Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Pyrimido[4,5-*d*]pyrimidin-4(1*H*)-one Derivatives as Selective Inhibitors of EGFR Threonine^{790} to Methionine^{790} (T790M) Mutants**

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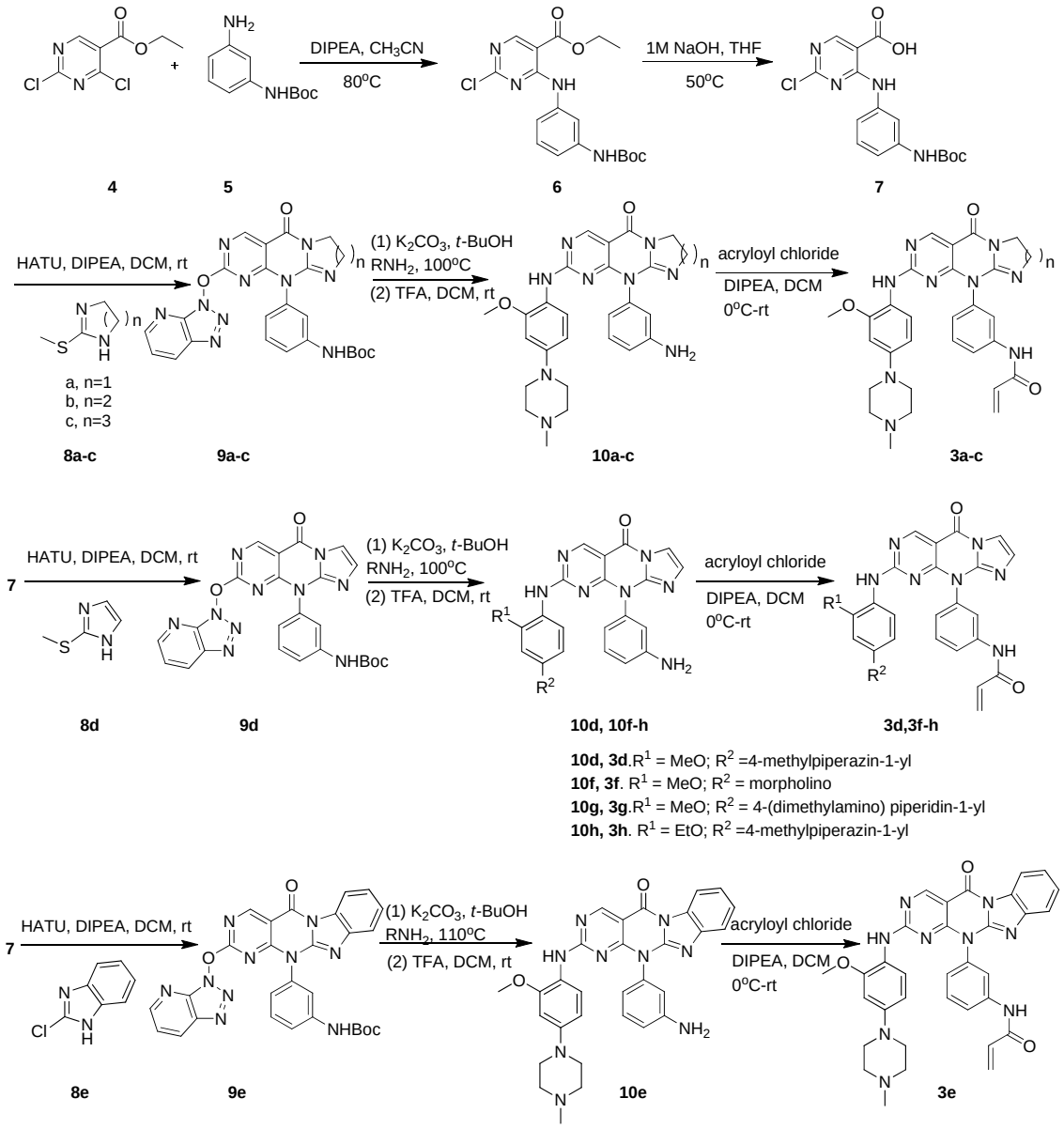
General Information

^{1}H NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 MHz. Chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to residual undeuterated solvent. The following abbreviations were used to describe peak splitting patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad signal), dd (doublet of doublets). Coupling constants (*J*) are expressed in hertz unit (Hz). High resolution mass spectra (HRMS) were obtained on a Q-STAR Elite ESI-LC-MS/MS Spectrometer. The purity of compounds was determined by reverse-phase high performance liquid chromatography (HPLC) analysis to be over 95% (>95%). HPLC instrument: Dionex Summit HPLC (Column: Diamonsil C18, 5.0 μm, 4.6× 250 mm (Dikma Technologies); detector: PDA-100 photodiode array; injector: ASI-100 autoinjector; pump: p-680A). Elution: MeOH in water; flow rate: 1.0 mL/min. Elemental analysis was used to determine the purity of the described compounds (SunYat-sen University, China). Where molecular formulas are given, elemental compositions were found to be within 0.4% of the theoretical values. The purities of the compounds were confirmed over 95% (≥ 95%). All reagents were purchased from suppliers without further purification.

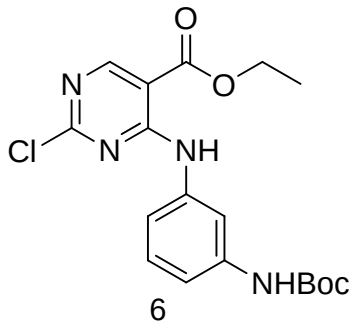
Synthesis of 3a-3h

The synthesis of designed compounds **3** was outlined in Scheme S1. Briefly, a direct nucleophilic coupling of commercially available ethyl 2, 4-dichloropyrimidine- 5-carboxylate (**4**) with *tert*-butyl 3-aminophenylcarbamate (**5**) produced ethyl 4-((3-((*tert*-butoxycarbonyl)amino)phenyl)amino)-2-chlor -opyrimidine-5-carboxylate (**6**) in 82% yield. Hydrolysis of compound **6** with 1 M NaOH in a H_2O-THF mixed solution yielded the carboxylic acid (**7**). The condensation of **7** and **8a-8e** in the presence of HATU and DIPEA in dry DCM gave the intermediates **9a-9e**, respectively. Compounds **9a-9e** were coupled with different substituted anilines *via* nucleophilic substitution and followed by deprotection with 50% trifluoroacetic acid in DCM to yield the key precursors **10a-10h**. The conformation-constrained EGFR inhibitors **3a**–**3h** were finally obtained by acryloylation of **10a-10h** with acryloyl chloride.

Scheme S1. Chemical synthesis of compounds **3a-3h**.

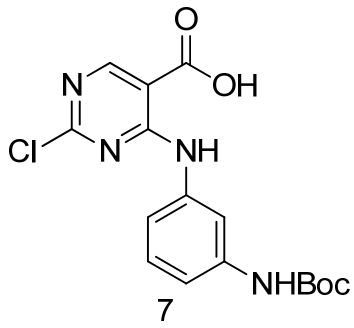


The synthetic procedures and characterization data of **3a-3h**



Ethyl 4-((3-((*tert*-butoxycarbonyl)amino)phenyl)amino)-2-chloropyrimidine -5-carboxylate(6)

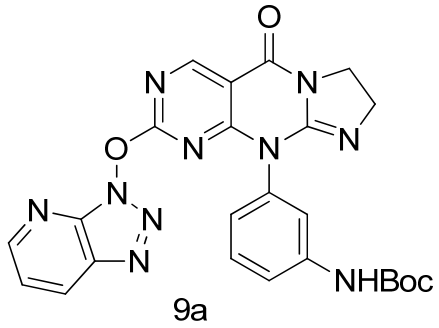
A mixture of ethyl 2, 4-dichlo-ropyrimidine-5-carboxylate (22.1g, 100mmol), tert-butyl (3-aminophenyl)carbamate (20.8g, 100mmol), and diisopropyl-ethyl amine(17.4 mL, 100mmol) in CH_3CN (500 mL) was refluxed for 2 hrs.[1] After being cooled to room temperature, the precipitate was filtered to give **9** as a white solid (32.2g, 82%). ^{1}H NMR (400Hz, $CDCl_3$) δ 10.44 (s, 1H), 8.82 (s, 1H), 7.79 (s, 1H), 7.37 (d, J = 7.6 Hz , 1H), 7.29 (t, J = 8.0Hz, 1H,), 7.18 (d, J=8.0Hz, 1H,), 6.55 (s, 1H), 4.45 (q, J=7.2Hz, 2H), 1.53 (s, 9H), 1.43 (t, J=7.2Hz, 3H).



7

4-((3-((*tert*-butoxycarbonyl)amino)phenyl)amino)-2-chloropyrimidine-5-carboxylic acid (7)

A solution of **6** (3.92g, 10mmol) in THF and 1 M NaOH (20mL, 20mmol) was stirred at 50 ^{o}C for 4 hrs, then the solvent was partly removed under reduced pressure. The solution was acidified with 1 M HCl (25mL, 25mmol) and cooled to give a solid, which was collected by filtration and dried in a vacuum oven to give **7** as a white solid (3.57g, 98%). ^{1}H NMR (400Hz, DMSO- d_6) δ 10.56 (s, 1H), 9.47 (s, 1H), 8.77 (s, 1H), 7.68 (t, J=2.0Hz, 1H), 7.39 (d, J=8.0Hz, 1H), 7.28 (t, J=8.0Hz, 1H), 7.24 (d, J=8.4Hz, 1H), 1.48 (s, 9H).

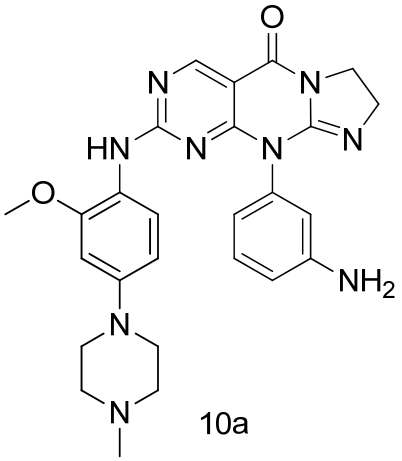


9a

***tert*-butyl(3-(2-((3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)oxy)-5-oxo-7,8-dihydroimidazo[1,2-a]pyrimido[4,5-d]pyrimidin-10(5*H*)-yl)phenyl)carbamate (9a)**

A mixture of **7** (91.2mg, 0.178mmol), HATU (190.11mg, 0.356mmol) and diisopropyl-ethyl amine (0.13ml, 0.534mmol) in DCM (2 mL) was stirred for 0.5 hr at room temperature, then 2-(methylthio)-4,5-dihydro-1*H*-imidazole (20.68mg, 0.178mmol) was added to the mixture. The reaction mixture was stirred for 24 hrs at room temperature before being partitioned between water and DCM. The organic layer was separated, dried over $MgSO_4$ and concentrated, and the crude product was purified by flash silica gel chromatography with dichloromethane/methanol (200/1 to 150/1, v/v) to give **9a** as a white solid (75mg, 58%). ^{1}H NMR (400Hz, DMSO- d_6) δ 9.47 (s, 1H), 8.85 (s, 1H), 8.68(dd, J=1.2Hz, 4.4Hz, 1H) , 8.56 (dd, J=0.8Hz, 8.0Hz, 1H), 7.56 (dd, J=4.4Hz, 8Hz, 1H),

7.37 (s, 1H), 7.23 (d, *J*=8.4Hz, 1H) 7.09 (t. *J*=8.0Hz, 1H) , 6.65 (d, *J*=8.0Hz, 1H), 3.97 (t, *J*=8.8Hz, 2H), 3.72 (t, *J*=8.8Hz, 2H), 1.51 (s,9H).

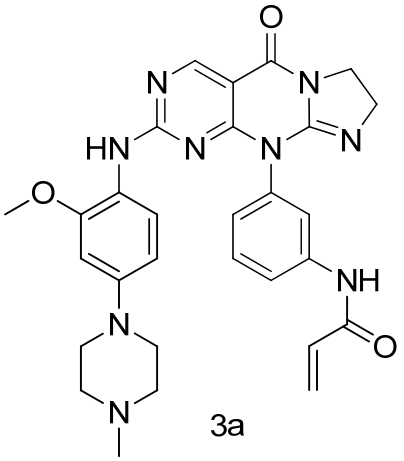


10a

10-(3-aminophenyl)-2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-7,8-dihydroimidazo[1,2-a]pyrimido[4,5-d]pyrimidin-5(10*H*)-one (10a)

To a solution of compound **9a** (257mg, 0.5mmol) in *tert*-Butanol (5 mL) were added 2-methoxy-4-(4-methylpiper azin-1-yl)aniline (110.6mg, 0.5 mmol) and potassium carbonate (207.3mg, 1.5mmol). The reaction mixture was stirred for 6 hrs at 100 ° C in a sealed tube, then the solvent was removed under reduced pressure. The residue was partitioned between water and dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give the crude product which was used without further purification.

To a mixture of the crude product in dichloromethane (2 mL) was added trifluoroaceticacid (TFA, 2mL). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, and neutralized the residue by slow addition of saturated $NaHCO_3$. The precipitate formed was collected by filtration and washed with water. The resulting crude product was purified by silica gel chromatography with dichloromethane/methanol (60/1 to 30/1, v/v) to give **10a** as a yellow solid (174.8mg, 70%). ^{1}H NMR (400Hz, $CDCl_3$) δ 8.80 (s, 1H), 7.96(s, 1H), 7.55(d, *J*=8.8Hz, 1H), 7.35(t, *J*=8.0Hz, 1H), 6.85(d, *J*=7.2Hz, 1H), 6.76(d, *J*=7.6Hz, 1H), 6.67(s, 1H), 6.44(s, 1H), 6.14(d, *J*=8.4Hz, 1H), 4.12(t, *J*=8.8Hz, 2H), 3.92(t, *J*=8.8Hz, 2H), 3.82(s, 3H), 3.16(m, 4H), 2.67(m, 4H), 2.42(s, 3H).



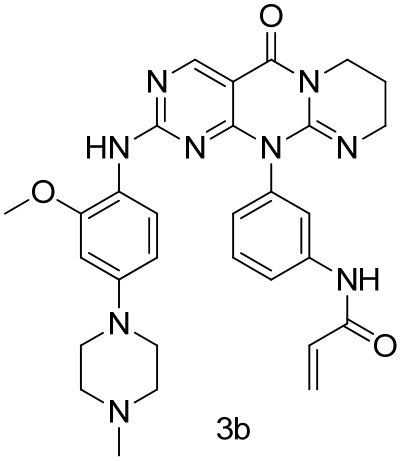
3a

***N*-(3-(2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-oxo-7,8-dihydroimidazo[1,2-a]pyrimido[4,5-d]pyrimidin-10(5*H*)-yl)phenyl)acrylamide (3a)**

Acryloyl chloride (24 μ L, 0.30 mmol) was added dropwise to a mixture of **10a** (100mg, 0.2mmol) and diisopropylethylamine (64 μL, 0.40 mmol) in dichloromethane (2 m L) at 0 ^{o}C, and then warmed to room temperature. The reaction mixture was stirred for 2 hrs. *n*-Hexane(3 mL) was then added to the mixture. The precipitate formed was collected by filtration and purified by silica gel chromatography with dichloromethane/methanol (60/1 to 30/1, v/v) to give **3a** as a yellow solid (71.9mg, 65%). ^{1}H NMR (400Hz, DMSO- d_6) δ 10.35 (s, 1H), 8.16 (s, 1H), 8.37 (brs, 1H), 7.83 (brs, 1H), 7.70 (s, 1H), 7.47 (t, *J*=8.0Hz, 1H), 7.21 (d, *J*=8.8Hz, 1H), 7.12 (d, *J*=8.0Hz, 1H) 6.51 (s, 1H), 6.48 (dd, *J*=10.0Hz, 16.8Hz, 1H), 6.28 (dd, *J*=2.0Hz, 16.8Hz, 1H), 5.95 (br s, 1H), 5.78 (dd, *J*=2.0Hz, 10.0Hz, 1H), 3,97 (t, *J*=8.4Hz, 2H), 3.71-3.75 (m, 5H), 3.05 (m, 4H), 2.26 (s, 3H). HRMS (ESI): exact mass calcd for $C_{29}H_{31}N_9O_3$ [M + H] $^{+}$, 554.2623, found 554.2616, HPLC analysis: 85:15 methanol−water, 5.16 min, 98.6%.

Compound **9b-9d** was synthesized from **7** and different aniline (**8b-8d**) with similar procedures to that of **9a**.

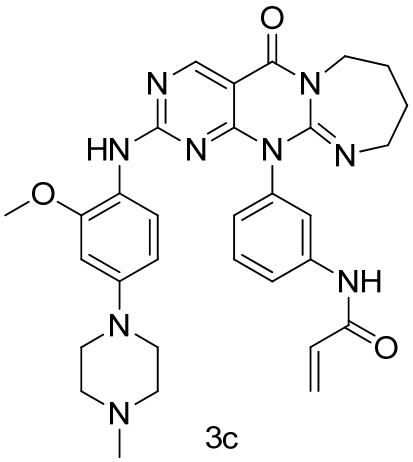
Compound **10b-10d**, **10f-10h** was synthesized from **9b-9d** with similar procedures to that of **10a**.



3b

***N*-(3-(2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-oxo-8,9-dihydro-5*H*-dipyrimido [1,2-a:4',5'-d]pyrimidin-11(7*H*)-yl)phenyl)acrylamide(3b)**

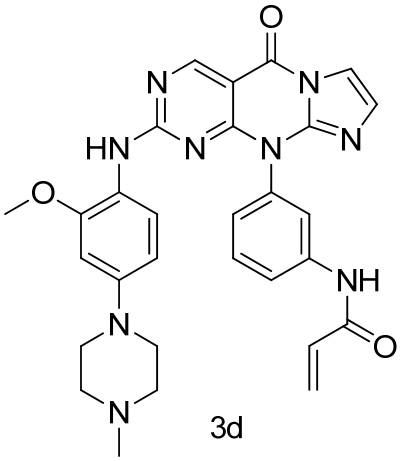
Compound **3b** was synthesized from **10b** with similar procedures to that of **3a**. ^{1}H NMR (400Hz, DMSO- d_6) δ 10.31(s, 1H), 8.64(s, 1H), 8.20(s, 1H), 7.85(s, 1H), 7.54(s, 1H), 7.44(t, *J*=8.0Hz, 1H), 7.16(d, *J*=8.8Hz, 1H), 7.00(d, *J*=8.0Hz, 1H), 6.49(s, 1H), 6.46(dd, *J*=10.0, 16.8Hz, 1H), 6.27(dd, *J*=2.0, 16.8Hz, 1H), 5.93(br s, 1H), 5.77(dd, *J*=10.0, 2.0Hz, 1H), 3.86(m, 2H), 3.76(s, 3H), 3.02(m, 4H), 2.42(t, *J*=4.4Hz, 4H), 2.22(s, 3H), 1.80(m, 2H). HRMS (ESI): exact mass calcd for $C_{30}H_{33}N_9O_3$ $[M + H]^+$, 568.2779, found 568.2771. HPLC analysis: 85:15 methanol−water, 6.29 min, 99.2%.



3c

***N*-(3-(2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-oxo-7,8,9,10-tetrahydropyrimid o[4',5':4,5]pyrimido[1,2-a][1,3]diazepin-12(5*H*)-yl)phenyl)acrylamide (3c)**

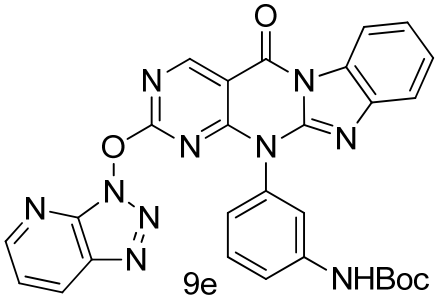
Compound **3c** was synthesized from **10c** with similar procedures to that of **3a**. ^{1}H NMR (400Hz, DMSO- d_6) δ 10.29 (s,1H), 8.61 (s, 1H), 8.18 (s, 1H), 7.85 (s, 1H), 7.50 (s, 1H), 7.42 (t, *J*=8.0Hz, 1H), 7.17 (d, *J*=8.8Hz, 1H), 6.99 (d, *J*=8.4Hz, 1H), 6.50 (s, 1H), 6.46 (dd, *J*=10.0Hz, 17.2Hz, 1H), 6.27 (dd, *J*=2.0Hz, 17.2Hz, 1H), 5.94 (br s, 1H), 5.77 (dd, *J*=2.0Hz, 10.0Hz, 1H), 4.09 (m, 2H), 3.76 (s, 3H), 3,55 (m, 2H), 3.02 (m, 4H), 2.42(m, 2H), 2.22 (s, 3H), 1.84-1.89 (m, 4H). HRMS (ESI): exact mass calcd for $C_{31}H_{35}N_9O_3$ $[M + H]^+$, 582.2936, found 582.2930. HPLC analysis: 80:20 methanol−water, 8.54 min, 97.8%.



3d

***N*-(3-(2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-oxoimidazo[1,2-a]pyrimido[4,5-d]pyrimidin-10(5*H*)-yl)phenyl)acrylamide (3d)**

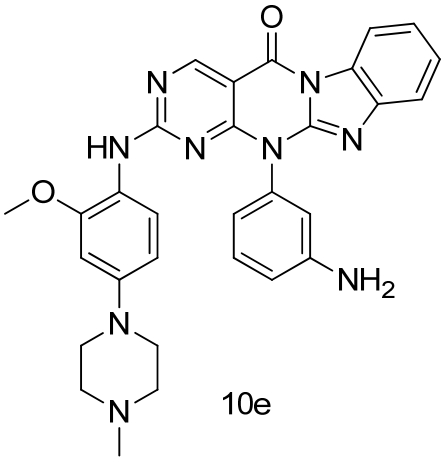
Compound **3d** was synthesized from **10d** with similar procedures to that of **3a**. ^{1}H NMR (400Hz, DMSO- d_6) δ 10.41(s, 1H), 8.99-9.07(m, 1H), 8.70(s, 1H), 7.90(s, 1H), 7.83(s, 1H), 7.74(s, 1H), 7.54(t, *J*=8.0Hz, 1H), 7.36(d, *J*=7.2Hz, 1H), 7.07(s, 1H), 6.53(s, 1H), 6.50(dd, *J*=10.0, 16.8Hz, 1H), 6.29(dd, *J*=2.0, 16.8Hz, 1H), 6.02(br s, 1H), 5.79(dd, *J*=2.0, 10.0Hz, 1H), 3.78(s, 3H), 3.07(m, 4H), 2.55(m, 4H), 2.31(s, 3H). HRMS (ESI): exact mass calcd for $C_{29}H_{29}N_9O_3$ $[M + H]^+$, 552.2466, found 552.2460. HPLC analysis: 85:15 methanol−water, 6.02 min, 97.8%.



9e

***tert*-butyl(3-(2-((3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)oxy)-5-oxobenzo[4,5]imidazo[1,2-a]pyrimido[4,5-d]pyrimidin-12(5*H*)-yl)phenyl)carbamate (9e)**

A mixture of **7** (364mg, 1mmol), HATU (760.4mg, 2mmol) and diisopropyl-ethyl amine (0.522ml, 3mmol) in DCM (10 mL) was stirred for 0.5 h at room temperature, then 2-chloro-1*H*-benzo[d]imidazole (152.5mg, 1mmol) was added to the mixture. The reaction mixture was stirred for 24 hrs at room temperature. The precipitate formed was collected by filtration and washed successively with DCM (5 mL), methanol (2 mL), water (5mL) to give **9e** as a light yellow solid (270mg, 48%). ^{1}H NMR (400Hz, DMSO- d_6) δ 9.80 (s, 1H), 9.61 (s, 1H), 8.74 (d, *J*=3.6Hz, 1H), 8.42 (d, *J*=7.6Hz, 1H) 7.99 (s, 1H) , 7.84 (d, *J*=8.0Hz, 1H), 7.64-7.68 (m, 3H), 7.59 (dd, *J*=4.4Hz, 8.4Hz, 1H), 7.42-7.50 (m, 2H), 7.29-7.30 (m, 1H), 1.43 (s, 9H).

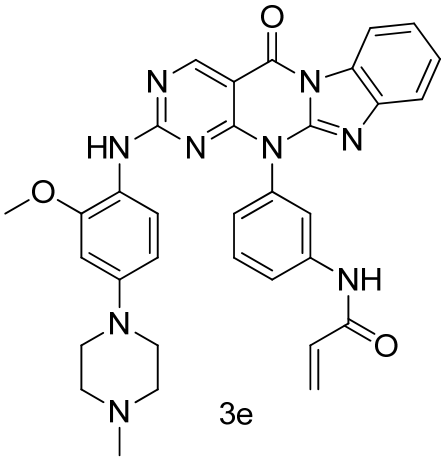


10e

12-(3-aminophenyl)-2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)benzo[4,5]imidazo[1,2-a]pyrimido[4,5-d]pyrimidin-5(12*H*)-one (10e)

To a solution of compound **9e** (323.8mg, 0.5mmol) in *tert*-Butanol (5 mL) were added 2 -methoxy-4-(4-met hylpiperazin-1-yl)aniline (110.6mg , 0.5 mmol) and potassium carbonate (207.3mg, 1.5mmol). The reaction mixture was stirred for 24 hrs at 110 ° C in a sealed tube, then the solvent was removed under reduced pressure. The residue was partitioned between water and dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give the crude product which was used without further purification.

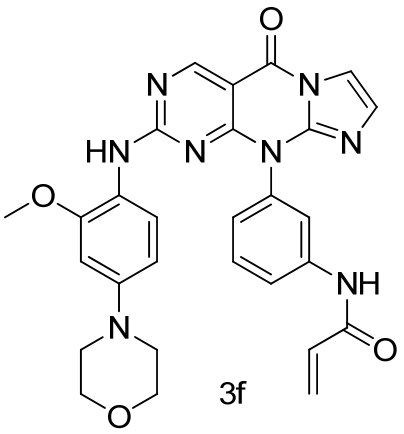
To a mixture of the crude product in dichloromethane (2 mL) was added trifluoroaceticacid (TFA) (2mL). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, and neutralized the residue by slow addition of saturated $NaHCO_3$. The precipitate formed was collected by filtration and washed with water. The resulting crude product was purified by silica gel chromatography with dichloromethane/methanol (60/1 to 30/1, v/v) to give **10e** as a yellow solid (82.1mg, 30%). ^{1}H NMR (400Hz, $CDCl_3$) δ 9.20(s, 1H), 8.49(d, *J*=8.0Hz, 1H), 8.16(s, 1H), 7.65-7.69(m, 2H), 7.45(t, *J*=8.0Hz, 1H), 7.34-7.41(m, 2H), 6.95(d, *J*=8.8Hz, 1H), 6.90(d, *J*=7.6Hz, 1H), 6.80(s, 1H), 6.47(d, *J*=2.0Hz, 1H), 6.21(d, *J*=6.8Hz, 1H), 3.86-3.89(m, 5H), 3.15(m, 4H), 2.60(t, *J*=5.2Hz, 4H), 2.38(s, 3H).



3e

***N*-(3-(2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-oxobenzo[4,5]imidazo[1,2-a]pyrimido[4,5-d]pyrimidin-12(5*H*)-yl)phenyl)acrylamide (3e).**

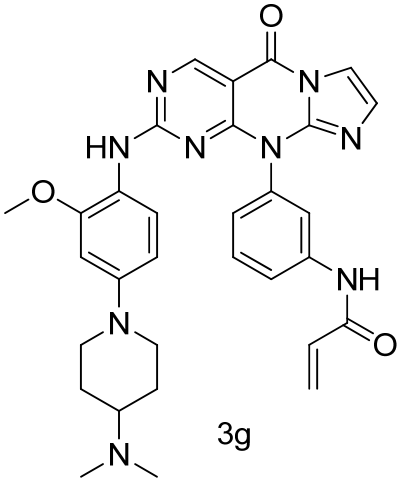
Compound **3e** was synthesized from **10e** with similar procedures to that of **3a**. ^{1}H NMR (400Hz, DMSO- d_6) δ 10.43(s, 1H), 9.00-9.09(m, 1H), 8.74(s, 1H), 8.35(d, *J*=7.2Hz, 1H), 7.89-7.95(m, 2H), 7.56-7.60(m, 2H), 7.27-7.40(m, 4H), 6.52(s, 1H), 6.50(dd, *J*=10.0, 16.8 Hz, 1H), 6.29(dd, *J*=1.6, 16.8H, 1H), 6.01(d, *J*=6.0Hz, 1H), 5.79(dd, *J*=1.6, 10.0Hz, 1H), 3.78(s, 3H), 3.04-3.13(m, 4H), 2.44(m, 4H), 2.23(s, 3H). HRMS (ESI): exact mass calcd for $C_{33}H_{31}N_9O_3$ $[M + H]^+$, 602.2623, found 602.2615. Anal. Calcd. For $C_{33}H_{31}N_9O_3 \cdot 3/2H_2O$: C, 63.05; H, 5.45; N, 20.05; found: C, 63.10; H, 5.40; N, 19.97.



3f

***N*-(3-(2-((2-methoxy-4-morpholinophenyl)amino)-5-oxoimidazo[1,2-a]pyrimido[4,5-d]pyrimidin-10(5*H*)-yl)phenyl)acrylamide(3f)**

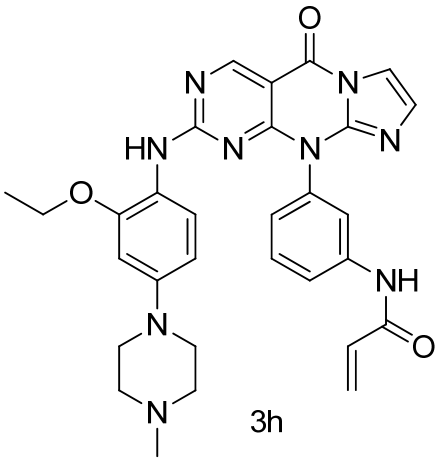
Compound **3f** was synthesized from **10f** with similar procedures to that of **3a**. ^{1}H NMR (400Hz, DMSO- d_6) δ 10.41(s, 1H), 9.07(s, 1H), 8.72(s, 1H), 7.89(m, 1H), 7.83(s, 1H), 7.75(s, 1H), 7.54(t, *J*=8.0Hz, 1H), 7.36(d, *J*=8.0Hz, 1H), 7.23(d, *J*=7.6Hz, 1H), 7.08(s, 1H), 6.54(s, 1H), 6.49(dd, *J*=10.0, 16.8Hz, 1H), 6.29(dd, *J*=1.6, 16.8Hz, 1H), 6.02(d, *J*=7.6Hz, 1H), 5.79(dd, *J*=2.0, 10.0Hz, 1H), 3.71-3.78(m, 7H), 3.01(m, 4H). HRMS (ESI): exact mass calcd for $C_{28}H_{26}N_8O_4$ $[M + H]^+$, 539.2150, found 539.2154. HPLC analysis: 90:10 methanol−water, 4.28 min, 95.2%.



3g

***N*-(3-(2-((4-(4-(dimethylamino)piperidin-1-yl)-2-methoxyphenyl)amino)-5-oxoimidazo[1,2-a]pyrimido[4,5-d]pyrimidin-10(5*H*)-yl)phenyl)acrylamide (3g)**

Compound **3g** was synthesized from **10g** with similar procedures to that of **3a**. ^{1}H NMR (400Hz, Acetic acid-d_4) δ 9.20(s, 1H), 8.17(d, *J*=7.6Hz, 1H), 7.80-7.82(m, 2H), 7.61-7.67(m, 2H), 7.33(d, *J*=7.6Hz, 1H), 7.23(m, 1H), 6.93(s, 1H), 6.46-6.47(m, 3H), 5.82(t, *J*=5.6Hz, 1H), 3.88(s, 3H), 3.78-3.81(m, 2H), 3.63(t, *J*=11.2Hz, 1H), 3.16(t, *J*=11.2Hz, 2H), 2.92(s, 6H), 2.28-2.31(m, 2H), 2.16-2.19(m, 2H). HRMS (ESI): exact mass calcd for $C_{31}H_{33}N_9O_3$ $[M + H]^+$, 580.2779, found580.2787. HPLC analysis: 90:10 methanol−water, 10.09 min, 98.0%.



***N*-(3-(2-((2-ethoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-oxoimidazo[1,2-a]pyrimido[4,5-d] pyrimidin-10(5*H*)-yl)phenyl)acrylamide (3h)**

Compound **3h** was synthesized from **10h** with similar procedures to that of **3a**. ^{1}H NMR (400Hz, DMSO- d_6) δ 10.42(s, 1H), 9.07(s, 1H), 8.58(s, 1H), 7.91-7.92(m, 1H), 7.84(s, 1H), 7.75(s, 1H), 7.54(t, *J*=8.0Hz, 1H), 7.37-7.39(m, 1H), 7.24(d, *J*=8.4Hz, 1H), 7.08(s, 1H), 6.51(s, 1H), 6.49(dd, *J*=10.0, 16.8Hz, 1H), 6.28(dd, *J*=1.6, 16.8Hz, 1H), 5.98-6.00(m, 1H), 5.79(dd, *J*=2.0, 10.0Hz, 1H), 4.00-4.04(m, 2H), 3.02(m, 4H), 2.43(m, 4H), 2.22(s, 3H), 1.33(m, 3H). HRMS (ESI): exact mass calcd for $C_{30}H_{31}N_9O_3$ $[M + H]^+$, 566.2623, found 566.2627. HPLC analysis: 90:10 methanol−water, 5.40 min, 99.3%.

In Vitro Enzymatic Activity Assay

The Z'-LYTE™ biochemical assay employs a FRET-based, coupled-enzyme format and is based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to proteolytic cleavage (Figure S1). The recommended excitation wavelength is 400 nm and the recommended emission wavelengths are 445 nm and 520 nm, respectively. The Emission Ratio is calculated by the equation below. This Kit provides a screening assay that yields Z'-factor values >0.7.

$$\text{Emission Ratio} = \frac{\text{Coumarin Emission (445 nm)}}{\text{Fluorescein Emission (520 nm)}}$$

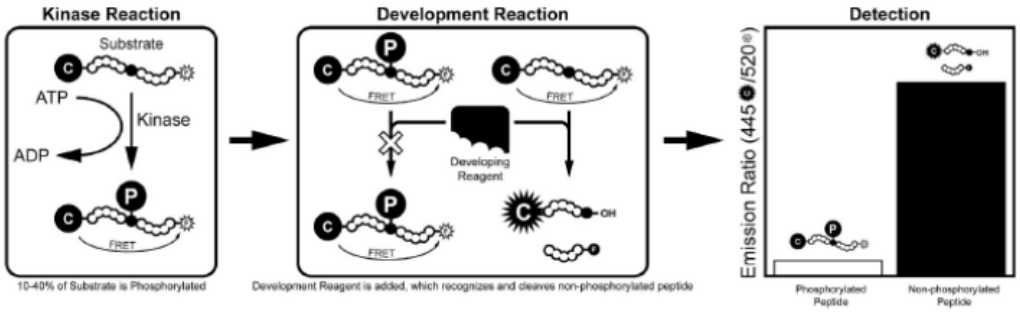


Figure S1. Schematic diagram of the Z'-LYTE™ biochemical assay (Invitrogen)

The concentrations of different kinase were determined by optimization experiments and the respective concentration was: EGFR-T790M (PV4803, Invitrogen) 0.174μg/μL, EGFR-L858R/T790M (PV4879, Invitrogen) 0.055μg/μL. The compounds were diluted three-fold from $5.1x10^{-9}$ M to $1x10^{-4}$ M in DMSO. Plate was measured on EnVision Multilabel Reader (Perkin Elmer). Curve fitting and data presentations were performed using Graph Pad Prism version 4.0. Every experiment was repeated at least 3 times.

Western Blotting

$1x10^{6}$ cells of H1975 were seed into 6-cm dishes. 24 hrs latter, medium was changed and 2.0, 0.4, 0.08, 0.016μM of **3d/3g** was added. Medium with 1‰ DMSO was used as control. Cells were exposed to treatment for 2 hrs. Washed the dishes twice using pre-cold PBS, removed the residuary PBS completely, and 400 μL 1x Cell Lysis Buffer was added. The lysis buffer was prepared according to CST protocol. After incubating plates on ice for 5 minutes, cells were scraped carefully and sonicated immediately. Centrifuged extract for 10 minutes at 14,000 x g at 4 ˚C, remained the supernatant and denatured it via boiling. Samples were maintained at -70˚C. 20μL sample was loaded. Proteins were transfered to PVDF membrane (Mili pore). PVDF membranes were blocked in 5% bovine serum albumin-TBST for 1h. The primary antibody EGFR (CST, 2232), phospho-EGFR (Tyr1068) (CST, 2234), AKT (CST, 9272), phospho-AKT (Ser 473) (CST, 9271), ERK (CST, 9102), phospho-ERK (t202/y204) (CST, 9101), GAPDH (KC-5G5, KangChen) were dilute 1:1000 with 5% BSA-TBST to use. The membrane was incubated in primary antibody for 2 hours at room temperature. Wash membrane three times for 10 minutes each with TBST. the membrane was incubated for 1h at room temperature with horseradish peroxidase (HRP, sigma) conjugated Rabbit secondary antibody, diluted to 1:2000 in 5% BSA-TBST. Wash membrane three times for 10 minutes each with TBST. Blots were developed by enhanced chemiluminescence (Thermo).

For selectivity assay, NCI-H820 (NSCLC, EGFR $^{\text{del E746-E749/T790M}}$),NCI-H446, NCI-H322, NCI-H1703, NCI-H1299, A549, 95D, NCI-H358, NCI-H661(NSCLC, $EGFR^{WT}$) cells were exposed to 0.5 μM of 3d/3g for 2 hrs,and then excited 0.5 hr with EGF(200ng/mL), and then excited 0.5 hr with EGF (200ng/mL) western blot was performed and pEGFR(Y1068) was tested, α-Tubulin was used as control.

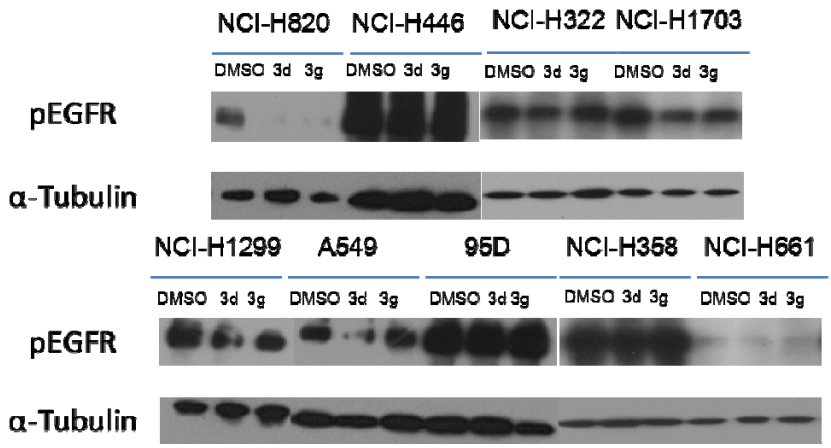


Figure S2. Compounds **3d/3g** shows low potency to inhibit the activation of EGFR in cancer cells with wide type EGFR.

Cell Proliferation and Growth Inhibition Assay

NCI-H1975, NCI-H322, A549, NCI-H1299, NCI-H1703, NCI-H661, 95D, NCI-H358, HCC827, HLF-1, HL-7702, A431 cells were cultured with respective growth medium. Before use, cells were at least passaged twice after thawing. Cells of log phase were trypsinized and resuspended in growth medium. 1000-3000 cells/well were seeded in 96-well plates with a 100 μL volume, 6 parallels and 7 rows were designed. Plates were maintained at 37 ℃ in a 5% CO_2 incubator overnight. Dissolved the compounds with DMSO to 10μM, and a five-fold serial dilution of the compounds from $1x10^{-5}$ M to $0.64x10^{-9}$ M was performed (10 μL compound solution plus 90 μL DMSO). 2μl of compound solution was added to 998 μL growth medium, the mixture was vortexes sufficiently. 100 μL mixture was correspondingly added to 96-well plate. 2 μL DMSO instead of compound solution was used as 0% inhibitor control. After co-incubation for 68 hrs, 20 μL MTT (5mg/ml) was added. 4hs later, discarded supernatant completely and added 150μL DMSO. After shaking for 10 min, the plates were read in the Synergy™ HT (Bio Tek) at 570nm. The data was calculated using Graph Pad Prism version 4.0. The IC_{50} were fitted using a non-linear regression model with a sigmoidal dose response.

Table S1. Antiproliferative activities of the new inhibitors **3** against cells Harboring different status of EGFR. [a]

Cpds	IC_{50}(μM)				
	HCC827	H1975	A431	A549	HL-7702
3a	0.033±0.011	1.107±0.343	2.656±1.423	12.012±3.716	35.058±5.947
3b	0.057±0.016	3.556±1.165	23.066±8.543	52.968±9.992	37.895±5.595
3c	0.019±0.006	0.648±0.081	1.536±0.693	7.129±2.850	9.128±1.676
3d	0.039±0.013	0.143±0.026	2.983±1.115	12.417±2.166	9.963±5.080
3e	0.023±0.008	0.307±0.089	3.567±0.373	3.132±1.373	3.230±0.423
3f	0.142±0.061	0.476±0.219	>30	>30	>30
3g	0.049±0.027	0.086±0.018	14.53±8.105	>30	>30
3h	0.046±0.0162	0.396±0.179	5.254±3.482	4.478±2.54	5.46±3.735
WZ4002	0.009±0.001	0.055±0.011	1.042±0.014	4.069±1.755	21.425±5.915
Gefinitib	0.005±0.002	13.125±0.925	1.199±0.473	17.091±6.37	11.840±2.533

[a] The antiproliferative activities of the compounds were evaluated using the MTS assay. The data were means from at least four independent experiments.

Cell Cycle Assay

H1975 cells were plated in 6-well plates overnight. Medium of none FBS was used for synchronization. 24 hrs latter, growth medium with **3d/3g** (0.01μM, 0.1μM, 1μM) were changed. Medium with 1‰ DMSO was used as control. After incubated for 24 hrs, cells were collected and centrifuged for 10 minutes at 300 x g. The samples were washed twice with washing buffer, discarded the supernatant completely. Cells were treated with 250μl solution A and 200 μL solution B for 10min respectively; 250μL PI (solution C) was added in the end (CYCLETEST PLUS DNA REAGENT KIT, BD Pharmingen). After 10 min staining, cell suspension was filtered via 200 mesh Filter mesh. Samples were analyzed on a FACS Calibur flow cytometer (Becton Dickinson), and Data were analyzed using the Modfit software package.

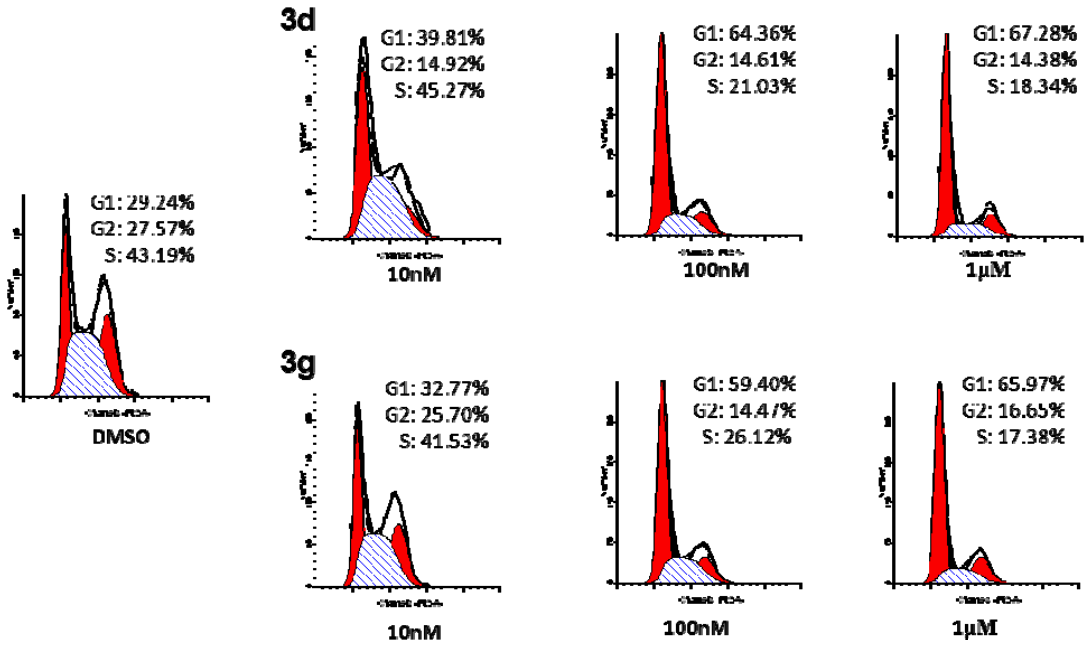


Figure S3. Compounds **3d/3g** dose-dependently induces G1/S arrest of NCI-H1975 NSCLC cells.

Cell Apoptosis Assay

H1975 cells were plated in 6-well plates overnight. Fresh growth medium with **3d/3g** (0.1μM, 0.5μM, 1μM) was added. Medium with 1‰ DMSO was used as control. After incubating for 24 hrs, growth medium was collected and cells were trypsined and collected correspondingly to the medium. Suspensions were centrifuged for 10 minutes at 300 x g at 4 ˚C. Removed the supernatant completely and washed cells twice with pre-cold PBS. 200μL 1 x Binding buffer and 2.5μL 7-AAD, 2.5 μL annexin-V were added (PE-Annexin V Kit,BD Pharmingen). Gently vortex the cells and incubate for 15 min at rt (25 °C) in the dark. Cells stained with 7-AAD, annexin-V alone were used as positive control. The samples were detected with FACS Calibur flow cytometer (Becton Dickinson).

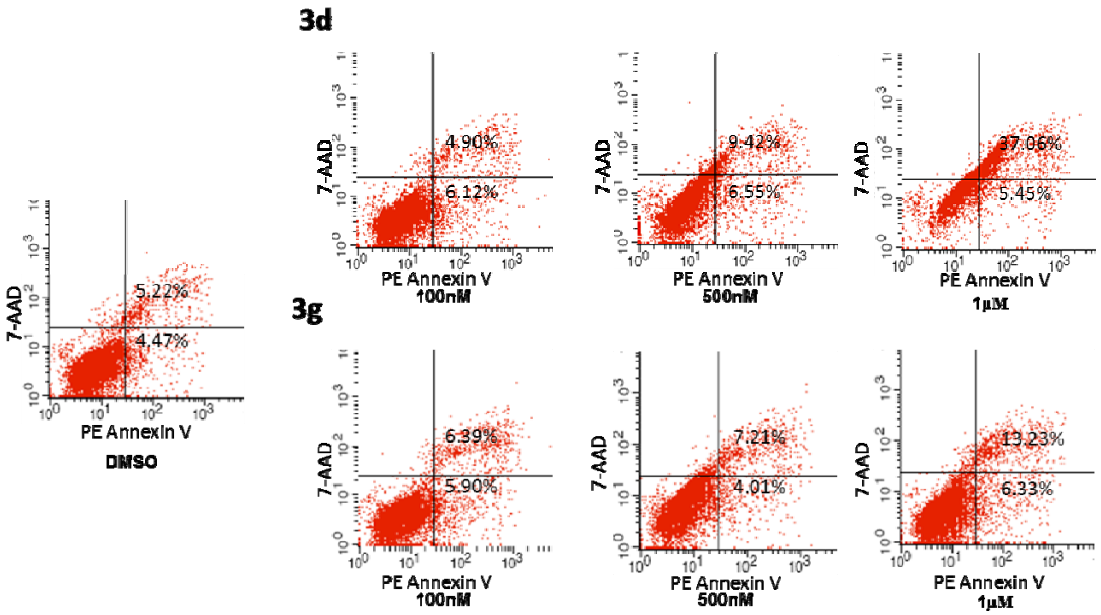
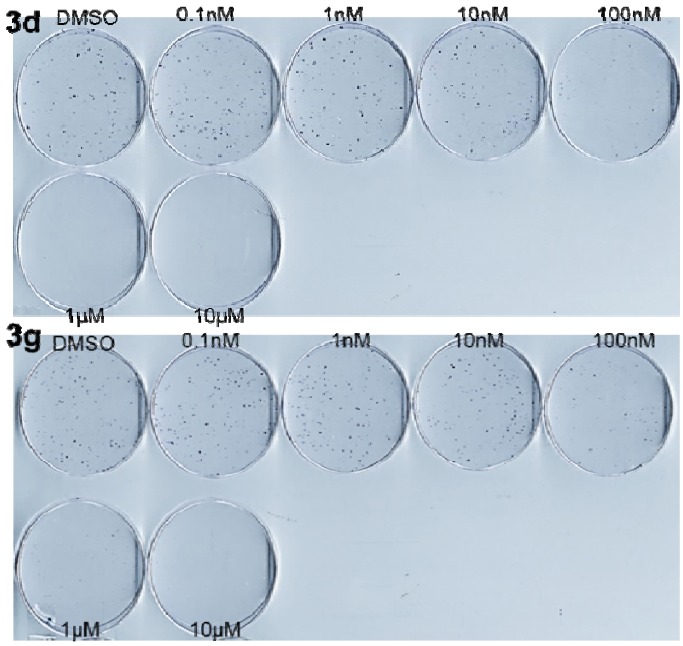
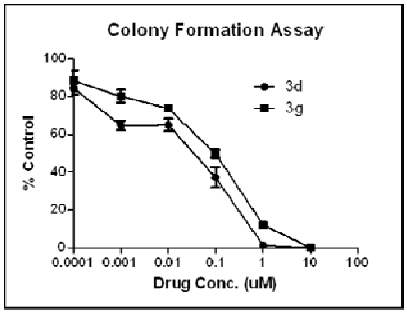


Figure S4. Compounds **3d/3g** dose-dependently induces NCI-H1975 NSCLC cell apoptosis.

Colony Formationassay

H1975 cells were cultured in RPMI 1640, supplemented by 10% FBS. Cells were passaged prior to achieving full confluence. Washed the cell twice with PBS, lifted by adding 1ml trypsin and incubated for 2 minutes at 37 ºC. The cell suspension was spun down in a centrifuge for 10 minutes at 500xg and was resuspended in 5 ml of culture medium. The concentration determined by counting using a haemocytometer. A cell suspension of 500 cells/3ml was performed by adding suitable growth medium. 3ml cell suspension was plated into a 6-cm dish. 4 parallels and 7 gradients were set. 3 μL different concentration (10μM, 1μM, 0.1μM, 0.01μM, 0.001μM, 0.0001μM) of compounds **3d/3g** dissolved in DMSO was added immediately. Plates with 1‰ DMSO added were used as control. Medium with **3d/3g** was changed every 3 days. 9 days later, the colonies were mainly greater than 50 cells. Removed suspension and wash the plates twice with PBS. 4% formaldehyde was used to fix the colonies for 10 min, washed twice with PBS, and stained the plates with 0.2% crystal violet for 10 min. Wash the plates with PBS until the background is clear. The plates were scanned with a HP scanner. The data was calculated using Graph Pad Prism version 4.0. The IC_{50} were calculated by using a non-linear regression model with a sigmoidal dose response.





compounds	3d	3g
IC_{50}(μM)	0.03306	0.07885

Figure S5. Compounds **3d/3g** inhibited the colony formation of NCI-1975 NSCLC cells in a dose dependent manner.

Cell Migration and Invasion Assay

Wound healing assay was used to evaluate the inhibitory effect on H1975 cell migration ability of **3d/3g**. Cells were plated 90% confluence overnight and scratched with a tip, **3d/3g** (50, 250nM) was added immediately. The scratch length at 0 h and 24 hrs was measured after microscopic photograph was taken and the migration ratio was set as 0 h/24 hrs. Trans-Well assay was also used, $6x10^4$ NCI-H1975 cells were plated in Trans-Well chamber, incubated 24 hrs with **3d/3g** (50, 250nM), cells were fixed and dyed and micrographs were taken. Cell number passed through was counted and column graph was made. In invasion assay, MaxGel™ ECM (Sigma, E0282) was used to simulate the extra-cellular matrix.

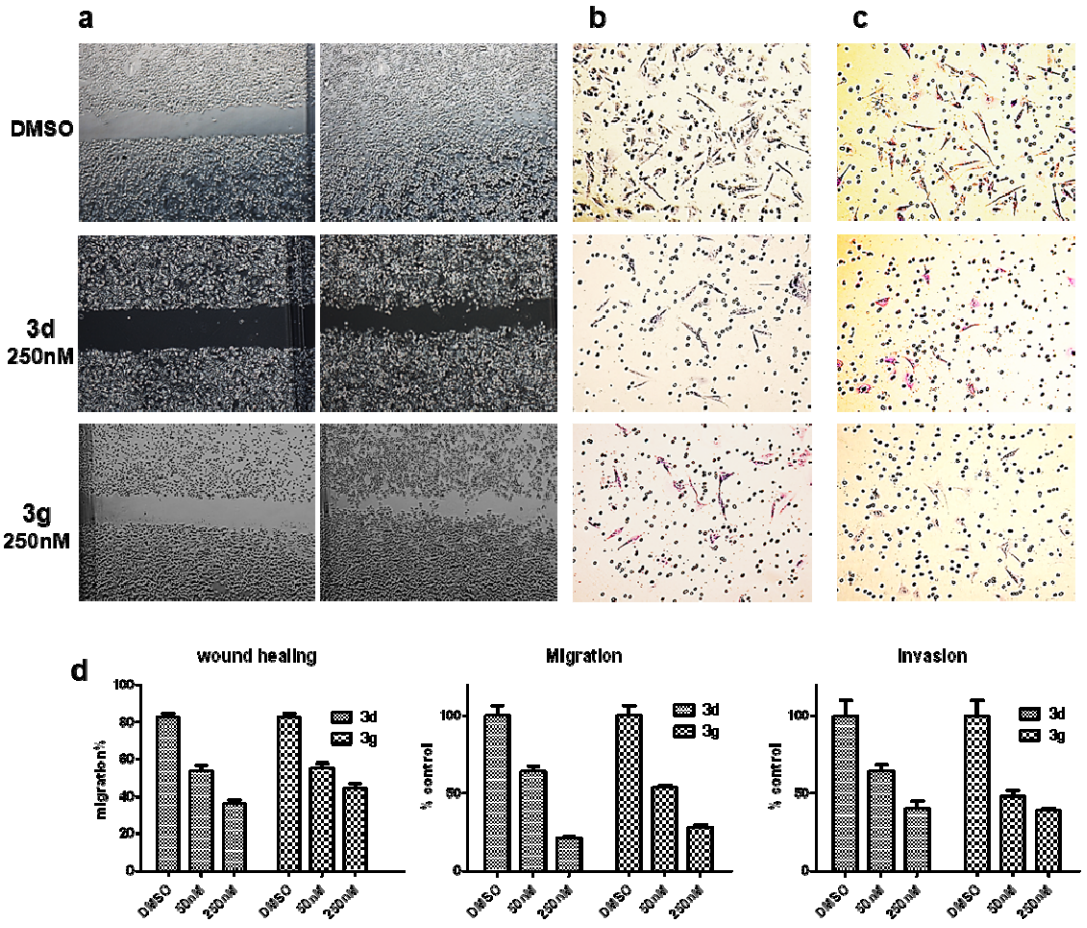


Figure S6. Compounds **3d/3g** inhibited the migration and invasion of NCI-1975 NSCLC cells in dose dependent manners. (a) Wound healing assay on NCI-H1975 cell. (b) Trans-well (migration) assay on NCI-H1975 cell. (c) Trans-well (invasion) assay on NCI-H1975 cell. (d) Statistical analysis of the results.

Kinase Profiling Results

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GUA020-01-p-00001 Study Results

Table 1 - Matrix of Compound Screen for GUA020-01-p-00001

Target	XTF-150
Gene Symbol	%Ctrl @ 100nM
AAK1	68
ABL1(E255K)-phosphorylated	85
ABL1(F317I)-nonphosphorylated	100
ABL1(F317I)-phosphorylated	76
ABL1(F317L)-nonphosphorylated	100
ABL1(F317L)-phosphorylated	100
ABL1(H396P)-nonphosphorylated	100
ABL1(H396P)-phosphorylated	100
ABL1(M351T)-phosphorylated	83
ABL1(Q252H)-nonphosphorylated	100
ABL1(Q252H)-phosphorylated	96
ABL1(T315I)-nonphosphorylated	100
ABL1(T315I)-phosphorylated	77
ABL1(Y253F)-phosphorylated	100
ABL1-nonphosphorylated	100
ABL1-phosphorylated	100
ABL2	86
ACVR1	100
ACVR1B	96
ACVR2A	74
ACVR2B	100
ACVRL1	100
ADCK3	100
ADCK4	100
AKT1	100
AKT2	92
AKT3	100
ALK	89
ALK(C1156Y)	100
ALK(L1196M)	98
AMPK-alpha1	100
AMPK-alpha2	100
ANKK1	46
ARK5	100
ASK1	100
ASK2	100
AURKA	100
AURKB	74
AURKC	94
AXL	85
BIKE	100
BLK	100
BMPR1A	88
BMPR1B	100
BMPR2	97
BMX	81
BRAF	90
BRAF(V600E)	100

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Table 1 - Assay Matrix (continued).

Target	XTF-150
Gene Symbol	%Ctrl @ 100nM
BRK	85
BRSK1	100
BRSK2	52
BTK	92
BUB1	100
CAMK1	80
CAMK1D	78
CAMK1G	64
CAMK2A	28
CAMK2B	37
CAMK2D	73
CAMK2G	70
CAMK4	95
CAMKK1	80
CAMKK2	61
CASK	78
CDC2L1	82
CDC2L2	100
CDC2L5	100
CDK11	100
CDK2	100
CDK3	99
CDK4-cyclinD1	100
CDK4-cyclinD3	87
CDK5	100
CDK7	100
CDK8	88
CDK9	78
CDKL1	100
CDKL2	100
CDKL3	88
CDKL5	100
CHEK1	100
CHEK2	100
CIT	68
CLK1	83
CLK2	43
CLK3	98
CLK4	100
CSF1R	100
CSF1R-autoinhibited	91
CSK	100
CSNK1A1	100
CSNK1A1L	93
CSNK1D	90
CSNK1E	100
CSNK1G1	100
CSNK1G2	100
CSNK1G3	83

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Table 1 - Assay Matrix (continued).

Target	XTF-150
Gene Symbol	%Ctrl @ 100nM
CSNK2A1	55
CSNK2A2	100
CTK	100
DAPK1	89
DAPK2	30
DAPK3	34
DCAMKL1	53
DCAMKL2	100
DCAMKL3	100
DDR1	100
DDR2	100
DLK	96
DMPK	100
DMPK2	69
DRAK1	100
DRAK2	99
DYRK1A	100
DYRK1B	100
DYRK2	77
EGFR	76
EGFR(E746-A750del)	89
EGFR(G719C)	88
EGFR(G719S)	100
EGFR(L747-E749del, A750P)	76
EGFR(L747-S752del, P753S)	72
EGFR(L747-T751del,Sins)	65
EGFR(L858R)	62
EGFR(L858R,T790M)	5
EGFR(L861Q)	75
EGFR(S752-I759del)	55
EGFR(T790M)	5.6
EIF2AK1	100
EPHA1	100
EPHA2	100
EPHA3	100
EPHA4	78
EPHA5	100
EPHA6	100
EPHA7	100
EPHA8	84
EPHB1	75
EPHB2	100
EPHB3	86
EPHB4	93
EPHB6	92
ERBB2	77
ERBB3	100
ERBB4	78
ERK1	100

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Table 1 - Assay Matrix (continued).

Target	XTF-150
Gene Symbol	%Ctrl @ 100nM
ERK2	89
ERK3	69
ERK4	89
ERK5	100
ERK8	100
ERN1	100
FAK	97
FER	78
FES	100
FGFR1	59
FGFR2	97
FGFR3	89
FGFR3(G697C)	82
FGFR4	95
FGR	77
FLT1	100
FLT3	87
FLT3(D835H)	86
FLT3(D835Y)	88
FLT3(ITD)	100
FLT3(K663Q)	100
FLT3(N841I)	100
FLT3(R834Q)	100
FLT3-autoinhibited	100
FLT4	98
FRK	100
FYN	87
GAK	81
GCN2(Kin.Dom.2,S808G)	100
GRK1	54
GRK4	90
GRK7	41
GSK3A	83
GSK3B	100
HASPIN	100
HCK	100
HIPK1	100
HIPK2	30
HIPK3	100
HIPK4	99
HPK1	75
HUNK	100
ICK	96
IGF1R	100
IKK-alpha	66
IKK-beta	83
IKK-epsilon	100
INSR	84
INSRR	81

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Table 1 - Assay Matrix (continued).

Target	XTF-150
Gene Symbol	%Ctrl @ 100nM
IRAK1	77
IRAK3	71
IRAK4	55
ITK	86
JAK1(JH1domain-catalytic)	100
JAK1(JH2domain-pseudokinase)	100
JAK2(JH1domain-catalytic)	46
JAK3(JH1domain-catalytic)	51
JNK1	84
JNK2	80
JNK3	93
KIT	100
KIT(A829P)	100
KIT(D816H)	97
KIT(D816V)	95
KIT(L576P)	100
KIT(V559D)	100
KIT(V559D,T670I)	100
KIT(V559D,V654A)	100
KIT-autoinhibited	100
LATS1	100
LATS2	100
LCK	86
LIMK1	100
LIMK2	56
LKB1	66
LOK	100
LRRK2	100
LRRK2(G2019S)	100
LTK	100
LYN	97
LZK	95
MAK	100
MAP3K1	100
MAP3K15	100
MAP3K2	75
MAP3K3	100
MAP3K4	97
MAP4K2	93
MAP4K3	80
MAP4K4	91
MAP4K5	95
MAPKAPK2	83
MAPKAPK5	97
MARK1	72
MARK2	93
MARK3	100
MARK4	78
MAST1	94

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Table 1 - Assay Matrix (continued).

Target	XTF-150
Gene Symbol	%Ctrl @ 100nM
MEK1	100
MEK2	100
MEK3	64
MEK4	67
MEK5	96
MEK6	90
MELK	100
MERTK	100
MET	89
MET(M1250T)	66
MET(Y1235D)	100
MINK	46
MKK7	95
MKNK1	91
MKNK2	93
MLCK	98
MLK1	100
MLK2	77
MLK3	75
MRCKA	98
MRCKB	91
MST1	100
MST1R	100
MST2	100
MST3	81
MST4	100
MTOR	100
MUSK	100
MYLK	68
MYLK2	90
MYLK4	100
MYO3A	99
MYO3B	100
NDR1	45
NDR2	82
NEK1	89
NEK10	100
NEK11	93
NEK2	90
NEK3	86
NEK4	96
NEK5	85
NEK6	99
NEK7	100
NEK9	100
NIK	69
NIM1	97
NLK	70
OSR1	67

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Table 1 - Assay Matrix (continued).

Target	XTF-150
Gene Symbol	%Ctrl @ 100nM
p38-alpha	92
p38-beta	100
p38-delta	100
p38-gamma	96
PAK1	81
PAK2	74
PAK3	100
PAK4	100
PAK6	83
PAK7	100
PCTK1	100
PCTK2	95
PCTK3	100
PDGFRA	100
PDGFRB	100
PDPK1	99
PFCDPK1(P.falciparum)	100
PFPK5(P.falciparum)	100
PFTAIRE2	100
PFTK1	92
PHKG1	100
PHKG2	35
PIK3C2B	100
PIK3C2G	100
PIK3CA	100
PIK3CA(C420R)	83
PIK3CA(E542K)	81
PIK3CA(E545A)	69
PIK3CA(E545K)	68
PIK3CA(H1047L)	70
PIK3CA(H1047Y)	90
PIK3CA(I800L)	99
PIK3CA(M1043I)	100
PIK3CA(Q546K)	82
PIK3CB	78
PIK3CD	100
PIK3CG	99
PIK4CB	100
PIM1	100
PIM2	99
PIM3	95
PIP5K1A	90
PIP5K1C	100
PIP5K2B	93
PIP5K2C	78
PKAC-alpha	100
PKAC-beta	100
PKMYT1	56
PKN1	94

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Table 1 - Assay Matrix (continued).

Target	XTF-150
Gene Symbol	%Ctrl @ 100nM
PKN2	100
PKNB(M.tuberculosis)	92
PLK1	89
PLK2	100
PLK3	84
PLK4	66
PRKCD	51
PRKCE	88
PRKCH	100
PRKCI	100
PRKCQ	100
PRKD1	100
PRKD2	100
PRKD3	100
PRKG1	100
PRKG2	100
PRKR	97
PRKX	93
PRP4	100
PYK2	100
QSK	97
RAF1	91
RET	84
RET(M918T)	100
RET(V804L)	100
RET(V804M)	100
RIOK1	100
RIOK2	88
RIOK3	86
RIPK1	75
RIPK2	62
RIPK4	74
RIPK5	62
ROCK1	100
ROCK2	100
ROS1	73
RPS6KA4(Kin.Dom.1-N-terminal)	100
RPS6KA4(Kin.Dom.2-C-terminal)	93
RPS6KA5(Kin.Dom.1-N-terminal)	100
RPS6KA5(Kin.Dom.2-C-terminal)	86
RSK1(Kin.Dom.1-N-terminal)	83
RSK1(Kin.Dom.2-C-terminal)	93
RSK2(Kin.Dom.1-N-terminal)	68
RSK2(Kin.Dom.2-C-terminal)	100
RSK3(Kin.Dom.1-N-terminal)	87
RSK3(Kin.Dom.2-C-terminal)	99
RSK4(Kin.Dom.1-N-terminal)	76
RSK4(Kin.Dom.2-C-terminal)	91
S6K1	100

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Table 1 - Assay Matrix (continued).

Target	XTF-150
Gene Symbol	%Ctrl @ 100nM
SBK1	81
SGK	100
SgK110	100
SGK2	100
SGK3	52
SIK	92
SIK2	85
SLK	78
SNARK	47
SNRK	48
SRC	98
SRMS	98
SRPK1	100
SRPK2	93
SRPK3	100
STK16	74
STK33	100
STK35	85
STK36	100
STK39	50
SYK	79
TAK1	100
TAOK1	100
TAOK2	93
TAOK3	92
TBK1	59
TEC	80
TESK1	97
TGFBR1	100
TGFBR2	100
TIE1	100
TIE2	94
TLK1	93
TLK2	84
TNIK	100
TNK1	100
TNK2	100
TNNI3K	90
TRKA	100
TRKB	100
TRKC	94
TRPM6	87
TSSK1B	100
TTK	80
TXK	85
TYK2(JH1domain-catalytic)	68
TYK2(JH2domain-pseudokinase)	100
TYRO3	100
ULK1	55

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Table 1 - Assay Matrix (continued).

Target	XTF-150
Gene Symbol	%Ctrl @ 100nM
ULK2	100
ULK3	51
VEGFR2	100
VRK2	100
WEE1	85
WEE2	96
WNK1	100
WNK3	100
YANK1	99
YANK2	92
YANK3	92
YES	100
YSK1	91
YSK4	100
ZAK	94
ZAP70	94

%Ctrl Legend

0≤x<.1	.1≤x<1	1≤x<10	10≤x<35	x≥35

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DiscoveRx

S-score Results

Table 2 - S-score Table for GUA020-01-p-00001

Compound Name	Selectivity Score Type	Number of Hits	Number of Non-Mutant Kinases	Screening Concentration (nM)	Selectivity Score
XTF-150	S(35)	4	395	100	0.01
XTF-150	S(10)	0	395	100	0
XTF-150	S(1)	0	395	100	0

Figure S7. Kinase profiling results performed by using the Ambit Kinome screening platform. The Ambit score is calculated as the percent of DMSO control. XTF-150 : **3g**. S = Number of hits / Number of assays, S(35) = (number of non-mutant kinases with %Ctrl <35)/(number of non-mutant kinases tested), S(10) = (number of non-mutant kinases with %Ctrl <10)/(number of non-mutant kinases tested), S(1) = (number of non-mutant kinases with %Ctrl <1)/(number of non-mutant kinases tested).

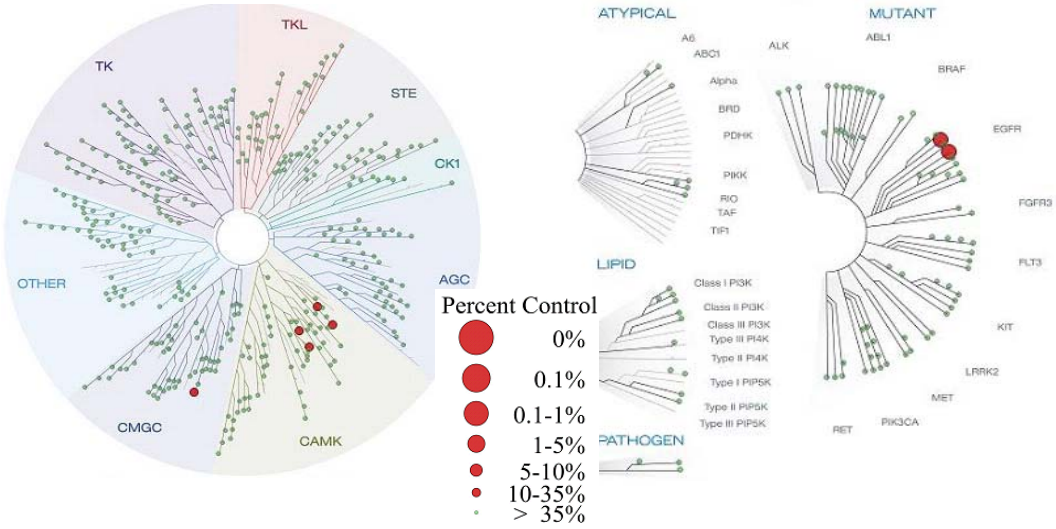
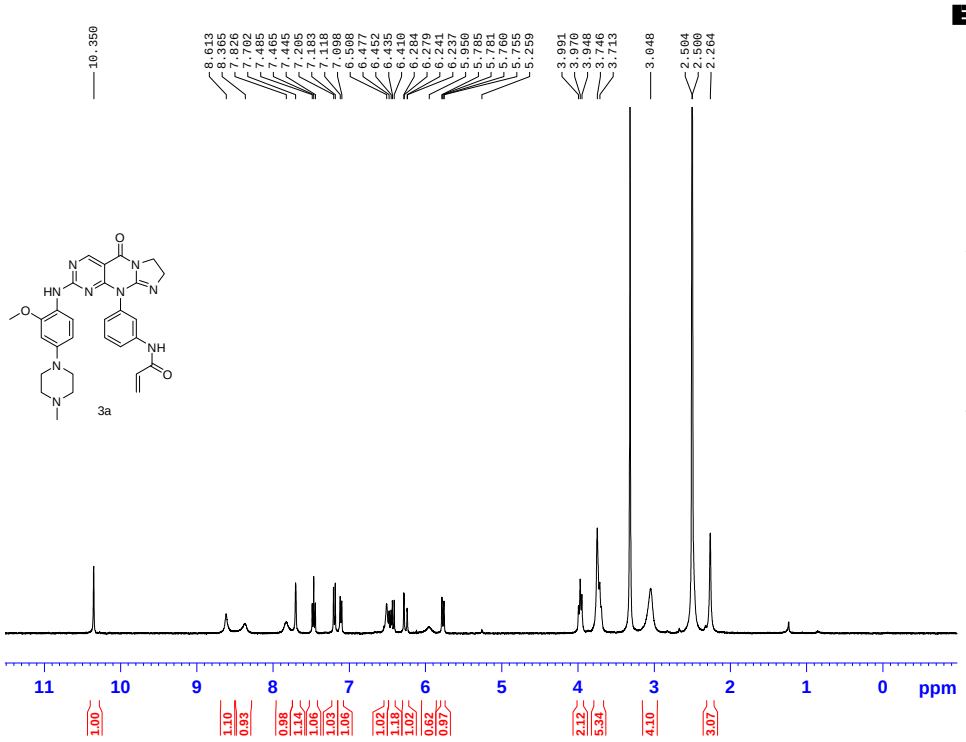


Figure S8. KINOMEsacn tree spot maps illustrating the selectivity profiles for compounds **3g** versus a panel of 456 kinase targets (including 395 wild-type kinases). The size of the red circle is proportional to the percent of DMSO control, where 0% and 35% of control equals 100% and 65% competition, respectively.

Copies of ^{1}H NMR Spectra

01-dcm




```
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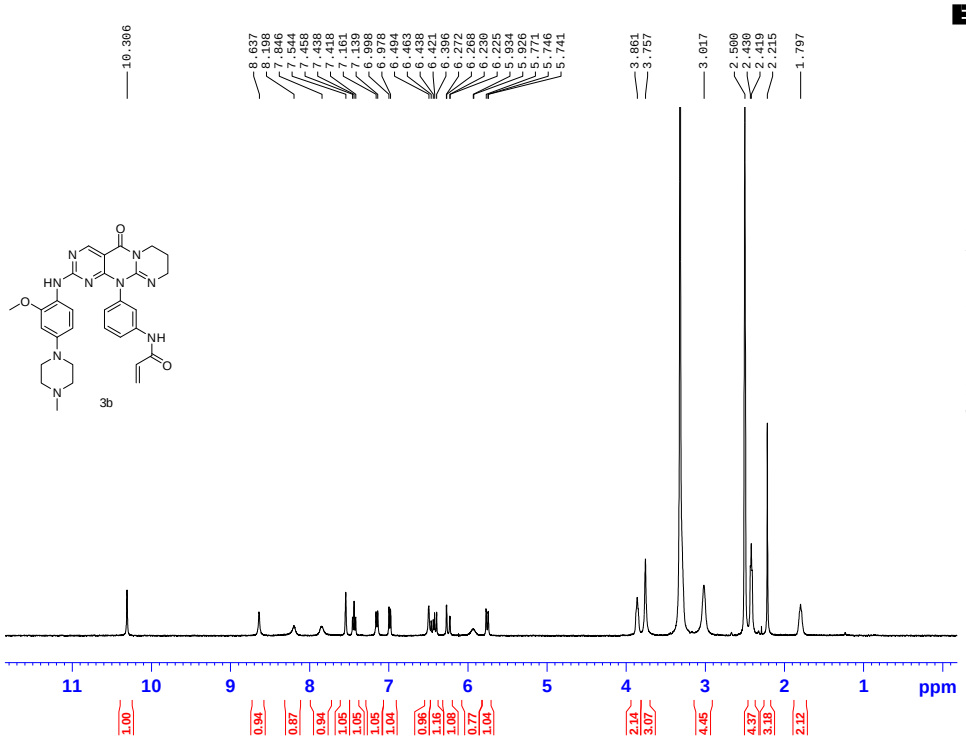
EXPNO                37
PROCNO                1
Date_             20110926
Time                13.54
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT              DMSO
NS                     16
DS                      2
SWH              8278.146 Hz
FIDRES           0.126314 Hz
AQ               3.9584243 sec
RG                    512
DW                60.400 usec
DE                 6.50 usec
TE                298.8 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 14.50 usec
PL1                0.00 dB
PL1W         10.87646866 W
SFO1         400.1324710 MHz
SI                 32768
SF           400.1300025 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                 1.00
```

XTF-02




```
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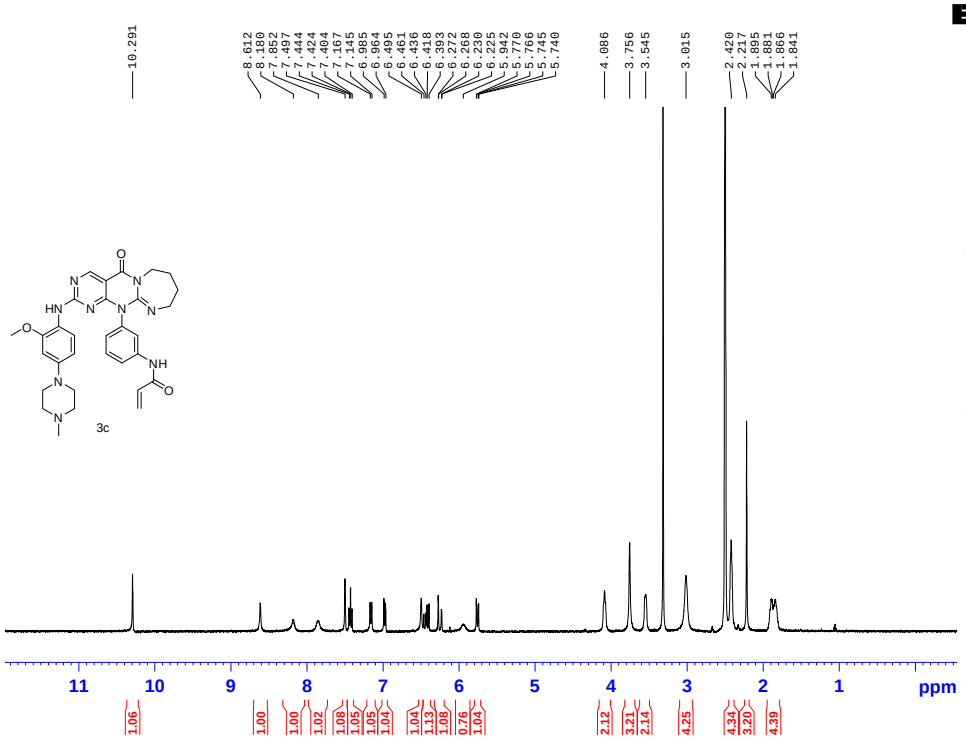
EXPNO                76
PROCNO                1
Date_             20120827
Time                19.25
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT              DMSO
NS                     16
DS                      2
SWH              8278.146 Hz
FIDRES           0.126314 Hz
AQ               3.9584243 sec
RG                    362
DW                60.400 usec
DE                 6.50 usec
TE                673.2 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 14.50 usec
PL1                0.00 dB
PL1W         10.87646866 W
SFO1         400.1324710 MHz
SI                 32768
SF           400.1300024 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                 1.00
```

XTF-23



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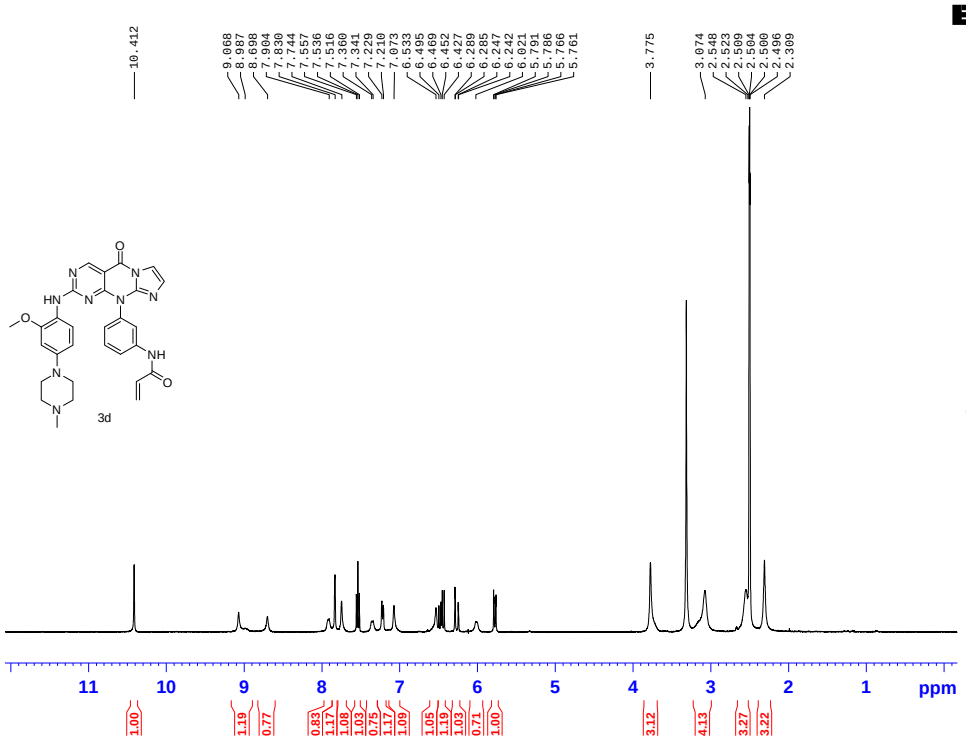
EXPNO                   43
PROCNO                   1
Date_             20111104
Time                 16.11
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG              zg30
TD                  65536
SOLVENT              DMSO
NS                      16
DS                       2
SWH              8278.146 Hz
FIDRES           0.126314 Hz
AQ               3.9584243 sec
RG                   456.1
DW                  60.400 usec
DE                   6.50 usec
TE                  298.0 K
D1               1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   14.50 usec
PL1                   0.00 dB
PL1W           10.87646866 W
SFO1           400.1324710 MHz
SI                    32768
SF             400.1300025 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

XTF-104



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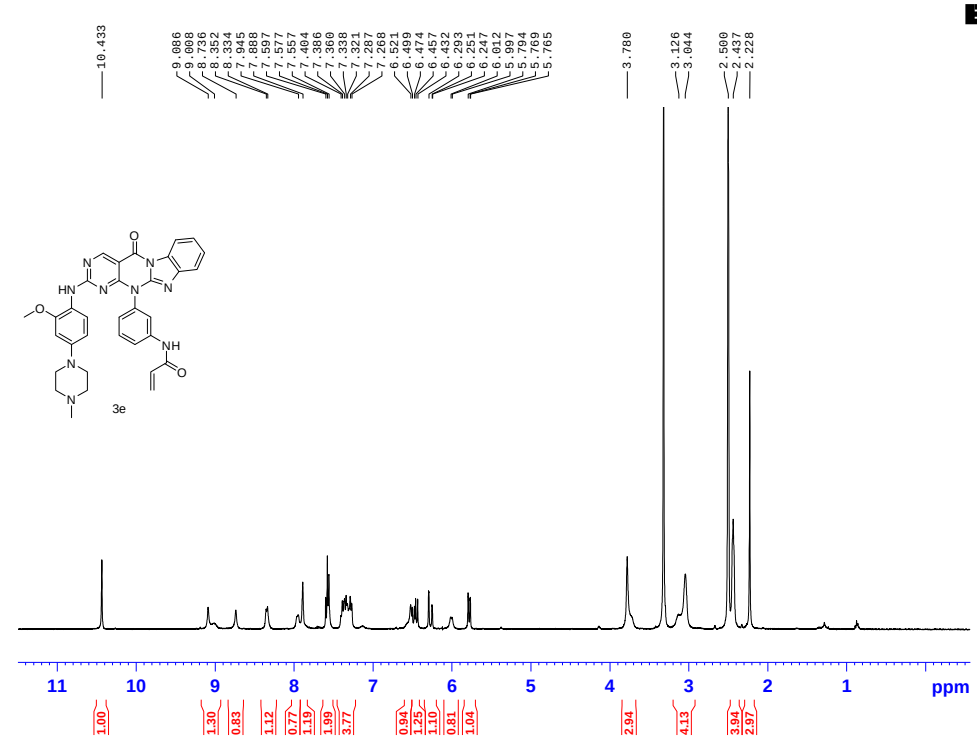
EXPNO                   61
PROCNO                   1
Date_             20120810
Time                 17.08
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG              zg30
TD                  65536
SOLVENT              DMSO
NS                      16
DS                       2
SWH              8278.146 Hz
FIDRES           0.126314 Hz
AQ               3.9584243 sec
RG                     362
DW                  60.400 usec
DE                   6.50 usec
TE                  673.2 K
D1               1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   14.50 usec
PL1                   0.00 dB
PL1W           10.87646866 W
SFO1           400.1324710 MHz
SI                    32768
SF             400.1300022 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

XTF

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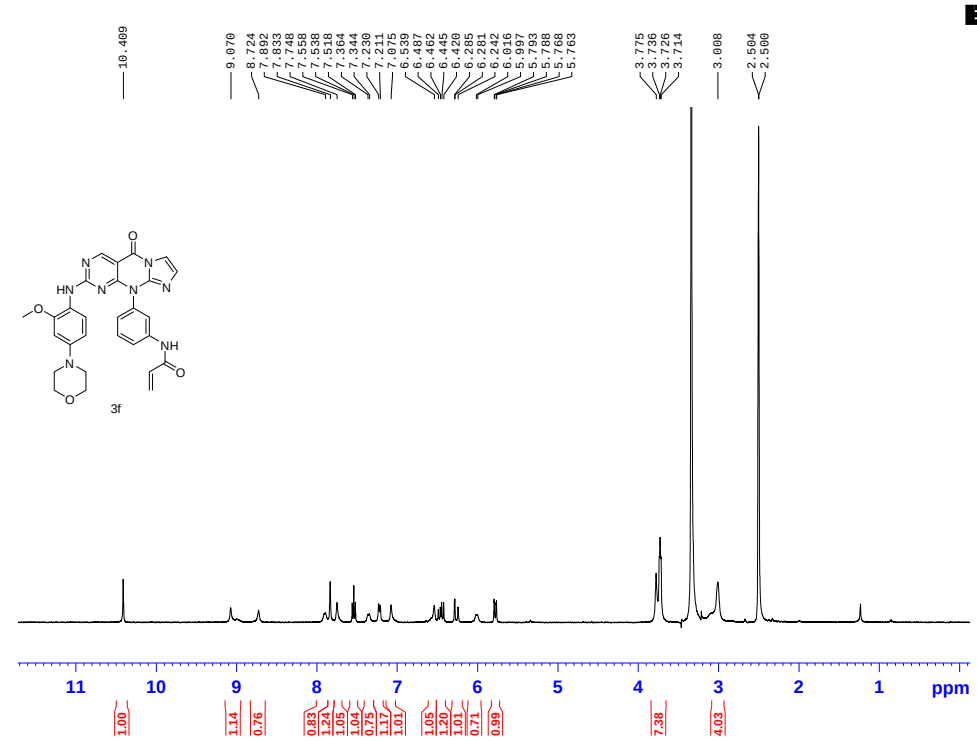
EXPNO                457
PROCNO                 1
Date_           20120712
Time                21.42
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH           8278.146 Hz
FIDRES        0.126314 Hz
AQ            3.9584243 sec
RG                322.5
DW              60.400 usec
DE               6.50 usec
TE              673.2 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                14.50 usec
PL1                0.00 dB
PL1W        10.87646866 W
SFO1        400.1324710 MHz
SI                 32768
SF          400.1300022 MHz
WDW                   EM
SSB                    0
LB                0.30 Hz
GB                     0
PC                  1.00
```

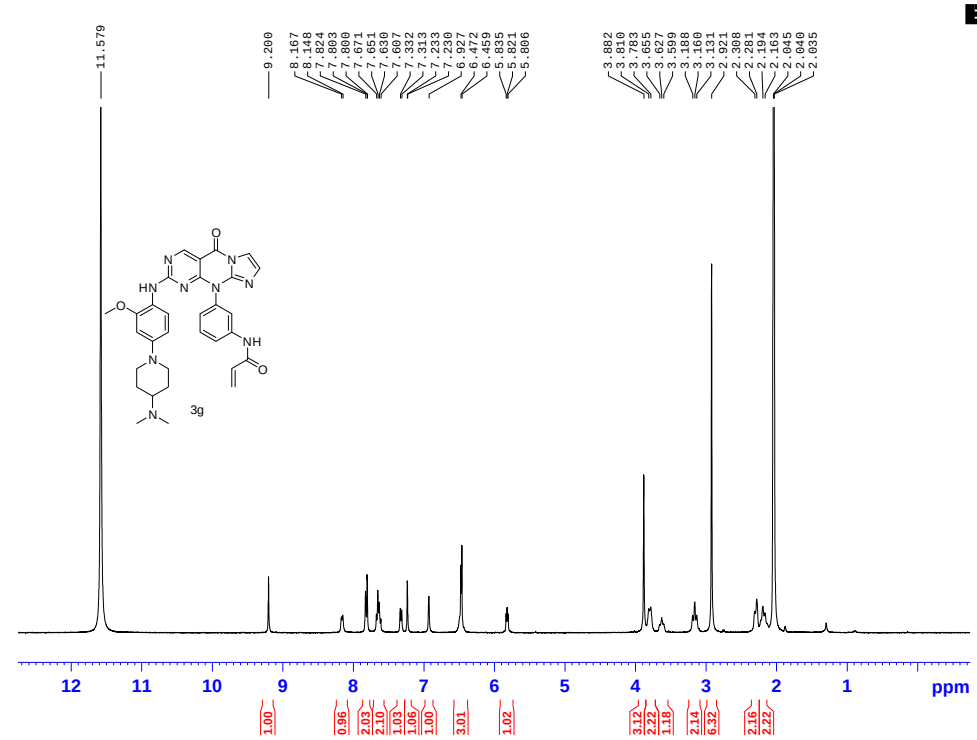
XTF-14

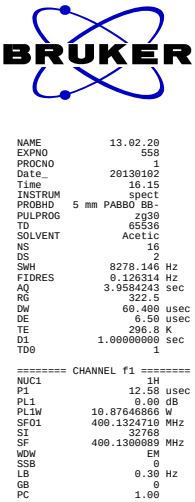



```
NAME            13.02.20
EXPNO                557
PROCNO                 1
Date_           20130102
Time                16.09
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH           8278.146 Hz
FIDRES        0.126314 Hz
AQ            3.9584243 sec
RG                322.5
DW              60.400 usec
DE               6.50 usec
TE              296.8 K
D1            1.00000000 sec
TD0                    1

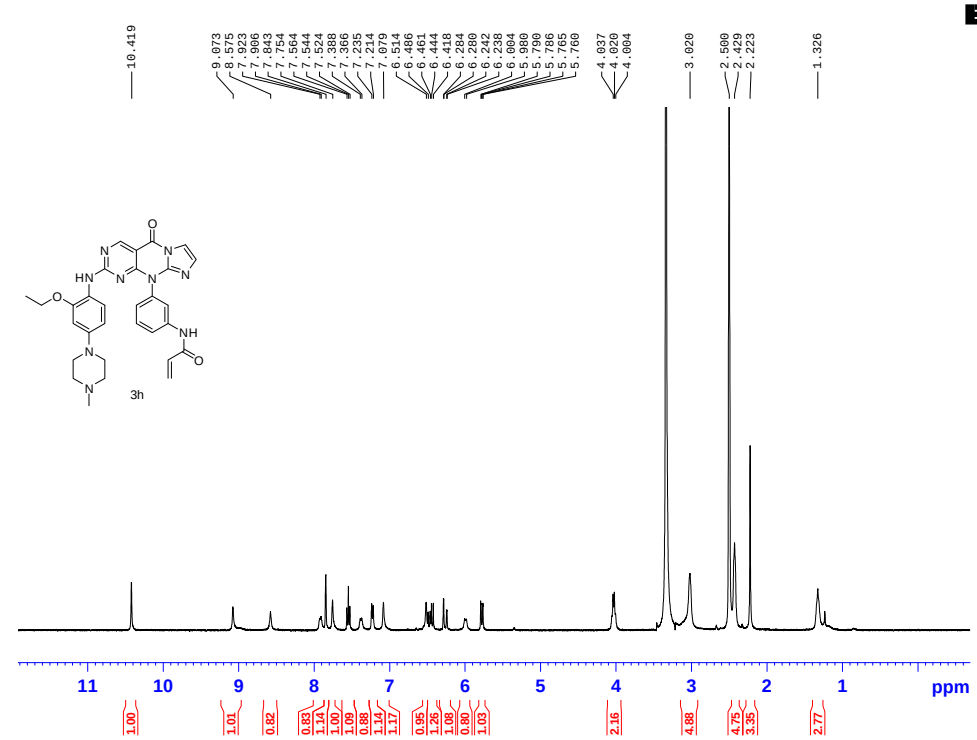
======== CHANNEL f1 ========
NUC1                  1H
P1                12.58 usec
PL1                0.00 dB
PL1W        10.87646866 W
SFO1        400.1324710 MHz
SI                 32768
SF          400.1300020 MHz
WDW                   EM
SSB                    0
LB                0.30 Hz
GB                     0
PC                  1.00
```

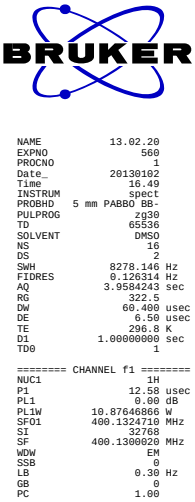
XTF-15





XTF-16





Supplemental References

[1] H. Hisamichi, R. Naito, A. Toyoshima, N. Kawano, A. Ichikawa, A. Orita, M. Orita, N. Hamada, M. Takeuchi, M. Ohta, S. I. Tsukamoto, *Bioorg. Med. Chem.* **2005**, *13*, 4936-4951.