

Supporting Information

Synthesis and evaluation of a radioiodinated tracer with specificity for poly(ADP-ribose) polymerase-1 (PARP-1) *in vivo*.

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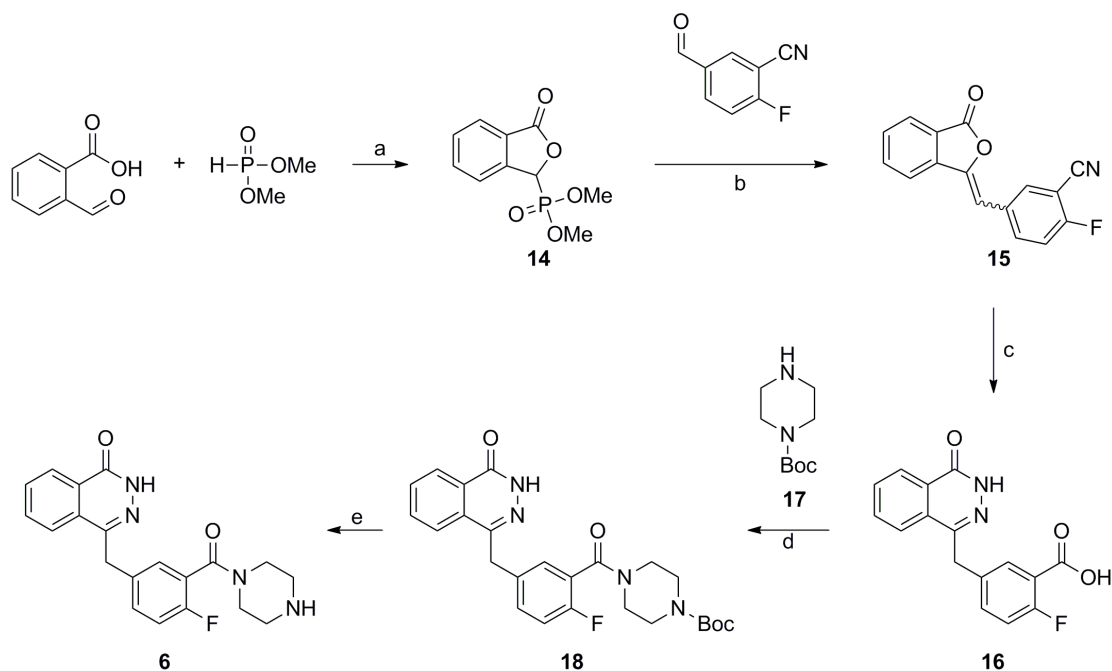
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Table of Contents

1. Synthesis of the penultimate compound 6 .	S2–6
2. ¹ H- and ¹³ C-NMR for all new compounds.	S7–22
3. Cell-free IC ₅₀ curves.	S23
4. Cellular IC ₅₀ curves for compounds 1 and 5 .	S24
5. Plasma stability graph for compound 5 .	S24
6. Metabolic stability graphs for compounds 1 and 5 .	S25
7. Radiochemistry HPLC chromatograms.	S26
8. References	S27

1. Synthesis of the penultimate compound 6.



Reagents and conditions: (a) NaOMe, MeOH, MeSO_3H , 0 °C to rt, 2 h, 96%; (b) Et_3N , THF, 0 °C to rt, 48 h, 98%; (c) (i) 13 M NaOH, 90 °C, 24 h; then (ii) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, 70 °C, 72 h, 96% over two steps; (d) HBTU, Et_3N , DMF, rt to 50 °C, 72 h, 71%; (e) 6 M HCl, EtOH, rt, 24 h, 71%.

All reagents and starting materials were obtained from commercial sources and used as received. All dry solvents were purified using a solvent purification system. All reactions were performed under an atmosphere of argon unless otherwise mentioned. Flash column chromatography was performed using Fisher matrix silica gel 60 (35–70 μm). Macherey-Nagel aluminium-backed plates pre-coated with silica gel 60F₂₅₄ were used for thin layer chromatography and were visualized with a UV lamp or by staining with potassium permanganate. ^1H NMR spectra were recorded on a Bruker DPX 400 spectrometer or a Bruker 500 spectrometer and data are reported as follows: chemical shift in ppm relative to Me_4Si or the solvent (CDCl_3 , δ 7.26 ppm, CD_3OD , δ 3.31 ppm or $\text{DMSO}-d_6$, δ 2.50 ppm) as

the internal standard, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or overlap of nonequivalent resonances, integration). ^{13}C NMR spectra were recorded on a Bruker DPX NMR spectrometer at either 101 or 126 MHz and data are reported as follows: chemical shift in ppm relative to Me_4Si or the solvent as internal standard (CDCl_3 , δ 77.2 ppm, CD_3OD , δ 49.0 ppm or $\text{DMSO}-d_6$, δ 39.5 ppm), multiplicity with respect to proton (deduced from DEPT experiments, C, CH, CH_2 or CH_3). Infrared spectra were recorded using a Shimadzu IRPrestige-21 spectrometer; wavenumbers are indicated in cm^{-1} . Mass spectra were recorded using electron impact, chemical ionization or electrospray techniques. HRMS spectra were recorded using a JEOL JMS-700 spectrometer. Melting points are uncorrected.

4-[4'-Fluoro-3'-(piperazine-1''-carbonyl)benzyl]-2H-phthalazin-1-one (6).¹ To a suspension of **18** (1.40 g, 3.00 mmol) in EtOH (30 mL) was added 6 M HCl (60 mL) and the mixture was stirred overnight at room temperature. EtOH was removed under reduced pressure and the aqueous layer was basified to approximately pH 10 using 4 M NaOH. The product was extracted into CH_2Cl_2 (3×30 mL) and the organic layer was washed with water (3×50 mL), dried with MgSO_4 and concentrated *in vacuo*. Purification by recrystallization with CH_2Cl_2 (5 mL) and petroleum ether (25 mL) gave **6** (0.776 g, 71%) as a yellow foam; ^1H NMR (400 MHz, CDCl_3) δ 2.25–3.15 (m, 4H), 3.16–3.96 (m, 4H), 4.25 (s, 2H), 5.99 (br s, 1H), 6.97 (t, J 8.8 Hz, 1H), 7.24–7.35 (m, 2H), 7.64–7.76 (m, 3H), 8.38–8.46 (m, 1H), 12.01 (br s, 1H); ^{13}C NMR (101 MHz, CDCl_3) δ 37.9 (CH_2), 43.3 (CH_2), 45.9 (CH_2), 46.4 (CH_2), 48.4 (CH_2), 116.1 (CH, d, $J_{\text{C-C-F}}$ 22.1 Hz), 124.4 (C, d, $J_{\text{C-C-F}}$ 18.4 Hz), 125.2 (CH), 127.3 (CH), 128.4 (C), 129.2 (CH, d, $J_{\text{C-C-C-F}}$ 3.8 Hz), 129.7 (C), 131.3 (CH, d, $J_{\text{C-C-C-F}}$ 8.0 Hz), 131.7 (CH), 133.8 (CH), 134.3 (C, d, $J_{\text{C-C-C-F}}$ 3.0 Hz), 145.7 (C), 157.2 (C, d, $J_{\text{C-F}}$ 247.7 Hz), 160.8 (C), 165.0 (C); HRMS (ESI) calcd for $\text{C}_{20}\text{H}_{20}\text{FN}_4\text{O}_2$ (MH^+), 367.1565, found 367.1553.

3-Dimethoxyphosphoryl-3*H*-2-benzofuran-1-one (14).¹ Dimethylphosphite (0.92 mL, 10.0 mmol) was added dropwise to a solution of NaOMe (0.583 g, 10.8 mmol) in MeOH (15 mL) at 0 °C. To the solution, 2-carboxybenzaldehyde (1.00 g, 6.66 mmol) was added portionwise while stirring. The mixture was gradually warmed to room temperature and stirred for 2 h. Methanesulfonic acid (0.77 mL, 11.9 mmol) was added dropwise and the mixture was allowed to stir for a further 0.5 h. The solution was concentrated *in vacuo* to produce a white solid, to which water was added (30 mL) and the crude product was extracted into CH₂Cl₂ (3 × 30 mL). The organic layer was washed with water (2 × 30 mL), dried with MgSO₄ and filtered. The filtrate was concentrated *in vacuo* and washed with Et₂O (3 × 20 mL) to give **14** (1.54 g, 96%) as a white solid. Mp 90–91 °C; ¹H NMR (400 MHz, CD₃OD) δ 3.72 (d, *J* 10.8 Hz, 3H), 3.91 (d, *J* 10.8 Hz, 3H), 6.10 (d, *J* 10.8 Hz, 1H), 7.68 (t, *J* 7.5 Hz, 1H), 7.75 (d, *J* 7.5 Hz, 1H), 7.83 (t, *J* 7.5 Hz, 1H), 7.94 (d, *J* 7.5 Hz, 1H); ¹³C NMR (101 MHz, CD₃OD) δ 54.9 (CH₃, d, *J*_{C-O-P} 6.8 Hz), 55.3 (CH₃, d, *J*_{C-O-P} 7.4 Hz), 76.4 (CH, d, *J*_{C-P} 167.0 Hz), 124.7 (CH, d, *J*_{C-C-C-P} 3.0 Hz), 126.2 (C, d, *J*_{C-C-P} 4.1 Hz), 126.8 (CH, d, *J*_{C-C-C-C-P} 1.9 Hz), 131.2 (CH, d, *J*_{C-C-C-C-P} 2.9 Hz), 135.9 (CH, d, *J*_{C-C-C-C-P} 2.8 Hz), 145.2 (C, d, *J*_{C-C-C-P} 4.0 Hz), 171.3 (C, d, *J*_{C-O-C-P} 2.7 Hz); MS (EI) *m/z* 242 (M⁺, 37), 213 (10), 199 (9), 133 (100), 105 (13), 77 (17), 51 (5).

2-Fluoro-5-[(3'-oxo-2'-benzofuran-1'-ylidene)methyl]benzonitrile (15).¹ A solution of **14** (3.87 g, 16.0 mmol) and 2-fluoro-5-formylbenzonitrile (2.26 g, 15.2 mmol) in THF (50 mL) was prepared at room temperature. The solution was then cooled to 0 °C followed by the addition of Et₃N (2.15 mL, 16.0 mmol). The reaction mixture was allowed to warm up to room temperature and was stirred for 48 h, followed by concentration *in vacuo* to produce a white solid. The solid was suspended in water, collected by vacuum filtration and washed with hexane (2 × 20 mL), Et₂O (2 × 20 mL), and MeOH (2 × 20 mL) to give **15** (3.77 g, 98%) as a white solid. NMR spectra showed a 3:1 mixture of *E* and *Z* isomers. Only data for the major isomer were recorded. Mp 164–167 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.98 (s, 1H), 7.65 (t, *J* 9.0 Hz, 1H), 7.67–7.75 (m, 1H), 7.92 (td, *J* 7.6, 1.0 Hz, 1H), 7.97–8.00 (m, 1H), 8.08 (dt, *J* 8.0, 1.0 Hz, 1H), 8.12–8.21 (m, 1H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 100.8 (C, d, *J*_{C-}

$_{\text{C-F}}$ 15.8 Hz), 103.4 (CH), 113.8 (C), 117.4 (CH, d, $J_{\text{C-C-F}}$ 20.0 Hz), 121.0 (CH), 122.6 (C), 125.4 (CH), 130.9 (CH), 131.0 (C, d, $J_{\text{C-C-C-C-F}}$ 3.7 Hz), 134.2 (CH), 135.4 (CH), 136.7 (CH, d, $J_{\text{C-C-C-F}}$ 9.0 Hz), 139.5 (C), 145.4 (C), 161.5 (C, d, $J_{\text{C-F}}$ 258.1 Hz), 165.9 (C); MS (EI) m/z 265 (M^+ , 100), 237 (12), 208 (64), 182 (10), 133 (13), 104 (14), 63 (29), 45 (8).

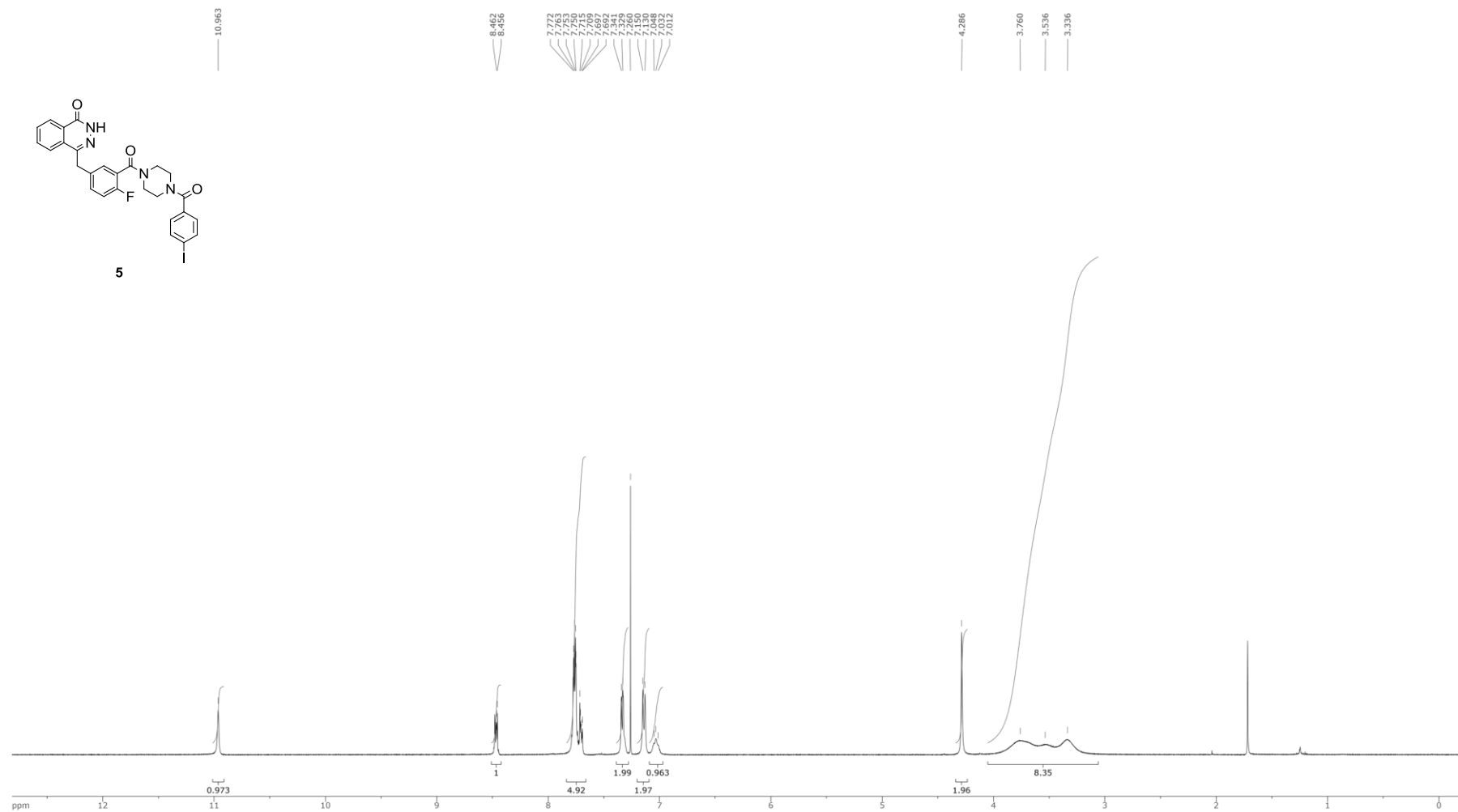
2-Fluoro-5-[(4'-oxo-3'-H-phthalazin-1'-yl)methyl]benzoic acid (16).¹ 2-Fluoro-5-[(3'-oxo-2'-benzofuran-1'-ylidene)methyl]benzonitrile (**15**) (1.77 g, 7.00 mmol) was suspended in water (30 mL) and 13 M NaOH was added (30 mL). The mixture was heated to 90 °C and stirred for 24 h, after which it was cooled to 70 °C, followed by the addition of hydrazine monohydrate (4.76 mL, 98.0 mmol) and a further 72 h of stirring. The mixture was then cooled to room temperature and acidified with 8 M HCl to an approximate pH of 4. The solid precipitate was collected by vacuum filtration and washed with water (3 \times 25 mL) and Et₂O (4 \times 25 mL) to give **16** (2.00 g, 96%) as a red solid. Mp >300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.36 (s, 2H), 7.24 (dd, J 10.8, 8.5 Hz, 1H), 7.54–7.62 (m, 1H), 7.79–7.87 (m, 2H), 7.90 (td, J 7.5, 1.3 Hz, 1H), 7.98 (d, J 7.5 Hz, 1H), 8.26 (dd, J 7.8, 1.3 Hz, 1H), 12.60 (s, 1H), 13.24 (br s, 1H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 36.3 (CH₂), 117.0 (CH, d, $J_{\text{C-C-F}}$ 22.8 Hz), 119.1 (C, d, $J_{\text{C-C-F}}$ 10.7 Hz), 125.5 (CH), 126.1 (CH), 127.9 (C), 129.1 (C), 131.6 (CH), 131.9 (CH), 133.6 (CH), 134.3 (C, d, $J_{\text{C-C-C-C-F}}$ 3.4 Hz), 134.9 (CH, d, $J_{\text{C-C-C-F}}$ 8.7 Hz), 144.9 (C), 159.4 (C), 160.3 (C, d, $J_{\text{C-F}}$ 255.5 Hz), 165.0 (C, d, $J_{\text{C-C-C-F}}$ 2.8 Hz); HRMS (ESI) calcd for C₁₆H₁₀FN₂O₃ (M-H^-), 297.0681, found 297.0671.

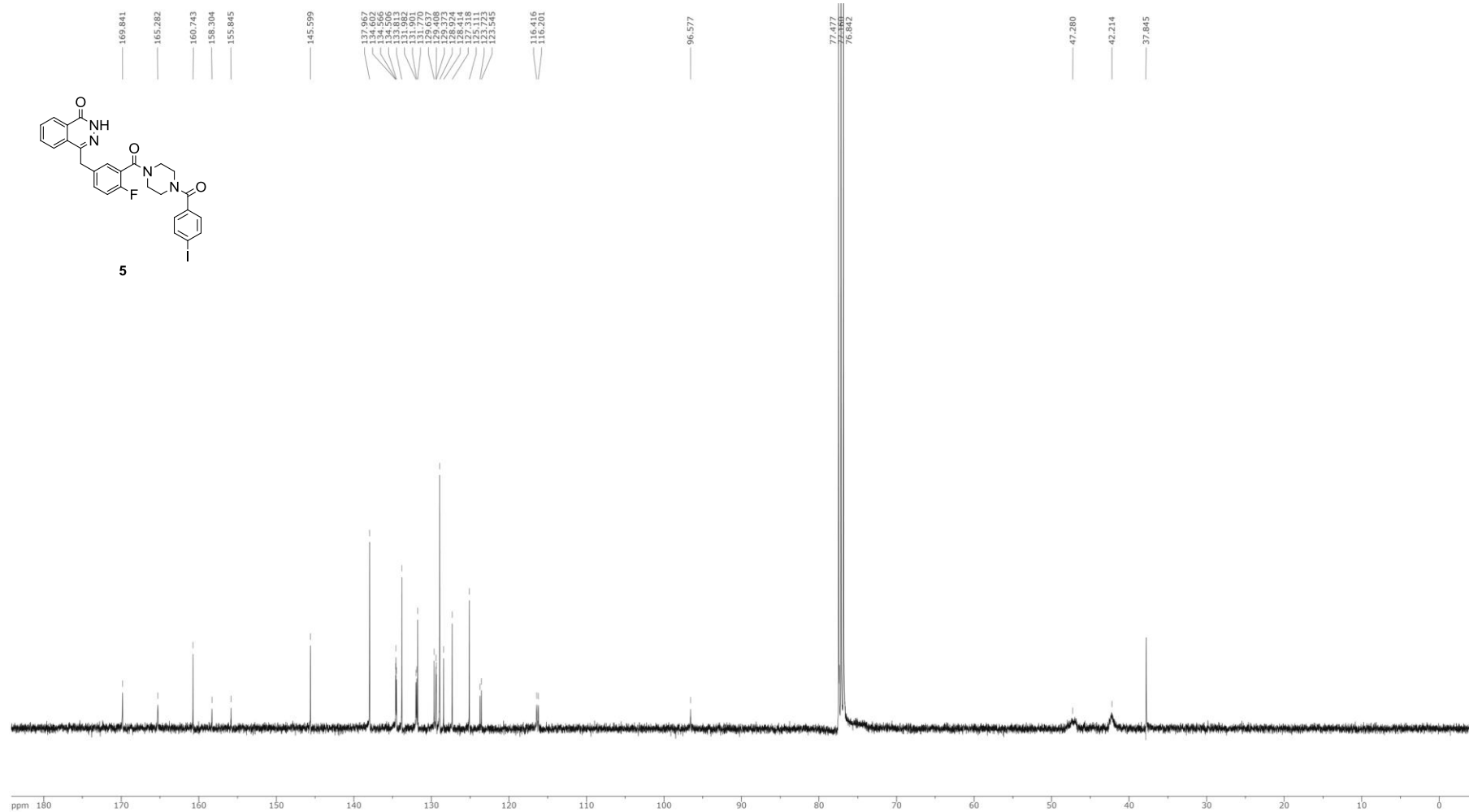
tert-Butyl piperazine-1-carboxylate (17). Di-*tert*-butyl dicarbonate (4.04 g, 18.5 mmol) was dissolved in glacial AcOH (20 mL) and this was added dropwise to a solution of piperazine (1.60 g, 18.5 mmol) in 50% AcOH (40 mL) at room temperature. The mixture was stirred for 2 h, followed by addition of water (50 mL) and removal of the precipitate by filtration. The filtrate was basified to approximately pH 10 using 6 M KOH and the product was extracted into CH₂Cl₂ (3 \times 25 mL). The organic layer was dried with Na₂SO₄ and concentrated *in vacuo* to give **17** (1.58 g, 46%) as a white solid. Mp 46–48 °C (lit.² 46–47 °C); ¹H NMR (400 MHz, CD₃OD) δ 1.46 (s, 9H), 2.75 (t, J 5.0 Hz, 4H), 3.38 (t, J 5.0 Hz,

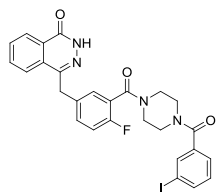
4H); ^{13}C NMR (101 MHz, CD_3OD) δ 28.7 ($3 \times \text{CH}_3$), 44.8 ($2 \times \text{CH}_2$), 46.3 ($2 \times \text{CH}_2$), 81.1 (C), 156.4 (C); MS (CI) m/z 187 (MH^+ , 78), 175 (5), 131 (100), 87 (20), 73 (10).

tert-Butyl 4-[2'-fluoro-5'-[(4''-oxo-3''*H*-phthalazin-1''-yl)methyl]benzoyl]piperazine-1-carboxylate (18).¹ To a solution of **16** (2.75 g, 9.23 mmol) in DMF (70 mL) was added Et_3N (2.00 mL, 14.8 mmol), followed by HBTU (3.85 g, 10.2 mmol). The mixture was stirred at room temperature for 1 h, after which it was warmed to 50 °C and **17** (1.72 g, 9.23 mmol) was added with continued stirring for 72 h. Water (70 mL) was then added and the mixture was stirred at 50 °C for a further 2 h, followed by cooling to 0 °C. The resulting precipitate was collected by vacuum filtration and washed with water (5×25 mL), Et_2O (2×25 mL), and *i*-PrOH (50 mL) to give **18** (3.04 g, 71%) as an orange solid. Mp 214–216 °C; ^1H NMR (400 MHz, CDCl_3) δ 1.46 (s, 9H), 3.27 (br s, 2H), 3.38 (t, J 5.1 Hz, 2H), 3.51 (br s, 2H), 3.75 (br s, 2H), 4.28 (s, 2H), 7.03 (t, J 8.8 Hz, 1H), 7.27–7.36 (m, 2H), 7.68–7.73 (m, 1H), 7.74–7.80 (m, 2H), 8.44–8.50 (m, 1H), 10.52 (s, 1H); ^{13}C NMR (101 MHz, CDCl_3) δ 28.5 ($3 \times \text{CH}_3$), 37.9 (CH_2), 42.1 ($2 \times \text{CH}_2$), 47.0 ($2 \times \text{CH}_2$), 80.5 (C), 116.3 (CH, d, $J_{\text{C-C-F}}$ 22.2 Hz), 124.1 (C, d, $J_{\text{C-C-F}}$ 17.9 Hz), 125.2 (CH), 127.3 (CH), 128.5 (C), 129.3 (CH, d, $J_{\text{C-C-C-F}}$ 3.7 Hz), 129.7 (C), 131.7 (CH, d, $J_{\text{C-C-C-F}}$ 8.1 Hz), 131.8 (CH), 133.8 (CH), 134.5 (C, d, $J_{\text{C-C-C-C-F}}$ 3.0 Hz), 145.7 (C), 154.6 (C), 157.1 (C, d, $J_{\text{C-F}}$ 246.1 Hz), 160.6 (C), 165.2 (C); MS (EI) m/z 466 (M^+ , 17), 365 (40), 311 (30), 281 (100), 254 (30), 196 (12), 178 (42), 165 (10), 85 (16), 57 (57).

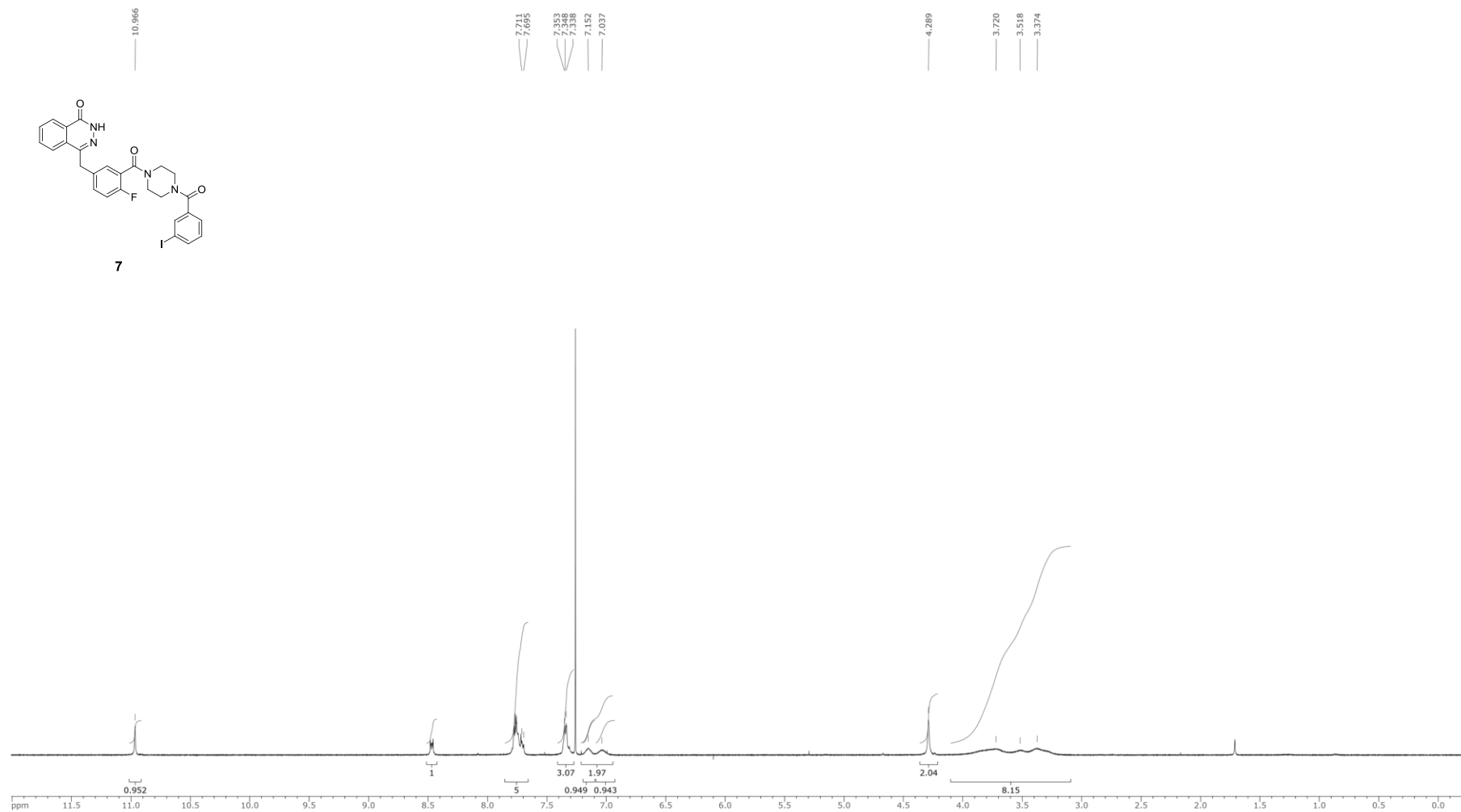
2. ^1H - and ^{13}C -NMR for all new compounds.

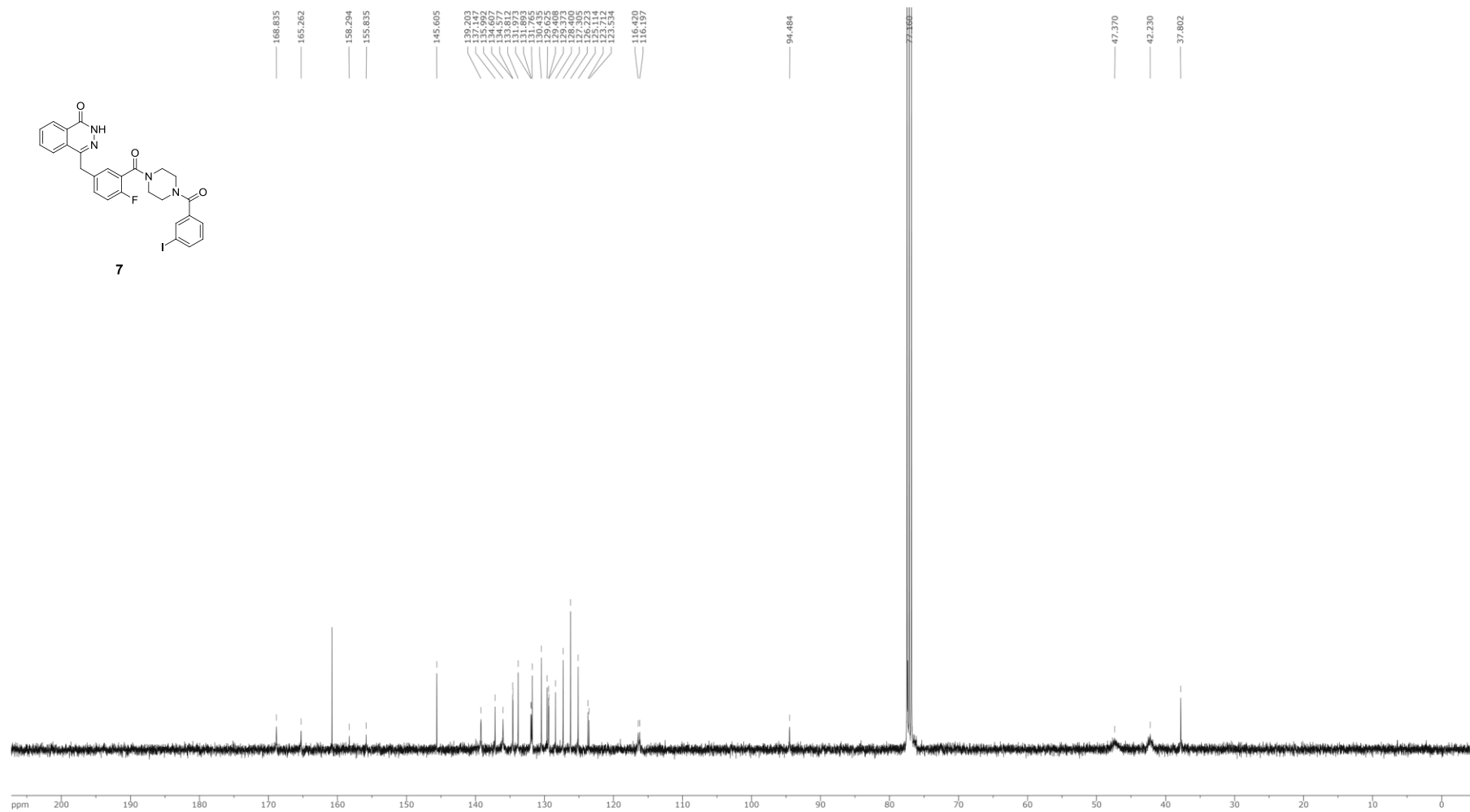


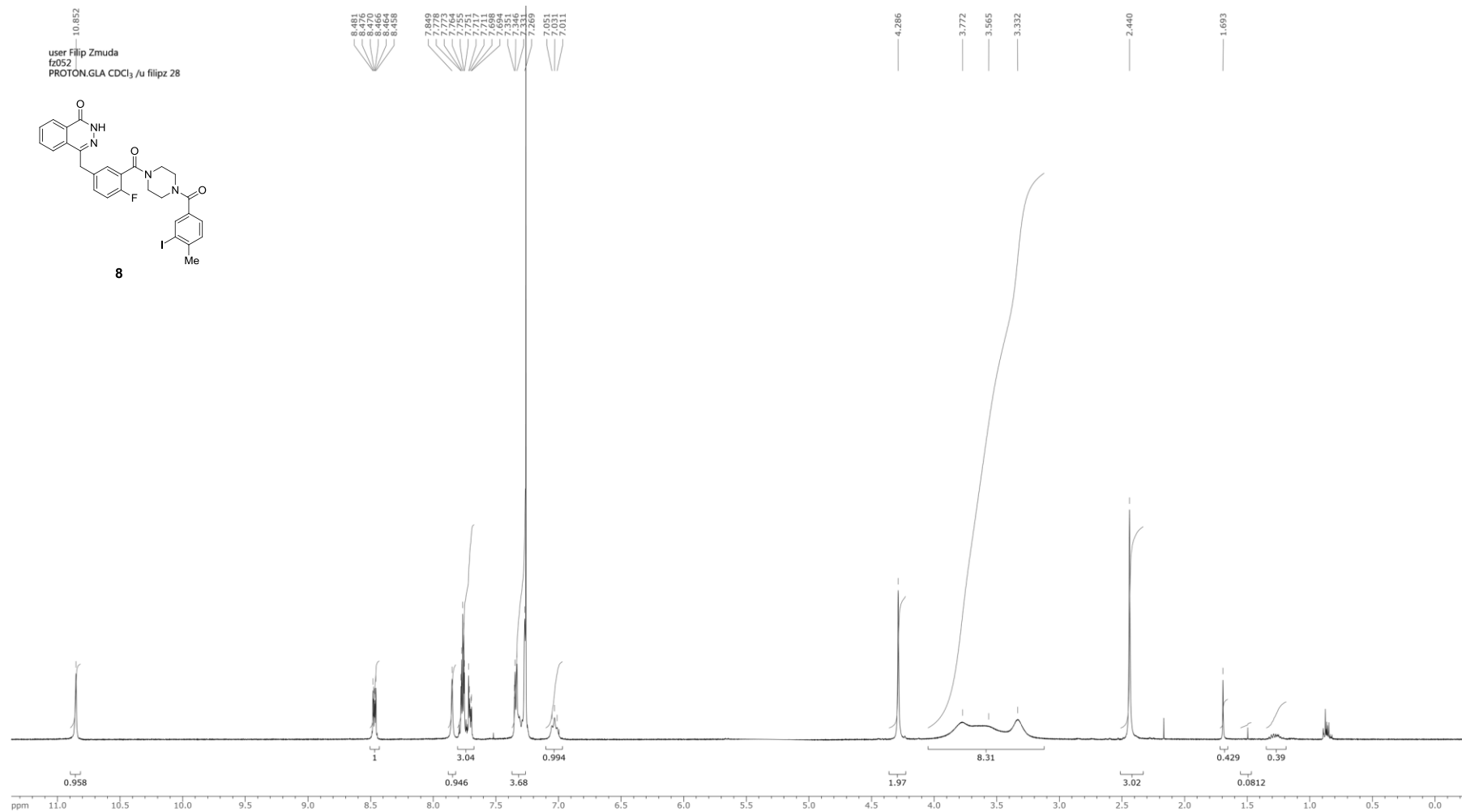


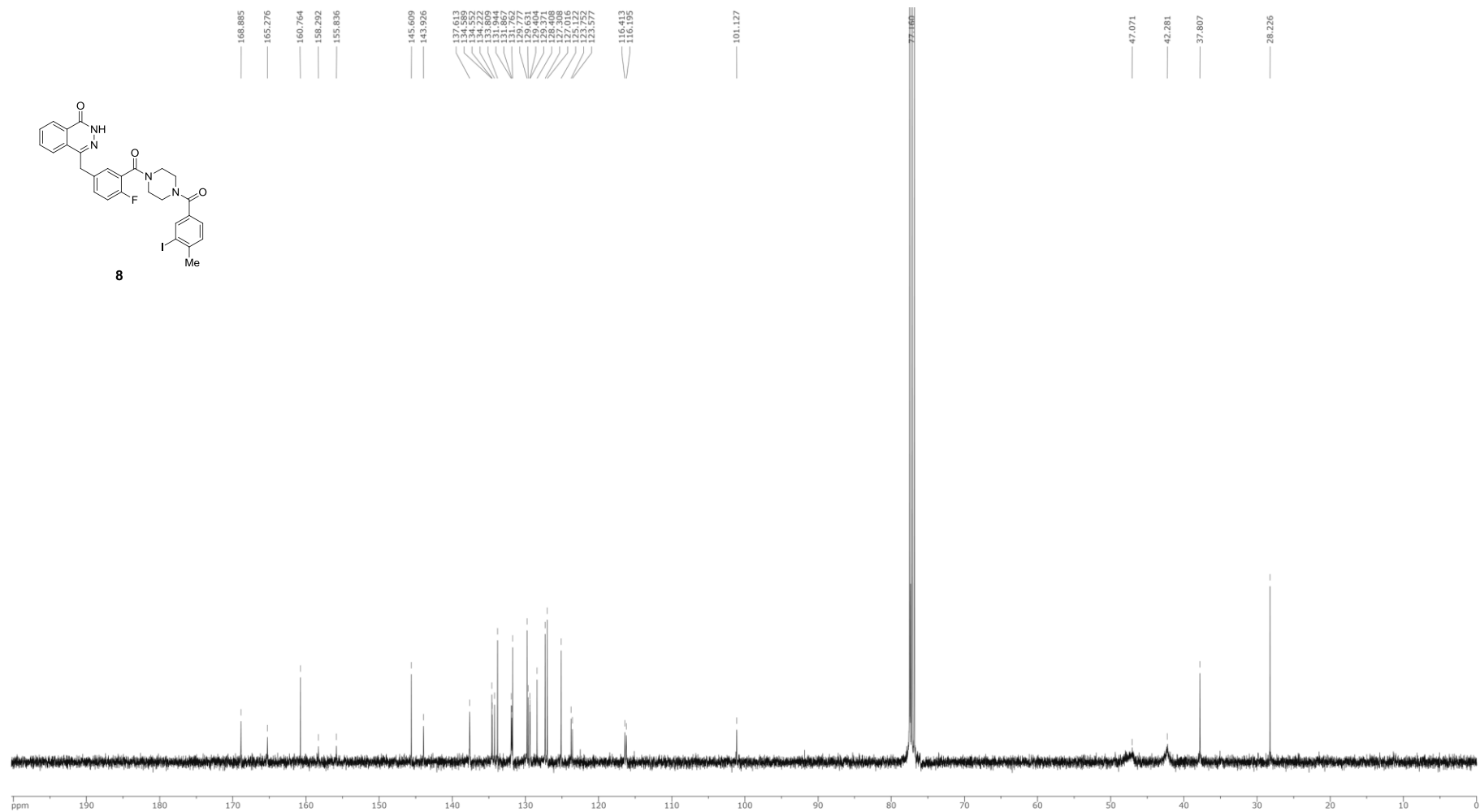


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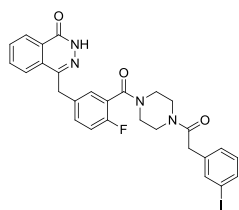




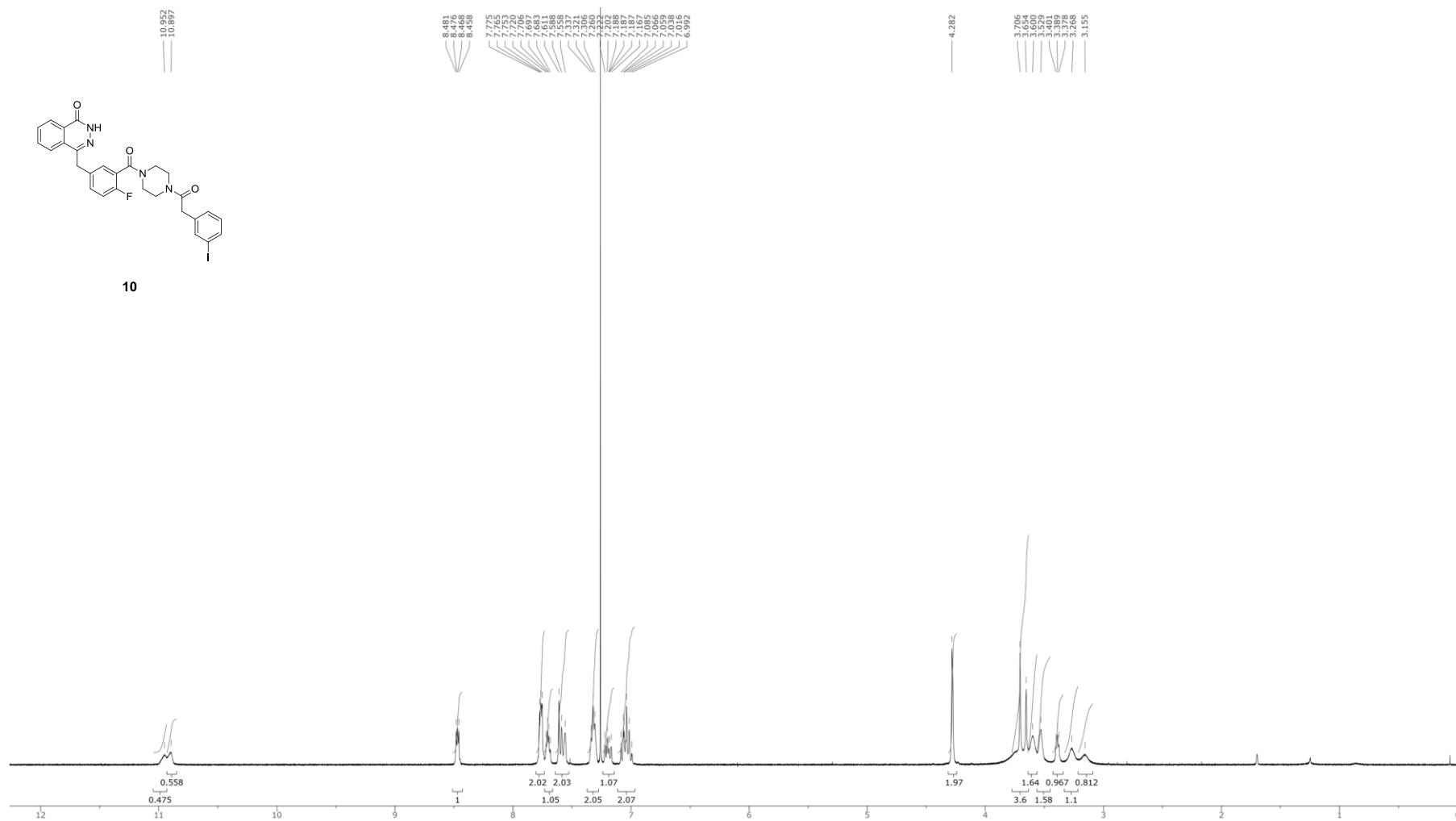


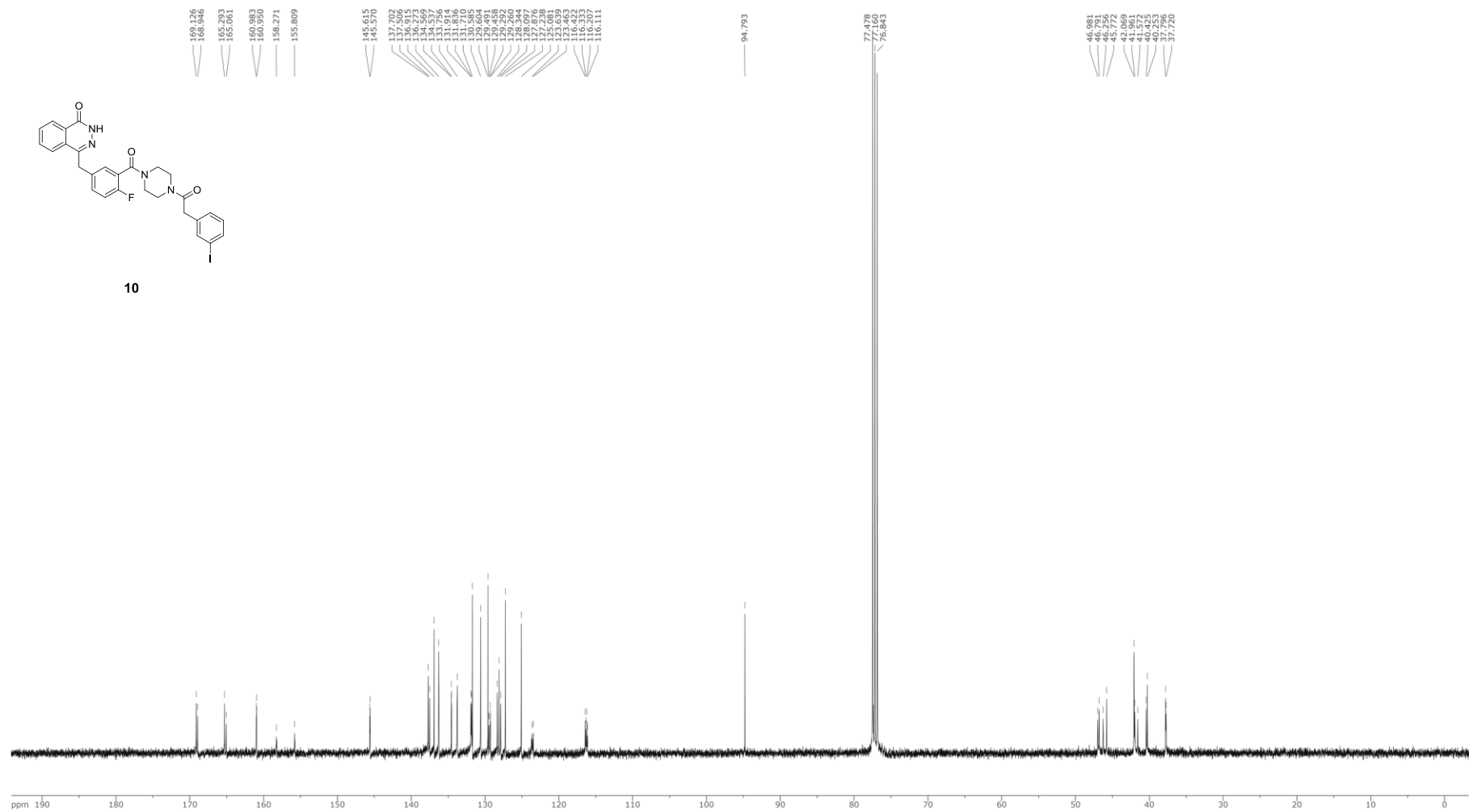


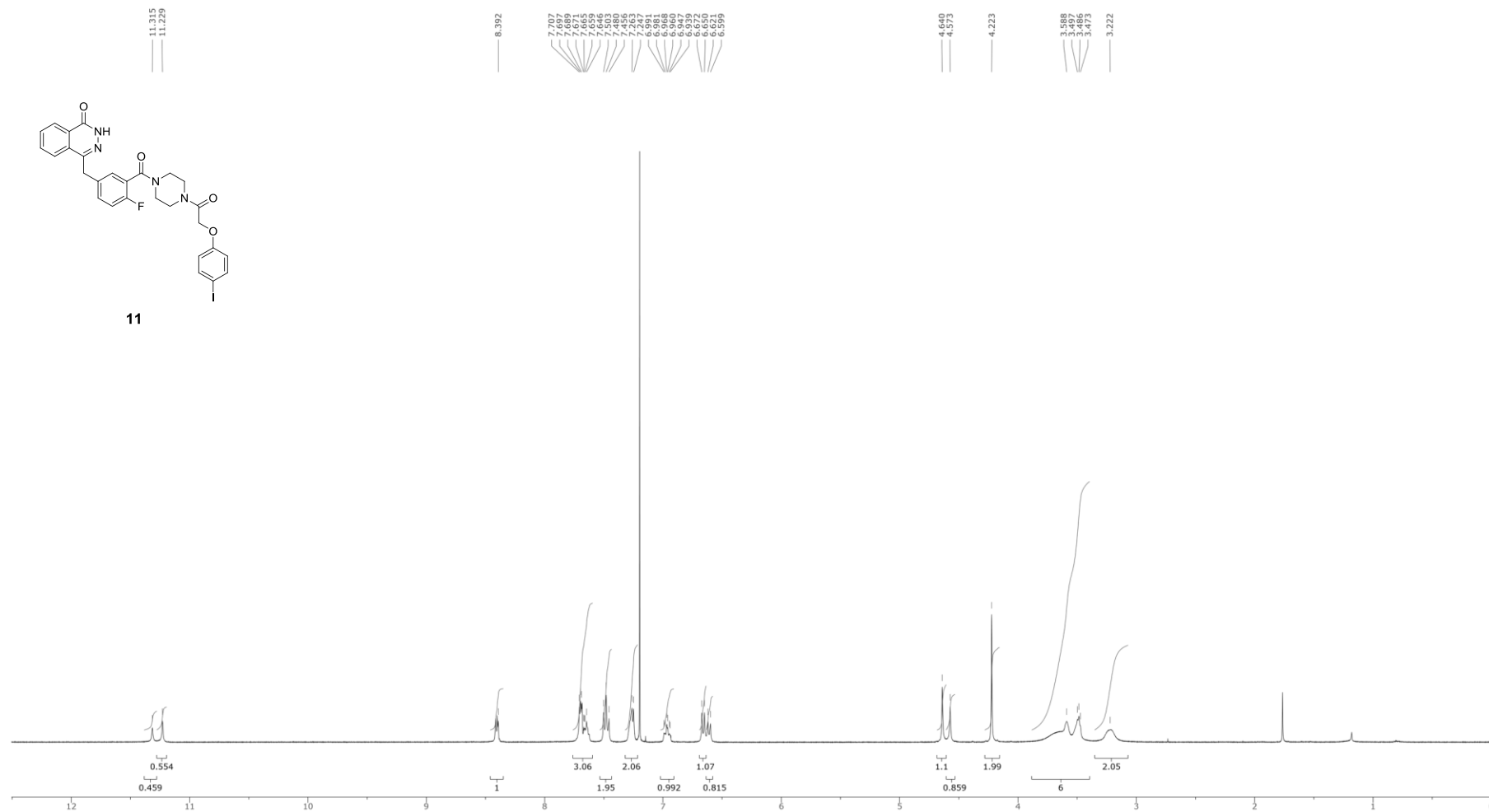


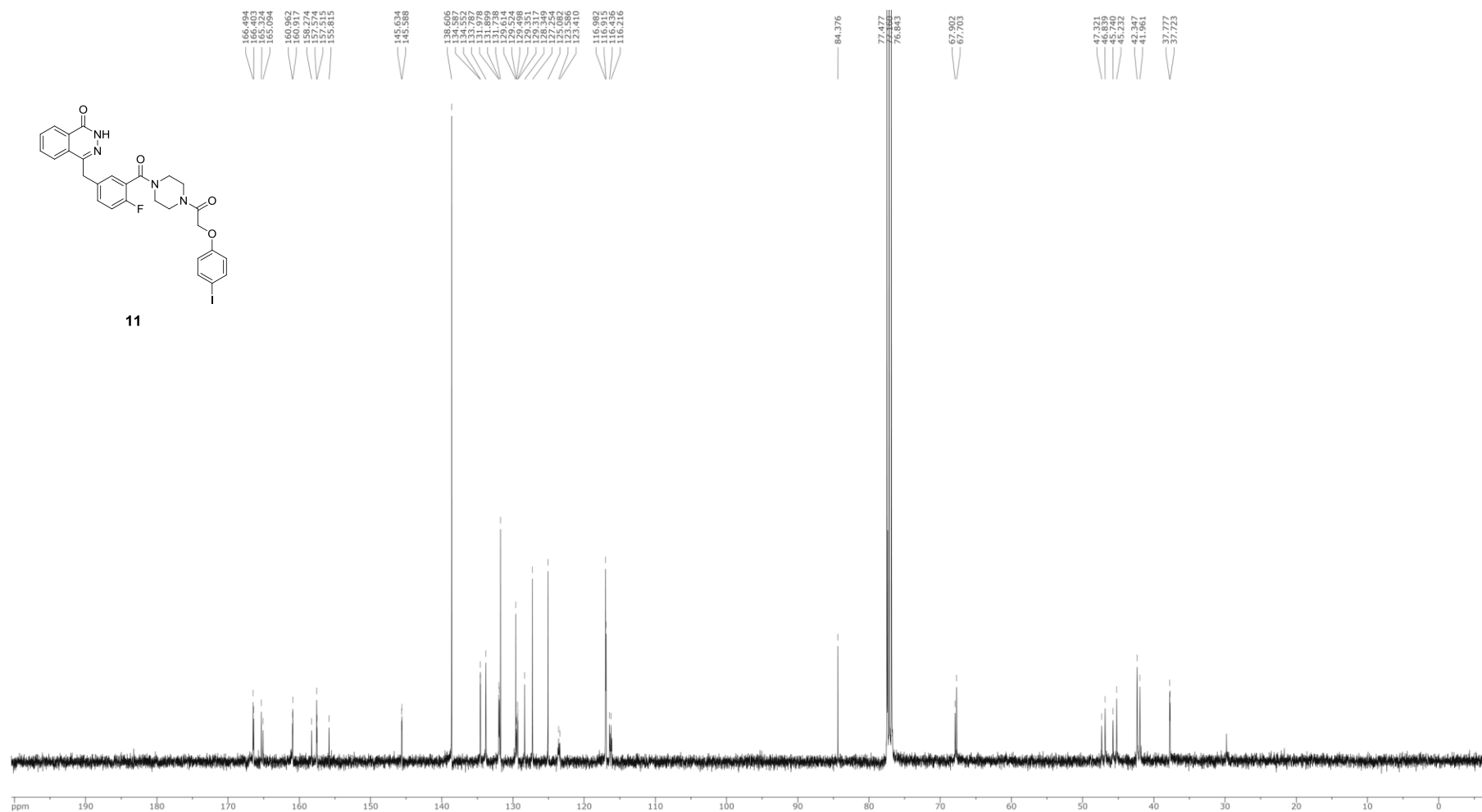


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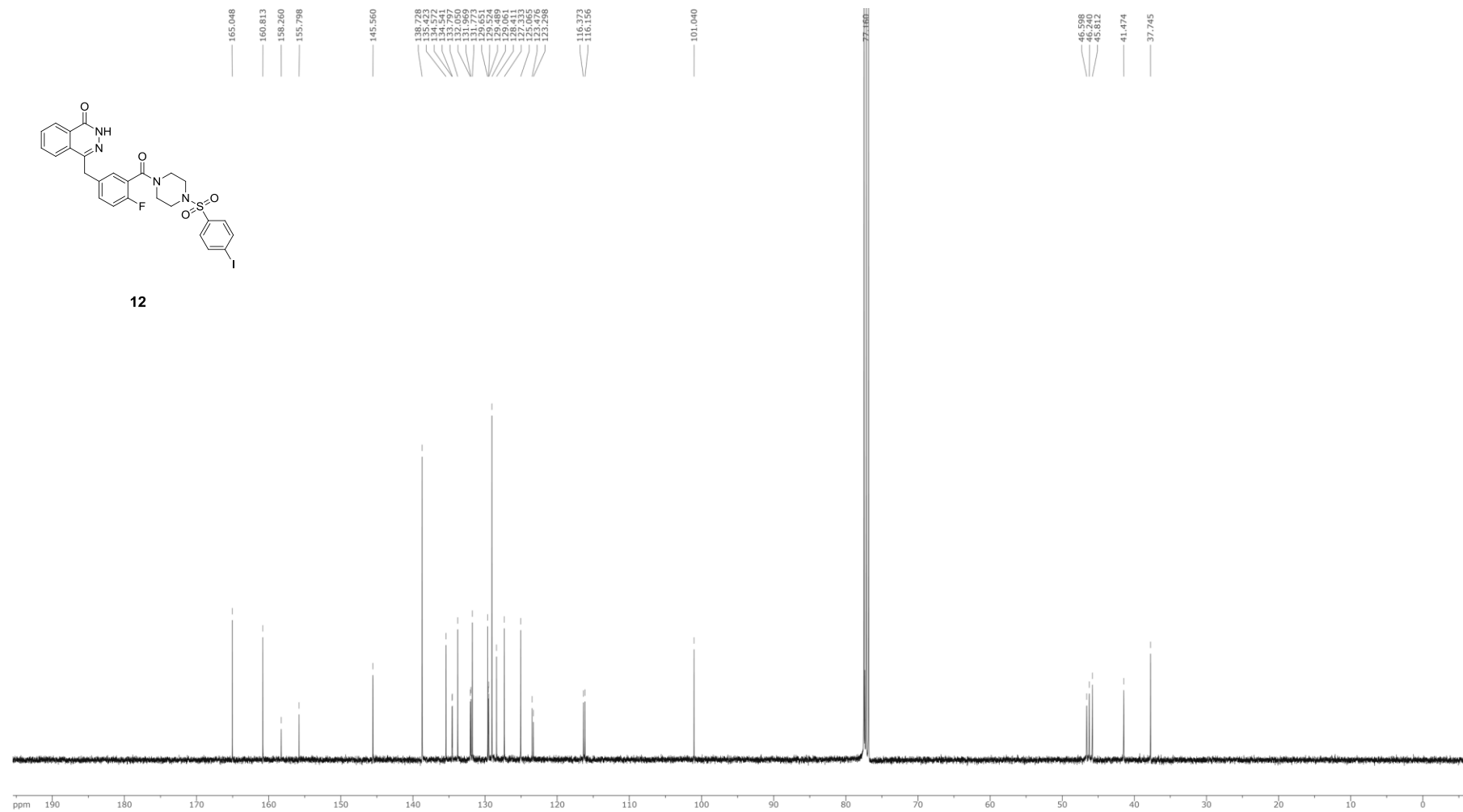


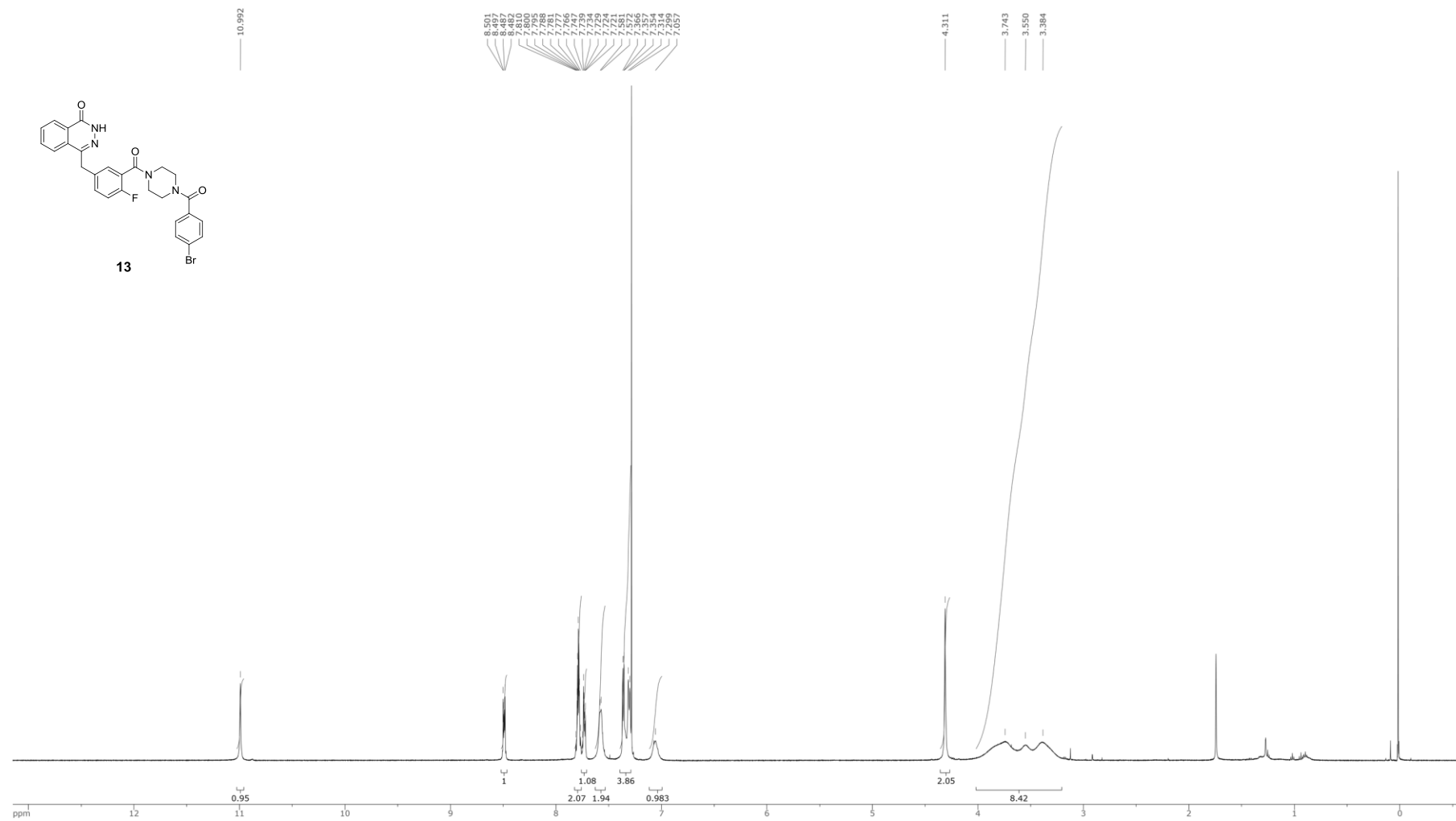


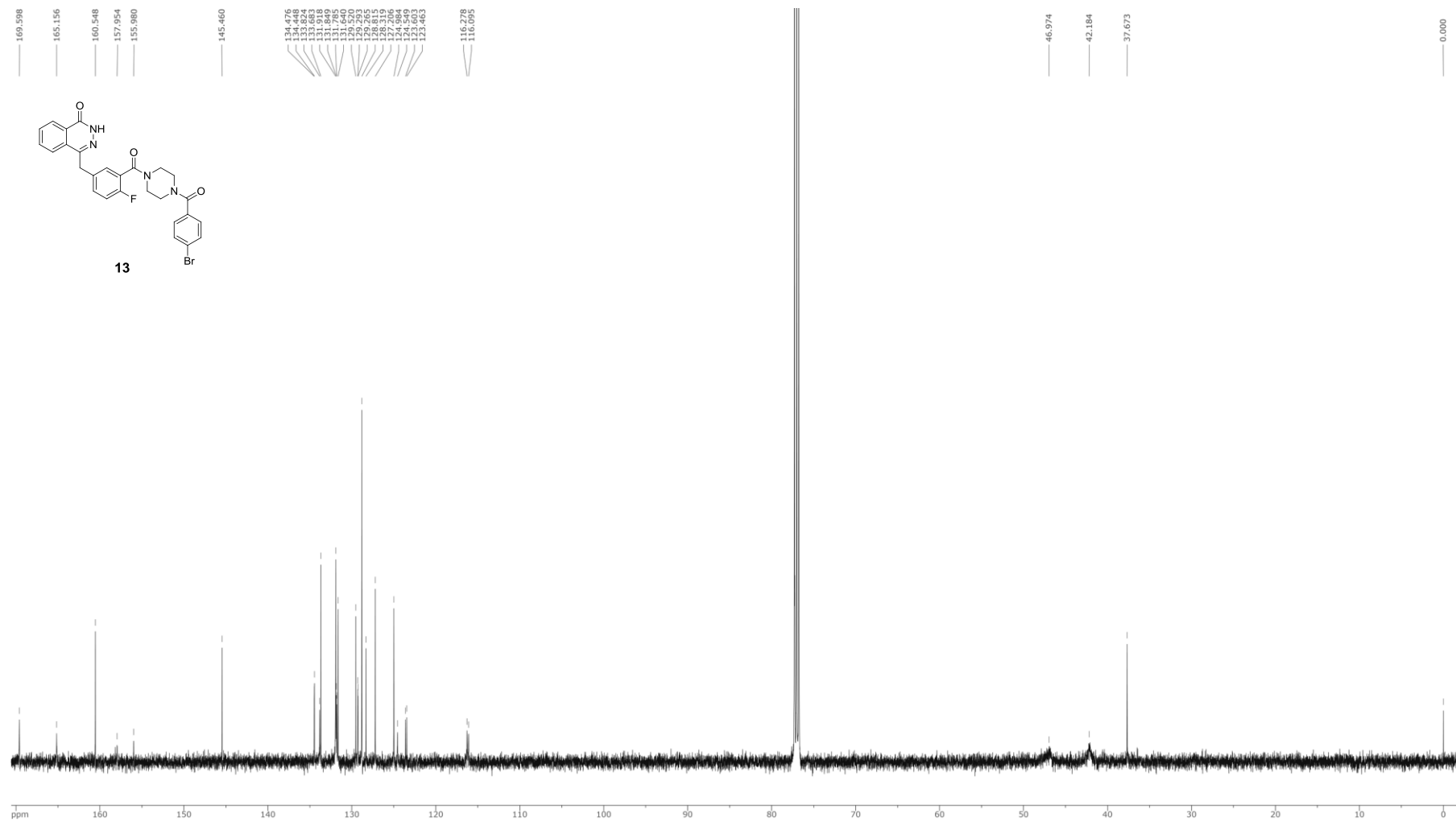




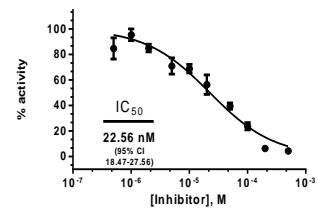
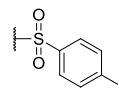
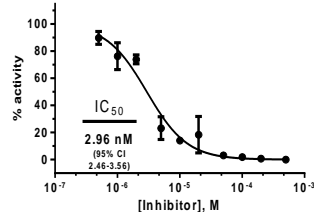
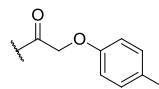
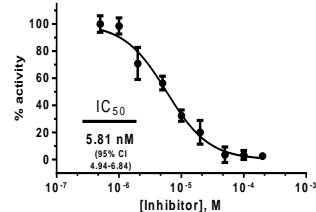
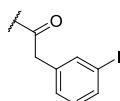
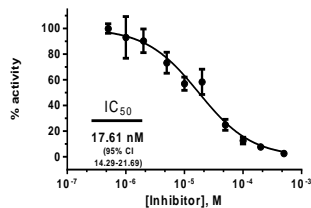
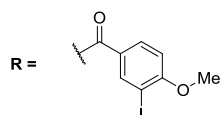
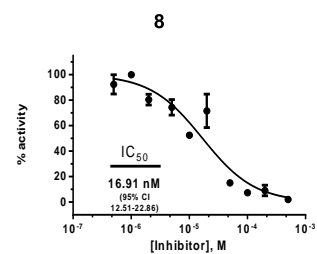
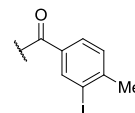
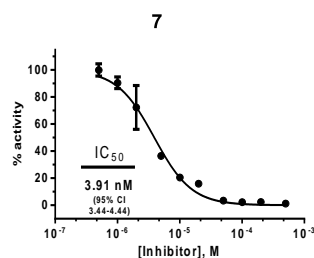
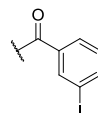
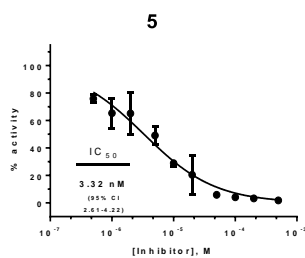
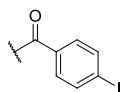
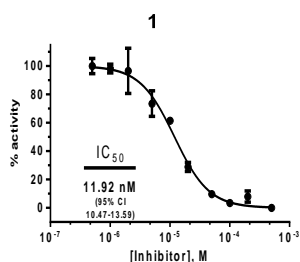
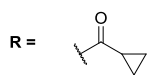
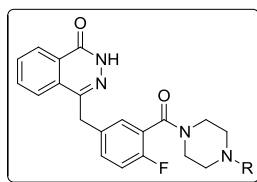






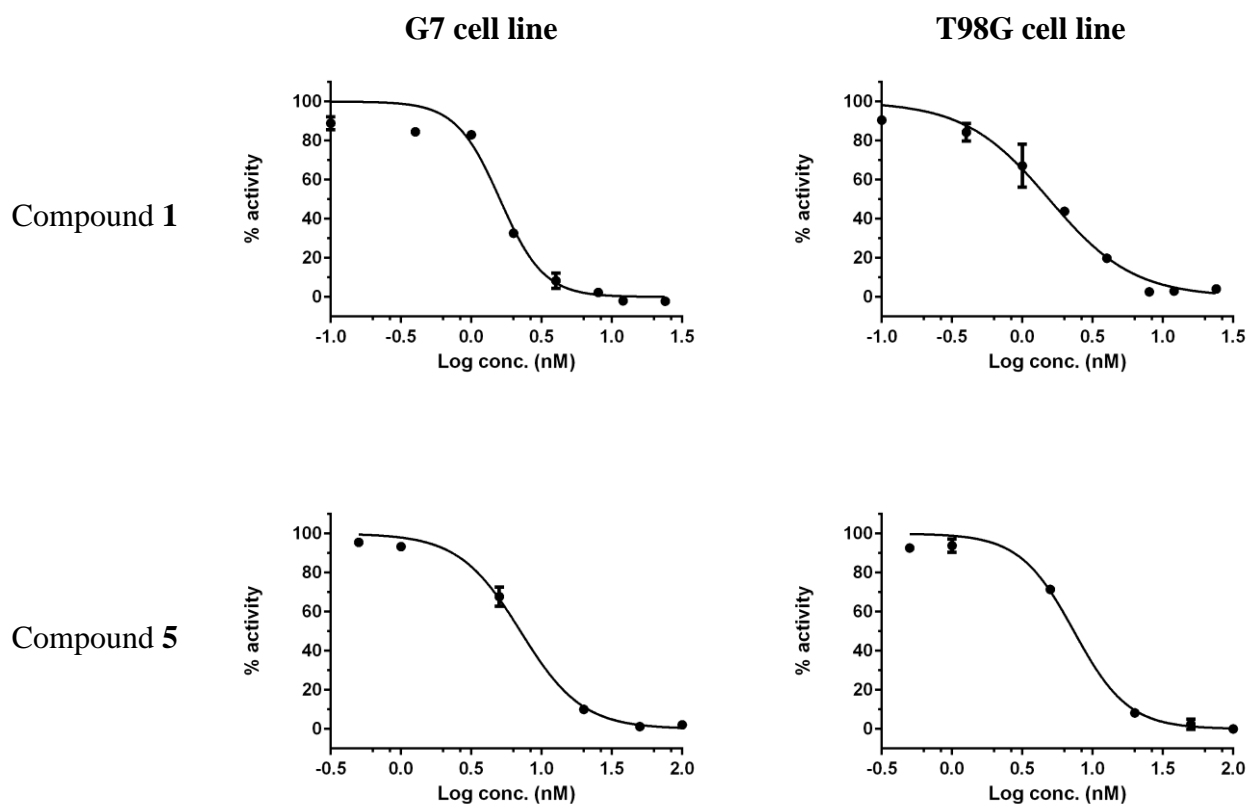


3. Cell-free IC₅₀ curves.



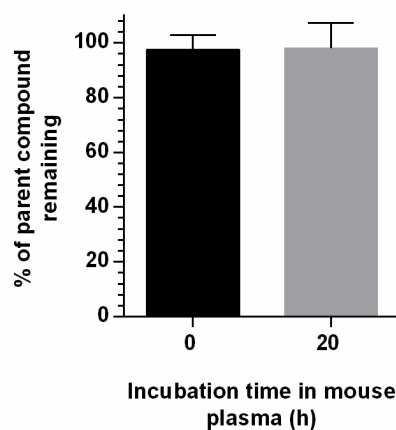
Each experiment was performed in triplicate. Error bars represent the mean \pm SD.

4. Cellular IC₅₀ curves for compounds 1 and 5.



Each experiment was performed in duplicate. Error bars represent the mean \pm SD.

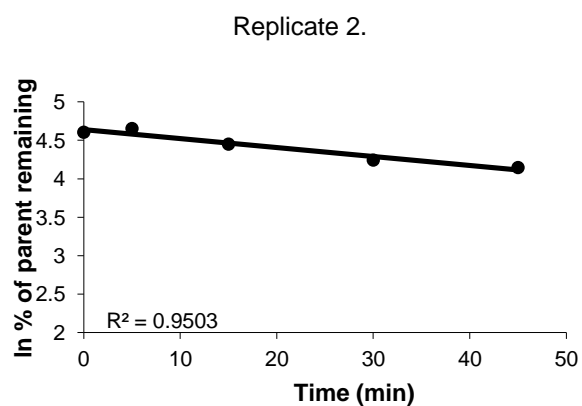
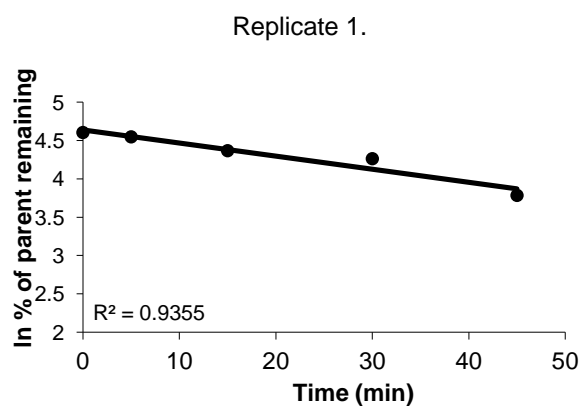
5. Plasma stability graph for compound 5.



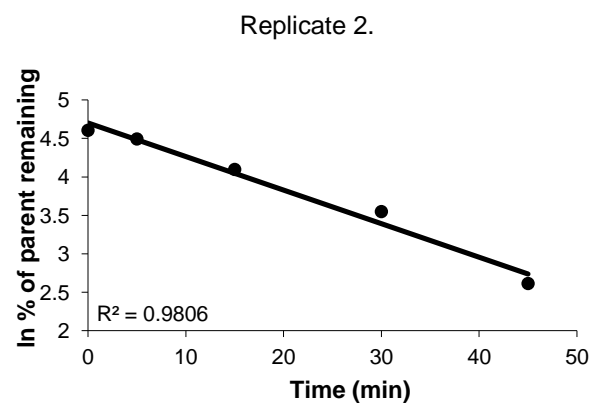
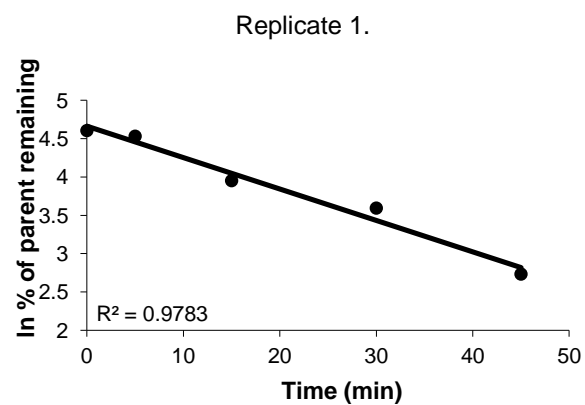
Each experiment was performed in triplicate. Error bars represent the mean \pm SD.

6. Metabolic stability graphs for compounds 1 and 5.

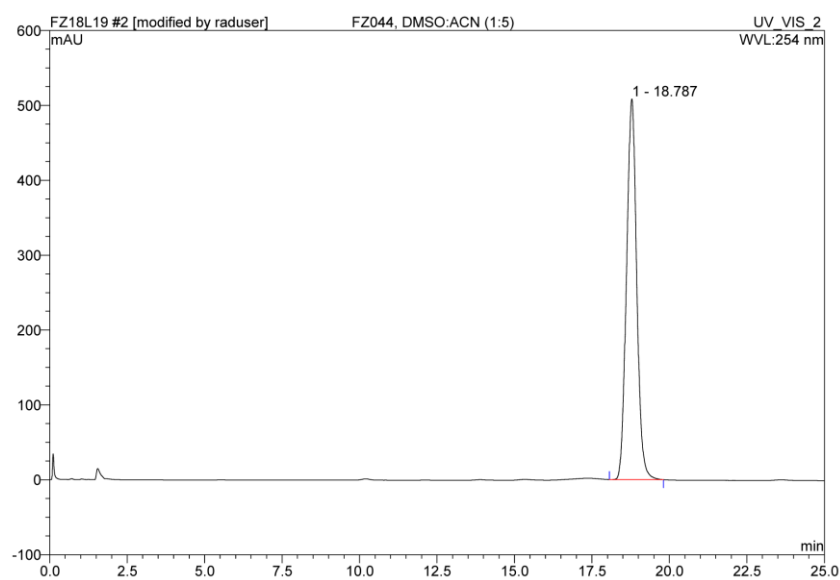
Compound 1



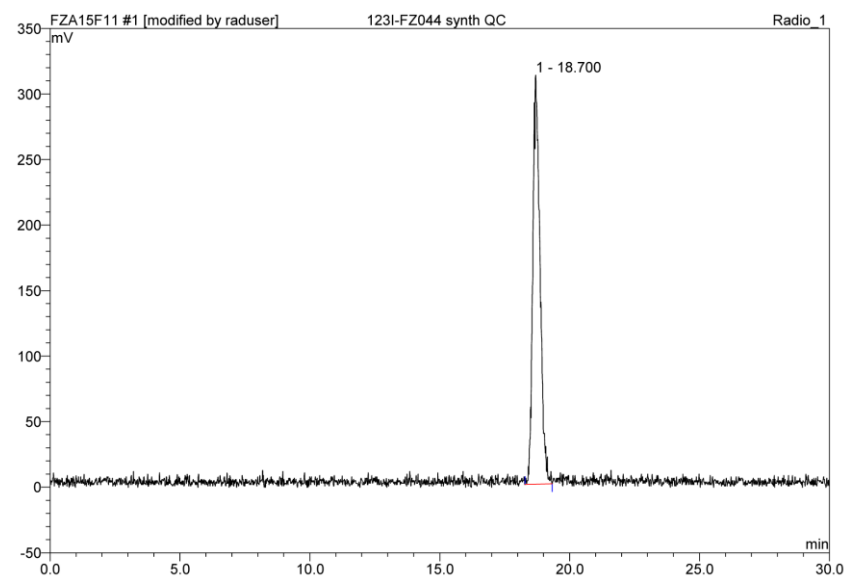
Compound 5



7. Radiochemistry HPLC chromatograms



UV (254 nm) HPLC chromatogram of non-radioactive compound 5.



Radio-HPLC chromatogram of [^{123}I]-5.

8. References

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