Research Article

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Flupyradifurone: a brief profile of a new butenolide insecticide

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Abstract

BACKGROUND: The development and commercialisation of new chemical classes of insecticides for efficient crop protection measures against destructive invertebrate pests is of utmost importance to overcome resistance issues and to secure sustainable crop yields. Flupyradifurone introduced here is the first representative of the novel butenolide class of insecticides active against various sucking pests and showing an excellent safety profile.

RESULTS: The discovery of flupyradifurone was inspired by the butenolide scaffold in naturally occurring stemofoline. Flupyradifurone acts reversibly as an agonist on insect nicotinic acetylcholine receptors but is structurally different from known agonists, as shown by chemical similarity analysis. It shows a fast action on a broad range of sucking pests, as demonstrated in laboratory bioassays, and exhibits excellent field efficacy on a number of crops with different application methods, including foliar, soil, seed treatment and drip irrigation. It is readily taken up by plants and translocated in the xylem, as demonstrated by phosphor imaging analysis. Flupyradifurone is active on resistant pests, including cotton whiteflies, and is not metabolised by recombinantly expressed CYP6CM1, a cytochrome P450 conferring metabolic resistance to neonicotinoids and pymetrozine.

CONCLUSION: The novel butenolide insecticide flupyradifurone shows unique properties and will become a new tool for integrated pest management around the globe, as demonstrated by its insecticidal, ecotoxicological and safety profile.

Keywords: flupyradifurone; Sivanto®; nicotinic acetylcholine receptor agonist; CYP6CM1; metabolic resistance; resistance management; sucking pests; butenolide

1 INTRODUCTION

The discovery and development of new chemical classes of insecticides is of utmost importance to keep key invertebrate pests under economic damage thresholds and thus to guarantee sustainable yields of commodities in order to supply affordable food for a growing world population.[1] The task of discovering a new class of insecticides or a new mode of action these days seems to be reserved for a fairly low number of R&D-based companies, particularly considering the recent consolidation process in the crop protection industry.[2] However, between 1990 and 2010 the costs for the development of a new insecticide increased by more than 100% to approximately $US 250 million, and the average development and registration process takes almost 10 years.[2] It must be borne in mind that during the last two decades strong cost/benefit measures have been implemented in industry, and that these days 140 000 compounds need to be screened for a new product on the market, in strong contrast to the 1 out of 20 000 compounds 35 years ago.[2,3] However, new selective insecticides compatible with modern integrated pest management (IPM) principles addressing the regulatory needs for an improved toxicological and environmental profile will stepwise replace older chemistry suffering from resistance development in many invertebrate pests frequently targeted by indispensable chemical treatments in some agricultural settings. The implementation of resistance management strategies based on mode-of-action rotation, including new chemical classes/modes of action, is a prerequisite in modern applied agriculture as promoted by the Insecticide Resistance Action Committee (IRAC).[4] In particular, hemipteran insect species such as aphids, whiteflies, stink bugs and psyllids are known to be among the most destructive pests in the world, transmitting plant pathogenic viruses/bacteria and causing feeding damage on numerous annual and perennial crops. Such pests are not yet accessible by trait technologies, so there is a need for corrective treatments to keep them under economic damage thresholds. Some sucking pest species have developed resistance to virtually all chemical classes of insecticides introduced to control them.[5] In order to diversify the toolbox necessary for appropriate resistance management measures

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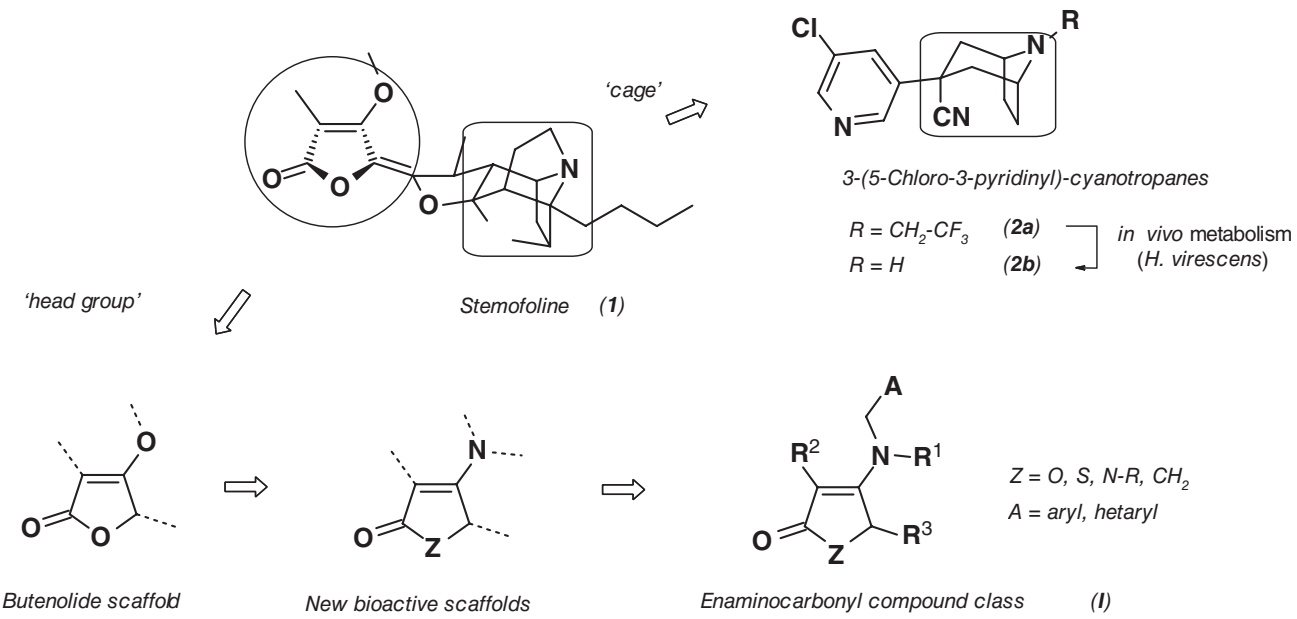

Figure 1. Natural product stemofoline **1** as the lead structure for novel ligands, e.g. **2a**, **2b** and **I**.

alternating with established compounds, new chemical classes of insecticides need to be introduced.[6]

In search of new chemical scaffolds leading to novel chemical classes of insecticides, particularly for sucking pest control, the natural product stemofoline **1** (Fig. 1), isolated from the leaves and stem of the oriental medicinal plant *Stemona japonica* (Blume) Miq. (Stemonaceae) and known as a potent agonist of insect nicotinic acetylcholine receptors (nAChRs), was considered as a good starting point.[7,8] Stemofoline shows fast-acting insecticidal, antifeedant and repellent activities, but its activity is significantly lower than that of commercial products acting on insect nAChRs.[9–11] Therefore, **1** was broadly used as a potent lead structure in order to identify novel active ingredients for modern crop protection.[11]

Recently, the design of smaller molecules has focused mainly on the stemofoline 2,6-methanofuro[2,3,4-gh]pyrrolizine cage structure. Based on this cage structure, the class of pyridinyl-cyanotropanes was found, for example the *in vivo* highly active 3-(5-chloro-3-pyridinyl)-8-(2,2,2-trifluoroethyl)-8-azabicyclo [3.2.1]octane-3-carbonitrile **2a** (R = CH_2CF_3) (Fig. 1), which is bioactivated in larval *Heliothis virescens* (Fabricius) by cleavage of the *N*-(2,2,2-trifluoroethyl) residue **2b** (R = H) (Fig. 1).[12,13] When studying the impact of the stemofoline head group on the efficacy of **1**, we became interested in the five-ring butenolide scaffold as a starting point for the preparation of new bioactive scaffolds and finally the identification of the biologically active enaminocarbonyl compound class (**I**) (Fig. 1).

At the same time, our concept was strongly supported by molecular modelling investigations with **1** and relevant nAChR ligands containing similar structural fragments like the azoles (**II**) and five-ring and six-ring heterocycles (**III**), as outlined in Fig. 2.[14–16] By stepwise chemical optimisation of enaminocarbonyl compounds (**I**) via forming different active butenolide subclasses like **IV** (Z = O) and **V** (A = Het), the butenolide lead structures **VI** ($R^1 = CH_2CHR-F$) were obtained (Fig. 3) (in all structures of subclasses **IV** to **VIII**, changes are highlighted by frames). Further exploration of substituted *N*-containing five-ring and six-ring heterocycles in **VI** resulted in butenolides **VII** (Het = X, Y, Z or

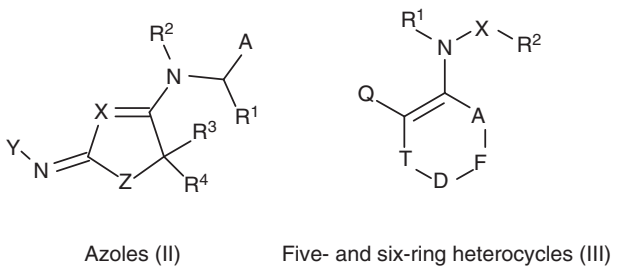

Figure 2. Relevant nAChR ligands **II** and **III** as lead structures for butenolide chemistry.

W-substituted 3-pyridinyl), which could be optimised by the introduction of the 6-chloropyridin-3-yl moiety (X, Y, Z, W = H) to form highly active insecticides such as **VIII**. Finally, the introduction of a second fluorine atom into the *N*-2-fluoroethyl side chain of **3** (R = H) led to the discovery of flupyradifurone **4** (R = F) (Fig. 3).[17]

Compared with already commercialised nAChR agonists such as *N*-cyanoamidines (acetamiprid, thiacloprid), nitroenamines (nitenpyram), *N*-nitroguanidines (imidacloprid, clothianidin, thiamethoxam or dinotefuran) or sulfoximines (sulfoxaflor), the butenolide flupyradifurone **4** (Z = O) contains a different pharmacophore system as a new bioactive scaffold (Fig. 4; Table 1) and was chosen for further global development by Bayer CropScience under the trade name Sivanto® [SL 200 g L^{-1} (soluble liquid)].[11] Flupyradifurone has a favourable toxicological and ecotoxicological safety profile (Table 2) and is under global development for foliar, drench and seed treatment applications, particularly against sucking pest insects in numerous agricultural and horticultural crops (Table 3). It received its first commercial registrations in Central America (Guatemala and Honduras) in April 2014 and is classified by IRAC as a new chemical subgroup 4D (butenolides) within the latest published version of the IRAC mode-of-action classification system (www.irac-online.org).

The present paper briefly reports on the chemistry, including chemical synthesis and similarity relations to established pharmacophores addressing the nAChR. It reflects on the profile and

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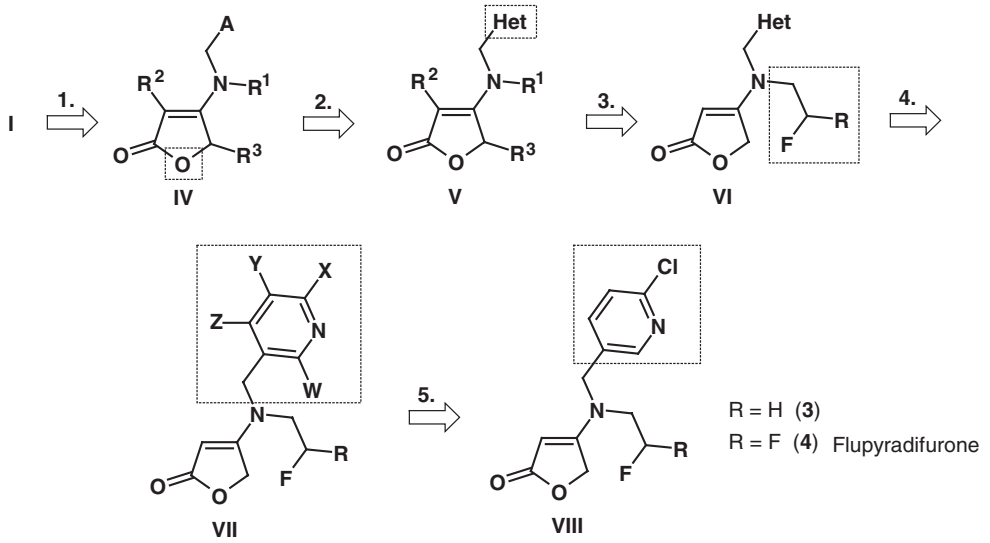


Figure 3. Stepwise chemical evolution of enaminocarbonyl compound **I** via the active butenolide subclasses **IV** to **VIII**, resulting in the discovery of flupyradifurone **4** (Sivanto®).

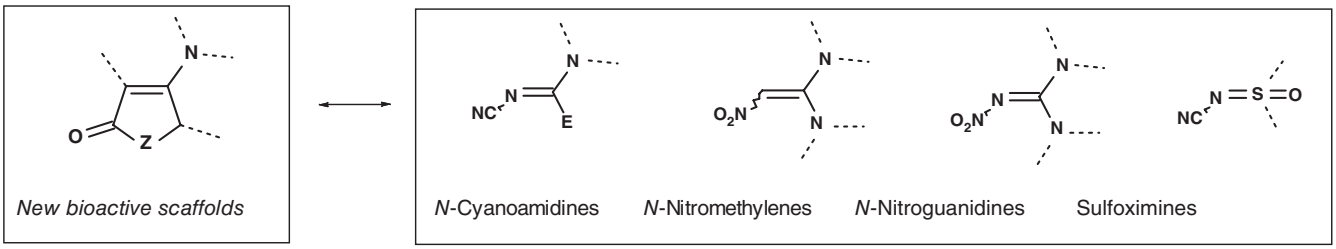


Figure 4. New bioactive scaffold versus pharmacophore systems of known nAChR agonists.

some of the technical properties of flupyradifurone, such as mode of action, translocation in plants, insecticidal efficacy against selected sucking pests in laboratory and field tests, speed of action and the lack of metabolic cross-resistance to neonicotinoids *in vivo* and *in vitro* by using recombinantly expressed CYP6CM1, a cytochrome P450 known to confer resistance to neonicotinoids and pymetrozine.[18]

2 EXPERIMENTAL METHODS

2.1 Chemical synthesis

All reagents were purchased from commercial suppliers and used without further purification. 4-[(2-Fluoroethyl) amino]furane-2(5*H*)-one **X** (R = H) or 4-[(2,2-difluoroethyl)amino] furane-2(5*H*)-one **X** (R = F) can be synthesised by a described route: 2-fluoroethylamine hydrochloride or 2,2-difluoroethylamine and tetronic acid (**5**). *N*-[(6-Chloropyridin-3-yl)methyl]-2,2-difluoro ethane-1-amine **XI** (R = F) was synthesised by a described route: 2-chloro-5-chloromethylpyridine and 2,2-difluoroethane -1-amine. Figure 5 shows general synthetic pathways to butenolides **VII** via tetronic acid **5** or five-ring lactone intermediates **X** by two different methods A and B.[19] Starting with **5**, the butenolides **VII** can be prepared either by treatment with 2-fluoro-containing ethylamine (R = H, F) following *N*-alkylation of the intermediates **X** with 2-chloro-5-chloromethylpyridine (method A) or by coupling of **5** with secondary amines of type **XI** (method B). Representative preparation methods of the insecticidally active butenolides **VII** are given below. ^{1}H NMR spectra were recorded in deuteroacetonitrile (CD_3CN), with tetramethylsilane as the internal standard, using a Bruker Avance 600 instrument (600 MHz) (Bruker Corporation, Billerica, MA).

2.1.1 4-{[(6-Chloropyridin-3-yl)methyl](2-fluoroethyl)amino} furan-2(5H)-one **3**; general procedure for butenolides **VII** according to method A

A quantity of 1.00 g (6.89 mmol) of 4-[(2-fluoroethyl)amino] furane-2(5*H*)-one **X** (R = H) and 0.55 g (13.78 mmol) of a 60% dispersion of sodium hydride in mineral oil in tetrahydrofuran (200 mL) were heated under reflux for 2 h. After cooling to room temperature, 2.23 g (13.78 mmol) of 2-chloro-5-chloromethylpyridine was added, and the mixture was heated under reflux for a further 4 h. The reaction mixture was cooled to room temperature, and methanol was added. After concentration of the reaction mixture under reduced pressure, the residue was taken up in ethyl acetate and the mixture was washed successively twice with 1 N aqueous hydrochloric acid, twice with 1 N aqueous sodium hydroxide solution and once with saturated sodium chloride solution. The organic phase was then dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by column chromatography on silica gel [silica gel 60, particle size 0.04–0.063 mm; Merck, Darmstadt, Germany) using the mobile phase mixture ethyl acetate:cyclohexane (9:1) resulted in 949 mg (50% of theory) of 4-{[6-chloropyridin-3-yl)methyl](2-fluoroethyl)amino}furan-2 (5*H*)-one **3**.

^{1}H NMR (CD_3CN, δ, ppm): 3.50 (td, 2H), 4.50 (s, 2H), 4.57 (dt, 2H), 4.65 (s, 1H), 4.79 (s, 2H), 7.38 (d, 1H), 7.65 (dd, 1H), 8.28 (d, 1H).

Table 1. Identity and physicochemical properties of flupyradifurone

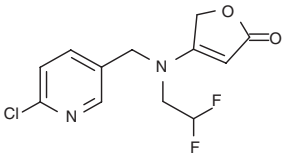
Common name (ISO)	Flupyradifurone
Chemical name (IUPAC)	4-{[(6-Chloropyridine-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5*H*)-one
Structural formula	
Empirical formula	$C_{12}H_{11}ClF_2N_2O_2$
Molecular weight	288.68 g mol^{-1}
Appearance	Solid powder
Colour	White to beige
Odour	Weak, not characteristic
Melting point	69 °C
Boiling point	No boiling point at atmospheric conditions
Vapour pressure	9.1×10^{-7} Pa (20 °C)
Density	D = 1.43 (purity 99.4%)
Solubility (at 20 °C)	Water 3.2 g L^{-1} (pH 4)
	Water 3.0 g L^{-1} (pH 7)
	n-Heptane 0.0005 g L^{-1}
	Methanol >250 g L^{-1}
Partition coefficient	log P_{ow} = 1.2 (at 25 °C and pH 7)

Table 2. Mammalian toxicological and ecotoxicological properties of technical flupyradifurone on non-target organisms

Acute oral toxicity, rat LD_{50}	>300 mg kg^{-1}, <2000 mg kg^{-1} body weight
Acute dermal toxicity, rat LD_{50}	>2000 mg kg^{-1} body weight
Acute inhalation toxicity, rat LC_{50}	>4671 mg m^{-3}
Skin irritation	Non-irritant
Eye irritation	Non-irritant
Mutagenicity	Not a mutagen
Carcinogenicity	Not a carcinogen
Reproductive and developmental toxicity	No concern
Birds (bobwhite quail) acute oral LD_{50}	232 mg kg^{-1} body weight
Fish (rainbow trout) acute LC_{50}	>74.2 mg L^{-1}
Aquatic invertebrates (*Daphnia*) acute EC_{50}	>77.6 mg L^{-1}
Aquatic plants (algae) EC_{50}	>80 mg L^{-1}
Earthworm (*Eisenia*) 14 day LC_{50}	193 mg kg^{-1} dry weight soil
Honey bee acute contact LD_{50}	>100 µg bee^{-1}
Honey bee acute oral LD_{50}	1200 ng bee^{-1}
Honey bee foliage residue	No effects at 205 g ha^{-1}
Honey bee long-term field studies at 205 g AI ha^{-1} in oilseed rape (full bloom and bees actively foraging)	No adverse effects
Bumblebee acute contact LD_{50}	>100 µg bee^{-1}

Table 3. Selected crops and pests targeted by flupyradifurone applications

Crop	Pest	Application method
Vegetables/potatoes	Whiteflies Aphids Potato psyllid	Foliar/drench
Pome fruits	Aphids Scales Psyllids Sawfly	Foliar
Grapes	Leafhoppers Grapevine mealybug	Foliar/drench
Citrus	Asian citrus psyllid Citricola scale Citrus thrips	Foliar/drench
Cotton	Cotton aphid *Lygus* bugs	Foliar
Soybean	Aphids	Seed treatment
Coffee	Coffee leafminer	Foliar/drench
Cocoa	Cocoa mirids	
Hops	Damson hop aphid	Foliar
Ornamentals	Aphids Whiteflies	Foliar

2.1.2 4-{[(6-Chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5H)-one **4**; flupyradifurone, Sivanto®; general procedure for butenolides **VII** according to method B

On a water separator, 21.90 g (106.0 mmol) of *N*-[(6-chloropyridin-3-yl)methyl]-2,2-difluoroethane-1-amine, 14.85 g (148.4 mmol) of tetronic acid **5** and 183 mg (1.1 mmol) of 4-toluenesulfonic acid (*p*-TSA) in toluene (250 mL) were heated under reflux for 2 h. The reaction mixture was concentrated under reduced pressure, the residue was then taken up in ethyl acetate and the mixture was washed successively twice with 1 N aqueous hydrochloric acid, twice with 1 N aqueous sodium hydroxide solution and once with saturated sodium chloride solution. The organic phase was dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by column chromatography on silica gel (silica gel 60 – Merck, particle size 0.04–0.063 mm) using the mobile phase ethyl acetate resulted in 15.9 g (52% of theory) of 4-{[6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5*H*)-one **4**.

^{1}H NMR (CD_3CN, δ, ppm): 3.59 (td, 2H), 4.51 (s, 2H), 4.76 (s, 1H), 4.80 (s, 2H), 6.03 (tt, 1H), 7.38 (d, 1H), 7.64 (dd, 1H), 8.28 (d, 1H).

2.2 Cheminformatics to compute chemical similarity relations

The full 30 × 30 similarity matrix was calculated for a set of compounds (Fig. 7), employing Tanimoto indices computed on Unity fingerprints using Sybylx2.0.[20] The full similarity table can also be interpreted as a connection table, connecting all pairs of molecules with a certain similarity value. Inspired by methods from social network analysis, the similarity-derived connection table reveals cliques (or clusters) of related compounds when only connections with a minimum similarity value are considered. In a first step, all pairs of compounds with a Tanimoto index greater than 0.5 were considered, resulting in five disconnected graphs, i.e. one for nicotinoids, one for sulfoximines, one for butenolides, one for furanyl-substituted neonicotinoids and one big cluster comprising all other neonicotinoid derivatives. In order to arrive at a single, connected graph, the nearest neighbour with a Tanimoto of ≤0.5 was searched for each member of the four small clusters. Only the pairs with the highest similarities were kept. Using the Mathematica software suite v.10.0 (Wolfram Research, Inc., Champaign, IL), the resulting connection tables were visualised using the spin electric embedding scheme, which treats the graph as a kind of 2D molecule and minimises assumed repulsive forces between all vertices.

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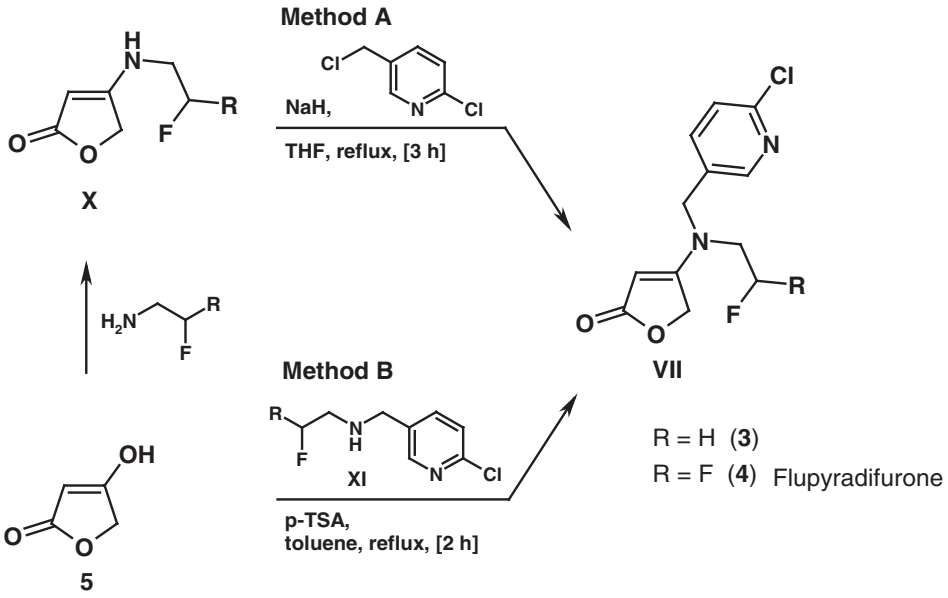

Figure 5. General synthetic pathways to butenolides **VII** by methods A and B.

2.3 Mode of action

2.3.1 Receptor binding studies

Radioligand [^{3}H]imidacloprid displacement studies were conducted according to established protocols by using membranes isolated from frozen (−80 °C) housefly (*Musca domestica* L.) heads.[21,22] Briefly, 5 g heads were homogenised in 100 mL of 0.1 M K-phosphate buffer, pH 7.4, 320 mM of sucrose and 1 mM of EDTA using an Ultra Turrax at 4 °C. After centrifugation for 15 min at 1200 × *g* and 4 °C, the pellet was resuspended and centrifuged again. Both supernatants were combined and filtered through Miracloth, and the filtrate was subsequently centrifuged at 105.000 × *g* for 60 min at 4 °C. The resulting pellet was resuspended in buffer and adjusted to approximately 0.5 mg protein mL^{-1}. The assay was conducted in a total volume of 1 mL, consisting of 850 µL of homogenate and 50 µL of [^{3}H]imidacloprid (25.000 dpm; 1.406 GBq µmol^{-1}) in 0.1 M K-phosphate buffer, pH 7.4, containing 1 g L^{-1} of BSA and 5% ethanol (0.25% final concentration). After 5 min, different flupyradifurone concentrations were added (1000, 100, 10, 3, 1, 0.3, 0.1 and 0.01 nM, containing up to 0.1% DMSO). After incubation for 60 min at 22 °C while shaking, the samples were filtered through prewetted Whatman GF/C glass fibre filters, followed by two rinses with 3 mL of ice-cold 0.1 M K-phosphate buffer (pH 7.4). Subsequently, the filters were dried (55 °C, 40 min), and 3.5 mL of scintillation cocktail was added. After 16 h at room temperature, the samples were subjected to liquid scintillation counting.

2.3.2 Electrophysiology

Electrophysiological recordings using whole-cell voltage clamp technology were done on isolated neurons of *Spodoptera frugiperda* (JE Smith) (fall armyworm), as recently described.[23] Briefly, isolated ganglia were treated with 4 mg L^{-1} of dispase, incubated for 5 min at 37 °C, centrifuged and resuspended in culture buffer by gentle aspiration with a fire-polished pasteur pipette, with slight modifications as described elsewhere.[24] Cell somata were plated onto glass cover slips previously coated with concanavalin-A (400 µg mL^{-1}) and laminin (4 µg mL^{-1}). The cells were kept at room temperature. Electrophysiological recordings were done using the whole-cell voltage clamp technique at a holding potential of −70 mV, as described elsewhere.[25] The external bath contained 150 mM of NaCl, 4 mM of KCl, 2 mM of $MgCl_2$, 2 mM of $CaCl_2$, 150 mM of trehalose and 10 mM of Hepes, pH 7.4 (adjusted with NaOH). The (internal) pipette solution contained 100 mM of CsF, 50 mM of CsCl, 10 mM of Cs-EGTA, 1 mM of $CaCl_2$, 150 mM of trehalose and 10 mM of Hepes, pH 7.4 (adjusted with CsOH). Flupyradifurone was applied at different concentrations to the cells using the U-tube reversed flow technique.[26] Currents were measured with an L/M-EPC 7 patch clamp amplifier (List, Darmstadt, Germany). Current records were low-pass Bessel filtered at 315 Hz and digitised at 1 kHz sample rate. The dose–response curve was fitted by the Hill equation. All currents were normalised to mean amplitudes elicited by 10 µM of acetylcholine before and after test concentrations were applied.

2.4 Plant uptake and translocation studies

Uptake and distribution of [pyridinylmethyl-^{14}C]-labelled flupyradifurone (specific activity 4.37 MBq mg^{-1}; purity >99%) formulated as SL200 (containing radiolabelled flupyradifurone at 13.4 KBq mg^{-1}) was qualitatively studied in 21-day-old tomato plants (*Solanum lycopersicum* L.) after soil drench application at 24 mg AI plant^{-1} in 5 L pots in dry sandy loam at 80–90% water-holding capacity. Plants were maintained at 60–70% relative humidity and a temperature of 21 and 16 °C during the day (14 h light) and night-time (8 h) respectively. At sampling dates (1, 3, 7, 14 and 24 days after application), shoots were cut near the soil surface, pressed between several layers of filter paper and dried for 24 h at 50 °C. After drying, the shoots (leaves) were exposed to a phosphor imaging plate placed into a Fuji-Fujix BAS cassette 2040. Cassettes were then stored for 6 days in a lead shielding box at ambient temperature. Afterwards, the phosphor imaging plates were measured with a Fujifilm BAS 2500 scanner, and obtained images were analysed using AIDA Image Analyzer 4.14 software (Raytest GmbH, Straubenhardt, Germany). In a second set of experiments, droplets of [pyridinylmethyl-^{14}C]flupyradifurone SL200 formulation were either applied to the midrib of leaf 7 (25 000 Bq droplet^{-1}) or directly onto the centre pinnate leaves of

leaf 8 (1000 Bq droplet^{-1}) of a tomato plant. Four days after application, plants were subjected to the same preparation and imaging procedure as described above.

2.5 Bioassays

2.5.1 Speed of action and suppression of aphid honeydew excretion

Green peach aphid, *Myzus persicae* (Sulzer), used in this bioassay were taken from an anholocyclic insecticide-susceptible laboratory strain (NS) maintained on cabbage (*Brassica oleracea* L.) at 22 °C, 60–70% relative humidity and 16:8 h photoperiod. In order to check the speed of action of flupyradifurone (formulated as SL200), we measured the suppression of honeydew excretion of *M. persicae* feeding on oilseed rape (*Brassica napus* L.) leaves after foliar treatment at a rate of 75 g AI ha^{-1}. As a comparison, a treatment with thiamethoxam (Actara®, 25 g AI ha^{-1}; Syngenta, Basel, Switzerland) was included in the trial. Ten-day-old greenhouse-grown oilseed rape plants (six leaves) were infested with a mixed population of 100 aphids feeding on the abaxial side of the leaves. Plants were treated 50 cm above the canopy with a purpose-built linear track sprayer with a flat-fan nozzle SS 8003-E at the above-mentioned rates using a water volume of 300 L ha^{-1}. Each treatment was replicated twice; control plants were treated with water only. The treated plants were placed at 22 °C and 60–70% relative humidity under neon light. Filter papers were mounted under each leaf during preapplication and post-application phases and replaced every 30 min for up to 240 min. Filter papers were then sprayed with ninhydrin at 1 g L^{-1} acetone and dried for 5 min at 100 °C. The area of purple honeydew spots was determined using standard image analysis software based on the Open CV Halcon library (Bayer Technology Services GmbH, Leverkusen, Germany), plotted per plant and compared with water-treated controls at different elapsed time intervals.

2.5.2 Aphid leaf-dip bioassays

All insecticide-susceptible aphid species tested were derived from laboratory cultures grown without insecticide selection pressure for at least 10 years. A leaf-dip bioassay procedure was recently designed to test diverse insecticidal classes of chemistry under the same conditions in six-well tissue culture plates or purpose-built ventilated petri dishes.[27] Wells of six-well tissue culture plates or petri dishes were filled with 2 mL of agar (10 g L^{-1}). Several plates/petri dishes were usually prepared and stored at 4 °C until use. Plates/petri dishes were equilibrated to room temperature, and leaf discs (30 mm diameter) were cut out of the first and second fully expanded true leaves from three-week-old plants such as *B. oleracea* (*M. persicae*), *Humulus lupulus* L. [*Phorodon humuli* (Schrank)], *Gossypium hirsutum* L. [*Aphis gossypii* (Glover)] and *Lactuca sativa* L. [*Nasonovia ribisnigri* (Mosley)]. The leaf discs were then dipped into serial aqueous dilutions of active ingredient in 0.02% (w/v) Triton X-100, and after drying on tissue paper the leaf discs were transferred to six-well plates/petri dishes. Flupyradifurone was tested at least 3 times, with up to six replicates per concentration. Ten adult aphids were confined to each well. Mortality was scored after 72 h unless otherwise stated. Aphids unable to move and showing strong symptoms of poisoning were scored as dead.

A similar set-up was used to test imidacloprid in comparison with flupyradifurone with metabolically resistant clones of *M. persicae* (F03-09) and *P. humuli* (Hallertau). *M. persicae* clone F03-09 was derived from an *M. persicae* strain collected in oilseed rape in France in 2009, and *P. humuli* clone Hallertau was derived from a field strain collected in German hops (Hallertau) in 2011.

2.5.3 Whitefly bioassays

Insecticide-susceptible strains of *Bemisia tabaci* (Gennadius) (SUD-S) and *Trialeurodes vaporariorum* (Westwood) (S-6230) were taken from laboratory cultures maintained without insecticide selection for at least 10 years. Whitefly adult leaf-dip bioassays followed an established experimental protocol.[28] Briefly, cotton leaf discs (3.5 cm in diameter) were dipped for 5 s into insecticide solutions (prepared in aqueous Triton X-100, 0.02% w/v) diluted to the required concentration, or into the diluent alone for controls. Leaf discs were then air dried on tissue paper and subsequently laid on an agar bed (12 g L^{-1}) held within a plastic petri dish or six-well tissue culture plate. Adult females were then placed in the dish and confined using a close-fitting ventilated lid. Bioassays consisted of three replicates per concentration, each with a group of 20–30 female insects. They were maintained at 22–24 °C, with adult mortality scored after 72 h.

The same bioassay type was used to compare the efficacy of imidacloprid and flupyradifurone against neonicotinoid-resistant strains of *B. tabaci*, i.e. biotype Q (strain Q, collected in Almeria, Spain, 2007) and biotype B (strain B, collected in Unai, Brazil, 2009). Furthermore, an imidacloprid-resistant strain of *T. vaporariorum* collected in 2007 on greenhouse ornamentals in the Netherlands was tested for flupyradifurone cross-resistance.

2.5.4 Field trials against lettuce aphids

As an example to show the field performance of flupyradifurone against aphids, seven field trials in lettuce against *N. ribisnigri* conducted in the EU maritime centre zone were analysed and combined. The trials were conducted in Belgium, France, Germany and the Netherlands at BBCH stage 15 to 19 using flupyradifurone (SL200) at 125 g AI ha^{-1} applied by foliar spray using a knapsack sprayer equipped with flat-fan nozzles and water volumes between 300 and 916 L ha^{-1}. Commercial formulated standards such as pymetrozine (200 g AI ha^{-1}), thiacloprid (96 g AI ha^{-1}) and spirotetramat (75 g AI ha^{-1}) were included in all trials for comparison. Treatments in each trial were replicated 3–4 times, and aphid infestations were above economic threshold levels. Assessments of efficacy were done at different elapsed time intervals after spraying by scoring surviving aphids. Percentage control was calculated in relation to the aphid infestation level of control plots.

2.6 Baculovirus-mediated P450 expression and insecticide metabolism

B. tabaci full-length CYP6CM1 sequence (GenBank accession number GQ214539) and *Drosophila melanogaster* (Meigen) NADPH CPR (cytochrome P450 reductase) sequence (GenBank accession number Q27597) were obtained by gene synthesis, and each was inserted into pDEST8 expression vector (Invitrogen, Darmstadt, Germany) for baculoviral expression. The heterologous expression in SF9 cells using baculovirus transfection and preparation of microsomes was described elsewhere.[18,29] CYP6CM1 activity was confirmed by its dealkylation ability relative to various fluorescence substrates such as 7-ethoxycoumarin (data not shown) and the successful hydroxylation of imidacloprid to 5-hydroxy-imidacloprid, as described recently.[30]

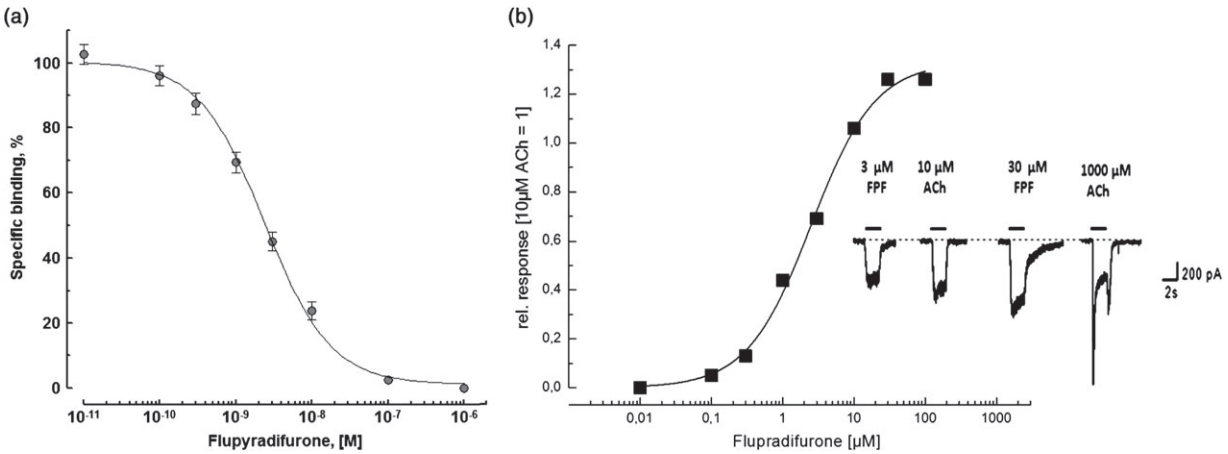

Figure 6. (a) Binding affinity of flupyradifurone to nicotinic acetylcholine receptors in head membrane preparations of *Musca domestica* using [^{3}H]imidacloprid as a probe in radioligand displacement studies. Data are mean values ± SEM (n =3). (b) Electrophysiological whole-cell current responses of a neuron isolated from the central nervous system of *Spodoptera frugiperda* after application of different concentrations of flupyradifurone (FPF) and acetylcholine (ACh) (holding potential −70 mV).

2.6.1 Metabolism of insecticides by CYP6CM1

The metabolism of technical insecticides, including imidacloprid, pymetrozine, flupyradifurone, spirotetramat and sulfoxaflor (obtained in-house; purity of each >99%), was assayed by incubation of the recombinant CYP6CM1/NADPH CPR microsomes (0.2 mg mL^{-1} total protein content) in 0.1 M potassium phosphate buffer with an NADPH-regenerating system (Promega; 1.3 mM of NADP+, 3.3 mM of glucose-6-phosphate, 3.3 mM of $MgCl_2$, 0.4 U mL^{-1} of glucose-6-phosphate dehydrogenase) and insecticide (20 µM) at 30 °C for 4 h. The total assay volume was 200 µL, using three replicates for each data point. Microsomes without NADPH served as a control. The assay was quenched by the addition of acetonitrile (to 50% final concentration) and centrifuged for 10 min at 3000 × g, and the supernatant was subsequently analysed by tandem mass spectrometry. Recovery rates of active ingredients using microsomal fractions without cofactor were normally close to 100%. All samples obtained from insecticide metabolism assays were analysed for parent compound degradation using ultraperformance liquid chromatography (Waters Acquity UPLC System; Waters, Eschborn, Germany) – mass spectrometry utilizing a TSQ Vantage triple quadrupole instrument with an H-ESI II source (Thermo, Dreieich, Germany) operating in positive ion mode, as recently described.[29]

2.7 Statistics

In radioligand binding studies, I_{50} values (concentration of unlabelled ligand displacing 50% of [^{3}H]imidacloprid from its binding site) were calculated using a four-parameter logistic non-linear fitting routine (GRAPHPAD-PRISM 5, www.graphpad.com). Lethal concentration (LC) values were calculated from log dose probit mortality regressions using Polo PC (LeOra Software, Berkeley, California). Whenever necessary, mortality figures were corrected using Abbott's formula.[31] Resistance ratios were calculated by dividing the LC_{50} value of the susceptible reference strain with the LC_{50} value of the resistant strain.

3 RESULTS

3.1 Mode of action

The novel butenolide insecticide flupyradifurone acts on insect nAChRs as shown in radioligand binding studies conducted with tritiated imidacloprid, a well-known nAChR agonist out of the different chemical class of neonicotinoids (Fig. 6). Flupyradifurone displaces [^{3}H]imidacloprid bound to *M. domestica* nAChRs from its binding site with nanomolar affinity, and an I_{50} value of 2.38 ± 1.93 nM was calculated. Whole-cell current responses after application of flupyradifurone and acetylcholine in a single neuron isolated from *S. frugiperda* CNS suggest reversible binding and are shown in Fig. 6. Flupyradifurone activates endogenously expressed insect nAChRs and acts as a partial agonist with a relative agonist efficacy of 0.56 relative to the amplitude elicited by 1 mM of acetylcholine. Using the same experimental set-up, imidacloprid exhibits a relative efficacy of 0.15 relative to acetylcholine (data not shown). The flupyradifurone dose–response curve fitted by the Hill equation revealed a Hill coefficient n_H of 0.97 ± 0.090 and suggests a single binding site (EC_{50} = 2.3 µM for half maximal activation of nAChRs).

3.2 Chemical similarity analysis

Flupyradifurone is shown to address insect nAChRs in a similar way as other commercialised chemical classes of insecticides such as sulfoximines, nicotinoids and neonicotinoids. In order to check its chemical relatedness to known effectors of insect nAChRs, an analysis in terms of Tanimoto indices of a given set of compounds for each subclass was conducted (Fig. 7). The resulting graph visualises all pairs of compounds with a similarity of more than 50%, plus some additional, lower similarities to create a connected graph. This graph may be read in an analogous way to graphs from social network analysis, revealing cliques of tightly connected, and thus related, individuals. Here, 'individuals' are chemical structures, and 'relations' are defined in terms of Tanimoto index. The majority of neonicotinoid compounds included in the analysis form a large clique or cluster, suggesting high chemical relatedness, with the exception of the tetrahydrofuryl (methyl)-substituted dinotefuran derivatives, which are clearly separated from heteroaromatic chloropyridyl- and chlorothiazolyl-substituted neonicotinoids, albeit they have the same pharmacophore system (*N*-nitroguanidine). Sulfoximines also clearly cluster outside the neonicotinoid substructures as well as the nicotine group. The novel butenolide chemistry represented by flupyradifurone and its derivatives also forms a separate cluster in terms of Tanimoto similarity, highlighting its chemical difference to neonicotinoids and other nAChR agonists.

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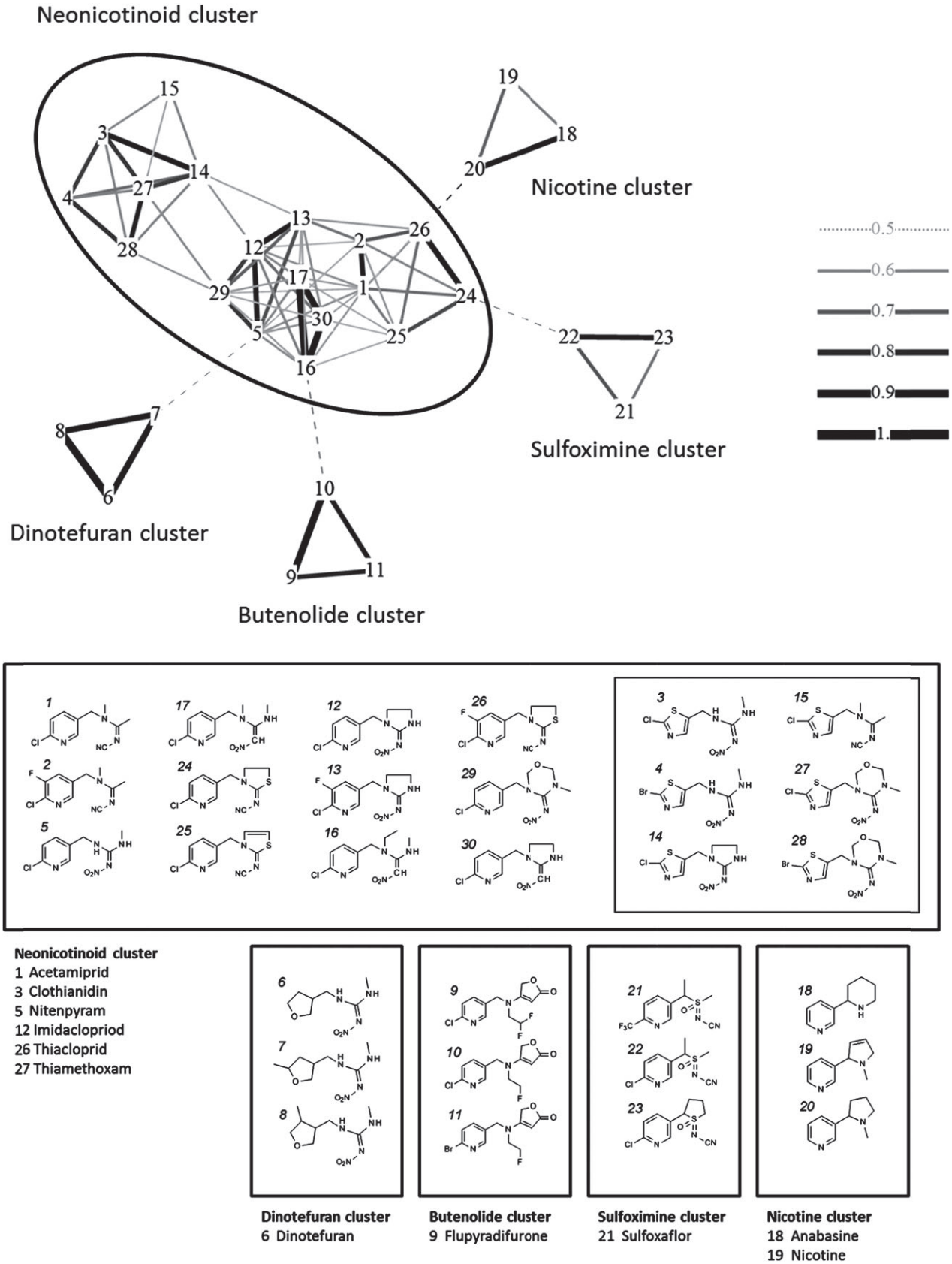

Figure 7. Chemoinformatic analysis of chemical similarity relations (Tanimoto indices) between different structural classes of nicotinic acetylcholine receptor agonists and their derivative-revealed structure-based clusters. Line thickness and grey level encode Tanimoto similarity values. Solid lines are used for similarities greater than 50%. In order to avoid a disconnected graph, the fourth highest similarity relationship for each of the small, triangular-shaped clusters is given as a dashed line.

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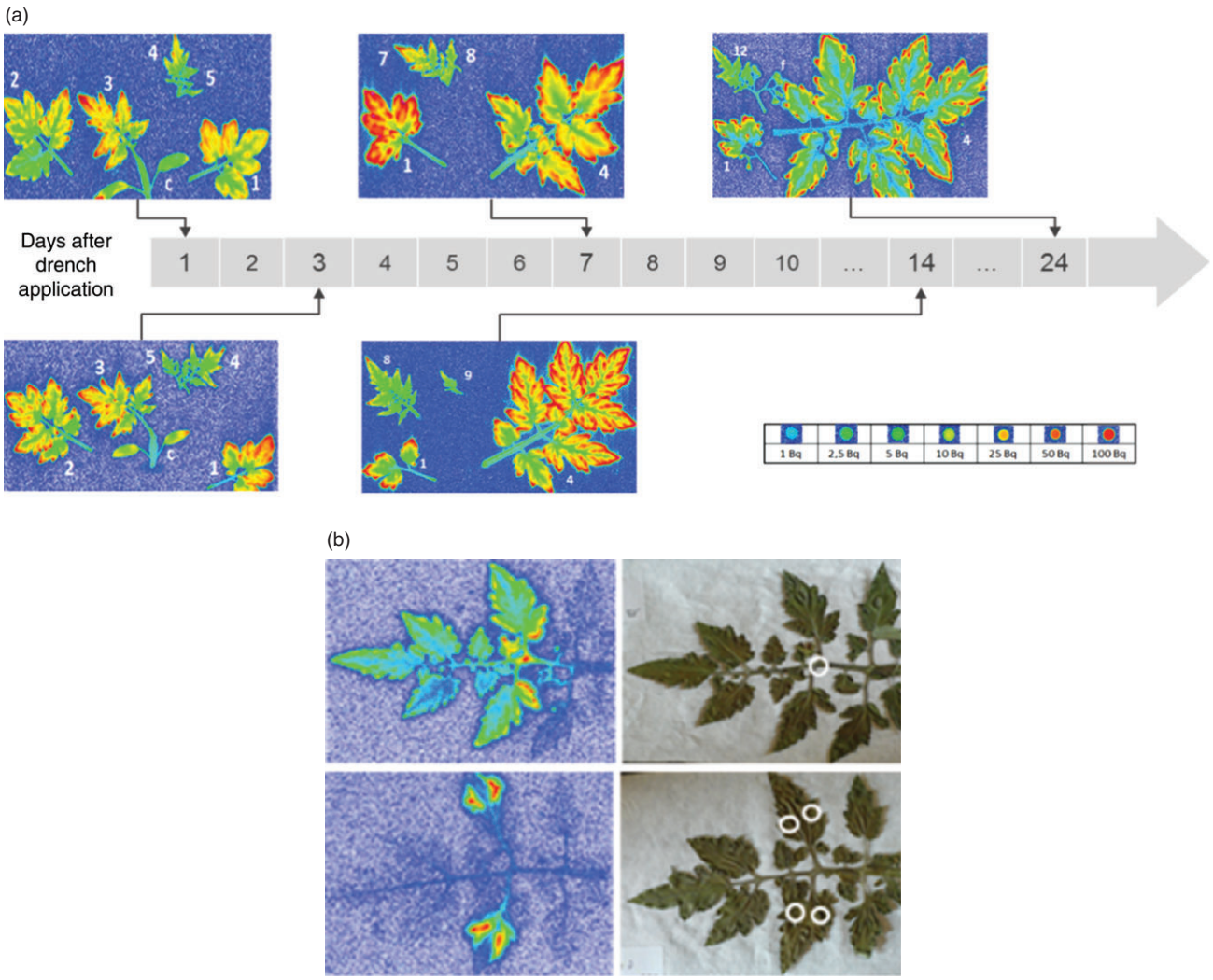

Figure 8. (a) Translocation of SL200 [^{14}C]flupyradifurone AI equivalents at different elapsed time intervals in tomato plants after root uptake via drench application. Numbers and letters indicate leaf positions: c = cotyledons; leaf 1 = oldest leaf; 12 = youngest leaf; f = flowers. (b) Uptake and translocation of SL200 [^{14}C]flupyradifurone AI equivalents into tomato plants 4 days after stem and foliar application respectively. Droplet application points are indicated by white circles.

3.3 Plant uptake and translocation

Uptake and translocation of [pyridinylmethyl-^{14}C]-labelled flupyradifurone was qualitatively studied in tomato plants using different treatment regimes, i.e. drench, stem and foliar application. Phosphor imaging analysis of tomato plants harvested 1 day after drench application revealed a fast root uptake and an even distribution of [^{14}C]-flupyradifurone equivalents within the entire plant via xylem translocation (Fig. 8a). Highest concentrations of [^{14}C]-flupyradifurone equivalents are seen 7 and 14 days after drench application, whereas 24 days after application a marked decline is observed. Furthermore the phosphor imaging autoradiographs clearly indicate the accumulation of flupyradifurone in the distal parts of the leaves at different time intervals after drench application, thus suggesting apoplastic transport via the xylem and the absence of significant phloem translocation (Fig. 8a). Flupyradifurone applied to the midrib of tomato plants or true leaves is also readily taken up and translocated via the transpiration stream (Fig. 8b), clearly indicating the systemic properties of the compound after foliar application.

3.4 Speed of action

The translaminar speed of action of flupyradifurone was investigated by checking the suppression of honeydew excretion of *M. persicae* feeding on treated oilseed rape plants. Flupyradifurone applied at recommended rates leads to a quick feeding (honeydew excretion) stop within 2 h of spray application, even on aphids feeding on the abaxial leaf side and not directly exposed to spray droplets (Fig. 9). Two days after application of flupyradifurone, all aphids were dead (data not shown). The action of flupyradifurone is superior to that of thiamethoxam which was included as a commercial standard in the same experiment.

3.5 Cross-resistance assessment and efficacy against aphids and whiteflies

Log dose probit mortality data of flupyradifurone against a number of strains of different aphid and whitefly species are shown in Table 4. Endpoint mortality was reached 72 h after leaf-dip application in all cases, and calculated LC_{50} values were often lower than those obtained for imidacloprid using the same bioassay set-up,

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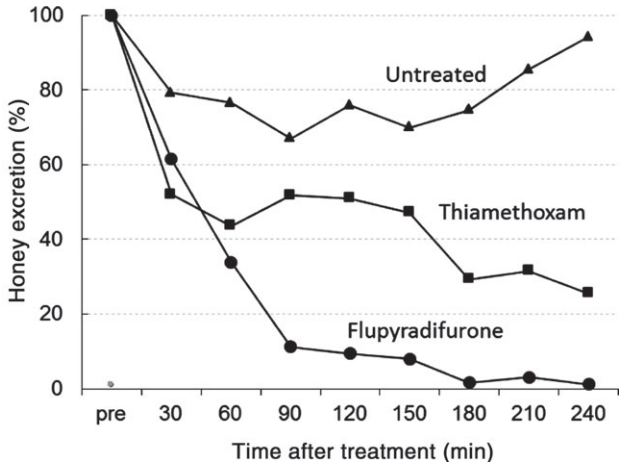

Figure 9. Kinetics of suppression of honeydew excretion in *Myzus persicae* feeding on oilseed rape and foliarly treated with flupyradifurone (Sivanto® SL200, 75 g AI ha^{-1}) and thiamethoxam (Actara®, 25 g AI ha^{-1}). Control plants remain untreated. Percentage suppression of honeydew excretion is relative to honeydew excretion levels preapplication.

indicating the high insecticidal activity of flupyradifurone, particularly against strains resistant to imidacloprid (Table 4). Some resistant strains included in the bioassays, such as the Q- and B-type *B. tabaci* strains, exhibit extremely high resistance ratios against imidacloprid, but virtually no or low resistance ratios to flupyradifurone, indicating a lack of high-level cross-resistance between neonicotinoids and the new butenolide insecticide flupyradifurone (Table 4; Fig. 10). Adult females of the B-type strain collected in Unai, Brazil, do not respond at all to imidacloprid concentrations of 1000 mg L^{-1}, whereas flupyradifurone at 16 mg L^{-1} resulted in 50% adult mortality, and 100% nymph mortality (data not shown).

3.6 Field trial results

Flupyradifurone (SL200) provided excellent field control of lettuce aphids at rates of 125 g ha^{-1} after foliar application, and shows at all assessment intervals an insecticidal potential greater than pymetrozine and thiacloprid included as commercial standards at rates of 200 g ha^{-1} and 96 g ha^{-1} respectively. Flupyradifurone provided the highest level of control against lettuce aphids at 6–10 days after application, i.e. 96% efficacy. Table 5 summarises the results obtained in seven field trials in the EU maritime zone. Flupyradifurone provided greater levels of control than spirotetramat at 2–3 and 6–10 days after application, but spirotetramat at 75 g ha^{-1} shows superior control 13–15 days after application.

3.7 CYP6CM1-mediated metabolism

Microsomal preparations containing *B. tabaci* CYP6CM1 functionally expressed in Sf9 cells do not metabolise flupyradifurone, but show significant degradation of imidacloprid and pymetrozine (Fig. 11). However, imidacloprid is different from flupyradifurone, as it belongs to the neonicotinoid class of chemistry. Two more insecticides of distinct chemical classes, spirotetramat (ketoenols) and sulfoxaflor (sulfoximines), were also incubated with the same microsomal preparations, and in both cases no detoxification was observed. After a microsomal incubation period of 4 h at 30 °C, quantitative UPLC-MS analysis revealed a degradation of $23 \pm 1\%$ and $70 \pm 6\%$ for imidacloprid and pymetrozine respectively, whereas flupyradifurone, spirotetramat and sulfoxaflor were not depleted at all (Fig. 11). Both imidacloprid and pymetrozine were hydroxylated by CYP6CM1 overexpressed in Sf9 cell microsomes (data not shown). No metabolisation was observed in the absence of NADPH, and all data shown in Fig. 11 were corrected for possible substrate depletion observed in Sf9 cells infected with baculovirus not expressing CYP6CM1.

4 DISCUSSION

New chemical classes of insecticides are considered to be one of the cornerstones in modern applied entomology in order to guarantee sustainable yields and to combat the development and spread of insecticide resistance, particularly in sucking pests such as aphids and whiteflies, which are known to include some of the most destructive global crop pest species within the order Homoptera.[32,33] Flupyradifurone is a new IPM-suitable insecticide developed by Bayer CropScience that will be globally launched, starting in 2014, as a new chemical option for the control of agricultural and horticultural pests, especially sucking pests of the order Homoptera. Some parameters of its chemical, ecotoxicological and safety profile are outlined in Tables 1 to 3, as well as in a recent regional conference paper in Italy.[34] The present paper aims to introduce flupyradifurone to a broader scientific audience and provide first sets of experimental data concerning its chemical and biological properties.

Flupyradifurone was shown to act on insect nAChRs by both radioligand binding studies using housefly head membranes and electrophysiological recordings using isolated insect neurons. As shown by alternating acetylcholine and flupyradifurone applications on the very same neuron, it reversibly binds to and activates endogenous insect nAChRs, similarly to other commercial insecticidal compounds such as sulfoxaflor and the neonicotinoids and nicotinoids.[11,35] However, although flupyradifurone shares the same mode of action with the chemical classes mentioned above, it is chemically different as it is the first nAChR insecticide containing the stemofoline-derived (natural compound) butenolide pharmacophore as a new bioactive scaffold agonistically addressing insect nAChRs. Its distinct chemical nature among commercial nAChR agonists is demonstrated here by a computational cheminformatics-driven approach, i.e. the analysis of chemical similarity relations (Tanimoto indices) between all commercially known classes of nAChR agonists and some non-commercial, but close derivatives. Butenolide substructures cluster separately from neonicotinoid insecticides, sulfoximines and nicotinoids. The neonicotinoid cluster includes 2-halogen-thiazol-5-yl methyl-substituted as well as 6-chloropyridin-3-yl methyl-substituted neonicotinoids, but not dinotefuran, which has a non-aromatic tetrahydrofuryl(methyl) substituent; however, the *N*-nitroguanidine pharmacophore system remains the same as in imidacloprid, clothianidin and thiamethoxam, thus justifying its recent pharmacophore-based classification as a neonicotinoid insecticide.[11,35] In terms of pharmacophore substructures, sulfoximines are also clearly distinct from neonicotinoids, as recently shown,[36] and likewise the nicotine derivatives owing to their basic nature.[37] It is interesting to note that chlorothiazolyl- and chloropyridyl-substituted neonicotinoids are still similar enough in terms of Tanimoto indices to form one large chemical group (Fig. 7). The distinct chemical structure of the novel butenolide pharmacophore and the lack of metabolic cross-resistance of flupyradifurone led to the formation of a new subgroup (4D) classification within the IRAC mode-of-action classification scheme, a tool for setting up resistance management strategies based on mode-of-action

Table 4. Log dose probit mortality data of flupyradifurone against different strains of aphid and whitefly species in leaf-dip laboratory bioassays (72 h). Strains Roznava (*P. humuli*), NS (*M. persicae*), SUD-S (*B. tabaci*) and 6230 (*T. vaporariorum*) are insecticide-susceptible laboratory reference strains. Strains Hallertau (*P. humuli*), F03-09 (*M. persicae*), Q and B type (*B. tabaci*) and NL-07 (*T. vaporariorum*) are field strains expressing moderate to high resistance to neonicotinoids

	Flupyradifurone		Imidacloprid	
Species/strain	LC_{50} (mg L^{-1})	95% CL	LC_{50} (mg L^{-1})	95% CL
Nasonovia ribisnigri	0.38	0.32–047	0.27	0.095–0.87
Phorodon humuli				
Roznava	0.040	0.059–0.077	0.14	0.12–0.14
Hallertau	0.087	0.064–0.12	4.4	2.2–8.9
Aphis gossypii	3.2	2.8–3.6	13	10–18
Myzus persicae				
NS	0.32	0.19–0.54	0.24	0.11–0.40
F03-09	0.64	0.18–2.5	4.7	3.8–5.9
Bemisia tabaci				
SUD-S	5.5	0.33–4.9	0.76	0.47–1.2
Q type (Almeria, Spain)	40	22–56	>200	nd
B type (Unai, Brazil)	16	9.6–23	>1000	nd
Trialeurodes vaporariorum				
6230	4.8	3.8–6.7	22	5.4–38
NL-07	4.3	2.1–7.4	140	47–540

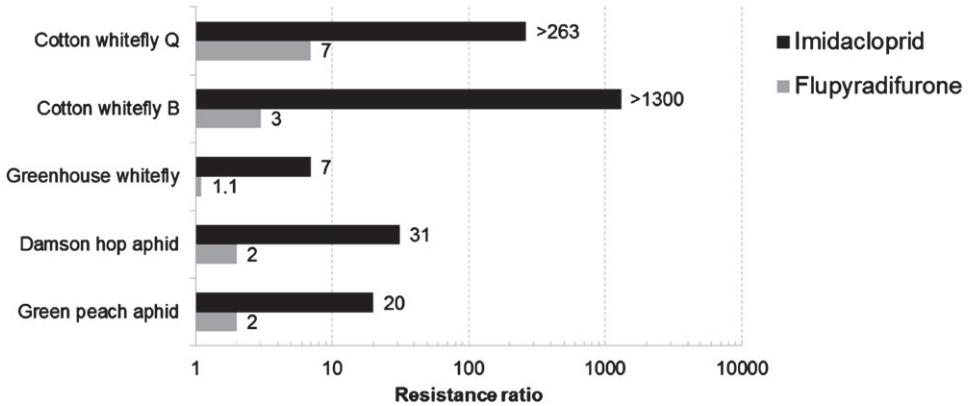

Figure 10. Comparison of resistance ratios for flupyradifurone and imidacloprid in different strains of selected sucking pest species. All data are based on leaf-dip tests using adults (72 h).

Table 5. Summary of seven lettuce field trials conducted in the EU central zone, comparing flupyradifurone and commercial standards against lettuce aphids (*Nasonovia ribisnigri*)

		Efficacy (%)[a]		
Compound	Rate (g ha^{-1})	2–3 DAA	6–10 DAA	13–15 DAA
Flupyradifurone	125	86	96	83
Pymetrozine	200	74	79	57
Thiacloprid	96	84	88	72
Spirotetramat	75	65	90	98

[a] DAA: days after application.

rotation.[38] In that scheme, neonicotinoids, nicotine and sulfoxaflor are separated as subgroups 4A, 4B and 4C respectively, a structure-based subclassification clearly supported by the chemical similarity analysis conducted here. However, it is worth mentioning here that subgroup rotation is only advisable if no other alternatives of different mode-of-action groups are available in certain agricultural settings.

Flupyradifurone shows excellent activity against different aphid and whitefly species in laboratory bioassays, and LC_{50} values for all tested susceptible strains are in a similar range to those published for other insecticides commercialised to control particularly sucking pests, e.g. neonicotinoids and homopteran feeding blockers.[39–41] Flupyradifurone acts quite rapidly on sucking pests, as exemplified by a translaminar study on the suppression of honeydew excretion in green peach aphid feeding on the abaxial site of adaxially treated oilseed rape leaves. Within a short time interval, most of the aphids stopped feeding and died 2 days later, suggesting a high potential of flupyradifurone to prevent the transmission of plant pathogenic viruses at recommended field rates. The good translaminar action is supported by phosphor imaging results of translocation studies with radiolabelled flupyradifurone, which is readily taken up by plant roots, leaves and stems shortly after application, suggesting a good systemic action once translocated *in planta*. It is mainly translocated in the xylem, as shown by its accumulation in distal leaf regions when taken up by the leaf lamina,

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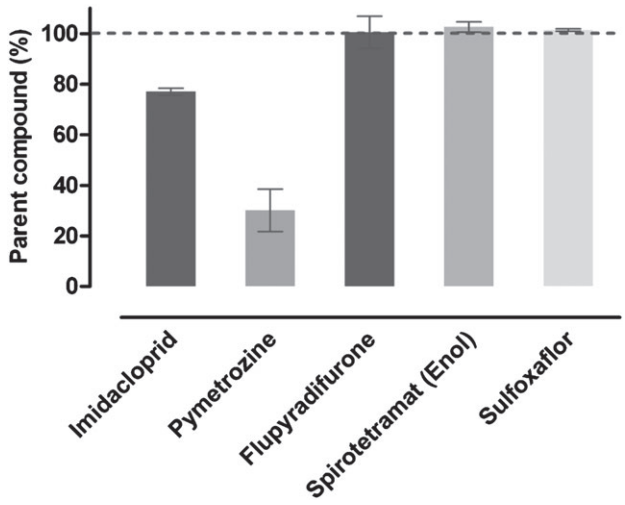

Figure 11. Detoxification of different insecticides (20 µM; 30 °C; 4 h; 200 µL) by CYP6CM1 of *Bemisia tabaci* functionally expressed in Sf9 cells using a baculovirus transfection system.

roots and stems. Such a pattern has recently also been demonstrated for other xylem systemic insecticides such as thiacloprid and cyantraniliprole.[42,43]

Field trials against lettuce aphids known to be difficult to control owing to their feeding sites deep in the canopy of lettuce plants demonstrated the excellent efficacy of flupyradifurone when compared with market standards such as thiacloprid, pymetrozine and thiamethoxam. This finding is strongly supported by recently published field results against other sucking pests such as *Dysaphis plantaginea* and *Aphis pomi* in apples and *A. gossypii*, *M. persicae*, *T. vaporariorum* and *B. tabaci* in vegetables, as well as *Empoasca flavescens* and *Scaphoideus titanus* in grapes.[34] The authors showed that flupyradifurone in many field trials is equal to or better than standard insecticides against many economically important sucking pest species. Flupyradifurone can be foliarly applied even during flowering, as it shows no adverse effects on actively foraging honey bees in long-term field trials in oilseed rape when applied at rates as high as 205 g ha^{-1} (Table 2).

Cross-resistance studies using resistant strains of aphids and whiteflies revealed that flupyradifurone lacks metabolic cross-resistance to the neonicotinoid insecticide imidacloprid. However, flupyradifurone should not be used against *M. persicae* carrying a R81T target-site mutation in the nAChR β1-subunit as recently described for populations collected in peaches in Spain and France.[5,11] In particular, neonicotinoid-resistant strains of *B. tabaci* belonging to groups MEAM-1 (Middle East–Asia Minor 1, B type) and MED (Mediterranean, Q type) were of interest in our studies, as both are known to express high metabolic resistance by overexpressing a specific detoxification enzyme, CYP6CM1.[44] The overexpression of this cytochrome P450 is known to confer extremely high resistance to a number of neonicotinoid insecticides and pymetrozine.[44,45] The B-type strain of *B. tabaci* originating from Brazil and tested in this study shows a resistance ratio of >1300-fold against imidacloprid, and a strong overexpression of CYP6CM1 (data not shown). Flupyradifurone exhibited virtually no cross-resistance in this strain (RR =3), and therefore we investigated the potential of CYP6CM1 to metabolise flupyradifurone when functionally expressed in an insect cell line. Similar studies were conducted earlier and clearly demonstrated the potential of CYP6CM1 to detoxify neonicotinoids such as imidacloprid by hydroxylation.[30,45] The metabolism studies presented here revealed that functionally expressed CYP6CM1 does not detoxify flupyradifurone, but imidacloprid and pymetrozine, as already shown earlier,[18] thus demonstrating that flupyradifurone is resistant to the major CYP6CM1-based metabolic mechanism conferring neonicotinoid resistance in whiteflies. Similarly to flupyradifurone, two other chemically distinct commercial insecticides, i.e. sulfoxaflor and spirotetramat, were also not detoxified, highlighting the dual specificity of CYP6CM1 to some neonicotinoids such as imidacloprid and pymetrozine, but not to other sucking-pest active insecticides, which are therefore potential partners in resistance management programmes. However, sulfoxaflor was already shown earlier to resist detoxification by another cytochrome P450, cyp6g1 of *Drosophila melanogaster*, which converts imidacloprid to 5-hydroxy-imidacloprid.[46] Our results support the usefulness of flupyradifurone as a new tool in resistance management strategies, even against sucking pest species such as B- and Q-type *B. tabaci* expressing metabolic resistance to neonicotinoid insecticides.

5 CONCLUSIONS

Flupyradifurone is a new butenolide insecticide discovered and developed by Bayer CropScience and offering a high potential for the rapid control of some of the most destructive sucking pest species in many crops. It is shown to have an excellent ecotoxicological and safety profile, fulfilling today's regulatory requirements for a modern IPM-suitable insecticide that offers versatile application methods to provide best possible pest/crop solutions in modern applied agriculture. It will be globally launched under the name Sivanto®, starting in 2014, and is a new resistance management tool shown to offer potential to control resistant whitefly and aphid species expressing metabolic mechanisms of resistance against neonicotinoid insecticides.

ACKNOWLEDGEMENTS

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