Peer-reviewed version available at *Molecules* **2018**, *23*, 2119; doi:10.3390/molecules23092119

Review Article

Linking Aromatic Hydroxy Metabolic Functionalization of Drug Molecules to Structure and Pharmacologic Activity

Babiker M. El-Haj [1,*], Samrein B.M. Ahmed [2], Mousa A. Qarawi [2], Heyam S.Ali [4]

[1] Department of Pharmaceutical Sciences, College of Pharmacy, Ajman University, UAE; b.elhag.ajman.ac.ae
[2] College of Medicine, Sharjah Institute for Medical Research, University of Sharjah, UAE; samreinahmed@sharjah. ac.ae
[3] Department of Pharmaceutical Sciences, College of Pharmacy, Ajman University, UAE; m.qarawi@ajman.ac.ae
[4] Department of Pharmaceutics, Dubai Pharmacy College, UAE; heyam57@hotmail.com

* Correspondence: e-mail: b.elhag.ajman.ac.ae; Tel.: 0071567204338

Abstract: Drug functionalization through formation of hydrophilic groups is the norm in phase I metabolism of drugs for modification of drug action. The reactions involved are mainly oxidative catalyzed mostly by CYP isoenzymes. The benzene ring, as phenyl or fused with other rings, is the most common hydrophobic pharmacophoric moiety in drug molecules. On the other hand the alkoxy group (mainly methoxy) bonded to the benzene ring assumes an important and sometimes essential pharmacophoric status in some drug classes. Upon metabolic oxidation, both moieties, i.e. the benzene ring and the alkoxy group, produce hydroxy groups; the products are arenolic in nature. Through a pharmacokinetic effect, the hydroxy group enhances the water solubility and elimination of the metabolite with the consequent termination of drug action. However, through hydrogen bonding, the hydroxy group may modify the pharmacodynamics of the interaction of the metabolite with the site of parent drug action (i.e. the receptor). Accordingly, the expected pharmacologic outcome will be enhancement, retaining, attenuation, or loss of activity of the metabolite relative to the parent drug. All the above issues have been presented and discussed in this review using selected members of different classes of drugs with inferences regarding mechanisms, drug design and drug development.

Keywords: Aromatic hydroxy metabolites; arenolic drug metabolites; metabolic *O*-dealkylation; metabolic aromatic ring hydroxylation; primary and auxiliary pharmacophores; auxophores; metabolic modification of drug activity.

Introduction

Phase I metabolism of drugs (also known as the non-synthetic or functionalization phase) is mainly brought about by microsomal enzymes by adding hydrophilicity to hydrophobic moieties in drug molecules. In most cases, this is attained metabolically by revealing or introducing the hydrophilic hydrogen bonding hydroxyl group. Generally, metabolic interference may take place at pharmacophoric and/or auxophoric groups. Accordingly, the location in a drug molecule at which the metabolic change takes place may determine the pharmacologic outcome of the metabolite.

In any case, the resulting drug metabolite may be pharmacologically inactive, less active, equiactive, or even more active with respect to the parent drug. In other cases, an inactive parent drug is metabolically converted to the active form, in which case the inactive parent drug is known as 'prodrug.' Cases where the metabolic changes are intermediary in drug activation are also known.

For some drug metabolites, the pharmacologic outcomes - pharmacodynamic, pharmacokinetic, and toxicologic - have proven to be substantially more favorable compared to their parent drugs. In such cases, the metabolites have been developed into drugs of their own rights. In this review, such drug metabolites will be referred to as "metabolite drugs".

Generally, in drug development, the pharmacophoric or auxophoric roles of functional groups in lead drug compounds or drug prototypes are modified in the synthetic laboratory in some ways.

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119

Introduction of a group in the framework, reduction of a group to framework status, or replacement of a group by another are the main strategies followed. Through biotransformation, the body may play an analogous in vivo role by changing pharmacophoric and/or auxophoric groups into others or by introducing groups at certain positions of the drug molecule with concomitant changes in pharmacodynamic and/or pharmacokinetic properties of the metabolite relative to the parent drug. Such metabolic changes of a drug molecule may lead to a variety of pharmacologic outcomes. The dependence of the metabolic change on the structure of the drug molecule and the pharmacologic outcomes of the resulting metabolites are the subjects of this review.

Objective of the Review

The objective of this review is to investigate the relationship between the metabolic change and modification of pharmacodynamic and/or pharmacokinetic properties of the drug in an endeavor to explain when the pharmacologic activity of the metabolite is retained, decreased, lost, or even enhanced with respect to the parent drug. The link of the metabolic functionalization and pharmacologic activity to the structure of the drug is also a prime interest. Such information, as described above, will be useful in drug designing, when contemplating new drug entities. As well, it should be useful in drug development when considering what chemical changes need to be made in a lead or prototype for better drug efficacy and metabolic stability

Methodology

The inclusion criteria of the selection of drugs to be reviewed include

(a) drugs that are metabolized by O-dealkylation or aromatic ring hydroxylation; and

(b) availability of data regarding the pharmacologic activity of the major metabolite(s).

The sources of information include:

- the published literature;
- drug manufacturers' data sheets; and
- reference books on drug metabolism and activity of metabolites

The selection of the case study drugs has been based on varying the pharmacologic and chemical classes, as will be described in each section.

Review Strategy

Although the selected drugs are mostly metabolized by multi routes, only metabolic oxidative reactions that result in one chemical functionality, the aromatic hydroxy group, will be considered. The representative drugs are selected based on both chemical structure and pharmacologic action. Each study/metabolite(s) case will be presented in a figure including the following information subject to availability in the literature:

- the chemical structure of the drug and metabolite(s), and the enzymes or isoenzymes involved;
- the status of the pharmacologic activity of the metabolite;
- the percentage concentration of the metabolite(s) with respect to the parent drug.

Each parent drug will be briefly reviewed. The arguments for the links of metabolic changes to pharmacologic activity and structure will be discussed for some drug/metabolite cases as they are presented with an overall discussion given for all the cases at the end of the section.

Aromatic-Hydroxy (Arenolic) Metabolites

Arenolic metabolites can result from one of two metabolic reactions: *O*-dealkylation of aralkoxy groups or hydroxylation of aromatic rings (mainly benzene); in the latter case, the positions of the hydroxy groups are determined by the prevailing electronic and steric effects on the aromatic ring. The benzene ring may be present in the drug molecule as a separate entity (i.e. as a phenyl group), or it may be fused to another benzene ring, or to an alicyclic or a heterocyclic ring. When the benzene

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119

ring is fused to either an alicyclic or heterocyclic ring the resulting ring system may be described as benzenoid. Arenolic metabolites will be presented and discussed in two sections, 1 and 2.

Section 1. Arenolic Metabolites Resulting from *O*-Dealkylation of Aralkoxy Groups

Metabolic CYP-catalyzed *O*-dealkylation of aralkoxy groups involves, as a first step, the hydroxylation of the carbon atom of the alkyl group that is linked to the oxygen atom. This hydroxylated metabolite is unstable. It breaks spontaneously into two molecules: the dealkylated metabolite (e.g., an alcohol or a phenol), and an aldehyde (e.g., formaldehyde after demethylation, or acetaldehyde after deethylation, etc.). The reaction is shown for a general case in Fig. 1.1., where the group R can be aliphatic or aromatic [1].

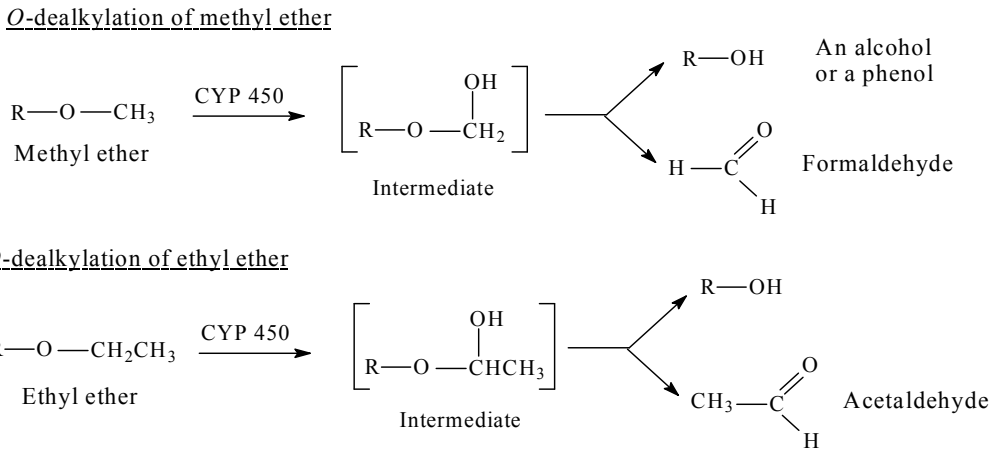


Fig. 1.1. CYP-catalyzed O-dealkylation of alkyl or aralkyl ethers

O-dealkylation of aralkyl ethers results in the production of arenolic metabolites of varying pharmacologic activities, as will be reviewed for the following selected cases:

(1) Enhancement of activity occurring in the opioid narcotic/analgesic drug codeine and its semisynthetic and synthetic congeners, all containing an aryl-methoxy group, and the analgesic/antipyretic drug phenacetin, which contains an aryl-ethoxy group
(2) Retaining of activity in the SSRI antidepressant venlafaxine, containing an aryl-methoxy group
(3) Loss of activity in the COX1/COX2-inhibiting NSAIDS naproxen, indomethacin, and nabumetone, each containing an aryl-methoxy group

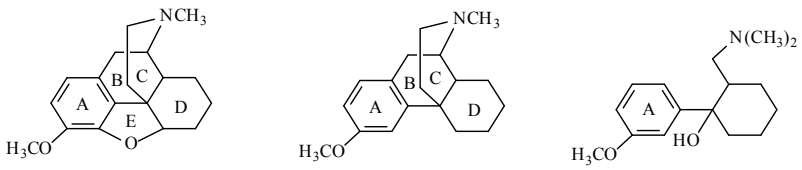
1.1. Metabolic Aralkoxy Cleavage Resulting in Enhancement of Pharmacologic Activity: Opioids and Phenacetin

1.1.1. Opioids. Opioid drugs can be defined as those acting on the different types of opioid receptors (mu, kappa, and delta) to produce a variety of biologic effects. Mu-receptor stimulation is associated with analgesic (antinociceptive) effects, respiratory depression, reduced GI motility (constipation), and euphoric and dependence effects, while kappa-receptor stimulation is associated with analgesia, psychomimetic effects, dysphoria, and diuresis [2, 3].

For the sake of discussion, methoxy-group-containing opioid drugs can be classified with regard to source and chemical build-up. Source includes natural origin (e.g. codeine), semisynthetic (e.g. codeine congeners), and synthetic (e.g. tramadol). Tramadol is considered to be a synthetic-codeine congener since it was developed using codeine as template. Chemically, codeine and its semisynthetic congeners are classified as pentacyclic or tetracyclic morphinans (Fig. 1.2.). On the other hand, tramadol is a non-morphinan bicyclic opioid devoid of rings B and C, as shown in Fig. 1.2.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 July 2018 doi:10.20944/preprints201807.0143.v1

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119



Pentacyclic morphinan (Codeine and its congeners) | Tetracyclic morphinan (Levomethorphan) | Tramadol

Fig. 1.2. Chemical classification of methoxy-group-containing morphinan and non-morphinan opioids

1.1.1.1. Codeine. Codeine (Fig. 1.3) is an opium alkaloid; ii is the methyl ether of morphine and is present in opium in 1-3% concentration [4]. It is obtained semisynthetically on a large scale by the methylation of morphine. Codeine is a weak mu-receptor agonist, being only about 0.1% as active as morphine [5]. The analgesic activity of codeine has been attributed mainly to its metabolites. Codeine is metabolized per the pathways shown in Fig. 1.3 to codeine-6-glucuronide, morphine, and norcodeine. The resulting morphine is further metabolized to the 3- and 6-glucuronide conjugates [6, 7]. As indicated in Fig. 1.3, reports in the literature on the concentrations of codeine metabolites are variable. Further, reports on the mu-receptor affinity and analgesic activity of codeine metabolites are even contradictory. It is a longstanding belief that the mu-receptor affinity and analgesic activity of codeine are mediated through its metabolite morphine, which has an affinity for the mu-opioid receptor 200-fold greater than that of codeine [8]. The analgesic activity of codeine has been suggested to be largely due its metabolite morphine [8-11]. However, of late, Vree et al. (2000) have developed a different view of codeine's affinity to the mu-opioid receptor and its analgesic activity. Here, we quote the abstract of their paper:

Eighty per cent of codeine is conjugated with glucuronic acid to codeine-6-glucuronide. Only 5% of the dose is *O*-demethylated to morphine, which in turn is immediately glucuronidated at the 3- and 6-positions and excreted renally. Based on the structural requirement of the opiate molecule for interaction with the mu-receptor to result in analgesia, codeine-6-glucuronide, in analogy to morphine-6-glucuronide, must be the active constituent of codeine. Poor metabolizers of codeine, those who lack the CYP450 2D6 isoenzyme for the O-demethylation to morphine, experience analgesia from codeine-6-glucuronide. Analgesia of codeine does not depend on the formation of morphine and the metabolizer phenotype. [12]

The literature's contradictory evidence on the mu-receptor affinity and the analgesic activity of codeine and its metabolites will be weighed in the discussion section.

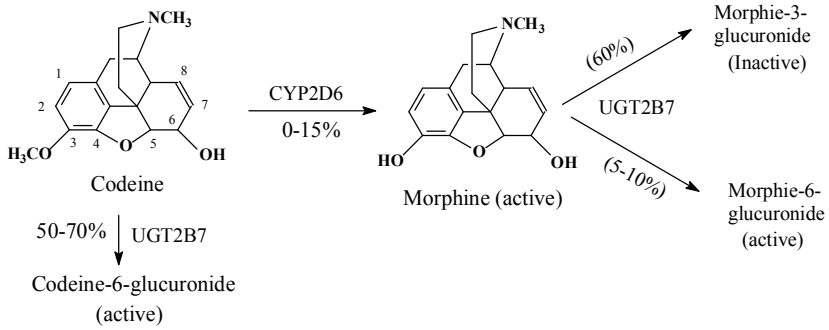


Fig. 1.3: Metabolic pathways of codeine

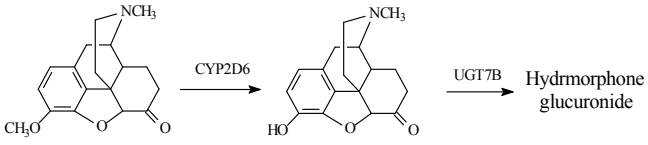
It may be of interest to note that codeine's metabolic pathways cover almost the entire spectrum of biological activity by including reactions that are augmenting, activating, inactivating, and attenuating of the analgesic activity of the metabolite with respect to the parent drug.

1.1.1.2. Codeine congeners. Codeine synthetic pentacyclic-morphinan congeners include hydrocodone, oxycodone, and the tetracyclic morphinan congener levomethorphan, which all have

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 July 2018 doi:10.20944/preprints201807.0143.v1

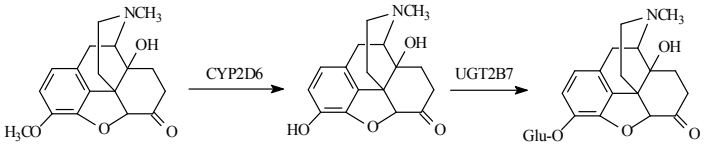
Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119

3-arylmethoxy groups. On the other hand, tramadol may be considered to be a non-morphinan codeine analogue since its development has been based on codeine as template. Tramadol contains an arylmethoxy group relative in position to that of codeine. While hydrocodone, oxycodone, and tramadol are currently clinically used in pain management [13, 14], levomethorphan has never been used. The four drugs are metabolized by the isozyme CYP2D6 to their corresponding O-desmethyl phenolic counterparts as shown in the corresponding figures: 1.4, 1.5, 1.6, and 1.7 [15-17]. Due to their substantially higher mu-receptor affinities and, accordingly, their higher analgesic activities compared to their parent drugs [15, 16], the four phenolic metabolites have been developed into drugs of their own rights. Presumably, the increase in mu-receptor affinity and analgesic activity is a result of the phenolic hydroxy groups in the four-metabolite drugs. We will present possible explanation of why this is the case in the discussion of this section.



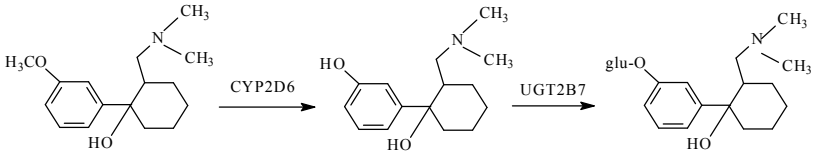
Hydrocodone Hydromorphone

Fig. 1.4.: Metabolic pathways of hydrocodone



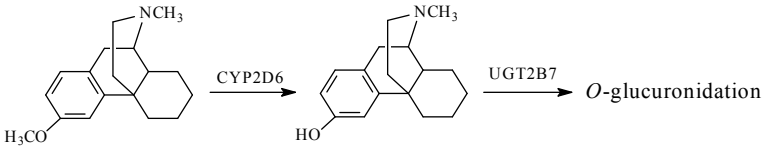
Oxycodone Oxymorphone Oxymorphone glcuronide

Fig. 1.5. Metabolic pathways of oxycodone



Tramadol *O*-Desmethyltramadol *O*-Desmethyltramadol glucuronide

Fig. 1.6. Metabolic pathways of tramadol



Levomethorphan Levorphanol

Fig. 1.7. Metabolic pathways of levomethorphan

Discussion of Section 1.1.1 on Opioids

Section 1.1.1 on the metabolic *O*-demethylation of the methoxy-group-containing opioids is discussed separately because of the availability of substantial supportive evidence.

A number of theories have been proposed to account for the role of the phenolic-hydroxy group in mu-receptor interaction and the consequent production of analgesia.

Foye (2013) has classified opioid drugs into two categories according to the pharmacophore responsible for mu-receptor interaction: the rigid multicyclic (morphinan) opioids (exemplified by morphine, codeine, and codeine congeners), and the flexible opioids (exemplified by

4-arylpyridinepethidine) [18]. To the latter category, we may add arylcyclohexylmethylamine, which is found in tramadol. The structures of the three categories are depicted in Fig. 1.8.

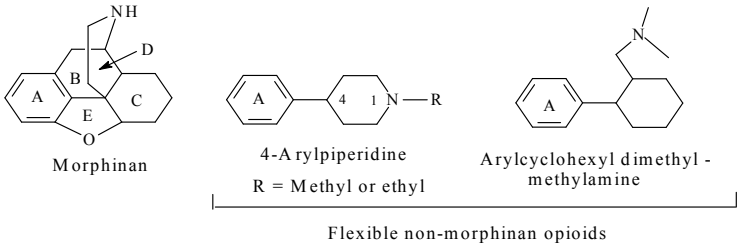


Fig. 1.8. Structures of the opioid drugs pharmacophores

Foye (2013) has argued the importance of a phenolic-hydroxy group on ring A (Fig. 1.1.8) to the mu-receptor interaction and analgesic activity of the rigid multicyclic morphinan opioids such as morphine and the *O*-desmethyl metabolites of codeine congeners [18]. The author also maintained that a phenolic-hydroxy group is not a requirement for the mu-receptor interaction and analgesic activity of the flexible non-morphinan opioids. It should be noted that none of the latter groups of drugs contain a phenolic hydroxy group and that, to our knowledge, aromatic-ring hydroxylation has not been reported as a metabolic route of any of them.

Foye's theory of "phenolic OH binding liability" for the flexible opioids (18) may be contested based on observations from the metabolic activity of *O*-desmethyltramadol (a metabolite of tramadol) (Fig. 1.6) and ketomebidone (an analogue of pethidine) (Fig. 1.9). Both drugs can be categorized as non-rigid flexible non-morphinan opioids. *O*-Desmethyltramadol exhibits a 200-fold increase in mu-receptor affinity and analgesic activity relevant to tramadol [19]. Ketomebidone, on the other hand, has four-fold mu-receptor affinity and analgesic activity compared to pethidine [20].The enhanced mu-receptor affinity and analgesic activity of both O-desmethyltramadol and ketomebidone can be attributed to the phenolic hydroxy group, which is reminiscent in position to that of morphine.

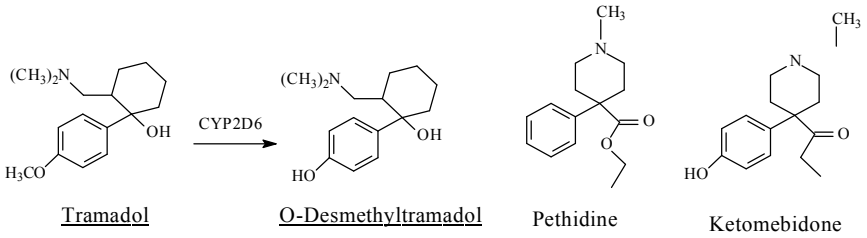


Fig.1.9. Tramadol/O-desmethyltramadol and pethidine/ketomebidone

Another opioid drug-receptor interaction theory has been suggested by Beckett and Casy(1959) [21] who stated that the macromolecule with which the analgesic interacts has, or attains, a certain conformation into which the phenolic group must fit before the biological effect (of analgesia) can occur. A "three-point" attachment of the macromolecule to a substrate's flat aromatic moiety, its basic center, and its hydrocarbon area was postulated.

Glucuronide and acetyl derivatives of morphine provide substantiating evidence of the phenolic hydroxy group's involvement in strengthening the mu-receptor affinity and, accordingly, the analgesic activity of the morphinan-opioid drugs that contain it, either intrinsically or metabolically. Further discussion of this point will follow.

By virtue of its phenolic-hydroxy group at position 3 and alcoholic hydroxy group at position 6 (Fig. 1.10), morphine forms two glucuronide conjugates: morphine-3-glucuronide and morphine-6-glucuronide (Fig.1.3). While morphine-6-glucuromide is a far more potent mu-receptor agonist and analgesic than morphine [22–24], morphine-3-glucuronide is devoid of both effects [25]. Furthermore, the *O*-glucuronide conjugates of both *O*-desmethyltramadol (Fig. 1.6) and levorphanol

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 July 2018 doi:10.20944/preprints201807.0143.v1

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119

(Fig. 1.7) are devoid of mu-receptor agonistic effects and, accordingly, analgesic activity [26-27]. The above data may be explained based on size and hydrogen-bonding ability differences between the hydroxy and glucuronide groups. In morphine, the hydrogen bonding provided by the 6-OH group is important for mu-receptor fitting and thus analgesic activity. With three hydroxy moieties, the glucuronide group at position 6 of morphine is capable of establishing more hydrogen bonds than the 6-OH. Further stronger interactions of the glucuronide group with the mu receptor involve ion-ion and ion-dipole binding provided by the carboxylate (COO-) moiety. Such state of affairs will most probably lead to stronger mu-receptor fit and hence higher analgesic activity of morphine-6-glucuronide than morphine. On the other hand, the size factor favors the hydroxy group at position 3 of morphine to anchor the aromatic ring in its hydrophobic pocket in the mu receptor rather than the considerably bigger glucuronide group. With reference to the tug-of-war hypothesis (to be discussed in section 1.1.3), the glucuronide group may even cause the dislodging of the aromatic ring from its hydrophobic pocket.

Upon comparing the mu-receptor affinity and analgesic activity of morphine, 6-acetylmorphine, and diamorphine (heroin) (Fig. 1.10), 6-acetylmorphine has been found to be the most active of the three opiates. It is four times as active as morphine. Heroin is also more active than morphine by a factor of two but less active than 6-acetylmorphine [28-31].

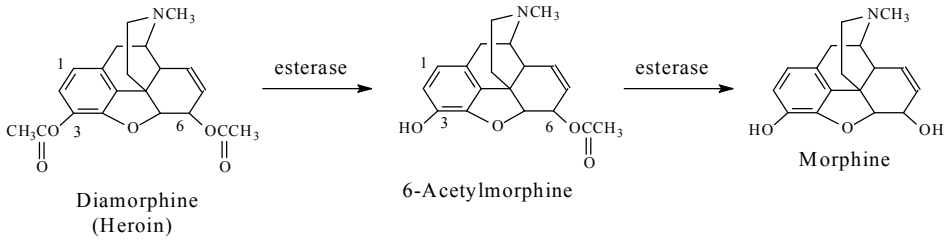


Fig. 1.10. Diamorphine, 6-acetylmorphine, and morphine

A plausible explanation of the above data is as follows: being more lipophilic than morphine, both 6-acetylmorphine and heroin (Fig. 1.1.10) will cross the blood-brain barrier faster and in higher concentrations than morphine. On the other hand, having a free phenolic hydroxy group, 6-acetylmorphine will interact with the opioid mu receptor more efficiently than diamorphine.In diamorphine, the free hydroxy group is generated metabolically in the brain by esterase hydrolysis, which will lead to a delayed effect and reduced efficacy. The effects of morphine and the two acetylated opiates (diamorphine and 6-acetylmorphine) can therefore be explained by a combination of pharmacodynamic and pharmacokinetic influences.

Extra substantiating evidence for the role of the free phenolic hydroxy group may be obtained from levomethorphan and its *O*-desmethyl metabolite levorphanol (Fig. 1.7). These two tetracyclic opiate drugs lack the 6-hydroxy group and hence the ability to form glucuronide conjugates at that position; yet, levorphanol has a stronger affinity for the mu receptor and analgesic activity than its parent drug levomethorphan [32].

In conclusion, we may summarize the role of the hydroxy group in arenolic opioidsinthe following statement: 'As shown in the opioid pharmacophores in Fig. 1.8, the aromatic rings labeled A, present in both morphinan and non-morphinan opioids, are essential components of the pharmacophore of the opioid-mu-receptor interaction. The high affinity of the phenolic opioids for the mu receptor is an indication of a logistic role played by the hydroxy group. Through hydrogen bonding with an adjacent amino-acid residue in the mu receptor, the hydroxy group plays the logistic role of anchoring the aromatic ring to the assigned hydrophobic pocket in the receptor, thus enhancing both affinity and efficacy. Substantiating evidence to the above statement is provided by the work of Sahu et al (2008) on the HIV-1-NNRT inhibitors, tetrahydroimidazobenzodiazepinones [33]. In this class of compounds, the predominant hydrophobic proper orientation for maximum effect has been found to be enhanced by hydrogen bonding and polar interactions.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 July 2018 doi:10.20944/preprints201807.0143.v1

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119

Vree's et al assertion that the analgesic activity of codeine being entirely due to its glucuronide conjugate [12] may now be reconsidered in view of the evidence so far presented about the role of phenolic hydroxy group in the opioid drugs. It is important to emphasize that Vree's et al. conclusion was abstract rather than experimental and, as such, may be subject to difference of opinion.

Because both codeine and tramadol have intrinsic analgesic activities, they can be viewed as prodrugs of morphine and *O*-desmethyltramadol respectively, both having sustained-release effects. Both morphine and *O*-desmethyltramadol are strong mu-receptor agonists used in the management of severe pain but having the disadvantage of causing dependence. Therefore, for the management of mild to moderate pain it would be advisable to use the corresponding prodrugs, codeine and tramadol, which have the advantage of sustained release. However, some people who are poor CYP2D6 metabolizers do not make use of the beneficial prodrug-sustained-release effect. For such people, it is advisable to adjust the dose of the parent drug, give the *O*-desmethyl metabolites, or seek alternative therapies. The frequency of the phenotype of poor metabolizers differs among ethnic groups. Less than 1% of Asians, 2-5% of African Americans, and 6-10% of Caucasians are poor metabolizers of CYP2D6 [34-36].

In addition to the pharmacodynamic receptor interactions of the methoxy opioids (occurring mainly through their polar hydroxy metabolites), the analgesic effects of hydrophobic opioid drugs, such as fentanyl, dextropropoxyphene, methadone, and pethidine (Fig. 1.11), have mainly been explained by pharmacokinetic effects. These drugs cross the blood-brain barrier more efficiently and, accordingly, they reach the mu-receptors in the brain in higher concentrations than the methoxy-group-containing members [18, 37].

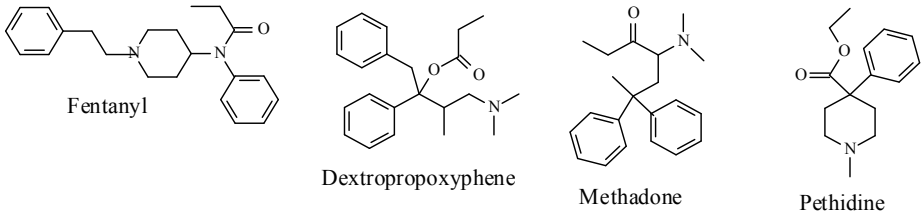


Fig. 1.11. Highly hydrophobic opioids

1.1.2. Acetanilide/Phenacetin/Paracetamol. The story of the development of the most commonly used analgesic antipyretic drug, paracetamol, as a metabolite drug of acetanilide and phenacetin, is depicted in Fig 1.12. Both the latter two drugs were once used as analgesics and antipyretics. Paracetamol results from phenacetin by metabolic *O*-deethylation [38] and from acetanilide by metabolic para-hydroxylation of the aromatic ring [39]. Compared to its two precursors, paracetamol has been found to have superior pharmacologic profiles, including toxicity and therapeutic index (38, 39). The free hydroxy group seems to give paracetamol the edge in inhibiting, through hydrogen-bonding interaction, two prostaglandin H2 synthases, now believed to be involved in the mechanism of action of paracetamol [40]. Besides, the hydroxy group may play the logistic role of anchoring the aromatic ring in the proper geometric orientation in the enzyme active cavity.

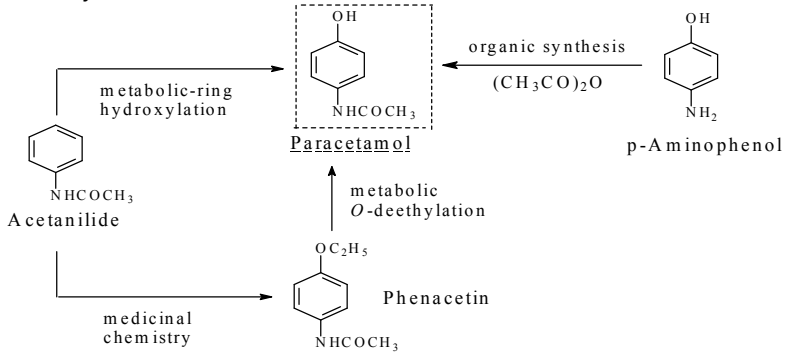


Fig. 1.12: From acetanilide to phenacetin to paracetamol

1.2. Metabolic *O*-Dealkylation of Aralkoxy Groups Resulting in Retaining of Pharmacologic Activity: Venlafaxine/Desvenlafaxine

Venlafaxine is an antidepressant in the SSRI category. It is mainly metabolized by O-demethylation to equiactive desvenlafaxine (*O*-desmethylvenlafaxine) (Fig. 1.13). Venlafaxine is further metabolized through N-demethylation and glucuronide conjugation to inactive products (Fig.1.1.13) [41-44]. Desvenlafaxine has been developed into a drug of its own right and approved by the FDA in the US for the treatment of major depressive disorder (MDD), similarly to its parent drug, venlafaxine, which is used for major depressive and anxiety disorders. However, the European Medicines Agency (EMA) had second thoughts and did not approve desvenlafaxine as a drug. They argue that venlafaxine is almost all metabolized to desvenlafaxine and that both compounds have essentially the same pharmacologic and pharmacokinetic profiles; hence, there is no strong reason to use desvenlafaxine as a separate drug [45].

Venlafaxine carries structural similarity to the analgesic drug tramadol. The latter drug exerts its analgesic effect mainly through serotonin-norepinephrine reuptake inhibition (SNRI) [46] and to a minor extent through blocking oh the mu-receptor. *O*-Demethylation of both venlafaxine and tramadol has resulted in active metabolites that have been developed into drugs of their own rights. The fact that neither venlafaxine nor desvenlafaxine has analgesic effects, and that neither tramadol nor *O*-desmethyltramadol has antidepressant effects, emphasizes the close link between drug action and drug structure.

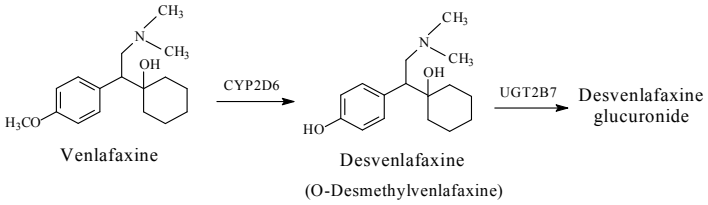


Fig.1.13: Metabolic pathway of venlafaxine

1.3. Metabolic *O*-Dealkylation of Aralkoxy Groups Resulting in Loss of Pharmacologic Activity: NSAIDS (Naproxen, Indomethacin and Nabumetone)

NSAIDS fall into five chemical classes: arylalkanoic acids, salicylates, fenamates, oxicams (cyclic sulfonamides), and diarylheteroaromatics. The benzene ring, either as a separate entity or fused with other rings, constitutes an integral part of the pharmacophore in all the NSAIDS chemical classes. The carboxyl group (in the form of carboxylate ion COO-) forms the other part of the pharmacophore in the arylalkanoic acid NSAIDS. With the exception of aspirin, the mechanism of action of the NSAIDS involves the competitive inhibition of arachidonic acid, the precursor of prostaglandins, from accessing the COX active cavity. The binding of the arylalkanoic acid NSAID to the amino-acid moieties in COX active cavity involves ion-ion and ion-dipole through the carboxylate group (COO-) and hydrophobic through alkyl and aryl groups [47-50] as depicted in Fig. 1.14.

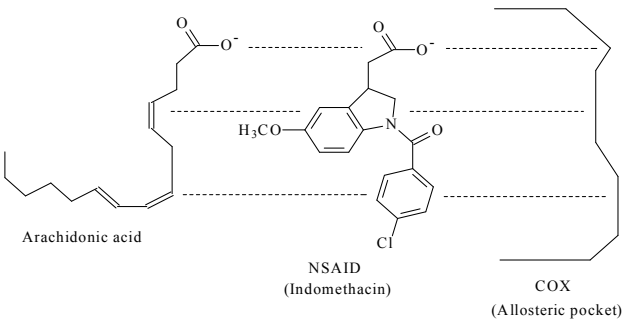


Fig. 1.14: An NSAID (Indomethacin) inside the COX receptor (adapted from [50])

Naproxen, indomethacin, and nabumetone (Figs. 1.15, 1.16, and 1.17 respectively) are NSAIDS that act through COX1/COX2 inhibition. To our knowledge, they are about the only three arylalkanoic acid NSAIDS that contain aromatic methoxy groups. Further, nabumetone is a prodrug NSAID, which must first be activated by metabolic oxidation to the carboxy derivative, 6-methoxynaphthylacetic acid, as shown in Fig. 1.17. Naproxen, indomethacin, and the active form of nabumetone are mainly metabolized through *O*-demethylation to give *O*-desmethylnaproxen, O-desmethylindomethacin, and 6-hydroxynaphthylacetic acid, respectively (Figs. 1.15, 1.16, and 1.17). The first two *O*-desmethyl metabolites are devoid of COX inhibitory effect and, accordingly, of NSAID activity [51-53]. By analogy, 6-hydroxynaphthylacetic acid, the *O*-desmethyl metabolite of nabumetone, is expected to be devoid of NSAID activity. According to Duggan et al. (1972), the p-hydroxy groups in the *O*-desmethyl metabolites place polar, hydrogen bond-donating properties within otherwise entirely aromatic-hydrophobic-pharmacophoric groups in the parent drugs [54]. Presumably, a poor metabolite-COX fit would result with a subsequent loss of pharmacologic activity. The loss of pharmacologic activity in the *O*-desmethyl arylalkanoic acid NSAIDS is further reflected on in the discussion section

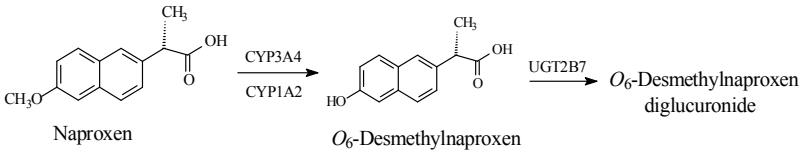


Fig. 1.15: Metabolic pathway of naproxen (51)

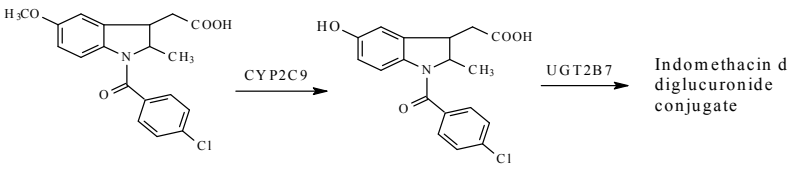


Fig. 1.16: Metabolic pathway of indomethacin (53, 54, 55)

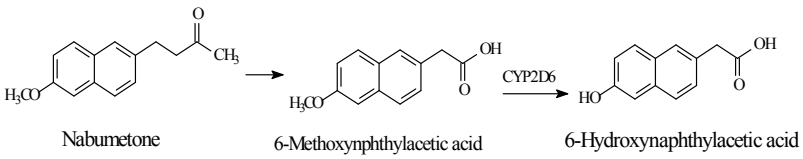


Fig. 1.17: Metabolic pathways of nabumetone (56, 57)

Overall Discussion of Section 1

Phase I metabolic *O*-dealkylation of aralkoxy groups almost invariably occurs in all the drugs containing these moieties.

From the cited drug examples in this section, metabolic *O*-dealkylation of aralkoxy groups has resulted in three situations regarding pharmacologic activity:

(1) Enhancement of activity is exhibited by the *O*-desmethyl morphinan opioids, *O*-desmethyltramadol and *O*-desethyl phenacetin,

(2) Retaining of activity is exhibited by *O*-desmethyl venlafaxine, and

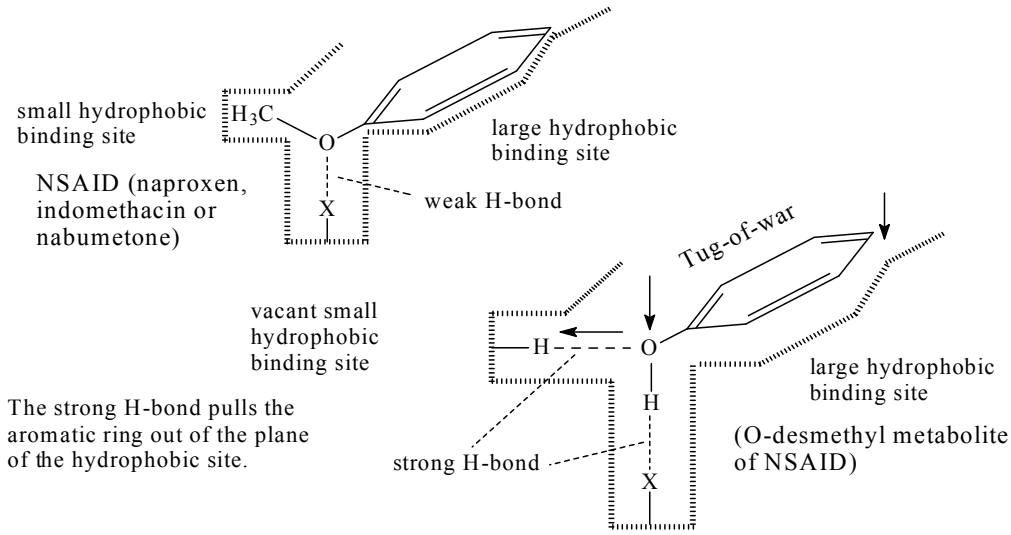
(3) Loss of activity is exhibited by *O*-desmethyl naproxen and O-desmethyl indomethacin.

Some inferences can be made from the effect on pharmacologic activity in the three NSAID cases upon considering the new state of structural affairs created by the loss of the hydrophobic methyl group and generation of the hydrophilic hydroxy group. The effect mostly depends on the site of drug action involved. The cases where the activity is enhanced involve the opioid drugs acting on the mu-receptor. In these drugs, the aromatic ring A, an integral part of the pharmacophore (Fig. 1.14), binds to the receptor through hydrophobic forces of attraction. The hydroxy group on the aromatic ring plays the logistic role of optimally anchoring the hydrophobic ring in its hydrophobic pocket in the mu-receptor [53, 58]. The same theory can be extended to the acetanilide/phenacetin/paracetamol case, where the sites of action are the H2 synthase enzymes used in the formation of prostaglandin, as has been recently proposed [39].

The loss of pharmacologic activity upon *O*-demethylation of the aryl-methoxy group is mainly observed in the NSAIDS indomethacin and naproxen and inferred for nabumetone. In this class of arylalkanoic acid NSAIDS, a hydrophobic moiety that fits into a hydrophobic pocket in COX is an essential requirement for activity as depicted in Fig. 1.14 (44, 50). *O*-demethylation of the aryl-methoxy group in these drugs results in two situations that may be explained in pharmacodynamic terms. Firstly, loss of the methoxy methyl group, which represents a small but essential hydrophobic interacting moiety with COX. Secondly, formation of a hydrophilic hydroxy group, which through hydrogen bonding with an adjacent amino-acid residue in COX can dislodge the hydrophobic moiety in the NSAID from its hydrophobic pocket in the enzyme. An analogy can be made to the tug-of-war hypothesis, as depicted in Fig. 1.18. The hydrogen-bonding and the hydrophobic binding site in COX respectively pull on the hydroxy group and the aromatic ring in the metabolite. Hence, both the hydroxy group and the aromatic ring in the metabolite represent the rope, while the two binding sites represent the players. The hydrogen-bonding interaction, being the stronger, wins the game and dislodges the aromatic ring out of the plane of interaction with the hydrophobic site in the enzyme. The situations described above will act synergistically with the loss of the methoxy methyl group to cause significant weakening of the drug-COX hydrophobic interaction, thus leading to loss of activity. However, a pharmacokinetic effect may not be excluded. Aromatic hydroxy groups are almost invariably metabolized in phase II to the highly water-soluble and rapidly eliminated glucuronide conjugates. Such effect will lead to reduction of the effective concentration of the metabolite at the receptor or enzyme active cavity thus resulting in curtailing or loss of activity. According to Fura (2006), attenuation or loss of pharmacologic activity is associated with biotransformation of pharmacophoric groups and the possible accompanying changes in physicochemical properties [59].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 July 2018 doi:10.20944/preprints201807.0143.v1

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119



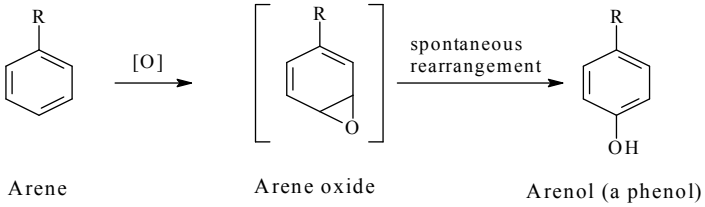
The arrows indicate the direction of the pull on the hydroxyl group by the H-bonding sites and on the aromatic ring by the large hydrophobic site. The strong H-bond pull causes the dislodging of the aromatic ring out of the plane of the hydrophobic pocket. The combined effect of the dislodging of the aromatic ring from its binding site and the loss of the methyl-group hydrophobic binding lead to loss of affinity of the NSAID to COX and hence loss of efficacy.

Fig. 1.18: Tug-of-war game between COX-active sites and NSAID/*O*-desmethyl NSAID

The retaining of antidepressant activity of desvenlafaxine indicates a non-essential hydrophobic binding role of the methoxy methyl group in venlafaxine. Nevertheless, a hydrogen-bonding role may not be excluded since it is provided by the methoxy oxygen in venlafaxine and by the hydroxy group in desvenlafaxine, however, with prominence in the latter case.

Section 2. Arenolic Metabolites Resulting from Aromatic Ring Hydroxylation

The mechanism of metabolic aromatic-ring hydroxylation involves, as a first step, the formation of an epoxide (arene oxide) intermediate, which rearranges rapidly and spontaneously to the arenol product in most instances (Fig. 2.1) [60, 61].



Aromatic-ring hydroxylation catalyzed by a variety of enzymes in different xenobiotic molecules

Fig. 2.1: Mechanism of aromatic-ring hydroxylation

Metabolic aromatic-ring hydroxylation assumes its importance from the fact that a large number of drug molecules contain the benzene ring as a separate entity (phenyl) or fused with other rings, alicyclic or heterocyclic. In drug molecules, aromatic rings serve the dual role of providing relatively large hydrophobic sites for interaction with receptors or acting as carriers for other functional groups. However, not all of the benzene rings in drug molecules are subject to metabolic hydroxylation. This is because certain electronic and steric effects dictate metabolic hydroxylation of aromatic rings. These effects include the following with possible anomalous events:

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119

(a) the least substituted aromatic ring will be favorably oxidized, especially at the least hindered carbon atom;
(b) the activated ring (i.e., the ring bearing an electron-donating group such as alkyl) will be better oxidized;
(c) ring-deactivating groups (generally groups with negative inductive effects such as halo and nitro groups) discourage ring hydroxylation;
(d) being the farthest from steric effects in di-substituted benzene rings, the para position is the favored site of hydroxylation;
(e) if two aromatic rings in a drug molecule have the same chemical environment, hydroxylation will occur in only one of them; and
(f) when the parent drug contains an aromatic hydroxy group, further metabolic hydroxylation is generally not favored even if there was more than one aromatic ring.

Aromatic-ring hydroxylation of drugs has led to the formation of inactive metabolites, metabolites with attenuated activity, and metabolites that are equiactive with the parent drugs. These situations have been reviewed using selected representative drugs. The basis for the choice of the candidate drugs is as follows:

- varying the chemical classes of the drugs;
- varying the pharmacologic class of the drugs and, accordingly, the type of drug-site-of-action interaction involved (i.e., the mechanism of action of the class of drugs); and
- varying the aromatic character in both number of rings and chemical environment.

Metabolic aromatic-ring hydroxylation leading to loss of activity is exemplified by:
(a) the CNS depressant anticonvulsant drugs, phenobarbital and phenytoin, of the imide chemical class (Fig. 2.2);
(b) the CNS depressant tranquilizer benzodiazepines diazepam (Fig. 2.3) and estazolam (Fig. 2.4);
(c) the arylalkanoic acid NSAIDs diclofenac, flurbiprofen, and ketorolac(Figs. 2.5-2.8) and the pyrazolone derivative phenylbutazone (Fig. 2.12);
(d) the anticoagulant warfarin (Fig. 2.9); and
(e) the proton-pump inhibitors (the 'prazoles').

Metabolic aromatic-ring hydroxylation leading to attenuation of activity is exemplified by the CNS depressant and major tranquilizer chlorpromazine (Fig. 2.10).

Metabolic aromatic-ring hydroxylation leading to the formation of equiactive products is exemplified by:
(a) the beta-blocker propranolol of the chemical class aryloxypropanolamine (Fig. 2.11); and
(b) the HMG-CoA reductase inhibitor atorvastatin (used in lowering blood cholesterol level) (Fig. 2.11).

Metabolic-ring hydroxylation leading to enhancement of activity is exemplified by acetanilide to paracetamol, which has already been discussed in section 1.1.

2.1. Metabolic aromatic-ring hydroxylation leading to loss of activity

2.1.1. Phenobarbital/Phenytoin. The anticonvulsant drug phenobarbital (Fig. 2.2) has been chosen as representative of the barbiturate group of drugs because it is the only member that contains an aromatic benzene ring susceptible to metabolic hydroxylation. On the other hand, the anticonvulsant drug phenytoin (Fig. 2.2) has been selected for its similarity to phenobarbital with respect to chemical structure, pharmacologic activity, and even mechanism of action. Phenytoin additionally contains two benzene rings with an identical chemical environment. Both phenobarbital and phenytoin are metabolized by aromatic-ring hydroxylation (Fig. 2.2), a process that has led to loss of pharmacologic activity [62-65].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 July 2018 doi:10.20944/preprints201807.0143.v1

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119

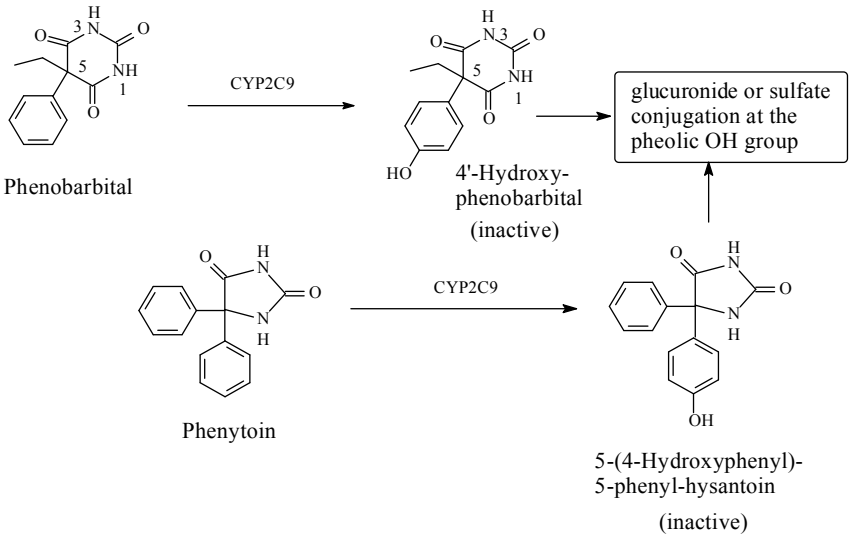


Fig. 2.2: Major metabolic pathways of phenobarbital and phenytoin

The barbiturates' CNS depressant activity (sedative, hypnotic, and anticonvulsant) and its termination are mainly dependent on the drugs' lipophilicity, which is imparted by the aromatic rings and the alkyl groups [66]. Lipophilicity helps the barbiturates to cross the blood-brain barrier, exert their effects, and again facilitate their distribution to other tissues, thus reducing their effective concentrations at the brain's GABA receptors. This redistribution process of the barbiturates is considered a deactivation process. In addition, metabolism plays a role in the deactivation of barbiturates. All barbiturates contain two alkyl groups at carbon 5 of the barbituric acid ring (Fig.2.2) with the exception of phenobarbital, which contains an alkyl group (ethyl) and a phenyl group. All the alkyl groups in barbiturates are metabolized by oxidation in phase I at the ω or ω-1 carbons to either primary or secondary alcohols, respectively. The primary alcoholic groups may further be oxidized to carboxyl (COOH) groups. On the other hand, the phenyl group in phenobarbital is metabolically oxidized to a phenolic group at the favored para position (Fig. 2.2). All such hydrophilic functionalities are detrimental to the essential hydrophobicity of the alkyl and phenyl groups and thus to the ability of the resulting metabolites to cross the blood-brain barrier. Enhancement of the water solubility of the barbiturate metabolites and their subsequent, fast elimination is a further cause of termination of pharmacologic activity resulting from the introduction of hydrophilic functionalities. Even more, the phase I metabolites, either carboxylic or phenolic, may further be conjugated in phase II to glucuronides and/or sulfates (Fig. 2.2) with a consequent further reduction in lipid solubility and inability to cross the blood-brain barrier. In addition, enhancement of water solubility and rapid elimination through the kidneys is a further deactivating process.

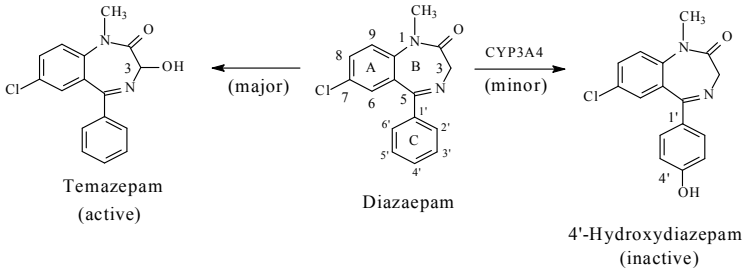
Despite the fact that the pharmacokinetics of the barbiturates play a major role in their deactivation, a pharmacodynamic dimension cannot be excluded: the introduction of a hydrogen-bonding functionality, such as the hydroxy (OH) group, on an essentially hydrophobic site, will most probably negatively affect binding to the GABA receptor, resulting in loss of pharmacologic activity. The tug-of-war analogy described for the NSAIDS in section 1.1.3 may be extended to barbiturates and phenytoin.

The two aromatic rings in phenytoin have identical chemical environments, and only one of them is hydroxylated, which is consistent with the rules of metabolic aromatic-ring hydroxylation. The same reasoning that applies for the loss of activity of the phenolic metabolite of phenobarbital discussed above should apply to the loss of activity of the phenolic metabolite of phenytoin.

2.1.2. Benzodiazepines: Diazepam and Estazolam. The benzodiazepines are CNS depressants used as minor tranquilizers, sedatives, hypnotics, and anticonvulsants; in these respects, they have largely superseded the barbiturates. Their mechanism of action involves binding to GABA receptors

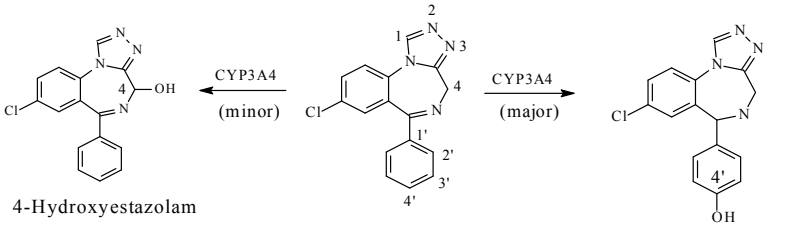
Peer-reviewed version available at *Molecules* **2018**, *23*, 2119; doi:10.3390/molecules23092119

[67-69]. The benzodiazepines are metabolized through several phase I oxidative reactions with some followed by phase II conjugative reactions. The metabolic pathways of the two benzodiazepine representative members, diazepam [70, 71] and estazolam [72, 73], are shown Figs. 2.3 and 2.4, respectively, with only aromatic-ring hydroxylation discussed in this section. The other metabolic pathways of diazepam and estazolam will be discussed where relevant.



The two hydroxy metabolites are further metabolized by glucuronide conjugation in phase II.

Fig. 2.3: Metabolic pathways of diazepam



The two hydroxy metabolites are further metabolized by glucuronide conjugation in phase II.

Fig. 2.4: Metabolic pathways of estazolam

Due to favorable electronic and steric structural environments, diazepam and estazolam, to our knowledge, are the only two members of the clinically used benzodiazepines to undergo metabolic aromatic-ring hydroxylation at the 4′ position (Fig. 2.3 and Fig. 2.4). In most of the other members, metabolic 4′-hydroxylationis is possibly disfavored by the presence of an electron-withdrawing halo group at position 2′ (fluoro in flurazepam, flunitrazepam, and quazepam, and chloro in triazolam and clonazepam). (For the numbering of the benzodiazepine ring system, refer to the structure of diazepam in Fig. 2.3.)

4′-Hydroxylation of both diazepam [70, 71] and estazolam [72, 73] has resulted in inactive metabolites. Foye (2013) has attributed the loss of sedative-hypnotic effects of 4′-hydroxyestazolam to two factors [72]. The first factor is of pharmacodynamic nature. It is explained by the 4′-hydroxy group weakening optimal binding of the aromatic ring to $GABA_A$ receptor by a steric effect. The result is decreased receptor affinity and drug potency. Interestingly, in its essence, the steric hindrance factor proposed by Foye (2013) [72] lends credence to our *tug-of-war* hypothesis discussed in section 1.3. The second factor is of a pharmacokinetic disposition and results from decreased hydrophobicity (i.e., increased hydrophilicity), which results in the decrease of the effective concentrations of the circulating 4′-hydroxy metabolites due to enhanced polarity, water solubility, and elimination, as per se and as glucuronide conjugates. In the absence of reports regarding the loss of activity of 4′-hydroxydiazepam, an analogy may be extrapolated from that of 4′-hydroxyestazolam.

In contrast to 4′ hydroxylation, metabolic hydroxylation at position 3 of the diazepine ring in diazepam (Fig. 2.3) has not affected activity but has rather introduced a pharmacokinetic dimension: the 3-hydroxy metabolite is more hydrophilic and is subject to glucuronide conjugation with

subsequent enhanced rate of elimination and hence shorter duration of action than diazepam. These observations tend to consolidate a major pharmacodynamic role of 4′-aromatic-ring hydroxyl group on causing loss of activity of diazepam as well as estazolam. The plausible explanation is that the introduction of the hydrogen-bonding group (the hydroxy) into an essentially pharmacophoric hydrophobic moiety (the benzene ring) is detrimental to the optimal $GABA_A$ receptor binding as can be inferred from Foye's (2013) explanation [72].

A halo group at position 2′ of the benzodiazepine backbone (ring C, Fig. 2.3) serves three purposes: it adds a welcomed hydrophobicity to the drug, disfavors metabolic ring hydroxylation, and imparts a conformation-locking effect on the aromatic ring through a steric effect. The consequence of these effects could be enhanced selectivity on drug-receptor interaction leading to higher efficacy and possibly higher potency. Increased hydrophobicity would tend to enhance blood-brain barrier penetration and therefore increased access to the $GABA_A$ receptor.

In the above context, it would be worthwhile to investigate the effect of another halo group at position 6′ (Fig. 2.3) on the conformation locking of the aromatic rings in comparison to the NSAID pair fenoprofen/diclofenac. In diclofenac (Fig. 2.5), the two ortho-positioned chloro groups have resulted in a restricted conformation with consequent enhanced selectivity, efficacy, and potency compared to fenoprofen, in which the two chloro groups are absent [74].

2.1.3. NSAIDS.

The chemical classes of the NSAIDS have been discussed in section 1.3.

2.1.3.1. Diclofenac. Diclofenac is a phenylacetic acid NSAID. It was developed as a variant of fenoprofen by introducing two ortho-positioned chloro groups in the anilino-aromatic ring to restrict its free rotation (74). This restriction of rotation increases selectivity and hence potency with respect to fenoprofen.The metabolism of diclofenac shown in Fig. 2.5 [75] represents one of the anomalies of aromatic-ring hydroxylation in that hydroxylation occurs at the meta position of two chloro groups. The hydroxy metabolites are pharmacologically inactive.

In accounting for the structure-activity relationship of diclofenac, Foye (2013) [75] has proposed that the function of the two ortho chloro groups was to force the anilino-phenyl ring out of the plane of the phenylacetic acid portion. Such twisting, as proposed by Foye (2013) [75], is important in the binding of diclofenac to the active site of COX. The introduction of a hydroxy group in the anilino-phenyl group would create a hydrogen-bonding character, which would weaken or hinder the necessary twisting, thus resulting in attenuation or loss of pharmacologic activity.

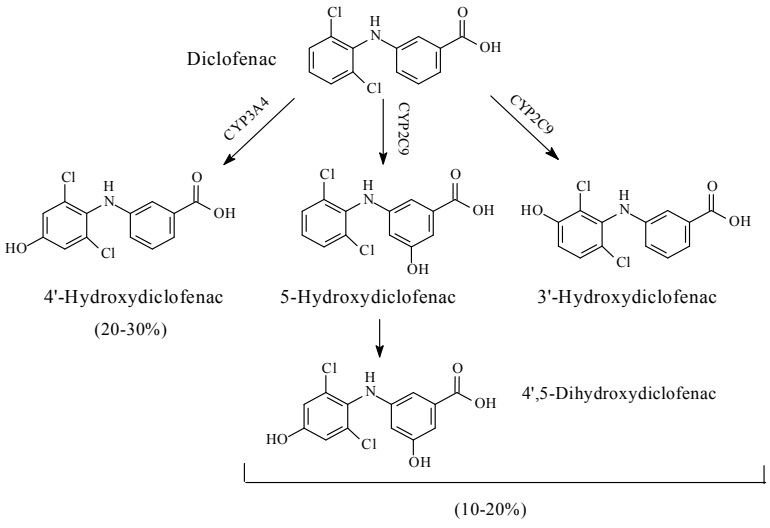


Fig. 2.5. Metabolic pathways of diclofenac

2.1.3.2. Ketorolac. Ketorolac (Fig. 2.6) is a pyrrole-acetic acid derivative structurally related to indomethacin and tolmetin. It is metabolized to p-hydroxyketorolac (Fig. 2.6), which is inactive.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 July 2018 doi:10.20944/preprints201807.0143.v1

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119

Both the carboxy and phenolic hydroxy groups are further metabolized by glucuronide conjugation to give pharmacologically inactive products [76]. In analogy to the previously discussed cases, the loss of activity of 4-hydroxy ketorolac may be attributed to both pharmacodynamic and pharmacokinetic effects.

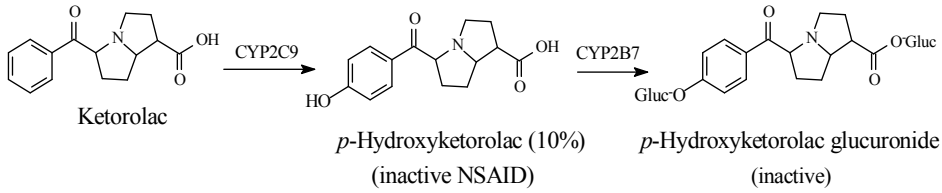


Fig. 2.6. Metabolic pathways of ketorolac

2.1.3.3. Flurbiprofen. Flurbiprofen is an arylpropionic acid COX1/COX2-inhibitor NSAID. It is mainly metabolized by aromatic-ring hydroxylation as shown in Fig.2.7with loss of activity [77]. The metabolism of flurbiprofen shows a rather interesting pattern in that a catechol ring is formed in which the 4′-position is anomalously methylated to yield a methoxy group with reduced polarity. This metabolic route is reminiscent of that of adrenaline, which is metabolized by the methylation of the para-hydroxy group by the enzyme catechol-O-methyl transferase (COMT). Furthermore, the metabolic double hydroxylation of flurbiprofen to yield 3′,4′-dihydroxy flurbiprofen is also an anomaly of metabolic-ring hydroxylation. We recall metabolic aromatic-ring dihydroxylation in the same molecule is generally disfavored [60, 61].

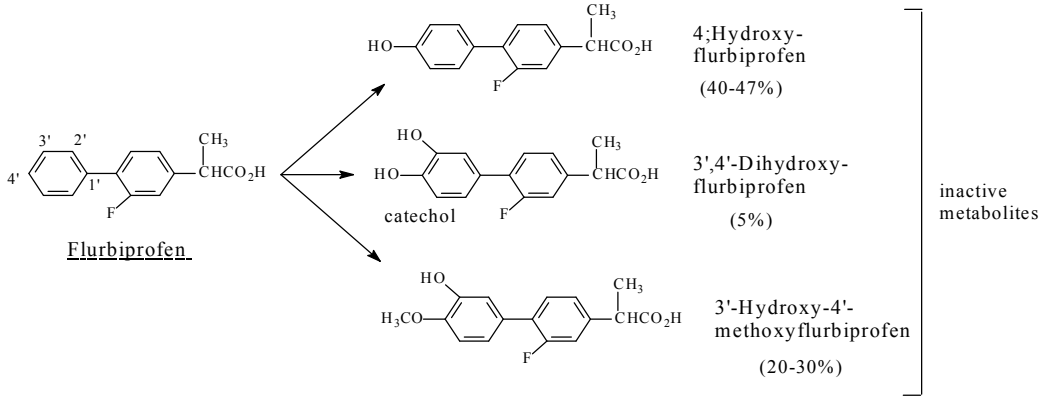


Fig. 2.7. Metabolic pathways of flurbiprofen

2.1.4. Miscellaneous

2.1.4.1. Warfarin. Warfarin (Fig. 2.8) is an anticoagulant drug used as prophylactic in preventing thrombus formation in susceptible patients. Warfarin is chiral with the *S*-enantiomer having five-fold the activity of the *R*-enantiomer [78]. Warfarin contains two aromatic groups: a benzopyran and a phenyl in addition to a 2-butanone chain (Fig. 2.8). The major metabolic pathway of warfarin is through hydroxylation of the benzene ring of the benzopyran moiety, in addition to a minor route through the reduction of the side-chain keto group to a secondary alcohol (Fig. 2.8) [79-82]. The acidic hydroxy group at C4, being in conjugation with the benzene ring, possibly explains the preference of metabolic hydroxylation of the benzopyran ring over the phenyl group since it increases the electron density toward hydroxylation through a positive inductive effect. It has been observed that the hydroxy group introduced metabolically on the aromatic ring has led to loss of anticoagulant activity while the hydroxy group resulting from side-chain keto reduction has resulted only in attenuation of activity [79]. Keto-group reduction and pharmacologic activity are the subject of alcoholic metabolites to be discussed in part II of this review series.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 July 2018 doi:10.20944/preprints201807.0143.v1

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119

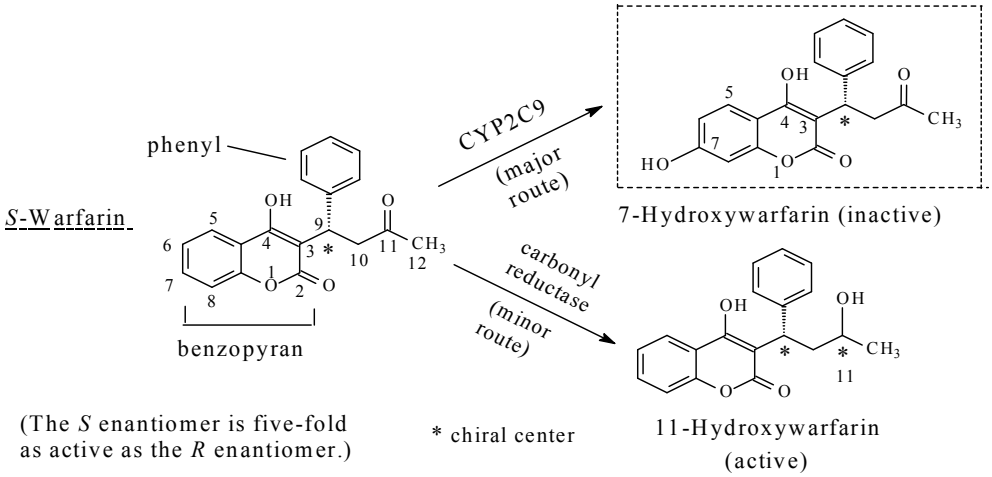


Fig. 2.8. Metabolic pathways of warfarin

2.2. Metabolic Aromatic-Ring Hydroxylation Resulting in Attenuation of Activity

2.2.1. Chlorpromazine. Chlorpromazine (Fig. 2.9) is a major tranquilizer used as an antipsychotic. It is metabolized in humans by two major routes [83-87]: (a) aromatic-ring hydroxylation at position 7 to a moderately active metabolite, and (b) sulfoxidation to an inactive metabolite (Fig. 2.9). A minor deactivating route through N-demethylation also occurs. It should be noted that the metabolic hydroxylation of chlorpromazine is in accordance with the rule that groups with negative inductive effects, such as chloro, deactivate the ring to hydroxylation.

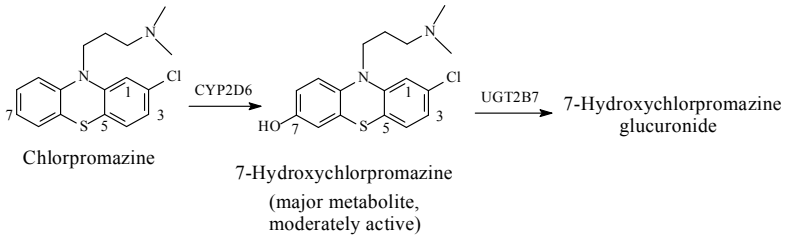


Fig. 2.9: Metabolic pathways of chlorpromazine

2.3. Aromatic-ring hydroxylation resulting in parent-drug equiactive metabolites

2.3.1. Propranolol. Propranolol (Fig. 2.10) is an aryloxypropanolamine β–adrenoceptor blocker used as an antihypertensive and antiangina agent. As shown in Fig. 2.10, it is metabolized in humans to three major metabolites, two of which are inactive and one of which is as active as the parent drug. In naphthoyloxyacetic acid (Fig. 2.10), the pharmacophore is ruptured, leading to loss of activity. In metabolite (II), glucuronide conjugation of the side-chain hydroxy group has led to loss of activity while in metabolite (III), activity is maintained upon introduction of a hydroxy group para to the side chain. The glucuronide conjugate of the aromatic-ring hydroxy metabolite is inactive [88, 89]. The metabolism of propranolol directs attention to two interesting points: (a) glucuronic-acid conjugation of both alcoholic and phenolic hydroxy groups leads to loss of pharmacologic activity, a phenomenon that is true for most cases, and (b) alkoxy groups are aromatic-ring activators and para-directors in metabolic hydroxylation.

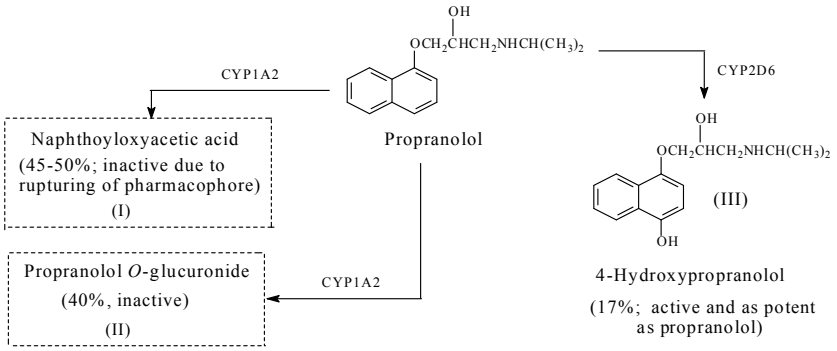


Fig. 2.10: Metabolic pathways of propranolol

2.3.2. Atorvastatin. Atorvastatin (Fig.2.11) is a HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase inhibitor used in lowering blood cholesterol and triglyceride levels. Atorvastatin is metabolized by CYP3A4 hydroxylation at the ortho- or para-position at ring D as shown in Fig. 2.11 [90, 91]. Being electron withdrawing, both the fluoro group and the pyrrole ring (C) disfavor metabolic hydroxylation of rings A and B, respectively. In accordance with the rule, the hydroxylation takes place at the least hindered benzene ring, i.e., ring D. The two metabolites are equiactive with the parent drug and account for about 70% of its overall circulating activity [90, 91].

The molecule of atorvastatin can be dissected into two parts: the dihydroxyheptanoic acid moiety and the aromatic ring system with its substituents. Dr. Philip Portoghese, a medicinal chemist from the University of Minnesota, has developed a concept called "Message-Address," which conceptually breaks a drug molecule up into two components: one, which "finds" the active site (the address), and the other, which actually delivers the drug's chemical message (92). In atorvastatin, the dihydroxyheptanoic acid moiety represents the message, while the aromatic-ring system with substituents represents the address. When the address is substantially large, a small-group metabolic change is not expected to result in a significant impact on its role.This is true for atorvastatin, which contains a four-ring system that mainly interacts with the active site of the enzyme via hydrophobic binding.

Since the term 'pharmacophore' is mostly used in the pharmacodynamics of drug action, we propose, an adaptation of Dr. Portuguese's concept by using the terms 'primary pharmacophore' and 'auxiliary (logistic) pharmacophore' as equivalent terms to 'message' and 'address', respectively. By binding to the receptor, the auxiliary (logistic) pharmacophore will facilitate the anchoring of the primary pharmacophore prior to its binding and competitive inhibition of the physiologic substrate.

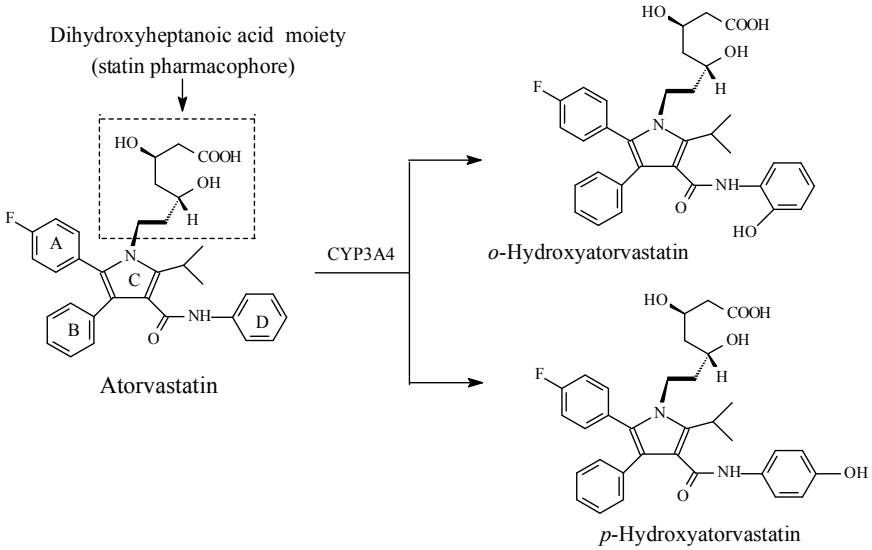


Fig. 2.11: Metabolism of atorvastatin

2.3.3. Phenylbutazone. Two metabolites of Phenylbutazone (Fig. 2.12) which were isolated from human urine possess some of the pharmacological activities of the parent drug. Metabolite I (oxyphenbutazone), formed by aromatic-ring hydroxylation, has the potent antirheumatic and sodium-retaining effects of phenylbutazone; it has been developed into a drug of its own. On the other hand, metabolite II, formed by the hydroxylation of the ω-1 carbon of the butyl side chain, also possesses reduced sodium-retaining and antirheumatic properties, but it is a considerably more potent uricosuric agent than phenylbutazone [93, 94].

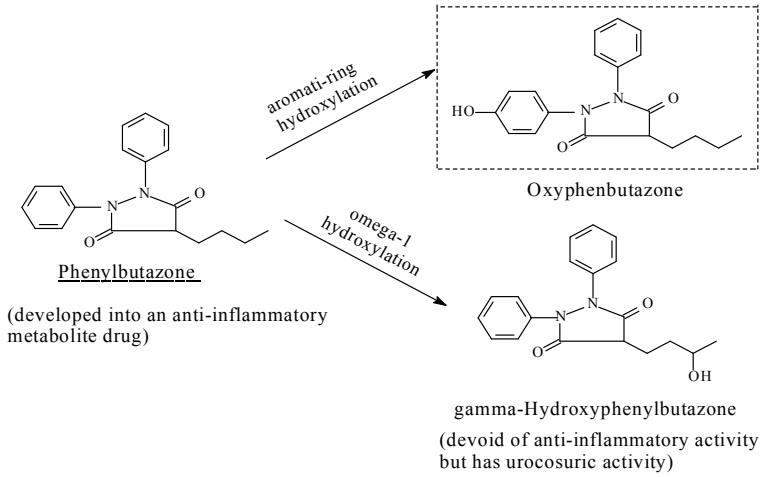


Fig. 2.12: Metabolism of phenylbutazone

Phenylbutazone binds to and deactivates prostaglandin H synthase and prostacyclin synthase through peroxide- (H_2O_2-) mediated deactivation. The reduced production of prostaglandin leads to reduced inflammation in the surrounding tissues [94]. It is also pertinent to note that γ-hydroxyphenylbutazone, which results from ω-1 hydroxylation of the butyl-side chain in phenylbutazone, is devoid of anti-inflammatory activity [95]. Metabolic-aliphatic-hydroxylation and pharmacologic activity of the resulting metabolites is the subject of Part II of this review series.

1.2.4. Discussion of Metabolic Aromatic-Ring Hydroxylation

For the drug-cases reviewed in this section, except for diclofenac, the structural features, either electronic or steric, set above for the occurrence of aromatic-ring hydroxylation, conform well to the rule of thumb.

Loss, decrease, or retention of pharmacologic activity upon aromatic-ring hydroxylation in the cited cases may reflect the status of the ring in the parent drug regarding its mechanism of interaction with the receptor. When hydrophobic binding of the aromatic ring with the receptor is essential for activity, introduction of the hydrophilic-hydrogen-bonding hydroxy group will compromise the fit and will not be tolerated. The established hydrogen bonding may force the ring out of the plane of interaction with the receptor; the result will be loss of activity. This has been the case with phenobarbital and phenytoin (Fig. 2.2), diazepam, (Fig. 2.3), estazolam (Fig. 2.4), NSAIDS (Figs. 2.5, 2.6, and 2.7), and warfarin (Fig. 2.8). The tug-of-war hypothesis suggested for the *O*-desmethyl-NSAIDS in section 1.1.3 may be extended to the above cases. Furthermore, the phase II glucuronide conjugation of the aromatic-hydroxy group is an important factor in causing loss of activity of the metabolite. It considerably enhances metabolite clearance and accordingly reduction of its effective concentration at the receptor.

When aromatic-ring hydroxylation results in decreased activity, as in 7-hydroxychlorpromazine (Fig. 2.9), four inferences present themselves: (a) the hydroxy group has resulted in increase of the optimum chlorpromazine molecular size; (b) the hydroxy group weakens

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 July 2018 doi:10.20944/preprints201807.0143.v1

Peer-reviewed version available at Molecules 2018, 23, 2119; doi:10.3390/molecules23092119

optimal binding of the aromatic ring to the receptor by a steric effect (c) the *tug-of-war* effect is only partially operative; and (d) the hydroxylated ring is playing an auxiliary role. In addition, reduction of effective concentration of the hydroxy metabolite at the receptor through glucuronide conjugation may be playing an important role.

When the hydroxy metabolite is equiactive with the parent drug, two inferences can be tentatively made. Firstly, the hydroxylated aromatic ring is auxiliary; i.e., it plays the role of the address. This is the case with the statin drug atorvastatin (Fig. 2.11), where the three aromatic rings are not part of the primary pharmacophore and are therefore not involved in essential binding to the enzyme [96]. Their role, however, is logistic, that of proper anchoring of the drug in the enzyme active cavity for optimum interaction of the primary pharmacophoric groups with the enzyme to take place. The second inference is associated with 4-hydroypropanolol (Fig. 2.10), the equiactive metabolite of propranolol. Propranolol is a nonselective β1/β2-adrenoceptor blocker; it belongs to the aryloxypropanolamine chemical class. In this class of compounds, hydrophilic amide substitution at position 4 of the aromatic ring imparts β1 antagonistic selectivity [97], such as in atenolol and practolol (Fig. 2.13). On the other hand, hydrophilic hydroxy substitution at the same position reverses the activity altogether, i.e., from antagonistic to agonistic, such as in prenalterol (Fig. 2. 13) [98]. It can hence be concluded that the nature of the hydrophilic group substitution at position 4 of the aryloxypropanolamines significantly dictates the pharmacologic outcome of the β1-receptor interaction. Based on the above findings, it may be inferred that 4-hydroxypropranolol is a tentative β1-adrenoceptor agonist pending experimental testing.

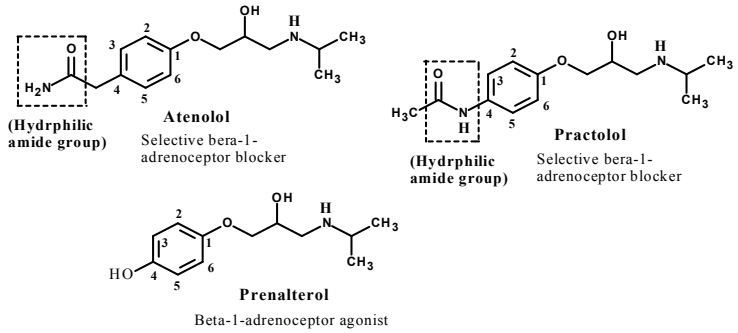


Fig. 2.13: Structures of atenolol and practolol

The atorvastatin equiactive hydroxy metabolites furnish a useful inference: pharmacologic equiactivity of metabolites relative to the parent drug occurs when the metabolic change takes place at the "address" or "auxiliary pharmacophore". We will provide further examples in review 2 of this series.

Of the NSAIDS, phenylbutazone stands in a class of its own in that its mechanism of action does not involve inhibition of COX but rather the inhibition of prostaglandin H synthase. As has been shown in this section and in section 1.3, a hydroxy group on the aromatic rings of arylalkanoic-acid COX1/COX2-inhibitor NSAIDS is detrimental to their pharmacologic activity. This is in contrast to phenylbutazone whose aromatic-hydroxy metabolite (oxyphenbutazone (Fig. 2.12)) is equiactive with the parent drug and has been developed into an anti-inflammatory drug of its own right. The different mechanisms of action and, accordingly, the varying sites of drug action involved may explain the disparity between the activities of the hydroxy-metabolites of phenylbutazone and the arylalkanoic-acid NSAIDS.

As pharmacodynamics may handsomely explain the pharmacological activity of drug hydroxy metabolites relative to the parent drugs, the role of the pharmacokinetics of these metabolites should not be excluded. In the cases where the pharmacologic activity of the hydroxy metabolite is either attenuated or lost relative to the parent drug, pharmacokinetic factors may come into perspective in two aspects. Firstly, the hydroxy metabolites are rapidly cleared by phase II conjugation, thus aiding in the termination of their action. Secondly, the hydroxy metabolites do not readily penetrate target

tissues due to a decrease in membrane permeability caused by an increase in polar surface area, a limitation that affects their active concentrations at the target site [99].

Glucuronide conjugation: prevalence and effect on pharmacologic activity

Glucuronide conjugation is the most common phase II metabolic process [100] though certain structural features control its occurrence. It occurs with almost all aromatic hydroxy (arenolic) groups, most carboxyl groups, unhindered alcoholic hydroxy groups and a few amino and sulfhydryl groups. Because of its relatively big size, glucuronic acid may not have easy access to active-hydrogen containing groups that are awkwardly situated in a molecule, i.e. sterically hindered groups. For instance, tertiary alcoholic groups such as 14-hydroxy in oxycodone (Fig.1.5) and 1'-hydroxy in tramadol (Fig. 1.6) are sterically hindered and are hence not susceptible to glucuronide conjugation.

With the exception of codeine glucuronide and morphine-6-glucuronide, glucuronide conjugate has led to loss of activity of all the cases presented in this review. With three hydroxy moieties and a completely ionized carboxyl moiety at physiologic pH, the glucuronide group considerably increases metabolite hydrophilicity, water solubility, elimination and termination of action. With this pharmacokinetic concept of loss of activity of most drug glucuronide conjugates, the door is open only to pharmacodynamic speculation to explain the activity of codeine glucuronide and morphine-6-glucuronide.

Conclusion

The hydroxy group is the most common hydrophilic group produced by metabolic functionalization in drug molecules. Aromatic hydroxy groups result in one of two ways: *O*-dealkylation of aralkoxy groups or hydroxylation of the aromatic ring. *O*-dealkylation of aralkoxy groups in drug molecules is an invariably predictable metabolic route. On the other hand, metabolic aromatic-ring hydroxylation is governed by electronic and steric factors prevailing in the ring. The pharmacologic activity of the resulting arenolic metabolites resulting from both processes depends on the site of drug action (i.e. the receptor) as well as on the pharmacophoric or auxophoric status of the aromatic ring to which the alkoxy or hydroxy group is bonded. In general terms, phase I metabolic functionalization may reveal the status of a group in a drug molecule, whether primary or auxiliary pharmacophoric or auxophoric. As well, if there is pre-knowledge of the pharmacophoric or auxophoric statuses of the rings, then the effect of the metabolically formed hydroxy group on activity of the metabolite may be predicted. In addition to pharmacodynamic effects, the attenuation or loss of activity of polar metabolites may be explained by pharmacokinetic effects, which by enhancing elimination lead to decreased metabolite effective concentration at the receptor.

When more active or equiactive metabolites have shown favorable pharmacodynamic and pharmacokinetic properties, they have been developed into drugs of their own rights. Nevertheless, not all equiactive metabolites have been developed into drugs of their own rights and may hence be classified as drug-action-extension forms.

Acknowledgement. We would like to thank Dr. J. DeRuiter for the use of the diagram in Fig. 50. We also would like to apologize to Dr. DeRuiter for using the diagram without securing his/her consent since we were not able to track his/her address.

References

1. Williams D.A. Drug Metabolism.In: Foye's Principles of Medicinal Chemistry.7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S. W., Eds.; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; p. 135; ISBN-13: 978-1-60913-345-0

2. Williams D.A., Roche V.E., Roche E.B. Central Analgesics, Opioid receptors. In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds.; Wolters

Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; pp. 666-670; ISBN-13: 978-1-60913-345-0

3. 15. Satoh M., Minami M. Molecular pharmacology of opioid receptors. Pharmacol. Ther. 1995, 68, 343-364

4. The opium alkaloids. UNDOC, creation date 1953/01/01, pp 13-14

5. Nogrady T. Medicinal Chemistry, A Biochemical Approach, 2nd ed.; Oxford University Press, Oxford, UK,1988; p. 316; ISBN 0-19-505368-0 0-19-505369-9

6. Mikus G., Weiss J. Influence of CYP2D6 on Opioid Kinetics, Metabolism and Response. Curr. Pharmacogenomics Person. Med. 2005, 3, 43-52

7. Codeine metabolism pathway. Pharmacokinetics. PharmGKB http://www.pharmgkb.org/patheay/PA146123006 (URL accesses on 20.11.2017)

8. Thompson C.M., Wojno H., Greiner E., May E.L., Rice K.C., Selley D.E. Activation of G-Proteins by Morphine and Codeine Congeners: Insights to the Relevance of O- and N-Demethylated Metabolites at μ- and δ-Opioid Receptors. J. Pharmacol. Exp. Therap. 2004, 208, 547-554

9. Patrick J.L. An Introduction to Medicinal Chemistry. 4th ed.; Oxford University Press, Oxford, UK, 2009; p. 634; ISBN 978-0-19-923447-9

10. Alder T.K. The comparative potencies of codeine and its demethylated metabolites after intravenous injection in the mouse. J. Pharmacol. Exp. Ther. 1963, 140, 155-161

11. Findlay J.W.A., Jones E.C., Butz R.F., Welch RM. Plasma codeine and morphine concentrations after therapeutic oral doses of codeine-containing analgesics. Clin. Pharmacol. Ther. 1978, 24, 60-68

12. Vree T.B.1., van Dongen R.T., Koopman-Kimenai P.M. Codeine analgesia is due to codeine-6-glucuronide, not morphine. Int. J. Clin. Pract. 2000, 54, 395-98

13. Vallejo R., Barkin R.L., Wang V.C.Pharmacology of Opioids in the Treatment of Pain Syndromes. Pain Physician. 2011, 14, E343-E360

14. Trescot A.M.,Datta S., Lee M. Opioid Pharmacology. Pain Physician. Opoiod Special Issue. 2008, 11, S133-S153

15. Chen Z.R,, Irvine RJ, Somogyi A.A., Bochner F.Mu receptor binding of some commonly used opioids and their metabolites. Life Sci. 1991, 48, 2165-71

16. Howard S., Smith M.D. Opioid Metabolism. Mayo Clin Proc · v.84(7); 2009 Jul; PMC2704133

17. Leppert W. CYP2D6 in the Metabolism of Opioids for Mild to Moderate Pain. Pharmacology, 2011, 87:274-285

18. Williams D.A., Roche V.E., Roche E.B.. Central Analgesics.Opioid receptors.In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; pp. 658-690; ISBN-13: 978-1-60913-345-0

19. Mario G. Tramadol Vs Tapentadol: Anew Horizon in Pain Treatment? Am. J. Animal. and Vet. Sci. 2012, 7, 7-11

20. Bergel I., Morrison A.L. Pethidine ketomebidone. Quart. Rev. Chem. Soc. 1948, 2, 349

21. Beckett and Casy Model, Pharmacological Sciences How Drugs Act. https://www.pharmacologicalsciences.us/how-drugs-act/och3.html. Last Updated on Sat, 15 Apr 2017 How Drugs Act. (URL accessed on 05.11.2017)

22. Carrupt P.A., Testa B., Bechalany A., Tayar N., Descas P., Perrissoud D. Morphine 6-glucuronide and morphine 3-glucuronide as molecular chameleons with unexpected lipophilicity. J. Med. Chem.1991, 34, 1272-5.

23. McCurdy C.R. Development of opioid-receptor ligands. In: Analogue-Based Drug Discovery. Fisher J.C., Robin Ganellin C.R. Eds; WILEY-VCH Verlag GmbH and Co. KGaA, Weinheim, Germany (on-line), 2006; p 263; ISBN 9783527608003

24. Paul D., Standifer K.M., Inturrisi C.E., Pasternak G.W. Pharmacological characterization of morphine-6-glucuonide, a very potent morphine metabolite. Pharmacol. Exp. Therap. 1989, 251, 477-83

25. Vindenes V., Ripel A., Handal M., Boix F.Mørland J. Interactions between morphine and the morphine-glucuronides measured by conditioned place preference and locomotor activity. Pharmacol. Biochem. Behav. 2009, 93, 1–9

26. Päivi L., Taina S., Olli A., Mika K., Katariina V., Moshe F., Risto K. Glucuronidation of racemic O-desmethyltramadol, the active metabolite of tramadol. Eur. J. Pharm. Sci. 2010, 41, 523-30

27. Dixon R., Crews T., Inturrisi C., Foley K. Levorphanol: pharmacokinetics and steady state plasma concentrations in patients with pain. Res. Commun. Chem. Pathol. Pharmacol. 1983, 41, 3-17

28. Hubner C.B., Kornetsky C. Heroin, 6-acetylmorphine and morphine effects on threshold for rewarding and aversive brain stimulation. J. Pharmacol. Exp.Ther.1992, 260, 562-7

29. Inturrisi C.E., Schultz M., Shin S., Umans J.G., Angel L., Simon E.J. Evidence from opiate binding studies that heroin acts through its metabolites. Life Sci. 1983, 1, 773-6.

30. Patrick G.L. An Introduction to Medicinal Chemistry, 4thed., Oxford University Press, Oxford, UK, 1995; p 637; ISBN: 978-0-19-923447-9

31. Hubner C.B.1., Kornetsky C. Heroin, 6-acetylmorphine and morphine effects on threshold for rewarding and aversive brain stimulation. J. Pharmacol. Exp. Ther. 1992, 260, 562-7

32. Gudin J., Fudin J., Nalamachu S. Levorphanol Use: Past, Present and Future. Postgraduate Medicine. 128, 2016, 1-24

33. Sahu V.K., Khan A.K.R., Singh R.K, Singh P.P. Hydrophobic, Polar and Hydrogen Bonding Based Drug-Receptor Interactions of Tetrahydroimidazobenzodiazepinones. Am. J. Immunol. 2008, 4, 33-42

34. Kalow W., Otton S.V., Kadar D. Ethnic difference in drug metabolism: debrisoquine 4-hydroxylation in Caucasians and Orientals. Can. J. Physiol. Pharmacol. 1980, 58, 1142-4

35. Johnson M.I., Radford H. CYP2D6 Polymorphisms and Response to Codeine and Tramadol. Analg., Resusc.: Curr Res, 2016, 5:1. doi:10.4172/2324-903X.1000e106

36. Yiannakopoulou E. Pharmacogenomics and Opioid Analgesics: Clinical Implications. Int. J. Genomics. 2015, 368-379

37. Hall A., Hardy J.R. The lipophilic opioids: fentanil, sufetanil and remifentanil. Chapter 11. In: Opioids in Cancer Pain, 2nd ed.; Davis M.P., Glare P.A., Hardy J., Quigley C. Eds; Oxford University Press, Oxford, UK, 2013; Chapter 11; ISBN-13:9780199236640

38. Prescott L.F. Kinetics and metabolism of paracetamol and phenacetin. Br. J. Clin. Pharmacol. 1980, 2, 291S-298S

39. Brodie B.B., Axelrod J.The fate of acetanilide in man. J. Pharmacol. Exptl. Therap. 1948, 94:29-38

40. Aronoff D.M., Oates J.A., Olivier Boutaud O. New insights into the mechanism of action of acetaminophen: Its clinical pharmacologic characteristics reflect its inhibition of the two prostaglandin H2 synthases. Clin. Pharmacol. Ther. 2006, 79, 9-19.

41. Howell S. R., Husbands G. E, Scatina J. A., Sisenwine S. F. Metabolic disposition of 14C-venlafaxine in mouse, rat, dog, rhesus monkey and man. Xenobiotica. 1993, 23, 349-359

42. Preskornl P., Silman H., Isler J.A., Burczynski M.E., Ahmed S., Paul J., Nichols A.I. Comparison of the pharmacokinetics of venlafaxine extended release and desvenlafaxine in extensive and poor cytochrome P450 2D6 metabolizers. J. Clin. Psychopharmacol. 2009, 29, 39-43

43. Sangkuhl K., Stingl J.C., Turpeinene M., Altman R.B., Kleina T.E. Venlafaxine metabolic pathway.2014, Pharmacogenet. Genomics. 24, 62-72

44. Colvard M.D. Key differences between Venlafaxine XR and Desvenlafaxine: An analysis of pharmacokinetic and clinical data. Mental Health Clinician. 2014, 4, 35-39

45. Williams D.A. Antidepressant Drugs. In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; pp. 608-609; ISBN-13: 978-1-60913-345-0

46. Tramadol Update Review Report Agenda item 6.1, Expert Committee on Drug Dependence, World Health Organization, Thirty-sixth Meeting, Geneva, 16-20 June 2014

47. Cashman J.N. The mechanism of action of NSAIDS in analgesia. Drug. 1996, 52, 13-23

48. Vance J.R., Botting R.M. 1998. Mechanism of action of nonsteroidal anti-inflammatory drugs. Am. J. Medicine.1998, 104, 2S-8S

49. Vance J.R. Introduction: Mechanism of action of NSAIDS. Br. J. Pharmacol.1996, 35, 1-3
50. DeRuiter J. www.auburn.edu/~deruija/nsaids_2002.pdf (URL accessed on 15.12. 2017)
51. Duggan K.C., Walters M.J., Musee J., Harp J.M., Kiefer J.R., Oates J.A., Marnett L.J..Molecular basis for byclooxygenaseinhibition by the nonsteroidal anti-inflammatory drug naproxen. J. Biol. Chem. 2010, 285, 34950-34959
52. Borne R., Levi M., Wilson N. Nonsteroidal Anti-Inflammatory Drugs. In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; p 1014; ISBN-13: 978-1-60913-345-0
53. Helleberg L. Clinical Pharmacokinetics of indomethacin. Clin. Pharmacokinet. 1981, 6, 245–58
54. Duggan D.E., Hogan A.F., Kuran K.C, McMahon F.G. The metabolism of indomethacin in man. J. Pharmacol. Exptl. Therap. 1972, 181, 563-575
55. Borne R., Levi M., Wilson N. Nonsteroidal Anti-Inflammatory Drugs. In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; p 1007; ISBN-13: 978-1-60913-345-0
56. Turpeinen M.1., Hofmann U., Klein K., Mürdter T., Schwab M., Zanger U.M. A predominate role of CYP1A2 for the metabolism of nabumetone to the active metabolite, 6-methoxy-2-naphthylacetic acid, in human liver microsomes. Drug Metab. Dispos. 2009, 37, 1017-24
57. Borne R., Levi M., Wilson N. Nonsteroidal Anti-Inflammatory Drugs. In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; p 1011; ISBN-13: 978-1-60913-345-0
58. Williams DA, Roche VE, Roche EB. Central Analgesics.Opioid receptors.In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK; 2013; pp. 675-679, ISBN-13: 978-1-60913-345-0
59. Fura A. Role of pharmacologically active metabolites in drug discovery and development. Drug Discovery Today. 2006, 11, 133-142
60. Sweety M. Phase I Metabolism – Oxidative Reactions –Oxidation of Aromatic Compounds. Medicinal Chemistry Notes February 15, 2014 PharmaXchange info www://pharmaxchange.info/press/2014/02/phase-i-metabolism-oxidative-reactions-oxidation-of-aromatic-compounds/ (URL accessed on March 15, 2017)
61. Morice C., Vermuth C.G. Mechanism of metabolic aromatic-ring hydroxylation: The Practice of Medicinal Chemistry, 2nd ed.; Vermuth CG, Ed.; Academic Press (Elsevier), CA, USA, 2003; pp 243-263; eBook ISBN: 9780080497778
62. Butler T.C. The metabolic hydroxylation of phenobarbital. J. Pharmacol. Exptl. Therapeutics. 1956, 116:326-336
63. Dasgupta A. 4-Hydroxyphenobarbital. In: A Health Educators Guide to Understanding Drugs of Abuse Testing. Jones and Bartkett Publishers, Huston, Texas, USA, 2010; p 19; ISBN-13: 978-0763765897
64. Lowry W.T.,GarriottJ.C..Phenobarbital. In: Forensic Toxicology: Controlled Substances and Dangerous Drugs, 17th ed.; Prenum Press, New York, USA, 2012; pp 347-348; ISBN 978-1-4684-3444-6
65. Veronese M.E., Doecke C.J., Mackenzie P.I., McManus M.E., Miners J.O., ReeD.L.P., Gasser R., Meyer U.A., Birkett D.J. Site-directed mutation studies of human liver cytochrome P-450 isoenzymes in the CYP2 subfamily. Biochem. J. 1993, 289, 533-538
66. Moniri N.H. Sedatives-Hypnotics. In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013, pp. 491-492, ISBN-13: 978-1-60913-345-0
67. Rudolph U., Crestani F., Benke D. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature, 1999, 401(6755):796–800.
68. Kaye A.M., Bueno F.R.,Kaye A.D. Benzodiazepine Pharmacology and Central Nervous System–Mediated Effects. Ochsner Journal. 2013, 13, 214-23

69. Moniri N.H. Sedatives-Hypnotics. In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; pp. 492-493; ISBN-13: 978-1-60913-345-0

70. Schwartz M.A., Koechlin B.A., Postma E., Palmer S., Krol G. Metabolism of diazepam in rat, dog, and man. J. Pharmacol. Exptl. Therap.1965, 149:423-435

71. Saito K., Kim S-H., Sakai N., Ishizuka M., Fujita S.Polymorphism in Diazepam Metabolism in Wistar Rats. J. Pharmaceutical. Sci. 2004, 93:1271-1278

72. Moniri N.H. Sedatives-Hypnotics. In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; pp. 494-496; ISBN-13: 978-1-60913-345-0

73. Miura M., Otani K., Ohkubo T. Identification of human cytochrome P450 enzymes involved in the formation of 4-hydroxyestazolam from estazolam. Xenobiotica. 2004, 35, 455-465

74. Harrold M.W., Zavod R.M. Basic Concepts in Medicinal Chemistry; eBook, 2013, pp. 26-61, ISBN ISBN 978-1-58528-266-1 (Accessed on 13.08.2017)

75. Borne R., Levi M., Wilson N. Nonsteroidal Anti-Inflammatory Drugs. In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; pp. 1009-1010; ISBN-13: 978-1-60913-345-0

76. Kuehl G.E., Lampe J.W., Potter J.D., Bigler J. Glucuronidation of nonsteroidal anti-inflammatory drugs: identifying the enzymes responsible in human liver microsomes. Drug Metab. Dispos. 2005, 33, 1027-1035

77. Borne R., Levi M., Wilson N. Nonsteroidal Anti-Inflammatory Drugs. In: Foye's Principles of Medicinal Chemistry. 7th ed.; Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; pp 1014-1015; ISBN-13: 978-1-60913-345-0

78. AkamineY., Uno T.Warfarin Enantiomers Pharmacokinetics by CYP2C19. www.intechopen.com (URL accessed on 30.05.2017)

79. Rettie A.E., Korzekwa K.L., Kunze K.L., Lawrence R.F., Eddy A.C., Aoyama H.,Gelboin H.V., Gonzalez F.J., Trager W.F. Hydroxylation of warfarin by human cDNA expressed cytochrome P-450. A role forP4502C9 in the etiology of (S)-warfarin drug intoxications. Chem. Res. Toxicol. 1992, 5, 54-59

80. Kaminsky L.S., de Morais S.M.F., Faletto D.A., Dunbar D.A., Goldstein J. Correlation of human P4502C substrate specificities with primary structure: warfarin as a probe. Mol. Pharmacol.1993, 43, 234-239

81. Kaminsky L.S., Zhang Z.Y. Human P450 metabolism of warfarin. Pharmacol. Ther. 1997, 73, 67-74

82. Trager W.F., Lewis R.J.Mass spectral analysis in the identification of human metabolites of warfarin. J. Med. Chem. 1970, 13, 1196-1204

83. Beckett A.H., Beaven M.A., Robinson A.F. Metabolism of chlorpromazine in humans. Biochem. Pharmacol. 1963, 12, 779-794

84. Mackay A.V.P., Healy A.F., Baker J.The relationship of plasma chlorpromazine to its 7-hydroxy and sulfoxide metabolites in a large population of chronic schizophrenics. Br. J. Clin. Pharmacol. 1974, 1, 425-30

85. Stevenson D., Reid E. Determination of Chlorpromazine and Its Sulfoxide and 7-Hydroxy Metabolites by Ion-Pair High Pressure Liquid Chromatography. Analytical Letters. 1981, 14, 1785-1805

86. Goldenberg H., Fishman V. Metabolism of chlorpromazine: V. Confirmation of position 7 as the major site of hydroxylation. Biochem. Biophys. Res. Comm. 1964, 14, 404-407

87. Chen B.M., Herschel M., Aoba A. Neuroleptic, antimuscarinic and antiadrenergic activity of chlorpromazine, thioridazine and their metabolites. Psychiatry Res. 1979, 1, 199-208

88. Mehvar R., Brocks R. Stereospecific Pharmacokinetics and Pharmacodynamics of Beta-Adrenergic Blockers in Humans. J. Pharm. Pharmceut. Sci. 2001, 4, 185-200

89. Paterson J.W., Conolly M.F., Dollery C.T. The Pharmacodynamics and Metabolism of Propranolol in Man. Pharmacologia Clinica. 1970, 2, 127-133

90. Harrold M. Antihyperlipoproteinemics and Inhibitors of Cholesterol Biosynthesis. In: Foye's Principles of Medicinal Chemistry. 7th ed., Lemke T.L., Williams D.A., Roche V.F., Zito S.W., Eds; Wolters Kluwer and Lippincott Williams and Wilkins, London, UK, 2013; pp. 826-830; ISBN-13: 978-1-60913-345-0

91. Drugbank: Atorvastatin (DB01076)), (URL accessed on 10.07.2017)

92. Portoghese P., Sultana M., Nagase H., Takemon A.E. Application of the message-address concept in the design of highly potent and selective. Delta.Opioid receptor antagonists. J. Med. Chem. 1988, 31, 281-282

93.Yü T.F., Burns J.J., Paton B.C., Brodie B.B. Phenylbutazone Metabolites: Antirheumatic, Sodium-Retaining andUricosuric Effects In Man. J. Pharmacol. Exptl. Therap. 1958, 123, 63-69

94. Aarbakke J., BakkeO.M., Milde E.J., Davies D.S. Disposition and oxidative metabolism of phenylbutazone in man. Eur. J. Clin. Pharmacol. 1977, 11, 359-366

95. Woolfe G., Deesi L., Uchleke H., Archer S., Harris L.S., Whitehouse M.W., Montgomery J.A.Y., "Progress in Drug Research" ; Jucker E., Ed; Basel und Stuttgart; Springer: 1965, Vol. 8; p. 381; ISBN 978-3-0348-7133-4

96. Sriram D., Yogeeswar P. Medicinal Chemistry. 2nd ed.; Pearson Publisher, Delhi, India, 2007, p 361; ISBN 10: 8131731448

97. Bagwell E.E., Williams E.M. Further studies regarding the structure activity relationships of beta-adrenoceptor antagonists. Br. J. Pharmacol. 1973, 58, 686-92

98. Hedberg A., CarlssoE., Fellenius E., Lundgren B.1982.Cardiostimulatory effects of prenalterol, a beta-1 adrenoceptor partial agonist, in vivo and in vitro. Naunyn Schmiedelberg's Archives of Pharmacology. 1982, 318, 185–191

99. Artursson P., Tavelin S. The Role of Permeability, Transport, and Absorption Prediction. Drug Discovery. Comprehensive Medicinal II. 2007, 5, 259-27

100. Hodgson E. Introduction to Biotansformation (Metabolism). In: Pesticide Biotransformation and Disposition; Hodgson E, Ed.; Academic Press, 2012 (Elsevier), Cambridge, Massachusetts, USA, 2012; Chapter 4, pp 53-72; ISBN: 9780123854810