Supporting Information

Analytic and chromatographic methods

Pre-coated Merck silica gel 60F-254 plates were used for thin layer chromatography and the spots were detected under UV light (254 nm). Column chromatography was performed using silica gel (0.040-0.063 mm) Fluka; glass column was slurry-packed under gravity. The ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded at 23 °C on a Bruker Avance 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz; ^{19}F, 376 MHz) spectrometer using $CDCl_3$ or DMSO-d_6 as the solvent. Chemical shifts (ppm) were determined relative to internal $CHCl_3$ (^{1}H, δ 7.24; $CDCl_3$), internal $CDCl_3$ (^{13}C, δ 77.0, $CDCl_3$), internal DMSO-d_5 (^{1}H, δ 2.49; DMSO-d_6), or interal DMSO-d_6 (^{13}C, δ 39.5, DMSO-d_6). For ^{19}F NMR measurements, $CFCl_3$ was used as the internal standard. Values of the coupling constant, *J*, are given in hertz (Hz). Low-resolution mass spectra (LRMS) were recorded with a Micromass Quattro micro API LC-ESI. High resolution mass spectra (HRMS) were recorded with a Bruker FTMS 4.7T BioAPEXII (ESI).

Analytical radio-high performance liquid chromatography (HPLC) of [^{18}F]FHOMP was performed on an Agilent 1100 series HPLC system equipped with a UV multi-wavelength detector and a Raytest Gabi Star detector using a Gina software. [^{18}F]FHOMP was analyzed on a reversed-phase column, (Gemini C18, 250 x 4.6 mm, particle size: 5 µm, Phenomenex) using a flow rate of 1 mL/min (UV detection at 267 nm) and a gradient as follows: Eluent A was water, eluent B was acetonitrile. The gradient was from 100% A to 90% A and 10% B at 0-15 min, then isocratic at 90% A from 15-20 min, then to 100% A from 20-21 min followed by 100% A isocratic from 21-25 min.

Semi-preparative radio-HPLC was performed on an HPLC system equipped with a Merck-Hitachi L-6200A intelligent pump, a Knauer variable-wavelength ultraviolet detector and an Eberline radiation monitor. [^{18}F]FHOMP was purified on a reversed phase column, (Gemini C18, 250 x 10 mm, particle size: 5 µm, Phenomenex) using a flow rate of 4 mL/min and a gradient as follows: Eluent A was water, eluent B was ethanol. After 10 min isocratic 100% A the gradient was from 100% A to 90% A and 10% B at 10-30 min.

For the determination of the microsomal stability of [^{18}F]FHOMP Ultra Performance Liquid Chromatography (UPLC) was performed using a Waters ACQUITY UPLC® system equipped with a Berthold FlowStar LB513 radioactivity flow through detector (coincidence detection) and a Waters ACQUITY UPLC® BEH 2.1 x 50 mm, 1.7 µm C_{18} reversed-phase analytic column. Elution was performed at a flow of 0.6 mL/min and a wavelength of 267 nm with a gradient from 100% A (sodium phosphate buffer, pH 7.0) and 0 % B (acetonitrile) to 90% A and 10% B over a 1.5-min period, then a gradient to 30% A and 70% B until 2 min followed by a constant flow of 30% A and 70% B until 3.0 min, then a gradient to 100% A and 0% B to 3.1 min followed by a constant flow of 100% A until 3.2 min.

*Synthesis of reference FHOMP (**6**)*

6-(Chloromethyl)-5-methylpyrimidine-2,4(1H,3H)-dione (1): Compound **1** was synthesized according to published protocols starting from 5,6-dimethylpyrimidine-2,4(1*H*,3*H*)-dione [1]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.82 (s, 3H, C*H*$_3$), 4.43 (s, 2H, C*H*$_2$), 10.87 (s, 1H, N1*H*), 11.16 (s, 1H, N3*H*) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.1, 39.4, 107.4, 145.3, 150.6, 164.8 ppm. LRMS (ESI+) m/z 174.71 (*M*+H)$^+$.

2-(Benzyloxymethyl)oxirane (3): Compound **3** was prepared by benzylation of (±)-glycidol (**2**) by following published methods [2]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.62 (dd, 1H, *J* = 5.0, 2.7 Hz, CH-C*H*$_2$-O), 2.80 (dd, 1H, *J* = 4.9, 4.1 Hz, CH-C*H*$_2$-O), 3.19 (m, 1H, CH), 3.45 (dd, 1H, *J* = 11.5, 5.7 Hz, O-C*H*$_2$), 3.72 (dd, 1H, *J* = 14.1, 7.1 Hz, O-C*H*$_2$), 4.60 (dd, 2H, *J* = 23.2, 11.9 Hz, Ph-C*H*$_2$-O), 7.26-7.38 (m, 5H, Ph) ppm. HRMS (ESI+) m/z found 164.0830, calcd 164.0837 for $C_{10}H_{12}O_2$.

1-(Benzyloxy)-3-fluoropropan-2-ol (4): Epoxide **3** was selectively opened via fluorination using TBABF-KHF_2 according to published methods [3]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.54 (bs, 1H, OH), 3.58 (m, 2H, O-C*H*$_2$-CH), 4.05 (m, 1H, O-CH$_2$-C*H*), 4.46 (ddd, 1H, $J_{H,F}$ = 47.5 Hz, $J_{H,H}$ = 18.3, 9.7 Hz, F-C*H*$_2$), 4.48 (ddd, 1H, $J_{H,F}$ = 47.5 Hz, $J_{H,H}$ = 18.3, 9.7 Hz, F-C*H*$_2$), 4.57 (s, 2H, Ph-C*H*$_2$-O), 7.29-7.39 (m, 5H, Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 69.3 (d, $J_{C,F}$ = 20.5 Hz), 70.0 (d, $J_{C,F}$ = 6.8), 73.5, 83.9 (d, $J_{C,F}$ = 169.9 Hz), 127.8,

127.9, 128.5, 137.6 ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ -232.1 (dt, 1F) ppm. HRMS (ESI+) m/z found 184.0893, calcd 184.0899 for $C_{10}H_{13}FO_2$.

6-((1-(Benzyloxy)-3-fluoropropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione (5): NaH (60% in mineral oil, 39 mg, 1.629 mmol) was added to a solution of **4** (100 mg, 0.586 mmol) in THF (17 mL) at 0 °C. The mixture was stirred for 30 min at room temperature. Compound **1** (55.4 mg, 0.317 mmol) was added at 0 °C and the reaction was stirred for 5 min at 0 °C. After warming to room temperature, the mixture was heated to reflux for 16 h. The reaction was cooled to room temperature, quenched with ice and water, diluted with ethylacetate and washed with water and brine. The organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2 : CH_3OH = 30 : 1) to afford **5** (46.6 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.75 (s, 3H, C*H*$_3$), 3.57 (dd, 2H, *J* = 5.5, 1.0 Hz, BnO-C*H*$_2$), 3.83 (m, 1H, C*H*), 4.41 (s, 2H, C6-C*H*$_2$), 4.52 (s, 2H, Ph-C*H*$_2$), 4.55 (m, 2H, C*H*$_2$-F), 7.32 (m, 5H, Ph), 10.46 (s, 1H, N1*H*), 11.07 (s, 1H, N3*H*) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 9.0, 65.2, 68.1 (d, $J_{C,F}$ = 8.5 Hz), 72.4, 77.2 (d, $J_{C,F}$ = 18.5 Hz), 82.7 (d, $J_{C,F}$ = 167.4 Hz), 106.0, 127.5, 128.3, 138.0, 145.9, 150.7, 164.9 ppm. ^{19}F NMR (376 MHz, DMSO-d_6): δ -230.1 (dt, 1F) ppm. LRMS (ESI+) m/z 322.68 (*M*+H)$^+$.

6-((1-Fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione (6): Palladium on activated carbon 10% (15 mg, 0.014 mmol Pd) was added to a 10 mL-reacti-vial containing **5** (46.6 mg, 0.145 mmol). Methanol (1 mL) was added and the system was flushed with argon. H_2 gas was bubbled into the system using a balloon. Having the system saturated with H_2, the balloon was removed and the closed system was heated to 80 °C for 20 h. After cooling to room temperature and filtration through celite (CH_3OH) the crude was purified by column chromatography on silica gel (CH_2Cl_2 : CH_3OH = 20 : 1) to obtain **6** (17 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.74 (s, 3H, C*H*$_3$), 3.51 (t, 2H, *J* = 10.4 Hz, C*H*$_2$-OH), 3.63 (m, 1H, C*H*), 4.41 (s, 2H, C6-C*H*$_2$), 4.47 (m, 2H, C*H*$_2$-F), 5.02 (t, 1H, *J* = 11.3 Hz, O*H*), 10.40 (s, 1H, N1*H*), 11.04 (s, 1H, N3*H*) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 8.8, 59.4 (d, $J_{C,F}$ = 6.7 Hz), 64.9, 79.2 (d, $J_{C,F}$ = 14.2 Hz), 82.1 (d, $J_{C,F}$ = 133.1 Hz), 105.2, 146.2, 150.6, 164.8 ppm. ^{19}F NMR (376 MHz, DMSO-d_6): δ -230.4 (1F) ppm. HRMS (ESI+) m/z found 233.0932, calcd 233.0932 for $C_9H_{14}FN_2O_4$.

*Synthesis of precursor **12***

6-((1,3-Bis(benzyloxy)propan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione (8): NaH (60% in mineral oil, 124.5 mg, 3.11 mmol) was added to a solution of 1,3-bis(benzyloxy)propan-2-ol (**7**) (213 µL, 0.86 mmol) in THF (10 mL) at 0 °C. The mixture was stirred for 60 min at room temperature. A solution of **1** (147.1 mg, 0.84 mmol) in THF (5 mL) was added at 0 °C over 40 min. The reaction was stirred at room temperature for 30 min and heated to reflux for 19 h. After this time, the mixture was concentrated, treated with ice water and neutralized. The solution was extracted with ethylacetate. The combined organic phases were dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2 : CH_3OH = 40 : 1) to afford **8** (168.5 mg, 49%) as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.72 (s, 3H, C*H*$_3$), 3.55 (m, 4H, BnO-C*H*$_2$), 3.78 (m, 1H, C*H*), 4.43 (s, 2H, C6-C*H*$_2$), 4.50 (s, 4H, Ph-C*H*$_2$), 7.32 (m, 10H, Ph), 10.36 (s, 1H, N1*H*), 11.04 (s, 1H, N3*H*) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 8.8, 65.2, 69.5, 72.2, 77.9, 105.1, 127.4, 127.4, 128.1, 138.1, 146.4, 150.5, 162.7 ppm. LRMS (ESI+) m/z 410.76 (*M*+H)$^+$.

6-((1,3-Bis(benzyloxy)propan-2-yloxy)methyl)-1,3-bis(methoxymethyl)-5-methylpyrimidine-2,4(1H,3H)-dione (9): Compound **8** (65 mg, 0.158 mmol) was dissolved in DIPEA (300 µL) and dichloromethane (450 µL) and the mixture was stirred for 30 min. Chlorotrimethylsilane (60 µL, 0.473 mmol) was added and the reaction was stirred for 30 min. After this time, methoxymethyl chloride (MOMCl) (70 µL, 0.92 mmol) was added. After 1 h, additional MOMCl (250 µL, 3.29 mmol) was added. After 22 h, the reaction was diluted with ethylacetate, washed with saturated aqueous $NaHCO_3$ solution and extracted with ethylacetate. The combined organic phases were combined, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (ethylacetate : hexane = 1:1) to yield **9** (77 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01 (s, 3H, C*H*$_3$), 3.34 (s, 3H, N3-MOMC*H*$_3$), 3.43 (s, 3H, N1-MOMC*H*$_3$), 3.57 (m, 4H, BnO-C*H*$_2$), 3.81 (m, 1H, C*H*), 4.52 (s, 4H, Ph-C*H*$_2$), 4.68 (s, 2H, C6-C*H*$_2$), 5.39 (s, 2H, N3-MOMC*H*$_2$), 5.42 (s, 2H, N1-MOMC*H*$_2$), 7.27-

7.37 (m, 10H, Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.1, 57.0, 57.9, 64.4, 70.5, 72.7, 73.7, 75.1, 78.6, 111.9, 127.8, 127.9, 128.5, 137.8, 145.0, 152.5, 163.6 ppm. LRMS (ESI+) m/z 498.80 (M+H)$^+$.

6-(((1,3-Dihydroxypropan-2-yl)oxy)methyl)-1,3-bis(methoxymethyl)-5-methylpyrimidine-2,4(1H,3H)-dione (10): To a solution of **9** (300 mg, 0.60 mmol) in ethanol (23 mL), $Pd(OH)_2$ on activated carbon 20% (382.5 mg, 0.54 mmol) and cyclohexene (4.5 mL) were added and the reaction was heated to reflux for 70 min. After cooling, the mixture was filtered through celite (ethylacetate) and the solvent was evaporated to yield **10** (179 mg, 93%) as light-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (s, 3H, CH_3), 2.32 (bs, 2H, OH), 3.45 (s, 3H, N3-MOMCH_3), 3.48 (s, 3H, N1-MOMCH_3), 3.62 (m, 1H, CH), 3.78 (ddd, 4H, J = 30.2, 11.8, 3.7 Hz, OH-CH_2), 4.70 (s, 2H, C6-CH_2), 5.41 (s, 2H, N3-MOMCH_2), 5.49 (s, 2H, N1-MOMCH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.3, 57.5, 58.0, 62.7, 64.0, 72.8, 75.4, 80.8, 112.0, 144.6, 152.4, 163.3 ppm. LRMS (ESI+) m/z 318.70 (M+H)$^+$.

6-(((1-Hydroxy-3-((4-methoxyphenyl)diphenylmethoxy)propan-2-yl)oxy)methyl)-1,3-bis(methoxymethyl)-5-methylpyrimidine-2,4(1H,3H)-dione (11): To a solution of **10** (20 mg, 0.063 mmol) in DMF (0.4 mL) triethylamine (9.5 mg, 0.094 mmol) was added and the mixture was stirred at room temperature for 10 min. 4-methoxytritylchloride (20 mg, 0.064 mmol) and catalytic amount of DMAP were added and the reaction was stirred for 2 h at room temperature. The mixture was diluted with ethylacetate, washed with water and brine, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (ethylacetate : hexane 1:4 to ethylacetate : hexane 1:1) to afford **11** (11.2 mg, 30%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.95 (s, 3H, CH_3), 2.31 (bs, 1H, OH), 3.21 (m, 2H, MMTrO-CH_2), 3.35 (s, 3H, N3-MOMCH_3), 3.36 (s, 3H, N1-MOMCH_3), 3.57 (m, 2H, OH-CH_2), 3.63 (m, 1H, CH), 3.73 (s, 3H, MMTrCH_3), 4.57 (dd, 2H, J = 52.6, 11.7 Hz, C6-CH_2), 5.34 (m, 4H, MOMCH_2), 6.77 (m, 2H, CH-C-O-CH_3), 7.14-7.36 (m, 12H, MMTrCH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.3, 55.2, 57.5, 57.9, 63.0, 63.5, 64.2, 72.7, 75.3, 80.5, 87.0, 111.8, 113.2, 127.1, 127.9, 128.3, 130.3, 135.1, 144.0, 144.7, 152.4, 158.7, 163.4 ppm. LRMS (ESI+) m/z 612.87 (M+H)$^+$.

2-((1,3-Bis(methoxymethyl)-5-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)methoxy)-3-((4-methoxyphenyl)diphenylmethoxy)propyl 4-methylbenzenesulfonate (12): Compound **11** (11.2 mg, 0.019 mmol) was suspended in pyridine (0.3 mL) and stirred at room temperature for 30 min. Tosylchloride (16.4 mg, 0.086 mmol) dissolved in dichloromethane (0.1 mL) was added and the mixture was stirred for 2 h at 30 °C. A catalytic amount of DMAP was added and the reaction was heated at 30 °C for 17 h. The mixture was quenched with water and diluted with ethylacetate. The mixture was washed with 1M $CuSO_4$ solution and extracted with ethylacetate. The combined organic phases were dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (ethylacetate : hexane = 1:1) to obtain **12** (6.4 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.92 (s, 3H, CH_3), 2.44 (s, 3H, TsCH_3), 3.21 (d, 2H, J = 19.1 Hz MMTrO-CH_2), 3.36 (s, 3H, N3-MOMCH_3), 3.43 (s, 3H, N1-MOMCH_3), 3.61-3.67 (m, 1H, CH), 3.80 (s, 3H, MMTrCH_3), 4.03 (dd, 1H, ^2J = 10.6, ^3J =3.2 Hz , TsO-CH_2), 4.10 (dd, 1H, ^2J = 10.6, ^3J = 3.2 Hz, TsO-CH_2), 4.53 (dd, 2H, J = 13.3, 11.7 Hz, C6-CH_2), 5.32 (m, 2H, N3-MOMCH_2), 5.39 (s, 2H, N1-MOMCH_2) 6.81 (m, 2H, S-C-CH-CH), 7.21-7.36 (m, 14H, tritylCH), 7.74 (m, 2H, S-C-CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.2, 21.7, 55.2, 57.1, 57.9, 62.5, 64.3, 69.6, 72.7, 75.0, 77.5, 87.0, 112.0, 113.3, 127.2, 127.9, 128.2, 129.9, 130.3, 132.7, 134.8, 143.8, 143.8, 144.3, 145.1, 152.3, 158.8, 163.4 ppm. HRMS (ESI+) m/z found 767.2618 for $[C_{40}H_{44}N_2NaO_{10}S]^+$, calcd 767.2609 for $[C_{40}H_{44}N_2NaO_{10}S]^+$.

*Radiosynthesis of [^{18}F]**FHOMP** ([^{18}F]-**6**)*

The no-carrier-added [^{18}F]-fluoride was trapped on the anion exchange cartridge and directly eluted into a 5-mL sealed reaction vessel using a solution of tetrabutylammonium hydroxide (27.8 mg) in 0.6 mL of methanol. The solvent was removed at 90 °C under reduced pressure and a stream of nitrogen. Subsequently, water was removed by azeotropic distillation with acetonitrile (3 x 1 mL) under reduced pressure and a stream of nitrogen at 90 °C. The vial was kept at room temperature for additional 5 min under vacuum. To this dried [^{18}F]TBAF salt was added a solution of the tosylate precursor **12** (4 mg) in 0.3 mL *tert*-butanol and anhydrous acetonitrile (4:1). The reaction mixture was heated for 30 min at 110 °C. After cooling, the crude product was passed through an SPE cartridge (Sep-Pak silica, Waters AG) to

remove TBA and unreacted fluoride. The cartridge was washed with acetonitrile (3 x 0.5 mL). The solvent was evaporated at 90 °C under a stream of nitrogen to dryness. For hydrolysis, 0.6 mL of conc. HCl was added and the mixture was heated for 10 min at 110 °C. The vial was cooled, neutralized with 4 M NaOH (1.5 mL) and diluted with 0.6 M phosphate buffer to a total volume of 5 mL. The crude product was injected onto the semi-preparative radio-HPLC column for HPLC purification. The desired product fraction was collected at approximately 19 min (max. 5% ethanol in water) and directly passed through a sterile filter into a sterile and pyrogen-free vial. Radiochemical yield was determined after purification and based on the amount of starting activity.

Determination of partition coefficient

The log $D_{pH7.4}$ of [^{18}F]FHOMP and [^{18}F]FHBG in 1-octanol/PBS was determined by the shake-flask method as previously described [20]. [^{18}F]FHOMP or [^{18}F]FHBG (~1.5 MBq) was added in 5 mL water containing 5% ethanol to vials containing 0.5 mL each of 1-octanol and PBS. The vials were shaken for 20 min at room temperature and the phases were separated by centrifugation. The radioactivity was measured in 50 µL of each phase with a gamma counter (Wizard, Perkin Elmer). Measurements were performed in triplicates and log $D_{pH7.4.}$ values were calculated as the logarithmic ratio of the counts in the octanol and PBS samples.

References

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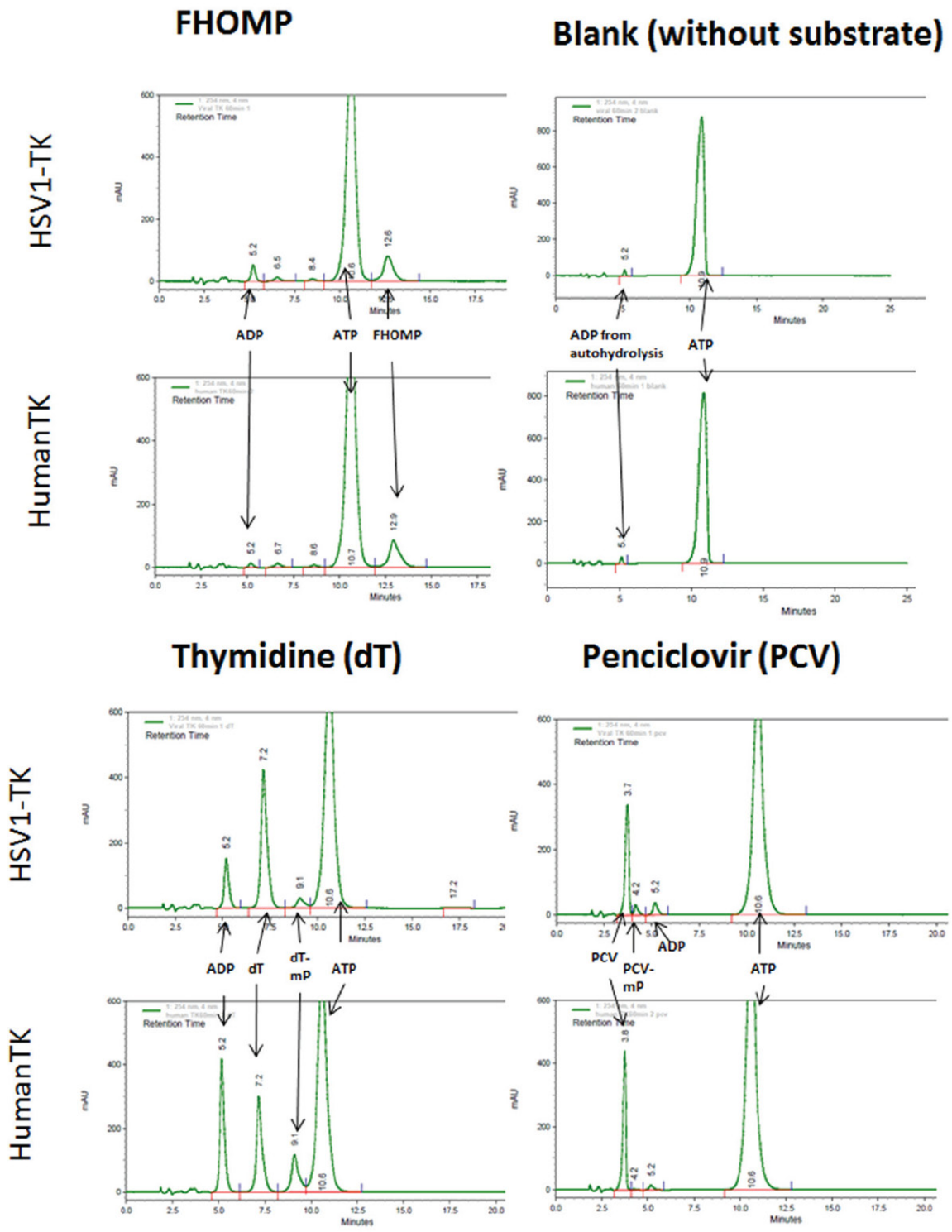

Figure S1. Phosphorylation pattern assay at 60 min of incubation: The formation of new peaks (ADP and some monophosporylated compounds) have been monitored by HPLC coupled with a UV detector at 254 nm. To assess the functionality of the HSV1-TK and the human-TK enzymes, thymidine (dT) was used as a positive control. It showed ADP formation for both enzymes as expected and monophosphorylated thymidine (dT-mP) could be detected. Penciclovir was used to compare with a well known HSV1-TK but not human-TK substrate. Very small substrate-independent ATP hydrolysis was observed for the blank (no substrate) for both enzymes. These chromatograms clearly show the formation of ADP for PCV (and some speculated monophosphorylated penciclovir (PCV-mP)) and FHOMP with HSV1-TK but not the human-TK. The data for dT, PCV and blank have been shown before in [20].