Topic 4 Proteins as Drug Targets

Receptors-Chapters 5 and 6
Patrick and Corey 78-80
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8. Non competitive (reversible) allosteric antagonists

9. Antagonists by umbrella effect

10. Agonists
1. **Structure and function of receptors**

- Globular proteins acting as a cell’s ‘letter boxes’
- Located mostly in the cell membrane
- Receive messages from chemical messengers coming from other cells
- Transmit a message into the cell leading to a cellular effect
- Different receptors specific for different chemical messengers
- Each cell has a range of receptors in the cell membrane making it responsive to different chemical messengers
1. Structure and function of receptors
1. Structure and function of receptors

Chemical Messengers

**Neurotransmitters**: Chemicals released from nerve endings which travel across a nerve synapse to bind with receptors on target cells, such as muscle cells or another nerve. Usually short lived and responsible for messages between individual cells.

**Hormones**: Chemicals released from cells or glands and which travel some distance to bind with receptors on target cells throughout the body.

- Chemical messengers ‘switch on’ receptors without undergoing a reaction.
1. **Structure and function of receptors**
1. **Structure and function of receptors**

**Mechanism**

- Receptors contain a binding site (hollow or cleft in the receptor surface) that is recognised by the chemical messenger.
- Binding of the messenger involves intermolecular bonds.
- Binding results in an induced fit of the receptor protein.
- Change in receptor shape results in a ‘domino’ effect.
- Domino effect is known as Signal Transduction, leading to a chemical signal being received inside the cell.
- Chemical messenger does not enter the cell. It departs the receptor unchanged and is not permanently bound.
1. Structure and function of receptors

Mechanism
2. The binding site

- A hydrophobic hollow or cleft on the receptor surface - equivalent to the active site of an enzyme
- Accepts and binds a chemical messenger
- Contains amino acids which bind the messenger
- No reaction or catalysis takes place
3. Messenger binding

3.1 Introduction

- Binding site is nearly the correct shape for the messenger
- Binding alters the shape of the receptor (induced fit)
- Altered receptor shape leads to further effects - signal transduction
3. **Messenger binding**

3.2 **Bonding forces**

- Ionic
- H-bonding
- van der Waals

**Example:**

![Diagram showing receptor binding site with bonding interactions]
3. Substrate binding

3.2 Bonding forces

- Induced fit - Binding site alters shape to maximise intermolecular bonding
4. Overall process of receptor/messenger interaction

- Binding interactions must be:
  - strong enough to hold the messenger sufficiently long for signal transduction to take place
  - weak enough to allow the messenger to depart
- Implies a fine balance
- Drug design - designing molecules with stronger binding interactions results in drugs that block the binding site - antagonists
5. **Signal transduction**

5.1 **Control of ion channels**

- Receptor protein is part of an ion channel protein complex
- Receptor binds a messenger leading to an induced fit
- Ion channel is opened or closed
- Ion channels are specific for specific ions (\(\text{Na}^+\), \(\text{Ca}^{2+}\), \(\text{Cl}^-\), \(\text{K}^+\))
- Ions flow across cell membrane down concentration gradient
- Polarises or depolarises nerve membranes
- Activates or deactivates enzyme catalysed reactions within cell
5. Signal transduction

5.1 Control of ion channels
5. Signal transduction

5.1 Control of ion channels

Cationic ion channels for $K^+$, $Na^+$, $Ca^{2+}$ (e.g. nicotinic) = excitatory
Anionic ion channels for $Cl^-$ (e.g. $GABA_A$) = inhibitory
5. Signal transduction

5.1 Control of ion channels:

Induced fit and opening of ion channel
5. Signal transduction

5.2 Activation of signal proteins

- Receptor binds a messenger leading to an induced fit
- Opens a binding site for a signal protein (G-protein)
- G-Protein binds, is destabilised then split
5. Signal transduction

5.2 Activation of signal proteins

- G-Protein subunit activates membrane bound enzyme
  - Binds to allosteric binding site
  - Induced fit results in opening of active site
- Intracellular reaction catalysed
5. Signal transduction

5.3 Activation of enzyme active site

- Protein serves dual role - receptor plus enzyme
- Receptor binds messenger leading to an induced fit
- Protein changes shape and opens active site
- Reaction catalysed within cell
6. Competitive (reversible) antagonists

- Antagonist binds reversibly to the binding site
- Intermolecular bonds involved in binding
- Different induced fit means receptor is not activated
- No reaction takes place on antagonist
- Level of antagonism depends on strength of antagonist binding and concentration
- Messenger is blocked from the binding site
- Increasing the messenger concentration reverses antagonism
7. Non competitive (irreversible) antagonists

- Antagonist binds irreversibly to the binding site
- Different induced fit means that the receptor is not activated
- Covalent bond is formed between the drug and the receptor
- Messenger is blocked from the binding site
- Increasing messenger concentration does not reverse antagonism
8. Non competitive (reversible) allosteric antagonists

- Antagonist binds reversibly to an allosteric site
- Intermolecular bonds formed between antagonist and binding site
- Induced fit alters the shape of the receptor
- Binding site is distorted and is not recognised by the messenger
- Increasing messenger concentration does not reverse antagonism
9. Antagonists by umbrella effect

- Antagonist binds reversibly to a neighbouring binding site
- Intermolecular bonds formed between antagonist and binding site
- Antagonist overlaps with the messenger binding site
- Messenger is blocked from the binding site
10. Agonists

- Agonist binds reversibly to the binding site
- Similar intermolecular bonds formed as to natural messenger
- Induced fit alters the shape of the receptor in the same way as the normal messenger
- Receptor is activated
- Agonists are often similar in structure to the natural messenger
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   2.3. Detailed structure of ion channel
   2.4. Gating
1. Receptor superfamilies

- ION CHANNEL RECEPTORS
- G-PROTEIN COUPLED RECEPTORS
- KINASE LINKED RECEPTORS
- INTRACELLULAR RECEPTORS

RESPONSE
TIME
msecs

MEMBRANE
BOUND
seconds

minutes
2. Ion channel receptors (Ligand gated ion channels)

2.1 General structure

Five glycoprotein subunits traversing cell membrane

Receptor Binding site Messenger

Cell membrane

INDUCED FIT ‘GATING’ (ion channel opens)

Cationic ion channels for $K^+$, $Na^+$, $Ca^{2+}$ (e.g. nicotinic) = excitatory
Anionic ion channels for $Cl^-$ (e.g. $GABA_A$) = inhibitory
2. Ion channel receptors (Ligand gated ion channels)

Transverse view (nicotinic receptor)

Ion channel

Binding sites

Cell membrane

2xα, β, γ, δ subunits

Two ligand binding sites mainly on α-subunits
2. Ion channel receptors (Ligand gated ion channels)

Transverse view (glycine receptor)

- Binding sites
- Cell membrane
- 3xα, 2xβ subunits
- Three ligand binding sites on α-subunits

Ion channel
2. Ion channel receptors (Ligand gated ion channels)

2.2 Structure of protein subunits (4-TM receptor subunits)

4 Transmembrane (TM) regions (hydrophobic)
2. Ion channel receptors (Ligand gated ion channels)

2.3 Detailed structure of ion channel

Note: TM2 of each protein subunit ‘lines’ the central pore
2. Ion channel receptors (Ligand gated ion channels)

2.4 Gating

- Neurotransmitter binds
- Induced fit at binding site
- ‘Domino effect’
- Rotation of 2TM regions of each protein subunit

Diagram:

- Cell membrane
- Transverse view of TM2 subunits
- Closed
- Open
- Ion flow

- TM2
- Transverse view of TM2 subunits
2. **Ion channel receptors (Ligand gated ion channels)**

2.4 **Gating**

- Fast response measured in msec
- Ideal for transmission between nerves
- Binding of messenger leads directly to ion flows across cell membrane
- Ion flow = secondary effect (signal transduction)
- Ion concentration within cell alters
- Leads to variation in cell chemistry
3. G-protein-coupled receptors (7-TM receptors)
   3.1. Structure - Single protein with 7 transmembrane regions
   3.2. Ligands
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3. G-protein-coupled receptors (7-TM receptors)

3.1 Structure - Single protein with 7 transmembrane regions
3. G-protein-coupled receptors (7-TM receptors)

3.2 Ligands

- **Monoamines**  e.g. dopamine, histamine, noradrenaline, acetylcholine (muscarinic)
- **Nucleotides**
- **Lipids**
- **Hormones**
- **Glutamate**
- **Ca**++
3. **G-protein-coupled receptors (7-TM receptors)**

3.3 **Ligand binding site - varies depending on receptor type**

A) **Monoamines** - pocket in TM helices

B) **Peptide hormones** - top of TM helices + extracellular loops
   + *N*-terminal chain

C) **Hormones** - extracellular loops + *N*-terminal chain

D) **Glutamate** - *N*-terminal chain
3. **G-protein-coupled receptors (7-TM receptors)**

3.4 **Bacteriorhodopsin & rhodopsin family**

- Rhodopsin = visual receptor
- Many common receptors belong to this same family
- Implications for drug selectivity depending on similarity (evolution)
- Membrane bound receptors difficult to crystallise
- X-Ray structure of bacteriorhodopsin solved - bacterial protein similar to rhodopsin
- Bacteriorhodopsin structure used as ‘template’ for other receptors
- Construct model receptors based on template and amino acid sequence
- Leads to model binding sites for drug design
- Crystal structure for rhodopsin now solved - better template
3. G-protein-coupled receptors (7-TM receptors)

3.4 Bacteriorhodopsin & rhodopsin family
3. G-protein-coupled receptors (7-TM receptors)

3.5 Receptor types and subtypes

Reflects differences in receptors which recognise the same ligand

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Types</th>
<th>Subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenergic</td>
<td>Alpha (α)</td>
<td>α₁, α₂A, α₂B, α₂C</td>
</tr>
<tr>
<td></td>
<td>Beta (β)</td>
<td>β₁, β₂, β₃</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Nicotinic</td>
<td>M₁–M₅</td>
</tr>
<tr>
<td></td>
<td>Muscarinic</td>
<td></td>
</tr>
</tbody>
</table>
3. G-protein-coupled receptors (7-TM receptors)

3.5 Receptor types and subtypes

- Receptor types and subtypes not equally distributed amongst tissues.
- Target selectivity leads to tissue selectivity

Heart muscle - $\beta_1$ adrenergic receptors
Fat cells - $\beta_3$ adrenergic receptors
Bronchial muscle - $\alpha_1$ & $\beta_2$ adrenergic receptors
GI-tract - $\alpha_1$ $\alpha_2$ & $\beta_2$ adrenergic receptors
3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

a) Interaction of receptor with $G_s$-protein

$G_s$-Protein - membrane bound protein of 3 subunits ($\alpha$, $\beta$, $\gamma$)
- $\alpha_s$ subunit has binding site for GDP
- GDP bound non covalently
3. **G-protein-coupled receptors (7-TM receptors)**

3.6 **Signal transduction pathway**

a) Interaction of receptor with $G_s$-protein

![Diagram showing the interaction of a receptor with a G-protein](image)

- **Ligand** binds to the receptor, inducing a fit.
- The binding site for the G-protein opens.
- The G-protein binds, with GDP binding site distorted and GDP binding weakened.
- GDP departs, allowing GTP to bind.

$\bullet = GDP$
3. **G-protein-coupled receptors (7-TM receptors)**

3.6 **Signal transduction pathway**

a) **Interaction of receptor with Gₚ-protein**

- Process repeated for as long as ligand bound to receptor
- Signal amplification - several G-proteins activated by one ligand
- $\alpha_s$ Subunit carries message to next stage
3. **G-protein-coupled receptors (7-TM receptors)**

3.6 **Signal transduction pathway**

b) Interaction of $\alpha_s$ with adenylate cyclase

- Binding site for $\alpha_s$ subunit
- Active site (closed)
- ATP → cyclic AMP
- Signal transduction (con)
- $\alpha_s$ Subunit changes shape
  - Weaker binding to enzyme
  - Departure of subunit
  - Enzyme reverts to inactive state
- $\alpha_s$-subunit
- Adenylate cyclase

- GTP hydrolysed to GDP catalysed by $\alpha_s$ subunit
- Active site (open)
- $\alpha_s$ Subunit recombines with $\beta,\gamma$ dimer to reform $G_s$ protein
3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

b) Interaction of $\alpha_s$ with adenylate cyclase

- Several-100 ATP molecules converted before $\alpha_s$-GTP deactivated
- Represents another signal amplification
- Cyclic AMP becomes next messenger (secondary messenger)
- Cyclic AMP enters cell cytoplasm with message
c) Interaction of cyclic AMP with protein kinase A (PKA)

- Protein kinase A = serine-threonine kinase
- Activated by cyclic AMP
- Catalyses phosphorylation of serine and threonine residues on protein substrates
- Phosphate unit provided by ATP

3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway
3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

c) Interaction of cyclic AMP with protein kinase A (PKA)
c) Interaction of cyclic AMP with protein kinase A (PKA)

Protein kinase A - 4 protein subunits
- 2 regulatory subunits (R) and 2 catalytic subunits (C)

Cyclic AMP binds to PKA
Induced fit destabilises complex
Catalytic units released and activated

Note
Cyclic AMP binds to PKA
Induced fit destabilises complex
Catalytic units released and activated
3. G-protein-coupled receptors (7-TM receptors)

3.6 Signal transduction pathway

c) Interaction of cyclic AMP with protein kinase A (PKA)

Phosphorylation of other proteins and enzymes
Signal continued by phosphorylated proteins
Further signal amplification
3. G-protein-coupled receptors (7-TM receptors)

3.7 Glycogen metabolism - triggered by adrenaline in liver cells

![Diagram showing the process of glycogen metabolism triggered by adrenaline.](image)
3. G-protein-coupled receptors (7-TM receptors)

3.7 Glycogen metabolism - triggered by adrenaline in liver cells

Coordinated effect
- activation of glycogen metabolism
- inhibition of glycogen synthesis

Adrenaline has different effects on different cells
- activates fat metabolism in fat cells
3. G-protein-coupled receptors (7-TM receptors)

3.8 G₁ proteins

- Binds to different receptors from those used by Gₛ protein
- Mechanism of activation by splitting is identical
- $\alpha_i$ subunit binds adenylate cyclase to inhibit it
- Adenylate cyclase under dual control (brake/accelerator)
- Background activity due to constant levels of $\alpha_s$ and $\alpha_i$
- Overall effect depends on dominant G-Protein
- Dominant G-protein depends on receptors activated
3. G-protein-coupled receptors (7-TM receptors)

3.9 Phosphorylation

- Prevalent in activation and deactivation of enzymes
- Phosphorylation radically alters intramolecular binding
- Results in altered conformations
3. G-protein-coupled receptors (7-TM receptors)

3.10 Drugs interacting with cyclic AMP signal transduction

Cholera toxin - constant activation of cAMP - diarrhea

Theophylline and caffeine
- inhibit phosphodiesterases
- phosphodiesterases responsible for metabolising cyclic AMP
- cyclic AMP activity prolonged

![Chemical structures](Theophylline.png)  ![Chemical structures](Caffeine.png)

Theophylline  Caffeine
3. G-protein-coupled receptors (7-TM receptors)

3.11 Signal transduction involving phospholipase C (PLC)

- $G_q$ proteins - interact with different receptors from $G_S$ and $G_I$
- Split by same mechanism to give $\alpha_q$ subunit
- $\alpha_q$ Subunit activates or deactivates PLC (membrane bound enzyme)
- Reaction catalysed for as long as $\alpha_q$ bound - signal amplification
- Brake and accelerator

\[ \text{Binding weakened} \]

\[ \text{GTP hydrolysis} \]

\[ \text{Phosphate} \]

\[ \text{Active site (closed)} \]

\[ \text{Active site (open)} \]

\[ \text{Active site (closed)} \]

\[ \text{enzyme deactivated} \]
3. G-protein-coupled receptors (7-TM receptors)

3.11 Signal transduction involving phospholipase C (PLC)

Phosphatidylinositol diphosphate (integral part of cell membrane)

Inositol triphosphate (polar and moves into cell cytoplasm)

Diacylglycerol (remains in membrane)

\[ R = \text{long chain hydrocarbons} \]

\[ \text{PO}_3^{2-} = \text{PO}_3^{2-} \]
3. G-protein-coupled receptors (7-TM receptors)

3.12 Action of diacylglycerol

- Activates protein kinase C (PKC)
- PKC moves from cytoplasm to membrane
- Phosphorylates enzymes at Ser & Thr residues
- Activates enzymes to catalyse intracellular reactions
- Linked to inflammation, tumour propagation, smooth muscle activity etc

![Diagram showing the action of diacylglycerol (DG) on protein kinase C (PKC)]
3. **G-protein-coupled receptors (7-TM receptors)**

3.12 *Action of diacylglycerol*

Drugs inhibiting PKC - potential anti cancer agents

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**Bryostatin** (from sea moss)
3. G-protein-coupled receptors (7-TM receptors)

3.13 Action of inositol triphosphate

- $\text{IP}_3$ - hydrophilic and enters cell cytoplasm
- Mobilises $\text{Ca}^{2+}$ release in cells by opening $\text{Ca}^{2+}$ ion channels
- $\text{Ca}^{2+}$ activates protein kinases
- Protein kinases activate intracellular enzymes
- Cell chemistry altered leading to biological effect
3. **G-protein-coupled receptors (7-TM receptors)**

3.13 **Action of inositol triphosphate**

![Diagram of calcium signaling](image)

- **Calcium stores**
- **IP$_3$**
- **Calmodulin**
- **Cell membrane**
- **Cytoplasm**
- **Enzyme (inactive)**
- **Enzyme (active)**
- **Protein kinase**
- **Chemical reaction**
- **Activation**
- **P**
3. G-protein-coupled receptors (7-TM receptors)

3.14 Resynthesis of PIP$_2$

$IP_3 + DG \xrightarrow{\text{several steps}} PIP_2$

Inhibition

Li$^+$ salts

Lithium salts used vs manic depression
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Part 3: Section 6.7

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   4.6. Signalling pathways
4. Tyrosine kinase linked receptors

- Bi-functional receptor / enzyme
- Activated by hormones
- Over-expression can result in cancer
4. Tyrosine kinase linked receptors

4.1 Structure
4. Tyrosine kinase linked receptors

4.2 Reaction catalysed by tyrosine kinase

\[
\text{Protein} \quad \text{Tyrosine residue} \quad \text{Protein} \quad \text{Tyrosine kinase} \quad \text{Mg}^{++} \quad \text{ATP} \quad \text{ADP} \quad \text{Protein} \quad \text{Phosphorylated tyrosine residue} \quad \text{Protein}
\]
4. Tyrosine kinase linked receptors

4.3 Epidermal growth factor receptor (EGF-R)

Binding site for EGF

EGF - protein hormone - bivalent ligand

Active site of tyrosine kinase
4. Tyrosine kinase linked receptors

4.3 Epidermal growth factor receptor (EGF-R)

- Active site on one half of dimer catalyses phosphorylation of Tyr residues on other half
- Dimerisation of receptor is crucial
- Phosphorylated regions act as binding sites for further proteins and enzymes
- Results in activation of signalling proteins and enzymes
- Message carried into cell
4. Tyrosine kinase linked receptors

4.4 Insulin receptor (tetrameric complex)

- Insulin binding site
- Kinase active site

Insulin binding site opened by induced fit

Phosphorylation

Kinase active site

ATP to ADP

PO to OP

OH to HO
4. **Tyrosine kinase linked receptors**

4.5 **Growth hormone receptor**

Tetrameric complex constructed in presence of growth hormone

- Growth hormone binding site
- Kinase active site (Janus, JAK kinase)

http://en.wikipedia.org/wiki/Cytokine_receptor
http://www.ebi.ac.uk/interpro/potm/2004_4/Page2.htm
Eltrombopag, PROMACTA
Binds to DIFFERENT site than thrombopoietin with Zn\(^{2+}\).

http://www.ligand.com/collaborations.php#Leading
Tales from the drug development trenches-Tucson

http://en.wikipedia.org/wiki/Cytokine_receptor

TPO and EPO receptors (cytokine type, also growth hormone) connected to Janus kinase (JAK) family of tyrosine kinases
4. Tyrosine kinase linked receptors

4.6 Signalling pathways

Ligand

P P P

P P P

P P P

Ligand

→

signalling protein
4. Tyrosine kinase linked receptors

4.6 Signalling pathways

1-TM Receptors

Tyrosine kinase inherent or associated

Signalling proteins

PLCγ
IP₃ kinase
GAP
Grb2
Others

IP₃
DG
PIP₃
Ca²⁺
PKC

Guanylate cyclase
cGMP
4. Tyrosine kinase linked receptors

4.6 Signalling pathways

[Diagram showing a growth factor receptor and its tyrosine kinase active site (inactive)]
4. Tyrosine kinase linked receptors

4.6 Signalling pathways

1) Binding of growth factor
2) Conformational change

Growth factor

Binding and phosphorylation of Grb2

Binding Ras and GTP/GDP exchange
4. Tyrosine kinase linked receptors

4.6 Signalling pathways

Raf (inactive) → Raf (active)

Mek (inactive) → Mek (active)

Map kinase (inactive) → Map kinase (active)

Transcription factor (inactive) → Transcription factor (active)

Gene transcription
Part 4: Section 6.8

5. Intracellular receptors
   5.1. Structure
   5.2. Mechanism
   5.3. Oestrogen receptor
5. **Intracellular receptors**

- Chemical messengers must cross cell membrane
- Chemical messengers must be hydrophobic
- Example - steroids and steroid receptors
5. Intracellular receptors

5.1 Structure

Zinc fingers contain Cys residues (SH)
Allow S-Zn interactions
5. Intracellular receptors

5.2 Mechanism

1. Messenger crosses membrane
2. Binds to receptor
3. Receptor dimerisation
4. Binds co-activator protein
5. Complex binds to DNA
6. Transcription switched on or off
7. Protein synthesis activated or inhibited
5. Intracellular receptors

5.3 Oestrogen receptor
5. Intracellular receptors

5.3 Oestrogen receptor

- Phenol and alcohol of oestradiol are important binding groups
- Binding site is spacious and hydrophobic
- Phenol group of oestradiol positioned in narrow slot
- Orientates rest of molecule
- Acts as agonist
5. Intracellular receptors

5.3 Oestrogen receptor

- Raloxifene is an antagonist (anticancer agent)
- Phenol groups mimic phenol and alcohol of oestradiol
- Interaction with Asp351 is important for antagonist activity
- Side chain prevents receptor helix H12 folding over as lid
- AF-2 binding region not revealed
- Co-activator cannot bind
5. Intracellular receptors

5.3 Oestrogen receptor

Tamoxifen (Nolvadex)
- anticancer agent which targets oestrogen receptor
Case Study-LATER
6. Case Study - Inhibitors of EGF Receptor Kinase
   6.1. The target
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6.1 The target
- Epidermal growth factor receptor
- Dual receptor / kinase enzyme role

![Diagram of EGF Receptor](image)

- Extracellular space
- Cell membrane
- Cell
- Receptor
- Binding site
- Kinase active site (closed)
6.1 The target

Overexpression of erbB1 gene

Excess receptor

Excess sensitivity to EGF

Excess signal from receptor

Excess cell growth and division

Tumours

KINASE INHIBITOR

Potential anticancer agent
6.1 The target

ATP

Tyrosine kinase

ADP

Phosphorylated tyrosine residue
6.1 The target

**Inhibitor Design**

Possible versus binding site for tyrosine region
Possible versus binding site for ATP

**Inhibitors of the ATP binding site**

**Aims:**
To design a potent but selective inhibitor versus EGF receptor kinase and not other protein kinases.
6.2 Testing procedures

*In vitro tests*

Enzyme assay

using kinase portion of the EGF receptor produced by recombinant DNA technology. Allows enzyme studies in solution.
6.2 Testing procedures

*In vitro tests*

Enzyme assay
Test inhibitors by ability to inhibit standard enzyme catalysed reaction

- Tests inhibitory activity only and not ability to cross cell membrane
- Most potent inhibitor may be inactive *in vivo*
6.2 Testing procedures

*In vitro tests*

**Cell assays**

- Use cancerous human epithelial cells which are sensitive to EGF for growth
- Measure inhibition by measuring effect on cell growth - blocking kinase activity blocks cell growth.
- Tests inhibitors for their ability to inhibit kinase and to cross cell membrane
- Assumes that enzyme inhibition is responsible for inhibition of cell growth

**Checks**

- Assay for tyrosine phosphorylation in cells - should fall with inhibition
- Assay for m-RNA produced by signal transduction - should fall with inhibition
- Assay fast growing mice cells which divide rapidly in presence of EGF
6.2 Testing procedures

*In vivo tests*

- Use cancerous human epithelial cells grafted onto mice
- Inject inhibitor into mice
- Inhibition should inhibit tumour growth
- Tests for inhibitory activity + favourable pharmacokinetics
Selectivity tests

Similar *in vitro* and *in vivo* tests carried out on serine-threonine kinases and other tyrosine kinases.
6.3 Lead compound - Staurosporine

- Microbial metabolite
- Highly potent kinase inhibitor but no selectivity
- Competes with ATP for ATP binding site
- Complex molecule with several rings and asymmetric centres
- Difficult to synthesise
6.4 Simplification of lead compound

Staurosporine

Simplification
Remove asymmetric ring

Symmetry

Arcyriaflavin A
- Symmetrical molecule
- Active and selective vs PKC but not EGF-R
6.4 Simplification of lead compound

Dianilinophthalimide (CGP 52411)
- Selective inhibitor for EGF receptor and not other kinases
- Reversal of selectivity

Bisindolylmaleimides
PKC selective

maleimide ring

indole ring

indole ring

Simplification

Simplification

Phthalimide

Aniline

Aniline
6.5 X-Ray crystallographic studies

Different shapes implicated in different selectivity

Arcyriaflavin

Bisindolyl-maleimides

Dianilino-phthalamides

Planar

Bowl shaped

Propellor shaped asymmetric
6.5 X-Ray crystallographic studies

Propeller conformation relieves steric clashes
6.6 Synthesis of analogues

\[
\begin{align*}
\text{Acetic acid, 120 }^\circ\text{C} & \rightarrow \text{Anilines, Acetic acid, 120 }^\circ\text{C} \\
\text{Diels Alder} & \rightarrow \\
\text{Toluene} & \rightarrow \\
\text{MeO}_2\text{C} & \rightarrow \\
\text{NH}_3 \text{ or formamides} & \rightarrow 140-150 \, ^\circ\text{C}
\end{align*}
\]
6.7 Structure Activity Relationships (SAR)

- **R=H**    Activity lost if N is substituted
- Aniline aromatic rings essential (activity lost if cyclohexane)
- **R\(^1\)=H or F** (small groups). Activity drops for Me and lost for Et
- **R\(^2\)=H** Activity drops if N substituted
- Aniline N’s essential. Activity lost if replaced with S
- Both carbonyl groups important. Activity drops for lactam
6.7 Structure Activity Relationships (SAR)

Parent Structure: $R = R^1 = R^2 = H$ chosen for preclinical trials
$IC_{50} = 0.7 \, \mu M$

![Chemical Structure](image)
6.8 Drug metabolism

**Excretion**

Metabolism (man, mouse, rat, dog)

Glucuronylation

Metabolism (monkey)

Glucuronylation

Excretion
6.8 Drug metabolism

Introduce F at para position as metabolic blocker

![Chemical structure of CGP 53353](image)
6.9 Further modifications

a) Chain extension

Activity drops
6.9 Further modifications

b) Ring extension / expansion

CGP 52411 (IC$_{50}$ 0.7µM)

CGP54690 (IC$_{50}$ 0.12µM)
Inactive in cellular assays due to polarity (unable to cross cell membrane)

CGP57198 (IC$_{50}$ 0.18µM)
Active in vitro and in vivo
6.9 Further modifications

c) Simplification

CGP52411  
Similar activity in enzyme assay  
Inactive in cellular assay  

CGP58522
6.10 Modelling studies on ATP binding

- No crystal structure for EGF-receptor available
- Make a model active site based on structure of an analogous protein which has been crystallised
- Cyclic AMP dependant protein kinase used as template
6.10 Modelling studies on ATP binding

Cyclic AMP dependant protein kinase + Mg + ATP + Inhibitor (bound at substrate site)

\[ \text{Crystallise} \]

Crystals

\[ \text{X-Ray Crystallography} \]

Structure of protein / inhibitor / ATP complex

\[ \text{Molecular modelling} \]

Identify active site and binding interactions for ATP
6.10 Modelling studies on ATP binding

- ATP bound into a cleft in the enzyme with adenine portion buried deep close to hydrophobic region.

- Ribose and phosphate extend outwards towards opening of cleft

- Identify binding interactions (measure distances between atoms of ATP and complementary atoms in binding site to see if they are correct distance for binding)

- Construct model ATP binding site for EGF-receptor kinase by replacing amino acid’s of cyclic AMP dependent protein kinase for those present in EGF receptor kinase
6.10 Modelling studies on ATP binding

1N is a H bond acceptor
6-NH$_2$ is a H-bond donor
Ribose forms H-bonds to Glu in ribose pocket
6.11 Model binding studies on Dianilinophthalimides
6.11 Model binding studies on Dianilinophthalimides

- Both imide carbonyls act as H-bond acceptors (disrupted if carbonyl reduced)
- Imide NH acts as H bond donor (disrupted if N is substituted)
- Aniline aromatic ring fits small tight ribose pocket
- Substitution on aromatic ring or chain extension prevents aromatic ring fitting pocket
- Bisindolylmaleimides form H-bond interactions but cannot fit aromatic ring into ribose pocket.
- Implies ribose pocket interaction is crucial for selectivity
6.11 Model binding studies on Dianilinophthalimides
6.11 Model binding studies on Dianilinophthalimides
6.12 Selectivity of action

POSERS?

• Ribose pocket normally accepts a polar ribose so why can it accept an aromatic ring?

• Why can’t other kinases bind dianilinophthalimides in the same manner?
6.12 Selectivity of action

Amino Acids present in the ribose pocket

Hydrophobic  Hydrophilic

Protein Kinase A  Leu,Gly,Val,Leu  Glu,Glu,Asn,Thr

EGF Receptor Kinase  Leu,Gly,Val,Leu,Cys  Arg,Asn,Thr
6.12 Selectivity of action

- Ribose pocket is more hydrophobic in EGF-receptor kinase
- Cys can stabilise and bind to aromatic rings (S-Ar interaction)

- Stabilisation by S-Ar interaction not present in other kinases
- Leads to selectivity of action
6.13 Pharmacophore for EGF-receptor kinase inhibitors

- Pharmacophore allows identification of other potential inhibitors
- Search databases for structures containing same pharmacophore
- Can rationalise activity of different structural classes of inhibitor
6.14 Phenylaminopyrrolopyrimidines

CGP 59326 - Two possible binding modes for H-bonding

Only mode II tallies with pharmacophore and explains activity and selectivity
6.14 Phenylaminopyrrolopyrimidines

Illustrates dangers in comparing structures and assuming similar interactions (e.g. comparing CGP59326 with ATP)

Binding Mode I  like ATP  
(not favoured)

Binding mode II  (favoured)

Illustrates dangers in comparing structures and assuming similar interactions (e.g. comparing CGP59326 with ATP)
6.14 Phenylaminopyrrolopyrimidines
6.15 Pyrazolopyrimidines

i) Lead compounds

- Both structures are selective EGF-receptor kinase inhibitors
- Both structures belong to same class of compounds
- Docking experiments reveal different binding modes to obey pharmacophore

(I) EC50 0.80\(\mu\)M

(II) EC50 0.22\(\mu\)M
6.15 Pyrazolopyrimidines

ii) Structure I

Extra binding interactions
6.15 Pyrazolopyrimidines

ii) Structure I

\[
\begin{align*}
\text{(I) } & \quad \text{EC}_{50} \ 0.80\mu\text{M} \\
\text{(III) } & \quad \text{EC}_{50} \ 2.7\mu\text{M}
\end{align*}
\]
6.15 Pyrazolopyrimidines

iii) Structure II

- Cannot bind in same mode since no fit to ribose pocket
- Binds in similar mode to phenylaminopyrrolopyrimidines
6.15 Pyrazolopyrimidines

iv) Drug design on structure II

- Upper binding pocket is larger than ribose pocket allowing greater variation of substituents on the ‘upper’ aromatic ring

<table>
<thead>
<tr>
<th>Structure</th>
<th>EC$_{50}$ Value</th>
<th>Activity Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>(II)</td>
<td>0.22 µM</td>
<td></td>
</tr>
<tr>
<td>(IV)</td>
<td>0.16 µM</td>
<td>Activity increases</td>
</tr>
<tr>
<td>(V)</td>
<td>0.033 µM</td>
<td>Activity increases, Ar fits ribose pocket</td>
</tr>
<tr>
<td>(VI)</td>
<td>0.001 µM</td>
<td>Activity increases</td>
</tr>
</tbody>
</table>

- Extra H-bonding interaction