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Structural Basis of Human Pregnane X Receptor Activation by the Hops Constituent Colupulone

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ABSTRACT

Hops extracts are used to alleviate menopausal symptoms and as an alternative to hormone replacement therapy, but they can produce potentially harmful drug-drug interactions. The nuclear xenobiotic receptor pregnane X receptor (PXR) is promiscuously activated by a range of structurally distinct chemicals. It has a key role in the transcriptional regulation of genes that encode xenobiotic metabolism enzymes. In this study, hops extracts are shown to induce the expression of numerous drug metabolism and excretion proteins. The β-bitter acid colupulone is demonstrated to be a bioactive component and direct activator of human PXR. The 2.8-Å resolution crystal structure of the ligand binding domain of human PXR in complex with colupulone was elucidated, and colupulone was observed to bind in a single orientation stabilized by both van der Waals and hydrogen bonding contacts. The crystal structure also indicates that related α- and β-bitter acids have the capacity to serve as PXR agonists as well. Taken together, these results reveal the structural basis for drug-drug interactions mediated by colupulone and related constituents of hops extracts.

Although more than 1500 botanically derived products are currently available in the U.S., herbal formulations are not subject to Food and Drug Administration approval, and there is often a lack of clinical data regarding efficacy and potential side effects (http://ntp.niehs.nih.gov/ntp/htdocs/liason/factsheets/HerbMedFacts.pdf). The flowers of the hops plant (*Humulus lupulus*) were historically used as a preservative and flavoring agent in beer. Hops extracts are currently marketed as a source of phytoestrogens to alleviate menopausal symptoms and as an alternative to hormone replacement therapy (Milligan et al., 1999; Milligan et al., 2000, 2002; Bowe et al., 2006), and they have also been applied to treating insomnia and anxiety (Hoffmann, 2003). In addition to plant fibrous material and proteins, hops contain many small molecules, including volatile oils, flavonoids, and, primarily, the so-called bitter resins or acids, which make up 12 to 15% of all components (Stevens, 1967). Bitter acids have exhibited several antineoplastic properties, including inhibition of tumor transition to malignancy (Chen and Lin, 2004). Bitter resins are classified as α- (e.g., humulone) or β-acids (e.g., lupulone) (Fig. 1). The β-acid colupulone has been reported to have antibacterial properties and to inhibit tumor cell proliferation (Manering et al., 1993). In addition, colupulone was shown to stimulate expression of hepatic CYP3A enzymes in rats and mice (Mannering et al., 1992).

The pregnane X receptor (PXR), a member of the nuclear receptor superfamily of proteins, modulates the expression of genes involved in the metabolism and clearance of a wide array of structurally diverse endogenous and exogenous compounds (Watkins et al., 2001, 2002, 2003a,b; Goodwin et al., 2002; Chrencik et al., 2005; Orans et al., 2005; Noble et al., 2006; Huang et al., 2007; Wang et al., 2007; Xue et al., 2007a,b). Ligand-regulated nuclear receptors contain canonical DNA binding domains and ligand binding domains (LBDs), respectively, the latter of which maintains a surface activation function (AF) region-2 groove that binds to transcriptional coregulator proteins. Genes regulated by PXR include those encoding cyto-

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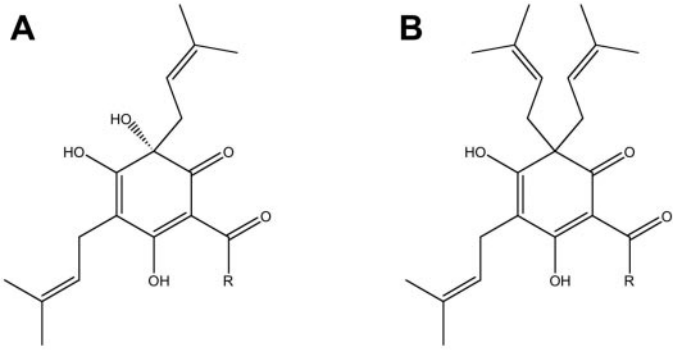
ABBREVIATIONS: PXR, pregnane X receptor; LBD, ligand binding domain; AF, activation function; RTQ-PCR, real-time quantitative-polymerase chain reaction; SRC, steroid receptor coactivator; DTT, dithiothreitol; MDR, multidrug resistance protein; Veh, vehicle; RMSD, mean square deviation; SR12813, 4-[2,2-bis(diethoxyphosphoryl)ethenyl]-2,6-ditert-butyl-phenol; T0901317, *N*-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxy-propan-2-yl)phenyl)-*N*-(2,2,2-trifluoroethyl)benzenesulfonamide; PPAR, peroxisome proliferator-activated receptor.

chromes P450, glutathione transferases, UDP-glucuronosyltransferases, sulfotransferases, and the multidrug resistance efflux pumps (Orans et al., 2005).



α-Acids	
Compound	**R-group**
Humulone	$CH_2CH(CH_3)_2$
Cohumulone	$CH_2(CH_3)_2$
Adhumulone	$CH(CH_3)CH_2CH_3$

β-Acids	
Compound	**R-group**
Lupulone	$CH_2CH(CH_3)_2$
Colupulone	**$CH_2(CH_3)_2$**
Adlupulone	$CH(CH_3)CH_2CH_3$

Fig. 1. Hops α- (A) and β (B)-bitter acids.

The PXR-LBD has been reported to bind to drugs such as phenobarbital (Guzelian et al., 1988a,b), dexamethasone (Maurel, 1996), avasimibe (Sahi et al., 2003; Sahi et al., 2004), and hyperforin, a bioactive compound in the herbal antidepressant St. John's wort (Watkins et al., 2003b). PXR activation by these compounds leads to the expression of drug metabolism enzymes that can lead to dangerous drug-drug interactions. For example, the presence of hyperforin has been shown to reduce the serum concentration and the efficacy of oral contraceptives, immunosuppressants, HIV protease inhibitors, and anticancer chemotherapeutics (Ernst et al., 1998b; Piscitelli et al., 2000; Mathijssen et al., 2002).

In addition to its potential for mediating drug-drug interactions, PXR plays a major role in protecting tissues from xenobiotic and endobiotic stress. For example, PXR activation has been shown to decrease the severity of ulcerative colitis and Crohn's disease by suppressing proinflammatory mediators (Shah et al., 2007). PXR offers hepatoprotection from the toxic accumulation of bile acids by inducing their clearance (Teng and Piquette-Miller, 2007). Neuroprotective effects are also mediated by PXR against neurodegenerative diseases such as Niemann-Pick C by clearing excess lipids and cholesterol (Langmade et al., 2006). In this study, the ability of human PXR to be activated by hops extracts is examined both structurally and functionally.

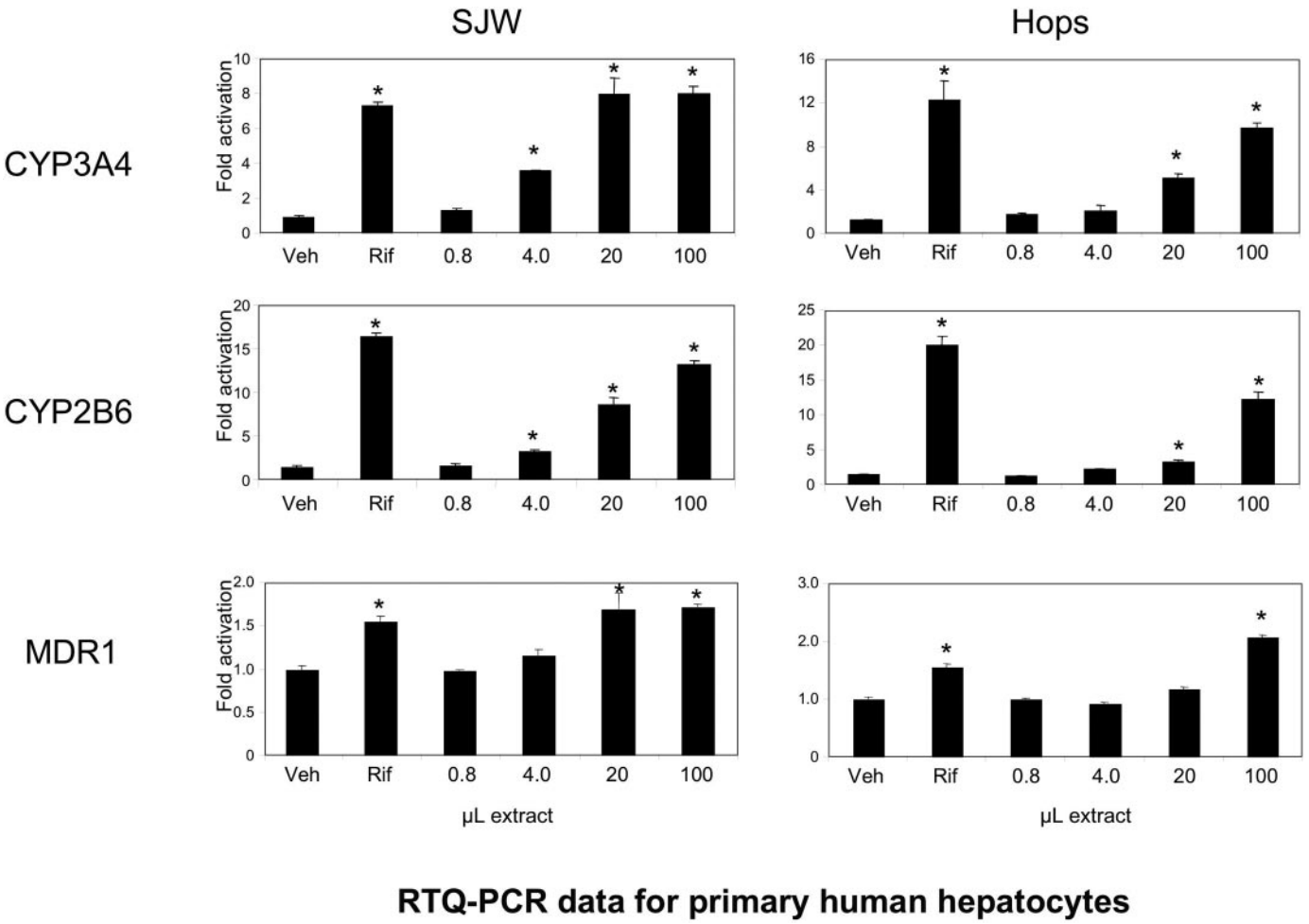


Fig. 2. Gene expression in primary human hepatocytes assessed by RTQ-PCR.

Materials and Methods

Colupulone, Herbs, and Preparation of Herbal Extracts. Colupulone was a gift from KALCEK, Inc. (Kalamazoo, MI). St. John's wort and guggulipid were purchased from General Nutrition Companies, Inc. (Pittsburgh, PA), and hops were purchased from Nature's Way Products, Inc. (Springville, UT). Before extraction, lyophilized hops and guggulipid were removed from their gelatin capsules, and St. John's wort tablets were ground into a fine powder with a mortar and pestle. The resultant powders were extracted by vortexing for 2 min in the presence of ethanol (1 g of herbal product/10 ml). A 1-ml aliquot of the mixture was transferred into a microcentrifuge tube and centrifuged for 15 min at 1500 rpm to remove the particulate material. The supernatant was transferred to a fresh microfuge tube and recentrifuged for 15 min at 1500 rpm. The resulting ethanol extracts were dried and weighed, and the residue was redissolved in dimethyl sulfoxide.

Human Hepatocytes. Human primary hepatocytes were obtained from the Liver Tissue Procurement and Distribution System as attached cells in six-well plates in human hepatocyte maintenance medium (Lonza Walkersville Inc., Walkersville, MD) supplemented with 100 nM dexamethasone, 100 nM insulin, 100 U/ml penicillin G, and 100 μg/ml streptomycin. Twelve hours after changing the culture medium to serum-free William's E medium, cells were treated with herbs, colupulone, rifampicin, or vehicle (0.1% dimethyl sulfoxide) for 24 h.

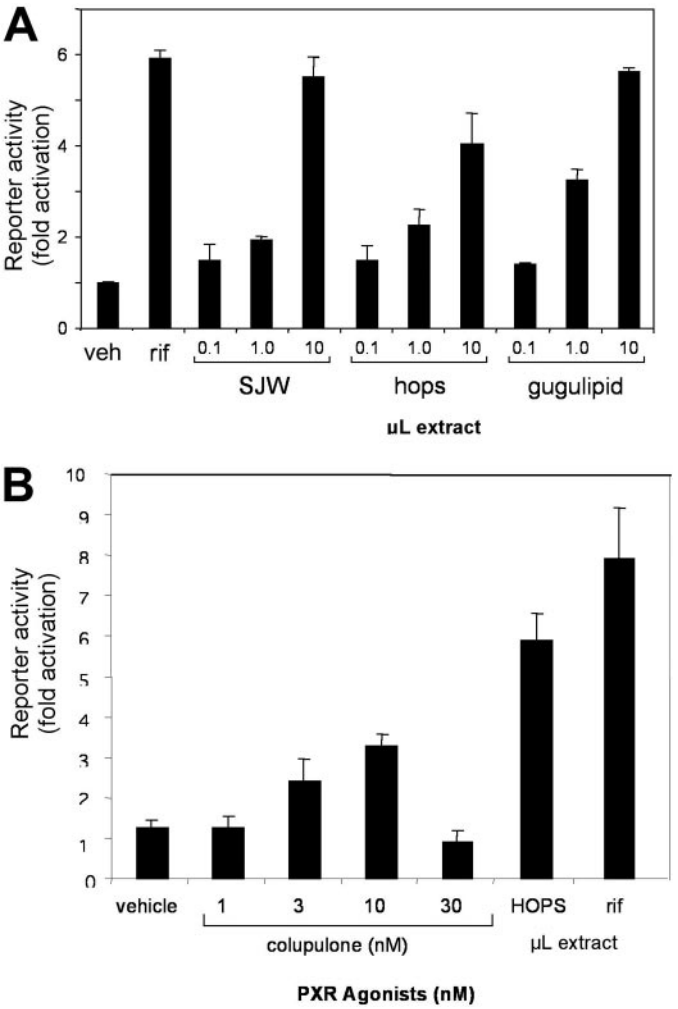


Fig. 3. Transient transfection assays in CV-1 cells. A, hops extracts induce reported gene expression in CV-1 cells at levels close to that of St. John's wort (SJW) extracts. Induction levels were compared with the known PXR agonist rifampicin. B, ability of the hops compound colupulone to active reporter gene activation under the control of human PXR is compared with that of hops extracts and rifampicin.

RNA Preparation and Real-Time Quantitative PCR Analysis. Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Real-time quantitative PCR (RTQ-PCR) was performed using an ABI Prism 7000 Sequence Detection system instrument and software (Applied Biosystems, Foster City, CA). Samples were assayed in triplicate 25-μl reactions using 25 ng of RNA per reaction. Primers were designed using Primer Express version 2.0.0 (Applied Biosystems) and synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). All primers and probes were entered into the National Center for Biotechnology Information Blast program to ensure specificity. -Fold induction values were calculated by subtracting the mean threshold cycle number for each treatment group from the mean threshold cycle number for the vehicle group and raising 2 to the power of this difference. RTQ-PCR primers were as follows: CYP2B6 (forward, AAGCGGATTTGTCTTGGTGAA and reverse, TGGAGGATGGTGGTGAAGAAG), CYP3A4 (forward, CAGGAGGAAATTGATGCAGTTTT and reverse, GTCAAGATACTCCATCTGTAGCACAGT), and MDR1 (forward, GTCCCAGGAGCCCATCCT and reverse, CCCGGCTGTTGTCTCCAT).

Cell-Based Reporter Assays. Transfection assays were performed in CV-1 cells plated in 96-well plates at a density of 20,000 cells/well in Dulbecco's modified Eagle's medium high-glucose medium supplemented with 10% charcoal/dextran-treated fetal bovine serum (HyClone Laboratories, Logan, UT). Transfection mixes included 5 ng of receptor expression vector, 20 ng of reporter plasmid, 12 ng of β-actin secreted placental alkaline phosphatase as an internal control, and 43 ng of carrier plasmid. Human PXR expression plasmids and the CYP3A4/XREM-luciferase reporter, containing the enhancer and promoter of CYP3A4 driving luciferase expression, were used as described previously (Watkins et al., 2003b). Transfections were performed with Lipofectamine (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Luciferase activity was normalized to secreted placental alkaline phosphatase expression.

Protein Expression and Purification. PXR-LBD (residues 140–434) was expressed in the N-terminal His-tagged expression vector pRSET-A (Invitrogen). As described previously, residue Cys284 was

TABLE 1
Crystallographic data collection and refinement statistics

	Statistic
X-ray data collection	
Space group	$P4_32_12$
Cell dimensions	
a, b, c (Å)	90.9, 90.9, 85.4
α, β, γ (°)	90.0, 90.0, 90.0
Resolution (Å) (highest shell)	50–2.8 (3.0–2.8)[a]
R_{sym} (%)[b]	10.5 (43.5)
I/σ	20 (2.8)
Completeness (%)	99.7 (97.6)
Redundancy	10 (6.3)
Crystallographic refinement	
Resolution (Å)	50–2.8 (3.0–2.8)
Unique reflections	8870 (1166)
Mean thermal displacement parameter (Å^2)	
Protein	48.1
Water	44.4
RMSD	
Bond lengths (Å)	0.008
Bond angles (°)	1.2
R_{work}/R_{free} (%)[c,d]	24.2 (34)/28.4 (38.7)

[a] Data for the outer shell are given in parentheses.
[b] $R_{sym} = (\Sigma_{hkl}\Sigma_i|\ I_i\ (hkl) - <I\ (hkl)>|)/\Sigma_{hkl}\Sigma_i\ I_i\ ((hkl))$ for n independent reflections and observations of a given reflection, $<I\ (hkl)>$ is the average intensity of the I observation.
[c] $R_{work} = \Sigma||Fo| - |Fc||/\Sigma|Fo|$, where Fo is the observed amplitude of an X-ray reflection, and Fc is the calculated amplitude of this refletion derived fromn the refined coordinates.
[d] R_{free} is the R-factor calculated using 10% of randomly selected reflections.

mutated to a serine using the QuikChange mutagenesis kit (Stratagene, LA Jolla, CA) to prevent formation of covalent complexes in the presence of DTT (Chrencik et al., 2005; Xue et al., 2007a,b). An 88-amino acid construct of the human SRC-1 gene (residues 623–710) in the pACYC184 vector was cotransformed with the PXR/pRSET-A plasmid into BL21(DE3) *Escherichia coli* cells. Fifteen liters of cell culture in Luria broth supplemented with ampicillin and chloramphenicol were inoculated with PXR/SRC-1 and grown overnight at 22°C. Harvested cells were centrifuged (20 min; 3500*g*; 4°C), and the resulting pellet was resuspended in nickel buffer A (50 mM Tris-Cl, pH 7.8, 250 mM NaCl, 50 mM imidazole, pH 7.5, and 5% glycerol). Cells were sonicated on ice for 20 min and centrifuged at 20,000*g* for 90 min at 4°C. The supernatant was loaded onto a 50-ml nickel column (ProBond; Invitrogen). The column was washed with 200 ml each of nickel buffer A and nickel buffer B (50 mM Tris-Cl, pH 7.8, 250 mM NaCl, 75 mM imidazole, pH 7.5, and 5% glycerol). On-column buffer exchange was achieved by washing the column with nickel buffer C (50 mM Tris-Cl, pH 7.8, 75 mM imidazole, 5% glycerol, and 50 mM NaCl) to prepare the sample for subsequent ion exchange chromatography. Protein was eluted off using nickel buffer D (50 mM Tris-Cl, pH 7.8, 250 mM imidazole, 5% glycerol, and 50 mM NaCl). Column fractions were pooled and immediately loaded onto a SP-cation exchange column (Bio-Rad, Hercules, CA) pre-equilibrated with SP buffer A (50 mM Tris-Cl, pH 7.8, 50 mM NaCl, 5 mM DTT, 2.5 mM EDTA, pH 8.0, and 5% glycerol). The protein sample was washed with 200 ml of SP buffer A and eluted with SP buffer B (50 mM Tris-Cl, pH 7.8, 400 mM NaCl, 5 mM DTT, 2.5 mM EDTA, pH 8.0, and 5% glycerol). Pooled fractions were diluted to double the volume and concentrated to 10 mg/ml using the Centri-prep 30K units (Millipore, Billerica, MA) in the presence of 25-fold molar excess of colupulone and 2-fold molar excess of SRC-1 peptide.

Crystallization, X-Ray Data Collection, and Structure Refinement. PXR-LBD was crystallized using hanging-drop vapor dif-

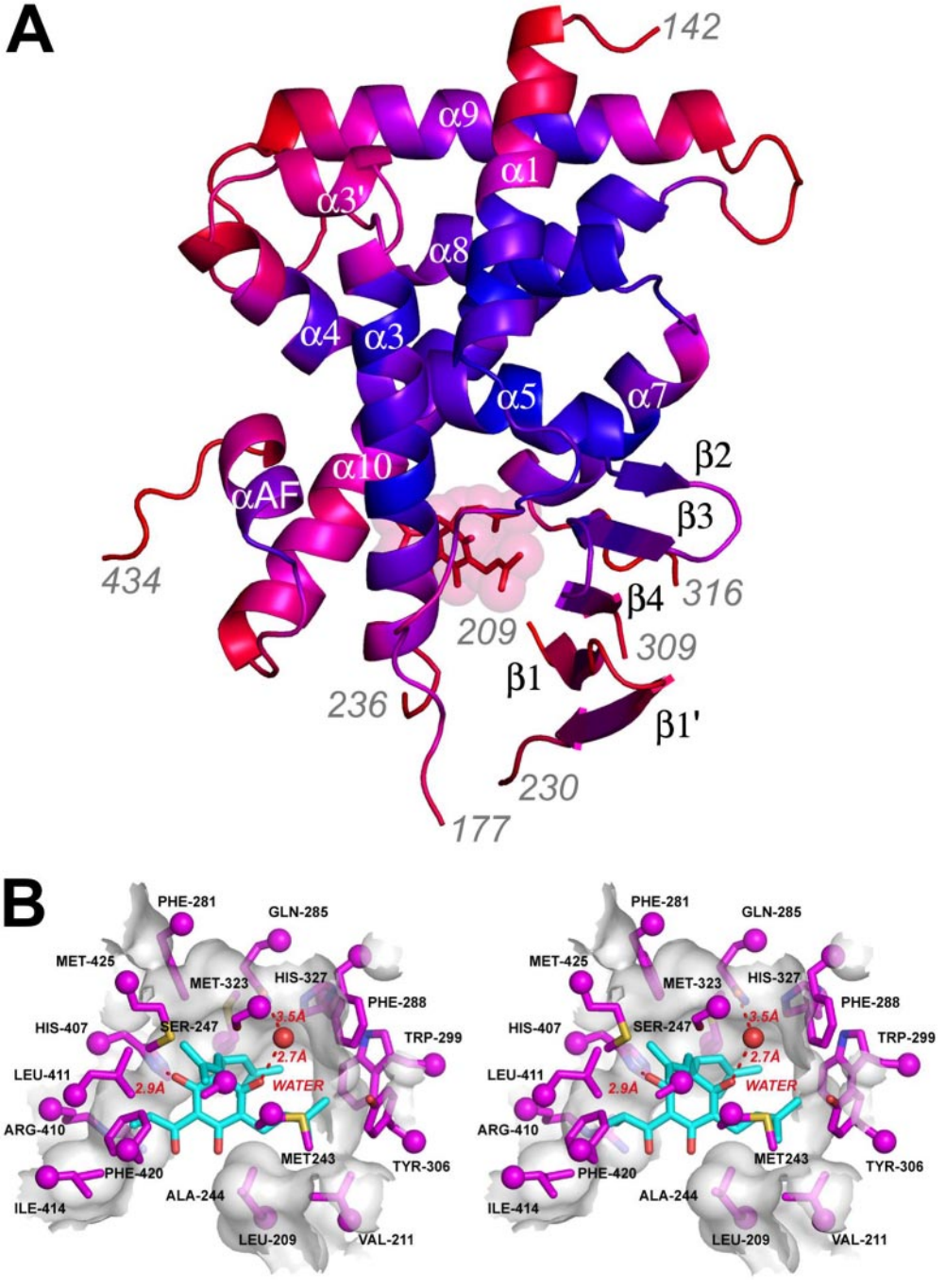


Fig. 4. Crystal structure of the PXR-colupulone complex. A, structure of the PXR LBD in complex with colupulone is rendered by thermal displacement parameter (B factor), ramping from blue (relatively low; 20 Å^2) to red (relatively high, 95 Å^2). Colupulone is shown in cyan. B, stereoview of colupulone-contacting residues in the PXR ligand binding pocket.

fusion methods at room temperature against a crystallant containing 50 mM imidazole at pH 8.0, 10% (v/v) isopropanol, and 50 mM DTT. Crystals were cryoprotected by serial dipping into 15, 25, and 35% ethylene glycol. Data collection was conducted at Southeast Regional Collaborative Access Team at the Advanced Photon Source in Argonne National Labs (Beamline 19-ID). Diffraction data were indexed, scaled, and integrated using HKL2000 (Otwinowski and Minor, 1997). Using the apo structure of PXR-LBD (Protein Data Bank ID: 1ILG) as a search model, molecular replacement was conducted with the MolRep module of CCP4 (Collaborative Computational Project Number 4, 1994; Winn et al., 2002). Clear molecular replacement solutions were obtained in the space group of $P4_32_12$. The structure was manually adjusted using a combination of O (Jones et al., 1991) and WinCoot 3.1 (Emsley and Cowtan, 2004) and was refined using CNS (Brünger et al., 1998) and CCP4 (Murshudov and Dodson, 1997). Molecular graphics figures were created using Pymol (DeLano, 2002).

Results

Hops Extracts Induce Expression of Drug Clearance Proteins. We sought to determine the effects of hops on metabolic gene regulation in hepatic tissues using RTQ-PCR methods. St. John's wort extracts and rifampicin, two established PXR activators, were used as positive controls. Hyperforin from St John's wort has been shown to have nanomolar affinity for PXR (Moore et al., 2000), whereas rifampicin is a micromolar affinity ligand (Kliewer et al., 1998; Watkins et al., 2001, 2003b). RTQ-PCR methods indicate that hops extracts increase mRNA levels for CYP3A4, CYP2B6, and MDR1 in a concentration-dependent manner (Fig. 2). The efficacy of hops in inducing these genes was comparable with that exhibited by rifampicin at 10 μM. Comparison of hops and St. John's wort results indicates that both herbal extracts affect CYP3A4, CYP2B6, and MDR1 levels. Activation of CYP3A4 is noteworthy because this gene product is the most abundant of all the cytochromes P450, clearing more than half of all prescription drugs (Kliewer et al., 2002; Kliewer, 2003).

A transient transfection assay was used to determine whether hops activated PXR. Guggulipid, a herbal extract from the guggul tree (*Commiphora mukul*) that reduces hyperlipidemia in humans (Brobst et al., 2004), was used as an additional positive control. The biotransformation of guggulipid has been linked to CYP3A4 oxidation in both rodent and human hepatocytes, via a PXR-regulated pathway (Brobst et al., 2004). Hops, guggulipid, and St. John's wort all activated PXR with comparable efficacy. Our data indicate that hops induces CYP3A4 and other drug-metabolizing genes by activating PXR.

Colupulone Up-Regulates Gene Expression via PXR. Because the hops constituent colupulone is known to activate the transcription of CYP3A genes in mice (Mannering et al., 1992), we hypothesized that it serves as the PXR agonist in hops extracts. Cotransfection data from CV-1 cells validated this hypothesis and demonstrated a dose-dependent transcriptional activation 2.0- to 2.5-fold above basal levels with only nanomolar (3–10 nM) concentrations of colupulone (Fig. 3B). Addition of 30 nM colupulone drops activation levels, possibly due to cell death. Indeed, α- and β-acids have been shown to activate the death receptor Fas, causing apoptosis (Chen and Lin, 2004). The efficacy of colupulone alone, however, was less than that of hops extract, suggesting that other bitter acids in hops may be binding to PXR to induce full transcriptional activity (as discussed below).

PXR-Colupulone Complex Crystal Structure. The crystal structure of the PXR-LBD in complex with colupulone was determined in space group $P4_32_12$ using molecular replacement and refined to a resolution of 2.8 Å (Table 1; Fig. 4A). Root-mean square deviations (RMSDs) between the PXR-colupulone complex and previously reported PXR structures are small, ranging from 0.27 to 0.54 Å over Cα positions (Watkins et al., 2001, 2003a,b; Chrencik et al., 2005; Xue et al., 2007a,b). Low RMSD values were observed regardless of whether the space group of the previously reported structure was $P4_32_12$ with one complex per asymmetric unit, like the PXR-colupulone structure reported here (Watkins et al., 2001, 2003b; Chrencik et al., 2005; Xue et al., 2007b), or $P2_12_12_1$ with two complexes per asymmetric unit (Watkins et al., 2003a; Xue et al., 2007a). The PXR LBD maintains the canonical nuclear receptor ligand binding fold with a seven-membered α-helical sandwich arranged in three layers (α1/α3, α4/α5, and α7/ α8). The PXR-colupulone complex structure also contains this five-stranded antiparallel β-sheet unique to PXR (Noble et al., 2006). The surface AF-2 groove maintains a conformation consistent with the agonist-bound form for nuclear receptors, wherein the αAF helix remains immobilized against the groove formed by α3, α3′, and α4.

The main core of the PXR LBD (made up of the bottom half

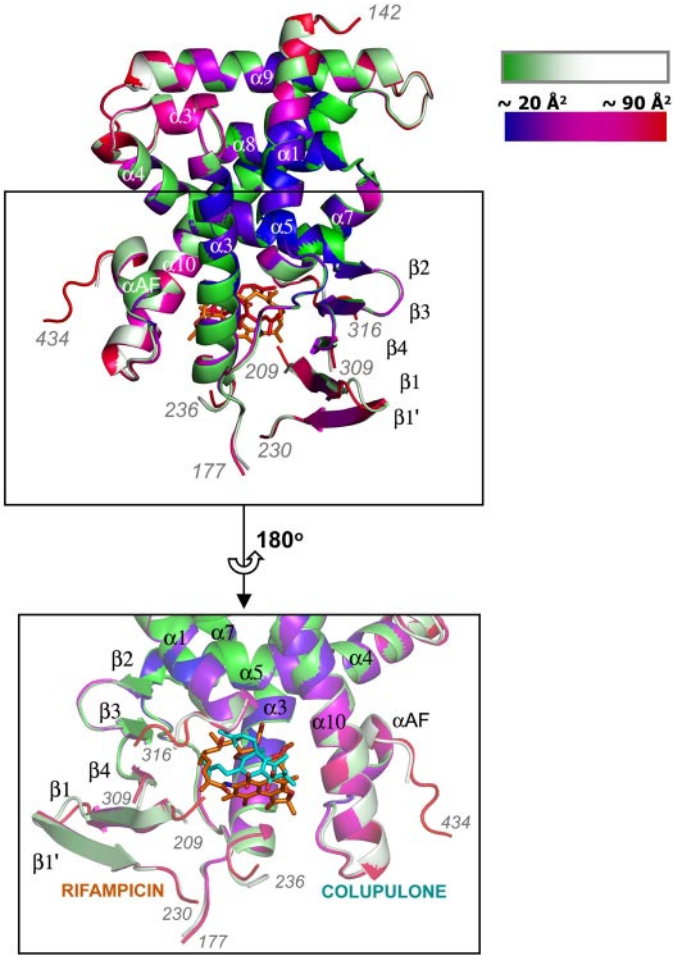
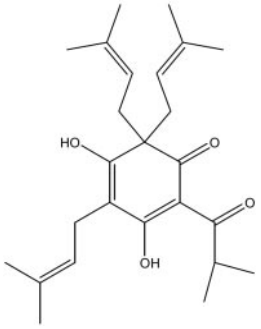
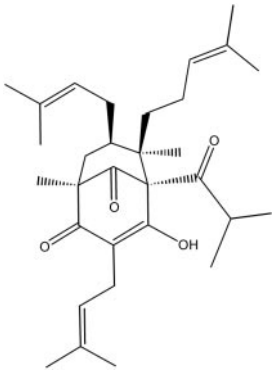
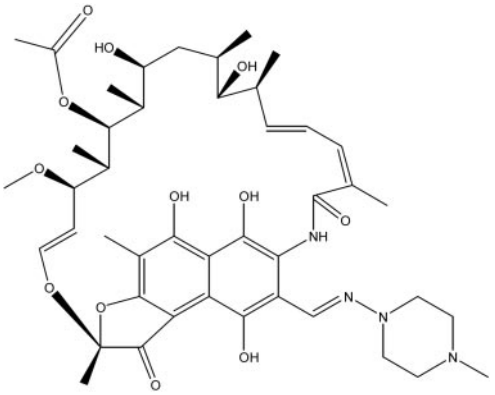


Fig. 5. PXR-colupulone complex superimposed on the PXR-rifampicin complex. The structures are colored by thermal displacement parameters ramping from green (relatively low; 20 Å^2) to white (relatively high, 95 Å^2).

of α1 and α3; bottom half of α4, α5, α7, and α8; top half of α10, β2, and β3) exhibits low thermal displacement parameters (B-factors; ~20 Å^2). However, as seen previously (Chrencik et al., 2005), higher degrees of thermal motion (thermal displacement parameters approaching 90 Å^2) are observed for the AF-2 region (α3, α3′, α4, and αAF), the bottom half of the ligand pocket (β1 and β1′) and other solvent exposed areas (top half of α1, top of half α4 α9, and bottom half α10) (Fig. 4A). Average thermal displacement parameters for ligand binding pocket residues of reported PXR complex crystal structures yield the following ranking, from highest to lowest: rifampicin (51.3 Å^2) > colupulone (47.3 Å^2) > SR12813 (47.2 Å^2) > T0901317 (42.2 Å^2) > hyperforin (35.1 Å^2) > SR12813 with SRC-1 peptide (24.3 Å^2) (Watkins et al., 2001, 2003a,b; Chrencik et al., 2005; Xue et al., 2007a,b). In both the rifampicin- and colupulone-PXR complexes' structures, α2 and the loops connecting β3 and β4 are disordered (Figs. 4A and 5). Thus, although portions of the ligand binding pocket of PXR remain relatively fixed regardless of the ligand bound, other elements (e.g., α2) are capable of a high degree of flexibility even when the LBD is complexed to established agonists (e.g., colupulone and rifampicin). In this way, PXR shares both similarities (ability to change the ligand binding pocket to adapt to ligands) and distinctions (one hemisphere of the pocket of PXR is fixed, whereas the other hemisphere is highly mobile) with other members of the nuclear receptor superfamily.

Difference electron density facilitated the positioning of central region of colupulone in the PXR ligand binding pocket, and subsequent refinement allowed the building of the remaining atoms of the isoprene units. Thirteen hydrophobic residues (Met425, Met323, Phe281, Phe288, Trp299, Tyr306, Val211, Leu209, Met243, Ala244, Phe420, Ile414, and Leu411) and two polar residues (Arg410 and His327)

TABLE 2
PXR ligand binding pocket residues contacted by colupulone, hyperforin, and rifampicin
Those not contacting colupulone are in bold.

Ligand	Binding Pocket Contact
 Colupulone	Met425, Met323, Phe281, Phe288, Trp299, Tyr306, Val211, Leu209, Met243, Ala244, Phe420, Ile414, Leu411, Arg 410, His327
 Hyperforin	Met425, Met323, Phe281, Phe288, Trp299, Tyr306, Val211, Leu209, Met243, Ala244, Phe420, Ile414, Leu411, Arg 410, His327, **Leu239**, **Leu240**, **Leu206**, **Cys284**, **Met250, Met246**, **Phe251**, **Leu324**
 Rifampicin	Met425, Met323, Phe281, Phe288, Trp299, Tyr306, Val211, Met243, Ala244, Phe420, Ile414, Leu411, Arg 410, His327, **Leu239**, **Leu240**, **Cys284**, **Met250**, **Met246**, **Met250**, **Phe251, Leu324**

contact carbon atoms of colupulone (Table 2; Fig. 4B). Note that residues Met425 and Phe420 are on αAF of the AF-2 region of the receptor. In addition, a direct hydrogen bond is formed between a colupulone hydroxyl and His407, and a water-mediated hydrogen bond is observed between another colupulone hydroxyl group and Gln285 (Fig. 4B).

Ligand Binding Pocket Analysis. The pocket of the PXR-colupulone complex was compared with other reported PXR crystal structures (Watkins et al., 2001; Watkins et al., 2003a,b; Chrencik et al., 2005; Xue et al., 2007a,b), and it was noted that the hyperforin (537-Da) and colupulone (400-Da) ligands exhibit some structural similarities. Both contain a cyclic core with isoprene extensions that are significantly involved contacting PXR. However, the hyperforin-PXR complex exhibits interactions in the ligand binding pocket that more closely resembles the PXR-rifampicin complex than the receptor with colupulone (Figs. 5 and 6; Table 2). Hyperforin contacts the same residues as colupulone, but it requires further stabilization provided by seven additional hydrophobic amino acids (Leu240, Leu206, Cys284, Met250, Met246, Phe251, and Leu324) that are also found in the rifampicin structure. Thus, although residues in the colupulone pocket have been observed to contact other ligands in previous structures, it seems difficult to predict the exact identity of residues that may contact a ligand.

Related Hops Constituents. Our functional data indicate that additional hops compounds beyond colupulone likely contribute to PXR activation (Figs. 2 and 3). Thus, because only purified colupulone was readily available, we superimposed the other bitter α- and β-acids found in hops onto the ligand in the PXR-colupulone structure and found that these compounds seem to be capable of binding to human PXR in an analogous manner (Fig. 7, A and B). Overlay of the largest and most substituted member of the bitter acids family, lupulone (414 Da), indicates the potential for improved hydrophobic packing

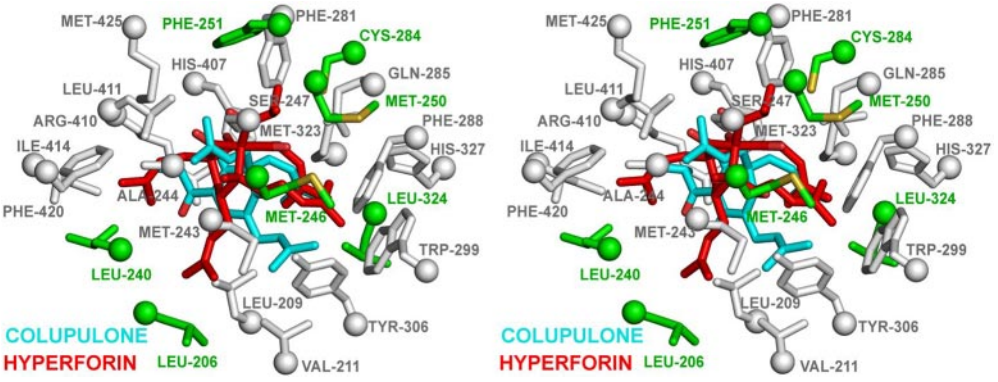


Fig. 6. Stereoview of residues in the ligand binding pocket of PXR-colupulone (magenta) superimposed onto the PXR-hyperforin pocket (green).

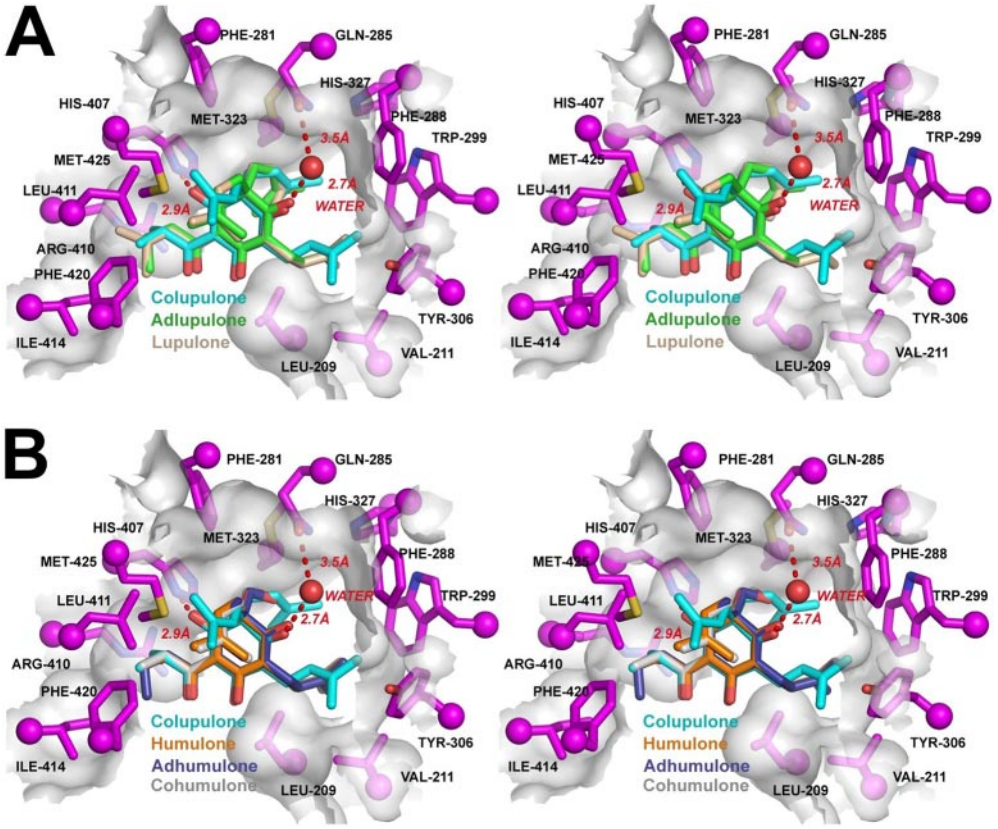


Fig. 7. Superimposition of α-bitter (A) and β-bitter (B) acids onto colupulone ligand within the colupulone-PXR crystal structure.

with PXR (e.g., the distance lupulone carbons and decreased from 5 to 3 Å) but no new polar or nonpolar contacts (Fig. 7B). Taken together, these modeling observations suggest that both the bitter α- and β-acids from hops have the potential to act as activators of human PXR.

Discussion

The use of herbal remedies and supplements together with prescribed medications increases the risk of potentially dangerous drug-herb interactions (Burka, 2003). Altered drug clearance due to changes in CYP450 expression profiles have been observed for cardiovascular drugs (e.g., digoxin), immunosuppressants (e.g., cyclosporine and tacrolimus) and anticancer drugs (e.g., imatinib and irinotecan) (Yang et al., 2006). Herbal therapies can also affect laboratory test results and interfere with proper diagnoses (Dasgupta and Bernard, 2006). Thus, we investigated the ability of hops extracts, which are used as herbal supplements, to induce gene transcription in primary human hepatocytes. We found that extracts activated the expression of drug metabolism and clearance genes (Fig. 2) in a manner similar to that of St. John's wort, an established mediator of herb-drug interactions (Ernst et al., 1998a,b; Piscitelli et al., 2000; Watkins et al., 2003b). We also establish that the human xenobiotic receptor PXR was activated by the hops β-bitter acid colupulone, which has been shown to up-regulate rodent CYP3A expression (Mannering et al., 1992) (Fig. 3). The human PXR LBD-colupulone complex crystal structure then facilitated a molecular understanding of the ability of other hops bitter acids to activate PXR (Figs. 4–7).

Although they may contribute to drug-drug interactions, activators of PXR have the potential to serve as therapeutic leads. For example, PXR agonists have been shown to attenuate inflammatory bowel disease through reducing nuclear factor-κB target gene expression (e.g., interleukin-1β, interleukin-10, inducible nitric-oxide synthase, and tumor necrosis factor-α) that mediate colonic inflammation. PXR activators may provide new avenues for the treatment of inflammatory bowel disease (Shah et al., 2007). PXR agonists are also hepatoprotective by promoting the elimination of toxic bile acids (Teng and Piquette-Miller, 2007). Likewise, PXR activation has been shown to be neuroprotective in Niemann-Pick Type C disease, characterized by cholesterol and lipid accumulation in the brain (Langmade et al., 2006). Concomitant use of the neurosteroid allopregnanolone and the PXR agonist T0901317 delays symptom onset and prolongs neural cell survival (Langmade et al., 2006; Mellon et al., 2008). PXR activation induces cerebellar CYP3A13 expression, increasing cholesterol clearance and attenuating neuronal injury (Ghoumari et al., 2003; Langmade et al., 2006).

For related nuclear receptors, peroxisome proliferator-activated receptor (PPAR)-γ agonists suppress inflammation by disrupting nuclear factor-κB function (Trifilieff et al., 2003). Nonsteroidal anti-inflammatory drugs were reported to reduce the risk of developing Alzheimer's disease by as much as 80% through mechanisms dependent on PPARγ activation (in t' Veld et al., 2001). Induction of PPARγ also reduced inflammation related to multiple sclerosis (Schmidt et al., 2004) and is now being used to treat central nervous system disorders (Heneka et al., 2007). Likewise, there is potential for developing new therapies that exploit the protective functions of PXR in various tissues, in addition to its role in xenobiotic metabolism and drug-drug interactions (Moore and Kliewer, 2000; Xie et al., 2000a,b; Staudinger et al., 2001; Watkins et al., 2001, 2002, 2003b; Ekins et al., 2002; Kliewer et al., 2002; Willson and Kliewer, 2002; Xie and Evans, 2002; Kliewer, 2003; Sahi et al., 2003; You, 2004). Several recent reports examine the possibility of PXR agonists as therapeutics (Stedman et al., 2005; Langmade et al., 2006; Shah et al., 2007; Teng and Piquette-Miller, 2007; Xue et al., 2007a). Thus, an expanded understanding of chemical scaffolds capable of activating PXR may facilitate the design of PXR-directed lead compounds.

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