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Agrochemistry/Insecticides

# Nicotinic Acetylcholine Receptor Agonists: A Milestone for Modern Crop Protection**

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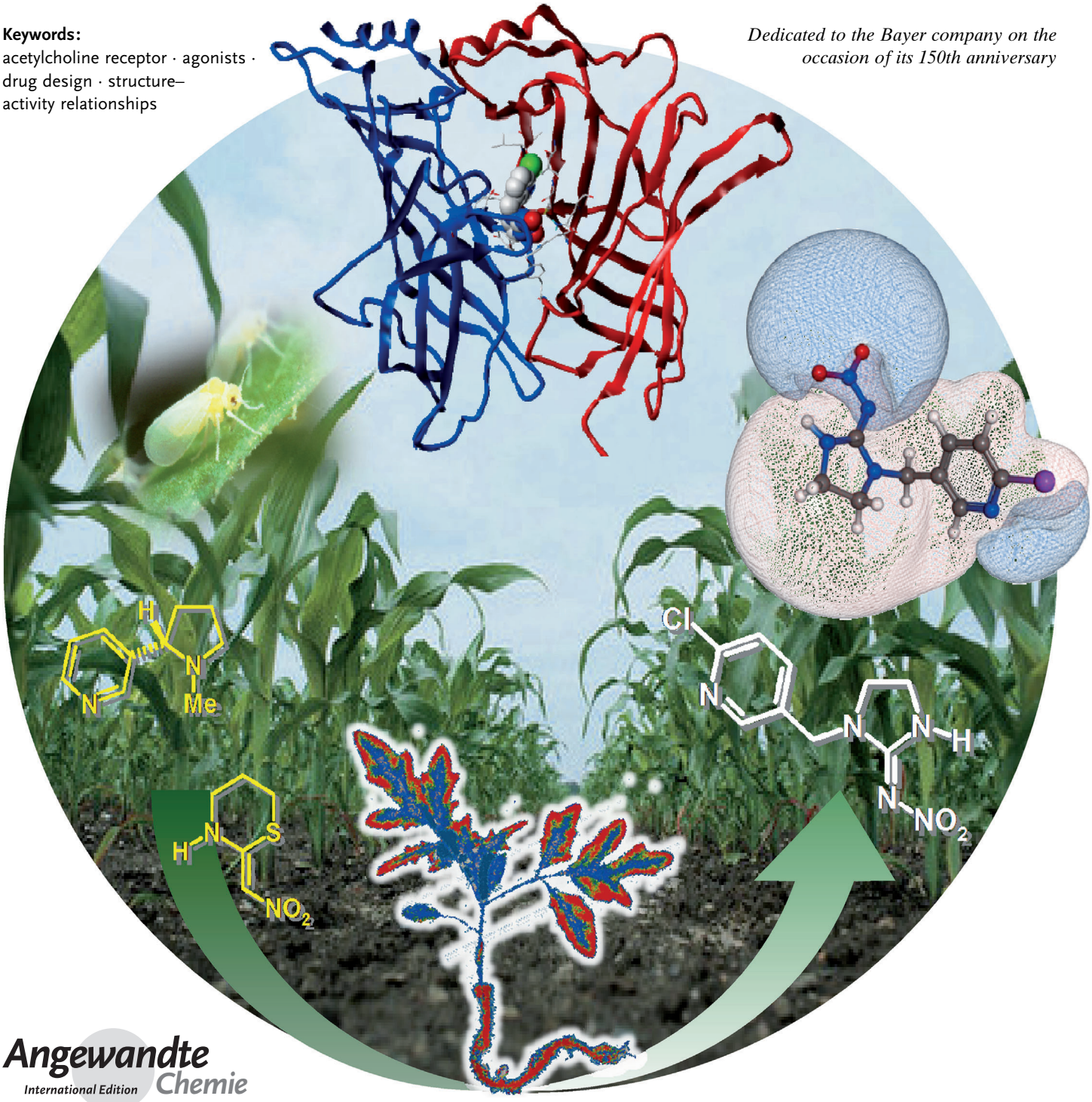
**Keywords:**
acetylcholine receptor · agonists · drug design · structure–activity relationships

*Dedicated to the Bayer company on the occasion of its 150th anniversary*



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*The destruction of crops by invertebrate pests is a major threat against a background of a continuously rising demand in food supply for a growing world population. Therefore, efficient crop protection measures in a vast range of agricultural settings are of utmost importance to guarantee sustainable yields. The discovery of synthetic agonists selectively addressing the nicotinic acetylcholine receptors (nAChRs), located in the central nervous system of insects, for use as insecticides was a major milestone in applied crop protection research. These compounds, as a result of their high target specificity and versatility in application methods, opened a new innovative era in the control of some of the world's most devastating insect pests. These insecticides also contributed massively to extending our knowledge of the biochemistry of insect nicotinic acetylcholine receptors. The global economic success of synthetic nAChR agonists as insecticides renders the nicotinic acetylcholine receptor still one of the most attractive target sites for exploration in insecticide discovery.*

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## 1. Introduction

Sustainable agriculture in the 21st century takes into account economic, ecological, and social aspects to produce sufficient high-quality and safe agricultural products for a continually growing world population in the face of dwindling resources, by minimizing the environmental impact of farming and biological diversity as far as possible. In this context, part of sustainable agriculture is the application of modern insecticides that address relevant target sites to protect crops from deleterious invertebrate pests feeding on plants cultivated for human nutrition.

The nicotinic acetylcholine receptor (nAChR) has been an insecticide molecular target site of growing importance for many years (total market share in 2011 for agricultural use: 30.8%). It plays a central role in the mediation of fast excitatory synaptic transmission in the central nervous system (CNS) of insects. Despite long-term use of the botanical insecticide (*S*)-(−)-nicotine (Figure 1), isolated as an agonistically active alkaloid from *Nicotiana* species and applied as aqueous tobacco extract (application of 2500 tons worldwide after 1945), the nAChR has been an underexploited biochemical target for new insecticides, reflected by an estimated total insecticide world market share of only 1.5% in 1987.[1] As a consequence of its high mammalian toxicity and limited insecticidal efficacy, no prominent class based on (*S*)-(−)-nicotine as a lead structure for novel nAChR agonists could be established for decades. However, more recently, the nAChR has become an important target site in modern crop protection with the discovery and commercialization of three classes of insecticides.

A small class of pro-insecticides (cartap hydrochloride, thiosultap sodium, bensultap, thiocyclam)[2] is based structurally on the neurotoxic and insecticidally active natural occurring insect-paralysing nereistoxin (Figure 1), an 1,2-dithiolane neurotoxic derivative isolated from the salivary glands of the marine annelid worm *Lumbriconer*cis *heteropoda* Marenz. These pro-insecticides are converted metabolically in the insect body into nereistoxin[3] and were classified by the Insecticide Resistance Action Committee (IRAC; an expert committee of CropLife International; http://www.irac.org) according to their mode of action (MoA) as nAChR-channel blockers in group 14.

The second class of insecticides is the fast-growing class of neonicotinoid insecticides, which belong to IRAC MoA group 4A and act selectively on the insect CNS as agonists of post-synaptic nAChRs (see Section 1.1, Figure 2). The third class are spinosyns, a family of fermentation-derived insecticidal macrocyclic lactones such as the naturally occurring bio-insecticide spinosad (a mixture of spinosyns A and D), produced by the actinomycete bacteria *Saccharopolyspora spinosa*, and the semisynthetic spinetoram (a mixture of

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[**] A list of important abbreviations can be found at the end of the article.

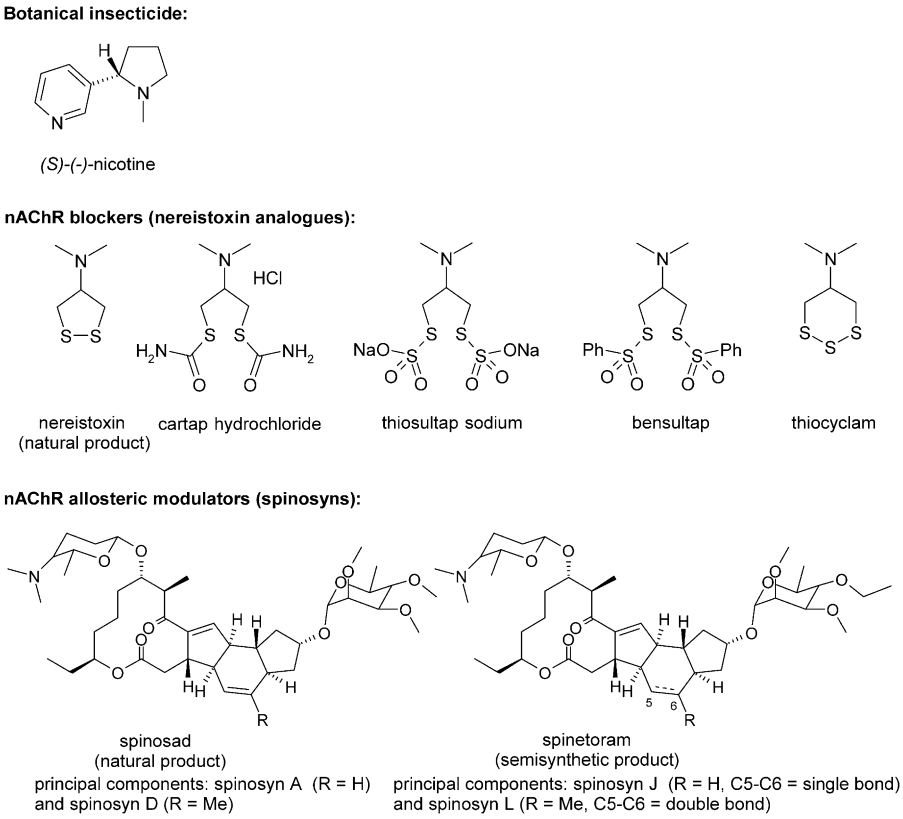


***Figure 1.*** Botanical insecticide (*S*)-(−)-nicotine, commercialized nAChR channel blockers (nereistoxin analogues), and nAChR allosteric activators (spinosyns).

spinosyns J and L; Figure 1).[4] As a result of their different binding sites compared to neonicotinoids, spinosyns have been classified by IRAC as nAChR allosteric modulators in MoA group 5 (sales in 2011: US$ 300 million).

In 2011, the market share of neonicotinoids in the total global market for insecticides (US$ 12.750 million) was 28.5%, as outlined in Table 1. Neonicotinoids are potent broad-spectrum insecticides that possess contact, stomach, and systemic activity. As these nAChR agonists have a new MoA, there was no cross-resistance to conventional long-established insecticide classes, and therefore the neonicotinoid class has begun to replace these older, and environmentally less-benign classes, such as sodium channel (SoCh) modulators (pyrethroids), acetylcholine esterase (AChE) inhibitors (organophosphates, carbamates), and several other classes of insecticides used in agriculture.

Neonicotinoids are especially active on hemipteran pest species such as aphids, bugs, whiteflies, and planthoppers, but they have also been commercialized to control different coleopteran and some lepidopteran pest species. As a consequence of their special physicochemical properties (see Section 2.3), these nAChR agonists are highly versatile in terms of application methods and they can be used with a wide range of different application techniques including foliar, seed treatment, soil drench, and stem application with several crops.

As a result of the competitive safety profile, the high target specificity, and versatility in application methods, the neonicotinoid class is globally an integral component in numerous pest and integrated pest management strategies (IPM). Innovative concepts for life-cycle management, jointly with the introduction of generic nAChR agonists, rendered neonicotinoids as the most important class introduced to the insecticide market. As a consequence of their

***Table 1:*** Sales of insecticide classes by MoA classification in 2011.[5]

| IRAC subgroup[a] | Mode of action classification[b] | Sales [in US$ million] (total insecticides [%])[c] |
|---|---|---|
| 4A | nAChR agonists | 3640 (28.5) |
| 3A | SoCh modulators | 2025 (15.9) |
| 1A, 1B | AChE inhibitors | 2330 (18.2) |

[a] 1A = Carbamates, 1B = OPs, organophosphates. [b] SoCh: sodium channel; AChE: acetylcholine esterase. [c] Total insecticide market, US$ 12 750 million.

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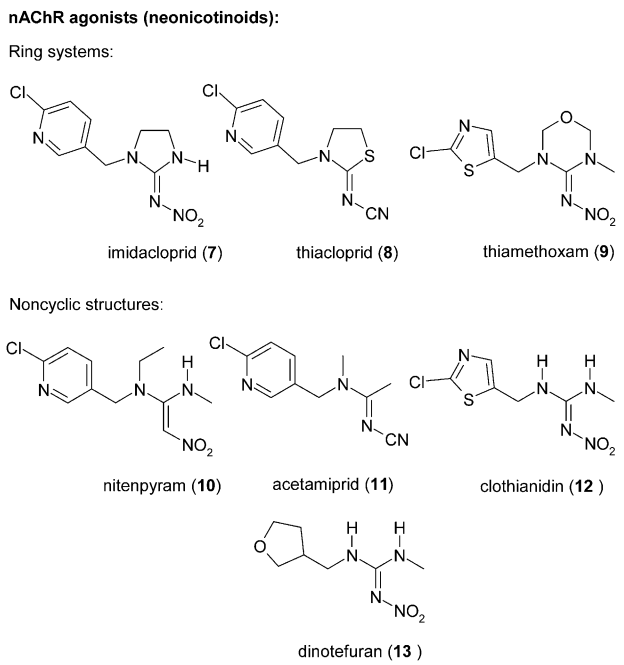


***Figure 2.*** Commercialized nAChR agonists (neonicotinoids) with ring systems and noncyclic structures.

favorable mammalian safety characteristics, some of them are also important in the control of subterranean pests such as termites and some veterinary pests.[6]

To date, it is impossible to arrive at perfect models of the native nAChRs of insects in terms of their functional architecture and molecular aspects of diversity. In particular, the use of neonicotinoids as highly effective probes have created a renaissance in the structural investigation of insect nAChRs as well as the understanding of ligand selectivity between insects and mammals. Within the past decade a wide variety of high-resolution crystal structures of soluble homopentameric acetylcholine-binding proteins (AChBPs) have provided the theoretical foundation for designing homology models of the corresponding receptor–ligand binding domains within nAChRs, thus becoming a useful basis for virtual screening of chemical libraries and the rational design of new nAChR agonists. This reflects the importance of the nAChR as a continuous source for the identification of modern insecticides with high efficacy and maximal safety.

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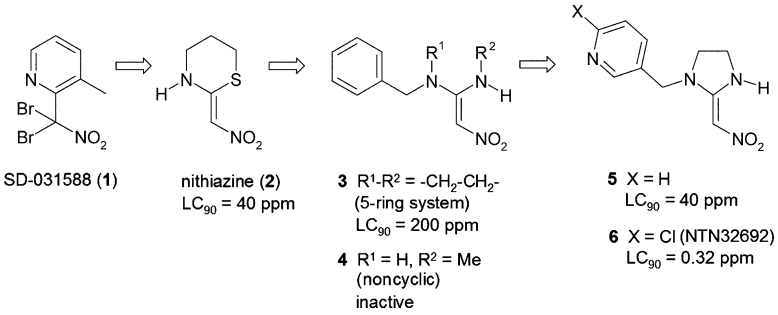
### *1.1. Historical Overview*

The discovery of the outstanding class of neonicotinoid insecticides and their introduction to the global markets are the result of generally applicable key factors for a long-term, successful design of active ingredients in combination with a well-focused and efficient optimization strategy. In the early 1970s, the Biological Research Center of the former Shell Development Company in Modesto, California, identified five- and six-membered, saturated nitromethylene-containing heterocycles as being active on insect nAChRs. Starting with a random screening to discover new lead structures from university sources,[7] Shell identified 2-(dibromonitromethyl)-3-methylpyridine (**1**, SD-031588), which showed an unexpected low-level insecticidal activity against houseflies (*Musca domestica* L.) and pea aphids (*Acyrtosiphum pisum* Harris). Rational and straightforward optimization cumulated in the finding of the nitroenamine nithiazine (**2**), the first neonicotinoid lead structure (Scheme 1).[8]




***Scheme 1.*** Development of the second lead structure (NTN32692, **6**); insecticidal activity ($LC_{90}$) against green rice leafhopper (*N. cincticeps*, third instar larvae); see Ref. [9].

Electrophysiological studies and radioligand binding assays revealed that **2** and structure-related nitromethylenes act on insect nAChRs in the same fashion as the (*S*)-(−)-nicotine.[10] Nithiazine (**2**) showed higher activity than parathion (OP) against adult houseflies, and was 1662 times more potent against corn earworm (*Helicoverpa zea* Boddie) larvae, combined with good systemic behavior in plants and low mammalian toxicity. However, because of its instability under hydrolytic and photolytic conditions (induced by the unstable [=CH-$NO_2$] chromophore, $\lambda_{max} = 343$ nm; $t_{1/2} = 0.5$ h) in field trials and its limited efficacy, **2** was never commercialized for broad agricultural use. In 1979, a synthesis project was started at Nihon Tokushu Noyaku Seizo KK (later Bayer CropScience KK, Japan) on the basis of nithiazine (**2**). However, it seemed at first there were no apparent structural similarities between (*S*)-(−)-nicotine and **2**. A new insecticide screening method using rice seedlings was developed for continuous monitoring of the combined systemic and contact

activities of all prepared compounds over a testing period of two weeks against rice leafhoppers (instead of formerly used *H. zea*), one of the major hemipteran pests of rice in Japan.[11] Replacement of the six-membered nithiazine (**2**) ring with different N-substituted ring systems showed that insecticidal activity against the green rice leafhopper (*Nephotettix cincticeps*) depended on ring size (seven- < six- < five-membered-ring systems). For example, the introduction of various substituents to the five-membered 2-nitromethylene-imidazolidine system demonstrated that only 1-benzyl-2-(nitromethylene)imidazolidine (**3**) enhanced the activity (Scheme 1). As a result of the complete loss of activity in the case of noncyclic structures such as **4** (Scheme 1), simple (five-membered) ring systems were preferred at that time. Replacement of the benzyl residue in **3**, for example by 4-chlorobenzyl or pyridine-3-ylmethyl in **5**, enhanced the insecticidal activity by a factor of 5 and 25, respectively, thus providing clear evidence that the whole molecule is the active structure and not just the 2-nitromethylene-imidazolidine system (if formed after metabolic cleavage of the *N*-benzyl group), similar to **2**. Further stepwise optimization resulted in the second lead structure NTN32692 (**6**) with a unique 6-chloropyridin-3-ylmethyl residue, which exhibited an over 100-fold greater activity than **2** against strains of green rice leafhoppers with resistance to OPs, carbamates, and pyrethroids. The introduction of a single chlorine (X = Cl) atom at the 6-position of the pyridin-3-ylmethyl residue of **5** resulted in the molecular properties and insecticidal activity being changed dramatically—this discovery was an epoch-making event.[9]

However, similar to **2**, its [=CH-$NO_2$] chromophore strongly absorbs sunlight, and decomposes rapidly under field conditions ($\lambda_{max} = 323$ nm; $t_{1/2} = 1$ h). After preparation of about 2000 compounds, including intensive research on more stable functional groups, imidacloprid (**7**) containing a [=N-$NO_2$] chromophore ($\lambda_{max} = 269$ nm; $t_{1/2} = 3$ h) was identified[12] (Figure 2).

Compared with **2**, the insecticidal activity of imidacloprid (**7**) against the green rice leafhopper (*N. cincticeps*) could be enhanced 125-fold. In addition, **7** is about 10000-fold more active than (*S*)-(−)-nicotine. The synthesis of this novel systemic insecticide **7** was achieved by combination of the unique 6-chloropyridin-3-ylmethyl residue with the 2-(*N*-nitroimino)imidazolidine system, new in this class of chemistry. Subsequently, a parallel change to the [=N-CN] chromophore ($\lambda_{max} = 242$ nm) led to the discovery of the neonicotinoid insecticide thiacloprid (**8**; Figure 2).

With the introduction of the insect *n*AChR agonist imidacloprid (**7**) to the market in 1991 by Bayer CropScience AG, the success story of the neonicotinoids started, and this was definitely also a milestone in insecticide research and for modern crop protection.[13] Imidacloprid (**7**) has become the most successful, highly efficacious, and best-selling insecticide worldwide, important for the control of invertebrate pests in numerous crops, and for veterinary use as well. Its extremely high insecticidal potency triggered extensive research programs within several other research-based agrochemical companies. They preferred to investigate scaffolds based on noncyclic structures as well as six-membered ring systems and developed further neonicotinoid insecticides (Figure 2).

### 1.2. Scope and Focus of the Review

This Review focuses on selected synthetic modifications of nAChR agonists, rather than on nAChR channel blockers and nAChR allosteric modulators. The sections of this Review cover structural characteristics of neonicotinoids and encompass their physicochemical properties, high biochemical target-site selectivity, biological profile and applied aspects, phytotonic effects, and quantitative structure–activity relationships (SARs), as well as novel candidates in agrochemical development, and recent trends in the search for new lead structures of nAChR agonists. Many aspects of the chemistry (including industrial processes), biochemistry, and biology of this successful substance class have been thoroughly investigated and reviewed in numerous articles and book chapters.[1,13,14] Therefore, we have limited the scope of this Review to work published mainly since 1998, covering highlights derived from chemo- and biorational approaches as influenced by the current knowledge about AChBPs and insect nAChR structure, target-based screening of substances, and development of new nAChR agonists during this time period. The intention of this Review is to highlight the importance of nAChRs as molecular target sites for insecticides and to focus on nAChR agonists as well as molecular tools in insect neurochemistry. However, increasing levels and cases of neonicotinoid resistance in major pests targeted by this chemistry underpin the need for continuing research to discover novel nAChR agonists with resistance-breaking properties, promising efficacy, and high target selectivity.

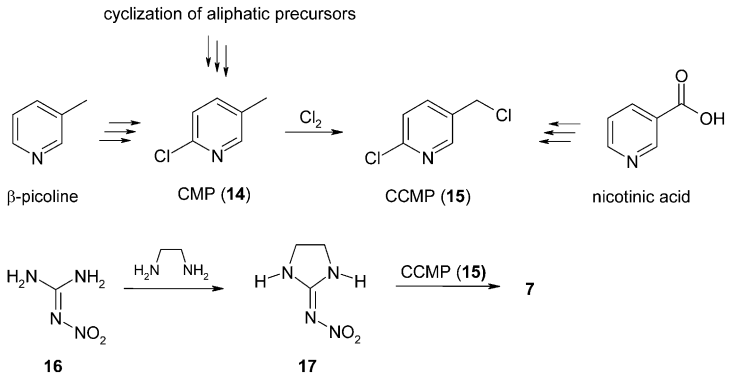
## 2. Commercial Agonists

Seven neonicotinoids are commercially well-established: the two five-membered-ring systems imidacloprid (**7**) and thiacloprid (**8**; Bayer CropScience AG) and one six-membered-ring system, thiamethoxam (**9**; Syngenta AG), as well as the four noncyclic structures nitenpyram (**10**; Sumitomo Chemical Takeda Agro Company), acetamiprid (**11**; Nippon Soda), clothianidin (**12**; Sumitomo Chemical Takeda Agro Company/Bayer CropScience AG), and dinotefuran (**13**; Mitsui Chemicals; Figure 2).[14]

### 2.1. Industrial Processes

Several overviews on the industrial preparation of the ring systems **7**–**9** and noncyclic structures **10**–**13** (Figure 2)[14a,b] have been described, as exemplified by two important nAChR agonists, the five-membered imidacloprid (**7**) and the noncyclic structure clothianidin (**12**).

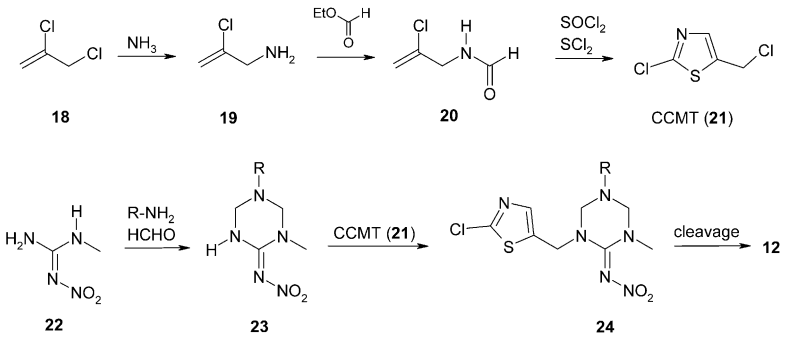
A number of methods are known for the syntheses of 2-chloro-5-methylpyridine (CMP, **14**) or its chlorinated derivative 2-chloro-5-(chloromethyl)pyridine (CCMP, **15**) for the synthesis of **7**. As outlined in Scheme 2, various routes based




***Scheme 2.*** Synthesis of the five-membered-ring imidacloprid (**7**).

on the commercially available starting materials β-picoline, nicotinic acid, or aliphatic precursors such as propionic aldehyde and acrylic acid lead to **14** or **15**. Finally, **7** can be obtained by N-alkylation of the 2-*N*-nitroiminoimidazolidine (**17**), prepared by cyclocondensation of *N*-nitroguanidine (**16**) and 1,2-ethylenediamine with CCMP (**15**; Scheme 2). Clothianidin (**12**) can be synthesized by a convergent pathway from N-alkylated 2-(*N*-nitroimino)hexahydro-1,3,5-triazine (**23**) and 2-chloro-5-(chloromethyl)-1,3-thiazole (CCMT, **21**).

Starting from *N*-methyl-*N*′-nitroguanidine (**22**; easily obtained by treatment of **16** with methylamine) and formaldehyde in the presence of a primary amine (Mannich reaction), the 6-membered ring intermediate **23** is formed. The 2-chloro-2-propene-1-amine (**19**) can be obtained from 1,2-dichloro-2-propene (**18**) and leads after N-formylation to the *N*-formyl-2-chloro-2-propene-1-amine (**20**). After its halogenation, followed by a cyclization, CCMT (**21**) is obtained. The final preparation of **12** is performed by N-alkylation of **23** with **21** and a subsequent ring cleavage reaction of the bis(aminal) structure **24** (Scheme 3).

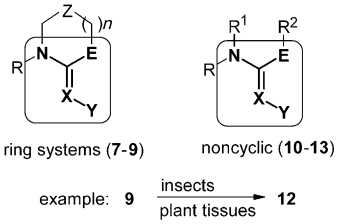



***Scheme 3.*** Synthesis of the noncyclic clothianidin (**12**).

### 2.2. Structural Diversity

The term neonicotinoid was originally proposed by Yamamoto[15] for imidacloprid (**7**) and related insecticides to differentiate these novel nAChR agonists from older nicotinoid insecticides such as (*S*)-(−)-nicotine. From consideration of the pharmacophore feature [-*N*-C(E)=X-Y], as outlined in Scheme 4, commercial neonicotinoids can be classified as *N*-nitroguanidines (**7**, **9**, **12**, **13**), nitromethylenes (**10**), and *N*-cyanoamidines (**8**, **11**; see also Figure 2).

The overall chemical feature of both ring systems and noncyclic structures consists of different segments: For the five- and six-membered-ring systems




***Scheme 4.*** Ring systems (**7**–**9**) versus noncyclic structures (**10**–**13**) and example of the transformation of **9** to **12** by ring cleavage.

(**7**–**9**), the bridging fragments [-$CH_2$-Z-$(CH_2)_n$-] ($n=0$, Z = $CH_2$; $n=1$, Z = O), and for noncyclic structures (**10**–**13**) the separate substituents $R^1$ (H, ethyl) and $R^2$ (methyl); the hetarylmethyl (6-chloropyridin-3-ylmethyl, 2-chloro-1,3-thiazol-5-ylmethyl) or heterocyclylmethyl ((*RS*)-(±)-tetrahydrofur-3-ylmethyl) group, and the functional group [=X-Y] (e.g. [=N-$NO_2$], [=CH-$NO_2$], and [=N-CN]) as part of the different pharmacophore types [-*N*-C(E)=X-Y]. By metabolic cleavage of the six-membered-ring system thiamethoxam (**9**) in vivo (e.g. bioactivation by oxidation), the noncyclic clothianidin (**12**) can be formed in insects and plant tissues (also see example in Scheme 4).[16a–d] On the other hand, **9** and **12** were described as noncompetitive inhibitors of the insect nAChR.[16e]

#### 2.2.1. Bioisosteric Segments

Compared with corresponding ring systems (**7**–**9**), the noncyclic structures (**10**–**13**) exhibit a similar broad-spectrum insecticidal activity, forming a so-called "quasi-cyclic" conformation when binding as agonists to the insect nAChRs.[17] In Figure 3 the superimpositions of van der Waals volumes of ring systems (**7**–**9**) and noncyclic structures (**10**–**13**), represented by Connolly surfaces, are shown. Compared with the nitrogen-containing hetarylmethyl groups, replacement by the bioisosteric (*RS*)-(±)-tetrahydrofur-3-ylmethyl residue resulted in a markedly weaker hydrogen-bond acceptor at the nAChR target site.[18]

On the other hand, both the electrostatics and the spatial relation of the pharmacophore feature [-*N*-C(E)=X-Y] are very much in line with the nAChR agonist binding. Besides its influence on biological activity, the pharmacophore group is not only responsible for the photolytic stability but also for some specific properties such as degradation in soil, metabolism in plants, and lack of toxicity to different animals and beneficial insects.[1]

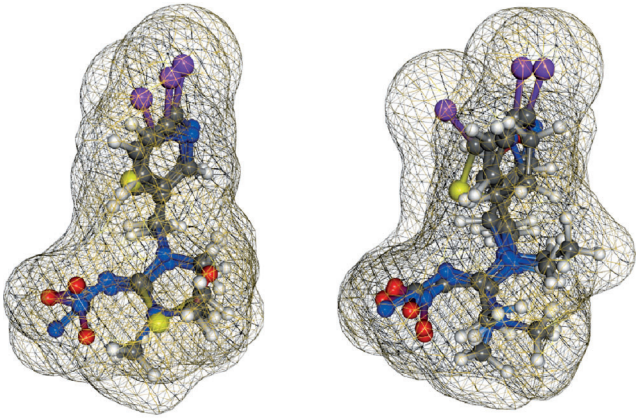



***Figure 3.*** Superimposition of the van der Waals volumes (represented by Connolly surfaces) of ring systems (**7**–**9**) in their minimum energy conformations versus noncyclic structures (**10**–**13**).

### 2.3. Physicochemical Properties

The physicochemical properties of nAChR agonists are considered of utmost importance for their successful development as modern insecticides. In this context, photostability is a significant factor in their field performance.[19] For technical application methods in the field, such as soil drench, seed treatment, and foliar application, their uptake, translaminar, and acropetal translocation in plants is crucial for their insecticidal activity against numerous sucking pests (see Section 2.3.1). Therefore, not only the bioisosteric segments such as the 6-chloropyridin-3-ylmethyl and 2-chloro-1,3-thiazol-5-ylmethyl group (see Section 2.2.1), but also the whole molecular shape has to be considered for their water solubility and lipophilicity, as described by their 1-octanol/water partition coefficient ($\log P_{ow}$; Table 2, where $P_{ow}$ is the partition coefficient in the 1-octanol/water system), is commonly used as a surrogate measure of lipophilicity and determines membrane permeation, transport, and translocation of compounds in living systems.[20]

***Table 2:*** Overview of nAChR agonists according to their pharmacophore type, solubility in water, and $\log P_{ow}$ values.

| nAChR agonists [pharmacophore type][a] | Solubility in water ($g\,L^{-1}$ at 20 °C)[b] | Log $P_{OW}$ (at 25 °C)[c] |
|---|---|---|
| **10** [*N*-C(N)=CH-$NO_2$] | 840 | −0.64 |
| **13** [*N*-C(N)=N-$NO_2$] | 54.3 ± 1.3 | −0.644 |
| **9** [*N*-C(N)=N-$NO_2$] | 4.10 | −0.13 |
| **7** [*N*-C(N)=N-$NO_2$] | 0.61 | 0.57 |
| **12** [*N*-C(N)=N-$NO_2$] | 0.327 | 0.7 |
| **11** [*N*-C(Me)=N-CN] | 4.20 | 0.8 |
| **8** [*N*-C(S)=N-CN] | 0.185 | 1.26 |
| **41** [*N*-cyansulfoximine] | 0.67 | 0.802 |
| **50** [butenolide] | 3.24 | 1.49 |

[a] Pharmacophore type [*N*-cyanosulfoximine] for sulfoxaflor (**41**; see Section 8.3.1) and [butenolide] for flupyradifurone (**50**; see Section 8.3.3). [b] Solubility in water for **10** at pH 7; **9** and **11** at 25 °C. [c] Log $P_{ow}$ for **7** at 22 °C, **8** at 20 °C.

Lipophilicity refers to the ability of active ingredients to dissolve in fats, oils, and lipids. Insecticides that are lipophilic ($\log P_{ow} > 4$) are generally not systemic, whereas those compounds considered moderately lipophilic have a $\log P_{ow}$ value between 0.5 and 3.5. These insecticides, once taken up, move within the plant xylem and are translocated to growing shoots. Uptake by the roots is greater when the insecticides are more lipophilic.[21]

Generally, commercial neonicotinoids have low $\log P_{ow}$ values (Table 2), which permit good plant systemically mediated control of piercing-sucking insects. Compared with nonpolar insecticides, the polar, nonvolatile compounds have greater water solubilities (e.g. in the case of **10**: $840\,g\,L^{-1}$) and lower $\log P_{OW}$ values (e.g. **13**: −0.644 at 25 °C). From these observations, the following conclusions can be drawn:

- In general, noncyclic structures are less lipophilic than the corresponding five- and six-membered ring systems.
- Water solubility is influenced by the functional group [=X-Y] within the pharmacophore moiety [-*N*-C(E)=X-Y] and increases in the order [=N-$NO_2$] < [=N-CN] < [=CH-$NO_2$].
- With regard to E, the lipophilicity increases in the order NH < O < C < S.[22]

According to Briggs et al.,[23] more lipophilic neonicotinoid insecticides should be favorable for seed treatment applications, because of their superior uptake by the roots and subsequent translocation. As a result of the higher lipophilicity, **8** and **12** show the best uptake by the roots, whereas **10** and **12** are more xylem-mobile than the other members. The extent of uptake by the root can be quantitatively described by the transpiration stream concentration factor (TSCF), which is defined as the ratio of the concentration of a compound in the transpiration stream (xylem) versus the concentration in the external solution.

By fitting a Gaussian curve to TSCFs observed experimentally for numerous compounds (as a function of $\log P_{ow}$) in barley shoots, Briggs et al.[23] found that to a good approximation $TSCF(\log P_{ow}) = 0.784\ \exp[-(\log P_{ow} - 1.78)^2/2.44]$. Figure 4 maps the compounds listed in Table 2 onto this relationship, clearly illustrating that relatively small differences in lipophilicity can have a rather pronounced impact on uptake by the root.

## 3. Selective Insecticides Are Significant

In the last three decades a number of new chemical classes of insecticides were introduced, not necessarily addressing new MoAs but being more selective in terms of toxicodynamic properties in insect pest species. Agonists of the nAChR are among the new chemical classes introduced and showing a favorable environmental and safety profile. Neonicotinoid insecticides, for example, address the cholinergic pathway, but their selectivity is much higher than other insecticides that either address the same target site or cover at least the same pathway by interfering with enzymes such as AChE (Table 3).

***Table 3:*** Selective toxicity of nAChR agonists and AChE inhibitors as a comparison of $LD_{50}$ values obtained after oral exposure of rats and oral application in green peach aphid (*M. persicae* Sulzer).

| Insecticide | Mode of action | Oral toxicity rat $LD_{50}$ [mg kg$^{-1}$] | Oral toxicity aphid $LD_{50}$ [mg kg$^{-1}$] | Selectivity (rat/aphid) |
|---|---|---|---|---|
| (*S*)-(−)-nicotine | *n*AChR | 50 | >5 | <10 |
| imidacloprid (**7**) | *n*AChR | 450 | 0.36 | 1300 |
| clothianidin (**12**) | *n*AChR | >5000 | 0.14 | 36000 |
| pirimicarb | AChE | 150 | 0.50 | 300 |
| oxydemetonmethyl | AChE | 70 | 0.98 | 71 |

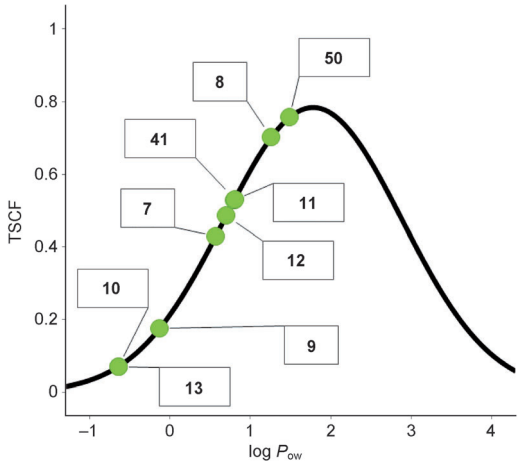



***Figure 4.*** Transpiration stream concentration factors (TSCF) of nAChR agonists taken from Table 2 as a function of log $P_{ow}$ values.

Insecticide selectivity has become increasingly important, particularly in regard to increasing environmental and human safety measures for regulatory approval, such as highly sophisticated safety constraints concerning application and consumer safety.

The selectivity of nAChR agonists such as imidacloprid (**7**) or clothianidin (**12**) based on their acute toxicity is extremely high compared to plant-derived (*S*)-(−)-nicotine preparations, as shown in Table 3. In the USA, for example, the last registrations of (*S*)-(−)-nicotine for food use were only cancelled in 1994, three years after the introduction of **7** as the first neonicotinoid insecticide. Neonicotinoids are also much more selective than AChE inhibitors, which were the most important chemical candidates for controlling sucking pests before the introduction of SoCh modulators and agonists of the nAChR (see also Table 1, Section 1). However, insecticide selectivity is also very important in IPM programs where chemical insecticides are combined with beneficial insects as biological control measures. Therefore, the IPM fitness of a compound is of utmost importance these days, and agonists of the nAChR, particularly when applied systemically, meet the requirements of modern IPM-friendly insecticides when compared with other chemical classes.[24,25] Selectivity for beneficials and pollinators has, in particular, been optimized by selectivity in space and time, thus allowing, for example, foliar applications against starting pest populations when beneficial arthropods are still absent.[26]

### *3.1. Target-Site Selectivity*

In contrast to (*S*)-(−)-nicotine, which is fairly toxic to mammals, neonicotinoid insecticides act selectively as reversible agonists of the post-synaptic nAChRs of the insect CNS. Insecticides such as **7** or **12** show either little or almost no binding affinity to mammalian nAChRs (e.g. α4β2-nAChRs) because of striking differences in the architecture of the nAChRs of insects and mammals, particularly in regard to amino acid residues presumed to be involved in ligand binding (see Section 4.3).[18a,27] The binding affinity of imidacloprid (**7**) was shown to be at least 1000-fold lower to mammalian neuronal α4β2-nAChRs than to insect nAChR preparations from different species. This is "*due to the interaction of imidacloprid with a unique subsite consisting of cationic amino acid residue(s) in insect receptors and lacking in mammalian nAChRs*", as outlined by Tomizawa and Casida.[28] The recent finding of a loss of neonicotinoid binding by an nAChR mutation in the *M. persicae* β1 subunit, which results in a substitution of arginine to threonine in loop D, nicely demonstrates the importance of a positively charged amino acid residue for ligand binding (see also Figure 7, Section 4.3). The so-called R81T mutation confers a loss of direct electrostatic interactions between the electronegative pharmacophore and the basic arginine residue at this key position within loop D, thereby resulting in a high resistance of such genotypes to neonicotinoids.[29]

All commercial nAChR agonists bind selectively to insect nAChRs and evoke the same effect as the natural neurotransmitter acetylcholine (ACh), that is, agonistic activation of the receptors by causing a transient inward current that leads to the generation of action potentials. Similar to ACh, neonicotinoid binding to nAChRs is reversible, as shown by their rapid desensitization/recovery during short-term exposure in electrophysiological whole-cell voltage clamp assays on isolated neurons from both mammals and insects.[18a] Radioligand binding studies conducted with [$^3$H]-**7** and using insect head membrane preparations revealed selective high-affinity and specific and reversible binding with fast kinetics.[30] Taking into consideration that mammalian nAChRs show none to low affinity to imidacloprid (**7**) at concentrations in the micromolar range at the target site, a high selectivity for insecticidal action is obtained with neonicotinoid insecticides. The broad knowledge generated on the functional architecture and molecular features of the insect and mammalian nAChRs and their ligand-binding site helped to explain the selectivity of this class of safe and effective insecticides.

## *4. Nicotinic Acetylcholine Receptors*

The cation-selective nAChRs belong to the "Cys-loop" superfamily of ligand-gated ion channels (LGICs) responsible for rapid excitatory neurotransmission. They are well-char-

acterized, complex, large pentameric transmembrane allosteric proteins (molecular weight ca. 290 kDa), and involved in the rapid gating of ions elicited by ACh at the vertebrate neuromuscular junction as well as in all animal central and peripheral nervous systems.[31]

The nAChRs are homo- or heteromeric pentamers of structurally related subunits that encompass an extracellular N-terminal domain which has six distinct regions (loops A–F) involved in ligand binding, as well as a Cys-Cys loop, four C-terminal transmembrane-spanning domains (TM1–TM4) that form the cation-permeable channel, and an intracellular region extending from TM3 to TM4.[32] Each subunit comprises approximately 500 amino acids, and is encoded by a separate gene, and possesses the four transmembrane domains (TM1–TM4). The subunits are oriented around the central pore, such that the resulting transmembrane ion channel is formed by a pentameric arrangement of the TM2 helical segments contributed by each of the five subunits. In vertebrates, 17 nAChR subunits have been identified (α1–α10, β1–β4, γ, δ, and ε), which can coassemble to generate a diverse family of nAChR subtypes. In insects, several genes have been identified that encode multiple nAChR subunits, which suggests the existence of a range of subtypes of insect receptors across species.[33] As an agonist-gated ion channel complex for excitatory neurotransmission, the nAChR is widely distributed in the insect CNS, and constitutes a major target for insecticide action. Compared to their vertebrate counterparts, the functional architecture, diversity, and three-dimensional (3D) tertiary structure of native insect nAChRs are poorly understood.[34]

Insect nAChR gene families are smaller than those known for vertebrates and even other invertebrates such as *Caenorhabditis elegans*.[36] To date, various complete insect nAChR gene families have been described as a result of completely sequenced insect genomes, for example, from *Drosophila melanogaster*,[37a] which includes 10 nAChR subunits (seven Dα1–7 and three Dβ1–3) similar to the malaria vector *Anopheles gambiae* (nine Agamα1–α9 and one Agamβ1). Eleven subunits have been described in the honey bee (*Apis mellifera*), which include nine Amelα (1α–α9) and two Amelβ (β1 and β2).[37b] As with many other integral membrane proteins, it has not yet been possible to obtain crystals of any nAChR of sufficient quality to conduct high-resolution X-ray crystallographic studies. Today, both the crystal structure of AChBPs and the refined model of the membrane-associated *Torpedo* AChR,[35a] are available to describe the interactions between nAChRs and corresponding nAChR agonists (see Figure 5).

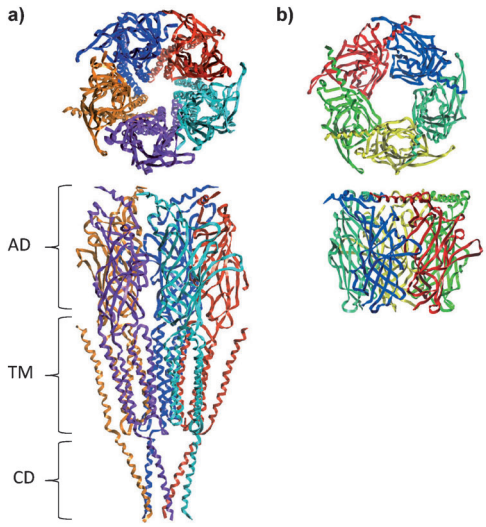



***Figure 5.*** a) 3D Structure of *Torpedo marmorata* nAChR and side view (top) and along the direction of the channel (bottom). The agonist binding domain, the transmembrane domain, and the cytoplasmic domain are denoted "AD", "TM", and "CD", respectively. b) The corresponding views of the 3D structure of *Ac*-AChBP. The pictures were created from PDB entries 2BG9 and 2BYN, see Refs. [35a] and [35b].

### 4.1. Acetylcholine-Binding Proteins

An important breakthrough in the understanding of the Cys-loop receptor (CLR) structure, in particular with respect to the ligand-binding mechanism, was derived from the characterization and structural determination of various soluble homopentameric AChBPs.

The AChBP has the same overall architecture as the extracellular portion of the nAChR[38] and the presence of the vicinal Cys pair characteristic of ligand-binding receptor subunits. AChBPs are secreted from glia cells in the CNS of molluscs, such as the fresh-water snail *Lymnea stagnalis* (*Ls*-AChBP)[39] and the seawater mollusc *Aplysia californica* (*Ac*-AChBP), which shares only 33% amino acid identity with *Ls*-AChBP but possesses all of the functional residues identified in *Ls*-AChBP.[40] The *Ac*-AChBP shows a similar high sensitivity for both neonicotinoids and nicotinoids such as (*S*)-(−)-nicotine, whereas *Ls*-AChBP exhibits lower neonicotinoid and higher nicotinoid sensitivities (Table 4).[41]

***Table 4:*** Crystal structures of mollusc AChBPs in the apo form and in complex with natural as well as commercial agonists of nAChR, sorted by resolution in Å.

| PDB-ID[a] | Organism[b] | Ligand[c] | Resolution | Year |
|---|---|---|---|---|
| 2XZ5 | *Ac* | ACh | 2.80 | 2010 |
| 2ZJV | *Ls* | **12** | 2.70 | 2008 |
| 2ZJU | *Ls* | **7** | 2.58 | 2008 |
| 3C79 | *Ac* | **7** | 2.48 | 2008 |
| 1UW6 | *Ls* | (*S*)-(−)-nicotine | 2.20 | 2004 |
| 2BYN | *Ac* | apo form | 2.02 | 2005 |
| 3C84 | *Ac* | **8** | 1.94 | 2008 |
| 2Y7Y | *Ac* | apo form | 1.90 | 2011 |

[a] Nomenclature follows the respective numbering in the PDB files. [b] Ac = *Aplysia californica*, LS = *Lymnea stagnalis*, [c] ACh = acetylcholine.

In 2005, Unwin described a refined (cryo)electron microscopy structure of the heteropentameric vertebrate muscle type receptor, which demonstrated considerable structural similarity to the *Ls*-AChBP ligand-binding domain (LBD; Figure 5).[35a]

### 4.2. Agonist Binding Sites

The ACh binding site of the nAChR is located on the hydrophilic extracellular domain of the nAChR at the interface of two adjacent subunits and is formed by six distinct regions (loops A–F) in the N-terminal extracellular domain with each of adjacent subunits contributing three loops.[42] Several studies have allowed the identification of key interactions that lead to the binding of ligands such as ACh and (*S*)-(−)-nicotine at the agonist binding site of nAChRs, which is also conserved in AChBP.[43]

### 4.3. Mode of Agonist Binding

Before intensive crystallographic studies on AChBP were conducted, the structure of the nAChR binding site(s) and the rational design of nAChR agonists was facilitated by the identification of a specific 3D arrangement of essential chemical groups common to nAChR ligands, so-called pharmacophores (see Section 2.2). Although several early "nicotinic pharmacophores" were described, these either did not take into consideration any specific binding data, or they were derived on the basis of pharmacological data.[44] More recently, AChBPs have been cocrystallized with bound nAChR agonists, thereby providing detailed structural information and identifying key residues involved in binding (Table 4). The high-resolution crystal structures of mollusc AChBPs in complex with nAChR agonists **7**, **8** and **12** (Table 4,[45] Figure 6) have shown the binding orientation and molecular recognition of their distinctive electronegative *N*-nitroimino or *N*-cyanimino pharmacophores, in contrast to a cationic functionality of nicotinic agonists such as (*S*)-(−)-nicotine.[46]

Figure 6 compares the modes of binding of (*S*)-(−)-nicotine and imidacloprid (**7**) to AChBP from *A. californica* and *L. stagnalis*. The figure has been created from PDB entries 1UW6 ((*S*)-(−)-nicotine in complex with *Ls*-AChBP[42b] (Figure 6b)), 2ZJU ((**7**) in complex with *Ls*-AChBP[45b] (Figure 6d)), and 3C79 ((**7**) in complex with *Ac*-AChBP,[45a] (Figure 6e)). Figure 6c shows a model of the binding mode of (*S*)-(−)-nicotine to Ac-AChBP, based on 3C79 and 1UW6. For ease of reading, hydrogen atoms taking part in ligand binding have been modeled for Figure 6b–e.

In all four cases, aromatic amino acids build a fence that interacts with either the protonated nitrogen atom in (*S*)-(−)-nicotine or the positively polarized *N*-nitroguanidine π system of **7**. In addition, a conserved water molecule mediates a hydrogen bridge between the pyridine nitrogen atoms of both **7** and (*S*)-(−)-nicotine and the loop D backbone. When overlaying multiple X-ray structures of AChBP in complex with nAChR agonists, the position of this water molecule appears to be strikingly conserved, thus hinting at its high importance for ligand binding. As a consequence of the protonation of (*S*)-(−)-nicotine under physiological conditions, a hydrogen bond to the backbone tryptophan (W143) is likely (Figure 6b,c). An analogous hydrogen bond is observed in the binding mode of clothianidin (**12**) in complex with *Ac*-AChBP, where the hydrogen bond to W143 involves an NH group of its guadinium center (not shown, Ihara et al.[45b]). Comparison of the binding modes of **7** to *Ls*- and *Ac*-AChBP reveals subtle but decisive differences in loop D. In *Ac*-AChBP, the *N*-nitroimino group builds a hydrogen bridge to a glutamine residue (Q57; see Figure 6e). No such interaction can be found in *Ls*-AChBP (Figure 6d,e).

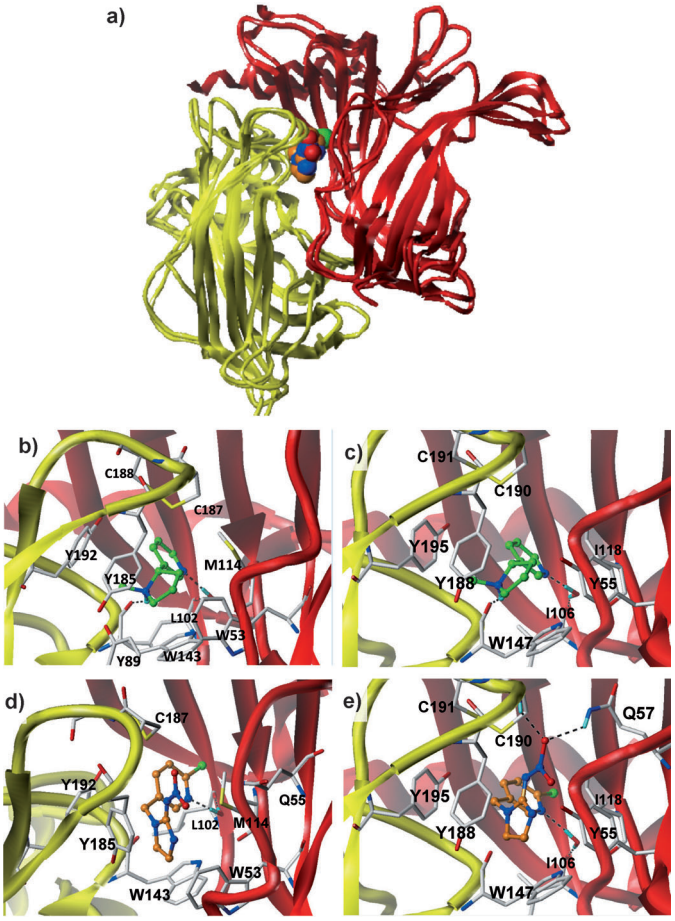



***Figure 6.*** Binding modes of imidacloprid (**7**) and (*S*)-(−)-nicotine to *Ac*-AChBP and *Ls*-AChBP. a) Overlay of two neighboring monomers, generated from PDB entries 3C79, 1UW6, and 2ZJU. Only for the latter, is the ligand **7** shown. The remaining panels zoom into the respective structures, but adopt similar perspectives. b) Binding of (*S*)-(−)-nicotine to *Ls*-AChBP (PDB-ID 1UW6). c) Binding of (*S*)-(−)-nicotine to *Ac*-AChBP (model generated from 3C79). d) Binding of **7** to *Ls*-AChBP (model generated from 2ZJU). Binding of **7** to *Ac*-AChBP (model generated from 3C79).

As a result from these interactions, the pyridine rings of imidacloprid (**7**) and (*S*)-(−)-nicotine align very well when overlaying their bound conformations to AChBP. The electronegative [-*N*-C(N)=N-$NO_2$] pharmacophore of **7** and the positively charged center of (*S*)-(−)-nicotine, however, point in opposite directions, which is referred to as "inverted pharmacophores" in the literature (compare Figure 6b–e), repectively. These binding modes are in line with observed binding affinities.[41a] The binding constants of (*S*)-(−)-nicotine to *Ls*- and *Ac*-AChBP are on the same order of magnitude, while **7** shows a tenfold increase in affinity going from *Ls*-AChBP to *Ac*-AChBP, thus reflecting the additional hydrogen bond to glutamine Q57.

It is often argued that because of the differential affinities of neonicotinoids and nicotinoids against *Ac*- and *Ls*-AChBP, these may serve as surrogates for understanding the selectivity against insect and vertebrate nAChR, respectively. In this context, homology models of the receptor domain of nAChR are worthwhile to investigate the validity and the limitations of this line of argument.

The crystal structures of AChBPs show a conserved architectural fold that has been already recognized as a useful template to construct homology models for extracellular domains of nAChRs.[40b,41a,47] These models allow structure-based understanding of the atomistic detail of subtype-selective nAChR agonist binding.

Following a similar procedure,[48] homology models for the β1 subunit of the green peach aphid (*Myzus persicae* Sulzer) of the receptor domain of nAChR were constructed on the basis of the crystal structure of *Ls*-AChBP (PDB-ID 1I9B).[99] The same procedure was applied to build models for the green peach aphid (*M. persicae* Sulzer) subunits $\alpha_{1-5}$. With the exact composition of the sensitive insect nAChR still being unknown, a consensus model of all five α subunits was deduced from a structure-based alignment of the constructed five α subunit models and the ß1 subunit onto a dimer extracted from the homopentameric AChBPs in complex with imidacloprid (**7**), thiacloprid (**8**), and clothianidin (**12**; see Table 4 for the respective PDB IDs).

The finally obtained dimer model of the receptor domain illustrates the binding mode of imidacloprid (**7**; Figure 7). A cluster of conserved residues from both the α and β subunits surround and stabilize the *N*-nitroguanidine group. The nitrogen atom of the 6-chloropyridin-3-ylmethyl moiety forms a water-mediated hydrogen bond to the backbone of the D loop of the β subunit. So far the situation is highly analogous to that in AChBP (Figure 6). In the sensitive wild-type of green peach aphid (*M. ersicae* Sulzer), an arginine side chain—again from loop D—can form an additional hydrogen bond to the *N*-nitroimino function of the neonicotinoids. The positive charge introduced by this residue adds to the binding effect, thus fortifying the π–π interaction to the aromatic amino acids through electron dispersion: This effect can be quantified by correlated ab inito calculations to be on the order of 4–6 kcal mol$^{-1}$ (Figure 7d).[49] Both the resistance mutant of green peach aphid (*M. persicae* Sulzer) nAChR as well as the human nAChR carry an uncharged threonine residue in this position, which is not only incapable of hydrogen-bond formation but also cannot intensify π–π interactions (Figure 7c). In both *Ac*- and *Ls*-AChBP, a glutamine occupies the respective position, which, in the case of *Ac*-AChBP, comes close enough to build a hydrogen bond to the *N*-nitroimino moiety (Figure 6e), but cannot exert an inductive effect on the π–π interaction, as could a cationic group (Figure 7b).

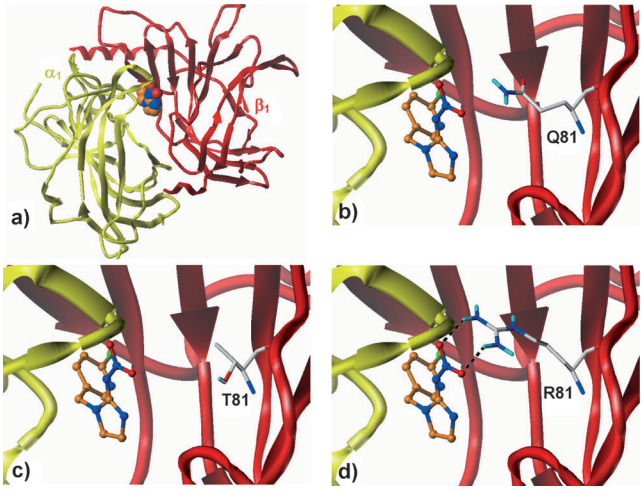



***Figure 7.*** Modeling the decisive R81T mutation in models of the nAChR agonist binding domains of green peach aphid (*M. persicae* Sulzer). The $\beta_1$ subunit is shown in red, only one out of the five possible $\alpha_1$ to $\alpha_5$ subunits is shown in yellow; (b) provides an overview with imidacloprid (**7**), highlighted as a space-fill model. All four panels adopt the same perspective. In (b)–(d), the ligand **7** is shown in a ball and stick model with orange carbon atoms and amino acids at the resistance mutation site 81 shown as capped stick models.

## 5. Biological Profile and Applied Aspects

The agricultural and horticultural use of nAChR agonists such as neonicotinoid insecticides is enormous, as reflected by a global sales volume well above US$ 2.6 billion for the entire chemical class. Therefore, it is beyond the scope of this Review to cover all applied aspects relevant to this substance class and their use as insecticides for more than 100 crops worldwide against agricultural pests as well as urban pest insects and ectoparasiticides in animal health. The agronomic profile as well as their efficacy has been reviewed in a number of comprehensive articles over the last 15 years; therefore this section briefly highlights a few aspects and we recommend that some of the recently published reviews should be consulted for further reading on the biological aspects.[13,14,18,24,50]

Neonicotinoids are the most versatile insecticides for a broad range of crops as a result of some important compound properties, that is, rapid foliar and root uptake, acropetal translocation, excellent acute and residual efficacy particularly against sucking, leaf mining, and coleopteran pests, including populations resistant to other chemical classes such as aphids, whiteflies, leafhoppers and planthoppers, leaf miners, Colorado potato beetles, rice water weevils. However, not only do these insecticides control crop pests, many are also used to target urban and structural insects including termites, ants, cockroaches, and houseflies, as well as turf pests such as white grubs (Figure 8).

In animal health, several neonicotinoids are important chemical tools to control, for example, cat and dog fleas (*Ctenocephalides felis* and *C. canis*).[6,18] Their physicochemical properties render them unique concerning the diversity of application methods, for example, soil treatments by granules or drench, seed treatment, stem injection, painting, dipping, baited gels, and of course spraying. Some of the application methods were only introduced because of the availability of the neonicotinoids and this revolutionized IPM.[50] The subsections below briefly cover some of the applied aspects highlighted above.

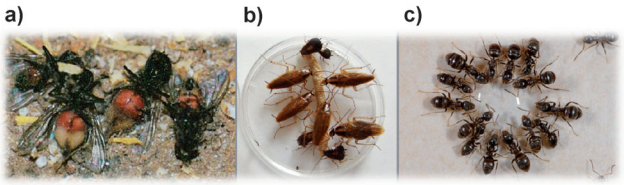



***Figure 8.*** Neonicotinoid applications in urban entomology. a) Control of houseflies by wall paintings, b) cockroaches using gels, and c) ants by employing attractive baits.

### 5.1. Control of Plant Virus Vectors

Viral as well as bacterial plant diseases are globally of increasing importance and could have a severe impact on quality and yield of crops once infested because of transmission by an insect pest, also called a vector. Phytopathogenic viruses are mainly transmitted by sucking insects such as aphids, whiteflies, thrips, and leafhoppers. Of course, aphids and other sucking pests such as whiteflies damage plants by feeding, but the indirect damage arising from the transmission of virus diseases is considered to be a greater threat in many cases. The green peach aphid (*M. persicae* Sulzer) is a known vector of more than 150 virus diseases. However, whiteflies such as *Bemisia tabaci* can reproduce on more than 500 host plants and are even more deleterious in vectoring plant diseases; for example, from 1997 to 2000 more than 11 000 jobs were lost in the Brazilian tomato industry because of whitefly-transmitted geminiviruses.[51] Virus diseases are broadly classified as nonpersistent (transmission within seconds) and persistent (longer feeding periods necessary). Many neonicotinoid insecticides are known to indirectly prevent the spread of plant viruses in treated plants, through their systemically driven rapid action on virus-vectoring sucking pest insects. Imidacloprid (**7**) shows excellent virus suppression effects for barley yellow dwarf virus (BYDV) transmitted by oat bird cherry aphid (*Rhopalosiphum padi* L.) and grain aphid (*Sitobion avenae* F.).[52] Other examples include sugar beet seed pelleting with **7**, which prevents transmission of beet mild yellows virus (BMYV),[53] and foliar applications in tomato against whiteflies to prevent the transmission of tomato yellow leaf curl virus (TYLCV).[54]

### 5.2. Innovative Formulation Concepts

The uptake and translocation in planta of systemically acting insecticides such as neonicotinoids and other new nAChR agonists depends on application conditions to obtain maximum efficacy, which can be influenced by modern formulation concepts tailored to achieve the best performance based on their physicochemical properties. Such concepts were extensively reviewed,[55] thus only two very recent examples of formulation innovation at Bayer CropScience AG are briefly described, that is, oil dispersion technology for foliar application and a technology that combines conventional seed treatments with biological control agents for improved crop protection and yield enhancement.

Bayer CropScience AG recently introduced a formulation concept that overcomes the incompatibility between water and oil. The technology is called O-TEQ (oil dispersion, OD) and was, for example, commercialized for imidacloprid (**7**; Confidor O-TEQ 200) and thiacloprid (**8**; Biscaya OD 200).[56]

The OD formulation type is described as "*a stable suspension of active ingredients in a water immiscible fluid, which may contain other dissolved active ingredients, intended for dilution with water before use*".[57] OD technology offers innovation in the uptake and transport of systemic agonists of the nAChR by plant tissue and pest insects. In particular, the rain-fastness as well as the penetration after foliar application is higher with OD formulation concepts than with other formulation types such as suspension concentrate (SC), as shown in Figure 9. In summary, innovative formulation

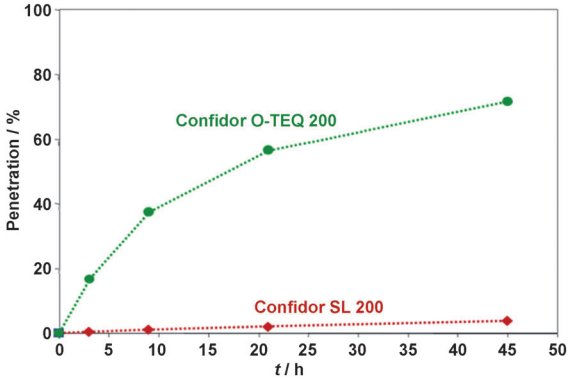



***Figure 9.*** Kinetics of the penetration of imidacloprid (**7**) through isolated apple leaf cuticles from SL 200 and O-TEQ 200 formulations (data kindly provided by R. Pontzen, Bayer CropScience AG).

concepts for neonicotinoids such as O-TEQ can help farmers to sustainably control pests by reducing the impact of environmental factors, even under unfavorable conditions such as rainfall shortly after treatment.

A second innovation introduced very recently (first registration in 2010) is a combination of clothianidin (**12**) and spores of the naturally occurring bacterium *Bacillus firmus*, which is applied as a seed treatment to corn, cotton, and soybean. The combination is marketed under the brand Poncho/VOTiVO, for example, for corn and protects young plants from pests such as black cutworm (*Agrotis ypsilon* Rottemberg) and wireworms (*Melanotus* spp., *Agriotes* spp.) during critical early development stages, thereby leading to healthier root development and stronger stands. Applied directly to the seed, the combination protects, for example, soybean plants above and below the ground, that is, preventing damage to early season seedlings by aphids (by **12**) and roots by soybean cyst nematode (by *B. firmus*).

## 6. Phytotonic Effects

Environmental conditions are known to influence crop growth and yield as well as product quality; for example,

adverse weather conditions such as drought stress or a deficiency in nutrients can have a considerable impact on plant growth depending on the time scale of exposure to such adverse conditions.[58] Adverse conditions promoting stress impair the energy balance of crops, and could result in the generation of less energy necessary for proper growth, subsequent yield, and post-harvest quality measures. Therefore, maximum growth, plant development, and highest possible yield potential are normally considered to be far from what can actually be obtained without stressors. After the introduction of imidacloprid (**7**) in crop protection, numerous field observations on plant-stimulating (so-called phytotonic) effects were made, which could not be linked to a reduction in pest pressure. It was found that compounds such as **7** and **9** could stimulate plant growth and thus lead to higher yields under conditions normally rendering plants less productive because of abiotic stress.[14c,59]

### 6.1. Plant Health Effects and Overcoming Environmental Stress

The number of studies thoroughly investigating the effects of neonicotinoids on abiotic stress is fairly limited; however, one of the most detailed studies was conducted with imidacloprid (**7**) on barley grown under short-term drought stress. The plants drenched with **7** showed better growth, and gene expression profiling analysis revealed that the expression of drought-stress marker genes is delayed, thereby resulting in a less-pronounced decline in photosynthetically driven energy production.[58] Greenhouse trials with *Arabidopsis thaliana* also clearly demonstrated the potential of **7** to improve the survival and growth rate under drought-stress conditions (Figure 10).

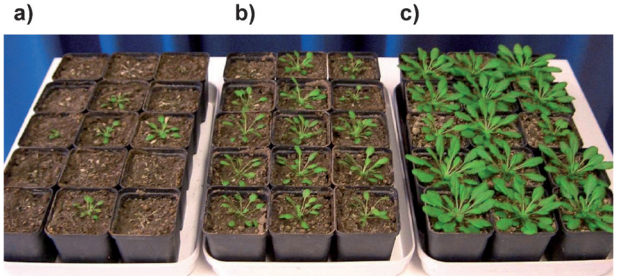



***Figure 10.*** Growth rate and survival of *Arabidopsis thaliana*. a) Drought-stressed, untreated. b) Drought-stressed, imidacloprid (**7**) treated (0.5 mg per pot). c) Unstressed.

Field experiments conducted in 2004 confirmed the potential of imidacloprid to moderate drought stress in plants, with an average lint yield increase of 10 percent observed in cotton.[60–62] These results clearly indicate that **7**, besides its insecticidal activity, supports plants in moderating the effects of abiotic and biotic stress, and it is speculated that the observed effects are possibly systemically induced by the phloem-mobile imidacloprid (**7**) metabolite 6-chloronicotinic acid (6-CNA). The first experimental evidence of the effects of 6-CNA was recently provided by the demonstration of the induction of salicylate-associated plant defense responses in *A. thaliana*.[63]

## 7. Quantitative Structure–Activity Relations

Before the availability of cocrystal structures, rational design was limited to ligand-based approaches. These were relatively successful, which may have been fostered by the fact that the binding of (*S*)-(−)-nicotin and its analogues is driven more by enthalpy than by entropy;[42b] enthalpic contributions are far more accostable towards computation than entropy.

### 7.1. Electrostatic Potentials

Electrostatics is a main driver of molecular recognition processes. In particular for agonists of nAChR, such as neonicotinoids, the electrostatics of these compounds was identified very early on as a driving factor, well-suited for qualitative understanding as well as QSAR purposes.[41b,64]

Figure 11 visualizes the electrostatic potentials (ESPs) of seven commercial agonists of nAChR (see also Figure 2)

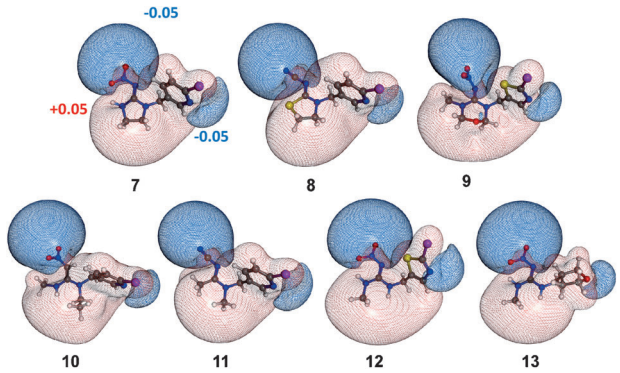



***Figure 11.*** Isosurfaces of the ESPs of nAChR agonists **7**–**13**. ESPs were calculated directly from DFT electron densities, rather than from atomic charges. One positive and one negative isolevel are displayed for each molecule; the respective values are given in atomic units (au). Calculation procedure as outlined in the literature.[65]

obtained at the DFT level of theory. All these molecules possess a pronounced dipole moment. The overall similarity of the ESPs is larger than might be expected given the range of chemical variation.

All the molecules exhibit two regions of negative ESP, one caused by the lone pair of electrons on the nitrogen atom in the 6-chloropyridin-3-yl-/2-chloro-1,3-thiazol-5-yl moiety, or the oxygen atom in the case of the tetrahydrofur-3-yl ring of dinotefuran (**13**). The second area of negative ESP corresponds to the electronegative pharmacophore. In the receptor, these areas of the molecules serve as hydrogen-bond acceptors, as discussed in Section 4. The large "bulb" of the positive ESP fits nicely into the aromatic pocket of the π system, spanned by aromatic residues in the binding niche of nAChR.

Alignments of the molecules based on maximizing the overlap of just the ESPs results in molecular overlays which resemble the overlays of cocrystal structures surprisingly well. The differences in the receptor binding of (*S*)-(−)-nicotin and imidacloprid (**7**) have already been discussed in Section 4.3 and Figure 6, highlighting the reversed orientations of the respective cationic and electronegative pharmacophores. Figure 12 emphasizes the differences of protonated (*S*)-(−)-nicotine and imidacloprid (**7**), which, as expected, do not share too many similarities in terms of electrostatics.

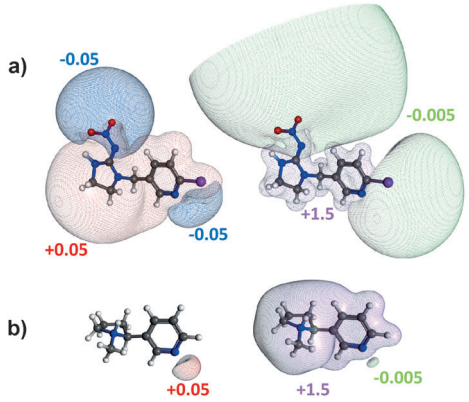



***Figure 12.*** a) Isosurfaces of the ESPs of imidacloprid (**7**). b) Protonated (*S*)-(−)-nicotine. See Figure 11 for computational details. Four isolevels are displayed for each molecule; the respective values are given in atomic units (au). The isosurface at −0.05 au (ESP) cannot be visualized for protonated species.

### *7.2. Local Reactivity Descriptors for Metabolism Studies*

Density functional theory (DFT), as pioneered by the studies of Parr and Yang[66] has provided a theoretical sound foundation to successful concepts of chemists such as electronegativity, hardness, and softness. The Fukui function $F(\mathbf{r})$[67] provides an easy way to calculate local reactivity. It is defined as the partial derivative of the electron density with respect to electron number $N$, given a fixed nuclear arrangement (= molecular geometry) $V[\mathbf{r}]$. $F(\mathbf{r})$ thus reflects the amenability of a molecule towards changes in the electron density at a certain point in space **r**. The right and left hand derivatives of the electron density are different, thus reflecting attack by a nucleophile or electrophile, respectively.

Fukui functions can be used to estimate sites of attack by electrophiles and nucleophiles on a given molecule. It has been shown that the maxima of the Fukui function for attack by an electrophile may allow the prediction of sites of oxidative metabolic attack, although their interpretation requires some experienced chemical intuition.[65] In addition to that, Fukui functions can also be used as molecular fields for 3D-QSAR, and have in particular been shown to be descriptors well-suited for 3D-QSAR models of nAChR activity.[68]

Figure 13 shows the Fukui functions of commercialized agonists of nAChR (see also Figure 2). The maxima of the Fukui function for attack of an electrophile at the $CH_2$ hydrogen atoms of the five-membered-ring system of imidacloprid (**7**), for example, correlate nicely to the observed metabolic hydroxylation at these positions (see green isosurfaces in Figure 13).

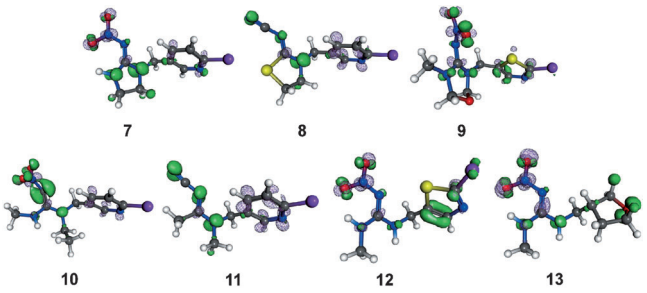



***Figure 13.*** Isosurfaces of the Fukui functions for attack by an electrophile (green, solid; isolevel 0.05 au and for attack by a nucleophile (purple, fishnet; 0.05 au), as calculated from DFT electron densities[65] for ring systems (**7**–**9**) and noncyclic structures (**10**–**13**). The ordering of the molecules follows Figure 2.

The same holds true for thiamethoxam (**9**), where metabolic ring cleavage towards the formation of clothianidin (**12**)[16a–d] can also be guessed from the Fukui functions in Figure 13.

## *8. Recent Trends*

Global sales value growth of nAChR agonists such as neonicotinoid insecticides is increasingly being affected by generic (off-patent) products. Imidacloprid (**7**) became generic in many countries in 2006, and patent protection for further commercial compounds such as **8**–**13** (see also Section 1.1) will expire in 2013. This has already resulted in the generic manufacture of patent-free products (China, India) and an increasing price erosion is likely to facilitate the development of pest resistance to the neonicotinoid insecticide class (see Section 5).[69] Therefore, the discovery and development of new nAChR agonists that overcome metabolic resistance is essential; however, mutations in nAChR subunits covering critical amino acids may complicate the situation for any new agonist showing a binding mode similar to neonicotinoids and nicotinoids.

### *8.1. Literature-Inspired Approach*

Patent-free key intermediates such as CCMP (**15**) and CCMT (**21**), building blocks such as **17** (Scheme 2) and methyl *N*-cyanoacetimidate (intermediate for **11**), as well as the nitromethylenes NTN32692 (**6**) and nitenpyram (**10**; see Section 1.1) have been used for some years to an even greater degree for fragment combination or pharmacophore-based modification (see Section 8.2.2).[70] At present, most neonicotinoid-based insecticides developed in China include only marginal structural variations of known commercial products and thus lack further innovation. For example, the combination of the bioisosteric 2-chloro-1,3-thiazol-5-ylmethyl frag-

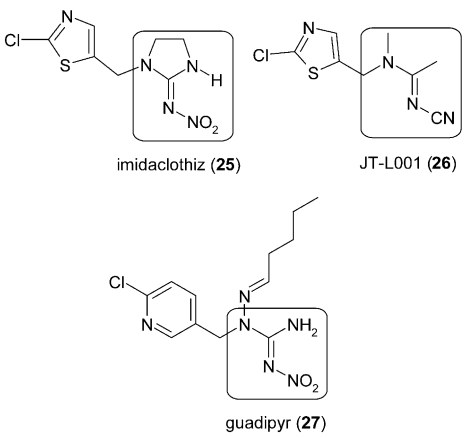



***Figure 14.*** Chemical structures of imidaclothiz (**25**), JT-L001 (**26**), and guadipyr (**27**).

ment with **17** or *N*-cyano-*N*′-methylethaneimidamide resulted in imidaclothiz (**25**; name is approved in China, no ISO common name) and JT-L001 (**26**; Figure 14).

Despite the fact that both chemical structures were already published together with those of imidacloprid (**7**)[71] and acetamiprid (**11**),[72] **25** has recently been commercialized in China as a so-called "new neonicotinoid insecticide" by Nantong Jiangshan Agrochemical and Chemicals Co. Ltd. (China) for control of tea green leafhopper (*Jacobiasca formosana*) in tea and aphids in vegetable crops. Compound **26** is also currently under development in China by Jiangsu Tianrong Group Co. Ltd. (China).[72b] Guadipyr[73] (**27**; name is approved in China by National Pesticide Standardization Technical Committee, no ISO common name) combines the 6-chloropyridin-3-ylmethyl fragment with *N*-nitroguanidine (**16**) as well as a semicarbazone-like chemistry (SoCh blocker, indoxacarb)[74] and was co-developed by China Agricultural University and Hefei Xingyu Chemical Co. Ltd. (China) for control of rice planthoppers and cabbage aphids. The registration and first launch of **27** (20% SC formulation) is scheduled for 2013.

### *8.2. Chemorational Approach*

The chemical and structural biology investigations on the 3D structure of AChBP in the neonicotinoid-bound state (see Section 4) can support the strategic ligand design of novel insecticidal nAChR agonists with unique pharmacophore modifications or replacements of the hetarylmethyl side chain, possibly showing distinct binding mechanisms. This may widen the known spectrum of commercial nAChR agonists or may prevent cross-resistance in insects which is normally caused by elevated in vivo metabolism (see Section 7) and (in very rare cases) target-site mutation.

#### *8.2.1. Rational Design*

Docking experiments of imidacloprid (**7**) into the aphid α2β1 interfacial agonist-binding pocket (based on the nAChR structural model of the green peach aphid (*M. persicae* Sulzer))[41a] led to the identification of a niche beyond its bound *N*-nitroimino function toward the loop D arginine (Arg). This space may provide room for (hetero)aromatic ring structures as novel pharmacophore modifications. Therefore, nAChR agonists with extended *N*-acylimino [=NC(O)-$R^1$] functions were designed that demonstrated specific binding to the loop D region (Figure 15).[75]

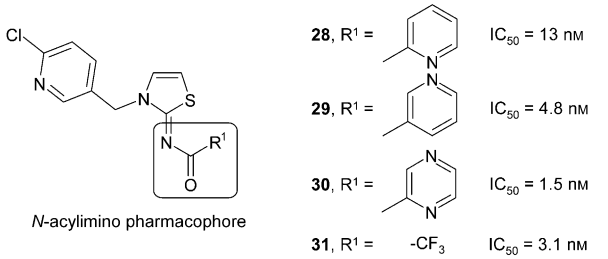



***Figure 15.*** nAChR agonists with *N*-acylimino pharmacophores.

The *N*-acylimino [=NC(O)-$R^1$] analogues, where $R^1$ is a hydrogen acceptor such as pyridin-2-yl (**28**), pyridin-3-yl (**29**), pyrazin-2-yl (**30**), or trifluoromethyl (**31**) shows high nAChR binding (binding affinity to nAChRs membrane preparations from *D. melanogaster*, assay with [$^3$H]-**7**; comparison thiacloprid (**8**): $IC_{50} = 2.7$ nM).[75a] In molecular dynamic simulations with the pyrazinyl analogue **30** it was shown that two pyrazine nitrogen atoms and the oxygen atom of the *N*-acylimino function undergo hydrogen bonding with the loop D Arg (basic guanidine $NH_2$) and tryptophan (Trp, indole-NH). Similarly, the [=NC(O)-$CF_3$] analogue **31** interacts with loops C and D regions through fluorine–hydrogen interaction (directly or through water bridges), respectively. The binding of the 6-chloropyridin-3-ylmethyl moiety is identical to those of imidacloprid (**7**) and thiacloprid (**8**).[75d]

On the other hand, special nAChR agonists were designed to understand the SARs at the insect nAChR target site, focusing on the water-mediated ligand–protein interactions. In this context, the 2-nitroiminoimidazolidine analogues with a 3-fluoropropyl residue **32** or carbonyl oxygen atom such as 3-oxobutyl residue **33** which is useful for hydrogen bonding were synthesized (Figure 16).[76]

The SAR predicted that fluorine and oxygen atoms at a specific position is essential for the formation of a hydrogen bond, presumably with a water bridge between the nAChR

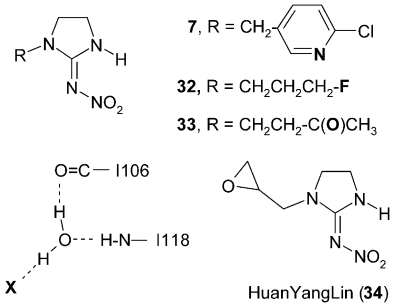



***Figure 16.*** Predictive binding site interactions of imidacloprid (**7**), 2-nitroiminoimidazolidine analogues (**32**, **33**), and HuanYangLin (**34**).

agonist and the relevant amino acid at the ligand binding pocket of the *Ac*-AChBP. The water forms a bridge between the backbone NH group of isoleucine (I118) and the 3-chloropyridin-3-yl nitrogen atom (X = N) in **7**, fluorine (X = F) in **32** or carbonyl oxygen atom (X = O) in **33**. The alternative water hydrogen atom undergoes hydrogen bonding to the isoleucine backbone carbonyl oxygen atom (I106), as outlined in Figure 16 (see Section 4.3).[76b]

However, none of the nAChR agonists reached the effectiveness of imidacloprid (**7**) and its close analogues. The in vivo insecticidal effect of the fluorocarbon moiety is not compatible with that of the 6-chloropyridin-3-ylmethyl residue and analogous groups. In 2005, the 1-(2,3-epoxypropyl)-*N*-nitro-2-imidazolidine HuanYangLin (**34**)[77] was synthesized at the Wuhan Institute of Technology, China. As a result of its sensitive structural moieties, a very violent exothermic effect with strong release of heat can be observed ($\Delta H = 4059\ J\,g^{-1}$) after heating it up to 104 °C (Bayer CropScience AG, unpublished results). Nevertheless, a manufacturing process is being developed by Wuhan Zhongshin Chemical Co. Ltd. (China) and the compound is under development (10% WP formulation water dispersible powder) for the control of sucking insect pests in vegetables, rice, and cotton.

#### 8.2.2. *Structurally Fixed cis-Nitromethylenes*

In 1988, first syntheses of insecticidally active, *cis*-configured nitromethylenes, such as BAY T9992, fixed through the 1,2,3,6-tetrahydropyrimidine (obtained by a Mannich reaction with formaldehyde and amines) or 1*H*-pyrrolo[1,2-*a*]imidazole systems (R = H, *t*Bu; Figure 17) were published by Nihon Tokushu Noyaku Seizo KK (later Bayer CropScience KK, Japan).[78]

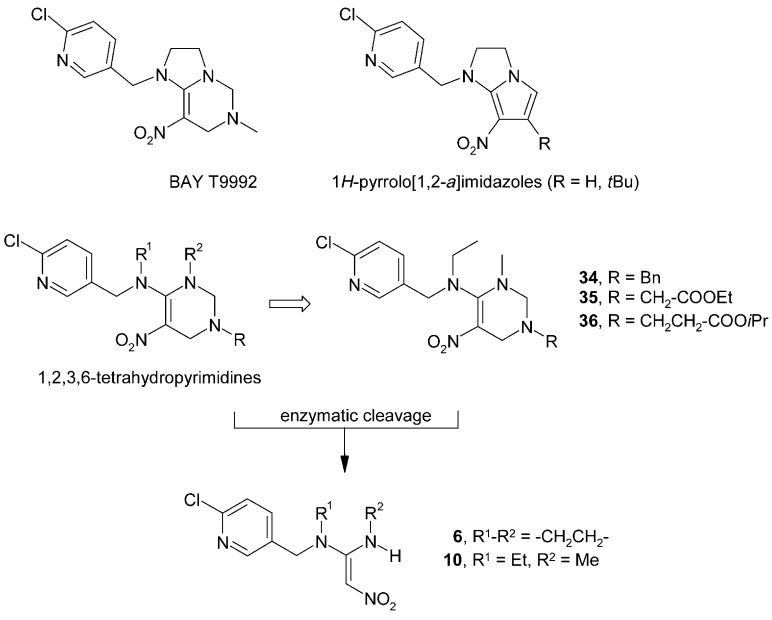



***Figure 17.*** First *cis*-configured nitromethylene structures, resulting in new 4-amino-1,2,3,6-tetrahydropyrimidines (**34**)–(**36**) and by cleavage of nitromethylenes **6** and **10**.

The 4-amino-1,2,3,6-tetrahydropyrimidine scaffolds can act directly, or after acidic or enzymatic cleavage by reverting to their highly insecticidal nitromethylene precursors, for example, NTN32692 (**6**; $R^1$-$R^2$ = -$CH_2CH_2$-) and nitenpyram (**10**; $R^1$ = Et, $R^2$ = Me; see Section 1.1).[79] The hydrolysis of Mannich adducts can be observed at different pH values (pH 4, 7, or 9), and the data are in full agreement with their insecticidal activity as prodrugs.[1]

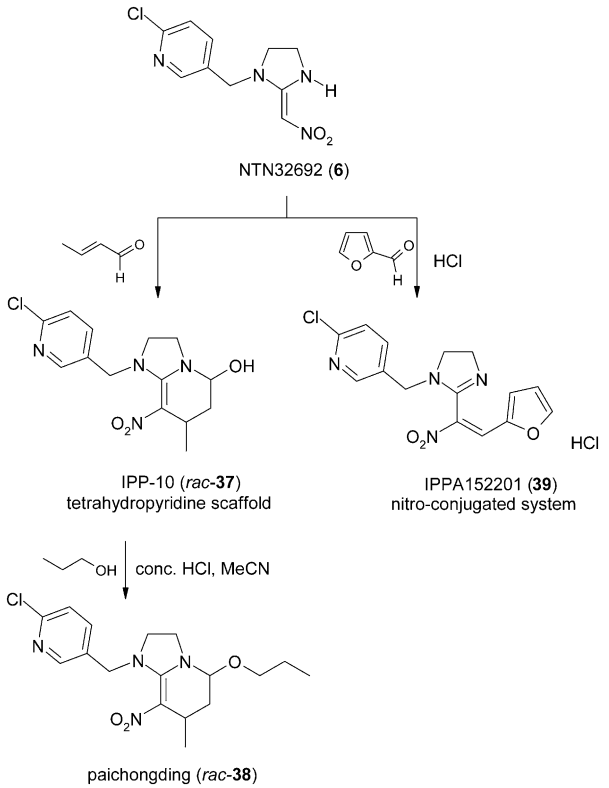
Recently, molecular docking studies of ligand–receptor complexes in the extracellular domain of *n*AChRs (subunit of *Ls*-AChBP as structural surrogate of the insect *n*AChR) were carried out with candidates **34** and **36** (Figure 17) to explain the SAR observed in in vitro assays.[80a] Despite their prodrug properties, the insecticidal potency seems to be influenced by the number of $CH_2$ groups and size of the ester function.[80b] So far, no commercial insecticide has been developed from this concept.

In addition, the β-nitroenamine structures **6** and **10** have been used more intensively as starting materials for the synthesis of novel nAChR agonists containing a structurally fixed *cis*-nitromethylene pharmacophore.[81] In this context it was demonstrated that the α-carbon atom of the nitromethylene group in **6** and **10** can be attacked by further electrophilic agents such as unsaturated or heterocyclic aldehydes, benzyl bromide, ethyl propiolate, diphenylnitrile imine, and diazonium salts.[82] The tetrahydropyridine scaffold and the formation of a nitro-conjugated system resulted in the most successful rigidizations, as exemplified by IPP-10 (*rac*-**37**), paichongding (*rac*-**38**), and IPAA152201 (**39**) in Scheme 5.

The 1,2,3,5,6,7-hexahydroimidazo[1,2-α]pyridine paichongding (*rac*-**38**) was obtained from **6** by cyclization with crotonaldehyde to form *rac*-**37** and subsequent etherification with 1-propanol. The intermediate IPP-10 (*rac*-**37**) has both contact and systemic activity and shows plant systemicity with lethal and sublethal effects on bird cherry oat aphid (*Rhopalosiphum padi* L.) on wheat.[83] Paichongding (*rac*-**38**; mixture of four diastereomers; two couples of enantiomers)[84] was jointly developed with Jiangsu Kwin Co. Ltd. and it has obtained a temporary pesticide registration (10% SC formulation) for the Chinese market.

IPAA152201 (**39**) is generated by a condensation reaction of the nitromethylene group in **6** with furfural catalyzed by concentrated hydrochloric acid (Scheme 5). Although **39** shows a good activity against Lepidoptera insects such as oriental armyworm (*Pseudaletia separata* Walker) and rice leafroller (*Cnaphalocro*cis *medinalis* Guenee), the development as an insecticide for crop protection was impeded by its poor stability in water (photostability: $t_{1/2} = 4$ h, i.e. lower than *rac*-**38** and **7**). Furthermore, it was found that both compounds displayed a 30-fold higher activity against imidacloprid (**7**) resistant strains of the brown planthopper (*Nilaparvata lugens* Stal). Both paichongding (*rac*-**38**) and IPAA152201 (**39**) are influenced by the Y151S mutation in N1α1 to some extent; however, the mutation has not yet been identified in any field-




***Scheme 5.*** Synthesis of IPP-10 (*rac*-**37**), paichongding (*rac*-**38**), and IPA152201 (**39**).

collected population.[85] In the case of paichongding (*rac*-**38**), its 2-chloro-1,3-thiazol-5-ylmethyl bioisostere, was recently shown to maintain high insecticidal activity against cowpea aphid (*A. craccivora*).[86]

### *8.3. Biorational Approach*

Novel chemical scaffolds, also known as privileged structures,[87] are of increasing importance in the search for new agrochemical motifs and active ingredients for use in modern crop protection. They can be a helpful starting point for the design of compounds containing underexplored chemical moieties or binding elements with preferable physicochemical properties. In this context, topological pharmacophore patterns of natural products active on the insect nAChRs are of interest, for example, *Stemona* alkaloids of the stemofoline group[88] such as stemofoline and 16,17-didehydro-16(*E*)-stemofoline, isolated from plants belonging to the Stemonaceae family (Figure 18).

Stemofoline (R = *n*Bu; isolated from the leaves and stem of *Stemona japonica*) has long been known as a potent nAChR agonist (α-BgTx displacement: $EC_{50} = 1.7$ nM)[90] with fast-acting insecticidal, rapid knock-down, antifeedant, and repellent activities.[91] In addition, it shows systemic (xylem mobility) and translaminar efficacy ($\log P_{ow} = 2.64$ at pH 7.5). On the other hand, 16,17-didehydro-16(*E*)-stemofoline (R = -HC=CH-Et; isolated from dried roots of *Stemona collinsae*) is even more potent than stemofoline against larvae

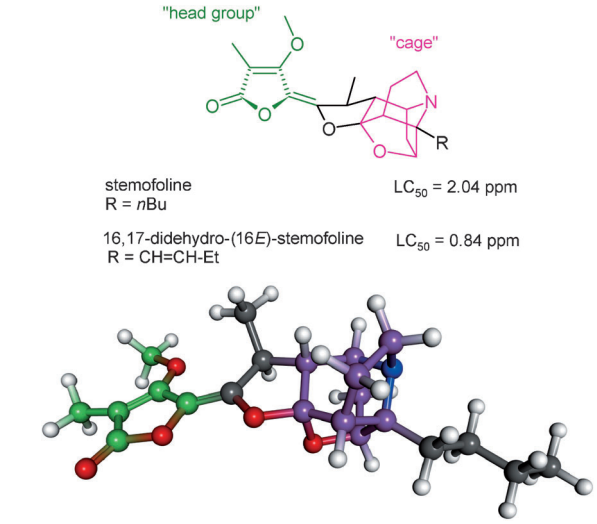


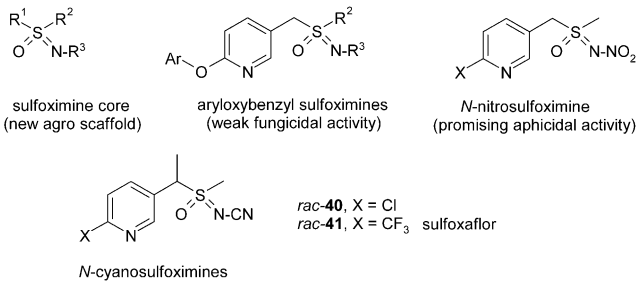

***Figure 18.*** Two *Stemona* alkaloids of the stemofoline group and their insecticidal activity against neonate larvae of cotton leaf worm (*S. littoralis*).[89] Bottom: Geometry of stemofoline (R = *n*Bu), optimized at the DFT level of theory. For further reference, the lactone "head group" is highlighted by coloring the respective carbon atoms in green; purple carbon atoms highlight stemofolin's "cage structure".

of cotton leafworm (*Spodoptera littoralis*) and shows a stronger antifeedant activity against diamondback moth (*Plutella xylostella*) larvae.[92] Therefore, the complex chemical scaffold of stemofoline is considered as a privileged structure to identify smaller, insecticidal-active *n*AChR agonists.

The value of privileged structures can be exemplified by the identification of the sulfoximine core as a new scaffold for agrochemicals (see Section 8.3.1). Simplification of the stemofoline structure (see Sections 8.3.2 and 8.3.3) resulted in the discovery of two insecticidal classes of novel nAChR agonists, that is, pyridinylcyanotropanes and butenolides.

#### *8.3.1. Sulfoximine Insecticides*

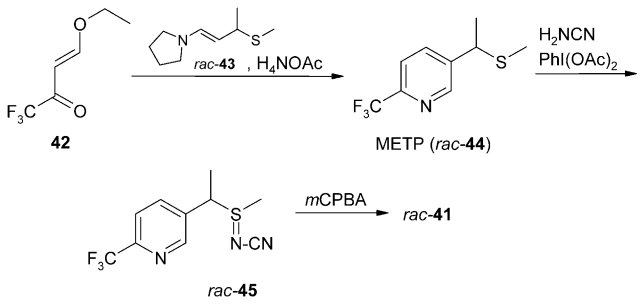
Although sulfoximines have been described in the literature for more than 65 years, the value of the small hydrophilic sulfoximine core (Scheme 6) in agrochemicals was only recently discovered.[93] According to researchers from Dow AgroSciences, several different sets of substituted sulfoximine scaffolds containing three diverse residues $R^1$, $R^2$, and $R^3$ were synthesized, guided by agrochemical-like parameters




***Scheme 6.*** Sulfoximine core driven optimization procedure.

known from Lipinski's Rule of 5.[94] Whereas the *S*-aryloxybenzyl substitution ($R^1$) initially resulted in sulfoximines with weak fungicidal activity, further investigation of the bioactive scaffold resulted in *N*-nitrosulfoximines (e.g. $R^1$ = 6-chloropyridin-3-ylmethyl, $R^2$ = Me, and $R^3$ = $NO_2$) as lead structures with promising insecticidal activity. Replacement of the *N*-nitro group by *N*-cyano ($R^3$ = CN) and introduction of a single mono-methyl-substituted linker (CHMe) between the pyridine ring and sulfoximine group resulted in *N*-cyanosulfoximines. Further investigation of the SAR of the pyridine ring revealed that their insecticidal activity can be remarkably increased by small, lipophilic, electron-withdrawing substituents at the 6-position, such as 6-trifluoromethyl (X = $CF_3$), which led to the discovery of sulfoxaflor (*rac*-**41**; Scheme 6).

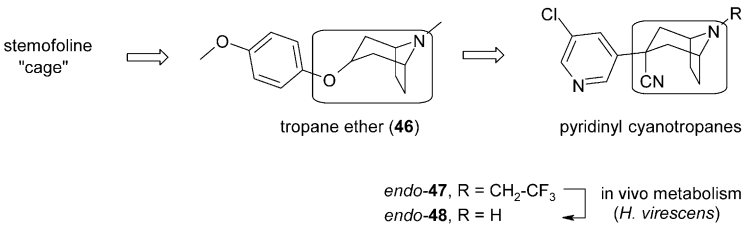
Starting from (3*E*)-4-ethoxy-1,1,1-trifluoro-3-but-2-one (**42**) the racemic 5-[(1-methylthio)ethyl]-2-(trifluoromethyl)-pyridine (METP, *rac*-**44**) can be synthesized by formation of a pyridine ring by using *rac*-**43** in the presence of ammonium acetate (Scheme 7). Oxidative addition of cyanamide to *rac*-**44** yields the *N*-cyanosulfimine (*rac*-**45**), which is further oxidized to give sulfoxaflor (*rac*-**41**) as a mixture of four diastereomers (two pairs of enantiomers). Compared to the 6-chloro substitution in *rac*-**40** (X = Cl), the 6-trifluoromethyl group in *rac*-**41** (X = $CF_3$) resulted in a stronger activity against cotton aphid (*Aphis gossypii* Glover) and green peach aphid (*M. persicae* Sulzer), whereas their activities against cotton whitefly (*B. tabaci* Gennadius) is lower. Sulfoxaflor (*rac*-**41**) was first registered in South Korea for use on apples, pears, and red peppers, and has been classified by IRAC as an nAChR agonist in group 4 (subgroup 4C).




***Scheme 7.*** Synthesis of sulfoxaflor (*rac*-**41**). *m*CPBA = *meta*-chloroperbenzoic acid.

### 8.3.2. *Pyridinylcyanotropane Insecticides*

The 3-*exo*-3-(4-methoxyphenoxy)-8-methyl-8-azabicyclo[3.2.1]octane (**46**), (α-BgTx displacement: $IC_{50}$ = 310.0 nM) was designed by Zeneca (later Syngenta AG) on the basis of the tetracyclic, rigid pyrrolo[1,2*a*]azepine ring system (stemofoline "cage"). This led to the discovery of the pyridinylcyanopropane insecticides, as exemplified by the 3-*endo*-3-(5-chloro-3-pyridinyl)-8-(2,2,2-trifluoroethyl)-8-azabicyclo-[3.2.1]octane-3-carbonitrile (**47**; $IC_{50}$ = 1.0 nM). This compound shows high nAChR agonist activity in vivo against sucking pests, such as aphids (Scheme 8).

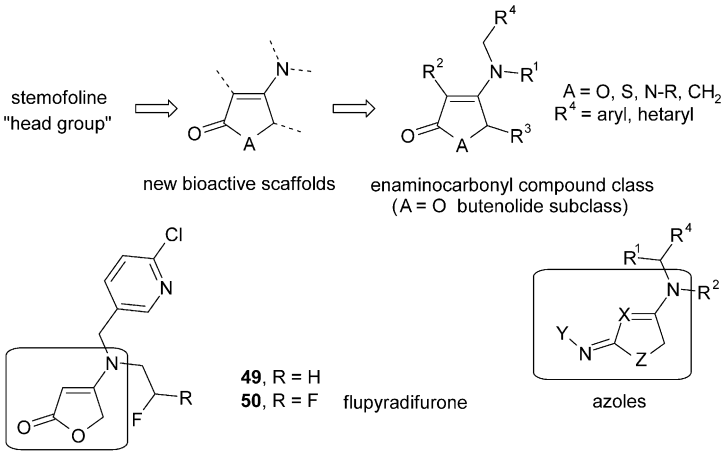



***Scheme 8.*** Stemofoline cage driven optimization procedure.

After injection into tobacco budworm larva (*Heliothis virescens* Hübner) a fast bioactivation of the prodrug *endo*-**47** can be observed by cleavage of its *N*-(2,2,2-trifluoroethyl) group within two hours under formation of *endo*-**48** (R = H).[95] The pyridinylcyanotropanes share a high-affinity binding site with α-BgTx, based on similarities in pharmacology, but which is distinct from the high-affinity imidacloprid (**7**) binding site. [$H^3$]-*endo*-**48** was shown to be a specific biochemical tool for defining a subpopulation of nAChRs in insects, and for investigating the binding behavior of novel nAChR agonists. However, no commercial nAChR agonist has yet been developed through the stemofoline "cage" simplification strategy.

### 8.3.3. *Butenolide Insecticides*

Inspired by the stemofoline lactone "head group" as a topological pharmacophore pattern, a new bioactive scaffold was found, which resulted in the identification of the insecticidally active enaminocarbonyl compound class (Scheme 9). This approach was strongly supported by molecular modeling studies with relevant nAChR agonists, that is, azoles with new pharmacophores and high binding affinity to the insect nAChR, for example, the 4-amino-2(5*H*)-thiazolidine-cyanamidine (X = N, Y = CN, Z = S),[96] as well as 5- and 6-membered-ring heterocycles including (thio)lactone, lac-

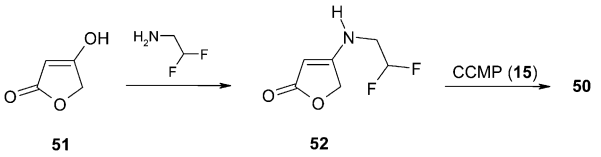



***Scheme 9.*** Stemofoline head group driven optimization procedure.

tame, and cycloalken-1-one moieties.[97] The insecticidally active butenolide subclasses (A = O) was identified by stepwise chemical evolution of the enaminocarbonyl compounds.

Further exploration of the hetarylmethyl moiety $R^4$ (5- and 6-membered-ring hetaryl residues) resulted finally in the discovery of highly potent nAChR agonists, as exemplified by **49** (R = H) and flupyradifurone (**50**; R = F).[98] The 4-[(2,2-difluorethyl)amino]-2(5*H*)-furanone (**52**) can be prepared by treatment of 4-hydroxy-2(5*H*)-furanone (β-tetronic acid; **51**) with 2,2-difluoroethylamine, and its subsequent N-alkylation with CCMP (**15**) resulted in the formation of **50** (Scheme 10).




51 52

***Scheme 10.*** Synthesis of flupyradifurone (**50**).

As a member of the novel class of butenolide insecticides, flupyradifurone (**50**; ISO common name provisionally approved in June 2011) is currently under development by Bayer CropScience AG for the control of a broad spectrum of sucking insects, in particular, various species of aphids, leafhoppers, psyllids, certain species of scales, thrips, and whiteflies. Its first launch is scheduled for 2015. Compared with *N*-nitroguanidines (**7**, **9**, **12**, **13**, **25**), *N*-cyanoamidines (**8**, **11**), or nitroenamines such as **10** (see Sections 1.1 and 8.1) and sulfoxaflor (**41**; see Section 8.3.1), the butenolide insecticide flupyradifurone (**50**) contains a different pharmacophore system as a new bioactive scaffold, which is responsible for its specific physicochemical properties, systemicity, metabolism in plants and insects, and for a favorable safety profile. As a result of its fast uptake, translocation, and distribution in plants (see Section 2.3), **50** is very versatile in terms of application methods to a variety of crops, and exhibits excellent and fast efficacy against a broad spectrum of sucking pests.

### 8.4. Homology Model Based Virtual Screening

The use of structural information on nAChR itself or mollusc AChBP surrogates for the understanding of binding modes has already been discussed in Section 4.3. Homology models for the agonist binding domains of insects, for which information from structural biology is not so far available, can also be used for in silico docking simulations, rational design, and virtual screening of chemical libraries to identify new insecticidally active nAChR ligands.[13c]

## 9. Summary and Outlook

The enormous global economic success of nAChR agonists such as neonicotinoid insecticides explains why nAChR is still one of the most attractive biochemical target sites for modern crop protection and for exploration in insecticide discovery. As a result of its competitive safety profile, the high target specificity, and versatility in application methods, this insecticide class is globally an integral component in numerous pest and IPM strategies.

Over the last 12 years various high-resolution crystal structures have been elucidated of homopentameric AChBPs from different species, such as *L. stagnalis* and *A. californica*, which are homologues to the extracellular N-terminal LBD of nAChRs. They can provide the theoretical foundation for constructing homology models for the ligand-binding domains of insect nAChRs, as a useful basis for a virtual screening of chemical libraries to identify novel insecticidal-active nAChR ligands. In this context, the identification and characterization of insect nAChR subtypes is an important research field, and may open up a new era for subtype-selective nAChR agonists with a specific biological profile and maximal safety.

In the future, the discovery and development of new nAChR agonists overcoming metabolic resistance in vivo is essential. Furthermore, it can be expected that mutations in nAChR subunits covering critical amino acids may reduce the efficacy of any new agonist showing a binding mode similar to well-established products. Therefore, current ligand design of novel insecticidal nAChR agonists is more focused on structural constraints such as unique pharmacophore modifications or replacements of the hetarylmethyl side chain, as outlined by the sulfoximine and butenolide insecticides.

## Abbreviations

| | |
|---|---|
| Ac | *Aplysia californica* |
| ACh | acetylcholine |
| AChBP | acetylcholine binding protein |
| α-BgTx | α-bungarotoxine |
| au | atomic units |
| CLR | cysteine-loop receptor |
| CNS | central nervous system |
| DFT | density functional theory |
| ESP | electrostatic potential |
| $IC_{50}$ | inhibitory concentration 50 % |
| IPM | insect pest management |
| $\lambda_{max}$ | electronic absorption |
| LBD | ligand binding domain |
| $LC_{90}$ | lethal concentration value in ppm killing 90 % of the treated population |
| LGIC | ligand-gated ion channel |
| $\log P_{OW}$ | logarithm of the 1-octanol and water partition coefficient |
| Ls | *Lymnea stagnalis* |
| nAChR | nicotinic acetylcholine receptor |
| OD | oil dispersion |
| QSAR | quantitative structure–activity relationship |
| SAR | structure–activity relationship |
| SC | suspension concentrate |
| $t_{1/2}$ | half-life (in water) by photolysis |
| TSC | transpiration stream concentration factor |
| WG | water-dispersible granular |
| WP | water-dispersible powder |

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