

Development of Novel Small Molecule Inhibitors of Neurotropic Alphaviruses

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October 28, 2014

- **Introduction**

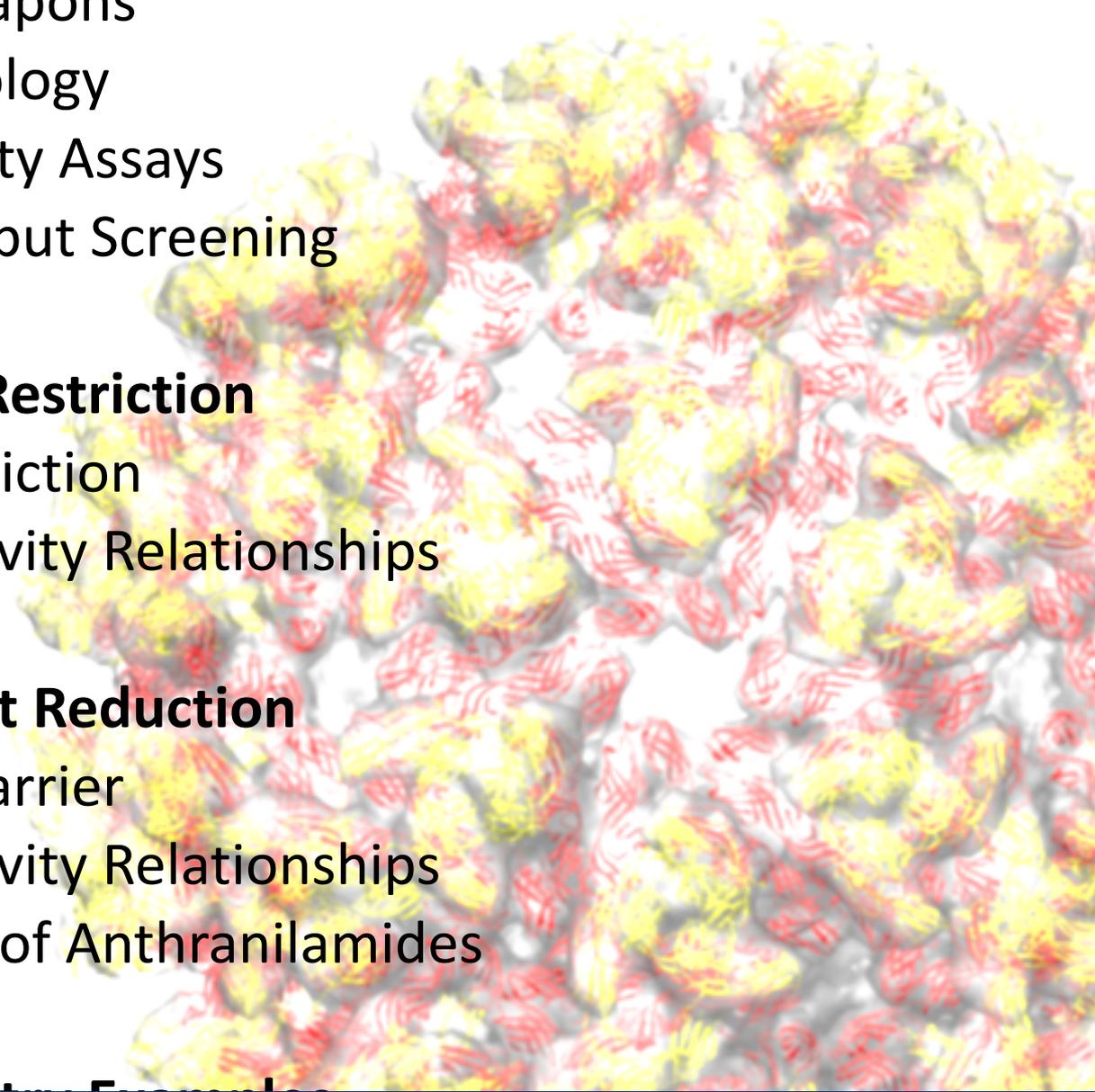
- Biological Weapons
- Alphavirus Biology
- Activity/Toxicity Assays
- High-Throughput Screening

- **Conformational Restriction**

- Basis for Restriction
- Structure-Activity Relationships

- **Molecular Weight Reduction**

- Blood Brain Barrier
- Structure-Activity Relationships
- Development of Anthranilamides



- **Synthetic Chemistry Examples**

National Select Agents List

- Examples:
 - Ebola Virus
 - Small Pox Virus
 - Bovine Spongiform Prion
 - *Yersinia pestis*

Characteristics of Viral Bioweapons

- Easily and cheaply grown in large amounts
- Highly stable and dispersible
- Simple genome amenable to genetic engineering
- Confound defensive vaccine development

Equine Encephalitis Viruses (EEVs)
Eastern EEV (EEEV)*
Venezuelan EEV (VEEV)*
Western EEV (WEEV)
* Select Agents



Figure 1

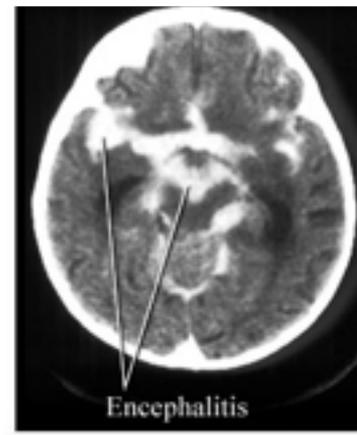
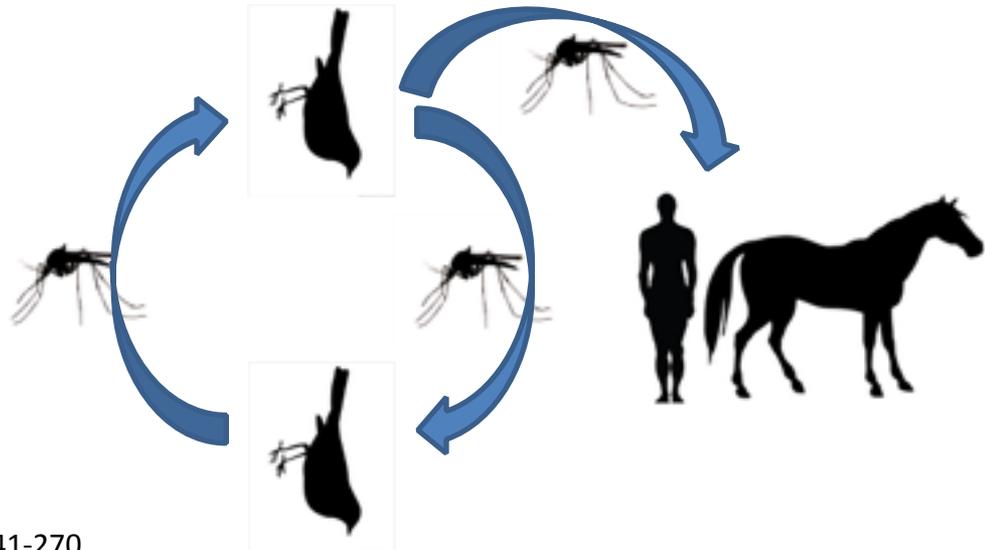


Figure 2

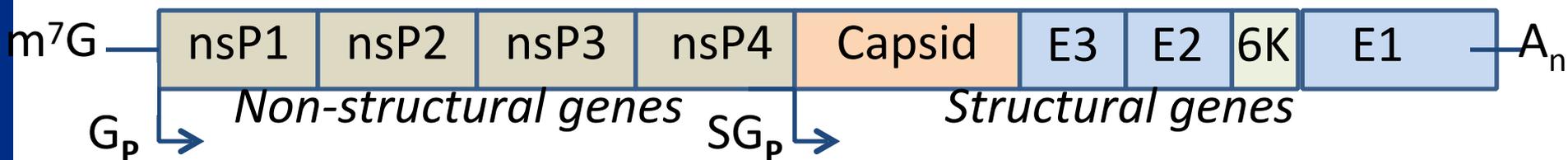


Forrester, Naomi L, et al. *Virology*. 2008, 170-172

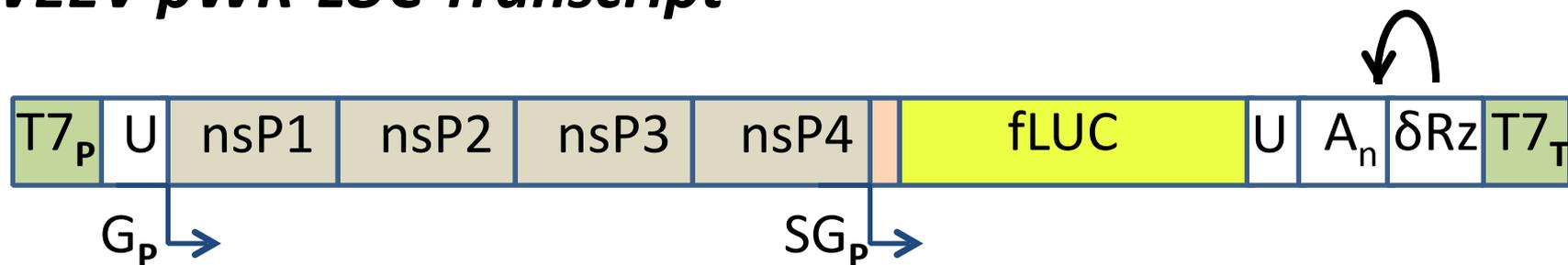
Steele, Keith E, et al. *Modern Aspects of Biological Warfare*. 2007, 241-270

eMedicine, Online: <<http://www.emedicinehealth.com/script/main/art.asp?articlekey=138806&ref=128399>>

Natural WEEV Genome



WEEV pWR-LUC Transcript



Cells: BSR-T7/5 cells transfected with pWR-LUC WEEV replicon
Controls: Ribavirin (+) and DMSO (-)

LUC Activity Assay

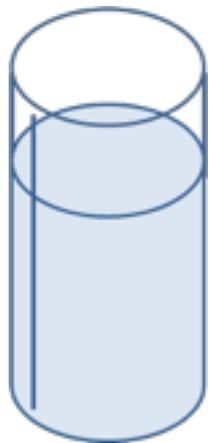
MTT Cytotoxicity Assay

Transfected Cells,
Compound,
Media

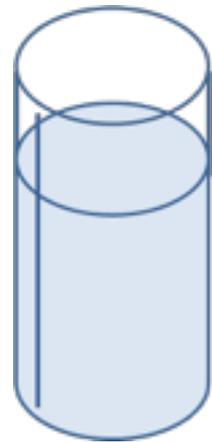
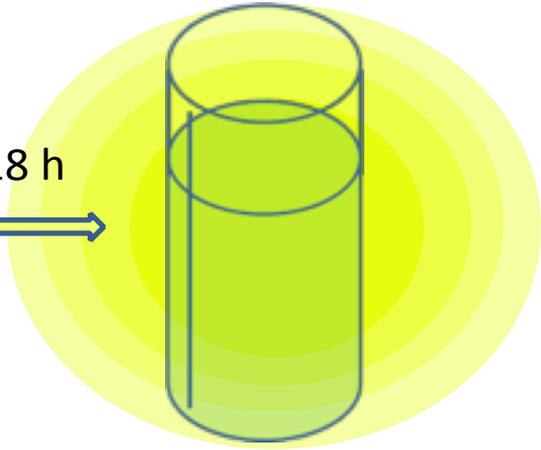
Measure
luminescence

MTT

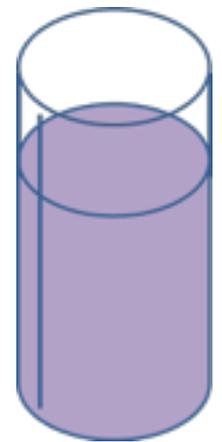
Measure
absorbance



37 °C, 18 h



RT, 2 h

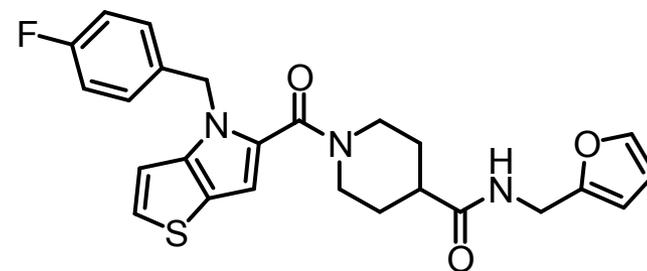


HTS / Validation Steps

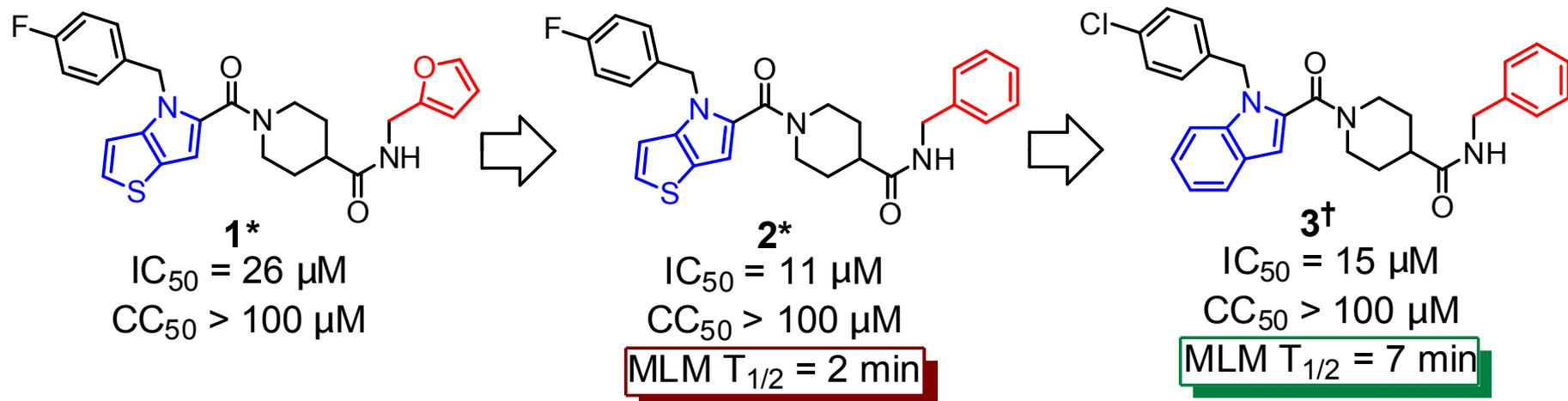
Step	Experimental System	Criterion	No. Compounds
n/a	n/a	n/a	51,028
HTS	pWR-LUC and BSR-T7/E cells	fLUC activity >2 SD below negative control or inhibition of fLUC activity >90% of positive control	196
HTS	pWR-LUC and BSR-T7/E cells	No activity in CCG LUC-based screens	114
1° Validation	pWR-LUC and BSR-T7/E cells	Dose-response, $IC_{50} < 100 \mu M$	77
2° Validation	EEEV/VEEV-SEAP and BHK cells	Dose-response, $IC_{50} < 100 \mu M$	11
3° Validation	pWR-LUC and BSR-T7/E cells	Repeat dose-response, Selectivity index >5	4

Verification

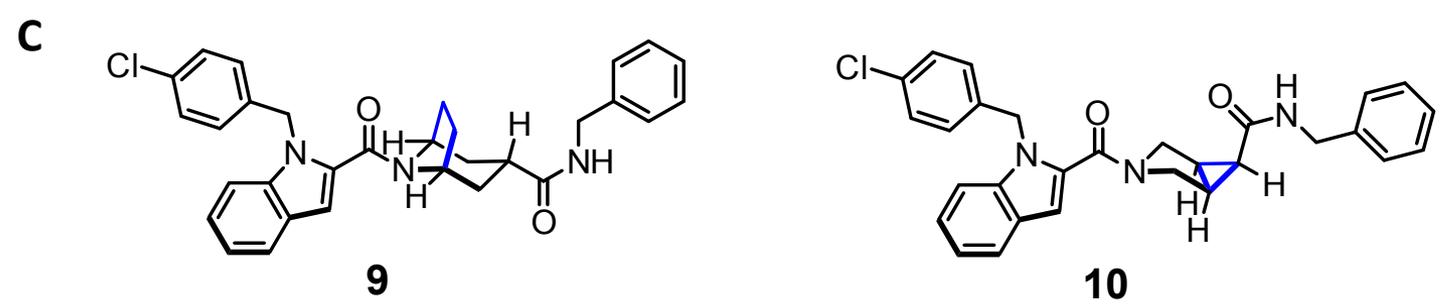
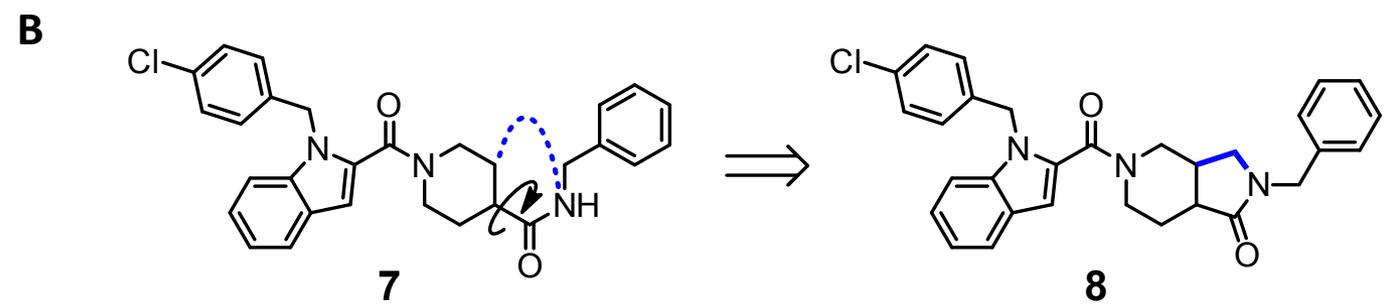
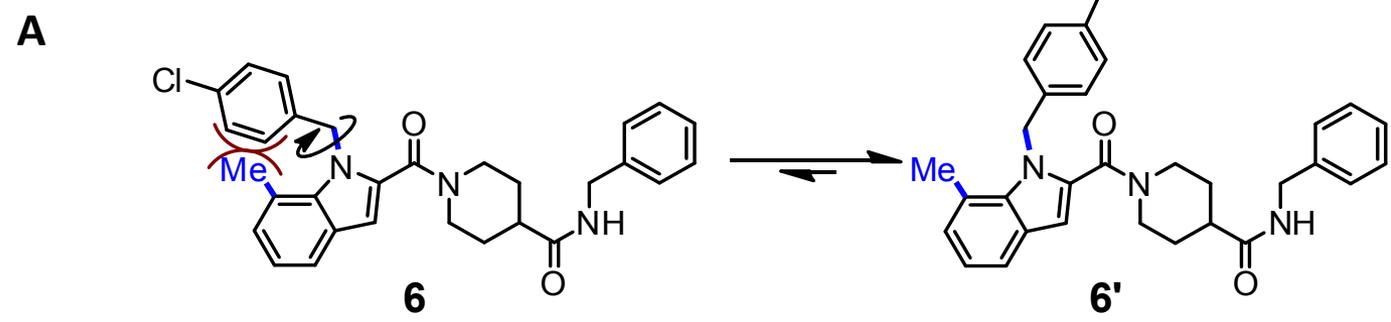
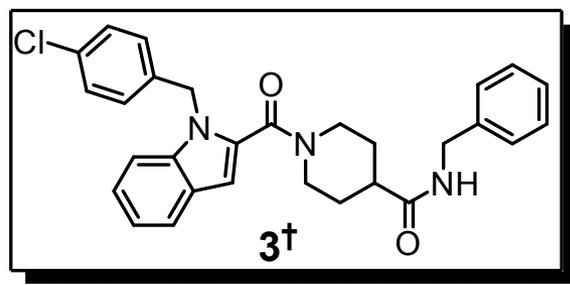
Experiment	Measure	Results
Live virus (SINV, FMV) in cultured neuronal cells	Cytopathic effect	Increased cell viability
Inhibition of virion production (FMV)	Viral titer	Decreased titer
Inhibition of viral RNA replication (RT-PCR)	RNA expression	Decreased expression

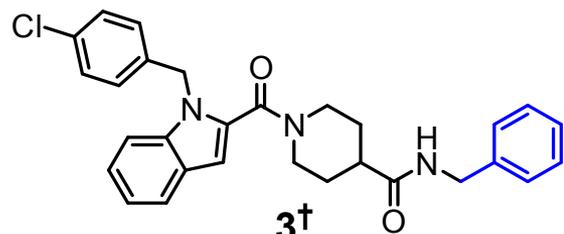


1*
 $IC_{50} = 26 \mu M$
 $CC_{50} > 100 \mu M$

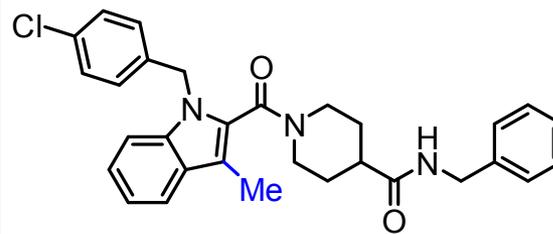


HTS discovery: *J Infect Dis* **2009** (199) 950-957
 Development of lead **3**: *J Med Chem* **2012** (55) 3535-3545
 Development of lead **4**: *J Med Chem* **2013** (56) 9222-9241

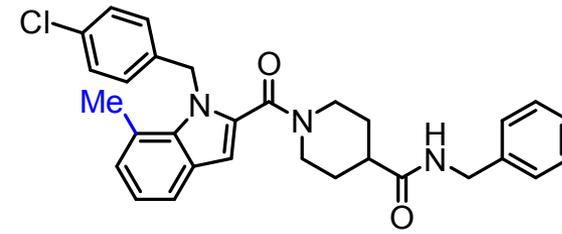




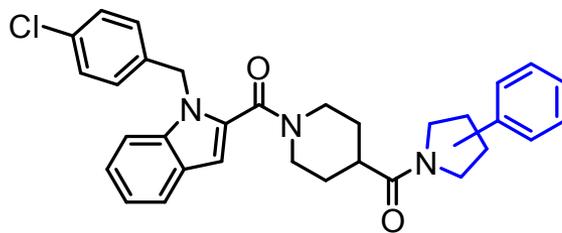
3t
 $IC_{50} = 15.6 \mu M$
 $CC_{50} > 100 \mu M$



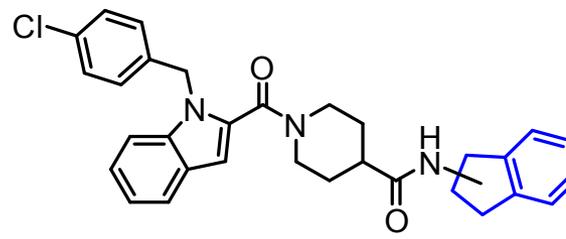
11
 $IC_{50} > 50 \mu M$



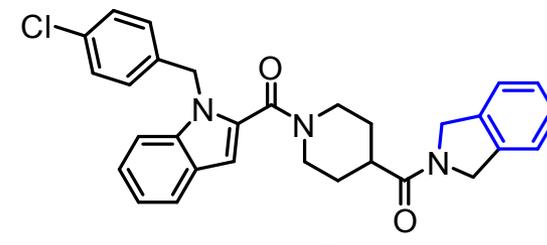
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 $IC_{50} > 50 \mu M$



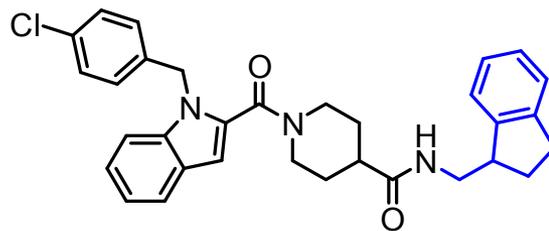
13
 $IC_{50} > 50 \mu M$



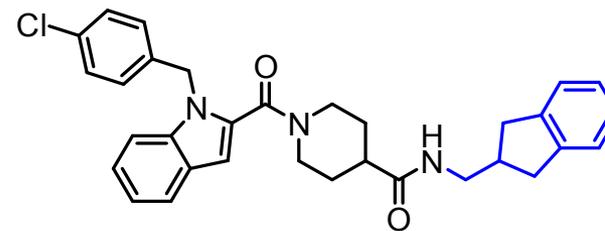
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 $IC_{50} > 50 \mu M$



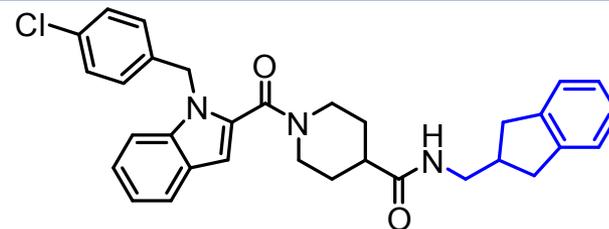
15
 $IC_{50} > 50 \mu M$



16
 $IC_{50} = 7.1 \mu M$
 $CC_{50} > 100 \mu M$



17
 $IC_{50} = 0.53 \mu M$
 $CC_{50} > 100 \mu M$

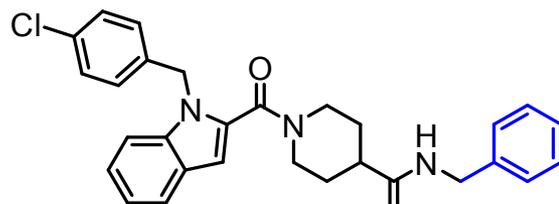


17

$IC_{50} = 0.53 \mu M$

$CC_{50} > 100 \mu M$

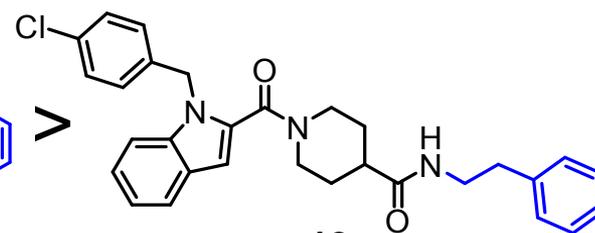
$ClogP = 4.72$



18

$IC_{50} = 15.6 \mu M$

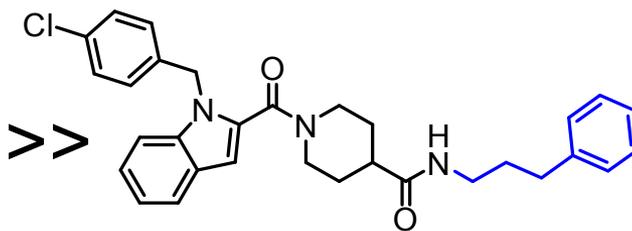
$ClogP = 4.30$



19

$IC_{50} = 15.2 \mu M$

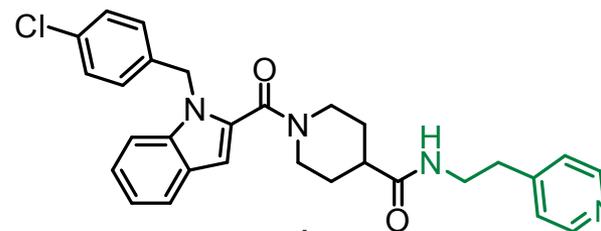
$ClogP = 4.37$



20

$IC_{50} > 100 \mu M$

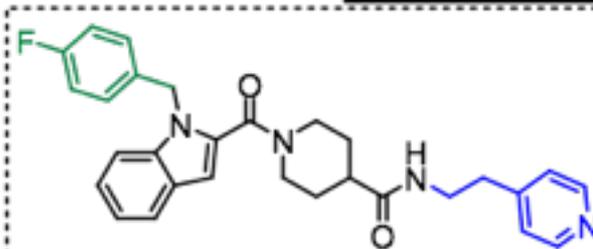
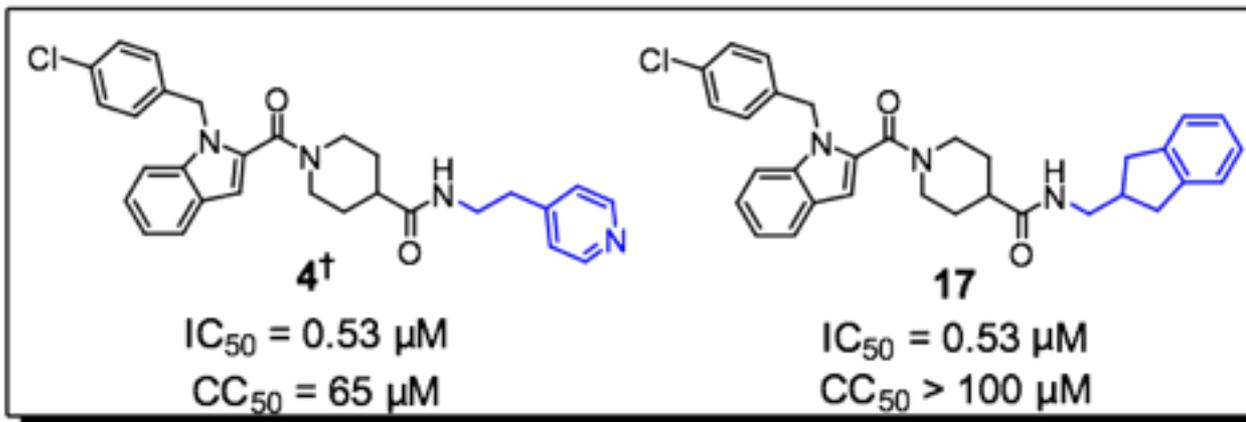
$ClogP = 4.75$



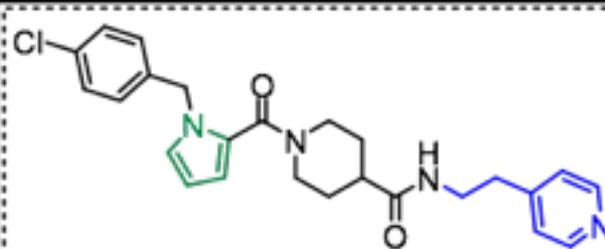
4†

$IC_{50} = 0.53 \mu M$

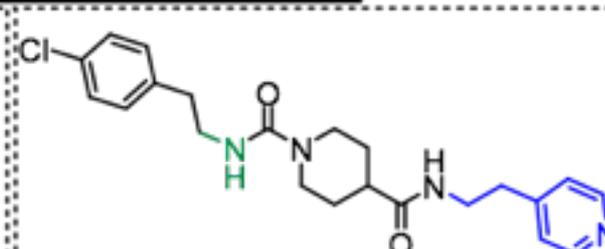
$CC_{50} = 65 \mu M$



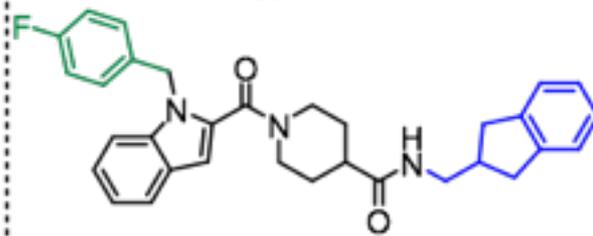
21
 $IC_{50} = 1.2 \mu M$
 $CC_{50} > 100 \mu M$



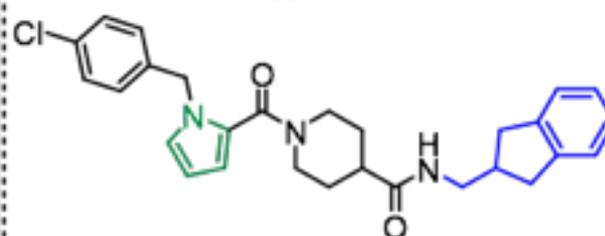
22
 $IC_{50} = 0.68 \mu M$
 $CC_{50} > 100 \mu M$



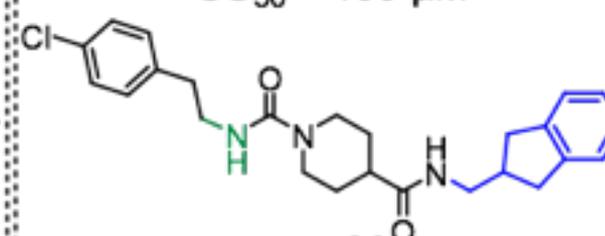
23
 $IC_{50} = 40.9 \mu M$
 $CC_{50} > 100 \mu M$



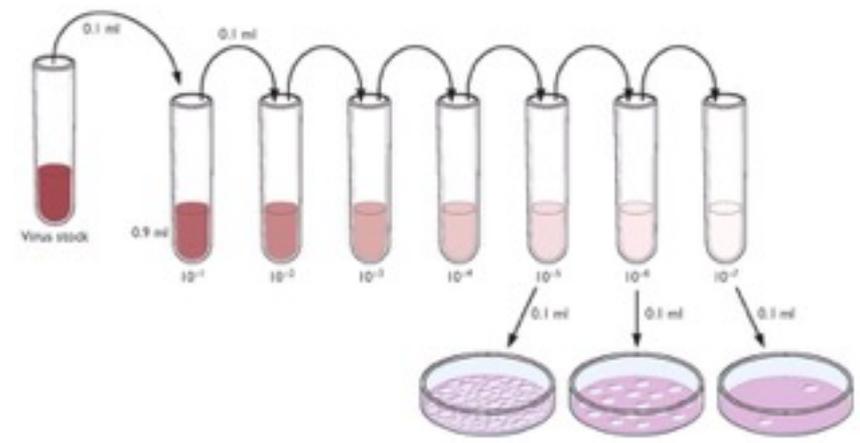
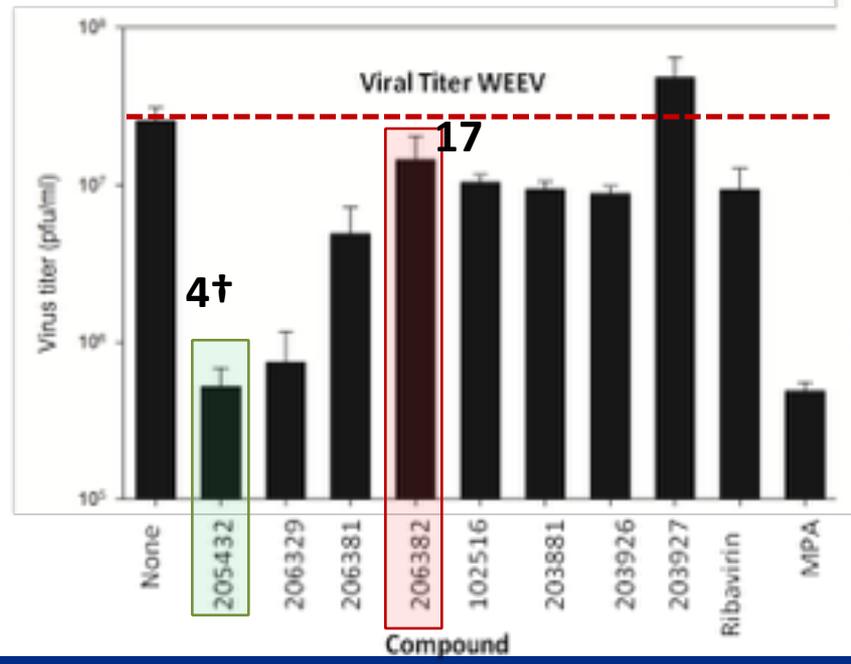
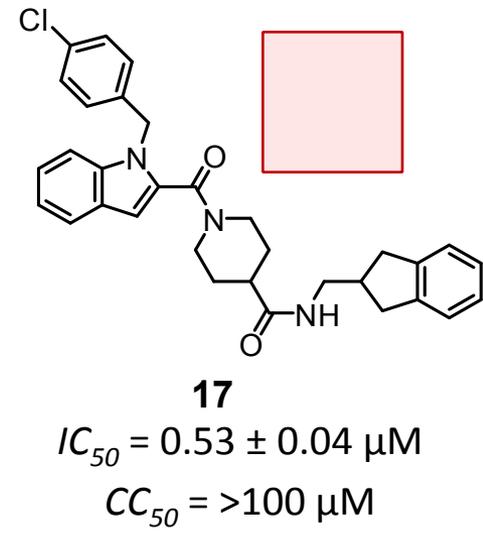
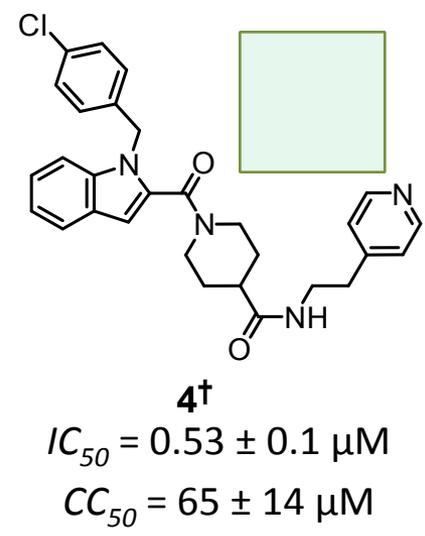
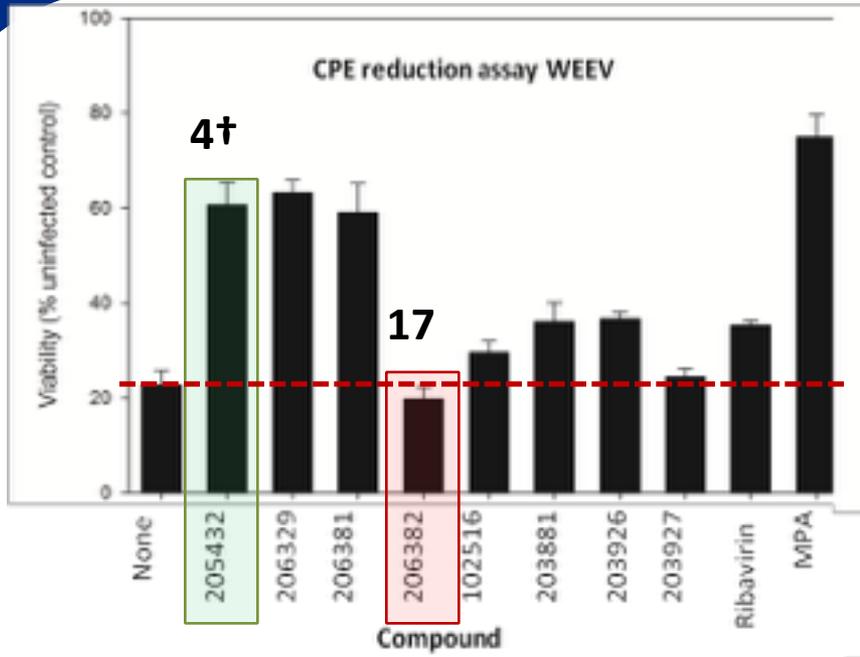
24
 $IC_{50} > 50 \mu M$
 $CC_{50} > 50 \mu M$



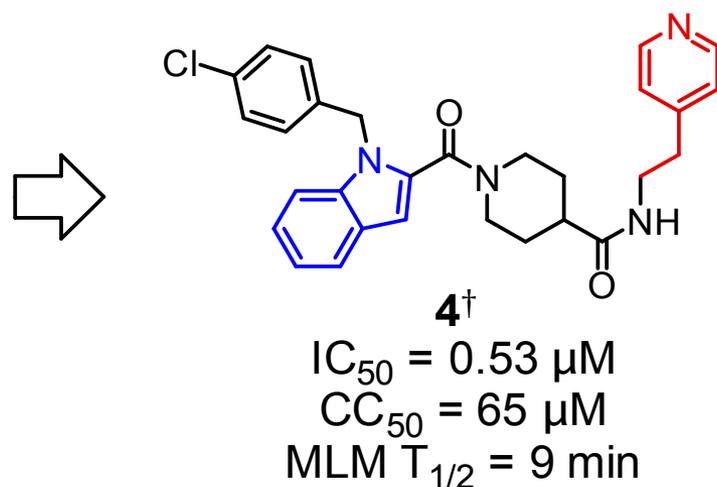
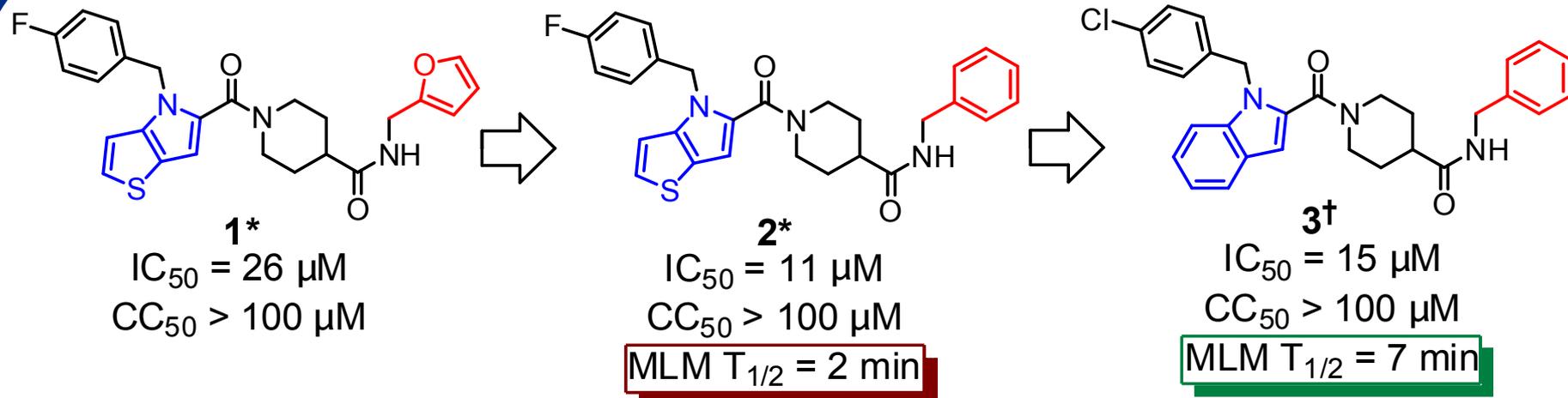
25
 $IC_{50} = 61.7 \mu M$
 $CC_{50} > 100 \mu M$



26
 $IC_{50} > 100 \mu M$
 $CC_{50} > 100 \mu M$



Data from the David Miller lab

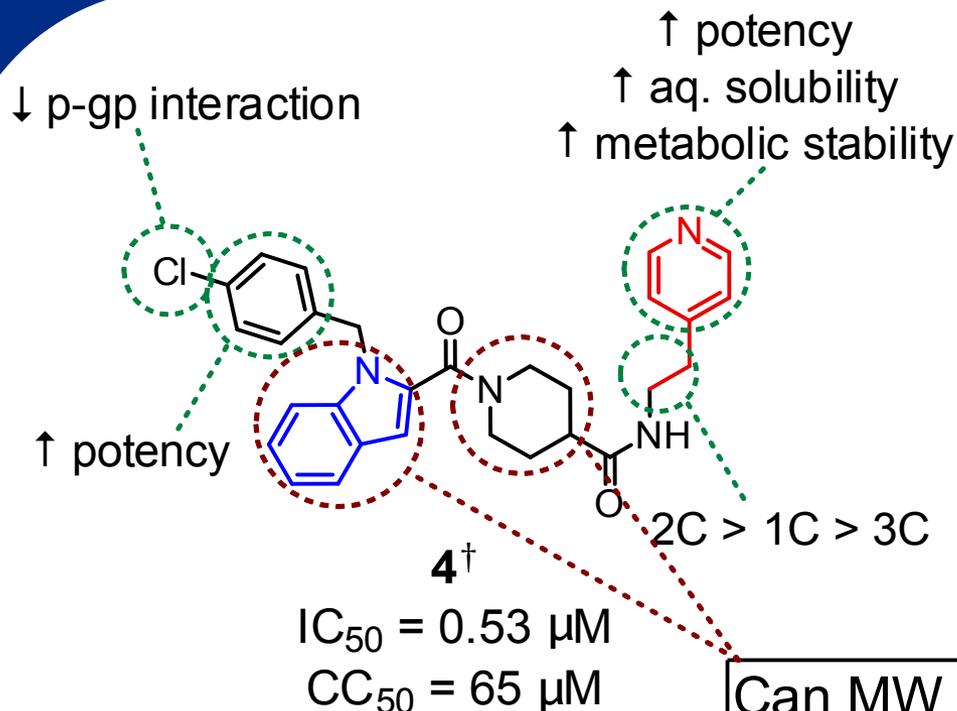


Successful CNS Drugs Properties		
Property	Mean CNS	4†
MW	319	501
TPSA	40.5	65
cLogP	3.43	3.1

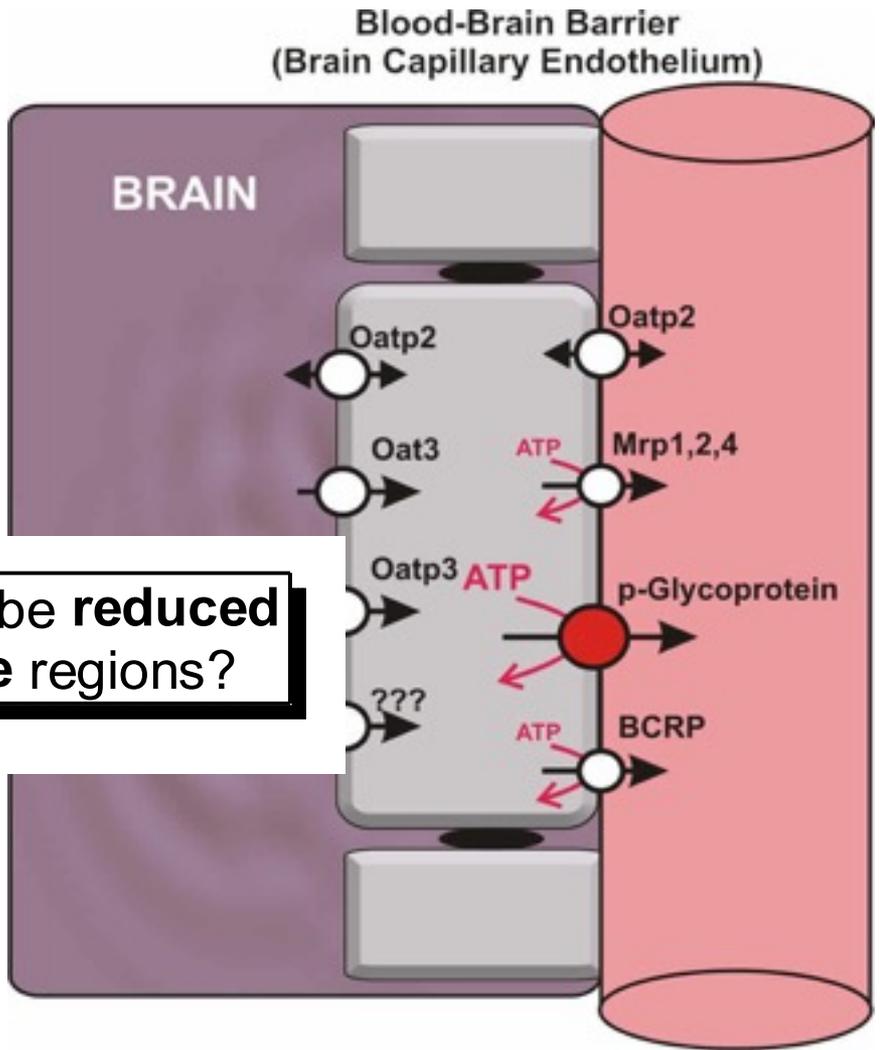
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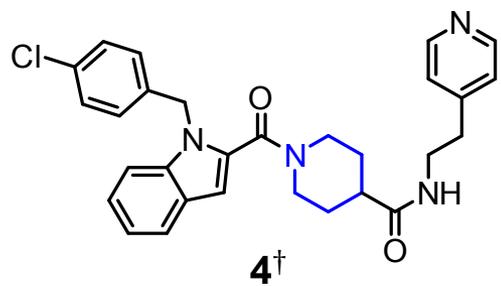


Can MW be **reduced** in **these** regions?

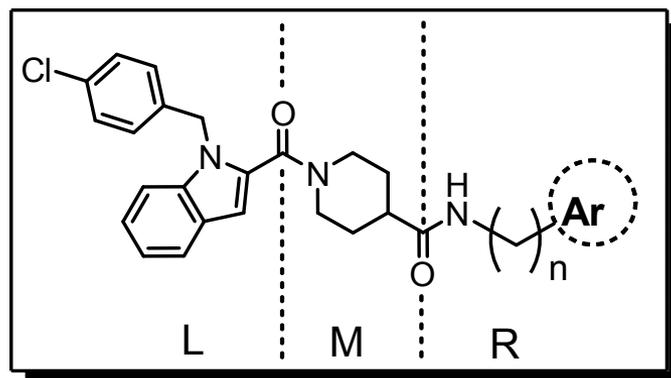
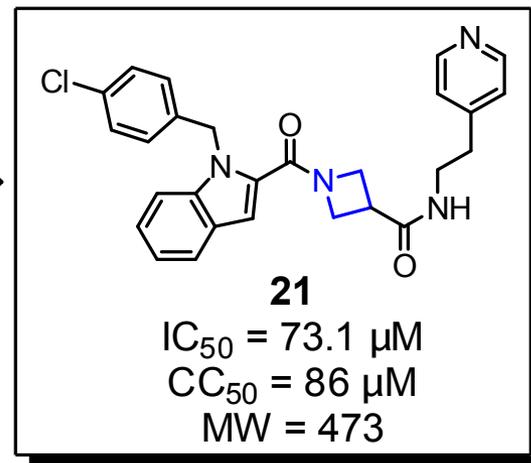
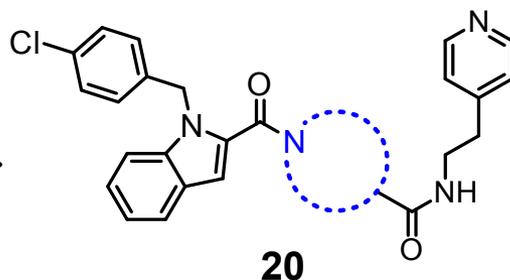


Successful CNS Drug Properties		
Property	Mean CNS Drug	4 [†]
MW	319	501
TPSA	40.5	65
cLogP	3.43	3.1

CNS drug properties: Pajouhesh, H., et al. *NeuroRX* 2005 (4) 541-553
 BBB figure: David S. Miller Lab, http://neuroscience.nih.gov/Lab.asp?Org_ID=547

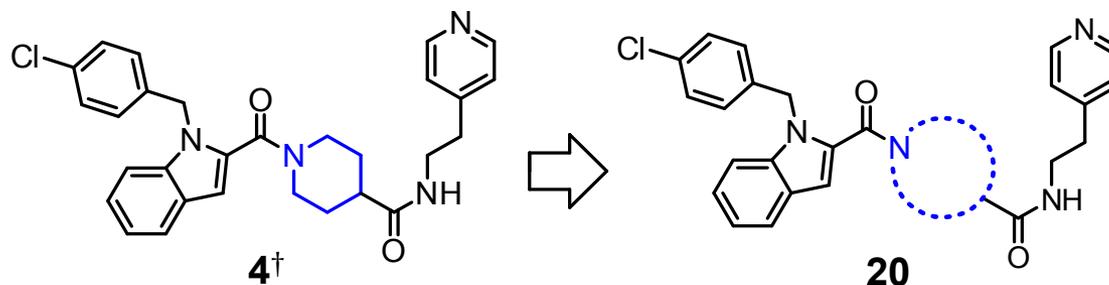


$IC_{50} = 0.53 \mu M$
 $CC_{50} = 65 \mu M$
 MW = 501

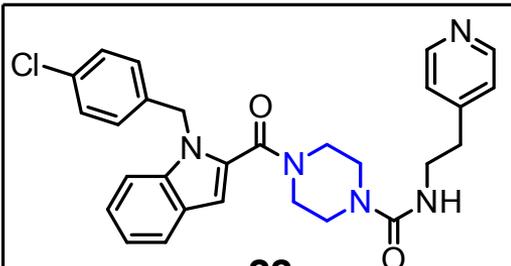


Activity/chain dependence:

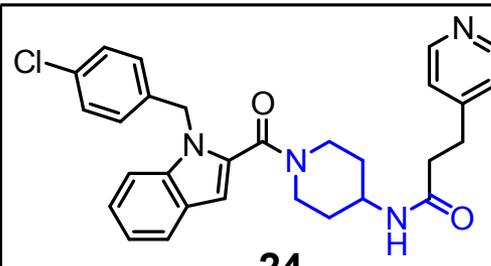
$n = 2 > 1 \gg 3$



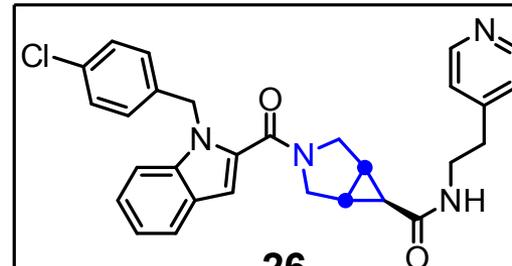
$IC_{50} = 0.53 \mu M$
 $CC_{50} = 65 \mu M$



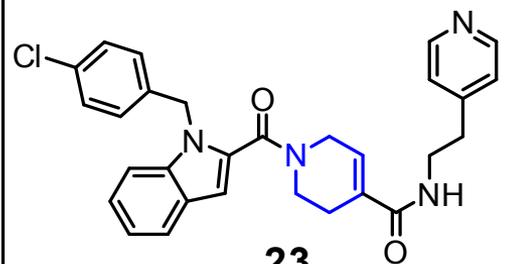
$IC_{50} = 24.4 \mu M$
 $CC_{50} = 78 \mu M$



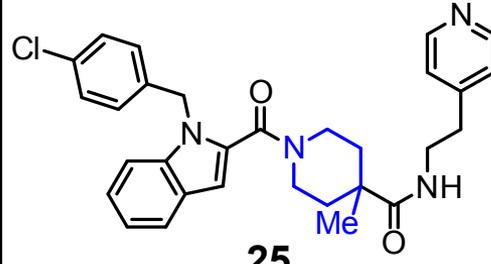
$IC_{50} = 17.6 \mu M$
 $CC_{50} = 67 \mu M$



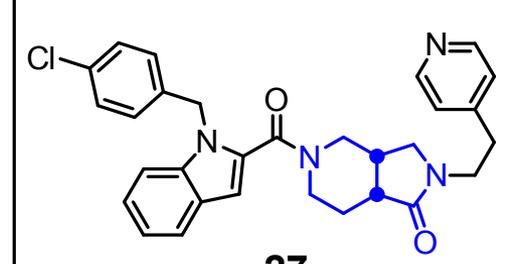
$IC_{50} = 24.6 \mu M$
 $CC_{50} > 100 \mu M$



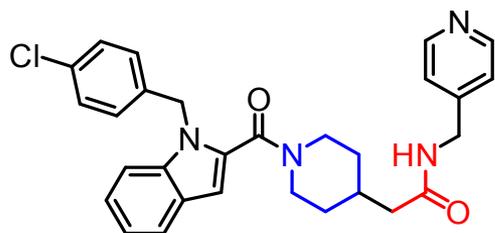
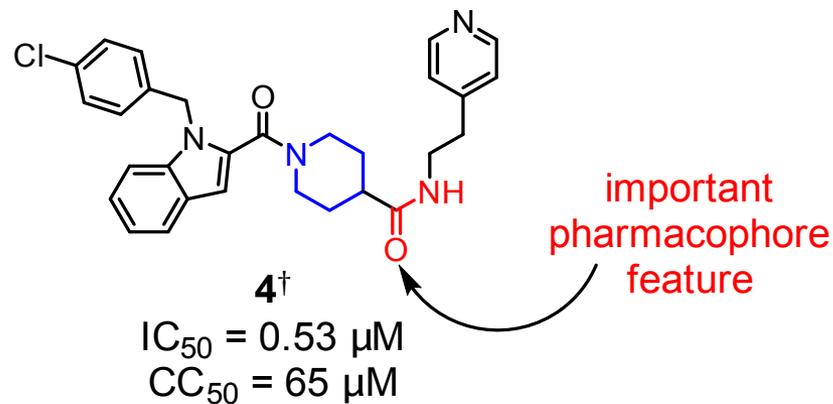
$IC_{50} = 3.9 \mu M$
 $CC_{50} = 74 \mu M$



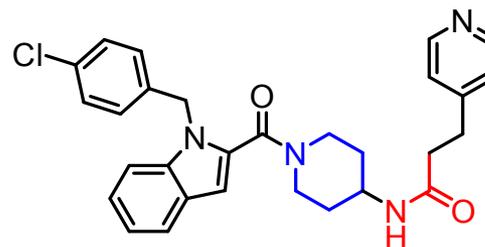
$IC_{50} > 50 \mu M$
 $CC_{50} > 50 \mu M$



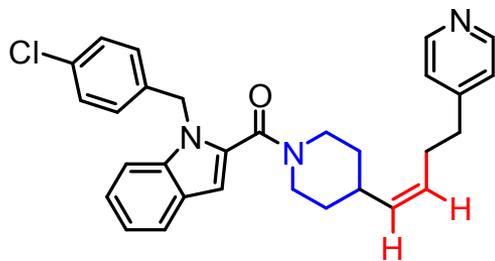
$IC_{50} = 24.4 \mu M$
 $CC_{50} = 62 \mu M$



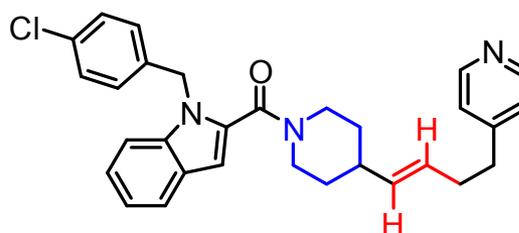
24
 $IC_{50} = 24.7 \mu M$
 $CC_{50} = 77 \mu M$



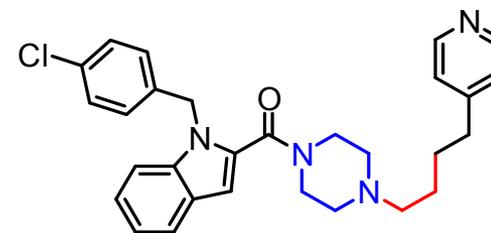
28
 $IC_{50} = 17.6 \mu M$
 $CC_{50} = 67 \mu M$



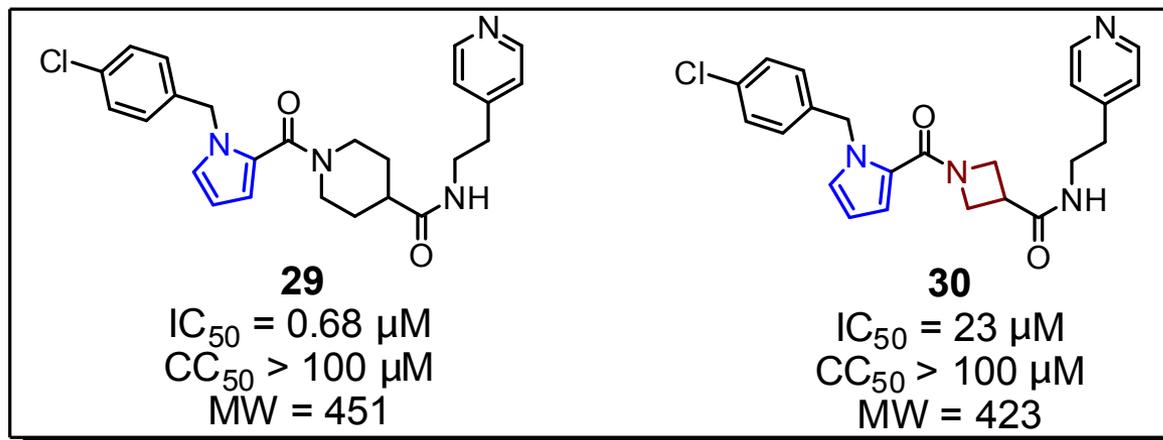
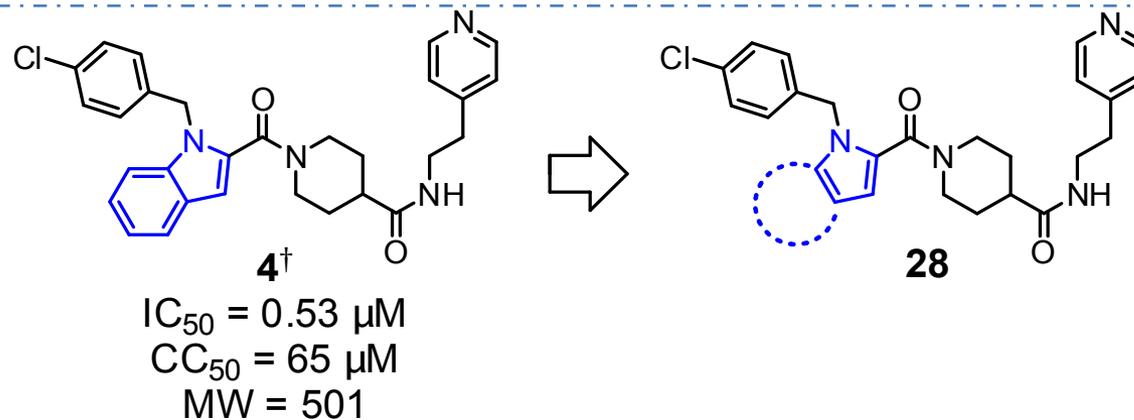
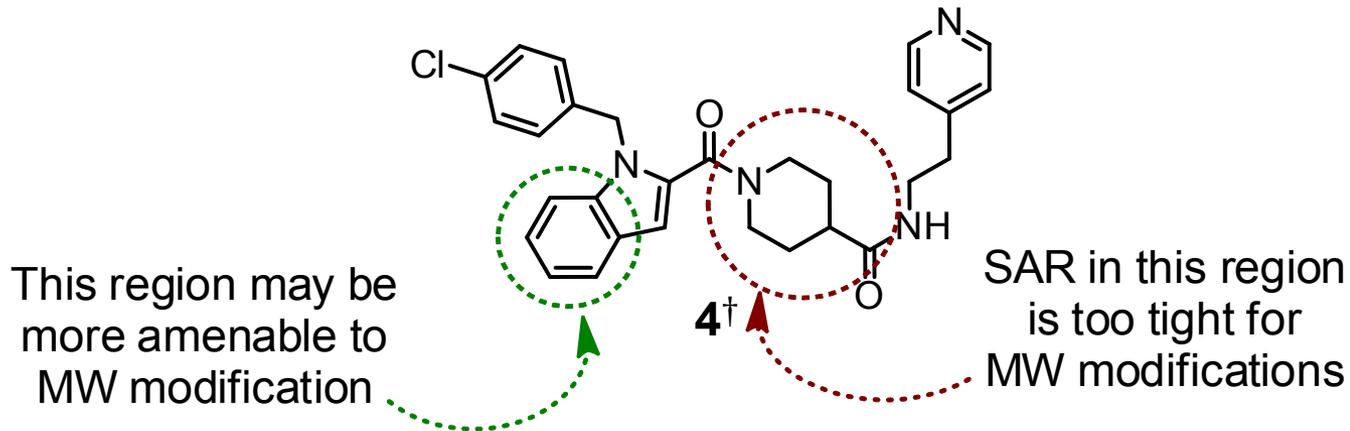
29
 $IC_{50} > 50 \mu M$
 $CC_{50} > 50 \mu M$

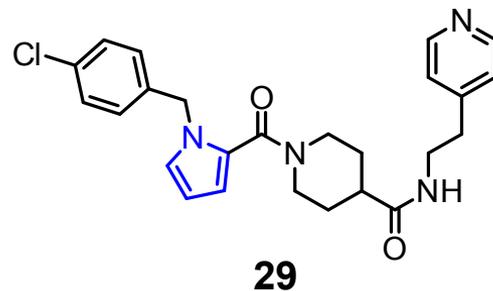
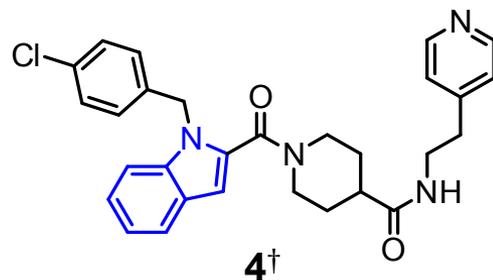


30
 $IC_{50} > 50 \mu M$
 $CC_{50} > 50 \mu M$

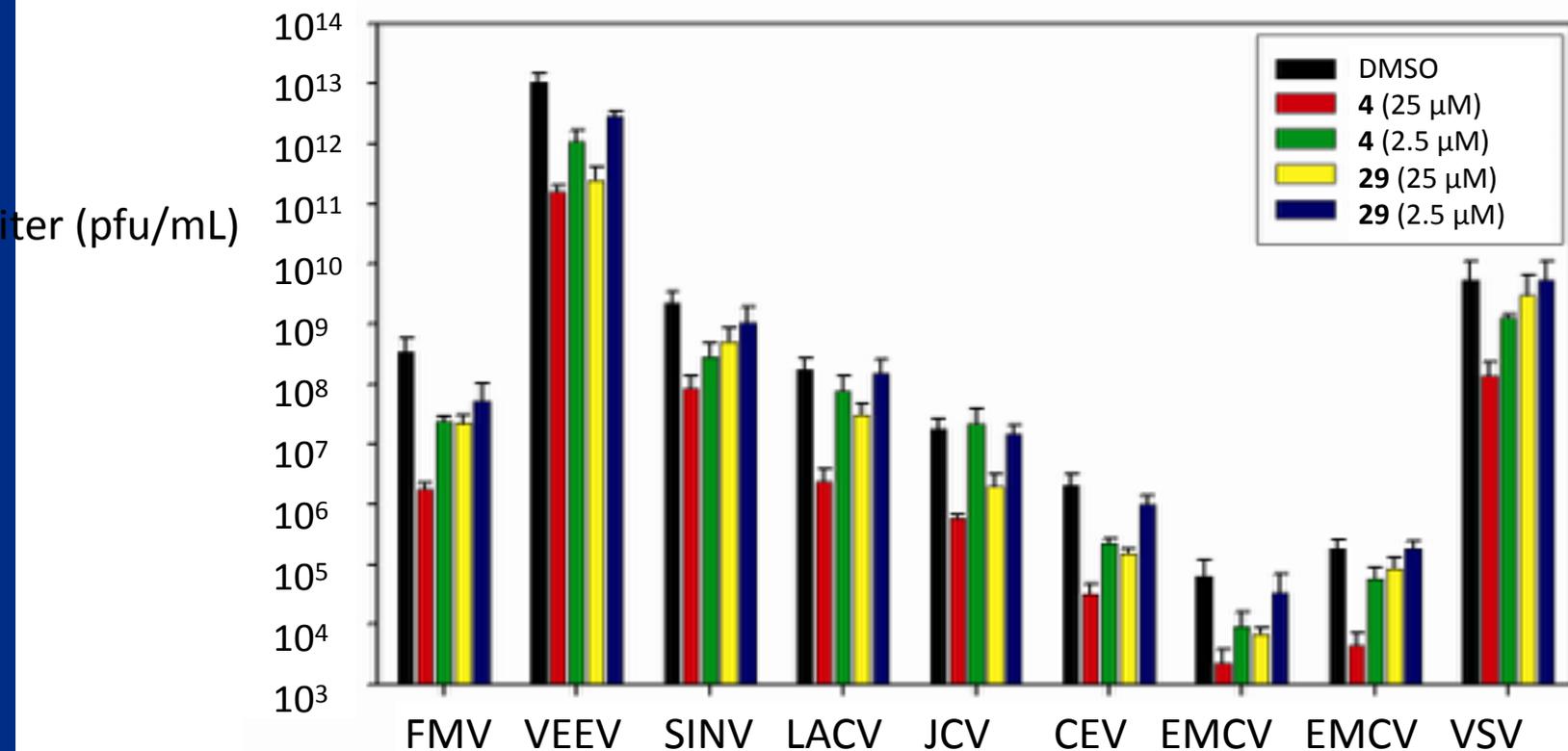


31
 $IC_{50} = 12.4 \mu M$
 $CC_{50} > 100 \mu M$

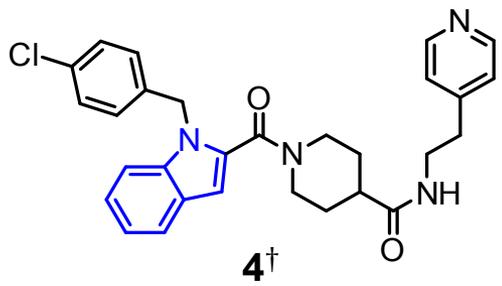




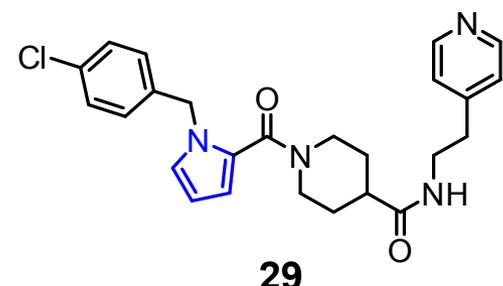
Antiviral Activity Against Various Live Viruses



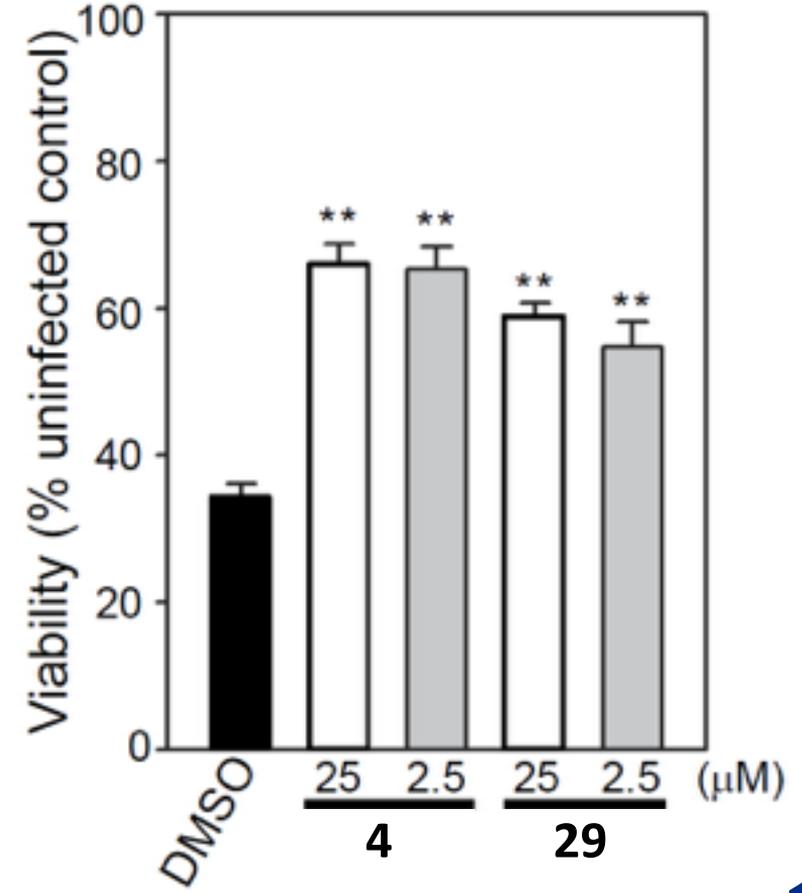
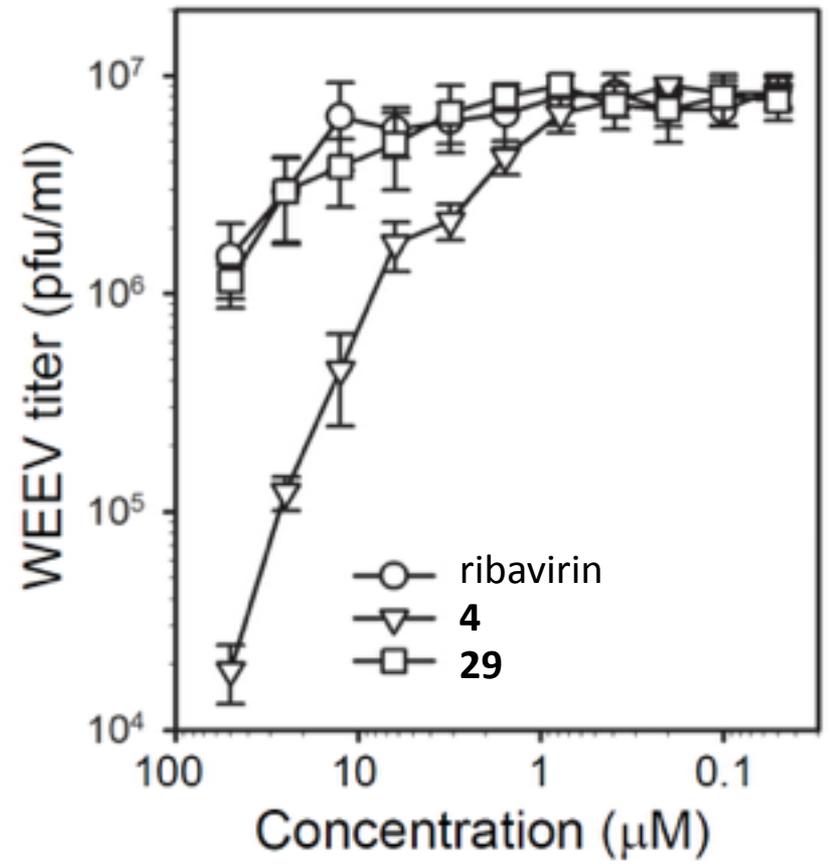
Data from the David Miller lab, U of M

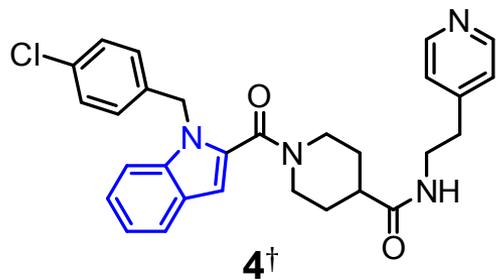


IC₅₀ = 0.53 μM
CC₅₀ = 65 μM

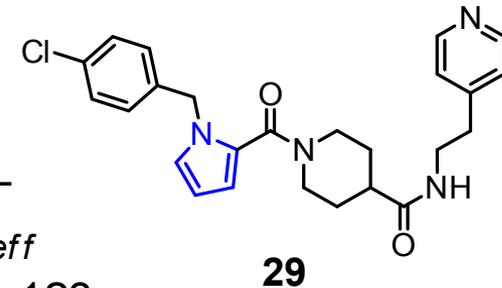


IC₅₀ = 0.68 μM
CC₅₀ > 100 μM

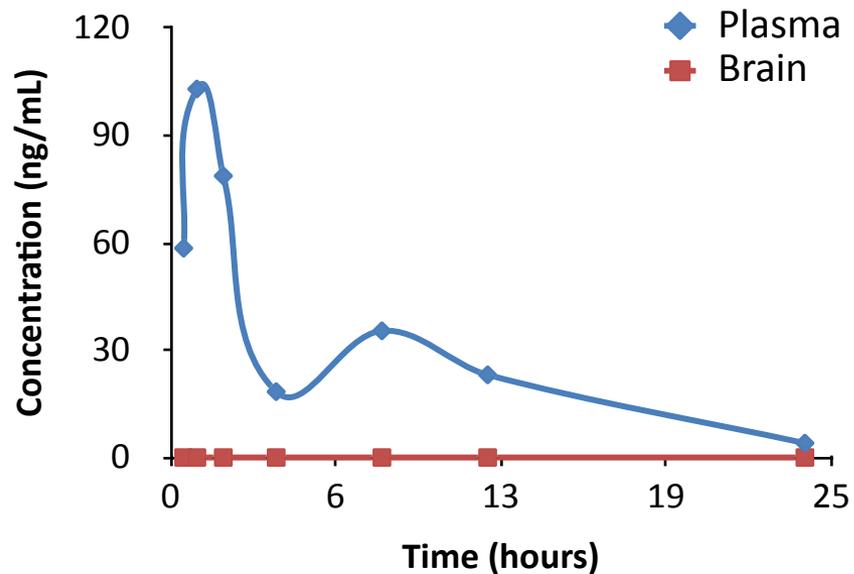




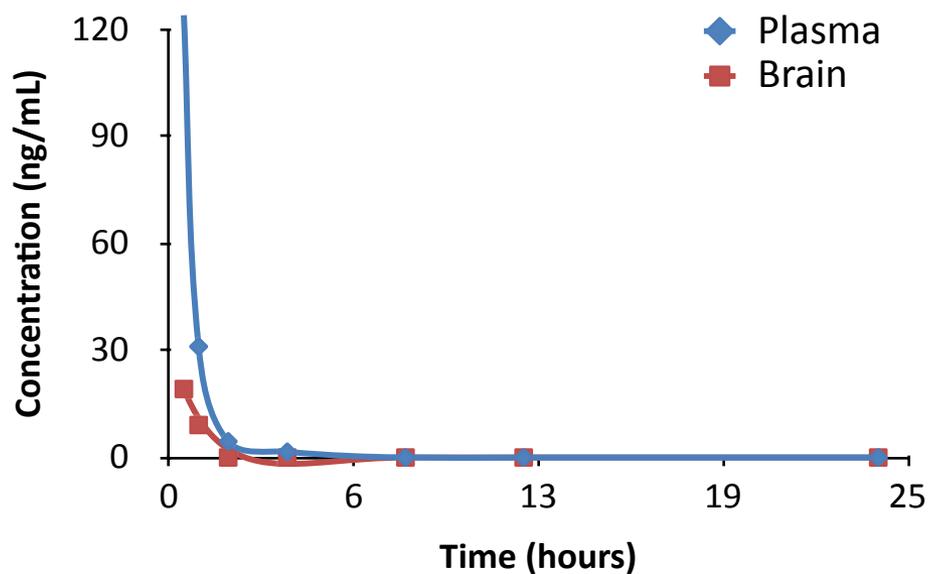
	4[†]	29	
IC ₅₀ =	0.53	0.68	μM
CC ₅₀ =	65	> 100	μM
MW =	501	451	g/mol
Aq. Sol =	31-63	125-250	μg/mL
PAMPA =	-4.18	-5.01	log P _{eff}
MDR1 =	30.9	-1.2	% Rho123
MLM T _{1/2} =	> 9	< 3	min

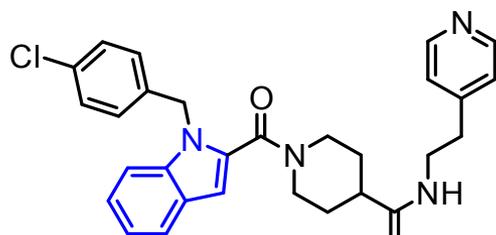


4
Plasma/Brain Concentration-Time



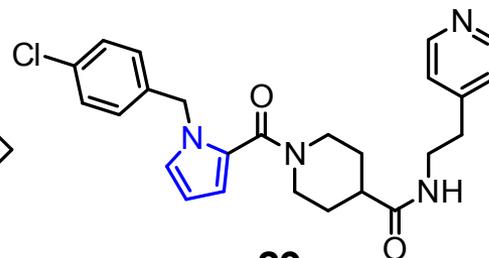
29
Plasma/Brain Concentration-Time





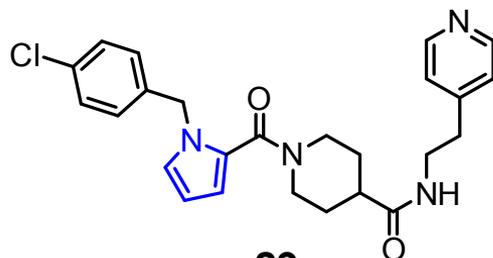
4†
 $IC_{50} = 0.53 \mu M$
 $CC_{50} = 65 \mu M$

MLM $T_{1/2} = 9 \text{ min}$

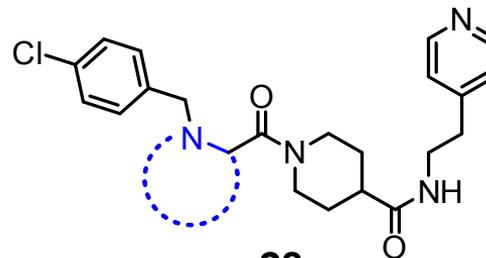


29
 $IC_{50} = 0.68 \mu M$
 $CC_{50} > 100 \mu M$

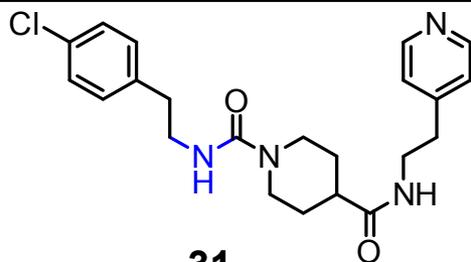
MLM $T_{1/2} = 3 \text{ min}$



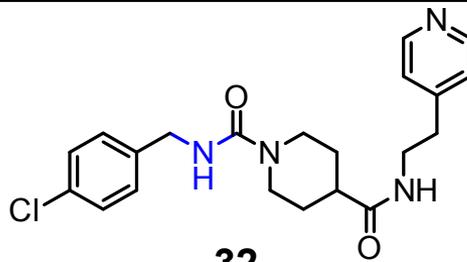
29



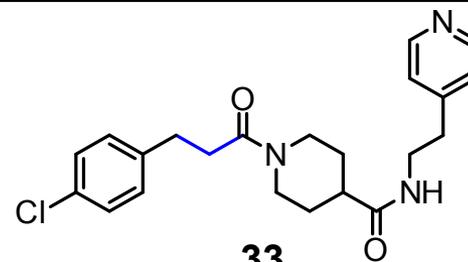
28



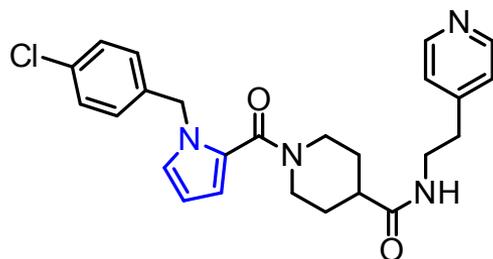
31
 $IC_{50} = 41 \mu M$
 $CC_{50} > 100 \mu M$
 MW = 414



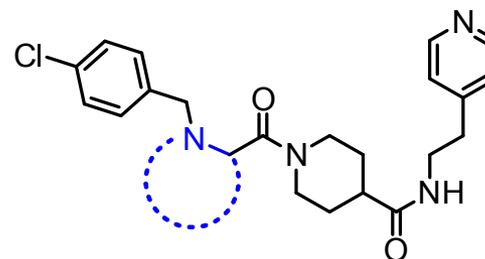
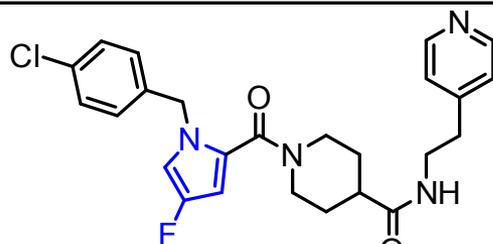
32
 $IC_{50} > 50 \mu M$
 $CC_{50} > 100 \mu M$
 MW = 401



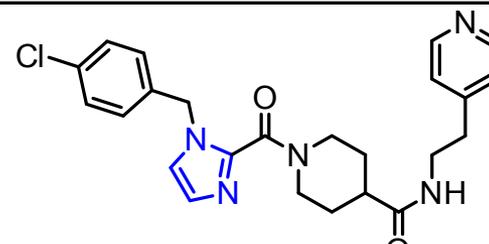
33
 $IC_{50} > 50 \mu M$
 $CC_{50} > 100 \mu M$
 MW = 400


29
 $IC_{50} = 0.68 \mu M$
 $CC_{50} > 100 \mu M$

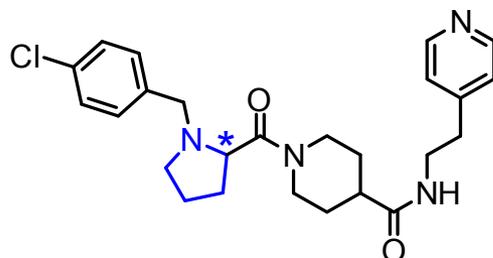
MW = 451


28

34
 $IC_{50} = 0.93 \mu M$
 $CC_{50} = 8.0 \mu M$

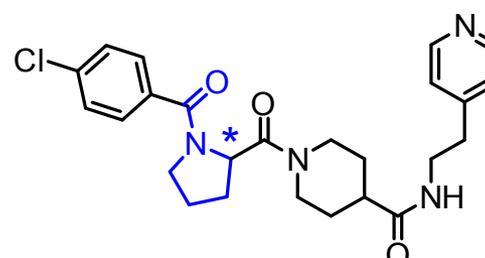
MW = 468


35
 $IC_{50} > 50 \mu M$
 $CC_{50} > 100 \mu M$

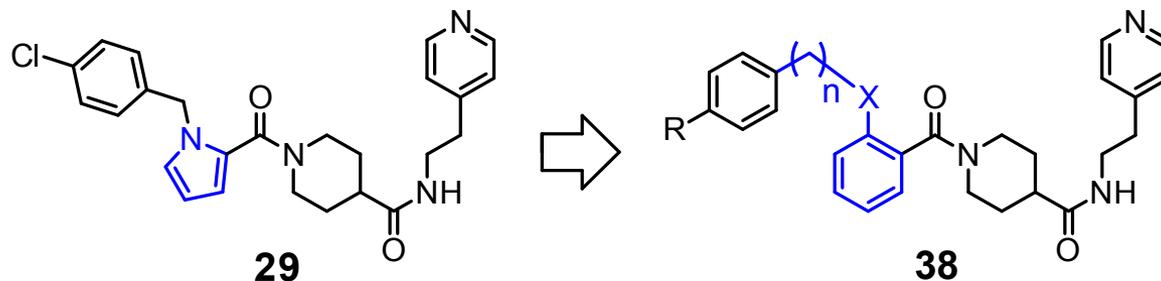
MW = 452


(R)-36 $IC_{50} = 13.5 \mu M$
(S)-36 $IC_{50} = 53.5 \mu M$
 $CC_{50} > 100 \mu M$

MW = 455

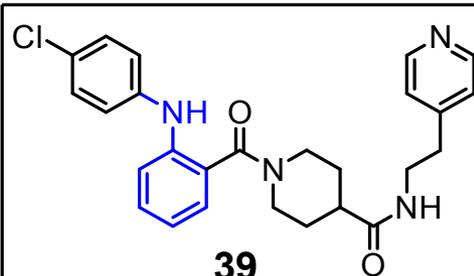

(R)-37 $IC_{50} > 100 \mu M$
(S)-37 $IC_{50} > 100 \mu M$
 $CC_{50} > 100 \mu M$

MW = 469



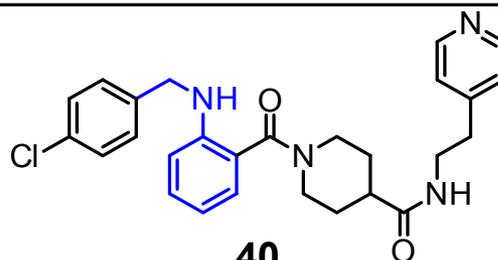
$IC_{50} = 0.68 \mu M$
 $CC_{50} > 100 \mu M$

MLM $T_{1/2} = 3 \text{ min}$



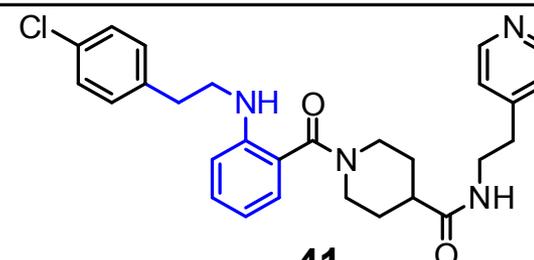
$IC_{50} = 1.6 \mu M$
 $CC_{50} > 100 \mu M$

MLM $T_{1/2} = 19 \text{ min}$

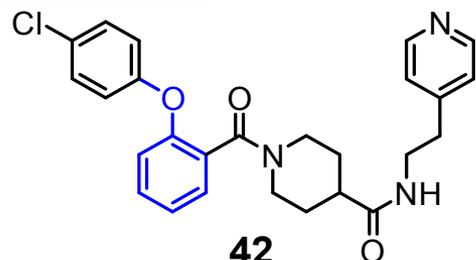


$IC_{50} = 0.56 \mu M$
 $CC_{50} = 75 \mu M$

MLM $T_{1/2} = 15 \text{ min}$

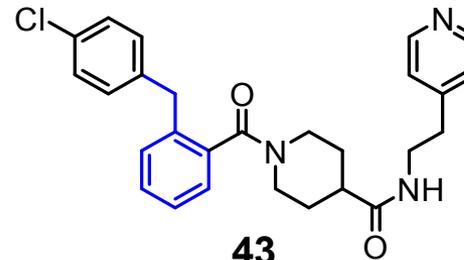


$IC_{50} = 3.5 \mu M$
 $CC_{50} = 86 \mu M$



$IC_{50} = 6.1 \mu M$
 $CC_{50} = 56 \mu M$

MLM $T_{1/2} = 18 \text{ min}$

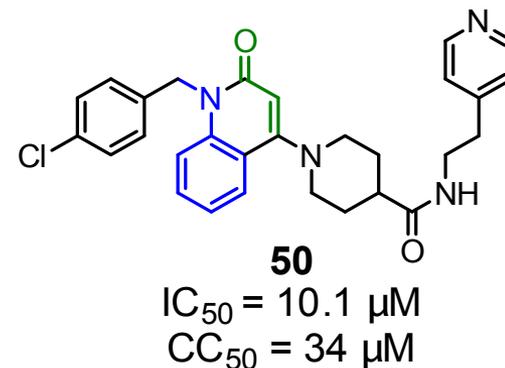
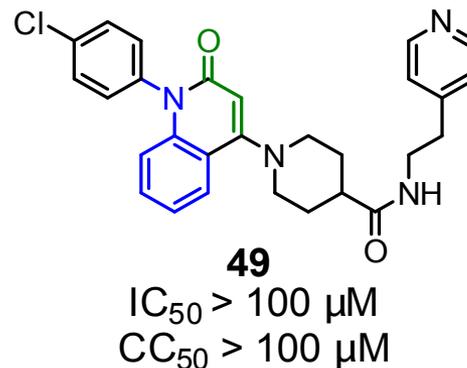
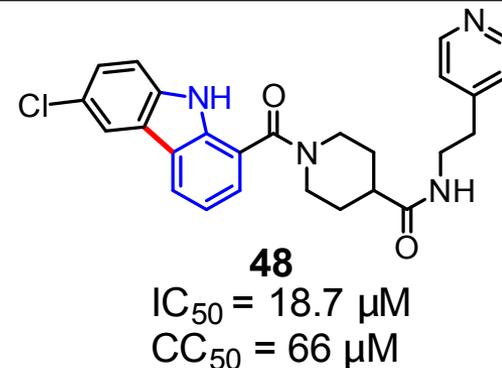
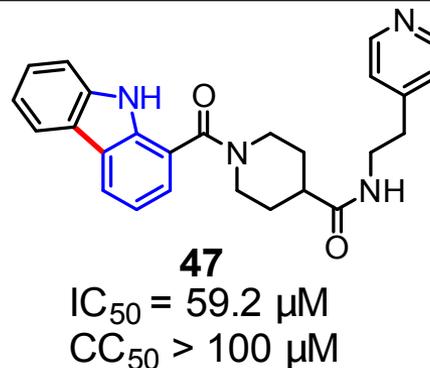
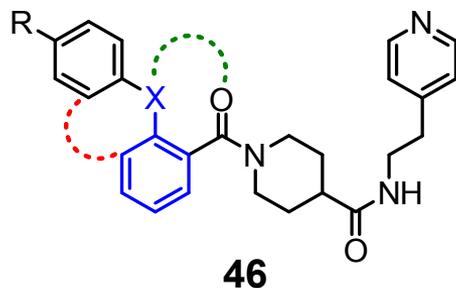
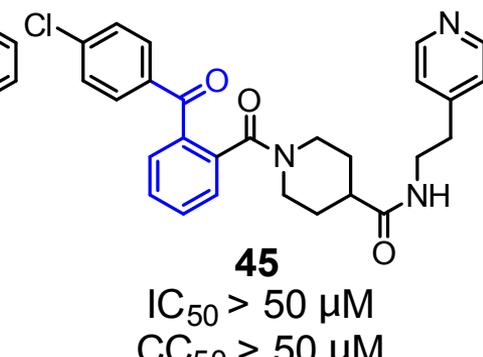
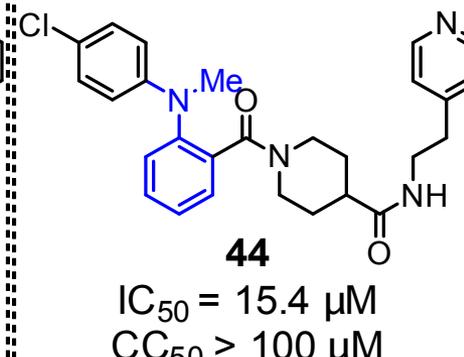
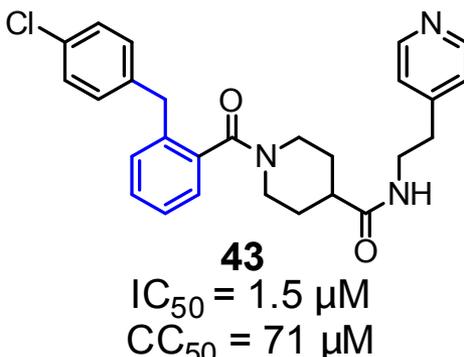
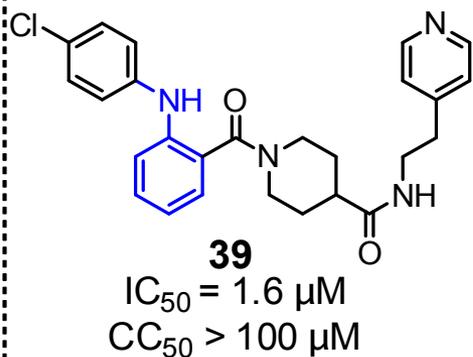


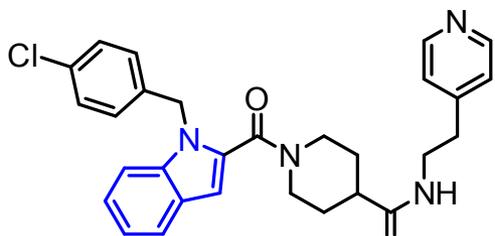
$IC_{50} = 1.5 \mu M$
 $CC_{50} = 71 \mu M$

MLM $T_{1/2} = 3 \text{ min}$

Active

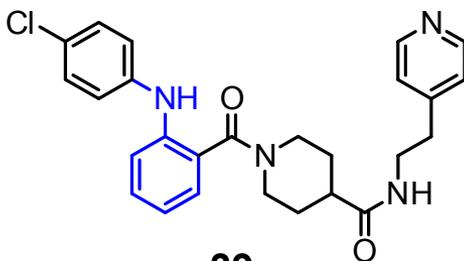
"Inactive"





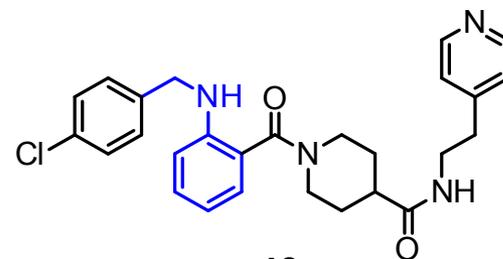
4†

IC₅₀ = 0.53 μM
 CC₅₀ = 65 μM
 MLM T_{1/2} = 9 min



39

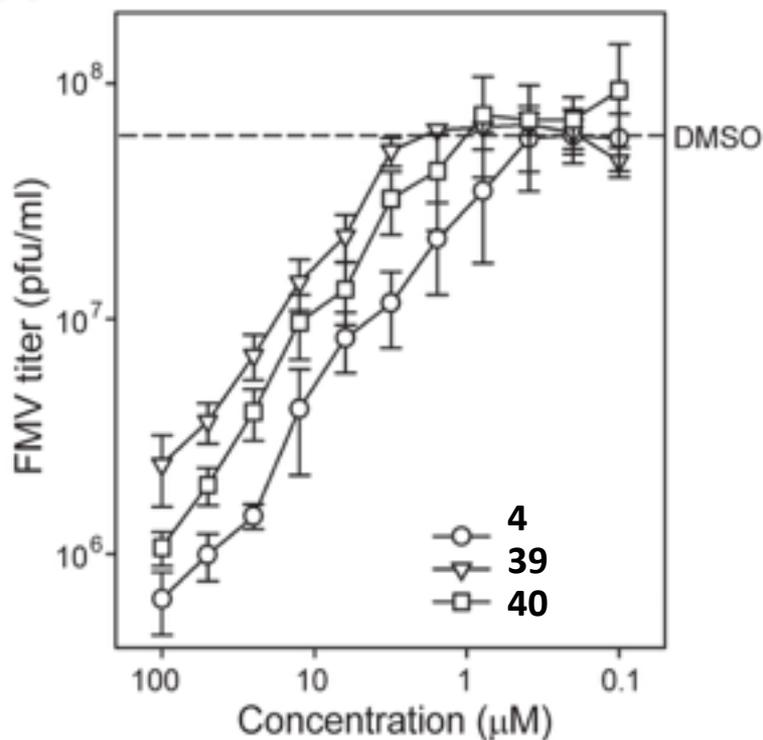
IC₅₀ = 1.6 μM
 CC₅₀ > 100 μM
 MLM T_{1/2} = 19 min



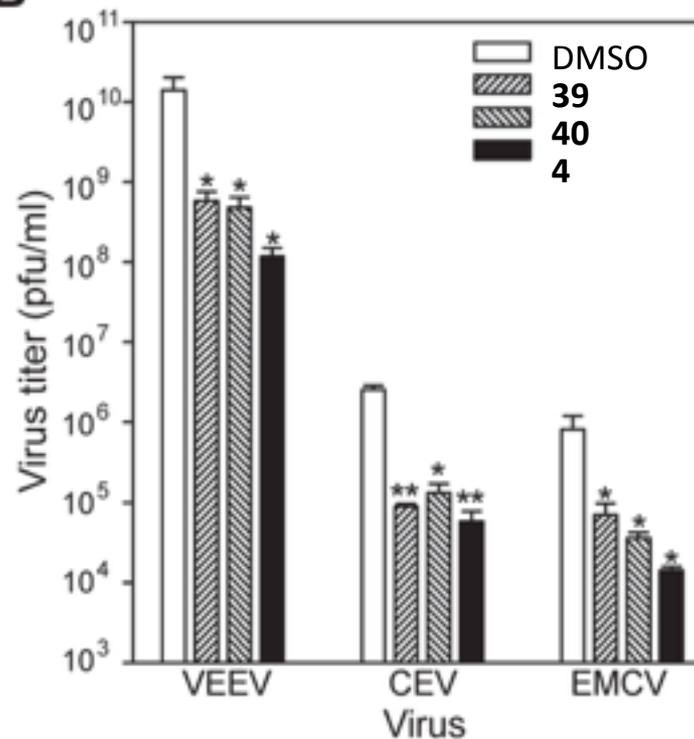
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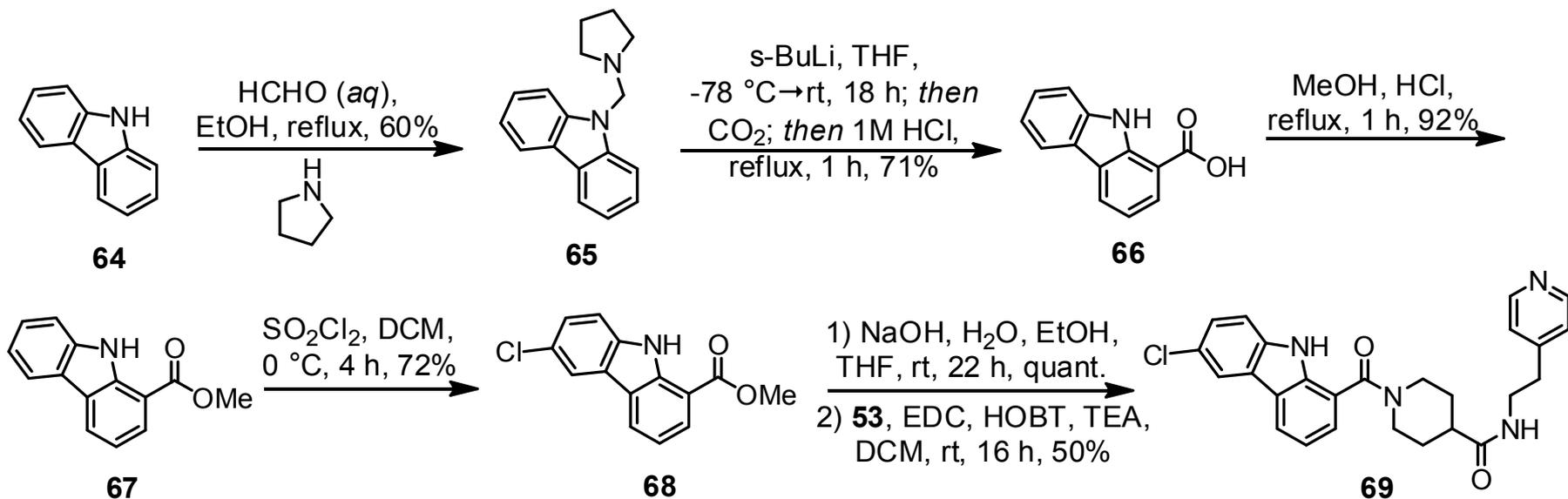
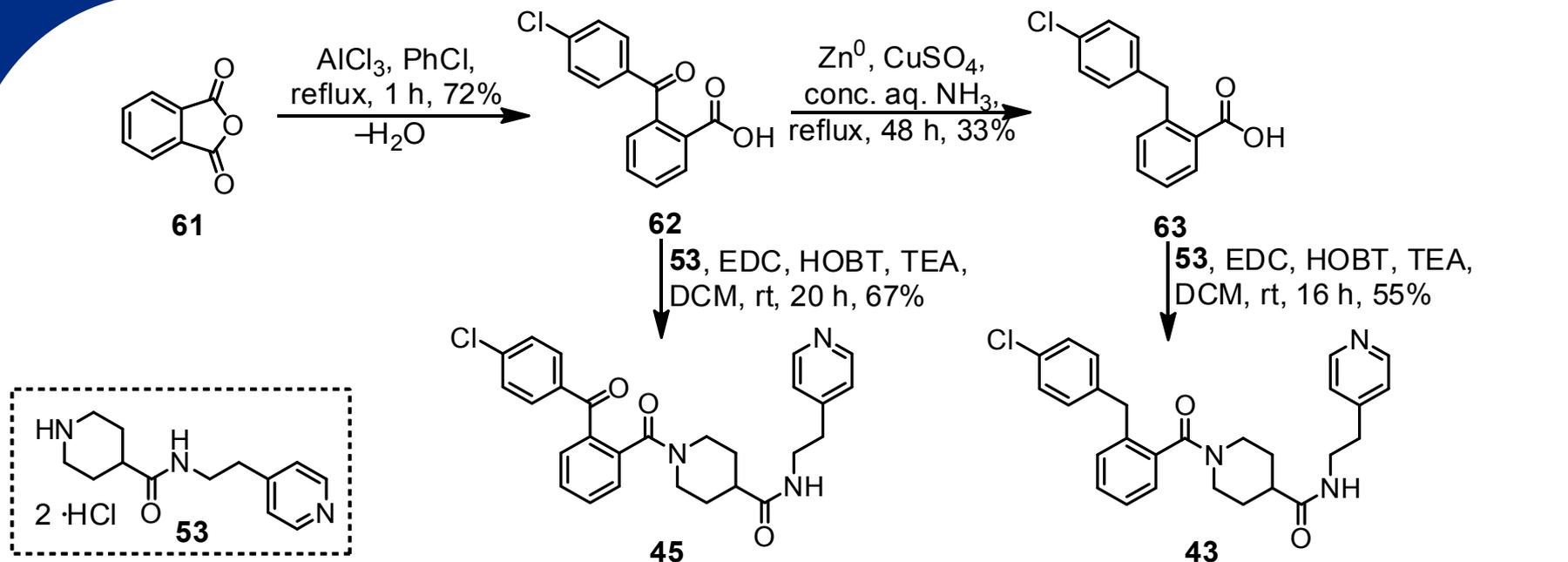
IC₅₀ = 0.56 μM
 CC₅₀ = 75 μM
 MLM T_{1/2} = 15 min

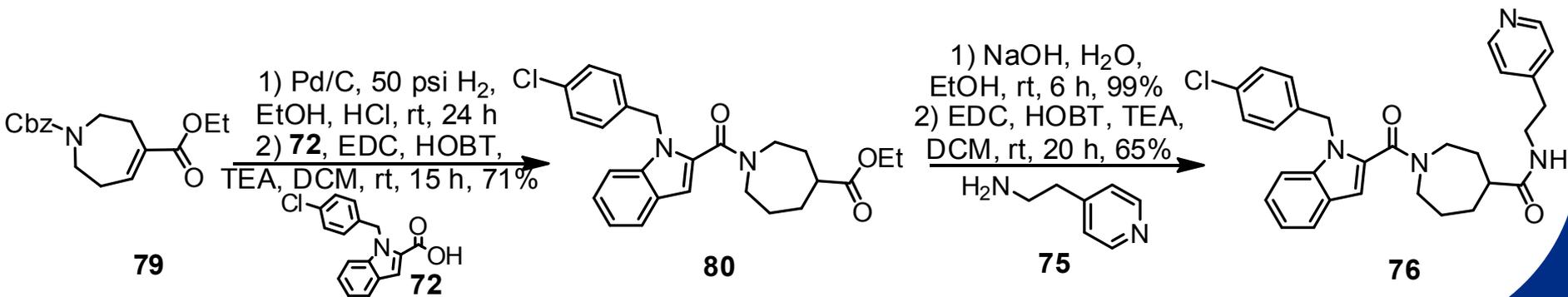
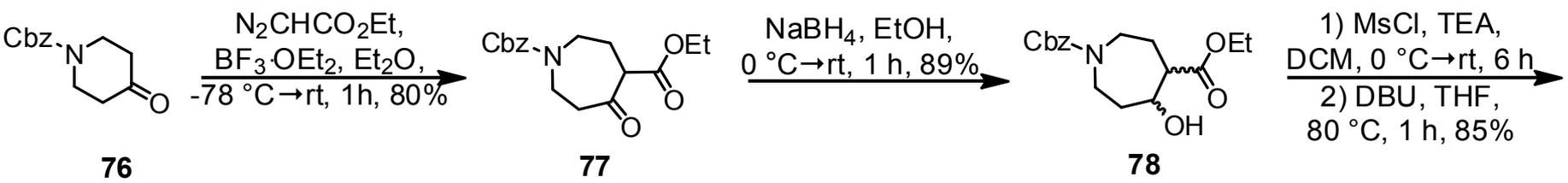
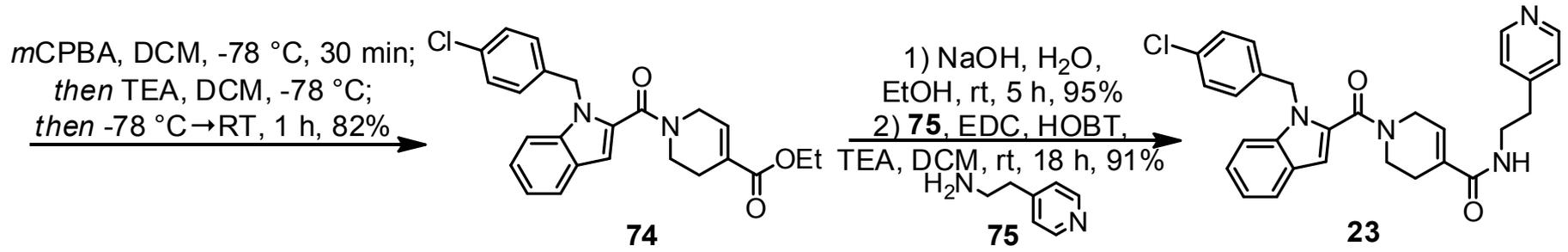
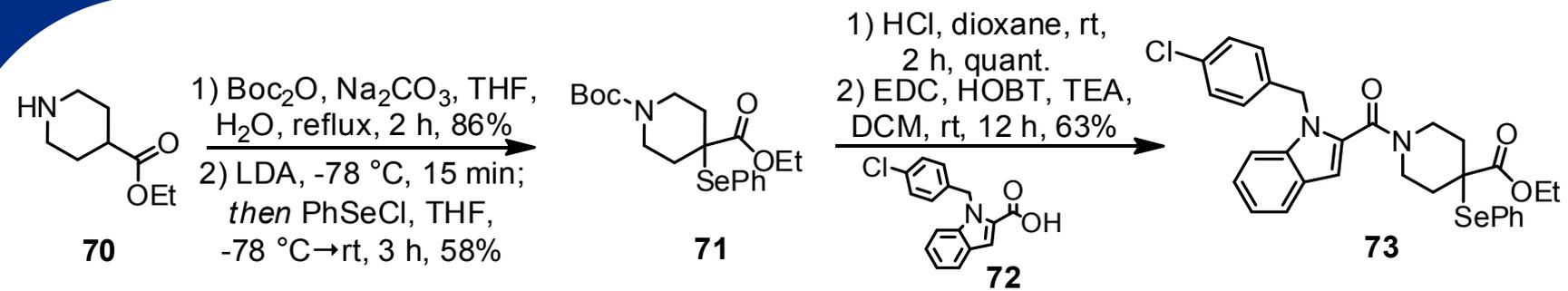
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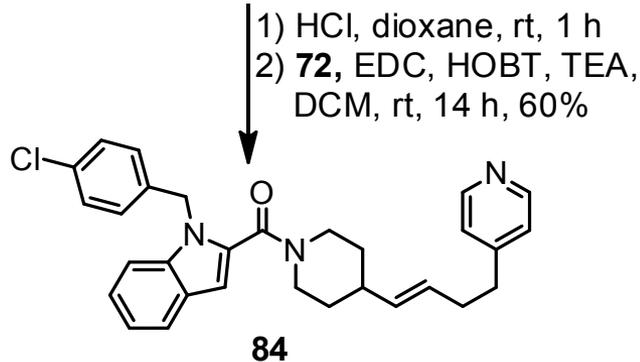
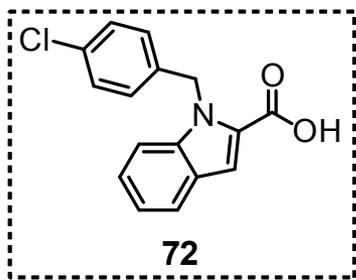
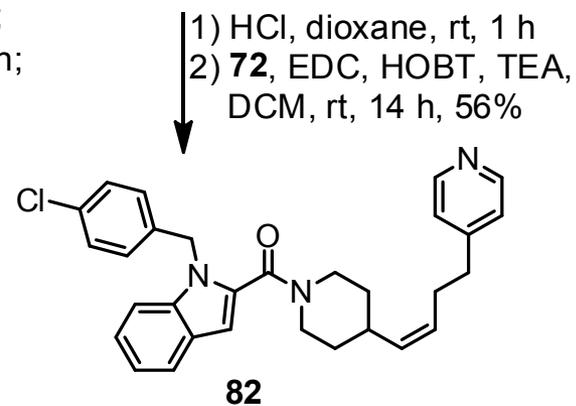
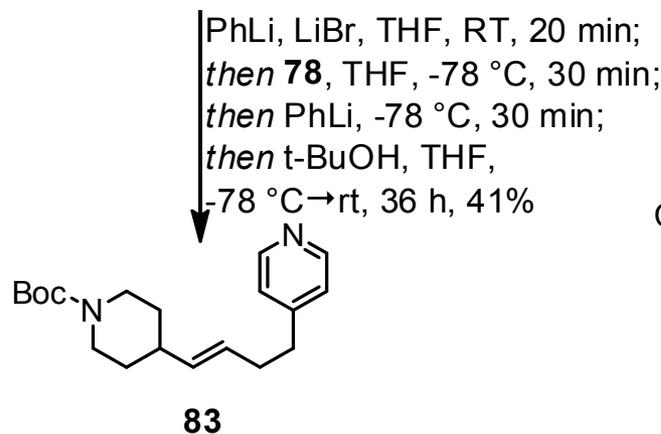
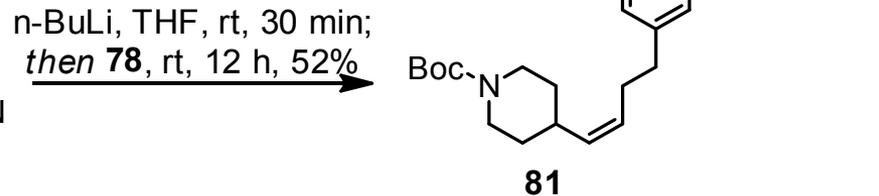
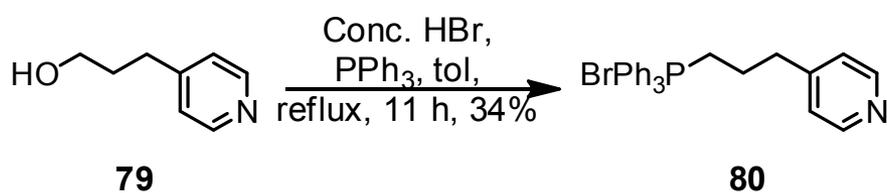
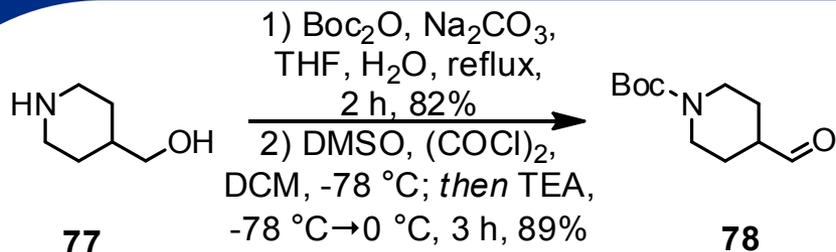


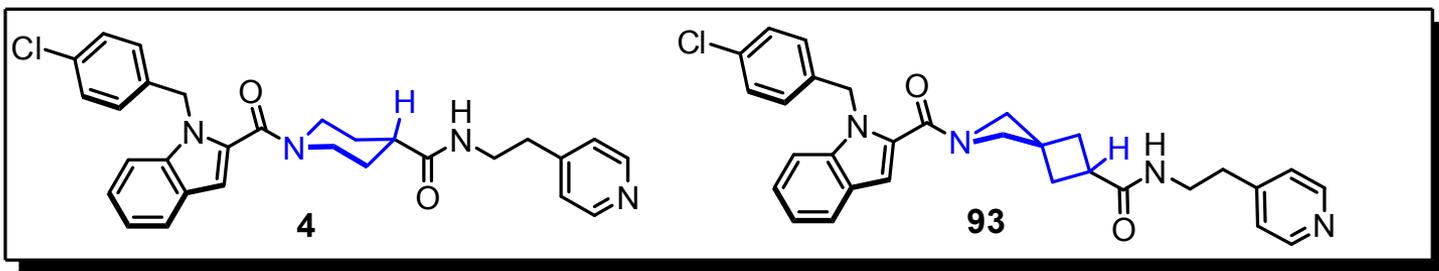
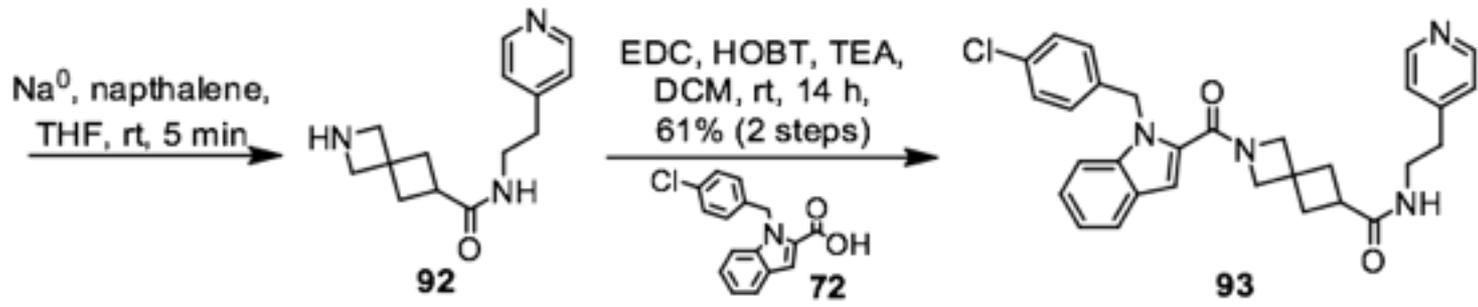
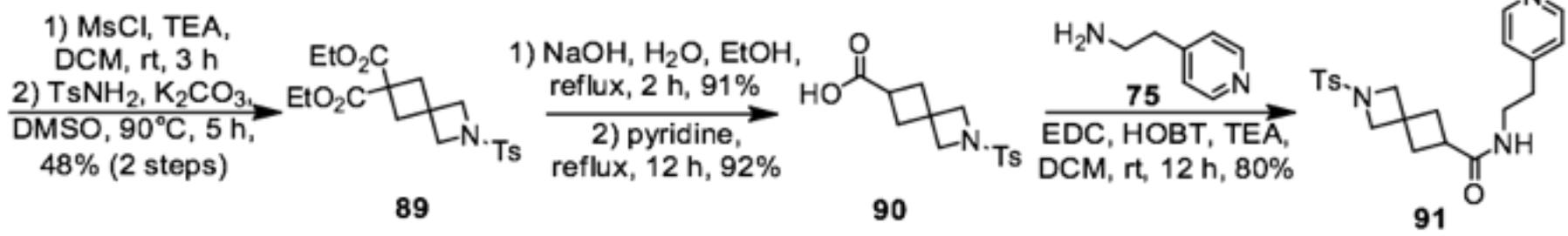
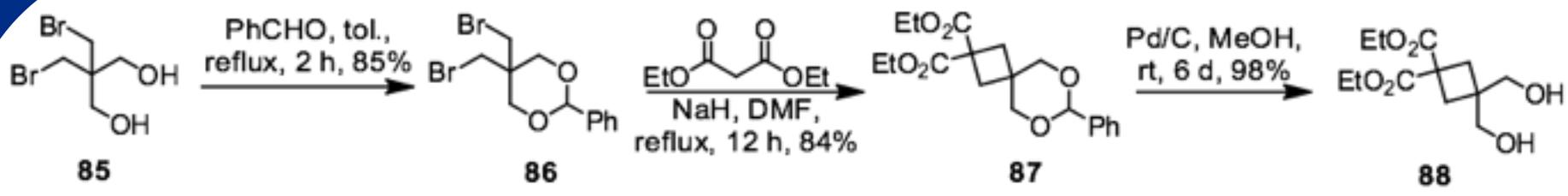
B

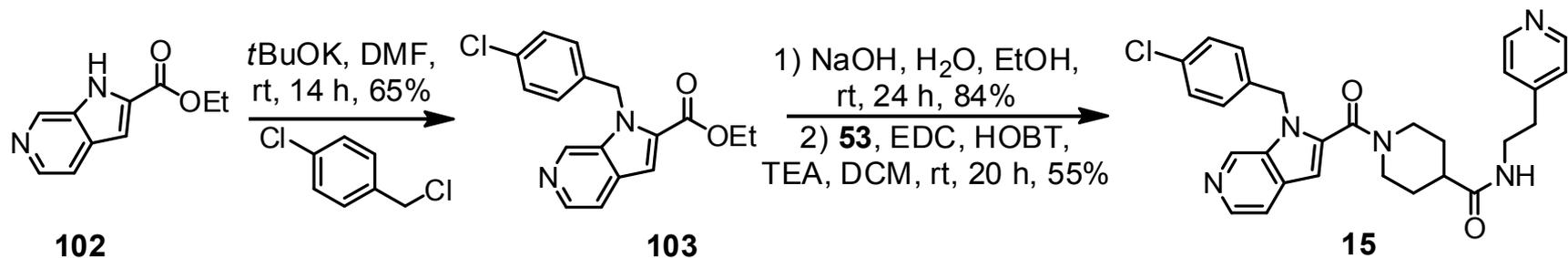
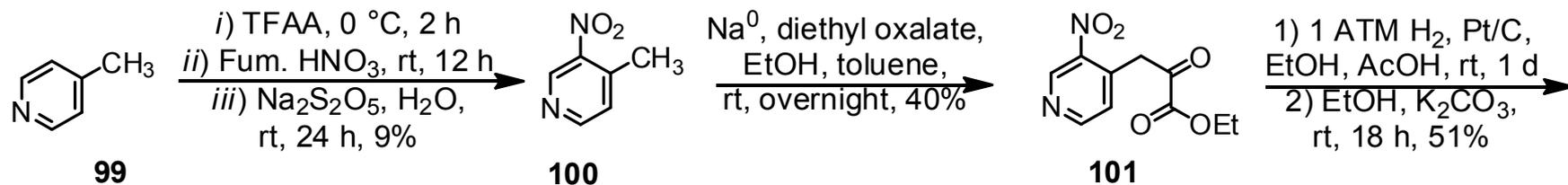
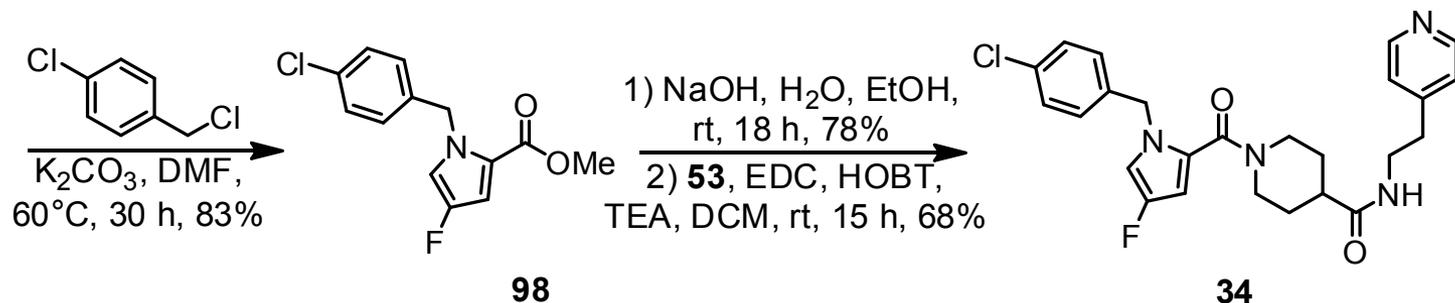








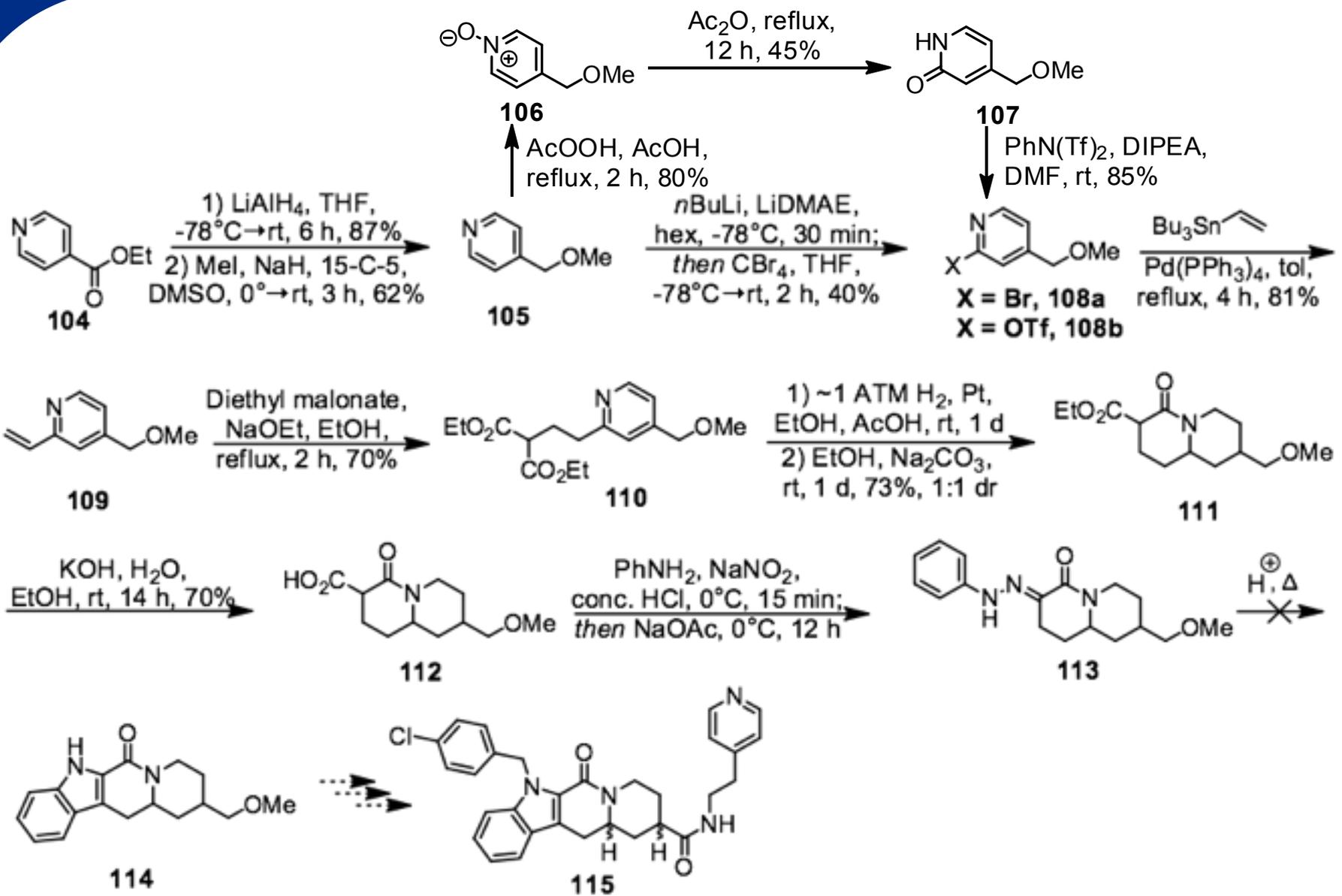




Fluoropyrrole core: *Bioorg Med Chem* **2009** (17) 1557-1572, *Tet* **2002** (58) 6713-6722

Nitration of pyridine: *Org Biomol Chem* **2005** (3) 538-541

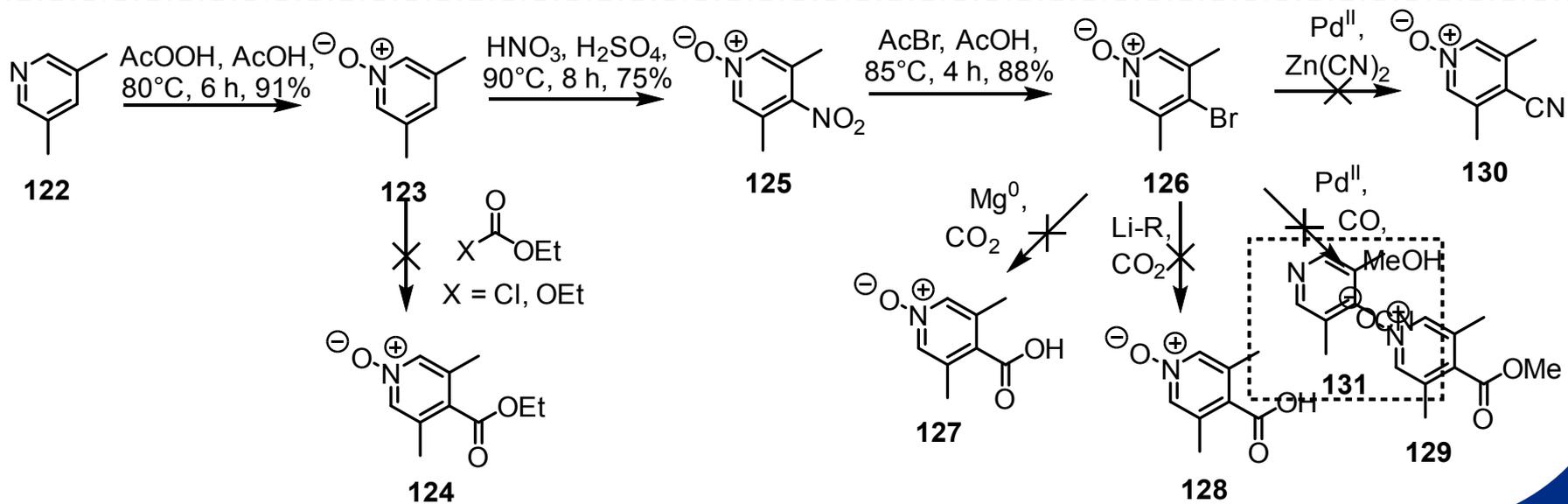
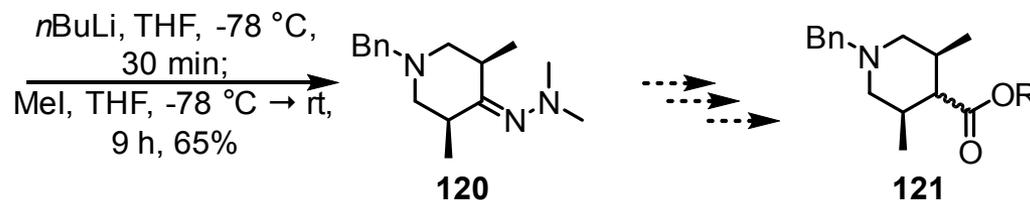
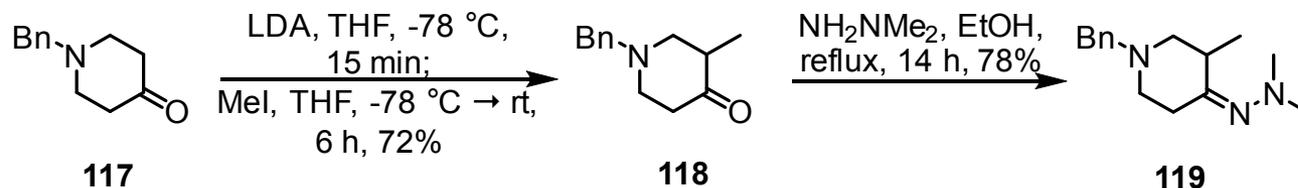
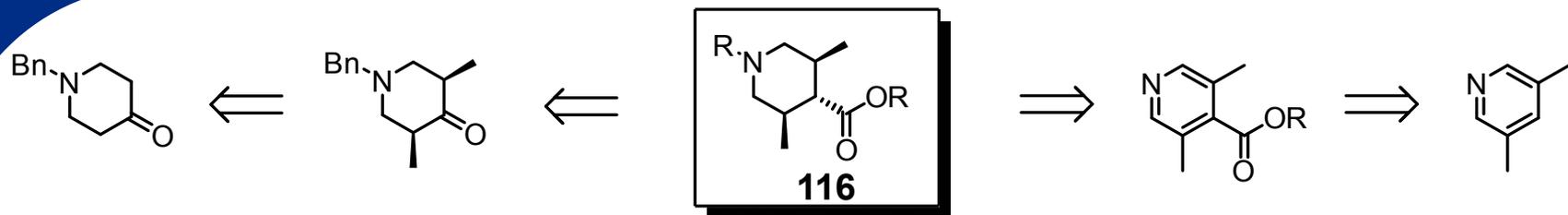
Azaindole core: *ARKIVOC* **2004** (v) 286-300

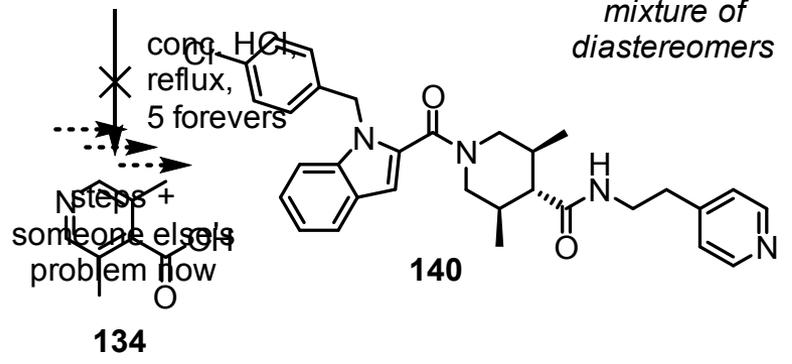
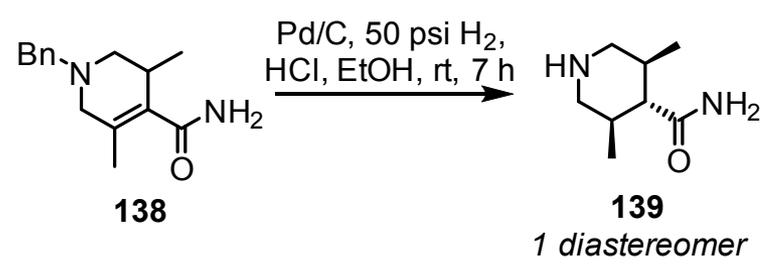
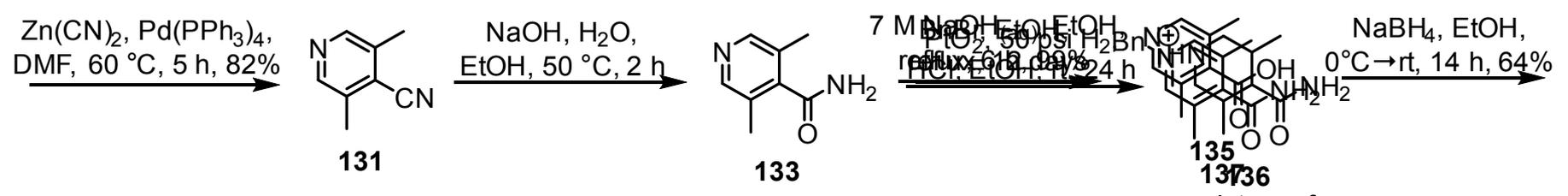
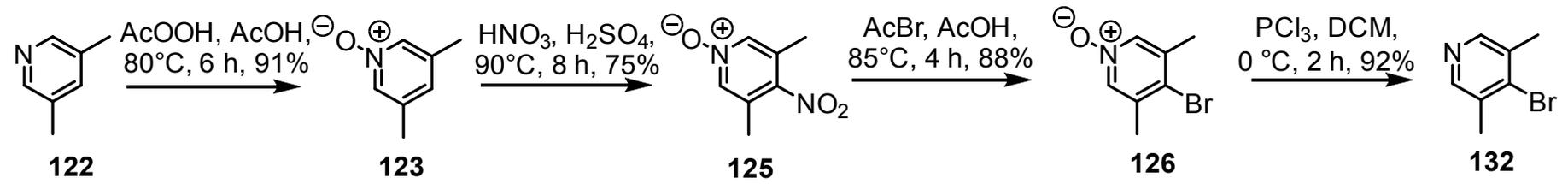


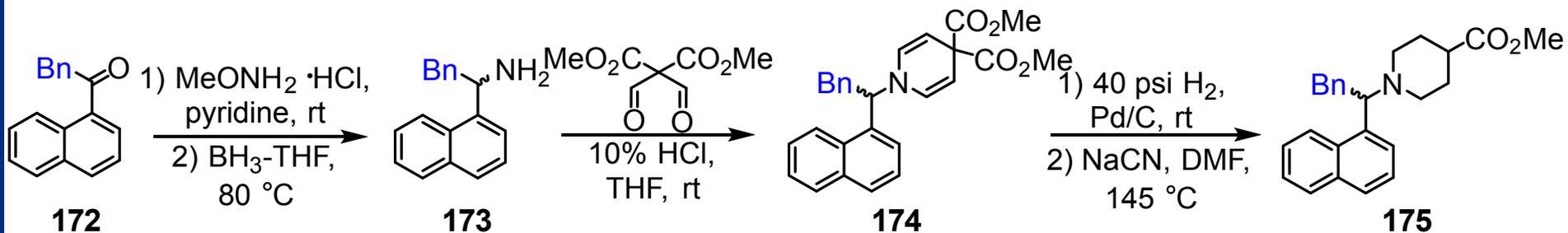
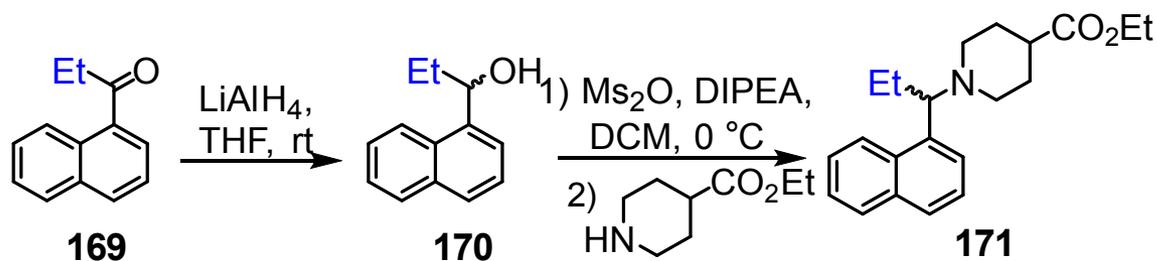
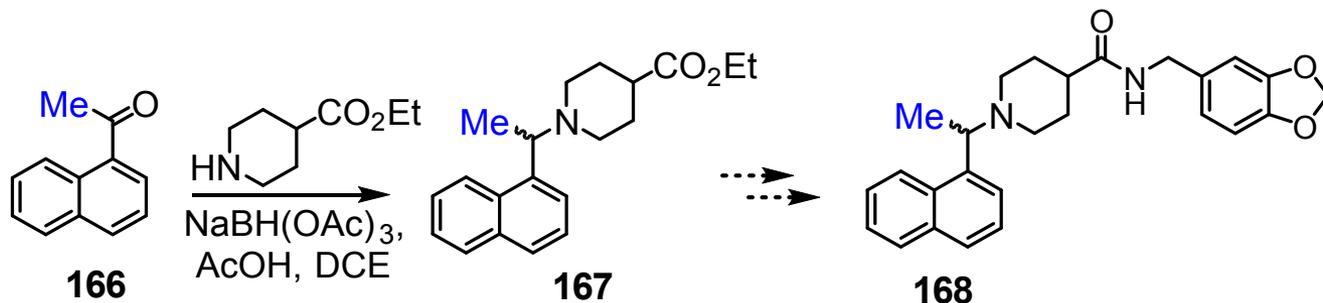
Directed lithiations of picolines: *Eur JOC* **2003** (19) 3855-3860

N-oxide rearrangements: *JOC* **1961** (26) 428-430, *JOC* **1953** (18) 534-551

Indole tetracycles: *Archiv Pharm* **1958** (291) 610-620

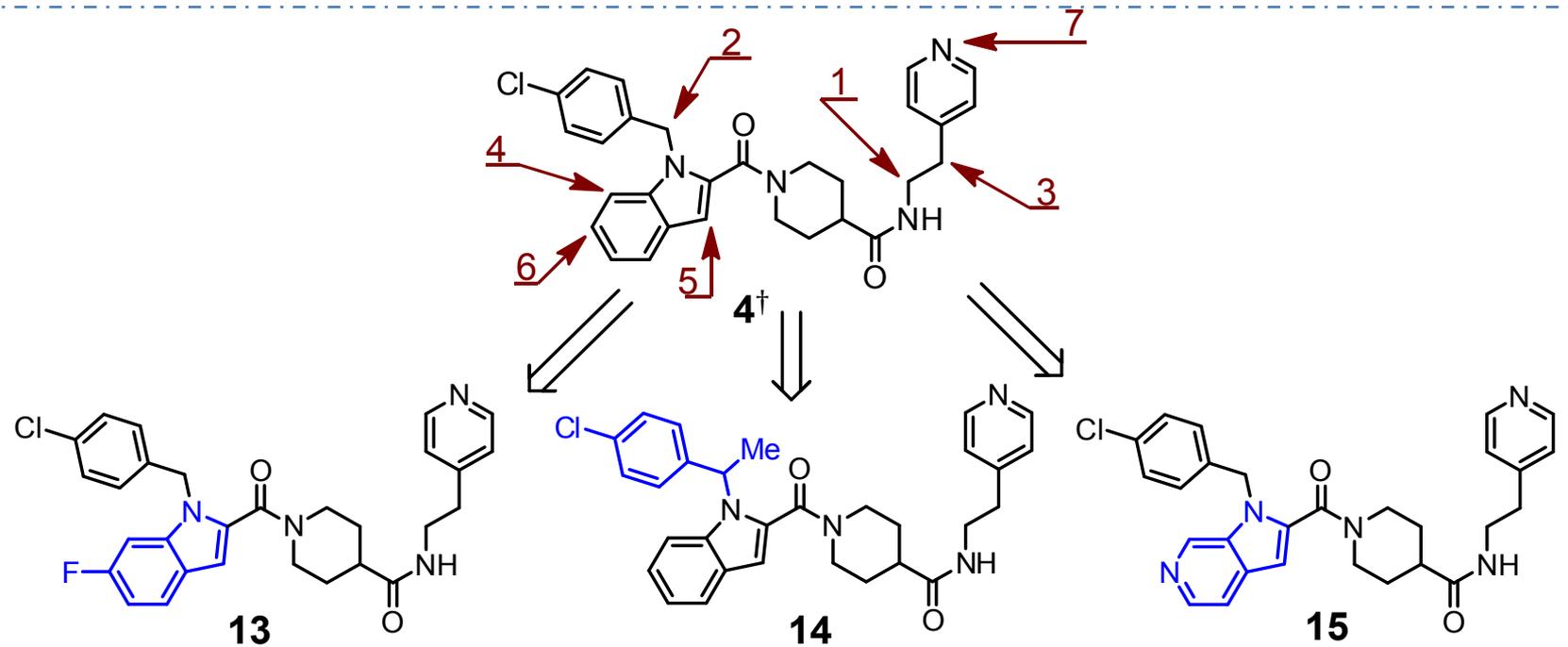
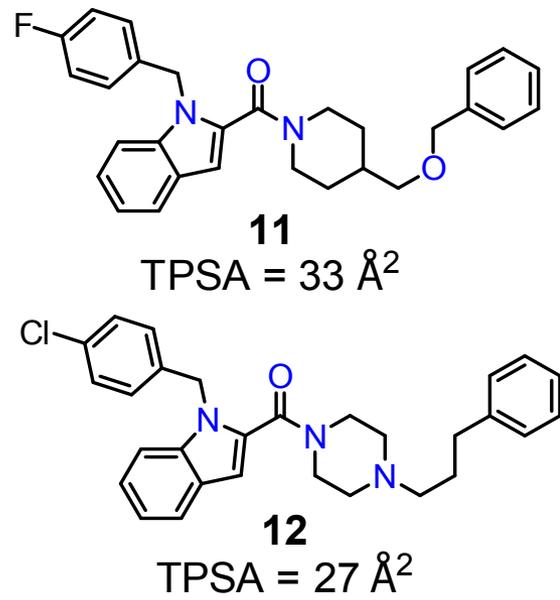
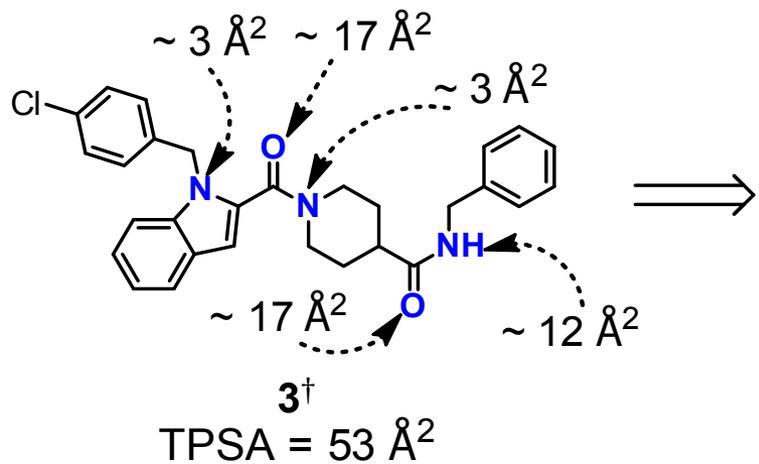






Báez-Santos, Y. M.; Barraza, S. J.; et al. *Journal of Medicinal Chemistry*. **2014**, *57*, 2393-2412

Gosh, A; et al. *Journal of Medicinal Chemistry*. **2010**, *53*, 4968-4979



Vahlteich Medicinal Chemistry Core

Prof. Scott Larsen*

Janice Sindac

Bryan Yestrepky

Kyle Bolduc

Paul Kirchhoff

In Vitro Work – Miller Lab

Prof. David Miller*

Craig Dobry

Phillip Delekta

In Vivo Work – Irani Lab

Prof. David Irani*

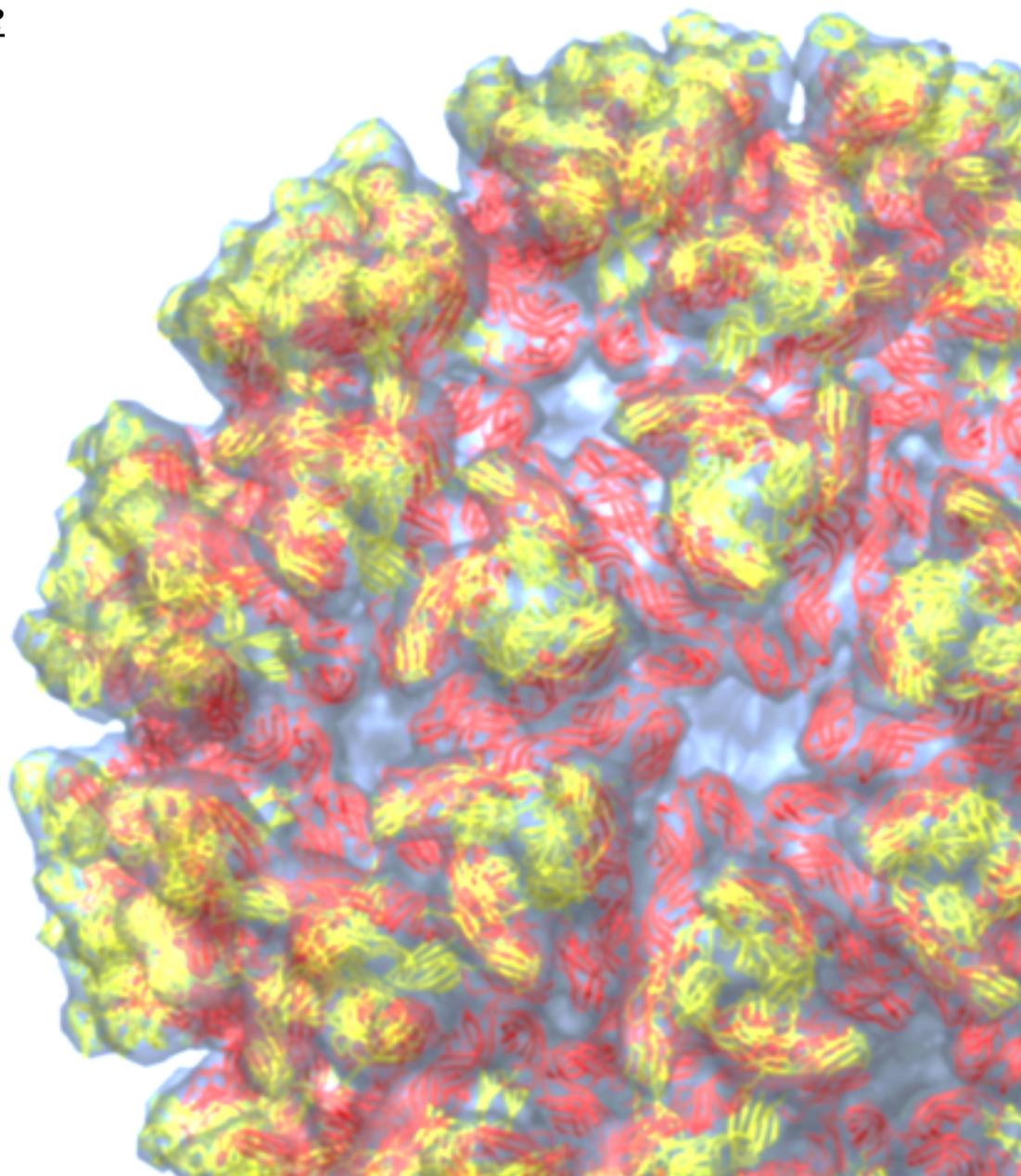
Penny Blakely

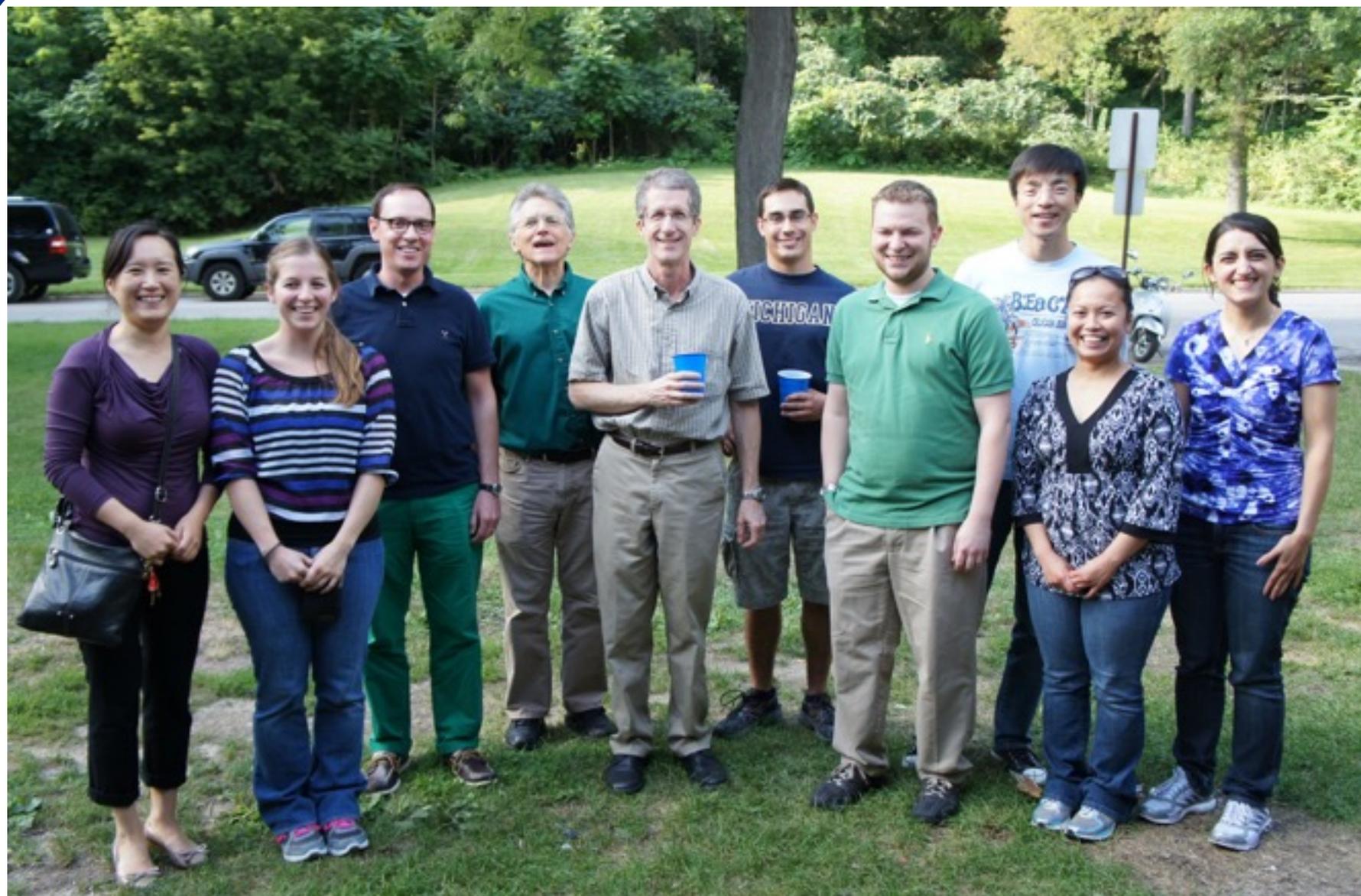
MDR1 Work – Keep Lab

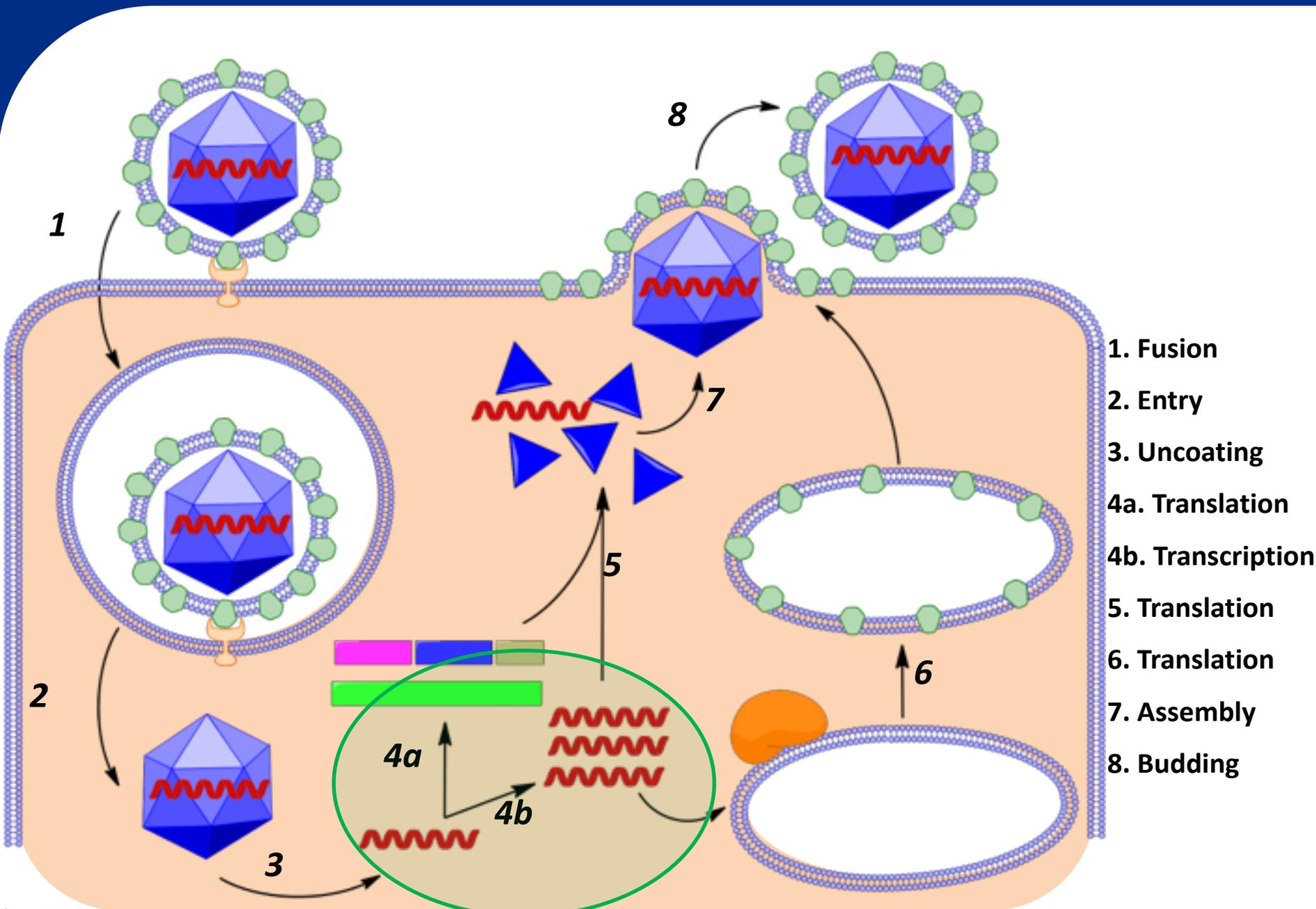
Prof. Richard Keep*

Jianming Xiang

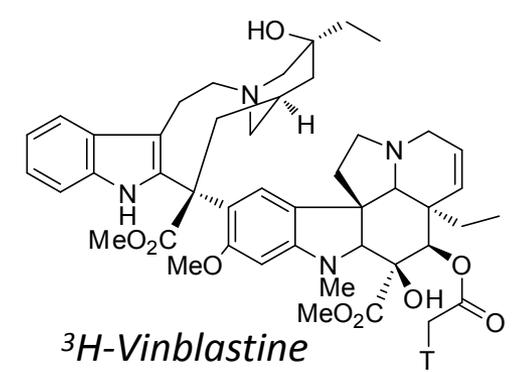
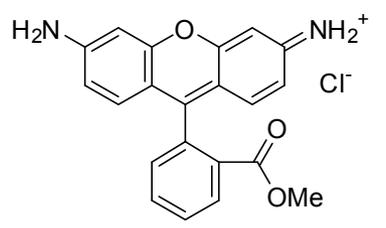
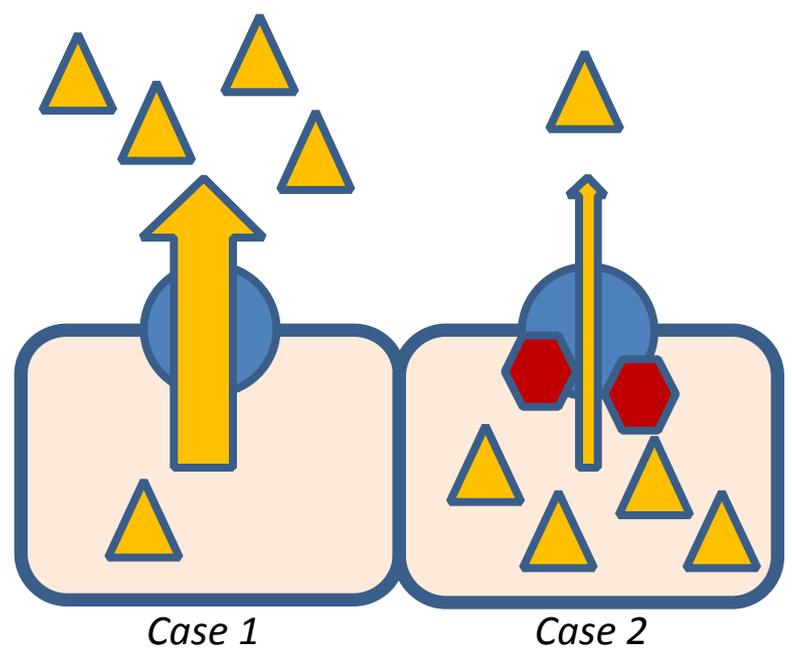
Biomedical Mass Spec Facility







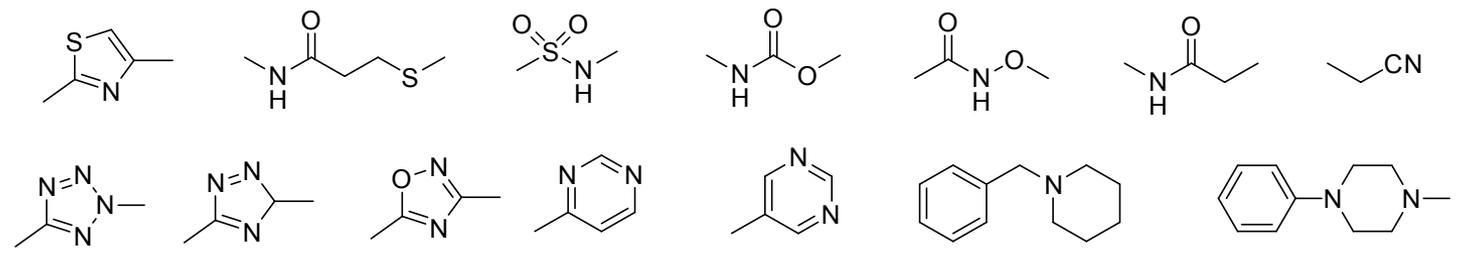
- 1. Fusion
- 2. Entry
- 3. Uncoating
- 4a. Translation
- 4b. Transcription
- 5. Translation
- 6. Translation
- 7. Assembly
- 8. Budding



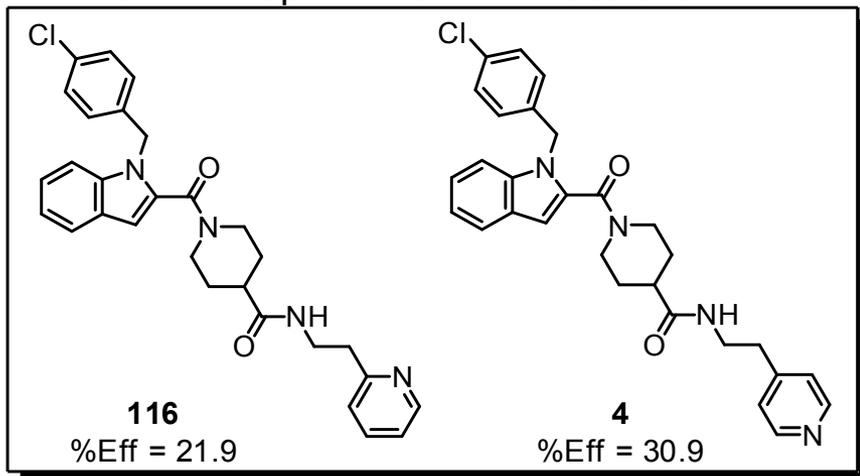
 ³H-vinblastine or Rhodamine 123

 Compound of interest

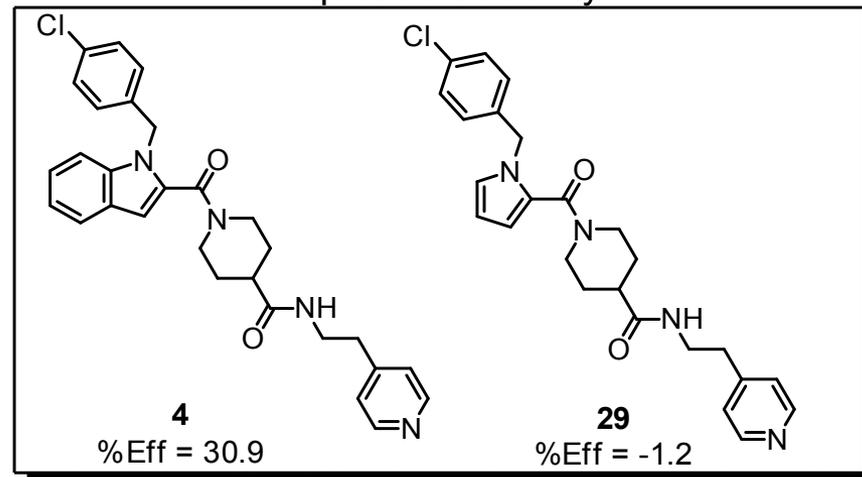
Recognition-Susceptible Structures



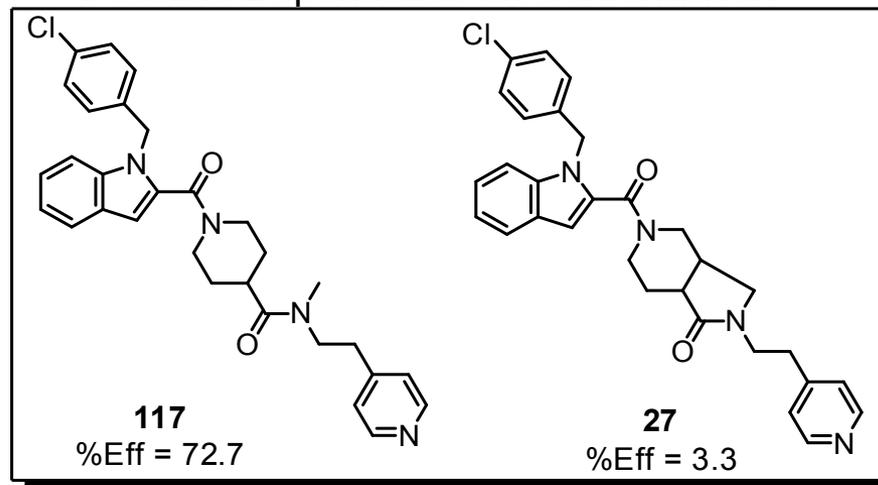
Subtle Dependence on Substitution

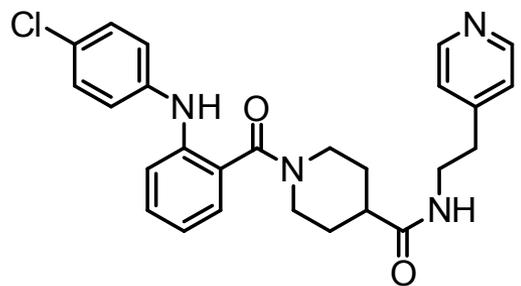
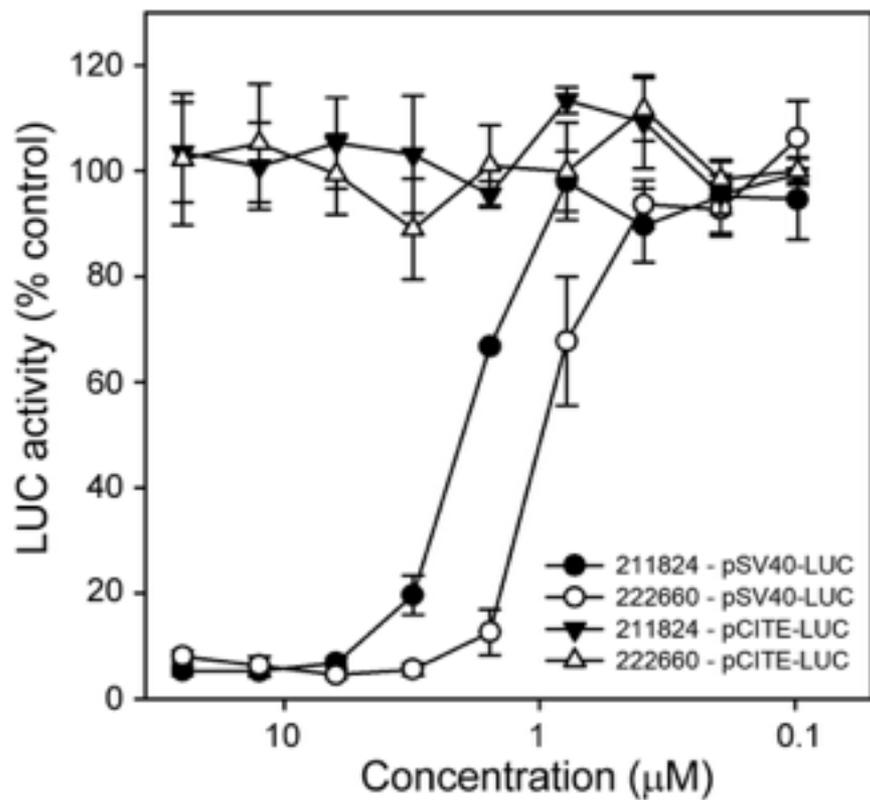


Dependence on Molecular Weight or Aqueous Solubility

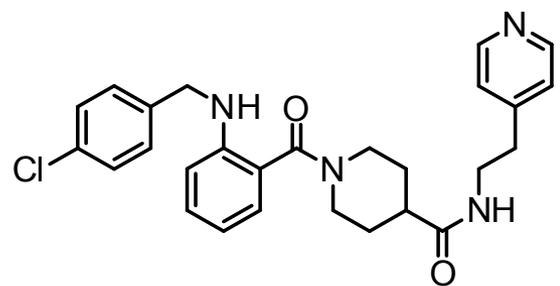


Possible Dependence on Conformation





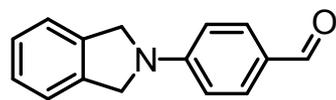
39
IC₅₀ = 1.6 µM



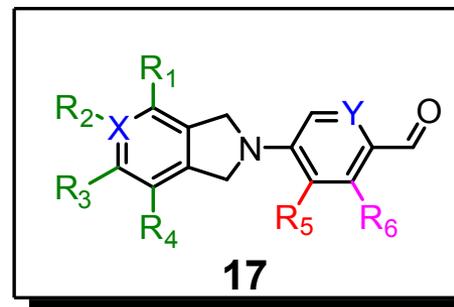
40
IC₅₀ = 0.56 µM

Data from the Miller Lab.

Inhibitors of ALDH1 (anti-cancer target)

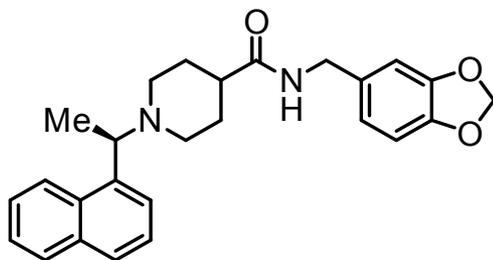


16

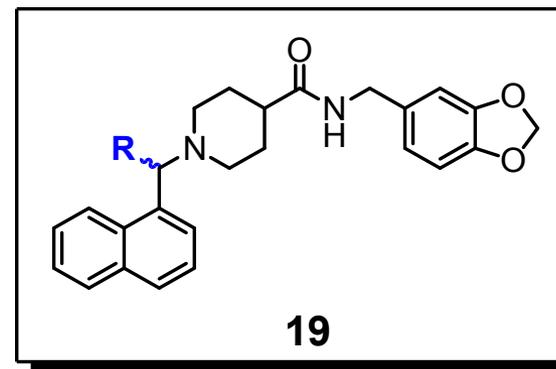


17

Inhibitors of coronavirus PI-Protease



18



19