TOXICOPHORE AND PHARMACOPHORE DEPENDENT TOXICITY: PERSPECTIVE REVIEW

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Summary

A toxicophore is responsible for the toxic property of a compound through interaction with a cellular macromolecule such as proteins or DNA. A pharmacophore carries all the essential features responsible for a drug's biological activity. Acetaminophen as a pharmacophore produces massive hepatic necrosis even after administration of a single toxic dose. To eliminate or reduce the toxic effects of a metabolite, the discovery of other novel compounds is very essential. Structurally diverse HIV-1 integrase inhibitors can be discovered by using functional feature pharmacophore model method. A structure-based approach to establish validated shape pharmacophore is used in which 3-D structure of the apoferritin is used as the basis for the development of several shape of pharmacophore models. This current review can emphasize a great deal of information about the cellular toxicity which depends on the specific pharmacophore as well as toxicophore.

Keywords: Pharmacophore, Toxicophore, Toxicity

Introduction

The toxic properties of compounds can be related to chemical structures, and more specifically, to particular substructures, called toxicophores. Reliability and accuracy of mutagenicity, hepatotoxicity or cardiotoxicity predictions may be achieved by identifying toxicophores [1]. A toxicophore exerts its toxicity through interaction (covalent bonding or oxidation) with a cellular macromolecule, such as a protein or DNA. This toxicity causes changes in the normal cellular biochemistry and physiology eliciting toxic effects. Occasionally, to produce a more reactive chemical species that is able to covalently bind to cellular macromolecules, the toxicophore requires bio activation, modified by an enzyme [2]. Adverse drug reactions (ADRs) are significant health problems that contribute to the morbidity and mortality of patients. There are many different types of ADRs, affecting every organ system in the body. Toxicophores are substrates that indicate an increased potential for mutagenicity, whether this is caused by DNA reactivity or not. A toxicophore can represent a reactive structure or a sub-structure that is prone to either metabolic activation or intercalation. In humans mutagenic compounds (*i.e.* nitroso, azo compounds, aromatic amines, hydroxylamines, and amides) poses some toxic risks. Therefore, prior to the drug approval the screening of drug candidate is essential [3]. The ability of a compound to cause DNA mutation is called as Mutagenicity. A mutagenic toxicophore containing compounds can be transformed into non mutagenic compounds by the action of detoxifying compounds because of their inhibition mechanisms such as DNA reactivity, metabolic activation or intercalation. This transformation effect in the mutagenic toxicophore containing compounds may be caused by steric hindrance in the toxicophore or by a disruption of the required electronic charge distribution near the toxicophore. The aromatic nitro and aromatic amine toxicophores are specific examples of how toxicophore accuracy could be improved by the introduction of electron withdrawing detoxifying substructures such as sulfonamide or trifluoromethyl groups [4].

Pharmacophore

A molecular framework that carries (*phoros*) the essential features or having the properties which are responsible for a drug's (*pharmacon's*) biological activity are known as a Pharmacophore. But later Peter Gund® defined Pharmacophore as "a set of structural features in a molecule that are recognized at a receptor site and is responsible for that molecule's biological activity" [5]. But presently a pharmacophore may be defined as "an ensemble of steric and electronic features that is necessary to ensure the optimal supra molecular interactions with a specific biological target and to trigger or block its biological responses. A typical pharmacophore must have some features i.e. hydrophobic, aromatic, a hydrogen bond acceptor, a hydrogen bond donor, a cation, or an anion which are needed to match different chemical groups with similar properties, in order to identify novel ligands [6].

Drug metabolism and drug toxicity

Liver is the primary site of drug metabolism in the body. The physiological role of drug metabolism is the biotransformation of lipophilic compounds into water-soluble derivatives that are more readily excreted out (figure 1).

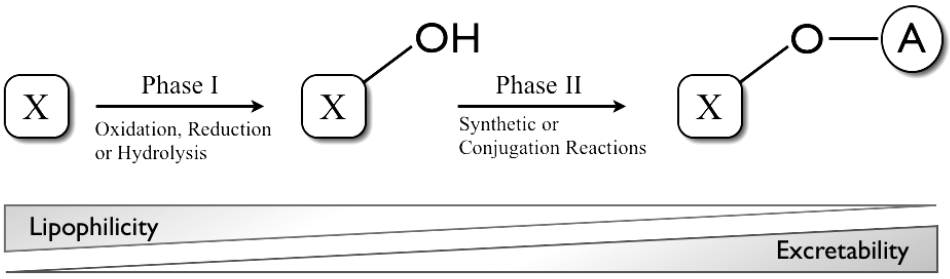


Figure 1: Physiological role of drug metabolism is the biotransformation

Drug metabolism is a major determinant in pharmacokinetic and pharmacodynamic studies, elimination/ detoxication, metabolic activation, pharmacokinetic variability (individuals, species etc), drug interactions, pharmacogenetic polymorphisms, physiological and pathological factors. The liver is exposed to xenobiotics

immediately after their absorption from the gastro-intestinal tract and has a high capacity for both phase I and phase II biotransformation.

Some therapeutic agents as well as diverse range of foreign compounds can be metabolised by cytochrome P450 (cytochrome P450 plays primary role in metabolism). Such foreign compounds get concentrated in the liver by various processes i.e. active transport systems, which contributes to the first pass elimination of drug by extracting foreign compounds from the blood. Variability in the rate of drug metabolism can influence both efficacy and safety of a drug. Factors which influence this process and are of clinical relevance are well documented and include pharmacogenetics, enzyme induction, enzyme inhibition, disease and age [7]. Detoxication and clearance are the major physiological role of drug metabolism, but certain bio transformations can act as an ‘‘intoxication’’ process. Thus, xenobiotics interfere with cellular functions by undergoing biotransformation to toxic metabolites which shows interference with cellular functions and intrinsic chemical reactivity with different types of macromolecules. The chemistry of the molecule is responsible for the propensity of a molecule to form either toxic and/or chemically reactive metabolites. Thus chemistry of a molecule is solely responsible for a molecule to form either toxic and/or chemically reactive metabolites (Figure 2) [8].

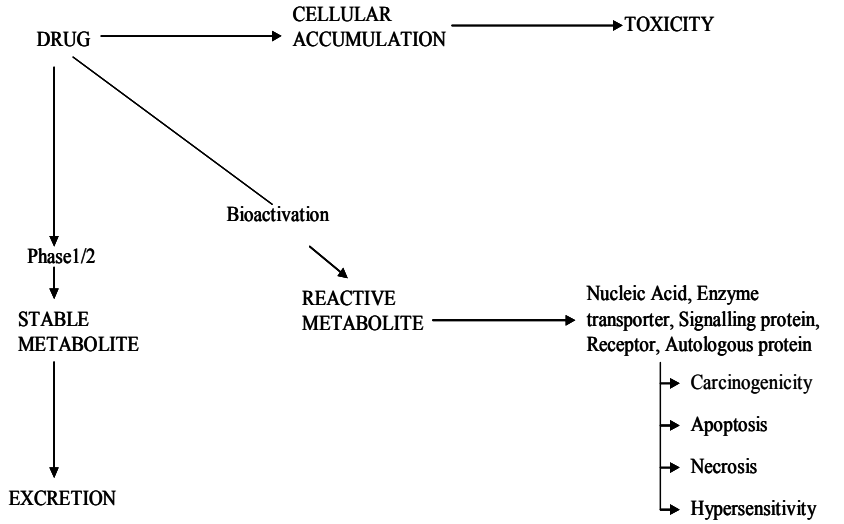


Figure 2. Schematic representation of the drug metabolism and toxicity.

Toxicophore inducing toxicity

A metabolic bioactivation of structural toxicophores plays a key role in initiating toxicity. So the elucidation of the mechanisms associated with each toxicophore is an important objective, because this will be required to underpin the safe clinical use of currently licensed drugs and to improve safety evaluation and risk assessment of the new candidate drugs [9]. An example of toxicophore induced toxicity is antihistaminic drug methapyrilene [N,N-dimethyl-N′-pyridyl-N′(2-thienylmethyl)-1,2-ethanediamine] which is hepatotoxic in the rat due to Cytochrome P450 mediated bioactivation of the thiophene ring, which has been identified as a structural alert for hepatotoxicity [10]. The detoxication of the S-oxide is also demonstrated via its conjugation with glutathione (GSH) in vivo (Figure 3) [11]. MP toxicity is of great interest because of two reasons, one is the unusual and distinctive pattern of liver injury observed (i.e. periportal hepatocellular hepatic injury accompanied by bile duct hyperplasia) and its progression to hepatic tumours and another is because of its definite dependence on the activation of the thiophene toxicophore to defined reactive metabolites. Tienilic acid (TA) is another drug containing thiophene functional groups which have been withdrawn due to toxicity. As MP and TA share similar mechanisms of thiophene S-oxidation so it suggests that thiophene toxicophore induces a potent, class-specific effect[12].

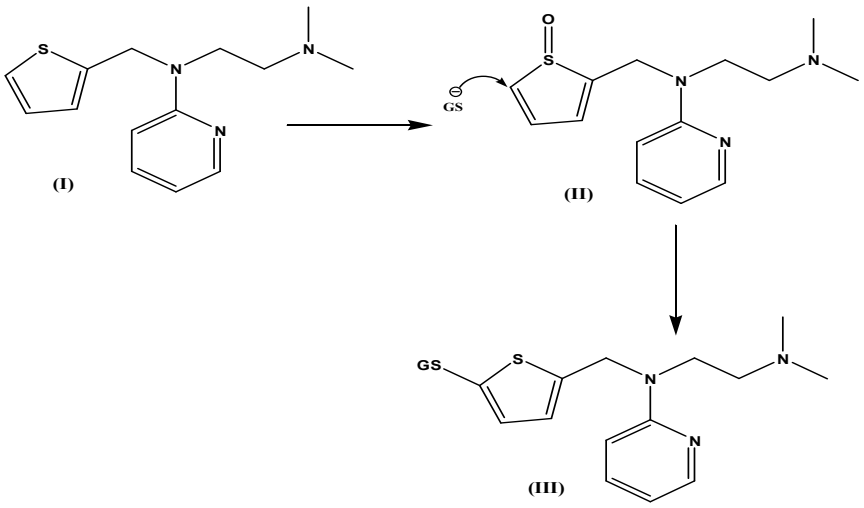


Figure 3. A schematic representation of the metabolism of methapyrilene (MP) to a toxic S-oxide intermediate before detoxification by glutathione (GSH). MP (I) undergoes cytochrome P450 activation to an S-oxide intermediate (II). The S-oxide intermediate (II) is detoxified via conjugation with glutathione.

Methods used for the detection of toxicophoric metabolites after bio-activation

Chemically reactive metabolites can be easily detected in the early stage of the drug development by utilising a number of techniques i.e. bioanalytical techniques, because of the potential hazards associated with such metabolites. This detection of glutathione adducts, DNA adducts, proteins and oxidative damage in systems of varying biological integrity such as microsomes, expressed enzymes, hepatocytes, cell lines and animal models including transgenic can be done by this technique. For the determination of covalent binding of drug to tissues and macromolecules the availability of radiolabeled compound is critical. In the presence of microsomes and NADPH the drugs which are perfectly safe in man, will undergo bioactivation. This is simply a result of repeated oxidation reactions (removal of electrons) making an inert molecule electrophilic. This helps to introduce a chemical marker of potential hazards that can be assessed further in progressively more integrated

biological systems. Ultimately, the chemistry of the process must be related to biological function [13].

The role of P450 enzymes in chemical-induced toxicity can be described by the use of transgenic animals. Studies on the relationship of xenobiotic-metabolizing enzymes with the induction of toxicity in whole animals have been limited and difficult to interpret due to the multiple forms of Cytochrome P450 expressed. By either introducing the expression through genetic manipulation of the cytochrome P450 enzymes in mice or by disrupting the expression of enzymes in mice the effect of single enzyme on chemical toxicity can be precisely determined [14]. Oxidative metabolism of paracetamol, bromobenzene, carbon tetrachloride, furosemide etc. gives some toxic chemically reactive toxic metabolites (Figure 4).

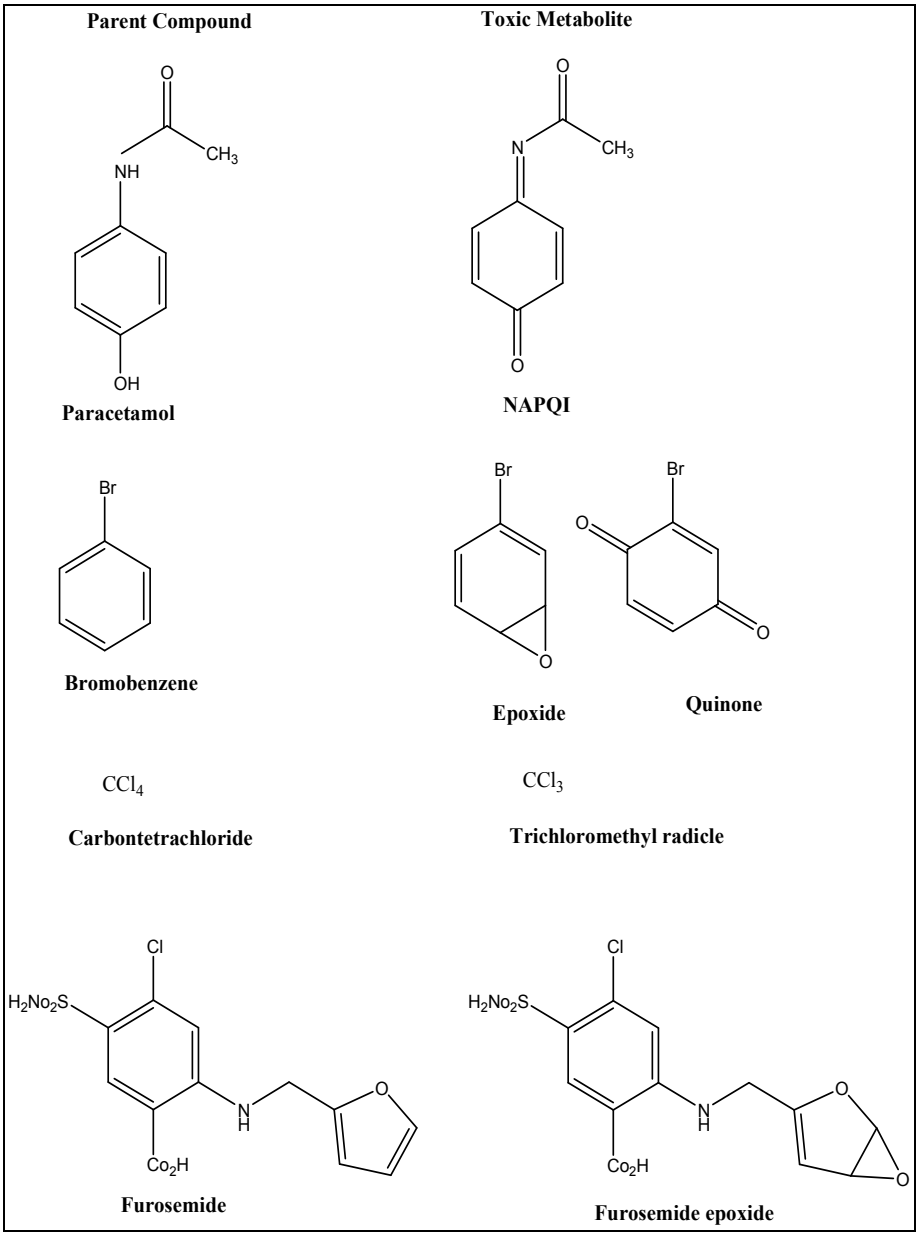


Figure 4. Examples of some toxic chemically reactive toxic metabolites, which are formed from oxidative metabolism of paracetamol, bromobenzene, carbon tetrachloride and furosemide.

Pharmacophore-induced toxicity

This involves findings relating to the pharmacology of the compound. Within this category the adverse effects are either a direct or an indirect extension of the pharmacology. With the indirect extension, at the elevated doses the original selectivity of the compound for a target is lost and the effects seen are triggered by effects on the proteins whose structure is closely similar to the original target. Doses in excess of therapeutic dose are mostly responsible for pharmacophore-induced toxicity. Pharmacophore-induced toxicity is usually seen at doses in excess of the therapeutic dose [15]. It is not necessary that pharmacophore-induced toxicity always occur at the site of intended therapy or within the organ. An example of this is the toxicity of loop diuretics [16]. The targets for this class of drugs are the Na-(K)-Cl co-transporters of the kidney. In the ion transport and fluid secretion of the utricle and semicircular canal of the ear these co transporters plays a major role. Perhaps not surprisingly loop diuretics are associated with ototoxicity. The different toxicity profiles of various diuretics can be explained by their selectivity and potency. In the thick ascending limb and distal convoluted tubule some kidney-specific co-transporters like ENCC1 and ENCC2 respectively can be easily expressed. Whereas ENCC3 is expressed in many tissues including the cochlea. Thiazide diuretics doesn't show any toxicity but shows major activity against ENCC1. Loop diuretics inhibit NKCC2 with potencies for bumetanide < 0.2 μM and NKCC3 > 0.5 μM. Under the conditions which causes ototoxicity after the intravenous administration of drug in high doses the selectivity of the compound for NKCC2 is lost. Unexpected or polypharmacology in a structure can occasionally lead to additional benefits in drugs. In the same way polypharmacology can have dramatic consequences in toxicity. Thalidomide was used as an anti-nausea drug to control morning sickness. Its use in pregnant women had terrible consequences due to the teratogenic nature of the drug [17].

Potential Pharmacophore: Example

After a single dose several simple chemicals produce selective hepatotoxicity where there is evidence that bio activation is essential for hepatotoxicity. The Major cause of drug-related morbidity and mortality in humans is Acetaminophen (APAP); it produces massive

hepatic necrosis even after administration of a single toxic dose. The standard treatment for acetaminophen intoxication is N-acetylcysteine, which replaces hepatic glutathione and prevents toxicity; this treatment is most effective if given within 16 hrs [18].

Glucuronylation and Sulphation deactivate acetaminophen to metabolites which are rapidly excreted in urine when given at therapeutic doses (Figure 5).

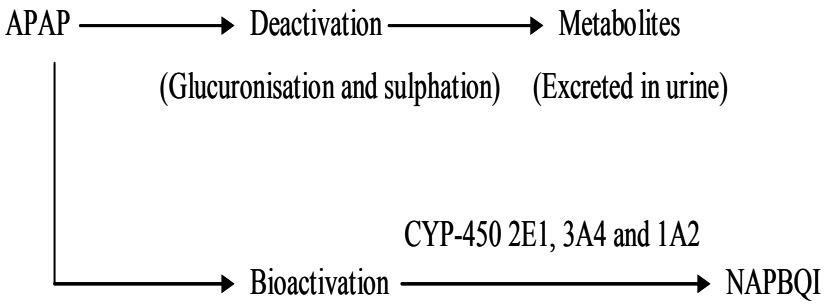


Figure 5. Formation of pharmacophore after deactivation and bioactivation of APAP.

However, some portion of the drug undergoes bioactivation to *N*-acetyl *p*- benzoquinoneimine (NAPQI) by CYP2E1, CYP1A2, and CYP3A4 [19, 20]. When acetaminophen is administered at therapeutic doses, NAPQI is rapidly quenched by a spontaneous reaction with hepatic glutathione (GSH), thus underlying the concept that drug bio activation does not always equate with drug toxicity. Oxidative stress reactions, dysfunction of mitochondria and DNA damage is caused by the excessive toxic dose of NAPQI which depletes hepatic GSH and covalently binds to cellular proteins. GSH depletion has been shown to be an obligatory step for covalent binding and toxicity [21] (Figure 6).

GSH DEPLETION	COVALENT BONDING	PATHOLOGY
Required	Yes	Massive Hepatocellular Necrosis (Multilobular)
Required	Yes	Centrilobular/portal Necrosis(Multifocal)
May Occur	Yes	Midzonal/Centrilobular Necrosis (Multifocal)
May occur	Yes	Midzonal/Centrilobular Necrosis Focal/Multifocal

Figure 6. Severiy and localisation of cellular toxicities varies from the level of glutathione (GSH) and co-valent binding affinity of reactive metabolites in the tissues.

AQ, like acetaminophen, undergoes extensive bioactivation to an electrophilic quinoneimine metabolite, amodiaquine quinoneimine (AQQI) (Figure 7), which has been easily detected *in vivo* in rats and *in vitro* by hepatic microsomes. Subsequent oxidative stress or conjugation to cysteinyl sulfydryl groups on proteins is likely to be involved in the induction of toxicity by either cytotoxic or immunological. In patients which shows adverse reactions to amodiaquine, IgG antibodies can be easily detected which are used to recognise the 5-cysteinyl group these antibodies. However, the factors determining individual susceptibility are unknown [22, 23].

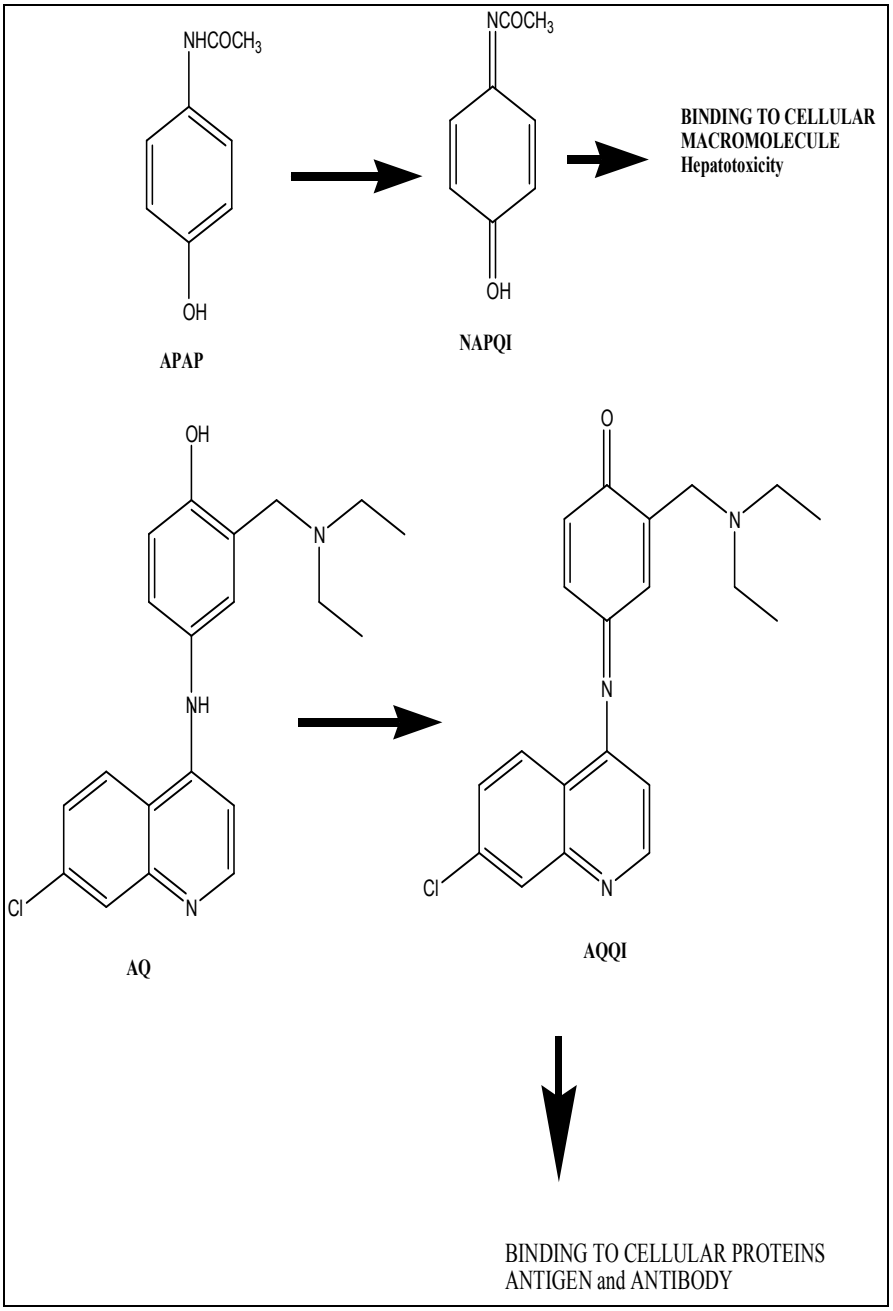
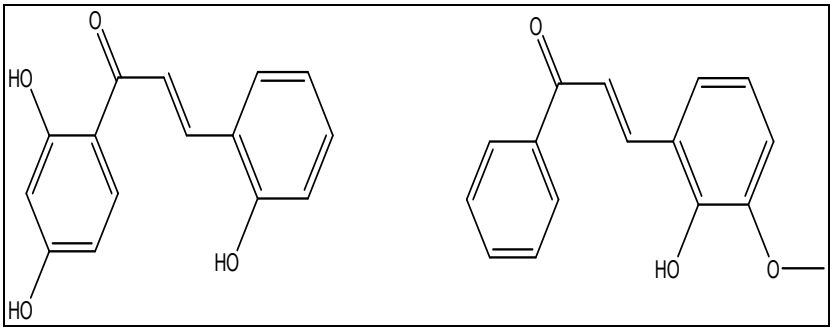


Figure 7. Metabolic bioactivation of acetaminophen (APAP) and amodiaquine (AQ) to their respective quinoneimines, like – NAPQI and AQQI. These reactive intermediates causes cellular toxicity.

Discovery of various pharmacophore with improved activity

1. Discovery of structurally diverse HIV-1 integrase inhibitors

This discovery is based on chalcones pharmacophore. Integrase (IN) is one of the three essential enzymes encoded by the human immunodeficiency virus (HIV) gene. Reverse transcriptase and protease are the other two target enzymes which have been widely exploited as antiretroviral drug targets. Currently there are 21 drugs are available for the treatment of HIV infected patient, but many patients experience unsatisfactory virologic, immunologic, or clinical outcomes from currently available therapies, limiting therapeutic options due to the development of drug resistance and cytotoxic side effects. These aspects require the identification of novel drugs targeting a different stage of the retroviral life cycle [24]. Recently 11 structurally novel compounds have been reported which shows antiviral activity in the the NCI drug screening program by enzyme based assays specific for IN [25]. Chalcones 1 and 2 were selected as potential leads (Figure 8).



(a) Chalcone 1
3'-Processing: 53 ± 5 μM
Strand transfer: 47 ± 4 μM

(b) Chalcone 2
3'-Processing: 2 ± 1 μM
Strand transfer: 2 ± 1 μM

Figure 8. Structures of the two recently reported chalcones (a) chalcone 1 and (b) chalcone 2 derivatives, that are used to designed a Pharmacophore, having HIV integrase inhibitor activity. The numerical value denoted as IC-50.

Unfortunately, in a large number of tumor cell lines many chalcones are non-specific inhibitors and possess cytotoxicity. Therefore for the design of pharmacophore models previously identified chalcones can be used as starting leads which is aimed to discover non-chalcones-based compounds. The Functional feature pharmacophore model method is used for development of a potent non toxic pharmacophore. The various which are used in this process, in the first step single conformation of either chalcones 1 or 2 are used for the generation of functional feature pharmacophore model by the use of Catalyst software package. H-bond donor, H-bond acceptor, and hydrophobic feature are the desired functional features that were mapped and added to the selected conformation. Then the energy-optimized conformation is generated by using poling algorithm with CHARMM force field. Next, we assigned the constraints to each feature e.g., coordinate and size of the feature). To avoid negative fitting values we chose the default value for each selected feature. Finally, all the selected features were merged into one pharmacophore model. This process can also be used for other chalcones also [26].

2. Discovery of novel anesthetic compounds

Due to durable changes in cognition current anesthetics, especially the inhaled ones shows troublesome side effect. So, the development of novel chemical entities that can reduce these effects while preserving or enhancing anesthetic potency is highly desirable. In spite of advancement toward identifying protein targets involved in anesthesia, we still do not have the necessary atomic level structural information to delineate their interactions with anesthetic molecules. Recently, a protein target, apoferritin has been studied, to which several anesthetics bind specifically and in a pharmacodynamically relevant manner. A structure-based approach to establish validated shape pharmacophore models for future application to virtual and high throughput screening of anesthetic compounds has been described, in which 3-D structure of the apoferritin is used as the basis for the development of several shape pharmacophore models [27].

Conclusion

In this review we have studied about the causes of toxic property and adverse drug reactions of compounds. Pharmacophores along with their pharmacological actions plays a very significant role in toxicological property of molecules. As toxicity is very frequently caused by the Pharmacophores, so there is a great need to discover novel drug molecule which can eliminate the risk of causing toxicity.

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