PXR-LBD

Adnectin-1
SRC-1

Developing Adnectins that Target SRC Co-Activator Binding to PXR; A Structural Approach Towards Understanding Promiscuity of PXR

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Abstract

The human pregnane X receptor (PXR) is a promiscuous nuclear receptor that functions as a sensor to a wide variety of xenobiotics and regulates expression of several drug metabolizing enzymes and transporters. We have generated “Adnectins,” derived from 10th fibronectin type III domain (¹⁰Fn3), that target the PXR ligand binding domain (LBD) interactions with the steroid receptor co-activator-1 (SRC-1) peptide, displacing SRC-1 binding. Adnectins are structurally homologous to the immunoglobulin superfamily. Three different co-crystal structures of PXR LBD with Adnectin-1 and a Chemokine Receptor-1 (CCR1) antagonist Compound-1 were determined. This structural information was used to modulate PXR affinity for a related CCR1 antagonist compound that entered into clinical trials for rheumatoid arthritis. The structures of PXR with Adnectin-1 reveal specificity of Adnectin-1 in targeting not only the interface of the SRC-1 interactions but also engaging the same set of residues that are involved in binding of SRC-1 to PXR. Substituting SRC-1 with Adnectin-1 does not alter the binding conformation of Compound-1 in the ligand binding pocket. The structure also reveals the possibility of using Adnectins as crystallization chaperones to generate structures of PXR with compounds of interest.

Abbreviations used: PXR, pregnane X receptor; LBD, ligand binding domain; SRC-1, steroid receptor coactivator-1; CCR1, Chemokine Receptor-1; NR, nuclear receptor; AF, activation function; CYP, cytochrome P450; MDR1, multi-drug resistance gene-1.

Introduction

The human pregnane X receptor (PXR) is a member of the nuclear receptor (NR) super family that includes the steroid, retinoid, and thyroid hormone receptors.^{1; 2; 3; 4; 5; 6} PXR acts as sensor to a broad spectrum of ligands, endogenous metabolites and chemotherapeutics and regulates a number of genes involved in drug metabolism. Human PXR activators include a wide range of commercially available drugs that can be involved in clinically relevant drug-drug interactions.^{7; 8} PXR functions as a heterodimer with retinoic X receptor α (RXR α) and binds to a variety of repeat elements in the regulatory region of target genes. Upon association with an activating agonist, a transcriptional co-activator protein like steroid receptor co-activator-1 (SRC-1) is recruited to the activation function-2 (AF-2) region of the PXR ligand binding domain (LBD). The PXR-RXR-co-activator-DNA complex then recruits and directs downstream members of the transcription machinery.^{9; 10} The two most important target genes of PXR activation are cytochrome P450 (CYP3A4) and multidrug resistance 1 (MDR1).^{9; 10; 11} Together these 2 genes are responsible for elimination of more than 50% of all drugs.^{4; 12; 13} The increase in expression of drug metabolizing genes mediated by PXR can reduce the clinical efficacy of the drug and cause dangerous drug-drug interactions by enhancing metabolism of other drugs.

Over the last decade a number of crystal structures of human PXR (hPXR) ligand binding domain (hPXR-LBD) have become available. This includes structures of apo-PXR,¹⁴ binary complexes of PXR with different activators,^{14; 15; 16; 17; 18; 19} a ternary complex of PXR with SRC-1 and activating agonist SR12813,²⁰ and a structure of PXR with tethered SRC-1 and SR12813.²¹ More recently Wallace *et al* have reported a structure of a heterotetramer complex of the PXR LBD in complex with the LBD of RXR α that advances our understanding of interactions of PXR with RXR.²²

[_ENREF_21](#) The elucidation of the three dimensional structure of the PXR (LBD) has provided important insights into the structural basis for the promiscuous ligand binding properties of this unusual NR. The promiscuity of PXR is greatly assisted by its relatively large, flexible and expandable pocket with a volume of $>1600\text{\AA}^3$.^{14; 15} The ligand binding cavity of PXR is largely hydrophobic and lined with ~ 20 hydrophobic amino acids plus four polar and four charged residues, but not all residues are found to be in contact with any given ligand. The flexibility of the pocket allows ligands to take on several simultaneous binding conformations¹⁴, which has been reported to be restricted by binding of a 25mer SRC-1 co-activator peptide.^{15; 20} [_ENREF_15](#) Almost all these structures were obtained by using protein generated by recombinant co-expression of PXR LBD with SRC-1 co-activator peptide using dual plasmids to maintain expression and solubility of the PXR LBD.^{14; 20} Alternatively, a single polypeptide chain of PXR LBD tethered with SRC-1 through a flexible linker was used to promote intramolecular PXR/SRC-1 complex formation.²¹

The SRC contains LxxLL motif (Where L is Leu and x is any amino acid) that adopts a helical conformation and binds to the surface of PXR-LBD forming a charge

clamp as observed in other NR-coactivator complexes.^{21; 23; 24} It has been reported that the interactions of PXR LBD with SRC-1 restrict structural flexibility of the ligand binding pocket, thereby stabilizing the bound ligand in an active orientation.²⁰ Molecular dynamics simulations have shown for PXR and other NR LBD structures that coactivator binding restricts the motions of the LBDs.²⁵ Although structures of PXR with agonist in a single orientation exist, the binding of SRC-1 has additional effects on the manner in which bound ligand interacts with PXR.^{14; 20} SR12813 is bound to the PXR/SRC-1 complex in a single orientation²⁰ that is distinct from the three orientations observed earlier.¹⁴ However, in several structures, especially ones in the P4₃2₁2 crystal form, the electron density of the SRC -1 was not visible. Here, we propose that a structurally bulkier PXR binding partner with higher binding affinity than SRC-1 can limit the ability of the promiscuous PXR receptor to flex and may help trap a single active conformation for compounds of interest. Such a molecule may also increase the crystallizability of PXR LBD. We also demonstrate that the SRC-1 replacement does not alter the agonist binding affinity or change the overall conformation of the PXR ligand.

The use of engineered binding proteins such as antibodies or antibody fragments, e.g. Fab, Fv, or V_HH (also termed nanobodies) as crystallization chaperones has been previously demonstrated.^{26; 27; 28; 29; 30} Adnectins (a trademark of Adnexus, a Bristol-Myers Squibb R&D company) are a family of targeted biologics derived from the tenth type III domain of human fibronectin (¹⁰F_n3), that are designed to bind protein or therapeutic targets with high affinity and specificity.^{31; 32; 33; 34; 35; 36} The ¹⁰F_n3 domain is a member of the immunoglobulin (Ig) superfamily and both antibody variable domains (V_L, V_H) and the ¹⁰F_n3 domain have a protein fold that resembles sandwiches of

antiparallel beta sheets (Figure 1).^{31; 34} In fact, the three loops BC, DE and FG of ¹⁰F_n3 are analogously located to the complementarity determining regions (CDRs) of an antibody variable domain (Figure 1b-c). Adnectins such as CT-322 that binds to vascular endothelial growth factor receptor-2 (VEGFR-2) are also being tested in clinical trials.^{37;}³⁸ Additional co-crystal structures of Adnectins in complex with different protein targets such as EGFR, IL-23 and PCSK9 have also been reported.^{34; 36} The small size and the shape of the binding surface of Adnectins allows them to bind to surfaces on their targets that would be sterically inaccessible to Fvs. Adnectins that bind to PXR/SRC-1 complex with high affinity were generated using an *in vitro* selection method involving mRNA display (described previously).³⁹ Two different Adnectin classes were identified; one that competes with SRC-1 for binding to PXR and another that binds at an allosteric site and does not affect SRC-1 interactions with PXR.

We took different biochemical, biophysical and structural approaches to test the molecular interactions of these Adnectins with the PXR ligand binding domain. We generated crystal structures with one of the compounds (Compound-1) that was designed to inhibit a G-protein coupled, CCR1 receptor (CC Chemokine Receptor-1) and to provide therapeutic benefits to individuals with inflammatory disease. However, in cell-based assays, Compound-1 and a related compound had demonstrated PXR agonism.^{40; 41} The information from structures of these compounds with PXR was then used to diminish PXR binding and activation for a third related compound “BMS-817399” which had entered in Phase II human clinical trials for the treatment of rheumatoid arthritis.[_ENREF_41](#)

We present here the selection and identification of high affinity Adnectins that could target SRC-1 binding to PXR. Three different co-crystal structures of PXR with Adnectin-1 and a CCR1 antagonist compound are reported. The structures reveal the specificity of Adnectin-1 in targeting not only the interface of the SRC-1 interactions but also engaging the same set of residues that are involved in binding of SRC-1 to PXR. We also show that substituting SRC-1 with Adnectin-1 in generating structures with PXR LBD does not alter the binding conformations of Compound-1 in the ligand binding pocket. Unlike previously published structures of PXR, we report the first structure of PXR in complex with a biologics (Adnectin-1), suggesting that an Adnectin can substitute for SRC-1 co-activator peptide and potentially be used as a crystallization chaperone to obtain structures of PXR with compounds of interest.

Results

Identification of Adnectins that compete with SRC-1 binding to PXR

Adnectins that bind to PXR were identified using the in vitro selection technique of mRNA display against PXR immobilized on beads.^{33; 34; 35; 39; 42} Eight different Adnectins were identified upon final selection (Figure 2a; Supplemental, Figure. S1). All 8 Adnectins showed high affinity to PXR with K_D values ranging from sub-nanomolar to 100 nM (Table 1). A few were competitive with SRC-1 (Figure 2b-c), while the remaining ones did not compete for the SRC-1 binding to PXR (Figure 2d) and thus could be inferred to have a different binding site. Adnectin-1 binds to PXR LBD with a K_D of 11 nM and competes with SRC-1 for binding to PXR (Figure 2e). SDS-PAGE of the eluate obtained after size exclusion chromatography clearly showed the displacement

of SRC-1 with Adnectin-1 (Figure 2b-c). PXR LBD complexes with each of the 8 Adnectins (Adnectin 1-8) were screened for crystallization conditions, but only Adnectin-1 gave crystal leads that could be optimized. Adnectin-1 was co-crystallized with PXR LBD to determine the structural basis of the competition with SRC-1.

The dimer of PXR/Adnectin-1 complex

Three different co-crystal structures of PXR LBD with Adnectin-1 and a CCR1 antagonist, Compound-1, were determined. These structures include a binary complex of PXR with Compound-1 (PXR/Compound-1), a binary complex with Adnectin-1 (PXR/Adnectin-1) and a ternary complex of PXR with Adnectin-1 and Compound-1 (PXR/Adnectin-1/Compound-1) (Table 2). The two complexes with Adnectin-1 (PXR/Adnectin-1 and PXR/Adnectin-1/Compound-1) crystallized with a dimer of PXR-Adnectin complex in the asymmetric unit (Table 2; Figure 3a). The atomic model of PXR/Adnectin-1 complex includes PXR residues 143-177, 192-429 (both molecules of PXR) and the Adnectin-1 model contains residues 7-79C, 83-94 (both molecules of Adnectin-1). The atomic model of PXR/Adnectin-1/Compound-1 complex contains for both molecules of PXR residues 143-177 and 186-429, and both molecules of Adnectin-1 contains residues 6-79C and 84-95.

The crystal structures of PXR/Adnectin-1 and PXR/Adnectin-1/Compound-1 reveal an intimately associated dimer. The formation of homodimer interface in the PXR complexes with Adnectin-1 buries $\sim 1600 \text{ \AA}^2$ of molecular surface area, which is similar to that reported earlier for homodimer interface of PXR/SRC-1 complexes.^{17; 20; 43} The formation of this dimer interface is mediated largely by the $\beta 1'$ residues from each monomer. The $\beta 1'$ strand interacts in an antiparallel manner to generate a 10 stranded

intermolecular β sheet (Figure 3). The interlocking tryptophan-zipper across the dimer interface involving conserved residues Trp223 and Tyr225 and reported earlier is also observed in the PXR/Adnectin-1 complexes (Figure 3c).^{20; 43}

Although structures of PXR in this crystal form have not been reported earlier, the overall orientation of the PXR dimer and the key intermolecular interactions between the PXR LBD in these two structures with Adnectin (PXR/Adnectin-1 and PXR/Adnectin-1/Compound-1) is similar to the interactions between PXR LBD reported earlier for 1NRL (Figure 3b).^{16; 20} The 1NRL is a complex of PXR with SRC-1 and a cholesterol lowering compound SR12813 that was reported in the $P2_12_12_1$ crystal form¹⁵. The superposition of the dimer of PXR LBDs excluding Adnectin-1 from PXR/Adnectin-1 structure and also excluding SRC-1 from 1NRL shows that the structures have the same conformation, with r.m.s. deviation of about 0.8Å between PXR/Adnectin-1 and 1NRL. The structure of PXR/Adnectin-1/Compound-1 was also superimposable with 1NRL and shows a r.m.s deviation of 0.9Å for the equivalent C α atoms.

Overall structure of PXR/Compound-1 and PXR/Adnectin-1 monomer

The PXR/Compound-1 complex crystallized similarly to the other structures reported earlier with 1 molecule of PXR LBD in the asymmetric unit (Table 2).^{14; 15; 16; 17; 18; 19; 44} The atomic model of PXR/Compound-1 contains residues 142-178, 197-309 and 314-431. Although PXR LBD was co expressed with co-activator fragment of SRC-1 (residues 623-710) to improve protein stability, the structure of SRC-1 was not visible in this complex.

The structures of PXR LBD monomer in complex with Compound-1 or Adnectin-1 show similar protein folds that include three sets of α -helical sandwich comprising $\alpha 1/\alpha 3$, $\alpha 4/\alpha 5/\alpha 8/\alpha 9$ and $\alpha 7/\alpha 10$ lined by a five-stranded antiparallel β -sheet comprising $\beta 2$, $\beta 3$, $\beta 4$, $\beta 1$ and $\beta 1'$ that frames a large ligand binding conformable cavity (Figure 4a). The PXR LBD contains an insert of ~50 residues (residues 177-228) comprising $\alpha 2$, $\beta 1$ and $\beta 1'$ that are unique to PXR and not observed in any other NR.^{19; 44; 45; 46} It is noteworthy that this PXR specific insert has a stretch of ~22 residues (residues 176-198) that is disordered in most other crystal structures,^{14; 15; 16; 17; 18; 19; 21; 44; 47} except for a SRC-1 bound structure where an additional six residues (192-197) are also visible (Figure 4a). However, in the structure of PXR/Adnectin-1/Compound-1 only 7 residues (179-185) of this region are missing. The lack of a major-symmetry related molecule in this region of the PXR suggests that it is unlikely a result of crystal packing interactions, but the high B factors observed for these residues (186-197) suggest that this region remains highly flexible.

The PXR LBD ends in a helix (α AF) that is involved in interactions with the transcriptional co-regulators including SRC-1. In the structures of PXR /Adnectin complexes, the Adnectin-1 binds to PXR at a site close to AF-2 helix (Figure 4a). The Adnectin binding site on PXR is ~15Å away from the ligand binding pocket and ~600 Å² of molecular surface are buried on each molecule (Figure 4a). The interface interactions between PXR and Adnectin-1 is driven by all three variable loops of Adnectin-1 (BC, DE and FG loops). The Adnectin-1 sequence has an insertion of 4 residues in the BC loop and 4 residues in the FG loop in addition to 9 extra residues at the N-terminus, relative to unmodified ¹⁰F_n3 (Figure 2a). To retain a consistent numbering scheme, residues were

given insertion letters analogous to those devised by Kabat, et al for antibody CDRs and used by others.^{34; 48}

Specific interactions of Adnectin-1 with PXR LBD.

The Adnectin-1 binds to PXR at a site close to AF-2 helix in both monomers of the PXR LBD homodimer (Figure 3a and Figure 4a). The interface is characterized by the presence of several polar and hydrophobic interactions (Figure 4b). The interface interactions between PXR and Adnectin-1 are driven by all three variable loops of Adnectin-1. The contribution towards the molecular interface in decreasing order of interactions are: DE loop ($\sim 230 \text{ \AA}^2$ of buried surface), the FG loop ($\sim 115 \text{ \AA}^2$), the BC loop ($\sim 140 \text{ \AA}^2$) and the D strand ($\sim 85 \text{ \AA}^2$). Five residues from the DE loop (Pro51, Tyr52, Trp53, Thr54 and Thr56), four from BC (Val26b, Glu26c, Thr28, Val29), two from FG (Tyr77 and Pro78) and three residues from D strand (Phe48, Thr49 and Val50) contact 21 residues from the PXR LBD. The residues of PXR LBD that are involved in contact with Adnectin-1 include Lys 160, Lys252, Ile255, Ser256, Lys259, Val260, Arg265, Ile269, Ile273, Leu276, Pro423, Leu424, Glu427 and Leu428.

A prominent feature of the interface interactions between PXR and Adnectin-1 is the extensive interactions of the DE loop with residues of the PXR LBD. The DE loop by itself contributes $> 1/3$ of the total buried surface area, with five of the six DE loop residues contributing to this interface. The side chains of Pro51, Tyr52 and Trp53 on Adnectin-1 insert deep into the pocket formed by the residues of $\alpha 3$ helix (Lys252, Ile255, Ser256, Lys259, Lys260), the loop connecting $\alpha 3$ with $\alpha 4$ (Arg265), residues of $\alpha 4$ (Ile269, Ile273, Leu276 and Lys277) and α -AF (Pro423, Leu424, Glu427 and Leu428). A comparison of the buried surface area contributions of the key residues of

PXR that are involved in binding to SRC-1 versus those involved in interactions with Adnectin-1 shows that these interactions are not only conserved at the secondary structural level involving residues from $\alpha 3$, $\alpha 4$ and αAF , but that the individual residues and their surface contributions are also conserved (Table 3). The two largest contributors from $\alpha 3$ (Ile255 and Lys259), $\alpha 4$ (Ile273 and Ile269) and αAF (Leu424, Glu-427) are the same for both SRC-1 and Adnectin-1 interfaces in their co-structures with PXR.

Comparison of Adnectin-1 versus SRC-1 interactions with PXR.

Crystal structure comparison of Adnectin-1 binding to PXR LBD against SRC-1 shows that both Adnectin-1 and SRC-1 compete for the same site on the surface of PXR. (Figure 5a). However, in contrast to the smaller interface of SRC-1 with PXR LBD ($\sim 420 \text{\AA}^2$), the Adnectin-1 has a relatively larger interface ($\sim 600 \text{\AA}^2$) (Figure 5c-d). The competitive binding of Adnectin-1 was also observed during purification of the ternary complex of PXR LBD with SRC-1 and Adnectin-1. Mixture of Adnectin-1 with the binary complex of co-expressed PXR LBD/SRC1 leads to displacement of SRC-1 from the mixture (Figure 2b-c). It had been reported earlier that the C-terminal 7 residues of SRC-1 (residues 685-691) has a LxxLL motif that forms an α -helix that binds to a groove on the surface of PXR composed of residues from $\alpha 3$, $\alpha 4$ and α -AF (Figure 4c and Figure 5d)²⁰. A detailed comparison of the molecular interactions at the PXR/Adnectin-1 interface with the interaction of the LHRLL-helix of SRC-1 shows that most of the residues coming from $\alpha 3$, $\alpha 4$ and α -AF of the PXR LBD that are involved in binding to BC, DE and FG loops of Adnectin-1 are also involved in the binding of the LxxLL motif of SRC-1 (Table 3; Figure 4b-c). The PXR residues which are common to interactions with both Adnectin-1 and SRC-1 include: Ile255, Lys252 and Lys259 (from $\alpha 3$), Ile269,

Glu270, Gln272, Ile273, Leu276 and Lys277 (from $\alpha 4$) and residues Pro423, Leu424, Glu427 and Leu428 (from αAF). It is noteworthy that the two major contributors of buried interface of SRC-1, Leu690 and Leu694, are analogously situated to two major contributors, Trp53 and Tyr52, of the DE loop of Adnectin-1 (Figure 5b). A summary of the residues of PXR involved in binding and their surface contributions are summarized in Table 3.

The only two polar contacts between PXR and SRC-1 involve residues Glu427 and Lys259, which are highly conserved in other nuclear receptors and form a charge clamp that plays an important role in nuclear-transcriptional co-activator interactions.^{20; 24; 49; 50; 51} These residues are also involved in interactions with Adnectin-1. The Glu427 forms contact with the side chain nitrogen on the indole of Trp53 and the side chain of Thr54 (both in the DE loop) and Lys259 of PXR α -3 form polar contacts with the backbone carbonyl atoms of Glu26C and Val27 (both from the BC loop). In short, the stabilization of AF-2 helix in the active conformation that forms a charge clamp pocket to facilitate binding with the co-activator helix of SRC-1 is also conserved when PXR is bound to Adnectin-1.

Binding mode of Compound-1

Binding of Adnectin-1 does not alter the overall protein conformation of PXR or change interactions with the small molecule in the ligand binding pocket. The structure of PXR/Adnectin-1 is similar to the structure of PXR/Compound-1 and PXR/Adnectin-1/Compound-1, with a r.m.s. deviation of 0.8Å and 0.5Å over all C $^{\alpha}$ atoms, respectively (Supplemental Figure S2). The structure of PXR/Adnectin-1 complex is also similar to the structure of unliganded PXR (PDB ID 1ILG) determined earlier¹⁴, with a r.m.s

deviation of 1.3 Å over all C^α atoms (Supplemental Figure S3). In the binary complex of PXR/Compound-1 and the ternary complex of PXR/Adnectin-1/Compound-1, Compound-1 has clearly defined electron density and is bound to the PXR LBD in a single orientation (Figure 6a). Compound-1 forms 2 polar contacts and 15 van der Waals contacts with amino acid side chains that line the ligand binding pocket (Figure 6b-c). The 2 direct polar contacts between Compound-1 and the PXR protein chain involve hydrogen bonds with the side chains Gln285 and His407. In addition three more hydrogen bonds involve interactions with water molecules in the ligand binding pocket. The side chain of His407 and a water molecule are positioned 2.8 Å from the 4-hydroxy group on the piperidine of Compound-1. The piperidine amide carbonyl of Compound-1 forms the second polar contact at 3.1 Å with Gln285; this carbonyl oxygen atom is also hydrogen bonded to neighboring water. The carbonyl oxygen of cyclopropyl acetamide is hydrogen bonded to a water molecule. The cyclopropylacetamide of Compound-1 is lodged in a tight pocket formed by Phe288, Trp299, Tyr306 and Cys201, such that the cyclopropyl of Compound-1 is sandwiched between Phe288 and Trp299 in parallel aromatic stacking interactions and an edge-to-face stacking contact with Tyr306. The chlorophenyl of Compound-1 forms a 3.8 Å interaction with Met-425, which is located on αAF and likely stabilizes the active conformation of the AF-2 surface for co-activator binding.

A detailed comparison of the interactions at the ligand binding pocket of the three structures shows that while most of the residues in the binding pocket align well, the noteworthy exceptions are the side chain conformations of Phe420, Leu206 and Leu240 observed in PXR/Adnectin-1 structure (Figure 6d). To accommodate the chlorophenyl

group of the Compound-1, the side chain of Phe420 in both compound-bound structures (PXR/Compound-1 and PXR/Compound-1/Adnectin-1) flips and adopts a rotamer conformation that is almost 120° to the conformation observed in the structure of PXR with Adnectin by itself (PXR/Adnectin-1). In addition the side chains of Leu206 and Leu240 move by 1.5Å and 1.2Å closer to the position of Compound-1 in the two Compound-1 structures (Figure 6d).

Modelling studies and modulation of PXR Affinity for CCR1 antagonist

The compound within the series exemplified by Compound-1 are very potent and selective CCR1 antagonists with good efficacy in chemotaxis assays⁵². However, these compounds were found to activate PXR at low micromolar concentrations, hindering their ability to progress as preclinical candidates. To address this issue, the PXR/Compound-1 crystal structure was utilized in the design of compounds predicted to lower PXR activity. The ligand binding site was analyzed using the program Sitemap (Sitemap, version 2.6, Schrodinger LLC, New York, NY, 2012) to determine the hydrophobic and hydrophilic characteristics of the sub-pockets within the site. A van der Waals surface was also generated to assess the size of the site. The Sitemap calculation revealed significant hydrophobic density surrounding the cyclopropyl methyl amide, isopropyl and chlorophenyl moieties of Compound-1 with no hydrophilic density in these regions (Figure 7a). In addition to the hydrophobic character of these subsites, the surface representation showed close packing between the protein, the cyclopropyl methyl amide and the isopropyl groups (Figure 7b). Collectively these findings suggested that addition of bulk and/or polarity to either the terminal amide or the isopropyl moiety would reduce PXR activity. Previous CCR1 structure-activity relationship (SAR) had shown that the

receptor was intolerant to polar substitution on the isopropyl moiety, thus focusing our efforts on replacement of the terminal amide cyclopropyl group.

Two strategies were explored in an effort to find groups that would be incompatible with PXR binding: specific placement of polar groups to create unfavorable interactions and rigidifying the terminal amide such that it creates steric clashes. The successful implementation of the first strategy resulted in BMS-776456 (Figure 7c-d), a single digit nanomolar CCR1 antagonist and an early pre-clinical candidate. Incorporation of a cyclopentyl amide forced the *trans* hydroxyl group into contact with the side chains of F288 and W299 (Figure 7d), resulting is a substantial reduction of PXR activity_{ENREF_41}. The second strategy was achieved by exchanging the terminal amide for a urea moiety, resulting in elongation of the group and increased conformational rigidity, thereby making the group unable to fit within the PXR ligand binding site. This series showed substantially reduced PXR activity and led to BMS-817399, a clinical candidate, which is completely inactive against PXR. The lack of activity can be explained structurally as there are no low energy conformations of BMS-817399 that do not produce significant steric clashes with the PXR binding site (Figure 7e).

Discussion

PXR activation upregulates cellular levels of several drug metabolizing enzymes such as cytochrome P450 (CYP) enzymes and MDR1. The increased expression of CYP enzymes can alter drug pharmacokinetics and lead to dangerous drug-drug interactions including loss of therapeutic efficacy and increased toxicity. To avoid late stage clinical failures

and high costs associated with bringing a new drug to market, many pharmaceutical companies have adopted screening assays for early detection of compounds that activate PXR.^{53; 54; 55} Additionally, *in silico* screening using known crystal structures of PXR is increasingly being used to predict potential PXR activity.⁵⁶ The large and flexible ligand binding pocket of PXR and the potential of compounds to bind to different locations and in multiple orientations within the ligand binding cavity of PXR complicate reliable prediction of PXR activity. This is especially true for more advanced compounds/chemotypes with desirable efficacy, selectivity and bioavailability towards the intended therapeutic target but with known PXR liability. Given these limitations, a co-crystal structure is often required to define exact binding interactions and to suggest specific modifications that can disturb crucial interactions related to PXR binding while maintaining activity against the primary target. In our CCR1 program, the information from the crystal structures of PXR with Compound-1 (Figure 7) and another related compound published recently were used to understand the lack of PXR activity for other compounds including BMS-776457 and BMS-817399, leading to the design of additional PXR inactive analogs which retained CCR1 activity, selectivity and oral bioavailability.^{40; 41} BMS-817399 has entered into Phase II human clinical trials for the treatment of rheumatoid arthritis.

Although the above work is a good example of using tools of crystallography to help mitigate PXR activity for a CCR1 inhibitor, the method is not always straightforward due to challenges in generating structures of PXR with compounds for other therapeutic targets. The large and flexible binding pocket of PXR further complicates the process of obtaining co-crystal structures of compounds. As not all

compounds that show PXR activity in screening assays will be tight binders of PXR, even some weak binders could elicit CYP activity and thus still be a liability. These compounds, when tried in crystallization, either do not show electron density for the compound, or show weak and ambiguous electron density which makes it difficult to predict the binding pose. This ambiguity limits the ability of structural biology to provide a direct structure-guided approach for modulating PXR affinity.

In order to investigate further the interface interactions of SRC-1 with the α AF of PXR ligand binding domain and to increase our likelihood of crystallization success, mRNA display technology³⁹ was used to identify Adnectins targeting the PXR interactions with the SRC-1 peptide to stabilize PXR and to facilitate co-crystallization of PXR with our proprietary compounds. The structure of PXR with Adnectin-1 clearly shows that the exclusion of SRC-1 from the complex of PXR, Adnectin-1 and SRC-1 was due to direct competition of Adnectin-1 with SRC-1 for binding to the remarkably similar region of PXR LBD (Figure 4 and Figure 5; Table 3).

Our structural studies of PXR/Adnectin complexes show that binding of Adnectin-1 does not change the overall conformation of the PXR LBD nor does it change the conformation of the residues lining the ligand binding pocket (Figure 6). The structures of the binary complex of PXR/Compound-1 and ternary complex of PXR/Adnectin-1/Compound-1 are essentially superimposable with no notable changes in the main-chain and side-chain conformations of the residues lining the ligand binding pocket (Figure 6c and Supplemental Figure S2). However, some movement of ligand binding residues is observed when the two Compound-1 bound structures are compared to the apo structure of PXR/Adnectin-1 complex (Figure 6d). The notable changes in

PXR/Adnectin-1 apo structure include the 120° flip of the side chain of Phe420 and the outward movement of the main chain of residues 205-211 by 1.2 Å. This finding reinforces the belief that replacing the natural binding partner SRC-1 with a bulkier and tight-binding Adnectin-1 does not rigidify the movement of the residues lining the ligand binding pocket; rather, the side chain residues remain flexible and can interact with ligand.

A comparison of the overall dimer of PXR/Adnectin complexes with the dimer of PXR/SRC-1/SR12813 reported earlier also reveals a similar orientation of the two molecules of PXR in the complex (Figure 3a-b). The dimer interface created by antiparallel association of $\beta 1'$ strands of each monomer generate 10 stranded antiparallel β -sheets (Figure 3). Earlier work by Noble, *et al*, has stressed the importance of residues lining the dimer interface where they studied the effect of mutagenesis of two key residues of the tryptophan zipper (Trp223Ala and Tyr225Ala) at the dimer interface⁴³. Mutagenesis of these residues results in a monomer of PXR and affects the response of full length PXR to agonist rifampicin and SR12813, but this does not prevent full length PXR from entering into the nucleus and binding to ligands or RXR α . However, mutagenesis of these residues does prevent PXR from associating with SRC-1 co-activator.⁴³ This tryptophan zipper previously reported in 1NRL was thought to have physiological relevance, and its presence in the PXR/Adnectin co-structures gives us additional confidence of the validity of PXR/Adnectin structures being a suitable surrogate for PXR/SRC-1 co-structures (Figure 3c).

Earlier reports comparing PXR structures with other nuclear receptors have all pointed to the presence of an extra 50 residue insertion (residues 177-228) observed in

PXR that is not present in any other nuclear receptor of this superfamily. This insertion comprises $\alpha 2$, $\beta 1$ and $\beta 1'$ that are unique to PXR (Figure 3). This insertion extends the β sheets of PXR to five strands and forms a novel $\alpha 2$ helix (residues 192-210) that forms the base of the conformable ligand binding pocket. The $\alpha 2$ helix is mobile and was reported as both pseudo helical in most structures and truly helical in two other structures including the SRC-1 bound PXR/SR12813 structure²⁰. The uniqueness and mobility of this region of the receptor, its involvement in ligand binding, plus an earlier hypothesis of $\alpha 2$ acting as some kind of trap door guiding movement of ligands into and out of the ligand binding pocket, has pointed to this region being an important determinant for expansion and promiscuity of the PXR ligand binding pocket. It is therefore interesting to observe that a portion of this insert comprising residues 178-198 is disordered in all PXR structures reported to date, except the ternary complex of PXR/SR12813 and SRC-1 co-activator peptide, where the additional six residues [192-197] were traced. However, in the PXR/Adnectin-1 structure reported here, 13 additional residues (185-199) when compared to most PXR structures and 7 additional residues (185-191) compared to 1NRL are visible. This region is not involved in any major crystal packing interactions, which supports the true representation of this region of the molecule in this structure.

Also, the other mobile regions of the PXR, including 229-235 and 310-314, which are reportedly disordered in many other PXR structures, are visible in the PXR/Adnectin-1 structure. The structure of PXR/Adnectin-1/Compound-1 complex reported here is the most complete structure of PXR for which most of the core residues of PXR LBD were resolved. Redinbo and co-workers, when reporting 1NRL, had argued that the binding of SRC-1 had restricted the breathing space for ligand binding residues and helped trap the

single conformation of the SR12813.²⁰ It is therefore not surprising that an Adnectin targeting the PXR/SRC-1 interface displaces SRC-1 while maintaining ligand binding.

In summary, we report here the first direct example of the use of structural information from a related compound to modulate PXR affinity for a CCR1 antagonist that had entered clinical trials for rheumatoid arthritis. It is also the first report of a crystal structure of PXR in presence of a biologic (Adnectin-1) that was used as a chaperone, displacing SRC-1 and binding to the same set of residues that are involved in SRC-1 interactions with PXR. The PXR/Adnectin complex structure reported here is the most complete structure of PXR reported to date in which most residues in the structure have been accounted. Our results provide new methodology that can potentially be used for generating structures of difficult to crystallize compounds with PXR.

Experimental Procedures.

Protein expression and purification.

The cloning and expression of PXR LBD and SRC-1 coactivator peptide was analogous to that described earlier.¹⁴ The human PXR LBD (residues 130-434) was subcloned into the pCO7⁵⁷ vector with a His₆ tag at the N-terminus. The SRC-1 (residue 623-710) was subcloned in pET28a vector (Novagen). The PXR LBD was coexpressed with SRC-1 fragment in *Escherichia coli* BL21(DE3) at 20 °C. The soluble complex of PXR LBD and SRC-1 was purified by nickel-agarose affinity chromatography followed by gel-filtration chromatography (Superdex 75). The His₆ fusion tag was not removed during purification. The protein was concentrated to 1.2 mg ml⁻¹ in a buffer containing 20

mM Tris (pH 8.0), 100 mM NaCl, 5 mM DTT, 1 mM EDTA and 6% (v/v) glycerol and stored at -80 °C.

Adnectins were cloned in pET9D vector (New England Biolabs) and expressed in *Escherichia coli* BL21(DE3) at 20 °C. The lysates were purified in a single step using Ni-agarose affinity chromatography. For preparation of PXR LBD/Adnectin complexes, a 4 fold molar excess of Adnectins was mixed with PXR/SRC-1 protein and the mixture was incubated on ice for 5 hours. The mixture was passed over a Superdex 75 column equilibrated with 20mM Tris pH 8.0, 100mM NaCl, 5mM DTT, 1mM EDTA and 6% (v/v) glycerol. SDS-PAGE analysis of the 3 eluted peaks indicated that peak 1 contained PXR and Adnectin-1 complex (Figure 2c). Peak 1 fractions were concentrated to 4.8 mg/ml, flash frozen in liquid nitrogen and stored at -80°C.

Surface Plasmon Resonance Determination of Adnectin Binding Constants

The affinity (K_D) values for Adnectins binding to PXR were determined by surface plasmon resonance (SPR) on a Biacore T100 instrument (GE Healthcare), by injecting a concentration series of the Adnectin over human PXR-GST (Invitrogen) that had been captured on the chip surface with an immobilized anti-GST antibody (GE Healthcare). Regeneration of the chip surface between kinetic cycles was performed using 10mM Glycine, pH 2. Kinetic parameters were calculated using Biacore T100 evaluation software and a 1:1 binding model.

Protein crystallization.

Crystals of PXR/SRC-1/Compound-1 complex were obtained by first growing crystals of apo PXR/SRC-1 in 15 well Qiagen trays by hanging drop vapor diffusion method at 20 °C. The PXR/SRC-1 complex was concentrated to 3mg/ml and equilibrated

against a reservoir solution containing 100 mM imidazole buffer (pH 7.8) and 10% (v/v) isopropanol. Crystals of PXR/SRC-1 complex were obtained after 48 hours. The crystals were transferred to a fresh drop of the same well solution containing 5mM Compound-1 and soaked overnight at 20°C. Crystals were cryoprotected in 32% (v/v) ethylene glycol and harvested by flash-cooling in liquid nitrogen.

Crystals of the ternary complex of PXR/Adnectin-1/Compound-1 were obtained by mixing 5 fold molar excess of Compound-1 to the complex of PXR/Adnectin-1. The crystals were grown by the sitting drop vapor diffusion method at 20°C. The crystals were obtained by equilibrating the complex against a reservoir solution containing 100mM Tris pH 7.5, 10% (v/v) isopropanol, 3.5% (v/v) 2-methyl-2, 4-pentanediol (MPD). Crystals were harvested after 10 days by flash-cooling in liquid nitrogen using Paratone-N as a cryoprotectant.

Crystals of PXR/Adnectin-1 were obtained by hanging drop vapor diffusion method at 12°C by equilibrating the complex against a reservoir solution containing 100mM HEPES pH 7.0, 18.3% (v/v) 1,6-hexanediol and 3.0% (v/v) 2-methyl-2,4-pentanediol(MPD). Crystals were harvested by flash-cooling in liquid nitrogen using mother liquor supplemented with 21% (v/v) 1, 6-hexane diol and 3% (v/v) MPD as a cryoprotectant.

Data collection and processing.

Data for the PXR/SRC-1/Compound-1 were collected at beamline 17BM at IMCA-CAT of the Advanced Photon Source at Argonne National Laboratory. The wavelength used was 1.00 Å and the detector was a MAR-165 CCD. The diffraction images were indexed, integrated, and scaled with HKL2000 (Otwinowski & Minor,

1997). Data for PXR/Adnectin-1/Compound-1 and PXR/Adnectin-1 were collected at beamline 17ID at IMCA-CAT of the Advanced Photon Source at Argonne National Laboratory. The wavelength used was 1.0 Å and the detector was a DECTRIS PILATUS 6M. Data were indexed, integrated, and scaled with D*TREK.⁵⁸ Space group, unit cell parameters and data collection statistics for all three data sets are listed in Table 2.

Structure determination and refinement.

The structure of PXR/Adnectin-1/Compound-1 was determined by molecular replacement using the program PHASER⁵⁹ with a homology model of PXR based on 2O9I¹⁷ as a search model. The model for the Adnectin was derived from PDB 1FNF⁶⁰ by deleting the BC, EF, and FG loops. The structure was examined and manually fitted using the program COOT⁶¹. The location of ligands in the PXR ligand binding site was easily identified by inspection of electron density maps. The ligand was automatically fit into the ligand density using the program AFITT (Open Eye Scientific software, Inc., Santa Fe, NM, USA) and refined using the program autoBUSTER (Global Phasing LTD, Cambridge, UK). Refinement statistics are listed in Table 2.⁶² Display graphics were produced with PyMOL v1.4 (Schrödinger, LLC). Buried surface area was calculated with the program MS⁶³ using a 1.7 Å probe sphere^{64; 65}.

BMS-817399 Conformational Generation and Compound Docking.

Conformations were generated using the Mixed Torsion/Low Mode method in Macromodel (Macromodel v. 9.9, Schrodinger LLC, New York, NY) and the OPLS-2.0 forcefield. All atoms were fixed except the terminal amino moiety, allowing free rotation of that group. Conformations within 10 kcal/mol of the global minimum were retained.

Each conformation of BMS-817399 was placed by superposition of the chlorophenyl-piperidine onto the crystallographic position of Compound-1.

Accession Numbers. Coordinates and structure amplitudes will be deposited in the RCSB Protein Data Bank under ID codes ### (PXR/Adnectin 1), ### (PXR/Adnectin 1/Compound-1) and ### (PXR/Compound-1).

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Table 1. SPR data for direct binding of Adnectins to immobilized PXR-GST at 25°C.

Adnectin	ka (1/Ms*10e4)	kd (1/s*10e-4)	KD (nM)
Adnectin-1	10.3 ± 0.9	11.7 ± 0.3	11.4 ± 0.7
Adnectin-2	4.7 ± 0.4	43.6 ± 4.6	93.7 ± 7.7
Adnectin-3	17.1 ± 5.0	3.7 ± 0.3	2.3 ± 0.8
Adnectin-4	2.2 ± 0.2	1.4 ± 0.4	6.3 ± 2.2
Adnectin-5	48.4 ± 8.6	26.5 ± 1.8	5.6 ± 0.5
Adnectin-6	67.0 ± 12.1	11.9 ± 1.7	1.8 ± 0.2
Adnectin-7	22.8 ± 10.4	1.0 ± 0.2	0.6 ± 0.5
Adnectin-8	4.1 ± 12.1	1.8 ± 0.2	4.7 ± 0.5

The 1:1 binding model was used to obtain the K_D values, n=3 for each Adnectin sample, ± standard error.

Table 2. Summary of crystallographic information

	PXR LBD + Compound-1	PXR LBD +Adnectin-1	PXR LBD + Adnectin-1 + Compound-1
Data collection			
Space group	<i>P4</i> ₃ <i>2</i> ₁ <i>2</i>	<i>P4</i>	<i>P4</i>
Cell dimensions			
<i>a</i> , <i>b</i> , <i>c</i> (Å)	91.5, 91.5, 85.6	119.2, 119.2, 83.7	119.8, 119.8, 84.1
α , β , γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	45-2.4	35-2.8	42-3.1
<i>R</i> _{merge} (%)	10.1 (68.5)	3.8 (56.6)	8.1 (47.2)
<i>I</i> / σI	20.9 (3.4)	26 (2.7)	13.5 (3.9)
Completeness (%)	99 (99)	100 (100)	99 (99)
Redundancy	10.4 (10.6)	6.8 (6.9)	6.6 (6.3)
Refinement			
Resolution (Å)	45-2.4	35-2.8	42.3-3.1
Number of reflections	13,906	29,083	20,742
<i>R</i> _{work} / <i>R</i> _{free} (%)	19.7 / 24.2	21.6 / 22.9	21.5 / 24.7
No. atoms			
Protein	2180	5,625	5,735
Ligand/ion	35	—	58
Water	68	—	—
<i>B</i> -factors (Å ²)			
Protein	45.0	92.9	92.9
Ligand/ion	35.9	—	93.6
Water	44.7	—	—

R.m.s. deviations			
Bond lengths (Å)	0.010	0.010	0.010
Bond angles (°)	1.07	1.21	1.27
Ramachandran Plot Stats			
Most allowed (%)	96.2	97.9	97.7
Additional allowed (%)	3.1	1.3	1.5
Outliers (%)	0.7	0.8	0.8

^aAs defined by Laskowski *et al.*, 1993.

The numbers in parentheses are for the highest-resolution shell. One crystal was used for each data collection.

Table 3. Comparison of key residues of PXR involved in interface interactions with Adnectin-1 and SRC-1

Residue Number	PXR/Adnectin-1 Interface			PXR/SRC-1 Interface		
	Main Chain($\text{\AA}^2$)	Side Chain($\text{\AA}^2$)	Total($\text{\AA}^2$)	Main chain($\text{\AA}^2$)	Side Chain($\text{\AA}^2$)	Total($\text{\AA}^2$)
$\alpha 3$						
Lys252	10	15	25	10	25	35
Ile255	0	50	50	0	50	50
Ser256	15	20	35	0	10	10
Lys259	10	70	80	0	50	50
Val260	10	30	40	0	0	0
Arg265	0	40	40	0	0	0
$\alpha 4$						
Ile269	0	30	30	0	35	35
Gln272	5	15	20	0	20	20
Ile273	10	40	50	5	50	55
Leu276	0	25	25	0	35	35
Lys277	0	15	15	0	10	10
αAF						
Pro423	10	35	45	5	15	20
Leu424	10	45	55	5	40	45
Glu427	5	50	55	0	45	45
Leu428	0	20	20	0	15	15

Area: calculated by the method of Connolly¹ and rounded to the nearest 5 $\text{\AA}^2$.

Figure legends

Figure 1: A comparison of antibody, antibody fragment (V_H) and Adnectin scaffolds (a) Cartoon representation of IgG1 structure (PDB; 1IGT), heavy chain is shown in blue, light chain in red, CDRs in orange. (b) The V_H scaffold with CDRs. (c) The Adnectin scaffold with the diversified loops. The CDRs of V_H and corresponding loops of Adnectin are shown in orange.

Figure 2: Sequence alignment and biophysical measurements of Adnectin-1 (a) Sequence alignment of Adnectin-1 and Adnectin-8 with the parent $^{10}\text{Fn}^3$ domain. Secondary structure is labeled below the alignment; diversified loops (BC, DE and FG) are shown in red, disordered residues from the Adnectin-1 structure are shown in lowercase blue. (b) Elution profile from gel filtration column (SuperdexTM 75) of the complex of PXR/SRC-1 mixed with purified Adnectin-1 (4-fold molar excess), three different peaks corresponding to PXR/Adnectin-1 (Peak-1), PXR/SRC-1 (Peak-2) and Adnectin1 (Peak-3) are obtained. (c) A SDS/PAGE gel of peak 1-3, showing displacement of SRC-1 by Adnectin-1. (d) A SDS/PAGE gel showing no displacement of SRC-1 by Adnectin-8. (e) Various concentrations of Adnectin-1 from 4.1 nM (light blue) to 1 μM (magenta) were injected over a surface composed of PXR-GST captured by an anti-GST antibody. The sensorgrams have been triple referenced.

Figure 3: Comparison of dimer of PXR/Adnectin-1 complex versus PXR/SRC-1 complex (a) Crystal structure of the homodimer in the asymmetric unit of PXR/Adnectin-1/Compound-1 ternary complex. One monomer of PXR LBD is shown in cyan (Mol-1), the other monomer of PXR LBD is in green (Mol-2). The Adnectin associated with Mol-1 is shown magenta; the other Adnectin associated with Mol-2 is in orange. Carbon

atoms of Compound-1 are shown as sticks in orange; nitrogen atoms are shown in blue, oxygen atoms are shown in red, and chlorine atom in splitpea green (b) Structure of ternary complex of PXR with SRC-1 and SR12813 (PDB: 1NRL), the SRC-1 associated with Mol-1 is shown magenta; the other SRC-1 associated with Mol-2 is in orange; the dimer of PXR is shown using same color and orientation as in **a**. (c) Close-up view of the superposed PXR LBD homodimer interface, the $\beta 1'$ strands of PXR LBD from PXR/Adnectin-1/Compound-1 ternary complex are shown as cyan (Mol-1) and Green (Mol-2) superposed with $\beta 1'$ from 1NRL (pink). The interlocking Trp223 and Tyr225 from each monomer are shown as sticks, other residues are shown as lines, and hydrogen bonds are indicated (black dash).

Figure 4: Structure of PXR/Adnectin-1 monomer and comparison with SRC-1 interface (a) PXR monomer in complex with Adnectin-1 and Compound-1. PXR is shown in cyan; Adnectin-1 in magenta, Compound-1 as orange sticks, the residues 185-198 that are disordered in other PXR structures are shown in yellow. (b) Close-up of the interface interactions between PXR and Adnectin-1 (c) Close-up of the interface interactions between PXR and SRC-1, using same orientation as **b**., PXR is shown in cyan; SRC-1 is in green.

Figure 5: Competition between Adnectin-1 and SRC-1 for binding to PXR (a) Overlap of the Adnectin-1 and SRC-1 contact surfaces on PXR ligand binding domain is shown. Adnectin-1 (magenta) and SRC-1(yellow) are shown as cartoons. The unique and overlapping contacting surface of PXR are shown as, Adnectin-1(cyan), SRC-1 (red) and for both(blue), the remaining residues of PXR not involved in interface interactions with either Adnectin-1 or SRC-1 are shown as white. (b) Close-up comparison of the key

residue involved in interface interactions; DE loop of Adnectin-1 is in magenta, SRC-1 in yellow, and PXR in blue. (c) Close-up of the PXR and Adnectin-1 interface interactions shown in **a**. The PXR surface residues contributing to the interface with Adnectin-1 are shown in cyan, the remaining residues of PXR are shown as white. The side-chain of the key residues from the diversified loops of Adnectin-1 (BC, DE and FG) that are at the interface is shown as magenta sticks. (d) Close-up of the PXR and SRC-1 interface interactions shown in **a**. The PXR surface residues contributing to interface interaction with SRC-1 are shown in red, the remaining residues of PXR are shown as white. The side-chain of the LHRLL motif of SRC-1 is shown as sticks (yellow).

Figure 6: Binding mode of Compound-1 and ligand binding pocket of PXR. (a) Final 2Fo-Fc electron density map for Compound-1 at 2.4Å resolution (contour level is 1 r.m.s.d.). (b) Schematic drawing of the interactions of Compound-1 and PXR. Water molecules are shown as W in red and hydrogen bonding interactions are shown as red dashed lines. (c) Stereo diagram showing superimposition of the structures of PXR/Compound-1(yellow), PXR/Adnectin-1/Compound-1 (cyan). (d) Sterodialogram showing structural comparison at the ligand binding pocket, between PXR/Compound-1(yellow), PXR/Adnectin-1/Compound-1(Cyan) and PXR/Adnectin-1(red) , the water molecules in PXR/Compound-1 structure are shown as red spheres.

Figure 7: Modeling studies of selected CCR1 compounds in PXR. (a) Sitemap density maps of the ligand binding site from the PXR/Compound-1 crystal structure. The grey and red mesh denote regions that are predicted to preferentially bind hydrophobic groups and hydrophilic groups, respectively. (b) Van-der-Walls surface of the ligand binding site. (c) Two dimensional representations of Compound-1, BMS-776457 and BMS-

817399. (d) BMS-776457 docked into the PXR ligand binding site. Residues that form unfavorable interactions with the hydroxyl are shown in sticks. (e) Placement of BMS-817399 low energy conformations into the PXR ligand binding site. Residues which form steric clashes with one or more conformations of BMS-817399 are shown as thin green sticks.

(a)

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Figure. 1

(a)

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Figure. 2

Figure. 3

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Figure. 4

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Figure. 5

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Figure. 6

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(e)

Figure. 7