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# Discovery and Characterization of Sulfoxaflor, a Novel Insecticide Targeting Sap-Feeding Pests

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**ABSTRACT:** The discovery of sulfoxaflor [*N*-[methyloxido[1-[6-(trifluoromethyl)-3-pyridinyl]ethyl]-$\lambda^4$-sulfanylidene] cyanamide] resulted from an investigation of the sulfoximine functional group as a novel bioactive scaffold for insecticidal activity and a subsequent extensive structure−activity relationship study. Sulfoxaflor, the first product from this new class (the sulfoximines) of insect control agents, exhibits broad-spectrum efficacy against many sap-feeding insect pests, including aphids, whiteflies, hoppers, and *Lygus*, with levels of activity that are comparable to those of other classes of insecticides targeting sap-feeding insects, including the neonicotinoids. However, no cross-resistance has been observed between sulfoxaflor and neonicotinoids such as imidacloprid, apparently the result of differences in susceptibility to oxidative metabolism. Available data are consistent with sulfoxaflor acting via the insect nicotinic receptor in a complex manner. These observations reflect the unique structure of the sulfoximines compared with neonicotinoids.

**KEYWORDS:** nicotinic acetylcholine receptor, sulfoximines, sulfoxaflor, insecticide resistance, *Myzus persicae*

## INTRODUCTION

Crop damage due to sap-feeding insects such as aphids and whiteflies can be extensive. Over time, there have been several classes of insecticides with different modes of action that have proven effective in the control of many sap-feeding pests. However, resistance to many of these insecticides has limited their utility.[1,2] In fact, 3 of the 10 species of insects that have developed resistance to the largest number of insecticides are sap-feeding insects.[1] These three sap-feeding insects, *Myzus persicae* (green peach aphid), *Aphis gossypii* (cotton aphid), and *Bemisia tabaci* (sweet potato whitefly), have developed resistance to a variety of organophosphate, carbamate, pyrethroid, and, in some cases, neonicotinoid insecticides.[2−6] Given the continuing development of insecticide resistance, there is an ongoing need for new insect control agents to provide effective control options for sap-feeding insect pests.

The discovery and development of new insect control agents can involve a wide variety of approaches including investigations of structural chemical scaffolds. Structural chemical scaffolds of interest, also known as privileged structures, can be associated with a certain type of biological activity or may involve a key molecular fragment or recognition element known or suspected to be essential for the activity of a compound or ligand.[7−9] Alternatively, privileged structures or scaffolds may simply be novel or underexplored chemical moieties with desired chemical or physical properties. As such, these privileged structures or scaffolds can be used as the basis for the design and synthesis of desired target sets of compounds that incorporate additional structural features such as putative carrier groups or binding elements.

Enticed by the potential of a scaffold-based approach for the generation of new chemistries, we initiated an effort to identify novel scaffolds for the development of novel crop protection agents. Candidate scaffolds included those that were small molecular weight entities, which possessed either a hydrogen bond donor or acceptor, that were novel or underexplored as agrochemicals, and that were amenable to synthetic modification.

One structural scaffold selected for investigation was the sulfoximine functionality (Figure 1). Although sulfoximines have been reported in the literature as early as the 1940s[10−13] they have not been extensively examined for use as agrochemicals. Sulfoximines have a small hydrophilic core, a hydrogen bond acceptor and, in cases where R3 = H, a hydrogen bond donor. They are also amenable to synthetic modifications because they possess, unlike the closely related sulfone, a third point of diversity at the imine nitrogen. These chemical characteristics made the sulfoximine functionality an appealing structural scaffold for further exploration.

## DISCOVERY OF SULFOXIMINE INSECTICIDES

Several different sets of substituted sulfoximine scaffolds were initially prepared with a relatively diverse array of R1, R2, and R3 substituents. Selection of substituents was guided by

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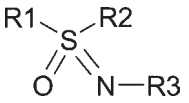



**Figure 1.** Sulfoximine moiety: three sites for diversity.

agrochemical-like parameters[14] working within the framework of available substituents and known synthetic methods. Synthetic efforts evolved from a broad search for entities with agrochemical utility to a more focused exploration of structural motifs thought to be associated with fungicidal activity such as the aryloxybenzyl sulfoximines (Figure 2, structure **A**). In the course of exploring various R3 substituents for the aryloxybenzyl sulfoximine series, an *N*-nitrosulfoximine was prepared using a literature method.[15] Recognizing the method might provide access to a broader set of *N*-nitrosulfoximines, the motif was targeted for follow-up as a second-generation structural scaffold (Figure 2, structure **B**). Further investigation of this structural scaffold eventually resulted in the synthesis and identification of the *N*-nitrosulfoximine **1**, which was found to have promising aphicidal activity (Figure 2). Sulfoximine **1** therefore represented a novel starting point for the optimization of the aphicidal activity.

The structure−activity relationship (SAR) investigation of sulfoximine **1** was greatly enabled by two synthetic routes, both shown in Figure 3. The first synthetic route (route A) is an adaptation of a procedure described by Johnson et al. by which sulfoxides are functionalized with sodium azide and concentrated sulfuric acid to give unsubstituted sulfoximines.[16] Subsequent nitration or cyanation provided targeted *N*-substituted sulfoximines.[15,17] A scalable route was subsequently identified in which the oxidation steps of route A are reversed, and the mild oxidant iodobenzene diacetate[18] is employed in the oxidative addition of cyanamide to disubstituted sulfides yielding *N*-cyanosulfilimines (Figure 3, route B). Subsequent oxidation of the intermediate sulfilimine gave targeted *N*-cyanosulfoximine analogues. Decyanation via treatment with trifluoroacetic anhydride followed by basic hydrolysis[19] provided access to the unsubstituted sulfoximine, a key intermediate in the exploration of different imine substituents.

These two general routes enabled the synthesis of a number of molecules that helped define the sulfoximine SAR, particularly related to a wide range of different substituents for both the imine nitrogen and the bridging methylene carbon linking the sulfoximine moiety to the pyridine ring. From this SAR, a compound with even greater aphicidal potency, the monomethyl substituted *N*-cyanosulfoximine **2**, was identified (Figure 4).

## DISCOVERY OF SULFOXAFLOR

From sulfoximine **2**, the effects of various modifications to the bridging methylene carbon linking the sulfoximine functionality and the pyridine ring were explored. Included in this investigation were various ring systems that conformationally biased the orientation of the sulfoximine functionality relative to the pyridine ring. These modifications employed a diverse set of synthetic schemes that allowed the synthesis of a variety of chemical targets.[17,20] Emerging from these efforts was the observation that potent aphicidal activity tended to coincide with systems that employed a single methylene linker between the sulfoximine and the pyridyl ring and a monosubstitution, preferably a methyl group, in an open-chain form.

An investigation of pyridyl ring SAR revealed that the better aphicidal activity was afforded by small, lipophilic, electron-withdrawing substituents at the 6-position, with 6-trifluoromethyl being one of the best substituents in terms of aphid control.[21,22] The combination of the best features from these investigations, namely, the *N*-cyano substitution, with a single monomethyl-substituted methylene linker and 6-trifluoromethyl substitution on the pyridine ring led to the discovery of sulfoxaflor (Figure 5). Sulfoxaflor was found to exhibit significantly better *M. persicae* activity than any other sulfoximine that had been prepared in the series. Below are brief descriptions of studies characterizing the insecticidal activity, the cross-resistance to known resistant insects, and the mode of action of sulfoxaflor. In total, the data indicate that sulfoxaflor represents a novel insecticide targeting sap-feeding pests with unique resistance and mode of action characteristics.

## MATERIALS AND METHODS

**Chemicals.** All chemicals were from conventional sources. Sulfoxaflor, sulfoximine **1**, and sulfoximine **2** were prepared at Dow Agro Sciences. Imidacloprid (IMI) and acetamiprid were purchased from Chem Service (West Chester, PA). [$^3$H]Imidacloprid ([$^3$H] IMI) was obtained from Amersham (Piscataway, NJ; specific activity = 37.2 Ci/mmol).

**Laboratory Bioassays.** Laboratory leaf disk bioassays for Rothamsted susceptible and resistant strains of *M. persicae* and *B. tabaci* (see Table 1) were conducted as described previously.[23] Bioassays of DAS strains of these same two species along with *A. gossypii* utilized whole plant bioassays as described previously.[24] Laboratory bioassays for *Lygus hesperus* (tarnished plant bug) on green beans were also conducted as described previously.[24]

**UV Stability and Residual.** Suspension concentrate (SC) formulations (1000 ppm) of sulfoxaflor and imidacloprid were applied to glass disks (10 $\mu$L/disk) held in a UV chamber for selected time intervals, extracted (acetonitile), and then analyzed by HPLC (Beckman Coulter, Brea CA; model 126, with a model 508 autosampler, and a model 168 photodiode array detector set at 270 nm) using a Gemini (Phenomenex, Torrence, CA) 5 $\mu$m, C6-phenyl column and water/acetonitrile 10−100% gradient, 2 mL/min. There were three replicates per time point for each compound.

Sulfoxaflor and imidacloprid (25 g/ha each; 125 ppm) were applied to young pepper plants, allowed to dry, and then held in a UV chamber for selected time intervals. At each interval, the plants were infested with a mixed population of *M. persicae* and then assessed for *M. persicae* control 3 days later. There were four replicates per treatment/time point.

**[$^3$H]Imidacloprid Binding Assays.** *M. persicae* were collected from leaf surfaces and frozen at −80 °C. Frozen *M. persicae* were placed in chilled homogenization buffer (200 mM sucrose, 50 mM Trizma-HCl, 1 mM ethylenediaminetetraacetic acid, and 0.1 mM phenylmethanesulfonyl fluoride, pH 7.2) and then homogenized using a cold (4 °C) blender. The homogenized mixture was then filtered through cheesecloth to remove large debris. The resulting effluent was then centrifuged at 3500 rpm for 15 min at 4 °C. The supernatant was collected and subjected to an additional centrifugation at 17500 rpm for 20 min at 4 °C. The supernatant was then discarded, and the remaining pellet of tissue was resuspended in binding buffer (120 mM NaCl, 50 mM Trizma-HCl, pH 7.4). The resulting protein preparation was aliquoted and frozen at −80 °C.

Radioligand binding assays were performed in 96-well microtiter plates, at a final assay volume of 0.1 mL. For each replicate, ∼2 nM [$^3$H]IMI, protein (70 $\mu$g/well), and any unlabeled competing

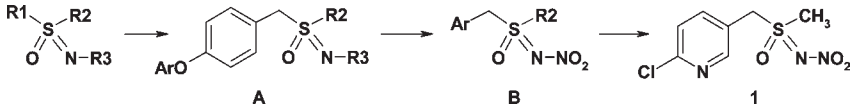
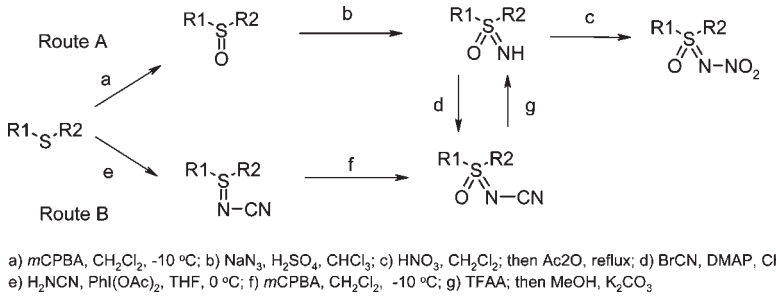



**Figure 2.** Temporal development leading to *N*-nitrosulfoximine insecticide lead. Using the sulfoximine structural scaffold (left), the aryloxyphenol sulfoximines (**A**) and the *N*-nitro-substituted sulfoximines (**B**) ultimately led to the discovery of sulfoximine **1**, which had promising aphicidal activity.




a) *m*CPBA, $CH_2Cl_2$, -10 °C; b) $NaN_3$, $H_2SO_4$, $CHCl_3$; c) $HNO_3$, $CH_2Cl_2$; then Ac2O, reflux; d) BrCN, DMAP, $CH_2Cl_2$
e) $H_2NCN$, $PhI(OAc)_2$, THF, 0 °C; f) *m*CPBA, $CH_2Cl_2$, -10 °C; g) TFAA; then MeOH, $K_2CO_3$

**Figure 3.** Synthesis of targeted sulfoximines. Route A features the formation of a sulfoximine from a sulfoxide, whereas route B utilizes a sulfilimine intermediate in route to the targeted sulfoximines.

compound were co-incubated for 60 min at room temperature (~22 °C). The binding reaction was initiated by the addition of protein and terminated by filtration using a TomTec Mach-II harvester (TomTec, Inc., Hampden, CT). Filter mats were dried in an oven, and solid scintillant was then melted onto the filter. Bound radioactivity was counted using a Wallac 1453 Microbeta Plus scintillation counter (Wallac/Perkin-Elmer, Waltham, MA). Total binding (in the absence of competing ligand), filter binding (in the absence of competing ligand and protein), and the binding of a positive control (i.e., unlabeled imidacloprid, unlabeled sulfoxaflor) were determined for each set of experiments. The resulting displacement data were fit by least-squares nonlinear regression using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA) and, when applicable, expressed as the concentration producing half-maximal displacement ($IC_{50}$, in nM).

**Cloning of Nicotinic Acetylcholine Receptor (nAChR) Subunits and cRNA Synthesis.** The *Drosophila melanogaster* α2 nAChR subunit (*Dα2*) was amplified from first-strand cDNA made from *D, melanogaster* embryo mRNA (Clontech Laboratories, Mountain View, CA) using the primers SADFW2 (5′ AGATCTCACCATGGCTCCTGGCTGCTGCAC 3′) and SADRV2 (5′ AGATCTTTAATTCTTCTTCTCGGTTA 3′). PCR was performed using the FailSafe PCR kit (Epicenter Biotechnologies, Madison, WI). A clone having a sequence similar to GenBank accession no. X53583 was identified. The clone had a two conservative single-base changes compared to the published sequence. This clone was isolated as a *Bgl*II fragment and ligated into pGH19. A clone having the *Dα2* gene in the correct orientation was identified by restriction digest.

The chicken *β2* (*β2*) nAChR subunit was amplified from first-strand cDNA made from chicken brain mRNA obtained from Clontech Laboratories, Inc. PCR was performed with the TaKaRa EX taq kit (TaKaRa Bio, Inc., Otsu, Japan) using the primers 5′ GGATCCACGGACACGGAGGAGCGCCTGGTGGAATACCT 3′ and 5′ GGATCCCTATTTGGAGGTGGGGGTGCCCTGGCCGA 3′. This amplified the coding region for *β2* without the signal peptide and resulted in a product of 1434 bp, which was cloned into pCR2.1-TOPO for sequencing. A clone having the *β2* sequence corresponding to GenBank accession no. AJ250362 was identified. The clone was amplified with the primer CK *β2*FL (5′ GGATCCATGGCGCTGCTCCGCGTCCTCTGCCTCCTCGCCGCGCTCCGACGCAGTCTGTGCACGGACACGGAGGAGCGCCTG GTGGAATAC 3′) to add the signal peptide sequence. The PCR product (1488 bp) was cloned into pCR2.1-TOPO and sequenced. A clone with the correct sequence was identified,

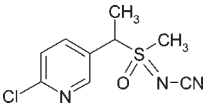



**Figure 4.** *N*-Cyanosulfoximine **2**.

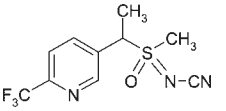



**Figure 5.** Sulfoxaflor.

and the full-length *β2* gene was removed as a *Bam*HI fragment and cloned into pGH19 (received from Cambria Biosciences, Boston, MA). A clone of pGH19/*CKβ2FL* was identified by restriction digest having the *CKβ2FL* gene in the correct orientation.

For cRNA synthesis, pGH19/CK*β*2FL was linearized with *Nhe*I and pGH19/Dα2 was linearized with *Xho*I. cRNA synthesis was carried out using the mMessage mMachine T7 Ultra kit (Ambion, Inc., Austin, TX). cRNAs were LiCl-precipitated, the pellets were redissolved (typically at 1 ng/nL) in The RNA Storage Solution (Ambion, Inc.), and the solution was stored at −80 °C until thawed for injection into *Xenopus leavis* oocytes.

***X. laevis* Oocyte Preparation, Expression, and Electrophysiology.** Gravid adult female *X. laevis* frogs were purchased from Nasco, Inc. (Fort Atkinson, WI) and maintained in dechlorinated water at room temperature. For oocyte removal, frogs were anesthetized by placing them in a water bath containing 0.2% tricaine methane sulfonate (pH 7.0) for 30 min. Following ovarectomy, harvested oocytes were placed in ND-96 medium (containing, in mM, 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES, pH 7.6) supplemented with 10000 units/L penicillin, 10 mg/mL streptomycin, and 2.5 mM sodium pyruvate. Oocytes were then defolliculated by a 2 h treatment with 1.5 mg/mL type 1A collagenase (Sigma Chemical, St. Louis, MO) in ND-96 medium without calcium. After defolliculation, oocytes were washed for 30 min in zero-calcium ND-96 medium without collagenase and then returned to standard ND-96 medium with calcium.

Stage V−VI oocytes were injected with individual or mixtures of cRNAs encoding *D. melanogaster* nicotinic receptor subunits and the *C.*

**Table 1. Laboratory Efficacies of Sulfoxaflor and Imidacloprid on Sap-Feeding Insects[a]**

| | $LC_{50}$ (95% fl[b]), ppm | | |
|---|---|---|---|
| insecticide | susceptible (strain) | resistant (strain) | RR[c] |
| | *M. persicae* (DAS Lab) | | |
| sulfoxaflor | 0.074 (0.049−0.101) | | |
| sulfoximine **2** | 0.374 (0.199−0.484) | | |
| imidacloprid | 0.090 (0.07−0.13) | | |
| | *M. persicae* (S-USIL)[d] | *M. persicae* (R-4013A)[e] | |
| sulfoxaflor | 4.13 (2.25−6.82) | 1.52 (0.644−2.65) | 0.37 |
| sulfoximine **2** | 62.3 (14.5−186.1) | 12.5 (3.44−23.4) | 0.20 |
| imidacloprid | 0.896 (0.620−1.15) | 15.3 (10.62−21.40) | 17.1 |
| | *A. gossypii* (DAS Lab) | | |
| sulfoxaflor | 0.20 (0.015−1.1) | | |
| sulfoximine **2** | 3.0 (0.6−7.0) | | |
| imidacloprid | 7.8 (2.4−15.6) | | |
| | *L. hesperus* (DAS Lab) | | |
| sulfoxaflor | 2.78 (1.41−4.95) | | |
| sulfoximine **2** | 1.69 (0.42−3.82) | | |
| imidacloprid | 1.32 (0.48−2.61) | | |
| | *B. tabaci* (DAS Lab) | | |
| sulfoxaflor | 0.85 (0.40−1.5) | | |
| sulfoximine **2** | 0.29 (0.083−0.66) | | |
| imidacloprid | 0.37 (0.18−0.63) | | |
| | *B. tabaci* (DAS S) | *B. tabaci* (R-PBI)[f] | |
| sulfoxaflor | 2.8 (1.2−5.5) | 6.4 (2.6−13.1) | 2.3 |
| imidacloprid | 0.20 (0.05−0.55) | 174 (24.6−>2000) | 870 |
| | *B. tabaci* (S-4971BT1)[g] | *B. tabaci* (R-4991BT1)[h] | |
| sulfoxaflor | 18 (13−24) | 28 (25−55) | 1.6 |
| imidacloprid | 4.4 (2.8−6.1) | >1000 (−) | >230 |
| | *B. tabaci* (S-4971BT1) | *B. tabaci* (R-4971BT9)[i] | |
| sulfoxaflor | 18 (13−24) | 39 (25−55) | 2.2 |
| imidacloprid | 4.4 (2.8−6.1) | 4500 (1900−29000) | 1022 |
| | *B. tabaci* (SUD-S)[j] | *B. tabaci* (R-CHLORAKA)[k] | |
| sulfoxaflor | 1.80 (0.84−3.13) | 5.0 (3.13−7.76) | 2.8 |
| sulfoximine **2** | 4.48 (2.01−8.16) | 13.2 (7.25−23.2) | 2.9 |
| imidacloprid | 1.23 (0.203−4.17) | >1000 | >833 |

[a] Some data adapted, in part, from Huang et al.[23] and Babcock et al.[24] [b] Fiducial limits. [c] Resistance ratio = $LC_{50}$ resistant strain/$LC_{50}$ of susceptible strain. [d] Rothamsted susceptible laboratory strain. [e] Rothamsted strain collected from tobacco in Greece in 2000 (resistant to pyrethroids, organophosphates, and carbamates as well neonicotinoids) shows high levels (>50-fold) of resistance to deltamethrin. [f] DAS insecticide resistant B-biotype strain. [g] DAS susceptible reference strain. [h] Rothamsted resistant strain collected from Spain in 2008. [i] Rothamsted resistant Q-biotype strain collected from Spain in 2007. [j] Rothamsted susceptible laboratory strain. [k] Rothamsted Q-biotype strain collected from Cyprus in 2003 shows resistance to pyrethroid, organophosphate, and neonicotinoid insecticides.

*elegans* chaperone protein ric-3. Each oocyte was injected with no more than 50 nL (1 ng/nL) total volume cRNA using a Nanoject II microinjector (Drummond Scientific, Broomall, PA). Oocytes were housed individually in 96-well plates in ND-96 medium and stored in an incubator maintained at 18 °C. Oocytes were assayed for receptor expression 1−4 days after cRNA injection.

Electrophysiological recordings were performed using the Roboocyte automated oocyte recording system (Multichannel Systems, Reutlingen, Germany). Modified Barth's saline (containing, in mM, 88 NaCl, 2.4 $NaHCO_3$ 1 KCl, 0.41 $CaCl_2$, 0.3 $Ca(NO_3)_2$, 0.82 $MgSO_4$, and 15 HEPES, pH 7.6) was used for all experiments. Oocytes were voltage-clamped to −60 mV with leak currents of <1000 nA. Responses to

nAChR agonists were measured at peak amplitude. Test compounds were first dissolved in DMSO at a high concentration and then diluted into MBS at the appropriate test concentration, with final DMSO levels never exceeding 0.1%. For dose−response studies, a 10 s application of 100 $\mu$M acetylcholine (ACh) was first applied to each oocyte, and then subsequent concentrations of test compounds were applied to oocytes at 10 min intervals, beginning with the highest tested dose (100 $\mu$M). The resulting data were expressed as percent of the initial response to ACh.

**CYP6G1-Mediated Metabolism in D.mel-2 Cells.** The *CYP6G1* gene was amplified from adult *D. melanogaster* first-strand cDNA. The primers added *Bam*HI sites to both ends of the gene and a 6X-His tag to the C-terminus. A product of 1608 bp was generated and ligated into pCR2.1-TOPO. Several clones containing the *CYP6G1* product were identified and sequenced. One sequence was found to match that of NCBI accession NM136899 except for four single-base changes, which did not affect the amino acids at those positions and the 6X-His tag. For expression in *D. melanogaster* D.mel-2 cells, the *CYP6G1* was amplified by PCR using primers to change the *Bam*HI sites to *Kpn*I sites for subcloning into pAc5.1/V5-HisA. The PCR product was ligated into pCR2.1-TOPO and sequenced to ensure no changes were introduced except the change in restriction sites. A clone was digested with *Kpn*I to isolate the CYP6G1, which was subsequently ligated into the pAc5.1/V5-HisA vector (Invitrogen). A clone containing the *CYP6G1* gene in the correct orientation was scaled up for plasmid isolation.

For transient expression, D.mel-2 cells were seeded 24 h prior to transfection in 12-well plates ($5 \times 10^5$ cells/well) and incubated at 27 °C. A transfection mix contained 2 $\mu$g of DNA and 8 $\mu$L of Cellfectin (100 $\mu$L total volume) per well. A time course study indicated maximal CYP6G1 expression at 48 h after transfection. Following 24 h of incubation, imidacloprid, acetamiprid, or sulfoxaflor [400 ppm in water; filter sterilized (0.33 $\mu$m)] was added to the cells and then harvested at 0 and 48 h after application of compound. At harvest time points, each well was scraped twice, and the extracts were transferred to Eppendorf tubes, where they were diluted with acetonitrile ($CH_3CN$, 450 $\mu$L total volume). HPLC (Agilent 1100 system, Agilent Technologies, Santa Clara, CA) analysis was carried out using a YMC J′ Sphere ODS-H80, 150 mm × 4.6 mm column (YMC Co. Kyoto, Japan) with UV detector set at 254 nm. For imidacloprid and acetamiprid, the HPLC employed a gradient from 50% $CH_3CN$ to 100% in 10 min at a flow rate of 1 mL/min using 1% AA in water phase. For sulfoxaflor the HPLC employed a gradient from 50% $CH_3CN$ to 100% in 5 min at a flow rate of 1 mL/min using 1% AA in water phase. The D.mel-2 extracts were evaluated by LC-MS (Agilent Technologies) with detection of extracted ion of the parent (256+) and the metabolite (272+). Separation was performed by a Luna C18 25 cm × 4.6 mm column using a generic gradient of 10% acetonitrile/10 mM ammonium acetate ascending to 100% in 20 min. Flow rate was 1.2 mL/min, and injection volume was 25 $\mu$L.

## RESULTS

**Bioassays.** Across a range of sap-feeding insect pests, sulfoxaflor exhibits activity that is on par with one of the leading sap-feeding pest insecticides, imidacloprid (Table 1). Sulfoxaflor was as active as imidacloprid against *M. persicae* and *L. hesperus* in laboratory bioassays and significantly more active than imidacloprid against *A. gossypii*. Sulfoxaflor was generally less active than imidacloprid in bioassays against *B. tabaci*.

Compared to chloropyridyl sulfoximine analogue **2**, sulfoxaflor was significantly more active against the aphids *M. persicae* and *A. gossypii* (Table 1). Interestingly, there was no significant difference in activity between sulfoxaflor and **2** in assays involving *B. tabaci* or *L. hesperus* (Table 1).

**Table 2. Effect of Photolysis and UV Light on the Stabilities of Sulfoxaflor and Imidacloprid**

| | photolysis | UV chamber efficacy (% control) | | |
|---|---|---|---|---|
| | $t_{1/2}$ (h) at 1000 ppm | 0 DAA | 3 DAA | 7 DAA |
| sulfoxaflor SC | 88 | 100 | 100 | 90 |
| imidacloprid SC | 7 | 100 | 42 | 21 |

**Table 3. Metabolism of Sulfoxaflor, Imidacloprid, and Acetamiprid by D.mel-2 Cells Expressing *CYP6G1***

| | mean % recovery[a] | |
|---|---|---|
| | − CYP6G1[b] | + CYP6G1[c] |
| sulfoxaflor | 105.3 (4.4) | 108.1 (2.5) |
| imidacloprid | 115.4 (8.6) | 4.5 (0.9) |
| acetamiprid | 122.7 (29.4) | 0.0 (0) |

[a] Percent recovery 24 h after incubation compared to time 0 (standard deviation). [b] Cells lacking CYP6G1. [c] Cells expressing CYP6G1.

Bioassays with several *B. tabaci* strains resistant to imidacloprid indicated that there was no appreciable cross-resistance to sulfoxaflor (Table 1). Likewise, a multiresistant strain (CHLORAKA) of *B. tabaci* that also has high levels of resistance to imidacloprid and other insecticides[23] showed no appreciable cross-resistance to both sulfoxaflor and **2**. Similarly, a multiresistant strain of *M. persicae* (R-4013A) that exhibits a high degree of resistance to deltamethrin and primicarb[23] and modest resistance to imidacloprid (17-fold) displayed no cross-resistance to either sulfoxaflor or sulfoximine **2** (Table 1).

**UV Stability.** In laboratory studies sulfoxaflor exhibited superior UV stability ($t_{1/2}$ = 88 h) compared to imidacloprid ($t_{1/2}$ = 7 h) (Table 2). Likewise, in efficacy studies under UV conditions, the control of *M. persicae* by sulfoxaflor was maintained at a high level over a period of 7 days (Table 2). In contrast, the efficacy of imidacloprid, when applied at the same rate under identical UV conditions, significantly declined over a 7 day period (Table 2).

**Metabolism Studies.** Incubation of sulfoxaflor, imidacloprid, or acetamiprid with D.mel-2 cells lacking the *CYP6G1* gene resulted in complete recovery of each of the three compounds (Table 3). However, when incubated with D.mel-2 cells expressing the *CYP6G1* gene, there was little recovery of either imidacloprid or acetamiprid (Table 3). In contrast, there was complete recovery of sulfoxaflor in cells expressing CYP6G1 (Table 3), suggesting that sulfoxaflor is a poor substrate for the CYP6G1.

**Mode of Action Studies.** Initial observations on the effects of sulfoxaflor on *M. persicae* showed excitatory symptoms such as tremors, followed by paralysis and mortality, suggesting that the sulfoximines act via the insect nervous system. Similar symptoms were also noted for *D. melanogaster* and the American cockroach (*Periplaneta americana*) (G. Watson, personal observations). After preliminary mode of action analyses, sulfoxaflor was subsequently found to have an interaction with insect nAChRs. Like imidacloprid, sulfoxaflor was found to activate D$\alpha$2/$\beta$2 expressed in oocytes (e.g., Figure 6A). However, the maximal currents induced by sulfoxaflor were significantly larger than those induced by imidacloprid (Figure 6B). Additionally, sulfoxaflor displaced [$^3$H]IMI in *M. persicae* tissue homogenates.

However, the affinity of sulfoxaflor for the [$^3$H]IMI binding site was substantially weaker than that of imidacloprid (Figure 6C).

## DISCUSSION

The sulfoximines, as exemplified by sulfoxaflor, represent a new class of insecticidal molecules that are chemically distinct. Sulfoxaflor is effective against a wide range of sap-feeding insects including aphids, whiteflies, *Lygus*, and plant hoppers (Table 1).[24] Furthermore, sulfoxaflor displays a high level of biological activity in the laboratory that is on par with, and in some instances superior to, the best current sap-feeding insecticides, the commercial neonicotinoids, such as imidacloprid (Table 1).[24]

Compared to sulfoximine **2**, sulfoxaflor is substantially more active against the two aphid species examined (Table 1), but was similar in activity against the whitefly (*B. tabaci*) and *Lygus*. Thus, for these insect species, the replacement of the pyiridyl chlorine with $CF_3$ produced a marked improvement in aphid activity while retaining the whitefly and *Lygus* activity of sulfoximine **2**. This observation is in contrast to the SARs for the nitromethylene analogues of imidacloprid on green rice leafhopper (*Nephotettix cincticeps*), where substitution of the pyridyl chlorine with a $CF_3$ resulted in a 25-fold decrease in activity.[25,26]

In addition to the high level of insecticidal activity toward sap-feeding insect pests, available data for sulfoxaflor indicate a broad lack of cross-resistance in a variety of imidacloprid-resistant insect strains (Table 1).[23,24] This same trend also appears to be true for species that exhibit resistance to multiple types of insecticides (i.e., organophosphates, carbamates, pyrethroids) (Table 1). For these multiresistant strains there was also no cross-resistance to sulfoxaflor, providing further support for the utility of sulfoxaflor against a broad range of insecticide-resistant pest insect species. Furthermore, this lack of cross-resistance also extends to sulfoximine **2**, providing additional evidence for the uniqueness of the sulfoximine insecticide class.

Sulfoxaflor displayed improved UV stability relative to imidacloprid. Furthermore, in laboratory studies, sulfoxaflor was found to provide better *M. persicae* residual activity than imidacloprid. It is likely that much of the improvement in residual activity is due to the enhanced UV stability of sulfoxaflor.

Cytochrome P450 monooxygenases have been shown to play a role in imidacloprid resistance in several species including *N. lugens*,[27,28] house fly (*Musca domestica*),[29] *M. persicae*,[30] and *B. tabaci*.[31,32] The lack of cross-resistance observed with sulfoxaflor suggests that it may not be susceptible to the same monooxygenases that are responsible for degrading the neonicotinoids and other insecticides. A monooxygenase (CYP6G1) from *D. melanogaster* is responsible for resistance to a range of insecticides including DDT and the neonicotinoids imidacloprid and nitenpyram.[33,34] As a model system, the *CYP6G1* gene was cloned and expressed in the D.mel-2 cell line. Incubation of imidacloprid or acetamiprid with D.mel-2 cells expressing the *CYP6G1* gene resulted in the complete metabolism (94−100%) of both neonicotinoids. In total contrast, sulfoxaflor remained intact following incubation (Table 3), indicating that this particular monooxygenase (CYP6G1) is incapable of metabolizing sulfoxaflor. These data support the concept that the sulfoximines may not be susceptible to the same metabolic mechanisms (e.g., monooxygenases) responsible for resistance to the neonicotinoids and possibly other insecticides. Thus, sulfoxaflor is a good fit for insecticide resistance management (IRM) programs not only by providing a high level of efficacy against a wide variety of sap-feeding insect pests but also by retaining efficacy against many insecticide-resistant sap-feeding insect strains.

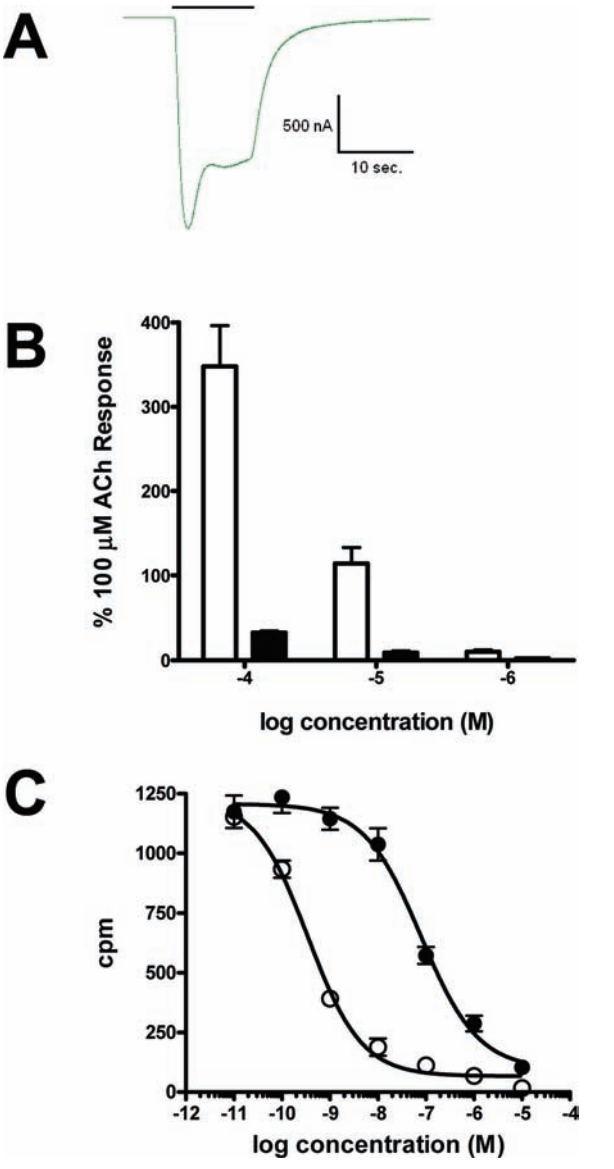



**Figure 6.** (A) Sulfoxaflor-induced current from Dα2/β2 receptors expressed in oocytes (sulfoxaflor applied to oocyte as indicated by horizontal line). (B) Dose dependence of sulfoxaflor (open bars) and imidacloprid (shaded bars) responses in Dα2/β2 receptors expressed in oocytes. (C) Representative experiment showing relative displacement of [$^3$H]imidacloprid from *M. persicae* homogenates by sulfoxaflor (●) and imidacloprid (○).

Initial observations of the effects of sulfoxaflor on *M. persicae* were excitatory symptoms such as tremors, followed by paralysis and mortality, suggesting that the sulfoximines act on the insect nervous system. These same observations were also noted for *Drosophila* and the American cockroach (*P. americana*) (G. Watson, personal observations). Sulfoxaflor was subsequently found to be a nAChR agonist, as evidenced by its ability to activate Dα2/β2 receptors expressed in oocytes (Figure 6A,B). Dose−response studies showed that the maximal currents induced by sulfoxaflor were greater than those induced by imidacloprid (Figure 6B). The relatively low efficacy of

imidacloprid has been observed in similar studies on both native (see, e.g., ref 35) and expressed insect nAChRs (see, e.g., ref 36). In addition, the affinity of sulfoxaflor for the [$^3$H]IMI binding site in *M. persicae* tissue was substantially weaker than that of imidacloprid. These results indicate that sulfoxaflor is a high-efficacy nicotinic receptor agonist with relatively low affinity for the imidacloprid binding site. These observations further suggest that the interaction of sulfoxaflor with the insect nAChR is unique and distinguishable from that of imidacloprid. Further studies will be necessary to gain insight into the potentially complex interaction of sulfoxaflor with the nAChR.

Sulfoxaflor is the first insecticide in a new, unique class of insect control agents, the sulfoximines. Discovered by a scaffold-based approach and subsequent SAR-based structural modifications, sulfoxaflor exhibits broad-spectrum, sap-feeding insect control at levels that are comparable to those of the best commercial standards, including the neonicotinoids. Compared to the neonicotinoid imidacloprid, sulfoxaflor exhibits greater UV stability and, as a consequence, improved residual insect control. Importantly, sulfoxaflor is highly effective against a variety of pest insect strains that are resistant to imidacloprid and a range of other insecticides. At least in part, the lack of cross-resistance appears to be associated with its novel chemistry in that sulfoxaflor is not susceptible to degradation by a cytochrome P450 monooxygenase such as CYP6G1 that is readily able to metabolize the neonicotinoids imidacloprid and acetamiprid. The novel sulfoximine chemistry of sulfoxaflor also translates to a unique set of interactions with nicotinic receptors that are distinct from those observed with the neonicotinoid imidacloprid. Thus, sulfoxaflor possesses a combination of distinctive and favorable attributes that suggest an excellent fit for many IRM programs.

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## ABBREVIATIONS USED

SAR, structure−activity relationships; IMI, imidacloprid; nAChR, nicotinic acetylcholine receptor; fi, fiducial limits; RR, resistance ratio.

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