Lecture 4: Drug targets (receptors, enzymes, antigens, DNA, tubulin)

Key objectives:
1. Be able to describe several receptor type drug targets
2. Be able to describe several enzyme type drug targets
3. Understand antigen type drug targets
4. Understand the nature of DNA type drug targets
5. Understand replication targets such as tubulin

In the discipline of oncology, anything can be viewed as a target to kill or inhibit cancer cells. Both FDA and drug companies will entertain the idea that anything could be targeted. However, solid scientific evidence is needed. The supposed joke at FDA is: *"In God We Trust, but everyone else bring data!"*

Receptors: Most commonly, receptors are proteins on the surface of cells that cause a signal following the binding of a specific ligand. Binding of the ligand can increase in the transport of specific ions (e.g. sodium, chloride, calcium, potassium) or the amplification of a signaling cascade (e.g. kinase cascade).

Enzymes: Enzymes are proteins on or inside (more often inside) of cells that perform specific metabolic transformations to convert substrates into products. The products formed possess different chemical properties than those of the substrates. Note: Receptors and enzymes can work together to create and amplify signals.

DNA: Deoxyribonucleic acid, which (as the component bases) make up the chromosomes within the nuclei of mammalian cells. All the information required for normal cellular homeostasis is encoded in the DNA.

Antigens: Antigens are proteins on the surface of cells; the protein might have a known function (or not) but the important thing is that it is expressed mainly on a specific cell type to allow targeting of a drug (usually an antibody).
Almost all the effects of drugs turn off or decrease a cellular signal. This is because it is easier to decrease a signal than to increase a signal. Gene therapy (e.g. gene insertion into DNA) can increase a signal, but this technology is much more complicated to implement. It remains an active research area however.

Ia. Extracellular receptors as targets

Acetylcholine (Ach; Figure 1) binds to cholinergic receptors and is important as a neurotransmitter in the brains of animals and humans. It is also important in the stimulation of skeletal muscles for physical movement and in autonomic stimulation. There are two major types of acetylcholine receptors which are classified as muscarinic (mAChR) or nicotinic (nAChR) due to the ability of these two compounds to act as selective agonists for the receptor in question (Figure 2).

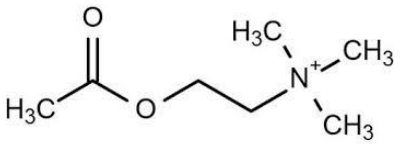


Figure 1. The structure of acetylcholine

These two major classes are further divided into subclasses, so the actual situation is rather complicated. We note right away that the muscarine molecule is unusual as it has a quaternary nitrogen (always positively charged just like acetylcholine itself) so we wouldn't expect it to cross membranes particularly well. Nicotine has two basic ionizable groups (pyridine pKa around 5) and an alkylamine (pKa around 9). Both compounds (muscarine and nicotine) can exist as stereoisomers, but only single enantiomers are found in nature. How many chiral centers does muscarine have? How many chiral centers does nicotine have?

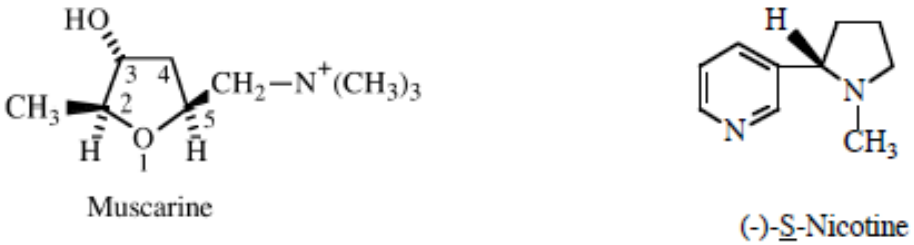


Figure 2. Structures of muscarine and nicotine

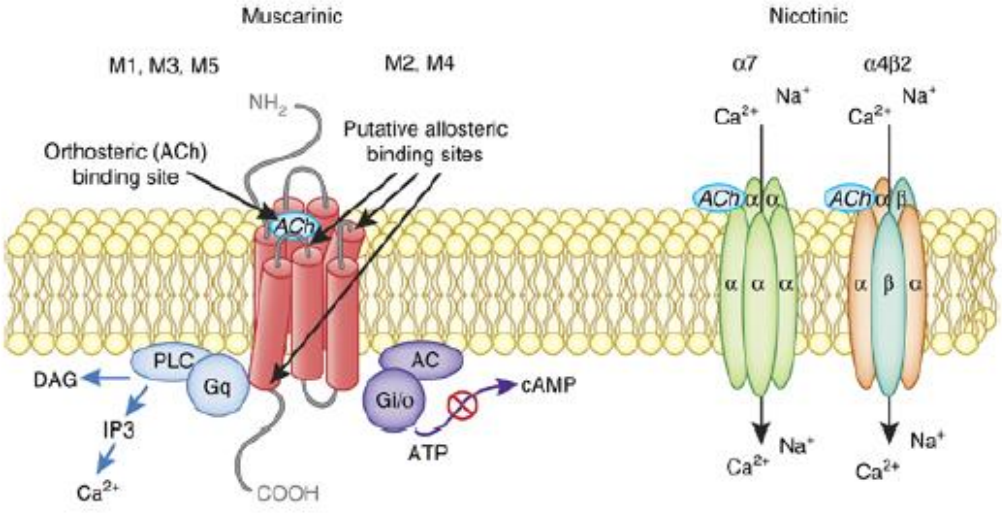


Figure 3. Muscarinic and nicotinic receptors embedded in a membrane

To make matters even more complicated, there are 5 types of muscarinic receptors (Figure 3) and all are G-coupled receptors. M1, M3 and M5 control intracellular free calcium concentrations via the phosphoinositol pathway. Binding to M2 and M4 inhibits the formation of the second messenger of the adrenergic system cAMP. The quantity and distribution of these receptor subtypes depends on the exact nerve under discussion.

Acetylcholinesterase (AChE) hydrolyses ACh (Figure 4). So inhibition of AChE causes increased levels of ACh and this causes agonist effects. Receptor selectivity can be enhanced by selective distribution of the inhibitors (e.g. the blood brain barrier). Also, drug companies have developed drugs that are selective agonists and antagonists of the major classes of acetylcholine receptors.

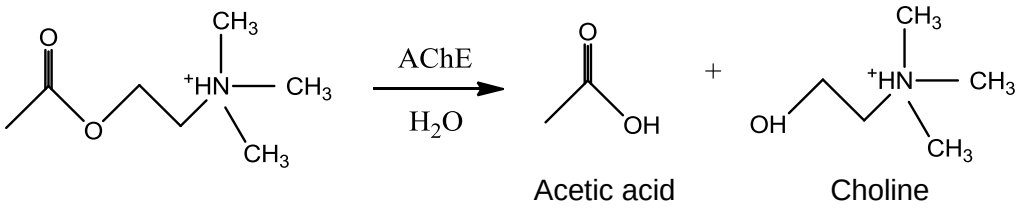
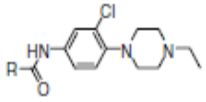


Figure 4. Hydrolysis of ACh to acetic acid and choline

Extensive structure activity relationship (SAR) studies have been carried out for cholinergic agonists and antagonists. The main goal of these kinds of studies is to develop ligands that differentiate between the various types of receptors.



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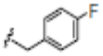
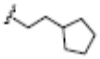
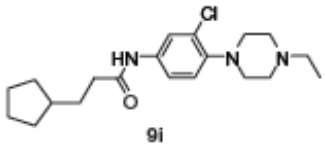
Compound	R	M1 IC_{50}[a] (μM)	M2 IC_{50}[a] (μM)	M3 IC_{50}[a] (μM)	M4 IC_{50}[a] (μM)	M5 IC_{50}[a] (μM)
5		13.2	>150	>150	>150	>150
9a		>150	>150	>150	>150	>150
9b		4.6	>150	>150	>150	>150
9c		5.0	>150	>150	6.6	>150
9d		5.6	>150	>150	>150	>150
9e		1.15	29	24	20	13
9f		1.1	52	70	18	7.6
9g		3.3	>150	>150	>150	>150
9h		18.8	>150	>150	>150	>150
9i		0.44	3.5	3.1	>150	1.1
9j		>150	>150	>150	>150	>150

Table 1. IC50 values for various analogs and muscarinic receptors M1-M5

Below we see the results of a study that sought to differentiate between the five mAChR receptor subtypes by developing selective antagonists for each subtype. Results are shown for the most selective inhibitor 9 (Table 2 at left shows the values; Figure 5 at right shows displacement curves) in the displacement of a radioactive ligand from each individual receptor subtype. Overall selectivity for M1 is significantly improved over the other receptor subtypes and over atropine.

Many analogs were tested in this experiment against the receptor subtypes to arrive at the structure of 9i. In these cases, size and lipophilicity of a single R group were varied often with dramatic and surprising effect on binding affinity.

Table 2. K_i determinations and binding fold selectivity for 9i



9i

mAChR	9i K_i[a] (nM)	Fold selectivity (vs M1)	Atropine K_i (nM)[a]
M1	12.7 ± 1.7		0.88 ± 0.04
M2	338.0 ± 13.5	27	2.69 ± 0.20
M3	74.8 ± 4.3	6	0.96 ± 0.03
M4	445.1 ± 23.8	35	0.56 ± 0.01
M5	85.7 ± 15.9	7	1.80 ± 0.11

[a] K_is are an average of three independent experiments using rat mAChR (CHO) cell lines.

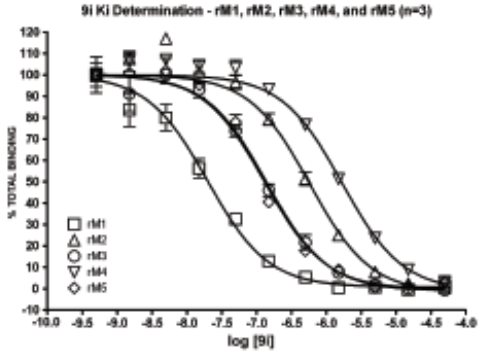


Figure 5. [^{3}H]-NMS competition binding experiments for 9i on rat M1–M5. Compound 9i displays 27-fold selectivity versus M2, 6-fold selectivity versus M3, 35-fold selectivity versus M4, and 7-fold selectivity versus M5. Curves represent the average of three separate experiments.

Figure 5. Determination of selectivity of compound 9i for different muscarinic receptors

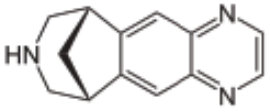
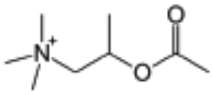
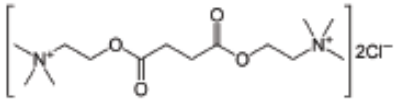
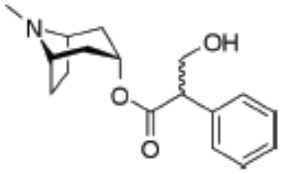
	Receptor Type	
Type of Action	Nicotinic (nAChR) Na/Ca channel Multiple subtypes	Muscarinic (mAChR) G-coupled receptor Five Subtypes
Agonist	 Chantix (α4β2 subtype Smoking cessation Issues with bizarre behavior	 Methanocholine Diagnostic for bronchial hypersensitivity in asthma (S) enantiomer more potent
Antagonist	 Succinyl Choline Depolarizing neuromuscular blocking agent	 Atropine Classic antagonist (S)-enantiomer 120 times more potent

Figure 6. Receptor subtypes and compounds optimized for activity

Ib. Intracellular receptors as drug targets

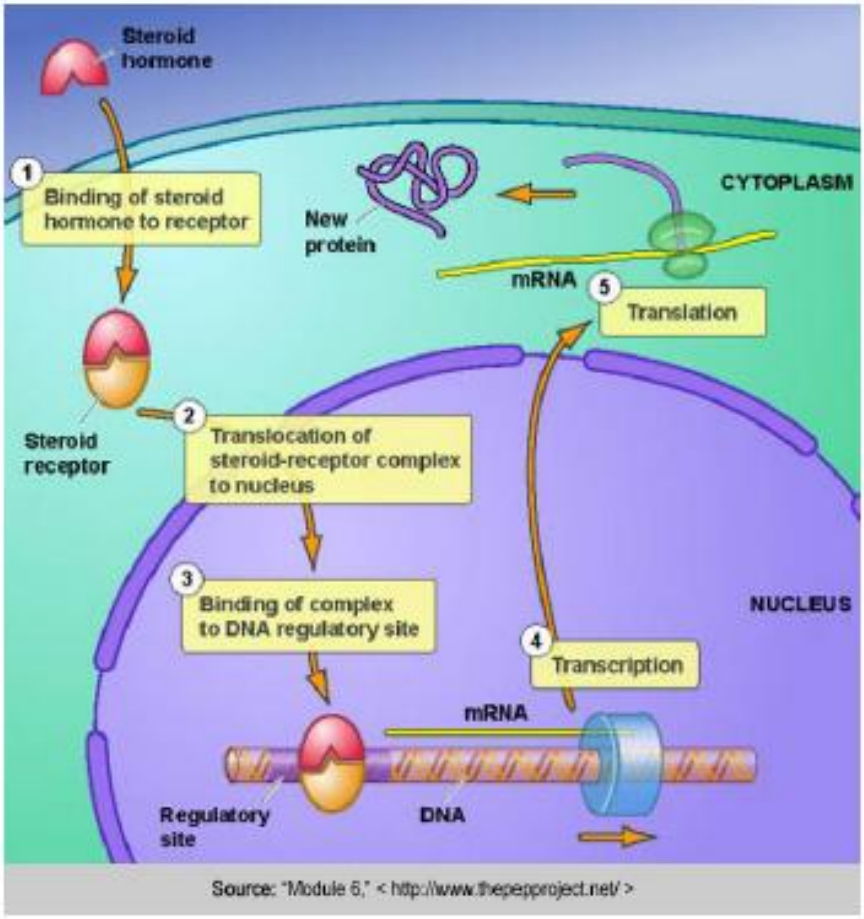


Figure 7. Action of steroids to enter the cell cytosol and translocation into the nucleus

Steroids readily enter cells and bind to receptors inside the cells (in the cell cytosol). They exert their effects by subsequent translocation of the receptor complexes into the nucleus (Figure 7) and binding to regulatory regions of DNA called response elements (GRE, MRE, ERE, ARE, PRE). Most often, this binding activates transcription of specific mRNA molecules that are exported to the cytosol and then translated into proteins. However, the binding to a response element can also inhibit transcription. For instance RU-486 is an antagonist of the progesterone response element (PRE) but has no effect on the mineralocorticoid response element (MRE).

Typically steroid antagonist-receptor complexes can also enter the nucleus, but they do not bind the response elements productively and are not active. So steroid type drugs may be either receptor agonists or antagonists – but usually antagonists.

All steroids are biosynthesized starting from cholesterol (Figure 8). Note the differences between the progestins, glucocorticoids, mineralocorticoids, androgens, and estrogens. The color shading in the figure below helps group these different steroid classes. Also note the large number of chiral centers in the steroids and the different functional groups. These differences give the different steroids the ability to bind selectively to their specific receptors and cause their specific (and markedly different) effects. Perhaps the best illustration of the differences between

steroids is between the androgens (male hormones) and estrogens (female hormones), which give rise to male and female sex characteristics.

Later in this course, we will discuss the importance of steroids and steroid antagonists in lectures that focus on anti-hormonal oncology agents.

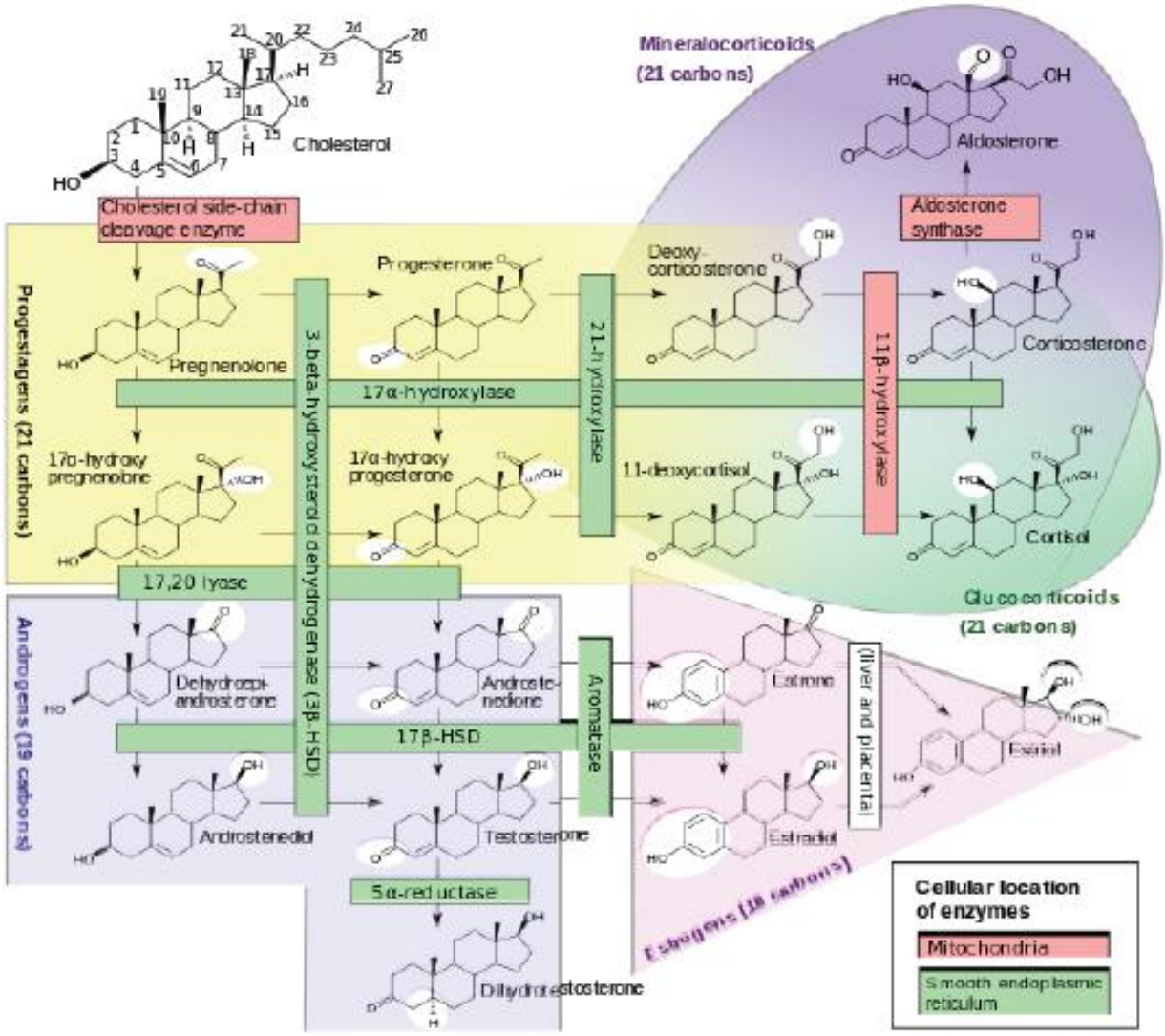


Figure 8. Biosynthetic scheme for the production of the steroid hormones