Development of a CCR5 antagonist for HIV therapy

Advanced Machine Learning for Innovative Drug Discovery (AIDD)

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- Overview on the drug development process
- Opportunities for computational chemistry
- Case study: Maraviroc

Target ID & Valid.

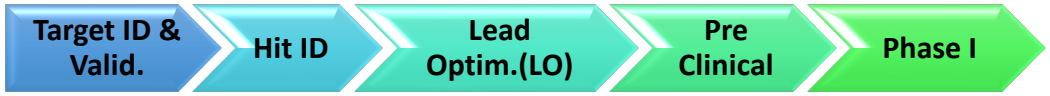
- Disease mechanism;
- Target type & drugability;
- Geno- & proteomics;
- Pathways;
- Chemical knock-out & transgenic models;
- In vitro/cell-based assays;
- In vivo/animal models.

- Primary screening (>10^6 cpds);
- In silico/CADD & SBDD;
- Potency & ADR;
- Counter screens;
- Mechanism of Action (MOA).

Target ID & Valid. → **Hit ID**

- Medicinal Chemistry;
- Animal PK/PD/ADME;
- Toxicity (Iterative process)
- Formulation & delivery.

- Pre-clinical data package;
- Process development/CMC/API/GMP;
- IND (investigational new drug application).

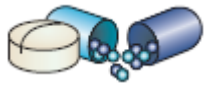


- Small group of healthy volunteers (20-80):
  - ✓Dose-escalation & safety;
  - ✓ Identification of side effects;
  - ✓ Early read-out of efficacy.

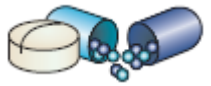
- Larger group of people (100-300):
  - ✓Effectiveness of drug for indication;
  - ✓ Effective dose range;
  - ✓ Further evaluation of safety.

- Even larger group of people (1-3k):
  - ✓Confirm effectiveness;
  - ✓ Monitor side effects;
  - ✓ Compare with existing therapies & assess overall risk / benefit of drug.

- NDA filing (New Drug Application);
- Review of clinical data by regulatory authorities (FDA, EMEA): approval/ approvable/ non-approvable.

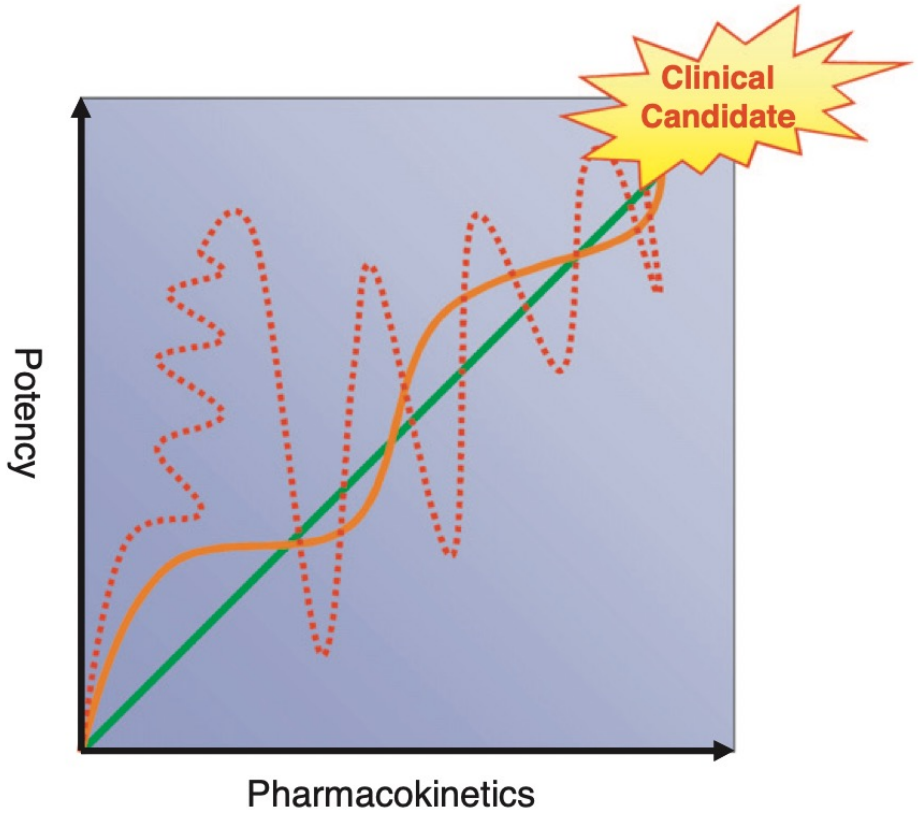


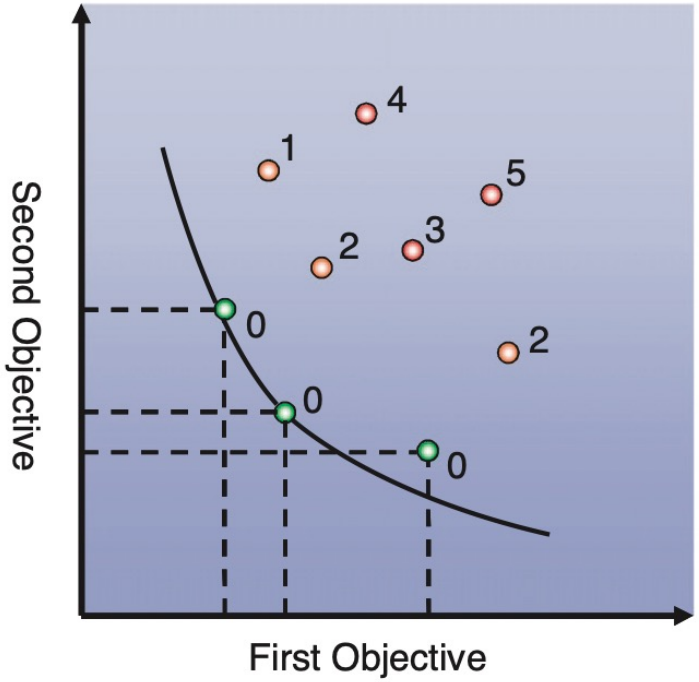
Target ID & Valid. → Hit ID → Lead Optim.(LO) → Pre Clinical → Phase I → Phase II → Phase III → Registration



- Adverse effects constantly monitored & reported after market approval; (market retraction- *e.g.* cesapride);
- Efficacy against other indications or improvement of existing formulations.

Not easy and requires testing many compounds



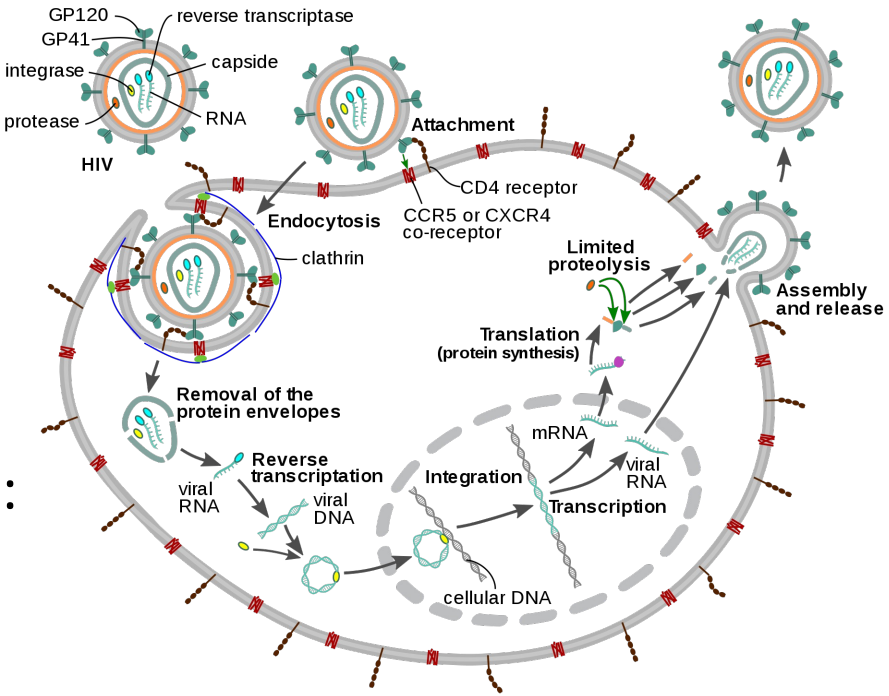


Optimizing potency is likely the easiest task
Optimizing conflicting objectives is when it gets challenging

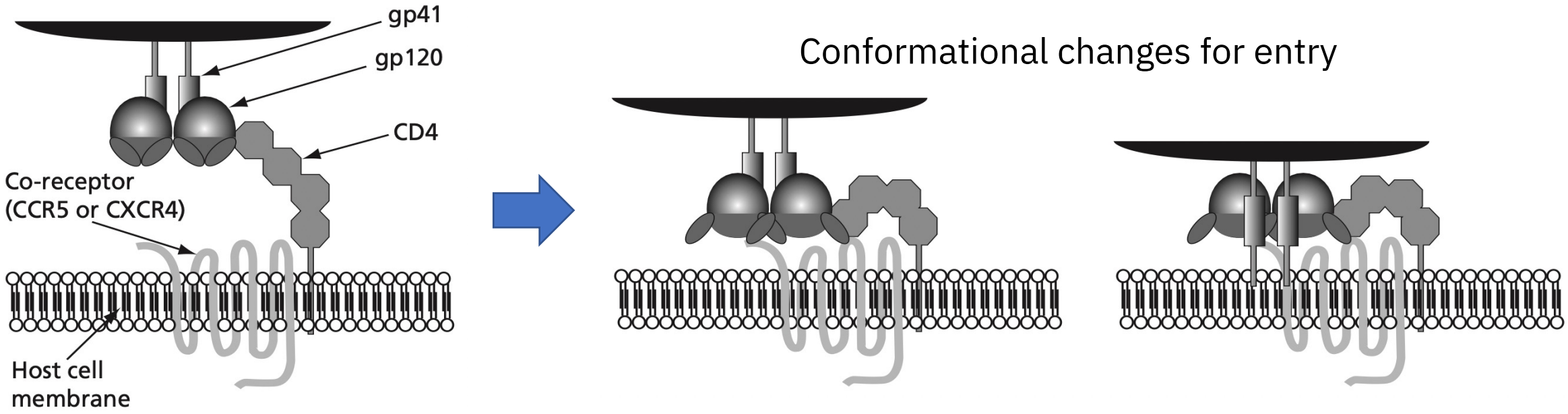
For review see: *Drug Discov Today Technol* **2013**, *10*, e427.

Primer and key concepts on HIV infection and therapy:

- HIV1 (most severe) and HIV2 (RNA viruses)
- Can stay dormant for several years
- Tropism for CD4+ cells, macrophages and glial cells
  - glycoprotein 120 is a key player for cellular entry
  - CCR5 and CXCR4 are key for infectivity (host cells)
- Highly active antiretroviral therapy (HAART), which include:
  - nucleoside/te reverse transcriptase inhibitors
  - non-nucleoside reverse transcriptase inhibitors
  - protease inhibitors
  - fusion inhibitors

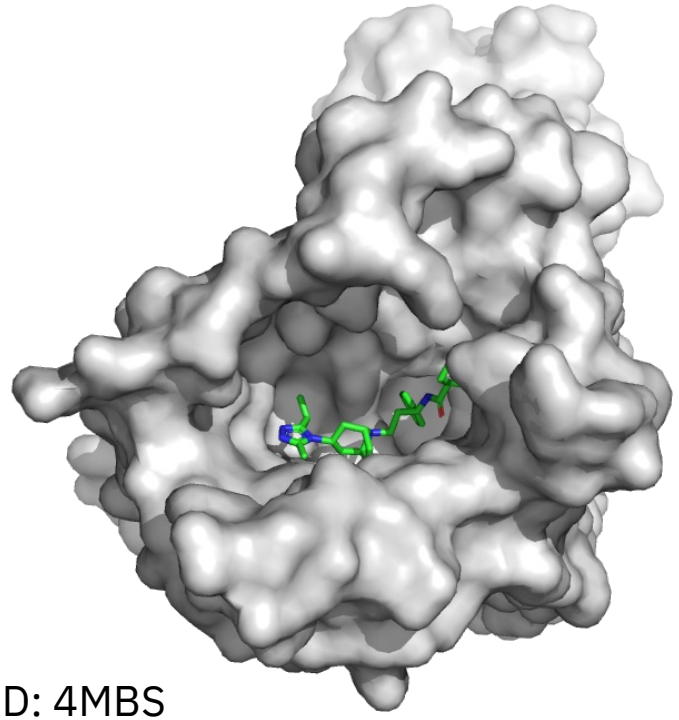


Targeting cellular entry:



For thorough review see: *Antivir Chem Chemother* **2005**, *16*, 339.

CCR5 as target:

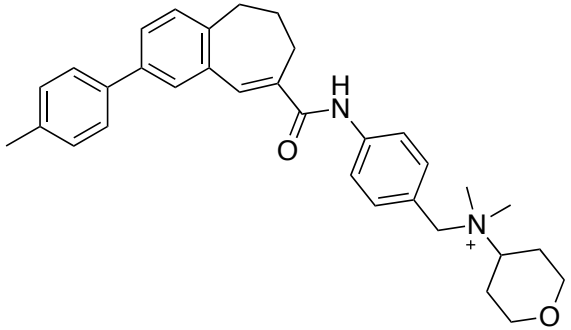


PDB ID: 4MBS

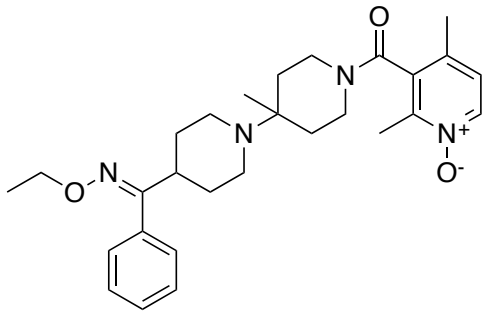
- Human GPCR in leukocytes (Mutation rate? Resistance?)
- Receptor to chemokines (trigger immune response)
- Co-receptor for cellular entry
- Inhibition of protein-protein interaction
  - small molecules
  - mAbs

For thorough review see: *Antivir Chem Chemother* **2005**, *16*, 339 & *Frontiers Immunol* **2022**, *12*, 816515.

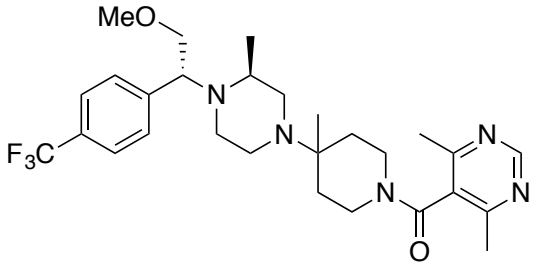
CCR5 antagonists (& allosteric):



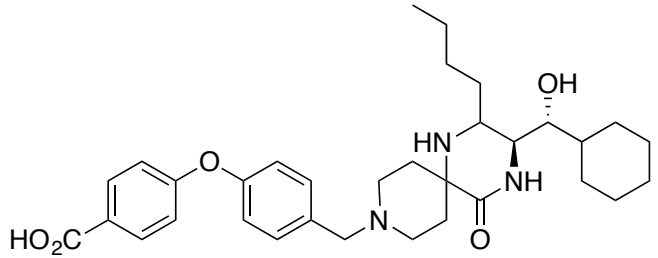
TAK-779
Not-bioavailable



SCH-351125
Optimized HTS hit
IC_{50} < 9 nM
QT prolongation



SCH-417690

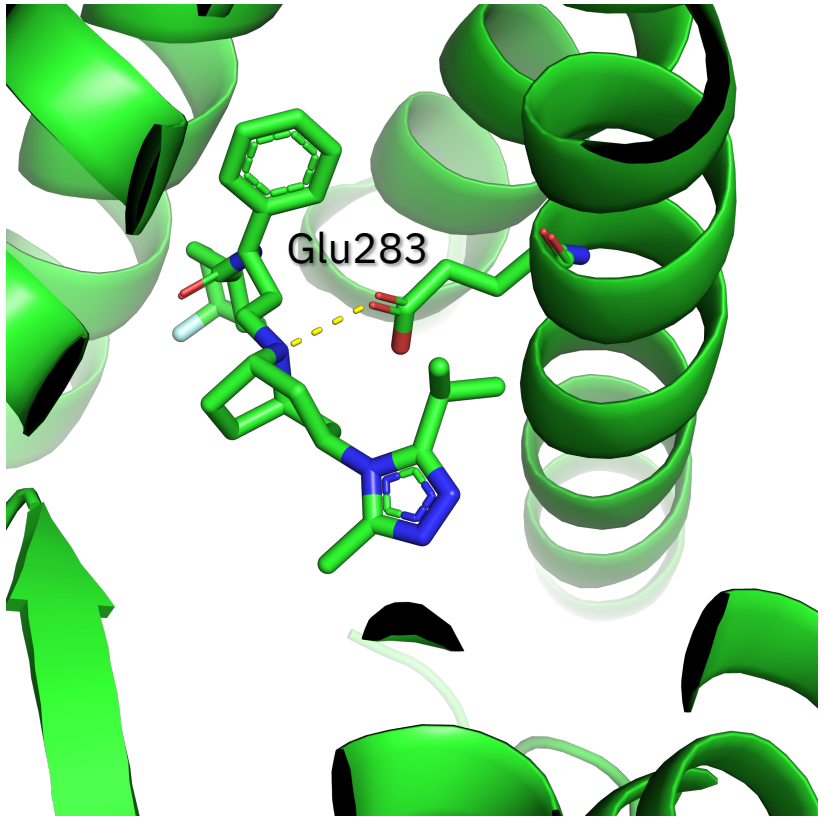


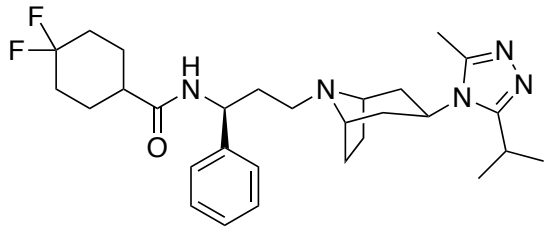
Aplaviroc (GSK)
IC_{50} < 0.6 nM

Q for your chemical intuition:
Can you spot similarities between ligands? Which?

For thorough review see: *Antivir Chem Chemother* **2005**, *16*, 339.

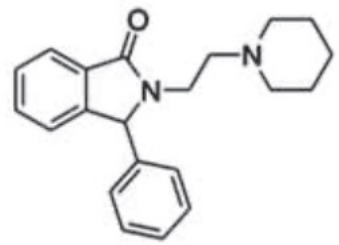
Maraviroc:



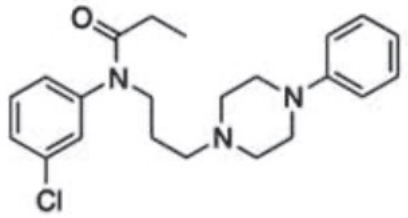


- Developed by Pfizer
- In clinical use (first-in-class) since 2007

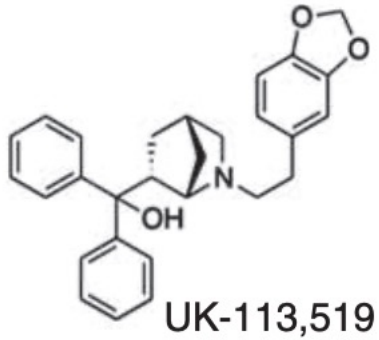
Hit finding:



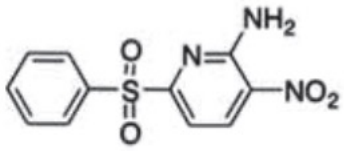
CP-88,326
IC_{50} = 2 µm



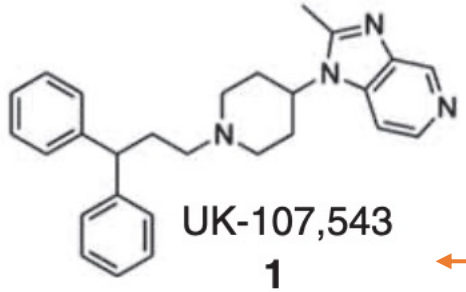
UK-239,737
IC_{50} = 1.28 µm



UK-113,519
IC_{50} = 0.27 µm



UK-33,400
IC_{50} = 1.87 µm



UK-107,543
1
IC_{50} = 0.6 µm

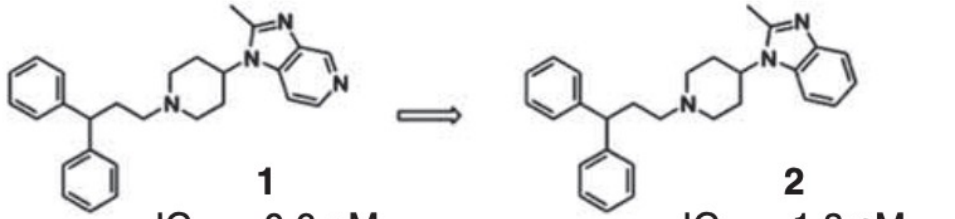
Library size: 500k molecules

Binding, cell-based assay (HEK)

You don't always prioritize the most potent
Did not have antiviral activity!
It inhibited CYP2D6 (IC_{50} = 40 nM)

For thorough review see: *Expert Opin Drug Discov* **2015**, *10*, 671.

Hit-to-lead:



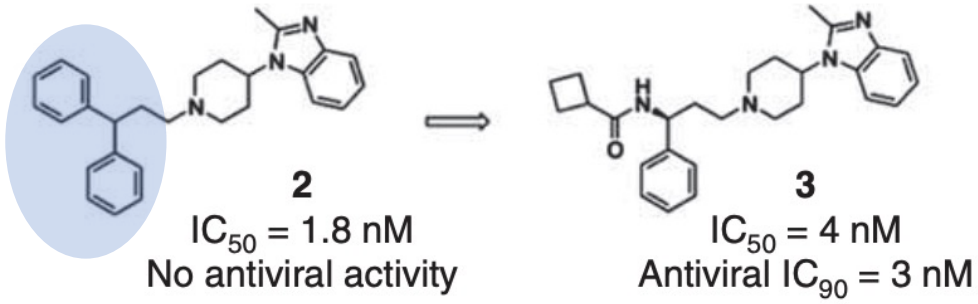
1
IC_{50} = 0.6 µM
No antiviral activity

2
IC_{50} = 1.8 nM
No antiviral activity

Attenuation of CYP2D6 inhibition (IC_{50} = 710 nM)
Increase of CCR5 binding affinity (IC_{50} = 1.8 nM)

For thorough review see: *Expert Opin Drug Discov* **2015**, *10*, 671.

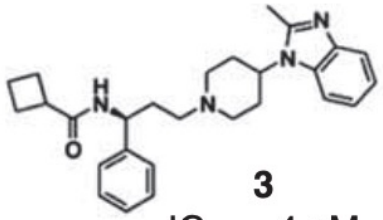
Hit-to-lead:



Distance between basic amine and phenyl ring is key for off-target modulation
Parallel synthesis of amides is a viable strategy at this point
Mind the stereocenter: (*S*) configuration
IC_{50} (CYP2D6): 5 μM

For thorough review see: *Expert Opin Drug Discov* **2015**, *10*, 671.

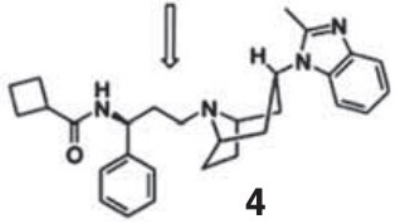
Hit-to-lead:



3

IC_{50} = 4 nM

Antiviral IC_{90} = 3 nM



4

IC_{50} = 6 nM

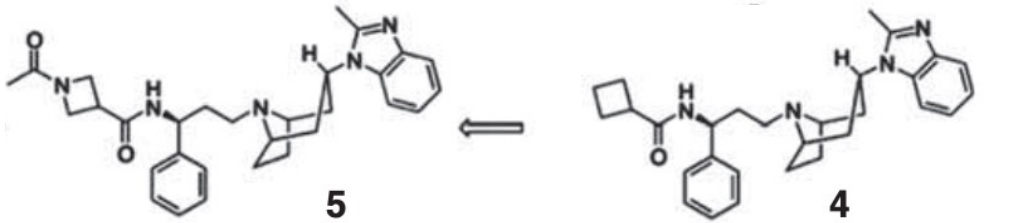
Antiviral IC_{90} = 3 nM

No activity against CYP
Endo/exo benzimidazole irrelevant
Poor metabolic stability
Potent inhibition of hERG (99% at 1 μM)

For thorough review see: *Expert Opin Drug Discov* **2015**, *10*, 671.

Hit-to-lead:

Increase polarity to decrease binding to hERG
IC_{50} (hERG) > 10 μM
Low cellular penetration (Caco-2 <1 cm/s)
No oral absorption in rat PK studies



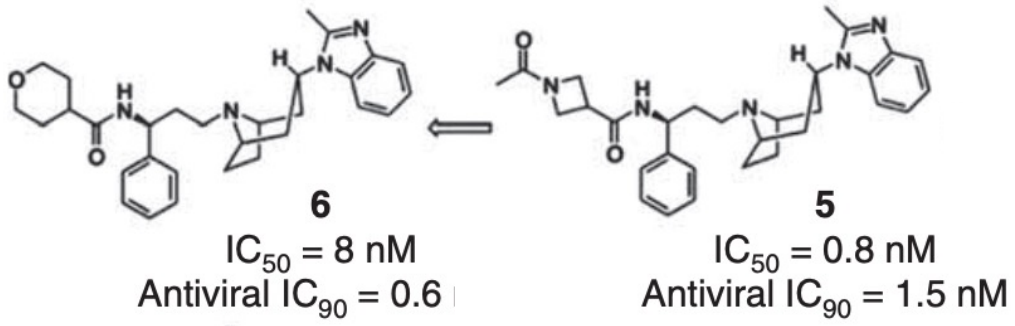
5
IC_{50} = 0.8 nM
Antiviral IC_{90} = 1.5 nM

4
IC_{50} = 6 nM
Antiviral IC_{90} = 3 nM

For thorough review see: *Expert Opin Drug Discov* **2015**, *10*, 671.

Hit-to-lead:

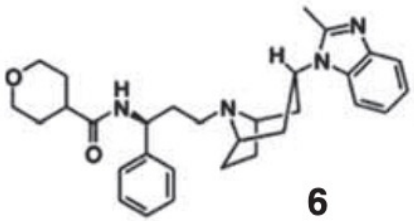
Good antiviral/hERG profile (it's a risk management issue)
P450 metabolism (human liver microsomes)
Bioavailability dogs: <10% (first pass effect)



Find compromise for 4 objectives:
Antiviral activity (maximize)
hERG inhibition (minimize)
Cellular permeability (maximize)
ADME (minimize some properties, maximize others)

For thorough review see: *Expert Opin Drug Discov* **2015**, *10*, 671.
For mitigation of hERG activity: *Bioorg Med Chem Lett* **2006**, *16*, 4633

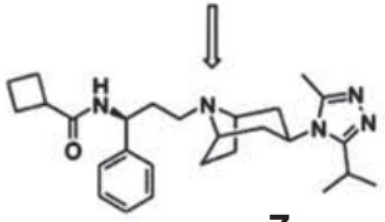
Hit-to-lead:



6

IC_{50} = 8 nM

Antiviral IC_{90} = 0.6 nM



7

IC_{50} = 7 nM

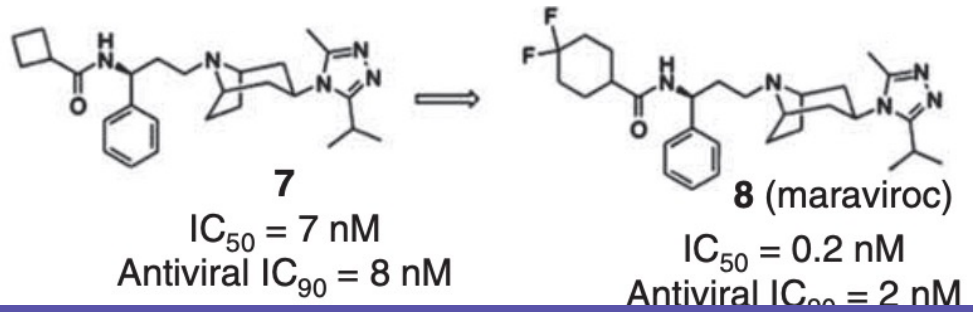
Antiviral IC_{90} = 8 nM

Good metabolic stability
30% inhibition of hERG at 300 nM
Abandoned due to QT interval prolongation

Clinical candidate:

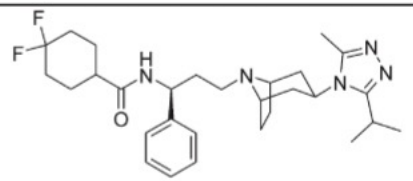
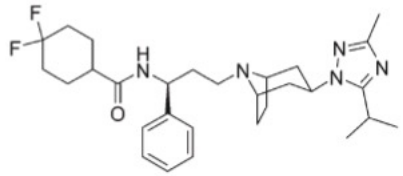
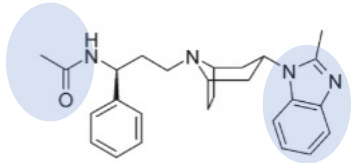
Approved but not the end of the story!

Clinical trials after profiling ~1000 molecules



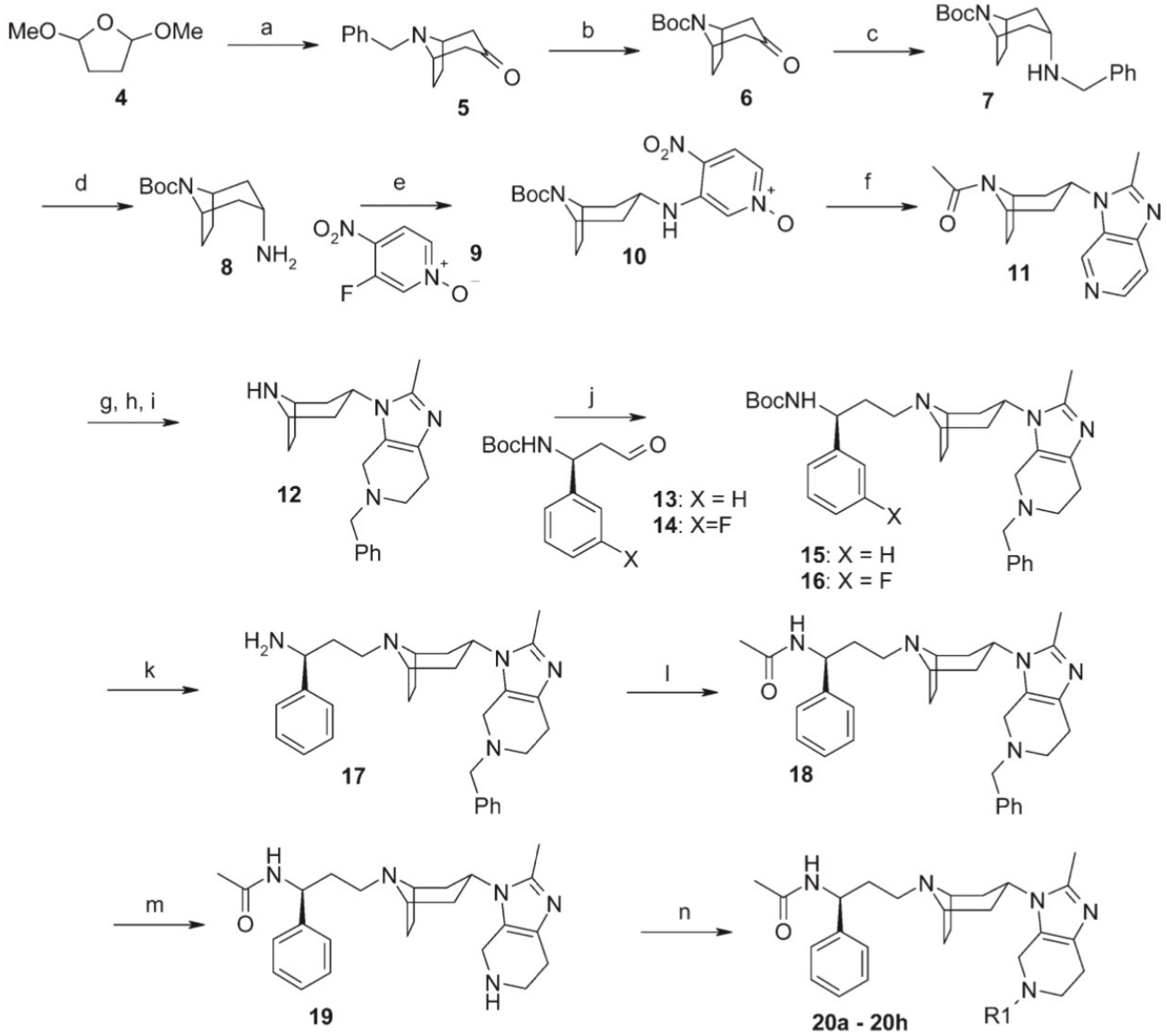
Fluorination increased metabolic stability
Selectivity for CCR5
No in vitro toxicity
IC_{50} (hERG) >10 μM
Good oral absorption in dogs

Maraviroc

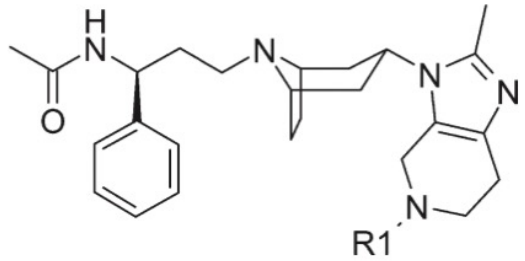
	Compound	gp160 Fusion IC_{50} (nM)[a]	hERG binding[b]	Caco-2 AB/BA[c]
1		0.2	0% @ 300 nM	<1/12
2		1.4	IC_{50} 4230 nM	11/26
3		0.5	IC_{50} 1500 nM	13/35

Changing the dipole led to reduced hydration and higher Permeability

J Med Chem **2011**, *54*, 67



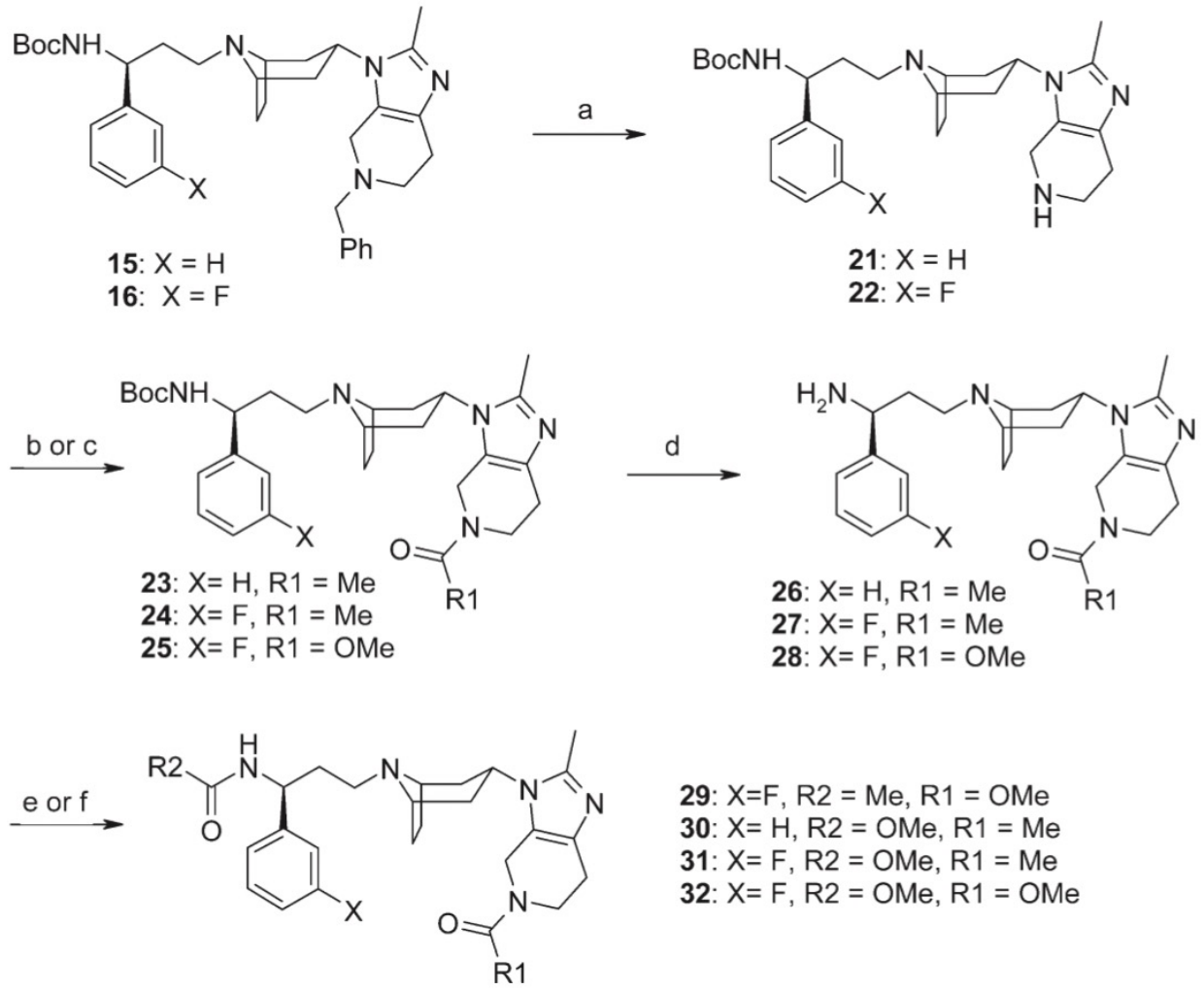
J Med Chem **2011**, *54*, 67



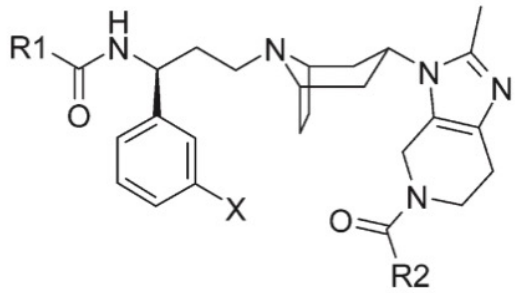
compd	R1	gp160, IC_{50} (nM)[a]	hERG binding (%)[b]	Caco-2 AB/BA[c]	HLM Cl_{int} (μL/min/mg)	logD[d]
18	$PhCH_2$	29				
19	H	9				
20a	Me	5	0	< 1/9	< 7	0.4
20b	*i*-Pr	3			46	0.8
20c	$MeSO_2$	2	10		24	0.9
20d	MeCO	0.4	0	< 1/5	< 7	0.6
20e	EtCO	0.4	13	< 1/17	< 7	1.2
20f	*i*-PrCO	0.4	0	< 1/20	42	1.6
20g	$MeCO_2$	0.3	20	3/16	18	1.5
20h	*i*-$PrCO_2$	< 0.1	75	5/28	44	2.7

[a] IC_{50} determinations were the mean of at least two replicates. [b] Inhibition of [^{3}H]-dofetilide binding to hERG stably expressed on HEK-293 cells with a compound concentration of 300 nM. [c] $P_{app} \times 10^{-6}$ cms^{-1}. [d] Experimentally determined (octanol/water).

J Med Chem **2011**, *54*, 67



J Med Chem **2011**, *54*, 67



compd	R1	R2	X	gp160 IC_{50} (nM)[a]	hERG binding (%)[b]	Caco-2 AB/BA[c]	HLM Cl_{int} (μL/min/mg)	logD[d]
29	Me	OMe	F	0.1	0	2/23	19	1.9
30	OMe	Me	H	0.6	57	4/27	42	1.4
31	OMe	Me	F	0.2	28	6/23	24	1.7
32	OMe	OMe	F	0.2	69	17/17	92	2.6

[a] IC_{50} determinations were the mean of at least two replicates. [b] Inhibition of [^{3}H]-dofetilide binding to hERG stably expressed on HEK-293 cells with a compound concentration of 300 nM. [c] $P_{app} \times 10^{-6}$ cms^{-1}. [d] Experimentally determined (octanol/water).

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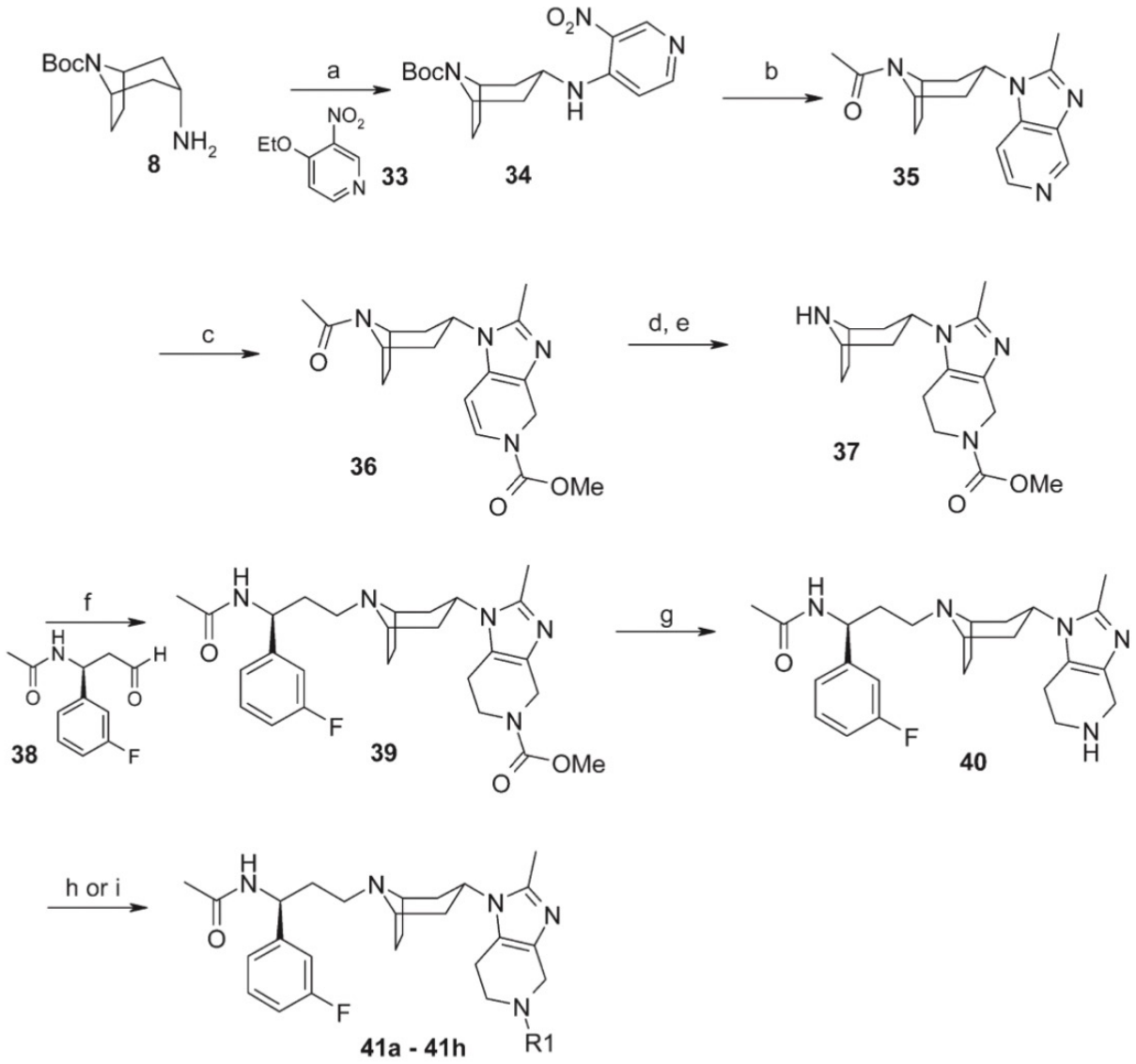
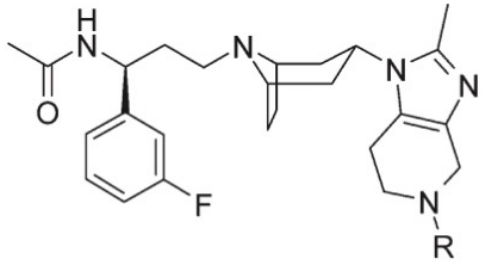


J Med Chem **2011**, *54*, 67



compd	R	gp160, IC_{50} (nM)[a]	hERG binding IC_{50} (μM)[b]	Caco-2 AB/BA[c]	HLM Cl_{int} (μL/min/mg)	logD[d]
41a	pyrimidin-4-yl	< 0.1	0.01	4/15	35	2.6
41b	pyrazin-2-yl	< 0.1	1		183	3.0
39	CO_2Me	< 0.1	2	3/11	< 8	1.9
41d	COMe	< 0.1	5	< 1/6	< 8	1.4
41e	COEt	< 0.1	5	2/13	11	1.7
41f	CO^iPr	< 0.1	12	2/8	13	2.0
41g	COPr	< 0.1	6	1/26	25	2.2
41h	CO^tBu	< 0.1	5	1/24	35	2.8

[a] IC_{50} determinations were the mean of at least two replicates. [b] Fluorescence polarization assay using a fluorescently labeled analogue of dofetilide.[26] [c] $P_{app} \times 10^{-6}$ cms^{-1}. [d] Experimentally determined (octanol/water).

J Med Chem **2011**, *54*, 67

Table 6. Pharmacokinetic Properties of Lead Compounds in Rat and Dog iv/po Studies[a]

	1[b]	29[c]	39[d]	41f[c]
rat PPB (%)[e]	46	71	68	62
rat Cl (mL/min/kg)	74	146	91	201
rat Cl_u (mL/min/kg)[f]	137	518	253	526
rat $t_{1/2}$ (h)	2.3	1.7	2.3	1.4
rat F (%)	6	8	20	< 5
estimated rat absorption (%)	20	complete	complete	
dog PPB (%)[e]	60	62	73	47
dog Cl (mL/min/kg)	21	36	36	36
dog Cl_u (mL/min/kg)[f]	53	93	150	63
dog V_d (L/kg)	4.3	11.8	9.9	10.5
dog $t_{1/2}$ (h)	2.3	3.8	3.3	3.4
dog F (%)	42	44	50	31
estimated dog absorption (%)	70	complete	complete	complete

[a] Assuming rat liver blood flow is 100 mL/min/kg and dog is 50 mL/min/kg. [b] Rat iv 1 mg/kg, po 10 mg/kg; dog iv 0.5 mg/kg, po 1 mg/kg. [c] Rat iv 1 mg/kg, po 2 mg/kg; dog iv 0.5 mg/kg, po 0.5 mg/kg. [d] Rat iv 2 mg/kg, po 2 mg/kg; dog iv 0.5 mg/kg, po 0.5 mg/kg. [e] % of drug bound to plasma proteins; blood:plasma partitioning is 1:1 for all compounds in rat and dog. [f] Cl_u is unbound clearance.

Table 7. Human, Rat and Dog Intrinsic Clearance Values from in Vitro Liver Microsome Assays

	1	29	39	41f
HLM Cl_{int}[a,b]	49		17	7
RLM Cl_{int}[a]	< 6	15	< 6	< 6
DLM Cl_{int}[a]	< 9	32	< 9	< 9

[a] Units: μL/min/mg protein. [b] Assay was performed using alternative batch of mircosomes to those used previously.

Nominated as clinical candidate

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- Drug development is inherently multi-disciplinary
- Iterative design-make-test cycles over multiple years
- Consider trade-off of conflicting objectives
- Computers can help prioritizing experiments and/or rationalizing results
- Validation only with experiments. Models get increasingly worse as we go from chemical/physical models to biological models
- Understanding the underlying biology is key