**Introduction**

- **G Protein-Coupled Receptors (GPCRs)** are very important for human biology
  - Largest family of membrane-bound receptors
  - Over 350 non-olfactory GPCRs in humans, ~1/3 of which have been drugged
  - Expressed on all cells in the body
  - Regulate numerous diverse physiological processes including intercellular communication and signal transmission
  - Over 30% of approved drugs target GPCRs
  - Between 2011-2015 represented ~27% of the global market share of therapeutic drugs and generated ~$890 billion

**Group Meeting Includes**

- Introduction to GPCRs, their structure, and basic functions
- Adrenergic and Angiotensin Receptors
- Histamine Receptors
- Dopamine and 5-HT (Serotonin) Receptors
- Muscarinic Receptors
- Adenosine Receptors
- Opioid Receptors
- Chemokine Receptors
- Gonadotropin-releasing Hormone Receptors
- List of Validated GPCR Drug Targets

**Group Meeting Doesn’t Include**

- An exhaustive discussion of any of these topics
- Many of the lesser-targeted GPCRs
- Orphan GPCRs
- In depth biology on individual GPCRs

**Definitions**

- **Agonist** - mimic function of natural ligands by binding to receptor and causing the normal response
- **Partial Agonist** - any agonist that produce the maximum response capable in a system even at saturating concentrations
- **Antagonist** - bind to receptor and prevent normal response by inhibiting natural ligand from binding; keeps response of receptor at basal levels. Could be either competitive (reversible) or non-competitive (irreversible).
- **Inverse Agonist** - different than simple antagonist as will switch a receptor into an “off” state; this cause a response that lowers response bellow basal levels
- **Allosteric Inhibitor** - acts by binding to a site that is topographically distinct from, but conformationally linked to, the active site
- **Potency** - the dose range over which a response is produced; from a dose-response curve the potency is the dose of the drug that produces 50% of the maximum response (ED50)
- **Efficacy** - the size/strength of a response produced by an agonist in a particular tissue

With GPCR Drugs it is not always clear exactly what kind of interaction they have with a particular receptor and there are many cases where a drug has been re-catagorized as a better understanding of the mechanism of action is gained from biological studies.
G Protein-Coupled Receptor Drugs

Lisa M. Barton

Structure of GPCRs

All share same transmembrane domain with common structure:
- 7 transmembrane domains that are hydrophobic α-helices
- 3 extracellular loops
- 3 intracellular loops
- N-terminus (responsible for ligand binding)
- C-terminus

GPCR Signaling Pathway

- GPCRs in constant motion
- Agonist binding stabalizes GPCR into “On” conformation
- Agonists can range from ions and small molecules to peptides and large proteins depending on the type of receptor
- “On” conformation further stabalized by binding of G-protein (made up of α, β and γ subunits)
- Note little is known about how GPCRs associate with specific G-proteins
- Once G protein encounters ligand-bound GPCR, GDP is exchanged with GTP; triggers release of G\textsubscript{βγ} dimer.
- Both free G\textsubscript{α} and G\textsubscript{βγ} can interact with effector molecules in cells, trigger downstream signaling
- Pathway stops when G\textsubscript{α} hydrolyzes GTP to GDP and reassociates with G\textsubscript{βγ}
- There are multiple pathways through which GPCRs can become desensitized, including receptor phosphorylation events, arresting-mediated internalization into endosomes, receptor recycling and lysosomal degradation
- Many GPCRs can trigger multiple different signalling pathways and specific ligands can produce different relative efficacies to different pathways (sometimes even opposite, ie acting as both an agonist for 1 and an antagonist for another)

Adrenergic Receptors (ARs)

- Also classified as α-1 Adrenergic Recep
- Epinephrine primarily released by the adrenal gland whereas Norepinephrin released by sympathetic nerve terminals in peripheral nervous system and brain
- Play key role in cardiac function
- 2 classes: β-ARs (~90% total ARs in heart) and α-ARs (~10%)
- Each classes can be further subdivided into α\textsubscript{1}, α\textsubscript{2}, β\textsubscript{1} and β\textsubscript{2}
- Targeting this GPCR class lead to development of Beta Blockers
- Drugs targeting these receptors also have indications in treatment of asthma and COPD

Select Examples:

Angiotensin Receptors (ATs)

- Angiotensin is a hormone made by the liver that is further converted to Angiotensin I and II by renin-angiotensin enzymatic cascade
- Angiotensin II plays an important role in cardiovascular system
- 3 classes: AT\textsubscript{1}, AT\textsubscript{2} and Mas which differ in their affinity for various angiotensin peptide fragments
- Targeting this GPCR class lead to development of Angiotensin II Receptor Blockers (ARBs): unlike ACE inhibitors (which only can prevent the formation of angiotensin by the renin-angiotensin enzymatic cascade) these can block all angiotensin as they prevent their binding to receptors

Select Examples:

For more details on drugs that target both these receptors see Cardiovascular (CV) Drugs Group Meeting (Merchant 2018)
G Protein-Coupled Receptor Drugs

Histamine Receptors

- Histamine is naturally produced from L-histidine by histidine decarboxylase in a number of different cell types and is involved in the regulation of a number of different body functions (e.g., cell proliferation, hematopoiesis, wound healing, sleep/wake cycle, cognition, memory, etc).
- 4 different classes: H₁-H₄.
- Indications: drugs that act as histamine antagonists range from treatment of allergic conditions (H₁), peptic ulcers (H₂), obesity and CNS disorders (H₃), and asthma and inflammation (H₄).

For a review on Histamine Receptors see Br. J. Pharmacol. 2006, 147, S127

H₁ Receptors

- H₁-receptor is widely expressed (nerves, respiratory epithelium, endothelial cells, hepatic cells, vascular smooth muscle cells, dendritic cells, lymphocytes).
- Involved in allergy and inflammation.
- Antihistamines all act as inverse agonists and lock into inactive state.
- 1st gen histadines: sedating
- 2nd gen: relatively non-sedating
- 6 Chemical Classes of Antihistamines: Ethanolamines, Phenothiazines, Piperazines, Ethylenediamines, Alkylamines, Piperidines

Select examples of H₁ Antihistamines

1st generation:
- Clemastine (Meclastin®)
- Diphenhydramine (Benadryl®)
- Chloropyramine (Phenergan®)
- Promethazine (Phenergan®)
- Astemizole (Hismanal®)
- Azelastine (Asterlin®)
- Acrivastine (Benadryl Relief®)
- H2O2C-(N-Phenyl)-N,N,N-trimethylammonium
- racemic: Cetirizine (Zyrtec®)
- chiral: Levocetirizine (Xyzal®)

2nd generation:
- Terfenadine (Seldane®)
- Fexofenadine (Allegra®)
- Desloratadine (Clarinex®)
- Racemic: Cetirizine
- Chiral: Levocetirizine

Crystal structure of H₁ receptor complexed with doxepin; Nature 2011, 475, 66

Ways to make chiral (Levocetirizine):

1. PhMgBr, Tol -20 to 0 °C 65%, >99% ee
2. HCl, MeOH 95%

Tetrahedron Lett. 1996, 37, 4837
G Protein-Coupled Receptor Drugs

**H₂ Receptors**

- Downstream signalling controls gastric acid secretion, vasodilator
- 1st potent H₂R antagonist was Burimamide
- 1st clinically approved was Cimetidine (Tagamet)

**H₃ Receptors**

- Found predominantly in the central nervous system
- Potential therapeutic applications including obesity, memory and learning deficits, Alzheimer's disease, epilepsy, schizophrenia and sleep disturbance
- No Selective H₃R drugs approved by FDA although multiple have been tested in the clinic
- For many years all antagonists with highest potency were imidazole derivatives but these are too polar to penetrate blood-brain barrier -> focus now on non-imidazole antagonists
- Similar structure found in many non-imidazole based scaffolds: basic tertiary amine (maintain ionic interaction had with imidazole) + flexible linker + lipophilic portion

Other approved H₂R antagonists:

- Ranitidine (Zantac)
- Famotidine (Pepcid)
- Nizatidine (Tazac/Axid)
- Roxatidine acetate (Roxil)

Other Clinical Candidates:

- GSK-189,254 (Alzheimer's/narcolepsy)
- Pitolisant (Wakix, approved in Europe 2016 for narcolepsy)

**Mechanism?**

\[ \text{[BMIM][Cl]} = 1\text{-butyl-3-methylimidazolium chloride} \]

\[ \text{Green Chem. 2011, 13, 3101} \]

**J. Med. Chem. 1972, 15, 750**
**JOC 1989, 54, 2242**

**Notes:**

- Not orally available so wasn't put into clinic
- dropped from clinical trials due to dangerous cardiac side effects
- 1. TsCl, TEA, DMAP
- 2. K₂CO₃, L-Tartaric acid
G Protein-Coupled Receptor Drugs

Dopamine Receptors

- Dopamine is a catecholaminergic neurotransmitter and regulates large variety of functions including voluntary movement, hormonal regulation, and hypertension.
- Indications of drugs targeting these receptors include Parkinson's, schizophrenia, bipolar disorder, Huntington's disease, ADHD, and Tourette's syndrome.
- 2 major classes that can be further divided into 5 subtypes: D1 (D1, D2, D3) and D2 (D2-D4).

Examples:
- Phenothiazine Antipsychotics - 1st generation, potent activity with pronounced side effects (examples include chlorpromazine and its derivatives, haloperidol, benperidol, clopenthixol, and droperidol).
- Atypical Antipsychotics - 2nd generation, lower propensity for side effects; typically have activity against other receptors especially 5-HT (examples include clozapine, sulpiride, and olanzapine).

For a review of Dopamine Receptors see *Pharmacol Rev. 2011*, 63, 182

Phenothiazine Antipsychotics

- Large number based on this core motif though typically not very selective (D1, D2, 5-HT, HRH, etc)
- Examples:
  - R = H, Promazine (Sparine®)
  - R = Cl, Chlorpromazine (Thorazine®)
  - R = Ac, Acrepromazine (Atrave®)
  - R = CF₃, Trifluoperazine (Vesprin®)

Ways to Synthesize of Core:

\[
\text{NH}_2 + \text{Cl} \quad \text{KOH} \quad \text{Ac}_2\text{O}
\]

\[
\text{Br}_2 \quad \text{NaOAc/HAc} \quad \text{POCl}_3 \quad \text{JOC 2003, 125, 12631}
\]

\[
\text{S} \quad \text{K}_2\text{CO}_3 \quad \text{90 - 110 °C}
\]

\[
\text{R}^1 \quad \text{R}^2 \quad \text{JOC 1950, 15, 1125}
\]

\[
\text{R}^1 \quad \text{R}^2 \quad \text{JACS 1955, 77, 2270}
\]

\[
\text{R}^1 \quad \text{R}^2 \quad \text{JOC 1956, 72, 6714}
\]

- Agonists:

\[
\text{HO} \quad \text{HO} \quad \text{Dopamine}
\]

Ergolines Agonists

\[
\text{MeS} \quad \text{Et}_2\text{N} \quad \text{NH}\text{Et}
\]

\[
\text{R} = \text{CH}_2\text{OH or CO}_2\text{H}
\]

Dibenzo Oxepines, Thiepine, and Azipines

\[
\text{S} \quad \text{N}
\]

\[
\text{MeN} \quad \text{NMe}
\]

\[
\text{TiCl}_4 \quad 50-80%
\]

Quetiapine Fumarate (Seroquel®)

Loxapine (Loxitane®)

Olanzapine (Zyprexa®)

Process Route
Tetrahedron **2010**, 66, 8203

\[
\text{Cu} \quad \text{K}_2\text{CO}_3 \quad \text{amyl alcohol}
\]

1. Na₂S₂O₄
2. Xylene

\[
\text{O} \quad >135 °C \quad 75-100%
\]

\[
\text{N} \quad 96%
\]

Thioxanthenes

Flupentixol (Depixol®)

Chlorprothixene (Truxal®)

Thiothixene (Navane®)

Zuclopenthixol (Clopixol®)

Typically synthesized through grignard addition to Thioxanthone derivatives (Note that they are sold as a mixture of Z/E isomers or isomers are separated)

\[
\text{O} \quad + \text{BrMg} \quad \text{NR}_2
\]

**Baran Group Meeting**
**5/4/19**
G Protein-Coupled Receptor Drugs

5-hydroxytryptamine receptors

- Also known as 5-HT or Serotonin receptors
- Largest family of GPCR Neurotransmitter receptors and likely predates evolution of muscarinic, dopaminergic, and adrenergic receptors and some of oldest receptors within the rhodopsin family
- Serotonin plays many roles (including development, cardiovascular, gastrointestinal, endocrine system, sensory perception, and range of brain functions)
- Indications of drugs targeting these receptors include analgesics and antipsychotics
- 5 Families of this GPCR: 5-HT1, 5-HT2, and 5HT2, 7
- Many drugs that target 5-HT receptors also target other GPCRs including Dopamine Receptors (see Clozapine, Olanzapine, Quetiapine, Pergolide and related compounds, Promazine and related compounds, Thioproperazine and related compounds)

For a review on 5-HT Receptors see Chem. Rev. 2008, 108, 1614

Muscarinic Receptors (mAChRs or CHRMs)

- Named because originally differentiated from nicotinic receptors by an increased sensitivity to Muscarine over Nicotine
- 5 different classes: M1, M3, M2 all couple with Gq Proteins and M2 and M4 couple with the Gi/Go family of G Proteins
- Clinically anticholinergic drugs targeting M1-M3 have found the most use towards the treatment of chronic obstructive pulmonary disease (COPD) and asthma
- Some of the first GPCRs to be purified and cloned
- Involved in central and parasympathetic nervous system
- Targets for multiple disease areas including Alzheimer's, schizophrenia, Parkinson's, and COPD.


Note: Nicotinic receptors are NOT GPCRs and instead work through a ligand-gated ion channel, differentiating them from mAChRs
Adenosine Receptors (ARs)

- Adenosine produced from metabolism of ATP and plays many roles in the body including modulation of neurotransmitter release, synaptic plasticity, neuroprotection (i.e., in cases of reduced blood flow, hypoxic conditions, or oxidative stress), vasodilatation, and reduced cell proliferation, cytokine production, and bronchoconstriction.
- 4 different subtypes: A_1, A_{2A}, A_{2B}, and A_3.
- A_1 (high-affinity for adenosine) and A_3 (low-affinity) are inhibitory towards adenylyl cyclase only where as A_{2A} (high-affinity) and A_{2B} (low-affinity) stimulate adenylyl cyclase and increases cAMP (cAMP).

For a review of Adenosine Receptors see Chem. Rev. 2008, 108, 238

A_1 Antagonists

Xanthines

- Some of best-known but limited success as a lot of off-target activity and typically suffer from low water solubility.
- One way to increase solubility of these types is to make tetracyclic imidazoline derivatives.

Non-Xanthine

- Examples of both pyrazolo[1,5-a]pyridine and adenine derivatives being selective for A_1

Process Route

2 equiv. (likely extra equiv. serves as oxidant)
Opioid Receptors
- Endogenous Opioid Peptides (Endorphins) are hormones expressed by the CNS system and serve as neuromodulators and promote a range of physiological effects including pain relief
- All Endorphins contain common motif: Tyr-Gly-Gly-Phe-Met/Leu sequence at the amino terminal
- Indications of drugs targeting these receptors mainly analgesic but also management of diarrhea, cough, and cancer
- Families of this GPCR: Mu (µ), Delta (δ), Kappa (κ)
- µ agonists are therapeutically the most common (ie morphine, codeine, methadone, fentanyl) but also typically have the most side effects as µ receptors mediate respiratory depression, sedation, reward/euphoria, nausea, urinary retention and constipation
- δ mediate dysphoric, aversive, sedative and diuretic side effects
- σ mediate reward, respiratory depression and convulsions
- Prolonged administration can also lead to tolerance and physical dependence
- The development of opioid specific drugs that do not have adverse side effects is of great interest for pharmaceutical companies

For a review on Opioid Receptors see *Annu. Rev. Med.* 2016, 67, 433

Examples of Opioid Agonists:

![Chemokine Receptors](image)

Chemokine Receptors
- Chemokines are signaling proteins secreted by cells and mediate cell migration
- 4 families of chemokines: CXC, CC, C, and CXC (classified by cysteine residues in the ligand) and receptors are named after the chemokine it binds
- CXCR3 and CCR5 both have indications for HIV and CCR5 has indications in for a number of autoimmune disorders

Case Study: Maraviroc (Selzentry®) - CCR5
- Was specifically developed for potency as a CCR5 antagonist
- Acts by stabilizing CCR5 into an inactive conformation, preventing viral entry into immune system cells and slowing progression of disease
- CCR5 acts as a co-receptor for the viral envelope glycoprotein gp120 of HIV

For more details on opioid drugs see *Opioid Drugs Group Meeting (Harwood 2019)*

![Bioorg. Med. Chem. Lett. 2004, 14, 5275](image)

**Maraviroc**

Hydrophobic pocket
Salt bridge with Glu-283

H-bond with Thr-195 and Thr-259

Initial high throughput screen hit:

1. Boc$_2$O, NaHCO$_3$
2. DIBAL-H, -78 ºC
3. 1. PrCO$_2$H, Et$_3$N, CDI, 53%
   2. POCl$_3$, pyr

1. NH$_2$OH·HCl pyr
2. Na, reflux

10:1 exo:end

Tetrahedron Lett. 2005, 46, 5005
G Protein-Coupled Receptor Drugs

Gonadotropin-releasing Hormone Receptors (GnRHRs)

- GnRH is the central regulator of the reproductive hormone cascade
- Targeting these GPCRs has indications for infertility, delayed puberty, contraception and hormone dependent diseases
- Approved agonists are all peptides (ex. Leuprolide, Goserelin)
- Approved antagonists are mix of peptides (ex. Cetrorelix, Abarelix) and small molecules (most in clinical trials)

Gonadotropin-releasing Hormone/Choriogonadotropin (FSHRs)

- Ovarian follicular stimulation
- Prevention of ovulation

Other GPCRs targeted by Pharmaceuticals

- Purinergic Receptors
- Cannabinoid Receptors
- Vasopressin Receptors
- GABA_B Receptors
- Glucagon-like Peptide Receptors
- Melanocortin Receptors
- Prostaglandin Receptors
- Oxytocin Receptors
- Endothelin Receptors
- Somatostatin Receptors
- Tachykinin Receptors
- Melatonin Receptors
- Bradykinin Receptors
- Calcium-sensing Receptors
- Calcitonin Receptors
- Metabotropic Glutamate Receptors
- Cholecystokinin Receptors
- Hydroxycarboxylic Acid Receptors
- Free Fatty Acid Receptors
- Neuropeptide Receptors
- Secretin Receptors
- Platelet-activating Factor Receptors
- Parathyroid Hormone Receptors
- Sphingosine-1-phosphate Receptors
- Growth-hormone-releasing Hormone Receptors (GHRHRs)
- Trace Amine-associated Receptors (TAARs)
- Luteinizing Hormone/Choriogonadotropin Receptors (LHCGRs)
- Hypocretin Receptors (also known as Orexin Receptors)
- Cysteinyl Leukotriene Receptor 1 (LYSLTR1)
- Thyrotropin-releasing Hormone Receptors
- Glutamate Receptors

Other small molecules:

- Linzagolix
  (Phase III trials for uterine fibroids and endometriosis)
- Elagolix (Orilissa®)
  (endometriosis)
- Relugolix (Relumina®)
  (Phase III trials for endometriosis and prostate cancer, approved in Japan)