

SUPPORTING INFORMATION

Targeting HER2-Positive HCC1954 Breast Cancer Cells by Novel Thiazole-Dihydrobenzisoaxazoles: In-Depth Design, Synthesis and Initial *In Vitro* Study

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ABSTRACT:

Background: The most aggressive forms of breast cancer are characterized by independence from steroid hormones but a strong dependence on growth factors. In such cancer cells, oncogenic receptors, including human epidermal growth factor receptor 2 (HER2), are activated, and their targeted inhibition represents an attractive therapeutic strategy. The study aimed to develop small-molecule potential dual heat shock protein 90 (HSP90)-HER2 inhibitors and evaluate them as anticancer agents in HER2-positive cells.

Methods: The research project involved obtaining a series of compounds with potential dual inhibitory activity against HSP90 and HER2 by targeted organic synthesis, which was preliminarily assessed using molecular modelling and calculation of key parameters of molecular dynamics. The potential therapeutic benefit of the obtained molecules was studied using basic molecular biological methods, including assessment of cytotoxic activity *in vitro* using the MTT test, as well as determination of a possible mechanism of action based on the expression of key participants in intracellular signaling (western blotting). Additionally, therapeutic combinations were developed and tested on a cellular model of the disease, including a lead compound and chemotherapeutic drugs used in clinical practice, in order to find synergistic pairs and improve the effectiveness of the treatment.

Results: In this work, novel dual HSP90-HER2 inhibitors, based on the fused thiazole-dihydrobenzisoaxazole polycyclic scaffold, were designed and synthesized. The resulting compounds exhibited strong antiproliferative activity against HER2-positive breast cancer cells with high selectivity. Among them, **ATF-2** demonstrated antiproliferative activity comparable to HER2 inhibitor lapatinib and significantly suppressed HER2 expression and activity, epidermal growth factor receptor (EGFR) activity, and cyclin-dependent kinase 6 (CDK6) expression in HCC1954 breast cancer cells.

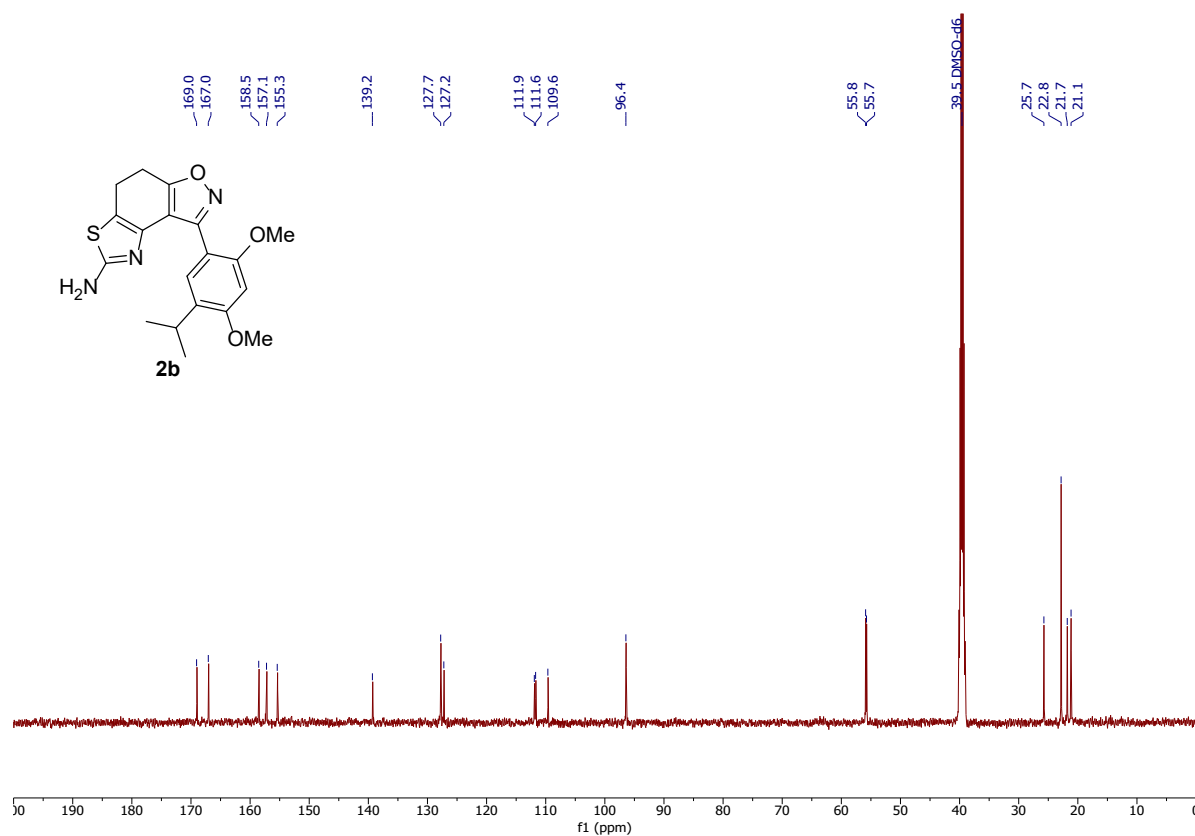
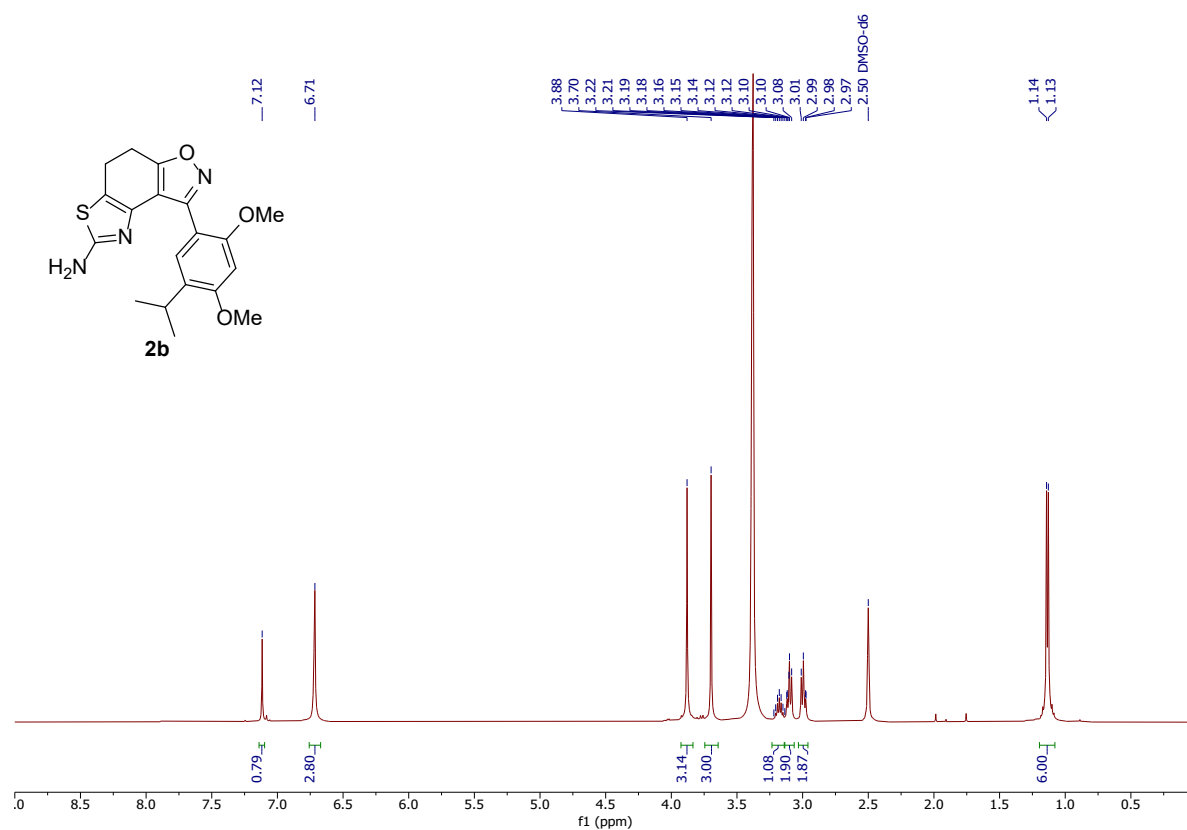
Conclusion: These findings highlight **ATF-2** as a promising dual HSP90-HER2 inhibitor with broader inhibitory effects on the HER2, EGFR, and CDK6 pathways.

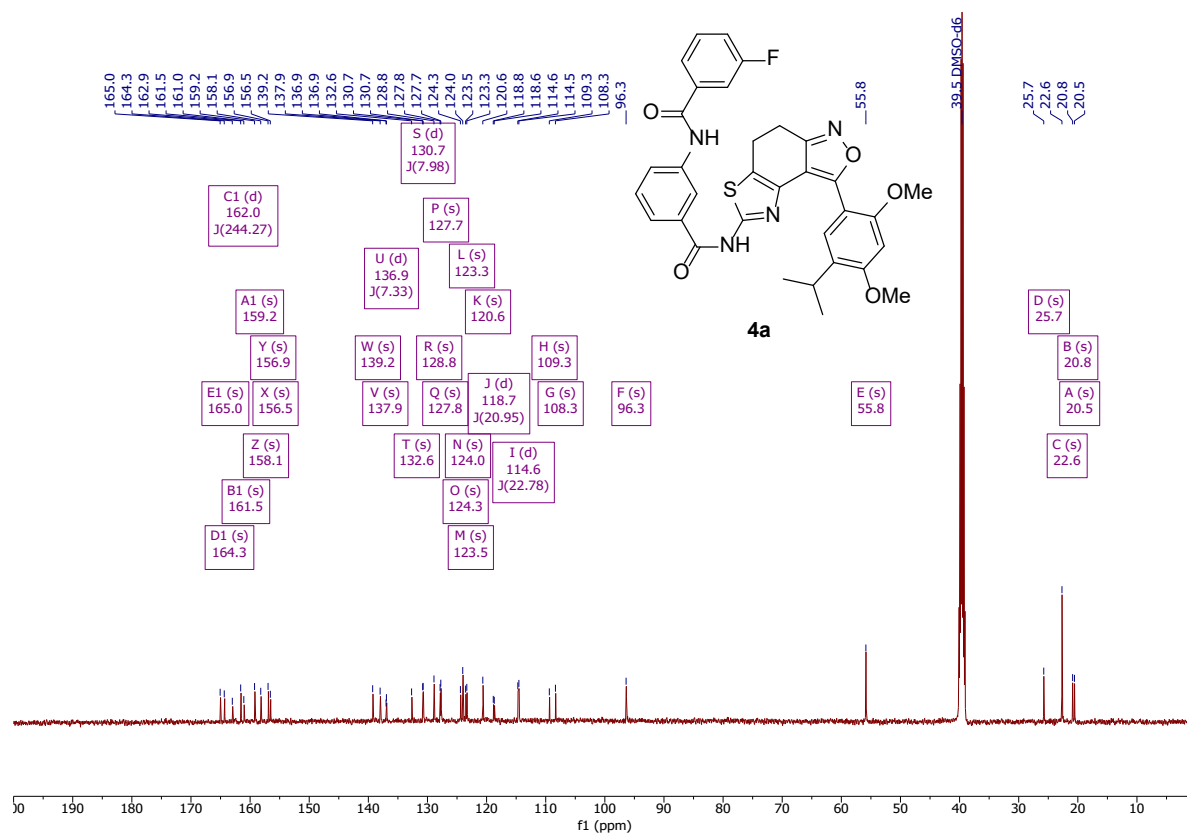
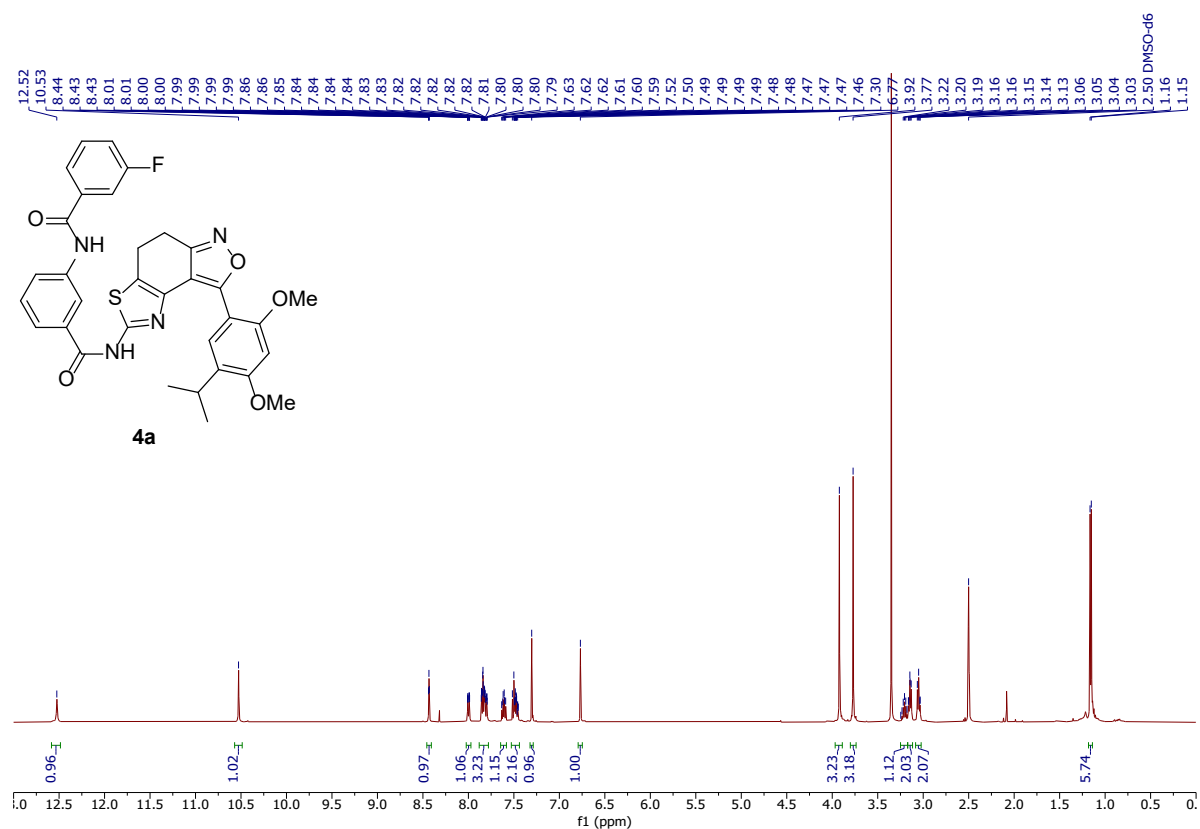
KEYWORDS: anticancer therapy; breast cancer; heat shock protein 90 (HSP90); human epidermal growth factor receptor 2 (HER2); epidermal growth factor receptor (EGFR); dual inhibitors

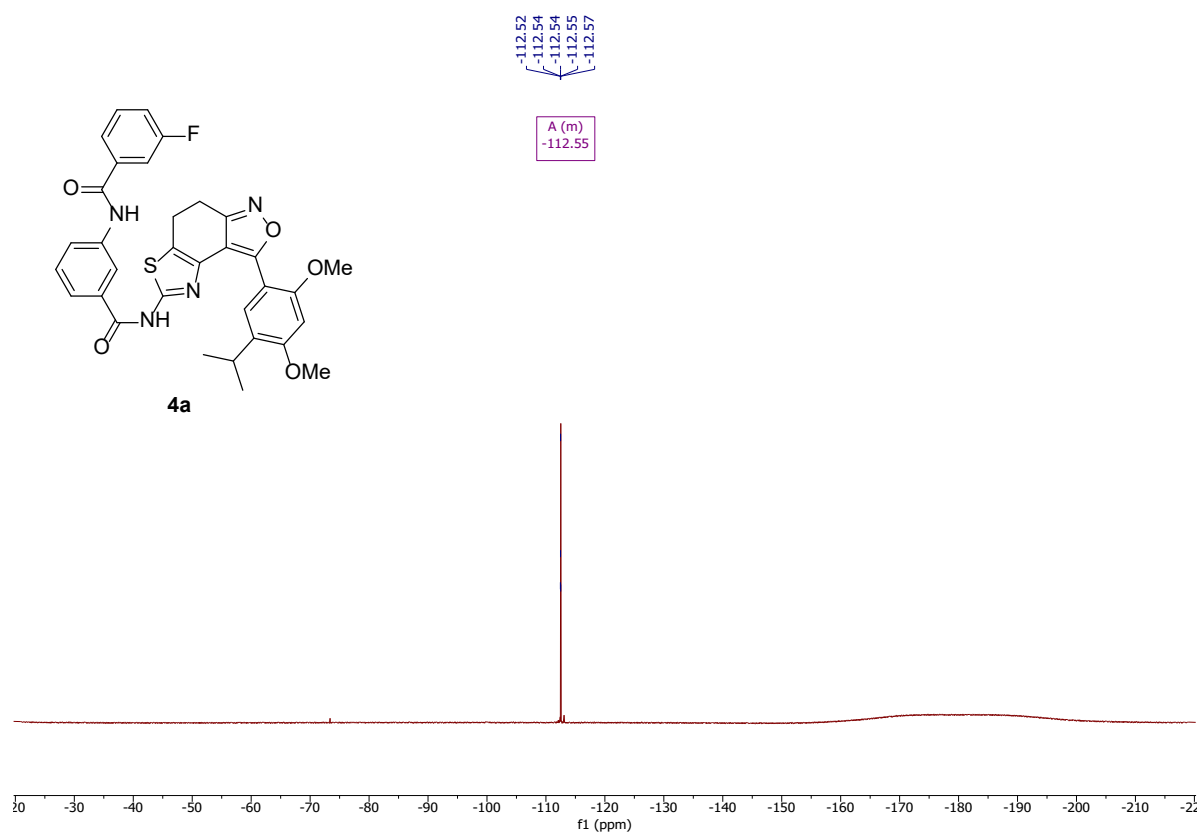
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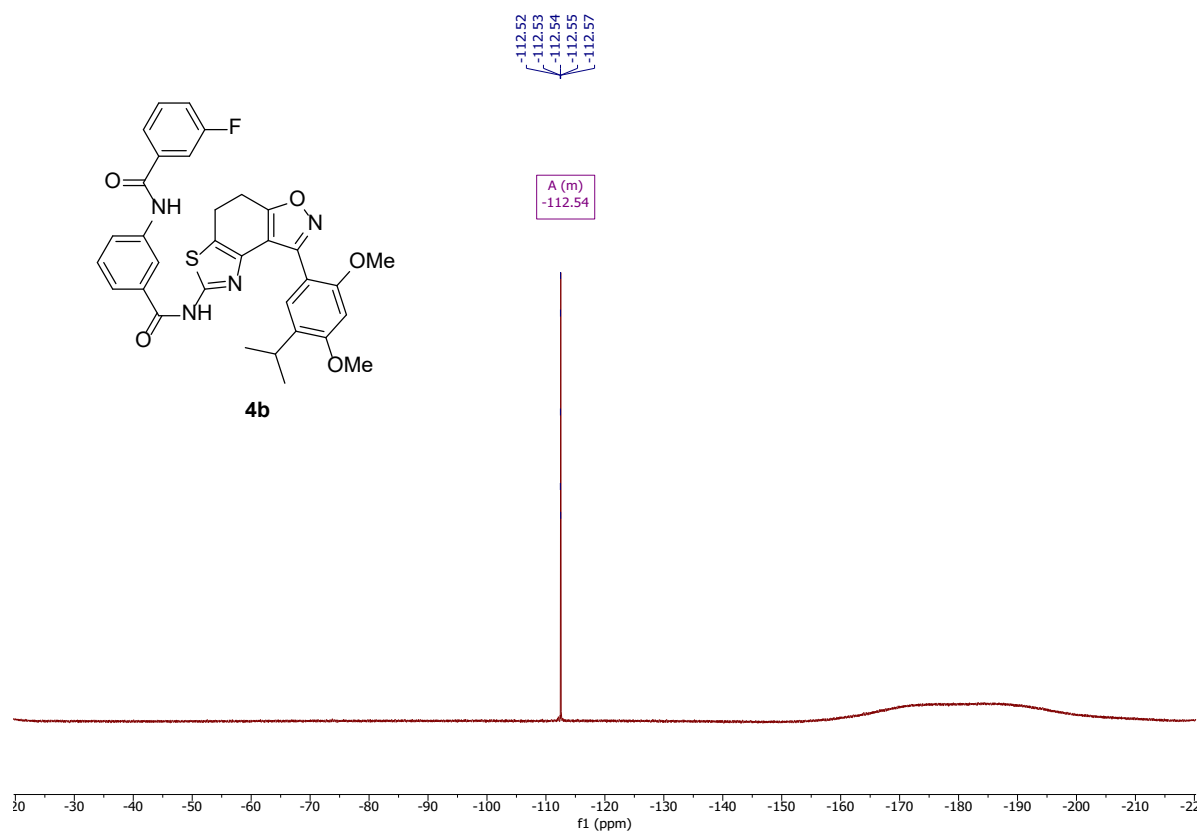
^1H NMR, ^{13}C NMR and ^{19}F NMR Spectra



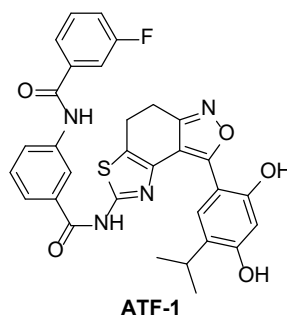
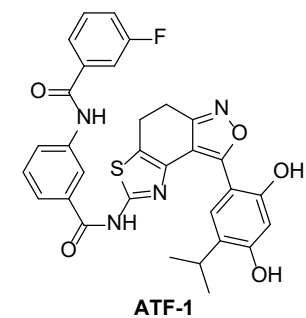


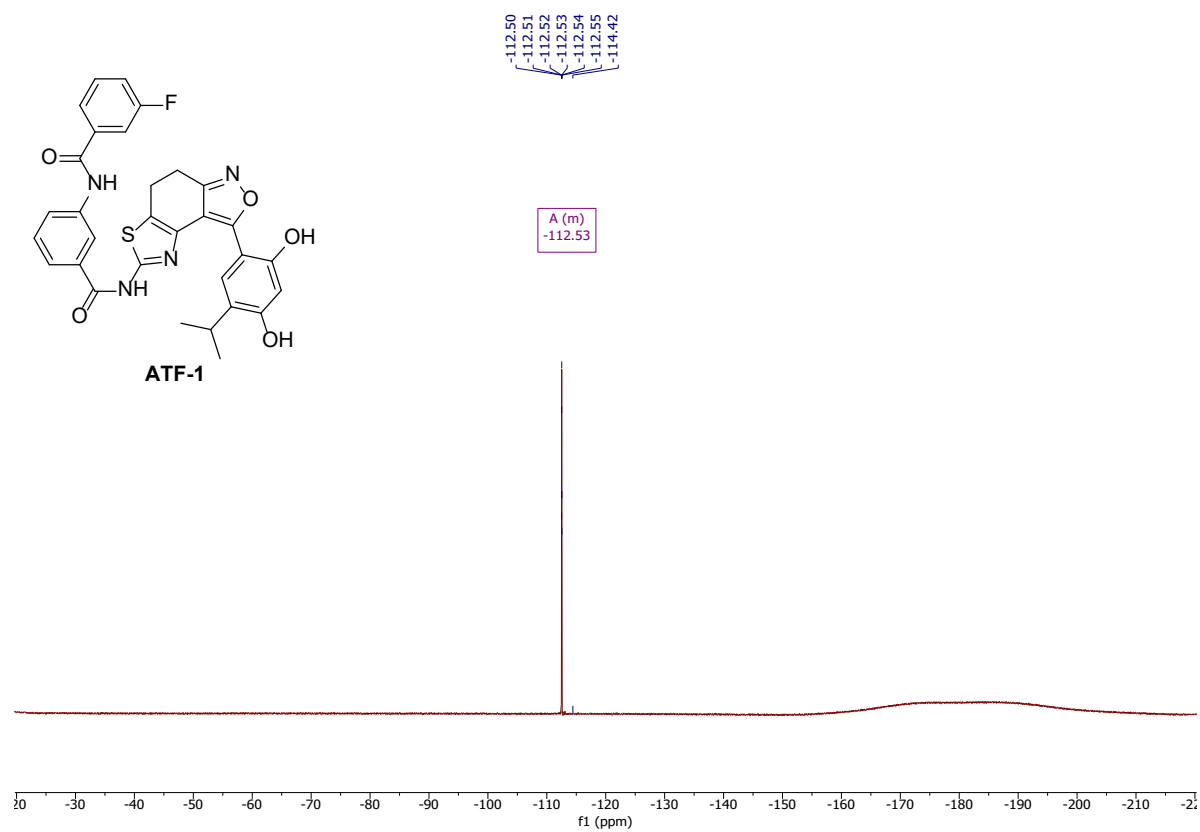


^{19}F NMR (470 MHz, DMSO- d_6) of compound **4a**

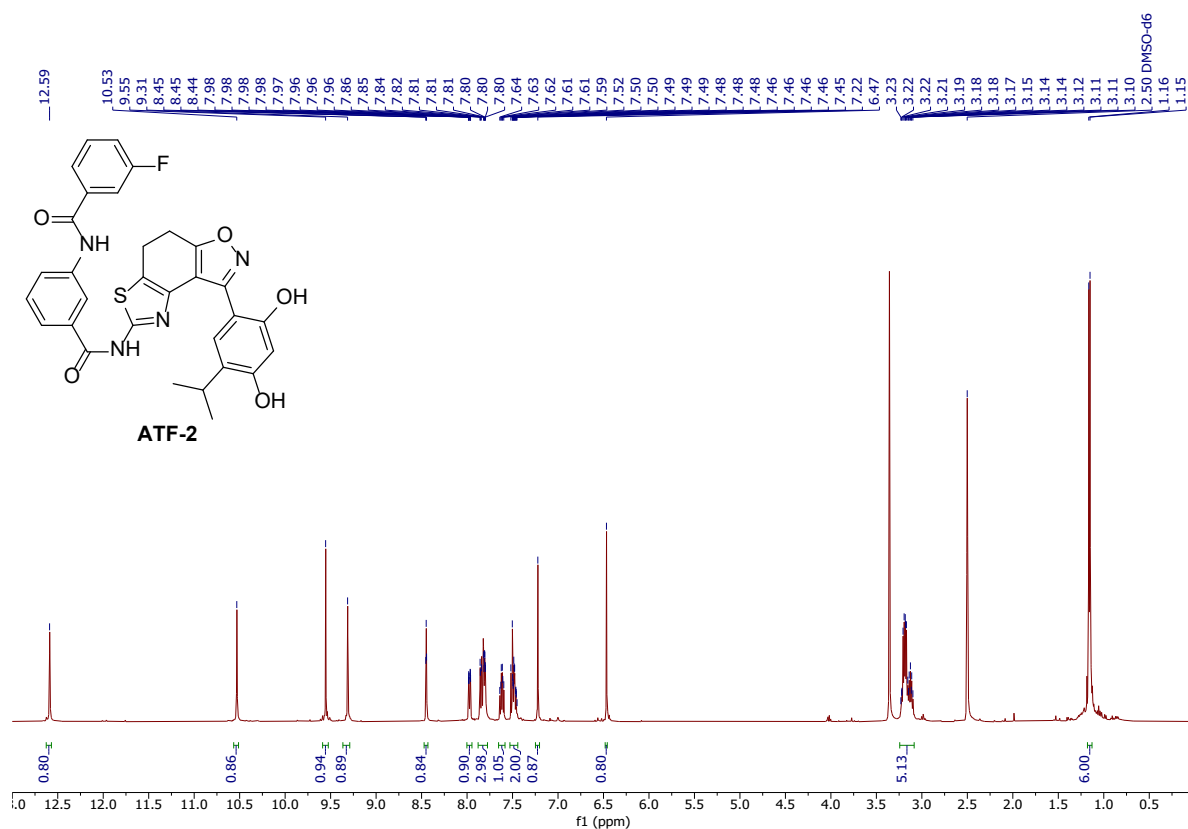


^{19}F NMR (470 MHz, DMSO- d_6) of compound **4b**

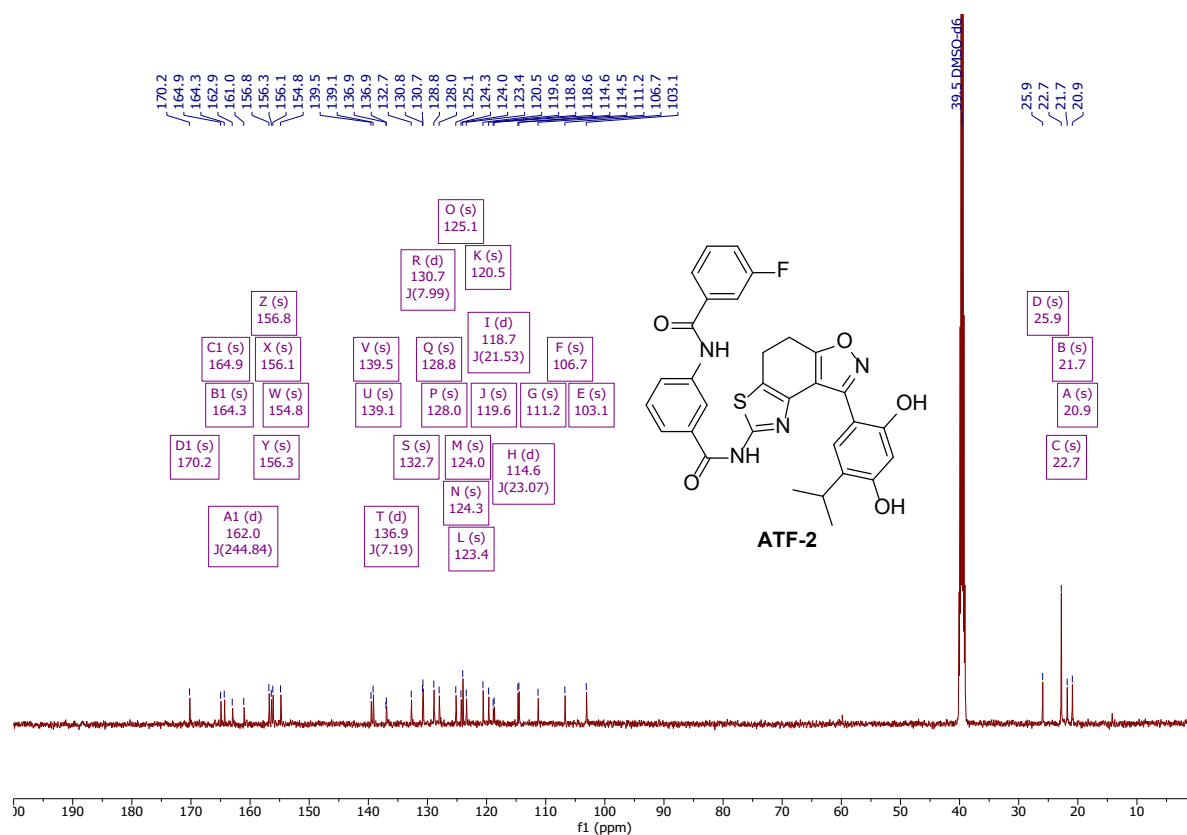


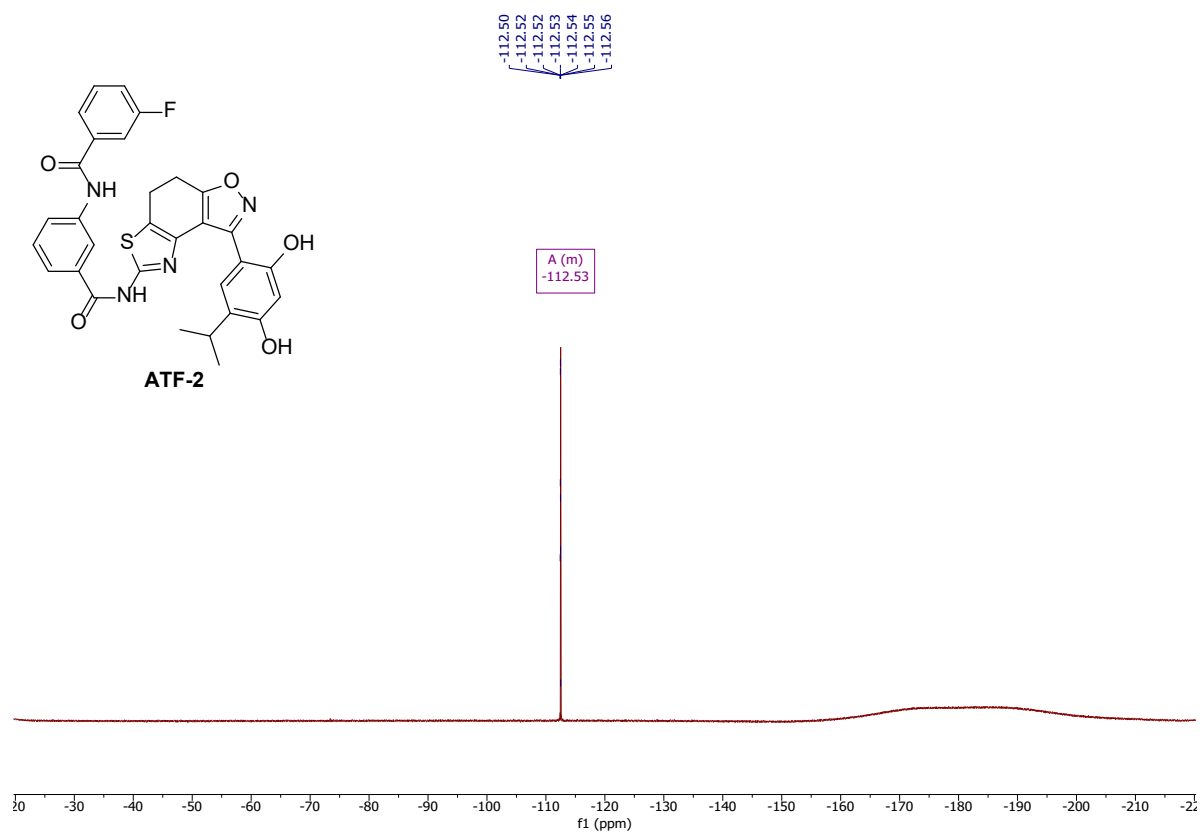


^{19}F NMR (470 MHz, DMSO- d_6) of compound **ATF-1**



¹H NMR (500 MHz, DMSO-d₆) of compound ATF-2

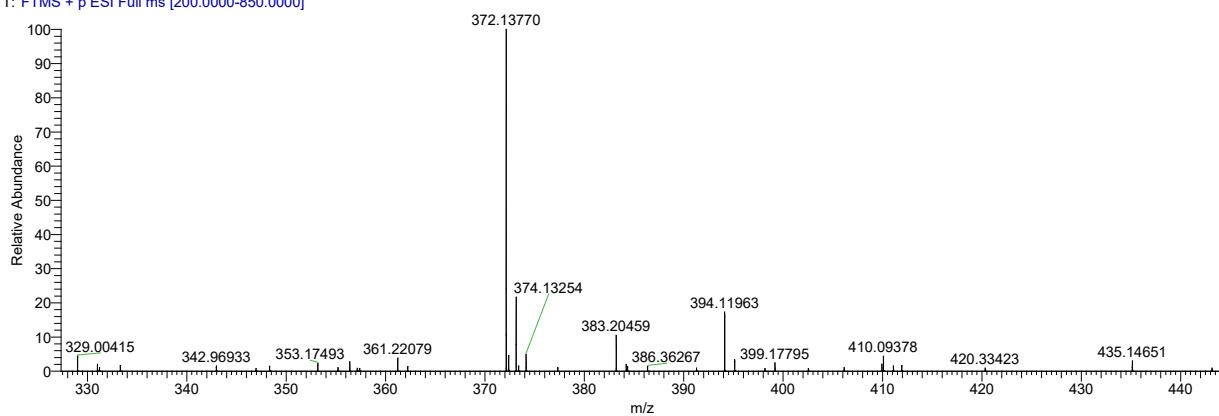




^{19}F NMR (470 MHz, DMSO- d_6) of compound **ATF-2**

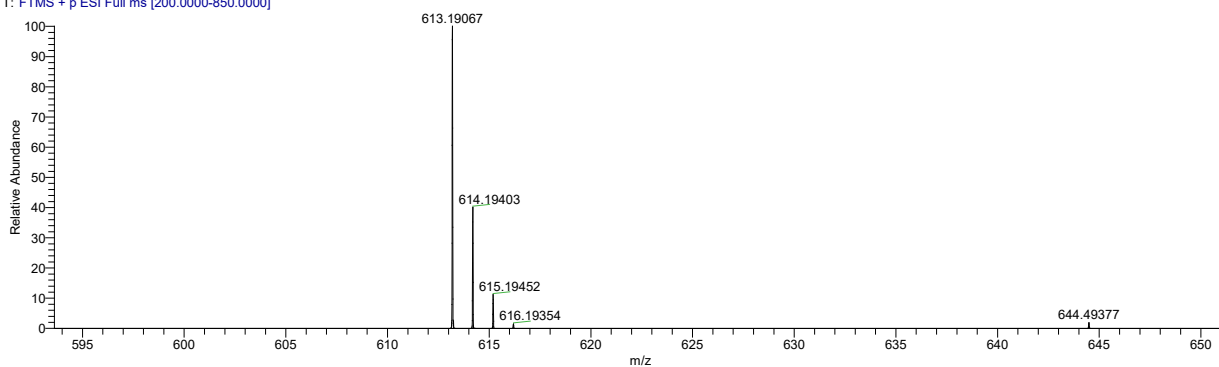
HRMS Spectra

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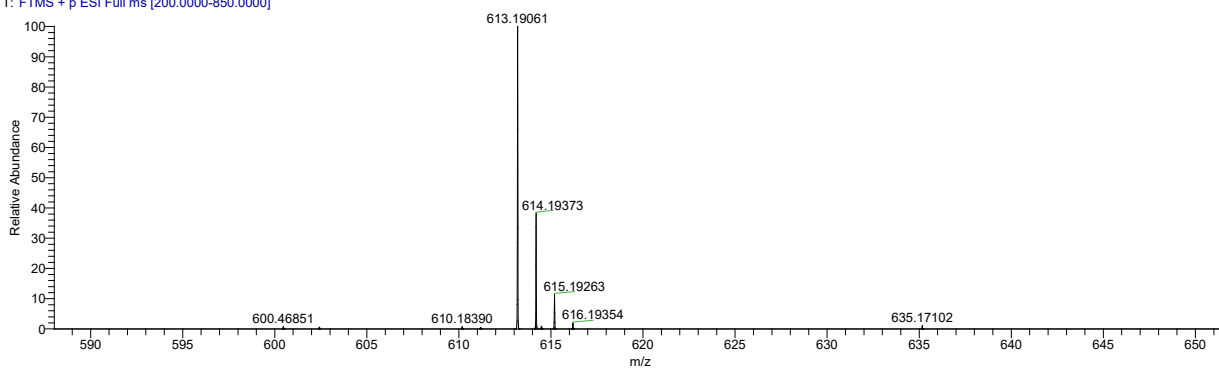
HRMS of 2b

V329 #369 RT: 2.81 AV: 1 NL: 1.55E7
T: FTMS + p ESI Full ms [200.0000-850.0000]



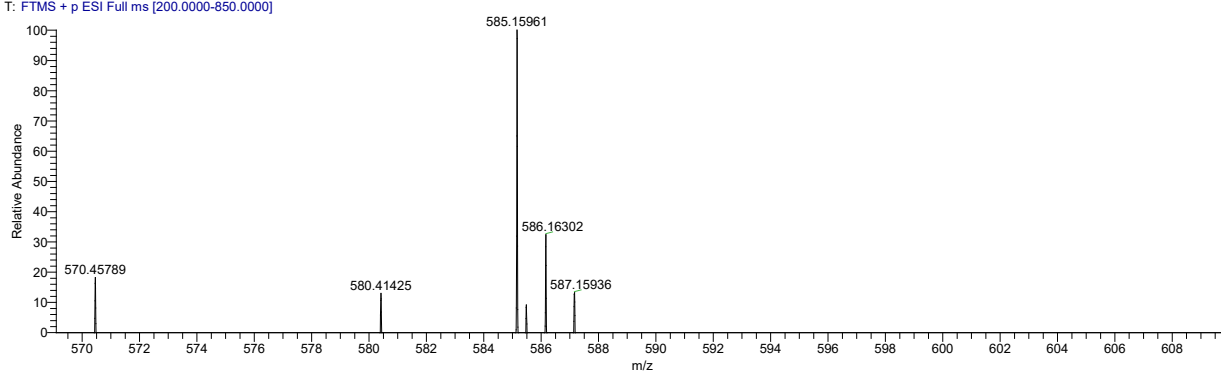
HRMS of 4a

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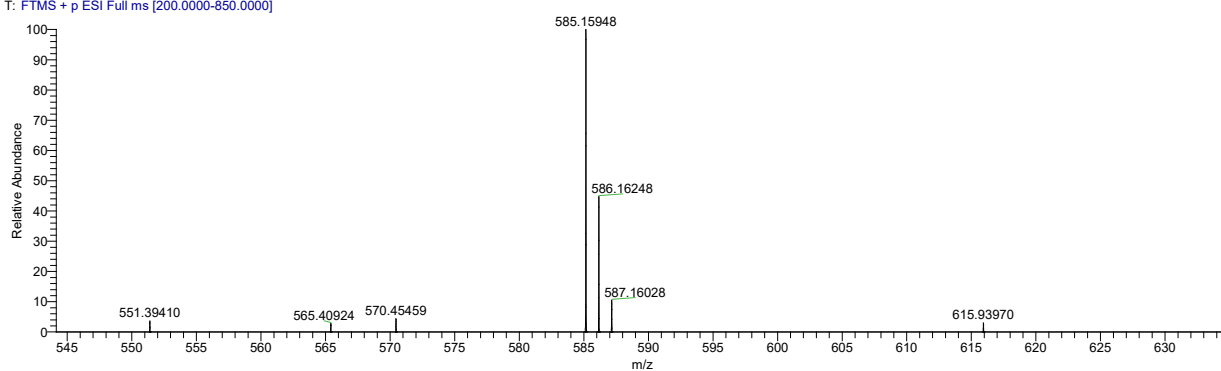
HRMS of 4b

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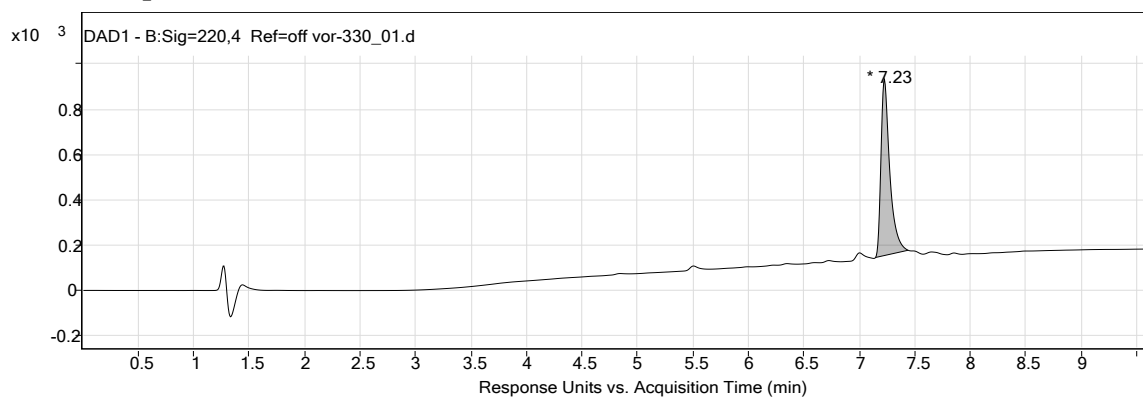
HRMS of ATF-1

V333 #347 RT: 2.65 AV: 1 NL: 4.79E6
T: FTMS + p ESI Full ms [200.0000-850.0000]



HRMS of ATF-2

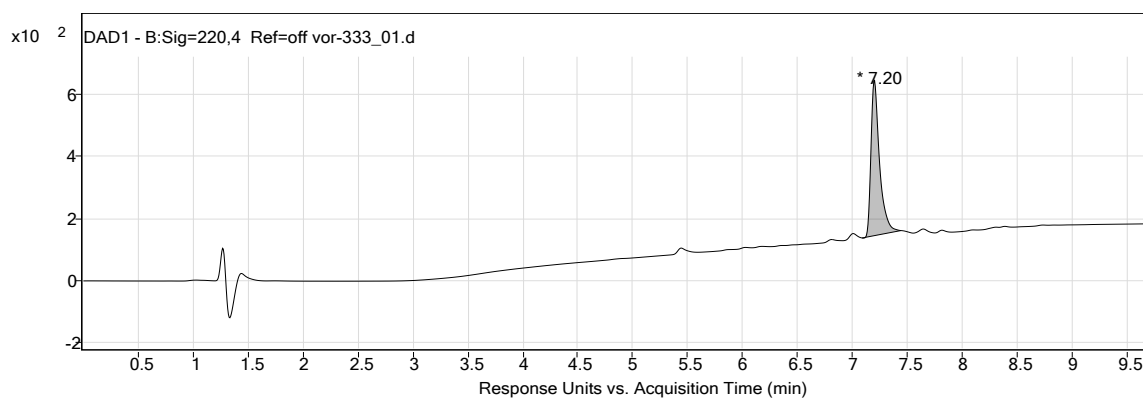
HPLC of compounds ATF-1 and ATF-2



Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	7,15	7,23	7,44	777,48	4291,66	100

HPLC of ATF-1

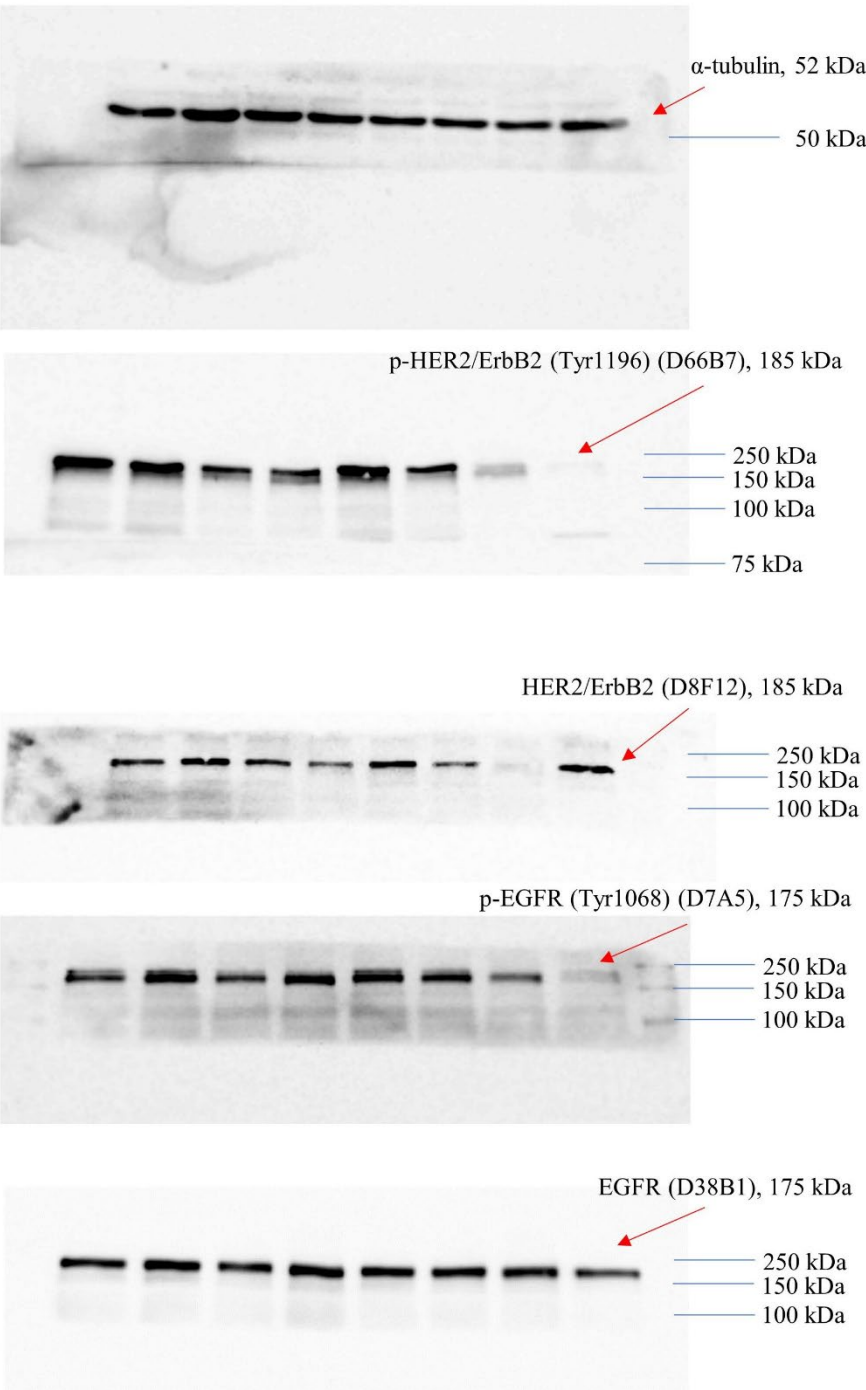


Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	7,09	7,2	7,44	499,09	2703,83	100

HPLC of ATF-2

Uncropped Immunoblots



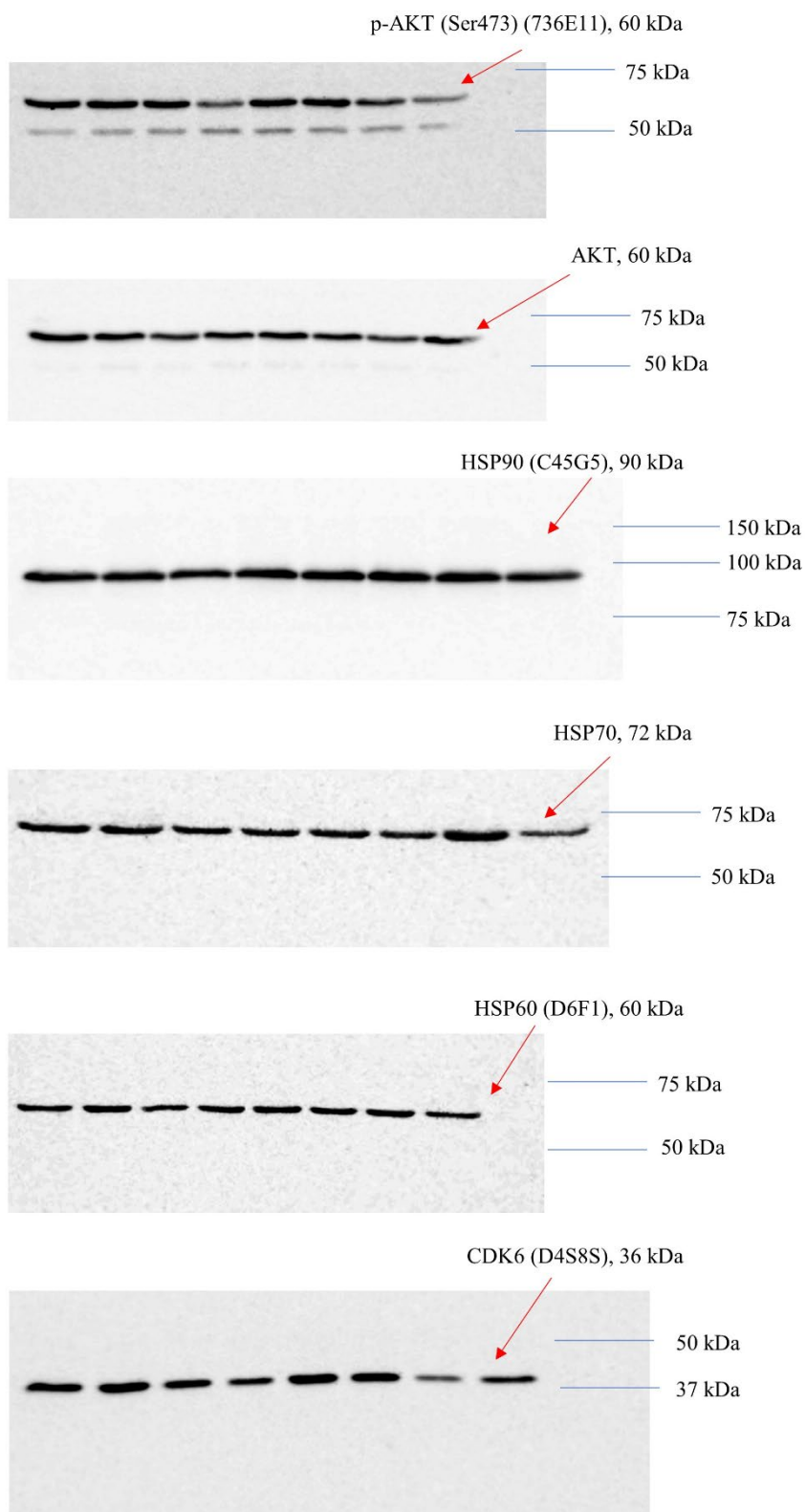


Figure S1. Uncropped immunoblots. On each blot, the first 7 tracks were cut out for Figure 4.