5.2: Treatments for Pain and Inflammation

Since the molecules involved in targeting the COX enzymes are not steroids but still reduce inflammation, they are referred to as **Non-Steroidal Anti-Inflammatory Drugs** or **NSAIDs**. Although the COX enzyme was first isolated and purified in 1976, it has been inhibited by the action of natural products used by multiple civilizations (such as the bark of white willow or meadowsweet trees). The active ingredient from these plants (salicylic acid) was eventually identified, and some of the more adverse reactions (such as GI discomfort) were ablated with the use of the esterified acetylsalicylic acid (aspirin). Aspirin was the first drug to be developed, patented, and marketed in 1899 and represents a large milestone in the history of pharmaceutical agents.

Aspirin

The anti-inflammatory effects of aspirin arise through inhibition of the COX enzyme. Acetylsalicylic acid binds the positively charged side chain of Arg120 on the COX enzyme and blocks arachidonic acid from entering the protein channel and being oxidized to inflammatory metabolites. (Figure 5.4) Aspirin also has a unique mechanism of action in which it covalently (or irreversibly) engages with the COX enzyme by reacting with Ser29. (Figure 5.6) The acetyl-group acts a leaving group, and the COX enzyme is permanently modified with the salicylic acid. Cells (such as those in the gastric lining) need to biosynthesize new COX1/COX2 enzymes to restore proper function. However, unnucleated cells (such as platelets) cannot re-synthesize these proteins and aspirin is a potent inhibitor of platelet aggregation.

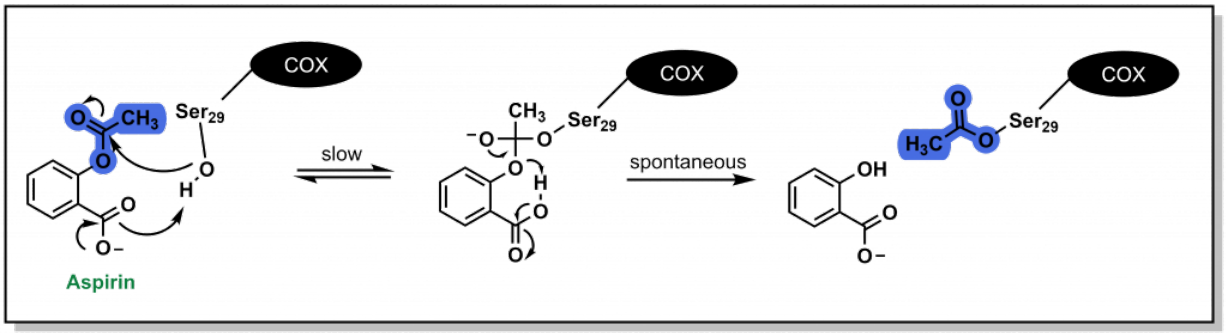

Figure 5.6 Aspirin is a covalent modifier of COX enzymes.

Other Non-steroidal Anti inflammatory Drugs

There are multiple NSAIDs that are employed (other than aspirin), although they differ in that they do not operate via a covalent mechanism of action. They can generally be classified into salicylic acids, arylalkanoic acids, and oxicams. (Figure 5.7) In all cases, they have an acidic functionality that is critical to engage with Arg120 of the COX enzyme and sterically block access to the pocket.

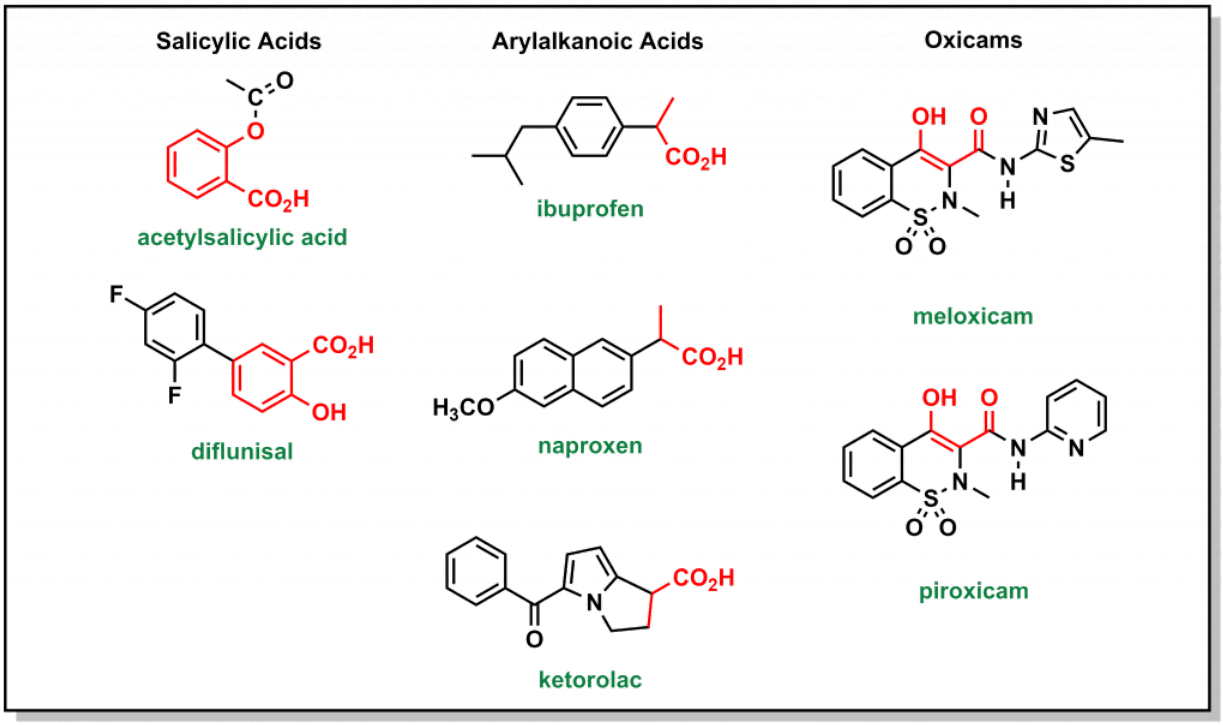

Figure 5.7 Structures of different classes of NSAIDs.

Tylenol

Tylenol or **acetaminophen** is a common analgesic that was previously thought to inhibit COX enzymes. However, it was shown to only weakly bind to the COX enzymes *in vitro* and as such is not an NSAID, likely due to low acidity of the hydroxyl group. Acetaminophen is thought to act centrally in the brain to block pain signals and directly engage with arachidonic acid. (Figure 5.8a) However, it is also capable of inducing anti-inflammatory responses. One of the largest concerns with Tylenol is the potential for toxicity with excessive doses. Nearly all (~95%) Tylenol is metabolized by glucuronidation and sulfonylation. (Figure 5.8b) However, a small amount will be metabolized by CYP2E1 which leads to generation of the superoxide and NAPQI (*N*-acetyl-*p*-benzoquinone imine). This intermediate is toxic because it is highly reactive and can covalently modify different residues or nucleotides. In the presence of glutathione, NAPQI is rapidly de-toxified. However, if there are substantial amounts of Tylenol, this will deplete the reservoir of liver glutathione leaving free NAPQI available to react with other biomolecules.

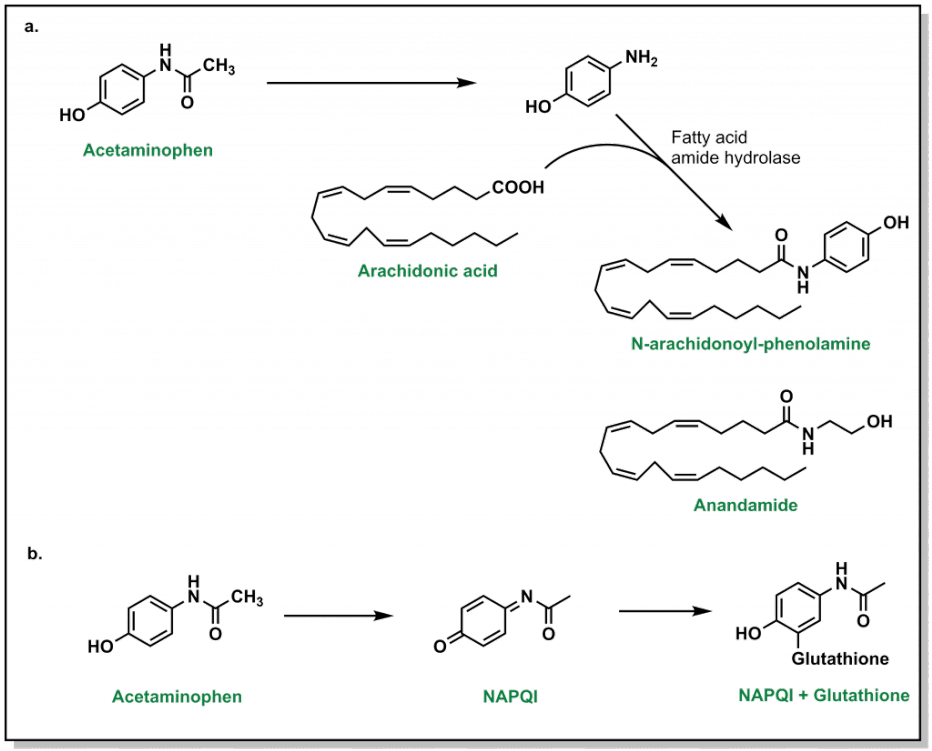

Figure 5.8 Mechanism of Tylenol metabolism and potential toxicity.

Opioids

Pain generally begins at the nociceptors, and the signal is transmitted to secondary neurons in the dorsal root ganglion and subsequently to the brain via the spinal cord nerves. The signal transmission occurs via action potentials that fire with increased frequency depending on the severity of the pain. Neurons release multiple neurotransmitters that help facilitate different responses:

- Glutamate: This activates different receptors on the post-synaptic neuron (such as NMDA, AMPA) and causes Ca^{2+} and Na^{+} to enter the cell (increasing the positive charge of the cell)
- Substance P: This leads to the release of substances of such as arachidonic acid, and reinforces the pain signal.
- CGRP: This alters GPCR expression which reinforces response to pain.

At the same time, the body also releases endogenous molecules to help alleviate some of the effects of pain such as dynorphins and endorphins. These molecules can bind to mu, delta, or kappa receptors. Euphoric effects arise upon binding and activation of the mu receptors in the brain. These receptors reduce the response of GABA receptors, and the net result is the removal of limiters on dopamine release. These natural ligands produce analgesic effects. However, opioids are substantially more potent binders and lead to more powerful analgesic effects.

Opioids have been used for thousands of years and they originate from the milky fluid (called latex) of the poppy plant. Morphine is the primary alkaloid of the opium family. (Figure 5.9) It contains multiple functional groups that are critical for analgesic activity. This includes:

- A tertiary nitrogen that is positively charged to engage with the side chain of an aspartic acid on the mu receptor.
- The tertiary nitrogen attached to a quaternary carbon by a 2-C bridge.
- The quaternary carbon has a phenyl group attached for pi-pi stacking interactions with the mu receptor.
- Functional groups on C_3 and C_6 are important for H-bonding interactions.

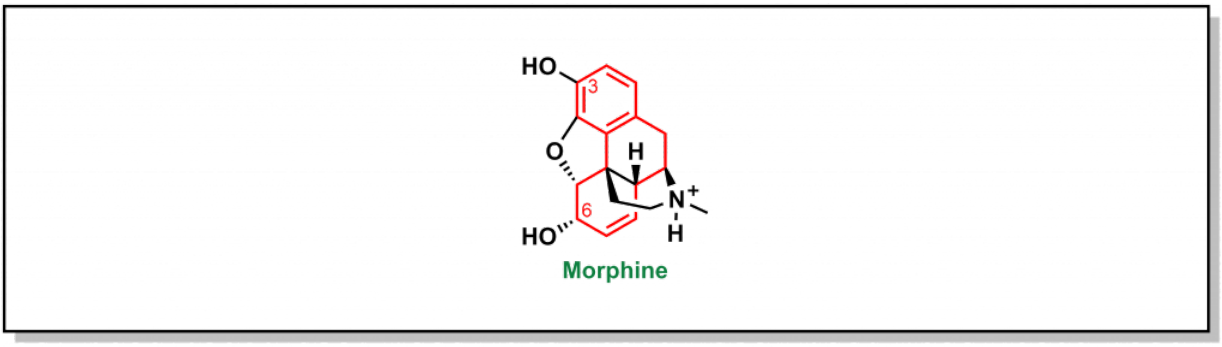

Figure 5.9 Structure of morphine.

Natural, synthetic, and semi-synthetic opioids have been explored which offer alternative properties in bioavailability. (Figure 5.10) For example, codeine is a naturally occurring opiate, and the only difference between morphine, is in the C_3 position (with a methoxy instead of the hydroxyl). This increases the lipophilicity of the molecule substantially. However, the C_3 methoxy can no longer participate in H-bonding and also has substantially lower binding to the mu receptor. Although codeine would be extremely potent if it was metabolized to morphine, this does not occur efficiently. In fact, even though the lipophilicity of codeine is higher than morphine, because of the slow rate of metabolism, an oral dose of 100 mg is required to achieve the same response as 10 mg of morphine.

Heroin is a version of morphine where the hydroxyl groups on C_3 and C_6 are both acetylated. This increases the lipid/membrane solubility leading to rapid CNS penetration. The acetyl groups are also readily metabolized to hydroxyls (unlike codeine) and heroin is approximately two-fold more potent than morphine.

To compensate for the low binding affinity of codeine, different derivatives were examined such as hydrocodone. In hydrocodone, the hydroxyl at C_6 is converted to a ketone which increases the lipophilicity while preserving the double bond. However, the carbonyl alters the positions of the oxygen relative to the hydrogen bond donor and removing the double bond at C_3-C_4 helps re-align the atoms. These changes result in similar potencies between hydrocodone and morphine. Hydrocodone can be metabolized to more potent hydromorphone which is similar with the exception of the hydroxyl instead of methoxy at C_6.

Another SAR modification was the introduction of a hydroxy at C_{14} to hydrocodone. Oxycodone has further improved bioavailability and is about two-fold more potent than morphine.

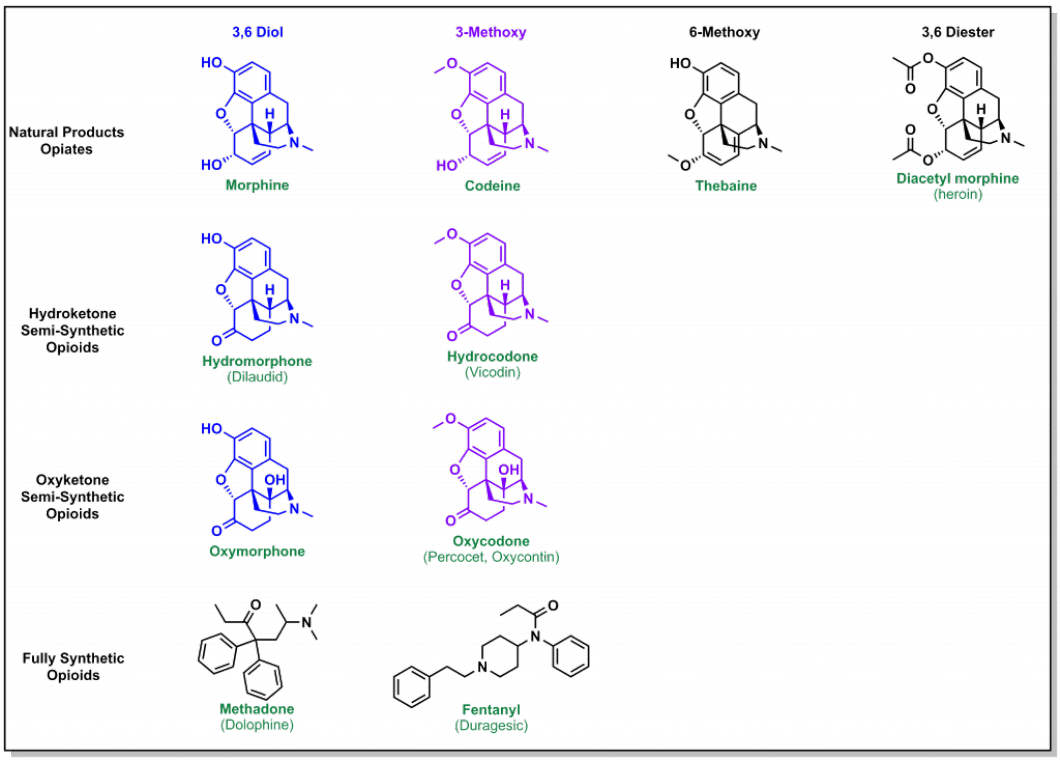

Figure 5.10 Natural, semi-synthetic, and synthetic opioids.
