Design, Synthesis and Biological Evaluation of Novel Conformationally Constrained Inhibitors Targeting EGFR

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Cell Proliferation Assay

The human epidermal carcinoma cell line A431 and human non-small cell lung cancer cell line NCI-H1975 $^{L858R/T790M}$ were used to evaluate the potency of synthesized analogues in cell-based level. Both cell lines were purchased from American type culture collection (ATCC). A431, K562 and A549 were cultured with RPMI 1640(GIBCO), and NCI-H1975 $^{L858R/T790M}$ was cultured with Dulbecco's Modified Eagle's Medium (GIBCO). Both mediums were supplemented with penicillin, streptomycin and 10% fetal bovine serum. The assays were performed using the CellTiter-Glo (Promega) Kit. A431 and NCI-H1975 $^{L858R/T790M}$ cells were seeded in density of 2000 cells/well and 1500 cells/well, respectively, in 384-well plates (Corning) for 24 hours. Duplicate wells were treated with test or reference compounds for 48 hours at various concentrations or DMSO (Sigma) as control. Plates were incubated at 37^{o}C in 5% CO_2 atmosphere. Cell proliferation was measured according to the manufacturer's protocol. The IC_{50} was calculated using GraphPad Prism 5.0.

Kinase Inhibition Assay

The assays were performed in vitro using Homogeneous time-resolved fluorescence (HTRF) method (Cisbio). EGFR was purchased from Sigma. The kinases and substrates were incubated first with synthesized analogues for 5 minutes in enzymatic buffer (for EGFR). Then ATP (Sigma) was added into the reaction mixture to start the enzyme reaction. The ATP concentrations used in each enzyme reaction were 1.65μM for EGFR, equivalent to the Km of ATP for the corresponding enzyme in this assay condition. The assays were conducted at room temperature for 30 minutes and stopped by detection reagents which contain EDTA. The detection step lasted for 1 hour. The IC_{50} was calculated using GraphPad Prism 5.0.

Western Blot Assays

A431 cells (5 x 10^5/well) were seeded in 6-well plates overnight. Cell were exposed to 1μM synthesized analogues for 1h at 37 ^{o}C and then either immediately treated with media containing EGF (20ng/ml) for 15min or thoroughly washed with fresh medium 10 times for 5h before EGF treatment. Whole cell lysates were prepared and total protein concentrations were determined. Proteins were extracted with lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 0.5% deoxycholic acid, 0.02% sodium

azide, 1% NP-40, 2.0 μg/ml aprotinin, 1 mM phenylmethylsulfonylfluoride). The lysates were centrifuged at 13,000 rpm for 30 min at 4 ^{o}C. Equivalent amounts of proteins were loaded on SDS–PAGE gels for electrophoresis and were subjected to transfer onto PVDF membranes. Appropriate antibodies to EGFR and p-EGFR from Cell signalling Technology (Danvers, MA) and anti-β-actin from Santa Cruz Biotech (Santa Cruz, CA) were used. Proteins were visualized with peroxidase-coupled secondary antibody from Southern Biotech (Birmingham, UK), using ECL-plus kit from Amersham Biosciences (UK) for detection.

Microsomal Stability Assays

All assays were conducted in single sample. The incubation mixtures were prepared in E-tube and were contained with 1 μM test analogues, 0.5 mg/mL hepatic microsomes (from rat and human), and 1 mM NADPH in 100 mM potassium phosphate buffer solution (pH 7.4). Reactions were initiated by the addition of NADPH and kept in a shaking water bath at 37 ^{o}C. After 0.5, 10, and 30 min incubations at 37 ^{o}C, the reactions were terminated by the addition of cold acetonitrile equivalent to the volume of the reaction mixture. The samples were vortexed for 10 min and then centrifuged at 10,000 rpm for 10 min. The supernatant was subjected to LC/MS/MS (Waters UPLC/API4000 Q Trap). In the determination of the *in vitro* $t_{1/2}$ (half-life, HL), the analyte peak areas were converted to percentage of drug remaining, using the T=0 peak area values as 100%. The slope of the linear regression from log percentage remaining versus incubation time relationships (−k) was used in the conversion to the *in vitro* $t_{1/2}$, values by the *in vitro* $t_{1/2}= -0.693/k$. The percent remaining of test compound is calculated compared to the initial quantity at 0 time.

Measurement of CYP Inhibition Activity

Inhibition activity of CYP was evaluated by incubating 100 μM human hepatic microsomes in the presence of 10 μM test compound. The incubation mixture was allowed to stand for 20 min at 37^{o}C. The incubation was terminated by the addition of acetonitrile equivalent to the volume of the incubation mixture. After the centrifugation, the supernatant was subjected to LC/MS/MS (Waters UPLC/API4000 Q Trap). The relative CYP activity is calculated via the percentage of metabolite product.

Pharmacokinetic Assays

Male rat (Sprague-Dawley rats, body-weight range of 180~220 g, i.v., n = 2, p.o., n = 3) were administered analogue intravenously via the tail vein at 3 mg/kg, respectively, or orally at 10 mg/kg, respectively. For the in vivo study, test compound was prepared as HCl salt. At predetermined times 24 h or more after dosing, 0.4 mL blood was collected, and the plasma was separated by centrifugation (8,000 rpm, 5 min, Sigma 3K15). The concentrations of the compound were measured in the plasma using LC/MS/MS after protein precipitation with acetonitrile. The relevant estimated pharmacokinetic parameters for plasma were derived using DAS 2.0.

Animals and Antitumor Activity in vivo

Human Epidermal Carcinoma A431 xenografts were established by 1 x 10^7 cells subcutaneously inoculated in nude mice. Treatments were initiated when tumors reached a mean group size of 50-150mm^3. The mice were randomized to Control (0.1 ml/10g 0.5% CMC-Na, i.g. administration), Gefitinb (200 mg/kg, i.g. administration), **9n** (200 mg/kg, i.g. administration) every 2 days for 12 days. The size of tumors were measured individually twice per week with microcalipers. Tumor volume (V) was calculated as V = (length x width) ^{2}/2. The individual relative tumor volume (RTV) was calculated as follows: RTV = V_t/V_0, where V_t is the volume on each day of measurement and V_0 is the volume on the day of initial treatment. Therapeutic effect of compound was expressed in terms of T/C% and the calculation formula is T/C (%) = mean RTV of the treated group/mean RTV of the control group x 100%.

Kinase Inhibitors

Gefitinib and Canertinib were obtained from commercial sources and were verified by LC-MS and ^{1}H NMR. Stock solution of all drugs were prepared in DMSO and stored at -20^{o}C.

Computational Methods for Molecular Docking

The molecular modeling simulations were performed using Tripos Sybyl x1.3 molecular modeling package. [1] The co-crystal structure of EGFR [T790M] in complex with dacomitinib was obtained from the RSC Protein Data Bank (http://www.rcsb.org) (PDB code: 4I24). The missing hydrogen atoms were added to the co-crystal structure using Biopolymer module in Sybyl x1.3. The initial structure of ***9n*** was generated by Sketch module in Sybyl x1.3. The geometries of the compound was subsequently optimized

using the Tripos force field with Gasteiger-Hückel charges. The produced conformation of ***9n*** was then inserted into the binding pocket of EGFR [T790M] to replace ligand dacomitinib for the initial structural model of ***9n*** binding to EGFR [T790M]. Molecular docking studies of ***9n*** with the EGFR [T790M] binding pocket was performed with FlexiDock module in Sybyl 6.9. [2] The docked complexes of inhibitor-enzyme were selected according to the criteria of interacting energy combined with geometrical matching quality. These complexes were used as the starting conformation for the geometrical optimization to achieve the final models of ***9n*** binding to EGFR [T790M].

Reference:

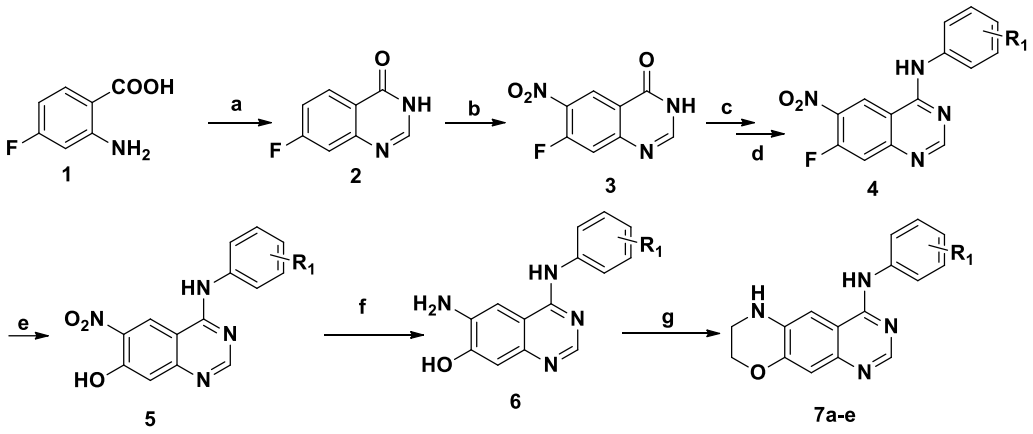
1. Sybyl X molecular modeling software packages, version 1.3; Tripos Associates, Inc.: St Louis, MO63114, 2011.
2. Sybyl molecular modeling software packages, 6.9; Tripos Associates, Inc., St Louis, MO63114, 2001.

Chemical Methods

All commercial chemicals and solvents were reagent grade and were used without further purification unless otherwise specified. All reported yields are isolated yields after flash column chromatography or crystallization. Melting points were determined on an SRS OptiMelt melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DRX-500 [Bruker Biospin, Germany]. Chemical shifts are reported in ppm relative to the residual solvent peak ($CDCl_3$, TMS: 0.00). Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublet); dt (triplet of doublet); td (doublet of triplet); brs (broad singlet); etc. Products were purified by flash column chromatography on silica gel (200-300 mesh). The purity of all biologically evaluated compounds was confirmed to be ≥95% by a Agilent 1290 HPLC-6224 Time of Fight Mass Spectrometer using PhenomenexLuna 5µ C18, 100 Å, 150 X 4.60 mm 5 micron column at a flow rate of 0.5ml/min using liner gradients buffer B in A (B: CH_3OH containing 0.1 % formic acid, A: H_2O containing 0.1% formic acid). Mobile phase B was increased linearly from 5% to 95% over 7 min and 95% over the next 2 min, after which the column was equilibrated to 5% for 1 min. Yields were not optimized. Thin-layer chromatography (TLC) was performed on precoated glass-backed

plates (silica gel 60 F254, 0.25 mm), and the components were visualized under UV light (254 and 365 nm). Distilled water was treated using ion exchange and filtration.

Synthetic Scheme S1 for Compound 7a-7e



Reagents and conditions: (a) formamidine acetate, reflux, 18 h; (b) HNO_3, H_2SO_4, 110 °C, 2 h; (c) $SOCl_2$, reflux, 4 h; (d) anilines, rt., 30 min.; (e) 50% NaOH, reflux, 3 h; (f) $SnCl_2$ $2H_2O$, 0 °C, 30 min., rt., 30 min.; (g) $Br(CH_2)_2Br$, Cs_2CO_3, 125 °C, 16 h;

Synthesis and Characterization of Intermediate

7-fluoro-3H-quinazolin-4-one (2)

A mixture of 2-amino-4-fluorobenzoic acid (126g, 0.82 mol) and formamidine acetate (170 g, 1.64 mol) in 2-methoxyethanol (800 mL) was heated under reflux for 18 h, and the solution was concentrated. The residue was diluted with water, and the suspension was filtered, washed with water, and dried to give pure product (yield 88%). M.p. 235-237 ℃. ^{1}H NMR (500 MHz, DMSO) δ 12.37 (s, 1H), 8.19 (dd, *J* = 8.5, 6.5 Hz, 1H), 8.15 (s, 1H), 7.46 (dd, *J* = 10.0, 2.5 Hz, 1H), 7.40 (td, *J* = 9.0, 2.5 Hz, 1H).

7-fluoro-6-nitro-3H-quinazolin-4-one (3)

7-Fluoro-3H-quinazolin-4-one (47.4 g, 0.29mmol) was added to a mixture of concentrated sulfuric acid (100 mL) and fuming nitric acid (105 mL) at 0 °C. The resulting mixture was heated at 110 ℃ for 2 h. The solution was cooled to room temperature, then poured onto ice-water (1.5 L) to give a mixture of 6- and 8-nitroquinazolin-4(3*H*)-ones. Recrystallization from AcOH gave pure 7-fluoro-6-nitroquinazolin-4(3*H*)-one (yield 53%): M.p. 283-285 ℃. ^{1}H NMR (500 MHz, DMSO) δ 12.81 (s, 1H), 8.71 (d, *J* = 8.5 Hz, 1H), 8.32 (s, 1H), 7.77 (d, *J* = 12.0 Hz, 1H).

4-Chloro-7-fluoro-6-nitro-quinazoline and 4-(3-bromophenylamino)-7-fluoro-6-nitro-quinazoline (4a)

A suspension of 7-fluoro-6-nitro-3H-quinazolin-4-one (10.45 g, 50 mmol) in $SOCl_2$ (200 mL) containing 3 drops of DMF was heated under reflux for 4 h to give a clear solution. The $SOCl_2$ was removed under reduced pressure to give crude 4-chloro-7-fluoro-6-nitroquinazoline. The crude chloro-substitute was dissolved in CH_2Cl_2 (200 mL), and a solution of aniline (10.5 g, 55 mmol) in EtOH (50 mL) was added. The resulting mixture was stirred at room temperature for 15 min when a precipitate of product hydrochloride formed. After a further 15 min, sufficient hexane was added to ensure complete precipitation, and the solid was collected by filtration, washed with petroleum ether, and dried to give pure product (yield 95%). ^{1}H NMR (500 MHz, DMSO) δ 11.27 (s, 1H), 9.78 (d, *J* = 7.5 Hz, 1H), 8.85 (s, 1H), 8.09 (s, 1H), 7.91 (d, *J* = 12.0 Hz, 1H), 7.82 (dd, *J* = 7.0, 1.5 Hz, 1H), 7.42-7.40 (m, 2H).

4-(3-bromophenylamino)-7-hydroxyl-6-nitro-quinazoline (5a)

A solution of 4-(3-bromophenylamino)-7-fluoro-6-nitro-quinazoline (1.0 mmol) in dioxane (5 ml) was refluxed with 50% aqueous NaOH (1 ml) for 2 h, cooled, acidified with 10% H_2SO_4 (10 ml) and extracted with EtOAc (3×10 ml). The combined organic layers were dried over anhydrous sodium sulfate and evaporated to dryness under reduce pressure. The residue was purified by flash column chromatography on silica gel eluting with petroleum ether/EtOAc=2:1 (yield 68%). ^{1}H NMR (500 MHz, DMSO) δ 11.97 (s, 1H), 10.14 (s, 1H), 9.25 (s, 1H), 8.61 (s, 1H), 8.18 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.37-7.33 (m, 2H), 7.25 (s, 1H).

4-(3-bromophenylamino)-7-hydroxyl-6-amino-quinazoline (6a)

To a solution of 4-(3-bromophenylamino)-7-hydroxyl-6-nitro-quinazoline (1.0 mmol) in 10 ml MeOH was added $SnCl_2 \cdot 2H_2O$ (10 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 minutes then at room temperature for 30 minutes. The mixture was evaporated to dryness under reduce pressure. The residue was added saturated Na_2CO_3, adjusted pH to 7-8 and extracted with EtOAc (3×20 ml). The combined organic phase was dried over anhydrous sodium sulfate and evaporated to dryness under reduce pressure. The residue was purified by flash column chromatography on silica gel eluting with CH_2Cl_2/MeOH=10:1(yield 90%). ^{1}H NMR (500 MHz, DMSO) δ 10.71 (s, 1H), 9.32 (s, 1H), 8.33 (s, 1H), 8.22 (t, *J* = 2.0 Hz, 1H), 7.85 (d, *J* = 9.0 Hz, 1H), 7.37 (s, 1H), 7.29 (t, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 9.0 Hz, 1H), 7.00 (s, 1H), 5.24 (s, 2H).

Synthesis and Characterization of 7a-7e

N-(3-bromophenyl)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-4-amine (7a)

To a suspension of 4-(3-bromophenylamino)-7-hydroxyl-6-amino-quinazoline (1 mmol) and cesium carbonate (5 mmol) in dry DMF (2 ml), 1, 2-dibromoethane (1.5 mmol) was added. The mixture was heated at 125 ^{o}C for 15 h. After cooling, the mixture was treated with crushed ice and then extracted with ethyl acetate (3×10 ml). The organic extracts were dried over Na_2SO_4 and evaporated. The residue was purified by chromatography on a silica gel column using CH_2Cl_2: MeOH (50:1) as the eluent (yield 11%). M.p. 246-247 ℃. ^{1}H NMR (500 MHz, DMSO) δ 9.41 (s, 1H), 8.35 (s, 1H), 8.21 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.44 (s, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.01 (s, 1H), 6.45 (s, 1H), 4.29 (t, *J* = 4.0 Hz, 2H), 3.40 (t, *J* = 4.0 Hz, 2H); LC-MS (ESI) : m/z ([M+ H]) $^{+}$: 357.2.

N-(3-chloro-4-fluorophenyl)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-4-amine (7b)

The similar procedure of 7a was repeated to obtain the compound 7b (yield 13%). M.p.>250 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.44 (s, 1H), 8.34 (s, 1H), 8.19 (dd, *J* = 7.0, 2.5 Hz, 1H), 7.83-7.80 (m, 1H), 7.42-7.38 (m, 2H), 7.01 (s, 1H), 6.46 (s, 1H), 4.29 (t, *J* = 4.0 Hz, 2H), 4.40 (t, *J* = 4.0 Hz, 2H). LC-MS (ESI) : m/z ([M+ H]) $^{+}$: 331.2.

N-(4-fluorophenyl)-7,8-dihydro-6H-[1,4]oxazino[3,2-g]quinazolin-4-amine (7c). The similar procedure of 7a was repeated to obtain the compound 7c (yield 11 %). M.p. 226-227 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.33 (s, 1H), 8.27 (s, 1H), 7.83-7.81 (m, 2H), 7.43 (s, 1H), 7.18 (t, *J* = 9.0 Hz, 2H), 6.98 (s, 1H), 6.38 (s, 1H), 4.28 (t, *J* = 4.5 Hz, 2H), 3.39 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI): m/z ([M+ H]) $^{+}$: 297.3.

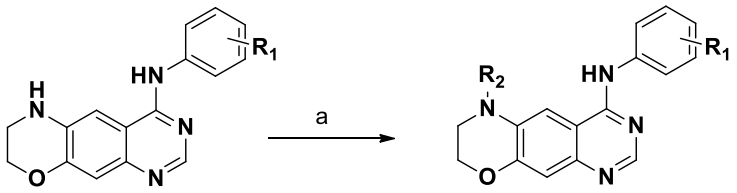
N-(3-(trifluoromethyl) phenyl)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-4-amine (7d).

The similar procedure of 7a was repeated to obtain the compound 7d (yield 10 %). Mp>250 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.57 (s, 1H), 8.36 (s, 1H), 8.31 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.58 (t, *J* = 8.0 Hz, 1H), 7.46 (s, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.02 (s, 1H), 6.47 (s, 1H), 4.29 (t, *J* = 4.5 Hz, 2H), 3.40 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI): m/z ([M+ H]) $^{+}$: 347.3.

N-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2 g]quinazolin-4-amine (7e)

The similar procedure of 7a was repeated to obtain the compound 7e (yield 14%). mp 250-251 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.31 (s, 1H), 8.29 (s, 1H), 8.01 (d, *J* = 2.5 Hz, 1H), 7.71 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.49-7.45 (m, 1H), 7.40 (s, 1H), 7.34-7.30 (m, 2H), 7.23-7.18 (m, 2H), 6.98 (s, 1H), 6.41 (s, 1H), 5.24 (s, 2H), 4.28 (t, *J* = 4.0 Hz, 2H), 3.39 (t, *J* = 4.0 Hz, 2H). LC-MS (ESI): m/z ([M+ H]) $^{+}$: 437.3.

Synthetic Scheme S2 for Compound 7f-7o



Regents and conditions: a) CH_3CH_2COCl or CH_3SO_2Cl or C_2H_5NCO, Et_3N, DCM-DMF. overnight;

Synthesis and Characterization of 7f-7o

1-(4-((3-bromophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-6-yl)propan-1-one (7f)

N-(3-bromophenyl)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-4-amine(50mg) was dissolved in 6 ml CH_2Cl_2 and a suitable volume of DMF. The solution was cooled to 0 ^{o}C. Acyl chloride (3 eq.) was added then Et_3N (4 eq.) was added dropwise. The mixture was stirred at 0-10 ^{o}C for 20 minutes and at room temperature for 2-3 h. The reaction was monitored by TLC. The reaction was quenched with saturated Na_2CO_3, extracted with EtOAc (3×20 ml) and dried over anhydrous sodium sulfate and evaporated to dryness under reduce pressure. The residue was crystallized from diethyl ether (Et_2O)/ ethyl acetate (EtOAc). The suspension was collected by filtration and washed with diethyl ether, and dried to give pure product (yield 95%). M.p.>250 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.93 (s, 1H), 8.58 (s, 1H), 8.51 (s, 1H), 8.15 (s, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.20 (s, 1H), 6.88 (dd, *J* = 16.5, 10.0 Hz, 1H), 6.38 (dd, *J* = 16.5, 1.5 Hz, 1H), 5.90 (dd, *J* = 10.0, 1.5 Hz, 1H), 4.44 (t, *J* = 4.5 Hz, 2H), 4.09 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI): m/z ([M+ H]) $^{+}$: 411.2.

1-(4-((3-chloro-4-fluorophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-6-yl)prop-2-en-1-one (7g)

The similar procedure of 7f was repeated to obtain the compound 7g (yield 75%). M.p.>250 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.81 (s, 1H), 8.54 (s, 1H), 8.50 (s, 1H), 8.12 (d, *J* = 4.5 Hz, 1H), 7.80-7.78 (m, 1H), 7.43 (t, *J* = 9.0 Hz, 1H), 7.21 (s, 1H), 6.88 (dd, *J* = 16.5, 10.5 Hz, 1H), 6.39 (dd, *J* = 16.5, 1.5 Hz, 1H), 5.91 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.44 (t, *J* = 4.5 Hz, 2H), 4.09 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI) : m/z ([M+ H]) $^{+}$: 385.2.

1-(4-((3-(trifluoromethyl) phenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-6-yl)prop-2-en-1-one (7h)

The similar procedure of 7f was repeated to obtain the compound 7h (yield 67%). M.p.>250 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 10.01 (s, 1H), 8.54 (s, 1H), 8.45 (s, 1H), 8.18 (s, 1H), 8.12 (d, *J* = 6.7 Hz, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 7.2 Hz, 1H), 7.16 (s, 1H), 6.89 (dd, *J* = 16.5, 10.0 Hz, 1H), 6.38 (dd, *J* = 16.5, 1.0 Hz, 1H), 5.90 (dd, *J* = 10.4, 1.0 Hz, 1H), 4.43 (t, *J* = 4.5 Hz, 2H), 4.08 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI): m/z ([M+ H]) $^{+}$: 401.3.

1-(4-(3-chloro-4-((3-fluorobenzyl) oxy) phenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-6-yl)prop-2-en-1-one (7i)

The similar procedure of 7f was repeated to obtain the compound 7i (yield 79%). M.p.>250 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.71 (s, 1H), 8.51 (s, 1H), 8.45 (s, 1H), 7.95 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.49-7.45 (m, 1H), 7.34-7.30 (m, 2H), 7.25 (d, *J* = 9.0 Hz, 1H), 7.20-7.16 (m, 2H), 6.87 (dd, *J* = 16.5, 10.0 Hz, 1H), 6.38 (dd, *J* = 16.5, 2.0 Hz, 1H), 5.90 (dd, *J* = 10.0, 2.0 Hz, 1H), 5.25 (s, 2H), 4.43 (t, *J* = 4.5 Hz, 2H), 4.08 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI): m/z ([M+ H]) $^{+}$: 491.4.

N-(3-bromophenyl)-6-(methylsulfonyl)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-4-amine (7j)

The similar procedure of 7f was repeated to obtain the compound 7j (yield 66%). ^{1}H NMR (500 MHz, DMSO) δ 9.94 (s, 1H), 8.50 (s, 1H), 8.40 (s, 1H), 8.01 (s, 1H), 7.70 (s, 1H), 7.30-7.15 (m, 3H), 4.42 (t, *J* = 4.5 Hz, 2H), 3.94 (t, *J* = 4.5 Hz, 2H), 3.26 (s, 3H). MS (ESI): m/z ([M-H]) $^{+}$: 435.2.

N-(3-chloro-4-fluorophenyl)-6-(methylsulfonyl)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-4-amine (7k)

The similar procedure of 7f was repeated to obtain the compound 7k (yield 65%). ^{1}H NMR (500 MHz, DMSO) δ 9.94 (s, 1H), 8.49 (s, 2H), 8.05 (dd, *J* = 6.5, 2.5 Hz, 1H), 7.75-7.72 (m, 1H), 7.45 (t, *J* = 9.0 Hz, 1H), 7.23 (s, 1H), 4.44 (t, *J* = 4.5 Hz, 2H), 3.95 (t, *J* = 4.5 Hz, 2H), 3.29 (s, 3H). MS (ESI): m/z ([M+H]) +: 409.2.

N-(3-chloro-4-((3-fluorobenzyl) oxy) phenyl)-6-(methylsulfonyl)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-4-amine (7l)

The similar procedure of 7f was repeated to obtain the compound 7l (yield 67%). ^{1}H NMR (500 MHz, DMSO) δ 9.90 (s, 1H), 8.50 (s, 1H), 8.43 (s, 1H), 7.91 (s, 1H), 7.66 (d, *J* = 7.5 Hz, 1H), 7.48 (dd, *J* = 13.5, 7.0 Hz, 1H), 7.33 (t, *J* = 7.0 Hz, 2H), 7.26 (d, *J* = 9.0 Hz, 1H), 7.20-7.17 (m, 2H), 5.26 (s, 2H), 4.43 (t, *J* = 4.5 Hz, 2H), 3.94 (t, *J* = 4.5 Hz, 2H), 3.33 (s, 3H). MS (ESI): m/z ([M+H]) +: 515.2.

4-((3-bromophenyl) amino)-N-ethyl-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazoline-6-carboxamide (7m)

The similar procedure of 7f was repeated to obtain the compound 7m (yield 78%). ^{1}H NMR (500 MHz, DMSO) δ 9.86 (s, 1H), 8.60 (s, 1H), 8.48 (s, 1H), 8.20 (s, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.33 (t, *J* = 9.0 Hz, 1H), 7.29-7.25 (m, 2H), 7.15 (s, 1H), 4.32 (t, *J* = 4.5 Hz, 2H), 3.85 (t, *J* = 4.5 Hz, 2H), 3.23-3.17 (m, 2H), 1.10 (t, *J* = 7.0 Hz, 3H). MS (ESI): m/z ([M+H]) +: 428.2.

4-((3-chloro-4-fluorophenyl) amino)-N-ethyl-7, 8-dihydro-6H-[1, 4]oxazino[3, 2 g]quinazoline-6-carboxamide (7n)

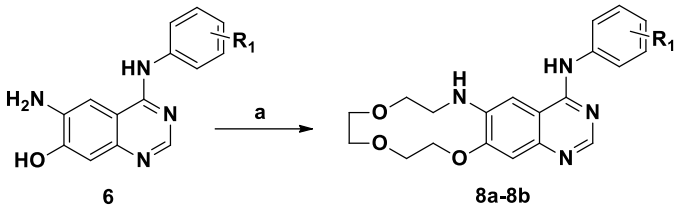
The similar procedure of 7f was repeated to obtain the compound 7n (yield 47%). ^{1}H NMR (500 MHz, DMSO) δ 9.86 (s, 1H), 8.58 (s, 1H), 8.46 (s, 1H), 8.17 (d, *J* = 4.5 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.42 (t, *J* = 9.0 Hz, 1H), 7.25 (t, *J* = 5.0 Hz, 1H), 7.14 (s, 1H), 4.31 (t, *J* = 4.5 Hz, 2H), 3.85 (t, *J* = 4.5 Hz, 2H), 3.22-3.17 (m, 2H), 1.10 (t, *J* = 7.0, 3H). MS (ESI): m/z ([M+H]) +: 402.2.

4-((3-chloro-4-((3-fluorobenzyl) oxy) phenyl) amino)-N-ethyl-7, 8-dihydro-6H [1, 4]oxazino[3,2-g]quinazoline-6-carboxamide (7o)

The similar procedure of 7f was repeated to obtain the compound 7o (yield 69%). ^{1}H NMR (500 MHz, DMSO) δ 9.62 (s, 1H), 8.53 (s, 1H), 8.41 (s, 1H), 8.00 (s, 1H), 7.70 (s, 1H), 7.47 (s, 1H), 7.33-7.12 (m, 6H), 5.25 (s, 2H), 4.31 (t, *J* = 4.5 Hz, 2H), 3.84 (t, *J* =

4.5 Hz, 2H), 3.20-3.17 (m, 2H), 1.10 (t, *J* = 7.0 Hz, 3H). MS (ESI): m/z ([M+H]) +: 508.2.

Synthetic Scheme S3 for Compound 8a-8b



Reagents and conditions: (a) Tri(ethylene glycol)di-p-toluenesulfonate, Cs_2CO_3, 125 °C, 16 h;

Synthesis and Characterization of 8a-8b

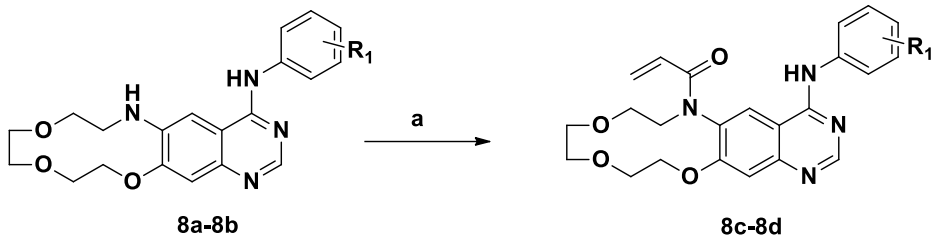
N-(3-(trifluoromethyl) phenyl)-7, 8, 10, 11, 13, 14-hexahydro-6H [1, 4, 7, 10] trioxaazacyclododecino[9, 8-g] quinazolin-4-amine (8a)

To a suspension of 4-(3-trfluoromethylphenylamino)-7-hydroxyl-6-amino-quinazoline (1 mmol) and cesium carbonate (5 mmol) in dry DMF (2 ml), tri(ethylene glycol)di-toluenesulfonate (1.5 mmol) was added. The mixture was heated at 125 °C for 15 h. After cooling, the mixture was treated with crushed ice and then extracted with ethyl acetate (3×10 ml). The organic extracts were dried over Na_2SO_4 and evaporated. The residue was purified by chromatography on a silica gel column using CH_2Cl_2: MeOH (50:1) as the eluent (yield 4%). M.p.111-112 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.02-7.99 (m, 2H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.42-7.37 (m, 3H), 7.27 (s, 1H), 6.77 (s, 1H), 6.07 (s, 1H), 4.40-4.39 (m, 2H), 3.75-3.74 (m, 2H), 3.69-3.67 (m, 2H), 3.64-3.62 (m, 2H), 3.58-3.56 (m, 2H), 3.46-3.39 (m, 2H). LC-MS (ESI): m/z ([M-H]) $^+$: 435.4.

N-(3-chloro-4-fluorophenyl)-7, 8, 10, 11, 13, 14-hexahydro-6H-[1, 4, 7, 10]trioxaazacyclododecino[9, 8-g]quinazolin-4-amine (8b)

The similar procedure of 8a was repeated to obtain the compound 8b (yield 3%). M.p. 185-186 °C.^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.87 (dd, *J* = 6.0, 2.0 Hz, 1H), 7.54-7.51 (m, 1H), 7.44 (s, 1H), 7.37 (s, 1H),7.14 (t, *J* = 9.0 Hz, 1H), 6.78 (s, 1H), 6.04 (s, 1H), 4.38-4.36 (m, 2H), 3.72-3.70 (m,2H), 3.67-3.66 (m, 2H), 3.62-3.61 (m, 2H), 3.57-3.56 (m, 2H), 3.41-3.33 (m, 2H). LC-MS (ESI): m/z ([M-H]) $^+$: 419.4.

Synthetic Scheme S4 for Compound 8c-8d



8a-8b 8c-8d

Regents and conditions: a) $CH_2CHCOCl$, Et_3N, DCM-DMF. overnight

Synthesis and Characterization of 8c-8d

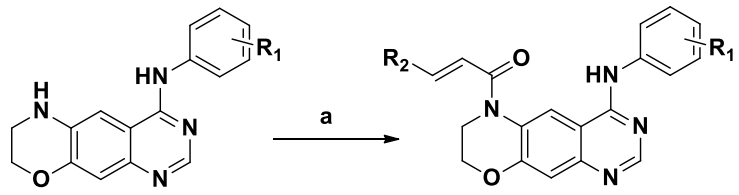
1-(4-((3-(trifluoromethyl) phenyl) amino)-7, 8, 10, 11, 13, 14-hexahydro-6H-[1, 4, 7, 10]trioxaazacyclododecino[9, 8-g]quinazolin-6-yl)prop-2-en-1-one (8c)

The similar procedure of 7f was repeated to obtain the compound 8c (yield 49%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.63 (s, 1H), 7.52-7.47 (m, 4H), 6.60 (s, 1H), 6.56 (dd, *J* = 16.5, 1.5 Hz, 1H), 6.20 (t, *J* = 5.5 Hz, 1H), 6.07 (dd, *J* = 16.5, 10.5 Hz, 1H), 5.70 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.44-4.42 (m, 2H), 3.69-3.67 (m, 2H), 3.57-3.54 (m, 6H), 3.23 (dd, *J* = 10.5, 5.5 Hz, 2H). LC-MS (ESI): m/z ([M+H]) +: 489.4.

1-(4-((3-chloro-4-fluorophenyl) amino)-7, 8, 10, 11, 13, 14-hexahydro-6H-[1, 4, 7, 10]trioxaazacyclododecino[9, 8-g]quinazolin-6-yl)prop-2-en-1-one (8d)

The similar procedure of 7f was repeated to obtain the compound 8d (yield 42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.94 (s, 1H), 7.51 (s, 1H), 7.42 (dd, *J* = 6.5, 2.5 Hz, 1H), 7.26-7.23 (m, 1H), 7.14 (t, *J* = 9.0 Hz, 1H), 6.63 (s, 1H), 6.55 (dd, *J* = 16.5, 1.5 Hz, 1H), 6.25 (t, *J* = 5.5 Hz, 1H), 6.07 (dd, *J* = 16.5, 10.0 Hz, 1H), 5.70 (dd, *J* = 10.0, 1.0 Hz, 1H), 4.45-4.43 (m, 2H), 3.72-3.70 (m, 2H), 3.66 (t, *J* = 4.5 Hz, 2H), 3.60-3.57 (m, 4H), 3.31-3.28 (m, 2H). MS (ESI): m/z ([M+H]) +: 473.2.

Synthetic Scheme S5 for Compound 9a-9p



7a-e 9a-9p

Regents and conditions: a) $R_2(CH)_2COCl$, Et_3N, DCM-DMF. overnight or $R_2(CH)_2COOH$, $(COCl)_2$, DMF, Et_3N, DCM-DMF, 0 oC-rt, overnight

Synthesis and Characterization of 9a-9p

1-(4-((3-bromophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-6-yl)prop-2-en-1-one (9a)

The similar procedure of 7f was repeated to obtain the compound 9a (yield 95%). ^{1}H NMR (500 MHz, DMSO) δ 9.93 (s, 1H), 8.58 (s, 1H), 8.51 (s, 1H), 8.15 (s, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.20 (s, 1H), 6.88 (dd, *J* = 16.5, 10.0 Hz, 1H), 6.38 (dd, *J* = 16.5, 1.5 Hz, 1H), 5.90 (dd, *J* = 10.0, 1.5 Hz, 1H), 4.44 (t, *J* = 4.5 Hz, 2H), 4.09 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI): m/z ([M+H]) +: 411.2.

1-(4-((4-fluorophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-6-yl)prop-2-en-1-one (9b)

The similar procedure of 7f was repeated to obtain the compound 9b (yield 72%). ^{1}H NMR (500 MHz, DMSO) δ 9.76 (s, 1H), 8.58-8.54 (m, 1H), 8.44 (s, 1H), 7.80-7.77 (m, 2H), 7.24-7.19 (m, 3H), 6.88 (dd, *J* = 16.5, 10.0 Hz, 1H), 6.38 (d, *J* = 16.5 Hz, 1H), 5.90 (d, *J* = 10.0 Hz, 1H), 4.44 (t, *J* = 4.0 Hz, 2H), 4.09 (t, *J* = 4.0 Hz, 2H). LC-MS (ESI): m/z ([M+H]) +: 351.3.

1-(4-((3-chloro-4-fluorophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-6-yl)prop-2-en-1-one (9c)

The similar procedure of 7f was repeated to obtain the compound 9c (yield 75%). ^{1}H NMR (500 MHz, DMSO) δ 9.81 (s, 1H), 8.54 (s, 1H), 8.50 (s, 1H), 8.12 (d, *J* = 4.5 Hz, 1H), 7.80-7.78 (m, 1H), 7.43 (t, *J* = 9.0 Hz, 1H), 7.21 (s, 1H), 6.88 (dd, *J* = 16.5, 10.5 Hz, 1H), 6.39 (dd, *J* = 16.5, 1.5 Hz, 1H), 5.91 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.44 (t, *J* = 4.5 Hz, 2H), 4.09 (t, *J* = 4.5 Hz, 2H). LCMS (ESI): m/z ([M+H]) +: 385.2.

1-(4-((3-(trifluoromethyl) phenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-6-yl)prop-2-en-1-one (9d)

The similar procedure of 7f was repeated to obtain the compound 9d (yield 67%). ^{1}H NMR (500 MHz, DMSO) δ 10.01 (s, 1H), 8.54 (s, 1H), 8.45 (s, 1H), 8.18 (s, 1H), 8.12 (d, *J* = 6.7 Hz, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 7.2 Hz, 1H), 7.16 (s, 1H), 6.89 (dd, *J* = 16.5, 10.0 Hz, 1H), 6.38 (dd, *J* = 16.5, 1.0 Hz, 1H), 5.90 (dd, *J* = 10.4, 1.0 Hz, 1H), 4.43 (t, *J* = 4.5 Hz, 2H), 4.08 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI): m/z ([M+H]) +: 401.3.

1-(4-((3-chloro-4-((3-fluorobenzyl) oxy) phenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-6-yl)prop-2-en-1-one (9e)

The similar procedure of 7f was repeated to obtain the compound 9e (yield 79%). ^{1}H NMR (500 MHz, DMSO) δ 9.71 (s, 1H), 8.51 (s, 1H), 8.45 (s, 1H), 7.95 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.49-7.45 (m, 1H), 7.34-7.30 (m, 2H), 7.25 (d, *J* = 9.0 Hz, 1H), 7.20-7.16 (m, 2H), 6.87 (dd, *J* = 16.5, 10.0 Hz, 1H), 6.38 (dd, *J* = 16.5, 2.0 Hz, 1H), 5.90 (dd, *J* = 10.0, 2.0 Hz, 1H), 5.25 (s, 2H), 4.43 (t, *J* = 4.5 Hz, 2H), 4.08 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI): m/z ([M+H]) +: 491.4.

(*E*)-1-(4-((3-bromophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-6-yl)-4-phenylbut-2-en-1-one (9f)

The similar procedure of 7f was repeated to obtain the compound 9f (yield 76%). ^{1}H NMR (500 MHz, DMSO) δ 9.78 (s, 1H), 8.65 (s, 1H), 8.48 (s, 1H), 8.03 (s, 1H), 7.76-7.69 (m, 5H), 7.38-7.37 (m, 3H), 7.32-7.19 (m, 5H), 4.46 (t, *J* = 4.5 Hz, 2H), 4.17 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI): m/z ([M+H]) +: 487.2.

(*E*)-1-(4-((3-chloro-4-fluorophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-6-yl)-4-phenylbut-2-en-1-one (9g)

The similar procedure of 7f was repeated to obtain the compound 9g (yield 55%). ^{1}H NMR (500 MHz, DMSO) δ 9.79 (s,1H), 8.66 (s, 1H), 8.52 (s, 1H), 8.05 (d, *J* = 5.0 Hz, 1H), 7.76-7.70 (m, 4H), 7.43-7.29 (m, 5H), 7.23 (s, 1H), 4.47 (t, *J* = 4.0 Hz, 2H), 4.19 (t, *J* = 4.0 Hz, 2H). LC-MS (ESI): m/z ([M+H]) +: 461.3.

(*E*)-4-phenyl-1-(4-((3-(trifluoromethyl) phenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-6-yl)but-2-en-1-one (9h)

The similar procedure of 7f was repeated to obtain the compound 9h (yield 62%). ^{1}H NMR (500 MHz, DMSO) δ 8.76 (s, 1H), 8.56 (s, 1H), 8.19-8.16 (m, 2H), 7.81-7.74 (m, 3H), 7.65-7.60 (m, 2H), 7.46-7.36 (m, 5H), 7.27 (s, 1H), 4.52 (t, *J* = 4.5 Hz, 2H), 4.24 (t, *J* = 4.5 Hz, 2H). LC-MS (ESI): m/z ([M+H]) +: 477.3.

(*E*)-1-(4-((3-bromophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-6-yl)-4-morpholinobut-2-en-1-one (9i)

(*E*)-4-morpholinobut-2-enoic acid hydrochloride (4 eq.) was dissolved in DCM, followed by adding 3 drops DMF. The suspension was cooled to 0 ^{o}C and oxalyl chloride (3.47 eq.) was added dropwise. The mixture was stirred at 0-10 ^{o}C for 20 minutes and at 22-26 ^{o}C for 2 h, then the temperature of reaction mixture is adjusted to 40-45 ^{o}C for 5 minutes. The reaction mixture was checked for complete consumption of oxalyl chloride by high-

pressure liquid chromatography (HPLC) then cooled to 0 ^{o}C. A solution of N-(3-bromophenyl)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-4-amine (50mg) in 6 ml DCM and a suitable volume of DMF was added dropwise then added Et_3N(5 eq.). The mixture was stirred at 0-10 ^{o}C for 20 minutes then at room temperature for 3-4 h. The reaction was monitored by TLC. The reaction was quenched with saturated Na_2CO_3, extracted with EtOAc (3×20 ml) and dried over anhydrous sodium sulfate and evaporated to dryness under reduce pressure. The residue was crystallized from diethyl ether (Et_2O)/ ethyl acetate (EtOAc). The suspension was collected by filtration and washed with diethyl ether, and dried to give pure product (yield 79%). M.p. 238-239 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.88 (s, 1H), 8.48-8.46 (m, 2H), 8.20-8.16 (m, 2H), 7.58 (s, 1H), 7.38 (s, 1H), 7.17 (s, 1H), 6.86 (d, *J* = 15.0 Hz, 1H), 6.63 (d, *J* = 15.0 Hz, 1H), 4.43 (s, 3H), 4.06 (s, 3H), 3.10 (s, 3H), 2.34 (s, 6H). MS (ESI): m/z ([M+H]) +: 510.1.

(*E*)-1-(4-((3-chloro-4-fluorophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3, 2-g]quinazolin-6-yl)-4-morpholinobut-2-en-1-one (9j)

The similar procedure of 9i was repeated to obtain the compound 9j (yield 90%). M.p. 241-242 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.77 (s, 1H), 8.43-8.37 (m, 2H), 8.11 (s, 1H), 7.72 (s, 1H), 7.39 (s, 1H), 7.14 (s, 1H), 6.86-6.84 (m, 1H), 6.62 (d, *J* = 11.0 Hz, 1H), 4.42 (s, 2H), 4.05 (s, 2H), 3.10 (s, 2H), 2.36 (s, 4H). LC-MS (ESI): m/z ([M-H]) +: 482.3.

(*E*)-4-morpholino-1-(4-((3-(trifluoromethyl) phenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-6-yl)but-2-en-1-one (9k).

The similar procedure of 9i was repeated to obtain the compound 9k (yield 94%). M.p. 232-233 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.88 (s, 1H), 8.48-8.46 (m, 2H), 8.20-8.16 (m, 2H), 7.58 (s, 1H), 7.38 (s, 1H), 7.17 (s, 1H), 6.86 (d, *J* = 15.0 Hz, 1H), 6.63 (d, *J* = 15.0 Hz, 1H), 4.43 (s, 2H), 4.06 (s, 2H), 3.10 (s, 2H), 2.34 (s, 4H). LC-MS (ESI): m/z ([M-H]) +: 498.3.

(*E*)-1-(4-((3-chloro-4-((3-fluorobenzyl) oxy) phenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-6-yl)-4-morpholinobut-2-en-1-one (9l)

The similar procedure of 9i was repeated to obtain the compound 9l (yield 98%). M.p. 235-236 ^{o}C. ^{1}H NMR (500 MHz, DMSO) δ 9.61 (s, 1H), 8.45 (s, 1H), 8.39 (s, 1H), 7.98 (s, 1H), 7.71 (s, 1H), 7.46 (s, 1H), 7.33-7.17 (m, 5H), 6.85 (d, *J* = 17.0 Hz, 1H), 6.61 (d, *J*

= 17.0 Hz, 1H), 5.23 (s, 2H), 4.42 (s, 2H), 4.05 (s, 2H), 3.37 (s, 4H), 3.11 (s, 2H), 2.35 (s, 4H). LC-MS (ESI): m/z ([M+ H]) +: 590.3.

(*E*)-1-(4-((3-bromophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-6-yl)-4-(dimethylamino)but-2-en-1-one (9m)

The similar procedure of 9i was repeated to obtain the compound 9m (yield 49%). M.p.202-203 ºC. ^{1}H NMR (500 MHz, DMSO) δ 9.70 (s, 1H), 8.45 (s, 2H), 8.09 (s, 1H), 7.77 (s, 1H), 7.28-7.14 (m, 3H), 6.86 (d, *J* = 14.5 Hz, 1H), 6.61 (d, *J* = 14.5 Hz, 1H), 4.42 (s, 2H), 4.05 (s, 2H), 3.04 (s, 2H), 2.11 (s, 6H). MS (ESI): m/z ([M+ H]) +: 468.1.

(*E*)-1-(4-((3-chloro-4-fluorophenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2 g]quinazolin-6- yl)-4-(dimethylamino)but-2-en-1-one (9n)

The similar procedure of 9i was repeated to obtain the compound 9n (yield 75 %). M.p. 220-221 ºC. ^{1}H NMR (500 MHz, DMSO) δ 9.79 (s, 1H), 8.41 (s, 2H), 8.07 (s, 1H), 7.66 (s, 1H), 7.37 (t, *J* = 8.0 Hz, 1H), 7.12 (s, 1H), 6.88-6.83 (m, 1H), 6.61 (d, *J* = 15.0 Hz, 1H), 4.41 (t, *J* = 4.0 Hz, 2H), 4.05 (t, *J* = 4.0 Hz, 2H), 3.04 (d, *J* = 5.0 Hz, 2H), 2.11 (s, 6 H). LC-MS (ESI) : m/z ([M- H]) +: 440.3.

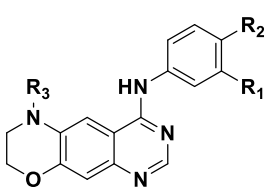
(*E*)-4-(dimethylamino)-1-(4-((3-(trifluoromethyl) phenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-6-yl)but-2-en-1-one (9o)

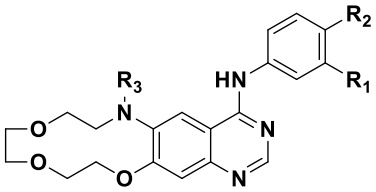
The similar procedure of 9i was repeated to obtain the compound 9o (yield 62 %). M.p. 201-202 ºC. ^{1}H NMR (500 MHz, DMSO) δ 9.87 (s, 1H), 8.47-8.45 (m, 2H), 8.13 (s, 2H), 7.55 (s, 1H), 7.34 (s, 1H), 7.14 (s, 1H), 6.86 (d, *J* = 15.0 Hz, 1H), 6.62 (d, *J* = 15.0 Hz, 1H), 4.42 (s, 2H), 4.06 (s, 2H), 3.04 (s, 2H), 2.11 (s, 6H). LC-MS (ESI): m/z ([M-H]) +: 456.3.

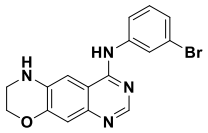
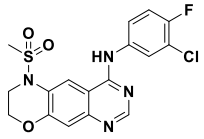
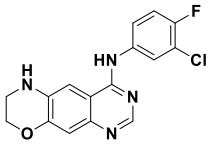
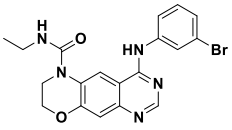
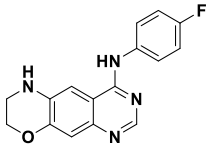
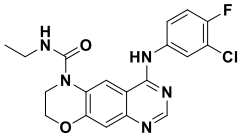
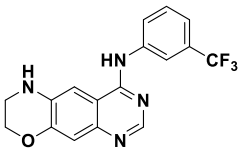
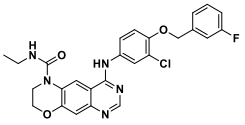
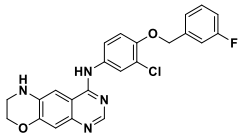
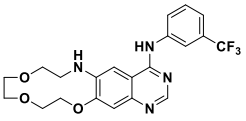
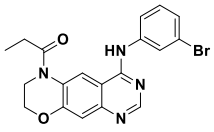
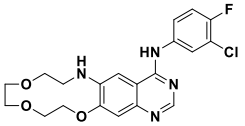
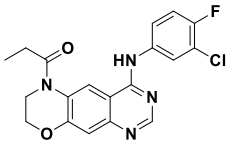
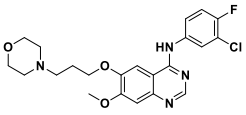
(E)-1-(4-((3-chloro-4-((3-fluorobenzyl) oxy) phenyl) amino)-7, 8-dihydro-6H-[1, 4]oxazino[3,2-g]quinazolin-6-yl)-4-(dimethylamino)but-2-en-1-one (9p)

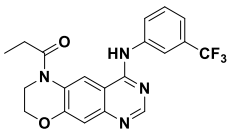
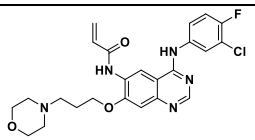
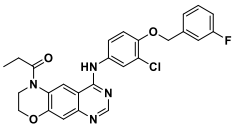
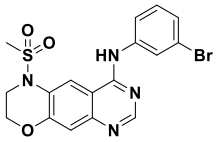
The similar procedure of 9i was repeated to obtain the compound 9p (yield 89 %). M.p. 223-224 ºC. ^{1}H NMR (500 MHz, DMSO) δ 9.66 (s, 1H), 8.44 (s, 2H), 7.96 (s, 1H), 7.66 (d, *J* = 7.5 Hz, 1H), 7.47 (dd, *J* = 14.5, 8.0 Hz, 1H), 7.34-7.17 (m, 5H), 6.89-6.83 (m, 1H), 6.61 (d, *J* = 15.5 Hz, 1H), 5.24 (s, 2H), 4.42 (t, *J* = 4.0 Hz, 2H), 4.06 (t, *J* = 4.0 Hz, 2H), 3.04 (d, *J* = 5.0 Hz, 2H), 2.11 (s, 6H). LC-MS (ESI): m/z ([M+ H]) +: 548.2.

Table S1. Efficacy (IC_{50} values) of kinase inhibitors in A431 (WT, overexpression) and wild-type EGFR [a]



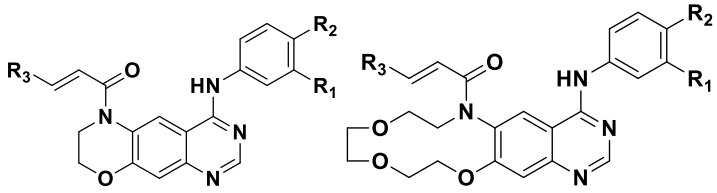


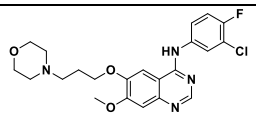
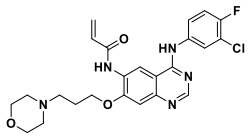
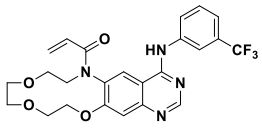
No.	Structure	A431/ μM	EGFR/ nM	No.	Structure	A431/ μM	EGFR/ nM
7a		>30	2.89	**7k**		>30	1.2
7b		>30	1.29	**7m**		>30	5.8
7c		>30	>20	**7n**		>30	8.2
7d		23.0	11.45	**7o**		11.8	7.5
7e		7.1	2.89	**8a**		29.5	>20
7f		23.0	2.43	**8b**		22.2	>20
7g		>30	2.05	**Gefitinib**		4.0	1.5

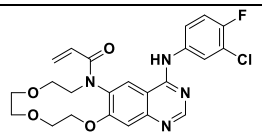
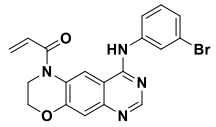
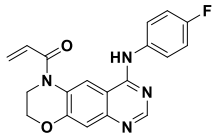
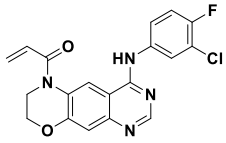
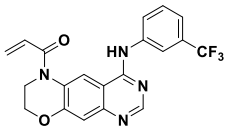
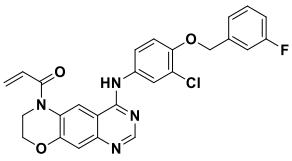
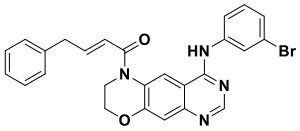
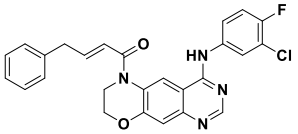
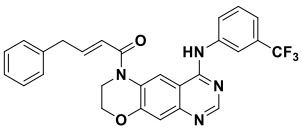
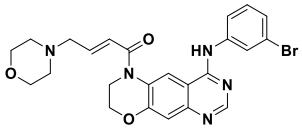
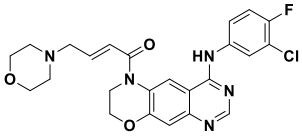
7h		>30	15.47	 **Canertinib**	4.3	3.0
7i		>30	14.56			
7j		25.8	0.6			

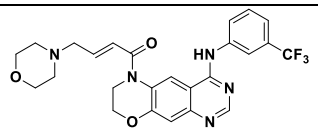
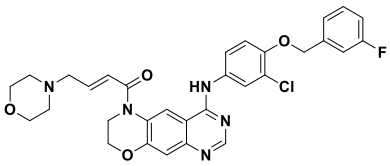
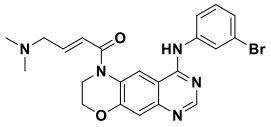
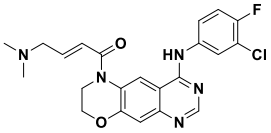
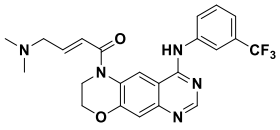
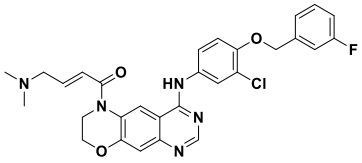
[a].The antiproliferative activities of the compounds were evaluated using the MTT assay. The data were means from three independent experiments.

Table S2. Efficacy (IC_{50} values) of kinase inhibitors in cell lines with different EGFR genotypes and wild-type EGFR [a]



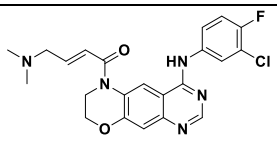
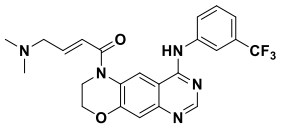
No.	Structure	A431/μM	H1975/μM	EGFR/nM
		4.0	10.48	1.5
		4.30	5.50	3.0
8c		6.69	6.94	>20

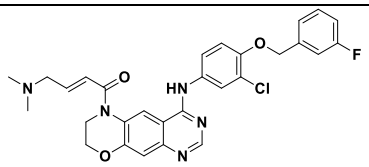
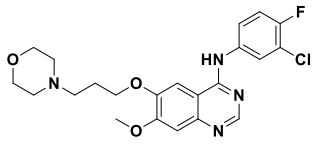
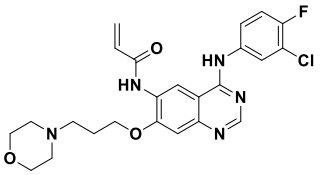
8d		10.30	9.03	>20
9a		7.2	37.5	0.49
9b		6.2	11.1	7.83
9c		5.2	22.5	0.4
9d		7.2	10.0	1.9
9e		8.7	21.4	12.5
9f		>30	>30	7.5
9g		>30	>30	>20
9h		>30	>30	>20
9i		5.7	15.19	3.6
9j		22.0	26.4	4.02

9k		17.8	13.2	15.9
9l		9.4	11.5	7.47
9m		2.3	>30	0.32
9n		2.2	4.90	0.56
9o		5.3	7.60	2.56
9p		0.9	3.61	2.62

a. The antiproliferative activities of the compounds were evaluated using the MTT assay. The data were means from three independent experiments.

Table S3. In Vitro Enzymatic Inhibitory Activities of Compound 9n-9p against Different Types of EGFR [a]

No.	Structure	EGFR IC50 (nM)	
		WT	**T790M**
9n		0.56	8.69
9o		2.56	NR

9p		2.62	8.61
		3.1	173.0
		3.0	NR

[a].The antiproliferative activities of the compounds were evaluated using the MTT assay. The data were means from three independent experiments.

Table S4. Cardiac hERG Activities Assay of 9n and 9p

No.	# of cells	% of inhibition at 1μM	% of inhibition at 10μM
9n	3	2.02	32
9p	3	14.29	59.42
Cisapride	2	59.8 at 0.1μM	95.52 at 1.0μM

Table S5 Microsomal Stability Study and CYP Inhibition Study of 9n and 9p

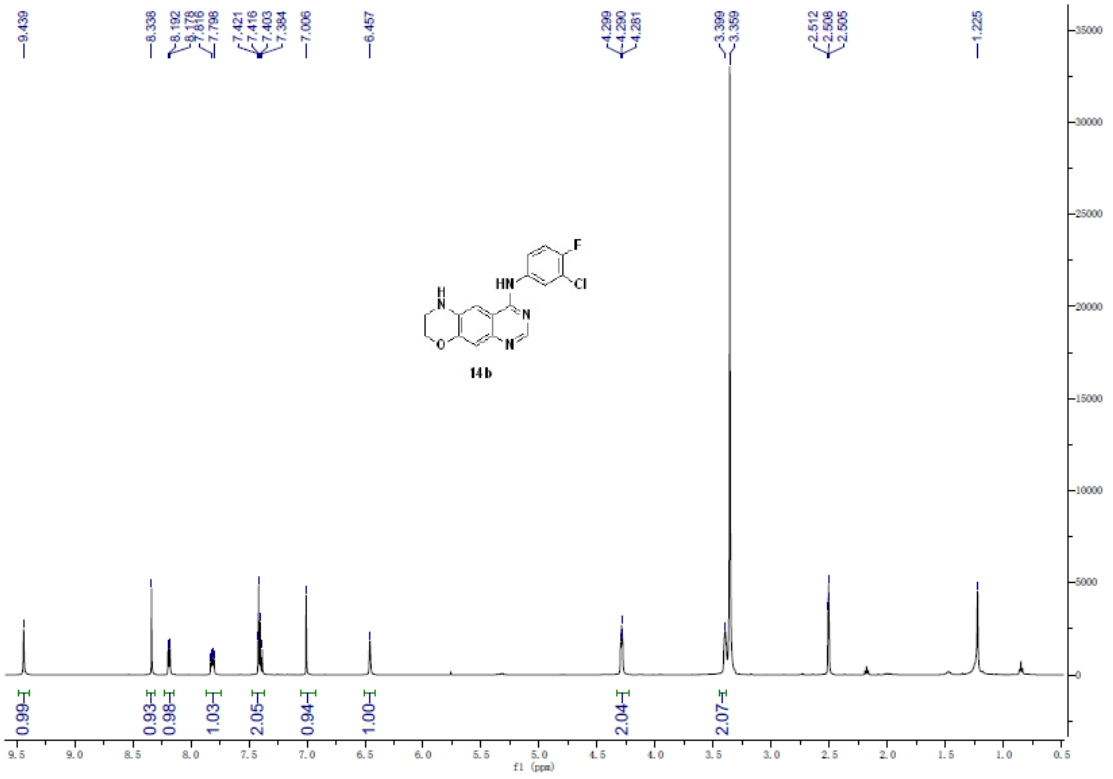
No.	Microsomal Stability		CYP % Inhibition at 10 μM			
	Rat	Human	3A4	2D6	2C9	1A2
9n	50	66	5	18	16	9
9p	188	66	8	19	30	7

Table S6 ***Pharmacokinetic Parameters for Compound 9p*** [a]

Route	AUC(0-24h)(μM*min)	t1/2 (min)	Tmax(min)	Cmax(μM)	F%
I.V.	60	119	2	1.34	
P.O.	13	212	130	0.04	6.42

[a].The pharmacokinetic parameters are obtained after a single i.v. (3mg/kg) or oral (10mg/kg) administration. The data are obtained from 12 mice in each treatment group.


9.413
8.352
8.209
7.880
7.864
7.438
7.291
7.230
7.215
7.005
6.448
4.296
4.290
4.281
3.397
3.344
2.508
2.505
2.502
1.232
0.000
HN
Br
N
H
O
N
14a
1.19
1.00
1.19
1.37
1.27
1.48
1.39
1.11
1.15
2.15
2.35
f1 (ppm)


9.439
8.338
8.192
8.178
7.816
7.798
7.421
7.416
7.403
7.384
7.006
6.457
4.299
4.290
4.281
3.399
3.359
2.512
2.508
2.505
1.225
F
HN
Cl
N
H
O
N
14b
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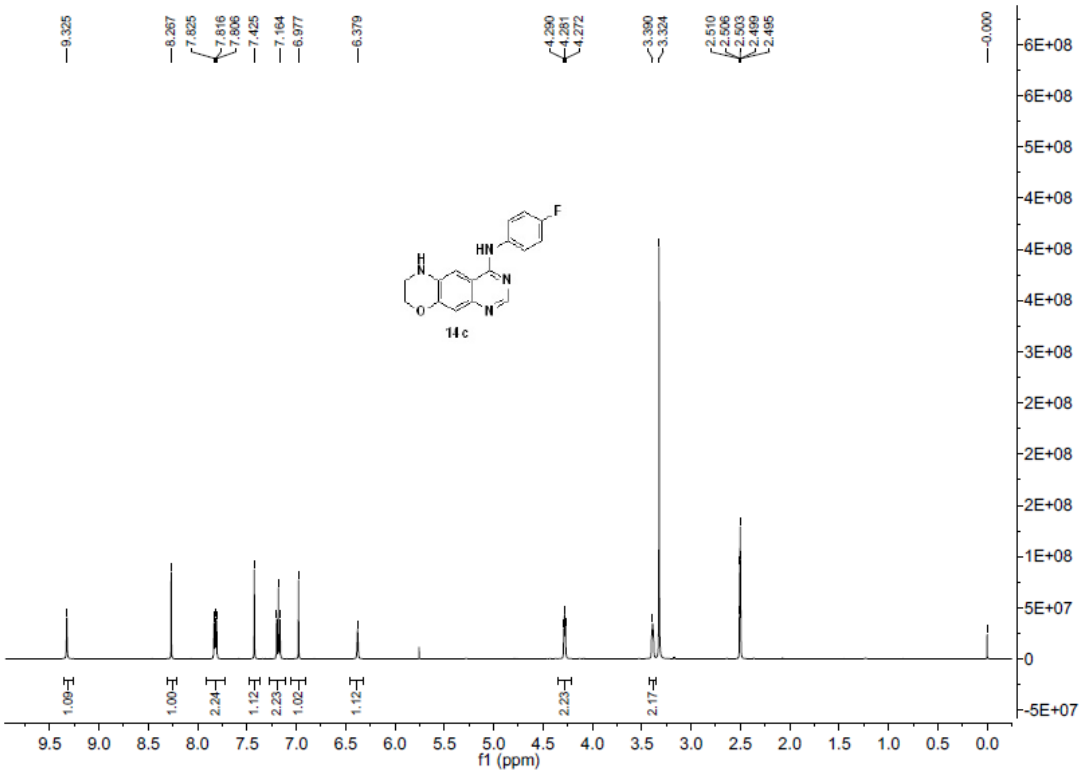
0.94
1.00
2.04
2.07
f1 (ppm)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6.977
6.379
4.290
4.281
4.272
3.390
3.324
2.510
2.506
2.503
2.499
2.495
0.000
F
HN
N
N
H
O
14c
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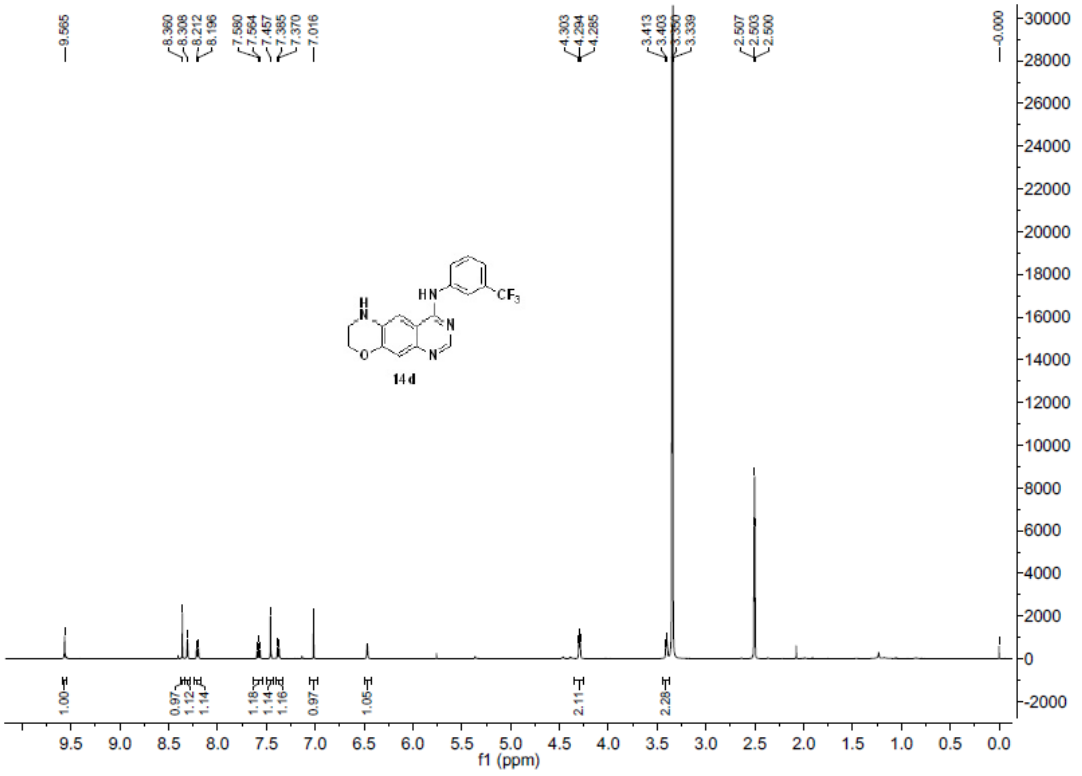
1.00
2.24
1.12
2.23
1.02
1.12
2.23
2.17
f1 (ppm)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7.457
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7.370
7.016
4.303
4.294
4.285
3.413
3.403
3.350
3.339
2.507
2.503
2.500
0.000
HN
CF3
N
N
H
O
14d
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1.14
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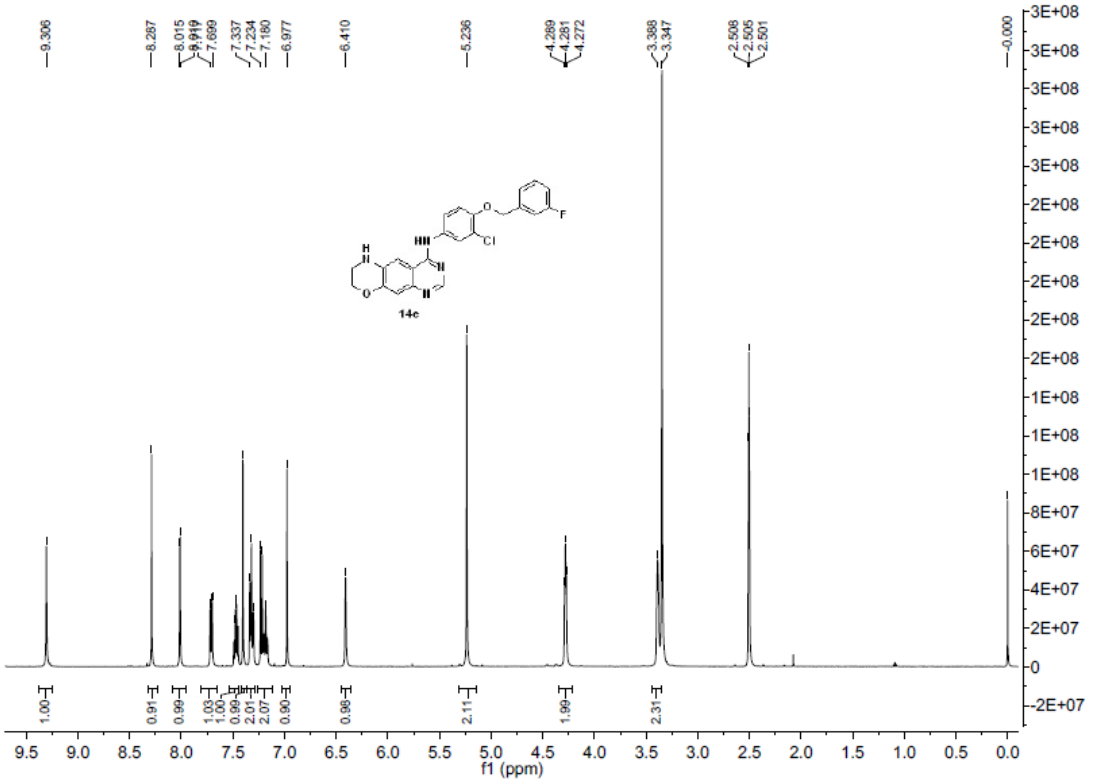
f1 (ppm)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7.180
6.977
6.410
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4.281
4.272
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3.347
2.508
2.505
2.501
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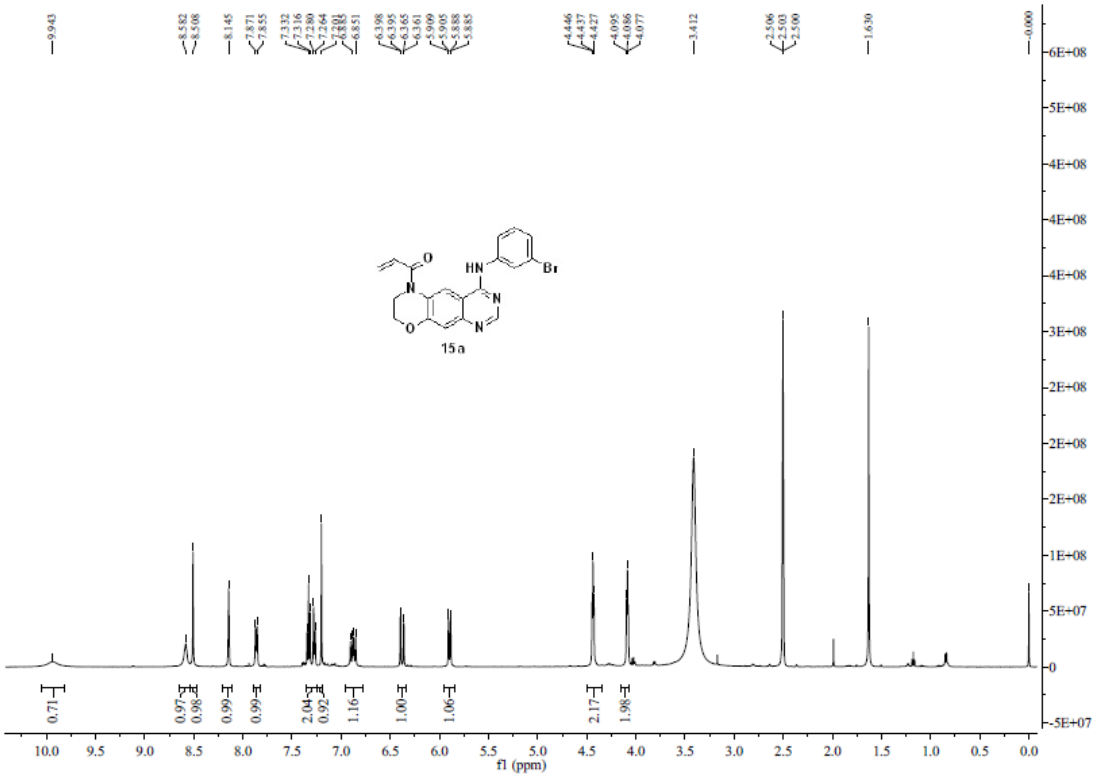
14e
f1 (ppm)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6.851
6.398
6.395
6.365
6.361
5.909
5.905
5.888
5.885
4.446
4.437
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4.095
4.086
4.077
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2.596
2.593
2.590
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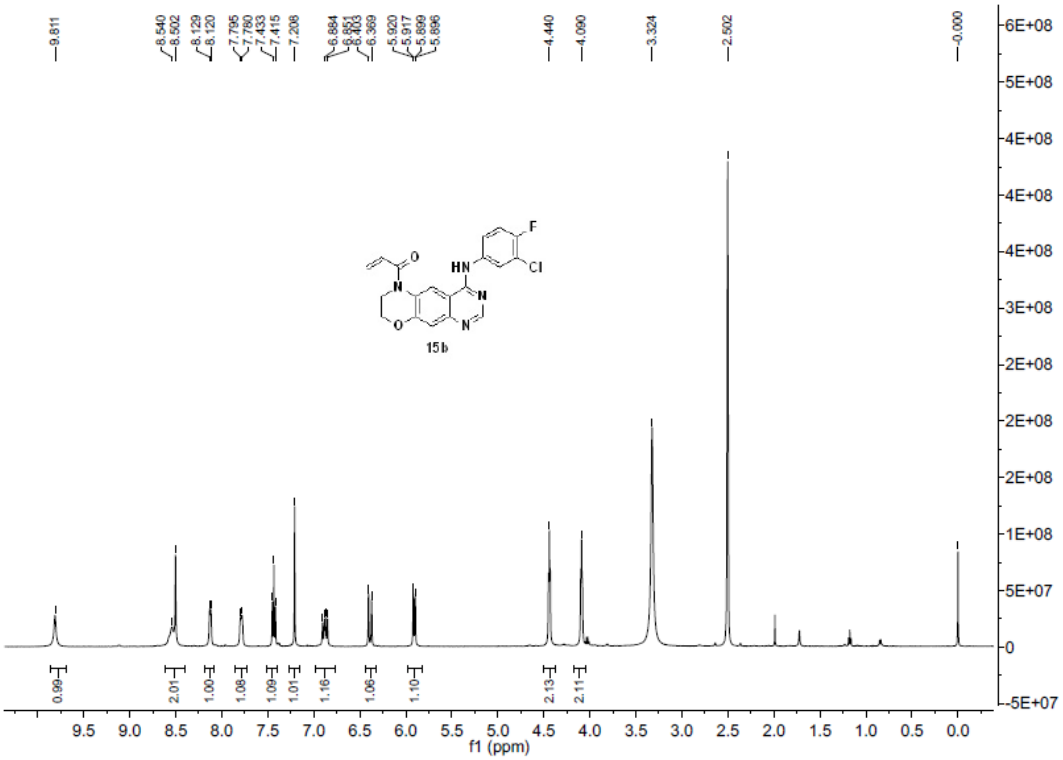
15a
f1 (ppm)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7.780
7.433
7.415
7.208
6.884
6.851
6.403
6.369
5.920
5.917
5.899
5.896
4.440
4.090
3.324
2.502
0.000
F
Cl
HN
O
N
N
O
15b
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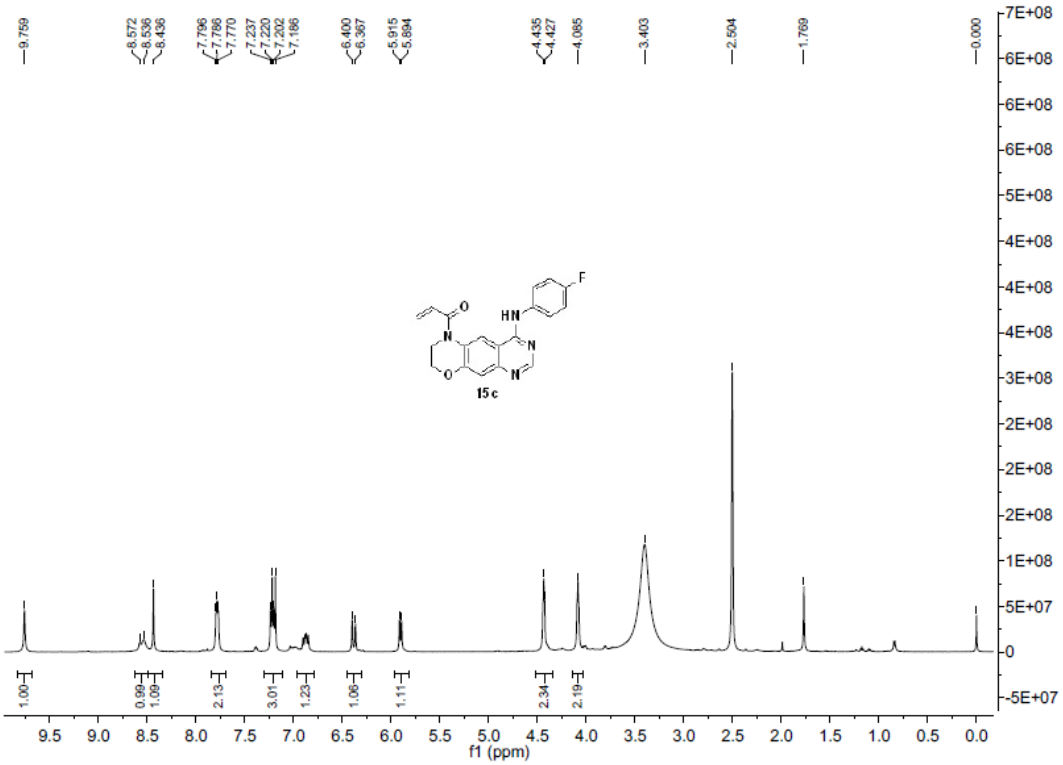
1.08
1.09
1.01
1.16
1.06
1.10
2.13
2.11
f1 (ppm)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8.536
8.436
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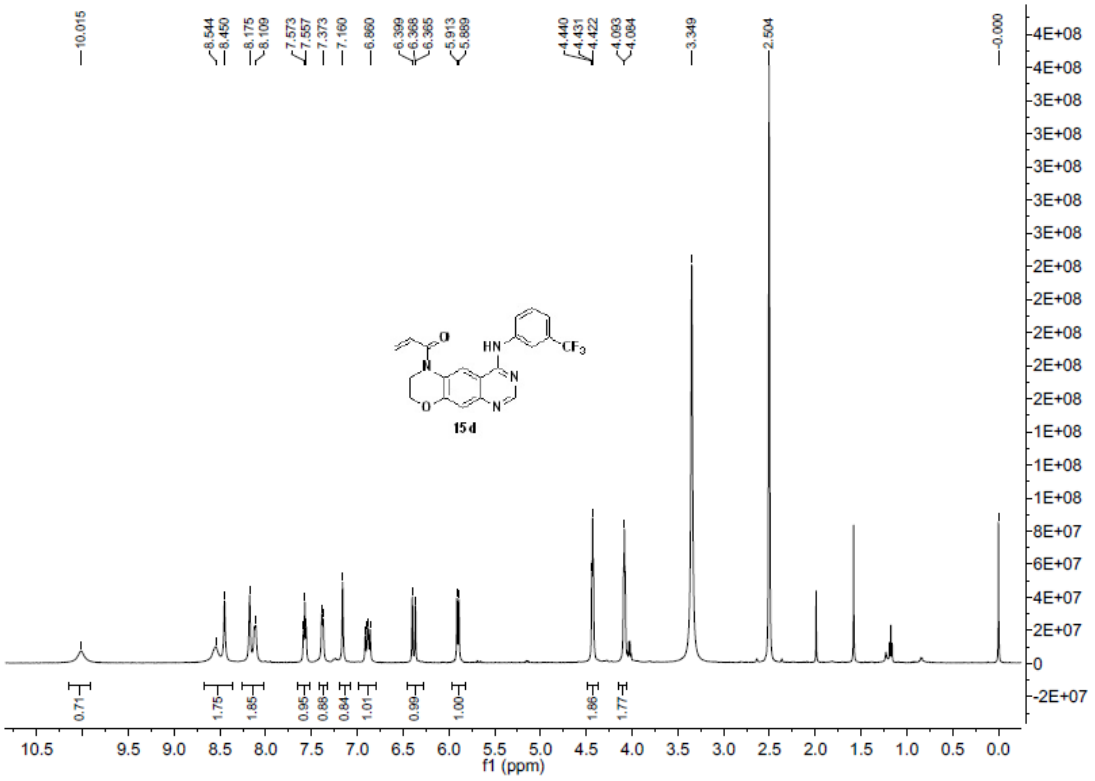
7.786
7.770
7.237
7.220
7.202
7.186
6.400
6.367
5.915
5.894
4.435
4.427
4.085
3.403
2.504
1.769
0.000
F
HN
O
N
N
O
15c
1.00
0.99
1.09
2.13
3.01
1.23
1.06
1.11
2.34
2.19
f1 (ppm)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8.450
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8.109
7.573
7.557
7.373
7.160
6.860
6.399
6.368
6.365
5.913
5.889
4.440
4.431
4.422
4.093
4.084
3.349
2.504
0.000
HN
CF3
O
N
O
N
15d
0.71
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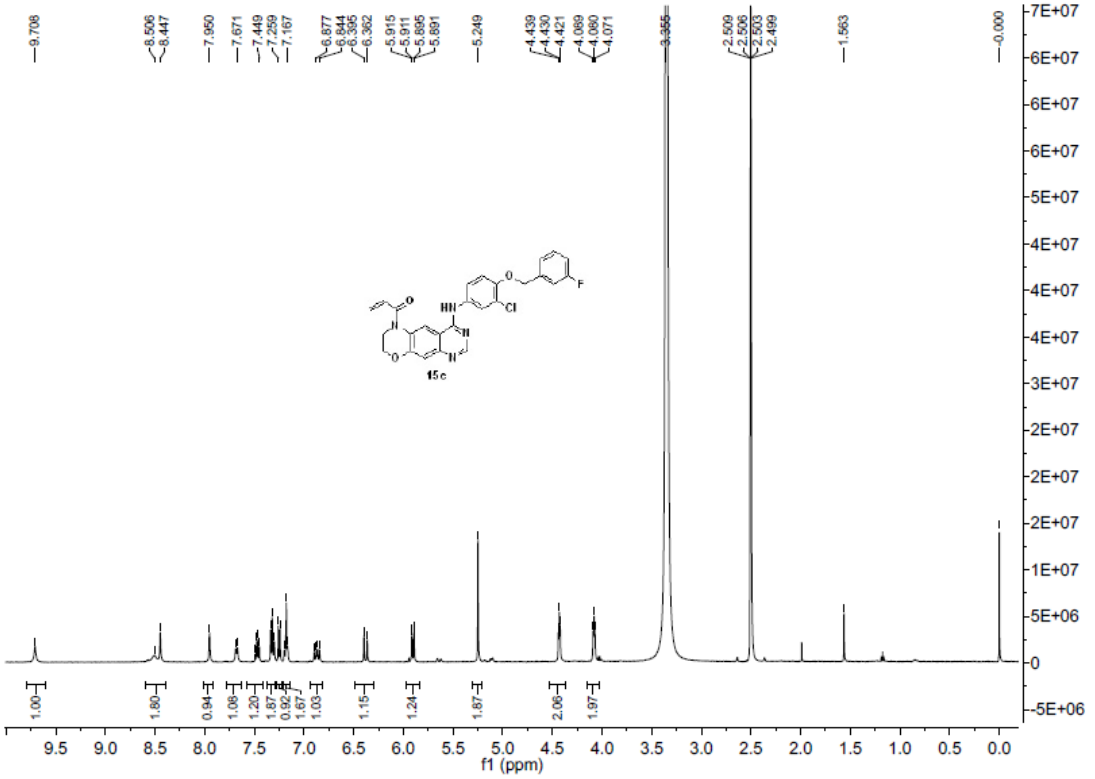
0.95
0.88
0.84
1.01
0.99
1.00
1.86
1.77
f1 (ppm)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7.671
7.449
7.259
7.167
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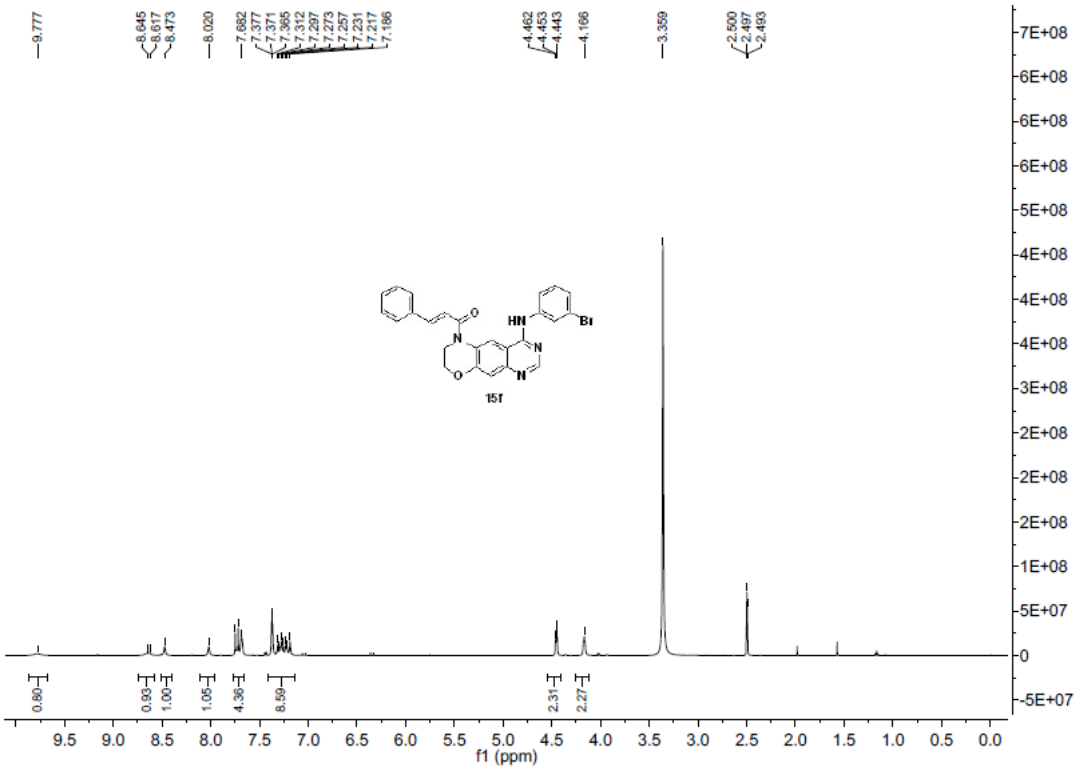
6.844
6.385
6.362
5.915
5.911
5.895
5.891
5.249
4.439
4.430
4.421
4.089
4.080
4.071
3.356
2.509
2.506
2.503
2.499
1.563
0.000
O
HN
Cl
O
F
N
N
15e
1.00
1.80
0.94
1.08
1.20
1.87
0.92
1.67
1.03
1.15
1.24
1.87
2.06
1.97
f1 (ppm)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7.682
7.377
7.371
7.365
7.312
7.297
7.273
7.257
7.231
7.217
7.186
4.462
4.453
4.443
4.166
3.359
2.500
2.497
2.493
HN
Br
N
O
15f
0.80
0.93
1.00
1.05
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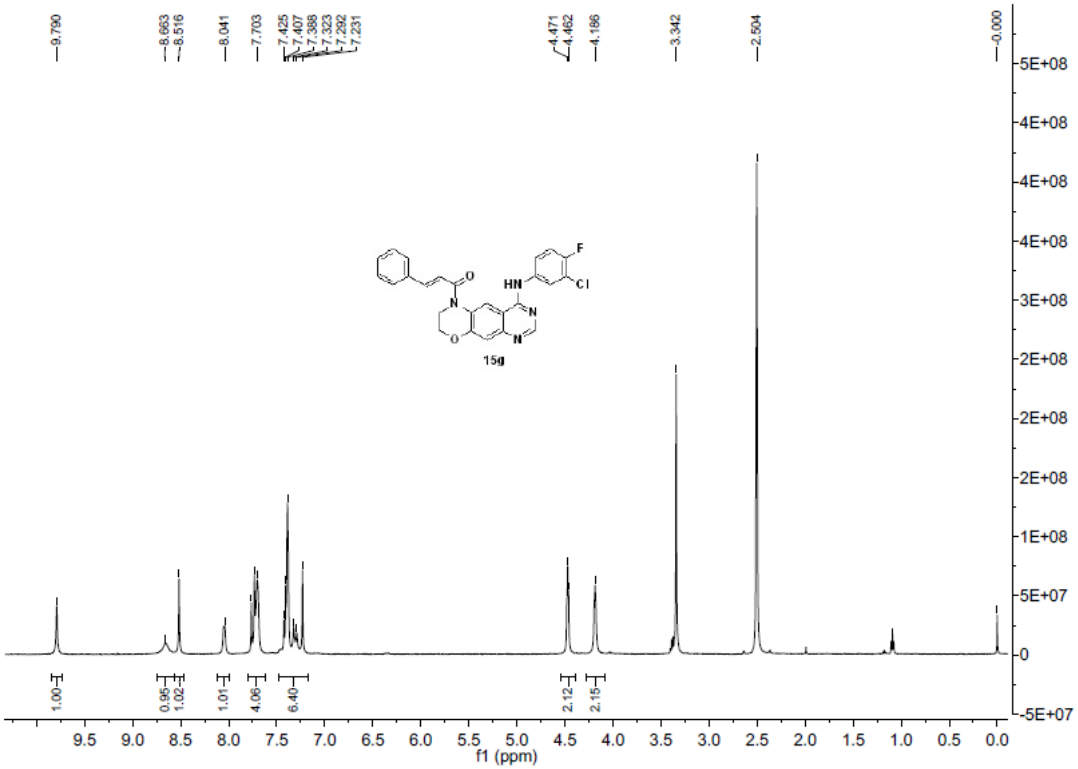
8.59
2.31
2.27
f1 (ppm)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7.425
7.407
7.388
7.323
7.292
7.231
4.471
4.462
4.186
3.342
2.504
0.000
F
HN
Cl
N
O
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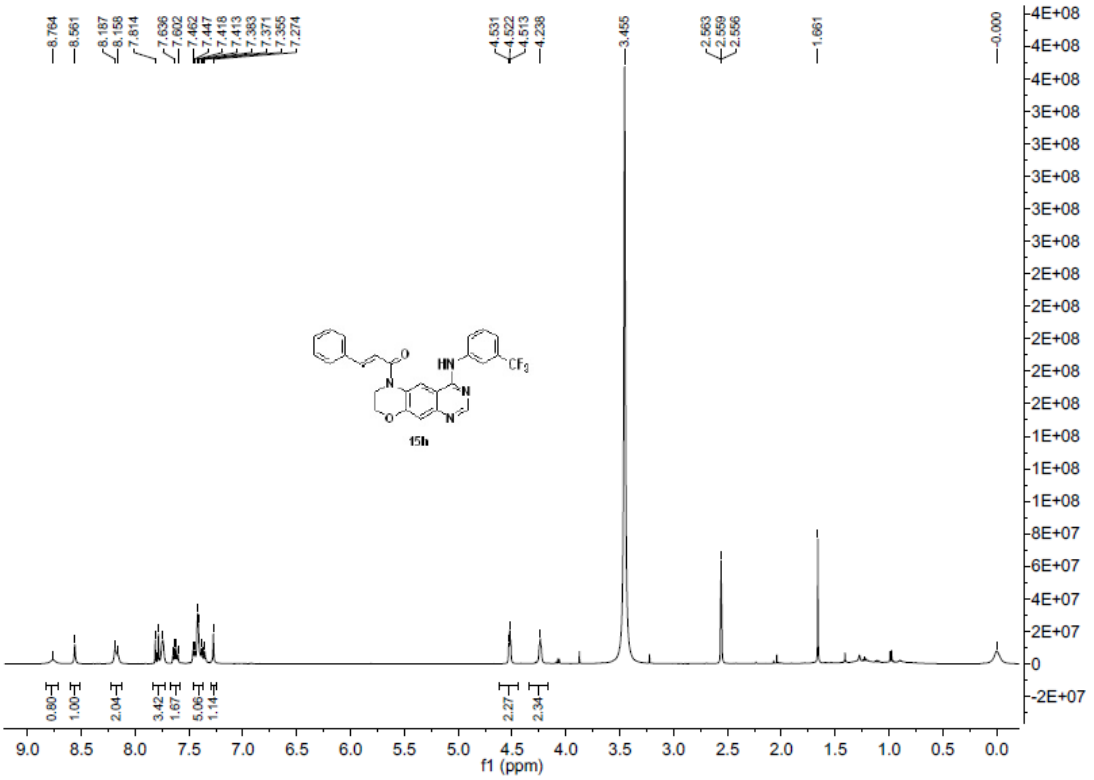
0.95
1.02
1.01
4.06
6.40
2.12
2.15
f1 (ppm)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8.561
8.187
8.158
7.814
7.636
7.602
7.462
7.447
7.418
7.413
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7.371
7.355
7.274
4.531
4.522
4.513
4.238
3.455
2.563
2.559
2.556
1.661
0.000
HN
CF3
O
N
O
15h
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1.00
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3.42
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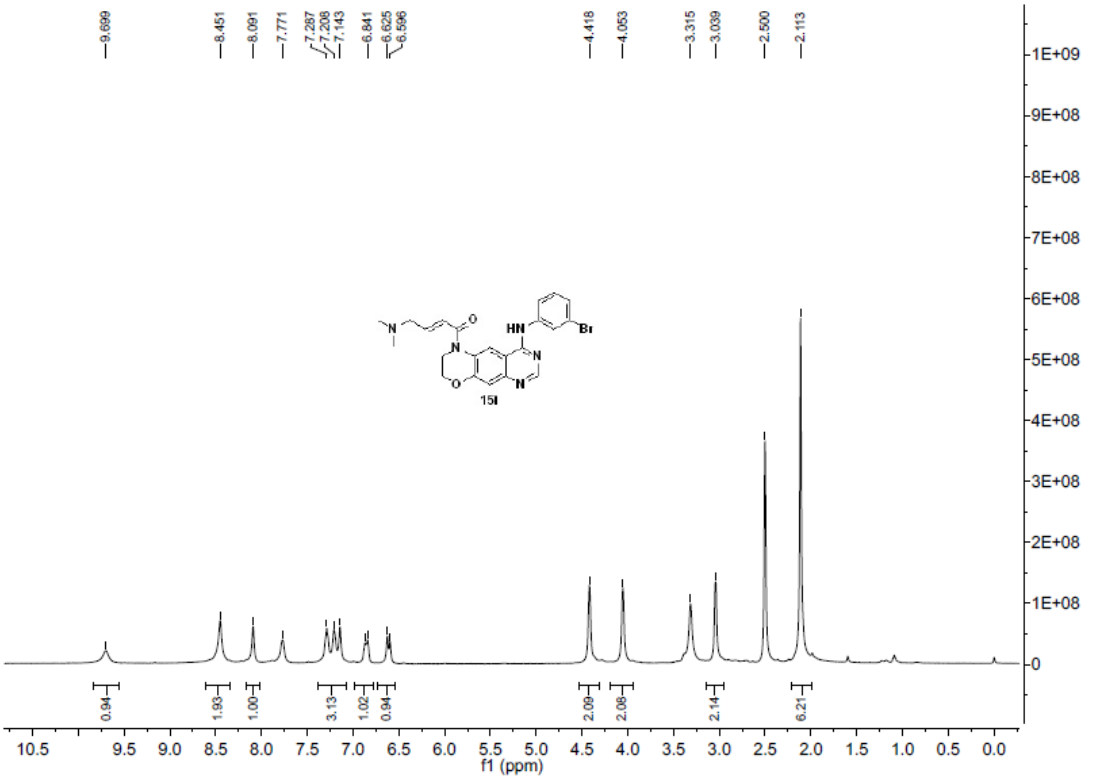
5.06
1.14
2.27
2.34
f1 (ppm)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7.208
7.143
6.841
6.625
6.596
4.418
4.053
3.315
3.039
2.500
2.113
HN
Br
O
N
O
15l
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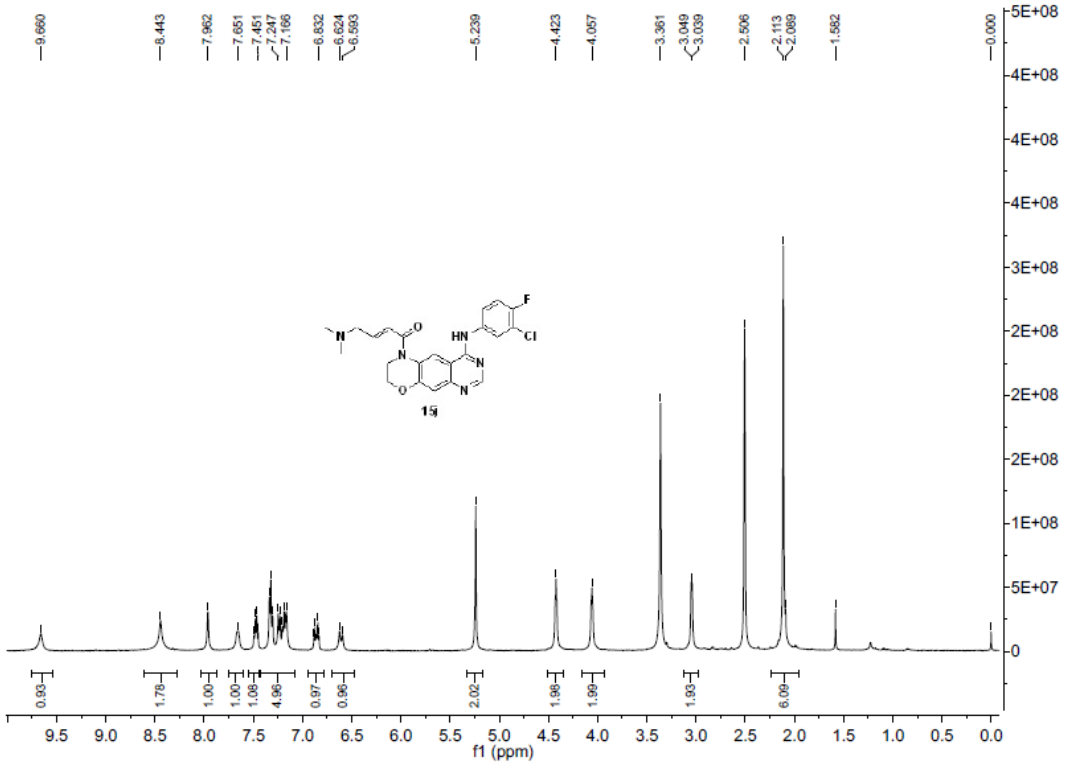
1.00
3.13
1.02
0.94
2.09
2.08
2.14
6.21
f1 (ppm)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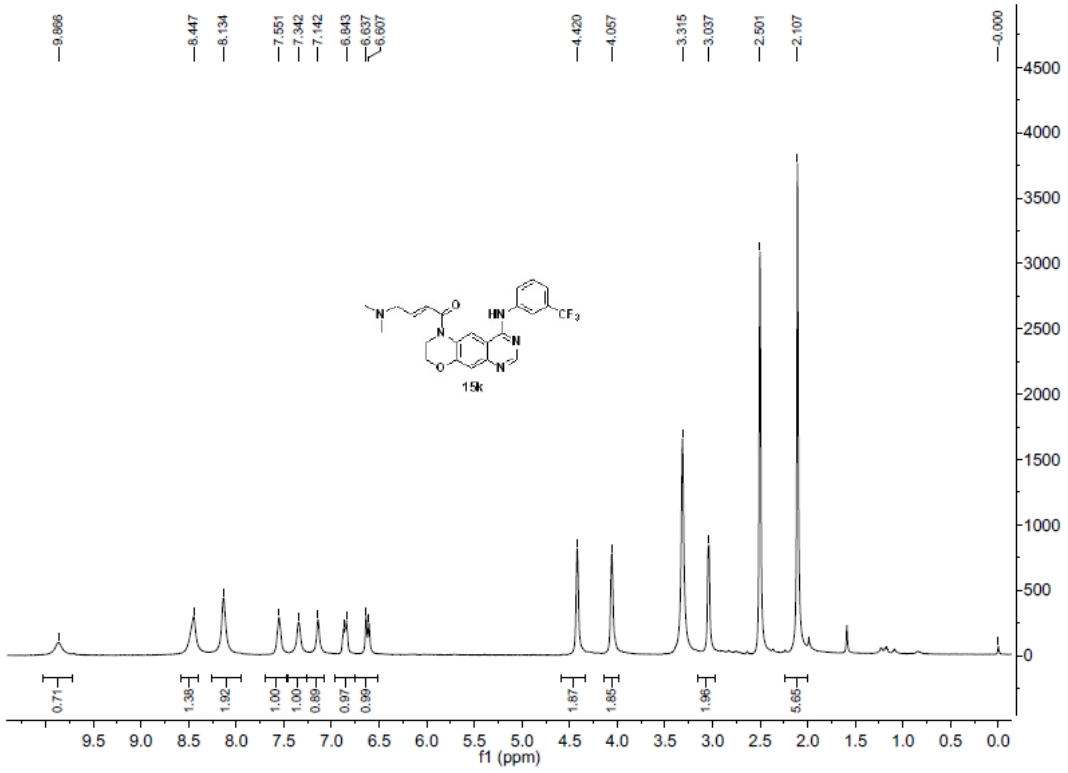
7.962
7.651
7.451
7.247
7.166
6.832
6.624
6.593
5.239
4.423
4.057
3.361
3.049
3.039
2.506
2.113
2.089
1.582
0.000
15j
f1 (ppm)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8.134
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6.637
6.607
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4.057
3.315
3.037
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2.107
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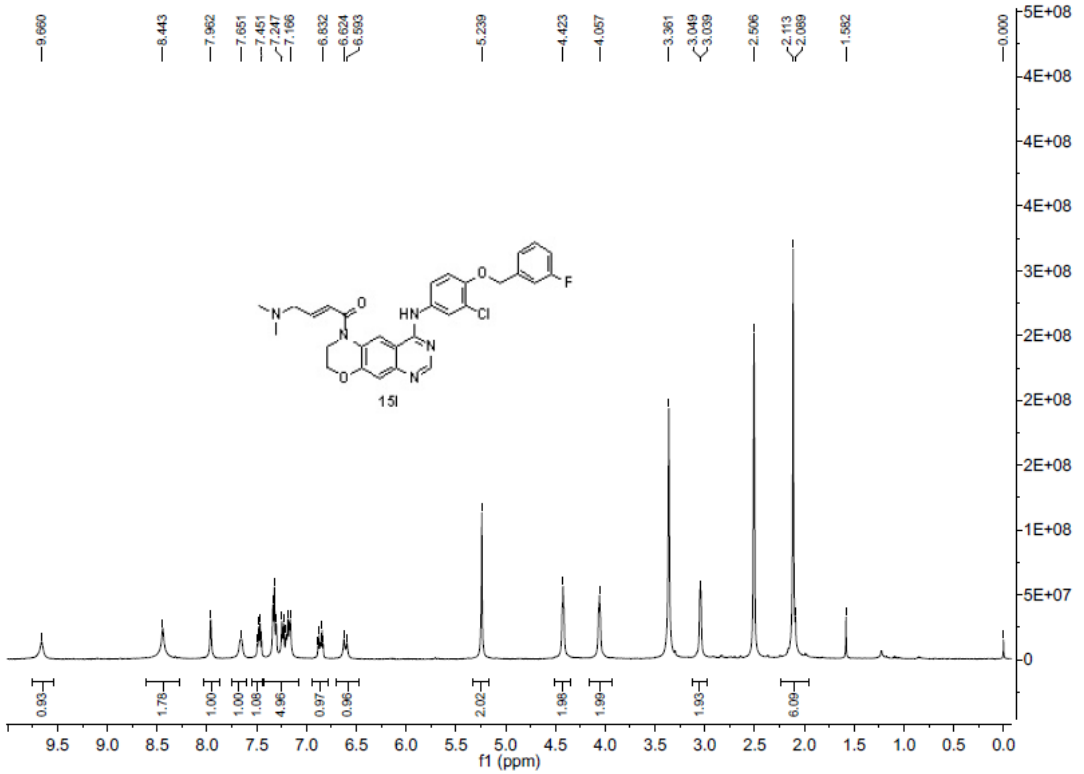
f1 (ppm)


9.660
8.443
7.962
7.651
7.451
7.247
7.166
6.832
6.624
6.593
5.239
4.423
4.057
3.361
3.049
3.039
2.506
2.113
2.089
1.582
0.000
15l
0.93
1.78
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0.97
0.96
2.02
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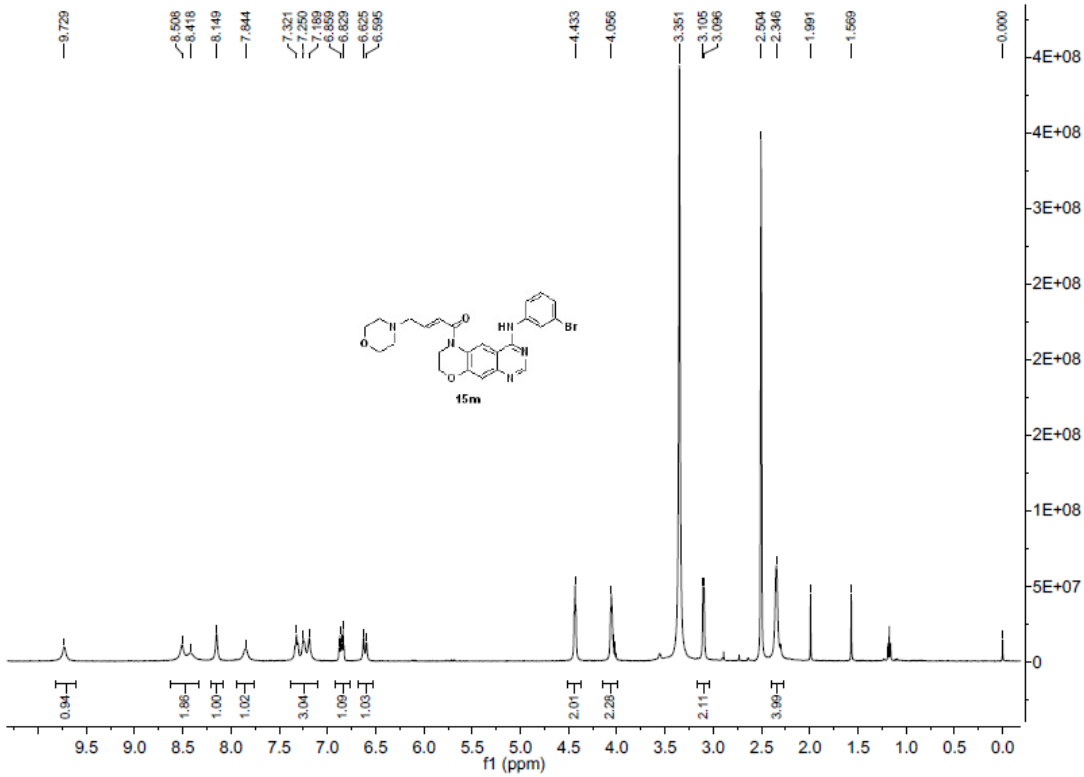
1.99
1.93
6.09
f1 (ppm)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6.829
6.625
6.595
4.433
4.056
3.351
3.105
3.096
2.504
2.346
1.991
1.569
0.000
15m
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1.02
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1.09
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2.28
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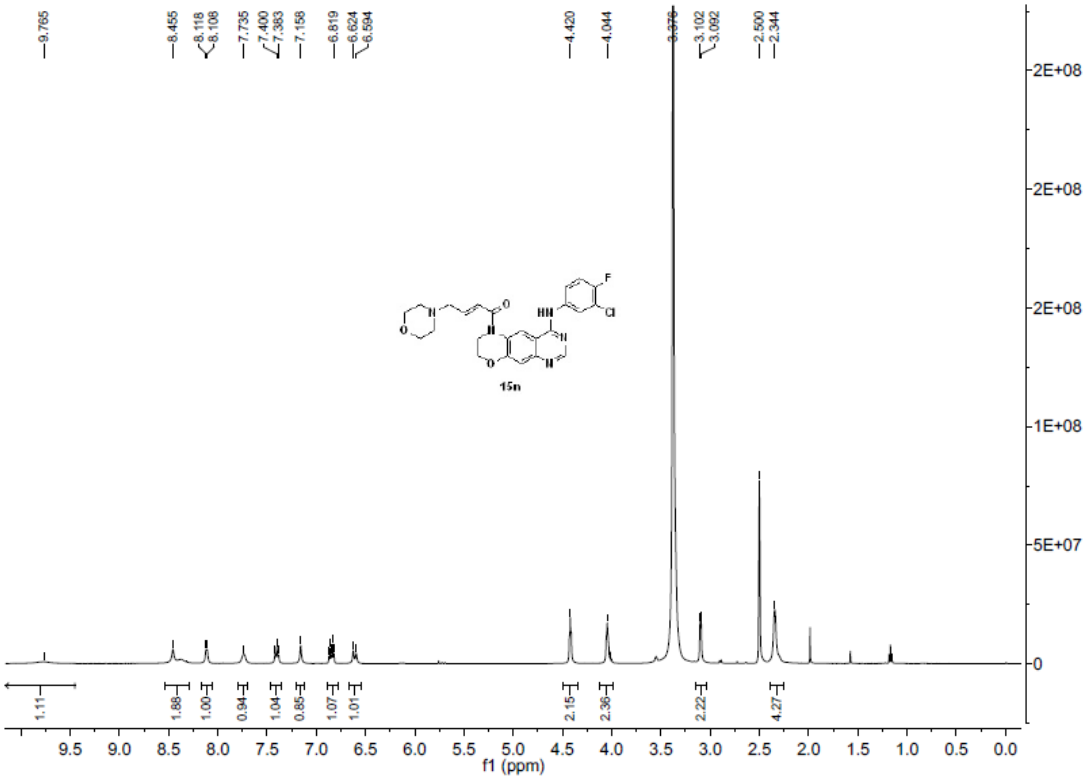
3.99
f1 (ppm)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7.158
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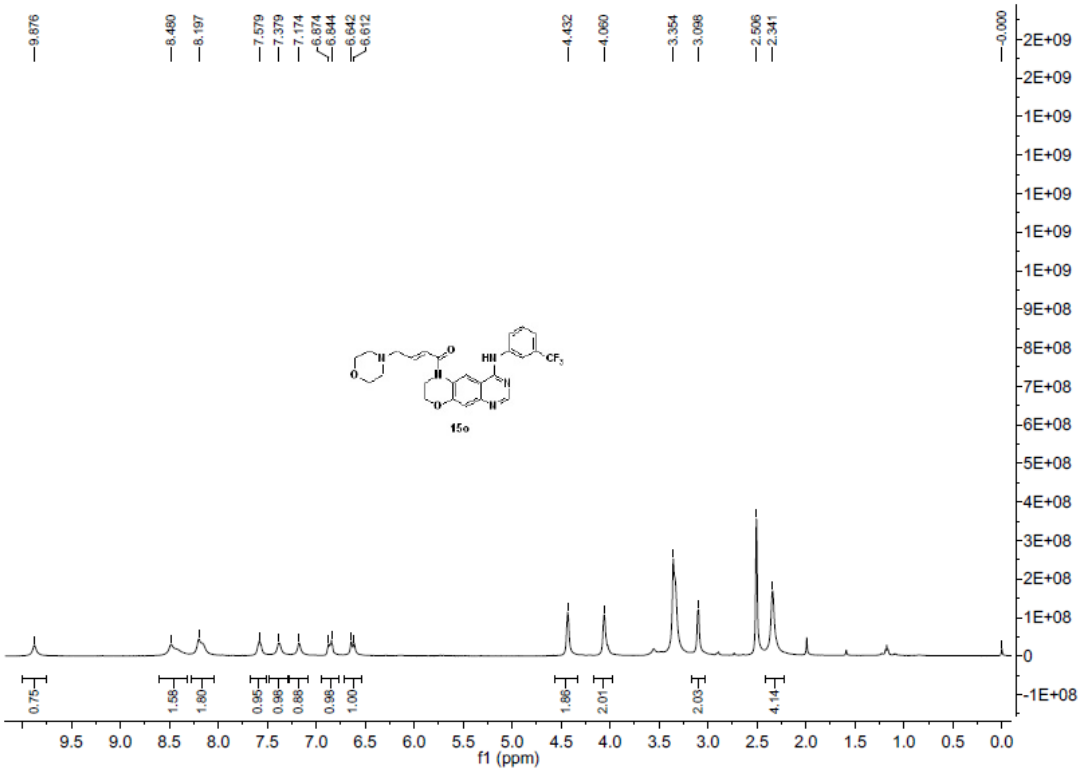
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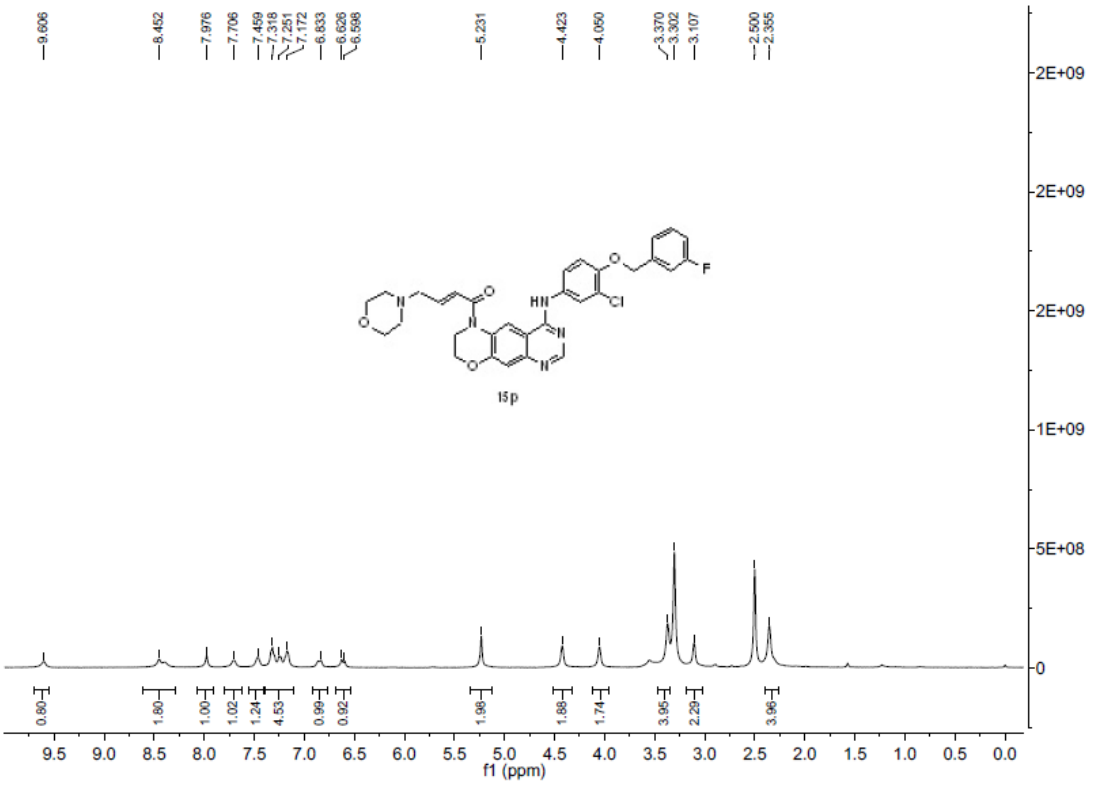
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7.172
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4.423
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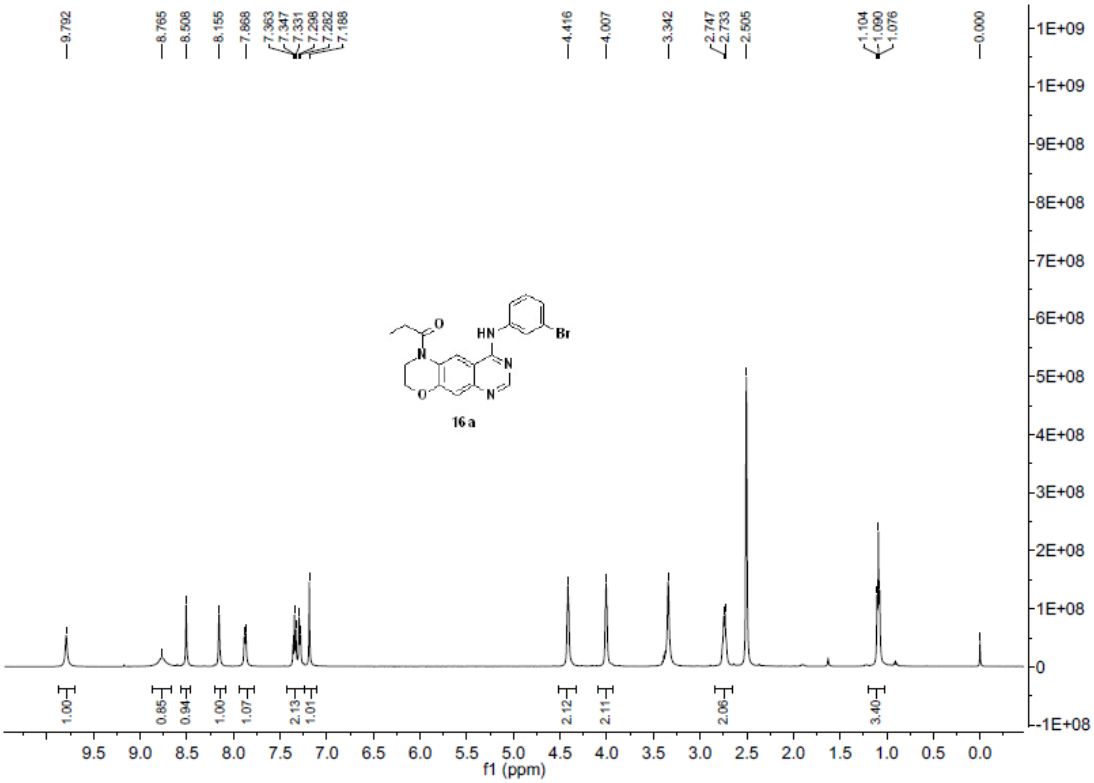
3.302
3.107
2.500
2.355
15p
f1 (ppm)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7.331
7.298
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7.188
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4.007
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2.747
2.733
2.505
1.104
1.090
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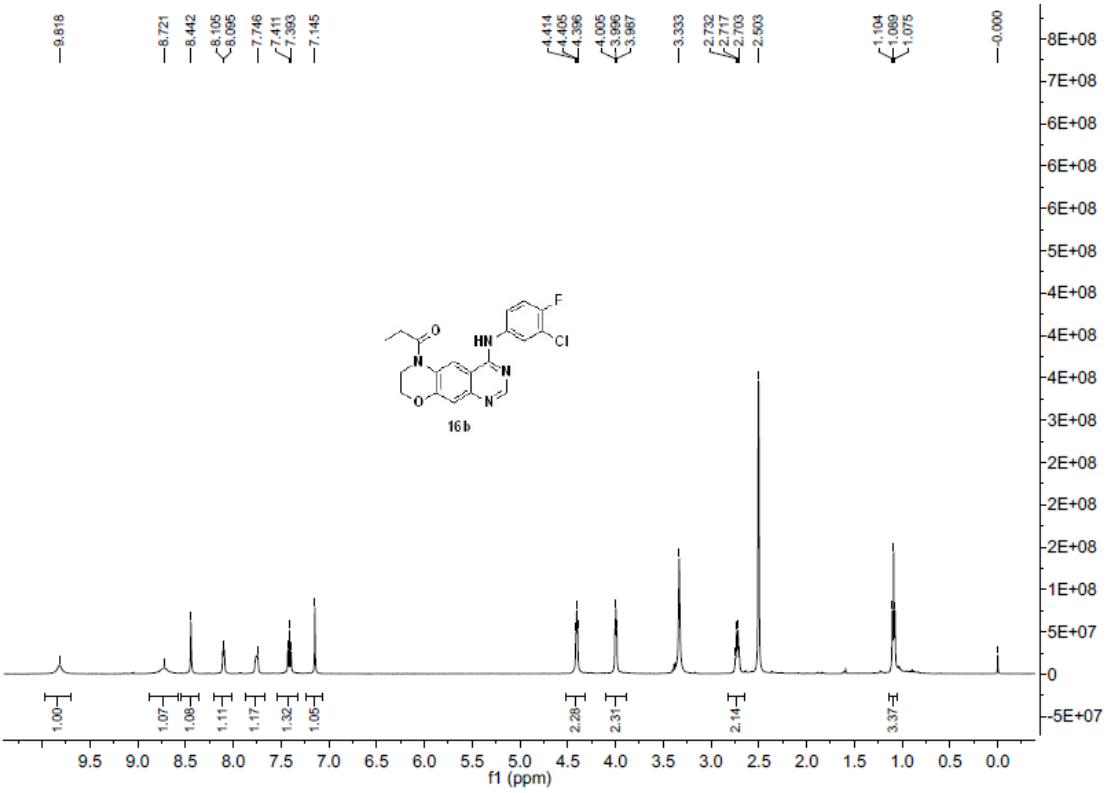
f1 (ppm)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7.393
7.145
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4.396
4.005
3.996
3.987
3.333
2.732
2.717
2.703
2.503
1.104
1.089
1.075
0.000
F
Cl
HN
O
N
16b
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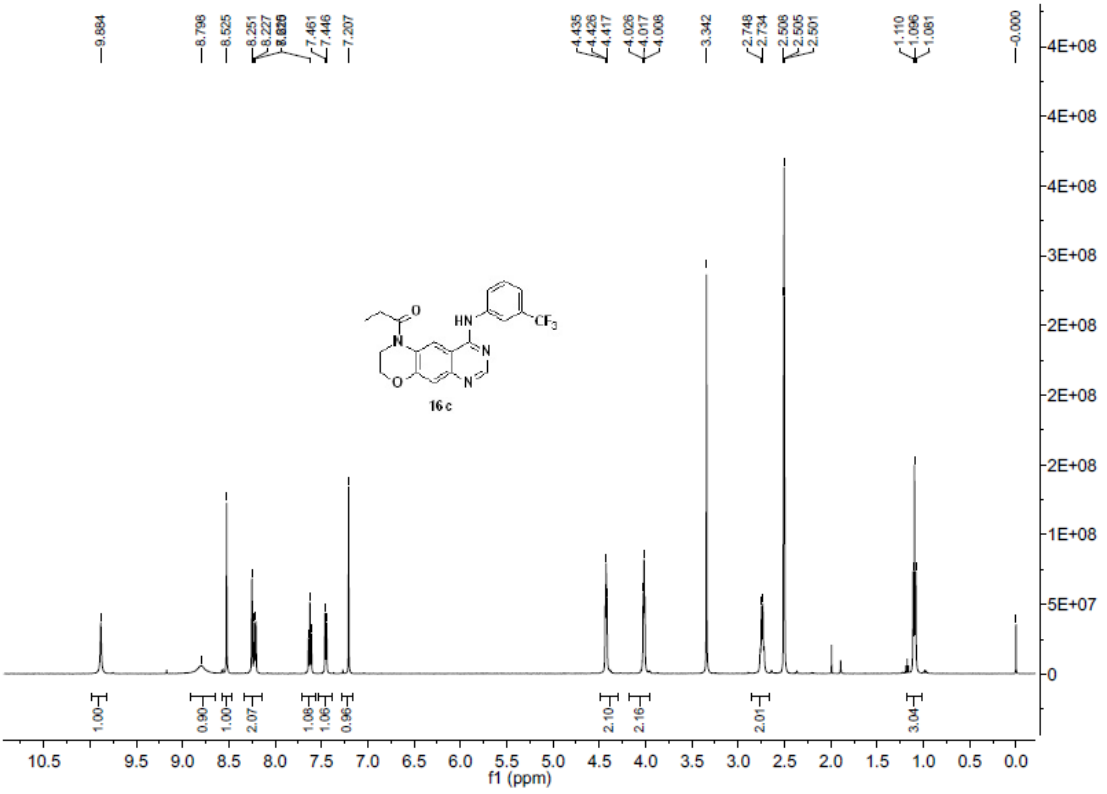
1.07
1.08
1.11
1.17
1.32
1.05
2.28
2.31
2.14
3.37
f1 (ppm)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4.426
4.417
4.026
4.017
4.008
3.342
2.748
2.734
2.506
2.505
2.501
1.110
1.096
1.081
0.000
HN
CF3
O
N
16c
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1.08
1.06
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2.16
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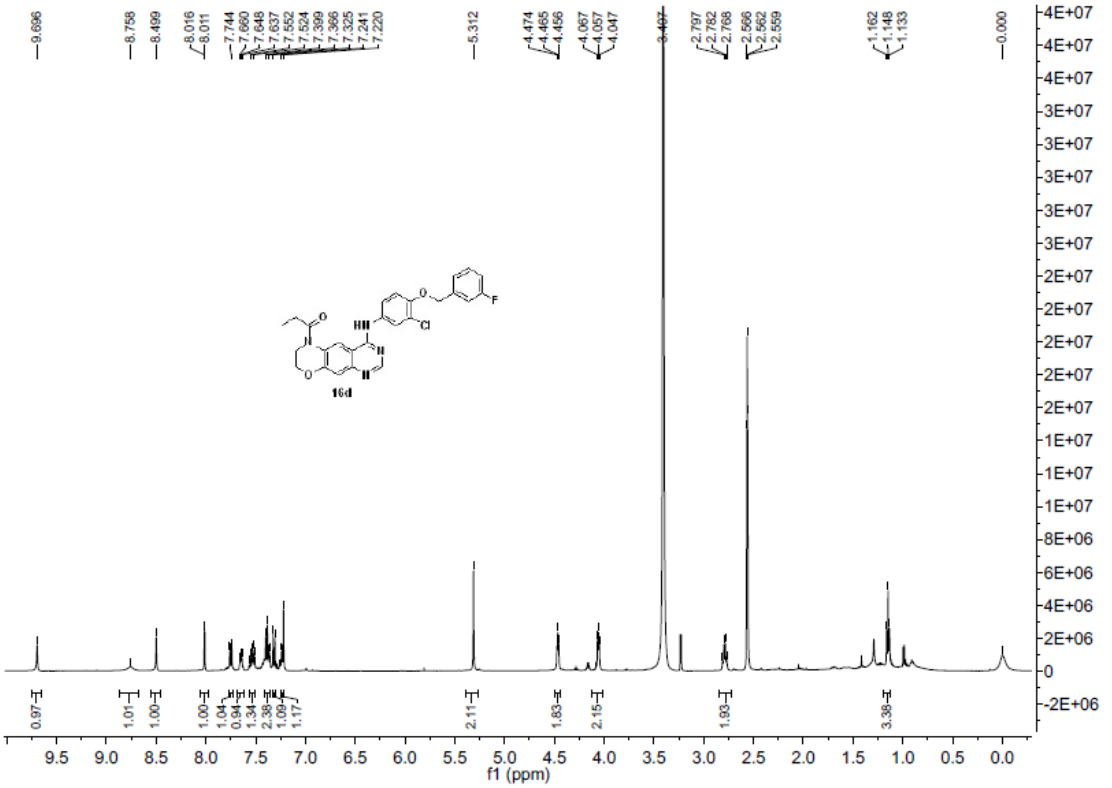
f1 (ppm)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7.524
7.399
7.366
7.325
7.241
7.220
5.312
4.474
4.465
4.456
4.067
4.057
4.047
3.497
2.797
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2.566
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2.559
1.162
1.148
1.133
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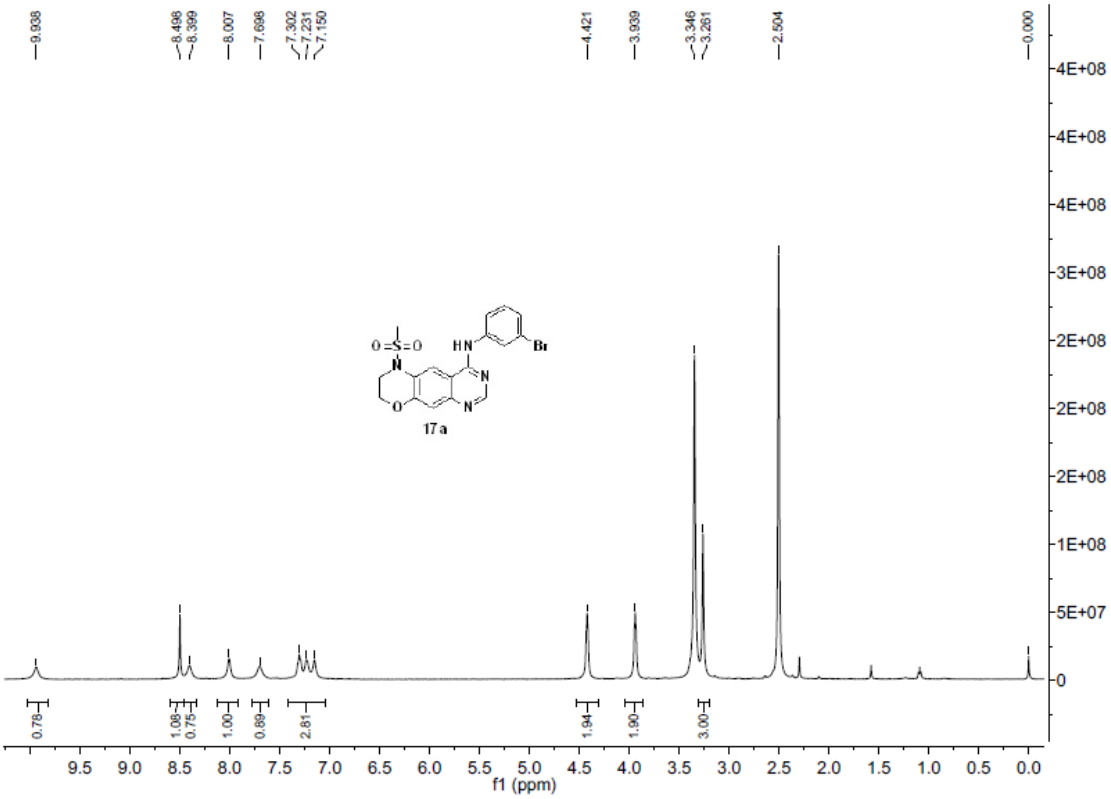
16d
f1 (ppm)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7.150
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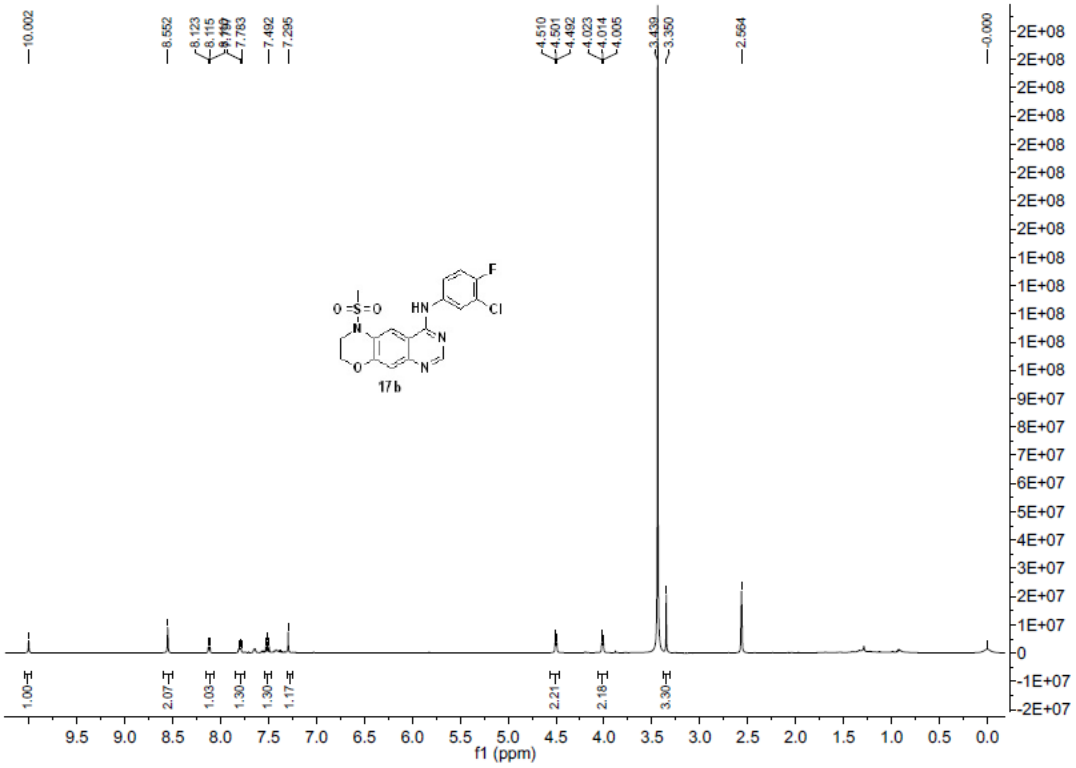
3.939
3.346
3.261
2.504
0.000
17a
f1 (ppm)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7.295
4.510
4.501
4.492
4.023
4.014
4.005
3.439
3.350
2.564
0.000
F
Cl
HN
N
O
S
17b
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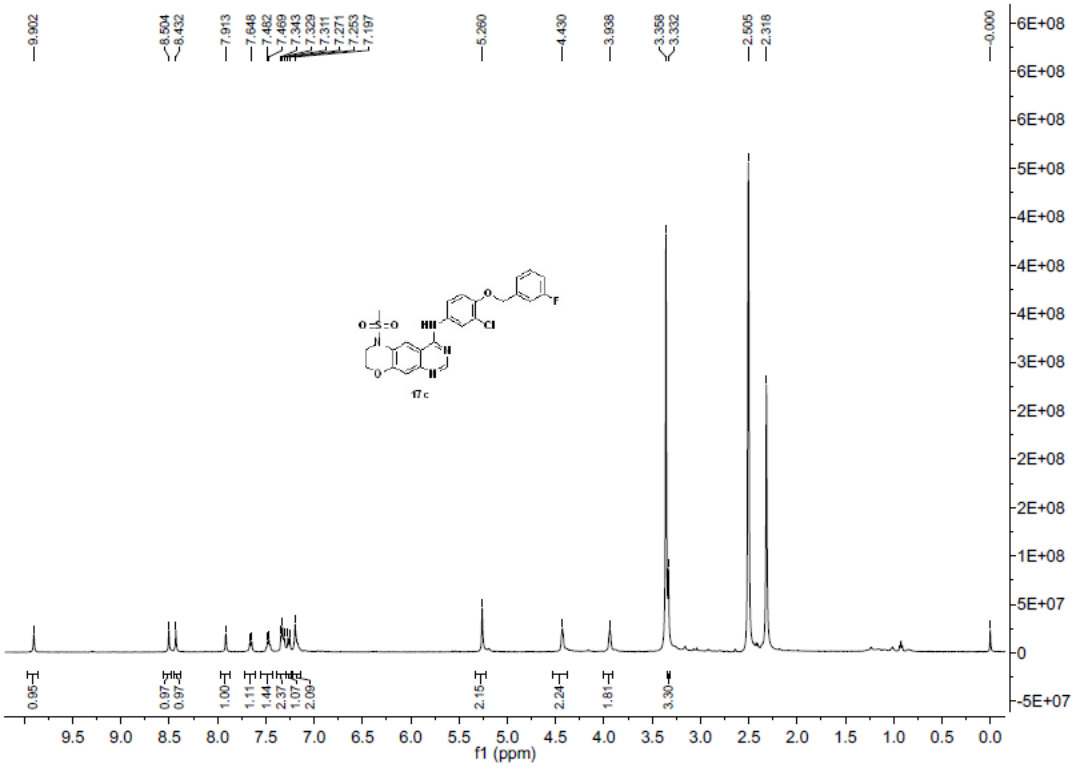
2.07
1.03
1.30
1.30
1.17
2.21
2.18
3.30
f1 (ppm)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7.311
7.271
7.253
7.197
5.260
4.430
3.938
3.358
3.332
2.505
2.318
0.000
F
Cl
HN
N
O
S
17c
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0.97
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2.24
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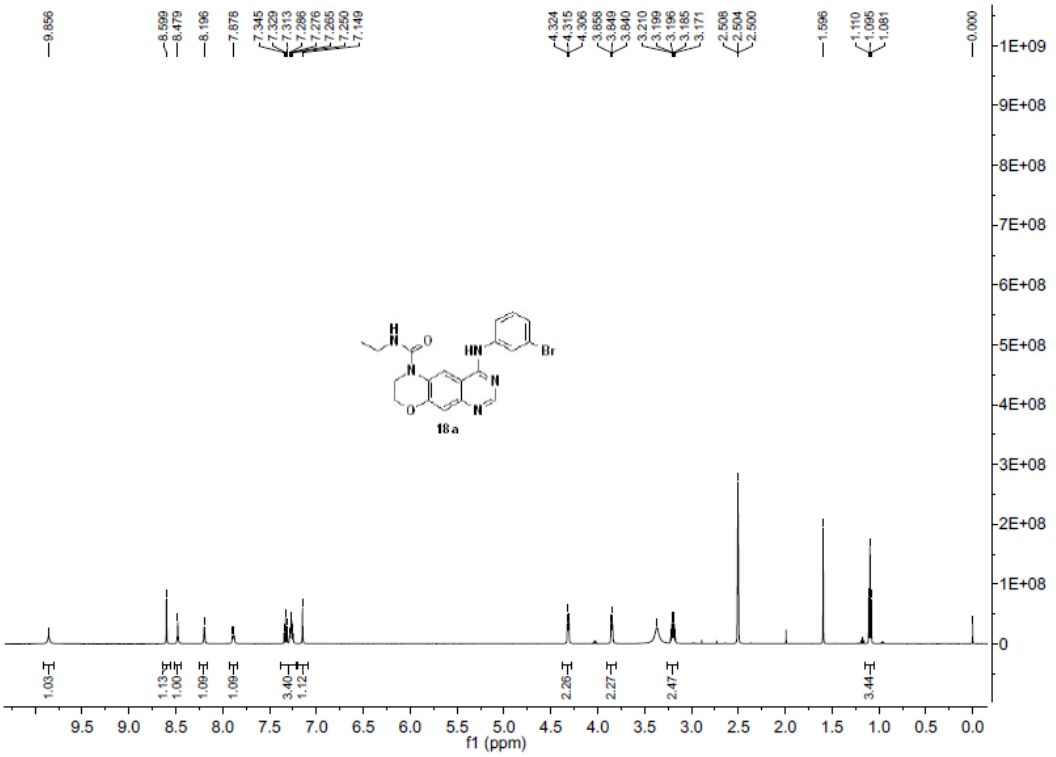
f1 (ppm)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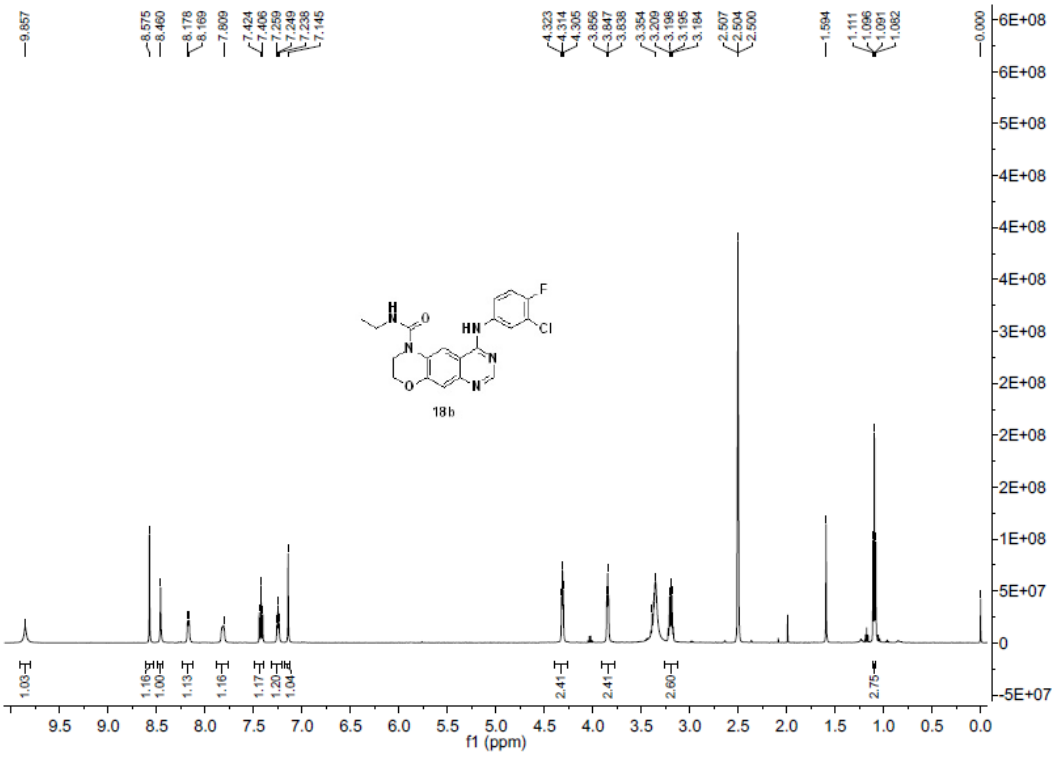
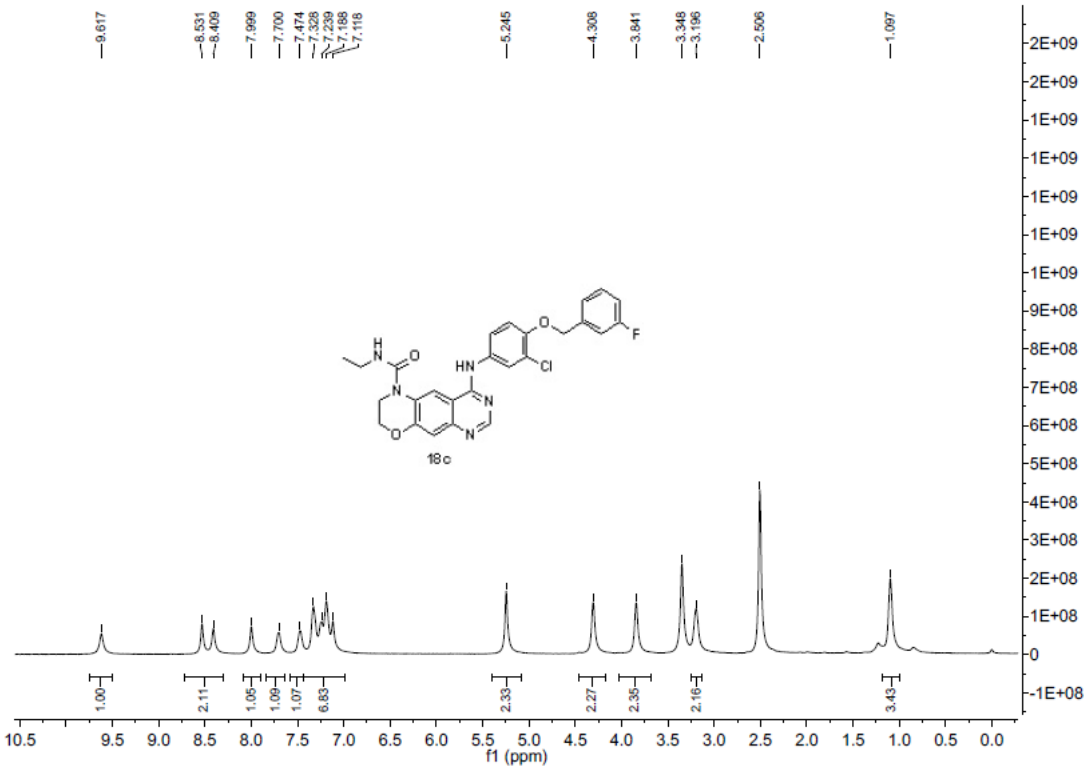
4.324
4.315
4.306
3.858
3.849
3.840
3.210
3.199
3.196
3.185
3.171
2.506
2.504
2.500
1.596
1.110
1.096
1.081
0.000
1.03
1.13
1.00
1.09
1.09
3.40
1.12
2.26
2.27
2.47
3.44
f1 (ppm)

18a


9.857
8.575
8.460
8.178
8.169
7.809
7.424
7.406
7.259
7.249
7.238
7.145
4.323
4.314
4.305
3.856
3.847
3.838
3.354
3.209
3.198
3.195
3.184
2.507
2.504
2.500
1.594
1.111
1.096
1.091
1.082
0.000
1.03
1.16
1.00
1.13
1.16
1.17
1.20
1.04
2.41
2.41
2.60
2.75
f1 (ppm)

18b


9.617
8.531
8.409
7.999
7.700
7.474
7.328
7.239
7.188
7.118
5.245
4.306
3.841
3.348
3.196
2.506
1.097
18c
1.00
2.11
1.05
1.09
1.07
6.83
2.33
2.27
2.35
2.16
3.43
f1 (ppm)