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Electronic Supplementary Information

for

Rapid Route Design of AZD7594

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1. General Experimental Details

General laboratory experiments were conducted using Mettler-Toledo EasyMax or OptiMax automated reaction vessels fitted with overhead stirrers, addition pumps, water condensers, and nitrogen inlets. Solvents and reagents were of typical laboratory grade and were used as received without additional drying or purification unless otherwise stated.

Proton (^{1}H) and carbon-13 (^{13}C) NMR data were collected using a Bruker Ultrashield DRX500 spectrometer fitted with a BBFO probe, operating at 500 and 125 MHz respectively, using Topspin3.5pl5 software. Chemical shift values (δ) are reported in parts per million (ppm) using tetramethylsilane as an internal standard. Coupling constants (J) are reported in Hz and quoted to the nearest 0.1 Hz.

Mass spectra were collected using a Waters Synapt G2-Si High Definition Mass Spectrometer with electrospray ionisation (ESI) in positive mode, time-of-flight (TOF) mass analyser, and operated with MassLynx V4.1 software. Sample introduction was via a Waters Acquity H-Class UHPLC equipped with a Waters BEH C18 column (100 × 2.1 mm, 1.7 µm). Mass-to-charge ratios (m/z) are reported in Daltons with less than 5 ppm error.

Melting points were measured using a Mettler-Toledo differential scanning calorimeter (DSC).

Ultra-high-performance liquid chromatography (UHPLC) was performed using a Waters Acquity H-class chromatograph equipped with a Phenomenex Kinetex C18 (75 × 3 mm, 2.6 µm) column maintained at 40 °C and a photodiode array detector monitoring at 220 or 254 nm.

2. General Procedures

2.1 Salt screening

The salt screen was carried out in a high-throughput manner using glass 96-well plates. Equimolar solutions (0.025 M) of intermediate and each acidic counterion (see Table 1) were prepared in methanol. From these solutions, 100 µL of intermediate and 100 µL of each acid were added to each well of a glass 96-well plate (equimolar stoichiometry). The wells were sealed with foil, pin-holed to allow slow evaporation of the solvent, and the plates placed at 25 °C and gently shaken for 1–2 days until evaporation of the solvent was complete. Each well was subsequently analysed by polarised light microscopy (PLM) to detect the presence of crystalline, birefringent material (as opposed to liquid or non-birefringent amorphous solid). To increase the chance of crystallisation of any potential salt, four further rounds of solvent addition and evaporation was applied; 200 µL of ethyl acetate, acetonitrile, acetone and

propan-2-ol/water (50/50) were next added to the wells, allowed to evaporate and analysed again by PLM.

Table 1. Acids used in salt screening.

Acetic acid	1-Hydroxy-2-naphthoic acid
Adipic acid	2-Furoic acid
Benzenesulfonic acid	Ascorbic acid
Benzoic acid	2,5-Dihydroxybenzoic acid
Cinnamic acid	Glutaric acid
Citric acid	Glucuronic acid
DL-Lactic acid	Glycolic acid
Ethanedisulfonic acid	Hippuric acid
Ethanesulfonic acid	Ketoglutaric acid
Fumaric acid	Mandelic acid
Hydrochloric acid	Oxalic acid
Methanesulfonic acid	Propionic acid
Napadisylic acid	L-pyroglutamic acid
Phosphoric acid	Vanillic acid
L-Tartaric acid	Camphoric acid
Maleic acid	2,4,6-Trimethylbenzenesulfonic acid
Malic acid	Malonic acid
Saccharin	Succinic acid
Sulfuric acid	Toluenesulfonic acid

2.2 Solubility measurements

In a typical screen, 50 mg of the material to be measured was dispensed into each of 24 GE Whatman filter vials[1] via a Mettler-Toledo Quantos QB-5 weighing robot.[2] Vials were transferred to 48-well microtitre plate, and aliquots (500 µL) of 22 different solvents (Table 2) were then dispensed into the vials via a multichannel pipette;[3] the last two vials were reserved for one solvent to be run in triplicate to confirm repeatability. The top half of the filter vials were put into place and compressed half way; the plate was then agitated on a temperature-controlled shaker (20 °C, 600 rpm) for 20 h to allow any turnover in solid form and to ensure that the thermodynamic solubility is obtained. After incubation each vial was observed to determine the physical characteristics of the contents (i.e. solution or suspension). The filter top was then fully compressed to effect filtration, and a 40-µL sample was taken from the vial using a positive displacement pipette[4] and transferred into a 2-mL HPLC vial, to which was added 1.2 mL MeCN diluent. This sample process was repeated three times, resulting in 72 samples from the 24 initial vials. Five calibration standards were individually prepared, in order to negate errors in weighing and solvent addition. Typically a one-minute UHPLC method was developed and all standards and samples were run in a continuous sequence. The injection

order includes five injections of each standard at the beginning of the run followed by the prepared samples, the run is concluded with three injections of each of the standards. The area counts for the standards were plotted against the known masses and a line of best fit drawn, from which a gradient was reported. The average relative standard deviation (%RSD) for the standard injections was noted, as this is an indication of the precision of the UHPLC and the processing method. For each sample the area counts were converted to a solubility value (in mg/mL) using the gradient from the calibration plot. An average of the three samples (nine for the triplicate) for each solvent was reported as the solubility; %RSD was also noted as a measure of precision in the whole process. For any vials that contained solid, the filter vial was split and solid analysed by x-ray powder diffraction to determine if any physical form change had occurred in the input material. All process metadata was recorded alongside the result and stored in a central database which is linked to a TIBCO Spotfire visualisation tool accessible by all scientists in the department.

Table 2. Typical solvents used for solubility screening.

MeTHF	Butyronitrile	Ethanol	Isopropanol
Acetic Acid	CPME	Ethyl Acetate	MTBE
Acetone	Diethyl carbonate	Isopropyl acetate	THF
Acetonitrile	DMSO	Methanol	
n-Butanol	Water	DMF	
MEK	Toluene	Heptane	

2.3 Solubility predictions

Solubility predictions have been made using the program COSMOtherm (2016 version, BP_TZVPD_FINE_C30_1601 parameterisation).

In all cases the accuracy of the predictions was optimized by choosing the most appropriate experimental reference solubility to estimate the free energy of fusion. This approach, in our experience, yields more accurate results than the commonly used approach of estimating the free energy of fusion from DSC data, but with the limitation that in the 2016 version of the software, solubility can be predicted only at the same temperature as the reference experiment (this restriction does not apply to later versions, as long as the melting point is known).

Reference solubilities were taken from experimental values measured in-house at 20 °C (see Section 2.2).

Determination of the optimal reference solubility:

For each solute, solubility predictions in all the solvents for which there were valid experimental measurements were repeated several times, each time choosing a different solvent as the reference.

Statistical analysis was then performed on the results to compare the performance of the different sets of predictions. The program JMP (SAS, version 12) was used for this task.

For each solute we compared the root mean square error (RMSE) of Log(predicted) *vs* Log(measured), the standard deviation of Log(predicted/measured) and the mean of Log(predicted/measured) obtained from each reference solvent. The best reference solvent is the one for which the lowest RMSE is observed.

When the best reference solvent was identified, solubility predictions were made for all remaining pure solvents in our solvent database.

In general, it was found that for a given solute the standard deviation of Log(predicted/measured) (*i.e.* the width of the distribution) hardly varied when changing reference solvent. In all cases the main factor for differentiating reference solvents was the average of Log(predicted/measured), or, in other words, the systematic bias offsetting Log(predicted) from Log(measured).

In summary, the main reason for choosing a reference solvent is that it causes less bias in the predictions, *i.e.* its distribution of Ln(Predicted/Actual) for the entire data set is the one that is centred closest to 0. The mean of the distribution seems to be a more important factor than the tightness of the distribution (described by the standard deviation), which is not significantly different for most of the reference solvents.

Solubilities computed by COSMOtherm are expressed in g (solute) per g (solvent) and are reported to projects as mg (solute) per mL (solvent), making use of the known density of pure solvents at 20 °C. For compounds with a low solubility, the error of using this value instead of mg (solute) per mL (solution) is usually negligible. At higher concentrations the error from neglecting the contribution of the solute to the overall volume of the solution, and the non-additivity of volumes (excess volume) of non-ideal solutions grows rapidly.

For example, assuming a solute true density of 1.2 g/mL, a concentration of 10 mg/mL (solvent) [100 “relative volumes” in process development parlance] corresponds to ~99.2 mg/mL (solution), assuming perfect additivity of volumes, *i.e.* 0.8% error.

The same error grows to ~8% for a concentration of 100 mg/mL (solvent) [10 “relative volumes”] and to >80% for a concentration of 1000 mg/ml (solvent) [1 “relative volume” or a

50/50 v/v solution – at this stage the distinction between solute and solvent is a dubious concept and excess volume effects should no longer be ignored].

2.4 High-throughput experiments

Reactions were performed on a 0.1–0.4 mmol scale, either in 1-mL or 4-mL glass vials containing Parylene-coated magnetic stir discs and situated in an aluminium heating block inside a glovebox (<10 ppm of O_2, <1 ppm of H_2O). In a typical reaction, a stock solution of copper iodide and internal standard (m-terphenyl, 10 mol%) in MeCN (0.02 M) was dispensed to each vial via pipette, and the carrier solvent was evaporated using a Genevac EZ-2 centrifugal evaporator situated inside the glovebox. This process was then repeated with stock solutions of the relevant ligands in 1,2-dichloroethane. The reactants and bases were then weighed and dispensed directly into the vials using a Mettler-Toledo Quantos QB-5 weighing robot.[2] Dry, degassed organic solvents were added at ambient temperature, and the vials were then sealed, heated to 100 °C and left to stir (400 rpm) on a V&P Scientific VP 710C5-7A tumble stirrer. Samples (15 µL of reaction mixture diluted into 1.2 mL MeCN/water, 4:1) were taken for UHPLC analysis at 1 h and 20 h. Samples were analysed by gradient chromatography using a flow rate of 1.2 mL/min, ammonium acetate (0.1% m/v) in water as eluent A and ammonium acetate (0.1% m/v) in acetonitrile/water (95:5) as eluent B. The following gradient was applied: 5 to 95% B over 4.0 min, hold for 0.5 min, 5% B for 0.5 min. The resulting data was processed using Microsoft Excel.

2.5 Process Analytical Technologies

Raman spectra were recorded using a non-contact Raman probe (NCO-1.3-NIR, SN: 2004066-601) focused through the viewing window of an EasyMax unit and fitted to a Kaiser Optical Systems Raman RXN1-785 spectrometer. See Figure 1 for a photograph of the set-up.

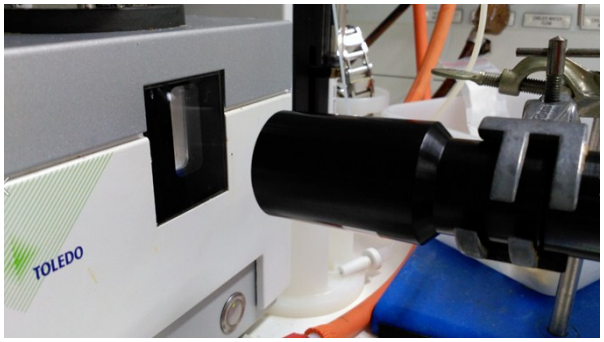


Figure 1. Non-contact Raman set up

Data was collected every minute using an exposure time of 0.4 seconds and 50 accumulations.

The raw data was taken into PLS Toolbox 8.2 (Eigenvector Research Inc.). To remove any minor baseline deviations a Whittaker filter was applied (λ = 3000; p = 0.001) and to remove any laser fluctuations an infinity normalisation was applied using the solvent band at 1003 cm^{-1}

Thionyl chloride band was tracked using a peak height centred on 485 cm^{-1}.

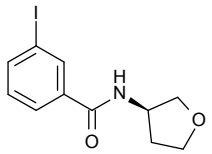
Sulfur dioxide band was tracked using a peak height centred on 1145 cm^{-1}.

A Multivariate Curve Resolution model was applied over the spectral range of 1820–1660 cm^{-1} (carbonyl region) using the default settings of non-negativity constraints in the spectral and concentration domains.

3. Compound Characterisation Data

The preparation and characterisation of compounds **1**,[5] **4**,[6] **6–8,**[7] **20**[5] and **23–24**[5] have been previously described.

3-Iodo-*N*-[(3*R*)-tetrahydrofuran-3-yl]benzamide (22)

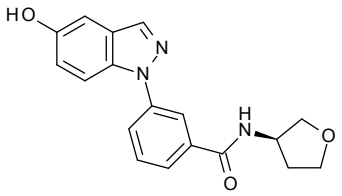


3-Iodobenzoic acid **11** (4.25 g, 17.1 mmol, 1.0 equiv.), toluene (43 mL), pyridine (70 µL, 0.86 mmol, 0.05 equiv.), and thionyl chloride (2.12 mL, 29.1 mmol, 1.7 equiv.) were charged to a 250 mL reactor vessel fitted with an overhead stirrer, fin condenser, N_2 inlet, and NaOH scrubber. The reaction mixture was stirred for 4 h at reflux before being removed from vessel to afford a yellow solution of acid chloride **43** (45 mL), which was used in the next step without purification. (3*R*)-3-Aminotetrahydrofuran hydrochloride **16** (2.12 g, 17.2 mmol, 1.0 equiv.), toluene (137 mL), and K_2CO_3 (2 M aqueous, 21.4 mL, 42.9 mmol, 2.5 equiv.) were charged to a 250 mL reactor vessel fitted with an overhead stirrer, N_2 inlet, and NaOH scrubber at 0 °C. A solution of acid chloride **43** in toluene (45 mL) was charged *via* dropping funnel over 1 h. The reaction slurry was left to stir at 500 rpm for 6 h before being filtered under reduced pressure. The resulting solution was washed with water (30 mL) and toluene (30 mL), and evaporated under reduced pressure to yield the desired product **22** as a white solid (3.55 g, 65%).

M.P. 144–146 °C; IR (ATR) ν_{max} 1629, 2849, 3212 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, 27 °C) δ 1.86–1.96 (m, 1 H), 2.09–2.20 (m, 1 H), 3.58 (dd, J = 9.0, 4.2 Hz, 1 H), 3.71 (app td, J = 8.1, 5.8 Hz, 1 H), 3.84 (dd, J = 8.9, 6.2 Hz, 2 H), 4.40–4.49 (m, 1 H), 7.27 (app t, J = 7.8 Hz, 1 H),

7.84–7.92 (m, 2 H), 8.22 (app t, J = 1.6 Hz, 1 H), 8.63 (br d, J = 6.4 Hz, 1 H) ppm; ^{13}C NMR (125 MHz, 27 °C, DMSO-d_6) δ 31.7 (CH_2), 50.3 (CH), 66.4 (CH_2), 72.1 (CH_2), 94.5 (C), 126.8 (CH), 130.3 (CH), 135.6 (C), 136.2 (CH), 139.6 (CH), 164.7 (C) ppm; HRMS (ESI) m/z: $[M+H]^+$ calcd. for $C_{11}H_{13}INO_2$ 317.9958, found 317.9988.

3-(5-Hydroxyindazol-1-yl)-*N*-[(3*R*)-tetrahydrofuran-3-yl]benzamide (17)[8]

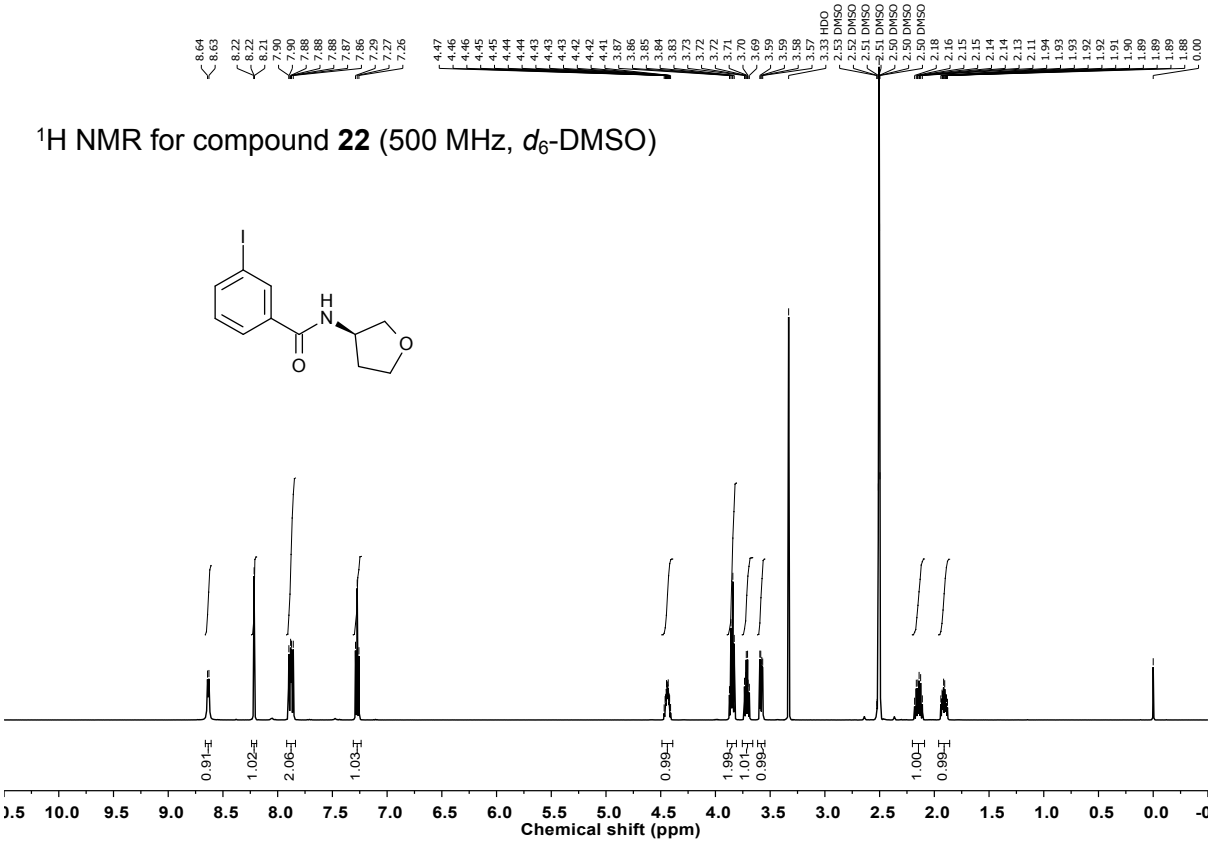


5-Hydroxyindazole **13** (499 mg, 3.72 mmol, 1.0 equiv.), amide **22** (1.19 g, 3.72 mmol, 1.0 equiv.), copper(I) iodide (35.1 mg, 0.18 mmol, 5.0 mol%), *trans*-cyclohexane-1,2-diamine (45 µL, 0.37 mmol, 10 mol%) and K_2CO_3 (520 mg, 3.76 mmol, 1.0 equiv.) were charged to a Stem block tube equipped with a magnetic stirrer. The tube was then evacuated (10 mins) and backfilled with nitrogen (10 mins); this process was repeated twice. Degassed butyronitrile (10 mL) was added *via* syringe and the reaction mixture heated to 110 °C and stirred at 500 rpm for 24 h. After this time, the reaction was cooled to RT and filtered; the filter was washed with butyronitrile (5 mL). The combined filtrate was washed with aq. NH_4Cl (25% w/w, 3 × 5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting solid was dissolved in THF (20 mL) at reflux and stirred for 30 mins. The hot solution was then filtered, allowed to cool and concentrated *in vacuo* to approximately 6 mL to effect crystallisation. The resulting solid was collected by filtration, washed with THF (5 mL) and dried in a vacuum oven to afford the product **17** as a brown solid (400 mg, 33%).

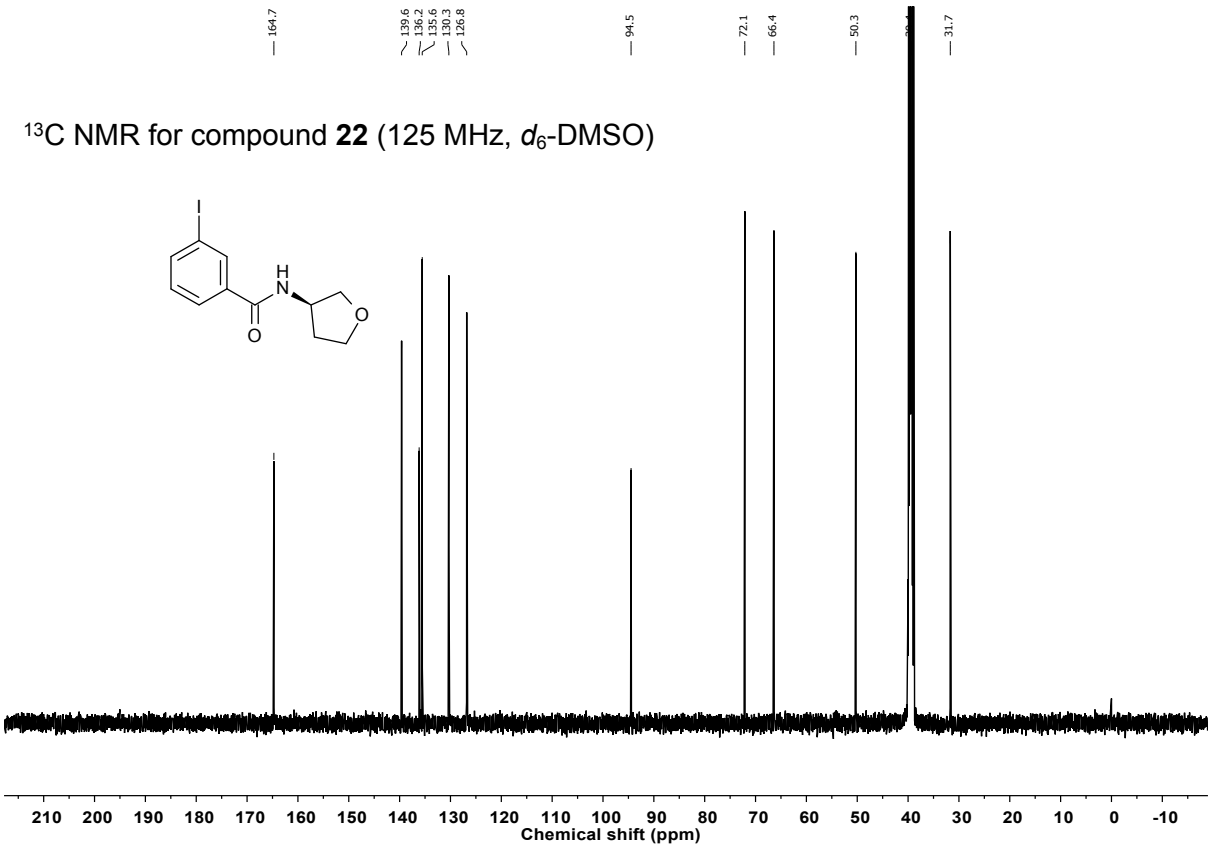
M.P. 234–235 °C; IR (ATR) v_{max} 1636, 2898, 3212, 3318 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, 27 °C) δ 1.92–2.00 (m, 1 H), 2.12–2.22 (m, 1 H), 3.36 (br s, 1 H), 3.63 (dd, J = 8.9, 4.3 Hz, 1 H), 3.73 (app td, J = 8.1, 5.9 Hz, 1 H), 3.83–3.92 (m, 2 H), 4.50 (dddd, J = 7.9, 6.4, 4.1, 2.0 Hz, 1 H), 7.07 (dd, J = 9.0, 2.3 Hz, 1 H), 7.14 (d, J = 2.1 Hz, 1 H), 7.66 (app t, J = 7.9 Hz, 1 H), 7.73 (d, J = 9.0 Hz, 1 H), 7.86 (d, J = 7.9 Hz, 1 H), 7.89–7.93 (m, 1 H), 8.22 (d, J = 0.8 Hz, 2 H), 8.74 (br d, J = 6.4 Hz, 1 H) ppm; ^{13}C NMR (126 MHz, 27 °C, DMSO-d_6) δ 31.7 (CH_2), 50.3 (CH), 66.4 (CH_2), 72.2 (CH_2), 103.3 (CH), 111.2 (CH), 118.8 (CH), 120.3 (CH) 123.9 (CH), 124.9 (CH), 126.2 (C), 129.5 (CH), 133.1 (C), 134.9 (CH), 135.7 (C), 139.8 (C), 152.4 (C), 165.6 (C) ppm; HRMS (ESI) m/z: $[M+H]^+$ calcd. for $C_{18}H_{18}N_3O_3$ 324.1366, found 324.1346.

4. NMR Spectra

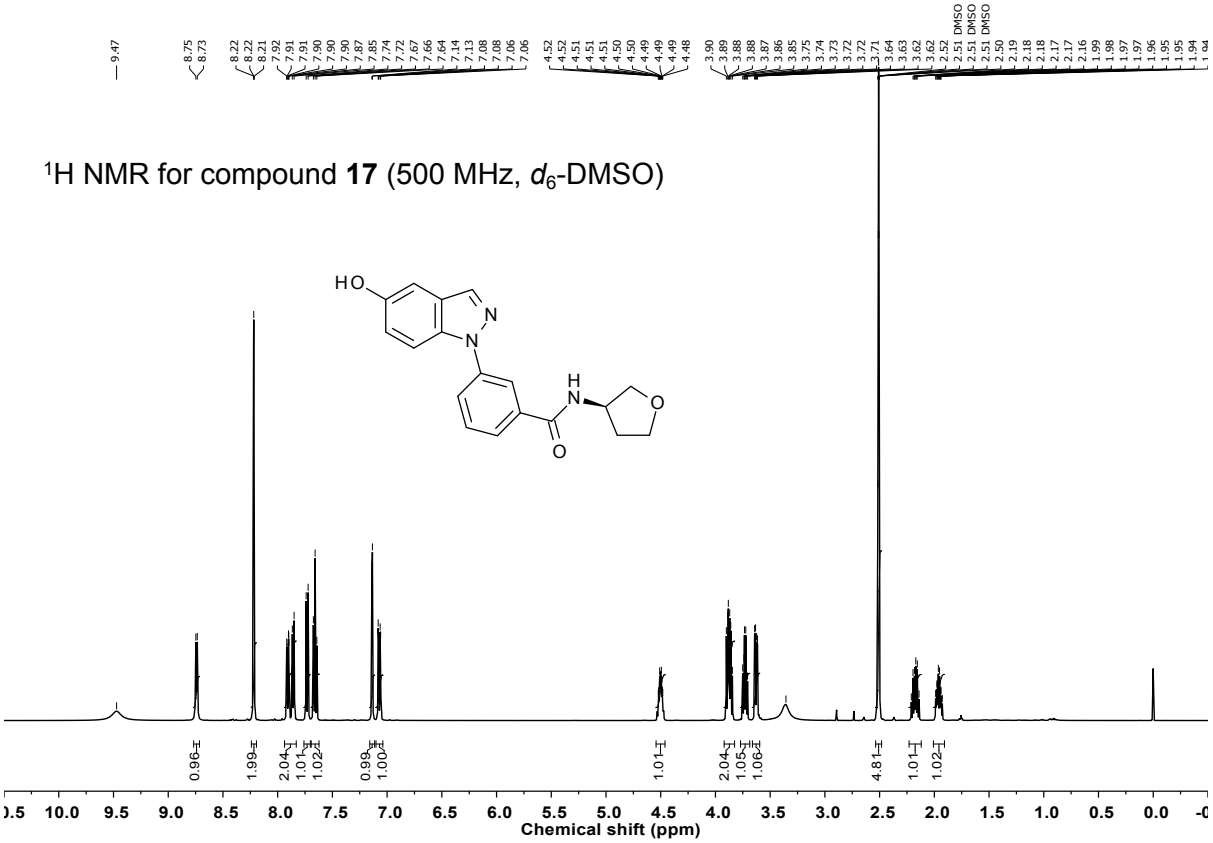
^{1}H NMR for compound **22** (500 MHz, d_6-DMSO)



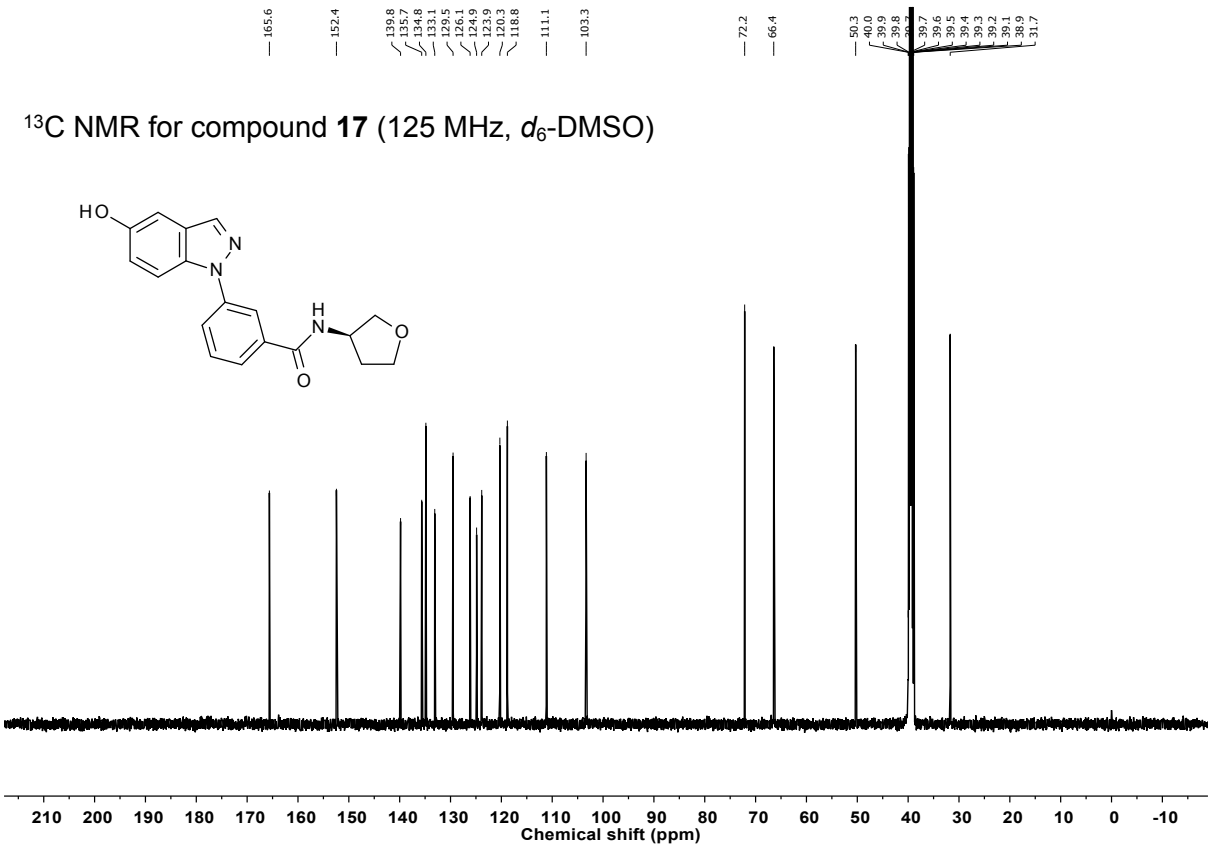
^{13}C NMR for compound **22** (125 MHz, d_6-DMSO)



^{1}H NMR for compound **17** (500 MHz, d_6-DMSO)



^{13}C NMR for compound **17** (125 MHz, d_6-DMSO)



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