

Current Status of the Cytosolic Sulfotransferases in the Metabolic Activation of Promutagens and Procarcinogens

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Abstract: Cytosolic sulfotransferases (SULT) catalyze the sulfation of structurally diverse drugs, endogenous compounds and xenobiotics. These reactions involve the transfer of a sulfuryl group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to the hydroxyl/amino groups of acceptor molecules. Although sulfate conjugation is generally considered as a detoxication pathway producing more water-soluble and often less toxic metabolites, sulfation of certain classes of compounds produce sufficiently electrophilic metabolites that can covalently bind to cellular macromolecules, DNA and RNA. The important roles of electrophilic sulfate ester metabolites in the metabolic activation, mutagenicity and ultimate carcinogenicity of many xenobiotics have been considerably elucidated. Examples include the class of hydroxymethyl polycyclic aromatic hydrocarbons, allylic alcohols, N-hydroxy derivatives of carcinogenic arylamines and heterocyclic amines. Results obtained by many scientists during the last two decade correlate with a hypothesis that electrophilic sulfate esters may be the major ultimate carcinogenic forms of many, if not most, procarcinogens derived from benzylic/allylic alcohols and hydroxy arylamines. Careful analysis of these results suggest that the activities of human hydroxysteroid sulfotransferase (hHST), and a related form in rat liver, rat hydroxysteroid sulfotransferase a (STa), as well as aryl sulfotransferases both from rat and human liver, account for a substantial portion of the activation of benzylic/allylic alcohols in these species. Moreover, aryl sulfotransferases have also been indicated as the responsible SULT family in the bioactivation of hydroxy arylamines in the liver of different species including human. Molecular cloning of the individual sulfotransferases and expression of these individual forms in heterologous expression systems have allowed us to better understand the role of SULTs in the bioactivation of different procarcinogens and the form of sulfotransferase involved in their bioactivation. Additional structure-activity studies with homogeneous forms of rat liver STa and AST IV have also yielded comparative insight into some of the parameters important in recognition of substrates and inhibitors by these enzymes.

INTRODUCTION

Sulfation (*O*-sulfonation) catalyzed by cytosolic sulfotransferases (SULTs)¹(EC 2.8.2) is an important biotransformation reaction in the metabolism of a large number of structurally diverse drugs, endogenous compounds and xenobiotics [1-6]. Cytosolic SULTs catalyze the transfer of a sulfuryl group from the endogenous sulfate donor 3'-phosphoadenosine 5'-phos-phosulfate (PAPS) to hydroxyl or amino groups of acceptor molecules thereby forming sulfuric acid esters and sulfamates, respectively, as shown in (Fig. 1). In general, sulfate conjugation of xenobiotics is a detoxication process that converts them into more water soluble and less toxic metabolites, therefore aiding their excretion in urine or bile. For example, biotransformation of phenolic drugs and xenobiotics yields products that are more

water soluble, less biologically active, and in most cases stable enough to be readily excreted from the body. Sulfation is also an important factor in the regulation of steroid biosynthesis in the body, since the sulfated dehydroepiandrosterone (DHEA) and estrone are the precursors of androgen and estrogen biosyntheses [7-10]. Sulfation also facilitates the inactivation and excretion of endogenous compounds such as thyroid hormones [11-14] and catechols [15-19]. There have been a few sulfuric acid ester metabolites reported to be more pharmacologically active than the corresponding parent molecules. Examples include minoxidil [20,21] and cicletanine [22] wherein the sulfated metabolites are the pharmacologically active forms.

However, formation of sulfuric acid ester metabolites of certain xenobiotics such as hydroxymethyl polycyclic aromatic hydrocarbons (PAHs) and N-hydroxy arylamines (N-OH-AAs) converts them into more toxic metabolites that cause mutagenic and ultimate carcinogenic responses in organisms including rodents and human [23-27].

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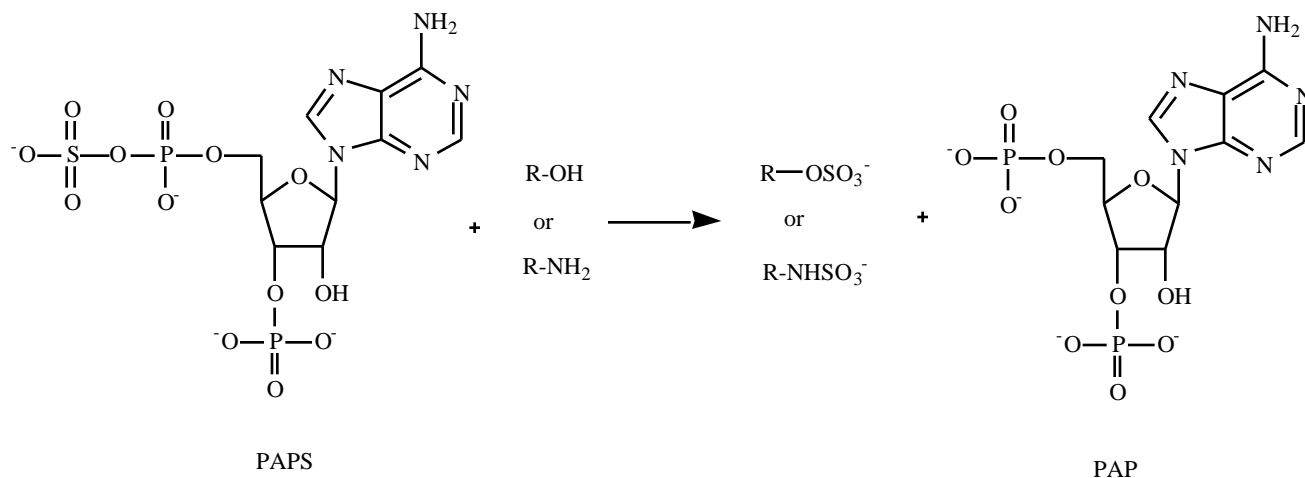


Fig. (1). The general sulfation reaction catalyzed by the cytosolic sulfotransferases.

Therefore, these examples emphasize that sulfate conjugation is an important phase-II metabolic activation reaction as well as a detoxication reaction. At the moment, it is hard to predict the answer of how significant the role of sulfation in metabolic activation processes leading to ultimately mutagenic and carcinogenic outcomes as compared to other metabolic pathways-mediated by this superfamily. However, rapid progress in the SULT research started to give some accurate answers to this question.

Although many aspects of SULTs have been reviewed previously [2,23-25,27,28], the present article will emphasize the role of sulfate conjugation in the metabolic activation of promutagens and procarcinogens. It will consider the scientific literature on the examples of chemicals wherein the sulfation is an integral part in their metabolic activation to form DNA-adducts and initiate mutagenic responses. Furthermore, studies on the substrate specificities of responsible SULT isoforms that are reported to have roles on the activation of certain classes of chemicals will also be reviewed.

CHEMICAL CARCINOGENESIS AND SULTs

Cancer has been one of the most menacing diseases threatening the human race. Although some types of cancer are regulated by genetic factors, epidemiological data on cancer cases strongly suggest that exogenous factors such as exposure to chemicals may influence the rate of developing cancer among individuals [29-33]. Due to a rapidly developing industrial world, humans are being exposed to new chemicals almost every

day. For example, PAHs present in urban air, auto exhaust, and cigarette smoke are widespread environmental pollutants [33-35], and some of them, such as benzo[a]pyrene (BP) are proved to be relatively potent carcinogens [36-38]. On the other hand, methylene-bridged derivatives of these PAHs have been cautiously identified as major components of automobile exhaust, and relatively high ratios of these are present in crude petroleum. Therefore, significant levels of these PAHs may occur as environmental pollutants [30,32,39].

Another common way of being exposed to chemicals is through the dietary products [29,40-42]. Many researchers have reported on a number of food-derived carcinogens and mutagens from grilled or fried meat. These compounds mostly included the derivatives of heterocyclic aromatic amines (HAAs) that result from the pyrolysis of amino acids, sugar and creatinine [42]. These chemicals are sometimes directly mutagenic and carcinogenic, but in most cases they require metabolic activation [43-45]. The metabolic pathways underlining these activations have been of interest for many research laboratories.

Studies over many years have provided evidence that many procarcinogens such as PAHs and AAs may undergo metabolic activation to mutagenic and/or carcinogenic metabolites via a metabolic mechanism which involves enzymatic hydroxylation by cytochromes P-450 monooxygenase (cyp P450) [46-48] followed by enzymatic conversion to reactive sulfate ester conjugates [2,23,26,49]. Work from many different laboratories has established that aryl (phenol) sulfotransferases (aryl-SULT, SULT1, EC 2.8.2.1) and alcohol (hydroxysteroid) sulfotransferases (alcohol-SULT, SULT2, EC 2.8.2.2) are the responsible

SULT families for further metabolic activation of some hydroxylated metabolites of PAHs and N-OH-AAAs to mutagenic and carcinogenic intermediates [25,50-56]. The role of sulfooxy metabolites of these procarcinogens for covalent binding with DNA, and other cell components is thought to be due to the ability of sulfate anion to serve as a good leaving group, thereby forming intermediate resonance-stabilized electrophilic benzylic carbocations or arylnitrenium ions that subsequently will react with nucleophiles in cells as generalized in (Fig. 2). Among the most studied chemical compounds activated by SULTs are benzylic alcohols derived from PAHs, allylic alcohols, N-OH-AAAs, N-OH-HAAs and secondary nitroalkanes, and these will be reviewed herein.

SULT-mediated Bioactivation of Benzylic Alcohols

Benzylic alcohols are commonly formed by the metabolic hydroxylation of benzylic positions on drug and xenobiotic molecules [47,57]. PAH molecules that are carcinogenic and mutagenic may bear a primary or secondary benzylic hydroxyl function on the molecule. Flesher and his colleagues proposed the following hypothetical mechanism for the ultimate carcinogenicity of PAHs

in early 1970s [38,58]. First of all, they speculated that the methylation of an unsubstituted PAH at the electron-dense meso position was the first step required for the formation of procarcinogenic hydroxylated metabolite from pre-procarcinogen parent PAH molecule. Subsequent esterification by phase-II conjugation enzymes would form a highly reactive aralkylating ester metabolite that is the ultimate carcinogenic metabolite, see (Fig. 3). This last step was important in that the SULT superfamily might have a profound effect on the metabolic activation of these molecules into more toxic metabolites. Results from many laboratories over the years have already proven the metabolic hydroxylation at the benzylic position and the last step, sulfuric acid ester formation, as the required metabolic processes for ultimate carcinogenicity of many alkyl-substituted PAHs.

PAHs Bearing Primary Benzylic Alcohols

Watabe *et al.* reported in 1982 the first concrete experimental evidence that the reactive sulfuric acid ester formed from 7-hydroxymethyl-12-methylbenz[a]anthracene (7-HM-12-MBA) was the mutagenic and carcinogenic metabolite of the parent compound [59]. After that, further studies elucidated that SULTs are the main enzymes catalyzing the metabolic activation of

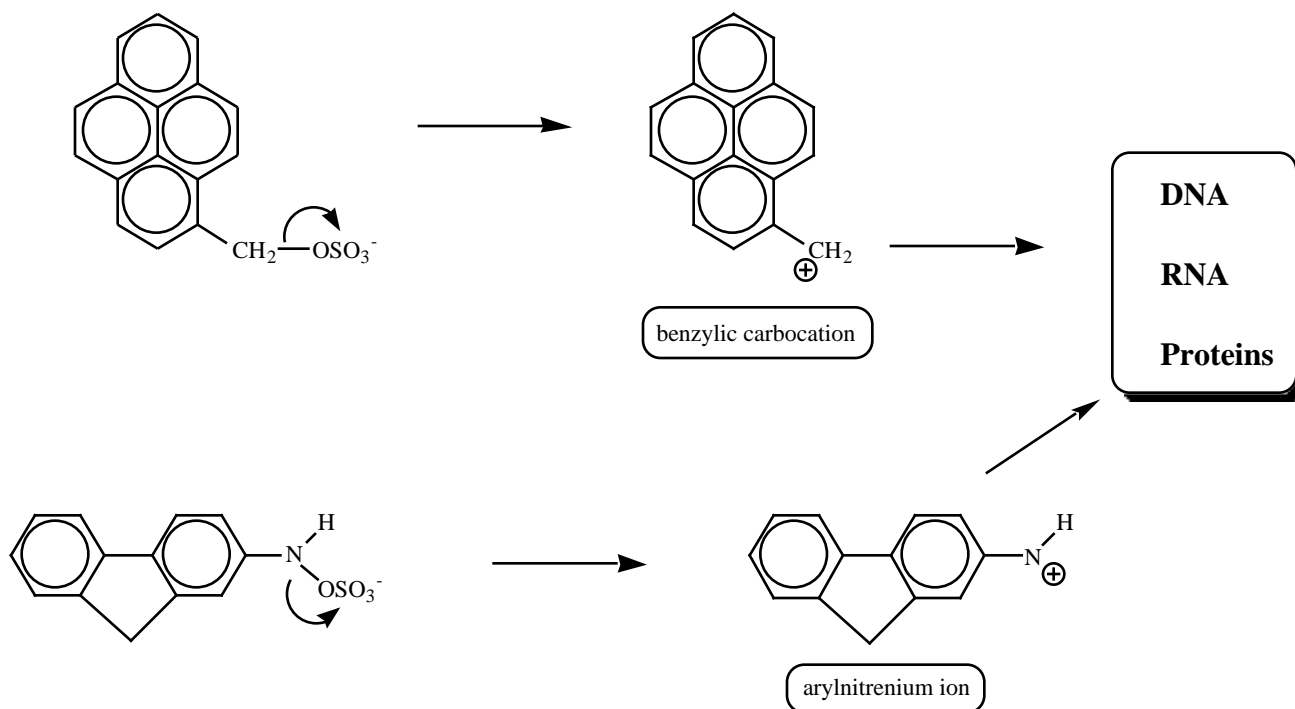


Fig. (2). Formation of reactive electrophilic carbocation or nitrenium ion intermediates from PAHs and N-OH-AAAs, respectively, through sulfated metabolites.

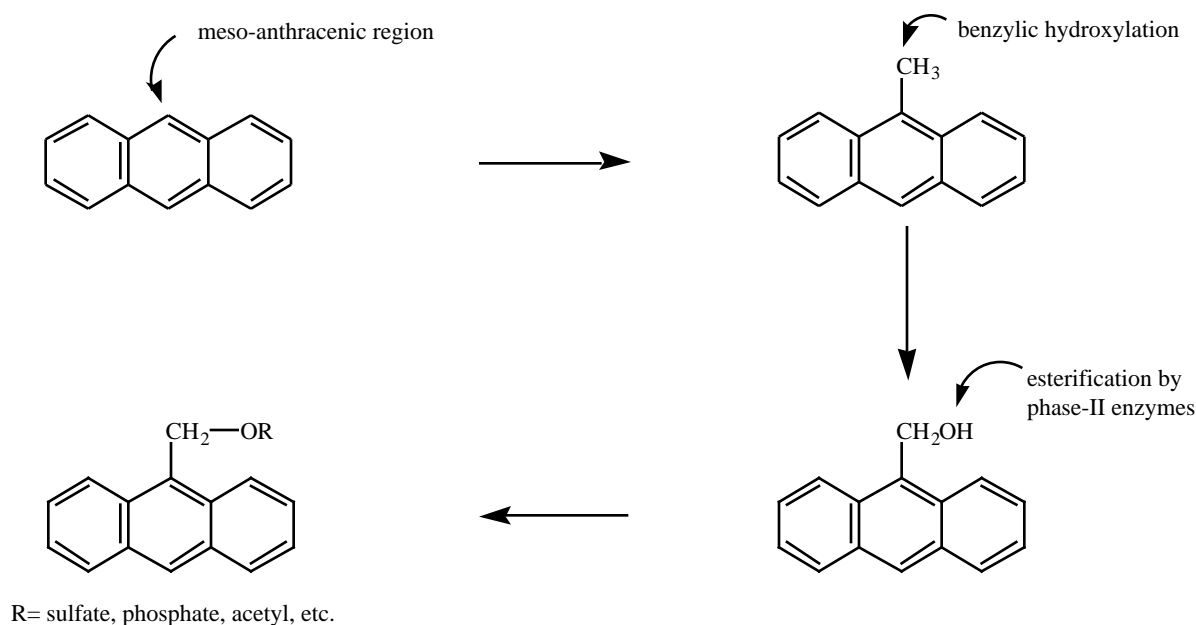


Fig. (3). Proposed hypothetical mechanism for the ultimate mutagenic and carcinogenic ester metabolites from unsubstituted parent PAH molecule demonstrated by anthracene as the model PAH (adapted from references [70-75]).

hydroxymethyl PAHs. Examples include 7-hydroxymethylbenz[a]anthracene (7-HMBA) [60], 7,12-dihydroxymethylbenz[a]anthracene (7,12-DMBA) [61], 5-hydroxymethylchrysene (5-HMC) [62-64], 6-hydroxymethylbenzo[a]pyrene (6-HMBP) [36-38], 9-hydroxymethyl-10-methylanthracene (9-HMMA) [65], and 1-hydroxymethylpyrene (1-HMP) [65]; see structures in (Fig. 4). In these studies, SULT-mediated activation of these hydroxymethyl PAHs was demonstrated in rodent cytosols with a PAPS-generating system. These studies showed that the metabolically formed sulfuric acid ester conjugates of above PAHs induced His⁺ revertants in Ames *Salmonella typhimurium* TA98. They also found that the sulfuric acid ester metabolites formed stable deoxyguanosine and deoxyadenosine DNA-adducts in calf thymus DNA. To determine the SULT family responsible for this activation process, inhibition studies of mutagenic responses were carried out using specific SULT inhibitors. These studies indicated that aryl-SULT inhibitors such as pentachlorophenol (PCP) and 2,6-dichloro-4-nitrophenol (DCNP) did not affect the mutagenic responses. However, DHEA, a typical physiologic substrate for alcohol-SULTs, greatly inhibited the mutagenic responses of these PAHs indicating the important role of this SULT family in the formation of mutagenic sulfate conjugates from these hydroxymethyl PAHs [62,66].

In a comparison of *in vivo* adduct formation with the *in vitro* formed adducts, Surh *et al.* intraperitoneally injected some of the above hydroxymethyl PAHs in rodents and observed the same pattern of DNA-adduct formation in liver as seen in *in vitro* experiments [37,65-67]. They concluded that these hydroxymethyl PAHs afforded DNA adducts at the exocyclic amino groups of guanine, adenine, and cytosine bases through the benzylic carbon on PAH molecules, probably with concomitant loss of a sulfate anion. Absence of mutagenic responses in DHEA-pretreated rats was a good indicator that alcohol-SULTs were responsible for metabolic activation of these PAH molecules [36,37,65-67]. Subsequently, Ogura *et al.* reported that these hydroxymethyl PAHs were substrates for a homogeneous rat alcohol-SULT (named as rSTa), and this enzyme catalyzed the formation of reactive sulfuric acid esters from these hydroxymethyl PAHs [53,68,69].

Recent reports indicate that sulfuric acid ester metabolites of PAHs are the ultimate carcinogenic forms of the parent molecules, and the electrophilic sulfoxy metabolites of hydroxymethyl PAHs account for the complete carcinogenicity of these intermediary metabolites following repeated subcutaneous injection in female Sprague-Dawley rats [70-73]. Most of the rats developed sarcomas at the injection site at the end of 52 weeks after administration of 1-sulfoxy-3-methylcholanthrene

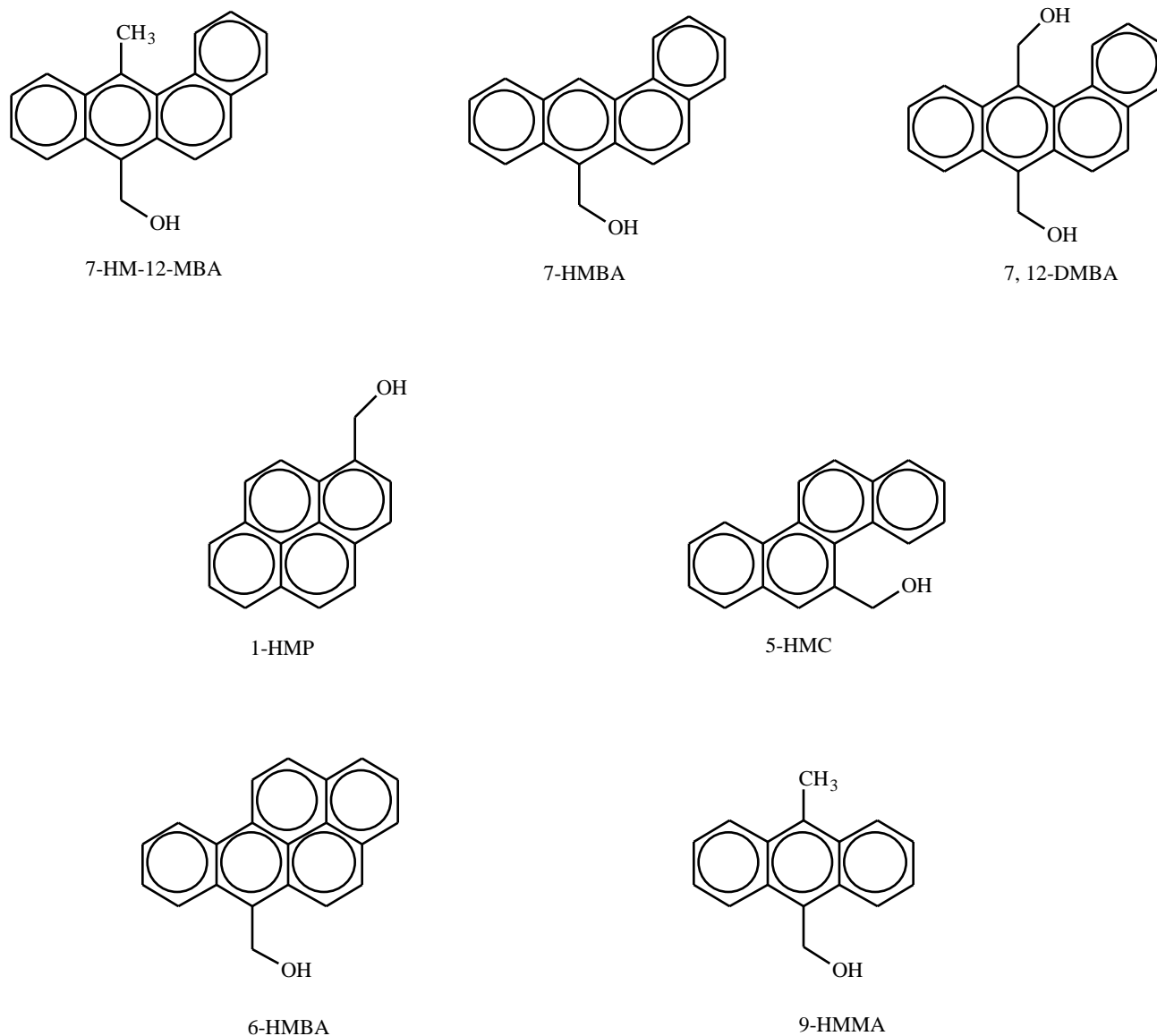


Fig. (4). Structures of PAHs bearing primary benzylic alcohol group(s) that are shown to be mutagenic and carcinogenic.

[70], 9-sulfooxymethylanthracene [71], 6-sulfooxymethylbenzo[a]pyrene [73], 1-sulfooxymethylpyrene [74], 7-sulfooxymethyl-12-methyl-benz[a]anthracene [72], or 7-sulfooxymethyl-benz[a]anthracene [75]. 6-Sulfooxymethylbenzo[a]pyrene was also reported as direct mutagen in *Salmonella* and Chinese hamster V79 cells [76]. These results were in good correlation with the complete carcinogenicity hypothesis of Flesher *et al.* [38,58] indicating that the SULT superfamily may play a final role for the formation of ultimate carcinogenic species from these PAH molecules.

PAHs Bearing Secondary Benzylic Alcohols

Most of the PAHs bearing secondary a benzylic hydroxyl group are in the form of cyclic derivatives as shown in (Fig. 5), and many have been reported to be mutagenic to *Salmonella typhimurium* in the Ames test in the presence of hepatic cytosols and PAPS [27,51,77]. The most studied PAHs in this class are the mono- and dihydroxy derivatives of the ubiquitous environmental and occupational pollutant cyclopenta[cd]pyrene (CPP) [51,78-80]. In the case of this molecule, DNA-adduct formation

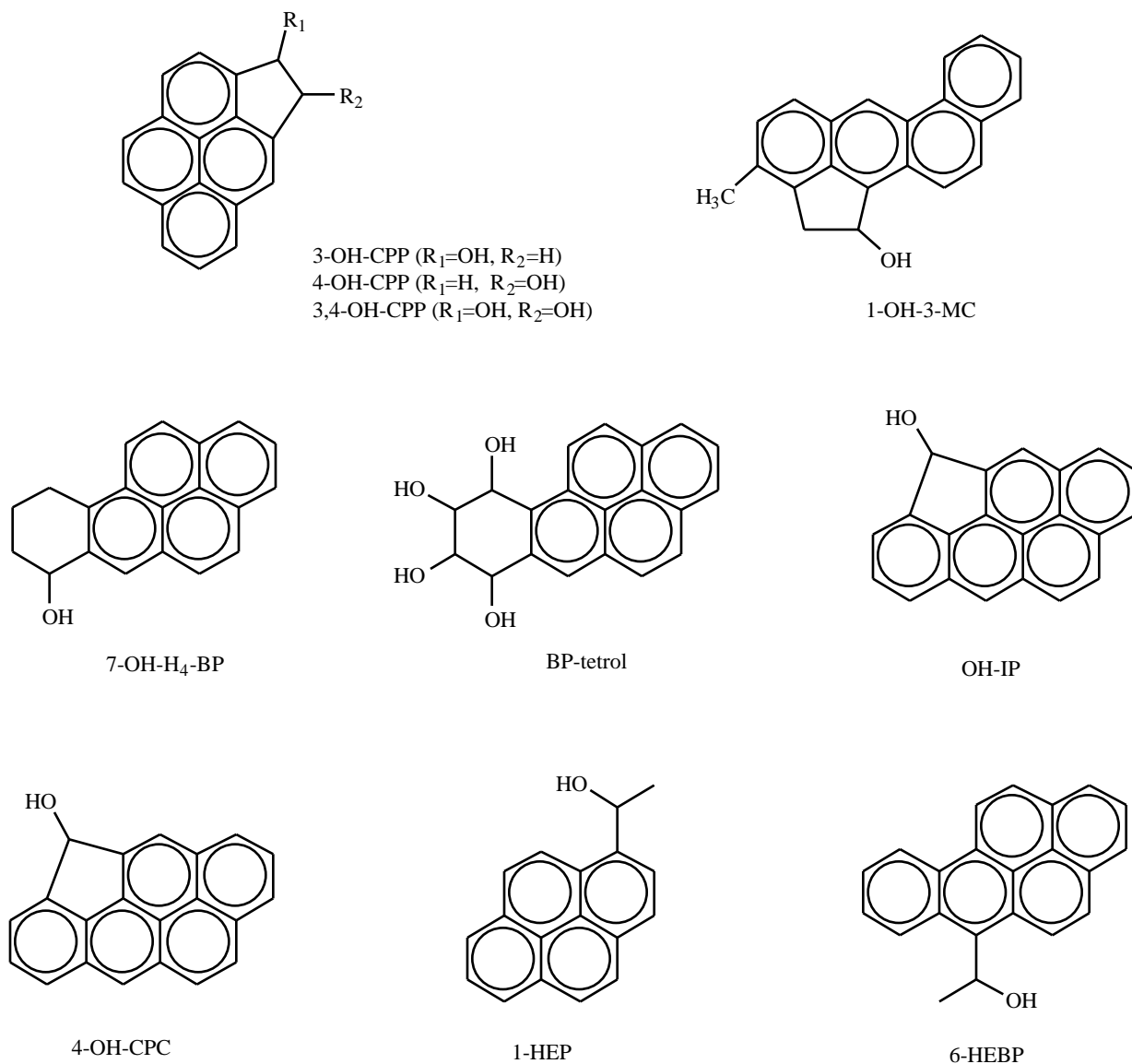


Fig. (5). Structures of PAHs bearing secondary benzylic alcohol group(s) that are found to be mutagenic and carcinogenic.

with calf thymus DNA was more strongly reduced by DCNP than DHEA, pointing out that aryl-SULT activity likely plays a more important role than alcohol-SULTs [80]. 1-OH-3-methylcholanthrene (1-OH-3-MC) is another example wherein the sulfoxy derivative of this molecule directly produces mutagenicity in bacteria [81].

In addition, enzymatic oxidation of BP produces such secondary alcohols as dihydrodiol and tetrol derivatives as primary metabolites [23,82]. Although possible sulfuric acid ester metabolite formed with benzylic-OH group of each of these metabolites would be expected to generate an electrophilic sulfuric acid ester capable of covalently binding to DNA, no such SULT-dependent mutagenicity was observed with dihydrodiol or

tetrol derivatives of BP [82]. However, the model benzo-ring reduced secondary benzylic alcohol, 7-OH-7,8,9,10-tetrahydro-BP (7-OH- H_4 -BP), covalently bound to DNA and exerted mutagenicity in the presence of rodent hepatic cytosols and PAPS [51,82]. Furthermore, another study indicated that BP-tetrols were activated by SULTs only at high substrate concentrations, and thus the importance of these metabolites to BP-mediated carcinogenicity was questioned [83].

Methylene bridged PAHs are universal pollutants, and they also represent a subclass of cyclic PAHs having secondary benzylic alcohol group. There are considerable number of reports indicating the SULT-mediated mutagenicity of these PAHs [51,77,78,84,85]. Incubations of these

PAHs with rat and/or human liver cytosol fortified with PAPS, or with recombinant rat SULT from *E. coli*, produced mutagenicity in Ames tests utilizing *S. typhimurium*. Examples include 4H-cyclopenta[def]chrysen-4-ol (4-OH-CPC) [85], 10H-indeno[1,2,7,8a-bcd]pyren-10-ol (OH-IP); see (Fig. 5) for structures. In addition, non-cyclic secondary benzylic alcohols derived from PAHs such as 1-hydroxyethylpyrene (1-HEP) and 6-hydroxyethylbenzo[a]pyrene (6-HEBP) were also reported to induce mutagenicity that was mediated by SULTs [51].

Allylic Alcohols

It has long been suggested that compounds with allylic alcohol moieties can form alkylating species through their sulfate esters [86,87]. The most extensively studied of these are 1'-hydroxysafrole, 1'-hydroxyestragole, 5-hydroxymethylfurfural (5-HF) and very recently -hydroxytamoxifen (-OH-TAM) will be reviewed in this section. Structures of these compounds are shown in (Fig. 6).

Safrole (3,4-methylenedioxy-allylbenzene) and estragole (4-methoxy-allylbenzene) are the natural ingredients of many essential oils and flavors, and the greater carcinogenicity of their 1'-hydroxy metabolites has been reported [88-90]. In addition, DNA adducts produced by these molecules have also been characterized [90-92], and the major role of hepatic aryl-SULT activity in the metabolic activation and DNA adduct formation has been reported [50,90-93] and reviewed [23,24].

Recent work by Daimon *et al.* further demonstrated the SULT-mediated *in vivo* genotoxicity of safrole [94]. The data from their study indicated that metabolic activation of safrole by hepatic SULTs contributed to the induction of sister chromatid exchanges (SCE), chromosomal aberrations, and replicative DNA synthesis (RSD). They also showed the SULT-mediated formation of safrole-DNA adducts. During this process, an aryl-SULT inhibitor, PCP, significantly decreased the formation of safrole-induced genotoxic effects, and also the amount of DNA adducts formed by safrole. Based on previous experimental evidence, as well

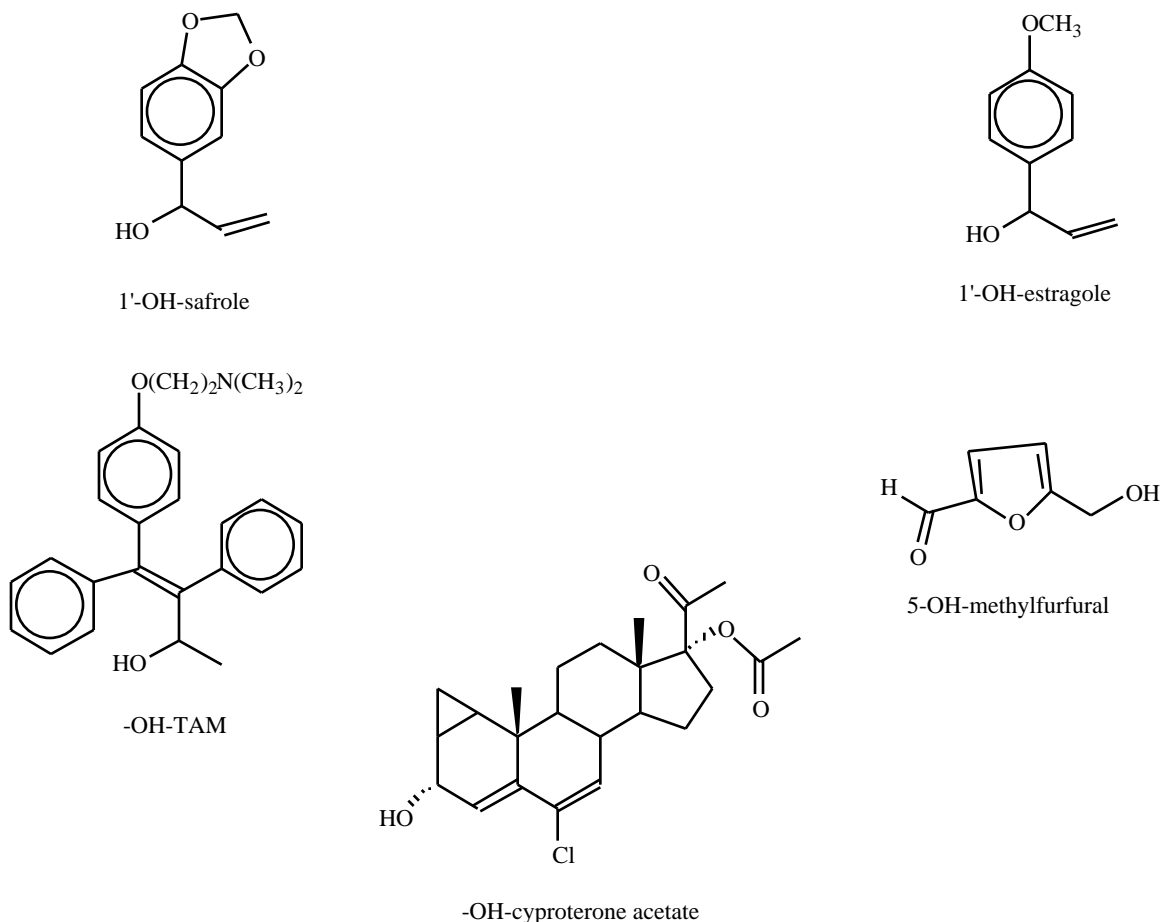


Fig. (6). Structures of allylic alcohols that are found to be mutagenic and carcinogenic.

as their *in vivo* genotoxicity results, they proposed that the formation of *SULT*-mediated safrole carcinogenesis seems to be the result of modification of DNA, followed by DNA replication which converts a repairable lesion into an inheritable chromosomal aberration or SCE [94]. They also added that the sulfate conjugate of safrole exhibits a cell proliferative activity which is required in the process of tumor promotion. Therefore, it can be concluded that genotoxicity and possibly carcinogenicity of safrole might be mediated by its sulfuric acid ester metabolite formed by aryl-*SULTs*.

Estragole (1-allyl-4-methoxybenzene) is also known to be hepatotoxic and hepatocarcinogenic when given to rodents at high doses [90,95,96]. Previous findings indicate that metabolic activation of 1'-hydroxy metabolite of estragole requires hepatic *SULT* activity to form covalently bound DNA adducts [90,93,97]. Recently, Wakazono *et al.* also determined hepatic protein adducts derived from estragole [98]. They determined similar pattern of protein adducts by using estragole pretreated rats and human monoamine phenol *SULT* (hM-PST, TL-PST)-expressing V79 cells. The combination of their studies with previous evidence clearly implicates 1'-hydroxylation of estragole [90,99], followed by sulfation catalyzed by aryl-*SULTs*, in the formation of protein adducts as well as DNA adducts. Because the formation of protein adducts is important in the toxic mechanism of various chemicals and drugs [100,101], it is possible that the protein adducts formed by estragole contribute to its toxicity.

5-HF is commonly found in food products and parenteral solutions that are subjected to heat treatment [102,103]. The mutagenicity of this molecule in *Salmonella* was reported by using rat liver cytosol fortified with PAPS, and 5-sulfoxymethylfurfural exhibited direct mutagenicity in bacteria and in cultured human lymphoblast cells [104-106]. In addition, another allylic compound, 3'-hydroxycyproterone acetate, a major metabolite of an anti-androgenic drug, also produced stable DNA adducts [107].

Tamoxifen (TAM) is a non-steroidal antiestrogen that has widely been used for the treatment of breast cancer [108,109]. Moreover, its prophylactic use for women with a genetic history of breast cancer is under investigation. However, its established carcinogenicity in rat liver [110-112] and a small but increased risk of endometrial cancer in long-term TAM-treated breast cancer patients [113] remains of concern. -OH-TAM is the major allylic alcohol

metabolite of TAM, and it is hypothesized to be the metabolite responsible for the adverse carcinogenic effects of this molecule [114-116]. Shibutani *et al.* recently demonstrated the diastereomeric -(N²-deoxyguanosyl)tamoxifen (dG-N²-TAM) DNA-adducts that have been formed via the sulfuric acid ester metabolite of -OH-TAM in the presence of rSTa [117,118]. Their results clearly show that -OH-TAM is a substrate for rat STa, and also the synthetic sulfate ester metabolite directly formed the same dG-N²-TAM-DNA adducts determined by ³²P-post-labeling and HPLC analysis [118]. They have indicated that these adducts are highly miscoding, and that they may result in G → T and G → C transversions and deletions in mammalian cells. Further studies with recombinant human alcohol-*SULT* (hHST) have found the formation of the same dG-N²-TAM DNA-adducts as was observed with rSTa [117]. However, the quantitative comparison of the amount of TAM-adducts indicated that three times less DNA adducts formed in the presence of hHST than that of formed with rSTa [117]. These results also demonstrated that the major DNA-adduct is formed with the *trans*-isomer of -OH-TAM, while a minor DNA adduct formation is observed with the *cis*-isomer. This was in good correlation with the finding that *trans*- -OH-TAM was better substrate for rSTa [118]. They have proposed that the minor *cis*-TAM-DNA adducts might be produced due to intramolecular conversion between the *cis* and *trans* forms of -OH-TAM which may occur through a carbocation intermediate; see (Fig. 7). Others have also reported the formation of TAM-adducts from both rat and human hepatocytes. In these cases, the DNA-adduct formation from human hepatocytes was considerably lower when compared to rat hepatocytes indicating that either the rate of formation of -OH- and subsequent sulfate metabolites could be lower in the case of human hepatocytes or the expression of the respective enzymes in human tissues could also be lower [115,116,119]. Furthermore, Glatt and his colleagues used heterologously expressed rSTa and hHST in bacteria and they found that the -OH-TAM showed pronounced mutagenic effect in cells expressing rSTa. The human correspondent of this enzyme in their system, hHST, caused weaker mutagenic effect (i.e., 20 times less active) [83,120].

SULT-mediated Bioactivation of N-OH-AAs and N-OH-HAAs

It is well established previously that N-OH metabolites of carcinogenic AAs and HAAs require phase-II enzymes to exert cellular and genetic

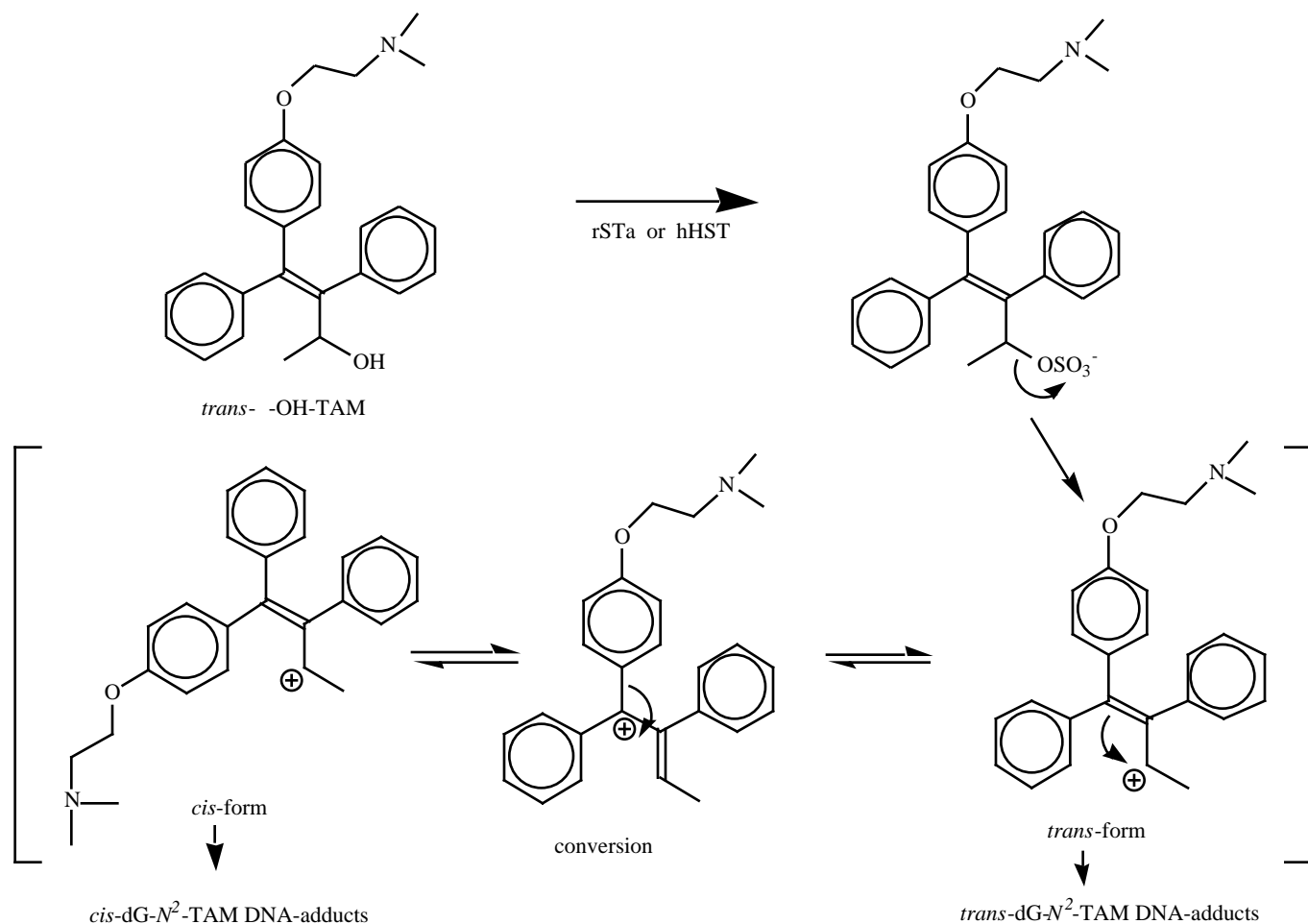


Fig. (7). Proposed mechanism for the formation of *cis*- and *trans*-TAM-DNA adducts from *trans*-*OH*-TAM (adapted from reference [118]).

damage [55,121-128]. Among these phase-II enzymes, human liver SULTs were suggested to have significant roles for chemical carcinogenesis of aromatic amines in humans [55,122,123,125,128,129].

The most extensively studied of these compounds are 2-aminofluorene (2-AF) and its amide derivative 2-acetylaminofluorene (2-AAF) [130,131]. Studies with these molecules opened the doors and acted as models to understand the mechanisms of metabolic activation of this class of compounds in chemical carcinogenesis and mutagenesis. Early studies with 2-AAF demonstrated that the hepatic SULT activity was responsible for the formation of short-lived electrophilic sulfuric acid ester metabolite of this molecule [132,133]. After these pioneering works, extensive studies have been done with this molecule as a model for elucidation of metabolic activation of N-OH-aromatic amines and amides [131]. When

we look at the major pathways involved in the metabolic activation of 2-AF and 2-AAF, we see that N-OH-metabolites of these compounds resulting from phase-I metabolism are further metabolized to esters (acetyl and sulfate esters), and, as demonstrated in (Fig. 8), two of the five elucidated activation pathways are mediated by SULTs. Sulfate esters formed from N-OH-AAF and N-OH-AF are the ultimate metabolites to produce DNA-adducts [134-136]. In addition, these sulfate metabolites of 2-AAF and 2-AF were elucidated to be the ultimate metabolites for development of hepatocarcinogenesis in male B6C3F1 mice [49,135]. In mice, N-OH-AAF and N-OH-AF formed only N-(deoxyguanosin-8-yl)-AF DNA-adduct [135-137]. Due to the excellent leaving group abilities of sulfate esters, highly reactive nitrenium ion species are produced, and these nitrenium ions are the reactive intermediates to form covalent bonds at nucleophilic sites on DNA. The reader is directed to cited reviews for the

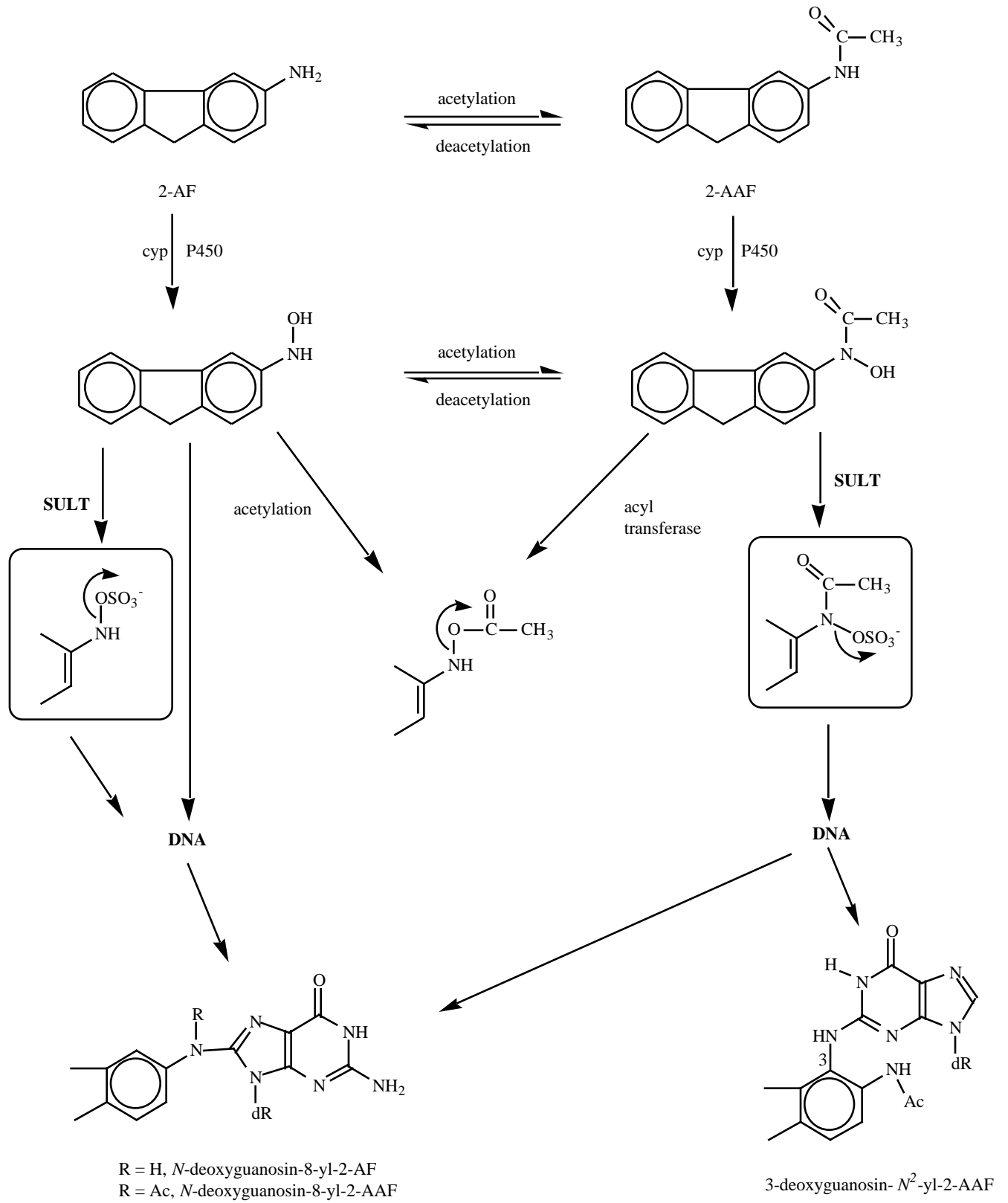


Fig. (8). Established metabolic activation pathways of N-OH-AF and N-OH-AAF (adapted from reference [131]).

primary references on the metabolic activation of 2-AAF and 2-AF [130,131].

In addition, other AAs have also been shown to be specifically activated by SULTs. For example, N-OH-4-aminobiphenol (N-OH-4-ABP) has been specifically activated to electrophilic species by the polymorphic human thermostable phenol-SULT (TS-PST) [122]. Following this report, Chou *et al.* also sought the role of human SULTs in the metabolic activation of several other carcinogenic AAs [55]. These carcinogenic compounds included N-OH-AF, N-OH-AAF, N-OH-4,4'-methylene-bis(2-chloroaniline) (N-OH-MOCA) as well as N-OH-ABP, see the structures in (Fig. 9). They again indicated that human polymorphic TS-PST is primarily responsible for the *in vitro* PAPS-dependent bioactivation of these compounds. Their study with other human tissues also concluded that PAPS-dependent DNA binding activity has also been observed in cytosols from human colon [55]. With regard to polymorphism in the expression of

human TS-PST [138-140], their results emphasized that the ability of human TS-PST to activate these N-OH-AAs might have an important role to determine interindividual susceptibility to such environmental carcinogens.

Some N-OH metabolites of HAAs that are formed from certain amino acids during the cooking of food are also classified as food mutagens. These include the N-hydroxy metabolites of 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), 2-amino-3-methyl(4,5-f)quinoline (IQ), 2-amino-6-methyl-dipyrido(1,2-a:3',2'-d)imidazole (Glu-P-1) and 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline (MeIQx) [42,141-144]. Structures of these N-OH-HAAs are shown in (Fig. 9). Subsequent esterification of these N-OH metabolites by conjugation enzymes including SULTs appears to be the ultimate step for the carcinogenicity and mutagenicity of these molecules [49,145]. Among these carcinogens, N-OH-PhIP and N-OH-Glu-P-1 require SULT-mediated metabolic activation,

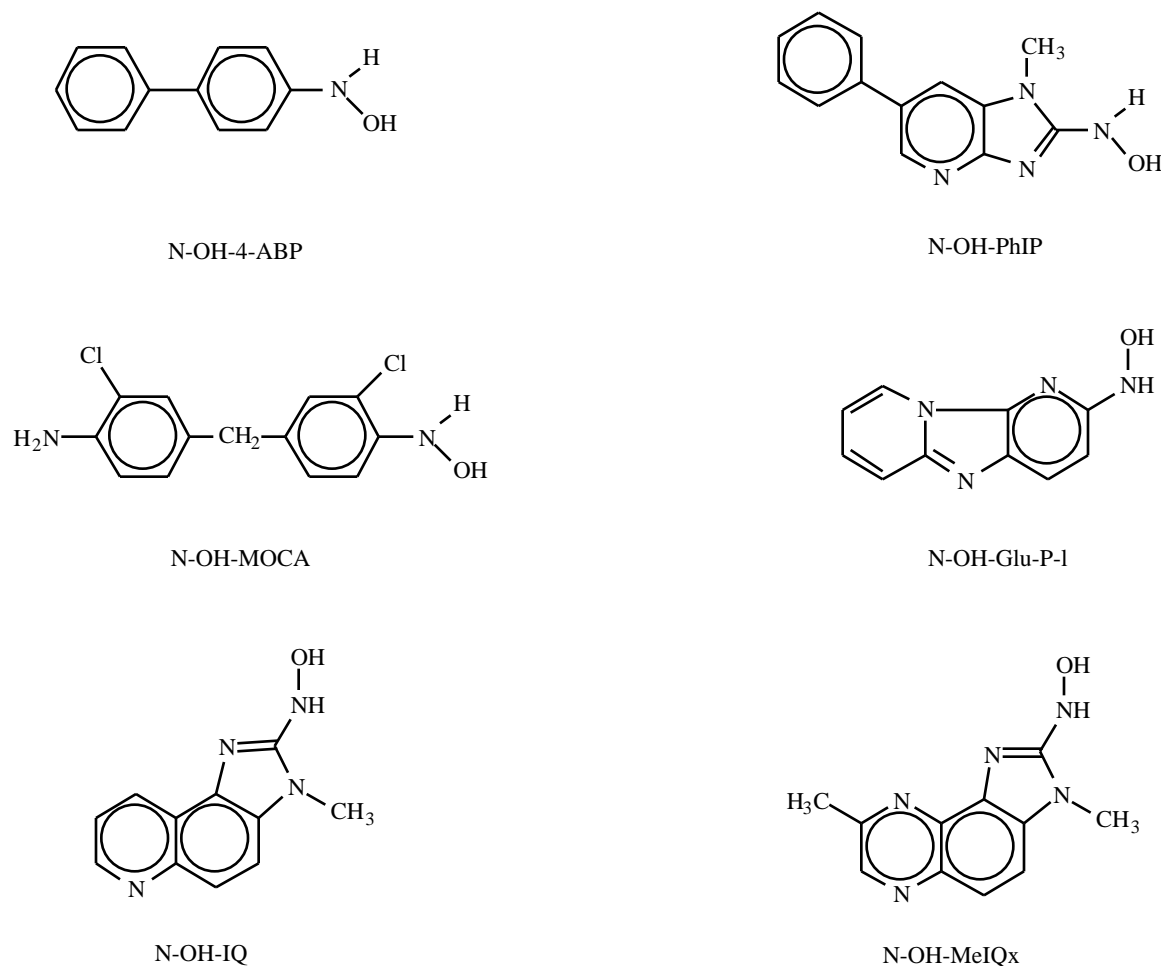


Fig. (9). Structures of N-OH-AAAs and N-OH-HAAs that are found to be mutagenic.

whereas cytosolic SULTs were not effective in the activation of N-OH-IQ and N-OH-MeIQx.

Among these heterocyclic amines, PhIP is the most prevalent and most studied mutagenic one [146,147] that forms during the cooking of meat products [40]. A study that compared human aryl-SULTs (ST1A2 and ST1A3) and rat aryl-SULTs (ST1A1, ST1B1 and ST1C1) in PAPS-dependent activation of N-OH-PhIP demonstrated that human SULT isoforms mediated the covalent binding of this molecule to calf thymus DNA 5.2 and 6.2 times higher than that of rat ST1C1 (i.e., the main isoform in rat liver responsible for activation of N-OH-2-AAF) [52]. Although human aryl-SULT isoforms potentially activated this molecule when compared to the corresponding enzymes in the rat, considerable interindividual differences were also present in bioactivation of PhIP [52]. In the case of N-AAF and N-OH-Glu-P-1, the situation was opposite (i.e., the rat isoforms mediated the activation of these N-OH metabolites at least five times higher than human counterparts) [128]. This was an indication of the presence of different substrate specificities among mammalian SULTs. Since these aryl-SULTs are also expressed in extrahepatic tissues such as brain, platelets and intestine, they become important in tissue specific metabolic activation of these carcinogens.

Recent findings also suggest that metabolic activation of these dietary carcinogens increase the risk factor in the etiology of human breast cancer [41,148-150]. *In vivo*, PhIP has been indicated to induce both colon and mammary tumors in rodents [151,152]. To determine the role of PhIP in the etiology of breast cancer, Dubuisson *et al.* studied the metabolic activation of this food carcinogen by human mammary gland enzymes including acetyltransferase, tRNA synthetase/kinase and SULT. They have shown by using a ^{32}P -postlabeling method that all of the three human breast enzymes converted the promutagen N-OH-PhIP to PhIP-DNA adducts, although each individual donor possessed a variable combination of one or more of the enzyme activities [148]. While the SULT activity played a less prominent role when compared with the other two-enzymes in the bioactivation of PhIP in human breast tissue, interindividual differences in the expression of these enzymes may still indicate the importance of SULT isoforms in the initiation of breast cancer. Another study showing the involvement of SULT in the bioactivation of PhIP was reported by Lewis *et al.* [149]. By using human mammary epithelial cells (HME) and human recombinant estrogen-SULT (EST), they investigated the DNA-adduct formation

properties of PhIP. Since the EST is the only SULT isoform expressed in HME cells [153], this isoform may also possess a significant role in the etiology of breast cancer. In support of this hypothesis, their results indicated that EST catalyzed the sulfation of N-OH-PhIP resulting in a more reactive sulfated metabolite that was capable of binding to DNA. Furthermore, they also observed variability for formation of PhIP-DNA adducts among studied individuals reflecting a difference in EST expression [149]. Because the EST expression is hormonally regulated in the human [154], high progesterone levels during the menstrual cycle might have a role in the susceptibility of women to chemical carcinogenesis through metabolic activation by high levels of EST.

SULT-mediated Bioactivation of 2-nitropropane and other Secondary Nitroalkanes

2-Nitropropane (2-NP) is a constituent of cigarette smoke [155] and a widely used industrial solvent that is reported to be a potent hepatocarcinogen in rats [156,157]. Its genotoxicity has also been reported by numerous researchers [158-161]. Investigations on the carcinogenicity and genotoxicity of 2-NP eventually revealed that 2-NP is metabolically activated by specific liver enzymes [161-163]. Recent evidence proved that hepatic SULT activity is required for the metabolic activation of 2-NP and its anionic form, propane 2-nitronate (P2N), and also other secondary nitroalkanes such as nitrocyclohexane and nitrocyclopentane, to reactive species that are capable of inducing genotoxic effects [164-167]. For example, Sodom *et al.* determined an aminated-DNA adduct, 8-aminoguanine, and postulated that aryl-SULT-mediated sulfation might have a role in the formation of this adduct [168]. They proposed a mechanism, shown in (Fig. 10), wherein an unsubstituted nitrenium ion (NH_2^+) could possibly be formed and aminate proteins and nucleic acids [165,168,169]. They also showed that *in vitro* sulfation of 2-NP catalyzed by aryl-SULT resulted in 8-aminoguanine and 8-oxoguanine adducts, as well as an unidentified modified guanosine nucleoside [165,167,168]. They subsequently characterized the previously unidentified modified nucleoside as 2-hydrazinohypoxanthine (N^2 -aminoguanine) [170]. Another study by Kreis *et al.* further demonstrated the importance of SULTs in the bioactivation of 2-NP and P2N by using cultured ovine seminal vesicle cells (OSV cell) [166]. OSV cells express high levels of aryl-SULT activity [171], and these studies were the first to show that 2-NP could be activated by cells other

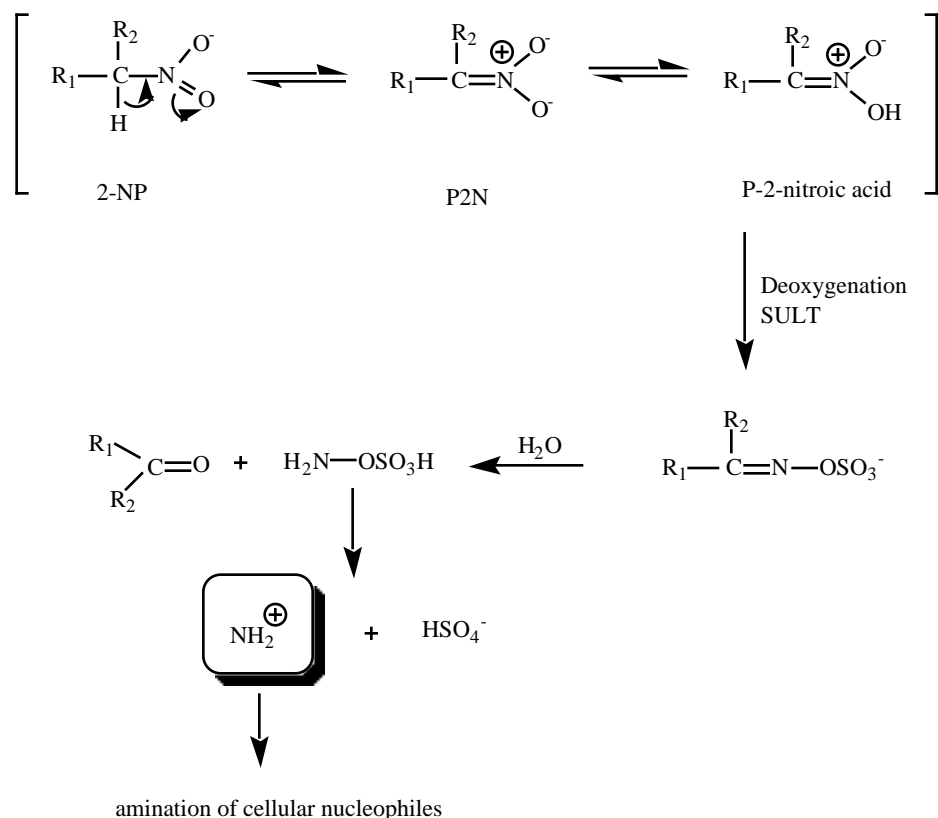


Fig. (10). Proposed pathway for the formation of unsubstituted nitrenium ion (NH_2^+) from secondary nitroalkanes (adapted from reference [168]).

than hepatic cells. They found that the aryl-SULT inhibitor, PCP, strongly reduced the genotoxic effects of 2-NP and other secondary nitroalkanes. Based on this, Andrae and his colleagues have investigated the genotoxicity of P2N in V79-cell lines that are engineered for expression of individual SULT isoforms. They have demonstrated that cell lines expressing SULT1A1 (rAST IV) and SULT1C1 (an N-OH-AF-sulfating SULT form in rat) clearly showed a concentration-dependent induction of DNA repair synthesis [164]. Therefore, they concluded that these two SULT isoforms were able to catalyze the activation of 2-NP/P2N. The above results are in line with the conclusion that aryl-SULTs may be responsible for the genotoxicity and carcinogenicity of 2-NP/P2N and other secondary nitroalkanes in rat. However, whether or not human SULTs can metabolically activate these secondary nitroalkanes remains to be determined.

SUBSTRATE SPECIFICITY OF SULTs

We currently understand from the metabolic activation studies depicted above that benzylic/allylic alcohols and N-OH derivatives of

arylamines and heterocyclic amines could serve as substrates for SULTs. However, in most of these studies, SULTs have been indicated to have a role in the bioactivation of these compounds by using hepatic cytosols and on the basis of the sensitivity of these transformations to specific SULT inhibitors. Elucidation of the involvement of individual isoforms of SULTs in these processes has usually required studies with homogeneous preparations of SULT isoforms or heterologously expressed individual SULT isoforms. Various aspects of the substrate specificities of individual SULTs in the metabolism of mutagens and carcinogens will now be reviewed.

Heterologous Expression Systems Expressing Rat and Human SULT Isoforms in the Activation of Mutagens

Evidence for bioactivation of PAHs by SULTs was obtained by Glatt and his colleagues using heterologous systems that expressed individual rat and human SULT isoforms, and this has been recently reviewed [25,26,83]. In most cases, the *in vitro* mutagenicity of the PAHs was studied using

hepatic cytosols fortified by PAPS as an external activating system. However, Glatt *et al.* discovered during their studies that externally generated sulfate conjugates of some PAHs may not be stable enough to penetrate the bacteria and are therefore inefficient in producing mutagenic responses in standard mutagenicity test systems. They also detected secondary reactive species created by the displacement of sulfoxy-group with medium components such as chloride and acetate ions [172-175]. Therefore, they proposed that formation and penetration of possible secondary species into the bacteria in standard test systems may mask the intrinsic mutagenicity of sulfuric acid ester metabolites of PAHs. To overcome these problems, they have engineered the bacterial and mammalian indicator cells expressing individual SULTs [25,26,83]. Thus, they have constructed *Salmonella* strains to express the following SULTs: rat hydroxysteroid sulfotransferase a (rSTa), rat aryl sulfotransferase IV (rAST IV), human hydroxysteroid sulfotransferase (hHST), human phenol-sulfating (thermostable) phenol sulfotransferase (hP-PST or TS-PST), human monoamine-sulfating (thermolabile) phenol sulfotransferase (hM-PST or TL-PST), and human estrogen sulfotransferase (hEST). In addition, they have constructed mammalian cell lines (Chinese hamster V79-derived cell lines) expressing rSTa, rAST IV, hHST, hP-PST, hM-PST, rat hydroxysteroid sulfotransferase 20 (rST20) and rat hydroxamic acid sulfotransferase (rST1C1) [26,83].

According to mutagenicity studies performed with these bacterial and mammalian cell lines, 1-HMP elucidated mutagenic activity with each of the SULT strains tested, although the degree of mutagenicity was the strongest with strain expressing hEST. The mutagenic response of 6-HMBP was strongly detected only in strains

expressing hHST and rSTa. Strains hEST, hP-PST, rSTa, and hHST were able to activate the 7-OH- H_4 -BP. However, mutagenicity was strongest for the strain expressing hEST. 9-HMMA and 9-HMA also exhibited strong mutagenicity in the strain expressing hHST, and they showed lower mutagenicity in the strain expressing the corresponding rat homolog of this enzyme (rSTa). 1-OH- and 2-OH metabolites of 3-MC were also mutagenic to the hHST-expressing strain. However, 2-OH-3-MC resulted in a stronger mutagenic response in the strain expressing hP-PST than that of the hHST. Hycanthone, a benzylic alcohol-containing drug molecule that has been withdrawn from market, exhibited stronger mutagenicity for the strain expressing hHST than the corresponding rat strain, rSTa [26,83]. 1'-Hydroxysafrole, an allylic alcohol that is the carcinogenic metabolite of safrole, also tested positive for mutagenicity against these SULT-strains [83]. Strains engineered for hHST and rSTa showed positive results, but not the strains expressing aryl-SULTs. This result conflicted with previously reported data wherein the selective aryl-SULT inhibitor, PCP, decreased the carcinogenicity of 1'-hydroxysafrole in mice [23,93,176]. In previous studies, the inhibitory effect of PCP was studied extensively by using hepatic cytosols as the source of SULT activity and suggested that the aryl-SULT activity was responsible for the carcinogenicity of this compound [24,93,96,97]. However, Glatt's results may suggest that in previous studies with rodents, additional SULTs might be involved in the activation of this compound. A summary of the mutagenicity of benzylic alcohols derived from PAHs can be found in Table 1.

Glatt *et al.* also tested the mutagenicity and genotoxicity of a series of closely related PAH-

Table 1. Substrate Specificity of Individual SULTs Expressed in *Salmonella* TA1538 toward Promutagens¹

Compound	rSTa	hHST	hP-PST	hM-PST	hEST
1-HMP	+stronger	+strong	+moderate	+moderate	++strongest
(R)-(+)-1-HEP	++strongest ^a	++strongest ^b			+weak
(S)-(-)-1-HEP	+strong	+strong			++strongest ^c
6-HMBP	++strongest	++strongest	-ND ^d		+weak
7-OH-H_4-BP	+moderate ^e	+moderate ^e	+strong ^f	-ND	++strong

(Table-1). contd....

Compound	rSTa	hHST	hP-PST	hM-PST	hEST
10-OH-H₄-BP	- ND	+borderline ^g	- ND	- ND	++strong ^h
9-HMMA	++strongest	+weak	- ND	- ND	- ND
9-HMA	++strongest	+weak	- ND	- ND	- ND
1-OH-MC	- ND	+ strong	- ND	- ND	- ND
2-OH-MC	- ND	+strong	++strongest	- ND	- ND
-OH-TAM	++strongest	+borderline ^g	- ND	- ND	- ND
hycanthone	+strong	++strongest	- ND	- ND	+borderline
1'-OH-safrole	+moderate	+moderate	- ND	- ND	+weak

¹All data taken from references [25] and [83]. ^a moderate (3-fold) stereoselectivity in favor of (R)-(+)-enantiomer. ^b strong stereoselectivity (15-fold) in favor of (R)-(+)-enantiomer. ^c 150-fold stereoselectivity in favor of (S)-(-)-enantiomer. ^d non detectable. ^e higher substrate concentrations required for mutagenicity. ^f mutagenicity was equally strong with hEST at low substrate conc., but considerably decreased at high substrate conc. ^g activation close to the limit of detection. ^h mutagenicity was weaker than that caused by positional isomer, 7-OH-H₄-BP.

derived benzylic alcohols in structure by using Chinese hamster V79-derived mammalian indicator cells. The structures of these compounds are seen in (Fig. 11). The capability of these compounds to induce SCEs and to cause gene mutations at the *hprt* locus in V79-SULT cells is summarized in Table 2.

Substrate Specificity of Aryl (phenol)-SULTs

AST IV is the most extensively studied aryl-SULT isoform in rat liver. It has a very broad substrate specificity including phenols [1,4,177, 178], oximes [179,180], benzylic alcohols [181-183], arylhydroxamic acids, and N-OH-AAs [184-

Table 2. Mutagenic and Genotoxic Effects in V79 cells expressing rSTa, rST20 and rST1C1¹

Compound	V79-rSTa ^b	V79-rST1C1 ^b	V79-rST20 ^b
1-HMP^a	++ ^c	+ ^c	ND
2-HMP	++	+weak	+weak
1-HEP	++	+	ND
2-HEP	ND or borderline	ND or borderline	ND or borderline
10-OH-H₄-BP	++	+	+
7-OH-H₄-BP	ND or borderline	ND or borderline	ND or borderline
BP-7,10/8,9-tetrol	++ ^d	++ ^d	++ ^d
BP-7/8,9,10-tetrol	++ ^d	+ ^d	+ ^d
6-HMBP^e			
-OH-TAM^f			

(Table-2). contd....

Compound	V79-rSTa ^b	V79-rST1C1 ^b	V79-rST20 ^b
N-OH-2-AAF ^h			

^l All data taken from reference [83].^a did not induce gene mutations at the *hprt* locus in V79 cells expressing rSTa and rST20.^b induction of SCE in V79p cells expressing rSTa, rST1C1, and rST20.^c strongest effect detected at low concentrations for induction of SCE.^d strongest effect detected at high concentrations for induction of SCE.^e induction of gene mutations at the *hprt* locus in V79 cells expressing rSTa.^f induction of gene mutations at the *hprt* locus in V79 cells expressing rSTa and rST20.^h induction of gene mutations at the *hprt* locus in V79 cells coexpressing *cyp* P450 1A2 and rST1C1.

186]. Since the aryl-SULTs are indicated in the metabolic activation of certain N-OH-AAs and PAH-derived benzylic alcohols, investigations on the molecular interactions of the homogeneous AST IV with the series of model compounds are of value to determine and predict the involvement of this SULT family in the metabolism of these xenobiotics.

Studies with a series of benzylic alcohols revealed that hydrophobicity was a significant factor in determining the catalytic efficiency of AST IV. Binder *et al.* demonstrated a linear decrease in the apparent K_m value as a function of increasing lipophilicity of benzylic alcohols [182]. Subsequently, Rao *et al.* confirmed that stereochemistry at the benzylic carbon atom determined the nature of the interaction of the chiral

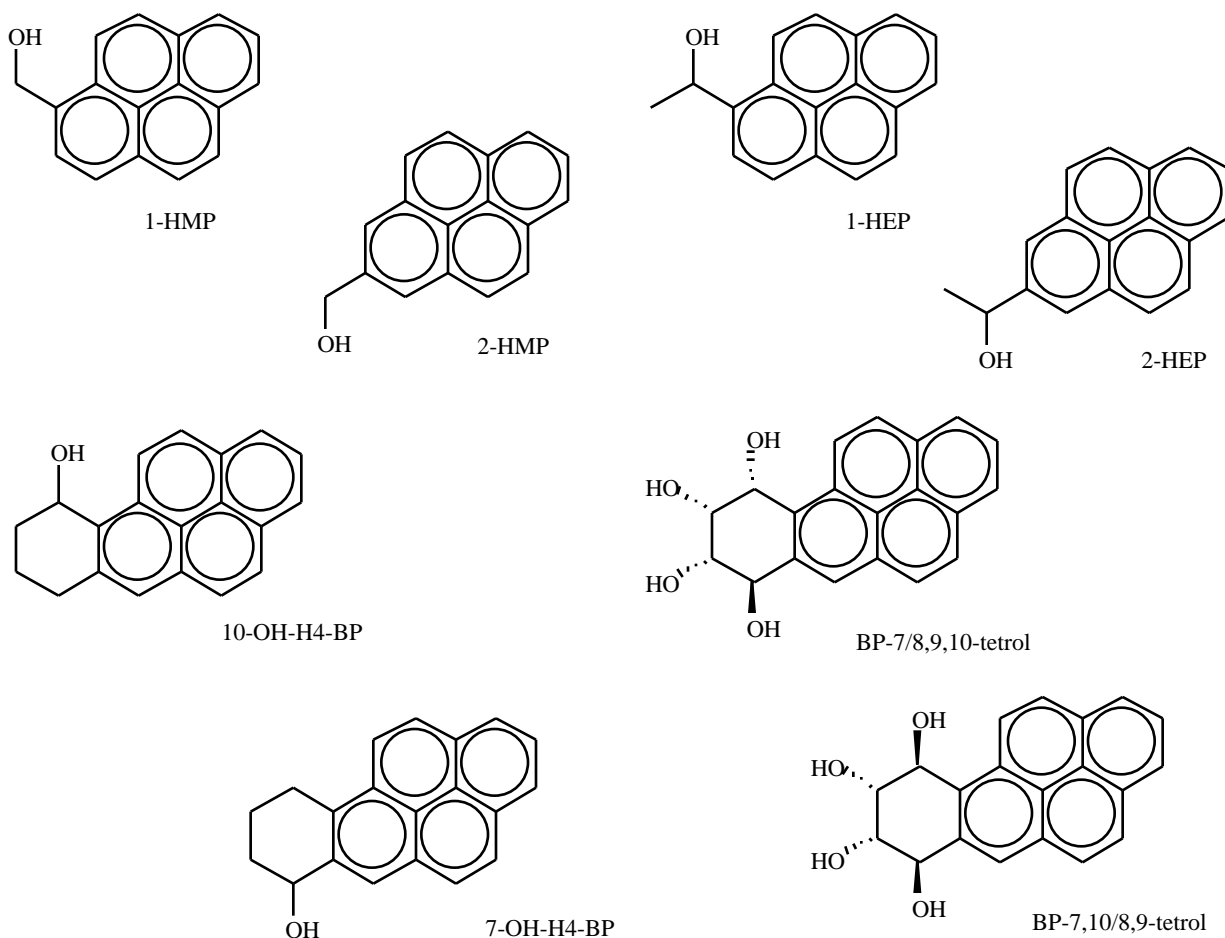
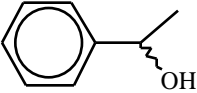
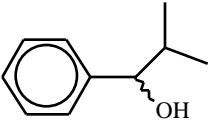
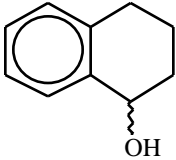
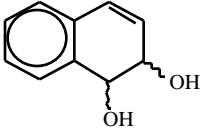
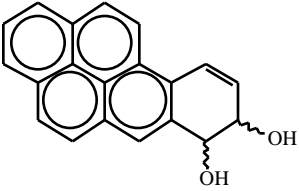


Fig. (11). Structures of closely related PAHs that are tested for their mutagenicity and genotoxicity in V79-cells expressing individual SULT isoforms.

benzylic alcohol with the enzyme [183]. In their study, presence of bulky substituents on the chiral benzylic carbon or being the benzylic carbon as a part of saturated ring led to a discrimination between enantiomers by AST IV (Table 3).

For instance, in the cases of 2-methyl-1-phenyl-1-propanol and 1,2,3,4-tetrahydronaphthol, only one enantiomer was substrate while the other was a competitive inhibitor of the AST IV [183]. Based on these results and previous kinetic evidence, they proposed that the active site of AST IV includes a

Table 3. Kinetic constants for AST IV-catalyzed Sulfation of Chiral Benzylic Alcohols and Dihydrodiols^a

Benzylic alcohol/dihydrodiol	(R) or (S)	K_m	V_{max}	k_{cat}/K_m	K_i
 (±)-1-phenylethanol	(R)-(+)-	0.6	18.8	1.91	-
	(S)-(-)-	0.36	22.0	3.73	-
 (±)-1-phenyl-2-methyl-1-propanol	(R)-(+)-	-	-	-	2.1
	(S)-(-)-	1.36	6.1	0.3	-
 (±)-1,2,3,4-tetrahydro-1-naphthol	(R)-(-)-	0.3	28.7	58.4	-
	(S)-(+)-	-	-	-	0.05
 (±)-trans-naphthalene-1,2-dihydrodiol	(1R, 2R)-(-)-	-	-	-	1.1
	(1S, 2S)-(+)-	-	-	-	1.7
 (±)-trans-benzo[a]pyrene-7,8-dihydrodiol	(7R, 8R)-(-)-	-	-	-	0.0044
	(7S, 8S)-(+)-	-	-	-	0.0037

^a Values for apparent K_m , V_{max} , k_{cat}/K_m , and K_i are expressed in mM, nmol min^{-1} (mg of STA^{-1}), $\text{mM}^{-1}\text{min}^{-1}$, and mM respectively. Values are taken from references [183] and [192].

lipophilic binding site for sulfonyl acceptor benzylic alcohol and a specific binding site for sulfonyl donor PAPS. According to their model, orientation of the sulfonyl group to be transferred with respect to the oxygen atom of benzylic alcohol would be critical, and the steric interactions that interferes with this determine the interaction of AST IV with its substrates [181]. Based on the stereoselectivity observed with chiral benzylic alcohols, interactions of chiral BP-dihydrodiols with AST IV were also investigated. As reported previously, BP-dihydrodiols are reactive intermediates that are formed stereoselectively, and further metabolized by oxidation to dihydrodiol epoxides that are the major carcinogenic and mutagenic metabolites of BP [57,187-189]. In addition, low levels of sulfate conjugates of BP-dihydrodiols were also reported [190,191]. However, studies with the *trans*-dihydrodiol derivatives of BP and naphthalene revealed that these compounds were not substrates for homogeneous AST IV, but were competitive inhibitors of this enzyme [192], see Table 3. This suggested that while these aromatic dihydrodiols were not substrates for AST IV, they still interacted with the active site of enzyme although either the binding orientation of these molecules or the presence of an extra OH group next to the OH to be sulfated did not allow the sulfation. However, this data should carefully be interpreted with regard to interactions of aromatic dihydrodiols with this isoform, since the sulfation of another dihydrodiol structure, 3,4-dihydroxy-3,4-dihydro-CPP, by aryl SULTs was reported [80]. This study demonstrated

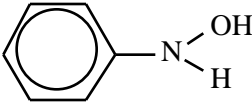
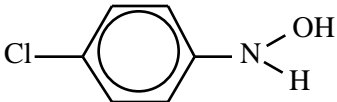
that aryl-SULTs more efficiently activated this dihydrodiol than alcohol-SULTs suggesting that some aromatic dihydrodiols might bind at the active site of aryl-SULTs in the proper orientation for sulfation.

Further studies on the interactions of different oxidation states of benzylic alcohols (i.e., benzaldehydes and benzylic carboxylic acids) with AST IV found that these molecules competitively inhibited this isoform [193,194]. Since these structures are commonly formed as intermediary metabolites from various drugs and xenobiotics, their interactions with SULTs are of importance because of the possibility that such metabolites of drugs and other xenobiotics may inhibit this superfamily of enzymes. The effects of such inhibition could involve both the detoxification of xenobiotics and the regulation of some physiological processes that are dependent upon sulfation.

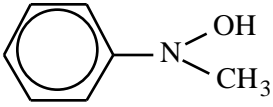
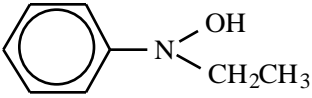
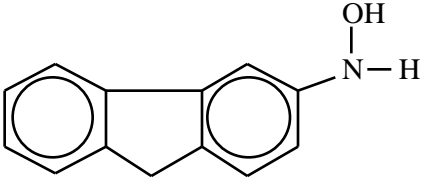
In addition to the benzylic alcohols, as explained previously, aryl-SULTs are also involved in the bioactivation of N-OH-AAs and N-OH-HAAs. Detailed investigation of the interactions of these compounds with AST IV has resulted in important conclusions for the involvement of this isoform in the bioactivation of these chemicals (Table 4).

For instance, while the model compound 4-chloro-N-OH-aniline was shown to be a substrate for AST IV at low concentrations, it also acted as a

Table 4. Summary of Kinetic Constants for Homogeneous AST IV Catalyzed Sulfation of N-OH-AAs^a

N-OH-AA	K_m (app)	V_{max}
 N-OH-aniline	230 ± 18	43 ± 1
 4-chloro-N-OH-aniline	26 ± 3	35 ± 2

(Table-4). contd....

N-OH-AA	K_m (app)	V_{max}
 <p data-bbox="406 506 646 533">N-methyl-N-OH-aniline</p>	120 ± 8	86 ± 6
 <p data-bbox="418 762 639 789">N-ethyl-N-OH-aniline</p>	650 ± 75	270 ± 12
 <p data-bbox="472 1087 586 1115">N-OH-2-AF</p>	-	0

^a Values for apparent K_m and V_{max} are expressed in mM and nmol min⁻¹ (mg of STa)⁻¹, respectively. Kinetic constants are taken from references [181] and [186].

time-dependent inhibitor [184]. Subsequently, N-alkyl substituted derivatives of these chemicals, N-methyl-N-OH-aniline and N-ethyl-N-OH-aniline, acted as substrates for the purified AST IV [181]. Lastly, King *et al.* demonstrated that N-OH-AAs are not stable and may undergo non-enzymatic oxidation during the *in vitro* enzyme assays. They reported that N-OH-aniline and N-OH-2-AF resulted in time-dependent and irreversible inactivation of homogeneous AST IV [186]. This inactivation was not dependent upon the presence of cofactor PAPS, and investigations with UV spectroscopy disclosed that nitrosobenzene and 2-nitrosofluorene intermediates were most likely the responsible oxidation products for the time dependent inactivation, presumably through interaction with the active site of enzyme. They additionally ascertained that under conditions where the oxidative degradation of N-OH-AAs was prevented, N-OH-aniline was substrate for the AST

IV. However, even under these conditions, N-OH-2-AF was still neither substrate nor inhibitor of this enzyme [186]. Although this was conflicting with some previously reported data on the sulfation-dependent bioactivation of N-OH-AF [123,125, 129,135], others have also reported that sulfation of N-OH-AF was not observed in their investigations [132,195]. In rat liver, another aryl-SULT isoform, ST1C1, has now been identified as a major N-OH-AF sulfating-SULT [196,197].

In addition to the above studies on aryl-SULTs in the rat, the human counterparts of these enzymes have also been extensively studied. Two aryl-SULT isoforms that are well characterized from human liver include the thermostable (phenol sulfating, TS-PST) and thermolabile (monoamine sulfating, TL-PST) aryl-SULTs [198,199]. Both of these forms have been reported to catalyze sulfation and metabolic activation of N-OH-AAs and

arylhydroxamic acids [129]. Therefore, it is proper to say that in general, sulfation of N-OH-AAs by aryl-SULTs in rat and human has a potential role for the *in vivo* toxicity of these chemicals.

Substrate Specificity of Alcohol (Hydroxysteroid)-SULTs

Previous studies have proved that alcohol-SULTs are responsible for the metabolic activation of PAHs bearing primary or secondary benzylic hydroxyl groups [24,25,53]. The first direct evidence for this activation with a pure isoform of alcohol-SULT was obtained by Ogura *et al.* wherein they demonstrated a rat liver cytosolic SULT, STa, catalyzed the formation of electrophilic sulfate esters from carcinogenic hydroxymethyl PAHs [53]. This investigation led to a more detailed SAR studies with this isoform to elucidate the active site characteristics in interactions with benzylic alcohols.

As shown with the AST IV, hydrophobic characteristics of the benzylic alcohols were also important influences on the catalytic efficiency of STa. Good correlation has been demonstrated between the partition coefficients ($\log P$) and the catalytic efficiency of the enzyme (k_{cat}/K_m) [200]. This important finding was in good agreement with the conclusion that very lipophilic PAHs are metabolically activated by this family of enzymes. However, further investigations also indicated that there was a limitation on the optimum size of the hydrophobic substrates that could be accommodated at the active site. For instance, the limitation on the extended carbon chain of *n*-alkanols to serve as substrates for STa was demonstrated. In that case, optimum catalytic efficiency of STa was obtained by *n*-alkanols with carbon chain length of C9-C11. For carbon chains above C11, there was a steady decrease in the catalytic efficiency of the enzyme, and with 1-pentadecanol and 1-hexadecanol the activity of enzyme was no longer detectable. This result also correlated well with studies on *p*-alkyl substituted benzyl alcohols in which 4-pentylbenzyl alcohol showed the optimum catalytic activity, and a steady decline in catalytic efficiency of the STa was achieved with substituents larger than pentyl at para position on the phenyl ring. Comparison of the molecular models of optimum alcohol substrates, *n*-decanol and 4-pentylbenzyl alcohol, with the molecular model of the DHEA, a physiological substrate for STa, displayed the analogous molecular dimensions for these three molecules in which the distance between the terminal carbon atom and the oxygen of the hydroxyl group was very similar [200]. The effect of the steric environment of

the hydroxyl group to be sulfated to the specificity of STa was also achieved by testing primary, secondary and tertiary alcohols as substrates for STa. In general, STa displayed the highest catalytic activity for primary alcohols, and this decreased in the order of secondary and tertiary alcohols. This was an indication of the steric effects that may play some role in these alterations of catalytic efficiency [200].

Following these results, Banoglu *et al.* investigated the stereochemistry of chiral benzylic alcohols that might influence the catalytic efficiency of the enzyme. This was important, because mutagenicity of some racemic PAHs bearing secondary benzylic alcohols [51] and also the enantiomers of 1-hydroxymethylpyrene were reported to involve activation by alcohol-SULTs [25,201]. Banoglu *et al.* investigated both stereochemical and steric interactions at the active site of STa by the use of *n*-alkyl-substituted chiral benzylic alcohols as model substrates [202]. Their results indicated that as the length of *n*-alkyl chain on the chiral benzyl alcohol was increased, the extent of the stereoselectivity of STa also increased. This means that benzylic alcohol with longer *n*-alkyl substituent led to a discrimination in catalytic activity by the enzyme in favor of one enantiomer, in these cases the (*R*)-(+)-enantiomer. Moreover, with the sterically bulky *n*-cyclohexyl substituted benzylic alcohol, STa showed absolute stereospecificity. In this case only the (*R*)-(+)-enantiomer was a substrate, whereas the (*S*)-(-)-enantiomer was a competitive inhibitor of the enzyme; see (Fig. 12). Based on these results they proposed that a specific orientation of the benzylic hydroxyl on the molecule must be achieved at the active site, and that steric factors may combine with the stereochemical configuration and the hydrophobicity of the molecule to determine the interaction of the enantiomers as substrates or inhibitors [202].

Banoglu *et al.* further extended their studies on the stereochemical interactions to model chiral PAH compounds having secondary benzylic hydroxyl group, see structures in Fig. (13) [203]. Their results with 1-hydroxyethylnaphthalene, 1-hydroxyethylpyrene and 9-hydroxyethylphenanthrene demonstrated that in all cases only the (*R*)-(+)-enantiomers were substrates and the (*S*)-(-)-enantiomers were competitive inhibitors of STa (Table 5).

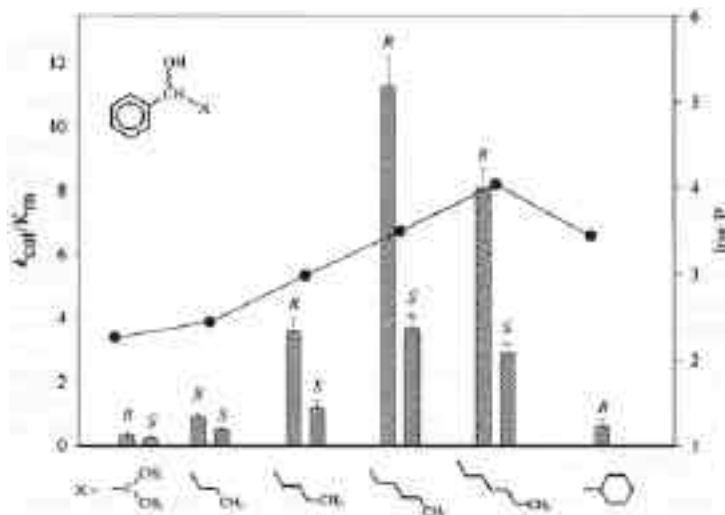


Fig. (12). Effects of α -alkyl-substituent on the stereoselectivity in the sulfation of chiral benzyl alcohols by STA demonstrated as a bar graph. In the case of α -cyclohexyl substituent, only the (R)-enantiomer was substrate whereas the (S)-enantiomer was a competitive inhibitor of STA with a K_i value of 0.75 ± 0.1 mM. Effect of hydrophobicity of compounds on the catalytic efficiency of STA shown as a line graph. All kinetic constants and hydrophobicity values are adapted from reference [202].

They also demonstrated that conformational changes of the benzylic alcohol moiety in these molecules are restricted due to steric interactions between the *peri*-hydrogen (H8) and the methyl and hydroxyl groups of the hydroxymethyl substituent at the 1-position on naphthalene and pyrene, and the corresponding 9-position on phenanthrene. Therefore, in PAHs with *peri*-substituent interactions, conformational restrictions combined

with the hydrophobic interactions between the enzyme and substrate to determine the specificity of STA for these *peri*-substituted PAHs. Hence, these results should help to elucidate and predict the molecular interactions that are required for SULT-mediated activation of PAHs. Indeed, Glatt and his colleagues reported that heterologously expressed rat STA discriminated in the metabolic activation of the stereoisomers of 1-hydroxyethylpyrene [25].

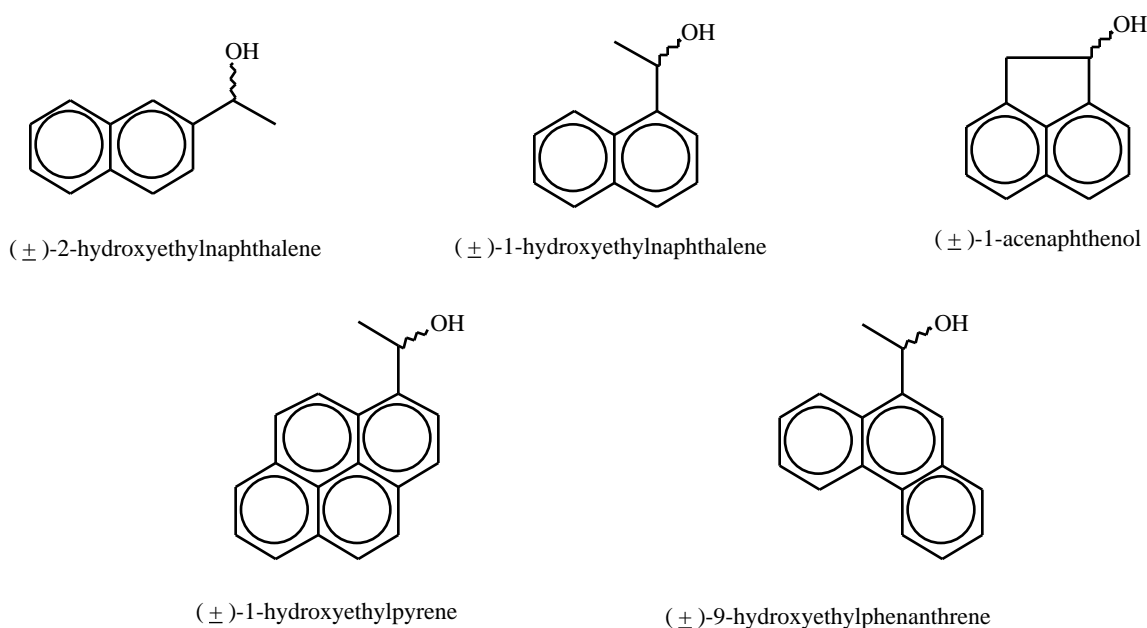


Fig. (13). Structures of model PAH-derived benzylic alcohols that are substrates and inhibitors for STA.

Table 5. Summary of Kinetic constants for Homogeneous rStA-catalyzed Sulfation of Model Benzylic Alcohols^a

Benzylic Alcohol	K_m	V_{max}	k_{cat}/K_m	K_i
(R)-(+)-2-hydroxyethylnaphthalene	1000 ± 170	34.0 ± 2.2	1.1 ± 0.19	-
(S)-(-)-2-hydroxyethylnaphthalene	1200 ± 200	28.5 ± 1.8	0.8 ± 0.13	-
(R)-(+)-1-hydroxyethylnaphthalene	800 ± 100	52.0 ± 2.9	2.2 ± 0.3	-
(S)-(-)-1-hydroxyethylnaphthalene	-	-	-	250 ± 20
(R)-(+)-9-hydroxyethylphenanthrene	90 ± 20	4.5 ± 0.5	1.7 ± 0.4	-
(S)-(-)-9-hydroxyethylphenanthrene	-	-	-	10 ± 1
(R)-(+)-1-hydroxyethylpyrene	7 ± 0.6	11.0 ± 1.0	49.2 ± 6.0	-
(S)-(-)-1-hydroxyethylpyrene	-	-	-	2 ± 0.2
(R)-(-)-1-acenaphthenol	560 ± 60	83.0 ± 3.3	4.8 ± 0.5	-
(S)-(+)-1-acenaphthenol	400 ± 40	121.0 ± 3.7	9.7 ± 0.9	-

^a All kinetic constants are taken from reference [203]. Values for apparent K_m , V_{max} , k_{cat}/K_m , and K_i are expressed in μM , $\text{nmol min}^{-1} (\text{mg of STa})^{-1}$, $\text{min}^{-1} \text{mM}^{-1}$, and mM , respectively.

Their results showed that (R)-(+)-1-hydroxyethylpyrene caused mutagenic response 2-fold greater than that caused by the (S)-(-)-enantiomer of this molecule correlating with Banoglu's results [25,203]. They also reported enantioselectivity with the same molecule by using heterologously expressed human HST-SULT and human EST-SULT [201].

CONCLUSIONS

An important concept that has emerged in recent years is that SULTs play an important role in the metabolic activation of certain chemicals to mutagenic and carcinogenic intermediates. Over the years, applications of recently developed techniques such as recombinant DNA technology have made great advances in SULT research in many aspects and facilitated to study the substrate specificity and

pharmacogenetics of these enzymes in relation to the role that they play in chemical carcinogenesis.

First of all, preparation of homogeneous SULT isoforms from original tissues and heterologous expression of individual SULTs in bacterial and mammalian indicator cells have provided us considerable information to understand the mechanism of metabolic activation of certain chemicals. For instance, the SAR studies described above with the homogeneous forms of rat AST IV and STa as representatives of their SULT families, have indicated important parameters for the recognition of substrates and inhibitors for these SULT families. These insights will be useful for understanding the potential roles of these SULT families in the metabolic activation of certain chemicals such as benzylic alcohols derived from PAHs and N-OH-AAs. For example, the enantioselectivity observed in the metabolic activation of 1-HEP shown by Glatt *et al.* with

heterologously expressed rSTa and hHST [25] correlated well with the stereospecificity observed for this compound with homogeneous STa [203]. Furthermore, the discovery that the presence of *peri*-substituent interactions was an important factor affecting the catalytic efficiency of STa may contribute to the prediction of analogous interactions with other PAHs where their racemates were shown to be mutagenic, but were not examined for contributions of individual enantiomers to their mutagenicity [51]. Additionally, sulfation of the phenolic metabolites of BP, a detoxication pathway for this molecule, may possibly be impaired by the *trans*-dihydrodiol metabolites of the same molecule as established by the inhibitory interactions of these metabolites with AST IV [192].

A further contribution of the research reviewed here is that it has provided us with insight into the specificity of rat and human isoforms of these SULTs that are important in metabolism of mutagens and carcinogens. Studies with experimental animals such as rats have resulted in identification of these SULTs as enzymes that contribute to metabolic activation and subsequent mutagenesis and carcinogenesis, and these findings may have relevance to the understanding of these events in humans. However, it is important to note that while many studies have focussed on the liver, alcohol-SULTs are also expressed at high levels in extrahepatic tissues such as adrenal gland in humans [204,205], and aryl-SULTs are likewise commonly expressed in other tissues such as gut and brain [206,207]. Therefore, it is necessary to keep in mind that, in both humans and rats, differences in extrahepatic expression of SULTs may contribute to the targeting of tissues other than liver for toxic effects.

In conclusion, it is more clear now that two-main SULT families are currently well evidenced to be responsible for the formation of mutagenic and, in some cases, carcinogenic responses from certain chemicals including PAH-derived benzylic alcohols and N-OH-AAs. However, since the role of oxygenase enzymes in the formation of procarcinogenic metabolites may also affect the SULT-dependent metabolic activation, the balance between oxidative and conjugative pathways is very important in determining levels of reactive metabolites that may be formed within tissues. Therefore, it is likely that the results obtained from these multifaced research on the role of SULTs in metabolic activation of chemicals reviewed herein will furnish a good foundation for more extensive investigations.

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ABBREVIATIONS

SULT	=	Sulfotransferase
PAPS	=	3'-Phosphoadenosine 5'-phosphosulfate
DHEA	=	Dehydroepiandrosterone
PAH	=	Polycyclic aromatic hydrocarbon
N-OH-AA	=	N-hydroxy arylamine
BP	=	Benzo[a]pyrene
aryl-SULT	=	Aryl (phenol) sulfotransferase
alcohol-SULT	=	Alcohol (hydroxysteroid) sulfotransferase
7-HM-12-MBA	=	7-Hydroxymethyl-12-methylbenz[a]anthracene;
7-HMBA	=	7-Hydroxymethylbenz[a]-anthracene
7,12-DMBA	=	7,12-Dihydroxymethylbenz[a] anthracene
5-HMC	=	5-Hydroxymethylchrysene
6-HMBP	=	6-Hydroxymethylbenzo[a]-pyrene
9-HMMA	=	9-Hydroxymethyl-10-methylanthracene
1-HMP	=	1-Hydroxymethylpyrene
PCP	=	Pentachlorophenol
DCNP	=	2,6-Dichloro-4-nitrophenol
CPP	=	Cyclopenta[cd]pyrene
3-MC	=	3-Methylcholanthrene
7-OH- <i>H₄</i> -BP	=	7-OH-7,8,9,10-Tetrahydrobenzo[a]pyrene

4-OH-CPC	=	4H-cyclopenta[def]chrysen-4-ol	ST1A2	=	Human thermostable phenol sulfotransferase
OH-IP	=	10H-indeno[1,2,7,8a-bcd]pyren-10-ol	ST1A3	=	Human thermostable phenol sulfotransferase
1-HEP	=	1-Hydroxyethylpyrene	ST1A1	=	Rat aryl sulfotransferase IV;
6-HEBP	=	6-Hydroxyethylbenzo[a]pyrene	ST1B1	=	Rat aryl sulfotransferase;
5-HF	=	5-Hydroxymethylfurfural	ST1C1	=	Rat hydroxylamine sulfotransferase
-OH-TAM	=	-Hydroxytamoxifen	HEST	=	Human estrogen sulfotransferase
SCE	=	Sister chromatid exchange	2-NP	=	2-Nitropropane
hM-PST or TL-PST	=	Human monoamine sulfating (thermolabile) phenol sulfotransferase	P2N	=	Propane-2-nitronate
dG-N ² -TAM	=	-(N ² -deoxyguanosinyl)-tamoxifen-DNA adduct	rST20	=	Rat hydroxysteroid sulfotransferase 20
hHST	=	Human alcohol (hydroxysteroid) sulfotransferase	REFERENCES		
rSTa	=	Rat alcohol sulfotransferase a	[1]	Duffel, M.W. (1997) in <i>Comprehensive Toxicology Vol. 3, Biotransformation</i> , (Guengerich, F.P. Ed.), Elsevier, Oxford, pp. 365-383.	
hP-PST or TS-PST	=	Human phenol sulfating (thermostable) phenol sulfotransferase	[2]	Coughtrie, M.W.; Sharp, S.; Maxwell, K. and Innes, N.P. (1998) <i>Chem. Biol. Interact.</i> , 109 , 3-27.	
2-AF	=	2-Aminofluorene	[3]	Weinshilboum, R. and Otterness, D. (1994) in <i>Handbook of Experimental Pharmacology Vol. 112</i> , (Kauffman, F.C. Ed.), Springer-Verlag, Berlin, pp. 45-78.	
2-AAF	=	2-Acetylaminofluorene	[4]	Mulder, G.J. and Jakoby, W.B. (1990) in <i>Conjugation reactions in drug metabolism</i> , (Mulder, G.J. Ed.), Taylor & Francis, New York, pp. 107-161.	
N-OH-4-ABP	=	N-hydroxy-4-amino-biphenol	[5]	Strott, C.A. (1996) <i>Endocr. Rev.</i> , 17 , 670-697.	
N-OH-MOCA	=	N-hydroxy-4,4'-methylene-bis(2-chloroaniline)	[6]	Jakoby, W.B.; Duffel, M.W.; Lyon, E.S. and Ramaswamy, S. (1984) in <i>Progress in Drug Metabolism Vol.8</i> , (Bridges, J.W. and Chasseaud, L.F. Eds.), Taylor & Francis, New York, pp. 11-33.	
PhIP	=	2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine	[7]	Falany, C.N.; Wheeler, J.; Oh, T.S. and Falany, J.L. (1994) <i>J. Steroid Biochem. Molec. Biol.</i> , 48 , 369-375.	
IQ	=	2-Amino-3-methyl(4,5-f)quinoline	[8]	Falany, J.L. and Falany, C.N. (1997) <i>Oncol. Res.</i> , 9 , 589-596.	
Glu-P-1	=	2-Amino-6-methyl-dipyrido(1,2-a:3',2'-d)imidazole	[9]	Luu-Thee, V.; Bernier, F. and Dufort, I. (1996) <i>J. Endocrinol.</i> , 150 , S87-S97.	
MeIQx	=	2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline			

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