Lecture 3: Stereochemistry and drugs

Key objectives:
1. Be able to explain the role of stereochemistry in drug action or metabolism
2. Be able to identify a chiral center (or centers) in a drug
3. Be able to explain the difference between enantiomers and diastereomers

Value of chirality and stereochemistry: Chirality as expressed through stereoisomers increases the specificity of molecule recognition and signaling. Like a key in a lock, or a hand in a glove.

Some useful definitions:
Chirality (and chiral centers): An object (such as a molecule) that is asymmetric and therefore not superimposable on its own image. Chiral centers are the most common features within molecules that give rise to chirality.
Stereoisomer: A molecule that possesses at least one chiral center (or center of chirality).
Enantiomers: A type of stereoisomer that exists in mirror image forms. See Figure 1.
Diastereomers: A type of stereoisomer that possesses more than one chiral center.
Stereospecific: A term used to describe a stereochemical property that one isomer possesses but the other does not. For example, the stereospecific metabolism of the S-enantiomer of a compound by an enzyme but the R-enantiomer is not metabolized by that enzyme.
Stereoselective: A term used to describe a stereochemical property that is shared by both stereoisomers, but the property is greater in one isomer than the other. For example, the stereoselective binding of the R-enantiomer for a receptor indicates that the S-enantiomer also binds but not as avidly as the R-enantiomer.

Enantiomers

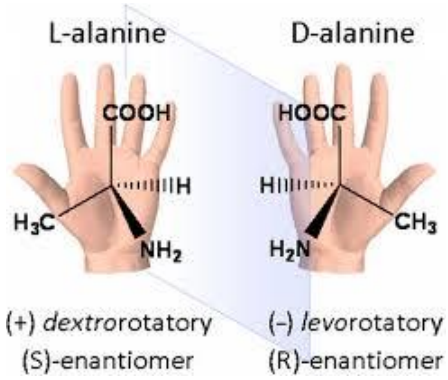


Figure 1: For enantiomers, many times we use the example of our left and right hands to demonstrate their asymmetry. Also shown are the two enantiomers of the amino acid alanine; L-alanine is the form found most common in nature.

Note: You should be able to identify chiral centers in a molecule, assign chemical group priority and deduce stereochemical identifiers [S (left) and R (right)] in a structure like alanine shown above.

Concept of “assumed” hydrogen bonds attached to carbon. Sometimes (or often) a hydrogen atom attached to carbon is not drawn – it is just assumed (See Figure 1 above). Carbon atoms always have 4 bonds to them and oftentimes an attached hydrogen is just left off. Be able to recognize this. We will see more of this in future slides.

The vast majority of drugs contain at least one stereo-center (like Figure 1) or other type of site of asymmetry. All biological receptors and enzymes have complex 3-dimensional structures and are inherently asymmetric. Therefore, important pharmacological differences between drug isomers are expected.

Most often, the chiral center(s) in drugs are located at carbon atoms that are tetrahedral (4 single bonds to carbon). In all cases, four different groups must be attached to the carbon to make it chiral (asymmetric).

When we study the ADME (absorption, distribution, metabolism, and excretion) and therapeutic properties of individual drug isomers, we find that they often behave differently. Thus, when we administer a mixture of two stereoisomers we are, in effect, giving a combination of two separate drugs with overlapping properties. As a result, when stereoisomers of a drug are given separately the biological response, side effects, protein binding, and pharmacokinetics of each isomer is often different, and sometimes strikingly so.

Historically, many drugs were developed and approved as mixtures of stereoisomers because making these drugs is easier and therefore the cost of making them is lower. However, the use of single isomers in drug development is strongly encouraged by the FDA today. In fact, in some cases the therapeutics and safety of old drugs that were given as mixtures of stereoisomers has been improved substantially by using single isomers. Patent protection for these products has been extended in these cases.

The FDA now requires that the properties of stereoisomers of drugs that are intended to be given as stereoisomeric mixtures be determined separately whenever possible. And with the advancement of technologies that allow for the synthesis of single stereoisomers, the cost of making them has dropped considerably.

Clearly, describing and identifying stereoisomers is important to avoid confusion in the literature.

Cahn-Ingold-Prelog rules for assigning stereochemistry (slightly abbreviated)

1. First, compare the atomic number (Z) for each of the atoms directly attached to the stereocenter (chiral center); the group having the atom of higher atomic number receives higher priority. In many cases, it is no more complicated than this and we can assign R or S.

2. Second, if there is a tie, then consider the atoms at distance 2 from the stereocenter - and a list is made for each group of the atoms bonded to the one directly attached to the stereocenter. Each list is arranged in order of decreasing atomic number. Then the lists are compared atom by atom; at the earliest difference, the group containing the atom of higher atomic number receives higher priority.

3. Third, if there is still a tie, each atom in each of the two lists is replaced with a sub-list of the other atoms bonded to it (at distance 3 from the stereocenter); the sub-lists are arranged in decreasing order of atomic number, and the entire structure is again compared atom by atom. This process is repeated, each time with atoms one bond farther from the stereocenter, until the tie is broken.

4. Finally, if an atom *A* is double-bonded to an atom *B*, *A* is treated as being singly bonded to two B atoms.
So, for the alanine example in Figure 1: -NH_2 = 1st priority; COOH = 2nd priority; CH_3 = 3rd priority and the hydrogen (facing back) is 4 and is the lowest priority and directed back out of the plane.
-When counting, if the sequence of 1, 2, 3 is clockwise, it is designated as R configuration
-When counting, if the sequence of 1, 2, 3 is counterclockwise it is designated as S configuration

Some real examples and their consequences

Enantiomeric drugs

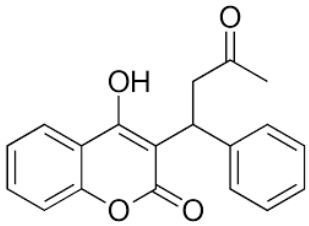


Figure 2. Structure of warfarin (Coumadin; anticoagulant)

1. Warfarin is an old drug and is given as the racemate [50:50 mixture of (R) and (S)]. It has a very low therapeutic index and individualized dosing with monitoring (INR: international normalized ratio) is required. The drug has a narrow therapeutic margin and its PK and pharmacodynamics are very sensitive to drug-drug interactions with CYP2C9 inhibitors and inducers.
2. Stereoselective (not stereospecific) inhibition Vitamin K epoxide reductase (favors the S isomer).
3. The (S) isomer (CYP2C9 substrate) is 3 fold more potent *in vivo* than the (R) enantiomer based on a similar dose, so potency is stereoselective for (S).
4. The (S) isomer is a substrate for CYP2C9 (fm 2C9 = 0.85). The (R) enantiomer is not a substrate for CYP2C9. Metabolism by CYP2C9 is stereospecific.
4. Stereoselective plasma half-life: (S) is 24 hours; (R) is 45 hours that is believed to be related to stereoselective protein binding.
5. Development of the single isomers was attempted. Interestingly, in this case it failed to improve clinical outcomes. Therefore, the racemate is still used.

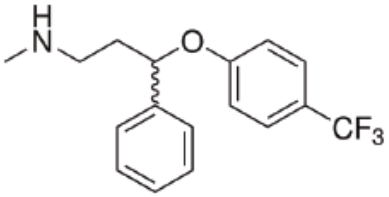


Figure 3. Fluoxetine (Prozac; selective serotonin reuptake inhibitor)

1. Note the use of a wavy bond to indicate the chiral center that is not defined (fairly common).

2. Fluoxetine is the only SSRI approved for use during pregnancy.
3. N-demethylated metabolite concentrations of both isomers can exceed parent concentrations during multiple dosing due to extremely long half-lives. The metabolites are pharmacologically active and stereoselectively inhibit the metabolism of the parent isomers, so the overall metabolism is pretty complicated. The parent and metabolites are potent inhibitors of CYP2D6 and CYP2C19. The parent and metabolites accumulate with repeated dosing.
4. Considerable efforts to develop and market a single enantiomer by Lilly failed.
5. More success has been achieved by development of newer generation SSRIs (Lexapro, S-enantiomer).

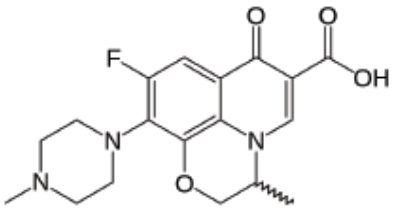


Figure 4. Fluoroquinoline (antibiotic)

1. (S) enantiomer (Levofloxacin (Levaquin)) is much more active than the (R) enantiomer and is marketed as a separate drug. Quinolones are infamous for causing spontaneous tendon ruptures. There are two black box warnings on Levofloxacin (Levaquin).
2. Note that there are multiple ionizable groups and the molecule is drawn as the neutral species. Therefore, it is zwitterionic at pH 7. However, bioavailability is good (>95%). Identify the functional groups that would be charged at pH 7.

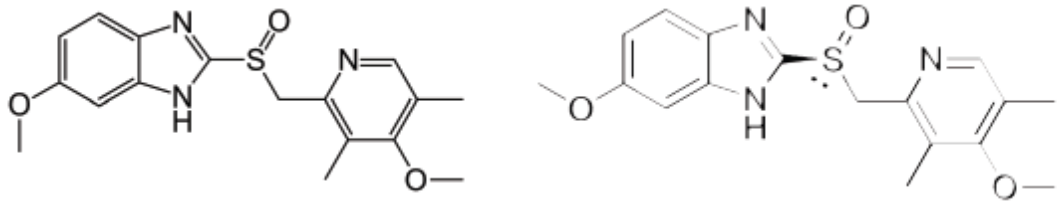


Figure 5. Omeprazole (prilosec; proton pump inhibitor) along with Esomeprazole (Nexium)

1. Note the unusual chiral center in this case. It is at sulfur, which is why I chose it. The lowest priority group here is the lone electron pair on sulfur. This center does no invert so the drug does not undergo racemization and can exist as enantiomers. All of the PPI's (Lanzoprazole for instance) have this structural feature and are chiral.
2. Metabolism and clearance is by CYP2C19 which is a polymorphic enzyme. Evidence suggests that administration of (S)-omeprazole (Nexium) provides better AUC and less individual variability. For some other PPI's, the (R) enantiomer appears superior. So there is some PK stereoselective differences but this is not always the case with this the class of drugs.
3. Overall, it is controversial whether Nexium was worth the extra cost of development.
4. The PPI's are unstable to the acid pH in the stomach, are formulated for release to cause irreversible inhibition of the proton pump.

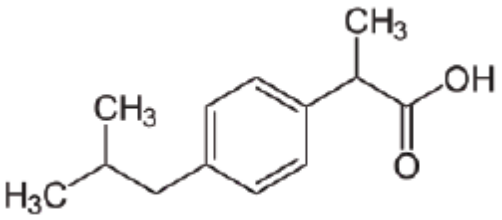


Figure 6. Ibuprofen (Advil; non-steroidal anti-inflammatory agent that inhibits cyclo-oxygenase)

1. The (S) enantiomer is the active form and is a stereospecific inhibitor of the enzyme.
2. Efforts to develop the single enantiomer to improve potency were discontinued when it was realized that an acyl-CoA racemase enzyme converts the (R)-enantiomer to the (S). Other NSAIDs like ketoprofen also undergo this type of racemization (also called epimerization). In the mechanism of racemization the hydrogen is removed and simply re-attached. As an exercise, be sure to be able to identify the chiral center and the ionizable group.

Diastereomers

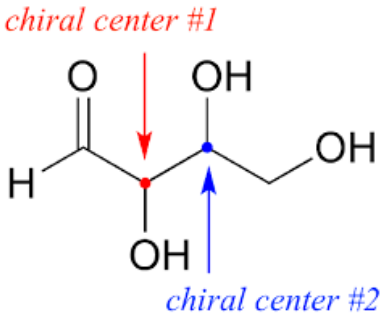


Figure 7. For diastereomers, there are at least two chiral centers. In this case, the hydrogen (H) attached to the chiral centers are not shown but still understood to exist.

We can apply the Cahn-Ingold-Prelog rules to diastereomers and arrive at the different stereoisomers (RS, RR, SR, and SS). Note that RS and SR are enantiomers and so are RR and SS.

The total number of isomers possible is $2^{(n)}$ where “n” is the number of chiral centers present in the molecule. A large number of drugs have multiple chiral centers. Steroid and protein type drugs possess many chiral centers.

Diastereomeric drugs (multiple chiral centers)

In the example below (Figure 8), each isomer has one enantiomer where the stereochemistry at both chiral centers are inverted. As discussed previously, these isomers have different properties in a chiral environment due to the 3-dimensional structures of enzymes and receptors. In an achiral environment, they have identical properties, for example in a simple solution of water.

(-) Ephedrine and (+) ephedrine are an enantiomeric pair because both stereocenters are inverted.

The carbon next to the benzene ring in (-)-ephedrine is (R) and the other stereocenter in the molecule is (S). So this enantiomer is RS. The other enantiomer is (+) ephedrine and is SR.

There is another enantiomeric pair and it is called (+) and (-) pseudoephedrine (lower structures). Note that these molecules are diastereomeric in their relation to (+) and (-) ephedrine. These molecules must be RR and SS by definition. Diastereomeric isomers differ in chemical properties even in an achiral environment. Obviously they can have different properties as well as in a chiral environment.

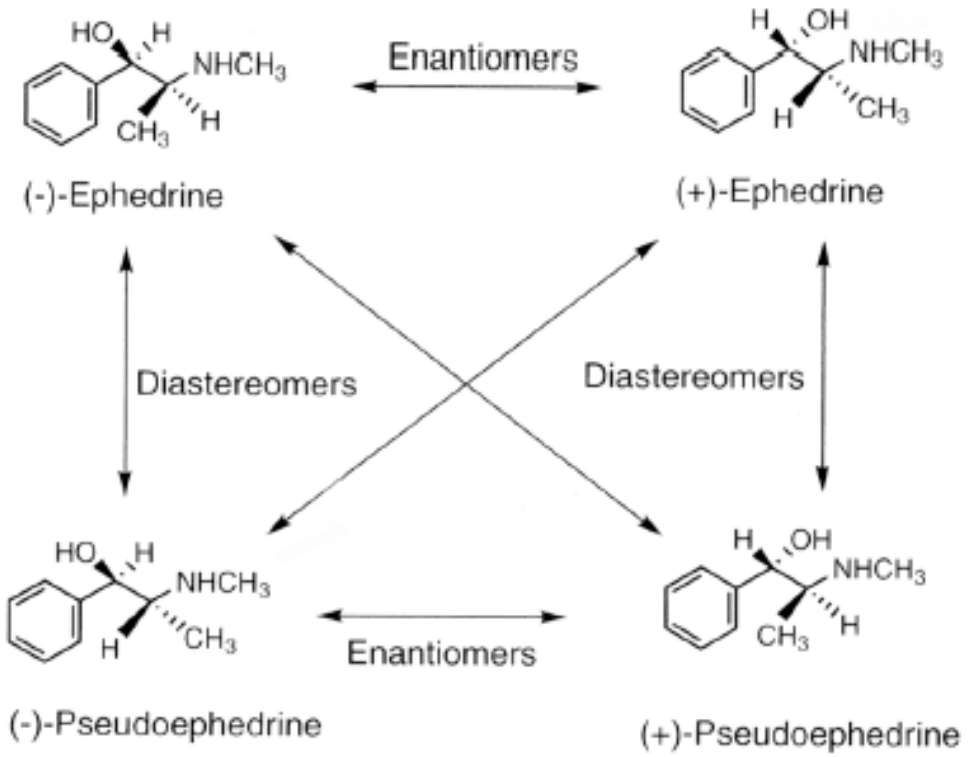


Figure 8. The 4 stereoisomers of ephedrine and pseudoephedrine.

In the example above, all hydrogens attached to the chiral centers are shown for clarity. Remember the rule that if 2 chiral centers are present, the total number of stereoisomers will be calculated as 2^2 (equals 4) because n=2.

Multiple stereocenters are common particularly in steroids (Figure 9) and drugs that bind to enzyme or receptors of peptides. Drugs derived from natural products usually have multiple chiral centers. However, only a single stereoisomer (one enantiomer) is made by nature. Again, the reason for this is to achieve specificity.

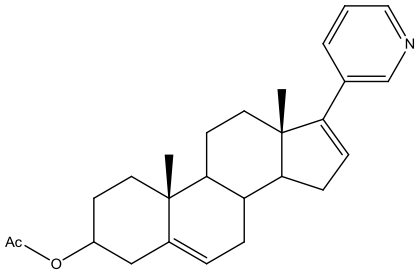
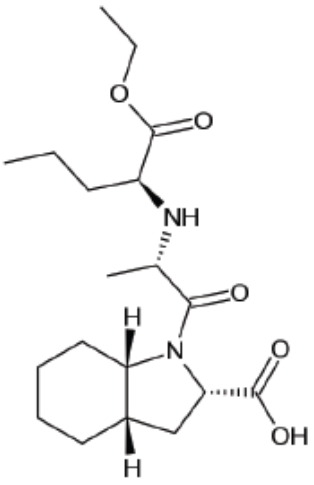


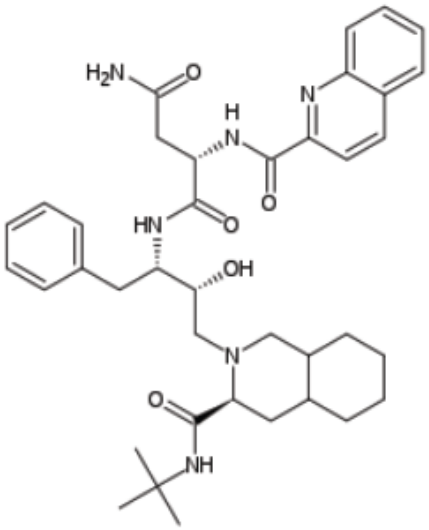
Figure 9. Structure of Abiraterone (Zytiga; anti-androgen drug for prostate cancer); notice that none of the hydrogen atoms are explicitly shown

Finally, a checklist of things to consider when isomeric drugs are compared:
1. Different ADME properties are almost always observed since transporters, metabolic enzymes, and serum albumin are all complex 3D structures. So the drugs can interact stereoselectively or stereospecifically with these other molecules.
2. Different pharmacology at receptors (e.g. cases where one isomer is an agonist and another isomer is an antagonist of the same receptor).
3. Different off-target effects and different side effects.
4. Racemization of stereocenters can sometimes occur and this can obviate the need for single isomers.
5. Sometimes industry markets a mixture (racemate) and later does a follow-on drug of a single isomer. Typically the racemate goes generic first and becomes inexpensive. The drug company must demonstrate that the follow-on single isomer (enantiomer) is different enough to provide true benefit (cost effectiveness), and warrant a separate patent (novelty).

As an exercise, circle the chiral centers in each of the following compounds. There are several in each drug. Note some of the missing (assumed hydrogens) in each example. Notice when bold or hatched bonds are shown. These indicate chiral centers, but these are not always shown.



Perindopril (ACE inhibitor)



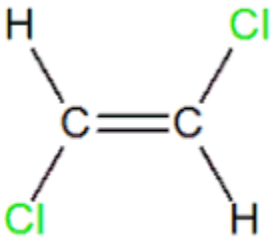
Saquinavir (HIV protease inhibitor)

Geometric isomers (cis and trans isomers)

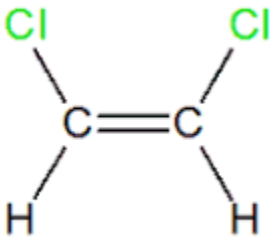
These types of isomers don't possess chiral centers but can still be non-superimposable by virtue of the geometric arrangement of the atoms. To determine if a molecule is cis or trans, we simply:

1. Identify the atoms of highest priority (atomic number)
2. Determine if the highest priority atoms are on the same (cis) or opposite (trans) side of the double bond.

In the example below, the chlorine atoms are clearly of highest priority. The molecule at left is trans, while that at right is cis.



trans-1,2-dichloroethene



cis-1,2-dichloroethene