

Design and Synthesis of Selective Modulators for N3-Subunit-Containing NMDA Receptors

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Design och syntes av selektiva modulatorer för N3-subenhetsinnehållande NMDA-receptorer

För kemister världen över är neurologiska och psykiatriska sjukdomar viktiga forskningsområden. Den ökande efterfrågan på läkemedel som kan hjälpa mot dessa sjukdomar är en växande utmaning. Därför har vi i detta projekt valt att arbeta med en typ av receptor i hjärnan som är involverad i många av dessa sjukdomar, nämligen NMDA-receptorer. Man kan jämföra NMDA-receptorer med de tre skandinaviska språken, svenska, danska och norska. Trots de många likheterna finns det distinkta skillnader som utmärker de tre språken. På samma sätt kan de olika NMDA-receptorerna, GLuN1, GLuN2 och GLuN3, beskrivas dela grund men ändå har distinkta egenskaper som utmärker var och en. Detta arbete kommer att fokusera specifikt på GluN3-subtypen av NMDA-receptorn. I vår roll som kemister har vi för avsikt att designa och syntetisera molekyler som kan hjälpa oss att förstå hur NMDA-receptorer bidrar till utvecklingen av neurologiska och psykiatriska sjukdomar.

Design av molekyler är som skapandet av nya maträtter. Man har ingredienserna och tidigare recept, vilket i detta fall speglar tidigare forskning om GluN3-subtypen. Med dessa komponenter kan man börja experimentera utifrån vad som har gjorts tidigare och vad som kan modifieras för att göra rätten unik. I detta projekt har därmed 19 nya molekyler skapats för att selektivt rikta sig mot GluN3 NMDA-receptorn, med syftet att få en bättre förståelse för dess funktion i kroppen och förhoppningsvis bidra till framtida behandling av relaterade sjukdomar.

Abstract

NMDA receptors are ligand-gated ion channels assembled as heterotetramers composed of glycine-binding GluN1 and GluN3 subunits (GluN3A-B), and glutamate-binding GluN2 subunits (GluN2A-D). Their involvement in various psychiatric and neurological disorders makes them potential therapeutic targets. However, compared to the extensively studied GluN1/2 NMDARs, the functional, physiological, and pharmacological characteristics of GluN1/3 NMDARs remain poorly understood. Here, we describe the design and synthesis of a series of analogues derived from two previously identified allosteric modulators (**A** and **C**) developed in the Rasmus group, followed by two novel competitive antagonists derived from the previously reported compounds, **DCKA** and **MDL-104,653**, to target GluN3-containing NMDA receptors. Different synthetic routes have been developed to efficiently synthesise the allosteric modulators and competitive antagonists, with varying degrees of difficulty regarding chemo- and regioselectivity. Further studies to evaluate the pharmacological activity of the 19 novel analogues are still ongoing.

Keywords:

Allosteric modulator

Competitive antagonist

GluN3 subunit

NMDA receptor

Acknowledgement

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List of abbreviations

ABD	Agonist binding domain
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
Aq.	Aqueous
ATD	Amino-terminal domain
BFMO	<i>N</i> ¹ , <i>N</i> ² -Bis(furan-2-ylmethyl)oxalamide
Bu ₄ N ⁺ SCN ⁻	Tetrabutylammonium thiocyanate
CNS	Central nervous system
CTD	Carboxy-terminal domain
DCE	1,2-Dichloroethane
DMAD	Dimethyl acetylenedicarboxylate
DMF	Dimethylformamide
DMG	N,N-Dimethylglycine
DMSO	Dimethyl sulfoxide
EAS	Electrophilic aromatic substitution
EC ₅₀	Half maximal effective concentration
EDG	Electron-donating group
Equiv.	Equivalent
EtOH	Ethanol
EWG	Electron-withdrawing group
HRMS	High-resolution mass spectrometry
Hz	Hertz
<i>i</i> -AmylONO	Isoamyl nitrite
iGluRs	Ionotropic glutamate receptors
<i>i</i> -PrOH	Isopropanol
LBDs	Ligand-binding domains
LCMS	Liquid chromatography mass spectrometry
LiHMDS	Lithium bis(trimethylsilyl)amide
MeCN	Acetonitrile
MeOH	Methanol
MTBE	Methyl <i>tert</i> -butyl ether
NBS	N-Bromosuccinimide

NMR	Nuclear Magnetic Resonance
NMDA	<i>N</i> -Methyl- <i>D</i> -aspartic acid
o/n	Overnight
PhMe	Toluene
Ph ₂ C=NNH ₂	Benzophenone hydrazone
PIFA	[Bis(trifluoroacetoxy)iodo]benzene
ppm	Parts per million
PTBP	Para- <i>tert</i> -butylphenol
Prep. HPLC	Preparative high-performance liquid chromatography
rt	Room temperature
S _N Ar	Nucleophilic aromatic substitution
TBDPSCl	<i>tert</i> -Butyldiphenylsilyl chloride
TEA	Triethylamine
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
THMD	2,2,6,6-Tetramethyl-3,5-heptanedione
TMD	Transmembrane domain

1. Introduction

N-Methyl-*D*-aspartic acid (NMDA) receptors are ionotropic glutamate receptors (iGluR) with high calcium permeability that are highly expressed throughout the central nervous system (CNS) and are involved in various physiological processes, including cognition, learning, and memory [1]. Dysfunction of NMDA receptors is involved in various neurological and psychiatric disorders, including stroke, Alzheimer's disease, Parkinson's disease, pathological pain, schizophrenia and depression [2-4]. All known functional NMDA receptors are assembled as heterotetramers composed of two GluN1 subunits together with either two GluN2 (GluN2A-D) subunits or a combination of GluN2 and GluN3 (GluN3A-B) subunits [5, 6]. Activation of GluN1/GluN2 NMDA receptors requires two molecules of glycine or D-serine and two molecules of L-glutamate, the major excitatory neurotransmitter in the central nervous system (CNS). Meanwhile, GluN1/GluN3 NMDA receptors are activated by the binding of four molecules of glycine or D-serine [6, 7]. Among the various NMDA receptor subtypes, the glycine-binding GluN1/GluN3 assemblies exhibit unique physiological, trafficking, and signalling properties compared to the classical, well-characterized GluN1/GluN2 subtypes. Their differences have promoted increasing research interest in recent years to investigate their role in synaptic plasticity, neuroprotection, and various neurological and psychiatric disorders [8, 9].

1.1 Background

The study of ionotropic glutamate receptors (iGluRs) has remained a central focus of neuroscience research for decades [2, 10, 11]. iGluRs are ligand-gated, cation-permeable ion channel receptors that are categorized into four structurally distinct subfamilies: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, Delta (GluD) receptors, kainate receptors, and *N*-methyl-*D*-aspartate (NMDA) receptors [2]. Within the ionotropic glutamate receptor family, NMDA receptors are characterized by several unique properties, including a voltage-dependent block by extracellular Mg^{2+} , high permeability to Ca^{2+} , and the requirement for co-agonists binding specifically, the simultaneous presence of L-glutamate and either glycine or D-serine, for channel activation [6]. *N*-methyl-*D*-aspartate (NMDA) receptors are tetrameric ion channel complexes composed of four subunits, typically containing at least one obligatory GluN1 subunit in combination with GluN2 (A-D) and/or GluN3 (A-B) subunits [9].

Every NMDA receptor subunit is composed of four distinct domains, ATD, ABD, TMD, and CTD (Figure 1). The extracellular amino-terminal domain (ATD) is important for subunit interactions and is key in forming binding pockets for allosteric modulators. The agonist-binding domain (ABD), composed of two polypeptide segments (S1 and S2), folds into a bilobed, kidney-shaped structure with an upper lobe (D1) and a lower lobe (D2), where L-glutamate or glycine (or D-serine) binds to initiate the receptor activation. The transmembrane domain (TMD), comprising three membrane-spanning helices (M1, M3, and M4) and a membrane-reentrant loop (M2), is responsible for forming and shaping the ion-conducting pore of the receptor. Finally, the intracellular carboxy-terminal domain (CTD) is involved in receptor trafficking and links the receptor to intracellular signalling cascades [2, 7, 10]. At resting membrane potential, the NMDA receptor channels are blocked by extracellular Mg^{2+} in a voltage-dependent manner. Upon postsynaptic depolarization, this block is relieved, allowing Ca^{2+} influx [2].

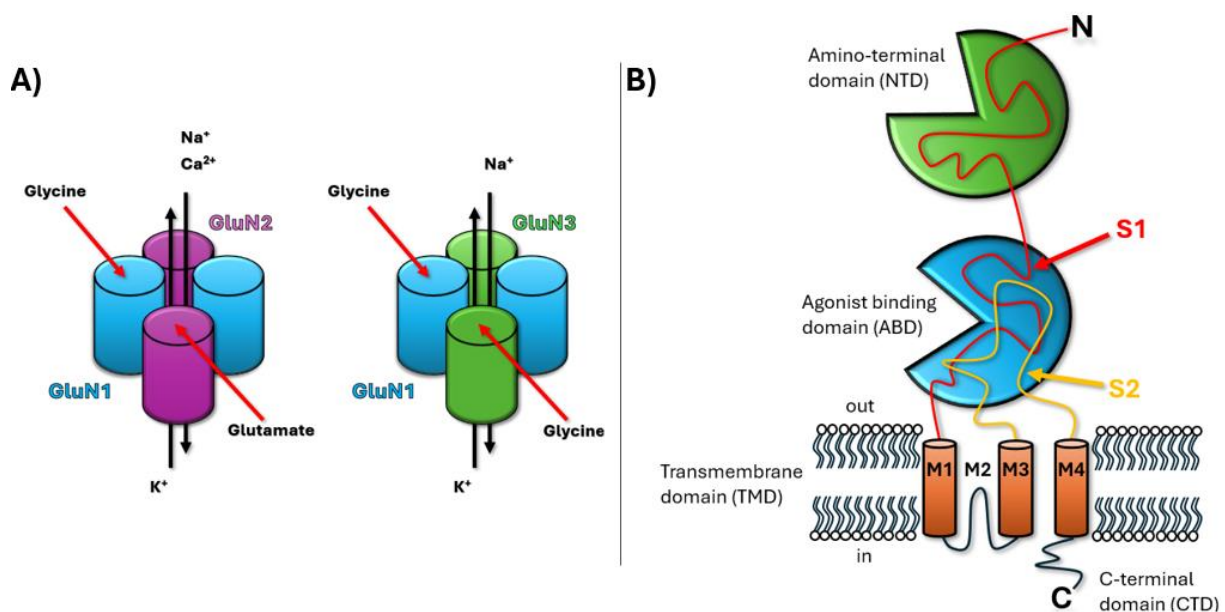
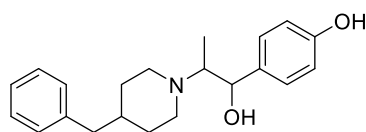


Figure 1. (A) Cartoon representation of subunit stoichiometry and subunit arrangement of NMDA receptors, shown as a tetrameric dimer-dimer complex. The alternating subunit composition is indicated by colour (Blue = GluN1, Purple = GluN2, Green = GluN3) [7]. (B) Cartoon representation of the domain architecture of NMDA receptors [12].

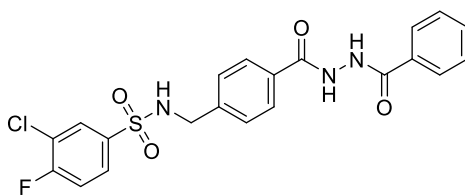
The GluN1/2 NMDARs, referred to as classical NMDARs, are defined by their high calcium permeability and their requirement for the simultaneous binding of two co-agonists, L-glutamate and glycine (or D-serine). Over the past three decades, these NMDARs have been extensively investigated and characterized regarding their functional diversity and signalling

properties [8, 13-15]. In contrast, relatively little is known about the glycine-binding subunits GluN3A and GluN3B. Accordingly, the past decade has seen a growing body of research aimed at elucidating their functional roles. GluN3-containing NMDARs are insensitive to the voltage-dependent Mg^{2+} block and display reduced Ca^{2+} permeability compared to classical NMDARs. Furthermore, depending on their subunit composition, GluN3-containing NMDA receptors can function either as glutamate/glycine-activated triheteromeric receptors composed of GluN1, GluN2, and GluN3 subunits or glycine-activated diheteromeric receptors composed of GluN1 and GluN3 subunits [7-10, 16]. Notably, these NMDARs only require the glycine (or D-serine) as agonist for activation [8, 17]. However, glycine-evoked currents mediated by GluN1/GluN3 receptors are relatively weak and challenging to measure accurately, which hinders the investigation of these receptors [16, 18].

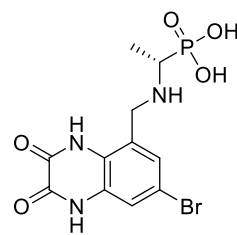
Several subunit-selective allosteric modulators and competitive antagonists have been developed to target GluN1/GluN2 NMDA receptors, including ifenprodil, a GluN2B-selective negative allosteric modulator [19], TCN-201, a GluN2A-selective antagonist [20], GNE-0723, a GluN2A-selective positive allosteric modulator [21], and glycine site competitive antagonists targeting GluN1, such as DCKA, CGP-78608 and MDL-104,653 [22-24]. In comparison, selective compounds targeting the glycine-binding subunits GluN3A and GluN3B remain largely unexplored [9]. Therefore, the development of selective small molecules targeting GluN3-containing NMDARs is crucial, as it holds the potential to provide important insights into the functional diversity and signalling mechanisms of these receptors in the context of different CNS disorders.



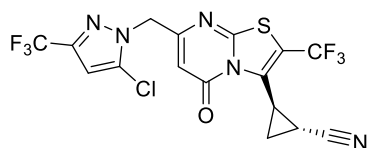
Ifenprodil



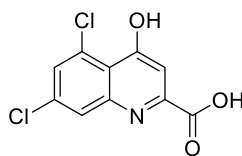
TCN-201



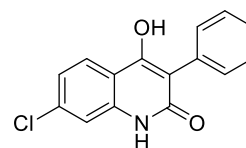
CGP-78608



GNE-0723



DCKA



MDL-104,653

Figure 2. Chemical structures of representative subunit-selective allosteric modulators and competitive antagonists targeting GluN1/2 NMDARs.

2. Research Aim

Due to their critical involvement in central nervous system (CNS) development and function, *N*-methyl-D-aspartate (NMDA) receptors are highly attractive targets for drug development. In particular, ligands that selectively modulate GluN3-containing NMDA receptor subtypes hold therapeutic potential for a range of neurological and psychiatric disorders. Two novel allosteric modulators (**A** and **B**) with selectivity for GluN1/N3 over GluN1/N2 NMDA receptors were previously identified and synthesized by the Rasmus group. These compounds exhibited micromolar potency at GluN1/3A subtypes. The primary aim of this study was to further enhance the selectivity and potency of these modulators through a ligand-based drug design approach. In parallel, structure-based ligand design was implemented based on two previously reported competitive antagonists (**DCKA** and **MDL-104,653**).

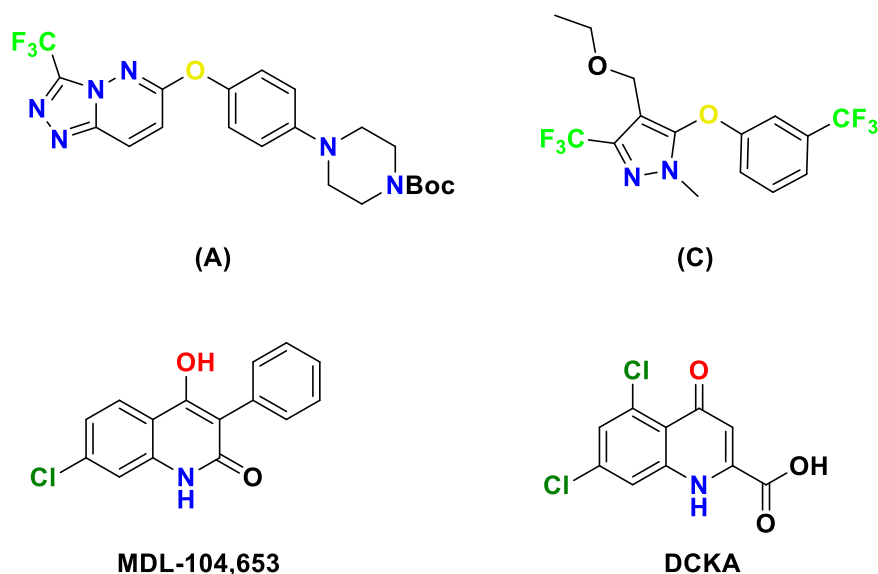


Figure 3. Chemical structures of GluN1/3-selective allosteric modulators **A** and **B** (developed by the Rasmus group) and two competitive antagonists (**MDL-104,653** and **DCKA**) targeting the glycine binding site on the GluN1 subunit.

3. Results and Discussion

3.1 Allosteric modulator **A**

Previous studies carried out by the Rasmus group on the pharmacological properties of the allosteric modulator **A** indicated that the CF₃ substituent on the triazole-fused pyridazine ring is important for potency, since its replacement with various other groups generally caused a loss of activity (**Figure 4**). Furthermore, among the different linkers tested (ether, thioether, amine, and methylene) at position 6 on the pyridazine moiety, the analogue containing the ether bridge was found to be the most potent. Finally, a series of analogues featuring various aryl groups connected via the ether linker at the 6-position were synthesized and tested. The derivative bearing a phenyl substituent with a Boc-protected piperazine group showed promising activity at the NMDA receptor subtype GluN1/3A ($EC_{50} = 27 \mu\text{M}$) and was therefore selected for further optimization. However, its potency at GluN1/3B receptors was low, with an EC_{50} value above $100 \mu\text{M}$.

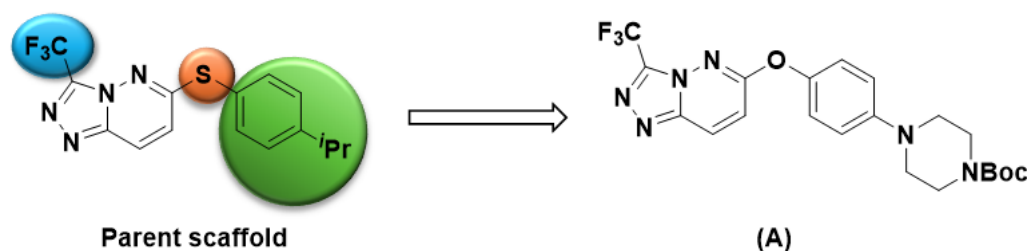
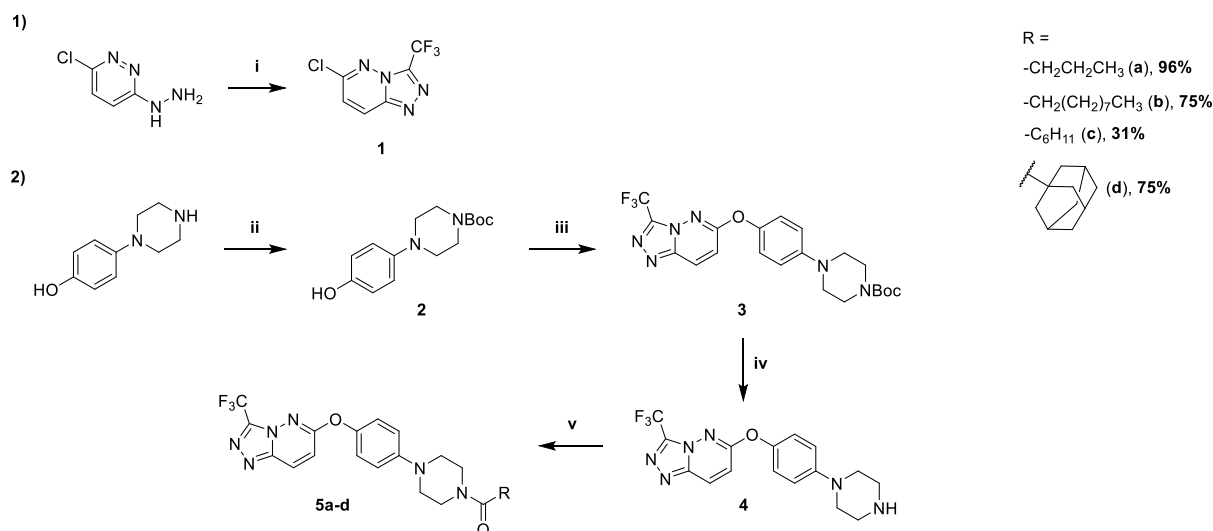


Figure 4. Design optimization of the parent scaffold leading to allosteric modulator **A**, targeting glycine-binding GluN1/GluN3 NMDA receptor subtypes.

To evaluate steric tolerance and potential potency shifts towards GluN1/3 NMDA receptor subtypes, the Boc protecting group was replaced by aliphatic chains with varying lengths and different hydrocarbon substituents. This involved synthesising newly designed analogues **5a-d** (**Scheme 1**).



Scheme 1. Synthesis route of analogues **5a-d**^a.

^a Reagents and conditions: (i) TFA, reflux, o/n, yield: 98%; (ii) Boc₂O, MeOH, rt, o/n, yield: 85%; (iii) **1**, K₂CO₃, MeCN, 65 °C, o/n, yield: 82%; (iv) 4 N HCl in dioxane, 65 °C, o/n, yield: 99%; (v) RCOCl, TEA, DCM, rt, o/n, yield: 31-96%.

The synthesis of the newly designed analogues **5a-d** began with the *in situ* cyclocondensation of 3-chloro-6-hydrazinylpyridazine with trifluoroacetic acid, yielding the triazole-fused pyridazine ring **1** (**Figure 6**, plausible mechanism). The intermediate was purified by recrystallization from a mixture of *n*-heptane and ethyl acetate and was obtained in complete conversion. Next, intermediate **2** was obtained by protecting 4-(piperazine-1-yl)phenol with di-*tert*-butyl decarbonate (Boc₂O) in MeOH at room temperature. This protection step was essential due to the presence of two nucleophilic positions on the compound, where the secondary amine is more nucleophilic. Thereafter, a nucleophilic aromatic substitution (S_NAr) reaction was carried out under mild conditions between compounds **1** and **2**, forming intermediate **3**. Deprotection of **3** using 4 N HCl in dioxane, followed by acylation with various acyl chlorides in the presence of triethylamine (TEA), afforded the final compounds **5a-d**. However, certain acyl chlorides, including hexanoyl chloride and pentanoyl chloride, did not afford the desired products, likely due to the degradation of aged reagents.

The final compounds **5a-d** were purified through manual column chromatography, achieving 90-97% purity. When looking at the ¹³C NMR for these compounds (**Figure 5**), due to the strong carbon-fluorine bond, the trifluoromethyl carbon is split into a quartet with a coupling constant of 270 Hz, and the adjacent carbon is also split into a quartet with a coupling constant around 40 Hz.

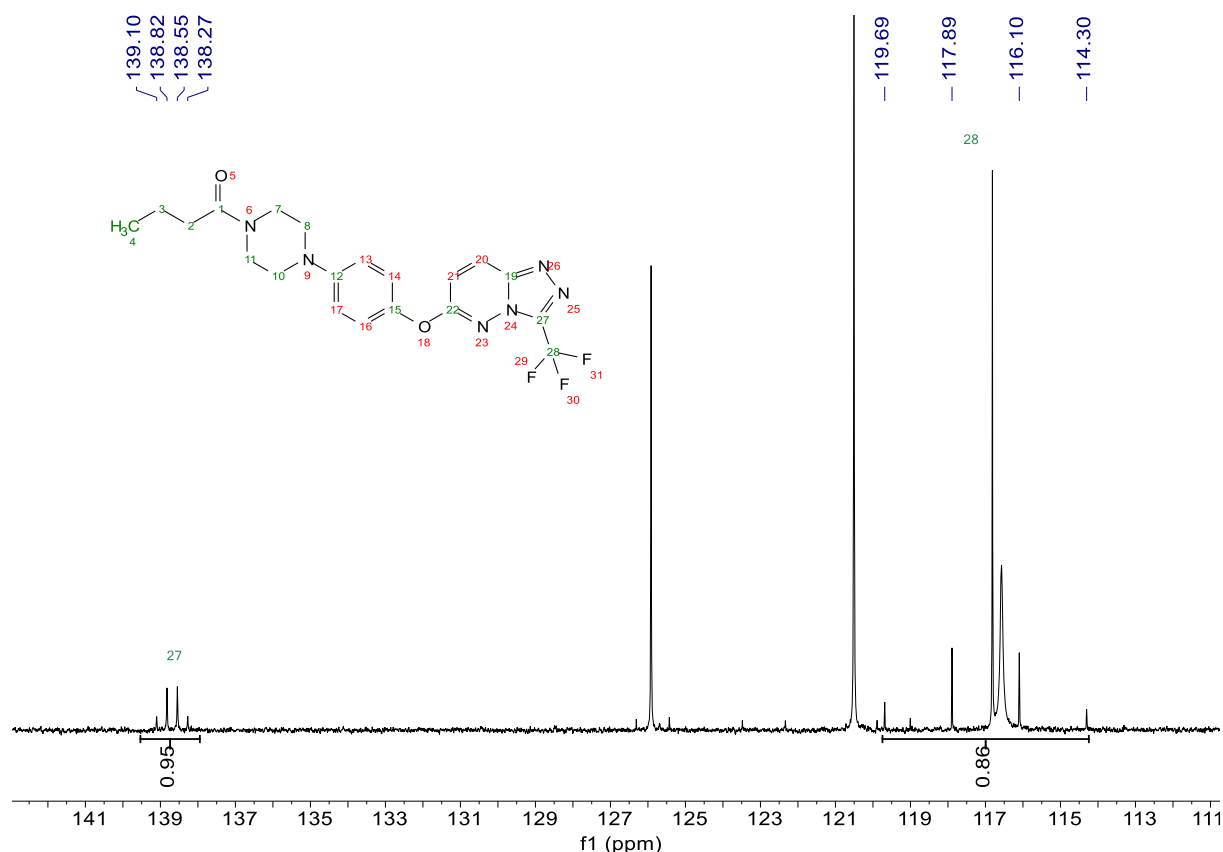


Figure 5. ^{13}C NMR of analogue **5d** showing the quartet splitting of the trifluoromethyl carbon and the adjacent carbon.

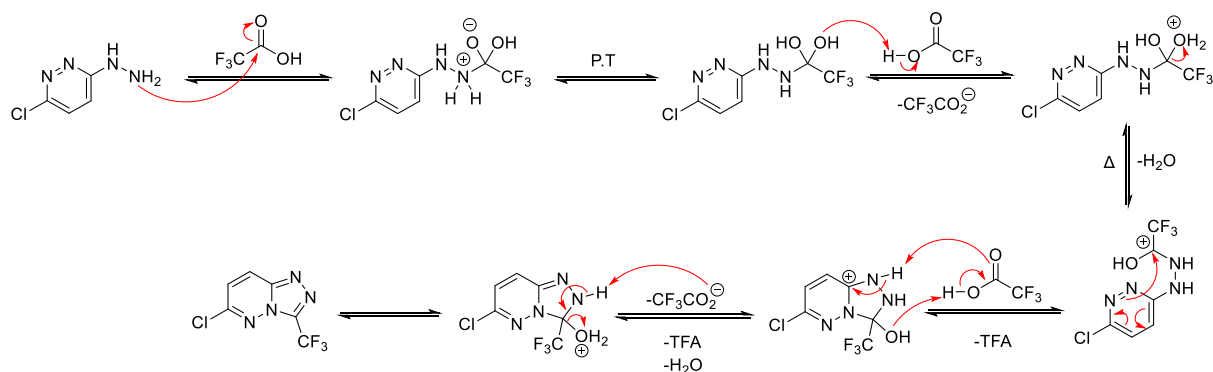


Figure 6. Plausible mechanism for the *in situ* cyclocondensation of compound **1**.

Furthermore, to investigate the importance of the piperazine moiety for activity and selectivity toward GluN3-containing NMDA receptors, a new series of compounds **8a-d** (Scheme 2) was designed and synthesized based on the structure of **TK67** (Figure 7). **TK67** has previously been reported to show antagonism at GluN1/GluN3 receptors while exhibiting only minor inhibition at GluN1/GluN2 receptors [9]. This suggests that this compound cannot access the GluN1 binding site to inhibit the receptor, highlighting its preference for the GluN3-containing receptor. The phenyl ring on the parent scaffold was replaced with a 2-aminobenzothiazole ring

to introduce hydrogen bond donor and acceptor groups capable of engaging in potential polar interactions.

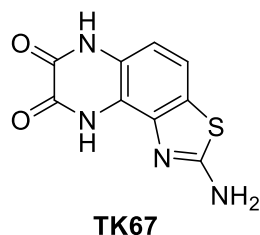
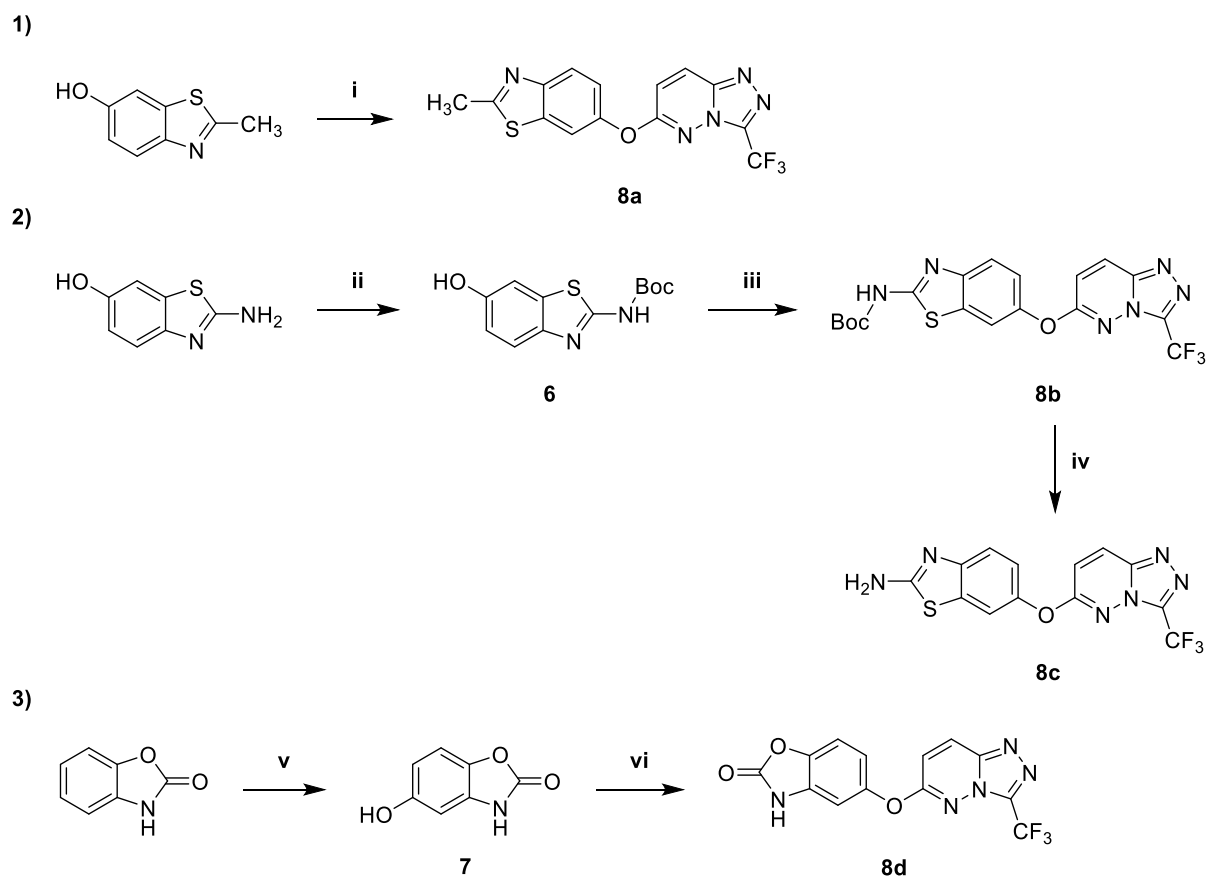


Figure 7. Chemical structure of GluN1/3-selective antagonist **TK67** [9].

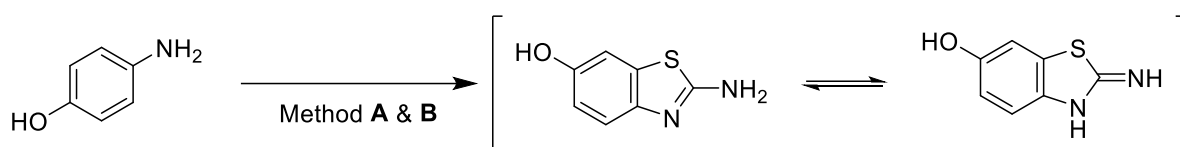


Scheme 2. Synthesis route of analogues **8a-d**^a.

^a Reagents and conditions: (i) **1**, K₂CO₃, MeCN, 65 °C, o/n, yield: 98%; (ii) Boc₂O, MeOH, rt, o/n, yield: 51%; (iii) **1**, K₂CO₃, MeCN, 65 °C, o/n, yield: 66%; (iv) 4 N HCl in dioxane, 65 °C, 6 h, yield: 14%; (v) PIFA, TFA, 75 °C, o/n, dr 81:19, yield: 17%; (vi) **1**, K₂CO₃, MeCN, 65 °C, o/n, yield: 33%.

The synthesis of analogue **8a** commenced with an S_NAr reaction between commercially available 2-methylbenzothiazol-6-ol and compound **1**. Furthermore, for the synthesis of compound **8b**, Boc protection of the starting material 2-aminobenzothiazol-6-ol was considered necessary to ensure chemoselectivity. The primary amine is more nucleophilic because nitrogen

is less electronegative than oxygen, making its lone pair more available for nucleophilic attack than the phenol group [25]. If left unprotected, the following step could lead to unwanted side reactions. However, the Boc protection conditions were not optimal, leading to some partial protection of the phenol group, resulting in a mixture of mono- and di-protected products. The next step involved an S_NAr reaction between intermediate **6** and **1**, followed by deprotection with 4 N HCl in dioxane to obtain compound **8c**. A significant drawback of the synthetic pathway for **8c** lies in the final deprotection step, which resulted in low yield and instability of the fused ring. One possible explanation for this issue is that the 2-amino benzothiazole ring can be expressed as a tautomerism of enamines (**Scheme 3**) [26]. This tautomerization may be favoured under acidic conditions, leading to instability of the ring due to the loss of aromaticity. An earlier synthetic approach toward **8c** involved starting with 4-aminophenol (**Scheme 3**) and attempting to synthesize the 2-aminobenzothiazole ring via the Hegerschoff method [27], but this was unsuccessful.



Scheme 3. Synthesis route of 2-aminobenzothiazol-6-ol^a.

^a Reagents and conditions: (A) $\text{PhCH}_2\text{NMe}_3\text{Br}_3$, Bu_4NSCN , DCM, rt, o/n, yield: 0%; (B) $\text{PhCH}_2\text{NMe}_3\text{Cl}_2\text{I}$, NH_4SCN , DMSO:H₂O (9:1), 70 °C, o/n, yield: 0%.

Lastly, for the synthesis of compound **8d**, a hydroxyl group was introduced onto the benzannulated cyclic carbamate through oxidation using PIFA. The hydroxyl group was predominantly introduced at the meta position to the nitrogen, affording intermediate **7**. This regioselectivity is due to the strong electronic influence of the carbamate oxygen (**Figure 8**, plausible mechanism) [28]. In contrast, if the starting material were a benzannulated lactam, hydroxylation would occur primarily at the para position to the nitrogen. Thereafter, an S_NAr reaction between intermediate **7** and **1** afforded the final compound **8d**.

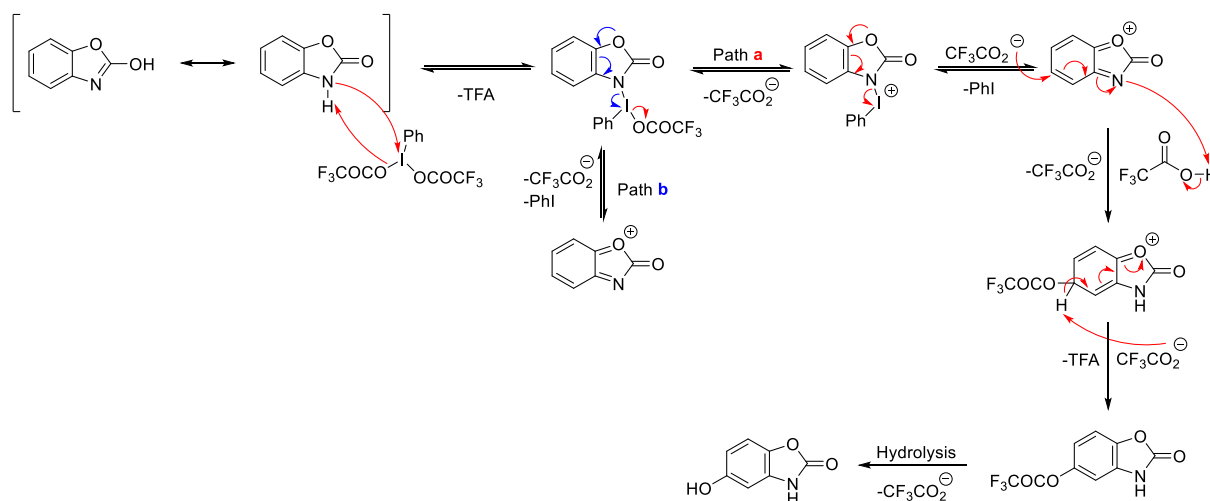


Figure 8. Plausible mechanism for the introduction of a hydroxy group to a benzannulated cyclic carbamate using PIFA. Pathway a = red, b = blue.

Compound **8a** was synthesized to determine whether substituting the primary amine in analogue **8b** with a methyl group on the benzothiazole ring would affect pharmacological activity. Additionally, compound **8d** was synthesised to incorporate a benzannulated cyclic carbamate capable of acting as both a hydrogen bond donor and acceptor. Analogues **8a-d** were purified by prep. HPLC, achieving 95-99% purity. However, prep. HPLC of compound **8b** isolated only a small amount of the protected compound due to the presence of TFA in the mobile phase.

3.2 Allosteric modulator **B**

Another analogue derived from the parent scaffold, investigated by the Rasmus group, is compound **B** (**Figure 9**), which demonstrated good potency at the NMDA receptor subtype GluN1/3A ($EC_{50} = 10 \mu\text{M}$). To further optimize this compound, the strategy involved modifying the triazole-fused pyridazine ring by deleting or altering the position of nitrogen atoms to explore whether selectivity towards GluN1/3 NMDA receptor subtypes could be improved. Alternatively, replacing this ring system entirely with one composed of two fused 6-membered rings was also considered (**Scheme 4**).

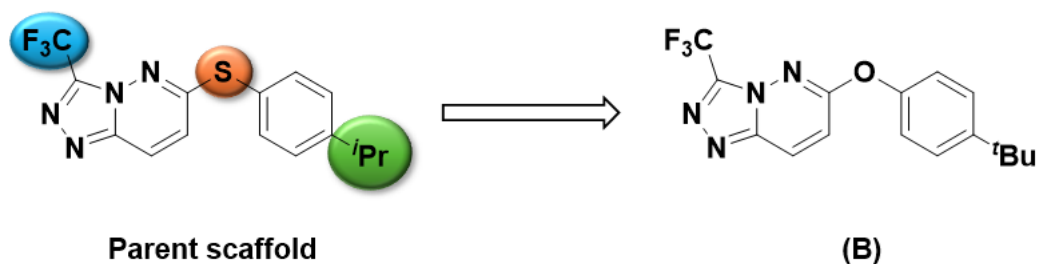
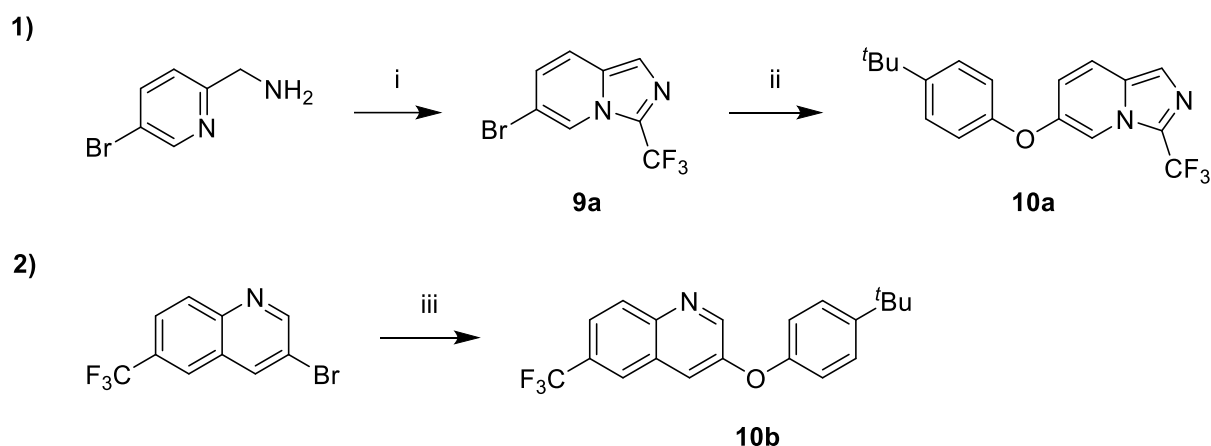


Figure 9. Design optimization of the parent scaffold leading to compound **B**, targeting glycine-binding GluN1/GluN3 NMDA receptor subtypes.

The first modification involved deleting two nitrogen atoms, resulting in an imidazo-fused pyrimidine ring (**Scheme 4, 10a**). This modification is expected to reduce the electron deficiency of the heterocyclic ring, potentially enhancing the binding affinity and selectivity of the ligand [29]. Furthermore, removing nitrogen atoms incapable of forming polar interactions can lower the associated solvation penalty.

The synthesis of the analogue **10a** (**Scheme 4**) began with the *in situ* cyclocondensation of 5-bromo-2-aminomethylpyridine with TFA in the presence of TEA, yielding the imidazole-fused pyridine ring **9a**. This was followed by a copper-catalysed Ullman coupling between intermediate **9a** and 4-*tert*-butylphenol, in the presence of caesium carbonate, dimethylglycine, and copper(I)iodide, to yield analogue **10a**. Furthermore, a second modification involved replacing the original heterocyclic core with a quinoline ring system. For this approach, 3-bromo-6-(trifluoromethyl)quinoline was selected as the starting material due to its structural resemblance to the initial scaffold. The synthesis of analogue **10b** was then obtained in one step through a copper-catalysed Ullman coupling with 4-*tert*-butylphenol under the same conditions used for **10a**.

For the *in situ* cyclocondensation reaction of analogue **10a**, a slightly excess amount of TFA compared to TEA was necessary to ensure sufficient free TFA remained to react with the primary amine. An interesting observation regarding this analogue is its increased hydrophobicity, which can be directly correlated to the deletion of nitrogen atoms, as indicated by the longer retention times in analytical HPLC compared to the parent scaffold.

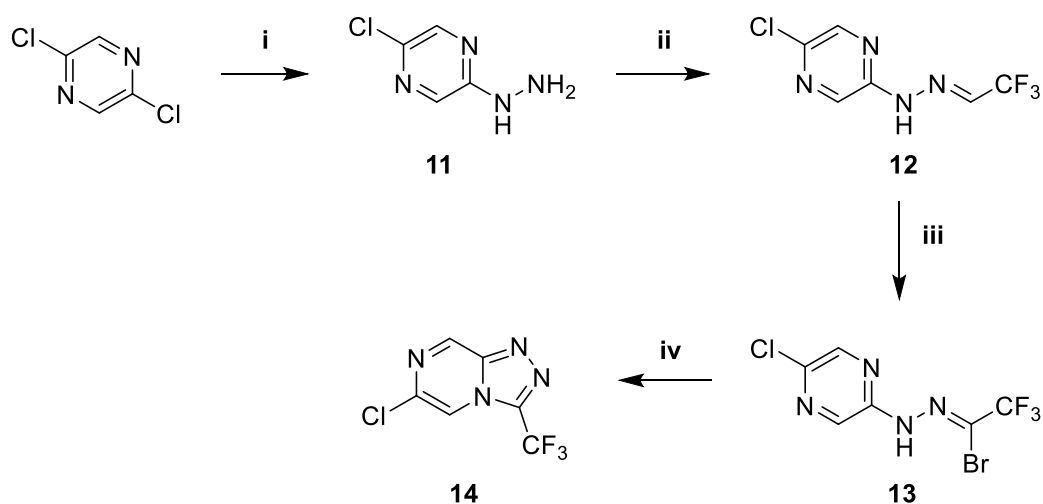


Scheme 4. Synthesis route of analogue **10a-b**^a

^a Reagents and conditions: (i) TFAA, TEA, THF, 0 °C to rt, 20 h, yield: 99%; (ii) PTBP, Cs₂CO₃, DMG, CuI, PhMe, 115 °C, o/n, yield: 89%; (iii) PTBP, Cs₂CO₃, DMG, CuI, PhMe, 115 °C, o/n, yield: 34%.

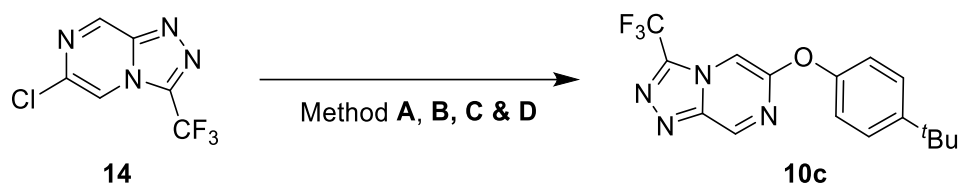
The third modification on compound **B** involved altering the position of one nitrogen atom, resulting in a triazole-fused pyrimidine ring (**Scheme 5**, intermediate **14**). The synthesis of intermediate **14** was straightforward, beginning with an S_NAr reaction between commercially available 2,5-dichloropyrazine and hydrazine monohydrate, affording intermediate **11**. This was followed by condensation with 2,2,2-trifluoroethane-1,1-diol to give **12**, and bromination of the resulting alkene with NBS under mild conditions provided **13**. Finally, key intermediate **14** was afforded by an *in situ* cyclocondensation of **13** in the presence of TEA, with the reaction typically completed within 2 hours. However, if the reaction proceeds overnight, decomposition of the triazole-fused pyrimidine ring is observed.

In order to synthesize analogue **10c**, intermediate **14** was screened under various S_NAr conditions (**Scheme 6**). All conditions screened led to the decomposition of **14**, even as short as 1 hour under mild conditions. The ¹⁹F NMR spectrum indicated ring fragmentation, suggesting a decomposition pathway by deprotonation of the pyrimidine moiety followed by ring opening. The key difference between compound **13** and the parent scaffold is that, in compound **13**, the two hydrogen atoms on the pyrimidine moiety that can be abstracted during deprotonation are close to a nitrogen atom capable of stabilizing the electron density (**Figure 10**), thereby promoting ring opening. In contrast, the corresponding hydrogens on the pyridazine ring of the allosteric modulator **B** are not near any nitrogen atom that can stabilize the electron density, making the process unfavourable and explaining why it does not decompose under the same conditions.



Scheme 5. Synthesis route of intermediate **14**.^a

^a Reagents and conditions: (i) Hydrazine monohydrate, 32 wt% NH₃ (aq), H₂O, 100 °C, o/n, yield: 80%; (ii) CF₃CH(OH)₂, MeOH, 4Å MS, 75 °C, o/n, yield: 53%; (iii) NBS, DMF, rt, o/n, yield: 87%; (iv) TEA, PhMe, rt, 2h, yield: 87%.



Scheme 6. Screening of various S_NAr conditions for the synthesis of analogue **10c**.^a

^a Reagents and conditions: (A) PTBP, K₂CO₃, MeCN, 65 °C, o/n, yield: 0%; (B) PTBP, Cs₂CO₃, MeCN, 65 °C, 1 h, yield: 0%; (C) PTBP, NaH, DMF, 75 °C, o/n, yield: 0%; (D) PTBP, NaO^tBu, DMI, 100 °C, 0.5 h, yield: 0%.

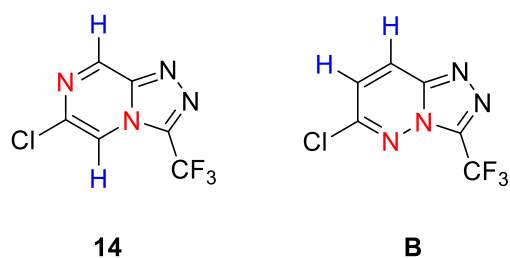
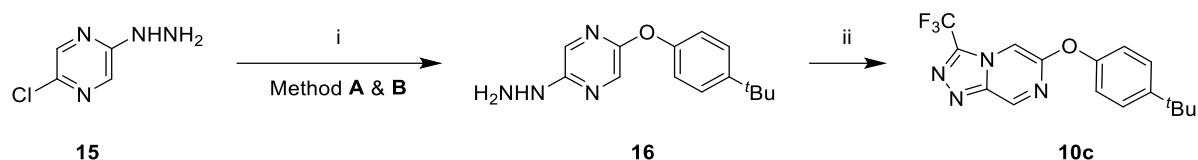


Figure 10. The positions of the hydrogen atoms on the fused ring of the allosteric modulator **B** and intermediate **14** that can be deprotonated.

Given that attempts to synthesise analogue **10c** via various S_NAr conditions were unsuccessful, an alternative synthetic route was used (**Scheme 7**). In this approach, the 4-*tert*-butylphenol group was proposed to be introduced first, followed by an *in situ* cyclization as previously established for analogues **5a-d**. However, the S_NAr and copper-catalyzed Ullmann coupling

reactions between compound **15** and 4-*tert*-butylphenol failed to afford the desired intermediate **16**. This is due to a regioselectivity problem, as the major side product indicates that substitution occurred at the hydrazine rather than at the chloride.

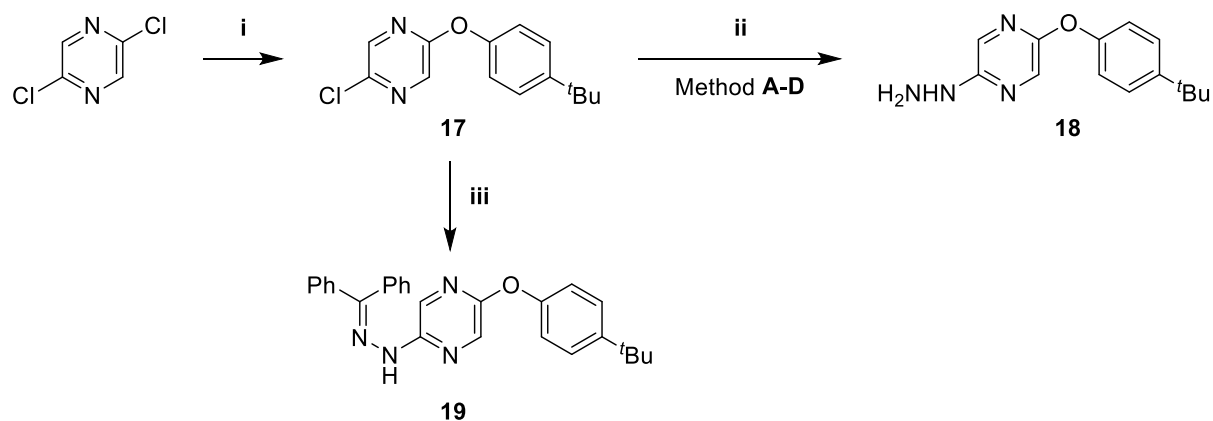


Scheme 7. Synthesis route of analogue **10c**.^a

^a Reagents and conditions: (i) (A) PTBP, Cs₂CO₃, MeCN, 65 °C, o/n, yield: 0%; (B) PTBP, Cs₂CO₃, DMG, CuI, PhMe, 115 °C, o/n, yield: 0%.

Regioselectivity issues in forming compound **16** were likely due to the carbon adjacent to the hydrazine being more electrophilic than the carbon adjacent to the chloride. Instead, starting with 2,5-dichloropyrazine and reacting it with 4-*tert*-butylphenol proved to be a better approach (**Scheme 8**, compound **17**). Nonetheless, the next step, attempting to install the hydrazine functional group under various conditions with compound **17**, was unsuccessful. This was not entirely unexpected, as intermediate **17** lacks any electron-withdrawing groups (EWGs) capable of stabilizing the negative charge during the formation of the Meisenheimer intermediate [25]. Instead, the ether bridge, acting as an electron-donating group (EDG), likely hinders the formation of this intermediate.

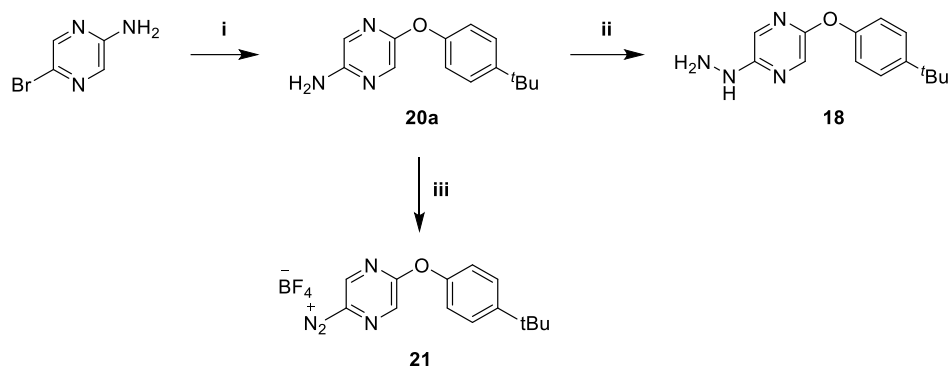
Another strategy was forming a hydrazone intermediate, compound **19**, through a palladium-catalyzed C-N coupling reaction with benzophenone hydrazone [30]. This hydrazone intermediate can easily be converted to hydrazine using acid-catalyzed hydrolysis [31]. However, this reaction did not proceed as planned, resulting in the decomposition of the starting material instead.



Scheme 8. Synthesis route for analogue **10c**.^a

^a Reagents and conditions: (i) PTBP, Cs₂CO₃, MeCN, 65 °C, o/n, yield: 93%; (ii) (A) Hydrazine monohydrate, 32 wt% NH₃ (aq), H₂O, 100 °C, o/n, yield: 0%; (B) Hydrazine monohydrate, *i*PrOH, 70 °C, o/n, yield: 0%; (C) Hydrazine monohydrate, 110 °C, o/n, yield: 0%; (D) Hydrazine monohydrate, EtOH, 120 °C, o/n, yield: 0%. (iii) Ph₂C=NNH₂, Pd(OAc)₂, Davephos, NaO^tBu, PhMe, 120 °C, o/n, yield: 0%.

The final approach toward synthesising analogue **10c** started with 5-bromopyrazin-2-amine, which underwent copper-catalyzed Ullmann coupling with 4-*tert*-butylphenol to give intermediate **20a**. The plan was to generate the corresponding diazonium salt, followed by reduction to afford the hydrazine intermediate **18**. However, this transformation did not proceed as expected and resulted in decomposition. An alternative approach, isolating the diazonium salt as the tetrafluoroborate salt (**21**) before reduction, also failed to give the desired product. The reason for the unsuccessful formation of the diazonium salt is due to its instability. From the LCMS, it can be seen that the diazonium salt was formed during the reaction but was cleaved immediately. This was also evident from the bubbling observed during the reaction, indicating the formation of nitrogen gas. Furthermore, the major byproduct formed can be explained by the plausible mechanism (**Figure 11**). Upon cleavage of the diazonium group, water rapidly attacks the positively charged aryl ring, leading to the byproduct with *m/z* 245. This is also the typical pathway by which diazonium salts are converted to phenols.



Scheme 9. Synthesis route for analogue **10c**.^a

^a Reagents and conditions: (i) PTBP, Cs₂CO₃, DMG, CuI, PhMe, 115 °C, o/n, yield: 98%; (ii) (1) NaNO₂, 37 wt% HCl, 0 °C, 0.5 h, (2) SnCl₂ · HCl, 37 wt% HCl, 0 °C, 4 h, yield: 0%; (iii) *i*AmylONO, HBF₄ · Et₂O, EtOH, -5 °C, 3 h, yield: 0%.

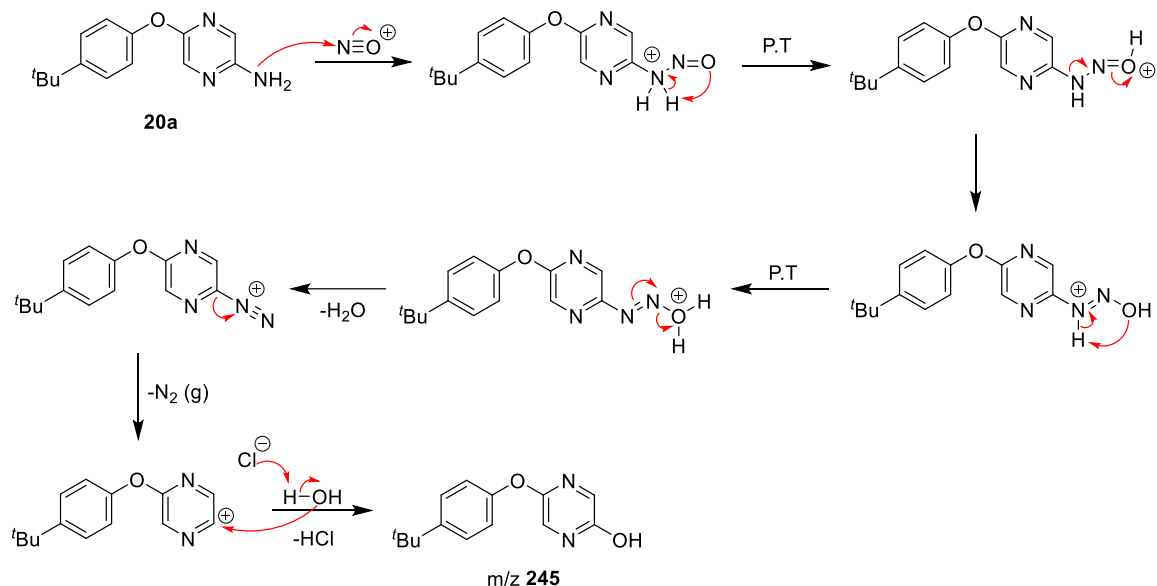


Figure 11. Plausible mechanism for the formation of the byproduct for compounds **20a** and **21**.

The challenging synthetic feasibility and instability of analogue **10c** led to a new design modification, **10d**, which again involved altering the position of one nitrogen atom in allosteric modulator **B** (**Figure 12**). This resulted in a different triazole-fused pyrimidine ring. Most of the reaction conditions previously tested for **10c** were also applied to this analogue, but they were similarly unsuccessful. However, trace product formation was detected by LCMS when analogue **10d** was synthesised through the established *in situ* cyclization, followed by a copper-catalyzed Ullmann coupling (**Scheme 10**). Even after multiple attempts and careful monitoring, only trace amounts of the desired product were detected. Consequently, both analogues **10c** and **10d** were considered unstable, and no further experiments were carried out.

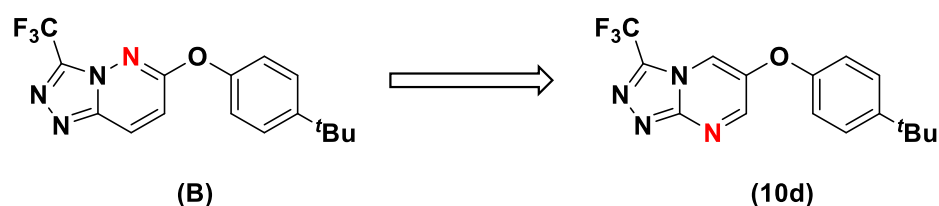
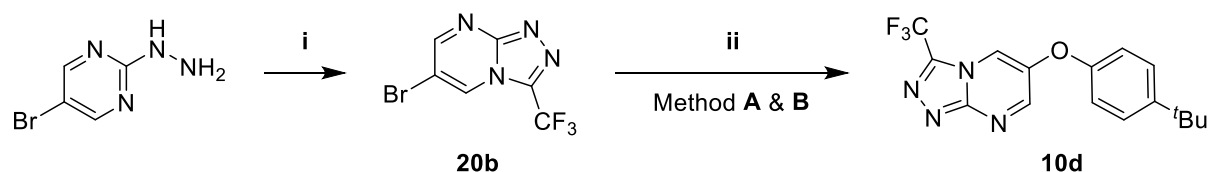


Figure 12. Design optimization of the allosteric modulator **B** to analogue **10d**, targeting glycine-binding GluN1/GluN3 NMDA receptor subtypes.



Scheme 10. Synthesis route for analogue **10d**.^a

^a Reagents and conditions: (i) TFAA, TEA, THF, 0 °C to rt, o/n, yield: 44%; (ii) (A) PTBP, Cs₂CO₃, DMG, CuI, PhMe, 80-115 °C, 2-20 h, yield: 0%; (B) PTBP, Cs₂CO₃, BFMO, CuI, PhMe, 80 °C, 2 h, yield: 0%.

3.3 Allosteric modulator **C**

As part of continued efforts to develop selective small molecules targeting GluN1/N3 NMDA receptor subtypes, the Rasmus group investigated compound **D** as a novel allosteric modulator derived from a distinct parent scaffold (**Figure 13**). Previous studies indicated that a *m*-CF₃ substituent on the aryl ring is important for potency, since its replacement with various other groups generally caused a loss of activity. Furthermore, among the different linkers tested at position 5 on the pyrazole ring (ether, thioether, amine, and methylene), the analogue containing the ether linker was found to be the most potent. Similarly, the analogue bearing an ether functional group at the 4-position on the pyrazole ring showed the highest activity toward the NMDA receptor subtype GluN1/GluN3A (EC₅₀ = 10 μM) and was therefore selected for further optimization.

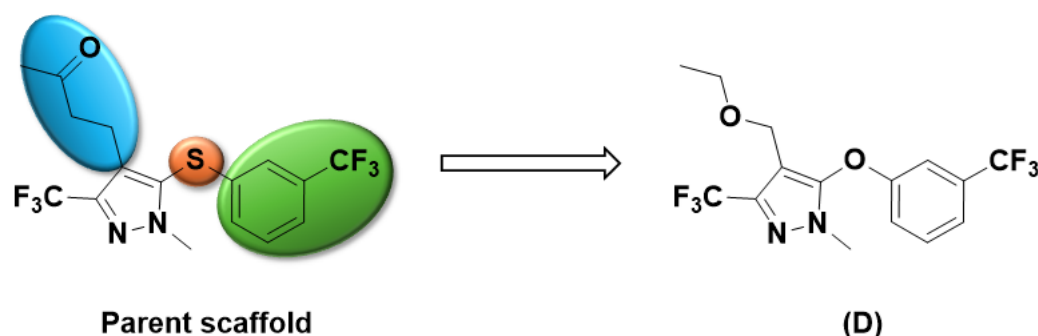
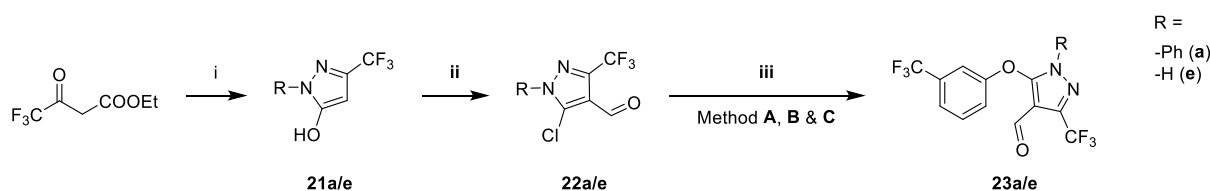


Figure 13. Design optimization of the parent scaffold leading to allosteric modulator **D**, targeting glycine-binding GluN1/GluN3 NMDA receptor subtypes.

Since all previous analogues of allosteric modulator **D** contained a methyl substituent on the pyrazole nitrogen (**Figure 13**), the first modification involved introducing different functional groups at this position to investigate any potential shift in potency at the GluN1/3 NMDA receptor subtypes. The synthesis of this series commenced with a Knorr pyrazole reaction, introducing various substituents, including a phenyl group at the pyrazole ring, to yield intermediate **21a** (**Scheme 11**). The Knorr pyrazole reaction generally proceeded very well for most substituents. However, in the previously synthesized intermediate **21f** (**Scheme 12**), significant impurities formed, likely due to cyclopropyl ring strain, which led to ring opening and decomposition of the compound.

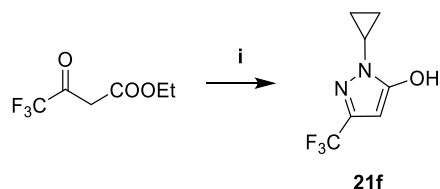
The next step in this synthesis route was a Vilsmeier-Haack formylation (plausible mechanism, **Figure 105**; see Appendix 7.2). Initially, the Vilsmeier-Haack reaction was unsuccessful due to incomplete *in situ* formation of the Vilsmeier reagent from DMF and phosphorus oxychloride [25]. Furthermore, the acidic nature of the Vilsmeier-Haack conditions posed an additional challenge, as previous work had shown that acid-sensitive groups, such as *tert*-butyl (**Scheme 13**), were cleaved under such conditions, highlighting the need for an acid-resistant substituent.

Attempts to introduce the aryl substituent in the following step via S_NAr or Buchwald-Hartwig cross-coupling reactions resulted in only trace amounts of the desired product **23a**. The problem was believed to arise from steric hindrance caused by the phenyl group at the pyrazole nitrogen, which interfered with the subsequent steps. Starting from an unsubstituted pyrazole ring (**Scheme 11**, **22e**) was not an option either, as the desired product was not formed. What happened is that the arylation occurred, but the LCMS showed complete conversion to a side product with an m/z of 169, indicating that the ether bridge was cleaved, leading to a hydroxyl group on the pyrazole ring (**Figure 14**).



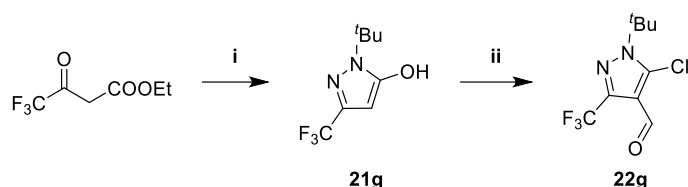
Scheme 11. The initial synthesis route of analogue **28a**.^a

^a Reagents and conditions: **(i)** PhCNHNH₂, CH₃COOH, 80 °C, o/n, yield: 86%; **(ii)** **(1)** POCl₃, DMF, 0 °C, 0.5 h, **(2)** 80 °C, o/n, yield: 76%; **(iii)** **(A, 23a)** 3-CF₃PhOH, Cs₂CO₃, DMSO, 100 °C, o/n, yield: trace amounts; **(B, 23a)** 3-CF₃PhOH, K₃PO₄, Pd(OAc)₂, ^tBuPhos, PhMe, 100 °C, o/n, yield: 12%; **(C, 23e)** 3-CF₃PhOH, K₂CO₃, THF, 80 °C, o/n, yield: 0%.



Scheme 12. Synthesis of compound **21f**.^a

^a Reagents and conditions: **(i)** Cyclopropylhydrazine hydrochloride, EtOH or DCE, 80 °C, o/n, yield: trace amount.



Scheme 13. Synthesis route of compound **22g**.^a

^a Reagents and conditions: **(i)** $(\text{CH}_3)_3\text{CNHNH}_2 \cdot \text{HCl}$, EtOH, 100 °C, 48 h, yield: 53%; **(ii)** **(1)** POCl_3 , DMF, 0 °C, 0.5 h, **(2)** 80 °C, o/n, yield: trace amount.

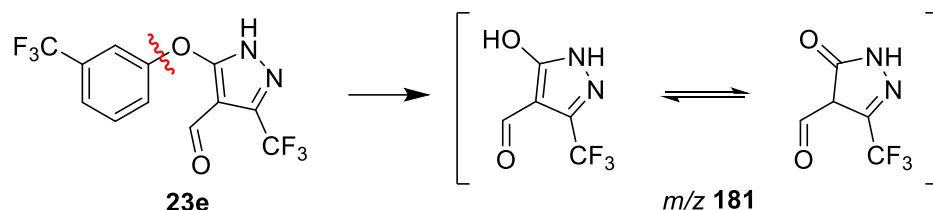
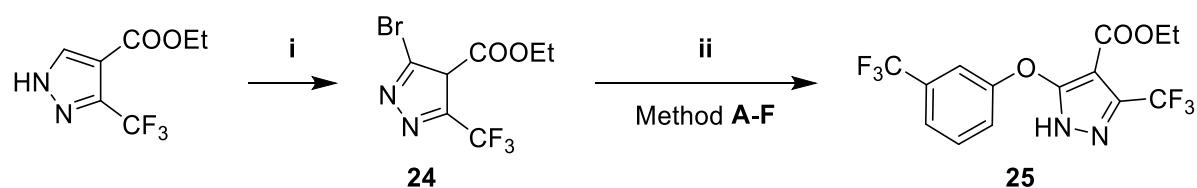


Figure 14. Cleavage of the ether bridge during the arylation reaction of compound **22e**.

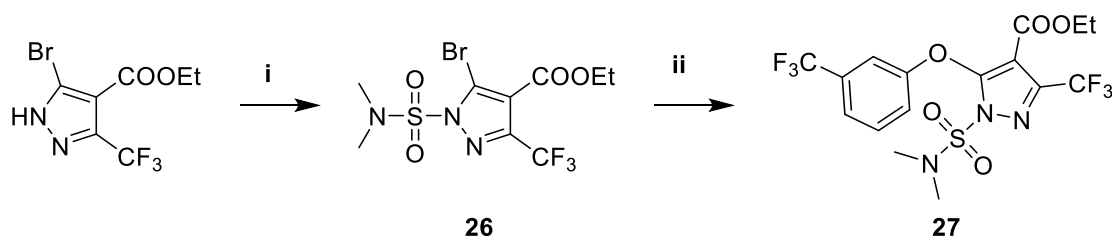
To address this, an alternative synthetic route was developed (**Scheme 14**), starting from ethyl 3-(trifluoromethyl)-1H-pyrazole-4-carboxylate, which allowed for early introduction of the aryl substituent. This new synthetic route began with electrophilic aromatic bromination at the 5-position of the pyrazole ring, followed by a series of screened reaction conditions to introduce the aryl substituent 3-(trifluoromethyl)phenol.

However, the introduction of the aryl substituent proved challenging. The reaction was time-consuming and required high temperatures. Moreover, complete conversion was not achieved under any of the tested conditions. As a result, separating compound **25** from the starting material 3-(trifluoromethyl)phenol was impossible using normal-phase chromatography. Instead, it required reversed-phase purification because of their close retention times (3.09 min for compound **25** and 3.15 min for 3-(trifluoromethyl)phenol). Due to the poor yields and complex purification, this route was discontinued. The next step would have involved a Chan-Lam coupling to introduce the phenyl substituent [32], followed by reduction of the ester to the corresponding ether using ammonia borane with TiCl_4 as the catalyst [33]. The reason the Chan-Lam coupling was thought to be performed after the introduction of the aryl substituent is that certain functional groups could not tolerate the high temperatures (**Scheme 15**, compound **27**).



Scheme 14. New synthesis route of analogues **28a-d**.^a

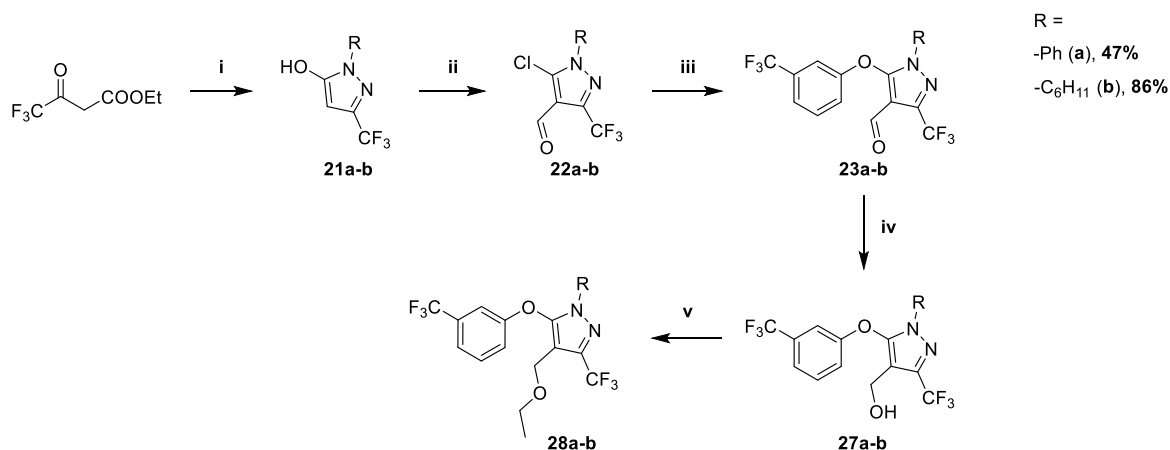
^a Reagents and conditions: **(i)** NaOAc, Br₂, AcOH, 100 °C, 7 h, yield: 99%; **(ii)** **(A)** 3-CF₃PhOH, Cs₂CO₃, DMSO, 85 °C, o/n, yield: 0%; **(B)** 3-CF₃PhOH, K₃PO₄, Pd(OAc)₂, ^tBuXPhos, PhMe, o/n, yield: 0%; **(C)** 3-CF₃PhOH, K₂CO₃, DMSO, 130 °C, 48 h, yield: 6%; **(D)** 3-CF₃PhOH, K₃PO₄, THMD, CuI, DMSO, 135 °C, 48 h, yield: 21%; **(E)** 3-CF₃PhOH, K₂PO₄, picolinic acid, CuI, DMSO, 135 °C, 48 h, yield: 26%; **(F)** 3-CF₃PhOH, NaO^tBu, DMSO, 140 °C, 3 h to 180 °C, 6 h, yield: 30%.



Scheme 15. Synthesis route of compound **27**.^a

^a Reagents and conditions: **(i)** (CH₃)₂NSO₂Cl, TEA, MeCN, 50 °C, o/n, yield: 87%; **(ii)** 3-CF₃PhOH, K₂CO₃, DMSO, 100 °C, o/n, yield: 0%.

In light of these limitations, a revised synthetic strategy was developed (**Scheme 16**) and proved effective for introducing various aryl substituents at the 5-position of the pyrazole ring via a copper-catalyzed Ullmann reaction. The new series of analogues **28a-b** followed the early steps outlined in **Scheme 11**, after which a copper-catalyzed coupling was carried out using copper(I) iodide and dimethylglycine, a bidentate ligand, to introduce various aryl substituents. This type of ligand has been reported to stabilize on-cycle copper species via the chelate effect [34], which may explain the improved performance observed compared to previous attempts (plausible mechanism, **Figure 106**; see Appendix 7.2). Furthermore, the next step involved reducing the aldehyde functional group with sodium borohydride to yield the corresponding alcohol, which was then alkylated with ethyl iodide in the presence of LiHMDS to obtain analogues **28a-b**. Heating was required for the alkylation step, as only minimal product formation occurred at room temperature. For analogue **28b**, almost complete conversion was achieved after 20 h. In contrast, the reaction for analogue **28a** was refluxed for 2 days and stopped when the conversion reached approximately 50%. Both analogues were purified by prep. HPLC, affording the products with 99% purity.



Scheme 16. Synthesis route of analogues **28a-b**.^a

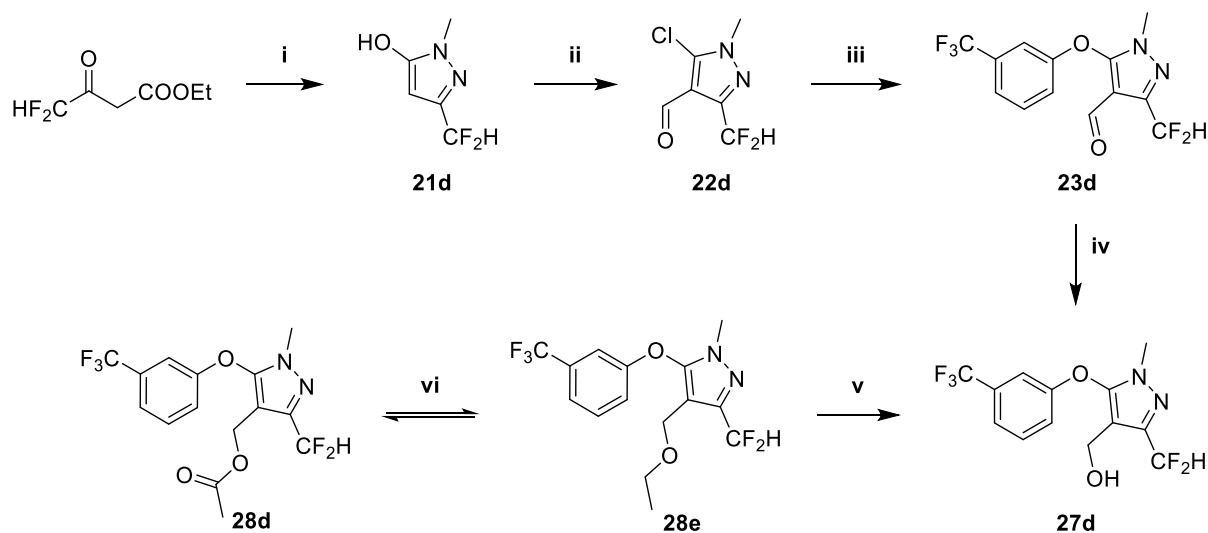
^a Reagents and conditions: (i) (**21a**) PhCNHNH₂, CH₃COOH, 80 °C, o/n, yield: 86%; (**21b**) Cyclohexylhydrazine hydrochloride, EtOH, 80 °C, 18 h, yield: 59%; (ii) (1) POCl₃, DMF, 0 °C, 0.5 h, (2) 80 °C, o/n, yield: 57-76%; (iii) 3-CF₃PhOH, Cs₂CO₃, DMG, CuI, PhMe, 115 °C, o/n, yield: 45-82%; (iv) NaBH₄, MeOH, 0 °C to rt, 3 h, yield: 58-91%; (v) EtI, LiHMDS, THF, 0 to 80 °C, 20-48 h, yield: 47-86%.

As part of further optimization of allosteric modulator **D**, the trifluoromethyl group at the 3-position of the pyrazole ring was replaced with a difluoromethyl substituent (**Scheme 17**). This minor structural modification may be significant, as the CHF₂ group can act as a weak hydrogen bond donor, potentially enhancing interactions within the binding pocket of NMDA receptor subtypes. This new analogue **28e** was prepared using the established synthetic route in **Scheme 16**. Due to the absence of a bulky group on the pyrazole ring, as in previous analogues, it was possible to introduce the aryl substituent 3-(trifluoromethyl)phenol via an S_NAr reaction under mild conditions.

Additionally, the synthesis of intermediate **21d** under the conditions used for **21b** gave poor yields and difficult separation. Improved results were obtained under modified conditions at room temperature with formic acid. The higher yield may be due to the low temperature and mild conditions, allowing the hydrazine derivative to selectively attack the more electrophilic carbonyl carbon adjacent to the difluoromethyl group. This selectivity results in the formation of only one isomer (**Figure 15**, plausible mechanism). On the other hand, at higher temperatures and without a strong electron-withdrawing group, such as the trifluoromethyl group in previous examples, both isomers may form, or other side reactions may occur, leading to many impurities.

Moreover, as the reaction mixture for the final step was transferred and stored in ethyl acetate without an acidic workup, transesterification of the ether to the corresponding acetate ester

occurred, resulting in the formation of a different compound, **28d** (Scheme 17). However, this issue was easily addressed by using a catalytic amount of p-toluenesulfonic acid and an excess of ethanol, resulting in the reformation of the intended analogue **28e**.



Scheme 17. Synthesis route of analogue **28e**.^a

^a Reagents and conditions: (i) CH_3NHNH_2 (40% aq), MTBE, HCOOH , 5 °C to rt, o/n, yield: 84%; (ii) (1) POCl_3 , DMF, 0 °C, 0.5 h, (2) 80 °C, 20 h, yield: 94%; (iii) 3- CF_3PhOH , K_2CO_3 , THF, 80 °C, o/n, yield: 77%; (iv) NaBH_4 , MeOH, 0 °C to rt, 4 h, yield: 92%; (v) EtI, LiHMDS, THF, 0 to 80 °C, 30 h, yield: 96%; (vi) p-TsOH, EtOH, 85 °C, 20 h, yield: 73%.

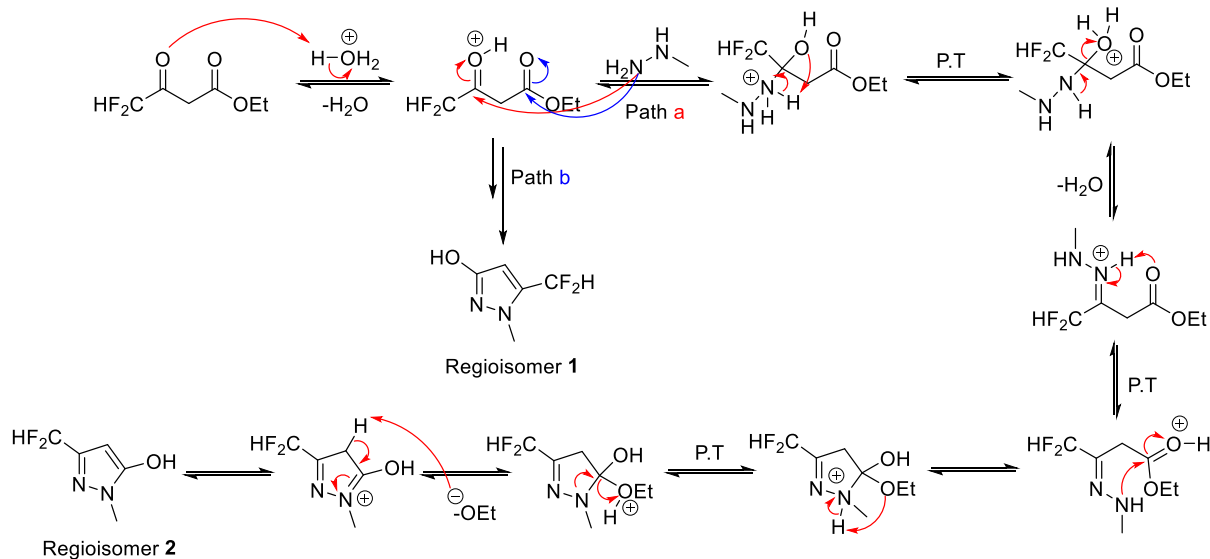


Figure 15. Plausible mechanism for the formation of the two regioisomers for compound **21d**. Pathway a = red, b = blue.

To further explore the role of the trifluoromethyl group at the 3-position of the pyrazole ring, it was replaced with a phenyl ring (Figure 16). Unlike the strongly electron-withdrawing CF_3

group, the phenyl ring is a planar, hydrophobic structure capable of engaging in van der Waals and π - π interactions within the binding pocket of GluN1/3 NMDA receptor subtypes. It may also interact with ammonium or quaternary ammonium ions through induced dipole interactions or hydrogen bonding, potentially improving binding affinity [35]. The synthesis of analogue **28c** was prepared using the established synthetic route outlined in **Scheme 16**. As previously stated, a copper-catalyzed Ullmann coupling was required to install the aryl substituent, since no product formation was observed under S_NAr conditions for compound **28c**.

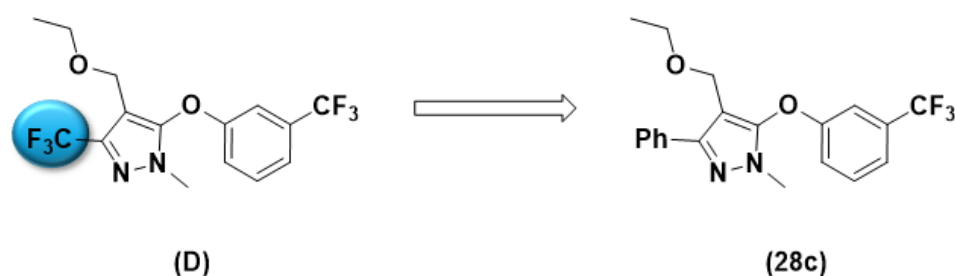


Figure 16. Design optimization of the allosteric modulator **D** to analogue **28c**, targeting glycine-binding GluN1/GluN3 NMDA receptor subtypes.

3.4 Competitive antagonist DCKA

To design selective competitive antagonists for the GluN3 subtype of NMDA receptors, 5,7-dichlorokynurenic acid (DCKA) was selected as a lead structure. DCKA has previously been reported as a selective competitive antagonist at the glycine-binding site of the GluN1 subunit [22]. The presence of a chloride atom at position 7 is crucial for both the activity and selectivity of the compound toward NMDA receptors [24, 36]. The DCKA scaffold is characterized by four key interaction regions, as illustrated in **Figure 17** [36].

Furthermore, the superposition of the GluN1 and GluN3A LBDs in complex with a DCKA analogue (**Figure 18**) reveals differences in the binding pockets. In the GluN1 LBD, a tryptophan residue (Trp223) sterically clashes with bulky substituents at the 5-position of the DCKA scaffold, such as a phenyl group, thereby reducing binding affinity [36]. On the other hand, this steric hindrance is not present in the GluN3A LBD, allowing the binding of bulkier substituents. This suggests that introducing bulky substituents at the 5-position could shift selectivity toward GluN3-containing NMDA receptors by taking advantage of these structural differences.

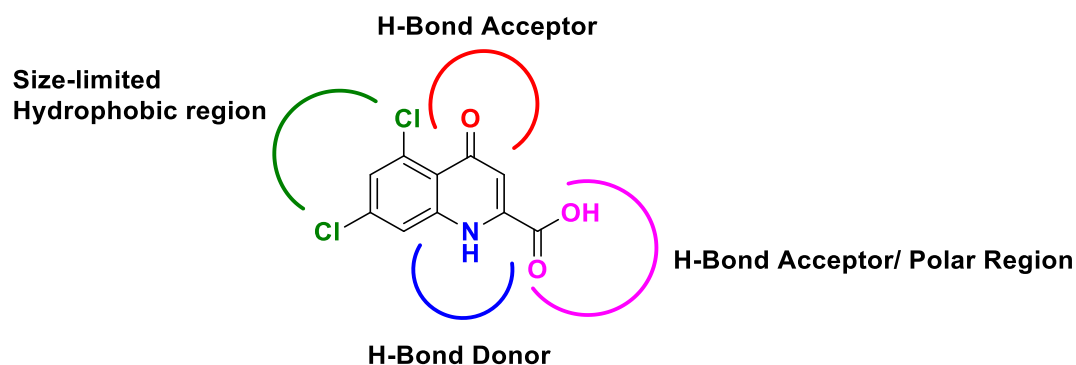


Figure 17. Key interaction regions of the **DCKA** scaffold.

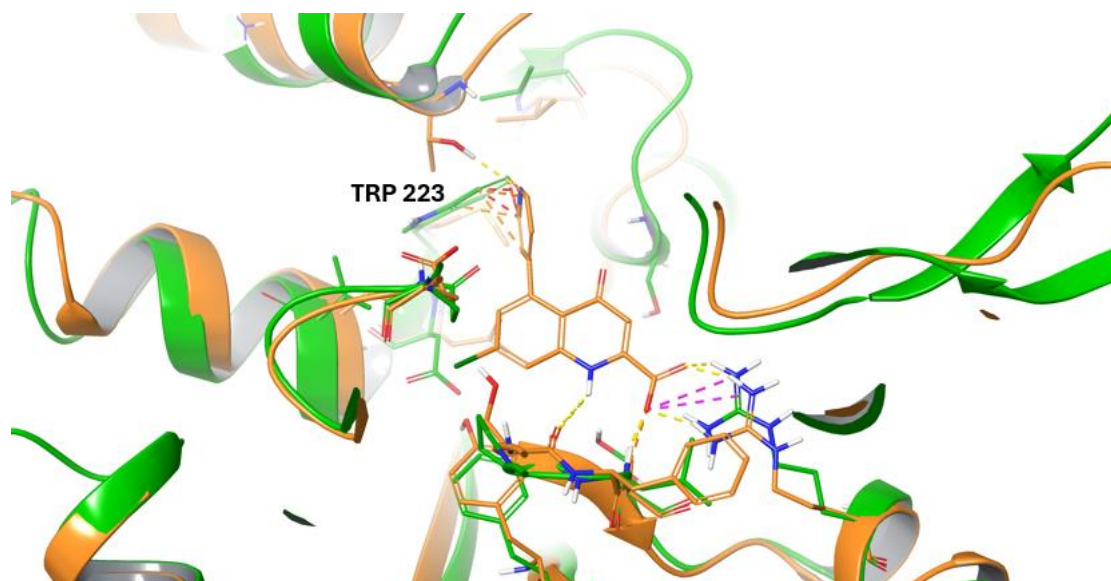
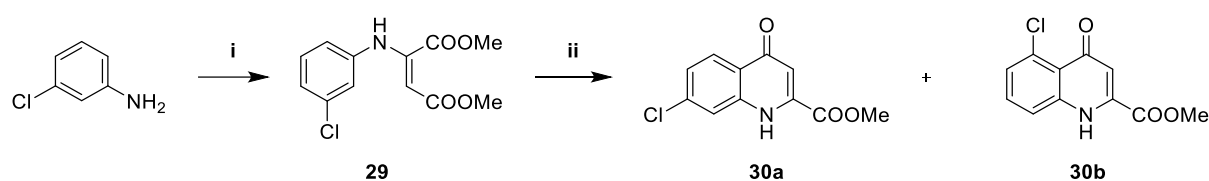


Figure 18. Superposition of GluN1 and GluN3A LBDs in complex with the **DCKA** analogue. The GluN3A LBD is shown in orange, and the GluN1 LBD in green. The GluN1-DCKA complex is derived from the crystal structure (PDB ID: 1PBQ). The GluN3A-DCKA complex is built from homology modeling and molecular docking.

The synthesis of intermediate **30a** (**Scheme 18**) has previously been reported by the group. The initial step involved a Michael condensation between 3-chloroaniline and dimethyl 2-butynedioate (DMAD), yielding compound **29**, followed by cyclization with Eaton's reagent to afford a mixture of regioisomers **30a** and **30b** (plausible mechanism, **Figure 107**; see Appendix 7.2) [37, 38]. Thereafter, the regioisomers were treated with sodium carbonate under monitored conditions, selectively hydrolysing one isomer and allowing separation, yielding pure **30a**. The final step involved Pd-catalyzed regioselective arylation of **30a** using diaryliodonium salts, followed by hydrolysis, affording analogous **15a-c** (**Scheme 19**) (plausible mechanism, **Figure 108**; see Appendix 7.2)[39]. Although it has been reported that 5 mol% catalyst loading is sufficient to promote regioselective arylation, no reaction was observed under these conditions.

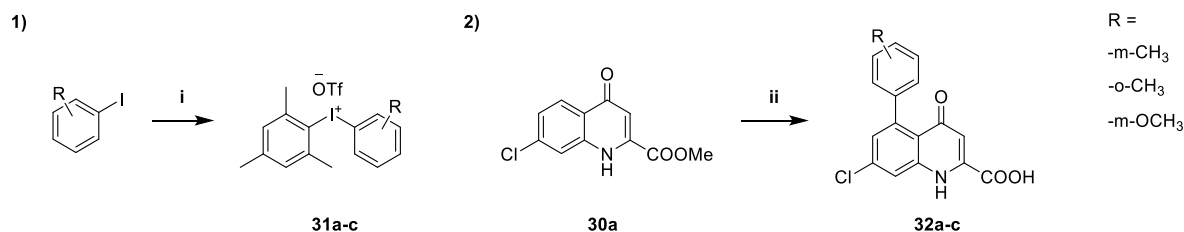
Stoichiometric amounts of catalyst were required to achieve around 33-41% conversion after 24 hours.

However, the final hydrolysis step with 2 M NaOH in THF was the biggest problem in this synthetic route, resulting in many impurities that were difficult to separate from the final compounds. This could be due to the reaction mixture from the previous step being taken directly into the final step without purification, as it was a small-scale reaction, starting from 50 mg of the starting material **30a**. As a result, some of the catalyst and other impurities remained in the reaction mixture, complicating the purification process. Analogues **32a-c** are therefore still under purification.



Scheme 18. Previously reported synthesis route of **30a**.^a

^a Reagents and conditions: (i) DMAD, EtOH, reflux, o/n, yield: 82%; (ii) Eaton's reagent, 60 °C, 2 h, yield: 30%, dr 50:50.



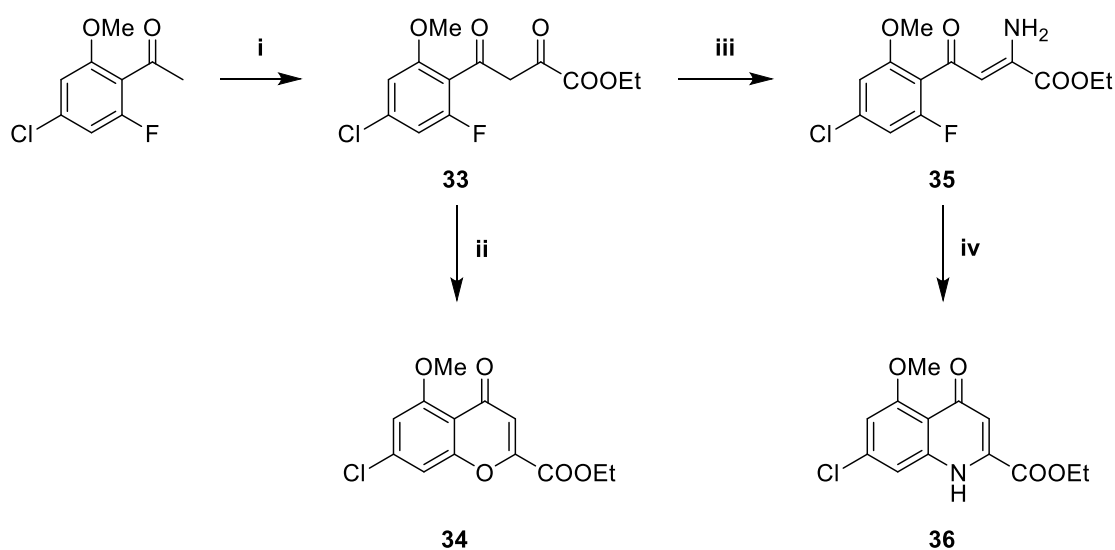
Scheme 19. Synthesis of diaryliodonium salts **31a-c** and analogues **32a-c**.^a

^a Reagents and conditions: (i) m-CPBA, mesitylene, TfOH, DCM, 0 °C to rt, 2 h, yield: 23-68%; (ii) (1) **31a-c**, Pd(OAc)₂, AcOH, 100 °C, o/n, (2) THF:2M NaOH (aq), rt, o/n.

Since only regioselective arylation could be achieved with the existing method, a new synthetic route was developed to enable the cross-coupling of various C-O bonds. This route began with a Claisen condensation between the commercially available starting material 1-(4-chloro-2-fluoro-6-methoxyphenyl)ethan-1-one and diethyl oxalate in the presence of LiHMDS, affording intermediate **33** (**Scheme 20**). A previous attempt using methoxide generated *in situ* from NaH and ethanol was also tested, but this condition did not produce the desired product. Although the next step was intended to be an amination followed by intramolecular nucleophilic substitution (plausible mechanism, **Figure 109**; see Appendix 7.2), during the acidic workup,

the majority of compound **33** underwent cyclization. This led to the formation of a chromene ring (compound **34**) [40, 41]. No procedure has been reported for converting the chromene ring to the quinoline ring within this specific scaffold. However, a potential solution could involve acidifying the reaction mixture under milder acidic conditions, such as in 1 M ammonium chloride, to prevent intramolecular cyclization before the amination step.

Another approach to this problem involves treating the chromene intermediate with a primary amine, leading to ring opening (**Figure 18**, plausible mechanism) [42]. This reaction is reported to occur with or without the presence of a base. However, the ester also has the potential to react with the primary amine. Furthermore, in the next step, to make the phenol a good leaving group, p-toluenesulfonyl chloride can be used to convert it into a sulfonate *in situ*. This sulfonate can then promote intramolecular nucleophilic substitution, forming the desired C-N bond and regenerating the quinoline ring [43]. The main challenge will then be to cleave the benzyl group from the quinoline nitrogen, which is not straightforward and will likely require specific conditions.



Scheme 20. Synthesis route for compound **36**.^a

^a Reagents and conditions: (i) (1) LiHMDS, THF, -78 °C, 30 min; (2) (COOEt)₂, -78 °C to rt, o/n; (ii) acidic workup with 1 M aq HCl; (iii) NH₄OAc, AcOH, PhMe, 115-155 °C, 5 h [44]; (iv) KOH, H₂O, 90-95 °C, 1 h [45].

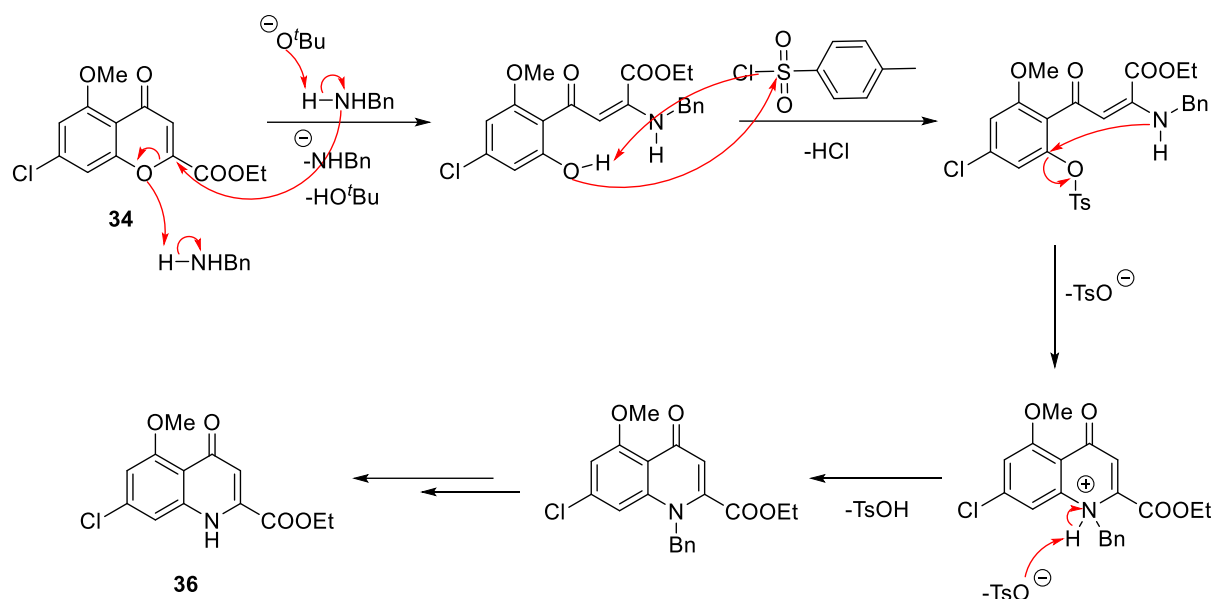


Figure 19. Plausible mechanism for the conversion of intermediate **34** to **36**.

3.5 Competitive antagonist **MDL-104,653**

The second competitive antagonist selected as a lead structure is **MDL-104,653**. This compound has also been previously reported as a selective competitive antagonist for the Gly/NMDA receptor, shown to inhibit NMDA receptor overactivation by selectively binding to the glycine site. Extensive research has focused on the bulk tolerance and hydrogen-bond donor regions to enhance potency at the Gly/NMDA receptor (**Figure 20**) [46, 47]. On the other hand, limited studies have been carried out on the size-limited hydrophobic region, which may offer possibilities for structural optimisation.

During the early literature research, we investigated different kinds of NMDARs glycine binding site competitive antagonists. Compound **TK67** was one of the few selective GluN3 competitive antagonists. Docking result of **TK67** with the LBDs of GluN1 and GluN3A revealed that a hydrogen bond formed between the nitrogen atom of the 2-aminobenzothiol ring and the Ser123 side chain in the GluN3A LBD (**Figure 21**). However, this interaction is not observed in the GluN1 LBD, where the corresponding residue is Pro124. We hypothesize that we can achieve GluN3A selectivity by combining the 2-aminothiazole with the **MDL-104,563**. Superimposing **TK67** and **MDL-104,653** (**Figure 22**) reveals that the 2-aminothiazole ring of **TK67** aligns well with the benzene ring of **MDL-104,653**. This modification could preserve key hydrogen-bonding interactions, potentially enhancing selectivity toward GluN3-containing NMDA receptors.

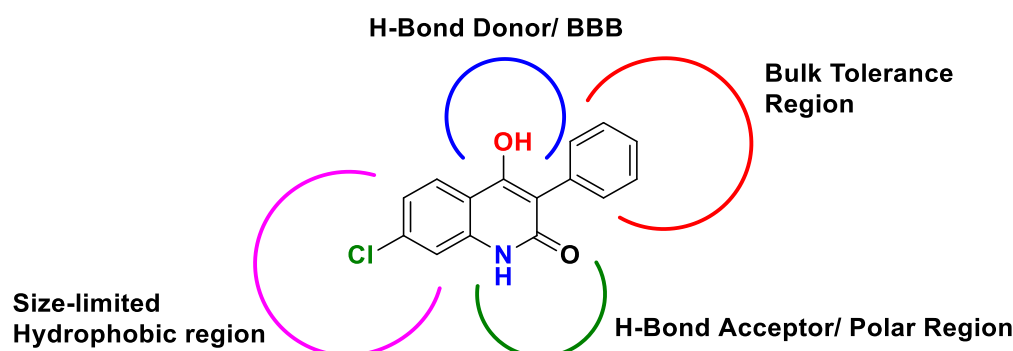


Figure 20. Key interaction regions of the **MDL-104,653** scaffold.

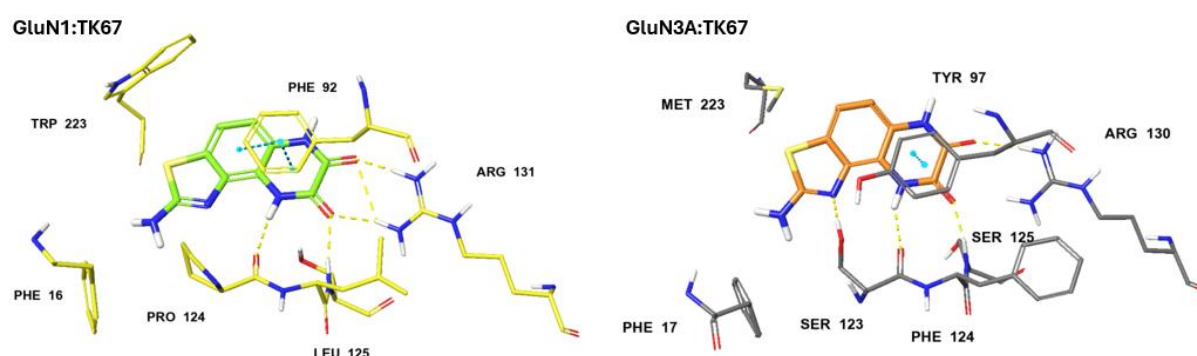


Figure 21. Docking analysis of the LBDs of GluN1 and GluN3A in complex with the TK67 scaffold. The GluN1 LBD is derived from the crystal structure (PDB ID: 1PBQ), while the GluN3A LBD is from homology modeling.

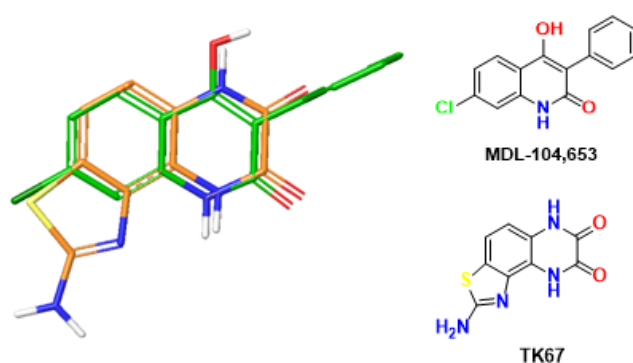
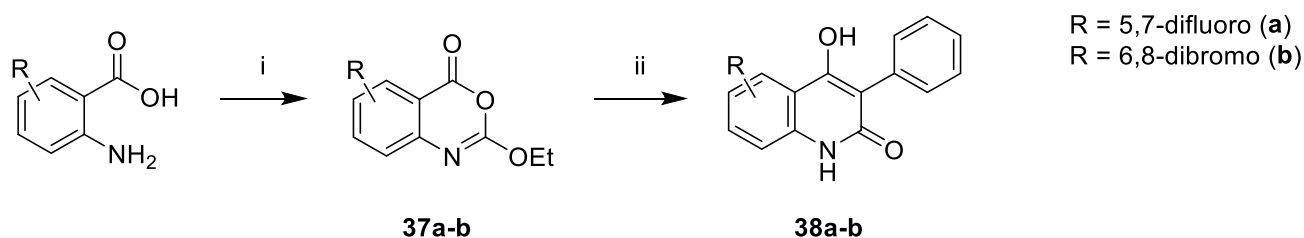


Figure 22. Superposition of **TK67** and **MDL-104,653** scaffolds. The TK67 scaffold is shown in orange, and MDL-104,653 in green.

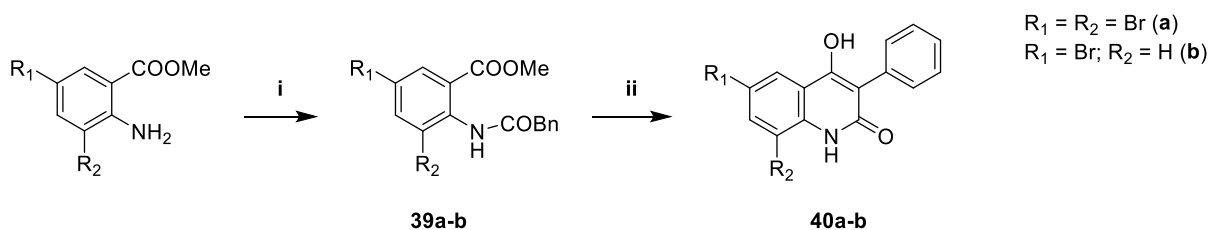
The previous reported synthesis of this competitive antagonist involved cyclization of substituted anthranilic acids with ethyl chloroacetate to form benzoxazinones (**37a-b**), followed by nucleophilic ring opening with arylacetic ester enolates and intramolecular cyclization to afford the desired compounds (**38a-b**) (**Scheme 21**) [48, 49]. However, despite multiple attempts with different substituted anthranilic acids, the first cyclization failed to yield the benzoxazinone intermediates, and the synthetic route was therefore abandoned.



Scheme 21. Previous reported synthesis route of the MDL-104,653 scaffold.^a

^a Reagents and conditions: (i) ClCOOEt, pyridine, rt, 3 h, yield: 0%; (ii) (1) LDA, -78 °C, 0.5 h; (2) BnCO₂Et, -78 °C, 0.5 h; (3) NaOCH₃, PhMe, 110 °C, 3h.

The new synthetic route began instead with the acylation of appropriate methyl anthranilates with 2-phenylacetyl chloride, followed by intramolecular cyclization in the presence of a strong base, yielding the desired compounds **40a-b** (Scheme 22). Compound **40b** was synthesized to confirm the efficiency of the new synthetic pathway. Compound **40a**, on the other hand, was prepared because the corresponding 6,8-dibromo analogue had not been previously reported for NMDA receptors, and the aim was to investigate whether replacing the key chlorine atom at the 7-position with two bromine atoms would affect receptor activity. Furthermore, analogue **40a** was obtained pure after workup, and no further purification was needed. This synthetic route is expected to yield pure intermediates and final compounds, as only specific positions and functional groups can undergo reaction, making it a selective reaction route for obtaining this type of scaffold.

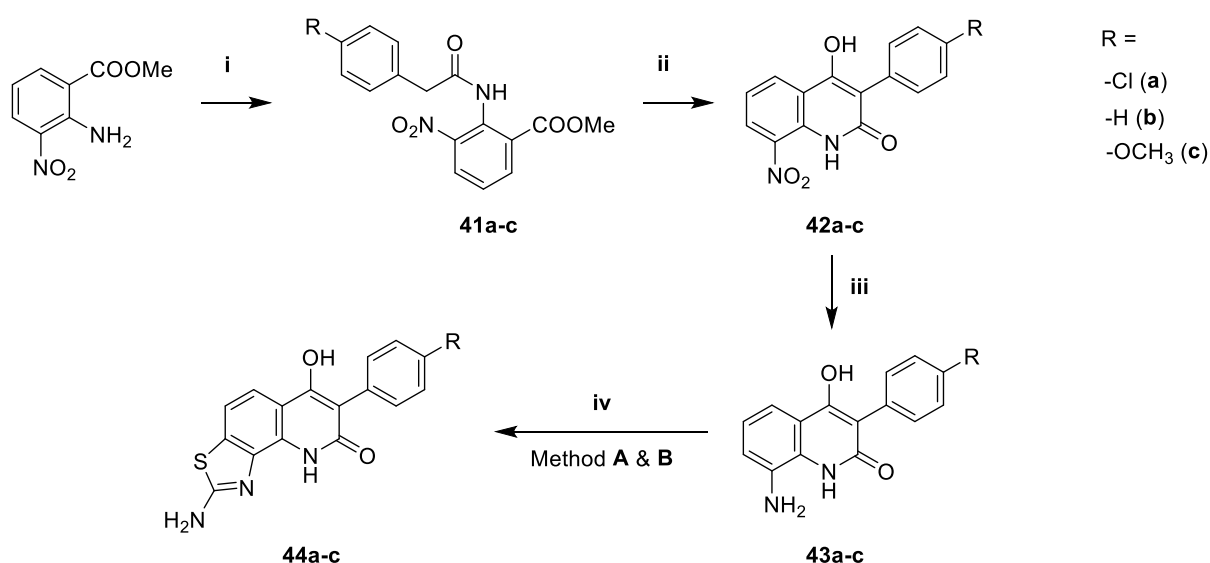


Scheme 22. Synthesis route of compounds **40a-b**.^a

^a Reagents and conditions: (i) BnCOCl, DCE, 90 °C, 3 h, yield: 54-60%; (ii) NaHMDS, THF, 0 °C to rt, o/n, yield: 35-39%.

To further develop the current scaffold and improve its activity for the specific NMDA subtypes, the introduction of the 2-aminobenzothiazole ring, inspired by the previous structure **TK67**, was considered again. The synthesis of the new analogues **44a-c** began with the acylation of methyl 3-nitroanthranilate using the appropriate phenylacetyl chlorides, followed by intramolecular cyclization in the presence of a strong base to afford intermediates **42a-c**

(**Scheme 23**). Thereafter, the nitro group was reduced, leading to the formation of the corresponding anilines **43a-c**. However, attempts to form the 2-aminobenzothiazole ring through the Hegerschoff method [27], using bromine as the oxidant, proved unsuccessful due to the harsh reaction conditions, resulting in no product formation. Alternatively, using benzyltrimethylammonium dichloroiodate and ammonium thiocyanate in DMSO:H₂O (9:1) gave slightly improved results, yielding the desired product **44a-c** in trace amounts [50]. However, the reaction lacked chemoselectivity, resulting in the formation of several side products.



Scheme 23. Synthesis route of analogues **44a-c**.^a

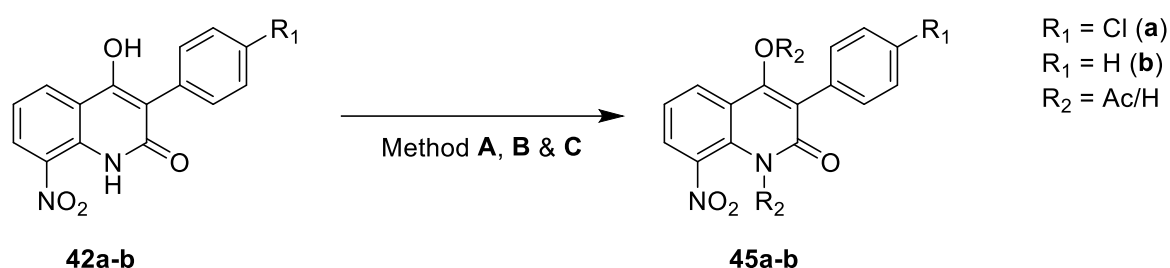
^a Reagents and conditions: (i) appropriate BnCOCl, DCE, 90 °C, 3 h, yield: 83-91%; (ii) LiHMDS, THF, 0 °C to rt, o/n, yield: 50-77%; (iii) 10% Pd/C, THF, 45 °C, 3-4 h, yield: 41-99%; (iv) (A) Br₂, KCNS, AcOH, 0 °C to rt, 22 h, yield: 0%; (B) PhCH₂NMe₃Cl₂I, NH₄SCN, DMSO:H₂O (9:1), 70 °C, 1 h, yield: 3-7 %.

The issue with chemoselectivity was later traced to the enol functional group formed via tautomerisation (**Figure 23**, plausible mechanism), which interfered with the formation of the 2-aminobenzothiazole ring. The first step in the plausible reaction mechanism is the oxidation of the thiourea sulfur to generate an electrophilic thiocarbonyl, followed by intramolecular EAS. The intramolecular EAS is probably the step where the enol functional group interferes and prevents the cyclization from happening.

Protecting the enol prior to the Hegerschoff method proved successful. Only acyl chlorides were effective among the screened protecting groups, resulting in a mixture of mono- and di-protected products where the enol and the amide group on the quinoline ring were protected (**Scheme 24**). Because of that, the ¹H NMR spectrum becomes complex and challenging, even

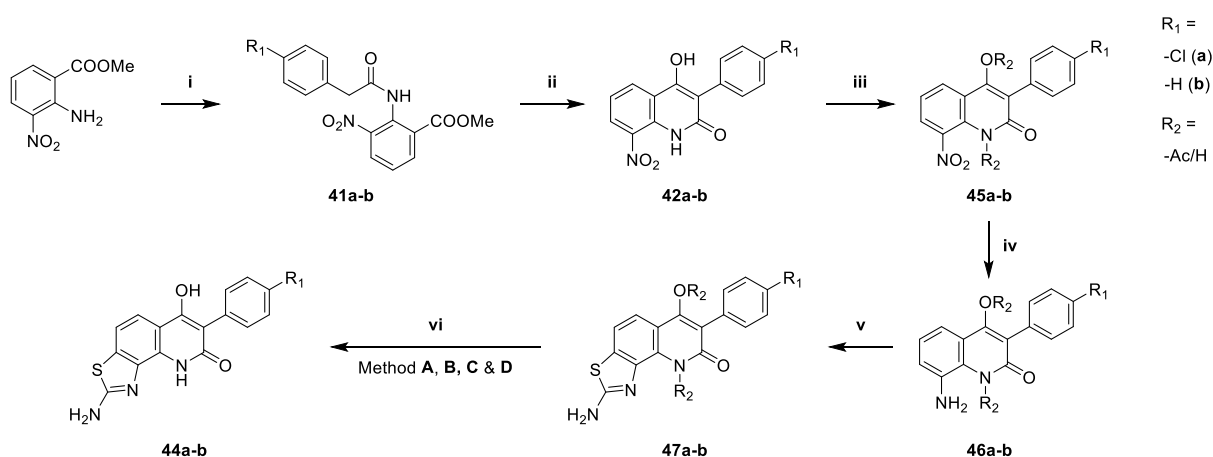
with 2D NMR, to interpret. However, with LCMS, the formation of the compounds can be proven. Furthermore, the final deprotection step in this synthetic route (**Scheme 25**) proved to be the main challenge, as acidic conditions led to decomposition of the intermediates, whereas mildly basic conditions, such as 5 M aqueous NaOH, proved ineffective. Only ammonium acetate in MeOH:H₂O (1:4) afforded partial product formation, although significant decomposition was still observed. Further investigation of the final deprotection step is required to obtain the desired compounds in higher yields.

After the deprotection step, analogues **44a-b** were purified using prep. HPLC. However, only **44b** was obtained, with 3 mg of isolated product at 94% purity. Due to the small quantity, the signal-to-noise ratio in the ¹H NMR spectrum is poor, and the compound could not be correctly assigned. Regardless, LCMS and HRMS analyses confirm the formation of the product, and no further optimization will be done until biological activity data are obtained.



Scheme 24. Screening of various protecting group conditions for intermediates **42a-b**.^a

^a Reagents and conditions: (**A**) TBDPSCl, LiHMDS, THF, -75 °C to rt, o/n, yield: 0%; (**B**) BnBr, LiHMDS, THF, 0 °C to rt, o/n, yield: 0%; (**C**) PMBCl, NaH or Cs₂CO₃, THF, 75 °C, o/n, yield: 0%; (**D**) CH₃COCl, TEA, DCM, rt, o/n, yield: 69-81%.



Scheme 25. Reaction route for analogue **44a-b**.^a

^a Reagents and conditions: **(i)** appropriate BnCOCl , DCE, 90 °C, o/n, yield: 83-91%; **(ii)** LiHMDS, THF, 0 °C to rt, o/n, yield: 50-77%; **(iii)** CH_3COCl , TEA, DCM, rt, o/n, yield: 69-81%; **(iv)** $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, EtOAc, 80 °C, o/n, yield: 45-98%; **(v)** $\text{PhCH}_2\text{NMe}_3\text{Cl}_2\text{I}$, NH_4SCN , DMSO: H_2O (9:1), 70 °C, 2.5 h, yield: 59-72%; **(vi)** **(A)** 4 N HCl in MeOH, rt, 1h, yield: 0%; **(B)** TFA, rt to 70 °C, 1-6 h, yield: 0%; **(C)** 5 M aq NaOH, rt to 100 °C, o/n, yield: 0%; **(D)** NH_4OAc , MeOH: H_2O (1:4), rt, o/n, yield: 2%.

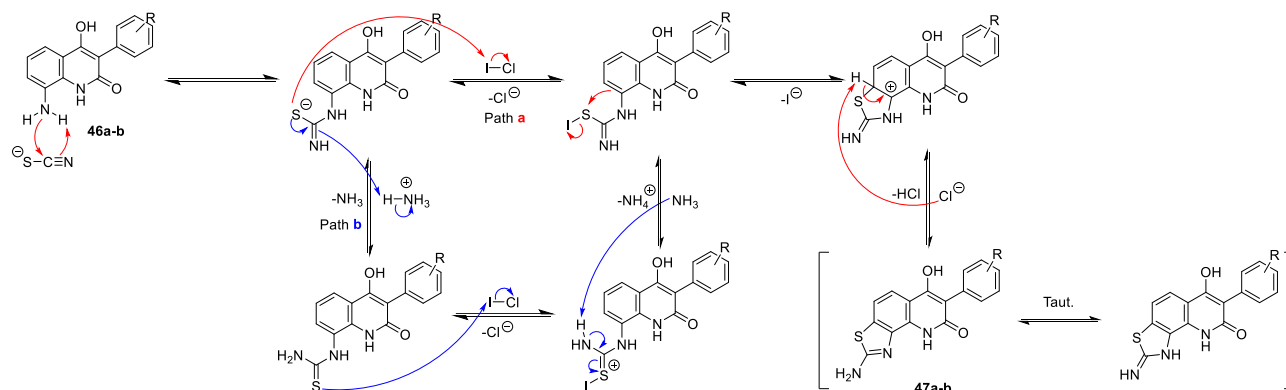


Figure 23. Plausible mechanism for the Hegerschoff method. Pathway a = red, b = blue.

4. Conclusions

The roles of GluN3 NMDARs in the central nervous system are still poorly understood. However, selective modulators of GluN3 containing NMDRs gave powerful tools for further investigating their physiological, functional, and pharmacological characteristics. In this master's thesis, a total of 19 novel compounds, derived from two parent allosteric modulators and two established competitive antagonists, were synthesized to target the GluN3-containing NMDARs.

We explored the steric tolerance of allosteric modulator **A** and introduced new fused ring systems to identify potential lead compounds. Additionally, efforts were made to modify the fused ring moiety of allosteric modulator **B**. However, the synthesis of certain compounds was unsuccessful due to their instability. Furthermore, a new synthetic route was successfully established to effectively introduce substituents at previously unexplored positions on allosteric modulator **C**. On the other hand, a series of non-selective competitive antagonists were selected to develop novel competitive antagonists for GluN3-containing NMDARs. A structure-based ligand design strategy was widely applied to guide the modifications.

For the **DCKA** scaffold, a superposition of the GluN1 and GluN3A LBDs in complex with the DCKA analogue was performed, revealing differences in the binding pockets. The GluN3A LBD did not exhibit the steric clash observed in the GluN1 LBD with the Trp223 residue. Hence, introducing bulky substituents at the 5-position was considered a strategy to enhance selectivity toward GluN3-containing NMDA receptors. This introduction of bulky groups was successfully achieved through Pd-catalyzed regioselective arylation using diaryliodonium salts.

Docking analysis of the LBDs of GluN1 and GluN3A with the competitive antagonist **TK67** revealed that the GluN3 selectivity of **TK67** could be explained by a potential hydrogen bond between the nitrogen of the 2-aminobenzothiol ring and Ser123 in GluN3A. This interaction is absent in GluN1, where the corresponding residue is Pro124. Superimposing the two scaffolds suggests that incorporating the 2-aminobenzothiol ring into the **MDL-104,653** scaffold could enhance selectivity for GluN3-containing NMDA receptors. Therefore, significant efforts have been dedicated to developing a selective synthetic route to obtain the desired compounds, with optimization of the final steps still ongoing.

5. Future aspects

The primary aim of this project was to synthesise novel ligands that selectively target GluN3-containing NMDARs. The next stage involves evaluating these compounds through pharmacological assays, which are being conducted in a collaborating department in the USA. We will apply these results to guide further optimization of the ligands. For allosteric modulators **A** and **B**, more detailed information about the binding pocket is needed before proceeding to the next stage of modification. We look forward to future structural elucidation of the receptor to better guide our ligand design.

For the competitive antagonist, we plan to develop a scaffold-based library built around several synthetically accessible core structures. A virtual screening workflow using machine learning and molecular docking will predict binding affinity toward the GluN3 LBD. Top-ranked hits will be refined through flexible or ensemble docking, and selected compounds will be synthesised and tested in biological assays. In our previous ligand design strategy, we developed the ligand via visual inspection and docking to suggest ligand candidates and integrate the bioactivity results to refine our ligand design, which can work well when ligand binding behaviour is well understood and bioactivity results are returned promptly. However, in this case, the binding mode of the competitive antagonist with the GluN3A LBD remains unclear. Therefore, all our hypotheses are highly subjective and risk leading the project in the wrong direction and potentially wasting time until bioactivity results are available. By adopting this new workflow, we can eliminate subjective speculation and explore a broader chemical space, increasing the likelihood of identifying effective substituents that might not have been considered otherwise.

6. Materials and Methods

6.1 General Procedures

Unless otherwise stated, all air- or moisture-sensitive reactions were carried out under an Ar atmosphere using standard Schlenk and syringe–septum techniques within flame-dried glassware. Anhydrous solvents for reactions were pre dried using a glass contour solvent system (SG Water, USA LLC). When necessary, solvents were dried over 4 Å molecular sieves prior to use. All chemical reagents were obtained from commercial sources and used without further purification. Thin-layer chromatography (TLC) was performed on Merck Silica Gel 60 F₂₅₄ aluminium plates (0.25 mm) to monitor reactions and chromatography fractions, with visualization by UV irradiation and potassium permanganate staining. Flash column chromatography was performed on silica gel 60 (Merck, 230–400 mesh, 0.040–0.063 mm). All nuclear magnetic resonance (NMR) spectra (¹H, ¹³C, ¹⁹F) were recorded at 300 K in the solvents indicated using Bruker Avance spectrometers operating at 400 or 600 MHz for ¹H, 100 or 150 MHz for ¹³C, and 376 MHz for ¹⁹F. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual solvent signals as internal standards. LCMS was performed using an Agilent 1200 HPLC system with a Zorbax Eclipse XBD-C₁₈ column (4.6 mm × 50 mm) coupled to a Hewlett Packard 1100 series mass spectrometer with an electrospray ionization source. A linear gradient of the binary solvent system H₂O/MeCN/formic acid (A = 95/5/0.1 and B = 5/95/0.1) was applied with a flow rate of 1 mL/min (0–100% B over 4 min, followed by 100% B for 0.5 min) for analysis. A 30 mg/mL solution of 2,5-Dihydroxybenzoic acid (DHB) in methanol was used as the MALDI matrix and mixed with the samples. High-resolution mass spectrometry (HRMS) was performed on a QExactive Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a SMALDI5 ion source (TransMIT GmbH, Giessen, Germany). The sample was analysed in the positive ion mode at 140,000 mass resolving power, using a peak from the DHB matrix for internal mass calibration, whereby a mass accuracy of 3 ppm or better was achieved. Analytical HPLC was performed on a system composed of an Ultimate 3000 pump with a PDA detector and a TSP AS-3000 autosampler, using a Gemini-NX C₁₈ column (4.6 mm × 250 mm). A linear gradient of the binary solvent system H₂O/MeCN/TFA (A = 95:5:0.1 and B = 5:95:0.1) was applied, increasing the concentration of B from 0 to 100% over 20 min at a flow rate of 1.0 mL/min. Final compounds were purified by preparative HPLC using an Ultimate 3000 system equipped with a Gemini-NX C₁₈ column (21 mm × 250 mm), with the same binary solvent system at a flow rate of 20 mL/min. Unless

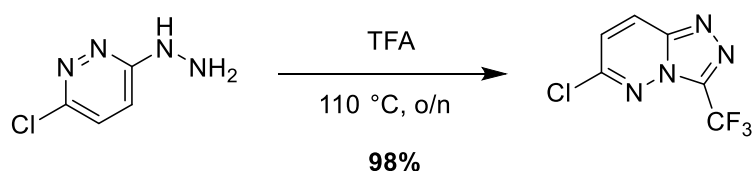
otherwise noted, the purities of all final compounds were determined by analytical HPLC to be $\geq 95\%$.

6.2 Molecular Modeling

All modeling was carried out in the Schrodinger Maestro program, Maestro version 14.0, Release 2024-2. Since the antagonist-bound LBD structure of GluN3A has not been reported, we constructed a homology model based on a previously published protocol [9]. Specifically, we adapted the crystal structure of the agonist-bound GluN3A:D-serine complex (PDB ID: 2RC8) to the receptor conformation of the GluN1:DCKA complex (PDB ID: 1PBQ), in order to generate an open-state model of the GluN3A LBD suitable for subsequent docking studies.

The GluN1:DCKA ABD (PDB ID: 1PBQ) and the GluN3A:D-serine ABD (PDB ID: 2RC8) crystal structures were downloaded from the RCSB Protein Data Bank and prepared using the Protein Preparation Workflow in the Schrödinger Suite. Protein bond orders were reassigned using the CCD database. All water molecules were removed, and the protonation states of ligands and receptors were set to pH 7.0. Restrained minimization was performed using the OPLS4 force field. The resulting protein complex structures were then used for docking. Ligand structures were prepared with the LigPrep module in the Schrödinger suites. Ligands were prepared with the OPLS4 force field, ionization states were generated with Epik at pH 7.0, and the energy-minimized conformation was used for docking. The agonist binding pockets of GluN1 and GluN3A were defined using the Receptor Grid Generation module, based on the location of the co-crystallized DCKA and the geometric centre of key binding site residues: SER123, PHE124, SER125, ARG130, TYR97, MET223, and PHE17, respectively.

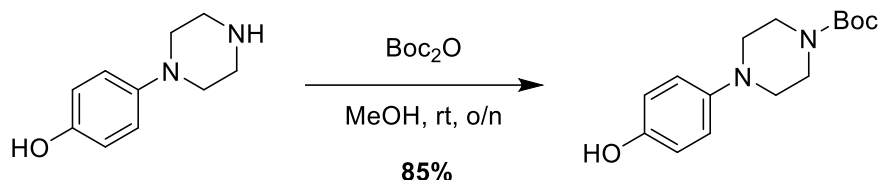
6.3 Chemistry



6-chloro-3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazine (1)

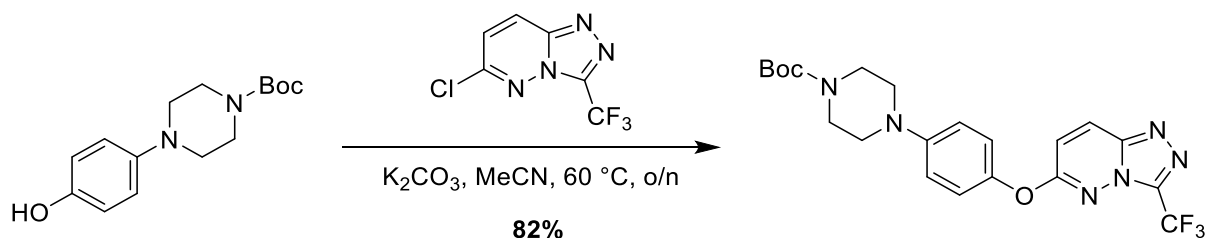
A suspension of 3-chloro-6-hydrazinylpyridazine (1.01 g, 6.98 mmol, 1.0 equiv) and TFA (2.65 mL, 34.63 mmol, 5.0 equiv) was heated at 110 °C overnight. The reaction mixture was cooled to rt, and TFA was removed in vacuo. The residue was diluted with sat. NaHCO₃ (10 mL) and the product was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced

pressure. The resulting crude compound was purified by recrystallization (*n*-heptane/EtOAc = 10:1) to yield the desired compound (1.53 g, 98.3%) as pale brown crystals. ¹H NMR (600 MHz, CDCl₃-*d*) δ 8.17 (d, *J* = 9.5 Hz, 1H), 7.27 (d, *J* = 0.7 Hz, 1H). LCMS (ESI, *m/z*) Calcd for C₆H₂ClF₃N₄ 222.0, Found 223.0 [M + H]⁺; retention time 3.85 min, LCMS purity > 98%.



Tert-butyl 4-(4-hydroxyphenyl)piperazine-1-carboxylate (2)

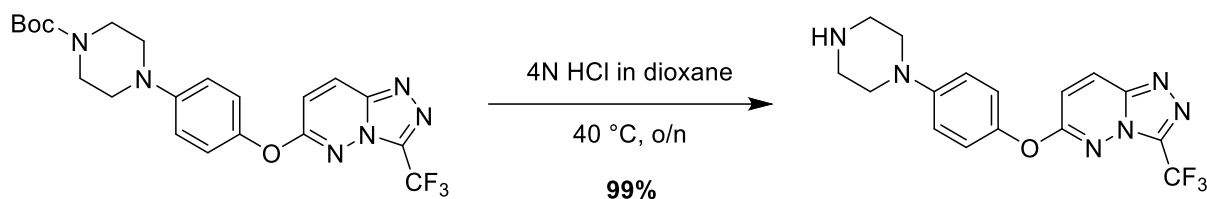
The synthesis was carried out in a round-bottom flask by adding 0.503 g (2.82 mmol, 1.0 equiv) of 4-(piperazin-1-yl)phenol to a solution of Boc₂O (0.747 g, 3.42 mmol, 1.2 equiv) in 20 mL of MeOH. The reaction mixture was then stirred at rt overnight. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (SiO₂, *n*-heptane/EtOAc = 3:1, *R_f* = 0.25), yielding the desired compound (0.66 g, 84.5%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.85 (s, 1H), 6.83 – 6.77 (m, 2H), 6.69 – 6.62 (m, 2H), 3.43 (t, *J* = 5.1 Hz, 4H), 2.92 – 2.85 (m, 4H), 1.42 (s, 9H). LCMS (ESI, *m/z*) Calcd for C₁₅H₂₂N₂O₃ 278.2, Found 279.3 [M + H]⁺; retention time 3.98 min, LCMS purity > 98%.



Tert-butyl 4-((3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)oxy)phenyl)piperazine-1-carboxylate (3)

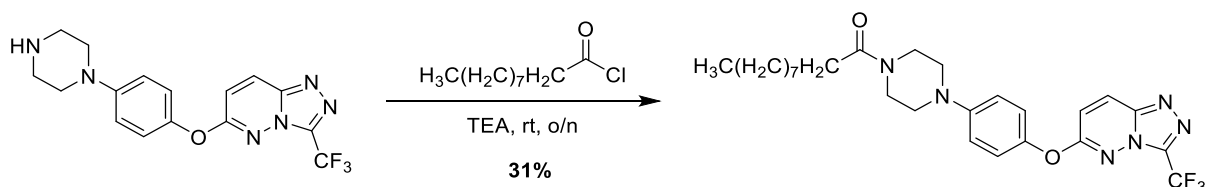
A mixture of **1** (0.534 g, 2.40 mmol), **2** (0.664 g, 2.38 mmol), and K₂CO₃ (0.991 g, 7.17 mmol) was dissolved in MeCN (10 mL) in a sealed reaction vial and heated at 60 °C overnight. After cooling to rt, the mixture was filtered through a pad of Celite and concentrated under reduced pressure. The crude residue was diluted with water (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na₂SO₄, and concentrated. The crude product was purified by column chromatography (SiO₂, *n*-heptane/EtOAc = 1:1, *R_f* = 0.22), yielding the desired compound (0.91 g, 82.3%) as a white solid. ¹H NMR (600 MHz, CDCl₃-*d*) δ 8.09 (d, *J* = 9.9 Hz, 1H), 7.12 – 7.06 (m, 3H), 6.91 (d, *J*

= 8.6 Hz, 2H), 3.55 (t, J = 5.1 Hz, 4H), 3.10 (t, J = 5.1 Hz, 4H), 1.43 (d, J = 0.8 Hz, 9H). LCMS (ESI, m/z) Calcd for $C_{21}H_{23}F_3N_6O_3$: 464.2, Found: 465.2 $[M + H]^+$; retention time 4.81 min, LCMS purity > 99%.



6-(4-(piperazin-1-yl)phenoxy)-3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazine (4)

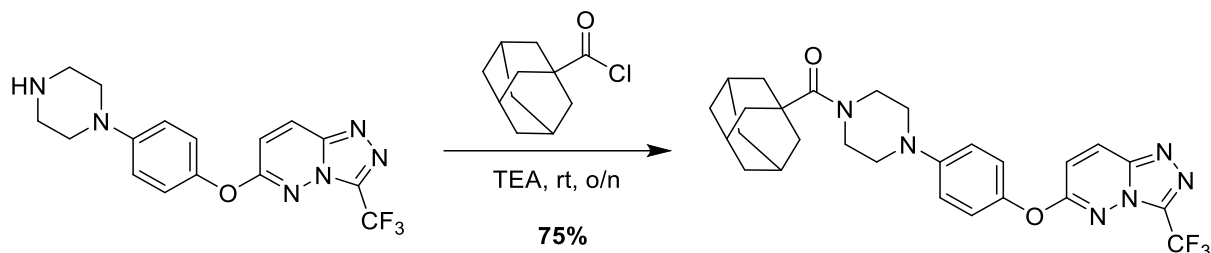
In a 50 mL round-bottom flask, **3** (0.911 g, 1.96 mmol, 1.0 equiv) was dissolved in 4N HCl in dioxane (20 mL). The reaction mixture was stirred at 40 °C overnight. After completion, the solvent was removed under reduced pressure to afford the desired compound (0.71 g, 98.9%) as a white solid. 1H NMR (600 MHz, $CDCl_3-d$) δ 8.15 (d, J = 9.9 Hz, 1H), 7.17 – 7.11 (m, 3H), 6.99 – 6.93 (m, 2H), 3.47 (s, 1H), 3.20 – 3.15 (m, 4H), 3.09 – 3.04 (m, 4H). LCMS (ESI, m/z) Calcd for $C_{16}H_{15}F_3N_6O$: 364.1, Found: 365.1 $[M + H]^+$; retention time 3.35 min, LCMS purity > 98%.



1-(4-(4-((3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)oxy)phenyl)piperazin-1-yl)decan-1-one (5a)

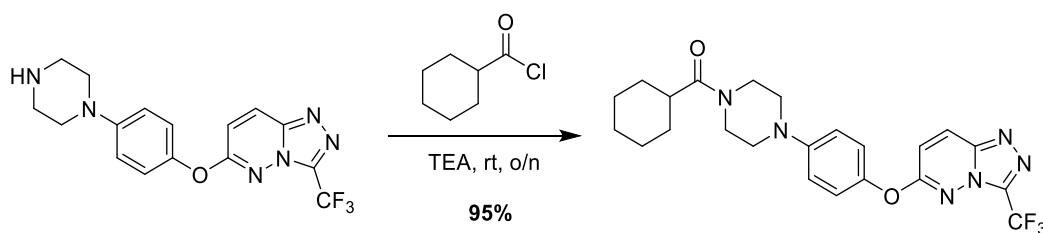
A mixture of **4** (50.0 mg, 0.14 mmol, 1.0 equiv), decanoyl chloride (72.6 μ L, 0.35 mmol, 2.5 equiv), and TEA (66.6 μ L, 0.48 mmol, 3.5 equiv) in 6 mL DCM were stirred at rt overnight. The reaction mixture was concentrated, diluted with 1 M HCl (10 mL), and extracted with EtOAc (3×20). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2 , n -heptane/EtOAc = 3:1, R_f = 0.30), yielding the desired compound (21.8 mg, 30.6%) as a colorless oil. 1H NMR (600 MHz, $CDCl_3-d$) δ 8.15 (d, J = 9.9 Hz, 1H), 7.15 – 7.07 (m, 3H), 6.93 (d, J = 8.5 Hz, 2H), 3.85 – 3.57 (m, 4H), 3.14 (dt, J = 20.1, 5.1 Hz, 4H), 2.34 – 2.30 (m, 2H), 2.27 (t, J = 7.5 Hz, 4H), 1.90 – 1.83 (m, 2H), 1.73 – 1.65 (m, 2H), 1.63 – 1.52 (m, 7H), 1.43 – 1.34 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3-d$) δ 180.7, 178.4, 171.1, 160.4, 126.0, 120.5, 116.9, 116.6, 33.1, 32.3, 30.9 (d, J = 2.9 Hz), 28.5 (d, J = 2.9 Hz), 28.4 (d, J = 5.5 Hz), 28.3 (d, J = 4.8 Hz), 28.1, 27.8, 24.7, 24.4 (d, J = 6.5 Hz),

23.7, 21.6, 13.1. LCMS (ESI, m/z) Calcd for $C_{26}H_{33}F_3N_6O_2$: 518.3, Found: 519.3 $[M + H]^+$; retention time 5.41 min. HPLC purity > 90%.



Adamantan-1-yl(4-(4-((3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)oxy)phenyl)piperazin-1-yl)methanone (5b)

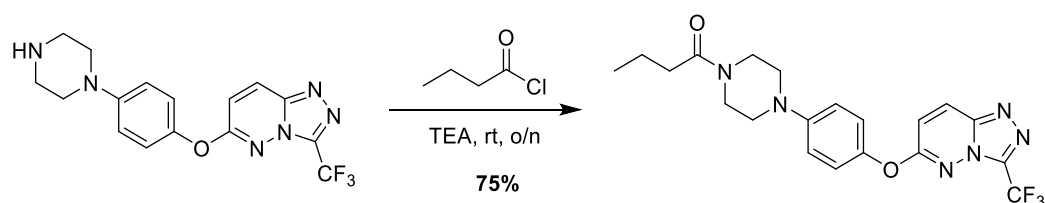
Prepared following the procedure described for **5a**, starting from **4** (50.0 mg, 0.14 mmol, 1.0 equiv), adamantane-1-carbonyl chloride (71.1 mg, 0.36 mmol, 2.5 equiv), and TEA (66.6 μ L, 0.48 mmol, 3.5 equiv). The crude product was purified by column chromatography (SiO_2 , n -heptane/EtOAc = 3:1, R_f = 0.34), yielding the desired compound (54.0 mg, 74.7%) as a white solid. 1H NMR (600 MHz, $CDCl_3$ - d) δ 8.10 (d, J = 9.9 Hz, 1H), 7.14 – 7.06 (m, 3H), 6.94 (d, J = 8.0 Hz, 2H), 3.84 (t, J = 5.2 Hz, 4H), 3.14 (t, J = 5.0 Hz, 4H), 2.02 – 1.95 (m, 9H), 1.72 – 1.64 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$ - d) δ 174.9, 160.3, 143.8, 138.7 (q, J = 41.7 Hz), 125.9, 120.5, 117.0 (q, J = 270.5 Hz), 116.8, 40.7, 38.1, 35.6, 27.5. LCMS (ESI, m/z) Calcd for $C_{27}H_{29}F_3N_6O_2$: 526.2, Found: 527.2 $[M + H]^+$; retention time 5.13 min. HPLC purity > 95%. HRMS (ESI) m/z : $[M + H]^+$ Calcd for $C_{27}H_{29}F_3N_6O_2$ 527.2376; Found 527.2365.



Cyclohexyl(4-(4-((3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)oxy)phenyl)piperazin-1-yl)methanone (5c)

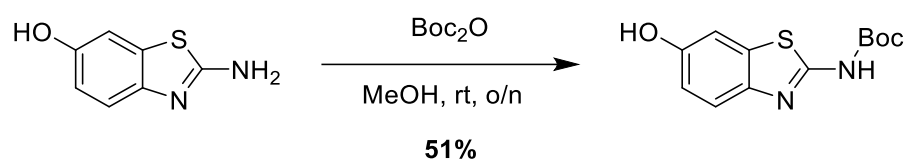
Prepared following the procedure described for **5a**, starting from **4** (69.0 mg, 0.19 mmol, 1.0 equiv), cyclohexanecarbonyl chloride (69.3 μ L, 0.36 mmol, 2.5 equiv), and TEA (91.9 μ L, 0.66 mmol, 3.5 equiv). The crude product was purified by column chromatography (SiO_2 , n -heptane/EtOAc = 3:1, R_f = 0.29), yielding the desired compound (86.3 mg, 96.0%) as a white solid. 1H NMR (600 MHz, $CDCl_3$ - d) δ 8.10 (d, J = 9.9 Hz, 1H), 7.13 (d, J = 8.5 Hz, 2H), 7.08 (d, J = 9.8 Hz, 1H), 6.98 (s, 2H), 3.72 (d, J = 63.5 Hz, 4H), 3.15 (d, J = 21.4 Hz, 4H), 2.45 (tt, J = 11.6, 3.4 Hz, 1H), 1.80 – 1.45 (m, 5H), 1.21 (m, 5H). ^{13}C NMR (151 MHz, $CDCl_3$ - d) δ

173.7, 160.4, 138.7 (q, $J = 42.2$ Hz), 125.9, 120.5, 117.0 (q, $J = 270.7$ Hz), 116.8, 39.5, 28.4, 24.8 (d, $J = 4.7$ Hz). LCMS (ESI, m/z) Calcd for $C_{23}H_{25}F_3N_6O_2$: 474.2, Found: 475.2 $[M + H]^+$; retention time 4.83 min. HPLC purity > 95%. HRMS (ESI) m/z : $[M + H]^+$ Calcd for $C_{23}H_{25}F_3N_6O_2$ 475.2063; Found 475.2056.



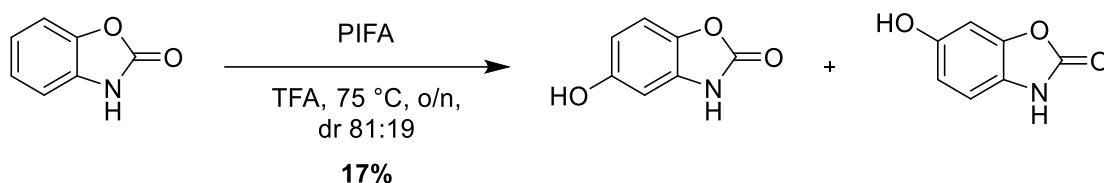
1-(4-(4-((3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)oxy)phenyl)piperazin-1-yl)butan-1-one (5d)

Prepared following the procedure described for **5a**, starting from **4** (114.3 mg, 0.31 mmol, 1.0 equiv), butyryl chloride (81.1 μ L, 0.78 mmol, 2.5 equiv), and TEA (152.2 μ L, 1.10 mmol, 3.5 equiv). The crude product was purified by column chromatography (SiO_2 , n -heptane/EtOAc = 3:1, $R_f = 0.32$), yielding the desired compound (102.1 mg, 74.9%) as a colorless oil. 1H NMR (600 MHz, $CDCl_3-d$) δ 8.10 (d, $J = 9.9$ Hz, 1H), 7.15 – 7.06 (m, 3H), 6.94 (d, $J = 8.4$ Hz, 2H), 3.76 (t, $J = 5.0$ Hz, 2H), 3.64 – 3.59 (m, 2H), 3.14 (dt, $J = 18.1, 5.2$ Hz, 4H), 2.33 – 2.27 (m, 2H), 1.63 (h, $J = 7.4$ Hz, 2H), 0.93 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3-d$) δ 170.6, 160.4, 143.8, 138.7 (q, $J = 41.6$ Hz), 125.9, 120.5, 117.0 (q, $J = 270.5$ Hz), 116.8, 44.4, 40.2, 34.2, 17.7, 13.0. LCMS (ESI, m/z) Calcd for $C_{20}H_{21}F_3N_6O_2$: 434.2, Found: 435.2 $[M + H]^+$; retention time 4.52 min. HPLC purity > 97%. HRMS (ESI) m/z : $[M + H]^+$ Calcd for $C_{20}H_{21}F_3N_6O_2$ 435.1750; Found 435.1740.



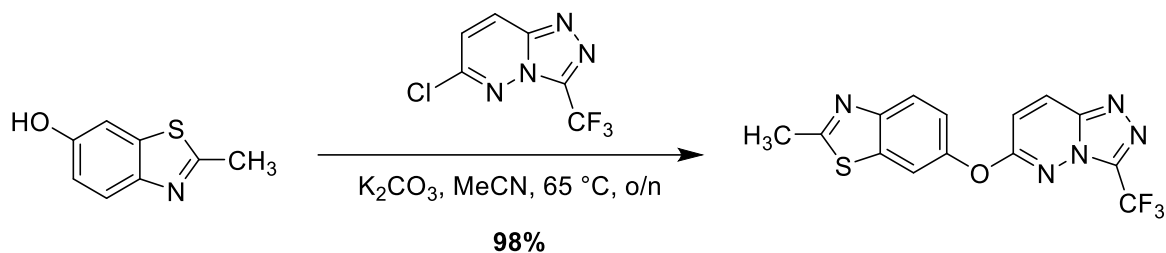
Tert-butyl (6-hydroxybenzo[d]thiazol-2-yl)carbamate (6)

Prepared following the procedure described for **1**, starting from 2-aminobenzo[d]thiazol-6-ol (0.589 g, 3.54 mmol, 1.0 equiv) and Boc_2O (0.773 g, 3.54 mmol, 1.0 equiv). The crude product was purified by column chromatography (SiO_2 , n -heptane/EtOAc = 3:2, $R_f = 0.15$), yielding the desired compound (0.485 g, 51.4%) as a pink solid. 1H NMR (400 MHz, $CDCl_3-d$) δ 11.47 (s, 1H), 9.44 (s, 1H), 7.46 (d, $J = 8.7$ Hz, 1H), 7.22 (d, $J = 2.4$ Hz, 1H), 6.83 (dd, $J = 8.7, 2.5$ Hz, 1H), 1.50 (s, 9H). LCMS (ESI, m/z) Calcd for $C_{12}H_{14}N_2O_3S$: 266.1, Found: 267.2 $[M + H]^+$; retention time 4.07 min, LCMS purity > 99%.



5-hydroxybenzo[d]oxazol-2(3H)-one (7)

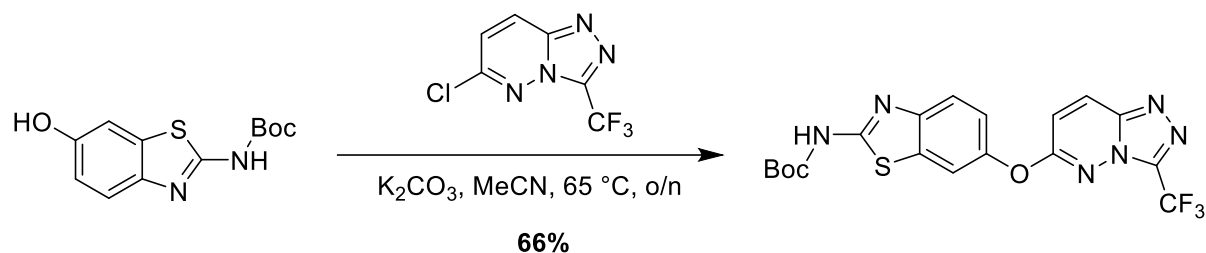
To a stirred solution of 2-benzoxazoline (150 mg, 1.11 mmol, 1.0 equiv) in trifluoroacetic acid (10 mL), PIFA (572.9 mg, 1.33 mmol, 1.2 equiv) was added. The reaction mixture was then refluxed overnight. Upon completion, the mixture was quenched with sat. NaHCO_3 solution (10 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product (28.9 mg, 17.2%) was used directly in the next step without further purification. ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 11.36 (s, 1H), 9.36 (s, 1H), 7.05 (d, $J = 8.6$ Hz, 1H), 6.49 (d, $J = 2.4$ Hz, 1H), 6.44 (dd, $J = 8.6, 2.5$ Hz, 1H). LCMS (ESI, m/z) Calcd for $\text{C}_7\text{H}_5\text{NO}_3$: 151.0, Found: 152.0 $[\text{M} + \text{H}]^+$; retention time 2.55 min (minor) and 2.86 (major), LCMS purity > 86%.



2-methyl-6-((3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)oxy)benzo[d]thiazole (8a)

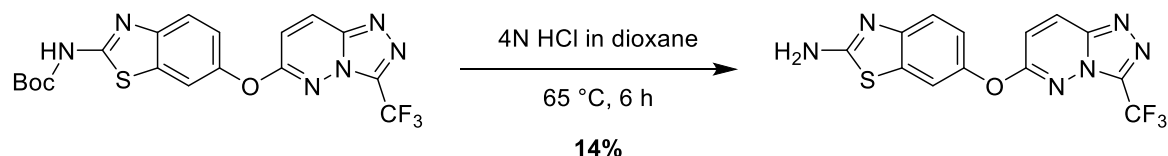
2-Methylbenzo[d]thiazol-6-ol (0.100 g, 0.605 mmol, 1.0 equiv) was dissolved in MeCN (6 mL). To this solution, **2** (0.135 g, 0.605 mmol, 1.0 equiv) and K_2CO_3 (0.251 g, 1.82 mmol, 3.0 equiv) were added. The reaction mixture was stirred at 65 °C overnight. Upon completion, the mixture was filtered through a pad of Celite and concentrated under reduced pressure. The resulting crude product was purified by recrystallization from a mixture of ethyl acetate and *n*-heptane to afford the desired compound as a light beige solid (0.208 g, 97.8%). ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 8.63 (d, $J = 9.9$ Hz, 1H), 8.15 (d, $J = 8.7$ Hz, 1H), 7.94 (d, $J = 2.3$ Hz, 1H), 7.58 (d, $J = 10.0$ Hz, 1H), 7.43 (dd, $J = 8.7, 2.4$ Hz, 1H), 2.83 (s, 3H). ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ 170.0, 162.1, 154.2, 151.1, 146.0, 138.4 (q, $J = 40.5$ Hz), 133.1, 128.2, 123.3, 119.3, 118.7, 118.6 (q, $J = 270.3$ Hz), 114.4, 20.4. LCMS (ESI, m/z) Calcd for $\text{C}_{14}\text{H}_8\text{F}_3\text{N}_5\text{OS}$: 351.0, Found:

352.1 [M + H]⁺; retention time 4.28 min. HPLC purity > 99%. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₁₄H₈F₃N₅OS 352.0474; Found 352.0478.



Tert-butyl (6-((3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)oxy)benzo[d]thiazol-2-yl)carbamate (8b)

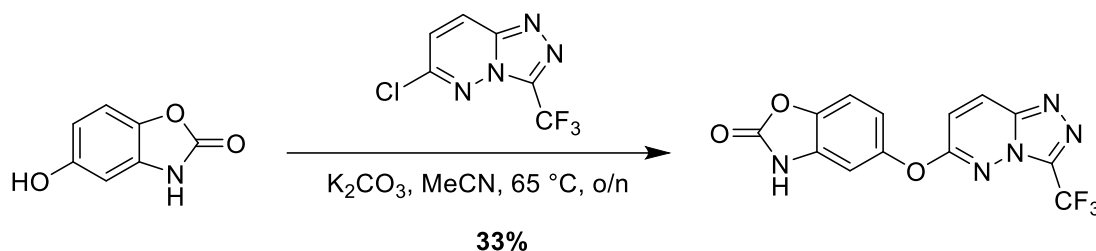
Prepared following the procedure described for **8a**, starting from **6** (0.178 g, 0.669 mmol, 1.0 equiv), **2** (0.194 g, 0.870 mmol, 1.3 equiv), and K₂CO₃ (0.324 g, 2.34 mmol, 3.5 equiv). The crude product was purified by pre-HPLC (isocratic elution, 55% B, *R_t* = 9.41 min) to afford the desired compound (0.200 g, 66.1%) as a white solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 11.86 (s, 1H), 8.62 (d, *J* = 9.9 Hz, 1H), 7.98 (d, *J* = 2.5 Hz, 1H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.57 (d, *J* = 10.0 Hz, 1H), 7.40 (dd, *J* = 8.7, 2.5 Hz, 1H), 1.53 (s, 9H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 162.3, 160.6, 148.2, 147.8, 145.9, 138.4 (q, *J* = 40.0 Hz), 132.8, 128.2, 121.3, 119.9, 119.2, 118.6 (q, *J* = 269.5 Hz), 114.3, 82.4, 28.3. LCMS (ESI, *m/z*) Calcd for C₁₈H₁₅F₃N₆O₃S: 452.1, Found: 453.1 [M + H]⁺; retention time 4.57 min. HPLC purity > 95%. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₁₈H₁₅F₃N₆O₃S 453.0951; Found 453.0941.



6-((3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)oxy)benzo[d]thiazol-2-amine (8c)

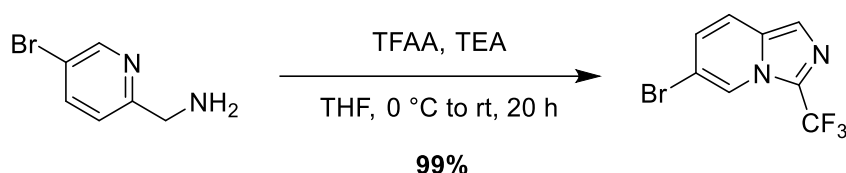
Prepared following the procedure described for **4**, starting from **8b** (0.200 g, 0.442 mmol, 1.0 equiv). The crude product was purified by pre-HPLC (isocratic elution, 30% B, *R_t* = 4.72 min) to afford the desired compound (22.4 mg, 14.4%) as a white solid. ¹H NMR (600 MHz, methanol-*d*₄) δ 8.30 (d, *J* = 9.9 Hz, 1H), 7.70 (d, *J* = 2.4 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 1H), 7.37 (d, *J* = 9.9 Hz, 1H), 7.35 (dd, *J* = 8.8, 2.4 Hz, 1H). ¹³C NMR (151 MHz, methanol-*d*₄) δ 170.3, 162.1, 148.5, 145.5, 139.4, 139.0 (q, *J* = 41.4 Hz), 126.9, 126.3, 120.9, 118.7, 118.1 (q, *J* = 269.6 Hz), 115.3. LCMS (ESI, *m/z*) Calcd for C₁₃H₇F₃N₆OS: 352.0, Found: 353.1 [M + H]⁺;

retention time 3.38 min. HPLC purity > 98%. HRMS (ESI) m/z : $[M + H]^+$ Calcd for $C_{13}H_7F_3N_6OS$ 353.0427; Found 353.0419.



5-((3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)oxy)benzo[d]oxazol-2(3H)-one (8d)

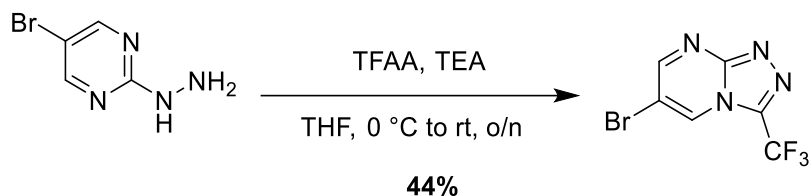
Prepared following the procedure described for **8a**, starting from **7** (28.90 mg, 0.191 mmol, 1.0 equiv), **2** (42.56 mg, 0.191 mmol, 1.0 equiv), and K_2CO_3 (79.29 mg, 0.574 mmol, 3.0 equiv). The crude product was purified by pre-HPLC (isocratic elution, 30% B, R_t = 14.29 min) to afford the desired compound (21.4 mg, 33.2%) as a white solid. 1H NMR (600 MHz, $DMSO-d_6$) δ 11.88 (s, 1H), 8.61 (d, J = 9.9 Hz, 1H), 7.52 (d, J = 9.9 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.18 (d, J = 2.4 Hz, 1H), 7.06 (dd, J = 8.6, 2.5 Hz, 1H). ^{13}C NMR (151 MHz, $DMSO-d_6$) δ 162.2, 155.1, 148.6, 145.9, 141.4, 138.4 (q, J = 40.6 Hz), 131.5, 128.1, 119.2, 118.6 (q, J = 270.3 Hz), 114.6, 110.6, 103.9. LCMS (ESI, m/z) Calcd for $C_{13}H_6F_3N_5OS$: 337.0, Found: 338.1 $[M + H]^+$; retention time 3.78 min. HPLC purity > 98%. HRMS (ESI) m/z : $[M + H]^+$ Calcd for $C_{13}H_6F_3N_5OS$ 339.0529; Found 339.0526.



6-bromo-3-(trifluoromethyl)imidazo[1,5-a]pyridine (9a)

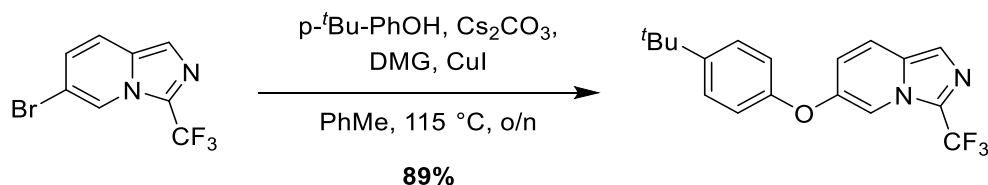
To a flame-dried sealed reaction vial equipped with a magnetic stir bar and sealed under an argon atmosphere, (5-bromopyridin-2-yl)methanamine (0.426 g, 2.28 mmol, 1.0 equiv) was added in anhydrous THF (5 mL). The solution was cooled to 0 °C, followed by the dropwise addition of TEA (0.79 mL, 5.69 mmol, 2.5 equiv). After stirring at 0 °C for 5 minutes, trifluoroacetic anhydride (0.87 mL, 6.15 mmol, 2.7 equiv) was added. The reaction mixture was stirred at 0 °C for an additional 30 minutes, then allowed to warm to rt and stirred overnight. The solvent was then removed under reduced pressure, and the resulting residue was diluted with water (25 mL) and extracted with EtOAc (3 $\times$ 20 mL). The combined organic layers were

washed with brine (25 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product (0.599 g, 99.3%) was used directly in the next step without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.63 (s, 1H), 7.87 – 7.79 (m, 1H), 7.72 (d, *J* = 1.0 Hz, 1H), 7.25 (dd, *J* = 9.6, 1.5 Hz, 1H). LCMS (ESI, *m/z*) Calcd for C₈H₄BrF₃N₂: 264.0, Found: 265.1 [M + H]⁺; retention time 4.37 min, LCMS purity >91%.



6-bromo-3-(trifluoromethyl)-[1,2,4]triazolo[4,3-a]pyrimidine (9b)

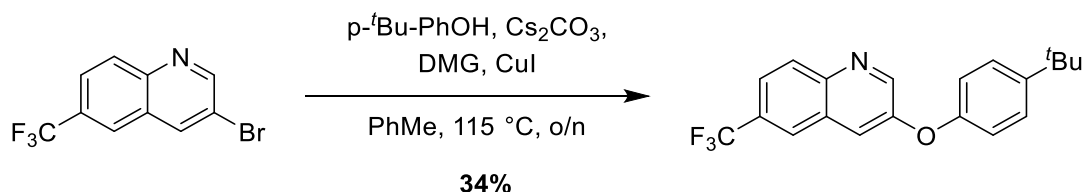
Prepared following the procedure described for **9a**, starting from 5-bromo-2-hydrazinepyrimidine (2.0 g, 10.58 mmol, 1.0 equiv), trifluoroacetic anhydride (6.0 mL, 42.32 mmol, 4.0 equiv), TEA (11.7 mL, 84.65 mmol, 8.0 equiv). The crude product (1.25 g, 44.4%) was used directly in the next step without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.49 (s, 1H), 9.03 (s, 1H). LCMS (ESI, *m/z*) Calcd for C₆H₂BrF₃N₄: 266.0, Found: 267.0 [M + H]⁺; retention time 3.40 min, LCMS purity > 85%.



6-(4-(tert-butyl)phenoxy)-3-(trifluoromethyl)imidazo[1,5-a]pyridine (10a)

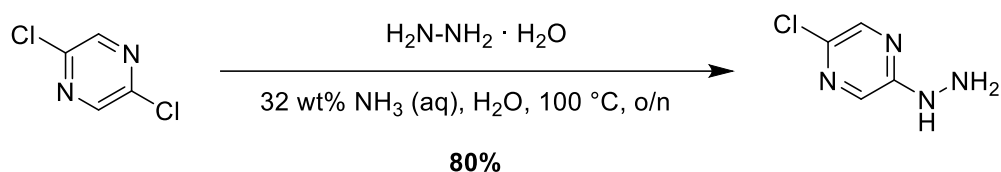
To a flame-dried sealed reaction vial equipped with a magnetic stir bar were added **9a** (0.300 g, 1.13 mmol, 1.0 equiv), 4-(tert-butyl)phenol (0.187 g, 1.25 mmol, 1.1 equiv), Cs₂CO₃ (0.516 g, 1.58 mmol, 1.4 equiv), dimethylglycine (11.7 mg, 0.113 mmol, 0.1 equiv), and CuI (35.9 mg, 0.113 mmol, 0.1 equiv) in dry toluene (6 mL). The mixture was degassed and purged with argon and then stirred at 115 °C overnight. The reaction mixture was diluted with water (25 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by pre-HPLC (isocratic elution, 80% B, *R*_t = 7.78 min) to afford the desired compound (0.338 g, 89.3%) as a colorless oil. ¹H NMR (600 MHz, methanol-*d*₄) δ 7.79 (dt, *J* = 1.8, 0.9 Hz, 1H), 7.65 (dd, *J* = 9.8, 1.0 Hz, 1H), 7.49 (s, 1H), 7.38 – 7.34 (m, 2H), 6.95 – 6.90 (m, 2H), 6.86 (dd, *J* = 9.8, 1.9 Hz, 1H), 1.23 (s, 9H). ¹³C NMR (151 MHz, methanol-

d_4) δ 153.6, 149.1, 147.5, 131.3, 125.6 (q, $J = 40.9$ Hz), 120.8, 119.7 (q, $J = 267.4$ Hz), 119.2, 118.3, 118.1, 110.0 (q, $J = 3.1$ Hz), 33.9, 30.4. LCMS (ESI, m/z) Calcd for $C_{16}H_{17}F_3N_2O$: 334.1, Found: 335.1 $[M + H]^+$; retention time 5.44 min. HPLC purity >99 HRMS (ESI) m/z : $[M + H]^+$ Calcd for $C_{16}H_{17}F_3N_2O$ 335.1366; Found 335.1359.



3-(4-(tert-butyl)phenoxy)-6-(trifluoromethyl)quinoline (10b)

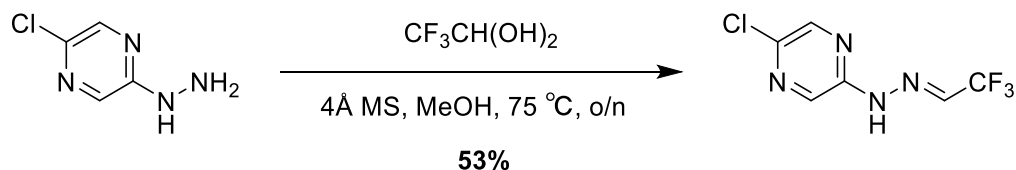
Prepared following the procedure described for **10a**, starting from 3-bromo-6-(trifluoromethyl)quinoline (120.3 mg, 0.436 mmol, 1.0 equiv), 4-(tert-butyl)phenol (72.0 mg, 0.479 mmol, 1.1 equiv), CS_2CO_3 (198.8 mg, 0.610 mmol, 1.4 equiv), dimethylglycine (8.99 mg, 0.087 mmol, 0.2 equiv), and CuI (27.7 mg, 0.087 mmol, 0.2 equiv). The crude product was purified by pre-HPLC (isocratic elution, 80% B, $R_t = 11.19$ min) to afford the desired compound (51.6 mg, 34.3%) as an orange solid. 1H NMR (600 MHz, methanol- d_4) δ 8.84 (d, $J = 2.8$ Hz, 1H), 8.16 – 8.11 (m, 2H), 7.80 (dd, $J = 8.8, 2.1$ Hz, 1H), 7.73 (dd, $J = 2.8, 0.7$ Hz, 1H), 7.51 – 7.45 (m, 2H), 7.10 – 7.04 (m, 2H), 1.33 (s, 9H). ^{13}C NMR (151 MHz, methanol- d_4) δ 152.9 (d, $J = 5.1$ Hz), 148.0, 146.5, 144.3, 129.2, 128.9 (q, $J = 32.6$ Hz), 128.1, 127.0, 125.1 (q, $J = 4.5$ Hz), 124.0 (q, $J = 271.8$ Hz), 123.1 (q, $J = 3.6$ Hz), 119.9, 119.1, 34.0, 30.5. LCMS (ESI, m/z) Calcd for $C_{20}H_{18}F_3NO$: 345.1, Found: 346.2 $[M + H]^+$; retention time 5.84 min. HPLC purity >99%. HRMS (ESI) m/z : $[M + H]^+$ Calcd for $C_{20}H_{18}F_3NO$ 346.1413; Found 346.1404.



2-chloro-5-hydrazineylpyrazine (11)

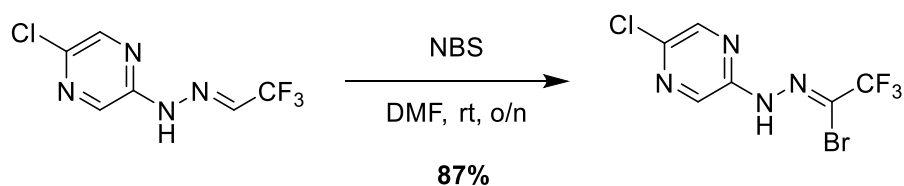
2,5-Dichloropyrazine (0.69 mL, 6.71 mmol, 1.0 equiv) was added to a sealed reaction vial, followed by water (6.5 mL), 32 wt% aqueous ammonia (1.14 mL, 19.47 mmol, 2.9 equiv), and hydrazine monohydrate (0.78 mL, 16.11 mmol, 2.4 equiv). The reaction mixture was heated at reflux overnight. Upon completion, the mixture was cooled to rt, resulting in precipitation of the product. The solid was collected by filtration through a sintered glass funnel, washed with cold H_2O (3×25 mL), and dried under reduced pressure to afford the desired compound as an

orange solid (0.778 g, 80.2%). ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 8.16 (s, 1H), 8.04 (d, $J = 1.4$ Hz, 1H), 7.94 (d, $J = 1.4$ Hz, 1H), 4.33 (s, 2H). LCMS (ESI, m/z) Calcd for $\text{C}_4\text{H}_5\text{ClN}_4$: 144.0, Found: 145.1 $[\text{M} + \text{H}]^+$; retention time 0.829 min, LCMS purity > 99%.



2-chloro-5-(2-(2,2,2-trifluoroethylidene)hydrazineyl)pyrazine (12)

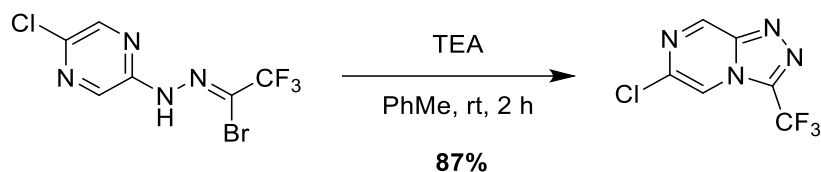
To a flame-dried sealed reaction vial equipped with a magnetic stir bar, **11** (0.692 g, 4.79 mmol, 1.0 equiv) and activated 4 Å molecular sieves (150 mg) were added, followed by anhydrous MeOH (8 mL). 2,2,2-Trifluoroethane-1,1-diol (1.0 mL, 14.36 mmol, 3.0 equiv) was then introduced, and the reaction mixture was stirred at 75 °C overnight. Upon completion, the reaction was filtered through a pad of Celite and washed thoroughly with MeOH (3 × 30 mL). The filtrate was concentrated, and the resulting residue was diluted with H_2O (30 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography, yielding the desired compound (0.573 g, 53.3%) as a brown oil. ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 11.96 (s, 1H), 8.37 (d, $J = 1.4$ Hz, 1H), 8.27 (d, $J = 1.4$ Hz, 1H), 7.58 (q, $J = 4.4$ Hz, 1H). LCMS (ESI, m/z) Calcd for $\text{C}_6\text{H}_4\text{ClF}_3\text{N}_4$: 224.0, Found: 225.0 $[\text{M} + \text{H}]^+$; retention time 4.35 min, LCMS purity > 99%.



N-(5-chloropyrazin-2-yl)-2,2,2-trifluoroacetohydrazonoyl bromide (13)

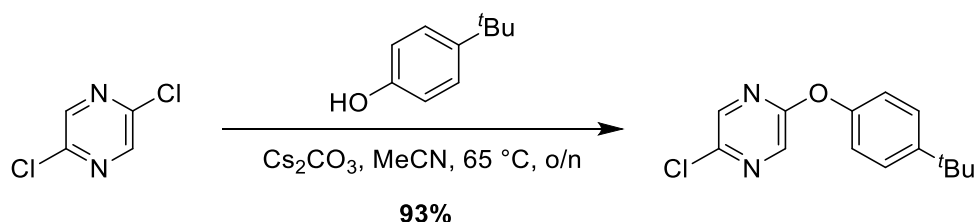
Compound **12** (0.573 g, 2.55 mmol, 1.0 equiv) was dissolved in DMF (6 mL), and NBS (0.477 g, 2.68 mmol, 1.05 equiv) was added in one portion. The resulting solution was stirred at rt overnight. After completion, the reaction mixture was poured into ice-cold water (50 mL) and extracted with *n*-heptane (3 × 25 mL). The organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. The crude product was purified by column, yielding the desired compound (0.672 g, 86.8%) as a brown oil. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.40 (s, 1H), 8.48 (d, $J = 1.4$ Hz, 1H), 8.41 (d, $J = 1.4$ Hz, 1H). LCMS

(ESI, m/z) Calcd for $C_6H_3BrClF_3N_4$: 302.0, Found: 303.0 $[M + H]^+$; retention time 4.79 min, LCMS purity > 98%.



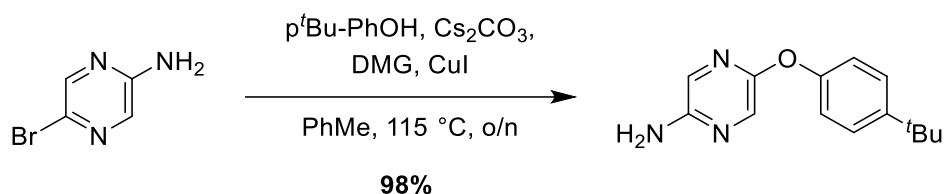
6-chloro-3-(trifluoromethyl)-[1,2,4]triazolo[4,3-a]pyrazine (14)

Compound **13** (0.215 g, 0.710 mmol, 1.0 equiv) was dissolved in toluene (6 mL), and TEA (0.20 mL, 1.42 mmol, 2.0 equiv) was added. The reaction mixture was stirred at rt for 2 h. Upon completion, the reaction was quenched with 1 M HCl (25 mL) and extracted with EtOAc (3×25 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography, affording the desired compound (0.138 g, 87.3%) as an orange solid. 1H NMR (600 MHz, $CDCl_3-d$) δ 9.29 (d, $J = 1.5$ Hz, 1H), 8.20 (dd, $J = 1.6, 0.8$ Hz, 1H). LCMS (ESI, m/z) Calcd for $C_6H_2ClF_3N_4$: 222.0, Found: 223.0 $[M + H]^+$; retention time 3.56 min, LCMS purity > 95%.



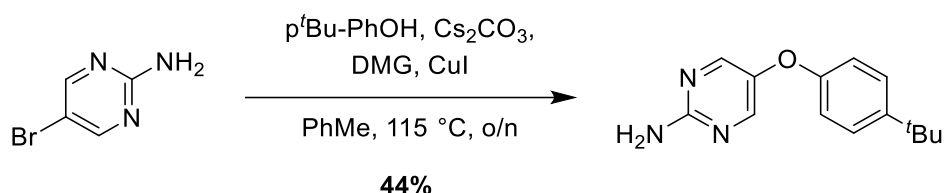
2-(4-(tert-butyl)phenoxy)-5-chloropyrazine (18)

Prepared following the procedure described for **8a**, starting from 2,5-dichloropyrazine (0.46 mL, 4.50 mmol, 1.0 equiv), 4-(tert-butyl)phenol (0.676 mg, 4.50 mmol, 1.0 equiv), and Cs_2CO_3 (2.93 g, 8.99 mmol, 2.0 equiv). The crude product (1.10 g, 93.1%) was used directly in the next step without further purification. 1H NMR (400 MHz, methanol- d_4) δ 9.22 (d, $J = 1.3$ Hz, 1H), 9.16 (d, $J = 1.3$ Hz, 1H), 8.31 – 8.23 (m, 2H), 7.99 – 7.91 (m, 2H), 2.12 (s, 9H). LCMS (ESI, m/z) Calcd for $C_{14}H_{15}ClN_2O$: 262.1, Found: 263.1 $[M + H]^+$; retention time 5.59 min, LCMS purity > 94%.



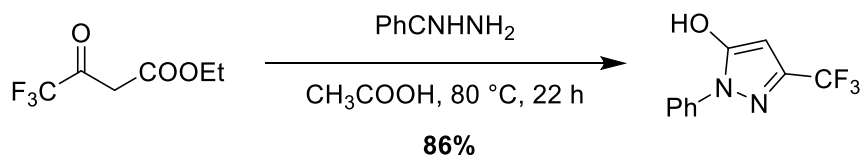
5-(4-(tert-butyl)phenoxy)pyrazin-2-amine (20a)

Prepared following the procedure described for **10a**, starting from 5-bromopyrazin-2-amine (0.500 g, 2.87 mmol, 1.0 equiv), 4-(tert-butyl)phenol (0.475 g, 3.16 mmol, 1.1 equiv), Cs₂CO₃ (1.31 g, 4.02 mmol, 1.4 equiv), dimethylglycine (29.6 mg, 0.287 mmol, 0.1 equiv), and CuI (91.2 mg, 0.287 mmol, 0.1 equiv). The crude product (0.688 g, 98.4%) was used directly in the next step without further purification. ¹H NMR (400 MHz, methanol-*d*₄) δ 7.74 (s, 1H), 7.63 (s, 1H), 7.43 – 7.35 (m, 2H), 6.97 – 6.89 (m, 2H), 1.33 (s, 9H). LCMS (ESI, *m/z*) Calcd for C₁₄H₁₇N₃O: 243.1, Found: 244.1 [M + H]⁺; retention time 4.90 min, LCMS purity > 93%.



5-(4-(tert-butyl)phenoxy)pyrimidin-2-amine (20b)

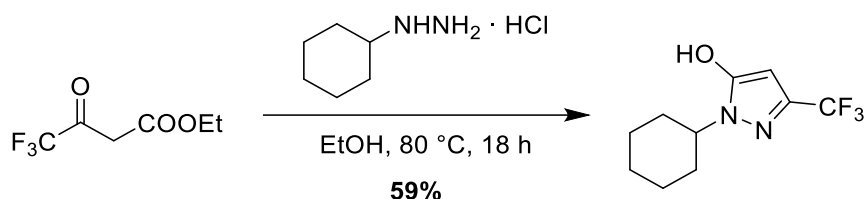
Prepared following the procedure described for **10a**, starting from 5-bromopyrimidin-2-amine (0.605 g, 3.48 mmol, 1.0 equiv), 4-(tert-butyl)phenol (0.575 g, 3.82 mmol, 1.1 equiv), Cs₂CO₃ (1.59 g, 4.87 mmol, 1.4 equiv), dimethylglycine (35.9 mg, 0.348 mmol, 0.1 equiv), and CuI (0.110 g, 0.348 mmol, 0.1 equiv). The crude product (0.369 g, 43.6%) was used directly in the next step without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.13 (s, 2H), 7.39 – 7.30 (m, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.61 (s, 2H), 1.26 (s, 9H). LCMS (ESI, *m/z*) Calcd for C₁₄H₁₇N₃O: 243.1, Found: 244.2 [M + H]⁺; retention time 4.82 min, LCMS purity > 90%.



1-phenyl-3-(trifluoromethyl)-1H-pyrazol-5-ol (21a)

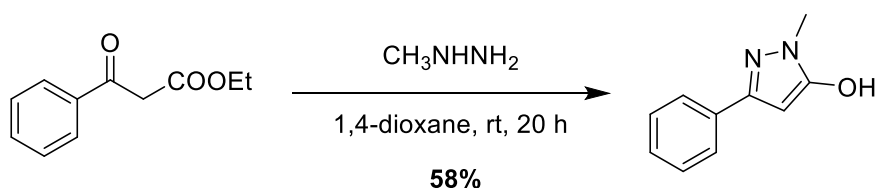
The synthesis was performed in a sealed reaction vial by adding phenylhydrazine (0.36 mL, 3.70 mmol, 1.1 equiv) to 10 mL of acetic acid at 0 °C, followed by the dropwise addition of ethyl 4,4,4-trifluoro-3-oxobutanoate (0.52 mL, 3.52 mmol, 1.0 equiv). The reaction mixture

was heated at 80 °C for 22 hours and then allowed to cool to rt. The resulting mixture was quenched by adding 100 mL of water, and the precipitate was collected by filtration through a sintered glass funnel. The solid was washed thoroughly with cold water (3 × 25 mL) and dried under reduced pressure to yield the desired compound (0.694 g, 86.3%) as a pale-yellow solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 12.43 (s, 1H), 7.74 – 7.69 (m, 2H), 7.55 – 7.49 (m, 2H), 7.42 – 7.36 (m, 1H), 5.94 (s, 1H). LCMS (ESI, *m/z*) Calcd for C₁₀H₇F₃N₂O: 228.1, Found: 229.2 [*M* + *H*]⁺; retention time 4.39 min, LCMS purity > 99%.



1-cyclohexyl-3-(trifluoromethyl)-1H-pyrazol-5-ol (21b)

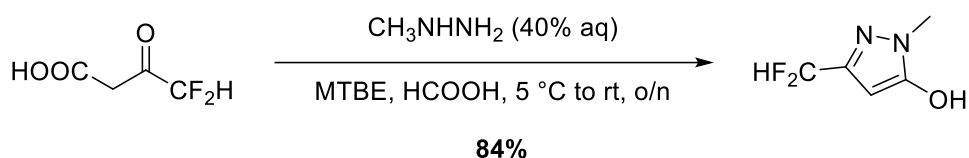
To a sealed reaction vial equipped with a magnetic stir bar was added cyclohexylhydrazine hydrochloride (1.23 g, 8.16 mmol, 1.05 equiv) in absolute EtOH (10 mL), followed by the dropwise addition of ethyl 4,4,4-trifluoro-3-oxobutanoate (1.14 mL, 7.78 mmol, 1.0 equiv). The reaction mixture was heated at 90 °C for 18 hours, then allowed to cool to rt. The solvent was removed under reduced pressure, and the resulting solid was collected by filtration through a sintered glass funnel. The solid was washed thoroughly with *n*-heptane (3 × 25 mL) and dried under reduced pressure to afford the desired compound (1.08 g, 59.3%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.61 (s, 1H), 5.70 (s, 1H), 4.11 (tt, *J* = 11.5, 3.8 Hz, 1H), 1.89 – 1.65 (m, 7H), 1.44 – 1.29 (m, 2H), 1.25 – 1.15 (m, 1H). LCMS (ESI, *m/z*) Calcd for C₁₀H₁₃F₃N₂O: 234.1, Found: 235.2 [*M* + *H*]⁺; retention time 4.43 min, LCMS purity > 96%.



1-methyl-3-phenyl-1H-pyrazol-5-ol (21c)

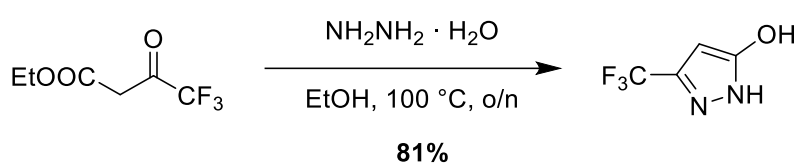
Ethyl 3-oxo-3-phenylpropanoate (1.34 mL, 7.80 mmol, 1.0 equiv) was dissolved in 1,4-dioxane (8 mL), followed by the dropwise addition of methylhydrazine (0.45 mL, 8.58 mmol, 1.1 equiv). The reaction mixture was stirred at rt overnight. Upon completion, a white precipitate was formed and collected by filtration through a sintered glass funnel. The solid was washed thoroughly with *n*-heptane (3 × 25 mL) and dried under reduced pressure to afford the desired

compound (0.790 g, 58.1%) as a white solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 10.99 (s, 1H), 7.72 – 7.65 (m, 2H), 7.35 (t, J = 7.6 Hz, 2H), 7.29 – 7.20 (m, 1H), 5.78 (s, 1H), 3.56 (s, 3H). LCMS (ESI, m/z) Calcd for $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}$: 174.1, Found: 175.2 $[\text{M} + \text{H}]^+$; retention time 3.10 min. LCMS purity > 99%.



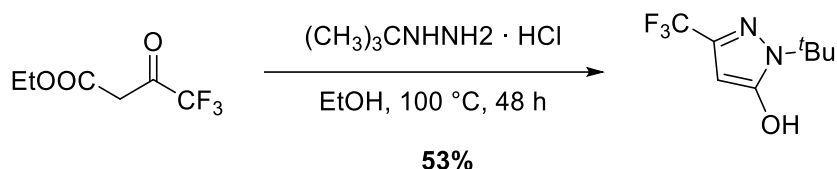
3-(difluoromethyl)-1-methyl-1H-pyrazol-5-ol (21d)

Ethyl 4,4-difluoro-3-oxobutanoate (0.93 mL, 9.03 mmol, 1.0 equiv) was added to a 50 mL round-bottom flask, followed by 5 mL of methyl tert-butyl ether and 1.1 mL of formic acid. The reaction mixture was cooled to 5 °C, after which methylhydrazine (1.10 mL, 9.48 mmol, 1.05 equiv, 40% aqueous solution) was added dropwise. The mixture was stirred at rt overnight. The solvent was then removed under reduced pressure, and the resulting residue was diluted with water (25 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2 , n -heptane/EtOAc = 1:1, R_f = 0.30), yielding the desired compound (1.13 g, 84.1%) as an orange solid. ^1H NMR (600 MHz, DMSO) δ 11.34 (s, 1H), 6.70 (t, J = 54.9 Hz, 1H), 5.56 (d, J = 1.0 Hz, 1H), 3.55 (t, J = 1.1 Hz, 3H). LCMS (ESI, m/z) Calcd for $\text{C}_5\text{H}_6\text{F}_2\text{N}_2\text{O}$: 148.0, Found: 149.1 $[\text{M} + \text{H}]^+$; retention time 2.77 min, LCMS purity > 99%.



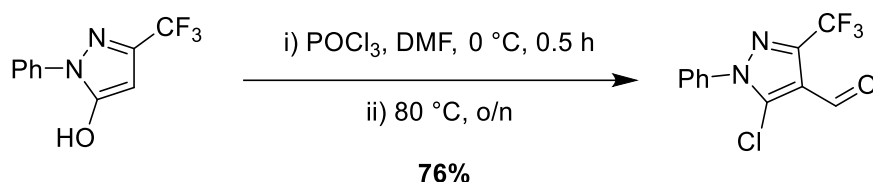
3-(trifluoromethyl)-1H-pyrazol-5-ol (21e)

Prepared following the procedure described for **21b**, starting from hydrazine hydrate (0.95 mL, 19.55 mmol, 1.2 equiv), and ethyl 4,4,4-trifluoro-3-oxobutanoate (2.38 mL, 16.29 mmol, 1.0 equiv), except that the reaction was stirred at 100 °C overnight. The crude product (2.02 g, 81.5%) was used directly in the next step without further purification. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 12.82 (s, 1H), 11.20 (s, 1H), 5.66 (s, 1H). ^{19}F NMR (376 MHz, $\text{DMSO-}d_6$) δ -61.38. LCMS (ESI, m/z) Calcd for $\text{C}_4\text{H}_3\text{F}_3\text{N}_2$: 152.0, Found: 153.2 $[\text{M} + \text{H}]^+$; retention time 2.94 min, LCMS purity > 95%.



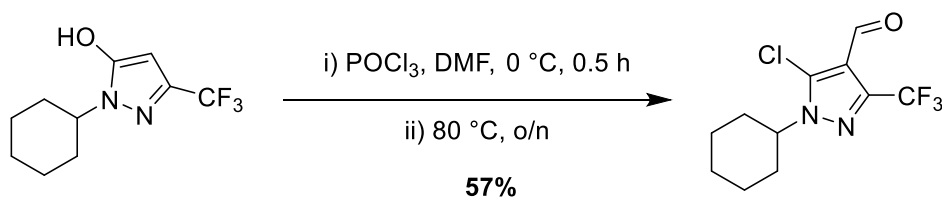
1-(tert-butyl)-3-(trifluoromethyl)-1H-pyrazol-5-ol (21g)

Prepared following the procedure described for **21b**, starting from tert-butylhydrazine hydrochloride (0.626 g, 5.02 mmol, 1.0 equiv) and ethyl 4,4,4-trifluoro-3-oxobutanoate (0.61 mL, 4.18 mmol, 1.0 equiv), except that the reaction was stirred at 100 °C for 2 days. The crude product was purified by recrystallization from a mixture of EtOAc and *n*-heptane to afford the product as a white solid (0.463 g, 53.2% ¹H NMR (600 MHz, DMSO-*d*₆) δ 11.5 (s, 1H), 5.7 (s, 1H), 1.5 (s, 9H). LCMS (ESI, *m/z*) Calcd for C₈H₁₁F₃N₂O: 208.1, Found: 209.1 [M + H]⁺; retention time 4.61 min, LCMS purity > 98%.



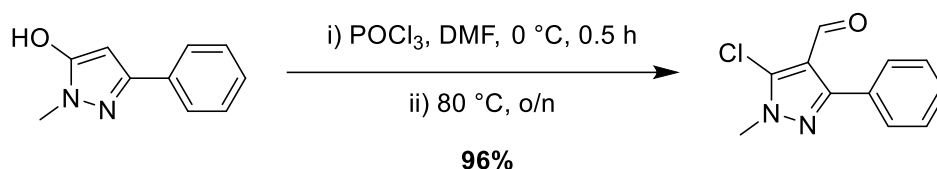
5-chloro-1-phenyl-3-(trifluoromethyl)-1H-pyrazole-4-carbaldehyde (22a)

Dry DMF (0.41 mL, 5.26 mmol, 3.0 equiv) was transferred to a flame-dried sealed reaction vial under an inert atmosphere. Phosphorus oxychloride (1.14 mL, 12.27 mmol, 7.0 equiv) was added dropwise at 0-10 °C to the violently stirred cold solution. The mixture was stirred at this temperature for 30 minutes. Subsequently, **21a** (0.400 g, 1.75 mmol, 1.0 equiv) was introduced into the reaction mixture, and the solution was refluxed overnight. The reaction was quenched with ice water (25 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were washed with 1 M HCl (15 mL) and brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography, yielding the desired compound (0.365 g, 75.7%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃-*d*) δ 10.08 (s, 1H), 7.63 – 7.55 (m, 5H). LCMS (ESI, *m/z*) Calcd for C₁₁H₆ClF₃N₂O: 274.0, Found: 275.0 [M + H]⁺; retention time 4.65 min, LCMS purity > 97%.



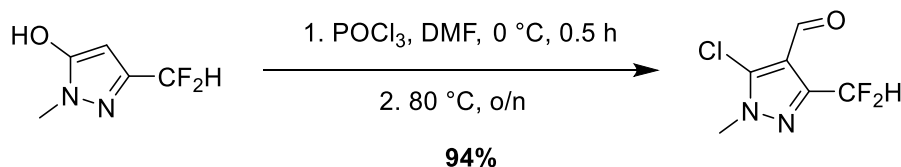
5-chloro-1-cyclohexyl-3-(trifluoromethyl)-1H-pyrazole-4-carbaldehyde (22b)

Prepared following the procedure described for **22a**, starting from **21b** (1.08 g, 4.61 mmol, 1.0 equiv), dry DMF (1.1 mL, 13.83 mmol, 3.0 equiv), and phosphorus oxychloride (3.0 mL, 32.28 mmol, 7.0 equiv). The crude product (0.734 g, 56.7%) was used directly in the next step without further purification. LCMS (ESI, m/z) Calcd for $C_{11}H_{12}ClF_3N_2O$: 280.1, Found: 281.1 $[M + H]^+$; retention time 5.15 min, LCMS purity > 90%.



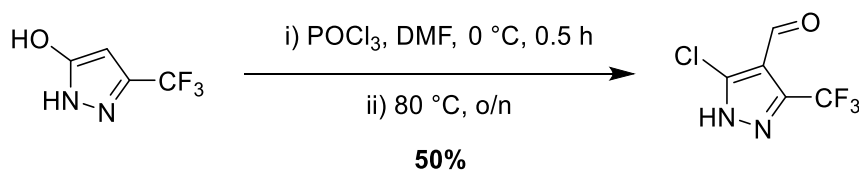
5-chloro-1-methyl-3-phenyl-1H-pyrazole-4-carbaldehyde (22c)

Prepared following the procedure described for **22a**, starting from **21c** (0.790 g, 4.53 mmol, 1.0 equiv), dry DMF (1.1 mL, 13.60 mmol, 3.0 equiv), and phosphorus oxychloride (3.0 mL, 31.74 mmol, 7.0 equiv). The crude product was purified by column chromatography, yielding the desired compound (0.961 g, 96.0%) as a colorless oil. 1H NMR (400 MHz, $DMSO-d_6$) δ 9.85 (s, 1H), 7.78 – 7.74 (m, 2H), 7.48 (dd, J = 5.1, 2.0 Hz, 3H), 3.92 (s, 3H). LCMS (ESI, m/z) Calcd for $C_{11}H_9ClN_2O$: 220.0, Found: 221.2 $[M + H]^+$; retention time 4.12 min, LCMS purity > 96%.



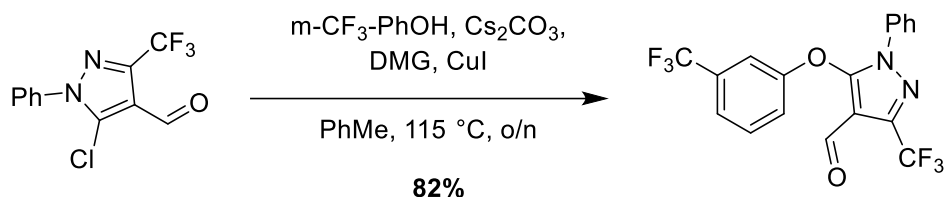
5-chloro-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carbaldehyde (22d)

Prepared following the procedure described for **22a**, starting from **21d** (0.500 g, 3.38 mmol, 1 equiv), dry DMF (0.78 mL, 10.13 mmol, 3.0 equiv), and phosphorus oxychloride (2.20 mL, 23.67 mmol, 7.0 equiv). The crude product was purified by column chromatography, yielding the desired compound (0.620 g, 94.4%) as an orange oil. 1H NMR (600 MHz, $DMSO-d_6$) δ 9.86 (s, 1H), 7.20 (t, J = 53.2 Hz, 1H), 3.91 (s, 3H). LCMS (ESI, m/z) Calcd for $C_6H_5ClF_2N_2O$: 194.0, Found: 195.1 $[M + H]^+$; retention time 3.62 min, LCMS purity > 94%.



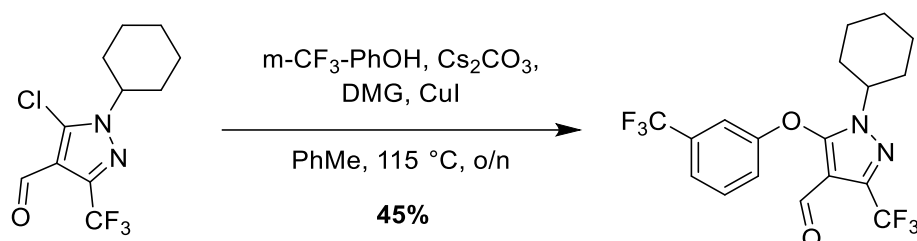
5-chloro-3-(trifluoromethyl)-1H-pyrazole-4-carbaldehyde (22e)

Prepared following the procedure described for **22a**, starting from **21g** (0.30 g, 1.97 mmol, 1.0 equiv), dry DMF (0.46 mL, 5.92 mmol, 3.0 equiv), and phosphorus oxychloride (1.28 mL, 13.81 mmol, 7.0 equiv). The crude product (0.197 g, 50.4%) was used directly in the next step without further purification. ^1H NMR (400 MHz, DMSO- d_6) δ 13.33 (s, 1H), 9.72 (s, 1H). ^{19}F NMR (376 MHz, DMSO- d_6) δ -62.38. LCMS (ESI, m/z) Calcd for $\text{C}_5\text{H}_2\text{ClF}_3\text{N}_2\text{O}$: 198.0, Found: 199.1 $[\text{M} + \text{H}]^+$; retention time 2.25 min, LCMS purity > 95%.



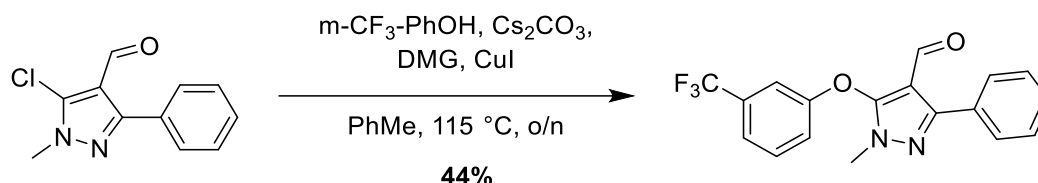
1-phenyl-3-(trifluoromethyl)-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazole-4-carbaldehyde (23a)

To a flame-dried sealed reaction vial equipped with a magnetic stir bar were added **22a** (0.924 g, 3.36 mmol, 1.0 equiv), 3-(trifluoromethyl)phenol (0.44 mL, 3.70 mmol, 1.1 equiv), Cs_2CO_3 (1.53 g, 4.71 mmol, 1.4 equiv), dimethylglycine (0.173 g, 1.68 mmol, 0.5 equiv), and CuI (0.534 g, 1.68 mmol, 0.5 equiv) in dry toluene (10 mL). The mixture was degassed and purged with argon and then stirred at 115 °C overnight. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (3 $\times$ 30 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography, yielding the desired compound (1.10 g, 81.7%) as a colorless oil. ^1H NMR (400 MHz, DMSO- d_6) δ 9.66 (s, 1H), 7.69 – 7.65 (m, 3H), 7.61 – 7.49 (m, 6H). LCMS (ESI, m/z) Calcd for $\text{C}_{18}\text{H}_{10}\text{F}_6\text{N}_2\text{O}_2$: 400.1, Found: 401.1 $[\text{M} + \text{H}]^+$; retention time 5.12 min, LCMS purity > 91%.



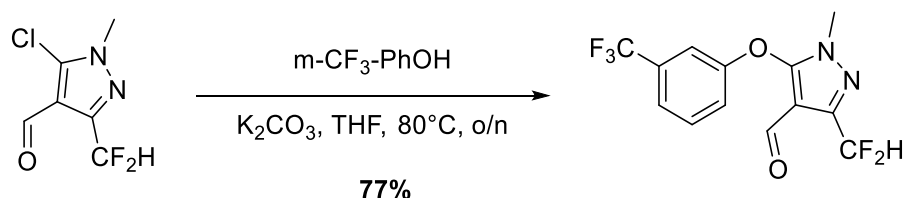
1-cyclohexyl-3-(trifluoromethyl)-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazole-4-carbaldehyde (23b)

Prepared following the procedure described for **23a**, starting from **22b** (0.734 g, 2.61 mmol, 1.0 equiv), 3-(trifluoromethyl)phenol (0.35 mL, 2.88 mmol, 1.1 equiv), Cs₂CO₃ (1.19 g, 3.66 mmol, 1.4 equiv), dimethylglycine (0.135 mg, 1.31 mmol, 0.5 equiv), and CuI (0.415 g, 1.31 mmol, 0.5 equiv). The crude product (0.478 g, 45.0%) was used directly in the next step without further purification. LCMS (ESI, m/z) Calcd for C₁₈H₁₆F₆N₂O₂: 406.1, Found: 407.1 [M + H]⁺; retention time 5.45 min.



1-methyl-3-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazole-4-carbaldehyde (**23c**)

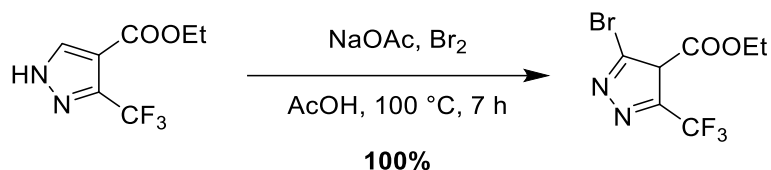
Prepared following the procedure described for **23a**, starting from **22c** (0.961 g, 4.35 mmol, 1.0 equiv), 3-(trifluoromethyl)phenol (0.57 mL, 4.79 mmol, 1.1 equiv), Cs₂CO₃ (2.0 g, 6.09 mmol, 1.4 equiv), dimethylglycine (0.224 g, 2.18 mmol, 0.5 equiv), and CuI (0.691 g, 2.18 mmol, 0.5 equiv). The crude product was purified by column chromatography, yielding the desired compound (0.670 g, 44.5%) as a colorless oil. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.68 (s, 1H), 7.83 – 7.79 (m, 2H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.60 – 7.55 (m, 1H), 7.55 – 7.45 (m, 4H), 7.39 (dd, *J* = 8.2, 2.7 Hz, 1H), 3.78 (s, 3H). LCMS (ESI, m/z) Calcd for C₁₈H₁₃F₃N₂O₂: 346.1, Found: 347.2 [M + H]⁺; retention time 4.82 min, LCMS purity > 95%.



3-(difluoromethyl)-1-methyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazole-4-carbaldehyde (**23d**)

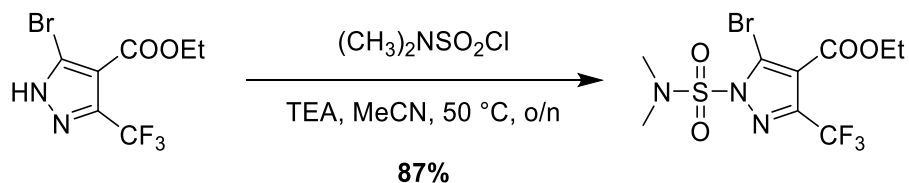
Compound **22d** (0.325 g, 1.67 mmol, 1.0 equiv) was dissolved in THF (8 mL) at ambient temperature. To this solution, 3-(trifluoromethyl)phenol (0.325 g, 2.00 mmol, 1.2 equiv) and potassium carbonate (0.693 g, 5.01 mmol, 3.0 equiv) were added. The reaction mixture was stirred at 80 °C overnight. After cooling to rt, the mixture was filtered through a pad of Celite, and the solvent was removed under reduced pressure. The resulting residue was diluted with water (25 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under

reduced pressure. The crude product (0.412 g, 77.0%) was used directly in the next step without further purification. LCMS (ESI, m/z) Calcd for $C_{13}H_9F_5N_2O_2$: 320.1, Found: 321.1 $[M + H]^+$; retention time 4.63 min, LCMS purity > 92%.



Ethyl 5-bromo-3-(trifluoromethyl)-1H-pyrazole-4-carboxylate (**25**)

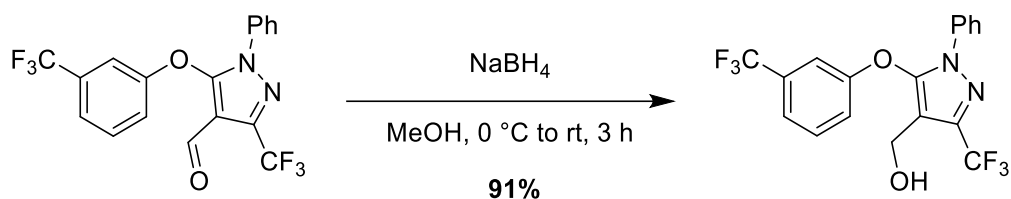
Sodium acetate (3.55 g, 43.24 mmol, 3.0 equiv) was added to a solution of ethyl 3-(trifluoromethyl)-1H-pyrazole-4-carboxylate (3.00 g, 14.41 mmol, 1.0 equiv) in acetic acid (20 mL) at ambient temperature. Bromine (2.20 mL, 43.24 mmol, 3.0 equiv) was then added dropwise to the suspended solution. The resulting mixture was stirred at rt for 10 minutes and subsequently heated to 100 °C for 7 hours. After completion, the reaction was quenched with saturated sodium carbonate (35 mL) and saturated sodium dithionite solutions (15 mL), followed by extraction with EtOAc (3×25 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product (4.13 g, 99.8%) was used directly in the next step without further purification. 1H NMR (400 MHz, $DMSO-d_6$) δ 4.24 (q, $J = 7.1$ Hz, 2H), 2.09 (s, 1H), 1.27 (t, $J = 7.1$ Hz, 3H). LCMS (ESI, m/z) Calcd for $C_7H_6BrF_3N_2O_2$: 286.0, Found: 287.0 $[M + H]^+$; retention time 4.15 min, LCMS purity > 97%.



Ethyl 5-bromo-1-(N,N-dimethylsulfamoyl)-3-(trifluoromethyl)-1H-pyrazole-4-carboxylate (**26**)

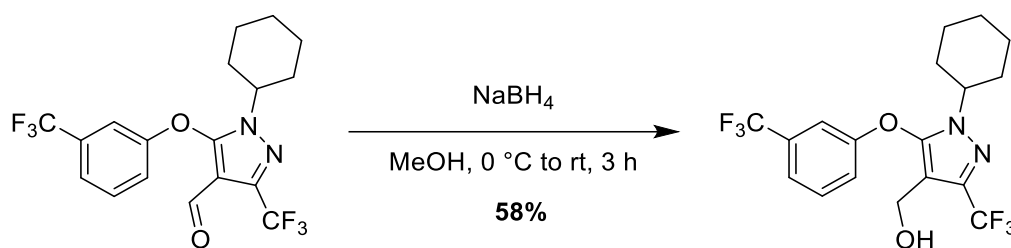
Compound **25** (209 mg, 0.729 mmol, 1.0 equiv) was dissolved in MeCN (6 mL) at ambient temperature. Dimethylsulfamoyl chloride (86 μ L, 0.802 mmol, 1.10 equiv) and TEA (163 μ L, 1.17 mmol, 1.60 equiv) were then added to the solution. The reaction mixture was stirred at 50 °C overnight. Upon completion, the mixture was quenched with 1 M aq. HCl (25 mL) and extracted with EtOAc (3×25 mL). The combined organic extracts were washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product (0.250 g, 87.0%) was used directly in the next step without further purification.

^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 4.33 (q, $J = 7.1$ Hz, 2H), 3.11 (s, 6H), 1.30 (t, $J = 7.1$ Hz, 3H). LCMS (ESI, m/z) Calcd for $\text{C}_9\text{H}_{11}\text{BrF}_3\text{N}_3\text{O}_4\text{S}$: 393.0, Found: 394.0 $[\text{M} + \text{H}]^+$; retention time 4.87 min, LCMS purity > 96%.



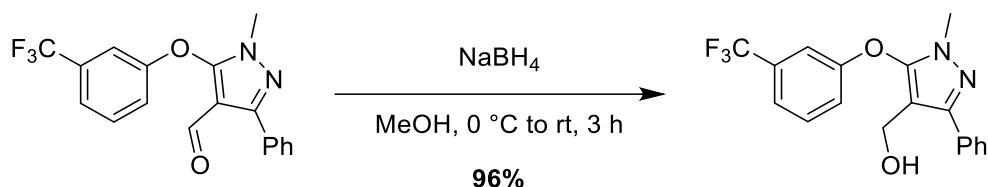
(1-phenyl-3-(trifluoromethyl)-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methanol (27a)

Sodium borohydride (0.312 g, 8.24 mmol, 3.0 equiv) was added in portions to a solution of **23a** (1.10 g, 2.75 mmol, 1.0 equiv) in MeOH (10 mL) at 0 °C. The reaction mixture was stirred at this temperature for 30 minutes, then allowed to warm to rt and stirred for 3 hours. Water (10 mL) was added to quench the reaction, and the mixture was extracted with EtOAc (3 $\times$ 25 mL). The combined organic layers were washed with brine (50 mL) and sat. NaHCO_3 solution (25 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product (1.01 g, 91.4%) was used directly in the next step without further purification. ^1H NMR (400 MHz, methanol- d_4) δ 7.53 – 7.44 (m, 2H), 7.37 – 7.27 (m, 5H), 7.17 (s, 1H), 7.06 (dd, $J = 8.4, 2.6$ Hz, 1H), 4.36 (s, 2H). LCMS (ESI, m/z) Calcd for $\text{C}_{18}\text{H}_{12}\text{F}_6\text{N}_2\text{O}_2$: 402.1, Found: 403.1 $[\text{M} + \text{H}]^+$; retention time 4.86 min, LCMS purity > 99%.



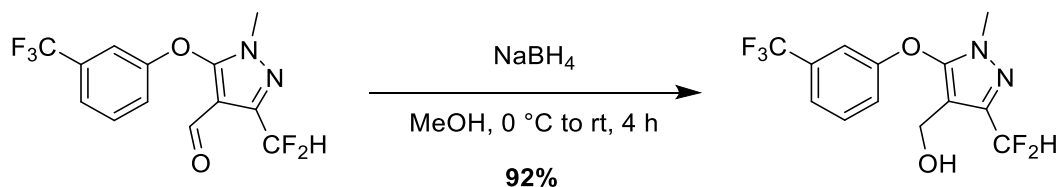
(1-cyclohexyl-3-(trifluoromethyl)-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methanol (27b)

Prepared following the procedure described for **27a**, starting from **23b** (0.478 g, 1.18 mmol, 1.0 equiv) and sodium borohydride (0.134 g, 3.53 mmol, 3.0 equiv). The crude product (0.177 g, 57.7%) was used directly in the next step without further purification. LCMS (ESI, m/z) Calcd for $\text{C}_{18}\text{H}_{12}\text{F}_6\text{N}_2\text{O}_2$: 408.1, Found: 409.2 $[\text{M} + \text{H}]^+$; retention time 5.20 min, LCMS purity > 92%.



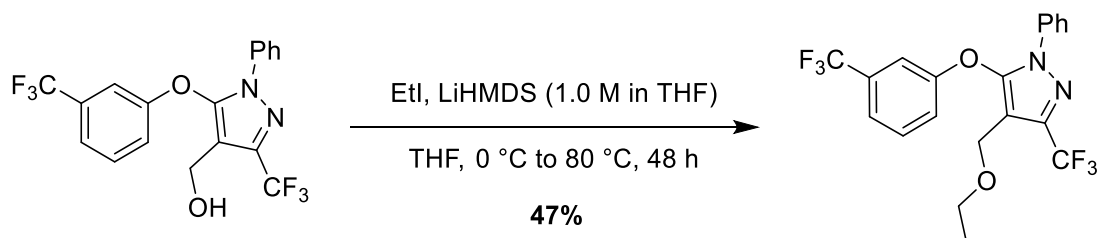
(1-methyl-3-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methanol (27c)

Prepared following the procedure described for **27a**, starting from **23c** (0.670 g, 1.93 mmol, 1.0 equiv) and sodium borohydride (0.220 g, 5.80 mmol, 3.0 equiv). The crude product (0.645 g, 95.7%) was used directly in the next step without further purification. ^1H NMR (400 MHz, DMSO- d_6) δ 7.92 – 7.84 (m, 2H), 7.65 (t, J = 8.0 Hz, 1H), 7.57 – 7.51 (m, 1H), 7.47 – 7.42 (m, 3H), 7.39 – 7.31 (m, 2H), 4.26 (s, 2H), 3.65 (s, 3H). LCMS (ESI, m/z) Calcd for $\text{C}_{18}\text{H}_{15}\text{F}_3\text{N}_2\text{O}_2$: 348.1, Found: 349.2 $[\text{M} + \text{H}]^+$; retention time 4.53 min, LCMS purity > 93%.



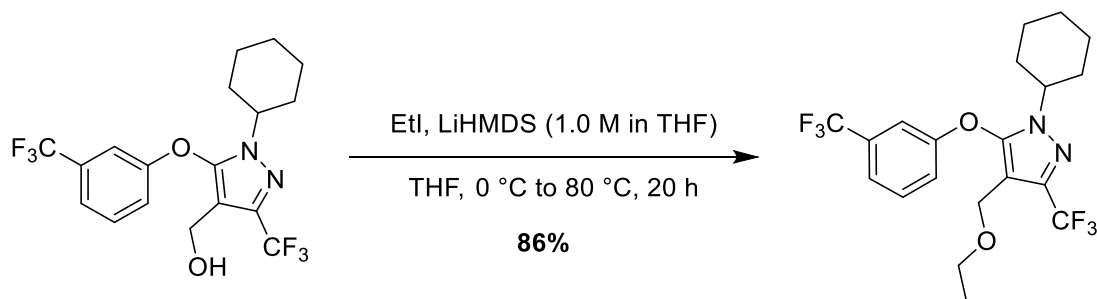
(3-(difluoromethyl)-1-methyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methanol (27d)

Prepared following the procedure described for **27a**, starting from **23d** (412 mg, 1.29 mmol, 1.0 equiv) and sodium borohydride (73.01 mg, 1.93 mmol, 1.5 equiv). The crude product (0.381 g, 92.0%) was used directly in the next step without further purification. ^1H NMR (400 MHz, DMSO- d_6) δ 7.64 (t, J = 8.0 Hz, 1H), 7.55 (d, J = 7.7 Hz, 1H), 7.43 (s, 1H), 7.29 (dd, J = 8.2, 2.6 Hz, 1H), 6.97 (t, J = 53.9 Hz, 1H), 4.91 (t, J = 4.9 Hz, 1H), 4.22 (d, J = 4.8 Hz, 2H), 3.65 (s, 3H). LCMS (ESI, m/z) Calcd for $\text{C}_{13}\text{H}_{11}\text{F}_5\text{N}_2\text{O}_2$: 322.1, Found: 323.2 $[\text{M} + \text{H}]^+$; retention time 4.23 min, LCMS purity > 89%.



4-(ethoxymethyl)-1-phenyl-3-(trifluoromethyl)-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazole (28a)

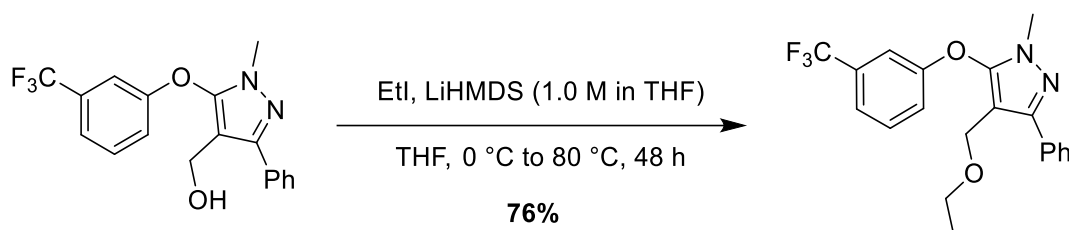
To a flame-dried sealed reaction vial under an argon atmosphere, equipped with a magnetic stir bar, was added **27a** (1.01 g, 2.51 mmol, 1.0 equiv) in anhydrous THF (8 mL). The solution was cooled to 0 °C, followed by dropwise addition of lithium bis(trimethylsilyl)amide (10.0 mL, 10.0 mmol, 4.0 equiv). After stirring at 0 °C for 5 min, ethyl iodide (2.0 mL, 25.1 mmol, 10.0 equiv) was added. The reaction mixture was stirred at 80 °C for 2 days. The reaction was then quenched with 1 M HCl (15 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by pre-HPLC (isocratic elution, 70% B, *R_t* = 11.17 min) to afford the desired compound (0.512 g, 47.4%) as a colorless oil. ¹H NMR (600 MHz, methanol-*d*₄) δ 7.64 – 7.60 (m, 2H), 7.53 – 7.46 (m, 3H), 7.44 – 7.39 (m, 2H), 7.31 (t, *J* = 2.2 Hz, 1H), 7.22 (dd, *J* = 8.4, 2.6 Hz, 1H), 4.33 (d, *J* = 1.0 Hz, 2H), 3.38 (q, *J* = 7.0 Hz, 2H), 1.05 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (151 MHz, methanol-*d*₄) δ 158.3, 149.2, 142.4 (q, *J* = 37.8 Hz), 138.7, 133.8 (q, *J* = 32.8 Hz), 132.6, 131.0, 130.4, 125.0, 124.9 (q, *J* = 271.7 Hz), 122.6 (q, *J* = 269.2 Hz), 122.4 (q, *J* = 3.9 Hz), 121.1, 114.7 (q, *J* = 3.9 Hz), 107.6, 67.4, 61.2, 15.5. LCMS (ESI, *m/z*) Calcd for C₂₀H₁₆F₆N₂O₂: 430.1, Found: 431.1 [M + H]⁺; retention time 5.46 min. HPLC purity >99%. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₂₀H₁₆F₆N₂O₂ 431.1188; Found 431.1181.



1-cyclohexyl-4-(ethoxymethyl)-3-(trifluoromethyl)-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazole (**28b**)

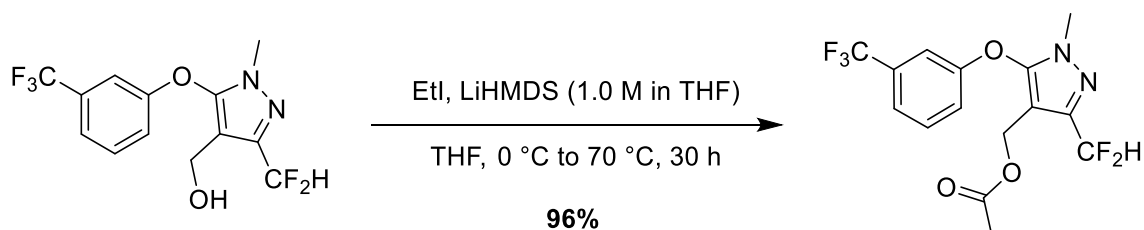
Prepared following the procedure described for **28a**, starting from **27b** (0.277 g, 0.679 mmol, 1.0 equiv), lithium bis(trimethylsilyl)amide (2.72 mL, 2.72 mmol, 4.0 equiv), and ethyl iodide (0.55 mL, 6.79 mmol, 10.0 equiv), except that the reaction was stirred for only 20 h. The crude product was purified by pre-HPLC (isocratic elution, 75% B, *R_t* = 12.06 min) to afford the desired compound (0.255 g, 86.2%) as a colorless oil. ¹H NMR (600 MHz, methanol-*d*₄) δ 7.50 (t, *J* = 8.1 Hz, 1H), 7.41 – 7.38 (m, 1H), 7.25 (t, *J* = 2.2 Hz, 1H), 7.18 (dd, *J* = 8.4, 2.6 Hz, 1H), 4.09 (d, *J* = 0.8 Hz, 2H), 4.07 – 4.00 (m, 1H), 3.19 (q, 2H), 1.81 – 1.71 (m, 6H), 1.60 – 1.55 (m, 1H), 1.28 – 1.11 (m, 3H), 0.90 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (151 MHz, methanol-*d*₄) δ

158.4, 147.9, 140.3 (q, $J = 37.4$ Hz), 133.5 (q, $J = 32.8$ Hz), 132.3, 123.6 (q, $J = 271.6$ Hz), 122.8 (q, $J = 268.8$ Hz), 121.9 (q, $J = 4.0$ Hz), 120.6, 114.1 (q, $J = 3.9$ Hz), 105.4, 66.9, 60.8, 58.9, 33.3, 26.3, 26.1, 15.1. LCMS (ESI, m/z) Calcd for $C_{20}H_{22}F_6N_2O_2$: 436.2, Found: 437.2 $[M + H]^+$; retention time 5.75 min. HPLC purity >99%. HRMS (ESI) m/z : $[M + H]^+$ Calcd for $C_{20}H_{22}F_6N_2O_2$ 437.1658; Found 437.1651.



4-(ethoxymethyl)-1-methyl-3-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazole (28c)

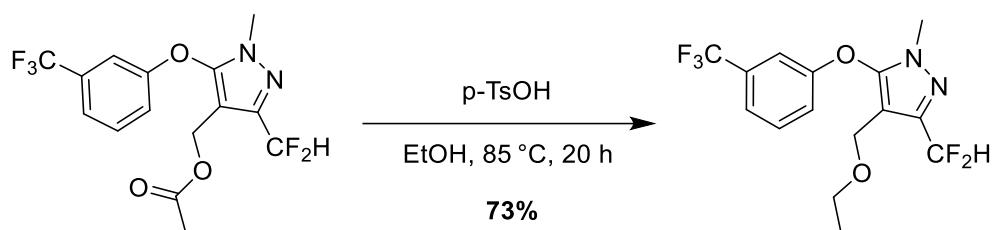
Prepared following the procedure described for **28a**, starting from **27c** (0.645 g, 1.85 mmol, 1.0 equiv), lithium bis(trimethylsilyl)amide (7.41 mL, 7.41 mmol, 4.0 equiv), and ethyl iodide (1.49 mL, 18.52 mmol, 10.0 equiv). The crude product was purified by pre-HPLC (isocratic elution, 75% B, $R_t = 9.28$ min) to afford the desired compound (0.528 g, 75.7%) as a colorless oil. 1H NMR (600 MHz, methanol- d_4) δ 7.67 – 7.63 (m, 2H), 7.47 (t, $J = 0.9$ Hz, 1H), 7.36 (ddt, $J = 7.7, 1.6, 0.8$ Hz, 1H), 7.34 – 7.30 (m, 3H), 7.29 – 7.23 (m, 1H), 7.21 – 7.18 (m, 1H), 4.07 (s, 2H), 3.59 (s, 3H), 3.23 (q, $J = 7.0$ Hz, 2H), 0.95 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (151 MHz, methanol- d_4) δ 156.47, 147.42, 140.62 (q, $J = 37.8$ Hz), 136.82, 131.96 (q, $J = 32.8$ Hz), 130.79, 129.12, 128.59, 123.50 (q, $J = 271.7$ Hz), 123.22, 121.19 (q, $J = 269.2$ Hz), 119.31, 112.91 (q, $J = 4.0$ Hz), 105.79, 65.60, 59.34, 13.69. LCMS (ESI, m/z) Calcd for $C_{20}H_{19}F_3N_2O_2$: 376.1, Found: 377.3 $[M + H]^+$; retention time 5.32 min. HPLC purity >99%. HRMS (ESI) m/z : $[M + H]^+$ Calcd for $C_{20}H_{19}F_3N_2O_2$ 377.1471; Found 377.1462.



(3-(difluoromethyl)-1-methyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methyl acetate (28d)

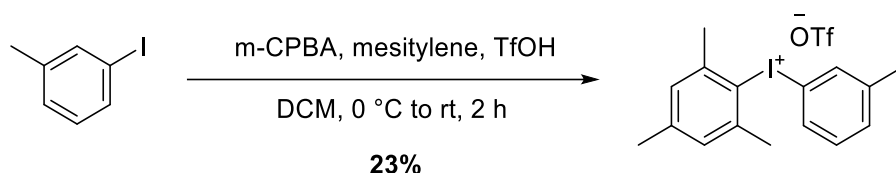
Prepared following the procedure described for **28a**, starting from **27d** (0.381 g, 1.18 mmol, 1.0 equiv), lithium bis(trimethylsilyl)amide (1.96 mL, 8.28 mmol, 7.0 equiv), and ethyl iodide (0.951 mL, 11.83 mmol, 10.0 equiv), except that the reaction was stirred for only 30 h. After

completion, the reaction mixture was transferred and stored in ethyl acetate, resulting in the transesterification of the ether to the corresponding methyl acetate. The crude product was purified by pre-HPLC (isocratic elution, 60% B, R_t = 9.55 min) to afford the desired compound (0.398 g, 95.5%) as a colorless oil. ^{19}F NMR (376 MHz, $\text{DMSO-}d_6$) δ -61.20, -112.68. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.67 (t, J = 8.0 Hz, 1H), 7.62 – 7.55 (m, 1H), 7.44 (s, 1H), 7.33 (dd, J = 8.3, 2.6 Hz, 1H), 7.01 (t, J = 53.6 Hz, 1H), 4.77 (s, 2H), 3.70 (d, J = 1.1 Hz, 3H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) δ 170.1, 156.7, 147.8, 132.1, 131.4, 131.1, 121.5 (t), 120.6, 113.6 (t), 112.1, 101.4, 54.0, 35.5, 20.6. LCMS (ESI, m/z) Calcd for $\text{C}_{14}\text{H}_{13}\text{F}_5\text{N}_2\text{O}_2$: 336.1, Found: 337.2 $[\text{M} + \text{H}]^+$; retention time 4.73 min. HPLC purity >99%.



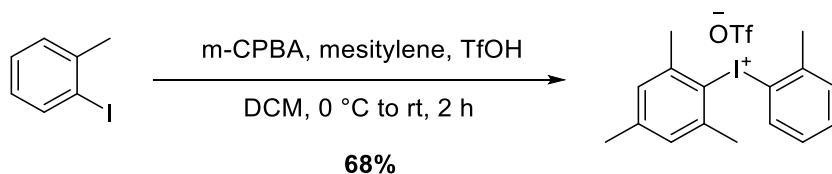
3-(difluoromethyl)-4-(ethoxymethyl)-1-methyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazole (28e)

28d (0.400 g, 1.19 mmol, 1.0 equiv) was dissolved in absolute EtOH (10 mL) at ambient temperature. To this solution, 4-methylbenzenesulfonic acid (20.5 mg, 0.119 mmol, 0.1 equiv) was added. The reaction mixture was stirred at 85 °C overnight. The solvent was evaporated under reduced pressure, and the resulting residue was diluted with water (25 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product was purified by pre-HPLC (isocratic elution, 70% B, R_t = 6.05 min) to afford the desired compound (0.415 g, 73.1%) as a colorless oil. ^1H NMR (600 MHz, $\text{methanol-}d_4$) δ 7.61 (t, J = 0.7 Hz, 1H), 7.50 (ddt, J = 7.8, 1.6, 0.8 Hz, 1H), 7.38 – 7.35 (m, 1H), 7.28 (dd, J = 8.3, 0.7 Hz, 1H), 6.73 (t, J = 54.1 Hz, 1H), 4.25 (s, 2H), 3.72 (t, J = 1.0 Hz, 3H), 3.33 (q, J = 7.0 Hz, 2H), 1.02 (t, J = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, $\text{methanol-}d_4$) δ 156.7, 147.6, 143.5 (t, J = 27.9 Hz), 132.1 (q, J = 32.9 Hz), 130.9, 123.6 (q, J = 271.7 Hz), 120.4 (q, J = 3.8 Hz), 119.3, 112.8 (q, J = 3.9 Hz), 111.9 (t, J = 234.1 Hz), 103.6, 65.3, 59.6, 33.8, 13.7. LCMS (ESI, m/z) Calcd for $\text{C}_{15}\text{H}_{15}\text{F}_5\text{N}_2\text{O}_2$: 350.1, Found: 351.2 $[\text{M} + \text{H}]^+$; retention time 4.99 min. HPLC purity >99%. HRMS (ESI) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{15}\text{H}_{15}\text{F}_5\text{N}_2\text{O}_2$ 351.1126; Found 351.1118.



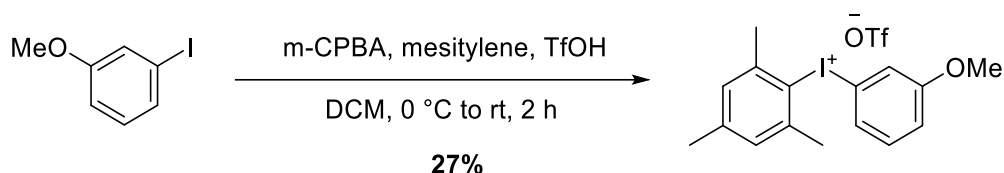
Mesityl(m-tolyl)iodonium trifluoromethanesulfonate (**31a**)

To a stirred solution of 1-iodo-3-methylbenzene (0.44 mL, 3.44 mmol, 1.0 equiv) in dry DCM (10 mL) was added m-Chloroperbenzoic acid (65% active oxidant, 0.665 g, 3.85 mmol, 1.12 equiv) and mesitylene (0.54 mL, 3.85 mmol, 1.12 equiv). The reaction mixture was cooled to 0 °C, and trifluoromethanesulfonic acid (0.34 mL, 3.85 mmol, 1.12 equiv) was added dropwise. The solution was allowed to warm to rt and stirred for 2 hours. The solvent was removed under reduced pressure, and the resulting residue was collected by filtration through a sintered glass funnel. The solid was washed thoroughly with diethyl ether (3 × 25 mL) and dried under reduced pressure to afford the desired aryl-mesityliodonium triflate as a white solid (0.391 g, 23.4%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.85 (s, 1H), 7.76 (d, *J* = 7.9 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.22 (s, 2H), 2.61 (s, 6H), 2.32 (d, *J* = 11.6 Hz, 6H). ¹⁹F NMR (376 MHz, DMSO-*d*₆) δ -77.72. LCMS (ESI, *m/z*) Calcd for C₁₇H₁₈F₃IO₃S: 486.0, Found: 337.0 [M – OTf]⁺; retention time 3.24 min, LCMS purity > 99%.



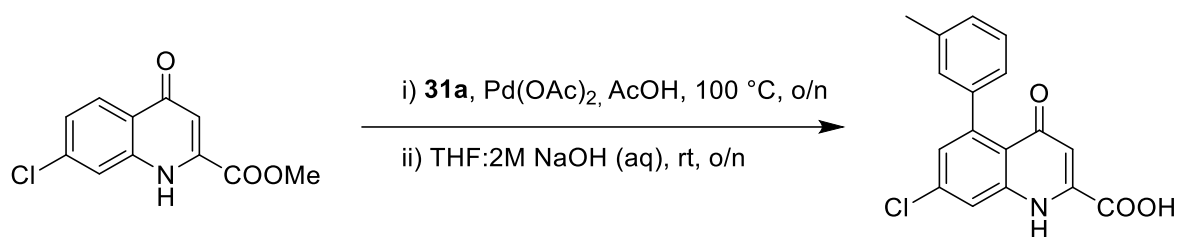
Mesityl(o-tolyl)iodonium trifluoromethanesulfonate (**31b**)

Prepared following the procedure described for **31a**, starting from 1-iodo-2-methylbenzene (0.23 mL, 1.83 mmol, 1.0 equiv), m-Chloroperbenzoic acid (65% active oxidant, 0.355 g, 2.08 mmol, 1.12 equiv) and mesitylene (0.29 mL, 2.05 mmol, 1.12 equiv), and trifluoromethanesulfonic acid (0.18 mL, 2.05 mmol, 1.12 equiv). The product was obtained as a white solid (0.605 g, 67.8%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.96 (d, *J* = 8.1 Hz, 1H), 7.55 (q, *J* = 3.2 Hz, 2H), 7.26 (m, 1H), 7.22 (s, 2H), 2.57 (d, *J* = 2.9 Hz, 9H), 2.30 (s, 3H). ¹⁹F NMR (376 MHz, methanol-*d*₄) δ -80.06. LCMS (ESI, *m/z*) Calcd for C₁₇H₁₈F₃IO₃S: 486.0, Found: 337.0 [M – OTf]⁺; retention time 3.05 min, LCMS purity > 99%.



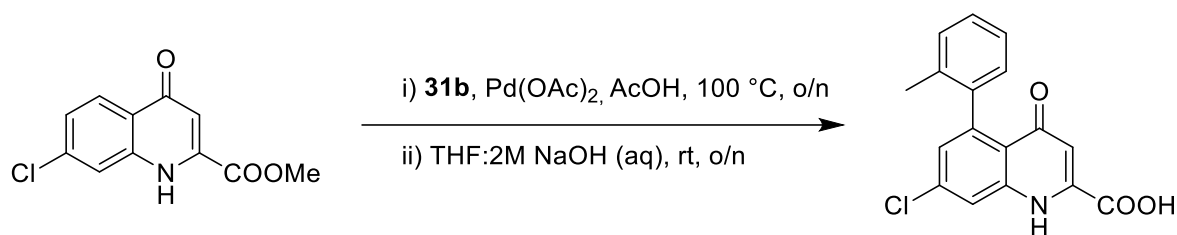
Mesityl(3-methoxyphenyl)iodonium trifluoromethanesulfonate (**31c**)

Prepared following the procedure described for **31a**, starting from 1-iodo-3-methoxybenzene (0.20 mL, 1.71 mmol, 1.0 equiv), m-Chloroperbenzoic acid (65% active oxidant, 0.330 g, 1.91 mmol, 1.12 equiv) and mesitylene (0.27 mL, 1.91 mmol, 1.12 equiv), and trifluoromethanesulfonic acid (0.17 mL, 1.91 mmol, 1.12 equiv). The product was obtained as a white solid (0.233 g, 27.1%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.56 (s, 1H), 7.42 (d, *J* = 7.2 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 3H), 3.78 (s, 3H), 2.61 (s, 6H), 2.31 (s, 3H). ¹⁹F NMR (376 MHz, methanol-*d*₄) δ -80.06. LCMS (ESI, *m/z*) Calcd for C₁₇H₁₈F₃IO₄S: 502.0, Found: 352.9 [*M* – OTf]⁺; retention time 3.16 min, LCMS purity > 99%.



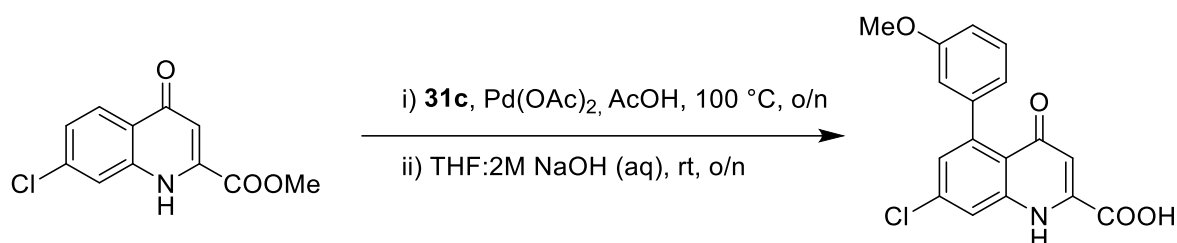
Methyl 7-chloro-4-oxo-5-(*m*-tolyl)-1,4-dihydroquinoline-2-carboxylic acid (**32a**)

To a stirred solution of methyl 7-chloro-4-oxo-1,4-dihydroquinoline-2-carboxylate (0.100g, 0.421 mmol, 1.0 equiv) in glacial acetic acid (8 mL) was added Pd(OAc)₂ (94.47 mg, 0.421 mmol, 1.0 equiv) and **31a** (0.246 g, 0.505 mmol, 1.2 equiv). The reaction mixture was heated to 100 °C and stirred overnight under an inert atmosphere. The solvent was removed under reduced pressure, and the resulting residue was dissolved in THF (4 mL) and 2 M aqueous NaOH (4 mL) and stirred at ambient temperature overnight. The solvent was evaporated, and the residue was diluted with water (25 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. LCMS (ESI, *m/z*) Calcd for C₁₇H₁₂ClNO₃: 313.1, Found: 314.3 [*M* + H]⁺; retention time 4.37 min. HRMS (ESI) *m/z*: [*M* + H]⁺ Calcd for C₁₇H₁₂ClNO₃ 314.0578; Found 314.0572.



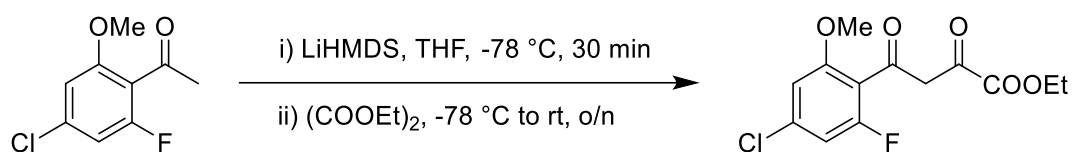
7-chloro-4-oxo-5-(o-tolyl)-1,4-dihydroquinoline-2-carboxylic acid (**32b**)

Prepared following the procedure described for **32a**, starting from 7-chloro-4-oxo-1,4-dihydroquinoline-2-carboxylate (50.0 mg, 0.210 mmol, 1.0 equiv), **31b** (122.8 mg, 0.252 mmol, 1.2 equiv), Pd(OAc)₂ (47.2 mg, 0.210 mmol, 1.0 equiv). LCMS (ESI, *m/z*) Calcd for C₁₇H₁₂ClNO₃: 313.1, Found: 314.0 [M + H]⁺; retention time 4.10 min. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₁₇H₁₂ClNO₃ 314.0578; Found 314.0572.



7-chloro-5-(3-methoxyphenyl)-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (**32c**)

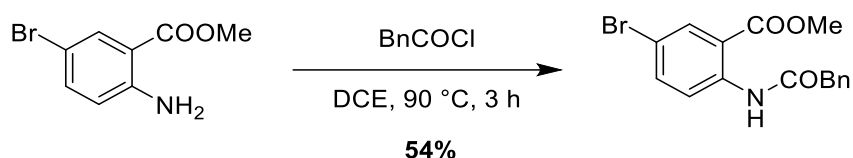
Prepared following the procedure described for **32a**, starting from 7-chloro-4-oxo-1,4-dihydroquinoline-2-carboxylate (50.0 mg, 0.210 mmol, 1.0 equiv), **31c** (126.8 mg, 0.252 mmol, 1.2 equiv), Pd(OAc)₂ (47.2 mg, 0.210 mmol, 1.0 equiv). LCMS (ESI, *m/z*) Calcd for C₁₇H₁₂ClNO₄: 329.0, Found: 330.0 [M + H]⁺; retention time 3.76 min. HRMS (ESI) *m/z*: [M + H]⁺ Calcd for C₁₇H₁₂ClNO₄ 330.0527; Found 330.0521.



Ethyl 4-(4-chloro-2-fluoro-6-methoxyphenyl)-2,4-dioxobutanoate (**33**)

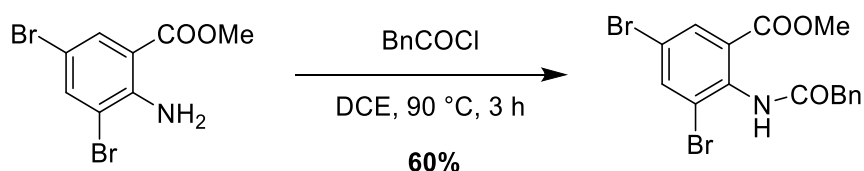
To a flame-dried sealed reaction vial equipped with a magnetic stir bar was added a solution of 1-(4-chloro-2-fluoro-6-methoxyphenyl)ethan-1-one (0.205 g, 1.01 mmol, 1.0 equiv) in anhydrous THF (6 mL). The reaction mixture was cooled to -75 °C, followed by dropwise addition of lithium bis(trimethylsilyl)amide (1.16 mL, 1.16 mmol, 1.15 equiv, 1 M in THF). Diethyl oxalate (0.14 mL, 1.01 mmol, 1.0 equiv) was then added, and the mixture was stirred at -75 °C for 30 min, then allowed to warm to rt and stirred overnight. The reaction was diluted

with water (20 mL) and extracted with EtOAc (3 × 25 mL). The organic layers were washed with 1 M HCl (20 mL) and brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. LCMS (ESI, m/z) Calcd for C₁₃H₁₂ClFO₅: 302.0, Found: 303.1 [M + H]⁺; retention time 4.77 min.



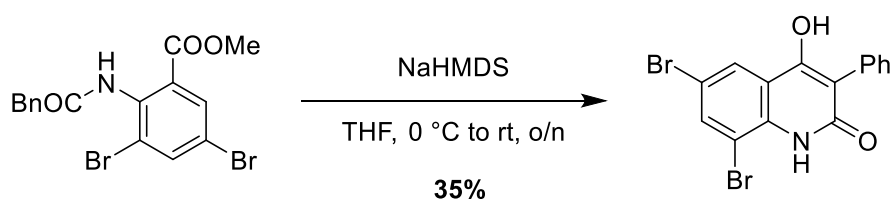
Methyl 5-bromo-2-(2-phenylacetamido)benzoate (39a)

A mixture of methyl 2-amino-5-bromobenzoate (0.21 g, 0.89 mmol, 1.0 equiv) and 2-phenylacetyl chloride (236 μL, 1.78 mmol, 2.0 equiv) in anhydrous DCE (5 mL) was heated to reflux for 3 hours. Upon completion of the reaction, the solvent was removed under reduced pressure, and the resulting crude product was purified by recrystallization from methanol, yielding the desired compound as a white solid (0.167 g, 53.8%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.56 (s, 1H), 8.16 (d, *J* = 8.9 Hz, 1H), 7.96 (d, *J* = 2.5 Hz, 1H), 7.79 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.36 (d, *J* = 5.0 Hz, 4H), 7.31 – 7.27 (m, 1H), 3.76 (s, 3H), 3.75 (s, 2H). LCMS (ESI, m/z) Calcd for C₁₆H₁₄BrNO₃: 347.0, Found: 348.0 [M + H]⁺; retention time 5.28 min, LCMS purity > 99%.



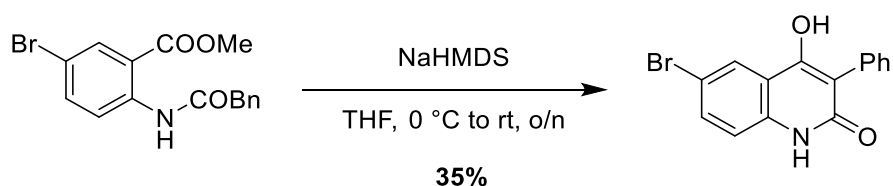
Methyl 3,5-dibromo-2-(2-phenylacetamido)benzoate (39b)

Prepared following the procedure described for **39a**, starting from methyl 2-amino-3,5-dibromobenzoate (0.20 g, 0.65 mmol, 1.0 equiv) and 2-phenylacetyl chloride (0.17 mL, 1.31 mmol, 2.0 equiv). The product was obtained as a white solid (0.168 g, 60.1%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.09 (s, 1H), 8.18 (d, *J* = 2.3 Hz, 1H), 7.84 (d, *J* = 2.3 Hz, 1H), 7.38 – 7.31 (m, 4H), 7.28 – 7.22 (m, 1H), 3.67 (s, 2H), 3.57 (s, 3H). LCMS (ESI, m/z) Calcd for C₁₆H₁₄Br₂NO₃: 424.9, Found: 425.9 [M + H]⁺; retention time 4.79 min, LCMS purity > 99%.



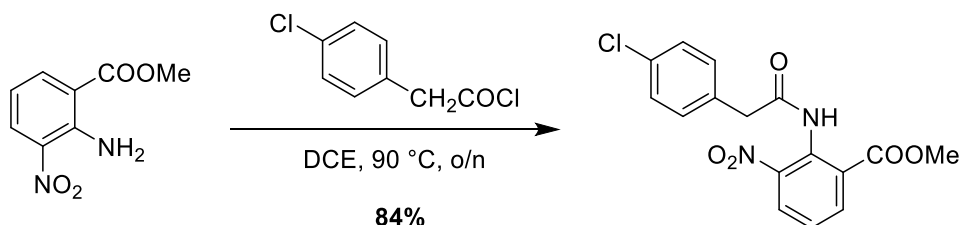
6,8-dibromo-4-hydroxy-3-phenylquinolin-2(1H)-one (40a)

To a flame-dried, sealed reaction vial under argon, equipped with a magnetic stir bar, was added methyl 3,5-dibromo-2-(2-phenylacetamido)benzoate (0.109 g, 0.255 mmol, 1.0 equiv) dissolved in anhydrous THF (8 mL). The solution was cooled to 0 °C and treated dropwise with sodium bis(trimethylsilyl)amide (161.2 μ L, 0.765 mmol, 3.0 equiv). After stirring at 0 °C for 3 h, the reaction mixture was allowed to warm to rt and stirred overnight. Upon completion, the reaction was quenched with 6 M aqueous HCl (30 mL). The resulting precipitate was collected by vacuum filtration and washed thoroughly with water (3 $\times$ 25 mL), followed by ice-cold acetonitrile (3 $\times$ 25 mL). The crude product was dried under vacuum to afford the desired compound as a white solid (35.2 mg, 34.9%). ^1H NMR (600 MHz, DMSO- d_6) δ 10.62 (s, 1H), 10.30 (s, 1H), 8.09 (d, J = 26.9 Hz, 2H), 7.51 – 7.29 (m, 5H). ^{13}C NMR (151 MHz, DMSO) δ 162.7, 156.5, 136.1, 135.3, 132.9, 131.5, 128.4, 127.8, 125.8, 119.4, 114.5, 113.5, 109.5. LCMS (ESI, m/z) Calcd for $\text{C}_{15}\text{H}_9\text{Br}_2\text{NO}_2$: 392.9, Found: 393.9 $[\text{M} + \text{H}]^+$; retention time 4.81 min. HPLC purity > 99%. HRMS (ESI) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{15}\text{H}_9\text{Br}_2\text{NO}_2$ 393.9073; Found 393.9074.



6-bromo-4-hydroxy-3-phenylquinolin-2(1H)-one (40b)

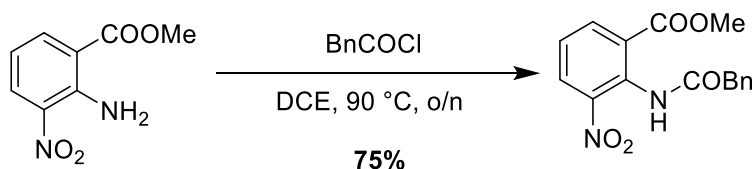
Prepared following the procedure described for **40a**, starting from **39a** (90 mg, 0.258 mmol, 1.0 equiv) and sodium bis(trimethylsilyl)amide (0.16 mL, 0.775 mmol, 3.0 equiv). The product was obtained as a white solid (32.1 mg, 39.2%). LCMS (ESI, m/z) Calcd for $\text{C}_{15}\text{H}_{10}\text{BrNO}_2$: 315.0, Found: 316.0 $[\text{M} + \text{H}]^+$; retention time 4.62 min.



Methyl 2-(2-(4-chlorophenyl)acetamido)-3-nitrobenzoate (41a)

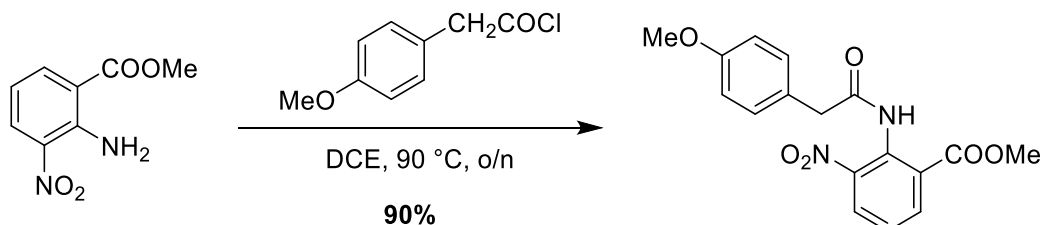
Prepared following the procedure described for **39a**, starting from methyl 2-amino-3-nitrobenzoate (0.51 g, 2.57 mmol, 1.0 equiv) and 2-(4-chlorophenyl)acetyl chloride (0.75 mL,

5.13 mmol, 2.0 equiv). The product was obtained as a white solid (0.754 g, 84.3%). ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 10.41 (s, 1H), 8.13 (dd, J = 8.1, 1.6 Hz, 1H), 8.07 (dd, J = 7.8, 1.6 Hz, 1H), 7.55 (t, J = 8.0 Hz, 1H), 7.44 – 7.39 (m, 2H), 7.36 – 7.31 (m, 2H), 3.76 (s, 3H), 3.70 (s, 2H). LCMS (ESI, m/z) Calcd for $\text{C}_{16}\text{H}_{13}\text{ClN}_2\text{O}_5$: 348.1, Found: 349.1 $[\text{M} + \text{H}]^+$; retention time 4.61 min, LCMS purity > 99%.



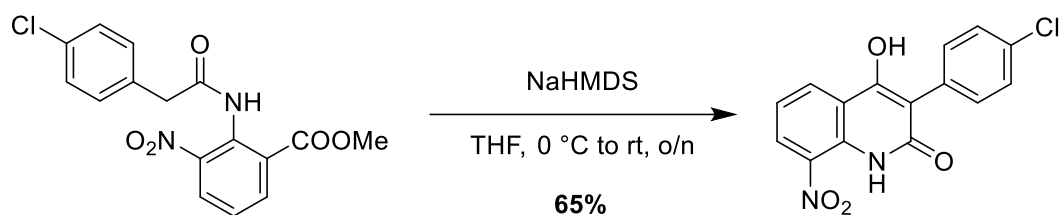
Methyl 3-nitro-2-(2-phenylacetamido)benzoate (41b)

Prepared following the procedure described for **39a**, starting from methyl 2-amino-3-nitrobenzoate (0.50 g, 2.56 mmol, 1.0 equiv) and 2-phenylacetyl chloride (0.68 mL, 5.12 mmol, 2.0 equiv). The product was obtained as a white solid (0.616 g, 74.5%). ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 10.39 (s, 1H), 8.13 (dd, J = 8.2, 1.6 Hz, 1H), 8.06 (dd, J = 7.9, 1.5 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.37 – 7.30 (m, 4H), 7.27 (tt, J = 6.6, 1.6 Hz, 1H), 3.73 (s, 3H), 3.69 (s, 2H). LCMS (ESI, m/z) Calcd for $\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_5$: 314.1, Found: 315.1 $[\text{M} + \text{H}]^+$; retention time 4.49 min, LCMS purity > 99%.



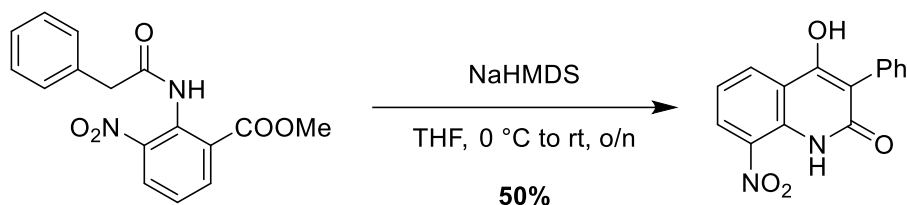
Methyl 2-(2-(4-methoxyphenyl)acetamido)-3-nitrobenzoate (41c)

Prepared following the procedure described for **39a**, starting from methyl 2-amino-3-nitrobenzoate (0.20 g, 1.03 mmol, 1.0 equiv) and 2-(4-methoxyphenyl)acetyl chloride (0.31 mL, 2.06 mmol, 2.0 equiv). The product was obtained as a white solid (0.319 g, 90.3%). ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 10.32 (s, 1H), 8.12 (dd, J = 8.2, 1.5 Hz, 1H), 8.06 (dd, J = 7.8, 1.5 Hz, 1H), 7.53 (t, J = 8.0 Hz, 1H), 7.26 – 7.20 (m, 2H), 6.94 – 6.88 (m, 2H), 3.74 (s, 6H), 3.61 (s, 2H). LCMS (ESI, m/z) Calcd for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_6$: 344.1, Found: 345.1 $[\text{M} + \text{H}]^+$; retention time 4.48 min, LCMS purity > 99%.



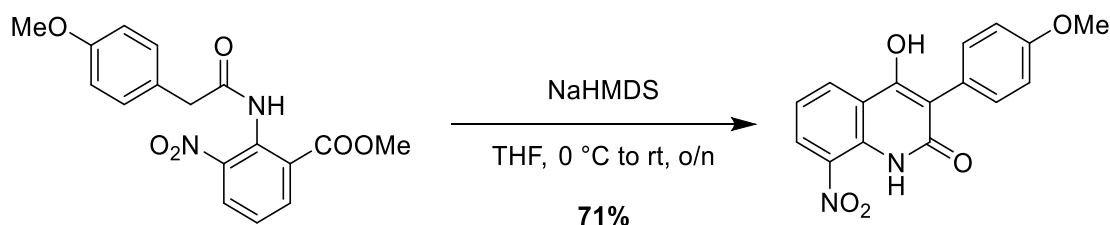
3-(4-chlorophenyl)-4-hydroxy-8-nitroquinolin-2(1H)-one (42a)

Prepared following the procedure described for **40a**, starting from **41a** (0.603 g, 1.73 mmol, 1 equiv) and sodium bis(trimethylsilyl)amide (1.09 mL, 5.19 mmol, 3 equiv). The product was obtained as a light beige solid (0.354 g, 64.7%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 11.11 (s, 1H), 10.84 (s, 1H), 8.52 – 8.48 (m, 1H), 8.47 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.51 – 7.48 (m, 2H), 7.45 – 7.40 (m, 3H). LCMS (ESI, *m/z*) Calcd for C₁₅H₉ClN₂O₄: 316.0, Found: 317.0 [M + H]⁺; retention time 4.30 min, LCMS purity > 98%.



4-hydroxy-8-nitro-3-phenylquinolin-2(1H)-one (42b)

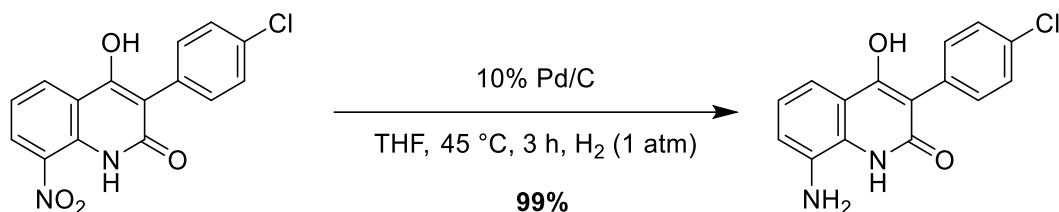
Prepared following the procedure described for **40a**, starting from **41b** (0.146 g, 0.464 mmol, 1 equiv) and sodium bis(trimethylsilyl)amide (0.29 mL, 1.39 mmol, 3 equiv). The product was obtained as a yellow solid (65.9 mg, 50.4%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.83 (s, 1H), 8.47 (dd, *J* = 8.3, 1.4 Hz, 1H), 8.41 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.47 – 7.35 (m, 6H). LCMS (ESI, *m/z*) Calcd for C₁₅H₁₀N₂O₄: 282.1, Found: 283.1 [M + H]⁺; retention time 4.03 min, LCMS purity > 99%.



4-hydroxy-3-(4-methoxyphenyl)-8-nitroquinolin-2(1H)-one (42c)

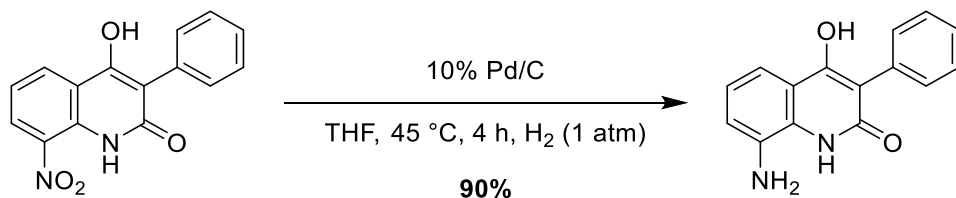
Prepared following the procedure described for **40a**, starting from **41c** (0.415 g, 1.21 mmol, 1 equiv) and sodium bis(trimethylsilyl)amide (0.76 mL, 3.62 mmol, 3 equiv). The product was obtained as a yellow solid (0.268 g, 71.3%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.81 (s, 1H),

10.75 (s, 1H), 8.46 (dd, $J = 8.3, 1.4$ Hz, 1H), 8.43 (dd, $J = 7.9, 1.5$ Hz, 1H), 7.41 (t, $J = 8.1$ Hz, 1H), 7.36 – 7.31 (m, 2H), 7.03 – 6.99 (m, 2H), 3.81 (s, 3H). LCMS (ESI, m/z) Calcd for $C_{16}H_{12}N_2O_5$: 312.1, Found: 313.1 $[M + H]^+$; retention time 4.61 min, LCMS purity > 99%.



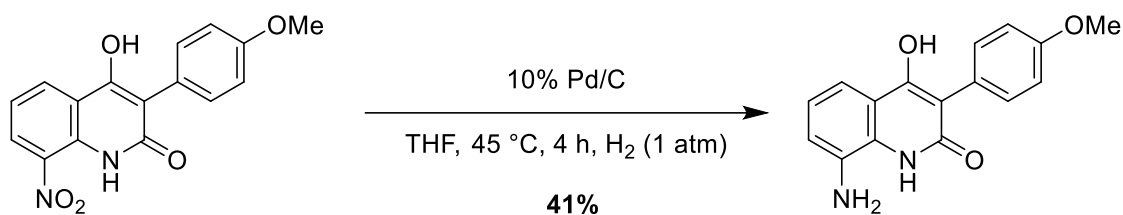
8-amino-3-(4-chlorophenyl)-4-hydroxyquinolin-2(1H)-one (43a)

To a flame-dried, sealed reaction vial under a hydrogen atmosphere, equipped with a magnetic stir bar, was added a mixture of **42a** (112 mg, 0.396 mmol, 1.0 equiv) and 10% Pd/C (16.8 mg, 15 wt%) in anhydrous THF (10 mL). The reaction mixture was stirred at 45 °C for 4 h. Upon completion, the mixture was filtered through a pad of Celite and washed thoroughly with methanol (3 × 30 mL). The filtrate was concentrated under reduced pressure to afford the desired product as a light pink solid (106 mg, 98.8%), which was used directly in the next step without further purification. ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.59 (s, 1H), 10.02 (s, 1H), 7.47 – 7.40 (m, 4H), 7.24 (dd, $J = 8.1, 1.3$ Hz, 1H), 6.95 (t, $J = 7.9$ Hz, 1H), 6.83 (dd, $J = 7.7, 1.3$ Hz, 1H), 5.52 (s, 2H). LCMS (ESI, m/z) Calcd for $C_{15}H_{11}ClN_2O_2$: 286.1, Found: 287.1 $[M + H]^+$; retention time 3.79 min, LCMS purity > 96%.



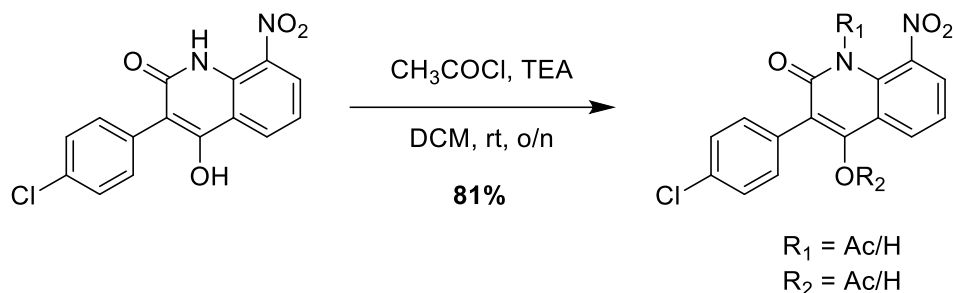
8-amino-4-hydroxy-3-phenylquinolin-2(1H)-one (43b)

Prepared following the procedure described for **43a**, starting from **42b** (112 mg, 0.396 mmol, 1.0 equiv) and 10% Pd/C (16.8 mg, 15 wt%). The product was obtained as a pink solid (93.3 mg, 90.4%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.52 (s, 1H), 9.87 (s, 1H), 7.40 (d, $J = 5.3$ Hz, 4H), 7.30 (tt, $J = 4.8, 3.3$ Hz, 1H), 7.24 (dd, $J = 8.1, 1.3$ Hz, 1H), 6.93 (t, $J = 7.8$ Hz, 1H), 6.82 (dd, $J = 7.7, 1.3$ Hz, 1H), 5.50 (s, 2H). LCMS (ESI, m/z) Calcd for $C_{15}H_{12}N_2O_2$: 252.1, Found: 253.2 $[M + H]^+$; retention time 3.46 min, LCMS purity > 92%.



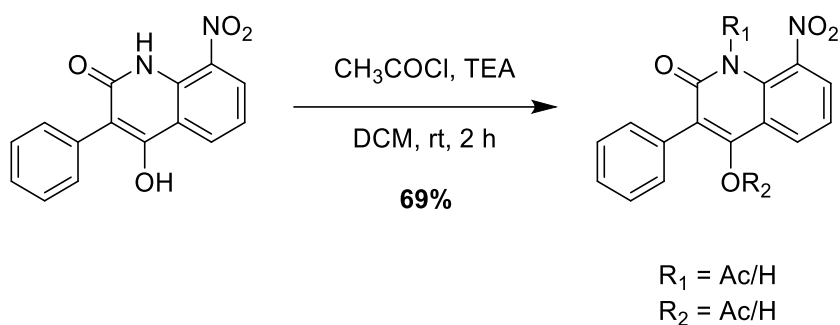
8-amino-4-hydroxy-3-(4-methoxyphenyl)quinolin-2(1H)-one (43c)

Prepared following the procedure described for **43a**, starting from **42c** (225 mg, 0.721 mmol, 1.0 equiv) and 10% Pd/C (33.8 mg, 15 wt%). The product was obtained as a light grey solid (93.3 mg, 90.4%). ^1H NMR (600 MHz, DMSO- d_6) δ 10.50 (s, 1H), 9.74 (s, 1H), 7.30 (d, J = 8.2 Hz, 2H), 7.24 (d, J = 8.1 Hz, 1H), 6.96 (d, J = 8.3 Hz, 2H), 6.91 (t, J = 7.8 Hz, 1H), 6.80 (d, J = 7.7 Hz, 1H), 5.51 (s, 2H), 3.79 (s, 3H). LCMS (ESI, m/z) Calcd for $\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_3$: 282.1, Found: 283.1 $[\text{M} + \text{H}]^+$; retention time 3.79 min, LCMS purity > 88%.



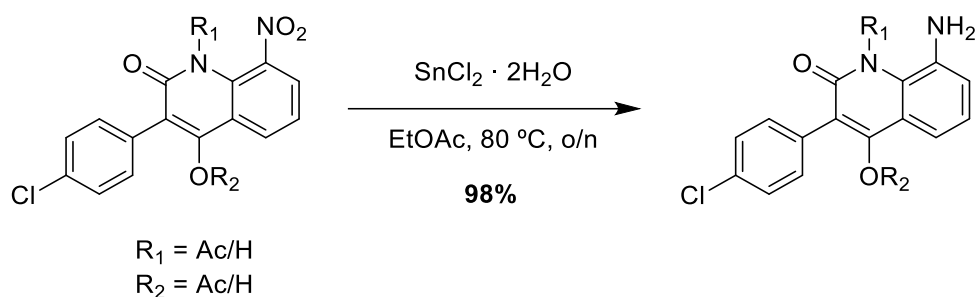
3-(4-chlorophenyl)-8-nitro-2-oxo-1,2-dihydroquinolin-4-yl acetate & 1-acetyl-3-(4-chlorophenyl)-4-hydroxy-8-nitroquinolin-2(1H)-one (45a)

To a solution of **42a** (200 mg, 0.632 mmol, 1.0 equiv) in DCM (8 mL), cooled to 0 °C, was added TEA (0.18 mL, 1.26 mmol, 2.0 equiv). After 5 min of stirring at 0 °C, acetyl chloride (0.05 mL, 0.695 mmol, 1.10 equiv) was introduced dropwise. The reaction mixture was stirred at ambient temperature overnight. Upon completion, the mixture was quenched with 1 M aq. HCl (25 mL) and extracted with EtOAc (3 $\times$ 25 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product (0.184 g, 81.1%) was used directly in the next step without further purification. LCMS (ESI, m/z) Calcd for $\text{C}_{17}\text{H}_{11}\text{ClN}_2\text{O}_5$: 358.0, Found: 359.0 $[\text{M} + \text{H}]^+$; retention time 4.55 min and 4.84 min, LCMS purity > 80%.



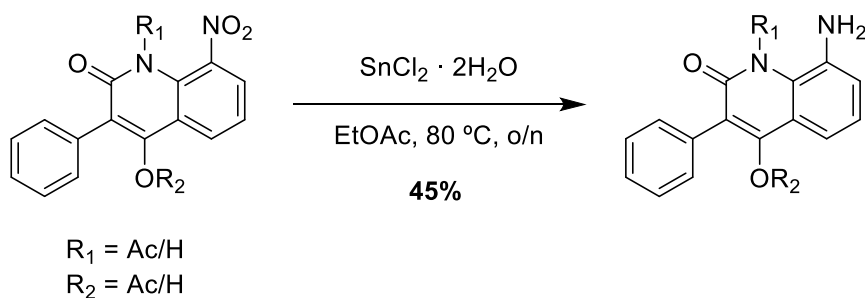
8-nitro-2-oxo-3-phenyl-1,2-dihydroquinolin-4-yl acetate & 1-acetyl-4-hydroxy-8-nitro-3-phenylquinolin-2(1H)-one (45b)

Prepared following the procedure described for **45a**, starting from **42b** (0.627 g, 2.22 mmol, 1.0 equiv), TEA (0.62 mL, 4.44 mmol, 2.0 equiv), and acetyl chloride (0.17 mL, 2.44 mmol, 1.1 equiv). The crude product (0.499 g, 69.3%) was used directly in the next step without further purification. LCMS (ESI, m/z) Calcd for $\text{C}_{17}\text{H}_{12}\text{N}_2\text{O}_5$: 324.1, Found: missing $[\text{M} + \text{H}]^+$.



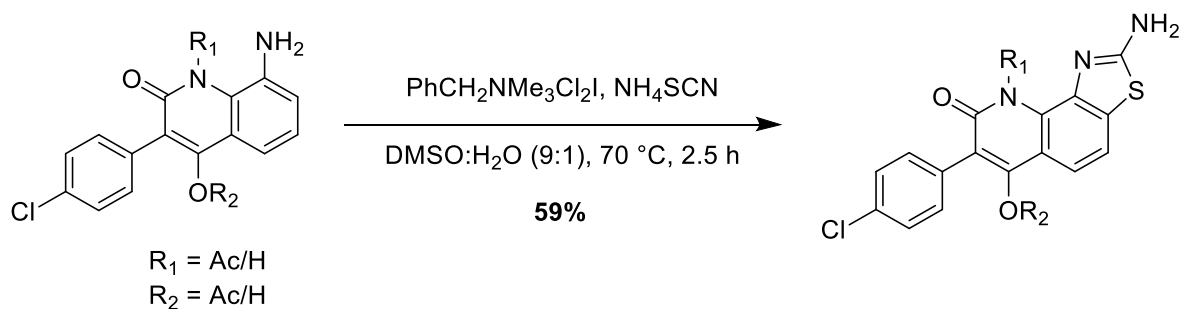
8-amino-3-(4-chlorophenyl)-2-oxo-1,2-dihydroquinolin-4-yl acetate & 1-acetyl-8-amino-3-(4-chlorophenyl)-4-hydroxyquinolin-2(1H)-one (46a)

A mixture of **45a** (0.166 g, 0.462 mmol, 1.0 equiv) and tin(II) chloride dihydrate (0.521 g, 2.31 mmol, 5.0 equiv) in EtOAc (6 mL) was stirred at 80 °C overnight. Upon completion, the reaction was cooled to rt and quenched by the addition of ice-cooled sat. NaHCO_3 solution (10 mL). The resulting suspension was filtered through a sintered glass funnel, and the filtrate was extracted with EtOAc (3×25 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product (0.150 g, 98.5%) was used directly in the next step without further purification. LCMS (ESI, m/z) Calcd for $\text{C}_{17}\text{H}_{13}\text{ClN}_2\text{O}_3$: 328.1, Found: 329.1 $[\text{M} + \text{H}]^+$; retention time 3.87 min and 4.12 min, LCMS purity > 75%.



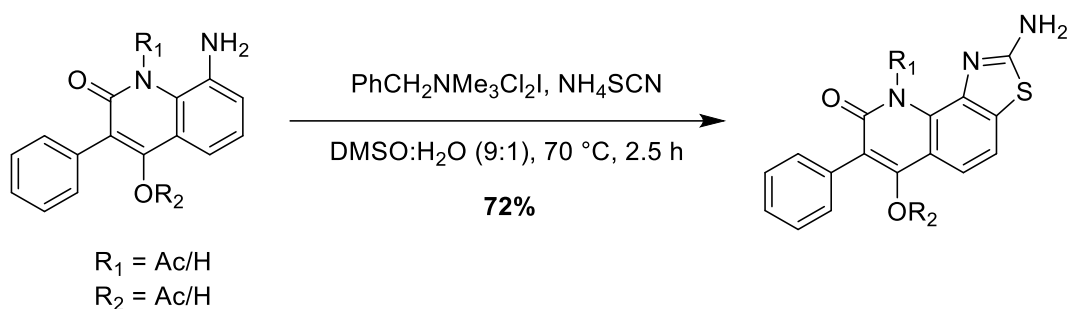
8-amino-2-oxo-3-phenyl-1,2-dihydroquinolin-4-yl acetate & 1-acetyl-8-amino-4-hydroxy-3-phenylquinolin-2(1H)-one (46b)

Prepared following the procedure described for **46a**, starting from **45b** (0.499 g, 1.54 mmol, 1.0 equiv) and tin(II) chloride dihydrate (1.74 g, 7.70 mmol, 5.0 equiv). The crude product (0.499 g, 69.3%) was used directly in the next step without further purification. LCMS (ESI, m/z) Calcd for $\text{C}_{17}\text{H}_{12}\text{N}_2\text{O}_5$: 294.1, Found: 295.1 $[\text{M} + \text{H}]^+$; retention time 3.13 min and 3.82 min.



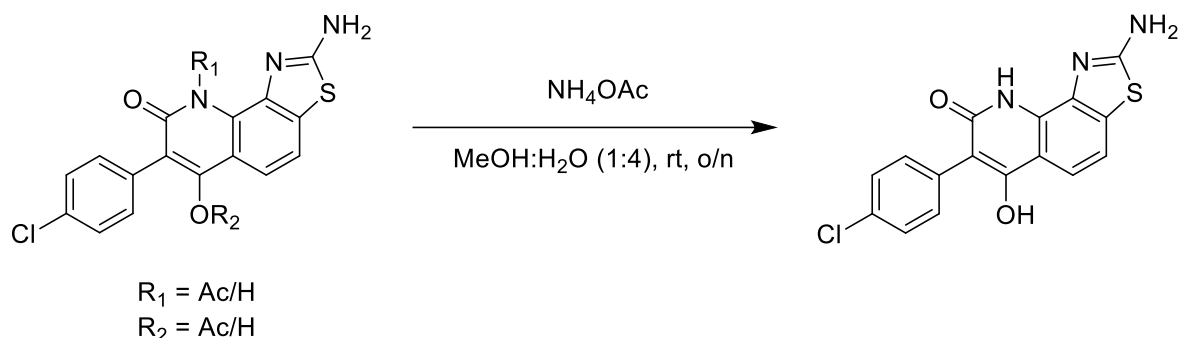
2-amino-7-(4-chlorophenyl)-8-oxo-8,9-dihydrothiazolo[5,4-h]quinolin-6-yl acetate & 9-acetyl-2-amino-7-(4-chlorophenyl)-6-hydroxythiazolo[5,4-h]quinolin-8(9H)-one (47a)

To a stirred mixture of **46a** (149.0 mg, 0.453 mmol, 1.0 equiv) and ammonium thiocyanate (38.0 mg, 0.499 mmol, 1.1 equiv) in 9:1 DMSO: H_2O (7 mL), benzyltrimethylammonium dichloroiodate (189.3 mg, 0.544 mmol, 1.2 equiv) was added. The reaction mixture was stirred at 70 °C for 2.5 hours. Afterward, the reaction was diluted with sat. sodium dithionite (25 mL) and extracted with EtOAc (3×25 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product (0.103 g, 58.6%) was used directly in the next step without further purification. LCMS (ESI, m/z) Calcd for $\text{C}_{18}\text{H}_{12}\text{ClN}_3\text{O}_3\text{S}$: 385.0, Found: 386.0 $[\text{M} + \text{H}]^+$; retention time 4.14 min and 4.28 min.



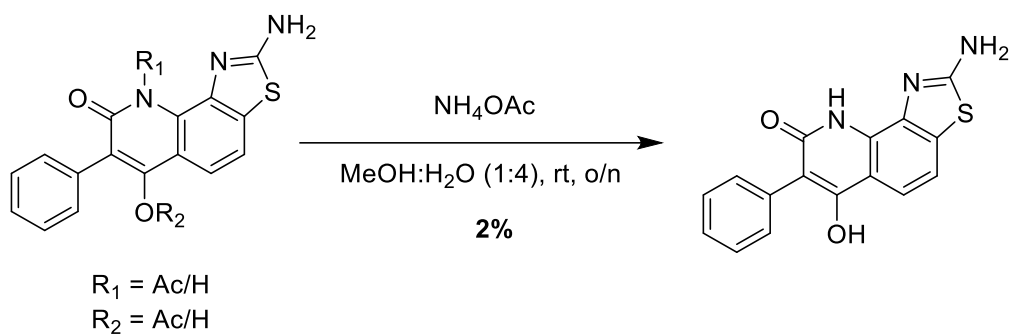
2-amino-8-oxo-7-phenyl-8,9-dihydrothiazolo[5,4-h]quinolin-6-yl acetate & 9-acetyl-2-amino-6-hydroxy-7-phenylthiazolo[5,4-h]quinolin-8(9H)-one (47b)

Prepared following the procedure described for **47a**, starting from **46b** (205 mg, 0.697 mmol, 1.0 equiv), ammonium thiocyanate (58.3 mg, 0.766 mmol, 1.1 equiv), and benzyltrimethylammonium dichloroiodate (291 mg, 0.544 mmol, 1.2 equiv). The crude product (0.175 g, 71.6%) was used directly in the next step without further purification. LCMS (ESI, m/z) Calcd for $\text{C}_{18}\text{H}_{13}\text{N}_3\text{O}_3\text{S}$: 351.1, Found: 352.1 $[\text{M} + \text{H}]^+$; retention time 3.33 min.



2-amino-7-(4-chlorophenyl)-6-hydroxythiazolo[5,4-h]quinolin-8(9H)-one (44a)

To a solution of **47a** (0.103 g, 0.266 mmol, 1.0 equiv) in aq. MeOH (1:4, 15 mL) was added ammonium acetate (0.164 g, 2.13 mmol, 8.0 equiv). The resulting reaction mixture was stirred at rt overnight. Upon completion, the mixture was concentrated under reduced pressure, and the residue was extracted with EtOAc (3×25 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. LCMS (ESI, m/z) Calcd for $\text{C}_{16}\text{H}_{10}\text{ClN}_3\text{O}_2\text{S}$: 343.0, Found: 344.0 $[\text{M} + \text{H}]^+$; retention time 3.86 min.



2-amino-6-hydroxy-7-phenylthiazolo[5,4-h]quinolin-8(9H)-one (**44b**)

Prepared following the procedure described for **44a**, starting from **47b** (0.175 g, 0.499 mmol, 1.0 equiv), and ammonium acetate (0.307 g, 3.99 mmol, 8.0 equiv). The crude product was purified by prep. HPLC (isocratic elution, 30% B, $R_t = 9.07$ min) to afford the desired compound (3.05 mg, 1.98%) as a white solid. LCMS (ESI, m/z) Calcd for $\text{C}_{16}\text{H}_{11}\text{N}_3\text{O}_2\text{S}$: 309.1, Found: 310.1 $[\text{M} + \text{H}]^+$; retention time 3.42 min. HPLC purity > 94%. HRMS (ESI) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{16}\text{H}_{11}\text{N}_3\text{O}_2\text{S}$ 310.0645; Found 310.0638.

6 References

1. Lemoine, D., et al., *Ligand-Gated Ion Channels: New Insights into Neurological Disorders and Ligand Recognition*. Chemical Reviews, 2012. **112**(12): p. 6285-6318.
2. Traynelis, S.F., et al., *Glutamate Receptor Ion Channels: Structure, Regulation, and Function*. Pharmacological Reviews, 2010. **62**(3): p. 405-496.
3. Dupuis, J.P., O. Nicole, and L. Groc, *NMDA receptor functions in health and disease: Old actor, new dimensions*. Neuron, 2023. **111**(15): p. 2312-2328.
4. Hanson, J.E., et al., *Therapeutic potential of N-methyl-D-aspartate receptor modulators in psychiatry*. Neuropsychopharmacology, 2024. **49**(1): p. 51-66.
5. Ulbrich, M.H. and E.Y. Isacoff, *Rules of engagement for NMDA receptor subunits*. Proceedings of the National Academy of Sciences, 2008. **105**(37): p. 14163-14168.
6. Zhou, C. and N. Tajima, *Structural insights into NMDA receptor pharmacology*. Biochemical Society Transactions, 2023. **51**(4): p. 1713-1731.
7. Hansen, K.B., et al., *Structure, function, and allosteric modulation of NMDA receptors*. J Gen Physiol, 2018. **150**(8): p. 1081-1105.
8. Pérez-Otaño, I., R.S. Larsen, and J.F. Wesseling, *Emerging roles of GluN3-containing NMDA receptors in the CNS*. Nature Reviews Neuroscience, 2016. **17**(10): p. 623-635.
9. Kvist, T., et al., *Structure-based discovery of antagonists for GluN3-containing N-methyl-d-aspartate receptors*. Neuropharmacology, 2013. **75**: p. 324-336.
10. Paoletti, P., C. Bellone, and Q. Zhou, *NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease*. Nature Reviews Neuroscience, 2013. **14**(6): p. 383-400.
11. Vyklicky, V., et al., *Structure, function, and pharmacology of NMDA receptor channels*. Physiol Res, 2014. **63**(Suppl 1): p. S191-203.
12. Hansen, K.B., et al., *NMDA Receptors in the Central Nervous System*. Methods Mol Biol, 2017. **1677**: p. 1-80.
13. Grand, T., et al., *Unmasking GluN1/GluN3A excitatory glycine NMDA receptors*. Nature Communications, 2018. **9**(1): p. 4769.
14. Zhao, F., et al., *Derivatives of (R)-3-(5-Furanyl)carboxamido-2-aminopropanoic Acid as Potent NMDA Receptor Glycine Site Agonists with GluN2 Subunit-Specific Activity*. Journal of Medicinal Chemistry, 2022. **65**(1): p. 734-746.
15. Zhao, F., et al., *Design of (R)-3-(5-Thienyl)carboxamido-2-aminopropanoic Acid Derivatives as Novel NMDA Receptor Glycine Site Agonists: Variation in Molecular Geometry to Improve Potency and Augment GluN2 Subunit-Specific Activity*. Journal of Medicinal Chemistry, 2025. **68**(3): p. 3572-3590.
16. Awobuluyi, M., et al., *Subunit-specific roles of glycine-binding domains in activation of NR1/NR3 N-methyl-D-aspartate receptors*. Mol Pharmacol, 2007. **71**(1): p. 112-22.
17. Yao, Y. and M.L. Mayer, *Characterization of a Soluble Ligand Binding Domain of the NMDA Receptor Regulatory Subunit NR3A*. The Journal of Neuroscience, 2006. **26**(17): p. 4559-4566.
18. Chatterton, J.E., et al., *Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits*. Nature, 2002. **415**(6873): p. 793-8.
19. Williams, K., *Ifenprodil discriminates subtypes of the N-methyl-D-aspartate receptor: selectivity and mechanisms at recombinant heteromeric receptors*. Molecular pharmacology, 1993. **44**(4): p. 851-859.
20. Edman, S., et al., *TCN 201 selectively blocks GluN2A-containing NMDARs in a GluN1 co-agonist dependent but non-competitive manner*. Neuropharmacology, 2012. **63**(3): p. 441-449.
21. Volgraf, M., et al., *Discovery of GluN2A-selective NMDA receptor positive allosteric modulators (PAMs): tuning deactivation kinetics via structure-based design*. Journal of medicinal chemistry, 2016. **59**(6): p. 2760-2779.
22. Baron, B.M., et al., *Activity of 5,7-dichlorokynurenic acid, a potent antagonist at the N-methyl-D-aspartate receptor-associated glycine binding site*. Mol Pharmacol, 1990. **38**(4): p. 554-61.
23. Rouzbeh, N., et al., *Allosteric modulation of GluN1/GluN3 NMDA receptors by GluN1-selective competitive antagonists*. Journal of General Physiology, 2023. **155**(6).
24. Catarzi, D., V. Colotta, and F. Varano, *Competitive Gly/NMDA receptor antagonists*. Curr Top Med Chem, 2006. **6**(8): p. 809-21.

25. Wärnmark, K., *Lecture notes for ORGANIC CHEMISTRY ADVANCED COURSE KEMM21*. 2012.
26. Javahershenas, R., et al., *Recent Advances in the Application of 2-Aminobenzothiazole to the Multicomponent Synthesis of Heterocycles*. ChemistryOpen, 2024. **13**(11): p. e202400185.
27. Hegerschoff, H., *Effect of bromine on aromatic thioureas*. Chem Ber, 1903. **36**: p. 3121-34.
28. Itoh, N., et al., *Introduction of a Hydroxy Group at the Para Position and N-Iodophenylation of N-Arylamides Using Phenylodine(III) Bis(Trifluoroacetate)*. The Journal of Organic Chemistry, 2002. **67**(21): p. 7424-7428.
29. Jiang, L., et al., *Discovery of GluN2A subtype-selective N-methyl-D-aspartate (NMDA) receptor ligands*. Acta Pharm Sin B, 2024. **14**(5): p. 1987-2005.
30. Mauger, C. and G. Mignani, *The Synthesis of Important Pharmaceutical Building Blocks by Palladium-Catalyzed Coupling Reaction: Access to Various Arylhydrazines*. Advanced Synthesis & Catalysis, 2005. **347**(6): p. 773-782.
31. Yildiz, I., *A DFT Approach to the Mechanistic Study of Hydrozone Hydrolysis*. The Journal of Physical Chemistry A, 2016. **120**(20): p. 3683-3692.
32. Sanjeeva Rao, K. and T.-S. Wu, *Chan–Lam coupling reactions: synthesis of heterocycles*. Tetrahedron, 2012. **68**(38): p. 7735-7754.
33. Ramachandran, P.V., A.A. Alawaed, and H.J. Hamann, *Catalyst- and Stoichiometry-Dependent Deoxygenative Reduction of Esters to Ethers or Alcohols with Borane–Ammonia*. Organic Letters, 2023. **25**(37): p. 6902-6906.
34. Richards, E.M., et al., *Bidentate NHC-Containing Ligands for Copper Catalysed Synthesis of Functionalised Diaryl Ethers*. Advanced Synthesis & Catalysis, 2025. **367**(6): p. e202400909.
35. Patrick, G.L., *An Introduction to Medicinal Chemistry*. 2023: Oxford University Press.
36. Leeson, P.D., et al., *Kynurenic acid derivatives. Structure-activity relationships for excitatory amino acid antagonism and identification of potent and selective antagonists at the glycine site on the N-methyl-D-aspartate receptor*. Journal of Medicinal Chemistry, 1991. **34**(4): p. 1243-1252.
37. Heindel, N.D., et al., *Cyclization of aniline-acetylenedicarboxylate adducts. Improved synthesis of 8-nitro-2-carbomethoxy-4(1H)-quinolones*. The Journal of Organic Chemistry, 1967. **32**(12): p. 4155-4157.
38. Heindel, N.D., et al., *Cyclization of aniline-acetylenedicarboxylate adducts. A modified Conrad-Limpach method for the synthesis of potential antimalarials*. Journal of Medicinal Chemistry, 1968. **11**(6): p. 1218-1221.
39. Mehra, M.K., et al., *Substrate or Solvent-Controlled PdII-Catalyzed Regioselective Arylation of Quinolin-4(1H)-ones Using Diaryliodonium Salts: Facile Access to Benzoxocine and Aaptamine Analogues*. European Journal of Organic Chemistry, 2020. **2020**(16): p. 2409-2413.
40. Liu, Q., et al., *Design, synthesis and evaluation of chromone-2-carboxamido-alkylbenzylamines as multifunctional agents for the treatment of Alzheimer's disease*. Bioorganic & Medicinal Chemistry, 2015. **23**(5): p. 911-923.
41. Walenzyk, T., et al., *Chromone derivatives which bind to human hair*. Tetrahedron, 2005. **61**(31): p. 7366-7377.
42. Myannik, K.A., et al., *Synthesis of 3-(N-arylcarbonyl)chromones from 2-hydroxyarylaminoenones and isocyanates*. Russian Chemical Bulletin, 2019. **68**(1): p. 104-109.
43. Lei, J., et al., *Practical synthesis of quinolone drugs via a novel TsCl-mediated domino reaction sequence*. Green Chemistry, 2022. **24**(15): p. 5755-5759.
44. Drysdale, M.J., et al., *Synthesis and SAR of 4-Aryl-2-hydroxy-4-oxobut-2-enoic Acids and Esters and 2-Amino-4-aryl-4-oxobut-2-enoic Acids and Esters: Potent Inhibitors of Kynurenine-3-hydroxylase as Potential Neuroprotective Agents*. Journal of Medicinal Chemistry, 2000. **43**(1): p. 123-127.
45. Saloutin, V.I., et al., *Reaction of pentafluorobenzoylpyruvic acid with amines*. Russian Chemical Bulletin, 1994. **43**(2): p. 279-282.
46. Chapman, A.G., et al., *Anticonvulsant activity of a novel NMDA/glycine site antagonist, MDL 104,653, against kindled and sound-induced seizures*. European Journal of Pharmacology, 1995. **274**(1): p. 83-88.
47. Carling, R.W., et al., *4-Substituted-3-phenylquinolin-2(1H)-ones: Acidic and Nonacidic Glycine Site N-Methyl-D-aspartate Antagonists with in Vivo Activity*. Journal of Medicinal Chemistry, 1997. **40**(5): p. 754-765.

48. Brnardic, E.J., et al., *Optimization of a pyrazoloquinolinone class of Chk1 kinase inhibitors*. Bioorganic & Medicinal Chemistry Letters, 2007. **17**(21): p. 5989-5994.
49. Mitsos, C.A., A.L. Zografos, and O. Igglessi-Markopoulou, *An Efficient Route to 3-Aryl-Substituted Quinolin-2-one and 1,8-Naphthyridin-2-one Derivatives of Pharmaceutical Interest*. The Journal of Organic Chemistry, 2003. **68**(11): p. 4567-4569.
50. Dass, R. and M.A. Peterson, *An efficient one-pot synthesis of 2-aminobenzothiazoles from substituted anilines using benzyltrimethylammonium dichloroiodate and ammonium thiocyanate in DMSO:H₂O*. Tetrahedron Letters, 2021. **83**: p. 153388.

7. Appendix

7.1 NMR Spectra

Shown below are the ^1H , ^{13}C , and ^{19}F NMR spectra of the intermediates and final compounds, numbered according to the experimental procedures.

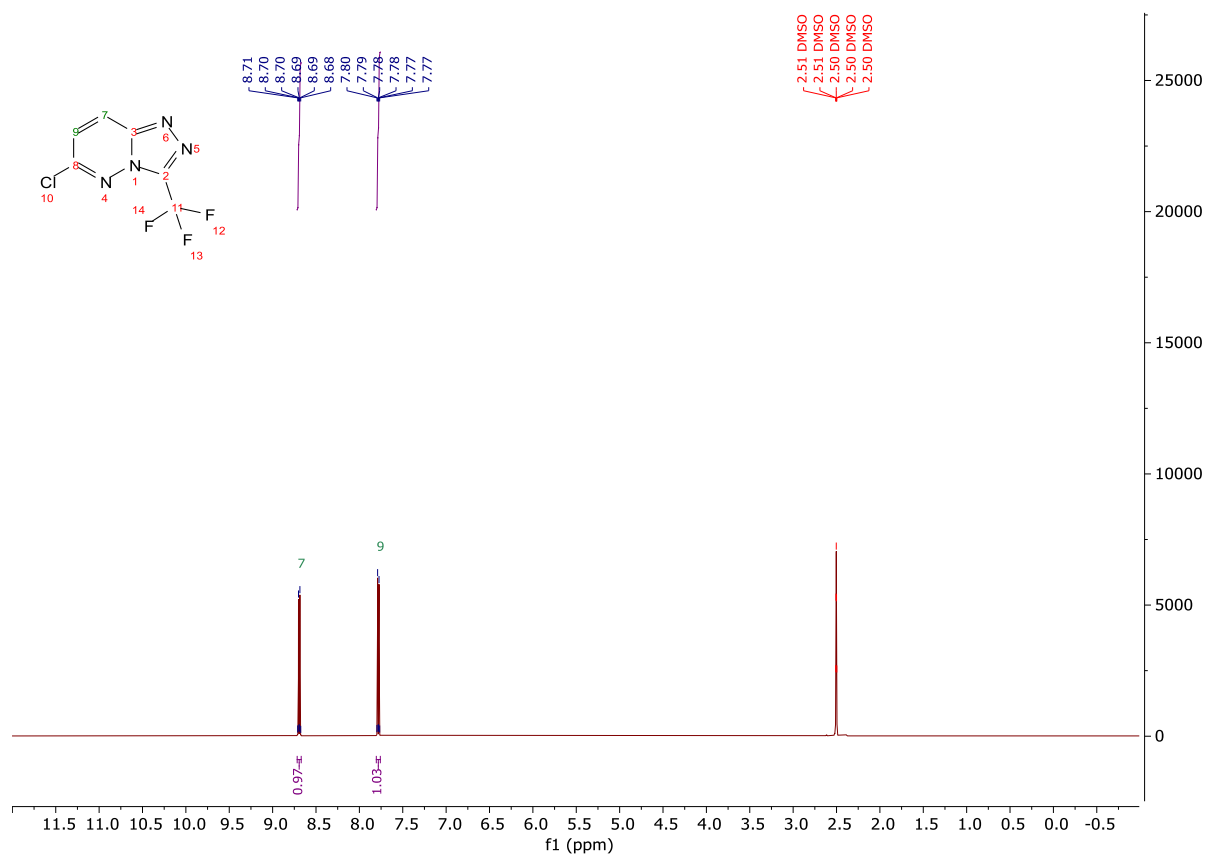


Figure 24. ^1H NMR spectrum for compound 1.

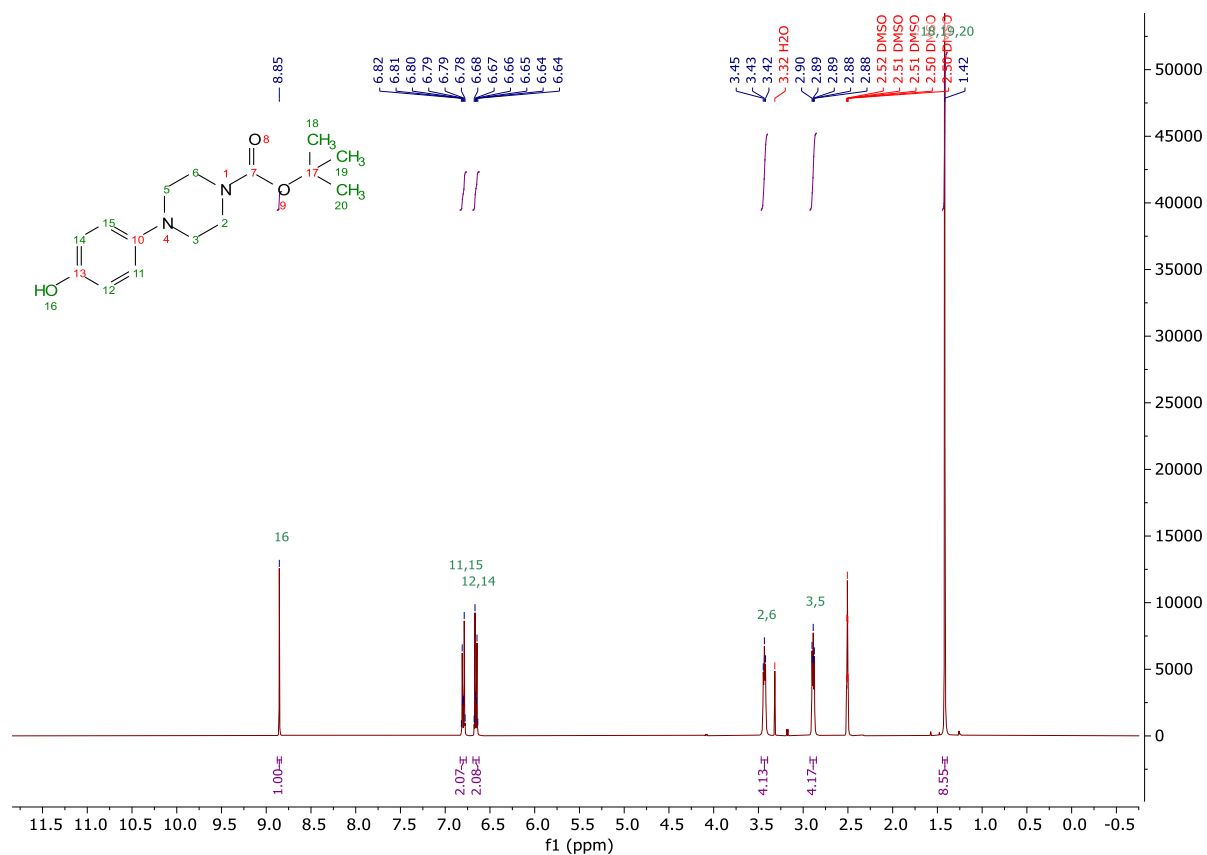


Figure 25. ^1H NMR spectrum for compound **2**.

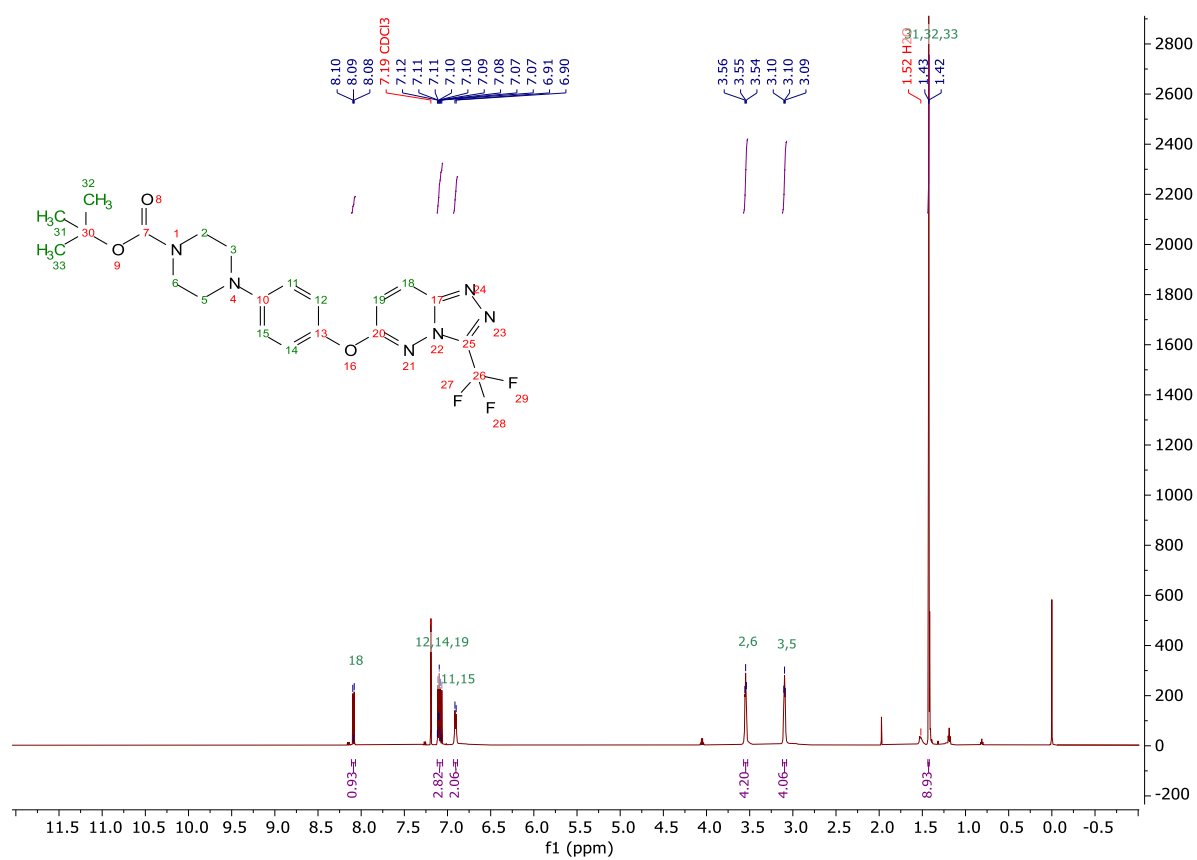


Figure 26. ^1H NMR spectrum for compound **3**.

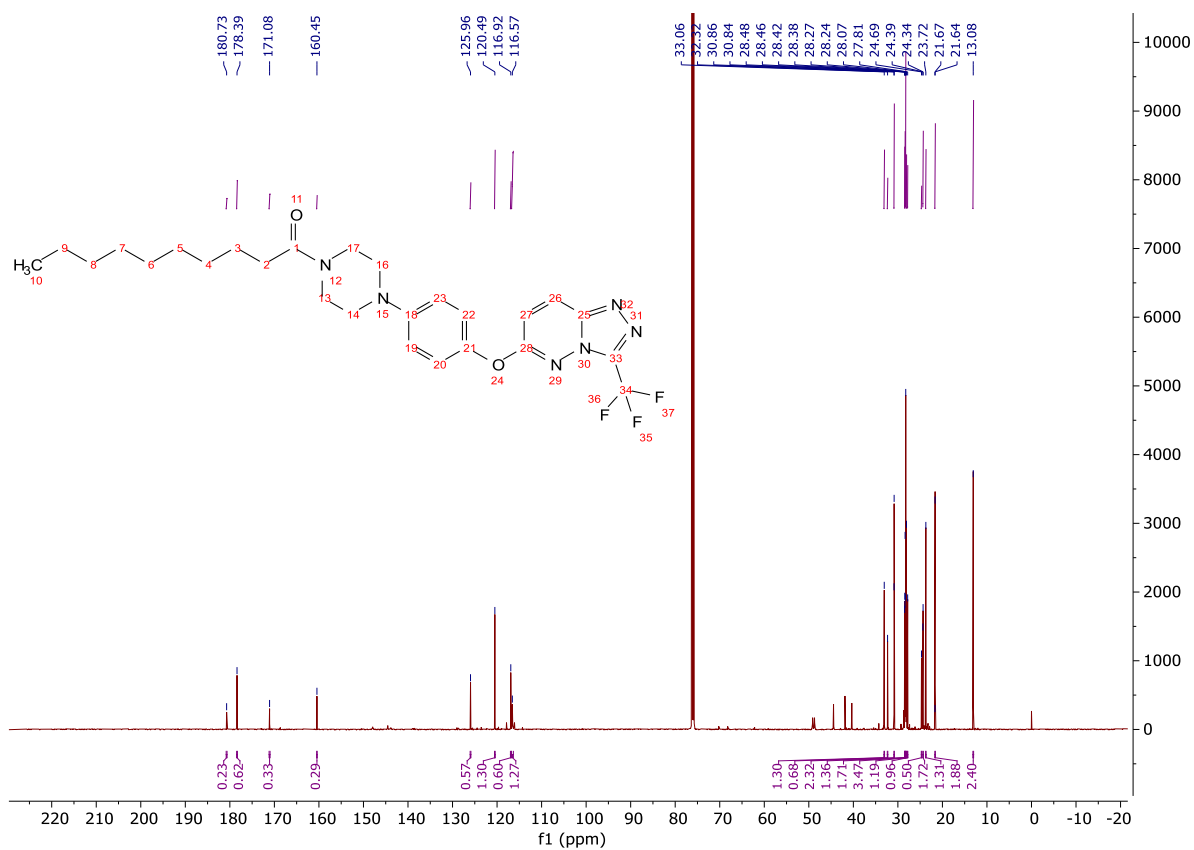


Figure 29. ^{13}C NMR spectrum for compound **5a**.

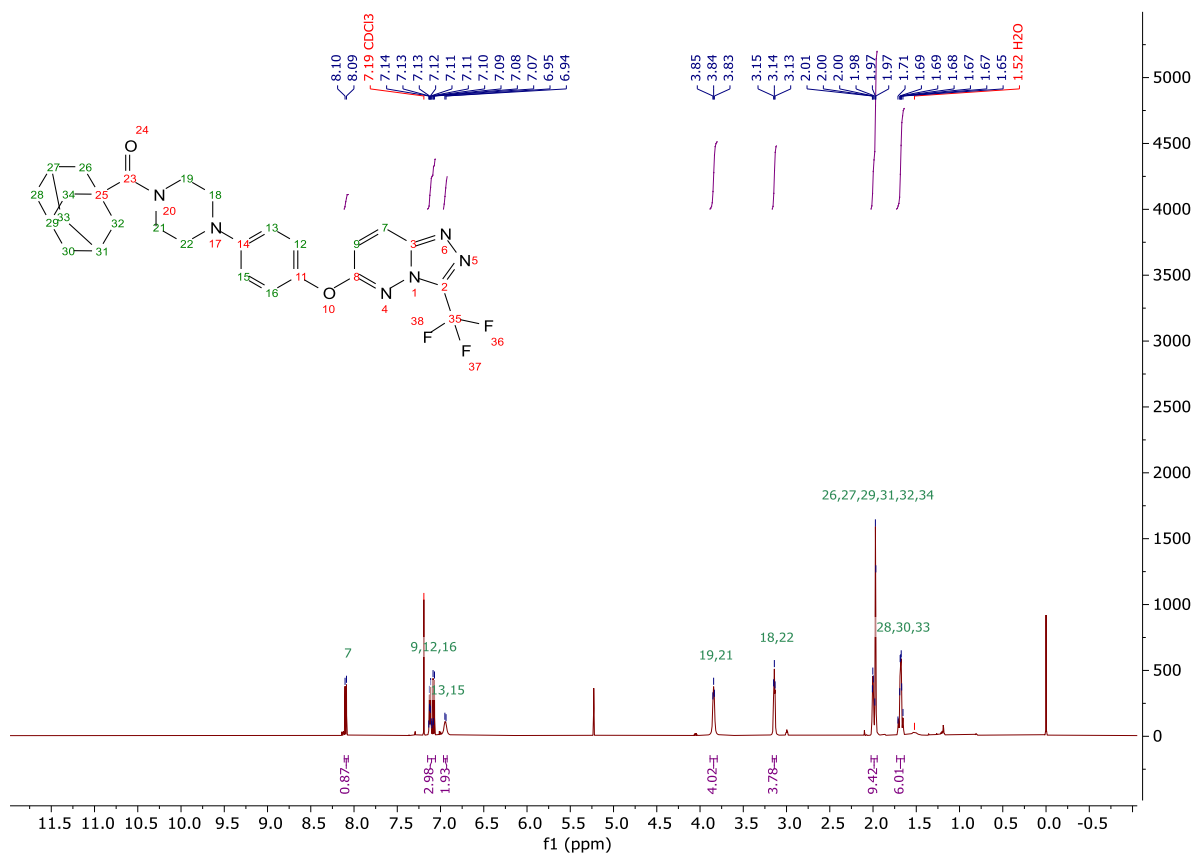
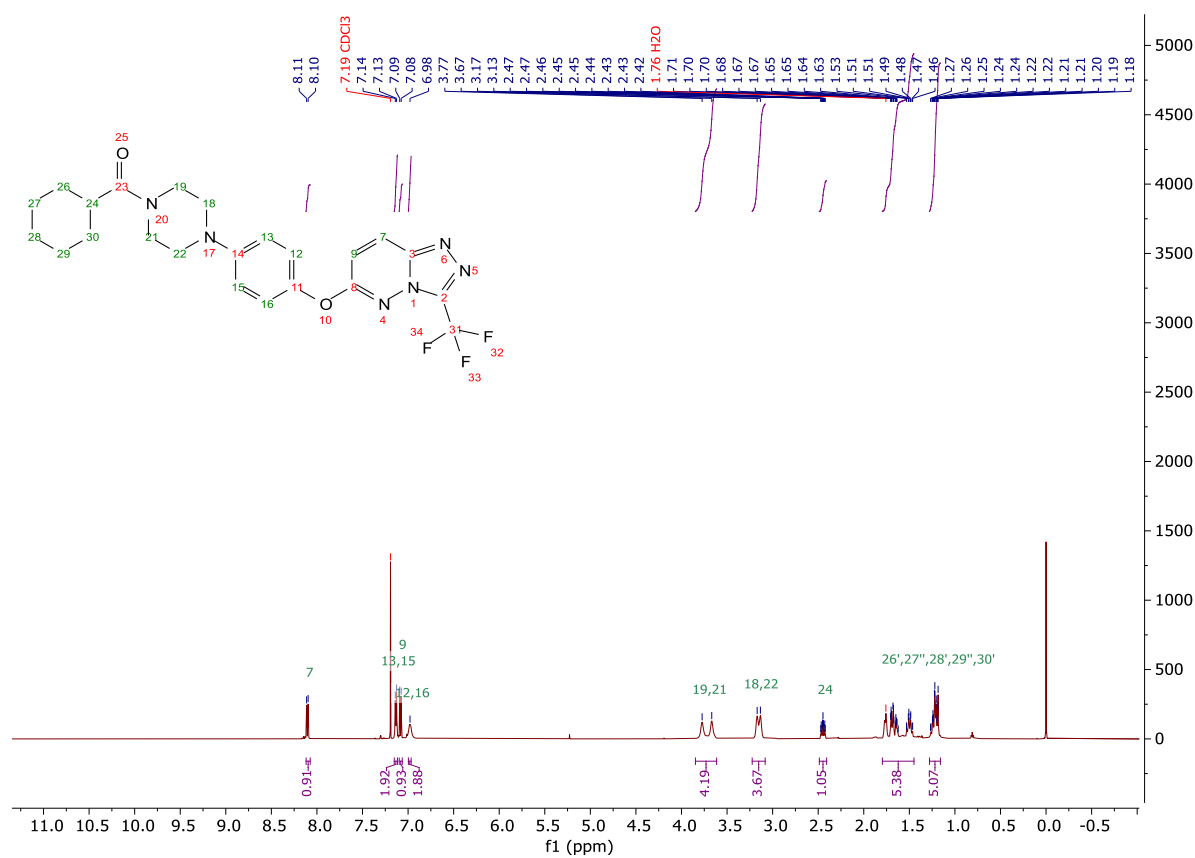
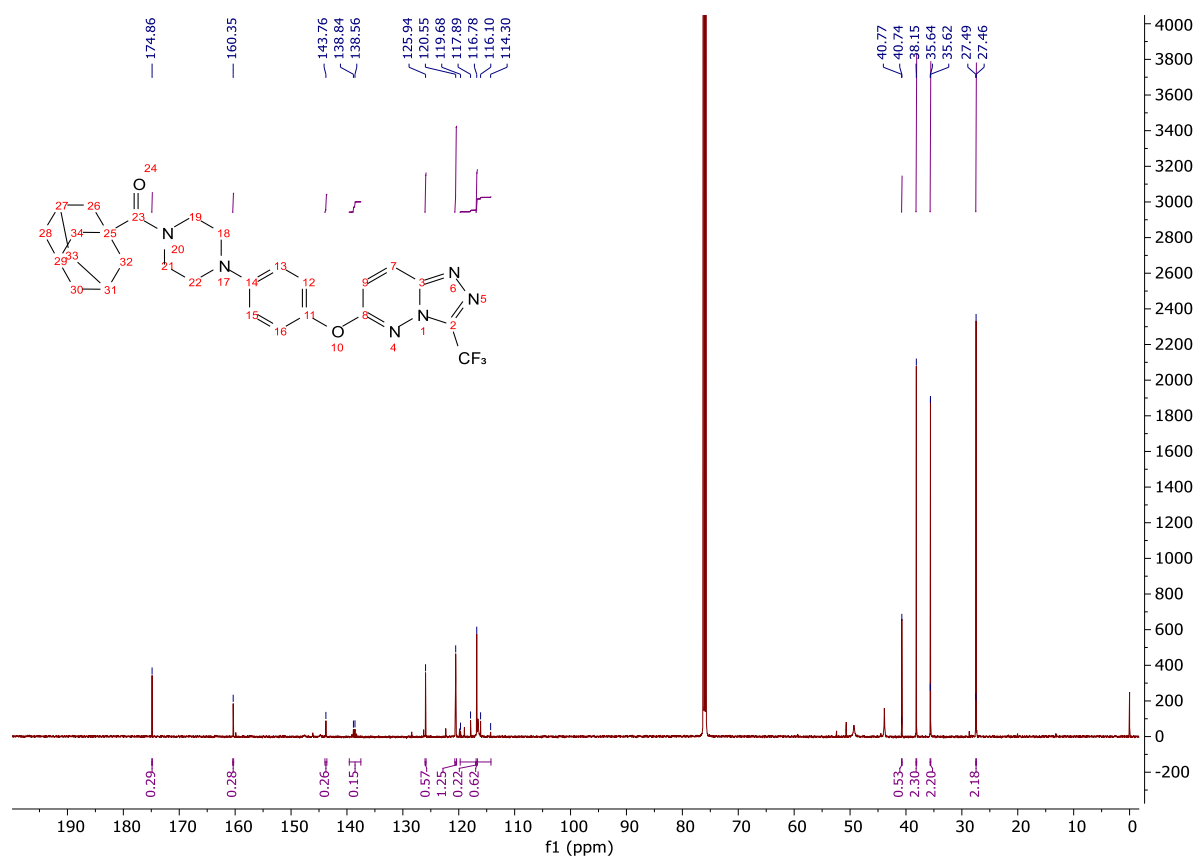
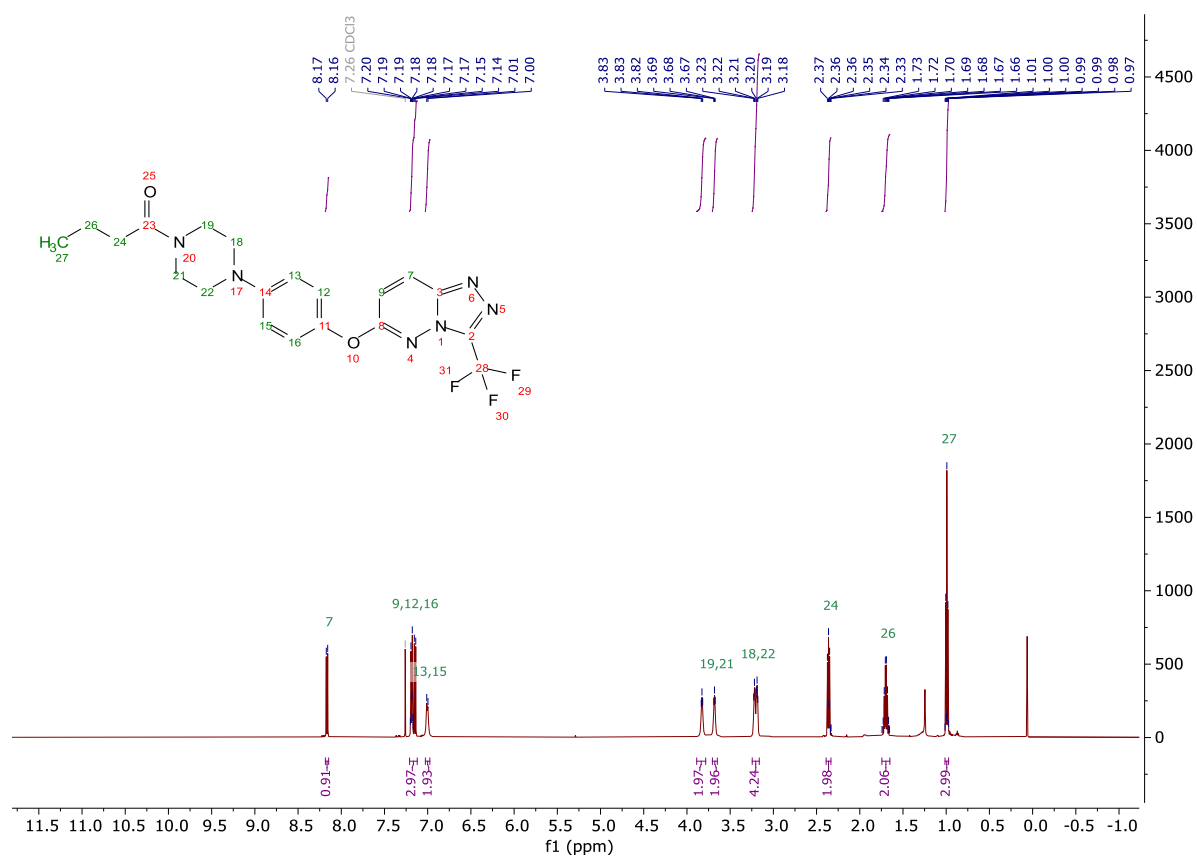
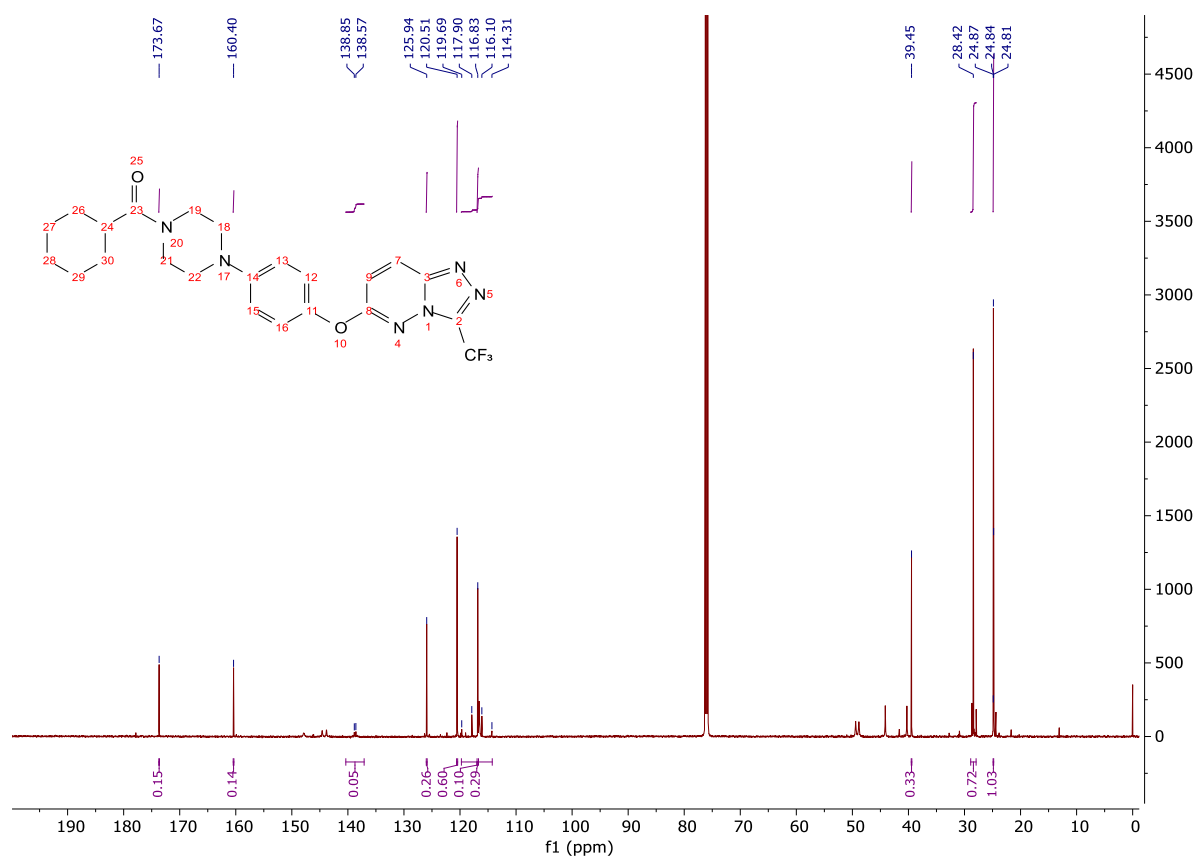


Figure 30. ^1H NMR spectrum for compound **5b**.





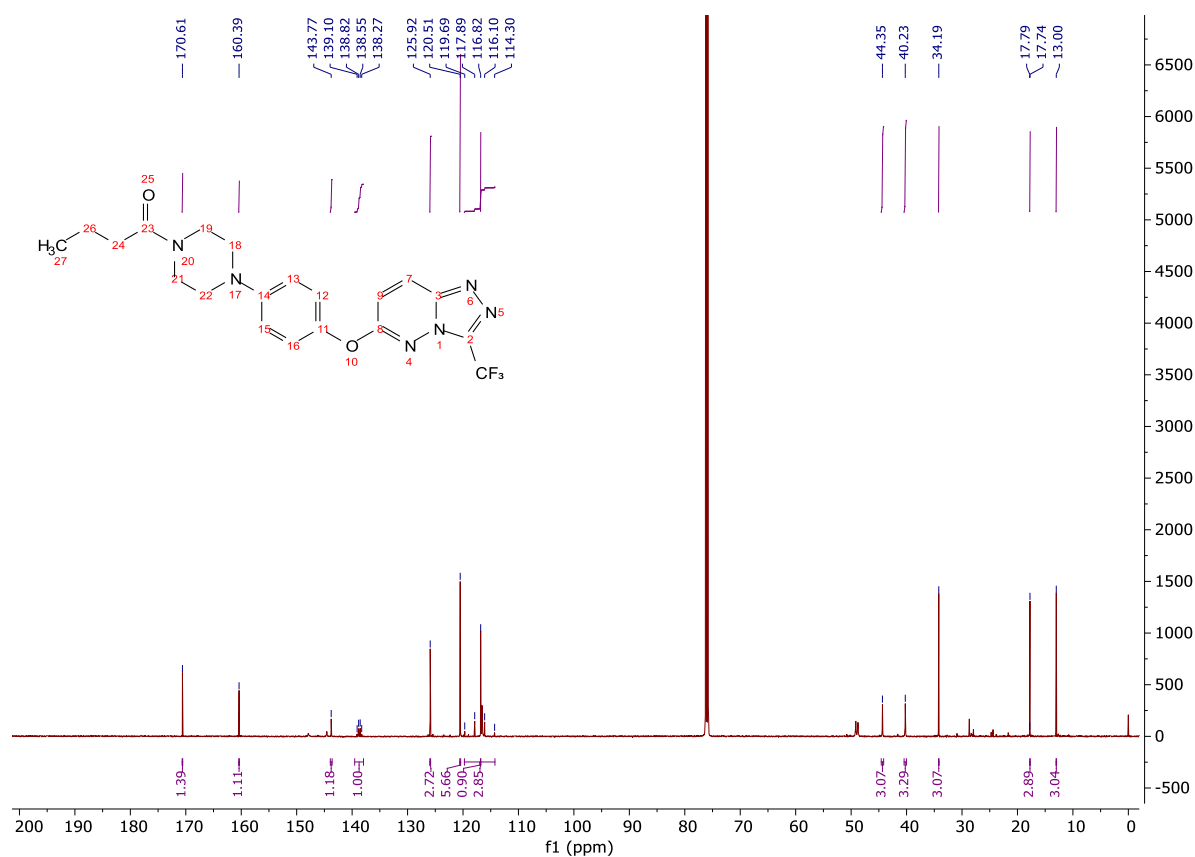


Figure 35. ^{13}C NMR spectrum for compound 5d.

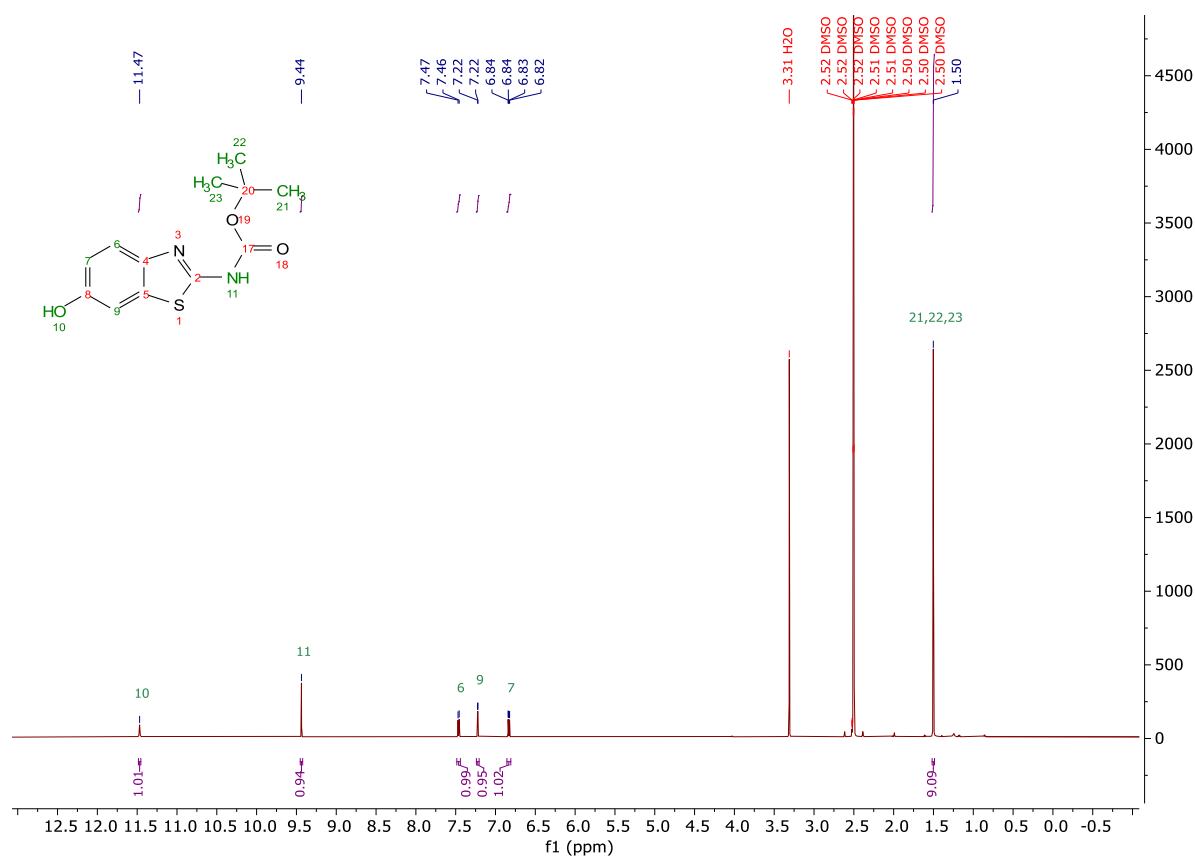


Figure 36. ^1H NMR spectrum for compound 6.

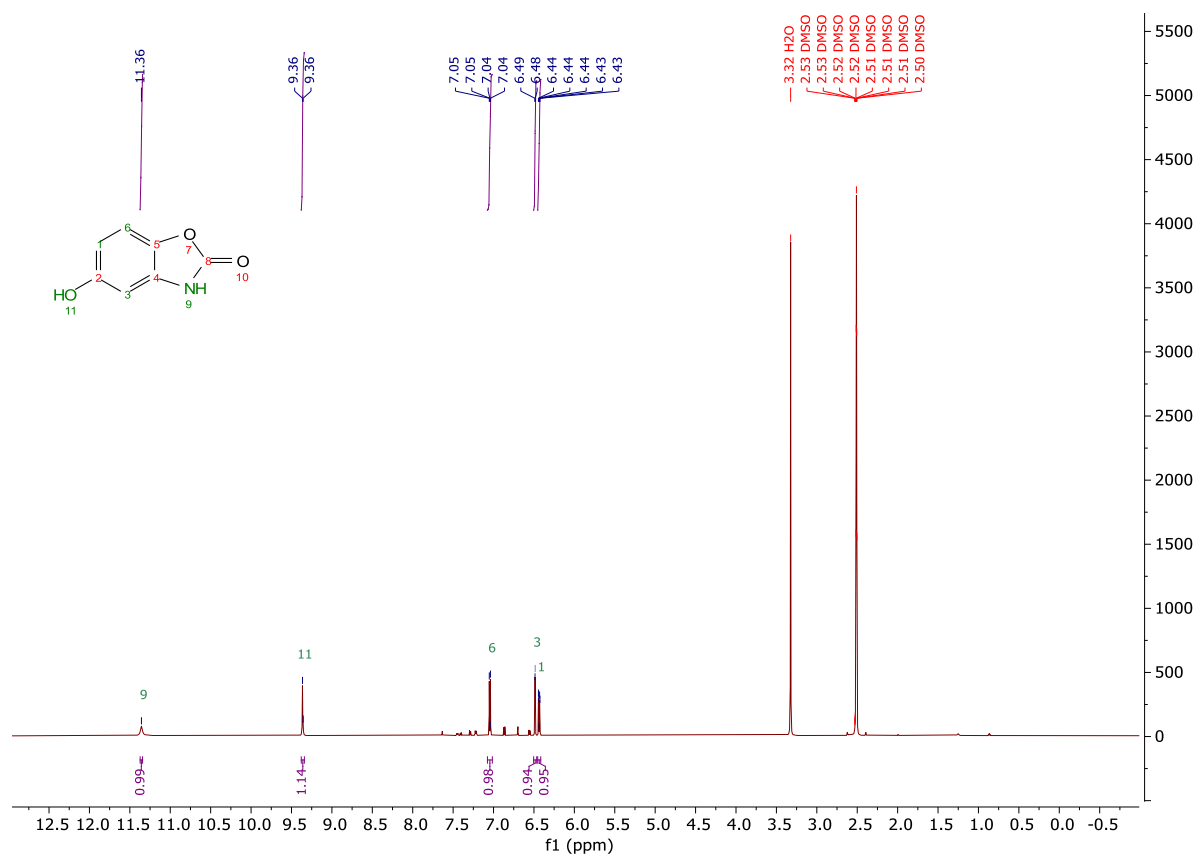


Figure 37. ¹H NMR spectrum for compound 7.

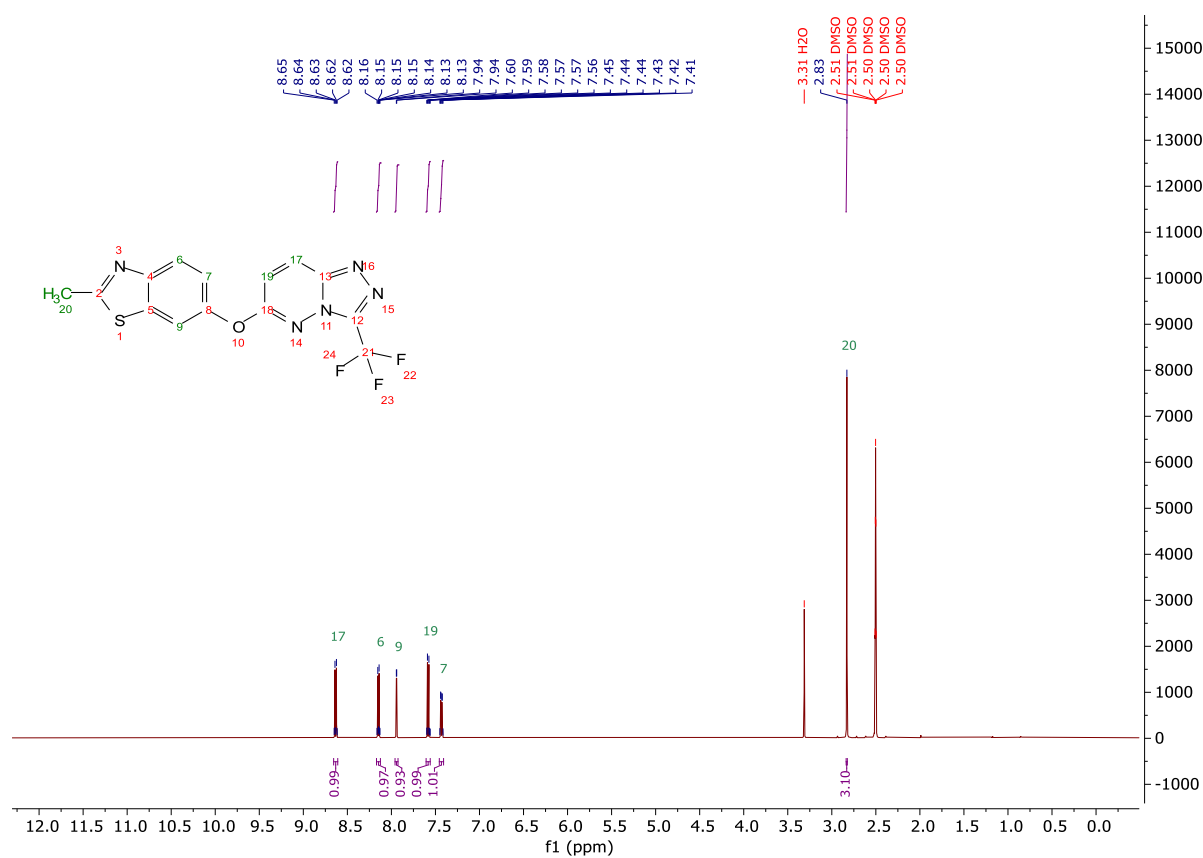


Figure 38. ¹H NMR spectrum for compound 8a.

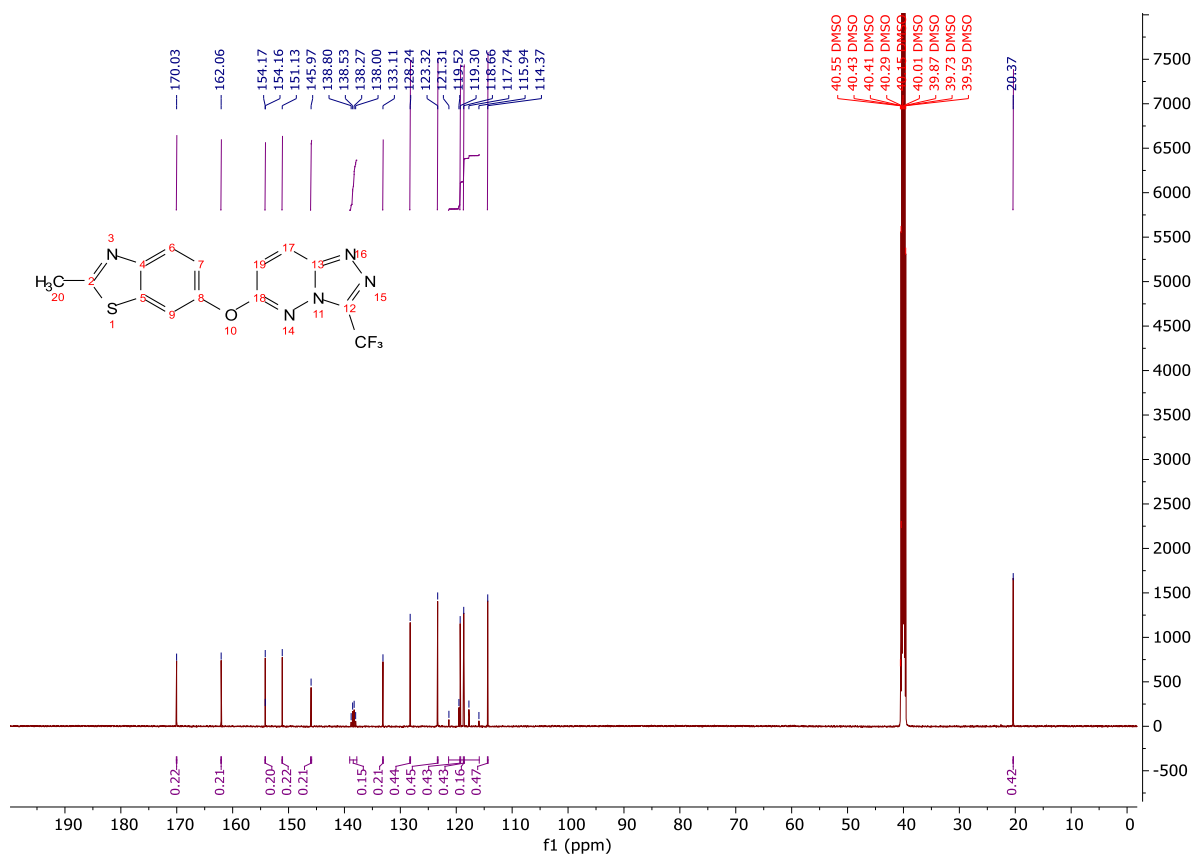


Figure 39. ^{13}C NMR spectrum for compound 8a.

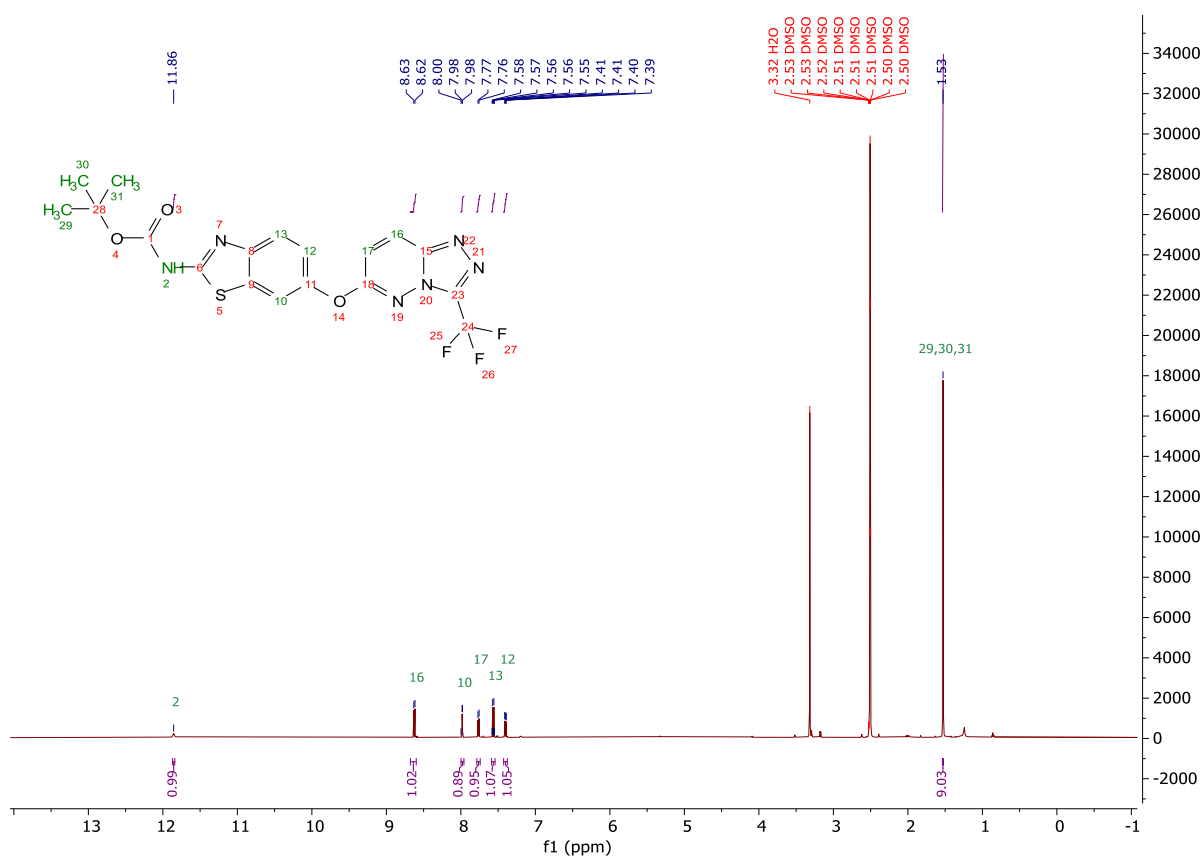
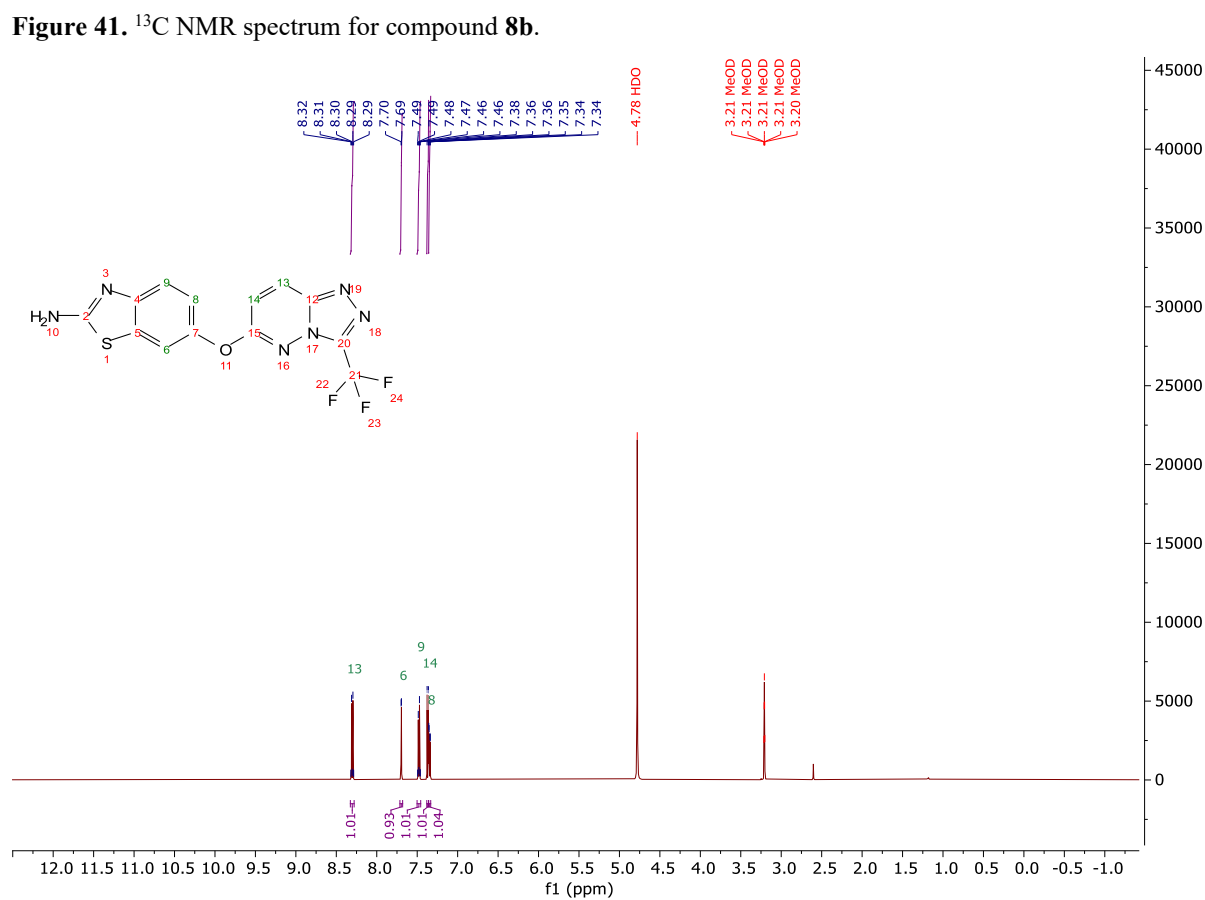
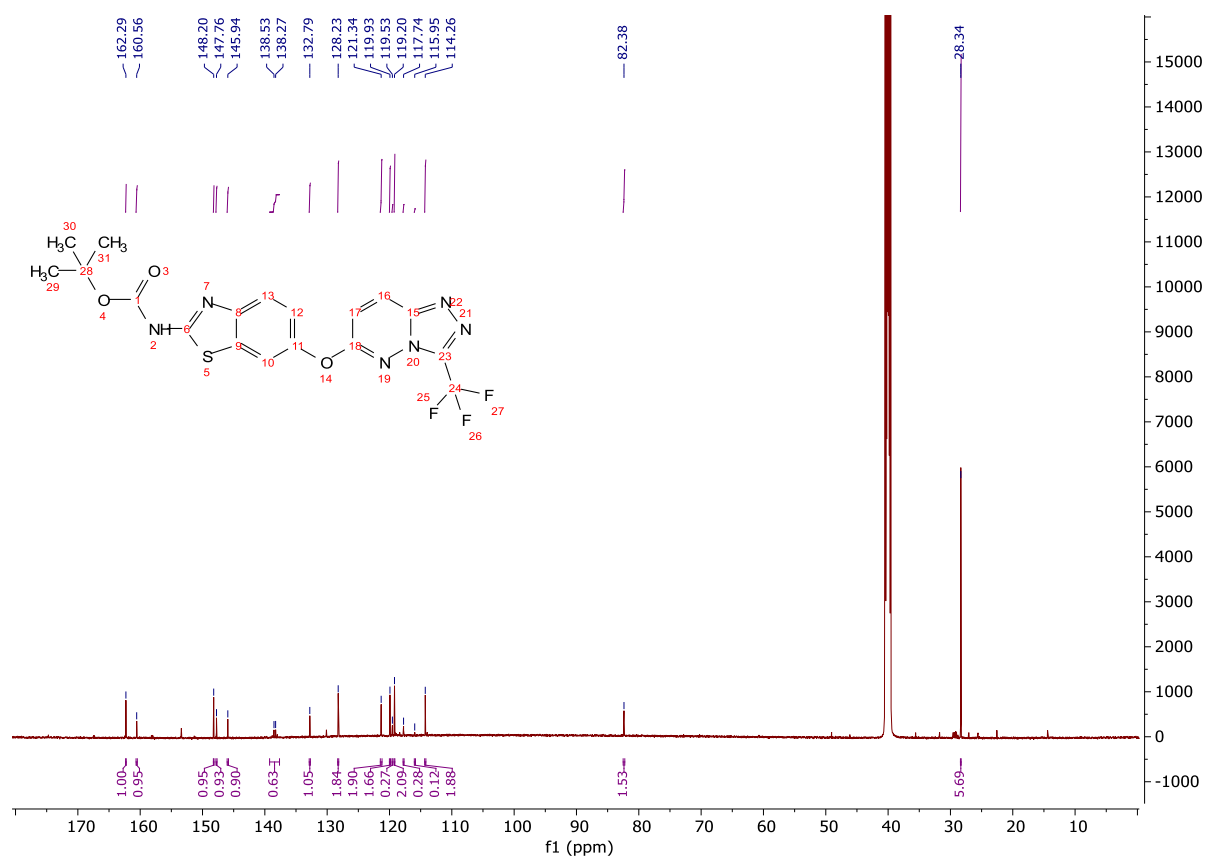


Figure 40. ^1H NMR spectrum for compound 8b.



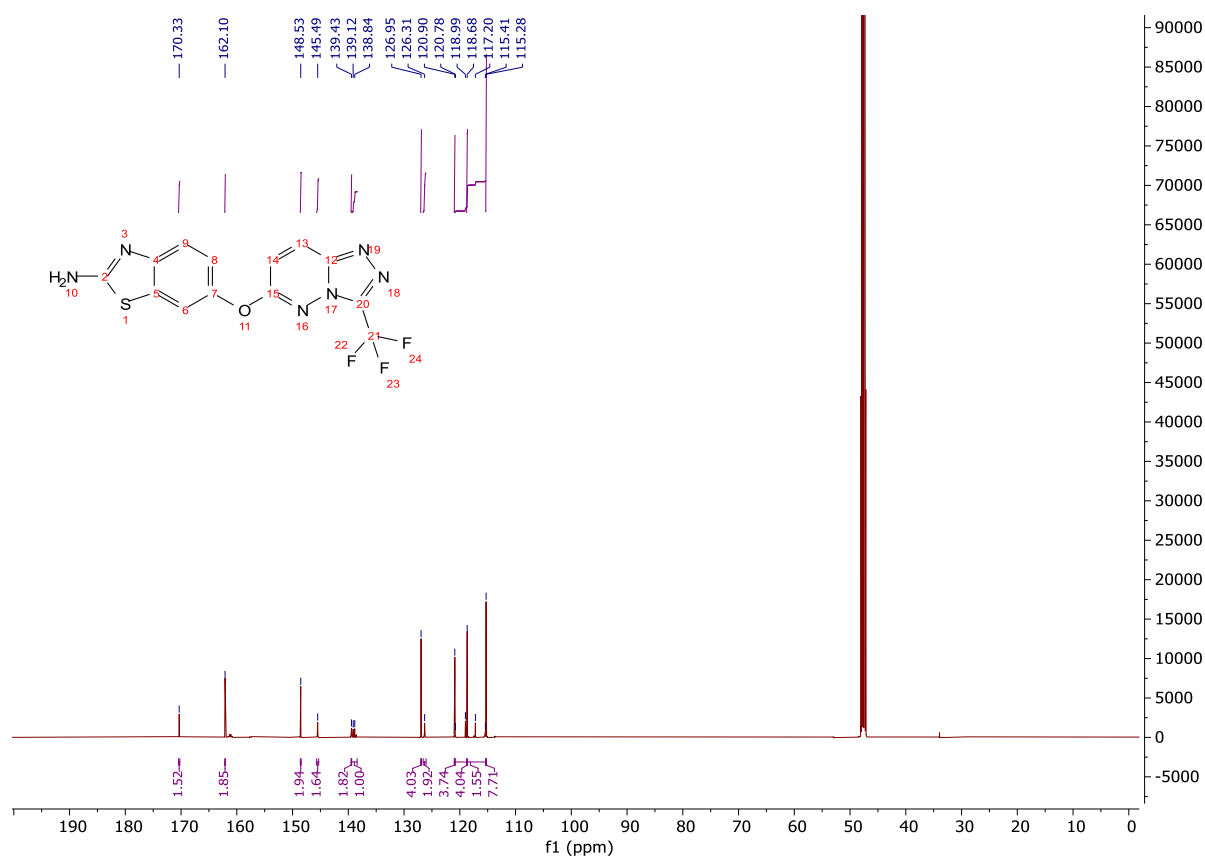


Figure 43. ¹³C NMR spectrum for compound **8c**.

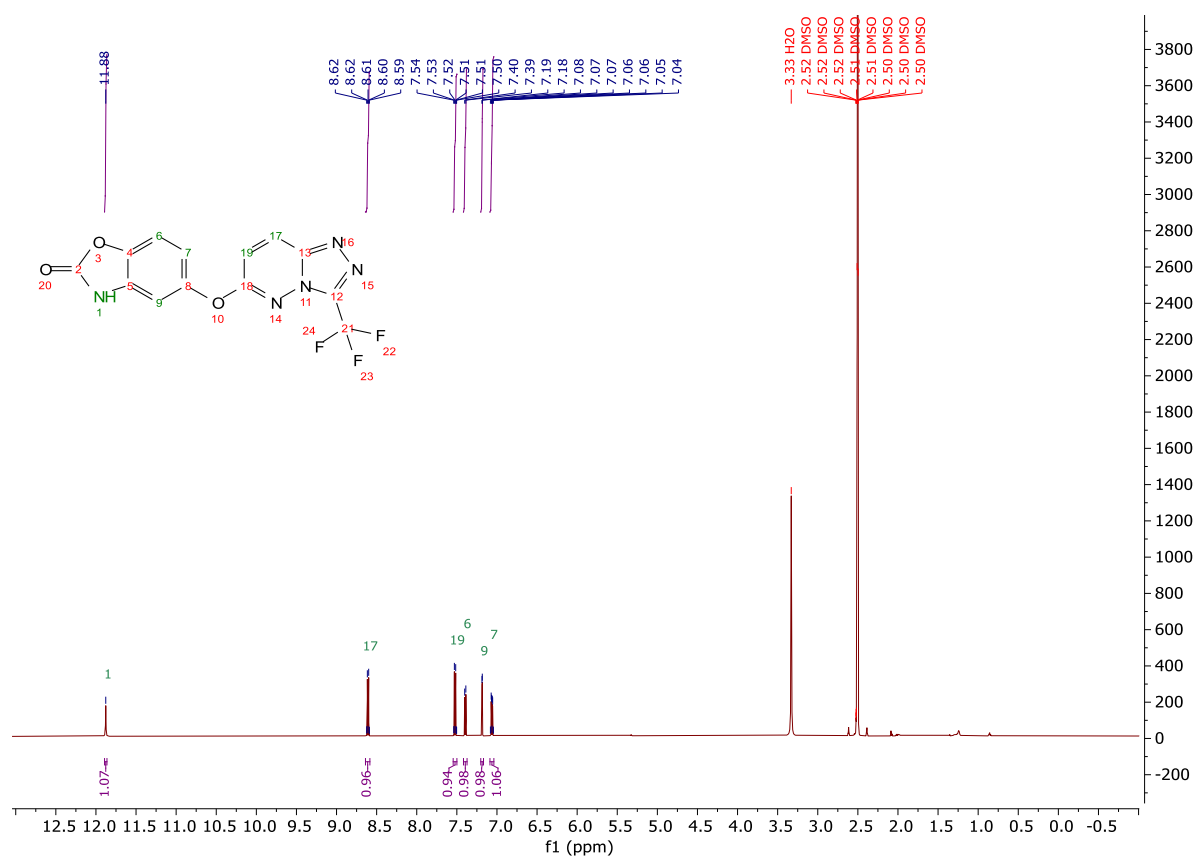


Figure 44. ¹H NMR spectrum for compound **8d**.

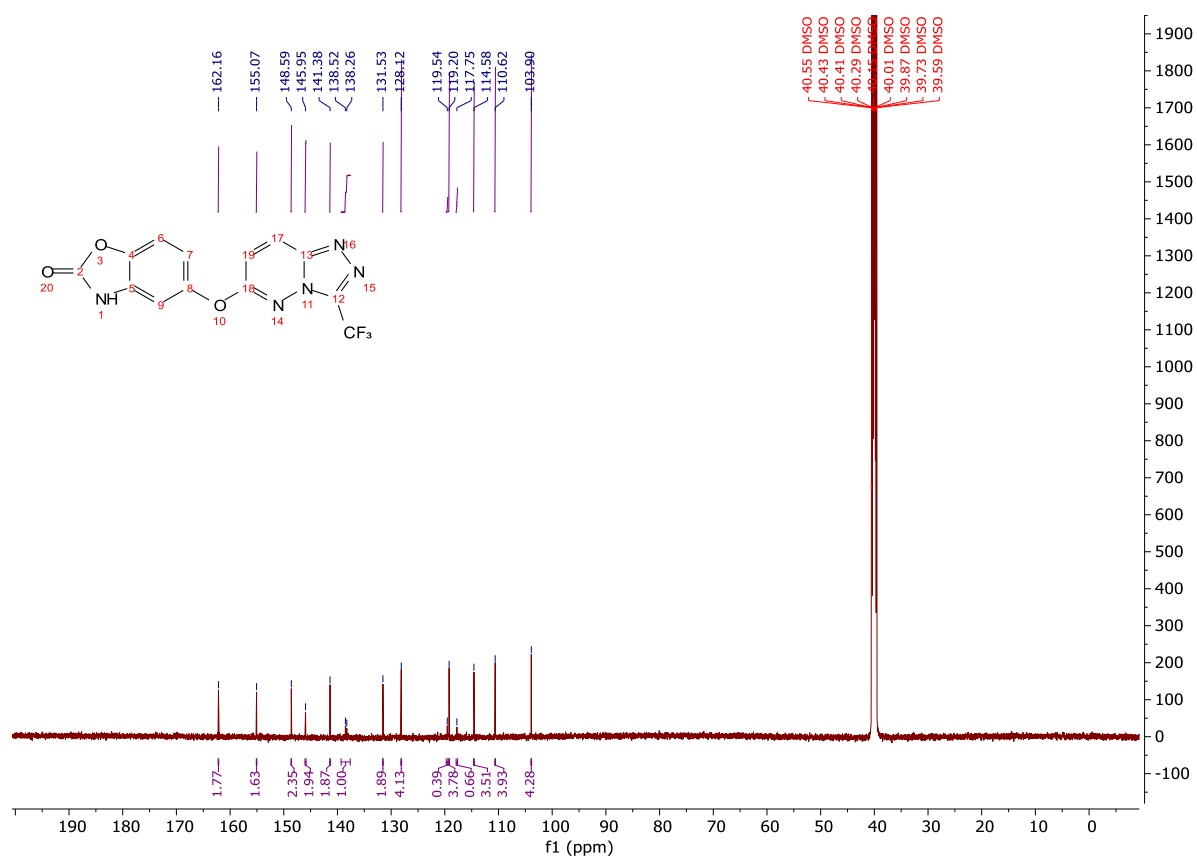


Figure 45. ^{13}C NMR spectrum for compound 8d.

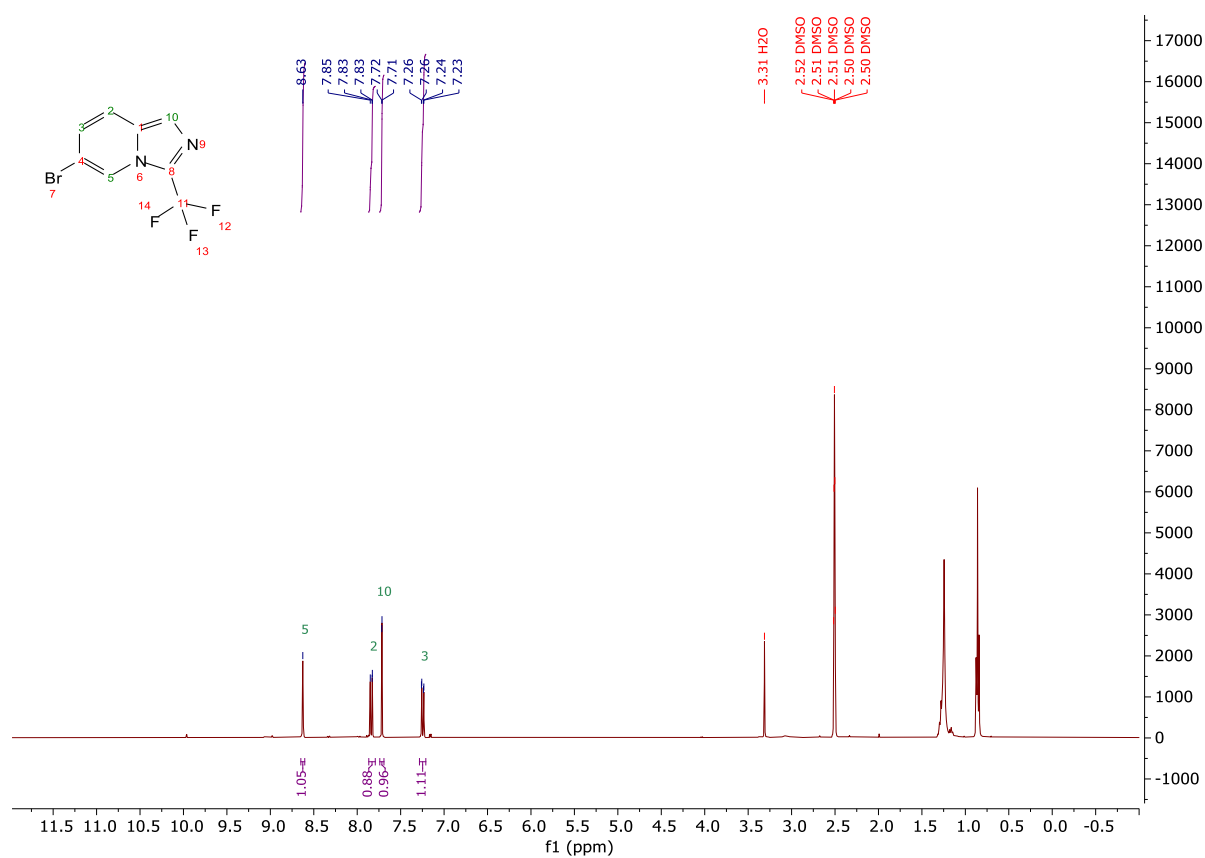


Figure 46. ^1H NMR spectrum for compound 9a.

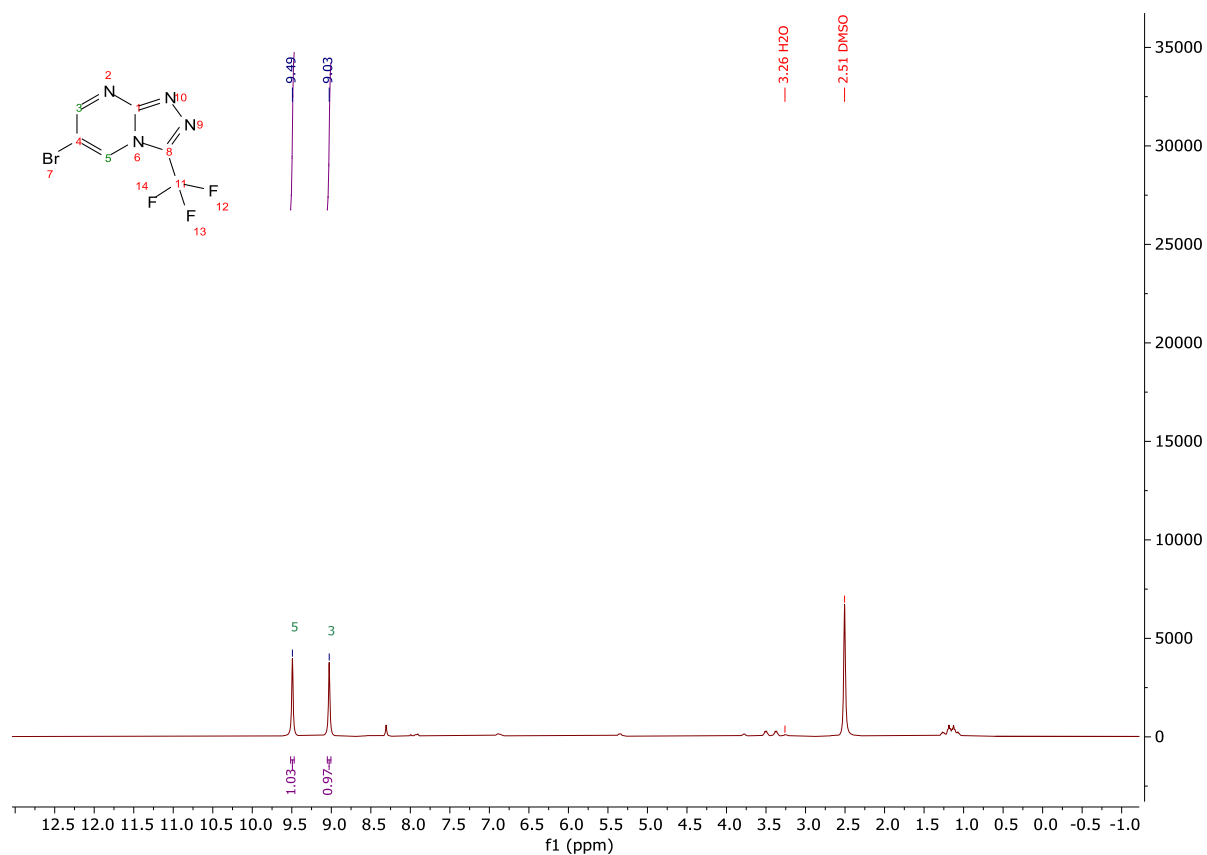


Figure 47. ¹H NMR spectrum for compound 9b.

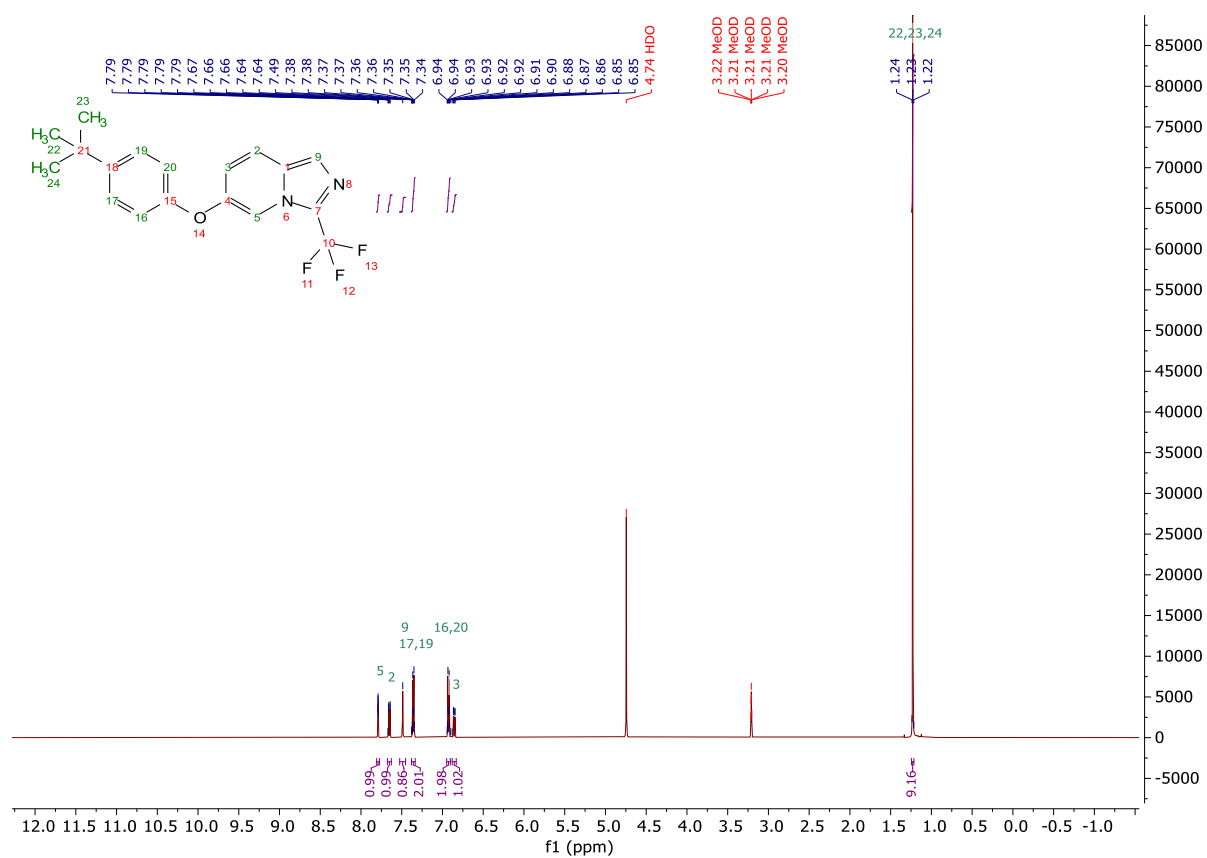


Figure 48. ¹H NMR spectrum for compound 10a.

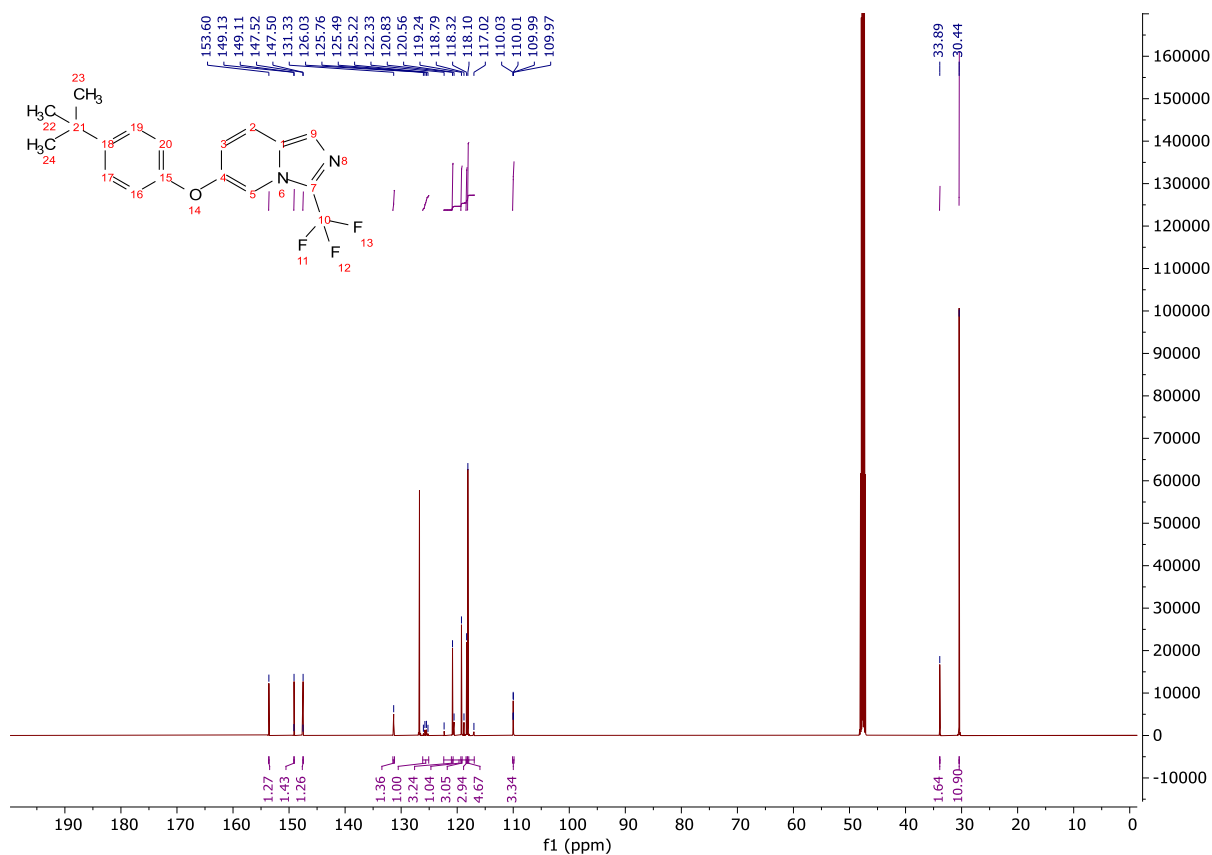


Figure 49. ^{13}C NMR spectrum for compound **10a**.

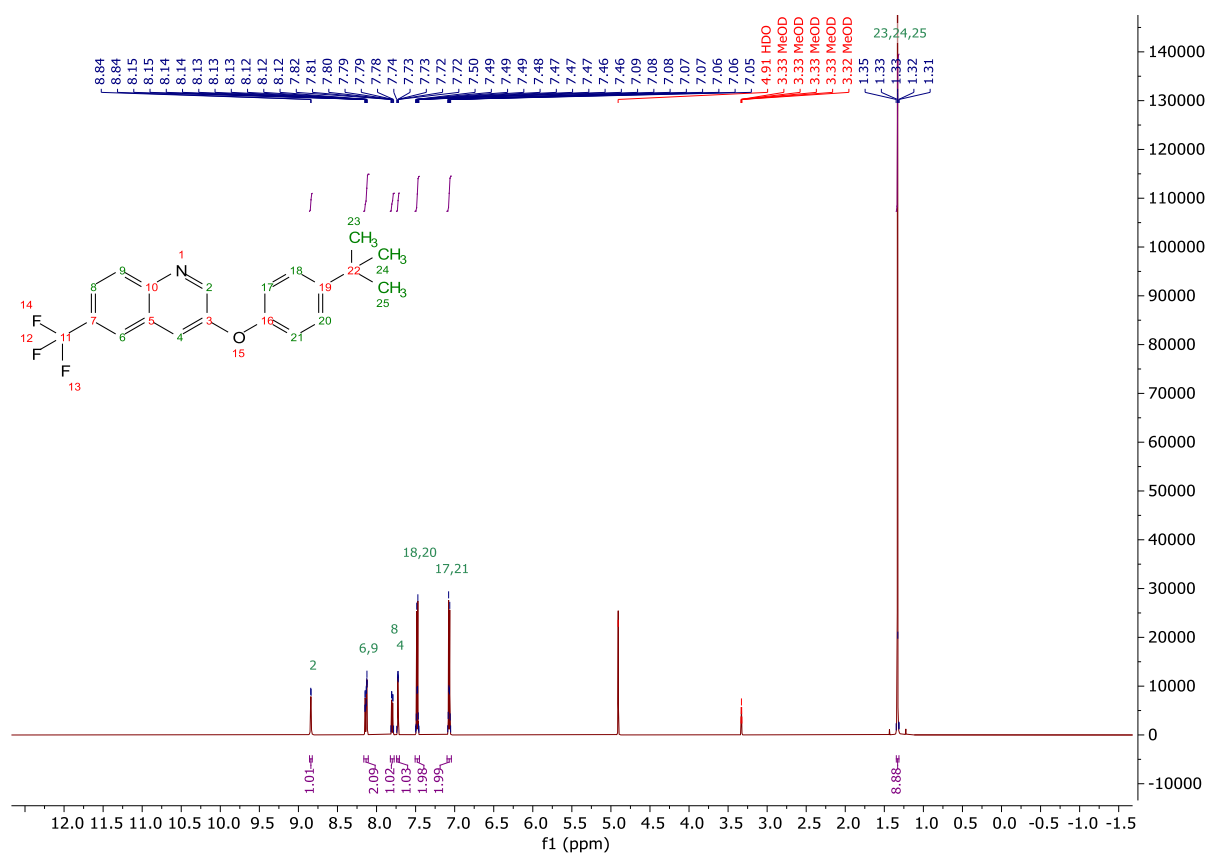


Figure 50. ^1H NMR spectrum for compound **10b**.

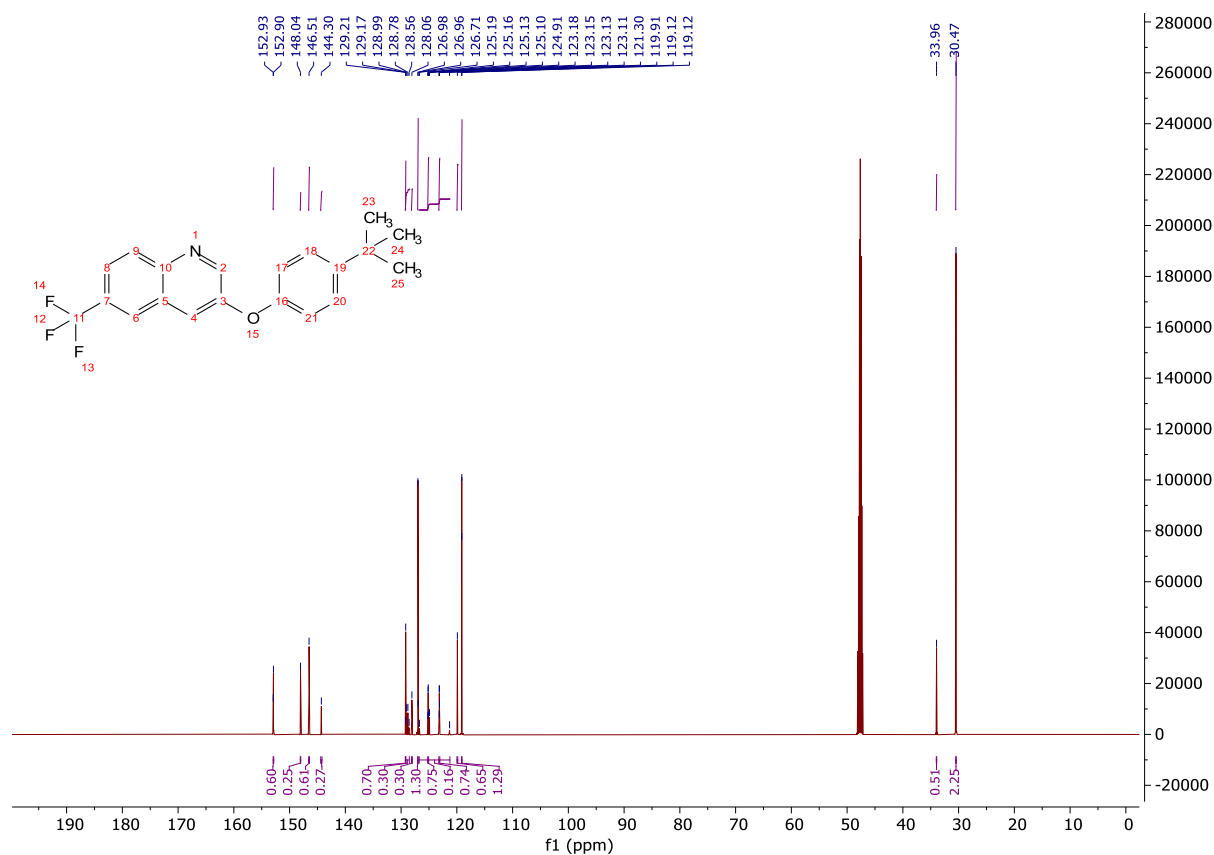


Figure 51. ^{13}C NMR spectrum for compound **10b**.

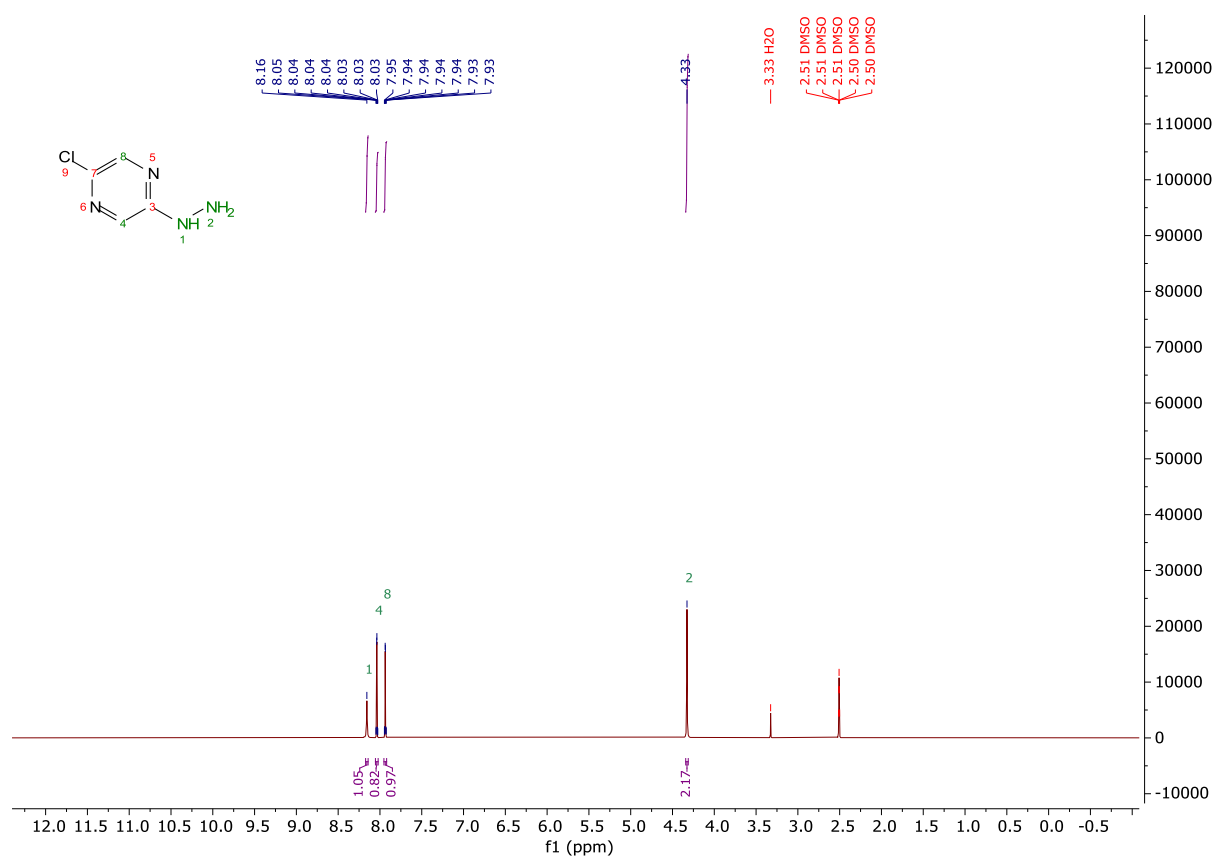


Figure 52. ^1H NMR spectrum for compound **11**.

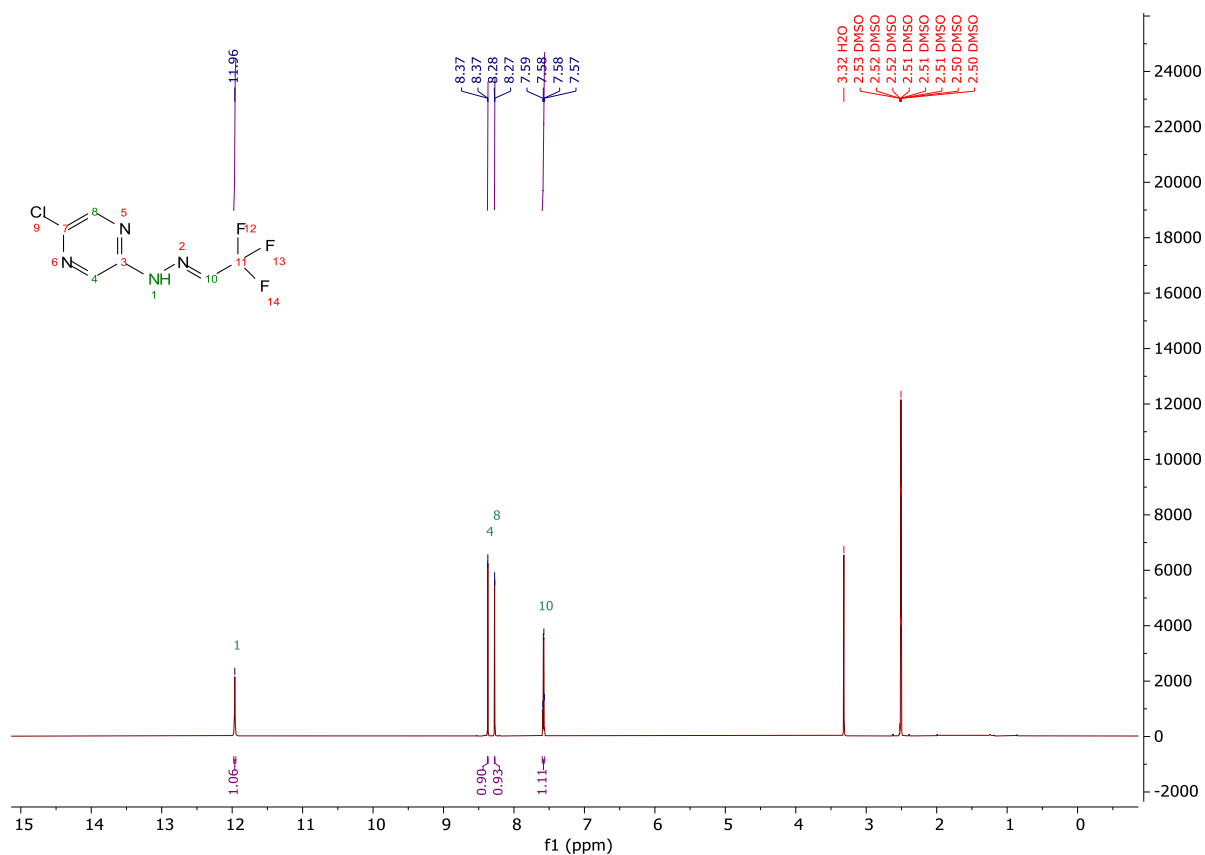


Figure 53. ¹H NMR spectrum for compound 12.

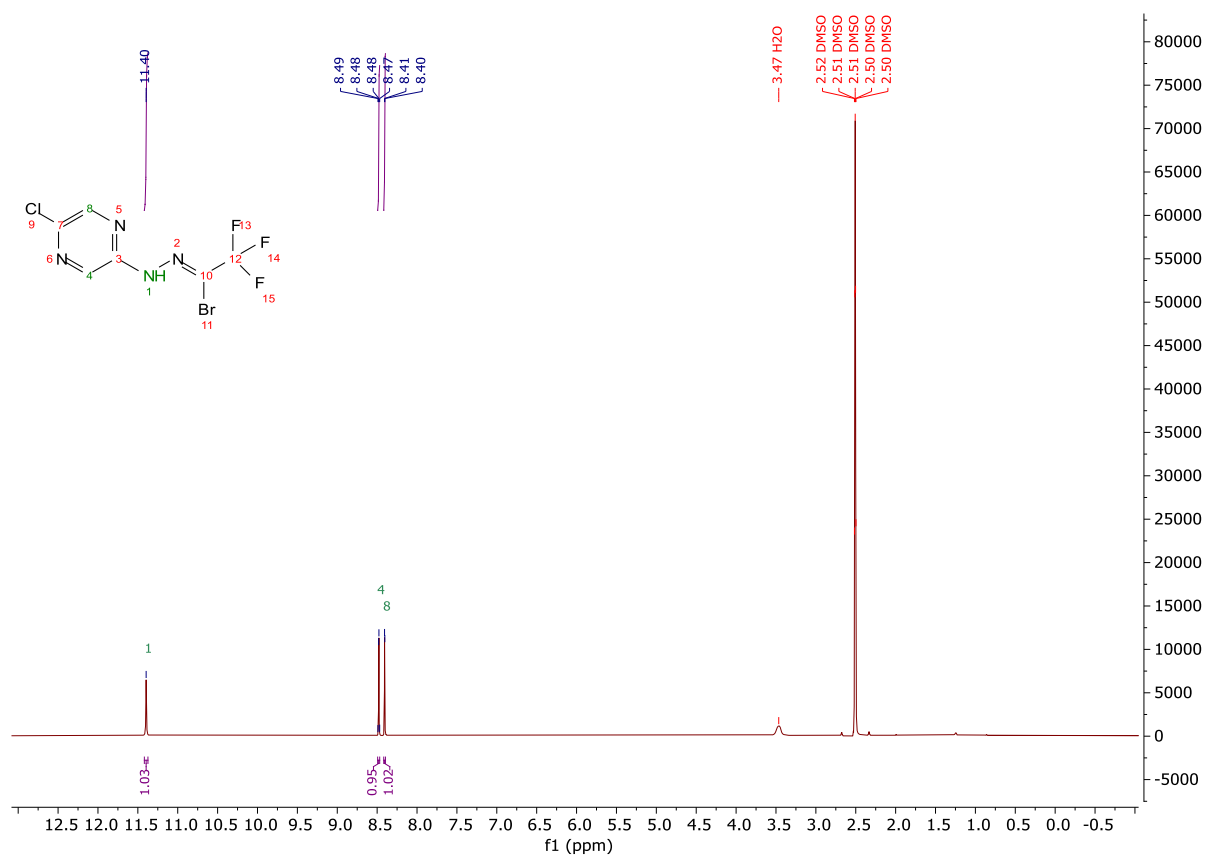


Figure 54. ¹H NMR spectrum for compound 13.

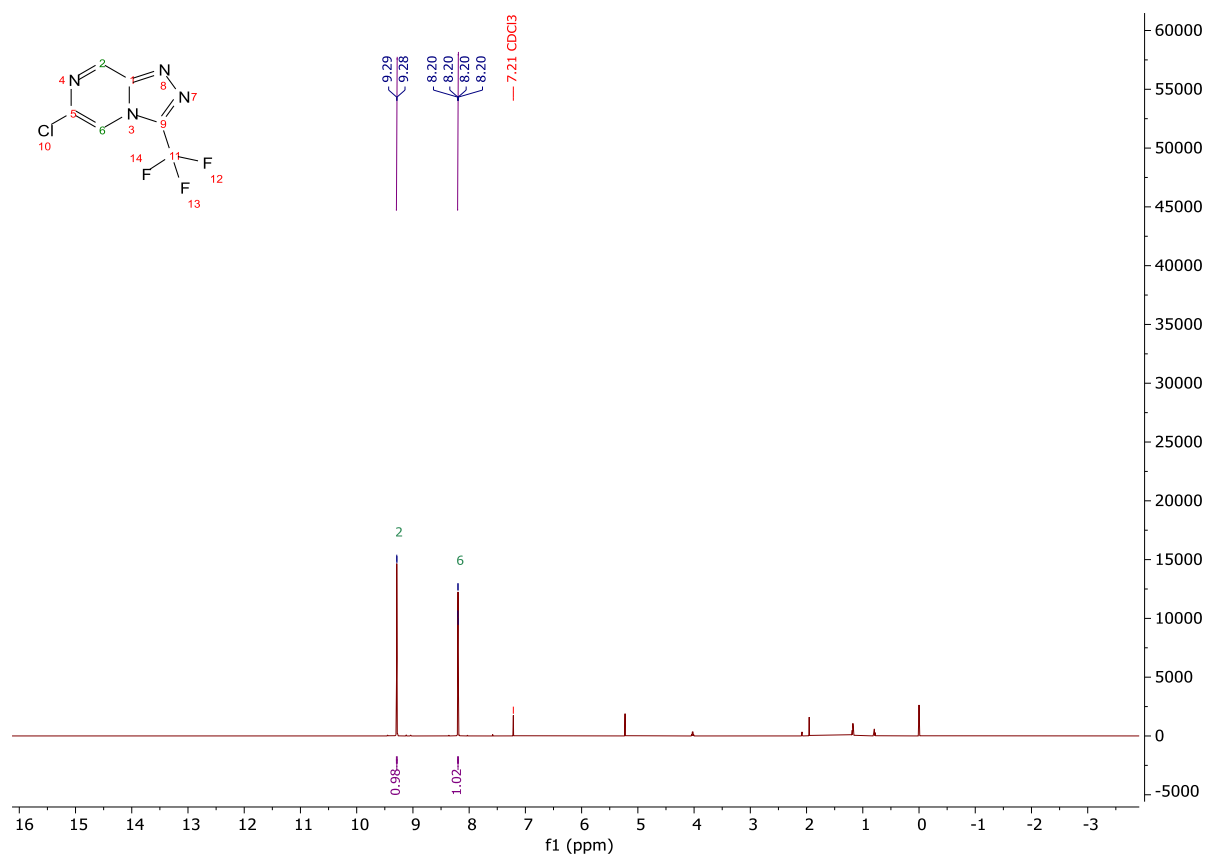


Figure 55. ¹H NMR spectrum for compound 14.

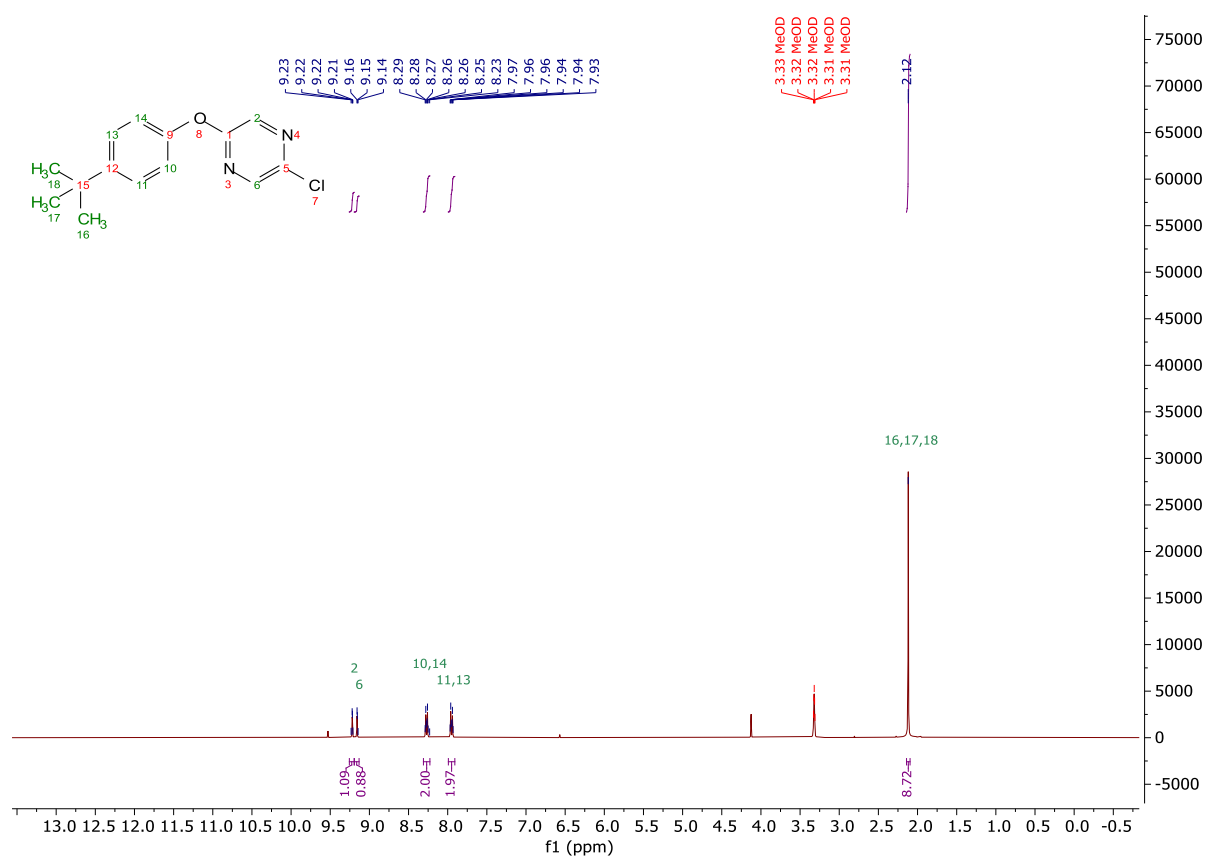


Figure 56. ¹H NMR spectrum for compound 18.

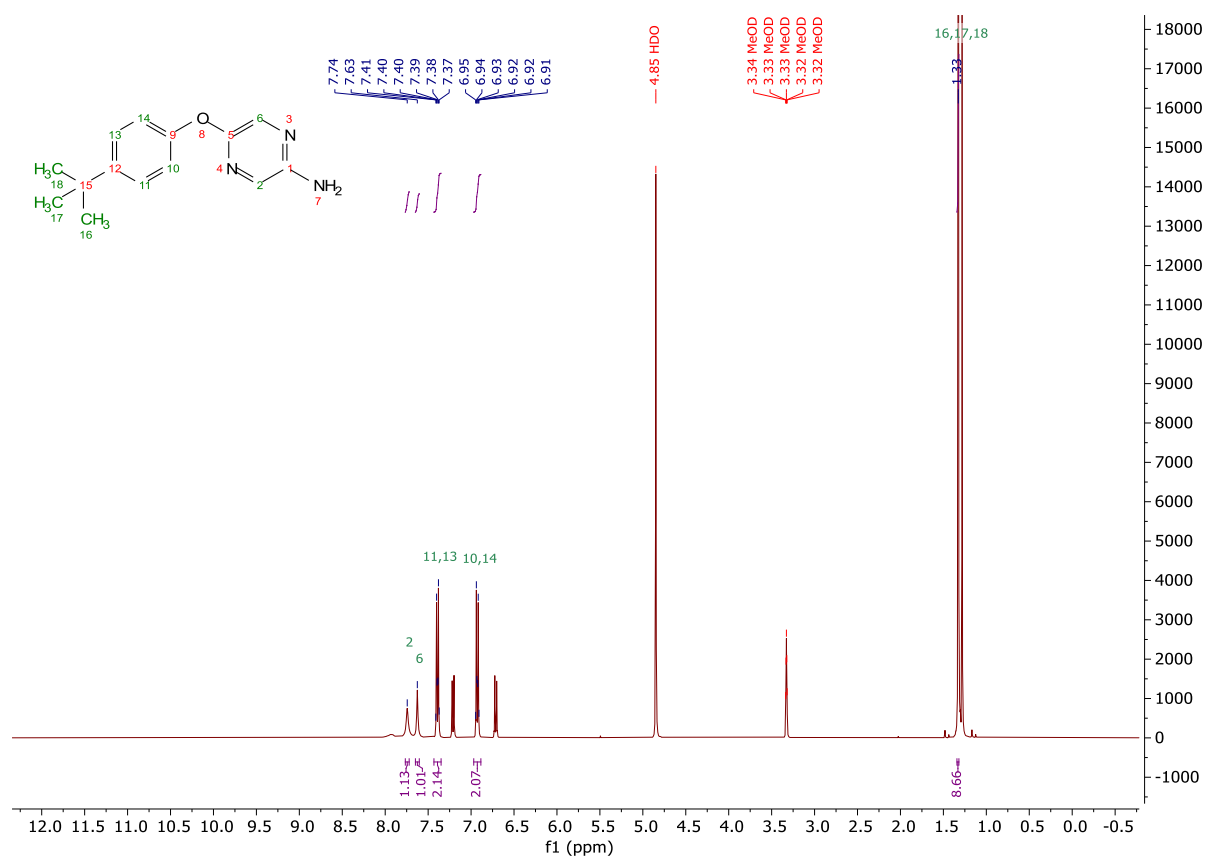


Figure 57. ¹H NMR spectrum for compound **20a**.

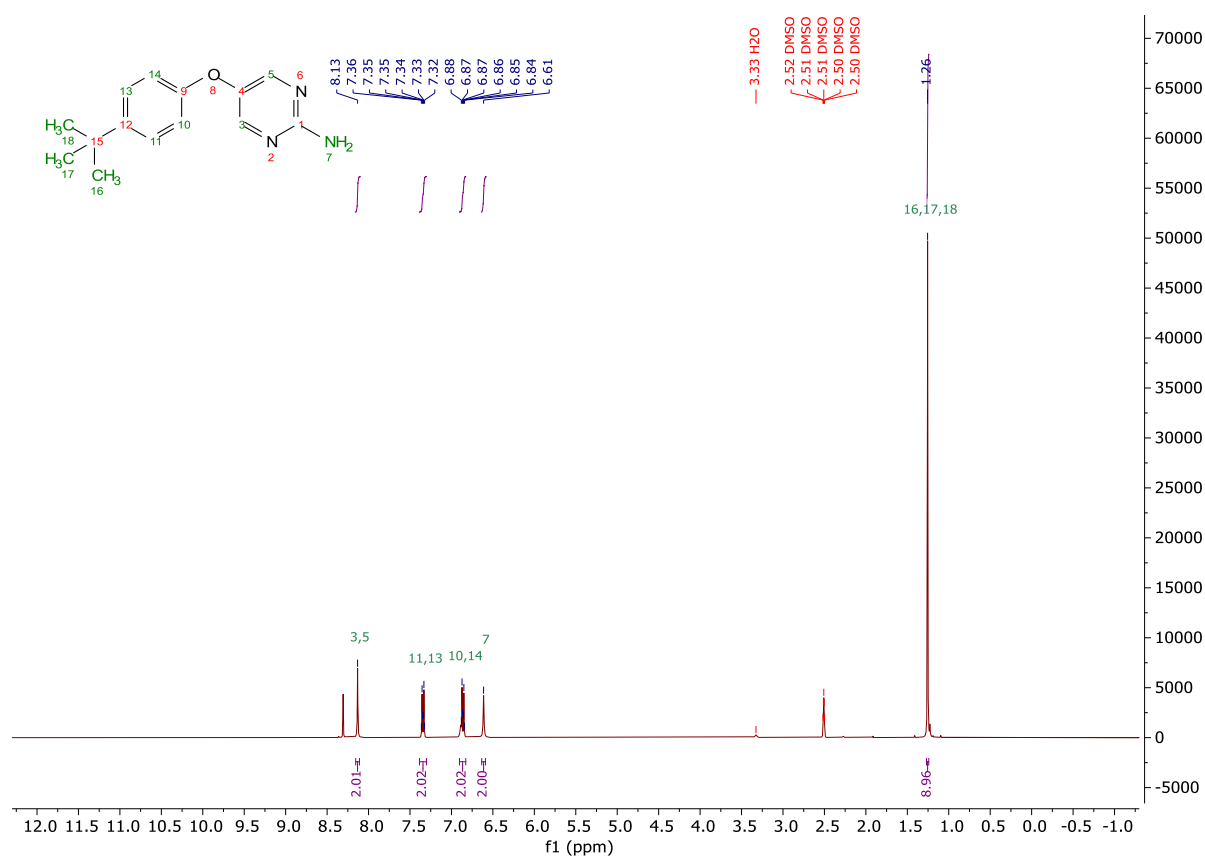


Figure 58. ¹H NMR spectrum for compound **20b**.

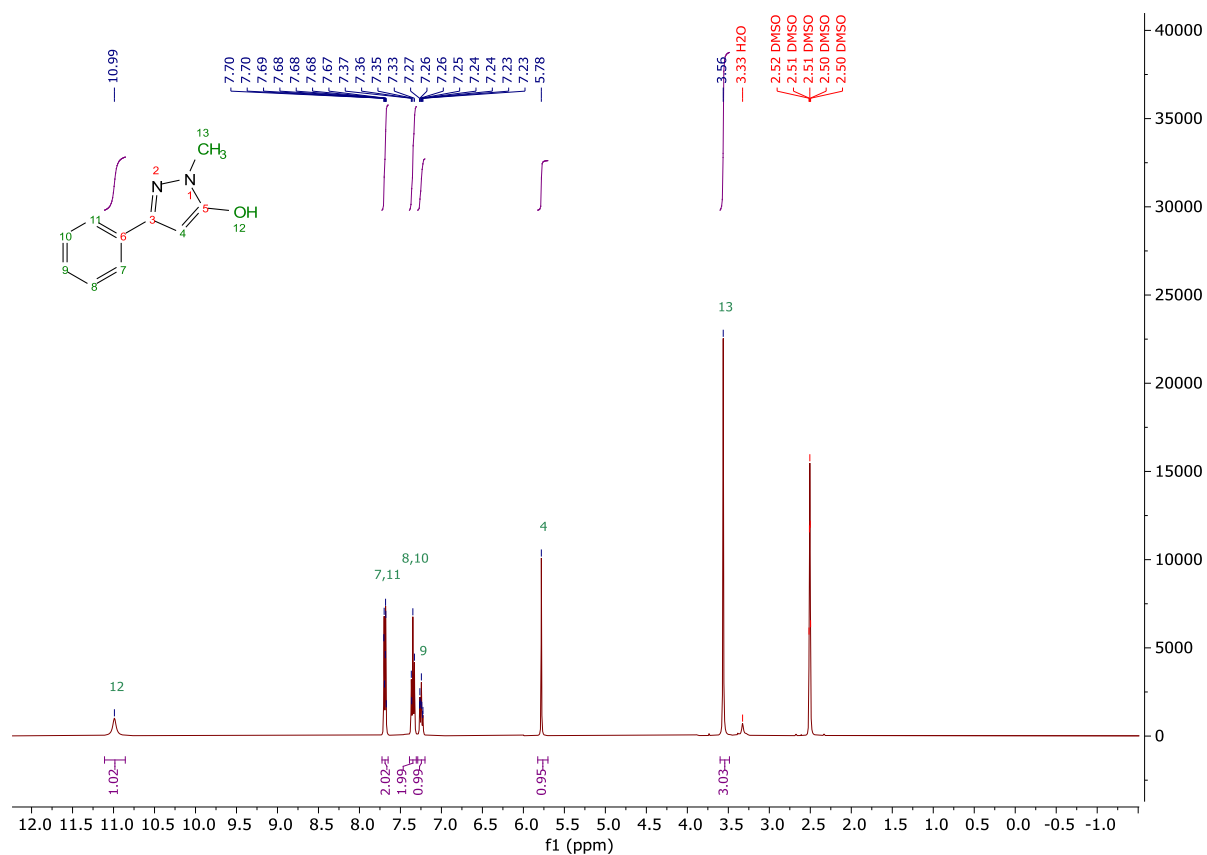


Figure 59. ^1H NMR spectrum for compound **21a**.

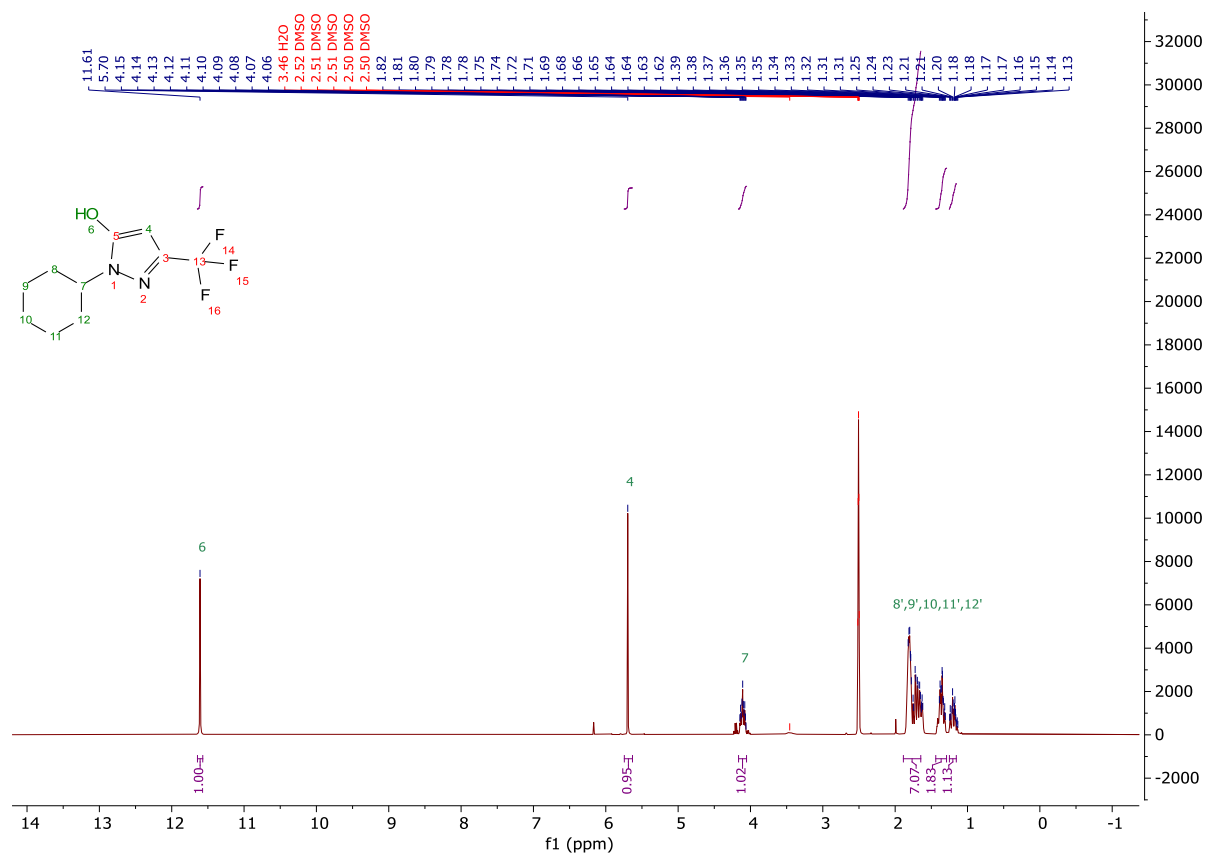


Figure 60. ^1H NMR spectrum for compound **21b**.

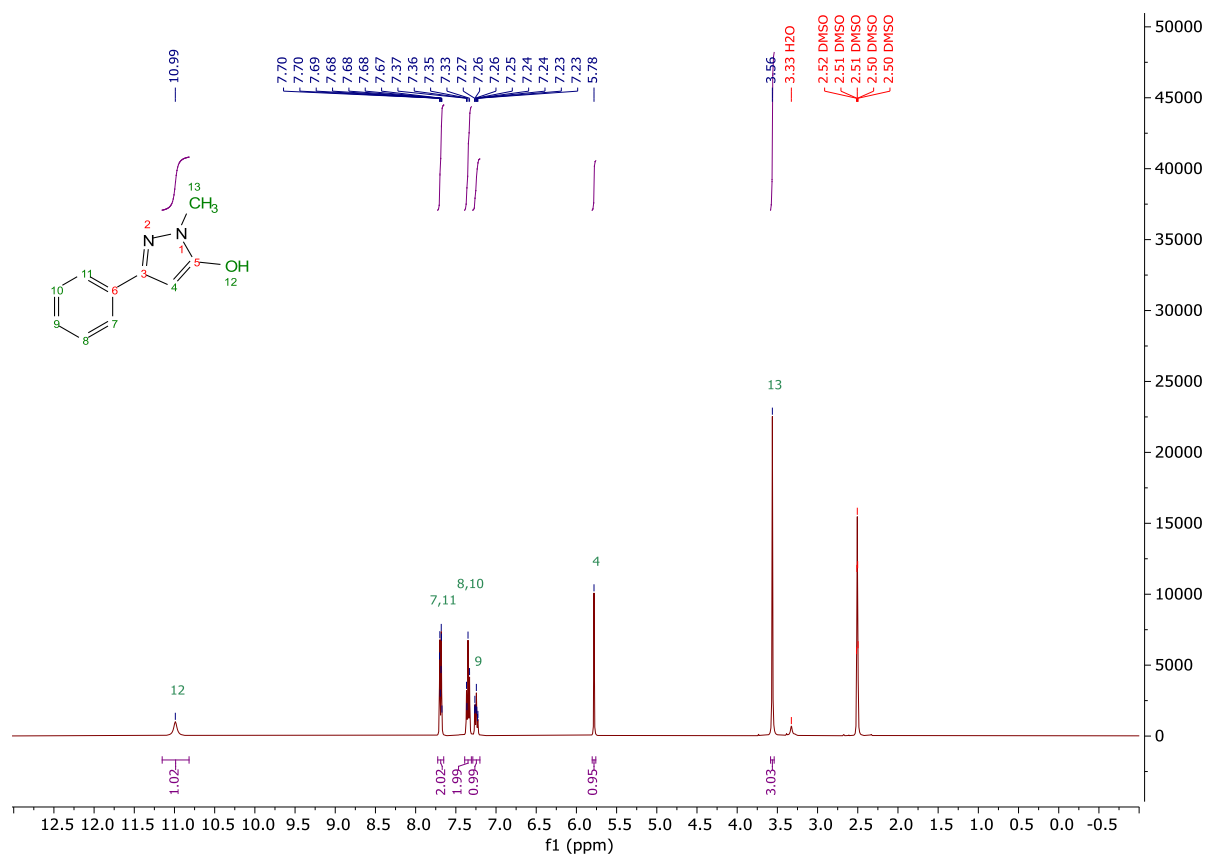


Figure 61. ¹H NMR spectrum for compound 21c.

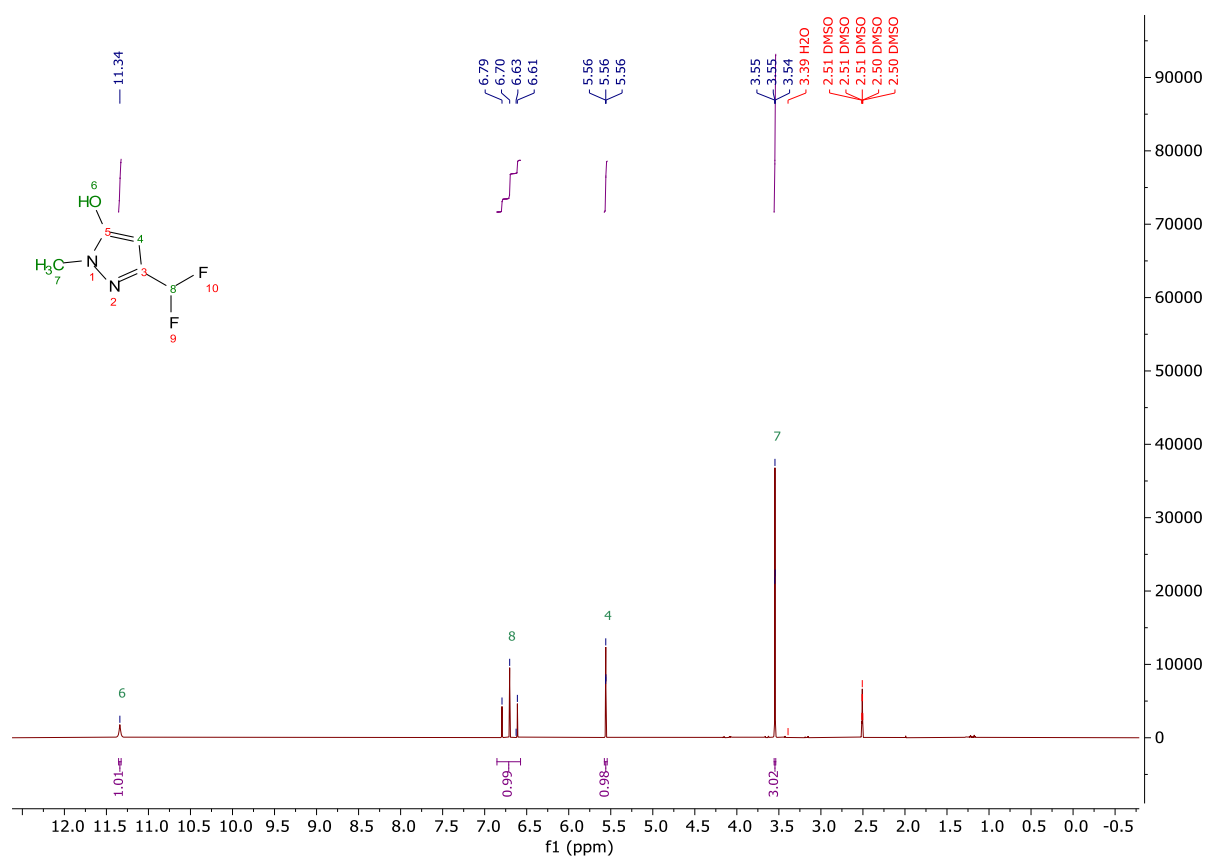


Figure 62. ¹H NMR spectrum for compound 21d.

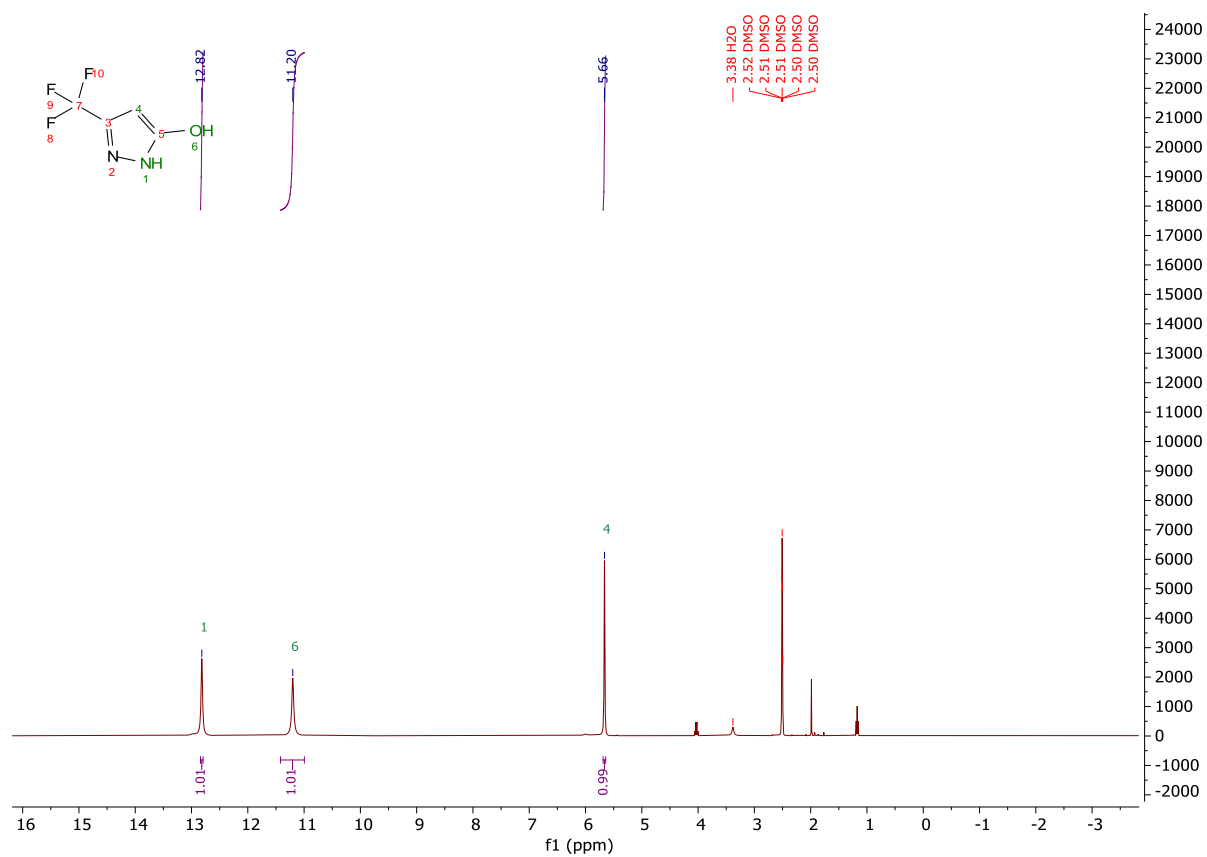


Figure 63. ¹H NMR spectrum for compound **21e**.

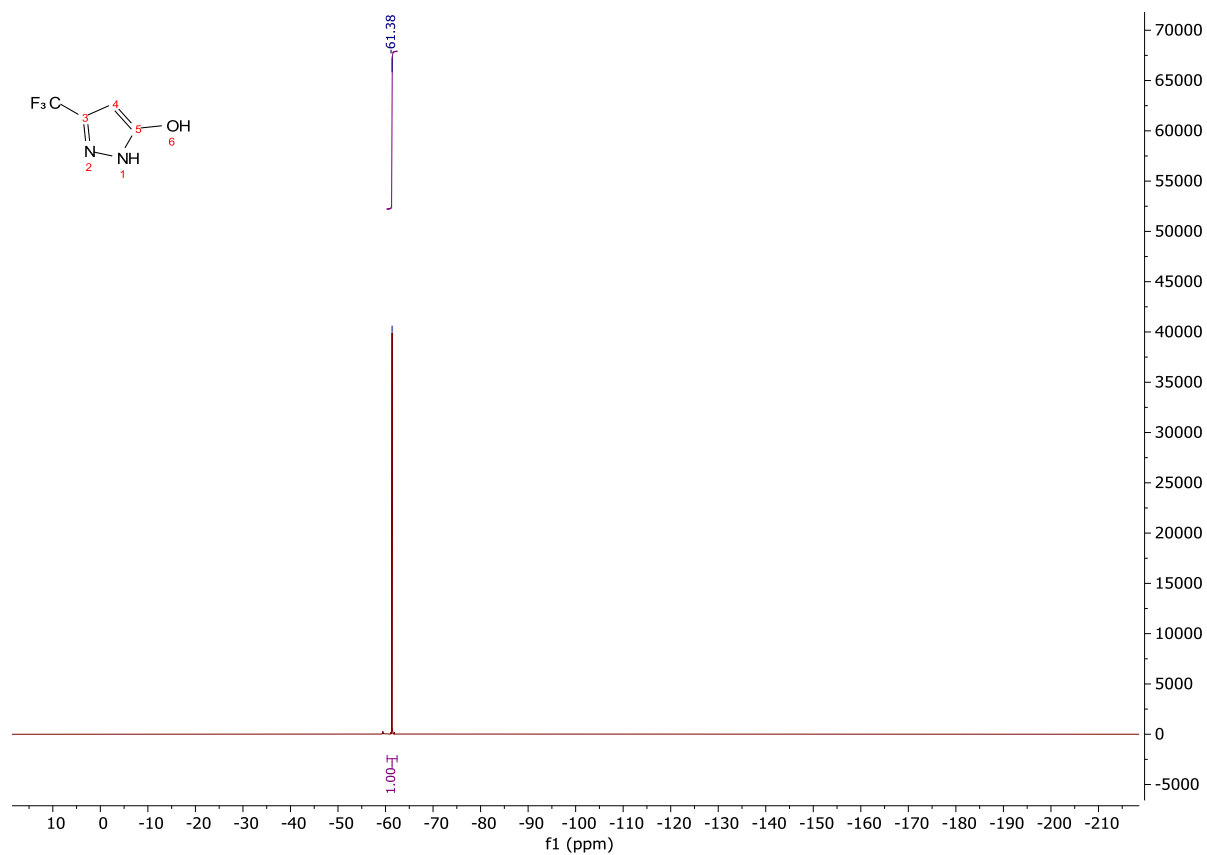


Figure 64. ¹⁹F NMR spectrum for compound **21e**.

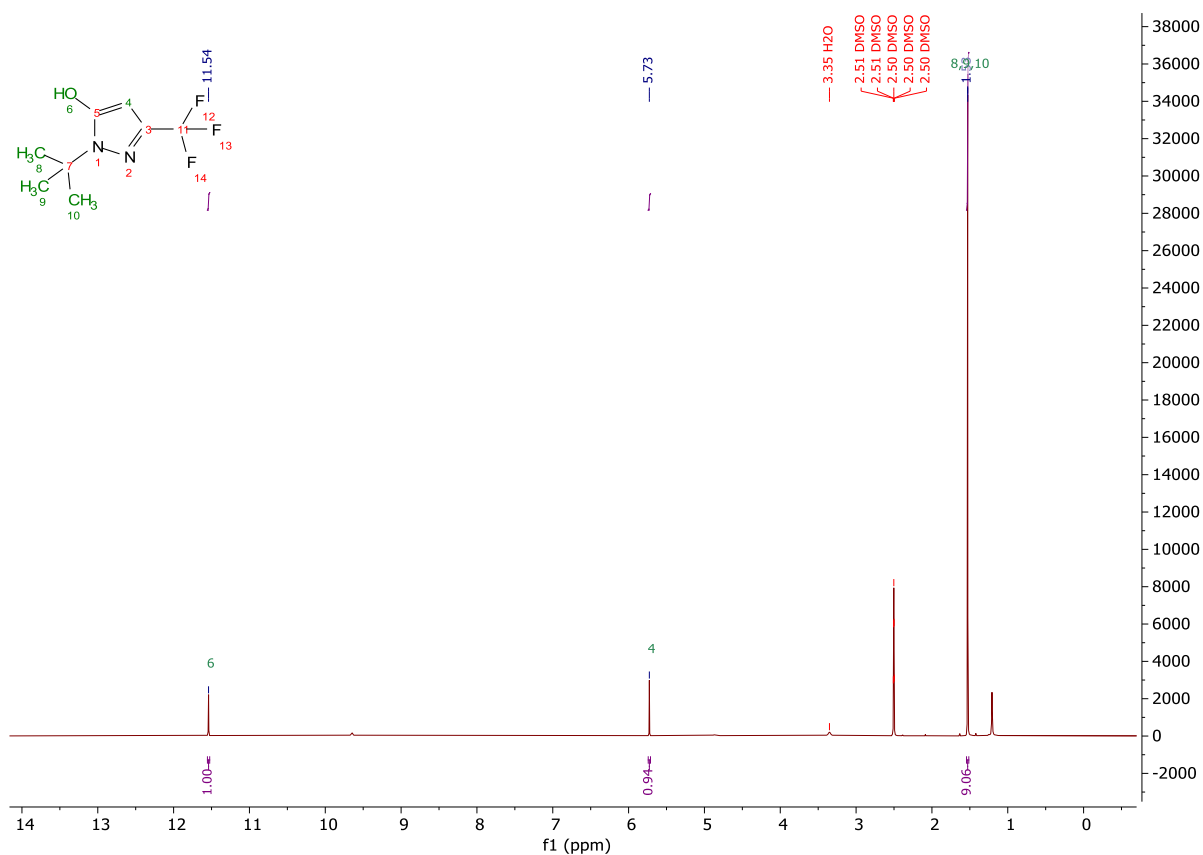


Figure 65. ¹H NMR spectrum for compound 21g.

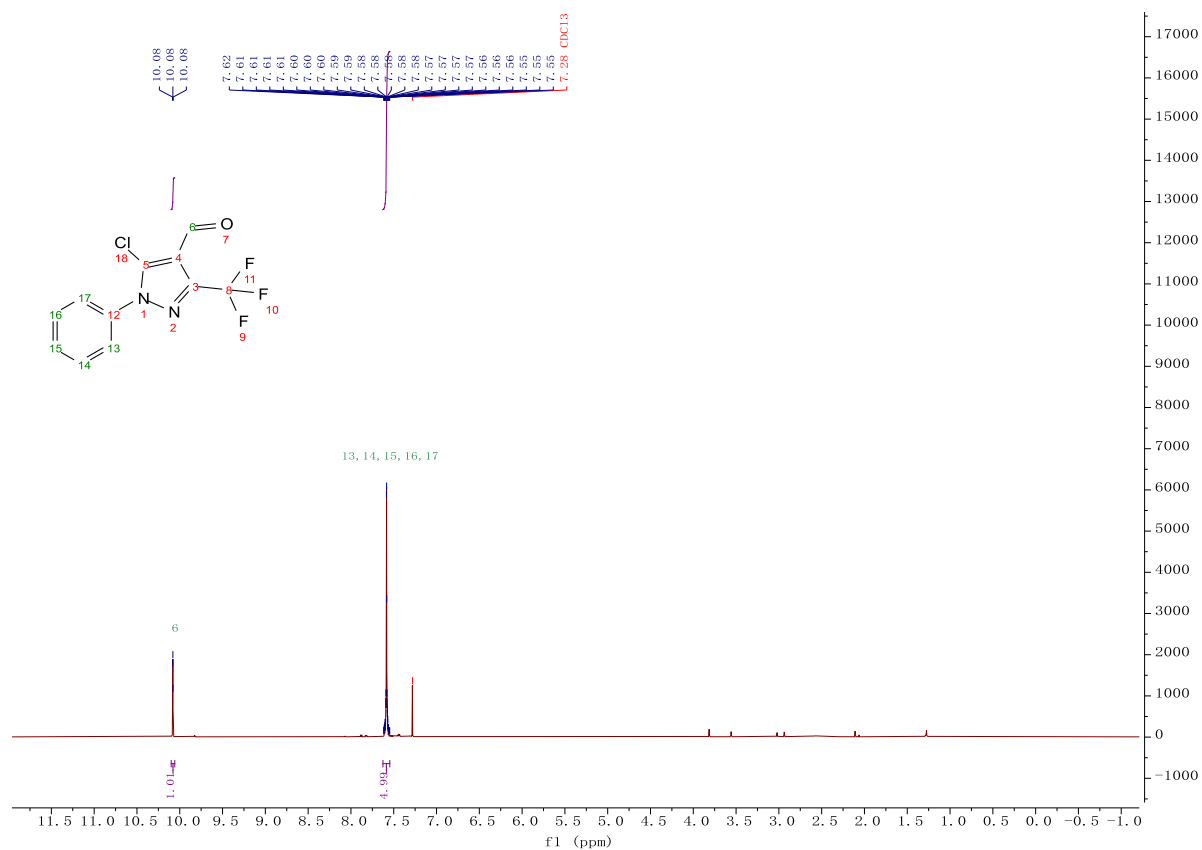


Figure 66. ¹H NMR spectrum for compound 22a.

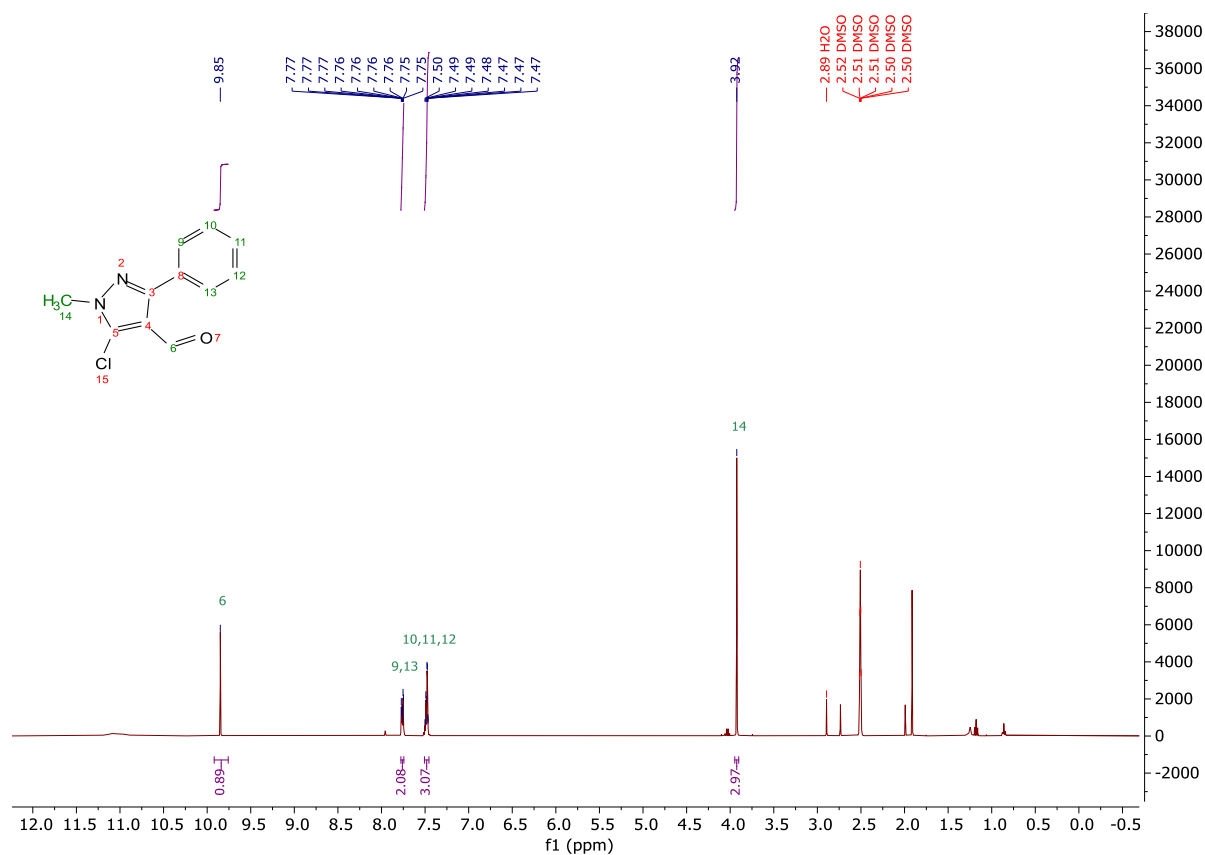


Figure 67. ¹H NMR spectrum for compound 22c.

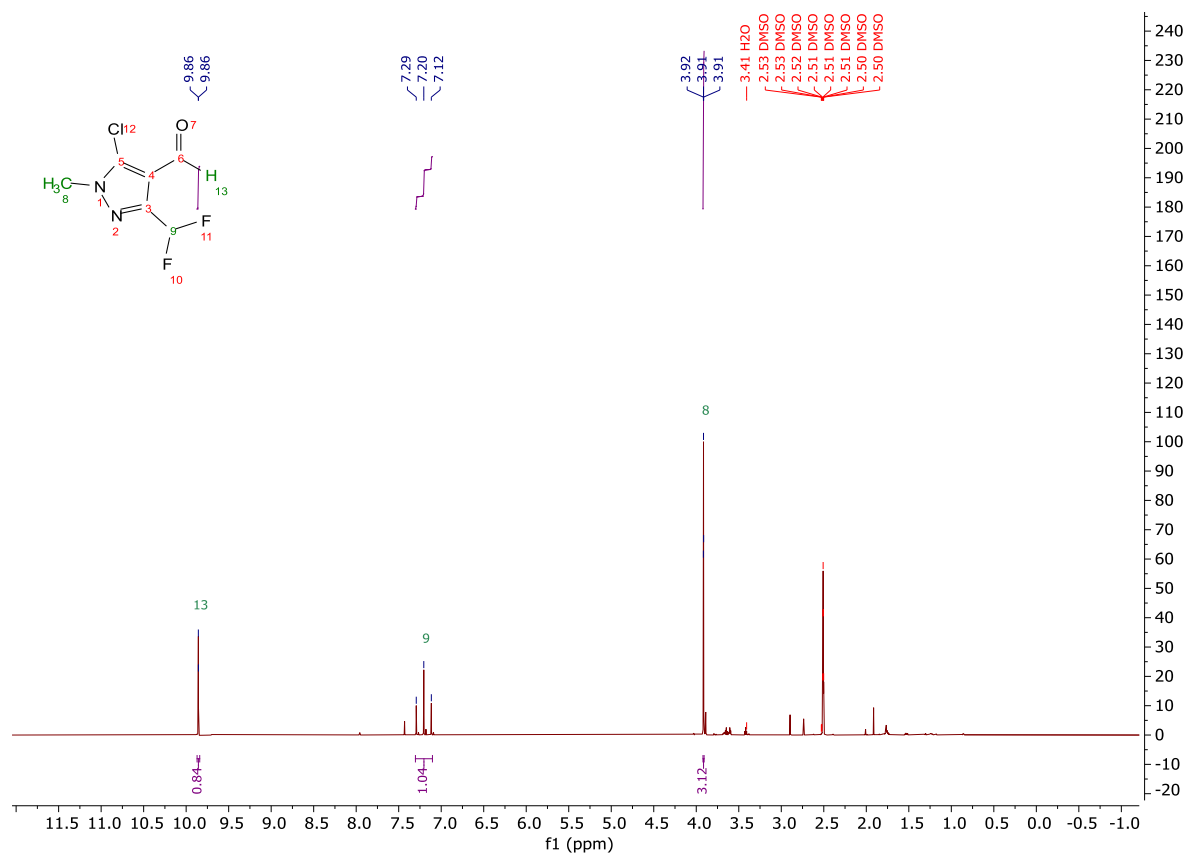


Figure 68. ¹H NMR spectrum for compound 22d.

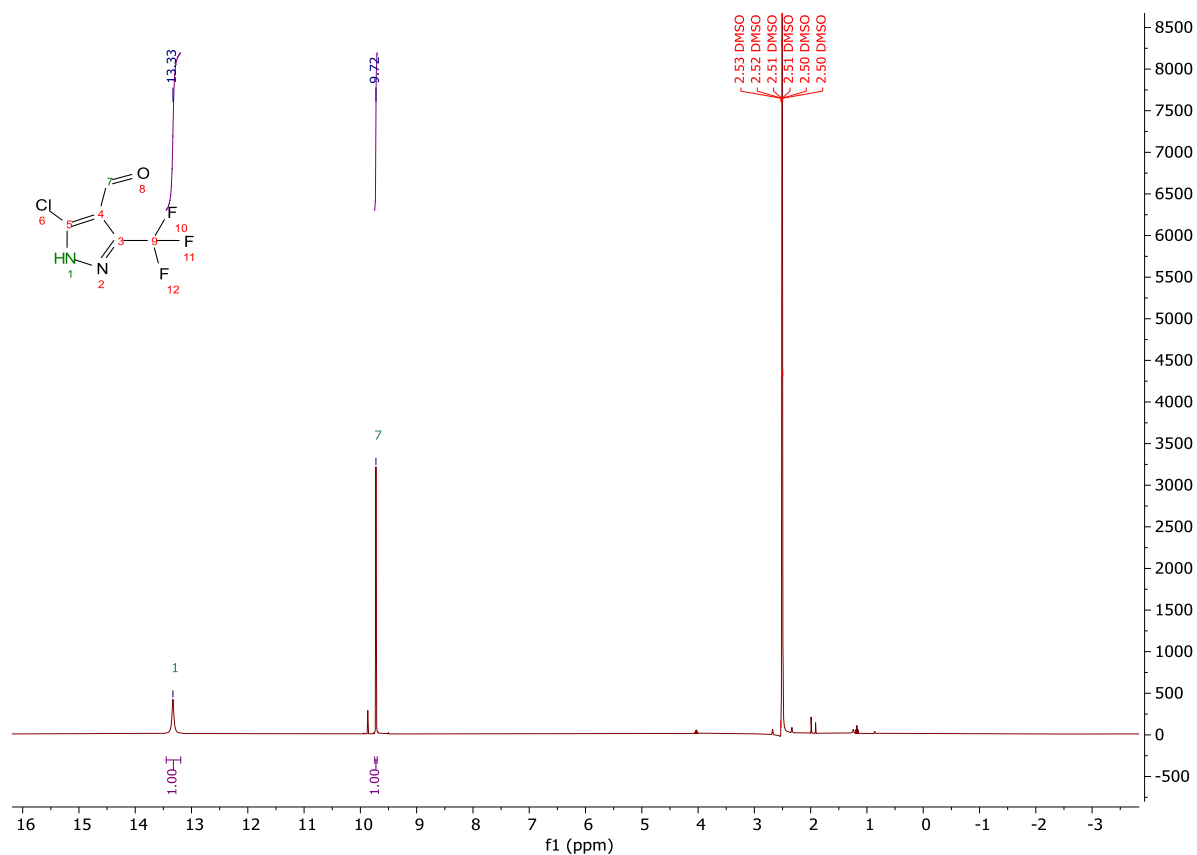


Figure 69. ^1H NMR spectrum for compound **22e**.

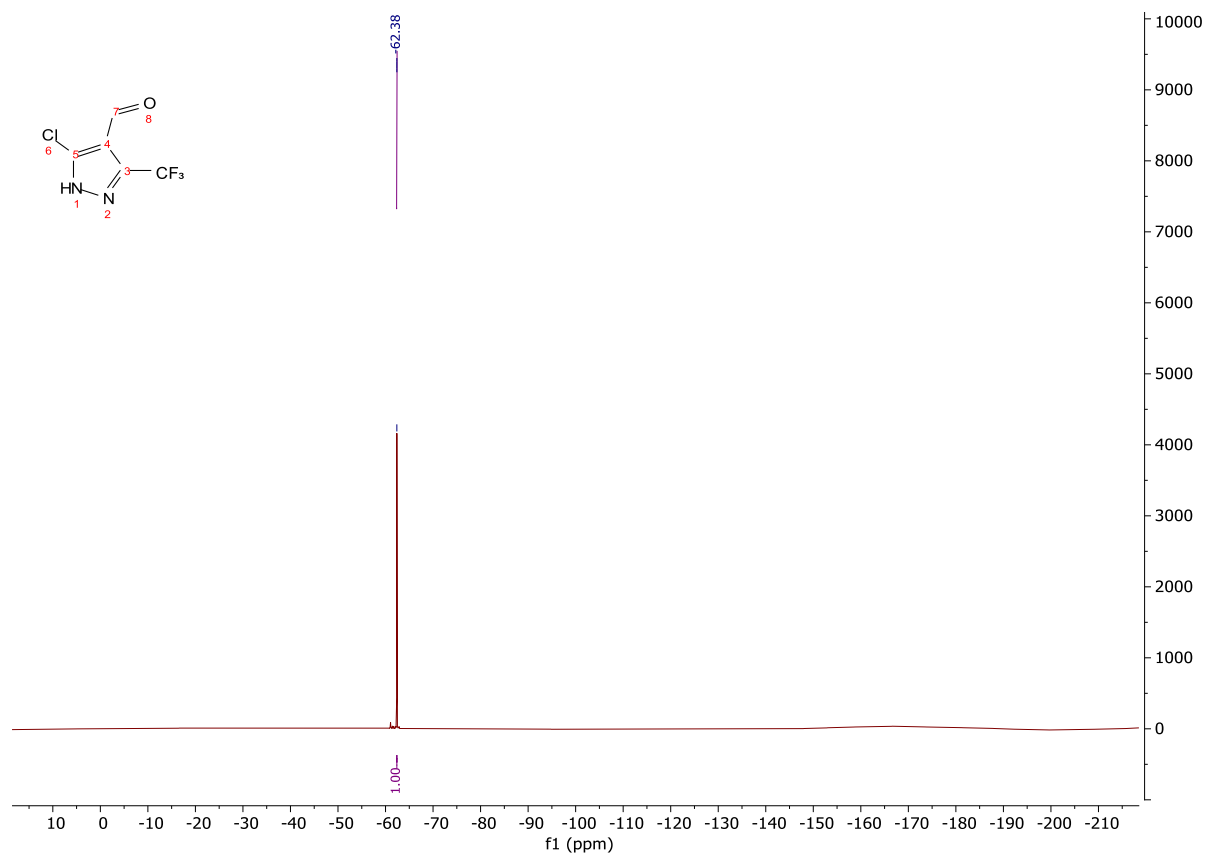
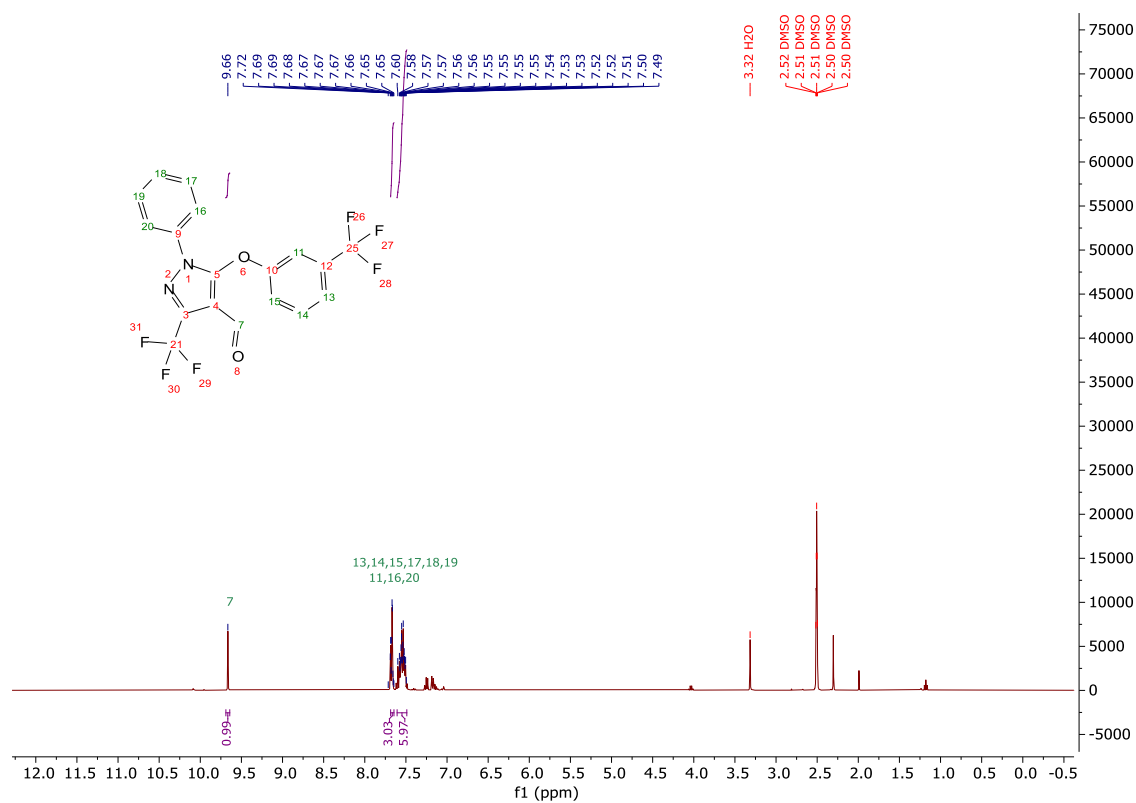


Figure 70. ^{19}F NMR spectrum for compound **22e**.



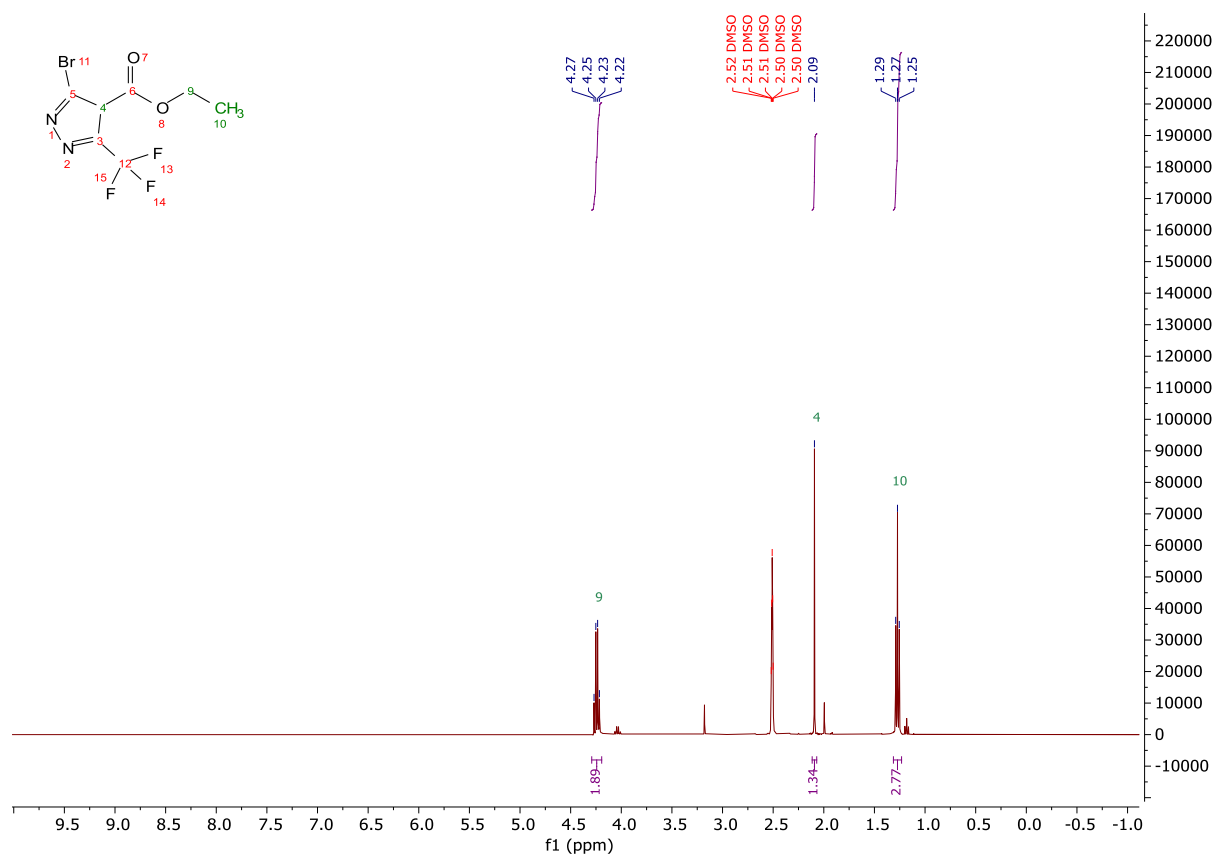


Figure 73. ¹H NMR spectrum for compound 25.

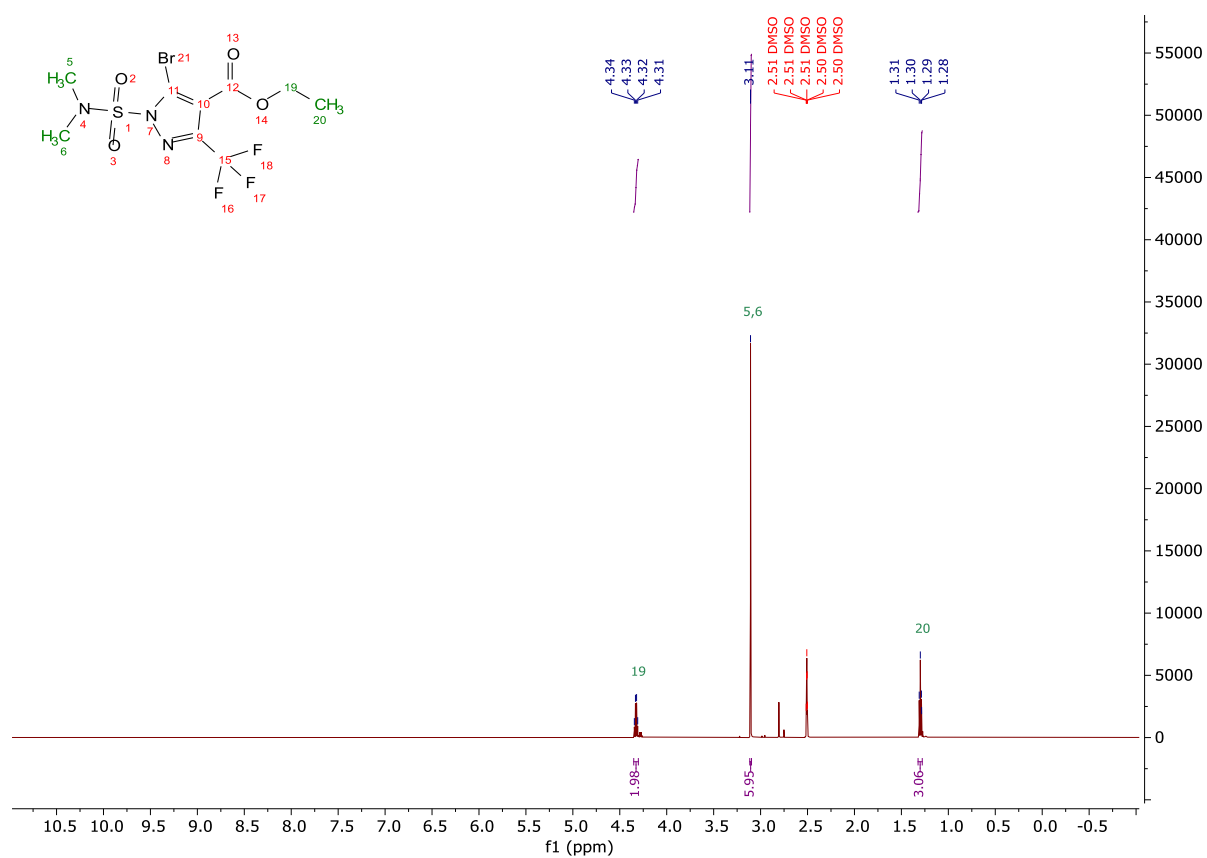


Figure 74. ¹H NMR spectrum for compound 26.

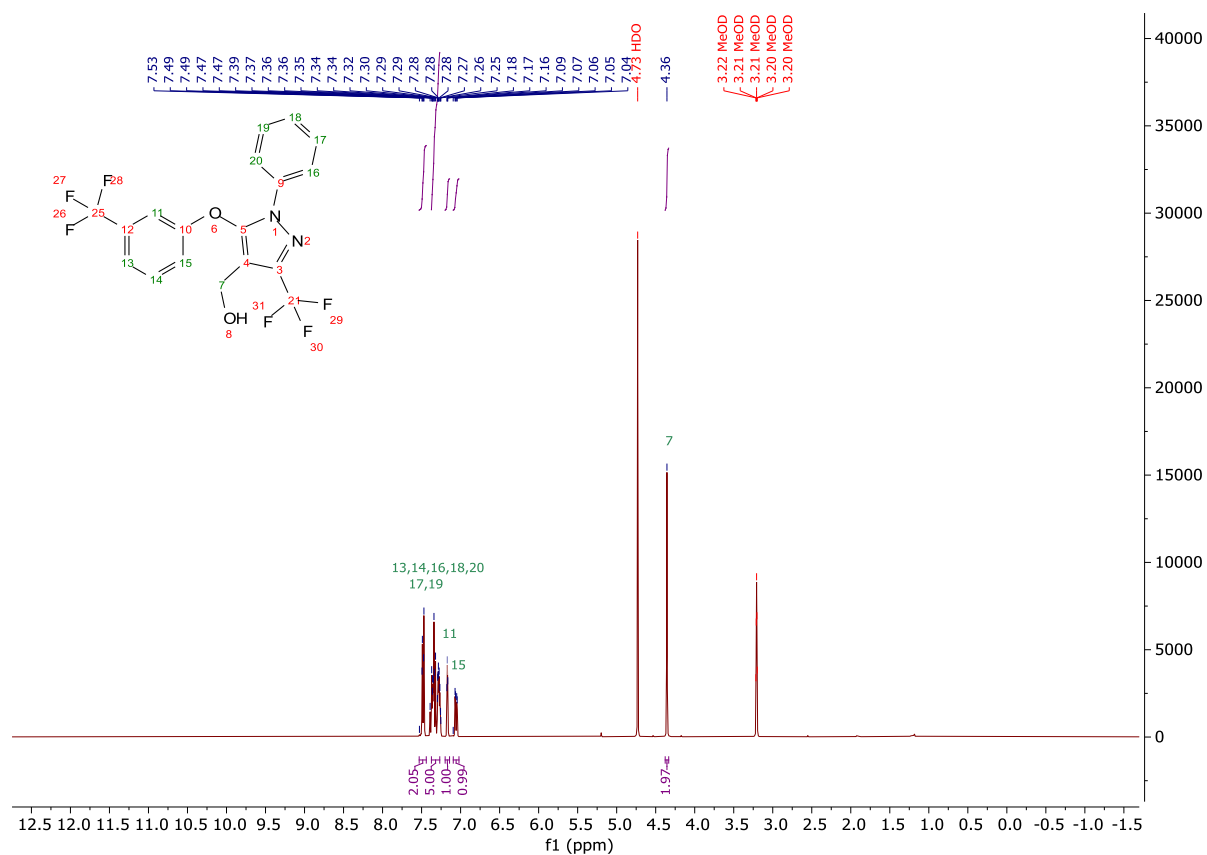


Figure 75. ¹H NMR spectrum for compound 27a.

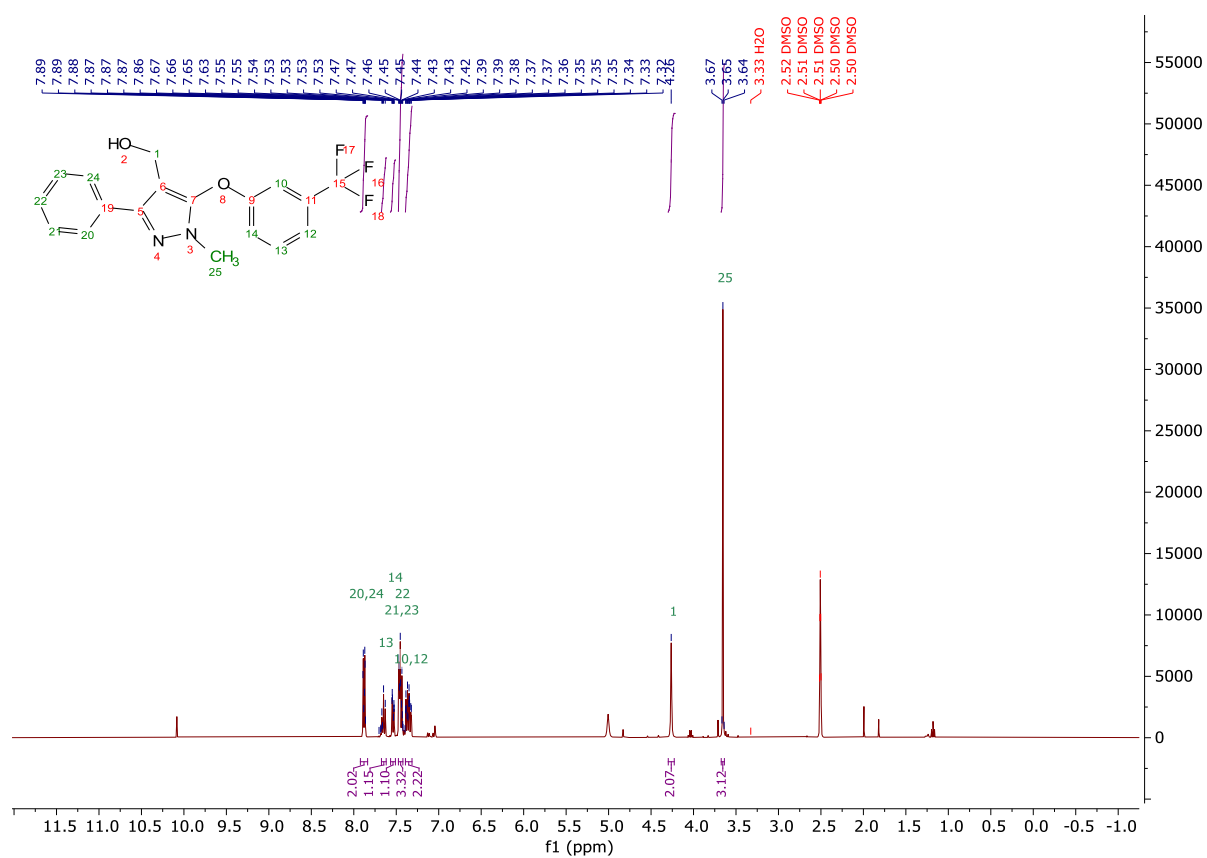


Figure 76. ¹H NMR spectrum for compound 27c.

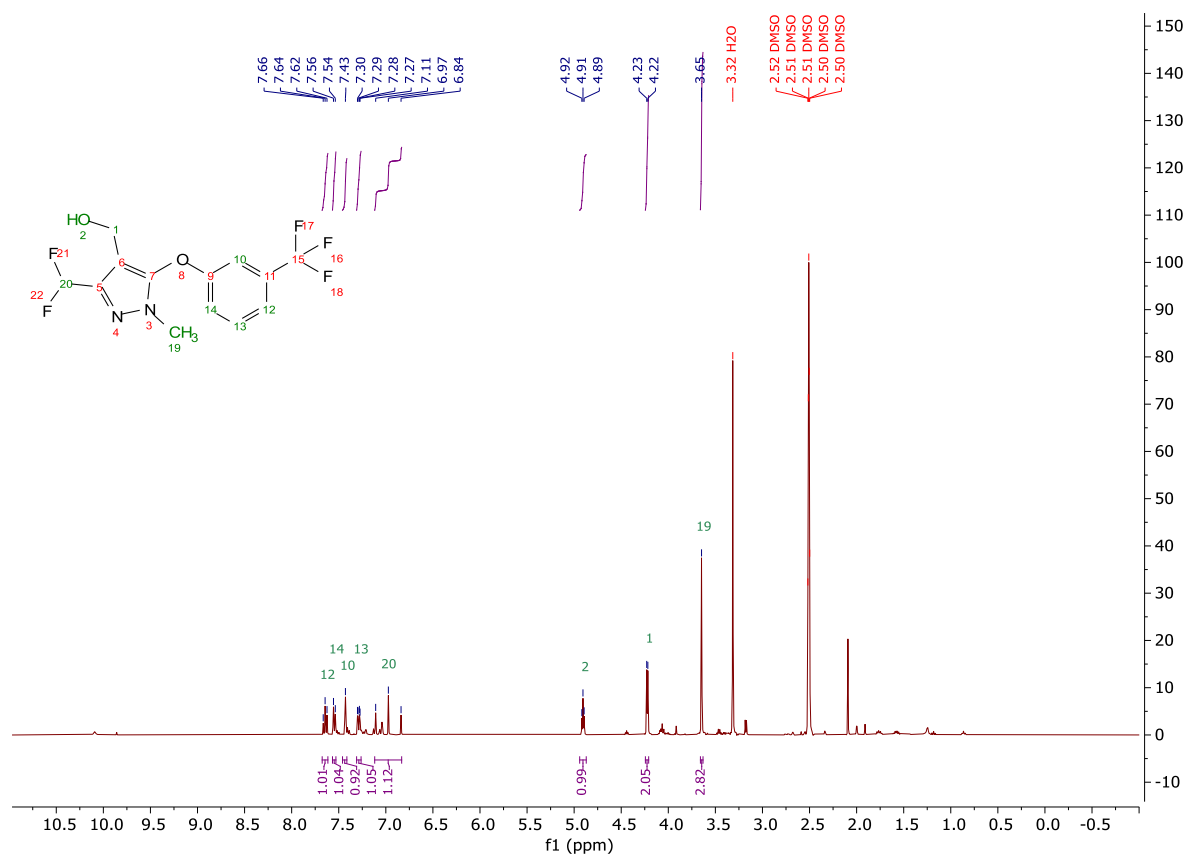


Figure 77. ^1H NMR spectrum for compound 27d.

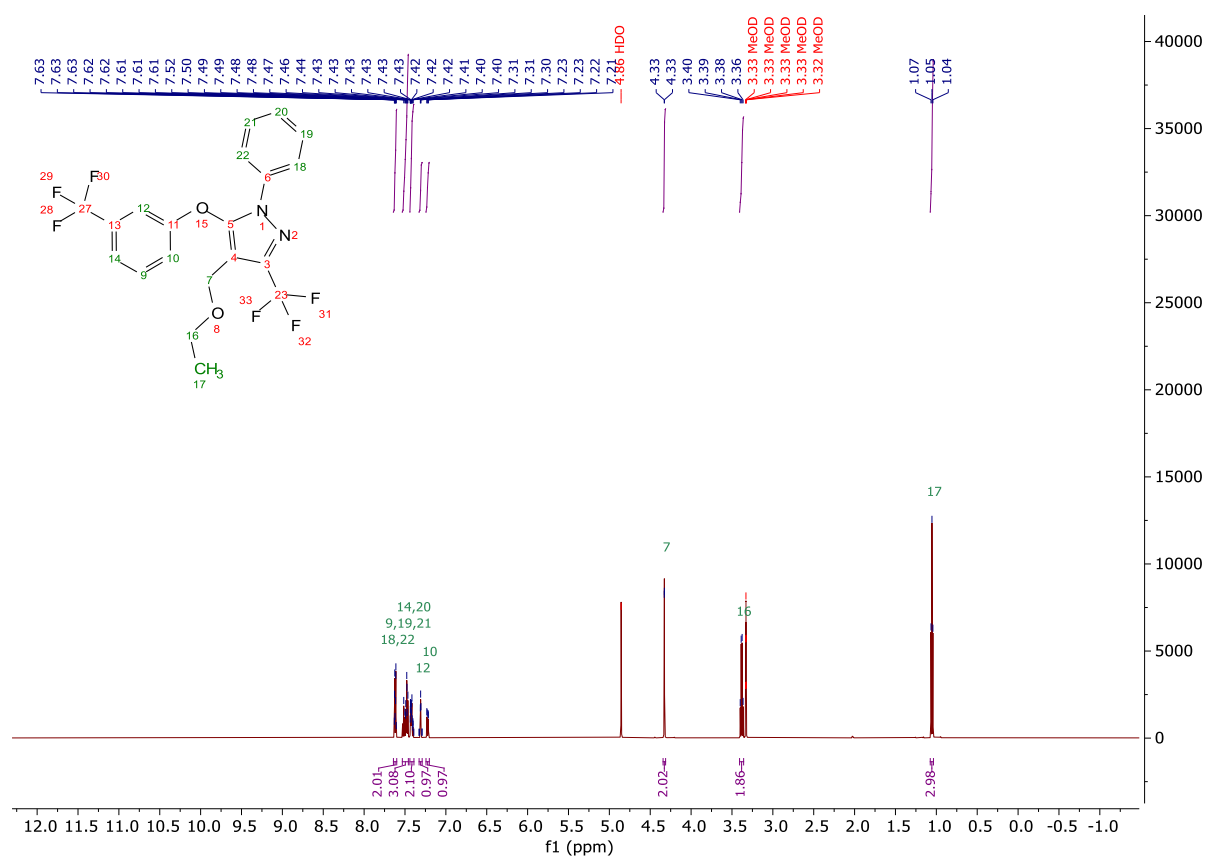


Figure 78. ^1H NMR spectrum for compound 28a.

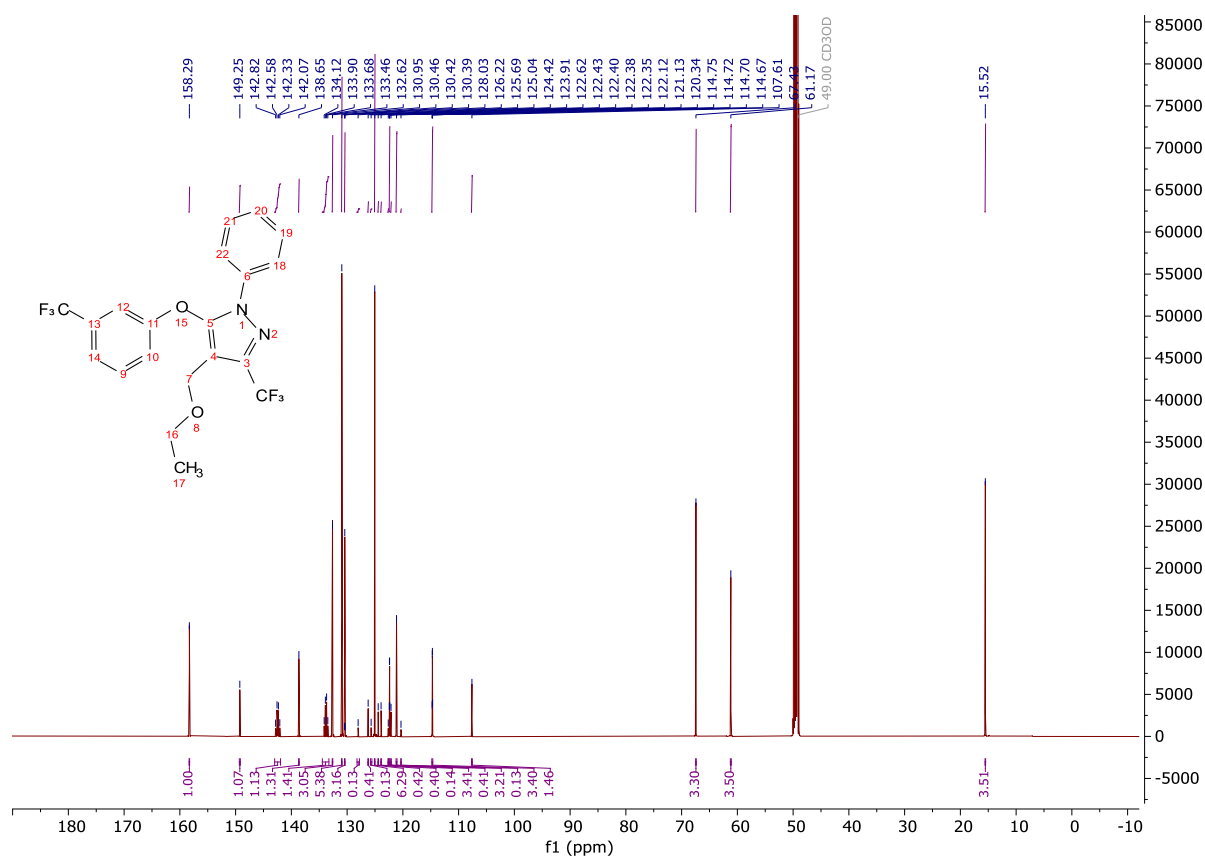


Figure 79. ^{13}C NMR spectrum for compound **28a**.

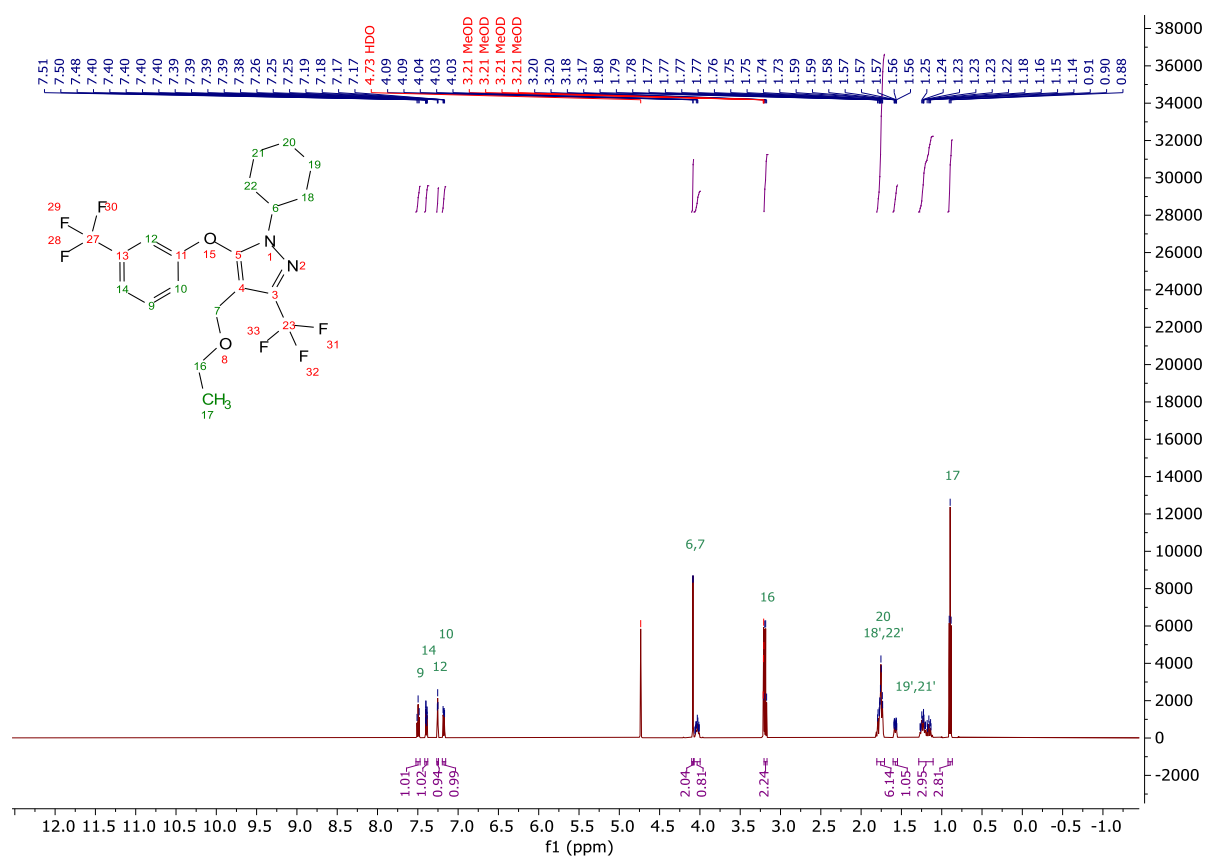
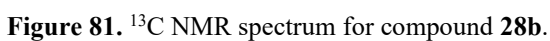


Figure 80. ^1H NMR spectrum for compound **28b**.



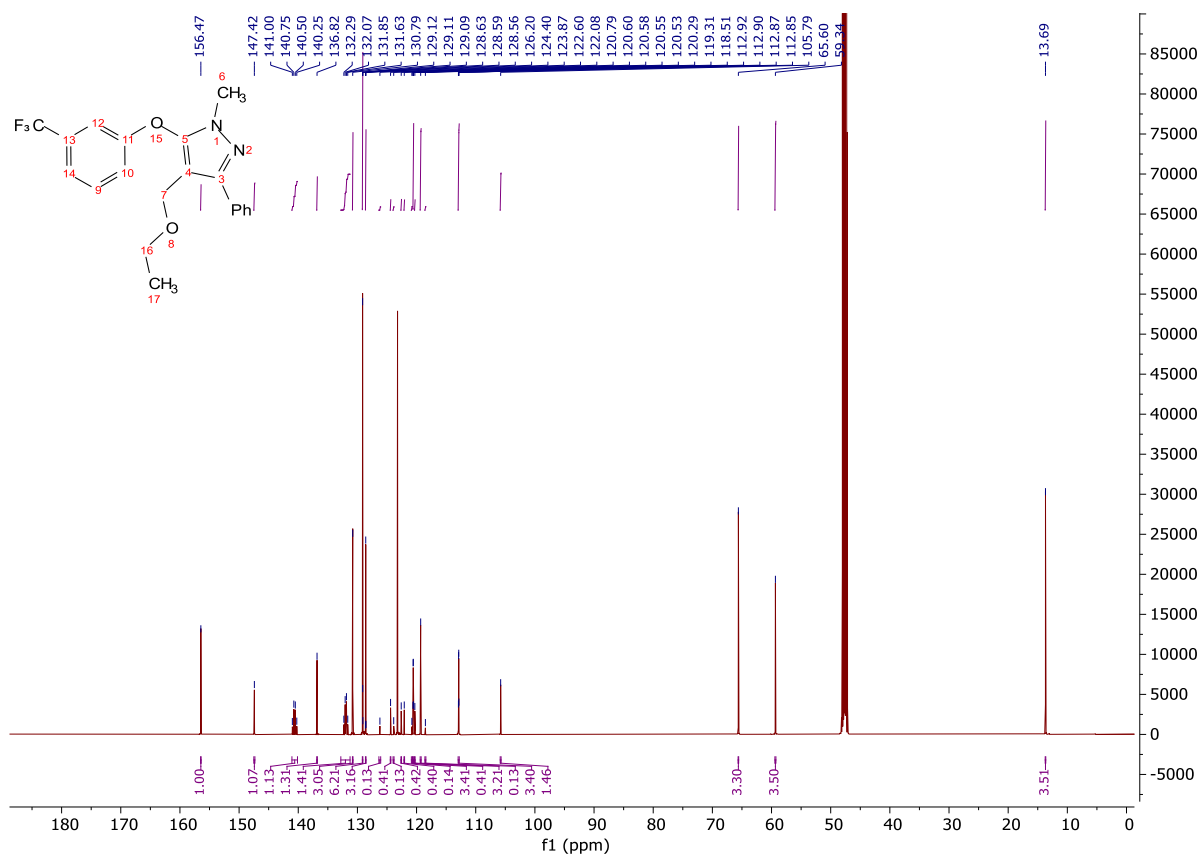


Figure 83. ¹³C NMR spectrum for compound **28c**.

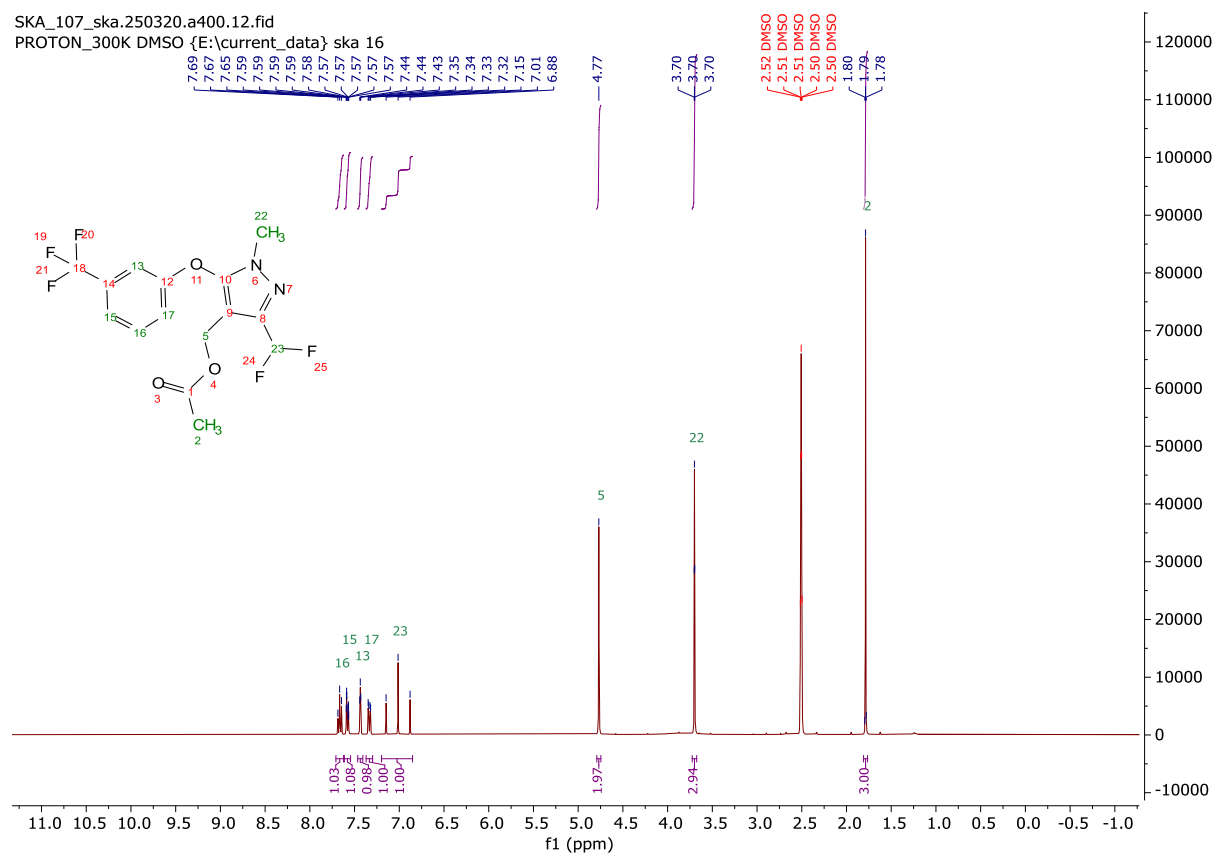


Figure 84. ¹H NMR spectrum for compound **28d**.

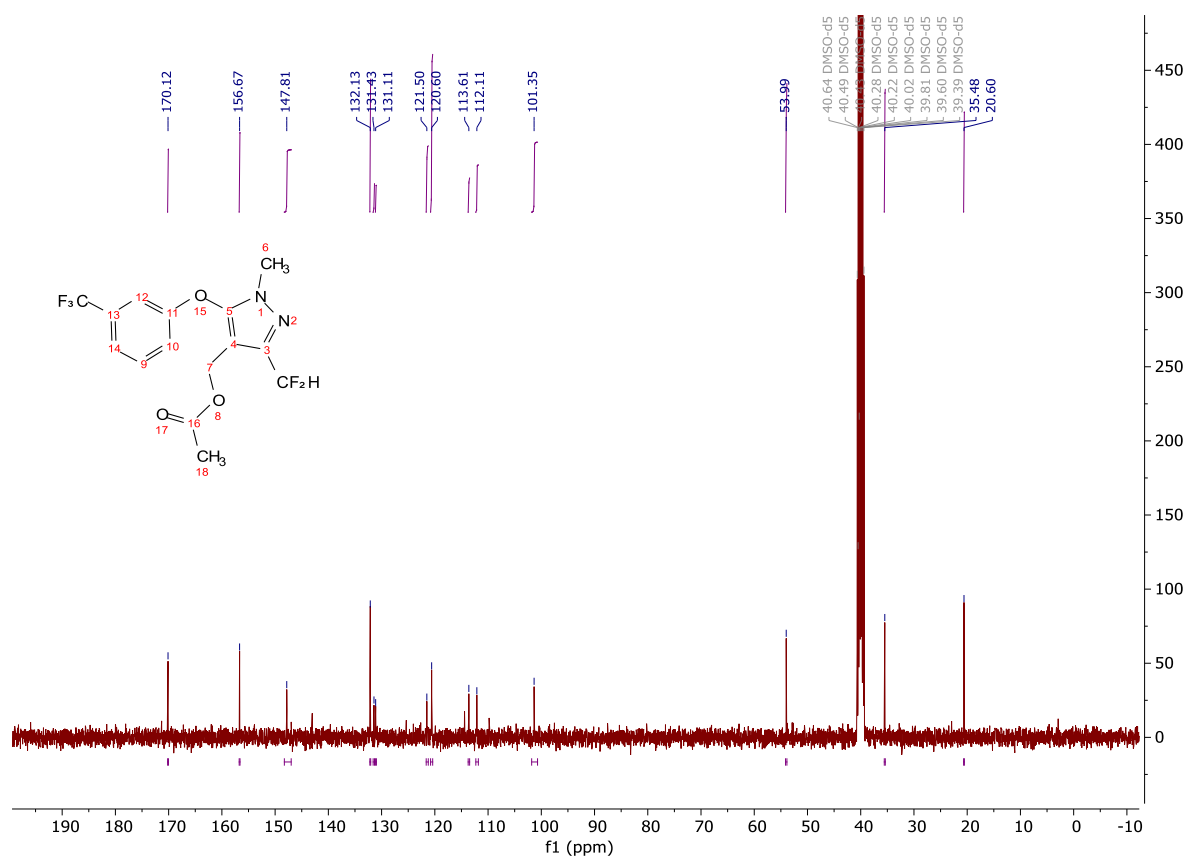


Figure 85. ^{13}C NMR spectrum for compound 28d.

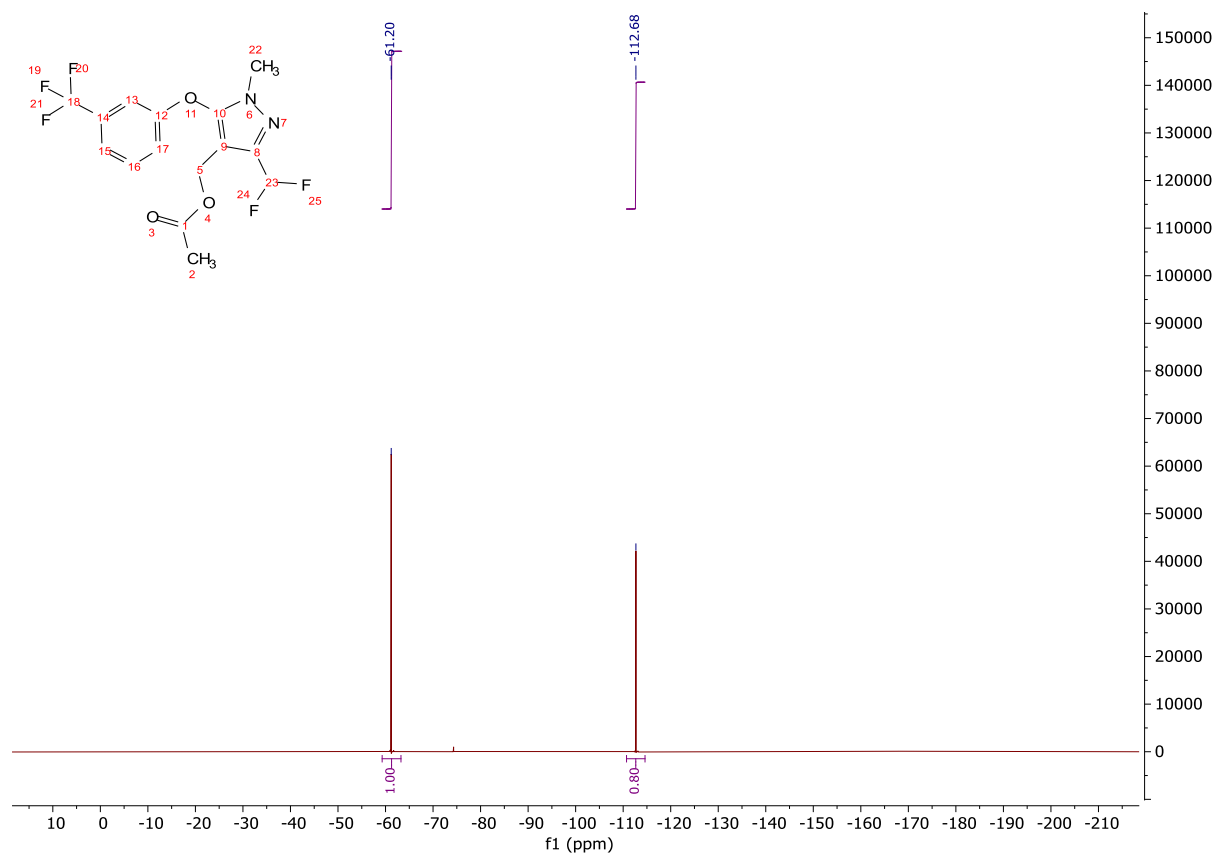


Figure 86. ^{19}F NMR spectrum for compound 28d.

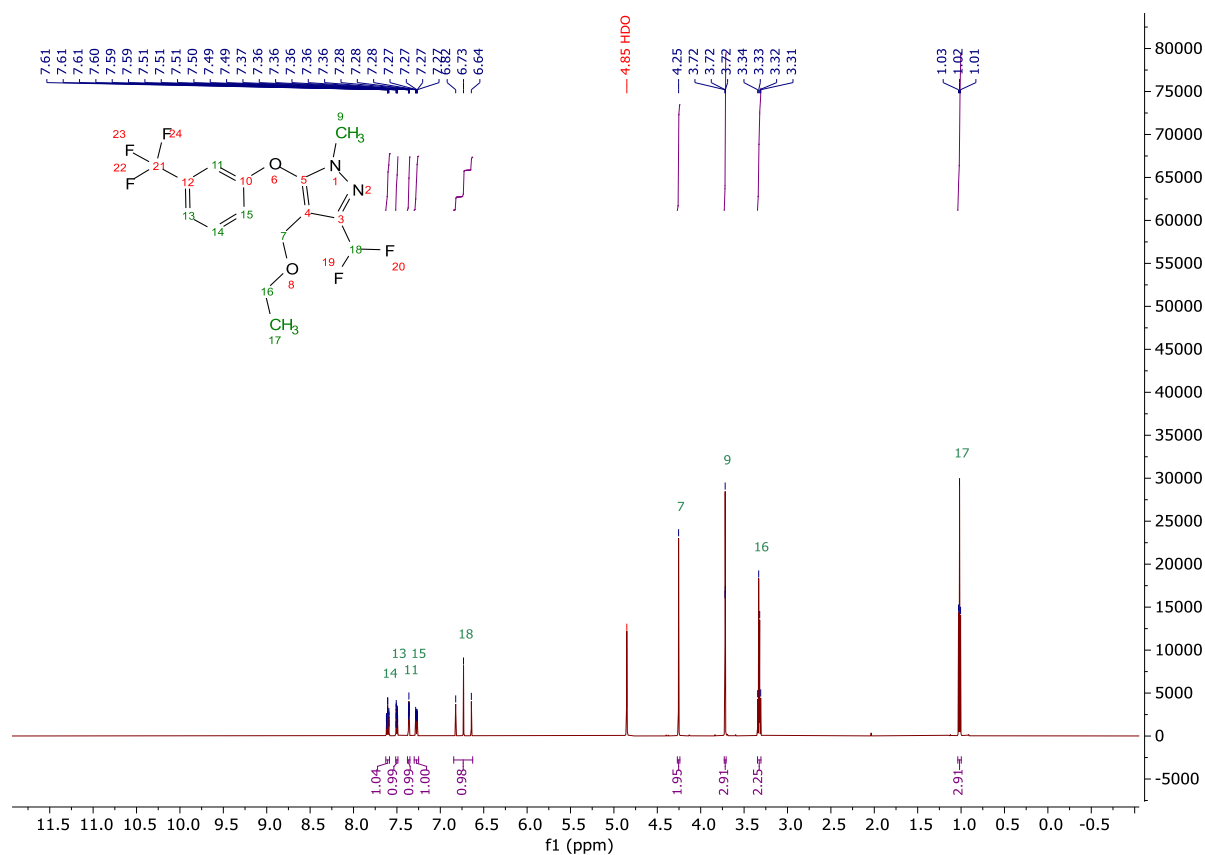


Figure 87. ¹H NMR spectrum for compound 28e.

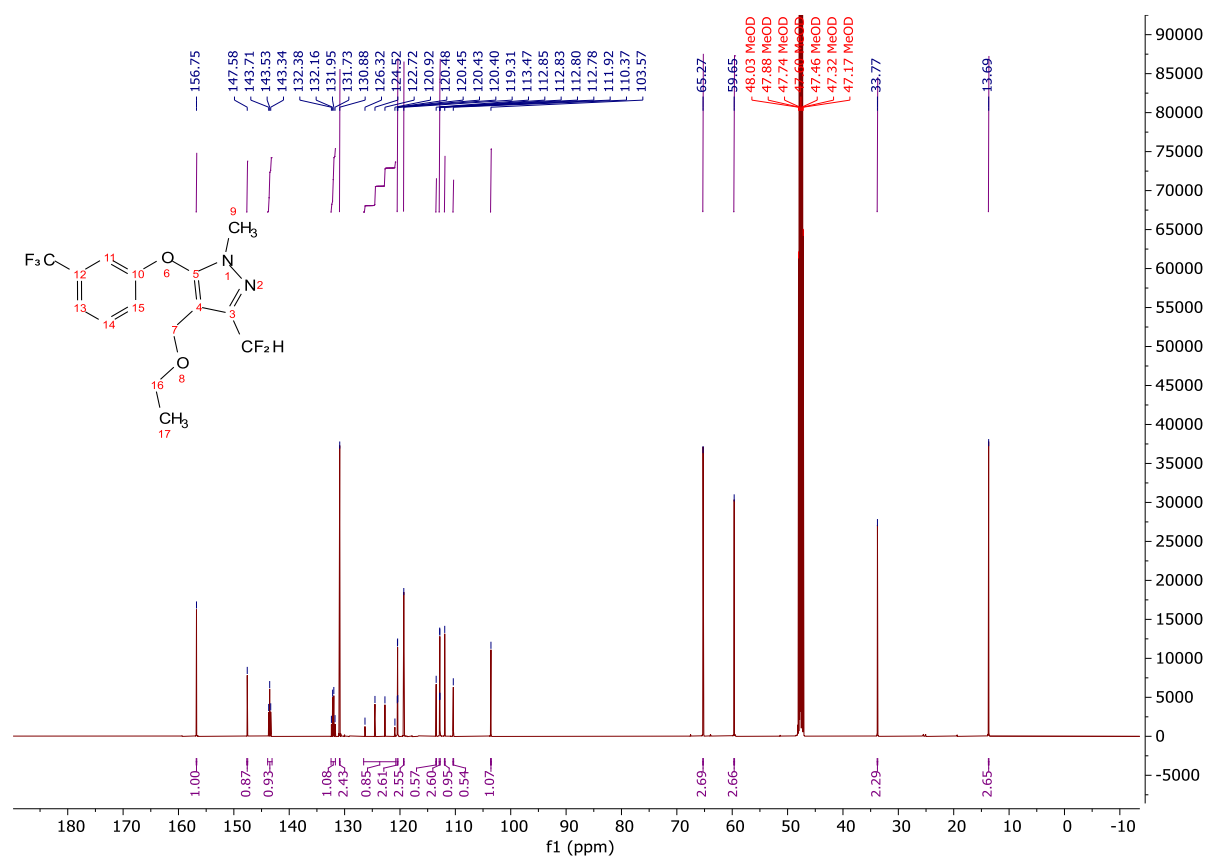


Figure 88. ¹³C NMR spectrum for compound 28e.

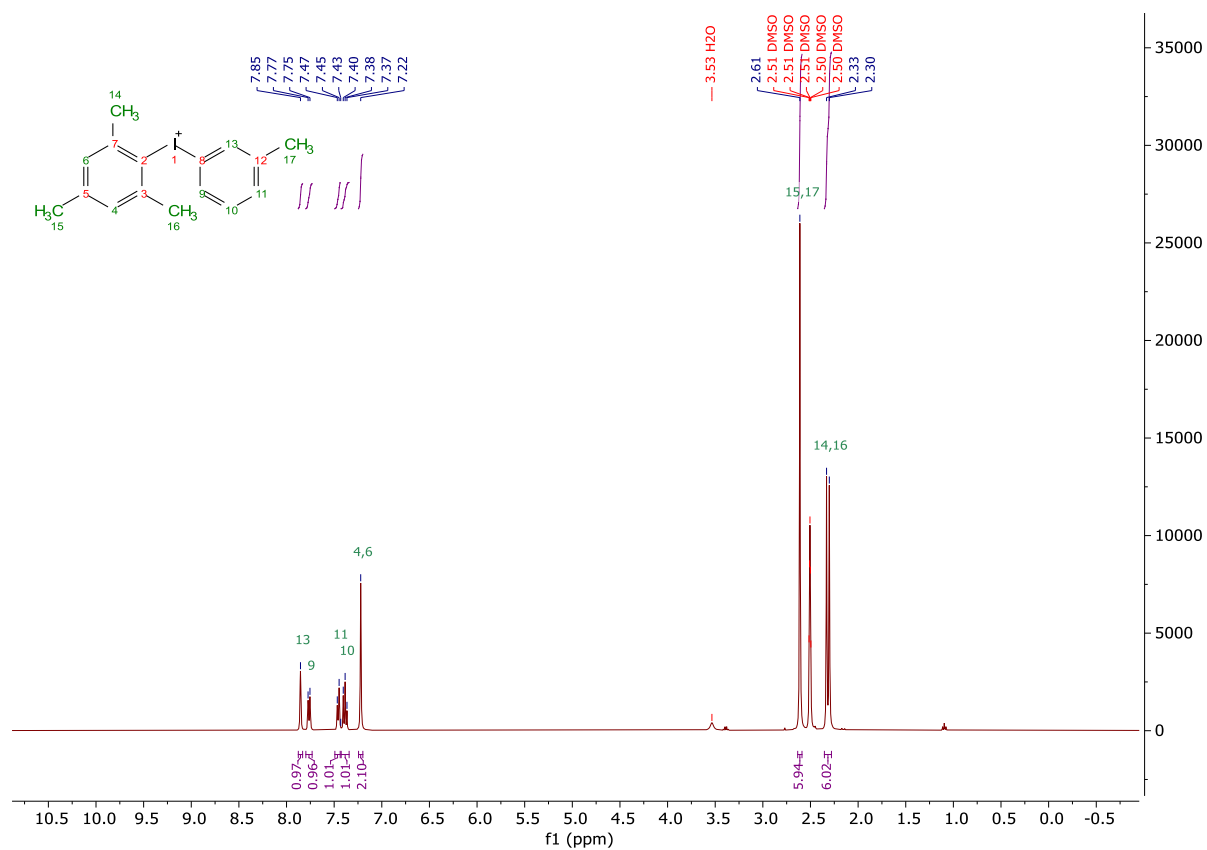


Figure 89. ¹H NMR spectrum for compound **31a**.

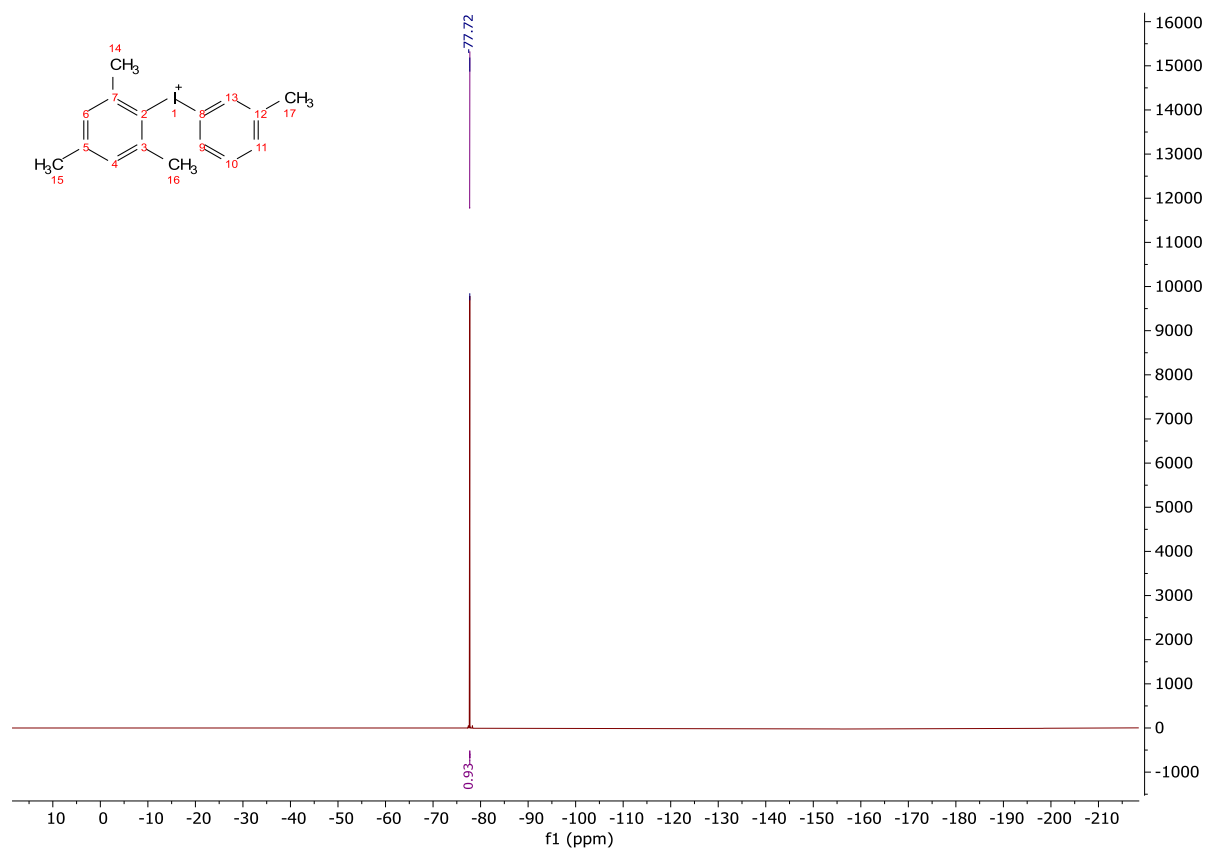


Figure 90. ¹⁹F NMR spectrum for compound **31a**.

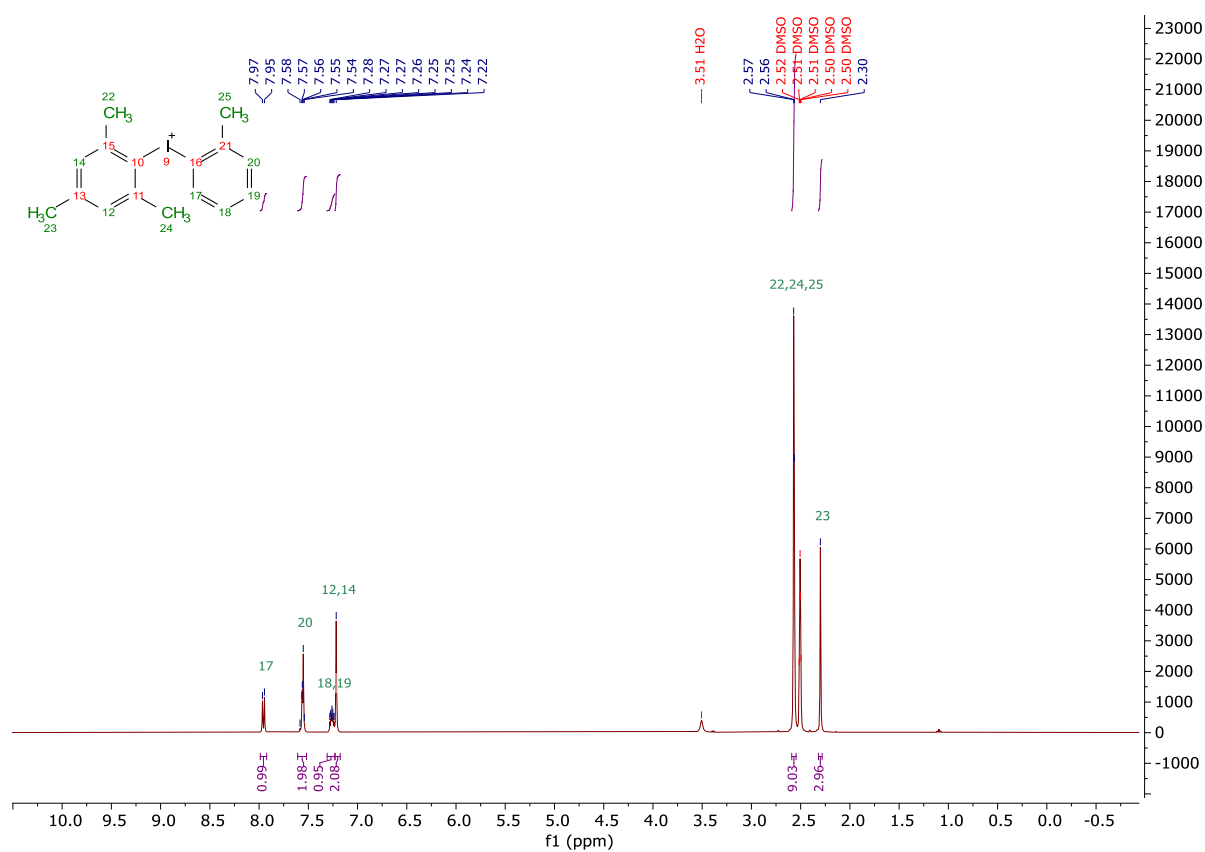


Figure 91. ^1H NMR spectrum for compound **31b**.

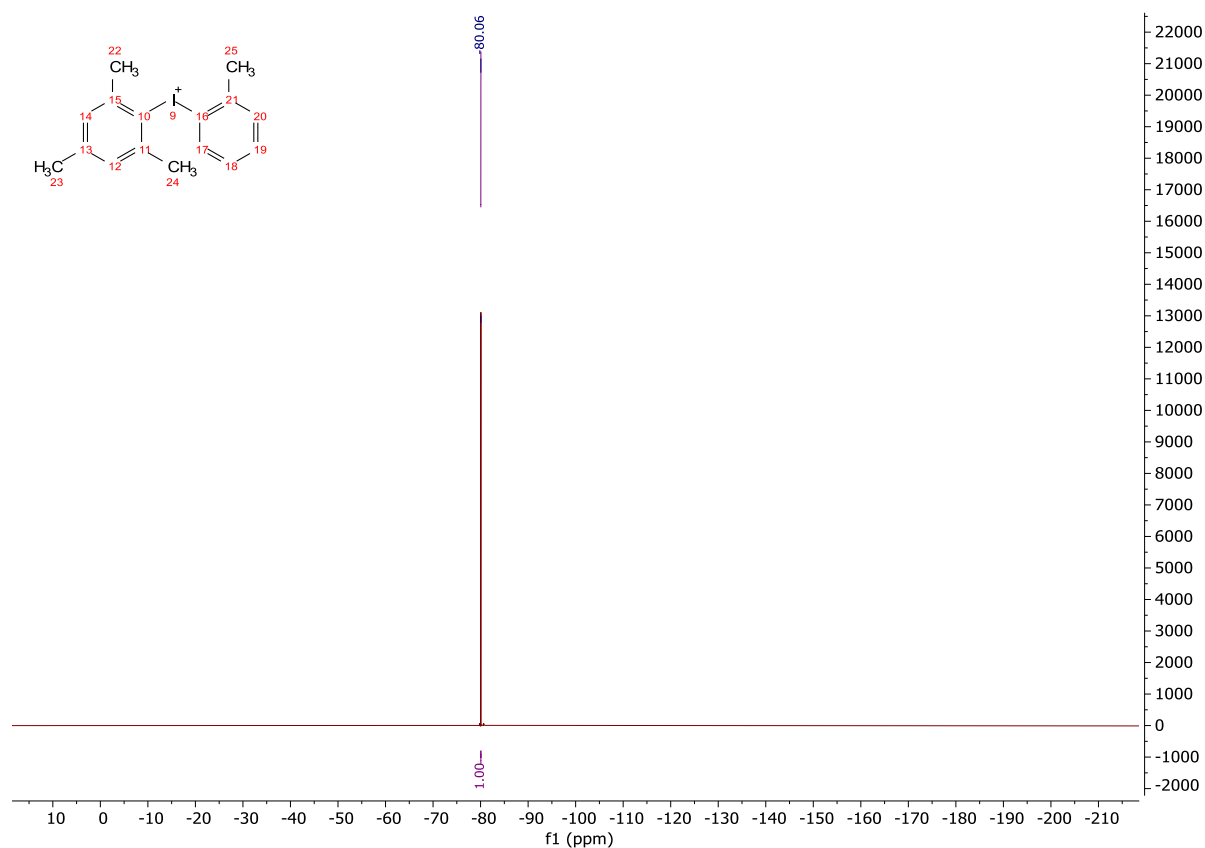


Figure 92. ^{19}F NMR spectrum for compound **31b**.

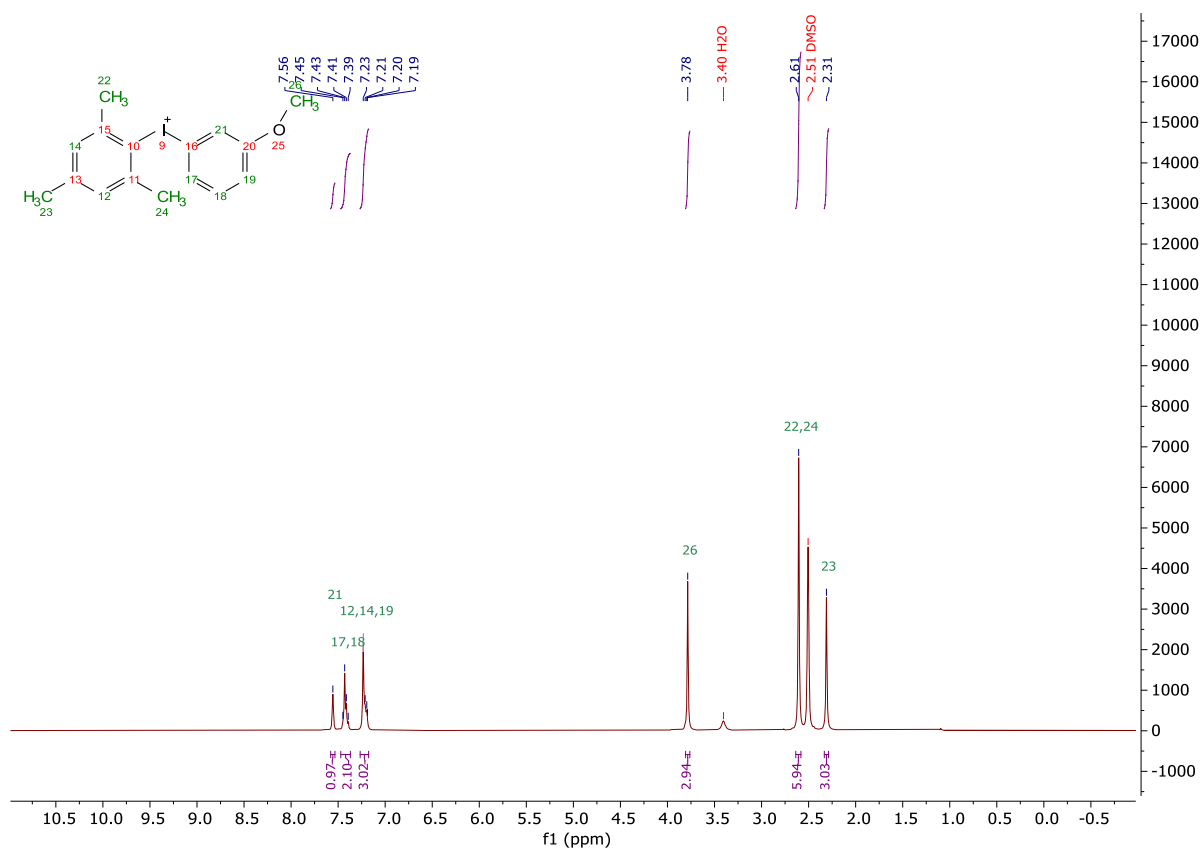


Figure 93. ^1H NMR spectrum for compound **31c**.

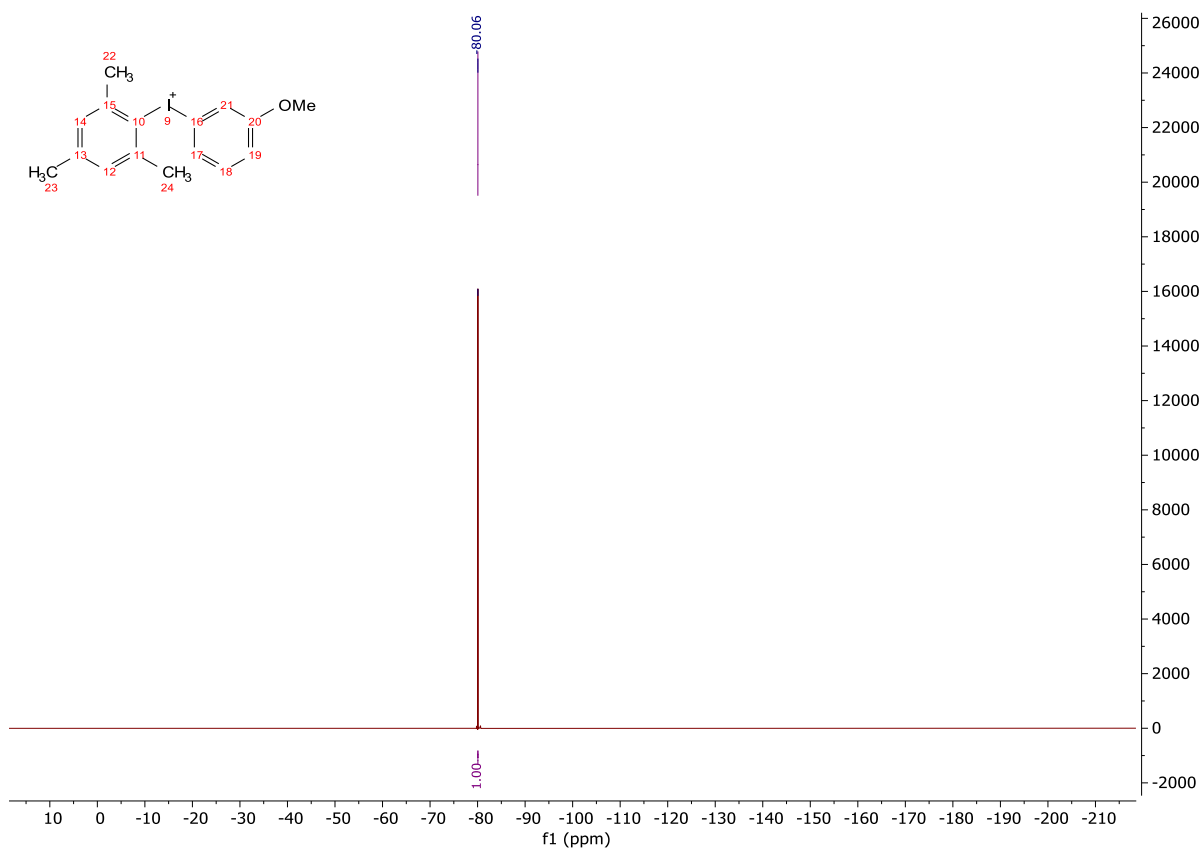


Figure 94. ^{19}F NMR spectrum for compound **31c**.

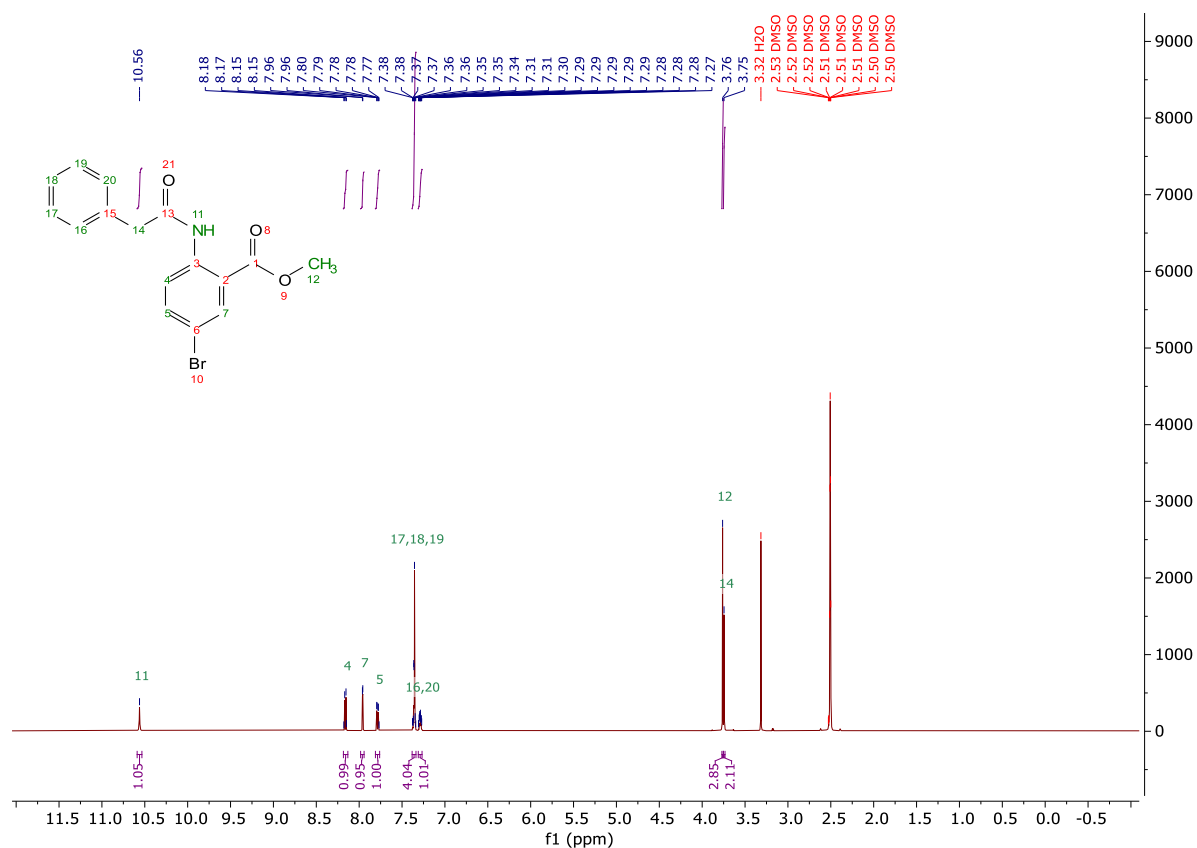


Figure 95. ¹H NMR spectrum for compound **39a**.

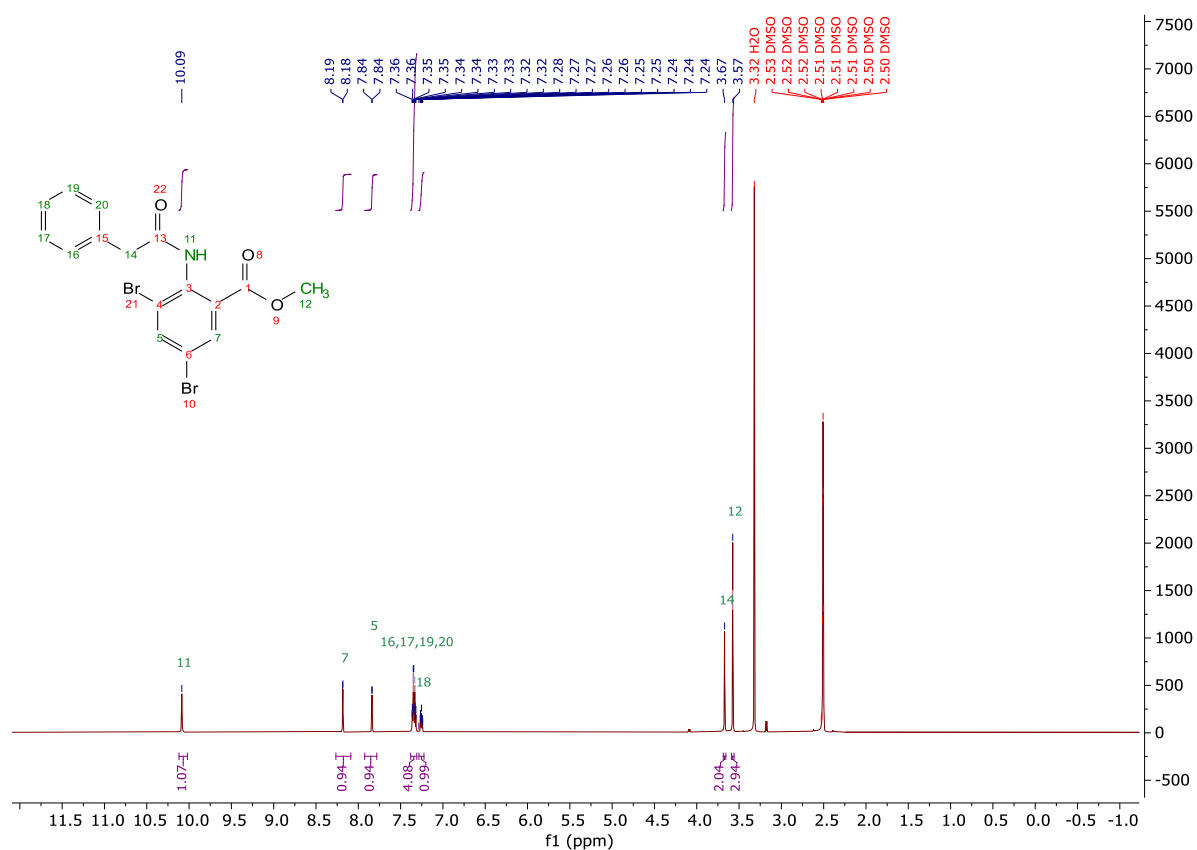


Figure 96. ¹H NMR spectrum for compound **39b**.

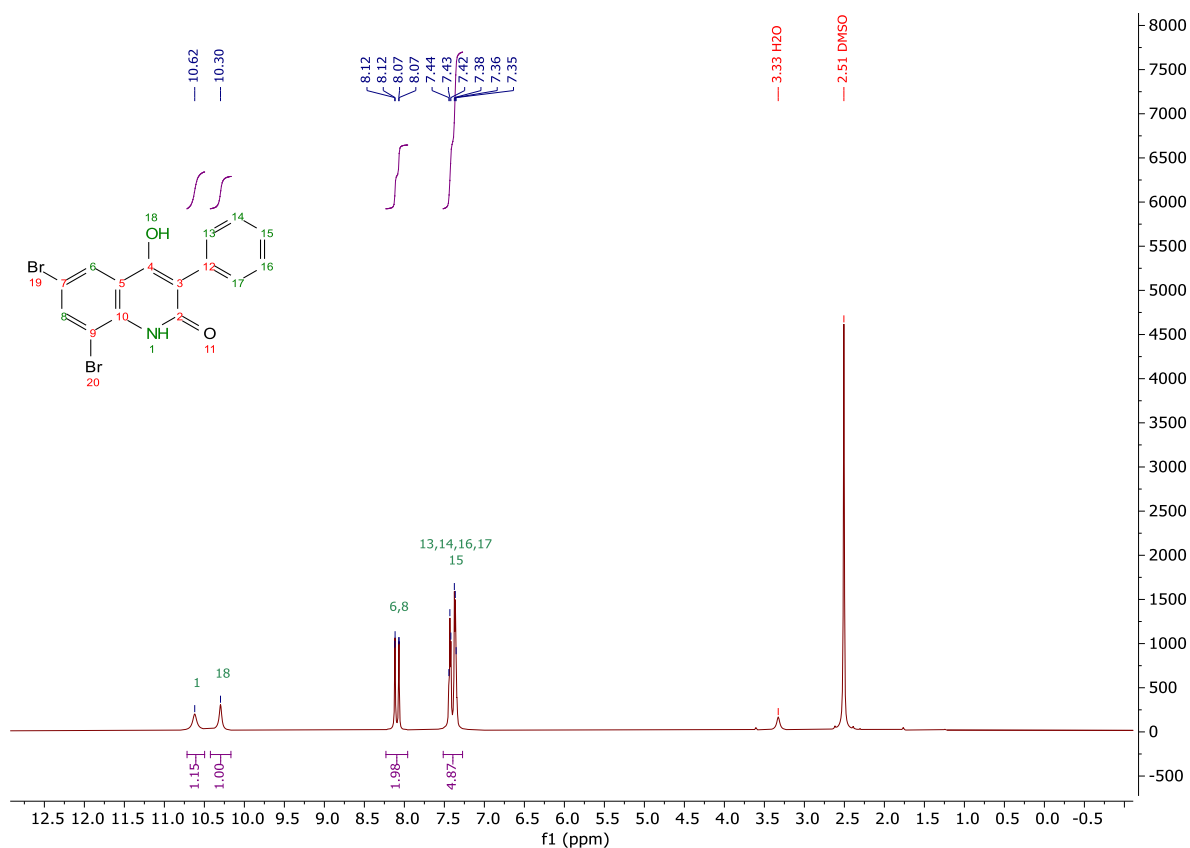


Figure 97. ¹H NMR spectrum for compound 40a.

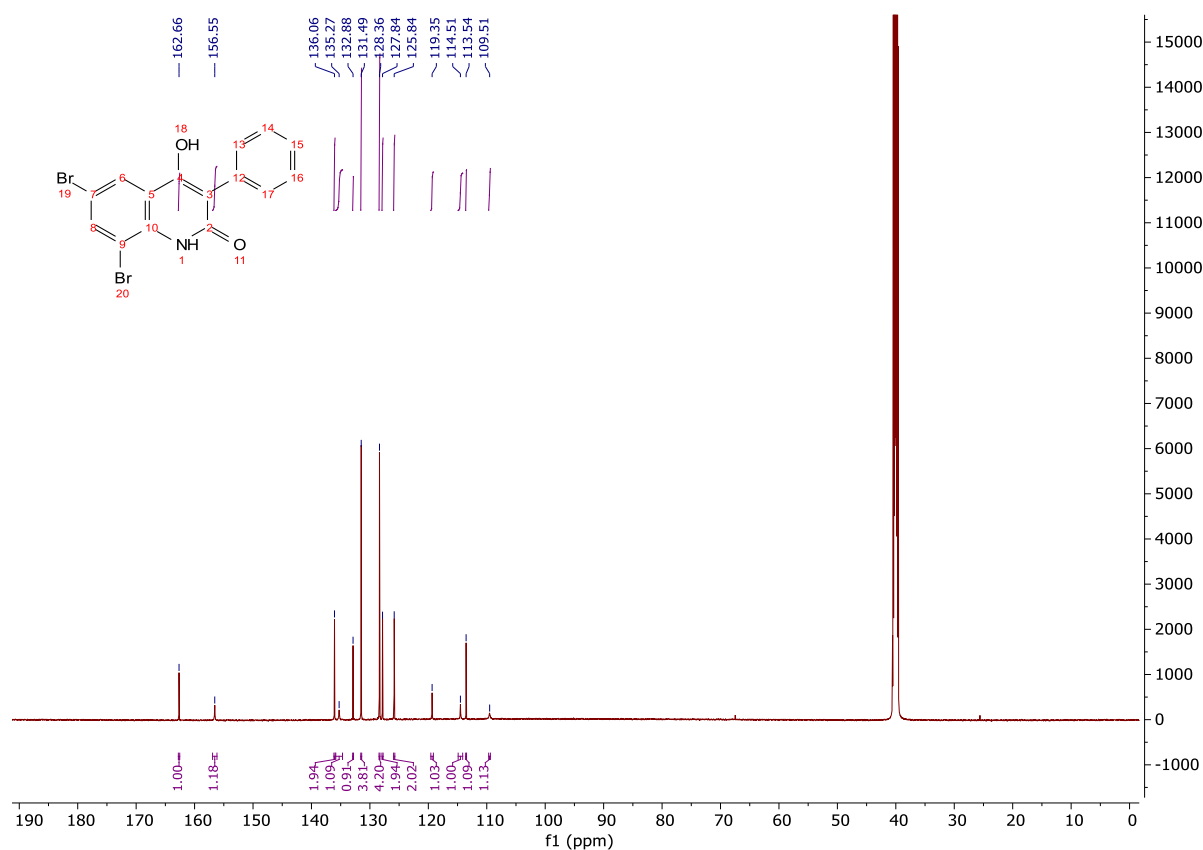


Figure 98. ¹³C NMR spectrum for compound 40a.

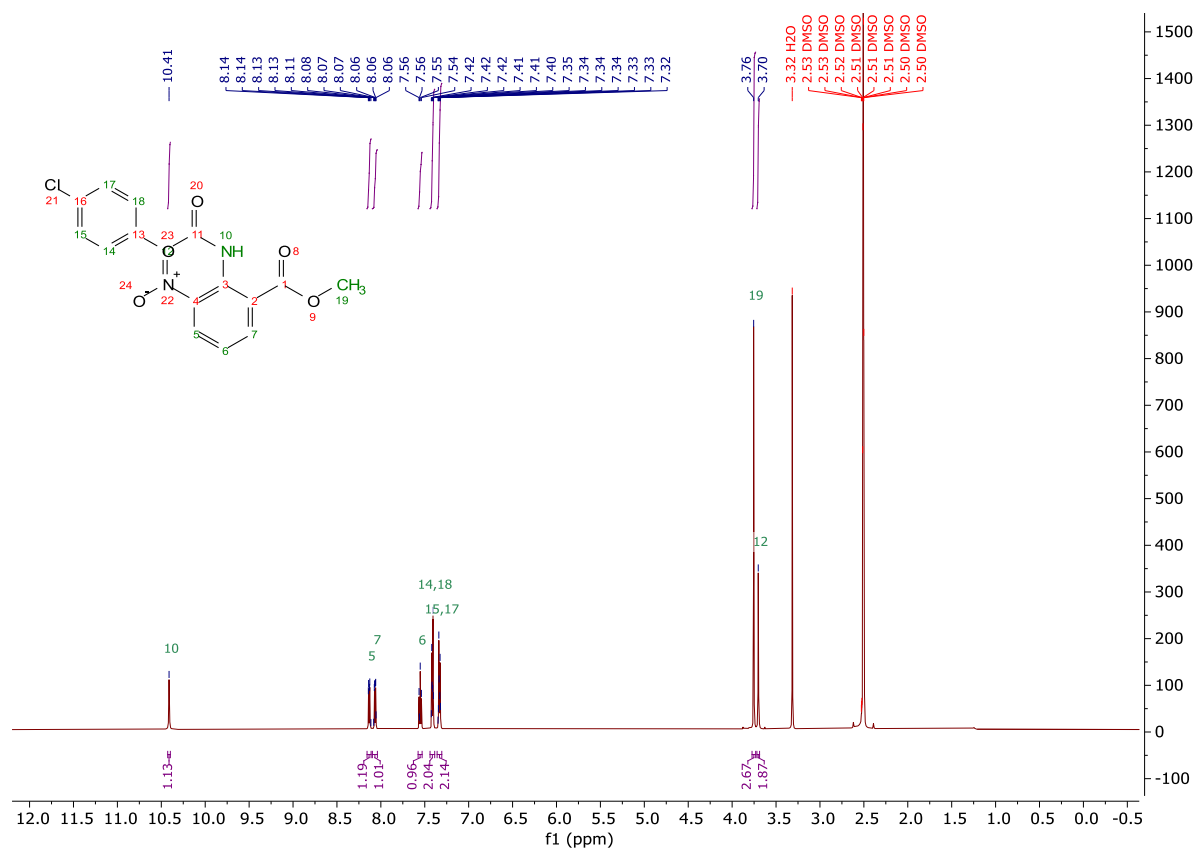


Figure 99. ¹H NMR spectrum for compound **41a**.

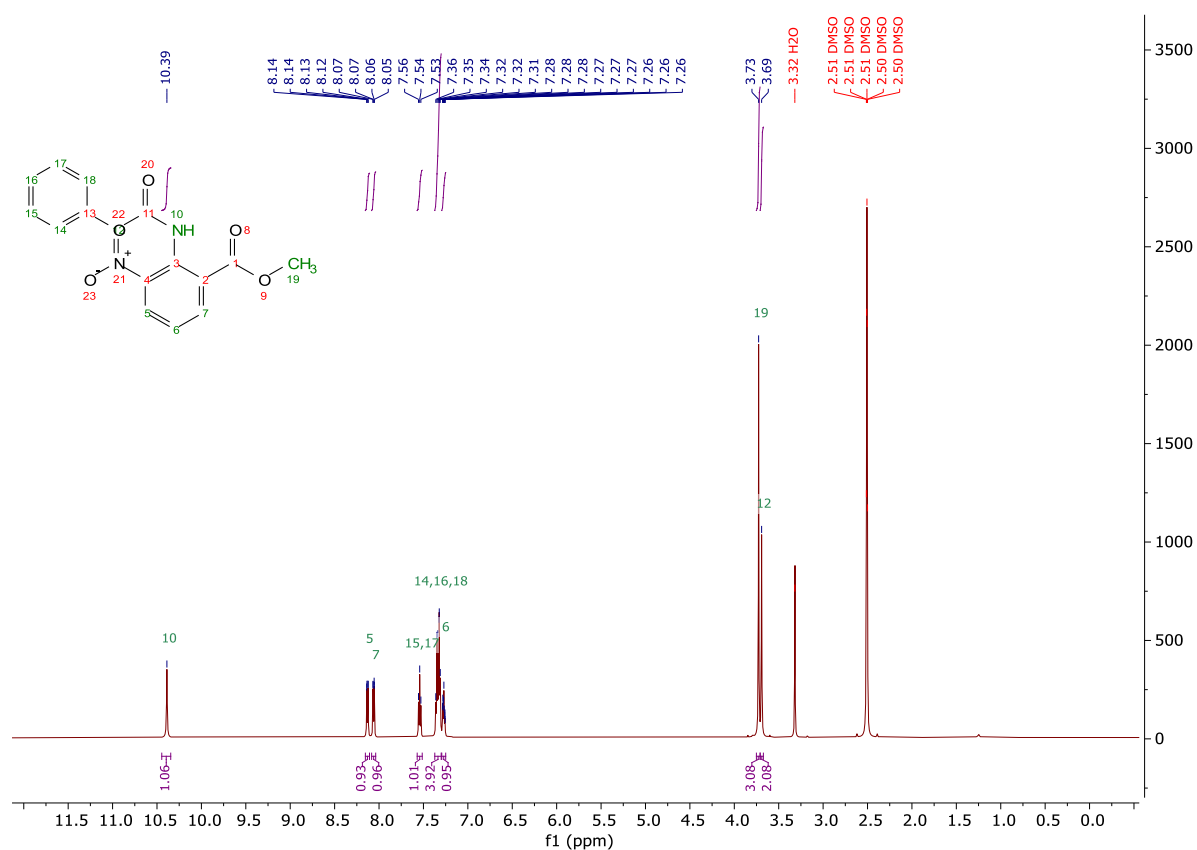


Figure 100. ¹H NMR spectrum for compound **41b**.

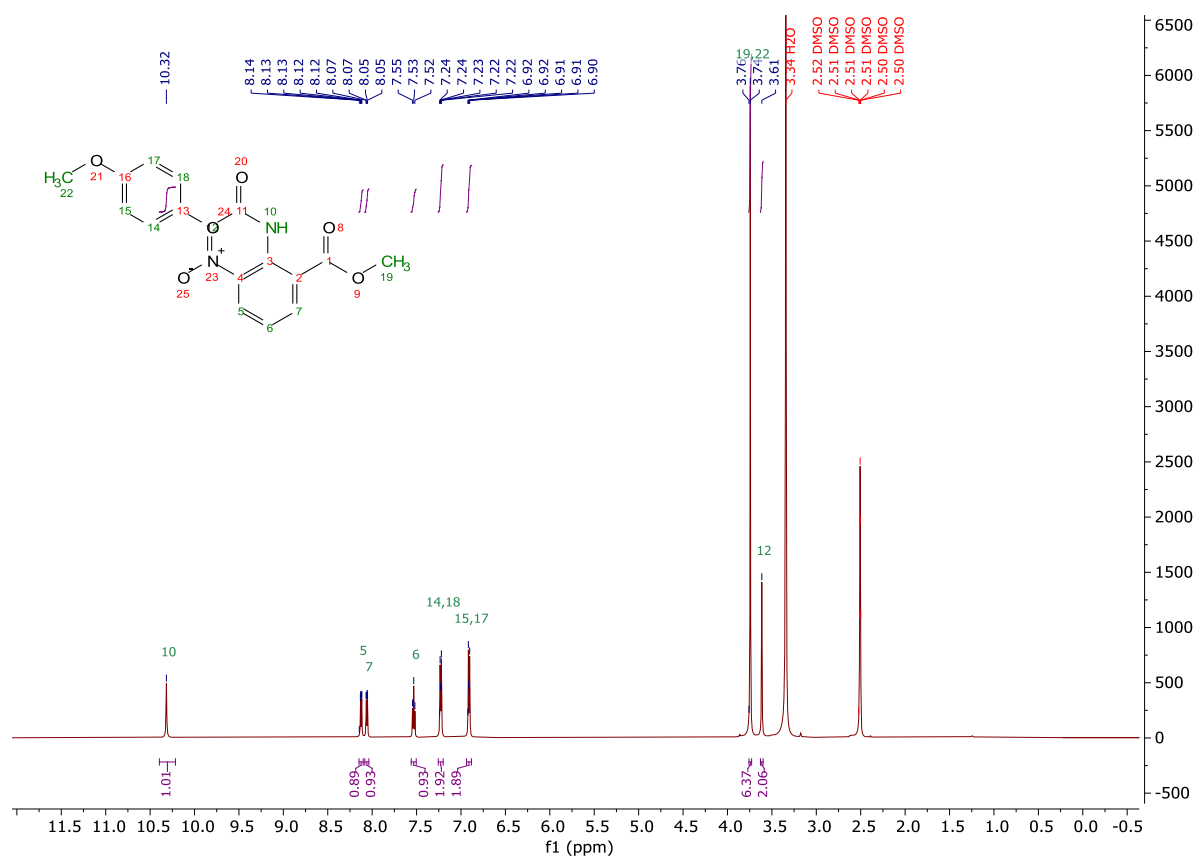


Figure 101. ¹H NMR spectrum for compound **41c**.

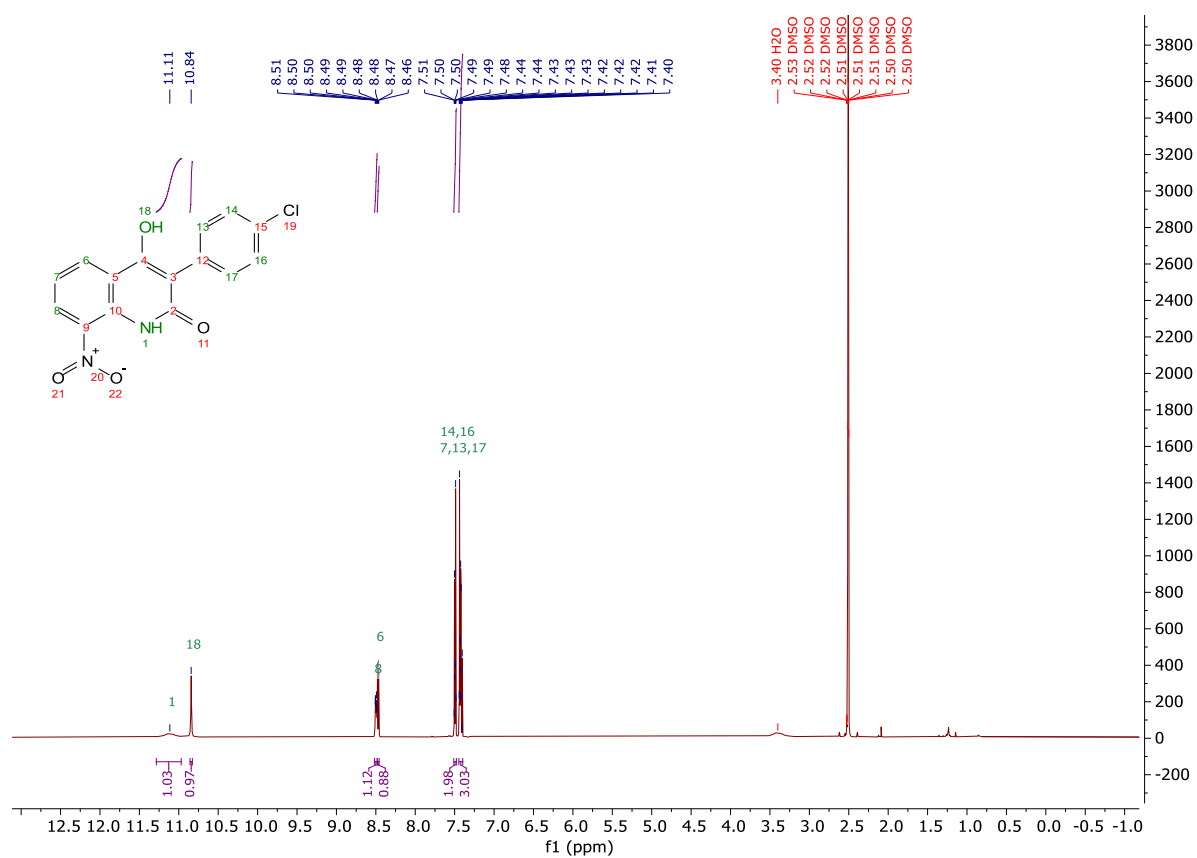


Figure 102. ¹H NMR spectrum for compound **42a**.

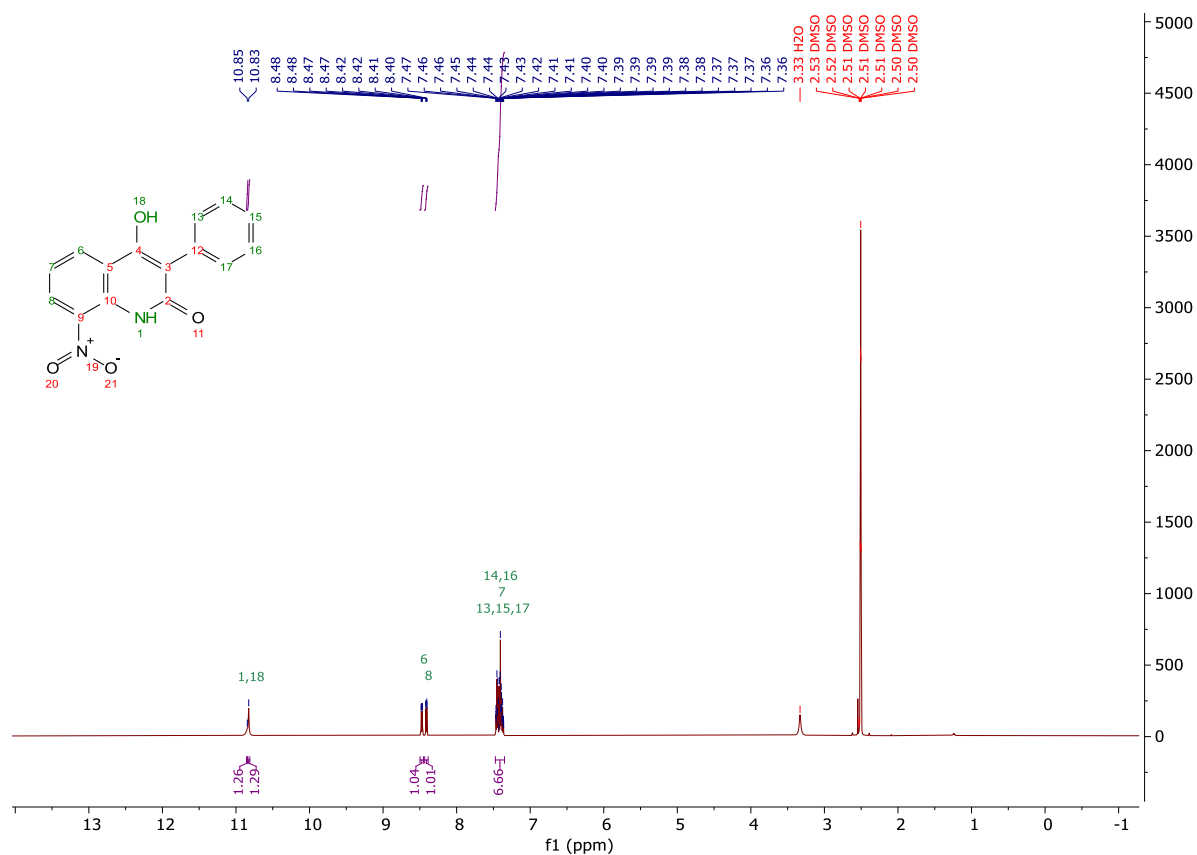


Figure 103. ¹H NMR spectrum for compound 42b.

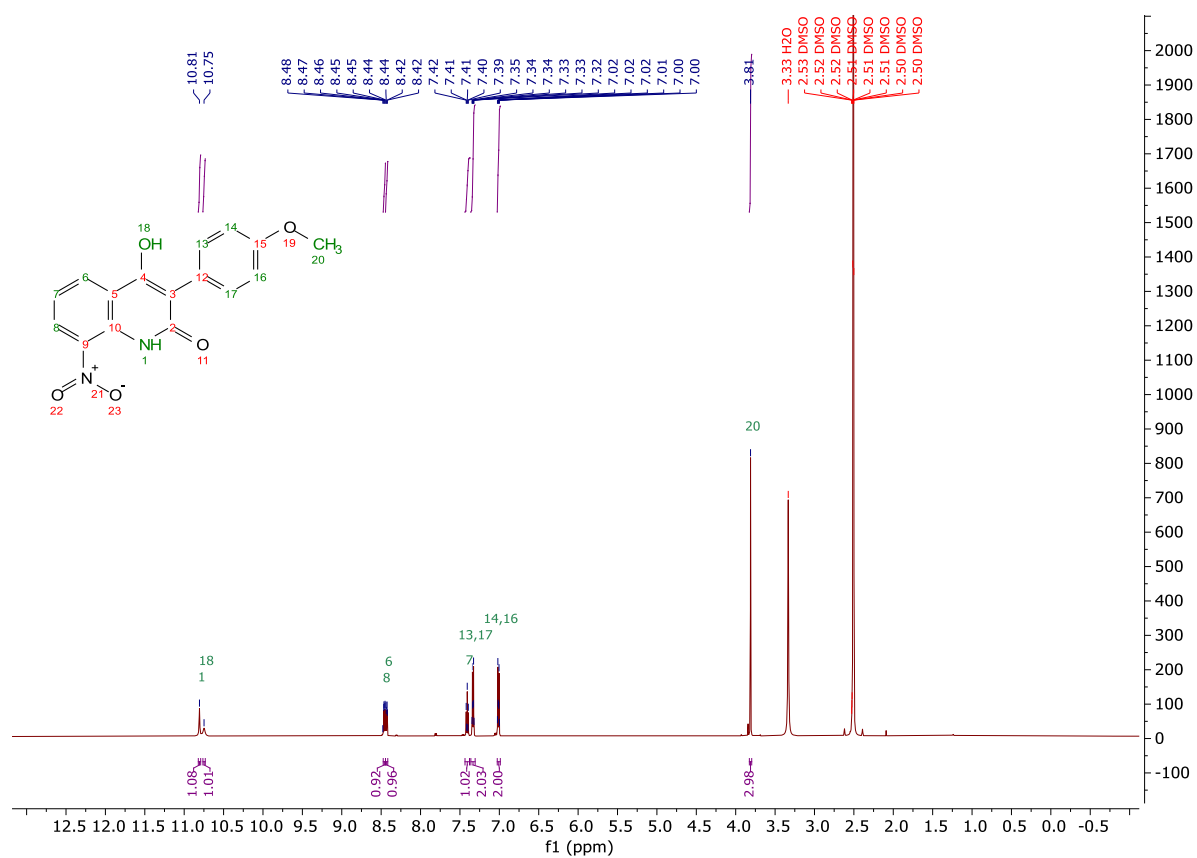


Figure 104. ¹H NMR spectrum for compound 42c.

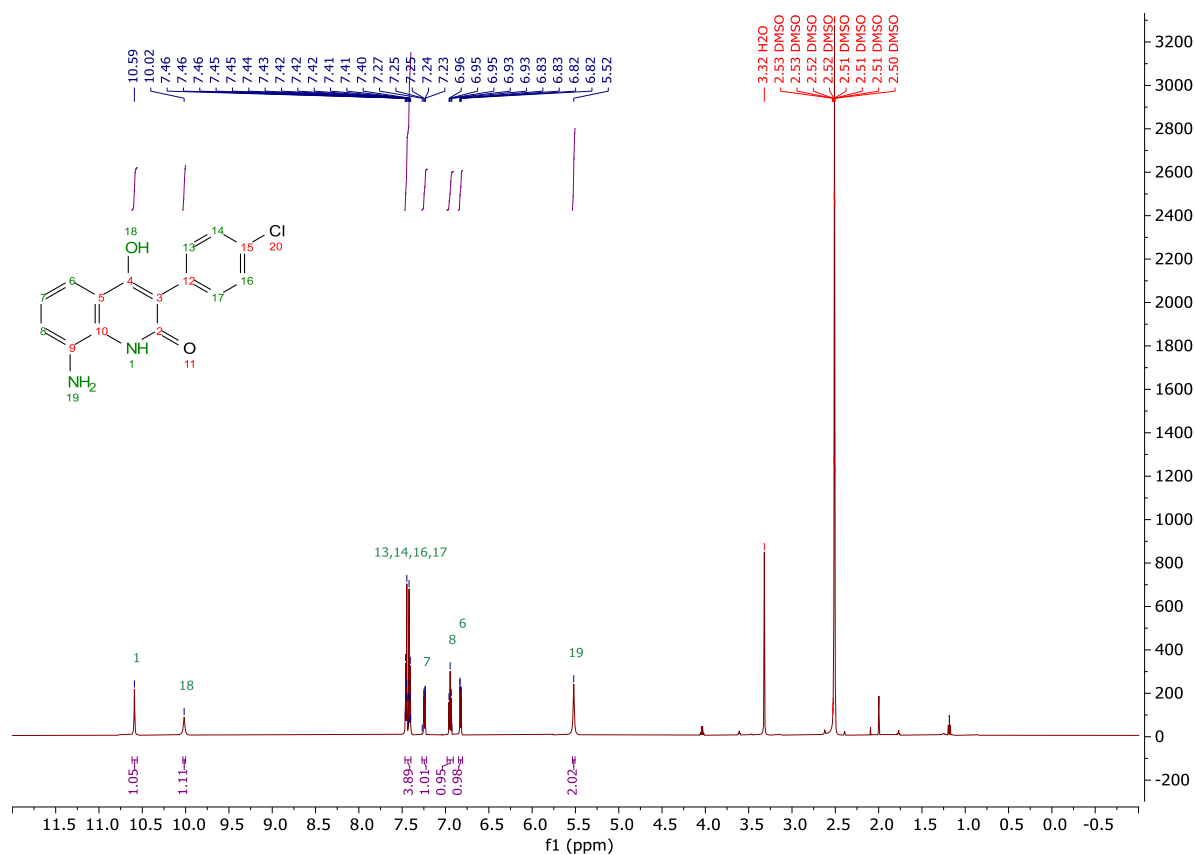


Figure 105. ¹H NMR spectrum for compound 43a.

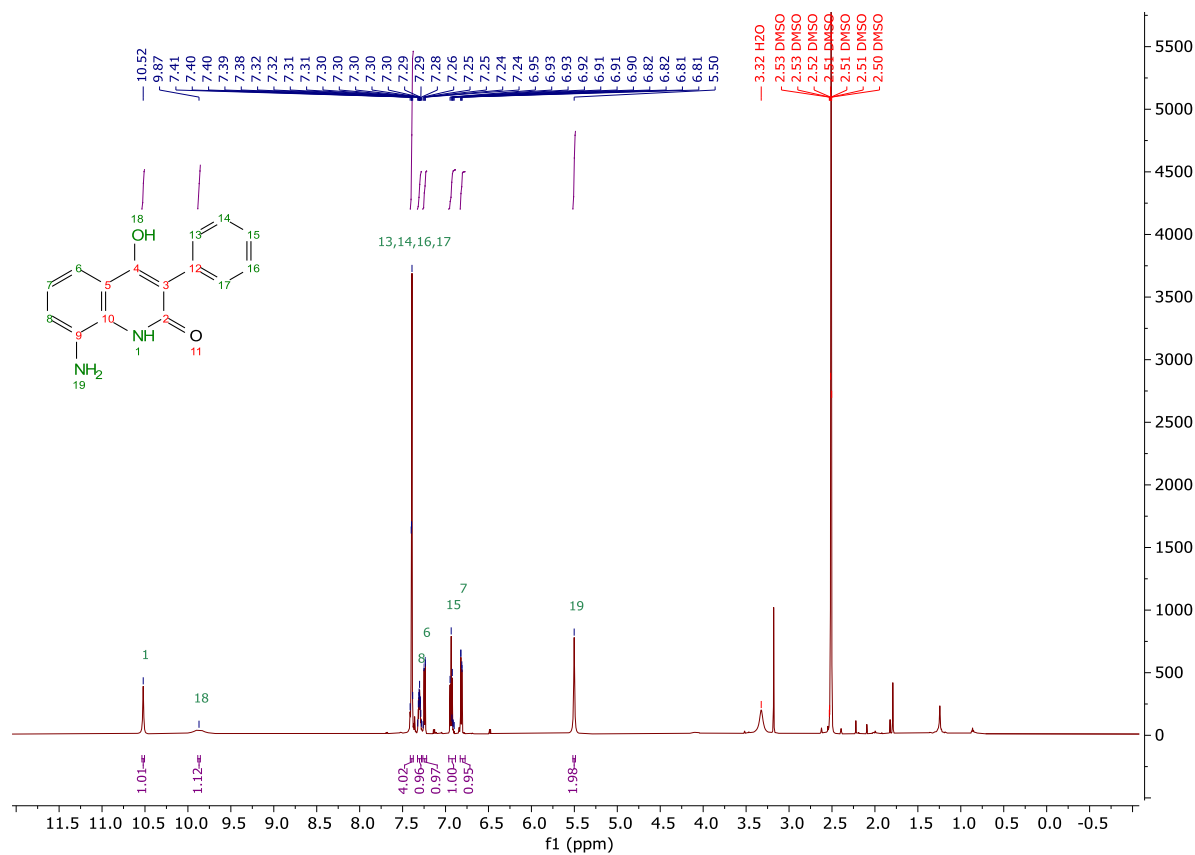


Figure 106. ¹H NMR spectrum for compound 43b.

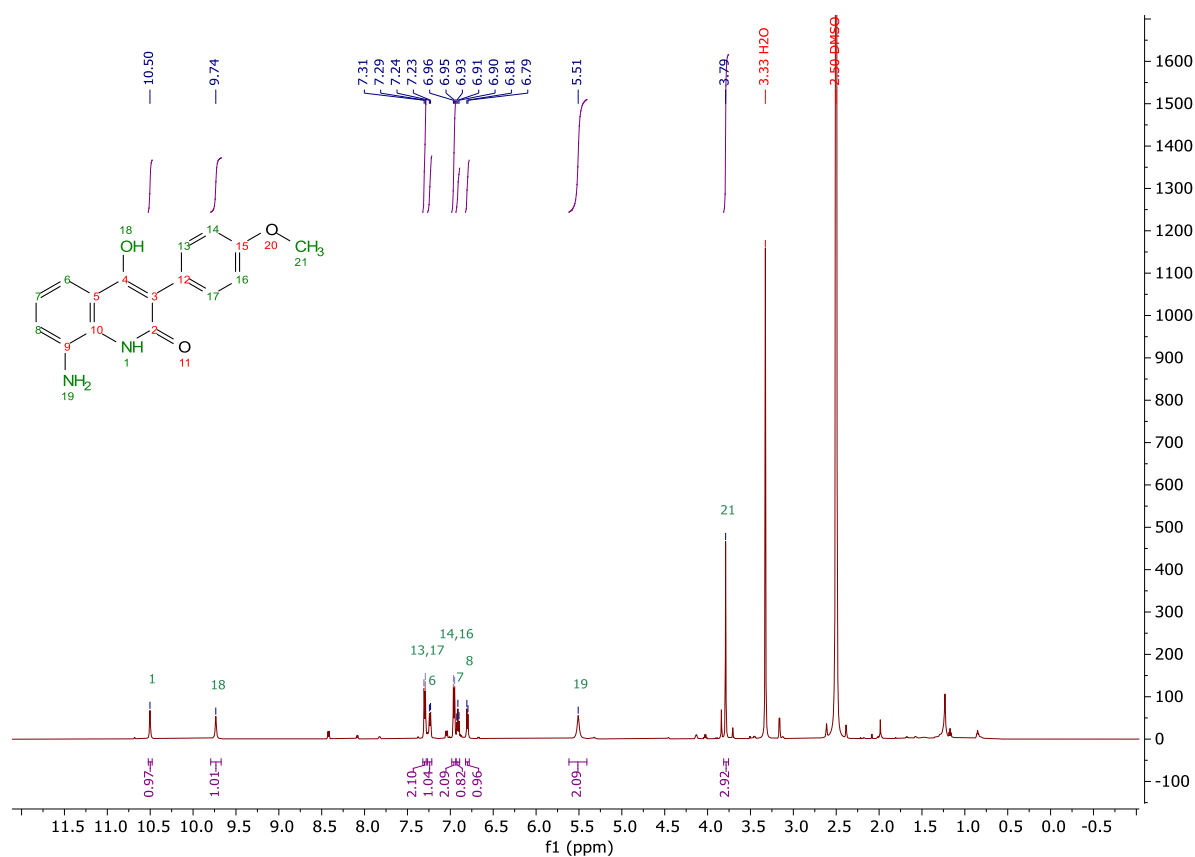


Figure 107. ^1H NMR spectrum for compound **43c**.

7.2 Plausible mechanisms

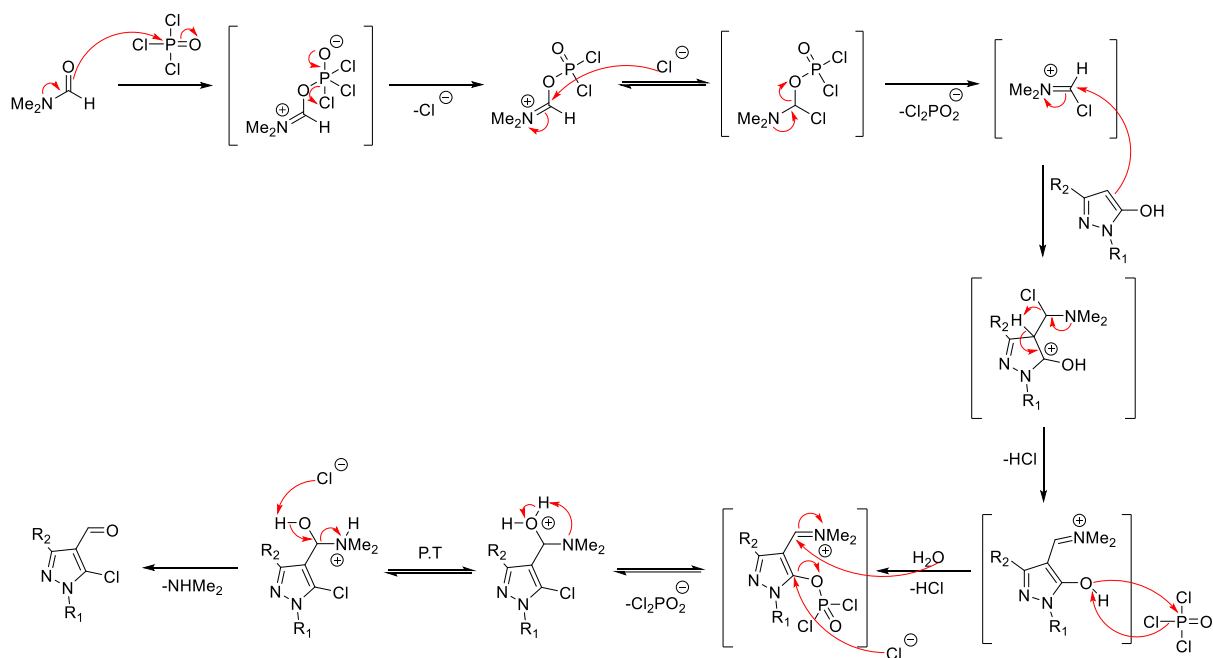


Figure 108. Plausible mechanism for the Vilsmeier-Haack formylation of intermediates **22a-c**.

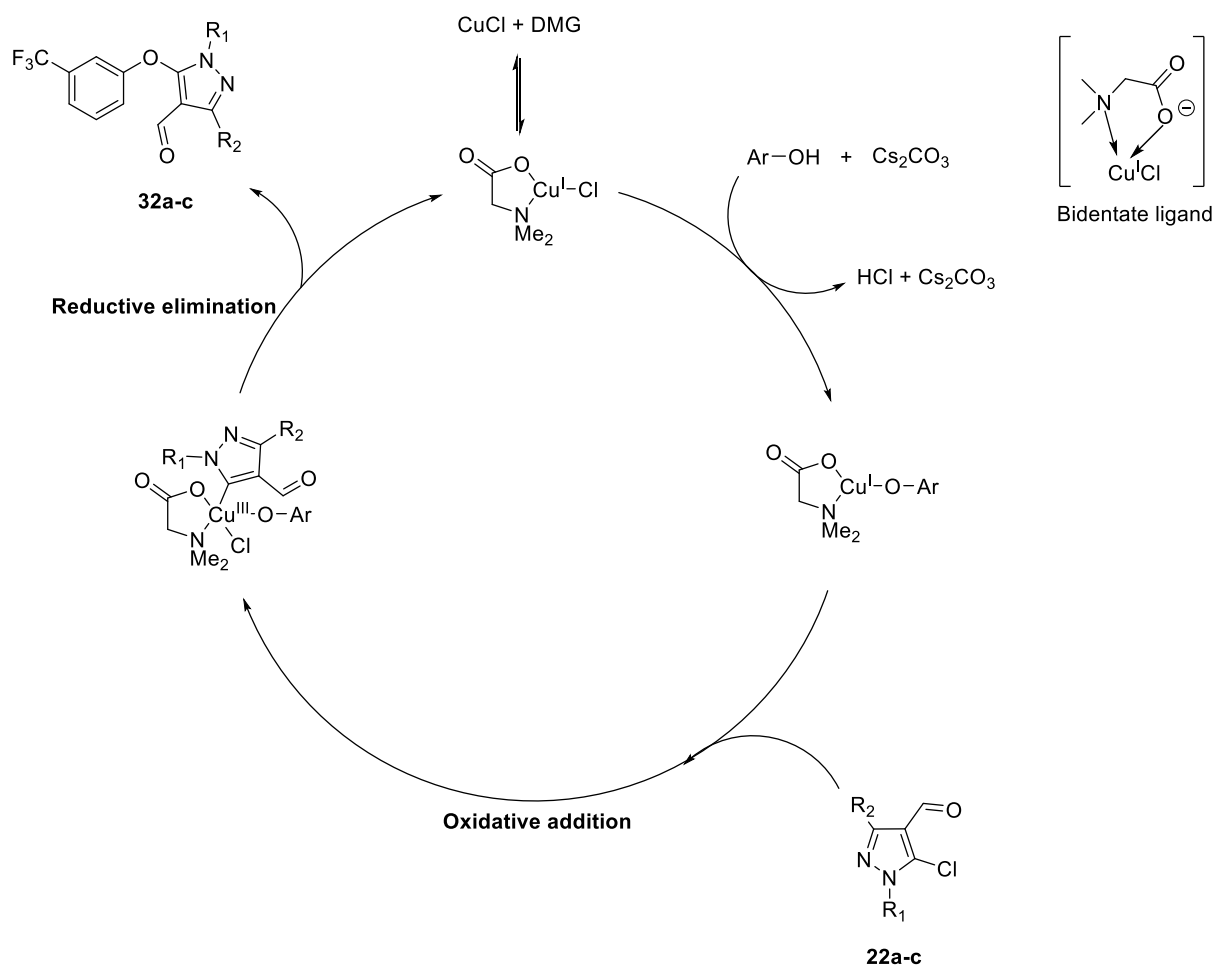


Figure 109. Plausible mechanism for the Ullmann copper-catalysed coupling reaction for compounds **32a-c**.

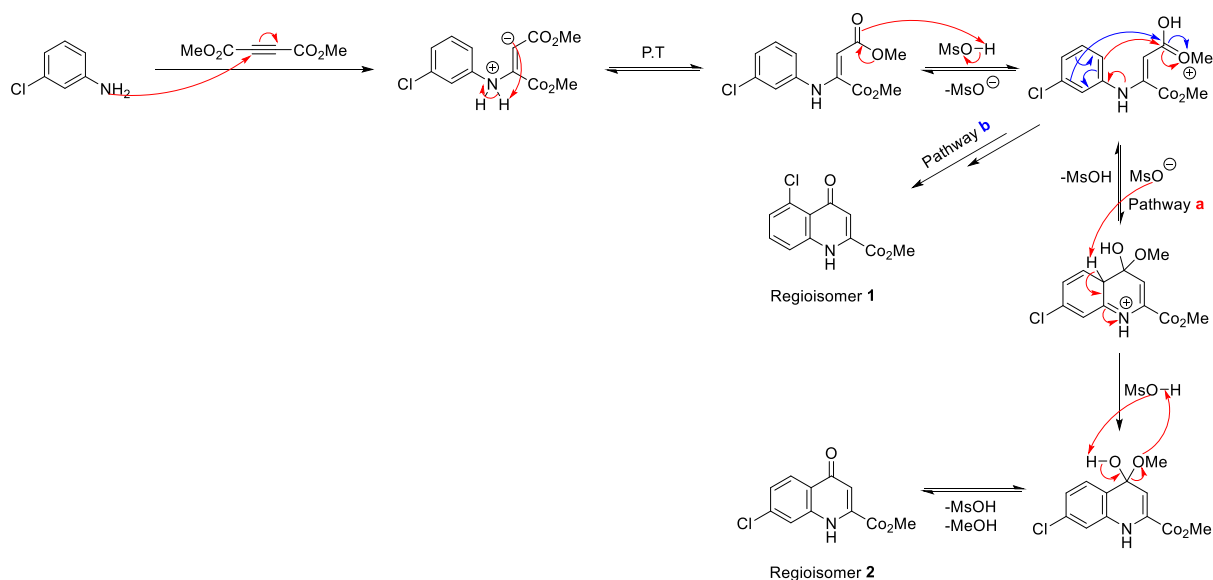


Figure 110. Plausible mechanism for the synthesis of compound **30a-b**. Pathway a = red, b = blue.

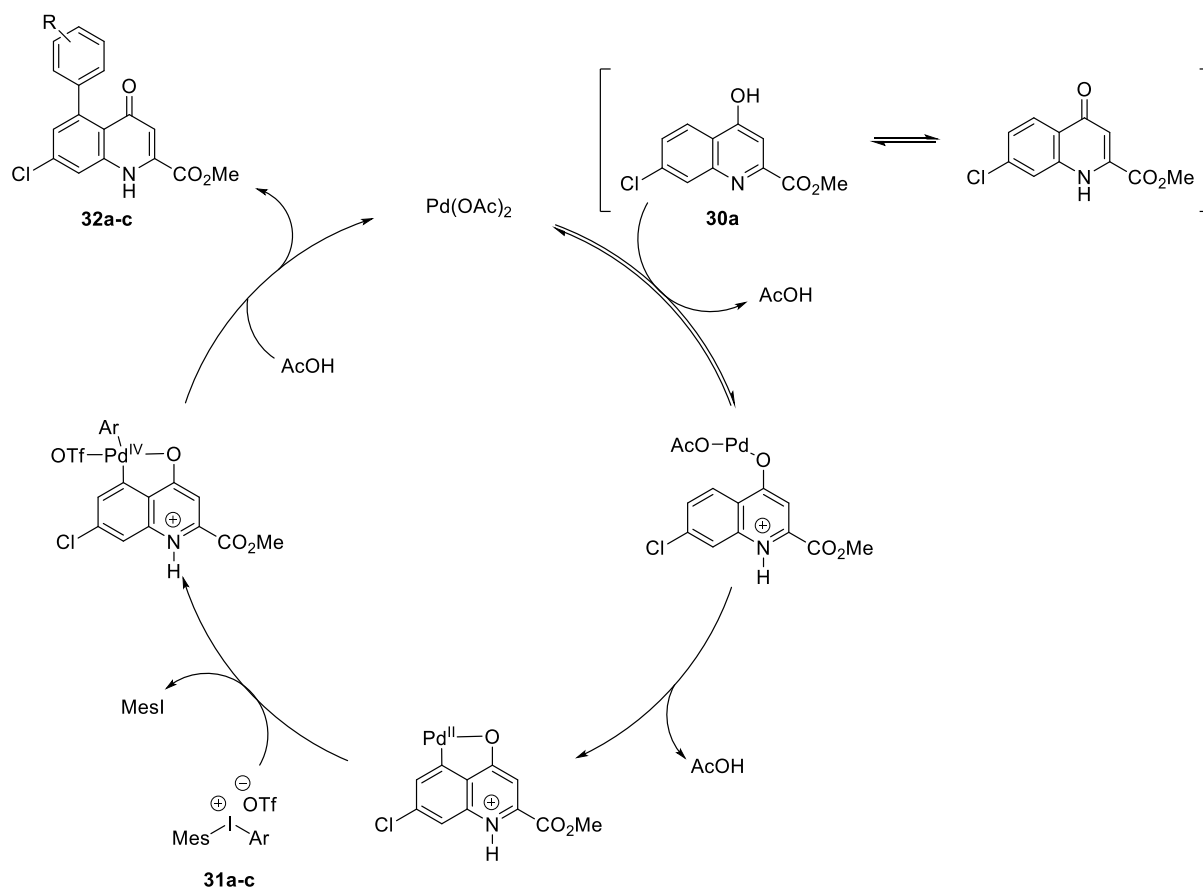


Figure 111. Plausible mechanism for the synthesis of compound **32a-c** [39].

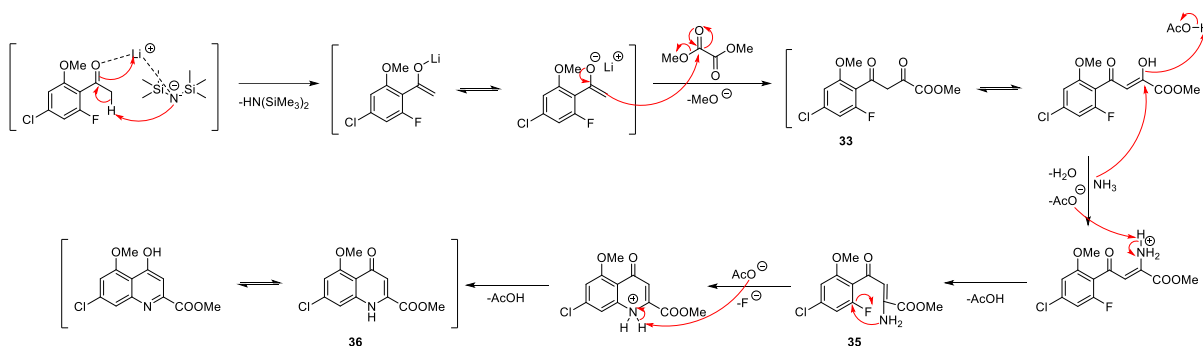


Figure 112. Plausible mechanism for the synthesis of compound **36**.