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REVIEW

Nuclear receptor ligand-binding domains: three-dimensional structures, molecular interactions and pharmacological implications

William Bourguet, Pierre Germain and Hinrich Gronemeyer

Nuclear receptors are members of a large family of ligand-inducible transcription factors that regulate gene programs underlying a plethora of (patho)physiological phenomena. The recent determination of the crystal structures of nuclear receptor ligand-binding domains has provided an extremely detailed insight into the intra- and intermolecular mechanisms that constitute the initial events of receptor activation and signal transduction. Here, a comprehensive mechanistic view of agonist and antagonist action will be presented. Furthermore, the novel class of partial agonists–antagonists will be described and the multiple challenges and novel perspectives for nuclear-receptor-based drug design will be discussed.

Multicellular organisms require a specific intercellular communication to organize the complex body plan properly during embryogenesis and maintain its physiological properties and functions throughout life. Although growth factors, neurotransmitters and peptide hormones bind to membrane receptors thereby inducing the activity of intracellular signalling pathways, other small hydrophobic signalling molecules such as steroid hormones, certain vitamins and metabolic intermediates enter target cells and bind to cognate members of a large family of nuclear receptors. Nuclear receptors are of major importance for intercellular signalling in animals because they converge different intra- and extracellular signals on the regulation of genetic programs. Such nuclear receptors are transcription factors that: (1) respond directly through physical association with a large variety of hormonal and metabolic signals; (2) integrate diverse signalling pathways because they correspond themselves to targets of post-translational modifications; and (3) regulate the activities of other major signalling cascades (commonly referred to as 'signal transduction crosstalk'). The genetic programs that these receptors establish or modify affect virtually all aspects of the life of multicellular organisms, covering such diverse aspects as, for example, embryogenesis, homeostasis, reproduction, cell growth or death. Their gene-regulatory power and selectivity has prompted intense research on these key factors, which is now starting to decipher the complex network of molecular events that account for their capacity to regulate transcription. The study of these molecular processes also sheds light on general mecha-nisms of transcription regulation, and it will be a future challenge to uncover the molecular rules that define spatial and temporal control of gene expression.

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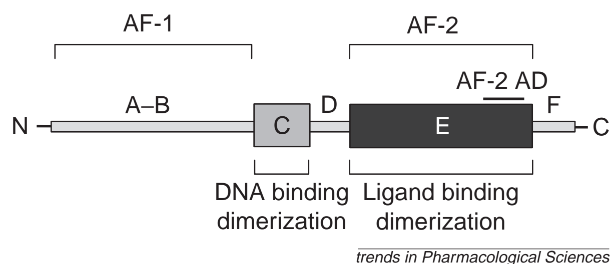


Fig. 1. Structural and functional organization of nuclear receptors. Nuclear receptors consist of six domains (A–F) based on regions of conserved sequence and function. The DNA-binding domain (DBD; region C) is the most highly conserved domain and encodes two zinc finger modules. The ligand-binding domain (LBD; region E) is less conserved and mediates ligand binding, dimerization and a ligand-dependent transactivation function, termed AF-2. Within the AF-2, the integrity of a conserved amphipathic α-helix termed AF-2 activation domain (AD) has been shown to be required for ligand-dependent transactivation. The N-terminal A–B region contains a cell- and promoter-specific transactivation function termed AF-1. The region D is considered as a hinge domain. The F region is not present in all receptors and its function is poorly understood.

All nuclear receptors are modular proteins (Fig. 1) that harbour one DNA-binding domain and one ligand-binding domain (LBD). The LBD also comprises the ligand-dependent activation function 2 (AF-2) whereas the activation function 1 (AF-1) operates autonomously and in a ligand-independent manner when placed outside of the receptor. However, in the context of its own receptor, the activity of AF-1 is also controlled by the cognate ligand (for further details see, for example, Ref. 1). Nuclear receptors act as agonist-induced factors that enhance the transcription of their target genes, and certain nuclear receptors, such as thyroid and retinoic acid receptors, can act as silencers of transcription in the absence of ligands or in the presence of certain antagonists. The silencing activity of nuclear receptors is due to their ability to recruit co-repressors and establish, by virtue of this interaction, a co-repressor complex at the promoters of target genes, which can affect the chromatin structure as a result of the associated histone deacetylase (HDAC) activity of the receptor[2]. The subsequent condensation of chromatin is believed to cause the gene repression. By contrast, agonists induce a change in the structure of the receptor that allows nuclear receptors to establish a co-activator complex that can acetylate histones, which is believed to prepare target gene promoters for transactivation by decondensation of the corresponding chromatin[2,3]. Following decondensation of chromatin, a second complex, variously termed TRAP (thyroid receptor-associated proteins) or DRIP (vitamin D receptor-interacting proteins), appears to take over and establish the link to the basal transcriptional machinery, which results in activation of the target gene[3,4].

The initial steps of ligand action, the mechanistic and molecular details of agonism, antagonism and partial agonism–antagonism will be discussed, and possibilities, challenges and perspectives for nuclear-receptor-based drug design will be presented.

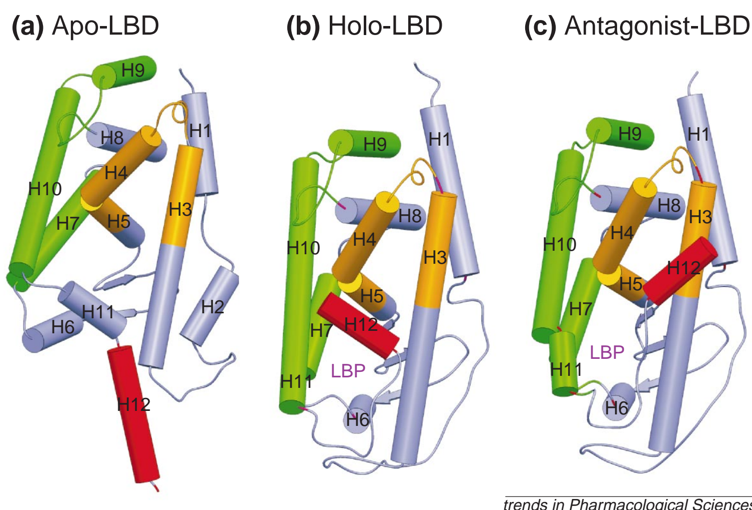


Fig. 2. Schematic drawing of three different conformational states of nuclear receptor ligand-binding domains (LBDs). **(a)** The unliganded (apo) retinoid X receptor (RXR) LBD. **(b)** The agonist-bound (holo) retinoic acid receptor (RAR) LBD. **(c)** The antagonist-bound RAR LBD. The α-helices (H1–H12) are depicted as rods whereas broad arrows represent the β-turn. The various regions of the LBD are coloured depending on their function: the dimerization surface is shown in green, the co-activator and co-repressor binding site, which also encompasses the nuclear receptor LBD signature motif[6], is shown in orange and the activation helix H12 that harbours the residues of the core activation function 2 (AF-2) activation domain (AD) is shown in red; other structural elements are shown in mauve. Abbreviation: LBP, ligand-binding pocket.

Allosteric effects induced by agonists

It is well known that ligand binding induces a conformational change in nuclear receptors, and protease digestion and antibody accessibility studies reveal that agonists and antagonists trigger distinct structural alterations of nuclear receptor LBDs. The resolution of the crystal structures of several ligand-free (apo) and ligand-occupied (holo) nuclear receptor LBDs alone or in a complex with co-activator fragments have provided molecular details of the various ligand-induced changes and, moreover, have shown how these structural alterations translate into protein–protein interactions. The first structure of a nuclear receptor LBD, the unliganded retinoid X receptor α (RXRα)[5], revealed a previously undescribed fold comprising 12 α-helices (H) and a short β-turn (s1–s2), arranged in three layers to form an anti-parallel 'α-helical sandwich' (Fig. 2a). Helices H1–H3 form one face of the LBD, H4, H5, s1–s2, H8 and H9 correspond to the central layer of the domain and helices H6, H7 and H10 constitute the second face. The overall fold has proven to be prototypic for the LBDs of other nuclear receptors following the determination of the structure of several liganded nuclear receptor LBDs (Ref. 6). Table 1 lists all presently available three-dimensional structures of nuclear receptor LBDs together with their Protein Data Bank (PDB) assignments. Superposition of these structures shows a clear overall similarity, particularly in the top half of the LBD, which comprises the helices H1, H4, H5 and H7–H10 and corresponds to a structurally rather invariable region of the LBD. The lower part of the LBD comprises the variable region, which contains the ligand-binding pocket (LBP). A comparison between apo-RXR and holo-nuclear-receptor LBDs reveals several differences in the variable region. The most striking difference is the repositioning of the C-terminal

helices. In the apo-RXRα (Fig. 2a), helix H11 is almost perpendicular to H10 and points towards the LBP, and some of the hydrophobic residues of H11 partially fill and stabilize the LBP. Helix H12, which contains the residues of the AF-2 activation domain (AD) core (Fig. 1), extends away from the LBD. In all holo-LBDs, helix H11 is positioned in the continuity of H10, and H12 folds back over the LBP (Fig. 2b). The crystal structures of all presently known holo-nuclear-receptor LBDs adopt a very similar structure (Table 1) with the ligand entirely buried in a predominantly hydrophobic pocket that is generated by residues originating from various secondary structural elements: helices H3, H5, H11, H12, the β-hairpin s1–s2, and loops L6–7 and L11–12 (Fig. 2b). The recent determination of the RXRα LBD bound to its agonist 9-*cis*-retinoic acid[7,8] (Fig. 3), allowed, for the first time, the comparison of the liganded and unliganded structures of RXR and confirmed the previously proposed model of an agonist-induced LBD transconformation[6]. This mechanism involves the generation of a LBP as a result of the repositioning of helix H11 along H10, the concomitant swinging of H12 underneath H4 and the bending of H3 allowing the N-terminal part to pack on the ligand (Figs 2a,b). To what extent this activation model can be applied to other nuclear receptors is unclear because only two other apo-LBD structures have been solved. In apo-PPAR-γ [peroxisome proliferator activated receptor γ (Refs 9,10)] and apo-PPAR-δ (Ref. 11), the AF-2 helix adopts a conformation similar to, but not identical with, that of their holo-LBDs. However, irrespective of whether more or less divergent nuclear receptor apo structures might exist, the ligand-triggered activation mechanism implies a stabiliz-ation of the canonical holo-LBD conformation that was previously described (Fig. 2b).

Agonists induce a cognate surface for co-activator interaction

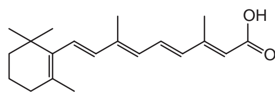
Co-activator recruitment is the second essential step by which the information generated by the agonist–receptor interaction is propagated along a cascade of events that finally leads to the activation of the transcriptional machinery. This recruitment is the direct consequence of the agonist-induced conformational changes that generate the surface to which the nuclear-receptor-interacting domain (NID) of co-activators bind. Several studies have revealed the structural basis of nuclear-receptor–co-activator interaction: PPAR-γ (Refs 7,9), RXRα (Ref. 7), oestrogen receptor α (ERα)[12] and thyroid hormone receptor β (TRβ)[13] were co-crystallized together with their cognate agonists and a short peptide from the NID that contained the so-called co-activator nuclear receptor box LxxLL motif[14]. In all cases, the nuclear receptor box peptide is bound to a hydrophobic groove generated by the carboxy terminal part of H3, the loop L3–4 and H4 (Fig. 4a). Note that this groove encompasses the highly conserved nuclear receptor LBDs signature motif[6] (Fig. 2), which suggests that the majority of nuclear receptors have the potential to interact with helices of the nuclear receptor box-type. The peptide is held in place via the interactions of its leucine residues with the hydrophobic groove constituents but also by hydrogen bonds that involve two conserved residues of the nuclear receptor LBDs (Fig. 4a). These amino acids are a lysine at the C-terminus of H3 and a glutamate in H12. Both are hydrogen-bonded to a main-chain peptide bond of the LxxLL motif and together form a 'charge clamp' that in addition to the stabilization of the peptide–receptor interaction defines the precise length of the helical motif that can be docked to the cleft. Biochemical experiments suggest that non-conserved residues adjacent to the LxxLL motif of co-activators make additional contacts with the nuclear receptor LBD and might determine the specificity of nuclear-receptor–co-activator interaction[13,15]. Fully supporting the original model[6], all the structural data discussed above have shown how nuclear receptor activity is regulated by ligand-binding via the alteration of LBD surface topology. It appears that the induction of the AF-2 upon ligand binding involves the proper repositioning of structural elements (helices H3, H4, loop L3–4 and the 'activation helix' H12) such that a defined nuclear receptor interaction surface for co-activators is generated.

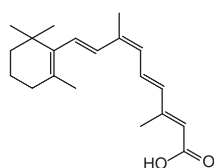
Table 1. Three-dimensional structures of nuclear receptor ligand-binding domains together with their PDB assignments[a]

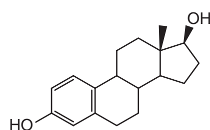
Receptors	Ligands	Remarks	PDB ID	Refs
Monomers				
RARγ	T-RA	Agonist	2lbd	18
	9C-RA	Agonist	3lbd	30
	BMS961	Agonist	4lbd	30
	BMS394	Agonist	1exa	32
	BMS395	Agonist	1exx	32
RXRα	9C-RA	Agonist	1fby	8
TRα	T3	Agonist	–	56
TRβ	T3	NR box complex	1bsx	13
PPAR-δ	GW2433	Agonist	1gwx	11
	Apo	–	2gwx	11
	EPA	Agonist	3gwx	11
VDR	Vitamin D3	Agonist	1db1	57
PR	Progesterone	Agonist	1a28	58
PPAR-γ	Apo	–	3prg	10
Homodimers				
RXRα	Apo	–	1lbd	5
ERα	Oestradiol	Agonist	1ere	16
	Raloxifene	Antagonist	1err	16
	Diethylstilbestrol	NR box complex	3erd	12
	4-Hydroxytamoxifen	Antagonist	3ert	12
	Oestradiol	Agonist	1a52	59
ERβ	Raloxifene	Antagonist	1qkn	19
	Genistein	Partial agonist	1qkm	19
PPAR-γ	Apo	–	1prg	9
	Rosiglitazone	NR box complex	2prg	9
	GW0072	Partial agonist	4prg	21
Heterodimers				
RARα–RXRα	BMS614, oleic acid	Antagonist, partial agonist	1dkf	17
PPAR-γ–RXRα	Rosiglit, 9C-RA	NR box complex	–	7
	GI262570, 9C-RA	NR box complex	–	7

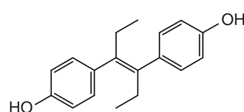
[a]Abbreviations: Apo, unliganded receptor; 9C-RA, 9-*cis* retinoic acid; EPA, eicosapentaenoic acid; ER, oestrogen receptor; NR, nuclear receptor; PDB, Protein Data Bank; PPAR, peroxisome proliferator activated receptor; PR, progesterone receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; T3, thyroid hormone; T-RA, all-*trans* retinoic acid; TR, thyroid hormone receptor; VDR, vitamin D receptor.

(a) Pure agonists

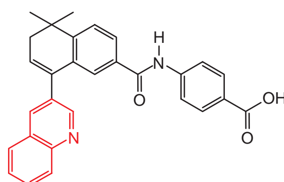


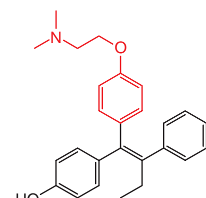


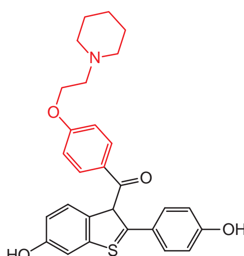


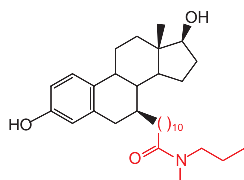


(b) Pure AF-2 antagonists

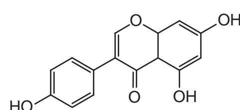








(c) Partial AF-2 antagonists



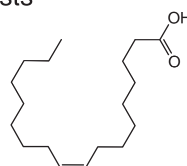


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Fig. 3. The structures of **(a)** pure agonists, **(b)** pure activation function 2 (AF-2) antagonists and **(c)** partial AF-2 antagonists, discussed in the text, are shown with the receptor to which they bind in parentheses. 'Antagonistic' substitutions are represented in red. Abbreviations: ER, oestrogen receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor.

Structural basis of antagonist action

To date, three crystals of nuclear receptor LBDs bound to pure AF-2 antagonists (Fig. 3) have been reported. The structural determination of the ERα LBD in complexes with the selective anti-oestrogens raloxifen[16] and 4-hydroxytamoxifen[12], provided the first structural evidence for the structural basis of antagonism. This principle was recently extended to another subgroup of the nuclear receptor family with the determination of the structure of the retinoic acid receptor α (RARα) LBD bound to BMS614, a RARα selective antagonist[17]. Together, all these structures revealed a well-conserved overall fold as compared to the canonical holo-nuclear-receptor LBD conformations, with one exception: due to the particular chemical structure of pure antagonists (see below), helix H12 is unable to adopt the holo position. Following a clockwise rotation of ~120°, combined with a shift towards the amino terminus of the LBD, helix H12 packs on the groove formed by the carboxy terminal part of H3, the loop L3–4 and H4, which also corresponds to the co-activator nuclear receptor box LxxLL motif binding site (Fig. 2c). Note that helix H12 harbours conserved hydrophobic residues that define a degenerated LxxLL motif that mimicks the nuclear receptor box of co-activators and mediates the interaction with the cleft (Fig. 4b). Importantly, antagonist-induced repositioning of the AF-2 helix immediately suggests a possible mechanism for antagonist action, because the interaction surface with co-activators, of which helix H12 is an integral part, is not formed.

A general feature common to all AF-2 antagonist molecules crystallized to date is the presence of a bulky side-chain that cannot be accommodated within the agonist-binding cavity. As exemplified by the recently reported RARα–BMS614 LBD complex[17], the antagonist extension points towards helix H12 and exits the binding pocket between helices H3 and H11 (Fig. 5a). This particular position prevents the positioning of the activation helix H12 in the 'active' conformation as seen in the RARγ–all-*trans*-retinoic-acid complex[18]. Indeed, the superposition of these two structures (Fig. 5a) shows that there would be a steric clash between the antagonist extension of BMS614 and helix H12 in its holo position. In the antagonist conformation, the lengthening of the loop L11–12, resulting from the unwinding of the C-terminus of helix H11 enables helix H12 to adopt a second low-energy position by binding to the co-activator LxxLL recognition cleft (compare Figs 5b and 5c). In contrast to agonists that stabilize a long H11 helical conformation, different ligand–receptor interactions at the level of H11 and of the surrounding regions (loop L6–7 and H3) most probably explain the antagonist-induced unwinding of the C-terminal part of this helix. Note that these structural features are found in all antagonist-bound LBD complexes crystallized so far. Hence, it appears that the type of action of pure AF-2 antagonists described above originates from at least two structural principles. The major feature is the presence of a large 'antagonistic' ligand extension that sterically prevents the alignment of helix H12 in the holo position. Without a holo-H12, no LBD–co-activator interface can be formed. The second structural principle is the unwinding of helix H11, which allows H12 to bind to the co-activator nuclear receptor box LxxLL motif binding groove. Thus, the second feature of antagonism is the competition between H12 and the nuclear receptor boxes of co-activators for a common LBD surface.

Structural basis for full and partial AF-2 antagonism

In addition to complete antagonists of AF-2 function (e.g. raloxifen and 4-hydroxytamoxifen), AF-2 partial

agonists–antagonists (Fig. 3) have been crystallized with the corresponding receptors. All the structures discussed above show a strict correlation between the orientation of the AF-2 helix and their biological activity. However, the ERβ–genistein[19] and RXRα–F318A–oleic acid[17] LBD structures show that H12 can adopt the antagonist conformation even though the corresponding ligand elicits a weak but clear transcriptional AF-2 activity. A probable explanation for the discrepancy between the antagonist location of H12 and the transcriptional activity of these complexes is that these ligands display some but not all features of pure AF-2 agonists or pure AF-2 antagonists. Both oleic acid and genistein can be classified as partial or mixed AF-2 agonists–antagonists[17,20]. A major difference between pure and partial antagonists lies in their steric properties. In contrast to full antagonists genistein and oleic acid do not bear a bulky extension. Thus, they do not sterically preclude the agonist position of H12 and are, in this respect, similar to agonists. However, these compounds induce unwinding of helix H11, which permits the positioning of helix H12 in the antagonist groove; in this respect, these ligands are similar to antagonists. The recently solved structure of PPAR-γ bound to the mixed agonist–antagonist GW0072 (Ref. 21) suggests that an additional mechanism might account for the particular biological properties of such ligands. In this case, the partial activity of the ligand is attributed to a poor stabilization of the holo position of H12 as a result of a lack of contact between the ligand and the AF-2 helix. In the presence of such mixed ligands, the active holo conformation of nuclear receptor LBDs is not firmly stabilized, and the position of H12 probably depends on the intracellular concentration of co-activators and co-repressors. Therefore, these ligands might act as either AF-2 agonists or antagonists depending on the cellular context (Fig. 6).

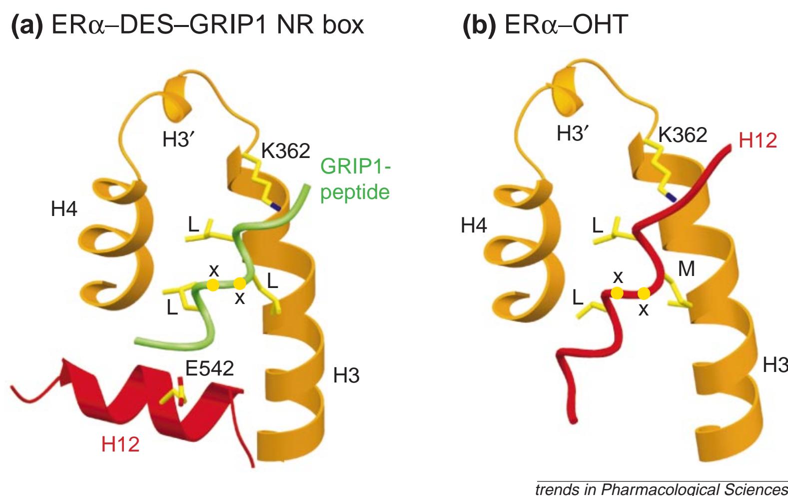

Fig. 4. Interfaces between the oestrogen receptor α (ERα) ligand-binding domain (LBD) and both the nuclear receptor box peptide and helix H12. **(a)** Close-up view of the co-activator [GRIP1–TIF2 (mouse and human homologues, respectively)] peptide bound to the ERα–diethylstilbestrol (DES) LBD complex. The nuclear receptor box peptide is represented as a green Cα 'worm' with the leucine side-chains of the LxxLL motif shown. For clarity, the hydrophobic residues of the groove that interacts with the co-activator peptide have been removed. The two ERα residues K362 and E542, which define the 'charge clamp', are depicted. The static part of the receptor-interacting surface (helices H3–H4) is shown in orange, whereas helix H12, the mobile part is shown in red. **(b)** Close-up view of the ERα–4-hydroxytamoxifen (OHT) antagonist complex showing helix H12 bound to the static part of the co-activator binding site. Helix H12 is represented as a red Cα 'worm' with the residue side-chains that mimic the LxxLL motif of co-activators depicted. Abbreviations: GRIP1, glucocorticoid receptor-interacting protein 1; TIF2, transcriptional intermediary factor 2. Adapted from Ref. 12 using the coordinates 3erd and 3ert deposited in the PDB.

Perspective for pharmacological drug design

Orphan receptors as new pharmacological targets

Because of its implication in virtually all fundamental physiological functions, the nuclear receptor superfamily offers an exceptional spectrum of targets for the development of therapeutics. In addition, the rapidly growing number of nuclear receptor superfamily members raises the prospects of new targets and new ligands. Indeed, recently, ligands for several of these new nuclear receptors, the so-called orphan receptors[1,22], have been identified. One example are PPARs whose α isotype was originally identified as binding to synthetic compounds that stimulated the proliferation of peroxisomes in liver cells, such as Wy14643 (Ref. 23). Now, we know that PPAR-α binds leukotrienes and might be involved in the control of inflammation. PPAR-γ controls adipogenesis, binds certain fatty acids and prostaglandins, mediates the action of insulin sensitizers (thiazolidinediones) used in the treatment of non-insulin-dependent diabetes and might have a perspective in the 'differentiation' therapy of liposarcoma[24] and breast cancer[25]. (For further details and references on PPARs, the reader is referred to a recent comprehensive review by Desvergne and Wahli[26].) A recent example of an 'intracrine' regulation by orphan receptors is cholesterol metabolism, which is under feedback and feed-forward control of the previous orphan receptors SF1 (steroidogenic factor 1), LXRα (liver X receptor α) and FXR (farnesoid X receptor). In this case, SF1 and LXRα act as oxysterol receptors that stimulate bile acid synthesizing enzymes, whereas FXR is the cheno-deoxycholate receptor, which exerts a feedback control in the same pathway. (For further details the reader is referred to the review by Repa and Mangelsdorf[27].) Another exciting progress is the recent identification of a new steroid receptor, PXR (pregnane X receptor), which interacts with several natural and synthetic steroids[28,29] and controls steroid catabolism by activating the cytochrome P450 isoform CYP3A and other P450 hydroxylases. Interestingly, PXR can bind to a large variety of compounds that all act as agonists, including, in addition to steroids agonists and antagonists, rifampicin or taxol. Obviously, this receptor plays an important role in determining the concentration of, for example, steroids and taxol used in contraception and tumour therapy, respectively.

Pharmacological action of nuclear receptor ligands

The pharmacological potential of nuclear receptors lies, in part, in the ability of synthetic derivatives to partially reproduce or inhibit the activities of natural ligands. Indeed, synthetic agonists and antagonists of several nuclear receptors are currently used as anticancer agents in chemotherapies of acute promyelocytic leukaemia (retinoid agonists), and prostate (androgen antagonists) and breast (oestrogen antagonists) cancers. Moreover, glucocorticoids are used as immunosuppressive

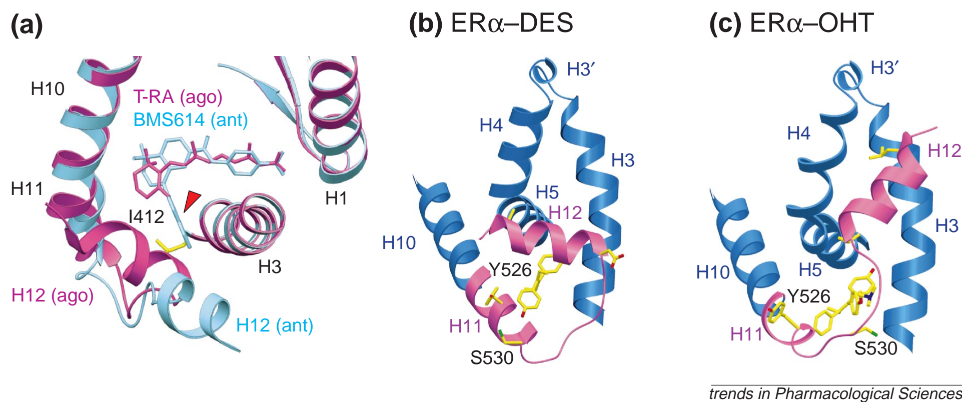

Fig. 5. Structural basis of antagonist action. **(a)** Superposition of the ligand-binding sites of the retinoic acid receptor α (RARα)–BMS614 (light blue) and the RARγ–all-*trans*-retinoic-acid (T-RA) (pink) complexes illustrating the steric clash between the BMS614 antagonist extension (red arrow) and I412 of holo-H12, which prevents the positioning of the AF-2 activation domain (AD) core in the agonist orientation. **(b,c)** Comparison between the conformations triggered by an agonist [diethylstilbestrol (DES)] and an antagonist [4-hydroxytamoxifen (OHT)] in the region H11–H12 of ERα LBD. This highlights the different positioning of helix H12 and the unwinding of helix H11 in the ERα–OHT complex when compared with the ERα–DES complex. S530 and Y526 indicate the C-terminal residues of H11 in the diethylstilbestrol and 4-hydroxytamoxifen complexes, respectively. Adapted from Refs 12 and 17 using the coordinates 1dkf, 3erd and 3erf deposited in the PDB. Abbreviations: ago, agonist; ant, antagonist.

or anti-inflammatory agents. However, the use of natural ligands for therapeutic purposes is often limited by their relatively low specificity that results in toxicity when these ligands are used at pharmacological doses. Therefore, the knowledge of holo-nuclear-receptor LBD structures is of great value for rational design of more selective nuclear receptor ligands with improved pharmacological properties. Although highly related, distinct receptor isotypes might display amino acid differences in their LBPs, allowing the generation of selective ligands. For example, three divergent amino acids in the LBPs of RARα, β or γ have been shown to mediate the isotype recognition by specific ligands[18,30–32]. Indeed, specific ligands for the α, β or γ RAR isotypes have been synthesized. In a similar way, the recent cloning of a second oestrogen receptor, ERβ, which presents an expression pattern that is different from that of ERα (Ref. 33), offers the opportunity to look for new oestrogen receptor isotype-selective ligands with higher specificity and reduced side-effects[34,60].

Many nuclear receptors function as either homo- or heterodimers with RXR as a promiscuous heterodimerization partner. In contrast to homodimerization, heterodimerization among nuclear receptor superfamily members allows for the fine-tuning of nuclear receptor action by using combinatorial sets of ligands. In this way, RXR ligands can be used to enhance various signalling pathways as demonstrated by the ability of RXR agonists to synergize with RAR ligands, including some RAR antagonists[35]. It has been suggested that in the context of the PPAR-γ–RXR heterodimer, RXR ligands stimulate insulin action in non-insulin dependent diabetes, and enhance the action of thiazolidinediones[36]. Thus, the generation of signalling pathway-specific RXR ligands would be an important achievement.

The generation of nuclear-receptor-based drugs with cell or tissue specificity is of significant interest to limit side-effects. To some extent, partial agonists–antagonists can display such a specificity because the agonistic or antagonistic activity of such compounds might be expressed in a cell-specific manner. In their N-terminal region, nuclear receptors harbour another activation function, AF-1 (Fig. 1), whose activity is also mediated by binding to co-activators. Moreover, the activity of several nuclear receptor AF-1s can be modulated by phosphorylation, which most probably alters nuclear receptor AF-1–co-activator interaction[37]. However, to date no crystal structure of the AF-1-containing region of a nuclear receptor has been reported and no interaction motifs homologous to the LxxLL nuclear receptor boxes could be determined. Antagonists inactivate AF-2 but not necessarily AF-1. As an example, the selective oestrogen receptor modulators (SERMs) 4-hydroxytamoxifen and raloxifen, which are used clinically in the treatment of osteoporosis and hormone-dependent breast cancer, act as anti-oestrogens in breast and endometrium but are agonists in bone. This is in contrast to the pure oestrogen receptor antagonist

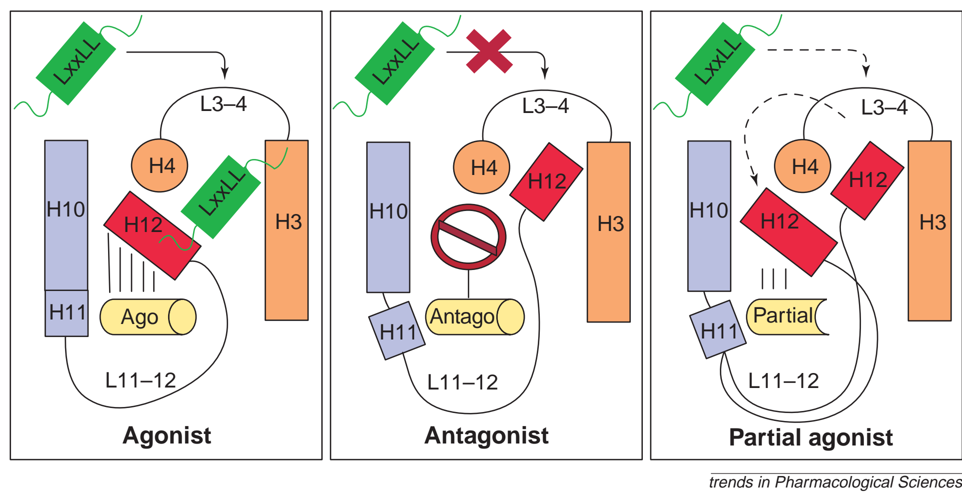

Fig. 6. The crystal structures discussed in this review have provided a structural view on how the binding of various ligands can induce different nuclear receptor conformations, thereby modulating their transcriptional activity. Agonist ligands (left) induce a conformation of nuclear receptor ligand-binding domains (LBDs) in which the holo-position of helix H12 is firmly stabilized (note that the black lines between the ligand and H12 indicate that the overall holo-LBD conformation is strongly stabilized by the ligand, which does not necessarily have to directly interact with H12). This active conformation provides a surface to which co-activators can bind via their nuclear receptor boxes that contain LxxLL motifs. By contrast, antagonists with bulky substitutions (centre panel) prevent the proper positioning of H12 in its agonistic site and therefore destabilize the interaction surface. The antagonist-induced unwinding of the C-terminal part of helix H11 allows helix H12 to bind to the static part of the co-activator binding site. In the presence of partial AF-2 agonists–antagonists (right), the holo-form is poorly stabilized (black lines). However, the agonist position of H12 is not precluded by a steric hindrance of the ligand and the active conformation might, at least transiently, be adopted. Consequently, the biological activity of such ligands might be highly dependent on the cellular concentration of co-activators and co-repressors. Abbreviations: Ago, agonist; Ant, antagonist.

ICI164384 (Ref. 38). It has been shown that ICI164384 antagonizes both the oestrogen receptor activation functions AF-1 and AF-2 whereas 4-hydroxytamoxifen antagonizes only AF-2 and functions as an AF-1 agonist[39]. Antagonists that block both AF-1 and AF-2 activity, such as the anti-oestrogen ICI164384, are normally referred to as complete or full oestrogen receptor antagonists, whereas 4-hydroxytamoxifen and raloxifen are partial oestrogen receptor agonists–antagonists. Recently, the crystal structure of the ERβ–ICI164384 LBD complex revealed a mechanism of antagonistic action that is similar, in principle, to that of 4-hydroxytamoxifen and raloxifen. However, in that case, because of the presence of the long antagonist substituent in the co-activator recruitment site, helix H12 cannot adopt a defined position[60]. It has remained unclear, however, why the N-terminal AF-1 remains silent in the presence of this antagonist. Along the same lines, Gehin and colleagues[31] have shown that some synthetic retinoids that display cell specificity, act as mixed RAR agonists–antagonists. However, in this case, the partial activity triggered by these ligands would be mediated only through the AF-2 function by the flip-flop mechanism of helix H12 described above. Nuclear receptors are also able to affect, positively or negatively, other signalling pathways such as those involving activator protein 1 (AP-1), nuclear factor κB (NF-κB) or signal transducer and activator of transcription 5 (STAT5) transcription factors[40,41]. Such signalling crosstalks have been shown to be important targets for drug design. For example, like RAR agonists, some RAR antagonists also induce AP-1 repression[42]. Such retinoid antagonists are therefore able to dissociate RAR-mediated transactivation from transrepression of AP-1 activity. Interestingly, the first 'dissociated' glucocorticoids have been reported and might display reduced side-effects[43].

Co-activators as pharmacological targets

Transcriptional regulation requires the recruitment by nuclear receptors of multiple enzyme activities (e.g. acetylases, deacetylases, kinases and ATPases), each of them representing a potential therapeutic target. A plethora of potential nuclear receptor co-activators that interact with transcriptionally active receptors in a ligand-dependent manner have been reported[2,3,44]. Although their function is not fully understood, some of these co-activators might play a role in disorders of the endocrine system and in diseases such as steroidal cancers. For example, the co-activator AIB1 (amplified in breast cancer-1) is found to be amplified in oestrogen-receptor-positive cell lines as well as in a high proportion of oestrogen-receptor-positive tumours[45]. The possibility that some co-activators present nuclear-receptor- or cell-specificities is an attractive view. For example, PGC-1 (PPAR-γ co-activator-1), which is preferentially expressed in skeletal muscle and brown fat, enhances transactivation by PPAR-γ and TR (Ref. 46). ARA70 (androgen receptor co-activator 7) was reported to exhibit a preference for androgen receptors, and enhance androgen-dependent transactivation[47]; however, this is controversial[48].

Both structural and biochemical data have shown that the LxxLL motif is part of co-activator surfaces that interact with nuclear receptor LBDs. Moreover, it has been shown that co-repressors N-CoR (nuclear receptor co-repressor) and SMRT (silencing mediator of RAR and TR) bind to a nuclear receptor surface overlapping the co-activator binding site via similar but not identical LxxLL motifs[49–51]. Therefore, these findings raise the possibility to preclude nuclear-receptor–co-regulator interactions with small peptidomimetic molecules. Using a phage display approach, Chang and colleagues[52] have screened combinatorial peptide libraries that contain the core LxxLL motif of co-activators. Some peptides have been found to selectively disrupt ERβ- but not ERα-mediated reporter gene expression. In a similar way, peptides were identified that discriminate between ERα and ERβ agonist and antagonist complexes[53]. Histone acetyltransferases (HATs) represent another potential pharmacological target. In this context, Lau and colleagues[54] recently described the design of peptide CoA conjugates that selectively inhibit the transcriptional co-activators p300 and PCAF [p300/CREB (cAMP response element-binding protein) binding protein-associated factor].

In addition to the nuclear receptor co-activators present in the HAT complex, a second type of complex, termed TRAP or DRIP, mediates the transcription stimulatory effect of nuclear receptors on the basal transcriptional machinery. Interestingly, ligands can be found that differentially stimulate recruitment of HAT and DRIP co-activators to the receptor, as shown for the vitamin D receptor[55]. Thus, ligand design can differentially target these two complexes. However, the specific roles of the HAT and TRAP–DRIP complexes in nuclear-receptor-mediated transcription have yet to be determined.

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Chemical names

BMS394 and BMS395: *R*- and *S*-3-fluoro-4-[2-hydroxy-2-(5,5,8,8-tetramethyl-5,6,7,8,-tetrahydro-naphthalen-2-yl)-acetylamino]-benzoic acid

BMS614: 4-[(4,4-dimethyl-1,2,3,4-tetrahydro-[1, 2′]binaphthalenyl-7-carbonyl)-amino]-benzoic acid

BMS961: racemic mixture of BMS394 and BMS395

G1262570: (2*S*)-((2-benzoylphenyl)amino)-3-{4-[2-(5-methyl-2-phenyloxazol-4-yl)ethoxy]phenyl} propionic acid

GW0072: (+/−)-(2*S*, 5*S*)-4-(4-(4-carboxyphenyl) butyl)-2-heptyl-4-oxo-5-thiazolidine *N*,*N*-dibenzylacetamide

GW2433: 2-(4-{3-[1-[2-(2-chloro-6-fluoro-phenyl)-ethyl]-3-(2, 3-dichloro-phenyl)-ureido]-propyl}-phenoxy)-2-methyl-propionic acid

ICI164384: *N*-*n*-butyl-*N*-methyl-11-[3,17β-dihydroxyoestra-1,3,5-(10)-trien-7α-yl] undecanamide

Wy14643: [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid