Electronic Supplementary Material (ESI) for MedChemComm.

Supporting Information

for

Synthesis and *in vitro* antiproliferative activity of novel pyrazolo[3,4-*d*]pyrimidine Derivatives

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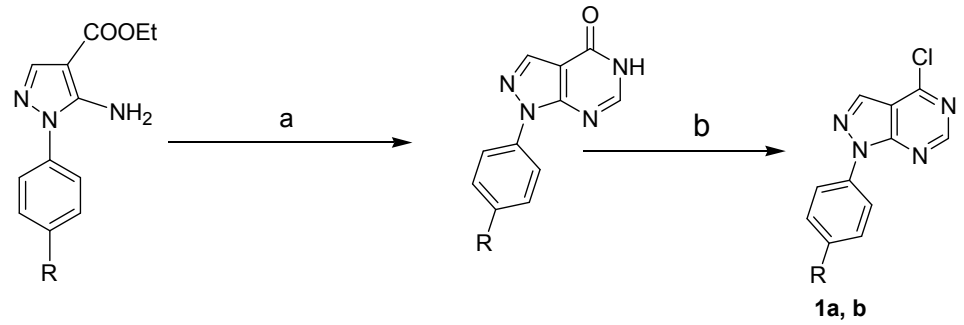
Scheme S1; Synthesis of intermediates 1a, b.



Reagents and conditions:
(a) formamide, reflux, overnight.
(b) $POCl_3$, TEA, reflux, 4 h.

Table (S1): Percentage enzymatic activity change of VEGFR-2 in the presence of synthesized compounds.

Compound ID	% activity change VEGFR-2	Compound ID	% activity change VEGFR-2
3a	-5	**7c**	-5
3b	-3	**7d**	1
3c	-3	**7e**	1
3d	3	**9a**	-5
3e	1	**9c**	0
4a	1	**11a**	7
4b	-3	**11b**	2
7a	-7	**11d**	-4
7b	2	**11e**	-8
		13a	-1

Table (S2): Percentage change of enzymatic activity of EGFR, AURORA, SRC, P38α, C-MET, PDGFR, and HER2 kinases in the presence of selected compounds.

Compound ID	% activity change EGFR	% activity change Aurora-A	% activity change SRC	% activity change P38α	% activity change C-MET	% activity change PDGFR	% activity change HER2
3b	8	-28[b]	-7	-15	ND[a]	ND[a]	ND[a]
3c	ND[a]	ND[a]	ND[a]	ND[a]	-2	-10	0
3d	5	-7	-8	9	ND[a]	ND[a]	ND[a]
3e	-6	6	-2	-18	ND[a]	ND[a]	ND[a]
7a	3	0	9	-4	-16	-24[b]	-17
7d	-1	-11	7	-5	ND[a]	ND[a]	ND[a]
9c	1	0	4	8	ND[a]	ND[a]	ND[a]
11a	4	7	-11	4	1	-9	-4
11b	-33[b]	-14	3	-2	ND[a]	ND[a]	ND[a]
13a	-23[b]	1	1	9	ND[a]	ND[a]	ND[a]

[a]ND = not determined

[b]highlighted underlined values represent the highest results.

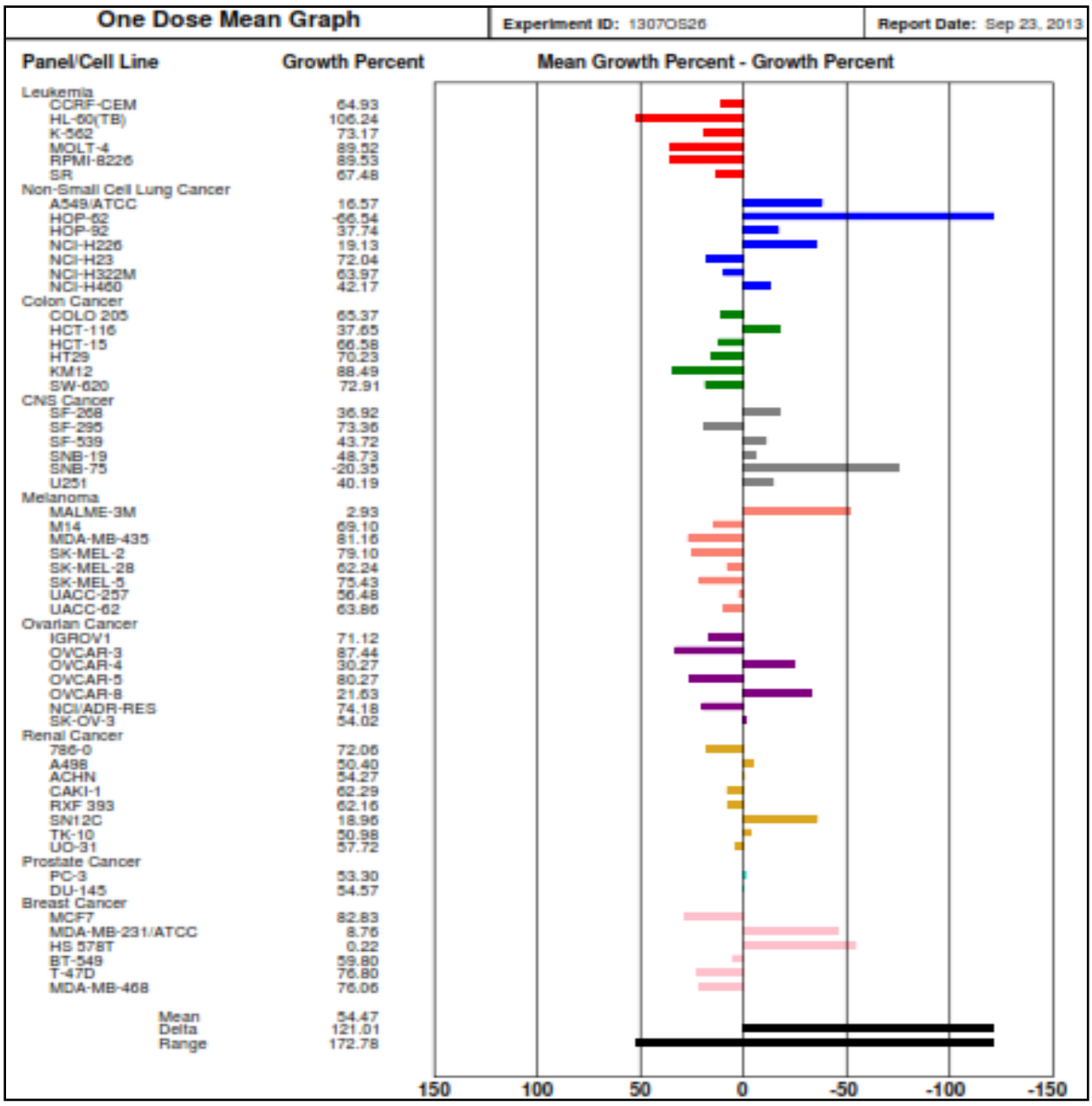


Fig. S1: One dose mean graph of compound (11a) produced from NCI 60 cell line screening program. codes are given for each cell line.[34]

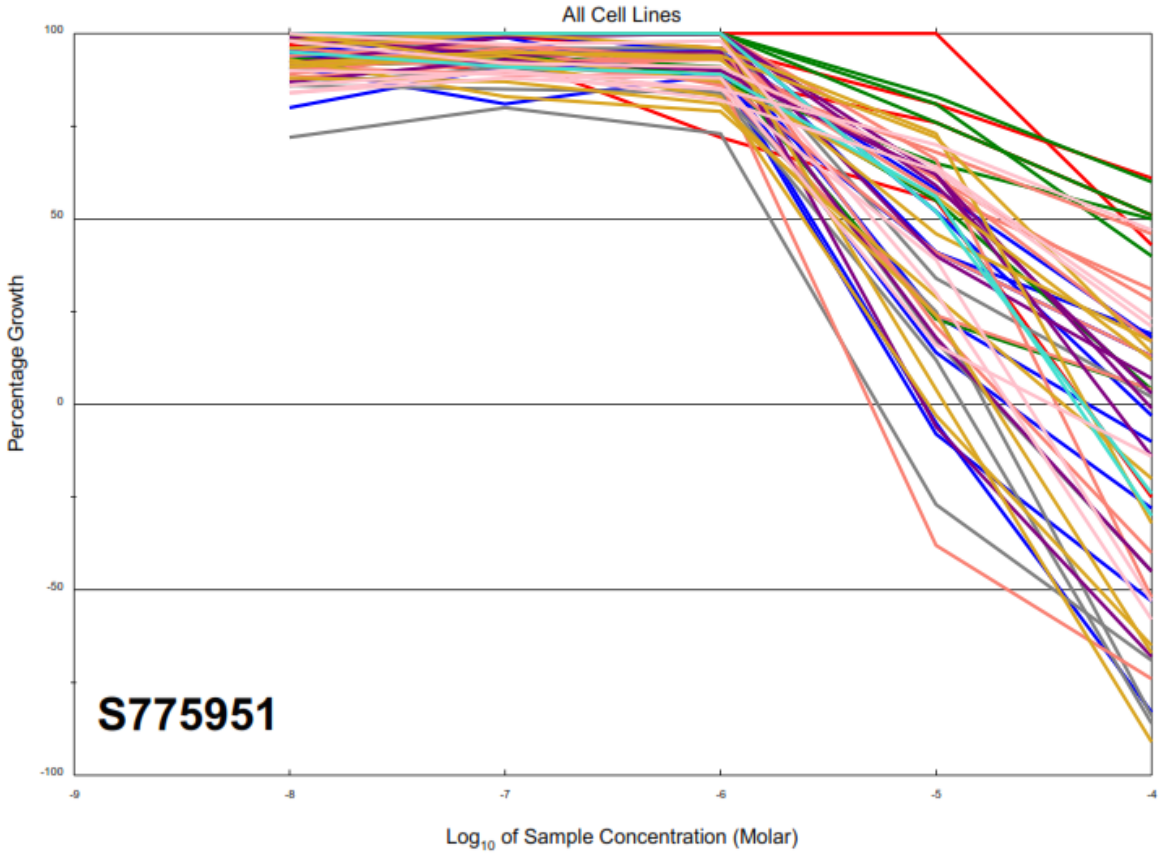
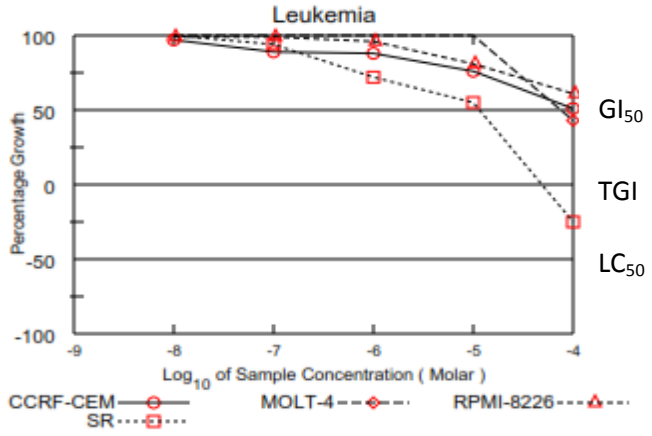
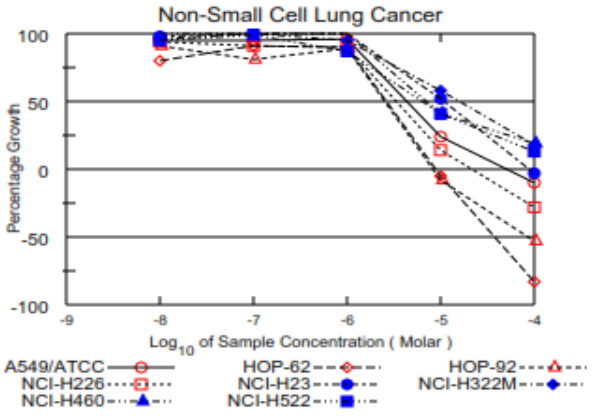


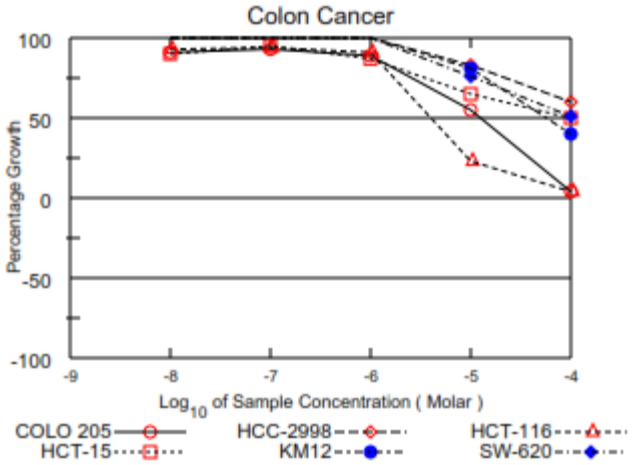
Fig. S2: Collective dose response curves for all NCI 60 cell lines of *in vitro* 5 dose assay



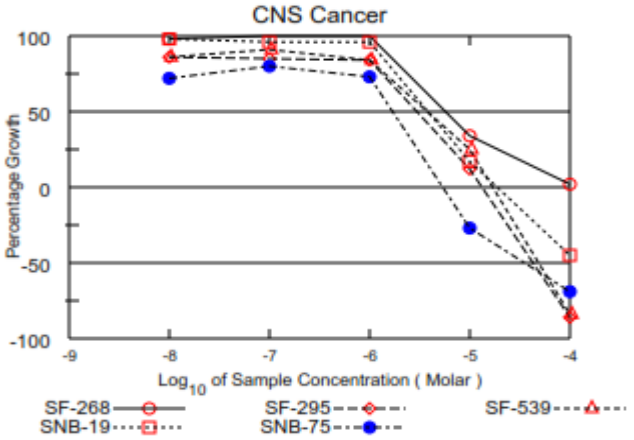
(a)



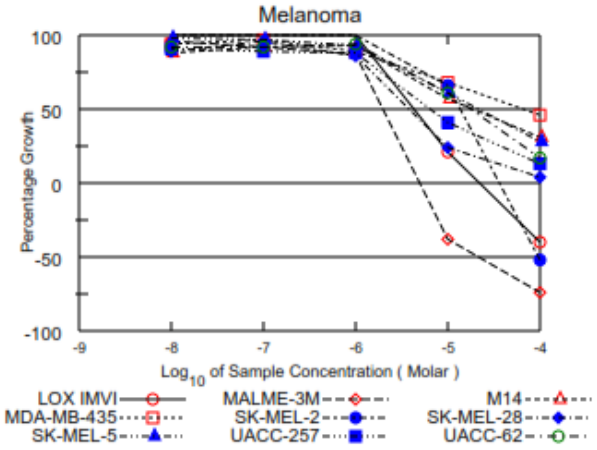
(b)


Colon Cancer
Percentage Growth
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
COLO 205
HCC-2998
HCT-116
HCT-15
KM12
SW-620

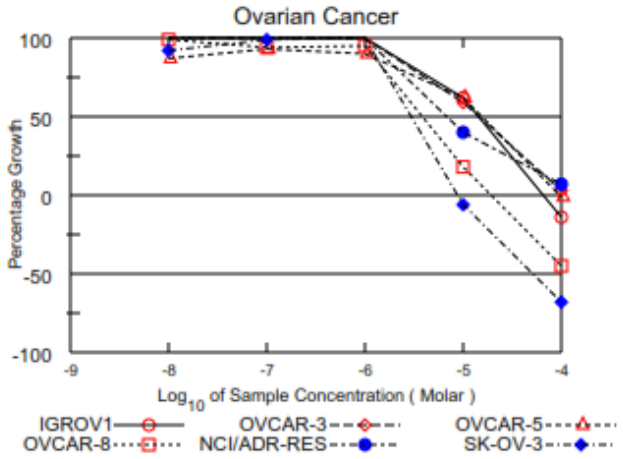
I


CNS Cancer
Percentage Growth
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
SF-268
SF-295
SF-539
SNB-19
SNB-75

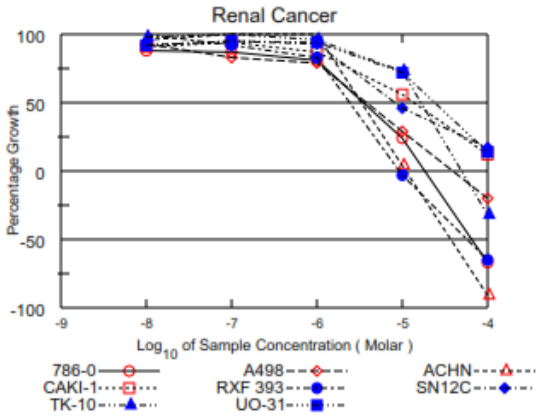
(d)


Melanoma
Percentage Growth
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
LOX IMVI
MALME-3M
M14
MDA-MB-435
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62

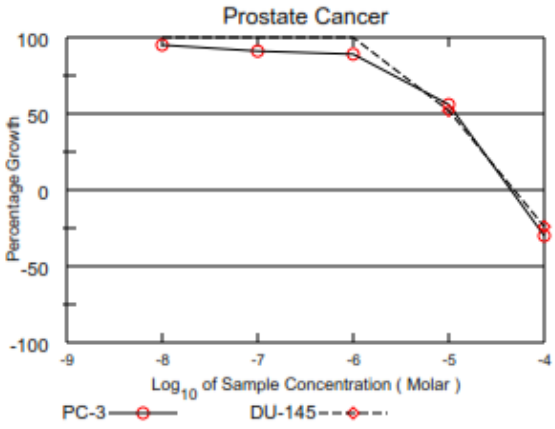
(e)


Ovarian Cancer
Percentage Growth
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
IGROV1
OVCAR-3
OVCAR-5
OVCAR-8
NCI/ADR-RES
SK-OV-3

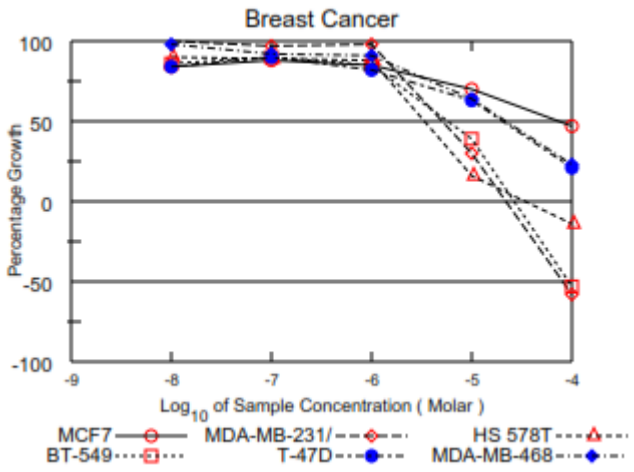
(f)


Renal Cancer
Percentage Growth
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
786-0
A498
ACHN
CAKI-1
RXF 393
SN12C
TK-10
UO-31

(g)


Prostate Cancer
Percentage Growth
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
PC-3
DU-145

(h)



(i)

Fig. S3: Dose response curves of (11a) against (a) leukemia, (b) non-small cell lung cancer, (c) colon cancer cell lines, (d) CNS cancer cell lines, (e) melanoma, (f) ovarian cancer cell lines, (g) renal cancer, (h) prostate cancer, and (i) breast cancer cell lines .

Experimental characterization analysis data for:

1-Compounds (3b-e)

1-(3-Chlorophenyl)-3-(4-((1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)phenyl)urea (3b): Yield 46.38%; m.p.: 197-199 ^{o}C; FT-IR (ύ max, cm^{-1}): 3300 (NH), 1670 (C=O), 1610 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.16 (s, 1H, NH, D_2O exchangeable), 8.90 (s, 1H, NH, D_2O exchangeable), 8.79 (s, 1H, NH, D_2O exchangeable), 8.50 (s, 2H, heterocyclic H) , 8.21 (d, *J* = 8.0 Hz, 2H, ArH), 7.58 -7.72 (m, 3H, ArH), 7.49 – 7.56 (m, 4H, ArH), 7.28 – 7.38 (m, 3H, ArH), 7.02 (t, *J* = 9.0 Hz, 1H, ArH); ^{13}C-NMR (400 MHz,DMSO-d_6): 155.57, 153.18, 152.96, 141.86, 139.05, 134.63, 133.66, 130.87, 129.70, 127.03, 121.79, 121.46, 119.24, 117.85, 116.95, 102.72; MS (Mwt.: 455.13): *m/z* (% rel. Int.) 457.10 (M^{+}+2, 26.67), 456.10 (M^{+}+1, 61.11), 455.10 (M^{+}, 82.22), 288.10 (100); Anal. Calcd for $C_{24}H_{18}ClN_7O$: C, 63.23; H, 3.98; N, 21.51; Found: C, 63.37; H, 3.95; N, 21.64.

1-(3-Bromophenyl)-3-(4-((1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)phenyl)urea (3c): Yield 66.4%; m.p. >280 ^{o}C; FT-IR (ύ max, cm^{-1}): 3320 (NH), 1680 (C=O), 1613 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.15 (s, 1H, NH, D_2O exchangeable), 8.86 (s, 1H, NH, D_2O exchangeable), 8.77 (s, 1H, NH, D_2O exchangeable), 8.50 (s, 2H, heterocyclic H) , 8.21 (d, *J* = 8.0 Hz, 2H, ArH) , 7.87 (s, 1H, ArH), 7.73 (d, *J* = 7.4 Hz, 2H, ArH), 7.59 – 7.49 (m, 4H, ArH), 7.36 – 7.16 (m, 4H, ArH); MS (Mwt.: 499.08): *m/z* (% rel. Int.) 501.10 (M^{+}+2, 17.55), 500.10 (M^{+}+1, 14.42), 499.10 (M+, 17.61), 302.10 (51.92), 91.10 (100); Anal. Calcd for $C_{24}H_{18}BrN_7O$: C, 57.61; H, 3.63; N, 19.60; Found: C, 57.79; H, 3.68; N, 19.82.

1-(4-((1-(4-Chlorophenyl)-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)phenyl)-3-(o-tolyl)urea (3d): Yield 64%; m.p. >300 ^{o}C; FT-IR (ύ max, cm^{-1}): 3305 (NH), 2985 (C-H aliphatic), 1690 (C=O), 1635 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.16 (s, 1H, NH D_2O exchangeable), 9.04 (s, 1H, NH, D_2O exchangeable), 8.50 (s, 2H, heterocyclic H), 8.28 (d, *J* = 6.0 Hz, 2H, ArH), 7.91 (s, 1H, NH, D_2O exchangeable), 7.85 (d, *J* = 7.3 Hz, 1H, ArH), 7.71 (d, *J* = 8.0 Hz, 2H, ArH), 7.62 (d, *J* = 7.4 Hz, 2H, ArH), 7.51 (d, *J* = 7.4 Hz, 2H, ArH), 7.16 – 7.12 (m, 2H, ArH), 6.94 (t, *J* = 6.0 Hz, 1H, ArH), 2.25 (s, 1H, CH_3); MS (Mwt.: 469.14): *m/z* (% rel. Int.) 471.00 (M^{+}+2, 1.39), 469.00 (M^{+}, 4.18), 101.00 (100); Anal. Calcd for $C_{25}H_{20}ClN_7O$: C, 63.90; H, 4.29; N, 20.86; Found: C, 63.97; H, 4.33; N, 20.97.

1-(2-Chlorophenyl)-3-(4-((1-(4-chlorophenyl)-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino) phenyl) urea (3e): Yield 48.6%; m.p. change in colour to brown at 250 ^{o}C and charring at 270 ^{o}C; FT-IR (ύ max, cm^{-1}): 3300 (NH), 1670 (C=O), 1615 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.18 (s, 1H, NH, D_2O exchangeable), 9.44 (s, 1H, NH, D_2O exchangeable), 8.51 (s, 2H, heterocyclic H), 8.30 - 8.27 (m, 3H, NH and 2ArH), 8.18 (d, *J* = 8.0 Hz, 1H, ArH), 7.74 (d, *J* = 6.0 Hz, 2H, ArH), 7.63 (d, *J* = 7.4 Hz, 2H, ArH), 7.52 (d, *J* = 6.0 Hz, 2H, ArH), 7.45 (d, *J* = 7.4 Hz, 1H, ArH), 7.30 (t, *J* = 6.0 Hz, 1H, ArH), 7.03 (t, *J* = 6.0 Hz, 1H, ArH); MS (Mwt.: 489.09): *m/z* (% rel. Int.) 491.10 (M^{+}+2, 8.94), 490.01 (M^{+}+1, 3.31), 489.10 (M+, 13.99), 336.15 (54.19), 151 (100); Anal. Calcd for $C_{24}H_{17}Cl_2N_7O$: C, 58.79; H, 3.49; N, 20.00; Found: C, 58.86; H, 3.53; N, 20.12.

2-Compounds (7b-e)

3-(3-Bromobenzyl)-6-((1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)benzo[*d*]thiazol-2(3H)-one (7b): Yield 55.7%; m.p. 230 ^{o}C; FT-IR (ύ max, cm^{-1}): 3320 (NH), 1690 (C=O), 1620 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.30 (s, 1H, NH, D_2O exchangeable), 8.51 (s, 2H, heterocyclic H), 8.27 (s. 1H, ArH), 8.19 (d, *J* = 7.6 Hz, 2H, ArH), 7.64 – 7.50 (m, 5H, ArH), 7.36 – 7.31 (m, 4H, ArH), 5.21 (s, 2H, aliphatic CH_2); MS (Mwt.: 528.04): *m/z* (% rel. Int.) 530.05 (M^{+}+2, 54.78), 529.10 (M^{+}+1, 19.92), 528.05 (M^{+}, (51.14), 359.05 (100); Anal. Calcd for $C_{25}H_{17}BrN_6OS$: C, 56.72; H, 3.24; N, 15.87; Found: C, 56.88; H, 3.25; N, 15.99.

3-(2,4-Dichlorobenzyl)-6-((1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)benzo[*d*]thiazol-2(3H)-one (7c):Yield 20%; m.p. 260 ^{o}C; FT-IR (ύ max, cm^{-1}): 3330 (NH), 1685 (C=O), 1630 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.29 (s, 1H, NH, D_2O exchangeable), 8.51 (s, 2H, heterocyclic H), 8.31 (s. 1H, ArH), 8.20 (d, *J* = 6.0 Hz, 2H, ArH), 7.61 – 7.53 (m, 4H, ArH), 7.37 (d, *J* = 6.0 Hz, 1H, ArH), 7.16 – 7.01 (m, 3 H, ArH), 5.21 (s, 2H, aliphatic CH_2); MS (Mwt.: 518.05): *m/z* (% rel. Int.) 520.10 (M^{+}+2, 24.74), 519.10 (M^{+}+1, 20.91), 518.10 (M^{+}, 38.59%), 517 (M^{+}-1, 35.89), 395.10 (82.93), 143.00 (100); Anal. Calcd for $C_{25}H_{16}Cl_2N_6OS$: C, 57.81; H, 3.10; N, 16.18; Found: C, 57.92; H, 3.12; N, 16.34.

3-(3,4-Dichlorobenzyl)-6-((1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino) benzo[d]thiazol-2(3H)-one (7d): Yield (70.7%); m.p. 255 ^{o}C; FT-IR (ύ max, cm^{-1}): 3305 (NH), 1680 (C=O), 1635 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.30 (s, 1H, NH, D_2O

exchangeable), 8.51 (s, 2H, heterocyclic H), 8.28 (s. 1H, ArH), 8.20 (d, *J* = 8.0 Hz, 2H, ArH), 7.66 – 7.54 (m, 5H, ArH), 7.38 – 7.35 (m, 2H, ArH), 7.24 (d, *J* = 7.6 Hz, 1 H, ArH), 5.21 (s, 2H, aliphatic CH_2); ^{13}C-NMR (400 MHz,DMSO-d_6): 169.60, 156.58, 153.52, 139.18, 137.49, 135.18, 134.17, 131.81, 131.54, 130.90, 129.89, 129.68, 127.88, 126.87, 122.23, 121.28, 117.02, 112.19, 102.80, 44.71; MS (Mwt.: 518.05): *m/z* (% rel. Int.) 520.20 (M^++2, 24.19), 519.20 (M^++1, 12.07), 518.20 (M^+, 31.64), 359 (95.32), 159 (100); Anal. Calcd for $C_{25}H_{16}Cl_2N_6OS$: C, 57.81; H, 3.10; N, 16.18; Found: C, 57.93; H, 3.14; N, 16.32.

6-((1-(4-Chlorophenyl)-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)-3-(2,4-dichlorobenzyl) benzo[*d*]thiazol-2(3H)-one (7e): Yield 66.06%; m.p. 267-268 ^{o}C; FT-IR (ύ max, cm^{-1}): 3300 (NH), 1670 (C=O), 1640 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.33 (s, 1H, NH, D_2O exchangeable), 8.53 (s, 2H, heterocyclic H), 8.30 (s. 1H, ArH), 8.28 (d, *J* = 7.6 Hz, 2H, ArH), 7.64 – 7.54 (m, 4H, ArH), 7.20 – 7.17 (m, 2H, ArH), 7.04 (d, *J* = 7.3 Hz, 1 H, ArH), 5.22 (s, 2H, aliphatic CH_2); MS (Mwt.: 552.01): *m/z* (% rel. Int.) 554.10 (M^++2, 19.53), 552.10 (M^+, 19.78),143 (100); Anal. Calcd for $C_{25}H_{15}Cl_3N_6OS$: C, 54.21; H, 2.73; N, 15.17; Found: C, 54.33; H, 2.71; N, 15.34.

3-Compounds (9b-d)

N-(4-((4-Chlorobenzyl)oxy)phenyl)-1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-amine (9b): Yield 64%; m.p. 212-214 ^{o}C; FT-IR (ύ max, cm^{-1}): 3315 (NH), 1635 (C=N), ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.11 (s, 1H, NH, D_2O exchangeable), 8.47 (s, 2H, heterocyclic H), 8.20 (d, *J* = 6.0 Hz, 2H, ArH), 7.70 (d, *J* = 7.6 Hz, 2H, ArH), 7.58 – 7.44 (m, 6 H, ArH), 7.35 (t, *J* = 6.0 Hz, 1H, ArH), 7.07 (d, *J* = 7.3 Hz, 2H, ArH), 5.13 (s, 2H, aliphatic CH_2); MS (Mwt.: 427.89): *m/z* (% rel. Int.) 429.05 (M^++2, 4.88), 427.05 (M^+, 14.64), 302.15 (100), 125.05 (66.27); Anal. Calcd for $C_{24}H_{18}ClN_5O$: C, 67.37; H, 4.24; N, 16.37; Found: C, 67.52; H, 4.36; N, 16.36.

N-(4-(Benzyloxy)phenyl)-1-(4-chlorophenyl)-1H-pyrazolo[3,4-*d*]pyrimidin-4-amine (9c): Yield 53%; m.p. 200-202 ^{o}C; FT-IR (ύ max, cm^{-1}): 3300 (NH), 1620 (C=N), ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.11 (s, 1H, NH, D_2O exchangeable), 8.47 (s, 2H, heterocyclic H), 8.26 (d, *J* = 7.6 Hz, 2H, ArH), 7.68 (d, *J* =7.6 Hz, 2H, ArH), 7.62 (d, *J* =7.6 Hz, 2H, ArH), 7.48 – 7.31 (m, 5 H, ArH), 7.07 (d, *J* = 7.4 Hz, 2H, ArH), 5.12 (s, 2H, aliphatic CH_2); ^{13}C-NMR (400

MHz,DMSO-d_6): 156.77, 155.49, 153.64, 138.15, 137.58, 134.56, 132.22, 130.69, 129.61, 128.89, 128.28, 128.16, 123.78, 122.41, 115.43, 102.70, 69.90; MS (Mwt.: 427.89): *m/z* (% rel. Int.) 429.05 (M^++2, 8.72), 428.10 (M^++1, 8.01), 427.05 (M^+, 26.18), 336.05 (88.80), 91.05 (100); Anal. Calcd for $C_{24}H_{18}ClN_5O$: C, 67.37; H, 4.24; N, 16.37; Found: C, 67.45; H, 4.27; N, 16.51.

N-(4-((4-Chlorobenzyl)oxy)phenyl)-1-(4-chlorophenyl)-1H-pyrazolo[3,4-*d*]pyrimidin-4-amine (9d): Yield 42%; m.p. 205-207 ^oC; FT-IR (ύ max, cm^{-1}): 3305 (NH), 1640 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.16 (s, 1H, NH, D_2O exchangeable), 8.49 (s, 2H, heterocyclic H), 8.27 (d, *J* = 7.4 Hz, 2H, ArH), 7.70 – 7.62 (m, 4H, ArH), 7.52 – 7.45 (m, 4H, ArH), 7.07 (d, *J* = 6.0 Hz, 2H, ArH), 5.13 (s, 2H, aliphatic CH_2); MS (Mwt.: 461.08): *m/z* (% rel. Int.) 463.20 (M^++2, 3.69), 462.20 (M^++1, 2.69), 461.20 (M^+, 6.08), 336.20 (34.35), 125.05 (100); Anal. Calcd for $C_{24}H_{17}Cl_2N_5O$: C, 62.35; H, 3.71; N, 15.15; Found: C, 62.52; H, 3.69; N, 15.18.

4-Compounds (11b-f)

N-(3-Chlorophenyl)-4-((1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)benzamide (11b): Yield 57.7%; m.p. 244-256 ^oC; FT-IR (ύ max, cm^{-1}): 1708 (C=O), 1660 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.47 (s, 1H, NH D_2O exchangeable), 10.31 (s, 1H, NH, D_2O exchangeable), 8.65 (s, 1H, heterocyclic H), 8.63 (s, 1H, heterocyclic H), 8.22 (d, *J* = 6.0 Hz, 2H, ArH), 8.10 – 7.98 (m, 5H, ArH), 7.72 (d, *J* = 6.0 Hz, 1H, ArH), 7.58 (t, *J* = 6.0 Hz, 2H, ArH), 7.38 (t, *J* = 6.0 Hz, 2H, ArH), 7.61 (d, *J* = 6.0 Hz, 1H, ArH); MS (Mwt.: 440.88): *m/z* (% rel. Int.) 442.10 (M^++2, 4.23), 440.10 (M^+, 12.70), 314.10 (100); Anal. Calcd for $C_{24}H_{17}ClN_6O$: C, 65.38; H, 3.89; N, 19.06; Found: C, 65.44; H, 3.92; N, 19.18.

N-(4-Chloro-3-(trifluoromethyl)phenyl)-4-((1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)benzamide (11c): Yield 19%; m.p. 124-126 ^oC; FT-IR (ύ max, cm^{-1}): 3300 (N-H), 1670 (C=O), 1610 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.55 (s, 1H, NH D_2O exchangeable), 10.49 (s, 1H, NH D_2O exchangeable), 8.66 (s, 1H, heterocyclic H), 8.64 (s, 1H, heterocyclic H), 8.38 (s, 1H, ArH), 8.22 (d, *J* = 6.0 Hz, 2H, ArH), 8.14 – 8.03 (m, 5H, ArH), 7.72 (d, *J* = 7.6 Hz, 1H, ArH), 7.58 (t, *J* = 6.0 Hz, 2H, ArH), 7.37 (t, *J* = 6.0 Hz, 1H, ArH); MS (Mwt.: 508.88): *m/z* (% rel. Int.) 510.10 (M^++2, 6.40), 508.10 (M^+, 19.18), 314.15 (100); Anal. Calcd for $C_{25}H_{16}ClF_3N_6O$: C, 59.01; H, 3.17; N, 16.51; Found: C, 58.95; H, 3.17; N, 16.52.

4-((1-(4-Chlorophenyl)-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)-N-cyclohexylbenzamide (11d): Yield 68.6%; m.p. >300 ^{o}C; FT-IR (ύ max, cm^{-1}): 2985 (CH aliphatic),3310 (N-H), 1708 (C=O), 1675 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.38 (s, 1H, NH, D_2O exchangeable), 8.61 (s, 1H, heterocyclic H), 8.60 (s, 1H, heterocyclic H), 8.27 (d, *J* = 7.6 Hz, 2H, ArH), 7.92 (dd, *J* = 6.0, 1.8 Hz, 4H, ArH), 7.62 (d, *J* = 7.6 Hz, 2H, ArH), 3.77 (p, 1H, NH-CH-$(CH_2)_2$), 1.82 – 1.59 (m, 6H, cyclohexyl), 1.31 (p, 4H, cyclohexyl); MS (Mwt.: 446.93): *m/z* (% rel. Int.) 448.15 (M^{+}+2, 15.97), 446.15 (M^{+}, 47.89), 364.05 (100), 363.10 (97.87), 348.05 (72.36); Anal. Calcd for $C_{24}H_{23}ClN_6O$: C, 64.50; H, 5.19; N, 18.80; Found: C, 64.63; H, 5.16; N, 18.94.

N-(2-Chlorophenyl)-4-((1-(4-chlorophenyl)-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)benzamide (11e):Yield 65.69%; m.p Charring at 260 ^{o}C; FT-IR (ύ max, cm^{-1}): 3325 (N-H), 1670 (C=O), 1615 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.49 (s, 1H, NH, D_2O exchangeable), 10.93 (s, 1H, NH, D_2O exchangeable), 8.66 (s, 1H, heterocyclic H), 8.64 (s, 1H, heterocyclic H), 8.28 (d, *J* = 7.6 Hz, 2H, ArH), 8.06 – 7.97 (m, 4H, ArH), 7.65 – 7.56 (m, 3H, ArH), 7.56 (d, *J* = 6.0 Hz, 1H, ArH), 7.39 (t, *J* = 6.0 Hz, 1H, ArH), 7.29 (t, *J* = 6.0 Hz, 1H, ArH); MS (Mwt.: 474.08): *m/z* (% rel. Int.) 476.00 (M^{+}+2, 20.60), 475.00 (M^{+}+1, 7.93), 474.00 (M^{+}, 30.50), 348.00 (100); Anal. Calcd for $C_{24}H_{16}Cl_2N_6O$: C, 60.64; H, 3.39; N, 17.68; Found: C, 60.68; H, 3.43; N, 17.82.

N-(4-Chloro-3-(trifluoromethyl)phenyl)-4-((1-(4-chlorophenyl)-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)benzamide (11f): Yield 60%; m.p 130-132 ^{o}C; FT-IR (ύ max, cm^{-1}): 3305 (N-H), 1690 (C=O), 1635 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 10.54 (s, 1H, NH D_2O exchangeable), 10.50 (s, 1H, NH D_2O exchangeable), 8.66 (s, 1H, heterocyclic H), 8.65 (s, 1H, heterocyclic H), 8.38 (s, 1H, ArH), 8.28 (d, *J* = 6.0 Hz, 2H, ArH), 8.14 – 8.02 (m, 5H, ArH), 7.71 (d, *J* = 7.6 Hz, 1H, ArH), 7.64 (d, *J* = 6.0 Hz, 2H, ArH); MS (Mwt.: 542.06): *m/z* (% rel. Int.) 544.10 (M^{+}+2, 5.87), 543.10 (M^{+}+1, 2.48), 542.10 (M^{+}, 9.11), 348.15 (100); Anal. Calcd for $C_{25}H_{15}Cl_2F_3N_6O$: C, 55.26; H, 2.78; N, 15.47; Found: C, 55.37; H, 2.59; N, 15.66.

5-Compounds (13b-d)

5,6-Dimethyl-N-(1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)benzo[d]thiazol-2-amine (13b): Yield 10.7%; m.p.: 290-292 ^{o}C; FT-IR (ύ max, cm^{-1}): 3310 (N-H), 2975 (CH aliphatic), 1675 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 9.27 (s, 1H, heterocyclic H), 8.82 (s, 1H, heterocyclic H), 8.60 (s, 1H, ArH), 8.13 (d, *J* = 8.0 Hz, 2H, ArH), 7.73 – 7.42 (m, 4H, ArH), 2.37 (s, 6H, CH_3 aliphatic); MS (Mwt.: 372.45): *m/z* (% rel. Int.) 373.10 (M^++1, 7.01), 372.15 (M^+, 31.98), 77.15 (100); Anal. Calcd for $C_{20}H_{16}N_6S$: C, 64.50; H, 4.33; N, 22.56; Found: C, 64.50; H, 4.45; N, 22.73.

6-Chloro-N-(1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)benzo[d]thiazol-2-amine (13c): Yield 12.6%; m.p.: 278-282 ^{o}C; FT-IR (ύ max, cm^{-1}): 3300 (N-H), 1655 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, ppm 9.27 (s, 1H, heterocyclic H), 8.83 (s, 1H, heterocyclic H), 8.60 (s, 1H, ArH), 8.21 – 8.05 (m, 4H, ArH), 7.65 – 7.49 (m, 3H, ArH); MS (Mwt.: 378.84): *m/z* (% rel. Int.) 380.05 (M^++2, 4.56), 379.05 (M^++1, 2.60), 378.10 (M^+, 13.68), 77.10 (100); Anal. Calcd for $C_{18}H_{11}ClN_6S$: C, 57.07; H, 2.93; N, 22.18; Found: C, 57.12; H, 3.01; N, 22.33.

6-Chloro-N-(1-(4-chlorophenyl)-1H-pyrazolo[3,4-*d*]pyrimidin-4-yl)benzo[*d*]thiazol-2-amine (13d): Yield 10.2%; m.p.: 289-291 ^{o}C; FT-IR (ύ max, cm^{-1}): 3310 (N-H), 1640 (C=N); ^{1}H-NMR (300 MHz, *DMSO-d_6*) δ, 12.48 (s, 1H,NH, D_2O exchangeable), 8.71 (s, 1H, heterocyclic H), 8.52 (s, 1H, heterocyclic H), 8.34 (s, 1H, ArH), 8.26 – 8.07 (m, 3H, ArH), 7.66 – 7.62 (m, 3H, ArH); MS (Mwt.: 412.01): *m/z* (% rel. Int.) 414.10 (M^++2, 10.76), 413.10 (M^++1, 3.01), 412.15 (M^+, 15.73), 77.05 (100); Anal. Calcd for $C_{18}H_{10}Cl_2N_6S$: C, 52.31; H, 2.44; N, 20.33; Found: C, 52.30; H, 2.63; N, 20.30.

Experimental procedure for biological assays

3.2.2.2. Cell Culture

Human lung cancer cell line A549 and Human breast cancer cell line (MCF-7) were obtained from the National Cancer Institute (Cairo, Egypt). A 549 cells were maintained in DMEM medium while MCF-7 was maintained in RPMI-1640 medium. Both media were supplemented with 100 mg/ mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37 ºC.

3.2.2.3. SRB Cytotoxicity Assay

The tested compound was dissolved in DMSO and kept at a stock concentration of 100 mM. Seeding was done at a density of 3,000 cells/well in 96-well plates and the cells were incubated overnight to allow for attachment. The cells were exposed to the tested agent for 72 h during which 5 different drug concentrations (0-10^3 μM) were tested. Cytotoxicity was assessed at the end of drug exposure using SRB assay as described previously.[43] Absorbance was measured at 545 nm using a microplate reader (ChroMate- 4300, FL, USA). Results were expressed as the relative percentage of absorbance compared to control. Experimental conditions were tested using six replicates (six wells of the 96-well plate per experimental condition) and all experiments were performed in triplicates. Half-maximal inhibitory concentration (IC_{50}), the drug concentration at which 50% growth inhibition is achieved, was calculated using Sigma Plot software, version 12.0 (Systat Software, San Jose, CA).

3.2.2.6. Western Blot Analysis

Primary antibody against $p27^{kip}$ (Cell Signaling, Danvers, MA) was used to assess the protein expression of this marker in the tested cells as described previously[44]. Cells were seeded, cultured and exposed to the tested agent for 72 h. Whole-cell protein lysates were prepared according to standard protocol using RIPA buffer (Cell Signaling, Danvers, MA). Protein (50 mg) was loaded per well of a 10% SDS-PAGE gel using electrophoresis buffer (0.192 M glycine, 25 mM Tris and 0.1% SDS). After electrophoresis, the gel was transferred onto a PVDF membrane (Bio-Rad Laboratorie, Hercules, CA) using transfer buffer (0.192 M glycine, 25 mM Tris, 0.025% SDS and 10% methanol). Membranes were blocked in TBS-T with 5% BSA and incubated overnight with the primary antibody (1:1,000) then incubated with secondary HRP-linked antibody (1:5,000). Development was done using Optiplot chemiluminescent substrate Abcam plc, Cambridge, MA). Anti-β-actin antibody (dilution, 1: 5000; Abcam plc, Cambridge, MA) was used to for loading correction.