

UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
FACULTAD DE CIENCIAS QUÍMICAS E INGENIERÍA



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“Una nueva perspectiva estructural para ligandos selectivos del receptor nicotínico de acetilcolina $\alpha 7$ ”

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Presenta:

M.C. Gisela Andrea Camacho Hernández

DIRECTOR DE TESIS:
Dr. José Manuel Cornejo Bravo

CO-DIRECTOR DE TESIS:
Dr. Palmer W. Taylor

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C. Gisela Andrea Camacho Hernández
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El tema de trabajo y/o tesis para su examen profesional, en la
Opción TESIS

Es propuesto, por los C. Dres. José Manuel Cornejo Bravo y Palmer W. Taylor

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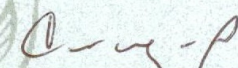
el cual deberá usted desarrollar, de acuerdo con el siguiente orden:

- I.- INTRODUCCIÓN
- II.- REFINAMIENTO QUÍMICO SINTÉTICO
- III.- EVALUACION FARMACOLÓGICA
- IV.- ESTUDIOS CRISTALOGRAFICOS
- V.- ANÁLISIS QUÍMICOS COMPARATIVO DE AGONISTAS CLÁSICOS
Y LA FAMILIA DE PIRIMINAS
- VI.- RESULTADO Y DISCUSIÓN
- VII.- CONCLUSIONES

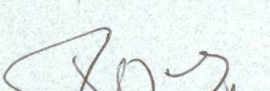
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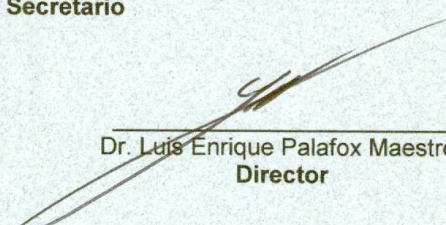


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Dr. José Manuel Cornejo Bravo
Director de Tesis


Dr. José Luis González Vázquez
Sub-Director Secretario


Dr. Palmer W. Taylor
Co-Director de Tesis


Dr. Luis Enrique Palafox Maestre
Director

ÍNDICE

AGRADECIMIENTOS	I
RESUMEN (ESPAÑOL)	III
RESUMEN (INGLES)	IV
ÍNDICE DE TABLAS	V
ÍNDICE DE FIGURAS	VII
ÍNDICE DE ESQUEMAS	XIII
ÍNDICE DE ABREVIACIONES	XV
OBJETIVOS GENERALES Y PARTICULARES	XIX
CAPÍTULO I. INTRODUCCIÓN	1
1.1. Canales iónicos pentaméricos regulados por ligando	2
1.2. Receptores nicotínicos de acetilcolina	2
1.3. Farmacología de $\alpha 7$ nAChR, enfermedades y desórdenes asociadas	5
1.3.1. Rol de $\alpha 7$ nAChR en Alzheimer	7
1.3.2. Rol de $\alpha 7$ nAChR en esquizofrenia	8
1.4. Ligandos selectivos de $\alpha 7$ nAChR	10
1.5. Proteínas de unión a acetilcolina	13
CHAPTER II. REFINAMIENTO QUÍMICO SINTÉTICO	16
CHAPTER III. PHARMACOLOGICAL EVALUATION	58
3.1. Materiales y métodos	59
3.1.1. Ensayo de unión a radioligando	60
3.1.1.1. Antecedentes	60
3.1.1.2. Expresión proteica y purificación	61
3.1.1.3. Ensayo rápido	61
3.1.1.4. Curvas concentración-respuesta y determinación de K_d	61
3.1.2. Ensayo celular funcional	62
3.1.2.1. Antecedentes	62
3.1.2.2. Ensayo rápido	63
3.1.2.3. Curvas concentración-respuesta, determinación de EC_{50} y K_A	64
3.1.3. Electrofisiología	65
3.1.3.1. Antecedentes	65

3.1.3.2. Expresión de nAChR en <i>Xenopus laevis</i> ovocitos	66
3.1.3.3. Curvas concentración-respuesta	67
CAPÍTULO. ESTUDIOS CRISTALOGRAFICOS	68
4.1. Materiales y métodos	69
4.2. Formación de complejos y cristalización	70
4.3. Recolección de datos, ajuste de fase y refinamiento	70
4.4. Estadísticas de refinamiento	72
CAPÍTULO V. ANÁLISIS QUÍMICOS COMPARATIVO DE AGONISTAS CLÁSICOS Y LA FAMILIA DE PIRIMIDINAS	73
5.1. Química estructural de agonistas clásicos de nAChR	74
5.2. Experimentos ¹ H RMN pKa	75
CAPÍTULO VI. RESULTADOS Y DISCUSIÓN	78
CAPÍTULO VII. CONCLUSIONES	122
REFERENCIAS	125
ANEXOS	135
AP 1. ¹ H RMN	136
AP 2. ¹³ C APT RMN	149
AP 3. HR-MS	158

CONTENTS

ACKNOWLEDGMENTS	I
ABSTRACT (SPANISH)	III
ABSTRACT (ENGLISH)	IV
LIST OF TABLES	V
LIST OF FIGURES	VII
LIST OF SCHEMES	XIII
LIST OF ABBREVIATIONS	XV
GENERAL AND MAJOR GOALS	XIX
CHAPTER I. INTRODUCTION	1
1.1. Pentameric ligand-gated ion channels	2
1.2. Nicotinic acetylcholine receptors	2
1.3. Pharmacology of the $\alpha 7$ nAChR and associated disease and disorder	5
1.3.1. Role of $\alpha 7$ nAChR in Alzheimer's disease	7
1.3.2. Role of $\alpha 7$ nAChR in schizophrenia	8
1.4. Selective ligands of the $\alpha 7$ nAChR	10
1.5. Acetylcholine binding proteins	13
CHAPTER II. CHEMICAL REFINEMENT	16
2.1. Chemicals	17
2.2. Synthetic strategies	19
2.2.1. Nucleophilic aromatic substitution: substitution of the di-picoly System	19
2.2.2. Cross coupling Suzuki reaction: Modulation at the 6 th position of pyrimidine system	20
2.2.3. Direct reductive amination: preparing an amine bulding block	21
2.2.4. Synthesis of sulfoxides and sulfones: Modulation at 2 nd position of pyrimidine system	22
2.3. Synthetic procedures	23
2.3.1 General procedure for the synthesis of 4-chloro-6-aminopyrimidin-2-amines.	23
2.3.2. General procedure for synthesis of 6-aryl-2,4-diaminopyrimidine.	24
2.3.3. General procedure for the synthesis of 6-aryl-2-methylthio-4-	24

aminopyrimidines.	
2.3.4. 6-chloro- N^4,N^4 -bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17).	25
2.3.5. N^4,N^4 -dibenzyl-6-chloropyrimidine-2,4-diamine(AC-19).	26
2.3.6. 6-chloro- N^4,N^4 -bis(pyridin-3-ylmethyl)pyrimidine-2,4-diamine (AC-20).	27
2.3.7. N^4,N^4 -bis(pyridin-2-ylmethyl)-6-(4 (trifluoromethyl) phenyl) pyrimidine-2,4-diamine (AC-17-2).	28
2.3.8. 6-(4-methoxyphenyl)- N^4,N^4 -bis(pyridin-2-ylmethyl) pyrimidine-2,4-diamine (AC-171A).	29
2.3.9. 6-(2-methoxyphenyl)- N^4,N^4 -bis(pyridin-2-ylmethyl) pyrimidine-2,4-diamine (AC-171A2)	30
2.3.10. 6-(3-methoxyphenyl)- N^4,N^4 -bis(pyridin-2-ylmethyl) pyrimidine-2,4-diamine (AC-171A3)	31
2.3.11. 6-(4-(dimethylamino)phenyl)- N^4,N^4 -bis(pyridin-2-ylmethyl) pyrimidine-2,4-diamine (AC-171B).	32
2.3.12. 4-(2-amino-6-(bis(pyridin-2-ylmethyl)amino)pyrimidin-4-yl)phenol (AC-171C).	33
2.3.13. N^4,N^4 -bis(pyridin-2-ylmethyl)-6-(thiophen-3-yl)pyrimidine-2,4-diamine (AC-171D).	34
2.3.14. 6-(furan-3-yl)- N^4,N^4 -bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-171E).	35
2.3.15. N^4,N^4 -bis(pyridin-2-ylmethyl)-6-(pyridin-4-yl)pyrimidine-2,4-diamine (AC-171F).	36
2.3.16. N^4,N^4 -bis(pyridin-2-ylmethyl)-6-(pyridin-3-yl)pyrimidine-2,4-diamine (AC-171F3).	37
2.3.17. 6-(3,4-dimethoxyphenyl)- N^4,N^4 -bis(pyridin-2-ylmethyl) pyrimidine-2,4-diamine (AC-171G).	38
2.3.18. 6-(2,4-dimethoxyphenyl)- N^4,N^4 -bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-171H).	39
2.3.19. N^4,N^4 -bis(pyridin-2-ylmethyl)-6-(3,4,5-trimethoxyphenyl) pyrimidine-2,4-diamine (AC-171I).	40
2.3.20. 6-(1 <i>H</i> -indol-5-yl)- N^4,N^4 -bis(pyridin-2-ylmethyl)pyrimidine-2,4-	41

diamine (AC-171J).	
2.3.21. 6-(4-fluoro-2-methoxyphenyl)- <i>N</i> ⁴ , <i>N</i> ⁴ -bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-171M).	42
2.3.22. <i>N</i> ⁴ , <i>N</i> ⁴ -dibenzyl-6-(4-(trifluoromethyl)phenyl)pyrimidine-2,4-diamine (AC-19-2).	43
2.3.23. <i>N</i> ⁴ , <i>N</i> ⁴ -bis(pyridin-3-ylmethyl)-6-(4-(trifluoromethyl)phenyl)pyrimidine-2,4-diamine (AC-20-2).	44
2.3.24. 6-(2-methoxyphenyl)- <i>N</i> ⁴ , <i>N</i> ⁴ -bis(pyridin-3-ylmethyl)pyrimidine-2,4-diamine (AC-201A2).	45
2.3.25. <i>N</i> -benzyl-1-(pyridin-2-yl)methanamine.	46
2.3.26. 6-chloro-2-(methylthio)- <i>N,N</i> -bis(pyridin-2-ylmethyl)pyrimidin-4-amine (AL-1).	47
2.3.27. <i>N</i> ⁴ -benzyl-6-chloro- <i>N</i> ⁴ -(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AL-2).	48
2.3.28 6-chloro- <i>N,N</i> -bis(pyridin-2-ylmethyl)pyrimidin-4-amine (AL-4).	49
2.3.29. 6-chloro- <i>N,N</i> -bis(pyridin-2-ylmethyl)-2-(trifluoromethyl)pyrimidin-4-amine (AL-6).	50
3.2.30. 6-(2-methoxyphenyl)-2-(methylthio)- <i>N,N</i> -bis(pyridin-2-ylmethyl)pyrimidin-4-amine(AL-1A).	51
2.3.31. 2-(methylthio)- <i>N,N</i> -bis(pyridin-2-ylmethyl)-6-(thiophen-3-yl)pyrimidin-4-amine(AL-1B).	52
2.3.32. 6-(2-methoxyphenyl)-2-(methylsulfonyl)- <i>N,N</i> -bis(pyridin-2-ylmethyl)pyrimidin-4-amine(AL-1A2).	53
2.3.33. 6-(2-methoxyphenyl)-2-(methylsulfinyl)- <i>N,N</i> -bis(pyridin-2-ylmethyl)pyrimidin-4-amine(AL-1A3).	54
2.3.34. <i>N</i> ⁴ -benzyl-6-(2-methoxyphenyl)- <i>N</i> ⁴ -(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AL-2A).	55
2.3.35. 6-(2-methoxyphenyl)- <i>N,N</i> -bis(pyridin-2-ylmethyl)pyrimidin-4-amine (AL-4A).	56
2.3.36. 6-(2-methoxyphenyl)- <i>N,N</i> -bis(pyridin-2-ylmethyl)-2(trifluoromethyl) pyrimidin-4-amine(AL-6A).	57
CHAPTER III. PHARMACOLOGICAL EVALUATION	58
3.1. Material and methods	59

3.1.1. Radioligand binding assay	60
3.1.1.1. Background	60
3.1.1.2. Protein expression and purification	61
3.1.1.3. Quick screen	61
3.1.1.4. Titration curves and determination of K_d	61
3.1.2. Cell-based functional assay	62
3.1.2.1. Background	62
3.1.2.2. Quick screen	63
3.1.2.3. Titration curves, determination of EC_{50} and K_A values.	64
3.1.3. Two-electrode voltage clamp electrophysiology	65
3.1.3.1. Background	65
3.1.3.2. Expression of nAChR in <i>Xenopus laevis</i> oocytes	66
3.1.3.3. Titration curves	67
CHAPTER IV. CRYSTALLOGRAPHIC STUDIES	68
4.1. Material and methods	69
4.1.1. Vapor diffusion: Hanging drop method.	69
4.2. Complex formation and crystallization	70
4.3. Data collection, phasing, and model refinement.	70
4.4. Refinement statistics.	72
CHAPTER V. CHEMICAL COMPARATIVE ANALYSIS OF CLASSICAL AGONIST AND THE PYRIMIDINES FAMILY	73
5.1. Chemistry of the classical nAChRs agonist	74
5.2. 1H NMR pKa experiments	75
CHAPTER VI. RESULTS AND DISCUSSION	78
6.1. Chemical synthesis	79
6.1.1. Synthetic rationel and synthetic pathways of series AC	79
6.1.2. Synthetic rationel and synthetic pathways of series AL	82
6.1.3. Structure elucidation	86
6.2. <i>in vitro</i> pharmacological analysis	98
6.2.1. Cell-based functional assay results	98
6.2.2. Radioligand binding assay results	110
6.2.3. Two-electrode voltage clamp electrophysiology	113

6.3. X-ray Crystallographic Analysis of the Ligands AChBP Complexes	116
CHAPTER VII. CONCLUSIONS	122
REFERENCES	125
APENDIX. ¹H AND ¹³C APT NMR AND HIGH-RESOLUTION MASS SPECTROMETRY SPECTRA.	135
AP 1. ¹H NMR	136
AP 2. ¹³C APT NMR	149
AP 3. HR-MS spectra	158
CV Gisela Andrea Camacho Hernández	174

“I love those who can smile in trouble, who can gather strength from distress, and grow brave by reflection. ‘Tis the business of little minds to shrink, but they whose heart is firm and whose conscience approves their conduct, will pursue their principles unto death” Leonardo da Vinci

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A través de estudios de unión de ligandos a la proteína de unión a acetilcolina (AChBP), previamente identificamos una serie de 4-aminopirimidinas disustituidas que se asocian con este sustituto soluble del receptor nicotínico de acetilcolina (nAChR) de forma cooperativa, interacción nunca observada antes en la literatura por clásicos agonistas y antagonistas nicotínicos. Para evaluar la interacción de esta familia de compuestos en canales iónicos regulados por ligando, utilizamos células HEK transfectadas por ADNc's que codifican para uno de los tres subtipos a evaluar: $\alpha 7$ -nAChR, $\alpha 4\beta 2$ -nAChR y 5HT_{3A}R, junto con un reportero fluorescente. El proceso de selección inicial de una serie de más de cincuenta compuestos 2-aminopirimidinas con afinidad por AChBP mostró que solo dos compuestos activaron de manera parcial $\alpha 7$ -nAChR. El objetivo principal de esta tesis fue diseñar y sintetizar agonistas selectivos de $\alpha 7$ -nAChR tomando 2-aminopirimidas como modelo. Dieciocho nuevos compuestos fueron sintetizados y caracterizados en la primera fase del Proyecto generando la serie AC, los ligandos más potente son AC-171A2, AC-171H para $\alpha 7$ -nAChR lo cuales tuvieron EC₅₀'s 70nM EC₅₀'s y K_d's para AChBP en un rango similar. Algunos compuestos de esta serie exhiben especificidad por $\alpha 7$ -nAChR, mostrando no activación o antagonismo por $\alpha 4\beta 2$ -nAChR o 5HT₃R mientras que otros mostraron débil respuesta antagonista (o agonista parcial) en estos mismos receptores. Para evaluar la activación rápida de los candidatos principales, utilizamos un sistema de ovocitos que revela un comportamiento agonista que los distingue de los agonistas silenciosos y los ago-PAM. El análisis posterior a la co-cristalización de cuatro ligandos con *Ls*-AChBP muestra interacción del compuesto en la interfaz de la subunidad, con una orientación o una posición de unión que difiere de los agonistas y antagonistas nicotínicos clásicos y de las 2-aminopirimidinas previamente sintetizadas las cuales mostraron una distinta interacción con AChBP. Las orientaciones de las cadenas laterales aromáticas de estos complejos son distintivas, lo que sugiere nuevos modos de unión en el sitio agonista-antagonista y quizás un modo alostérico de acción para nAChR heteroméricas. En la segunda etapa del proyecto, ampliamos la serie, compuestos AL, lo que nos permitió definir los parámetros estructurales determinantes de la activación selectiva de $\alpha 7$ -nAChR de estos nuevos tipos de ligandos selectivos. Hemos encontrado: 1) el grupo dipicolyl amina en la posición 4 del anillo pirimidínico es fundamental para la activación de la $\alpha 7$ -nAChR, 2) el 2-amino no es crucial para la activación de $\alpha 7$ -nAChR pero afecta la selectividad entre $\alpha 4\beta 2$ -nAChR y 5HT₃AR, 3) la modulación en la posición 6 del anillo pirimidínico afecta la potencia y la selectividad de los ligandos.

Through studies with ligand binding to the acetylcholine binding protein (AChBP), we previously identified a series of 4,6 substituted 2-aminopyrimidines that associate with this soluble surrogate of the nicotinic acetylcholine receptor (nAChR) in a cooperative fashion, not seen for classical nicotinic agonists and antagonists. To examine receptor interactions of this family of compounds on ligand-gated ion channels, we employed HEK cells transfected with cDNA's encoding one of three requisite receptor subtypes: $\alpha 7$ -nAChR, $\alpha 4\beta 2$ -nAChR and 5HT_{3A}R, along with a fluorescent reporter. Initial screening of a series of over 50 2-aminopyrimidines with affinity for AChBP showed only two of the compounds to be partial agonists on the $\alpha 7$ -nAChR below 10 μ M concentration. The main goal of this dissertation was to design and synthesize selective agonists of the $\alpha 7$ -nAChR taking advantage of previous findings. Eighteen new compounds were synthesized and characterized at the first phase of the project leading to the AC series, the most potent ligands AC-171A2, AC-171H had $\alpha 7$ -nAChR EC₅₀'s 70nM EC₅₀'s and K_d's for AChBP in a similar range. Several compounds within this series exhibit specificity for the $\alpha 7$ -nAChR, showing no activation or antagonism of $\alpha 4\beta 2$ -nAChR or 5HT_{3R} while others were weaker antagonists (or partial agonists) on these receptors. To assess fast activation of lead candidates we use an oocyte system, revealing an agonist behavior distinguishing them from silent agonists and ago-PAM's. Analysis following co-crystallization of four ligand complexes with AChBP show binding at the subunit interface, but with an orientation or binding pose that differs from classical nicotinic agonists and antagonists and from the previously analyzed set of 2-aminopyrimidines that displayed distinct cooperative interactions with AChBP. Orientations of the aromatic side chains of these complexes are distinctive, suggesting new modes of binding at the agonist-antagonist site and perhaps an allosteric mode of action for heteromeric nAChRs. In the second phase of the project we expanded the series, AL compounds, enabling us to define the molecular determinants of the selective activation of $\alpha 7$ -nAChR of these new types of selective ligands.

We have found: 1) the di-picolylyl moiety in the 4-position of the pyrimidine ring is fundamental for the activation of the $\alpha 7$ -nAChR, 2) the 2-amino is not crucial for the activation of $\alpha 7$ -nAChR but affects selectivity among $\alpha 4\beta 2$ -nAChR and 5HT_{3A}R, 3) modulation in the 6-position affects the potency and selectivity of the ligands. As we continue to increase our library we expect more information to come.

LIST OF TABLES

Table 1.	$\alpha 7$ -nAChR agonists and PAM's recently or currently in clinical trials. (Information found in clinicaltrials.gov 2017, Beinat, C. <i>et al.</i> 2015, Bertrand, D. <i>et al.</i> 2009, Hashimoto, K. 2015).	11	
Table 2.	Data collection and refinement statistics.	72	
Table 3.	Characteristics and yield of AC series.	81	
Table 4.	Characteristics and yield of AL series.	85	
Table 5.	Molecular weight detected by HR-MS of AC and AL series, all spectra are found in APENDIX.	87	
Table 6.	Summarized ^1H -NMR data of AC-17 in CDCl_3 and $\text{DMSO}-d_6$ at different temperatures.	88	
Table 7.	^1H NMR assignments of compounds of AC series.	97	
Table 8.	EC_{50} values for $\alpha 7$ -nAChR agonist activation and antagonist dissociation constants for $\alpha 4\beta 2$ -nAChR and $5\text{HT}_{3\text{A}}$ receptor antagonism.	102	
Table 9.	EC_{50} values for $\alpha 7$ -nAChR agonist activation.	107	
Table 10.	Physicochemical and pharmacokinetic properties predicted of two series.	109	
Table 11.	Dissociation constants for 4,6 disubstituted 2-aminopyrimidines determined by competitive back titrations against ^3H -epibatidine on SPA beads linked to purified <i>Lymnaea stagnalis</i> (<i>Ls</i>) and <i>Aplysia californica</i> (<i>Ac</i>) AChBPs (Talley, T. T. <i>et al.</i> 2006).	112	

LIST OF FIGURES

Figure 1.	a) Parallel view of the $\alpha 4\beta 2$ nAChR. b) Apical view of the $\alpha 4\beta 2$ nAChR (figure taken from Morales-Perez, C. 2016).	3
Figure 2.	Ligand overlay of complexes including nicotine (plum) in <i>L. stagnalis</i> -AChBP (magenta) (Celie et al., 2004) and epibatidine (salmon) in <i>A. californica</i> AChBP (beige) (Hansen et al., 2005) (PDB 1UW6 and 2BYQ), Triazole 4 in turquoise and tropisetron in yellow. Nitrogen atom distances from the quaternary tropane of 4, the tertiary tropane of tropisetron, the pyrrolidine of nicotine, and the azabicycloheptane of epibatidine to the backbone carbonyl oxygen of Trp147 are shown. A hydrogen bond between the N3 atom of triazole 4 and a water molecule (red sphere) is comparable to the hydrogen bond between water (magenta sphere) and the pyridine ring of nicotine (Figure and text taken from Yamauchi, J. et al. 2012).	4
Figure 3.	Possible subunit assemblies to form functional subtypes, the triangles represent the principal face of the binding site whereas the C or the semi-circle represents the complementary face (Gotti, C. <i>et al.</i> 2006, figure taken from Taly, A. <i>et al.</i> 2009).	5
Figure 4.	Broad distribution of nAChRs in the Brain (Gotti, C. <i>et al.</i> 2006, figure taken from Taly, A. <i>et al.</i> 2009).	6
Figure 5.	Smoking prevalence among individuals with schizophrenia, bipolar disorder, or no psychiatric disorder (N=1938), by age and smoking status at year of enrollment (Figure and text taken from Dickerson, F. <i>et al.</i> 2017).	9
Figure 6.	Model of the role of AChBP in neurotransmission. A basal level of AChBP is present in the synaptic cleft. Presynaptic ACh release can lead to activation of postsynaptic receptors and to EPSPs. In parallel, nAChRs on glia are activated, causing increased release of AChBP into the synapse, which leads to suppression of cholinergic transmission (figure and text taken from Smit, A. B. et al. 2001).	14

Figure 7.	Apical view of apo <i>Ac</i> -AChBP (blue) overlap with the muscle-type nAChR (gray) (Unwin, N. 2005) (Structure taken of Hansen, S.B. <i>et al.</i> 2005).	15
Figure 8.	The scintillation proximity assay. When the AChBP is bound to a SPA-beads the proximity of radiolabel ligand will activate the bead and this will result in emission of light. When ligand displace radiolabel epibatidine, the energy of unbound ligand will be absorb and scatter.	60
Figure 9.	Upon activation of calcium permeable receptor, Ca^{+2} enters the cell and upon binding to TN-XXL produces a conformational change eliciting a fluorescence change reflected in quenching of the donor fluorescence and excitation of the acceptor (figure and text taken from Yamauchi, J. <i>et al.</i> 2011).	63
Figure 10.	a) <i>Xenopus laevis</i> oocyte impaled by two glass electrodes. b) OpusXpress system, has automatic impalement, and can measure eight oocytes at the same time (Papke, R. L.; Stokes, C. 2010).	66
Figure 11.	Process of vapor diffusion.	69
Figure 12.	Crystal of AC-171A2.	70
Figure 13.	Data collection via remote connection at the Advanced Light Source Synchrotron in Berkeley, California.	71
Figure 14.	Structures of classical agonist of the nAChRs compared to 2-amino pyrimidines	74
Figure 15.	^1H NMR of 171A2 in phosphate buffer at different pH.	75
Figure 16.	^1H NMR peak position of proton of pyrimidine system as a function of pH.	76
Figure 17.	^1H NMR peak position of one proton of dipicolyl system as a function of pH.	77
Figure 18.	Structures of final compounds of AC series.	80
Figure 19.	Structures of final compounds of AL series.	84
Figure 20.	High resolution-Mass Spectrometry (HR-ESI-MS) of AC-171A2 .	86

Figure 21.	¹ H NMR spectra of AC-17, experiment conditions: A) CDCl ₃ at 25 °C, B) DMSO- <i>d</i> ₆ at 50 °C, C) DMSO- <i>d</i> ₆ at 25 °C.	89
Figure 22.	¹³ C (APT mode) NMR spectra of AC-17.	90
Figure 23.	HMBC NMR spectra of AC-17.	91
Figure 24.	HSQC NMR spectra of AC-17.	92
Figure 25.	¹ H NMR spectra of AC-171A2.	93
Figure 26.	¹³ C (APT mode) NMR spectra of AC-171A2.	94
Figure 27.	COSY NMR spectra of AC-171A2.	95
Figure 28.	HMBC NMR spectra of AC-171A2.	96
Figure 29.	HSQC NMR spectra of AC-171A2	96
Figure 30.	Functional quick screen of compounds of the AC series with cell line expressing $\alpha 7$ -nAChR. The FRET ratio (Δ/Δ_{max}) was normalized, when compounds showed agonist response higher than 0.2 they were further characterized as agonist. When compounds showed inhibition higher the 0.5, they were further characterized as antagonist.	99
Figure 31.	Concentration-response curve of compounds AC-171A, AC-171A2, AC-171C AC-171D and AC-171H, utilizing HEK cell line expressing $\alpha 7$ -nAChR.	100
Figure 32.	(A) Concentration-response curve of compounds AC-171A3, AC-171B, AC-171E, AC-171F, utilizing HEK cell line expressing $\alpha 7$ -nAChR. (B) Concentration-response curve of compounds AC-171F3, AC-171G, AC-171J, AC-171M, utilizing HEK cell line expressing $\alpha 7$ -nAChR.	101
Figure 33.	$\alpha 4\beta 2$ -nAChR selected antagonist concentration-response curves of AC series. A) Compound AC-171B, and B) Compound AC-171H.	103
Figure 34.	$\alpha 4\beta 2$ -nAChR antagonist concentration-response curves of lead compound AC-171A2.	104
Figure 35.	5HT _{3A} serotonin receptor antagonist concentration-response curves of AC series. A) Compound AC-20-2, B) Compound AC-171A, and C) AC-171J.	105

Figure 36.	Concentration-response curve of compound AC-171A2, AL-1A, AL-4A, and AL-6A, utilizing HEK cell line expressing $\alpha 7$ -nAChR.	106
Figure 37.	Functional quick screen $\alpha 7$ -nAChR comparing activity between AC-171A2 (lead compound of series AC) and AL-2A.	107
Figure 38.	Titration of the <i>Ls</i> -AChBP at various concentration of ligand competing againsts - ^3H -epibatidine binding by the respective 4-dibenzyl and 4-dipicolyl substituted 2-aminopyrimidines measured using a scintillation proximity assay (Talley, T. T. <i>et al.</i> 2006). K_d values are determined from the K_d of epibatidine and the concentrations of epibatidine and competing ligand.	111
Figure 39.	Activation of $\alpha 7$ -nAChR expressed in oocyte system. Activation by acetylcholine and AC-171A2; washing periods of 3.5 min were spaced between application of tested compounds.	113
Figure 40.	30 μM of AC-171A2 and AC-171H converts $\alpha 7$ -nAChR to a PNU-120596 sensitive state in an oocyte system; washing periods of 3.5 min were spaced between application of tested compounds.	114
Figure 41.	Almost full recovery of $\alpha 7$ -nAChR when exposed to 3 μM concentration of AC-171C as well as AC-171D. But a much slower recovery was detected when exposed to 30 μM of AC-171D.	115
Figure 42.	(A) Radial view of <i>Ls</i> -AChBP pentameric structure in complex with AC-171A2. A principal, C loop-containing, and complementary face are shown in violet and fushia. (B) Expanded radial view of AC-171A2 in the binding site. Side chain carbons of the principal (grey) and complementary (fusia) subunits are designated with black and red numbers, respectively. Ligand carbons displayed in yellow, nitrogens in blue, oxygen in red.	117

Figure 43.	(C) Superimposition of X-ray crystal structure of AC-171A2, that interacts more extensively with Gln55 and the Cys-loop S-S bridge in the <i>Ls</i> -AChBP with X-ray crystal structures of AC-171C, AC-171D and AC-171A. (D) Superimposition of AC-171A2 with nicotine (PDB code 1UW6, in orange).	119
Figure 44.	(E) Superposition of AC-171A2 with positively cooperative ligand 15 (PDB code 4QAA; ligand carbons shown in blue, protein in turquoise). (F) Superposition of AC-171A2 with negatively cooperative ligand 33 (PDB code 4QAC).	121

LIST OF SCHEMES

Scheme 1.	First step in the synthetic pathway. Conditions: (a) Amine, DIPEA, DMF, 2-6 h.	19
Scheme 2.	Mechanism of a nucleophilic aromatic substitution reaction (Joule, J. A.; Mills, K. 2013).	19
Scheme 3.	Second step of the synthetic pathway: (b) Boronic acid, Na ₂ CO ₃ , Pd(PPh ₃) ₄ , Toluene/Ethanol, 95 °C for 8 h-overnight. (c) Boronic acid, K ₂ CO ₃ 2M, Pd(dppf)Cl ₂ , DMA, 95 °C for 2 h-overnight.	20
Scheme 4.	Cross coupling Suzuki reaction general mechanism.	21
Scheme 5.	Reductive amination of aldehydes and ketones yield primary, secondary or tertiary amines.	22
Scheme 6.	Thioether oxidation utilizing <i>m</i> -CPBA as oxidant reagent.	22
Scheme 7.	Proposed mechanism of oxidation of sulfide by <i>m</i> -CPBA. The subsequent oxidation of the sulfoxide to afford the sulfone follows the same path, it will need another equivalent of <i>m</i> -CPBA.	23
Scheme 8.	Synthetic pathway leading to 4,6-substituted 2-aminopyrimidines. Conditions: (a) Amine, DIPEA, DMF, 2-6 h; (b) Boronic acid, Na ₂ CO ₃ , Pd(PPh ₃) ₄ , Toluene/Ethanol, 95 °C for 8 h-overnight. (c) Boronic acid, K ₂ CO ₃ 2M, Pd(dppf)Cl ₂ , DMA, 95 °C for 2 h-overnight.	78
Scheme 9.	Reductive amination; (1) pyridine-2-carboxaldehyde, EtOH, over night. (2) NaBH ₄ , 0 °C, 2 hrs.	82
Scheme 10.	Synthetic pathway leading to 2,4,6-substituted pyrimidines. Conditions: (a) 2-Dipicolyl amine, DIPEA, DMF, 3.5 h; (b) 2-methoxyphenyl boronic acid, K ₂ CO ₃ 2M, Pd(dppf)Cl ₂ , DMA, 95 °C for 18 h.	82
Scheme 11.	Synthetic pathway leading to 4,6-substituted 2-thiomethyl pyrimidines. Conditions: (a) 2-Dipicolyl amine, DIPEA, DMF, 3.5 h; (b) Boronic acid, K ₂ CO ₃ 2M, Pd(dppf)Cl ₂ , DMA, 95 °C for 18 h. (c) <i>m</i> -CPBA, CH ₂ Cl ₂ , 0°C, 45 min.	83

LIST OF ABBREVIATIONS

^{13}C-APT	Attached proton test
5-HT₃Rs	Serotonin type 3 receptors
Aβ	Amyloid- β
Ac	Aplysia californica
ACh	Acetylcholine
AChBP	Acetylcholine binding proteins
aCSF	Artificial cerebrospinal fluid
AD	Alzheimer's disease
BBB	Blood Brain Barrier
cDNA	Complementary deoxyribonucleic acid
CFP	Cyan Fluorescent protein
CNiFERs	Cell-based neurotransmitter fluorescent engineered reporters
CNS	Central nervous system
COSY	Homonuclear correlation spectroscopy
cRNA	Complementary ribonucleic acid
Cys	Cysteine
DIPEA	N,N-Diisopropylethylamine
DMA	<i>N,N</i> -dimethylacetamide
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EC₅₀	Half maximal effective concentration
ECD	Extracellular domain
EPI	Epibatidine
EtOAc	Ethylacetate
FBS	Fetal bovine serum
FRET	Förster resonance energy transfer
GABA	γ -aminobutyric acid
Gln	Glutamine
GlyRs	Glycine receptors
GnTI⁻	<i>N</i> -acetylglucosaminyltransferase I

H-bond	Hydrogen bond
HEK	Human embryonic kidney
HMBC	Heteronuclear multiple bond correlation
HR-ESI-MS	High resolution electrospray ionisation mass spectrometry
HR-ESI-TOFMS	High resolution electrospray ionisation time-of-flight mass spectrometry
HR-MS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
LBD	Ligand binding domain
Leu	Leucine
Ls	Lymnaea stagnalis
<i>m</i>-CPBA	Meta chloroperbenzoic acid
MLA	Methyllycaconitine
MW	Molecular weight
nAChRs	Nicotinic acetylcholine receptors
NMR	Nuclear magnetic resonance
PAM	Positive allosteric modulator
PB	Phosphate buffer
pH	Potential of hydrogen
pLGIC	Pentameric ligand gated ion channels
PNS	Peripheral nervous system
ppm	Parts-per-million
SD	Standard deviation
S_NAr	Nucleophilic aromatic substitution
SPA	Scintillation proximity assay
Thr	Threonine
TLC	Thin layer chromatography
TMD	Transmembrane domain
TnC	Troponin C
Trp	Tryptophan
Tyr	Tyrosine

LIST OF ABBREVIATIONS

α-Btx	α -bungarotoxin
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GENERAL AND MAJOR GOALS

General Goal

To generate lead nAChRs modulators that interact selectively with $\alpha 7$ -nAChR and to understand the determinants of activity.

Major Goals

To synthesize a library of 4,6-disubstituted 2-aminopyrimidines for screening against AChBPs and stable cell lines expressing $\alpha 7$ -nAChR, $\alpha 4\beta 2$ -nAChR and 5-HT_{3A} serotonin receptor.

To characterize the intermediates and final products of the synthesis by NMR, Mass Spectrometry and X-ray crystallography.

To perform radioligand binding assays using acetylcholine binding proteins: *Lymnea stagnalis*, *Aplysia californica* and *Aplysia californica* Y55W mutant.

To perform cell based assays (LGIC-CNIFERS) to study functional responses of nAChRs and serotonin receptors elicited by generated agents.

X-ray crystallographic studies of representative ligand-acetylcholine binding protein complexes to understand the basis of the interaction.

CHAPTER I. INTRODUCTION

1.1. Pentameric Ligand-gated ion channels

Ion channels are transmembrane proteins that regulate in a selective mode the movement of ions across cellular membranes (Green, W. N; Millar, N. S. 1995). Pentameric ligand gated-ion channels (pLGIC) respond primarily to chemical signals and secondarily to changes in the membrane potential (voltage gated ion channels), their activation happens when a specific neurotransmitter binds to the extracellular surface causing an opening of a central channel, allowing the passing of ions across the membrane with the subsequent depolarization or hyperpolarization (Purves, D. 2001). Fast synaptic transmission results in two main mechanisms: excitation mediated mainly by glutamate; acetylcholine and serotonin, and inhibition mediate by GABA and glycine (Smart, T. G. 2012). Nicotinic acetylcholine receptors (nAChRs), serotonin type 3 receptors (5-HT₃Rs), aminobutyric acid type A receptors (GABA_ARs), glycine receptors (GlyRs) and zinc-activated ion channels are the five mammalian receptors that comprises pLGIC (Nemecz, A. *et al.* 2016).

1.2. Nicotinic acetylcholine receptors

Nicotinic acetylcholine receptors (nAChRs) are members of the Cys-loop family, they are excitatory neurotransmitter receptors, activated by the endogenous neurotransmitter acetylcholine (ACh), nAChRs are present in the peripheral (PNS) and central nervous system (CNS). In the CNS, they are found presynaptically where they modulate the release of several neurotransmitters: dopamine, noradrenaline, GABA, glutamate (Turner, J. R. And Kellar, K. J. 2005) and postsynaptically; they are also present on non-neuronal cells where they control multiple functions.

These pentameric integral proteins have an approximate molecular mass of 290 kDa. Based in structural studies, the neuronal (Morales-Perez, C. *et al.* 2016) and muscle (Unwin, N. 2005) nAChRs consist in five subunits arrange around a central membrane spanning pore that is permeable to cations. This cationic channel allows Na⁺, K⁺ and Ca⁺² ions diffuse through the membrane, resulting in depolarization of the membrane (Figure 1a-b). Each subunit has a large N-terminal extracellular domain (ECD), a transmembrane domain (TMD), that comprise four transmembrane spanning segments and a variable cytoplasmic domain (Morales-Perez, C. *et al.* 2016; Unwin, N. 2005; Taly, A. *et al.* 2009) (**Figure 1a**).

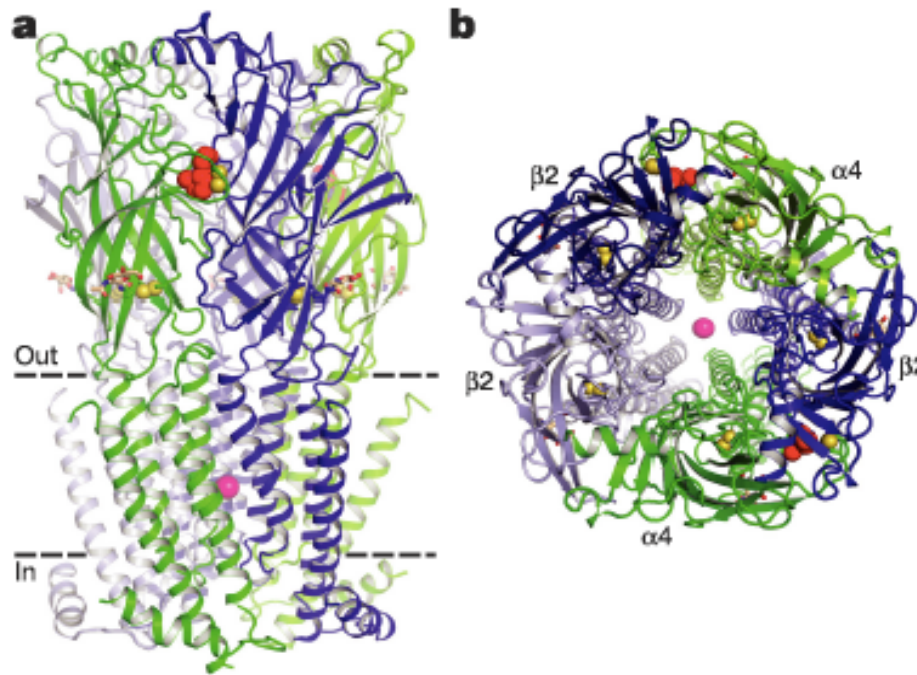


Figure 1. a) Parallel view of the $\alpha 4\beta 2$ nAChR. **b)** Apical view of the $\alpha 4\beta 2$ nAChR (figure taken from Morales-Perez, C. 2016).

Seventeen nAChR subunits have been identified in vertebrate species, $\alpha 1$ - $\alpha 10$, $\beta 1$ - $\beta 4$, γ , δ and ϵ . All of them with the exception of $\alpha 8$ (avian species) are found in humans (Millar, N.S; Gotti, C. 2009). Of the seventeen possible subunits, nine α ($\alpha 2$ - $\alpha 10$) and three β ($\beta 2$ - $\beta 4$) are neuronal. 2012). Neuronal nAChRs are comprised of only α and β subunits, and they can assemble in homopentamers or heteropentamers (Figure 3); this confers them a variety of possible ways of assembly which leads to a broad diversity of nAChR subtypes, each subtype has different pharmacology, physiology and distribution throughout the CNS (Gotti, C. *et al.* 2006, Taly, A. *et al.* 2009) (**Figure 4**), the $\alpha 7$ -nAChR for instance, is highly expressed in the hippocampus and cortex, where it modulates cognition, sensory process, attention and working memory.

There are between two and five ACh binding sites within the ECD, that is topographically different from the cationic ion channel (**figure 1b**), located within the symmetry axis of the TMD, to which it is functionally connected (Taly, A.; Charon, S, 2012). Nicotinic agonists binding takes place between subunit interface, consist in a cation- π interaction between a highly conserved tryptophan and the cationic moiety of the nicotine pharmacophore or the acetylcholine, a hydrogen bond of a protonated amine (N^+H) from the agonist and the backbone carbonyl of the same tryptophan, this last interaction it is

not true for acetylcholine, which lacks of N^+H , and a hydrogen bond between a molecule of water and the pyridine nitrogen of nicotine (**Figure 2**).

Acute exposure to ACh results in transient μ to msec opening of the channel. Prolonged exposure can lead to a gradual decrease in the ionic response, from msec to min, that can be attributed to a higher affinity close state of the receptor called, desensitized state. Chronic exposure to nAChRs ligands results in changes in receptor responses and expression and localization (up-regulation).

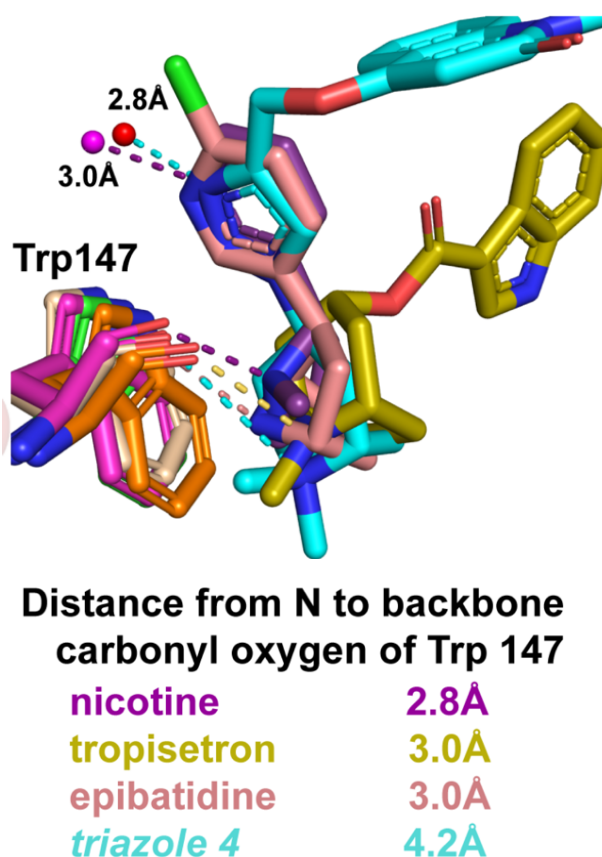


Figure 2. Ligand overlay of complexes including nicotine (plum) in *L. stagnalis*-AChBP (magenta) (Celie et al., 2004) and epibatidine (salmon) in *A. californica* AChBP (beige) (Hansen et al., 2005) (PDB 1UW6 and 2BYQ), Triazole **4** in turquoise and tropisetron in yellow. Nitrogen atom distances from the quaternary tropane of **4**, the tertiary tropane of tropisetron, the pyrrolidine of nicotine, and the azabicycloheptane of epibatidine to the backbone carbonyl oxygen of Trp147 are shown. A hydrogen bond between the N3 atom of triazole **4** and a water molecule (red sphere) is comparable to the hydrogen bond between water (magenta sphere) and the pyridine ring of nicotine

(Figure and text taken from Yamauchi, J. *et al.* 2012).

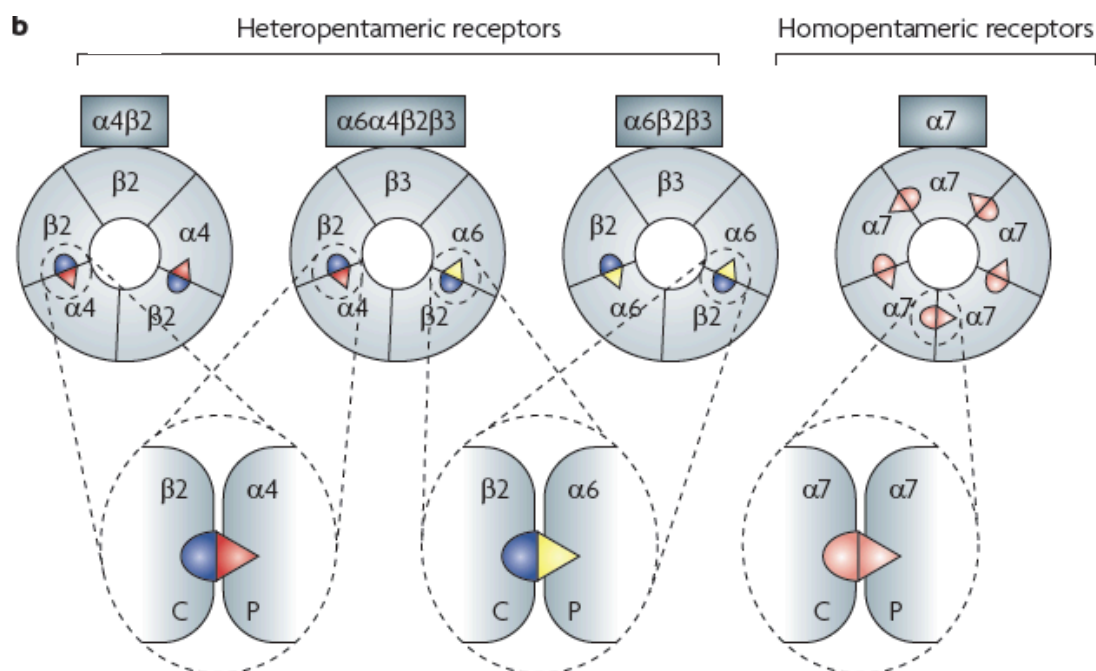


Figure 3. Possible subunit assemblies to form functional subtypes, the triangles represent the principal face of the binding site whereas the C or the semi-circle represents the complementary face (Gotti, C. *et al.* 2006, figure taken from Taly, A. *et al.* 2009).

1.3. Pharmacology of the $\alpha 7$ nAChR and the associated diseases and disorders

One of the most abundant neuronal nAChRs is the homopentameric $\alpha 7$. This subtype comprises of five equal $\alpha 7$ subunits being the only neural nAChR that has the ability to assemble without other subunits. It has five binding sites that are located between the subunit interfaces. $\alpha 7$ -nAChR has a relative low affinity for nicotine and acetylcholine, high permeability for Ca^{+2} , full activation from choline, submillisecond time scale desensitization upon prolonged application of agonist (Corradi, J. 2016), high sensitivity to α -bungarotoxin (α -Btx) (Cuevas, J and Berg, D. K. 1998), and methyllycaconitine (MLA). $\alpha 7$ nAChR on presynaptic terminals mediate release of other neurotransmitters (Wonnacott, S. *et al.* 2006). With regards to upregulation, there is information suggesting that $\alpha 7$ -nAChR requires activation to upregulate or enhance expression, unlike $\alpha 4 \beta 2$ -nAChR that upregulates by a different mechanism (see review Henderson, B. J.; Lester, H. A. 2015).

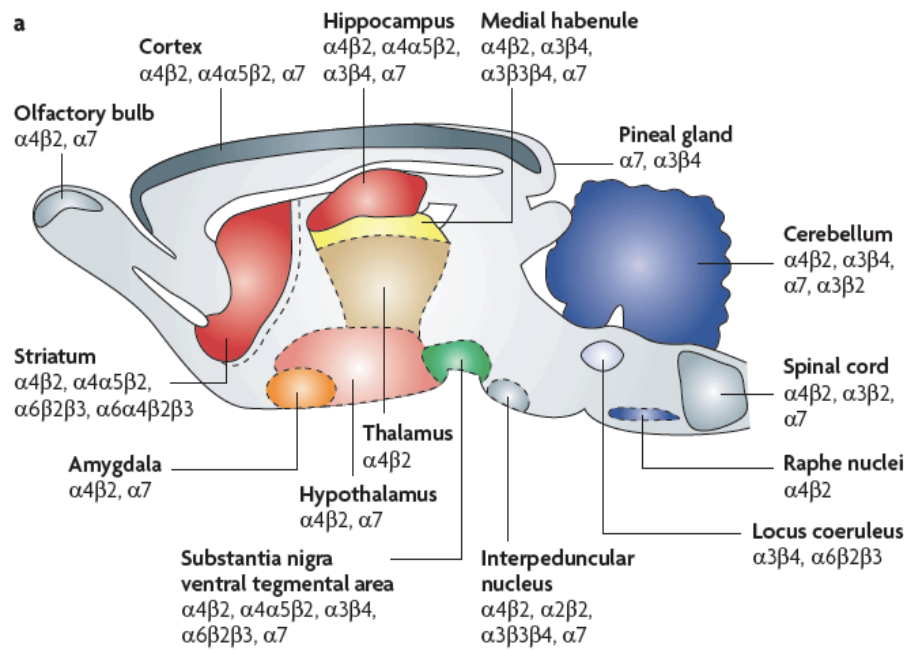


Figure 4. Broad distribution of nAChRs in the Brain (Gotti, C. *et al.* 2006, figure taken from Taly, A. *et al.* 2009).

Two of the interesting and distinct aspects of the $\alpha 7$ -nAChR are the ability to conduct Ca^{+2} currents and rapid desensitization. A proposed hypothesis, rapid desensitization of the receptor is a protective mechanism that will do not allow excessive concentrations of Ca^{+2} into the cell, high concentrations of this divalent cation could result in cytotoxicity (Bertrand, D. 2015). Another important feature of the high concentration of Ca^{+2} permeability, will be activation of a second messenger pathways mediating cellular processes such as gene expression.

The $\alpha 7$ -nAChR is the only homopentameric receptor expressed in the brain, and is found to be express in amygdala, olfactory region, cortex, spinal cord, hippocampus, cerebellum and hypothalamus (**figure 4**). Were it controls different processes such as: cognition, learning, memory, emotion including anxiety and depression, sensory processing.

The $\alpha 7$ -nAChR is also expressed in non-neuronal cells, such as pyramidal and interneurons, immature granule cells, astrocytes and microglia, and endothelial cells, immune cells such as, monocytes (Rosas-Ballina, M. *et al.*, 2009, Yamada-Nomoto, K. *et al.* 2016; Zakrzewicz, A. *et al.*, 2017), macrophages (Nemethova, A. *et al.* 2013, Báez-Pagán, C. A. *et al.* 2015), T-cells (Bencherif, M. *et al.* 2011). The non-neuronal $\alpha 7$ -

nAChR appears involved in controlling proliferation, adhesion, migration, secretion, survival and apoptosis (Taly, A.; Charon, S. 2012). As I describe before, they are present and play a fundamental role in key physiological functions emerging as an important target for the treatment of several diseases and disorders, like Alzheimer's disease and Schizophrenia.

1.3.1. Role of $\alpha 7$ nAChR in Alzheimer's disease

Alzheimer's disease (AD) is the most common worldwide cause of dementia and its frequency thought to double every 20 years. It accounts 70% of all the dementia cases. Dementia is a neurodegenerative disease that is characterized by deterioration in cognition and memory, function, (including motor coordination), and behavior (Reitz, C. *et al.* 2011, Buckingham, S. D. *et al.* 2009). AD is characterized by accumulation of deposits of the 39-42 amino acid amyloid- β (A β) plaques, and neurofibrillary tangles, composed of hyper-phosphorylated tau protein, neuronal loss, and a prominent synaptic loss (Spires-Jones, T. L.; Hyman, B. T., 2014).

The cholinergic system plays an important role in cognition, studies have report that is important in long-term memory. The cholinergic neurons will degenerate with aging, leading to a cholinergic hypofunction this will result in a progressive cognition decline, playing a key role in early stages of the disease. The nAChRs are important mediators of cholinergic signaling in modulation of learning and memory function.

The $\alpha 7$ nAChR is highly expressed in the hippocampus and the cortex, the first region affected primarily in AD (Gotti, C. *et al.* 2006). Interaction between A β and $\alpha 7$ nAChR has been widely demonstrated (Wang, H. Y. *et al.* 2000; Dineley, K. T. 2007; Oz, M. *et al.* 2013). Early experiments showed interaction between $\alpha 7$ nAChR and A β in senile plaques of brain slices from patients that suffered AD (Wang, H. Y. *et al.* 2000; Lombardo, S. and Maskos U. 20015). Competition studies performed by incubating $\alpha 7$ nAChR with A β and using ^{125}I - α -Bungarotoxin to displace A β , reveals decreased A β binding to $\alpha 7$ nAChR, indicating the both, the toxin and the peptide are competing for a commun orthosteric site.

There are discrepancies reported in the literature on functional consequences of nAChR-A β interaction, some of them report inhibition, for instance, Pettit, D. L. *et al.* reported in 2001 that A β 1-42 inhibit responses by blocking $\alpha 7$ nAChR postsynaptically in rat hippocampal interneurons. On the other hand, activation was reported in picomolar

concentrations of A β 1-42 by Dineley, K. T. *et al.* in 2002 using a *Xenopus oocyte* system expressing α 7 nAChR. Before we try to conclude anything, it is important to be aware that the findings vary because they employ different approaches and different concentrations of A β .

When we try to translate the information from mice to humans, of more clinical relevance, uncertainties arise. What it is clear, is that the α 7 nAChR plays a role in AD. Further studies should be conducted in order to better understand the disease. Considering the heterogeneity of AD, it is high possible that a multitarget approach will be necessary.

The only approved therapy available for AD that unfortunately offer limited relief are acetylcholinesterase inhibitors (galantamine, donepezil and rivastigmine), and memantine, a NMDA receptor blocker. With estimated health costs reaching billions of dollars per year and affecting 36 million people worldwide, according to the NIH, the disease incidence is expected to rise to 115 million by 2050 unless effective therapies are developed.

1.3.2. Role of α 7 nAChR in schizophrenia

Patients diagnosed with schizophrenia smoke tobacco more than the regular population (Dickerson, F. 2017, **Figure 5**). It is known that between 70-90% schizophrenics smoke, thus the elevation of the consumption of daily cigarettes is much higher than the general smoking population, α 7-nAChR is less sensitive to nicotine compare to other subtypes, which means that only very intense smoking is likely to activate this receptor, suggesting that smoking can be a way of self-medication.

Schizophrenia is a very complex disorder, it is not only chronic, it is disabling; it affects 1% of the worldwide population (Insel, T. R. 2010). Normally accompanied symptoms that are divided in three mayor categories: positive, negative and cognitive.

The positive symptoms are: tactile, auditory, visual, and gustatory hallucinations and delusions, movement disorders, (Pearlson, G. D. and Ford, J. M. 2014).

Negative symptoms: psychosocial impairments, disorganized speech, loss motivation, anhedonia, reduce expression of emotions.

Cognitive symptoms: attention and working memory deficits, trouble focusing or paying attention and poor executive functioning (Parikh, V. *et al.* 2016, Bertrand D. *et al.* 2015, NIH, mental illness web page 2017).

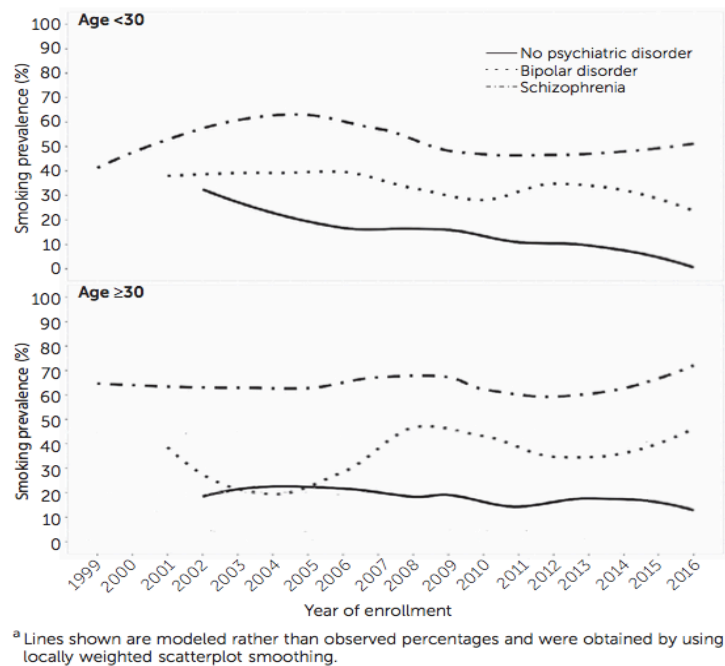


Figure 5. Smoking prevalence among individuals with schizophrenia, bipolar disorder, or no psychiatric disorder (N=1938), by age and smoking status at year of enrollment (Figure and text taken from Dickerson, F. *et al* 2017).

There are no currently medications approved by the US Food and Drug Administration (FDA) for the treatment of negative and cognitive symptoms, and the use of antipsychotics is the only type of therapy available for the treatment of schizophrenia, this treatment can be effective for positive symptoms, and the only strategy for the possible treatment of negative and cognitive symptoms will be the combination of antipsychotics, so far this has met with very limited success.

Cognitive impairment in patients with schizophrenia is relate to the P50 auditory evoked potential. P50 is a positive wave emerging around 50 ms after an auditory stimulus. P50 sensory gating is a phenomenon where the CNS will modulate its sensitivity to the incoming stimulus (Shan, J. C. *et al.* 2013). The poor ability to gate this stimulus in schizophrenic patients contributes to a sensory overload, cognitive fragmentation and social anhedonia (Shan, J. C. *et al.* 2013, Parikh, V. *et al.* 2016, Arnfred, S. M. And Chen A. C. 2004). Nicotine has positive neurocognitive effect in schizophrenia, where smoking significantly improves P50 gating (Adler, L. E. *et al.* 1993).

An atypical antipsychotic use for the treatment of schizophrenia is clozapine, it is known to work by blocking the 5-HT₃ receptor, that results in release of ACh. Clonazepine is

known to normalize the P50 on schizophrenia patients and also induces the reduction on smoking. Unfortunately, clozapine has a lot of side effects and the use of this antipsychotic is limited. A therapeutic approach to improve cognitive symptoms are $\alpha 7$ nAChR selective agonist and positive allosteric modulators (PAMs) (Olinicy, A. *et al.*, 2006; Freedman, R. *et al.*, 2008).

1.4. Selective ligands of the $\alpha 7$ nAChR.

There are four main types of compounds, acting on the $\alpha 7$ -nAChR; (1) classical agonist, that will bind to the principal binding site (orthosteric site) between the subunit interfaces, and where endogenous ligand, acetylcholine binds; (2) allosteric modulators that bind in a different site. (3) Silent agonist, that will only activate the receptor in the presence of a type II positive allosteric modulator and (4) agonist-positive allosteric modulators (ago-PAM), these compounds have the ability to potentiate orthosteric activation by binding to the orthosteric site, the type II PAM site and an additional site located in the vestibule of the ion channel (Horenstein, N. A. *et al.* 2016).

Strong efforts in the last 20 years from medicinal chemists have developed selective agonist and positive allosteric modulators due to the therapeutic importance of this receptor. Several of the highly studied compounds made it to clinical trials for schizophrenia, Alzheimer's disease, attention deficit disorder and smoking cessation (Table 2). There have been many studies implicated in the design of novel selective $\alpha 7$ nAChR agonist and positive allosteric modulators (Horenstein, N. A. *et al.* 2008, Wang, J. *et al.* 2012, Arunrungvichian, K. *et al.* 2015), that gave sufficient information to establish the primary pharmacophoric features: a cationic center, a hydrogen-bond acceptor, and a hydrophobic moiety (Arunrungvichian, K. *et al.* 2015).

PAMs are classified for $\alpha 7$ -nAChR into type I and type II, and they differ, not only in where they bind, but also in how they affect the kinetics of the receptor. Type I PAM's enhance agonist-induced peak currents, without affecting desensitization; type II PAM's will delay desensitization by increasing the energetic barrier for desensitization, which allows successive opening/close events, and/or will change desensitized receptor to an active one, by reversing an agonist-induced desensitization. Data suggest that the binding site for type II PAMs is in the transmembrane domain of the receptor (Young, G.T. 2008).

The large rationale that has been used as motivation to develop $\alpha 7$ nAChR selective agonist and positive allosteric modulators for the treatment of diseases and disorder are listed below (Kem, W. R. 2000):

Stimulation of this receptor enhance cognition: performance in learning and memory task as well as improving sensory processing information.

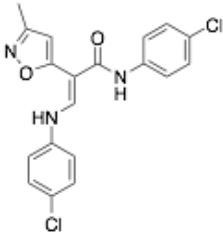
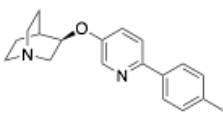
High permeability to Ca^{+2} , that will act as a second messenger stimulating downstream signaling and neurotransmitter release.

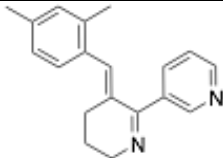
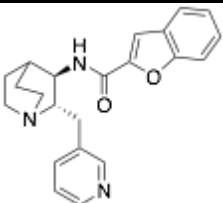
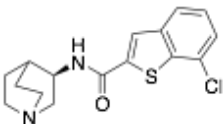
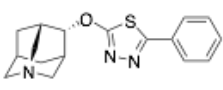
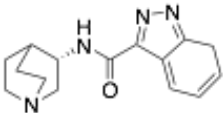
$\alpha 7$ -nAChR agonists do not possess significant drug dependence (self-administration) potential.

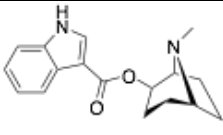
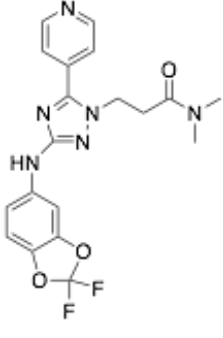
Stimulation a non-neuronal $\alpha 7$ -nAChR elicit analgesic and anti- inflammatory effects.

Table 1. $\alpha 7$ -nAChR agonists and PAM's recently or currently in clinical trials.

(Information found in clinicaltrials.gov 2017, Beinatz, C. *et al.* 2015, Bertrand, D. *et al.* 2009, Hashimoto, K. 2015).

Compound	Type of compound	Clinical phase	Disease	Reference
 AVL-3288 (Anvyl)	Positive allosteric modulator type I	I	Schizophrenia, cognition disorders	Gee, K. W. <i>et al.</i> , 2017; Ng, H. J. <i>et al.</i> , 2007.
 AQW-051 (Norvatis)	Selective agonist	II	Mild Alzheimer's Disease and Amnesic Mild Cognitive Impairment	Feuerbach, D. <i>et al.</i> , 2015.

 GTS-21 or DMXB-A	Partial agonist	II	Schizophrenia	Olincy, A. <i>et al.</i> , 2006; Freedman, R. <i>et al.</i> , 2008.
 TC-5619 (Targacept Inc)	Selective agonist	II	ADHD	Hauser <i>et al.</i> , 2009; Mazurov <i>et al.</i> , 2012; Lieberman <i>et al.</i> , 2013.
 EVP-6124, Encenicline or MT-4666 (FORUM pharmaceuticals Inc)	Selective agonist	III	Schizophrenia, Impaired Cognition	Prickaerts <i>et al.</i> , 2012.
 ABT-126 (Abb Vie)	Selective Agonist	II	Alzheimer's disease.	Bitner <i>et al.</i> , 2013; Gault, L. M, 2016.
 RG3487, RO5313534 or MEM3454 (Roche/Memory)	Partial agonist	II	Alzheimer's disease.	Rezvani A. H. <i>et al.</i> 2009; Wallace, T. L. <i>et al.</i> 2011.

 Tropisetron	Partial agonist	III	Schizophrenia	Zhang, X. Y. <i>et al.</i> 2012.
 JNJ-39393406 or UH3-P2 (Johnson & Johnson is collaborator)	Positive allosteric modulator type I	II	Smoking cessation, depression	Winterer, G. <i>et al.</i> , 2013; Hashimoto, K. 2015.

The potential therapeutic use of $\alpha 7$ -nAChR ligands has been documented (**Table 1**), unfortunately, no drug has passed phase III of clinical trials, all of the agonists mention in table 1, have the same pharmacophoric features. One of the aims of our project was to developed a new subset of ligands that differ from classical agonist in their structure.

1.5 Acetylcholine binding proteins.

Acetylcholine binding proteins (AChBPs) are secreted by fresh water snails *Lymnaea stagnalis* (Smit, A. B. *et al* 2001) *Bulinus truncatus* (Celie, P.H.N. *et al* 2005) and the saltwater snail *Aplysia californica* (Hansen, S.B. *et al.* 2004). AChBPs are soluble proteins thought to modulate synaptic transmission by capturing presynaptically released ACh acting as a buffering system, this will cause suppression of synaptic transmission in the mollusk synapses (Figure 5A, Smit, A. B. *et al* 2001). They are produced and stored in glial cells, and the release of these proteins into the synaptic cleft is mediated by acetylcholine (**Figure 6**).

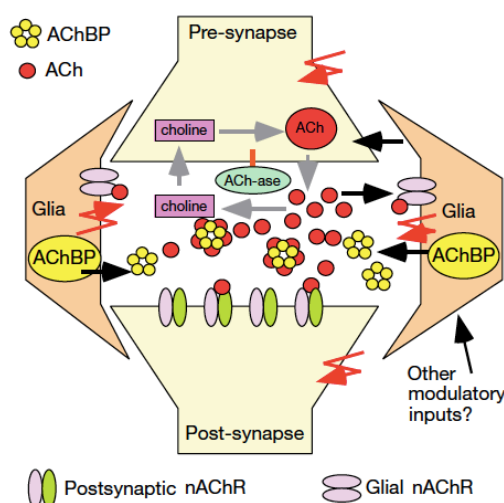


Figure 6. Model of the role of AChBP in neurotransmission. A basal level of AChBP is present in the synaptic cleft. Presynaptic ACh release can lead to activation of postsynaptic receptors and to EPSPs. In parallel, nAChRs on glia are activated, causing increased release of AChBP into the synapse, which leads to suppression of cholinergic transmission (figure and text taken from Smit, A. B. *et al.* 2001).

Characterization of the ligand recognition characteristics of *Lymnaea stagnalis* AChBP was first made by Brejc *et al* on 2001. They report for the first time the crystal structure of homopentameric *Lymnaea stagnalis* AChBP at 2.7 Å resolution bound with HEPES. AChBPs has a significant sequence homology with extracellular domain of the nAChRs. Few years later, in 2004 Hansen *et al.* first synthesized and expressed *Aplysia californica* AChBP using a mammalian expression system and cDNAs constructed from oligonucleotides. In both studies they showed that AChBPs assemble as homopentamers, they have stoichiometry of one ligand binding site per subunit and exhibit a general profile of ligand binding affinities that are characteristic for nAChRs, including the characteristic aromatic nest of the ligand binding domain (LBD) made of Tyr93, Trp147, Tyr188 and Tyr195 in the principal face and Tyr55 in the complementary face. The *Ac*-AChBP subunit consist of a N-terminal, 20-residues α -helices and 10 β -sheets, 6 inner (β 1, β 2, β 3, β 5, β 6, β 8) and 4 outer sheets (β 4, β 7, β 9, β 10) to form a sandwich core (Hansen, S. B. *et al.* 2005). The main differences between the LBD of both binding proteins are rearrangements in the complementary face.

AChBPs has been a source of information about atomic resolution structure complex with classical agonist and antagonist. These proteins have a similar folding of an α subunit (**figure 7**), they have five equal binding sites characterized. They lack transmembrane and intracellular domains, so it crystallizes more easily than the receptor,

therefore it has been used as a structure surrogate of the extracellular domain of the $\alpha 7$ -nAChR. The binding protein has proved to be a useful template for drug design of $\alpha 7$ -nAChR selective agonist.

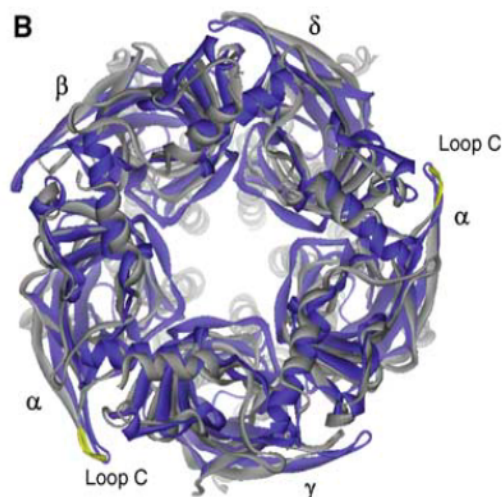


Figure 7. Apical view of apo *Ac*-AChBP (blue) overlap with the muscle-type nAChR (gray) (Unwin, N. 2005) (Structure taken of Hansen, S.B. *et al.* 2005).

CHAPTER II. CHEMICAL REFINEMENT

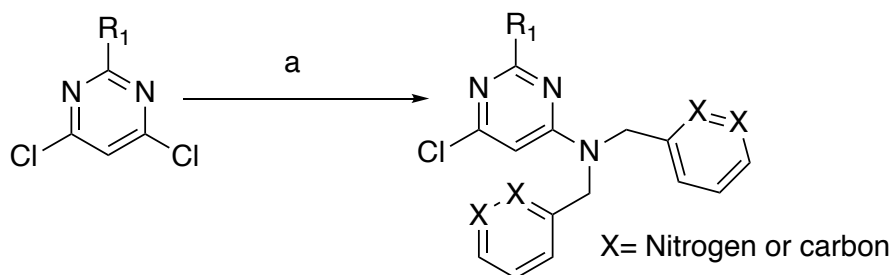
2.1. Chemicals

2-amino-4,6-dichloropyrimidine	Oakwood chemicals
2-Methoxyphenylboronic acid	Acros organics
2,4,-dimethoxy benzene boronic acid	Alfa aesar
3-Chloroperbenzoic acid	Sigma-Aldrich
3-Methoxyphenylboronic acid	Sigma-Aldrich
3-Pyridinyl- <i>N</i> -(3-pyridinylmethyl)methanamine	Oakwood chemicals
3-Thienylboronic acid	Aldrich
3,4-Dimethoxybenzene boronic acid	Alfa aesar
3,4,5-trimethoxybenzene boronic acid	Alfa aesar
4-(Dimethylamino) benzene boronic acid	Alfa aesar
4-(Trifluoromethyl)benzyl boronic acid	Oakwood chemicals
4-fluoro-2-methoxyphenyl boronic acid	Sigma-Aldrich
4-Hydroxyphenyl boronic acid	Acros organics
4-Methoxyphenylboronic acid	Aldrich
4-Pyridinylboronic acid	Aldrich-Sigma
4,6-dichloro-2-(methylthio)pyrimidine	Aldrich-Sigma
4,6-Dichloropyrimide	Alfa aesar
5-Indol boronic acid	Acros organics
Acetone	J.T. Baker
Ammonium choride	EMD
Benzylamine	Aldrich
Bis(diphenylphosphino)ferrocene]dichloropalladium(II)	Oakwood chemicals
Chloroform- <i>d</i>	Cambridge isotopes laboratories Inc.
Di-(2-picolyl) amine	Sigma-Aldrich
Dibenzylamine	Sigma-Aldrich
Dichloromethane	Fisher scientific/Acros organics
Dimethylformamide	EMD
DMSO- <i>d</i> ₆	Cambridge isotopes laboratories Inc.
Ethanol	Acros organics
Ethyl acetate	Fisher scientific

Furan-3-boronic acid	Oakwood chemical
Hexanes	Fisher scientific
Magnesium sulfate anhydrous	Honeywell
Methanol	Fisher scientific
<i>N,N</i> -diisopropylethylamine	Alfa aesar
<i>N,N</i> -dimethylacetamide	Aldrich
Oxalic acid	Spectrum
Potassium carbonate	Fisher scientific
Pyridine-2-carboxylaldehyde	Aldrich
Pyridine-3-boronic acid	Combi-Blocks Inc.
Silica gel	Alfa Aesar
Sodium borohydride	Sigma-Aldrich
Sodium carbonate	Fisher scientific
Sodium chloride (Brine)	Fisher scientific
Sodium sulfate anhydrous	Acros organics
tetrakis(triphenylphosphine)palladium(0)	Strem chemicals
Toluene	EMD

2.2. Synthetic strategies

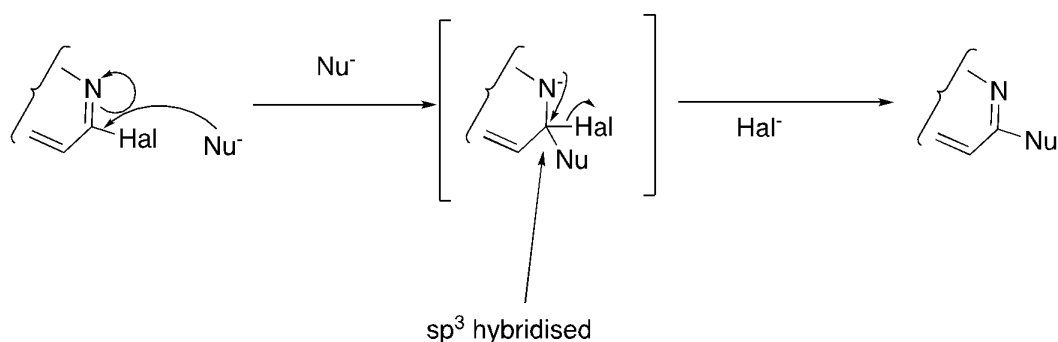
2.2.1. Nucleophilic aromatic substitution: substitution of the di-picolyl system



Scheme 1. First step in the synthetic pathway. Conditions: (a) Amine, DIPEA, DMF, 2-6 h.

The nucleophilic aromatic substitution (S_NAr) is a reaction that requires a two-step sequence of addition and elimination: 1) nucleophilic addition to a favoured electrophilic carbon, to form a non-aromatic and delocalised anion; 2) loss of a leaving group that was attached to that same carbon; this will return the system to aromaticity (**scheme 2**). In benzene chemistry, there must be a group that will facilitate the nucleophilic addition, and it has to be located *ortho*- or *para*- to the leaving group. A leaving group will be the departing entity in the electrophile (X^-) in this particular case, i use a halide.

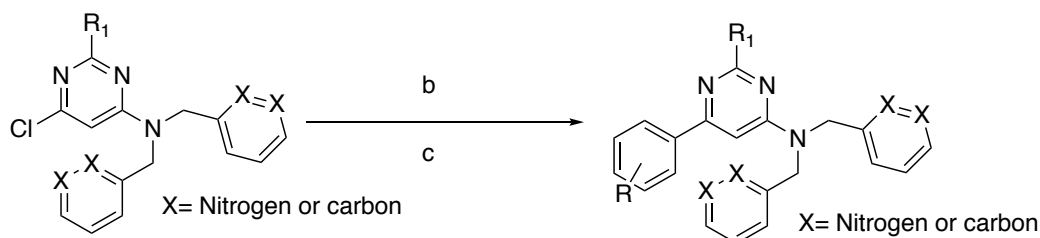
In the case of N-heterocycles, the group that facilitates the nucleophilic addition and accepts the negative charge intermediate is one of the nitrogens. Nitrogen presence makes it much easier to undergo a nucleophilic substitution than the equivalent benzene compounds.



Scheme 2. Mechanism of a nucleophilic aromatic substitution reaction (Joule, J. A.; Mills, K. 2013)

We used the di-picolyamine as the nucleophile to substitute the halide of the 2-amino-4,6-dichloropyrimidine in the α position since 4/6 positions of pyrimidines easily undergo S_NAr one equivalent was carefully added to yield a mono-substitution.

2.2.2. Cross coupling Suzuki reaction: Modulation at the 6th position of pyrimidine system

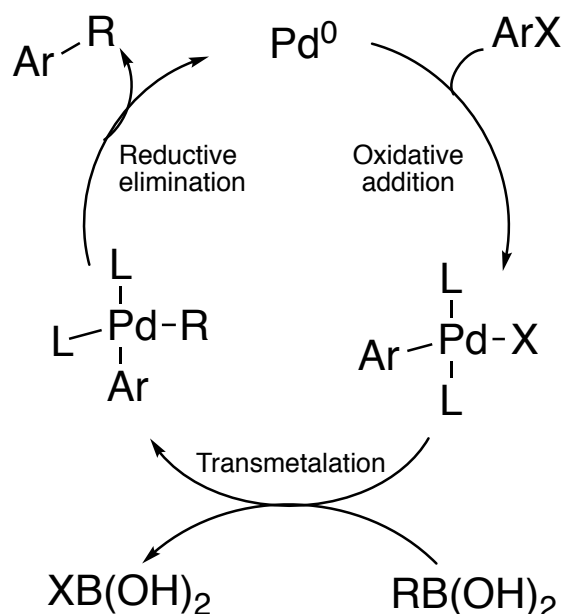


Scheme 3. Second step of the synthetic pathway: (b) Boronic acid, Na_2CO_3 , $\text{Pd}(\text{PPh}_3)_4$, Toluene/Ethanol, 95 °C for 8 h-overnight. (c) Boronic acid, K_2CO_3 2M, $\text{Pd}(\text{dppf})\text{Cl}_2$, DMA, 95 °C for 2 h-overnight.

The cross-coupling Suzuki reaction is one of the most important catalytic reactions. It is widely used in the pharmaceutical industry, because of its many advantages, such as robustness and high diversity of commercially available compatible reagents (i.e. halides and boronic acids). The latter is critical to medicinal chemistry where diversity of chemical libraries is a key to an exhaustive SAR. The reaction is a palladium-catalyzed cross coupling reaction between organoboronic acid and aryl halides. The general mechanism consists of three main steps (**scheme 3**): 1) oxidative addition of the aryl halide to the catalytically active L_nPd^0 ; 2) transmetalation with the organometallic reagent; and, 3) reductive elimination to yield the product and regenerate the active Pd^0 catalyst (Wu, X.F. *et al.* 2010).

In the pyrimidine ring, the cross-coupling reactions occur faster in the fourth and sixth positions (Conde-Ceide, S.; Garrido-Montalban, A. 2006). We proceeded with the nucleophilic aromatic substitution prior to the coupling, to avoid di-coupled byproducts excess of boronic acid is always added to the reaction which may cause disubstitution when to coupling positions are available. Initially we used $\text{Pd}(\text{PPh}_3)_4$ as the catalyst. It is a relatively inexpensive, stable and powerful catalyst most commonly used in Suzuki cross-coupling reactions. $\text{PdCl}_2(\text{dppf})$ is reported to be a better catalyst for $\text{sp}^3\text{-sp}^2$

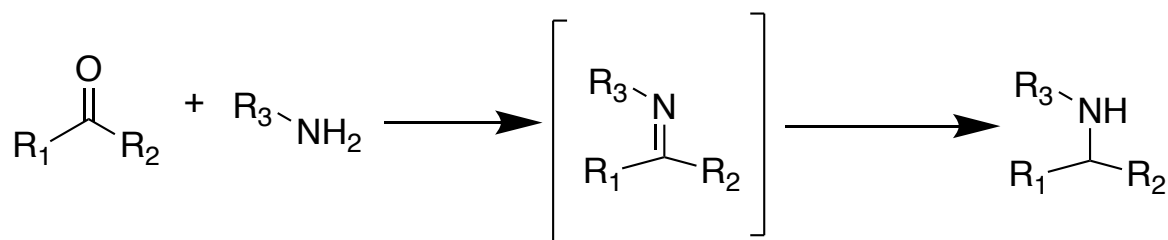
reactions, and we successfully used this reagent in our synthesis as an alternative catalyst when $\text{Pd}(\text{PPh}_3)_4$ was not affording good results.



Scheme 4. Cross coupling Suzuki reaction general mechanism (Wu, X.F. *et al.* 2010).

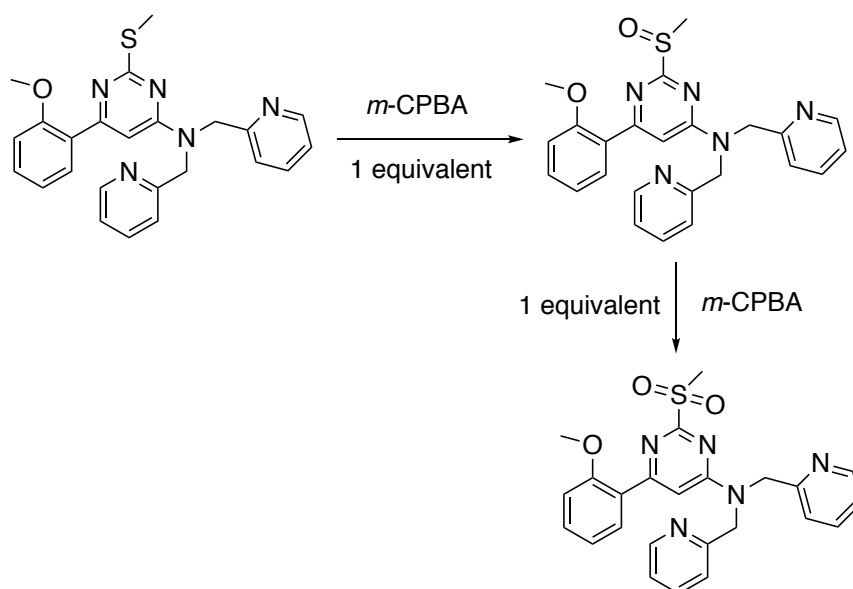
2.2.3. Direct reductive amination: preparing an amine bulding block

Reductive amination is an approach for the formation of C-N bonds. It is used extensively to prepare primary, secondary, and tertiary amines. In this reaction an amine and a carbonyl group condense to afford an imine that is later reduced to an amine (**Scheme 5**). There are two ways to perform a reductive amination; the so-called one pot procedure that after the addition of a carbonyl group to an amine and imine formation proceeds with a subsequent addition of a reducing reagent to afford the target amine. In the indirect approach, the first step of the reaction remains the same but unlike the direct reductive amination, here isolation and purification of the imine is required and is followed by a separate step of the imine reduction. NaBH_4 is a safe, green, environment-friendly and inexpensive reducing agent that has been choice as the reductive reagent of this type of reaction (Apodaca, R. and Xiao W. 2001; Hamadi, H. and Javadi, S. 2017). We used this strategy to react benzylamine and pyridine-2-carboxaldehyde generating a secondary amine that was used after purification to produce one of the AL intermediates, AL-2.



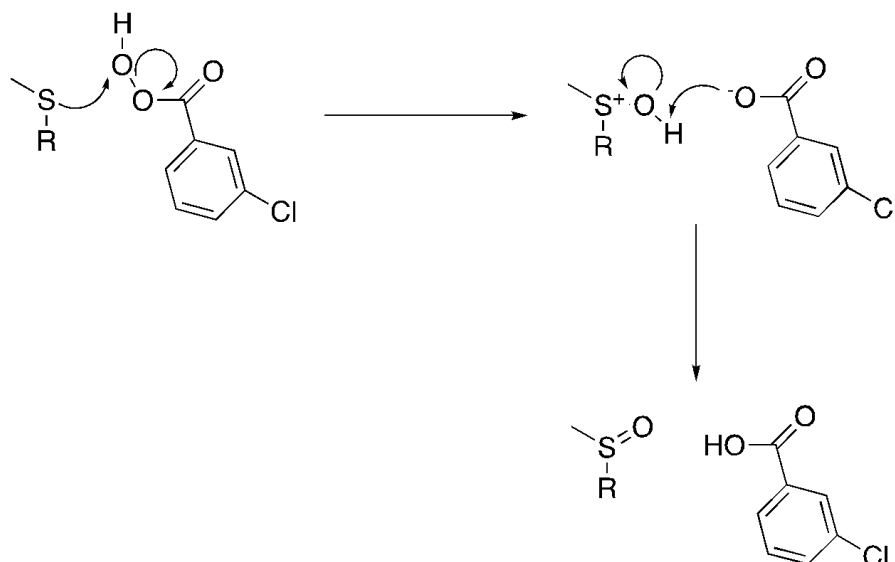
Scheme 5. Reductive amination of aldehydes and ketones yield primary, secondary or tertiary amines.

2.2.4. Synthesis of sulfoxides and sulfones: Modulation at 2nd position of pyrimidine system



Scheme 6. Thioether oxidation utilizing *m*-CPBA as oxidant reagent.

In an attempt to expand the library of modified compounds at position 2 in the pyrimidine ring, we decided to use an intermediate with a good leaving group at the corresponding position in the pyrimidine ring. To do so, a commercially available 2-methylthiopyrimidine was used as a starting material, an oxidation of the thioether group with *m*-CPBA led to the corresponding sulfoxide and sulfone compound AL-1A, t. Of the possible oxidation conditions, I chose to use *m*-CPBA, it has higher selectivity avoiding oxidation of the di-picolyl system. Such overoxidation would be highly possible with H₂O₂, the most commonly used oxidant for thioethers oxidation.



Scheme 7. Proposed mechanism for the oxidation of a thioether by *m*-CPBA. The subsequent oxidation of the sulfoxide to afford the sulfone follows the same reaction path and requires an additional equivalent of *m*-CPBA.

The reaction starts when the sulfur atom of the thioether attacks the terminal oxygen of the peroxide. Consequently a carboxylate and a sulfoxide protonated at the oxygen atom is formed. The carboxylate deprotonates the intermediate to yield the sulfoxide and *m*-Chlorobenzoic acid (**Scheme 7**). The oxidation of sulfoxide follows the same mechanism described for the oxidation of the thioether. Under basic conditions, a nucleophilic attack would be carried by the peroxy anion at the sulfur atom of the sulfoxide.

2.3. Synthetic procedures

2.3.1 General procedure for the synthesis of 4-chloro-6-aminopyrimidin-2-amines.

An appropriate amine (2.20 mmol) was added to a solution of 2-amino-2,4-dichloropyrimidine (0.328 g, 2.00 mmol) and *N,N*-diisopropylethylamine (0.383 mL, 2.20 mmol) in DMF (3 mL) and the solution was stirred at 80 °C for 2-6 hours (reaction monitored by TLC). The solvents were removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated on a rotary evaporator, and the product was purified by flash chromatography using silica gel or by crystallization.

2.3.2. General procedure for the synthesis of 6-aryl-2,4-diaminopyrimidine.

Conditions a.

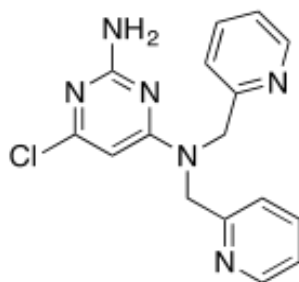
Appropriate boronic acid (0.73 mmol) was added to a solution of a derivative AC-17, AC-19, AC-20 (0.6 mmol) in toluene/ethanol (1:1, 4 mL). Sodium carbonate decahydrate (0.286 g, 1.0 mmol) was added following the addition of tetrakis(triphenylphosphine)palladium(0) [Pd (PPh₃)₄] (57.8 mg, 0.050 mmol). The resulting mixture was stirred in a capped glass vial at 95 °C for 6-12 h. After cooling the mixture to room temperature, water was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel.

Conditions b.

Appropriate boronic acid (0.46 mmol) was added to a solution of an appropriate chloropyrimidine (0.3 mmol) in *N,N*-dimethylacetamide (2.5 mL). 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) [PdCl₂(dppf)] (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C for 2 h-overnight. After cooling the mixture to room temperature, water was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel.

2.3.3. General procedure for the synthesis of 6-aryl-2-methylthio-4-aminopyrimidines.

Appropriate boronic acid (0.46 mmol) was added to a solution of 3-chloro-5-(methylthio)-*N,N*-bis(pyridin-2-ylmethyl)aniline (AL-1) (0.3 mmol) in *N,N*-dimethylacetamide (2.5 mL). 2M aqueous potassium carbonate (3 equivalents) was added following the addition of PdCl₂(dppf) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C for 8 h-overnight. After cooling the mixture to room temperature, water was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel.

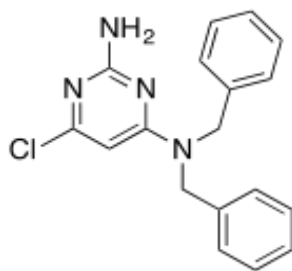
2.3.4. 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17).

Di-(2-picolyl)amine (0.438, 2.20 mmol) was added to a solution of 2-amino-4,6-dichloropyrimidine (0.328 g, 2.00 mmol) and *N,N*-diisopropylethylamine (0.383 mL, 2.20 mmol) in DMF (3 mL) and the solution was stirred at 80 °C for 3.5 h (reaction monitored by TLC). Solvent was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were washed with brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated on a rotary evaporator, and the product was purified by column chromatography using silica gel (hexanes/EtOAc 30%) or by crystallization (the compound crystalized using ethyl acetate as solvent), after purification a white solid was obtained with a 63% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 4.74 (bs, 2H), 4.99 (s, 4H), 5.94 (s, 1H), 7.12-7.23 (m, 4H), 7.55–7.69 (m, 2H), 8.52–8.57 (d, *J* = 4.0 Hz, 2H).

¹³C NMR (150 MHz, Chloroform-*d*) δ 53.2 (CH₂), 92.7 (CH), 122.4 (CH), 136.8 (CH), 149.6 (CH), 160.4 (C), 162.1 (C), 164.1 (C), 170.1 (C).

HR-ESI-MS: [C₁₆ H₁₆ N₆ Cl]⁺ 327.1124

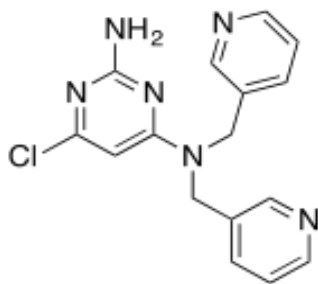
2.3.5. *N*⁴,*N*⁴-dibenzyl-6-chloropyrimidine-2,4-diamine(AC-19).

Dibenzylamine (0.434, 2.20 mmol) was added to a solution of 2-amino-4,6-dichloropyrimidine (0.328 g, 2.00 mmol) and *N,N*-diisopropylethylamine (0.383 mL, 2.20 mmol) in DMF (3 mL), and the solution was stirred at 80 °C for 6 h (reaction monitored by TLC). The solvent was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated on a rotary evaporator. The product (white solid, 69% yield) was obtained by column chromatography on silica gel (hexanes/EtOAc 5%).

¹H NMR (600 MHz, Chloroform-*d*) δ 4.68 (bs, 4H), 5.02 (s, 2H), 5.95 (s, 1H), 7.20 (d, *J* = 7.4 Hz, 3H), 7.27-7.31 (m, 2H) 7.32 – 7.40 (m, 5H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 50.1 (CH₂), 92.4 (CH), 127.2 (CH), 127.5 (CH), 128.4 (CH), 128.5 (CH), 128.8 (CH), 132.5 (C), 160.4 (C), 162.2 (C), 164.3 (C).

HR-ESI-TOFMS: [C₁₈H₁₈ClN₄]⁺ 325.1215

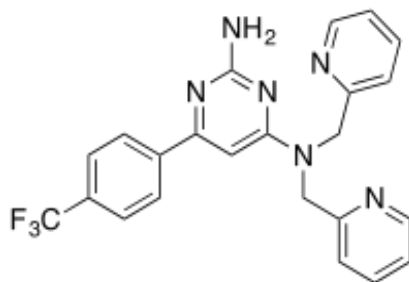
2.3.6. 6-chloro-*N*⁴,*N*⁴-bis(pyridin-3-ylmethyl)pyrimidine-2,4-diamine (AC-20).

3-Pyridinyl-*N*-(3-pyridinylmethyl)methanamine (0.438, 2.20 mmol) was added to a solution of 2-amino-4,6-dichloropyrimidine (0.328 g, 2.00 mmol) and *N,N*-diisopropylethylamine (0.383 mL, 2.20 mmol) in DMF (3 mL) and the solution was stirred at 80 °C for 4 h (reaction monitored by TLC). The solvent was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated on a rotary evaporator and filtered on silica gel with EtOAc as eluent; the product was recrystallized using EtOAc (orange needles, 12% yield).

¹H NMR (600 MHz, DMSO-*d*₆) δ 4.78 (s, 4H), 5.99 (s, 1H), 6.65 (s, 2H) 7.33 (m, 2H), 7.63 (s, 2H), 8.45 (m, 2H), 8.48 (s, 2H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 48.5 (CH₂), 91.1 (CH), 123.8 (CH), 124.0 (CH), 133.7 (C), 148.8 (CH), 148.8 (CH), 159.9 (C), 162.9 (C), 164.0 (C).

HR- ESI-TOFMS: [C₁₆H₁₆ClN₆]⁺ 327.1121

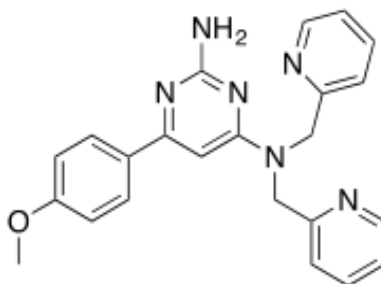
2.3.7. *N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)-6-(4 (trifluoromethyl) phenyl) pyrimidine-2,4-diamine (AC-17-2).

4-(Trifluoromethyl)benzene boronic acid (0.136, 0.72 mmol) was added to a solution of a 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.195g, 0.6 mmol) in toluene/ethanol (1:1, 4 mL). Sodium carbonate decahydrate (0.286 g, 1.0 mmol) was added following the addition of tetrakis(triphenylphosphine)palladium(0) (57.8 mg, 0.050 mmol). The resulting mixture was stirred in a capped glass vial at 95 °C for 8 h. After cooling to room temperature, water was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel. The final product was obtained in 50% yield by column chromatography on silica gel with hexanes /EtOAc gradient (5-100%).

¹H NMR (600 MHz, Chloroform-*d*) δ 5.00 (m, 6H), 6.36 (s, 1H), 7.2 (m, 2H), 7.65 (m, 6H), 7.89-7.93 (m, 2H), 8.57-8.60 (m, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 53.3 (CH₂), 91.4 (CH), 119.2 (CH), 120.7 (CH), 122.3 (CH), 125.4 (q, ³*J*_{C-F} = 3.83 Hz, CH), 127.2 (CH), 131.5 (q, ²*J*_{C-F} = 32.56 Hz, C), 136.8 (CH), 142.0 (C), 149.6 (CH), 160.4 (C), 162.9 (C), 163.5 (C), 164.1 (C).

HR-ESI-MS: [C₂₃H₂₀F₃ N₆]⁺ 437.1698

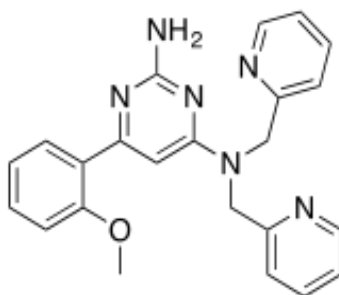
2.3.8. 6-(4-methoxyphenyl)-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl) pyrimidine-2,4-diamine (AC-171A).

4-methoxyphenyl boronic acid (0.07g, 0.45 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.1g, 0.3mmol) in toluene/etanol (1:1, 2mL) and sodium carbonate decahydrate was added (0.174g, 0.6 mmol) with the following addition of tetrakis(triphenylphosphine)palladium(0) (0.07g, 0.6 mmol) as catalyst. The resulting mixture was stirred in a capped glass vial at 95°C over night. Water was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure. Silica gel chromatography column using hexanes/ethyl acetate gradient (5-100%) afforded the product (white solid) in 88% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 3.82 (s, 3H), 4.82 (s, 2H), 5.0-5.13 (bs, 4H), 6.28 (s, 1H), 6.87-6.93 (m, 2H), 7.15-7.27 (m, 4H), 7.63 (td, $J=7.7$, 1.8Hz, 2H), 7.75-7.80 (m, 2H), 8.52-8.62 (m, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 53.3 (CH₂), 55.3 (CH₃), 90.0 (CH), 113.8 (CH), 120.0 (CH), 122.2 (CH), 128.3 (CH), 131.1 (C), 136.8 (CH), 149.5 (CH), 161.0 (C), 162.9 (C), 164.0 (C), 164.6 (C).

HR-ESI-MS: [C₂₃H₂₃N₆O]⁺ 399.1931

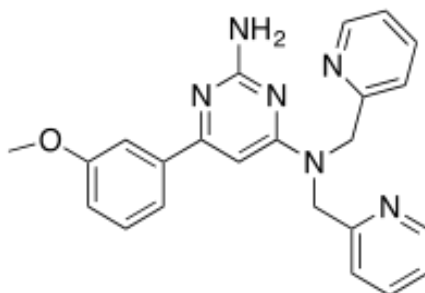
2.3.9. 6-(2-methoxyphenyl)-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl) pyrimidine-2,4-diamine (AC-171A2)

2-Methoxyphenylboronic acid (0.07g, 0.45 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.1 g, 0.3 mmol) in *N,N*-dimethylacetamide (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C for 2.5 h. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure, and the crude product was purified by column chromatography on silica gel with hexanes-EtOAc gradient (5 – 90%) to give the product as a white solid, in 80% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 3.62 (s, 3H), 4.80 (s, 2H), 4.9 (s, 4H), 6.5 (s, 1H), 6.9 (d, J = 8.2 Hz, 1H), 7.00 (t, J = 7.5 Hz, 1H), 7.15- 7.20 (m, 2H), 7.27 – 7.34 (m, 3H), 7.61-7.67 (m, 2H), 7.71 (dd, J = 7.6, 1.6 Hz, 1H), 8.55 (d, J = 4.3, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 55.4 (CH₃), 95.4 (CH), 111.3 (CH), 120.7 (CH), 122.1 (CH), 122.1 (CH), 130.2 (CH), 130.4 (CH), 136.7 (CH), 149.4 (CH), 157.2 (C), 157.2 (C), 162.7 (C), 163.0 (C), 164.0 (C).

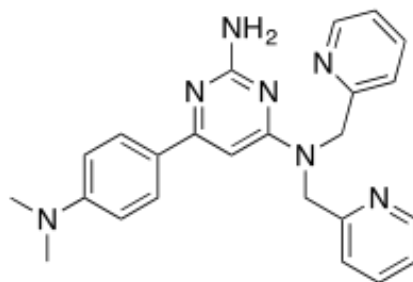
HR-ESI-MS: [C₂₃ H₂₃ N₆ O]⁺ 399.1929

2.3.10. 6-(3-methoxyphenyl)-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl) pyrimidine-2,4-diamine (AC-171A3).

(0.1g, 0.3 mmol) of chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) was added to *N,N*-dimethylacetamide (DMA) (2.5mL) following, the addition of 3-methoxyphenyl boronic acid (0.07g, 0.45 mmol) 2M aqueous solution of K₂CO₃ (0.3 mL) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (0.22g, 0.030 mmol) as catalyst. The resulting mixture was stirred in a capped glass vial at 149 °C for 6 h. DMA was removed under reduced pressure, brine was added, and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure. The pure compound was obtained by silica gel filtration using hexanes/EtOAc (80%) as eluent to afford the product, (white solid) 67% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 3.82 (s, 3H), 4.96 (s, 6H), 6.31 (s, 1H), 6.92 (d, *J* = 7.4 Hz, 1H), 7.13 – 7.19 (m, 2H), 7.27 (d, *J* = 15.8 Hz, 3H), 7.33 (d, *J* = 7.4 Hz, 1H), 7.38 (s, 1H), 7.54 – 7.68 (m, 2H), 8.48 – 8.64 (m, 2H).

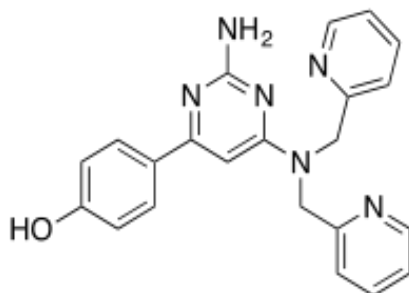
HR-ESI-MS: [C₂₃ H₂₃ N₆ O]⁺ 399.1927

2.3.11. 6-(4-(dimethylamino)phenyl)-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl) pyrimidine-2,4-diamine (AC-171B).

4-(Dimethylamino) benzene boronic acid (0.084g, 0.612 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.100 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C for overnight. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure. Purification using flash chromatography on silica gel (hexanes/ethyl acetate 5-100% gradient) afforded the product (pale solid) in 83% yield.

¹H NMR (600 MHz, CDCl₃) δ 2.99 (s, 6H), 4.75 (s, 2H), 4.98 (s, 4H), 6.26 (s, 1H), 6.69 (d, *J* = 9.1 Hz, 2H), 7.14-7.20 (m, 2H), 7.25-7.27 (m, 2H), 7.59-7.65 (m, 2H), 7.75 (d, *J* = 9.0 Hz, 2H), 8.56 (dd, *J* = 4.8, 1.0 Hz, 2H).

HR-ESI-MS: [C₂₄H₂₆N₇]⁺ 412.2245

2.3.12. 4-(2-amino-6-(bis(pyridin-2-ylmethyl)amino)pyrimidin-4-yl)phenol (AC-171C).

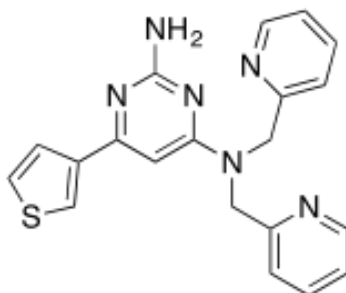
4-Hydroxyphenyl boronic acid (0.084g, 0.612 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.100 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C for overnight. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure and the purification was performed using column chromatography on silica gel with hexanes/ethyl acetate gradient (5-100%) to give the product (white solid) in 46% yield.

¹H NMR (600 MHz, DMSO-*d*₆) δ 4.92 (bs, 4H), 6.01 (s, 2H), 6.36 (s, 1H), 6.78 (d, *J* = 8.7 Hz, 2H), 7.26-7.31 (m, 4H), 7.74-7.78 (m, 4H), 8.51-8.55 (m, 2H), 9.74 (s, 1H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 88.0 (CH), 115.5 (CH), 121.8 (CH), 122.7 (CH), 128.5 (CH), 129.5 (C), 137.2 (CH), 148.8 (C), 149.6 (CH), 159.4 (C), 163.5 (C), 163.5 (C), 164.0 (C).

HR-ESI-TOFMS: [C₂₂ H₂₁ N₆ O]⁺ 385.1773

2.3.13. N^4,N^4 -bis(pyridin-2-ylmethyl)-6-(thiophen-3-yl)pyrimidine-2,4-diamine (AC-171D).

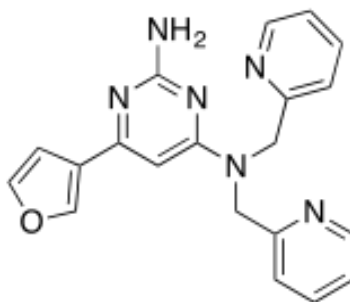


3-Thienylboronic acid (0.058g, 0.45 mmol) was added to a solution of 6-chloro- N^4,N^4 -bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.100 g, 0.3 mmol) in N,N -dimethylacetamide (DMA) (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C over night. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure and the product was isolated by column chromatography on silica gel using hexanes/ethyl acetate (80%) as eluent (white solid, 57% yield).

^1H NMR (600 MHz, Methanol- d_4) δ 5.01 (s, 4H), 6.35 (s, 1H), 7.27-7.31(m, 2H), 7.36 (d, J = 7.6, 2H), 7.39-7.47 (m, 2H), 7.77 (td, J = 7.7, 1.6 Hz, 2H), 7.87 (m, 1H), 8.49 (d, J = 4.5 Hz, 2H).

^{13}C NMR (151 MHz, MeOD) δ 52.7 (CH₂), 89.9 (CH), 121.7 (CH), 122.5 (CH), 124.7 (CH), 125.7 (CH), 127.9 (CH), 137.5 (CH), 140.8 (C), 148.6 (CH), 159.9 (C), 163.3 (C), 164.0 (C).

HR-ESI-MS: [C₂₀H₁₉N₆S]⁺ 375.1387

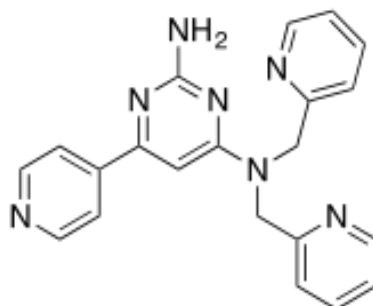
2.3.14. 6-(furan-3-yl)-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-171E).

Furan-2-boronic acid (0.051g, 0.46 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.100 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C over night. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure and the product was isolated by column chromatography on silica gel using hexanes/ethyl acetate (80%) as eluent (pale solid, 86% yield).

¹H NMR (600 MHz, Chloroform-*d*) δ 5.01 (m, 6H), 6.10 (s, 1H), 6.65– 6.68 (m, 1H), 7.18 (dd, *J* = 7.1, 5.2 Hz, 2H), 7.22–7.28 (m, 2H), 7.40 (m, 1H), 7.63 (td, *J* = 7.7, 1.7 Hz, 2H), 7.99 (s, 1H), 8.56 (d, *J* = 4.5 Hz, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 53.3 (CH₂), 90.3 (CH), 108.4 (CH) 118.2 (CH), 122.3 (CH), 125.5 (C), 136.9 (CH), 142.7 (CH), 143.6 (CH), 149.5 (CH), 157.8 (C), 162.3 (C), 163.8 (C).

HR-ESI-MS: [C₂₀H₁₉N₆O]⁺ 359.1616

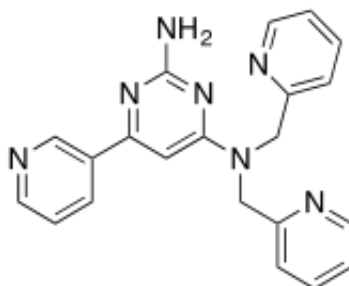
2.3.15. *N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)-6-(pyridin-4-yl)pyrimidine-2,4-diamine (AC-171F).

4-Pyridinylboronic acid (0.056g, 0.45 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.100 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C over night. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure and the crude product (brown solid, 82% yield) was obtained by silica gel filtration with hexanes/ethyl acetate gradient (5-100%).

¹H NMR (600 MHz, Chloroform-*d*) δ 4.94 (s, 4H), 5.17(bs, 2H), 6.38 (s, 1H), 7.2 (m, 4H), 7.65 (m, 4H), 8.58 (m, 2H), 8.64 (m, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 55.4 (CH₂), 91.5 (CH), 120.7 (CH), 121.1 (CH), 122.4 (CH), 136.8 (CH), 145.9 (C), 149.6 (CH), 150.2 (CH), 162.5 (C), 163.1 (C), 164.1 (C).

HR-ESI-MS: [C₂₁ H₂₀ N₇]⁺ 370.1779

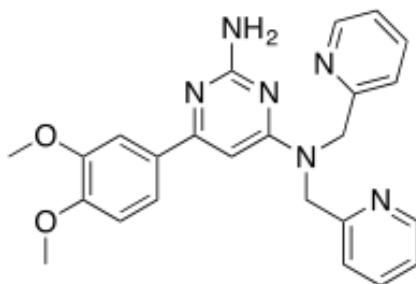
2.3.16. *N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)-6-(pyridin-3-yl)pyrimidine-2,4-diamine (AC-171F3).

3-Pyridinylboronic acid (0.056g, 0.45 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.100 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C over night. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure. The product was isolated by silica gel chromatography with hexanes/ethyl acetate gradient (5-100%) to give the product (pale solid) in 76% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 4.90 (s, 2H), 6.34 (s, 1H), 7.18 (dd, *J* = 7.2, 5.1 Hz, 2H), 7.25–7.34 (m, 2H), 7.60–7.66 (m, 2H), 8.12 (dt, *J* = 7.9, 1.9 Hz, 1H), 8.55–8.63 (m, 3H), 8.96 (d, *J* = 2.0 Hz, 1H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 53.3 (CH₂), 91.2 (CH), 122.3 (CH), 122.3 (CH), 123.2 (CH), 134.2 (C), 134.4 (CH), 136.8 (CH), 148.4 (CH), 149.6 (CH), 150.5 (CH), 162.4 (C), 163.0 (C), 164.0 (C).

HR-ESI-MS: [C₂₁ H₂₀ N₇]⁺ 370.1779

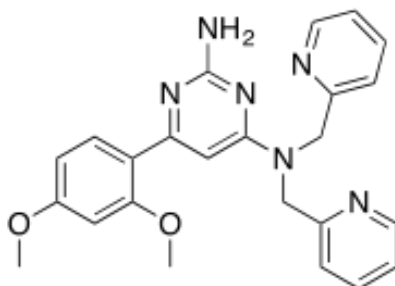
2.3.17. 6-(3,4-dimethoxyphenyl)-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl) pyrimidine-2,4-diamine (AC-171G).

3,4-dimethoxy benzene boronic acid (0.084g, 0.45 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.100 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.03 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C for 8 h or over night. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure. Purification using column chromatography on silica gel (hexanes/EtOAc 70%) afforded the product (brown solid) in 87% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 3.9 (s, 3H), 3.94 (s, 3H), 4.85-5.13 (bs, 4H), 6.28 (s, 1H), 6.86 (d, *J*= 8.4 Hz, 1H), 7.19 (m, 4H), 7.33 (dd, *J*=8.4, 2.0 Hz, 1H), 7.46 (d, *J*=1.8 Hz, 1H), 7.64 (m, 2H), 8.56-8.58 (m, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 53.4 (CH₂), 55.9 (CH₃), 55.91 (CH₃), 90.1 (CH), 110.1 (CH), 110.8 (CH), 119.6 (CH), 120.0 (C), 122.2 (CH), 131.5 (C), 136.7 (CH), 148.9 (C), 149.5 (CH), 150.5 (C), 158.0 (C), 162.9 (C), 164.0 (C), 164.6 (C).

HR-ESI-MS: [C₂₄H₂₅N₆O₂]⁺ 429.2039

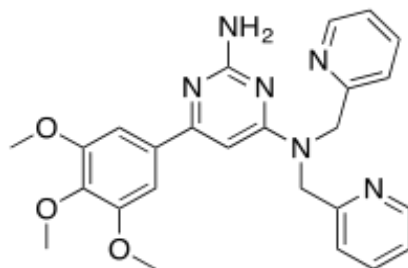
2.3.18. 6-(2,4-dimethoxyphenyl)-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-171H).

2,4-Dimethoxyphenylboronic acid (0.084g, 0.45 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.100 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.03 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C for 8 h or over night. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel with EtOAc/MeOH (2%) as eluent to give pale solid in 56% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 3.59 (s, 3H), 3.82 (s, 3H), 4.75 (s, 2H), 4.96 (bs, 4H), 6.43 (s, 1H), 6.57 – 6.52 (m, 2H), 7.17 (ddd, J = 7.6, 4.8, 1.2 Hz, 2H), 7.25-7.34 (m, 2H) 7.63 (td, J = 7.7, 1.9 Hz, 2H), 7.79 (d, J = 8.6 Hz, 1H), 8.53-8.58 (m, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 53.2 (CH₂), 55.3 (CH₃), 55.4 (CH₃), 94.9 (CH), 98.8 (CH), 104.8 (CH), 120.8 (C), 122.0 (CH), 122.1 (CH), 131.5 (CH), 136.7 (CH), 149.4 (CH), 158.6 (C), 161.6 (C), 162.6 (C), 162.6 (C), 163.5 (C).

HR-ESI-MS: [C₂₄ H₂₅ N₆ O₂]⁺ 429.2034

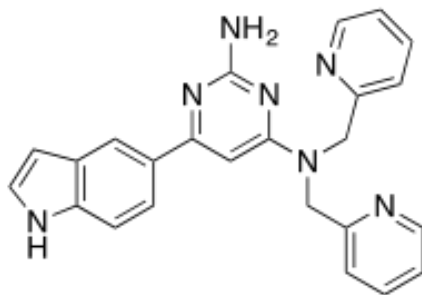
2.3.19. *N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)-6-(3,4,5-trimethoxyphenyl) pyrimidine-2,4-diamine (AC-171I).

3,4,5-trimethoxybenzene boronic acid (0.097g, 0.45 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.100 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.03 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C over night. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure. Silica gel filtration with hexanes/ethyl acetate gradient (5-100%) afforded the product (brown solid) in 55% yield.

¹H NMR (600 MHz, DMSO-*d*₆) δ 3.69 (s, 3H), 3.8 (s, 6H), 4.97 (bs, 3H), 6.12 (s, 2H), 6.45 (s, 1H), 7.15 (s, 2H), 7.25-7.32 (m, 2H), 7.33 (d, *J* = 7.8 Hz, 2H), 7.73–7.79 (m, 2H), 8.53 (d, *J* = 4.3 Hz, 2H).

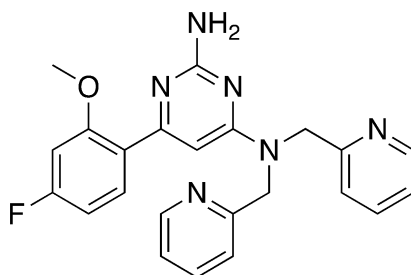
HR-ESI-TOFMS: [C₂₅H₂₇N₆O₃]⁺ 459.2142

2.3.20. 6-(1*H*-indol-5-yl)-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-171J).



5-Indolboronic acid (0.064g, 0.39 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.100 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.03 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C overnight. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure and the purification was performed by silica gel filtration (EtOAc/MeOH 5%) to give AC-171J (brown solid) in 52% yield.

¹H NMR (600 MHz, MeOD) δ 5.03-5.12 (bs, 4H), 6.37 (s, 1H), 6.49 (m, 1H), 7.26 (d, 1H), 7.3 (m, 2H), 7.38 (m, 3H), 7.49 (m, 1H), 7.77 (m, 2H), 7.98 (d, 1H), 8.5 (d, 2H). **HR-ESI-MS:** [C₂₄H₂₂ N₇]⁺ 408.1934

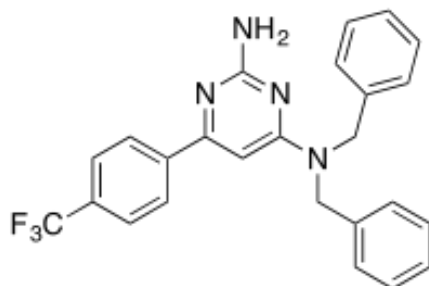
2.3.21. 6-(4-fluoro-2-methoxyphenyl)-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-171M).

4-fluoro-2-methoxyphenyl boronic acid (0.104g, 0.6 mmol) was added to a solution of 6-chloro-*N*⁴,*N*⁴-bis(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AC-17) (0.15 g, 0.45 mmol) in *N,N*-dimethylacetamide (DMA) (3 mL), 2M aqueous potassium carbonate (0.35mL) was added following the addition of [1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.036 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C overnight. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure and the crude product was isolated by silica gel chromatography with hexanes/ethyl acetate gradient (5-100%) to give the product (white solid) in 76% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 3.59 (s, 3H), 4.82 (s, 6H), 6.44 (s, 1H), 6.59 (dd, *J* = 11.0, 2.4 Hz, 1H), 6.69 (td, *J* = 8.3, 2.4 Hz, 1H), 7.18 (dd, *J* = 7.4, 5.7 Hz, 2H), 7.27 (d, *J* = 30.5 Hz, 2H), 7.64 (td, *J* = 7.7, 1.7 Hz, 2H), 7.74 (dd, *J* = 8.6, 7.1 Hz, 1H), 8.55 (d, *J* = 4.4 Hz, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 55.8 (CH₂), 55.8 (CH₃), 95.5 (CH), 95.5 (CH), 99.7 (CH), 107.4 (CH), 107.6 (CH), 122.4 (CH), 124.2 (C), 131.9 (CH), 132.0 (CH), 137.0 (CH), 149.7 (CH), 158.8 (C), 158.8 (C), 162.4 (C), 162.9 (C), 163.4 (C), 163.7 (C), 165.07 (C).

HR-ESI-MS: [C₂₃H₂₂N₆O F]⁺ 417.1832

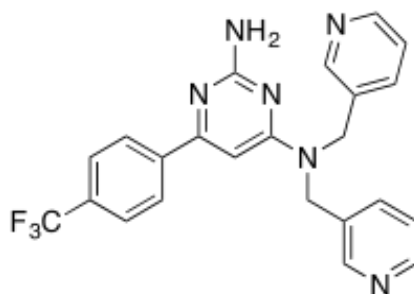
2.3.22. *N*⁴,*N*⁴-dibenzyl-6-(4-(trifluoromethyl)phenyl)pyrimidine-2,4-diamine (AC-19-2).

4-(Trifluoromethyl)benzenel boronic acid (0.07 g, 0.36 mmol) was added to a solution of *N*⁴,*N*⁴-dibenzyl-6-chloropyrimidine-2,4-diamine (AC-19) (0.100 g, 0.3 mmol) in toluene/ethanol (1:1, 2 mL). Sodium carbonate decahydrate (0.176 g, 0.6 mmol) was added following the addition of *tetrakis*(triphenylphosphine)palladium(0) (35 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 95 °C overnight. After cooling the mixture to room temperature, water was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by Silica gel filtration with hexanes/EtOAc (5%) as eluent afforded the product (white solid) in 77% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 4.79 (bs, 2H), 4.88 (s, 4H), 6.32 (s, 1H), 7.20-7.35 (m, 10H), 7.63-7.67 (m, 2H), 7.91 (d, *J* = 8.2 Hz, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 50.1 (CH₂), 91.1 (CH), 119.2 (CH), 120.0 (CH), 125.4 (q, ³*J*_{C-F} = 3.79 Hz, CH), 127.2 (CH), 127.4 (CH), 127.4 (CH), 128.7 (CH), 128.8 (CH), 131.2 (q, ²*J*_{C-F} = 32.38 Hz, C), 142.2 (C), 142.3 (C), 163.0 (C), 163.5 (C), 164.3 (C).

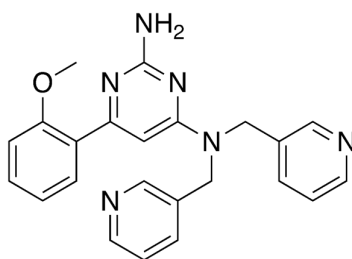
HR-ESI-MS: [C₂₅H₂₂F₃N₄]⁺ 435.1793

2.3.23. N^4,N^4 -bis(pyridin-3-ylmethyl)-6-(4-(trifluoromethyl)phenyl) pyrimidine-2,4-diamine (AC-20-2).

4-(Trifluoromethyl)benzenel boronic acid (0.014g, 0.07 mmol) was added to a solution of 6-chloro- N^4,N^4 -bis(pyridin-3-ylmethyl)pyrimidine-2,4-diamine (AC-20). (20mg g, 0.06 mmol) in in toluene/ethanol (1:1, 1 mL). Sodium carbonate decahydrate (0.035 g, 0.12 mmol) was added following the addition of *tetrakis*(triphenylphosphine)palladium(0) (7 mg, 0.006 mmol). The resulting mixture was stirred in a capped glass vial at 95 °C overnight. After cooling the mixture to room temperature, water was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by Silica gel filtration with hexanes/EtOAc (5%) as eluent afforded white solid in 77% yield.

^1H NMR (600 MHz, Chloroform-*d*) δ 4.8 (s, 4H), 4.99 (s, 2H), 6.29 (s, 1H), 7.26-7.26-7.29 (m, 2H), 7.57 (d, J = 7.8 Hz, 2H), 7.65 (d, J = 8.3 Hz, 2H), 7.92 (d, J =8.1Hz, 2H), 8.55-8.56 (m, 4H).

HR-ESI-TOFMS: $[\text{C}_{23}\text{H}_{20}\text{F}_3\text{N}_6]^+$ 437.1693

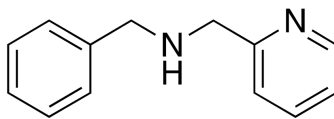
2.3.24. 6-(2-methoxyphenyl)-*N*⁴,*N*⁴-bis(pyridin-3-ylmethyl)pyrimidine-2,4-diamine (AC-201A2).

2-Methoxyphenylboronic acid (0.07g, 0.45 mmol) was added to a solution of 6-chloro-6-chloro-*N*⁴,*N*⁴-bis(pyridin-3-ylmethyl)pyrimidine-2,4-diamine (AC-20) (0.1 g, 0.3 mmol) in *N,N*-dimethylacetamide (2.5 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C for 2.5 h. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate and filtered. Purification using silica gel filtration (EtOAc/MeOH 2%) afforder the product (brown solid).

¹H NMR (600 MHz, Chloroform-*d*) δ 3.67 (s, 3H), 4.77 (s, 4H), 4.87 (s, 2H), 6.52 (s, 1H), 6.91 (d, 1H), 7.03 (s, 1H), 7.26 (m, 2H), 7.35 (m, 1H), 7.62 (d, *J* = 7.9 Hz, 2H), 7.75 (d, *J* = 7.5 Hz, 1H), 8.55 (dd, *J* = 1.9 Hz, 4H).

¹³C NMR (151 MHz, Chloroform- *d*) δ 48.2 (CH₂), 55.2 (CH₃), 95.2 (CH), 111.4 (CH), 119.2 (C), 120.0 (C), 120.7 (CH), 120.9 (CH), 123.6 (CH), 130.4 (CH), 135.1 (CH), 149.0 (CH), 149.1 (CH), 157.2 (C), 163.3 (C), 163.6 (C), 164.0 (C).

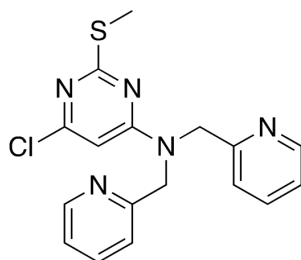
HR-ESI-TOFMS: [C₂₃H₂₃N₆O]⁺ 399.1930

2.3.25. *N*-benzyl-1-(pyridin-2-yl)methanamine.

To a solution of benzylamine (0.54 g, 5mmol) in 4 mL of ethanol, pyridine-2-carboxaldehyde (0.54 g, 5mmol) in 4 mL of ethanol was added. Heat was evolved and the solution got yellow. The solution was stirred overnight and boiled gently for 30 min. NaBH_4 (0.25 g, 6.5mmol) was slowly added at 0 °C. After 2 hrs, 7mL of H_2O was slowly added following by addition of 20 mL of a saturated solution of NH_4Cl , bring to pH 7 the solution, the mixture was extracted with ethyl acetate (3 x 30 mL). The filtrate was concentrated under reduced pressure and the crude oil was purified. Oxalic acid was added (0.636 g, 5.04 mmol) in 15 mL of ethanol. The salt was filtered and dissolved in 20 mL of H_2O following by addition of NaOH (30%), extractions with EtOAc was made. The organic solvent was removed and 67% of yield of oil compound was isolated.

^1H NMR (600 MHz, Chloroform-*d*) δ 2.52 (s, 1H), 3.86 (s, 2H), 3.94 (s, 2H), 7.20 – 7.14 (m, 1H), 7.26 (t, $J = 7.2$ Hz, 1H), 7.34 (td, $J = 8.4, 7.9, 2.0$ Hz, 3H), 7.38 (d, $J = 7.5$ Hz, 2H), 7.65 (td, $J = 7.6, 1.8$ Hz, 1H), 8.57 (ddd, $J = 4.8, 1.6, 0.8$ Hz, 1H).

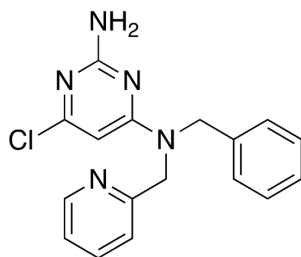
HR- ESI-TOFMS: $[\text{C}_{13}\text{H}_{15}\text{N}_2]^+$ 199.1223

2.3.26. 6-chloro-2-(methylthio)-*N,N*-bis(pyridin-2-ylmethyl)pyrimidin-4-amine (AL-1).

Di-(2-picolyl)amine (1.123g, 5.63 mmol) was added to a solution 4,6-dichloro-2-(methylthio)pyrimidine (1.0 g, 5.01 mmol) and *N,N*-diisopropylethylamine (0.732 mL, 5.63 mmol) in DMF (8 mL) and the solution was stirred at 80 °C for 3.5 hours (reaction monitored by TLC). The solvent was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 40 mL). The organic layers were washed with brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated on a rotary evaporator, and the product was purified by column chromatography using silica gel (hexanes/EtOAc 3:2 or by crystallization after purification a white solid was obtained with a 91% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 2.37 (s, 3H), 4.74 (s, 2H), 5.14 (s, 2H), 6.22 (s, 1H), 7.26 – 7.12 (m, 4H), 7.64 (td, *J* = 7.7, 1.6 Hz, 2H), 8.54 (s, 2H).

HR-ESI-TOFMS: [C₁₇H₁₇ClN₅S]⁺ 358.0885

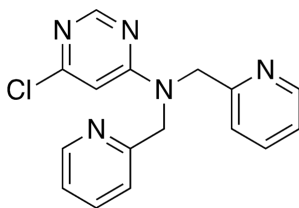
2.3.27. *N*⁴-benzyl-6-chloro-*N*⁴-(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AL-2).

N-benzyl-1-(pyridin-2-yl)methanamine (0.5, 2.5 mmol) was added to a solution of 2-amino-4,6-dichloropyrimidine (0.37, 2.2 mmol) and *N,N*-diisopropylethylamine (0.44 mL, 2.5 mmol) in DMF (3.4 mL) and the solution was stirred at 80 °C overnight. The solvent was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 40 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated on the rotary evaporator, and the product was purified by column chromatography using silica gel (EtOAc/MeOH 9:1) after purification a white solid was obtained with a 36% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 4.96 (s, 6H), 5.94 (s, 1H), 7.20 (dd, *J* = 13.8, 6.6 Hz, 3H), 7.28 (t, *J* = 7.2 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 2H), 7.64 (td, *J* = 7.7, 1.8 Hz, 1H), 8.57 (d, *J* = 4.3 Hz, 1H).

¹³C NMR (151 MHz, Chloroform- *d*) δ 52.7 (CH₂), 92.8 (CH), 110.4 (CH), 120.2 (CH), 122.6 (CH), 127.7 (CH), 129.0 (CH), 137.1 (CH), 149.8 (CH), 160.6 (C), 162.3 (C), 164.4(C).

HR-ESI-TOFMS: [C₁₇ H₁₇ Cl N₅]⁺ 326.1165

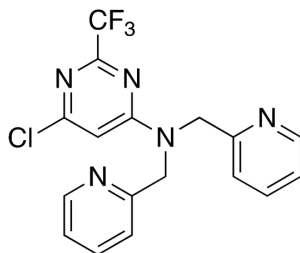
2.3.28 6-chloro-*N,N*-bis(pyridin-2-ylmethyl)pyrimidin-4-amine (AL-4).

Di-(2-picolyl)amine (1.45 mL, 8.0 mmol) was added to a solution of 4,6-dichloropyrimidine (1.0 g, 6.70 mmol) and *N,N*-diisopropylethylamine (1.4 mL, 8.0 mmol) in DMF (9 mL) and the solution was stirred at 80 °C for 1.5 hours (reaction monitored by TLC). The solvent was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were washed with brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated on a rotary evaporator, and the product was purified by column chromatography using silica gel and hexanes-EtOAc (75%). After purification a white solid was obtained with a 64% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 4.77 (s, 2H), 5.12 (s, 2H), 6.54 (s, 1H), 7.30 – 7.11 (m, 4H), 7.65 (td, *J* = 7.7, 1.7 Hz, 2H), 8.44 (s, 1H), 8.57 (s, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) 53.7 (CH₂), 102.2 (CH), 102.2 (CH), 122.9 (CH), 137.2 (CH), 158.3 (CH), 160.0 (C), 160.3 (C), 163.4 (C).

HR-ESI-TOFMS: [C₁₆ H₁₅ Cl N₅]⁺ 312.1007

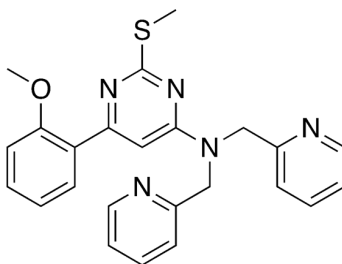
2.3.29. 6-chloro-*N,N*-bis(pyridin-2-ylmethyl)-2-(trifluoromethyl)pyrimidin-4-amine (AL-6).

Di-(2-picolyl)amine (0.215 mL, 1.20 mmol) was added to a solution of 4,6-dichloro-2-(trifluoromethyl)pyrimidine (0.216 g, 1.00 mmol) and *N,N*-diisopropylethylamine (0.208 mL, 1.20 mmol) in DMF (1.5 mL) and the solution was stirred at 80 °C for 3.5 hours (reaction monitored by TLC). The solvent was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were washed with brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated on a rotary evaporator, and the product was purified by column chromatography using silica gel (hexanes/EtOAc 30%). After purification a white solid was obtained with a 93% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 4.87 (s, 2H), 5.15 (s, 2H), 6.66 (s, 1H), 7.17 (d, J = 7.1 Hz, 1H), 7.26 – 7.19 (m, 2H), 7.42 (d, J = 7.1 Hz, 1H), 7.70 – 7.62 (m, 2H), 8.57 (d, J = 38.8 Hz, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 55.2 (CH₂), 104.0 (CH), 116.5-121.95 (q, $^1J_{C-F}$ = 276.3 Hz, CF₃), 121.3 (CH), 123.1 (CH), 123.2 (CH), 123.5 (CH), 149.6 (CH), 150.4 (CH), 155.3 (C), 156.5-155.7 (q, $^2J_{C-F}$ = 36.96 Hz, C), 156.4 (C), 160.8 (C), 163.6 (C).

HR-ESI-TOFMS: [C₁₇H₁₄ClF₃N₅]⁺ 380.0879

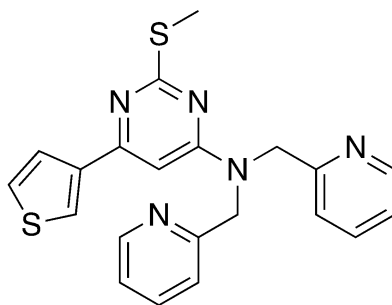
3.2.30. 6-(2-methoxyphenyl)-2-(methylthio)-*N,N*-bis(pyridin-2-ylmethyl)pyrimidin-4-amine(AL-1A).

2-Methoxyphenylboronic acid (0.552g, 3.6 mmol) was added to a solution of 6-chloro-2-(methylthio)-*N,N*-bis(pyridin-2-ylmethyl)pyrimidin-4-amine (AL-1) (1.0 g, 2.8 mmol) in *N,N*-dimethylacetamide (DMA) (22 mL), 2M aqueous potassium carbonate (2.8mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (0.2 g, 0.28 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C overnight. After cooling the mixture to room temperature, water was added and the mixture was extracted with ethyl acetate (3 x 50 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was filtrate through silica gel and recrystallized in EtAcO to give white solid, in 42% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 2.47 (s, 3H), 3.61 (s, 3H), 4.85 (s, 2H) , 5.22 (s, 2H), 6.85 (s, 1H), 6.88 (d, J = 8.3 Hz, 1H), 7.02 (t, J = 7.5 Hz, 1H), 7.20 (dd, J = 7.1, 5.1 Hz, 2H), 7.34 (t, J = 7.8 Hz, 2H), 7.65 (t, J = 7.6 Hz, 2H), 7.93 (dd, J = 7.7, 1.7 Hz, 1H), 8.57 (s, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 14.2 (CH₃), 53.6 (CH₂), 55.3 (CH₃), 99.5 (CH), 111.3 (CH), 114.2 (C), 120.0 (CH), 120.9 (CH), 122.3 (CH), 126.9 (C), 130.8 (CH), 130.9 (CH), 136.9 (CH), 157.6 (C), 161.5 (C), 162.0 (C), 170.63 (C).

HR-ESI-TOFMS: [C₂₄H₂₄N₅O S]⁺ 430.1692.

2.3.31. 2-(methylthio)-*N,N*-bis(pyridin-2-ylmethyl)pyrimidin-4-amine(AL-1B).

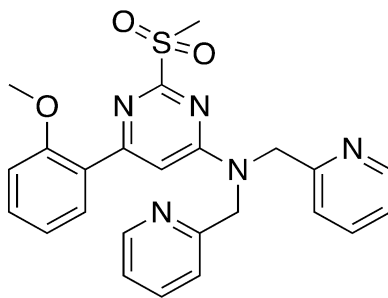
3-Thienylboronic acid (0.039g, 0.3 mmol) was added to a solution of 6-chloro-2-(methylthio)-*N,N*-bis(pyridin-2-ylmethyl)pyrimidin-4-amine (AL-1) (0.100 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.0 mL), 2M aqueous potassium carbonate (0.3mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C over night. After cooling the mixture to room temperature, water was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the product was isolated by column chromatography on silica gel using hexanes-EtOAc gradient (5 – 90%) (pale solid, 40% yield).

¹H NMR (600 MHz, Chloroform-*d*) δ 2.47 (s, 3H), 4.86 (s, 2H), 5.18 (s, 2H), 6.47 (s, 1H), 7.19 (dd, J = 7.0, 5.2 Hz, 3H), 7.36 – 7.26 (m, 2H), 7.49 – 7.44 (m, 1H), 7.63 (t, J = 7.2 Hz, 2H), 7.94 (d, J = 2.1 Hz, 1H), 8.57 (s, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 14.2 (CH₃), 53.7 (CH₂), 94.2 (CH), 122.5 (CH), 125.9 (CH), 126.0 (CH), 126.2 (CH), 127.3 (CH), 127.8 (CH), 136.9 (CH), 140.7 (C), 159.1 (C), 162.4 (C), 171.3 (C).

HR-ESI-TOFMS: [C₂₁H₂₀N₅S₂]⁺ 406.1152

2.3.32. 6-(2-methoxyphenyl)-2-(methylsulfonyl)-*N,N*-bis(pyridin-2-ylmethyl)pyrimidin-4-amine(AL-1A2).

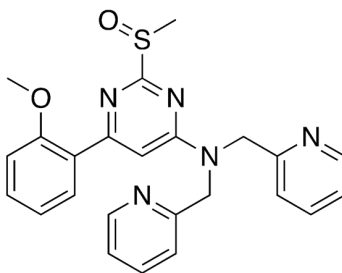


AL-1A (0.3 g, 0.7 mmol) was dissolved in 1.5mL of DCM, following by slow addition of m-CPBA (0.25g, 1.5 mmol) at 0°C. After 3 hrs, saturated solution of Na₂S₂O₃ was added, and the mixture was extracted with ethyl acetate (2 x 40 mL). The organic layers were washed with saturated solution of NaHCO₃ the mixture was extracted with ethyl acetate (3 x 40 mL). The organic layers were dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel with hexanes/EtOAc 80% to give white solid, in 31% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 3.28 (s, 3H), 3.63 (s, 3H), 4.95 (s, 2H), 5.24 (s, 2H), 6.91 (dd, *J* = 8.4, 1.1 Hz, 1H), 7.05 (td, *J* = 7.6, 1.1 Hz, 1H), 7.25 – 7.14 (m, 3H), 7.36 (s, 1H), 7.41 – 7.36 (m, 1H), 7.56 (d, *J* = 6.2 Hz, 1H), 7.68 (s, 2H), 8.08 – 8.04 (m, 1H), 8.58 (d, *J* = 45.3 Hz, 2H).

HR-ESI-TOFMS: [C₂₄ H₂₄ N₅ O₃ S]⁺ 462.1592

2.3.33. .6-(2-methoxyphenyl)-2-(methylsulfinyl)-*N,N*-bis(pyridin-2-ylmethyl)pyrimidin-4-amine(AL-1A3).

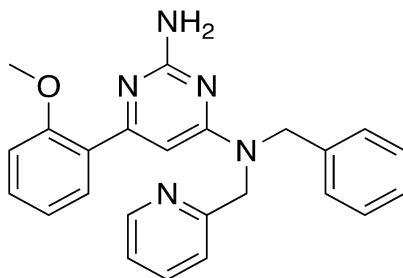


AL-1A (0.1 g, 0.23 mmol) was dissolved in 1.0 mL of DCM, followed by slow addition of *m*-CPBA (0.04g, 0.23 mmol) at 0°C. After 3 hrs, saturated solution of Na₂S₂O₃ was added and the mixture was extracted with ethyl acetate (2 x 20 mL). The organic layers were washed with saturated solution of NaHCO₃ and the mixture was extracted with ethyl acetate (3 x 20 mL). The organic layers were dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel with EtOAc/MeOH 10% to give white solid in 79% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 2.88 (s, 3H) 3.64 (s, 3H), 4.92 (s, 2H), 5.27 (d, *J* = 27.6 Hz, 2H), 6.90 (d, *J* = 8.2 Hz, 1H), 7.04 (t, *J* = 8.0 Hz, 1H), 7.21 (s, 2H), 7.23 (s, 2H), 7.39 – 7.34 (m, 1H), 7.53 (s, 1H), 7.67 (t, *J* = 7.1 Hz, 2H), 8.06 (dd, *J* = 7.8, 1.8 Hz, 1H), 8.61 (s, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 40.0 (CH₃), 54.0 (CH₂), 54.4 (CH₂) 55.6 (CH₃), 104.3 (CH), 111.5 (CH), 121.2 (CH), 122.8 (CH), 125.8 (C), 131.4 (CH), 131.7 (CH), 137.1 (CH), 157.9 (C), 162.1 (C), 163.2 (C), 172.0 (C).

HR-ESI-TOFMS [C₂₄H₂₄N₅O₂S]⁺ 446.1645

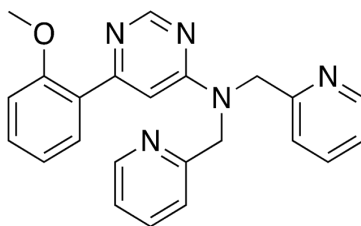
2.3.34. *N*⁴-benzyl-6-(2-methoxyphenyl)-*N*⁴-(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AL-2A).

2-Methoxyphenylboronic acid (0.056g, 0.37 mmol) was added to a solution of *N*⁴-benzyl-6-chloro-*N*⁴-(pyridin-2-ylmethyl)pyrimidine-2,4-diamine (AL-2) (0.1 g, 0.3 mmol) in *N,N*-dimethylacetamide (DMA) (2.0 mL), 2M aqueous potassium carbonate (0.45mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C for 3.5 h. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was filtrate through silica using ethyl acetate. After purification by column chromatography on silica gel was performed utilizing EtOAc to give the product as a white solid, in 83% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 3.62 (s, 3H), 4.79 (s, 6H), 6.48 (s, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 7.01 (t, *J* = 8.0 Hz, 1H), 7.18 (dd, *J* = 7.0, 5.2 Hz, 1H), 7.26 (s, 2H), 7.35 – 7.28 (m, 6H), 7.64 (td, *J* = 7.7, 1.7 Hz, 1H), 7.71 (dd, *J* = 7.6, 1.8 Hz, 1H), 8.56 (d, *J* = 5.3 Hz, 1H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 52.8 (CH₂), 55.6 (CH₃), 95.74 (CH), 111.6 (CH), 120.2 (CH), 120.2 (CH), 121.0 (CH), 121.5 (CH), 122.0 (CH), 122.3 (CH), 127.4 (CH), 128.4 (C), 128.8 (CH), 130.4 (CH), 130.6 (CH), 137.0 (CH), 145.0 (C), 149.7 (CH), 157.4 (C), 162.9 (C), 163.2 (C), 163.7 (C).

HR-ESI-TOFMS: [C₂₄H₂₄N₅O]⁺ 398.1977

2.3.35. 6-(2-methoxyphenyl)-*N,N*-bis(pyridin-2-ylmethyl)pyrimidin-4-amine (AL-4A).

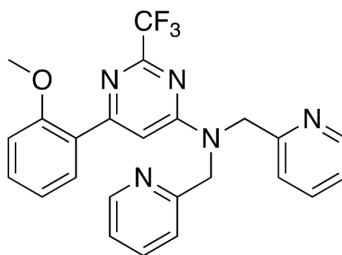
2-Methoxyphenylboronic acid (0.05g, 0.3 mmol) was added to a solution of 2.3.9 6-chloro-*N,N*-bis(pyridin-2-ylmethyl)pyrimidin-4-amine (AL-4) (0.075 g, 0.24 mmol) in *N,N*-dimethylacetamide (DMA) (2.0 mL), 2M aqueous potassium carbonate (0.25mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (17 mg, 0.024 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C overnight. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel with CH₃Cl/MeOH/NH₄OH 100/1.2/0.2 to give the product as a white solid, in 49% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 3.62 (s, 3H), 4.95 (s, 3H), 6.92 – 6.87 (m, 1H), 7.10 (d, J = 1.1 Hz, 1H), 7.04 (t, J = 8.0 Hz, 1H), 7.20 (dd, J = 7.0, 5.3 Hz, 2H), 7.40 – 7.28 (m, 2H), 7.65 (td, J = 7.7, 1.7 Hz, 2H), 7.86 (dd, J = 7.7, 1.8 Hz, 1H), 8.58 (d, J = 4.4 Hz, 2H), 8.78 (s, 1H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 53.7 (CH₂), 55.5 (CH₃), 104.0 (CH), 111.5 (CH), 121.1 (CH), 122.6 (CH), 127.3 (C), 129.1 (CH), 131.0 (CH), 131.0 (CH), 137.1 (CH), 149.8 (CH), 156.6 (C), 157.6 (C), 158.3 (CH), 161.6 (C), 162.4 (C).

HR-ESI-TOFMS: [C₂₃H₂₂N₅O]⁺ 384.1820

2.3.36. 6-(2-methoxyphenyl)-*N,N*-bis(pyridin-2-ylmethyl)-2-(trifluoromethyl)pyrimidin-4-amine(AL-6A).



2-Methoxyphenylboronic acid (0.05g, 0.34 mmol) was added to a solution of 6-chloro-*N,N*-bis(pyridin-2-ylmethyl)-2-(trifluoromethyl)pyrimidin-4-amine (AL-6) (0.1 g, 0.34 mmol) in *N,N*-dimethylacetamide (DMA) (2.0 mL), 2M aqueous potassium carbonate (0.2mL) was added following the addition of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (19 mg, 0.030 mmol). The resulting mixture was stirred in a capped glass vial at 149 °C overnight. DMA was removed under reduced pressure, brine was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel with hexanes-EtOAc 80% to give the product as a white solid, in 38% yield.

¹H NMR (600 MHz, Chloroform-*d*) δ 3.61 (s, 3H), 4.92 (s, 2H), 5.23 (s, 2H), 6.90 (d, *J* = 8.2 Hz, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 7.21 (s, 3H), 7.25 (s, 1H), 7.40 – 7.33 (m, 1H), 7.52 (s, 1H), 7.67 (s, 2H), 8.02 – 7.98 (m, 1H), 8.58 (d, *J* = 25.7 Hz, 2H).

¹³C NMR (151 MHz, Chloroform-*d*) δ 54.26 (CH₂), 55.6 (CH₃), 105.3 (CH), 105.5 (CH), 111.6 (CH), 118.4-121.8 (q, ¹*J*_{C-F} = 276.3 Hz, CF₃), 121.3 (CH), 122.8 (CH), 123.0 (CH), 126.1 (C), 131.3 (CH), 131.6 (C), 137.1 (CH), 155.6-156.3 (q, ²*J*_{C-F} = 36.2 Hz, C) 156.2 (CH), 157.8 (C), 161.9 (C), 162.9 (C).

HR-ESI-TOFMS: [C₂₄ H₂₁ F₃ N₅ O]⁺ 452.1689

CHAPTER III. PHARMACOLOGICAL EVALUATION

3.1. Material and methods

(±)-Epibatidine	Tocris
[³ H]- (±)-Epibatidine	Perkin Elmer
5-Hydroxytryptamine	Tocris
Calcium chloride	Fisher scientific
D-Glucose	Fisher scientific
DMEM	Corning
FBS	Gibco
Glutamine	Gibco
HEPES	Sigma-Aldrich
Hydrochloric acid, 37% solution	EMD
Magnesium chloride	Fisher scientific
MLA	Tocris
Monoclonal mouse anti-FLAG M2 antibody	Sigma-Aldrich
MQ-Water	Millipore
Nicotine	Tocris
PNU-120596	Tocris
Potassium chloride	Fisher scientific
Sodium bicarbonate	Fisher scientific
Sodium chloride	Fisher scientific
Sodium hydroxide, 33% solution	Ricca chemical company
Sodium phosphate dibasic anhydrous	Fisher scientific
Sodium phosphate monobasic monohydrate	Fisher scientific
SPA-Beads	Perkin Elmer
Tropisetron	Tocris

3.1.1. Radioligand binding assay

3.1.1.1. Background

Radioligand binding techniques are one of the most sensitive quantitative techniques to assess binding affinity and receptor density. There are three main types of radioligand binding assays: saturation, competition, and kinetics. The K_D or equilibrium dissociation constant is the concentration of ligand that will occupy 50% of the receptors, and this can be calculated directly or by using a competitive assay (Maguire, J.J. *et al.* 2012). The affinity or strength of interaction of a ligand for a receptor is given by the reciprocal of K_D , the K_a .

The Scintillation proximity assay (SPA) is a competitive radioligand binding technique used to assess drug-target binding affinity. SPA has been designed for radioisotope detection bound to a polymeric scintillant coated bead. The principle of this technique relies in the isotope being close or “in close proximity” to a scintillant bead (SPA-bead) so it can be activated. In a receptor-binding studies, the receptor is linked to a SPA-bead. Upon binding of radiolabelled ligand sufficiently near to the SPA-bead, activation will occur. The light emitted by the scintillant bead is detected by scintillation counter. Unbound radiolabelled ligand energy is then absorbed in the buffer and scattered.

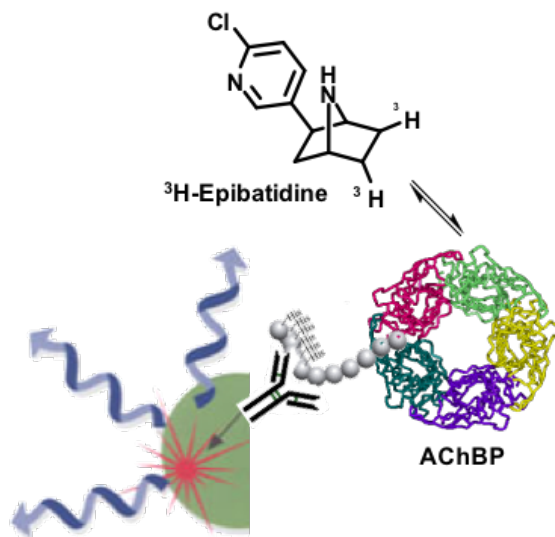


Figure 8. The scintillation proximity assay. When the AChBP is bound to a SPA-beads the proximity of radiolabel ligand will activate the bead and this will result in emission of light. When ligand displace radiolabel epibatidine, the energy of unbound ligand will be absorbed or scattered and will not activate the scintillant.

3.1.1.2. Protein expression and purification.

AChBPs were expressed and purified as previously reported (Hansen, S. B. *et al.* 2005; Talley, T. T. *et al.* 2016). Briefly, AChBP was expressed with an amino-terminal FLAG epitope tag and secreted from stably transfected HEK 293S cells lacking the *N*-acetylglucosaminyltransferase I (GnT1⁻) gene to limit variable processing of the attached oligosaccharide termini. Protein was purified using FLAG-antibody resin, eluted with FLAG peptide (Sigma Aldrich) and further characterized as a pentamer by size-exclusion chromatography [Superose 6 10/300GL column (GE Healthcare) in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.02% NaN₃]. The latter fractionation removes traces of contaminants and monomer. Protein concentrations were determined by UV absorbance at 280 nm NanoDrop 2000c Spectrophotometer (ThermoScientific).

3.1.1.3. Quick screen

A scintillation proximity assay was employed to measure ligand binding to AChBP using [³H]-(±)-epibatidine (5 nM for *Ls*-AChBP and 20 nM for *Ac*-AChBP final concentration) as the labeled ligand. Polyvinyltoluene anti-mouse SPA scintillation beads (0.17 mg/mL final concentration, GE Healthcare), monoclonal anti-FLAG M2 antibody from mouse 1:8000 dilution, and 0.50 nM AChBP in binding sites were prepared in phosphate buffer (PB) (SPA-cocktail) (). Combination of SPA-cocktail and 10 μM final concentration of ligands were tested after 15 minutes of incubation for displacement of [³H]-(±)-epibatidine, after incubation for 1 hour, the well were measured on a 1450 Microbeta. Nicotine was used as positive control and PB as a negative control. Passing initial screens for further analyses required dissociation of at least 50% of the bound epibatidine by the candidate competitive ligand.

3.1.1.4. Titration curves and determination of K_d.

Full concentration curves were then generated for the higher affinity ligands. Nonspecific binding was determined in parallel by adding a saturating concentration (10 μM) of MLA, different concentrations of the compounds were tested by adding the SPA-cocktail for 15 minutes following by addition of [³H]-(±)-epibatidine to generate the concentration-response curves. The resulting mixtures were allowed to equilibrate at room temperature for a minimum of 1 h and measured on a 1450 MicroBeta TriLux liquid scintillation counter (Wallac). Dissociation constants and Hill coefficients were determined from the competition profiles, using an epibatidine K_d of 0.30 nM (GraphPad

Prism 4), the data were normalized and fit to estimate the K_d . All measurements were completed in triplicate and the results were reported as means $\pm$ standard deviation (SD). Nicotine dissociation of labeled epibatidine was employed as a frame of reference for competing ligands.

3.1.2. Cell-based functional assay.

3.1.2.1. Background

Genetically encoded calcium indicators have become widely used to understand intracellular signaling in intact tissues or neuronal activity in living organisms. Förster or fluorescence resonance energy transfer (FRET) is a distance-dependent physical process by which energy is transferred from the fluorophore (donor) to another fluorophore (acceptor) (Sekar, R. B.; Periasamy, A. 2003). The two most used fluorophores are cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP).

Troponin C (TnC) is a calcium-binding protein specialized in regulating muscle constraction. The protein consist of a N-terminal regulatory lobe with two low affinity calcium binding sites, a C-terminal structural lobe with two high affinity calcium binding sites and one magnesium binding site; the last one was engineered to be eliminated. TN-XXL is a TnC mutant variant. Two copies of the C-terminal lobe of chicken skeletal TnC were linked to each other and sandwiched between CFP and Citrine cp174. Initially, the incorporation of TN-XLL to a G-protein-couple receptor GPCR express system produced a cell-based neurotransmitter fluorescent engineered reported (CNiFERS) that was developed mainly for detection of neurotransmitter activity (Nguyen, Q-T *et al.* 2009), Yamauchi and colaborators (2010) developed nAChR CNiFERS that allow us to detected ion-channel activation via Ca^{+2} flux.

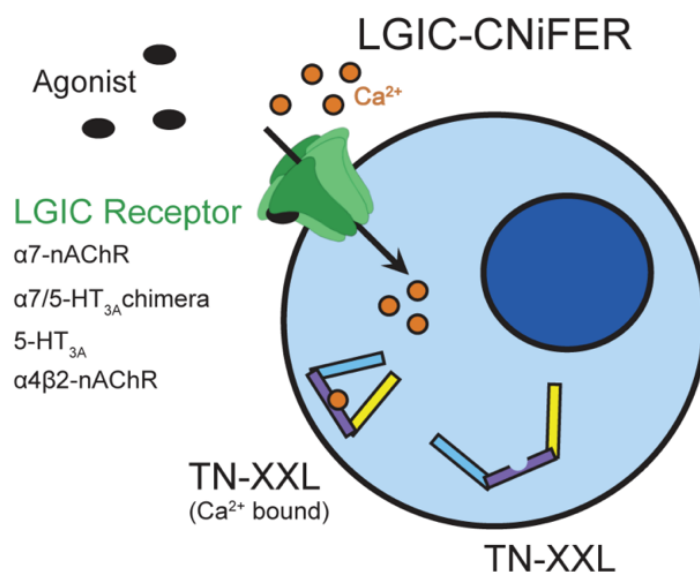


Figure 9. Upon activation of calcium permeable receptor, Ca²⁺ enters the cell and upon binding to TN-XXL produces a conformational change eliciting a fluorescence energy transfer reflected in quenching of the donor fluorescence and excitation of the acceptor (figure and text taken from Yamauchi, J. *et al.* 2011).

To functionally characterize the ligands, we use HEK293 cell lines expressing TN-XXL-FRET biosensor. The principle of this assay is based on binding of agonist ligand to the LGIC expressed in the membrane elicits influx of extracellular calcium, Ca²⁺ will bind to the modified-TnC (TN-XXL) that will cause a conformational change in this protein allowing the FRET to occur. This occurs by virtue of donor and acceptor coming on closer proximity resulting on quenching of donor fluorescence and excitation of the acceptor .

3.1.2.2. Quick screen

Cells selected to express high levels of the specified receptor α7 and α4β2 nAChRs and 5-HT_{3A} containing the CNiFER, were cultured in 10 cm plates with DMEM supplemented with 10% FBS and 1% Glutamine, and incubated at 37 °C with 10% CO₂. Cells were selected at 70-90% confluency and plated in a clear-bottom TC treated 96 well black plates the day before using 100 µl volumes per well.

On the next day, media were replaced with 100 ml of artificial cerebral spinal fluid (aCSF, 121mM NaCl, 5mM KCl, 26mM NaHCO₃, 1.2mM NaH₂PO₄·H₂O, 10mM glucose, 2.4mM CaCl₂, 1.3mM MgSO₄, 5mM HEPES, pH 7.4). For all assays performed

on $\alpha 7$ - nAChR CNiFERs aCSF with 10 μ M of the PAM, PNU-120596 was used. Plates were incubated with buffer for 30 min at 37 °C and 10% CO₂.

Agonist responses were measured in triplicate wells with the FlexStation III (SoftMax Pro 5.2, Molecular Devices) and run at 37 °C by monitoring TN-XXL FRET ratios, emissions of citrine cp174 (527 nm) to eCFP (485 nm), over 120 seconds with agonist injections at 30 sec. Initial testing of agonist properties was evaluated at final concentration of 13.3 μ M , then antagonism was subsequently evaluated at final concentration of 10 μ M by adding an agonist (100nM of ($\pm$)-epibatidine for $\alpha 7$ and $\alpha 4\beta 2$ -nAChRs and 3 μ M of 5-hydroxytryptamine for the 5-HT_{3A} receptors). Compounds having $\geq 20\%$ agonist response were following tested to determine EC₅₀ and compounds having $\geq 50\%$ of inhibition were following tested to determine K_A values.

3.1.2.3. Titration curves, determination of EC₅₀ and K_A values.

A sigmoidal concentration-response (variable slope) regression of the mean peak FRET ratios was fit to generate a concentration-response curves and obtain EC₅₀ values (GraphPad Prism 4). Different concentrations of the compounds were used to construct the dose-response curve using the method describe above (Functional cell-based assay). The EC₅₀ was reported as means $\pm$ standard deviation (SD) of at least three different experiments.

K_A values of tested compounds that passed the antagonistic quick screen, were determined by incubation of different concentrations of compound in aCSF 37 °C for 30 min. The agonist compounds, ($\pm$)-Epibatidine for $\alpha 4\beta 2$ -nAChR and 5-HT for 5-HT_{3A}-serotonin receptors were titrate at various concetration and added in the Flexstation in a 96 wells plate. GraphPad prism 4 was used to normalize and fit the data to a sigmoidal dose-response curve (variable slope) to obtain EC₅₀. The K_A value for competitive antagonists and noncompetitive antagonists was calculated from Shild equations 1 adn 2, respectively:

$$K_A = [A] / [DR-1] \text{ eq.1}$$

$$K_A = [A] / [(\Delta_{\max} / \Delta) - 1] \text{ eq.2}$$

3.1.3. Two-electrode voltage clamp electrophysiology

3.1.3.1. Background

Oocytes from the South African clawed frog *Xenopus laevis* are the most commonly used for studying the properties, organization, and cellular roles of Ca^{+2} permeable channels, like the nAChRs, and transporters. The Oocyte is a embryonic precursor of differentiated cells. There are several reasons of why this system allows recording multiple signals (Lin-Moshier, Y.; Marchant, J. S. 2013):

1. The oocytes are easy to obtain.
2. The oocytes are capable of being maintained *in vitro* for extended periods of time.
3. The size of an oocyte is large compared to other cells (1.0-1.2 mm of diameter), this allows manual electrode manipulations.
4. LGIC Functional assays are ease to perform. The large size of the oocyte allows two-electrode voltage clamp.

The two-electrode voltage clamp is a method for measuring and controlling the electrical potential across the cell membrane. This technique consists in the cell been impaled by two glass electrodes. One of them will measure the voltage across the cell membrane, relative to a reference electrode in the bath. Initially, the second electrode will also measure the voltage of the cell membrane. To switch to a “voltage clamp mode” a command potential will be set, this will be measure by the first electrode, and then current will be inject using the second electrode until the difference between them is zero (Papke, R. L.; Stokes, C. 2010).

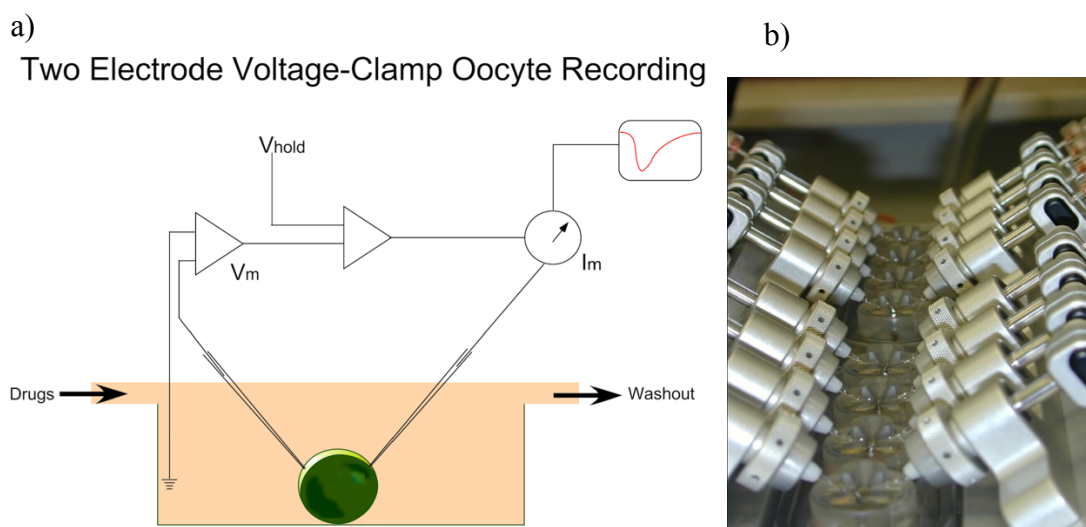


Figure 10. a) *Xenopus laevis* oocyte impaled by two glass electrodes. b) OpusXpress system, has automatic impalement, and can measure eight oocytes at the same time (Papke, R. L.; Stokes, C. 2010).

3.1.3.2. Expression of nAChR in *Xenopus laevis* oocytes

The human $\alpha 7$ nAChR clone was obtained from Dr. J. Lindstrom (University of Pennsylvania, Philadelphia, PA). The human resistance-to-cholinesterase 3 (RIC-3) clone was co-injected with $\alpha 7$ to improve the level and speed of $\alpha 7$ receptor expression without affecting the pharmacological properties of the receptors (Halevi, S. *et al.* 2003). Subsequent to linearization and purification of the plasmid cDNAs, cRNAs were prepared using the mMessage mMachine in vitro RNA transcription kit (Ambion, Austin, TX). Mutants were made as previously described (Papke, R. L. *et al.* 2011).

Oocytes were surgically removed from mature *Xenopus laevis* frogs (Nasco, Ft. Atkinson, WI) and injected with appropriate nAChR subunit cRNAs as described previously (Papke, R. L.; Stokes, C. 2010). Frogs were maintained in the Animal Care Service facility of the University of Florida, and all procedures were approved by the University of Florida Institutional Animal Care and Use Committee. In brief, the frog was first anesthetized for 15-20 min in 1.5 l frog tank water containing 1 g of 3-aminobenzoate methanesulfonate buffered with sodium bicarbonate. The harvested oocytes were treated with 1.25 mg/ml collagenase (Worthington Biochemicals, Freehold, NJ) for 2 h at room temperature in calcium-free Barth's solution (88 mM NaCl, 1 mM KCl, 2.38 mM

NaHCO₃, 0.82 mM MgSO₄, 15 mM HEPES, and 12 mg/l tetracycline, pH 7.6) to remove the follicular layer. Stage V oocytes were subsequently isolated and injected with 50 nl of 5-20 ng nAChR subunit cRNA. Oocytes were maintained in Barth's solution with calcium and recordings were carried out 1-11 days after injection.

3.1.3.3. Titration curves

Experiments were conducted using OpusXpress 6000A (Molecular Devices, Union City, CA) (Papke, R. L.; Stokes, C. 2010). Both the voltage and current electrodes were filled with 3 M KCl. Oocytes were voltage-clamped at -60 mV. The oocytes were bath-perfused with Ringer's solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 10 mM HEPES, and 1 μ M atropine, pH 7.2) at 2 ml/min for 3.5 min. To evaluate the effects of experimental compounds compared to ACh-evoked responses of various nAChR subtypes expressed in oocytes, control responses were defined as the average of two initial applications of ACh made before test applications. The solutions were applied from a 96-well plate via disposable tips. Drug applications were 12 s in duration followed by a 181s washout period. A typical recording for each set of oocytes constituted two initial control applications of ACh, one or more experimental compound applications, and then a follow-up control application(s) of ACh. The control ACh concentration was 60 μ M. The responses were calculated as both peak current amplitudes and net charge, as previously described (Papke, R. L.; Papke, J. K. P. 2002), and the average of the peak current amplitudes of the two initial controls were used for normalization purposes. Statistical comparisons were based on t-tests of the normalized data where indicated. All the oocyte experiments were conducted by Claire Stokes on Dr. Roger Papke's laboratory at the University of Florida.

CHAPTER IV. CRYSTALLOGRAPHIC STUDIES

4.1 Material and methods.

DMSO	Sigma-Aldrich
Liquid nitrogen	Prax air
Reagent 3 (0.26 M ammonium phosphate monobasic, 35% v/v glycerol).	Hampton Crystal Screen Cryo solutions (Hampton Research).
Siliconized thick glass square cover slides (22 mm).	Hampton Research
VDX with sealant (24 wells for crystallization).	Hampton Research

4.1.1. Vapor diffusion: Hanging drop method.

Vapor diffusion is the more frequently use technique for crystallization of macromolecules. This technic consists in the mixing of 1-10 μ L of protein with the same or more volume of the crystallizing buffer. This mixture is added to a cover slip that is left inverter sealed into a well/reservoir that has between 500 and 1000 μ L of the same buffer solution, so the drop is “hanging down”. Typically, the drop contains a lower reagent concentration that the reservoir. This difference in the concentration of the crystallization solution (buffer) between the drop and the well lead to an equilibrium that it is achieve by vapor phase (Unge, T. 1999).

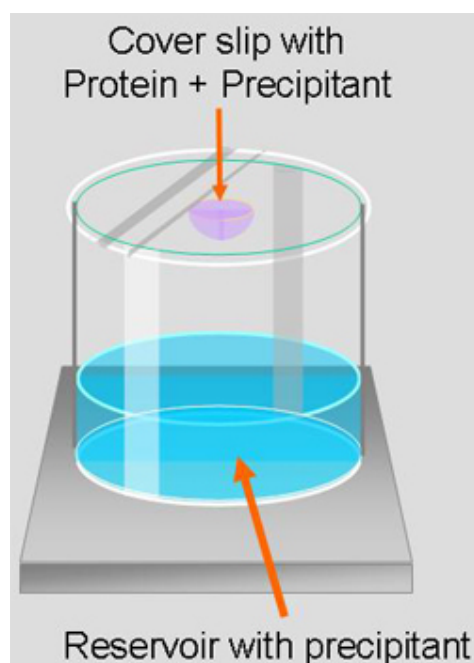


Figure 11. Process of vapor diffusion.

4.2 Complex formation and crystallization.

Complexes of multiple substituted 2-aminopyrimidines were prepared for crystallization utilizing the hanging drop method describe above. Ligand – *Ls*-AChBP complexes was formed by combining 2 μ L of solution of the ligand (10 mM dissolved in DMSO) with 48 μ L of the protein at a concentration of 5 mg/mL in order to achieve a stoichiometric excess of ligand to binding sites. Co-crystals were obtained by the vapor diffusion hanging drop method. The ligand – *Ls*-AChBP complex was mixed in a 1 μ L/1 μ L ratio with selected Hampton Crystal Screen Cryo solutions. Crystals of final size 0.3 mm $\times$ 0.3 mm $\times$ 0.2 mm were obtained using Reagent #3 (0.26 M ammonium phosphate monobasic, 35% v/v glycerol) and flash-cooled in liquid nitrogen.



Figure 12. Crystal of AC-171A2.

4.3 Data collection, phasing, and model Refinement.

A full set of X-ray diffraction data was collected at 100 K at the Advanced Light Source Synchrotron in Berkeley, California (BL 5.0.1; wavelength 1.0 Å). Data were processed using the HKL2000 program. Ligand – *Ls*-AChBP complex structures were solved by molecular replacement method with Phaser-MR using an ensemble of AChBP structures (PDB entry 4QAC) as the search model. The electron density maps were fitted with COOT and program Phenix-1.9-1692 was used for structure refinement. Refinement statistics are listed in **Table 2**. Atomic coordinates and structure factors of the complex have been deposited in the Protein Data Bank (PDB entries 5J5F, 5J5G, 5J5H, 5J5I). The structural figures were generated using PyMOL (PyMOL, 2010).

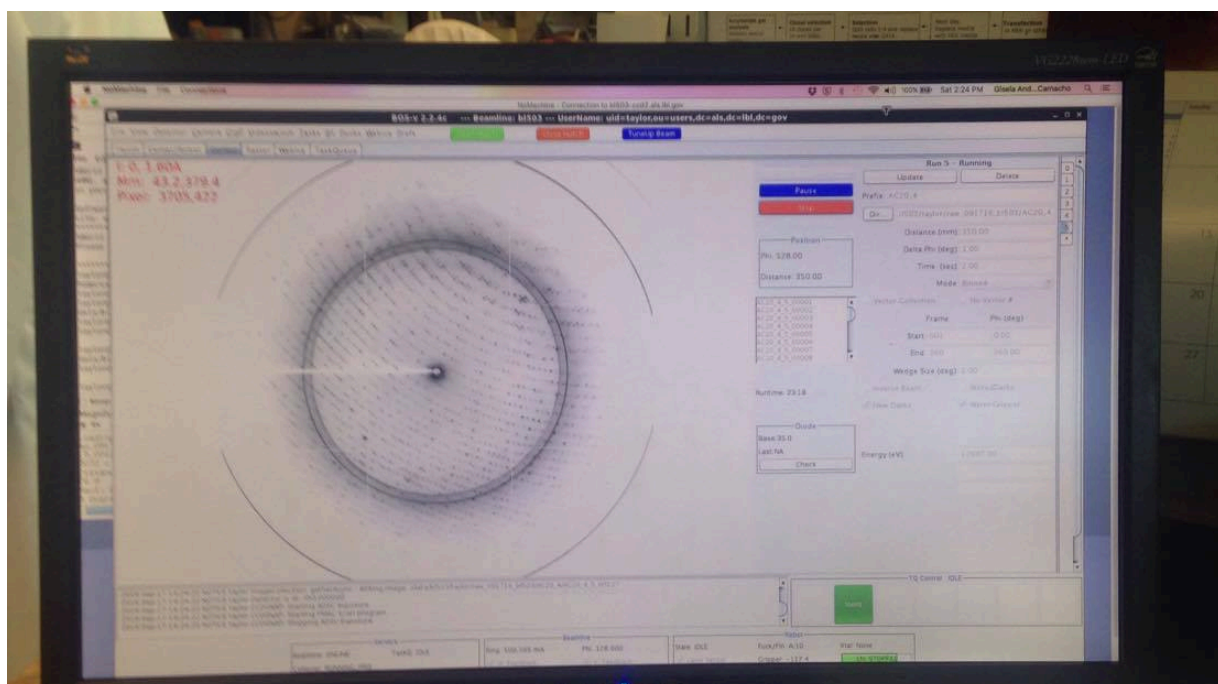


Figure 13. Data collection via remote connection at the Advanced Light Source Synchrotron in Berkeley, California.

4.4 Refinement statistics.

Table 2. Data collection and refinement statistics.

Ligand (PDB code)	44 (5J5F)	52 (5J5G)	40 (5J5H)	43 (5J5I)
DATA COLLECTION				
Space group	P 1 2 ₁ 1	P 1 2 ₁ 1	P 1 2 ₁ 1	P 1 2 ₁ 1
Cell dimensions				
a, b, c (Å)	86.76, 125.92, 121.07	87.72, 125.75, 118.73	107.73, 130.44, 107.69	87.14, 127.01, 122.12
α, β, γ (°)	90.00, 110.03, 90.00	90.00, 109.17, 90.00	90.00, 103.32, 90.00	90.00, 110.49, 90.00
Resolution (Å)	50.00-2.04 (2.08-2.04)	50.00-2.04 (2.08-2.04)	50.00-2.68 (2.75-2.68)	50.00-2.33 (2.37-2.33)
R _{sym} or R _{merge}	8.2 (41.9)	12.4 (58.8)	10.5 (75.0)	20.0 (69.0)
$I/\sigma(I)$	28.3 (4.6)	21.7 (2.9)	18.8 (2.4)	12.7 (1.4)
Completeness (%)	99.9 (99.1)	99.7 (94.2)	96.7 (100)	98.0 (89.8)
CC _{1/2} (%)	99.3 (94.5)	98.5 (88.7)	98.1 (87.3)	95.6 (75.8)
Redundancy	7.6 (7.1)	7.4 (6.1)	7.2 (7.7)	6.2 (3.6)
REFINEMENT STATISTICS				
Resolution (Å)	49.83-2.04	49.54-2.04	48.64-2.70	43.57-2.33
No. reflections	154,877	154,489	77,581	104,452
R _{work} / R _{free}	16.7/20.2	16.7/20.9	22.5/30.0	18.7/25.0
Ramachandran plot, residues in most favored regions (%)	99.1	98.5	94.4	98.2
No. molecules				
Atoms (non-solvent)	17,764	17,906	17,391	17,653
Ligand	10	10	10	10
Water	1,266	1,322	150	470
B-factors (Å ²)				
Protein (main/sidechain)	22.5/27.0	24.0/28.8	27.8/30.4	38.8/41.6

Ligand	20.1	25.2	21.8	36.4
Water	29.0	30.2	21.3	38.0
Wilson B-factor	22.4	24.1	58.8	35.0
R.m.s. deviations				
Bond lengths (Å)	0.008	0.008	0.010	0.008
Bond angles (°)	1.2	1.1	1.3	1.2

*Values in parentheses are for highest-resolution shell.

**CHAPTER V. CHEMICAL COMPARATIVE ANALYSIS OF CLASSICAL
AGONIST AND THE PYRIMIDINES FAMILY**

5.1. Chemistry of the classical nAChRs agonists.

It has been well established that nicotine has the capacity to permeate membranes, Henry Lester and collaborators have been working in demonstrate that nicotine is able to enter the cell and act as a chemical chaperon in the endoplasmic reticulum (Lester, H. A. *et al.* 2009) promoting the assembly of the high sensitivity form of $\alpha 4\beta 2$ -nAChR resulting in an up-regulation of the receptor. This is possible because the pKa of nicotine is around 8 and the log P 1.1, the pKa of nicotine it is highly conserve among all classical nicotinic agonist, the charge species that will result as part of the equilibrium, is responsible for a cation- π that has been well established as s key aspect of the binding.

Previous studies implicated in the design of novel selective $\alpha 7$ nAChR agonist and positive allosteric modulators (Horenstein, N. A. *et al.* 2008, Wang, J. *et al.* 2012, Arunrungvichian, K. *et al.* 2015), gave enough information to stablish the primary pharmacophoric features of: a cationic center, a hydrogen-bond acceptor, and a hydrophobic moiety (Arunrungvichian, K. *et al.* 2015). We have predicted by the chemistry of our structure that the protonation that has been so well characterized and stablished on the agonist of the nicotinic receptors it is not present in our landscape, therefore we approached this hypothesis using NMR spectroscopy to assess the pKa of our compounds.

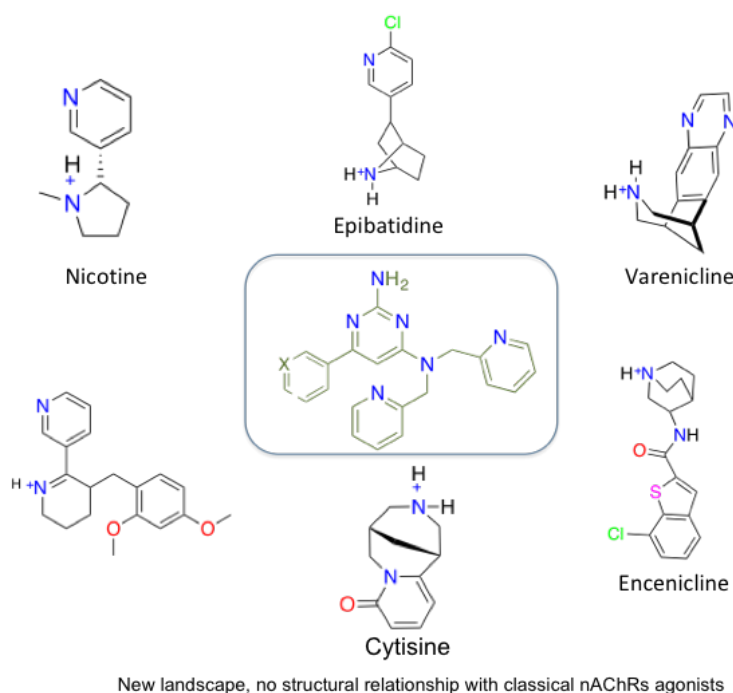


Figure 14. Structures of classical agonist of the nAChRs compared to 2-amino pyrimidines

5.2. ^1H NMR pKa experiments

In medicinal chemistry, specifically in drug development and design, pKa values have a crucial role providing important pharmacokinetics and pharmacodynamics predictions. To determine pKa, numerous approaches could be used such as UV-spectroscopy, capillary electrophoresis, the commonly used potentiometric measurements and proton NMR. Depending on the pH of a solution, the equilibrium between a base and its conjugated acid will shift. In NMR spectroscopy the chemical shift of a proton will change of position depending on the pH of the solution. The pKa can be determined using the next equation:

$$pKa = pH + \log \frac{X_{BH} +}{X_B}$$

^1H NMR spectroscopy is a sensitive and reliable technique that can be used with a small amount of compound, and it has proved that results obtained by ^1H NMR correlate well when compared to results from other techniques such as UV-spectroscopy (Benzecont, J. *et al.* 2014).

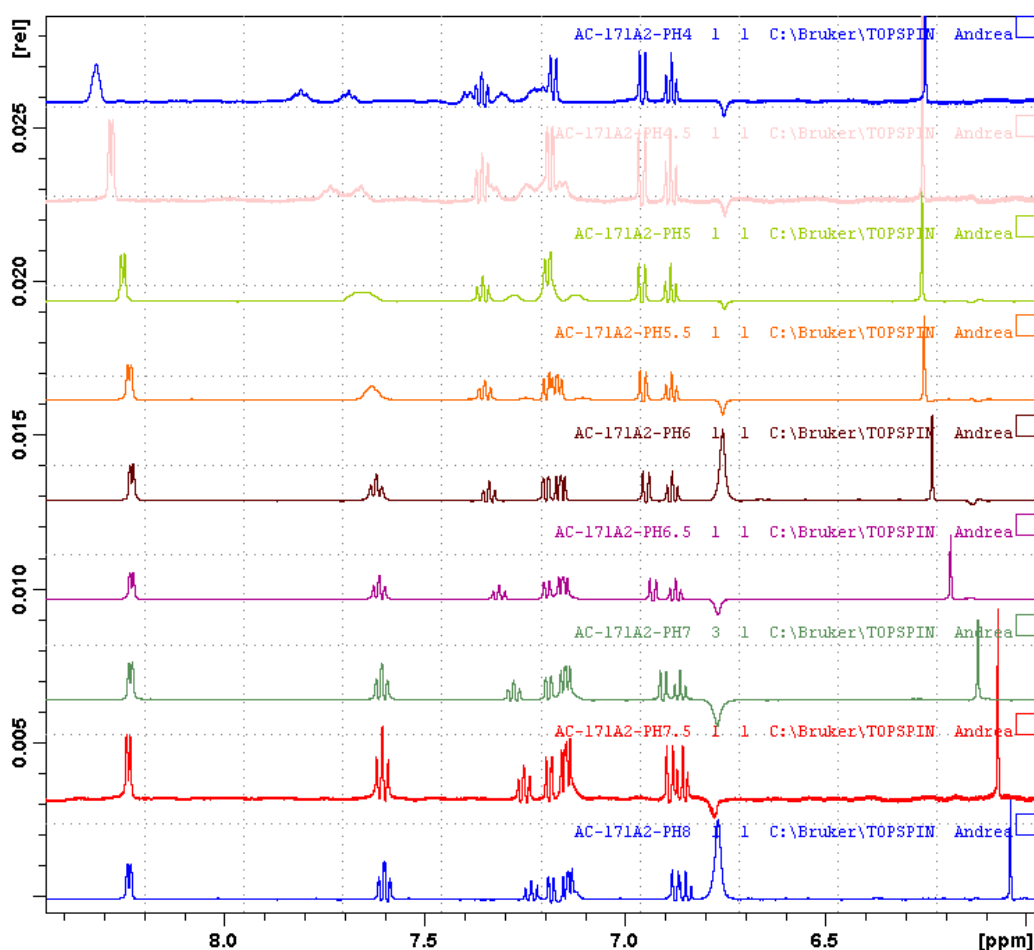


Figure 15. ^1H NMR of 171A2 in phosphate buffer at different pH.

The titrations were made using compound AC-171A2 at 1mM concentration in phosphate buffer at different pH, from 4 to 8.5. Then the 0.5 mL of the solution was added to a 5mm NMR tube that contained DMSO-*d*₆ in a 1.7 mm tube inside, this way we were able to eliminate the buffer signal. As we can observe in **figure 15** there is change of position in the chemical shifts at different pH, each peak is moving to the left, plus, at neutral pH the protons of the di-picolyl system are detected as equal, thing that seems to be affected by the pH, since in the more acidic samples we can see how a signal is splitting in two.

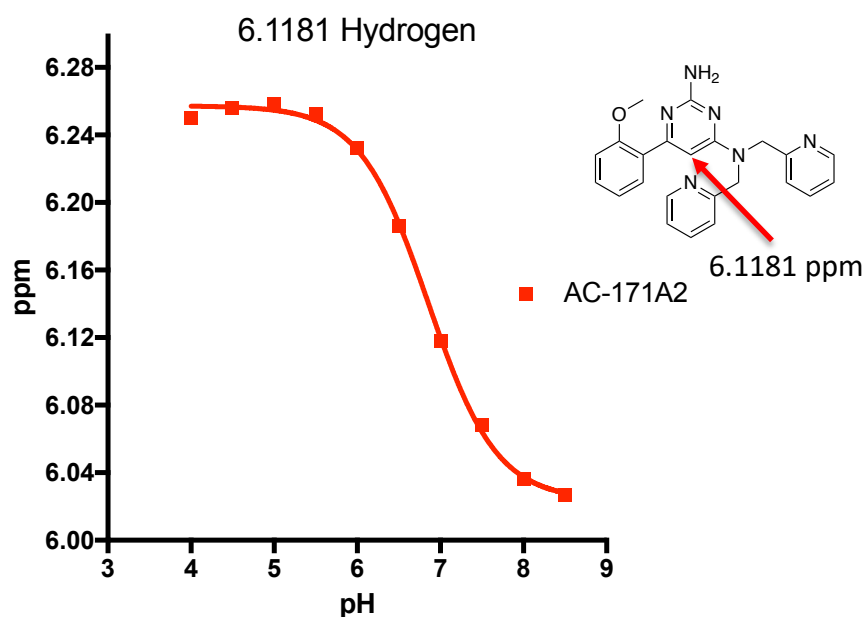


Figure 16. ¹H NMR peak position of proton of pyrimidine system as a function of pH.

The pK_a of the pyrimidine system I used the only proton available, the graph was created by plotting the ppm versus the pH, to form a sigmoidal curve **figure 16**. The point of inflection corresponds to the pK_a which in this particular proton will be 6.85. To asses pK_a of di-picolyl system we chose the proton next to the nitrogen of the pyrimidine, mainly because it was the one that did not split under acidic conditions. When we plotted the ppm versus the pH (**figure 17**) we were able to determined that the pK_a was around 4.4.

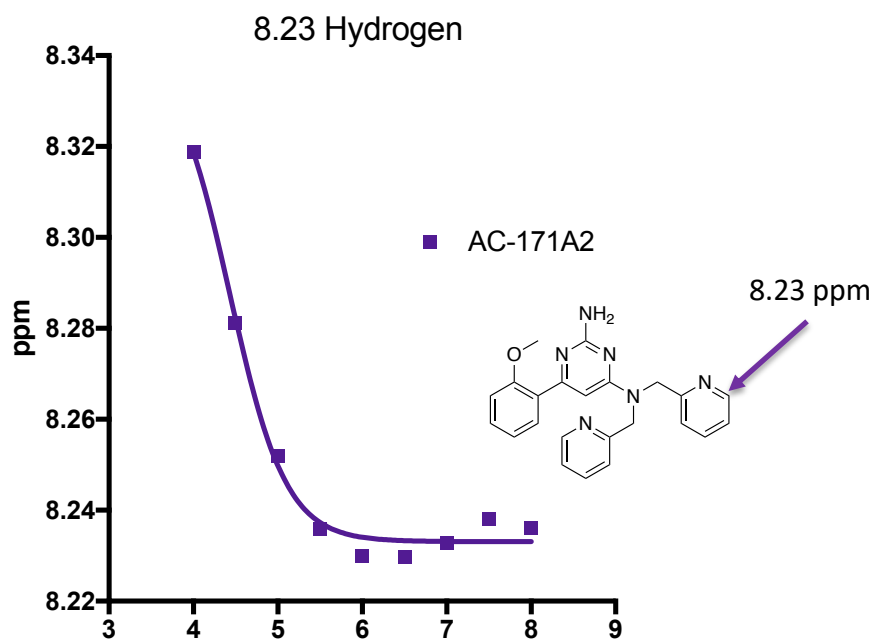


Figure 17. ^1H NMR peak position of one proton of dipicolyl system as a function of pH.

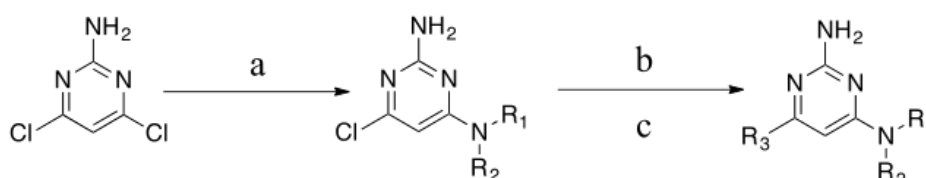
^1H -NMR allowed us to successfully determined the pKa of our lead compound, showing that this unique group of compounds differ from the prototypic agonist by not protonating at physiological pH.

CHAPTER VI. RESULTS AND DISCUSSION

6.1. Chemical synthesis

6.1.1. Synthetic rational and synthetic pathways of series AC.

Based on previous structure-activity considerations and X-ray crystal structures with AChBP and our lead cooperative ligands (Kaczanowska, K. *et al.* 2014), we identified the three 2-aminopyrimidine nitrogens as key positional determinants essential for ligand-AChBP molecular recognition. In order to accomplish synthesis of new potential activators of $\alpha 7$ -nAChR we use a straightforward synthetic pathway that allowed access to multiple 2-aminopyrimidine analogues varying in substitution at positions four and six in the pyrimidine ring as the first stage of the project (**Scheme 8**).



Scheme 8. Synthetic pathway leading to 4,6-substituted 2-aminopyrimidines.

Conditions: (a) Amine, DIPEA, DMF, 2-6 h; (b) Boronic acid, Na₂CO₃, Pd(PPh₃)₄, Toluene/Ethanol, 95 °C for 8 h-overnight. (c) Boronic acid, K₂CO₃ 2M, Pd(dppf)Cl₂, DMA, 95 °C for 2 h-overnight.

Commercially available 2-amino-4,6-dichloropyrimidine was reacted with appropriate amines to afford intermediates AC-17, AC-19, AC-20. In the subsequent step, compounds were subjected to a cross coupling reaction with various boronic acids using *tetrakis*(triphenylphosphine)palladium(0) or [1,1'-*Bis*(diphenylphosphino)ferrocene]dichloropalladium(II) as a catalyst to give final products in moderated to excellent yields. This synthetic route enabled us to synthesize eighteen final products (**Figure 18**), with separate modifications at the four and six positions in the pyrimidine system. The electronic influence of the 6-aryl substituent was considered, therefore boronic acids were used in syntheses encompassing different heterocycles. The aromatic rings at the sixth position were also substituted with electron donating or electron withdrawing substituents.

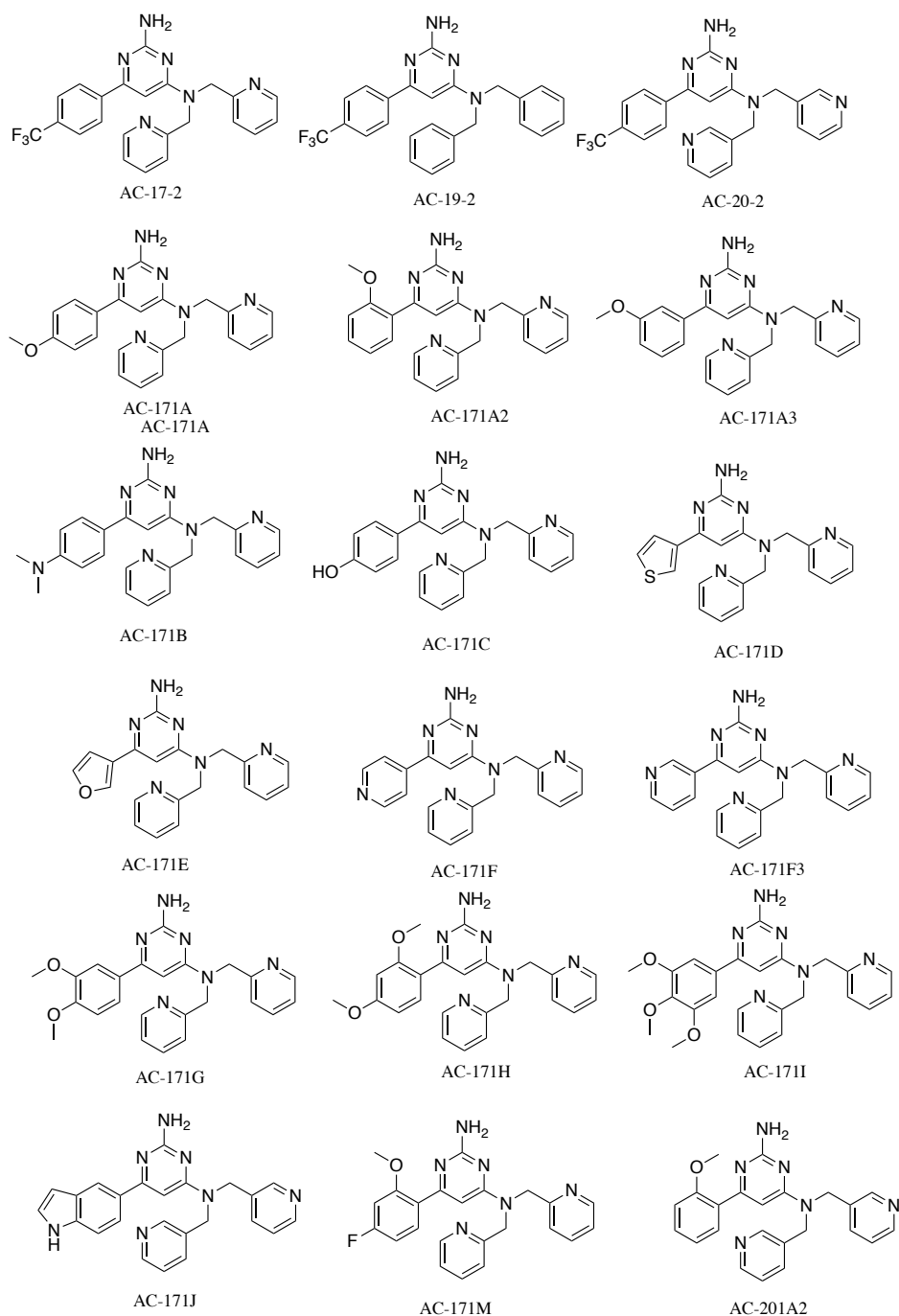
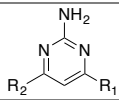
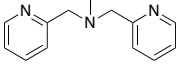
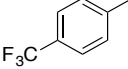
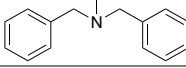
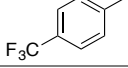
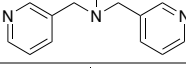
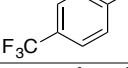
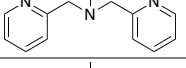
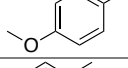
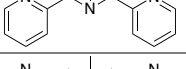
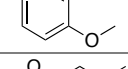
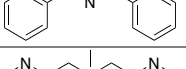
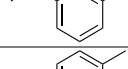
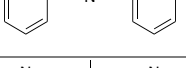
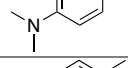
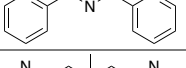
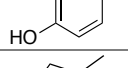
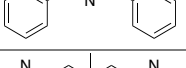
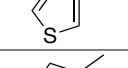
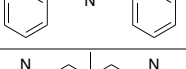
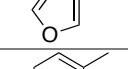
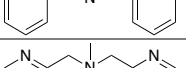
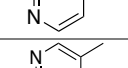
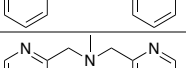
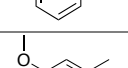
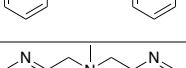
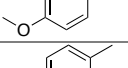
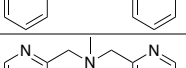
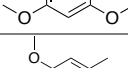
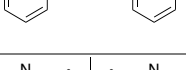
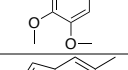
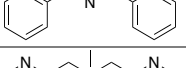
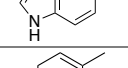
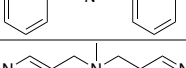
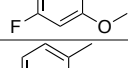
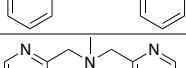
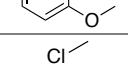
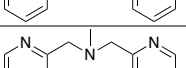
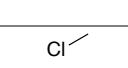
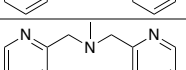
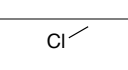


Figure 18. Structures of final compounds of AC series.

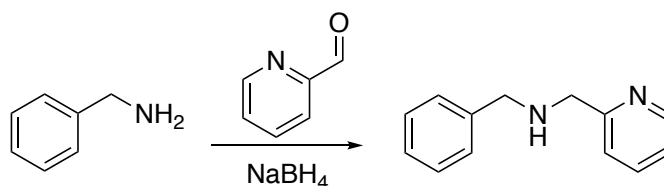
The products were functionally characterized in the first phase of the project; additional syntheses were later performed based on the *in vitro* results. Characteristics of the compounds and yields are shown in **table 3**.

Table 3. Characteristics and yield of AC series.

	R ₁	R ₂	Characteristic	% Yield
AC-17-2			White solid	50%
AC-19-2			White solid	69%
AC-20-2			White solid	12%
AC-171A			White solid	88%
AC-171A2			White solid	80%
AC-171A3			White solid	67%
AC-171B			Pale solid	83%
AC-171C			White solid	46%
AC-171D			White solid	57%
AC-171E			Pale solid	86%
AC-171F			Brown solid	82%
AC-171F3			Pale solid	76%
AC-171G			Brown solid	87%
AC-171H			Pale solid	56%
AC-171I			Brown solid	55%
AC-171J			Brown solid	52%
AC-171M			White solid	76%
AC-201A2			Pale sholid	n.d.
AC-17			White solid	73%
AC-19			White solid	69%
AC-20			Orange needles	12%

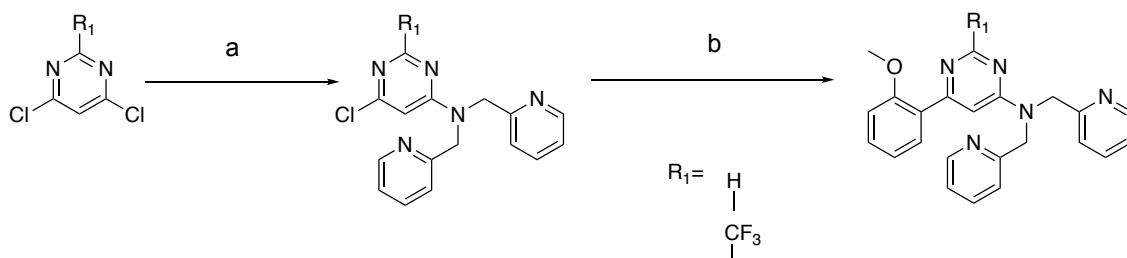
6.1.2. Synthetic rational and synthetic pathways of series AL.

On additional direct reductive amination was performed reacting benzyl amine and picolinaldehyde, following addition of NaBH₄ to yield N-benzyl-1-(pyridin-2-yl)methanamine (**Scheme 9**). This amine was used in the first step S_NAr reaction with 2-amino-4,6-dichloropyrimidine to yield an intermediary with an asymmetric system in the fourth position. A cross coupling Suzuki reaction as final step yielded the final compound AC-2A.



Scheme 9. Reductive amination; (1) pyridine-2-carboxaldehyde, EtOH, over night. (2) NaBH₄, 0 °C, 2 hrs.

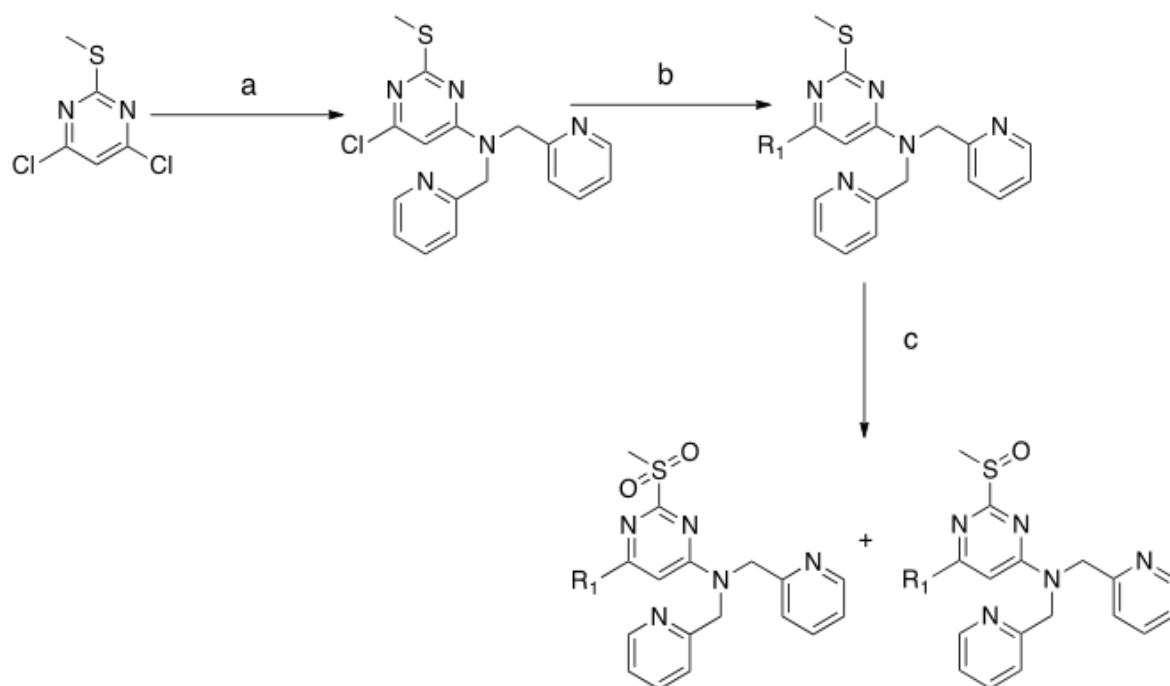
Based on results from the first eighteen compounds and the crystallography, what I will discuss in much more detail later, I decided to synthesize additional compounds based mainly in compound AC-171A2, but with modifications in the second position. The pyrimidine precursor was chosen depending on the substitution as seen in **scheme 7**.



Scheme 10. Synthetic pathway leading to 2,4,6-substituted pyrimidines. Conditions: (a) 2-Dipicolyl amine, DIPEA, DMF, 3.5 h; (b) 2-methoxyphenyl boronic acid, K₂CO₃ 2M, Pd(dppf)Cl₂, DMA, 95 °C for 18 h.

The same two step procedure was used to synthesize 2,4,6-disubstituted pyrimidines, commercially available 4,6-dichloro-2-(methylthio) pyrimidine, 4,6-dichloropyrimidine, 4,6-dichloro-2-(trifluoromethyl)pyrimidine, were used. Additional oxidation steps using only the 2-(methylthio)-4,6-disubstituted pyrimidine derivative was used, using m-CPBA as the oxidation reagent, to yield sulfoxide and 2-sulfonyl

pyrimidines AL-1A2 and AL-1A3 (**Scheme 11**). Characteristics of the compounds and yields are shown in **table 4**.



Scheme 11. Synthetic pathway leading to 4,6-substituted 2-thiomethyl pyrimidines.

Conditions: (a) 2-Dipicolyl amine, DIPEA, DMF, 3.5 h; (b) Boronic acid, K₂CO₃ 2M, Pd(dppf)Cl₂, DMA, 95 °C for 18 h. (c) m-CPBA, CH₂Cl₂, 0°C, 45 min.

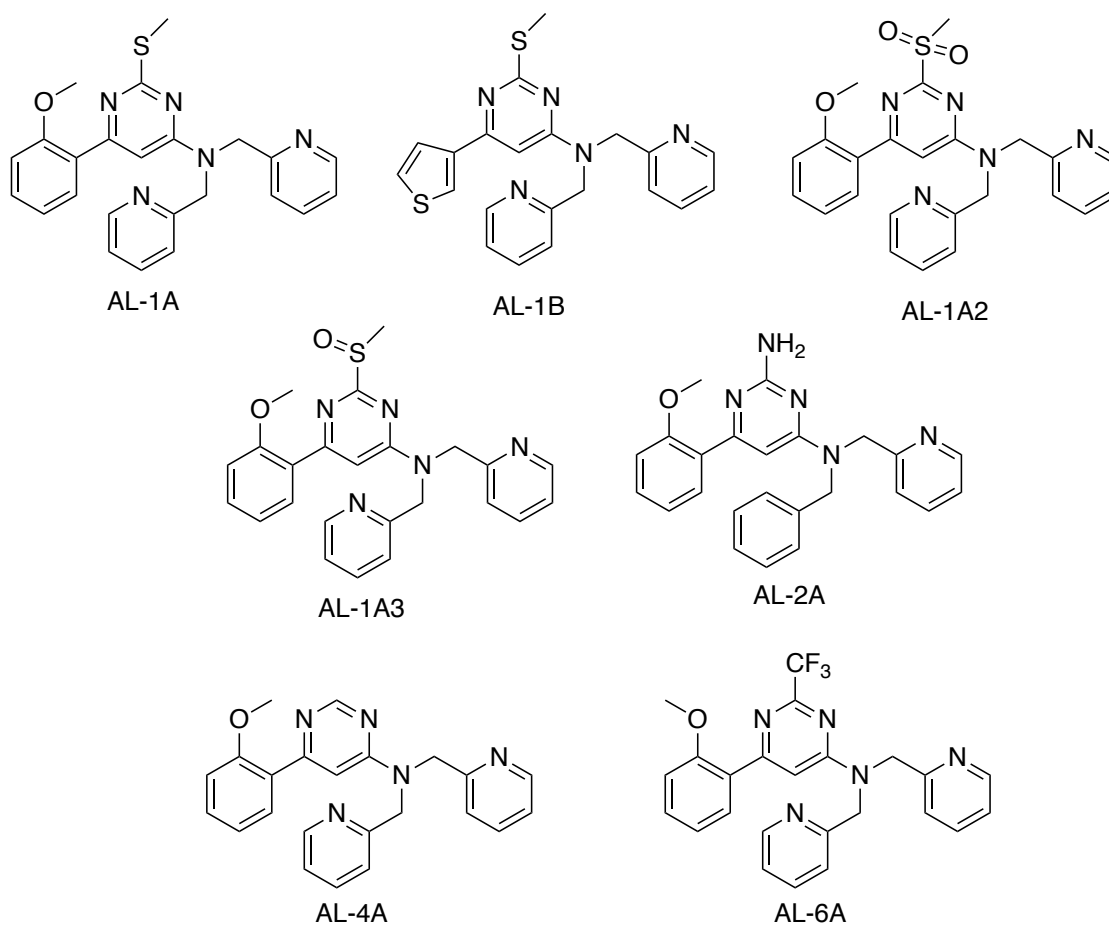
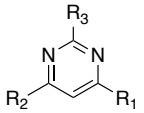
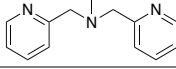
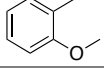
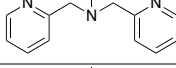
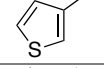
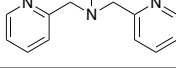
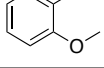
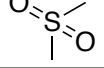
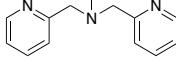
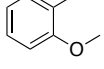
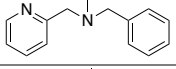
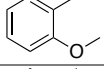
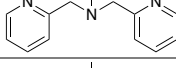
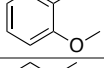
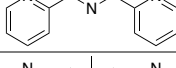
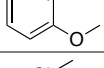
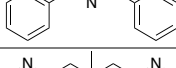
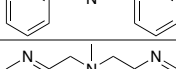
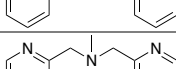
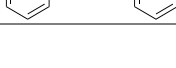


Figure 19. Structures of final compounds of AL series.

Table 4. Characteristics and yield of AL series.

	R ₁	R ₂	R ₃	Characteristic	% Yield
AL-1A				White solid	50%
AL-1B				White solid	69%
AL-1A2				White solid	31%
AL-1A3				White solid	79%
AL-2A				Pale solid	12%
AL-4A				White solid	88%
AL-6A				White solid	80%
AL-1				White solid	67%
AL-2				Pale solid	83%
AL-4				White solid	46%
AL-6				White solid	93%

6.1.3. Structure elucidation

Several techniques were used to characterize organic products (e.g. IR, LC-MS); the two techniques used to confirm the structure are protons and ^{13}C carbon nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HR-MS).

To confirm the molecular weight of the compounds we use HR-MS, Agilent 6230 Accurate-Mass TOFMS, all spectra of all compounds are found in APENDIX, and a summary of all mass information is shown in **Table 5**. Representative HR-MS spectrum of lead compound AC-171A2 is shown in **figure 20**.

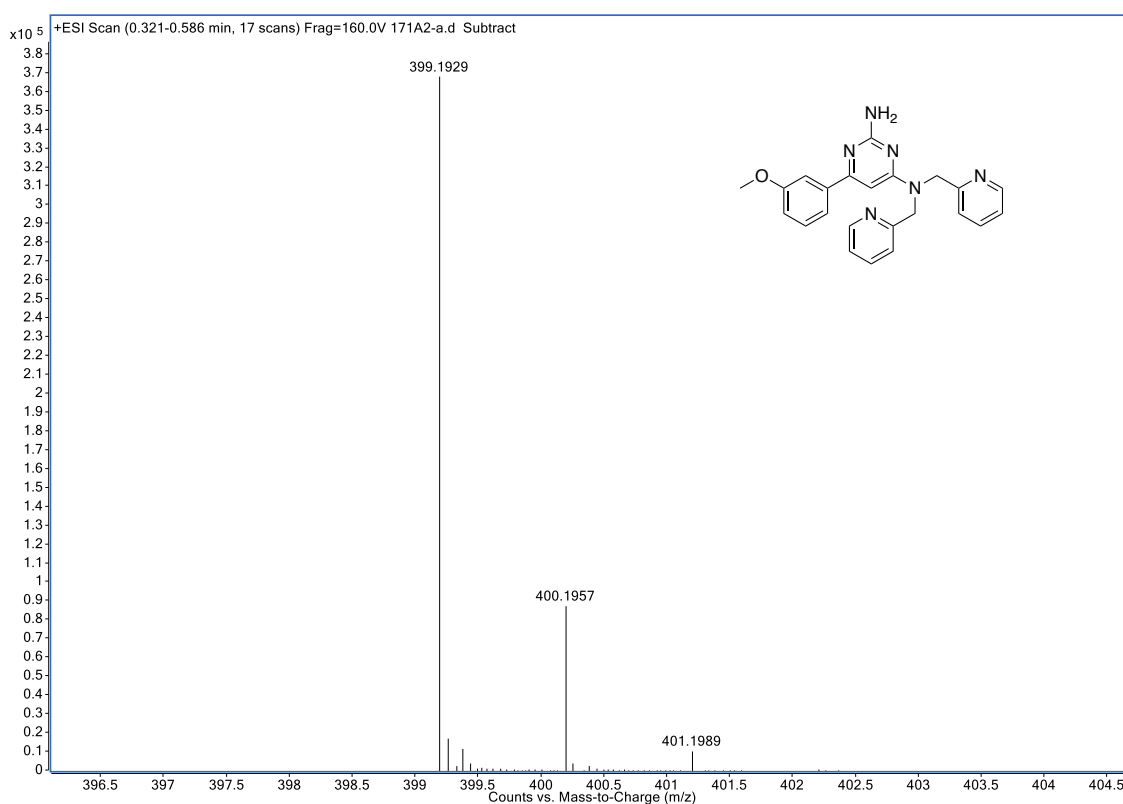


Figure 20. High resolution-Mass Spectrometry (HR-ESI-MS) of AC-171A2.

Table 5. Molecular weight detected by HR-MS of AC and AL series, all spectra are found in APENDIX.

Compound	MW	MH ⁺	Compound	MW	MH ⁺
AC-17-2	436.44	437.1698	AC-171M	416.46	417.1832
AC-19-2	434.47	435.1793	AC-201A2	398.47	399.1930
AC-20-2	436.44	437.1693	AC-17	326.79	327.1124
AC-171A	398.47	399.1931	AC-19	324.84	325.1215
AC-171A2	398.47	399.1929	AC-20	326.79	327.1121
AC-171A3	398.47	399.1927	AL-1A	429.54	430.1692
AC-171B	411.51	412.2245	AL-1B	405.54	406.1152
AC-171C	384.44	385.1773	AL-1A2	461.54	462.1592
AC-171D	374.47	375.1387	AL-1A3	445.54	446.1645
AC-171E	358.41	359.1616	AL-2A	397.48	398.1977
AC-171F	369.43	370.1779	AL-4A	383.46	384.1820
AC-171F3	369.43	370.1779	AL-6A	451.45	452.1689
AC-171G	428.50	429.2039	AL-1	357.86	358.0885
AC-171H	428.50	429.2034	AL-2	325.80	326.1165
AC-171I	458.52	459.2142	AL-4	311.77	312.1007
AC-171J	407.48	408.1934	AL-6	379.77	380.0879

Nuclear magnetic resonance (NMR) spectra were obtained on Brüker Avance III 600 MHz instruments and referenced to the signals of residual 1 protium in the NMR solvent. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), doublet of doublet of doublets (ddd), doublet of triplets (dt), triplet of doublets (td) and broad (b); the value of chemical shifts (δ) are given in ppm and coupling constants (J) in hertz (Hz) and ¹³C-DEPT (proton and carbon spectra are found in APPENDIX).

To determine and properly assign the chemical shifts to the structure, I analyzed the precursor AC-17 in different solvents and at different temperatures. (**Figure 21**). The compounds that I synthesized are high conjugated systems, and most have a symmetry axis in the di-picolyl system. This characteristic confers unusual behavior to them, specially to the hydrogens in position 7,7''. Analyzing the precursor in different

temperatures provided information to assign the protons. Unfortunately we were unable to separate hydrogens 4,4' and 6,6'.

The hydrogens in the position 7,7' overlap with the hydrogens of the amino group when the sample was analyzed in CDCl_3 at 25 °C. Different behavior of this overlapping occur when samples were diluted with $\text{DMSO}-d_6$, the singulate of hydrogens of the amino group appeared at 6.54 and 6.41 ppm when samples were analyzed at 25°C and 50°C respectively. With regards to hydrogens of position 7 and 7'', two broad peaks appear at 4.73 and 5.00 ppm in the $\text{DMSO}-d_6$ 25°C sample, when temperature increased to 50°C an average of the two peaks appeared as a broadened singulate at 4.87 ppm. (**Figure 21, Table 6**).

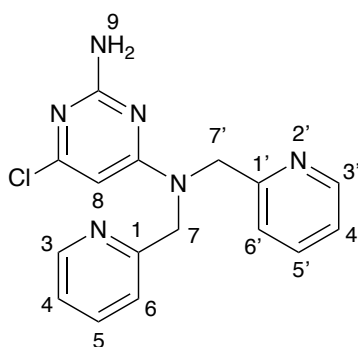


Table 6. Summarized ^1H -NMR data of AC-17 in CDCl_3 and $\text{DMSO}-d_6$ at different temperatures.

Solvent and Temperature	3,3'	4,4'	5,5'	6,6'	7,7'	8	9
CDCl_3 25 °C	8.55	7.16-7.18	7.61-7.64	7.16-7.18	4.73-5.05	5.95	4.99
$\text{DMSO}-d_6$ 50 °C	8.52-8.53	7.27-7.30	7.73-7.76	7.27-7.30	4.87	6.01	6.41
$\text{DMSO}-d_6$ 25 °C	8.52-8.53	7.28-7.3	7.74-7.77	7.28-7.3	4.73, 5.00	6.01	6.54

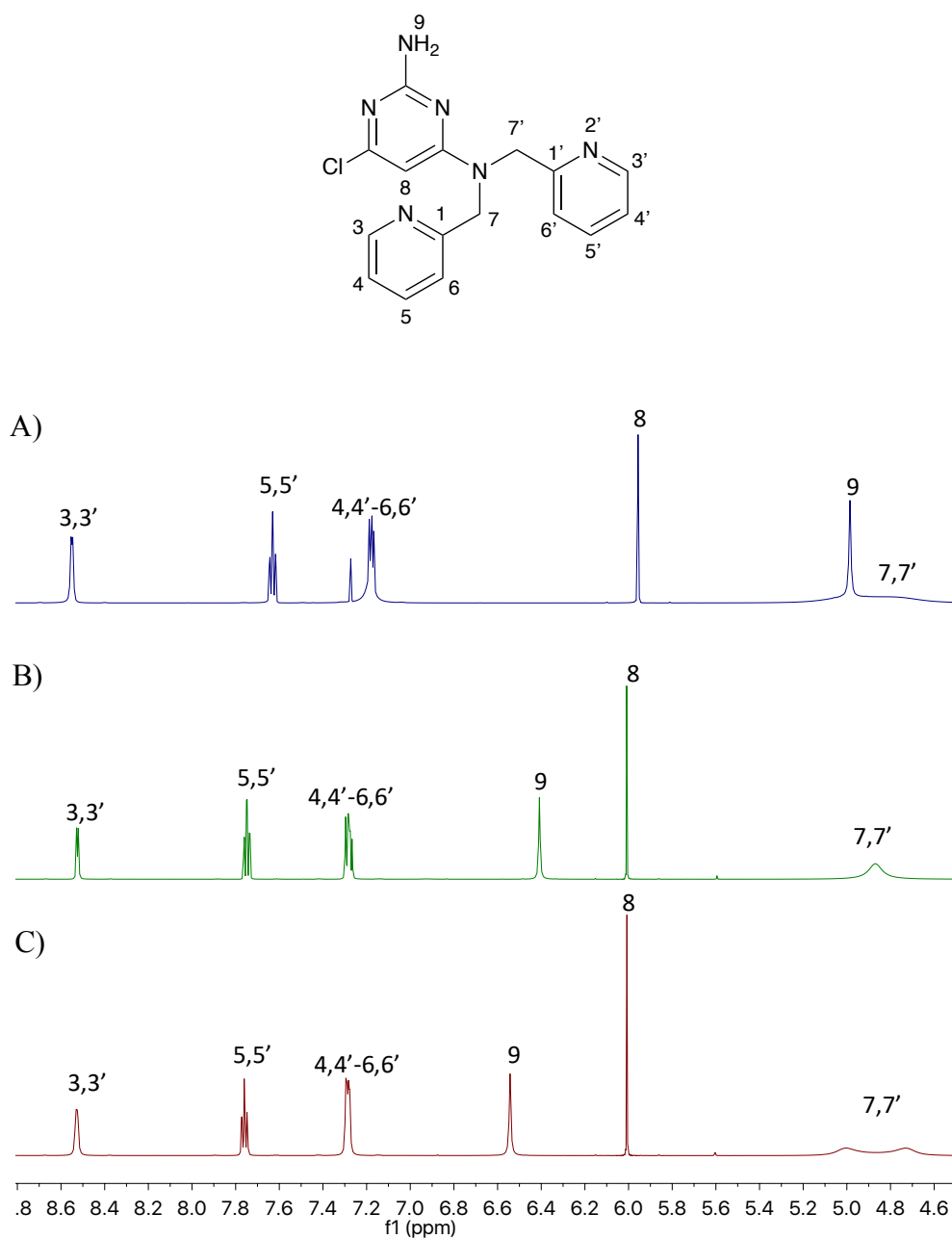


Figure 21. ^1H NMR spectra of AC-17, experiment conditions: A) CDCl_3 at 25 $^\circ\text{C}$, B) $\text{DMSO}-d_6$ at 50 $^\circ\text{C}$, C) $\text{DMSO}-d_6$ at 25 $^\circ\text{C}$.

The ^{13}C -APT technique was used to determine the nature of the carbon, in the positive range CH_3 and CH carbons, and in negative CH_2 and C . In addition, other 2D-NMR experiments were performed such as COSY, HMBC and HSQC to properly assign the signals and to confirm the structure. We also took advantage of chemdraw NMR prediction, especially in carbon, which has the tendency to be more accurate. The chemical shifts assigned in AC-17 were used in AC-171A2 and the remaining compounds that have the AC-17 as synthetic precursor (**table 7**).

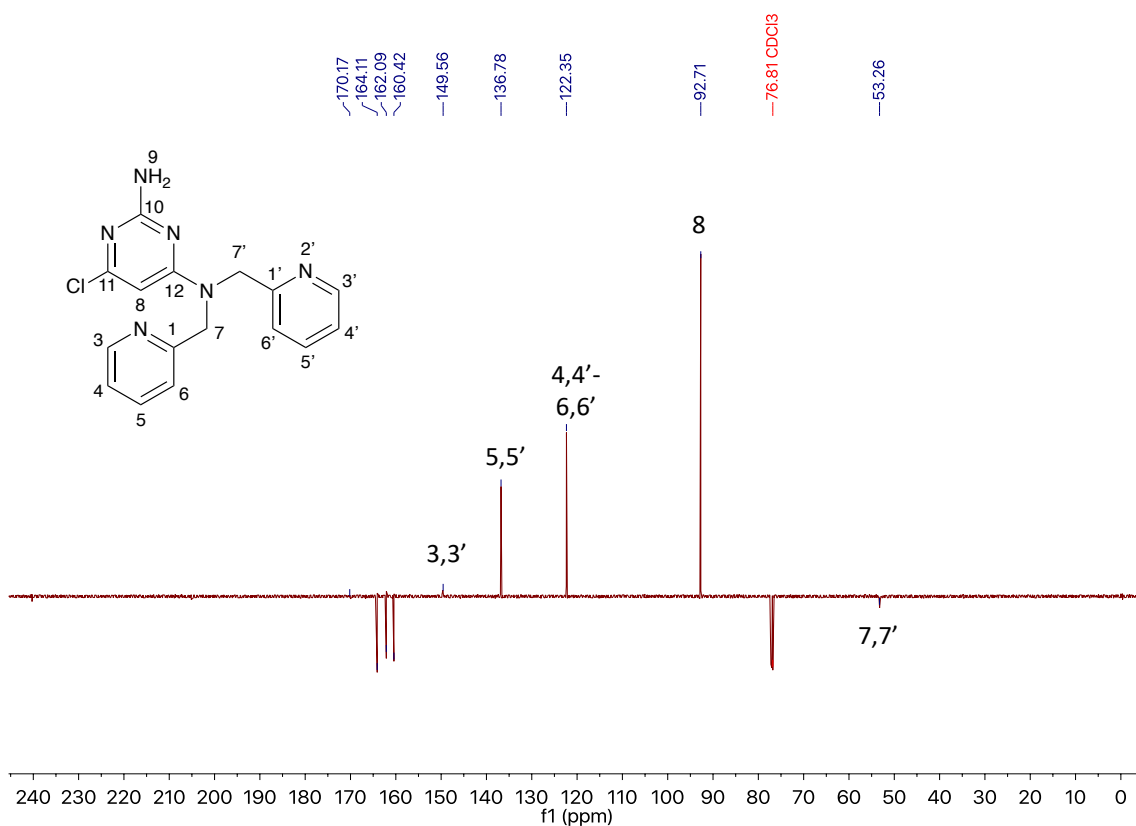


Figure 22. ^{13}C (APT mode) NMR spectra of AC-17.

The ^1H - ^{13}C HMBC (heteronuclear multiple bond correlation) provided us a correlation between carbon and proton that are separated by 2-4 bonds, all direct one-bond correlations are suppressed in this type of experiment. The ^1H - ^{13}C HSQC (heteronuclear single quantum coherence) experiment provided us the correlation between carbon and proton atoms that are directly bonded. These three techniques help us analyzed and assign were accurately the carbon chemical shifts.

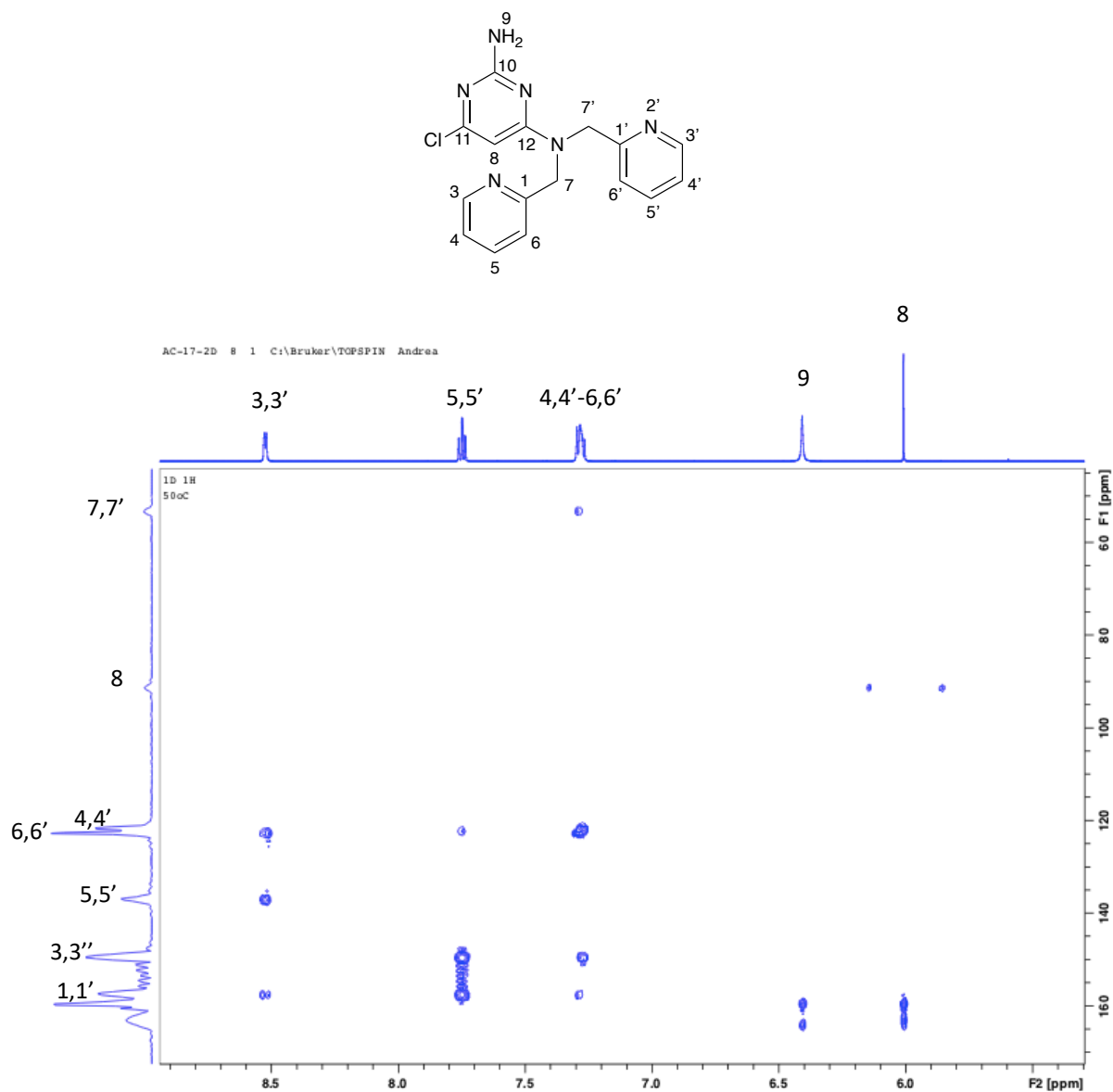
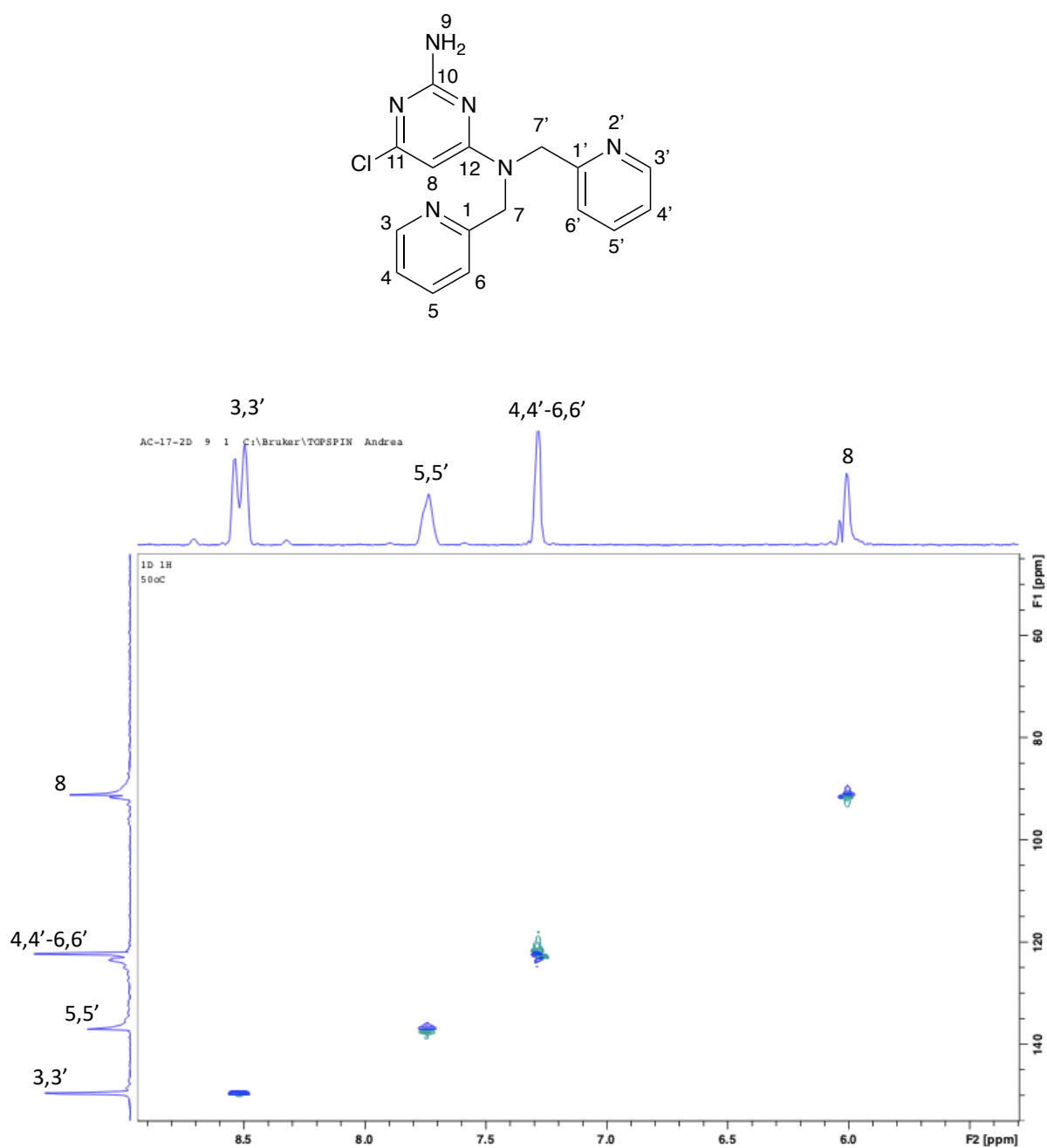
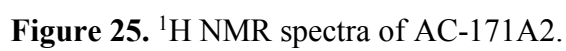


Figure 23. HMBC NMR spectra of AC-17.

**Figure 24.** HSQC NMR spectra of AC-17



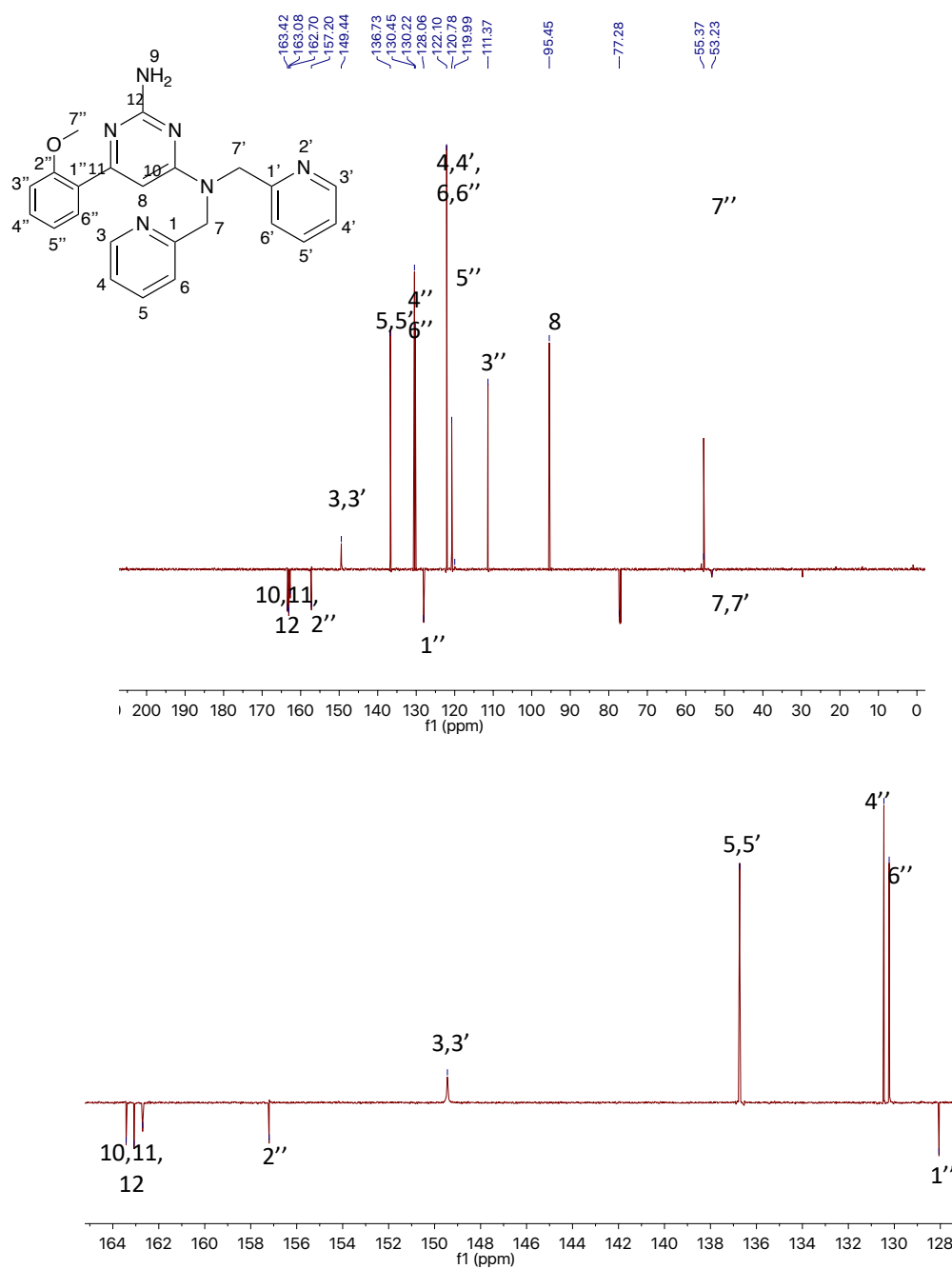


Figure 26. ¹³C (APT mode) NMR spectra of AC-171A2.

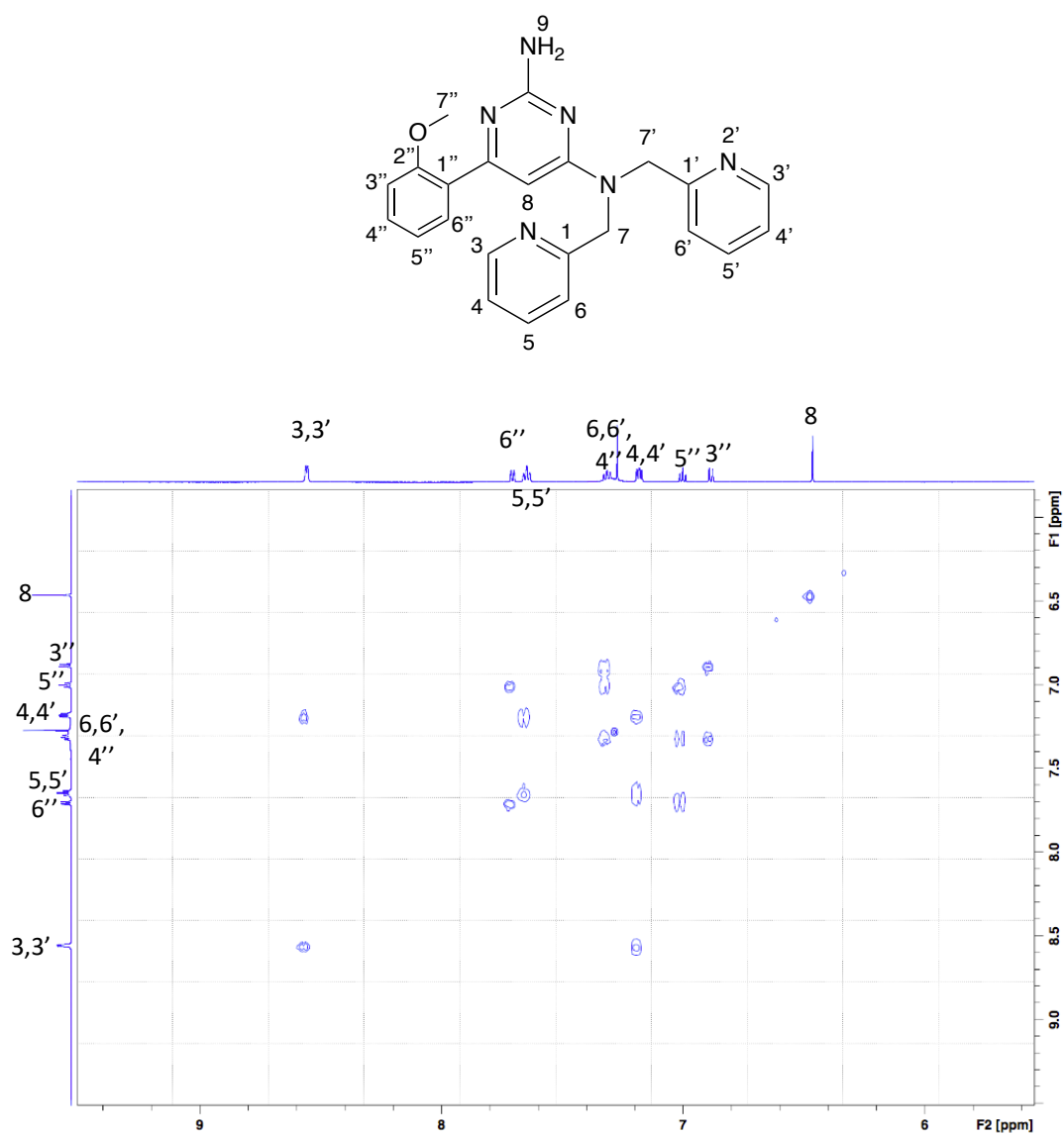


Figure 27. COSY NMR spectra of AC-171A2.

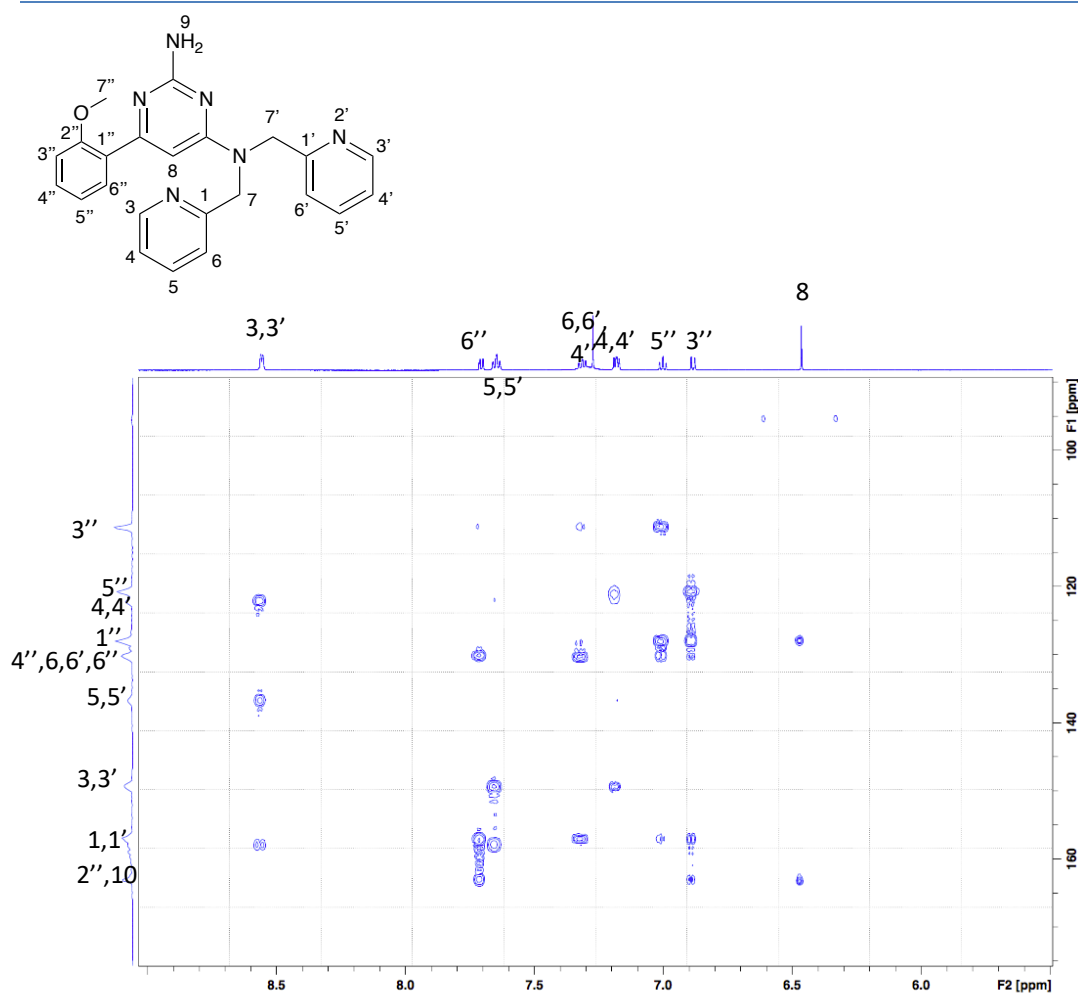


Figure 28. HMBC NMR spectra of AC-171A2.

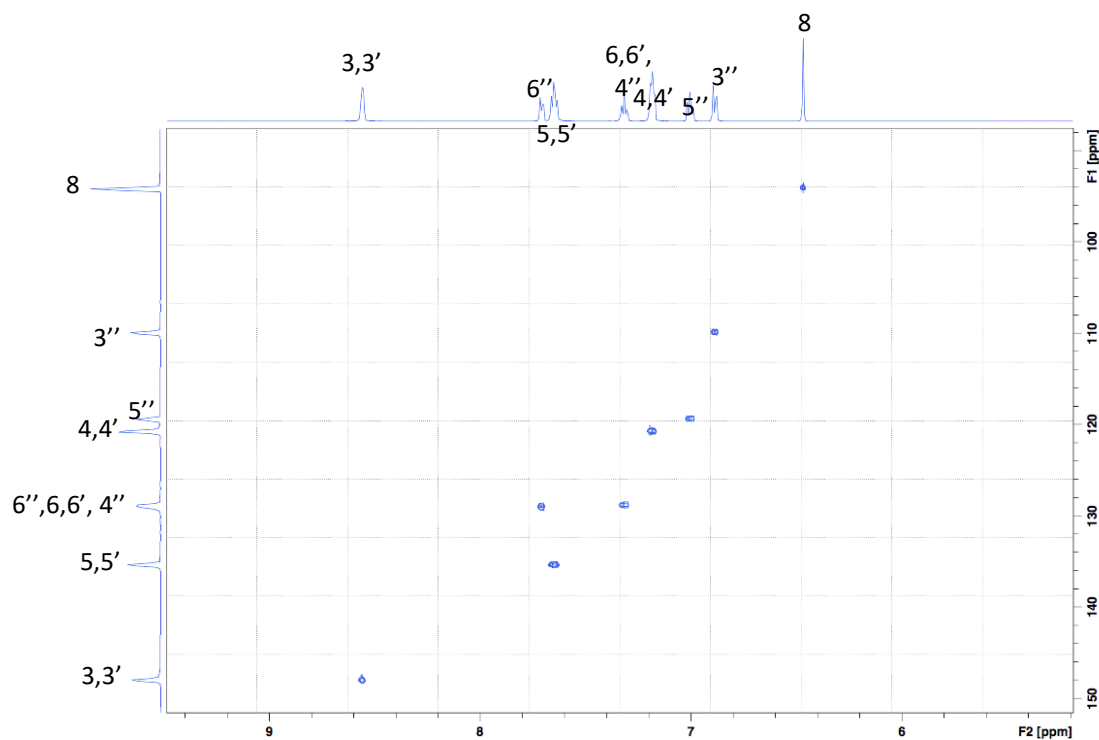


Figure 29. HSQC NMR spectra of AC-171A2

Table 7. ¹H NMR assignments of compounds of AC series.

	3,3'	4,4'	5,5'	6,6'	7,7'	8	9	2''	3''	4''	5''	6''	7''
17	8.52-8.57	7.12-7.23	7.55-7.69	7.12-7.23	4.99	5.94	4.74	-	-	-	-	-	-
17A	8.52-8.62	7.15-7.27	7.63	7.15-7.27	5.0-5.1	6.28	4.82	7.75-7.8	6.87-6.93	-	6.87-6.93	7.75-7.8	3.82
17A2	8.55	7.15-7.29	7.61-7.67	7.27-7.34	4.9	6.5	4.8	-	6.9	7.27-7.34	7.00	7.71	3.62
17A3	8.55-8.56	7.15-7.17	7.60-7.62	7.28	4.96	6.31	4.96	7.34	-	6.93	7.38	7.38	3.82
17B	8.56	7.14-7.2	7.59-7.65	7.25-7.27	4.98	6.26	4.75	7.75	6.69	-	6.69	7.75	2.99
17C*	8.51-8.55	7.26-7.31	7.74-7.78	7.26-7.31	4.92	6.31	6.01	7.74-7.78	6.78	-	6.78	7.74-7.78	9.74
17D**	8.49	7.28-7.3	7.77	7.35	5.01	6.35	7.33	7.46	7.42	7.87-7.91	-	-	-
17E	8.56	7.18	7.63	7.22-7.28	5.01	6.1	5.01	6.65-6.68	7.4	7.99	-	-	-
17F	8.58	7.2	7.65	7.2	4.94	6.38	5.17	8.64	7.65	-	7.65	8.64	-
17F3**	8.49-8.50	7.29-7.31	7.76-7.79	7.37-7.38	5.04-5.13	6.47	-	8.94-8.85	-	8.55-8.57	7.46-7.49	8.21-8.23	-
17G	8.56-8.58	7.19	7.64	7.19	4.85-5.13	6.28	-	7.46	-	-	6.86	7.33	3.9
17H	8.53-8.58	7.17	7.63	7.25-7.34	4.96	6.43	4.75	-	6.57-6.52	-	6.57-6.52	7.79	3.82
17I*	8.53	7.15	7.73-7.79	7.25-7.32	4.97	6.45	6.12	7.33	-	-	-	7.33	3.69
17J**	8.5	7.3	7.77	7.38	5.03-5.12	6.37	-	7.49	-	6.49	7.26	-	7.38
17M	8.55	7.18	7.64	7.27	4.82	6.44	4.82	-	6.69	-	6.59	7.74	3.59

Solvent: CDCl₃ standard solvent, *= DMSO- *d*₆, **=CD₃OD

6.2. *in vitro* pharmacological analysis

The synthetic compounds were evaluated by three distinct methods, each of them gave us different insights of the properties of the compounds. Functional characterizations of ligands were performed utilizing stable cell lines expressing $\alpha 7$ -nAChR, $\alpha 4\beta 2$ -nAChR and 5-HT_{3A}, serotonin receptor. Binding constants with the acetylcholine binding proteins were determined by scintillation proximity assay and fast activation of the $\alpha 7$ -nAChR was determined in an oocyte system utilizing two-electrode voltage clamp electrophysiology.

6.2.1. Cell-based functional assay results

Based on previous structure-activity considerations and the previous X-ray crystal structures with *Ls*-AChBP, Katarzyna Kaczanoswka in her analysis of the library of existing compounds detected two ligands that activated the $\alpha 7$ -nAChR. The chemical characteristics of this ligands was: 1) pyrimidine ring, 2) NH₂ substitution in the second position, 3) an apparent influence of a 2-dipicolyl system in the fourth position, and 4) an aromatic system with an electron-withdrawing moiety in the *-para* position. We then synthesized analogs of the previous compound by moving the nitrogen of the di-picolyl system to the third position or by removing the nitrogen of them. Results showed that by moving the nitrogen no significant activation was not achieved and by removing the nitrogen no activation was observed (**Table 8**).

Then, we decide to focus on the modulation of the six position, by adding electron-donating or electron-withdrawing groups to the aromatic system that was linked to the position or by coupling heterocycles to this position. Interesting by modification in this position we were able to achieve full activation of the $\alpha 7$ -nAChR, as we show in the quick screen graph in **figure 30**, all of the compound that fully or partially activated the $\alpha 7$ -nAChR also had the di-picolyl system.

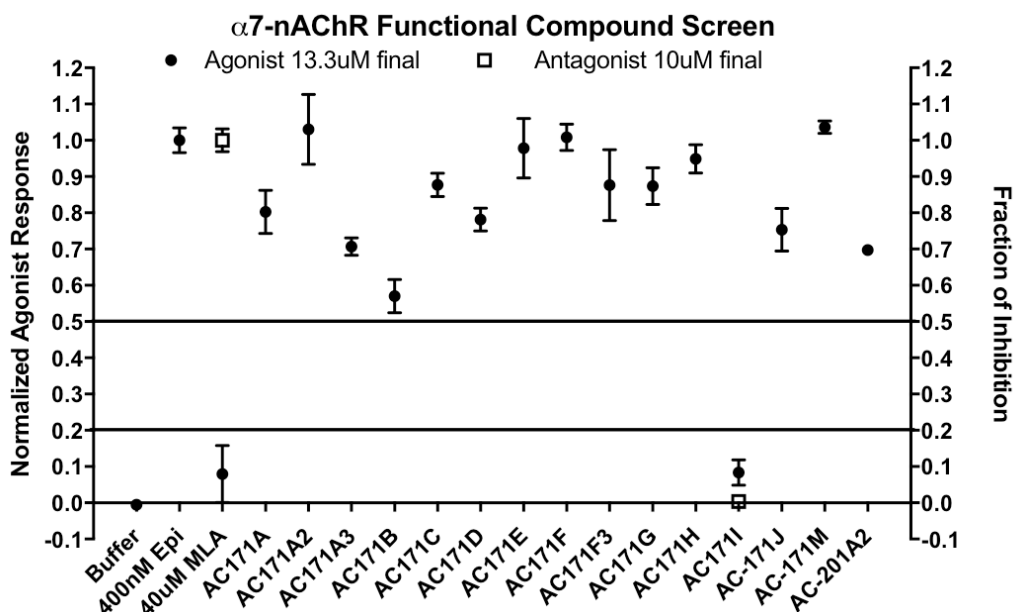


Figure 30. Functional quick screen of compounds of the AC series with cell line expressing $\alpha 7$ -nAChR. The FRET ratio ($\Delta/\Delta_{\text{max}}$) was normalized, when compounds showed agonist response higher than 0.2 they were further characterized as agonist. When compounds showed inhibition higher the 0.5, they were further characterized as antagonist.

Compounds containing electron-donating groups and heterocycles in the sixth position were the most potent activators of $\alpha 7$ -nAChR (figure 30, table). Concentration-response curves of lead compounds are shown in Figure 30; two of lead compounds, AC-171A2 and AC-171H elicited EC_{50} values of 70 nM. According to the crystallographic information that is later discussed in much more detail, the 2-methoxy of the aromatic substituent in the sixth position of the pyrimidine extends the contacts to the C loop cysteines also the methoxy oxygen located 3.5 Å from both sulfur atoms, seems to be very important in the potency of this compounds, since both AC-171A2 and AC-171H have the same substitution in the second position, AC-171H has additional 4-methoxy group, removing the 2-methoxy and having only the 4-methoxy as it is in AC-171A, reduces the potency from 70 nM to 4.1 μM (Table 8).

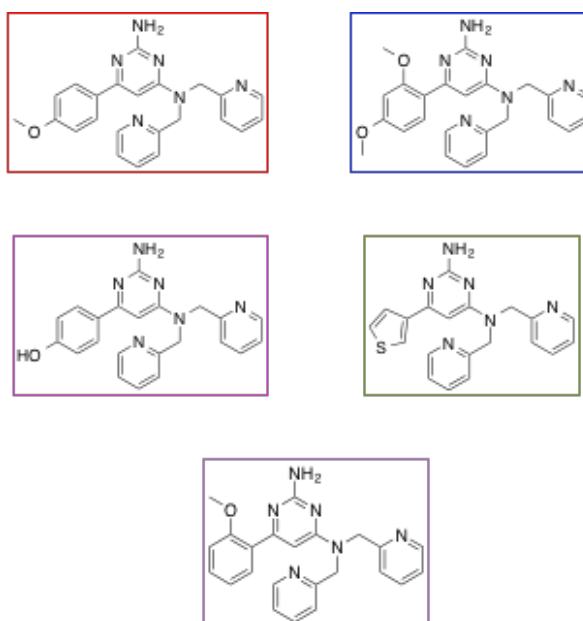
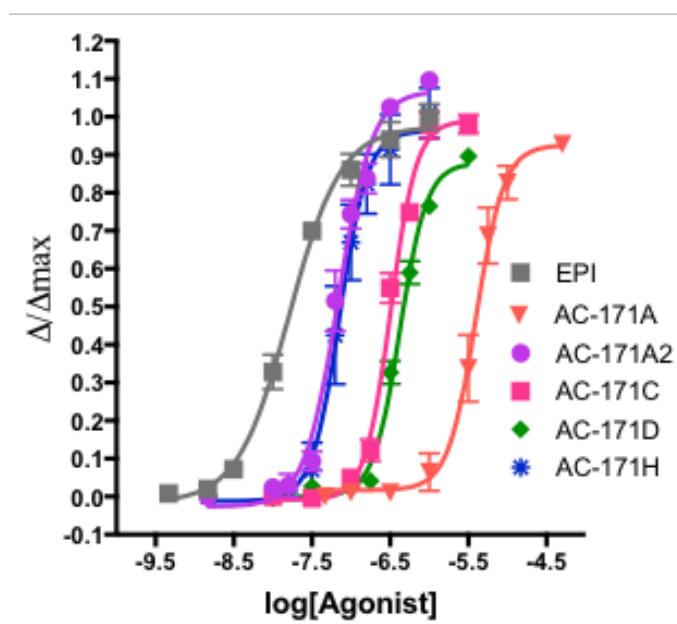


Figure 31. Concentration-response curves of compounds AC-171A, AC-171A2, AC-171C AC-171D and AC-171H, utilizing HEK cell line expressing $\alpha 7$ -nAChR.

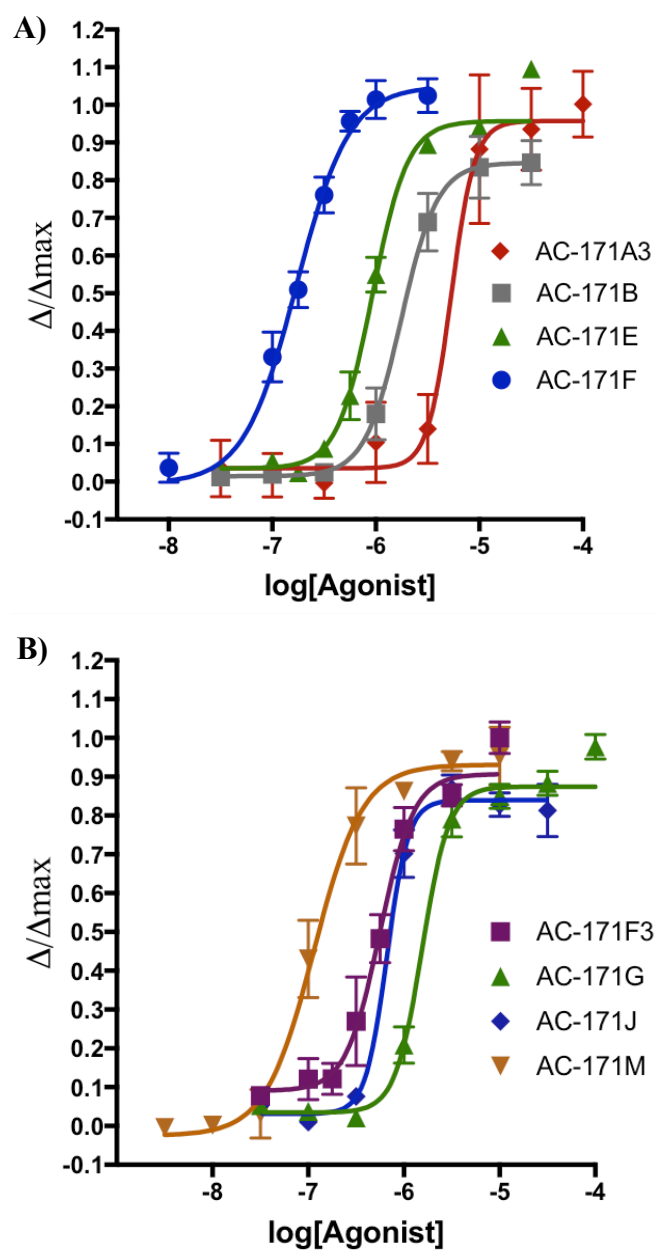
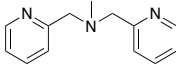
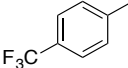
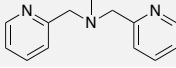
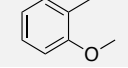
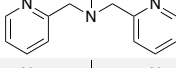
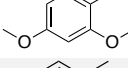
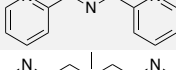
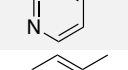
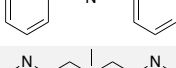
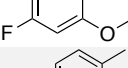
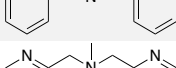
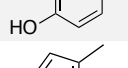
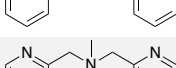
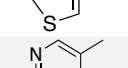
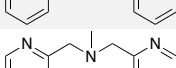
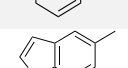
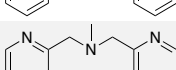
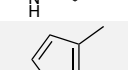
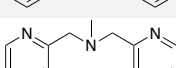
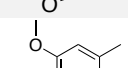
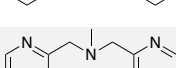
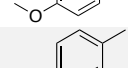
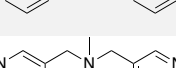
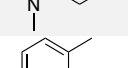
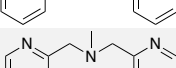
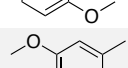
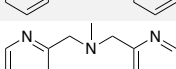
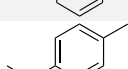
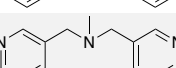
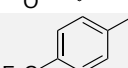
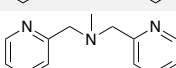
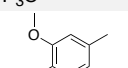
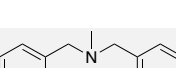
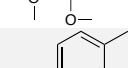
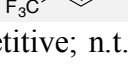


Figure 32. (A) Concentration-response curve of compounds AC-171A3, AC-171B, AC-171E, AC-171F, utilizing HEK cell line expressing $\alpha 7$ -nAChR. (B) Concentration-response curve of compounds AC-171F3, AC-171G, AC-171J, AC-171M, utilizing HEK cell line expressing $\alpha 7$ -nAChR.

Table 8. EC₅₀ values for $\alpha 7$ -nAChR agonist activation and antagonist dissociation constants for $\alpha 4\beta 2$ -nAChR and 5HT_{3A} receptor antagonism.

	STRUCTURE		AGONIST EC ₅₀ ± SD (μM) (N=3)	ANTAGONIST K _A ± SD (MM) (N=3)	
	R ₁	R ₂	$\alpha 7$	$\alpha 4\beta 2$	5HT _{3A}
AC-17-2			2.5 ± 0.3*	5.7 ± 0.9 ^{NC}	>10
AC-171A2			0.07 ± 0.01	6.3 ± 1.5 ^{NC} 6.3 ± 1.4 ^C	>10
AC-171H			0.07 ± 0.01	2.5 ± 0.8 ^{NC}	>10
AC-171F			0.14 ± 0.06	>10	>10
AC-171M			0.13 ± 0.04	n.t.	n.t.
AC-171C			0.26 ± 0.03	>10	>10
AC-171D			0.5 ± 0.1	>10	>10
AC-171F3			0.55 ± 0.10	>10	>10
AC-171J			0.6 ± 0.13	3.9 ± 0.7 ^C 2.9 ± 0.9 ^{NC}	15.2 ± 3.1 ^C 7.5 ± 1.2 ^{NC}
AC-171E			0.8 ± 0.2	>10	>10
AC-171G			1.4 ± 0.4	3.2 ± 0.5 ^{NC} 9.2 ± 1.2 ^C	>10
AC-171B			1.6 ± 0.2	2.4 ± 1.0 ^{NC}	>10
AC-201A2			2.7 ± 0.3	>10	>10
AC-171A3			3.9 ± 1.9	>10	>10
AC-171A			4.1 ± 1.3	n.t.	11.8 ± 1.4 ^{NC}
AC-20-2			10.3 ± 1.4*	2.3 ± 0.8 ^{NC}	7.2 ± 0.8 ^{NC}
AC-171I			>10	>10	>10
AC-19-2			>10	5.8 ± 0.7 ^{NC}	>10

NC: non competitive; C: Competitive; n.t. not tested; *: Partial agonist. Responses are measured from HEK cells containing a fluorescent reporter and a plate as monolayers on 96 well plates (Yamauchi, J. G. *et al.* 2011; Yamauchi, J. G. *et al.* 2012). The EC₅₀ represents the concentration giving half-maximal responses.

No activation of $\alpha 4\beta 2$ -nAChR of 5-HT_{3A} serotonin receptor was observed by the AC nor the ALseries. However, antagonism $\alpha 4\beta 2$ -nAChR was achieved by AC-17-2, AC-171A2, AC-171H, AC-171J, AC-171G, AC-171B and AC-20-2 compounds. Most of them behave as non-competitive antagonism (**figure 33**), AC-171A2 and AC-171H showed a wide window between the concentration achieving $\alpha 7$ activation and the $\alpha 4\beta 2$ inhibition (**table 8**).

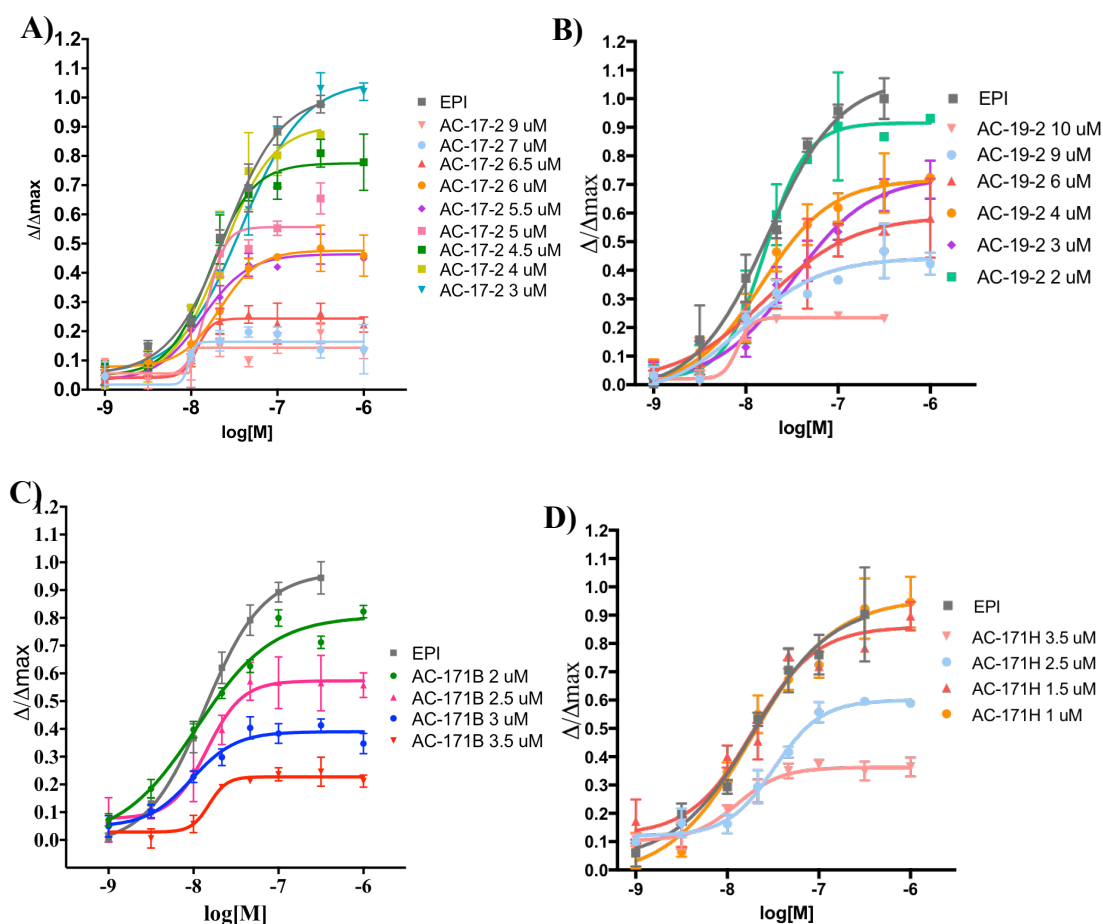


Figure 33. $\alpha 4\beta 2$ -nAChR selected antagonist concentration-response curves of AC series. A) Compound AC-17-2, B) Compound AC-19-2, C) Compound AC-171B, and AC-171H.

Compound AC-171A2 at submicromolar concentrations appears to enhance epibatidine activity on the $\alpha 4\beta 2$ -nAChR (**figure 34**). Under the conditions we used for transfection, it is likely the $\alpha 4\beta 2$ -nAChR is assembled in two pentameric stoichiometries $\{(\alpha 4)_3(\beta 2)_2\}$ and $\{(\alpha 4)_2(\beta 2)_3\}$ --the former forming an $\alpha 4:\alpha 4$ interface (Da Silva-Tavares, X. *et al.* 2012; Eaton, J. B. *et al.* 2014; Mazzaferro, S. *et al.* 2014) At higher concentrations, ($>3 \mu\text{M}$) non-competitive antagonism is evident. Analysis of the activation and inhibition

behaviour will require future investigation in more depth with fixed stoichiometries of these heteromeric subunit combinations forming $\alpha 4\beta 2$ - nAChR pentamers.

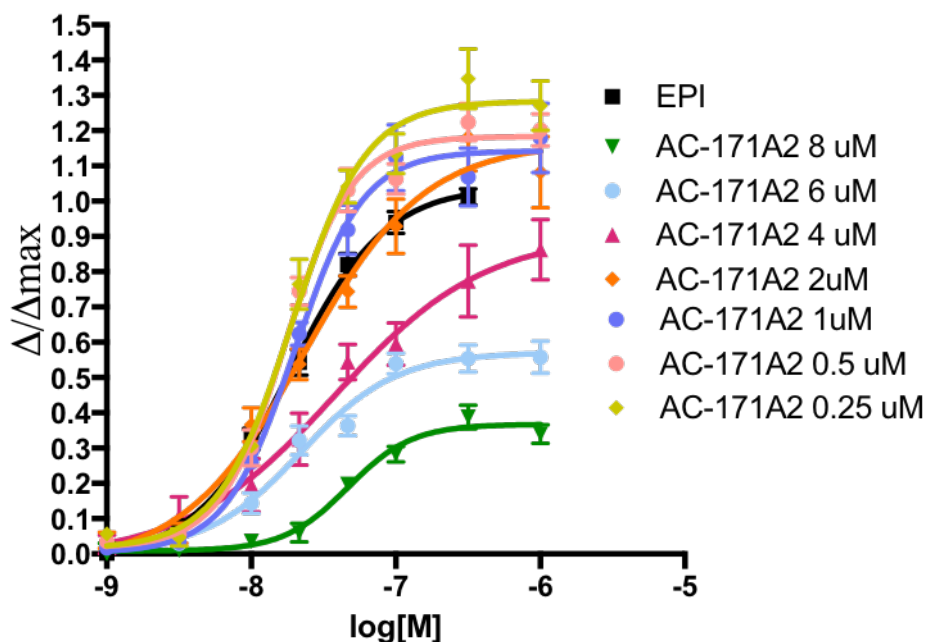


Figure 34. $\alpha 4\beta 2$ -nAChR antagonist concentration-response curves of lead compound AC-171A2.

Only three compounds out of the AC series showed non competitive antagonist behaviour when screened against cell line expressing the 5HT_{A3} serotonin receptor (**table 8, figure 35**). When compared antagonism K_A with $\alpha 7$ -nAChR activation, a broad window in the concentration was demonstrated, showing a different behaviour than tropisetron, a known partial agonist of $\alpha 7$ -nAChR and competitive antagonist of 5HT_{3A} receptor.

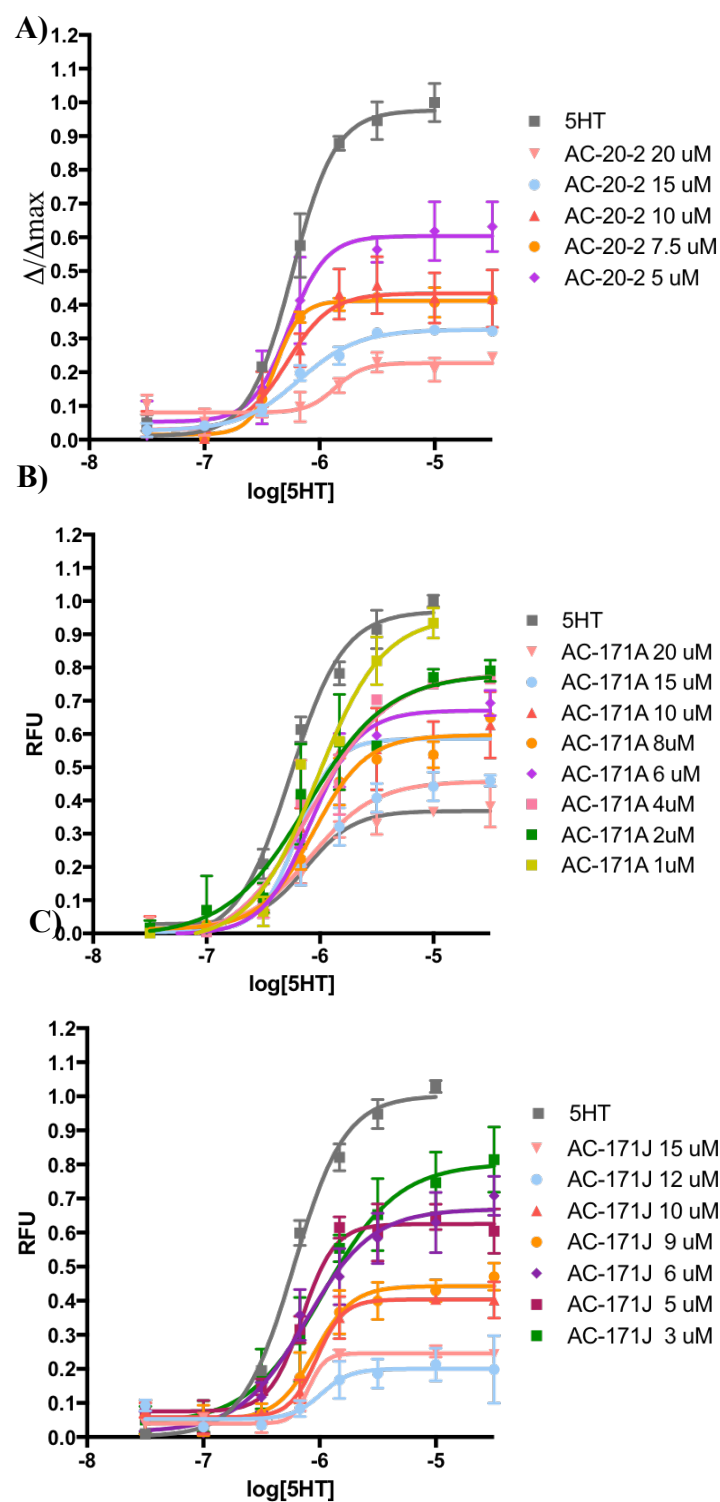


Figure 35. 5HT_{3A} serotonin receptor antagonist concentration-response curves of AC series. A) Compound AC-20-2, B) Compound AC-171A, and C) AC-171J.

After the results of the AC series, we decided to expand the library and design new compounds for activation of $\alpha 7$ -nAChR with specific modifications based primarily on AC-171A2. The AL series, is mostly based on modification in the second position of the pyrimidine system with the purpose of assess how by modifying this area the potency of the lead compound was affected, this specific position was not changed before because of the crystallographic information that we had from previous compounds (Kaczanowska, K. *et al.* 2014), but after the resulsits from the AC series and the crystallography information, we decided to modify this position.

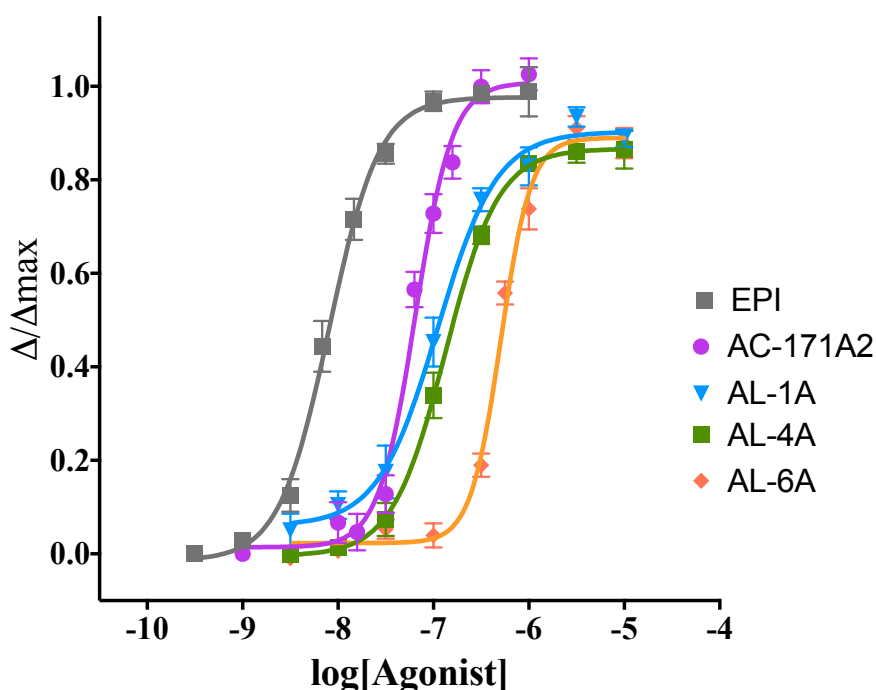
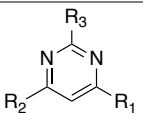
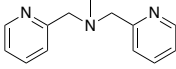
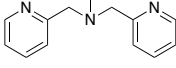
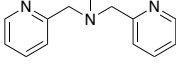
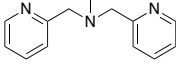
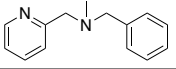
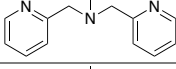
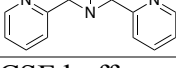


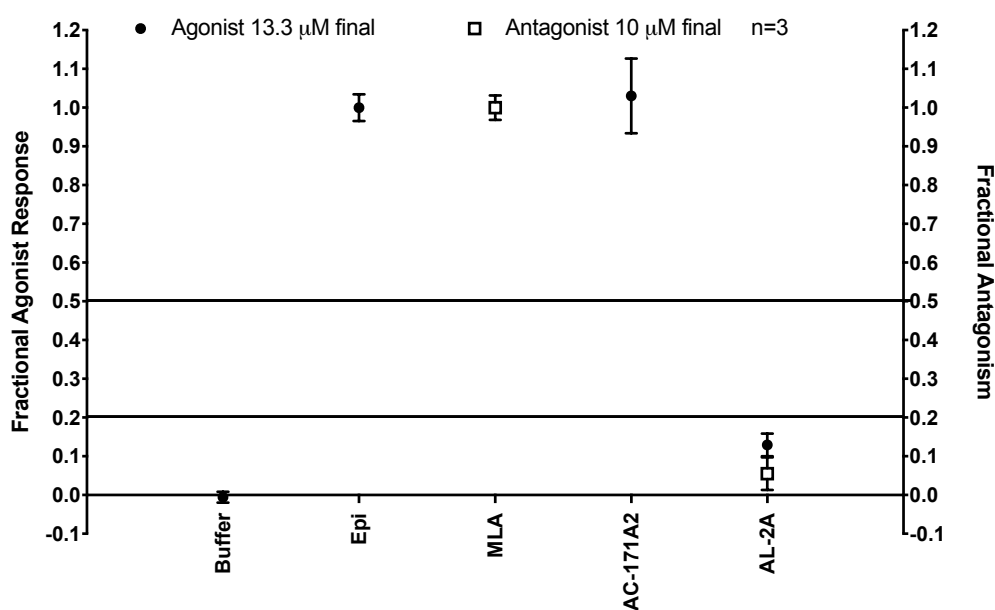
Figure 36. Concentration-response curve of compound AC-171A2, AL-1A, AL-4A, and AL-6A, utilizing HEK cell line expressing $\alpha 7$ -nAChR.

As we can see in the **table 9**, and **figure 36**, no crutial impact in the potency of the compounds when they were modified in the second position of the pyrimidine ring, adjacent to the two cyclic nitrogens, this information is critical on understanding structure-activity relationships for future modifications of the structure. Compound AL-1A2 showed to be unstable in buffer conditions, therefore no titration curve was achieved.

Table 9. EC₅₀ values for $\alpha 7$ -nAChR agonist activation.

	R ₁	R ₂	R ₃	Agonist EC ₅₀ ± SD (μM) (n=3)
AL-1A				0.11±0.02
AL-1B				1.8 ± 0.56
AL-1A2				Uns.
AL-1A3				0.9 ± 0.18
AL-2A				>10
AL-4A				0.14±0.02
AL-6A				0.53±0.06

Uns.= Unstable in aCSF buffer

**Figure 37.** Functional quick screen $\alpha 7$ -nAChR comparing activity between AC-171A2 (lead compound of series AC) and AL-2A.

In the AC series we showed: 1) moving the ring nitrogen of the di-picolyl system was not critical for the activation of $\alpha 7$ -nAChR. 2) Removing the nitrogen by substituting the pyridines for benzyl groups, activation was lost. I was curious to assess whether both pyridine rings were important for activation as well or if it was enough by having only

one of them and a benzyl group instead. We synthesized AL-2A as we describe previously and our screen demonstrates when compared to lead compound AC-171A2 (**Figure 37**), AL-2A shows no agonist activity. We concluded that the two pyridines of the di-picolyl system are decisive for the agonist profile of the compounds.

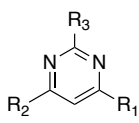
There are some important properties for drug design, such as lipophilicity, that could have impact on drug discovery. One of most used rules by medicinal chemists is the “Lipinski rule” also known as “rule of 5”. This rule was first established by Pfizer. The rule of 5 consists in:

- $\text{Log } P > 5$ (or $\text{Mlog } P > 4.15$)
- >5 H-bond donors
- >10 H-bond acceptors
- $\text{MW} > 500$
- Substrates for biological transporters are exceptions to the rule

“Violation” of one rule will not necessarily lead to a poor absorption or permeation, but it will increase with the number of rules broken (Kerns, E. H.; Di, L. 2008). Our synthetic compounds passed all rules including the Veber rule ($\text{PSA} < 140$, rotatable bonds < 10).

According to the Swiss ADME software that we used, only one of our compounds, AL-4A, has the possibility to cross the blood-brain barrier (BBB) (**table 10**). This prediction was done utilizing the BOILED-Egg (Brain Or IntestinaL EstimateD) method, this method only predicts passive diffusion and has an internal (by the creators) accuracy of 90% (Daina, A.; Zoete, V. 2016). If this interesting prediction turns out to be true, AL-4A could be used to develop a new library of compounds, additionally, the EC_{50} of AL-4A is close to the lead compound AC-171A2.

Table 10. Physicochemical and pharmacokinetic properties predicted of two series.

	MLog P	HBD	HBA	PSA	Rotable bonds	Lipinski	BBB permeant
AC-17-2	2.23	1	7	80.82	7	Yes	No
AC-171A	1.12	1	5	90.05	7	Yes	No
AC-171A2	1.12	1	5	90.54	7	Yes	No
AC-171A3	1.12	1	5	90.05	7	Yes	No
AC-171B	1.33	1	4	84.06	7	Yes	No
AC-171C	0.90	2	5	101.05	6	Yes	No
AC-171D	1.03	1	4	109.06	6	Yes	No
AC-171E	0.23	1	5	93.96	6	Yes	No
AC-171F	0.41	1	5	93.71	6	Yes	No
AC-171F3	0.41	1	5	93.71	6	Yes	No
AC-171G	0.81	1	6	99.28	8	Yes	No
AC-171H	0.81	1	6	99.28	8	Yes	No
AC-171I	0.50	1	7	108.51	9	Yes	No
AC-171J	1.19	2	4	96.61	6	Yes	No
AC-171M	1.49	1	6	90.05	7	Yes	No
AC-201A2	1.12	1	5	90.05	7	Yes	No
AC-19-2	4.28	1	5	55.04	7	No	No
AC-20-2	2.23	1	7	80.82	7	Yes	No
AL-1A	2.13	0	5	89.33	8	Yes	No
AL-1B	1.66	0	4	108.34	7	Yes	No
AL-1A2	1.21	0	7	106.55	8	Yes	No
AL-1A3	1.26	0	6	100.31	8	Yes	No
AL-2A	2.13	1	4	77.16	7	Yes	No
AL-4A	1.65	0	5	64.03	7	Yes	Yes
AL-6A	2.44	0	8	64.03	8	Yes	No

HBD= Hydrogen bond donor; HBA= Hydrogen bond acceptor; PSA= Polar surface area; BBB= blood-brain barrier. Predictions were made utilizing SwissADME software.

6.2.2. Radioligand binding assay results

Binding characterization of the series was performed by applying a radioligand competition assay against ^3H -epibatidine binding at a single, 10 μM concentration of the competing ligand at the quick screen first stage. Evaluation of their binding affinity was crucial to establish which of the compounds were co-crystallized with the binding proteins. Both epibatidine and the competing 2-aminopyrimidines were in excess of the total AChBP sites on two molluscan AChBP's from *Lymnaea stagnalis* (*Ls*- AChBP) and *Aplysia californica* (*Ac*-AChBP). Analysis of the binding constants was determined from titrations of the compounds that passed the quick screen.

The concentration dependency reveals that the compounds in large part show high affinities for *Ls*-AChBP (**Figure 38**) and moderately high affinities for *Ac*-AChBP (**Table 11**). It is likely that the Tyr to Trp side chain difference at residue 55 is one, but scarcely the sole, determining factor (Nemecz, A.; Taylor, P. 2011). Some of the synthesized ligands showed dissociation constants as low as 30nM, establishing that the di-picolyl substitution in and off itself was not compromising binding to the surrogate AChBP proteins, since we know from previous work (Kaczanowska, K. *et al.* 2014), that compounds without the di-picolyl system were able to bind *Ls*-AChBP.

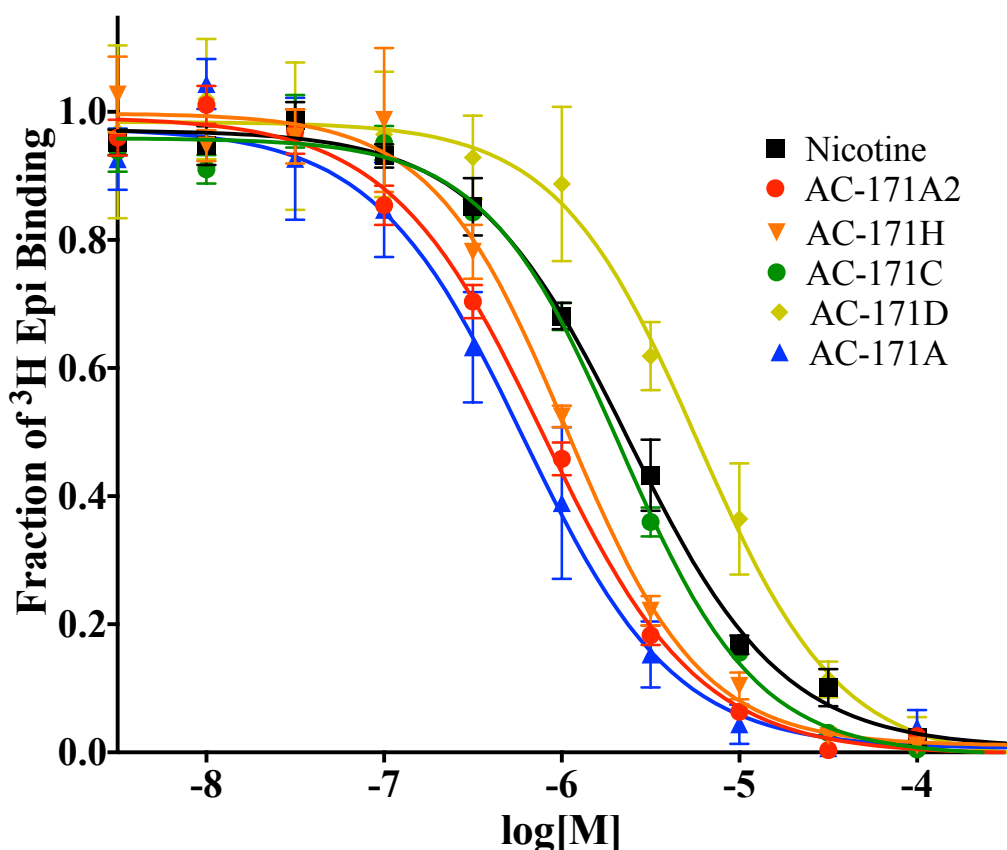
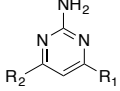


Figure 38. Titration of the *Ls*-AChBP at various concentration of ligand competing againsts - ³H-epibatidine binding by the respective 4-dibenzyl and 4-dipicolyl substituted 2-aminopyrimidines measured using a scintillation proximity assay (Talley, T. T. *et al.* 2006). K_d values are determined from the K_d of epibatidine and the concentrations of epibatidine and competing ligand.

Four of the eleven compounds that show affinity for *Ls*-AChBP were co-crystallized. Their binding orientations will be discussed in much more detail later on, and so a possible reason why only the compounds with the di-picolyl system activate the $\alpha 7$ -nAChR is proposed based on the binding orientations of the compounds. AL series binding affinity will be characterized from **table 11** when further molecules are characterized.

Table 11. Dissociation constants for 4,6-disubstituted 2-aminopyrimidines determined by competitive back titrations against ^3H -epibatidine on SPA beads linked to purified *Lymnaea stagnalis* (*Ls*) and *Aplysia californica* (*Ac*) AChBPs (Talley, T. T. *et al.* 2006).

	ACHBPS BINDING ASSAY			
	<i>Ls</i> -AChBP		<i>Ac</i> -AChBP	
	$K_d \pm \text{SD}$ (μM)	$n_H \pm \text{SD}$	$K_d \pm \text{SD}$ (μM)	$n_H \pm \text{SD}$
AC-17-2	0.16 $\pm$ 0.03	1.07 $\pm$ 0.15	0.31 $\pm$ 0.07	1.8 $\pm$ 0.4
AC-171A2*	0.040 $\pm$ 0.003	0.91 $\pm$ 0.03	0.60 $\pm$ 0.018	1.5 $\pm$ 0.2
AC-171H	0.060 $\pm$ 0.005	1.16 $\pm$ 0.05	>10	-
AC-171F	>10	-	>10	-
AC-171C*	0.130 $\pm$ 0.006	1.11 $\pm$ 0.11	0.33 $\pm$ 0.05	1.25 $\pm$ 0.24
AC-171D*	0.33 $\pm$ 0.02	1.13 $\pm$ 0.16	>10	-
AC-171F3	>10	-	0.083 $\pm$ 0.005	1.24 $\pm$ 0.04
AC-171J	0.27 $\pm$ 0.03	0.85 $\pm$ 0.01	0.062 $\pm$ 0.006	0.84 $\pm$ 0.07
AC-171E	>10	-	>10	-
AC-171G	>10	-	0.63 $\pm$ 0.09	1.12 $\pm$ 0.14
AC-171B	0.065 $\pm$ 0.006	1.08 $\pm$ 0.12	>10	-
AC-201A2	0.19 $\pm$ 0.06	0.96 $\pm$ 0.02	0.07 $\pm$ 0.007	0.84 $\pm$ 0.08
AC-171A3	>10	-	>10	-
AC-171A*	0.030 $\pm$ 0.001	1.00 $\pm$ 0.05	0.12 $\pm$ 0.02	1.4 $\pm$ 0.2
AC-20-2	0.030 $\pm$ 0.004	1.09 $\pm$ 0.09	0.18 $\pm$ 0.02	0.83 $\pm$ 0.05
AC-171I	>10	-	>10	-
AC-19-2	0.070 $\pm$ 0.005	1.05 $\pm$ 0.02	>10	-
AC-17	>10	-	>10	-
AC-20	>10	-	0.090 $\pm$ 0.004	0.80 $\pm$ 0.05
AC-19	>10	-	>10	-

Dissociation constants (K_d) and Hill coefficients (n_H) are reported as means ($\pm$ SD). Each experiment was done in triplicate. *: Solved crystal structures for *Lymnaea stagnalis* AChBP ligand complexes.

6.2.3. Two-electrode voltage clamp electrophysiology

As we described previously (chapter III), because of the characteristics of our $\alpha 7$ -nAChR cell based assay, there is a need for using a positive allosteric modulator PNU-120596 in order to detect activation signal. This arises because of the fast desensitization characteristic of the receptor. Therefore, we took advantage of the two-electrode voltage clamp electrophysiology (Papke, R. L. *et al.* 2010) technique, this was possible thanks to a collaboration that was established with Dr. Roger L. Papke at the University of Florida. This technique not only allows us to assess fast activation of the receptor by our compounds, but also will help us ascertain whether our compounds are silent agonists instead of full or partial agonists of the $\alpha 7$ -nAChR as we presume.

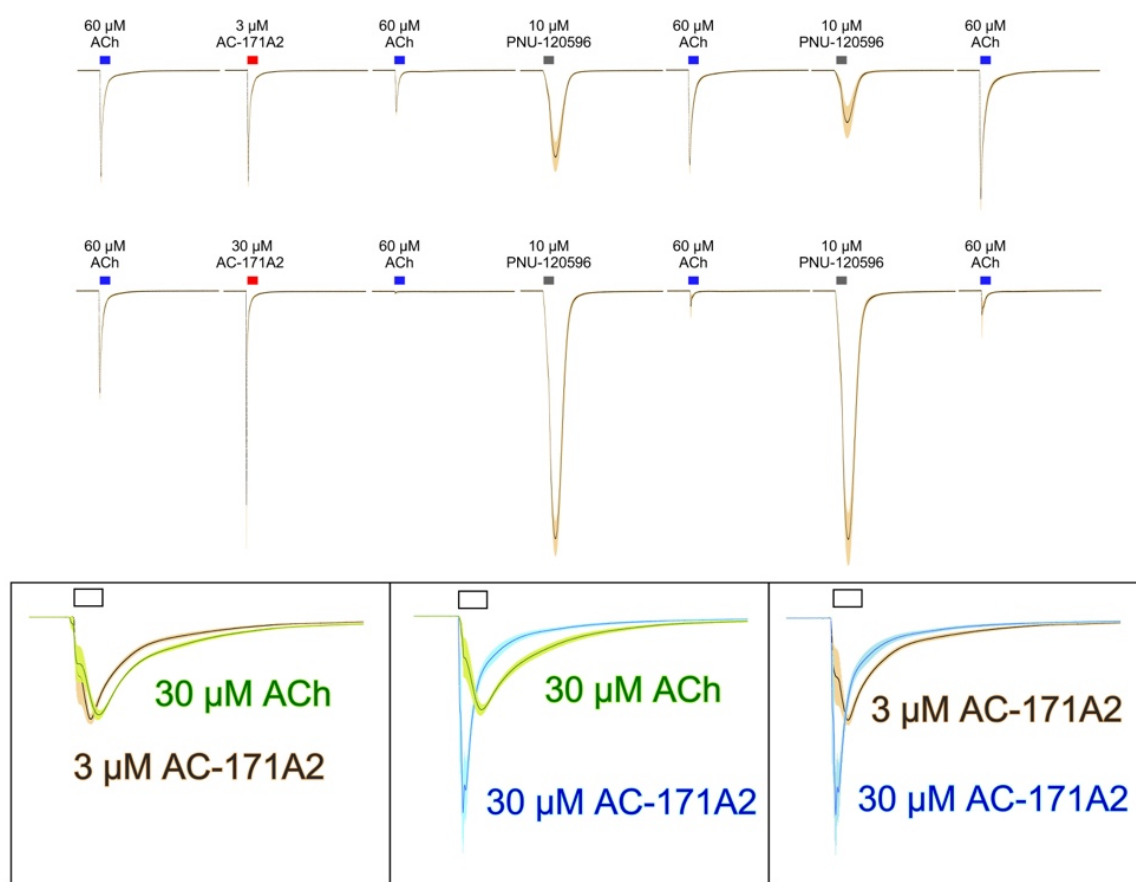


Figure 39. Activation of $\alpha 7$ -nAChR expressed in oocyte system. Activation by acetylcholine and AC-171A2; washing periods of 3.5 min were spaced between application of tested compounds.

Full activation was detected by addition of 3 μM of AC-171A2 without the presence of PNU-120596 in an oocyte system expressing the $\alpha 7$ -nAChR (**figure 39**), after

a wash period of 3.5 minutes, acetylcholine was added to assess recovery of the receptor, as we can appreciate in **figure 39**, slow recovery of the receptor occurs and it is dependent of the concentration of AC-171A2. By application of 30 μ M the full recovery of the receptor was not achieved by the end of the experiment, interesting we can appreciate a full activation, almost 10 times higher in the by the addition of PNU-120596 (**figure 40**), this same behavior was also appreciated with lead compound AC-171H.

One of the possibilities that we think is happening is that our two compounds stabilized a PNU-sensible desensitized state, either by a high affinity to the binding site or by stabilizing a change in the conformation a sort of “hit and run”, where the compound is binding to the orthosteric site promoting a change in the conformation, and then leaves the binding pocket. Then after the washing period and when is exposed to PNU-120596 will switch to an open state.

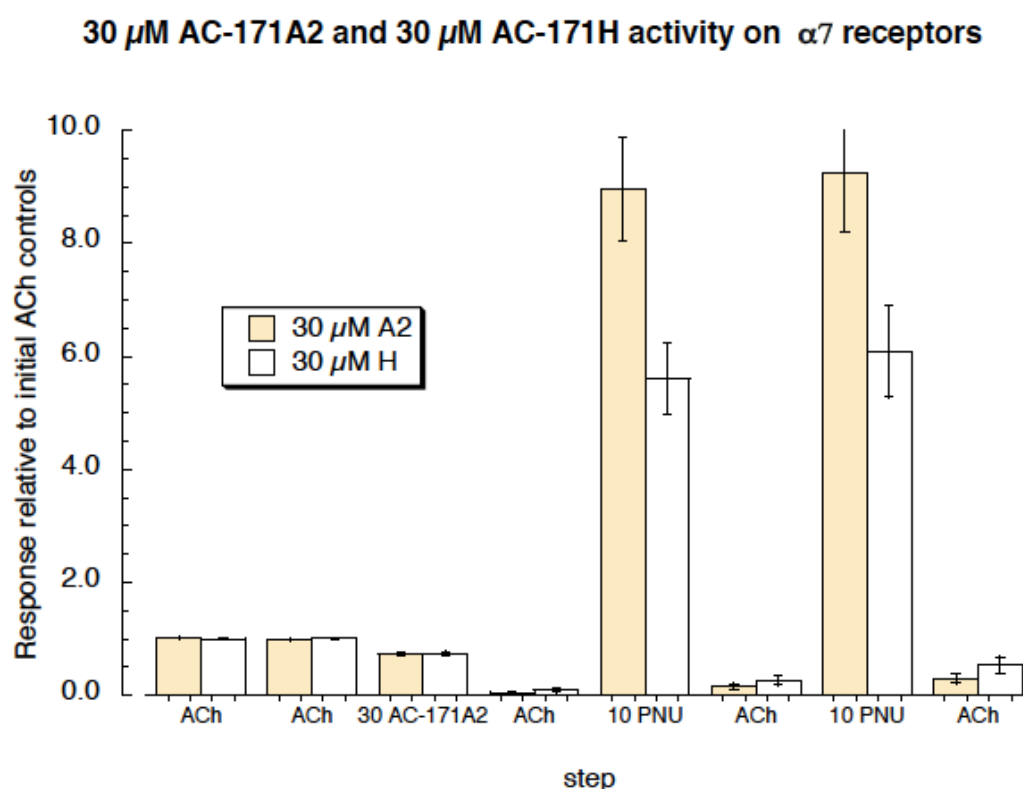


Figure 40. 30 μ M of AC-171A2 and AC-171H converts $\alpha 7$ -nAChR to a PNU-120596 sensitive state in an oocyte system; washing periods of 3.5 min were spaced between application of tested compounds.

On the other hand, AC-171C and AC-171D showed different profiles when compared to previous compounds. The $\alpha 7$ -nAChR expressed in oocytes recover much faster when exposed to AC-171C and AC-171D as we can see in **figure 41** at 3 μ M concentration, although, increasing the concentration to 30 μ M of AC-171D slowed down the recovery of the receptor, promoting a PNU-120596 sensitive state, not as potent as AC-171A2 and AC-171H. Pointing that not only substitutions in position six contributes to the full activation of $\alpha 7$ -nAChR as we demonstrate with the CNiFERs but it also affects the kinetics of the receptor.

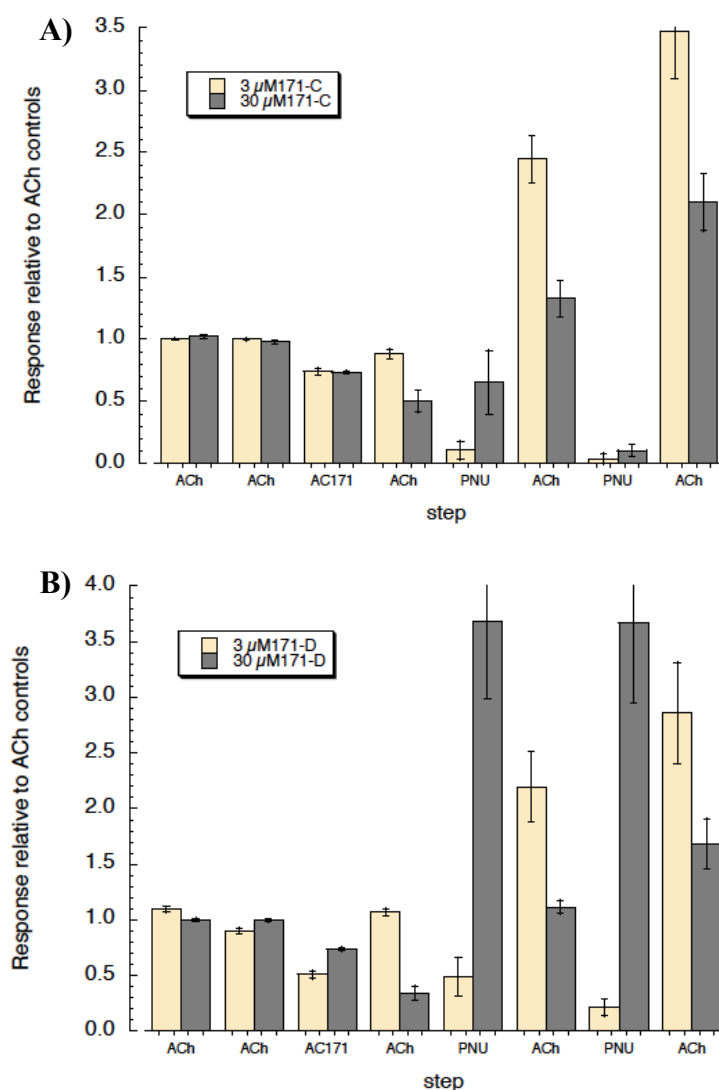


Figure 41. Almost full recovery of $\alpha 7$ -nAChR when exposed to 3 μ M concentration of AC-171C as well as AC-171D. But a much slower recovery was detected when exposed to 30 μ M of AC-171D.

Our compounds were shown to activate the $\alpha 7$ -nAChR without the presence of PNU-120596. The information that was collected through this experiment provide sufficient evidence that this new family of ligands are full agonist of the $\alpha 7$ -nAChR differing from silent agonist.

6.3. X-ray Crystallographic Analysis of the Ligands AChBP Complexes

Members of the AC series were co-crystallized with *Ls*-AChBP, despite the attempts only four full X-ray data sets were collected (Figure x): AC-171A2 ($K_d = 0.040 \mu\text{M}$), AC-171C ($K_d = 0.13 \mu\text{M}$), AC-171D ($K_d = 0.33 \mu\text{M}$), AC-171A ($K_d = 0.030 \mu\text{M}$). The structures were solved at 2.7 Å, 2.3 Å, 2.0 Å and 2.0 Å resolution respectively in P1211 space group. The structure of *Ls*-AChBP in complex with AC-171A2 (using chains A) superimposed on AC-171C with an RMS deviation of 0.3 Å for 183 C α atoms, on AC-171D with an RMS deviation of 0.26 Å for 186 C α atoms, and on AC-171A with RMS deviation 0.26 for 176 C α atoms. Side chain orientations show a high degree of similarity at all five binding sites in each pentamer and a similar orientation of the bound ligands. All four compounds were bound underneath of a closed C loop of the principal face at subunit junctures between the five subunits. Loop closure in X-ray data set of AC-171A2, as measured from the backbone carbonyl of W143 in the A loop to the γ -sulfur atom of the first vicinal Cys disulfide-linked residue in the C loop (C187 in *Ls*-AChBP), varies between 8.8-11.1 Å in the ten binding sites analyzed (average of 10.3 Å).

The X-ray crystal structures showed full occupancy of the protein pentamer and well defined ligand electron densities in all five binding pockets (AC-171A2 is used as an example: **Figure 42-A**), the protein crystallized in a dimer of pentamers. The compound showed full occupancy of the electron density (**Figure 42-B**).

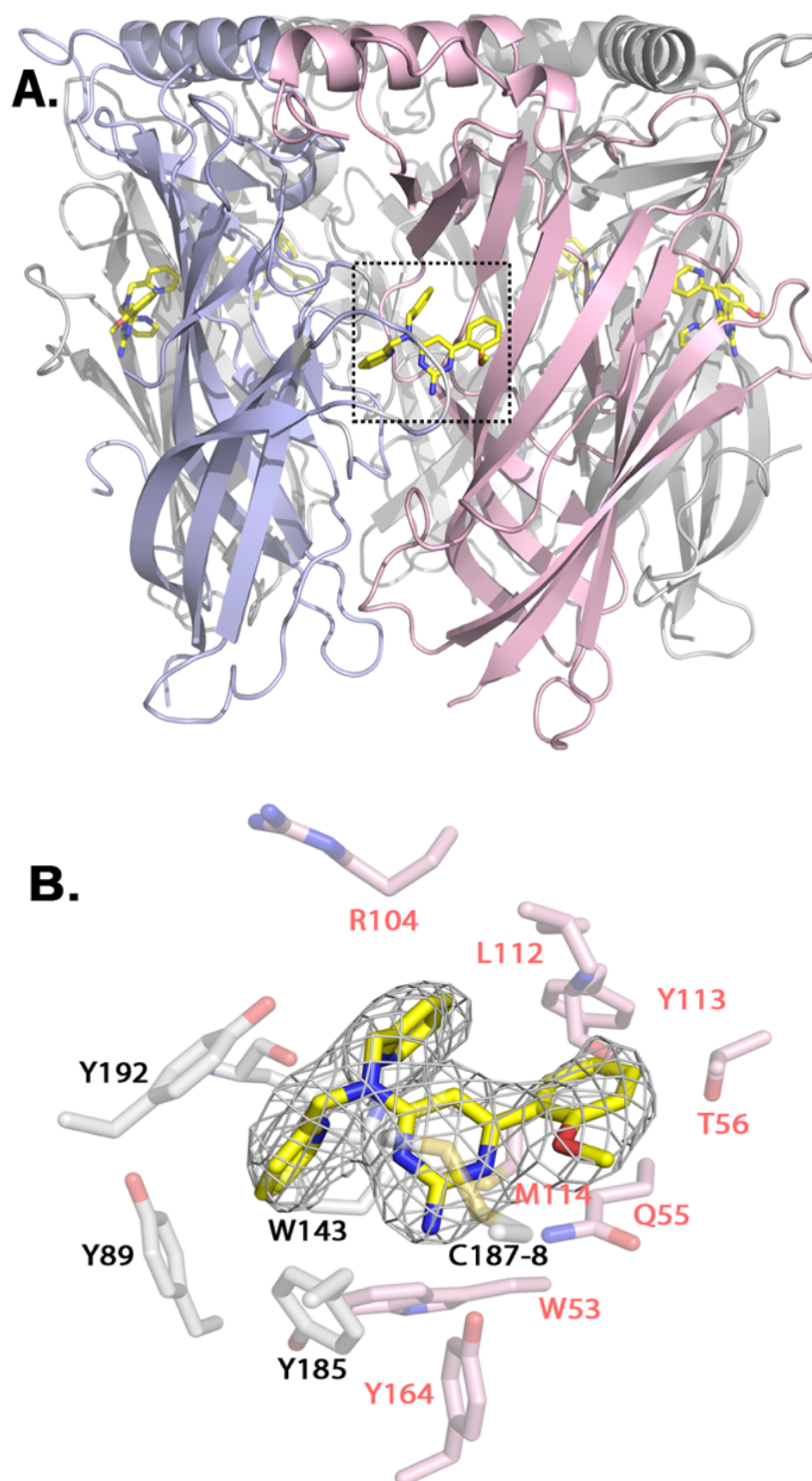


Figure 42. (A) Radial view of *Ls*-AChBP pentameric structure in complex with AC-171A2. A principal, C loop-containing, and complementary face are shown in violet and fushia. (B) Expanded radial view of AC-171A2 in the binding site. Side chain carbons of the principal (grey) and complementary (fushia) subunits are designated with black and red numbers, respectively. Ligand carbons displayed in yellow, nitrogens in blue, oxygen in red.

Strong polar interactions were observed for residues on the complementary face where ligand nitrogen N1, is not likely protonated at physiological pH, as we showed in the pKa experiments. It hydrogen bonds to the Gln55 side chain (distance 2.7 Å; all distances measured for the binding pocket formed by chains C and D) and its free amino group at the position 2 hydrogen bonds to Tyr164 hydroxyl group (3.2 Å distance). In addition, the aromatic rings of the branched *bis*-pyridyl (picolyl) substituent may likely interact with the carbonyl of Trp143 on the principal subunit interface. Such unconventional intermolecular C=O... π interactions have been studied in supramolecular frameworks, but have rarely been reported in protein crystals (Wan, C-Q. *et al.* 2008) Additional stabilization could be achieved by a surrounding peptide bond framework at the binding interface.

A T-shaped π -stacking interaction is shown between pyridines of the dipicolyl moiety and the aromatic ring of Trp143 (3.5-3.9 Å). The nitrogens of these systems are positioned 2.7 Å away from each other and they form dihedral angles (57 and 62 degrees) with the carbonyl oxygen of Trp 143. Other distinctive interactions are observed with Met114 sulfur atom and both sulfur atoms of the Cys187-Cys188 disulfide bridge in the C loop. These residues together align in a sandwich-type S/ π stacking system with the pyrimidine N1 atom being 3.3 Å away from the large methionine sulfur atom. The 2-methoxyphenyl ring in 40 resides on the complementary face in proximity with main hydrophobic interactions observed for Gln55, Thr56, Leu112, Tyr113 and Met114 (up to 3.2 Å for Gln55 and 3.4 Å for Thr56). The 2-methoxy substituent extends the contacts to the C loop cysteines with the methoxy oxygen located 3.5 Å from both sulfur atoms.

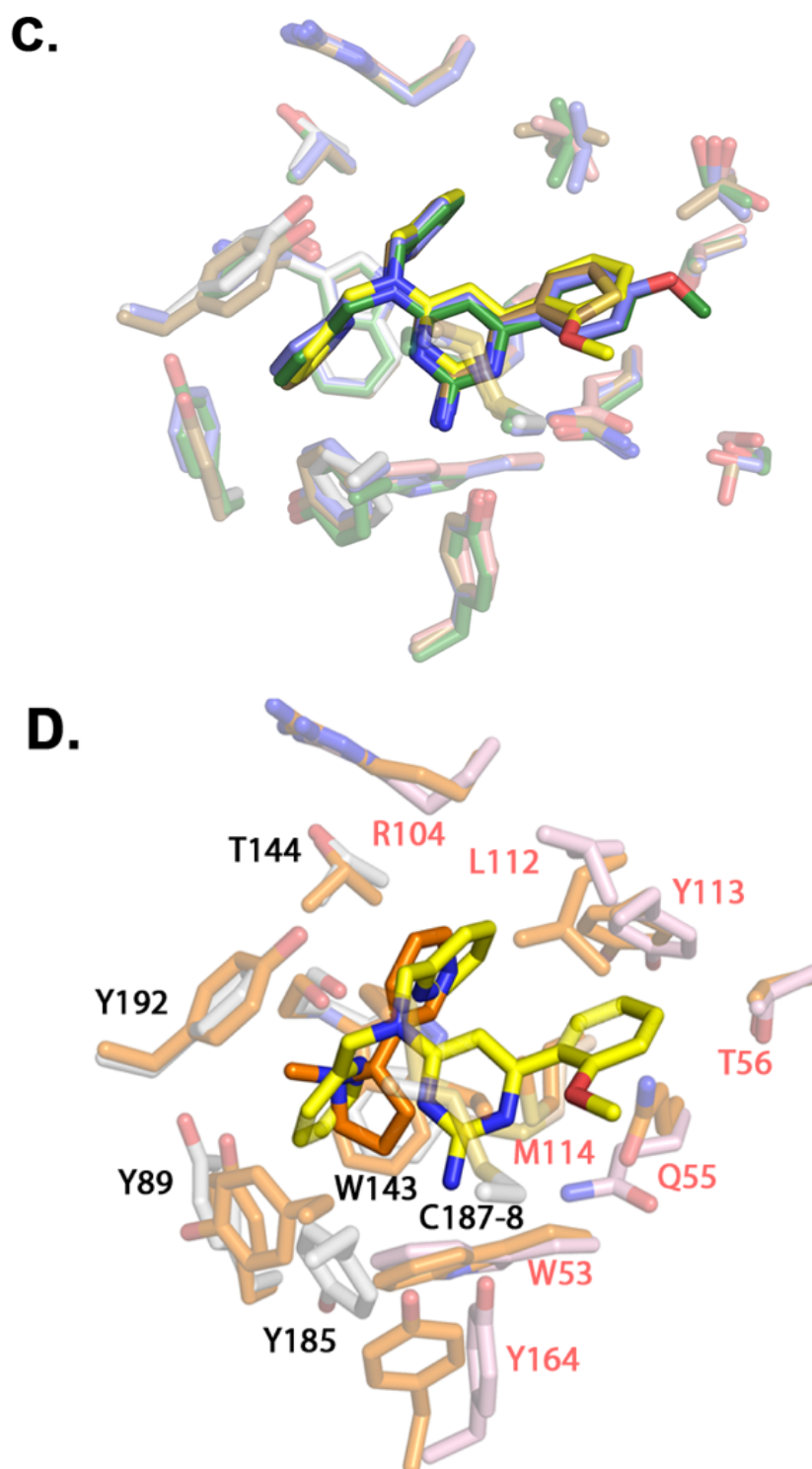


Figure 43. (C) Superimposition of X-ray crystal structure of AC-171A2, that interacts more extensively with Gln55 and the Cys-loop S-S bridge in the *Ls*-AChBP with X-ray crystal structures of AC-171C, AC-171D and AC-171A. (D) Superimposition of AC-171A2 with nicotine (PDB code 1UW6, in orange).

When the X-ray crystal structure of AC-171A2 was aligned with the *Ls*-AChBP:nicotine complex (PDB code: 1UW6), striking differences in ligand positioning along with some differences in protein side chain orientations were observed (**Figure 43**, panel D). Both pyridyl rings of AC-171A2 occupy the nicotine site where ring nitrogens overlap with those of nicotine. Most significant side chain differences are observed on the principal subunit for Tyr89, Tyr185, Cys 187-188, and for Gln55, Leu112, Met114 and Tyr164 on the complementary face. The cation-binding selectivity of nAChRs (Changeux, J. P. 2012; Dineley, K. T. *et al.* 2015, Thompson, A. J. *et al.* 2010; Bertrand, D. *et al.* 2015; Nemecz, A. and Taylor, P. 2011) is distinctive. Quaternary amines are stabilized by cation- π interactions with a nest of surrounding aromatic side chains, whereas other interactions with protonated secondary and tertiary amines and imines are often stabilized by hydrogen bonding to the backbone carbonyl of W143 (Hibbs, R. E. *et al.* 2009; Grimster, N. P. *et al.* 2012). Typically the quaternary amine or a strongly basic tertiary amine or imine is accompanied another ring system or region of the molecule that can serve as a hydrogen bond acceptor, the pyridine in nicotine, epibatidine or anabaseine or the keto group in cytosine or anatoxin. Hence, a common chemotype is also evident for the less basic nitrogen and candidate other H-bond acceptors.

The 2-aminopyrimidines appear to diverge from this pattern as distinctly different orientations of this core ring are evident in the bound structures (compare **Figure 44** panels D-F) and the three nitrogens of the 2-aminopyrimidine motif are only weakly basic. Thus, a cationic form of the 2-aminopyrimidine is not likely to be the bound species as found for the more strongly basic pyrrole, aza-bicyclic and imine ring systems. Moreover, the orientation of the 2-aminopyrimidine ring differs in the structures of compounds that bind only to AChBP vs. those that bind to both AChBP and activate the $\alpha 7$ -nAChR (**Figure 44**, panels E and F). It was only when I added the di-picolyl amine that we achieve greater $\alpha 7$ -activation. We suggest that the bulky, branched nature of the dibenzylamine or picolylamine substituents drives this change, leading to an important difference in eliciting $\alpha 7$ agonist activity. The necessity for adopting two distinct orientations for the 2-aminopyrimidine ring perhaps explains why only 2 of nearly 40 initial compounds elicited $\alpha 7$ -nAChR responses. With this landscape sub-structure in mind, I could then pursue a wider of SAR studies, and have a lead into centrally active $\alpha 7$ agonists.

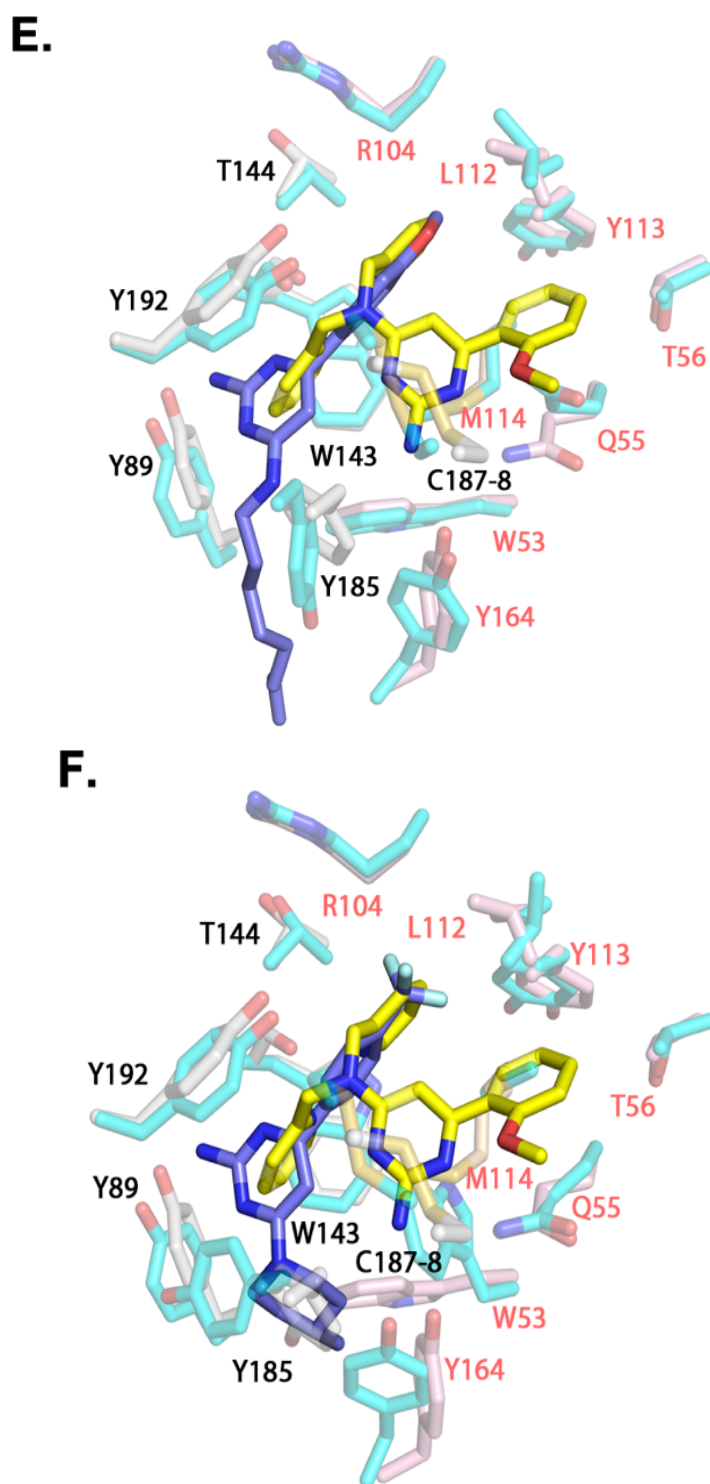


Figure 44. (E) Superposition of AC-171A2 with positively cooperative ligand **15** (PDB code 4QAA; ligand carbons shown in blue, protein in turquoise). (F) Superposition of AC-171A2 with negatively cooperative ligand **33** (PDB code 4QAC).

CHAPTER VII. CONCLUSIONS

At the initial stage of the project, I was able to synthesize a small library of a unique selective agonists of $\alpha 7$ -nAChR based on previous studies by Katarzyna Kaczanowska (AC series). The di-picolyl system in the fourth position of the pyrimidine system proved to be a requirement to achieve full or partial activation of the $\alpha 7$ -nAChR. Modulation of position sixth in the pyrimidine ring was important for ligand selectivity against $\alpha 4\beta 2$ -nAChR and 5-HT_{3A} as we describe in the discussion. AC-171A2 and AC-171H are our two lead compounds with 70 nM $\alpha 7$ -nAChR EC₅₀. In both cases substitution in the sixth position have a two methoxy moiety in the phenyl group, in the case of AC-171H additional methoxy group in the para-position. Same two compounds did not show activation nor inhibition of serotonin 5-HT_{3A}, both of the lead compounds showed inhibition of $\alpha 4\beta 2$ -nAChR with different competitive and non-competitive components.

Crystallographic studies of four of the first twenty synthesized compounds showed a unique binding pose when compared with classical agonist, nicotine. Having more interaction in the complementary face and high differences in the orientations of most of the residues of the principal and complementary face. The only oriented conserved residue was tryptophan 143, known to be important in classical agonist interaction. Previous cooperative ligands suggested that amino group of second position of the pyrimidine system was somehow important in the interaction, when compared to new crystalized ligands the difference in the binding pose suggested that modification in this position might be possible.

The AL series compounds synthesized based on cell and crystallographic results of AC series. A change in the amino group in position two results in a moderated decrease of the agonist response, suggesting that this position is important but not crucial as the di-picolyl system of position four. We were interested in assess whether one pyridine on the di-picolyl system was enough to achieve activation. AL-2A has no agonist response against $\alpha 7$ -nAChR proving that the two pyridines in the di-picolyl system are required for activation of the $\alpha 7$ -nAChR.

Compounds AC-171A2, AC-171H, AC-171C, and AC-171D activated $\alpha 7$ -nAChR express in *Xenopus* oocytes without the positive allosteric modulator PNU-120596 and promoted a PNU sensitive state showing a different agonist profile from classical agonist acetylcholine, in the case of the first two. This information was crucial to distinguish them from silent agonists and ago-PAMs and ensure they were full agonists of the $\alpha 7$ -nAChR.

Our compounds differ from the classical nicotine-agonist structure compounds, they lack a high pKa nitrogen that protonates at physiological pH and the responsible of the main cation- π interaction so well established in nicotine and congeners, and a low pKa nitrogen that interacts with positions on the complementary subunit at the subunit interface. We decided to prove our hypothesis utilizing ^1H -NMR, based on the experiments, AC-171A2 is largely a neutral species at physiological pH.

In summary, our approach to structure-guided drug design in this study employs a soluble surrogate of the nAChR to obtain leads of unique structure with an assay of through-put comparable to synthetic generation of newly designed agents. With candidate leads, more laborious cell-based screens of functional responses and crystallographic studies enable one to determine binding poses. Utilizing this approach we were able to discover novel $\alpha 7$ -nAChRs selective and potent agonists based on previous structure-activity relationship (SAR) studies, these compounds differ from classical agonists not only in the structure, but also in the binding orientation. Hence, high resolution structure-guided ligand design through a receptor surrogate appears to provide novel landscapes for $\alpha 7$ -nicotinic agonist leads.

REFERENCES

1. Adams, P. D. *et al.* PHENIX: A comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **66**, 213–221 (2010).
2. Adler, E., Hoffer, L. D. & Wiser, A. Normalization of auditory physiology by cigarette smoking in schizophrenia patients. *Am. J. Psychiatry* **150**, 1856–1861 (1993).
3. Apodaca, R. & Xiao, W. Direct Reductive Amination of Aldehydes and Ketones Using Phenylsilane: Catalysis by Dibutyltin Dichloride. *Org. Lett.* **3**, 1745–1748 (2001).
4. Arnfred, S. M. & Chen, A. C. N. Exploration of somatosensory P50 gating in schizophrenia spectrum patients: Reduced P50 amplitude correlates to social anhedonia. *Psychiatry Res.* **125**, 147–160 (2004).
5. Arunrungvichian, K., Fokin, V. V., Vajragupta, O. & Taylor, P. Selectivity Optimization of Substituted 1,2,3-Triazoles as $\alpha 7$ Nicotinic Acetylcholine Receptor Agonists. *ACS Chem. Neurosci.* **6**, 1317–1330 (2015).
6. Báez-Pagán, C. A., Delgado-Vélez, M. & Lasalde-Dominicci, J. A. Activation of the Macrophage $\alpha 7$ Nicotinic Acetylcholine Receptor and Control of Inflammation. *J. Neuroimmune Pharmacol.* **10**, 468–476 (2015).
7. Beinat, C., Banister, S. D., Herrera, M., Law, V. & Kassiou, M. The Therapeutic Potential of $\alpha 7$ Nicotinic Acetylcholine Receptor ($\alpha 7$ nAChR) Agonists for the Treatment of the Cognitive Deficits Associated with Schizophrenia. *CNS Drugs* **29**, 529–542 (2015).
8. M. Bencherif, P. M. Lippiello, R. Lucas, M. B. M. Alpha7 nicotinic receptors as novel therapeutic targets for inflammation-based diseases. **68**, 931–949 (2013).
9. Bertrand, D., Lee, C.-H. L., Flood, D., Marger, F. & Donnelly-Roberts, D. Therapeutic Potential of $\alpha 7$ Nicotinic Acetylcholine Receptors. *Pharmacol. Rev.* **67**, 1025–1073 (2015).
10. Bezençon, J. *et al.* pKa determination by ^1H NMR spectroscopy - An old methodology revisited. *J. Pharm. Biomed. Anal.* **93**, 147–155 (2014).
11. Bitner, R. *et al.* Preclinical characterization of a selective $\alpha 7$ neuronal nicotinic acetylcholine receptor agonist ABT-126: A novel therapeutic agent for the treatment of cognitive impairment in Alzheimer's disease and schizophrenia. *Alzheimer's Dement.* **9**, P817–P818 (2013).
12. Blum, A. P., Van Arnam, E. B., German, L. A., Lester, H. A. & Dougherty, D. A.

- Binding interactions with the complementary subunit of nicotinic receptors. *J. Biol. Chem.* **288**, 6991–6997 (2013).
13. Brejc, K. *et al.* Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature* **411**, 269–276 (2001).
 14. Celie, P. H. N. *et al.* Crystal structure of acetylcholine-binding protein from *Bulinus truncatus* reveals the conserved structural scaffold and sites of variation in nicotinic acetylcholine receptors. *J. Biol. Chem.* **280**, 26457–26466 (2005).
 15. Changeux, J. P. The nicotinic acetylcholine receptor: The founding father of the pentameric ligand-gated ion channel superfamily. *J. Biol. Chem.* **287**, 40207–40215 (2012).
 16. Ceide, S. C. & Montalban, A. G. Microwave-assisted, efficient and regioselective Pd-catalyzed C-phenylation of halopyrimidines. *Tetrahedron Lett.* **47**, 4415–4418 (2006).
 17. Corradi, J. & Bouzat, C. Understanding the Bases of Function and Modulation of $\alpha 7$ Nicotinic Receptors: Implications for Drug Discovery. *Mol. Pharmacol.* **90**, 288–299 (2016).
 18. Cuevas, J. & Berg, D. K. Mammalian nicotinic receptors with $\alpha 7$ subunits that slowly desensitize and rapidly recover from alpha-bungarotoxin blockade. *J. Neurosci.* **18**, 10335–10344 (1998).
 19. Tavares, X. D. S. *et al.* Variations in binding among several agonists at two stoichiometries of the neuronal, $\alpha 4\beta 2$ nicotinic receptor. *J. Am. Chem. Soc.* **134**, 11474–11480 (2012).
 20. Daina, A. & Zoete, V. A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. *ChemMedChem* 1117–1121 (2016).
 21. Dickerson, F. *et al.* Cigarette Smoking by Patients With Serious Mental Illness, 1999–2016: An Increasing Disparity. *Psychiatr. Serv.* appi.ps.2017001 (2017).
 22. Dineley, K. T. Beta-amyloid peptide – nicotinic acetylcholine receptor interaction: the two faces of health and disease. *Front. Biosci. A J. Virtual Libr.* **12**, 5030–5038 (2007).
 23. Dineley, K. T., Bell, K. A., Bui, D. & Sweatt, J. D. β -amyloid peptide activates $\alpha 7$ nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. *J. Biol. Chem.* **277**, 25056–25061 (2002).
 24. Dineley, Kelly T. Pandya, A. A. Y. J. L. Nicotinic ACh Receptors as Therapeutic Targets in CNS Disorders. **14**, 384–399 (2010).

25. Eaton, J. B. *et al.* The Unique 4(+)/(-) 4 Agonist Binding Site in (α 4) β 2 Subtype Nicotinic Acetylcholine Receptors Permits Differential Agonist Desensitization Pharmacology versus the (α 4) β 3 Subtype. *J. Pharmacol. Exp. Ther.* **348**, 46–58 (2013).
26. Emsley, P. & Cowtan, K. Coot: Model-building tools for molecular graphics. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **60**, 2126–2132 (2004).
27. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **66**, 486–501 (2010).
28. Feuerbach, D. *et al.* AQW051, a novel, potent and selective α 7 nicotinic ACh receptor partial agonist: Pharmacological characterization and phase i evaluation. *Br. J. Pharmacol.* **172**, 1292–1304 (2015).
29. Freedman, R.; Olincy, A.; Buchanan RW, Harris JG, Gold JM, Johnson L, Allensworth D, Guzman-Bonilla A, Clement B, Ball MP, Kutnick J, Pender V, Martin LF, Stevens KE, Wagner BD, Zerbe GO, Soti F, K. W. Initial phase 2 trial of a nicotinic agonist in schizophrenia. **14**, 571–581 (2014).
30. Gault, L. M. *et al.* ABT-126 monotherapy in mild-to-moderate Alzheimer's dementia: Randomized double-blind, placebo and active controlled adaptive trial and open-label extension. *Alzheimer's Res. Ther.* **8**, 1–13 (2016).
31. Gee, K. W. *et al.* First in human trial of a type i positive allosteric modulator of α 7-nicotinic acetylcholine receptors: Pharmacokinetics, safety, and evidence for neurocognitive effect of AVL-3288. *J. Psychopharmacol.* **31**, 434–441 (2017).
32. Geiger, A. *et al.* Correlating calcium binding, förster resonance energy transfer, and conformational change in the biosensor TN-XXL. *Biophys. J.* **102**, 2401–2410 (2012).
33. Gotti, C., Zoli, M. & Clementi, F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol. Sci.* **27**, 482–491 (2006).
34. Green, W. N. & Millar, N. S. Ion-channel assembly. *Trends Neurosci.* **18**, 280–287 (1995).
35. Grimster, N. P. *et al.* Generation of candidate ligands for nicotinic acetylcholine receptors via in situ click chemistry with a soluble acetylcholine binding protein template. *J. Am. Chem. Soc.* **134**, 6732–6740 (2012).
36. Halevi, S. *et al.* Conservation within the RIC-3 gene family: Effectors of mammalian nicotinic acetylcholine receptor expression. *J. Biol. Chem.* **278**, 34411–34417 (2003).

37. Hamadi, H. & Javadi, S. One-pot Reductive Amination of Carbonyl Compounds with NaBH₄-B(OSO₃H)₃/SiO₂ in Acetonitrile and in Solvent-free Condition. *J. Chem. Sci.* **129**, 75–80 (2017).
38. Hansen, S. B., Talley, T. T., Radić, Z. & Taylor, P. Structural and ligand recognition characteristics of an acetylcholine-binding protein from *Aplysia californica*. *J. Biol. Chem.* **279**, 24197–24202 (2004).
39. Hansen, Scott B., Sulzenbacher, G., Huxford, T., Marchot, P., Bourne, Y., Taylor, P. Structural Characterization of Agonist and Antagonist-Bound Acetylcholine-Binding Protein From *Aplysia californica*. *J. Mol. Neurosci.* **30**, 101–102 (2006).
40. Hashimoto, K. Targeting of $\alpha 7$ Nicotinic Acetylcholine Receptors in the Treatment of Schizophrenia and the Use of Auditory Sensory Gating as a Translational Biomarker. *Curr. Pharm. Des.* **21**, 3797–3806 (2015).
41. Hauser, T. A. *et al.* TC-5619: An $\alpha 7$ neuronal nicotinic receptor-selective agonist that demonstrates efficacy in animal models of the positive and negative symptoms and cognitive dysfunction of schizophrenia. *Biochem. Pharmacol.* **78**, 803–812 (2009).
42. Henderson, B. J. & Lester, H. A. Inside-out neuropharmacology of nicotinic drugs. *Neuropharmacology* **96**, 178–193 (2015).
43. Hibbs, R. E. *et al.* Structural determinants for interaction of partial agonists with acetylcholine binding protein and neuronal $\alpha 7$ nicotinic acetylcholine receptor. *EMBO J.* **28**, 3040–3051 (2009).
44. Horenstein, N. A., Leonik, F. M. & Papke, R. L. Multiple Pharmacophores for the Selective Activation of Nicotinic γ -Type Acetylcholine Receptors. *Mol. Pharmacol.* **74**, 1496–1511 (2008).
45. Insel, T. R. Rethinking schizophrenia. *Nature* **468**, 187–193 (2010).
46. Kaczanowska, K. *et al.* Substituted 2-Aminopyrimidines Selective for $\alpha 7$ -Nicotinic Acetylcholine Receptor Activation and Association with Acetylcholine Binding Proteins. *J. Am. Chem. Soc.* **139**, 3676–3684 (2017).
47. Kaczanowska, K. *et al.* Structural basis for cooperative interactions of substituted 2-aminopyrimidines with the acetylcholine binding protein. *Proc. Natl. Acad. Sci.* **111**, 10749–10754 (2014).
48. Kem, W. R. The brain $\alpha 7$ nicotinic receptor may be an important therapeutic target for the treatment of Alzheimer's disease: Studies with DMXBA (GTS-21). *Behav. Brain Res.* **113**, 169–181 (2000).

49. Lester, H. A. *et al.* Nicotine is a Selective Pharmacological Chaperone of Acetylcholine Receptor Number and Stoichiometry. Implications for Drug Discovery. *AAPS J.* **11**, 167–177 (2009).
50. Lieberman, J. A. *et al.* A randomized exploratory trial of an alpha-7 nicotinic receptor agonist (TC-5619) for cognitive enhancement in schizophrenia. *Neuropsychopharmacology* **38**, 968–975 (2013).
51. Lin-Moshier, Y. & Marchant, J. S. The *Xenopus* oocyte: A single-cell model for studying Ca^{2+} signaling. *Cold Spring Harb. Protoc.* **8**, 185–191 (2013).
52. Lombardo, S. & Maskos, U. Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment. *Neuropharmacology* **96**, 255–262 (2015).
53. Maguire, J. J., Kuc, R. E., Davenport, A. P. *Radioligand binding assays and their analysis. Methods in Molecular Biology* **897**, (2012).
54. Mazurov, A. A. *et al.* Discovery of (2*S*,3*R*)-*N*-[2-(Pyridin-3-ylmethyl)-1-azabicyclo[2.2.2]oct-3-yl]benzo[*b*]furan-2-carboxamide (TC-5619), a Selective $\alpha 7$ Nicotinic Acetylcholine Receptor Agonist, for the Treatment of Cognitive Disorders. *J. Med. Chem.* **55**, 9793–9809 (2012).
55. Mazzaferro, S. *et al.* Non-equivalent ligand selectivity of agonist sites in ($\alpha 4\beta 2$) $2\alpha 4$ nicotinic acetylcholine receptors: A key determinant of agonist efficacy. *J. Biol. Chem.* **289**, 21795–21806 (2014).
56. Millar, N. S. & Gotti, C. Diversity of vertebrate nicotinic acetylcholine receptors. *Neuropharmacology* **56**, 237–246 (2009).
57. Nemezc, Á., Prevost, M. S., Menny, A. & Corringer, P. J. Emerging Molecular Mechanisms of Signal Transduction in Pentameric Ligand-Gated Ion Channels. *Neuron* **90**, 452–470 (2016).
58. Nemezc, Á. & Taylor, P. Creating an $\alpha 7$ nicotinic acetylcholine recognition domain from the acetylcholine-binding protein: Crystallographic and ligand selectivity analyses. *J. Biol. Chem.* **286**, 42555–42565 (2011).
59. Nemethova, A., Michel, K., Gomez-Pinilla, P. J., Boeckxstaens, G. E. & Schemann, M. Nicotine attenuates activation of tissue resident macrophages in the mouse stomach through the $\beta 2$ nicotinic acetylcholine receptor. *PLoS One* **8**, 1–10 (2013).
60. Ng, H. J. *et al.* Nootropic alpha7 nicotinic receptor allosteric modulator derived from GABAA receptor modulators. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 8059–64

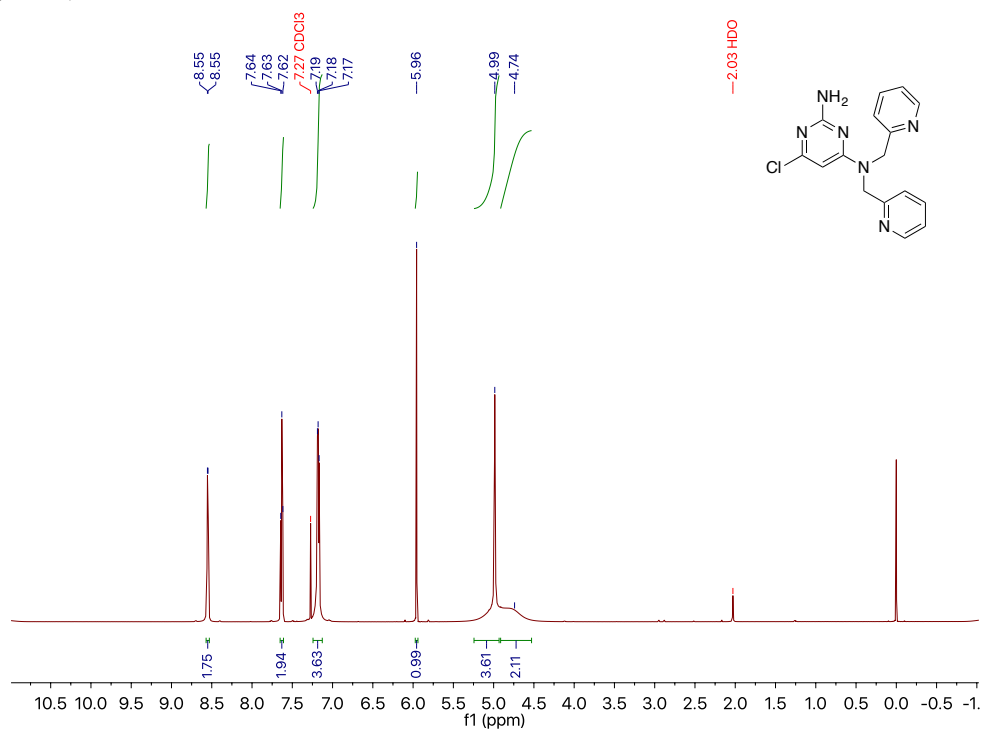
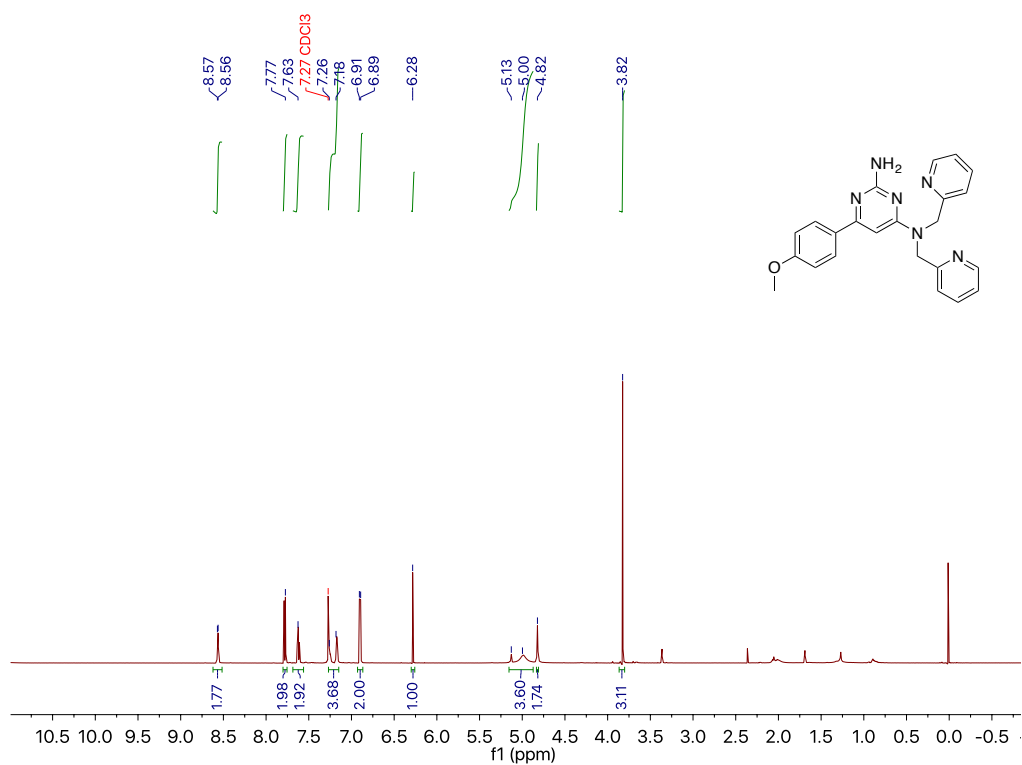
- (2007).
61. Nguyen, Q. T. *et al.* An in vivo biosensor for neurotransmitter release and in situ receptor activity. *Nat. Neurosci.* **13**, 127–132 (2010).
 62. Olincy, A. *et al.* Proof-of-concept trial of an $\alpha 7$ nicotinic agonist in schizophrenia. *Arch. Gen. Psychiatry* **63**, 630–638 (2006).
 63. Oz, M., Lorke, D. E., Yang, K. H. & Petroianu, G. On the interaction of beta-amyloid peptides and $\alpha 7$ -nicotinic acetylcholine receptors in Alzheimer's disease. *Curr. alzheimer Res.* **10**, 618–630 (2018).
 64. Papke, R. L. & Porter Papke, J. K. Comparative pharmacology of rat and human $\alpha 7$ nAChR conducted with net charge analysis. *Br. J. Pharmacol.* **137**, 49–61 (2002).
 65. Papke, R. L. & Stokes, C. Working with OpusXpress: Methods for high volume oocyte experiments. *Methods* **51**, 121–133 (2010).
 66. Papke, R. L., Stokes, C., Williams, D. K., Wang, J. & Horenstein, N. A. Cysteine accessibility analysis of the human $\alpha 7$ nicotinic acetylcholine receptor ligand-binding domain identifies L119 as a gatekeeper. *Neuropharmacology* **60**, 159–171 (2011).
 67. Pearlson, G. D. & Ford, J. M. Distinguishing between schizophrenia and other psychotic disorders. *Schizophr. Bull.* **40**, 501–503 (2014).
 68. Pettit, D. L., Shao, Z. & Yakel, J. L. β -Amyloid(1-42) peptide directly modulates nicotinic receptors in the rat hippocampal slice. *J. Neurosci.* **21**, NIL_10-NIL_14 (2001).
 69. Potterton, E., Briggs, P., Turkenburg, M. & Dodson, E. A graphical user interface to the CCP4 program suite. *Acta Crystallogr. - Sect. D Biol. Crystallogr.* **59**, 1131–1137 (2003).
 70. Prickaerts, J. *et al.* EVP-6124, a novel and selective $\alpha 7$ nicotinic acetylcholine receptor partial agonist, improves memory performance by potentiating the acetylcholine response of $\alpha 7$ nicotinic acetylcholine receptors. *Neuropharmacology* **62**, 1099–1110 (2012).
 71. Reitz, C., Brayne, C. & Mayeux, R. Genetic Epidemiology of Alzheimer Disease. *Nat. Rev. Neurol.* **7**, 137–152 (2011).
 72. Rezvani, A. H. *et al.* Effect of R3487/MEM3454, a novel nicotinic $\alpha 7$ receptor partial agonist and 5-HT3 antagonist on sustained attention in rats. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **33**, 269–275 (2009).

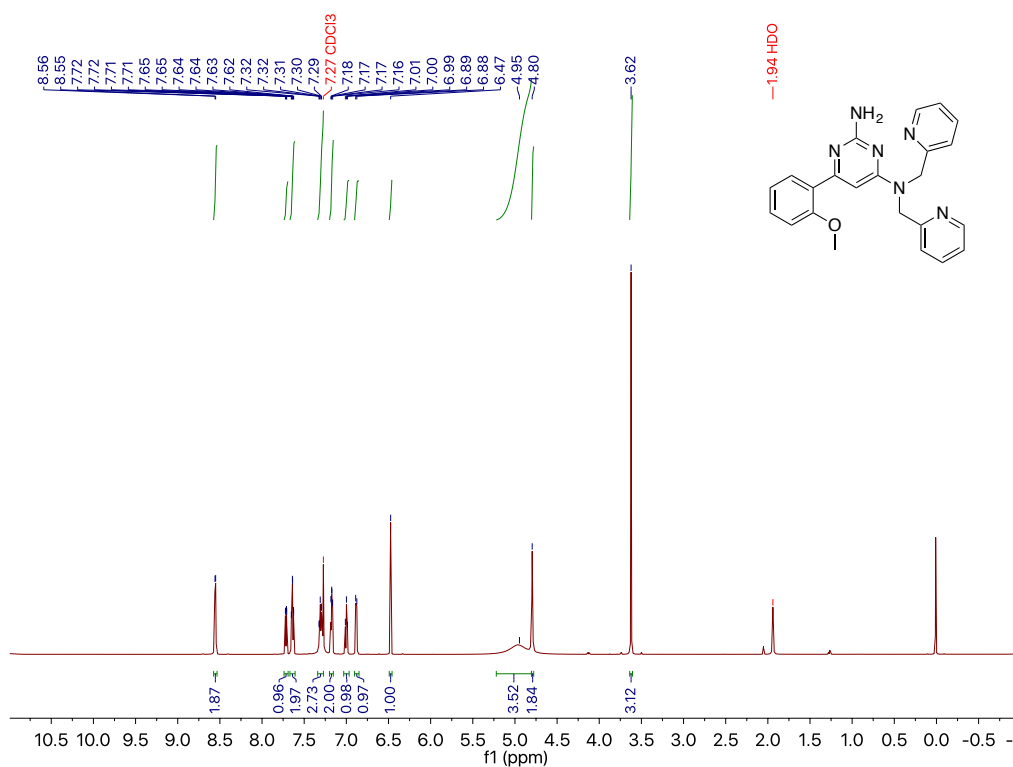
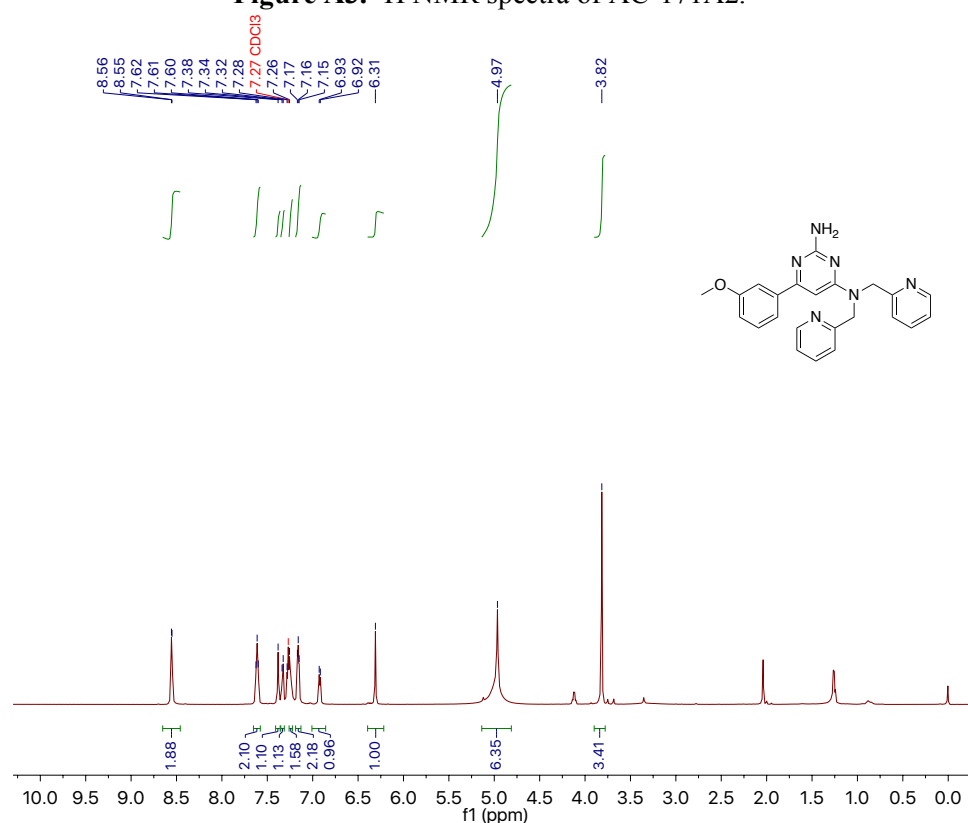
73. Rosas-Ballina, M. *et al.* The selective $\alpha 7$ agonist GTS-21 attenuates cytokine production in human whole blood and human monocytes activated by ligands for TLR2, TLR3, TLR4, TLR9, and RAGE. *Mol. Med.* **15**, 195–202 (2009).
74. Russo, P., Cardinale, A. & Shuller, H. A New “ Era ” for the 7-nAChR. 721–725 (2012).
75. Schliebs, R. & Arendt, T. The cholinergic system in aging and neuronal degeneration. *Behav. Brain Res.* **221**, 555–563 (2011).
76. Sekar, R. B. & Periasamy, A. Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations. *J. Cell Biol.* **160**, 629–633 (2003).
77. Shan, J. C. *et al.* A Diagnostic Model Incorporating P50 Sensory Gating and Neuropsychological Tests for Schizophrenia. *PLoS One* **8**, 1–7 (2013).
78. Smart, T. G. & Paoletti, P. Synaptic neurotransmitter-gated receptors. *Cold Spring Harb. Perspect. Biol.* **4**, (2012).
79. Smit, A. B. *et al.* A glia-derived acetylcholine-binding protein that modulates synaptic transmission. *Nature* **411**, 261–268 (2001).
80. Spires-Jones, T. L. & Hyman, B. T. The Intersection of Amyloid Beta and Tau at Synapses in Alzheimer’s Disease. *Neuron* **82**, 756–771 (2014).
81. Talley, T. T. *et al.* Spectroscopic analysis of benzylidene anabaseine complexes with acetylcholine binding proteins as models for ligand-nicotinic receptor interactions. *Biochemistry* **45**, 8894–8902 (2006).
82. Taly, A. & Charon, S. $\alpha 7$ Nicotinic Acetylcholine Receptors: A Therapeutic Target in the Structure Era. *Curr. Drug Targets* **13**, 695–706 (2012).
83. Taly, A., Corringer, P. J., Guedin, D., Lestage, P. & Changeux, J. P. Nicotinic receptors: Allosteric transitions and therapeutic targets in the nervous system. *Nat. Rev. Drug Discov.* **8**, 733–750 (2009).
84. Thompson, A. J., Lester, H. A. & Lummis, S. C. R. *The structural basis of function in Cys-loop receptors. Quarterly Reviews of Biophysics* **43**, (2010).
85. Turner, J. R. Nicotinic Cholinergic Receptors in the Rat Cerebellum: Multiple Heteromeric Subtypes. *J. Neurosci.* **25**, 9258–9265 (2005).
86. Unwin, N. Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *J. Mol. Biol.* **346**, 967–989 (2005).
87. Vallés, A. S., Borroni, M. V. & Barrantes, F. J. Targeting brain $\alpha 7$ nicotinic acetylcholine receptors in alzheimer’s disease: Rationale and current status. *CNS*

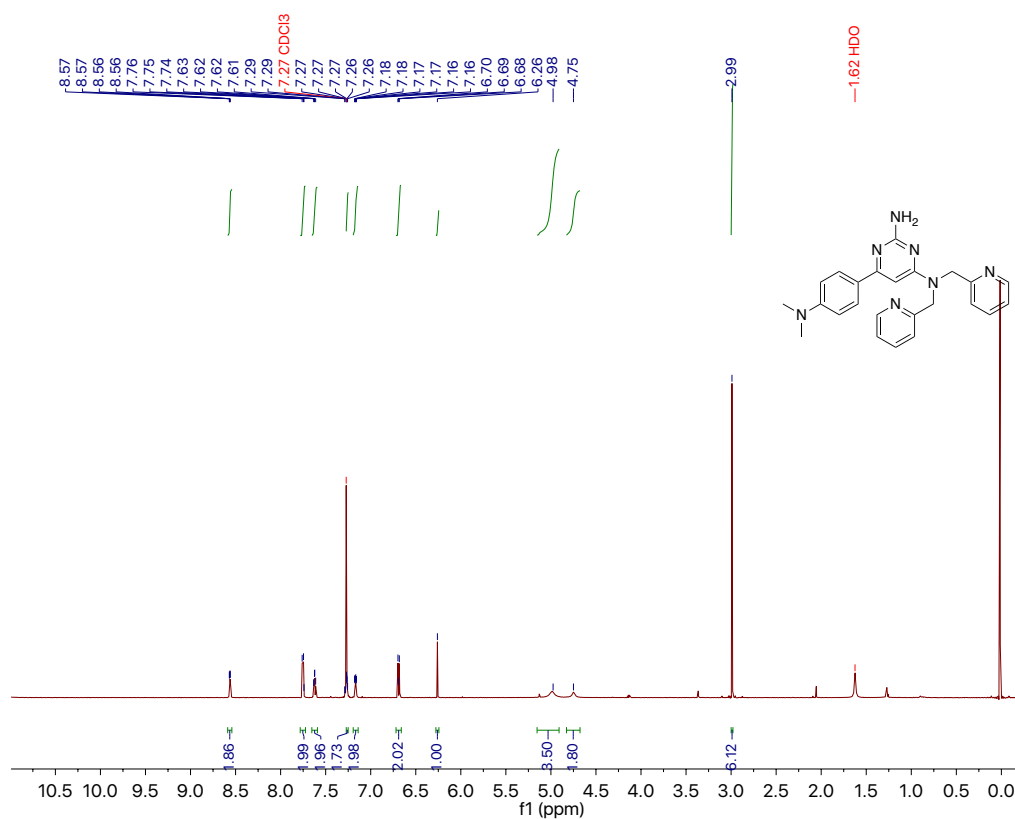
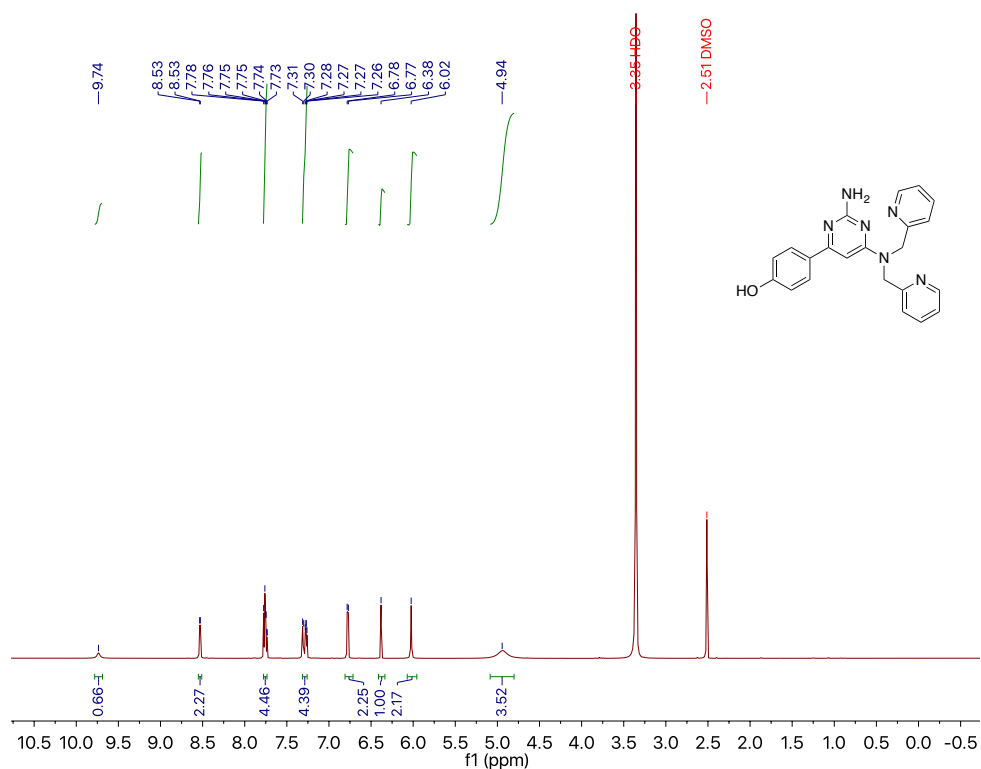
- Drugs* **28**, 975–987 (2014).
88. Wallace, T. L. *et al.* RG3487, a Novel Nicotinic $\alpha 7$ Receptor Partial Agonist, Improves Cognition and Sensorimotor Gating in Rodents. *J. Pharmacol. Exp. Ther.* **336**, 242–253 (2011).
89. Wan, C.-Q., Chen, X.-D. & Mak, T. C. W. Supramolecular frameworks assembled via intermolecular lone pair-aromatic interaction between carbonyl and pyridyl groups. *CrystEngComm* **10**, 475 (2008).
90. Wang, H. Y., Lee, D. H., Davis, C. B. & Shank, R. P. Amyloid peptide Abeta(1-42) binds selectively and with picomolar affinity to $\alpha 7$ nicotinic acetylcholine receptors. *J. Neurochem.* **75**, 1155–61 (2000).
91. Wang, J., Papke, R. L., Stokes, C. & Horenstein, N. A. Potential state-selective hydrogen bond formation can modulate activation and desensitization of the $\alpha 7$ nicotinic acetylcholine receptor. *J. Biol. Chem.* **287**, 21957–21969 (2012).
92. Williams, J. M. *et al.* Shorter interpuff interval is associated with higher nicotine intake in smokers with schizophrenia. *Drug Alcohol Depend.* **118**, 313–319 (2011).
93. Winterer, G. *et al.* Allosteric $\alpha 7$ nicotinic receptor modulation and P50 sensory gating in schizophrenia: A proof-of-mechanism study. *Neuropharmacology* **64**, 197–204 (2013).
94. Wonnacott, S., Barik, J., Dickinson, J. & Jones, I. W. Nicotinic receptors modulate transmitter cross talk in the CNS: nicotinic modulation of transmitters. *J. Mol. Neurosci.* **30**, 137–140 (2006).
95. Wu, X. F., Anbarasan, P., Neumann, H. & Beller, M. From noble metal to Nobel Prize: Palladium-catalyzed coupling reactions as key methods in organic synthesis. *Angew. Chemie - Int. Ed.* **49**, 9047–9050 (2010).
96. Xiu, X., Puskar, N. L., Shanata, J. A. P., Lester, H. A. & Dougherty, D. A. Nicotine binding to brain receptors requires a strong cation- interaction. *Nature* **458**, 534–537 (2009).
97. Yamada-Nomoto, K. *et al.* $\alpha 7$ nicotinic acetylcholine receptor (nAChR) agonist inhibits the development of endometriosis by regulating inflammation. *Am. J. Reprod. Immunol.* **76**, 491–498 (2016).
98. Yamauchi, J. G. *et al.* Synthesis of Selective Agonists for the $\alpha 7$ Nicotinic Acetylcholine Receptor with In Situ Click-Chemistry on Acetylcholine-Binding Protein Templates. *Mol. Pharmacol.* **82**, 687–699 (2012).
99. Yamauchi, J. G. *et al.* Characterizing Ligand-Gated Ion Channel Receptors with

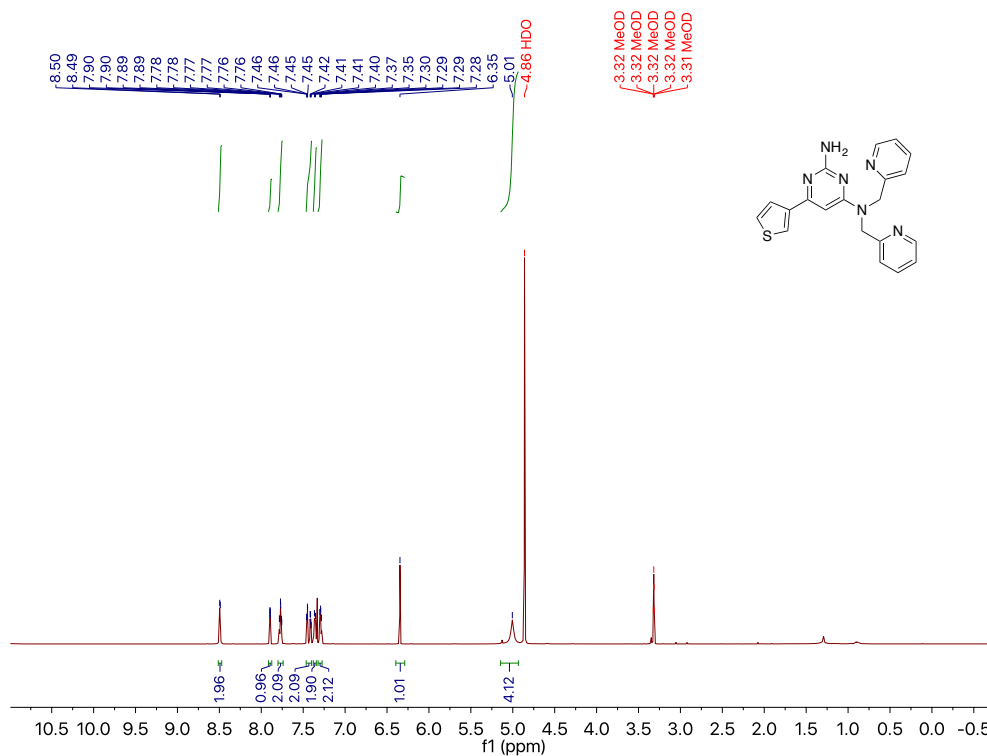
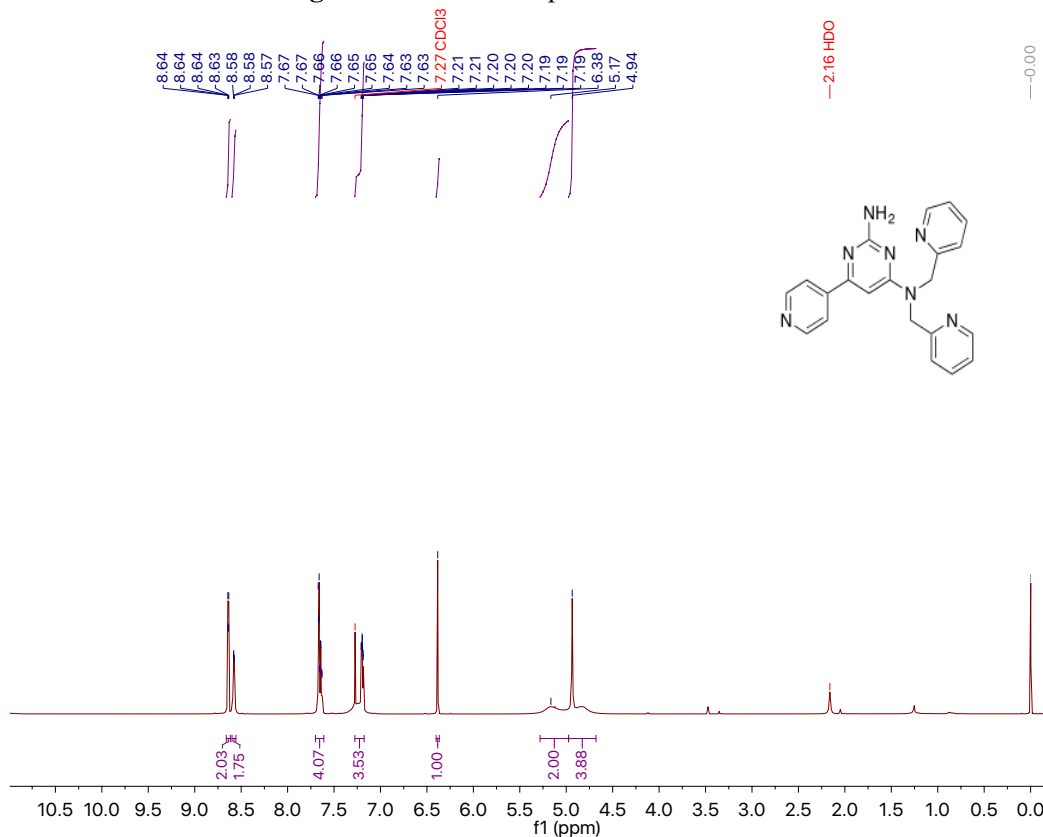
- Genetically Encoded Ca^{++} Sensors. *PLoS One* **6**, e16519 (2011).
100. Young, G. T., Zwart, R., Walker, A. S., Sher, E. & Millar, N. S. Potentiation of alpha $\alpha 7$ nicotinic acetylcholine receptors via an allosteric transmembrane site. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 14686–14691 (2008).
 101. Zakrzewicz, A. *et al.* Canonical and Novel Non-Canonical Cholinergic Agonists Inhibit ATP-Induced Release of Monocytic Interleukin-1 β via Different Combinations of Nicotinic Acetylcholine Receptor Subunits $\alpha 7$, $\alpha 9$ and $\alpha 10$. *Front. Cell. Neurosci.* **11**, 1–16 (2017).
 102. Zhang, X. Y. *et al.* Short-term tropisetron treatment and cognitive and p50 auditory gating deficits in schizophrenia. *Am. J. Psychiatry* **169**, 974–981 (2012).
 103. Zhong, W. From ab initio quantum mechanics to molecular neurobiology: A cation – binding site in the nicotinic receptor. *Proc. Natl. Acad. Sci.* **95**, 12088–12093 (1998).

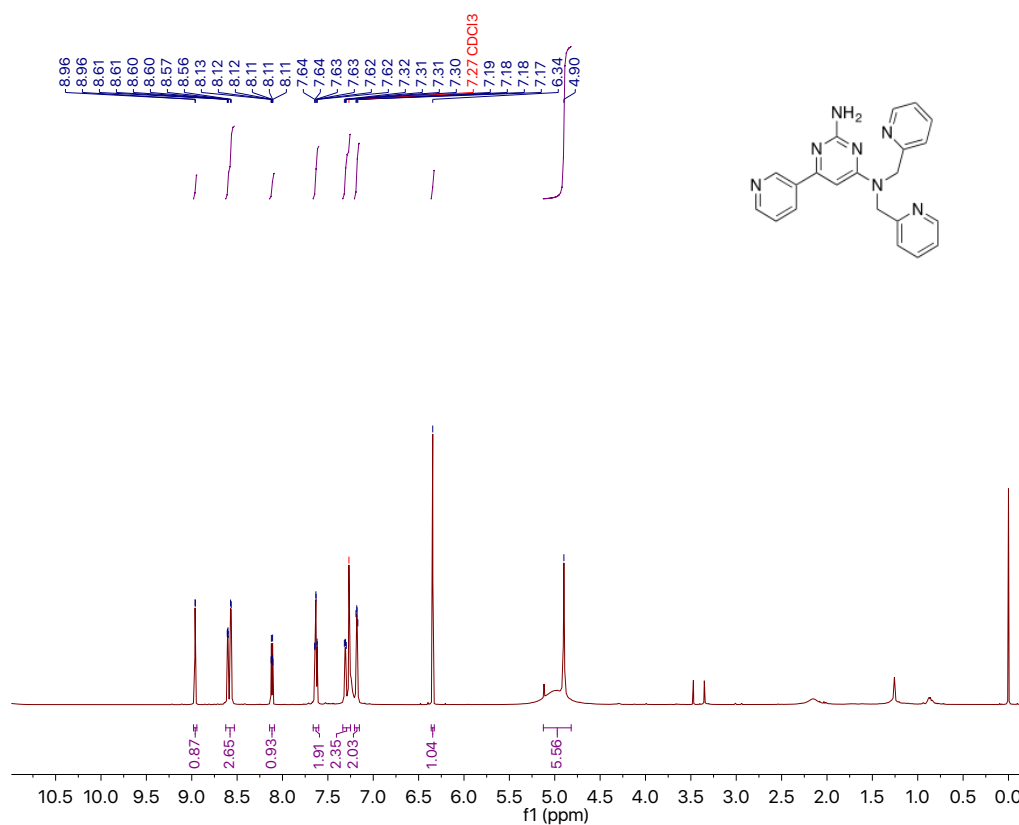
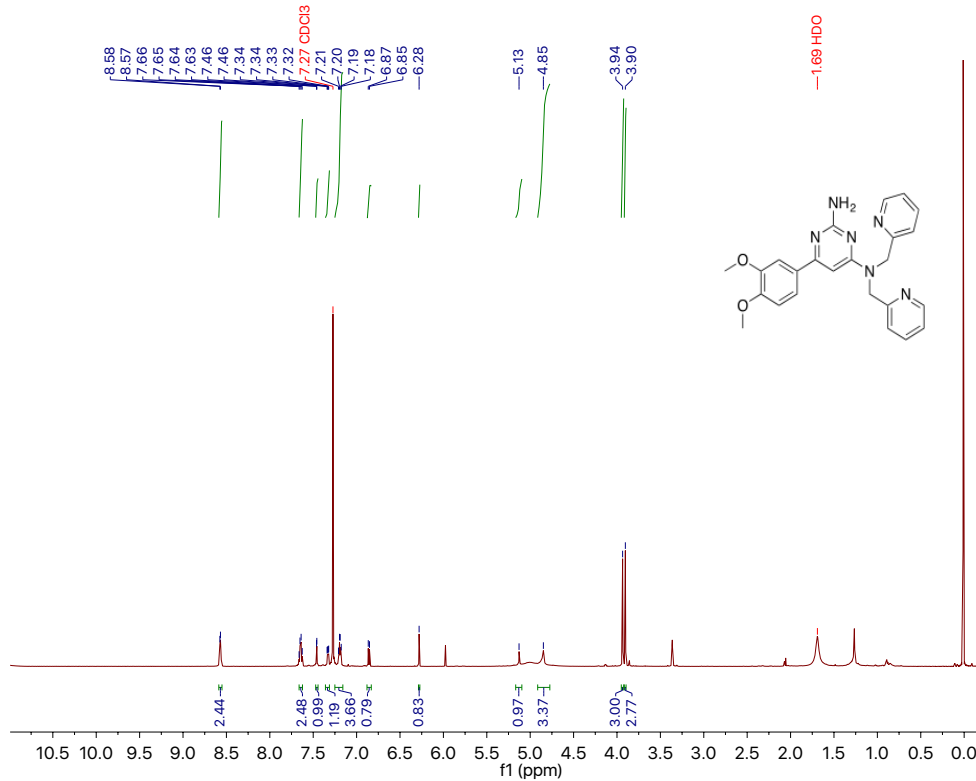
**APPENDIX. ^1H AND ^{13}C APT NMR AND HIGH-RESOLUTION MASS
SPECTROMETRY SPECTRA**

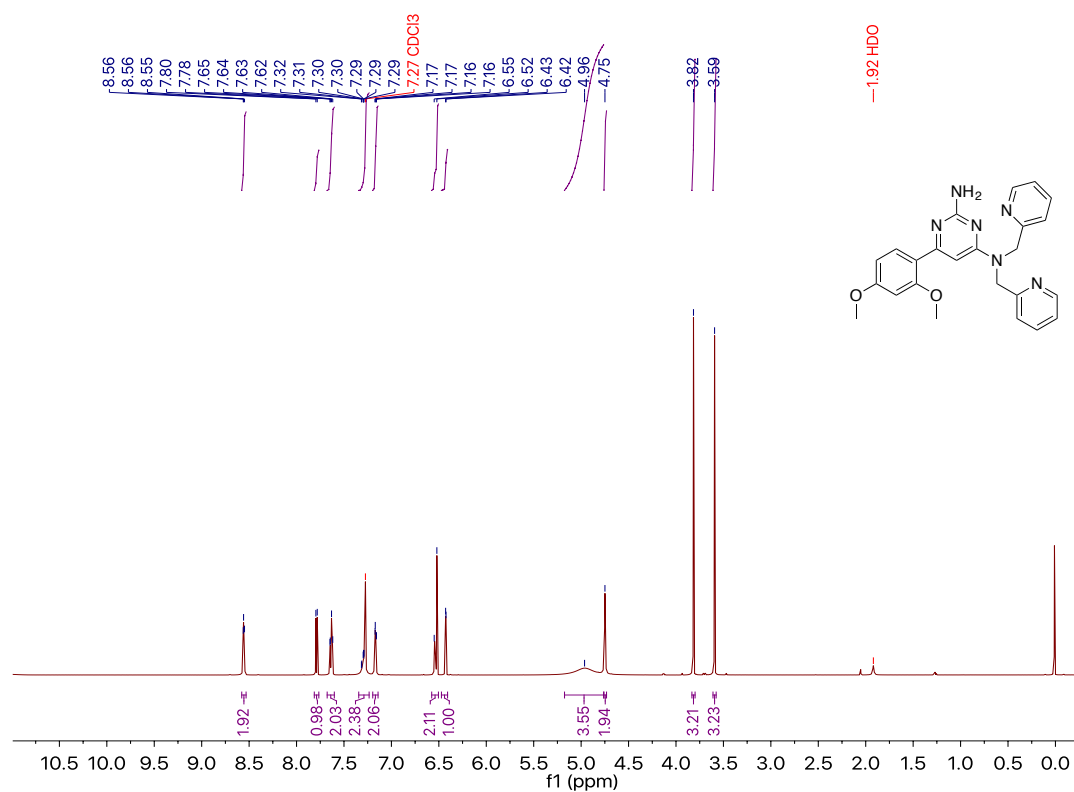
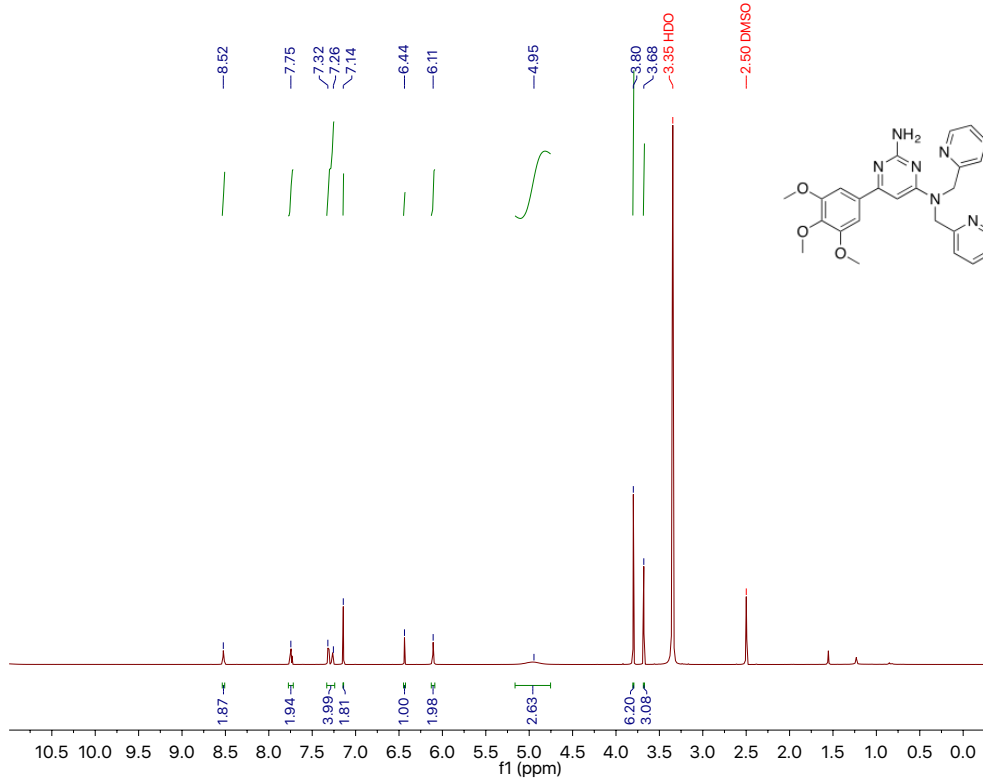
AP 1. ^1H NMRFigure A1. ^1H NMR spectra of AC-17.Figure A2. ^1H NMR spectra of AC-171A.

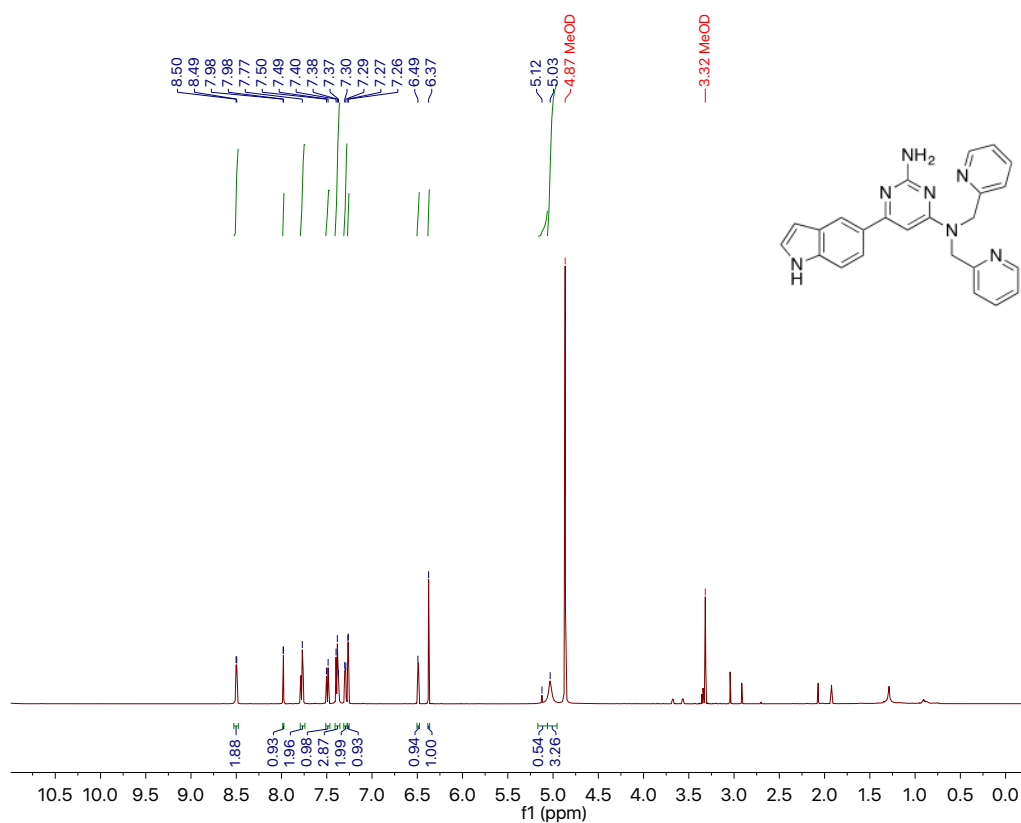
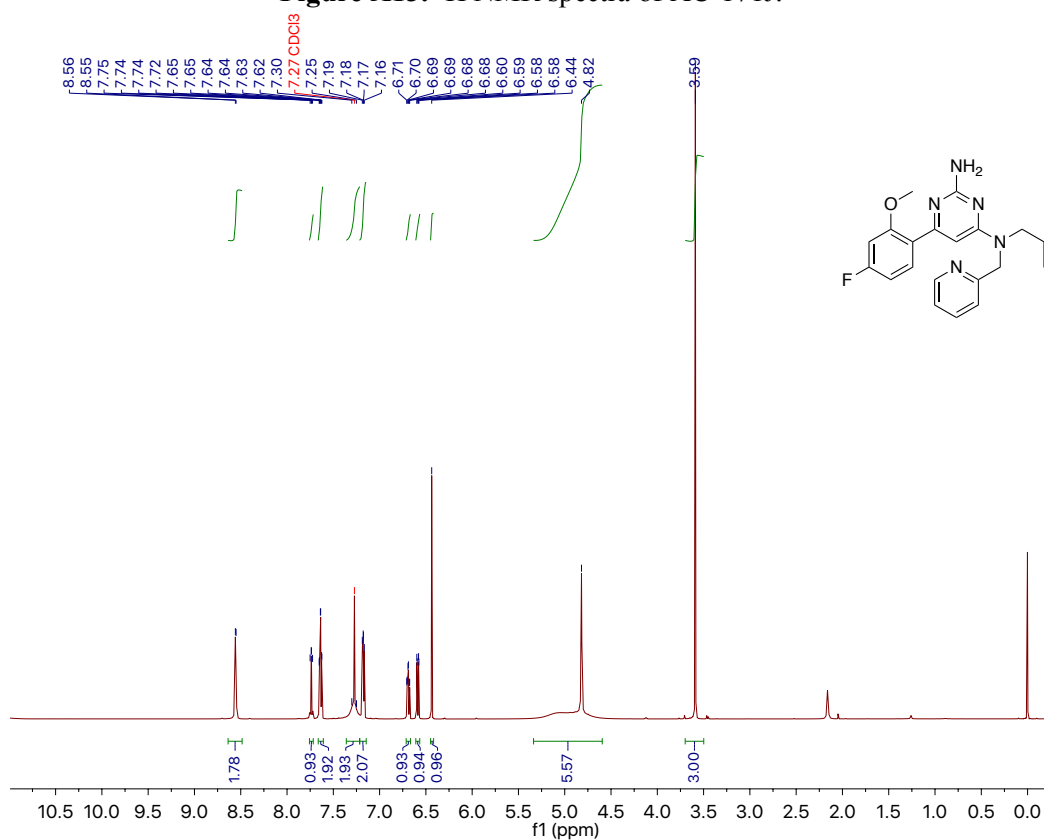
Figure A3. ^1H NMR spectra of AC-171A2.Figure A4. ^1H NMR spectra of AC-171A3.

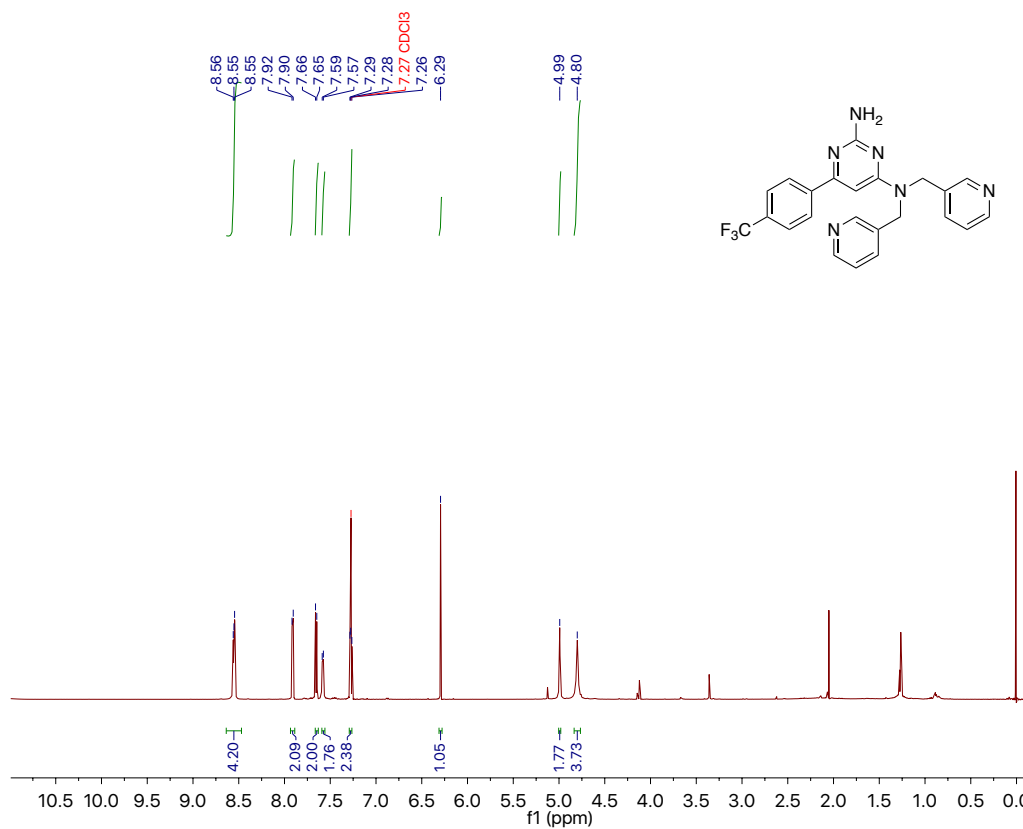
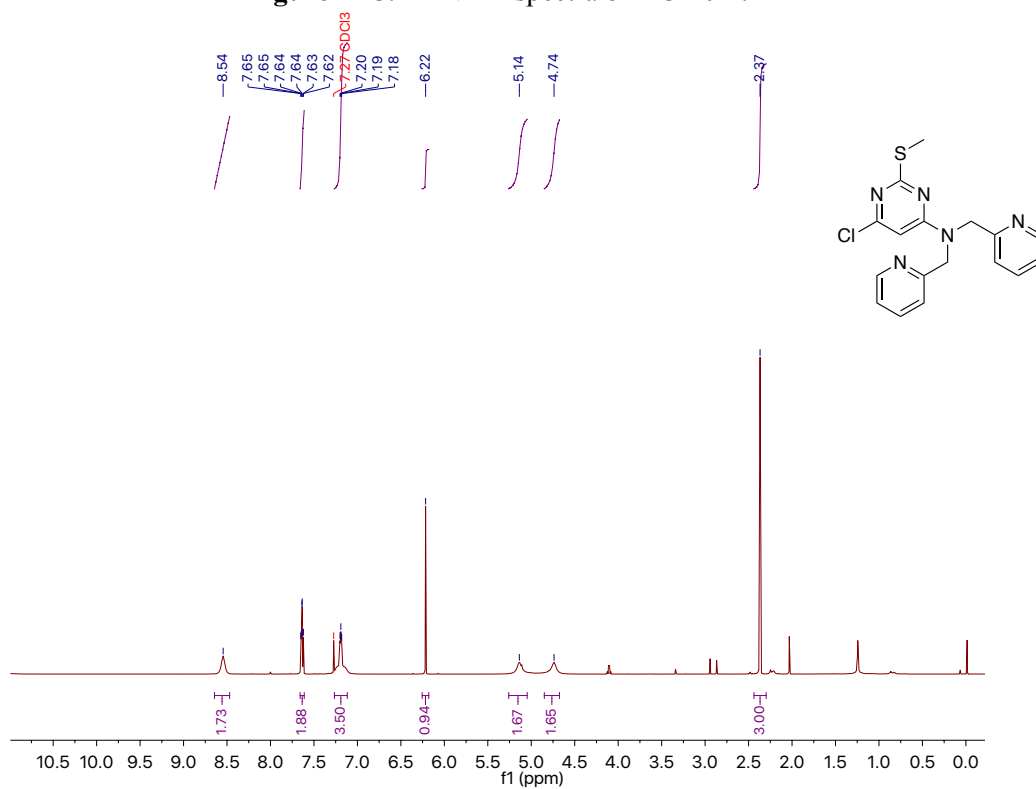
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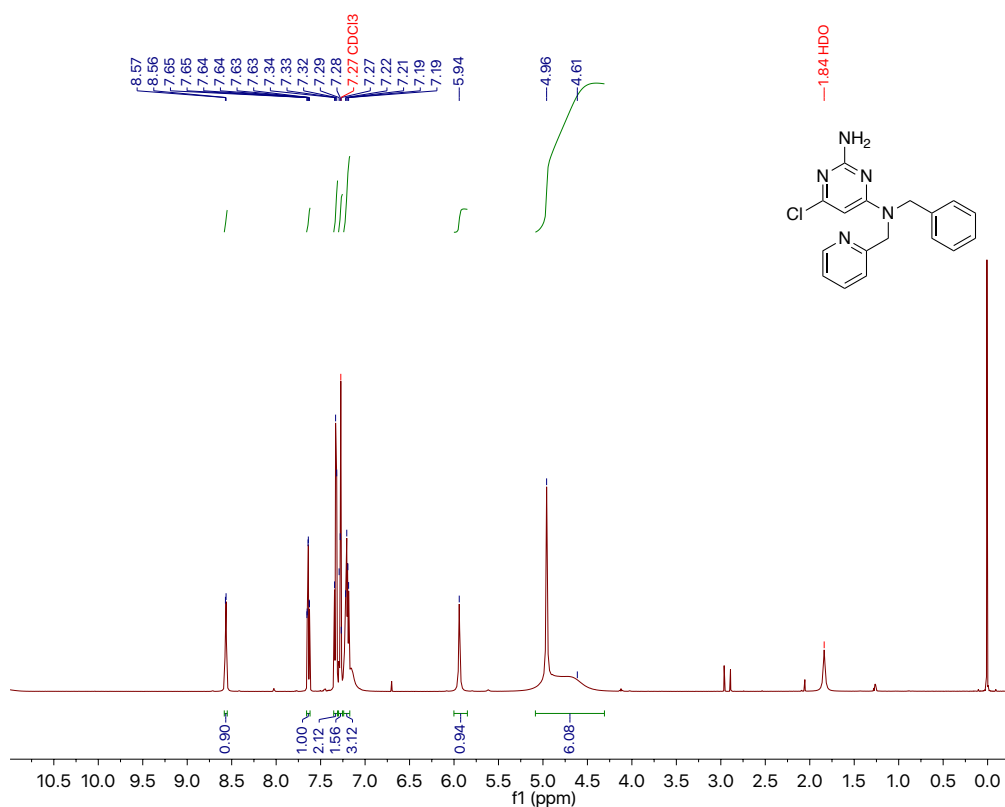
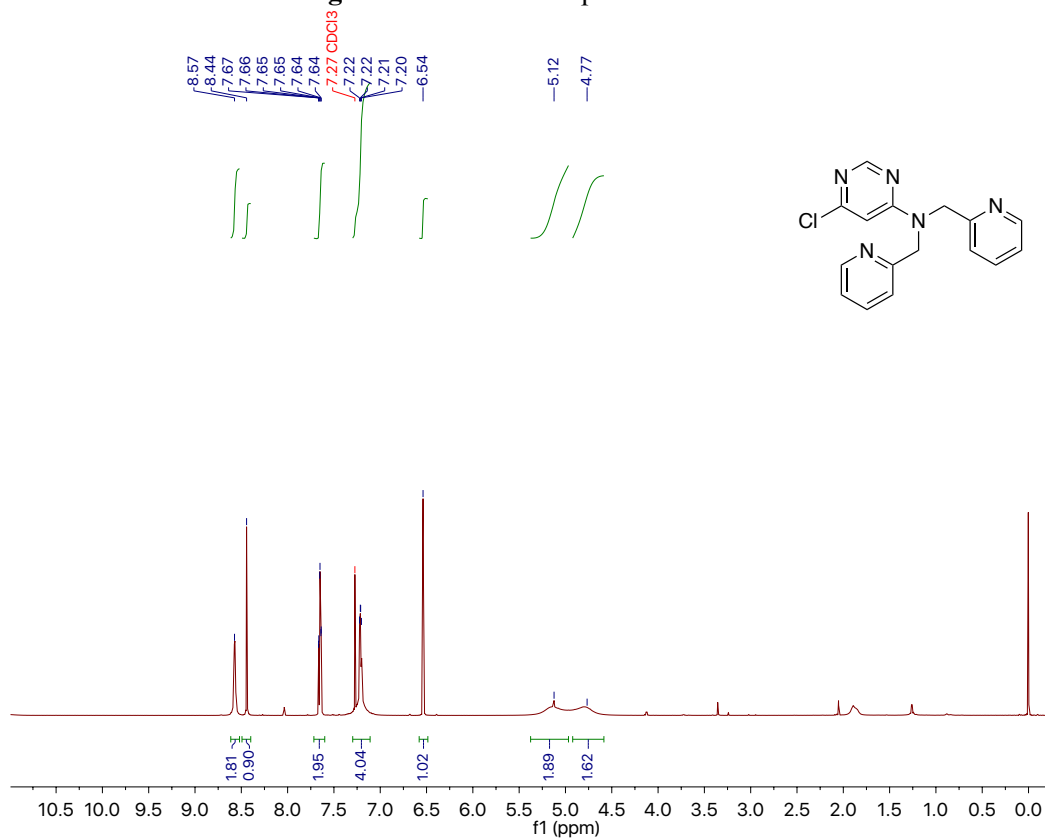
Figure A7. ^1H NMR spectra of AC-171D.Figure A8. ^1H NMR spectra of AC-171F.

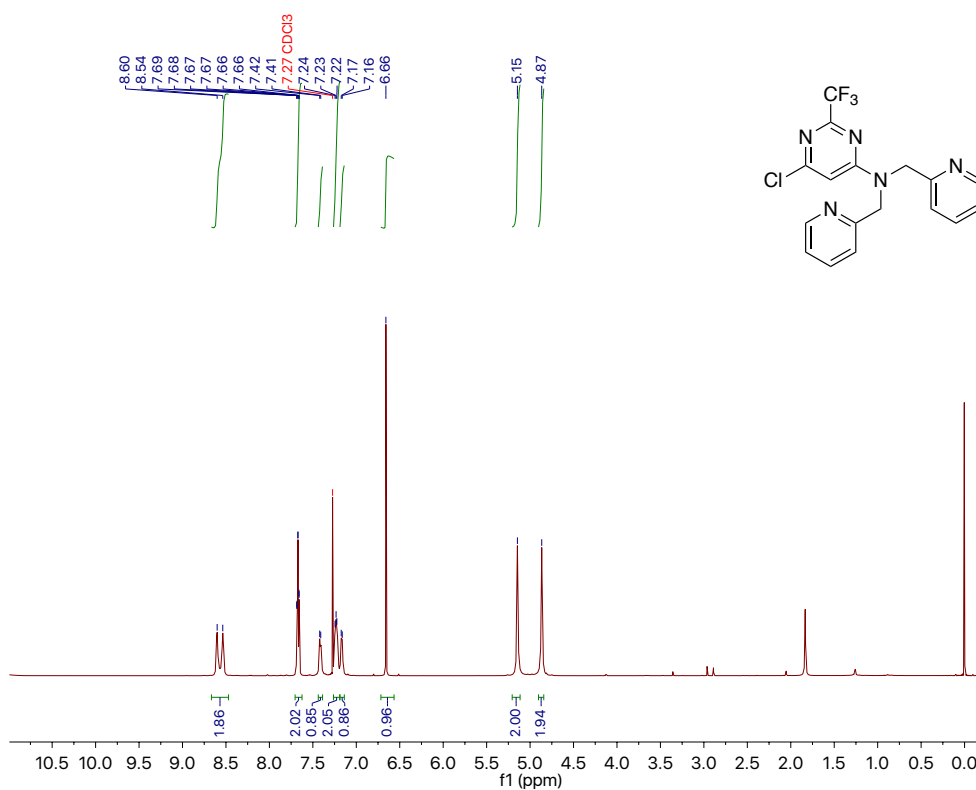
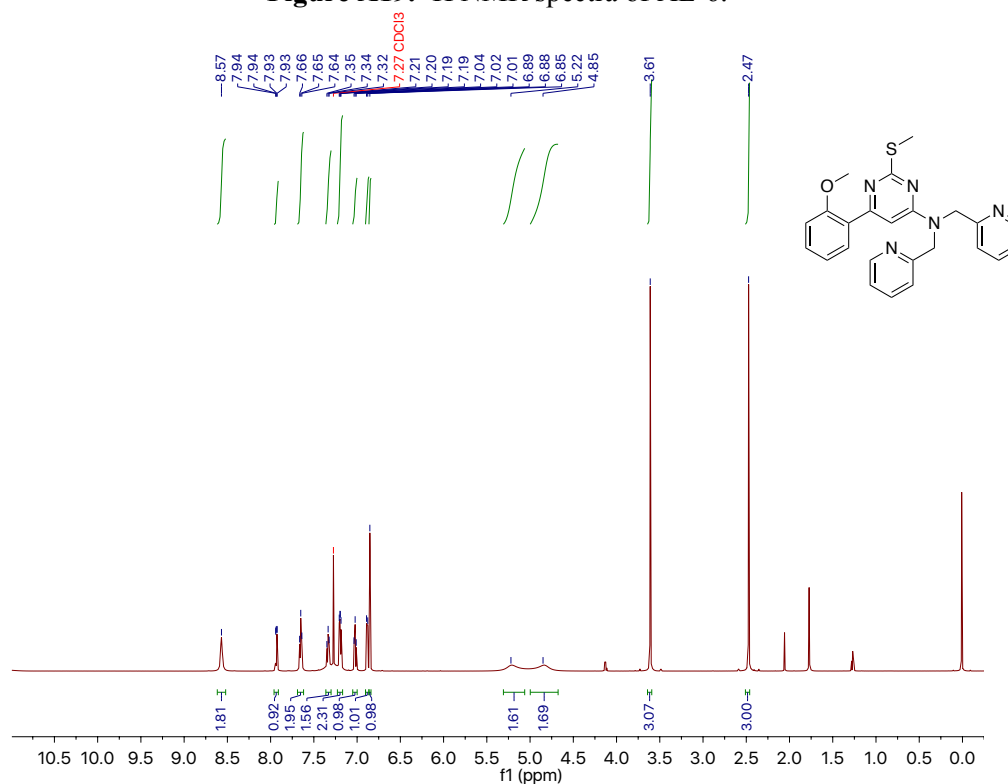
Figure A9. ^1H NMR spectra of AC-171F3.Figure A10. ^1H NMR spectra of AC-171G.

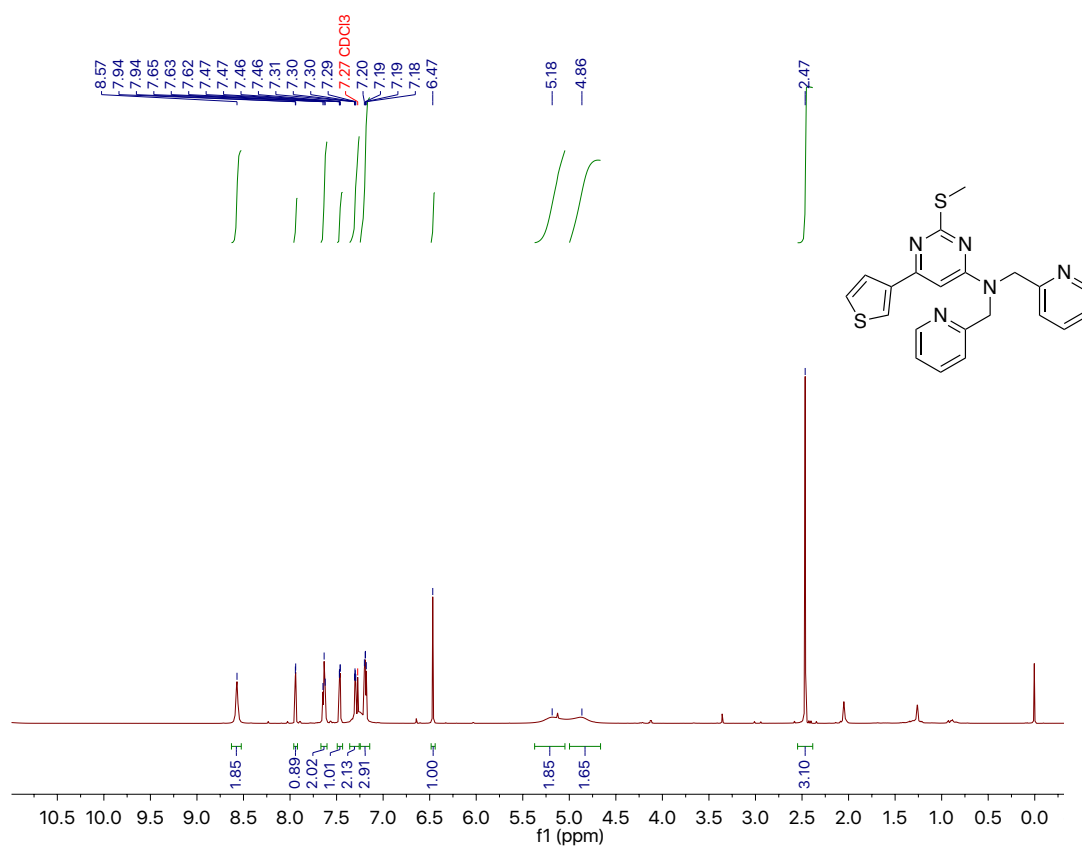
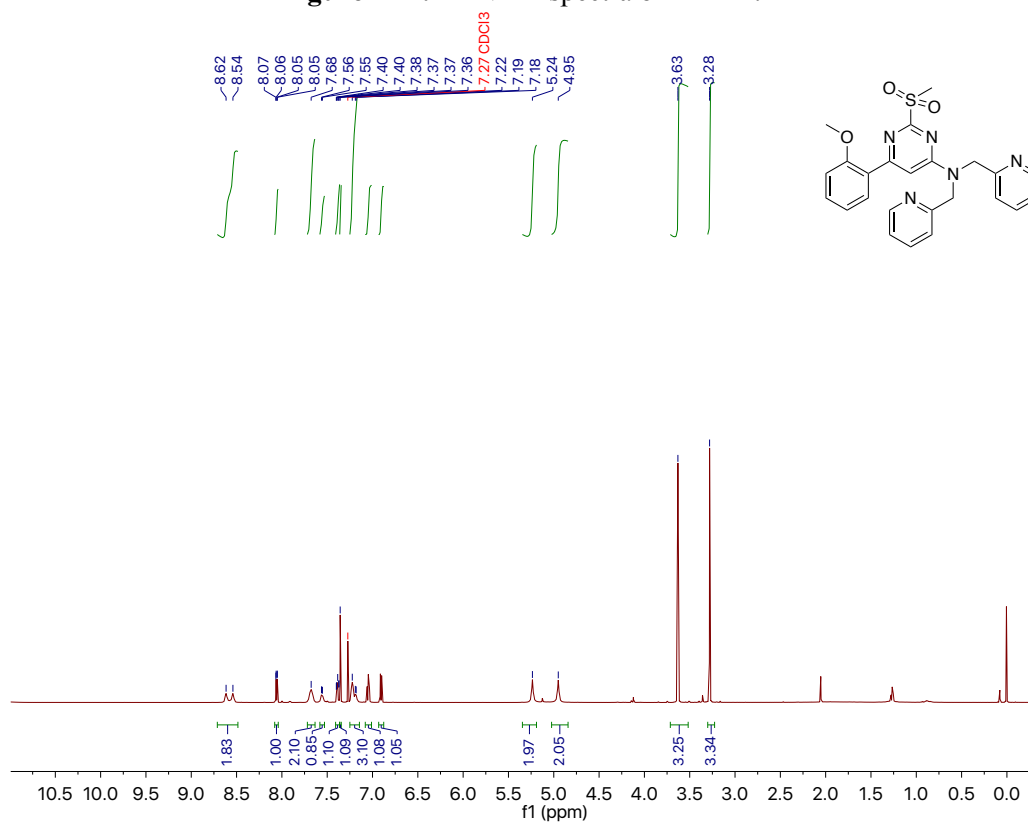
Figure A11. ^1H NMR spectra of AC-171H.Figure A12. ^1H NMR spectra of AC-171I.

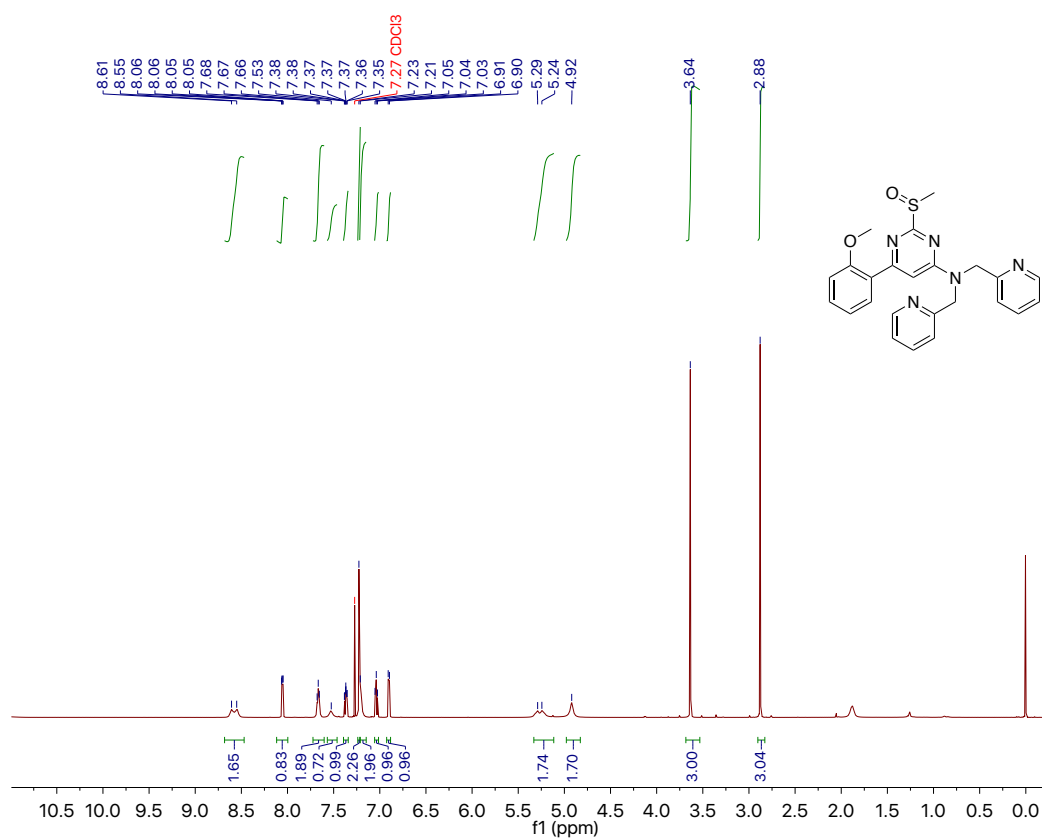
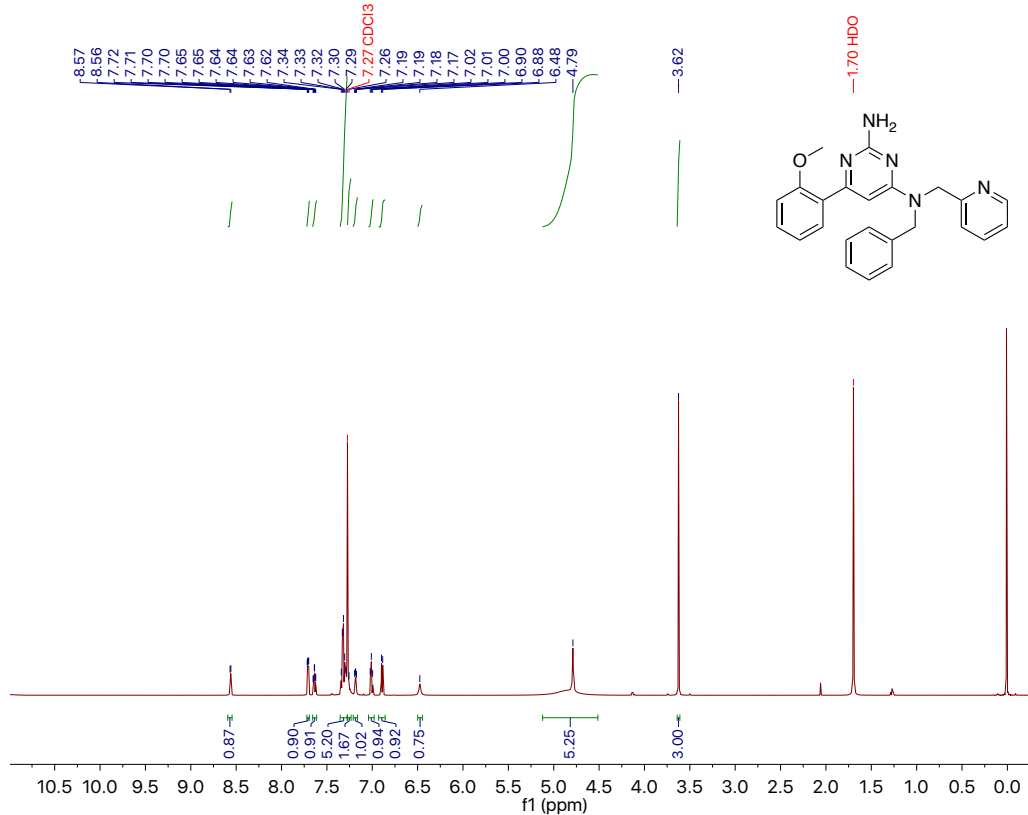
Figure A13. ^1H NMR spectra of AC-171J.Figure A14. ^1H NMR spectra of AC-171M.

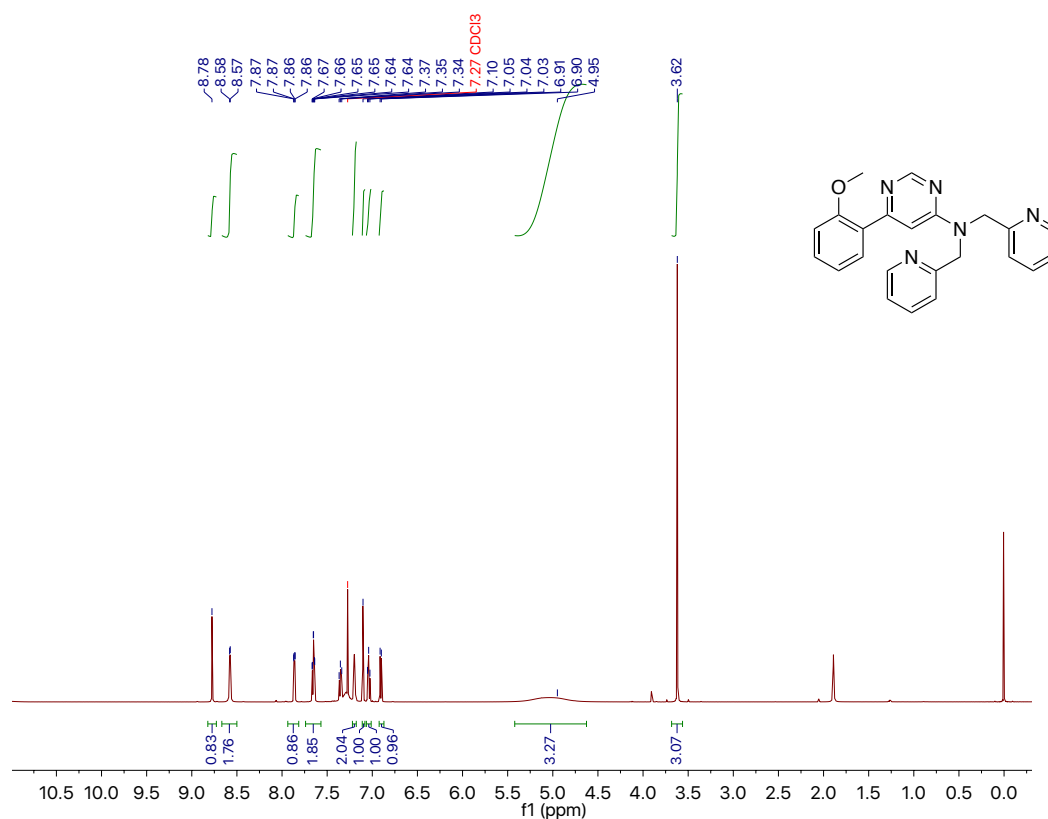
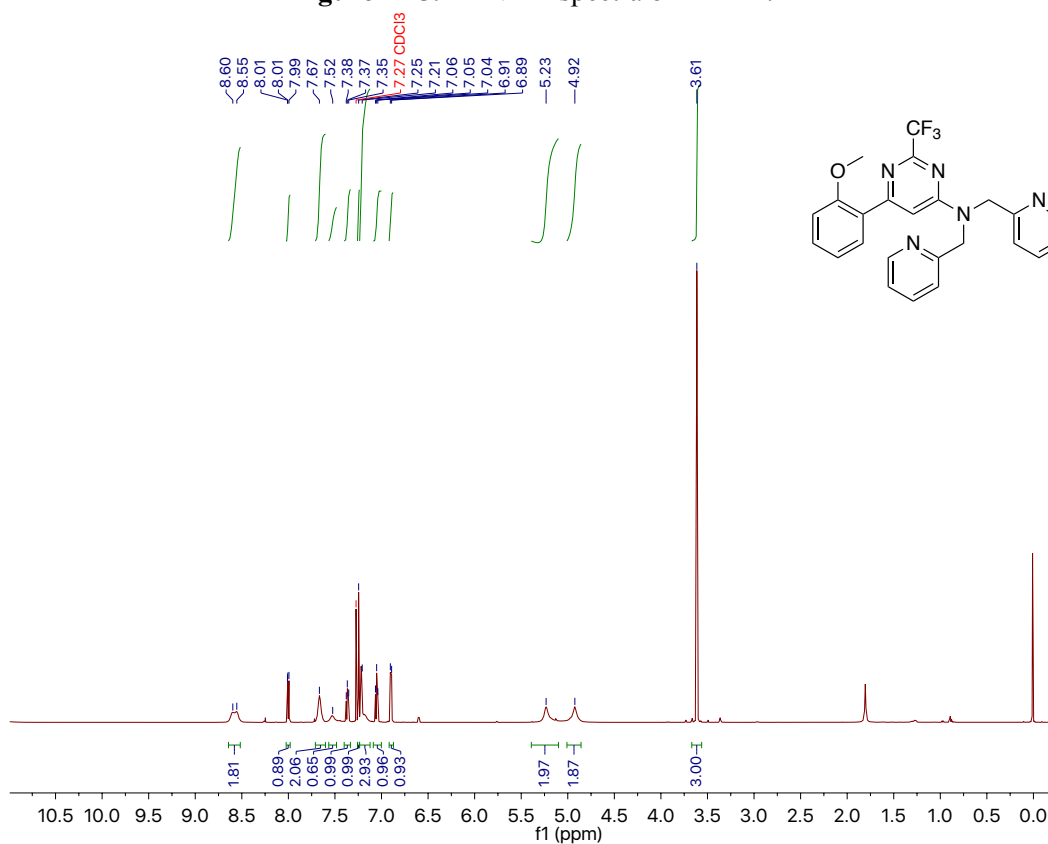
Figure A15. ^1H NMR spectra of AC-20-2.Figure A16. ^1H NMR spectra of AL-1.

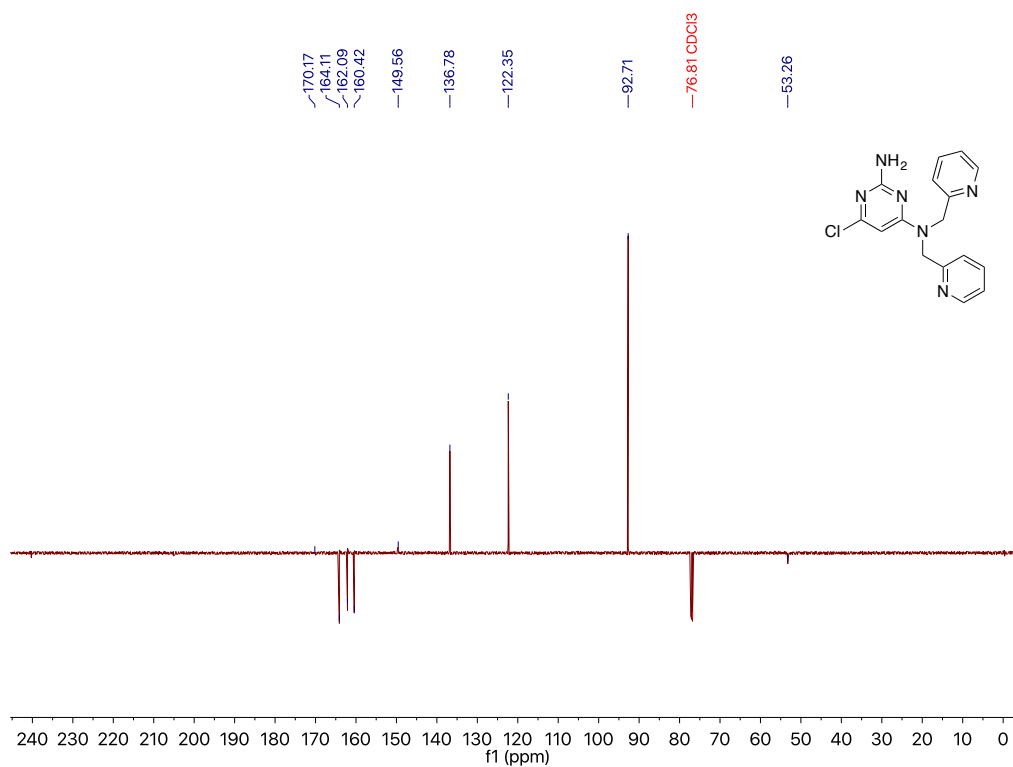
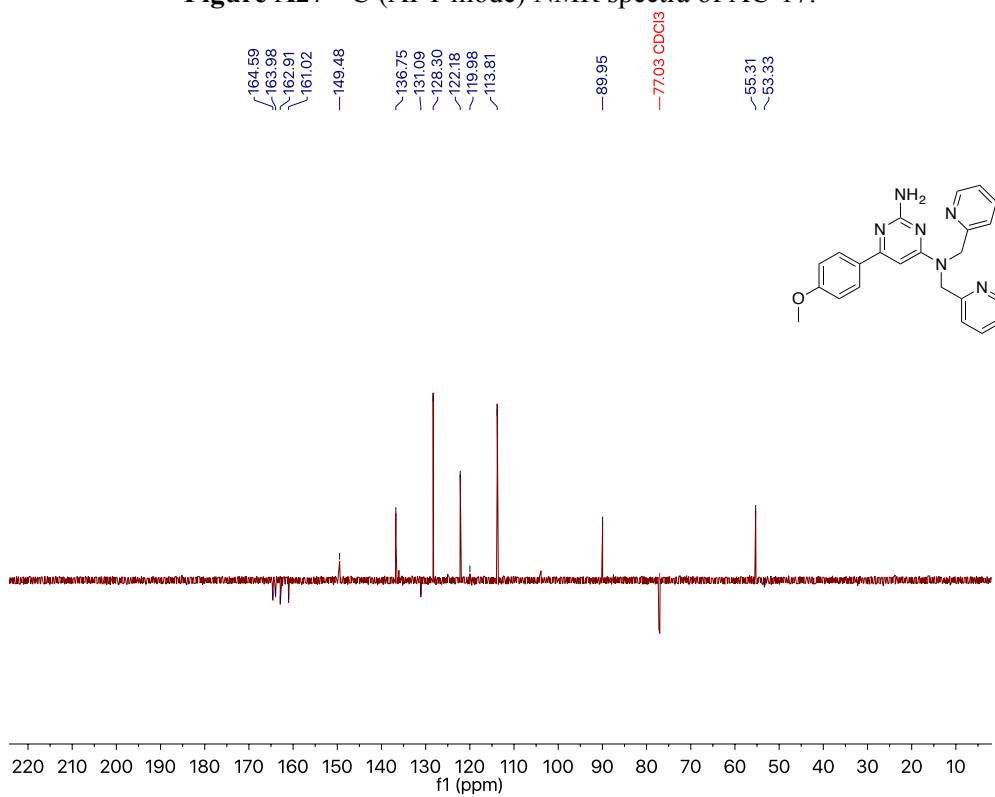
Figure A17. ^1H NMR spectra of AL-2.Figure A18. ^1H NMR spectra of AL-4.

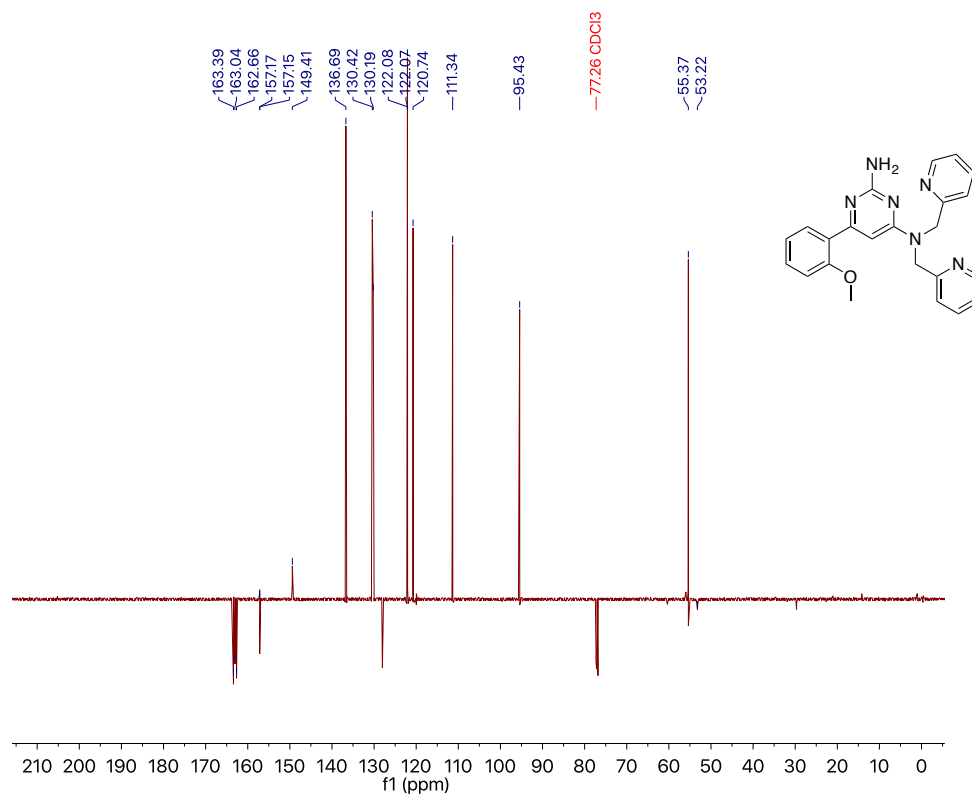
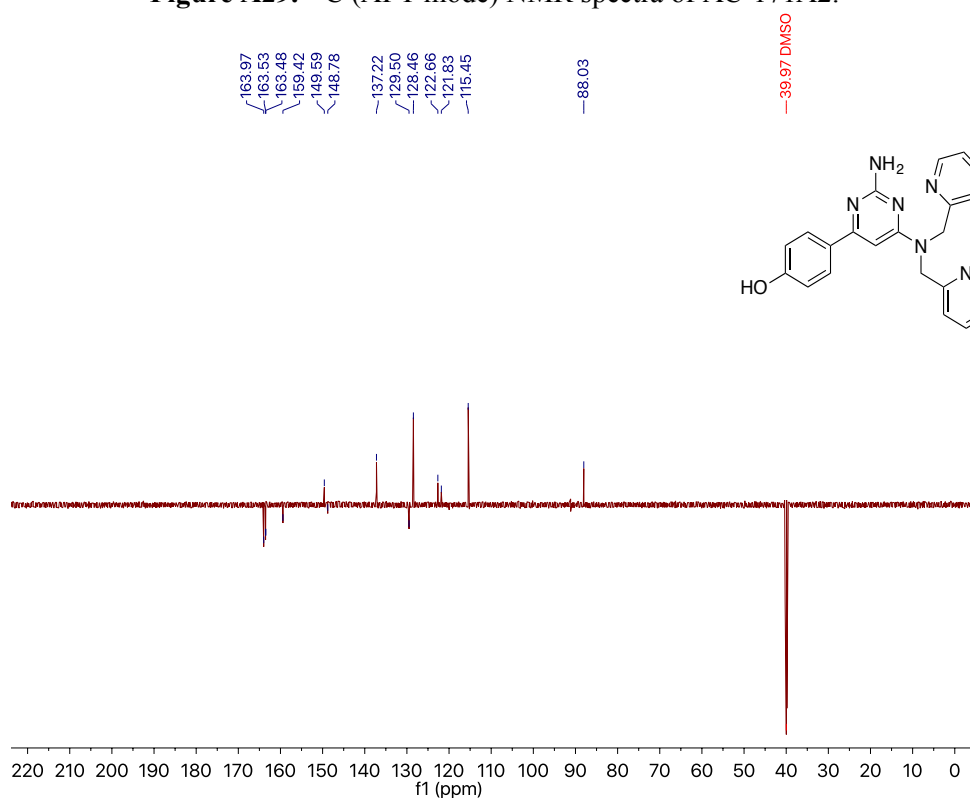
Figure A19. ^1H NMR spectra of AL-6.Figure A20. ^1H NMR spectra of AL-1A.

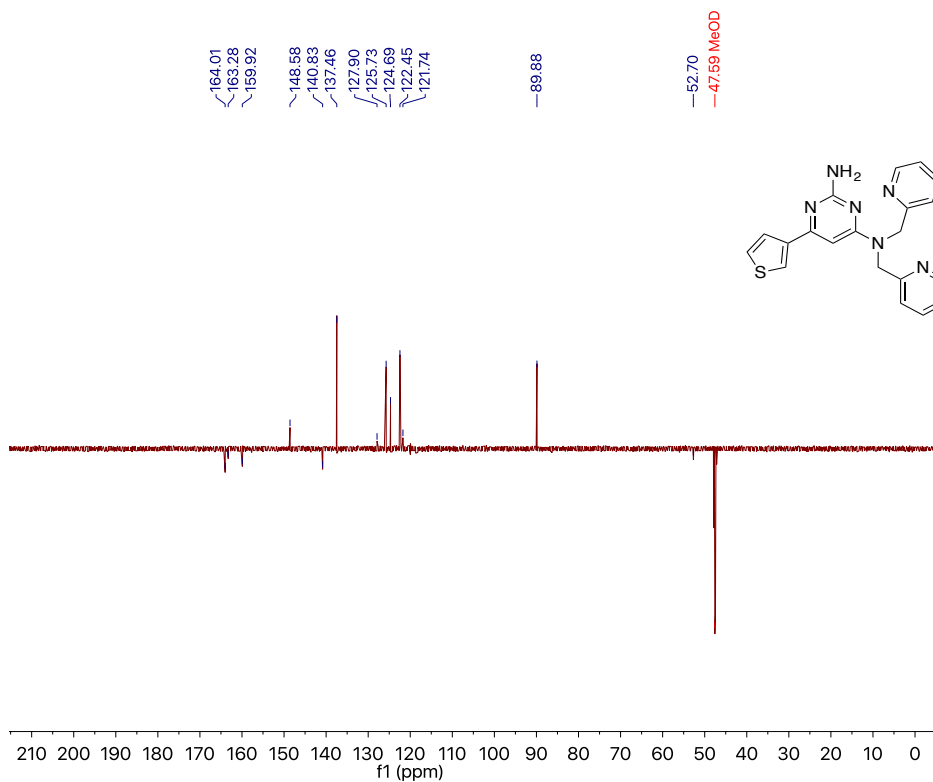
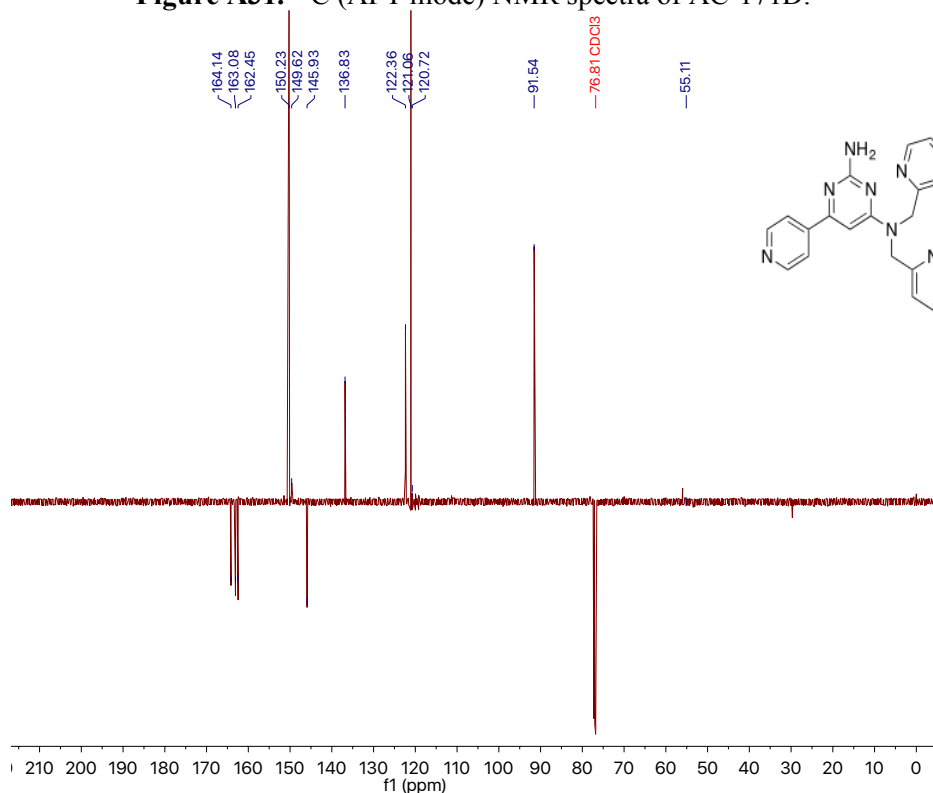
Figure A21. ^1H NMR spectra of AL-1B.Figure A22. ^1H NMR spectra of AL-1A2.

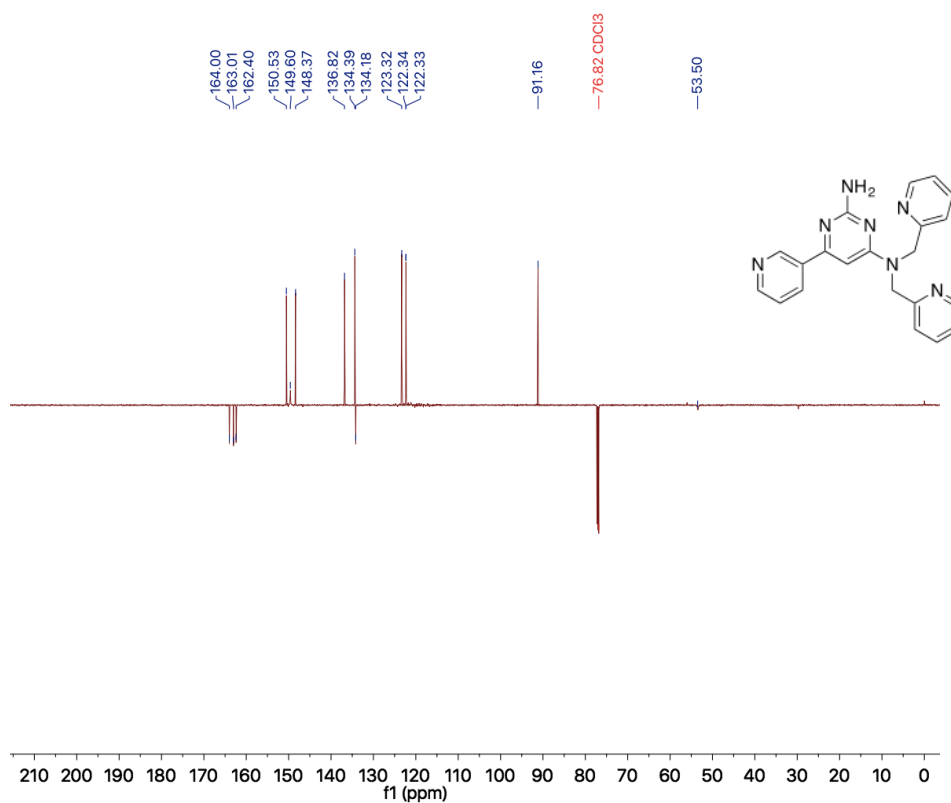
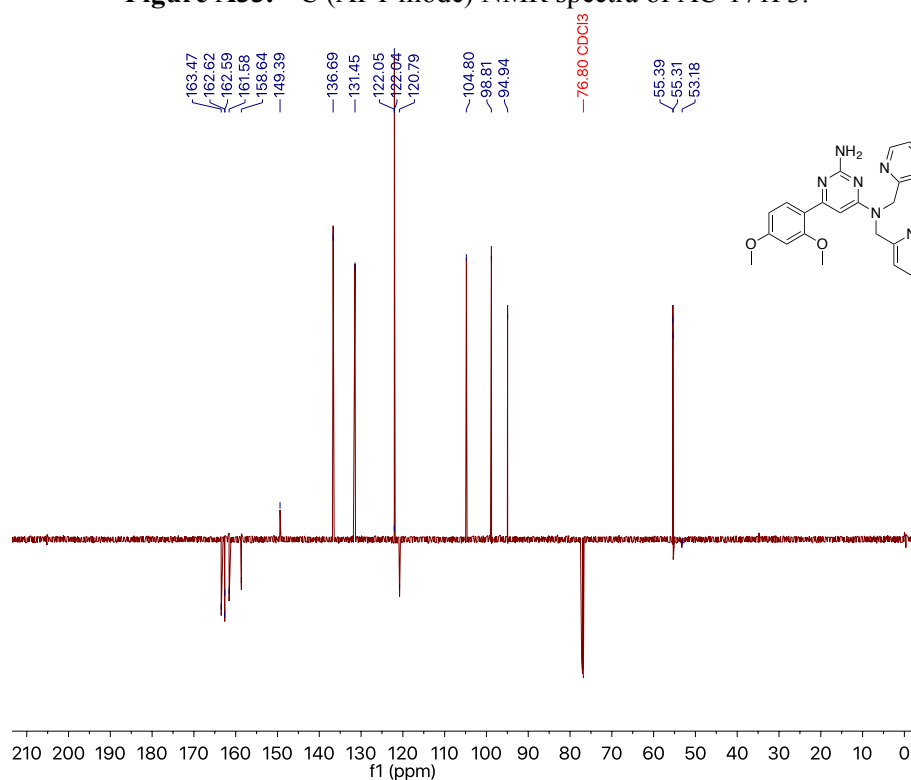
Figure A23. ^1H NMR spectra of AL-1A3.Figure A24. ^1H NMR spectra of AL-2A.

Figure A25. ^1H NMR spectra of AL-4A.Figure A26. ^1H NMR spectra of AL-6A.

AP 2. ^{13}C APT NMRFigure A27 ^{13}C (APT mode) NMR spectra of AC-17.Figure A28. ^{13}C (APT mode) NMR spectra of AC-17A.

**Figure A29.** ^{13}C (APT mode) NMR spectra of AC-171A2.**Figure A30.** ^{13}C (APT mode) NMR spectra of AC-171C.

**Figure A31.** ^{13}C (APT mode) NMR spectra of AC-171D.**Figure A32.** ^{13}C (APT mode) NMR spectra of AC-171F.

**Figure A33.** ^{13}C (APT mode) NMR spectra of AC-171F3.**Figure A34.** ^{13}C (APT mode) NMR spectra of AC-171H.

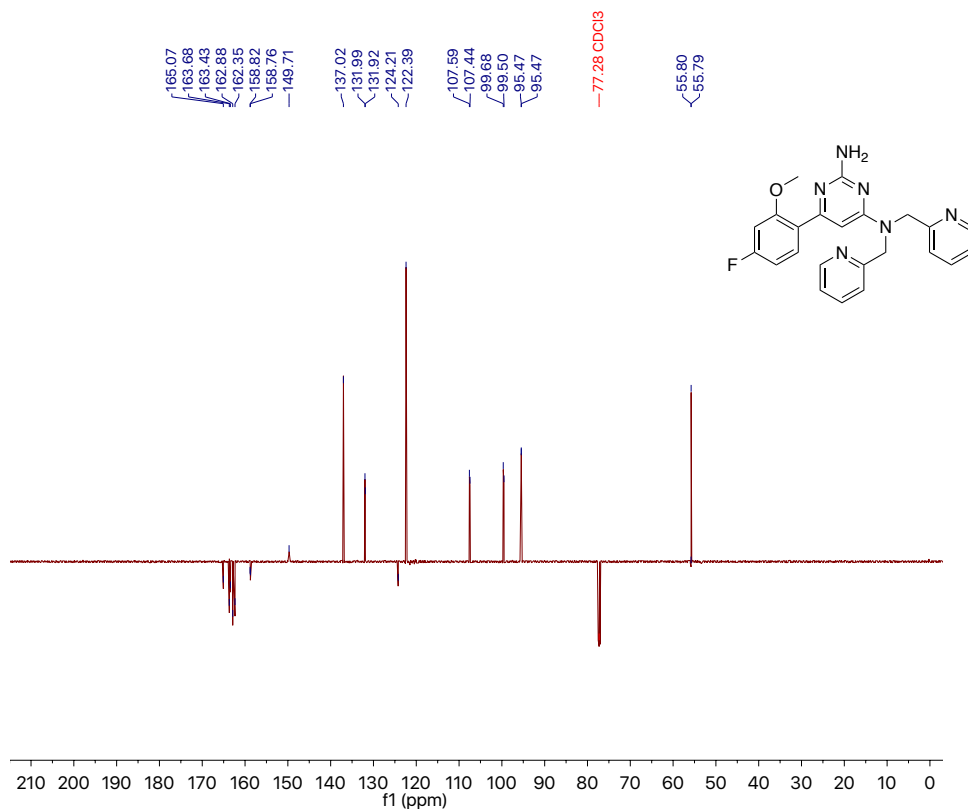


Figure 35. ^{13}C (APT mode) NMR spectra of AC-171M.

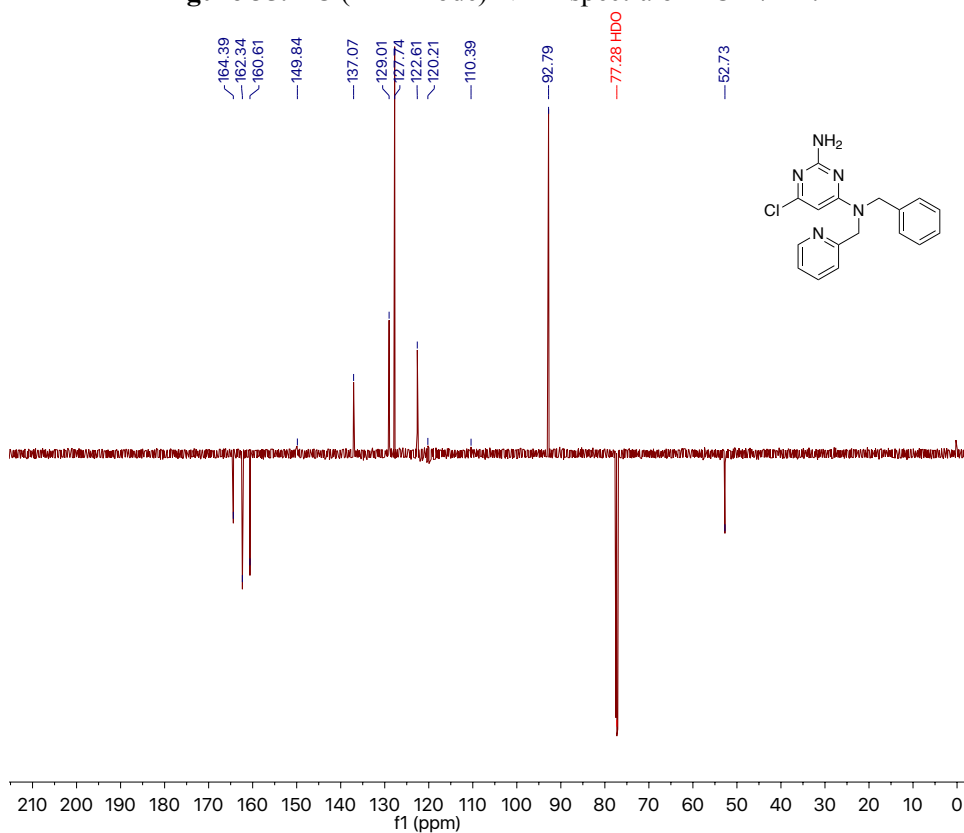
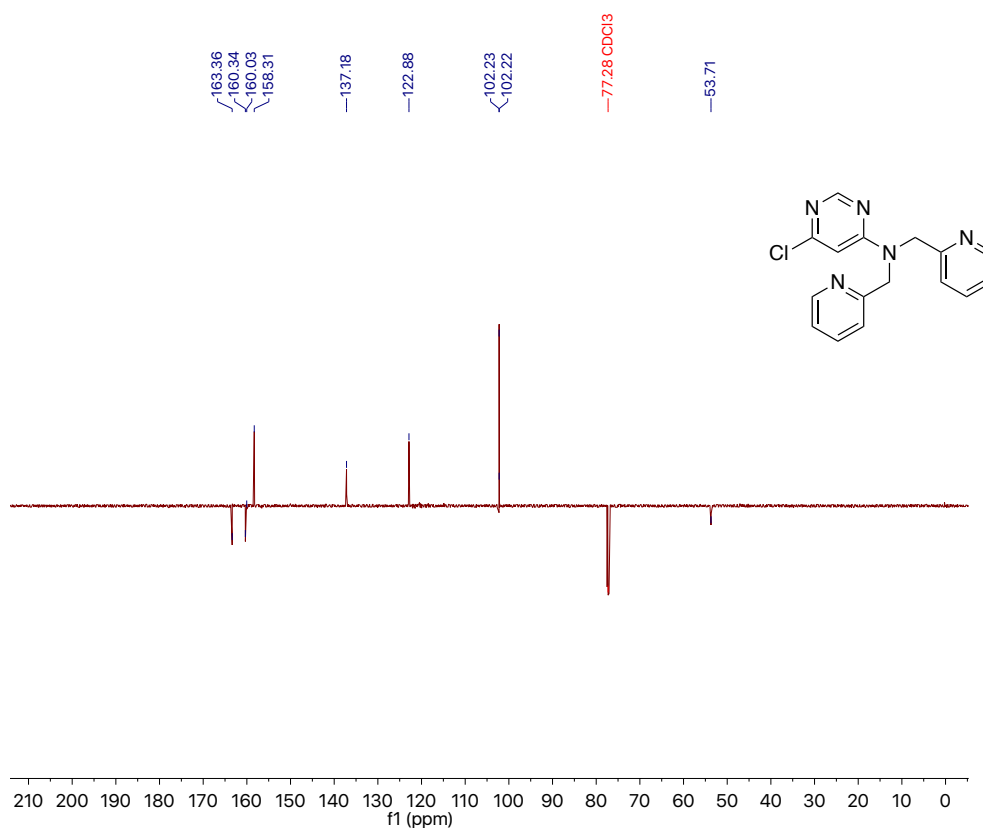
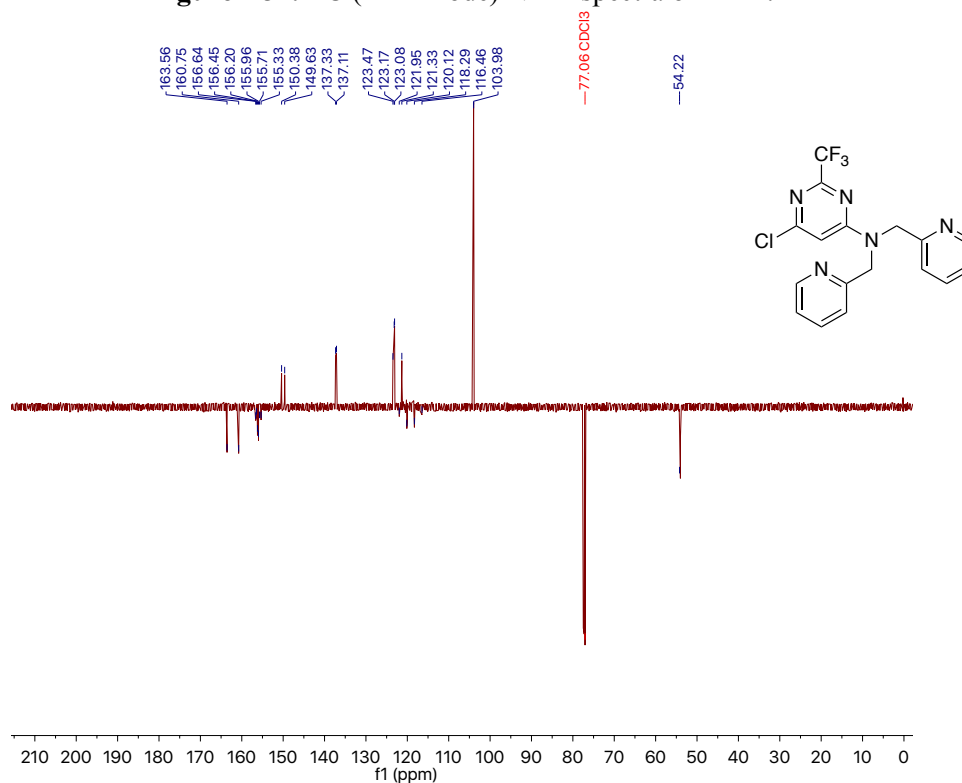


Figure 36. ^{13}C (APT mode) NMR spectra of AL-2.

**Figure A37.** ^{13}C (APT mode) NMR spectra of AL-4.**Figure A38.** ^{13}C (APT mode) NMR spectra of AL-6.

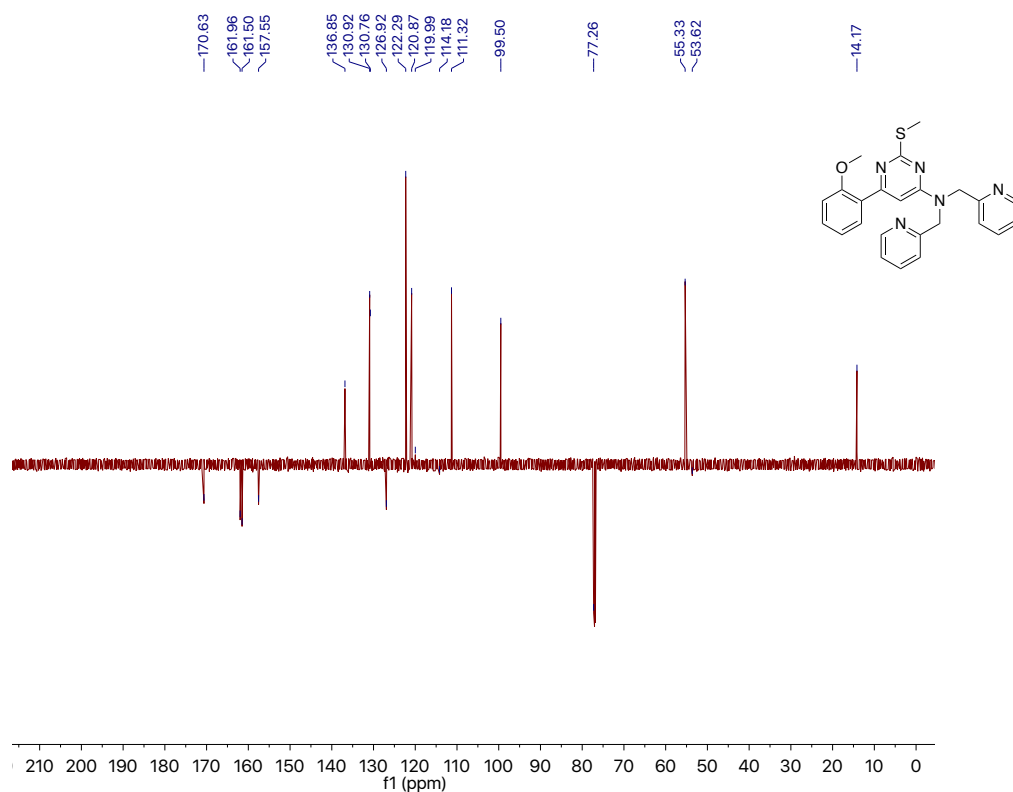


Figure A39. ^{13}C (APT mode) NMR spectra of AL-1A.

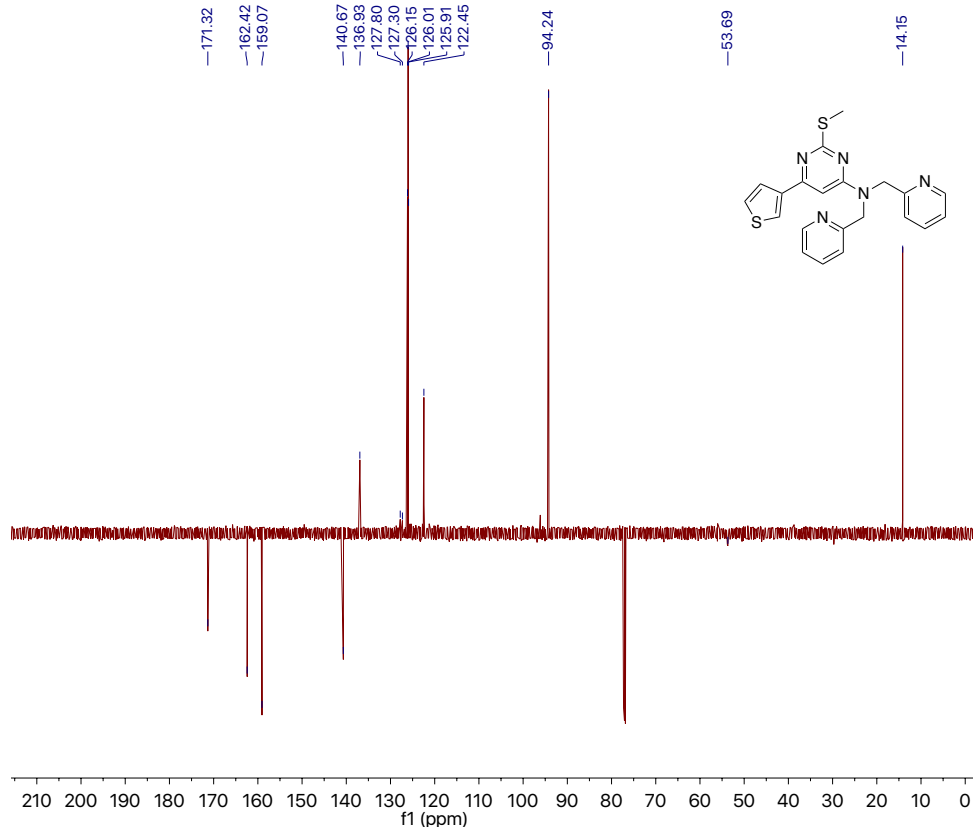
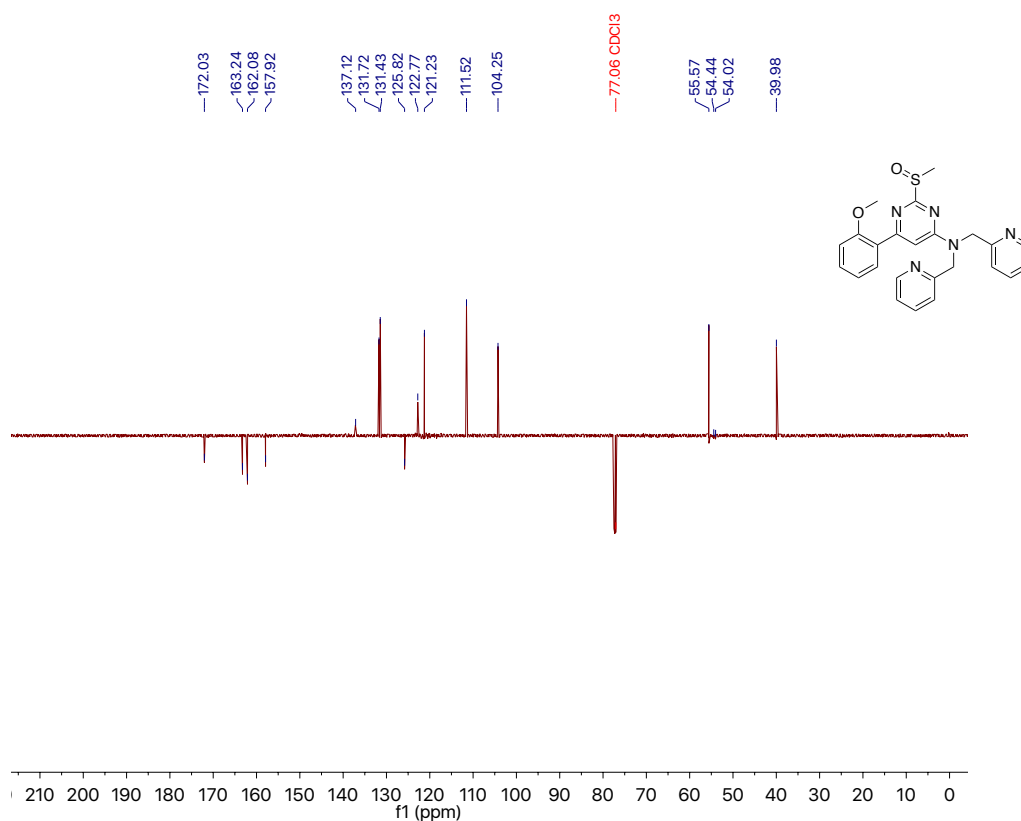
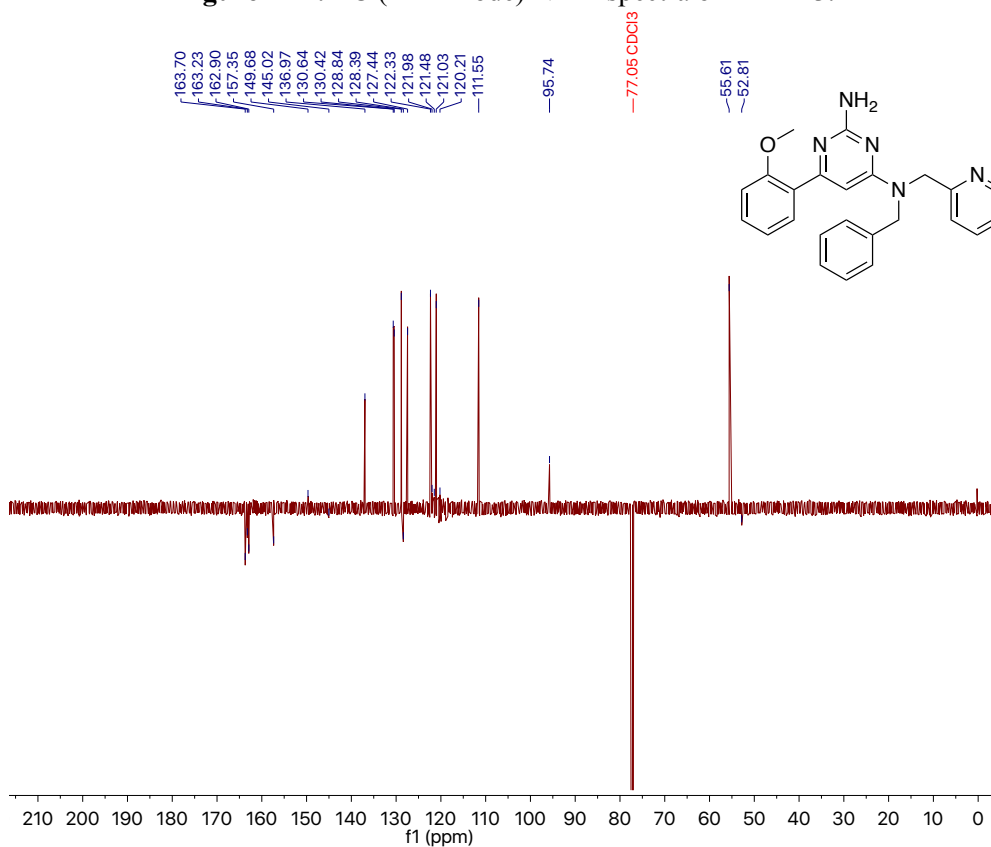
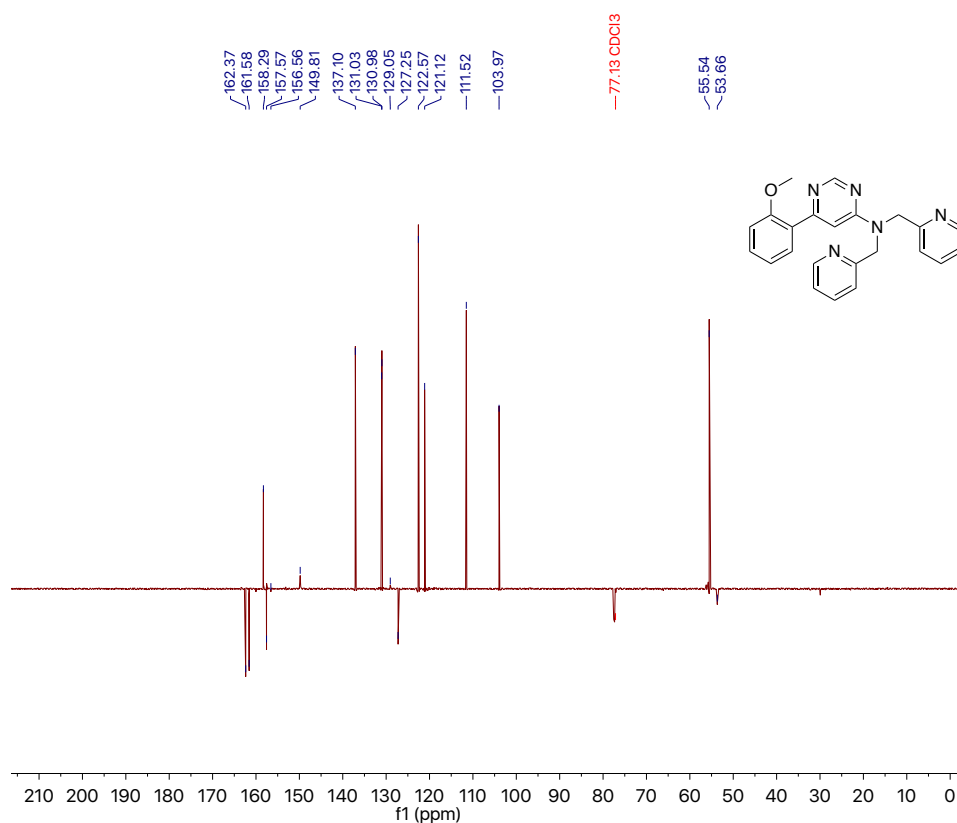
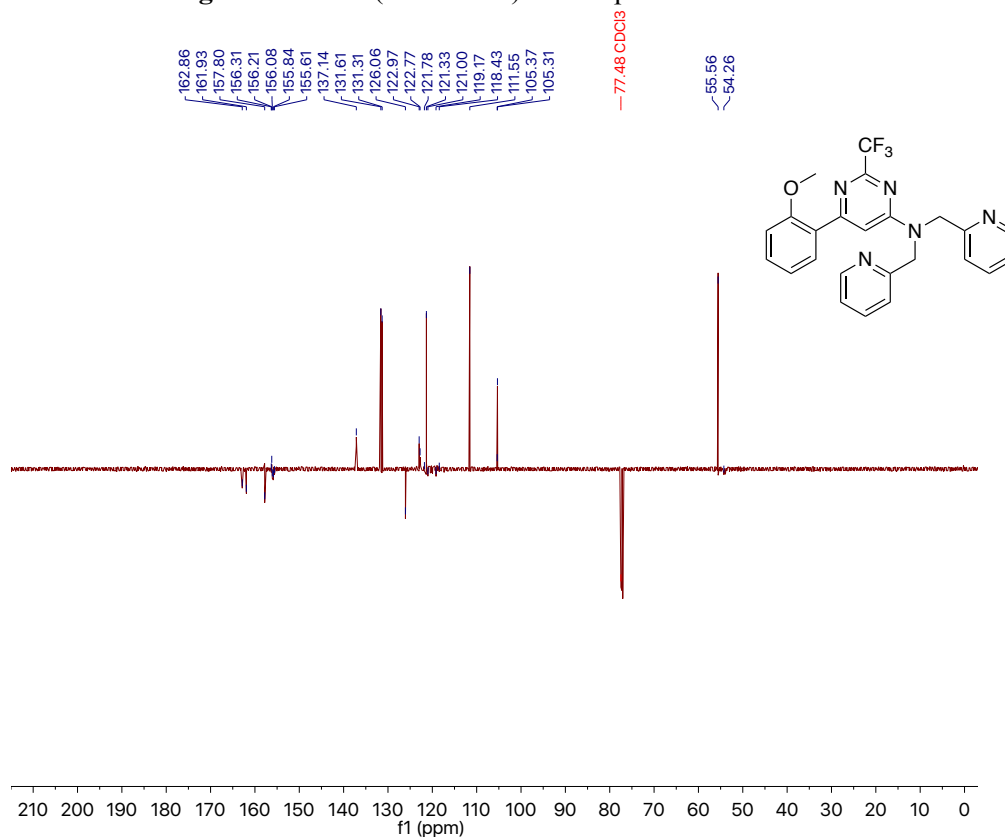


Figure A40. ^{13}C (APT mode) NMR spectra of AL-1B.

**Figure A41.** ^{13}C (APT mode) NMR spectra of AL-1A3.**Figure A42.** ^{13}C (APT mode) NMR spectra of AL-2A.

Figure A43. ^{13}C (APT mode) NMR spectra of AL-4A.Figure A44. ^{13}C (APT mode) NMR spectra of AL-6A.

AP 3. HR-MS spectra

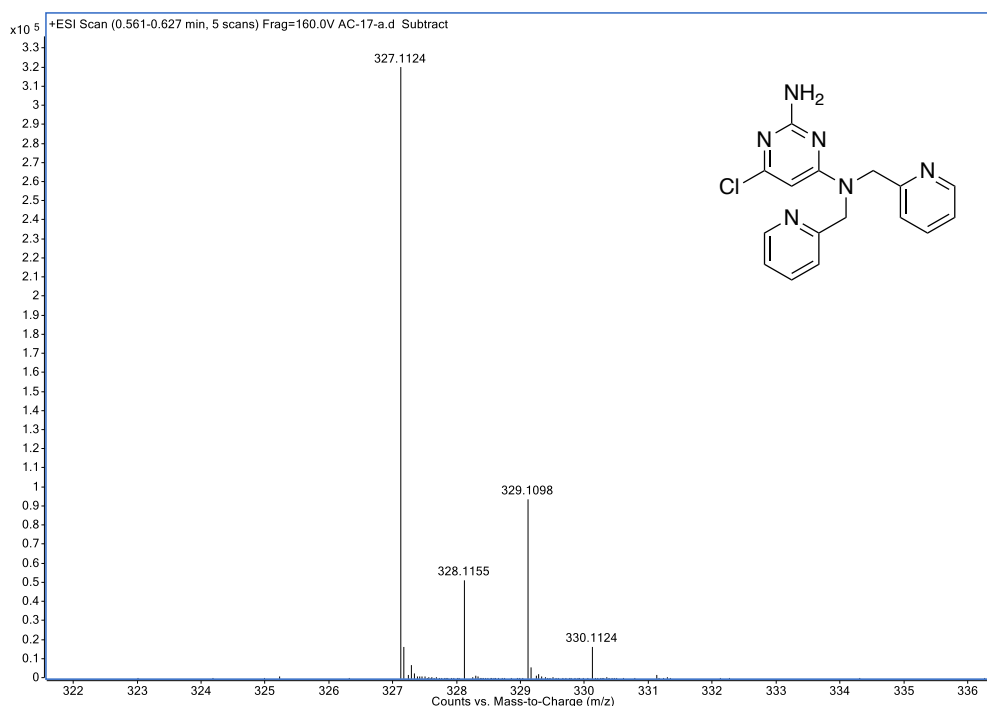


Figure A45. High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-17.

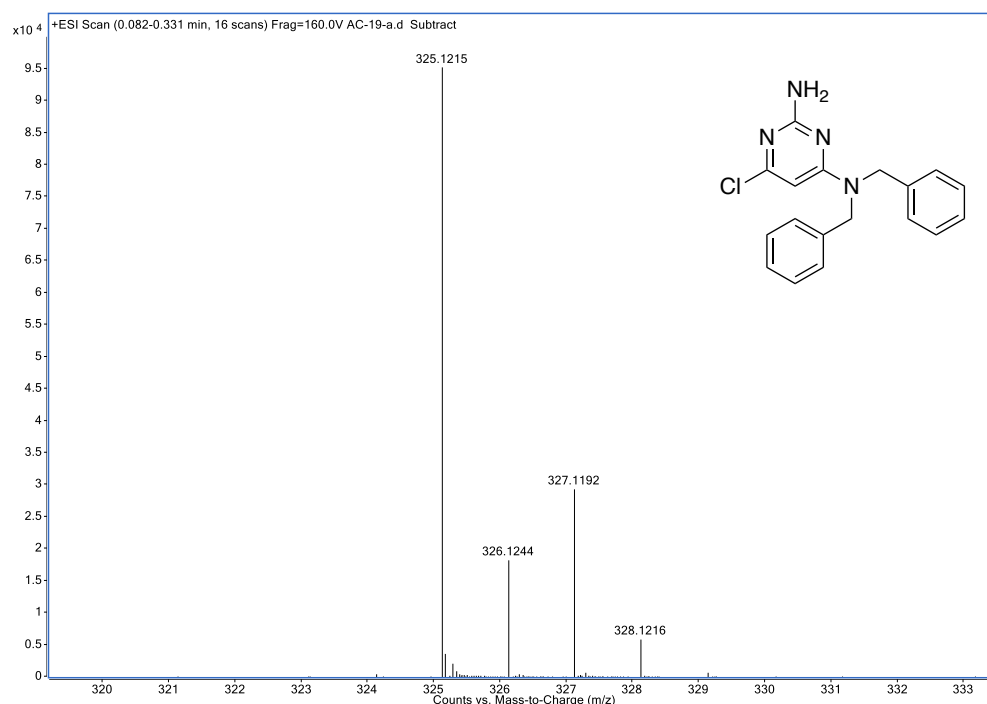
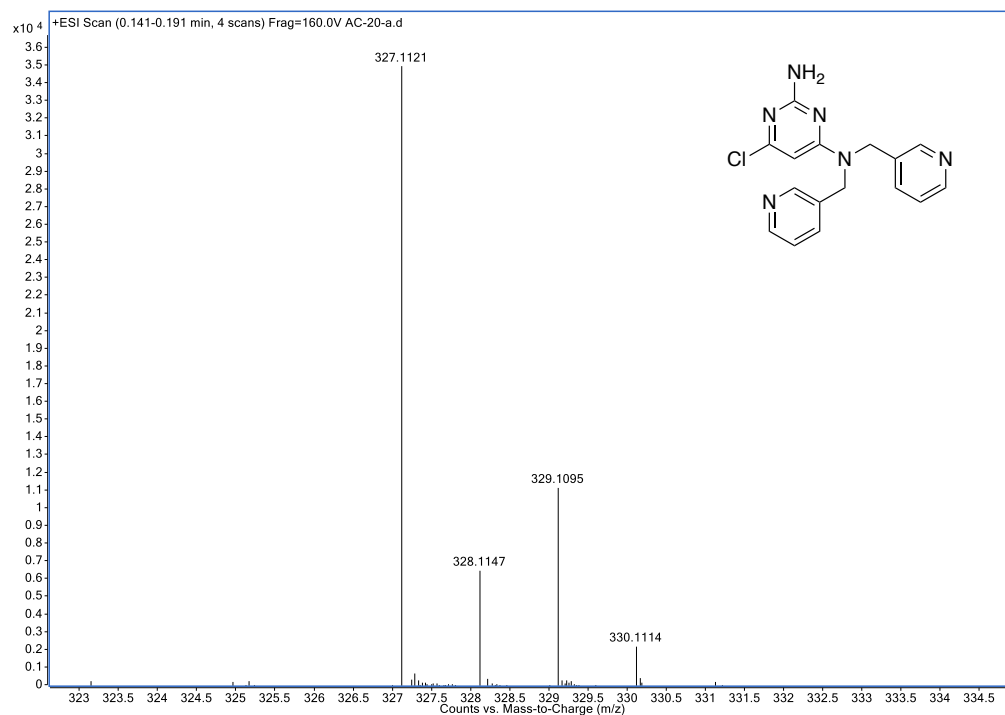
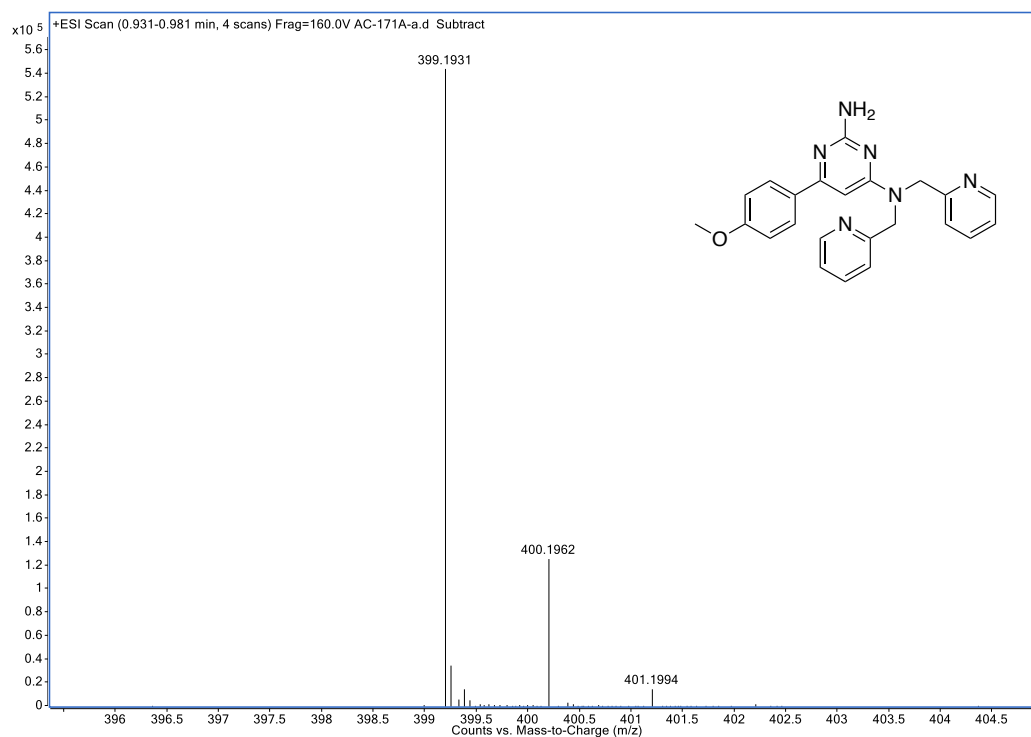
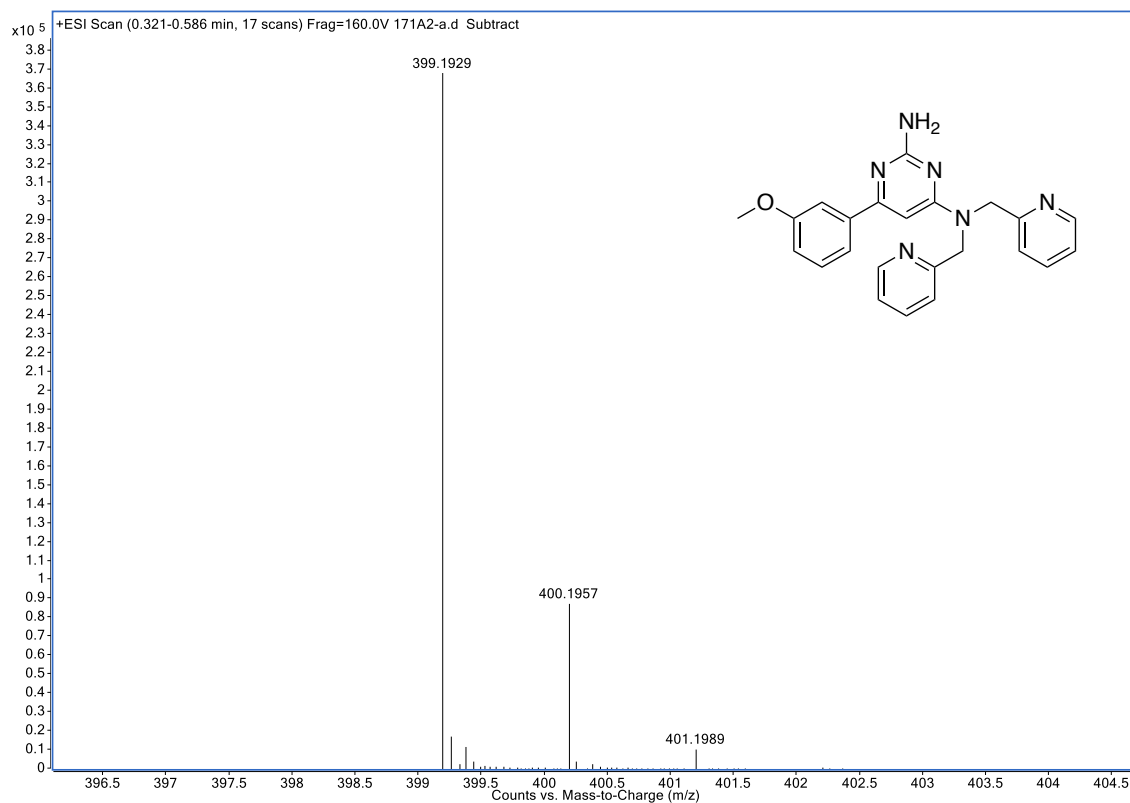
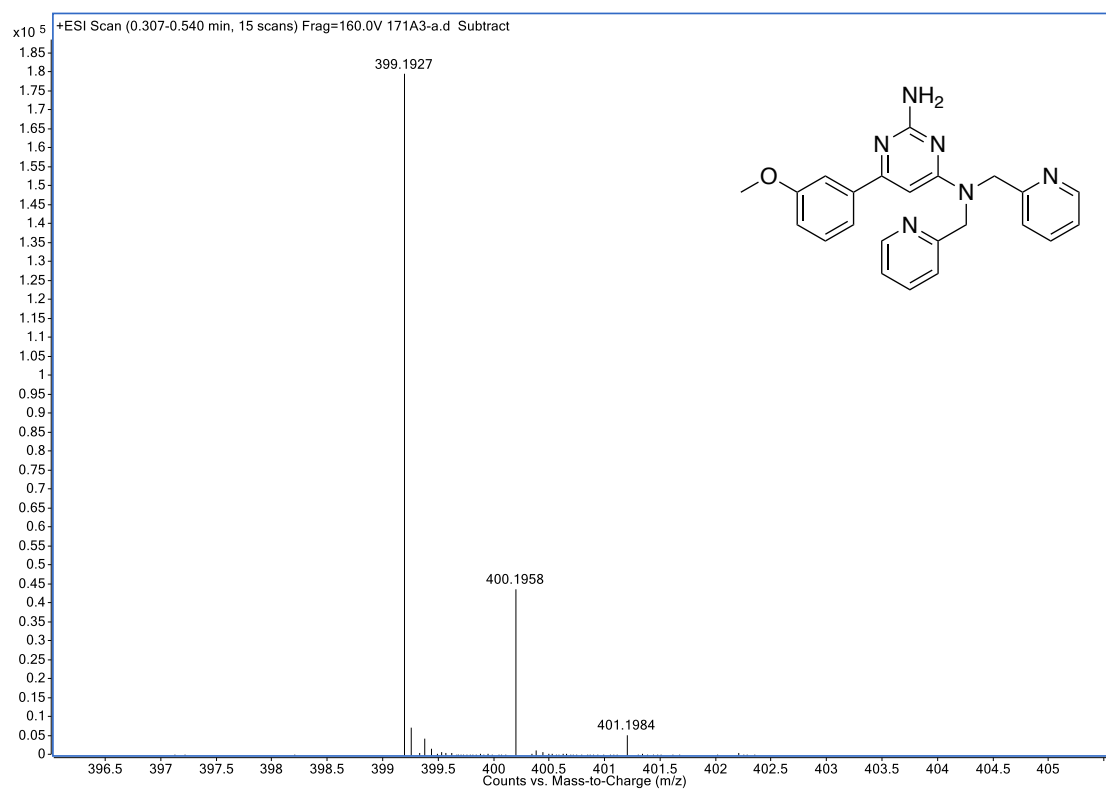
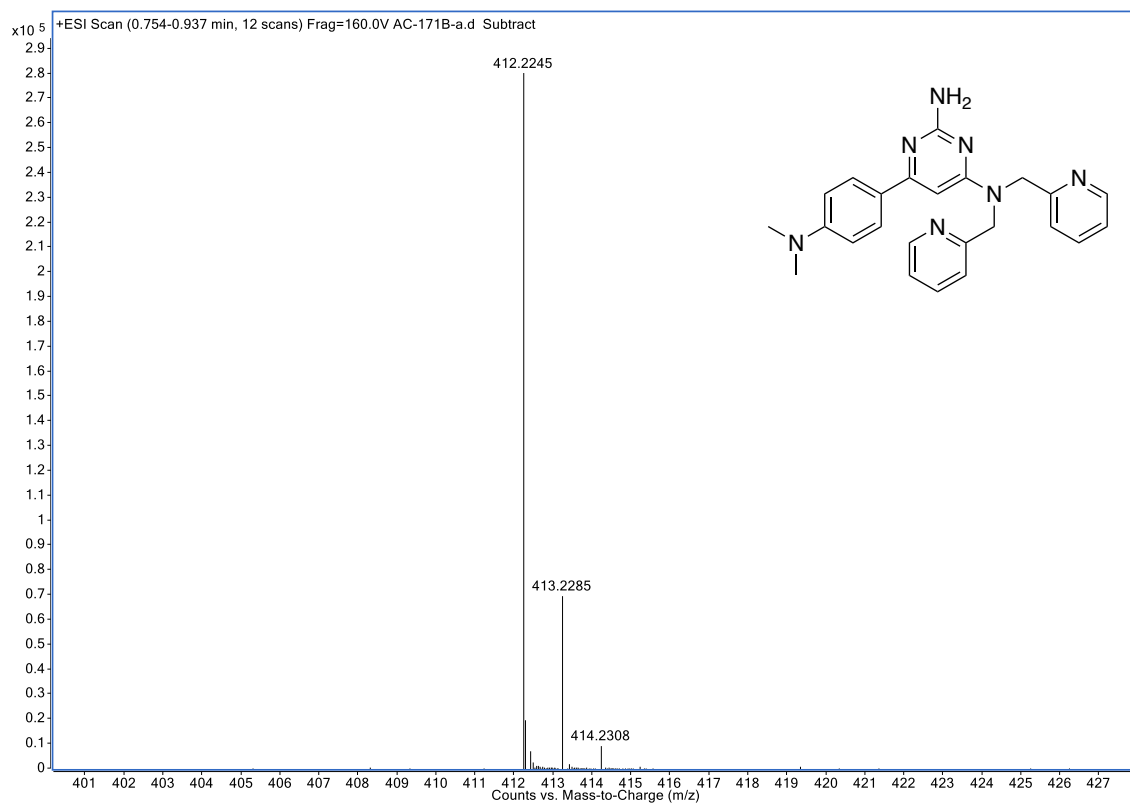
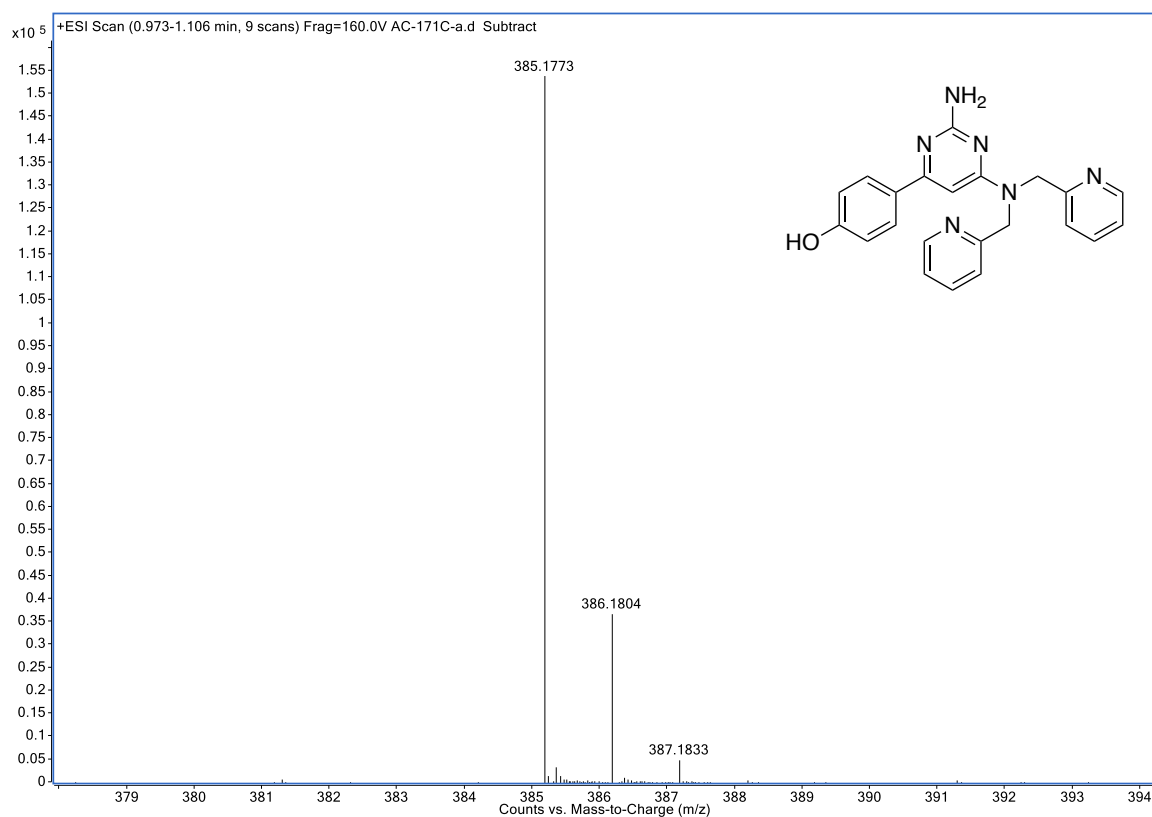


Figure A46. High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-19.

**Figure A47.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-20.**Figure A48.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-171A.

**Figure A49.** High resolution-Mass Spectrometry (HR-ESI-MS) of AC-171A2.**Figure A50.** High resolution-Mass Spectrometry (HR-ESI-MS) of AC-171A3.

**Figure A51.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-171B.**Figure A52.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-171C.

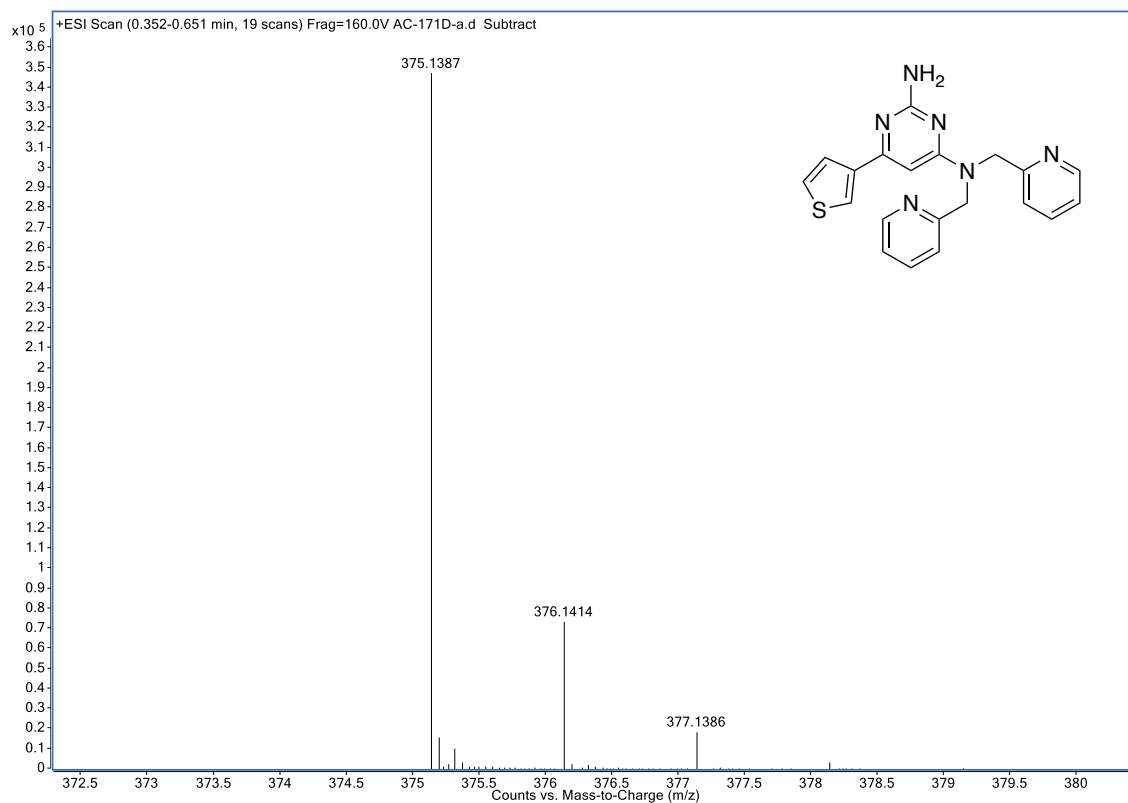


Figure A53. High resolution-Mass Spectrometry (HR-ESI-MS) of AC-171D.

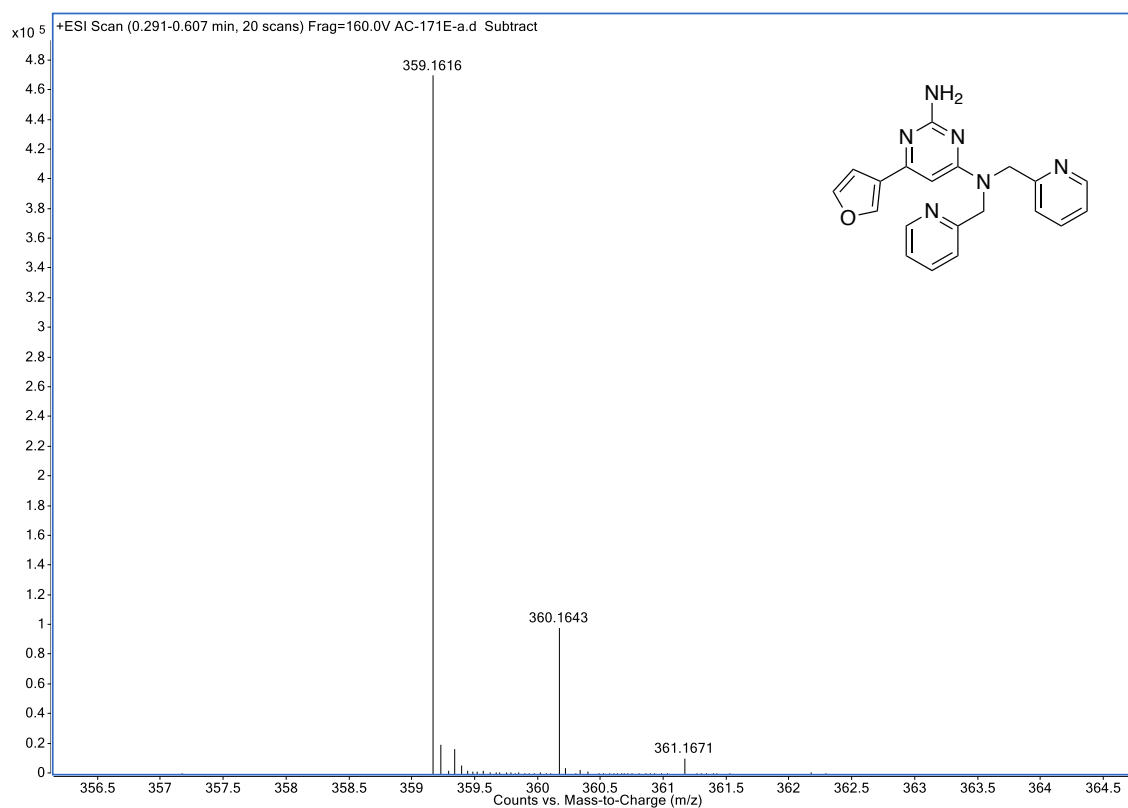
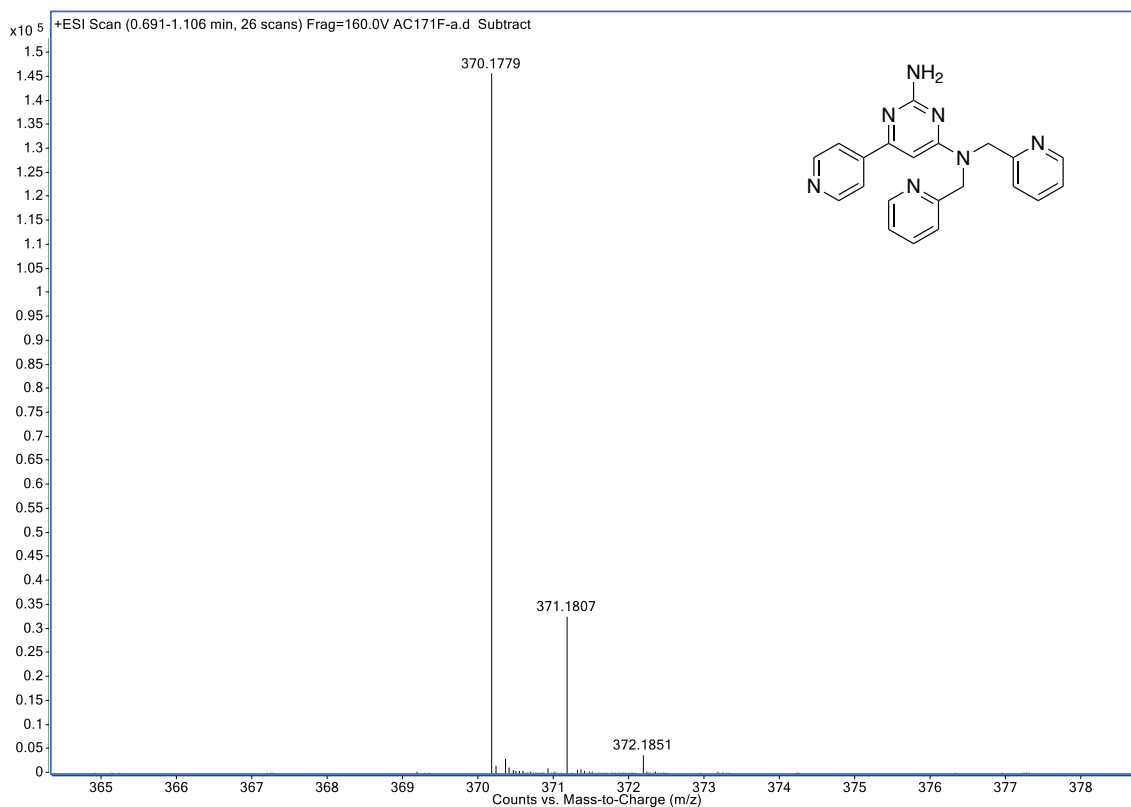
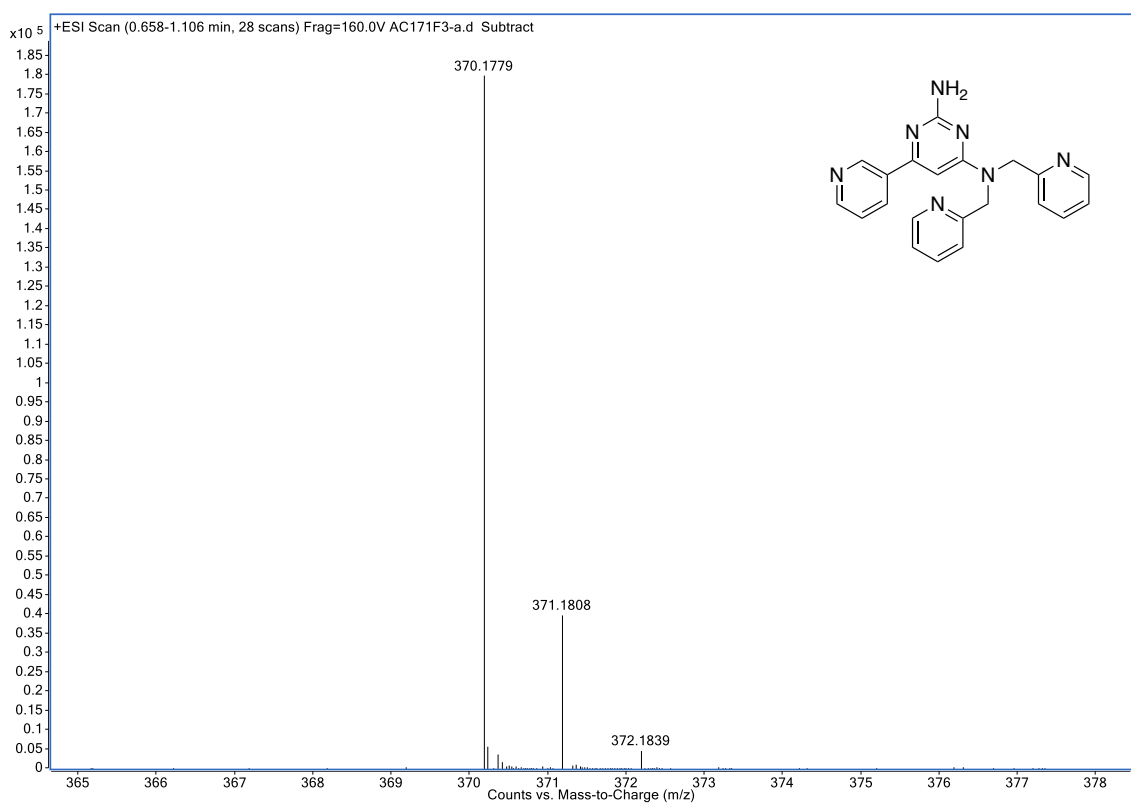
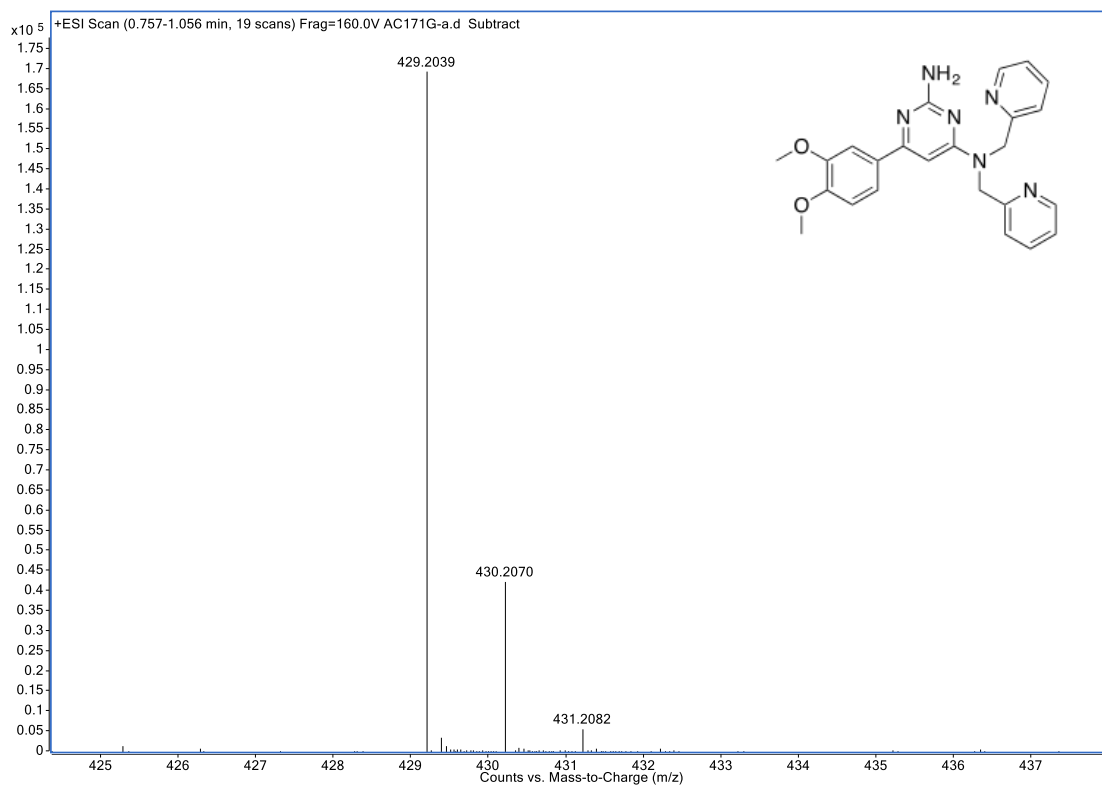
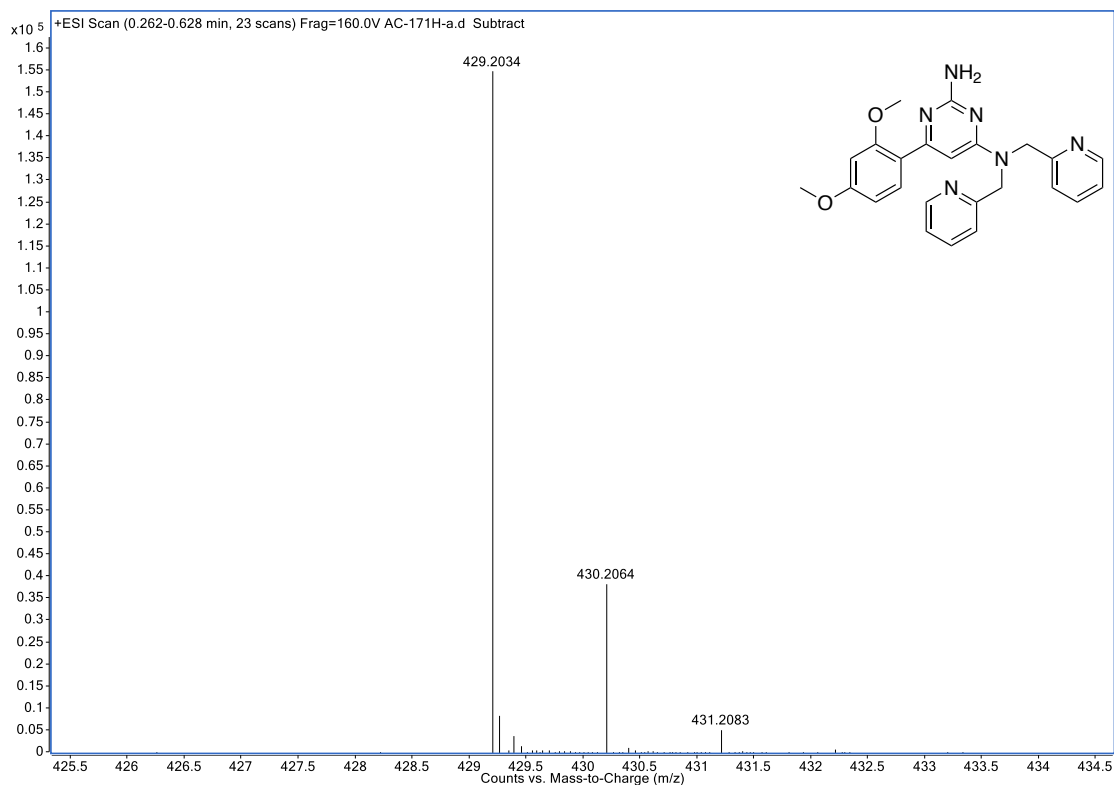


Figure A54. High resolution-Mass Spectrometry (HR-ESI-MS) of AC-171E.

**Figure A55.** High resolution-Mass Spectrometry (HR-ESI-MS) of AC-171F.**Figure A56.** High resolution-Mass Spectrometry (HR-ESI-MS) of AC-171F3.

**Figure A57.** High resolution-Mass Spectrometry (HR-ESI-MS) of AC-171G.**Figure A58.** High resolution-Mass Spectrometry (HR-ESI-MS) of AC-171H.

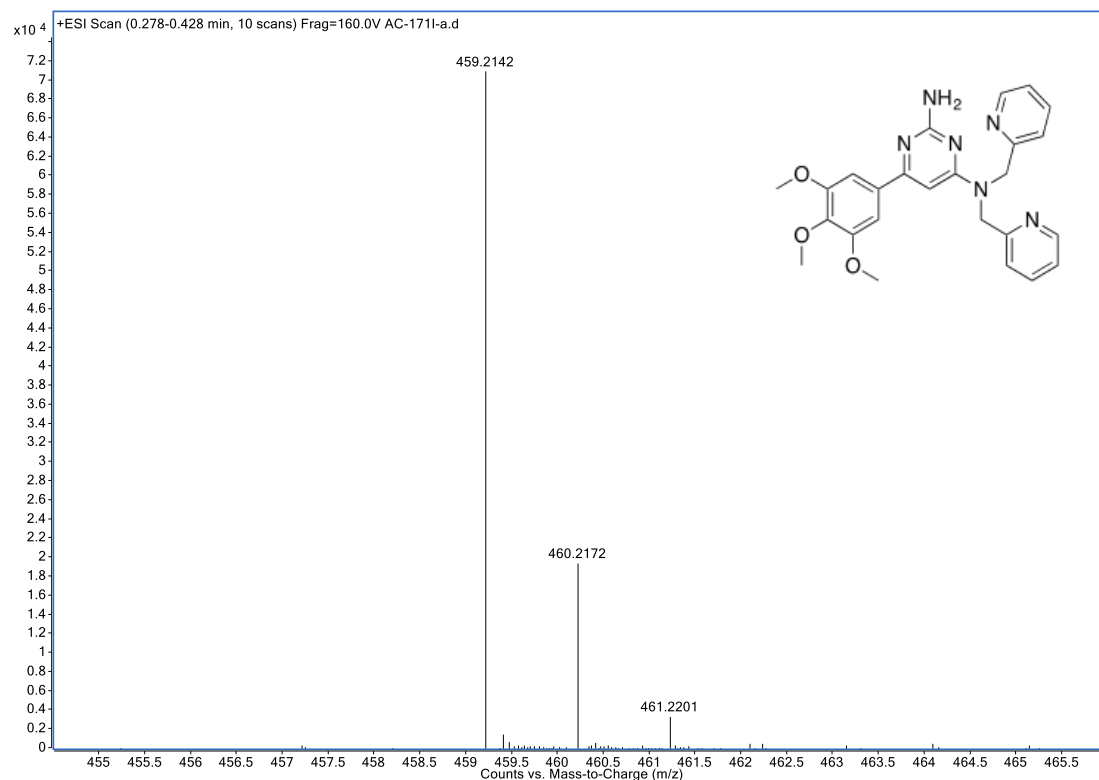


Figure A59. High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-171I.

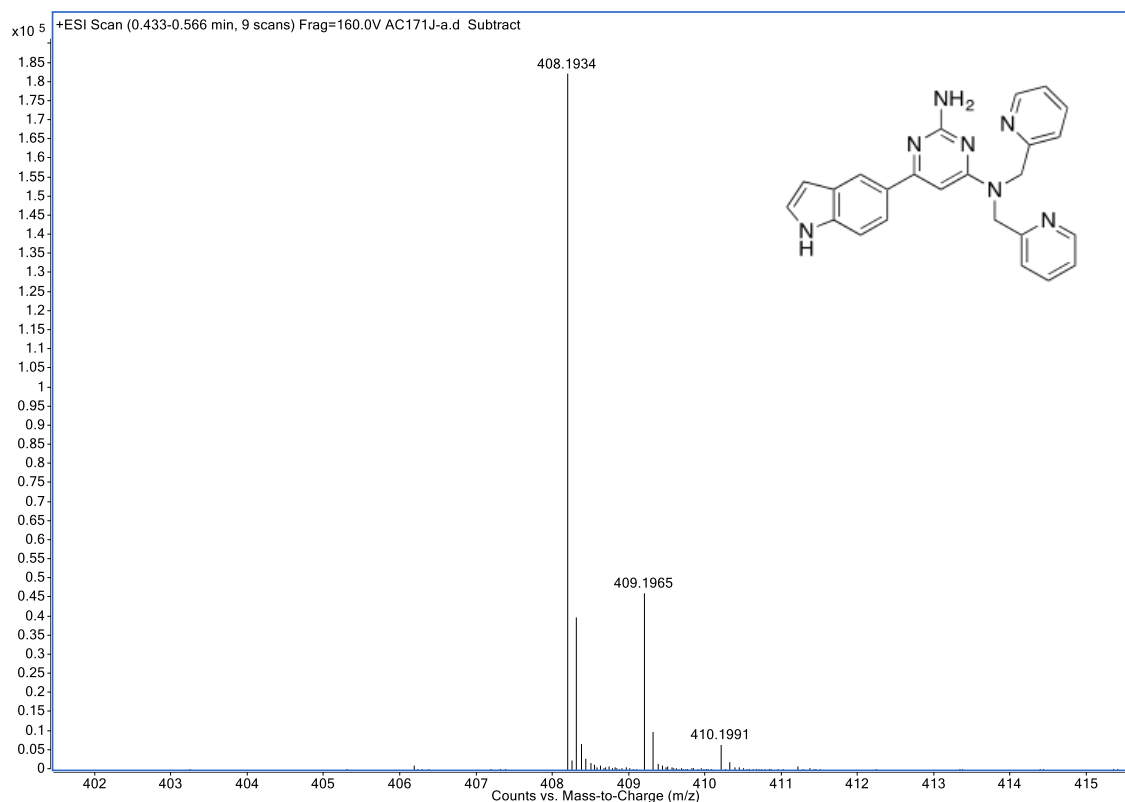
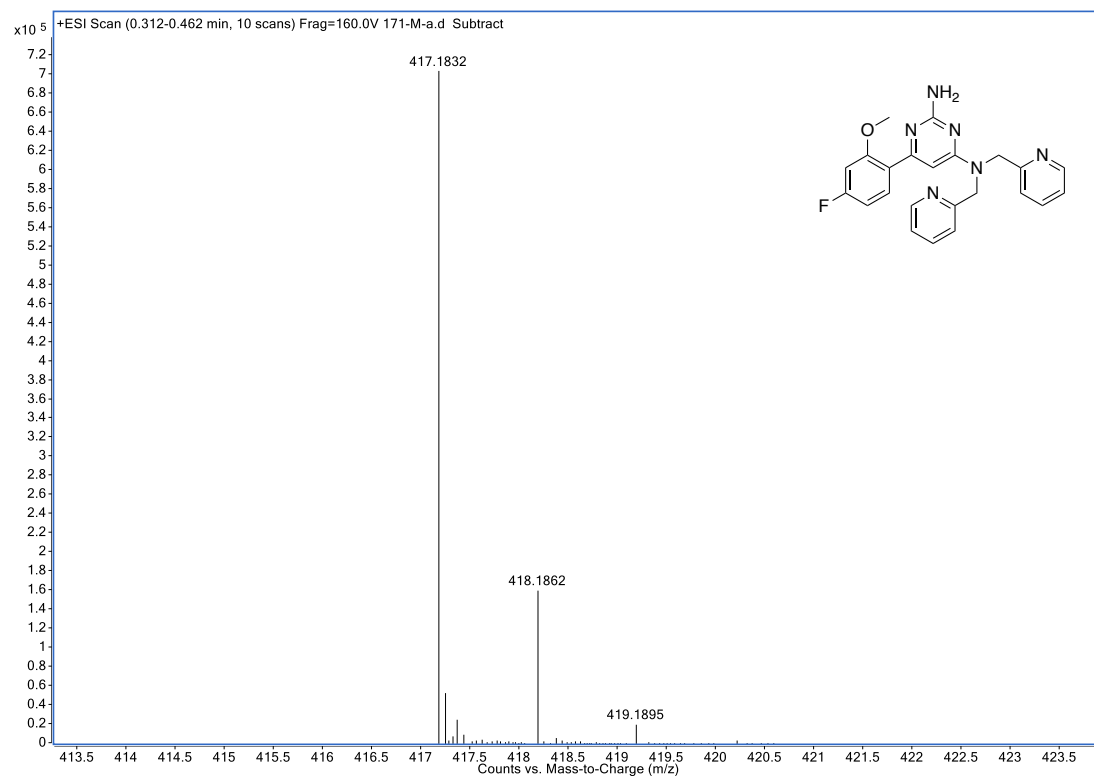
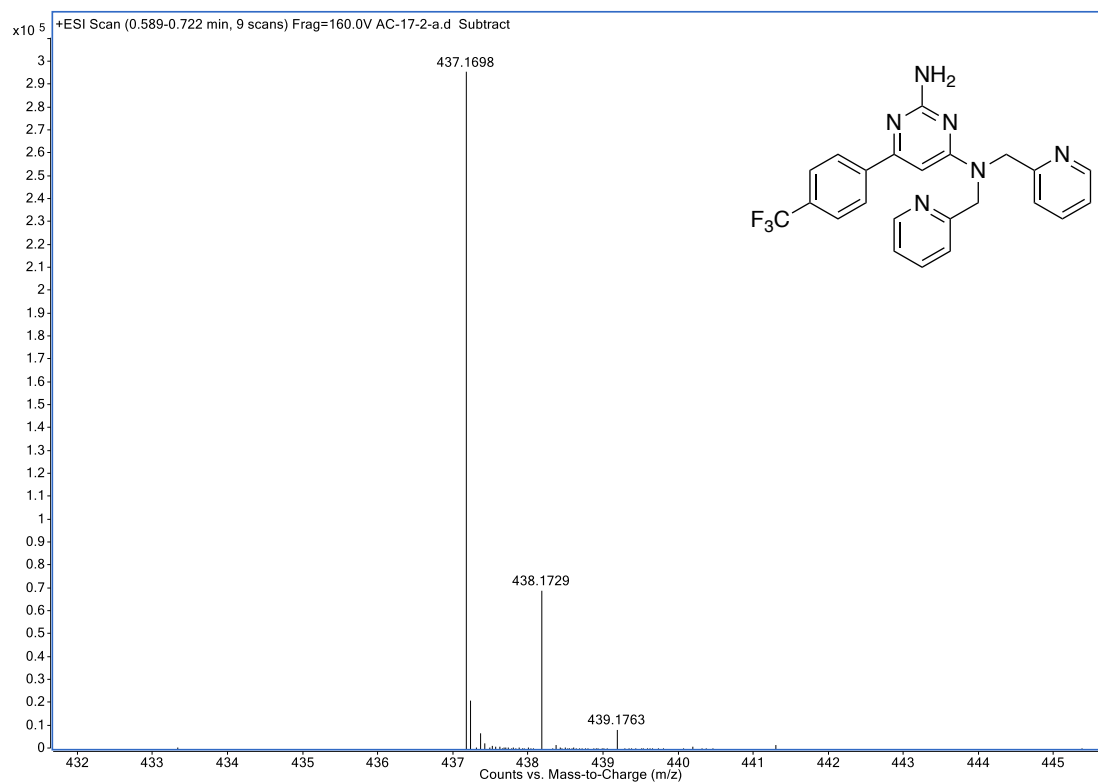
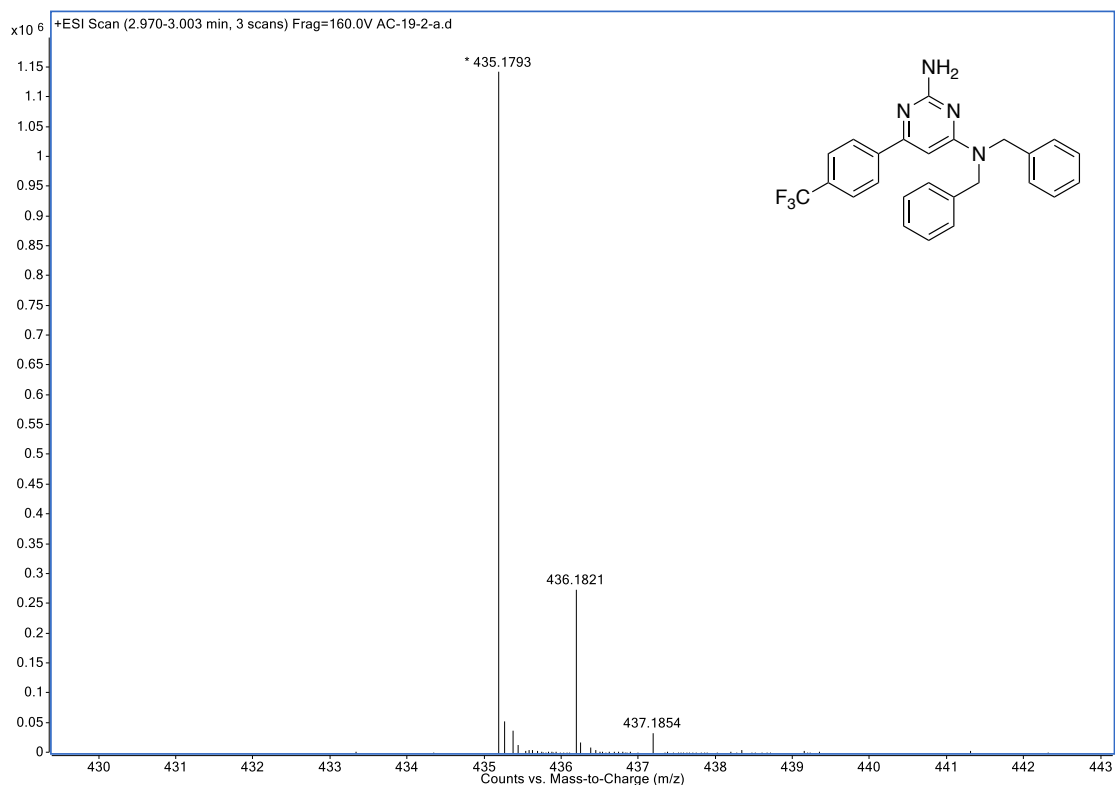
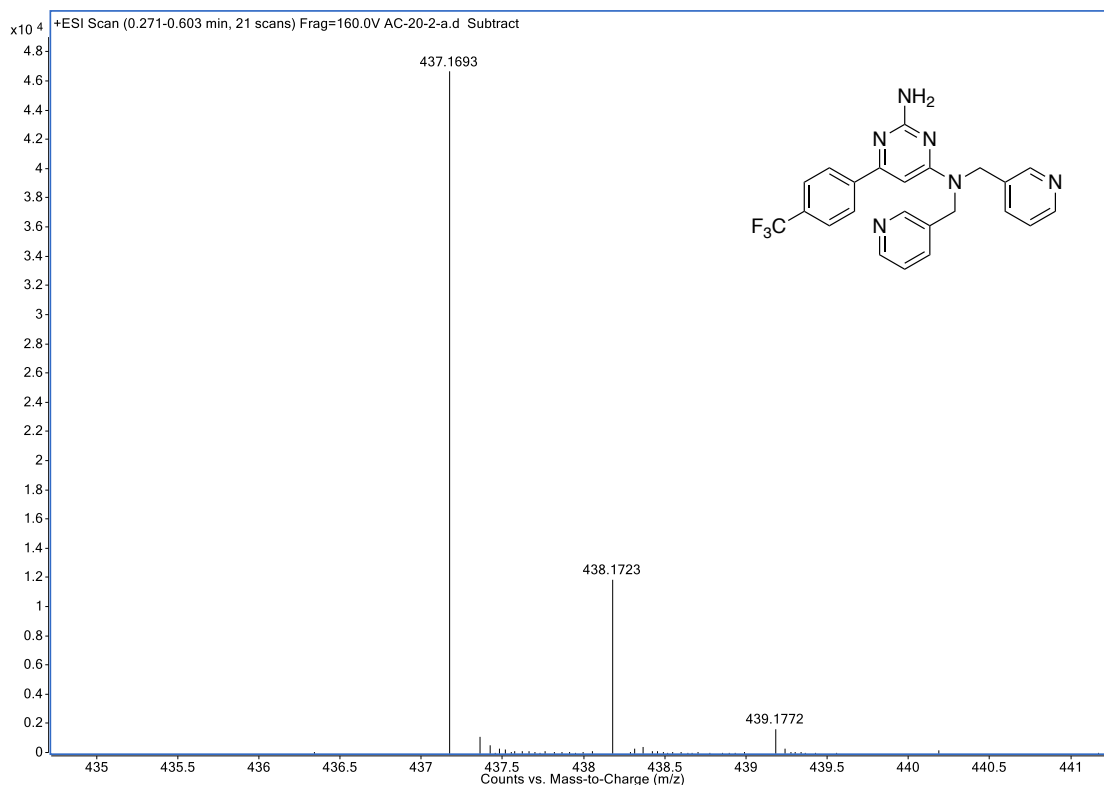
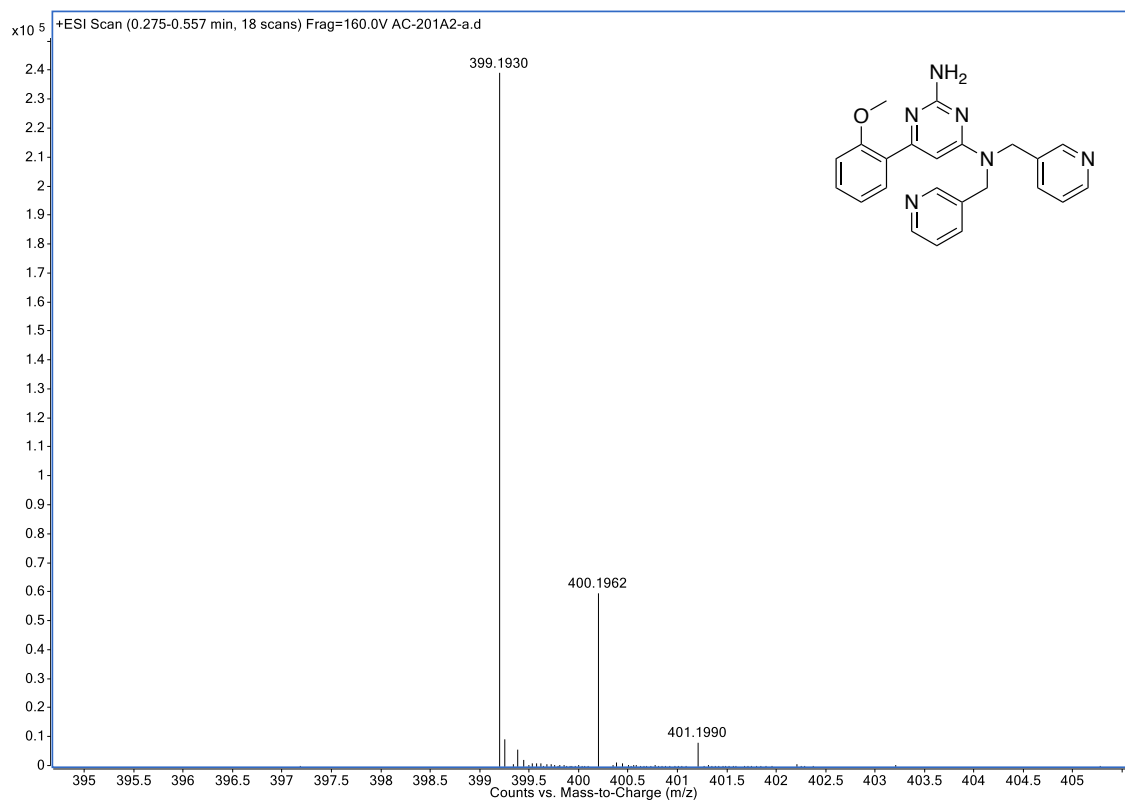
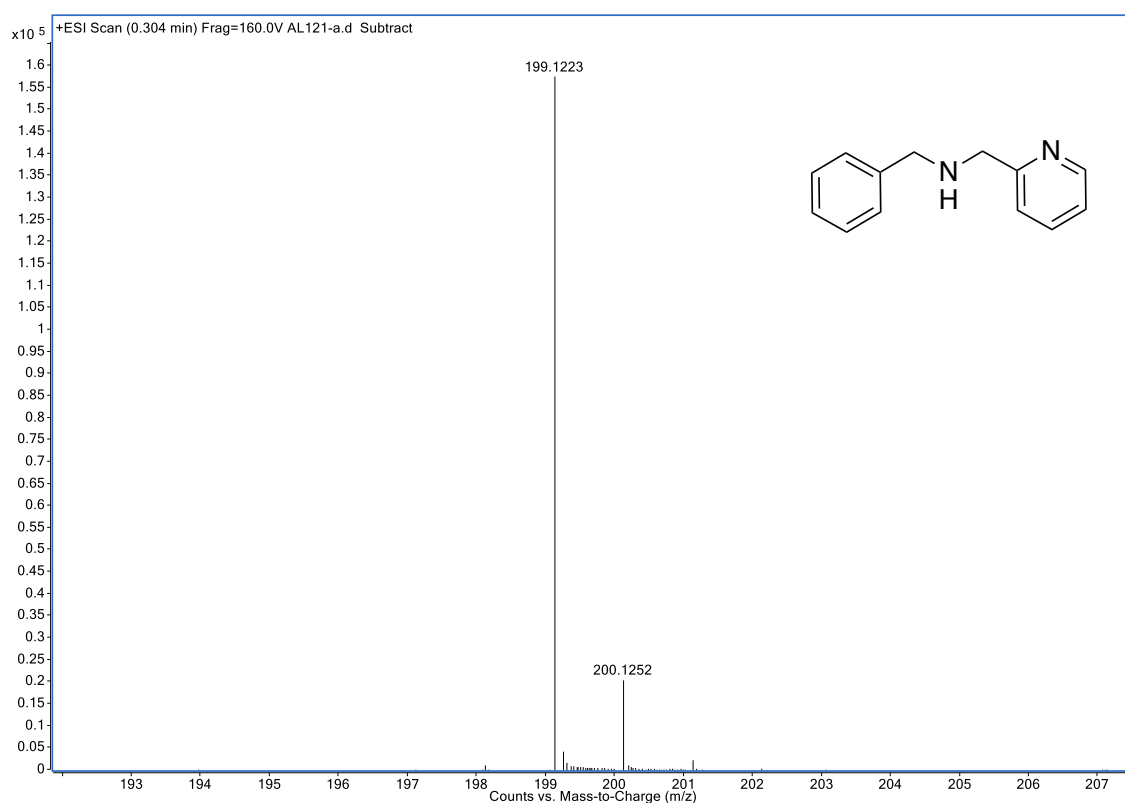
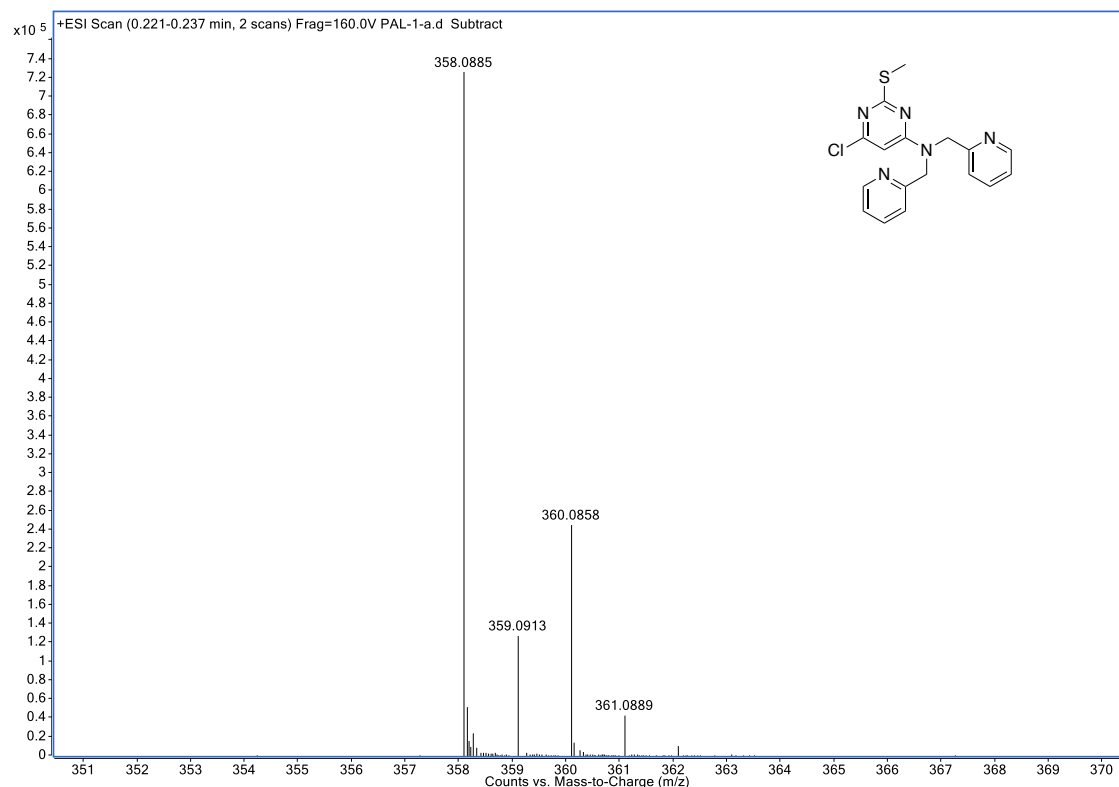
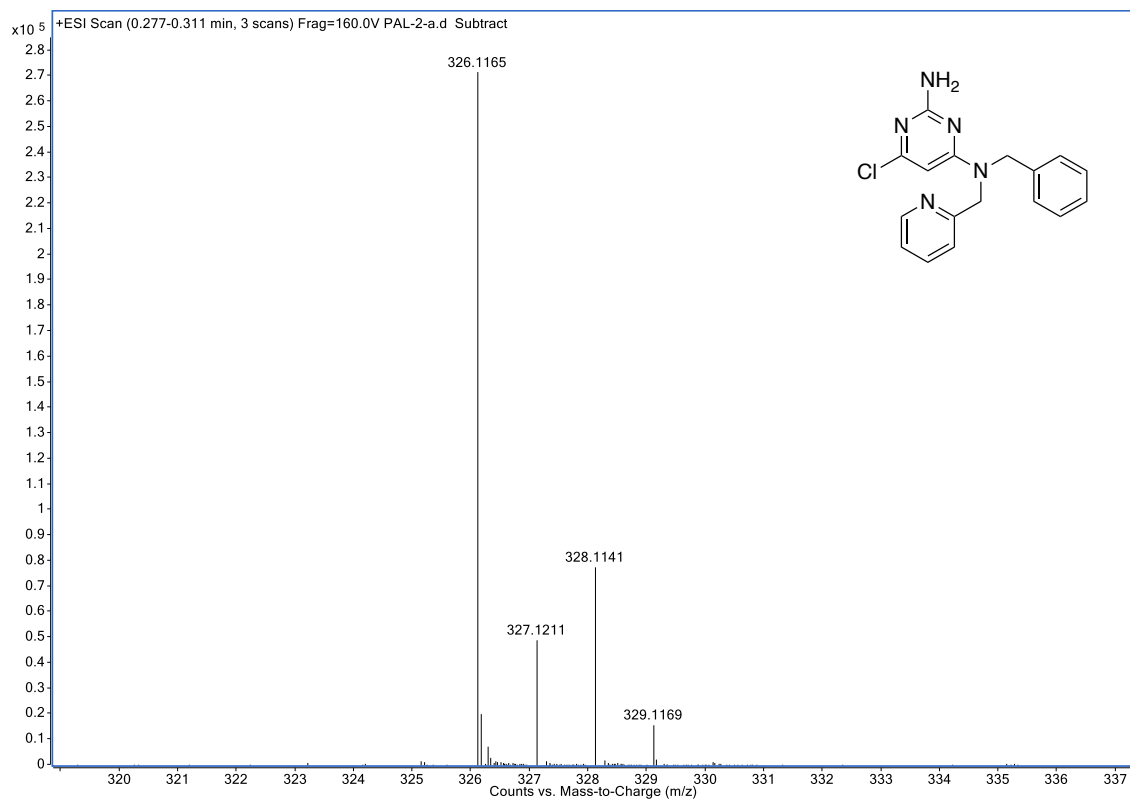


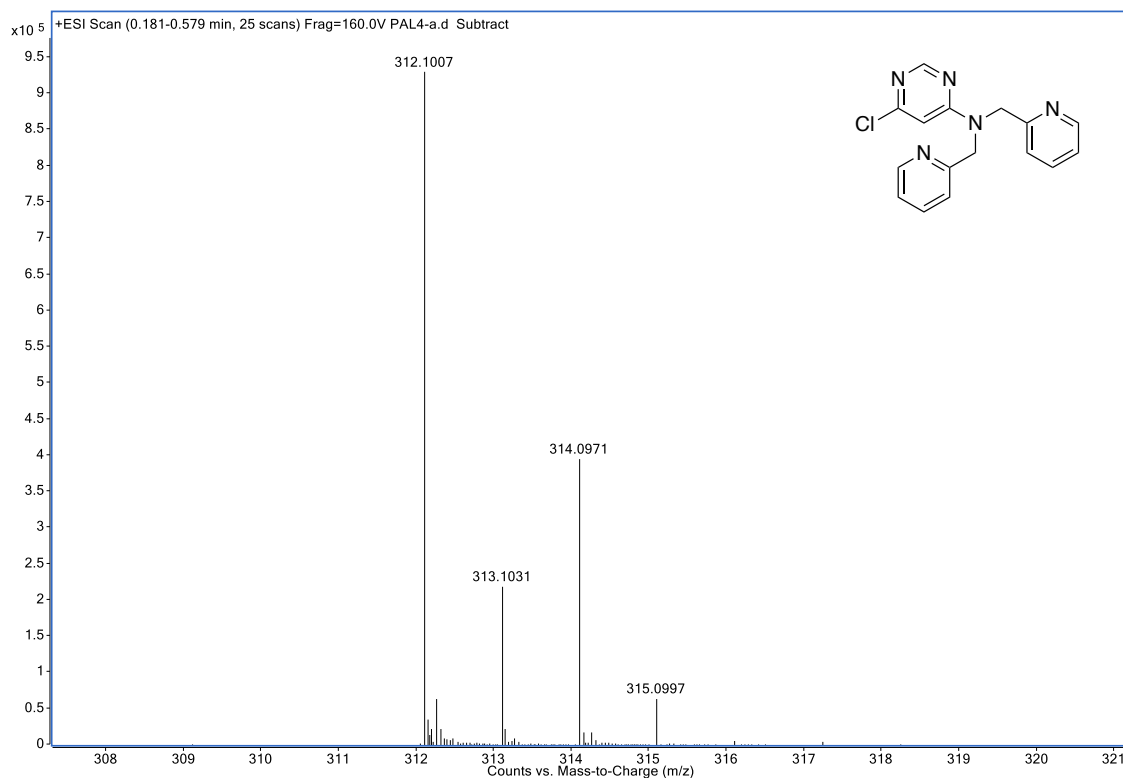
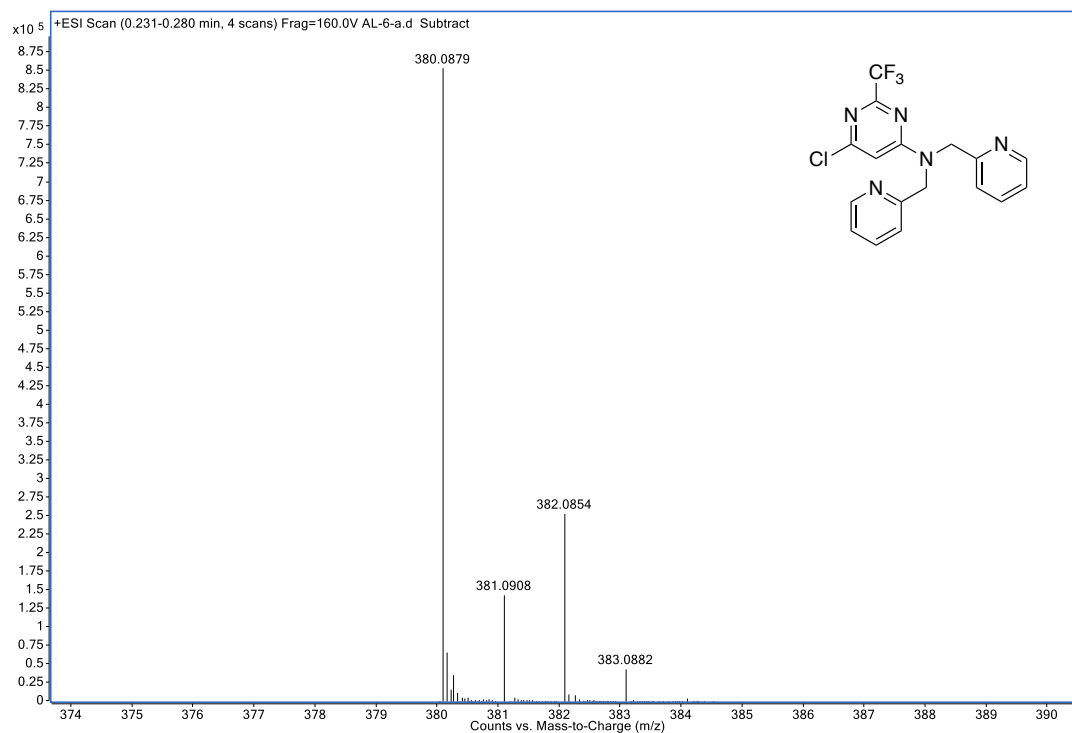
Figure A60. High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-171J.

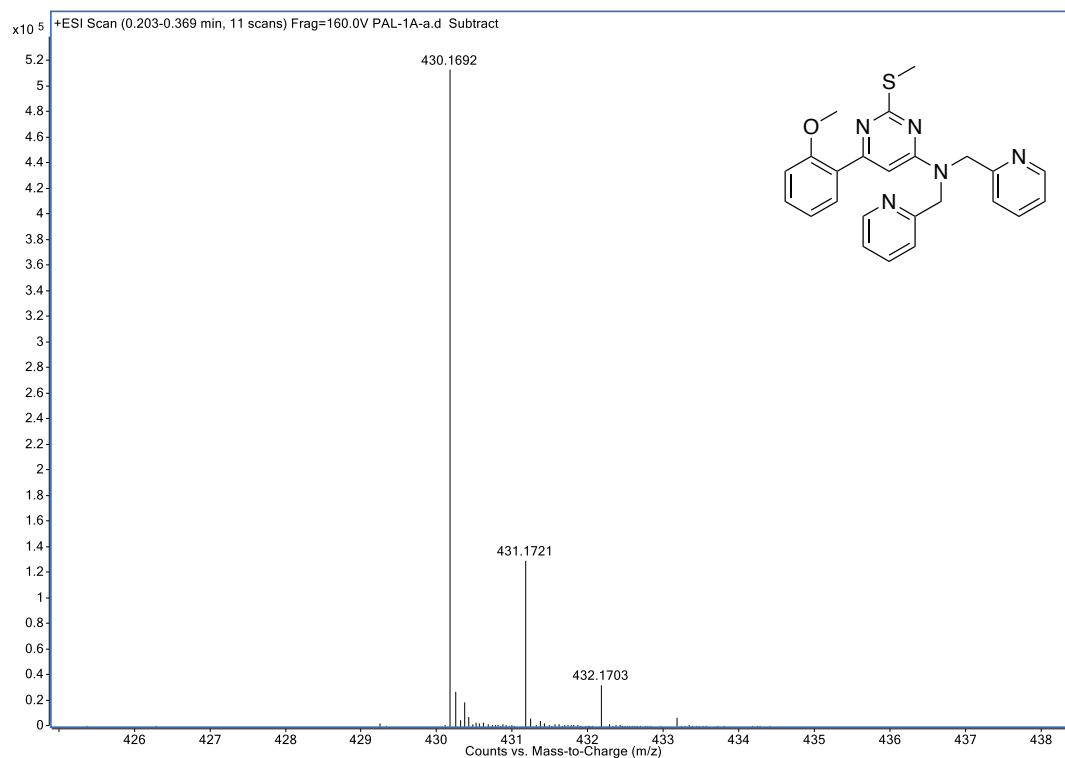
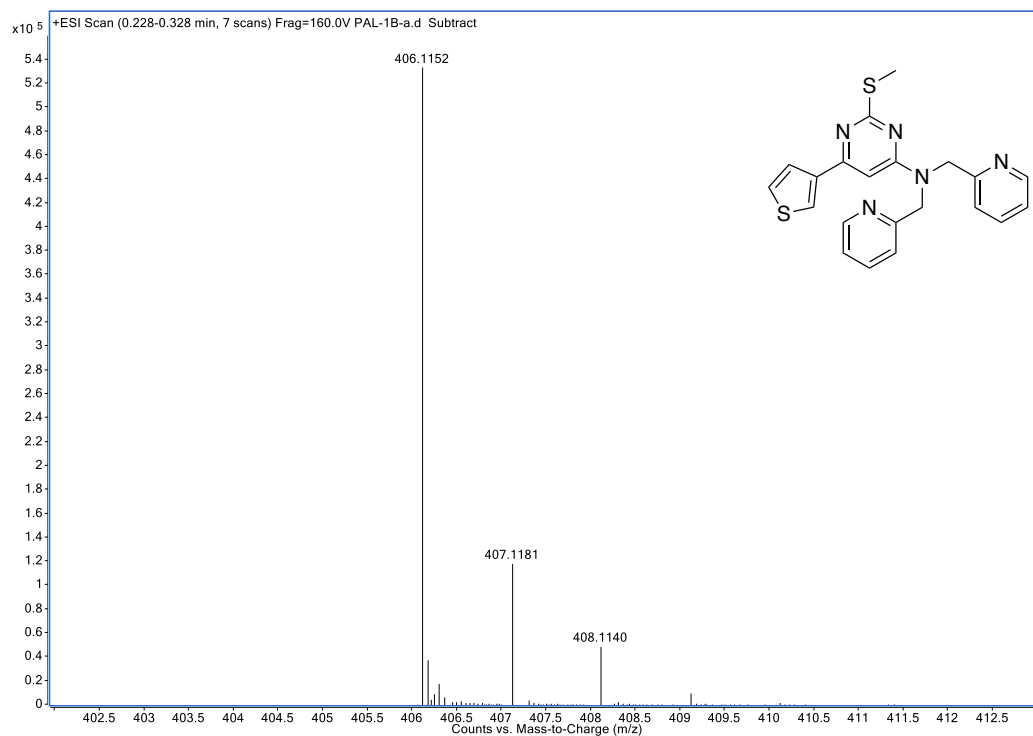
**Figure A61.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-171M.**Figure A62.** High resolution-Mass Spectrometry (HR-ESI-MS) of AC-17-2.

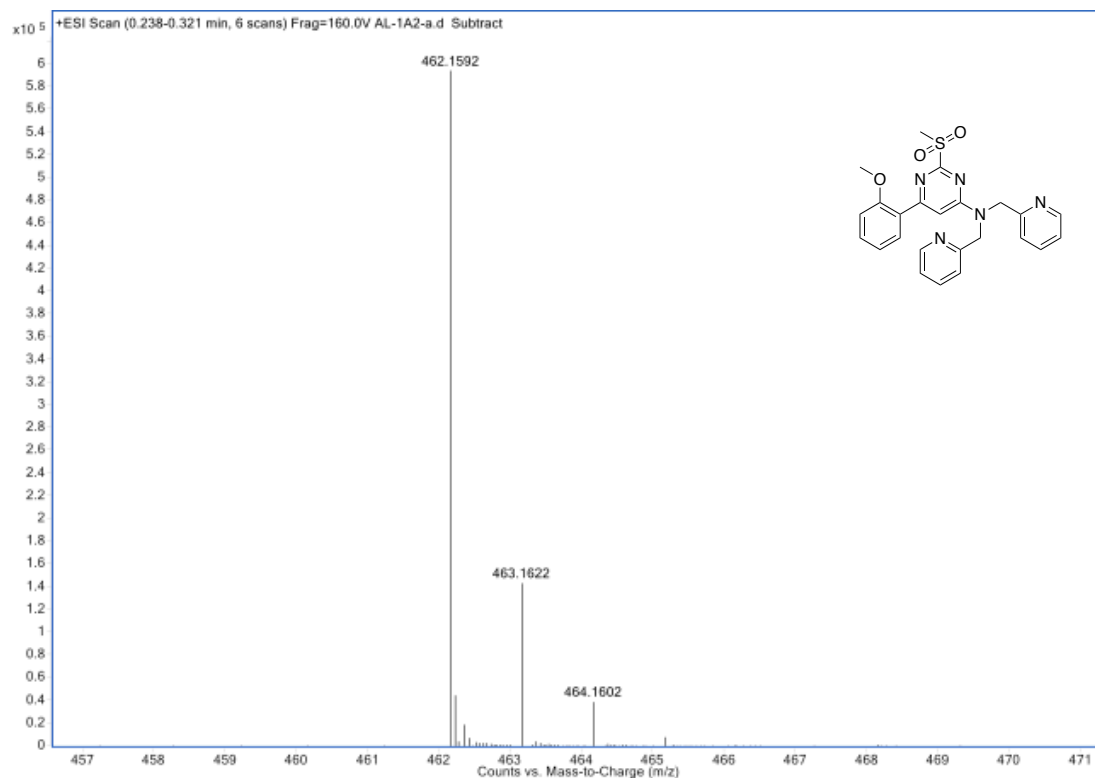
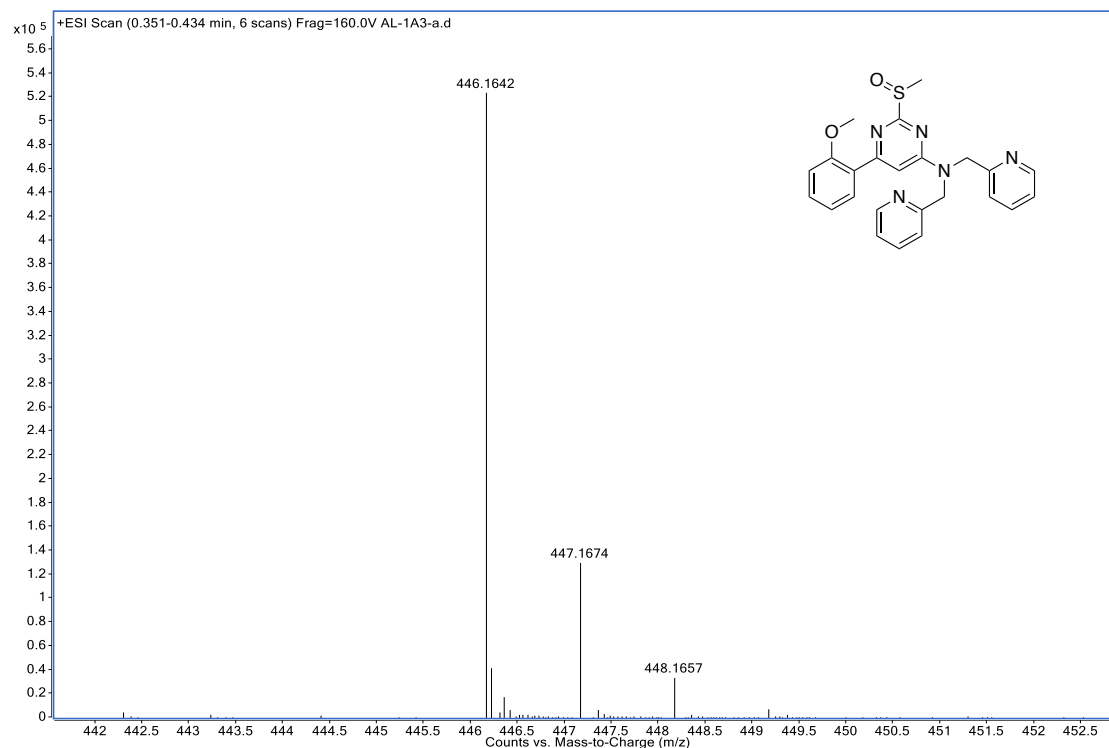
**Figure A63.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-19-2.**Figure A64.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-20-2.

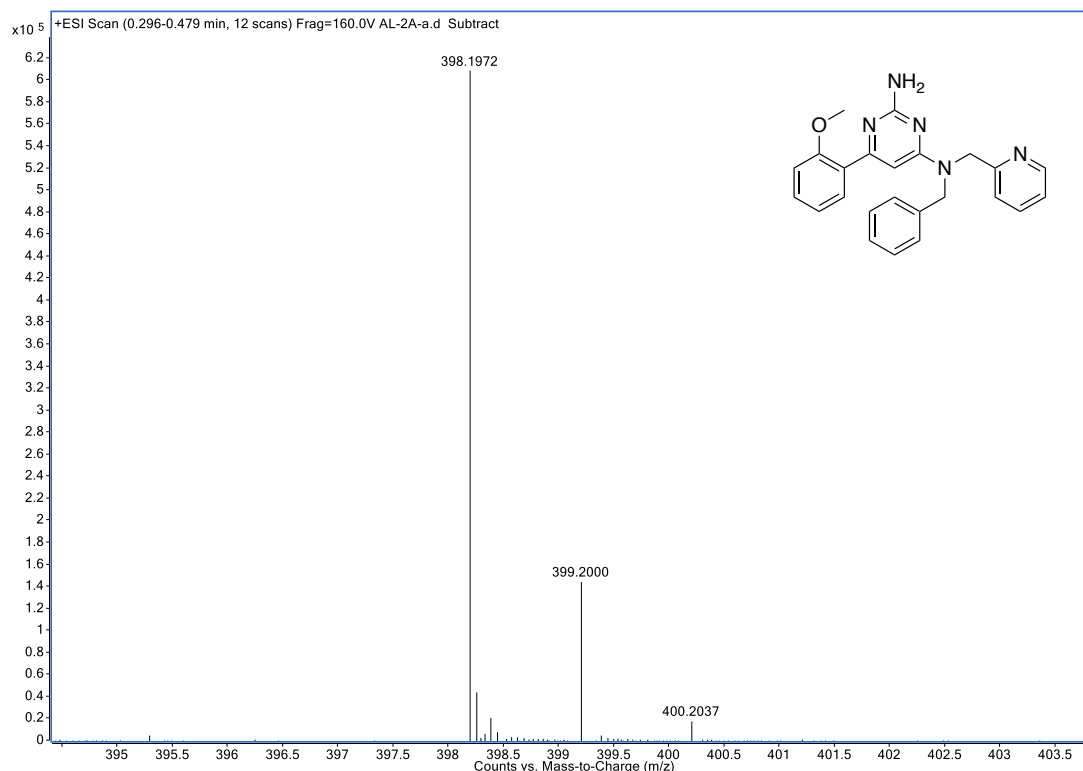
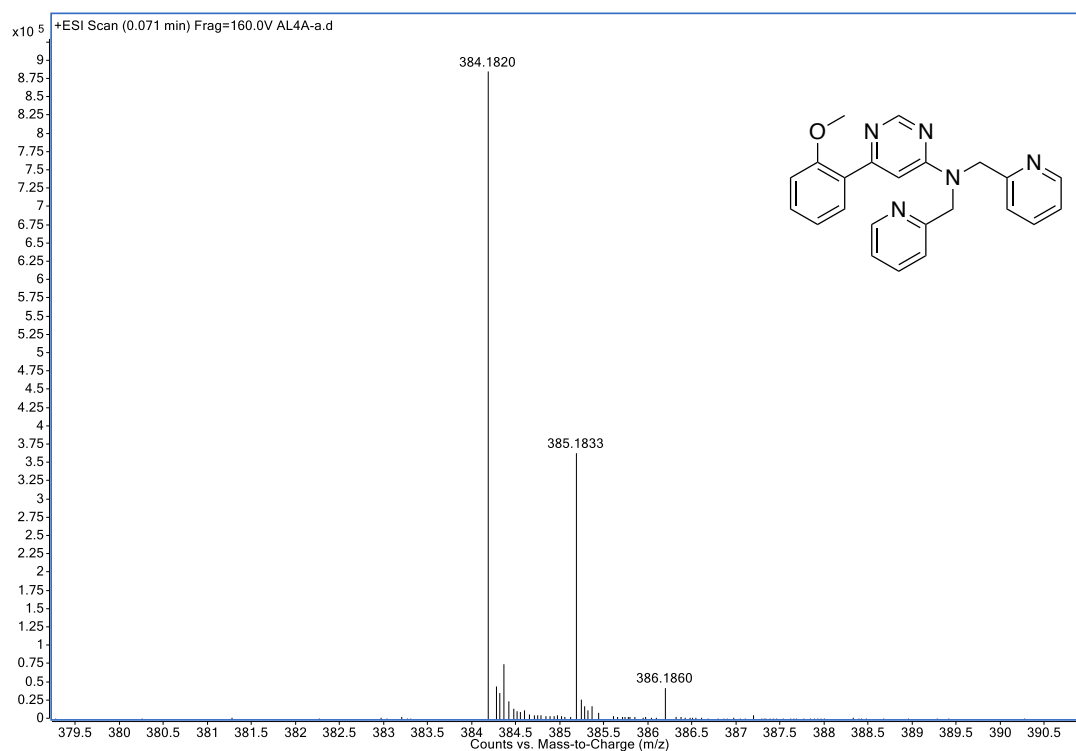
**Figure A65.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AC-201A2.**Figure A66.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AL-P2.

**Figure A67.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of **AL-1**.**Figure A68.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of **AL-2**.

**Figure A69.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AL-4.**Figure A70.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AL-6.

**Figure A71.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AL-1A.**Figure A72.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AL-1B.

**Figure A73.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AL-1A2.**Figure A74.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AL-1A3.

**Figure A75.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of **AL-2A**.**Figure A76.** High resolution-Mass Spectrometry (HR-ESI-TOFMS) of **AL-4A**.

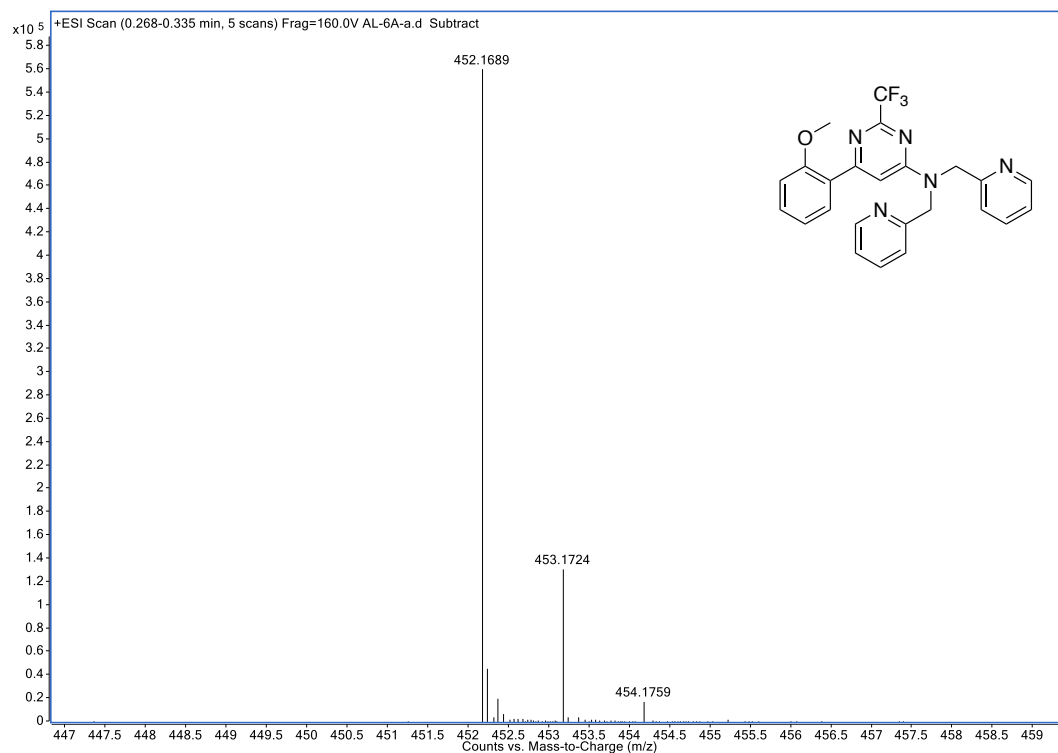


Figure A77. High resolution-Mass Spectrometry (HR-ESI-TOFMS) of AL-6A.

Gisela Andrea Camacho Hernández, MS

gicamacho@ucsd.edu
andrea.camacho16@uabc.edu.mx

2014-Present **Doctor of Philosophy in Science.** Universidad Autónoma de Baja California / University of California San Diego, Joint program.
Advisor: Dr. Palmer Taylor / Dr. José Manuel Cornejo-Bravo.

LANGUAGES

Spanish (Native language)
English (Proficient, fluent)
French (Beginner)

EDUCATION

2011-2013 **Master in Science.** Universidad Autónoma de Baja California (UABC)
Graduated with honors (*cum laude*).

2006-2010 **Bachelor of Science.** Químico Farmacobiólogo (Biopharmaceutical Chemistry). Universidad Autónoma de Baja California (UABC)
Graduated with honors (*cum laude*).

RESEARCH EXPERIENCE

2014-2018 **Ph. D. Candidate,** Universidad Autónoma de Baja California / University of California San Diego, Joint program.
Advisor: Dr. Palmer Taylor / Dr. José Manuel Cornejo-Bravo.

2011-2013 **Master in science research,** Universidad Autónoma de Baja California.
Advisor: Dr. Juan Cruz Reyes/ Dr. Raúl Romero Rivera

2007-2010 **Undergraduate Research,** Universidad Autónoma de Baja California.
Advisor: Dr. Iván Cordova Guerrero

2007-2009 **Undergraduate Research,** Universidad Autónoma de Baja California.
Advisor: Dr. Juan Cruz Reyes/ Dr. Raúl Romero Rivera

POSTERS PRESENTATION AND ABSTRACTS

1. Structure-activity relationships of a distinct family of selective agonists of $\alpha 7$ -nAChRs. Experimental Biology, San Diego California, April 21-25 2018.
2. Inhibition of pentameric ligand-gated ion channels by lophotoxin, a diterpenoid from coral *Lophogorgia chilensis*. Experimental Biology, San Diego California, April 21-25 2018.
3. Structure-guided design for agonist selectivity of the $\alpha 7$ -nAChRs. 2nd ASPET/ADDC Academic drug discovery colloquium. Bethesda, Maryland, October 12-13 2017.
4. Structure-guided design for ligand agonist selectivity of the $\alpha 7$ -nAChRs. Experimental Biology, Chicago, Illinois, April 22-26 2017.

5. A new structural landscape for ligand binding to the $\alpha 7$ nicotinic acetylcholine receptor. XVth International Symposium on Cholinergic Mechanisms. Marseille, France, October 16-20, 2016.
6. A new structural landscape for ligand binding to the $\alpha 7$ nicotinic acetylcholine receptor. Pharmacology and training grant program retreat, May 26, 2016.
7. A new structural landscape for ligand binding to the $\alpha 7$ nicotinic acetylcholine receptor. ASPET meeting in the Experimental Biology Meeting, San Diego California, April 2-6, 2016.
8. A new structural landscape for ligand binding to the $\alpha 7$ nicotinic acetylcholine receptor. TRDRP Meeting. Sacramento, California, October 27, 2015.
9. A new structural landscape for ligand binding to the $\alpha 7$ nicotinic acetylcholine receptor. Molecular Pharmacology Track Orientation. University of California San Diego, September 21, 2015.
10. A new structural landscape for ligand binding to the $\alpha 7$ nicotinic acetylcholine receptor. XLVIII Congress of Pharmaceutical Sciences, held in Cancun Quintana Roo, Mexico, September 6-9 2015.
11. Synthesis, characterization and catalytic activity molybdenum sulfide, Catalysts molybdenum sulfide derivatives alkyltrimethylammonium thiomolybdates. IX International Simposium: Chemical Research in the border region, Held in Tijuana B. C. November 20 – 22, 2013.
12. Antioxidant Structure-activity relationship of new analogs of capsaicinoids and capsinoids. The 8th International Meeting on Natural Products Research in Huatulco, Oaxaca from 23 to 25 May 2012.
13. Synthesis and Antioxidant Activity of Capsaicin Analogs and Compounds Derived from Vanillin. 8th Spanish-Italian Symposium on Organic Chemistry (SISOC-VIII). Held in Padova, Italy, July 3rd-6th 2010.
14. Antioxidant Activity and Antitumor Evaluation of tetraalkylammonium thiomolybdates against different tumor cell lines of human origin. XLII Congress of Pharmaceutical Sciences, held in Cancun Quintana Roo, Mexico from October 18th-22nd 2009.
15. Synthesis, characterization and catalytic activity molybdenum sulfide in high surface area of the hydrogenation reaction of cyclohexane. II International Symposium on Science and Technology Expo & T, School of Chemical Sciences and Engineering, Tijuana B. C. 22 and November 23, 2007.

ORAL PRESENTATION

1. A new structural landscape for ligand binding to the $\alpha 7$ nicotinic acetylcholine receptor. XVth International Symposium on Cholinergic Mechanisms. Marseille, France, October 18, 2016.
2. A new structural landscape for ligand binding to the $\alpha 7$ nicotinic acetylcholine receptor. Research discussions, Pharmacology Department UCSD, San Diego California, April 22, 2016.
3. A new structural landscape for ligand binding to the $\alpha 7$ nicotinic acetylcholine receptor. ASPET meeting in the Experimental Biology Meeting, San Diego California, April 5, 2016.
4. A new structural landscape for ligand binding to the $\alpha 7$ nicotinic acetylcholine receptor. TRDRP Meeting. Sacramento, October 27, 2015.
5. Molybdenum sulfide catalysts alkyltrimethylammonium derivatives thiomolybdates. Seminar program 2010-2 Faculty of Chemical Sciences and Engineering, UABC. Tijuana B.C. Mexico, May 2, 2013.

6. Synthesis and structure-activity relationship of a series of new analogues of capsaicinoids and capsinoids with antioxidant profile. Short talk on the VIII International symposium chemical research in the border. Held in Tijuana B. C. November 17, 2011.
7. Correlation Study of Structure-Antioxidant Activity of Capsaicin Analogs. Seminar program 2010-2 Faculty of Chemical Sciences and Engineering, UABC. Tijuana B.C. Mexico, November 11, 2010.

PUBLICATIONS

1. Substituted 2-Aminopyrimidines Selective for $\alpha 7$ -Nicotinic Acetylcholine Receptor Activation and Association with Acetylcholine Binding Proteins. Katarzyna Kaczanowska*, **Gisela Andrea Camacho-Hernandez***, Larissa Bendiks, Larissa Kohs, Michal Harel, M.G. Finn, Palmer Taylor. J. Am Chem Soc. 2017 Mar 15;139(10):3676-3684. *Equal contribution.
2. HDS of DBT with molybdenum disulfide catalysts prepared by in situ decomposition of alkyltrimethylammonium thiomolybdates. R. Romero-Rivera, **A. G. Camacho**, et al. *Top Catal* (2011) 54:561-567.

FELLOWSHIPS AND AWARDS

2017	Akira E. Takemori Graduate Student Travel Award. Experimental Biology, Chicago, Illinois, US.
2016	B.P. Doctor Young Investigator Award. XVth International Symposium on Cholinergic Mechanisms. Marseille, France.
2016	Best Neuropharmacology Poster. Pharmacology and training grant program retreat UCSD, San Diego, Ca.
2014-2018	National Fellowship CONACYT-Mexico (Ph D)
2014	Centro de Investigacion y Estudios Avanzados del IPN, Bio-CINVES Travel Award 2014 Conferences Mexico, DF.
2011-2013	National Fellowship CONACYT-Mexico (Master degree).
2008	Summer Research Program (Dolphin program) Training Grant.

TEACHING EXPERIENCE

Class and laboratory courses:

1. General Chemistry, Universidad Autonoma de Baja California School of Chemical Sciences and Engineering, Undergrad Laboratory Period: 02/2013-06/2015
2. Biochemistry, Universidad Iberoamericana Nutrition degree, Undergrad Class Period: 08/2013-05/2014
3. Organic Chemistry, Universidad Iberoamericana Nutrition degree, Undergrad Class Period: 01/2014-05/2014
4. Metabolism Biochemistry, Universidad Iberoamericana Nutrition degree, Undergrad Class Period: 05/2014-07/2014
5. Analytical Chemistry, Universidad Autonoma de Baja California School of Chemical Sciences and Engineering, Undergrad Laboratory and Class Period: 08/2013-12/2013

Mentoring:

- 2009-2012 Karina Granados Vega: Undergraduate research student, UABC, Presently: Graduate student, Universidad de Granada, Spain.
- 2010-2012 Lucia Castillo Garibay: Undergraduate research student, UABC.
- 2015-2016 Larissa Kohs: Pharmacy research student, Friedrich-Alexander University of Erlangen-Nurnber. (6 months)
- 2016-2016 Christina Beck: Pharmacy research student, Ludwig Maximilian University of Munich, Presently: Graduate student at LMU. (6 months)
- 2016-2017 Lisa Doan: Research Staff, UCSD. Presently: Pharmacy student University of Utah.
- 2016-2017 Hanna Binding: Pharmacy research student, University of Regensburg. (6 months)
- 2017-2017 Laura Huber: Pharmacy research student, Pharmacy research student, Ludwig Maximilian University of Munich. (6 months)
- 2018-2018 Nila Rahmatyan: Pharmacy research student, Heinrich-Heine University of Dusseldorf. (6 months)

AFFILIATION

2016-Present Member of the American Society for Pharmacology and Experimental Therapeutics (ASPET).