Supplementary Information

Synthesis, Modeling and Evaluation of 3'-(1-Aryl-1H-tetrazol-5-ylamino)-substituted 3'-Deoxythymidine Derivatives as Potent and Selective Human Mitochondrial Thymidine Kinase Inhibitors

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TK2 Dm-dNK HSV1-TK	::	* * * * MGAFCQRPSSDKEQEKEKKSVICVEGNIAGGKTTC MAEASCARKGTKYAEGTQPFTVLIEGNIGSGKTTY MASYPCHQHASAFDQAARSRGHNNRRTALRPRRQQEATEVRPEQKMPTLLRVYIDGPHGMGKTTT	::	35 36 65
TK2 Dm-dNK HSV1-TK	::	* * * * * LEFESNATDVEVLTEPVSKWRNVRGHNPLGLMYHDASRNGLTLQTYVQ- NHFEKYKNDICLITEPVEKWRNVNGVNLLELMYKDPKKWAMPFQSVVT- TQLLVALGSRDDIVYVPERMTYWRVLGASETIANIYTTQHRLDQGEISAGDAAVVMTSAQITMGM	::	83 85 130
TK2 Dm-dNK HSV1-TK	::	* * * -LTMLDRHTREQVSSVRLMERSIHSARYIFVENLYRSEKMPEVDYVVLSEMFD -LTMLQSHTAETNKKLKIMERSIFSARYCFVENMRRNESLEQGMYNTLEEWYK PYAVTDAVLAEHIGGEAGSSHAPPPALTLIFDRHPIAALLCYPAARYLMESMTPQAVLAFVAL	::	135 137 193
TK2 Dm-dNK HSV1-TK	::	* * * * * * WILRNMDVSVDIVYLRINPETCYQ-RIKKRCREDEKVIPIEYIEAIHHLHEEWIKGSLF- FIEESIHVQADIIYLRISPEV-AYERIRQRARSDESCVPIKYIQELELHEDWIHQRRP- -IPPTIPGTNIVIGALPEDRHIDRIAKRQRPGER-LDIAMIAAIRRVYG-LIANTVRYL	::	195 197 249
TK2 Dm-dNK HSV1-TK	::	* * * QCGGSWREDWGQLSGTAVPPQGAEPQSNAGPRPHIGDTLFTLFRAPELLAPNGDLYNVFAWALDV	::	- - 314
TK2 Dm-dNK HSV1-TK	::	* * * PMAAPVEVIEADHHMERMLELFEQNRDRILTPENRKHCP	::	234 250 376

Fig. S1. Sequence alignment of TK2 with *Dm*-dNK and HSV-1 TK. Black boxes indicate completely conserved amino acid residues while gray boxes indicate semi-conserved. TK2 and *D*m-dNK have approximately 55 % sequence homology whereas TK2 and HSV-1 TK have approximately 20 % sequence homology.

Copies of ¹H NMR spectra

Figure S2: 6a, DMSO-d₆, 25 °C, 300 MHz.



Figure S3: 6b, DMSO-d₆, 25 °C, 300 MHz.



Figure S4: 6c, DMSO-d₆, 25 °C, 300 MHz.



Figure S5: 9a, DMSO-d₆, 25 °C, 300 MHz.



Figure S6: 9b, DMSO-d₆, 25 °C, 300 MHz.



Figure S7: 9c, DMSO-d₆, 25 °C, 300 MHz.



Figure S8: 12a, DMSO-d₆, 25 °C, 300 MHz.



Figure S9: 12b, DMSO-d₆, 25 °C, 300 MHz.



Figure S10: 13b, DMSO-d₆, 25 °C, 300 MHz.



Figure S11: 18, DMSO-d₆, 25 °C, 300 MHz.



Figure S12: 19, DMSO-d₆, 25 °C, 300 MHz.



Copies of ¹³C NMR spectra

Figure S13: 6c, DMSO-d₆, 25 °C, 75 MHz.



Figure S14: 9b, DMSO-d₆, 25 °C, 75 MHz.



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Figure S15: 12a, DMSO-d₆, 25 °C, 75 MHz.



Figure S16: **12b**, DMSO-*d*₆, 25 °C, 75 MHz.



Figure S17: **13b**, DMSO-*d*₆, 25 °C, 75 MHz.



Figure S18: 18, DMSO-d₆, 25 °C, 75 MHz.



The purity of the final analogues was assessed by HPLC and PDA detection (190-400 nm) using a reverse phase column (Phenomenex Luna 3.0 μ m C18, 100 x 2.00 mm) with a linear gradient of 10–100% B over 9 min, where A is 0.1% formic acid in H₂O and B is 0.1% formic acid in CH₃CN at a flow rate of 0.4 mL/min).

Compound 6b



Compound 6c



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Compound 9a



Compound 9b



Compound 9c



Compund 12a



Compound 12b



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Compound 13a



Compound 13b



Compounds 18 and 19

Typical chromatogram of the preparative purification on RP-HPLC (Phenomenex Luna C-18, $H_2O/0.1\%$ HCOOH in CH₃CN, 90:10 \rightarrow 0:100 in 29 min, flow: 17.5 mL/min) of compounds **18** and **19**. Collected fractions are coloured in blue (**18**) and green (**19**).

