Spring 4-27-2023

Synthesis and evaluation of 1-substituted imidazo[4,5-c] quinoline TLR7 agonists with improved potency

Emma Grace DeYoung
Binghamton University--SUNY, edeyoun1@binghamton.edu

SYNTHESIS AND EVALUATION OF 1-SUBSTITUTED IMIDAZO[4,5-C] QUINOLINE TLR7 AGONISTS WITH IMPROVED POTENCY

BY

EMMA GRACE DEYOUNG

THESIS

Submitted in partial fulfillment of the requirement for
Honors Thesis in Chemistry
In Harpur College of Arts and Sciences of Binghamton University
State University of New York
2023

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2023

Dr. L. Nathan Tumey, Advisor
Department of Pharmacy and Pharmaceutical Sciences, Binghamton University

Dr. Christof T. Grewer, Member
Department of Chemistry, Binghamton University

Dr. Clarice Kelleher, Member
Department of Chemistry, Binghamton University

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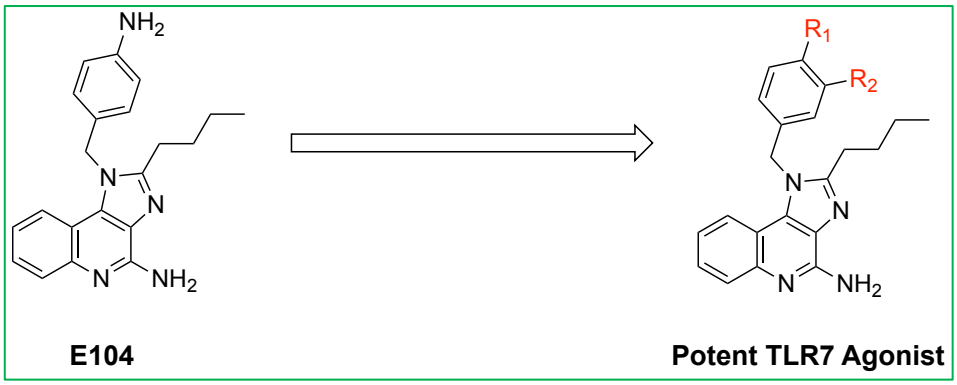
Abstract

TLR7 agonists are small molecules that are useful within cancer immunotherapy due to their ability to stimulate the TLR7 pathway resulting in NFκB activation, and cytokine release.[1] Due to the risk of toxicity when delivered systemically, our team has employed a particular type of drug-delivery technology, Antibody Drug Conjugates (ADCs) to deliver these payloads directly to tumor tissue. The initial focus of this work was on E104 (**8a**), an imidazoquinoline TLR7 agonist, which was synthesized by the Tumey lab.[1] However, the agonist was not sufficiently potent for many of the proposed applications of this technology, prompting a need to develop more potent derivatives of this compound. Initial derivatives were synthesized through amine couplings at the benzylic substituent and were characterized using HPLC and NMR. The derivatives were further evaluated using Ramos Blue Cell activation assays as well as PAMPA permeability assays, resulting in the identification of compounds **9b**, **11b** and **12b** as potent payloads with sufficient permeability for ADC applications. Generally, hydrophobic appendages attached to the benzyl group significantly enhanced the potency of the payloads and multiple analogs were found to be ~10x more potent than the parent E104 molecule. Although these specific payloads proved to be sufficiently potent, they proved to have limited reactivity and our initial studies showed them to be incompatible with typical ADC linkers. Linker payload synthesis difficulties were overcome by attempting to create new payloads using the reductive amination method in which a secondary



amine was a part of the functional group alteration. This allowed for linker attachment with the more reactive amine as compared to the amine attached onto the quinoline which is more poorly nucleophilic. These new generation payloads were also characterized by HPLC, UPLC and NMR and additionally evaluated for their ability to stimulate the TLR7 pathway and induce NFκB activation. Although they proved not to have improved potency to E104, compound **13a** proved to have modest potency and this new type of payload proved compatibility with the cleavable mcValCitPABC linker, paving the way for future efforts surrounding optimization of these payloads.

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List of Abbreviations

General Biology
ADC – Antibody Drug Conjugate
DAMP – Danger associated molecular pattern
HEK – Human embryonic kidney
hMDM – Human Monocyte-Derived Macrophages
IFN – Interferon
IL – Interleukins
NFκB – Nuclear factor kappa light chain enhancer of activated B cells
PAMP – Pattern associated molecular pattern
PBMC – Peripheral blood mononuclear cells
SEAP – Secreted embryonic alkaline phosphatase
TLR – Toll-Like Receptors
TNF – Tumor Necrosis Factor

Linker Component Chemistry
mc – 6-maleimidohexanoic acid
mcValCitPABC-PNP – Maleimidocaproyl-L-Valine-L-Citrulline-p-Aminobenzyl Alcohol p-Nitrophenyl Carbonate

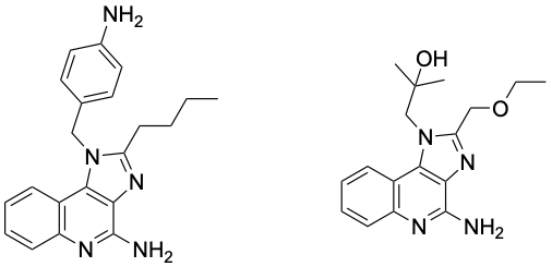
Organic Solvents
ACN – Acetonitrile

DCM – Dichloromethane
DMA – Dimethylacetamide
DMF – Dimethylforamide

Organic Reagents
DIEA – N, N-Diisopropylethylamine
DIPEA – Diisopropylethylamine
EDC – 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
EEDQ – 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinolin
HATU – Hexa fluorophosphate Azabenzotriazole Tetramethyl Uronium
HOAt – 1-hydroxy-7-azabenzotriazole
HOBt – Hydroxybenzotriazole
T3P – Propylphosphonic anhydride

1. Introduction

Antibody Drug Conjugates (ADCs) are a new drug delivery technology for the treatment of cancer and immunological diseases.[2,3] ADCs are composed of three parts: the antibody, the linker, and the payload. The linker is cleaved, and the payload is released upon internalization into antigen-expressing cells. One particular type of technology consists of an antibody which is conjugated to an immune activating payload. In this approach, the antibody delivers the immune stimulant to the cancer tissue resulting in activation of the tumor-associated immune cells in the tumor microenvironment, thus resulting in an innate and adaptive immune response that drives tumor regression.[1] The focus of our immune-stimulant work is on TLR7 agonists. Toll-like receptors (TLRs) play a critical role in activating the immune system by recognizing danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs).[3] When a TLR engages a DAMP or PAMP, it activates various downstream pathways (particularly the NFκB and IRF) that result in cytokine release and increased proliferation.[5] The Tumey Lab has developed a TLR7 agonist known as E104 (**8a**) which has been demonstrated to activate various human and mouse lymphocytes.[1] Moreover, it can be delivered using ADC technology. Immune activating ADCs require a potent payload to sufficiently decrease tumor volume and cause cancer regression. Specifically, my research focuses upon the synthesis and characterization of TLR7 agonists with improved potency as compared to E104 (**8a**).

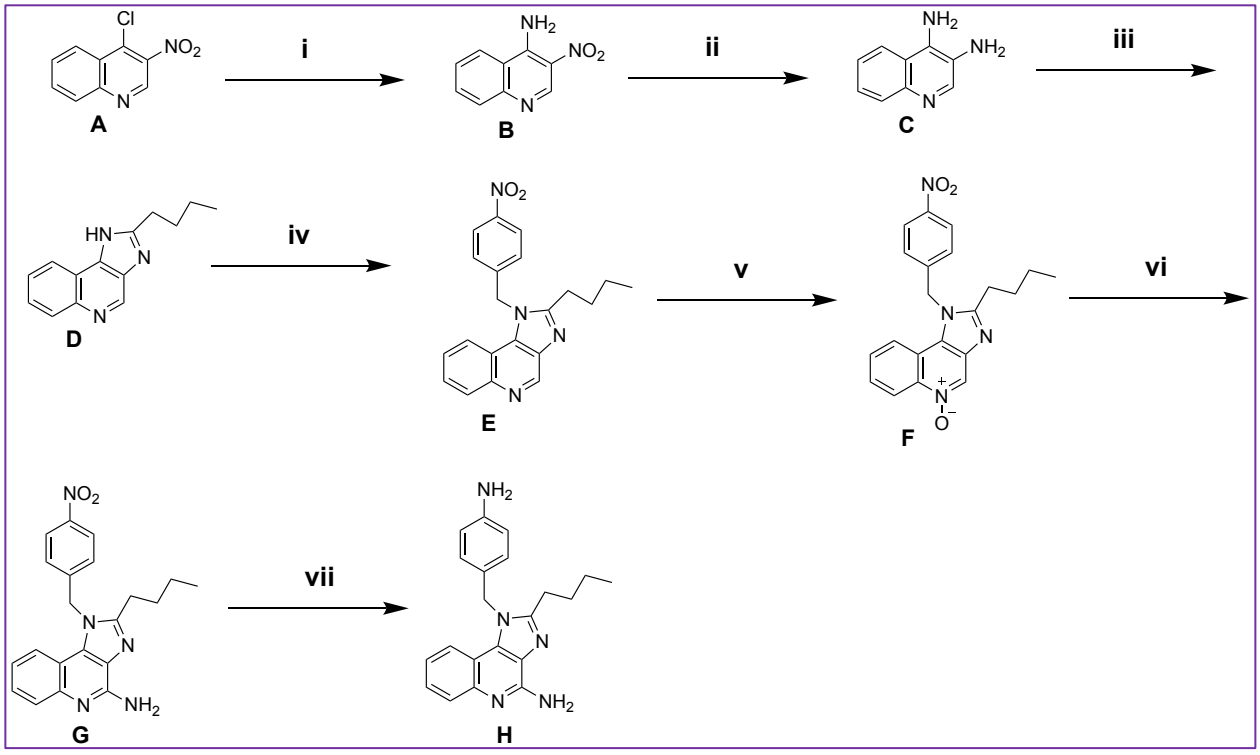


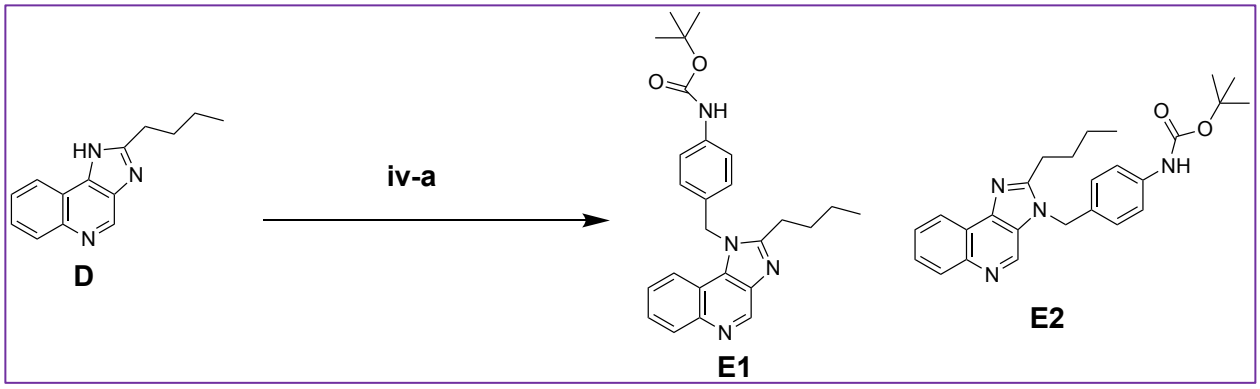
E104 (8a) **R848**

Figure 1: Structure of Known TLR7 Agonists

2. Failed Synthetic Pathway

Initial efforts consisted of an alternate synthetic pathway of E104 (**8a**) as shown below in Scheme 1. This pathway was attempted to postpone the benzylic functionalization until later in the synthetic sequence, allowing for easier and more time efficient synthesis of derivatives. Although this was proposed, the major and minor products in the step iv alkylation of the imidazole tricycle were in fact opposite of what was desired, yielding compound **E2** rather than **E1** as the major product, which was undesirable. In fact, there was only a 14.1% conversion to desired compound **E1**, providing a need for a different synthetic pathway of E104 (**8a**) and its derivatives. This overall synthetic route is shown below in the top image of Scheme 1 with my particular attempt at functionalization in the bottom image.





Scheme 1: Failed Synthetic Pathway, Top Image = Overall E104 (**8a**) Synthetic Route, Bottom Image = Specific Attempt at Functionalization, Reagents Top (i) NH_4OH, dioxane, 120°C; (ii) NH_4Cl, Fe, EtOH, H_2O; (iii) Valeric Acid, 130°C; (iv) p-nitrobenzyl bromide, cesium carbonate, DMF; (v) mCPBA, $CHCl_3$;(vi) NH_4OH, $CHCl_3$; (vii) NH_4COOH, Zn, MeOH. Reagents Bottom (iv-a) tert-butyl (4-(bromomethyl)phenyl)carbamate, cesium carbonate, DMF

3. Synthesis of Initial TLR7 Agonist Payloads

After optimizing the synthetic pathway of E104, the focus of our work was to explore replacing the amine of E104 with alternative H-bond donor/acceptor functional groups. The synthetic pathway used for the preparation of these derivatives can be seen below in the top image of Scheme 2. Additionally, I individually prepared compounds **7b** and **8b** and this synthetic scheme is shown in the bottom image of Scheme 2.

Firstly, within my synthetic pathway, a nucleophilic aromatic substitution reaction of compound **1** was completed at the 4-chloro position with 84% yield. Compound **2b** was extracted through vacuum filtration. This was followed by a nitro reduction with zinc to produce compound **3b** that often resulted in varying yields and the presence of a major impurity with molecular weight 276Da. During this we found that limiting reaction time to 25 minutes, using 10 equivalents of ammonium chloride and zinc, and completing the reaction in an ice bath were

optimized conditions in which the presence of the major impurity was limited, for an overall yield of 58%. A workup consisting of a basic extraction using sodium hydroxide was also helpful in removing this impurity. Additionally, the compound was vacuum filtrated previously to the extraction to remove the presence of excess zinc in the reaction. The proposed structure of this impurity is shown below in Figure 2.

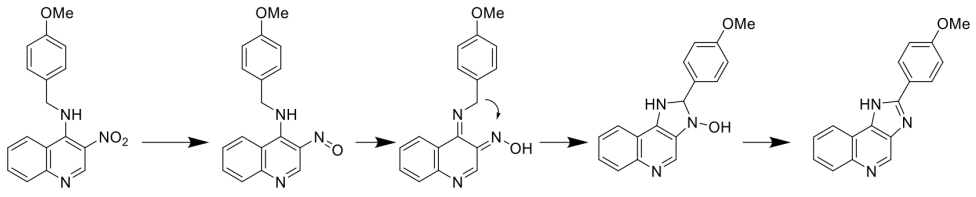
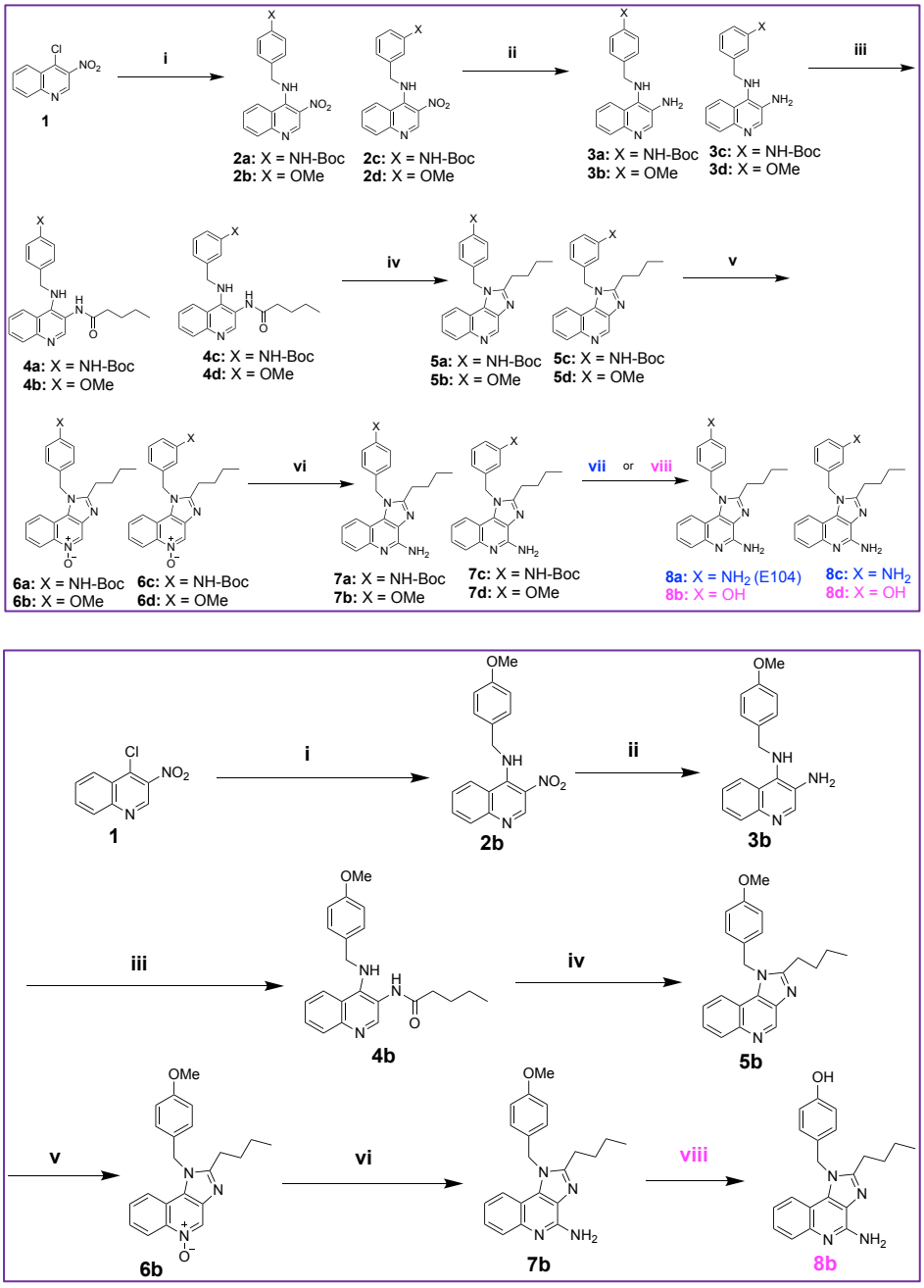


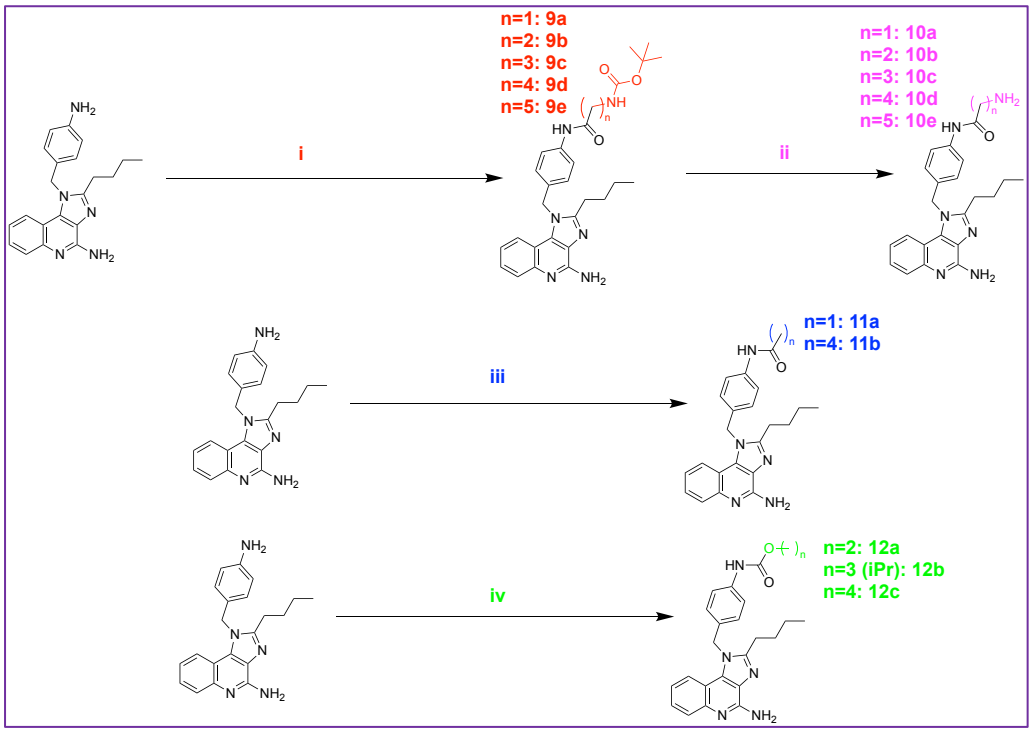
Figure 2: Proposed mechanism and structucture of the nitro reduction impurity

The next step of the synthesis consisted of an acylation of the 3-amino group to yield compound **4b** directly followed by a cyclization to yield compound **5b.** These two steps were completed without purification in between and resulted yield of 45%. Compound **5b** was purified by silica gel chromatography. Furthermore, an oxidation at the 2-position of the core quinoline was completed to yield compound **6b**. This was carried forward without purification. To yield the first final derivative compound **7b,** an amination was completed on the quinoline, with 45% yield. During this step, it was found that reducing the temperature from 40°C to room temperature after the oxidation along with limiting reaction time to 1.5 hours was important. The order of addition was also significant, with the ammonium chloride being added first and having this stir vigorously for an hour before the addition of the TsCl. To complete the functionalization a demethylation using BBr_3 was completed, although with low yield of 23%. These two final derivatives were purified using preparative HPLC. Overall, these functional group alterations in the meta and para position of the phenyl were synthesized but none of these original alterations proved to be sufficiently potent, compared to the parent small molecule E104 (**8a**).



Scheme 2: Reagents (i) Et_3N, 3-methoxybenzylamine OR 4-methoxybenzylamine OR tBu(3-(aminomethyl)phenyl)carbamate OR tBu(4-(aminomethyl)phenyl)carbamate, DCM, 30-40C (ii) Zn, NH_4Cl, MeOH, 0C (iii) C_5H_9ClO, Et_3N, EtOAc, 0C (iv) NaOH, EtOH, H_2O, 80C (v) mCPBA, $CHCl_3$, 40C (vi) NH_4OH, TsCl, $CHCl_3$ (vii) TFA, DCM, 23C (viii) BBr_3, DCM, under N_2, 0C -> RT.

A library of compounds derived from E104 were developed to expand our understanding of structure activity relationships (SAR) of this molecule. Coupling of various boc-amino acids using EDC coupling conditions yielded compounds **9a-e.** This was followed with a TFA deprotection of the Boc group resulting in derivatives with a primary amine (**10a-e**). Furthermore, amides and carbamates of E104 (**8a**) were prepared from acyl chlorides and chloroformates, respectively yielding compounds **11a-b** and **12a-c**. Compounds were purified by preparative HPLC and were generally obtained in yields of 20-30%, 80-100%, 24-28% and 30-46% for compounds **9a-e, 10a-e, 11a-b,** and **12a-c** respectively. The synthesis of these is shown below in Scheme 3. In all, 21 analogs of E104 (**8a**) were prepared, with the overall synthesis of these being shown both in Scheme 2 and 3. The goal of these efforts was to extend hydrophobic chains from the benzylic nitrogen in order to increase potency.



Scheme 3: Reagents (i) RCOOH, HOBt, DIEA, EDC, DMA (ii) TFA, DCM (iii) RCOCl, Et_3N, DCM (iv) ClCOOR, Et_3N, DCM

One unanticipated difficulty in performing this set of reactions was the nonspecific reactivity with the two amino functional groups. Although we anticipated that the core quinoline amine should be less reactive due to its poor nucleophilicity, we found that two different isomers and a double acylation product formed during the coupling reaction. The two isomers were differentiated using tandem quadrupole mass spectroscopy by identifying daughter ion fragments specific to each isomer, with an example of the fragmentation patterns of compound **9a** shown below in Figure 3. The double acylation contaminants were generally removable by preparative HPLC, although peak retention times were considerably close causing the need for careful separation of pure derivatives.

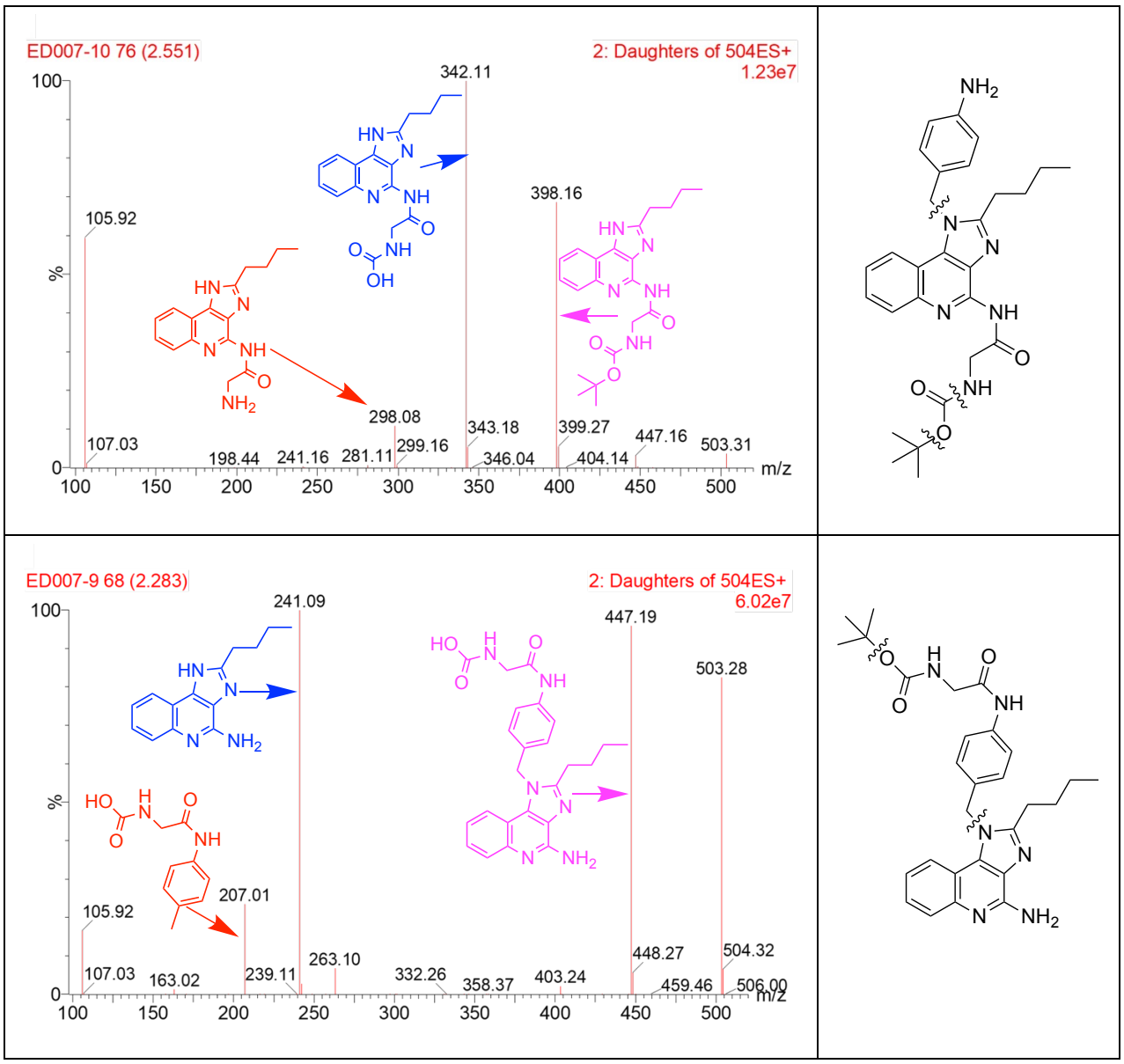


Figure 3: Fragmentation Patterns of Compound 9a with the major isomer being shown on the bottom. Squiggly lines within parent compounds represent fragmentation points within the molecules.

To reduce the amount of undesired double acylation, a comparison was performed between different coupling reagents, HATU, EDC, T3P and EEDQ. Both T3P and EEDQ resulted in extremely slow reactions and little conversion to desired product occurred even after a reaction time of 24 hours. EDC exhibited considerable conversion to desired product, but also

resulted in the formation of significant amounts of the double acylation product, especially with longer reaction times. Gratifyingly, HATU coupling resulted in minimal double acylation compared to other coupling reagents even with extended reaction time, thus allowing for more conversion of starting material to desired product. In fact, almost all of the E104 (**8a**) was consumed while using HATU as a coupling reagent. The HPLC comparison traces are represented below in Figure 4.

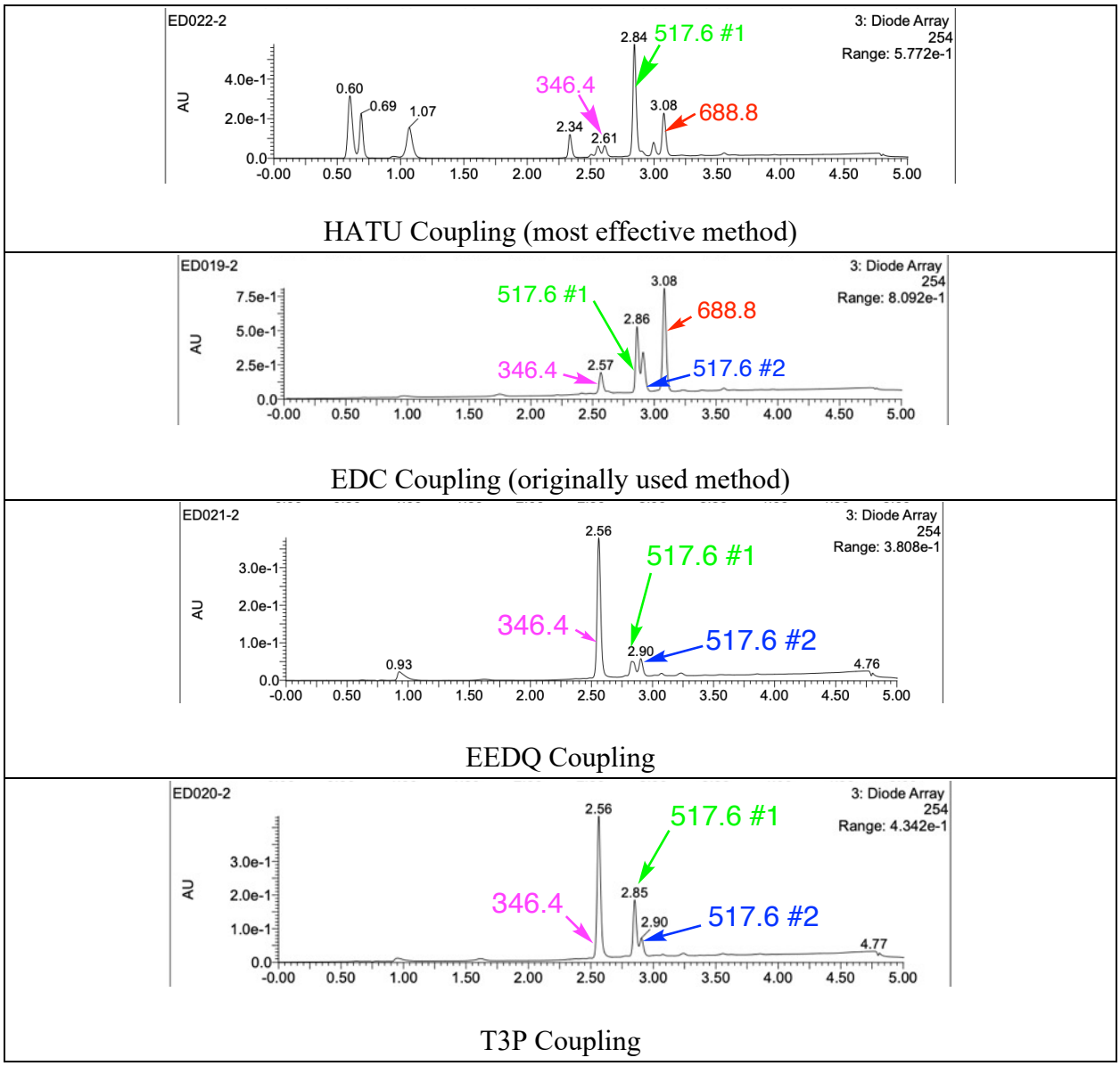


Figure 4: HPLC Traces after 24 hours for HATU, EDC, EEDQ and T3P Method Comparison

4. Evaluation of Initial TLR7 Agonist Payloads

4.1. Immune Activation of Ramos Blue Cells by Synthesized TLR7 Agonists

The potency of each compound was established in Ramos Blue Cells by measuring the amount of SEAP production in response to a 72 h incubation at 37 ℃ with each compound. The Ramos Blue cell line is engineered to express SEAP (secreted embryonic alkaline phosphatase) in response to NFκB activation by TLR ligands. Importantly, Ramos cells (the parental cell line) express large amounts of endogenous TLR7. The EC50 of these compounds are shown later in Table 1. The EC50 is defined as the point at which a compound exhibits half of its maximum activity. An ideal TLR7 agonist has an EC50 in the low nM range. These initial assays were performed by Justin Howe and results are shown below in Figure 5.

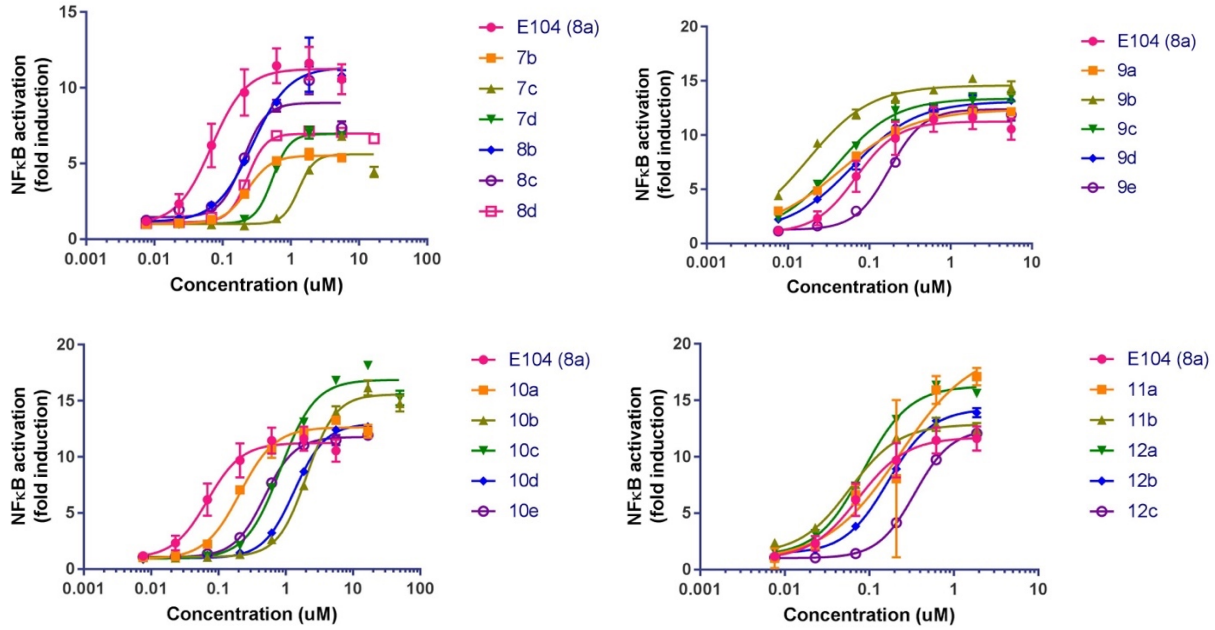


Figure 5: Ramos-Blue NFκB Activation of Initial TLR7 Agonist Payloads

Multiple compounds were seen to have enhanced potency compared to E104. Highly potent compounds were found to have Boc and alkyl chains at the end of the benzylic substituent. For example, compounds **9a, 9b, 9c** and **9d** all contained a Boc group at the end of

the benzylic substituent, indicating the significant influence of Boc structure on potency. In contrast, the primary amine (deprotected) versions of these compounds exhibited the lowest potency such as compound **10b** and **10d**, perhaps due to the decrease in hydrophobicity caused by this functional group. Interestingly, the Boc group attached directly to the benzene (**7c**) showed very poor potency and the Boc group containing a 6-carbon chain (**9e**) showed semi poor potency, indicating carbon chains of 2-5 being ideal for improving potency in TLR7 agonists. Compound **11b** consisted of a 4-carbon chain and compound **12b** consisted of an isopropyl group.

4.2.PAMPA Permeability of Synthesized TLR7 Agonists

In addition to potency, one key design factor for the TLR7 agonists is permeability. Permeability determines the amount of compound that is able to pass through a cell membrane which is required for the activation of nearby tumor-associated immune cells. To test the permeability of each derivative, PAMPA (Parallel Artificial Membrane Permeability Assay) was employed. PAMPA consists of acceptor and donor wells with a lipid/oil/lipid trilayer artificial membrane. The permeability is measured based upon the amount of compound that passes through the pre-coated filter plate from the donor solution to the acceptor solution. The plates were coupled and incubated at 37 ℃ for 5 hours without agitation and the amount of compound in both the donor and acceptor solutions were measured. The overall mechanism of this assay is shown below in Figure 6.

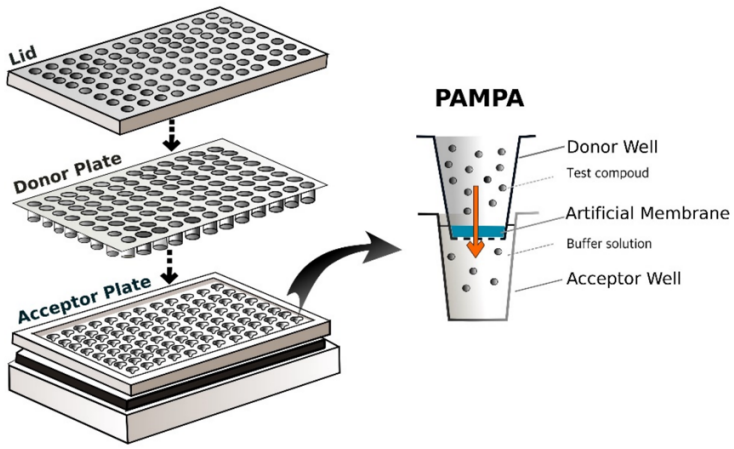


Figure 6: PAMPA mechanism with overall layout shown on the left and an individual well highlighted on the right.[6] Image reprinted from reference 6.

Additionally, to quantify the amount of material that passed through the artificial membrane, calculations were performed on the basis of the manufacturer's instructions.[8] This calculation is represented below.

Permeability: $\mathrm{Pe} = -\ln(1 - CA(t)Ceq)\, A \times (1VD + 1VA) \times t$

Where, A = filter area (0.3cm^2), VD = donor well volume (0.3mL), VA = acceptor well volume (0.2mL), t = incubation time (18000 seconds), CA(t) = compound concentration in acceptor well at time t, CD(t) = compound concentration in donor well at time t and Ceq = (CD(t)*VD +CA(t)*VA)(VD +VA)

Using calculated values found from this equation, average permeability for each compound is reported below as an average of the three triplicate values, as shown below in Figure 7.

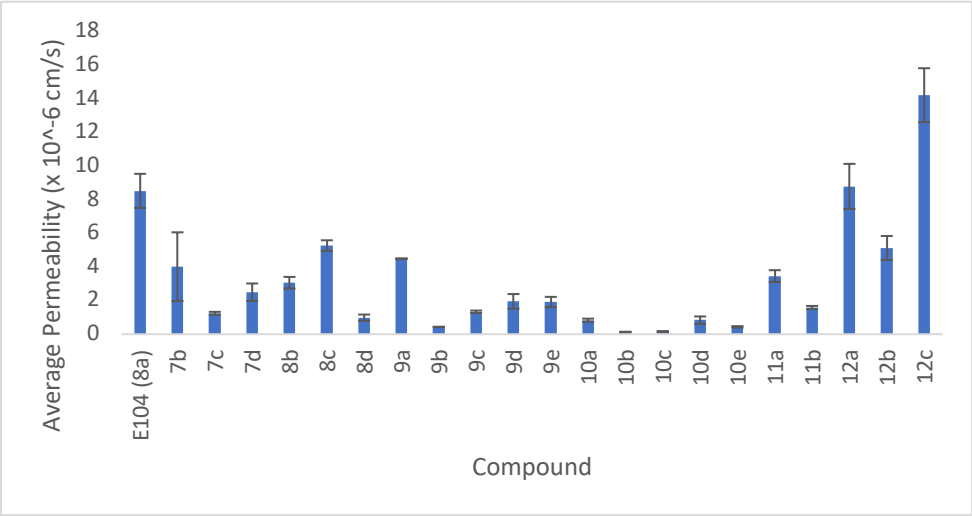


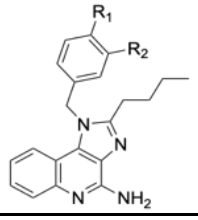
Figure 7: Permeability Evaluation as determined from PAMPA.

Derivatives with poor permeability generally contained amines at the end of their benzylic substituent. This was anticipated due to amines hydrophilicity and cationic nature, which decreases the ability of the compound to pass through the membrane. Derivatives with significantly high permeability consisted of E104 (**8a**) and derivatives made from chloroformates as its benzylic substituent (**12a-c**). Compounds **7b, 8c,** and **9a** were also seen to have adequate permeability.

4.3. SAR Evaluation of Synthesized TLR7 Agonists

Although there was no observed correlation between potency and permeability, three specific compounds were identified for further study based upon the permeability and potency assessment. **9b** and **11b** were chosen because of their high potency whereas **12b** was chosen as a semi potent but permeable compound. These are highlighted in green below in Table 1. These three agonists were additionally evaluated for cytokine release such as INFα and TNFα in PBMCs, HEK Blue TLR7 versus TLR8 specificity, TNFα and IL-6 production as a result of hMDM stimulation and Raw Dual NFκB activation. These further evaluative assays were performed by Siteng Fang.

Table 1[4]: Results of Ramos-Blue™ NFκB reporter assay and PAMPA permeability screening. All experiments were performed in triplicate and the number shown represents the average of three experiments.

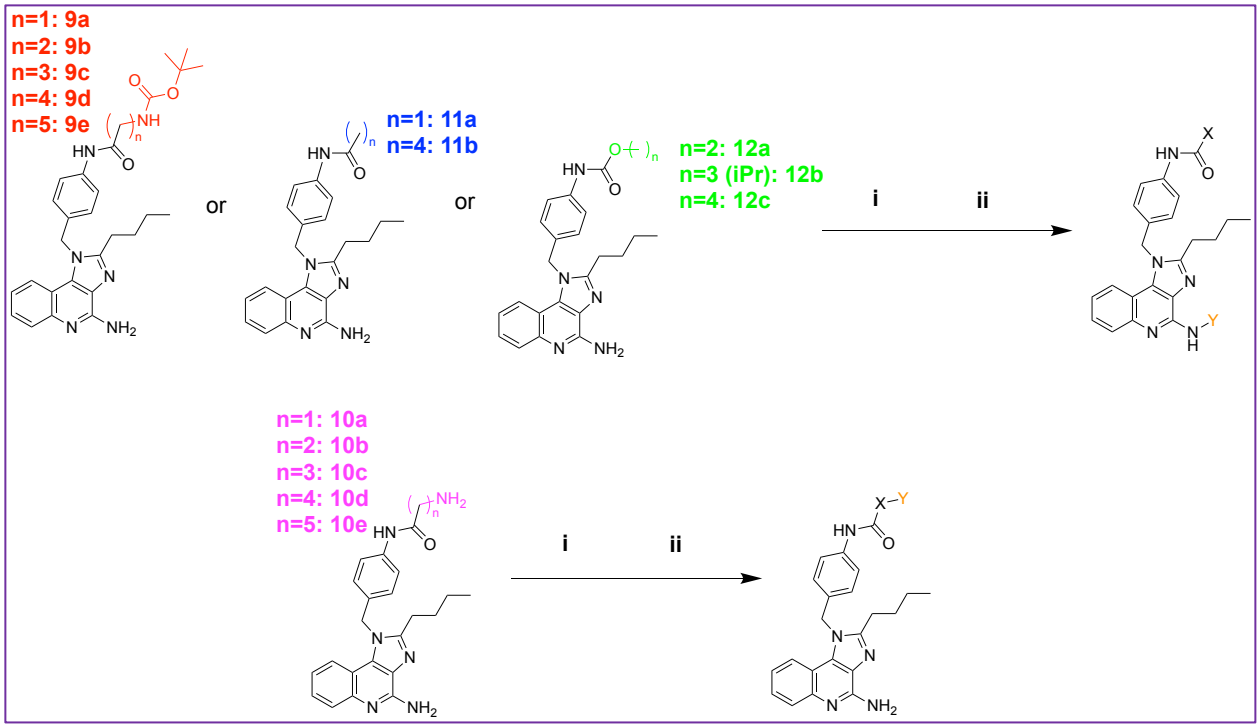


Compound	R_1	R_2	EC_{50} (μM)	P_{app} (cm/s)
E104 (8a)	-NH_2	-H	0.069 ±0.010	8.51E-06
7b	-OMe	-H	0.232 ±0.009	4.01E-06
7c	-H	-NHBoc	1.31 ±0.23	1.24E-06
7d	-H	-OMe	0.54 ±0.014	2.50E-06
8b	-OH	-H	0.275 ±0.029	3.06E-06
8c	-H	-NH_2	0.199 ±0.026	5.26E-06
8d	-H	-OH	0.231 ±0.007	9.80E-07
9a	-NHC(O)CH_2-NHBoc	-H	0.044 ±0.002	4.48E-06
9b	-NHC(O)$(CH_2)_2$-NHBoc	-H	0.018 ±0.001	4.34E-07
9c	-NHC(O)$(CH_2)_3$-NHBoc	-H	0.039 ±0.003	1.33E-06
9d	-NHC(O)$(CH_2)_4$-NHBoc	-H	0.0622 ±0.005	1.95E-06
9e	-NHC(O)$(CH_2)_5$-NHBoc	-H	0.176 ±0.009	1.91E-06
10a	-NHC(O)CH_2NH_2	-H	0.209 ±0.012	8.34E-07
10b	-NHC(O)$(CH_2)_2$-NH_2	-H	2.06 ±0.092	1.31E-07
10c	-NHC(O)$(CH_2)_3$-NH_2	-H	0.84 ±0.060	1.59E-07
10d	-NHC(O)$(CH_2)_4$-NH_2	-H	1.34 ±0.051	8.38E-07
10e	-NHC(O)$(CH_2)_5$-NH_2	-H	0.485 ±0.014	4.36E-07
11a	-NHAc	-H	0.107 ±0.010	3.45E-06
11b	-NHC(O)-*n*Bu	-H	0.059 ±0.004	1.59E-06
12a	-NHC(O)O-Et	-H	0.084 ±004	8.78E-06
12b	-NHC(O)O-iPr	-H	0.168 ±0.007	5.12E-06
12c	-NHC(O)O-*n*Bu	-H	0.344 ±0.009	1.42E-05

5. Synthesis of Initial Cleavable and Non-Cleavable Linker Payloads

Having identified a few payloads with improved potency compared to E104, the next step was attaching linkers to these more potent payloads in order to facilitate ADC preparation. Two types of linkers were used when synthesizing immune activating linker payloads. The first linker is the cleavable peptide linker mcValCitPABC which is cleaved in the lysosome by the cathepsin B protease for controlled payload release. Secondly, there is the 6-maleimidohexanoic acid linker which is non cleavable and releases the payload only after internalization and complete catabolism of the ADC. The two types of linker payloads were synthesized for different functionality in the delivery of payloads by ADCs.

Our previous linker payload synthesis using E104 was fairly easy, as both linker reagents were readily reactive with the aniline. However, due to different chemical structures of these newly developed agonists, many of which contained a hydrophobic acyl tail attached to the aniline, linker payload synthesis became complicated. Some of the agonists still contained a primary amine on the end of the benzylic substituent, resulting in easy attachment of the linker to the highly reactive amine for compounds such as **10a-e.** Potent compounds **9b, 11b** and **12b**, all contained a hydrophobic tail attached to the aniline, thus necessitating addition of the linker to the poorly nucleophilic core amine. Development of new methods for the successful synthesis of linker payloads was necessary, to develop a more versatile approach for attaching payloads to linkers. The comparison between successful (bottom) and failed (top) linker payload reactions are shown below in Scheme 4.



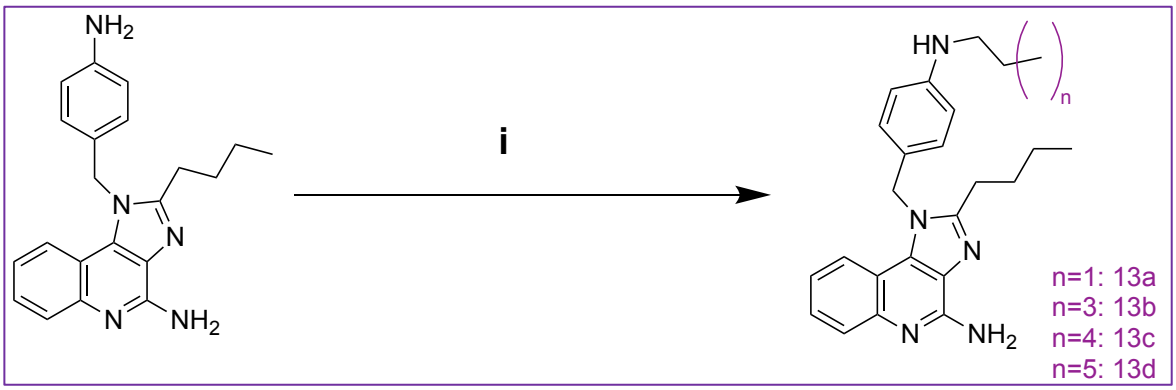
Scheme 4: Reagents (i) HOBt, 2,6-lutidine, mcValCitPABC-PNP, (ii) HOBt, 2,6-lutidine, HATU, 6-maleimidohexanoic acid, X = corresponding benzylic functionalization, Y = linker attachment point

6. Synthesis of New TLR7 Agonist Payloads for Linker Payload Compatibility

The 3 initial potent payloads of interest consisted of hydrophobic tails of $NHC(O)(CH_2)_2$-NHBoc, NHC(O)-*n*Bu and NHC(O)O-iPr and lack the aniline in which to serve as a linker attachment point, which is vital for ADC applications. Therefore, a new approach was explored for the development of potent TLR7 agonists that retained a nucleophilic aniline for linker attachment.

4 new compounds (**13a-c**) were synthesized with the goal of retaining a nucleophilic aniline for linker attachment. We envisioned attaching the hydrophobic appendage and the linker onto the same nitrogen by eliminating the carbonyl of the hydrophobic group. This was accomplished

using a reductive amination with sodium cyanoborohydride in the presence of acetic acid will be attempted as shown below in Scheme 5. This resulted in the production of a N-C bond between the aldehyde and an amine, while still leaving the ability of a linker to be attached at the same position.



Scheme 5: Reagents (i) CH_3COOH, $NaBH_3CN$, MeOH

Initially the reductive amination reactions for compounds **13c** and **13d** were completed using 3 equivalents of aldehyde. However, these conditions resulted in the formation of double addition and even slight amounts of triple addition product. Thus, the reaction was optimized to be performed with 1 equivalent of aldehyde which was seen to reduce double addition and triple addition. Using these conditions, two smaller aldehydes were employed for the preparation of **13a** and **13b** and little or no multi-addition side-products were observed. Additionally, the lower equivalents of aldehyde resulted in improved yields of around 40-50% with compounds **13a** and **13b** as compared to yields of 10-20% with compounds **13c** and **13d**.

Additionally, we found that the particular purification method was important for the isolation of these compounds. Typically, a preparative HPLC method containing TFA modifier was used to purify most derivatives described above. However, this method proved to not be suitable for **13a-d** due to the reaction of the compounds with the TFA within the mobile phase solvents. This issue was resolved by using a formic acid modifier in place of the TFA.

7. Immune Activation of Ramos Blue Cells by New TLR7 Agonist Payloads

Using the assay conditions developed by my coworker Justin Howe, I evaluated the compounds for their ability to activate the NFκB pathway in Ramos Blue Cells. Derivatives **13c** and **13d** were synthesized using more bulky aldehydes such as hexanal and heptanal, which resulted in poor potency. Derivatives **13a** and **13b** were synthesized using shorter alkyl chains which were previously seen to yield higher potency in the initial set of E104 derivatives. However, smaller aldehydes such as propionaldehyde and pentanal also did not have improved potency as compared to the parent molecule E104 (**8a**). Although the smaller aldehyde, in compound **13a,** was more potent than more bulky aldehydes, it also did not provide the desired potency for TLR7 agonist ADC technology. The activation of these 4 compounds is shown below in Figure 8.

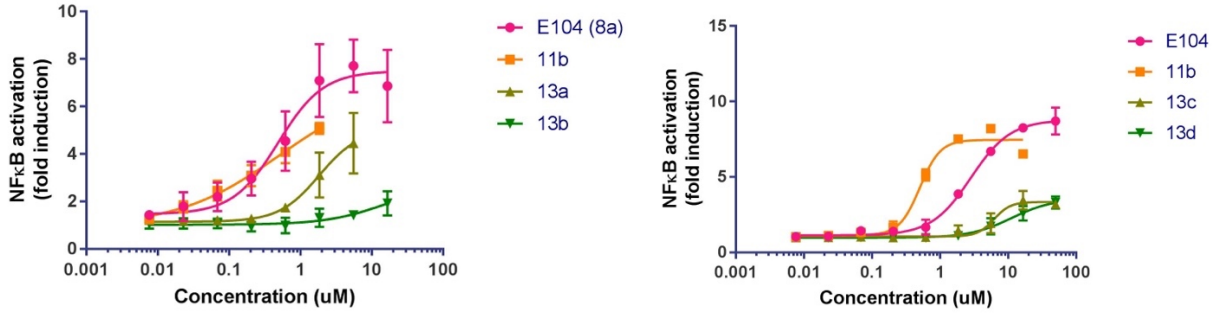
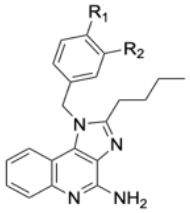


Figure 8: Ramos-Blue NFκB Activation of New TLR7 Agonist Payloads

Unfortunately, these new N-alkyl derivatives were far less potent than E104. Moreover, they were far less potent than the analogous N-acyl derivative **11b,** illustrating the importance of amide functionality. In fact, all compounds that exhibited improved potency as compared to E104 incorporated this amide.

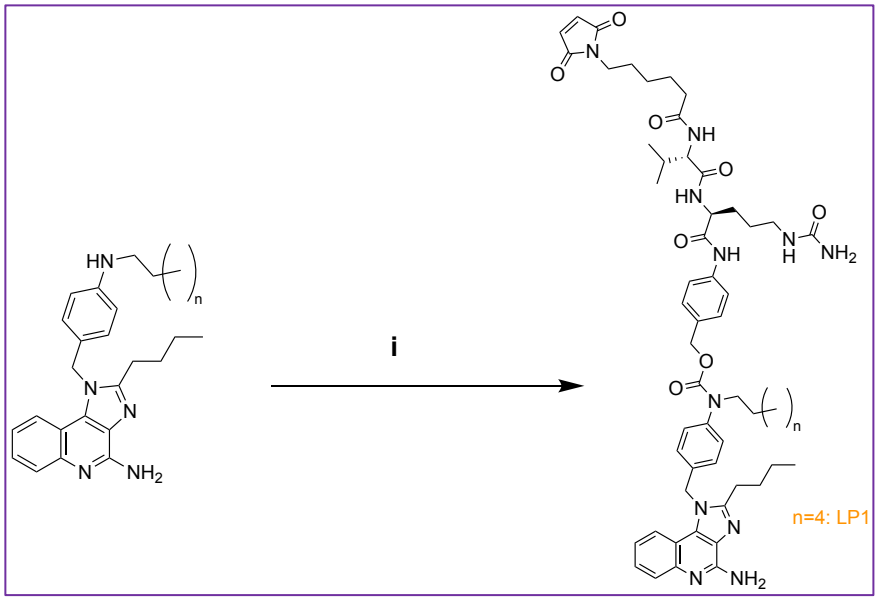
Table 2[4]: Results of Ramos-Blue™ NFκB reporter assay. All experiments were performed in triplicate and the number shown represents the average of three experiments.



Compound #	**R_1**	**R_2**	**EC_{50} (μM)**
E104 (8a)	- NH_2	-H	0.490 ± 0.136
11b (potent payload)	-NHC(O)-*n*Bu	-H	0.510 ± 0.040
13a	$-NH(CH_2)_2CH_3$	-H	1.85 ± 0.926
13b	$-NH(CH_2)_4CH_3$	-H	15.51 ± 72.95
13c	$-NH(CH_2)_5CH_3$	-H	6.23 ± 0.980
13d	$-NH(CH_2)_6CH_3$	-H	11.2 ± 3.054

8. Synthesis of New Cleavable Linker Payloads

Although these aldehyde containing derivatives were not significantly potent, they did exhibit improved reactivity towards linker-addition. For example, compound 13c readily reacted with mcValCitPABC-PNP to form compound **LP1** in around 23% conversion to desired product, indicating better ability than initial payloads. This reaction is shown below in Scheme 6.



Scheme 6: Reagents (i) mcValCitPABC-PNP, DIPEA, 2,6-lutidine, HOAt

9. Conclusion

Structure-activity relationships were studied in order to develop an understanding of the functionality of potential derivatives of E104 (**8a**). In the initial set of derivatives, specific compounds were identified with improved potency and permeability relative to parent small molecule E104 (**8a**). Compounds (**9a, 9b, 9c, 9d, and 11b**) showed improved potency and compounds (**12a** and **12c**) showed improved permeability. Although no specific derivatives showed both improved potency and permeability, potency was focused upon in order to determine efficacy of derivatives, with permeability being examined secondarily. Considering this, compounds **9b, 11b** and **12b** would all be sufficient E104 (**8a**) derivatives to serve as TLR7 agonist small molecules for further evaluation as ADC payloads. Building on these results, we also evaluated the SAR of a new set of N-alkyl payloads. Although, they proved not to have improved potency to E104 (**8a**), we demonstrate that these compounds are able to be easily functionalized with linkers such as mcValCitPABC.

Additional future efforts of this project will involve the exploration of TLR7 antagonists. TLR7 antagonists are relevant in their ability to inhibit the TLR7 pathway and reduce NFκB activation. Therefore, they can be used to treat diseases where there is overstimulation of the TLR7 pathway, such as lupus.[7] Compounds that were synthesized and proved to have extremely low potency will be evaluated in Ramos Blue Cells for their ability to inhibit the TLR7 pathway as potential starting point for immune-suppressing drug discover efforts.

10. Experimental Methods

10.1. Synthetic Methods

tert-Butyl (3-(((3-nitroquinolin-4-yl)amino)methyl)phenyl)carbamate (2c): To a solution of tert-butyl (3-(aminomethyl)phenyl)carbamate (1080 mg, 1 Eq, 4.85 mmol) in DCM (27.0 mL) was added **1** (1000 mg, 1 Eq, 4.82mmol) and triethylamine (975 mg, 1.34 mL, 2 Eq, 9.64 mmol). The mixture was brown and became yellow once the triethylamine was added. The mixture was brought to reflux at 40°C for 1 h. A yellow precipitate formed, and LCMS indicated near-complete conversion by 60 min. The reaction was cooled to room temperature and concentrated to dryness. The yellow solid was suspended in water (140 mL), filtered under vacuum through a sintered funnel and dried in a desiccator to obtain the desired compound. (1550 mg, 82% yield) LCMS rt =3.50 min (polar gradient); m/z = 394.43 [M+H].

tert-Butyl (3-(((3-aminoquinolin-4-yl)amino)methyl)phenyl)carbamate (3c): A suspension of **2c** (650 mg, 1 Eq, 1.65 mmol) in MeOH (20 mL) was treated with a pre-cooled suspension of zinc (1100 mg, 10.2 Eq, 16.8 mmol) and ammonium chloride (900 mg, 10.2 Eq, 16.8 mmol) in MeOH (6 mL). The mixture was stirred at 0°C for 20 min and monitored by HPLC. The mixture

rapidly turned to a grey/green suspension. After 20 min the reaction mixture was filtered through celite, and the solvent was evaporated in vacuo. The residue was dissolved in DCM, washed with water, and back-extracted into DCM. The organic layer was dried over $MgSO_4$ .The solvent was removed under vacuum to obtain the desired compound (549 mg, 91% yield). LCMS rt = 2.66 min (polar gradient); m/z = 365.45 [M+H].

tert-Butyl (3-((2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)carbamate (5c):
Step 1: **3c** (549 mg, 1.0 Eq, 1.51 mmol) in anhydrous EtOAc (16 mL), cooled to 0°C, was added to triethylamine (198 mg, 273 µL, 1.3 Eq, 1.96 mmol) also at 0°C. This mixture was stirred for 10 min. Valeryl chloride (236 mg, 232 µL, 1.3 Eq, 1.96 mmol) in EtOAc (5 mL) was added dropwise at 0°C to the reaction mixture. The mixture was stirred for 40 min and monitored by HPLC. The solution was concentrated to dryness under reduced pressure and carried forward without purification. LCMS rt = 2.79 min (polar gradient) m/z = 449.57 [M+H].
Step 2: The crude product from the previous step, **4c** (676 mg, 1 Eq, 1.51 mmol) was dissolved in EtOH (9 mL) and treated with sodium hydroxide (121 mg, 2 Eq, 3.01 mmol) in H_2O (1.38 mL). The mixture was refluxed for 4 h and was monitored by HPLC. The reaction was diluted with water and the product was extracted with DCM. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The amber-red product was purified using silica gel chromatography (MeOH/DCM) providing 647 mg (99.8% yield) of the title product (5c). LCMS rt = 3.15 min (polar gradient) m/z = 431.55 [M+H].

tert-Butyl (3-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)carbamate (7c): 3-Chlorobenzoperoxoic acid (382 mg, 2.90 Eq, 1.55 mmol) was added to a solution of

5c (230 mg, 1.0 Eq, 0.535 mmol) in $CHCl_3$ (3 mL). The reaction mixture was stirred at 40°C for 1 h. The material was re-dissolved in $CHCl_3$ (10 mL) and warmed to 50°C. Ammonium hydroxide (20%) (2.81 g, 30 Eq, 16.0 mmol) was added dropwise and the mixture was left to stir for 1 h at 50°C. 4-Methylbenzenesulfonyl chloride (204 mg, 2 Eq, 1.07 mmol) was added to this mixture and the resulting reaction was stirred rapidly at 50°C for 4 h. The reaction was further diluted with $CHCl_3$ (40 mL) and washed with aqueous bicarbonate and brine. The organic layer was dried with $MgSO_4$ and dried under reduced pressure. The crude product was purified by silica gel chromatography (20% DCM/MeOH). The pure fractions were dried under reduced pressure giving 165 mg of the title compound. (69% yield) LCMS rt = 2.94 min (69%) (polar gradient); m/z = 446.57 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 8.94 (bs, 2H), 7.96 (d, J = 8.1Hz, 1H), 7.82 (d, J = 8.1Hz, 1H), 7.65 (t, J = 7.5Hz, 1H), 7.47-7.35 (m, 3H), 7.25 (t, J = 7.7Hz, 1H), 6.73 (d, J = 7.7Hz, 1H), 5.93 (s, 2H), 2.98 (t, J = 7.5Hz, 2H), 1.75 (quint, J = 7.5Hz, 2H), 1.52-1.36 (m, 11H), 0.89 (t, J = 7.0Hz, 3H).

1-(3-Aminobenzyl)-2-butyl-1H-imidazo[4,5-c]quinoline-4-amine (8c): TFA (78 μL, 15 eq) was added to a solution of **7c** (30 mg, 67 μmol) in DCM. The reaction mixture was stirred at 23℃ for 3 h. The resulting product mixture was dried under reduced pressure giving the desired material as a dark red-amber solid. (16.3 mg, 71% yield) LCMS rt = 2.61 min (97.9%) (polar gradient) m/z = 346.45 [M+H]. 1 H NMR (400 MHz, DMSO) δH/ppm 9.00 (bs, 2H), 7.98 (d, J = 8.5Hz, 1H), 7.81 (d, J = 8.5Hz, 1H), 7.65 (t, J = 7.9Hz, 1H), 7.39 (t, J = 7.5Hz, 1H), 7.08 (t, J = 7.7Hz, 1H), 6.59 (d, J = 8.1Hz, 1H), 6.43 (d, J = 6.9Hz, 1H), 6.29 (s, 1H), 5.84 (s, 2H), 1.75 (quint, J = 7.7Hz, 2H), 1.41 (sextet, J = 7.7Hz, 2H), 0.89 (t, J = 7.7Hz, 3H)

N-(4-Methoxybenzyl)-3-nitroquinolin-4-amine (2b): 1 (1000 mg, 1 Eq, 4.81 mol) was dissolved in DCM (15.0 mL) and treated with (4-methoxyphenyl)methanamine (725 mg, 690 µL, 1.10 Eq, 5.28 mmol) followed by triethylamine (973 mg, 1.34 mL, 2 Eq, 9.62 mmol). The reaction was stirred at 30℃ for 1 h, concentrated to dryness, then titrated with water. The title compound was obtained by vacuum filtration (1250 mg, 4.04 mmol, 83.7% yield) as a bright yellow powder. LCMS rt = 3.23 min; m/z = 310.1 [M+H]. 1 H NMR (400 MHz, DMSO) δH/ppm 9.40 (t, J = 5.4Hz, 1H), 9.02 (s, 1H), 8.54 (d, J = 8.4Hz, 1H), 7.90 (d, J = 8.4Hz, 1H), 7.83 (t, J = 7.5Hz, 1H), 7.58 (t, J = 7.6Hz, 1H), 7.27 (d, J = 8.6Hz, 2H), 6.91 (d, J = 8.4Hz, 2H), 4.84 (d, J = 5.7Hz, 2H), 3.73 (s, 3H)

N4-(4-Methoxybenzyl)quinoline-3,4-diamine (3b): An approximately 500 mg portion of **2b** (501 mg, 1 Eq, 1.62 mmol) was suspended in Methanol (30.0 mL). The solution was stirred and ammonium chloride (872 mg, 10.0 Eq, 16.3 mmol) was added. This was then put into an ice bath and then Zinc (1063 mg, 10.0 Eq, 16.3 mmol) was added. The reaction was kept for a total of 25 minutes and was monitored by UPLC, giving the desired product (534 mg, 118.0% recovery). This was then washed with a 50:50 Methanol and DCM mixture through vacuum filtration over celite for removal of the excess Zinc. The solvent was then evaporated. This was now dissolved in 10% Methanol in DCM (40.0 mL) and partitioned against NaOH (40.0 mL). This was repeated 3 times and then the organic layer was dried using $MgSO_4$. This was then dried to recover the desired compound (308 mg, 57.6% yield). LCMS rt = 1.64 min; m/z = 280.4 [M+H].

2-Butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-c]quinoline (5b):

Step 1: 308 mg of **3b** was dissolved in ethyl acetate (5 mL). Triethylamine (145 mg, 200 µL, 1.3 Eq, 1.43 mmol) was added and the reaction was chilled to 0°C and stirred. Valeryl chloride (152 mg, 150 µL, 1.15 Eq, 1.26 mmol) was dissolved in 1.25 mL of ethyl acetate and cooled to 0°C then added dropwise to the reaction mixture. After stirring for 20 minutes at 0°C, the reaction was concentrated to dryness for a final weight of 576 mg of N-(4-((4-methoxybenzyl)amino)quinolin-3-yl)pentanamide that was used in the next step without further purification. LCMS rt = 2.59 min; m/z = 364.4 [M+H].

Step 2: The crude **4b** from above (576 mg) was dissolved in ethanol (13 mL) and heated to 80°C. The reaction was treated with NaOH (127 mg, 2.89 Eq, 3.19 mmol) dissolved in water (1.8 mL), also at 80°C. The reaction was heated at 80°C for three and a half hours. After cooling, 25 mL of water was added, and the product was extracted (3x) into DCM. The crude product was dried over magnesium sulfate, concentrated to dryness, and purified using silica gel chromatography (0% -> 10% MeOH in DCM) giving 154 mg of the title compound (44.9% yield). LCMS rt = 2.95 min; m/z = 346.3 [M+H]. 1 H NMR (400 MHz, DMSO) δ H /ppm 9.20 (s, 1H), 8.17-8.10 (m, 2H), 7.62 (t, J = 7.7Hz, 1H), 7.51 (t, J = 7.7Hz, 1H), 6.97 (d, J = 8.5Hz, 2H), 6.88 (d, J = 8.6Hz, 2H), 5.90 (s, 2H), 3.68 (s, 3H), 2.97 (t, J = 7.8Hz, 2H), 1.79 (quint, J = 7.4Hz, 2H), 1.41 (sextet, J = 7.5Hz, 2H), 0.90 (t, J = 7.3Hz, 3H).

2-Butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-c]quinolin-4-amine (7b): Compound **5b** (141 mg, 1 Eq, 409 µmol) was dissolved in chloroform (2.5 mL) and treated with 3-chlorobenzoperoxoic acid (216 mg, 3.05 Eq, 1.24 mmol). After vortexing, the reaction was heated to 40°C and stirred for 1 hour. The intermediate N-oxide was observed by LCMS (rt =

3.24 min; m/z = 362.3 [M+H]). The reaction was cooled to room temperature and then stirred vigorously and treated with ammonium hydroxide (405 mg, 460 µL, 28.2 Eq, 11.5 mmol). After stirring vigorously for 1h, 4-methylbenzenesulfonyl chloride (161 mg, 2.01 Eq, 846 µmol) was added and stirring was continued for 30 minutes. The reaction was concentrated to dryness, dissolved in 40 mL of chloroform, and washed with 40 mL of aqueous sodium bicarbonate and 40 mL of brine giving 229 mg of crude product. A portion of the product (70.6 mg) was further purified by prep HPLC providing a total of 31.9 mg of the title compound. LCMS rt = 2.77 min (90.9%); m/z = 361.5 [M+H]. 1 H NMR (400 MHz, DMSO) δH/ppm 8.01 (d, J = 8.2Hz, 1H), 7.81 (d, J = 8.6Hz, 1H), 7.64 (t, J = 7.5Hz, 1H), 7.40 (t, J = 7.5Hz, 1H), 7.02 (d, J = 8.5Hz, 2H), 6.90 (d, J = 8.6Hz, 2H), 5.89 (s, 2H), 3.70 (s, 3H), 2.98 (t, J = 7.7Hz, 2H), 1.73 (quint, J = 7.6Hz, 2H), 1.39 (sextet, J = 7.4Hz, 2H), 0.88 (t, J = 7.2Hz, 3H)

4-((4-Amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenol (8b): Compound **7b** (23.5 mg) was dissolved in DCM (500 µL) under a nitrogen balloon. Upon cooling to 0°C, a solution of BBr_3 (20 µL) in 500 µL of DCM and was then added to the reaction dropwise. After warming to room temperature, the reaction was stirred overnight and treated with additional BBr_3 (3 Eq) and stirred for additional 2h. The reaction was carefully quenched with 1 mL water and 1 mL of sodium bicarbonate. The product was extracted into DCM giving 5.2 mg of crude material which was further purified by prep HPLC giving 3.3 mg of the title compound (14.6% yield). LCMS rt = 2.13 min (100%); m/z = 347.4 [M+H]. 1 H NMR (400 MHz, DMSO) δH/ppm 9.46 (s, 1H), 8.00 (d, J = 8.0Hz, 1H), 7.78 (d, J = 8.0Hz, 1H), 7.61 (t, J = 7.4Hz, 1H), 7.37 (t, J = 7.0Hz, 1H), 6.89 (d, J = 8.5Hz, 2H), 6.72 (d, J = 8.4Hz, 2H), 6.52 (s, 1H), 5.82 (s, 2H), 2.96 (t, J = 7.6Hz, 2H), 1.72 (quint, J = 7.6Hz, 2H), 1.39 (sextet, J = 7.3Hz, 2H), 0.88 (t, J =

7.3Hz, 3H).

N-(3-Methoxybenzyl)-3-nitroquinolin-4-amine (2d): A suspension of **1** (1.50 g, 1.0 Eq, 7.20 mmol) in DCM (22.0 mL) was treated with (3- methoxyphenyl)methanamine (986 mg, 0.92 mL, 1.0 Eq, 7.20 mmol) and triethylamine (1.09 g, 1.50 mL, 1.50 Eq, 10.8 mmol). The mixture was refluxed at 40°C for 1h resulting in the formation of a bright yellow suspension. Upon cooling to rt, the solid was washed with water, filtered, and dried under vacuum to give the title compound (2.18 g) as a bright yellow powder. LC/MS rt = 3.25 min; m/z = 310.1 [M+H].

N4-(3-Methoxybenzyl)quinoline-3,4-diamine (3d): A suspension of **2d** (2.18 g, 1.0 Eq, 7.04 mmol) in MeOH (25.0 mL) was treated with zinc (1.49 g, 4.0 Eq, 28.0 mmol) and ammonium chloride (1.80 g, 4.0 Eq, 28.0 mmol). The reaction mixture was stirred at room temperature for 10 min to give a grey suspension. The reaction mixture was filtered through celite and the solvent was evaporated in vacuo. The residue was dissolved in DCM and treated with 1M NaOH. The product was extracted (3x) into 10% MeOH in DCM, dried over $MgSO_4$, and concentrated to give 1.36 g (70%, yield) of the title compound as a black-brown oil that was used without further purification. LC/MS rt = 2.44; m/z = 280.1 [M+H].

2-Butyl-1-(3-methoxybenzyl)-1H-imidazo[4,5-c]quinoline (5d):

Step 1: Compound **3d** (1.36 g, 1.0 Eq, 4.88 mmol) was dissolved in anhydrous EtOAc (45.0 mL), cooled to 0°C, and treated with pre-chilled triethylamine (642 mg, 884 µL, 1.3 Eq, 6.34 mmol). After stirring for 5 mins, valeryl chloride (883 mg, 868 µL, 1.5 Eq, 7.32 mmol) in EtOAc (15.0 mL) was added dropwise at 0°C and the reaction mixture was further stirred for 15

min. The reaction was quenched with ethanol and concentrated under reduced pressure forming a yellow-brown crystal that was used directly in the next step. LC/MS rt = 2.56 min; m/z = 364.4 [M+H].

Step 2: The crude product of step 1, **4d** (1.77 g, 1.0 Eq, 4.88 mmol) was dissolved in EtOH (26.0 mL) and treated with sodium hydroxide (464 mg, 1.0 Eq, 11.6 mmol) in H_2O (4.00 mL). The reaction mixture was refluxed at 80°C for 24 h. Upon cooling, the reaction was treated with water (75 mL) and the product was extracted into EtOAc (75 mL x 3). The combined organic extracts were dried over $MgSO_4$ and evaporated to dryness and the title product was purified by silica gel chromatography (10% MeOH in DCM) providing the title compound as an amber resin (856mg, 51% yield). LC/MS rt = 2.97 min; m/z = 346.2 [M+H]. 1 H NMR (400 MHz, DMSO) δ H /ppm 9.21 (s, 1H), 8.11 (d, J = 8.5 Hz, 2H), 7.61 (t, J = 7.7 Hz, 1H), 7.50 (t, J = 7.7 Hz, 1H), 7.21 (t, J = 8.0 Hz, 1H), 6.83 (d, J = 8.2 Hz, 1H), 6.66 (s, 1H), 6.49 (d, J = 7.7 Hz, 1H), 5.93 (s, 2H), 3.67 (s, 3H), 2.96 (t, J = 7.5 Hz, 2H), 1.78 (quint, J = 7.5 Hz, 2H), 1.40 (sextet, J = 7.4 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H).

2-Butyl-1-(3-methoxybenzyl)-1H-imidazo[4,5-c]quinoline-4-amine (7d): Compound **5d** (156 mg, 1.0 Eq, 450 mmol) was dissolved in $CHCl_3$ (2.5 mL) and treated with 3-chlorobenzoperoxoic acid (173 mg, 1.6 Eq, 700 mmol) and stirred at 40°C for 1h. The temperature was increased to 50°C, stirred vigorously, and treated with ammonium hydroxide (2.2 g, 2.5 mL, 28 Eq, 13 mmol) dropwise followed by 4-methylbenzenesulfonyl chloride (174 mg, 2 Eq, 920 μmol). The mixture was cooled to rt and stirred for 1 h. The product was extracted with chloroform, washed with aqueous bicarbonate, and dried over $MgSO_4$ giving 183 mg (112% yield) of crude product which was carried forward without further purification. A portion

of the crude product was purified by preparative HPLC affording the title compound as a white residue. LC/MS rt = 2.78 min (98.5%); m/z = 361.2 [M+H]. 1 H NMR (400 MHz, DMSO) δ H /ppm 7.97 (d, J = 7.9 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.63 (t, J = 7.8 Hz, 1H), 7.38 (t, J = 7.8 Hz, 1H), 7.24 (t, J = 8.0 Hz, 1H), 6.86 (d, J = 8.2, 1H), 6.71 (s, 1H), 6.54 (d, J = 8.0 Hz, 1H), 5.93 (s, 2H), 3.70 (s, 3H), 2.97 (t, J = 7.7 Hz, 2H), 1.72 (quint, J = 7.6 Hz, 2H), 1.38 (sextet, J = 7.4 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H).

3-((4-Amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenol (8d): A crude suspension of compound **13d** (106 mg, 1.0 Eq, 293 μmol) in DCM (1.00 mL) was cooled to 0°C under nitrogen and treated dropwise with BBr_3 (220 mg, 83 μL, 3.0 Eq, 878 μmol) in DCM (0.70 mL). The mixture was stirred at 0°C for 5 minutes then warmed to rt and stirred for an additional 1.5 h. The reaction was quenched with ice water and aqueous bicarbonate and the product was extracted into DCM (10 mL x 2), dried over $MgSO_4$, and evaporated to dryness. The residue was purified by preparative HPLC affording the title compound as a fine white powder (35.0 mg, 35% yield) LC/MS: Retention time = 2.57 min (100%): m/z = 347.2 [M+1]. 1 H NMR (400 MHz, DMSO) δ H /ppm 7.97 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.64 (t, J = 7.8 Hz, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.14 (t, J = 7.8 Hz, 1H), 6.66 (d, J = 8.1 Hz, 1H), 6.52 (d, J = 7.9 Hz, 1H), 6.39 (s, 1H), 5.88 (s, 2H), 2.97 (t, J = 7.7 Hz, 2H), 1.72 (quint, J = 7.6 Hz, 2H), 1.39 (sextet, J = 7.4 Hz, 2H), 0.87 (t, J = 7.3, 3H).

tert-Butyl(2-((4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)amino)-2-oxoethyl)carbamate (9a): E104 (**8a**) (15.2 mg, 1 Eq, 44.0 μmol) was dissolved into DMA (2.0 mL) and treated with DIEA (5.6 mg, 7.6 μL, 0.99 Eq, 44 μmol) followed by boc-glycine

(7.7 mg, 1.0 Eq, 44 μmol). EDC (16.9 mg, 2 Eq, 88.0 μmol) was added and the reaction was stirred at room temperature for 12 hours then purified by preparative HPLC to obtain the title compound. (7.0 mg, 32% yield) LCMS rt = 2.74 min (86.2%); m/z = 503.6 [M+H] 1 H NMR (400 MHz, DMSO) δH/ppm 9.96 (s, 1H), 7.96 (d, J = 7.5Hz, 1H), 7.80 (d, J = 7.7Hz, 1H), 7.72 (t, J = 7.5Hz, 1H), 7.54 (d, J = 8.3Hz, 2H), 7.37 (t, J = 7.7Hz, 1H), 7.03 (d, J = 8.1Hz, 2H), 5.91 (s, 2H), 2.95 (t, J = 7.4Hz, 4H), 1.72 (quint, 7.3Hz, 2H), 1.48-1.22 (m, 11H), 0.87 (t, J = 7.5Hz, 3H).

tert-Butyl (3-((4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)amino)-3-oxopropyl)carbamate (9b): The title compound was prepared according to the general procedure described for compound **9a**. (4.8 mg, 22% yield) LCMS rt = 2.78 min (100%); m/z = 517.7 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 10.22 (s, 1H), 9.40 (bs, 1H), 9.08 (bs, 1H), 7.95 (d, J = 8.1Hz, 1H), 7.79 (d, J = 8.3Hz, 1H), 7.63 (t, J = 7.3Hz, 1H), 7.56 (d, J = 8.5Hz, 2H), 7.37 (t, J = 7.6Hz, 1H), 7.04 (d, J = 8.6Hz, 2H), 5.91 (s, 2H), 3.10 (t, J = 7.7Hz 11H), 2.98 (t, J = 7.6Hz, 2H), 2.67 (t, J = 7.0Hz, 2H), 1.73 (quint, J = 7.6Hz, 2H), 1.39 (sextet, J = 7.4Hz, 2H), 0.87 (t, J = 7.3Hz, 3H)

tert-Butyl (4-((4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)amino)-4-oxobutyl)carbamate (9c): The title compound was prepared according to the general procedure described for compound **9a**. (6.0 mg, 26% yield) LCMS rt = 2.80 min (89.8%); m/z = 531.6 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.90 (s, 1H), 8.91 (bs, 2H), 7.98 (d, J = 8.4Hz, 1H), 7.81 (d, J = 8.4Hz, 1H), 7.65 (t, J = 7.7Hz, 1H) 7.55 (d, J = 8.5Hz, 2H), 7.40 (t, J = 7.7Hz, 1H), 7.02 (d, J =8.5Hz, 2H), 5.91 (s, 2H), 3.01 (t, J = 7.7Hz, 4H), 2.27 (t, J =

7.3Hz, 2H), 1.75 (quint, J = 7.4Hz, 2H), 1.66 (quint, J = 7.2Hz, 2H), 1.46-1.33 (m, 11H), 0.89 (t, J = 7.3Hz, 3H)

tert-Butyl(5-((4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)amino)-5-oxopentyl)carbamate (9d): The title compound was prepared according to the general procedure described for compound **9a**. (3.2 mg, 14% yield) LCMS rt =2.87 min (84.3%); m/z = 545.6 [M+H] WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.90 (s, 1H), 7.95 (d, J = 8.3Hz, 1H), 7.77 (d, J = 8.3Hz, 1H), 7.59 (t, J = 7.4Hz, 1H), 7.55 (d, J = 8.3Hz, 2H), 7.34 (t, J = 7.3Hz, 1H) , 7.01 (d, J = 8.3Hz, 2H), 5.88 (s, 2H), 3.01 (s, 6H), 1.67 (quint, J = 7.3Hz, 2H), 1.46 (t, J = 7.4Hz, 2H), 1.37-1.27 (m, 15H), 0.82 (t, J = 7.3Hz, 3H)

tert-Butyl(6-((4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)amino)-6-oxohexyl)carbamate (9e): The title compound was prepared according to the general procedure described for compound 9a. (5.6 mg, 23% yield) LCMS rt =2.90 min (96.4%); m/z =559.7 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.82 (s, 1H), 8.81 (bs, 2H), 7.92 (d, J = 8.3Hz, 1H) 7.75 (d, J = 8.3Hz, 1H), 7.58 (t, J = 7.6Hz, 1H), 7.49 (d, J = 8.5Hz, 2H), 7.33 (t, 7.6Hz, 1H) 6.95 (d, J = 8.5Hz, 2H), 5.84 (s, 2H), 2.95 (t, J = 7.7Hz 6H), 1.68 (quint, J = 7.5Hz, 2H), 1.49 (quint, J = 7.5Hz, 2H), 1.38-1.28 (m, 15H), 0.83 (t, J = 7.2Hz, 3H)

2-Amino-N-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)acetamide (10a): Compound **9a** (4.7 mg, 1 Eq, 9.4 μmol) was dissolved in DCM (400 μL) and treated with TFA (59 mg, 40 μL, 56 Eq, 0.52 mmol). The reaction mixture was stirred for one hour then

concentrated to dryness to obtain the desired product. (2.4 mg, 64% yield). LCMS rt = 2.30 min (100%); m/z = 403.5 [M+H] 1 H NMR (400 MHz, DMSO) δH/ppm 10.50 (s, 1H), 7.94 (d, J = 7.5Hz, 1H), 7.76 (d, J = 7.5Hz, 1H), 7.63-7.57 (m, 1H), 7.55 (d, J = 8.6Hz, 2H), 7.37-7.26 (m, 1H), 7.07 (d, J = 8.5Hz, 2H) 5.91 (s, 2H), 2.95 (t, J = 7.2Hz, 4H), 1.72 (quint, J = 7.5Hz, 2H), 1.39 (sextet, J = 7.5Hz, 2H), 0.87 (t, J = 7.0Hz, 3H)

3-Amino-N-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)propenamide (10b): The title compound was prepared according to the general procedure described for compound **10a**. (1.9 mg, 81% yield) LCMS rt = 2.78 min (100%); m/z = 417.5 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 10.13 (s, 1H), 9.16 (bs, 2H), 7.86 (d, J = 8.2Hz, 1H), 7.69 (d, J = 8.2Hz, 1H), 7.55-7.47 (m, 3H), 7.24 (t, J = 7.4Hz, 1H), 6.97 (d, J = 8.5Hz, 2H), 5.84 (s, 2H), 4.05 (d, J = 4.8Hz, 2H), 3.13 (d, J = 4.3Hz, 2H), 2.91 (t, J = 7.7Hz, 2H), 1.67 (quint, J = 7.6Hz, 2H), 1.34 (sextet, J = 7.4Hz, 2H), 0.82 (t, J = 7.4Hz, 3H)

4-Amino-N-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)butanamide (10c): The title compound was prepared according to the general procedure described for compound **10a**. (2.8 mg, 91% yield) LCMS rt = 2.81 min (100%); m/z =431.3 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 10.01 (s, 1H), 9.03 (bs, 2H), 7.91 (d, J = 8.3Hz, 1H), 7.81 (d, J = 8.3Hz, 1H), 7.65 (t, J = 7.8Hz, 1H), 7.56 (d, J = 8.5Hz, 2H), 7.39 (t, J = 7.8Hz, 1H), 7.04 (d, J = 8.5Hz, 2H), 5.92 (s, 2H), 3.01 (t, J = 7.7Hz, 4H), 2.40 (t, J = 7.2Hz, 2H), 1.83 (quint, J = 7.6Hz, 2H), 1.75 (quint, J = 7.6Hz, 2H), 1.41 (sextet, J = 7.4Hz, 2H), 0.89 (t, J = 7.4Hz, 3H).

5-Amino-N-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)pentanamide (10d): The title compound was prepared according to the general procedure described for compound **10a**. (1.7 mg, 100% yield) LCMS rt = 3.42 min (100%); m/z =445.5 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.96 (s, 1H), 8.94 (bs, 2H), 7.97 (d, J = 8.4Hz, 1H) 7.81 (d, J = 8.4Hz, 1H), 7.65 (t, J = 7.5Hz, 1H), 7.56 (d, J = 8.4Hz, 2H), 7.39 (t, J = 7.5Hz, 1H), 7.03 (d, J = 8.4Hz, 2H), 5.91 (s, 2H), 3.01 (t, J = 7.7Hz, 6H), 1.75 (quint, J = 7.4Hz, 2H), 1.65-1.52 (m, 2H), 1.41 (sextet, J = 7.4Hz, 2H), 1.27 (quint, J = 6.0Hz, 2H), 0.89 (t, J = 7.2Hz, 3H).

6-Amino-N-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)hexanamide (10e): The title compound was prepared according to the general procedure described for compound **10a**. (2.5 mg, 100% yield) LCMS rt = 2.33 min (100%); m/z =459.5 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.92 (s, 1H), 9.01 (bs, 2H), 7.98 (d, J = 8.2Hz, 1H), 7.81 (d, J = 8.3Hz, 1H), 7.65 (t, J = 7.6Hz, 1H), 7.56 (d, J = 8.3Hz, 2H), 7.39 (t, J = 7.7Hz, 1H), 7.03 (d, J = 8.3Hz, 2H), 5.91 (s, 2H), 3.01 (t, J = 7.7Hz, 4H), 2.29 (t, J = 7.3Hz, 2H), 1.75 (quint, J = 7.6Hz, 2H), 1.64-1.48 (m, 4H), 1.41 (q, J = 7.4Hz, 2H) 1.33 (q, J = 8.1Hz, 2H), 0.90 (t, J = 7.3Hz, 3H)

N-(4-((4-Amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)acetamide (11a): E104 (**8a**) (10.5 mg, 1 Eq, 30.4 μmol) was dissolved in DCM (1.4 mL) and treated with acetic anhydride (9.31 mg, 10.2 μL, 3 Eq, 91.2 μmol). After stirring at room temperature for 3 hours, the product was purified by preparative HPLC to obtain the desired product (2.8 mg, 24% yield). LCMS rt =2.71 min (90.2%); m/z = 388.4 [M+H]. WETDC_1 NMR (400 MHz, DMSO)

δH/ppm 9.98 (s, 1H), 8.90 (bs, 2H), 7.98 (d, J = 8.3Hz, 1H), 7.80 (d, J = 8.3Hz, 1H), 7.65 (t, J = 7.6Hz, 1H), 7.53 (d, J = 8.6Hz, 2H), 7.40 (t, J = 7.6Hz, 1H), 7.02 (d, J = 8.4Hz, 2H), 5.90 (s, 2H), 2.98 (t, J = 7.8Hz, 2H), 2.01 (s, 3H), 1.74 (quint, J = 7.5Hz, 2H), 1.40 (sextet, J = 7.4Hz, 2H), 0.88 (t, J = 7.3Hz, 3H).

N-(4-((4-Amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)pentanamide (11b): E104 (**8a**) (15.0 mg, 1 Eq, 43.4 µmol) was dissolved in ethyl acetate (400 µL) and treated with triethylamine (5.7 mg, 7.9 µL, 1.3 Eq, 57 µmol) and cooled to 0°C. Valeryl chloride (5.8 mg, 5.7 µL, 1.1 Eq, 48 µmol) was dissolved in EtOAc (133 µL) and transferred to the reaction dropwise. The reaction was warmed to rt and stirred overnight. An additional 1.3 equivalents of triethylamine and 1.1 equivalents of valeryl chloride were added to the reaction in the same manner as above. After stirring for 2 h, the reaction was purified by prep HPLC to obtain the title product (5.1 mg, 28% yield). LCMS rt = 2.82 min (100%); m/z = 430.6 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.91 (s, 1H), 8.89 (bs, 2H), 7.98 (d, J = 8.2Hz, 1H), 7.80 (d, J = 8.2Hz, 1H), 7.64 (t, J = 7.7Hz, 1H), 7.55 (d, J = 8.6 Hz, 2H), 7.40 (t, J = 7.7Hz, 1H), 7.02 (d, J = 8.6Hz, 2H), 5.90 (s, 2H), 2.99 (t, J = 7.6Hz, 2H), 2.27 (t, J = 7.3 Hz, 2H), 1.74 (quint, J = 7.5Hz, 2H), 1.55 (quint, J = 7.4Hz, 2H), 1.40 (sextet, J = 7.4Hz, 2H), 1.30 (sextet, J = 7.4Hz, 2H), 0.89 (t, J = 7.3Hz, 3H) 0.88 (t, J = 7.3Hz, 3H).

Ethyl (4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)carbamate (12a): E104 (**8a**) (14.1 mg, 1 Eq, 40.8 µmol) was dissolved in DCM (2.0 mL) and treated with triethylamine (8.26 mg, 11.4 µL, 2 Eq, 82 µmol) followed by ethyl chloroformate (8.9 mg, 7.9 µL, 2.0 Eq, 82.0 µmol). After stirring for 12 hours at rt, an additional 2 equivalents of ethyl

chloroformate were added and the reaction was stirred for an additional 1 h. The crude mixture was then purified by preparative HPLC to obtain the title compound. (7.9 mg, 46% yield) LCMS rt = 2.77 min (95.6%); m/z = 418.5 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.63 (s, 1H), 8.94 (bs, 2H), 7.99 (d, J = 8.6Hz, 1H), 7.81 (d, J = 8.6Hz, 1H), 7.65 (t, J = 7.8Hz, 1H), 7.46-7.37 (m, 3H), 7.02 (d, J = 8.5Hz, 2H), 6.53 (bs, 1H), 5.90 (s, 2H), 4.11 (q, J = 7.0Hz, 2H), 2.98 (t, J = 7.6Hz, 2H), 1.75 (quint, J = 7.3Hz 2H), 1.42 (sextet, J = 7.4 Hz, 2H), 1.23 (t, J = 7.1Hz, 3H) 0.89 (t, J = 7.4Hz, 3H).

Isopropyl (4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)carbamate (12b): The title compound was prepared according to the procedure described for compound **12a**. (4.9 mg, 41% yield) LCMS rt = 2.91 min (100%); m/z = 431.5 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.56 (s, 1H), 8.96 (bs, 2H), 7.99 (d, J = 8.3Hz, 1H), 7.82 (d, J = 8.3Hz, 1H), 7.65 (t, J = 7.7Hz, 1H), 7.46-7.37 (m, 3H), 7.00 (d, J = 8.5Hz, 2H), 6.49 (bs, 1H), 5.90 (s, 2H), 4.86 (septet, J = 6.2Hz, 1H), 2.99 (t, J = 7.8Hz, 2H) 1.75 (quint, J = 7.5 Hz, 2H), 1.41 (sextet, J = 7.4Hz, 2H), 1.24 (d, J = 6.3Hz, 6H), 0.90 (t, J = 7.3 Hz, 3H).

Butyl 2-(4-((4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)methyl)phenyl)acetate (12c): The title compound was prepared according to the procedure described for compound **12a**. (3.8 mg, 29% yield) LCMS rt = 3.00 min (100%); m/z = 446.6 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 9.62 (s, 1H), 8.78 (bs, 2H), 7.97 (d, J = 8.3, 1H) 7.80 (d, J = 8.3Hz, 1H), 7.63 (t, J = 7.7Hz, 1H), 7.43 (d, J = 8.5Hz, 2H), 7.38 (t, J = 7.7Hz , 1H), 7.01 (d, J = 8.5Hz, 2H), 6.54 (bs, 1H), 5.89 (s, 2H), 4.06 (t, J = 6.5Hz, 2H), 2.99 (t, J = 7.6Hz, 2H), 1.75 (quint, J = 7.5Hz, 2H), 1.59 (quint, J = 6.7Hz, 2H), 1.46-1.32 (m, 4H), 0.91 (t, J = 7.6Hz, 3H), 0.89 (t, J = 7.3Hz,

3H).

2-butyl-1-(4-(propylamino)benzyl)-1H-imidazo[4,5-c]quinolin-4-amine (13a):

E104 (**8a**) (15.1 mg, 1 Eq, 43.7 μmol) and propionaldehyde (2.6 mg, 3.3 μL, 1.0 Eq, 44 μmol) were dissolved in anhydrous MeOH (500 μL) and then acetic acid (1.4 mg, 1.3 μL, 0.52 Eq, 23 μmol) was added. The reaction was left for 20 minutes and then the sodium cyanotrihydroborate (8.3 mg, 3.0 Eq, 0.13 mmol) was added and stirred overnight. An extraction in DCM and sodium bicarbonate was performed to quench the sodium cyanotrihydroborate. The organic layer was dried with magnesium sulfate, filtered, and then concentrated to dryness. The reaction was then purified by prep HPLC with the formic acid method to prevent the product reacting with the TFA to yield the title compound. (8.5 mg, 50% yield). LCMS rt = 2.99 (100%); m/z = 388.3 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 8.19 (s, 2H), 7.91 (d, J = 7.5Hz, 1H), 7.63 (d, J = 7.1Hz, 1H), 7.41 (t, J = 7.4Hz, 1H), 7.15 (t, J = 7.5Hz, 1H), 6.77 (d, J = 8.4Hz, 2H), 6.49 (d, J = 8.0Hz, 2H), 5.68 (s, 2H), 2.95-2.86 (m, 4H), 1.73 (quint, J = 7.7Hz, 2H), 1.50 (sextet, J = 7.5Hz, 2H), 1.39 (sextet, J = 8.0Hz, 2H), 0.89 (t, J = 7.5Hz, 6H).

2-butyl-1-(4-(pentylamino)benzyl)-1H-imidazo[4,5-c]quinolin-4-amine (13b):

The title compound was prepared according to the general procedure described for compound **13a**. (8.0 mg, 44% yield). LCMS rt = 3.19 (100%); m/z = 416.4 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 8.20 (s, 2H), 7.90 (d, J = 7.5Hz, 1H), 7.62 (d, J = 7.9Hz, 1H), 7.40 (t, J = 7.5Hz, 1H), 7.13 (t, J = 7.5Hz, 1H), 6.77 (d, J = 8.4Hz, 2H), 6.49 (d, J = 8.6Hz, 2H), 5.67 (s, 2H), 2.91 (t, J = 7.3Hz, 4H), 1.72 (quint, J = 7.4Hz, 2H), 1.49 (quint, J = 7.0Hz, 2H), 1.39 (sextet, J = 7.6Hz, 2H), 1.33-1.25 (m, 4H), 0.89 (t, J = 7.4Hz, 3H), 0.86 (t, J = 7.3Hz, 3H).

2-butyl-1-(4-(hexylamino)benzyl)-1H-imidazo[4,5-c]quinolin-4-amine (13c):

The title compound was prepared according to the general procedure described for compound **13a**, except using 3 equivalents of aldehyde, these methods were optimized with compounds 13a and **13b**. (8.4 mg, 23% yield). LCMS rt = 2.91 (100%); m/z = 430.4 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 8.21 (s, 1H), 7.89 (d, J = 7.4Hz, 1H), 7.61 (d, J = 7.4Hz, 1H), 7.39 (t, J = 7.4Hz, 1H), 7.11 (t, J = 7.4Hz, 1H), 6.77 (d, J = 8.2Hz, 2H), 6.49 (d, J = 8.0Hz, 2H), 5.67 (s, 2H), 1.72 (quint, J = 7.2Hz, 2H), 1.48 (quint, J = 7.0Hz, 2H), 1.39 (sextet, J = 7.2Hz, 2H), 1.34-1.20 (m, 6H), 0.89 (t, J = 7.4Hz, 3H) 0.85 (t, J = 7.2Hz, 3H).

2-butyl-1-(4-(heptylamino)benzyl)-1H-imidazo[4,5-c]quinolin-4-amine (13d):

The title compound was prepared according to the general procedure described for compound **13a** except using 3 equivalents of aldehyde, these methods were optimized with compounds 13a and **13b**. (2.3 mg, 12% yield). LCMS rt = 3.15 (90%); m/z = 444.4 [M+H]. WETDC_1 NMR (400 MHz, DMSO) δH/ppm 7.91 (d, J = 8.3Hz, 1H), 7.62 (d, J = 8.2Hz, 1H), 7.41 (t, J = 7.5Hz, 1H), 7.14 (t, J = 7.4Hz, 1H), 6.77 (d, J = 8.3Hz, 2H), 6.48 (d, J = 8.4Hz, 2H), 5.68 (s, 2H), 2.92 (t, J = 7.4Hz, 4H), 1.73 (quint, J = 7.5Hz, 2H), 1.48 (quint, J = 6.9Hz, 2H), 1.39 (sextet, J = 7.4Hz, 2H), 1.34-1.18 (m, 8H), 0.89 (t, J = 7.5Hz, 3H) 0.85 (t, J = 7.2Hz, 3H).

10.2 Analytical UPLC and HPLC Methods

10.2.1 HPLC-MS Method

Samples were analyzed during reactions and for purity using an analytical HPLC-MS method on a Waters Autopurification system containing a 2545 binary gradient module, 2767 sample manager, 2998 UV/PDA detector, and SQD2 mass spectrometer. 3 µl injections were

separated using an XBridge BEH C18 5 µm (4.6 x 100 mm) column at 80°C. Eluent was monitored by UV (210-600 nm), and mass spectrometry (150-1800 Da, ES+/ES-). Solvents for the mobile phase were water with 0.2% formic acid (solvent A) and acetonitrile (ACN) with 0.2% formic acid (solvent B). Flow rate was 2.00 ml/min. 25 Gradient Used: Isocratic solvent B for 1.0 min (0-1.0 min), then gradient from 5% to 99% solvent B over 2.8 min (1.0-3.8 min), then isocratic solvent B for 0.1 min (3.8-3.9 min), then gradient from 99% to 5% solvent B for 0.1 min (3.9-4.0 min), then isocratic solvent B for 1.0 min (4.0-5.0 min)

10.2.2 UPLC-MS Method

Samples were also analyzed during reactions and for purity using an analytical UPLC-MS method on a Water Acquity H-Class UPLC ® with TUV detector and QDa mass spectrometer. 3 µl injections were separated using an Acquity UPLC BEH C18 1.7 µm column (2.1 x 50 mm) at 80°C. Eluent was monitored by UV (220 and 254 nm) and mass spectrometry (150-1250 Da, ES+/ES-). Solvents for the mobile phase were water with 0.1% formic acid (solvent A) and acetonitrile (ACN) with 0.1% formic acid (solvent B). Flow rate was 0.8 ml/min. Gradient Used: Isocratic solvent B for 0.8 min (0-0.8 min), then gradient from 10%-90% Solvent B over 3.6 min (0.8-4.4 min), isocratic solvent B for 0.1 min (4.4-4.5 min), then gradient from 90% solvent B over 0.05 min (4.50-4.55 min), then isocratic solvent B for 0.45 min (4.55-5.0 min).

10.3 Preparative HPLC Methods

General purification of organic compounds was performed using a Waters Auto-purification system containing a 2545 binary gradient module, 2767 sample manager, 2998

UV/PDA detector, and SQD2 mass spectrometer. 50-500 µl injections were separated using an XBridge BEH C18 5 µm OBD (19 x 100 mm) prep column at room temperature. Eluent was monitored by UV (210-600 nm), and mass spectrometry (150-2500 Da, ES+/ES-). Solvent for the mobile phase was water with 0.05% trifluoroacetic acid (solvent A) and acetonitrile (ACN) with 0.05% trifluoroacetic acid (solvent B). Flow rate was 20.0 ml/min. Gradient Used: Isocratic solvent B for 1.0 min (0-1.0 min), then gradient from 15% to 95% solvent B over 7.0 min (1.0-8.0 min), then isocratic solvent B for 0.5 min (8.0-8.5 min), then gradient from 95% to 15% solvent B for 0.5 min (8.5-9.0 min), then isocratic solvent B for 1.0 min (9.0- 10.0 min)

10.4 NMR Methods

All derivatives were analyzed using a Bruker Avance III HD 400 MHz Proton NMR instrument, with a 9.1 Tesla shielded, superconducting magnet, 2 RF Channels, z-axis Pulsed Field Gradient, 24-sample SampleCase Automation and a 5mm Broadband/Fluorine Observe Probe. 4 derivatives were tested using 1H proton NMR. Small amounts of solid product of these derivatives were dissolved in 0.75 mL of D6-DMSO. The remaining derivatives were tested by using prepared 5 mM or 10 mM stock solutions in DMSO for WETDC_1 solvent suppression NMR, Derivatives initially in DMA interfered with the upfield region of the spectra and needed DMSO to be the solvent.

10.5 Ramos Blue Cell Activation Methods

Quantification of NFkB activation and subsequent production of secreted embryonic alkaline phosphatase production was measured in Ramos-Blue Cells (NF-κB/AP-1 Reporter B

lymphocytes) with experimental conditions consisting of a seeding density of 1e6 Cells/mL, serial dilutions with a factor of 3 and concentrations of 50 uM to 7.6 nM (in cells).

Cells were suspended in IMDM + 10% FBS media at a concentration of 1.11e6 cells/mL (1.00e6 cells/mL after derivatives added) and 135 uL of this solution was added to each well. After serial dilution was set up, 15 uL (1:10 dilution into cells) of various concentrations of small molecule was added to the cell solution in each well; this was done in triplicate. The original serial dilutions were done in PBS+10% v/v DMSO to ensure the derivatives dissolve fully. Because of the dilution into cells, the final percent organic in the solution with the cells was 1% v/v. The plates were then incubated for 72 hours at 37 ^{o}C before the readings were taken.

After incubation for 72 hours, the plates were centrifuged at 2000 rpm for 10 min. A Quanti-blue solution was created based on manufacturer instructions (for example, 100 uL Quanti-blue buffer + 100 uL Quanti-blue reagent = 9.8 mL Sterile water). 40 uL of supernatant was then added to 160 uL Quant-blue solution and incubated at 37 ^{o}C for 24 hours. Readings were taken on the plate reader at 4 and 24 hours for absorbance at 620 nm.

10.6 PAMPA Methods

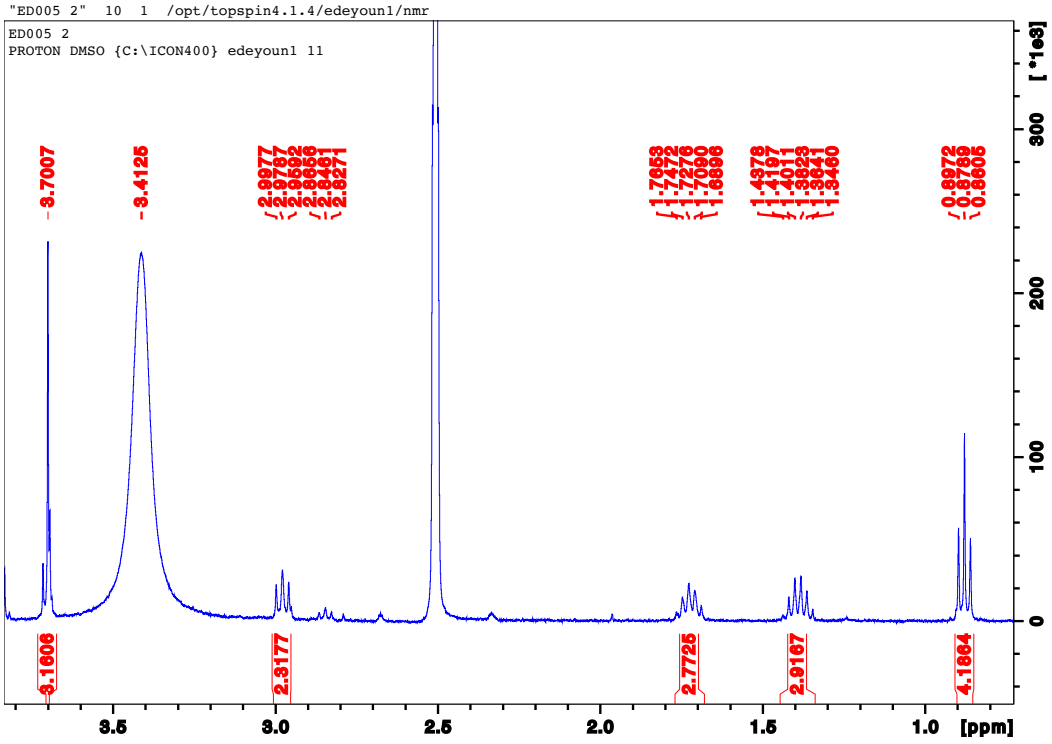
To analyze the payloads in a permeability assay, compounds were studied in a pre-analysis. 96 well plates were found insufficient for this type of experiment (in absence of cells) due to absorption of polystyrene in the plates. 384 well plates were used instead for a serial dilution of resiquimod with concentrations in the range 200 µM -> 0 µM. Preparation of this serial dilution consisted of taking an aliquot of the 10 mM in DMSO stock solution and diluting with 10% Methanol in PBS for a total of 30 µL (200 µM).

21 payloads and 4 standards (25 total) were analyzed in a permeability assay. The stock solutions (from 5 mM or 10 mM in DMSO) were diluted in PBS with 10% Methanol, yielding a total well volume of 300 µL and a total well concentration of 200 µM. A total percent organic component of 14% for 5 mM stock and 12% for 10 mM stock was added. The methanol was added to improve solubility. Each payload was analyzed in triplicate, with 3 wells of 300 µL for each payload. This 300 µL/well of DMSO stock solutions in PBS with 10% Methanol was added to the receiver plate (donor solution). 200 µL/well of 10% Methanol in PBS was added to the pre-coated filter plate (acceptor solution). The plates were then coupled and incubated at 37 ºC for 5 hours without agitation. 100 µL was also put into a 384 well plate to establish an absorbance vs. concentration relationship in the plate reader at 280 nM and 320 nM, for the known concentration of 200 µM.

The plates were then separated, and both the acceptor solution and donor solutions were transferred to 96 well plates for storage. These were covered and stored in the -80 degree freezer. For analysis 50 µL of each well was transferred to a 384 well plate. Every other well was used (A2 -> G6). Well H2 was used as a background well, to eliminate residual absorption of the plastic. This was completed for both the acceptor solution and the donor solution at wavelengths of 280 nm and 320 nm.

11. Supplemental Material

11.1 HPLC and NMR Characterization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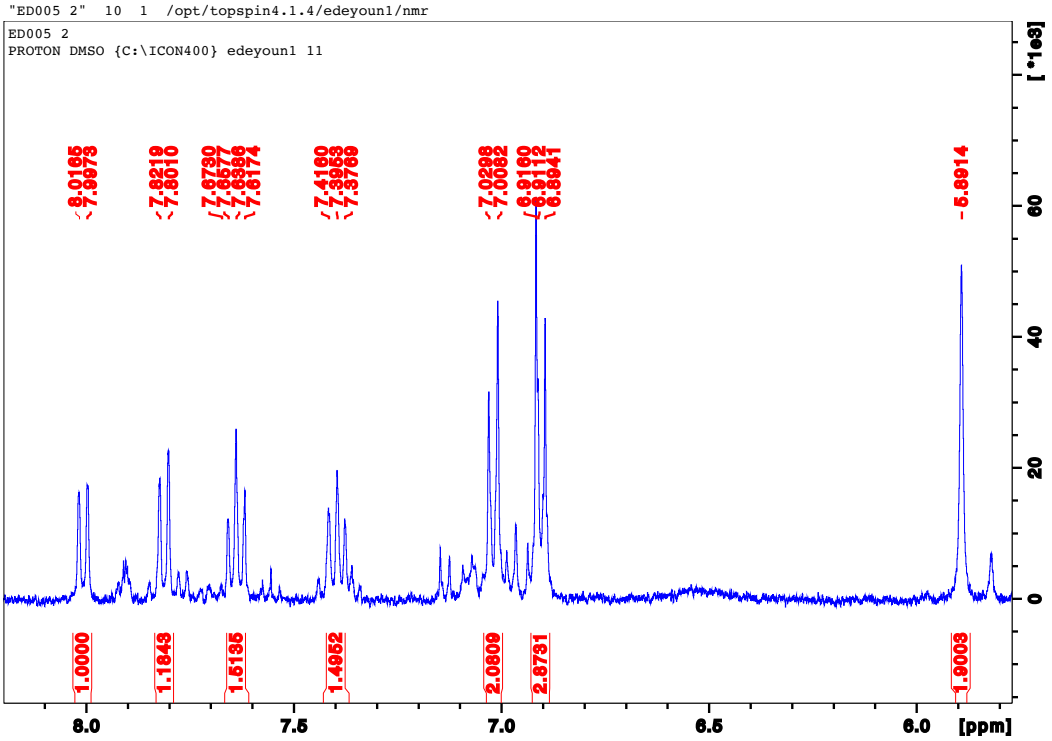
3.4125
2.9977
2.9787
2.9592
2.8656
2.8461
2.8271
1.7653
1.7472
1.7276
1.7090
1.6896
1.4378
1.4197
1.4011
1.3823
1.3641
1.3460
0.8972
0.8782
0.8605
3.1606
2.9177
2.7725
2.0167
4.1864
[*1e3]
300
200
100
0
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]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ED005 2
PROTON DMSO {C:\ICON400} edeyoun1 11
8.0165
7.9973
7.8219
7.8010
7.6730
7.6577
7.6396
7.6174
7.4160
7.3953
7.3769
7.0298
7.0082
6.9160
6.9112
6.8941
5.8914
1.0000
1.1843
1.5135
1.4952
2.0809
2.8731
1.0003
[*1e3]
60
40
20
0
8.0
7.5
7.0
6.5
6.0
[ppm]

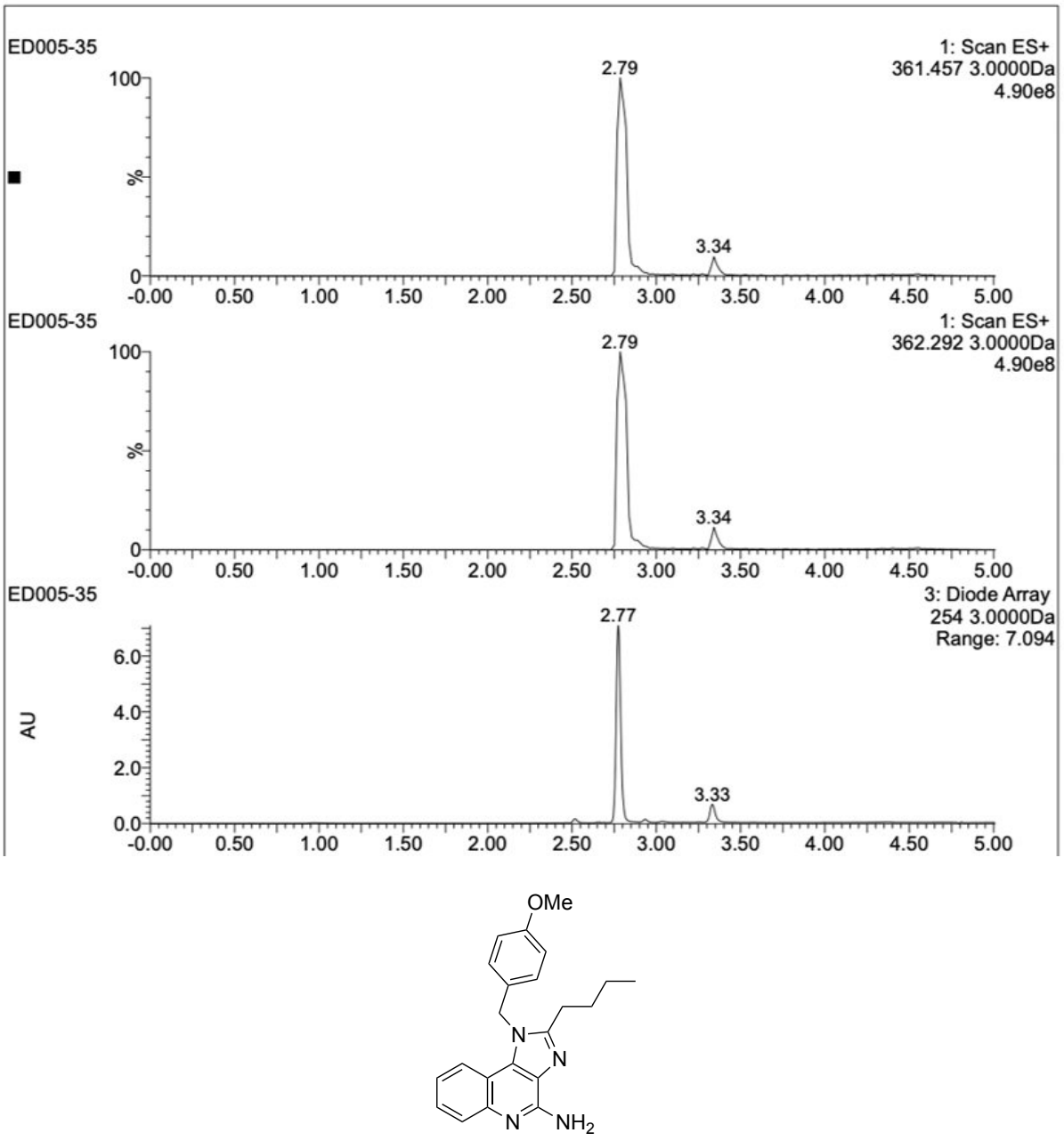
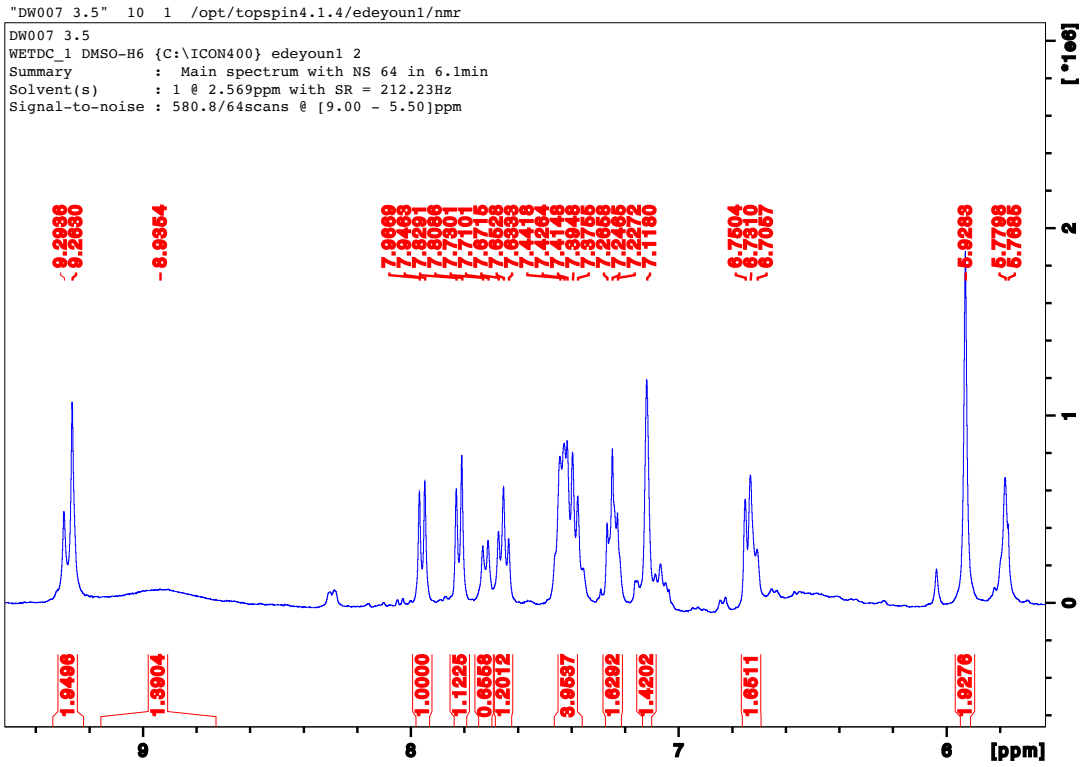
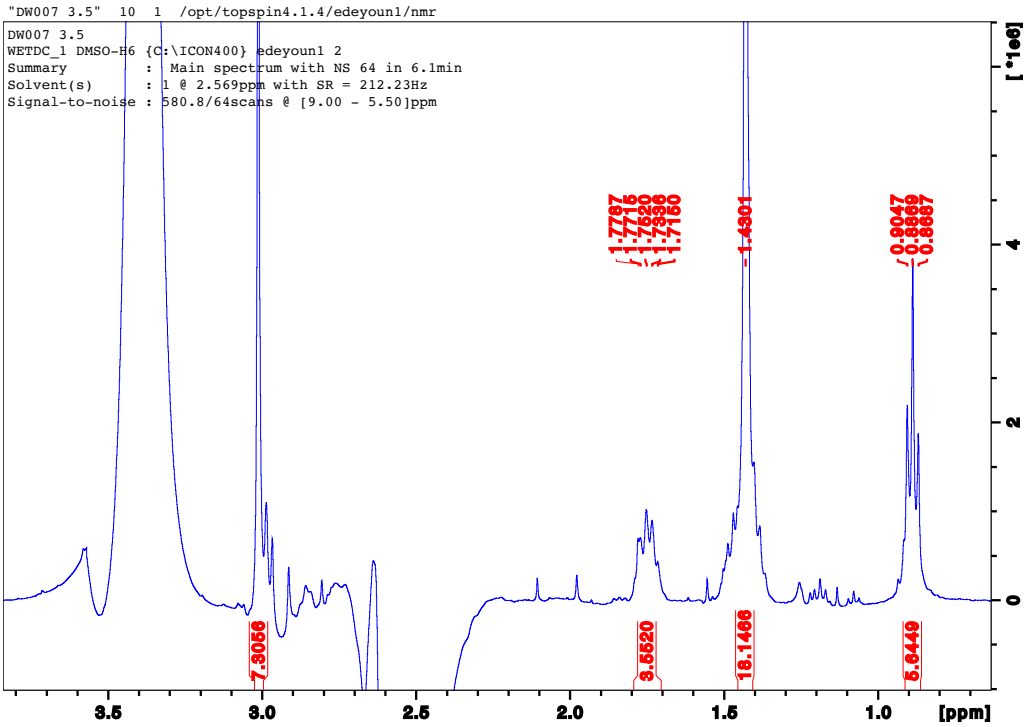


Figure S1: HPLC and NMR Characterization of Compound **7b**, MW = 361.5 [M+H]


"DW007 3.5" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
DW007 3.5
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 2
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.569ppm with SR = 212.23Hz
Signal-to-noise : 580.8/64scans @ [9.00 - 5.50]ppm
9.2938
9.2680
8.9354
7.9669
7.9483
7.8291
7.8086
7.7301
7.7101
7.6715
7.6628
7.6333
7.4416
7.4264
7.4148
7.3948
7.3755
7.2656
7.2496
7.2272
7.1180
6.7504
6.7310
6.7057
5.9283
5.7798
5.7685
1.9496
1.3904
1.0000
1.1225
0.8558
1.2012
3.9537
1.6292
1.4202
1.0511
1.9276
9
8
7
6
[ppm]
[*1e6]


"DW007 3.5" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
DW007 3.5
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 2
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.569ppm with SR = 212.23Hz
Signal-to-noise : 580.8/64scans @ [9.00 - 5.50]ppm
1.7787
1.7715
1.7620
1.7398
1.7180
1.4301
0.9047
0.8869
0.8687
7.3066
3.5620
16.1466
6.8449
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]
[*1e6]

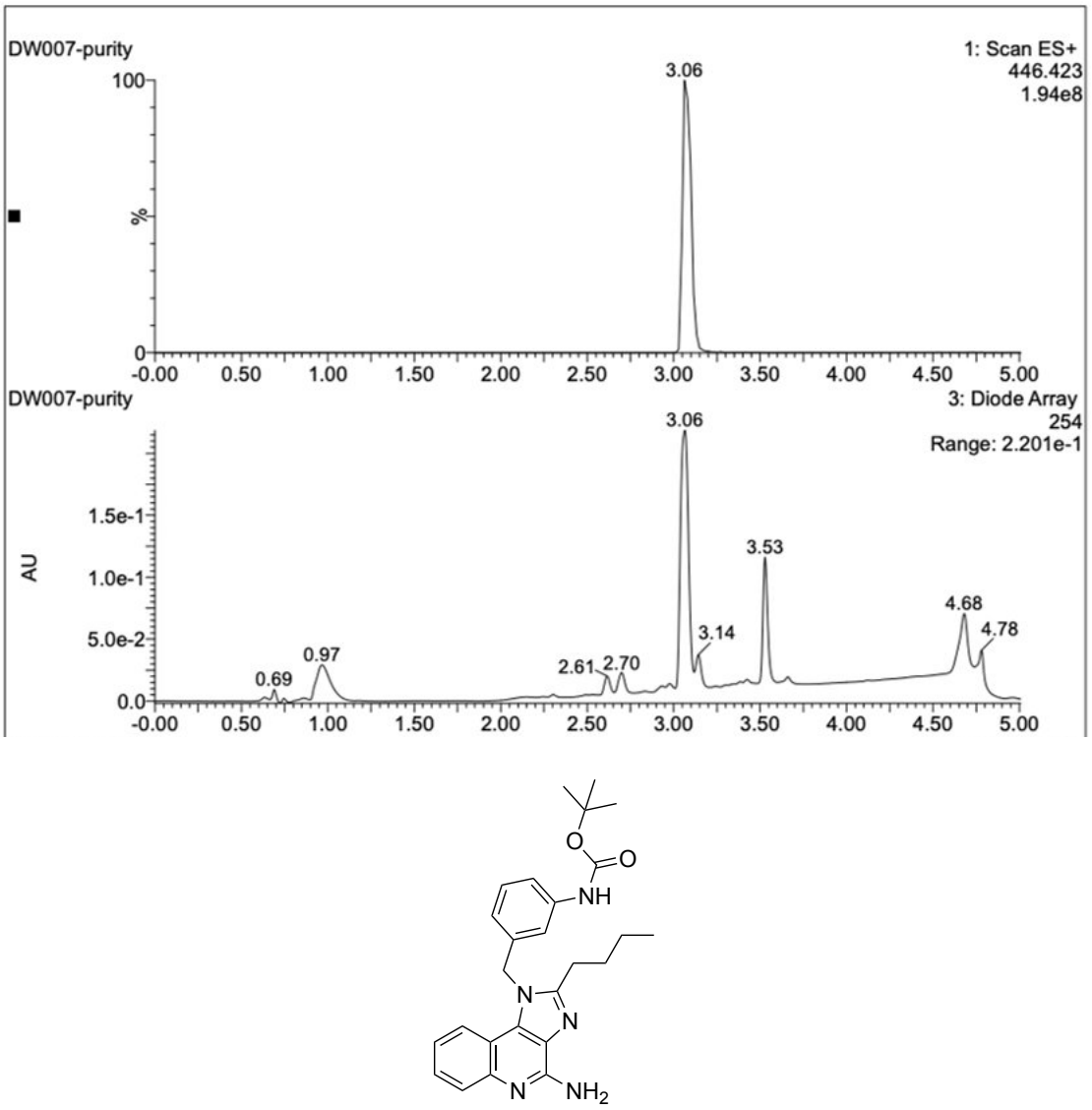


Figure S2: HPLC and NMR Characterization of Compound **7c,** MW = 446.4 [M+H]

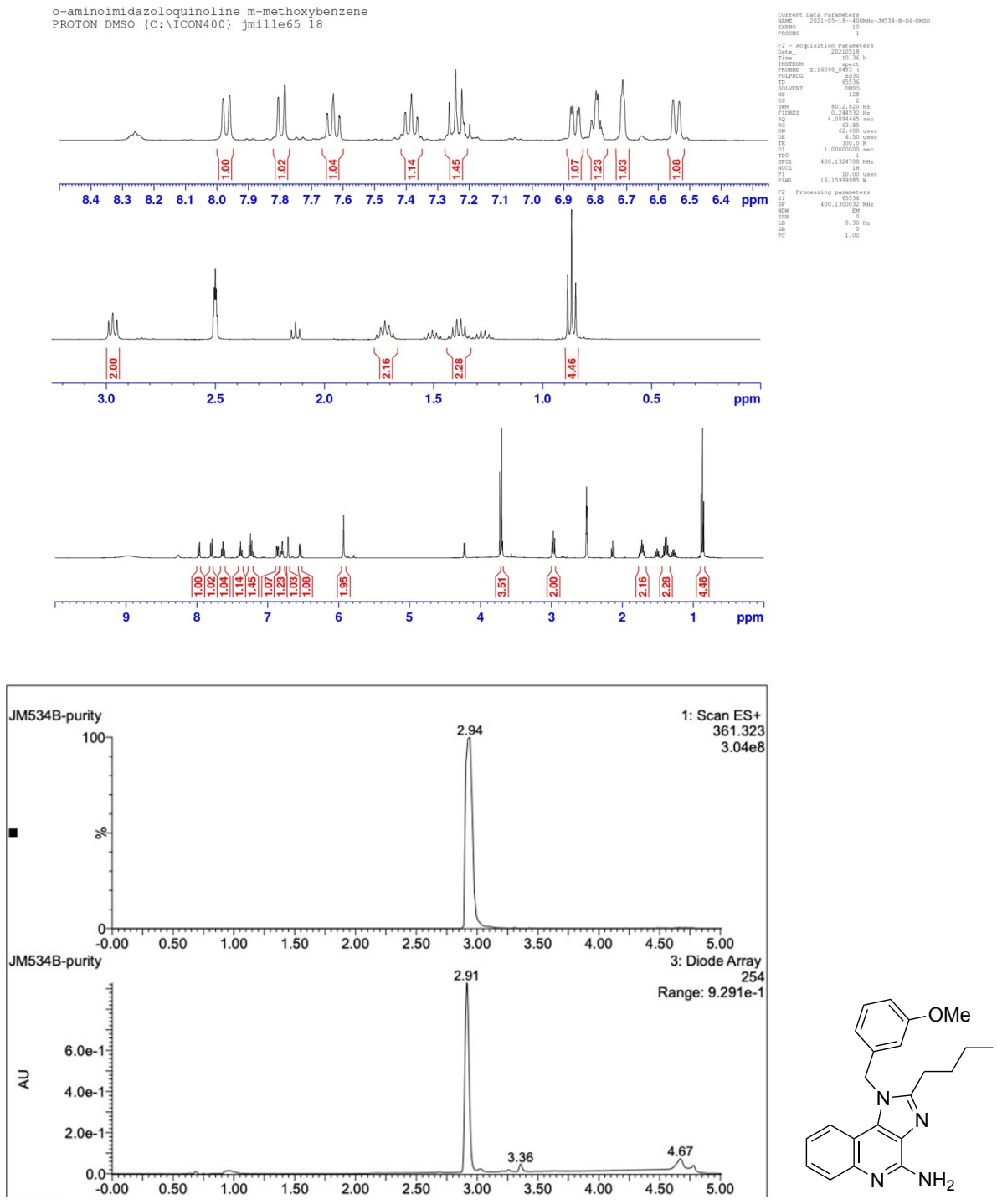
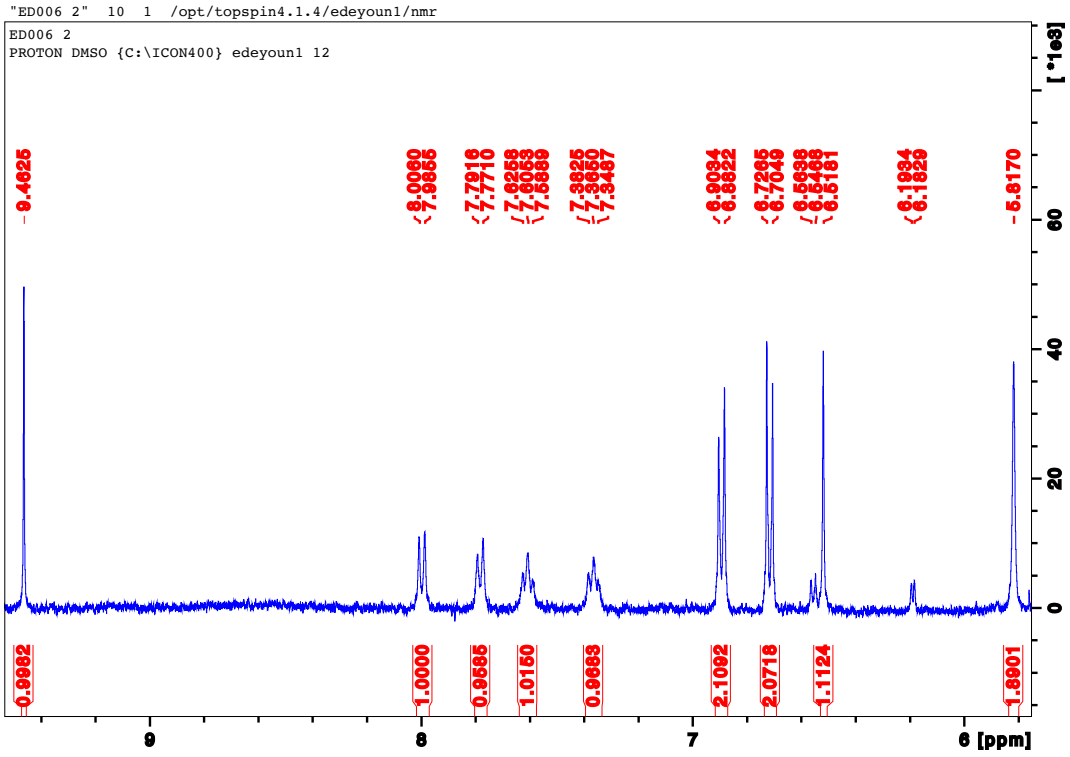


Figure S3: HPLC and NMR Characterization of Compound **7d,** MW = 361.3 [M+H]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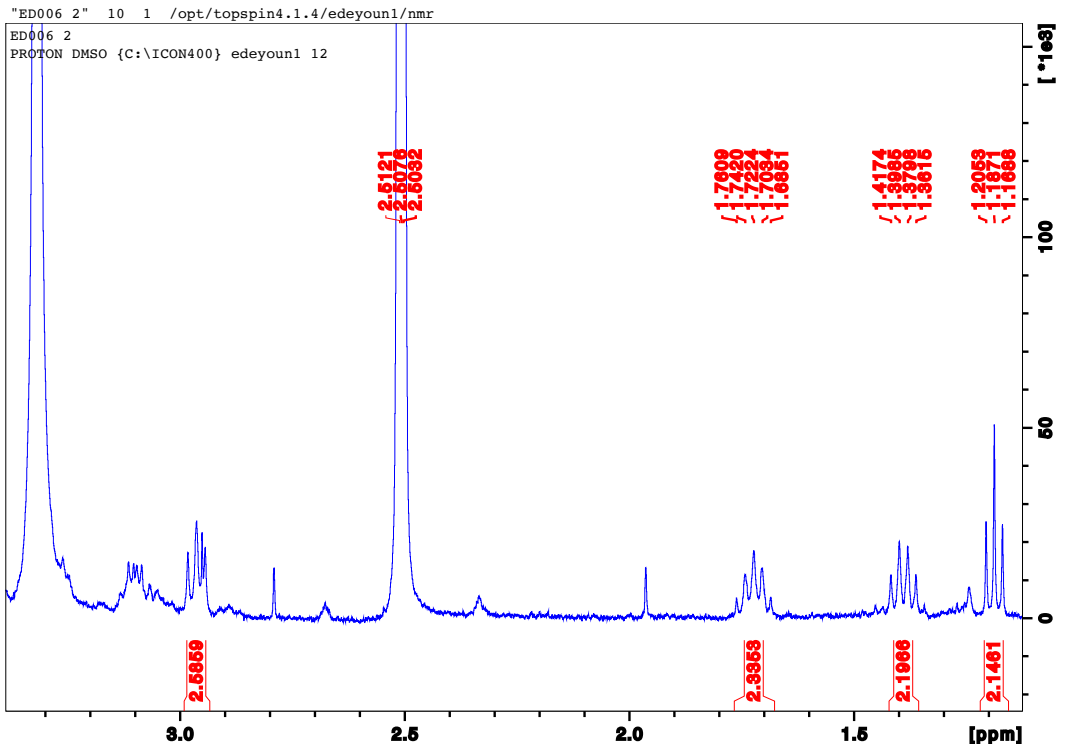
ED006 2
PROTON DMSO {C:\ICON400} edeyoun1 12
9.4025
9.0060
7.9855
7.7916
7.7710
7.6258
7.6053
7.5889
7.3825
7.3650
7.3487
6.9034
6.8822
6.7285
6.7040
6.5538
6.5488
6.5181
6.1934
6.1826
5.8170
0.9982
1.0000
0.9585
1.0150
0.9883
2.1002
2.0718
1.1124
1.8901
9
8
7
6 [ppm]
[*1e3]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ED006 2
PROTON DMSO {C:\ICON400} edeyoun1 12
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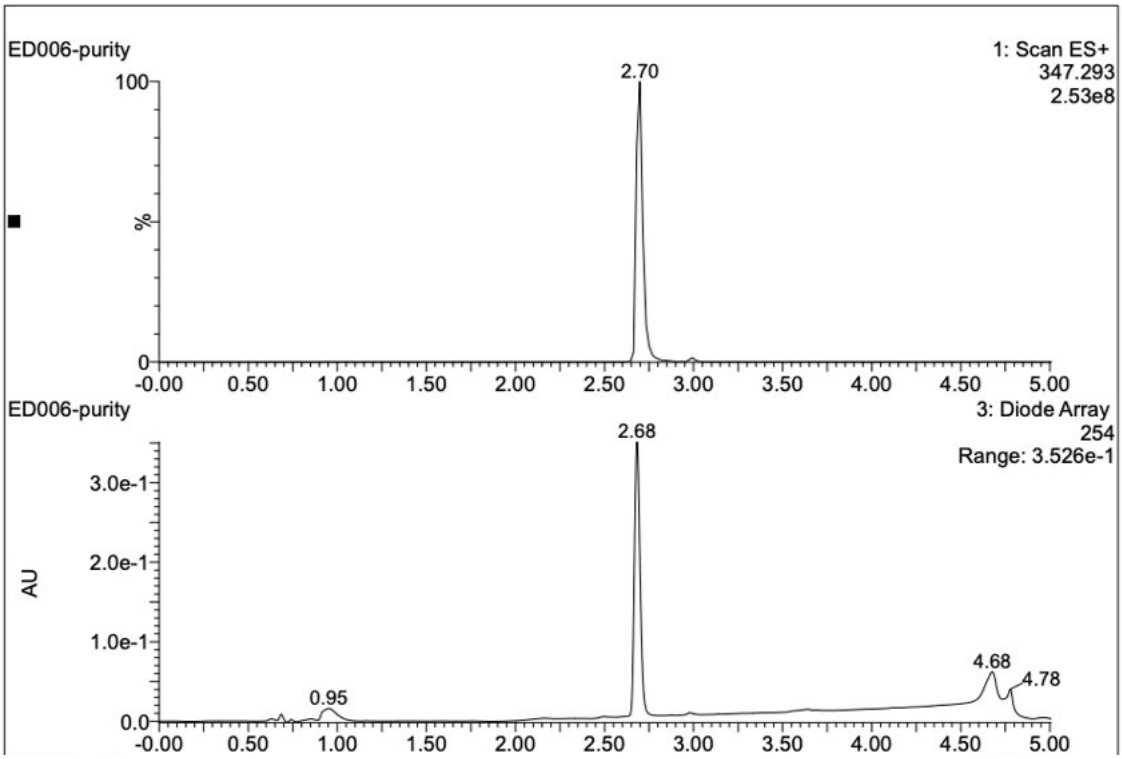
2.5076
2.5032
1.7900
1.7420
1.7224
1.7034
1.6851
1.4174
1.3995
1.3798
1.3615
1.2053
1.1871
1.1688
2.5859
2.3353
2.1906
2.1461
3.0
2.5
2.0
1.5
[ppm]
[*1e3]



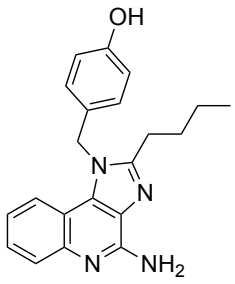
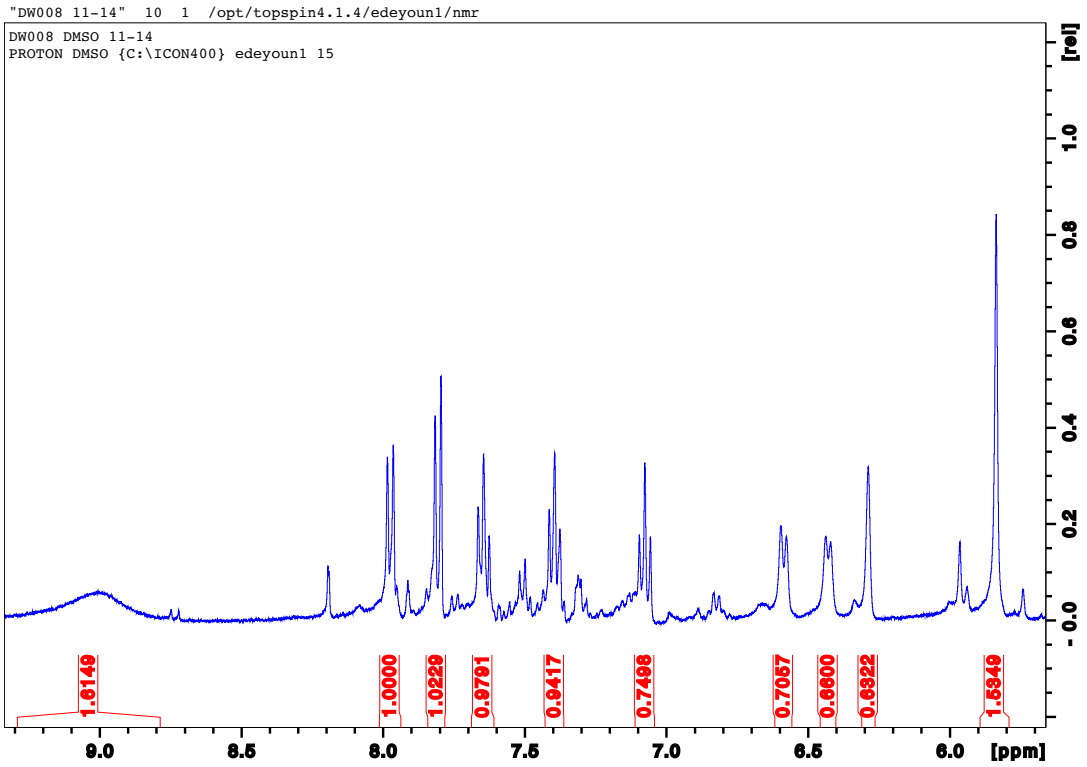


Figure S4: HPLC and NMR Characterization of Compound **8b,** MW = 347.3 [M+H]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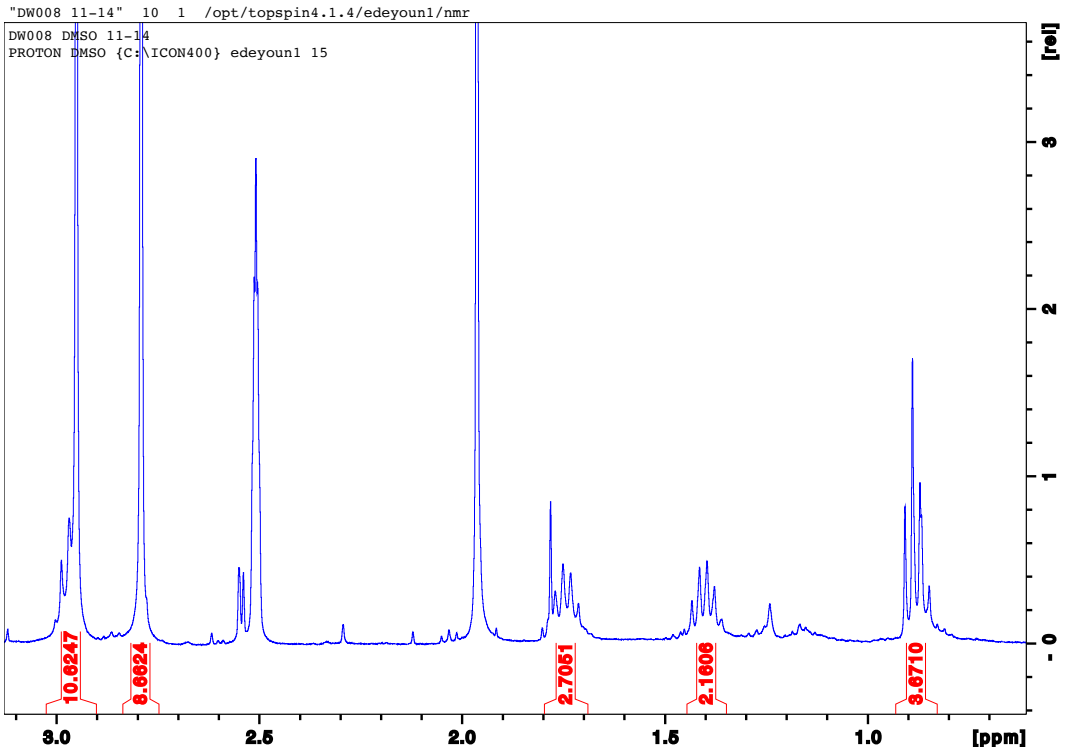
PROTON DMSO {C:\ICON400} edeyoun1 15
[rel]
1.0
0.8
0.6
0.4
0.2
- 0.0
1.6140
1.0000
1.0229
0.9791
0.9417
0.7498
0.7057
0.6600
0.6322
1.5349
9.0
8.5
8.0
7.5
7.0
6.5
6.0
[ppm]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DW008 DMSO 11-14
PROTON DMSO {C:\ICON400} edeyoun1 15
[rel]
3
2
1
- 0
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2.1606
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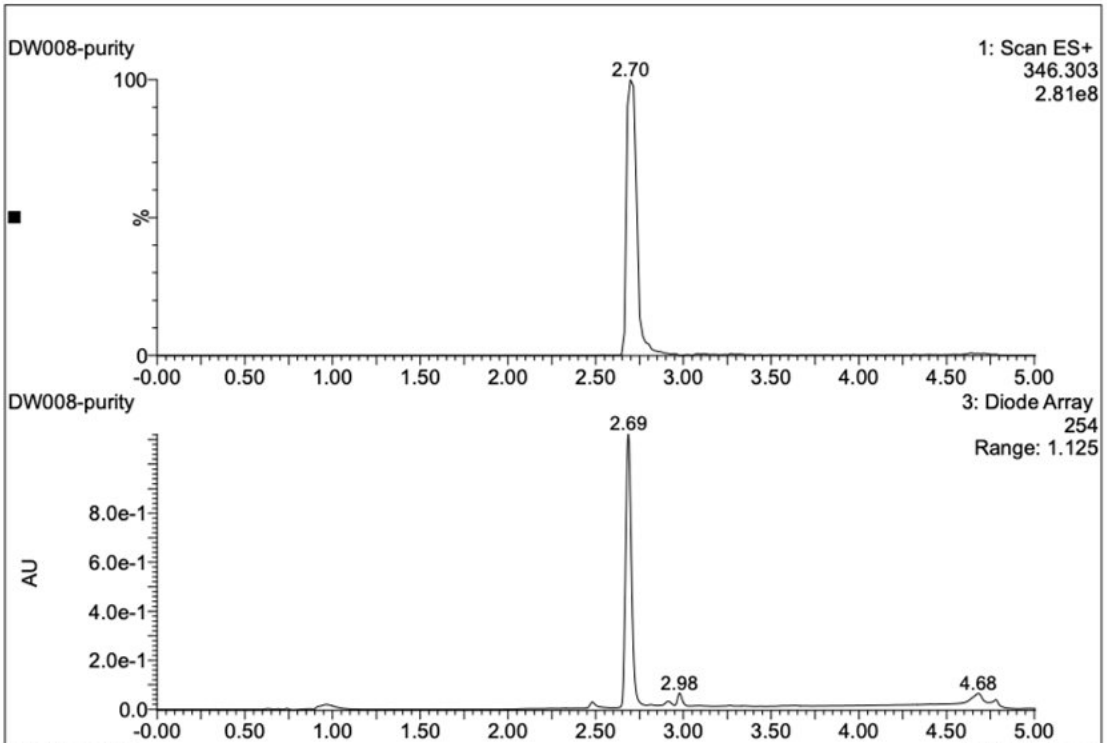
3.0
2.5
2.0
1.5
1.0
[ppm]



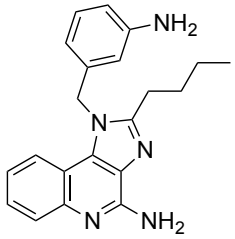
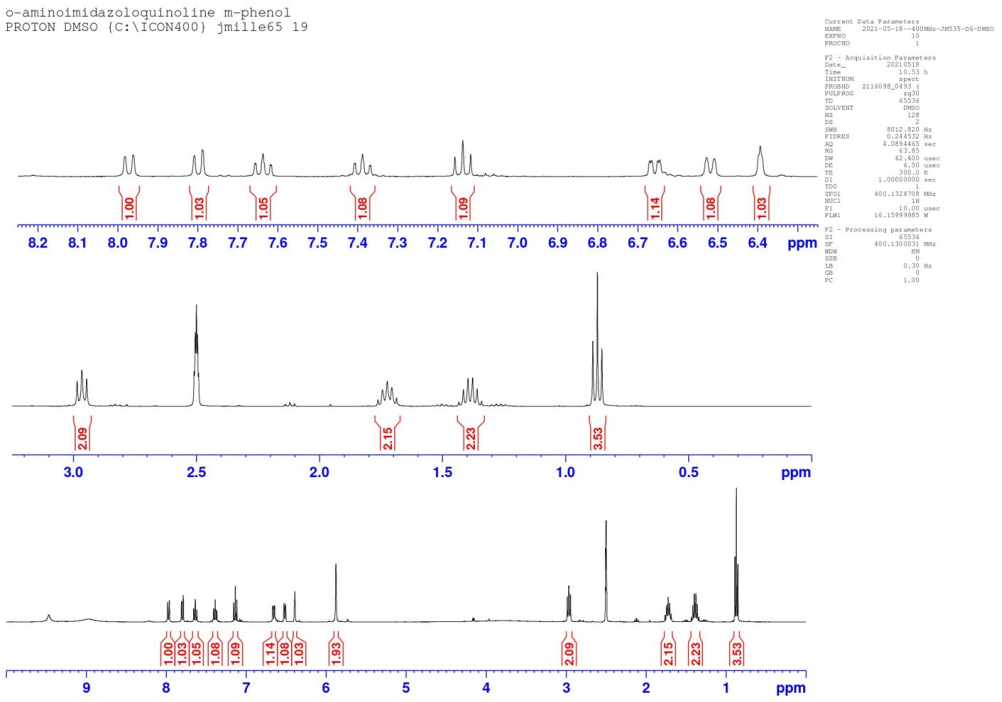
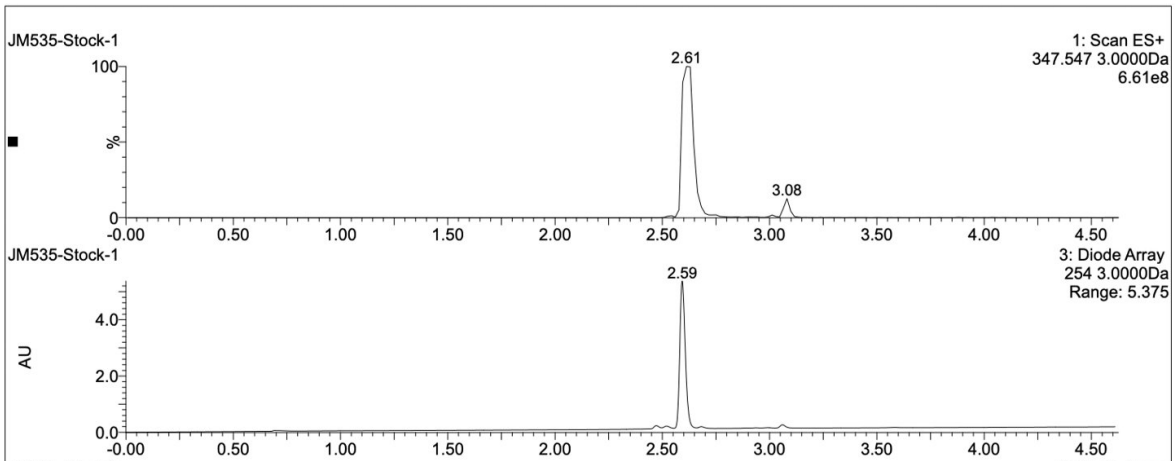


Figure S5: HPLC and NMR Characterization of Compound **8c,** MW = 346.3 [M+H]





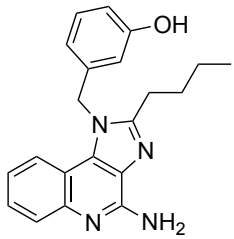
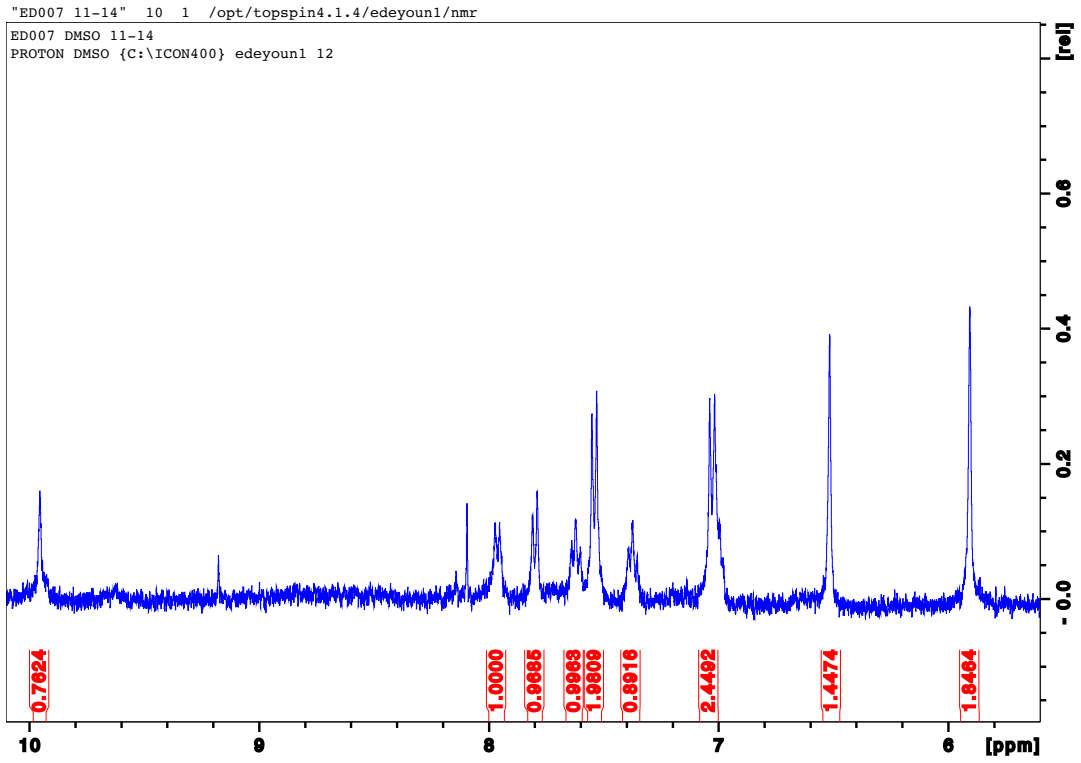


Figure S6: HPLC and NMR Characterization of Compound **8d,** MW = 347.5 [M+H]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PROTON DMSO {C:\ICON400} edeyoun1 12
[rel]
0.6
0.4
0.2
- 0.0
0.7624
1.0000
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0.9963
1.0000
0.8916
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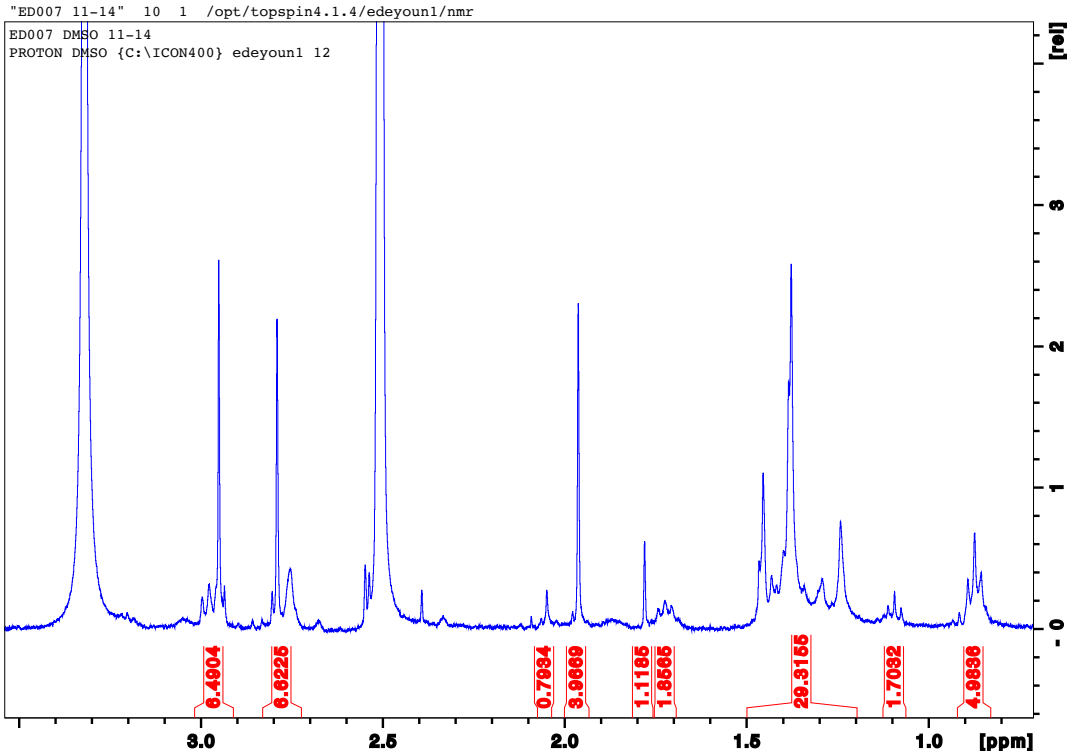
1.4474
1.8464
10
9
8
7
6
[ppm]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PROTON DMSO {C:\ICON400} edeyoun1 12
[rel]
3
2
1
- 0
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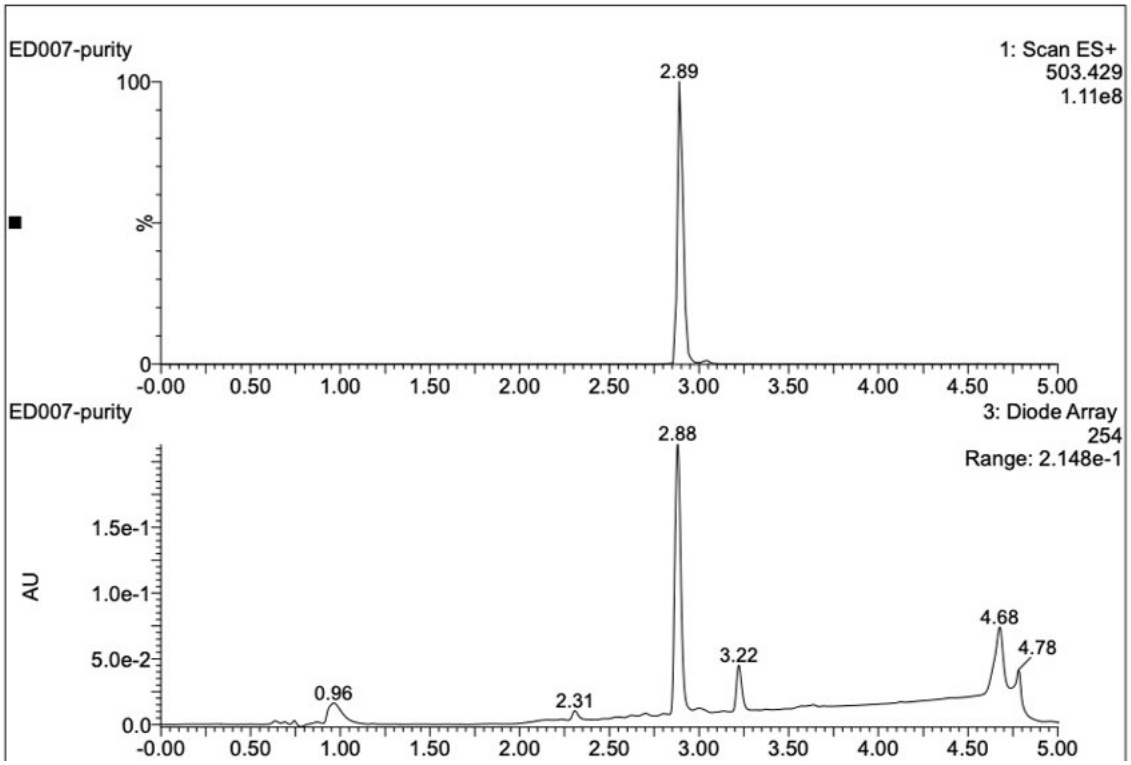
6.6225
0.7034
3.0069
1.1185
1.0565
20.3155
1.7032
4.0836
3.0
2.5
2.0
1.5
1.0
[ppm]



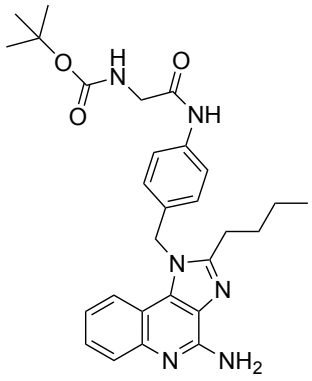
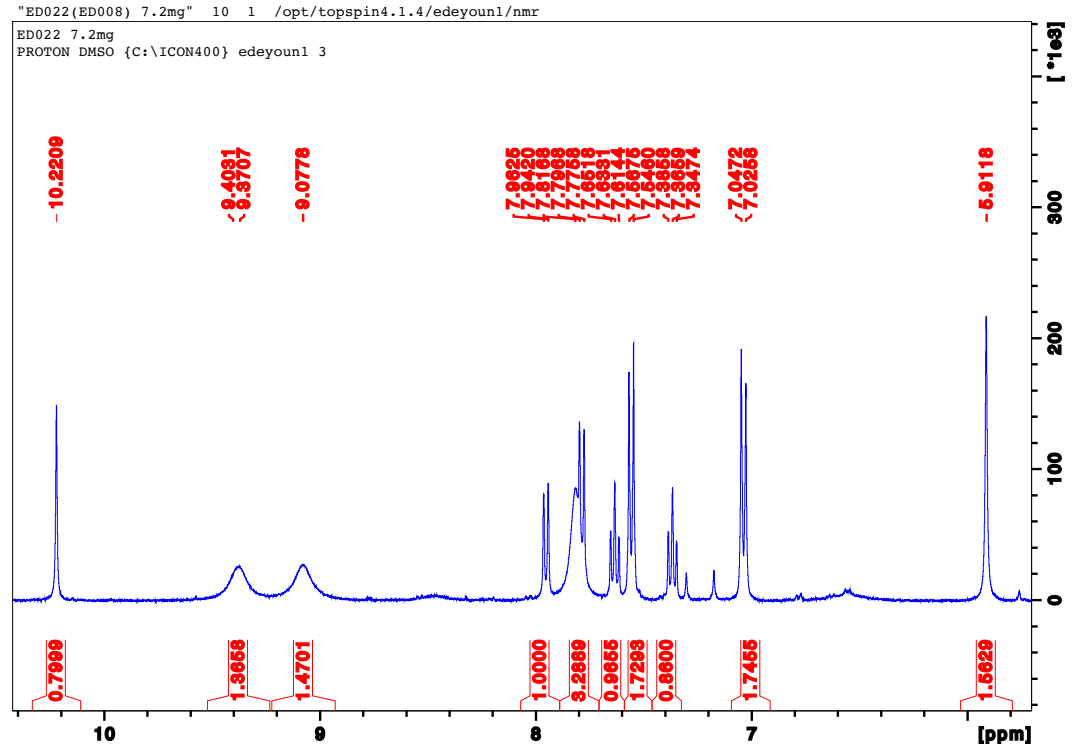


Figure S7: HPLC and NMR Characterization of Compound **9a,** MW = 503.4 [M+H]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[*1e3]
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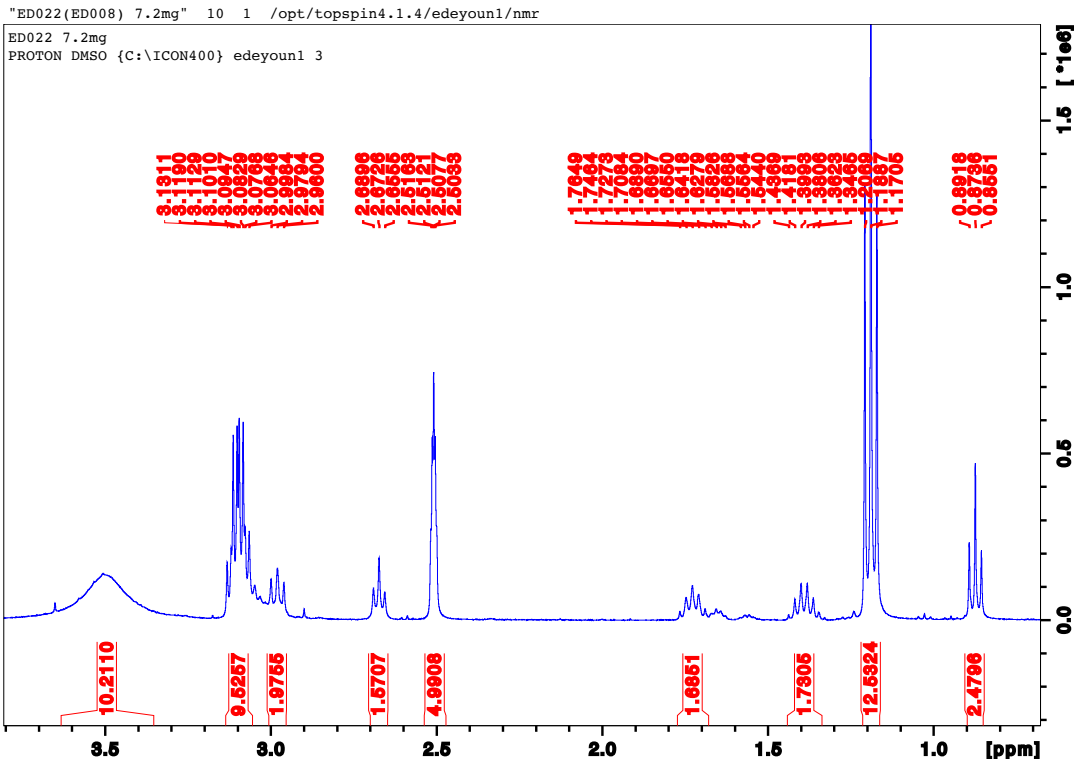
200
100
0
10.2200
9.4031
9.3707
9.0778
7.9625
7.9420
7.8168
7.7988
7.7768
7.6618
7.6331
7.6144
7.6075
7.6460
7.3656
7.3659
7.3474
7.0472
7.0258
5.9118
0.7999
1.9658
1.4701
1.0000
3.2889
0.9655
1.7293
0.8600
1.7455
1.5629
10
9
8
7
[ppm]


"ED022(ED008) 7.2mg" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED022 7.2mg
PROTON DMSO {C:\ICON400} edeyoun1 3
[*1e6]
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1.0
0.5
0.0
3.1311
3.1190
3.1129
3.1010
3.0947
3.0829
3.0766
3.0646
2.9964
2.9794
2.9600
2.6696
2.6726
2.6555
2.6168
2.6121
2.6077
2.6033
1.7649
1.7464
1.7273
1.7084
1.6897
1.6570
1.6418
1.6279
1.6028
1.5868
1.5664
1.5440
1.4369
1.4161
1.3993
1.3806
1.3623
1.3466
1.2069
1.1967
1.1705
0.8918
0.8736
0.8551
10.2110
9.5257
1.9755
1.5707
4.9908
1.6651
1.7305
12.5324
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3.0
2.5
2.0
1.5
1.0
[ppm]

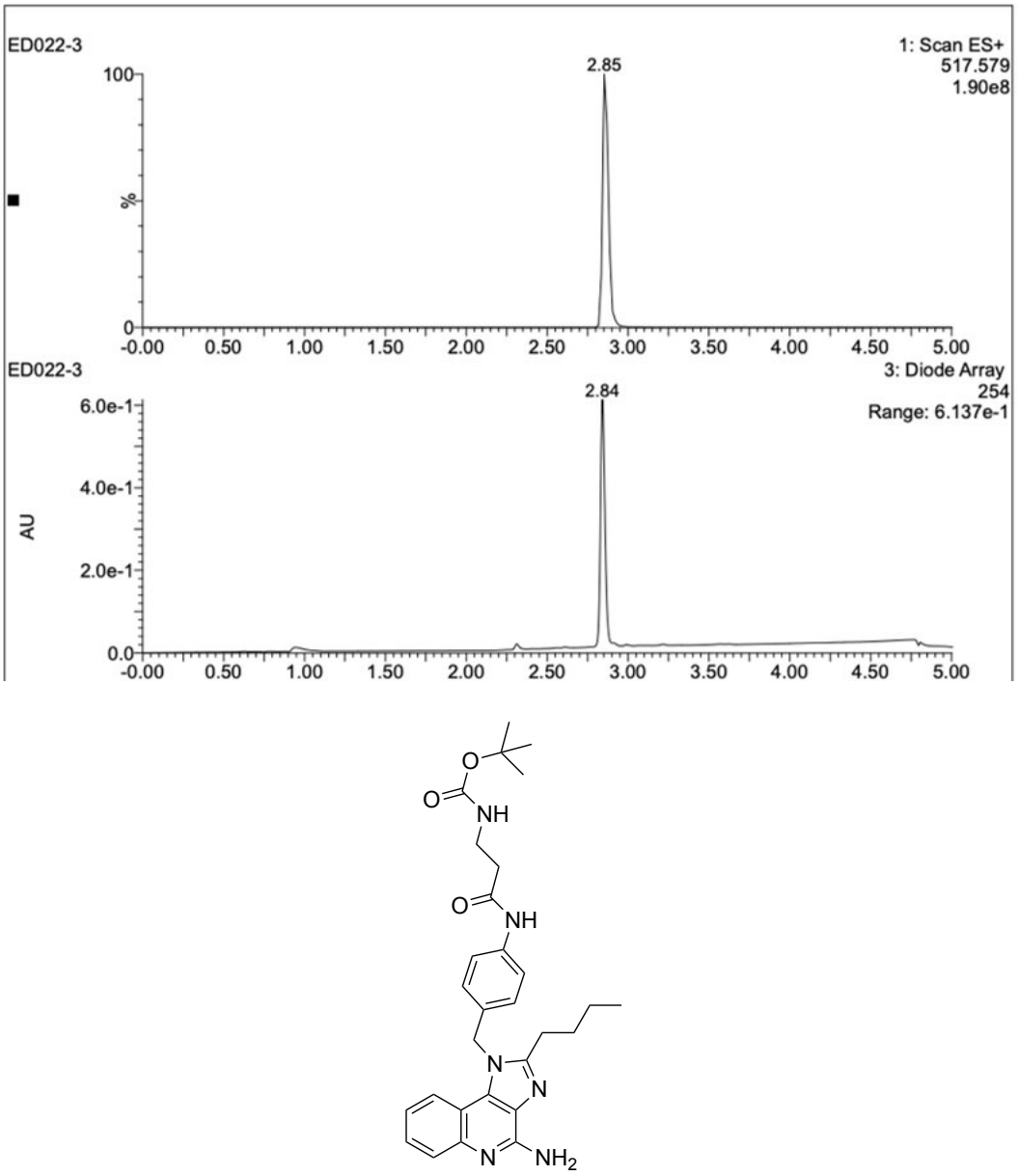
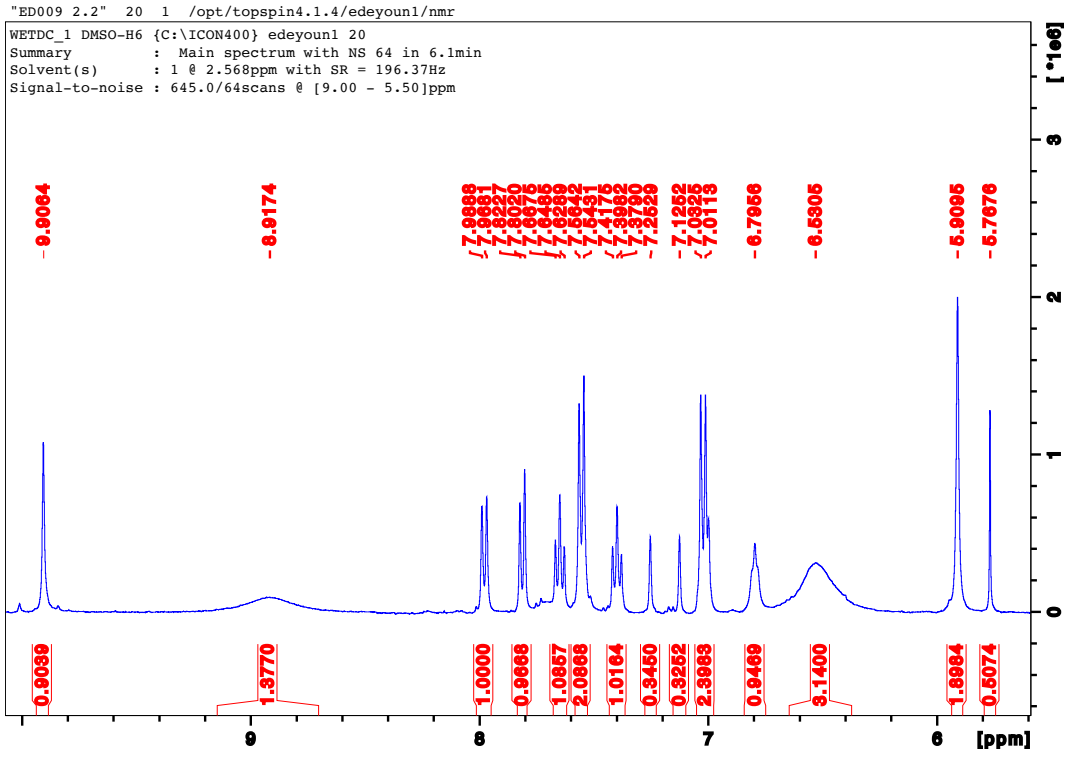


Figure S8: HPLC and NMR Characterization of Compound **9b,** MW = 517.6 [M+H]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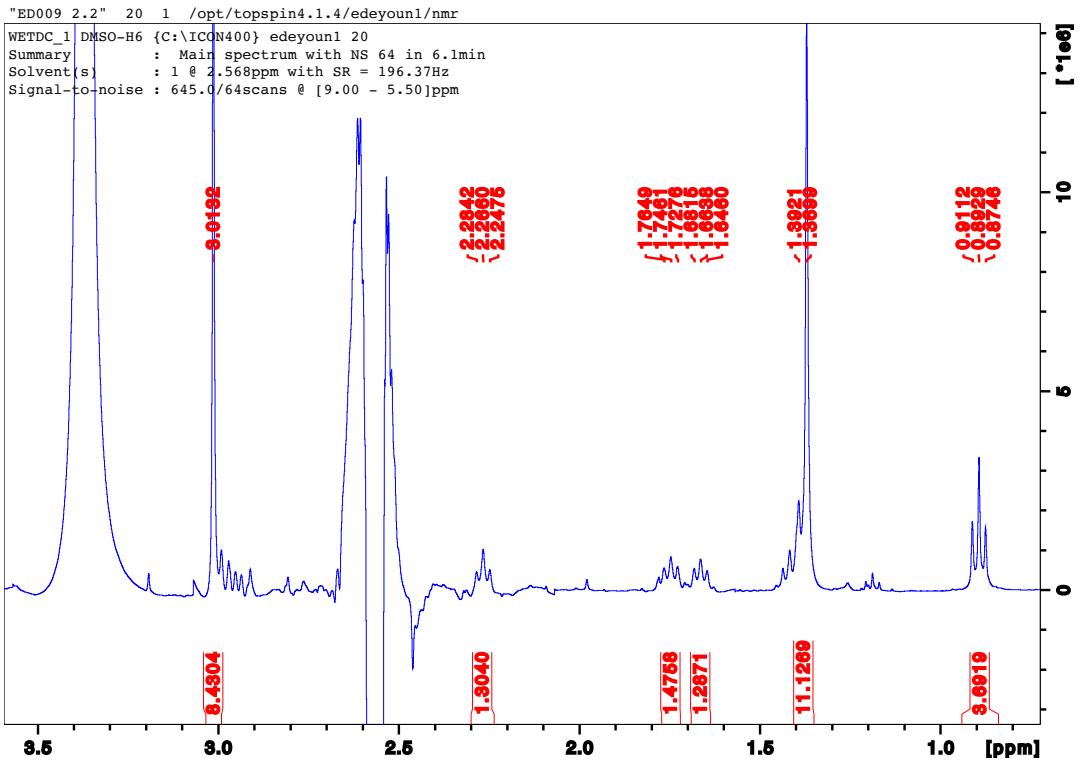
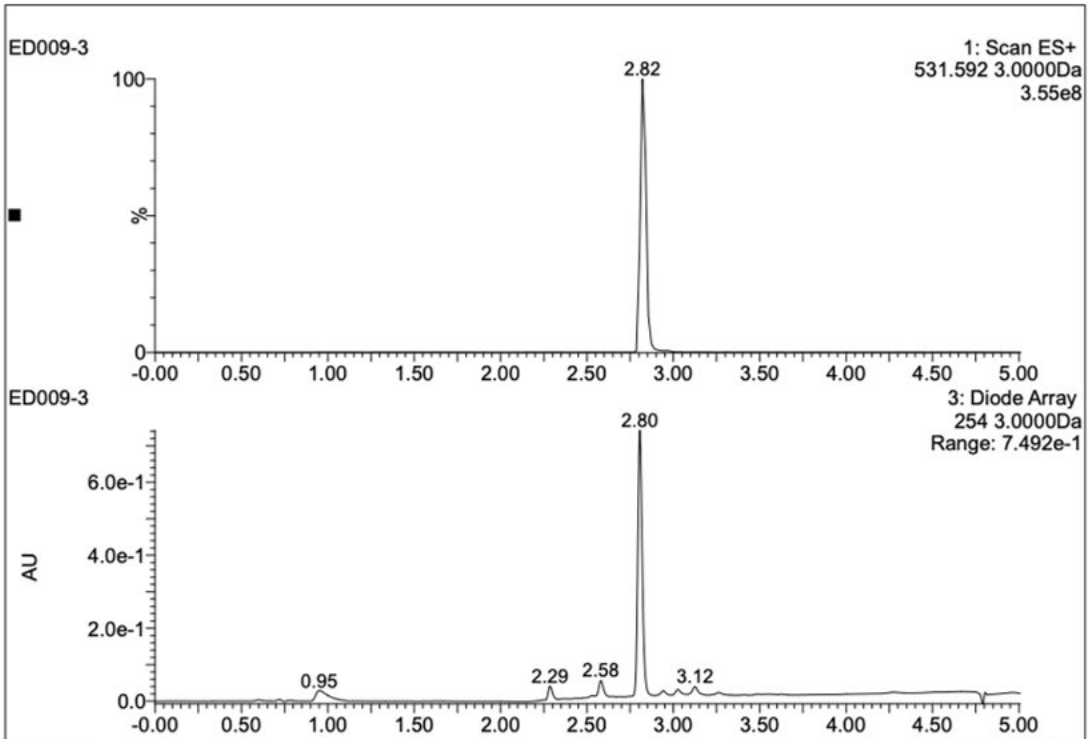
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 20
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 196.37Hz
Signal-to-noise : 645.0/64scans @ [9.00 - 5.50]ppm
[*1e6]
[ppm]


"ED009 2.2" 20 1 /opt/topspin4.1.4/edeyoun1/nmr
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 20
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 196.37Hz
Signal-to-noise : 645.0/64scans @ [9.00 - 5.50]ppm
[*1e6]
[ppm]



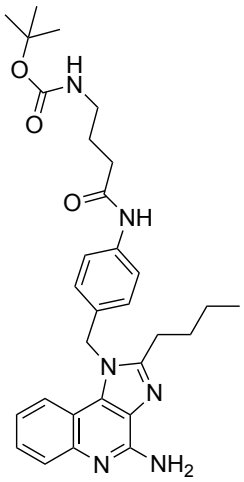
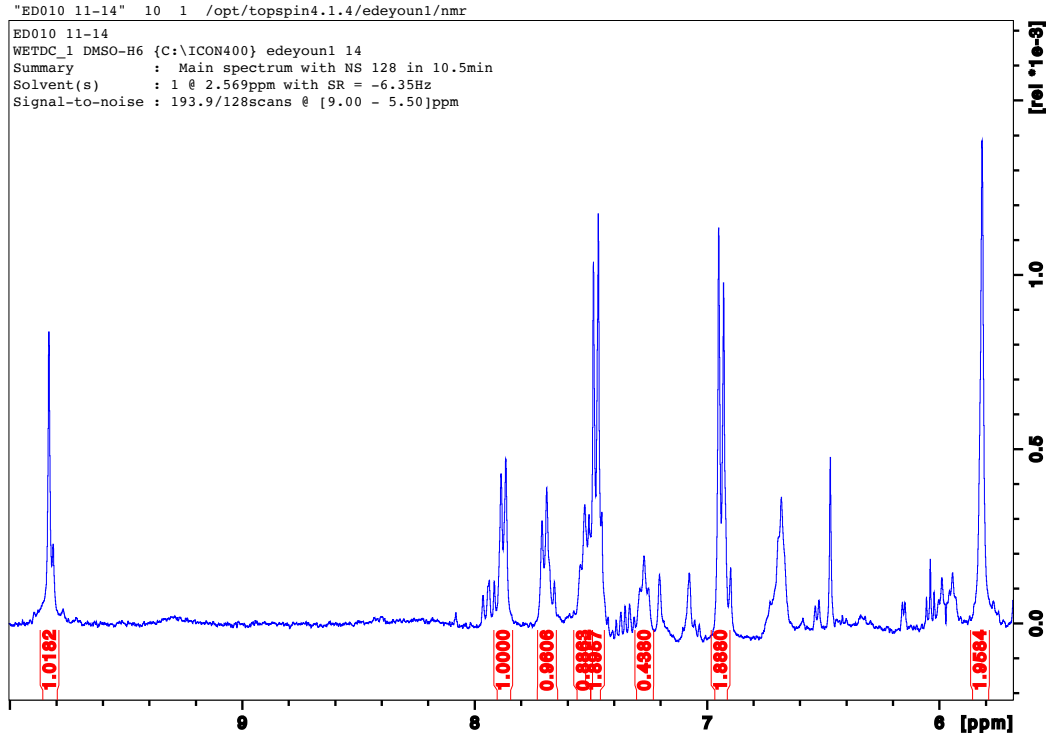
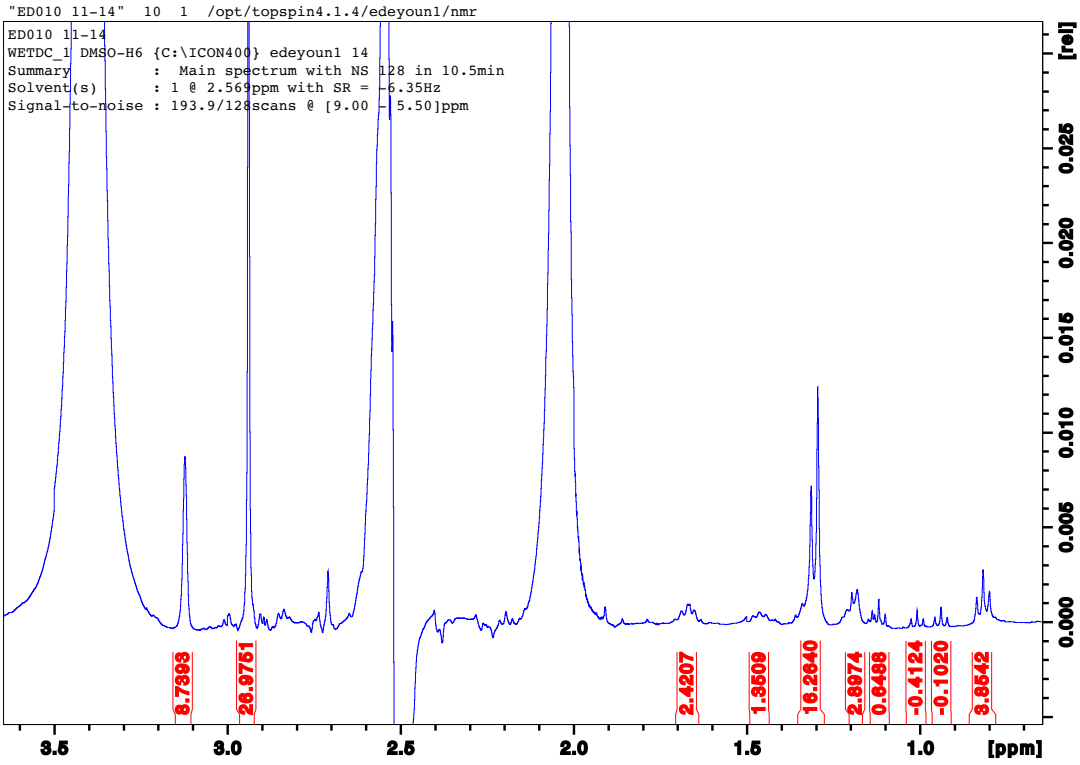


Figure S9: HPLC and NMR Characterization of Compound **9c,** MW = 531.6 [M+H]


"ED010 11-14" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED010 11-14
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 14
Summary : Main spectrum with NS 128 in 10.5min
Solvent(s) : 1 @ 2.569ppm with SR = -6.35Hz
Signal-to-noise : 193.9/128scans @ [9.00 - 5.50]ppm
[rel *1e-3]
1.0
0.5
0.0
1.0182
1.0000
0.9606
0.4980
1.8880
1.9584
9
8
7
6
[ppm]


"ED010 11-14" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED010 11-14
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 14
Summary : Main spectrum with NS 128 in 10.5min
Solvent(s) : 1 @ 2.569ppm with SR = -6.35Hz
Signal-to-noise : 193.9/128scans @ [9.00 - 5.50]ppm
[rel]
0.025
0.020
0.015
0.010
0.005
0.000
6.7393
26.9751
2.4207
1.9500
16.2640
2.8974
0.8488
-0.4124
-0.1020
3.8642
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]

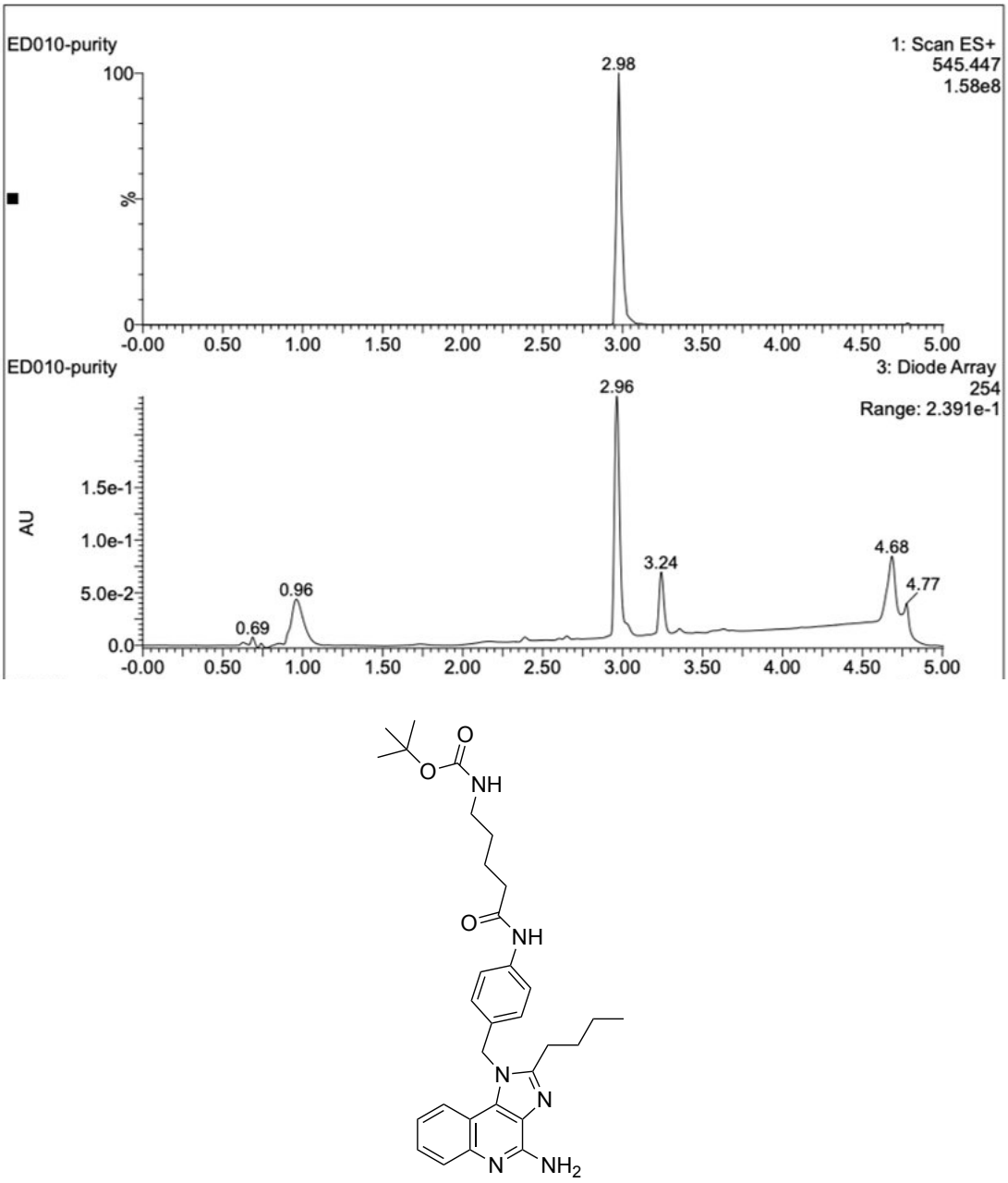
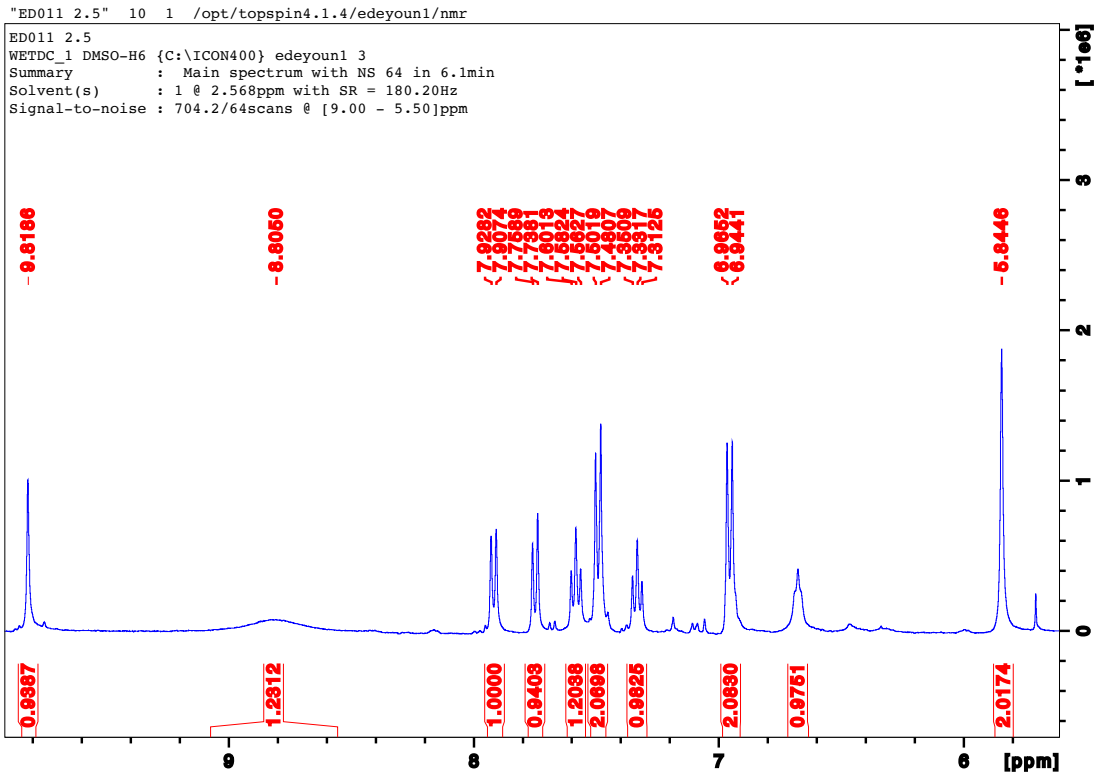
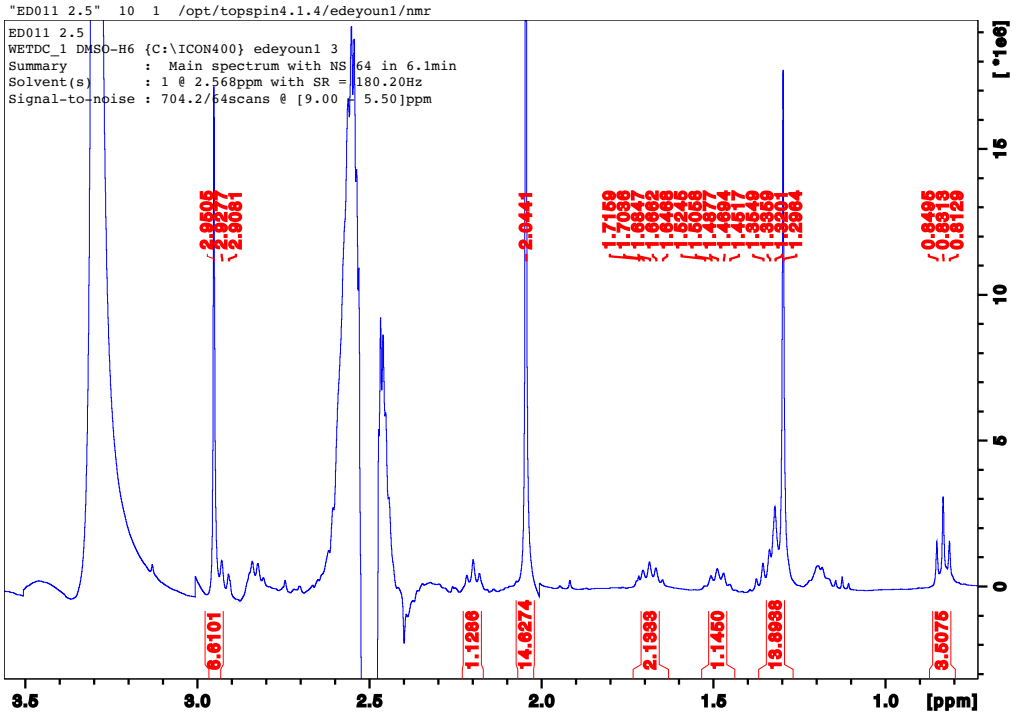


Figure S10: HPLC and NMR Characterization of Compound **9d,** MW = 545.4 [M+H]


"ED011 2.5" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED011 2.5
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 3
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 180.20Hz
Signal-to-noise : 704.2/64scans @ [9.00 - 5.50]ppm
9.8186
8.8060
7.9282
7.9074
7.7589
7.7381
7.6013
7.5824
7.5627
7.5019
7.4807
7.3509
7.3317
7.3125
6.9652
6.9441
5.8446
0.9387
1.2312
1.0000
0.9403
1.2038
2.0698
0.9825
2.0830
0.9751
2.0174
9
8
7
6
[ppm]
[*1e6]


"ED011 2.5" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED011 2.5
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 3
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 180.20Hz
Signal-to-noise : 704.2/64scans @ [9.00 - 5.50]ppm
2.9505
2.9277
2.9081
2.0441
1.7159
1.7039
1.6847
1.6662
1.6468
1.5245
1.5069
1.4877
1.4694
1.4617
1.3549
1.3369
1.3201
1.2984
0.8495
0.8313
0.8129
6.6101
1.1286
14.6274
2.1333
1.1450
13.8938
3.5076
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]
[*1e6]

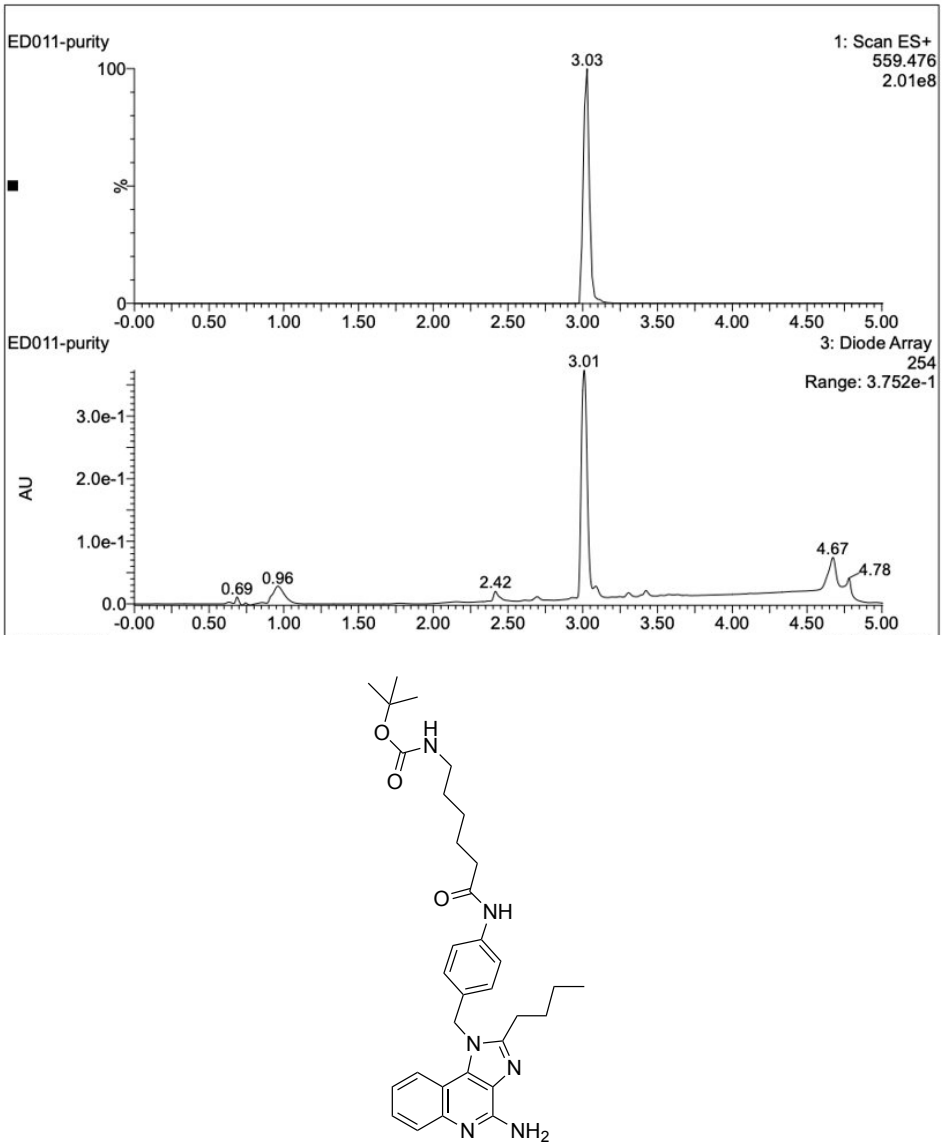
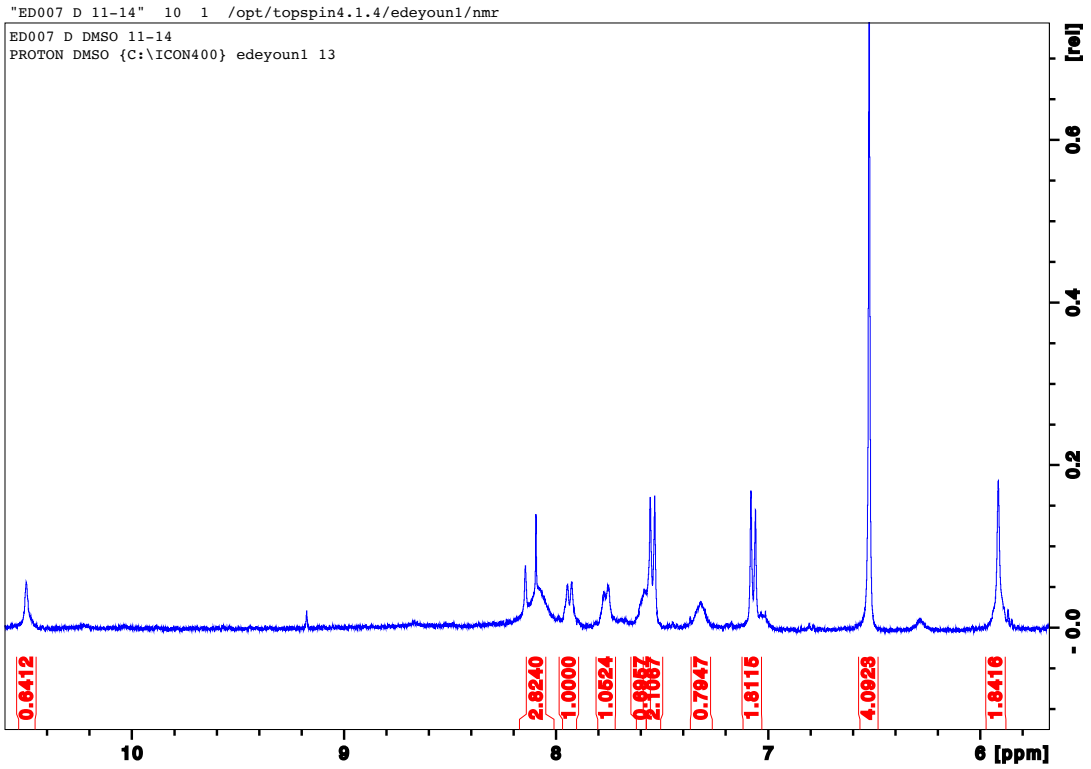
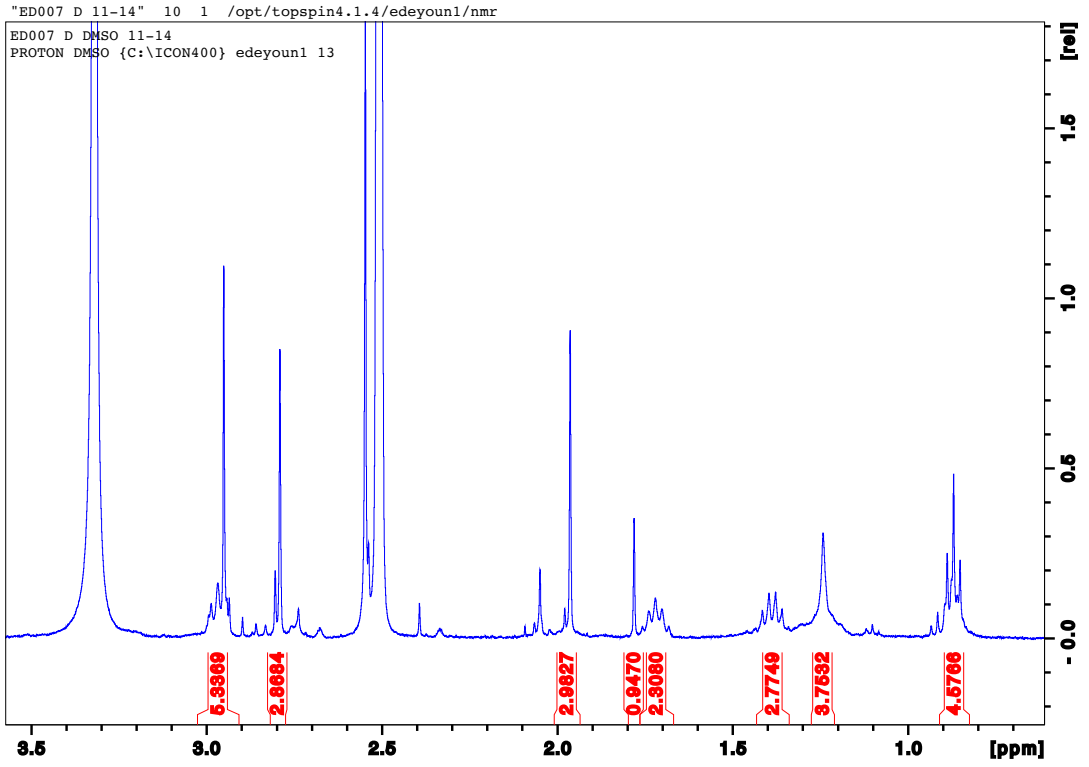


Figure S11: HPLC and NMR Characterization of Compound **9e,** MW = 559.5 [M+H]


"ED007 D 11-14" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED007 D DMSO 11-14
PROTON DMSO {C:\ICON400} edeyoun1 13
[rel]
0.6
0.4
0.2
- 0.0
0.6412
2.8240
1.0000
1.0624
0.7947
1.8115
4.0923
1.8416
10
9
8
7
6 [ppm]


"ED007 D 11-14" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED007 D DMSO 11-14
PROTON DMSO {C:\ICON400} edeyoun1 13
[rel]
1.5
1.0
0.5
- 0.0
5.3369
2.8694
2.9827
0.9470
2.3000
2.7749
3.7532
4.6766
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]

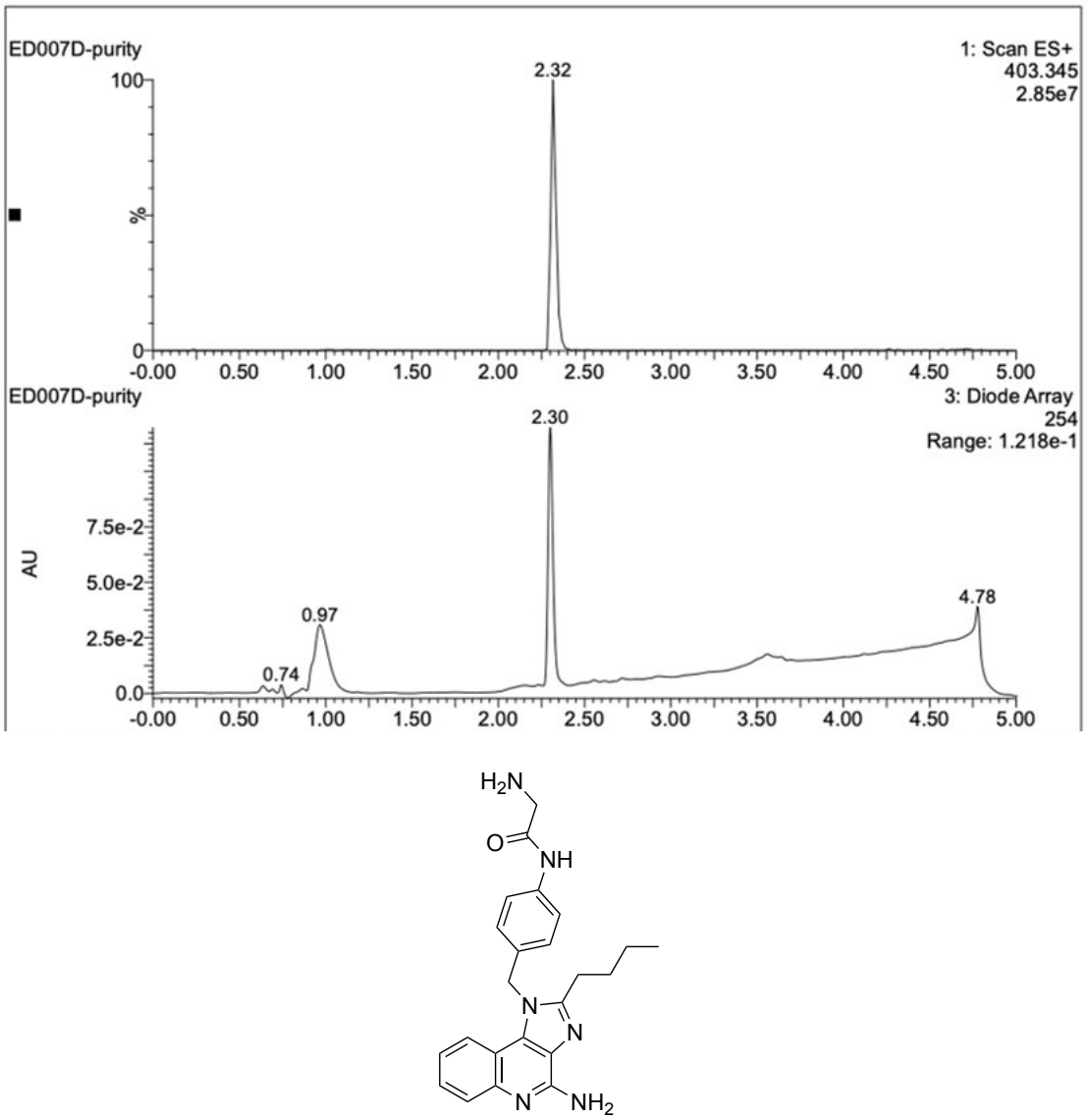
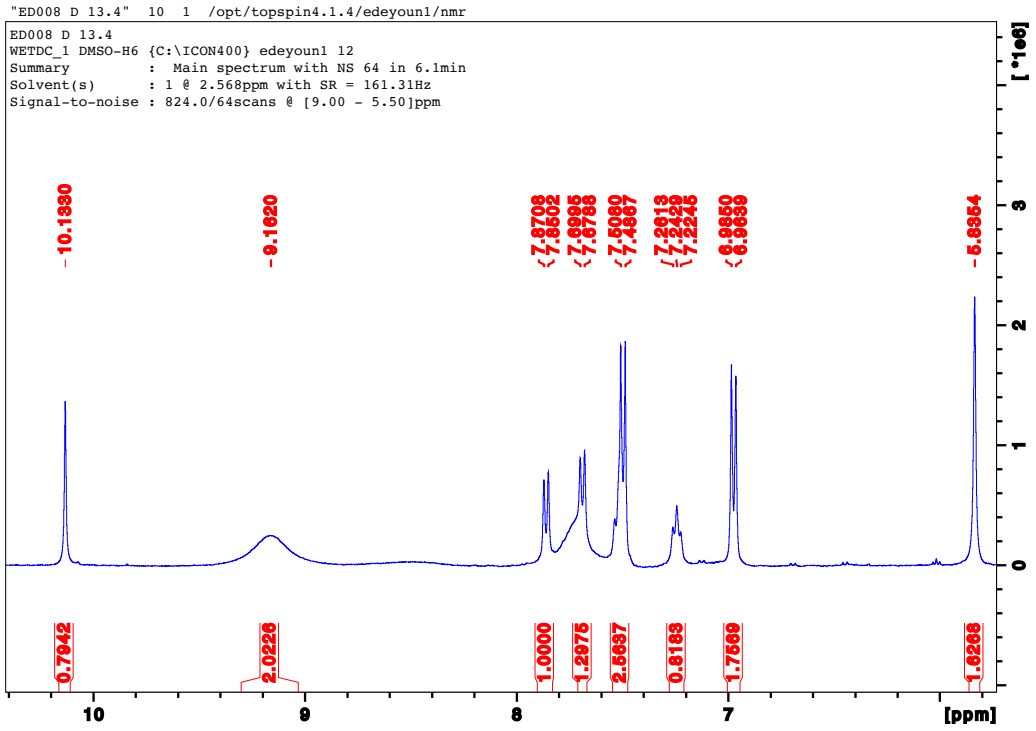
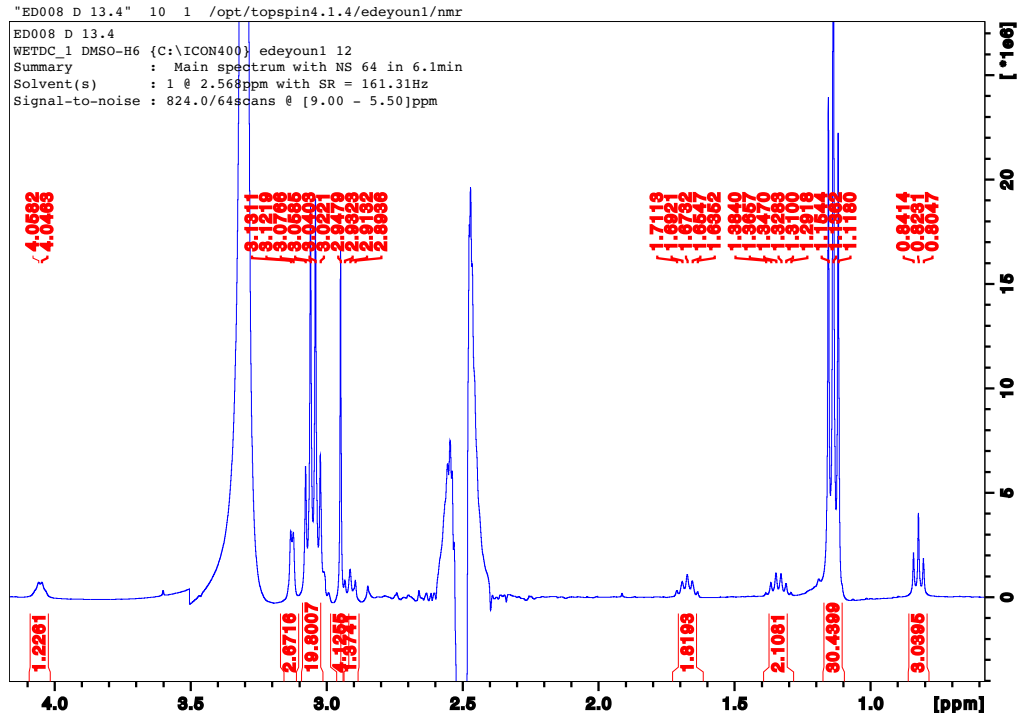


Figure S12: HPLC and NMR Characterization of Compound **10a,** MW = 403.3 [M+H]


"ED008 D 13.4" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED008 D 13.4
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 12
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 161.31Hz
Signal-to-noise : 824.0/64scans @ [9.00 - 5.50]ppm
10.1330
9.1620
7.8708
7.8502
7.6995
7.6788
7.5080
7.4867
7.2613
7.2429
7.2245
6.9850
6.9639
5.8354
0.7942
2.0226
1.0000
1.2975
2.5637
0.8183
1.7569
1.0266
10
9
8
7
[ppm]
[*1e6]


"ED008 D 13.4" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED008 D 13.4
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 12
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 161.31Hz
Signal-to-noise : 824.0/64scans @ [9.00 - 5.50]ppm
4.0682
4.0463
3.1311
3.1219
3.0766
3.0585
3.0403
3.0221
2.9479
2.9323
2.9132
2.8936
1.7113
1.6921
1.6792
1.6647
1.6552
1.3940
1.3857
1.3470
1.3283
1.3100
1.2916
1.2544
1.1363
1.1180
0.8414
0.8231
0.8047
1.2261
2.8716
10.8007
4.1255
1.3741
1.8183
2.1061
30.4399
3.0395
4.0
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]
[*1e6]

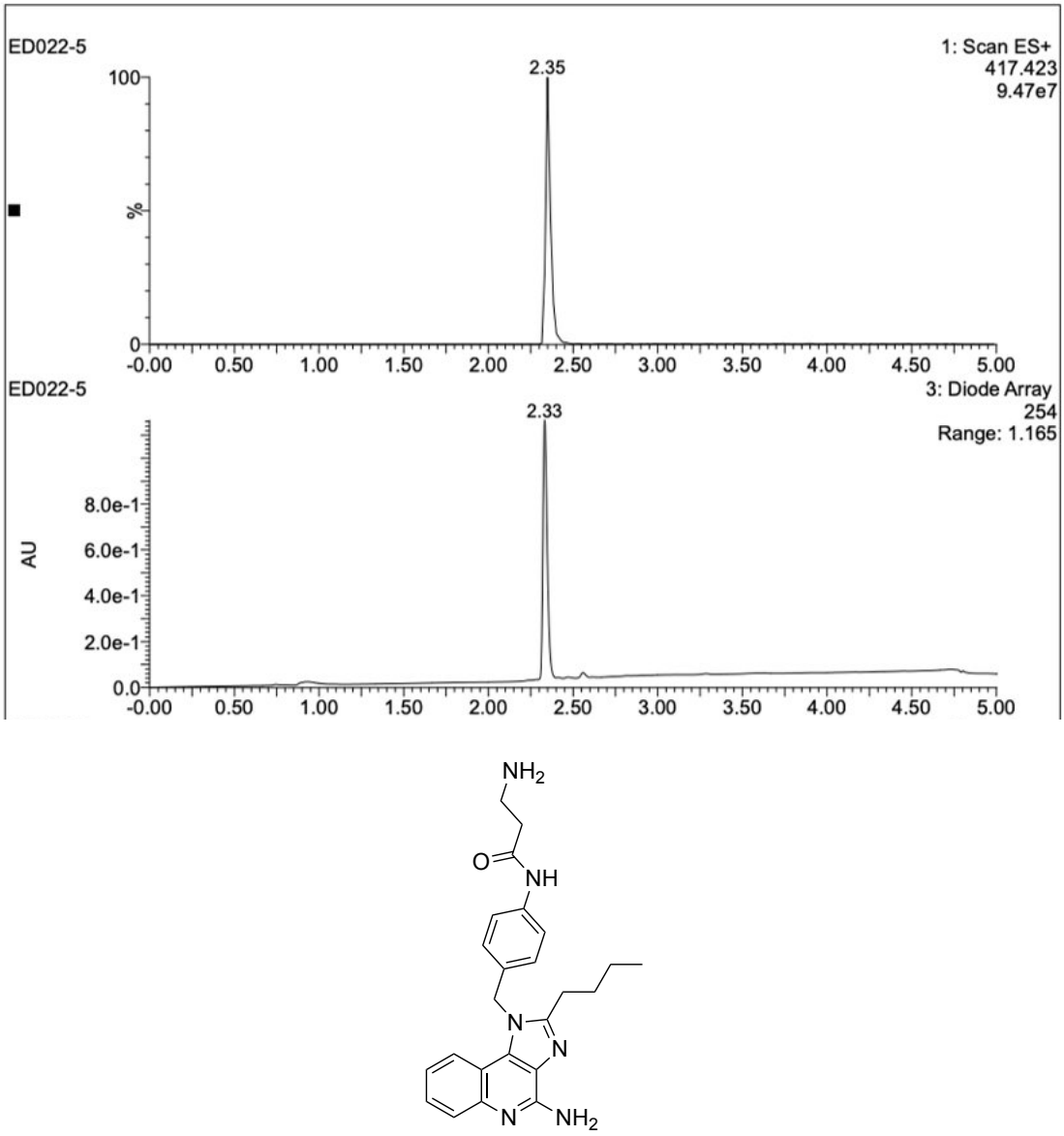
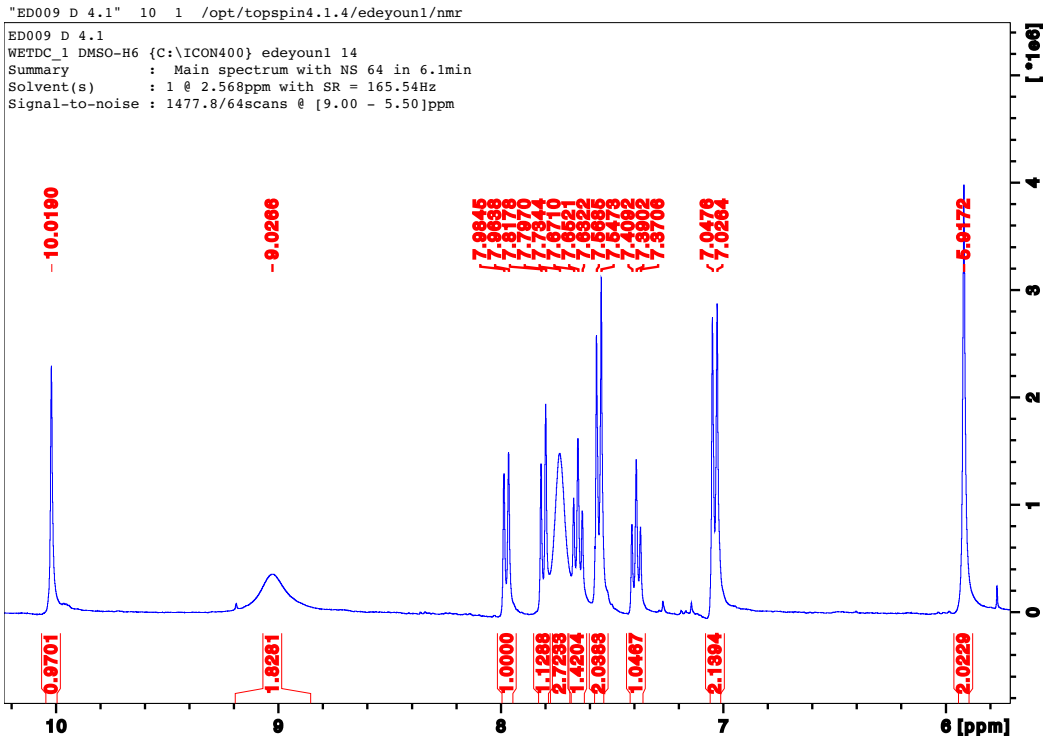
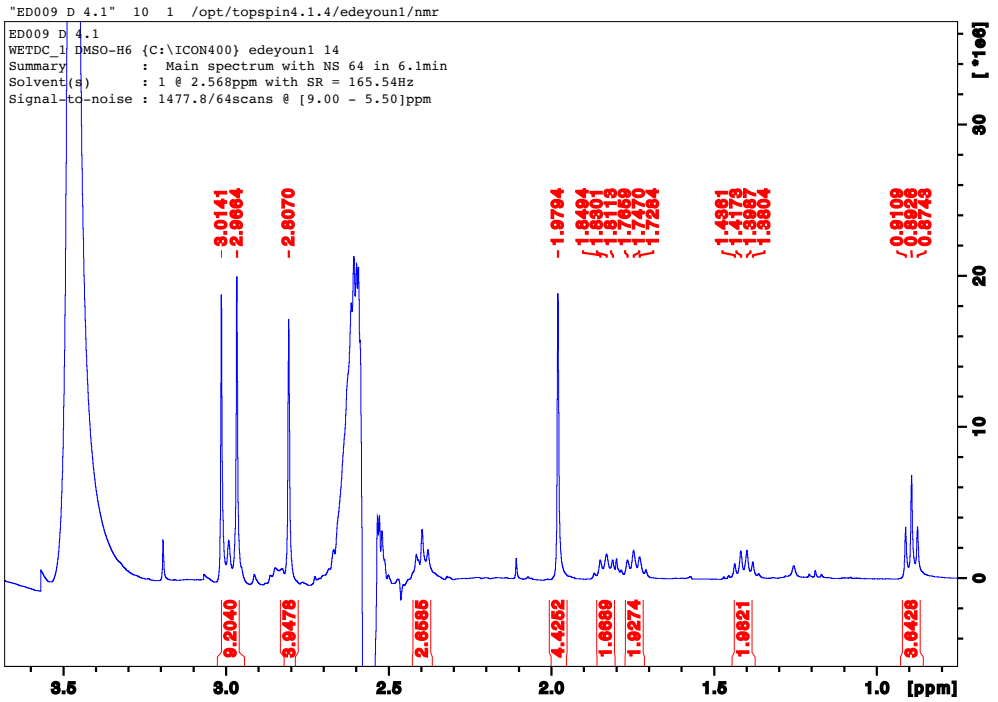


Figure S13: HPLC and NMR Characterization of Compound **10b,** MW = 417.4 [M+H]


"ED009 D 4.1" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED009 D 4.1
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 14
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 165.54Hz
Signal-to-noise : 1477.8/64scans @ [9.00 - 5.50]ppm
10.0190
9.0266
7.9845
7.9638
7.9178
7.8970
7.8744
7.8710
7.8521
7.8322
7.8165
7.5473
7.4092
7.3902
7.3706
7.0478
7.0284
5.9172
0.9701
1.8281
1.0000
1.1288
2.7253
1.4204
2.0383
1.0467
2.1394
2.0229
10
9
8
7
6 [ppm]
[*1e6]


"ED009 D 4.1" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED009 D 4.1
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 14
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 165.54Hz
Signal-to-noise : 1477.8/64scans @ [9.00 - 5.50]ppm
3.0141
2.9864
2.8070
1.9784
1.8494
1.8301
1.8113
1.7059
1.7470
1.7284
1.4361
1.4173
1.3987
1.3804
0.9109
0.8926
0.8743
9.2040
3.0478
2.0585
4.4252
1.0669
1.0274
1.9821
3.0428
3.5
3.0
2.5
2.0
1.5
1.0 [ppm]
[*1e6]

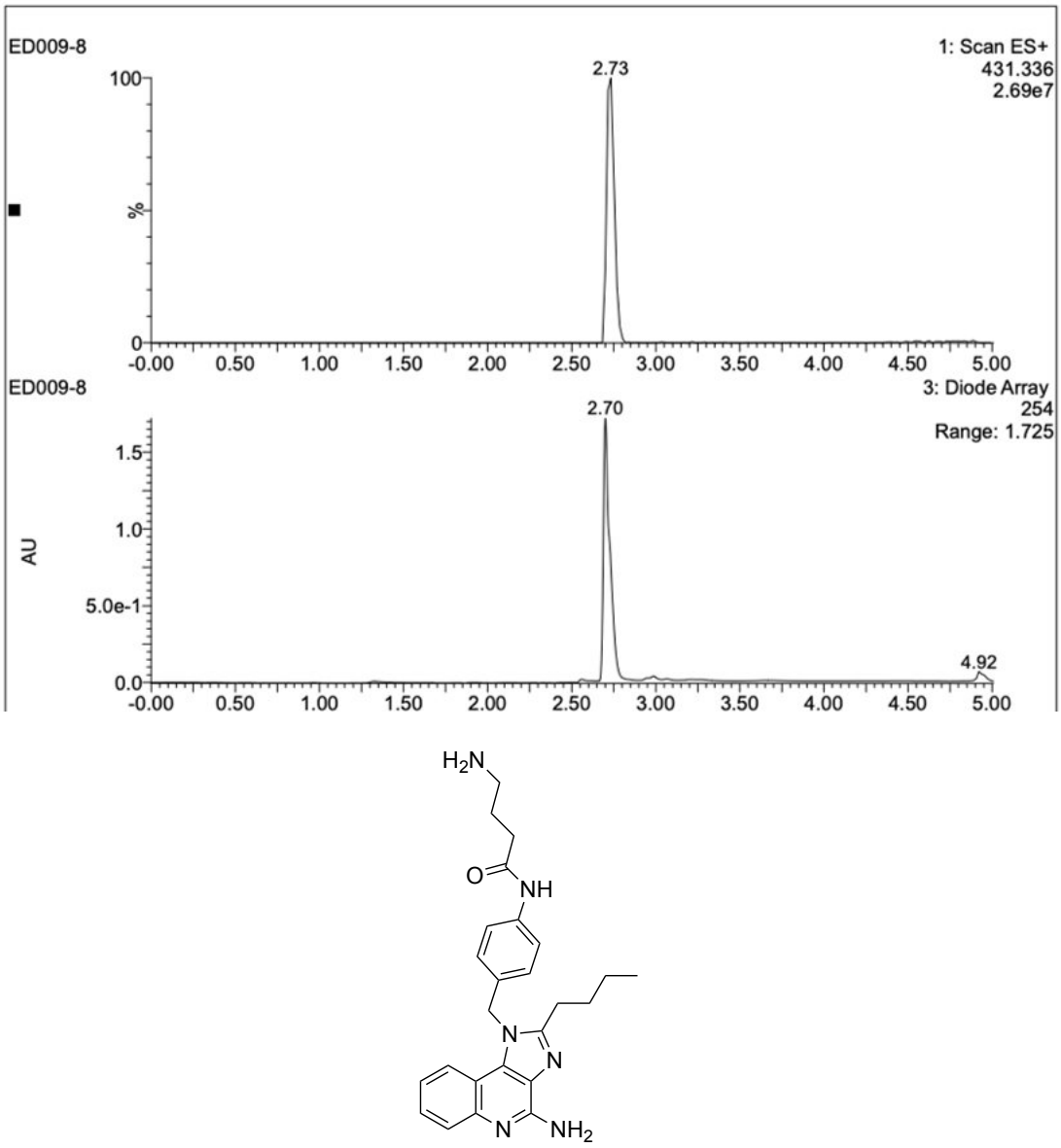
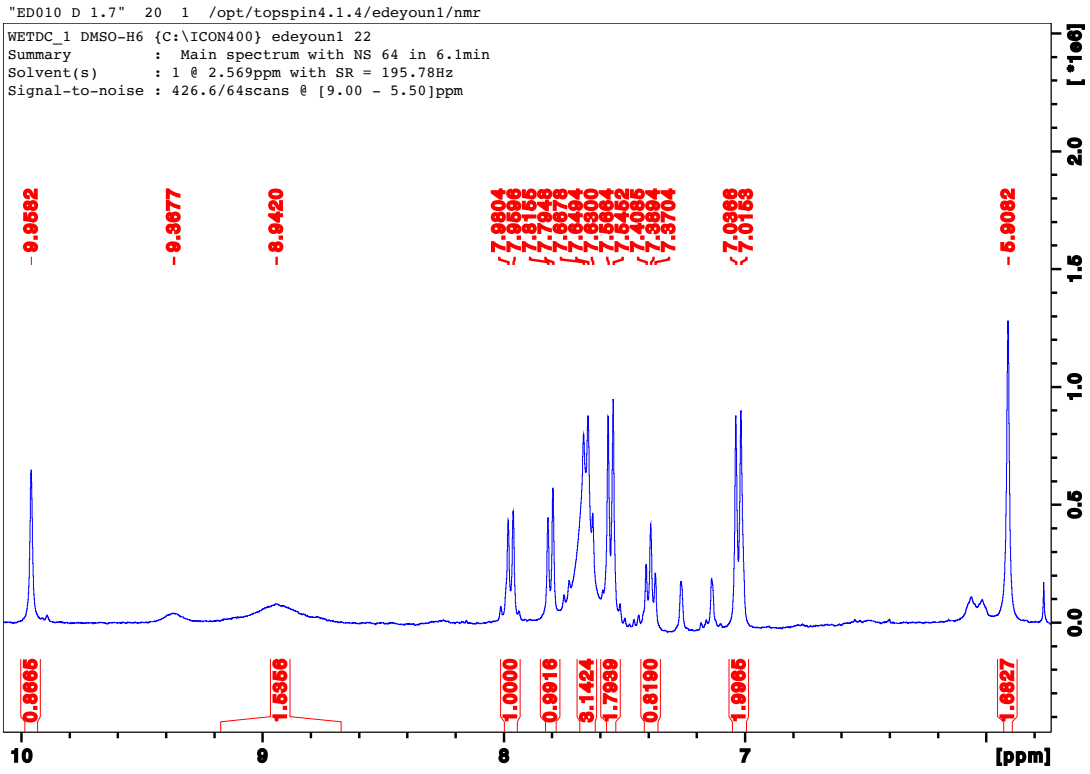
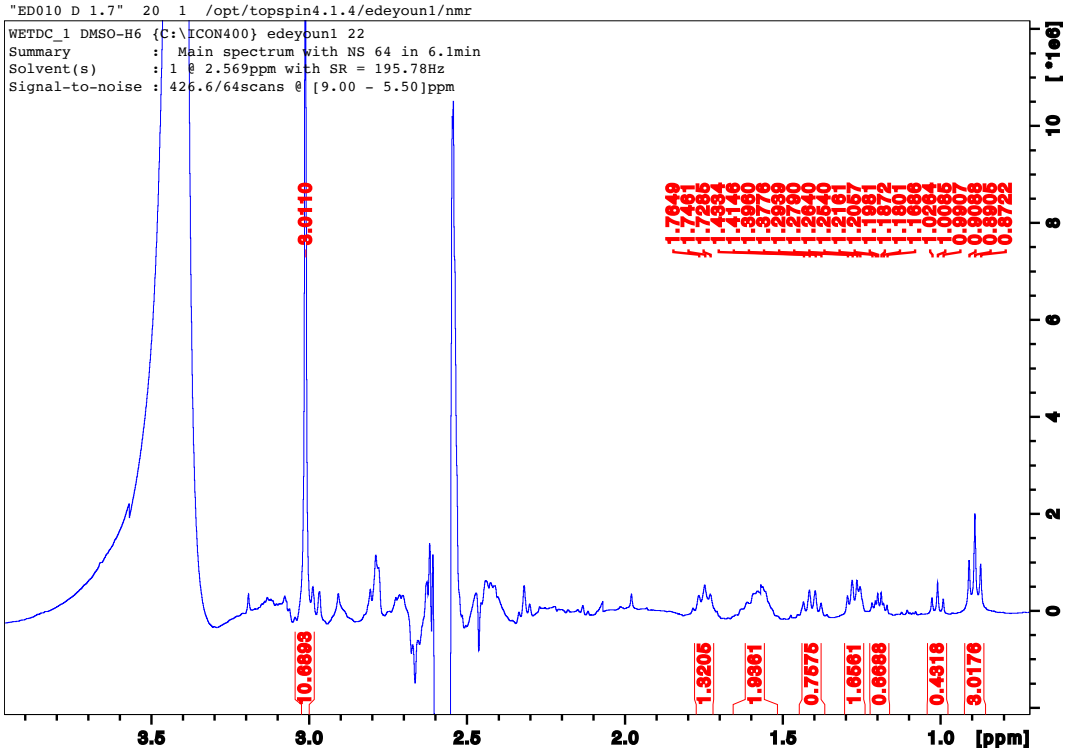


Figure S14: HPLC and NMR Characterization of Compound **10c,** MW = 431.3 [M+H]


"ED010 D 1.7" 20 1 /opt/topspin4.1.4/edeyoun1/nmr
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 22
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.569ppm with SR = 195.78Hz
Signal-to-noise : 426.6/64scans @ [9.00 - 5.50]ppm
9.9562
9.3677
8.9420
7.9804
7.9656
7.8155
7.7948
7.6678
7.6494
7.6300
7.5664
7.5452
7.4085
7.3894
7.3704
7.0366
7.0163
5.9062
0.8665
1.6556
1.0000
0.9916
3.1424
1.7939
0.8190
1.0965
1.6627
10
9
8
7
[ppm]
[*1e6]


"ED010 D 1.7" 20 1 /opt/topspin4.1.4/edeyoun1/nmr
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 22
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.569ppm with SR = 195.78Hz
Signal-to-noise : 426.6/64scans @ [9.00 - 5.50]ppm
3.0110
1.7940
1.7461
1.7285
1.4534
1.4174
1.4146
1.3960
1.3776
1.2939
1.2790
1.2540
1.2355
1.2161
1.2057
1.1961
1.1672
1.1507
1.1089
1.0939
1.0264
1.0066
0.9907
0.9058
0.8905
0.8722
10.6809
1.3205
1.9361
0.7575
1.6561
0.6868
0.4318
3.0176
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]
[*1e6]

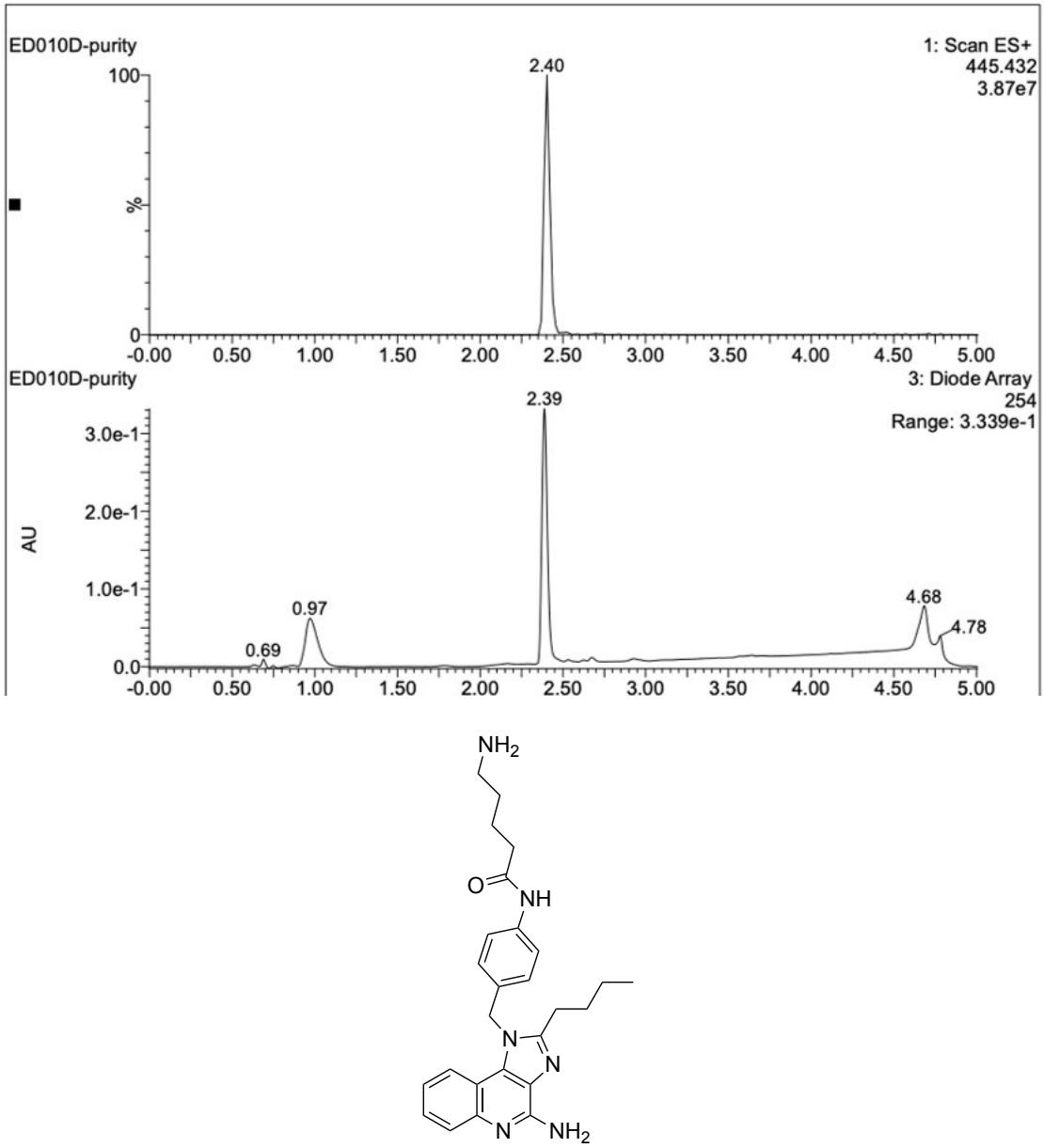
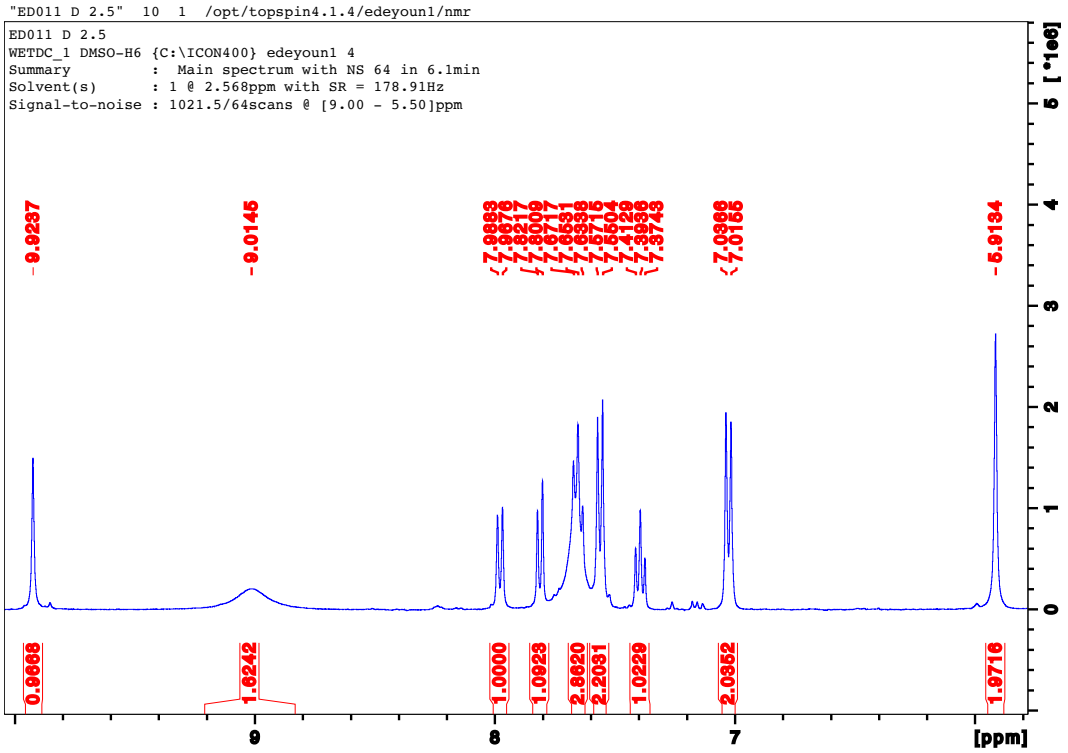
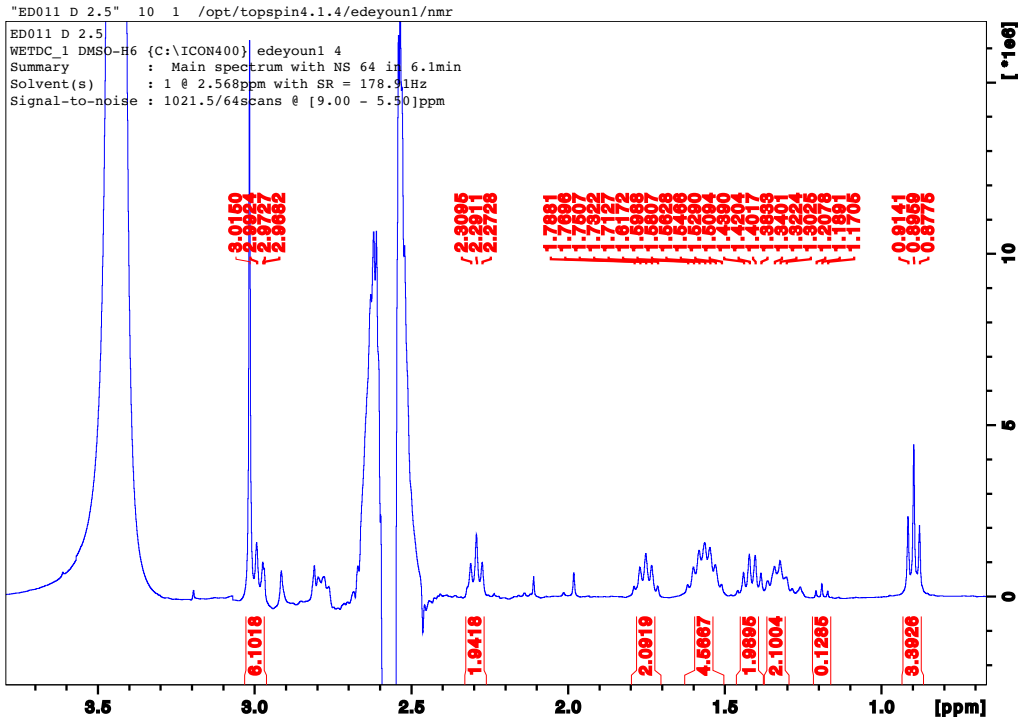


Figure S15: HPLC and NMR Characterization of Compound **10d,** MW = 445.4 [M+H]


"ED011 D 2.5" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED011 D 2.5
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 4
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 178.91Hz
Signal-to-noise : 1021.5/64scans @ [9.00 - 5.50]ppm
-9.9237
-9.0145
7.9883
7.9676
7.8217
7.8009
7.6717
7.6631
7.6338
7.5715
7.5504
7.4129
7.3936
7.3743
7.0366
7.0165
-5.9134
0.9868
1.6242
1.0000
1.0923
2.8820
2.2031
1.0229
2.0352
1.9716
9
8
7
[ppm]
[*1e6]


"ED011 D 2.5" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED011 D 2.5
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 4
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 178.91Hz
Signal-to-noise : 1021.5/64scans @ [9.00 - 5.50]ppm
3.0150
3.0047
2.9727
2.9582
2.3095
2.2911
2.2728
1.7981
1.7890
1.7807
1.7322
1.7127
1.6172
1.5988
1.5807
1.5628
1.5466
1.5290
1.5094
1.4890
1.4704
1.4017
1.3833
1.3651
1.3524
1.3026
1.2878
1.1991
1.1705
0.9141
0.8959
0.8776
6.1018
1.9418
2.0919
4.5667
1.0095
2.1004
0.1285
3.9926
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]
[*1e6]

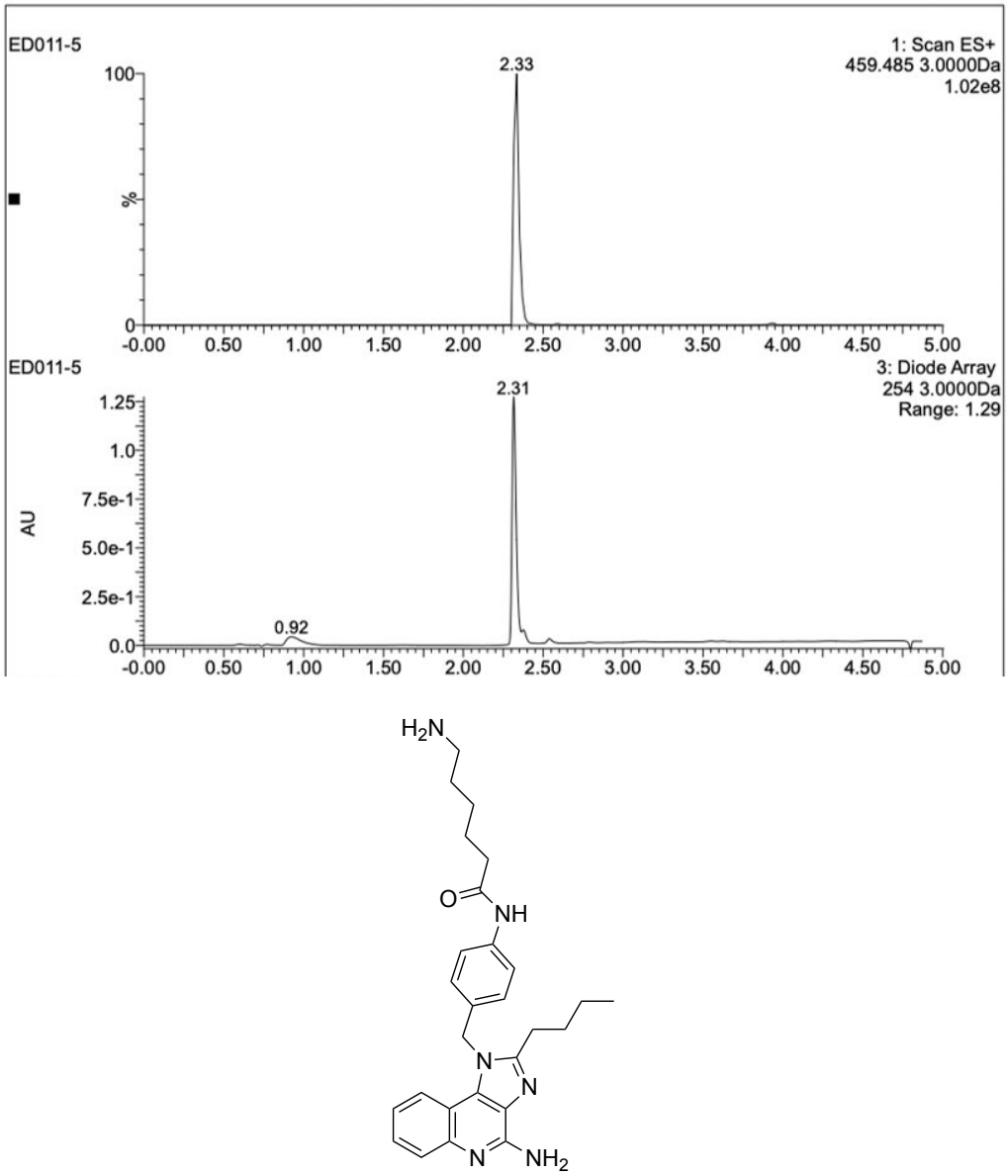
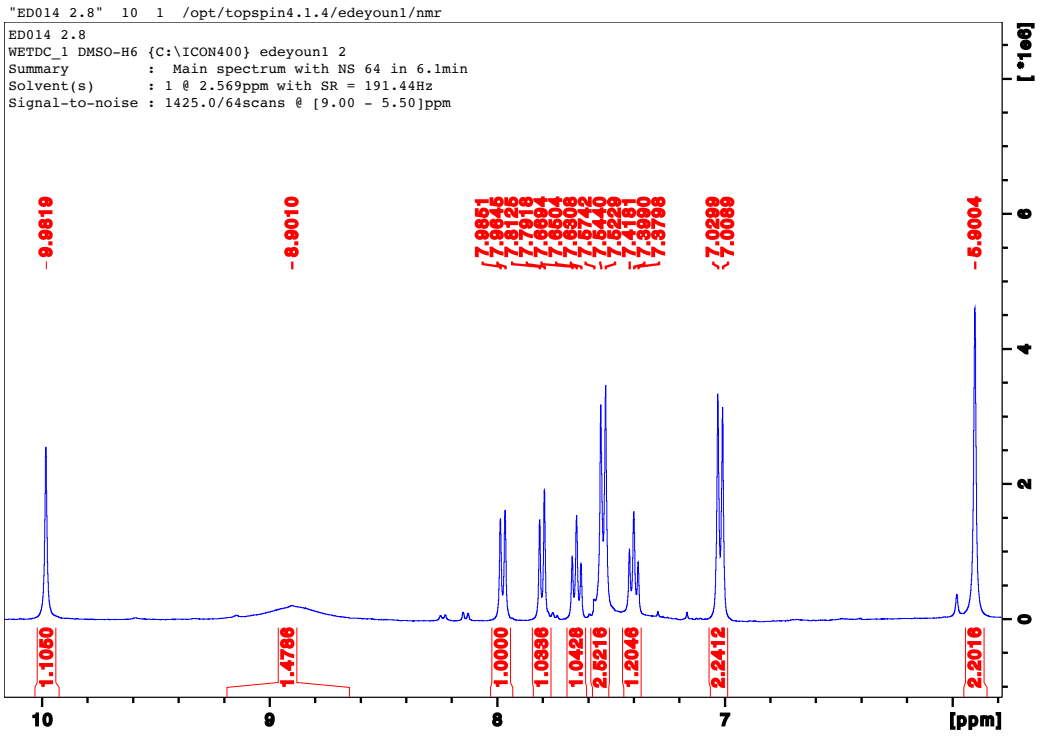
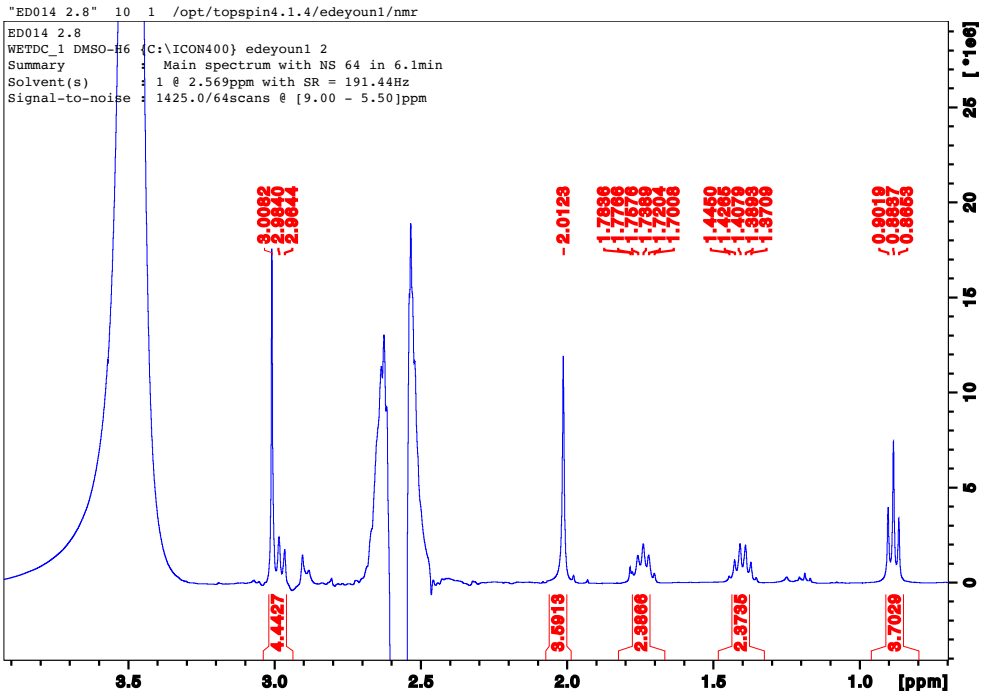


Figure S16: HPLC and NMR Characterization of Compound **10e,** MW = 459.5 [M+H]


"ED014 2.8" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED014 2.8
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 2
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.569ppm with SR = 191.44Hz
Signal-to-noise : 1425.0/64scans @ [9.00 - 5.50]ppm
9.9819
8.9010
7.9851
7.9646
7.8126
7.7918
7.6894
7.6504
7.6308
7.5742
7.5440
7.5229
7.4181
7.3990
7.3798
7.0289
7.0089
5.9004
1.1050
1.4786
1.0000
1.0396
1.0428
2.5216
1.2048
2.2412
2.2016
10
9
8
7
[ppm]
[*1e6]


"ED014 2.8" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED014 2.8
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 2
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.569ppm with SR = 191.44Hz
Signal-to-noise : 1425.0/64scans @ [9.00 - 5.50]ppm
3.0062
2.9840
2.9644
2.0123
1.7836
1.7766
1.7576
1.7389
1.7204
1.7008
1.4450
1.4295
1.4079
1.3863
1.3700
0.9019
0.8857
0.8663
4.4427
3.5913
2.3866
2.3735
3.7029
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]
[*1e6]

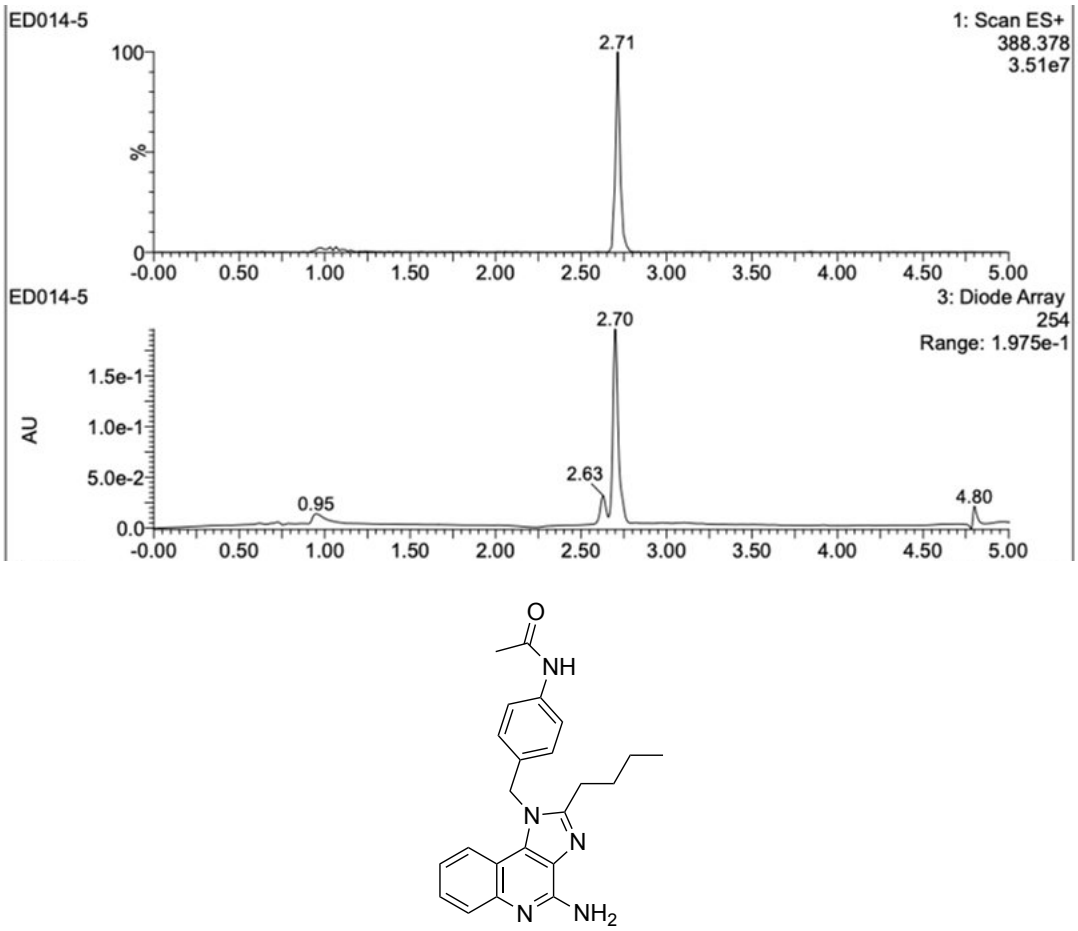
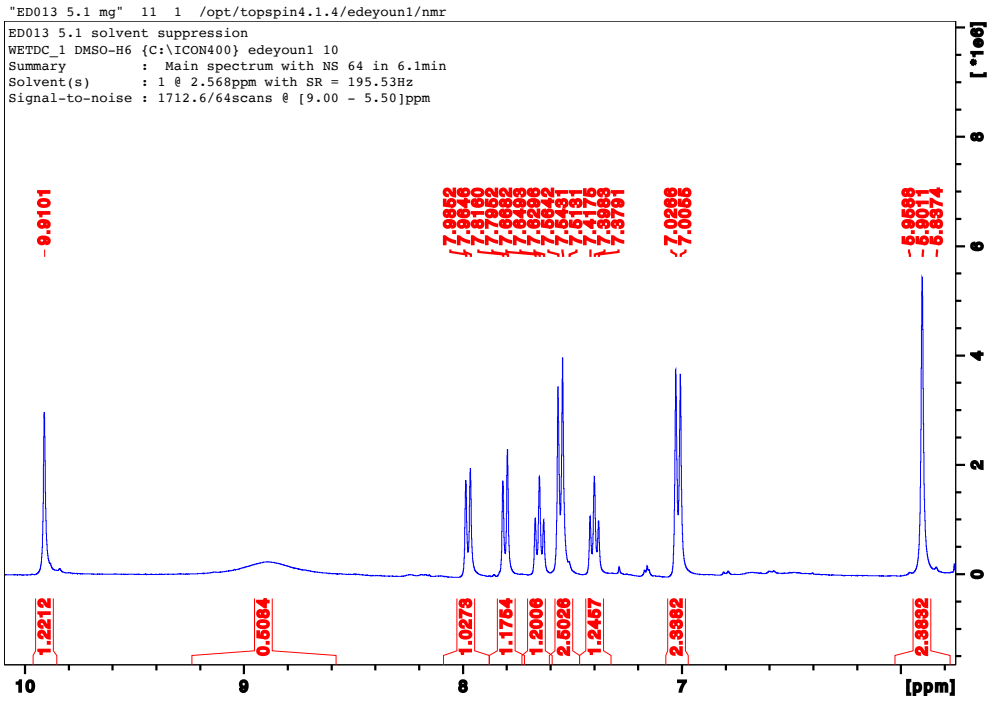
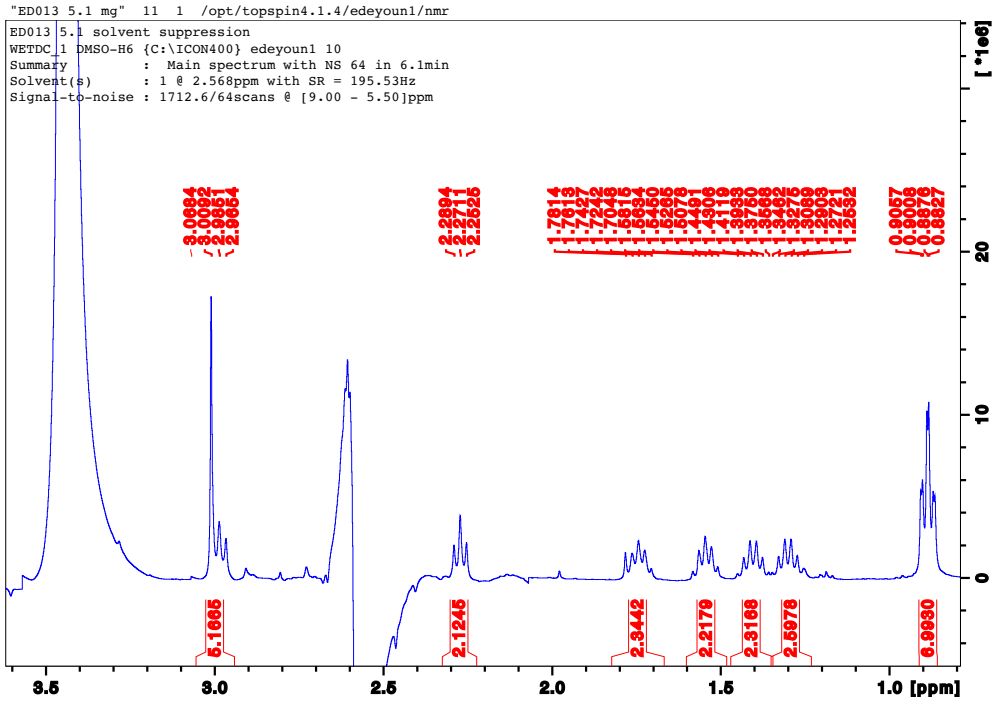


Figure S17: HPLC and NMR Characterization of Compound **11a,** MW = 388.4 [M+H]


"ED013 5.1 mg" 11 1 /opt/topspin4.1.4/edeyoun1/nmr
ED013 5.1 solvent suppression
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 10
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 195.53Hz
Signal-to-noise : 1712.6/64scans @ [9.00 - 5.50]ppm
[*1e6]
[ppm]


"ED013 5.1 mg" 11 1 /opt/topspin4.1.4/edeyoun1/nmr
ED013 5.1 solvent suppression
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 10
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 195.53Hz
Signal-to-noise : 1712.6/64scans @ [9.00 - 5.50]ppm
[*1e6]
[ppm]

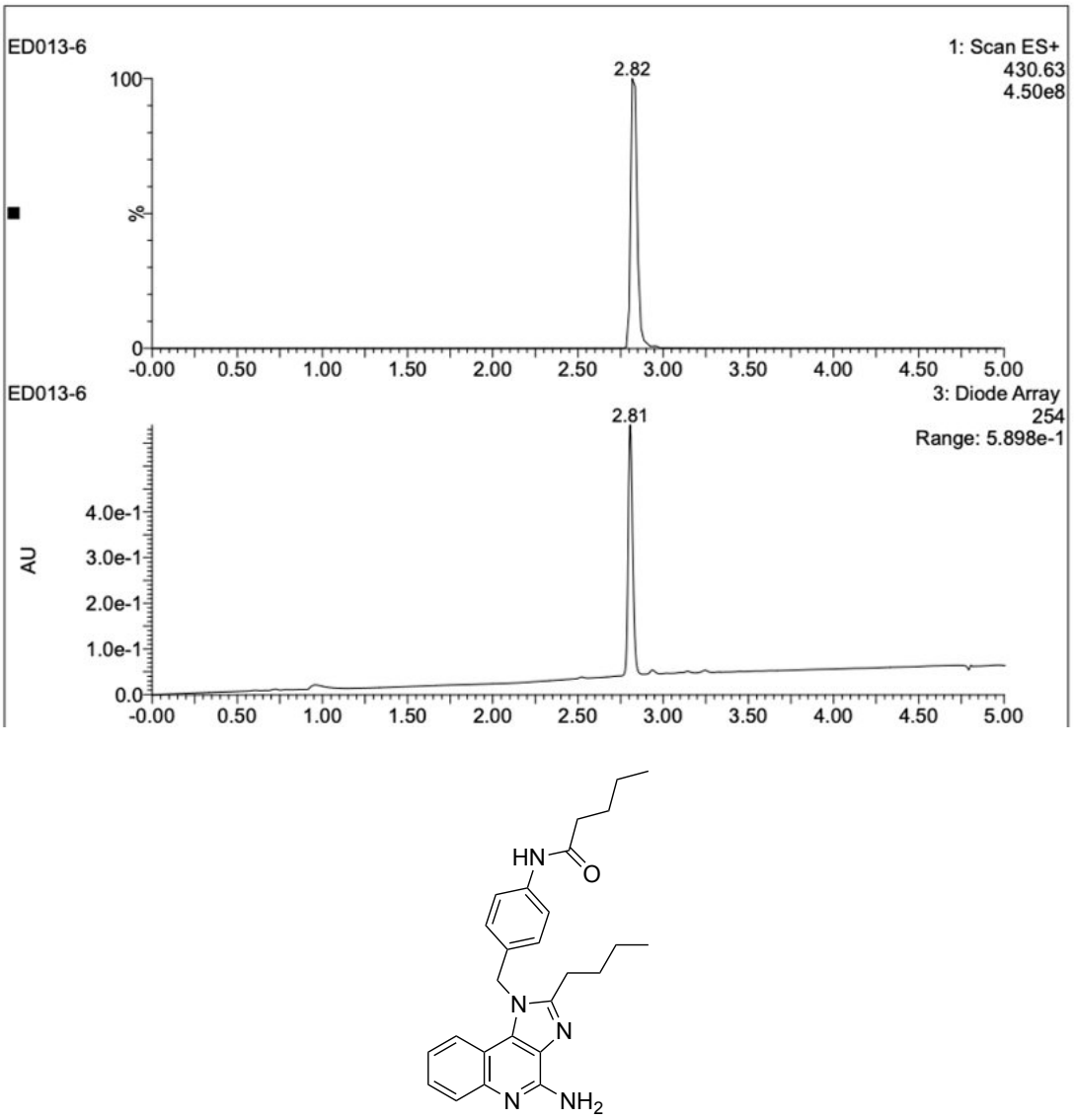
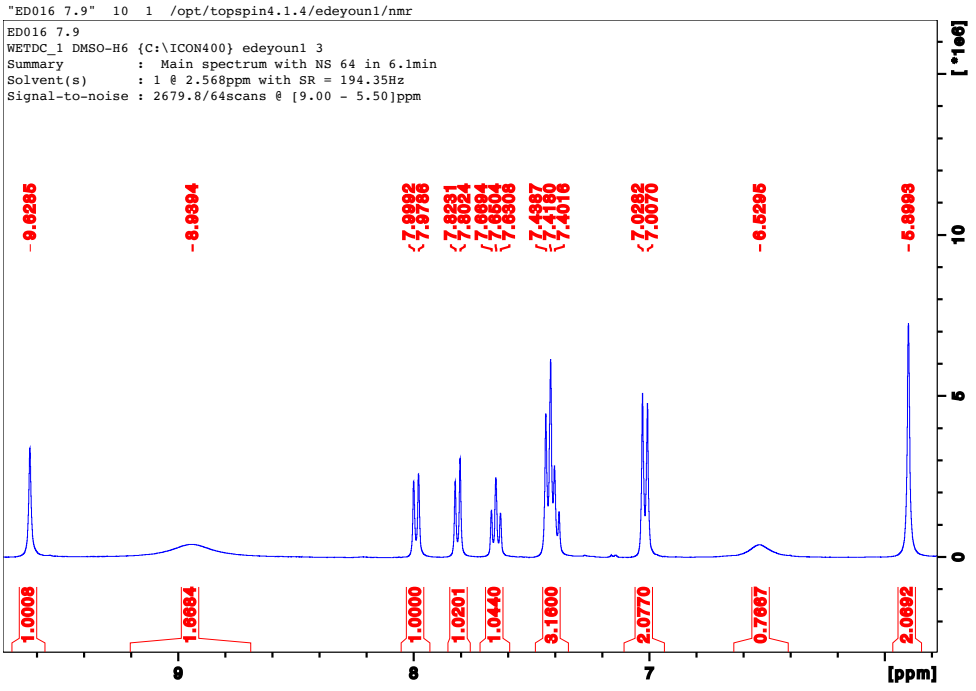
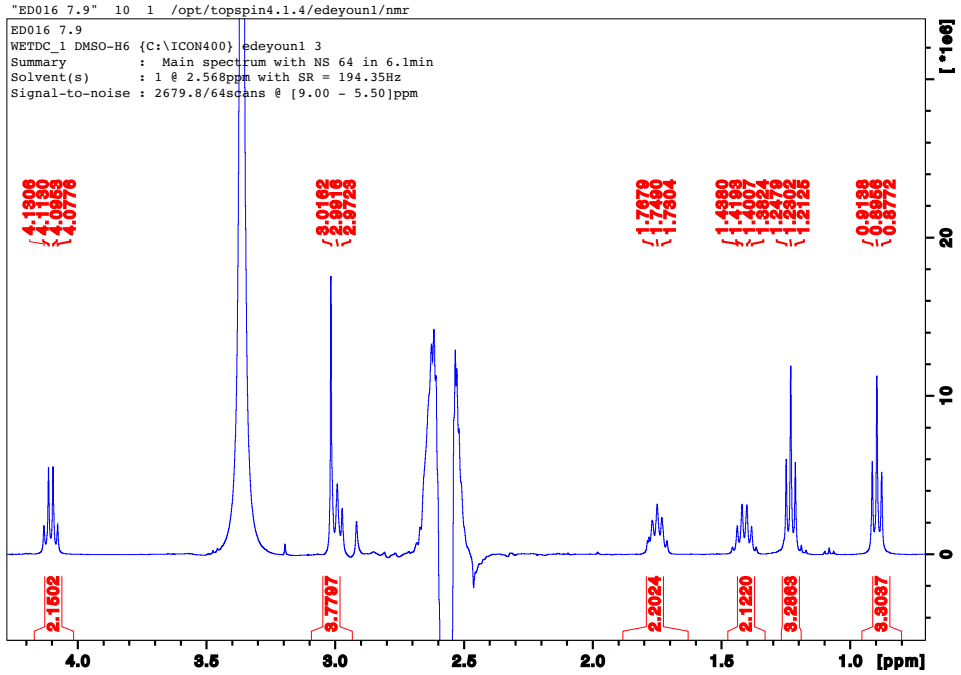


Figure S18: HPLC and NMR Characterization of Compound **11b,** MW = 430.6 [M+H]


"ED016 7.9" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED016 7.9
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 3
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 194.35Hz
Signal-to-noise : 2679.8/64scans @ [9.00 - 5.50]ppm
9.0285
8.0394
7.9992
7.9786
7.8231
7.8024
7.6694
7.6504
7.6306
7.4387
7.4180
7.4016
7.0282
7.0070
6.5295
5.8993
1.0008
1.6684
1.0000
1.0201
1.0440
3.1600
2.0770
0.7667
2.0692
9
8
7
[ppm]
[*1e6]


"ED016 7.9" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED016 7.9
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 3
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 194.35Hz
Signal-to-noise : 2679.8/64scans @ [9.00 - 5.50]ppm
4.1306
4.1180
4.0953
4.0776
3.0162
2.9916
2.9728
1.7679
1.7490
1.7304
1.4380
1.4189
1.4007
1.3824
1.2479
1.2302
1.2125
0.9138
0.8958
0.8772
2.1502
3.7797
2.2024
2.1220
3.2663
3.3037
4.0
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]
[*1e6]

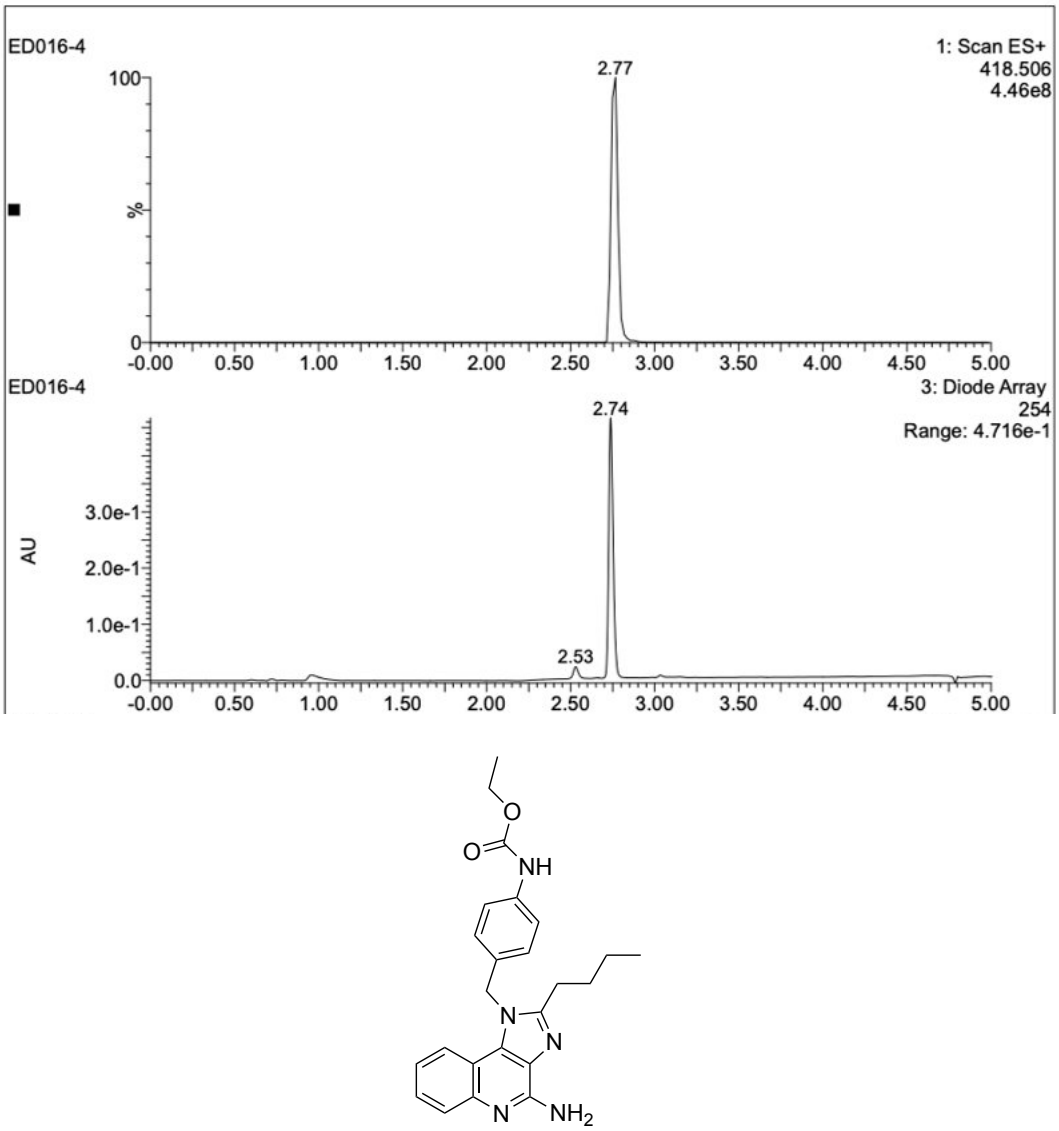
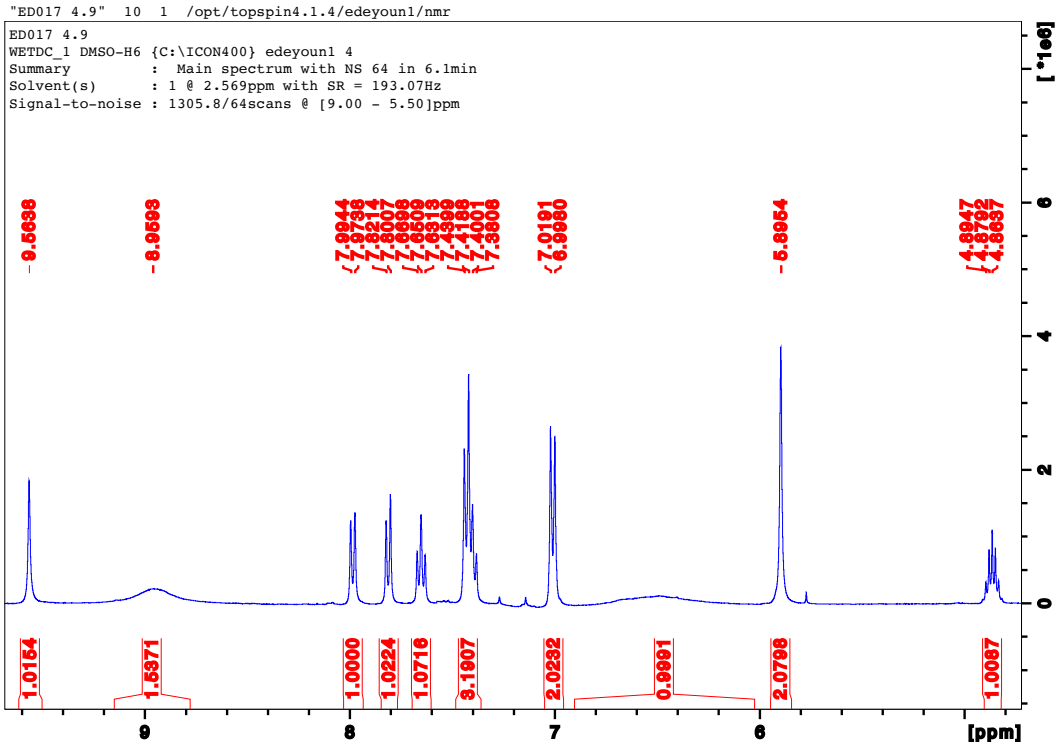
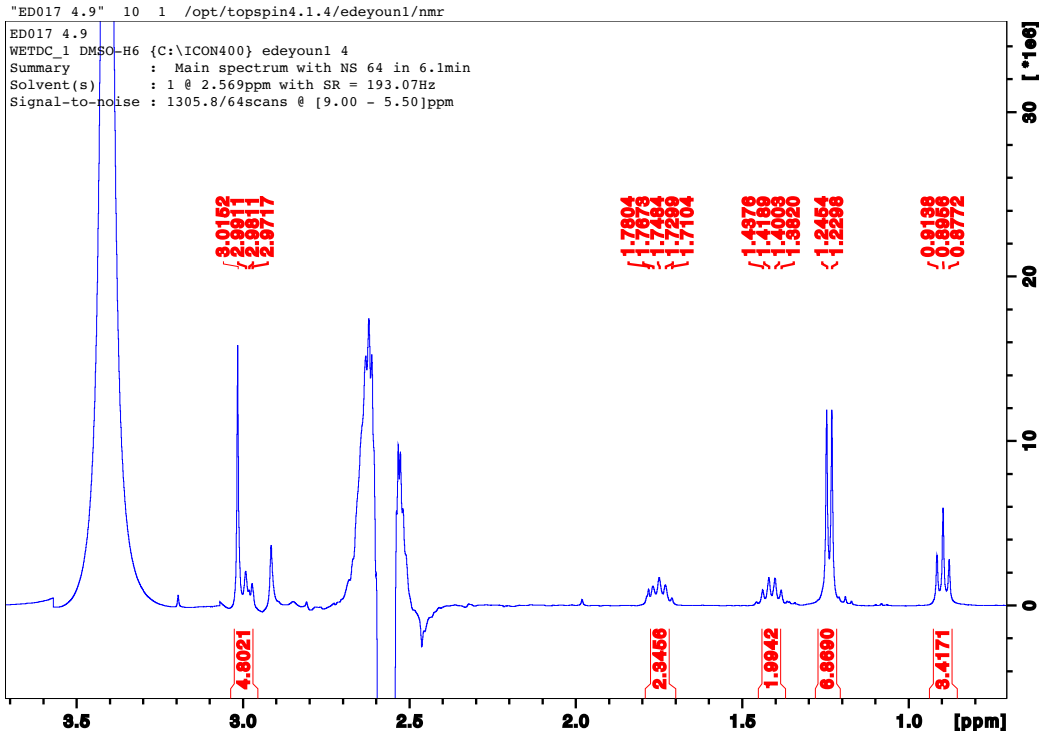


Figure S19: HPLC and NMR Characterization of Compound **12a,** MW = 418.5 [M+H]


"ED017 4.9" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED017 4.9
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 4
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.569ppm with SR = 193.07Hz
Signal-to-noise : 1305.8/64scans @ [9.00 - 5.50]ppm
[*1e6]
[ppm]


"ED017 4.9" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED017 4.9
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 4
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.569ppm with SR = 193.07Hz
Signal-to-noise : 1305.8/64scans @ [9.00 - 5.50]ppm
[*1e6]
[ppm]

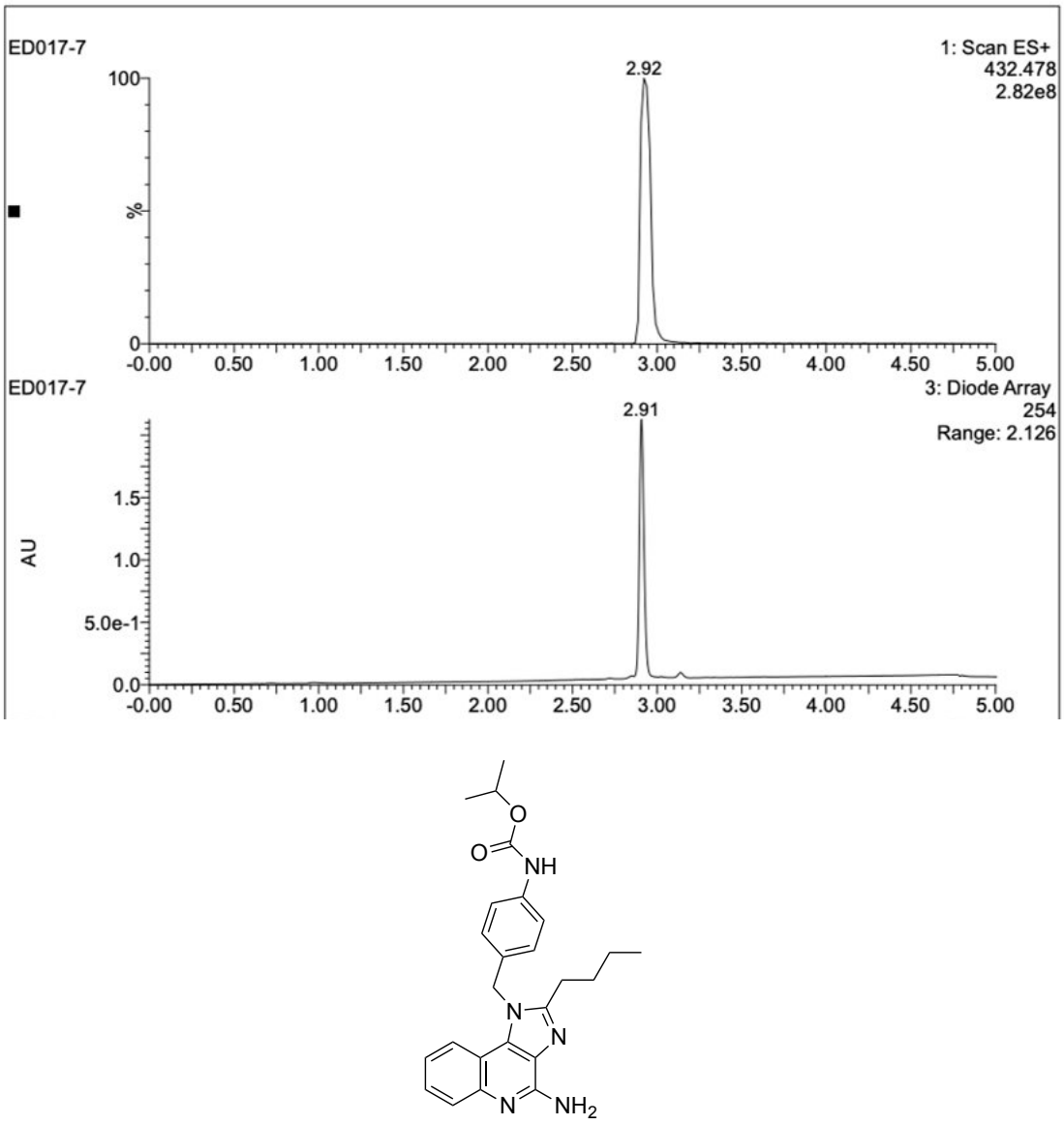
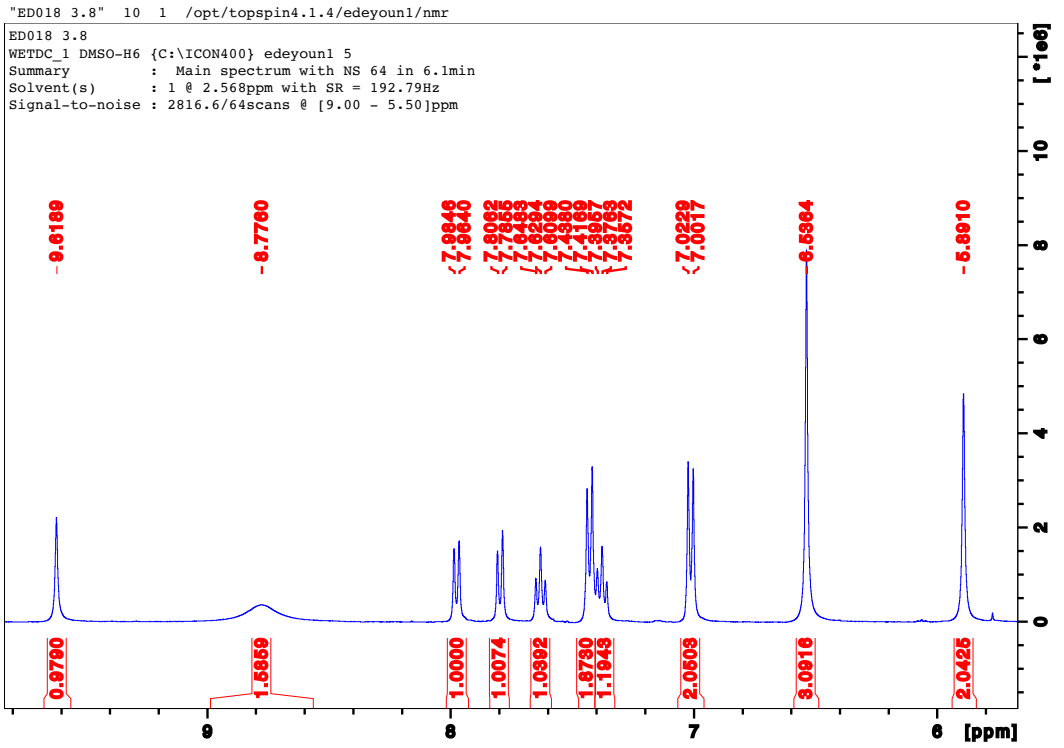
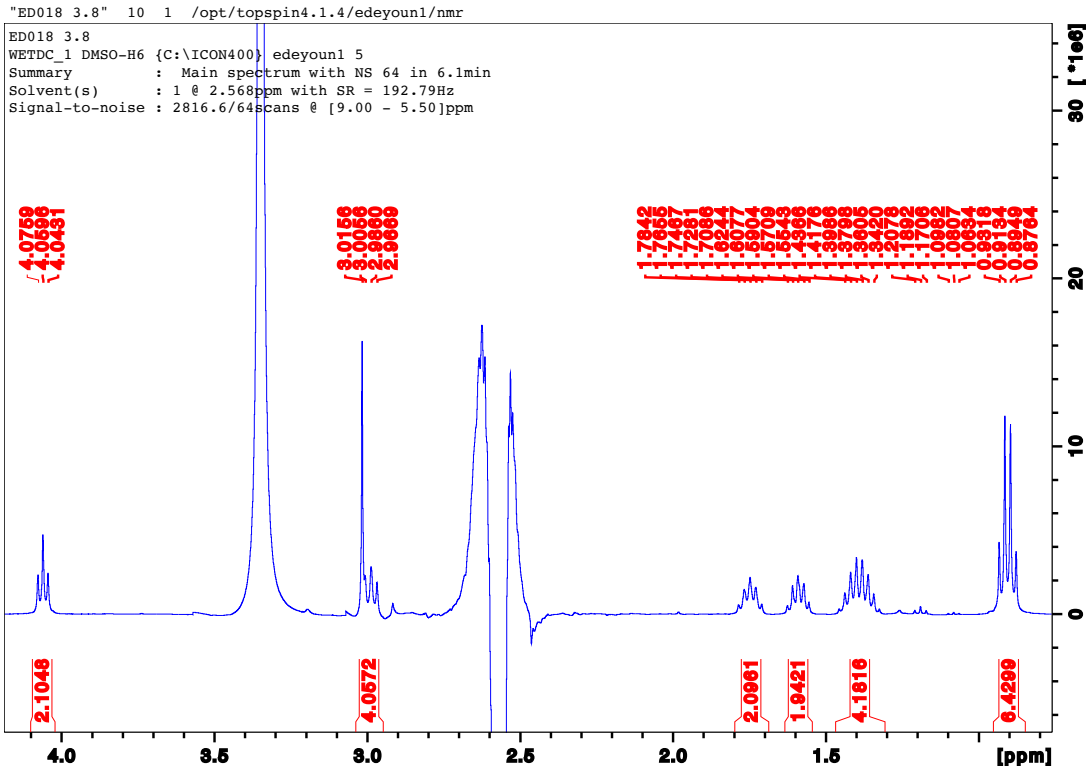


Figure S20: HPLC and NMR Characterization of Compound **12b,** MW = 432.5 [M+H]


"ED018 3.8" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED018 3.8
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 5
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 192.79Hz
Signal-to-noise : 2816.6/64scans @ [9.00 - 5.50]ppm
9.0180
8.7760
7.9846
7.9640
7.8062
7.7865
7.6483
7.6294
7.6099
7.4380
7.4169
7.3957
7.3763
7.3572
7.0229
7.0017
6.5364
5.8910
0.9790
1.5859
1.0000
1.0074
1.0392
1.8730
1.1943
2.0503
3.0916
2.0425
9
8
7
6
[ppm]
[*1e6]


"ED018 3.8" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED018 3.8
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 5
Summary : Main spectrum with NS 64 in 6.1min
Solvent(s) : 1 @ 2.568ppm with SR = 192.79Hz
Signal-to-noise : 2816.6/64scans @ [9.00 - 5.50]ppm
4.0759
4.0596
4.0431
3.0156
3.0066
2.9860
2.9669
1.7842
1.7656
1.7487
1.7281
1.7086
1.6244
1.6077
1.5904
1.5703
1.5543
1.4337
1.4178
1.3988
1.3798
1.3605
1.3420
1.2076
1.1892
1.1705
1.0983
1.0807
1.0669
0.9316
0.9134
0.8940
0.8764
2.1048
4.0672
2.0961
1.9421
4.1816
6.4299
4.0
3.5
3.0
2.5
2.0
1.5
[ppm]
[*1e6]

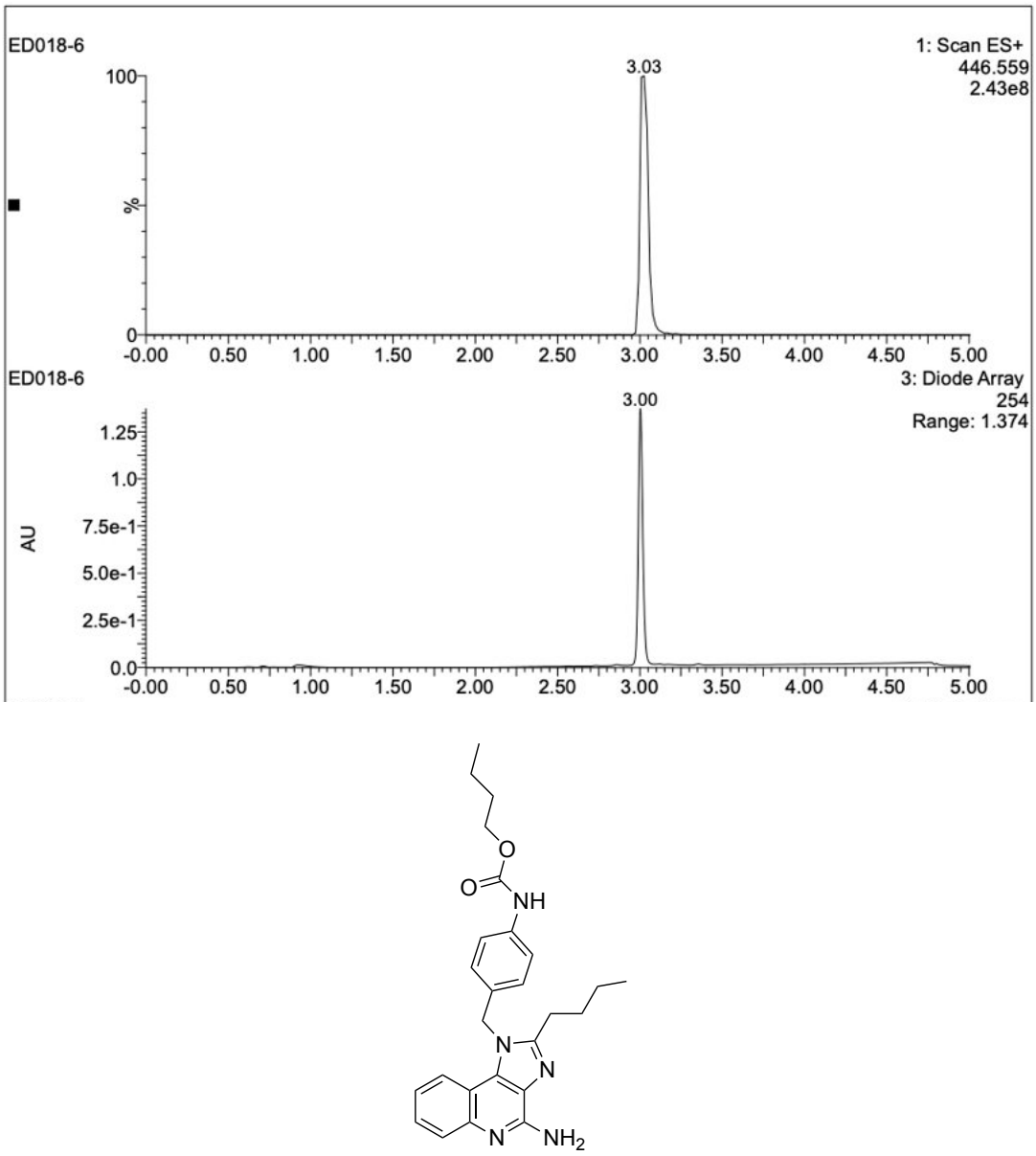
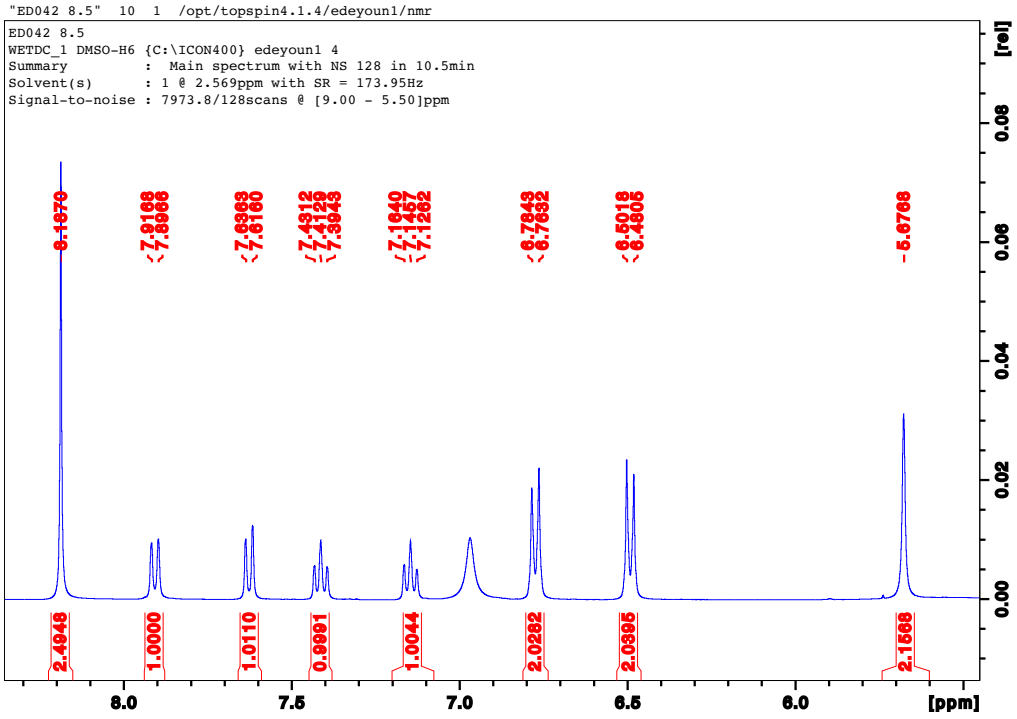
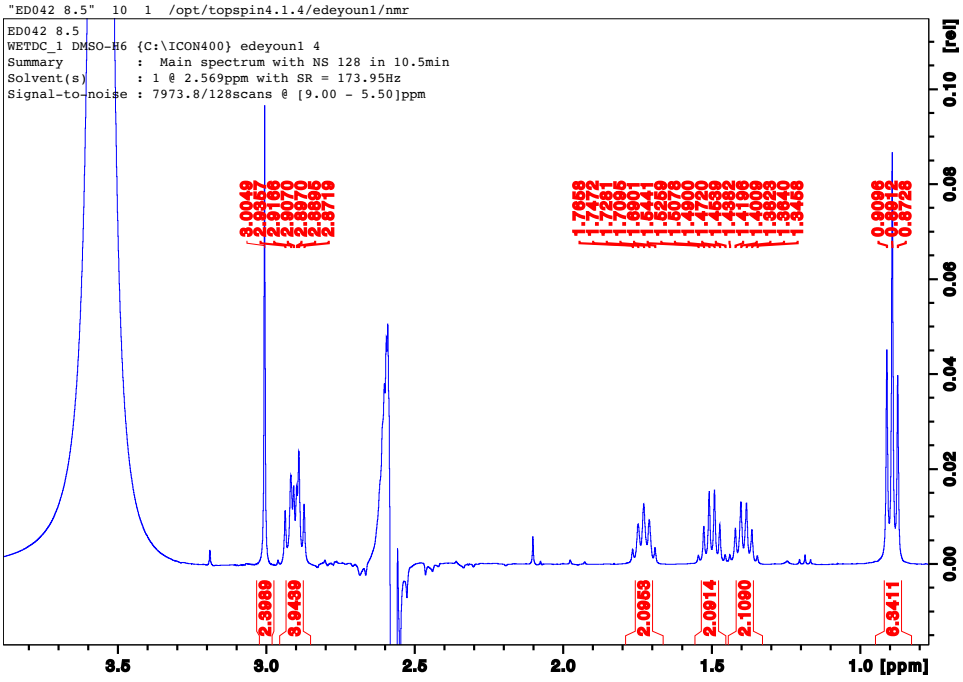


Figure S21: HPLC and NMR Characterization of Compound **12c,** MW = 446.6 [M+H]


"ED042 8.5" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED042 8.5
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 4
Summary : Main spectrum with NS 128 in 10.5min
Solvent(s) : 1 @ 2.569ppm with SR = 173.95Hz
Signal-to-noise : 7973.8/128scans @ [9.00 - 5.50]ppm
8.1670
7.9188
7.8988
7.6363
7.6160
7.4312
7.4129
7.3943
7.1640
7.1457
7.1262
6.7843
6.7632
6.5018
6.4806
5.0768
2.4946
1.0000
1.0110
0.9901
1.0044
2.0282
2.0395
2.1568
8.0
7.5
7.0
6.5
6.0
[ppm]
[rel]
0.00
0.02
0.04
0.06
0.08


"ED042 8.5" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED042 8.5
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 4
Summary : Main spectrum with NS 128 in 10.5min
Solvent(s) : 1 @ 2.569ppm with SR = 173.95Hz
Signal-to-noise : 7973.8/128scans @ [9.00 - 5.50]ppm
3.0049
3.0357
2.9166
2.9070
2.8895
2.8719
1.7658
1.7472
1.7281
1.7096
1.6901
1.6441
1.6259
1.6078
1.4900
1.4720
1.4539
1.4382
1.4196
1.4009
1.3829
1.3640
1.3468
0.9006
0.8912
0.8728
2.3989
3.9439
2.0053
2.0014
2.1000
6.3411
3.5
3.0
2.5
2.0
1.5
1.0 [ppm]
[rel]
0.00
0.02
0.04
0.06
0.08
0.10

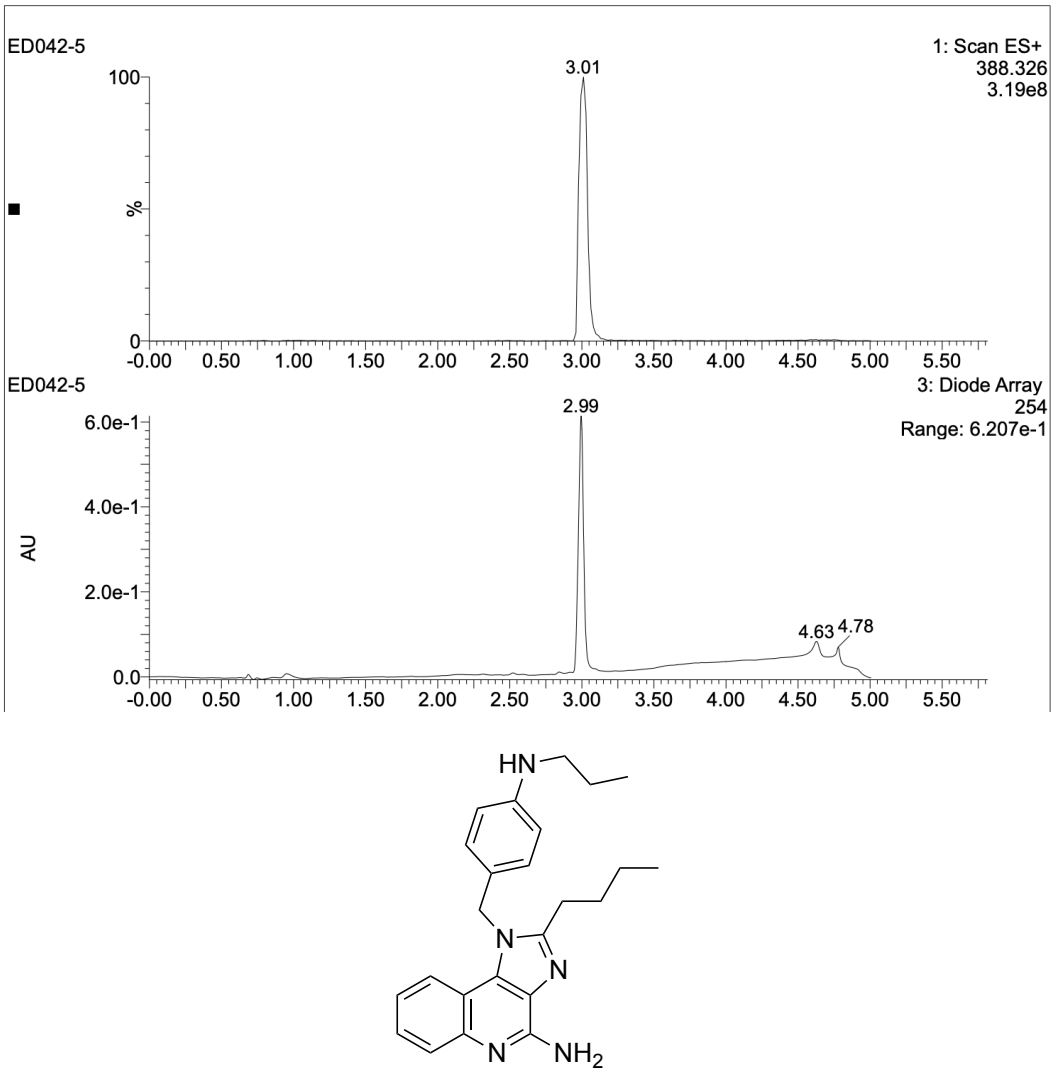
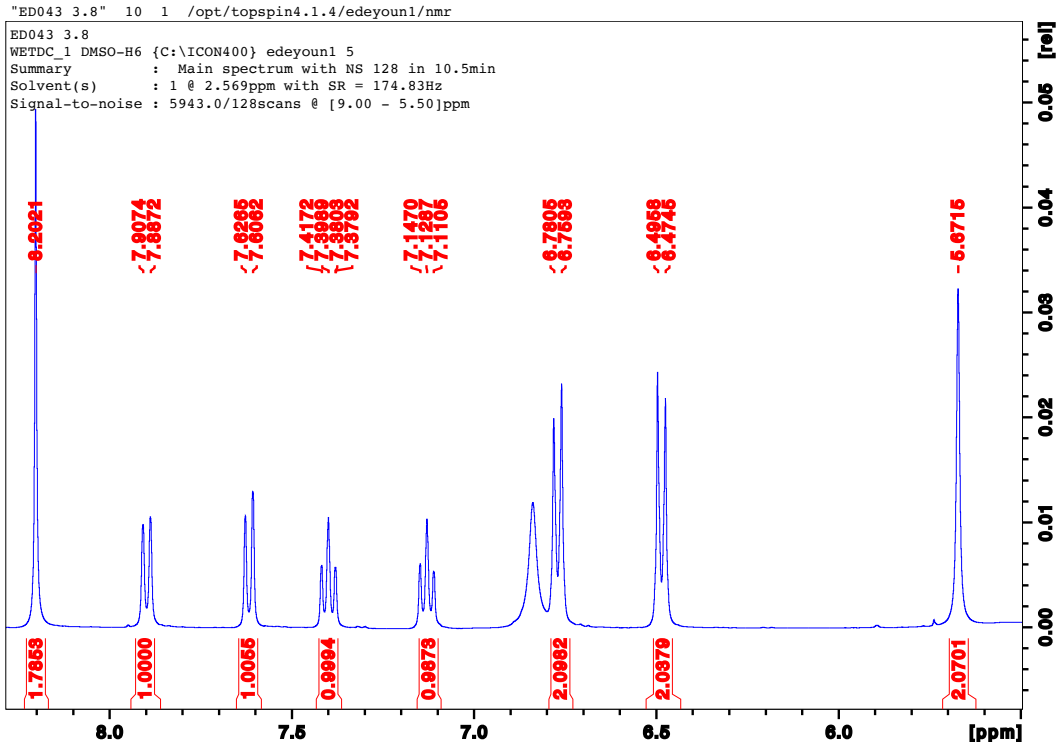
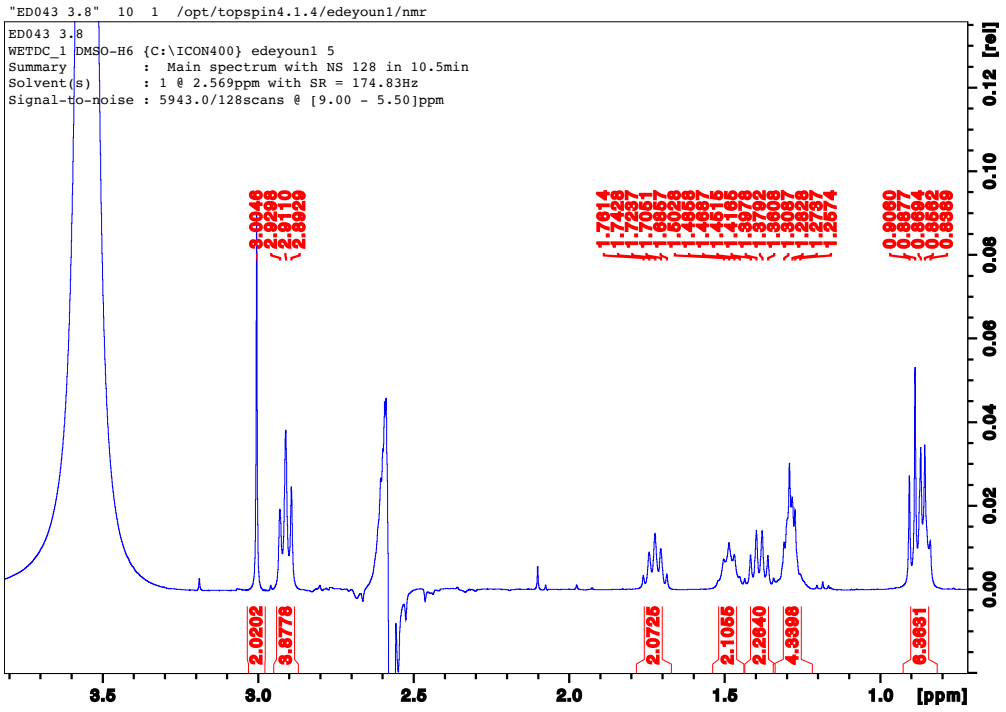


Figure S22: HPLC and NMR Characterization of Compound **13a,** MW = 388.3 [M+H]


"ED043 3.8" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED043 3.8
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 5
Summary : Main spectrum with NS 128 in 10.5min
Solvent(s) : 1 @ 2.569ppm with SR = 174.83Hz
Signal-to-noise : 5943.0/128scans @ [9.00 - 5.50]ppm
8.0
7.5
7.0
6.5
6.0
[ppm]
[rel]


"ED043 3.8" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED043 3.8
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 5
Summary : Main spectrum with NS 128 in 10.5min
Solvent(s) : 1 @ 2.569ppm with SR = 174.83Hz
Signal-to-noise : 5943.0/128scans @ [9.00 - 5.50]ppm
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]
[rel]

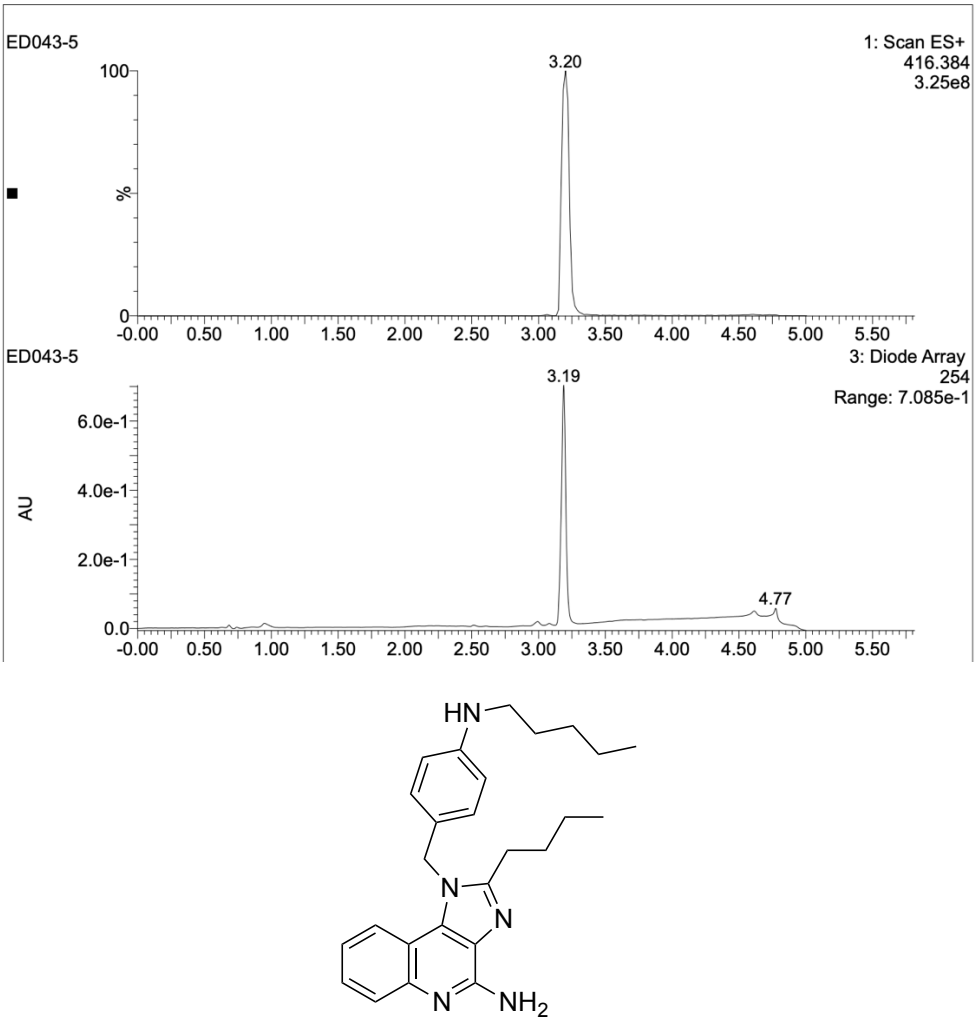
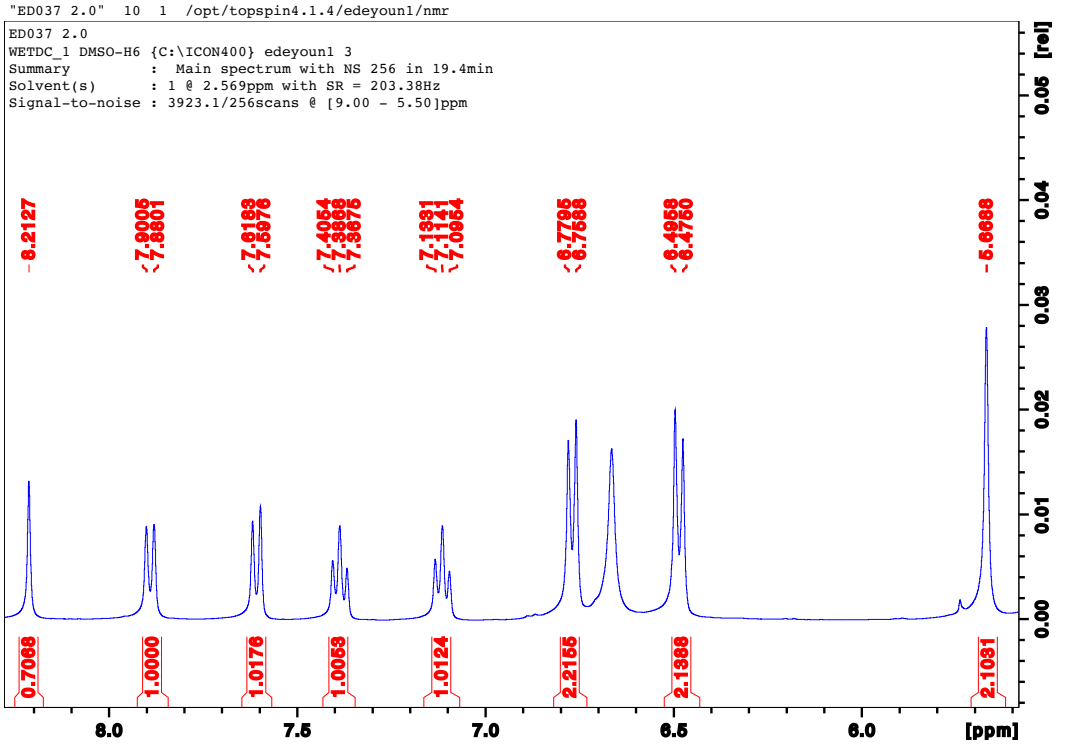
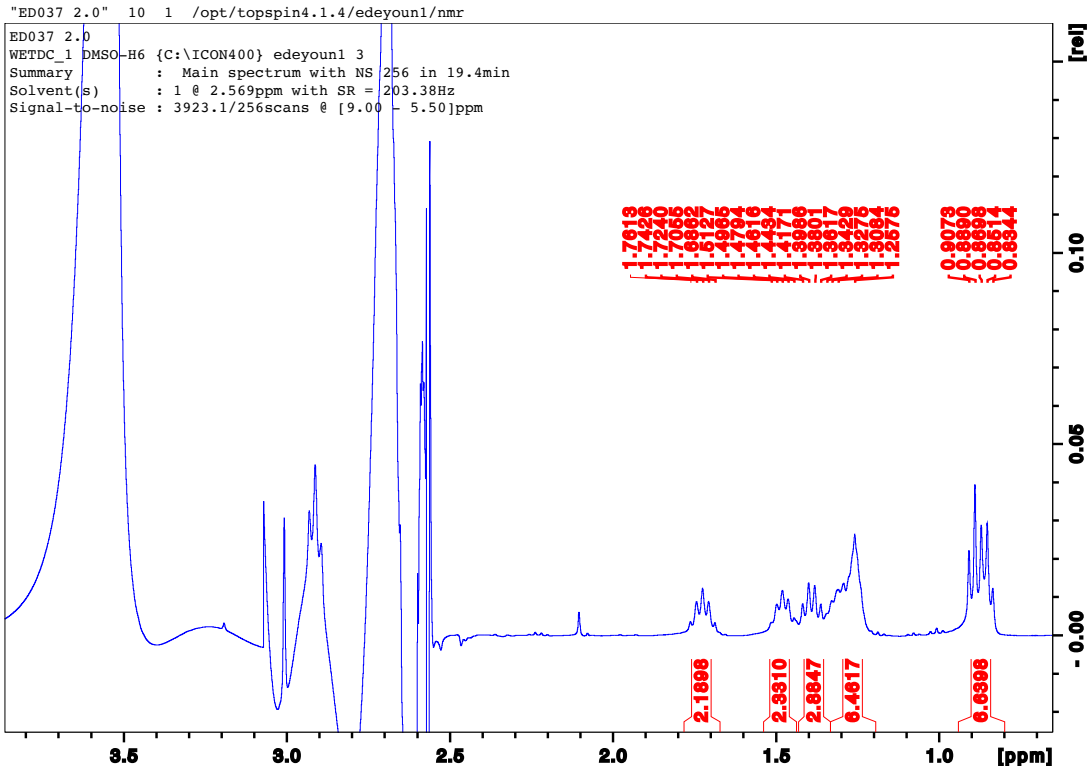


Figure S23: HPLC and NMR Characterization of Compound **13b,** MW = 416.4 [M+H]


"ED037 2.0" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED037 2.0
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 3
Summary : Main spectrum with NS 256 in 19.4min
Solvent(s) : 1 @ 2.569ppm with SR = 203.38Hz
Signal-to-noise : 3923.1/256scans @ [9.00 - 5.50]ppm
8.2127
7.9005
7.8801
7.6183
7.5978
7.4054
7.3868
7.3676
7.1331
7.1141
7.0964
6.7795
6.7688
6.4958
6.4760
5.6666
0.7068
1.0000
1.0176
1.0053
1.0124
2.2155
2.1388
2.1031
8.0
7.5
7.0
6.5
6.0
[ppm]
0.00
0.01
0.02
0.03
0.04
0.05
[rel]


"ED037 2.0" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED037 2.0
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 3
Summary : Main spectrum with NS 256 in 19.4min
Solvent(s) : 1 @ 2.569ppm with SR = 203.38Hz
Signal-to-noise : 3923.1/256scans @ [9.00 - 5.50]ppm
1.7613
1.7426
1.7240
1.7066
1.6862
1.5127
1.4965
1.4794
1.4616
1.4434
1.4171
1.3986
1.3801
1.3617
1.3429
1.3275
1.3084
1.2575
0.9073
0.8890
0.8696
0.8514
0.8344
2.1898
2.3310
2.8847
0.4617
0.6398
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]
- 0.00
0.05
0.10
[rel]

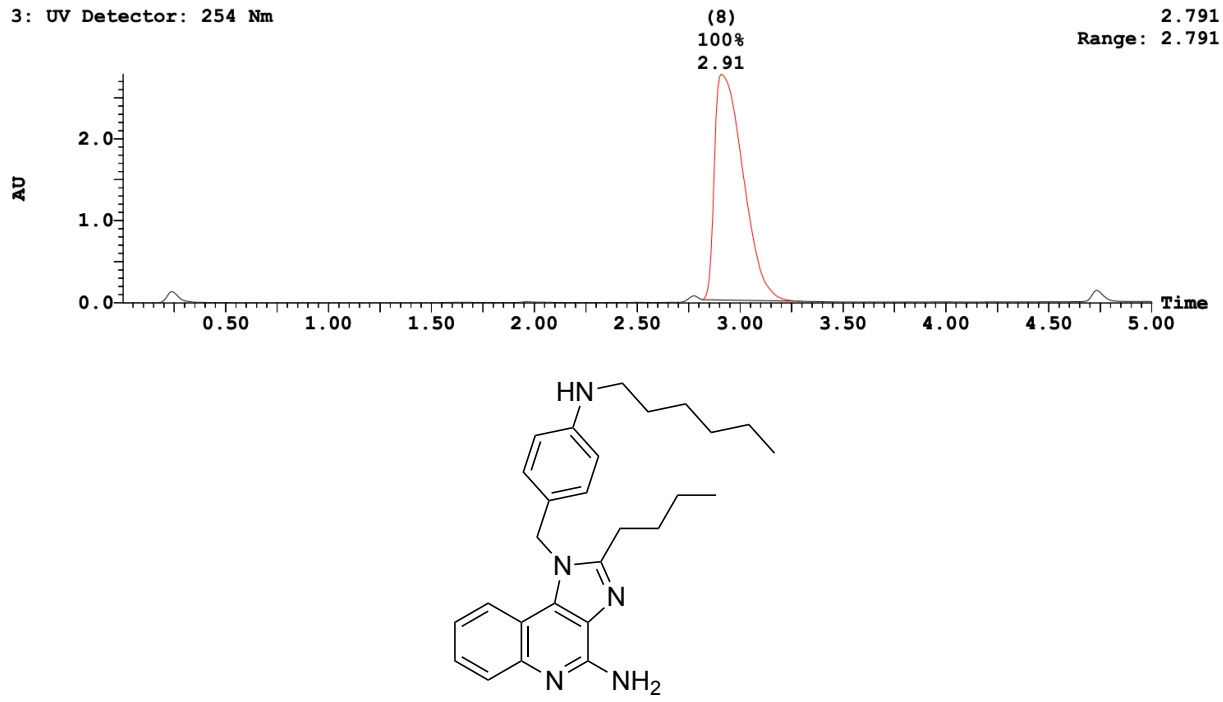
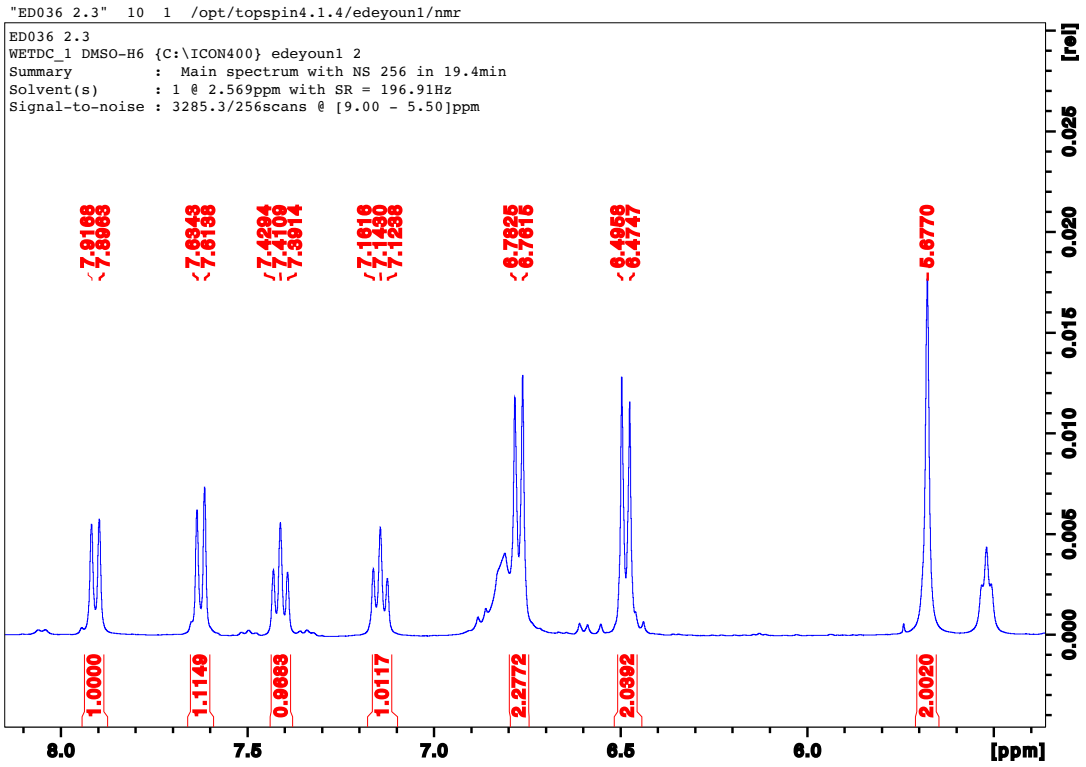
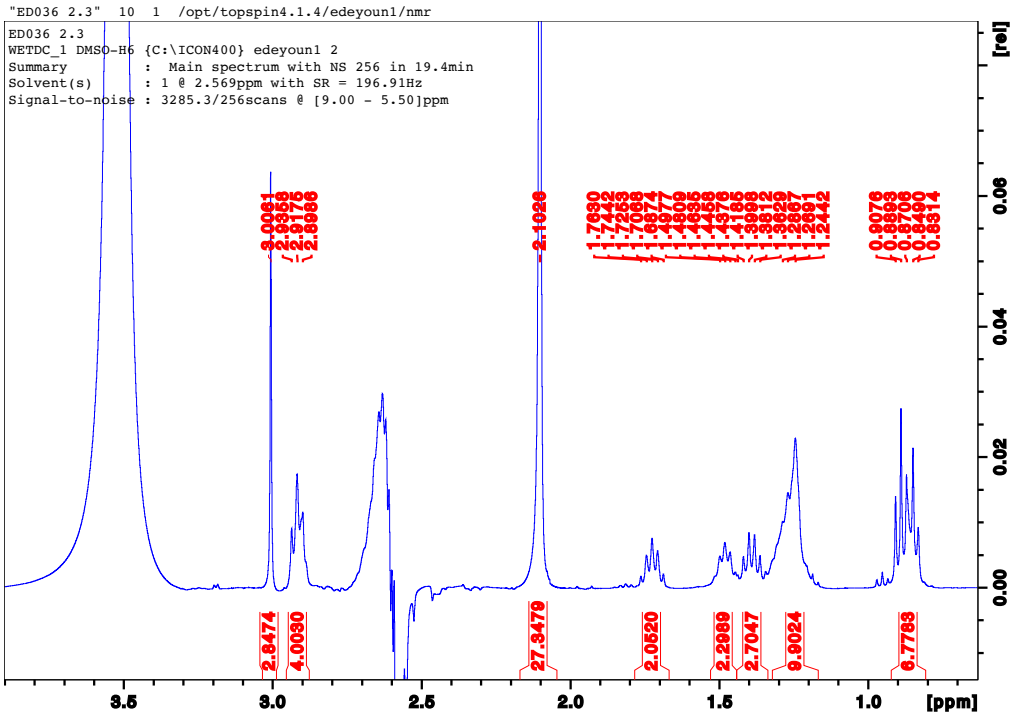


Figure S24: HPLC and NMR Characterization of Compound **13c,** MW = 429.6 [M+H]


"ED036 2.3" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED036 2.3
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 2
Summary : Main spectrum with NS 256 in 19.4min
Solvent(s) : 1 @ 2.569ppm with SR = 196.91Hz
Signal-to-noise : 3285.3/256scans @ [9.00 - 5.50]ppm
7.9168
7.8983
7.6343
7.6138
7.4294
7.4109
7.3914
7.1616
7.1430
7.1238
6.7825
6.7615
6.4956
6.4747
5.6770
1.0000
1.1140
0.9883
1.0117
2.2772
2.0392
2.0020
8.0
7.5
7.0
6.5
6.0
[ppm]
[rel]
0.025
0.020
0.015
0.010
0.005
0.000


"ED036 2.3" 10 1 /opt/topspin4.1.4/edeyoun1/nmr
ED036 2.3
WETDC_1 DMSO-H6 {C:\ICON400} edeyoun1 2
Summary : Main spectrum with NS 256 in 19.4min
Solvent(s) : 1 @ 2.569ppm with SR = 196.91Hz
Signal-to-noise : 3285.3/256scans @ [9.00 - 5.50]ppm
3.0061
2.9358
2.9175
2.8986
2.1020
1.7630
1.7443
1.7253
1.7058
1.6877
1.4809
1.4636
1.4458
1.4376
1.4185
1.3998
1.3812
1.3629
1.2867
1.2691
1.2442
0.8876
0.8693
0.8706
0.8490
0.8314
2.8474
4.0030
27.3479
2.0520
2.2980
2.7047
9.9024
6.7783
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]
[rel]
0.08
0.04
0.02
0.00

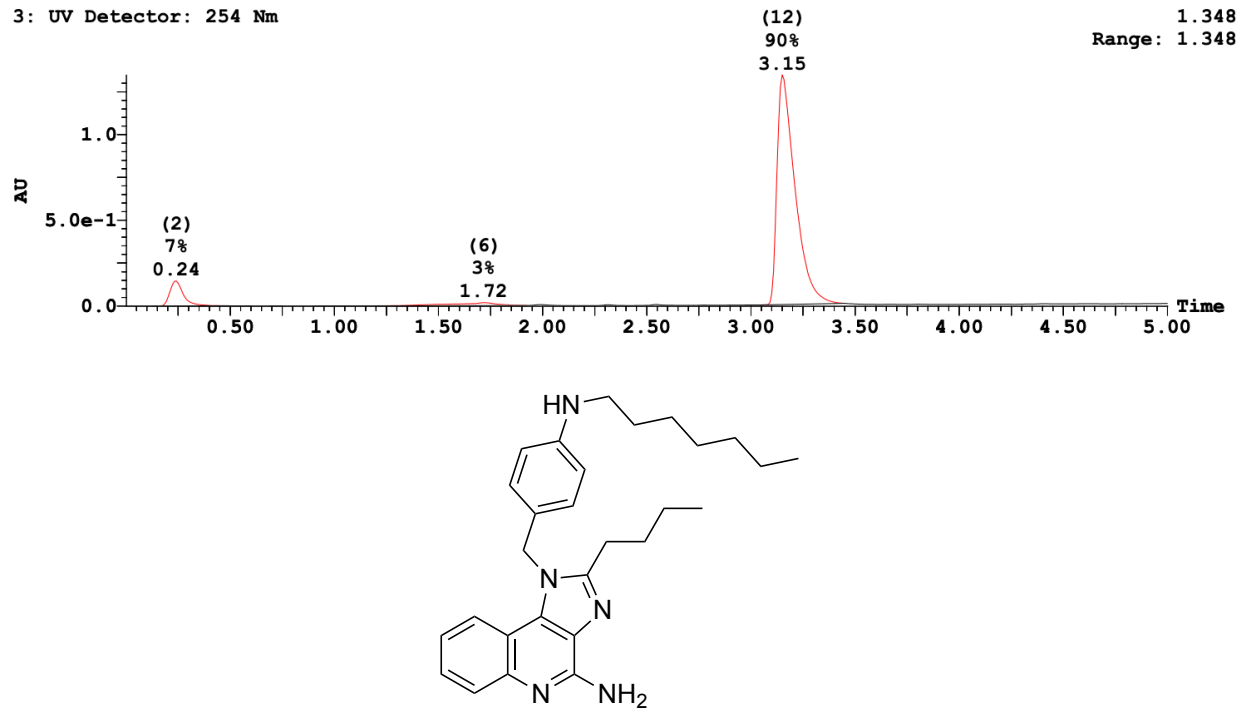


Figure S25: HPLC and NMR Characterization of Compound **13d,** MW = 443.6 [M+H]

11.2 Fragmentation Patterns

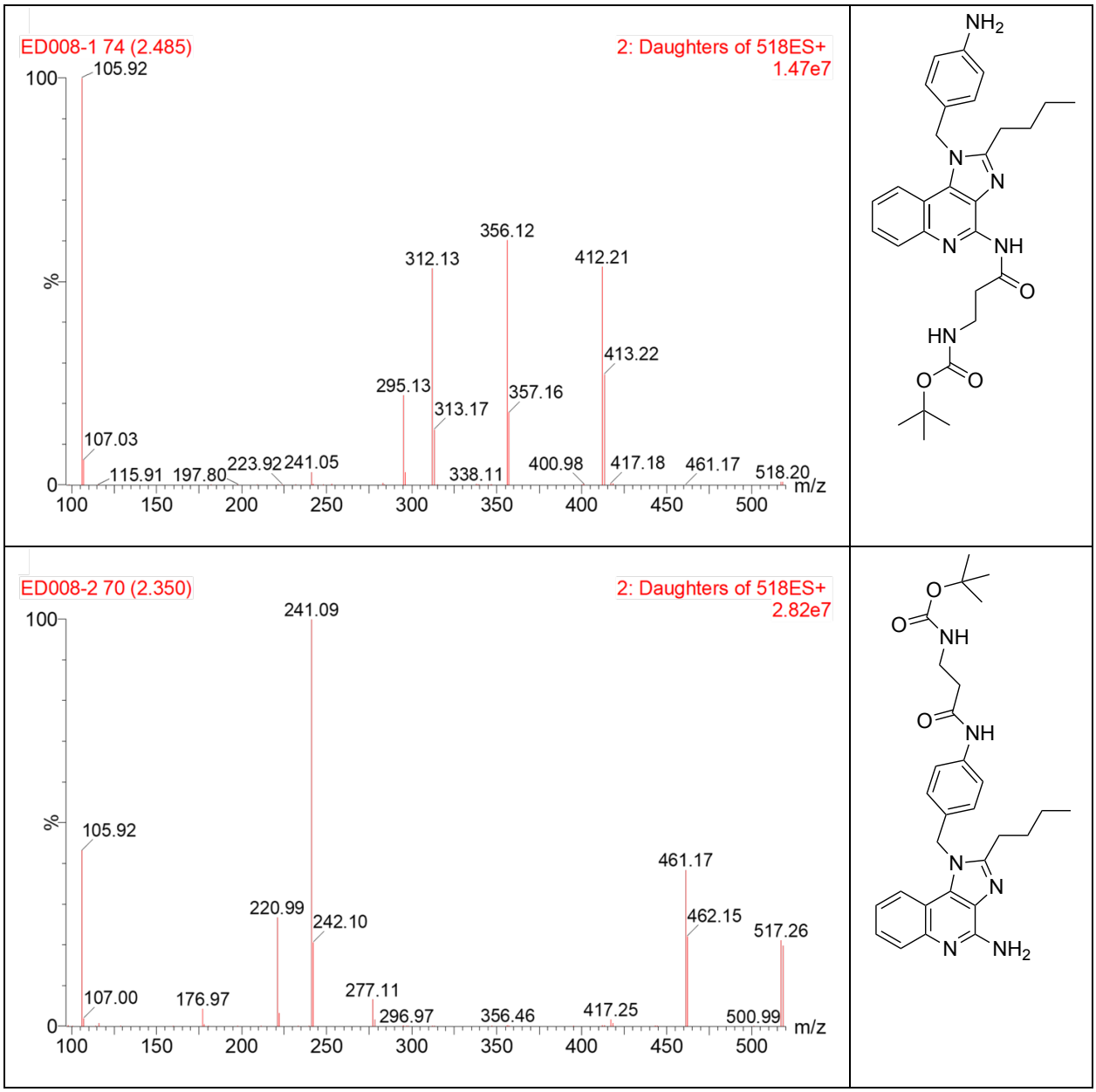


Figure S26: Fragmentation Patterns of Compound **9b** with the desired isomer shown on the bottom.

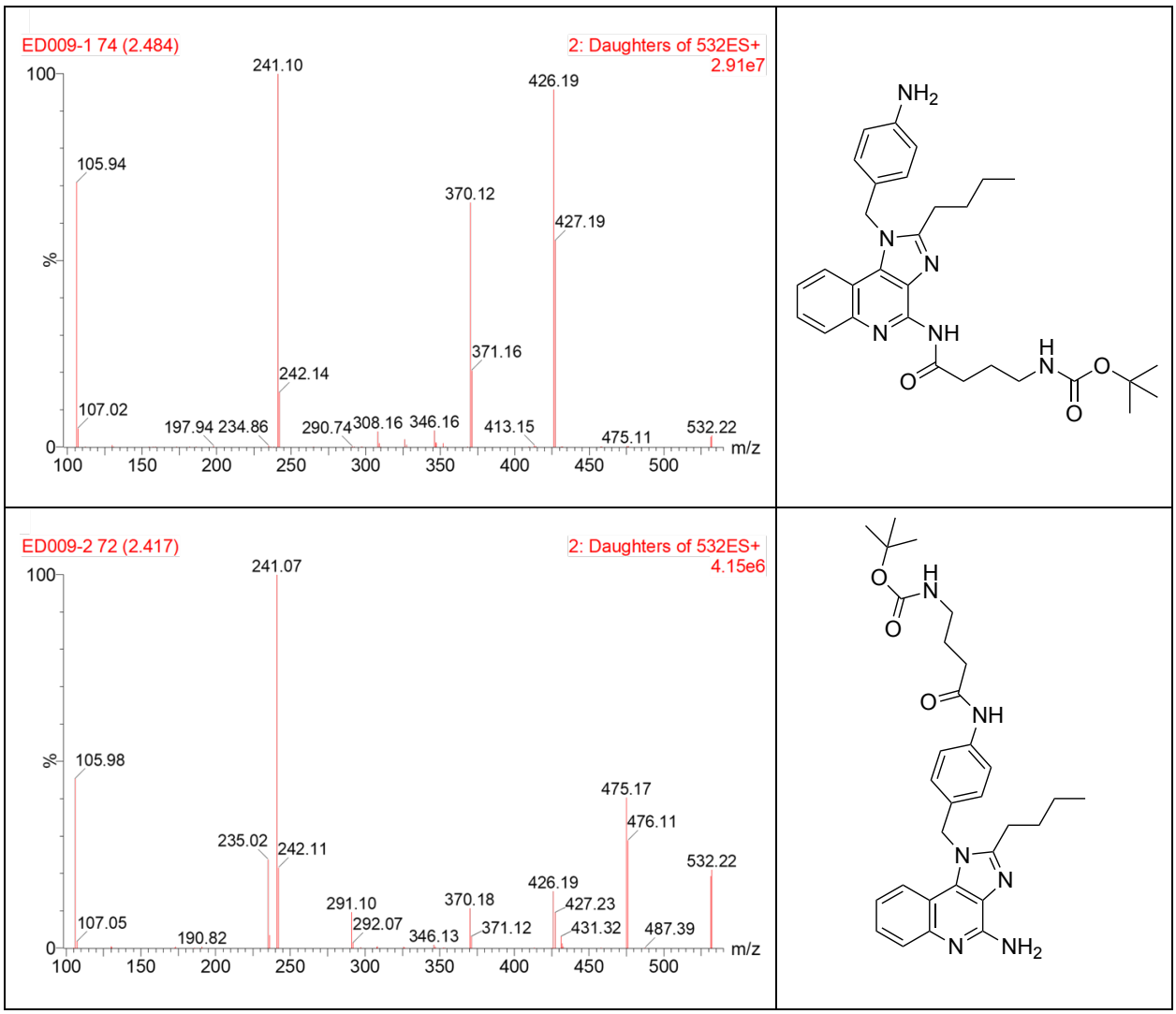


Figure S27: Fragmentation Patterns of Compound **9c** with the desired isomer shown on the bottom.

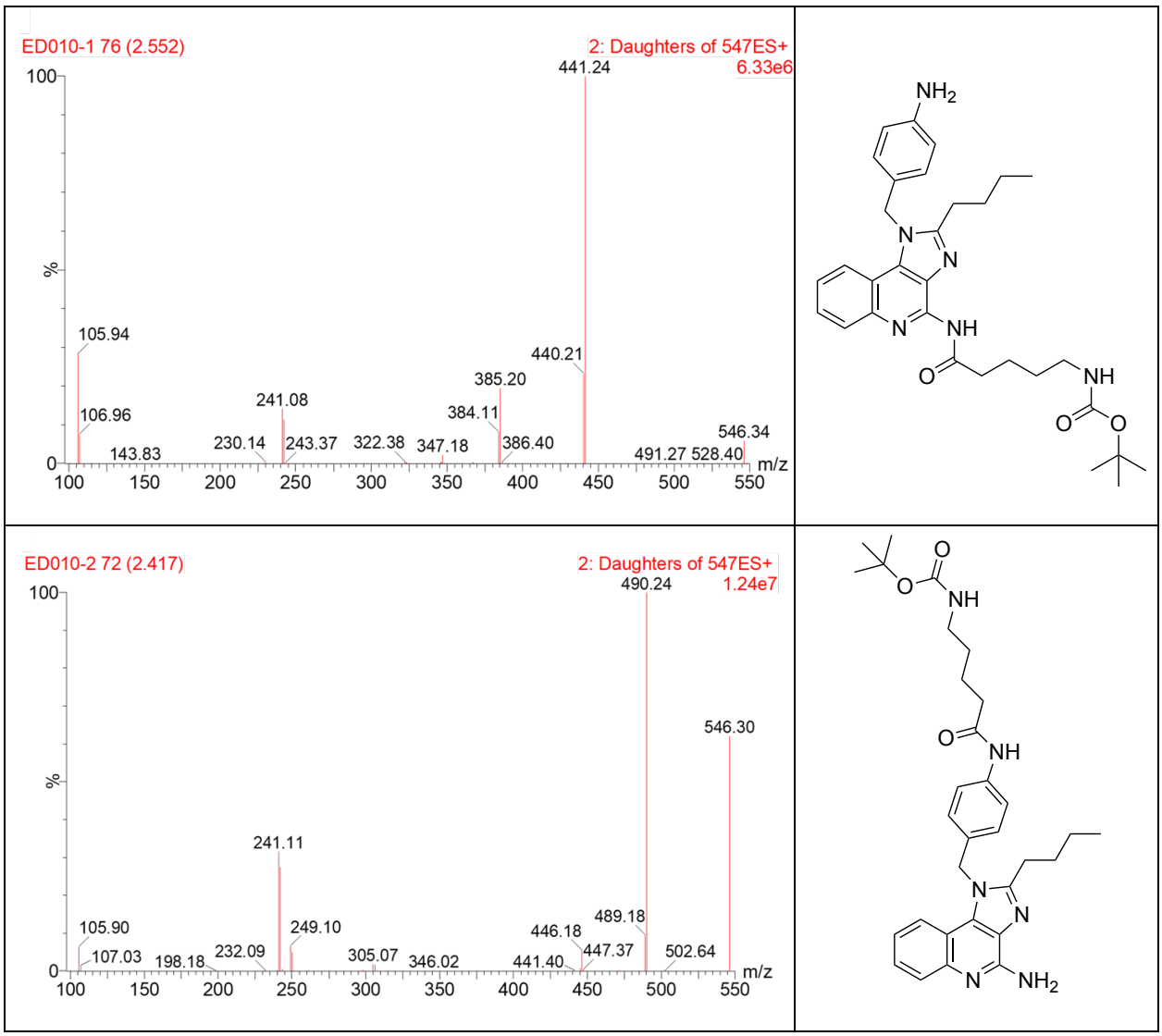


Figure S28: Fragmentation Patterns of Compound **9d** with the desired isomer shown on the bottom.

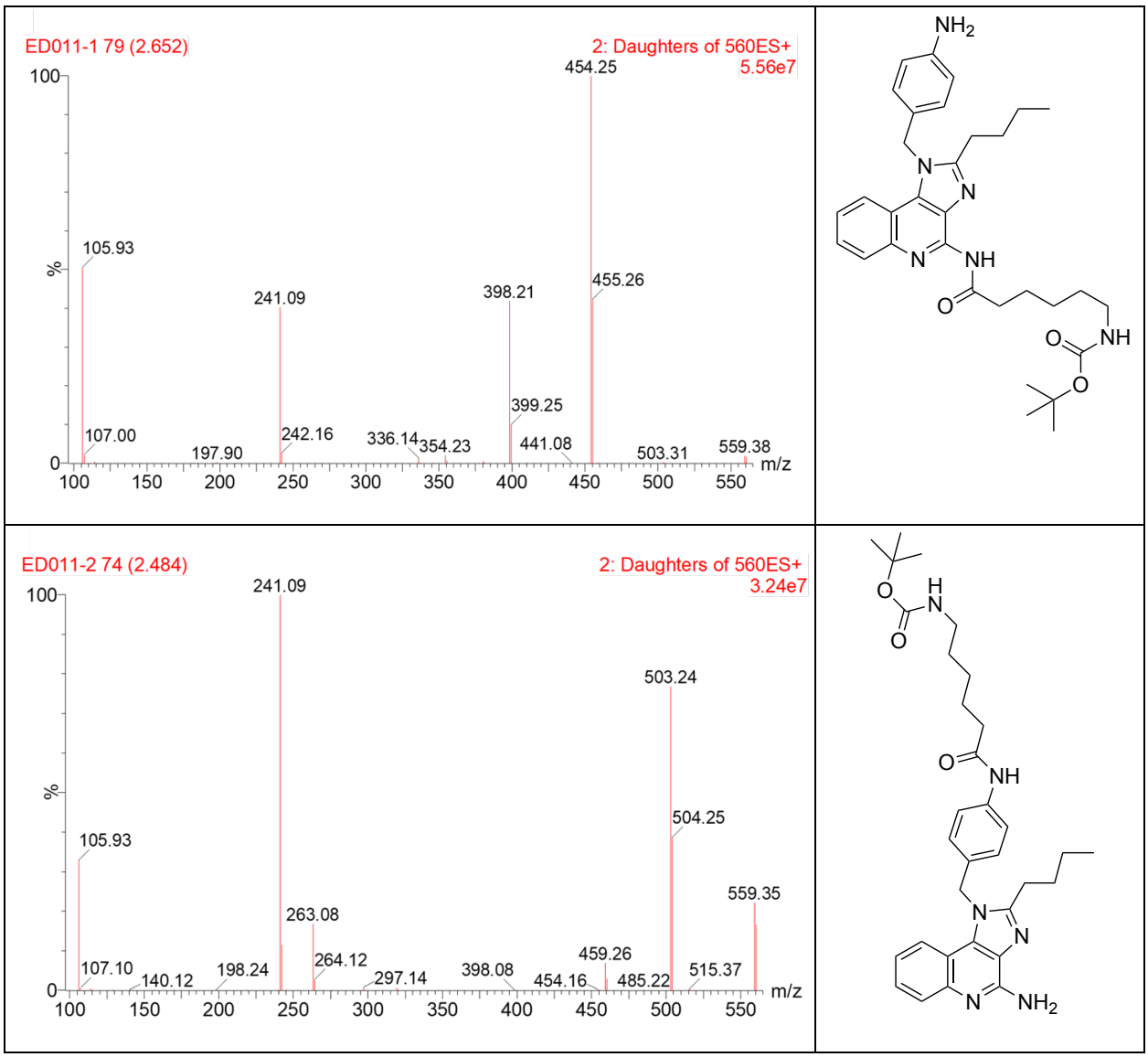


Figure S29: Fragmentation Patterns of Compound **9e** with the desired isomer shown on the bottom.

12. Acknowledgements

Firstly, I would like to thank Dr. Tumey for all the years of support and mentoring throughout my undergraduate research career. I would also like to thank my committee members for being willing to provide me with constructive criticism and feedback to further expand my knowledge and abilities as an independent researcher. I would also like to thank my research lab for being the best of friends and coworkers I could have asked for and helping me develop my passion for research. I would also like to thank Binghamton University and the SUNY Research Foundation for the bountiful opportunities and funding.

13. References

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