Supporting Information

Tariquidar-related chalcones and ketones as ABCG2 modulators

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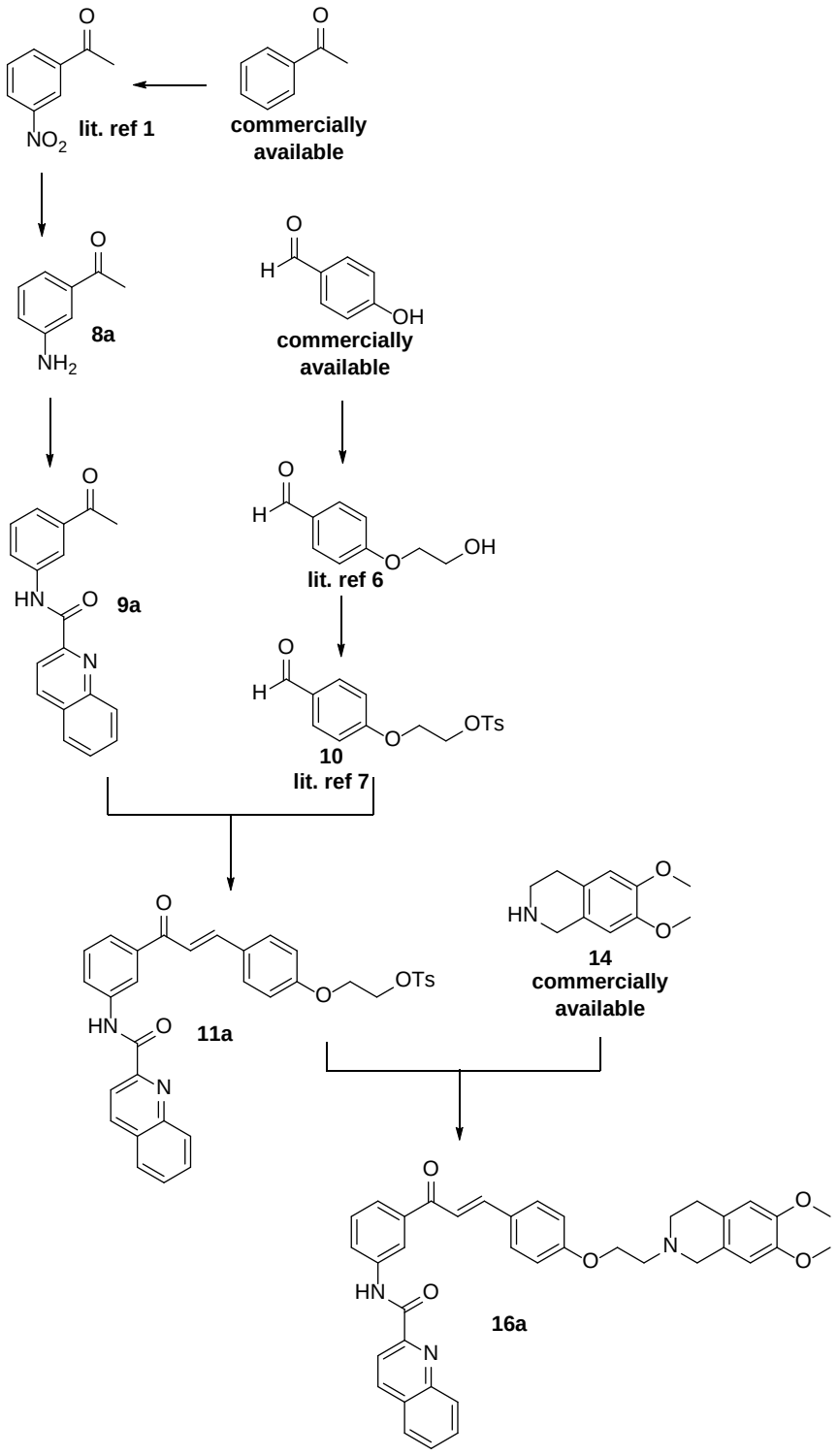
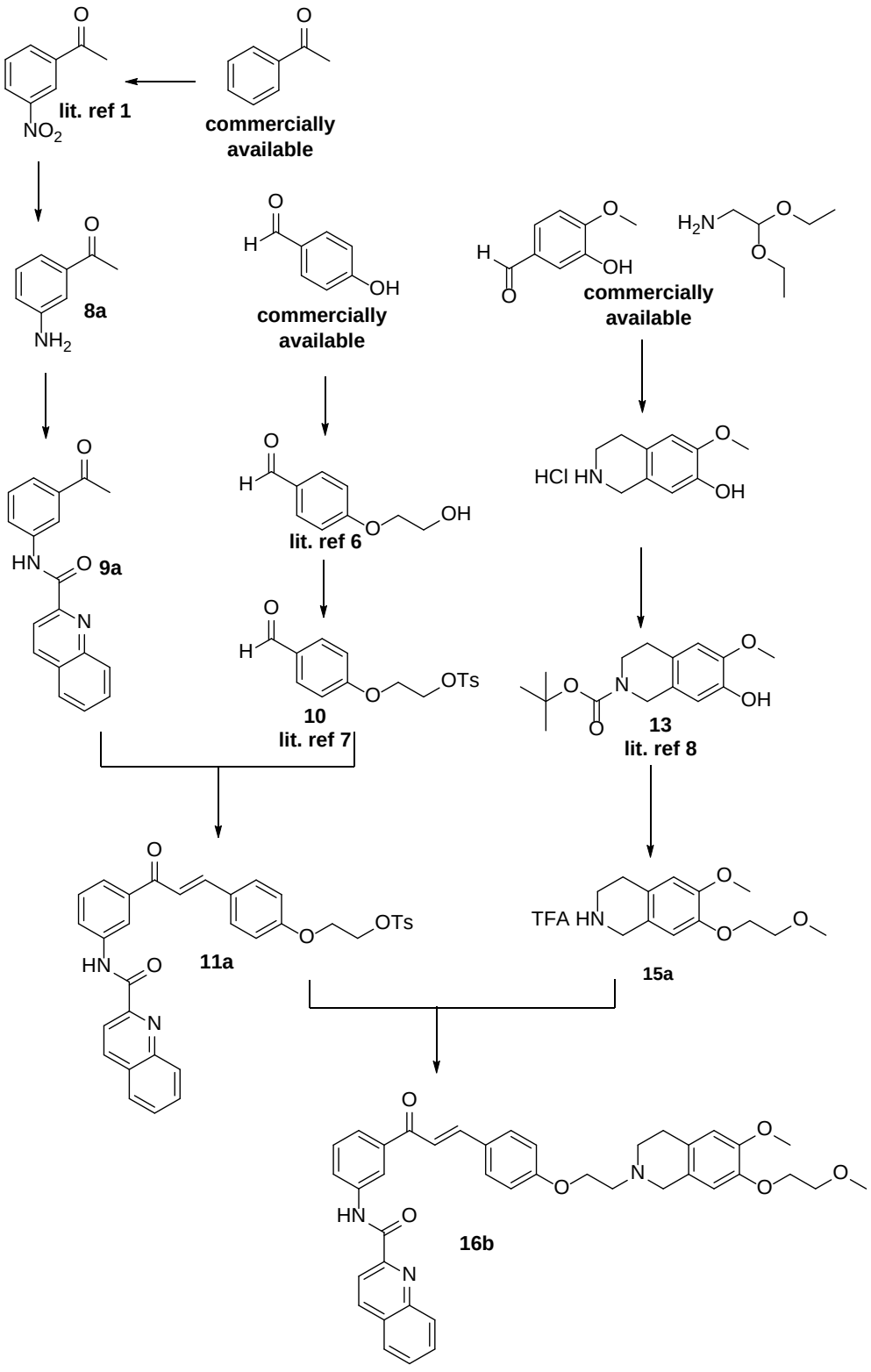
1. Experimental section

1.1 Synthetic pathways

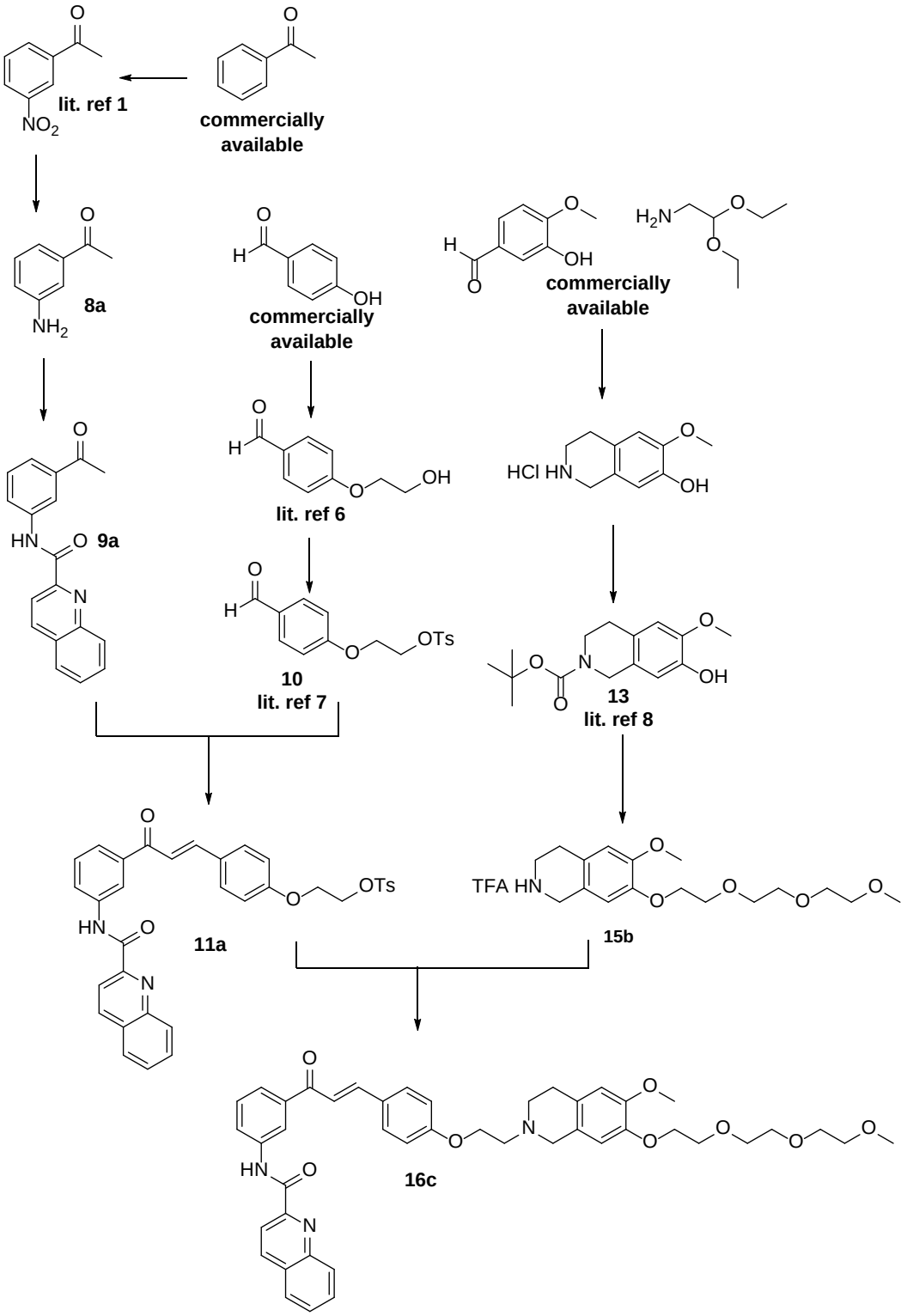
(E)-N-(3-(3-(4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)phenyl)quinoline-2-carboxamide



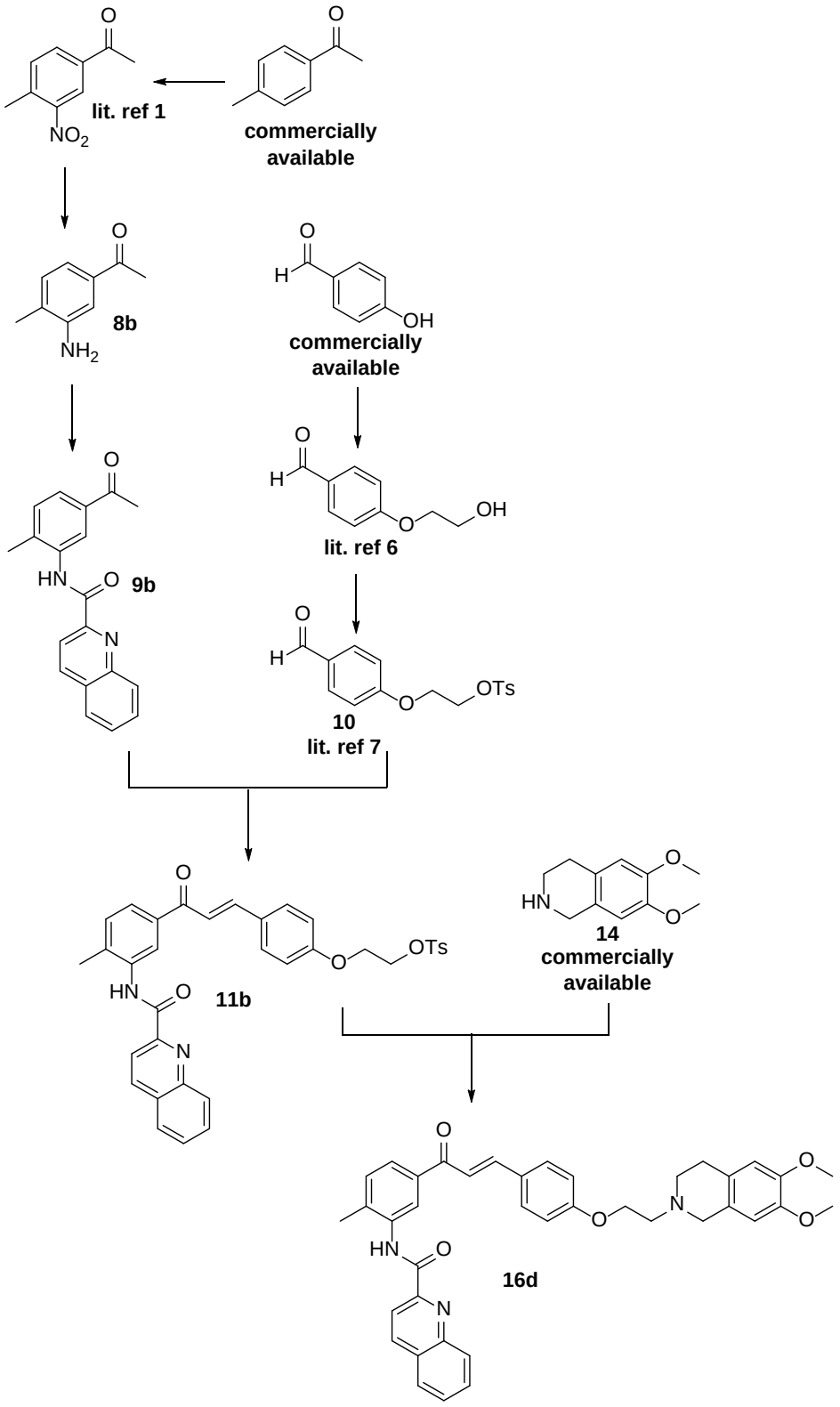
(E)-N-(3-(3-(4-(2-(6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)phenyl)quinoline-2-carboxamide



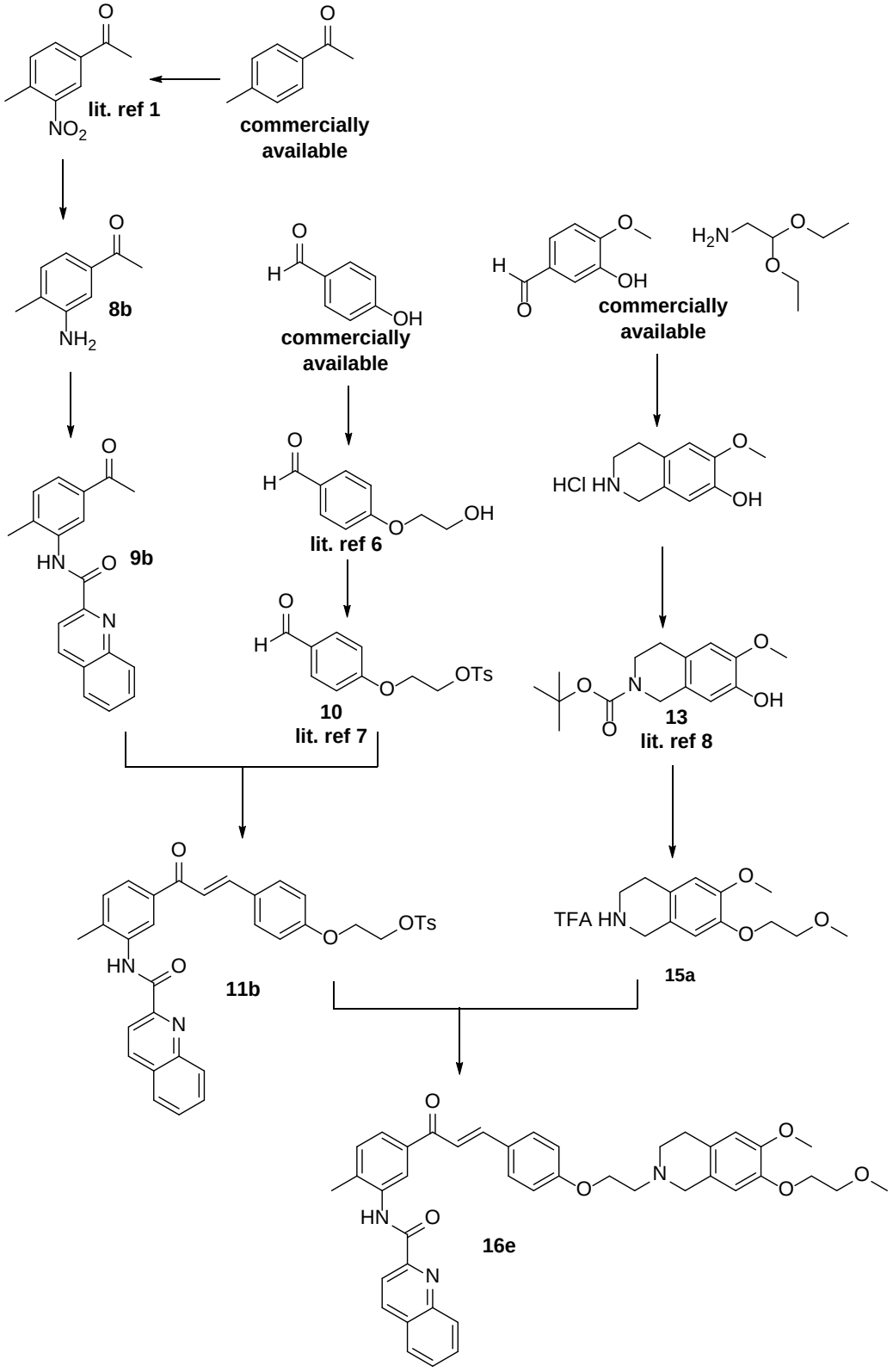
(E)-N-(3-(3-(4-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)phenyl)quinoline-2-carboxamide



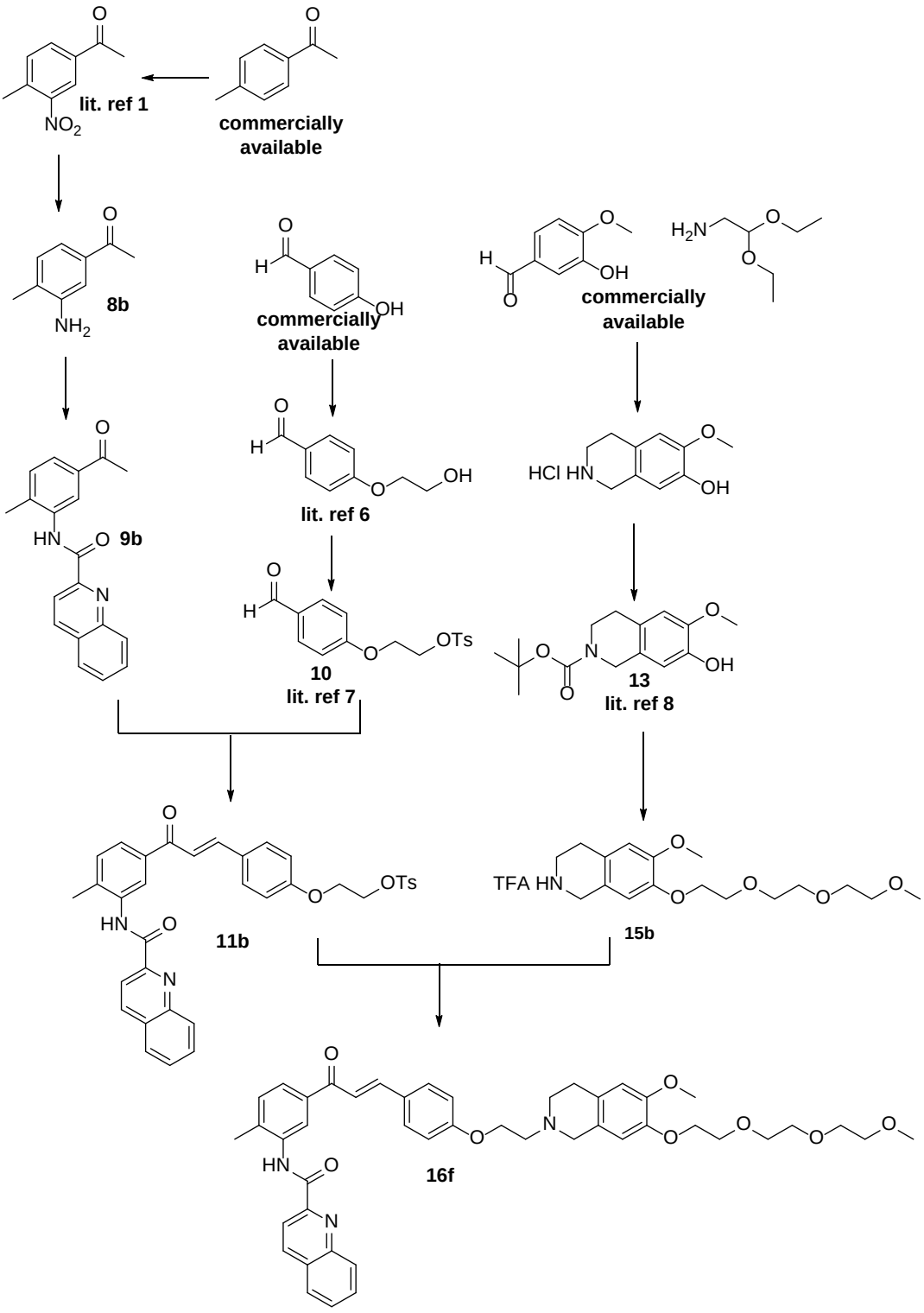
(E)-N-(5-(3-(4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)-2-methylphenyl)quinoline-2-carboxamide



(E)-N-(5-(3-(4-(2-(6,7-bis(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)-2-methylphenyl)quinoline-2-carboxamide



(E)-N-(5-(3-(4-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)-2-methylphenyl)quinoline-2-carboxamide



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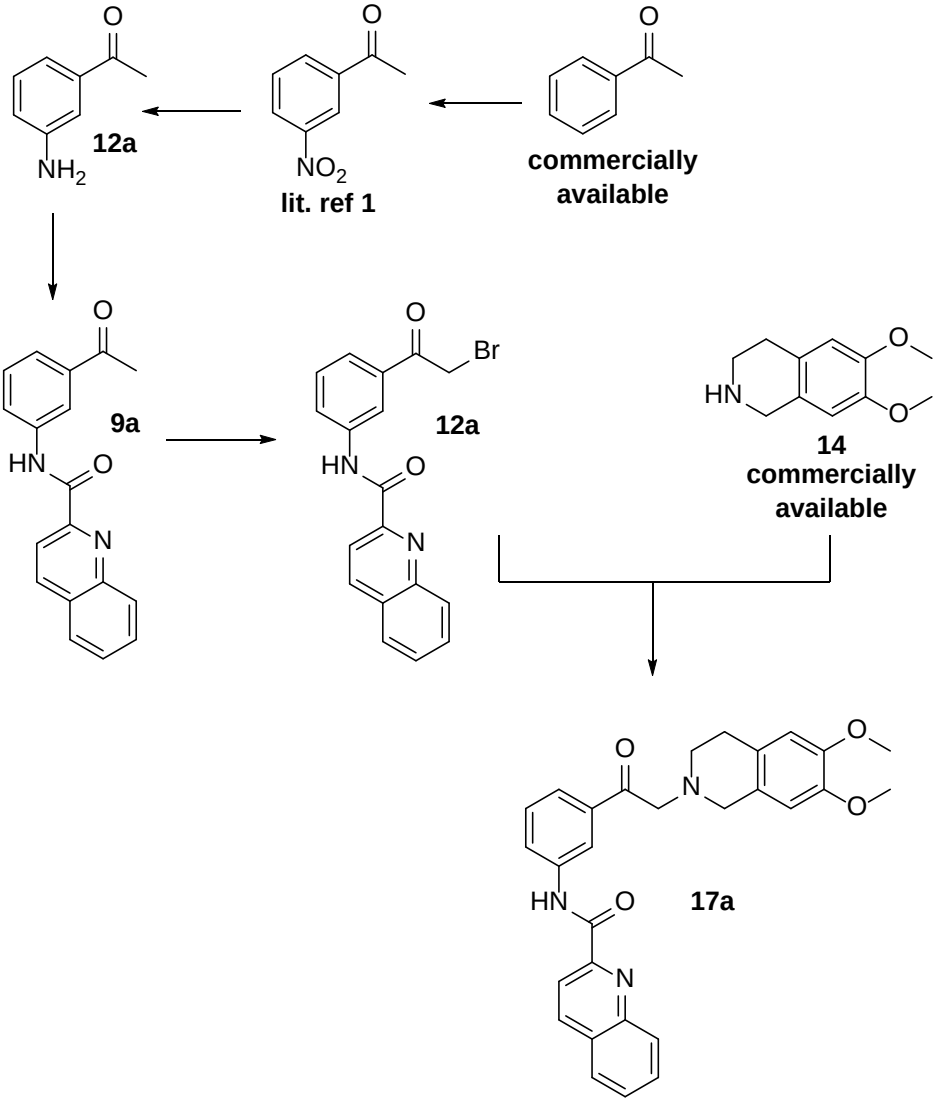

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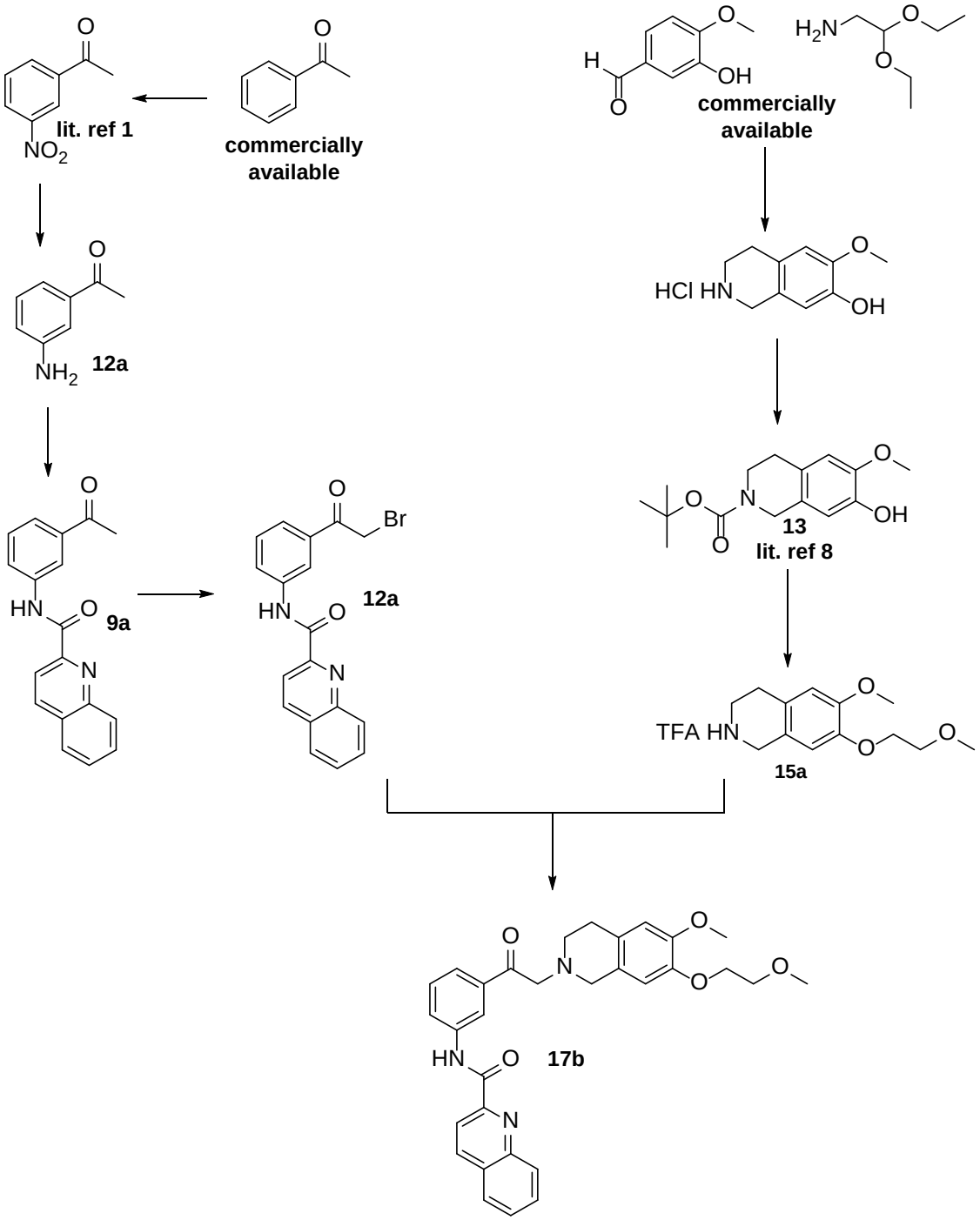
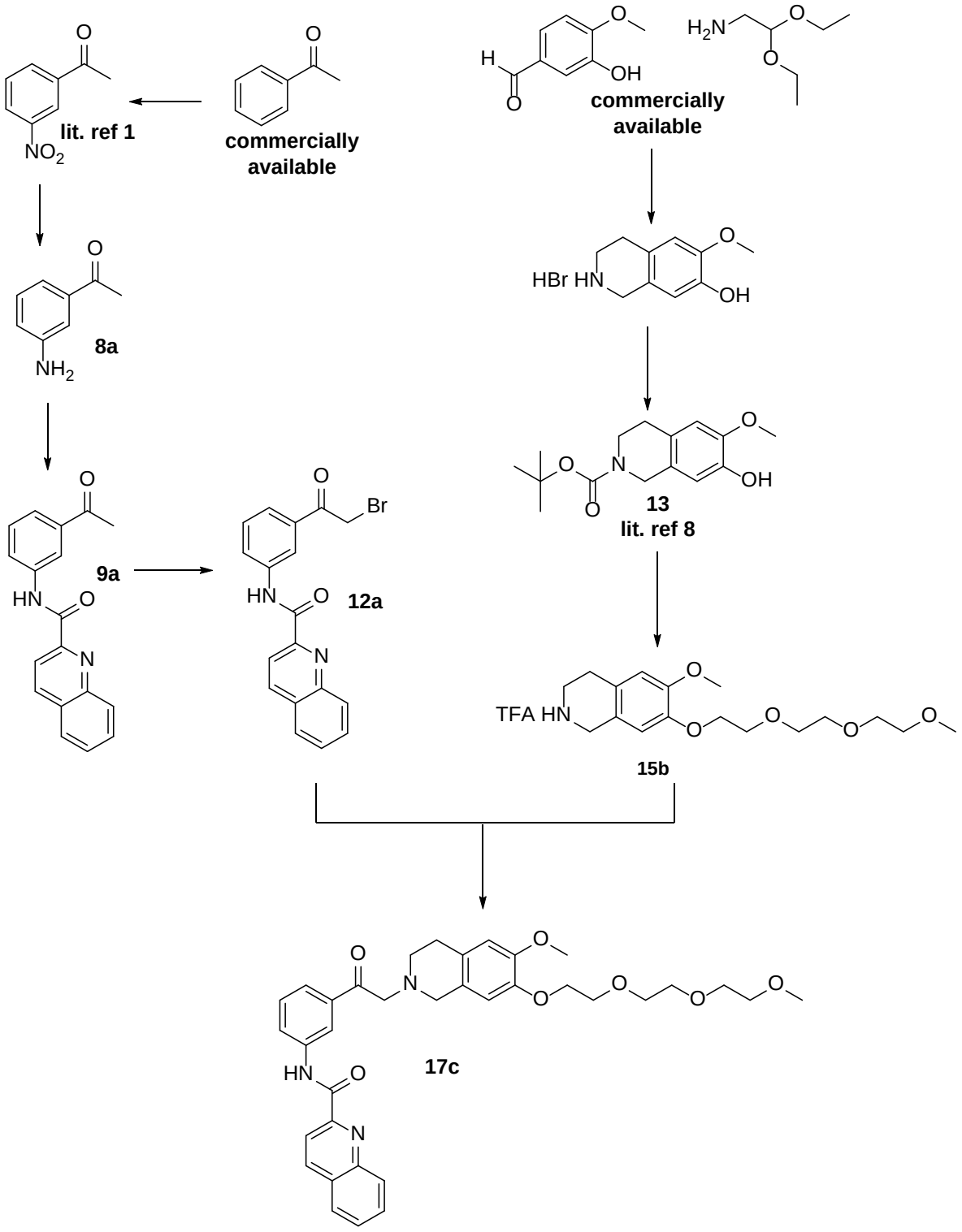
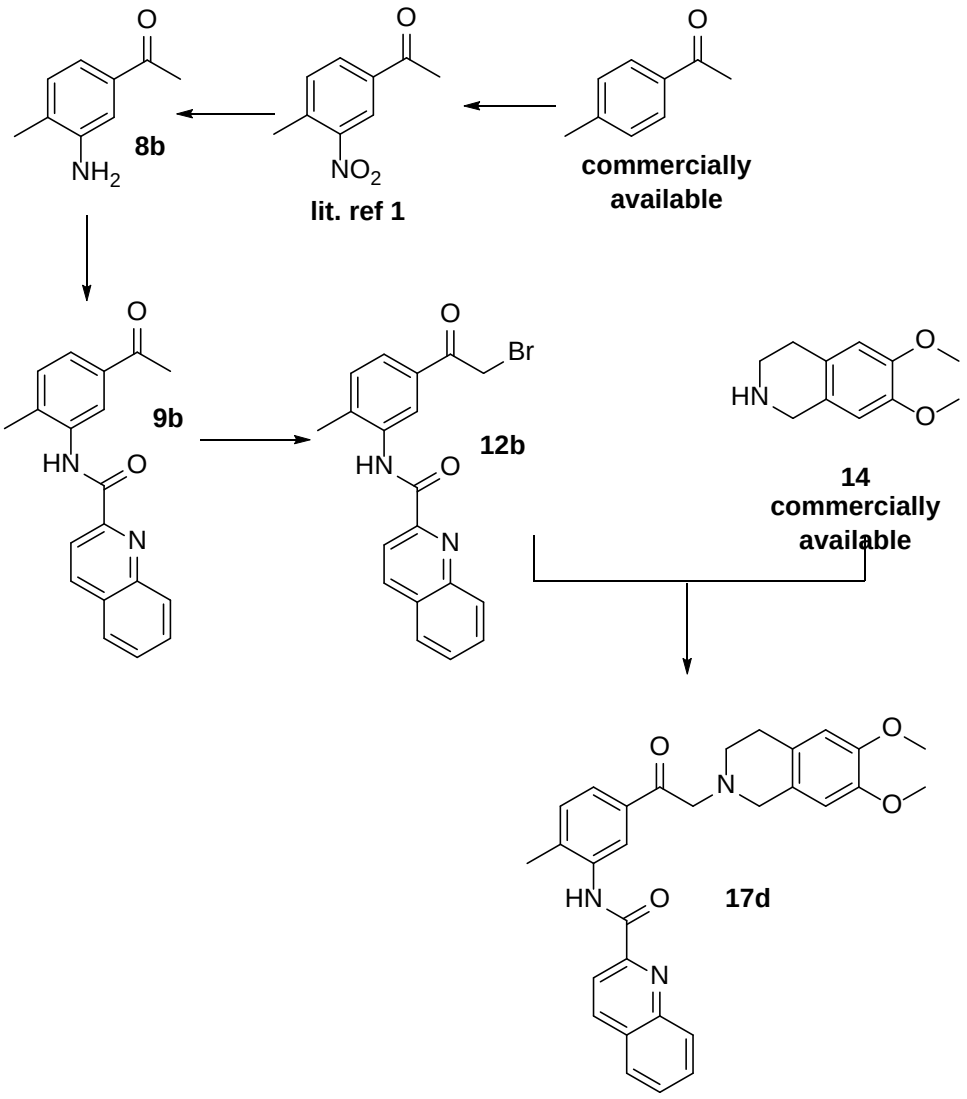


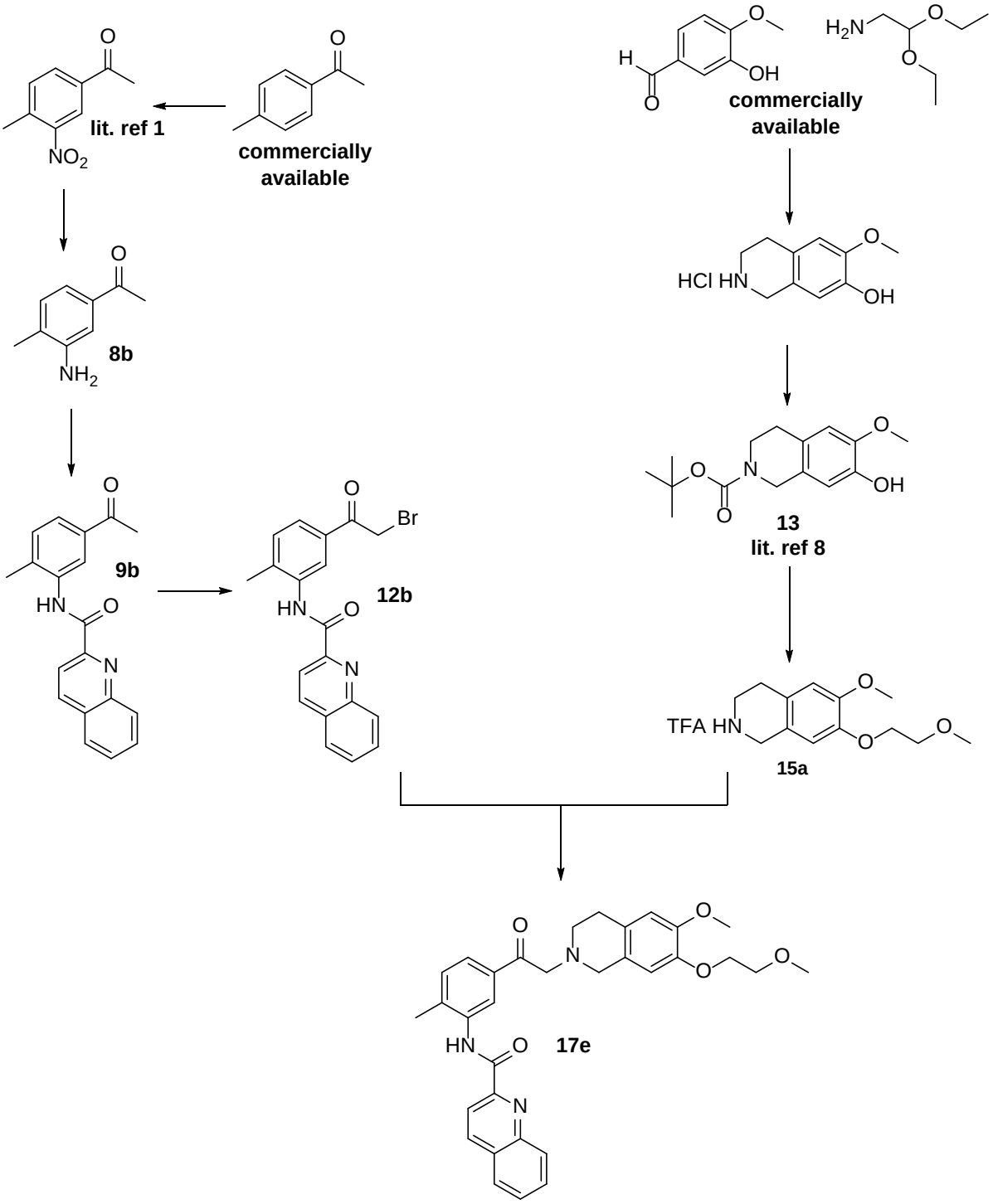
N-(3-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)phenyl)quinoline-2-carboxamide



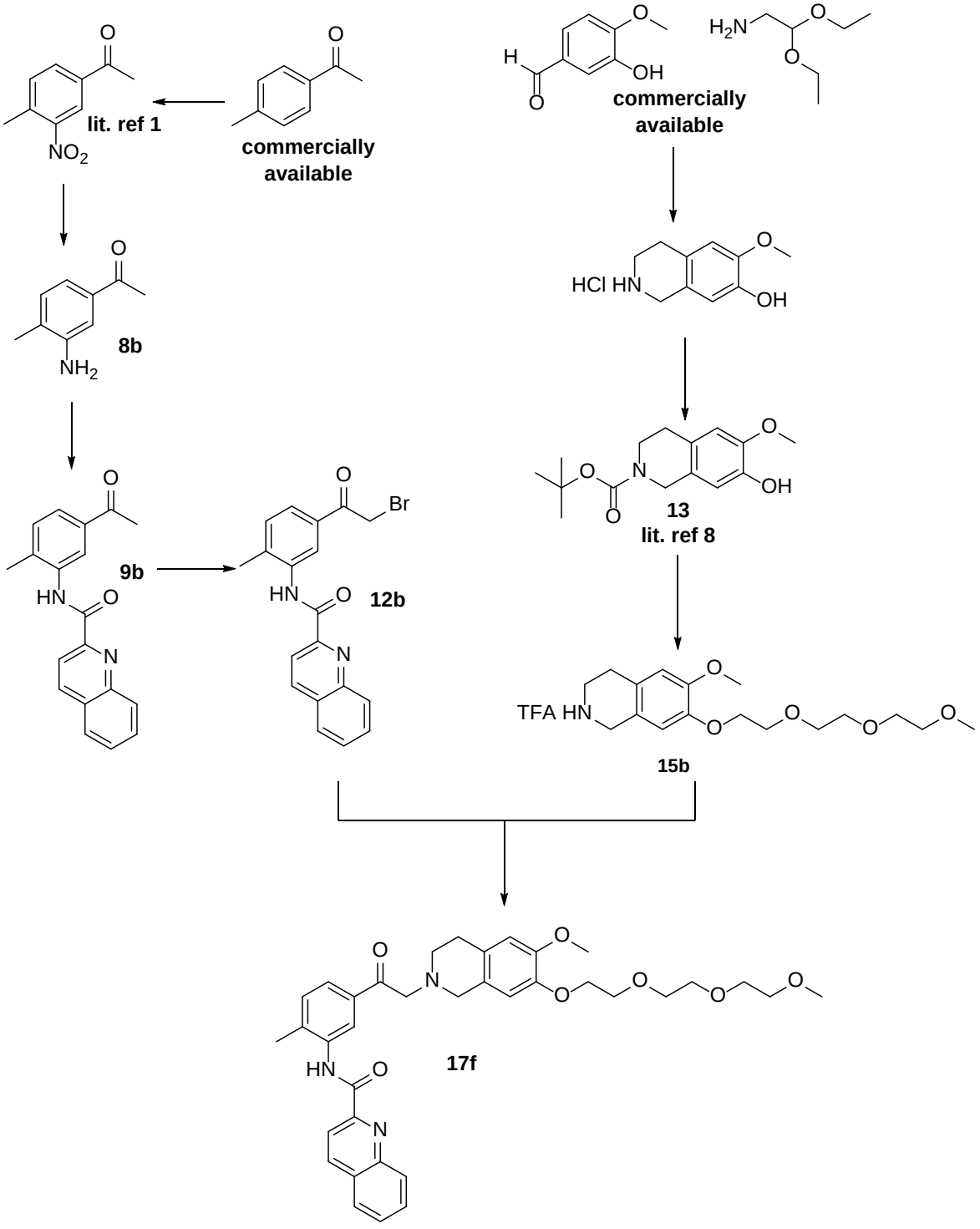
N-(5-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)-2-methylphenyl)quinoline-2-carboxamide



N-(3-(2-(6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)phenyl)quinoline-2-carboxamide



N-(5-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)-2-methylphenyl)quinoline-2-carboxamide



1.2 Reagents and devices

Reagents and solvents

Reagents Commercial reagents were used as provided. Solvents were used in per analysis grade for reaction mixtures and industrial grade for chromatography.

1.2.1 Devices

Automated Flash chromatography

Purification by column chromatography was performed with a Biotage® Isolera™ Spektra One device. Silica gel 60 M (40-63 µM, Merck) for flash column chromatography was used.

Thin layer chromatography

Analytical TLC on silica gel coated alumina plates (MN TLC sheets ALUGRAM® Xtra SIL G/UV254) was used for monitoring reaction progress and product visualization was conducted with UV-light (254 and 366 nm), and staining with potassium permanganate or Ehrlich's reagent.3

Nuclear magnetic resonance spectroscopy

NMR spectra were recorded at room temperature on a Bruker Avance 9.4 Tesla (^{1}H: 400 MHz, ^{13}C: 101 MHz) spectrometer with a Nanobay console and a BBFOPlus broadband ATMA probe or a Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150 MHz) instrument. The chemical shifts were reported in δ [ppm] relative to tetramethylsilane as external standard according to IUPAC recommendations. The relative number of protons was determined by integration. Coupling constants J were reported in Hertz [Hz]. Abbreviations for the characterization of the signals: s=singlet, d=doublet, t=triplet, m=multiplet, dd=doublet of doublets, q=quartet.

Mass spectrometry

High resolution mass spectra were recorded with an Agilent Tech 6540 UHD Accurate Mass Q-TOF LC/MS spectrometer.

Infrared spectroscopy

IR-spectra were recorded with a spectrometer FTIR Nicolete iS 10 (Thermo Fisher Scientific). Scans of 50 readings were performed by spectrum; the data were recorded in units of transmittance. The accessory ATR (PIKE) was used with a diamond crystal and an incidence angle of 45 °; samples were analyzed with the software IRsolution.

Reversed phase high-performance liquid chromatography

Purification with preparative HPLC was performed on a system from Knauer (Berlin, Germany; two K-1800 pumps, a K-2001 detector set to 280 nm). Column: Nucleodur 100-5 C18 ec (250 × 21 mm, 5 µm, Macherey-Nagel, Düren, Germany). Solvents: Millipore water (with 0.1 % (v/v) TFA) / acetonitrile (gradient grade). Flow rate: 15 mL/min at 26 °C. The products were collected manually, acetonitrile was removed from the eluate under reduced pressure, and lyophilization furnished the corresponding TFA salts.

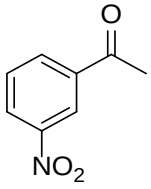
Analytical high-performance liquid chromatography

The purity of all final compounds (dissolved in DMSO) was determined by analytical HPLC (integration of the UV signal at 220 nm wavelength) using of a 1220 Infinity LC System from Agilent equipped with a reverse phase Phenomenex Luna® 3µ C18(2) column (150 × 2.0 mm, 100 Å) thermostatted at 40 °C. HPLC conditions were the following: solvent A = water (Millipore)/TFA (0.05% v/v), solvent B = MeCN (Merck, gradient grade); flow rate = 0.3 mL/min; injection volume 1 µL, elution with a gradient of 10% to 95% B in 30 min.

1.3 Synthesis and characterization

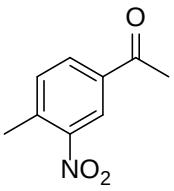
General procedure for the preparation of nitro compounds: compounds were prepared according to known procedures [1].

1-(3-nitrophenyl)ethan-1-one



Light yellow solid (1.8 g, yield 50%, R_f PE/EA 1:1 0.67). mp 75-78°C (76-78ºC lit) [1]. **^{1}H NMR ($CDCl_3$, 400 MHz): δ**= 8.81 (d, ^4J=1.9 Hz, 1H, ArH), 8.46 (ddd, ^3J=8.2, ^4J=2.2, ^4J=1.1 Hz, 1H, ArH), 8.32 (d, ^3J=7.8 Hz, 1H, ArH), 7.72 (s, 1H, ArH), 2.72 (s, 3H, CH_3CO). **^{13}C NMR ($CDCl_3$, 100 MHz): δ**= 195.8 (C_{quat}), 147.0 (C_{quat}), 138.4 (C_{quat}), 133.7 (+), 129.9 (+), 127.4 (+), 123.2 (+), 26.7 (+). **IR (KBr) [cm^{-1}]: ν** = 3237, 3090, 1691, 1520, 1428, 1348, 1250, 970, 821, 587.

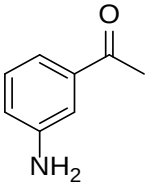
1-(4-methyl-3-nitrophenyl)ethan-1-one



Yellow solid (1.5 g, yield 65%, R_f PE/EA 7:3 0.43). mp 60-62°C (60-61 lit) [2]. **^{1}H NMR (400 MHz, $CDCl_3$): δ** 8.52 (d, ^3J=1.5 Hz, 1H, ArH), 8.08 (dd, ^3J=8.0, ^4J=1.5 Hz, 1H, ArH), 7.47 (s, 1H, ArH), 2.67 (s, 3H, CH_3CO), 2.65 (s, 3H, CH_3). **^{13}C NMR (100 MHz, $CDCl_3$): δ** 195.5 (C_{quat}), 149.2 (C_{quat}), 138.6 C_{quat}), 136.1 (C_{quat}), 133.3 (+), 131.9 (+), 124.6 (+), 26.5 (+), 20.6 (+). **IR [cm^{-1}]: v** = 1692, 1617, 1531, 1348, 1282, 1251, 833, 803.

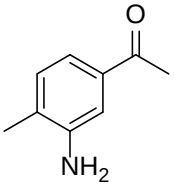
General procedure for the preparation of amino compounds (8a-b): The respective nitro derivative (1 equiv) was dissolved in a mixture of ethanol and water (2:1), iron powder (10 equiv) and conc. HCl (0.05 mL) were added and the mixture was refluxed for 90 min. Ethyl acetate was added and the mixture was filtered over celite, dried over sodium sulfate, filtered again and the solvent evaporated, resulted the corresponding amino compound.

1-(3-aminophenyl)ethan-1-one (8a)



Light brown solid (1.2 g, yield 85%, R_f Pentane:EA 1:1 0.55). mp 93-95°C (94-98ºC) [3]. **^{1}H NMR ($CDCl_3$, 400 MHz)**: **δ=** 6.97 (t, ^3J=7.7 Hz, 1H, ArH), 6.90 (m, 1H, ArH), 6.85 (m, 1H, ArH) 6.50 (dd, ^3J=7.8, ^4J=1.7 Hz, 1H, ArH), 2.19 (s, 3H, CH_3CO). **^{13}C NMR ($CDCl_3$, 100 MHz)**: **δ=** 198.6 (C_{quat}), 146.7 (C_{quat}), 138.1 (C_{quat}), 129.4 (+), 119.7 (+), 118.9 (+), 114.0 (+), 26.7 (+). **IR (KBr) [cm^{-1}]: v =**3550, 3473, 3415, 3236, 2925, 2810, 1639, 1618, 1458, 1384, 1358, 1323, 1238, 870, 621.

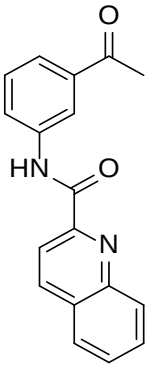
1-(3-amino-4-methylphenyl)ethan-1-one (8b)



Brown solid (1.4 g, yield 93%, R_f Hex/EA 4:1 0.24). mp 78-80ºC (80-81ºC lit) [2]. **^{1}H NMR (400 MHz, $CDCl_3$): δ** 7.28 (dd, ^3J=7.5, ^4J=2.0 Hz, 1H, ArH), 7.27 (d, ^3J=7.3, 1H, ArH), 7.12 (d, ^3J=7.5, 1H, ArH), 2.54 (s, 3H, $COCH_3$), 2.21 (s, 3H, CH_3). **^{13}C NMR (100 MHz, $CDCl_3$): δ** 198.3 (C_{quat}), 144.8 (C_{quat}), 136.2 (C_{quat}), 130.4 (C_{quat}), 128.0 (+), 119.1 (+), 113.8 (+), 26.5 (+), 17.5 (+). **IR [cm^{-1}]: v** = 3367, 1672, 1573, 1422, 1356, 1291, 1237, 1199.

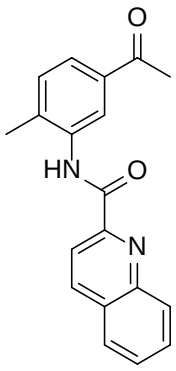
General procedure for the amide formation (9a-b)[4]: TsCl (1.2 equiv) and amine (**8a-b**) were added, in that order, at 0 °C under N_2 to a flask containing quinaldic acid (1.2 equiv), triethylamine (2 equiv), and CH_2Cl_2, and the mixture was stirred at 40-50°C overnight. AcOH (5% aq.) was added to quench the reaction, the organic phase was separated and washed three times with water, dried over sodium sulfate, filtered again and the solvent evaporated.The obtained solid was washed with ether furnishing the desired product.

N-(3-acetylphenyl)quinoline-2-carboxamide (9a)



White solid (0.500 g, yield quant, R_f PE:EA 2:1 0.40).**^{1}H NMR ($CDCl_3$, 400 MHz)**: **δ=** 8.43 (s, 2H, quinol), 8.39 (t, ^3J=1.9 Hz, 1H, quinol), 8.25 (m, 2H, ArH), 7.96 (d, ^3J=8.2 Hz, 1H, quinol), 7.87 (t, ^3J=8.3 Hz, 1H, ArH), 7.79 (d, ^3J=7.9 Hz, 1H, ArH), 7.71 (t, ^3J=7.5 Hz, 1H, quinol), 7.56 (t, ^3J=7.9 Hz, 1H, quinol), 2.70 (s, 3H, CH_3). **^{13}C NMR ($CDCl_3$, 100 MHz)**: **δ=** 197.8 (C_{quat}), 162.3 (C_{quat}), 149.1 (C_{quat}), 146.1 (C_{quat}), 138.3 (C_{quat}), 138.1 (C_{quat}), 137.9 (C_{quat}), 130.5 (+), 129.5 (+), 129.5 (+), 129.4 (+), 128.4 (+), 127.8 (+), 124.2 (+), 124.1 (+), 119.3 (+), 118.7 (+), 26.7 (+). **IR (KBr) [cm^{-1}]: v =**3483, 3416, 3341, 3082, 2923, 1687, 1591, 1530, 1504, 1442, 1365, 1295, 1126, 955, 846, 774, 686, 584. **HRMS (EI-MS)** calcd. For $C_{18}H_{15}N_2O_2$ $[M+H]^+$: 291.1133; found $[M+H]^+$: 291.1128.

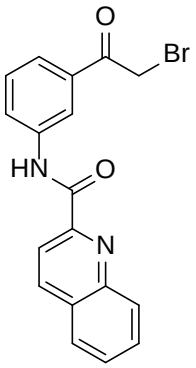
N-(5-acetyl-2-methylphenyl)quinoline-2-carboxamide (9b)



Light yellow solid (0.700 g, yield quant, R_f PE/EA 2:1 0.52). **^{1}H NMR (400 MHz, $CDCl_3$): δ** 8.95 (d, ^3J=7.7 Hz, 1H, quinol), 8.40 (s, 2H, ArH quinol), 8.17 (d, ^3J=8.5 Hz, 1H, ArH), 7.93 (d, ^3J=9.0 Hz, 1H, quinol), 7.82 (ddd, ^3J=8.4, ^3J=6.9 Hz, 1H, quinol), 7.73 (dd, ^3J=7.9, 1H, ArH), 7.67 (ddd, ^3J= 8.1, ^3J=6.9 Hz, 1H, quinol), 7.35 (d, ^3J=7.9 Hz, 1H, quinol), 2.65 (s, 3H, CH_3), 2.55 (s, 3H, $COCH_3$). **^{13}C NMR (100 MHz, $CDCl_3$): δ**= 197.8 (C_{quat}), 162.2 (C_{quat}), 149.5 (C_{quat}), 146.2 (C_{quat}), 138.0 (C_{quat}), 136.2 (C_{quat}), 133.5 (C_{quat}), 130.7 (C_{quat}), 130.4 (+), 129.8 (+), 129.5 (+), 128.3 (+), 127.8 (+), 124.1 (+), 121.5 (+), 118.6 (+), 26.7 (+), 18.0 (+). **IR [cm^{-1}]: v =** 3420, 3050, 2910, 1670, 1580, 1410, 1350, 1220, 1080, 760. **HRMS (EI-MS)** calcd. For $C_{19}H_{17}N_2O_2$ $[M+H]^+$: 305.1290; found $[M+H]^+$: 305.1285.

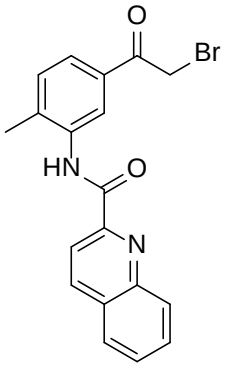
General procedure for the bromination of ketones 12a-b [5]: To a solution of acetophenone (10 mmol) in methanol at reflux conditions were added 10% (w/w) silica gel and slowly N-bromosuccinimide (12 mmol), the progress of the reaction was monitored by TLC until the disappearance of the substrate. The reaction was filtered after the completion of the reaction. The filtrate was concentrated under vacuum and the solid was washed twice with distilled water, then aqueous sodium thiosulfate was added to quench the reaction and the product was extracted with dichloromethane, organic layers were collected and washed thrice with water, dried over anhydrous Na_2SO_4, filtered and concentrated to give the desired product.

N-(3-(2-bromoacetyl)phenyl)quinoline-2-carboxamide (12a)



White solid (0.500 g, yield 84%, R_f PE:EA 2:1 0.67). **^{1}H NMR ($CDCl_3$, 400 MHz): δ** 8.46 (t, ^3J=7.9 Hz, 1H, quinol), 8.42 (s, 1H, quinol), 8.27 (d, ^3J=8.5 Hz, 1H, ArH), 8.22 (d, ^3J=7.7 Hz, 1H, quinol), 7.95 (d, ^3J=8.2 Hz, 1H, quinol), 7.86 (ddd, ^3J=8.4, ^3J=6.9 Hz, 1H, ArH), 7.81 (m, 1H, quinol), 7.72 (m, 2H, ArH), 7.56 (t, ^3J=7.9 Hz, 1H, quinol), 4.54 (s, 2H, CH_2Br).**^{13}C NMR ($CDCl_3$, 100 MHz): δ** 191.0 (C_{quat}), 162.2 (C_{quat}), 148.9 (C_{quat}), 145.9 (C_{quat}), 138.5 (C_{quat}), 138.4 (C_{quat}), 134.8 (+), 130.8 (+), 129.7 (+), 128.6 (+), 127.9 (+), 125.0 (+), 124.7 (+), 119.8 (+), 118.8 (+), 31.1 (-). **IR (KBr) [cm^{-1}]: ν** =3483, 3416, 3341, 3082, 2923, 1687, 1591, 1530, 1504, 1442, 1365, 1295, 1126, 955, 846, 774, 686, 584. **HRMS (EI-MS)** calcd. For $C_{18}H_{14}BrN_2O_2$ $[M+H]^+$: 369.0238; found $[M+H]^+$: 369.0233.

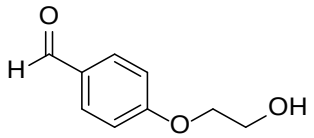
N-(5-(2-bromoacetyl)-2-methylphenyl)quinoline-2-carboxamide (12b)



Light yellow solid (0.720 g, yield 77%, R_f PE:EA 2:1 0.59). **^{1}H NMR ($CDCl_3$, 400 MHz): δ** 9.02 (d, ^3J=1.7 Hz, 1H, quinol), 8.41 (s, 1H, quinol), 8.18 (d, ^3J=8.5 Hz, 1H, ArH), 7.94 (d, ^3J=8.2 Hz, 1H, quinol), 7.86 (m, 1H, quinol), 7.77 (d, ^3J=7.9 Hz, 2H, ArH), 7.68 (dd, ^3J=8.1, ^3J=7.0 Hz, 1H, quinol), 7.39 (d, ^3J=7.9 Hz, 1H, quinol), 4.54 (s, 2H, CH_2Br), 2.57 (s, 3H, CH_3). **^{13}C NMR ($CDCl_3$, 100 MHz): δ** 192.6 (C_{quat}), 162.3 (C_{quat}), 149.6 (C_{quat}), 146.6 (C_{quat}), 138.1 (C_{quat}), 136.4 (C_{quat}), 134.8 (C_{quat}), 133.3 (+), 131.1 (+), 130.5 (+), 129.8 (+), 129.6 (+), 128.4 (+), 127.9 (+), 124.8 (+), 121.9 (+), 118.7 (+), 31.4 (-), 20.6 (+). **IR**

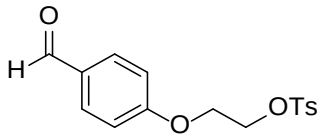
(KBr) [cm^{-1}]: v 3483, 3416, 3341, 3082, 2923, 1687, 1591, 1530, 1504, 1442, 1365, 1295, 1126, 955, 846, 774, 686, 584. **HRMS (EI-MS)** calcd. For $C_{19}H_{16}BrN_2O_2$ $[M+H]^+$: 383.0395; found $[M+H]^+$: 383.0396.

4-(2-hydroxyethoxy)benzaldehyde



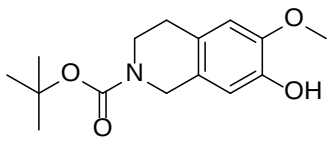
4-(2-hydroxyethoxy)benzaldehyde has already been described [6]. Colorless oil (1.2 g, yield 85%, R_f Hex:EA 1:1 0.20), **^{1}H NMR ($CDCl_3$, 400 MHz): δ**= 7.45 (d, ^3J=8.9 Hz, 2H, OCH_2CH_2), 6.64 (d, ^3J=8.7 Hz, 2H, OCH_2CH_2), 3.79 (t, ^3J=9.0 Hz, 2H, OCH_2), 3.73 (t, ^3J=9.3 Hz, 2H, CH_2OH). **^{13}C NMR ($CDCl_3$, 100 MHz): δ**= 190.99 (C_{quat}), 163.72 (C_{quat}), 131.80 (C_{quat}), 130.10 (+), 114.82 (+), 69.55 (-), 61.21 (-). **IR [cm^{-1}]: v** = 3443, 3274, 2873, 2842, 2747, 1690, 1580, 1427, 1044, 916, 653.

2-(4-formylphenoxy)ethyl 4-methylbenzenesulfonate (10)



Compound **3** has already been described [7] Light pink solid (0.700 g, yield 84%, R_f Pen: EA 1:1 0.62). mp: 102-104ºC (103-105ºC lit).[7] **^{1}H NMR ($CDCl_3$, 400 MHz)**: **δ=** 7.83 (d, ^3J=2.5 Hz, 2H, ArH), 7.81 (d, ^3J=7.0 Hz, 2H, ArH), 7.36 (d, ^3J=8.1 Hz, 2H, ArH), 6.91 (d, ^3J=8.7 Hz, 2H, ArH), 4,42 (m, 2H, OCH_2), 4,25 (m, 2H, CH_2OSO_2), 2.46 (s, 3H, CH_3). **^{13}C NMR ($CDCl_3$, 100 MHz)**: **δ=** 190.6 (C_{quat}), 162.8 (C_{quat}), 145.1 (C_{quat}), 127.9 (C_{quat}), 128.0 (C_{quat}), 129.9 (+), 129.9 (+), 130.4 (+), 131.8 (+), 131.9 (+), 132.8 (+), 114.7 (+), 114.7 (+), 67.6 (-), 65.6 (-), 21.6 (+). **IR (KBr) [cm^{-1}]: v =**3254, 2830, 2750, 1698, 1603, 1508, 1271, 1015, 915, 665.

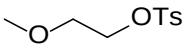
***tert*-butyl 7-hydroxy-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (13)**



1,2,3,4-Tetrahydroisoquinoline-6,7-diol hydrochloride (1 mmol) and triethylamine (2.5 equiv) were dissolved in 10 mL of dry DCM, the mixture was cooled in an ice bath and di-tert-butyl dicarbonate (1 equiv) was added slowly. The mixture was stirred at room temperature overnight, and then washed with water and brine, dried over magnesium sulphate, filtered and the solvent evaporated. The compound

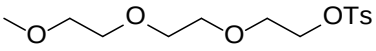
was purified by column chromatography (PE:EA 2:1), white solid (3.0 g, yield 89%, R_f PE:EA 1:1 0.55). mp 124-126 ºC (123.6-124.2ºC lit).[8] **^{1}H NMR ($CDCl_3$, 400 MHz): δ**= 6.85 (s, 1H, ArH), 6.70 (s, 1H, ArH), 4.47 (s, 2H, NCH_2), 3.81 (s, 3H, OCH_3), 3.60 (m, 2H, NCH_2CH_2), 2.75 (t, ^3J=5.6 Hz, 2H, NCH_2CH_2), 1.48 (s, 9H, $C(CH_3)_3$) **^{13}C NMR ($CDCl_3$, 100 MHz): δ**= 154.8 (C_{quat}), 151.7 (C_{quat}), 149.5 (C_{quat}), 138.5 (C_{quat}), 133.1 (C_{quat}), 120.1 (+), 112.5 (+), 83.4 (C_{quat}), 56.0 (+), 45.1 (-), 40.4 (-), 28.9 (-), 28.4 (+). **IR (KBr) [cm^{-1}]: ν** = 3024, 2976, 1685.

2-methoxyethyl 4-methylbenzenesulfonate[9]



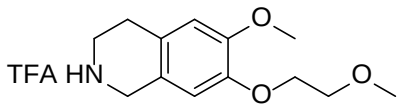
A solution of 4-toluenesulfonyl chloride (0.06 mol) in dichloromethane was added drop-wise to a solution of (2-methoxyethoxy)ethanol (0.057 mol) and triethylamine (0.114 mol) in dichloromethane at 0°C under a nitrogen atmosphere. The formation of a white precipitate was observed. The reaction mixture was stirred at room temperature for 18h and then poured into water (50 mL). The aqueous layer was extracted with dichloromethane. The organic layers were combined and washed with 3M HCl (50 mL), saturated solution of sodium bicarbonate (50 mL), and water (50 mL). The organic phase was dried over magnesium sulfate, filtered and the solvent was removed in vacuo. Pale yellow oil (1.45 g, yield 100%). **^{1}H NMR (CD_3OD, 400 MHz)**: **δ=** 7.82 (d, ^3J=8.3 Hz, 2H, ArH), 7.36 (d, ^3J=8.0 Hz, 2H, ArH), 4.17 (d, ^3J=1.2 Hz, 2H, OCH_2CH_2), 3.60 (s, 2H, CH_2OCH_3), 3.32 (s, 3H, CH_3), 2.46 (s, 3H, OCH_3).**^{13}C NMR (CD_3OD, 100 MHz)**: **δ=** 140.87 (C_{quat}), 129.12 (C_{quat}), 125.87 (+), 124.03 (+), 65.99 (-), 65.11 (-), 55.06 (+), 17.68 (+). **IR (KBr) [cm^{-1}]: ν =**3070, 2928, 2822, 1598, 1495, 1356, 1311, 1018, 817, 689.

2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate



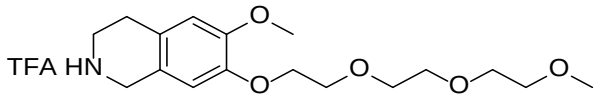
2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate was prepared according to known procedures [9]. Pale yellow oil (2.0 g, yield 100%). **^{1}H NMR (CD_3OD, 400 MHz)**: **δ=** 7.77 (d, ^3J=8.3, 2H, tosyl), 7.32 (d, ^3J=8.2, 2H, tosyl), 4.13 (t, ^3J=4.8, 2H, CH_2 PEG), 3.66 (t, ^4J=4.8, 2H, CH_2 PEG), 3.55 (m, 6H, $3CH_2$ PEG), 3.48 (m, 2H, CH_2 PEG), 3.36 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3 tosyl).

6-methoxy-7-(2-methoxyethoxy)-1,2,3,4-tetrahydroisoquinoline-2,2,2-trifluoroacetate (15a)



Compound **7a** was prepared according to known procedures with slight modifications [8]. (quantitative yield, R_f DCM:MeOH 19:1 0.50). **^{1}H NMR (CD_3OD, 400 MHz): δ=** 6.81 (s, 1H, ArH), 6.79 (s, 1H, ArH), 4.25 (s, 2H, CH_2), 4.12 (m, 2H, CH_2), 3.82 (s, 3H, CH_3), 3.76 (m, 2H, CH_2), 3.46 (t, ^3J=6.4 Hz, 2H, CH_2), 3.41 (s, 3H, CH_3), 3.04 (t, ^3J=6.3 Hz, 2H, CH_2). **^{13}C NMR (CD_3OD, 100 MHz): δ=** 149.6 (C_{quat}), 147.6 (C_{quat}), 124.0 (C_{quat}), 119.6 (C_{quat}), 111.6 (+), 70.7 (-), 68.4 (-), 57.8 (+), 55.0 (+), 44.0 (-), 41.5 (-), 24.2 (-).**HRMS (EI-MS)** calcd. For $C_{15}H_{21}F_3NO_5$ $[M+H]^+$: 352.1371; found $[M+H]^+$: 352.1347.

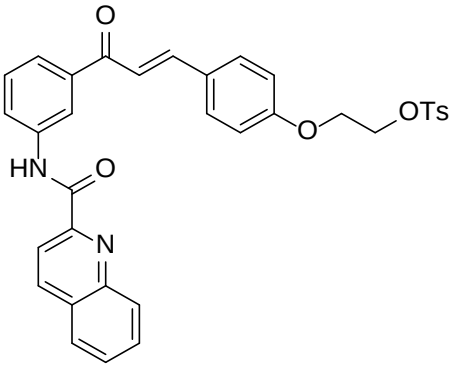
6-methoxy-7-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-1,2,3,4-tetrahydroisoquinoline-2,2,2-trifluoroacetate (15b)



Compound **7b** was prepared according to known procedures with slight modifications [8]. Sticky brown solid (Yield 79%, R_f DCM:MeOH 19:1 0.46). **^{1}H NMR ($CDCl_3$, 400 MHz): δ**= 6.60 (s, 1H, CH THIQ), 6.57 (s, 1H, CH THIQ), 4.39 (s, 2H, NCH_2 THIQ), 4.06 (t, ^3J=5.04 Hz, 4H, CH_2 PEG), 3.77 (t, ^3J=5.1 Hz, 4H, $2CH_2$ PEG), 3.65 (m, 4H, $2CH_2$ PEG), 3.56 (m, 8H, $4CH_2$ PEG), 3.51 (m, 2H, CH_2 THIQ), 3.46 (m, 4H, CH_2 PEG), 3.30 (s, 6H, $2OCH_3$), 2.65 (t, ^3J=5.3 Hz, 2H, CH_2 THIQ). **^{13}C NMR ($CDCl_3$, 100 MHz): δ**= 154.8 (+), 147.5 (C_{quat}), 147.4 (C_{quat}), 127.5 (C_{quat}), 126.2 (C_{quat}), 115.0 (+), 112.7 (+), 79.7 (C_{quat}), 71.9 (-),70.78 (-), 70.6 (-), 70.5 (-), 69.6 (-), 69.0 (-), 69.0 (-), 59.0 (+), 45.5 (-), 44.8 (+), 41.9 (-), 40.6 (-), 28.4 (+), 28.4 (-). **IR (KBr) [cm-1]: v** = 2920, 2875, 1670.

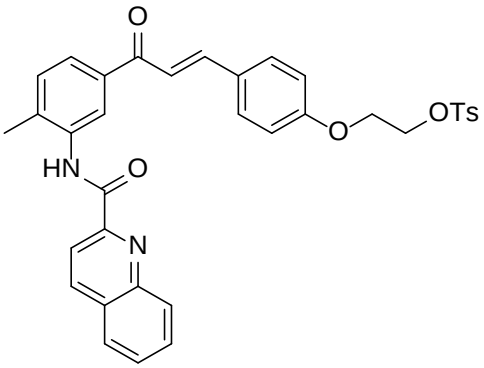
General procedure for the chalcone formation (11a-b): Chalcones were obtained by a Claisen-Schmidt condensation. The ketone **9a-b** (1equiv) and aldehyde **10** (1.3 equiv) were mixed and heated for 2 hours at 80 ºC, then the mixture was allowed to reach room temperature and at 0 ºC and 1 mL of NaOH 30% was added slowly. After 24 h at room temperature, ice was added to the mixture; the product was filtered and dried.

(*E*)-2-(4-(3-oxo-3-(3-(quinoline-2-carboxamido)phenyl)prop-1-en-1-yl)phenoxy)ethyl 4-methylbenzenesulfonate (11a)



Pale yellow solid (0.400 g, yield 72%, R_f Hex:EA 2:1 0.47). **^{1}H NMR (400 MHz, $CDCl_3$): δ** 8.40 (d, ^3J=8.3 Hz, 2H, ArH), 8.23 (t, ^3J=9.0 Hz, 2H, ArH), 7.93 (d, ^3J=8.2 Hz, 1H, ArH), 7.84 (m, 5H, ArH), 7.67 (t, ^3J=7.5 Hz, 1H, quinol), 7.60 (m, 2H, ArH), 7.55 (s, 1H, ArH), 7.47 (d, ^3J=15.6 Hz, 1H, COCH), 7.35 (d, ^3J=8.0 Hz, 2H, tosyl), 6.83 (d, ^3J=7.7 Hz, 2H, ArH), 4.44 (t, ^3J=4.0 Hz, 2H, $ArOCH_2$), 4.37 (t, ^3J=4.1 Hz, 2H, CH_2OSO_2), 2.45 (s, 3H, CH_3 Tosyl). **^{13}C NMR (100 MHz, $CDCl_3$): δ** 190.0 (C_{quat}), 162.4 (C_{quat}), 160.0 (+), 158.3 (C_{quat}) 154.7 (C_{quat}), 147.6 (C_{quat}), 145.0 (+), 143.8 (C_{quat}), 142.1 (C_{quat}), 141.6 (C_{quat}), 139.3 (C_{quat}), 138.9 (C_{quat}), 137.8 (+), 132.9 (+), 131.5 (C_{quat}), 130.5 (+), 130.3 (+), 129.9 (+), 129.7 (+), 129.5 (+), 128.3 (+), 127.9 (+), 124.4 (+), 114.9 (+), 67.8 (-), 65.5 (-), 21.6 (+). **IR (KBr) [cm-1]: v** = 1640, 1710, 730. **HRMS (EI-MS)** calcd. For $C_{34}H_{29}N_2O_6S$ $[M+H]^+$: 593.1746; found $[M+H]^+$: 593.1729.

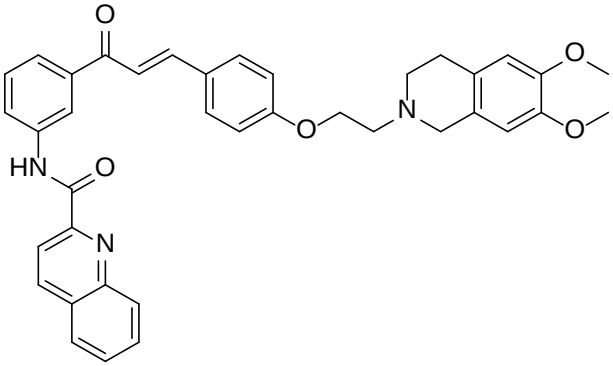
(*E*)-2-(4-(3-(4-methyl-3-(quinoline-2-carboxamido)phenyl)-3-oxoprop-1-en-1-yl)phenoxy)ethyl 4-methylbenzenesulfonate (11b)



Yellow solid. (0.600 g, yield 80%, R_f Hex:EA 2:1 0.50). **^{1}H-NMR (400 MHz, $CDCl_3$): δ** 9.00 (s, 1H, quinol), 8.42 (m, 2H, quinol), 8.18 (d, ^3J=8.4 Hz, 1H, ArH), 7.94 (d, ^3J=8.2 Hz, 1H, quinol), 7.89 (m, 2H, ArH), 7.68 (t, ^3J=7.6 Hz, 2H, quinol), 7.60 (d, ^3J=8.7 Hz, 2H, ArH), 7.50 (d, ^3J=15.6 Hz, 1H, COCH), 7.35 (d, ^3J=8.1 Hz, 2H, tosyl), 6.81 (d, ^3J=8.7 Hz, 2H, ArH), 4.39 (t, ^3J=5.5 Hz, 2H, OCH_2CH_2), 4.20 (t, ^3J=5.6 Hz, 2H, CH_2OSO_2), 2.58 (s, 3H, CH_3 tosyl), 2.45 (s, 3H, CH_3). **^{13}C-NMR (101 MHz, $CDCl_3$): δ** 190.6 (C_{quat}), 162.3 (C_{quat}), 146.3 (C_{quat}), 138.0 (C_{quat}), 136.2 (C_{quat}), 133.6 (C_{quat}), 131.9 (C_{quat}), 130.7 (C_{quat}), 130.4 (C_{quat}), 129.9 (+), 129.8 (C_{quat}), 129.6 (C_{quat}), 129.4 (+), 128.3 (+), 128.0 (+), 127.8 (+), 127.9 (+), 124.1 (+), 121.6 (+), 118.7 (+), 114.7 (+), 67.6 (-), 65.6 (-), 26.7 (+), 21.6 (+). **IR (KBr) [cm-1]: v** = 1635, 1693, 735. **HRMS (EI-MS)** calcd. For $C_{34}H_{29}N_2O_6S$ $[M+H]^+$: 607.1902; found $[M+H]^+$: 607.1906.

General procedure for the preparation of compounds 16a-f: A mixture of **11a-b** (1 mol), 1,2,3,4-tetrahydroisoquinoline **14**, **15a**-**b** (1.3 equiv) and K_2CO_3 (3 equiv) was refluxed in CH_3CN overnight. The solvent was evaporated, water was added and the compound was extracted with DCM, organic layer was separated, dried with Na_2SO_4, filtered and the solvent evaporated to give the crude product. Compounds were purified, first, with a flash column chromatography (DCM:MeOH) and second, reverse column chromatography and in some cases a preparative HPLC.

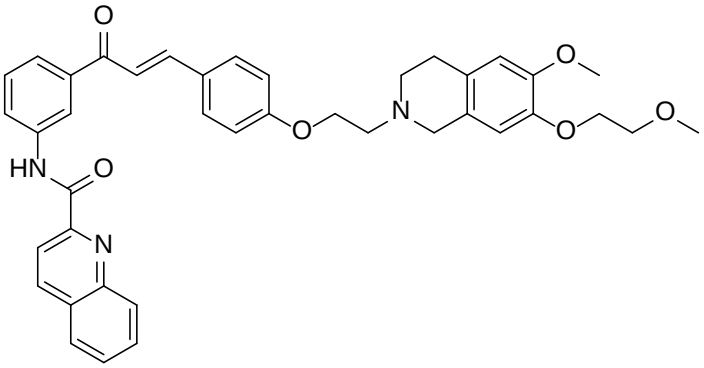
(*E*)-N-(3-(3-(4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)phenyl)quinoline-2-carboxamide (16a)



Yellow solid (0.030 g, yield 54%, R_f DCM:MeOH 20:1 0.42). **^{1}H-NMR (400 MHz, $CDCl_3$): δ** 8.38 (m, 3H, quinol), 8.24 (m, 2H, ArH), 7.93 (m, 2H, quinol), 7.80 (m, 2H, ArH), 7.69 (m, 1H, quinol), 7.52 (d, ^3J=15.5 Hz, 1H, C_qCOCH), 6.96 (d, ^3J=8.5 Hz, 2H, ArH), 6.65 (s, 1H, CH THIQ), 6.54 (s, 1H, CH THIQ), 4.53 (s, 2H, CH_2 THIQ), 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.81 (s, 2H, CH_2 THIQ), 3.65 (m, 2H, CH_2 THIQ), 3.42 (m, 2H, OCH_2CH_2), 2.65 (m, 2H, CH_2 THIQ). **^{13}C-NMR (100 MHz, $CDCl_3$): δ** 190.0 (C_{quat}), 162.5 (C_{quat}), 159.1 (C_{quat}), 149.2 (C_{quat}), 148.6 (C_{quat}), 146.3 (C_{quat}), 144.4 (+), 139.1 (C_{quat}), 138.3 (C_{quat}), 138.0 (C_{quat}), 130.5 (+), 129.6 (+), 129.0 (+), 128.3 (C_{quat}), 127.8 (+), 124.3 (+), 123.9 (C_{quat}), 122.2 (+), 120.5 (+), 119.5 (+), 118.4 (+), 118.2 (+), 115.0 (+), 109.2 (+), 63.2 (-), 56.0 (+), 53.0 (-), 49.6 (-), 23.8

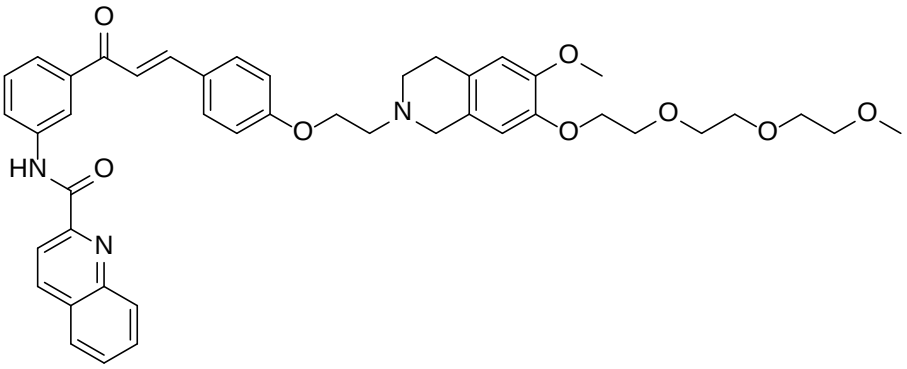
(-). **IR (KBr) [cm-1]: v** = 3137, 2810, 1642, 1689, 1660, 1140, 693. **HRMS (EI-MS)** calcd. For $C_{38}H_{36}N_3O_5$ $[M+H]^+$:614.2655; found $[M+H]^+$: 614.2657.

(*E*)-N-(3-(3-(4-(2-(6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)phenyl)quinoline-2-carboxamide (16b)



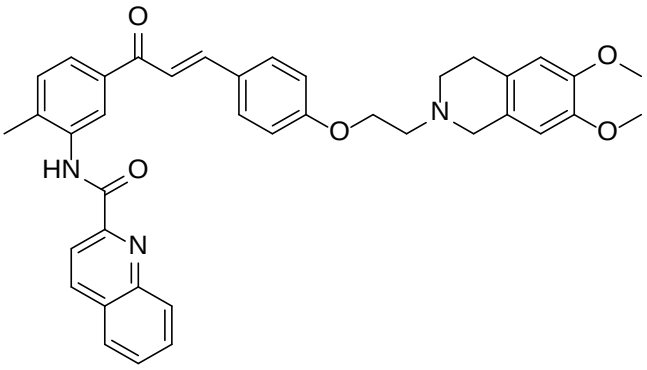
Pale yellow solid. (0.032 g, yield 63%, R_f DCM:MeOH 20:1 0.39). **^{1}H NMR (400 MHz, $CDCl_3$): δ** 8.43 (m, 2H, quinol), 8.22 (d, ^3J=8.4 Hz, 2H, ArH), 7.92 (d, ^3J=8.5 Hz, 1H, quinol), 7.87 (m, 2H, ArH), 7.63 (s, 2H, quinol), 7.55 (d, ^3J=15.7 Hz, 1H, COCH), 7.46 (t, ^3J=7.5 Hz, 1H, ArH), 6.96 (s, 1H, CH THIQ), 6.58 (s, 1H, CH THIQ), 4.28 (m, 2H, CH_2 PEG), 4.17 (m, 2H, CH_2 THIQ), 4.02 (m, 2H, OCH_2CH_2), 3.80 (s, 3H, OCH_3), 3.73 (m, 2H, CH_2CH_2O), 3.60 (m, 2H, CH_2 THIQ), 3.44 (s, 3H, CH_2OCH_3), 2.86 (m, 2H, CH_2 PEG), 2.44 (d, ^3J=3.8 Hz, 2H, CH_2 THIQ).**^{13}C NMR (100 MHz, $CDCl_3$): δ** 190.1 (C_{quat}), 162.5 (C_{quat}), 159.3 (C_{quat}), 154.5 (C_{quat}), 149.3 (C_{quat}), 146.3 (C_{quat}), 144.9 (+), 140.0 (C_{quat}), 139.3 (C_{quat}), 138.3 (C_{quat}), 138.0 (+), 132.0 (+), 130.4 (+), 130.2 (C_{quat}), 128.3 (+), 128.0 (+), 124.2 (C_{quat}), 119.8 (+), 119.4 (+), 118.7 (+), 115.0 (+), 71.0 (-), 68.6 (-), 65.7 (-), 59.1 (+), 58.9 (+), 55.9 (-), 51.3 (-), 29.7 (+). **IR (KBr) [cm-1]: v** = 3185, 2836, 1617, 1676, 1660, 1260, 710. **HRMS (EI-MS)** calcd. For $C_{40}H_{40}N_3O_6$ $[M+H]^+$: 658.2917; found $[M+H]^+$: 658.2920.

(*E*)-N-(3-(3-(4-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)phenyl)quinoline-2-carboxamide (16c)



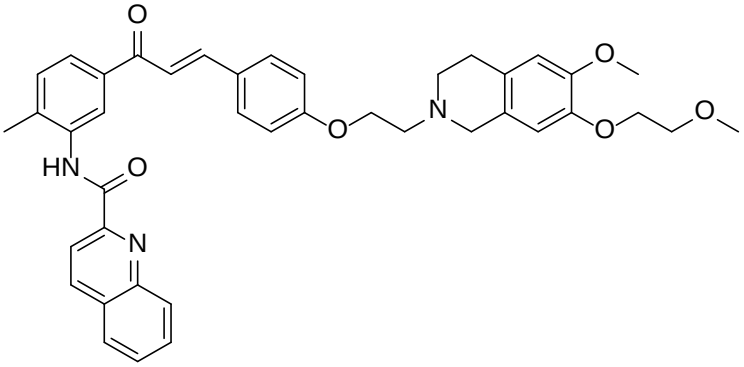
Pale yellow solid. (0.025 g, yield 58%, R_f DCM:MeOH 20:1 0.35). **^{1}H NMR (400 MHz, $CDCl_3$): δ** 8.43 (m, 3H, quinol), 8.27 (m, 1H, ArH), 7.94 (d, ^3J=8.2 Hz, 1H, quinol), 7.84 (dd, ^3J=8.2, ^3J=6.9, 3H, ArH), 7.68 (t, ^3J=7.2 Hz, 2H, quinol), 7.50 (d, ^3J=15.6 Hz, 1H, COCH), 6.98 (s,1H, CH THIQ), 6.66 (s,1H, CH THIQ), 4.52 (s, 2H, CH_2 THIQ), 4.17 (m, 2H, CH_2 THIQ), 3.94 (m, 2H, CH_2 THIQ), 3.87 (s, 3H, OCH_3), 3.73 (dd, ^3J=5.8, ^3J=3.1 Hz, 4H, 2CH_2 PEG), 3.70 (m, 4H, 2CH_2 PEG), 3.55 (dd, ^3J=5.7, ^3J=3.5 Hz, 4H, 2CH_2 PEG), 3.36 (m, 2H, CH_2OCH_3), 3.14 (m, 2H, CH_2CH_2N). **^{13}C NMR (100 MHz, $CDCl_3$): δ** 190.1 (C_{quat}), 162.4 (C_{quat}), 161.0 (C_{quat}), 149.3 (C_{quat}), 148.3 (C_{quat}), 146.8 (C_{quat}), 145.0 (+), 139.3 (C_{quat}), 138.3 (C_{quat}), 138.0 (C_{quat}), 132.0 (+), 130.4 (+), 129.6 (+), 128.3 (C_{quat}), 127.8 (+), 126.8 (+), 124.2 (C_{quat}), 119.4 (+), 118.7 (+), 115.0 (+), 112.5 (+), 111.9 (+), 71.9 (-), 70.9 (-), 70.3 (-), 69.6 (+), 68.7 (-), 59.0 (+), 56.6 (-), 56.0 (-), 28.5 (-). **IR (KBr) [cm-1]: v** = 3156, 2891, 1672, 1661, 1646, 1040, 682. **HRMS (EI-MS)** calcd. For $C_{44}H_{48}N_3O_8$ $[M+H]^+$: 746.3441; found $[M+H]^+$: 746.3444.

(*E*)-N-(5-(3-(4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)-2-methylphenyl)quinoline-2-carboxamide (16d)



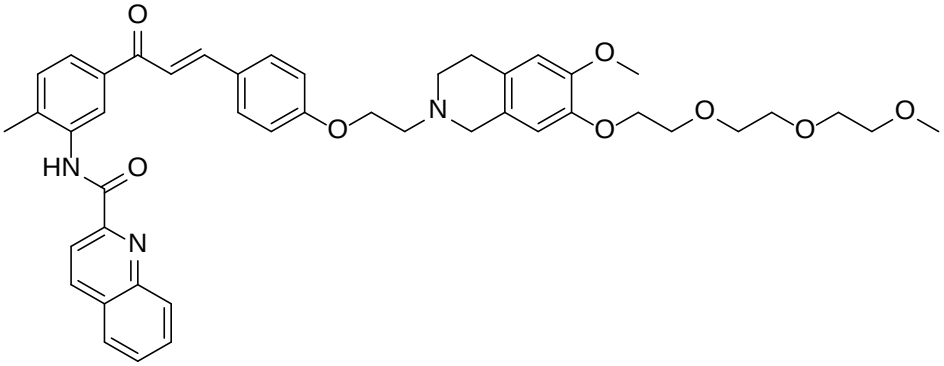
Brown yellow solid. (0.042 g, yield 68%, R_f DCM:MeOH 20:1 0.47). **^{1}H NMR (400 MHz, $CDCl_3$): δ** 8.99 (s, 2H, quinol), 8.41 (s, 2H, ArH), 8.18 (d, ^3J=8.4 Hz, 1H, ArH), 7.93 (d, ^3J=13.6 Hz, 1H, quinol), 7.83 (m, 1H, ArH), 7.65 (d, ^3J=8.7 Hz, 3H, quinol), 7.50 (d, ^3J=15.6 Hz, 1H, COCH), 7.38 (d, ^3J=8.0 Hz, 1H, ArH), 6.96 (d, ^3J=8.7 Hz, 2H, ArH), 6.61 (s, 1H, CH THIQ), 6.53 (s, 1H, CH THIQ), 4.31 (s, 2H, CH_2 THIQ), 3.85 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.66 (m, 2H, CH_2 THIQ), 3.16 (m, 2H, CH_2 THIQ), 2.58 (s, 3H, CH_3). **^{13}C NMR (100 MHz, $CDCl_3$): δ** 189.9 (C_{quat}), 162.3 (C_{quat}), 159.8 (C_{quat}), 154.6 (C_{quat}), 149.6 (C_{quat}), 146.3 (C_{quat}), 144.4 (C_{quat}), 142.4 (C_{quat}), 138.0 (C_{quat}), 137.4 (C_{quat}), 136.1 (+), 133.3 (+), 130.9 (+), 130.4 (+), 129.8 (+), 129.5 (+), 128.3 (C_{quat}), 127.8 (C_{quat}), 126.4 (C_{quat}) 124.7 (+), 121.4 (+), 118.7 (+), 115.0 (+), 111.3 (+), 109.4 (+), 68.0 (-), 58.1 (+), 55.9 (-), 51.0 (-), 27.2 (-), 18.0 (+). **IR (KBr) [cm-1]: v** = 3185, 2824, 1583, 1653, 1660, 1047, 698. **HRMS (EI-MS)** calcd. For $C_{39}H_{38}N_3O_5$ $[M+H]^+$: 628.2811; found $[M+H]^+$: 628.2814.

(*E*)-N-(5-(3-(4-(2-(6,7-bis(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)-2-methylphenyl)quinoline-2-carboxamide (16e)



Yellow solid. (0.036 g, yield 57%, R_f DCM:MeOH 20:1 0.45). **^{1}H NMR (400 MHz, $CDCl_3$): δ** 8.43 (m, 2H, ArH quinol), 8.21 (m, 2H, ArH), 7.93 (d, ^3J=8.1 Hz, 2H, ArH quinol), 7.81 (t, ^3J=7.2 Hz, 4H), 7.71 (m, 2H, ArH quinol), 7.57 (m, 1H, ArH), 7.36 (d, ^3J=8.3 Hz, 1H, COCH), 6.57 (s, 1H, CH THIQ), 6.56 (s, 1H, CH THIQ), 4.13 (m, 4H, OCH_2CH_2), 3.80 (s, 3H, OCH_3), 3.76 (t, ^3J=7.0 Hz, 2H, CH_2 PEG), 3.74 (d, ^3J=4.9 Hz, 2H, CH_2 PEG), 3.61 (m, 2H, CH_2 THIQ), 3.52 (m, 2H, CH_2 THIQ), 3.41 (m, 2H, CH_2 THIQ), 3.38 (s, 3H, CH_2OCH_3), 2.57 (s, 3H, CH_3). **^{13}C NMR (101 MHz, $CDCl_3$): δ** 191.5 (C_{quat}), 163.5 (C_{quat}), 149.9 (C_{quat}), 148.3 (C_{quat}), 146.8 (C_{quat}), 144.6 (+), 143.5 (+), 141,5 (C_{quat}), 138.0 (C_{quat}), 130.9 (+) 130.4 (+), 129.7 (+), 128.3 (C_{quat}), 127.9 (+), 126.9 (C_{quat}), 126.3 (C_{quat}), 125.3 (+), 115.0 (+), 112.4 (+), 111.8 (+), 71.0 (-), 70.6 (-), 68.6 (-), 59.1 (+), 58.9 (-), 57.4 (+), 55.9 (-), 51.3 (-), 29.7 (-), 28.4 (+). **IR (KBr) [cm-1]: ν** = 3158, 2823, 1631, 1596, 1632, 976, 683. **HRMS (EI-MS)** calcd. For $C_{41}H_{42}N_3O_6$ $[M+H]^+$: 672.3073; found $[M+H]^+$: 672.3072.

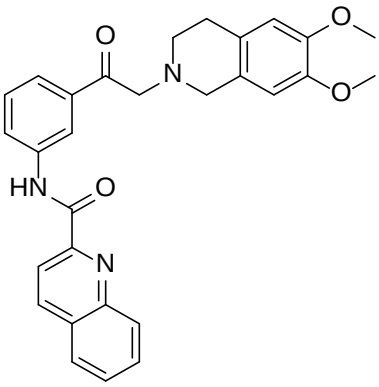
(*E*)-N-(5-(3-(4-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)-2-methylphenyl)quinoline-2-carboxamide (16f)



Yellow solid. (0.023 g, yield 61%, R_f DCM:MeOH 20:1 0.44). **^{1}H NMR (400 MHz, $CDCl_3$): δ** 8.41 (m, 2H, ArH quinol), 8.24 (m, 1H, ArH), 8.05 (s, 1H, ArH), 7.93 (d, ^3J=8.2 Hz, 2H, ArH quinol), 7.82 (t, ^3J=9.8 Hz, 1H, ArH), 7.66 (m, 2H, ArH quinol), 7.51 (d, ^3J=15.6 Hz, 1H, COCH), 7.35 (d, ^3J=8.1 Hz, 2H, ArH), 6.92 (d, ^3J=6.0 Hz, 1H, ArH), 6.57 (s, 1H, CH THIQ), 6.55 (s, 1H, CH THIQ), 4.19 (m, 4H, $2CH_2$ PEG), 3.90

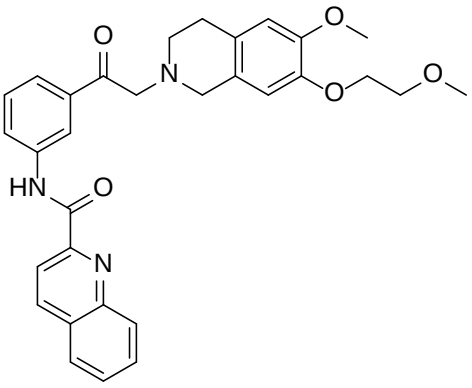
(m, 2H, CH_2 THIQ), 3.87 (m, 2H, CH_2 PEG), 3.81 (s, 3H, OCH_3), 3.68 (m, 2H, CH_2 PEG), 3.55 (d, ^3J=5.0 Hz, 2H, CH_2 PEG), 3.37 (m, 4H, $2CH_2$ PEG), 3.09 (t, ^3J=5.9 Hz, 2H, CH_2 THIQ), 2.69 (t, ^3J=5.8 Hz, 2H, CH_2 THIQ). **^{13}C NMR (100 MHz, $CDCl_3$): δ** 170.7 (C_{quat}), 162.3 (C_{quat}), 154.6 (C_{quat}), 153.6 (C_{quat}), 148.0 (C_{quat}), 146.4 (C_{quat}), 139.0 (C_{quat}), 138.0 (C_{quat}), 128.2 (C_{quat}), 127.9 (C_{quat}), 127.3 (C_{quat}), 118.7 (+), 115.0 (+), 112.6 (+), 111.9 (+), 71.9 (-), 70.7 (-), 70.6 (-), 70.5 (-), 69.6 (+), 68.7 (-), 59.0 (+), 56.0 (-), 43.9 (-), 28.7 (-), 18.0 (+). **IR (KBr) [cm-1]: v** = 3217, 2913, 1702, 1692, 1589, 984, 714. **HRMS (EI-MS)** calcd. For $C_{45}H_{50}N_3O_8$ $[M+H]^+$: 760.3597; found $[M+H]^+$: 760.3606.

N-(3-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)phenyl)quinoline-2-carboxamide (17a)



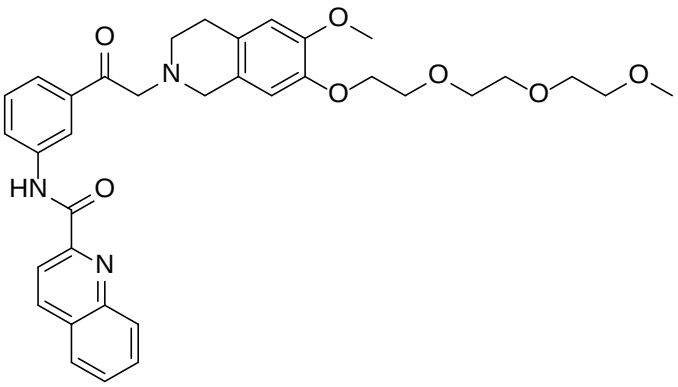
Light yellow solid. (0.025 g, yield 75%, R_f PE:EA 2 : 1 0.36). **^{1}H-NMR (400 MHz, $CDCl_3$): δ** 8.38 (m, 1H, quinol), 8.30 (dd, ^3J=7.6, ^3J=8.4 Hz, 2H, quinol), 8.12 (dd, ^3J=8.3, ^3J=7.9 Hz, 2H, ArH), 7.87 (d, ^3J=8.1 Hz, 1H, quinol), 7.78 (t, ^3J=7.4 Hz, 1H, quinol), 7.69 (m, 2H, ArH), 7.47 (t, ^3J=6.2 Hz, 1H, quinol), 6.62 (s, 1H, CH THIQ), 6.52 (s, 1H, CH THIQ), 4.90 (s, 2H, $COCH_2$), 4.56 (m, 2H, CH_2 THIQ), 3.83 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.13 (m, 4H, $2CH_2$ THIQ). **^{13}C-NMR (100 MHz, $CDCl_3$): δ** 194.5 (C_{quat}), 169.7 (C_{quat}), 149.2 (C_{quat}), 148.8 (C_{quat}), 148.5 (C_{quat}), 146.6 (C_{quat}), 138.6 (C_{quat}), 138.0 (C_{quat}), 136.2 (C_{quat}), 134.4 (C_{quat}), 130.5 (+), 129.9 (+), 129.7 (+), 128.4 (+), 127.8 (+), 125.8 (+), 123.7 (+), 122.3 (+), 118.5 (+), 111.2 (+), 55.9 (-), 52.8 (+), 50.0 (-), 45.7 (-), 24.1 (-). **IR (KBr) [cm^{-1}]: v =** 2880, 1689, 1635, 1425, 1150, 753, 726. **HRMS (EI-MS)** calcd. For $C_{29}H_{28}N_3O_4$ $[M+H]^+$: 482.2080; found $[M+H]^+$: 482.2048

N-(3-(2-(6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)phenyl)quinoline-2-carboxamide (17b)



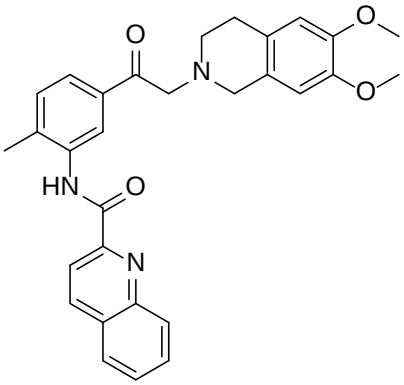
Light yellow solid. (0.030 g, yield 90%, R_f PE:EA 2:1 0.31). **^{1}H-NMR (400 MHz, $CDCl_3$): δ** 8.38 (m, 2H, quinol), 8.33 (s, 2H, quinol), 8.21 (d, ^3J=8.5 Hz, 2H, ArH), 7.93 (d, ^3J=8.3 Hz, 2H, quinol), 7.83 (dd, ^3J=8.4, ^3J=7.0 Hz, 2H, ArH), 7.67 (t, ^3J=7.9 Hz, 2H, quinol), 6.80 (s, 1H, CH THIQ), 6.62 (s, 1H, CH THIQ), 5.28 (s, 2H, $COCH_2$), 4.19 (s, 2H, CH_2 THIQ), 3.87 (d, ^3J=5.4 Hz, 2H, CH_2 PEG), 3.83 (s, 3H, OCH_3), 3.80 (d, ^3J=4.7 Hz, 2H, CH_2 PEG), 3.47 (s, 3H, CH_2OCH_3), 3.14 (m, 2H, CH_2 THIQ), 2.86 (t, ^3J=5.7 Hz, 2H, CH_2 THIQ). **^{13}C-NMR (100 MHz, $CDCl_3$): δ** 187.0 (C_{quat}), 163.4 (C_{quat}), 146.3 (C_{quat}), 138.6 (C_{quat}), 138.0 (C_{quat}), 134.3 (C_{quat}), 130.5 (C_{quat}), 129.8 (C_{quat}), 129.7 (C_{quat}), 129.5 (+), 128.4 (+), 127.8 (+), 125.8 (+), 125.6 (+), 125.6 (+), 122.8 (+), 120.6 (+), 118.7 (+), 111.8 (+), 111.6 (+), 71.0 (-), 68.7 (-), 59.6 (-), 55.8 (+), 49.5 (+), 49.3 (-), 45.8 (-), 29.7 (-). **IR (KBr) [cm^{-1}]: ν =** 2903, 1673, 1645, 1402, 1189, 764, 731. **HRMS (EI-MS)** calcd. For $C_{31}H_{32}N_3O_5$ $[M+H]^+$: 526.2342; found $[M+H]^+$: 526.2315

N-(3-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)phenyl)quinoline-2-carboxamide (17c)



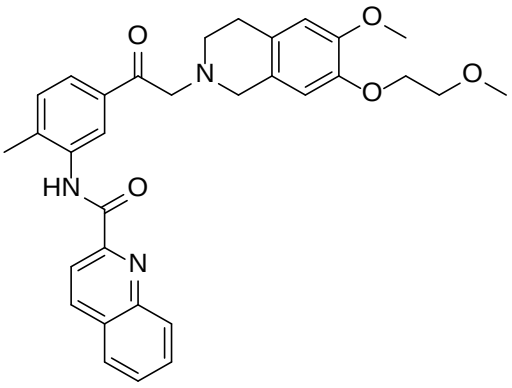
Light yellow solid. (0.026 g, yield 85%, R_f PE:EA 2:1 0.20). **^{1}H-NMR (400 MHz, $CDCl_3$): δ** 8.38 (m, 2H, quinol), 8.33 (s, 2H, quinol), 8.20 (d, ^3J=8.5 Hz, 1H, ArH), 7.91 (s, 1H, ArH quinol), 7.84 (m, 1H, quinol), 7.67 (t, ^3J=7.2 Hz, 1H, ArH), 7.53 (m, 2H, ArH), 6.81 (s, 1H, CH THIQ), 6.62 (s, 1H, CH THIQ), 5.27 (s, 2H, $COCH_2$), 4.21 (s, 2H, CH_2 THIQ), 3.91 (dd, ^3J=5.7, 4.3 Hz, 2H, CH_2 THIQ), 3.82 (s, 3H, OCH_3), 3.81 (s, 4H, CH_2 PEG), 3.78 (m, 4H, CH_2 PEG), 3.59 (m, 4H, CH_2 PEG), 3.39 (s, 3H, CH_2OCH_3), 3.32 (d, ^3J=3.6 Hz, 2H, CH_2 THIQ). **^{13}C-NMR (100 MHz, $CDCl_3$): δ** 195.0 (C_{quat}), 162.3 (C_{quat}), 149.0 (C_{quat}), 148.6 (C_{quat}), 146.3 (C_{quat}), 138.3 (C_{quat}), 137.8 (C_{quat}), 134.1 (C_{quat}), 130.5 (C_{quat}), 129.8 (C_{quat}), 129.7 (C_{quat}), 129.6 (+), 128.3 (+), 127.6 (+), 125.8 (+), 125.6 (+), 122.7 (+), 120.6 (+), 118.6 (+), 111.7 (+), 111.6 (+), 71.9 (-), 70.6 (-), 70.5 (-), 69.4 (-), 68.7 (-), 68.5 (-), 58.8 (+), 55.8 (+), 49.6 (-), 49.2 (-), 28.6 (-). **IR (KBr) [cm^{-1}]: v =** 2892, 1705, 1687, 1412, 1126, 749, 731. **HRMS (EI-MS)** calcd. For $C_{35}H_{40}N_3O_7$ $[M+H]^+$: 614.2866; found $[M+H]^+$: 614.2851

N-(5-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)-2-methylphenyl)quinoline-2-carboxamide (17d)



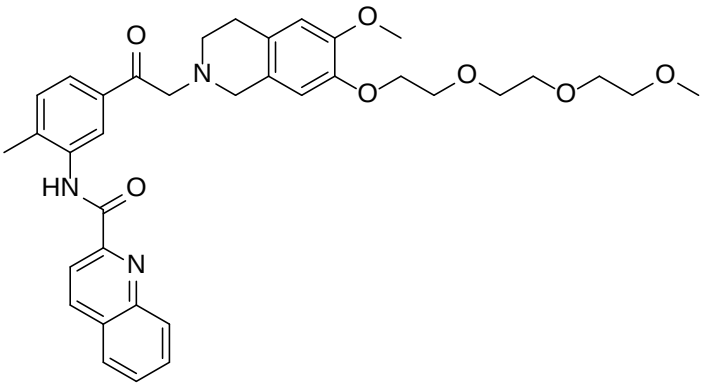
Light yellow solid. (0.024 g, yield 82%, R_f PE:EA 2:1 0.40). **^{1}H-NMR (400 MHz, $CDCl_3$): δ** 8.61 (m, 2H, quinol), 8.32 (t, ^3J=7.5 Hz, 2H, quinol), 8.16 (m, 1H, ArH), 7.87 (s, 1H, ArH), 7.75 (t, ^3J=8.4 Hz, 1H, ArH), 7.61 (t, ^3J=7.0 Hz, 1H, quinol), 7.46 (d, ^3J=8.3 Hz, 1H, quinol), 6.51 (s, 1H, CH THIQ), 6.44 (s, 1H, CH THIQ), 5.23 (s, 2H, $COCH_2$), 3.91 (s, 2H, CH_2 THIQ), 3.78 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.09 (t, ^3J=5.5 Hz, 2H, CH_2 THIQ), 2.68 (t, ^3J=5.7 Hz, 2H, CH_2 THIQ). **^{13}C-NMR (100 MHz, $CDCl_3$): δ** 200.2 (C_{quat}), 162.2 (C_{quat}), 149.2 (C_{quat}), 146.2 (C_{quat}), 138.9 (C_{quat}), 138.1 (C_{quat}), 135.6 (C_{quat}), 132.2 (C_{quat}), 130.5 (C_{quat}), 129.7 (C_{quat}), 128.4 (+), 127.8 (+), 121.4 (+), 119.6 (+), 118.6 (+), 113.6 (+), 111.9 (+), 110.4 (+), 109.1 (+), 56.0 (+), 55.2 (-), 53.4 (-), 47.2 (-), 29.7 (-), 17.3 (+). **IR (KBr) [cm^{-1}]: v =** 2873, 1712, 1696, 1402, 1127, 762, 734. **HRMS (EI-MS)** calcd. For $C_{30}H_{30}N_3O_4$ $[M+H]^+$: 496.2236; found $[M+H]^+$: 496.2224

N-(3-(2-(6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)phenyl)quinoline-2-carboxamide (17e)



Light yellow solid. (0.032 g, yield 88%, R_f PE:EA 2:1 0.33). **^{1}H-NMR (400 MHz, $CDCl_3$): δ** 8.95 (d, ^3J=7.7 Hz, 1H, quinol), 8.40 (s, 1H, quinol), 8.16 (d, ^3J=8.4 Hz, 1H, ArH), 7.93 (d, ^3J=8.2 Hz, 1H, quinol), 7.84 (m, 1H, quinol), 7.73 (d, ^3J=9.7 Hz, 2H, ArH), 7.67 (t, ^3J=7.5 Hz, 1H, quinol), 7.35 (d, ^3J=8.0 Hz, 1H, quinol), 6.57 (s, 1H, CH THIQ), 6.55 (s, 1H, CH THIQ), 4.94 (s, 2H, $COCH_2$) 4.12 (s, 2H, CH_2 THIQ), 3.88 (s, 3H, OCH_3), 3.60 (t, ^3J=5.6 Hz, 2H, CH_2 THIQ), 3.37 (s, 3H, CH_2OCH_3), 2.76 (t, ^3J=5.6 Hz, 2H, CH_2 THIQ), 2.55 (s, 3H, CH_3). **^{13}C-NMR (100 MHz, $CDCl_3$): δ** 200.2 (C_{quat}), 162.19 (C_{quat}), 149.2 (C_{quat}), 146.2 (C_{quat}), 138.9 (C_{quat}), 138.1 (C_{quat}), 135.6 (C_{quat}), 135.5 (C_{quat}), 132.2 (C_{quat}), 130.5 (C_{quat}), 129.8 (C_{quat}), 129.6 (C_{quat}), 128.4 (+), 127.8 (+), 127.6 (+), 127.0 (+), 121.4 (+), 118.5 (+), 113.6 (+), 76.7 (-), 70.9 (-), 68.6 (-), 59.1 (+), 56.0 (+), 55.6 (-), 51.2 (-), 30.9 (-), 17.3 (+). **IR (KBr) [cm^{-1}]: v =** 2905, 1652, 1624, 1423, 1151, 739, 725. **HRMS (EI-MS)** calcd. For $C_{32}H_{34}N_3O_5$ $[M+H]^+$: 540.2498; found $[M+H]^+$: 540.2487

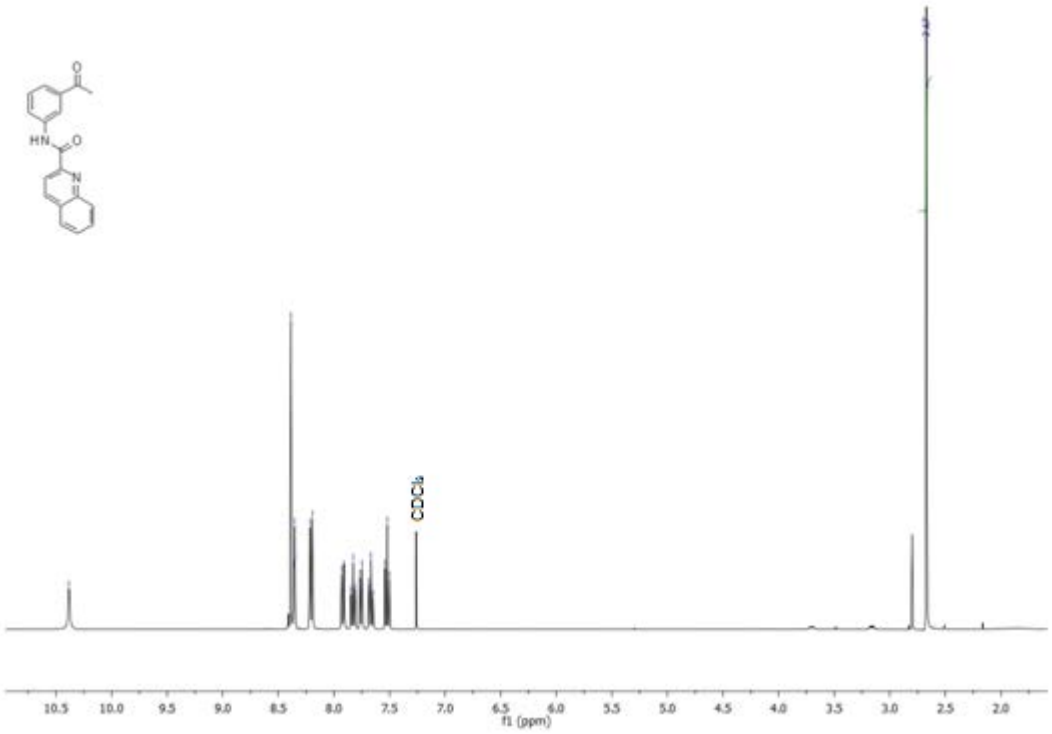
N-(5-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)-2-methylphenyl)quinoline-2-carboxamide (17f)

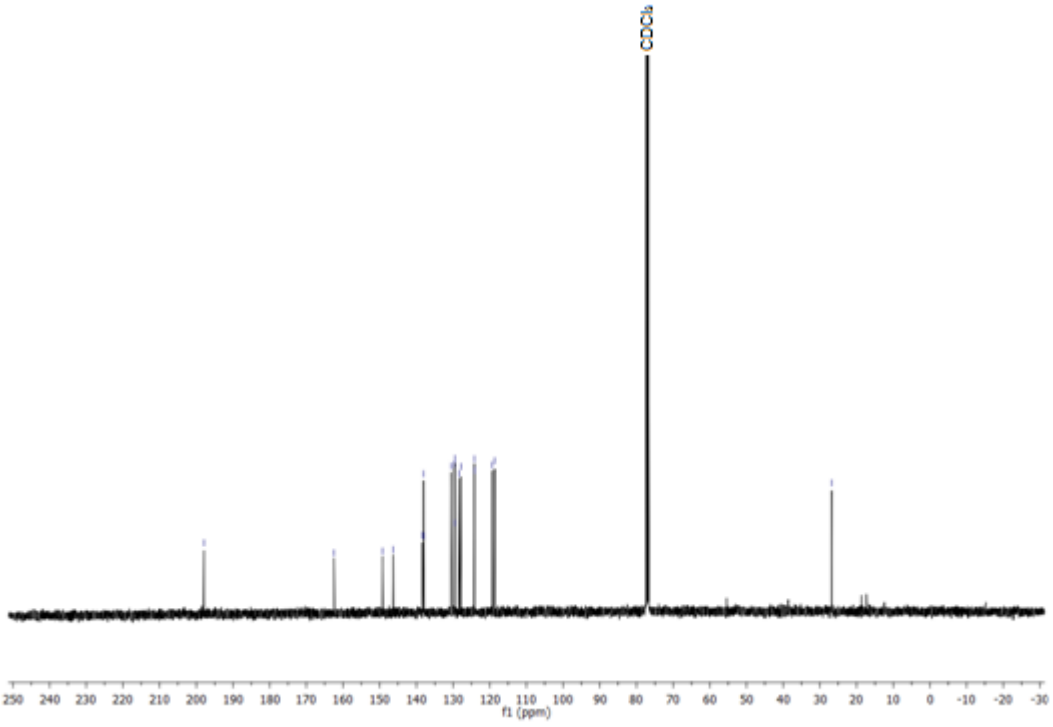


Light yellow solid. (0.031 g, yield 82%, R_f PE:EA 2:1 0.28). **^{1}H-NMR (400 MHz, $CDCl_3$): δ** 8.67 (m, 1H, quinol), 8.38 (d, ^3J=3.9 Hz, 1H, quinol), 8.15 (d, ^3J=8.5 Hz, 2H, ArH), 7.93 (d, ^3J=8.1 Hz, 1H, quinol), 7.74 (m, 1H, ArH), 7.84 (m, 1H, quinol), 7.69 (m, 1H, quinol), 7.52 (s, 1H, quinol), 6.87 (s, 1H, CH THIQ), 6.65 (s, 1H, CH THIQ), 5.29 (s, 2H, $COCH_2$), 4.20 (s, 2H, CH_2 THIQ), 4.14 (m, 16H, $4CH_2$ PEG), 3.87 (s, 3H, OCH_3), 3.72 (t, ^3J=5.2 Hz, 4H, $2CH_2$ PEG), 3.65 (d, ^3J=4.8 Hz, 4H, $2CH_2$ PEG), 3.55 (s, 3H, CH_2OCH_3), 2.50 (s, 3H, CH_3). **^{13}C-NMR (100 MHz, $CDCl_3$): δ** 199.7 (C_{quat}), 161.6 (C_{quat}), 148.7 (C_{quat}), 147.6 (C_{quat}), 146.1 (C_{quat}), 145.7 (C_{quat}), 138.4 (C_{quat}), 137.6 (C_{quat}), 136.4 (C_{quat}), 135.1 (C_{quat}), 131.7 (C_{quat}), 130.0 (C_{quat}), 129.2 (+), 127.9 (+), 127.3 (+), 120.9 (+), 118.1 (+), 113.1 (+), 112.8 (+), 112.0 (+), 111.4 (+), 110.2 (+), 71.4 (-), 70.3 (-), 70.1 (-), 70.0 (-), 69.1 (-), 68.4 (-), 68.2 (-), 58.5 (+), 55.5 (+), 46.7 (-), 29.2 (-), 16.8 (+). **IR (KBr) [cm^{-1}]: v =** 2873, 1675, 1649, 1392, 1129, 741, 716. **HRMS (EI-MS)** calcd. For $C_{36}H_{42}N_3O_7$ $[M+H]^+$: 628.3022; found $[M+H]^+$: 628.2994.

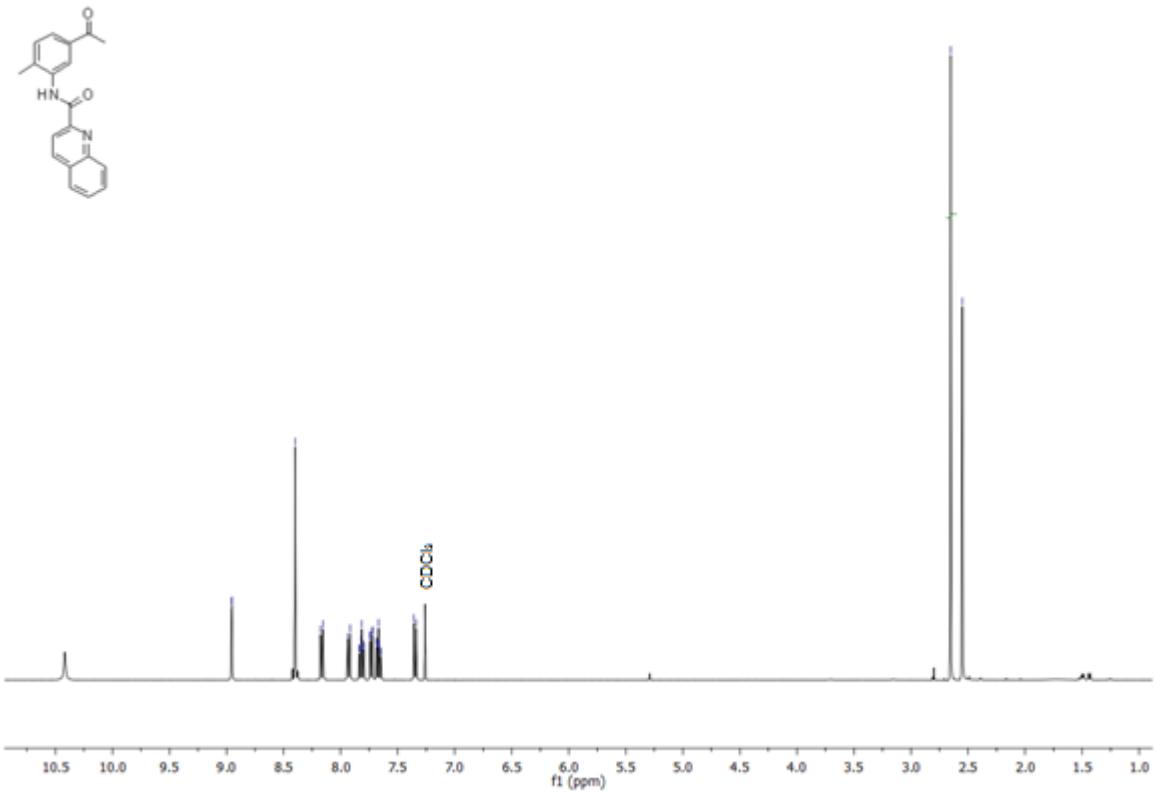
1.4 NMR spectra

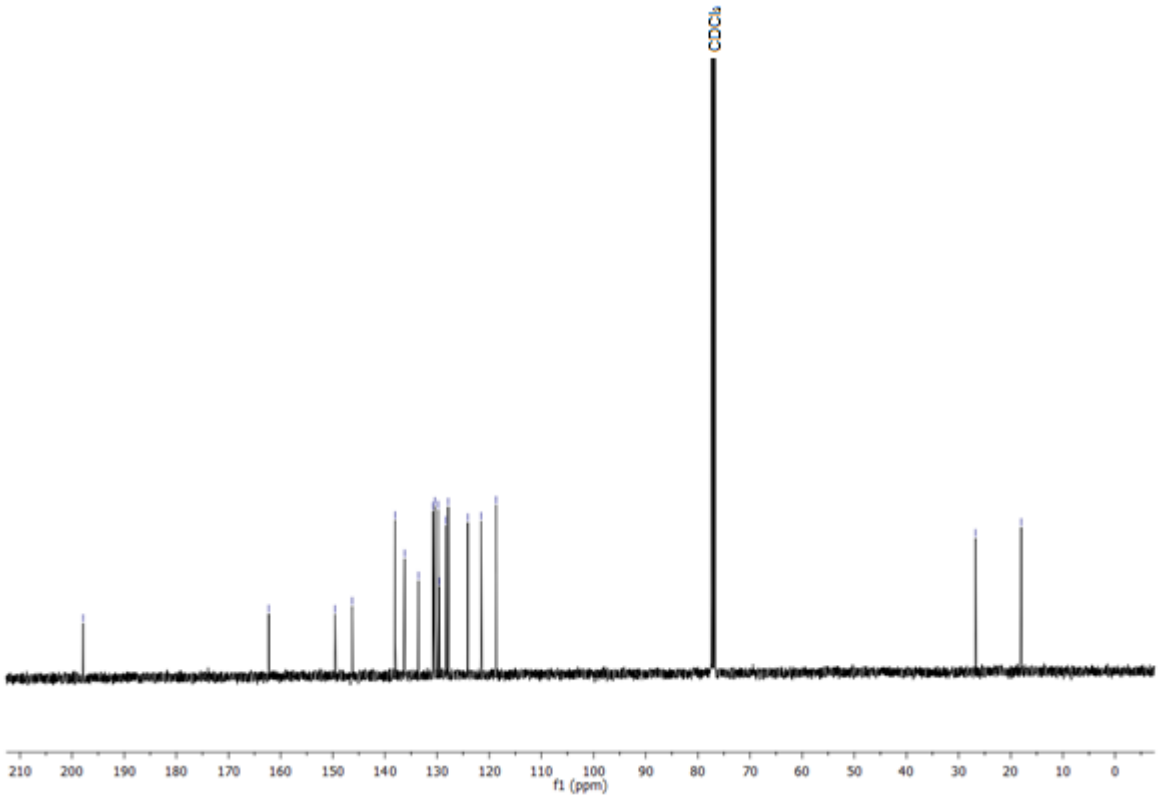
N-(3-acetylphenyl)quinoline-2-carboxamide (9a)



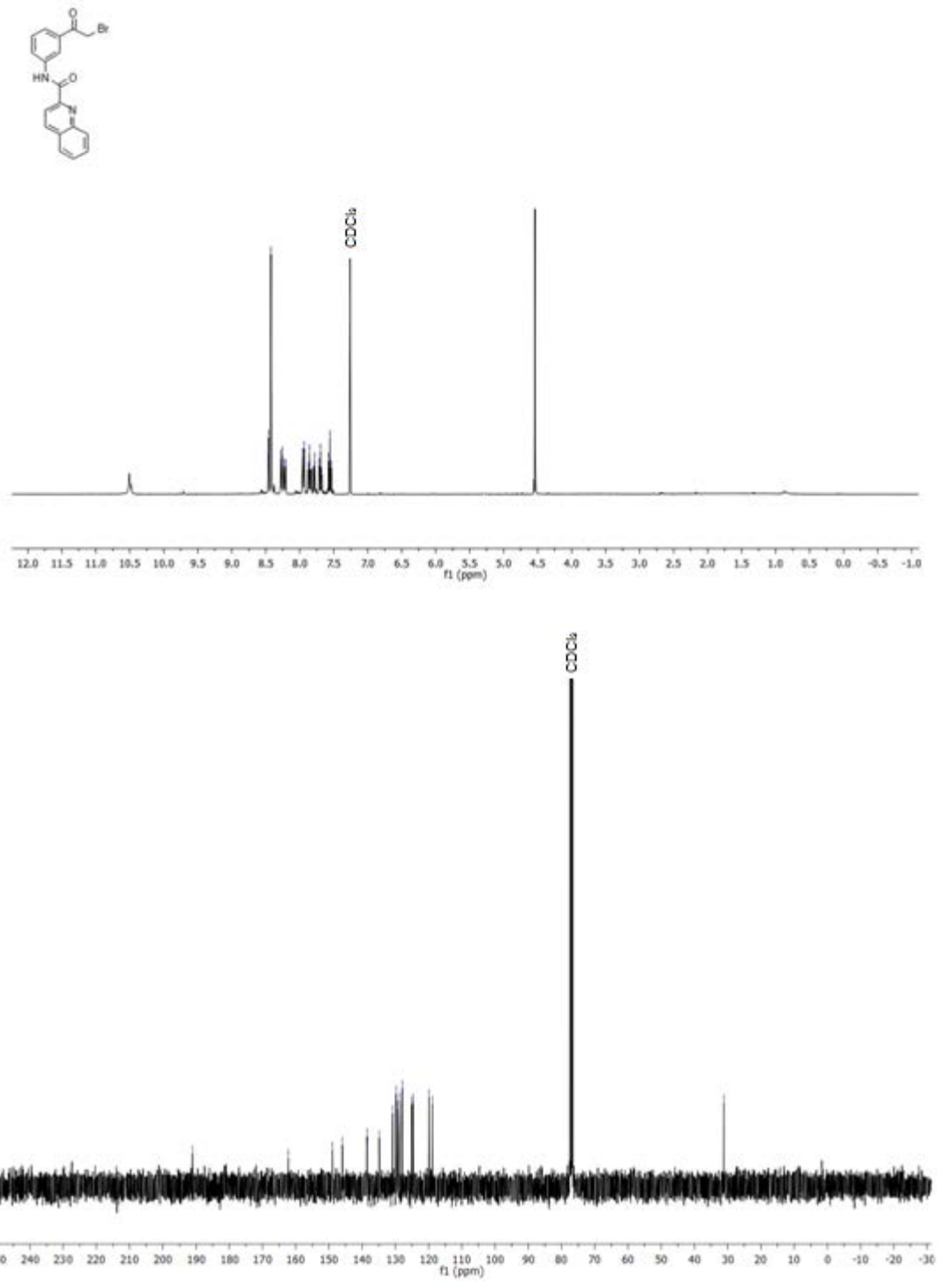


N-(5-acetyl-2-methylphenyl)quinoline-2-carboxamide (9b)

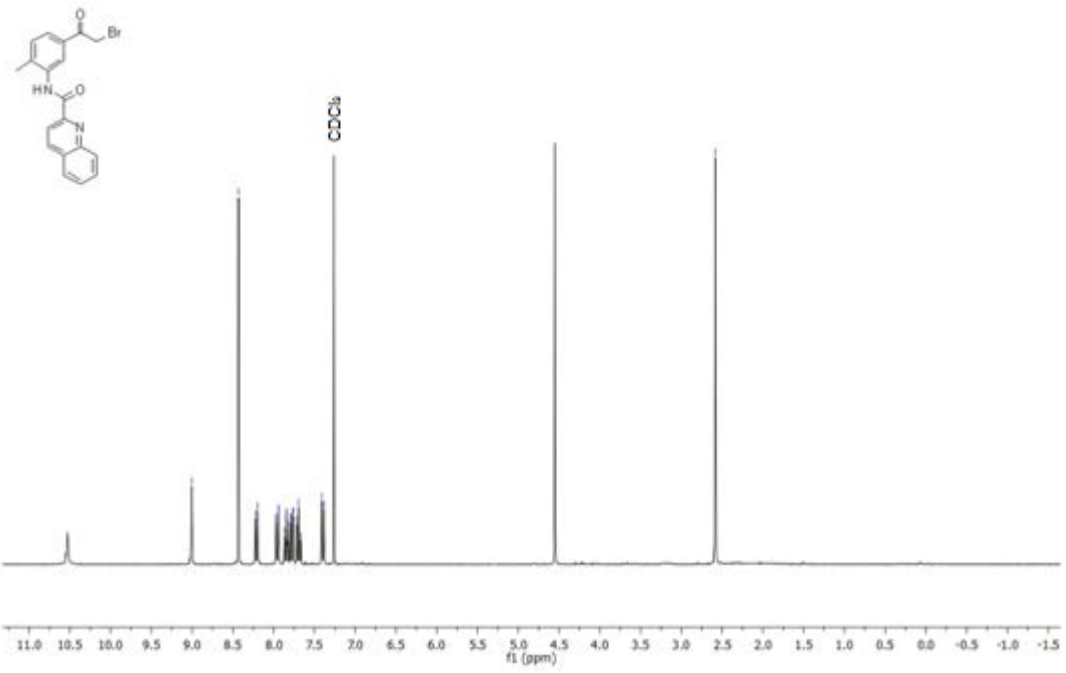


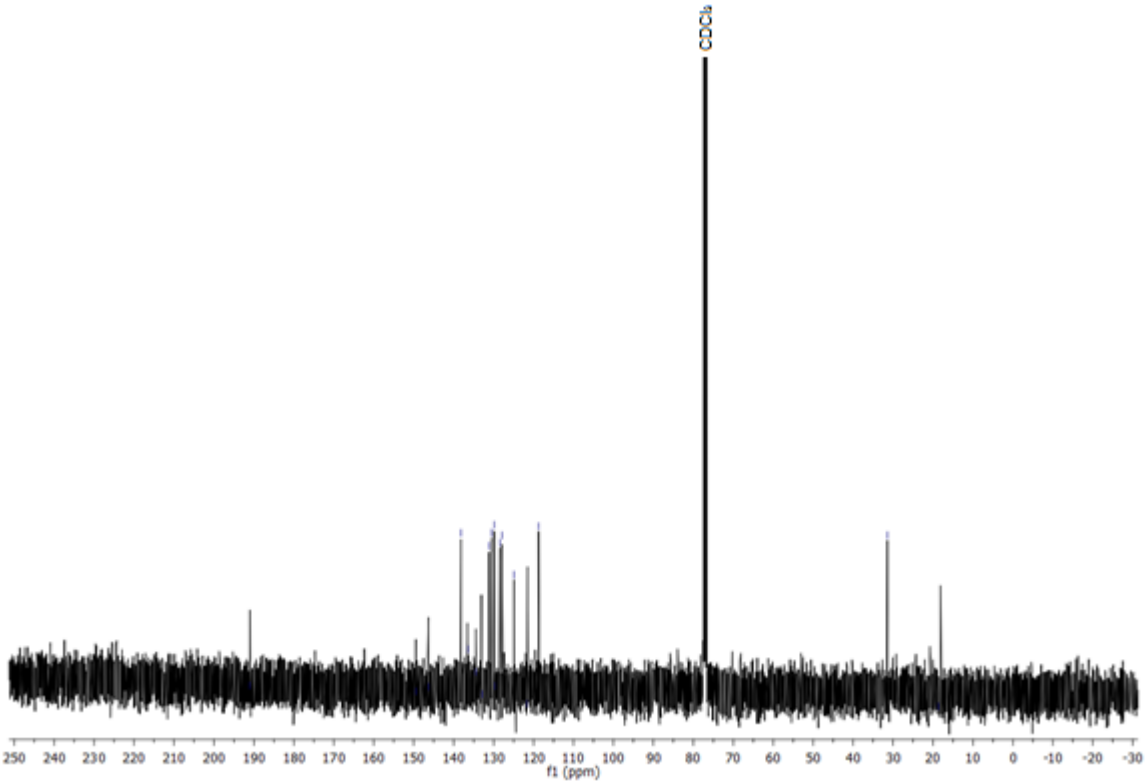


N-(3-(2-bromoacetyl)phenyl)quinoline-2-carboxamide (12a)

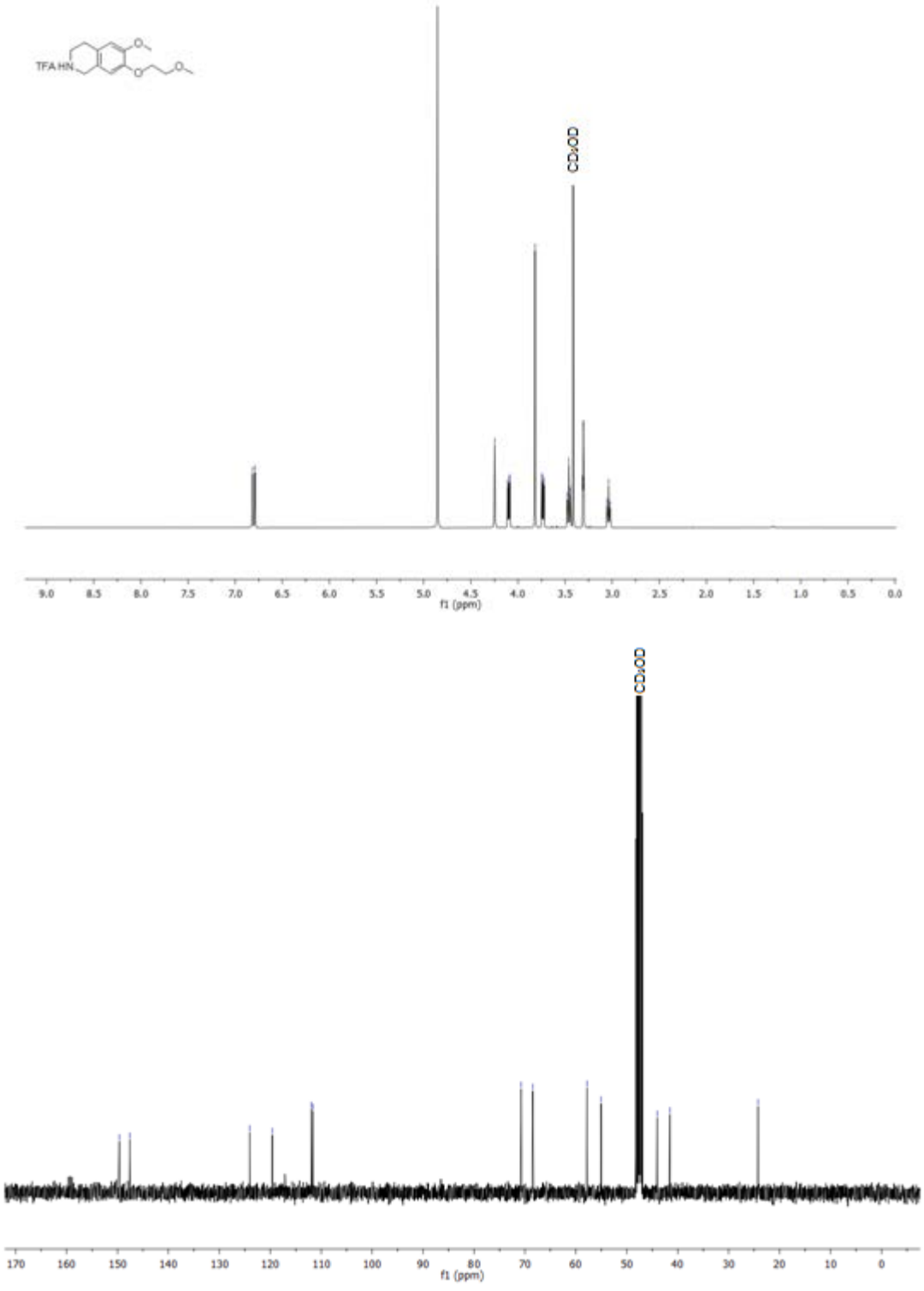


N-(5-(2-bromoacetyl)-2-methylphenyl)quinoline-2-carboxamide (12b)

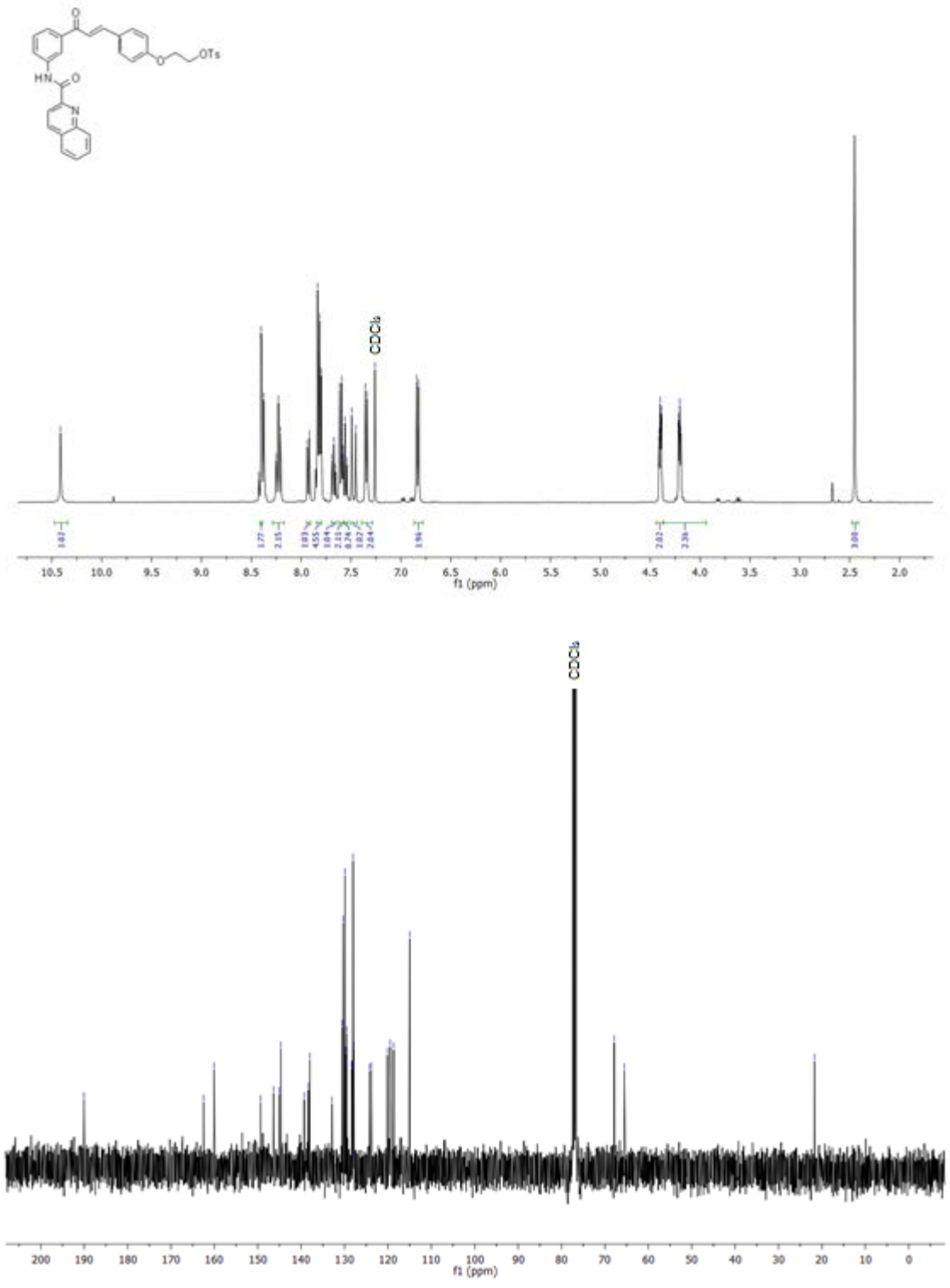




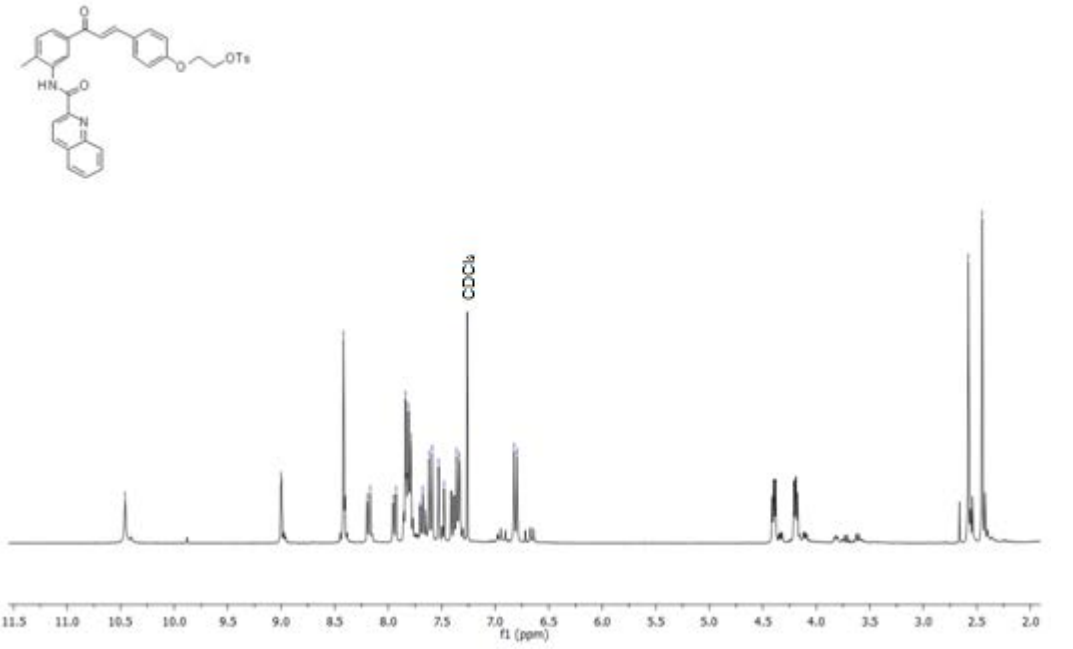
6-methoxy-7-(2-methoxyethoxy)-1,2,3,4-tetrahydroisoquinoline 2,2,2- Trifluoroacetate (15a)

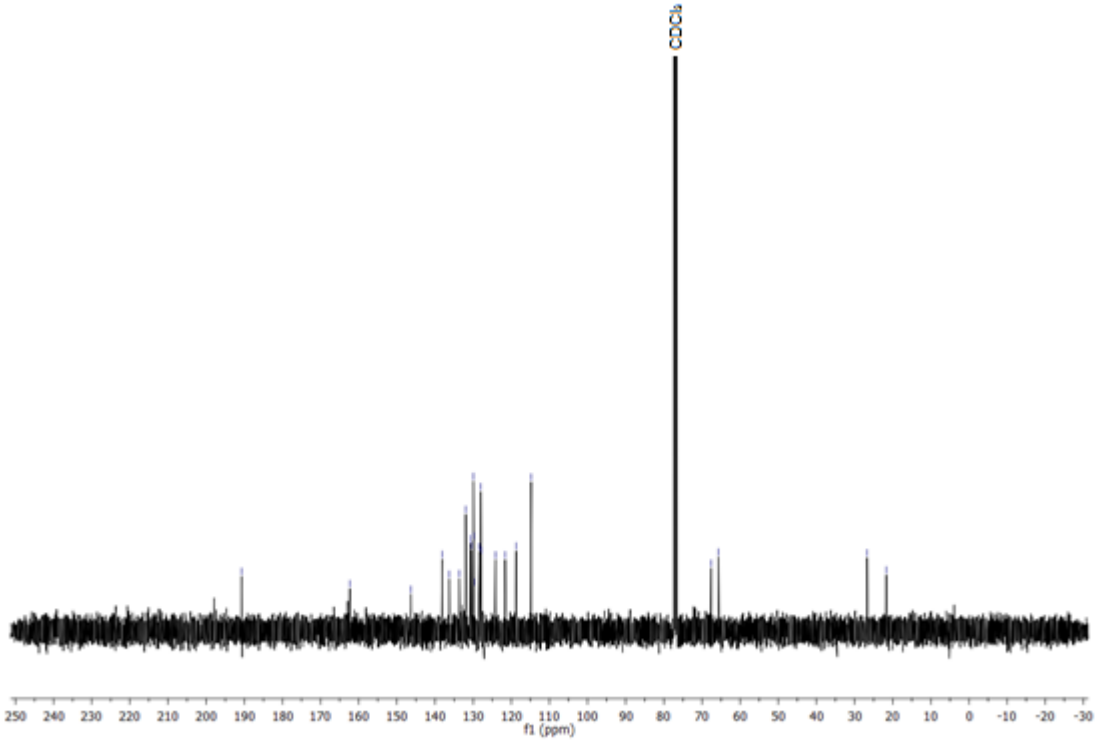


(E)-2-(4-(3-oxo-3-(3-(quinoline-2-carboxamido)phenyl)prop-1-en-1-yl)phenoxy)ethyl 4-methylbenzenesulfonate (16a)

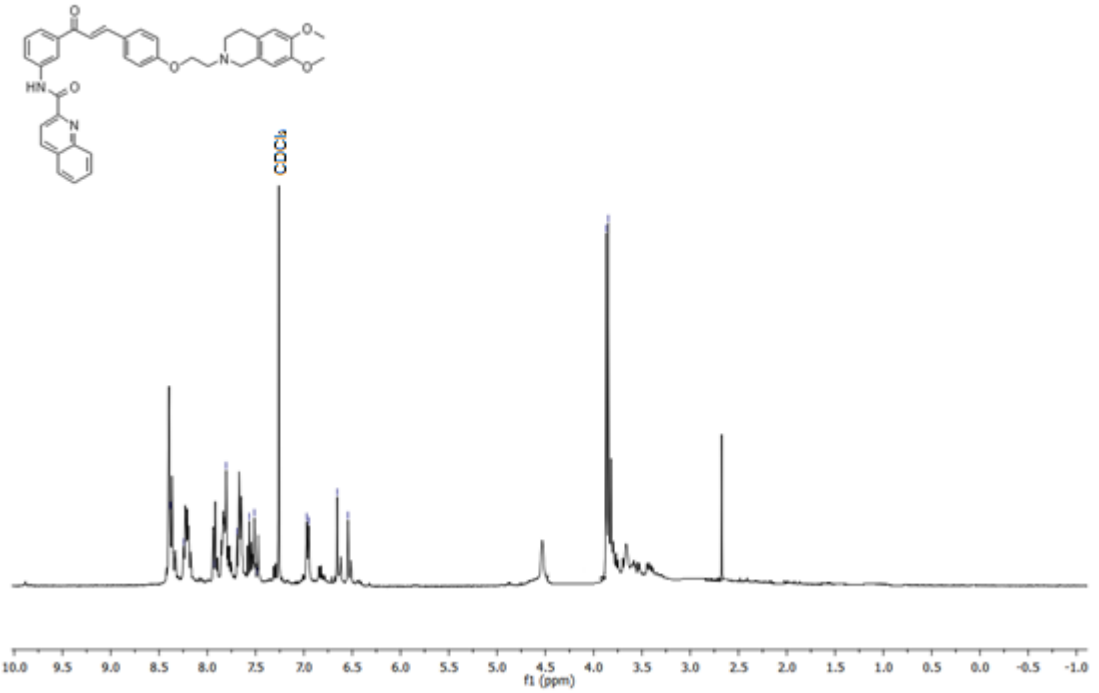


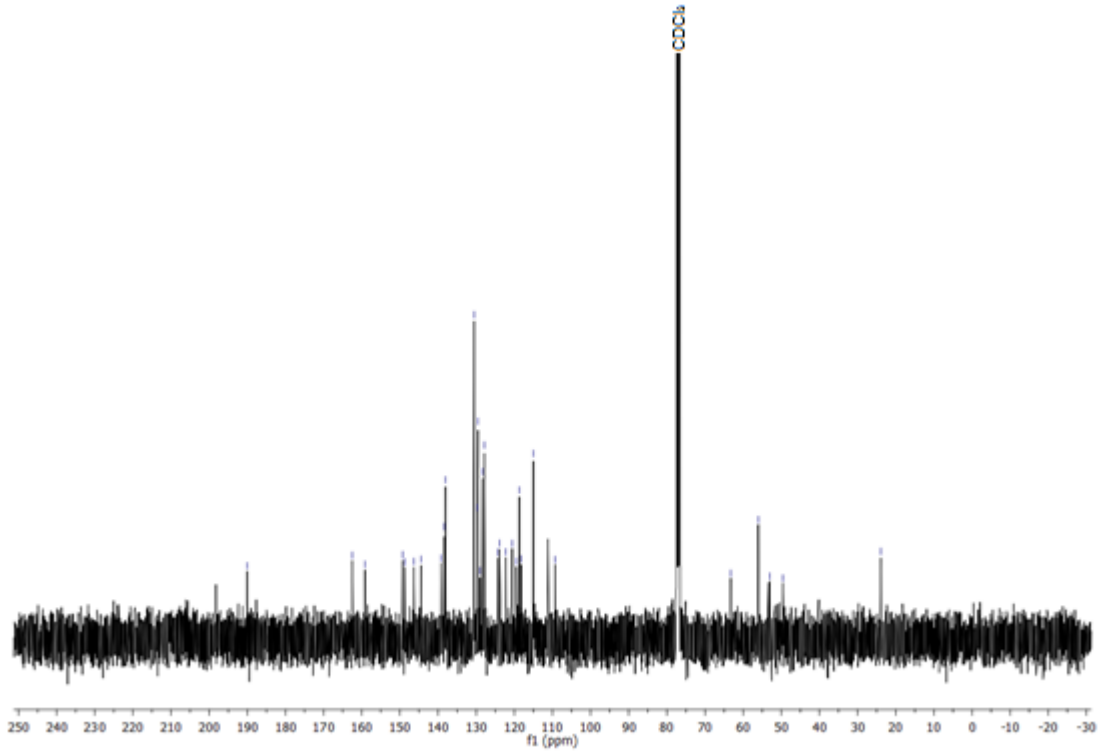
(E)-2-(4-(3-(4-methyl-3-(quinoline-2-carboxamido)phenyl)-3-oxoprop-1-en-1-yl)phenoxy)ethyl 4-methylbenzenesulfonate (16b)



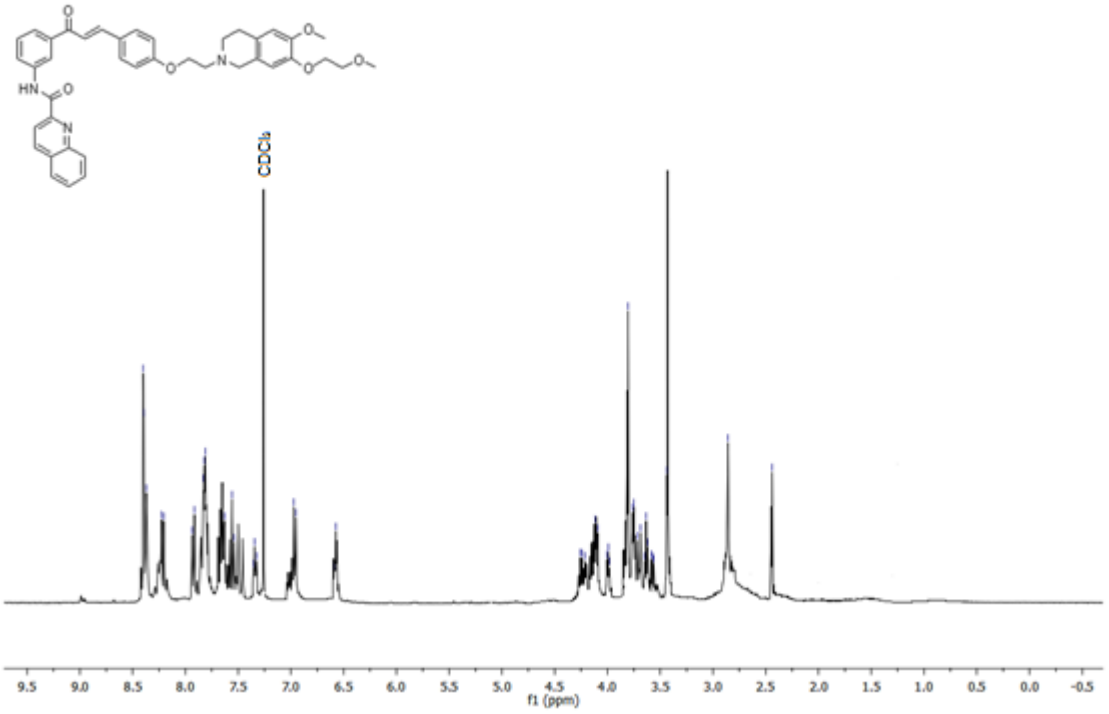


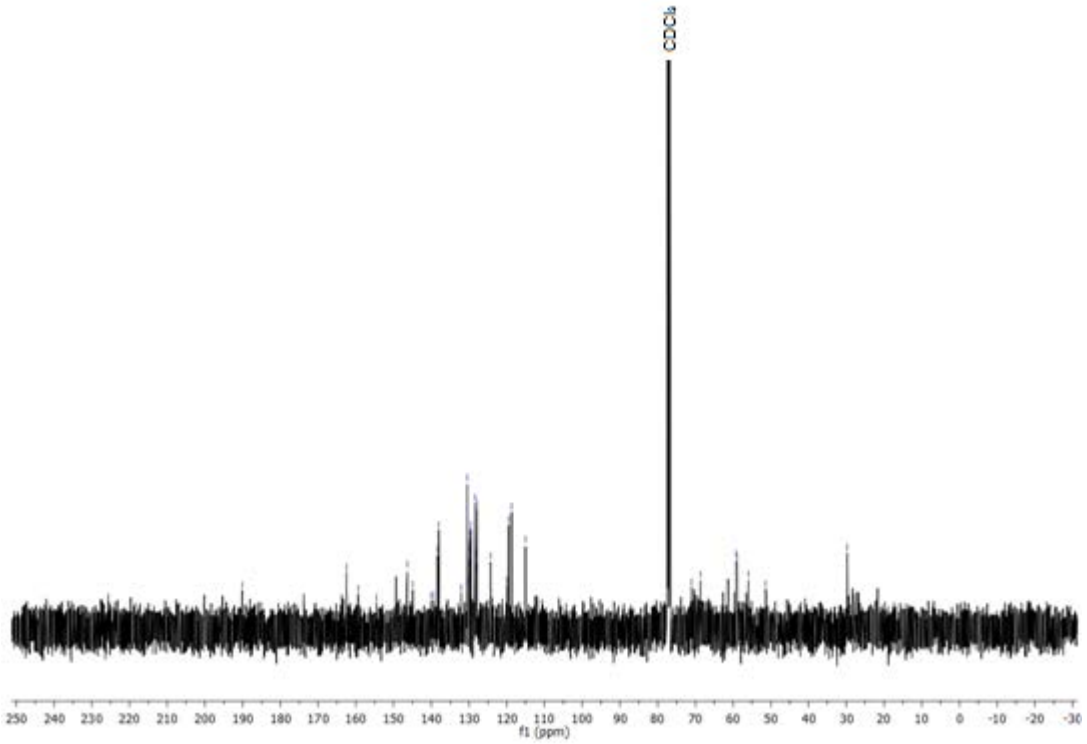
(E)-N-(3-(3-(4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)phenyl)quinoline-2-carboxamide (16a)



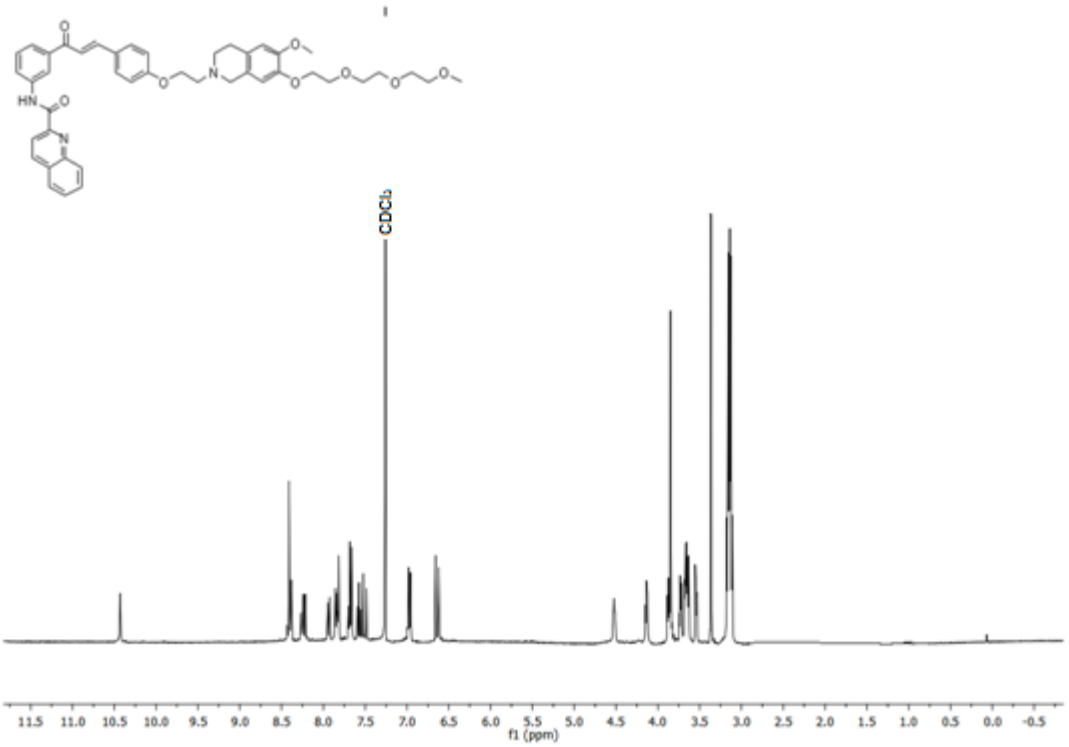


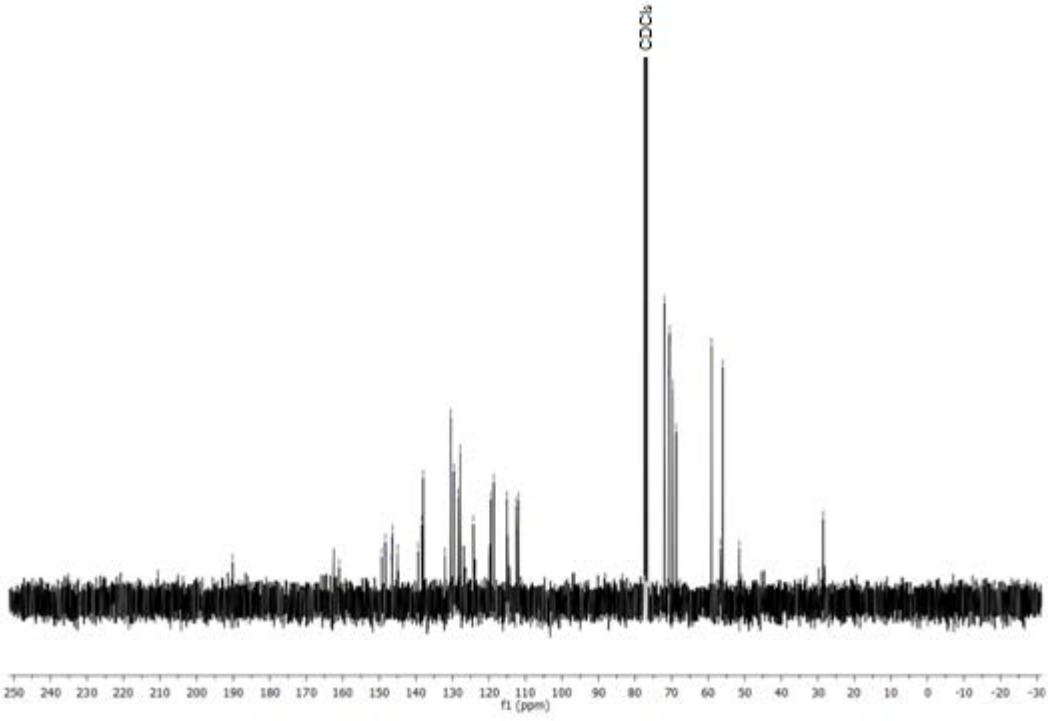
(E)-N-(3-(3-(4-(2-(6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)phenyl)quinoline-2-carboxamide (16b)



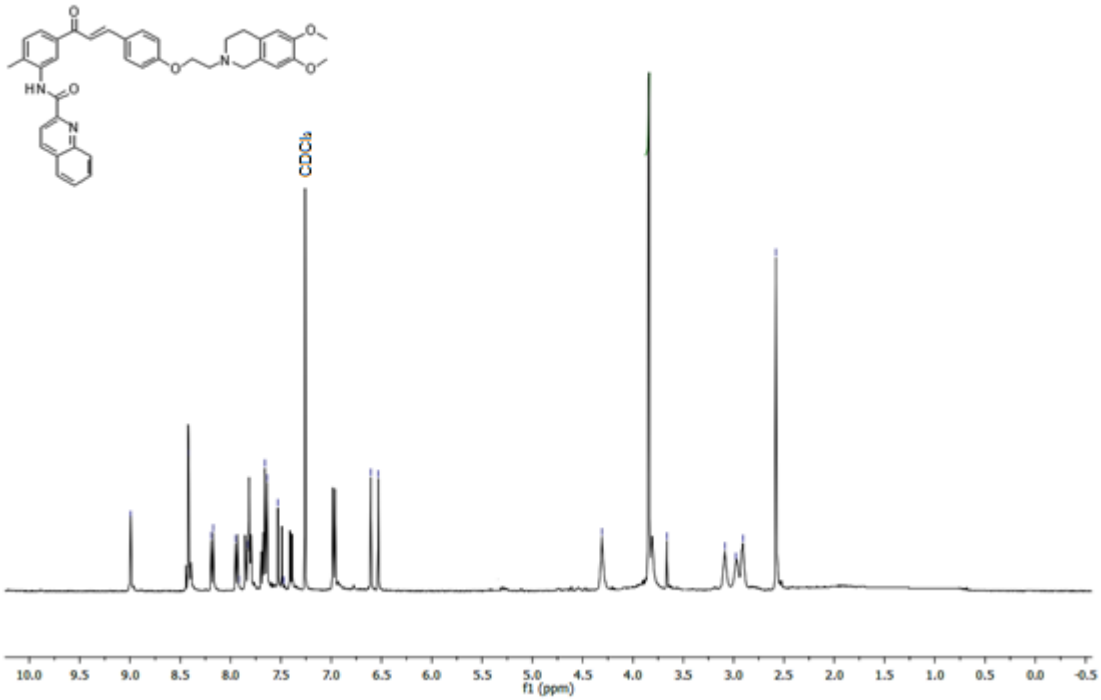


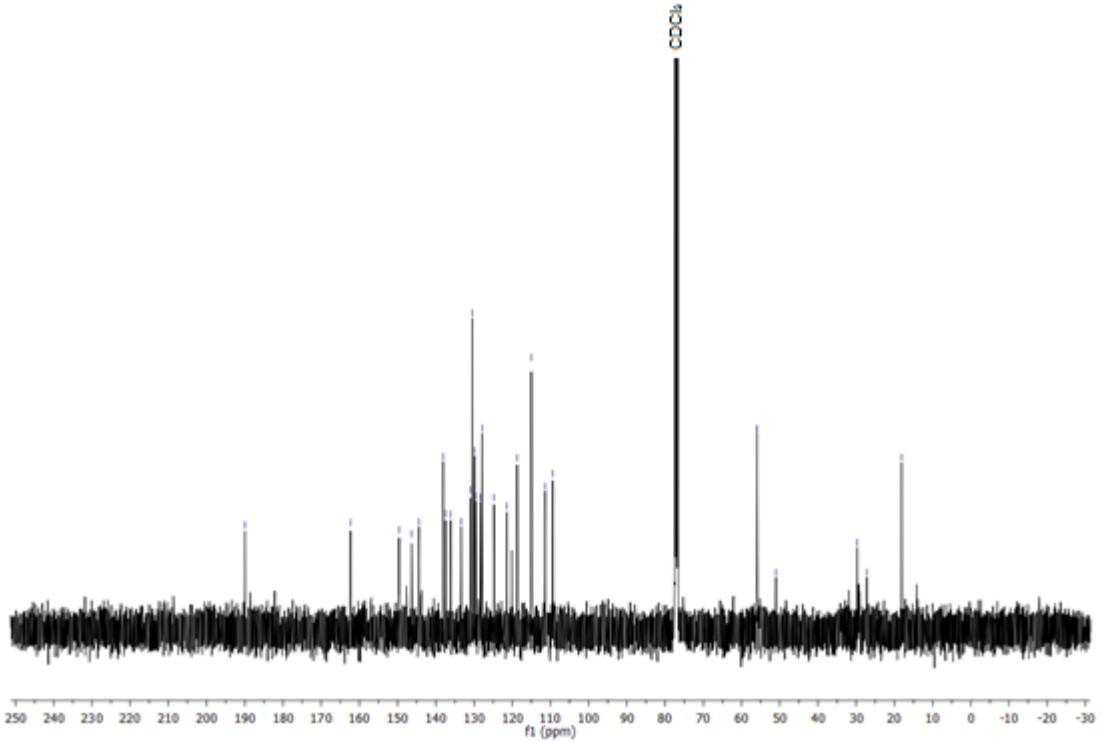
(E)-N-(3-(3-(4-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)phenyl)quinoline-2-carboxamide (16c)



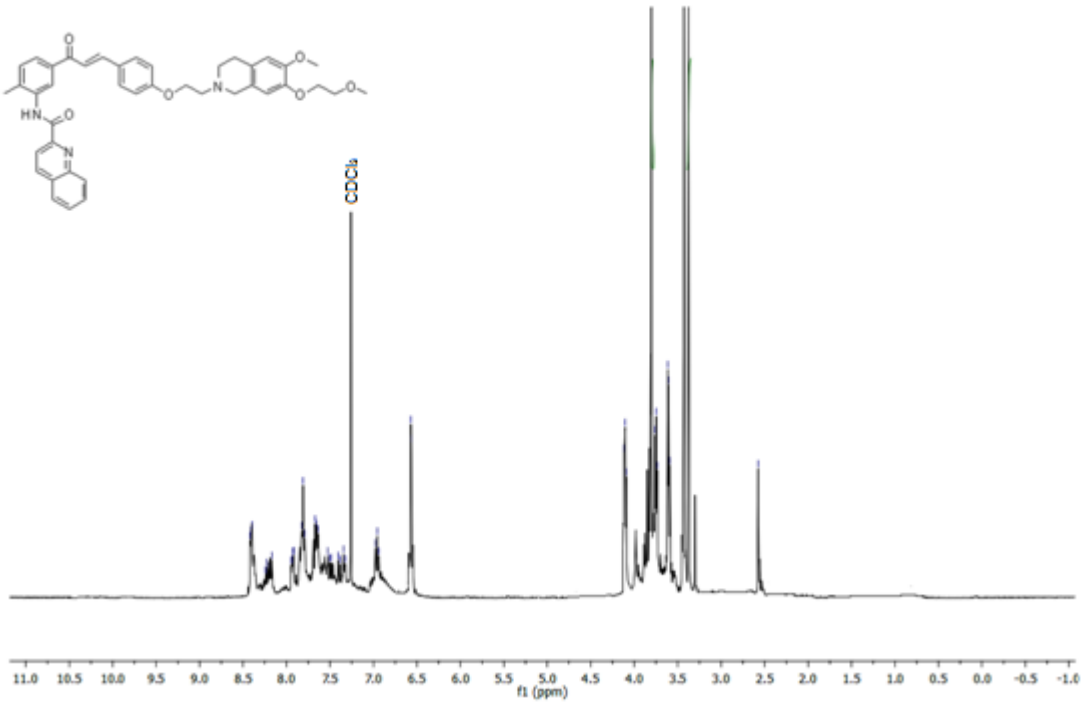


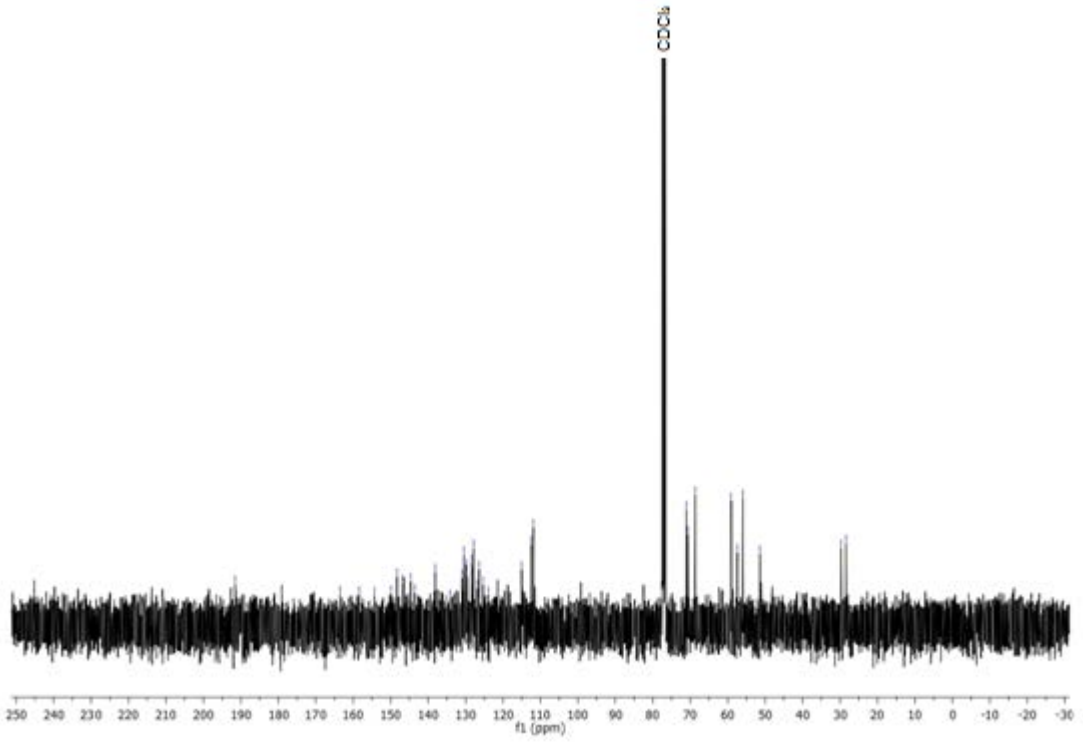
(E)-N-(5-(3-(4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)-2-methylphenyl)quinoline-2-carboxamide (16d)



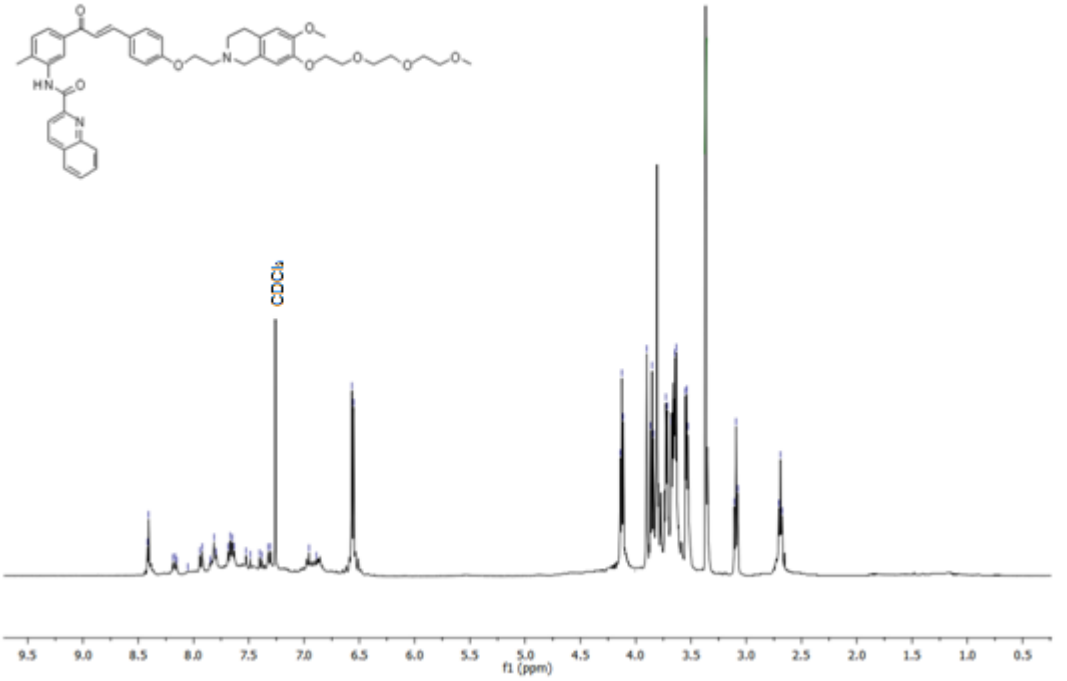


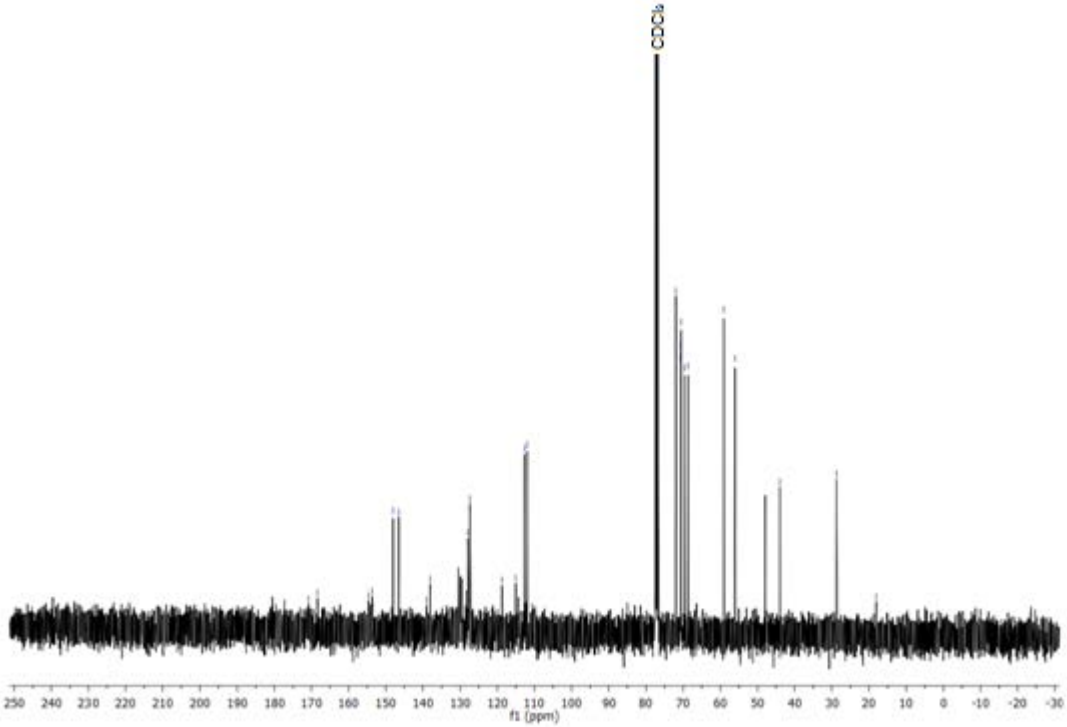
(E)-N-(5-(3-(4-(2-(6,7-bis(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)-2-methylphenyl)quinoline-2-carboxamide (16e)



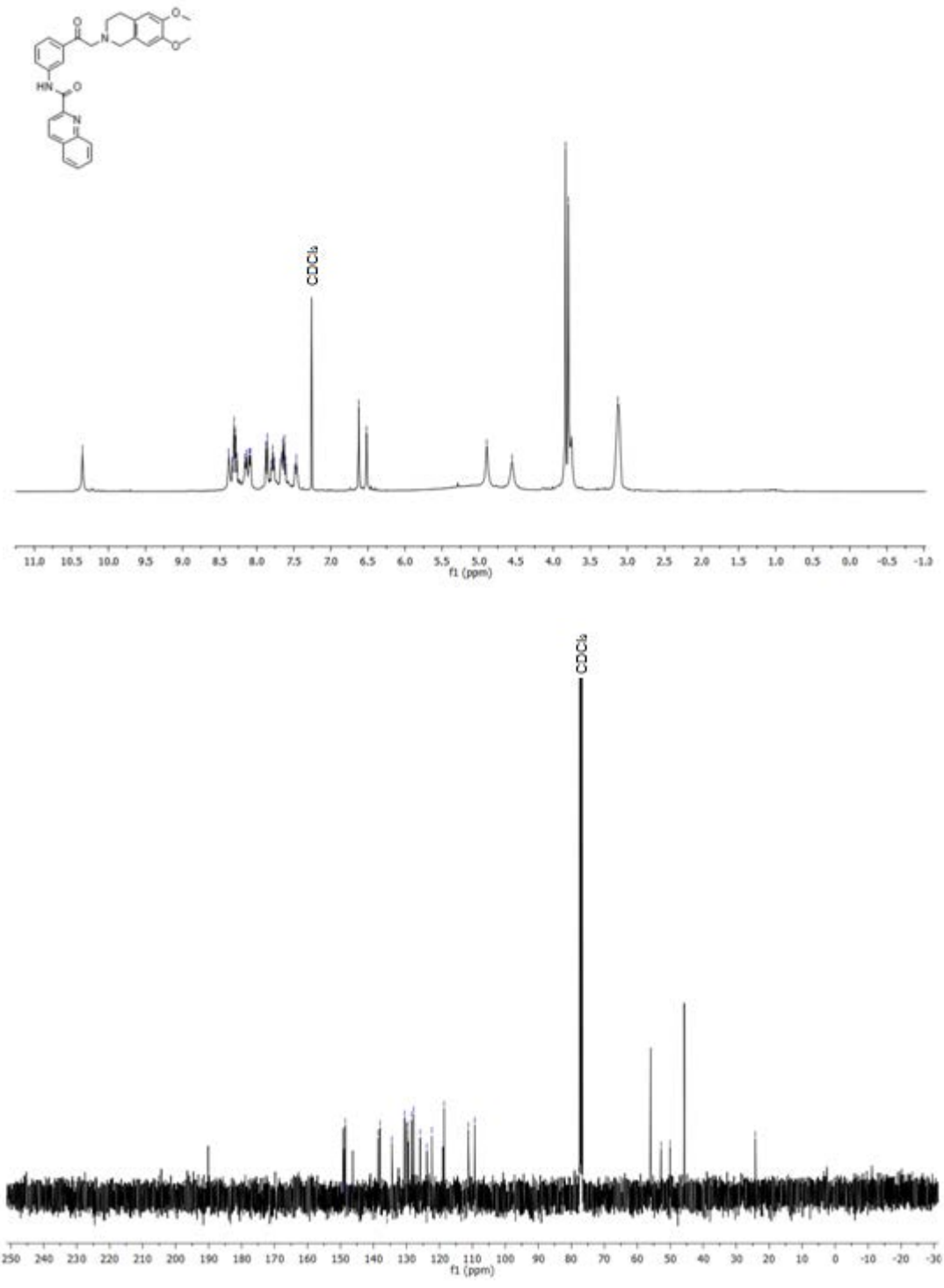


(E)-N-(5-(3-(4-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)ethoxy)phenyl)acryloyl)-2-methylphenyl)quinoline-2-carboxamide (16f)

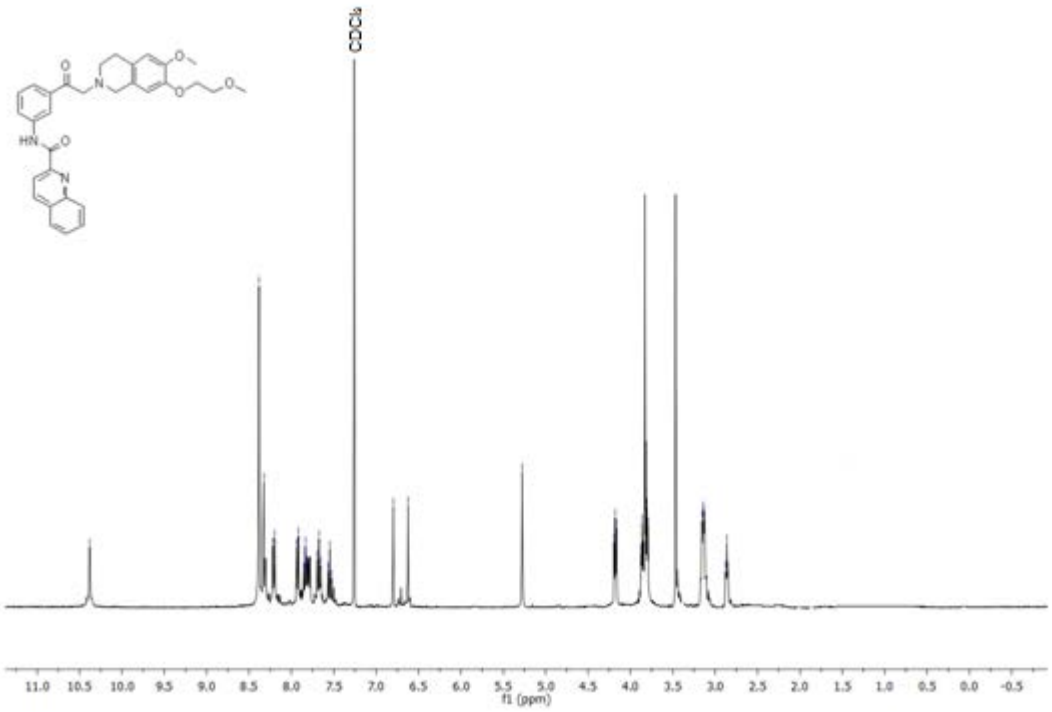


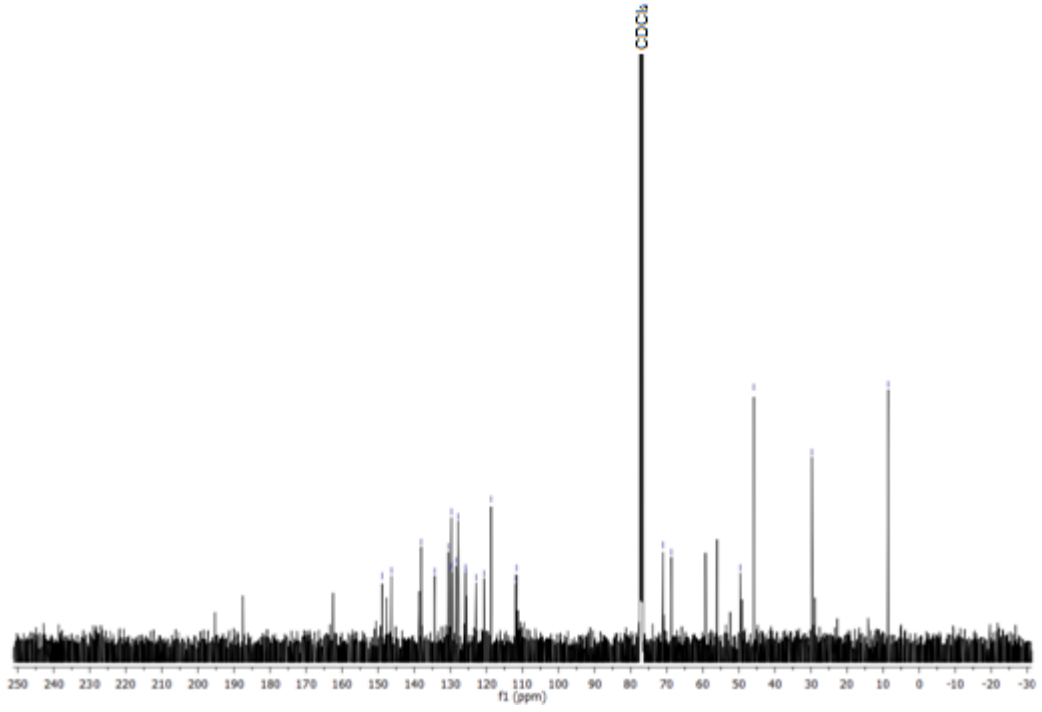


N-(3-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)phenyl)quinoline-2-carboxamide (17a)

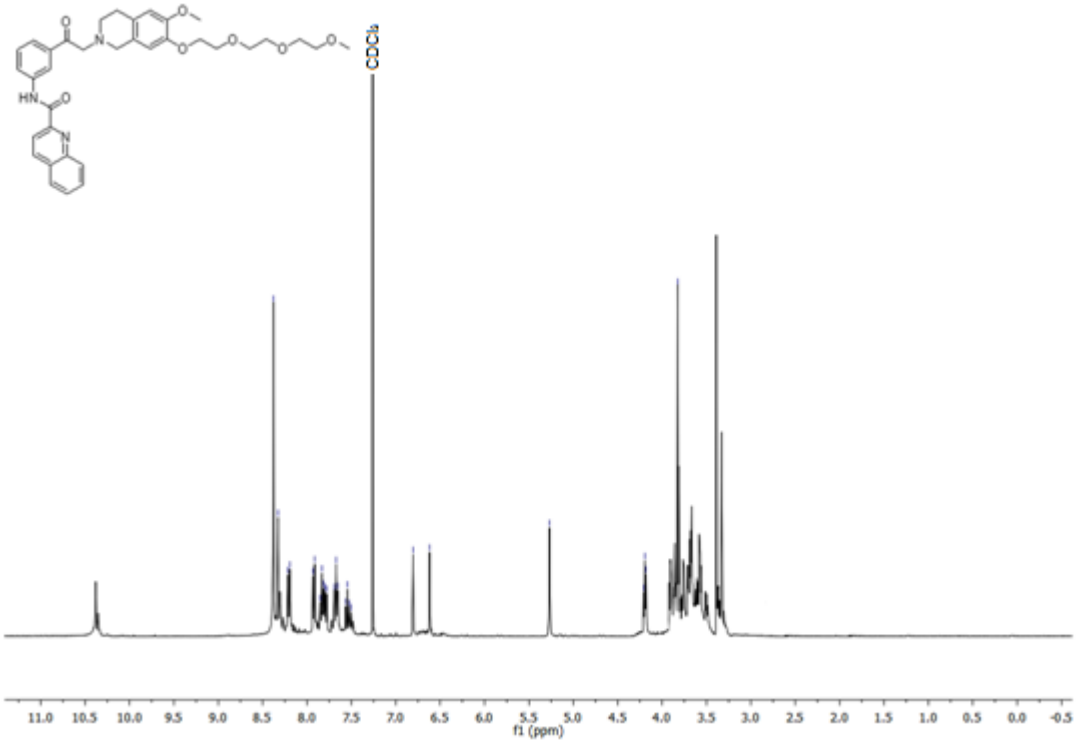


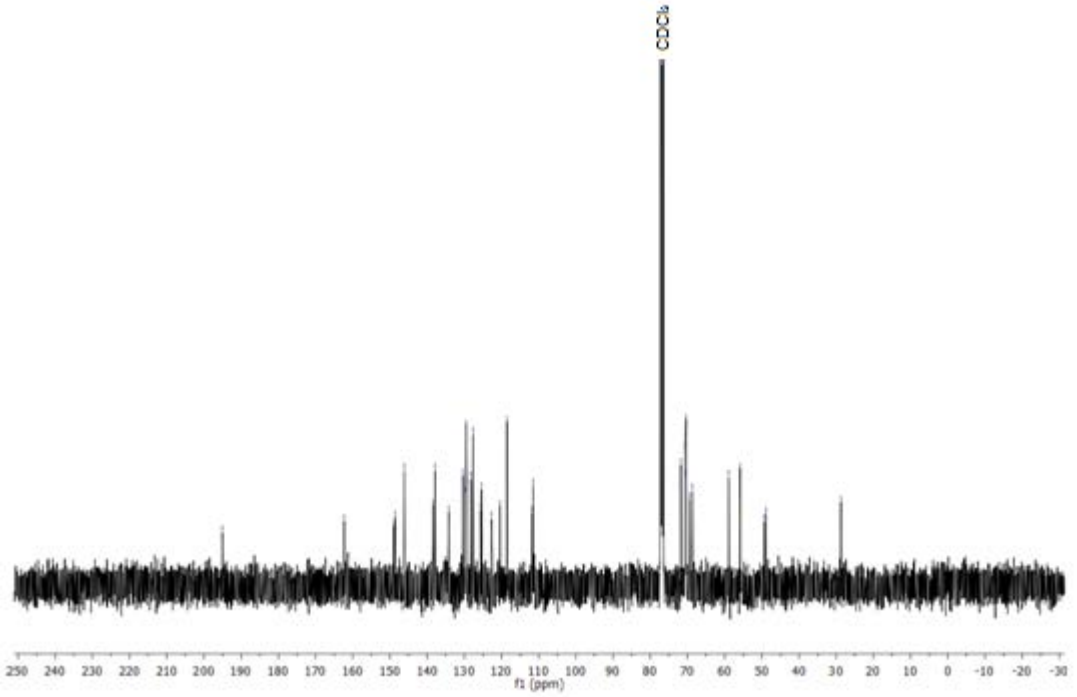
N-(3-(2-(6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)phenyl)quinoline-2-carboxamide (17b)



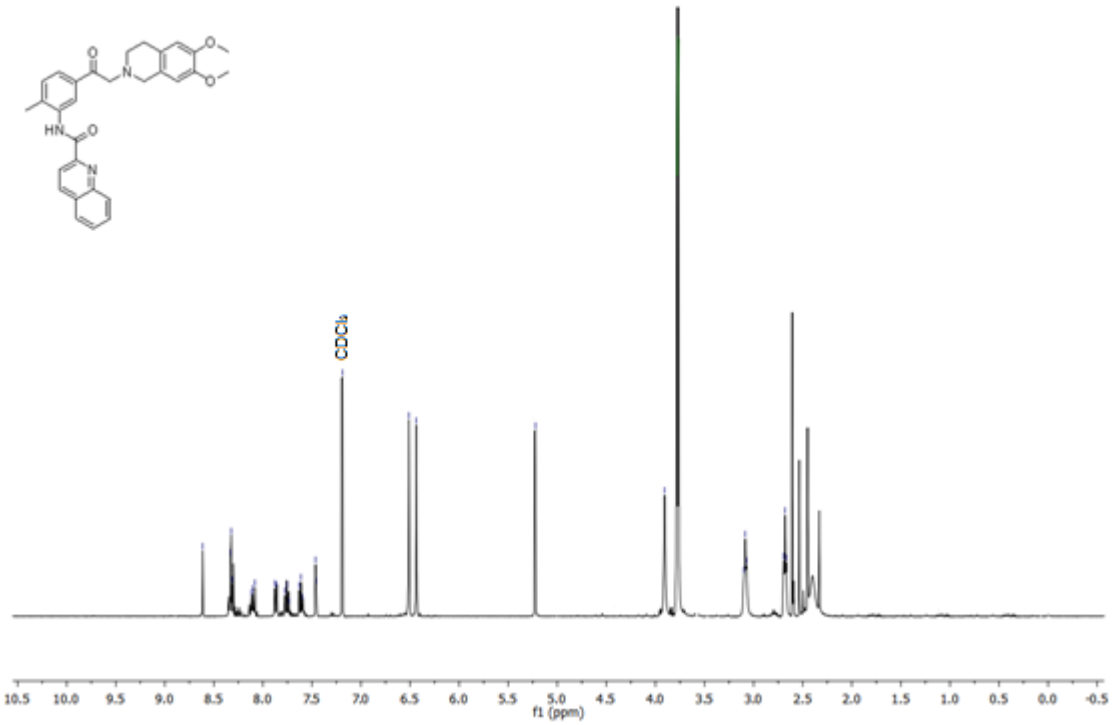


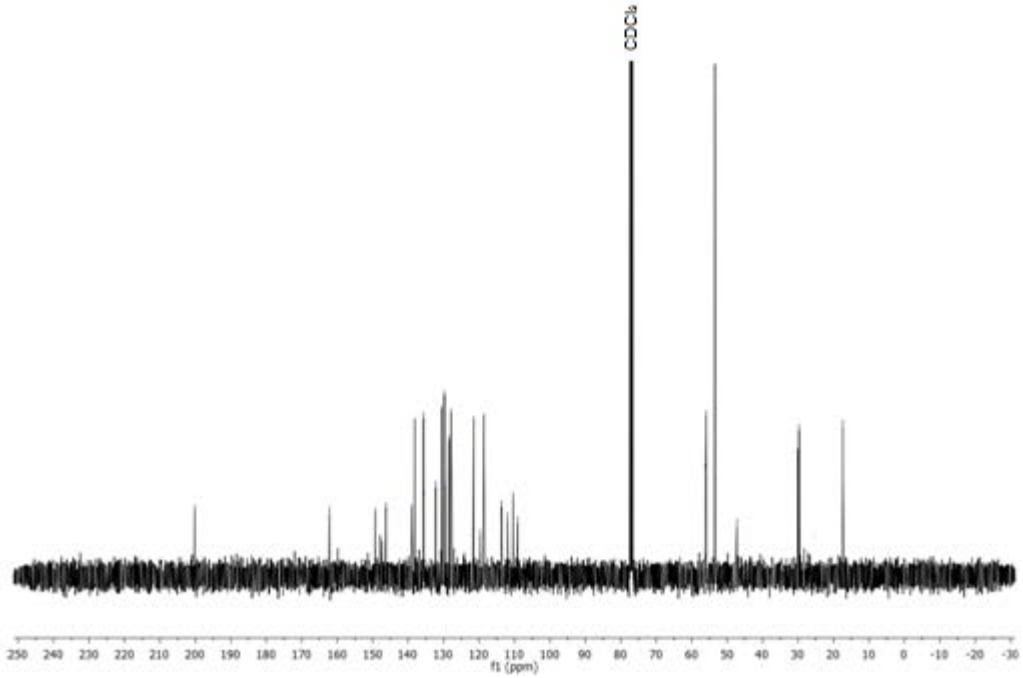
N-(3-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)phenyl)quinoline-2-carboxamide (17c)



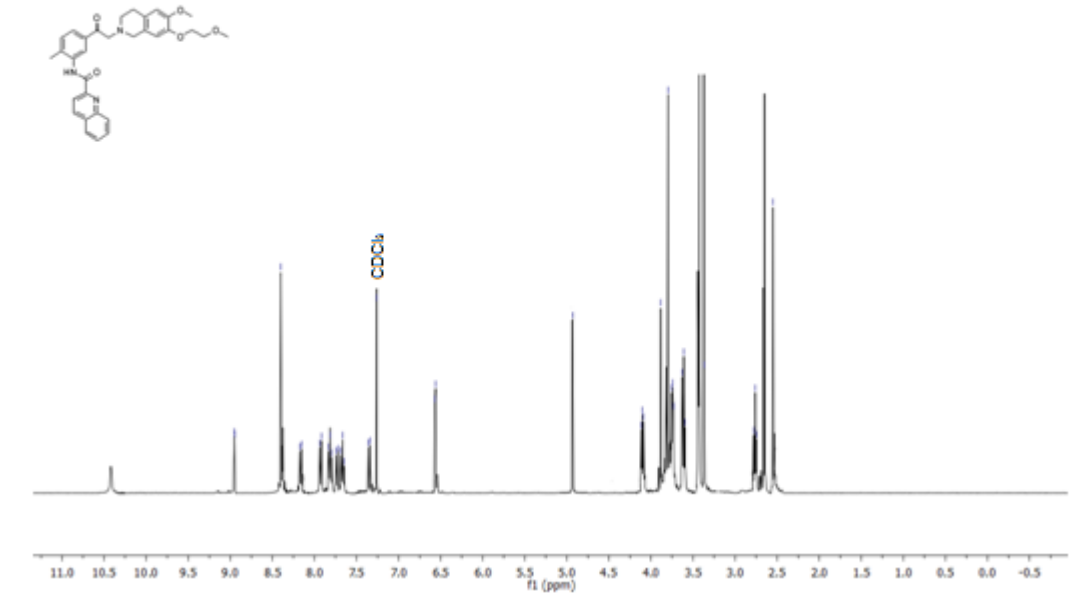


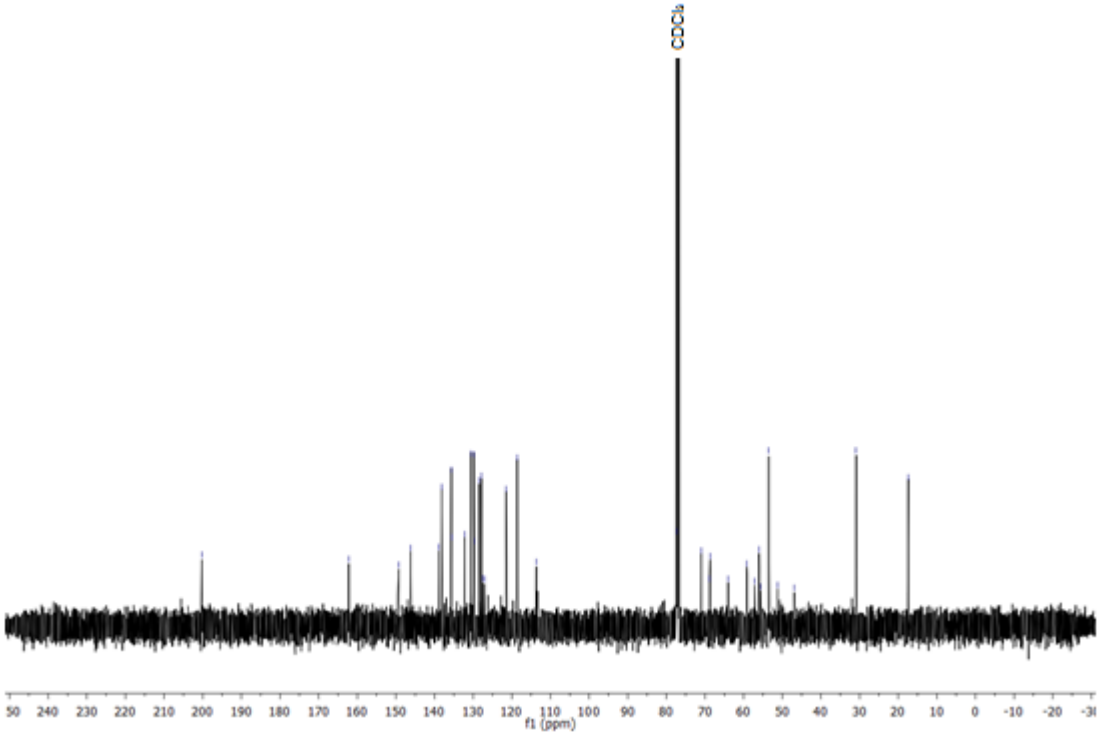
N-(5-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)-2-methylphenyl)quinoline-2-carboxamide (17d)



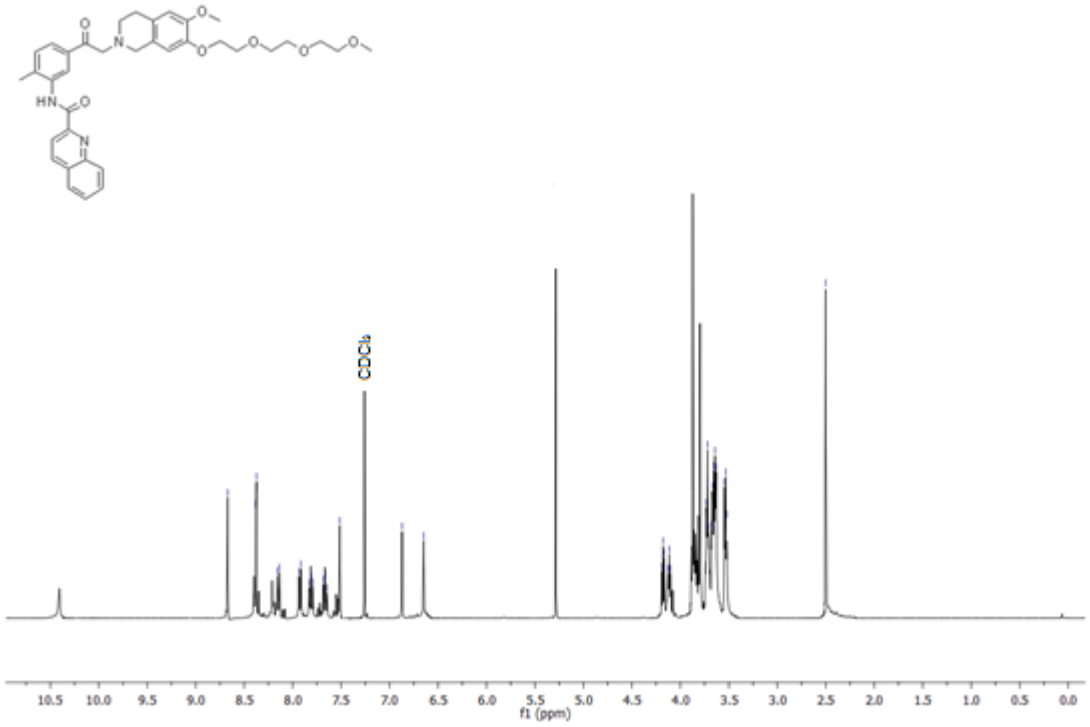


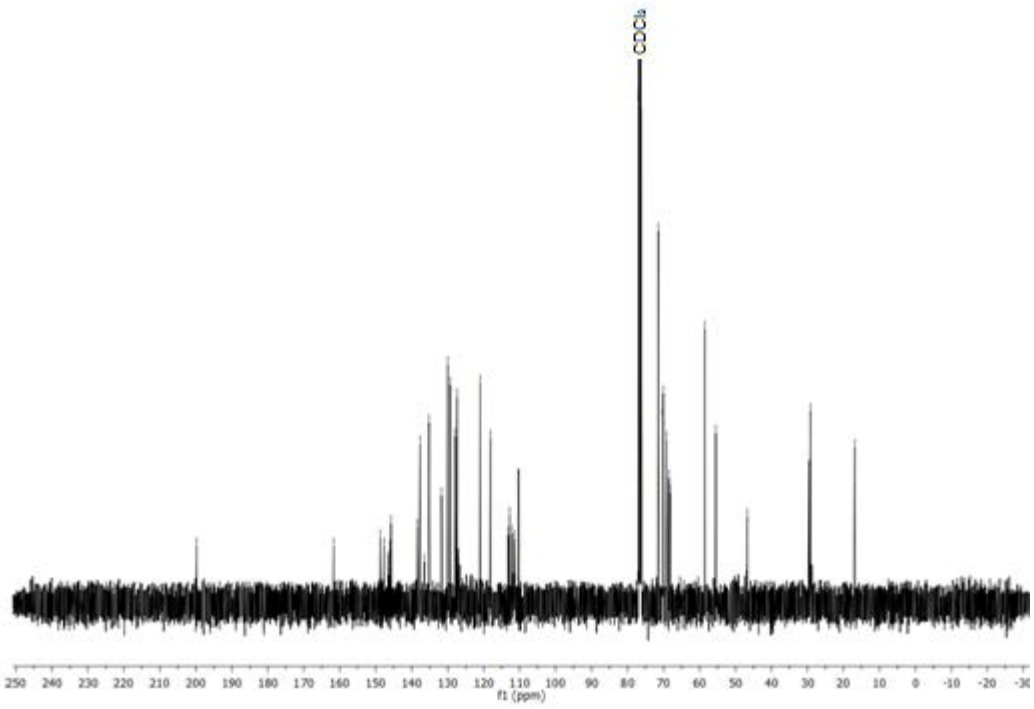
N-(3-(2-(6-methoxy-7-(2-methoxyethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)phenyl)quinoline-2-carboxamide (17e)





N-(5-(2-(6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)acetyl)-2-methylphenyl)quinoline-2-carboxamide (17f)



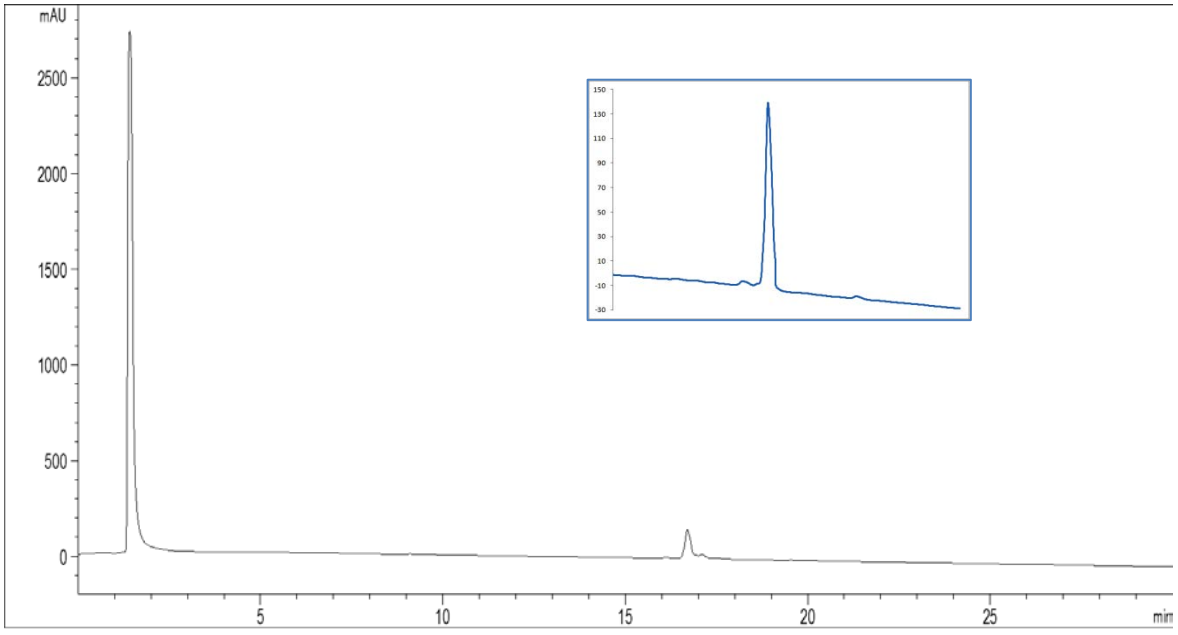


1.5 Purity (determined by HPLC)

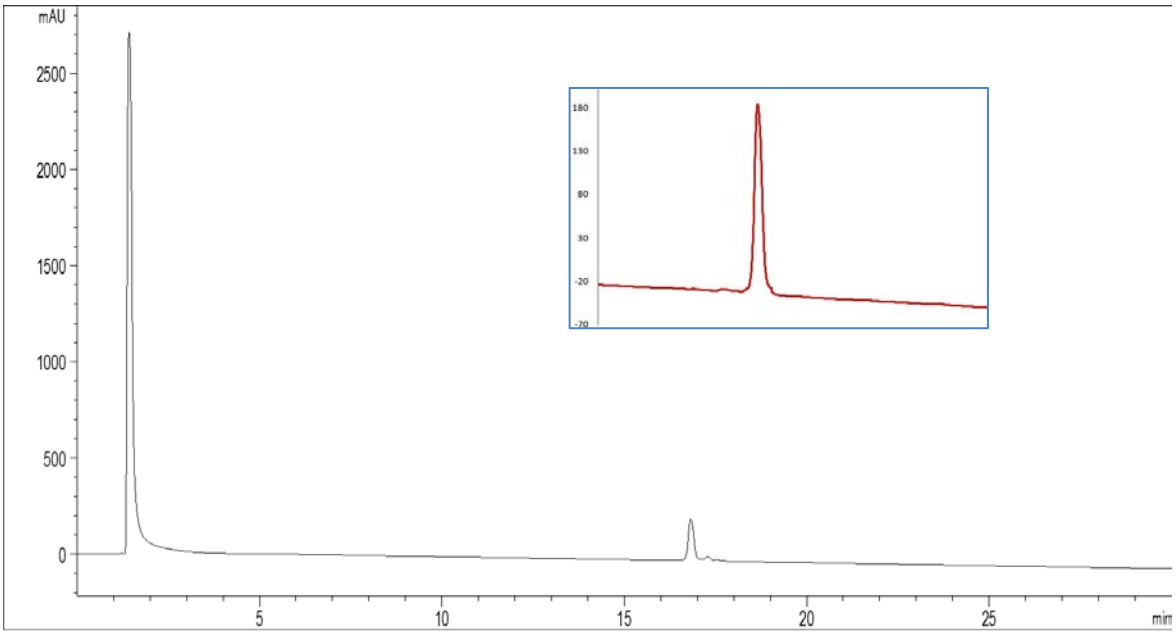
Table 1: Purities of key compounds

Compound	Purity (%)	Retention time (min)
16a	96	16.7
16b	96	16.8
16c	97	16.8
16d	95	17.7
16e	96	17.4
16f	95	17.4
17a	95	14.1
17b	97	14.1
17c	95	14.1
17d	95	15.1
17e	97	15.0
17f	95	14.5

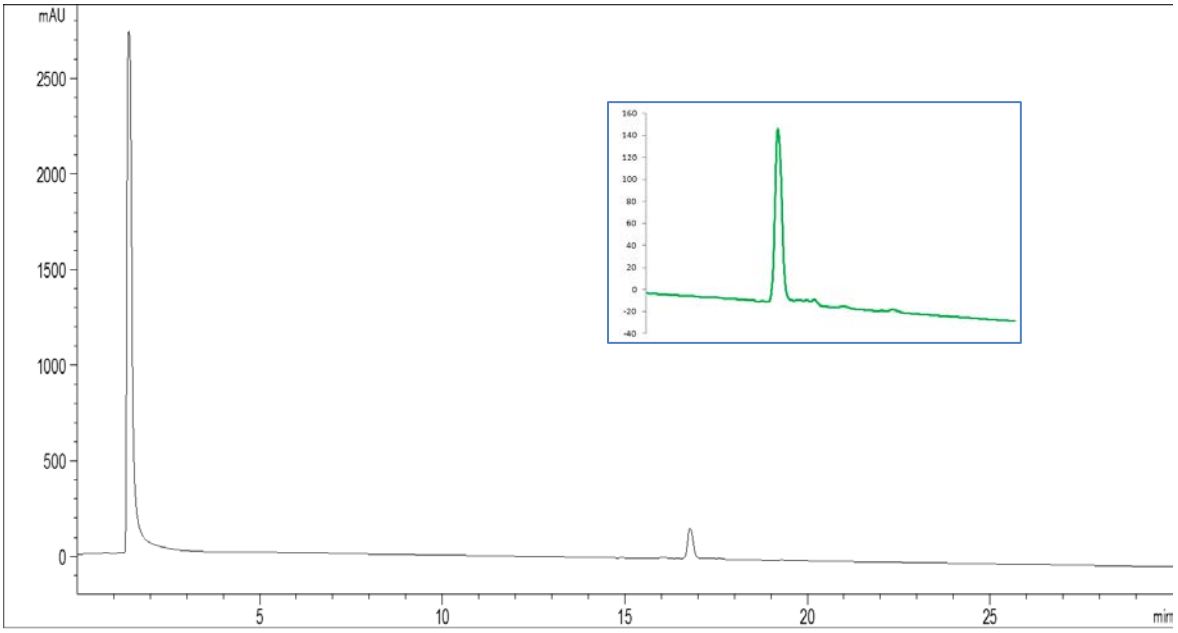
Compound **16a** in DMSO, 0.1 µL injected; Detector: 220 nm



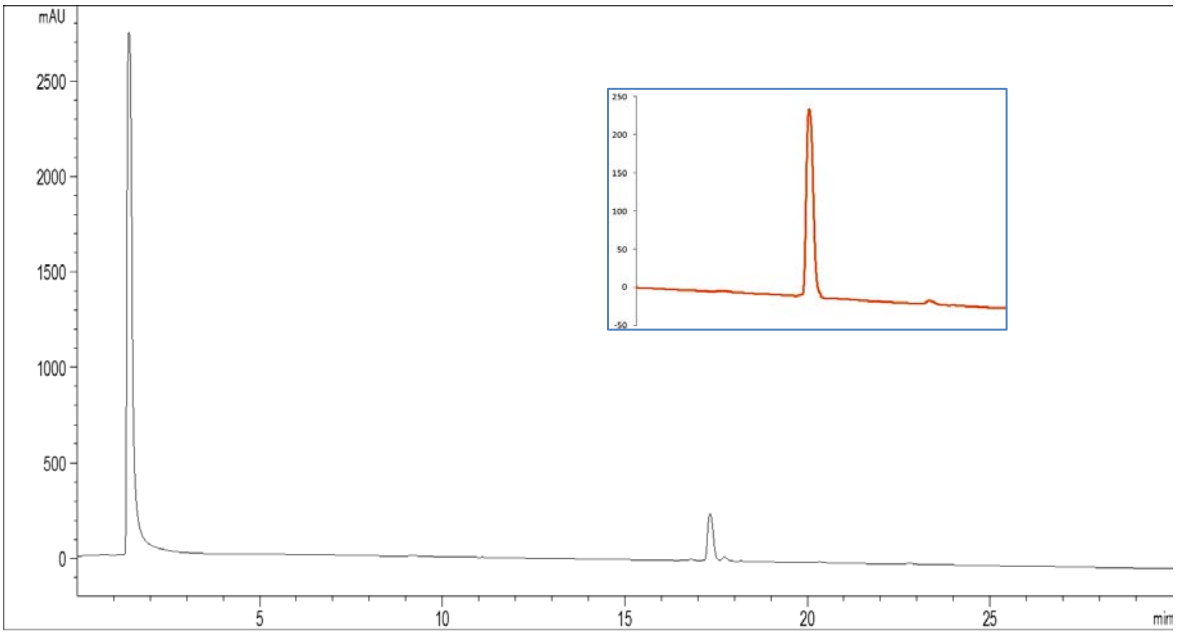
Compound **16b** in DMSO, 0.1 µL injected; Detector: 220 nm



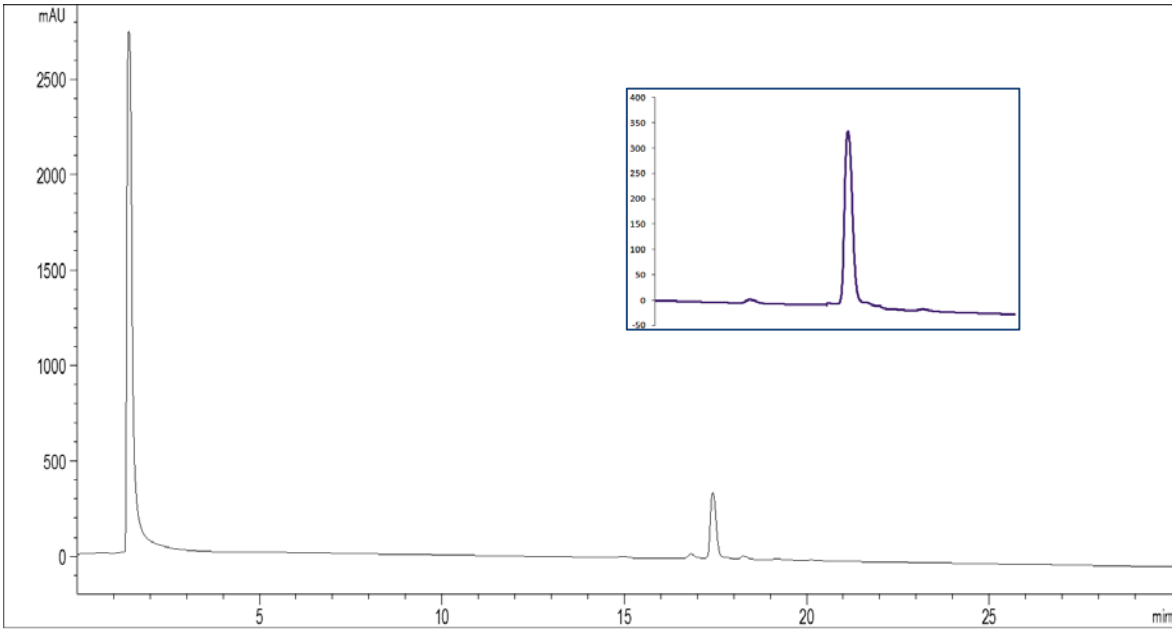
Compound **16c** in DMSO, 0.1 µL injected; Detector: 220 nm



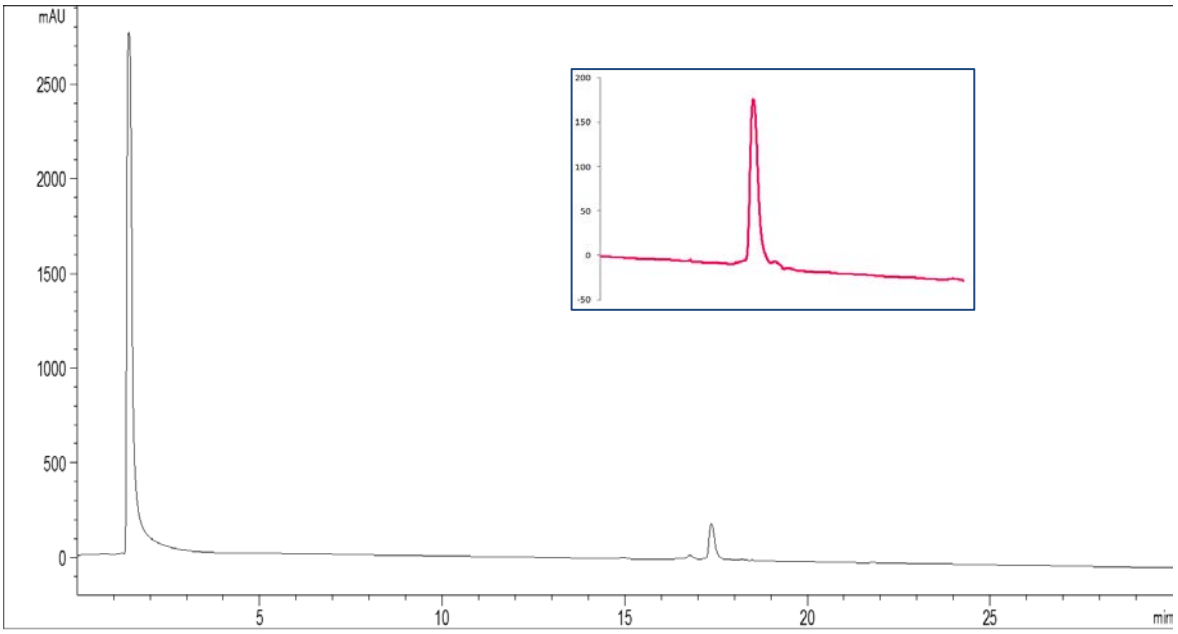
Compound **16d** in DMSO, 0.1 µL injected; Detector: 220 nm



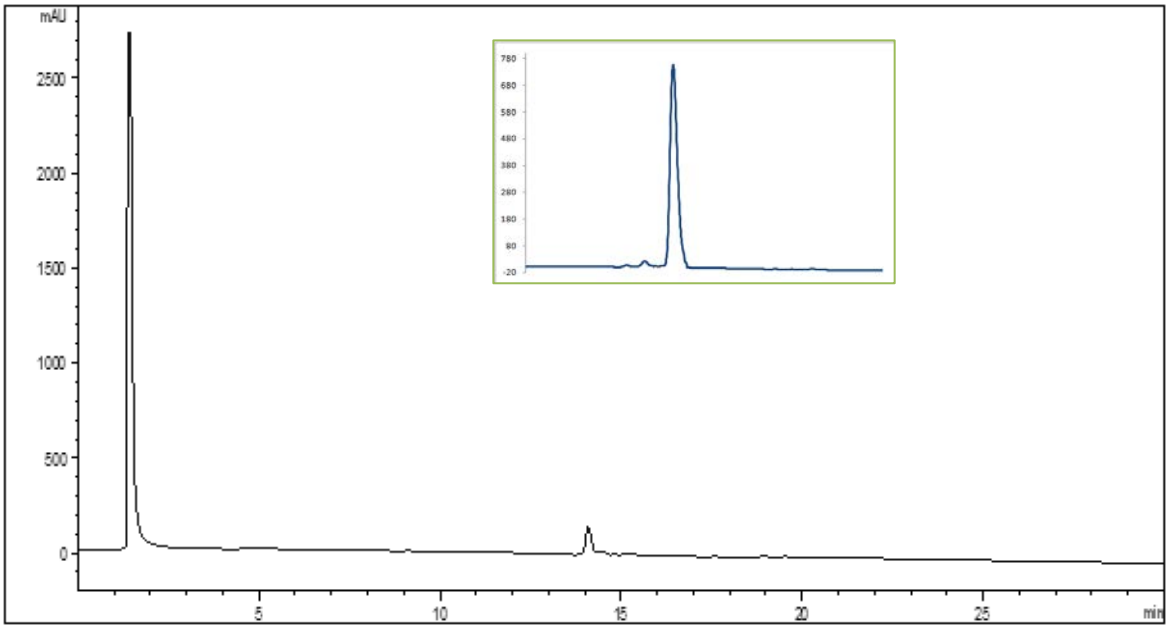
Compound **16e** in DMSO, 0.1 µL injected; Detector: 220 nm



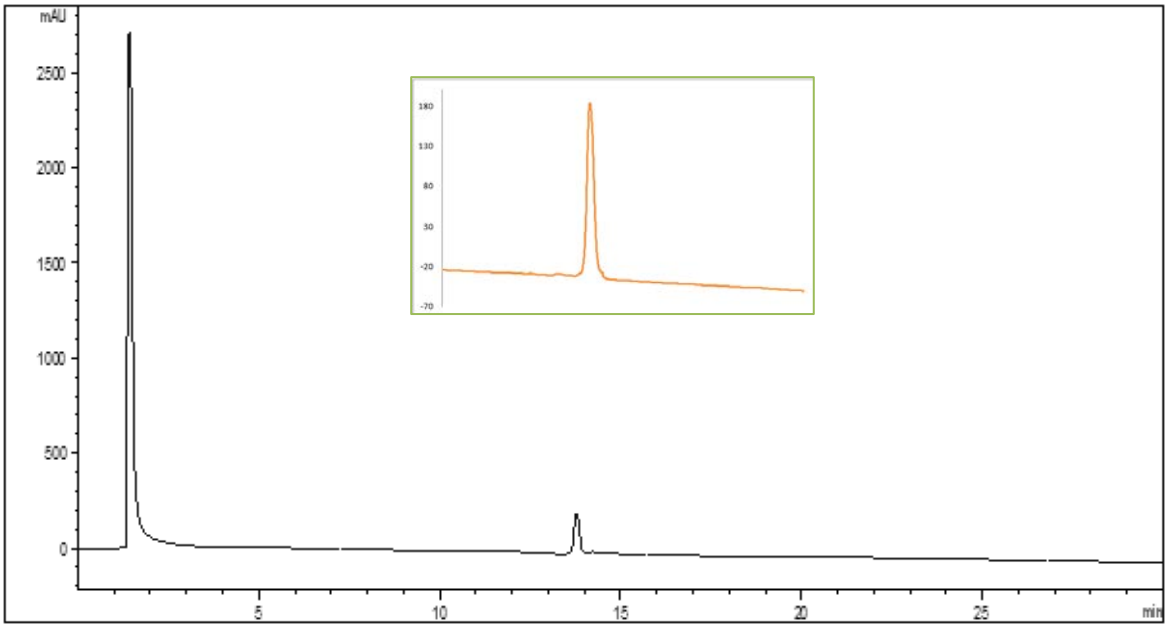
Compound **16f** in DMSO, 0.1 µL injected; Detector: 220 nm



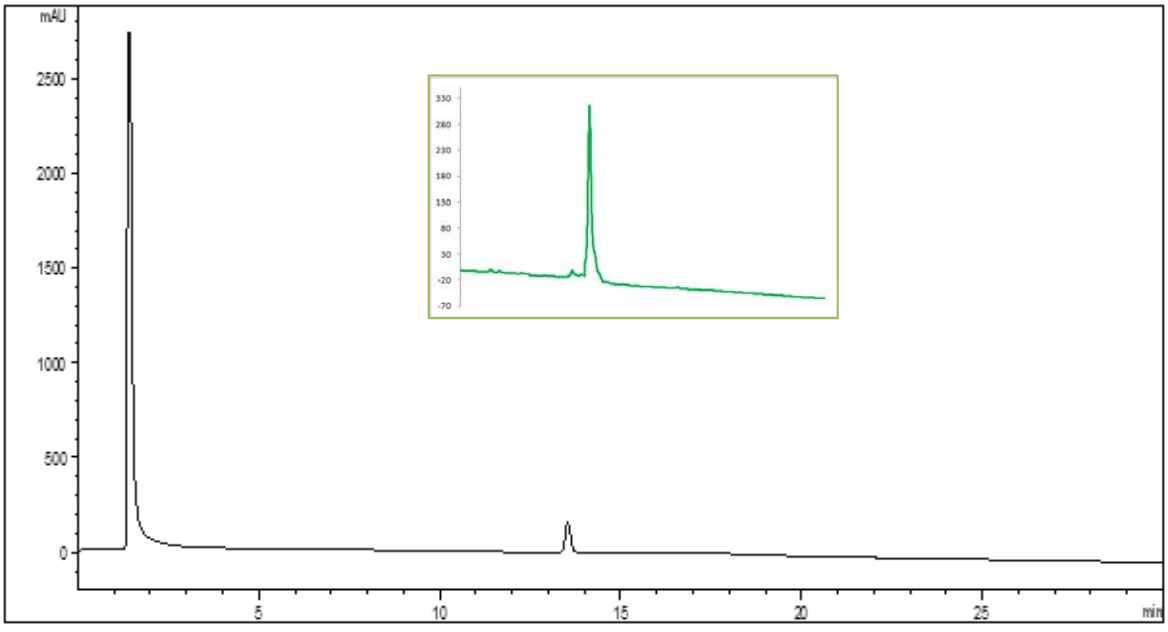
Compound **17a** in DMSO, 0.1 µL injected; Detector: 220 nm



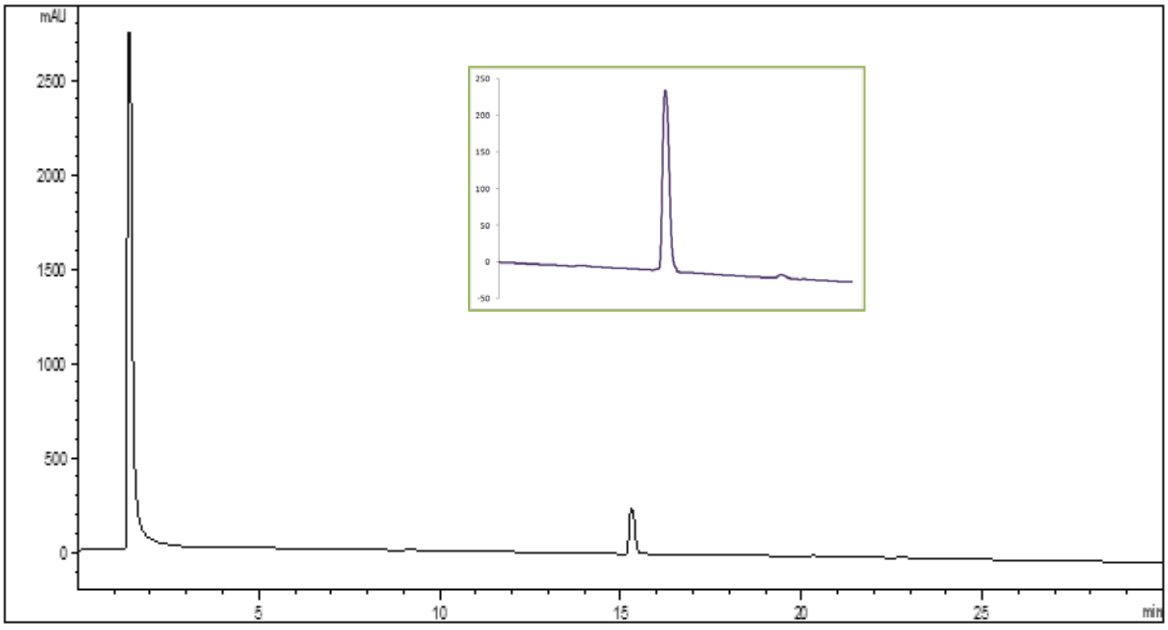
Compound **17b** in DMSO, 0.1 µL injected; Detector: 220 nm



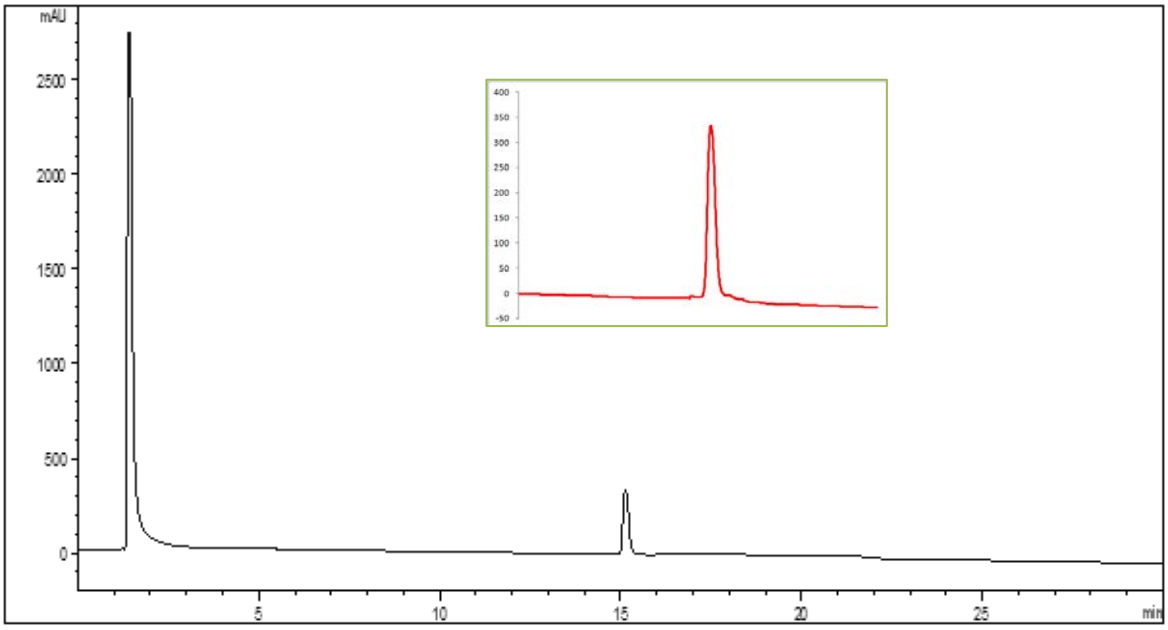
Compound **17c** in DMSO, 0.1 µL injected; Detector: 220 nm



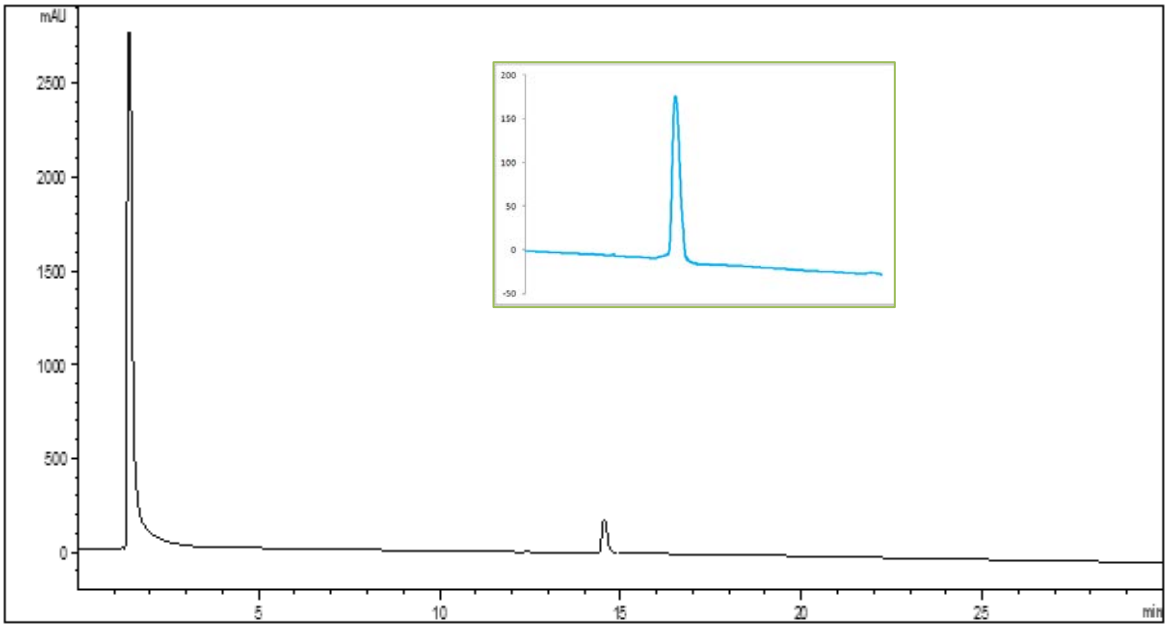
Compound **17d** in DMSO, 0.1 µL injected; Detector: 220 nm



Compound **17e** in DMSO, 0.1 µL injected; Detector: 220 nm



Compound **17f** in DMSO, 0.1 µL injected; Detector: 220 nm



2. Pharmacological evaluation

2.1 Chemicals and test compounds

Hoechst 33342 (Invitrogen, Karlsruhe, Germany) was dissolved in sterile water to produce a 0.8 mM working solution. Calcein-AM (4 mM in anhydrous DMSO) and pluronic F127 were obtained from Biotium (Hayward, CA, USA) and diluted to 100 µM. Bovine serum albumin (BSA) was purchased from Serva (Heidelberg, Germany). Fumitremorgin C (FTC; Merck, Darmstadt, Germany) was also dissolved in DMSO and diluted to a concentration of 1 mM. Reversan was purchased from Tocris Bioscience (Bristol, UK) and diluted to a 3 mM working solution in DMSO. Topotecan and vinblastine for cell culture supplementation were purchased from Sigma (Munich, Germany). Loading buffer (calcein-AM assay) was made of 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2.6H_2O$, 1.5 mM $CaCl_2.2H_2O$, 25 mM HEPES, 10 mM glucose, pH 7.4. PBS (phosphate buffered saline) was made of 8.0 g/L NaCl, 1.0 g/L $Na_2HPO_4.2H_2O$, 0.20 g/L KCl, 0.20 g/L KH_2PO_4 and 0.15 g/L $NaH_2PO_4.H_2O$. The pH value was adjusted to 7.4. A solution of 4% (m/m) paraformaldeyde (PFA) in PBS was made by stirring 2 g of PFA per 50 g total solution while heating on a magnetic stirrer for approximately 30 min. The test compounds were dissolved in DMSO at a concentration of 10 mM if possible, depending on the solubility of the compounds. All stock solutions were stored at −20 °C. If not otherwise stated, chemicals (p.a. quality) were obtained from Merck (Darmstadt, Germany). Purified water (Milli-Q system, Millipore, Eschborn, Germany) was used throughout.

2.2 Cells and culture conditions

MCF-7/Topo cells, an ABCG2-overexpressing subclone of MCF-7 cells (ATCC® HTB-22™, American Type Culture Collection, Manassas, VA), were obtained as described [10] and cultured in water-saturated atmosphere (95% air, 5% CO_2) at 37 °C in 75-cm^2 culture flasks from Nunc (Wiesbaden, Germany) in Dulbecco's Modified Eagle Medium (DMEM; Sigma, Munich, Germany) containing L-glutamine, 2.2 g/L $NaHCO_3$ and 110 mg/L sodium pyruvate, supplemented with 10% fetal calf serum (FCS; Biochrom, Berlin, Germany) and 550 nM topotecan to induce overexpression of the ABCG2 transporter. Human Kb-V1 cells, an ABCB1-overexpressing subclone of Kb cells (ATCC® CCL-17™), were obtained and cultured as described [10]. MDCKII-MRP1 cells: MDCKII cells (Madin-Darby Canine Kidney cells, strain II; a dog epithelial cell line; ATCC® CRL-2936™), transfected with human ABCC1, [11, 12] were a kind gift from Prof. Dr. P. Borst from the Netherland Cancer Institute (Amsterdam, NL).

The cells were cultured in DMEM supplemented with 10% FCS, 3.7 g/L $NaHCO_3$ and 110 mg/L sodium pyruvate. Trypsinization was performed at 37 °C (95% air, 5% CO_2) for 2 min for MCF/Topo and Kb-V1 cells using 0.1% trypsin /0.04% EDTA and for 20-30 min using 0.2% trypsin / 0.08% EDTA. All cells were routinely monitored for mycoplasma contamination by PCR (Venor GeM, Minerva Biolabs, Berlin, Germany) and only mycoplasma-negative cultures were used.

2.3 Calcein-AM microplate assay for the determination of ABCB1 modulation

Kb-V1 cells were seeded into flat-bottomed 96-well plates (Greiner, Frickenhausen, Germany) at a density of 20,000 cells per well. On the following day, cells were washed with loading buffer (120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2.6H_2O$, 1.5 mM $CaCl_2.2H_2O$, 25 mM HEPES, 10 mM glucose, pH 7.4) in order to remove unspecific serum esterases. Afterwards, cells were incubated with loading suspension (loading buffer, 5 mg/mL BSA, 1.25 µL/mL pluronic F127 (20% in DMSO)) containing 0.5 µM calcein-AM and the test compounds at increasing concentrations (10 nM – 100 µM) for 10 min (37 °C / 5% CO_2) to construct concentration-response curves. Tariquidar served as positive control at a final concentration of 10 µM corresponding to 100% inhibition of calcein-AM efflux. In general, test compounds were investigated as triplicates, controls as sextuplicates, respectively. Subsequently, the loading suspension was discarded, and cells were fixed with 4% PFA solution in PBS for 30 min. After three washing steps, fixed cells were overlaid with loading buffer and relative fluorescence intensities were determined with a GENios Pro microplate reader (Tecan Deutschland, Crailsheim, Germany). Measurement mode: fluorescence top; excitation filter: 485/20; emission filter: 535/25; number of reads: 10; integration time: 40 µs; lag time: 0 µs; mirror selection: Dichroic 3; plate definition file: GRE96ft.pdf; multiple reads per well (Circle): 3x3; time between move and flash: 100 ms. On each plate, the optimal gain was calculated by determination of the fluorescence intensity in the presence of the reference substance, tariquidar. Relative IC_{50} values were calculated using GraphPad Prism 7, “Four parameter curve” fitting. Errors were expressed as standard error of the mean (SEM) [13].

2.4 Calcein-AM microplate assay for the determination of ABCC1 modulation

MDCKII cells were seeded into flat-bottomed 96-well plates at a density of about 20,000 cells/well. On the following day, cells were washed with loading buffer in order to remove unspecific serum esterases. Afterwards, cells were incubated with loading suspension (loading buffer, 5 mg/mL BSA, 1.25 µL/mL

pluronic F127 (20% (m/v) in DMSO)) containing 0.5 µM calcein-AM and the test compound at increasing concentrations (10 nM-100 µM) for 60 min (37 °C / 5% CO_2). Reversan served as positive control at a final concentration of 30 µM corresponding to 100% ABCC1 inhibition. In general, test compounds were investigated as triplicates, controls as sextuplicates, respectively. Subsequently, the loading suspension was discarded, and the cells were fixed with 4% PFA solution for 30 min. After three washing circles (loading buffer), fixed cells were overlaid with loading buffer, and relative fluorescence intensities were determined with a GENios Pro microplate reader (Tecan Deutschland, Crailsheim, Germany). Measurement mode: fluorescence top; excitation filter: 485/20; emission filter: 535/25; number of reads: 10; integration time: 40 µs; lag time: 0 µs; mirror selection: Dichroic 3; plate definition file: GRE96ft.pdf; multiple reads per well (Circle): 3x3; time between move and flash: 100 ms. On each plate, the optimal gain was calculated by determination of the fluorescence intensity in the presence of the reference substance, tariquidar. Relative IC_{50} values were calculated using GraphPad Prism 7, “Four parameter curve” fitting. Errors were expressed as standard error of the mean (SEM) [13].

2.5 Hoechst 33342 microplate assay for the determination of ABCG2 modulation

Optimal conditions of the assay were systematically determined as described elsewhere [14] with slight modifications. MCF-7/Topo cells were seeded into 96-well plates at a density of 20,000 cells/well (total volume: 100 mL) and allowed to attach to the surface of the microplates overnight in a water-saturated atmosphere (95% air, 5% CO2) at 37 °C. The next day, the culture medium was removed, and the cells were incubated with loading suspension (DMEM, supplemented as described above, and 0.8 µM Hoechst 33342) in combination with the test compound at increasing concentrations (10 nM-100 µM) for 2 h (37 °C, 5% CO_2). FTC at a final concentration of 10 µM served as reference compound; under these conditions, the response was defined as 100% inhibition of Hoechst 33342 efflux. The supernatants were drained and the cells were fixed for 30 min under light protection using 100 mL per well of a 4% PFA solution. Finally, MCF-7/Topo cells were washed with PBS (2x250 mL per well) to remove residual dye. Cells were overlaid with PBS (100 mL), and the fluorescence intensities were determined using a GENios Pro microplate reader (TECAN Deutschland GmbH, Crailsheim, Germany). Measurement mode: fluorescence top; excitation filter: 340/35 nm; emission filter: 485/20 nm; number of reads: 10; integration time: 40 ms; lag time: 0 ms; mirror selection: automatic; plate definition file: GRE96ft.pdf; multiple reads per well (circle, 3x3); time between move and flash: 50 ms. On each plate, the optimal gain was calculated by determination of the fluorescence intensity in the presence of the reference compound FTC. Relative IC_{50} values were calculated using GraphPad Prism 7, “four parameter curve” fitting. Errors are expressed as standard error of the mean (SEM) [13].

2.6 Stability of test compounds Dulbecco's Modified Eagle Medium (DMEM)

Stock solutions of the test compounds (3 mM) were prepared in DMSO. A 1:50 dilution of the substances in DMEM (containing 10% FCS) was prepared in 1.5-mL polypropylene reaction vessels (Eppendorf, Hamburg, Germany). The samples were shortly vortexed and incubated at 37 °C. At different periods of time, aliquots were taken, and the samples were deproteinated by mixing with two parts of ice-cold acetonitrile. For quantitative precipitation, the samples were vortexed and stored at 4 °C for 30 min. Finally, samples were centrifuged for 5 min at 14,000 g, using an Eppendorf MiniSpin plus centrifuge, and the supernatants were transferred into new reaction vessels. Prior to HPLC analysis, the samples were diluted (1:1) with acetonitrile and stored at -80 °C. Subsequent RP-HPLC analysis were performed using a Agilent 1220 Infinity LC System equipped with a Phenomenex Luna® 3µ C18 column (150 × 2.0 mm, 100 Å) at 30°C. HPLC conditions: solvent A = water (Millipore)/TFA (0.05% v/v); solvent B = MeCN (Merck, gradient grade); flow rate = 0.3 mL/min; injection volume 100 µL; gradient: 10% to 95% B in 30 min. UV-detection at 220 nm.

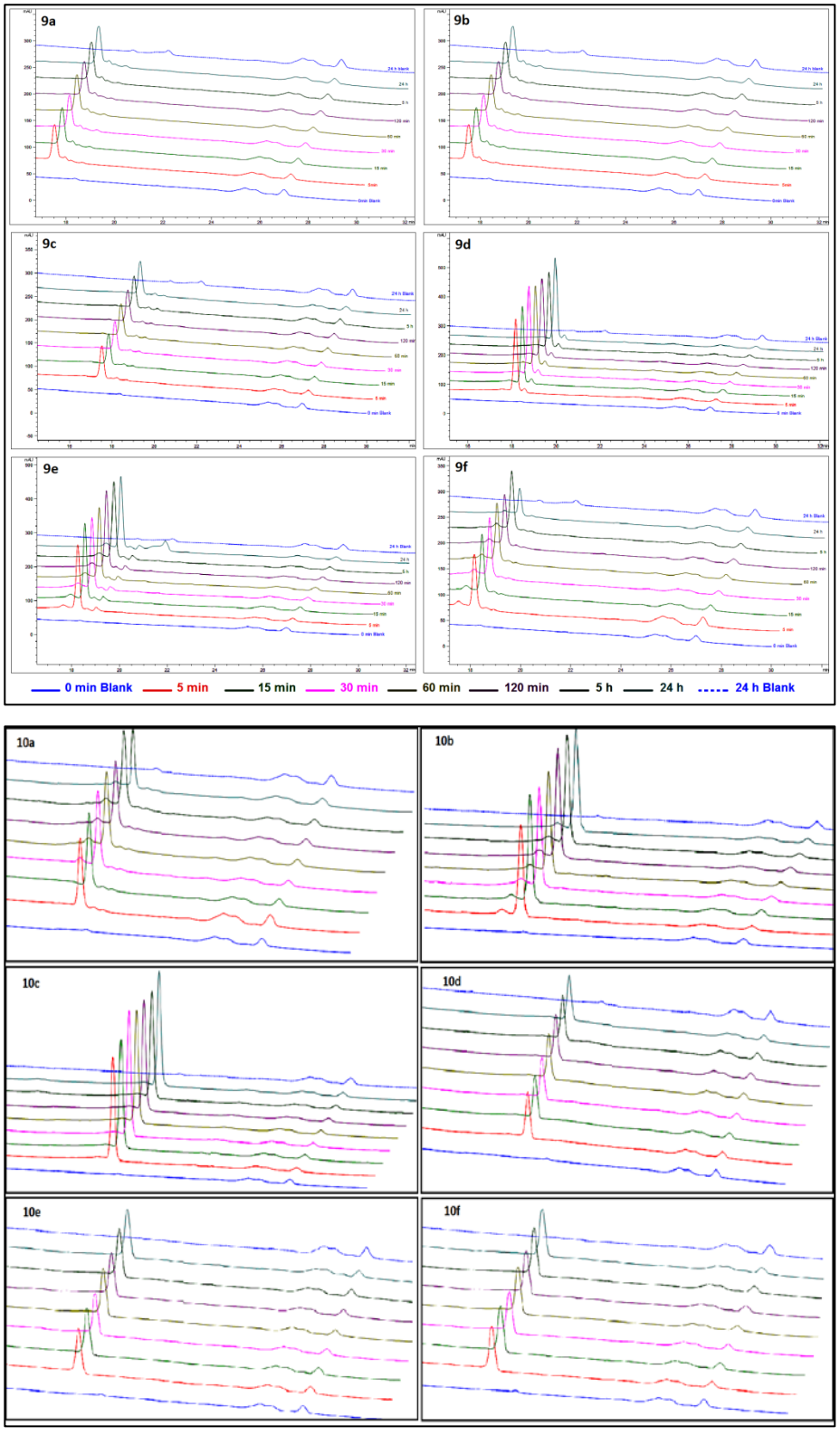


Figure 1 Chemical stability of compounds in DMEM for 24 h A) chalcones **16a-f** B) ketones **17a-f** (HPLC analysis, UV detection at 220 nm)

2.7 Chemosensitivity assay

The assays were performed as described previously [15] with slight modifications. Briefly, 100 mL/well of a tumor cell suspension, yielding a density of 10-20 cells per microscopic field (Olympus, CK2, 320x), were seeded into 96-well plates and incubated overnight at 37°C and 5% CO_2. The next day, 100 mL/well of fresh medium was added containing the test compounds in various concentrations or vehicle. In general, test compounds were added as 1000-fold concentrated feed solutions (16 wells per drug concentration). On every plate, untreated cells (solvent DMSO in a dilution of 1:1000) served as growth control (vehicle), cells treated with a reference cytostatic (vinblastine) as a positive control. Incubation of the cells was stopped after different periods of time by removal of medium and fixation with a solution of 2% glutardialdehyde (in PBS). All plates were stored at 4 °C until the end of the experiment and afterwards simultaneously stained with 100 mL/well of a 0.02% aq crystal violet solution for 20 min. The trays were rinsed with H_2O for 20 min in order to remove residual dye. Crystal violet bound by the fixed cells was extracted with 70% EtOH (180 mL/well) while shaking the microplates for 2 h. Absorbance (a parameter proportional to cell mass) was measured at 580 nm using the GENios Pro microplate reader.

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