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Targets Validation

- Background
  - What is involved in establishing a discovery project
  - Biological relevance and chemical tractability
    - Focus on small molecules
- Validated target
  - What is a validated target and how do you make that determination
  - Examples
- Druggable target
  - What this means and how it is used
  - Drug like
- Conclusion – Viability for drug discovery
  - Biological relevance and chemical tractability

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Drug Discovery & Development

Target Identification

Target Prioritization/Validation

Lead Identification

Lead Optimization

Preclinical Testing

Chemical Manufacturing Controls (CMC)/Pharmaceutics

Pharmacology/Toxicology

Investigational New Drug (IND)/CTX/CTA

Phase I

Phase II

Phase III

New Drug Approval (NDA)/MAA

Phase IIIb/IV

Post Market

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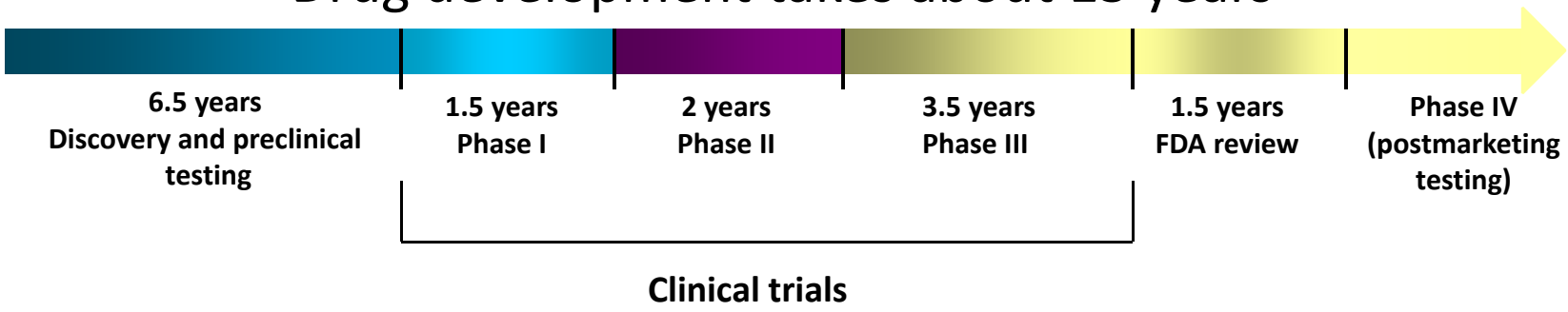
Drug Discovery and Development
A Long Process

Drug development takes about 15 years



$800 MM to 1.3 billion to develop a drug

Why is it so expensive?

- -More regulations and requirements
- -Large failure Rate
  - -1 in 10 compounds entering Phase 1make it to a drug
- -How many drug discovery programs are needed

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Typical Large Pharma Averages

- Start 100 new screening programs/assays
  - New targets
- About 50 programs find leads or acceptable chemotypes to pursue
  - Limited chemotypes is a liability
  - Backup compounds
- 20 - 25 Advance into late stage lead optimization
- Only 10 programs/compounds proceed into Phase 1
- Why?

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Why Compounds Fail

A Changing Paradigm

- Pharmacokinetics
  - Human ADME properties
    - Adsorption, distribution, metabolism, excretion
  - Rodent vs. dog vs. monkey vs. human
- Toxicology
  - Not predicted by animal studies
- Adverse effects in man
- Lack of efficacy
  - Biological rationale is incorrect
  - Relevance of animal models
    - Arthritis model in rats vs. rheumatoid arthritis in patients
    - Oncology models
- Commercial reasons

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Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework

Nature Reviews Drug Discovery June 2013

as the five 'R's: the right target, the right patient, the right tissue, the right safety and the right commercial potential. A sixth factor — the right culture — is also

Validating molecular targets in preclinical drug discovery

A recent analysis of failures in Phase II and III trials in the past two years confirmed earlier reports that more than half of the drugs fail due to insufficient efficacy[1-3]. In other words, the clinical target validation fails for about 50 per cent of therapeutic approaches. A retrospective analysis of drug development programs at Pfizer revealed some opportunities for optimisation of the drug development process[4]. Three knowledge pillars have been identified, which increase the likelihood of candidate survival in Phase II trials: deep understanding of the drug exposure at the site of action, target binding of the drug, and clear expression of functional pharmacological activity. The latest reached highest significance for prediction of success in clinical trials[4]. Hence, an in-depth biological understanding of a molecular target as one of the very early steps in the entire drug discovery and development process which can determine later success or failure of the emerging drug candidate is required.

The clinical target validation fails for about 50% of therapeutic approaches

European Drug Target Review 2014

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Preclinical Drug Discovery

- Target identification and validation 
  - High throughput screening and lead identification
- In vitro activity
  - IC_{50}, EC_{50}, chemical tractability
  - Liability targets
    - CNS, cardiovascular,
- Medicinal chemistry and lead optimization
- In vitro ADME
  - Stability, solubility, cyotochrome p450 enzymes, plasma protein binding, transporters
- Toxicology and additional liabilities
- In vivo assessment
  - In vivo optimization
    - Disease models, in vivo pharmacokinetics, bioavailability, safety
- Nomination candidate

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Target Identification

- Key steps for a target
  - Is the target validated and druggable
    - Small molecule
  - How will you screen for modulators?
    - Agonist, antagonist, inhibitor
    - Outcome looking for
    - Where is the target!!
  - Secondary assays and species differences
  - Animal models to test hypothesis
    - Standards or known compounds
    - Predictability of the model
    - Biomarkers
  - Clinical outcome

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Failure Rate Alzheimer's Disease

- Higher clinical failure rate than other therapeutic areas
- Not a single disease modifying drug approved
- Lack of a clear animal model that recreates the histopathological and neurodegeneration hallmarks of AD
- May have to target particular areas of the brain
- Targets not validated or better animal model?

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What is a Drug Discovery Target

- Proteins
- Receptors and enzymes
  - Either inside the cell or on the surface
    - G-protein coupled receptors
    - Protease or kinase
- Transcription factors
  - Gene regulation
- Ion channels and transporters
- Infectious diseases and cancer
  - Direct cell-based assays

- Protein-protein interactions
- Phenotypic screens

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A Case History -Senicapoc

- Potassium channel inhibitor for the treatment of sickle-cell anemia
  - IK1 (KCa3.1) inhibitor in RBC's to maintain hydration
  - In vivo efficacy in a mouse sickle cell model
- Advanced to a phase III clinical trial in sickle-cell patients
  - Positive Phase II trials
  - Phase III - Vaso-occlusive crisis rate was the approvable end-point
  - Three arms to the study
    - Senicapoc alone, hydroxyurea and combination
- Independent review board analyzed the data and concluded their would be no benefit
- The trial was stopped
  - There was an improvement in several hematological factors indicating biological activity
- Is this a validated/druggable target?

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Where Do Discovery Ideas (targets) Come From?

- Historically from natural products
  - Herbal medicines, snake venom
  - Observed in vivo effects in animals
- Clinical observation
  - Side-effects or desired effect
- Rationale approaches based upon biochemistry/biology
- Screening, systems biology
- Understanding genetic mutations in people
  - Precision medicine

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New Target Identification

- Newer approaches have identified more targets
- Genomics, proteomics, pharmacogenomics
- RNA interference and related technologies
  - Small interfering RNA (gene silencing)
  - Interference with the expression of a specific gene
- Pathway analysis
- Transgenic animals

- Key Question - Target Validation
  - Modulate a target and what effect does it have?

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What Constitutes a Validated target

- Genetic mutations in a protein leading to or associated with a disease
  - Alzheimer's and amyloid precursor protein and secretase
  - Human epidermal growth factor receptor 2 (HER2) amplified in breast cancer promotes cancer cell growth
- Up-regulation during a disease process
  - Inflammation and Cyclooxygenase 2
- Kinases

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KCNQ2/3 – Validated Targets

- Present at high levels in neurons including dorsal root ganglia (DRG). No significant expression in major peripheral organs.
- Mutations in KCNQ2 and KCNQ3 associated with a congenital seizure disorder in humans – *Benign Familial Neonatal Convulsions*
- Targeted deletion of KCNQ2 in mice increases sensitivity to chemoconvulsant induced seizures.
- KCNQ/M-current activators are efficacious in animal models and human diseases associated with excessive neuronal excitability.

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KCNQ Family

- **KCNQ1/KCNE1** contributes to cardiac action potential repolarization. Mutation can result in Long QT Syndrome
- **KCNQ2** - Forms heterotetramers with KCNQ3. Mutations in KCNQ2 cause the congenital seizure disorder benign familial neonatal convulsions (BFNC).
- **KCNQ3** -Expresses poorly as a homomultimer. Co-assembles with other KCNQ channels such as KCNQ2 and KCNQ5. Mutations in KCNQ3 also linked to BFNC.
- **KCNQ4** - Expressed primarily in inner ear. Mutation linked to one form of hereditary deafness.
- **KCNQ5** - Expressed in nervous system and co-assembles with KCNQ3

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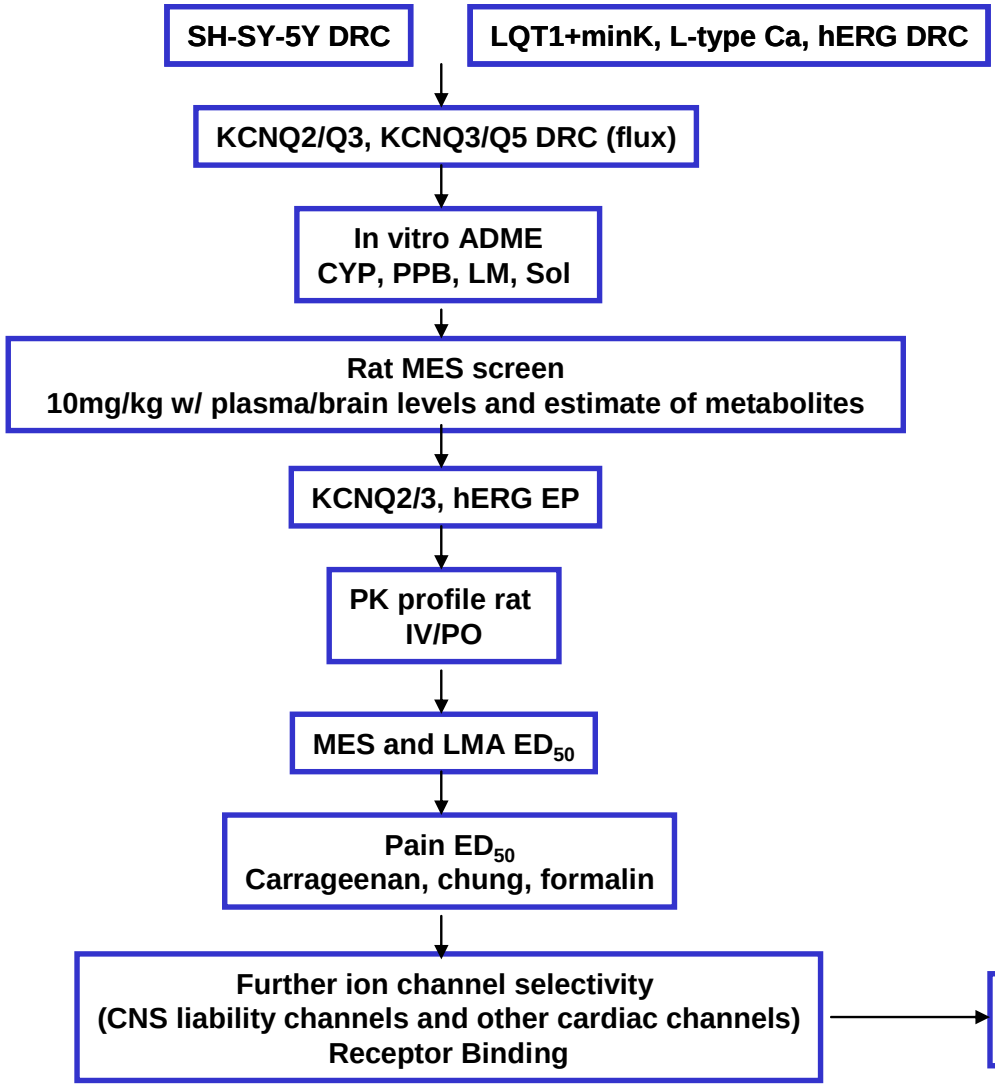
Typical Assay Progression Scheme

Epilepsy drug



***In vitro* selectivity**

Early assessment of *in vitro* ADME

***In vivo* activity and early *in vivo* PK**

Electrophysiology

Definitive *in vivo* PK

Therapeutic index

Efficacy in pain models

Safety

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Cyclooxygenase Inhibitors

Validated Target?

- The classical *COX* inhibitors are not selective and inhibit all types of *COX*. The resulting inhibition of prostaglandin and thromboxane synthesis has the effect of reduced inflammation, as well as antipyretic, antithrombotic and analgesic effects.

- The most frequent adverse effect of NSAIDs is irritation of the gastric mucosa as prostaglandins normally have a protective role in the gastrointestinal tract.

- Some NSAIDs are also acidic which may cause additional damage to the gastrointestinal tract.

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Cyclooxygenase Inhibitors
COX 1 and COX2

- In the 1990s, researchers discovered that two different COX enzymes existed, now known as COX-1 and COX-2
- COX-1 is known to be present in most tissues.
  - In the GI tract, COX-1 maintains the normal lining of the stomach. The enzyme is also involved in kidney and platelet function
- COX-2 is primarily present at sites of inflammation
- COX-1 and COX-2 convert arachidonic acid to prostaglandin, resulting in pain and inflammation, their other functions make inhibition of COX-1 undesirable while inhibition of COX-2 is considered desirable

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COX-2 Inhibitors

- Celecoxib, Rofecoxib,
  - COX-2 is usually specific to inflamed tissue, there is much less gastric irritation associated with COX-2 inhibitors, with a decreased risk of peptic ulceration.
- COX-2 inhibitors have been found to increase the risk of atherothrombosis
  - A 2006 analysis of 138 randomized trials and almost 150,000 participants showed that selective *COX-2* inhibitors are associated with a moderately increased risk of vascular events, mainly due to a twofold increased risk of myocardial infarction
- Validated, druggable
  - Viable – New data?

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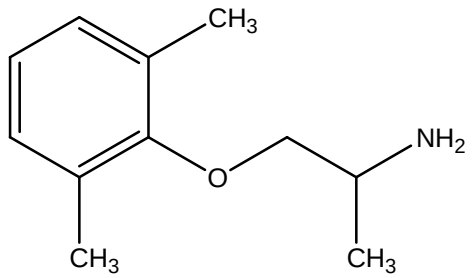
Sodium Channel Inhibitors
Therapeutic Applications

- Local anesthetic
  - Lidocaine or Procaine
    - Short acting
- Epilepsy
  - Phenytoin
- Antiarrythmics
  - TAMBOCOR™ (flecainide), Mexitil
- Neuropathic pain
- All are non-selective
  - Affect multiple channels
- Validated targets?



Mexilitene

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Protein name	Gene	Expression profile	Associated human channelopathies
$Na_v1.1$	*SCN1A*	Central neurons, [peripheral neurons] and cardiac myocytes	febrile epilepsy, GEFS+, Dravet syndrome (also known as *severe myclonic epilepsy of infancy* or SMEI), borderline SMEI (SMEB), West syndrome (also known as *infantile spasms*), Doose syndrome (also known as *myoclonic astatic epilepsy*), intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC), Panayiotopoulos syndrome, familial hemiplegic migraine (FHM), familial autism, Rasmussens's encephalitis and Lennox-Gastaut syndrome[7]
$Na_v1.2$	*SCN2A*	Central neurons, peripheral neurons	inherited febrile seizures and epilepsy
$Na_v1.3$	*SCN3A*	Central neurons, peripheral neurons and cardiac myocytes	none known
$Na_v1.4$	*SCN4A*	Skeletal muscle	hyperkalemic periodic paralysis, paramyotonia congenita, and potassium-aggravated myotonia
$Na_v1.5$	*SCN5A*	Cardiac myocytes, uninnervated skeletal muscle, central neurons	Long QT syndrome, Brugada syndrome, and idiopathic ventricular fibrillation
$Na_v1.6$	*SCN8A*	Central neurons, dorsal root ganglia, peripheral neurons, heart, glia cells	none known
$Na_v1.7$	*SCN9A*	Dorsal root ganglia, sympathetic neurons, Schwann cells, and neuroendocrine cells	erythromelalgia, PEPD, channelopathy-associated insensitivity to pain and recently discovered a disabling form of fibromyalgia (rs6754031 polymorphism - PMID: 22348792).
$Na_v1.8$	*SCN10A*	Dorsal root ganglia	none known
$Na_v1.9$	*SCN11A*	Dorsal root ganglia	none known
Na_x	*SCN7A*	heart, uterus, skeletal muscle, astrocytes, dorsal root ganglion cells	none known

Congenital Insensitivity to Pain: Novel *SCN9A*
Missense and In-Frame Deletion Mutations

- *SCN9A* encodes the voltage-gated sodium channel $Na_v1.7$, a protein highly expressed in pain-sensing neurons.

- Mutations in *SCN9A* cause **three human pain disorders**
  - bi-allelic loss of function mutations result **in Channelopathy-associated Insensitivity to Pain (CIP)**
  - whereas activating mutations cause **severe episodic pain in Paroxysmal Extreme Pain Disorder (PEPD) and Primary Erythermalgia (PE)**.

- To date, all mutations in *SCN9A* that cause a complete inability to experience pain are protein truncating and presumably lead to no protein being produced

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When is a Target Validated?

- Mechanistic studies – in vitro
  - Over expression, anti-sense, mutations
- Cell based activity
- Animal studies – knockout studies
  - Disease phenotype
- Therapeutic intervention
  - Small molecule or biological
- Phase 2 clinical results
- NDA approval

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ADDA Examples

- CD38 for Memory Disorders – Dr. Fran Lund
  - We crossed APPsweePS1ΔE9 (APP.PS) mice with *Cd38*$^{-/-}$ mice to generate AD-prone CD38-deficient animals (APP.PS.*Cd38*$^{-/-}$) and examined AD-related phenotypes in both groups
    - (Annals of Neurology Volume 78, Issue 1, pages 88–103, July 2015
- Heme-oxygenase 1 for chronic kidney disease- Dr. Anupam Aggarwal
  - Data published identifying the role of HO-1
  - Established a phenotypic screen to identify compounds that increase HO-1 levels
    - J Kim et al.: Humanized BAC mice rescue HO-1/ mice Kidney International April 2012

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Discussion

- Validated target vs. “druggable target”
- Is there a difference?
- A druggable target is?

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A New "Validated" Drug Target
Issues to Consider

- Is the target "druggable"
  - What evidence is there to support initiating a drug discovery program?
  - Small molecule inhibitors, animal models, species differences, biomarker (Phase 1)
  - Related to other known targets - Gene Families
    - Kinases, phosphatases, nuclear receptors
- Structural information
  - Protein crystallographic data, NMR structure
- Intellectual property
  - Competition

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Types of Drugs

- Natural products
- Steroids, antibiotics
- Peptides (smaller)
- Biologicals
  - Antibodies, proteins, antisense
- Small molecules
  - Oral bioavailability, ease of manufacture, stability, cost

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Gene Families

- A gene family is a group of genes that share important characteristics. In many cases, genes in a family share a similar sequence of DNA building blocks (nucleotides).
  - In other cases, dissimilar genes are grouped together in a family because proteins produced from these genes work together as a unit or participate in the same process

- Gene family drug discovery - Programs and expertise directed toward certain sets of targets
  - For example, assays, chemistry, modeling
  - Success in drug discovery

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Types of Gene Families

Druggable Targets

- G-protein coupled receptors
- Kinases
- Proteases
- Nuclear receptors
- Phosphatases
- Phosphodiesterases
- Ion channels

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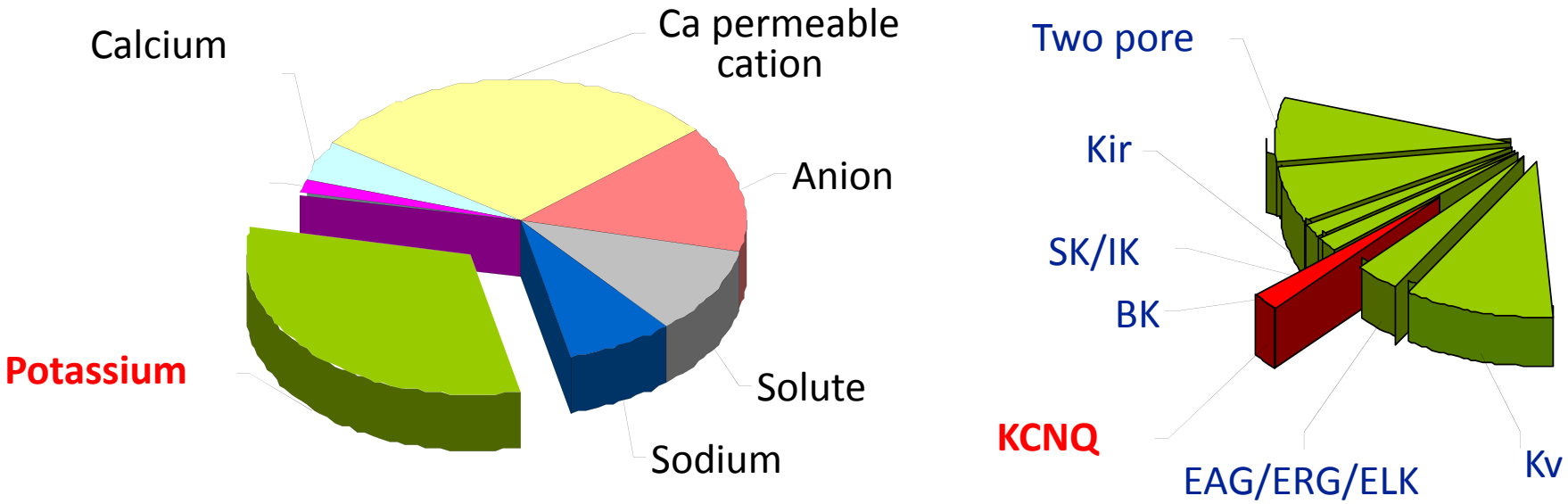
Ion Channel Gene Family



Interesting epilepsy and pain targets based on: Function, Distribution and Pharmacology

The IUPHAR name for the KCNQ family is Kv7.x

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G-Protein Coupled Receptors

- **World Market for G-Protein-Coupled Receptors (GPCRs) Targeting Drugs to Reach US$120.5 Billion By 2017, According to New Report by Global Industry Analysts, Inc.**

- GIA announces the release of a comprehensive global report on the 'G-Protein-Coupled-Receptors (GPCRs)' market. Global market for G-Protein-Coupled Receptors (GPCRs) is projected to reach US$120.5 billion by the year 2017. Major factors driving growth in the market include rising interest among researchers for GPCR drug targets, increased know-how of membrane structures of GPCR, and advancements in identification as well as crystallization of newer structures. In addition to these, emergence of efficient and powerful technologies used in GPCR screening is expected to stimulate market growth.

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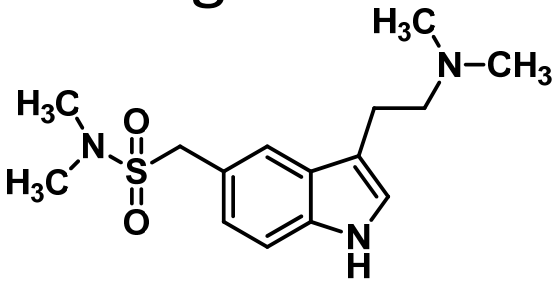
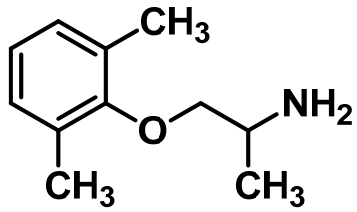
GPCR drugs

- H2 antagonists – Zantac
  - Ulcers
- Beta-blockers – Bystolic
  - Hypertension
- Beta-agonists – Symbicort
  - Asthma
- Serotonin Agonists – Sumatriptan
  - Migraine





Mexilitine

Focused libraries – GPCR's
Develop an expertise

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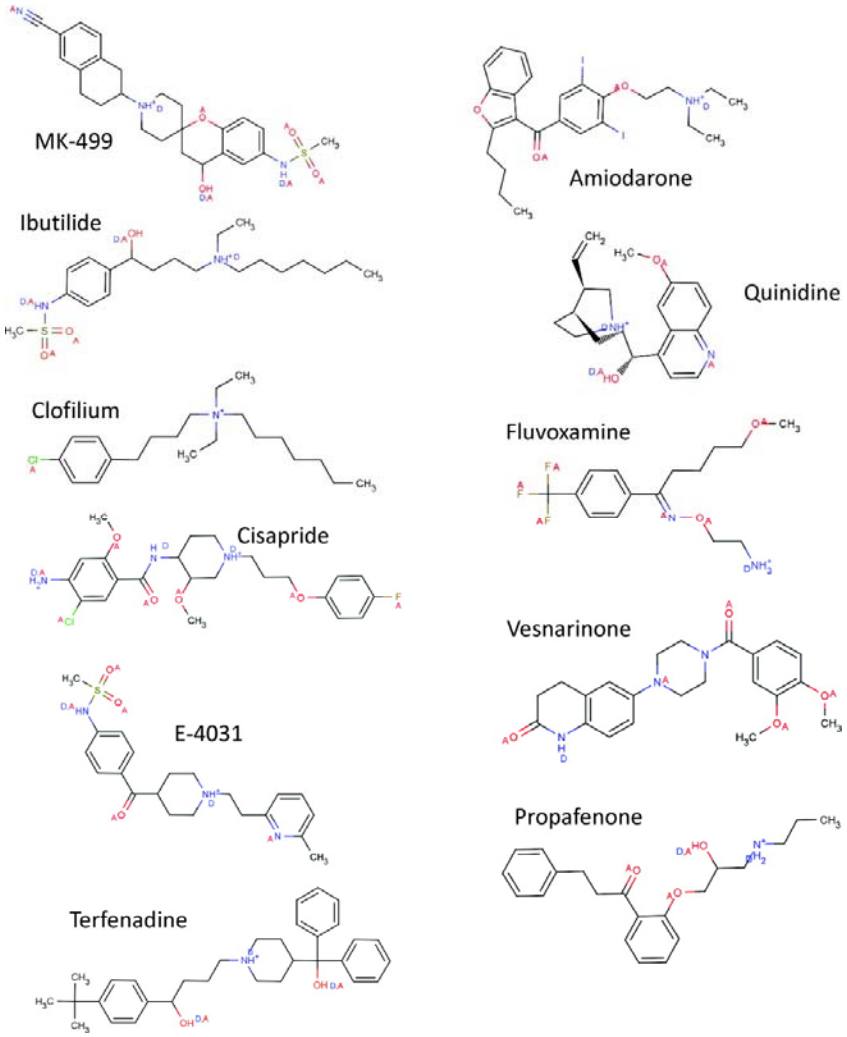
Chemical structure of hERG channel blockers.



Perry M et al. J Physiol 2010;588:3157-3167

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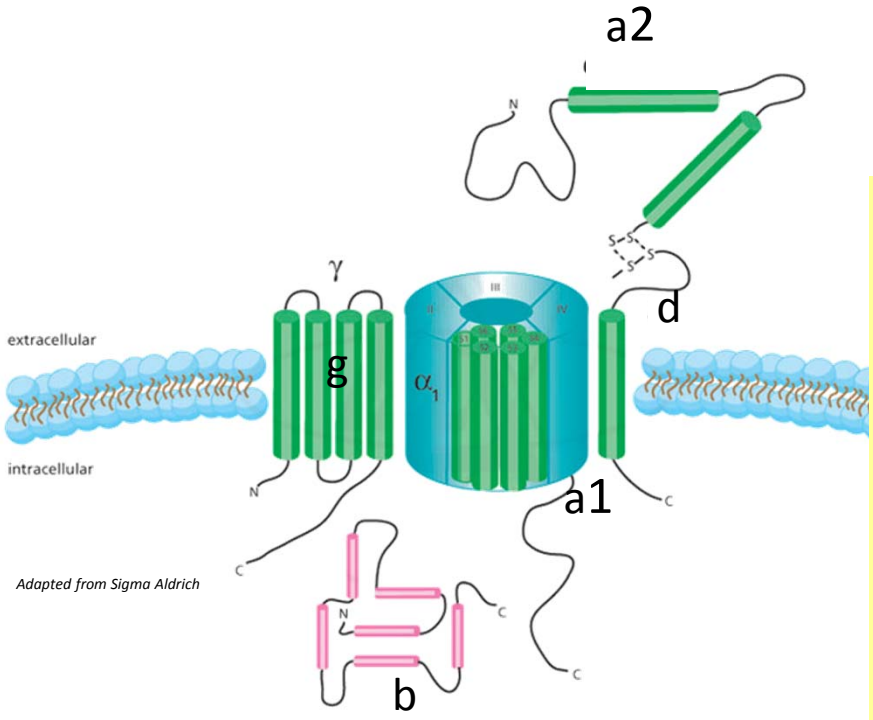
Calcium Channel Gene Family

Complex Problem But Druggable Targets



Clone	Ca_v	Gene	Type
a1S	1.1	CACNA1S	L-type
a1C	1.2	CACNA1C	L-type
a1D	1.3	CACNA1D	L-type
a1F	1.4	CACNA1F	L-type
a1A	2.1	CACNA1A	P/Q type
a1B	2.2	CACNA1B	N-type
a1E	2.3	CACNA1E	R-type
a1G	3.1	CACNA1G	T-type
a1H	3.2	CACNA1H	T-type
a1I	3.3	CACNA1I	T-type

Representative drugs that target different calcium channels

-L-type: nifedipine, verapamil, diltiazem for cardiovascular indications

-N-type: ziconitide for cancer pain (i.t. administration)

-T-type: zonisamide, ethosuximide, mibefradil (epilepsy, pain)

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T-Type Calcium Channel Antagonists
Target Validation

- Literature evidence suggests that T-type calcium channels are involved in certain pain and CNS disorders
  - i.e., small molecules, antisense, rodent knockout studies

- Identify a novel series of T-type calcium channel antagonists and evaluate in rodent models of pain
  - Target validation – IC_{50} vs. plasma (brain?) concentrations
    - T-type IC_{50}<50nM, 30-40-fold selective vs. L-type
    - Selective versus other relevant targets
    - Orally bioavailable, $t_{1/2}$ >1 hr (i.v. rat)
    - ~5-10-fold over IC_{50} at Cmax (1-2h)

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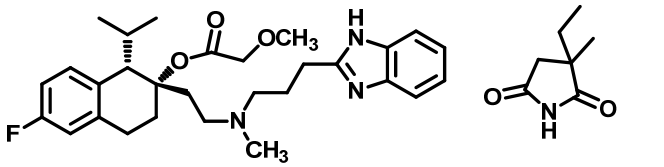
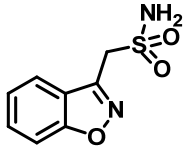
T-type Calcium Channel Blockers

Druggable - Small Molecules Antagonists

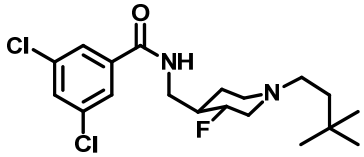


mebefradil

ethosuximide



zonisamide



Merck

- Pain
  - T-type calcium channel inhibitor ethosuximide reverses dorsal horn responses to mechanical and cold allodynia in Chung model and reverses paclitaxel (taxol) and vincrisitine-evoked neuropathy (Flatters S.J. et al, 2004, *Pain* 109:150-161)
  - "T-Type calcium channel inhibitors" mibefradil and ethosuximide reduce tactile and thermal hypersensitivity in Chung model of neuropathic pain (Dogrul A. et al, 2003, *Pain* 105:159-68)
- Epilepsy
  - Molecular targets for antiepileptic drug development (Meldrum B.S., et al 2007, *Neurotherapeutics,* 4: 18-61).
  - The role of T-type calcium channels in epilepsy and pain (Nelson, M.T. et al,2006, *Curr Pharm. Des*., 12, 2189-2197).
  - Design, synthesis and evaluation of a novel 4-aminomethyl-4-fluorpiperidine as a T-type $Ca^{+}2$ channel antagonist (Shipe D. et al, 2008, *J. Med Chem.*, *51*, 3692-3695.
- Arousal states
  - Lack of delta waves and sleep disturbances during non-rapid eye movement in mice lacking $\alpha 1_G$-subunit of T-Type calcium channel (Lee, J. et al, 2004, *PNAS*, 101, 18195-18199.
- Oncology
  - A role of functional T-Type calcium channel in hepatocellular carcinoma cell proliferation (Li Y et al, 2009, *Oncology Reports*, 22,1229 -1235).

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Summary

- Identified novel, potent, small molecule T-type antagonists
  - Pan antagonists
    - No selectivity versus T-type family sub-types
- Selective versus related gene family ion channels and cardiac channels
- Good in vitro properties can be achieved
  - Permeability, solubility, stability
- Oral bioavailability can be achieved
  - Caco2 - permeability assays used to guide synthesis
- Limited CNS exposure
- Plasma concentrations vs. T-type IC_{50} achieved

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Other Indications – T-Type Inhibitors

- Parkinson's disease
- Neruroprotection
- Sleep disorders

- Druggable, yes, validated, maybe

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Drug-like Molecules

- Rule of 5
  - Rule of 4.5?
- MW 500, ClogP 5, H-bond donors, 5 H-bond acceptors (sum of N and O atoms) 10
- Remarks: No more than one violation; not applicable for substrates of transporters and natural products
- Extensions
  - Polar surface area 140, sum of H-bond donors, and acceptors 12, rotatable bonds 10

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Druglikeness

- Optimal solubility to both water and fat
  - Orally administered drug has to go through the intestinal lining, carried in aqueous blood and penetrate the lipid cellular membrane to reach the inside of a cell.
    - cLogP, is used to estimate solubility.
- High potency (IC_{50} or EC_{50})
  - Reduces the risk of non-specific, off-target pharmacology at a given concentration
  - Low clearance, high potency also allows for low total dose, which lowers the risk of idiosyncratic drug reactions
  - The less you give the better

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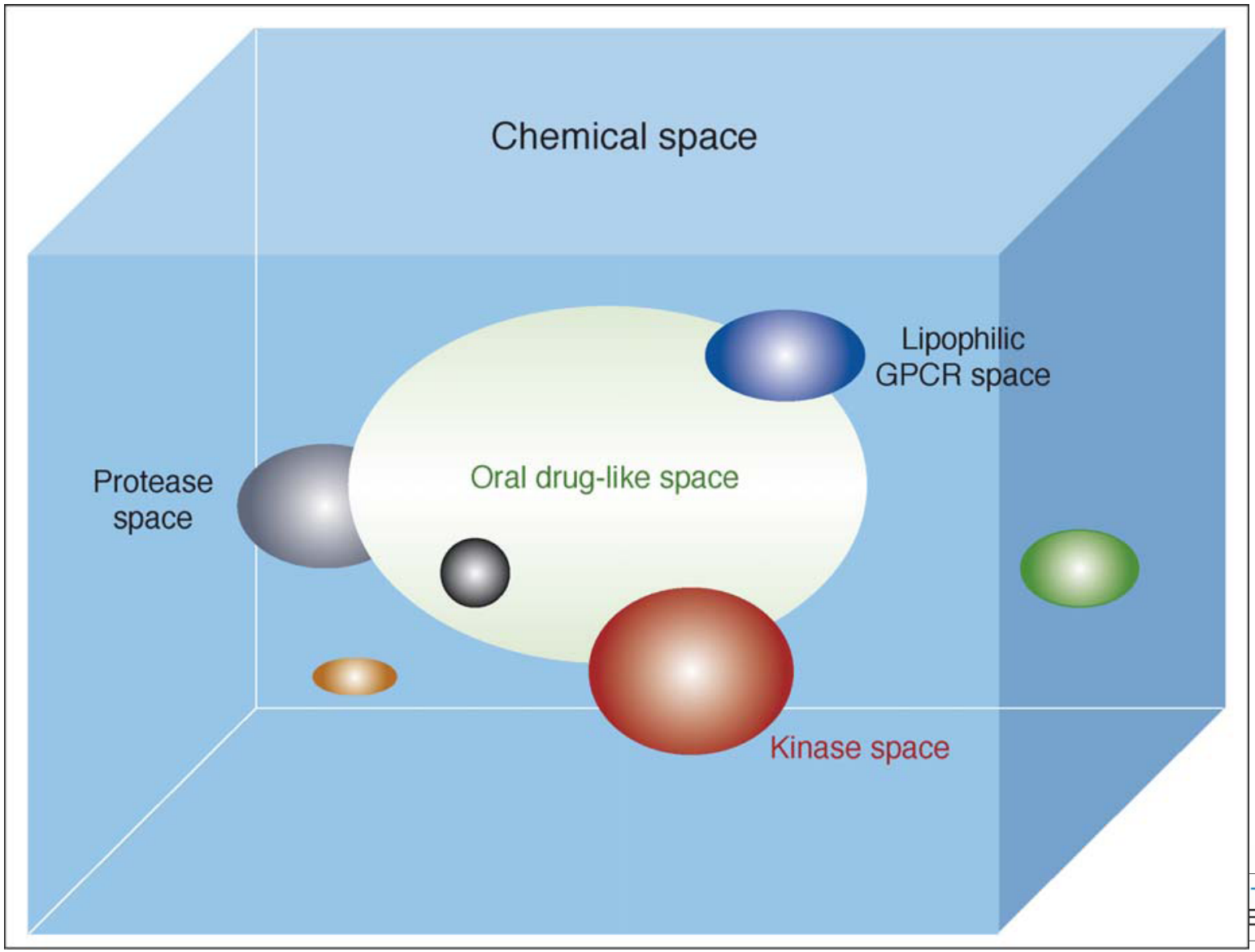
Drug-Like Space



Natural Products

- Very effective as drugs
- Optimized by nature
- Don’t fit the drug-like concept
  - Very complex
  - Many stereocenters
- More difficult to work with
- Making a comeback

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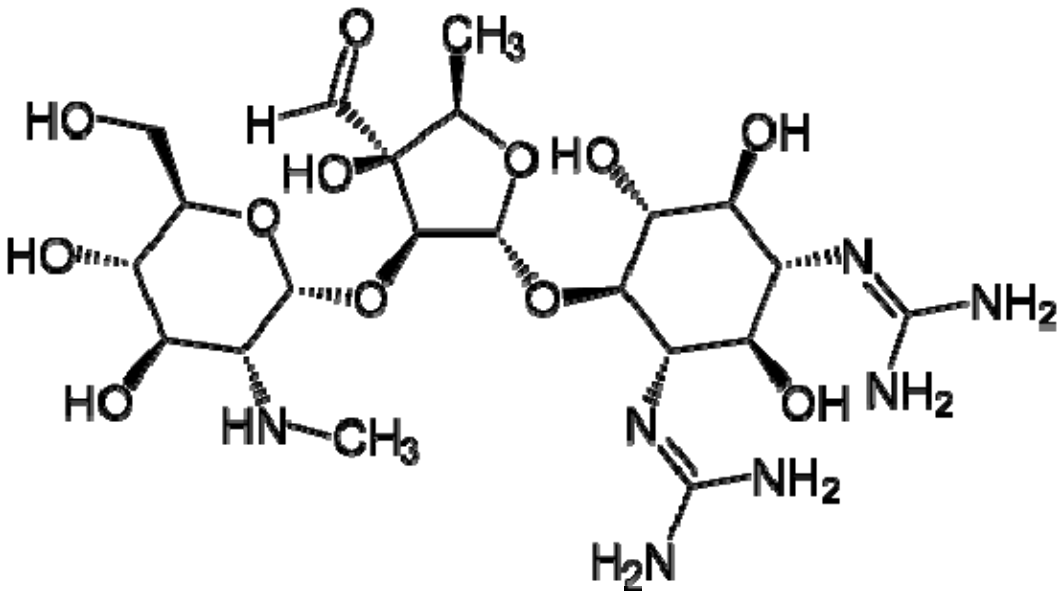
Streptomycin



Formula	$C_{21}H_{39}N_7O_{12}$
Mol. mass	581.574 g/mol

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