Endogenous and Exogenous Ligands of Aryl Hydrocarbon Receptor: Current State of Art

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Abstract: Aryl hydrocarbon receptor (AhR) is an important transcriptional regulator of drug metabolizing enzymes that dominantly controls the expression of cytochrome P450 CYP1 family genes and some phase II enzymes. AhR also has many endogenous functions including cell cycle control, immune response, and cell differentiation. In addition, AhR is well-known to be involved in chemically-induced carcinogenesis. AhR is activated by a variety of endogenous and exogenous ligands. While exogenous activation of AhR has deleterious effects on human organism, sustained activation of AhR by endogenous ligands is indispensable for proper cell functions. Therefore, the effects of exogenous and endogenous ligands on AhR resemble the Dr. Jekyll and Mr. Hyde story.

The aim of the current paper is to summarize and update the knowledge on exogenous and endogenous AhR ligands.

Keywords*:* Aryl hydrocarbon receptor, dietary ligands, ligand dependent activation, natural compounds, synthetic ligands, xenobiotics.

1. ARYL HYDROCARBON RECEPTOR

Aryl Hydrocarbon Receptor (AhR), also called the dioxin receptor, is a ligand-activated transcriptional factor of the basic helix-loop-helix/per-ARNT-Sim (bHLH/PAS) superfamily. AhR is activated by a variety of exogenous and endogenous compounds. In addition to the regulation of the CYP1 family of xenobiotic metabolizing enzymes by AhR *via* exogenous ligands, recent recognition of endogenous AhR ligands helped us to understand that AhR also plays a role in many physiological functions [1]. The examples are regulation of the cell cycle and proliferation [2, 3], immune response [4-6], circadian rhythm [7], tumor promotion [8, 9], the expression of lipid metabolism genes [10, 11] etc.

Chromosomal localization of the human AhR gene is assigned to chromosome 7p15 [12]. AhR gene is about 50 kb long and contains 11 exons. The AhR gene encodes a 96 kDa protein [13, 14].

In its resting state, AhR is sequestered in the cytosol in a multiprotein complex with heat shock protein 90 (hsp90) and other proteins. Hydrophobic ligands penetrate through the cell membrane and bind to AhR, which in turn undergoes cytosol to nucleus translocation. In the nucleus, AhR forms a heterodimer with AhR-nuclear translocator (ARNT) [15-19]. AhR/ARNT complex then binds to specific DNA sequence called dioxin responsive element (DRE) or xenobiotic responsive element (XRE) (consensus sequence 5'-T/GNGCGTGA/CG/CA-3´; core sequence 5'-GCGTG-3'**)** in the promoter of target genes and triggers their expression [15, 20]. As a negative feedback, AhR is exported from the nucleus to the cytosol here degraded by proteasome-ubiquitin system [21-23].

AhR was first identified in mouse liver by Poland *et al*. employing specific binding assay with radiolabeled 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [24]. AhR was also the first identified ligand-activated transcription factor that mediates increased expression of enzymes involved in drug metabolism and disposition [25].

AhR and ARNT genes are expressed ubiquitously in adult and fetal tissues. In particular, relatively high levels of AhR mRNA expression are observed in lung, placenta and spleen from humanadults and lung and spleen from fetuses [14, 27, 28]**.** Whereas relatively high ARNT mRNA expression is observed in ovary, lung, spleen, testis and pancreas from adults and lung and kidney from human fetuses [26]**.**

AhR protein contains several domains critical for its function. The bHLH domains of both AhR and ARNT are required for specific DNA-binding, whereas dimerization of the HLH domains forms a four-helix bundle structure that stabilizes the dimer [29, 30]. The PAS domain is itself made up of two repeats of approximately 50 amino acid residues, known as PAS-A and PAS-B [31]. Other important domain of AhR protein is the ligand binding domain (LBD) that is localized in the sequence of 230-421 amino acids encompassing PAS B. Interaction of this domain with hsp90 is essential in formation of a functional ligand binding entity [32]. Several naturally occurring mutations within this region reduce AhR ligand binding affinity [33].

Docking models of the complex of AhR with AhR ligands (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 3-methylcholanthrene, β-naphthoflavone) showed their extensive contact with Phenylalanine 318 (Phe318), Isoleucine 319 (Ile319), and Histidine 320 (His320). Extensive hydrophobic interaction can also be observed with Alanine 328 (Ala328), Methionine 342 (Met342), Leucine 347 (Leu347), and Leu348. Two lysine residues Lys284 and Lys286 suggest the formation of hydrogen bonds with oxygens on the aromatic rings of β-naphthoflavone (BNF) [34]**.**

It is suggested that Phe318 plays a critical role in ligand binding to AhR. Binding experiments corroborated that location of Phe318 at the ligand-binding surface of the receptor plays a key role in ligand-binding specificity. Mutation on Phe318 caused changes in binding activity of the receptor. Moreover, two amino acids, Ile319 and His320, neighboring Phe318, were both found to play an essential role in the binding activity of the receptor. Mutations of these amino acids affect the activity of the receptor. Ala375, whose allelic mutation was demonstrated to be responsible for the different ligand-binding affinity between C57BL/6 and DBA/2 mouse strains [35] is also exposed into the ligand-binding pocket [34]**.**

2. LIGAND-DEPENDENT ACTIVATION OF AhR

Interaction between a ligand and a receptor is characterized by several variables and the final cellular response is dependent on the combination of these variables. In other words, activation of the receptor by two different compounds will result in a different quantitative but also qualitative outcome of cell response.

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Affinity - a measure of affinity of a ligand for receptor is *equilibrium dissociation constant* K_D. The affinity defines the relation between concentration of a ligand and *fractional occupancy* of the receptor and it is usually the highest for hormones and natural ligands. Alternatively, a measure of affinity could be expressed as the *half maximal effective concentration* EC_{50} that corresponds to an ability of a drug to elicit 50% of maximal effect. The ability of drug to elicit measurable functional change is called *potency*. Relative maximal effect of a drug in certain tissue in comparison with natural (or another highly potent) ligand is called *intrinsic activity* (IA). According to intrinsic activity, we distinguish several types of ligands, including *full agonists* (IA = 1, 100% intrinsic activity), *partial agonists* (IA = 0~1), *antagonists* (IA = 0) and *inverse agonist* (IA < 0) [36-38].

The majority of AhR ligands are partial agonists. There is a lot of confusion in the literature when partial agonists are referred to as antagonists. While partial agonists have similar affinity as full agonists, the intrinsic activity of a partial agonist is lower than that of a full agonist. Thus partial agonists never elicit maximal response, even if all receptors are occupied by a partial agonist. Importantly, partial agonists behave as functional antagonists, i.e. when combining full agonist with partial agonist; the effect of full agonist is diminished by partial agonist, hence, displaying antagonistic behavior.

Most compounds that bind and activate AhR are hydrophobic molecules. Many AhR agonists represent planar chemicals; however, the SAR analysis showed that absolute planarity is not necessary for ligand binding of polychlorinated biphenyls. Coplanarity influences a ligand´s steric fit of the receptor. It was estimated that an AhR ligand would be between 12 Å-14 Å in lenght, less than 12 Å in width and no more than 5 Å deep. For halogenated aromatic ligand, increased receptor affinity and activation are controlled in part by the polarizability of the substituent group. A number of researches reported electronegativity, hydrophobic and hydrogen-bonding as properties that could also contribute to receptor interaction [39].

3. EXOGENOUS AHR LIGANDS

3.1. Synthetic Compounds

The best characterized high-affinity AhR ligands include a variety of toxic and hydrophobic chemicals, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and halogenated dioxins and related compounds [40, 41].

3.1.1. Polycyclic Aromatic Hydrocarbons

PAHs represent a large class of AhR ligands that contain aromatic hydrocarbons with two or more condensed benzene rings [42]. They are formed during thermal decomposition of organic compounds and their subsequent recombination. PAHs can be of both natural as well as of anthropogenic origin. Natural sources are forest and rangeland fires, oil seeps, volcanic eruptions and exudates from trees. Anthropogenic sources of PAHs include burning of fossil fuel, wood, coal tar, garbage, used lubricating oil and oil filters, municipal solid waste incineration and petroleum spills, charbroiled food, and cigarette smoke [43, 44]. As inducers of CYP xenobiotic-metabolizing and conjugating enzymes, these AhR agonists normally stimulate their own metabolism. In the first step, CYPs add an epoxide group to the PAHs, which can be converted to a dihydrodiol by epoxide hydrolase. This PAH metabolite may be further metabolized by CYPs to form a diol epoxide. However, the same enzymatic activities play a crucial role in tumorogenesis [45]. The chemical structure of some commonly studied PAHs are given in Fig. **1**.

On the other hand, several carcinogenic PAHs (e.g. benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, dibenzo[*a*,*c*]anthracene, 3-methylcholanthrene (3MC)) were also found to be potent inhibitors of CYP1A2 and CYP1B1, with IC_{50} values <150nM [46].

The well-studied examples of PAHs are 3-methylcholanthrene and benzo[*a*]pyrene.

Benzopyrenes are found in environmental mixtures, including air pollution (cigarette smoke, automobile exhaust fumes) [47]. Two isomeric species are known, benzo[*a*]pyrene and benzo[*e*]pyrene. Benzo[*e*]pyrene has been reported to be a weak carcinogen

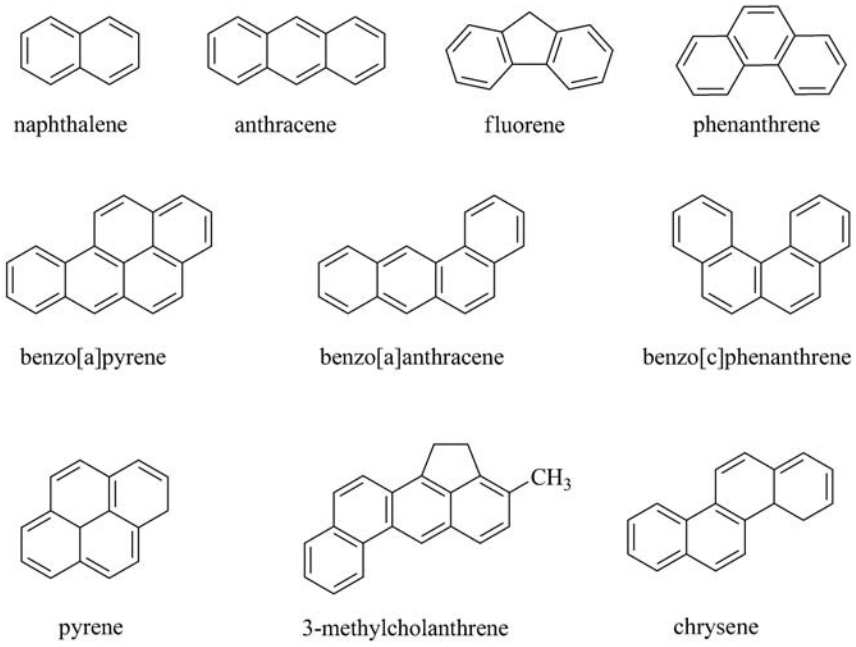

Fig. (1). Chemical structure of some commonly studied polycyclic aromatic hydrocarbons (PAHs): naphthalene, anthracene, fluorene, phenanthrene, benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*c*]phenanthrene, pyrene, 3-methylcholanthrene, chrysene.

[48, 49]. In contrast, benzo[*a*]pyrene is a prototype of carcinogenic PAHs activated by xenobiotic metabolizing enzymes (e.g. CYP1A1, CYP1A2, CYP1B1, and epoxide hydrolase). Its reactive metabolites initiate cell transformation *via* DNA damage and subsequent cellular response [50]**.**

3MC is the other highly carcinogenic polycyclic aromatic hydrocarbon produced by burning organic compounds. Like other PAHs, 3MC is genotoxic [51] and is a potent carcinogen for human cells [52, 53]. Recently, 3MC has been compared with TCDD, the most active AhR ligand which serves as the reference standard, but it has been suggested that 3MC is rather a selective AhR modulator [54].

3.1.2. Polychlorinated Biphenyls

PCBs cover a group of 209 different congeners, depending on the number and the position of chlorine atom substituents. Due to their chemical stability PCB mixtures are used largely in industry, notably as capacitor and transformer oils, hydraulic fluids, lubricating oils, and as plasticizers. PCB congeners can be divided into two groups according to their steric structure of molecule, i.e. planar dioxin-like PCB (DL-PCB) and non dioxin-like PCB (NDL-PCB), according to their toxicological properties. First group, including 12 congeners, shows toxicological properties similar to polychlorodibenzodioxins/polychlorodibenzofurans and is therefore termed DL-PCB. NDL-PCB group involves the vast majority of congeners of the PCB residues in the food chain. These compounds differ in the persistence, bioaccumulation and toxicity mechanisms [55]. Comparative structure-activity relationship studies have shown that the presence of halogens on lateral positions of the benzene rings contributes to the potency of PCBs to activate AhR. Maximal AhR activity is achieved when halogens bind at both para (4 and 4´) and two or more meta positions (3,3´,6 and 6´). The degree of substitution at the biphenyls bridge also influences the potency for AhR activation [56]. Due to the relative stability of highly chlorinated PCBs and their lipophilicity, PCBs are widely distributed and transported throughout the environment, and their residues have been identified in air, water, aquatic and marine sediments, fish and wildlife and human adipose tissue, serum and milk [57].

3.1.3. Halogenated Dioxins and Related Compounds

The halogenated dibenzo-*p*-dioxins, dibenzofurans, azo(xy) benzenes, naphthalenes represent a family of structurally related AhR agonists (Fig. **2**) [58]. They are formed by industrial processes such as combustion, bleaching of wood pulp and chlorination of phenols. The most potent AhR activators are TCDD and structurally related compounds that are widely distributed in the environment and have been shown to elicit a broad spectrum of biological responses in mammalian systems. At doses in the ng/kg range, TCDD leads to up-regulation of genes important for metabolism of xenobiotics and endogenous hormones. At doses in the µg/kg range, however; TCDD up-regulates the xenobiotic response while also causing a number of toxic effects such as hepatotoxicity, thymic involution, birth defects, cancer and lethality [59]. The structure-binding relationships for numerous halogenated congeners have been performed, and the results show that the most active congeners in competitive AhR binding assays are those compounds substituted only in their lateral positions (e.g. 2, 3, 7, and 8 for the polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans). The addition of nonlateral chlorine substituent or the removal of lateral chlorines tends to decrease AhR binding affinities for these compounds [60].

3.1.4. Other synthetic AhR Ligands

A significant amount of information has become available which suggest that AhR can be activated by „novel“ chemicals whose structural and physiochemical properties are inconsistent with currently defined structural requirements for AhR ligands (planarity, aromaticity and hydrophobic property). These chemicals show striking structural diversity in comparison each other and also

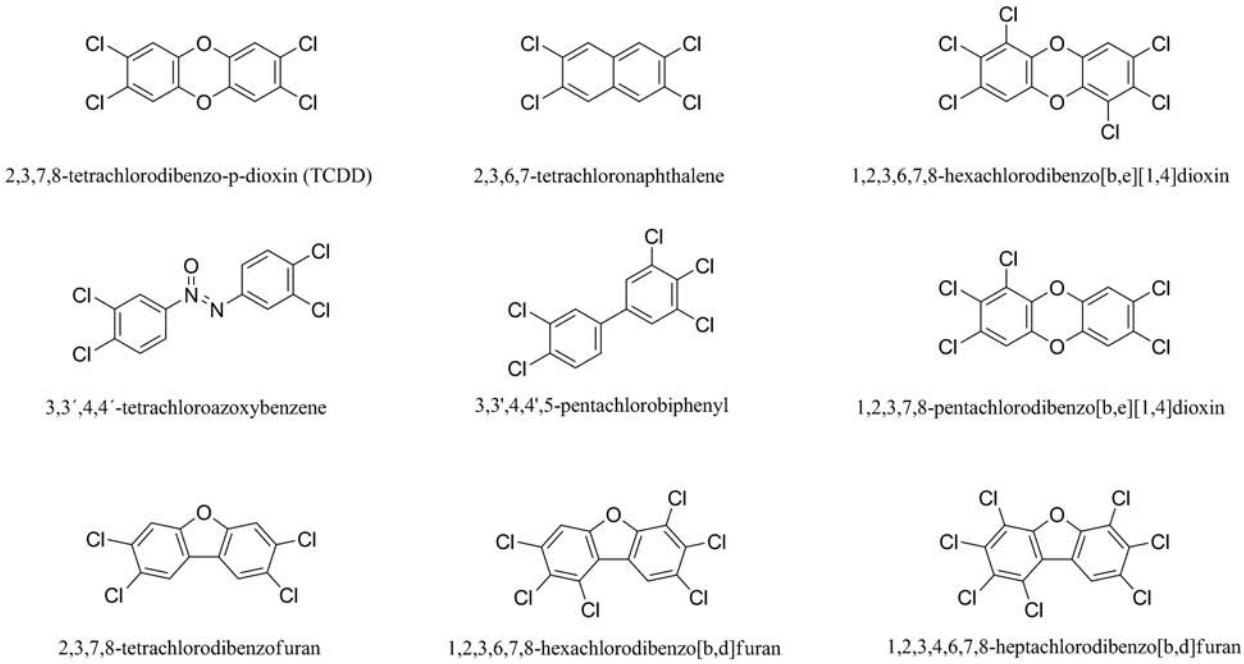

Fig. (2). Some halogenated compound that activate AhR receptor: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,6,7-tetrachloronaphthalene, 1,2,3,6,7,8-hexachlorodibenzo[*b,e*][1,4]dioxin, 3,3´,4,4´-tetrachloroazoxybenzene, 3,3',4,4',5-pentachlorobiphenyl, 1,2,3,7,8-pentachlorodibenzo[*b,e*][1,4]dioxin, 2,3,7,8-tetrachlorodibenzofuran, 1,2,3,6,7,8-hexachlorodibenzo[*b,d*]furan, 1,2,3,4,6,7,8-heptachlorodibenzo[*b,d*]furan.

in comparison to the structure of TCDD. The majority of these chemicals act as relatively weak inducers or AhR ligands, when compared with TCDD [61].

Benzimidazole

Benzimidazole derivates (e.g. antiulcer drug omeprazole and lansoprazole, Fig. **3**) represent an important class of compounds that are able to activate the transcription of CYP1A1 and CYP1A2 through the transformation of AhR into DNA (XRE) binding form. It was hypothesized that this transformation by benzimidazoles is due to the weakening of the interaction forces which, in absence of inducers, maintain the AhR ligand binding subunit-hsp90 complex in a silencing state [62].

Omeprazole (5-methoxy-2-{(4-methoxy-3,5-dimethyl-pyridin-2-yl)methyl-sulfinyl}-3H-benzoimidazole), a proton pump inhibitor, selectively inhibits the H+/K(+)-ATPase and is prescribed for treating peptic ulcer, Helicobacter pylori infection, gastrooesophageal reflux disease, nonsteroidal anti-inflammatory drug-induced gastrointestinal lesions (complications), and Zollinger-Ellison syndrome [63]. Omeprazole does not correlate with the classical structural requirements of AhR ligands, i.e. planar and polycyclic molecule. Thus, omeprazole is not considered to be a direct ligand for AhR [64]. Nonetheless, omeprazole does promote AhR nuclear translocation and association with ARNT together with subsequent DRE-dependent DNA binding, thus driving AhR-dependent gene expression [65]. Studies demonstrated that omeprazole could induce human AhR-dependent genes involved in xenobiotic metabolism, e.g., CYP1A1, CYP1A2 [64, 66]. Direct binding assays using 14C-labeled omeprazole failed to show a physical interaction with AhR [64]. Similarly, competitive ligand binding studies demonstrated that even at high micromolar concentrations omeprazole was incapable of displacing high-affinity 3H–labeled AhR ligands [67]. Interestingly, recent reports have suggested that omeprazole, as well as BNF and 3MC, activates both human CYP1A1 and CYP1A2 expression through the common regulatory region despite the fact that omeprazole affects different cellular signal(s) from BNF and 3MC [66]. Similarly to omeprazole, lansoprazole, a selective inhibitor of the H^+/K^+-ATPase with a similar chemical structure with omeprazole, was shown not to bind to human AhR [66, 68].

Primaquine

In addition to omeprazole/lansoprazole, primaquine (PQ) can cause an increase in CYP1A1 mRNA level and ethoxyresorufin-O-deethylase (EROD) activity can be also caused by primaquine (Fig. **3**) [69].

PQ, an 8-aminoquinoline, is playing a central role in malaria treatment especially because of its large spectrum of activities against all four species of plasmodia that are pathogenic to human [70].

Fontaine and his colleagues showed that PQ significantly induced CYP1A1 gene expression in hepatocytes and HepG2 cells. PQ was not able to displace [^{3}H]TCDD in competitive binding studies using 9S-enriched fractions of human cytosol. Thus, they suggested that CYP1A1 induction involved AhR, but no direct primaquine-receptor interaction [71]**.**

In contrast, Werlinder and coworkers found that PQ was capable of transforming cytosolic AhR in rat hepatoma H4IIE cells, indicating that PQ induced dissociation of AhR from the hsp90 complex and binding of the complex to XRE [72]**.**

Kinase Inhibitor

Only a limited number of kinase inhibitors have been reported to modulate AhR function. A mitogen–activated protein kinase kinase (MEK) 1 inhibitor, PD98059 [73], was reported to be a competitive antagonist of AhR, whereas U0126 [74], a MEK1/2 inhibitor, and SP600125 [75], a c-Jun N–terminal kinase (JNK) inhibitor, were demonstrated to be AhR partial agonist [76]. The chemical structures of the compounds are given in Fig. **3**.

Andrieux and coworkers showed that U0126, 1,4-diamino-2,3-dicyano-1,4-bis [2-aminophenylthio]butadiene, is a ligand and agonist of AhR, since it induces CYP1A1 mRNA and protein in primary rat hepatocytes and human hepatoma B16A2 cell line. They also demonstrated that U0126 is a substrate for several CYPs including human CYP1A2, CYP1A1 and CYP1B1 [74, 77]. The induction occurred independently of MEK/extracellular signal-regulated kinase (ERK) activation and in the absence of ERK1 and ERK2 expression [74].

SP600125 (anthra[1,9-cd]pyrazol-6(2H)-one), was reported as an AhR ligand that functions as an AhR antagonist at concentrations used to pharmacologically inhibit JNK [78]. Further research showed that SP600125 was not an antagonist but a partial agonist of human AhR. SP600125 significantly induced CYP1A1 and CYP1A2 mRNAs in primary human hepatocytes and CYP1A1 mRNA in human hepatoma cells HepG2. This effect was abolished by resveratrol, a partial agonist/antagonist of AhR. SP600125 dose-dependently inhibited CYP1A1 and CYP1A2 genes induction by a prototype AhR ligand TCDD in human hepatocytes [75].

PD98059, [2-(29-amino-39-methoxyphenyl)-oxanaphthalen-4-one], is a flavonoid and a potent inhibitor of MEK. Recent study has suggested that PD98059 is a ligand for AhR, which functions as an AhR antagonist at concentrations commonly used to inhibit MEK signaling [73]. The addition of PD98059 to rat liver cytosol just before addition of TCDD suppresses TCDD binding and AhR transformation [73]**.** PD98059 also inhibits AhR-mediated CYP1A1 induction by 3MC [79].

TSU-16, (Z)-3-[(2,4-dimethylpyrrol-5-yl)methylidenyl]-2-indolinone, is a potent anti-angiogenic agent that inhibits the tyrosine kinase of the vascular endothelial growth factor receptor-2 . In human hepatocytes, TSU-16 increased CYP1A1 and CYP1A2 mRNA levels. The extent and time-dependent changes in CYP1A1 and CYP1A2 mRNA levels after TSU-16 treatment were similar to those after treatment with 3MC. It was also suggested that TSU-16 binds to and activates AhR to enhance the expression of both human CYP1A1 and CYP1A2 in human hepatocytes with similar potency as 3MC [76]**.**

Synthetic Flavonoid

TMF (6,2',4'-trimethoxyflavone) and MNF (3'-methoxy-4'-nitroflavone), both synthetic flavonoids, were identified as AhR ligands that possess the characteristics of an antagonist having the capacity to reduced the ability of TCDD to stimulate AhR DNA binding. TMF also exhibits no species or promoter dependence with regard to AhR antagonism. In contrast to TMF, MNF exhibits species differences in activity. The differences in agonist/antagonist activity of MNF were observed between the rat and guinea pig in gene induction and DNA binding AhR bioassays. These results are also consistent with the previous report that showed differences in MNF effects on mouse (antagonist activity) and guinea pig (agonist activity) AhR activity [80-83].

Another synthetic flavone, 5,6-benzoflavone (β-naphthoflavone, BNF), binds to AhR and is known as inducer of AhR-mediated CYP1A1 and CYP1A2 gene expression [84]. In contrast, 7,8-benzoflavone (α-naphthoflavone, ANF) binds with moderate affinity to AhR and it was demonstrated ANF´s ability to inhibit CYP1A1 gene expression induced by AhR agonists through competitive interactions for binding with AhR [85-87]. It was also documented that ANF could act as weak AhR agonist and CYP1A1 inducers in cell culture, although the concentrations required to increased CYP1A1 mRNA were relatively high [88].

The research on variously substituted flavones suggests that within this structural class of compounds, various substituent groups can affect markedly the activity of each individual congener

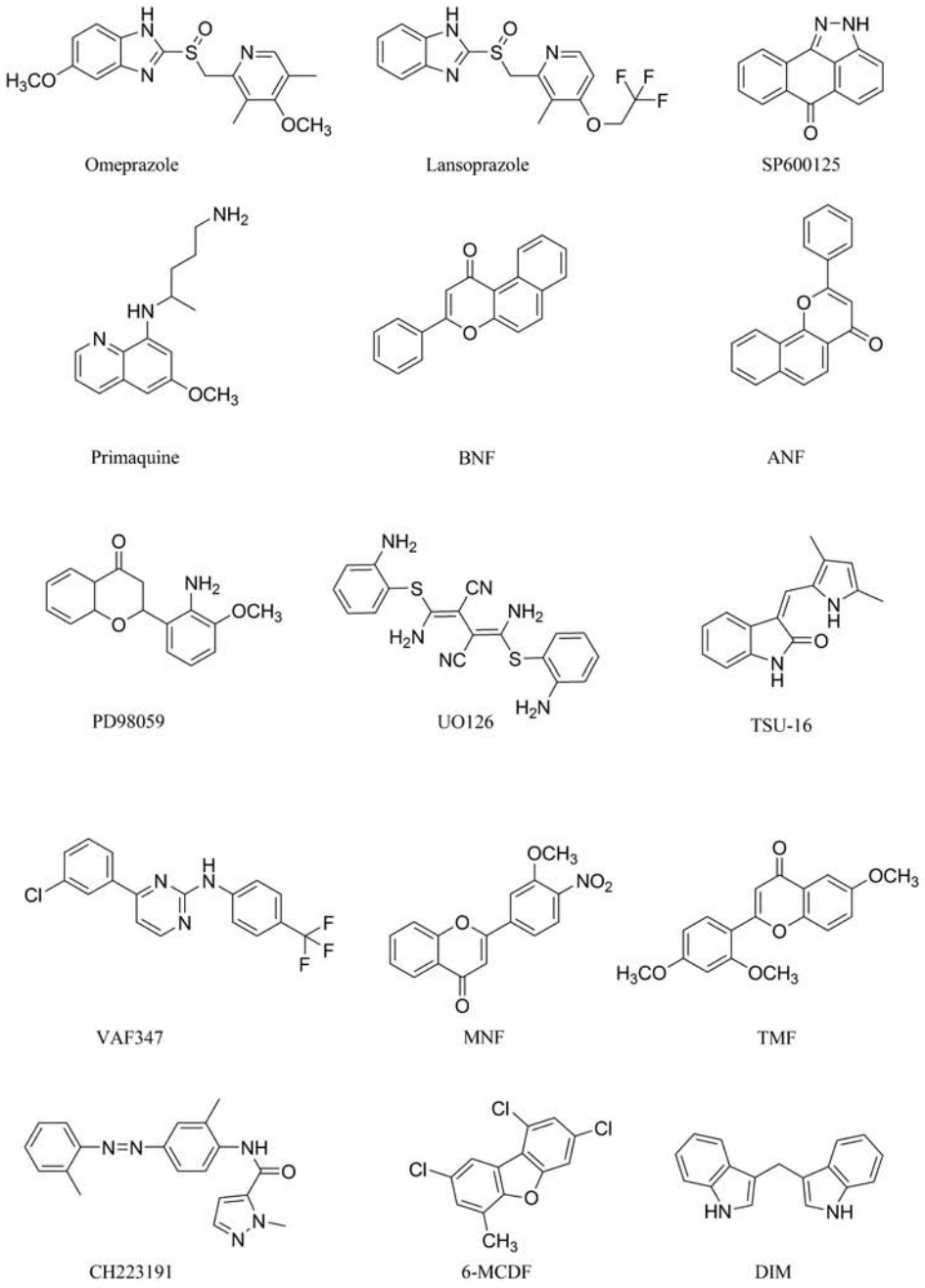

Fig. (3). Synthetic activators of AhR receptor (omeprazole, lansoprazole, SP600125, primaquine, BNF, ANF, PD98059, UO126, TSU-16, VAF347, MNF, TMF, CH223191, 6-MCDF, DIM).

as AhR agonist or antagonist. These substituent-dependent differences in activity may be related to ligand-induced conformational changes in AhR complex and/or support the existence of more than one form of AhR [89]. The chemical structures of mentioned flavonoids are given in Fig. **3**.

New Synthetic Ligands

Recently, novel compound 2-methyl-2*H*-pyrazole-3-carboxylic acid (2-methyl-4-*o*-tolylazo-phenyl)-amide (CH223191) has been identified. CH223191 potently inhibites TCDD-induced AhR-dependent transcription. In addition, CH223191 blocked the binding of TCDD to AhR and inhibited TCDD-mediated nuclear translocation and DNA binding of AhR. These inhibitory effects of CH223191 prevented the expression of CYPs, target genes of AhR and in this way the compound is supposed to inhibit TCDD-dependent toxicity [90].

It was suggested that CH223191 was a selective antagonist that preferentially inhibits the ability of some classes of AhR agonists (TCDD and related halogenated aromatic hydrocarbons (HAHs)), but not others (PAH, flavonoids or indirubin), to bind to and/or activate the AhR signal transduction. It was also shown that even though CH223191 binds to the AhR/AhR LBD, BNF could still bind and stimulated AhR transformation and DNA binding. The preferential antagonism of HAHs by CH223191 was consistent with the hypothesis that there were significant differences in the binding of the HAHs and non-HAH AhR agonists within ligand binding pocket. It is possible that CH223191 and other AhR an-

tagonists could act as selective AhR modulators (SAhRMs). Alternatively, CH223191 could bind to AhR outside of the LBD and act as an allosteric modulator, thereby affecting overall AhR structure that led to preferential inhibition of HAH access/binding to the LBD [82]. SAhRM is defined as an AhR ligand that is not capable of mediating a dioxin responsive element driven transcription. Although SAhRMs are relatively non-toxic in comparison with AhR ligands, they are able to mediate other activities such as repression of cytokine mediated acute-phase gene expression [91].

Recently, VAF347 ([4-(3-chloro-phenyl)-pyrimidin-2-yl]-(4-trifluoromethyl-phenyl)-amine), a novel low-molecular-weight compound with potent anti-inflammatory activity has been reported. VAF347 acts as immunomodulator by blocking the function of dendritic cells to generate functional T-helper (Th) cells *in vitro*. VAF347 was demonstrated to bind to AhR protein and consequently induced AhR driven signal transduction similar to the prototype AhR agonist TCDD. Most importantly, no anti-inflammatory activity of the compound was observed in AhR-deficient mice. The results proposed AhR protein as key molecular target mediating the anti-inflammatory phenotype of VAF347 *in vitro* and *in vivo* [92].

The antiallergic phenotype of VAF347 *in vivo* was reminiscent of that reported for M50367 (ethyl 3-hydroxy-3-[2-(2-phenylethyl)benzoimidazol-4-yl]propanoate). This compound, although structurally unrelated to VAF347, also exerted its anti-inflammatory effects by activating AhR signaling [92]. It was demonstrated that M50354 (3-[2-(2-phenylethyl)benzoimidazole-4-yl]-3-hydroxypropanoic acid) (an active metabolite of M50367) is an AhR agonist, and that the AhR signaling pathway activated by M50354 binding affected the Th1/Th2 balance toward Th1 dominance, resulting in immunological responses with antiallergic effects [93, 94].

Recently, it has been demonstrated that 4-hydroxy-tamoxifen (4OHT) (Selective estrogen receptor modulators (SERMs)), an active metabolite of tamoxifen, directly binds to and modulates the transcriptional activity of AhR. In the absence of estrogen receptor (ER), 4OHT can induce the expression of AhR target genes involved in estradiol metabolism, cellular proliferation, and metastasis in cellular models of breast cancer. These findings provide evidence that it is necessary to reevaluate the relative roles of ER and AhR receptors in pharmacological actions and therapeutic efficacy of tamoxifen and other SERMs [95].

6-Methyl-1,3,8-trichlorodibenzofuran (6-MCDF) and diindolylmethane (DIM) are two other SAhRMs. 6-MCDF is a synthetic compound, however; DIM is formed as condensation product of the phytochemical indole-3-carbinol. 6-MCDF was shown to reduce growth of estrogen receptor positive rodent mammary tumors [96].

Pesticides

Pesticides are another group of substances that bind AhR and adversely affect human health. Exposure to pesticides can be related to various diseases, including cancers, as well as neurological, mental and reproductive defects [97, 98].

About 200 pesticides that are intentionally released in a large quantity into the environment were discovered to elicit AhR agonistic activity. But only 11 of them (acifluorfen-methyl, bifenox, chlorpyrifos, isoxathion, quinalphos, chlorpropham, diethofencarb, propanil, diuron, linuron, prochloraz) showed AhR-mediated transcriptional activity *in vitro*. In particular, three herbicides (propanil, diuron, linuron) with similar chemical structure showed more potent agonistic activity than other pesticides.

These pesticides are relatively weak inducers or AhR ligands when compared with TCDD [99-101]. In addition, carbaryl, a carbamate insecticide, was also demonstrated to be a weak AhR ligand and inducer of AhR-dependent gene expression [102]. The chemical structures of the pesticides are given in Fig. **4**.

Ligands in Newspapers

It was also demonstrated, that newspapers and printing ink contain relatively potent agonists of AhR, e.g. PAHs derived from carbon black, a common ingredient in black printing inks; or soy-based ink with variety of several soy flavonoids, such as genistein, quercetin, and kaempferol [103, 104]. In guinea pig cells and mouse cells, solvent extracts of newspapers from countries around the world stimulated AhR signaling pathway. AhR agonist activity was observed for dimethyl sulfoxide (DMSO), ethanol, and water extracts of printed newspapers, unprinted paper, and black printing ink. DMSO and ethanol extracts also stimulated AhR transformation and DNA binding and also competed with [^{3}H]TCDD for binding to AhR [105, 106]. In previous research, ethanol and water extracts of recycled paper fibres have been shown to induce AhR receptor-dependent reporter gene expression in rat cells [107]. It is suggested that the ability of solvent extracts of newspapers from various countries around the world to stimulate AhR signaling pathway indicates that the source of AhR agonists in the samples is not unique to a particular paper or ink products, or printing company from one country, but is likely due to common constituents [105].

Ozone

Ozone (O3) is an air pollutant and the major oxidant of photochemical smog [108]. O3 pollution is now widespread in urban areas in many countries and has been associated with various harmful health effects, including increased rates of hospital admissions and exacerbation of respiratory illnesses. Increased numbers of motor vehicle and vehicle emission have resulted in increased level of ozone [109].

In addition to adverse effect to respiratory tract, O_3 also belongs to the most reactive environmental oxidant to which skin is exposed [110]. However, very little is known about the effects of O_3 exposure on human skin. Afaq and his colleagues used normal human epidermal epidermal keratinocytes (NHEKs) to determine the effects of attainable levels of O3 exposure on the family of cytochrome P450 isoforms. NHEK exposure to ozone (0,3 ppm) resulted in an increase in protein and mRNA expression of CYP1A1, CYP1A2, and CYP1B1. NHEK exposure to O_3 also resulted in nuclear translocation of AhR and in phosphorylation of epidermal growth factor receptor (EGFR). The induction of CYPs by O_3 was mediated through the activation of AhR and not by that of EGFR. Data also showed that ozone exposure resulted in an increased mRNA expression of AhR [111].

3.2. Natural Compounds

Perhaps the greatest exposure to AhR ligands comes from food. The diet is a source of naturally occurring ligands of AhR. There are numerous reports on naturally-occurring dietary chemicals, e.g. flavonoids [112], carotenoids (β-apo-8´carotenal, canthaxanthin, and astaxanthin) [113], berberine [114], and others, that activate AhR signaling pathway, although the majority of these chemicals appear to be relatively weak AhR ligands [113, 115-117].

It has been also known for long time that foods may contain naturally-occurring compounds, or contaminants, that tend to increase the frequency of cancer. On the other side, many foods, mostly fruits and vegetables, herbs and tea, contain compounds (flavonoids) that may protect against various kind of cancer [118].

Polyphenols

Flavonoids are widely studied polyphenols, which include several thousand compounds, divided into the following classes: flavones, flavonols, flavanones, isoflavones, and catechins.

The subclasses flavones and flavonols are usually received from vegetables, fruits, teas and red wine, and flavanones, isoflavones and catechins from citrus fruits, beans and teas, respectively [112].

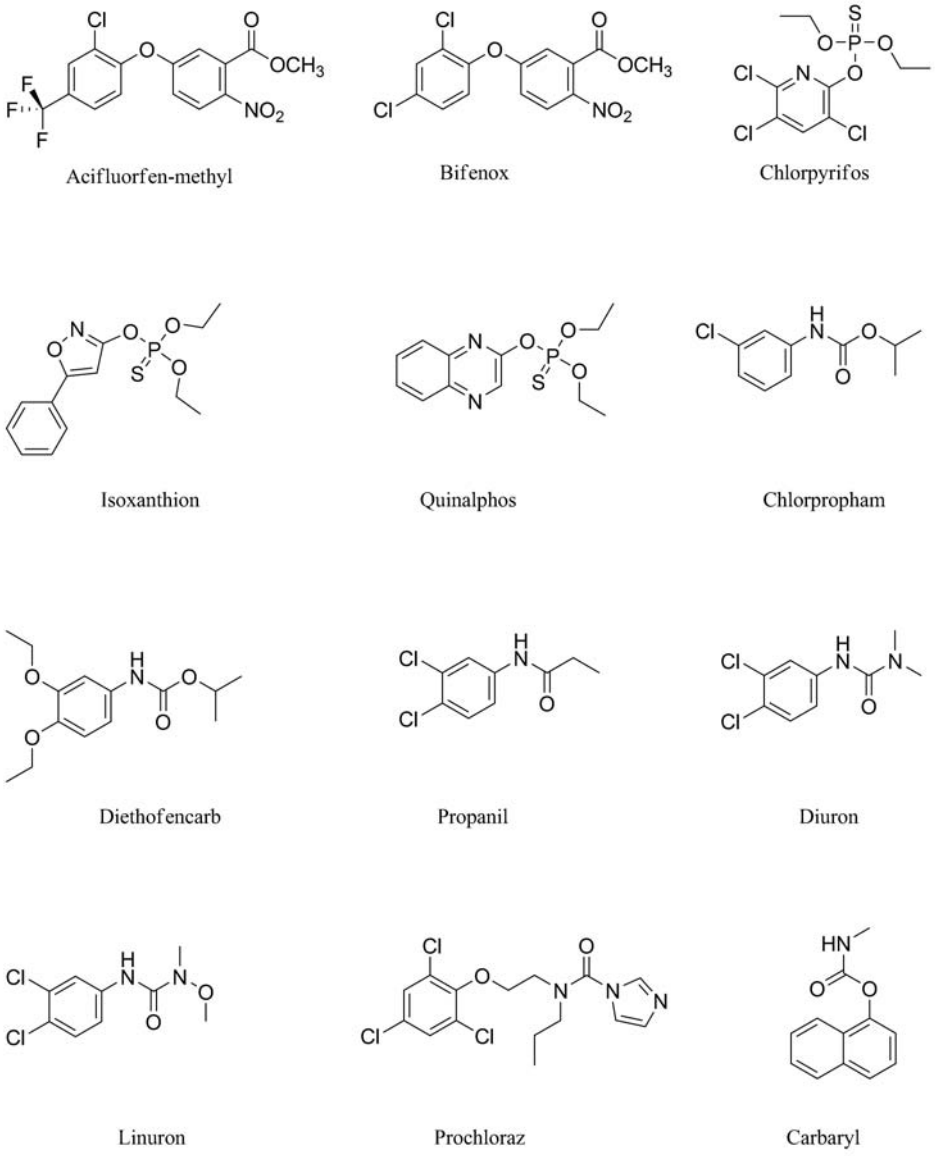

Fig. (4). Chemical structure of some pesticides (acifluorfen-methyl, bifenox, chlorpyrifos, isoxanthion, quinalphos, chlorpropham, diethofencarb, propanil, diuron, linuron, prochloraz, carbaryl).

The chemical structures of some studied polyphenols are given in Fig. **5**.

Among the tested polyphenols, marked AhR activation was exhibited by isoflavones such as daidzein, resveratrol, some flavanones such as naringenin, and flavones such as baicalein. On the other hand, some flavones such as apigenin, flavonols such as quercetin and antraquinones such as emodin, showed notable inhibitory effect on the *in vitro* activation of AhR induced by TCDD [119-121].

Green tea is an important source of polyphenolic compounds that show significant anticancer activity in numerous experimental animal models. Most of the polyphenols in green tea are flavonols, better known as catechins [122, 123]. The major catechins are (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epicatechin (EC) [124]. Of the tested catechins, EGCG and EGC were the most potent antagonists. These data suggested that EGCG and EGC were capable of altering AhR transcription and were responsible for most, if not all, of the AhR antagonist activity of green tea extract (GTE) [122]. Recent study suggested that GTE and only EGCG act as AhR antagonist and antagonized TCDD-induced binding of AhR to DNA and inhibited subsequent transcription of human CYP1A (CYP1A1, CYP1A2) [124]. Another recent research has suggested that EGCG does not bind to the AhR ligand binding side, indicating this compound functions through a mechanism unlike that of typical AhR antagonists. It has been demonstrated that EGCG directly binds to hsp90. This binding results in nuclear localization of an AhR form incapable of binding to DNA [125]. EGCG has been found to directly modulate the conformation of hsp90 and bind at or near to a C-terminal ATP binding site. Studies have showed that EGCG stabilizes AhR complex, and decreases the association of ARNT with ligand-activated AhR [126]. It has been also found that cacao polyphenol extract (CPE), rich in polyphenols, including catechins and procyanidin oligomers (e.g. (+)-catechin, (-)-epicatechin, procya-

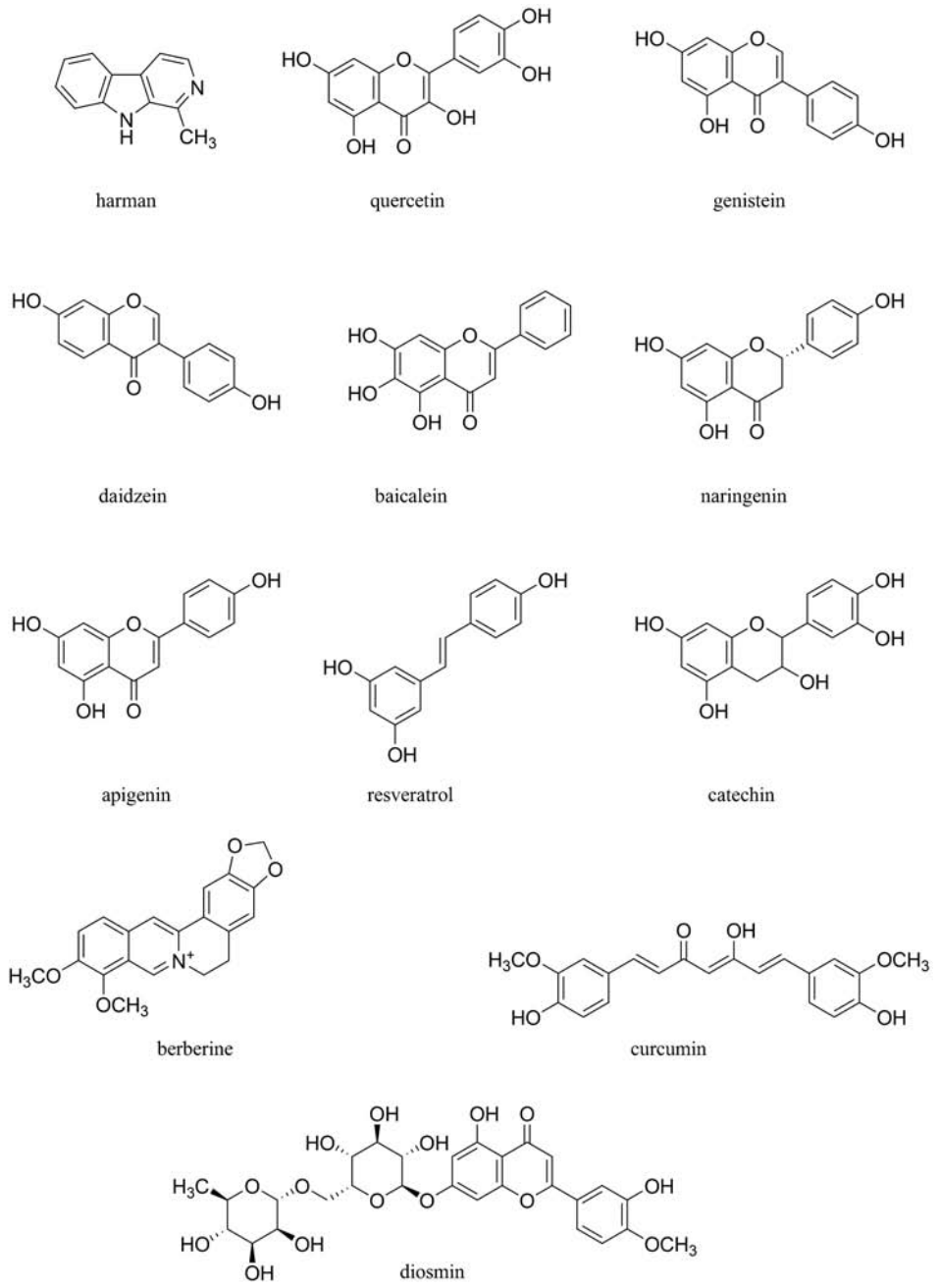

Fig. (5). Chemical structure of natural ligands (harman, quercetin, genistein, daidzein, baicalein, naringenin, apigenin, resveratrol, catechin, berberine, curcumin, diosmin).

nidin B2, procyanidin C1, cinnamtannin A2) [127], suppressed the 3MC-induced transformation by inhibiting the formation of a heterodimer between AhR and ARNT and abolished 3MC-induced CYP1A1 [128].

Fukuda *et al*. also discovered that chlorophylls in green tea, as well as lutein (carotenoid), could act as novel antagonists of AhR. Although the mechanism of the suppression has not yet been elucidated, the suppressive effect has been similar to that of catechins having gallate moieties. In green tea, other flavonoids including luteolin, quercetin, and kaempferol strongly suppressed AhR transformation. Xanthophylls, such as β-cryptoxanthin and zeaxanthin from carotenoid group, also showed a moderate suppressive effect, whereas β-carotene, lycopene, and astaxanthin showed a weaker effect [129].

Suppression of TCDD mediated AhR transformation has been also investigated in ethanolic extract of propolis [130] and in ethanolic extract from molokhia (Egyptian spinach) [131]. Propolis is a resinous substance collected by honeybees (*Apis mellifera*) from various plant sources and containing high concentration of flavonoids. Park *et al*. showed, that antagonistic effect of propolis was stronger than that of green tea extracts and vegetables [130].

Of 41 kinds of vegetables and fruits, such as apple, broccoli, cabbage, carrot, grape, soybean, potato, molokhia showed the strongest suppressive effect on AhR tranformation. Moreover, molokhia extract also suppressed translocation of AhR into the nucleus and subsequent binding of AhR to DRE both in the cultured cells and animal experiments [131].

Diosmin

Diosmin is an oral phlebotropic drug used in the treatment of venous disease, i.e., chronic venous insufficiency and hemorrhoidal disease, in acute or chronic hemorrhoids. Diosmin and its aglycone form, diosmetin, are natural dietary agonists of AhR, causing a potent increase in CYP1A1 transcription and CYP1A1 activity. However, only diosmetin is capable of inhibiting CYP1A1 enzyme activity, thus inhibiting carcinogen activation [116].

Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene), a plant antifungal and a phytoalexin present in some red wine, has been found to be a competitive inhibitor of AhR ligands. Resveratrol binds AhR and induces AhR nuclear translocation in a manner similar to that of dioxin, but inhibits the induction of DRE-driven transcription of AhR and blocks the induction of CYP1A1, both *ex vivo* and *in vivo* [132, 133]. It has been also suggested that resveratrol could prevent the adverse effects of PAHs *in vivo*, through antagonism of ligand binding to AhR and down-regulation of genes such as CYP1A1 [132, 134]. Our recent study indicated that resveratrol induces CYP1A1 and CYP1A2 mRNA and could act as partial agonist of AhR [75]**.**

Recently, it has been demonstrated that resveratrol is able to increase the cytotoxic activity of natural killer (NK) cells. The cytotoxic effect has been accompanied by increases in JNK and ERK activity and perforin expression. These resveratrol effects have been inhibited by JNK and ERK inhibitors (SP600125, PD98059) or by siRNAs (small interfering RNAs) against JNK-1 and ERK-2 [135].

Isosteric chemical modification of resveratrol led to synthesis of new synthetic stilbene derivates that have either higher affinity for AhR, selective TCDD antagonism or undetectable affinity for estrogen receptor [136]. This last property is important because it could eliminate estrogen-related risks, such as the increased risk of breast cancer. It was also found that some reported AhR antagonists (isoflavones-genistein, daidzein, and biochanin A; flavone-6,3',4'-trihydroxyflavone) have a high affinity for the estrogen receptor.

Curcumin

Curcumin (diferuloylmethane), a major component of turmeric, ligand of AhR, has attracted public attention because of its cancer preventative effect. Several animal studies have demonstrated that curcumin inhibits carcinogens initiation [137, 138]. It was demonstrated the inhibitory effects of dietary curcumin on benzo[a] pyrene-induced AhR activation, nuclear translocation and DNA binding, which subsequently resulted in decreased transcriptional activation of CYP1A and hence reduced protein expression and enzyme activity of CYP1A1/1A2 *in vivo* [139]. Curcumin was shown to functionally inhibit AhR by dephosphorylating AhR and ARNT [140]. Recently, it has been discovered that curcumin suppressed ARNT, the partner of AhR.

Alkaloids

Harman

Recently, it has been also reported that harman could be a ligand for AhR [141]. Harman (1-methyl-9H-pyrido-[3,4-*b*]indole), a aromatic β-carboline that is found in several food and drinks, and cigarette smoke, is known mutagenic, co-mutagenic and carcinogenic compound [142-144]. In comparison with TCDD, harman is a weak ligand of AhR, and can directly induce CYP1A1 gene expression and enzymatic activity in an AhR-dependent manner [141].

Another β-carboline alkaloids, rutaecarpine (found in Rutaceae and szechuan pepper), annomontine (found in Annonaceae and marine sponges of the order *Petrosiidae*) and xestomanzamine A (contained in *Petrosiidae* sponges), have been found to increase AhR-driven reporter gene activity as well as expression of two AhR target genes (CYP1A1, AHRR) in a dose-dependent and time-dependent manner. Additionally, these alkaloids have stimulated cytochrome CYP1 enzyme activity without showing any antagonistic effects regarding benzo[*a*]pyrene-stimulated CYP1 activation. The AhR-activating property of the β-carbolines is completely abrogated in AhR-deficient cells providing evidence that rutaecarpine, annomontine and xestomanzamine A are natural stimulators of the human AhR [145]. Recent research has also showed that rutaecarpine can stimulate the expression of CYP1A1 both through AhR-dependent mechanism and through calcium-dependent mechanism [146].

Berberin

Studies also showed that berberin, a quarternary isoquinoline alkaloid found in many different plants e.g. *Berveris vulgaris*, *Hydrastis canadensis* and used as a remedy for the treatment of diarrhea and gastroenteritis [147], activated AhR in high concentration (10-50μM) and short periods (6 and 24h) AhR in human hepatoma cells HepG2 and rat hepatoma cells (H4IIE.luc) stably transfected with a dioxin responsive element fused to the luciferase gene. In HepG2, berberin elevated the levels of CYP1A1 mRNA, but suppressed the CYP1A1 activity. In contrast, low doses (1μM) and prolonged times (48h) failed to produce any activation of AhR in H4IIE.luc cells. Induction of AhR nuclear translocation by berberin was confirmed by fluorescence microscopy [114].

4. ENDOGENOUS AhR LIGANDS

This category of ligands represents chemicals that are endogenously synthesized in higher organisms. Indigoids, equilenin, arachidonic acid metabolites, heme metabolites and tryptophan metabolites are examples of endogenous AhR ligands [39] (see Fig. **6**).

4.1. Indigoids

Indigo and indirubin have been suggested to be endogenous AhR activators. Indigo and indirubin were detected in bovine serum and human urine. Both were used as dyes for textile coloring and they are also used as ingredients in the traditional Chinese medicine. Indigo is equipotent activator of AhR as compared to TCDD and indirubin is even more potent than TCDD in the yeast system. Although, indigoids are not as potent as TCDD in mammalian systems, they were found to be AhR ligands and inducers of the AhR-mediated response [148-150].

4.2. Heme Metabolites

Bilirubin, biliverdin and hemin, the heme metabolites, are endogenous ligands of AhR receptor. Sinal and Bend reported that in mouse hematoma cells, bilirubin induces Cyp1a1 gene transcription through a direct activation of AhR, while biliverdin and hemin appeared to induce CYP1a1 indirectly by acting as precursors to the endogenous formation of bilirubin *via* normal heme metabolism pathways. In contrast to TCDD, heme metabolites have relatively weak affinity towards AhR [151]. However, given that the majority of plasma bilirubin exists as bound to serum albumin, combined with the fact that normal plasma bilirubin levels are in the range of 5–20 μM, it is unlikely that bilirubin influences AhR-dependent gene expression in normal physiological conditions. However, one may envision several scenarios in which abnormal elevations in plasma bilirubin can occur, including individuals with inborn errors of metabolism, such as Crigler–Najjar syndrome or Gilbert's syndrome where bilirubin levels can reach as high as 300–800 μM. Under the conditions increases in AhR-dependent responses can occur. In fact, increased plasma bilirubin levels observed after liver transplantation has been shown to be associated with elevated CYP1A1 levels [152].

4.3. Eicosanoids

Numerous studies indicated that lipoxin A4 (LXA_4), the metabolite of arachidonic acid that has an anti-inflammatory role, is a ligand for the AhR that can mediate the transformation of the receptor to a form that binds to the cognate DRE and activates transcription of CYP1A1. In addition, it has been suggested that LXA_4 is a substrate for CYP1A1. Thus, since the expression of CYP1A1 is

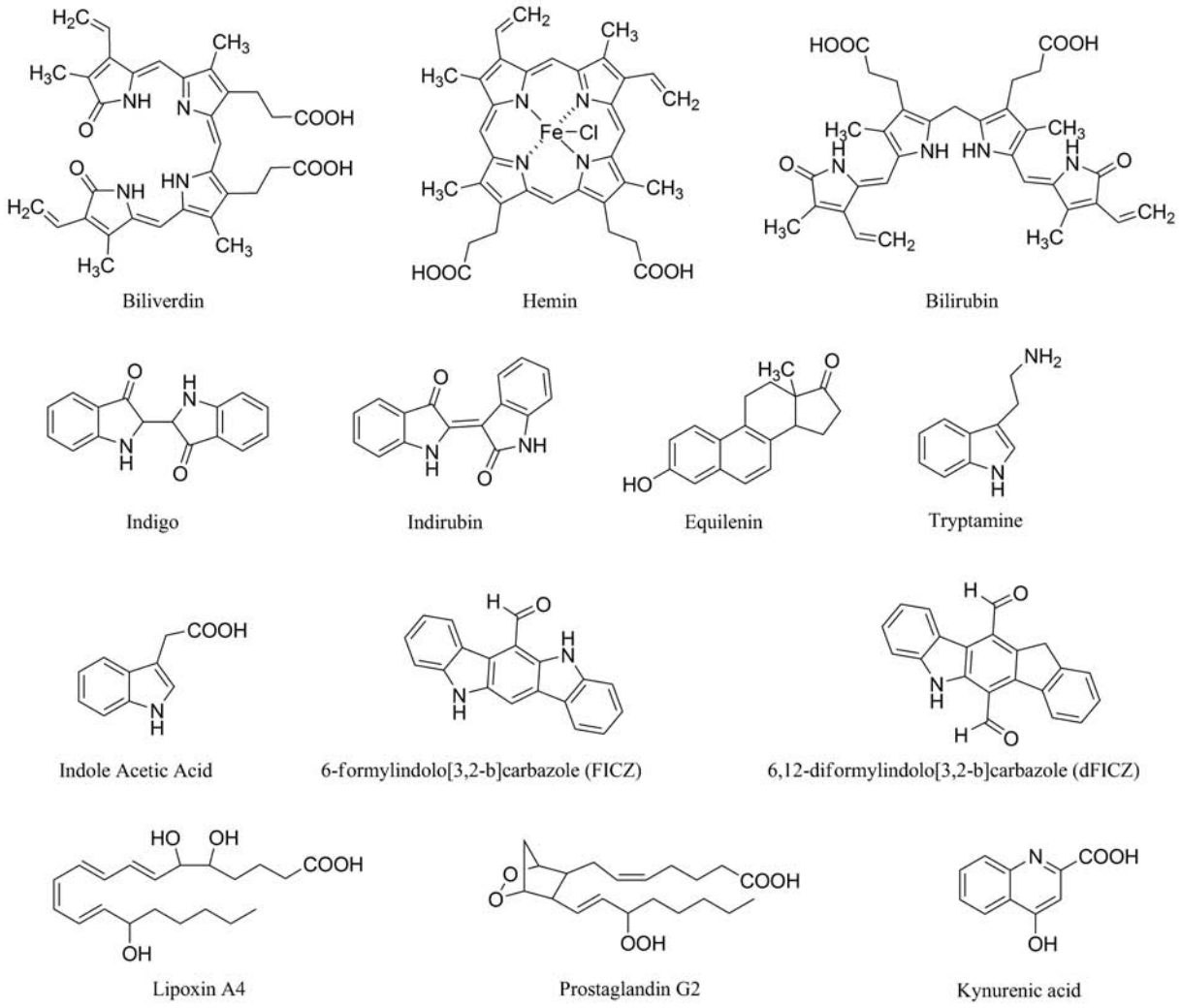

Fig. (6). Endogenous compounds that activate AhR receptor (biliverdin, hemin, bilirubin, indigo, indirubin, equilenin, tryptamine, indole acetic acid, 6-formylindolo[3,2-*b*]carbazole (FICZ), 6,12-diformylindolo[3,2-*b*]carbazole (dFICZ), lipoxin A4, prostaglandin G2, kynurenic acid).

regulated by the activated AhR, the metabolism of LXA_4 is autoregulated by this system. In contrast to classical AhR ligands, LXA_4 contains neither rings nor fully planar geometry, and has a negative charge at physiological pH [153]. Other eicosanoids that are able to bind AhR are prostaglandins (Prostagandins B_3, D_3, $F_{3\alpha}$, G_2, H_1 and H_2). These six compounds are able to stimulate AhR transformation and DNA binding form *in vitro* and induce AhR-dependent gene expression in intact cells.

4.4. Tryptophan Derivatives

The aromaticity of tryptophan structure has led to idea that its metabolites could be the AhR agonists. Indeed, it was confirmed that tryptamin (TA) and indole acetic acid (IAA), as well as other endogenous ligands, act as weak AhR activators as compared to TCDD. TA and IAA bind competitively to AhR and stimulate AhR transformation and DNA binding form, and induce expression of an AhR-dependent reporter gene in the cells. The physiological level of tryptophan ranges from 70 to 150 μM, while plasma IAA levels appear to be substantially lower. Thus, although tryptophan, TA and IAA can activate AhR and induce AhR – dependent gene expression *in vitro* and in mouse hepatoma cells in culture, it is not clear whether these compounds have capability to activate AhR *in vivo* under normal physiological conditions [154, 155].

UV irradiation of tryptophan generates compounds that exert high affinity for AhR and potency for induction of CYP1A1. For the two high affinity AhR ligands produced from tryptophan, the chemical structure was identified as 6-formylindolo[3,2-*b*]carbazole (FICZ) and 6,12-diformylindolo[3,2-*b*]carbazole (dFICZ). These two compounds show a close similarity to the indolecarbinol condensation product indolo[3,2-*b*]carbazole (ICZ) [156]. Some data provided indirect evidence that the synthesis of FICZ could occur *in vivo*. The exposure of human skin to UV-B was shown to induce CYP1A1 and CYP1A2 mRNAs and proteins in the epidermis and dermis [157]. Fritche *et al*. also demonstrated the intracellular formation of the AhR ligand FICZ from tryptophan and provided the evidence that UVB irradiation translocated the AhR into the nucleus and induced CYP1A1 gene expression [158]**.** Recent study also showed that FICZ could be formed from the prolonged exposure of tryptophan to sunlight [159]. Since AhR activation is associated with chemically-induced carcinogenesis, this makes a sense to enrich sun lotions with CYP1A1 inhibitors and cytoprotective compounds such as dehydrosilybin [160].

Another potential endogenous ligand derived from tryptophan and directly isolated from porcine lung tissue was 2-(1-*H*-indole-3-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) [161]. Compared with the prototypical toxic ligand, TCDD, ITE binds

AhR and elicits *in vitro* DNA binding with similar potency. It has been found that ITE is a potent AhR agonist in cell extracts, cultured cells, and intact animals, but does not cause the toxicity associated with the more stable xenobiotic ligand, TCDD [162, 163].

Recently, it has been found that the tryptophan derivates generated by the indoleamine-2,3-dioxygenase pathway represent endogenous activators of the human AhR. Furthermore, these products, kynurenic acid (KA) and xanthurenic acid (XA), are direct ligands of the AhR, with the capacity to stimulate AhR-dependent gene expression at physiologically attainable concentration [164]. KA has been found in the colon of pigs and rat at 1.5-16 μM, and at 0.8 μM in human bile [165, 166].

4.5. Equilenin

Equilenin (1,3,5-(10),6,8-estrapentaen-3-ol-17-one) is an equine estrogen, which is excreted into the urine of pregnant mares. It has a partially planar structure with aromatic A and B rings that is similar to 3MC, a known AhR ligand. Recent study performed in mice has suggested that it is highly likely that equilenin binds to AhR, induces CYP1A *via* AhR mediated mechanism, however the role of AhR has not been clarified in detail yet. It is suggested to be the weak AhR agonist [167].

CONCLUSION

Aryl hydrocarbon receptor is a ligand-dependent transcription factor that regulates the wide range of biological and toxic effects in many species and tissues. On one hand, AhR has been shown to be a mediator of toxicity of particular xenobiotics, such as dioxins, polycyclic aromatic hydrocarbons, and halogenated biphenyls that are involved in events such as tumor initiation, promotion, and progression. On the other hand, AhR plays multiple roles in fundamental cell biology, development and physiology.

Halogenated dioxins, polycyclic aromatic hydrocarbon and halogenated biphenyls represent the best characterized high affinity planar aromatic and hydrophobic ligands of AhR. Earlier, it was believed that AhR ligands had to share many of the common physiochemical characteristics of these chemicals. However, further extensive research has shown that this "dogma" is not entirely correct and that AhR can be activated by "novel" chemicals whose structural and physiochemical properties are inconsistent with defined structural requirement for AhR ligands. Relatively large number of structurally diverse natural, endogenous and synthetic AhR ligands has been discovered recently that have a little similarity with prototypical AhR ligands. These chemicals are reported to bind to AhR, and induce or inhibit AhR transformation. Because most of these "novel" ligands do not fulfill established structural characteristics of typical AhR ligands, re-evaluation of the current views of AhR ligands structure are supported. More importantly, the discovery of endogenous AhR ligands challenged numerous studies on physiological functions of AhR.

Various studies have been executed to find AhR ligands that possess the characteristic of an AhR antagonist, having the ability of compete with agonists, and inhibit AhR-mediated transactivation by exogenous compounds. Of the few antagonists currently available, the majority act as weak agonists that are able to induce the AhR target genes of the CYP1A family. These antagonists are reported to exhibit a number of beneficial properties (e.g. in cardiovascular diseases, inhibition of the progression of cancer). So it is possible that these inhibitors could serve as a chemoprotective agent in various type of cancer.

In conclusion, AhR can be activated by structurally diverse spectrum of chemicals. Elucidation whether a compound is a ligand/agonist of AhR can help us in understanding its toxicological potential. On the other hand, inhibition of AhR could be promising target for therapeutic intervention in future.

ACKNOWLEDGEMENTS

Our laboratories are supported by the grants from the Czech Scientific Agency GACR303/07/0128, GACR503/10/0579, GAUK No. 125709/C, SVV-2010-261-003 and GACR304/10/0149.

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Received: October 29, 2010 **Revised: January 2, 2011** **Accepted: February 21, 2011**