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Medicinal Chemistry

Open Access

ISSN: 2161-0444

Temple University School of Pharmacy

Department of Pharmaceutical Sciences

Moulder Center for Drug Discovery Research

Daniel Canney, Ph.D.

Chair and Director of Graduate Studies

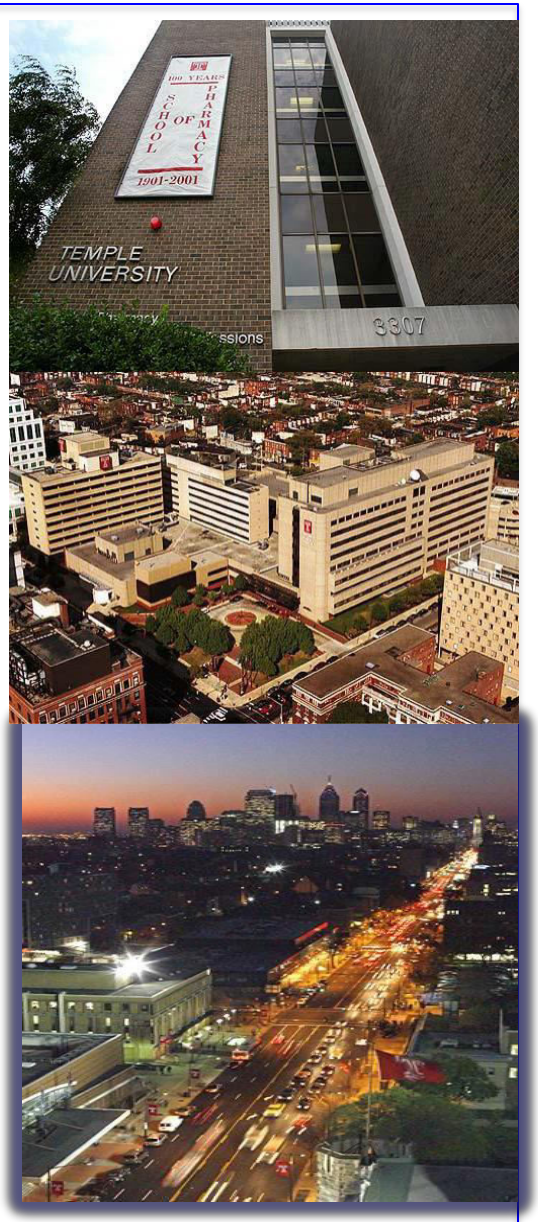
About TUSP …

- **Temple University School of Pharmacy** is one of the oldest pharmacy schools in the country (Founded 1901) and is part of a major educational and research institution.
- Located on a **comprehensive health sciences campus** which includes: Schools of Pharmacy, Medicine, Dentistry, Health Professions, Temple Hospital, Temple Children's Medical Center
- TUSP maintains high standards while adhering to the University's mission of providing affordable, high quality education to those who wish to learn regardless of income

- **Current T.U.S.P. Centers and Facilities:**
  - Moulder Center for Drug Discovery Research (MCDRR)
  - In Vitro ADME and Pharmacokinetics Laboratory
  - cGMP facility
  - Proteomics facility
  - Jayne Haines Center for Pharmacogenomics and Drug Safety
- **Current Programs**

– Doctor of Pharmacy (**PharmD**) degree
– **MS and PhD** in Pharmaceutical Sciences
  - Concentrations: Pharmaceutics, Medicinal Chemistry, & Pharmacodynamics

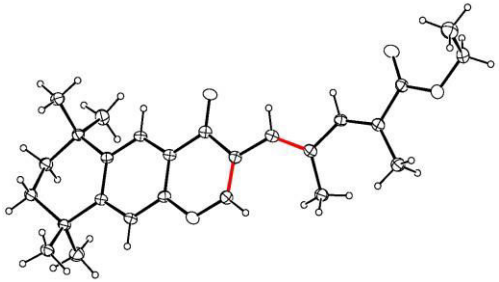
– **DBMD Program** through the Office of International Studies
– **MS - Quality Assurance/Regulatory Affairs** (premier US program)



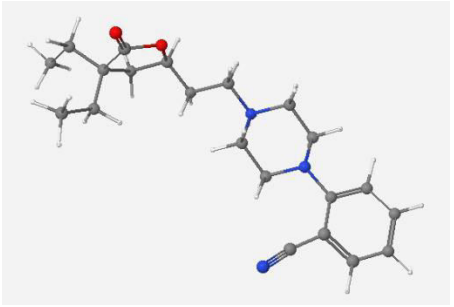
http://www.temple.edu/pharmacy/graduate.htm

Daniel J. Canney, Ph.D., R.Ph.

Chair, Department of Pharmaceutical Sciences
Director of Graduate Studies



RAR ligand
(IC_{50} = 40nM)



Muscarinic ligand
(IC_{50} = 17 nM)

Rong Gao, *Siva Annadurai, Safura Nantogma,* ***Richie Bhandare***, *Otito Iwuchukwu,*
(Shyam Desai, Weilin Sun; graduates of program)

Lead Optimization, Structure-Activity Relationship (SAR) Studies

- Cholinergic receptor (nicotinic and muscarinic) ligands
- Serotonergic receptor ligands and ligands for other GPCRs
- Retinoic acid receptors (RAR) ligands

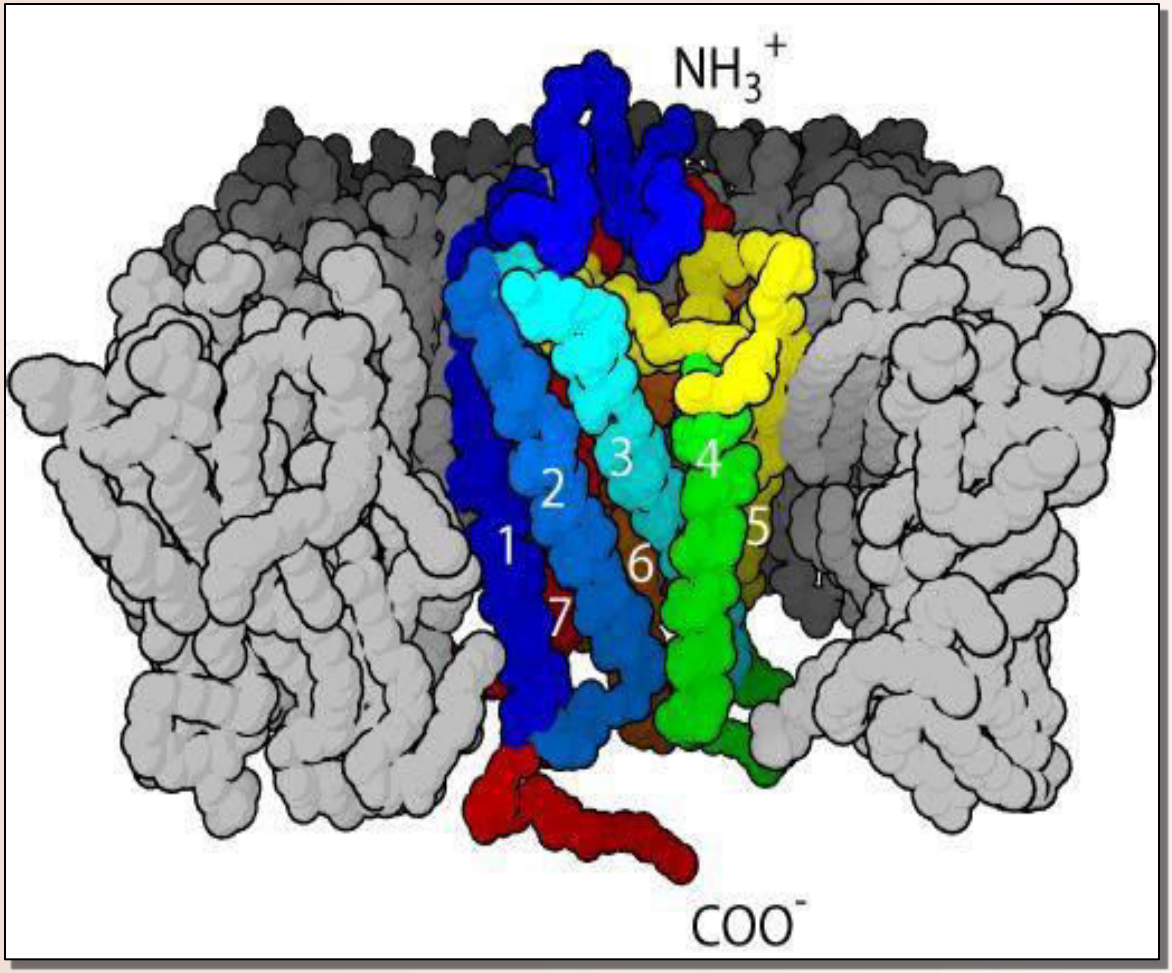
Lead modification approaches in the design, synthesis and evaluation of novel muscarinic ligands

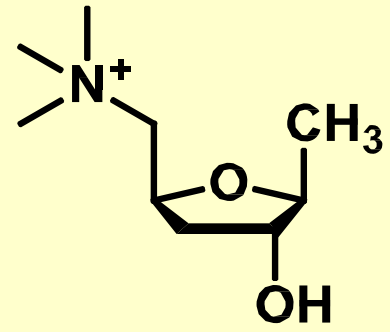
Dr. Daniel J. Canney

Lead Modification – Muscarinic Ligands

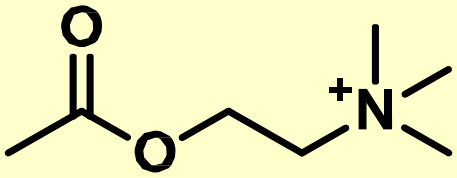
- **Background Information**
  - ❑ Muscarinic receptors
  - ❑ Crystal structures
  - ❑ Ligands and allosteric regulators
- **Research Design and Results**
  - ❑ Lead molecules and specific aims
  - ❑ Molecular modification strategy
    - Region 1: cationic center
    - Region 2: hydrogen bonding region
    - Region 3: linker
    - Region 4: carbonyl oxygen
- **Summary**
- **Acknowledgement**

Muscarinic receptors - G-Protein Coupled Receptors





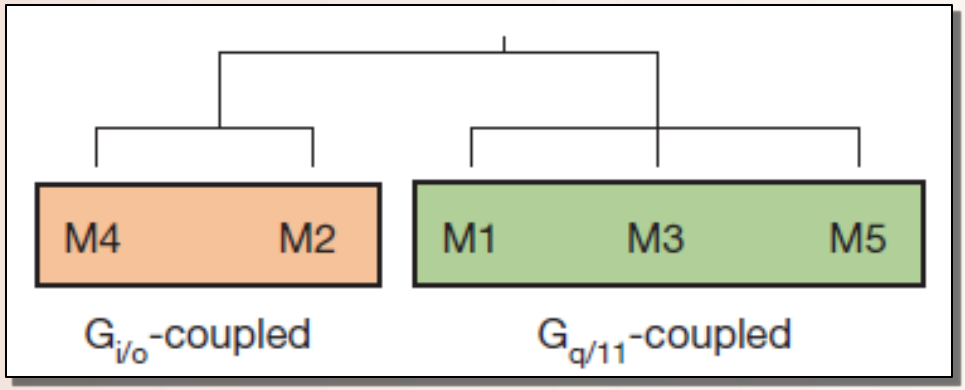
Muscarine



Acetylcholine

GPCR

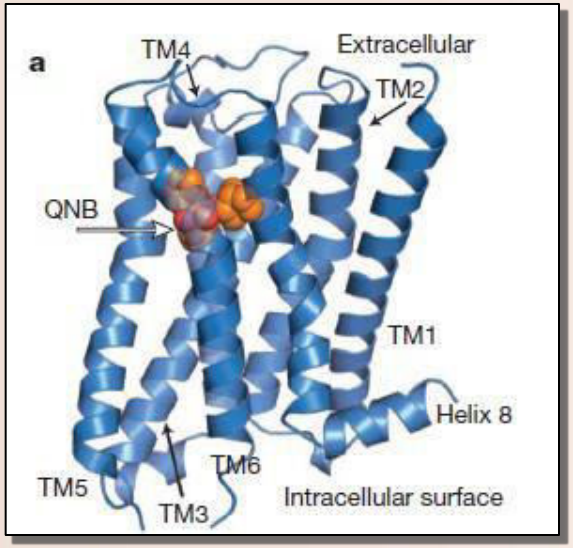
Muscarinic Receptors Families



Kruse AC and etc, *Nature.*, **2012**, 482, 552-559

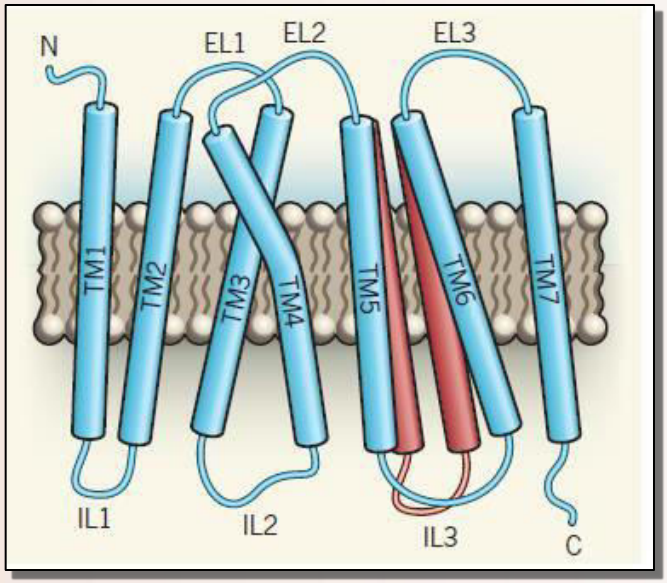
	Distribution	Therapeutic potential
M_1	CNS	Alzheimer's disease (agonist) Parkinson's disease (PD; antagonist)
M_2	CNS, Heart	Alzheimer's disease (antagonist)
M_3	CNS, smooth muscles	OAB, COPD, IBS (antagonist)
M_4	CNS	Parkinson's disease (PD: antagonist)
M_5	CNS	Parkinson's Disease: Addiction ?

Crystal Structures



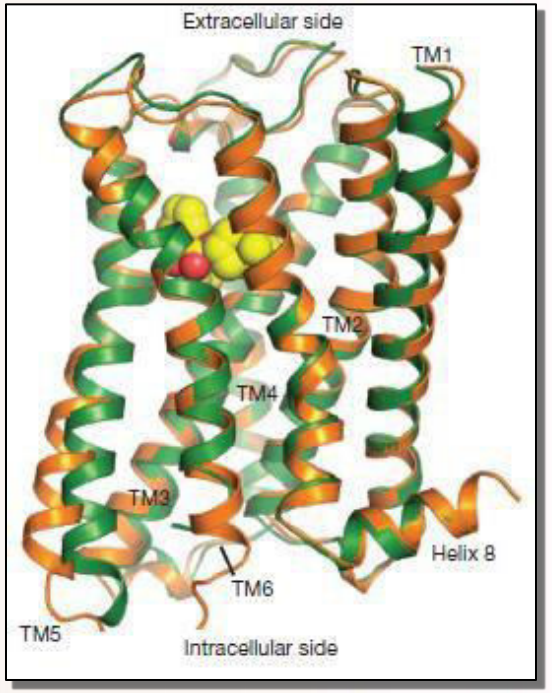
M_2 receptor with QNB

Haha K, Kruse AC and et. al.
Nature, **2012**, 482, 547-552



Differences between M_2 and M_3 receptor subtypes.

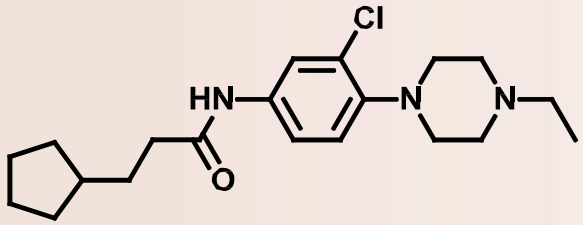
Kow, RL; Nathanson, NM
Nature., **2012**, 482, 480-481



Overlap of M3 receptor (green) and M2 receptor (orange)

Kruse AC and et al
Nature., **2012**, 482, 552-559

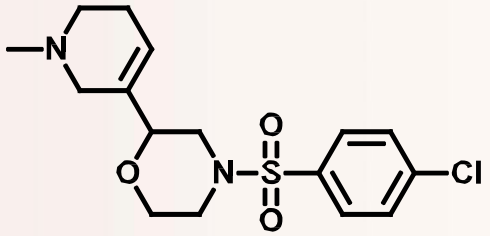
Muscarinic Ligands



3a

M_1 antagonist, Ki=12.7nM, 6-35 folds selectivity

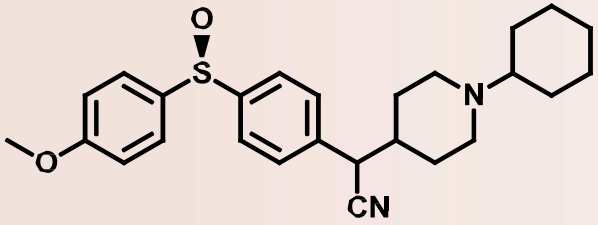
Lewis, L. M. and et.al. *Bioorg. & Med. Chem. Lett.* **2008**, 18, 885



7a

M_1 agonist, Ki=260nM

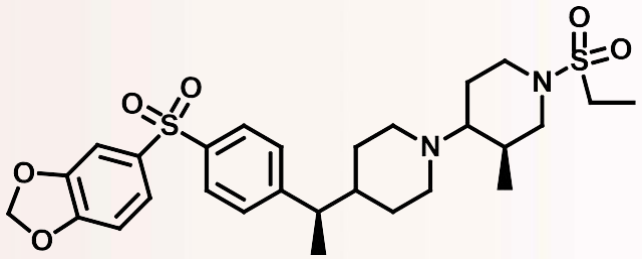
Malviya, M. and et.al. *Bioorg. & Med. Chem.* **2009**, 17, 5526



13a

M_2 antagonist
Ki=2.7nM, M_2/M_1 selectivity of 40-fold

Kozlowski, J. A.and et. al. *Bioorg. & Med. Chem. Lett.,* **2000**, 10, 2255

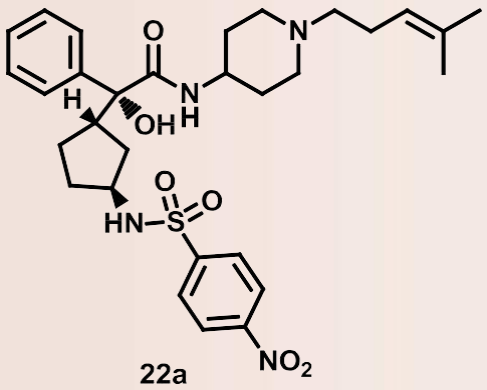


16a

M_2 antagonist
Ki=0.7nM, M_2/M_1 selectivity of 109 fold

Kozlowski, J. A. and et.al. *Bioorg. & Med. Chem. Lett.,* **2002**, 12, 791

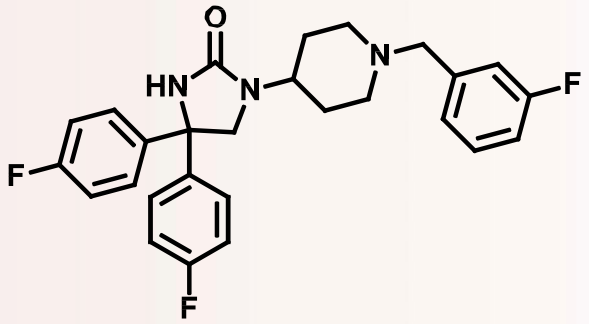
Muscarinic Ligands



22a

M_3 antagonist
Ki=2.5nM, M_3/M_2 selectivity of 1100 fold

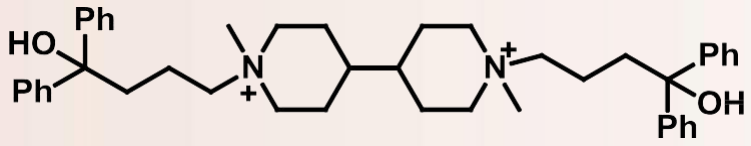
Mitsuya, M. and et.al. *Bioorg. & Med. Chem. Lett.,* **2000**, 8, 825



24a

M_3 antagonist
Ki<10nM, M_3/M_2 selectivity > 200 fold

Peretto, I. and et. al. *J. Med. Chem.* **2007,** *50,* 1571



34a

M_4 antagonist
pKi=7.9, selectivity 30-50 fold

Varoli, L. and et. al. *Bioorg. & Med. Chem. Lett.,* **2008**, 18, 2972

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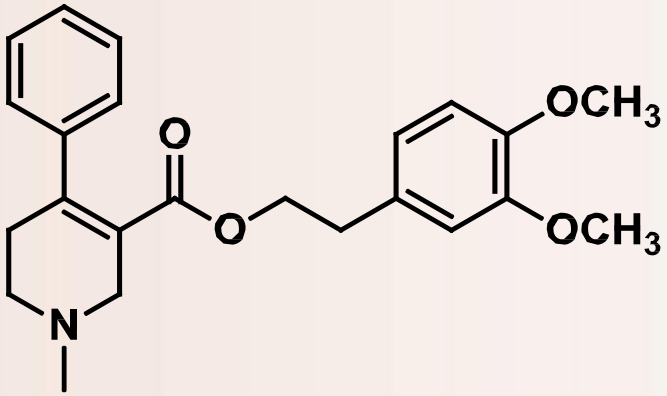
Muscarinic Ligands



40a

M_5 antagonist

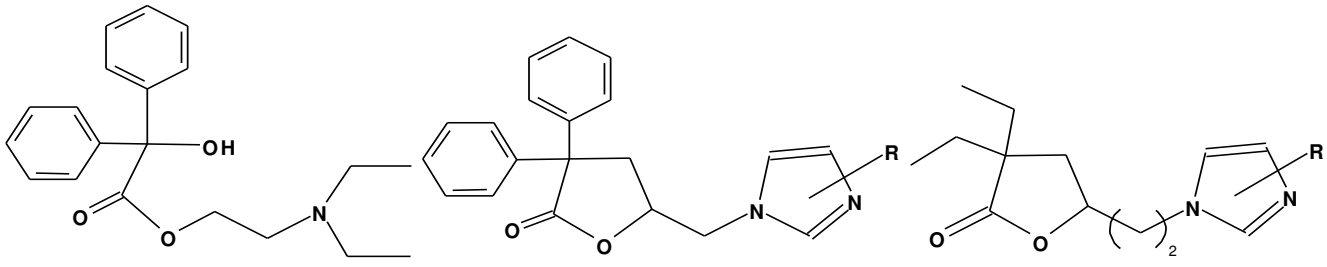
- 11-fold selectivity for M_5/ M_1, little activity at the M_2-M_4
- modest affinity (Ki = 2.24 μM), potent (IC_{50} = 0.45 nM)

Zheng, G., Smith, A. M., Dwoskin, L. P., *J. Med. Chem.*, **2013**, 56, 1693-1703

Lead Modification – Muscarinic Ligands

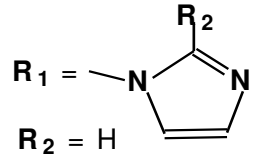
- **Background Information**
  - ❑ Muscarinic receptors
  - ❑ Crystal structures
  - ❑ Ligands and allosteric regulators
- **Research Design and Results**
  - ❑ **Lead molecules and specific aims**
  - ❑ **Molecular modification strategy**
    - **Region 1: cationic center**
    - **Region 2: hydrogen bonding region**
    - **Region 3: linker**
- **Summary**
- **Acknowledgement**

Lead Optimization – Addition of Aryl Rings

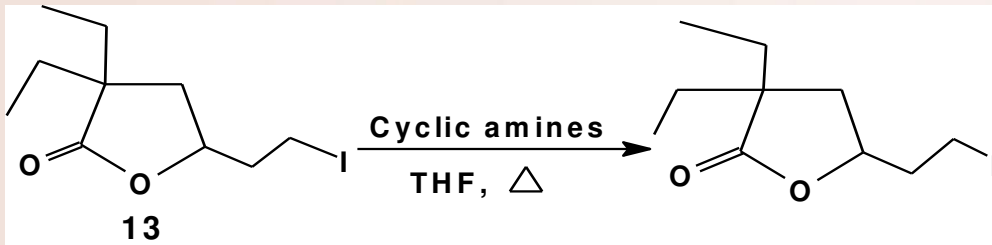


Benactyzine "Kaiser Lactones" Target Lactones

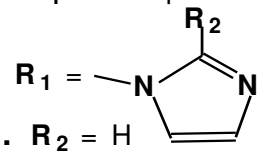
2. R_1 = piperidine
3. R_1 = morpholine



4. R_2 = H
5. R_2 = CH_3
6. R_2 = CH_2CH_3
7. R_2 = $CH_2CH_2CH_3$
8. R_2 = $CH(CH_3)_2$



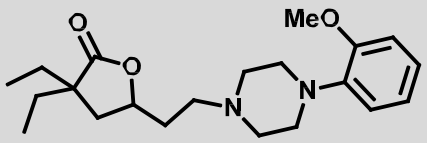
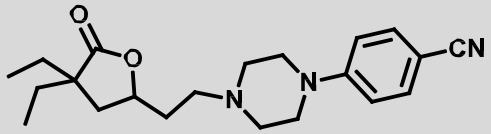
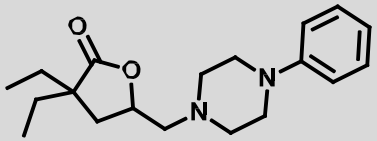
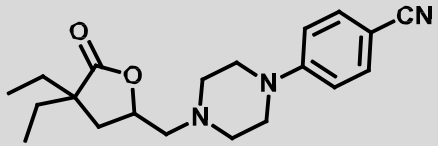
14. R_1 = Piperidine
15. R_1 = Morpholine



16. R_2 = H
17. R_2 = CH_3
18. R_2 = i-Propyl

Ahungena, A., Gabriel, J.L., Canney, D.J. *Med. Chem. Res.*, 12:9, **2003**, 481-511

Preliminary Data: Lead Modification and N-aryl-piperazines

#	structure	%inhib [1]
L5		82
L9		57
RB1		18
RB2		19

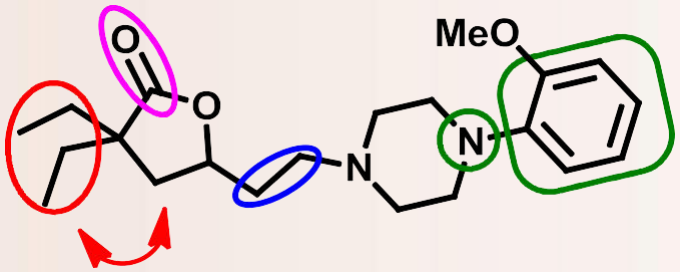
[1] % inhibition at 10 μM against muscarinic receptors

Hypothesis: systematic modification of H-bonding, cationic and linker regions of lactone-based leads will improve ligand affinity (selectivity?)

Ahungena, A., Gabriel, J.L., Canney, D.J. *Med. Chem. Res.,* 12:9, **2003**, 481-511
Bhandare, R., Canney, D. J. *Med Chem Res.,* 20**, 2010**, 558-565.

Specific Aims

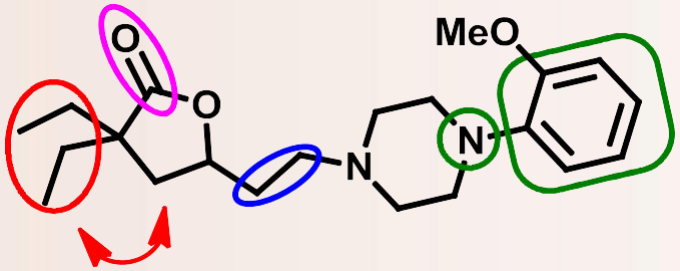
Specific Aim 1: **Design a novel series of muscarinic ligands through addition of aromatic substituents and** systematic modification of H-bonding, cationic and linker regions of lactone-based lead compounds to improve affinity, generate SAR data and ultimately develop subtype selective ligands.



Specific Aim 2: Develop **efficient synthetic routes** to the proposed ligands.

Specific Aim 3: **Evaluate** test compounds in muscarinic receptors binding assays

Lead Modification Strategy – The Specifics



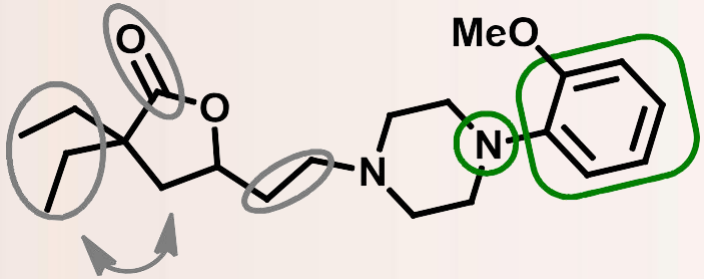
Hypothesis: It is possible to improve ligand affinity through systematic modification of H-bonding, cationic and linker regions of lactone-based lead compounds

Region 1: the physicochemical properties and position of the substituents on the cationic N-aryl piperazine region (and related heterocycles)

Region 2 the physicochemical properties and position of the substituents on the H-bonding lactone region

Region 3 the length and physicochemical properties of the linker

Lead Modification Strategy---Region 1



Hypothesis: It is possible to improve ligand affinity through systematic modification of H-bonding, cationic and linker regions of lactone-based lead compounds

Region 1:	the physicochemical properties and position of the substituents on the cationic N-aryl piperazine region (and related heterocycles)
Regioin 2	the physicochemical properties and position of the substituents on the H-bonding lactone region
Region 3	the length and physicochemical properties of the linker

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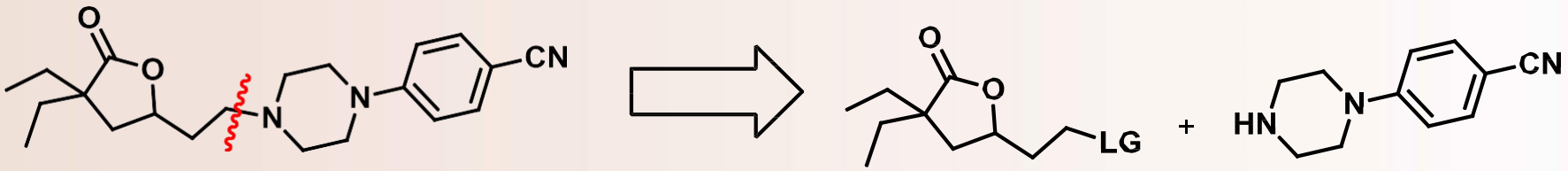
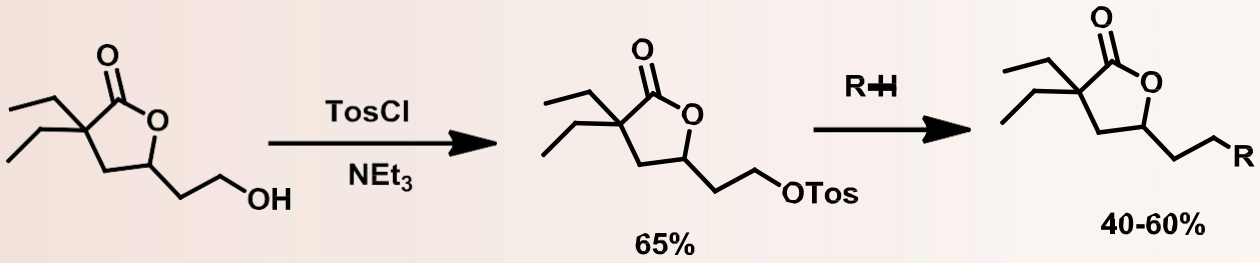
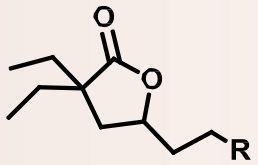
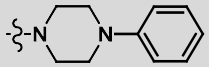
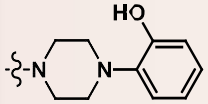
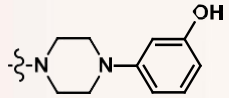
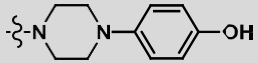
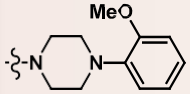
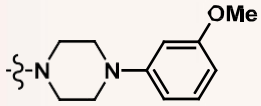
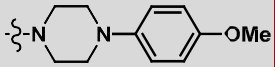
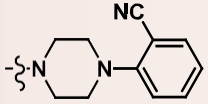
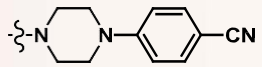
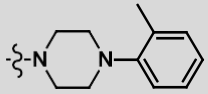
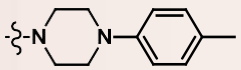
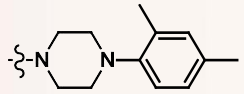
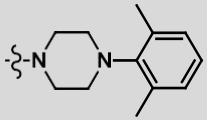
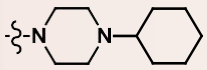
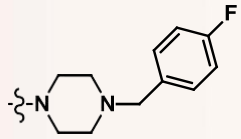
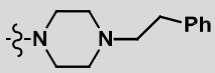
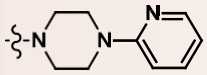
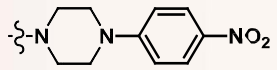
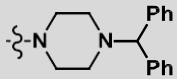
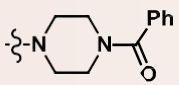
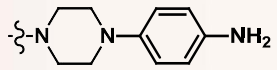
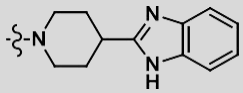
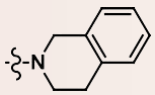
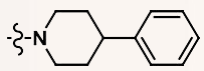
Region 1---Synthesis





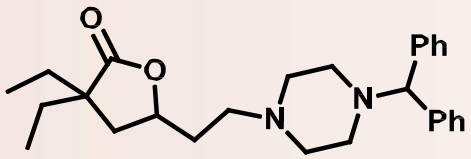
R = commercially available piperazine



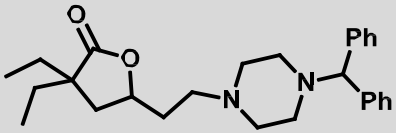
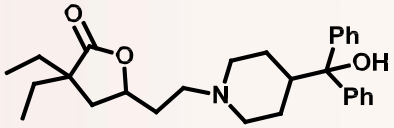
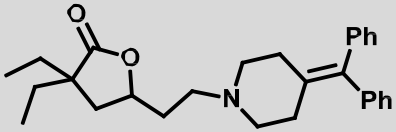
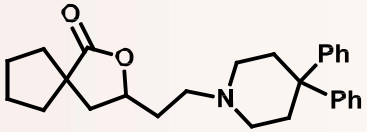
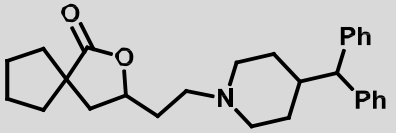
#	R	%inhib[1]	#	R	%inhib[1]	#	R	%inhib[1]
L1		74	L2		81	L3		61
L4		45	L5		82	L6		75
L7		56	L8		83	L9		57
L10		68	L11		67	L13		58
L14		58	L15		53	L16		63
L17		56	L18		64	L20		70
L21		99	L22		33	L23		47
L24		66	L25		57	L26		86

1. % inhibition at 10 μM against muscarinic receptors

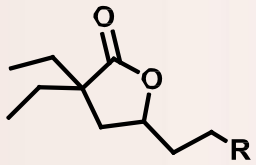
Results

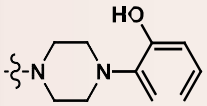
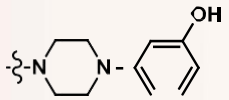
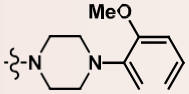
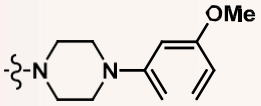
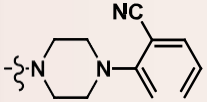
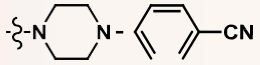
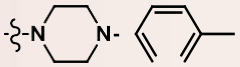
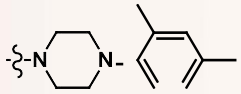
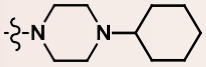
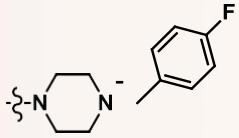
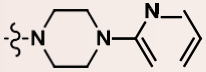
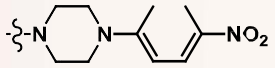
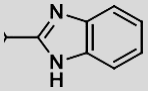
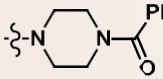
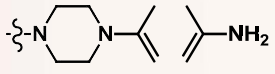
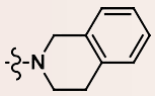
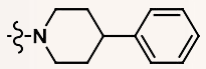


IC_{50} = 14nM[1]

#	structure	% inhib[2]	#	structure	% inhib[2]
L21		99	L27		96
L28		99	L30		94
L31		99			

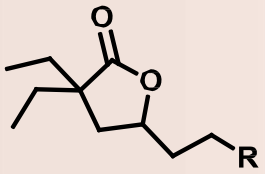
1. IC_{50} for muscarinic receptors
2. % inhibition at 10 μM for muscarinic receptors



#	R	%inhib[1]	#	R	%inhib[1]	#	R	%inhib[1]
L1		74	L2		81	L3		61
L4		45	L5		82	L6		75
L7		56	L8		83	L9		57
L10		68	L11		67	L13		58
L14		58	L15		53	L16		63
L17		56	L18		64	L20		70
L21		99	L22		33	L23		47
L24		66	L25		57	L26		86
								

1. % inhibition at 10 μM for muscarinic receptors

Region 1---Results



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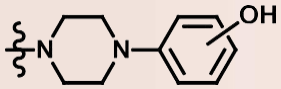
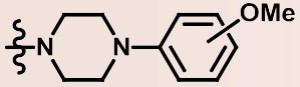
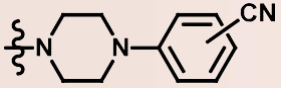
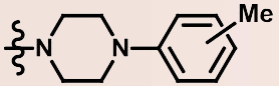
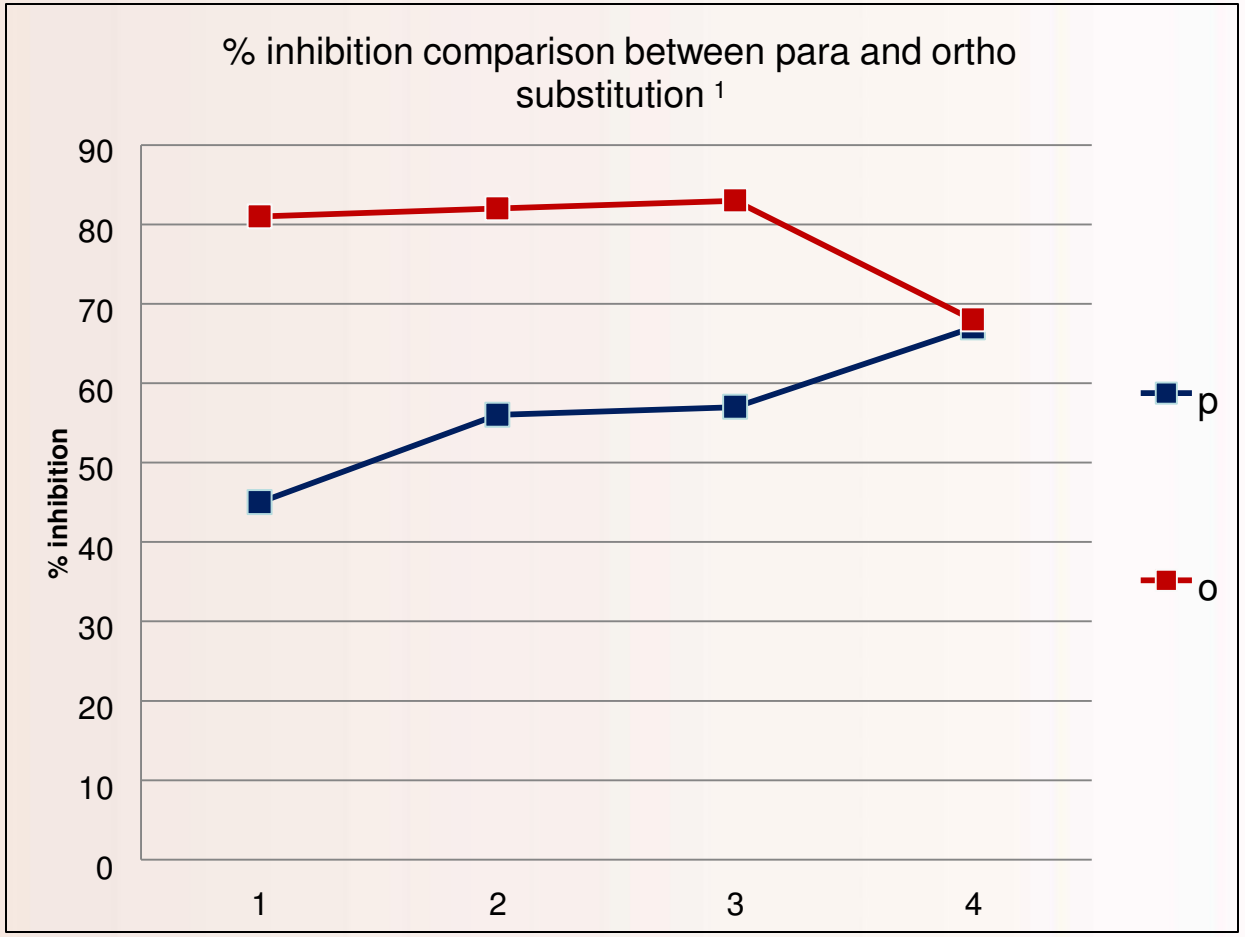
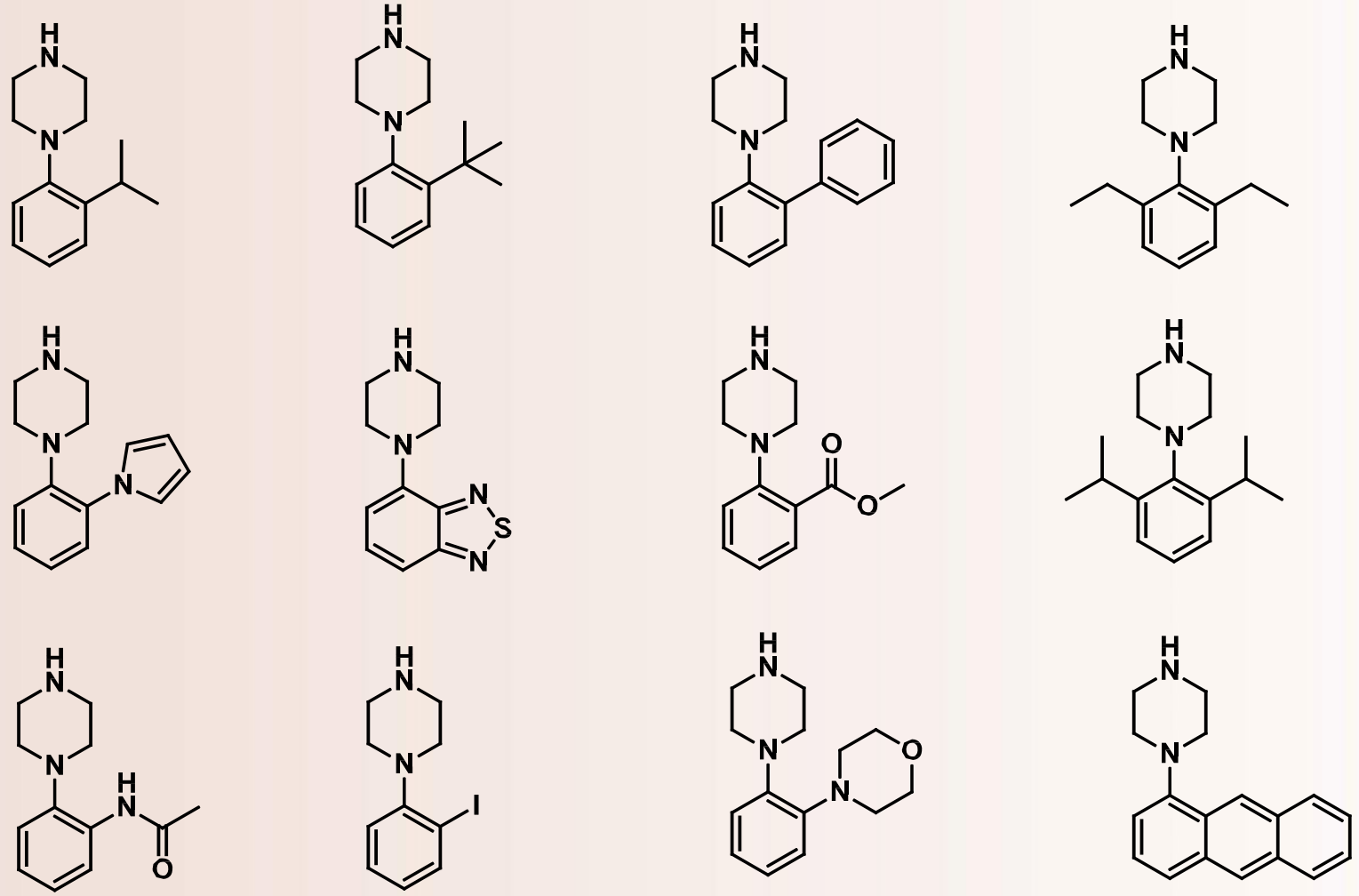
1	
2	
3	
4	

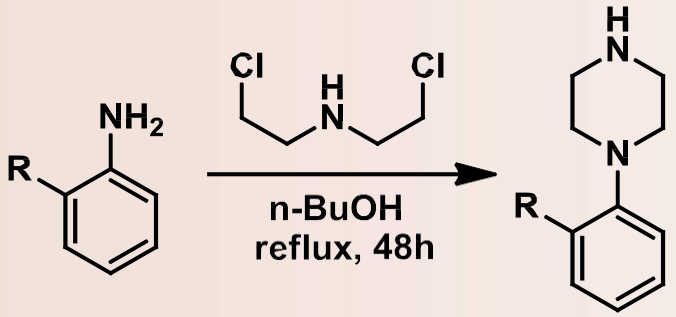


1. % inhibition at 10 μM against muscarinic receptors

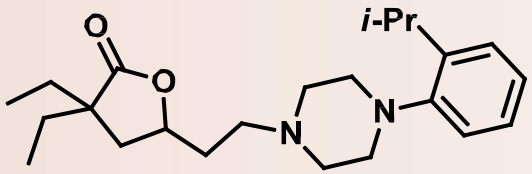
Target N-aryl Piperazines



Synthesis



R	Yield (%)
i-Pr	12
t-Bu	0
I	0

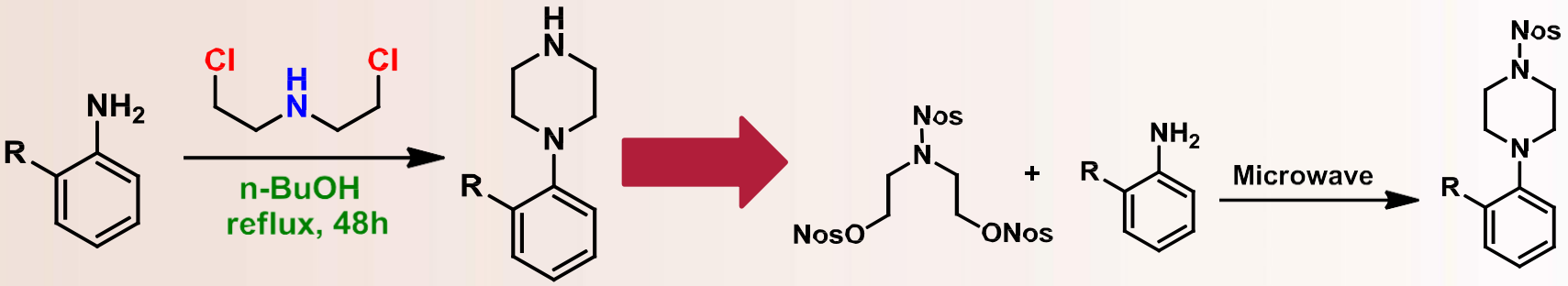


L19

Percent inhibition: 96% [1]

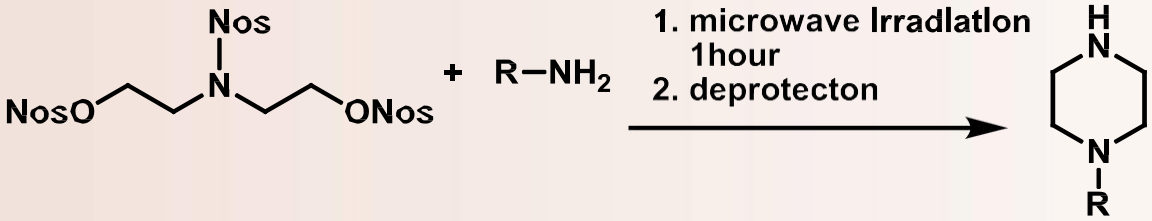
1. % inhibition at 10 μM against muscarinic receptors

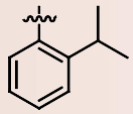
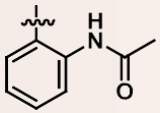
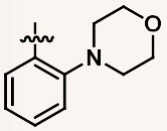
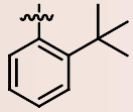
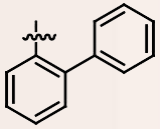
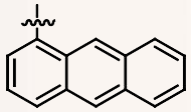
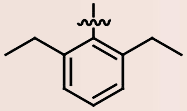
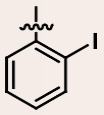
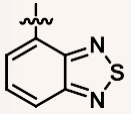
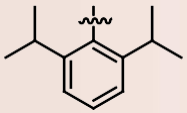
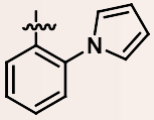
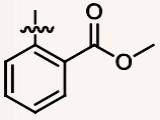
Synthesis of N-Aryl Piperazines



problems	solutions
Poor leaving group	Ns as leaving group
Unprotected amine	Ns as protecting group
High boiling point solvent	Microwave assisted reaction
Long reaction time	

Results

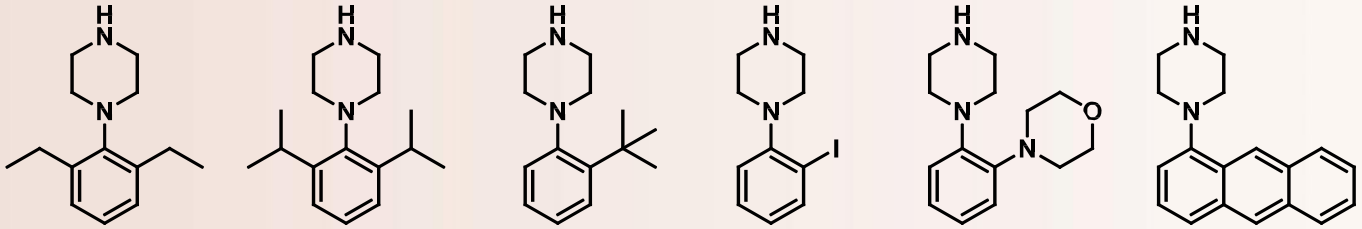


#	R	Yield	#	R	Yield	#	R	Yield
6a		80%	6e		68%	6i		81%
6b		71%	6f		86%	6j		71%
6c		72%	6g		65%	6k		60%
6d		61%	6h		71%	6l		66%

Gao, Rong.; Canney, Daniel., *J. Org. Chem.*, **2010**, 7451

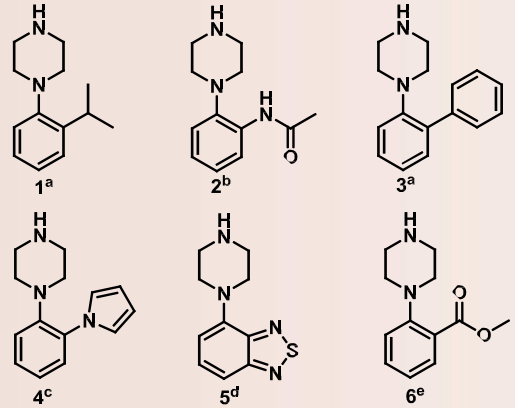
Results

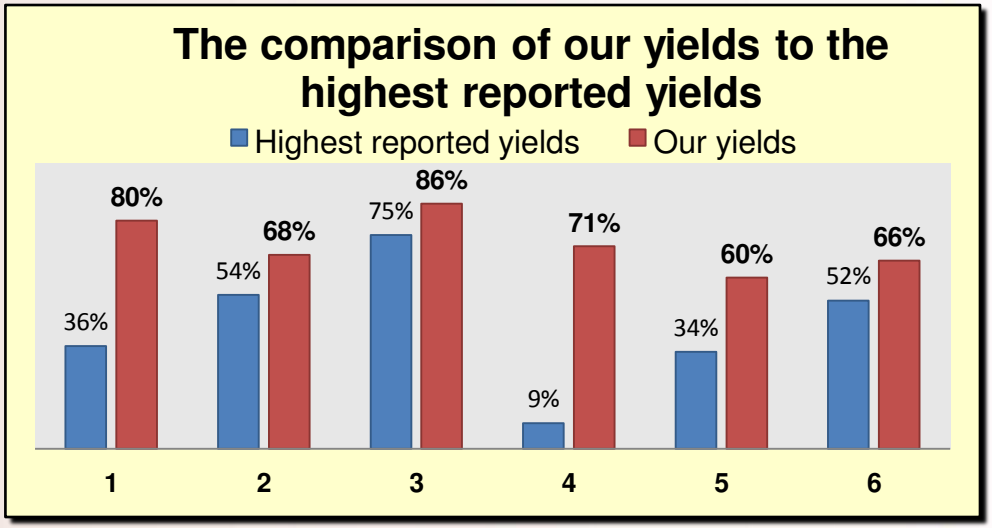
1. Provides the only facile route to access these molecules



Yield= 72% | Yield= 61% | Yield= 71% | Yield= 65% | Yield= 81% | Yield= 71%

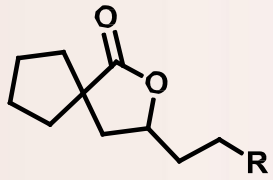
2. Provides an improved method to access these molecules in terms of yield and reaction time (24~72h → 1h).

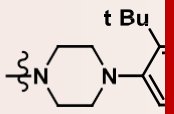
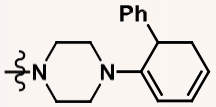
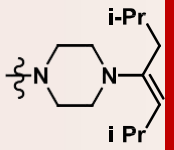
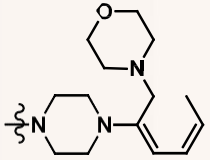
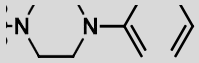
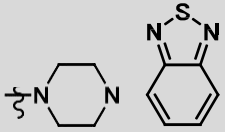
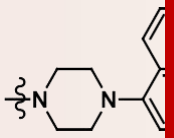
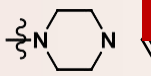
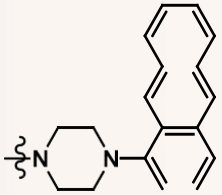




[a]Elworthy T. R. Bantle G. W.; *J. Med. Chem.* **1997,** *40,* 2674. [b]Mills, S. G.; MacCoss, M.; PCT Int. Appl. WO 9825617A1. [c]Roche, F. H.; Eur. Pat. Appl. EP 0748800A2, **1996;** [d]Heinrich, T.; Seyfried, C.; U.S. Pat. Appl. US 2006/0122191A1, **2006;** [e]Fotsch, C.; Norman, M. H.; PCT Int. Appl. WO 03009850, **2003**.

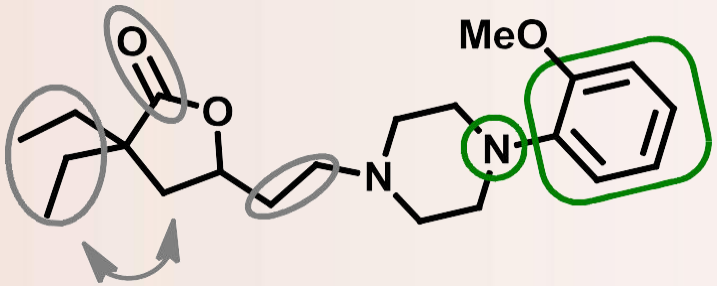
Region 1---Results



#	R	%inhib[1]	#	R	%inhib[1]	#	R	%inhib[1]
L33		96	L34		71	L35		94
L36		87	L37		52	L38		63
L39		34	L40		72	L41		48
L42	 	86	L43	 	90			

[1]. % inhibition at 10 μM for muscarinic receptors

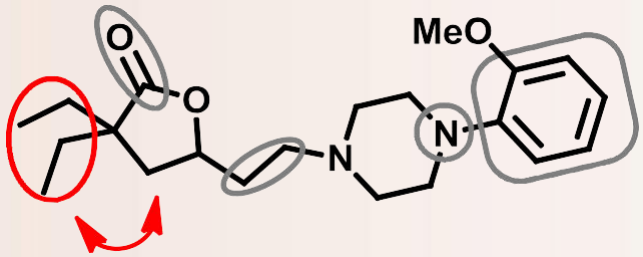
Region 1---Conclusions



Region 1: the property and position of the substituents on the cationic N-aryl piperazines (or similar N-heterocycles) affects the affinity of the ligands.

- Ortho substitution favored over para substitution
- Isopropyl, phenyl and iodo group are preferred groups for ortho substitution based on the series of compounds tested herein
- Biphenyl substitution provided the highest affinity compound (L21,99%; L28, 99%; L31, 99%) among those tested in the present study

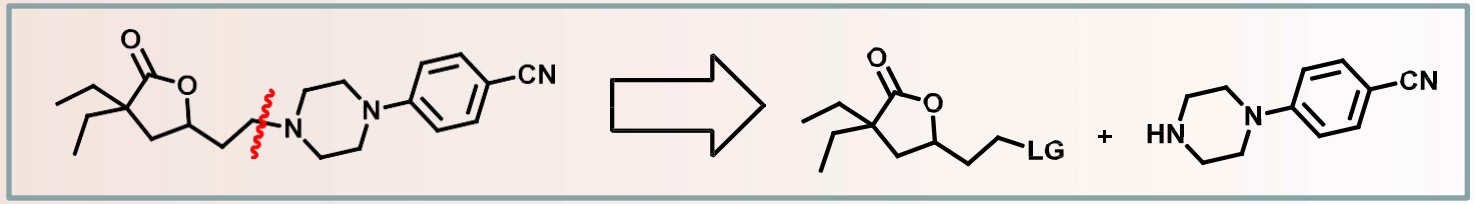
Lead Modification Strategy---Region 2

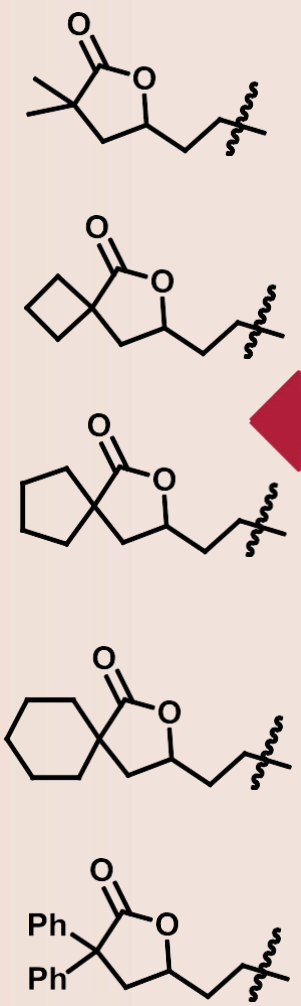


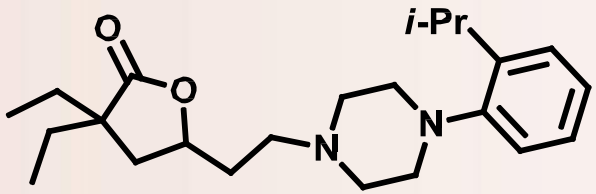
Hypothesis: It is possible to improve ligand affinity through systematic modification of H-bonding, cationic and linker regions of lactone-based lead compounds

Region 1:	the physicochemical properties and position of the substituents on the cationic N-aryl piperazine region (and related heterocycles)
Region 2	the physicochemical properties and position of the substituents on the H-bonding lactone region
Region 3	the length and physicochemical properties of the linker

Region 2--Design of Ligands

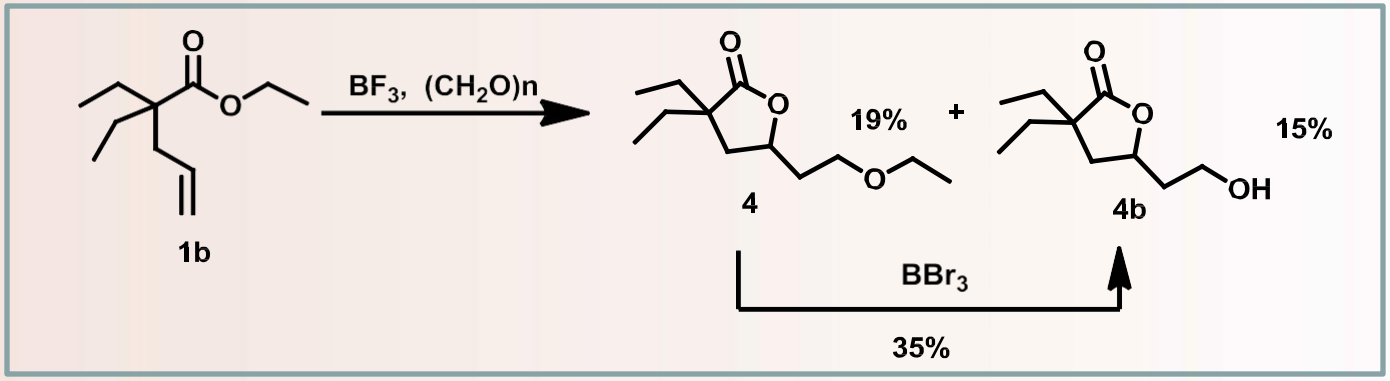






L19

Percent inhibition: 96%



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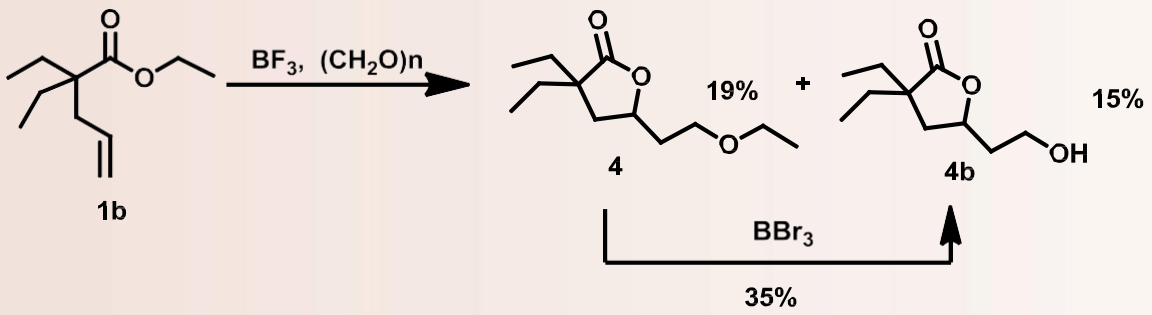
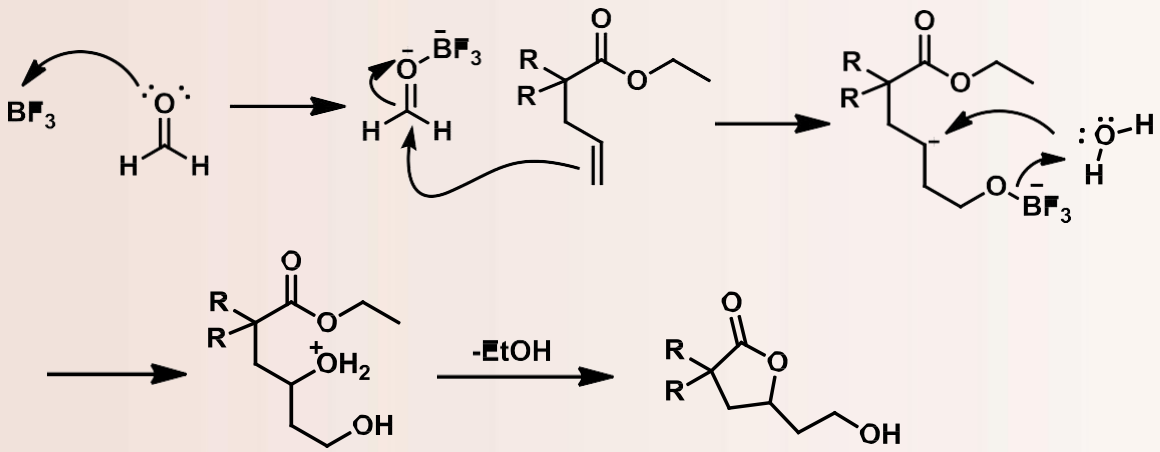
Region 2--Development of New Synthetic Route

	Reaction scheme	comment
Problematic reaction		• Low yield • Difficult separation
Mechanism		• Active cation • Unprotected hydroxyl group

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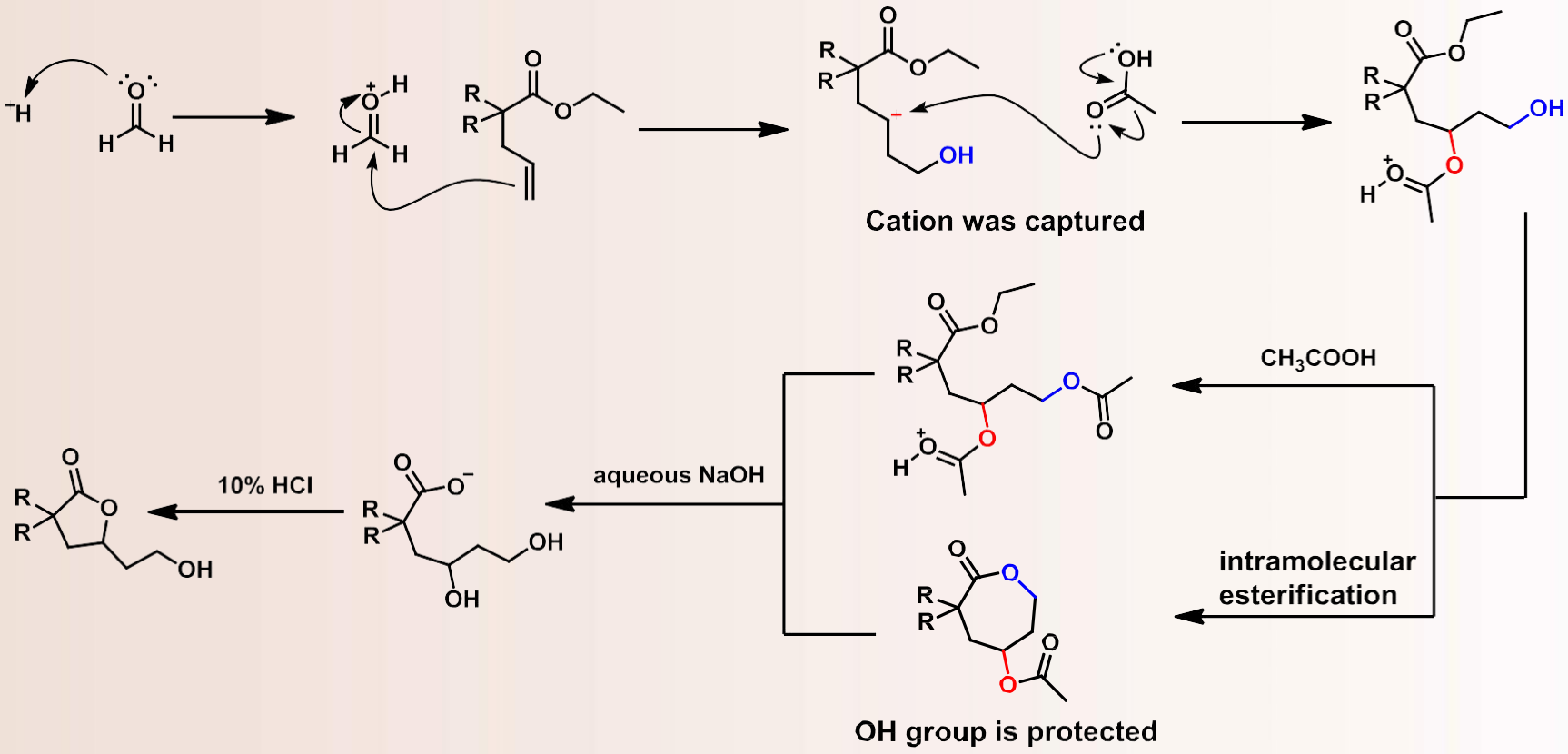
Solution – Modified Prins Reaction

- Use H_2SO_4 as catalyst instead of BF_3
- Use acetic acid as solvent instead of DCM



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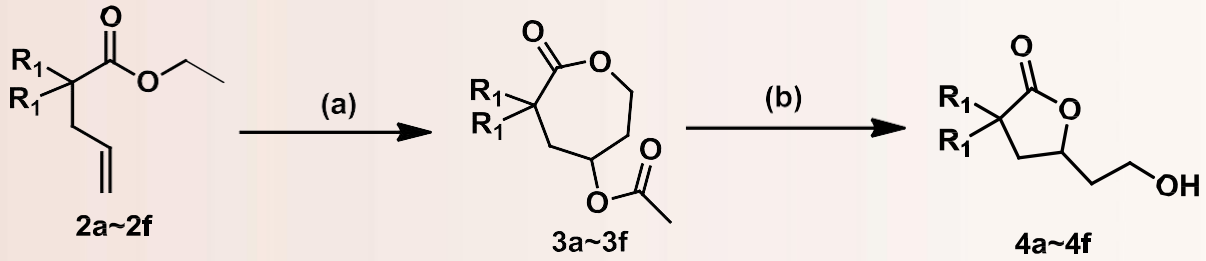
Region 2---Synthesis



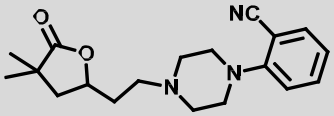
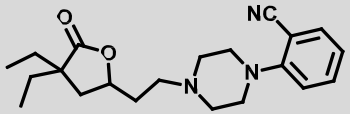
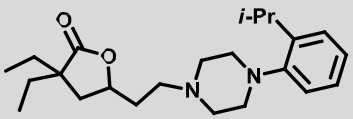
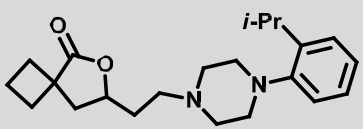
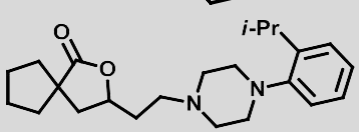
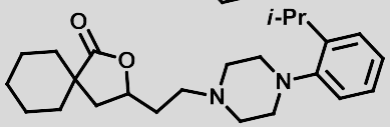
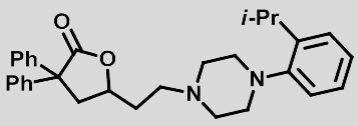
Reagents and conditions: (a) CH_3COOH, paraformaldehyde, H_2SO_4; (b) NaOH, H_2O, reflux, H_2SO_4;

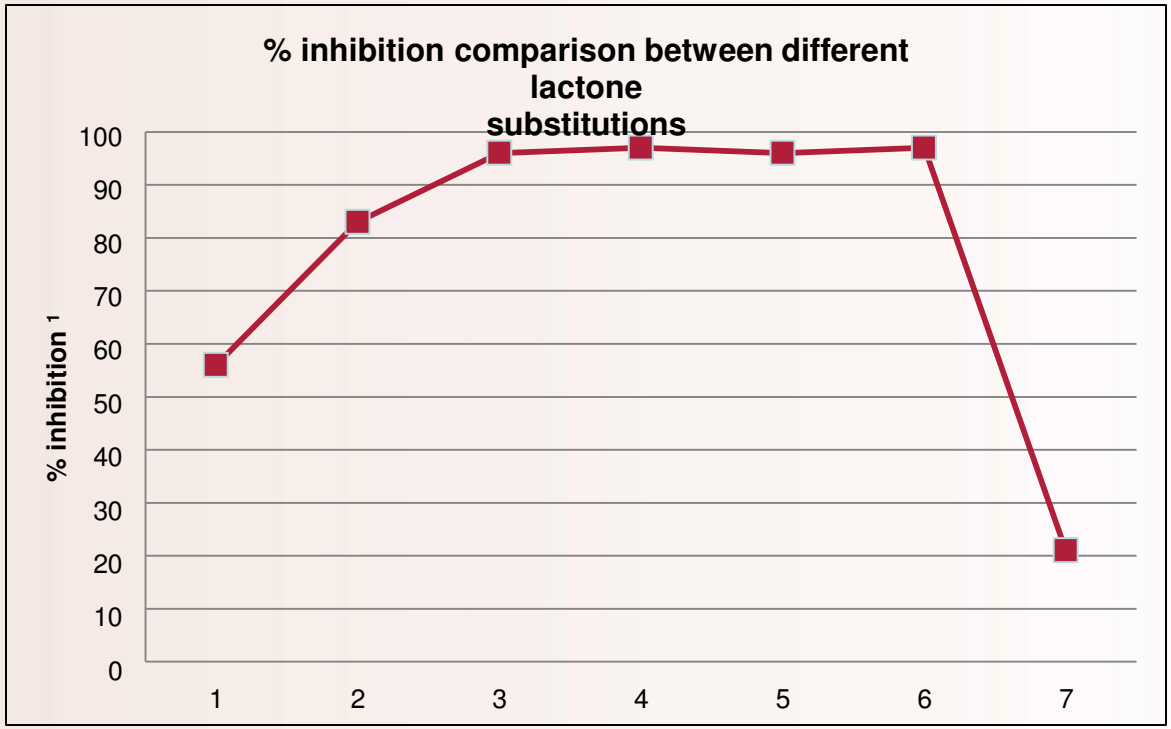
R_1	compound #	Yield (%)[a]
Methyl	4a	81
Ethyl	4b	76
Spiro (4)	4c	74
Spiro (5)	4d	73
Spiro (6)	4e	73
Phenyl	4f	66

[a] Isolated yield

Gao, Rong; Canney, Daniel; *Tetrahedron lett*, **2009**, 5914-5916

Region 2---Results

#	structure
1	
2	
3	
4	
5	
6	
7	



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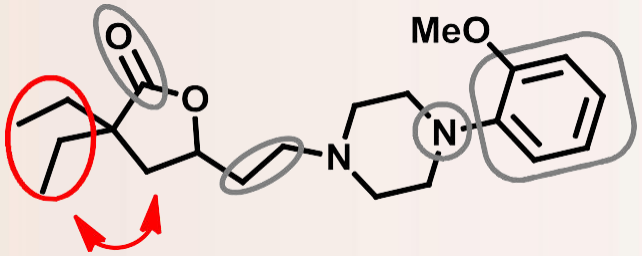
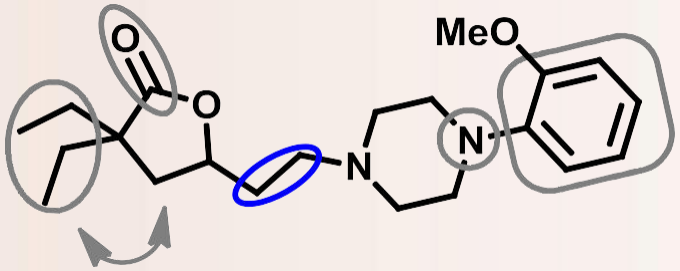
Region 2---Conclusions



Region 2: the property and position of the substituents on the H-bonding lactone region affects affinity of the ligands.

- Diethyl and spiro (4~6) substitution on the lactone region gave similar affinity
- In the alpha position, reducing the substitution size from diethyl to dimethyl or increasing the size to phenyl had negative effects on binding
- For substituents at the beta position, SAR data to be published soon

Structural Modification Strategy---Region 3



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Regioin 2	the physicochemical properties and position of the substituents on the H-bonding lactone region
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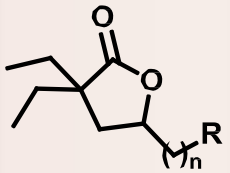
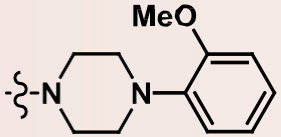
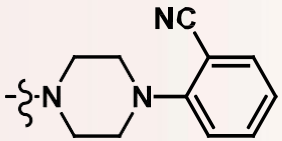
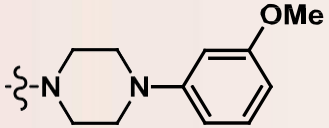
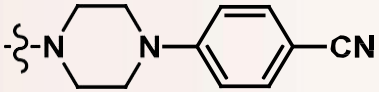
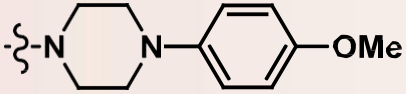
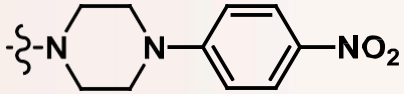
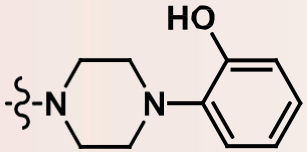
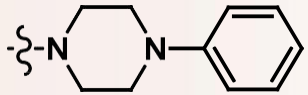
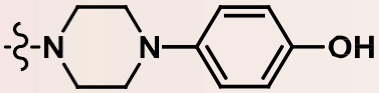
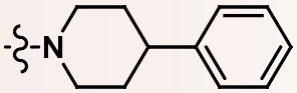
Region 3---Design of Ligands



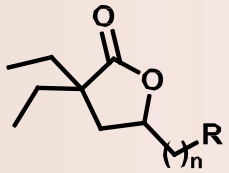
n=1 or 2

#	R	#	R
1		6	
2		7	
3		8	
4		9	
5		10	

[1] % inhibition at 10 μM against muscarinic receptors

Bhandare, R.R., **Canney, D.J**. *MedChem Res,* **2010**, *20* (5), 558-565.

Region 3---Results



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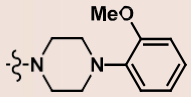
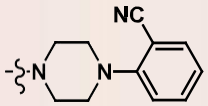
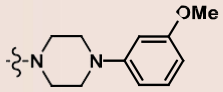
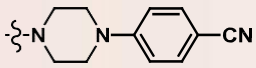
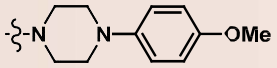
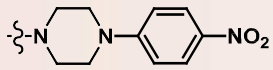
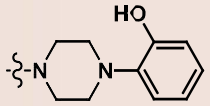
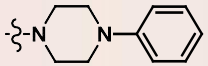
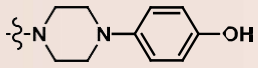
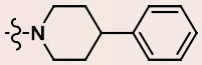
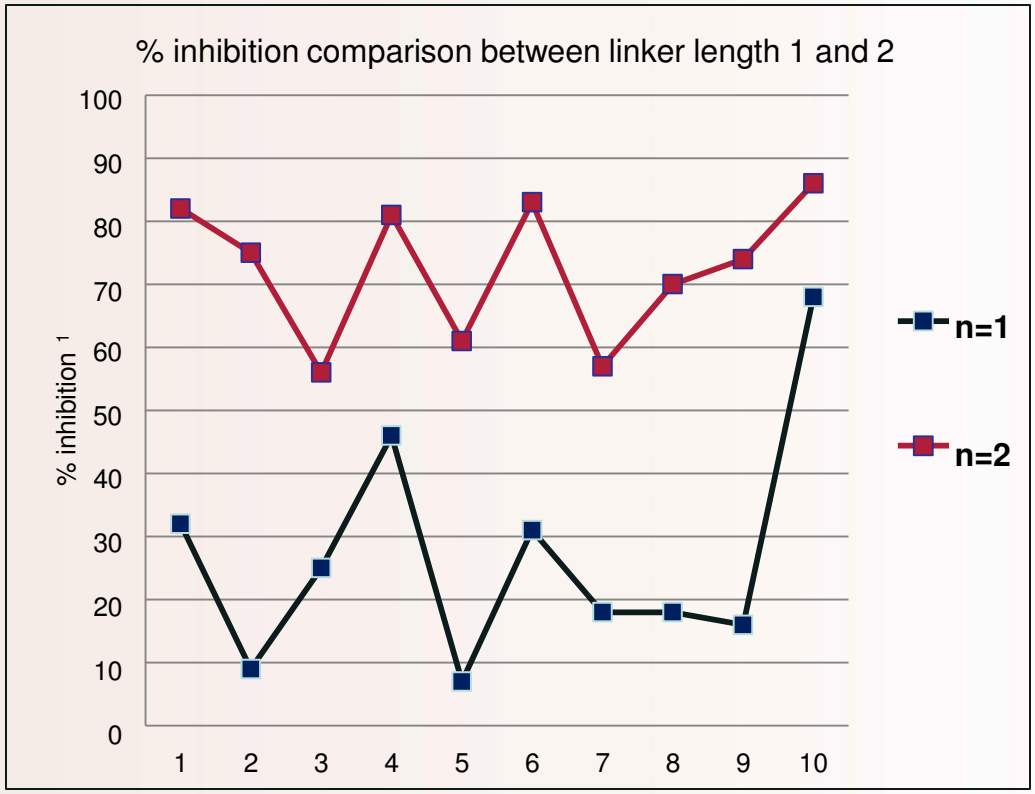
1		6	
2		7	
3		8	
4		9	
5		10	



1. % inhibition at 10 μM against muscarinic receptors

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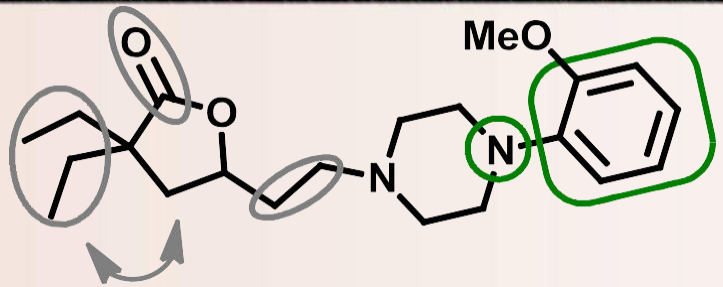
Regions 1, 2, 3. Summary of Conclusions



Region 1: the property and position of the substituents on the cationic N-aryl piperazines (or similar N-heterocycles) affects the affinity of the ligands.

- Ortho substitution favored over para substitution
- Isopropyl, phenyl and iodo group are preferred groups for ortho substitution based on the series of compounds tested herein
- Biphenyl substitution provided the highest affinity compound (L21,99%; L28, 99%; L31, 99%) among those tested in the present study

Region 2: the properties, position of substituents on H-bonding region affects ligand affinity.

- Diethyl and spiro (4~6) substitution on the lactone region gave similar affinity
- In the alpha position, reducing the substitution size from diethyl to dimethyl or increasing the size to phenyl had negative effects on binding
- For the beta position, data to be reported in the near future.

Region 3: the length, electronic nature of linker affects affinity. N=2 is favored over 1.

The series is being evaluated further as potenital subtype selective ligands for muscarinic subtypes.

Acknowledgements

Dean's Office, Temple University School of Pharmacy (Dr. Peter Doukas)

Department of Pharmaceutical Sciences, School of Pharmacy: faculty, staff members and graduate students.

Moulder Center for Drug Discovery Research: Dr. Magid Abou-Gharbia (Director), **Dr. Ben Blass** and Dr. Wayne Childers

Canney Lab Members, ***Rong Gao*** **(2013),** ***Richie Bhandare (2012),*** *Siva Annadurai (2011), Otito Iwuchukwu (2010), Safura Nantogma (2009), Shyam Desai (2009), Weilin Sun (2008)*

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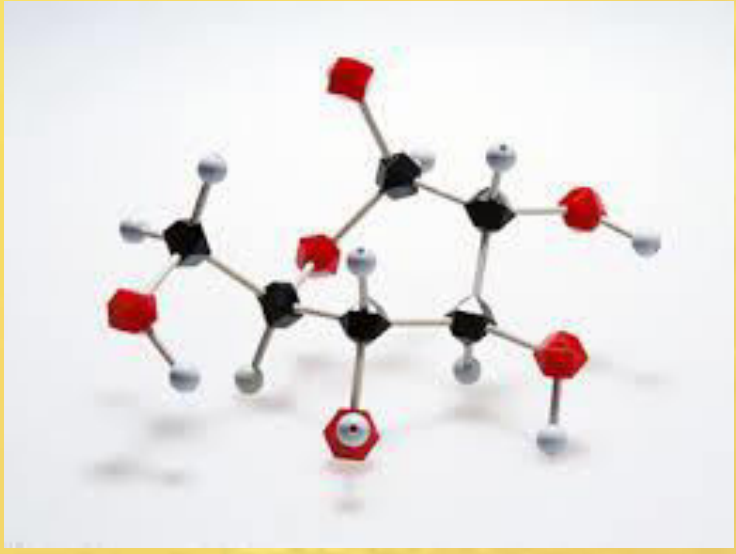
- Advances in Pharmacoepidemiology & Drug Safety



Medicinal chemistry Related Conferences

- 3rd International Conference on Medicinal Chemistry & Computer Aided Drug Designing
- 3rd International Conference and Exhibition on Pharmacognosy, Phytochemistry & Natural Products

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