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Protein structure based rational design of ecdysone agonists

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ABSTRACT

We review the impact of protein X-ray crystallography on the rational design of insecticides that act as agonists of the ligand-binding domain of the Ecdysone receptor (EcR). As the EcR is a target specific to insects, these compounds potentially constitute new chemical classes of safe insecticides. The increased insight relative to that from ligand-only based (Quantitative) Structure–Activity Relations (QSARs), classical 2D-Hansch type or 3D-CoMFA/CoMSIA (Comparative Molecular Field/Similarity Analysis), is discussed. The importance of protein X-ray structure determination in support of the discovery process is stressed as the simplistic lock-and-key picture fails due to the remarkable flexibility of the EcR ligand binding site. Several new non-steroidal chemical classes of ecdysone agonists, designed by guidance from protein X-ray studies, are described.

1. Introduction

The commercialization of the first dibenzoylhydrazine insecticides (DBHs) by the Rohm & Haas Company in 1988 introduced a new and invertebrate-specific molecular target to insecticide research, the ecdysone receptor (EcR). EcR belongs to the large class of Nuclear Receptors (NRs) which are known to act as transcription factors controlling a wide range of signaling pathways in many organisms.[1,2]

When activated by the steroid hormone ecdysone, or more precisely by its active metabolite 20-hydroxyecdysone (20E)(**1**), the EcR is responsible for initiating the moulting of insects by binding to ecdysteroid binding elements. Similar to the phytoecdysteroids, which are utilized by plants to protect themselves from insects, the new non-steroidal ecdysone agonists bind to EcR and induce premature moulting, which is finally lethal to the insect.

Shown below (Fig. 1) are the natural substrate 20E (**1**) and the first prototype dibenzoylhydrazine, RH-5849 (**2**), which was published in 1991. Astonishingly, RH-5849 and the commercialized DBHs Tebufenozide (**3**), Methoxyfenozide (**4**), Halofenozide (**5**), and later Chromafenozide (**6**) from Nippon Kayaku (see Fig. 2) bear no resemblance to the natural substrate 20E (**1**).[3–5]

It is hence not surprising that they were found not by target-based approaches but by classical screening. The fact that both classes of ligands act at the same molecular target presents a challenge to the rational design of new ecdysone agonists, as many Quantitative Structure Activity Relations (QSARs) are based on

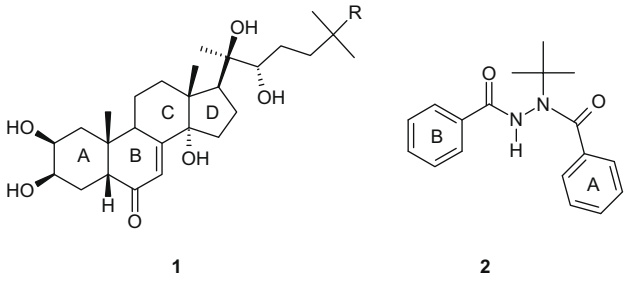

Figure 1. 20-Hydroxyecdysone (20E, **1**, R = OH), Ponasterone A (PonA, **1**, R = H) and RH-5849 (**2**).

pharmacophore models, that is, a set of functional groups arranged in a special orientation that is postulated to be responsible for the activity of a compound. The obvious dissimilarity between these steroidal and non-steroidal agonists was an invitation to speculate on the equivalence of characteristic parts of both types of molecules, and a plethora of pharmacophore models appeared in the literature. Conventional Hansch-type QSARs were augmented by 3D-models of CoMFA- or CoMSIA-type (Comparative Molecular Field or Comparative Molecular Similarity Analysis). The history of these approaches has been reviewed in several recent texts and will hence be sketched only briefly in this review.[3,6]

We shall start with a short overview on early QSARs on steroidal and non-steroidal ecdysone agonists and then concentrate on approaches based on the protein structure of the ligand-binding domain of the ecdysone receptor, beginning with a survey of nuclear receptors and their role in insect development, describing initial

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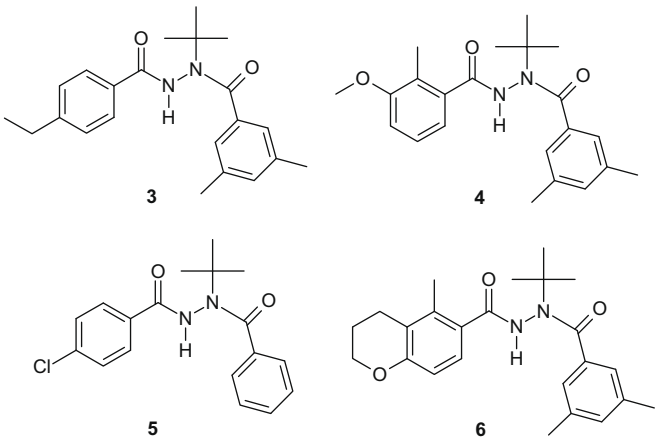


Figure 2. Commercialized diacylhydrazines Tebufenozide (**3**), Methoxyfenozide (**4**), Halofenozide (**5**) and Chromafenozide (**6**).

homology models and concentrate on experimentally determined prototype compounds co-crystallized with the ecdysone receptor.

2. Ligand based 2D- and 3D-QSAR of ecdysone agonists

The easiest way to establish a QSAR is to separate the steroidal agonists and the non-steroidal compound classes and to handle them separately. This was done by several authors from 1991 to 2006. The next step would be to combine the information gained from the separate QSARs and to build a combined model. As long as the 3D-structures of the DBHs do not come into play, the resulting SARs are meaningful. 3D-QSARs, however, require correct geometries of the ligands. This is especially true if artificial receptor cavity surfaces are used to discuss potential interactions with the protein. Unfortunately some authors did not take account of the fact that the *tert*-butyl group of RH-5849 (**2**) and its analogues requires a *cis*-amide bond as equilibrium geometry. Hence, conclusions drawn from 3D-QSARs based on other geometries should be treated with care. The SARs prior to the publication of the protein structure of the *Heliothis virescens* EcR have been summarized by Rohm & Haas scientists and by others.[3,7–10]

2.1. 2D-QSARs

The status of (Q)SAR studies of dibenzoylhydrazines starting in 1991 has been summarized recently.[11] Traditionally, the aromatic rings of the DBHs are denoted A and B, the ring close to the *tert*-butyl group being denoted A. Hansch-type equations for 27 A-ring substituted DBHs indicate a strong positive correlation of pLD_{50} values with increasing log*P* and σ_I^{ortho}, describing the inductive electronic effect of *ortho* substituents, and an adverse effect of bulky substituents in the *meta*- and *para*-positions. Keeping 2-Cl as the most potent substituent of the A-ring, substituents of the B-ring were varied, leading to a qualitatively similar Hansch equation for 30 singly B-substituted compounds, where the size of the substituents is characterized by the STERIMOL parameter L instead of a volume characterization. An equation relating the pIC_{50} values of 17 B-ring *para*-substituted DBHs, again leading to qualitatively similar results, led to the postulation of an artificial receptor cavity interacting with the agonists. There the danger of inferring 3D-structures from 2-dimensional formulae became obvious, as the *cis*-amide conformation of the *tert*-butyl group was not taken into account, despite the fact that the crystal structure, which shows this, had been published in 1990.[12]

2.2. 3D-QSARs

Ecdysteroids form a large class of steroid hormones in plants and invertebrates. Despite their structural diversity they possess a common core. This is a good starting point for 3D-QSAR approaches like CoMFA (Comparative Molecular Field Analysis), which need a superposition rule for the 3D-structures of the test molecules. In addition, since the first publication of the X-ray structure of ecdysone in 1965, even the influence of solvents on the steroid structure has been investigated.[13,14]

Two extensive CoMFA models for 71 ecdysteroids in *Drosophila melanogaster* cell lines led to the conclusion that the steroid hormone binding could be understood as a sum of 6 specific interactions.[15] A pharmacophore hypothesis with six elements was developed based on the CoMFA model and is awaiting its combination with CoMFA models for DBHs.

A CoMFA investigation of 37 DBHs, augmented by log*P* as an additional descriptor, resulted only in a modest correlation.[16] A combined CoMFA including 6 derivatives of 20E resulted in slightly better or slightly worse models, depending on the superposition rule. The situation did not improve by including a larger set of DBHs and different superposition rules.[6] One might argue that the set of molecules chosen was highly imbalanced, and that a combination of both sets would have been more satisfactory.

A QSAR based on MM2 optimized geometries found a good parabolic relation between the larvicidal activity of 23 DBHs and the distance between the carbonyl oxygen close to the A-ring and the halogen substituent at the B-ring.[17] Interestingly, this relation could only be established when the conformation of the *tert*-butyl group was assumed to be *cis*. These authors also suggested a possible superposition with the steroidal core of 20E without including it in their QSAR.

A thorough quantum mechanical MP2 analysis of small diacylhydrazines revealed that they are intrinsically nonplanar and that the torsional barrier of the N–N bond is rather high.[18] These findings are corroborated by our own DFT- and MP2-calculations on the rotational barrier of RH-5849 (**2**) (see Fig. 3). [19] Broad minima around perpendicular orientations at 45–120° and 240–315° are separated by DFT-barriers of 16 (19.6) kcal/mol at 180° and 12.5 (15.2) kcal/mol at 345° in vacuo (COSMO-RS energies in water are shown in parentheses).[22] The respective in vacuo MP2-barriers are somewhat higher, 19.0 and 16.3 kcal/mol.

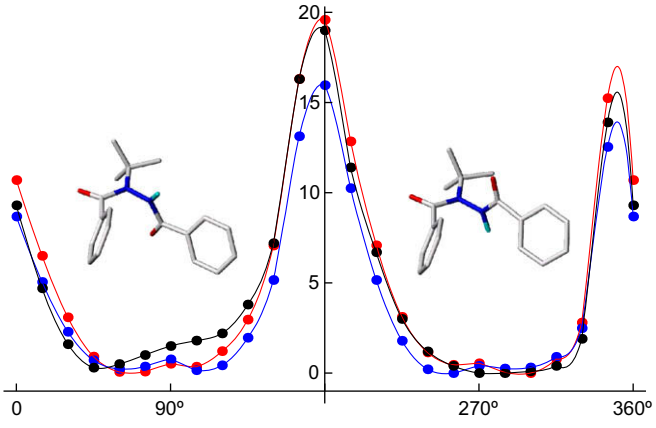


Figure 3. Rotation barrier of RH-5849 (**2**). Energy [kcal/mol] versus ∠CO–N–N–CO in vacuo DFT (blue), MP2 (black), and in water (COSMO-RS) DFT (red).

3. Target based approaches

3.1. Nuclear Receptors

Nuclear Receptors (NRs) belong to the large superfamily of transcription factors or steroid receptors that have been found in vertebrates, arthropods and nematodes. They can be activated by small hydrophobic molecules, which initiate or regulate gene expression in different signaling pathways such as homeostasis, differentiation, embryonic development, and organ physiology. In evolutionary terms it is assumed that the first NRs appeared after the divergence of metazoans from fungi.

NRs share a modular structure of five to six conserved domains, two of which, the DNA- and ligand-binding domains (DBDs and LBDs), are evolutionarily highly conserved. Several families of human retinoic, mineralocorticoid or farnesoid X receptors (FXR) are well characterized by protein X-ray crystallography. Crystallographic investigations on mammalian NRs revealed a so-called canonical structural organization for apo und holo LBDs consisting of 12 α helices and one β turn arranged in a 3-layer sandwich. The LBDs exist as monomers, as homo- or heterodimers and differ mainly in the relocation of the C-terminal helix 12, which is responsible for transactivation upon binding of the ligand. This conformational change permits cofactors/corepressors to bind to the coactivator binding-groove. Up to now, however, the X-ray structure of a complete NR, comprising DBD and LBD, is still unknown.

Important for pesticide research is the fact that the receptors acquired novel ligand affinities during their evolution. Together with the as yet unidentified juvenile hormone receptors, ecdysone receptors (EcRs) of arthropods are the key regulators of insect metamorphosis. Being steroid hormone receptors they belong to NR1 of the seven subfamilies NR0–NR6 of NRs together with vitamin D- (VDRs), retinoic acid- (RARs) or thyroid hormone- (ThRs) receptors.[23]

The obligatory heterodimer partner of the EcR has been shown to be the Ultraspiracle protein (USP).

Although the EcR of arthropods is orthologous to the FXR, LXRα, and LXRβ of vertebrates, vertebrate NRs do not respond to ecdysteroids.

3.2. Protein homology models

In view of the close sequence similarity of insectal EcRs and members of the mammalian NR family and their canonical structures, especially to the vitamin D and retinoic acid receptors, it was tempting to try to construct corresponding protein homology models in order to overcome the ambiguity of superimposing parts of dissimilar structures. Although it turned out later that the basic assumption on which these models were built, namely that 20E and the DBHs bind in the same binding niche, turned out to be wrong, some conclusions obtained from the homology models led to new active chemical agonist classes.

The first homology models of the LBD of the *Chironomus tentans* EcR in 2000 were based on human vitamin D (hVDR) and retinoic acid (hRARγ) receptor structures.[24] The proposed superposition of 20E and RH5849 (**2**) was similar in both models and was surprising in the sense that, for the first time, it was postulated that the *tert*-butyl group of RH5849 would occupy a groove not occupied by 20E. The ability to explain the known SAR of several ecdysteroids and DBHs was used to score the quality of the models. However, the ambiguity of how best to orient 20E in the binding niche could not be resolved.

A semiempirical PM3 study on complexes of DBHs and simplified homology models of lepidopteran and dipteran EcRs tried to explain ligand binding by calculated enthalpies of binding.[25] In 2003, another homology model of the LBD of *Heliothis virescens* EcR was published, based on the LBDs of RARγ and TRβ, and on the human estrogen receptor ERα and progesterone receptor PR.[26] Similar conclusions were drawn concerning the orientation of the bulky *tert*-butyl group of Chromafenozide (**6**).

3.3. Protein X-ray structures

All previously published structure-based investigations became obsolete when the first X-ray protein structures of the LBDs of *Heliothis virescens* EcR, co-crystallized with the steroid hormone PonA (**1**) and the DBH BYI06830 (**8**) were published in 2003 (see Fig. 4).[27]

To our great surprise, the binding niches of PonA and BYI06830 were different with only a partial overlap. Consequently, the interactions with the protein, especially in the steroid core region, are different. The hydroxyl groups of the core of PonA (and 20E) form hydrogen bonds with Arg^{383}, Glu^{309}, Tyr^{343} and Tyr^{346}, and the carbonyl group is H-bonded to Ala^{398}, while the alkyl chain hydroxyl groups are either directly bound to Tyr^{408} or interact via a water molecule. BYI06830, which overlaps partly in the alkyl-chain region, forms three hydrogen bonds, the carbonyls being acceptors for Thr^{343} and Asn^{504} and NH a donator to Tyr^{408}.

This means that all of the previous attempts, either to identify a common pharmacophore for steroidal- and non-steroidal agonists or to deduce a single binding site, were bound to fail. Even those QSARs, whether two- or three-dimensional, that combine different classes of diacylhydrazines, should be reviewed with care, as it is totally unclear whether these two partly overlapping binding niches are exceptional or whether they are just examples of a gen-

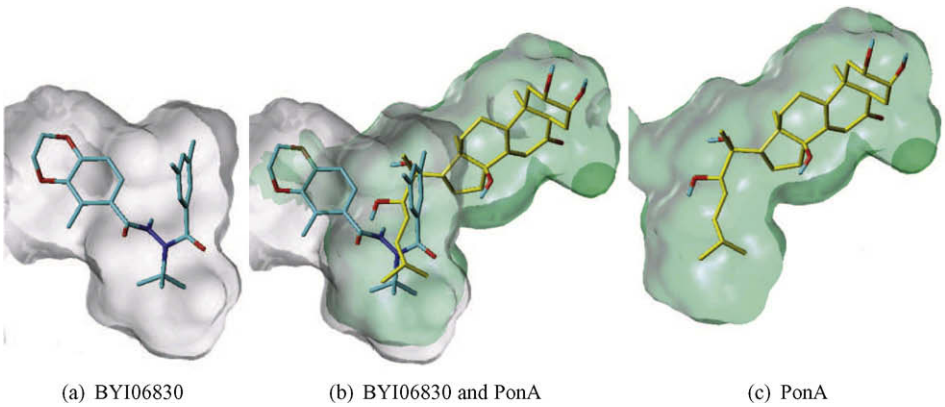


Figure 4. Bound agonists in the X-ray structure binding niches of the HvEcR-USP dimer.

eral principle of structural adaptability of the EcRs with respect to different chemical classes of agonists. In this review we shall present examples giving evidence for the second hypothesis, which severely limits predictions of new agonist classes. On the other hand, these new findings opened the door for the design of new chemical classes of agonists that could occupy both binding sites.

Bearing this in mind, it was mandatory to corroborate experimentally any proposal of a new chemical class significantly different from previous ones by protein X-ray crystallography. In the course of a joint project between Bayer CropScience and the IGBMC it turned out that the adaptability of the ligand-binding pocket of the EcR was not restricted to binding of PonA and BYI6830, instead every sufficiently different chemical class of compounds generated its own binding niche which in general overlapped only partly with the previous ones. Corresponding publications of the Moras group are in preparation.[28]

In this context, a recent 3D-QSAR of 77 closely related DBHs, differing only in the substitution pattern of the A- and B-rings, has to be mentioned, which uses the structure of BYI06830 (**8**) in its binding niche as reference conformation for CoMFA- and CoMSIA studies attempting to explain the different activities of DBHs in lepidoptera and coleoptera by postulating variations in contact residues, based on alignments of the respective EcR-LBD sequences.[29]

3.3.1. Steroidal agonists, and BYI06830

By the end of 2007 four protein structures of the EcR/USP heterodimer co-crystallized with ecdysteroids were known, three of the phytoecdysteroid PonA with *Heliothis virescens*, *Bemisia tabaci*, and *Tribolium castaneum* and one of the natural substrate 20E with *Heliothis virescens* (Fig. 5).[27,30–32] While the structures of the LBDs, the overall steroid orientation within the niches and their H-bonding patterns are very similar in all three species in that they adopt the canonical fold known from vertebrate complexes; the corresponding hetero-dimer partner (USP) structures are slightly different.

The binding niches are long and thin and slightly banana shaped, with the A-rings of the ligands being close to helix H1 and the β-sheet and the alkyl chains extending towards the C-terminal helix H12. With the exception of the C-22 OH-group, which needs a bridging water molecule for its interaction with the EcR, all OH-groups and the carbonyl function of 20E are directly H-bonded to amino acids in the ligand binding niche. The lower resolution of the PonA structures (2.9 Å) makes an assignment of water molecules difficult, but in view of the 20E structure (2.4 Å) corresponding peaks in the electron density difference maps can be interpreted as hints for water molecules.

Compared to PonA, 20E has one additional hydroxyl group at C-25, which is involved in a hydrogen bond to Asn^{504} of EcR. Surprisingly, the new H-bond leads to a lower affinity of 20E by up to two orders of magnitude. This effect is nicely explained by the higher desolvation cost of 20E in the subtle free-energy balance between internal H-bonds and solvation/desolvation.[32] For PonA itself the H-bond pattern does not change across the species.

BYI06830 creates its own binding niche that only partially overlaps with the steroid binding niche. It is located between helices H7 and H10 and forms three hydrogen bonds to the DBH agonist, stemming from helix 3 (Thr^{343}), helix 6 (Tyr^{408}), and helix 12 (Asn^{504}). This H-bond pattern differs considerably from that of PonA and 20E. As the natural substrate 20E is missing, the steroid binding niche is partially filled by two rotated aromatic sidechains from the β sheet. When superimposing the LBDs, the A-ring and the *tert*-butyl group of BYI6830 match the alkyl chain of PonA or 20E at C-17. For a detailed picture see Fig. 3 of the original publication.[27]

As all previous homology models were based on the assumption of a single ligand binding niche for steroidal and non-steroidal agonists, the first DBH-bound EcR structure rendered these models obsolete. Nevertheless it is interesting to note that the predicted orientation of 20E in the binding niche is not totally different from the experimental one. Of course, this is not true for the predicted DBH-orientations.

3.3.2. Nonsteriodal agonists

The commercialized non-steroidal ecdysone agonists have a rather narrow spectrum acting against some lepidopteran, dipteran and coleopteran pests, even though the natural substrate 20E is ubiquitously present in almost every arthropodal species. This fact gives rise to the hope that the mechanism of ecdysone agonism should indeed furnish broadly active insecticides.

One can imagine many reasons for the relatively selective action of DBHs, ranging from requirement of differing physico-chemical properties for differing species influencing, for example, uptake, transport and metabolism, to modified hydrogen-bond pattern in the binding niches and even to speculation about modified signaling mechanisms due to variation in the structure of the hetero-dimer partners.

The latter reason, the different structures of the EcR dimer partners USP, could be excluded, et least for *Chilo suppressalis*, *Drosophila melanogaster* and *Leptinotarsa decemlineata*, representing lepidoptera, diptera, and coleoptera, respectively.[33] The measured activity of diacylhydrazine agonists did not correlate with the exchange of the USPs among the different species.

Another reason for the rather narrow spectrum of DBHs might be the fact that the binding niches of DBHs and steroids differ in shape and size. Assuming that their specific binding niches are connected to their specific activity in contrast to the ubiquitously

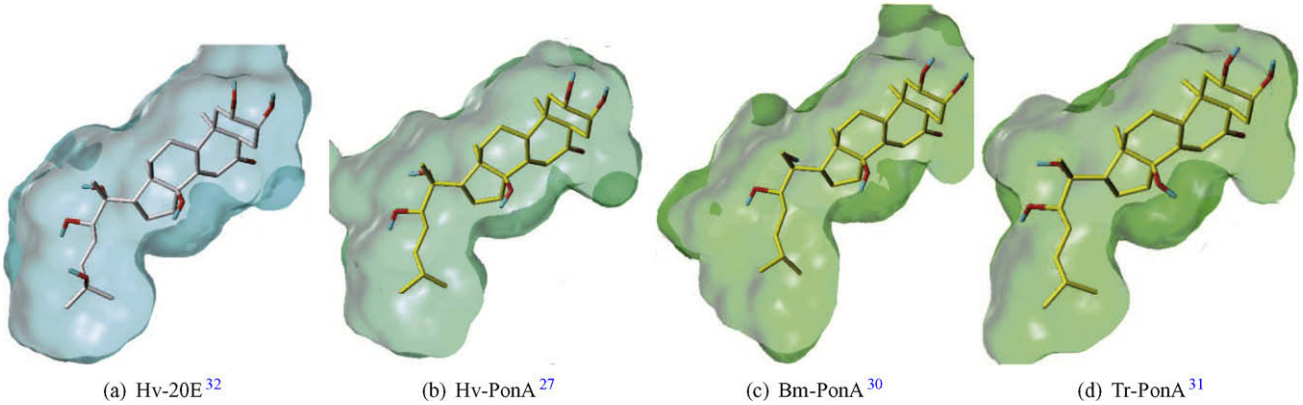


Figure 5. Binding niches of steroids in *Heliothis virescens*, *Bemisia tabaci*, and *Tribolium castaneum*.

active 20E binding to its canonical binding niche we aimed at finding agonists that would fit into the 20E/PonA pocket. If it were possible to design artificial agonists that occupy binding niches very close to those of PonA or 20E, one might hope to broaden their insecticidal spectrum.

3.4. Agonists designed by knowledge of binding niches

If the structural flexibility of EcR was not an exception but the rule for ecdysone agonists, it should be possible to explore the space of binding niches by new classes of chemically different molecules (Fig. 6).

Small variations of the DBHs should result in identical binding niches. This could be shown by the protein X-ray structure of BYI09181 (**7**) in the Hv-EcR/USP complex (see Fig. 9 Fig. 11). BYI09181 differs from Methoxyfenozide in that the 3,5-dimethylphenyl moiety, which hitherto had been the standard residue at this position, has been replaced by 2-methoxy-3-pyridyl.

The basic structure of the imidazole derivative **9** was originally conceived by drawing a ring between the *tert*-butyl group and the adjacent carbonyl function of **4**, as shown in Figure 7. At that time we regarded the function of the *tert*-butyl group as merely to provide steric bulk to force the molecule into the cramped *cis*-amide configuration. Modeling calculations showed that this function can be taken over by the imidazole ring such that the relative spatial positions of the phenyl rings in **4** and **8** are very similar, and therefore a bulky alkyl group should no longer be required.

A good synthesis route to the basic structure of **9** is shown in Figure 8: The keto-amide required for the hydrazone is normally readily available via the Dakin–West reaction (cf. p 69 in 37). However, for certain R^1 and R^2 substituents the reaction failed, even under Steglich conditions and it was easier to make this intermediate by acylation of the corresponding amino-alcohol and Swern oxidation of the alcohol group.[34] Synthesis of the hydrazone was straight-forward. Ring-closure to give the tosylated *N*-amino-imidazole had been described in principle but was also found to be critically dependent on the R^1 and R^2 substitution.[35,36] For $R^1 = H$ and $R^2 = CH_3$ the optimal conditions were addition of the hydrazone to a suspension of PCl_5 in abs $CHCl_3$ at −40 °C and allowing the reaction to come to RT over 1 h; for $R^1 = CH_3$ and $R^2 = i-$propyl the hydrazone was simply refluxed with $POCl_3$ in $CHCl_3$ for 2 h. The tosyl group was removed by stirring overnight at RT with conc H_2SO_4. Reaction with the appropriate benzoyl chloride provided the final product.[37]

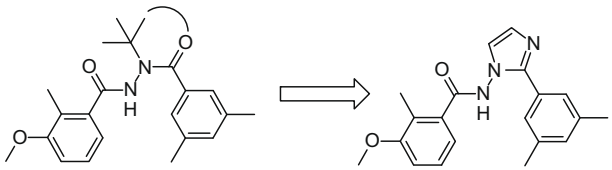


Figure 7. Conception of BYI08346(**9**) from methoxyfenozide (**4**).

The initial biological results were disappointing. All derivatives with the 'classical' 3,5-dimethylphenyl group had little activity. Fortunately our biochemical assay was showing there was indeed activity, albeit modest, at the receptor level. The protein homology model we were using at that time (see Section 3.2) in conjunction with the minimal energy configuration of **9** indicated that the 3,5-dimethylphenyl group was virtually coplanar with the imidazole ring but that this ring needed to turn out of plane to imitate the ring A of ecdysone. The introduction of the 2-chlorophenyl unit in its place gave us relevant biological activity. The development of the $pInd_{50}$ values (the EcR assay was an induction test) relative to that of 20E itself, which was run as a positive control, can be seen in Table 1.

Replacing the *tert*-butyl-amido unit by an isopropyl-substituted imidazole like in BYI08346 (**9**) is a rather severe structural change but should preserve the overall ligand geometry. This again is corroborated by the corresponding ligand-bound protein structure (Fig. 9). The original steroidal binding niche is superimposed as a mesh, colored by the electrostatic potential of the amino acids. Although the AAs involved in H-bonds are identical, the H-bond patterns of BYI09181 (**7**) and BYI08346 (**9**) differ from that of BYI06830 (**8**) in Figure 4, as the amide is rotated by 180° which is in line with the double minimum rotation potential of Figure 3, and hence donor and acceptor functionalities change. This aspect adds a further ambiguity to 3D-QSAR analyses, as mentioned by Horman et al. [29]. BYI09181 could possibly have a 3-point contact with Tyr^{408} compared to the 2-point interaction of BYI08346 and BYI06830, for the oxygen of its pyridine-methoxy group is very close to the phenolic OH-group of Tyr^{408}. Interestingly, the position of BYI08346 in its niche is slightly shifted relative to BYI06830 such that the 2-chloro substituent is now almost coincident with the 3-methyl group of BYI06830. What we originally posited as a necessity for the phenyl ring to move out of plane with the imidazole ring in order to imitate ring A of 20E has probably more to do with there being not enough room at the back of the niche to accommo-

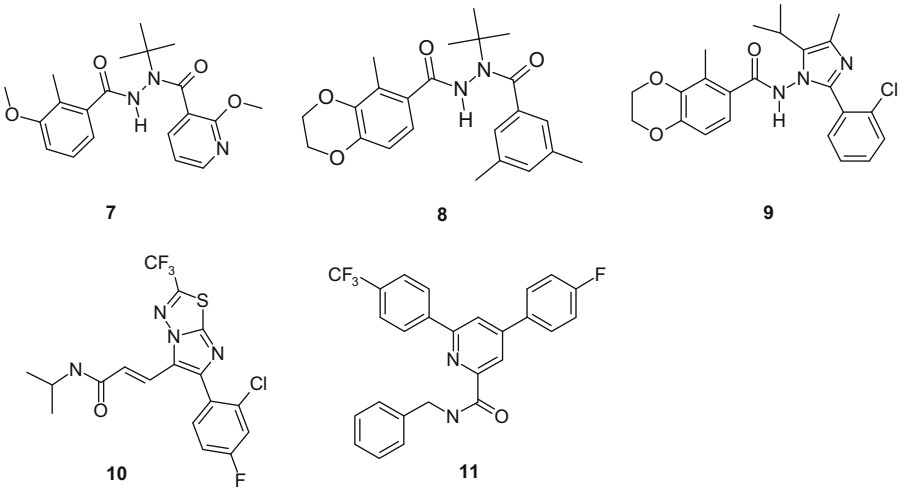


Figure 6. New EcR agonists BYI09181 (**7**), BYI6830 (**8**) BYI08346 (**9**), BYI06934 (**10**), and BYI08738 (**11**).

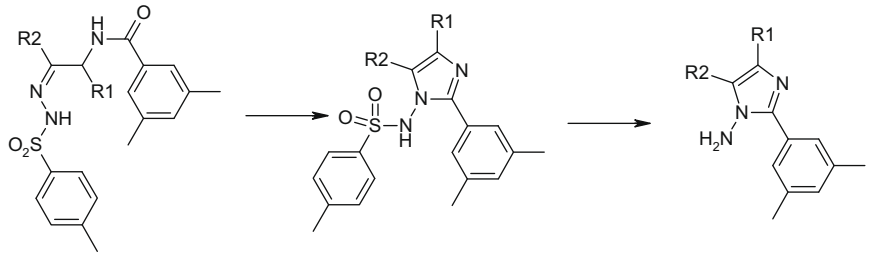
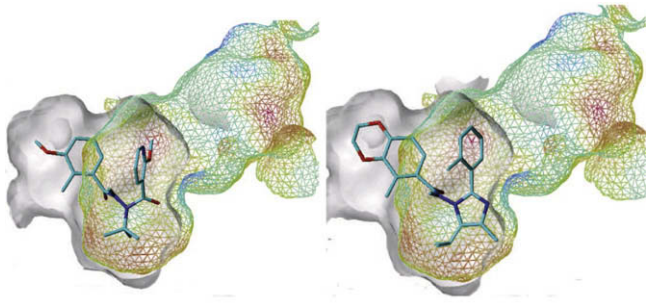


Figure 8. Synthesis route to BYI08346 (**9**).



(a) BYI09181 (**7**) (b) BYI08346 (**9**)

Figure 9. Overlap of HvEcR-USP 20E binding niche with Rohm & Haas type agonists.

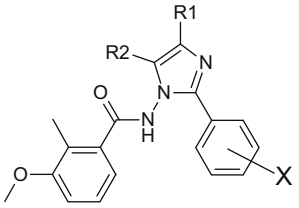


Figure 10. Imidazole-type agonists.

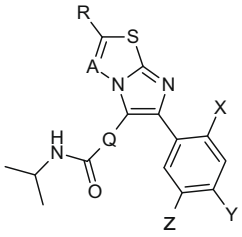


Figure 11. Thiadiazoloimidazole-type agonists.

Table 1
Receptor activity of agonists derived from Figure 10

No.	R^1	R^2	X	$pInd_{50}$	(20E)[a]
1	H	H	3,5-Dimethyl	4.3	(6.2)
2	H	CH_3	3,5-Dimethyl	4.8	(6.2)
3	H	CH_3	2,5-Dimethyl	5.6	(6.2)
4	CH_3	CH_3	3,5-Dimethyl	5.8	(6.2)
5	CH_3	CH_3	2-Chloro	6.1	(6.3)
6	CH_3	*i*-Propyl	2-Chloro	6.5	(6.3)

[a] Induction of reference compound 20-hydroxyecdysone.

date a 3,5-dimethyl substitution pattern - the X-ray structure subsequently showed that this unit is nowhere near the ring A position (see Section 3.3)

Some completely different chemical classes were designed and/or optimized from a knowledge of the shape of these binding niches. The precursors of BYI06934 (**10**) were originally noticed to have a similar qualitative SAR to the BYI08346 (**9**) class in biological screening (in particular the importance of the 2-chloro substituent), and their EcR agonistic activity was subsequently confirmed in a receptor test. The synthesis of this class has been published.[38] Again serendipity was involved, for, as it turned out, the position of the 2-chlorophenyl in the binding niche of BYI06934 does not coincide with that of BYI08346 in its niche.

The variation of intrinsic biological activity ($pInd_{50}$ values in the EcR induction assay) with structural changes is shown in Table 2. The basic structure with 2-chloro substitution performs very well. Moving the chloro substitution to the 4-position is severely punished. This was the similarity in SAR with the *N*-amino-imidazole class that was noticed originally. Activity is restored somewhat with 2,4-dichloro or 2-chloro-4-fluoro substitution but falls back with 2,5-dichloro. Hydrogenation of the double bond and replacement of a nitrogen in the thiadiazolo ring by CH are both disadvantageous. Replacement of the CF_3 group by CH_3 is catastrophic, but a C_2F_5 group gives a marked jump in intrinsic activity that is not really reflected in the biological screening results. The amido isopropyl group can be replaced within relatively wide limits so long as the amido group remains secondary.

The precursor of BYI08738 (**11**) was picked up in an EcR-HTS assay, and hit optimization led to this pyridine derivative, readily accessible via the Kröhnke synthesis.[39] In terms of their chemical structure these two compounds, the thiadiazoloimidazole **10** and the pyridine **11**, have only a distant resemblance, if at all, to the original DBHs.

It is therefore perhaps not surprising that their binding niches (see Fig. 12) differ both from those of the steroids and the DBHs. Somewhat related to the *tert*-butyl groups, the CF_3 groups of **7** and **8** explore a new hydrophobic region of the binding niche towards helix 12, and, in addition to occupying the typical DBH binding niche, the chain of BYI06934 (**10**) includes the D-ring region of the PonA niche while BYI08738 (**11**) even extends into to the C-ring region. While BYI06934 utilizes the same H-bond partners as the DBHs, BYI08738 has one less H-bond, as Asn^{504} finds no polar counterpart in the 4 – CF_3 – phenyl unit.

Table 2
Receptor activity of agonists derived from Figure 11

No	R	A	X	Y	Z	Q	$pInd_{50}$	(20E)[a]
1	CF_3	N	Cl	H	H	CH=CH	8.0	(6.7)
2	CF_3	N	H	Cl	H	CH=CH	6.4	(6.7)
3	CF_3	N	Cl	Cl	H	CH=CH	6.8	(6.7)
4	CF_3	N	Cl	H	Cl	CH=CH	6.6	(6.9)
6	CF_3	N	Cl	H	H	CH_2–CH_2	5.3	(6.7)
7	CF_3	CH	Cl	H	H	CH=CH	6.3	(7.0)
8	CH_3	N	Cl	H	H	CH=CH	–	(7.0)
9	C_2F_5	N	Cl	H	H	CH=CH	8.3	(6.6)

[a] Induction of reference compound 20-hydroxyecdysone.

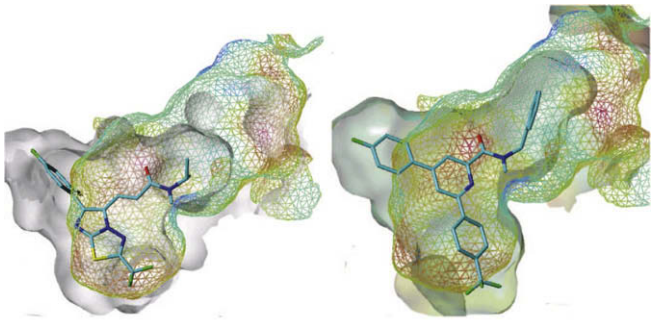


Figure 12. Overlap of HvEcR-USP 20E binding niche with non-Rohm & Haas type agonists.

Based on these results, one can conceive of further non-steroidal derivatives that could occupy even the steriodal A- and B-ring region of the binding niche.

4. Conclusion

Protein structure based design of ecdysone agonists revealed the tremendous structural adaptibility of the EcR LBD to artificial ligands which can create their own binding niches that are different from the steroidal ones. Based on the insights gained it was possible to design completely new chemical classes of non-steroidal and non-dibenzoylhydrazine agonists, some of which occupy almost the same binding niches as 20E or PonA. The insecticidal activity of these compounds was similar to those already introduced in the market, their relatively narrow spectrum, however, could not be widened. Thus we can conclude that the reasons for their selectivity are probably not connected to the shape and size of the binding niches.

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