Supplementary Material

Evaluation of [^{11}C]NMS-E973 as a PET tracer for *in vivo* visualisation of HSP90

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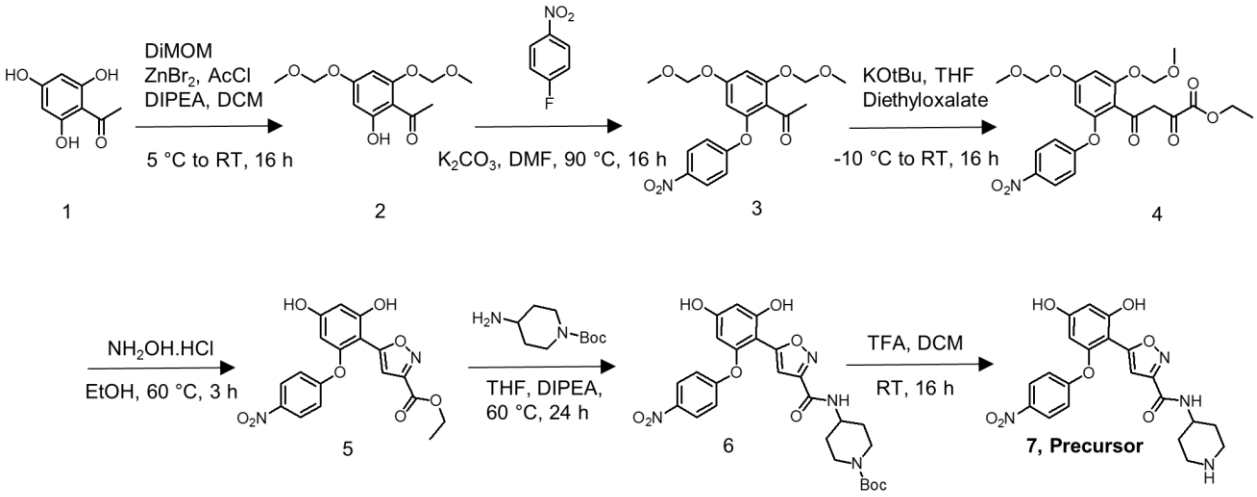

Figure S1. Synthesis of precursor compound **7**.

1-[2-hydroxy-4,6-*bis*(methoxymethoxy)phenyl]ethanone (2). To a stirred solution of dimethoxymethane (2.64 ml, 29.8 mmol) and zinc bromide (29.28 mg, 0.13 mmol) in DCM (23,2 mL), acetyl chloride (2.12 mL, 29.8 mmol) was added dropwise during 30 min maintaining the temperature below 30 °C. After stirring for 3 h at room temperature, the solution was diluted with DCM (48 mL), then cooled to 5 °C before the portion wise addition of 1-(2,4,6-trihydroxyphenyl)ethanone (**1**), 2.00 g, 11.89 mmol) followed by the dropwise addition of *N*,*N*-diisopropylethylamine (8.32 mL, 47.6 mmol). After 1 h the ice bath was removed and the temperature was allowed to rise to RT. The resulting solution was stirred for 16 h, and washed with NH_4Cl saturated solution, followed by washing with 10% citric acid solution. After drying over $MgSO_4$, the solvent was removed and purification was performed with column chromatography to yield (**2**) (Yield: 0.75 g, 37%) *Colorless oil*, ^{1}H NMR ($CDCl_3$-*d*): δ 2.66 (s, 3H, CH_3), 3.48 (s, 3H, CH_3), 3.52 (s, 3H, CH_3), 5.17 (s, 2H, CH_2), 5.26 (s, 2H, CH_2), 6.23-6.28 (m, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 33.2, 56.7, 56.9, 94.2 (2C), 94.7, 97.4, 107.2, 160.6, 163.7, 167.0, 200.4. HRMS (ESI) calculated for $C_{12}H_{17}O_6$ $[M+H]^+$: 257.1020. Found: 257.1023

1-[2,4-*Bis*(methoxymethoxy)-6-(4-nitrophenoxy-phenyl]ethanone (3). To a stirred solution of (**2**) (745 mg, 2.91 mmol) in DMF (6 mL) 4-nitro-1-fluorobenzene (0.34 mL, 3.20 mmol) was added, followed by K_2CO_3 (803 mg, 5.82 mmol). After stirring for 15 min at room temperature,

the resulting suspension was heated for 16 h at 90 °C. After cooling, the dark solution was diluted with EtOAc (20 mL) and thoroughly washed with 10% citric acid solution and brine and dried over $MgSO_4$. The solvent was removed and the residue was purified by column chromatography to yield (**3**). (Yield: 0.43 g, 58%). *Yellow powder*, ^{1}H NMR ($CDCl_3$): δ 2.43 (s, 3H, CH_3), 3.44 (s, 3H, CH_3), 3.49 (s, 3H, CH_3), 5.11 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 6.35 (s, 1H, Ar), 6.73 (s, 1H, Ar), 7.00 (d, 2H, *J* = 9.2 Hz, Ar), 8.18 (d, 2H *J* = 9.2 Hz, Ar). ^{13}C NMR ($CDCl_3$-*d*): δ 33.0, 57.0, 57.2, 95.1, 95.6, 101.3, 102.9, 117.8, 120.0, 126.6, 143.6, 153.3, 157.1, 160.6, 163.6, 200.0. HRMS (ESI) calculated for $C_{18}H_{19}NO_8$ $[M+H]^+$: 378.1184. Found: 378.1179.

Ethyl 5-[2,4-*bis*(methoxymethoxy)-6-(4-nitrophenoxy)phenyl]-2,4-dioxobutanoate (4). To a stirred solution of sodium *tert*-butoxide (0.284 g, 2.51 mmol) in THF (8 mL) at -10 °C, was added diethyl oxalate (0.5 mL, 3.42 mmol) in 2 mL of precooled THF. After 30 min a solution of (**3**) (430 mg, 1.14 mmol) in THF (4 mL) was added drop wise. The reaction mixture was stirred for 1 h at -10 °C and then further 16 h at room temperature. The solution was poured into a 10% citric acid solution (30 mL) and thoroughly extracted with EtOAc. After washing with brine and drying over $MgSO_4$, the combined organic fractions were evaporated to provide a yellow crude residue. Purification using column chromatography yielded (**4**). (Yield: 98%, proceeded to the next step without further characterization)

Ethyl 5-[2,4-*bis*(methoxymethoxy)-6-(4-nitrophenoxy)phenyl]-isoxazole-3-carboxylate (5). To a stirred solution of (**4**) (0. 42 g, 0.88 mmol) in EtOH (12 mL), hydroxylamine hydrochloride (2.67 mg, 4.02 mmol) was added. After stirring for 3 h at 60 °C, the solvent was evaporated, saturated $NaHCO_3$ (30 mL) was added and the suspension was taken up in DCM (30 mL) and thoroughly washed with water and dried over $MgSO_4$. The solvent was removed by evaporation and purification using column chromatography was performed to afford (**5**). (Yield: 0.15 g, 35%) *Yellow oil*, ^{1}H NMR (MeOD-d_4): δ 1.30-1.37 (m, 3H, CH_3), 4.32-4.36 (m, 2H, CH_2), 6.11 (s, 1H, Ar), 6.38 (s, 1H, Ar), 6.87 (s, 1H, Ar), 7.04 (d, 2H *J* = 8.8 Hz, Ar), 8.16 (d, 2H *J* = 8.8 Hz, Ar). ^{13}C NMR (MeOD-d_4): δ 13.6, 62.3, 100.6, 101.2, 104.7, 117.2, 120.1, 126.2, 143.4, 154.8, 156.4, 159.2, 160.7, 162.2, 163.7, 167.4. HRMS (ESI) calculated for $C_{18}H_{15}N_2O_8$ $[M+H]^+$: 387.0822. Found: 387.0841.

***Tert*-butyl-4-[({5-[2,4-*bis*(methoxymethoxy)-6-(4-nitrophenoxy)phenyl]-isoxazole-3-yl}carbonyl)amino]piperidin-1-carboxylate (6)**. (**5**) (149 mg, 0.3859 mmol) and *tert*-butyl 4-aminopiperidine-1-carboxylate (386 mg, 1.93 mmol) were dissolved in THF (12 mL). *N*,*N*-diisopropylethylamine (1.34 mL, 0.77 mmol) was added to the mixture. The reaction mixture was stirred at 60 °C for 24 h. After cooling to room temperature, the solvent was removed. The residue was purified by column chromatography to afford (**6**). *Brownish oil*, ^{1}H NMR (MeOD-d_4): δ 1.13-1.20 (m, 4H, CH_2), 1.40 (s, 9H, $3CH_3$), 3.93-4.01 (m, 5H, CH_2 & CH), 6.05 (s, 1H, Ar), 6.31 (s, 1H, Ar), 6.79 (s, 1H, Ar), 6.95 (d, 2H *J* = 8.6 Hz, Ar), 8.09 (d, 2H *J* = 8.6 Hz, Ar). ^{13}C NMR (MeOD-d_4): δ 13.8, 20.2, 28.0, 31.6, 80.5, 100.7, 100.9, 101.2, 103.7, 117.1, 126.2, 143.3, 154.7, 155.7, 158.7, 159.1, 160.3, 162.0, 163.8, 166.8, 172.3. HRMS (ESI) calculated for $C_{26}H_{29}N_4O_9$ $[M+H]^+$: 541.1929. Found: 541.1914.

5-[2,4-Dihydroxy-6-(4-nitrophenoxy) phenyl]-*N*-(piperidin-4-yl)-isoxazole-3-carboxamide (**7**). To a stirred solution of (**6**) (50 mg) in DCM (5 mL) was added TFA (3 eq), the reaction mixture was stirred for 16 h and monitored by LC-MS. After completion, the reaction mixture was diluted with DCM (10 mL) and washed with 0.1 M NaOH and saturated $NaHCO_3$. Purification over a short pad of silica gel yielded (**7**)**.** *Pale yellow oil*, ^{1}H NMR (DMSO-d_6): δ 1.32-1.38 (m, 4H), 2.57-3.59 (m, 2H), 2.91-2.93 (m, 2H), 3.98-4.01 (m, 1H), 6.07 (s, 1H), 6.33 (s, 1H), 6.80 (s, 1H), 6.96-6.98 (m, 2H), 8.12-8.14 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 27.8, 42.3, 44.0, 99.5, 100.4, 100.5, 103.3, 116.8, 126.1, 142.1, 153.4, 158.1, 158.3, 158.3, 161.2, 162.8, 165.4. HRMS (ESI) calculated for $C_{21}H_{21}N_4O_7$ $[M + H^+]$: 441.1404. Found: 441.1410.

Immunofluorescent staining of B16.F10 melanoma cells and high-content screening

Cells were plated in a 96-well plate (Greiner) at a density of 8000-10000 cells/well. At day 1, cells were treated with vehicle (DMSO; 0.001% final concentration), or with a compound (NMS-E973 or Ganetespib) at indicated final concentration (250 nM or 500 nM) for 16 hours. At day 2 cells were washed with PBS and fixed with 4% paraformaldehyde (Thermo Scientific, 16% PFA diluted in PBS) for 30 minutes and were either permeabilised or not by adding 0.2% Triton X100 to blocking buffer (1% Bovine Serum Albumine (BSA) in PBS) for 60 minutes. Primary antibodies were added to the cells in blocking buffer at a dilution of 1:500 (Anti-HSP90, AC88, Abcam) and incubated overnight at 4 °C while shaking gently. Cells were washed 3 times for 5 minutes in PBS and subsequently incubated with DAPI (0.1 µg/ml and/or secondary antibody (1:1000 goat-anti mouse alexa-594; PROMEGA) in blocking buffer for 60 minutes. Imaging was performed on the IN Cell Analyser 2000 (GE Healthcare). The IN Cell Developer package (v1.9.2) allows visualization, imaging, and quantification of staining intensity and quantification of inclusions in cells following immunofluorescent staining.

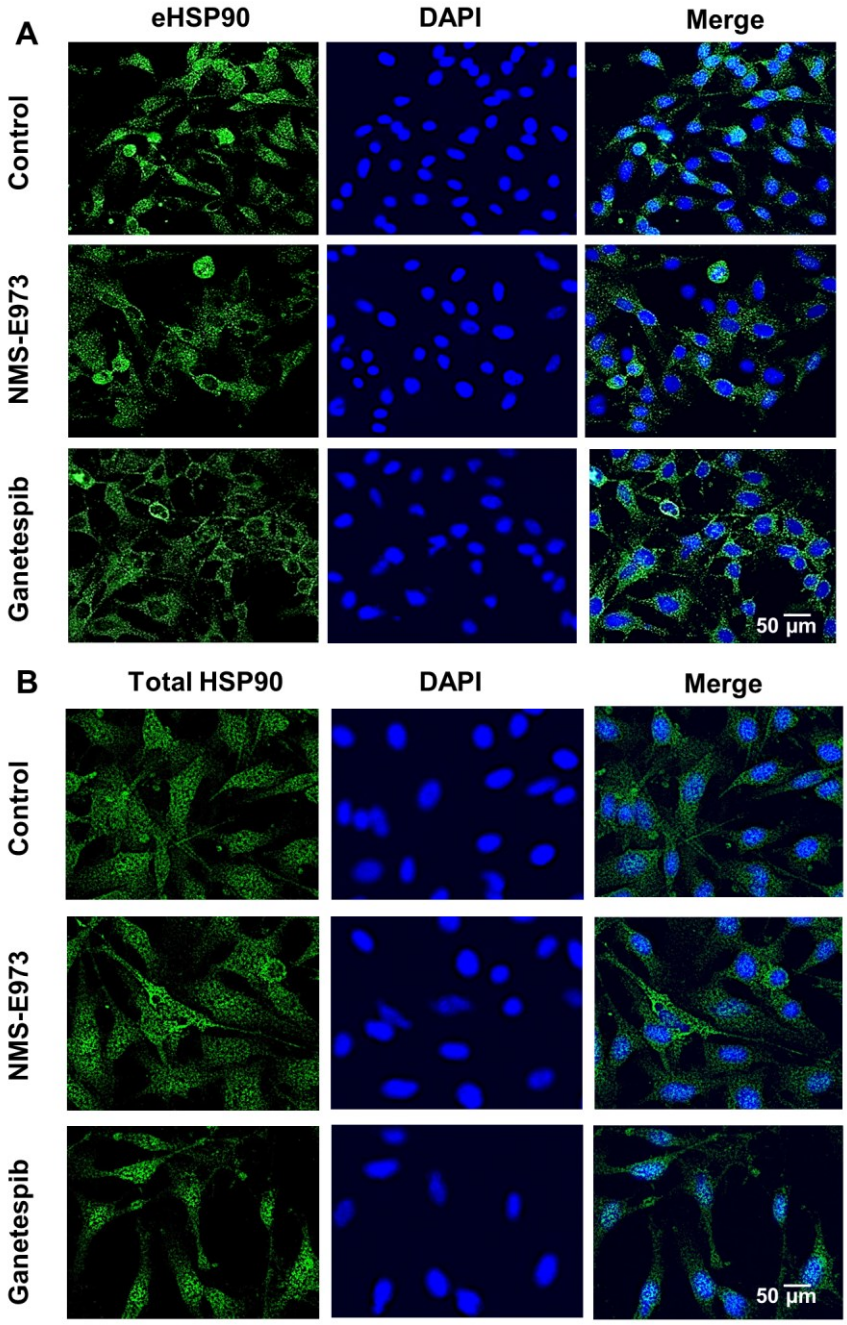

Figure S2. Immunofluorescent staining of eHSP90 and HSP90 of the B16.F10 melanoma cells. **(A)** Extracellular staining of HSP90 shows membrane staining that excludes the nucleus. FITC-Staining of HSP90 (left, Green), nuclear staining by DAPI (Middle, Blue) and the overlay (Right). Treatment with either Ganetespib or NMS-E973, both at 500 nM for 16 hours, shows no visual difference in phenotype (scale bar 50 µm). **(B)** Total staining of HSP90 after permeabilisation, shows homogeneous cellular staining. FITC-Staining of HSP90 (Left, Green), nuclear staining by

DAPI (Middle, Blue) and the overlay (Right). Treatments with either Ganetespib or NMS-E973, both at 500 nM for 16 hours, shows no visual difference in phenotype (scale bar 50 μm).

SDS/Western blot analysis on B16.F10 melanoma cells

Analysis of protein expression was carried out on all cell lines plated at a density of 250.000 cells in a 6-well plate at day 0. At day 1, cells were treated with vehicle (DMSO; 0.001% final concentration), or with 250 nM or 500 nM of NMS-E973 or Ganetespib for 16 hours. At day 2, cells were washed with PBS and lysed in 200 µl NP40 lysis buffer (150 mM NaCl, 50 mM Tris-HCl pH 8, 1% IGEPAL(NP40), containing a 1X PBS dissolved complete protease inhibitor cocktail (Roche) and 1U/µl Universal Nuclease (Pierce) for 30 minutes on ice. Lysates were subjected to regular SDS/Western blot analysis. Antibodies for detection include anti-Cyclin-dependent kinase 1 (CDK1) (Santa Cruz Biotechnology) and anti-GAPDH (6C5; Santa Cruz Biotechnology). Secondary HRP-linked antibodies were used (PROMEGA). Quantification is done by densitometry using the ImageJ software package. Normalization is corrected to GAPDH levels of the input.

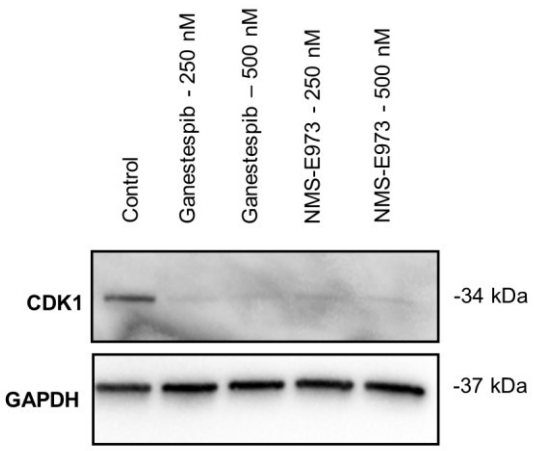

Figure S3. Effect of NMS-E973 and Ganetespib treatment on client protein CDK1 expression. Representative Western blot of decreased CDK1 expression after treatment shows that HSP90 is effectively inhibited by different concentrations of Ganetespib and NMS-E973.

QC chromatogram of [^{11}C]NMS-E973

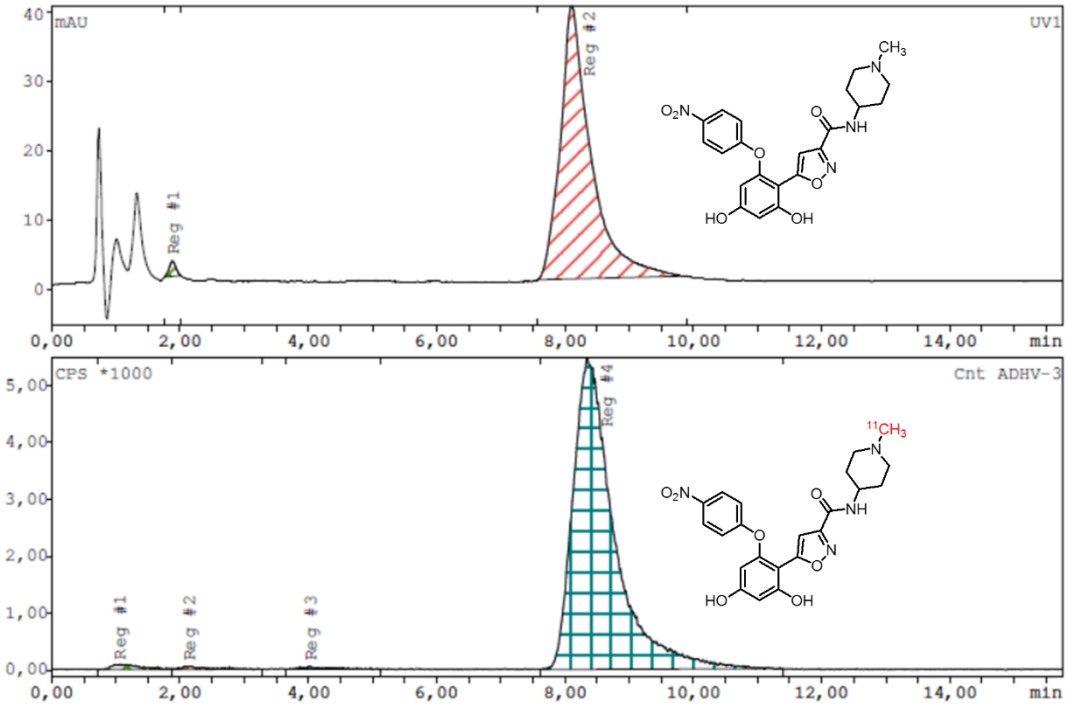

Figure S4. QC chromatogram of [^{11}C]NMS-E973 spiked with authentic reference compound NMS-E973 on an X-bridge RP-C_{18} column (100 x 3 mm 3.5 µm) (upper channel UV 254 nm, lower channel radioactivity)

Confirmation of N-methylation

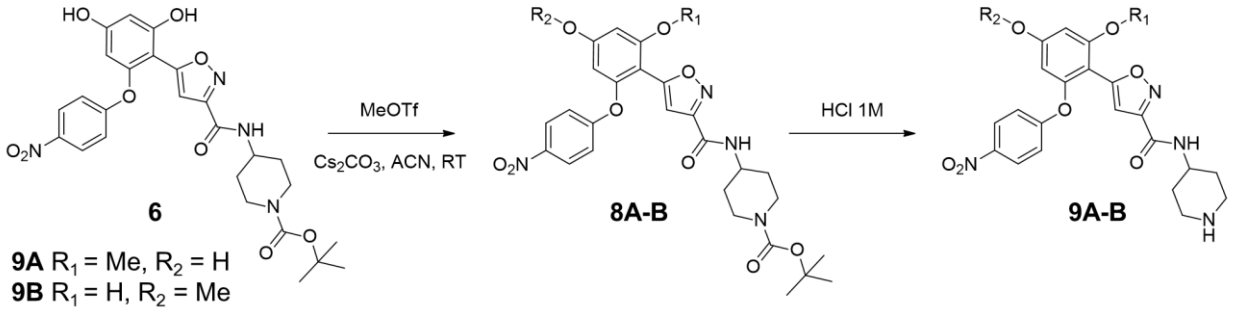

Figure S5. Synthesis of O-alkylated compound starting from (**6**) which is treated with Methyl triflate (MeOTf) under alkaline conditions to form **8A-B**, which is treated with 1 M HCl to remove the BOC-protection group to yield compounds **9A-B**.

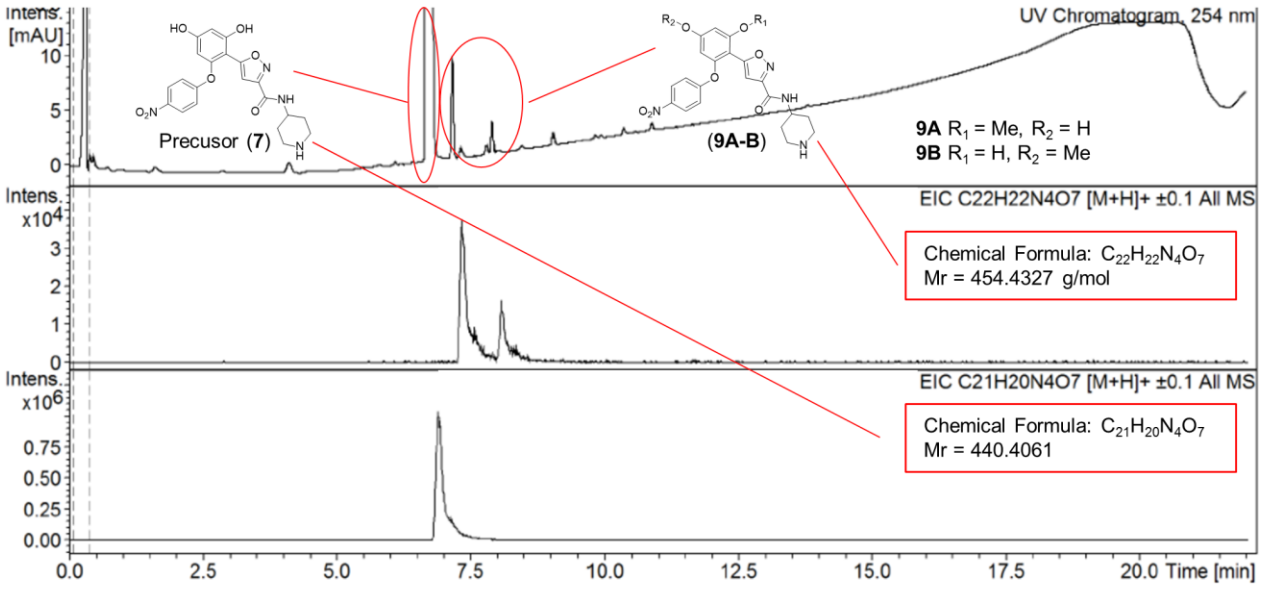

Figure S6. LC-MS-chromatograms of the reaction mixture yielding compound **9A-B**. Extracted ion chromatograms (EIC 454.4 ± 0.1 Da, Rt 7.7 min and 8.5 min) indicate formation of 2 O-methylated compounds **9A-B** that are present besides excess of deprotected precursor compound **7** (EIC 440.4 ± 0.1 Da, Rt 6.9 min). Samples were run on a LC/HRMS system, described in the materials and method section, over an Acquity UPLC BEH C_{18} column (1.7 µm, 2.1 mm x 150 mm, Waters), using a 22 min gradient containing H_2O + 0.1% HCOOH and ACN + 0.1% HCOOH (95/5 to 5/95) with a flow rate of 0.3 mL/min.

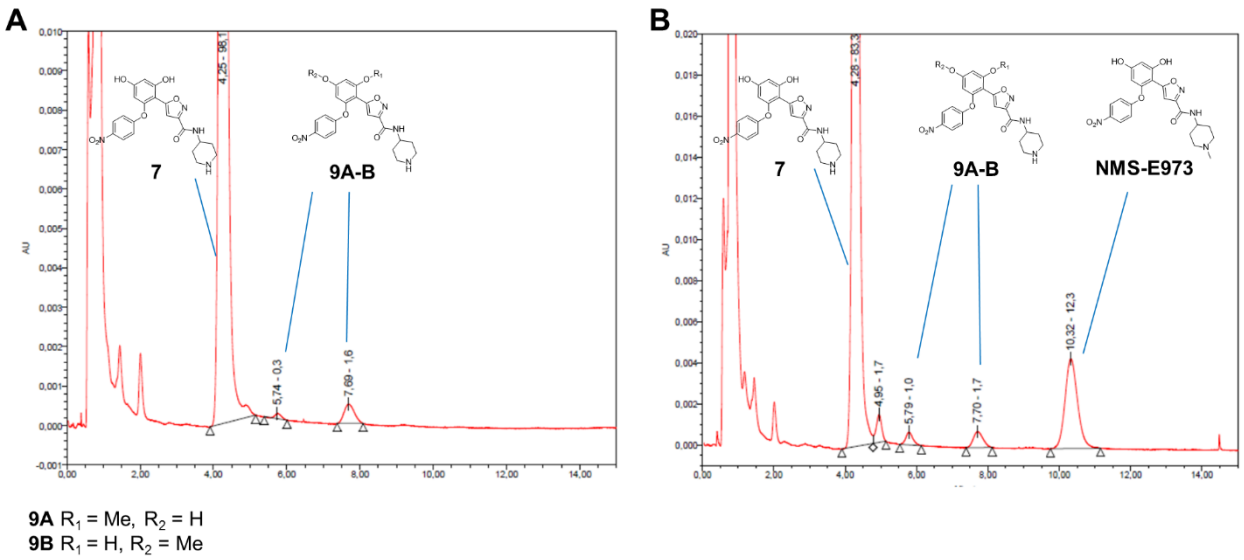

Figure S7. QC HPLC analysis (UV 254 nm) – chromatograms. **(A)** Injection of crude reaction mixture, containing precursor compound (**7**) (Rt 4.2 min) and **O-methylated compounds 9A-B** (Rt 5.7 min and 7.7 min) **(B)** Coinjection of crude reaction mixture with NMS-E973. Precursor (**7**) (Rt 4.2 min), O-methylated products **9A-B** (Rt 5.8 min and 7.7) and NMS-E973 (Rt 10.3 min) show a clear difference in retention time. UV detection was performed at 254 nm.

Plasma radiometabolite study and biodistribution studies

Table S1. Gradient mixture and flow rate used at given time points for radiometabolite study of [^{11}C]NMS-E973 with a Chromolith RP C_{18} column.

Time (min)	**A (ACN) (%)**	**B (NaOAc 0.05 M pH 5.5) (%)**	**Flow (mL/min)**
0	1	99	0.5
4	1	99	0.5
4.1	1	99	1.0
9	90	10	1.0
12	90	10	1.0
12.1	90	10	0.5
15	1	99	0.5

Table S2. Biodistribution data of [^{11}C]NMS-E973 in female Wistar rats at 2, 10, 30 and 60 min after tracer injection.

	%ID[a]			
	2 min	**10 min**	**30 min**	**60 min**
Blood	17.6 ± 1.5	9.7 ± 1.1	5.3 ± 1.1	2.6 ± 0.8
Bone	5.7 ± 1.0	3.7 ± 0.3	3.8 ± 0.2	1.6 ± 0.1
Brain	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Carcass	27.3 ± 1.9	23.2 ± 0.7	23.7 ± 1.0	17.2 ± 2.6
Heart	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	0.3 ±0.0
Intestines	9.3 ± 1.0	25.8 ± 2.9	41.8 ± 7.6	40.6 ± 4.2
Kidneys	14.8 ± 1.2	9.6 ±1.4	5.2 ± 0.4	2.1 ± 0.4
Liver	33.8 ± 3.7	25.9 ± 3.4	12.5 ± 4.0	11.3 ± 0.7
Lungs	1.2 ± 0.3	1.0 ± 0.3	0.6 ± 0.1	0.6 ± 0.1
Muscle	13.4 ± 0.7	12.7 ± 0.2	14.5 ± 1.5	10.2 ± 0.8
Pancreas	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.2
Spleen	0.6 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
Stomach	1.1 ± 1.1	3.9 ± 0.6	6.1 ± 5.1	14.0 ± 5.2
Urine	0.0 ± 0.0	2.7 ± 1.0	4.8 ± 1.2	11.3 ± 1.4
	SUV[b]			
	2 min	**10 min**	**30 min**	**60 min**
Blood	2.5 ± 0.2	1.4 ± 0.2	0.8 ± 0.2	0.4 ± 0.1
Bone	0.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
Brain	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Heart	1.6 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.1 ± 0.1
Kidneys	17.3 ± 0.6	12.5 ± 1.6	5.8 ± 0.5	3.0 ± 0.7
Liver	9.3 ± 0.3	7.9 ± 1.1	3.3 ± 1.1	3.5 ± 0.5
Lungs	1.8 ± 0.3	1.4 ± 0.1	1.2 ± 0.1	1.0 ± 0.1
Muscle	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
Pancreas	1.6 ± 0.3	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.2
Spleen	2.3 ± 0.2	2.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.1

[a] Percentage of injected dose calculated as cpm in organ/total cpm recovered) x 100. [b] SUV calculated as (radioactivity in cpm in organ/weight of organ in grams)/(total cpm recovered/body weight). Data expressed as mean ± SD; n = 3 per time point.

Table S3. Biodistribution data of [^{11}C]NMS-E973 in male NMRI-mice at 2, 10, 30 and 60 min after tracer injection.

	%ID[a]			
	2 min	**10 min**	**30 min**	**60 min**
Blood	8.8 ± 0.6	4.7 ± 0.9	2.1 ± 0.6	1.3 ± 0.1
Bone	2.8 ± 0.3	2.0 ± 0.3	1.8 ± 0.2	1.2 ± 0.1
Brain	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Carcass	15.6 ± 0.7	15.5 ± 1.1	14.8 ± 1.3	10.9 ± 2.3
Heart	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
Intestines	12.2 ± 1.5	35.7 ± 0.4	47.0 ± 0.7	56.2 ± 6.2
Kidneys	14.4 ± 0.6	7.8 ± 1.1	5.2 ± 1.9	2.1 ± 0.4
Liver	48.5 ± 1.8	24.1 ± 3.9	11.0 ± 2.3	7.9 ± 1.5
Lungs	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.1
Muscle	9.8 ± 0.5	8.6 ± 1.0	8.7 ± 1.2	5.3 ± 0.4
Pancreas	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.3 ± 0.0
Spleen	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
Stomach	0.4 ± 0.0	1.1 ± 1.1	0.7 ± 0.4	0.4 ± 0.2
Testes	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
Urine	0.2 ± 0.2	10.6 ± 2.7	16.7 ± 2.4	20.3 ± 3.5
	SUV[b]			
	2 min	**10 min**	**30 min**	**60 min**
Blood	1.3 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
Bone	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Brain	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Heart	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
Kidneys	7.7 ± 0.8	4.4 ± 0.2	2.7 ± 1.1	1.1 ± 0.2
Liver	8.6 ± 0.4	4.4 ± 0.7	2.1 ± 0.1	1.4 ± 0.3
Lungs	0.8 ± 0.1	0.8 ± 0.2	0.5 ± 0.1	0.5 ± 0.2
Muscle	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
Pancreas	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.5 ± 0.1
Spleen	0.6 ± 0.1	0.7 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
Testes	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0

[a] Percentage of injected dose calculated as (cpm in organ/total cpm recovered) x 100. [b] SUV calculated as (radioactivity in cpm in organ/weight of organ in grams)/(total cpm recovered/body weight). Data expressed as mean ± SD; n = 3 per time point.

Table S4. Biodistribution data of [^{11}C]NMS-E973 in male NMRI-mice pretreated with vehicle, NMS-E973 (25mg/kg) or PU-H71 (50 mg/kg) at 10 min after tracer injection.

%ID[a] 10 min p.i.			
	Control	**NMS-E973 pretreatment**	**PU-H71 pretreatment**
Blood	4.9 ± 0.8	2.5 ± 0.2	6.3 ± 0.2
Bone	2.6 ± 0.0	2.6 ± 0.5	3.1 ± 0.5
Brain	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
Carcass	20.0 ± 1.8	17.1 ± 2.2	28.0 ± 1.5
Heart	0.5 ± 0.2	0.3 ± 0.0	0.5 ± 0.1
Intestines	27.0 ± 0.5	33.3 ± 4.0	20.6 ± 0.6
Kidneys	12.8 ± 10.0	5.3 ± 0.4	17.2 ± 6.4
Liver	23.8 ± 2.3	24.6 ± 2.6	18.4 ± 2.6
Lungs	0.7 ± 0.1	0.5 ± 0.1	0.8 ± 0.2
Muscle	11.7 ± 0.4	10.2 ± 1.8	14.6 ± 3.1
Pancreas	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1
Spleen	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
Stomach	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.0
Testes	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Urine	10.8 ± 15.1	15.4 ± 1.7	7.2 ± 7.4
SUV[b] 10 min p.i.			
	Control	**NMS-E973 pretreatment**	**PU-H71 pretreatment**
Blood	0.7 ± 0.1	0.4 ± 0.0	0.9 ± 0.0
Bone	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
Brain	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
Heart	1.0 ± 0.2	0.8 ± 0.0	1.0 ± 0.1
Kidneys	7.0 ± 4.8	3.6 ± 0.2	9.2 ± 3.2
Liver	3.8 ± 0.2	4.3 ± 0.7	2.9 ± 0.4
Lungs	0.9 ± 0.0	0.6 ± 0.0	0.9 ± 0.3
Muscle	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
Pancreas	0.7 ± 0.0	0.7 ± 0.1	0.8 ± 0.1
Spleen	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.1
Testes	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

[a] Percentage of injected dose calculated as (cpm in organ/total cpm recovered) x 100. [b] SUV calculated as (radioactivity in cpm in organ/weight of organ in grams)/(total cpm recovered/body weight). Data expressed as mean ± SD; n = 2 (control) or 3 (NMS-E973 and PU-H71) per time point.

Table S5. Biodistribution data of [^{11}C]NMS-E973 in B16.F10 melanoma inoculated C57BL/6 mice at 60 min after tracer injection with pretreatment of vehicle, PU-H71 or Ganetespib.

	%ID[a]		
	60 min control	**PU-H71 pretreatment**	**Ganetespib pretreatment**
Blood	2.6 ± 0.7	0.6 ± 0.2	0.6 ± 0.3
Bone	2.1 ± 0.0	0.8 ± 0.3	0.7 ± 0.6
Brain	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Carcass	15.2 ± 0.5	9.2 ± 5.3	14.5 ± 8.0
Heart	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
Intestines	43.39 ± 2.0	53.9 ± 7.3	49.4 ± 4.4
Kidneys	1.8 ± 0.2	0.3 ± 0.1	1.2 ± 0.3
Liver	6.8 ± 2.0	8.6 ± 3.1	5.8 ± 2.4
Lungs	0.5 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
Muscle	8.2 ± 1.2	5.1 ± 1.0	6.4 ± 1.4
Pancreas	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.3
Spleen	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Stomach	1.0 ± 0.3	0.3 ± 0.2	0.3 ± 0.1
Tumor	0.8 ± 0.3	1.0 ± 0.4	1.2 ± 0.5
Urine	27.4 ± 3.5	28.9 ± 11.2	25.7 ± 7.4
	SUV[b]		
	60 min control	**PU-H71 pretreatment**	**Ganetespib pretreatment**
Blood	0.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
Bone	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Brain	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Heart	0.6 ± 0.0	0.3 ± 0.1	0.4 ± 0.1
Kidneys	1.4 ± 0.1	0.2 ± 0.0	0.9 ± 0.2
Liver	1.4 ± 0.3	1.6 ± 0.4	1.3 ± 0.6
Lungs	0.6 ± 0.1	0.3 ± 0.1	0.3 ± 0.0
Muscle	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Pancreas	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.2
Spleen	0.4 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
Tumour	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0

[a] Percentage of injected dose calculated as (cpm in organ/total cpm recovered) x 100. [b] SUV calculated as (radioactivity in cpm in organ/weight of organ in grams)/(total cpm recovered/body weight). Data expressed as mean ± SD; n = 3 per time point.

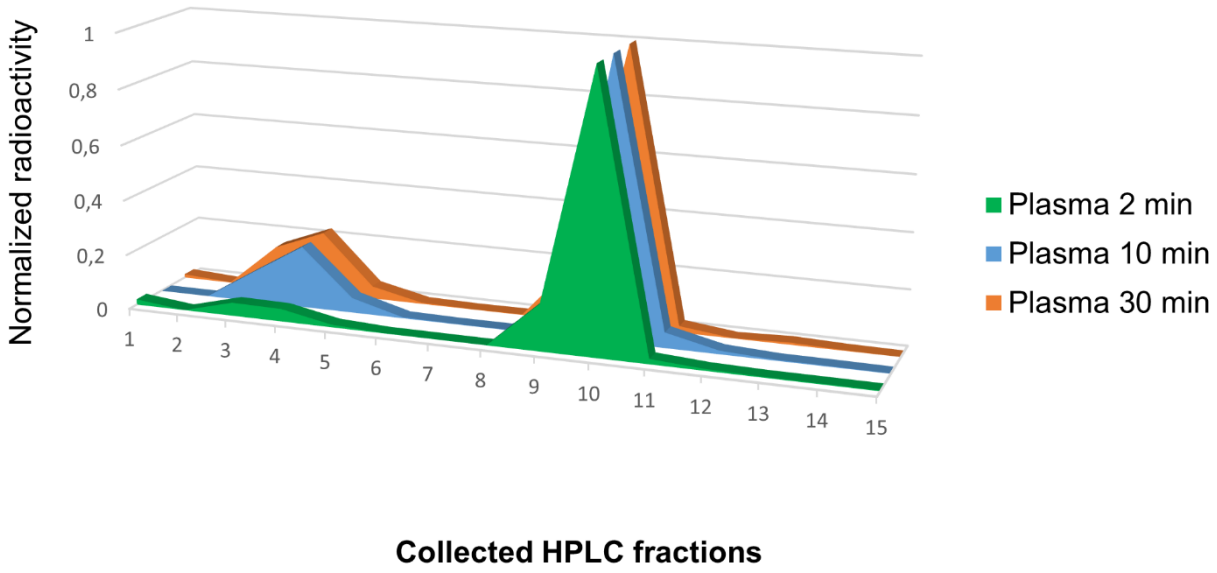

Figure S8. RadioHPLC analysis of plasma samples (radiometabolite study). Polar radiometabolite fractions for respectively 2, 10 and 30 min account for 11 ± 4%, 25 ± 6% and 28 ± 8% of total plasma activity.