Case histories in drug discovery

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Therapeutic indications

• Diabetes
  • Insulin
  • 20 other approved drugs in the UK

• Obesity
  • One approved drug, orlistat, in the UK

• Erectile dysfunction
  • 4 approved PDE5 inhibitors in the UK
  • Alprostadil or papaverine given by intracavernous injection
Strategies for lead discovery

- Play the chemical lottery

- Identify chemical space relevant to your target

- Pay attention to the rest of the world
Treatment of type II diabetes

• Diagnosis
  – levels of glycosylation of haemoglobin (HbA1c), proportional to glucose levels in the bloodstream
  – glucose tolerance test, clearance of glucose by the body

• Treatment- lifestyle changes
  – diet
  – weight
  – physical activity

• Treatment- medication
  – monotherapy with metformin or sulfonylureas
  – monotherapy with other oral drugs
  – combination of oral drugs
  – combination of oral drugs and insulin
Medication for Type II diabetes

• **Sulfonylureas**: glibenclamide, gliclazide, glimepiride, glipizide, tolbutamide; **Meglitinides**: nateglinide, repaglinide—Insulin secretagogues, block the ATP-sensitive $K^+(K_{ATP})$ ion channel in pancreatic beta cells.

• **Metformin**- Often first-line therapy, especially in overweight patients. Mechanism of action unclear, decreases glucose production in the liver.

• **Acarbose**- Inhibitor of $\alpha$-glucosidase, reduces glucose absorption in the intestine.

• **Pioglitazone**- A thiazolidinedione PPAR-\(\gamma\) agonist, increases glucose uptake in peripheral tissues.

• **Exenatide, liraglutide, lixisenatide**- Insulin secretagogues, GLP-1 agonists administered subcutaneously.

• **Alogliptin, linagliptin, saxagliptin, sitagliptin, vildagliptin**- Insulin secretagogues, dipeptidyl peptidase-4 inhibitors that increase GLP-1 levels.

• **Canagliflozin, dapagliflozin**- Inhibitors of renal subtype 2 sodium-glucose transport protein, reduce kidney reabsorption of glucose.
Biguanides- metformin

Inspiration from herbal remedies
**Discovery of metformin**

French lilac *Galega officinalis*, extracts used to treat painful and frequent urination in diabetics

- **Alkylguanidines**
  - Galegine, active constituent, briefly used in the 1920s, withdrawn due to toxicity

- **Diguanidines**
  - Synthalin, a diguanidine, withdrawn in the 1940s due to toxicity

- **Biguanidines**
  - Phenformin (USA)
    - Withdrawn in the 1970s, lactic acidosis
  - Buformin (Germany)
  - Metformin (France)
    - Reduced side effect profile.
    - UK approval 1958, on WHO list of Essential Medicines.
Synthesis and mechanism of action of metformin

- Primary effect of metformin- altered cell metabolism. Inhibition of mitochondrial complex I inhibits hepatic glucose production and opposes effects of glucagon.

- Stimulation of AMP-activated protein kinase modulates lipid metabolism and improves insulin sensitivity.
Metformin as a drug

• Inexpensive and well tolerated at the clinical dose (>1 g daily).

• Often more effective at reducing glucose levels than other antidiabetic drugs.

• Requires endogenous insulin for action, hence effective only if functional pancreatic islet cells are present.

• Does not cause hypoglycaemia or weight gain.

• Gastrointestinal side-effects and, in rare cases, lactic acidosis.

• Can be used in combination with other oral antidiabetic drugs or insulin.
Sulfonylureas - glibenclamide, gliclazide, glimepiride, glipizide, tolbutamide

Meglitinides - nateglinide, repaglinide

Drug repositioning based on a side-effect
Evolution of sulfonylurea antidiabetic drugs

Leads by serendipity

• 1942- side-effects and deaths in 2554 RP clinical trials attributed to hypoglycaemia.
• 1954- hypoglycaemia observed in normal subjects with carbutamide.
• Lilly begins clinical trials with carbutamide as an antidiabetic agent, discontinued due to toxicity.

MW 270
Clog P 2.0
HBA 3
HBD 2
nrot 4
TPSA 75 Å²

1st generation sulfonylurea

• Hypoglycaemic without antibacterial activity.
• Submitted by Upjohn for FDA approval in 1958.
• Short acting due to metabolism of $p$-methyl group to carboxylic acid.
• Concerns over potential cardiovascular side-effects.
Mechanism of action of sulfonylurea drugs

- Pancreatic $K_{ATP}$ channels are hetero-octamers of four Kir6.2 subunits, which form the $K^+$ conducting pore, and four regulatory SUR1 subunits.

- Upon glucose stimulation, the ion channel is closed, leading to influx of $Ca^{2+}$ and insulin secretion.

- Sulfonylureas and meglitinides bind to SUR1 and block the ion channel, having the same effect as glucose to stimulate insulin secretion.
Second and third generation sulfonylurea drugs

Sulfonylurea SAR:
- $p$-substituent on aromatic ring, not amine to avoid antibacterial activity
- alkyl substituent on urea.

2nd generation sulfonylureas
- Glibenclamide (glyburide)
  - Long-acting
  - Can bind to related cardiac SUR2 channel
- Gliclazide
  - Short-acting, SUR1 selective
  - On WHO list of Essential Medicines
- Glipizide
  - Short-acting

3rd generation sulfonylurea
- Glimepiride
  - Long-acting

MW 491
Clog P 2.8
HBA 5
HBD 3
nrot 6
TPSA 125 Å²
Synthesis of glimepiride

Tetrahedron Letters, 2003, 44, 4853
PCT Int. Appl., 200357131, 17 Jul 2003
Sulfonylureas as drugs

• Inexpensive, first class of oral antidiabetic drugs, in clinical use since the 1940s.

• Major side-effects are hypoglycaemia and weight gain.

• Duration of action (short, medium or long acting) can be tailored to patient response.

• Work by stimulating insulin secretion, hence effective only if functional pancreatic islet cells are present.

• Can be used in combination with other oral antidiabetic drugs or insulin.
Meglitinide antidiabetic drugs

- Discovered by medicinal chemistry efforts to find alternatives to sulfonylureas that have the same target (pancreatic $K_{ATP}$ channels).

- Weaker binding and faster dissociation compared to sulfonylureas.

- Similar side-effects of weight gain and hypoglycaemia but less pronounced than with sulfonylureas.

- Short-acting, rapid onset of action.

repaglinide (Boehringer-Ingelheim, licensed to NovoNordisk)  

MW 453  
Clog P 5.1  
HBA 5  
HBD 2  
nrot 10  
TPSA 79 Å²

nateglinide (Ajinomoto, licensed to Novartis)  

MW 317  
Clog P 3.6  
HBA 3  
HBD 2  
nrot 6  
TPSA 66 Å²
Glucosidase inhibitors- acarbose

High-throughput screening of natural product extracts
Hydrolysis of starch to glucose

- Starch is digested to glucose by two hydrolysing enzymes, \( \alpha \)-amylase and \( \alpha \)-glucosidase (\( \alpha \)-amyloglucosidase).

- The enzyme \( \alpha \)-glucosidase is inhibited by a number of clinical drugs among which acarbose is used in the UK.
Acarbose mechanism of action

- Starch oligosaccharides are broken down by $\alpha$-glucosidase in the membrane of the small intestine to glucose.

- Acarbose mimics the oligosaccharides formed from starch and acts as a competitive inhibitor of the enzyme $\alpha$-glucosidase.

- Inhibition of $\alpha$-glucosidase reduces absorption of dietary glucose.
Acarbose as a drug

- Discovered by high-throughput screening of microbial extracts by Bayer in the 1970s.

- Obtained by fermentation from a strain of *Actinoplanes*.

- GI side-effects such as diarrhoea and flatulence due to buildup of oligosaccharides in the colon and their bacterial digestion.

- Typically used when other antidiabetics are not tolerated or contra-indicated. Appears to be better tolerated in the Oriental population.

- Effect is independent of pancreatic insulin secretion.
PPAR$_{\gamma}$ agonists- pioglitazone

Drug discovery by phenotypic observation
Discovery of thiazolidinedione PPAR\(_\gamma\) agonists

Leads from phenotypic observation

1953, substituted acetic acids reported to have hypocholesterolemic effects in humans.


1975, Takeda SAR studies discovered compounds with hypolipidaemic and hypoglycaemic activity.

Mechanism of action of PPARγ agonists

• PPARs (peroxisome proliferator-activated receptors) are nuclear receptors.

• Upon ligand binding, they are activated and translocate to the nucleus, where they form heterodimers with the retinoid receptor.

• These bind to DNA and modulate the transcription of genes at the PPAR response element.
Glitazone and glitazar PPAR agonists

- **Glitazones**
  - PPAR\(\gamma\) agonists differ in safety profile. Development of ciglitazone and balaglitazone discontinued, troglitazone and rosiglitazone withdrawn from the market. Pioglitazone (Actos, Takeda) remains the only agent approved in the UK.
  - Rivoglitazone is the most potent agonist, currently in clinical trials.

- **Glitazars**
  - Dual PPAR\(\alpha\) and PPAR\(\gamma\) agonists for the treatment of metabolic syndrome. Saroglitazar approved in 2013 in India for the treatment of diabetic dyslipidaemia, dual reduction of glucose and lipid level.
Synthesis of pioglitazone

Synthesis of Pioglitazone hydrochloride

(II) \[ \text{PhCH}_2\text{Bu}_3\text{NCl NaOH p-TsCl} \]

(I) \[ \text{NaH} \]

(III) \[ \text{Ra-Ni HCOOH} \]

(IV) \[ \text{OH}^- \]

(V) \[ \text{H}_2 \]

(VI)
Pioglitazone as a drug

• Safety profile compares favourably to rosiglitazone that was withdrawn due to potential risk of cardiovascular side-effects.

• Can be used in combination with metformin, sulfonylureas and insulin.

• Effect is independent of pancreatic insulin secretion.

• Does not cause hypoglycaemia.

• Associated with weight gain and fluid retention.
GLP-1 agonists- exenatide, liraglutide, lixisenatide

Peptide engineering
Glucagon-like peptide 1 (GLP-1), an incretin

Incretins are GI hormones that are secreted in response to nutrient stimuli and stimulate glucose-dependent insulin secretion.

GLP-1, a peptide incretin, was first identified in 1983.

GLP-1 itself is rapidly cleaved ($t_{1/2}$ 1-2 min) to an inactive form by the serine protease dipeptidyl peptidase 4 (DPP-4).

Two forms of GLP-1: GLP-1-(7-37) and (7-36)-amide
- Increase insulin production and secretion.
- Decrease glucagon secretion.
- Slow gastric emptying.
- Promote weight loss by inducing satiety.

Incretins are GI hormones that are secreted in response to nutrient stimuli and stimulate glucose-dependent insulin secretion.

Two therapeutic solutions: GLP-1 agonists with higher stability or increase GLP-1 lifetime by inhibition of DPP-4.
GLP-1 signaling in the β-cell

- GLP-1 binds to the GLP-1 receptor, a GPCR coupled to adenylyl cyclase.
- Triggers Epac2, EGFR, PI3K and CHOP pathways to induce the following:
  - insulin secretion
  - proliferation of β-cells
  - suppression of apoptosis
Exenatide and lixisenatide, long-acting GLP-1 agonists

Exendin-4 - discovered in 1992 from the saliva of the Gila monster. Manufactured by Amylin as exenatide (Byetta).

- The 39-mer peptide has 53% homology with GLP-1. It is a potent GLP-1 agonist resistant to DPP-4 hydrolysis with a halflife in vivo of 2.4 h. Administered subcutaneously in μg doses 2x daily or 1x weekly.

- Lixisenatide (Lyxumia, Sanofi) - hexalysine tail further increases stability, halflife of 2-4 h, designed for 1x daily subcutaneous administration.

- Drugs are produced by solid-phase peptide synthesis.
Liraglutide, a modified analogue of human GLP-1

- Liraglutide (Victoza) peptidase degradation is insignificant, half-life 11-15 h suitable for once daily administration.

**Key residues**
- receptor binding
- conformational control

MW 3751
Clog P -3.4
HBA 55
HBD 54
nrot 132
TPSA 1510 Å²
Improving stability of liraglutide

- Novo Nordisk strategy - Avoid changes to key residues and improve stability by acylation as done with insulin.

- Lysine sidechain K26 chosen as acylation site and a second lysine present replaced by arginine to avoid competitive acylation.

- Albumin binding and solubility increased by adding a $\gamma$-Glu spacer with a negative charge between K26 and the C16 fatty acid.
DPP-4 inhibitors- alogliptin, linagliptin, saxagliptin, sitagliptin, vildagliptin

Protease target-based drug discovery
Irreversible DPP-4 inhibitors

Cyanopyrrolidines found to be proline mimetics that covalently bind to DPP-4 via the active site Ser. However, they were not very stable due to intramolecular cyclization.
Irreversible DPP-4 inhibitors

- Cyanopyrrolidine stability improved by steric shielding of the amine.
- Alkylation of the amine was the strategy that led to the drug vildagliptin (Novartis).
- Increasing the bulk of the side-chain was the strategy that led to the drug saxagliptin (Bristol-Myers Squibb)

vildagliptin (Galvus)
EMEA approval 2008, not FDA approved

MW 303
Clog P 1.1
HBA 4
HBD 2
nrot 3
TPSA 76 Å²

saxagliptin (Onglyza)
FDA/EMEA approval 2009

MW 315
Clog P 0.9
HBA 4
HBD 2
nrot 2
TPSA 90 Å²
Synthesis of vildagliptine and saxagliptine

Villhauer et al., J. Med. Chem. 2003, 46, 2774–2789

Reversible DPP-4 inhibitors - sitagliptin

**HTS leads**

- IC$_{50}$ 11 µM
- IC$_{50}$ 1.9 µM

**Hybrid**

- R = H, IC$_{50}$ 134 nM
- R = F, IC$_{50}$ 34 nM

**Optimisation**

- 2,4-5-tifluoroaryl improves potency
- CF$_3$ group improves bioavailability

- IC$_{50}$ 18 nM

**Simplification**

- IC$_{50}$ 139 nM

**MW 407**
**Clog P 2.0**
**HBA 4**
**HBD 1**
**nrot 5**
**TPSA 77 Å$^2$**

Sitagliptin (Januvia, Merck) - first in class DPP-4 inhibitor, launched in 2006, blockbuster drug.
Reversible DPP-4 inhibitors, linagliptin and alogliptin

**Patent disclosure**

Boehringer-Ingelheim patent 2002
IC$_{50}$ 82 nM

**Optimisation**

Off-target hERG and $M_1$ receptor effects
IC$_{50}$ 6 nM

MW 473
Clog P 2.6
HBA 7
HBD 1
nrot 5
TPSA 114 Å$^2$

linagliptin, Boehringer-Ingelheim
IC$_{50}$ 1 nM

FDA approval 2011, EMEA approval 2012

**Patent disclosure**

Novo Nordisk patent 2003

**Scaffold hopping**

Off-target hERG and CYP3A4 effects
Syrrx
IC$_{50}$ 4 nM

MW 339
Clog P 0.7
HBA 5
HBD 1
nrot 3
TPSA 94 Å$^2$

alogliptin, Takeda 2005
IC$_{50}$ 7 nM

FDA/EMEA approval 2013
GLP-1 agonists and DPP-4 inhibitors as drugs

- Dual effect of promoting insulin production in β-cells and inhibiting glucagon production in α-cells.
- Not associated with weight gain, in fact GLP-1 agonists are associated with weight loss.
- Low risk of hypoglycaemia.
- GLP-1 agonists are injectable whereas DPP-4 inhibitors are oral.
- Drug overdose is more problematic with GLP-1 agonists compared to DPP-4 inhibitors.
- Potential risk of pancreatitis and pancreatic cancer, ongoing class action lawsuit in the US.
SGLT2 inhibitors- canagliflozin, dapagliflozin

A target discovered by natural products
Discovery of SGLT1 and SGLT2

Phenotypic observation

Phlorizin, a flavonoid glycoside isolated in 1835 from apple tree bark
Used in 1899 to lower glucose levels in a diabetic patient, mechanism unknown.

Mechanism of action studies (1950s) led to discovery of the SGLTs

- The kidney normally reabsorbs all of the filtered glucose (~180 g/day).
- Reabsorption by two sodium-dependent glucose co-transporters (SGLTs) located on the luminal epithelium in the kidney tubules.
  - SGLT2: low affinity, high capacity, in the early proximal tubule, reabsorbs ~90% of the filtered glucose load.
  - SGLT1: low capacity, in the more distal regions of the tubule, absorbs the remaining ~10%.
- Rate of reabsorption 20-40% higher in diabetics compared to normal individuals.

Key: SGLT = sodium glucose co-transporter

Source: Br J Diabetes Vasc Dis © 2013 Sherbourne-Gibbs, Ltd
From phlorizin to selective SGLT2 inhibitors

- Nonselective inhibitor of SGLT1 and SGLT2.
- While SGLT2 is in the kidney, SGLT1 is widely expressed and its main function is glucose and galactose absorption in the intestine.
- SGLT1 inhibition thought to lead to side-effects.
- Poor metabolic stability, rapid hydrolysis by β-glucosidases.
- Hydrolysis produces phloretin which is toxic.

Tanabe clinical candidate, 1990s.
- Carbonate prodrug to reduce β-glucosidase hydrolysis.
- Moderate (4x) selectivity over SGLT1 inhibition, discontinued in phase II.

Other first-generation clinical candidates.
- Stable in rodent models.
- Poor plasma half-life in trials, attributed to higher O-glycoside hydrolysis in humans.
Dapagliflozin and canagliflozin

- **Dapagliflozin** (Forxiga, Bristol-Myers Squibb)
  - EMEA approval 2012, FDA 2014
  - MW 409
  - Clog P 2.5
  - HBA 6
  - HBD 4
  - nrot 6
  - TPSA 99 Å²

- **Canagliflozin** (Invokana, Tanabe)
  - EMEA and FDA approval 2013
  - MW 445
  - Clog P 3.1
  - HBA 5
  - HBD 4
  - nrot 5
  - TPSA 90 Å²

- C-Glycosides, not susceptible to β-glucosidase hydrolysis.

- High selectivity for SGLT2 over SGLT1 (1000x for dapagliflozin, 250x for canagliflozin).
Synthesis of dapagliflozin

SGLT2 inhibitors as drugs

• Not associated with weight gain, likely to promote some weight loss due to reduced glucose reabsorption.

• Not associated with hypglycaemia.

• Effect independent of insulin.

• Results in glycosuria (≥ 50 g/day).

• Potential for side-effects due to glycosuria - tiredness, dehydration, urinary tract infections.
Mechanism of action of antidiabetic drugs

**Intestine**
- Decrease glucose absorption
- $\alpha$-glucosidase inhibitors

**Pancreas**
- Insulin secretagogues
  - Sulfonylureas, metiglinides, GLP-1 agonists, DPP-4 inhibitors

**Liver**
- Decrease gluconeogenesis
- Metformin, PPAR$\gamma$ agonists

**Adipose tissue, muscle**
- Increase glucose uptake
- Metformin, PPAR$\gamma$ agonists

**Kidney**
- Decrease glucose reabsorption
- SGLT2 inhibitors
Targeting obesity via modern drugs

- **Appetite suppression**
  - CNS acting
    - neurotransmitter-like, hormones
    - potential CNS side effects
  - simulation of satiety
    - methylcellulose or hydrogels to physically occupy GI tract

- **Decreased absorption**
  - orlistat (Xenical/Alli), approved in the UK
  - fiber supplements

- **Increased metabolism**
  - activation of brown adipose tissue
Targeting absorption by binge and purge cycles

• Vomit the food immediately after consumption, practised at Roman feasts.

• Perhaps effective but not very pleasant.
Pancreatic lipase inhibitors- orlistat

*High-throughput screening of natural product extracts*
The composition of fats

- Triacylglycerides = esters of fatty acids and glycerol.

- They are the major source of fats in the Western diet.
Targeting fat absorption

- Triacylglycerols are solubilized by formation of micelles with the aid of detergents such as cholesterol and bile acids.

- Pancreatic lipase acts at the water/micelle interface to hydrolyze the ester linkages to give free fatty acids that are absorbed by the intestine.
Reducing fat absorption by pancreatic lipase inhibition

- Roche embarked upon high-throughput screening of actinomycete and fungal extracts for inhibitors of pancreatic lipase.

- This led to the discovery of the natural product lipstatin from *Streptomyces toxytricini*, disclosed in 1987.
Lipstatin mechanism of action

- Lipstatin is attacked by the active site serine residue in pancreatic lipase.

- With the natural triacylglyceride substrate, the acylserine intermediate would undergo hydrolysis to release the free fatty acid.

- With lipstatin, the acylserine intermediate is stable and hydrolysis requires ~24 h, making it essentially an irreversible inhibitor.
From lipstatin to tetrahydrolipstatin

- The alkenes in lipstatin make the molecule prone to oxidation upon storage and in vivo metabolism.
- Hydrogenation saturates the molecule and removes this problem.
- Orlistat has poor bioavailability but this is actually an advantage as it reduces systemic side effects.
Semisynthetic versus synthetic orlistat

There are two routes to orlistat:

- **Semisynthetic**
  - Production of lipstatin by fermentation followed by hydrogenation.
  - Method used by generic manufacturers.

- **Synthetic**
  - Totally synthetic from simple starting materials.
  - Roche process, gives higher purity orlistat.
Orlistat synthesis: β-lactone formation

1) protect OH
2) hydrolyse ester

remove first protecting group

ester formation
Orlistat: synthesis of key 6-membered ring lactone

- Ketone reduction gives one enantiomer due to chiral catalyst.

- Intramolecular enolate reaction leads to a six-membered ring ester.
Orlistat synthesis: stereoselective hydrogenation

- Hydrogenation of six-membered ring ester takes place from the less hindered face to set two new chiral centres.
- The ester is hydrolysed and converted in a series of steps to orlistat.
Orlistat as a drug

- FDA approval in 1999 for prescribed orlistat for obesity management in conjunction with a reduced caloric diet.

- At the prescription dose of 120 mg 3x daily before meals, ~30% of dietary fat is not absorbed. Mean weight loss of 4.2 kg in clinical trials.

- Approved in 2007 by FDA and 2009 by EMEA for OTC sales.

- For patients with BMI ≥ 30 as part of a managed care program and caloric reduction or patients with BMI ≥ 28 with other risk factors.

- Endorsed by NICE to be given free to patients who have lost at least 2.5 kg by dieting and exercise in the month prior to receiving the first prescription.

- Significant GI on-target side effects including steatorrhea, wind, faecal incontinence.

- An example of aversion therapy - the side effects encourage the patient to avoid eating fats.
PDE5 inhibitors- sildenafil, vardenafil, tadalafil, avanafil

*Drug repositioning based on a side-effect*
Sildenafil (Viagra) project at Pfizer

- **Target ID and validation**
  - Therapeutic indication: Hypertension
  - Treatment: Reduce blood pressure by relaxation of smooth muscle cells in kidney and blood vessels
  - Mechanism of action: Inhibition of PDE5, leading to increased levels of cGMP.

*PDE5: phosphodiesterase enzyme that specifically hydrolyzes cGMP to GMP*
Starting point for sildenafil discovery

**Medicinal chemistry strategy**
- Design substrate mimics that can bind to PDE5 as competitive reversible inhibitors
- Use lead from competitor: Zaprinast, PDE5 IC\textsubscript{50} 2000 nM, clinical candidate for asthma.

![cGMP substrate](image1.png)

![Zaprinast](image2.png)

**Pfizer objective:**
*Alter heterocycle and substituents to:*
1) *Improve potency*
2) *Improve pharmacokinetics*
3) *Achieve novel patentable series.*
Sildenafil - a beautiful compound

Key challenge:
Improve affinity by occupying hydrophobic pockets without substantial increase in log P

Solution:
Incorporate heteroatoms in sidechain and basic amine to improve aqueous solubility (Viagra is formulated as the citrate salt)

MW 474
C logP 1.5
HBD 1
HBA 7
Rot 7
TPSA 118

intramolecular H bond maintains coplanarity

key H bond interactions with Gln817 of PDE5

alkyl sidechains sit in hydrophobic pocket

propyl sidechain sits in hydrophobic pocket

PDE5 IC<sub>50</sub> 3.6 nM
From hypertension to angina

**Target ID and validation**
- Therapeutic rationale: Reduce blood pressure by PDE5 inhibition in kidneys.
- Experimental observation: Kidney does not contain PDE5.

**Target ID and validation (2\textsuperscript{nd} attempt)**
- Therapeutic indication: Angina
- Treatment: Increase blood flow to heart muscles by relaxation of smooth muscle cells in blood vessels
- Inhibition of PDE5 should lead to increased levels of cGMP.

![Chemical structure of the clinical candidate](image)

**Nomenclature:**
- *IUPAC*: 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)phenylsulfonyl]-4-methylpiperazine
- *Pfizer*: UK-92,480
- *Generic*: sildenafil
- *Trade*: Viagra
Sildenafil clinical trials and repositioning

- **Phase I clinical trials**
  - Sildenafil does not reach desired endpoint.
  - Penile erections observed as a side effect in second Phase I clinical trial.

- **Target ID and validation (3rd attempt)**
  - Therapeutic indication: Erectile dysfunction
  - Treatment: Increase blood flow to penis by relaxation of smooth muscle cells in blood vessels
  - Mechanism of action: Inhibition of PDE5, leading to increased levels of cGMP.
  - Project now becomes a first-in-man, first-in-class study.

**Viagra timeline**:
- 1989: UK-92,480 made at Pfizer UK
- 1991: First Phase I clinical trial for angina
- 1992: Second Phase I clinical trial, side effect identified
- 1993: Pilot study for erectile dysfunction in Bristol, 3x daily administration for one week
- 1994: Studies with once a day administration
- 1998: FDA approval
Sildenafil - retrosynthesis of the discovery route
**Sildenafil- diketone synthesis**

**Mechanism:**

- An example of the Claisen reaction.
- Driven by acidity of 1,3-dicarbonyl compound: EtOH \( pK_a \approx 16 \), product \( pK_a \approx 9 \).
Sildenafil- pyrazole synthesis

Mechanism:

An example of heterocycle synthesis.

The pyrazole heterocycle is made from a 1,3-dicarbonyl and hydrazine as a source of the two nitrogens.
Sildenafil- pyrazole functionalization

\[
\begin{align*}
\text{EtO} & \quad \text{HN-} \quad \text{N} \quad \text{Me} \quad \text{N-} \quad \text{N} \quad \text{Me} \quad \text{N-} \quad \text{N} \quad \text{Me} \\
\text{HN} & \quad \text{N} \quad \text{Me} \quad \text{N} \quad \text{Me} \quad \text{N} \quad \text{Me} \quad \text{N} \quad \text{Me} \\
\text{HN} & \quad \text{N} \quad \text{Me} \quad \text{N} \quad \text{Me} \quad \text{N} \quad \text{Me} \quad \text{N} \quad \text{Me} \\
\end{align*}
\]
Sildenafil- completion of the synthesis

Yields 30-70% byproduct- hydrolysis of starting material to carboxylic acid
Sildenafil - medicinal chemistry route

• The medicinal chemistry route with some improvements was used for initial toxicity and clinical studies.
• However, it was not optimal for large scale manufacture.
• The route is linear with 9-12 steps.
• The second last step is the chlorosulfonylation and multiple recrystallization is needed to remove potentially toxic impurities.
• Quenching of the chlorosulfonylation reaction without competing hydrolysis of the sulfonyl chloride is difficult to control on large scale.
• Late stage quenching of the chlorosulfonylation increases aqueous waste streams and the environmental burden.
Sildenafil- convergent process route

• **Early stage chlorosulfonylation.**

  - Early stage chlorosulfonylation.
  - 'Telescoped' reactions using the same solvent.
  - No aqueous waste stream.
  - Single solvent aids solvent recovery.
  - Amide product crystallizes directly from the reaction mixture.
  - Byproduct imidazole is highly soluble in ethyl acetate.
  - Robust process, reactions could be refluxed for days without loss of yield.
• Late stage cyclization.

• To produce 1,000 kg by the medicinal chemistry route, a total of 125,000L of six solvents (toluene, dichloromethane, ethyl acetate, acetone, 2-butanolone and pyridine) are needed.

• To produce 1,000 kg by the commercial route, a total of 13,500L of two solvents (toluene and ethyl acetate) are needed.

## Patenting strategies for sildenafil

<table>
<thead>
<tr>
<th><strong>Composition of matter</strong></th>
<th>patents a new chemical entity (NCE) and related structures</th>
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<tbody>
<tr>
<td><strong>Indication</strong></td>
<td>patents specific use of the NCE</td>
</tr>
<tr>
<td><strong>Process</strong></td>
<td>patents manufacturing process for NCE</td>
</tr>
<tr>
<td><strong>Formulation</strong></td>
<td>patents formulation of the NCE</td>
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<tr>
<td><strong>Target</strong></td>
<td>patents therapeutic target</td>
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</table>

- Pfizer patent on use of PDE5 inhibitors for the treatment of sexual dysfunction was challenged by GlaxoSmithKline and Eli Lilly. Patent invalidated in 2000.
- India, China and Egypt have permitted sildenafil manufacture for local use prior to patent expiration.
- Pfizer patent invalidated in 2012 in Canada due to lack of 'full disclosure'.
- Pfizer patent for sildenafil citrate manufacture expired in the UK in 2013.
Sildenafil polypharmacology

**Pulmonary arterial hypertension (PAH)**
- PDE5 mainly in arterial smooth muscles of the lung and penis
- PAH - rare disease where pulmonary artery is narrowed
- PDE5 inhibitors relax smooth muscle and increase blood flow
- Sildenafil approved by FDA for treatment of PAH in 2005.
- Marketed by Pfizer as Revatio.

Conversion of white fat to brown fat in obesity management

Performance enhancement in athletes

Delay of plant ripening
Fast followers of Viagra

Potential shortcomings of sildenafil
PDE5 IC$_{50}$ of 4 nM - can this be improved?

PDE6 IC$_{50}$ of 38 nM - off-target effect of ‘blue spots’ in the vision

Oral bioavailability is 41%, needs time to achieve effect, slow onset.

Halflife ~4 hours.

Urea prodrug broken down in vivo-outside Pfizer patents

Switch between one N and one C-outside Pfizer patents

Change in sulfonamide-outside Pfizer patents

Iodenafil (Helleva), Cristalia dimeric prodrug

Urea prodrug broken down in vivo-outside Pfizer patents

Change in sulfonamide-outside Pfizer patents

Lodenafil (Helleva), Bayer
PDE5 IC$_{50}$ 0.7 nM
15% oral bioavail.
$\tau_{1/2} \sim 4$ h

Vardenafil (Levitra), Bayer
PDE5 IC$_{50}$ 0.7 nM
15% oral bioavail.
$\tau_{1/2} \sim 4$ h

Udenafil (Zydena), Dong-A
PDE5 IC$_{50}$ 5 nM

Change in sulfonamide-outside Pfizer patents

Mirodenafil (Mvix), SK available as orally dissolving film
Development of tadalafil

- Discovered by Glaxo France and Icos.
- Icos partnered with Eli Lilly in 1998.
- Cialis receives FDA approval in 2003.
Tadalafil- key Interactions and retrosynthesis

interactions with hydrophobic pocket

key H bond interaction with Gln817 of PDE5

D-tryptophan

N-methylglycine

piperonal
Tadalafil medicinal chemistry route

- An example of the Pictet-Spengler reaction (intramolecular cyclization of iminium ion onto an aromatic ring).
- The reaction produces a mixture of two diastereomers.
  - 31% cis isomer + 31% trans isomer
  - cis is desired whereas trans is thermodynamic product
  - diastereomers separated by chromatography

- The reaction produces a mixture of two diastereomers.
Tadalafil medicinal chemistry route

- Three steps overall but wasteful in the Pictet-Spengler reaction as two diastereomers are produced.
Tadalafil process route

- The two diastereomers are in dynamic equilibrium.
- The desired product precipitates out of the reaction mixture, driving the equilibrium mixture towards formation of the cis diastereomer.
Avanafil: lead optimisation

**Isoquinolinone**

- PDE5 IC$_{50}$ 1 nM, PDE6 32 nM
- good potency, poor selectivity

**Isoquinoline**

- PDE5 IC$_{50}$ 34 nM, PDE6 >10,000 nM
- poor potency, high selectivity

**Scaffold hopping**

- replace isoquinoline by monocycles

- PDE5 IC$_{50}$ 3.5 nM
- PDE6 >10,000 nM
- good potency and selectivity
- unlikely to be oral
- clogP 7.4, MW 629
Avanafil: lead optimisation

PDE5/6 selectivity >3000
\( \text{clog P} \ 7.4 \)

\[
\text{HN-Cl} \quad \text{N-O} \quad \text{Cl}
\]
\[
\text{MeO} \quad \text{OMe} \quad \text{OMe}
\]
\[
\text{MeO} \quad \text{OMe} \quad \text{OMe}
\]

\[
\text{PDE5/6 selectivity 18000} \\
\text{clog P 5.3}
\]

- intramolecular H bond mimics isoquinoline ring

\[
\text{IC}_{50} \ 0.13 \text{ nM} \\
PDE5/6 selectivity 2400 \\
clog P 4.6
\]

\[
\text{EC}_{30} \ 11 \text{ nM} \\
PDE5/6 selectivity 2800 \\
clog P 3.2
\]
Synthesis of avanafil

Avanafil
IC$_{50}$ 5 nM
EC$_{30}$ 2 nM
PDE5/6 selectivity 4000
time to peak response 10 min
(30 min for sildenafil)
FDA approval 2012
EMEA approval 2013
