Synthesis of (*R*)-Roscovitine Analogs as Potential Treatments for Lambert-Eaton Myasthenic Syndrome

by

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Synthesis of (*R*)-Roscovitine Analogs as Potential Treatments for Lambert-Eaton Myasthenic Syndrome

Michael Frasso, M.S.

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This thesis describes the preparation of pyrazolo[1-5-*a*]-1,3,5-triazine analogs of the Ca^{2+} channel agonist and cyclin-dependant kinase inhibitor (*R*)-roscovitine. Initially, efforts to improve the published synthetic route to these analogs were investigated. Subsequently, several analogs with the pyrazolotriazine core were prepared and evaluated for agonist properties. Preliminary results suggest that this modified core retains and in some cases improves agonist behavior. A substantial increase in both the Ca^{2+} tail-current integral and the decay time constant tau were observed with the most active analogs **MF-06** and **MF-17**. Modifications to the C-8 alkyl and C-2 aminobutanol sidechains did not improve agonist activity.

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LIST OF ABBREVIATIONS

3,4-DAP 3,4-diaminopyridine

ACh .....acetylcholine

aq.....aqueous

ATR.....attenuated total reflectance

Bn.....benzyl

bs.....broad singlet

calcd .....calculated

cdk.....cyclin-dependant kinase

conc .....concentrated

d.....doublet

dd.....doublet of doublets

DIPEA.....*N*,*N*-diisopropylethylamine

DMAP .....*N*,*N*-dimethylaminopyridine

DMSO .....dimethylsulfoxide

dq.....doublet of quartets

eq.....equivalent(s)

ESI.....electrospray ionization

Et .....ethyl

HCCP .....hexachlorocyclophosphazene

HRMS .....high resolution mass spectroscopy

HVA.....high-voltage activated

IR.....infrared spectroscopy

LEMS.....Lambert-Eaton myasthenic syndrome

LVA .....low-voltage activated

m .....multiplet

m-CPBA.....*meta*-chloroperbenzoic acid

Me .....methyl

mp .....melting point

NMR .....nuclear magnetic resonance

$POCl_3$ .....phosphorus oxychloride

PTSA.....*para*-toluenesulfonic acid

PyBOP .....(benzotriazole-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

PyBrOP .....bromotripyrrolidinophosphonium hexafluorophosphate

q.....quartet

RIM.....Rab3 interacting molecule

RIM-BP.....RIM binding protein

rt .....room temperature

s .....singlet

SAR.....structure-activity relationship

sat .....saturated

sm.....starting material

SV .....synaptic vesicle

Syt .....synaptotagmin

t .....triplet

TFA .....trifluoroacetic acid

TFFH.....fluoro-*N,N,N′,N′*-tetramethylformamidinium hexafluorophosphate

THF .....tetrahydrofuran

TLC .....thin layer chromatography

VGCC .....voltage-gated Ca^{2+} channel

PREFACE

First, I would like to thank Professor Peter Wipf for granting me the opportunity to work in his laboratory and for being flexible with my somewhat unusual circumstances. I would also like to thank Dr. Seth Horne and Dr. Kazunori Koide for serving on my committee and the University of Pittsburgh for its financial support.

I would also like to thank current and past Wipf group members for their assistance, with a special thanks to Joe Salamoun and John Milligan for their companionship. Finally, I would like to extend my gratitude to my parents for their support through all of my academic endeavors and my fiancée Molly for her unabiding love and encouragement.

1.0 SYNTHESIS OF (*R*)-ROSCOVITINE ANALOGS AS POTENTIAL TREATMENTS FOR LAMBERT-EATON MYASTHENIC SYNDROME

1.1 INTRODUCTION

1.1.1. Synaptic Vesicle Release at the Neuromuscular Junction

Disorders of the neuromuscular junction (NMJ) affect approximately 20 per 100,000 people. The true occurrence rate is difficult to determine due to lack of rigorous diagnosis. These diseases decrease the quality of life for affected individuals by compromising muscle strength. Myasthenia gravis and Lambert-Eaton myasthenic syndrome (LEMS) are the most common NMJ disorders, affecting neurotransmitter reception and Ca^{2+} flow, respectively. Normal signal transmission at the skeletal NMJ is triggered by axon terminal depolarization, during which voltage-gated calcium channels (VGCCs) permit the influx of Ca^{2+}. This activates synaptic vesicle (SVs) binding to the axon terminal membrane and subsequent release of acetylcholine (ACh).

Due to the rapidity with which transmitter release takes place, SVs must already be docked near the axon terminal membrane at the time of Ca^{2+} influx.[1] Prior to Ca^{2+} entry, VGCCs must be recruited to the "active zone" where transmitter release takes place (Figure 1). Rab3 and Rab27 are vesicle membrane-bound GTP-binding proteins. These proteins bind to Rab3-

interacting molecule (RIM), which forms the vital tether between SV and VGCC. Ca^{2+} channel recruitment is aided by RIM-BP, which binds to both RIM and the channel. Absence of either RIM of RIM-BP results in decreased Ca^{2+} influx at active zones, probably due to decreased localization of the requisite channels.[2-4] RIM also binds an additional priming factor, Munc-13, which promotes SNARE complex assembly by altering the conformation of syntaxin-1 (*vide infra*).[5]

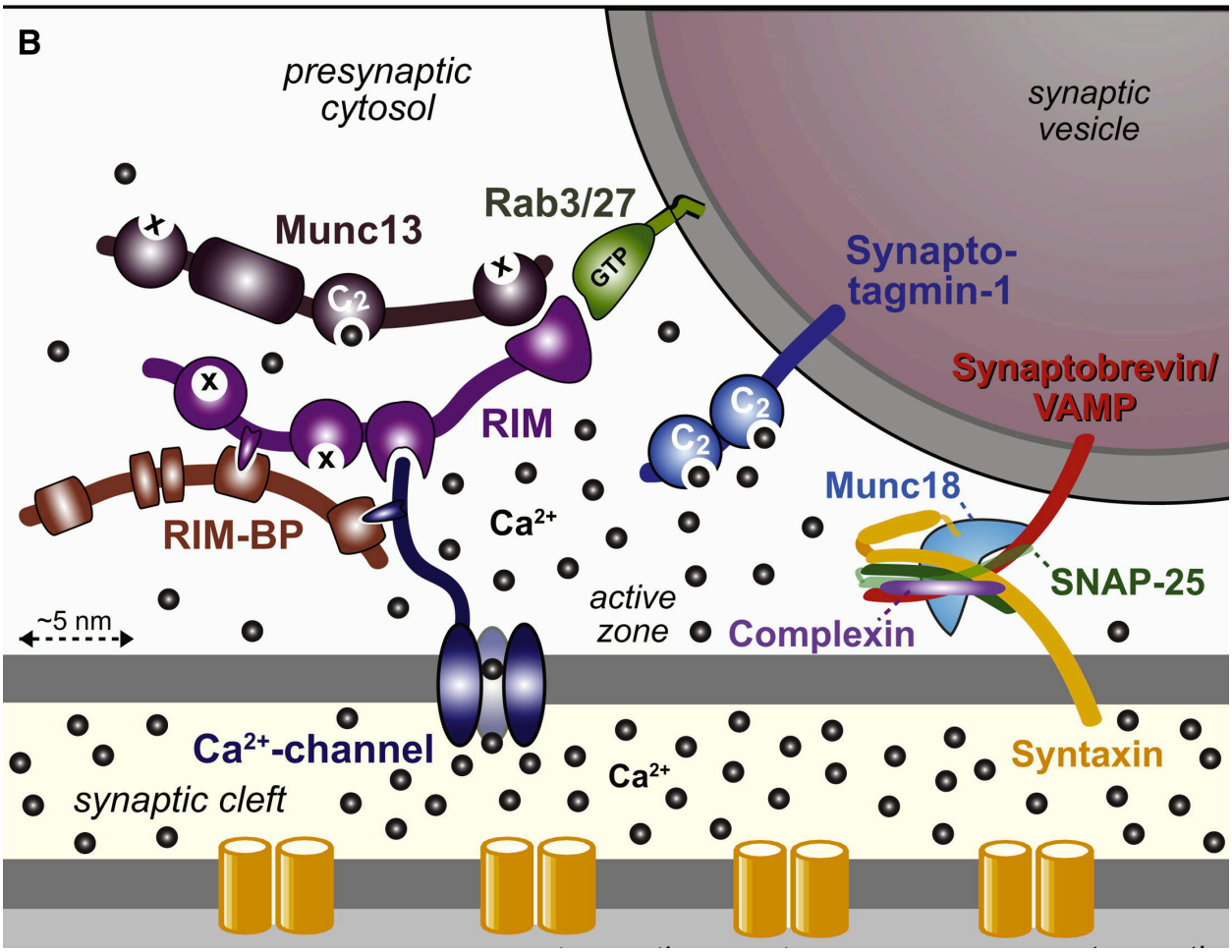


Figure 1: Proteins involved in neurotransmitter release at axon terminal active zones.[6] Reproduced with permission from Elsevier.

Additionally, the SV is tethered by the soluble *N*-ethylmaleimide sensitive fusion protein (NSF) attachment receptor protein (SNARE) complex (synaptobrevin/VAMP, syntaxin-1, and

SNAP-25), as well as Munc-18. Plasma membrane-bound syntaxin-1 in its closed conformation is initially bound to Munc-18. Munc-13 then promotes a conformational change in syntaxin-1 to the open state, which triggers SNAP-25 association, as well as interactions with synaptobrevin/VAMP. This action pulls the SV in close proximity to the plasma membrane.[7]

Upon entry of Ca^{2+} into the synaptic terminal, vesicular membrane-bound synaptotagmin-1 (Syt-1) is thought to act as a Ca^{2+} sensor and mediates fusion pore opening of the docked SVs. This may occur as a result of a conformational change that places the two membranes in close proximity.[8] Deletion of the Syt-1 gene has been shown to decouple rapid exocytosis from Ca^{2+} entry.[9-11] Additionally, complexin has also been shown to be important in this process, but its exact role has not been determined.[6]

1.1.2 Voltage-Gated Calcium Channels: Structure and Subtypes

The previous discussion shows that Ca^{2+} influx is the key event that triggers SV exocytosis. The channels responsible for this usually contain four major subunits, namely α_1, $\alpha_2\delta$, β, and γ (Figure 2). The α_1 subunit forms the central pore of the complex and is the primary determinate of the channel's properties. The other subunits all function to fine-tune the activation and deactivation kinetics, with the β subunit having the most substantial effect.[12,13] The α_1 subunit is comprised of four homologous domains with six membrane-spanning helices each. The fourth in each contains several positively charged arginine and lysine residues and is thus thought to be responsible for voltage sensing. The loop connecting the fifth and sixth transmembrane helix in each domain lines the interior of the pore and is thought to confer ion selectivity.[13,14] Intriguingly, three amino acid mutations in this region from a closely related Na^+ channel alters the ion selectivity from Na^+ to Ca^{2+}.[15,16]

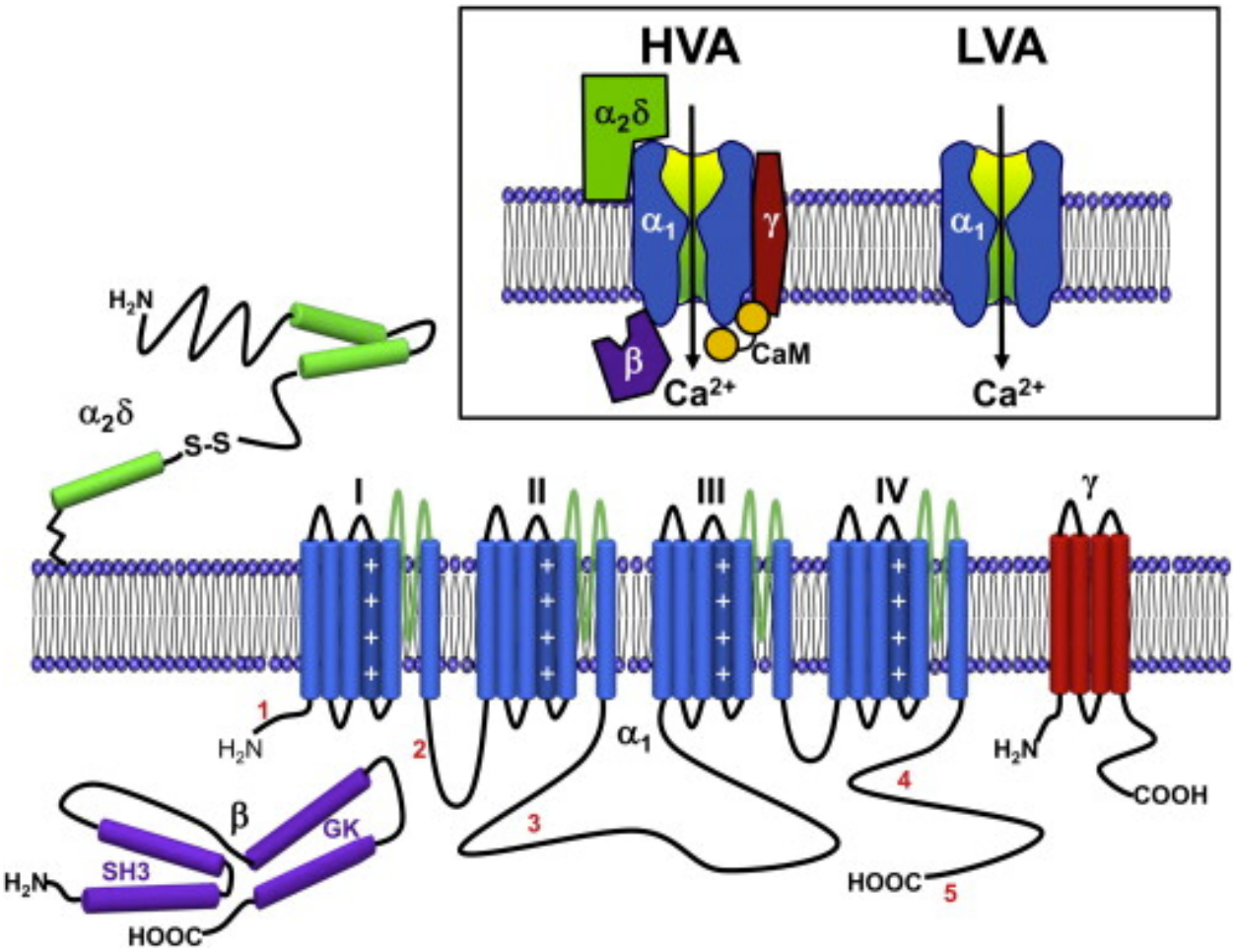


Figure 2: (Top) Schematic representation of high-voltage activate (HVA) and low-voltage activated (LVA) VGCCs. (Bottom) A more detailed view of the HVA channel.[13] Reproduced with permission from Elsevier.

At least ten different α_1 subunits have been identified to date and it is this variability that leads to the different VGCC subtypes.[14] There are two broad categories of VGCCs based on the requisite departure from the resting membrane potential. High voltage activated (HVA) channels require large departures from the resting membrane potential (from -70 mV to 30-50 mV), whereas low voltage activated (LVA) channels require much smaller perturbations for activation (-70 mV to -55 mV to -20 mV).[17] Each of these categories can then be subdivided into smaller groups as well. HVAs channel subtypes include L-, R-, N-, and P/Q-types. L- and R-type

channels are not thought to play a prominent role in synaptic transmission at adult NMJs, but have been implicated in vesicle release in developing muscle and memory, respectively.[18-20]

N-type channels are primarily found in neuronal tissue, particularly in dendrites and synaptic terminals.[21-23] Their localization suggests a role in neurotransmitter release, which is indeed the case at some synapses, mostly in the central nervous system.[24] Inhibition of these channels has been of interest, particularly in the treatment of neuropathic and chronic pain.[25,26] While their role in neurotransmitter release at the amphibian NMJ has been demonstrated,[27,28] they do not appear to facilitate the same process in mammals.[20]

P/Q-type channels are closely related to N-type channels and can be found in many of the same areas, and mice lacking the requisite gene suffer from various forms of ataxia.[13] These channels are primarily responsible for the release of neurotransmitters in the mammalian NMJ[20,29] and are thus of interest in the treatment of certain disorders, in particular LEMS.

1.1.3 Lambert-Eaton Myasthenic Syndrome

LEMS is an autoimmune disorder of the NMJ that results from an antibody mediated removal of presynaptic P/Q-type VGCCs.[20] This removal effectively decreases the Ca^{2+} concentration in the axon terminal following depolarization, and thus decreases ACh release. Consequently, patients typically exhibit muscle weakness, particularly proximal leg weakness and fatigability as the most common early symptoms. Dry mouth, impotence in men, and various other autonomic abnormalities can occur as later symptoms,[30,31] as well as oculobulbar (muscles of the eye and eyelid) weakness that commonly occurs in the related myasthenia gravis.[31,32] An upregulation of N- and L-type channels is also observed at affected NMJs, and this may be the reason that the

effects of LEMS are not more severe.[33] Repeated stimulation partially restores muscle function, due to the presumed accumulation of Ca^{2+} through the reduced number of channels.[34]

The first recorded case of LEMS was reported in 1953 by Anderson[35] in association with a lung tumor, and subsequently, it has been found that over half of the diagnosed cases appear as a paraneoplastic syndrome of small-cell lung carcinomas.[36] The majority of remaining cases are idiopathic in nature, and less commonly the disorder has been associated with other types of cancer.[30]

There is no cure for LEMS and treatment options are both limited and symptomatic only. The first strategy is to treat the tumor in relevant cases as this is a more serious threat to the patient's health and can provide symptomatic relief.[34,37,38] As LEMS is an autoimmune disorder, immunomodulation of various types can be used as well. Plasmapherisis (separating of patient's plasma from other blood components and replacing it with other plasma) and administration of immunoglobulin intravenously have been shown to be effective in a number of cases though these have obvious disadvantages such as inconvenience to the patient and limited effectiveness.[31,39,40] Oral dosage of prednisolone (Figure 3) has also been found to be effective.[31,41] Even with these treatments, impaired muscle function persists.[34]

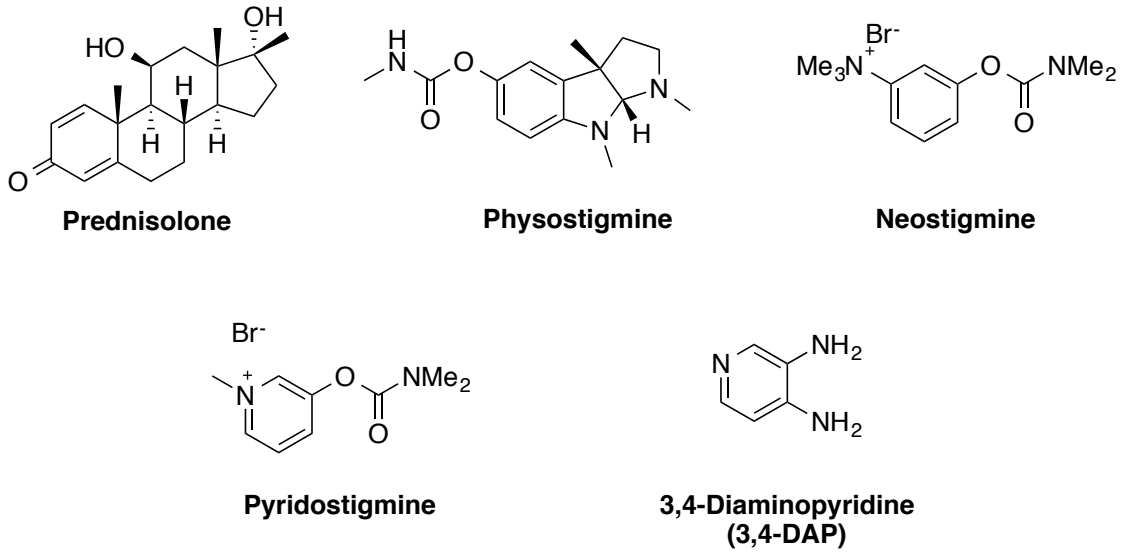


Figure 3: Structures of LEMS pharmaceuticals.

Blocking neurotransmitter degradation through acetyl cholinesterase inhibitors has also been used in the treatment of LEMS. Physostigmine, neostigmine and pyridostigmine (Figure 3) have been used most frequently, but this therapy seems to be rather ineffective if used alone. When used in combination with 3,4-diaminopyridine (3,4-DAP) (*vide infra*) (Figure 3), effectiveness is increased, but improvement is modest at best.[31]

The final and most widely successful treatment option is increasing neurotransmitter release. This has been achieved indirectly with K^+ channel blockers. A decrease in channel activity allows for prolonged axon terminal depolarization, thus longer VGCC channel open times and a larger influx of Ca^{2+}, resulting in increased neurotransmitter release.[42,43] Guanidine has been used but a low dose limit and a wide variety of side effects including bone marrow suppression and renal failure have prevented its widespread use.[44,45] The most successful treatment in this category is 3,4-DAP. At higher dosages, 3,4-DAP shows side effects including parethesia (numbness or "pins and needles" sensation), insomnia, and occasionally, seizures.[31,46] 3,4-DAP is currently available through a free distribution program, but this has been endangered

by clinical trials undertaken by a company that is pursuing patent protection. This has raised concerns about future affordability of the treatment for patients.[47]

1.1.4 Calcium Channel Agonists as Potential Therapeutics

One potential therapeutic strategy that has not yet been exploited is the direct activation of VGCCs. Since LEMS patients have fewer P/Q-type calcium channels at the NMJ, increasing flux through the remaining channels may mimic having a normal number of channels and reduce muscle weakness. (*R*)-Roscovitine (Figure 4) was initially developed as a cyclin-dependent kinase (cdk) inhibitor,[48] and is currently in various clinical trials as a cancer therapeutic. (*R*)-Roscovitine also shows mild VGCC agonist activity, specifically towards P/Q- and N-type channels, that is unrelated to its cdk inhibition properties.[49-51] It should also be noted that mild VGCC deactivation behavior has been observed, presumably due to a less favored binding to the closed state of the channel. Since these channels are primarily responsible for neurotransmitter release, (*R*)-roscovitine is a good lead for further development. As cdk inhibition would cause undesired side effects if (*R*)-roscovitine were used to treat LEMS, our group aims to develop analogs that would display both decreased cdk inhibition and increased VGCC agonist activity.[52]

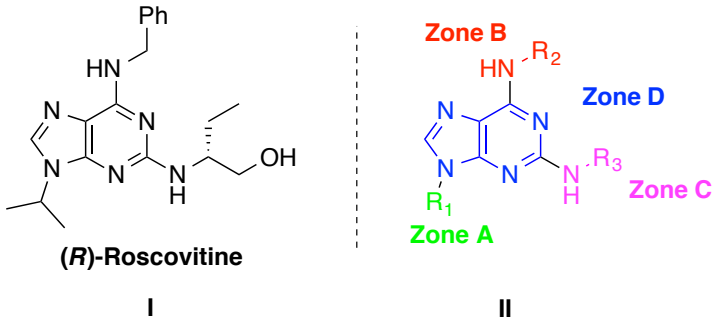


Figure 4: I) Structure of parent compound (*R*)-roscovitine. II) Zones for modification of parent compound.

Initially, zones A and B (Figure 4) were selected for modification. Only conservative modifications were tolerated in zone A, with many derivatives becoming too insoluble to test as the size of the substitution was increased. Subtle modification of the isopropyl group to the *n*-propyl group in zone A gave a 22-fold decrease in cdk 2 inhibition (Fig 5, **KB-08**), presumably due to greater flexibility, while giving a 2-fold increase in N-type channel agonist activity. Various alkyl and aryl replacements of the phenyl group in zone B with the exception of the (2-methyl)-5-thiophenyl derivative **ML-50** (Figure 5) completely abolished N-type channel agonist activity. Further modification in zone A showed the importance of the isopropyl group in cdk 2 inhibition, with methylated analog **GV-05** (Fig. 5) showing an 11–fold drop in activity compared to **ML-50**, although a 2-fold drop in N-type channel agonist activity was also observed. Ultimately, **GV-58** was found to be the most effective analog with a 22-fold decrease in cdk 2 inhibition and a 4-fold increase in N-type channel agonist activity. Additionally, **GV-58** was found to restore partial function at a LEMS passive transfer mouse model NMJ and to have a synergistic effect with 3,4-DAP.[53,54] For this reason, **GV-58** was selected for further development

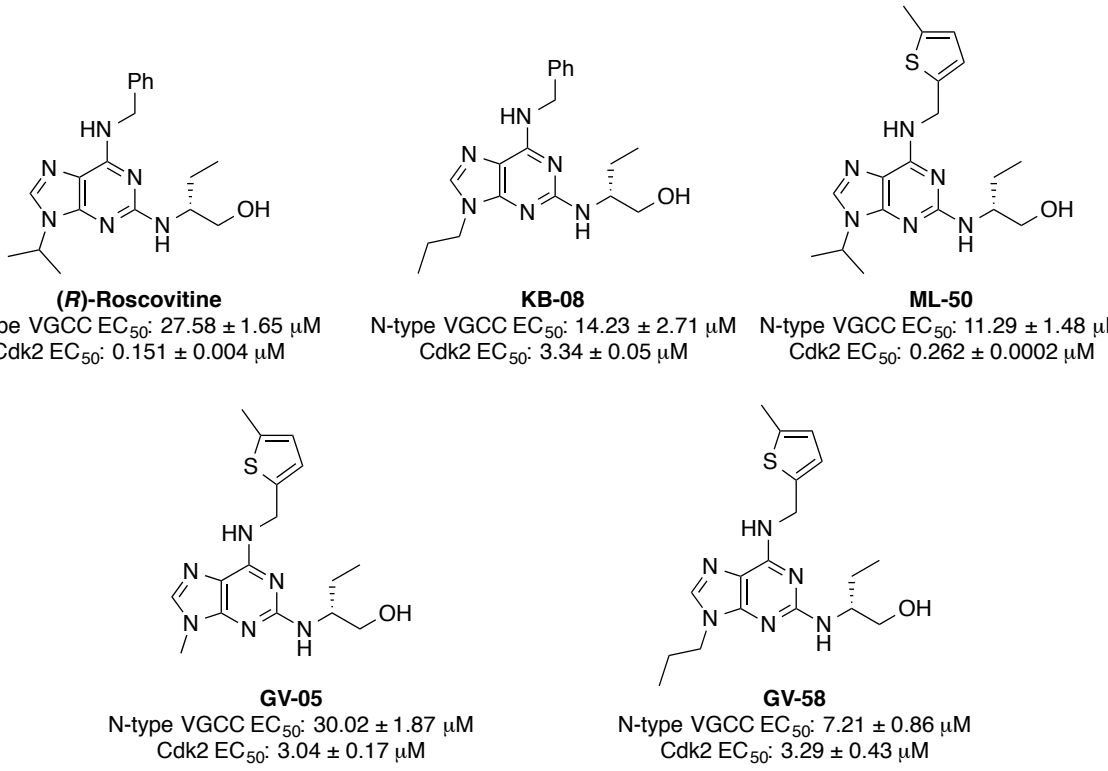


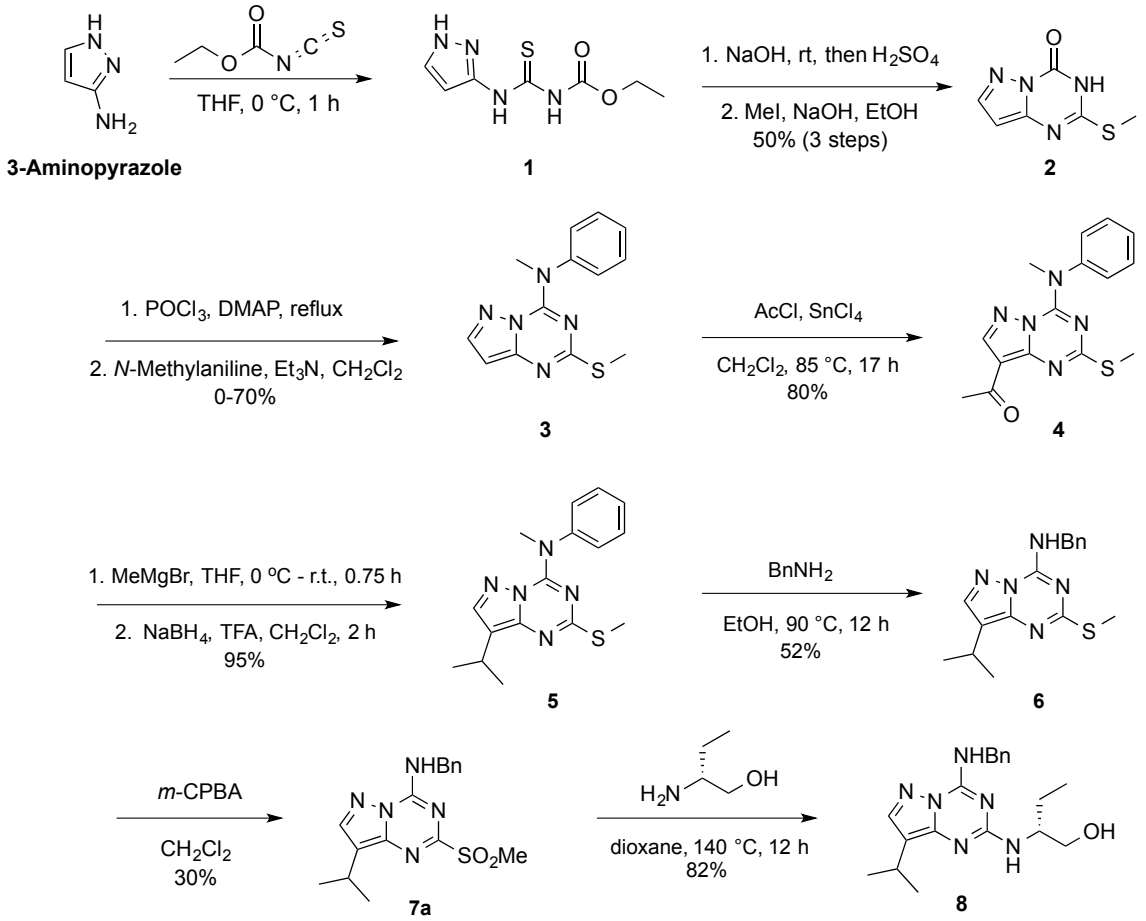
Figure 5: Structures of (*R*)-roscovitine and important previous analogs.[52]

1.2 RESULTS AND DISCUSSION

1.2.1 Chemistry

The pyrazolo[1,5-*a*]-1,3,5-triazine core has been shown to have comparable cdk inhibition properties in (*R*)-roscovitine analogs,[55] but its Ca^{2+} agonist properties are unknown. Since this scaffold has been shown to be a good bioisostere of the purine scaffold of (*R*)-roscovitine,[55,56] we elected to pursue it for structure-activity relationship (SAR) studies. Scheme 1 shows the published method for preparing a modified (*R*)-roscovitine analog.[55] Addition of 3-aminopyrazole into ethoxycarbonyl isothiocyanate gave thiourea **1** which was immediately

cyclized under basic conditions, then methylated with methyl iodide to intermediate **2**. The aniline activating group was installed via a phosphorus oxychloride ($POCl_3$) mediated chlorination followed by aminolysis (**3**). Friedel-Crafts acylation, Grignard addition to the resulting ketone, and a deoxygenation with $NaBH_4$/trifluoroacetic acid (TFA) allowed for the installation of the isopropyl moiety in compound **5**. Displacement of the aniline with benzylamine in ethanol at 90 ^oC, oxidation to the sulfone, and aminolysis gave the desired pyrazolo[1,5-*a*]-1,3,5-triazine analog of (*R*)-roscovitine (**8**).

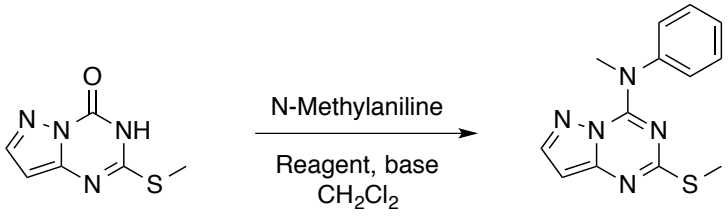


Scheme 1: Published preparation of a pyrazolo[1,5-a]-1,3,5-triazine analog of (*R*)-roscovitine.[55]

While preparing **8**, several weaknesses in the synthetic route became apparent. First, the preparation of **3** was found to give highly variable results, with yields ranging from 0-70%,

possibly due to the instability of the intermediate chloride. In order to circumvent this issue, a direct preparation of **3** was investigated. Various peptide coupling reagents were tested (Table 1). PyBOP, PyBrOP, and TFFH with Et_3N gave little to no conversion (entries 1-3). Changing the base to N,N-dimethylaminopyridine (DMAP) with PyBrOP showed slightly improved results (entry 4). Hexachlorocyclophospazene (HCCP) has been shown to be effective in similar systems,[57] and upon treating **2** with HCCP and *N*,*N*-diisopropylethylamine (DIPEA) in acetonitrile, a 65% yield of **3** was obtained (entry 5). Changing the base to DMAP did not improve the yield. The conditions in entry 5 were selected for further use and proved to be more reliable and operationally simple than the original conditions, as handling of large quantities of $POCl_3$ was not required.

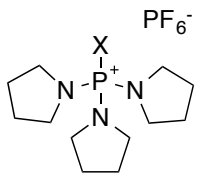
Table 1: Coupling reagents and conditions screen

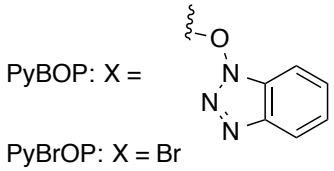


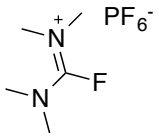
2 **3**

Entry	Reagent	Base	Yield (%)
1	PyBOP	Et_3N	trace
2	PyBrOP	Et_3N	trace
3	TFFH	Et_3N	0
4	PyBrOP	DMAP	15
5	HCCP	DIPEA	65[a]
6	HCCP	DMAP	47[a]

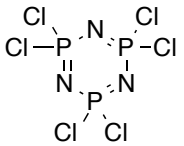
[a] MeCN used as solvent







TFHH



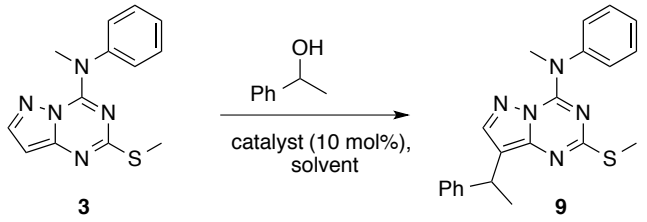
HCCP

The oxidation of **6** to the intermediate sulfone was also found to be inefficient, giving only a 30% yield using *m*-CPBA (Scheme 1). Altering the conditions to Oxone® with sodium

bicarbonate in acetone/water[58] gave the desired sulfone as the only product. This material could be used in the following aminolysis after a simple aqueous workup. Subsequently, it was found that the sodium bicarbonate was unnecessary.

The installation of the alkyl sidechain in zone A was targeted as the final area for improving the synthesis, as in the published process this requires three steps (Scheme 1). The previously used Friedel-Crafts acylation was high yielding, so a modified procedure leading directly to alkylated products was chosen. It had been shown that standard Friedel-Crafts alkylations using alkyl halides were ineffective,[55] therfore methods using alcohols were investigated. The first conditions used were 1,1-phenylethanol and *p*-toluenesulfonic acid (PTSA) in dichoromethane at 60 ^{o}C[59] and showed a trace of desired product **9** (Table 2, entry 1). Stochiometric Lewis acid conditions with $BF_3 \cdot Et_2O$ at 0 ^{o}C afforded none of the desired material (entry 2).[60] Catalytic Lewis acid processes using 1 mol% of $La(OTf)_3$ or $Sc(OTf)_3$ in nitromethane at 100 ^{o}C[60] showed slightly better results, with $Sc(OTf)_3$ being superior (entries 3-4). Increasing the catalyst loading to 10 mol% showed dramatically improved results, giving a 60% isolated yield (entry 5). Further increasing the catalyst loading to 20 mol% offered no improvement and increasing the amount of alcohol or changing the solvent resulted in decreased yield (entries 6-10).

Table 2: Conditions screen for Friedel-Crafts reaction.



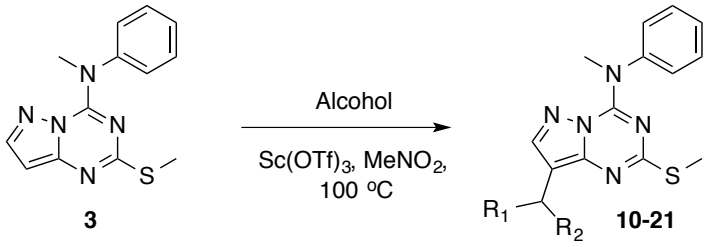
3 **9**

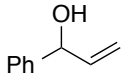
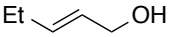
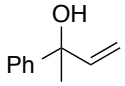
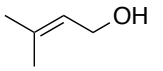
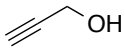
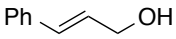
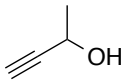
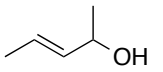
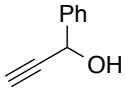
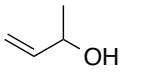
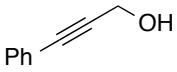
Entry	Catalyst	Temp (°C)	Solvent	Yield (%)	Entry	Catalyst	Temp (°C)	Solvent	Yield (%)
1	PTSA[a]	60	CH_2Cl_2	trace	6	$Sc(OTf)_3$	100	$MeNO_2$	----[c]
2	$BF_3{\cdot}Et_2O$[a]	0	CH_2Cl_2	0	7	$Sc(OTf)_3$	100	$MeNO_2$	33[d]
3	$La(OTf)_3$[b]	100	$MeNO_2$	5	8	$Sc(OTf)_3$	100	DCE	trace
4	$Sc(OTf)_3$[b]	100	$MeNO_2$	11	9	$Sc(OTf)_3$	100	MeCN	20
5	$Sc(OTf)_3$	100	$MeNO_2$	60					

[a] 100 mol%; [b] 1 mol%; [c] 20 mol% $Sc(OTf)_3$ conversion by ^1H NMR as previous entry; [d] 200 mol% alcohol.

With optimized conditions identified, we sought to explore the scope of the transformation (Table 3). Isopropanol showed no trace of product **10** even after an extended reaction time, probably due to lack of an adequately stabilized carbocation (entry 1). In an effort to increase intermediate carbocation stability, primary allylic alcohols were used and also proved to be ineffective, even with the added stabilization of a phenyl ring (entries 2-4). However, a secondary allylic alcohol gave a 53% yield of product **14** (entry 5). Surprisingly, two additional secondary allylic alcohols gave only trace amounts of product, with a phenyl ring again failing to aid the reaction (entries 6-7). A tertiary allylic alcohol (entry 8) and various propargylic alcohols failed as well (entries 9-12).

Table 3: Scope of Friedel-Crafts alkylation.

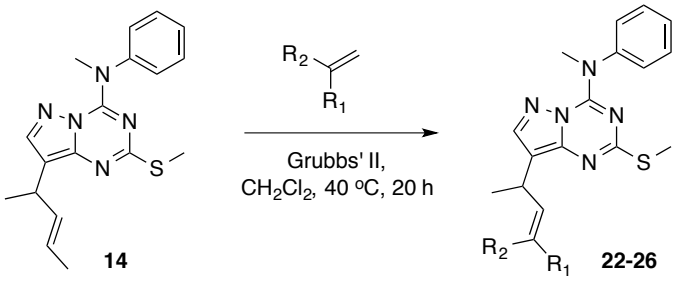


Entry	Alcohol	Product	Yield (%)	Entry	Alcohol	Product	Yield (%)
1		**10**	0	7		**16**	trace
2		**11**	trace	8		**17**	21
3		**12**	trace	9		**18**	0
4		**13**	5	10		**19**	0
5		**14**	53	11		**20**	26
6		**15**	trace	12		**21**	0

Since compound **14** could be prepared in moderate yield, we decided to investigate a cross metathesis to expand access to analogs at the 8-position (Table 4). A pendant double bond in this position following cross metathesis would allow for the preparation of several different analogs from a single starting material via hydrogenation, dihydroxylation, or epoxidation followed by nucleophilic ring opening. Unsaturated ring analogs have not been explored to date, so methylenecyclohexane was used in the initial trial with Grubbs second-generation catalyst in dichloromethane at 40 ^{o}C. (entry 1) No reaction was observed. Since 1,1-disubstituted alkenes

are known to be more difficult substrates for cross metathesis reactions,[61] simple terminal alkenes were used in further experiments to find optimal conditions. First, 1-hexene was used and showed some conversion under the same conditions (entry 2), but products were inseparable from the starting material. In order to both aid in product separation and remove the concern that 1-hexene evaporates during the reaction, 1-octene was used (b.p. ~60 ^{o}C vs. ~120 ^{o}C), but this gave similar results (entry 3). Ethylene gave no reaction (entry 4).

Table 4: Cross metathesis with **14**.

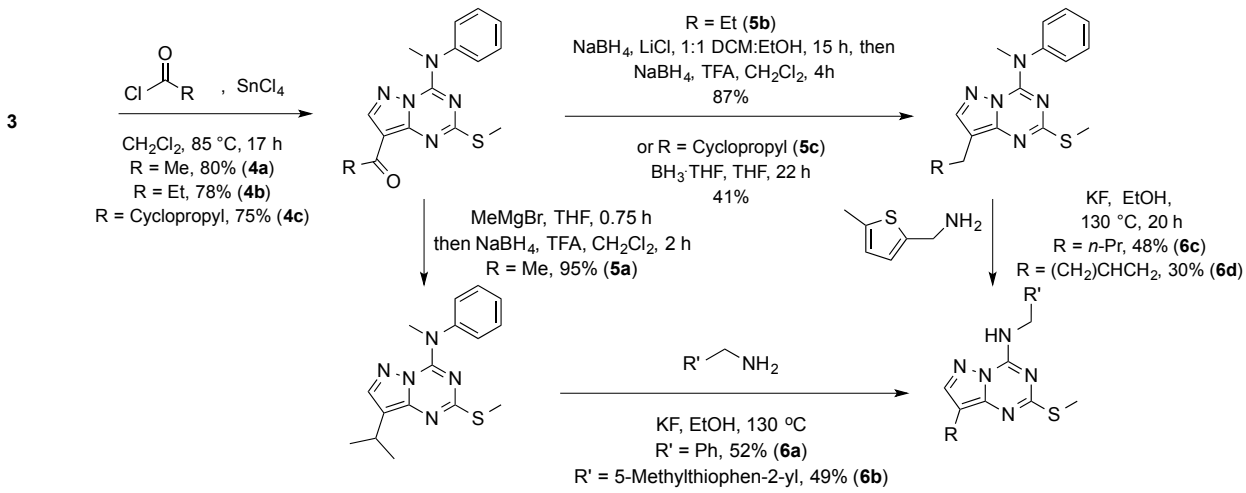


Entry	Alkene	Product	Yield (%)	Entry	Alkene	Product	Yield (%)
1		**22**	0	1	1-octene	**25**	----[a,b]
2	1-hexene	**23**	----[a]	2	ethylene	**26**	0
3	1-octene	**24**	----[a]				

[a] products could not be separated. [b] 75 ^{o}C, μW, 2 h.

It was hypothesized that the thioether was poisoning the catalyst, so similar cross metathesis reaction were performed with the corresponding sulfone (prepared using previously established oxidation conditions with Oxone®, *vide supra*). No significantly improved results were obtained. Due to these difficulties, cross metathesis efforts were abandoned and the original method for installing the alkyl sidechain was used in the analog synthesis.

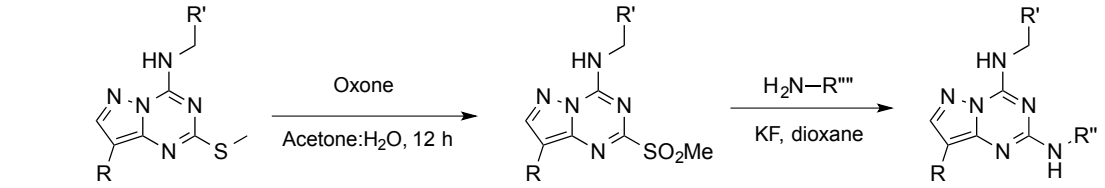
The final route toward pyrazolo[1,5-*a*]-1,3,5-triazine analogs can be seen in Scheme 2. The aniline activating group was installed by the modified HCCP coupling procedure giving **3** (Scheme 1) in good yield. Friedel-Crafts acylation was then performed using one of three acyl chlorides in good yields (75-80%). Reduction of ketones **4b** and **4c** proved to be unexpectedly difficult. Using TFA/$NaBH_4$ gave only 40% of **5b**, even after extended reaction time. It was noted that none of the intermediate alcohol was observed, suggesting that ketone reduction is sluggish in this system. Partial reduction of ketone **4b** using $LiBH_4$ generated *in-situ* followed by deoxygenation with TFA/$NaBH_4$ gave the desired product **5b** in 87% yield. Reduction of ketone **4c** proved to be even more challenging. The above method for reducing **4b** was found to be ineffective, giving only trace amounts of the desired product. Standard conditions using $LiAlH_4$ or $LiHBEt_3$ in THF or Wolff-Kishner reductions prompted decomposition. Luche reduction conditions gave recovered starting material, probably due to poor solubility of the starting material. Similarly, reductions with $LiAlH(OtBu)_3$ in THF or Et_3SiH in TFA were unsuccessful and also returned starting material. Finally, it was found that reduction with BH_3·THF gave an acceptable 41% yield of **5c**. Displacement of the aniline activating group with the desired amine gave compounds **6a-6d** in moderate yield.



Scheme 2: Preparation of intermediates **6a-6d**.

Oxidation of the thioethers **6a-6d** with Oxone® in acetone/water followed by aminolysis gave the desired final products in moderate to good yields (Table 5).

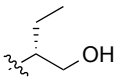
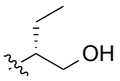
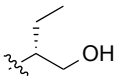
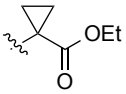
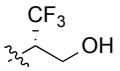
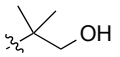
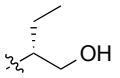
Table 5: Preparation of screening samples.



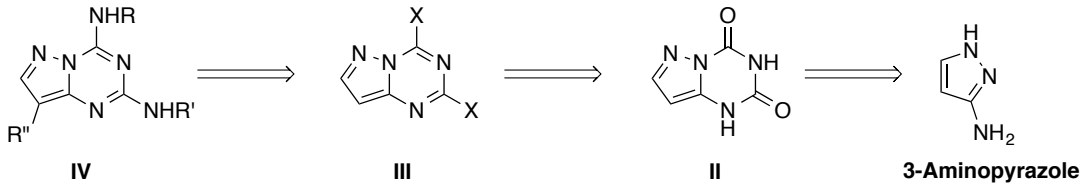
6a-6d
R = *i*-Pr, *n*-Pr, $(CH_2)_2CH_2$
R = Ph, 5-Methylthiophen-2-yl

7a-7d

8, MF-XX

Entry	Product	R	R'	R''	Temp (°C)	Yield (%)
1	**8**	*i*-Pr	Ph		140	82
2	**MF-06**	*i*-Pr	5-Methylthiophen-2-yl		140	68
3	**MF-17**	*n*-Pr	5-Methylthiophen-2-yl		140	57
4	**MF-27**	*n*-Pr	5-Methylthiophen-2-yl		140	11
5	**MF-59**	*n*-Pr	5-Methylthiophen-2-yl		120	43
6	**MF-21**	*n*-Pr	5-Methylthiophen-2-yl		120	57
7	**MF-71**	$(CH_2)_2CH_2$	5-Methylthiophen-2-yl		120	27

Future synthetic endeavors may be directed towards simplifying the synthesis in a different manner (Scheme 3). The two amine sidechains could be installed selectively with the same activating group at the 2- and 4-positions due to the difference in reactivity. This could potentially bypass the aniline activating group and the sulfur methylation/oxidation steps of the current synthetic route. The activation could be performed by either dichlorination or dimethylation of **II**, which can be prepared from the addition of the requisite 3-aminopyrazole into ethoxycarbonyl isocyanate followed by cyclization.



Scheme 3: Retrosynthesis for potential future synthetic route.

1.2.2 Biological Results

Preliminary results indicate that the pyrazolo[1,5-*a*]-1,3,5-triazine scaffold is beneficial for increasing Ca^{2+} current through N-type channels. Analogs were tested in whole-cell perforated patch-clamp assays of tsA-201 cells expressing N-type channels.[51,53] Cells were stimulated at +60 mV and tail-current integrals measured while holding the cells at -60 mV (integrals normalized to both peak tail-current amplitude for each compound and to control). Figure 6 shows normalized tail-current integrals for the prepared analogs. The pyrazolotriazine analog **MF-17** shows slightly improved activity compared to its purine analog **GV-58**, demonstrating that this modification is tolerated. Altering the alkyl sidechain in zone A to the cyclopropylmethylene in **MF-71** was found to be detrimental to agonist activity, whereas the isopropyl analog **MF-06** was found to be highly active. This is in agreement with prior data suggesting that activity is highly sensitive to zone A substitutions. Modifications of the aminobutanol sidechain in zone C proved to have a deleterious effect both in the case of trifluoromethyl analog **MF-59** and dimethyl analog **MF-21**. Cyclopropyl ester **MF-27** completely abolished activity and actually promoted channel deactivation (data not shown). Interestingly, **SCT-62**, the enantiomer of **GV-58**, retained some activity. To date, only the aminobutanol sidechain has been found to convey activity, with the (*R*)-enantiomer giving superior results.

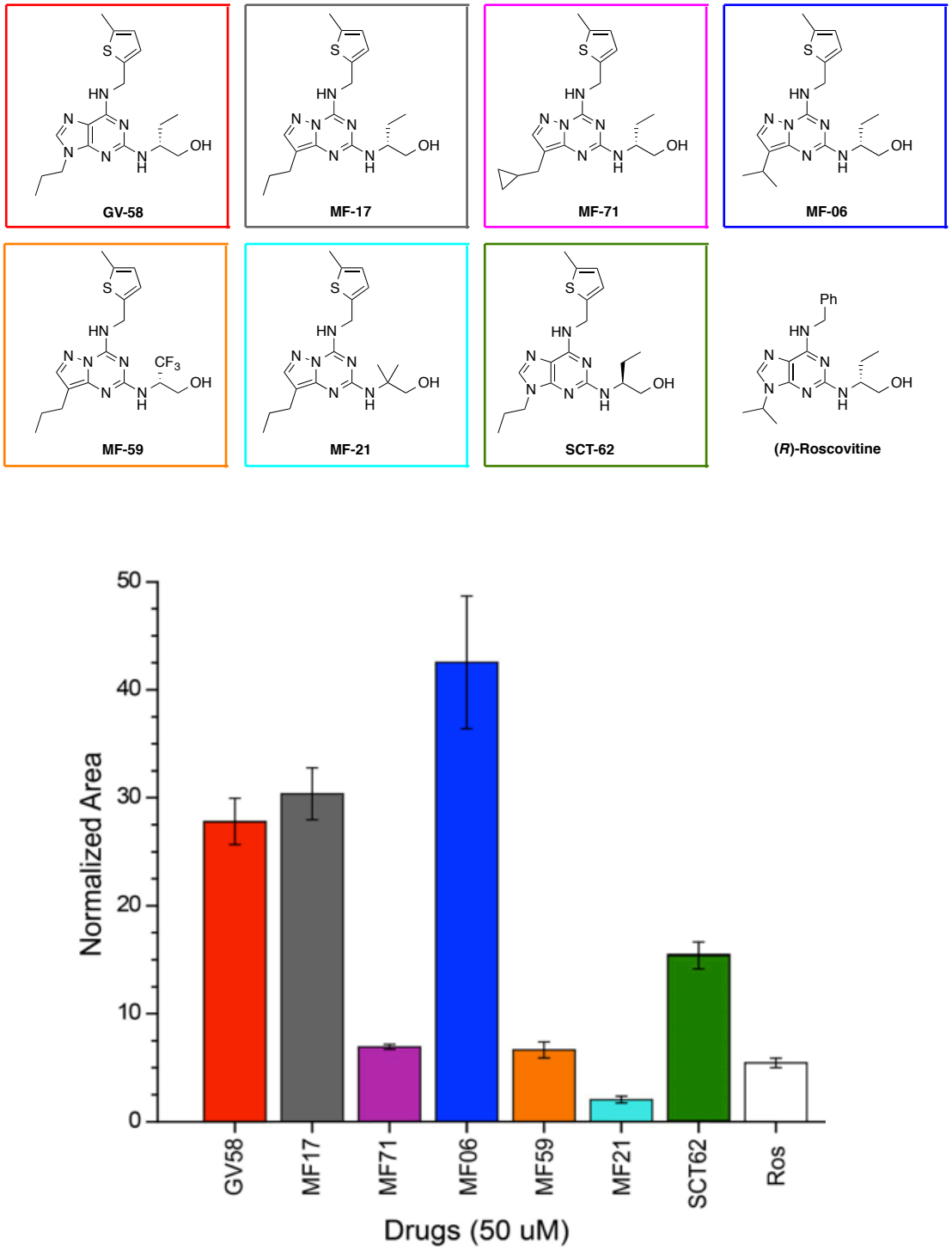


Figure 6: Normalized tail-current integrals for various analogs. All compounds were tested at 50 μM. Data for **GV-58** and (*R*)-roscovitine were previously published. All other data are unpublished.

Decay time constants (tau) were also investigated and provide a measure of mean channel open times (Figure 7).[51] The four most active compounds from Figure 6 all show significantly longer open times than both the control and (*R*)-roscovitine treated channels, with **MF-06** showing the largest tau value. Interestingly, enantiomers **GV-58** and **SCT-62** show similar tau values, yet noticeably different tail-current integral areas. Further investigation is needed to determine the origin of this effect.

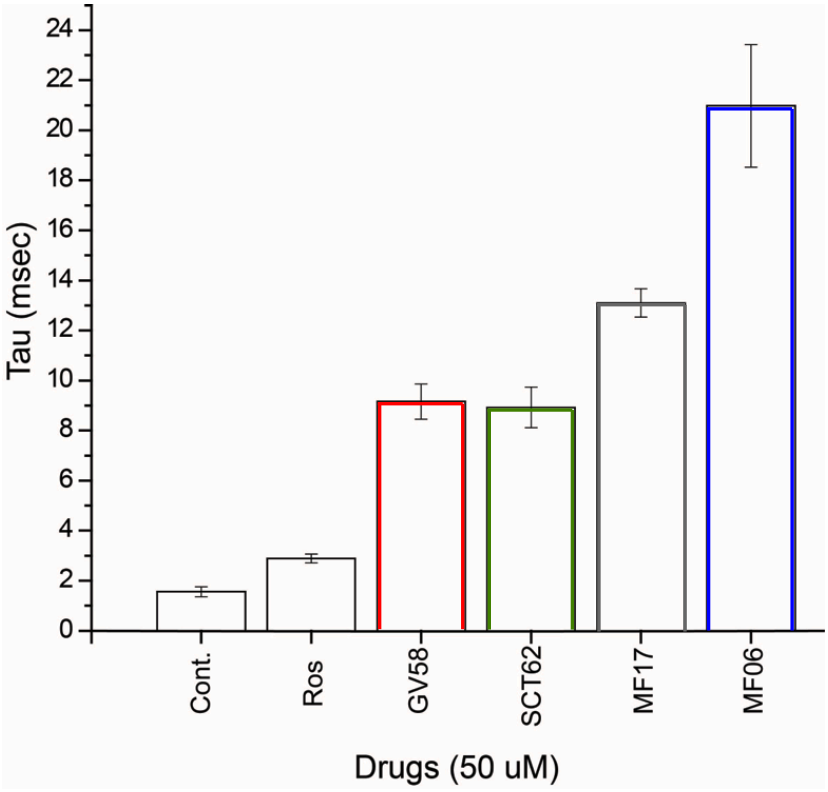


Figure 7: Decay time constants for most active analogs. All compounds were tested at 50 μM. Data are unpublished.

An interesting difference arises when comparing **MF-17** and **MF-06** with purine analogs **GV-58** and **ML-50** (Figure 8). The *n*-propyl analogs **MF-17** and **GV-58** show similar tail-current integrals, whereas isopropyl analog **MF-06** shows a ~67% increase over **ML-50**.

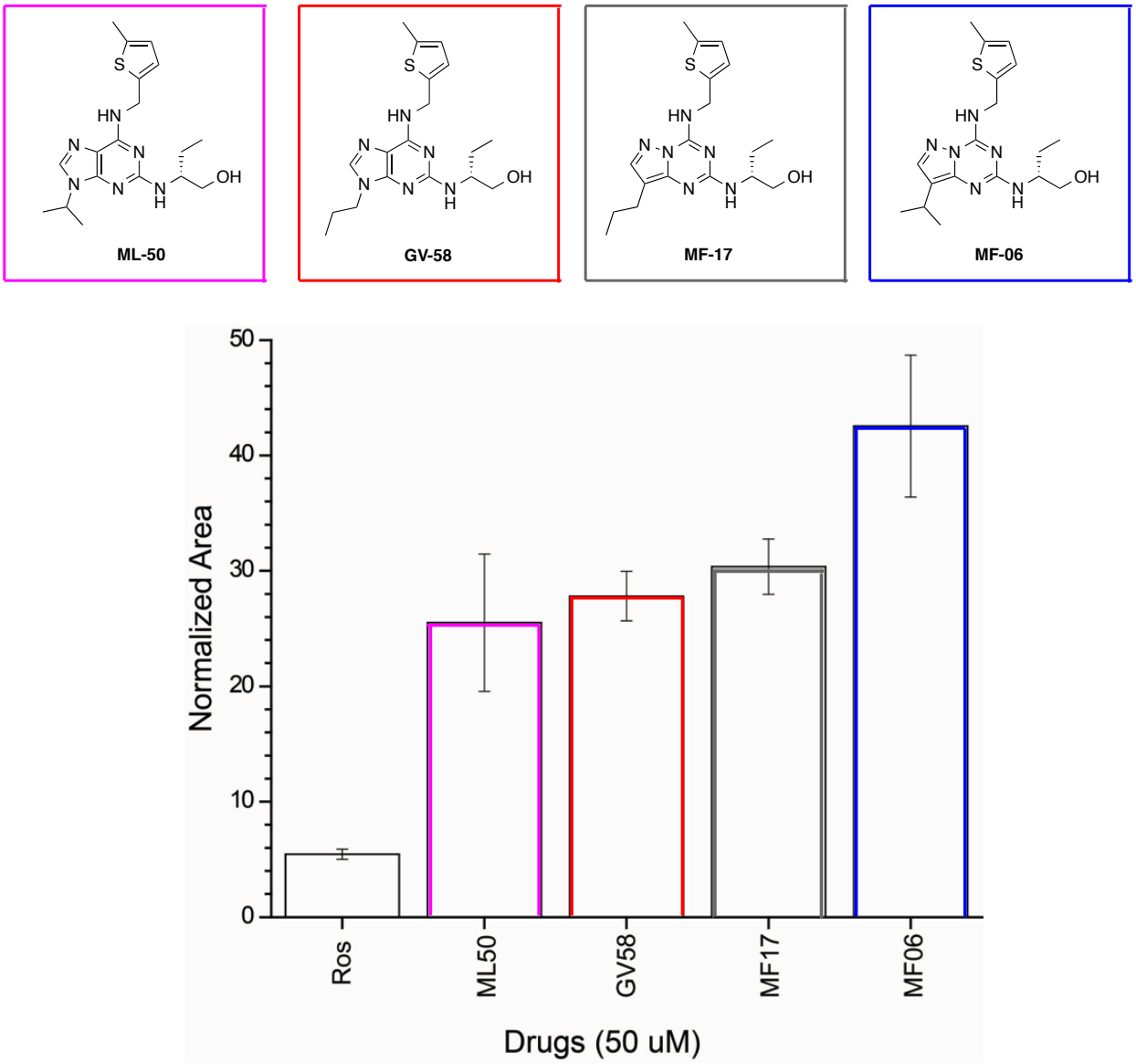


Figure 8: Comparison of agonist effects between zone D purine and pyrazolotriazine analogs. All compounds were tested at 50 μM except **ML-50**, which was tested at 100 μM. Data for (*R*)-roscovitine, **ML-50**, and **GV-58** have previously been published.[53] Other data are unpublished.

One potential explanation of the difference in activity between the two pairs of analogs (**ML-50**/**MF-06** have a large difference in activity, **GV-58**/**MF-17** have near identical activity) is the difference in the rotational energy barrier between the two sidechains. Molecular mechanics calculations were performed to investigate this possibility. Sidechains in zones B and C were truncated to methylamines to simplify calculations. The *n*-propyl substitutions in zone A were truncated to ethyl in order to eliminate artificial inflation of the rotation energy barrier due to locking the methyl-methylene bond in this sidechain. The conformer distribution was calculated and the minimum energy conformer selected. All torsion angles were then held constant and single-point energies calculated for rotation about the indicated bonds. Figure 9 and Figure 10 show the plot of relative energy versus the dihedral angle shown in either blue or red for the pairs of analogs.

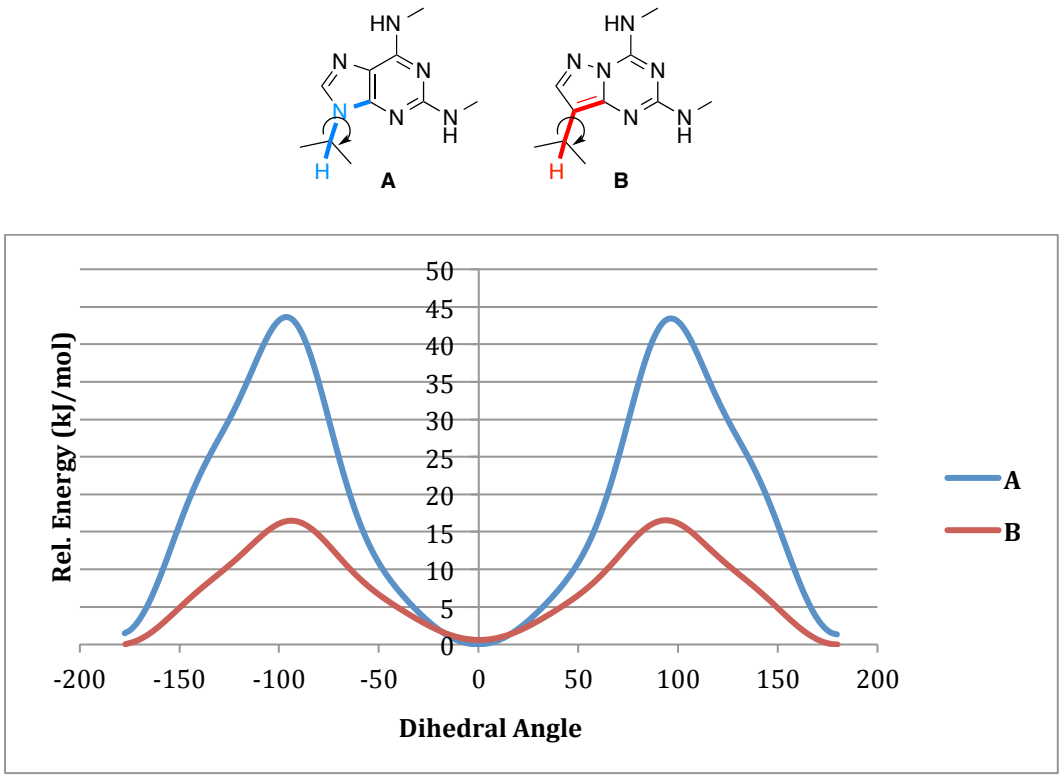


Figure 9: Steric energy of rotation for isopropyl sidechains with simplified structures. Torsion angle of interest is highlighted in either blue or red.

For purine derivatives **A** and **A'** (simplified structures for **ML-50** and **GV-58**) barriers are larger than in the corresponding pyrazolotriazine, possibly due to the decreased bond length (1.456 Å vs. 1.498 Å in **A** and **B**, Figure 9; 1.446 Å vs. 1.487 Å in **A'** and **B'**, Figure 10) between the ring and sidechain. However, the isopropyl derivatives **A** and **B** show a larger difference (~30 kJ/mol) in rotational energy barriers than the unbranched analogs **A'** and **B'** (~20 kJ/mol, ~7 kJ/mol, two different barriers). This increased energy penalty may prevent **ML-50** from attaining the preferred conformation for receptor binding. Since energy barriers are smaller in the *n*-propyl analogs, they may be able to fold into similar active conformations, and thus giving similar agonist activities.

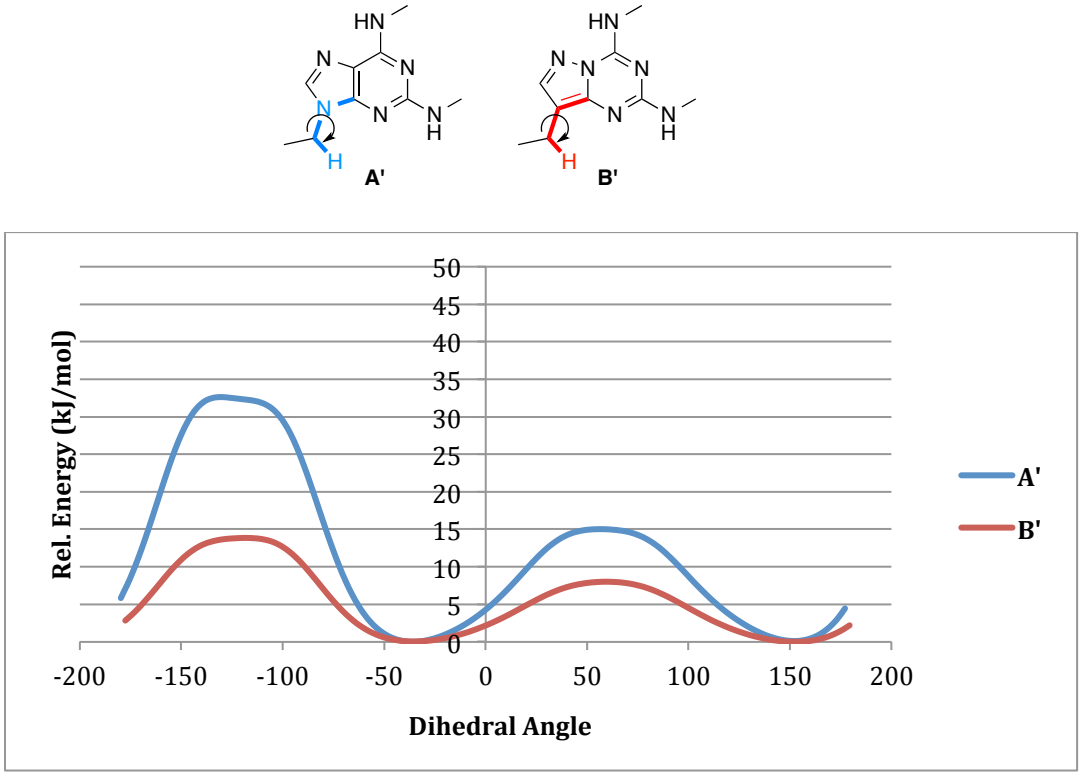


Figure 10: Steric energy of rotation for unbranched sidechains with simplified structures. Torsion angle of interest is highlighted in either blue or red.

One potential method to test this hypothesis would be to prepare the pyrazolotriazine of **GV-05** and compare agonist activities (methyl group in zone A, see Figure 5 for structure). The rotational energy barrier difference between these two compounds should be nearly identical, and should thus have very similar activities if the rotation energy barrier is a major determinant of activity.

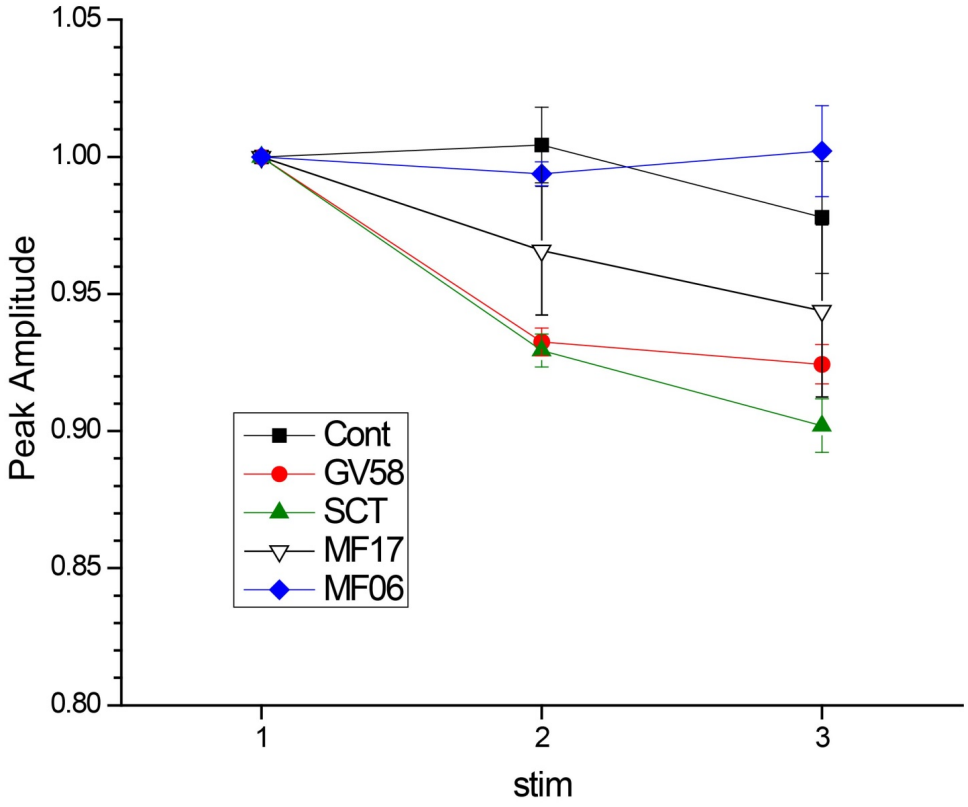


Figure 11: Decrease in peak amplitude of Ca^{2+} current with repeated stimuli for the four most active analogs. All compounds were tested at 50 μM. Data are unpublished. Cont = control. Contronl is peak amplitude without added compound.

Effects on peak current amplitudes over multiple stimuli were also investigated to determine the antagonist effects over time of the four most active analogs (Figure 11). Purine

analogs GV-58 and SCT-62 show stronger deactivation, with peak amplitude decreasing to ~92% of the first stimulus by the third stimulus. In contrast, the pyrazolotriazine analogs showed less deactivation, with **MF-06** having no significant effect on peak amplitude.

1.2.3 Conclusion

Several pyrazolo[1,5-a]-1,3,5-triazine analogs of (*R*)-roscovitine have been prepared. Preliminary results suggest this modification may beneficial for increasing Ca^{2+} current through N-type VGCCs and previous results show that this correlates well with a similar behavior in P/Q-type channels. Also, these analogs show decreased or absence of VGCC deactivation over time. Thus, these compounds may prove useful in treating Lambert-Eaton myasthenic syndrome. In the future, the most promising candidates will be tested for cdk2 inhibition to complete the SAR analysis.

2.0 EXPERIMENTAL SECTION

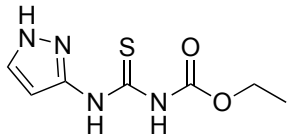
2.1 GENERAL

All moisture-sensitive reactions were performed using syringe-septum techniques under an atmosphere of either dry N_2 or Ar unless otherwise noted. Tetrahydrofuran and diethyl ether were purified by distillation over sodium/benzophenone under a dry N_2 atmosphere. Dichloromethane was purified by distillation from CaH_2. All other materials were obtained from commercial sources and used as received unless otherwise stated. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F_{254} plates, 250 μm layer thickness) and visualized by using UV lamp (254 nm) and/or by staining with $KMnO_4$ solution (3.0 g $KMnO_4$, 4.0 g K_2CO_3, in 200 mL H_2O and 4 mL 5% NaOH). Flash column chromatography was performed with 40-63 μm silica gel (Silicycle). Infrared spectra were measured on a Smiths Detection IdentifyIR FT-IR spectrometer (ATR). All NMR data was collected at room temperature in $CDCl_3$ on a 300, 400, or 500 MHz Bruker instrument. Chemical shift per million (ppm) with internal $CHCl_3$ (δ 7.26 ppm for ^1H and 77.00 ppm for ^{13}C) or $(CD_3)_2SO$ with internal $(CH_3)_2SO$ (δ 2.50 ppm for ^1H) as the reference. NMR data are reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, qd = quartet of doublets), integration, and coupling constant(s) (J) in Hertz (Hz).

Whole-cell Ca^{2+} currents were recorded using the perforated patch-clamp method of tsA-201 cells transfected with N-type channels.[53] The pipette solution contained 70 mM Cs_2SO_4, 1 mM $MgCl_2$, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 7.4. Cells were bathed in a saline solution composed of 130 mM choline chloride, 10 mM tetraethylammonium chloride, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, at pH 7.4. Patch pipettes were prepared with borosilicate glass pulled to a resistance of approximately 1 MΩ. The perforating agent was 65 μM amphotericin B. Passive membrane potentials and capacitive currents were subtracted form the data. Currents were activated using a tep delpolarization from -60 mV to +60 mV, amplified with an Axopatch 200B amplifier, and further analysis performed using pClamp 10 software. Tail-current integrals were measured before and after application of a compound, with each recording normalized to its peak. Compounds were bath applied at 50 μM (except **ML-50**, 100 μM) with a glass pipette in a 1.5 mL static bath chamber. Each compound tested 3-6 times. Control experiments were performed using 0.1-1% DMSO. All experiments were performed at room temperature. Tau values were determined by fitting a single exponential function to the Ca^{2+} current decay.

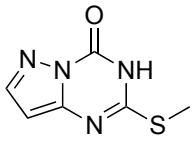
Molecular mechanics calculations were performed with the Spartan 10 software package using the Merck molecular force field (MMFF). Conformer distributions were calculated in the gas phase, and the minimum energy conformer selected. All torsion angles in this conformer were then locked except the dihedral angle indicated in the figures above and the single point energy calculated every 3 degrees of rotation about the indicated angle.

2.2 EXPERIMENTAL PROCEDURES



1

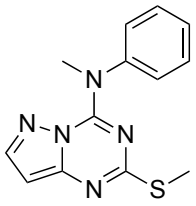
***N*-Ethoxycarbonyl-N'-(pyrazol-3-yl)thiourea (1)**.[62] A solution of 3-aminopyrazole (2.65 g, 31.6 mmol, 1.1 equiv.) in THF (37 mL) was cooled to 0 °C under nitrogen. In a dropwise fashion, ethoxycarbonyl isothiocyanate (3.5 mL, 28 mmol, 1.0 equiv.) was added over approximately 5 min. The reaction mixture was allowed to stir for 1 h at 0 °C. Solvent was then removed under reduced pressure giving crude **1** (6.80 g) as a yellow solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.9 (bs, 1 H), 8.28 (bs, 1 H), 7.55 (d, 2 H, J = 2.4 Hz), 7.04 (d, 2 H, J = 2.4 Hz), 4.30 (q, 2 H, J = 7.1 Hz), 1.34 (t, 3 H, J = 7.1 Hz).



2

2-(Methylthio)pyrazolo[1,5-*a*][1,3,5]triazin-4(3*H*)-one (2).[63] A solution of **1** (6.80 g, 25.4 mmol, 1.00 eq) in 2 N NaOH (52 mL, 104 mmol, 4.1 equiv.) was stirred at room temperature for 5 h. The reaction mixture was cooled in an ice bath and 2 N H_2SO_4 (75 mL, 150 mmol, 6.0 equiv.) was added in small aliquots. The resulting precipitate was collected by vacuum filtration, washed with water, and dried under vacuum to give an off white powder (5.05 g). This solid was suspended in EtOH (90 mL), and after addition of aqueous 2 N NaOH (23 mL, 46 mmol, 2.0 equiv.) and methyl iodide (1.4 mL, 22.5 mmol, 1.00 equiv.), the resulting slurry was stirred at room temperature for 1.5 h. The reaction mixture was then filtered and the solid

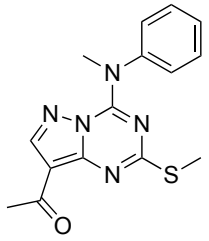
was dissolved in water (225 mL) by stirring for 10 min. After addition of 2 N H_2SO_4 (13 mL, 26 mmol, 1.2 equiv.), the resulting solid was filtered, washed with water, and air dried overnight giving **2** (2.99 g, 11.8 mmol, 50% over 3 steps) as a white solid that was used in the next step without further purification: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.89 (bs, 1 H) 7.97 (d, 1 H, J = 1.6 Hz), 6.35 (d, 1 H, J = 2.0 Hz), 2.53 (s, 3 H).



3

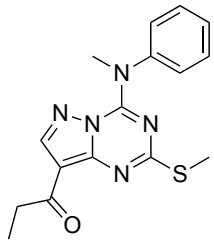
***N*-Methyl-2-(methylthio)-*N*-phenylpyrazolo[1,5-*a*][1,3,5]triazin-4-amine(3).**[64]

A flame-dried flask was charged with **2** (1.00 g, 5.38 mmol, 1.00 equiv.) and phosphonitrillic chloride trimer (2.27 g, 6.46 mmol, 1.20 equiv.). The vial was flushed with argon, and MeCN (50 mL) and DIPEA (4.7 mL, 26 mmol, 5.0 equiv.) were added. The reaction mixture was stirred for 1 h at room temperature and then treated with N-methylaniline (4.2 mL, 22 mmol, 4.0 equiv.). The suspension immediately cleared and stirring was continued for 6 h. The solvent was removed under reduced pressure and the residue dissolved in dichloromethane (50 mL). The organic phase was washed with 1 N HCl (2x20 mL), sat. $NaHCO_3$ (10 mL), and brine (20 mL), dried ($MgSO_4$) and concentrated. Purification by chromatography on SiO_2 (1:0 to 1:1 to 0:1, hexanes:dichloromethane) provided **3** (1.03 g, 3.72 mmol, 69%) as a pale yellow oil that slowly solidified: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (d, 1 H, J = 2.4 Hz), 7.41-7.36 (m, 3 H), 7.19-7.17 (m, 2 H), 6.16 (d, 1 H, J = 2.0 Hz), 3.73 (s, 3 H), 2.54 (s, 3 H).



4a

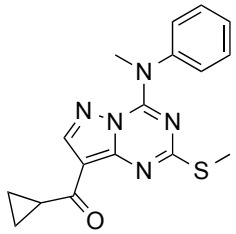
1-(4-(Methyl(phenyl)amino)-2-(methylthio)pyrazolo[1,5-*a*][1,3,5]triazin-8-yl)ethan-1-one (4a).[55] A flame-dried vial was charged with **3** (336 mg, 1.21 mmol, 1.00 equiv.), acetyl chloride (0.17 mL, 2.4 mmol, 2.0 equiv.) and 1 M tin(IV) chloride in dichloromethane (6.1 mL, 6.1 mmol, 5.0 equiv.). The vial was sealed and allowed to heat at 85 °C for 17 h. The reaction mixture was then poured over crushed ice, diluted with $CHCl_3$ (12 mL), extracted with $CHCl_3$ (3x8 mL), washed with water and brine (10 mL each), and dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (5:6:1, dichloromethane:hexanes:ethyl acetate) provided **4a** (310 mg, 0.969 mmol, 80%) as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.07 (s, 1 H), 7.40-7.37 (m, 3 H), 7.18-7.15 (m, 2 H), 3.74 (s, 3 H), 2.69 (s, 3 H), 2.58 (s, 3 H).



4b

1-(4-(Methyl(phenyl)amino)-2-(methylthio)pyrazolo[1,5-*a*][1,3,5]triazin-8-yl)propan-1-one (4b). A flame-dried vial was charged with **3** (737 mg, 2.67 mmol, 1.00 equiv.), dichloromethane (13 mL), propionyl chloride (0.47 mL, 5.3 mmol, 2.0 equiv.) and 1 M tin(IV) chloride (1.6 mL, 13 mmol, 5.0 equiv.). The vial was sealed and heated at 85 °C for 17 h. The reaction mixture was then poured over crushed ice, diluted with water (15 mL) and

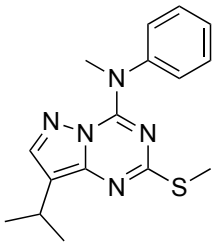
dichloromethane (10 mL) and allowed to stir for 30 min. The layers were separated, and the aqueous layer was extracted with dichloromethane (15 mL). The combined organic fractions were washed with brine (15 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (1:0 to 95:5 to 9:1, dichloromethane:ethyl acetate) provided **4b** (710 mg, 2.06 mmol, 78%) as a faintly brown solid: Mp 147.6-148.2 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.09 (s, 1 H), 7.43-7.35 (m, 3 H), 7.18-7.15 (m, 2 H), 3.74 (s, 3 H), 3.11 (q, 2 H, J = 5.5 Hz), 2.59 (s, 3 H), 1.20 (t, 3 H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 195.5, 170.7, 151.1, 148.2, 145.7, 144.2, 129.2, 127.6, 126.1, 108.9, 42.4, 34.7, 14.4, 8.3; IR ν 2934, 1652, 1501 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{16}H_{17}N_5OS$ $[M+H]^+$ 328.1232, found 328.1220.



4c

Cyclopropyl(4-(methyl(phenyl)amino)-2-(methylthio)pyrazolo[1,5-*a*][1,3,5]triazin-8-yl)methanone (4c). A flame-dried vial was charged with **3** (125 mg, 0.451 mmol, 1.00 equiv.), dichloromethane (2.3 mL), cyclopropanecarbonyl chloride (0.084 mL, 0.91 mmol, 2.0 equiv.), and tin(IV) chloride (0.27 mL, 2.2 mmol, 5.0 equiv.). The vial was sealed and allowed to heat at 85 °C for 17 h. The reaction mixture was then poured over crushed ice, diluted with water (8 mL) and dichloromethane (5 mL) and allowed to stir for 30 min. The layers were separated and the aqueous layer was extracted with dichloromethane (10 mL). The combined organic fractions were washed with water and brine (10 mL each), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (1:1, hexanes:dichloromethane, then 95:5 to 9:1, dichloromethane:ethyl acetate) provided **4c** (118 mg, 0.341 mmol, 75%) as a white solid: Mp

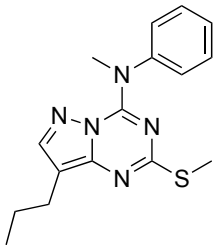
196.2-198.0 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (s, 1 H), 7.44-7.35 (m, 3 H), 7.19-7.15 (m, 2 H), 3.75 (s, 3 H), 3.46-3.38 (m, 1 H), 2.56 (s, 3 H), 1.22-1.17 (m, 2 H), 1.01-0.95 (m, 2H); ^{13}C NMR ($CDCl_3$,125 MHz) δ 194.6, 170.8, 151.5, 148.3, 145.5, 144.2, 129.2, 127.6, 126.1, 109.6, 42.4, 18.6, 14.4, 11.1; IR ν 3003, 2924, 1586, 1541, 1407 cm^{-1}; HRMS (ESI^{+}) *m/z* calculated for $C_{17}H_{17}N_5OS$ [M+H] 340.1232, found 340.1223.



5a

8-Isopropyl-*N*-methyl-2-(methylthio)-*N*-phenylpyrazolo[1,5-*a*][1,3,5]triazin-4-amine (5a).[55] To a solution of **4a** (378 mg, 1.15 mmol, 1.00 equiv.) in THF (10 mL) at 0 °C under nitrogen was added 3M methyl magnesium bromide in diethyl ether (1.2 mL, 3.6 mmol, 3.1 equiv.) in a dropwise fashion. The reaction was then allowed to stir for 44 min at room temperature, quenched with saturated NH_4Cl (aq.)(6 mL), and extracted with dichloromethane (5 mL) and ethyl acetate (2x5 mL). The combined organic layers were washed with brine (12 mL), dried ($MgSO_4$), and concentrated. The residue was dissolved in dichloromethane (12 mL) and added in a dropwise fashion to solution of $NaBH_4$ (158 mg, 4.18 mmol, 3.63 equiv.) in trifluoroacetic acid (12 mL) at 0 °C under nitrogen. The reaction was then allowed to stir for 2 h at room temperature and quenched with 1 M NaOH (aq.) (20 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (3x10 mL), rinsed with saturated sodium bicarbonate (2x20 mL), water (20 mL), and brine (20 mL), dried ($MgSO_4$), and concentrated giving **5a** (365 mg, 1.10 mmol, 95%) as a yellow-brown oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.54

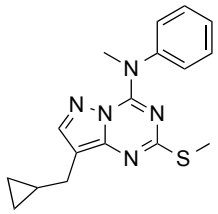
(s, 1 H) 7.41-7.28 (m, 3 H), 7.18-7.15 (m, 2 H), 3.70 (s, 3 H), 3.13 (septet, 1 H, J = 6.9 Hz), 2.55 (s, 3 H), 1.27 (d, 6 H, J = 6.9 Hz).



5b

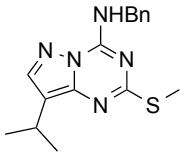
***N*-Methyl-2-(methylthio)-*N*-phenyl-8-propylpyrazolo[1,5-*a*][1,3,5]triazin-4-amine (5b).** To a solution of **4b** (399 mg, 1.16 mmol, 1.00 equiv.) in dichloromethane/EtOH (1:1, 6.8 mL) at 0 °C was added LiCl (147 mg, 3.47 mmol, 3.00 equiv.) and $NaBH_4$ (140 mg, 3.66 mmol, 3.16 equiv.). The reaction mixture was allowed to warm to room temperature, stirred for 17 h, quenched with water (5 mL), and extracted with dichloromethane (2x10 mL). The combined organic layers were washed with brine (5 mL), dried ($MgSO_4$), and concentrated. The residue was then dissolved in dichloromethane (12 mL) and added dropwise to a solution of $NaBH_4$ (442 mg, 11.5 mmol, 10 equiv.) in trifluoroacetic acid (12 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, stirred for 4 h, quenched with 1 M NaOH (20 mL) and diluted with dichloromethane (10 mL). The aqueous layer was then extracted with dichloromethane (2x3 mL). The combined organic fractions were washed with saturated sodium bicarbonate (2x5 mL) and brine (5 mL), dried ($MgSO_4$) and concentrated giving **5b** (331 mg, 1.00 mmol, 87%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (s, 1 H), 7.39-7.29 (m, 3 H), 7.18-7.15 (m, 2 H), 3.71 (s, 3 H), 2.60-2.55 (m, 2 H), 2.55 (s, 3 H), 1.63 (sextet, 2 H, J = 7.5 Hz), 0.93 (t, 3 H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.9, 148.3, 145.1, 144.8, 128.9,

126.9, 126.1, 107.3, 42.0, 24.5, 23.2,14.2, 13.9; IR ν 2954, 1534, 1508 cm^{-1}; HRMS (ESI^+) *m/z* calculated for $C_{16}H_{19}N_5S$ $[M+H]^+$ 314.1439, found 314.1427.



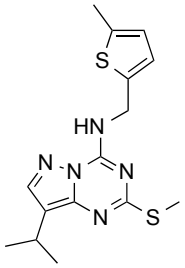
5c

8-(Cyclopropylmethyl)-*N*-methyl-2-(methylthio)-*N*-phenylpyrazolo[1,5-*a*][1,3,5]triazin-4-amine (5c). To a white slurry of **4b** (689 mg, 1.91 mmol, 1.0 equiv.) in THF (20 mL) was added at 0 °C a 1 M borane solution in THF (4.9 mL, 4.9 mmol, 2.6 equiv.). The reaction mixture was stirred for 21 h at room temperature, quenched by the slow addition of water (5 mL), and the volume was reduced by half under reduced pressure. The residue was extracted with dichloromethane (2x10 mL), and the combined organic fractions were rinsed with brine (10 mL), dried ($MgSO_4$), and concentrated giving **5c** (258 mg, 0.777 mmol, 41%) as a clear oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.63 (s, 1 H), 7.41-7.30 (m, 3 H), 7.19-7.16 (m, 2 H), 3.71 (s, 3 H), 2.55 (s, 3 H), 2.55-2.52 (m, 2 H), 0.99-0.92 (m, 1 H), 0.47-0.43 (m, 2 H), 0.21-0.17 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.1, 148.3, 145.1, 144.8, 129.2, 128.9, 127.0, 126.1, 107.0, 42.1, 27.3, 14.2, 11.3, 4.6; IR ν 3070, 2997, 2923, 1534, 1508 cm^{-1}; HRMS (ESI^+) *m/z* calculated for $C_{17}H_{19}N_5S$ $[M+H]^+$ 326.1439, found 326.1433.



6a

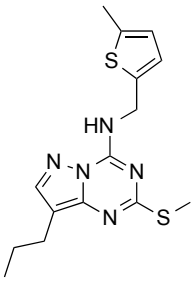
***N*-Benzyl-8-isopropyl-2-(methylthio)pyrazolo[1,5-*a*][1,3,5]triazin-4-amine (6a)**.[55] A flame-dried microwave vial was charged with **5a** (84 mg, 0.27 mmol, 1.0 equiv.), benzyl amine (0.15 mL, 1.4 mmol, 5.0 equiv.) and ethanol (0.72 mL). The vial was then sealed and allowed to heat at 120 °C for 2 h in the microwave, after which time the temperature was increased to 135 °C for 3 h. Following concentration, purification by chromatography (SiO_2, 1:0 to 9:1, hexanes:ethyl acetate) provided **6a** as a white solid (44 mg, 0.14 mmol, 52%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74 (s, 1 H), 7.39-7.28 (m, 5 H), 6.77 (broad s, 1H), 4.80 (d, 2 H, J = 6.0 Hz), 3.17 (septet, 1 H, J = 6.9 Hz), 2.58 (s, 3 H), 1.33 (d, 6 H, J = 6.9 Hz).



6b

8-Isopropyl-2-(methylthio)-*N*-((5-methylthiophen-2-yl)methyl)pyrazolo[1,5-*a*][1,3,5]triazin-4-amine (6b). A flame-dried microwave vial was charged with **5a** (80 mg, 0.24 mmol, 1.0 equiv.), triethylamine (0.67 mL, 0.48 mmol, 20 equiv.), (5-methylthien-2-yl)methylamine HCl (1935 mg, 1.170 mmol, 4.849 equiv.), KF (15 mg, 0.25 mmol 1.1 equiv.) and absolute ethanol (0.80 mL). The vial was then sealed and allowed to heat at 130 °C for 34 h. The reaction mixture was then diluted with water (2 mL) and extracted with dichloromethane (3x2 mL). The combined organic extracts were washed with brine (2 mL), dried ($MgSO_4$), and

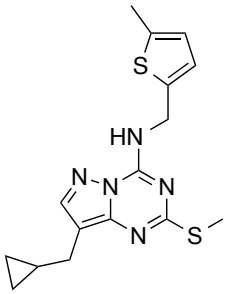
concentrated. Purification by chromatography on SiO_2 (98:2 to 95:5, hexanes:ethyl acetate) provided **6b** (41 mg, 0.062 mmol, 49%) as an off white solid: Mp 105.3-105.8°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.72 (s, 1 H), 6.84 (d, 1 H, *J* = 3.3 Hz), 6.74 (broad s, 1 H), 6.58 (m, 1 H), 4.85 (d, 2 H, 6.0 Hz), 3.13 (septet, 1 H, *J* = 6.9 Hz), 2.59 (s, 3 H), 2.44 (s, 3 H), 1.33 (d, 6 H, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.7, 145.5, 143.4, 140.6, 136.7, 126.8, 124.8, 115.3, 39.4, 23.6, 23.2, 15.3, 14.3; IR ν 3286, 2956, 2934,1592, 1564 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated $[M+H]^+$ for $C_{15}H_{19}N_5S_2$ 334.1160; found 334.1151.



6c

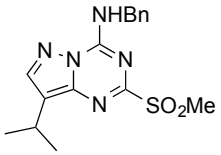
2-(Methylthio)-*N*-((5-methylthiophen-2-yl)methyl)-8-propylpyrazolo[1,5-*a*][1,3,5]triazin-4-amine (6c). A flame-dried microwave vial was charged with **5b** (0.813 g, 2.46 mmol, 1.00 equiv.), (5-methylthiophen-2-yl)methanamine (1.46 g, 10.9 mmol, 4.44 equiv.), KF (0.209 g, 3.56 mmol 1.45 equiv.), and absolute ethanol (8.2 mL). The vial was sealed and heated at 130 °C for 20 h. The reaction mixture was diluted with water (10 mL) and extracted with dichloromethane (2x10 mL). The combined organic extracts were washed with 1 N HCl (2x10 mL) and brine (10 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (98:2 then 95:5, hexanes:ethyl acetate) provided **6c** (405 mg, 1.19 mmol, 48%) as a white solid: Mp 84.9-85.3 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.71 (s, 1 H), 6.83 (d, 2 H, *J* = 1.0 Hz), 6.79, (bs, 1 H), 6.59-6.58 (m, 1 H), 4.86 (d, 2 H, *J* = 5.5 Hz), 2.63-2.58 (m, 2 H), 2.60 (s, 3 H), 2.45 (s, 3 H), 1.68 (sextet, 2 H, *J* = 7.5 Hz), 0.96 (t, 3 H, *J* = 7.3 Hz); ^{13}C NMR ($CDCl_3$, 125

MHz) δ 166.0, 147.2, 146.2, 145.3, 140.6, 136.7, 126.9, 124.9, 108.8, 39.4, 24.6, 23.3, 15.3, 14.3, 13.9; IR ν 3247, 2954, 1618, 1588 cm^{-1}; HRMS (ESI^+) *m/z* calculated for $C_{15}H_{19}N_5S_2$ $[M+H]^+$ 334.1160, found 334.1153.



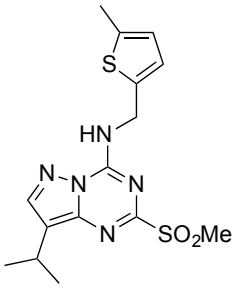
6d

8-(Cyclopropylmethyl)-2-(methylthio)-*N*-((5-methylthiophen-2-yl)methyl)pyrazolo[1,5-*a*][1,3,5]triazin-4-amine (6d). A flame-dried microwave vial was charged with **5c** (258 mg, 0.753 mmol, 1.00 equiv.), (5-methylthiophen-2-yl)methanamine (445 mg, 3.43 mmol, 4.55 equiv.), KF (66 mg, 1.13 mmol 1.50 equiv.), and absolute ethanol (2.5 mL). The vial was sealed and heated at 130 °C for 24 h. The reaction mixture was diluted with water (3 mL), extracted with dichloromethane (2x8 mL), washed with 1 N HCl (5 mL) and brine (5 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (95:5 then 92:8, hexanes:ethyl acetate) provided **6d** (79 mg, 0.22 mmol, 30%) as an off white solid: Mp 105.3-107.6 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.81 (s, 1 H), 6.85 (bs, 1 H), 6.67 (bs, 1 H), 6.60 (d, 1 H, *J* = 3.3 Hz), 4.87 (d, 2 H, *J* = 5.7 Hz), 2.59 (s, 3 H), 2.59-2.56 (m, 2 H), 2.44 (s, 3 H), 1.07-0.98 (m, 1 H), 0.26-0.21 (m, 2H), 0.52-0.46 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.2, 147.1, 146.3, 145.2, 140.4, 136.8, 126.6, 124.7, 108.4, 39.3, 27.3,15.2, 14.2, 11.4, 4.6; IR ν 3232, 2921, 1616, 1584, 12.75 cm^{-1}; HRMS (ESI^+) *m/z* calculated for $C_{16}H_{19}N_5S_2$ $[M+H]^+$ 346.1160, found 346.1154.



7a

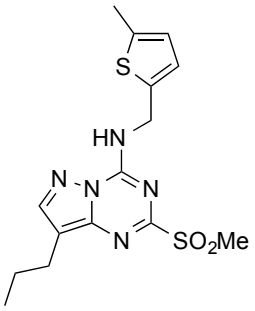
***N*-Benzyl-8-isopropyl-2-(methylsulfonyl)pyrazolo[1,5-*a*][1,3,5]triazin-4-amine (7a)**.[55] To a solution of **6a** (45 mg, 0.14 mmol, 1.00 equiv.) in acetone (1.0 mL) at 0 °C was added 3.17 M $NaHCO_3$ (0.36 mL, 1.1 mmol, 7.97 equiv.) followed by oxone in 4x10-4 M EDTA (119 mg in 0.43 mL, 1.4 equiv.). The reaction was then allowed to stir 5.5 h at room temperature. The reaction mixture was quenched with 50% aqueous sodium bisulfite (0.144 mL) and diluted with dichloromethane (5 mL). The organic layer was separated and the aqueous phase extracted with dichloromethane (2x1 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (4:1, hexanes:ethyl acetate) provided **7a** as a clear oil (38 mg, 0.10 mmol, 72%): ^1H NMR ($CDCl_3$, 300 MHz) δ 7.83 (s, 1 H), 7.30-7.25 (m, 5 H), 4.82 (d, 2 H, 6.0 Hz), 3.29 (s, 3 H), 3.17 (septet, 1 H, J = 6.9 Hz), 1.27 (d, 6 H, J = 6.9 Hz).



7b

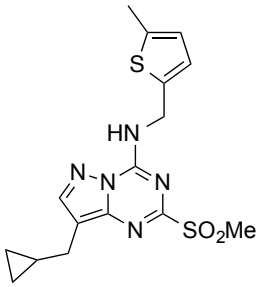
8-Isopropyl-2-(methylsulfonyl)-*N*-((5-methylthiophen-2-yl)methyl)pyrazolo[1,5-*a*][1,3,5]triazin-4-amine (7b).[8] A solution of **6b** (23 mg, 0.063 mmol, 1.00 equiv.) acetone (1.0 mL) at 0 °C was charged with 3.2 M $NaHCO_3$ (0.36 mL, 1.1 mmol, 8.0 equiv.) followed by 0.39

M oxone in water (232 mg in 0.8 mL, 3.0 equiv.). The reaction was then allowed to stir 5 h at room temperature, quenched with 50% aqueous sodium bisulfite (0.14 mL), and extracted with dichloromethane (4x2 mL). The organic layers were washed with brine (2 mL), dried ($MgSO_4$), and concentrated giving crude **7b** (14.4 mg, 0.0335 mmol, 53%) as a clear oil that was used without further purification.



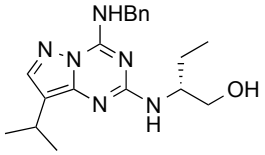
7c

2-(Methylsulfonyl)-*N*-((5-methylthiophen-2-yl)methyl)-8-propylpyrazolo[1,5-*a*][1,3,5]triazin-4-amine (7c). A solution of **6c** (303 mg, 0.890 mmol, 1.00 equiv.) in acetone (15 mL) at 0 °C was charged with 0.39 M oxone in water (1620 mg in 6.8 mL, 2.64 mmol 2.96 equiv.). The reaction mixture was stirred for 12 h at room temperature, quenched with 50% aqueous sodium bisulfite (0.48 mL), and the acetone was removed in vacuo. The residue was diluted with water (6 mL) and extracted with dichloromethane (3x6 mL). The combined organic layers were washed with brine (6 mL), dried ($MgSO_4$), and concentrated giving **7c** as a yellow oil that was used without further purification.



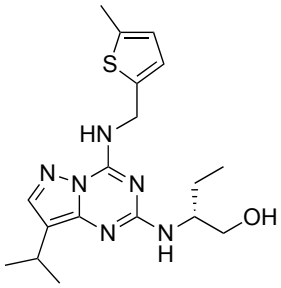
7d

8-(Cyclopropylmethyl)-2-(methylsulfonyl)-*N*-((5-methylthiophen-2-yl)methyl)pyrazolo[1,5-*a*][1,3,5]triazin-4-amine (7d) A solution of **6d** (79 mg, 0.22 mmol, 1.0 equiv.) in acetone (3.7 mL) at 0 °C was charged with 0.39 M oxone in water (1.7 mL, 0.66 mmol, 3.0 equiv.). The reaction mixture was stirred for 12 h at room temperature and quenched with 50% aqueous sodium bisulfite (0.12 mL) and the acetone was removed in vacuo. The residue was diluted with water (3 mL) and extracted with dichloromethane (3x3 mL). The combined organic layers were washed with brine (3 mL), dried ($MgSO_4$), and concentrated giving **7d** as a yellow oil that was used without further purification.



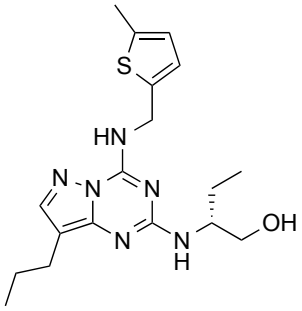
8

(*R*)-2-((4-(Benzylamino)-8-isopropylpyrazolo[1,5-*a*][1,3,5]triazin-2-yl)amino)butan-1-ol (8).[55] A flame-dried microwave vial was charged with **7a** (37 mg, 0.10 mmol, 1.0 equiv.), *R*-(-)-2-amino-1-butanol (0.048 mL, 0.51 mmol, 5.0 equiv.) and 1,4 dioxane (0.34 mL). The vial was sealed and the mixture allowed to heat at 140 °C in a sand bath for 12 h. The solvent was then removed and the crude mixture purified by chromatography on SiO_2 (2:1, hexanes:ethyl acetate) giving **8** as a white solid (30 mg, 0.083 mmol, 82%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (s, 1 H), 7.27-7.18 (m, 5 H), 6.77 (broad s, 1 H), 5.04 (broad s, 1 H), 4.62 (dd, 2 H, J = 2.4, 6 Hz), 3.83 (t, 1 H, 6.6 Hz), 3.75 (d, 1 H, J = 10.5 Hz), 3.59 (dd, 1 H, J = 7.5, 10.8 Hz), 2.94 (septet, 1 H, J = 6.9 Hz), 1.54 (m, 2 H), 1.20 (dd, 6 H, J = 0.9, 6.9 Hz), 0.95 (t, 3 H, J = 6.5 Hz),.



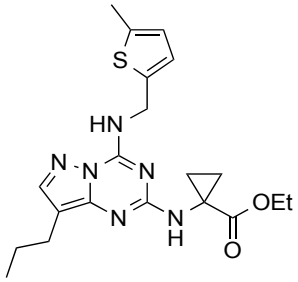
MF-06

(*R*)-2-((8-Isopropyl-4-(((5-methylthiophen-2-yl)methyl)amino)pyrazolo[1,5-*a*][1,3,5]triazin-2-yl)amino)butan-1-ol (MF-06). A flame-dried microwave vial was charged with **7b** (50 mg, 0.11 mmol, 1.00 equiv.), *R*-(-)-2-amino-1-butanol (0.053 mL, 0.055 mmol, 5.0 equiv.), KF (0.019 mg, 0.32 mmol, 3.0 equiv.) and 1,4 dioxane (0.36 mL). The vial was sealed and allowed to heat at 140 °C in a sand bath for 12 h. The reaction mixture was then quenched with water (1 mL) and extracted with dichloromethane (2x3 mL). The combined extracts were washed with brine (3 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (3:1, dichloromethane:ethyl acetate) provided **MF-06** as a viscous yellow oil (28 mg, 0.075 mmol, 68%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.59 (s, 1 H), 6.88 (broad s, 1H), 6.78 (d, 1 H, J = 3.3 Hz), 6.57 (m, 1 H), 5.15 (broad s, 1 H), 4.75 (d, 2 H, J = 6.0 Hz), 3.94 (broad s, 1H), 3.84 (dd, 1 H, J = 2.1, 10.8 Hz), 3.68 (dd, 1 H, J = 7.2, 10.8 Hz), 3.01 (septet, 1 H, J = 6.9 Hz), 1.70-1.53 (m, 2 H), 2.43 (s, 3 H), 1.27 (d, 6 H, J = 6.9 Hz), 1.04 (t, 3 H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ, 157.6, 148.5, 146.0, 143.6, 140.4, 137.0, 126.5, 124.8, 112.5, 67.8, 56.2, 39.3, 24.8, 23.4, 23.1, 15.3, 10.8; IR ν 3288, 2956, 2934, 1631, 1594, 1560 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated $[M+H]^+$ for $C_{18}H_{26}N_6OS$ 375.1967; found 375.1955.



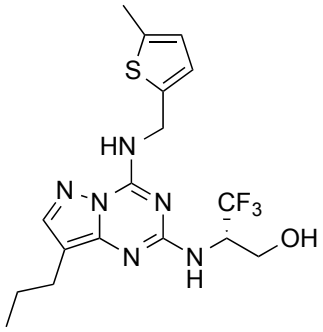
MF-17

(*R*)-2-((4-(((5-Methylthiophen-2-yl)methyl)amino)-8-propylpyrazolo[1,5-*a*][1,3,5]triazin-2-yl)amino)butan-1-ol (**MF-17).** A flame-dried microwave vial was charged with **7c** (47 mg, 0.011 mmol, 1.00 equiv.), *R*-(-)-2-amino-1-butanol (0.056 mL, 0.57 mmol, 5.0 equiv.), KF (34 mg, 0.0.57 mmol, 5.0 equiv.), and 1,4-dioxane (0.31 mL). The vial was sealed and heated at 140 °C in a sand bath for 12 h. The reaction mixture was quenched with water (2 mL) and extracted with dichloromethane (2x3 mL). The combined extracts were washed with brine (3 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (3:1, dichloromethane:ethyl acetate) provided **MF-17** (25 mg, 0.065 mmol, 57%) as a an off white solid: Mp 118.3-120.1 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.59 (s, 1 H), 6.82 (d, 1 H, J = 3.3 Hz), 6.70 (broad s, 1 H), 6.59 (m, 1 H), 4.78 (d, 2 H, J = 6.0 Hz), 3.95 (broad s, 1 H), 3.84 (d, 2 H, J = 9.6 Hz), 3.68 (dd, 2 H, J = 7.2, 10.8 Hz), 2.49 (t, 2 H, J = 7.4 Hz), 2.44 (s, 3 H), 1.68-1.57 (m, 4 H), 1.05, (t, 3 H, J = 7.5 Hz), 0.95 (t, 3 H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.7, 148.4, 146.7, 145.6, 140.4, 137.0, 126.6, 124.8, 105.8, 67.9, 56.3, 39.3, 24.8, 24.5, 23.3, 15.3, 13.8, 10.8; IR ν 3286, 2956, 2934,1592, 1564 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated $[M+H]^+$ for $C_{18}H_{26}N_6OS$ 375.1967, found 375.1960.



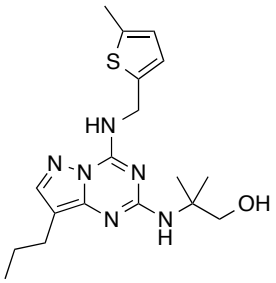
MF-27

Ethyl-1-((4-(((5-methylthiophen-2-yl)methyl)amino)-8-propylpyrazolo[1,5-*a*][1,3,5]triazin-2-yl)amino)cyclopropane-1-carboxylate (MF-27). A flame-dried microwave vial was charged with **7c** (92 mg, 0.24 mmol, 1.00 equiv.), ethyl-1-aminocyclopropanecarboxylate hydrochloride (203 mg, 1.19 mmol, 4.97 equiv.), KF (70 mg, 1.2 mmol, 5.0 equiv.), Et_3N (0.68 mL, 4.8 mmol, 20 equiv.) and 1,4-dioxane (0.8 mL). The vial was sealed and heated at 140 °C in a sand bath for 22 h; an additional portion of Et_3N (0.2 mL, 1.42 mmol, 5.9 equiv.) and 1,4-dioxane (0.4 mL) were added after 10 h. The reaction mixture was then treated with water (2 mL) and extracted with dichloromethane (3x3 mL). The combined organic extracts were washed with 1 N HCl (3 mL), with brine (3 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (98:2 to 96:4 to 95:5, dichloromethane:ethyl acetate) provided **MF-27** (12 mg, 0.026 mmol, 11%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.62 (s, 1 H), 6.80-6.70 (m, 2 H), 6.79 (d, 1 H, *J* = 3.3 Hz), 6.57 (d, 2 H, *J* = 3.3 Hz), 4.78 (d, 2 H, *J* = 5.7 Hz), 4.13, (q, 2 H, *J* = 7.1 Hz), 2.51 (t, 2 H, *J* = 6.0 Hz), 2.43 (s, 3 H), 1.67-1.59 (m, 4 H), 1.27-1.20 (m, 6 H), 1.20 (t, 3 H, J = 7.0 Hz) 0.93 (t, 3 H, *J* = 7.4 Hz)**;** ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.4, 157.4, 148.6, 145.7, 140.4, 137.0, 126.7, 124.8, 106.1, 61.1, 39.3, 35.4, 24.5, 23.3, 18.4, 15.3, 14.3, 13.8; IR ν 3188, 2953, 2923, 1726 1663, 1596 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{20}H_{26}N_6O_2S$ $[M+H]^+$ 415.1916, found 415.1909.



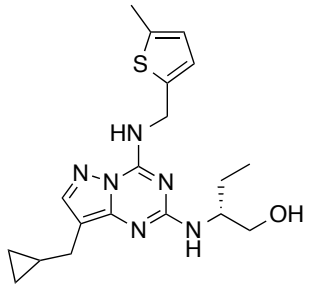
MF-59

(*S*)-3,3,3-Trifluoro-2-((4-(((5-methylthiophen-2-yl)methyl)amino)-8-propylpyrazolo[1,5-*a*][1,3,5]triazin-2-yl)amino)propan-1-ol (MF-59). A flame-dried microwave vial was charged with **7c** (49 mg, 0.12 mmol, 1.0 equiv.), (*S*)-trifluoroalaninol[5] (99 mg, 0.59 mmol, 4.9 eq.), KF (35 mg, 0.60 mmol, 5.0 equiv.), Et_3N (0.34 mL, 2.4 mmol, 20 equiv.), and 1,4 dioxane (0.48 mL). The vial was sealed and heated at 100 °C in an oil bath for 13 h and for 10 h at 120 °C. The reaction mixture was then treated with water (2 mL) and extracted with dichloromethane (2x3 mL). The combined organic extracts were washed with brine (3 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (1:0, to 9:1, to 5:1, dichloromethane:ethyl acetate) provided **MF-59** (22 mg, 0.052 mmol, 43%) as a clear oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.72 (s, 1 H), 6.91 (t, 1 H, J = 5.4 Hz), 6.83 (d, 2 H, J = 3.3 Hz), 6.60-6.58 (m, 1 H), 4.86 (d, 2 H, J = 5.7 Hz), 4.68 (dd, 1 H, J = 3.9, 11.0 Hz), 4.46 (dd, 1 H, J = 7.5 Hz, 11.0 Hz), 3.79 (ddq, 1 H, J = 3.9, 3.9, 7.5 Hz), 2.57 (t, 3 H, J = 7.5 Hz), 2.44 (s, 3 H), 1.76 (bs, 2 H), 1.65 (sextet, 2 H, J = 7.4 Hz), 0.95 (t, 3 H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.0, 149.7, 146.6, 146.1, 140.7, 136.3, 128.9, 126.9,125.5 (q, J = 280 Hz), 124.9, 108.6, 65.8, 53.3 (q, J = 28 Hz), 39.6, 24.4, 23.3, 15.3, 13.8; IR ν 3291, 2956, 2928, 2869, 1632, 1596, 1128 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{17}H_{21}F_3N_6OS$ $[M+H]^+$ 415.1528, found 415.1520.



MF-21

2-Methyl-2-((4-(((5-methylthiophen-2-yl)methyl)amino)-8-propylpyrazolo[1,5-*a*][1,3,5]triazin-2-yl)amino)propan-1-ol (MF-21). A flame-dried vial was charged with **7d** (107 mg, 0.26 mmol, 1.0 equiv.)**,** 2-amino-2-methyl-1-propanol (124 mg, 1.32 mmol, 5.0 equiv.), KF (77 mg, 1.32 mmol, 5.0 equiv.), and 1,4-dioxane (1.0 mL). The vial was sealed and heated in an oil bath for 18 h at 120 °C. The reaction mixture was treated with water (4 mL) and extracted with dichloromethane (2x10 mL). The combined organic layers were washed with brine (3 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (1:0 to 4:1, dichloromethane:ethyl acetate) provided **MF-71** (21 mg, 0.053 mmol, 27%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.59 (s, 1 H), 6.82 (d, 1 H, J = 3.2 Hz), 6.65 (bs, 1 H), 6.60 (d, 1 H, J = 2.4 Hz), 5.14 (bs, 1 H), 4.76 (d, 2 H, J = 6.0 Hz), 3.71 (d, 2 H, J = 6.0 Hz), 2.49 (t, 2 H, J = 6.6 Hz), 2.45 (s, 3 H), 1.65-1.59 (m, 3 H), 1.40 (s, 6 H), 0.94 (t, 3 H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.4, 148.3, 146.1, 145.5, 140.4, 136.8, 126.5, 124.8, 106.0, 71.9, 55.8, 39.3, 25.3, 24.5, 23.4, 15.3, 13.8; IR ν 3256, 2956, 2922, 1591, 1560 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{18}H_{27}N_6OS$ $[M+H]^+$ 375.1967, found 387.1956.



MF-71

(*R*)-2-((8-(Cyclopropylmethyl)-4-(((5-methylthiophen-2-yl)methyl)amino)pyrazolo[1,5-*a*][1,3,5]triazin-2-yl)amino)butan-1-ol (MF-71).

A flame-dried vial was charged with **7d** (84 mg, 0.20 mmol, 1.0 equiv.), (*R*)-(-)-2-amino-1-butanol (0.095 mL, 1.0 mmol, 5.0 equiv.), KF (59 mg, 1.0 mmol, 5.0 equiv.), and 1,4-dioxane (0.67 mL). The vial was sealed and heated in an oil bath for 10 h at 120 °C. The reaction mixture was treated with water (2 mL) and extracted with dichloromethane (2x3 mL). The combined organic layers were washed with 1 N HCl (2 mL) and brine (3 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on SiO_2 (5:1 to 3:1, dichloromethane:ethyl acetate) provided **MF-71** (21 mg, 0.053 mmol, 27%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (s, 1 H), 6.83 (bs, 1 H), 6.79 (d, 1 H, J = 3.2 Hz), 6.58 (d, 1 H, J = 2.4 Hz), 5.13 (bs, 1 H), 4.76 (d, 2 H, J = 5.6 Hz), 3.95 (bs, 1 H), 3.83 (dd, 2 H, J = 2.0, 11 Hz), 3.67 (dd, 2 H, J = 7.2, 11 Hz), 2.46-2.40 (m, 2 H), 2.42 (s, 3 H), 1.70-1.59 (m, 2 H), 1.06-0.94 (m, 4 H), 0.49-0.45 (m, 2 H), 0.20-0.16 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.9, 148.5, 146.8, 145.5, 140.4, 137.0, 126.5, 124.8, 105.4, 67.9, 56.2, 39.3, 27.2, 24.8, 15.3, 11.4, 10.8, 4.5, 4.5; IR ν 3340, 3292, 2960, 1633, 1592, 1562 cm^{-1}; HRMS (ESI$^+$) *m/z* calculated for $C_{19}H_{26}N_6OS$ $[M+H]^+$ 387.1967, found 387.1965.

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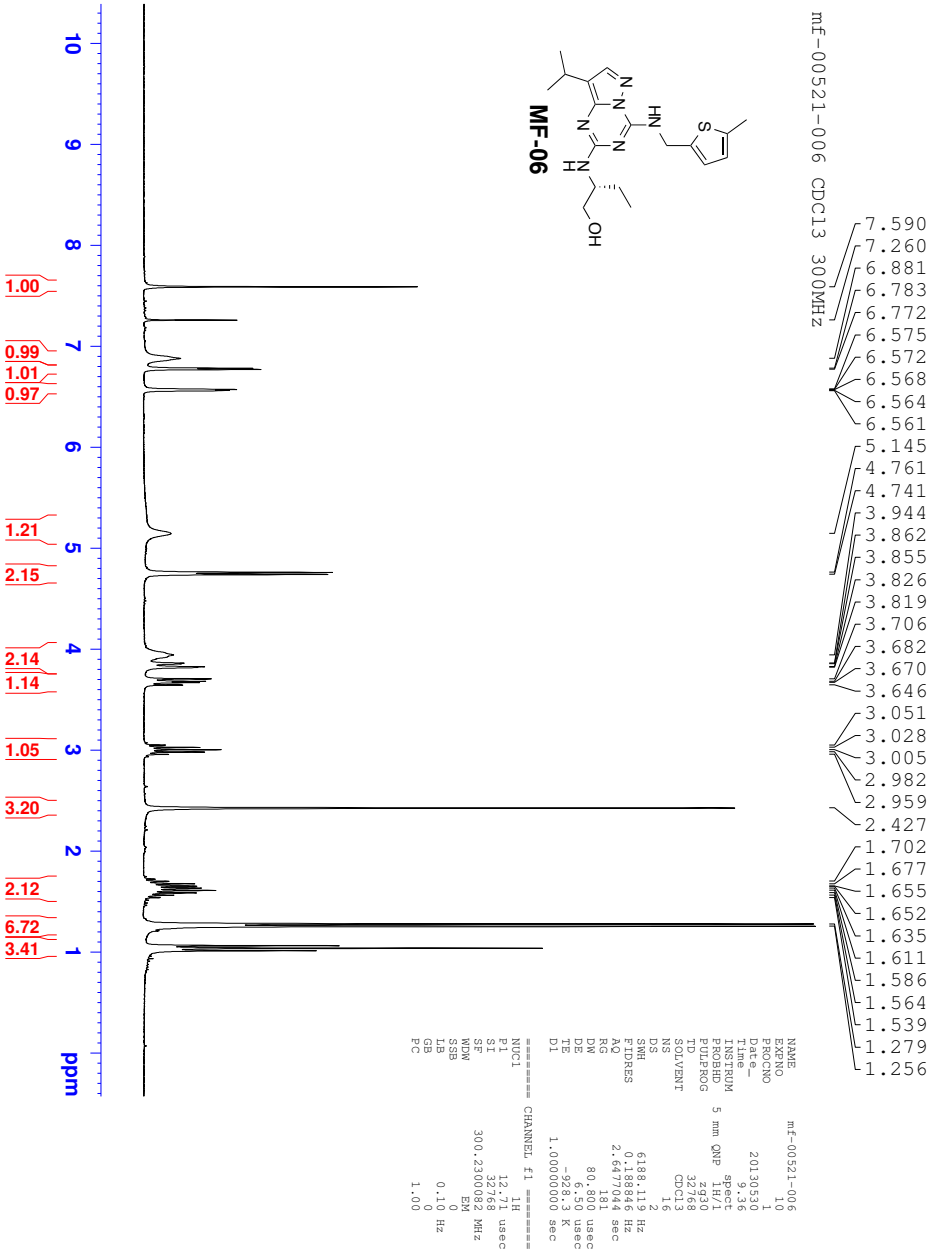
APPENDIX A

SELECTED NMR SPECTRA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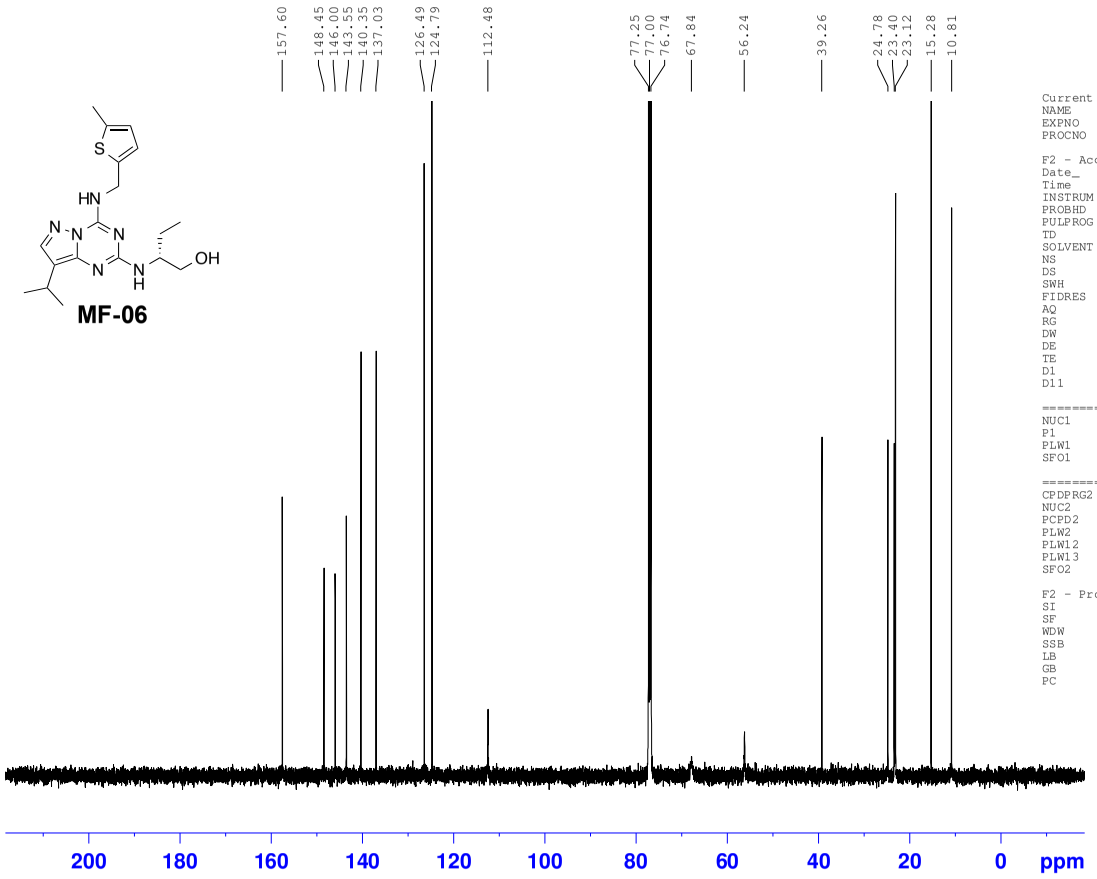
7.260
6.881
6.783
6.772
6.575
6.572
6.568
6.564
6.561
5.145
4.761
4.741
3.944
3.862
3.855
3.826
3.819
3.706
3.682
3.670
3.646
3.051
3.028
3.005
2.982
2.959
2.427
1.702
1.677
1.655
1.652
1.635
1.611
1.586
1.564
1.539
1.279
1.256
1.00
0.99
1.01
0.97
1.21
2.15
2.14
1.14
1.05
3.20
2.12
6.72
3.41
ppm

mf-00521-006 CDCl3 500MHz



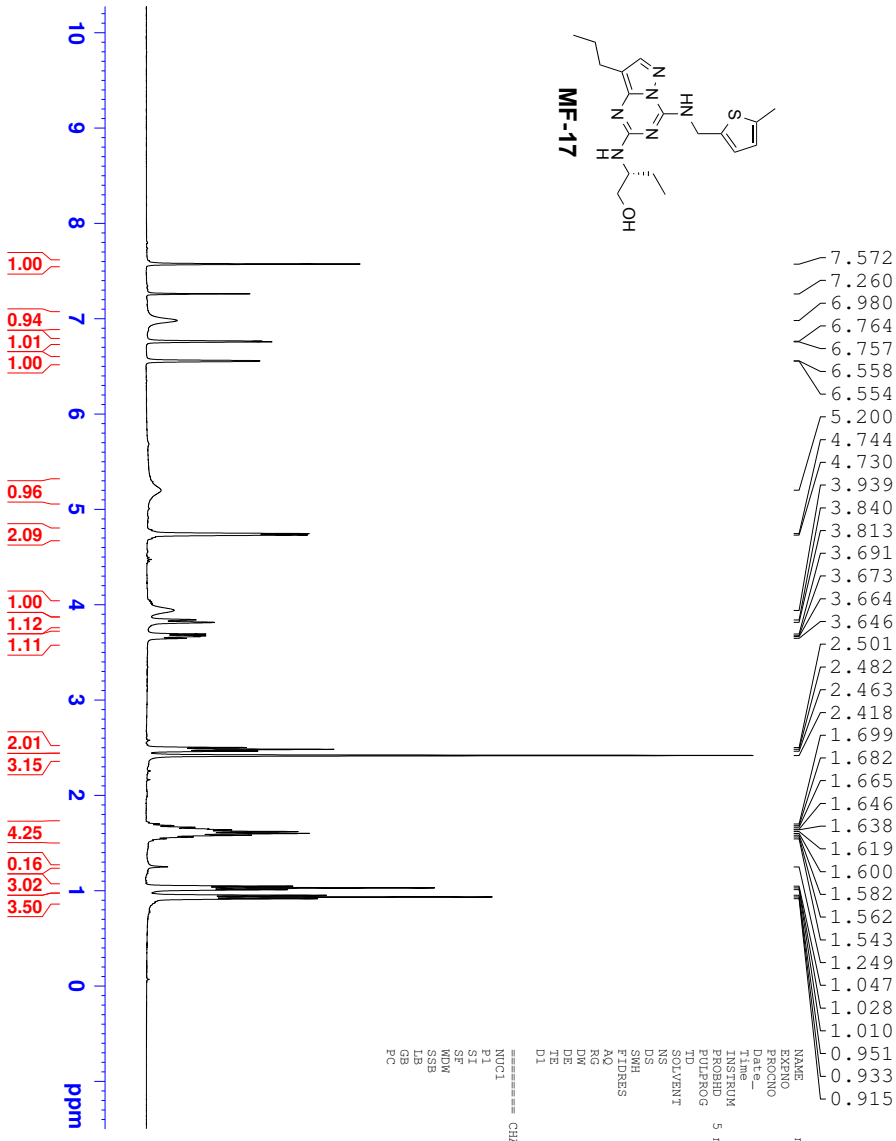

```
Current Data Parameters
NAME        mf-00521-006
EXPNO                   3
PROCNO                  1

F2 - Acquisition Parameters
Date_            20130530
Time                9.21
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 2457
DS                    4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ            1.1010548 sec
RG                  203
DW               16.800 usec
DE                6.50 usec
TE               300.0 K
D1          4.00000000 sec
D11         0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                 8.80 usec
PLW1       112.58000183 W
SFO1        125.7779080 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2            80.00 usec
PLW2        18.74300003 W
PLW12        0.36736000 W
PLW13    0 W
SFO2        500.1620006 MHz

F2 - Processing parameters
SI                 32768
SF          125.7653353 MHz
WDW                   EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.40
```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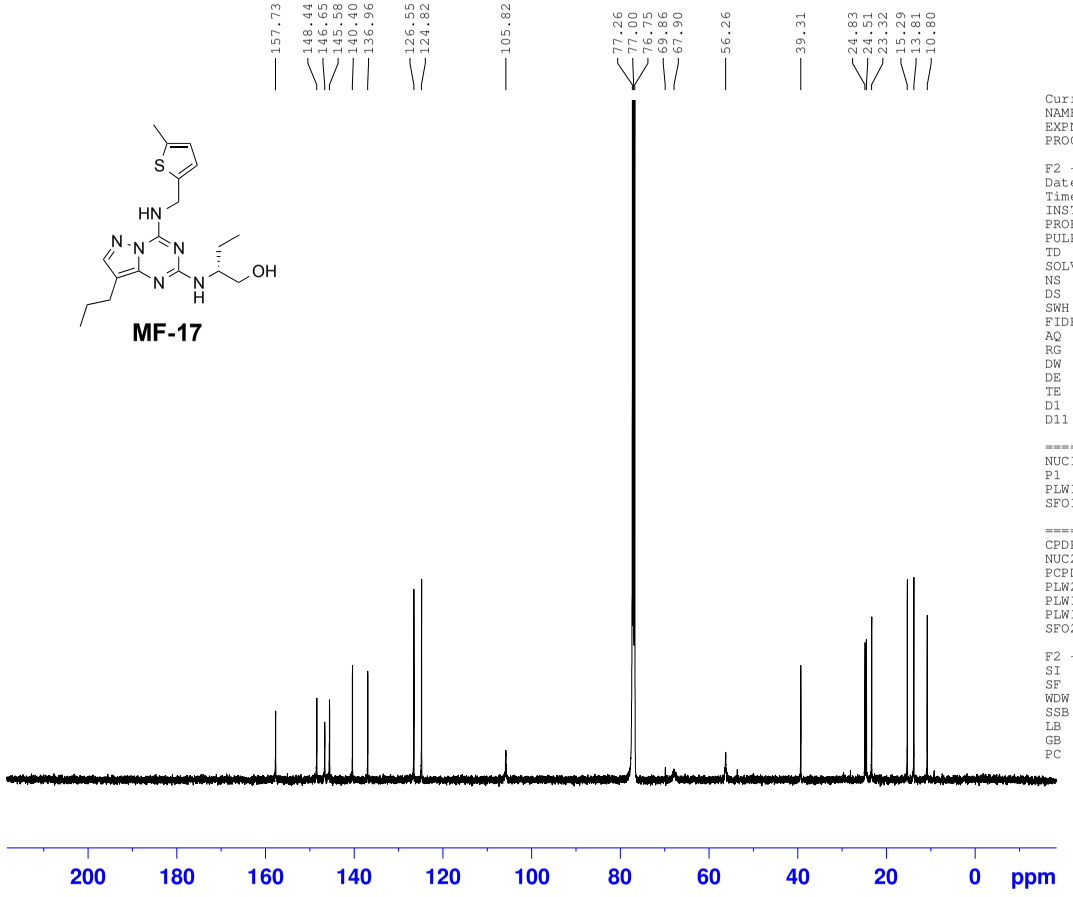
MF-17
7.572
7.260
6.980
6.764
6.757
6.558
6.554
5.200
4.744
4.730
3.939
3.840
3.813
3.691
3.673
3.664
3.646
2.501
2.482
2.463
2.418
1.699
1.682
1.665
1.646
1.638
1.619
1.600
1.582
1.562
1.543
1.249
1.047
1.028
1.010
0.951
0.933
0.915
1.00
0.94
1.01
1.00
0.96
2.09
1.00
1.12
1.11
2.01
3.15
4.25
0.16
3.02
3.50
ppm

mf-00521-063 125MHz



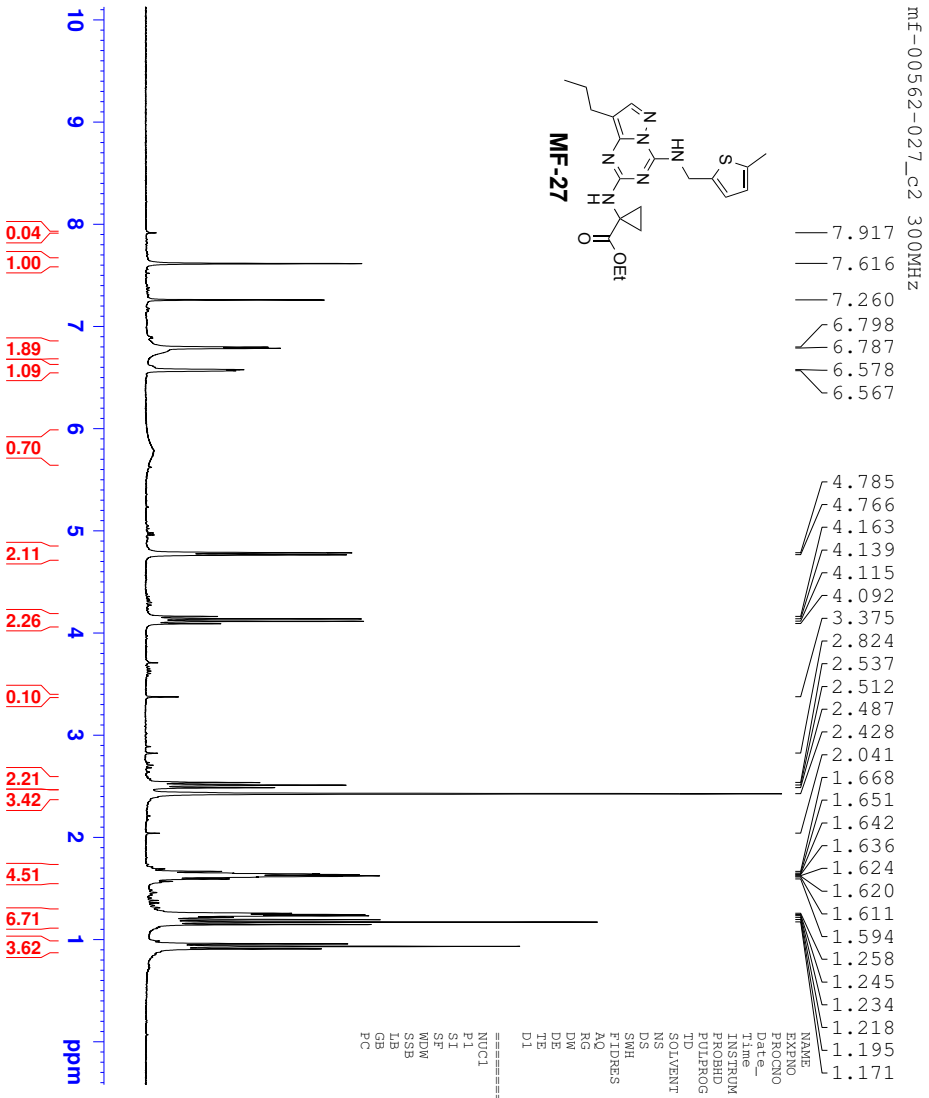

```
Current Data Parameters
NAME        mf-00521-063
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130806
Time                6.33
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  8000
DS                     4
SWH            29761.904 Hz
FIDRES         0.454131 Hz
AQ             1.1010548 sec
RG                   203
DW                16.800 usec
DE                 6.50 usec
TE                300.0 K
D1           4.00000000 sec
D11          0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                 8.80 usec
PLW1       112.58000183 W
SFO1        125.7779080 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PLW2        18.74300003 W
PLW12        0.36736000 W
PLW13    0 W
SFO2         500.1620006 MHz

F2 - Processing parameters
SI                 32768
SF          125.7653341 MHz
WDW                   EM
SSB      0
LB                 1.00 Hz
GB       0
PC                 1.40
```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MF-27
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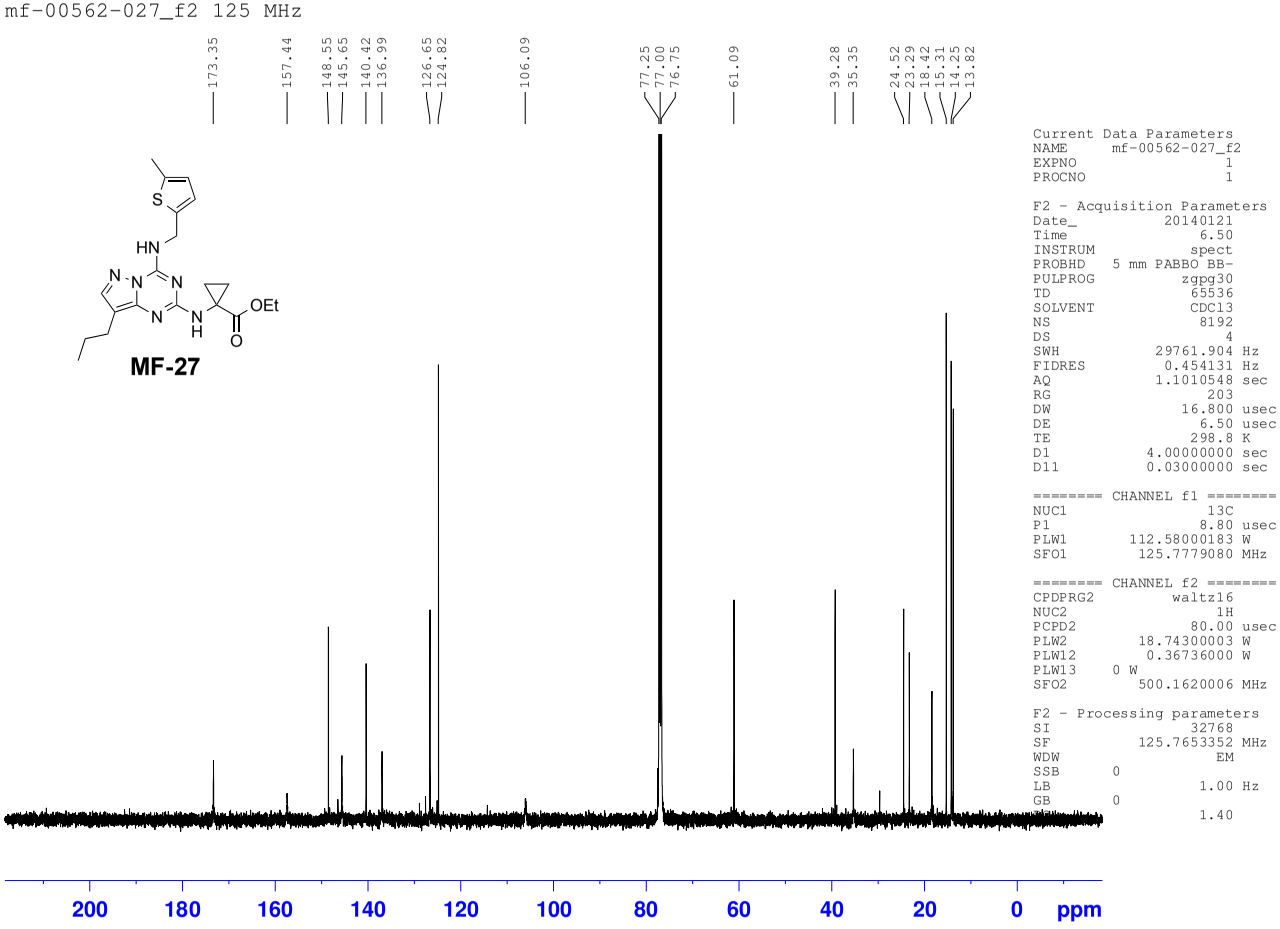
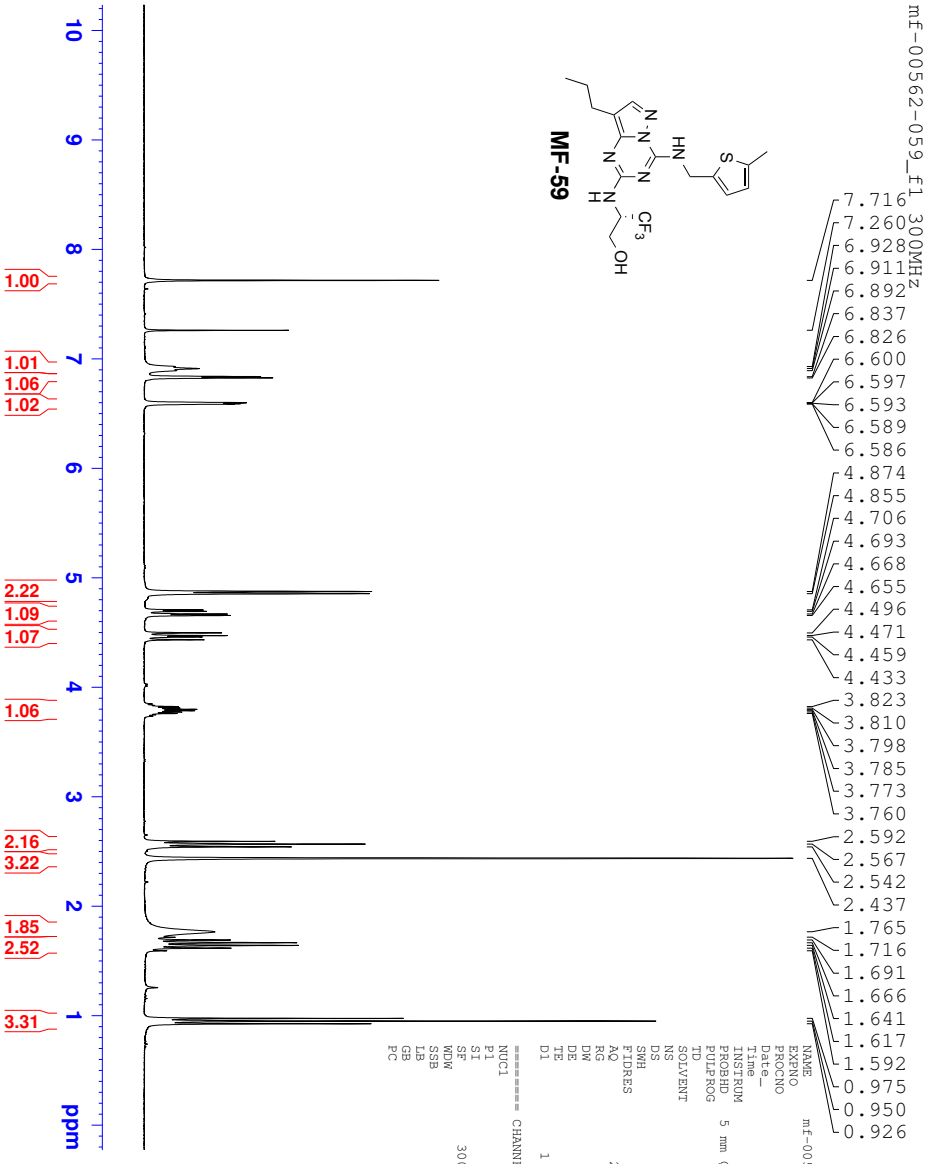
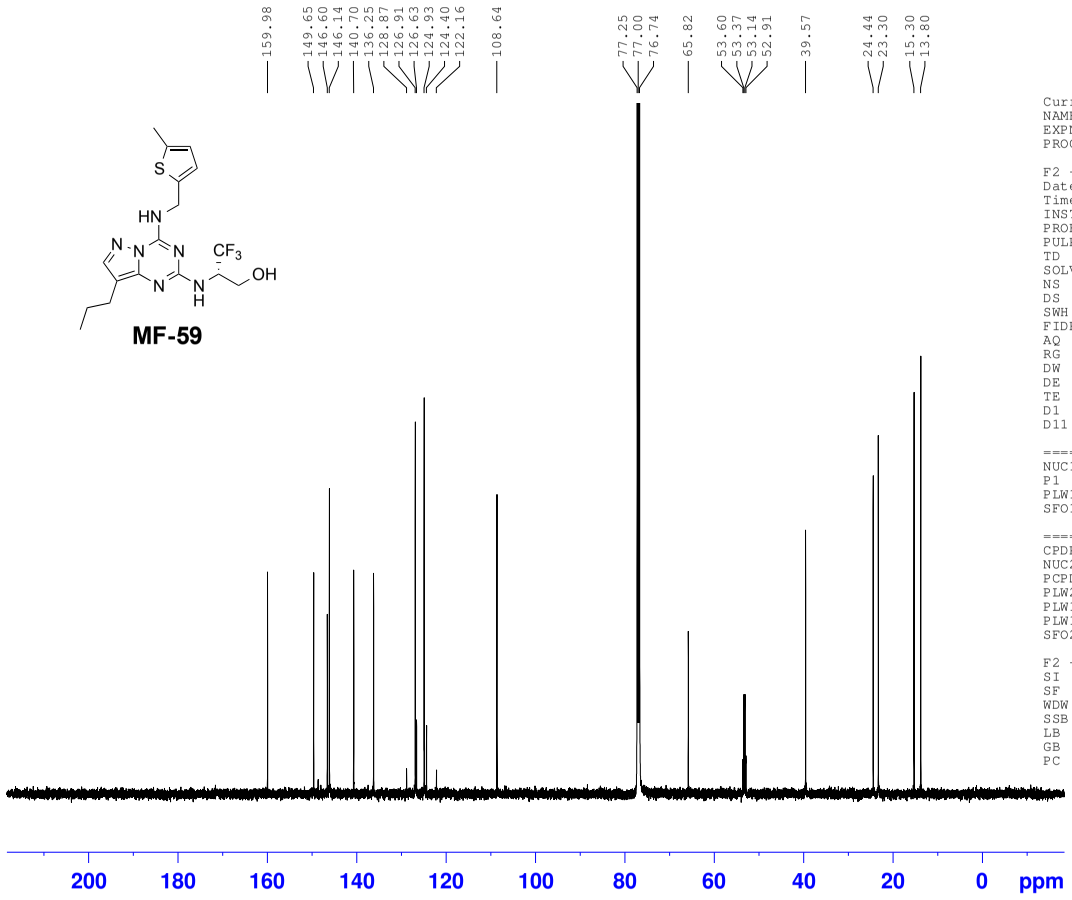
7.616
7.260
6.798
6.787
6.578
6.567
4.785
4.766
4.163
4.139
4.115
4.092
3.375
2.824
2.537
2.512
2.487
2.428
2.041
1.668
1.651
1.642
1.636
1.624
1.620
1.611
1.594
1.258
1.245
1.234
1.218
1.195
1.171
0.04
1.00
1.89
1.09
0.70
2.11
2.26
0.10
2.21
3.42
4.51
6.71
3.62
ppm



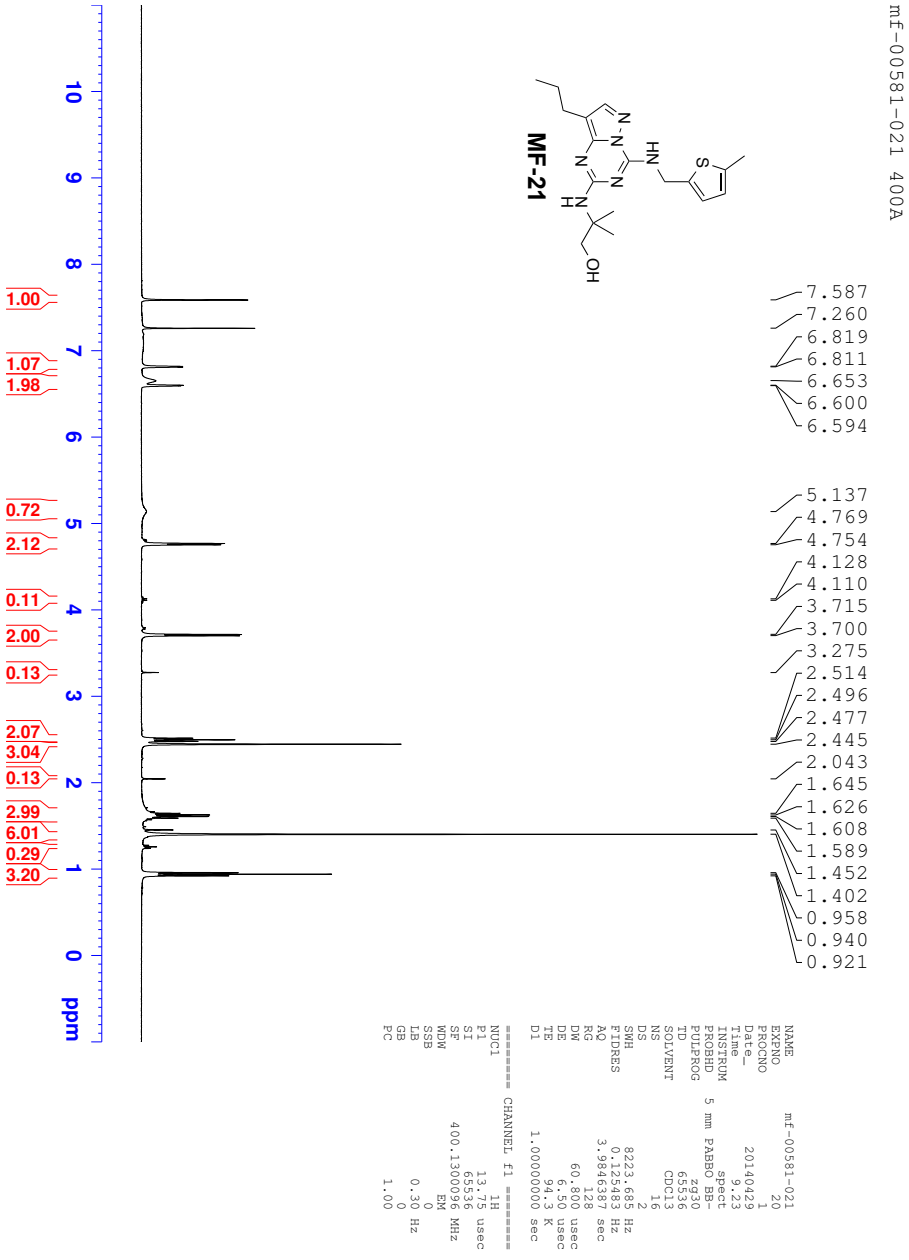

```
Current Data Parameters
NAME      mf-00562-027_f2
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20140121
Time                6.50
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  8192
DS                     4
SWH            29761.904 Hz
FIDRES         0.454131 Hz
AQ             1.1010548 sec
RG                   203
DW                16.800 usec
DE                 6.50 usec
TE                298.8 K
D1             4.00000000 sec
D11            0.03000000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                  8.80 usec
PLW1       112.58000183 W
SFO1        125.7779080 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PLW2        18.74300003 W
PLW12        0.36736000 W
PLW13    0 W
SFO2         500.1620006 MHz

F2 - Processing parameters
SI                  32768
SF           125.7653352 MHz
WDW                    EM
SSB      0
LB                  1.00 Hz
GB       0
                    1.40
```


mf-00562-059_f1 300MHz
7.716
7.260
6.928
6.911
6.892
6.837
6.826
6.600
6.597
6.593
6.589
6.586
4.874
4.855
4.706
4.693
4.668
4.655
4.496
4.471
4.459
4.433
3.823
3.810
3.798
3.785
3.773
3.760
2.592
2.567
2.542
2.437
1.765
1.716
1.691
1.666
1.641
1.617
1.592
0.975
0.950
0.926
MF-59
HN
S
N
N
N
N
H
CF3
OH
1.00
1.01
1.06
1.02
2.22
1.09
1.07
1.06
2.16
3.22
1.85
2.52
3.31
10
9
8
7
6
5
4
3
2
1
ppm
NAME mf-00562-059_f1
EXPNO 10
PROCNO 1
Date_ 20140213
Time_ 14.34
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg30
TD 32768
SOLVENT CDC13
NS 16
DS 2
SWH 6188.119 Hz
FIDRES 0.188846 Hz
AQ 2.6477044 sec
RG 128
DW 80.800 usec
DE 6.50 usec
TE -926.4 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.71 usec
SI 32768
SF 300.2300085 MHz
WDW EM
SSB 0
LB 0.10 Hz
GB 0
PC 1.00

mf-00562-059 125MHz

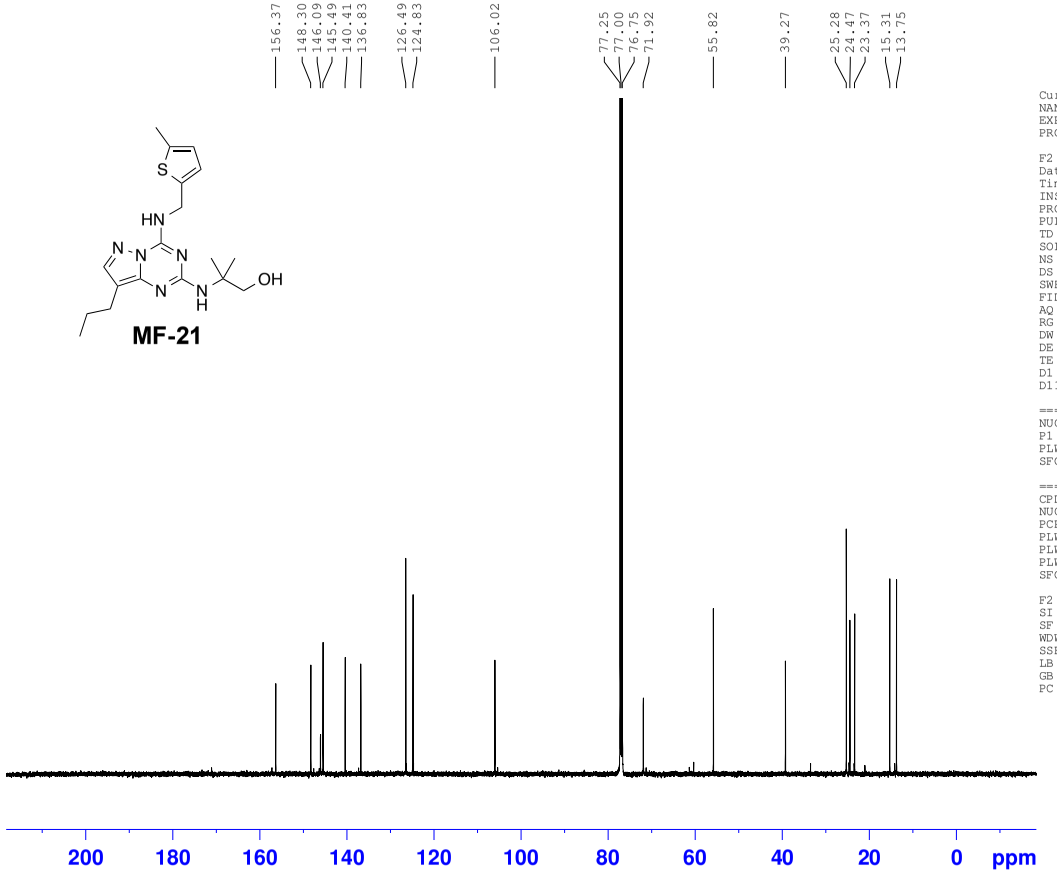



```
Current Data Parameters
NAME        mf-00562-059
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140215
Time                6.59
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT           CDC13
NS                 8344
DS                    4
SWH           29761.904 Hz
FIDRES        0.454131 Hz
AQ            1.1010548 sec
RG                  203
DW               16.800 usec
DE                6.50 usec
TE                298.4 K
D1           4.00000000 sec
D11          0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                 8.80 usec
PLW1       112.58000183 W
SFO1        125.7779080 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                1H
PCPD2            80.00 usec
PLW2        18.74300003 W
PLW12        0.36736000 W
PLW13    0 W
SFO2         500.1620006 MHz

F2 - Processing parameters
SI                 32768
SF          125.7653362 MHz
WDW                   EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.40
```

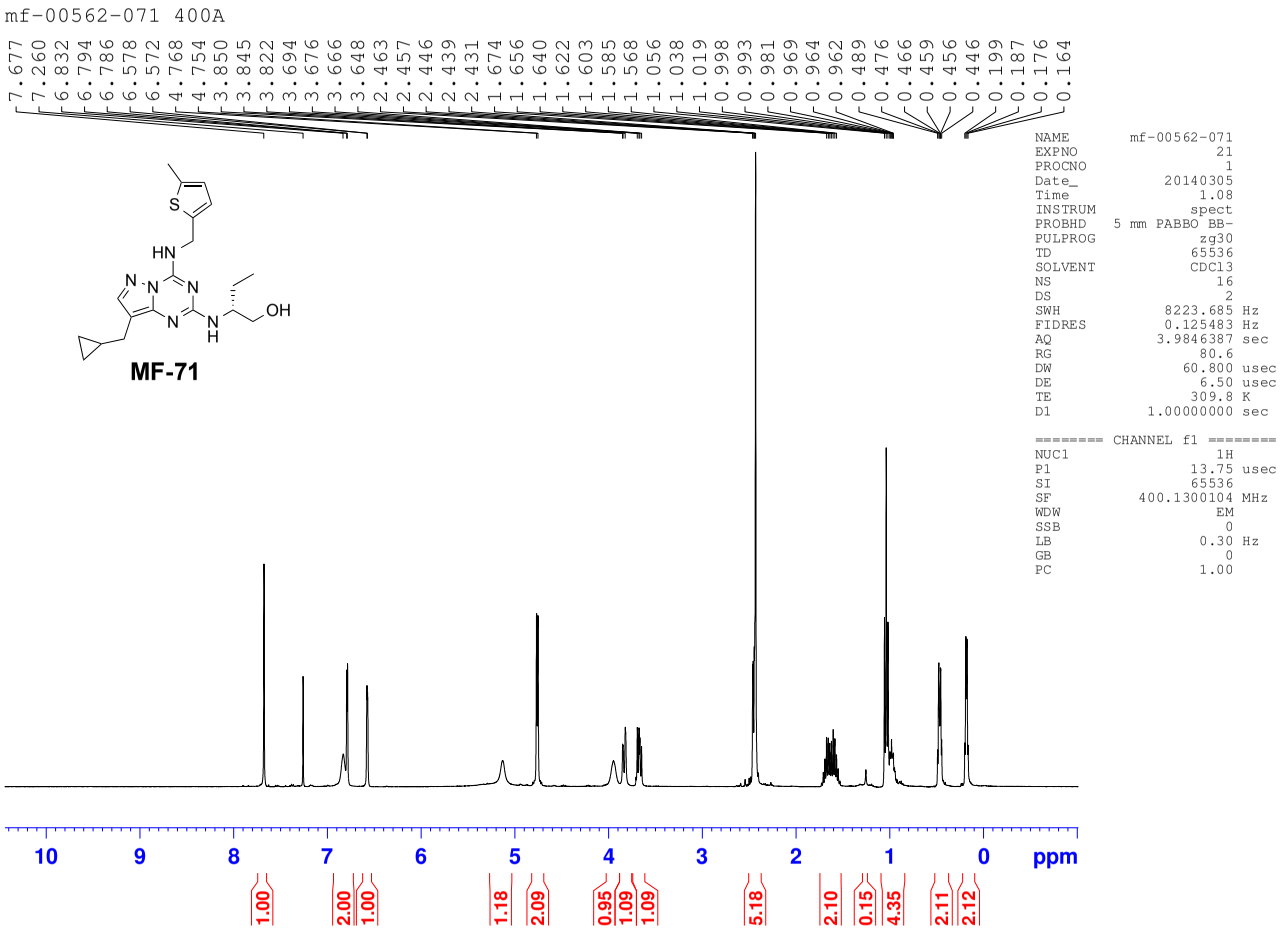


NAME mf-00581-021
EXPNO 20
PROCNO 1
Date_ 20140429
Time 9.23
INSTRUM spect
PROBHD 5 mm PABBO BB
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 128
DW 60.800 usec
DE 6.50 usec
TE 94.3 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 13.75 usec
SI 65536
SF 400.1300096 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

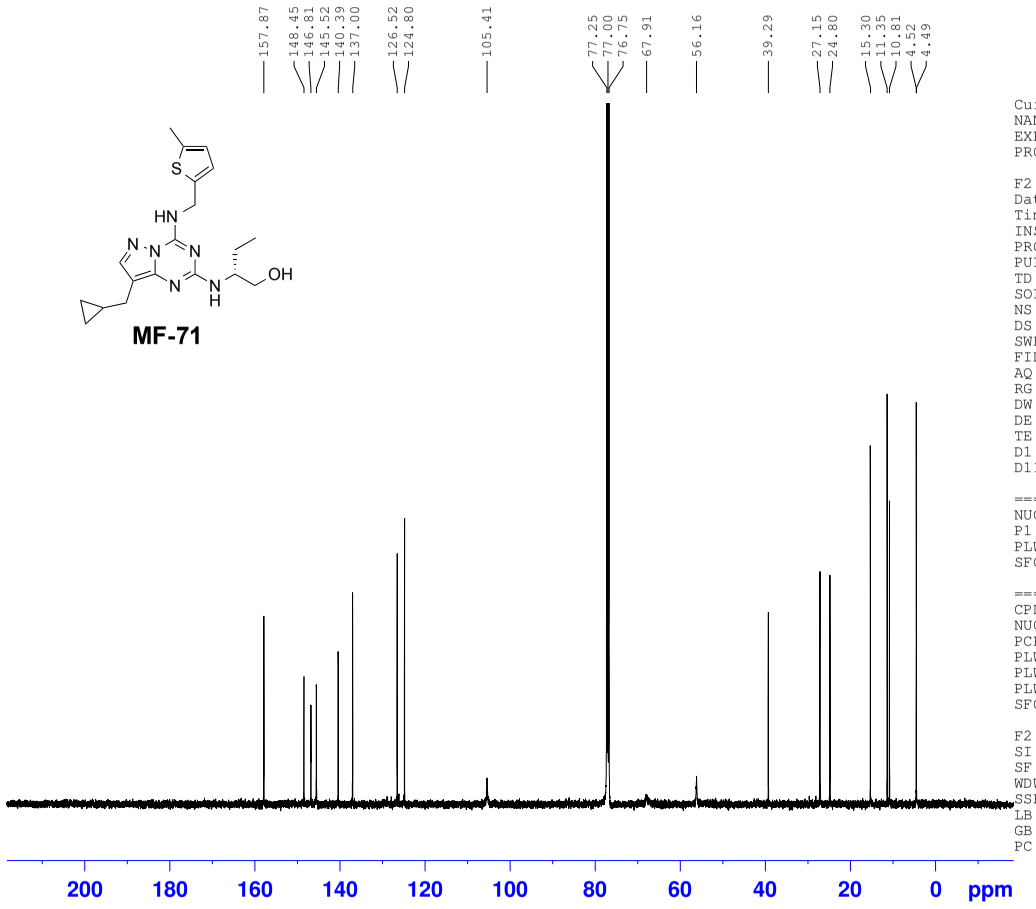
mf-00581-021 125 MHz




```
Current Data Parameters
NAME         mf-00581-020
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20140429
Time                8.58
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 2617
DS                    4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ             1.1010548 sec
RG                  203
DW                16.800 usec
DE                 6.50 usec
TE                298.0 K
D1            3.00000000 sec
D11           0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                 8.80 usec
PLW1       112.58000183 W
SFO1        125.7779080 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2              80.00 usec
PLW2        18.74300003 W
PLW12        0.36736000 W
PLW13     0 W
SFO2         500.1620006 MHz

F2 - Processing parameters
SI                 32768
SF          125.7653371 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```


mf-00562-071 400A
7.677
7.260
6.832
6.794
6.786
6.578
6.572
4.768
4.754
3.850
3.845
3.822
3.694
3.676
3.666
3.648
2.463
2.457
2.446
2.439
2.431
1.674
1.656
1.640
1.622
1.603
1.585
1.568
1.056
1.038
1.019
0.998
0.993
0.981
0.969
0.964
0.962
0.489
0.476
0.466
0.459
0.456
0.446
0.199
0.187
0.176
0.164
MF-71
NAME mf-00562-071
EXPNO 21
PROCNO 1
Date_ 20140305
Time 1.08
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 80.6
DW 60.800 usec
DE 6.50 usec
TE 309.8 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 13.75 usec
SI 65536
SF 400.1300104 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
2.00
1.00
1.18
2.09
0.95
1.09
1.09
5.18
2.10
0.15
4.35
2.11
2.12

mf-00562-071




```
Current Data Parameters
NAME        MF-00562-071
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140306
Time                7.13
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  7024
DS                     4
SWH            29761.904 Hz
FIDRES          0.454131 Hz
AQ             1.1010548 sec
RG                   203
DW                16.800 usec
DE                 6.50 usec
TE                299.3 K
D1             4.00000000 sec
D11            0.03000000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                  8.80 usec
PLW1        112.58000183 W
SFO1         125.7779080 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PLW2         18.74300003 W
PLW12         0.36736000 W
PLW13     0 W
SFO2         500.1620006 MHz

F2 - Processing parameters
SI                  32768
SF           125.7653352 MHz
WDW                  EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```