7.80–7.92 (1H, m, 3′-NH), 8.02 (1H, m, N′H), 11.35 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.79 (5-CH_3), 30.80 (CH_2Ph), 35.81 (C-2′), 47.04 (C-3′), 63.76 (C-5′), 83.63 (C-1′ and C-4′), 86.51 (Tr), 109.55 (C-5), 126.86, 127.17, 127.23, 128.01, 128.27, 128.32 (Tr and CH_2Ph), 135.51 (CH_2Ph), 139.13 (C-6′), 143.47 (Tr), 150.38 (C-2), 162.34 (C-4). Exact mass (ESI-MS) for $C_{37}H_{37}N_4O_4S$ [M + H]$^+$ found, 633.2505; calcd. 633,2530.

5-(3′-Amino-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′*N*-yl)-1-benzyl-tetrazole 10b and 5-aminobenzyl-(3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′*N*-yl)-tetrazole 11b

Reaction of compound **7b** (207 mg, 0.33 mmol), NaN_3 (64 mg, 0.98 mmol), $HgCl_2$ (98 mg, 0.36 mmol) and Et_3N (136 µL, 0.98 mmol) in 1.3 mL DMF was performed as described in the general synthesis of 1-substituted-5-(3′-amino-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′-yl)-tetrazole analogues. A mixture of compounds **10b** and **11b** was obtained as white solid (108 mg, 52%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (isomer 1: 5-CH_3, app s), 1.49 (isomer 2: 5-CH_3, app s), 2.40–2.50 (isomer 1: H-2′a and H-2′b, m), 2.79–2.88 (isomer 2: H-2′a and H-2′b, m), 3.34-3.45 (isomer 2: H-5′a and H-5′b, m), 3.48-3.54 (isomer 1: H-5′a and H-5′b, m), 4.07–4.10 (isomer 1: H-4′, m), 4.33–4.47 (isomer 2: CH_2Ph, m), 4.51–4.56 (isomer 2: H-4′, m), 4.69–4.71 (isomer 1: H-3′, m), 5.07–5.15 (isomer 2: H-3′, m), 5.32 (isomer 1: CH_2Ph, s), 6.09 (isomer 2: H-1′, app t, J = 5.4 Hz), 6.18 (isomer 1: H-1′ & isomer 2: N′H, m), 6.57 (isomer 1: 3′-NH, d, J = 7.2 Hz), 7.07–7.56 (20H, m, Tr, CH_2Ph & 6-H), 10.15 (isomer 1: 3-NH, s), 10.27 (isomer 2: 3-NH, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.72 and 12.13 (5-CH_3), 37.66 (C-2′), 48.15 and 48.72 (CH_2Ph), 54.31 and 55.24 (C-3′), 62.59 (C-5′), 82.25 (C-1′), 84.17 (C-4′), 87.48 and 87.69 (Tr), 110.69 and 111.54 (C-5), 127.36–128.90 (Tr), 133.91 (CH_2Ph), 135.75 and 136.72 (C-6), 137.86 (CH_2Ph), 143.15 and 143.34 (Tr), 150.70 and 151.04 (C-2), 154.92 and 155.81 (C═N), 164.04 (C-4). Exact mass (ESI-MS) for $C_{37}H_{36}N_7O_4$ [M + H]$^+$ found, 642.2827; calcd. 642,2823.

5-(3′-Amino-3′-deoxy-β-D-thymidin-3′*N*-yl)-1-benzyl-tetrazole 12b and 5-aminobenzyl-(3′-deoxy-β-D-thymidin-3′*N*-yl)-tetrazole 13b

Reaction of a mixture of compounds **10b** and **11b** (199 mg, 0.31 mmol) under the same conditions as described for the synthesis of **12a** and **13a** and purification on a RP-HPLC (Phenomenex Luna C-18, H_2O/0.1% HCOOH in CH_3CN, 90 : 10 → 0 : 100 in 29 min, flow: 17.5 mL min^{-1}) afforded compounds **12b** and **13b** as white powders (17 mg, 54%, 1 : 3). **12b**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.79 (3H, d, J = 0.9 Hz, app s, 5-CH_3), 2.22–2.40 (2H, m, H-2′a and H-2′b), 3.62–3.72 (2H, m, H-5′a and H-5′b), 3.97 (1H, app dt, J = 3,3 Hz, H-4′), 4.30–4.36 (1H, m, H-3′), 5.19 (1H, br s, 5′-OH), 5.43 (2H, s, CH_2Ph), 6.29 (1H, t, J = 6,9 Hz, H-1′), 7.22–7.25 (2H, m, CH_2Ph), 7.30–7.41 (3H, m, CH_2Ph), 7.51 (1H, d, J = 6,9 Hz, 3′-NH), 7,81 (1H, d, J = 0,9 Hz, 6-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.30 (5-CH_3), 37.08 (C-2′), 47.66 (CH_2Ph), 54.26 (C-3′), 61.56 (C-5′), 83.62 (C-1′), 84.89 (C-4′), 109.48 (C-5), 127.42, 127.97, 128.77 and 135.30 (CH_2Ph), 136.09 (C-6), 150.53 (C-2), 154.95 (C═N), 163.82 (C-4). Exact mass (ESI-MS) for $C_{18}H_{22}N_7O_4$ [M + H]$^+$ found, 400.1738; calcd. 400,1728. **13b**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.81 (3H, d, J = 0.9 Hz, app s, 5-CH_3), 2.52–2.60 (2H, m, H-2′a and H-2′b), 3.68 (2H, app dq, J = 3.0 Hz, J = 11.9 Hz, H-5′a and H-5′b), 4.32 (1H, app q, J = 3.6 Hz, H-4′), 4.51 (2H, d, J = 5.7 Hz, CH_2Ph), 5.10–5.16 (1H, m, H-3′), 5.39 (1H, br s, 5′-OH), 6.39 (1H, t, J = 6.6 Hz, H-1′), 7.23–7.40 (5H, m, CH_2Ph), 7.60 (1H, t, J = 5.7 Hz, 5′-NH), 7,81 (1H, d, J = 1.5 Hz, 6-NH), 11.35 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.36 (5-CH_3), 36.66 (C-2′), 46.87 (CH_2Ph), 55.04 (C-3′), 60.90 (C-5′), 83.60 (C-4′), 84.03 (C-1′), 109.51 (C-5), 127.08, 127.40 and 128.32 (CH_2Ph), 136.05 (C-6), 138.92 (CH_2Ph), 150.45 (C-2), 155.40 (C═N), 163.76 (C-4). Exact mass (ESI-MS) for $C_{18}H_{22}N_7O_4$ [M + H]$^+$ found, 400.1709; calcd. 400.1728.

3′-Amino-3′-deoxy-β-(*E*)-5-(2-bromovinyl)-2′-deoxyuridine 15

Water (40 µL) and triphenylphosphine (318 mg, 1.21 mmol) were added to a solution of 3′-azido-3′-deoxy-β-(*E*)-5-(2-bromovinyl)-2′-deoxyuridine (**14**)[9] (154 mg, 0.26 mmol) in THF (6.6 mL). The solution was stirred for 24 h at room temperature. Then the solvent was evaporated and the crude residue was purified by column chromatography (CH_2Cl_2–MeOH 93 : 7) affording compound **15** as a yellow solid (104 mg, 71%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.03–2.12 (1H, m, H-2′a), 2.212.29 (1H, m, H-2′b), 3.17-3.29 (2H, m, H-5′a and H-5′b), 3.46 (1H, app dt, J = 7.2 Hz, H-3′), 3.69-3.74 (1H, m, H-4′), 4.09 (1H, br s, 3′-NH), 6.13 (1H, dd, J = 4.5 Hz, J = 6.9 Hz, H-1′), 6.53 (1H, d, J = 13.2 Hz, Br–CH═CH), 7.19–7.42 (16H, m, Br–CH═CH and Tr), 7.74 (1H, s, 6-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.34 (C-3′), 63.94 (C-5′), 84.20 (C-1′), 85.70 (C-4′), 86.11 (Tr), 106.80 (Br–CH═CH), 109.73 (C-5), 127.09, 127.93, 128.27, 129.85 (Br–CH═CH and Tr), 139.45 (C-6), 143.63 (Tr), 149.23 (C-2), 161.70 (C-4). HRMS (ESI-MS) for $C_{30}H_{28}BrN_3NaO_4$ [M+Na]$^+$ found, 596.1162; calcd, 596.1155.

N-(3′-deoxy-5′-*O*-triphenylmethyl-β-(*E*)-5-(2-bromovinyl)-2′-deoxyuridin-3′-yl)-*N*′-(4-chloro-3-trifluoromethylphenyl)-thiourea 16

Reaction of compound **15** (104 mg, 0.18 mmol) with 4-chloro-3-(trifluoromethyl)-phenylisothiocyanate (37 µL, 0.24 mmol) was performed as described for the synthesis of compound **7a** affording compound **16** in a 82% yield (119 mg, colourless solid). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27–2.33 (1H, m, H-2′a), 2.49–2.60 (1H, m, H-2′b), 3.27–3.32 (1H, m, H-5′a), 3.41–3.46 (1H, m,

H-5′b), 4.12–4.13 (1H, m, H-4′), 5.08 (1H, app br s, H-3′), 6.25 (1H, app t, J = 6.6 Hz, H-1′), 6.41 (1H, d, J = 13.8 Hz, Br–CH═CH), 7.19–7.42 (16H, m, Br–CH═CH and Tr), 7.63–7.70 (2H, m, subs Ph), 7.85 (1H, s, 6-H), 8.02 (1H, s, subs Ph), 8.62 (1H, m, 3′-NH), 9.94 (1H, s, N′H), 11.65 (H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 36.83 (C-2′), 54.23 (C-3′), 64.18 (C-5′), 83.07 (C-1′), 84.35 (C-4′), 86.40 (Tr), 107.12 (Br–CH═CH), 110.02 (C-5), 120.88, 121.60, 124.50, 124.74, 125.29, 126.56, 127.17, 127.96, 128.28, 129.51, 130.42, 131.67 (CF_3, Br–CH═CH, subs Ph and Tr), 139.14 (C-6′), 143.39 (Tr), 149.24 (C-2), 161.67 (C-4), 180.57 (C═S). Exact mass (ESI-MS) for $C_{38}H_{31}BrClF_3N_4NaO_4S$ [M + H]$^+$ found, 833.0752; calcd. 833.0782.

5-(3′-Amino-3′-deoxy-5′-*O*-triphenylmethyl-β-(*E*)-5-(2-bromovinyl)-2′-deoxyuridin-3′*N*-yl)-1-(4-chloro-3-trifluoromethylphenyl)-tetrazole 17

Reaction of compound **16** (90 mg, 0.11 mmol), NaN_3 (22 mg, 0.34 mmol), $HgCl_2$ (33 mg, 0.12 mmol) and Et_3N (45 µL, 0.32 mmol) in 0.5 mL DMF was performed as described in the general synthesis of 1-substituted-5-(3′-amino-3′-deoxy-5′-*O*-triphenylmethyl-β-D-thymidin-3′-yl)-tetrazole analogues. Compound **17** was obtained as white foam (68 mg, 75%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.36–2.45 (1H, m, H-2′a), 2.49–2.58 (1H, m, H-2′b), 3.27–3.32 (1H, m, H-5′a), 3.43–3.48 (1H, m, H-5′b), 4.14 (1H, ddd, J = 2.7 Hz, J = 5.4 Hz, J = 5.4 Hz, H-4′), 4.43-4.55 (1H, m, H-3′), 6.29 (1H, app t, J = 6.9 Hz, H-1′), 6.50 (1H, m, J = 13.5 Hz, Br–CH═CH), 7.21–7.46 (17H, m, Br–CH═CH and 3′-NH and Tr), 7.87–7.91 (2H, m, subs Ph), 8.00 (1H, s, 6-H), 8.03–8.04 (1H, subs Ph), 11.65 (1H, s, 3-NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 36.65 (C-2′), 54.40 (C-3′), 64.28 (C-5′), 82.75 (C-1′), 84.35 (C-4′), 86.36 (Tr), 107.11 (Br–CH═CH), 110.13 (C-5), 120.36, 123.98, 124.93, 127.15, 127.68, 127.93–128.26, 129.53, 130.83, 131.89, 132.19 and 133.23 (CF_3, Br–CH═CH, subs Ph and Tr), 139.42 (C-6), 143.49 (Tr), 149.24 (C-2), 154.48 (C═N), 161.63 (C-4). Exact mass (ESI-MS) for $C_{38}H_{30}BrClF_3N_7NaO_4$ [M + Na]$^+$ found, 842.1042; calcd. 842.1076.

5-(3′-Amino-3′-deoxy-β-(*E*)-5-(2-bromovinyl)-2′-deoxyuridin-3′*N*-yl)-1-(4-chloro-3-trifluoromethylphenyl)-tetrazole 18 and 5-[amino-(4-chloro-3-trifluoromethylphenyl)]-1-(3′-deoxy-β-(*E*)-5-(2-bromovinyl)-2′-deoxyuridin-3′*N*-yl)-tetrazole 19

Reaction of compound **17** (80 mg, 0.11 mmol) under the same conditions as described for the synthesis of **12a** and **13a** and purification on a RP-HPLC (Phenomenex Luna C-18, H_2O/0.1% HCOOH in CH_3CN, 90 : 10 → 0 : 100 in 29 min, flow: 17.5 mL min^{-1}) afforded compounds **18** and **19** as white powders (17 mg, 32%, 3 : 1). **18**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.38 (2H, app t, J = 6.6 Hz, H-2′a and H-2′b), 3.67–3.77 (2H, m, H-5′a and H-5′b), 4.02–4.06 (1H, m, H-4′), 4.40 (1H, app dd, J = 5.7 Hz, J = 6.0 Hz, H-3′), 5.23 (1H, br s, 5′-OH), 6.22 (1H, app t, J = 6.9 Hz, H-1′), 6.86 (1H, d, J = 13.5 Hz, Br–CH═CH), 7.26 (1H, d, J = 13.5 Hz, Br–CH═CH), 7.95–8.09 (2H, m, subs Ph), 8.13–8.14 (1H, m, subs Ph), 8.19 (1H, s, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 37.36 (C-2′), 54.06 and 61.27 (C-3′ and C-5′), 84.43 (C-1′), 84.91 (C-4′), 106.59 (C-5), 109.79 (Br–CH═CH), 124.03–133.20 (CF_3, Br–CH═CH and subs Ph), 139.49 (C-6), 149.29 (C-2), 154.67 (C═N), 161.68 (C-4). HRMS (ESI-MS) for $C_{19}H_{17}BrClF_3N_7O_4$ [M+H]$^+$ found, 578.0168; calcd, 578,0161. **19**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.65≥2.71 (2H, m, H-2′a and H-2′b), 3.70–3.3.74 (2H, m, H-5′a and H-5′b), 3.90–4.03 (1H, m, H-4′), 4.34–4.40 (1H, m, H-3′), 5.55–5.57 (1H, m, 5′-OH), 6.42 (1H, app t, J = 6.3 Hz, H-1′), 6.93 (1H, d, J = 13.8 Hz, Br–CH═CH), 7.29 (1H, d, J = 13.5 Hz, Br–CH═CH), 7.69 (2H, d, J = 8.7 Hz, subs Ph), 7.99–8.01 (1H, m, subs Ph), 8.22 (1H, s, H-6). ^{13}C NMR (175 MHz, DMSO-d_6): δ 56.70 and 61.46 (C-3′ and C-5′), 84.80 (C-1′), 85.25 (C-4′), 110.49 (C-5), 116.92–132.73 (CF_3, Br–CH═CH and subs Ph), 139.91 (C-6), 149.78 (C-2), 162.16 (C-4). HRMS (ESI-MS) for $C_{19}H_{17}BrClF_3N_7O_4$ [M+H]$^+$ found, 578.0173; calcd, 578,0161.

Thymidine kinase assay using [CH_3-^{3}H]dThd as the natural substrate.[8,9]

The activity of recombinant thymidine kinase 1 (TK1), TK2, herpes simplex virus-1 (HSV-1) TK, and *Drosophila melanogaster* deoxynucleoside kinase (Dm-dNK) and the 50% inhibitory concentration of the test compounds were assayed in a 50-µl reaction mixture containing 50 mM Tris/HCl, pH 8.0, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM CHAPS, 3 mg ml^{-1} bovine serum albumin, 2.5 mM ATP, 1 µM [methyl-^{3}H]dThd, and enzyme (4 nM). The samples were incubated at 37 °C for 30 min in the presence or absence of different concentrations (5-fold dilutions) of the test compounds. At this time point, the enzyme reaction still proceeded linearly and did not convert more than 15% of the natural substrate under all experimental conditions. Aliquots of 45 µl of the reaction mixtures were spotted on Whatman DE-81 filter paper disks (Whatman, Clifton, NJ). The filters were washed three times for 5 min each in 1 mM ammonium formate, once for 1 min in water, and once for 5 min in ethanol. The radioactivity was determined by scintillation counting. The 50%-inhibitory concentration (IC_{50}) of the test compounds was determined using the formula $IC_{50} = C_1 - [(50 - x/y - x)(C_1 - C_2)]$ where C_1 is the compound concentration giving more than 50% inhibition; C_2 is the next compound concentration giving less than 50% inhibition; x is the percent substrate conversion of the enzyme reaction at C_1 and y is the percent substrate conversion of the enzyme reaction at C_2. The radioactivity found in blank samples (without enzyme) was subtracted from the test values prior to the calculations.

Acknowledgements

We thank the UGent Research Fund (BOF, Ghent University), the Fund for Scientific Research-Flanders (F.W.O.-Vlaanderen) for funding and the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT) for providing a scholarships to SVP. Financial support to F.G. from Comision Interministerial de Ciencia y Tecnología (SAF2006-12713-C02-02) and Comunidad de Madrid (S-BIO/0214/2006) and to JB from the Concerted Research Actions (GOA 10/14) is gratefully acknowledged.

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