Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 599–613

BIOORGANIC & MEDICINAL CHEMISTRY

Tricyclic Cyanoguanidines: Synthesis, Site of Action and Insecticidal Activity of a Novel Class of Reversible Acetylcholinesterase Inhibitors

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Received 23 June 2001; accepted 28 August 2001

Abstract—Bridged-tricyclic cyanoguanidines **1** were found to be active as insecticides. The preparation and structure–activity relationships of oxacyclic (X = O) and carbocyclic ($X = CH_2$) analogues of 1 is described. Compounds **1** were found to inhibit acetylcholinesterase with IC_{50} values comparable to the organophosphate Paraoxon. Unlike organophosphates, cyanoguanidines **1** were shown to reversibly bind acetylcholinesterase. This mode of action is shared by the structurally-related natural product Huperzine A.

Introduction

Acetylcholinesterase (AChE) inhibitors are the most widely used class of insecticides. All of the compounds used today fall into two groups, organophosphates and carbamates. Activity of these compounds results from deactivation of the AChE enzyme by irreversible phosphorylation or slowly reversible carbamoylation of the active serine hydroxyl.[1] These compounds are effective insecticides, but their extensive use for over 40 years has given rise to strains of resistant insects. In addition, the acute mammalian toxicity of some of these compounds (particularly the organophosphates) has raised safety concerns and many of these compounds are under review by regulatory agencies.

Recently there has also been much interest in anticholinergic compounds as therapies for cognitive disorders.[2] To date, four drugs have been approved by the United States Food and Drug Administration for treatment of Alzheimer's disease. These are tacrine,[3] donepezil,[4] galanthamine,[5] and rivastigmine[6] (Fig. 1). These compounds all inhibit AChE, the first three of these do so reversibly. The natural product Huperzine A shares this mode of action and is currently the subject of clinical studies towards the treatment of Alzheimer's disease.[7]

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We were interested in preparing novel insecticides which might overcome some of the limitations of the organophosphates and carbamates, particularly for the control of homopteran pests. Screening of our compound collection against *Peregrinus maidis* (corn planthopper) led to the discovery of compound **1a** as a lead structure (Fig. 2). We report herein on the synthesis, site of action and insecticidal activity of a novel class of insecticides.

Chemistry

The synthesis of bridged benzoxadiazocines **1** (X = O) followed the procedure of Svetlik[8] who originally described the synthesis of **1a** in 1988 (Scheme 1). In the first step, Claisen-Schmidt condensation of salicylaldehyde and acetone gave the hydroxyphenyl-enone **2a**. Treatment of **2a** with 2 equiv of cyanamide and catalytic piperidine in refluxing 1,2-dimethoxyethane gave **1a**.

Preparation of analogues **1** (X = O) with variations in the benzo substituent (Y), the bridgehead substituent (R^4), or the methano-bridge substituent (R^1) involved preparations of the appropriate α,β-unsaturated ketones and aldehydes **2** as shown in Schemes 2 and 3.

In Scheme 2, Claisen-Schmidt condensation[8,9] of a ketone with a salicylaldehyde in the presence of NaOH gave the (2-hydroxyphenyl) enones **2** in generally good yields (highest for acetone). Alternatively, compounds **2**

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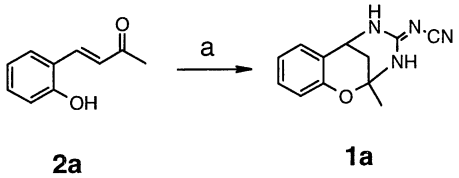
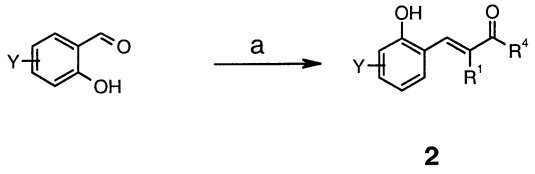
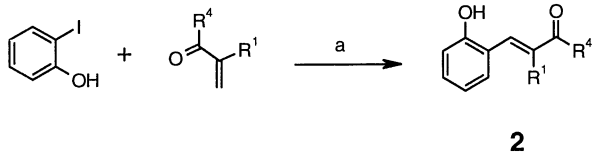


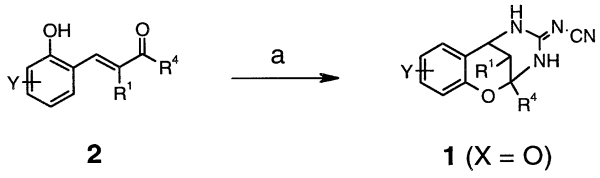
Scheme 1. (a) H_2NCN (2 equiv), piperidine (cat), DME, reflux (**1a**, 46%).



Scheme 2. (a) $R^1CH_2C(O)R^4$, NaOH, H_2O, rt (15–97% yields).



Scheme 3. (a) $Pd(OAc)_2$, $NaHCO_3$, $(n\text{-}Bu)_4NI$, DMF, 60 °C (11–45% yields).



Scheme 4. (a) H_2NCN (2 equiv), piperidine (cat), DME, reflux (3–85% yields).

could be prepared by a tetrabutylammonium chloride assisted[10] Heck reaction[11] of 2-iodo phenol with an α,β-unsaturated ketone or aldehyde (Scheme 3). Table 1 lists the compounds **2** that were prepared.

Cyclization of compounds **2** with cyanamide using the procedure of Svetlik[8] gave the benzoxadiazocines **1** (X = O) as shown in Scheme 4.

Compounds **1** (X = O) with substitution at N-5 (R^2 not equal to H) were prepared by reaction of **1** (R^2 = H) with an alkylating, acylating or sulfonylating agent in the presence of NaH (Scheme 5). In most cases, reaction at N-3 was not observed, even in the presence of excess electrophile and base. The exception was methyl iodide, which, when used in excess and in the presence of >2 equiv of NaH, gave the dimethylated compound (**1l**) in 15% yield, along with 33% of the mono-methyl compound (**1g**) (Scheme 6).

The regiochemical assignments of the products obtained in the alkylations/acylations described in Schemes 5 and 6 were made by difference NOE experiments. For example, the assignment of structure for **1f** was made on the basis of an observed NOE between the angular methyl group and the N–H proton, while the regiochemistry of **1g** was determined by observed NOE's between the angular methyl group and the N–H proton as well as between the angular proton and the *N*-methyl group (see Fig. 3).

Table 2 lists the bridged-benzoxadiazocines **1** (X = O) which were prepared using the methods described in Schemes 4–6.

Bridged-benzodiazocines **1** (X = CH_2) were prepared by cyclization of the appropriate *cis* 1,2,3,4-tetrahydronaphthalene-1,3-diamine with diphenyl cyanocarbonimidate. The diamines were prepared by the method of Shiotani and Mitsuhashi[12] (Scheme 7). In the instances were diastereomers were obtained they were separated at the lactam stage. The relative configurations were determined by NOE difference experiments. Benzodiazocines **1** (X = CH_2) that were prepared and tested for insecticidal activity are shown in Table 3.

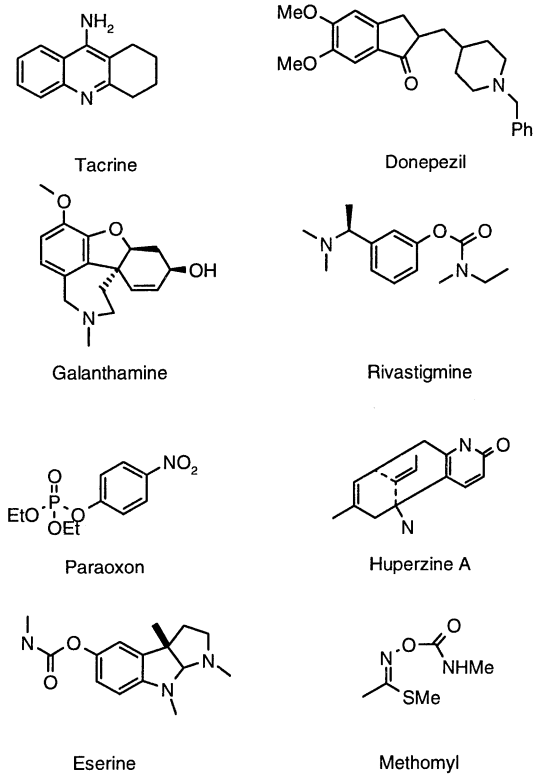


Figure 1. Acetylcholinesterase (AChE) inhibitors.

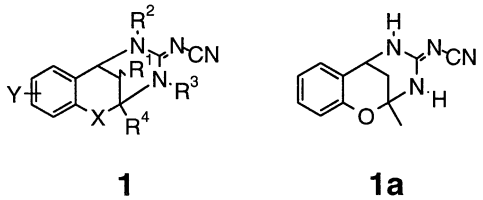
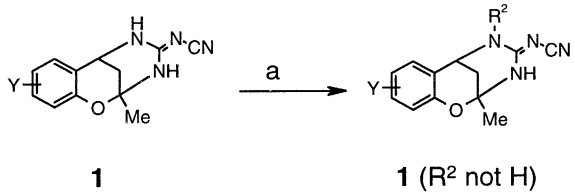
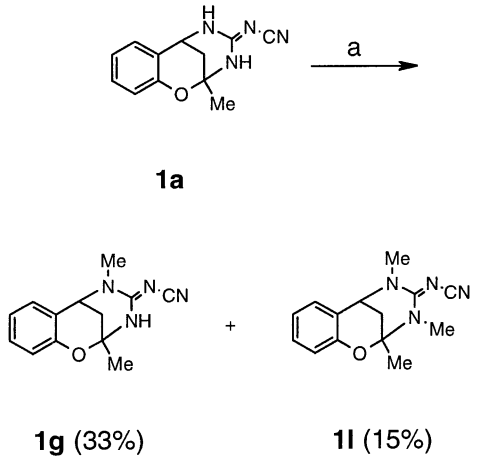


Figure 2.



Scheme 5. (a) NaH (1.1 equiv), R^2-X, (1.1 equiv), DMF, rt.



Scheme 6. (a) NaH (2.2 equiv), MeI, (4 equiv), DMF, rt.

Results and Discussion

Insecticidal activity

Cyanoguanidines **1** were tested for insecticidal activity against corn planthopper (CPH) (*Peregrinus maidis*), brown planthopper (BPH) (*Nilaparvata lugens*), and green leafhopper (GLH) (*Nephotettix cincticeps*). The latter two species are economically-important rice pests. All three species belong to the order *Homoptera* and, as such, typically feed upon the phloem or xylem sap of the host plant. Due to this feeding characteristic, it is advantageous to use systemic-acting insect control agents to combat *Homopteran* pest insects. In this way, the insect is exposed to the control agent when it feeds upon the plant sap. Another advantage to using systemic insecticides is that they can be applied to the soil where they are taken-up by the plant roots, obviating the need for a spray application.

The active analogues **1** typically displayed greater activity via systemic application than by contact (spray) application, although in most cases some degree of contact activity was also observed. Tables 2 and 3 summarize the systemic activity for compounds **1** versus

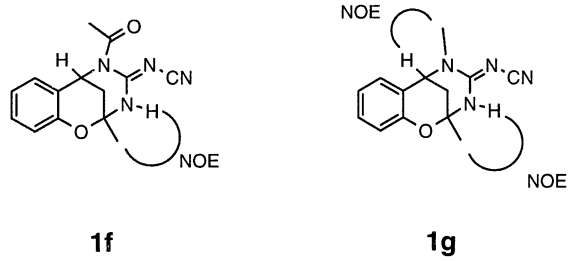
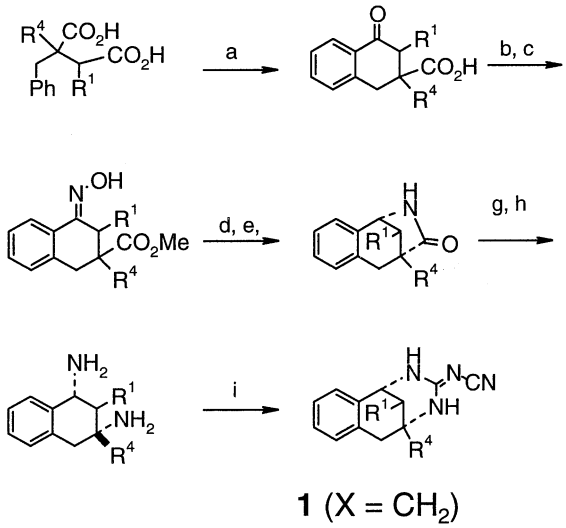


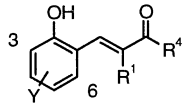
Figure 3. Observed NOE's for **1f** and **1g**.



Scheme 7. (a) H_2SO_4; (b) MeOH, HCl, reflux; (c) NH_2OH; (d) H_2 (50 psi), PtO_2, MeOH; (e) 120 °C, 4 h; (f) separate diastereomers (when R^1, R^3 other than H); (g) HCl, reflux; (h) HN_3; (i) $(PhO)_2C{=}NCN$, *i*-PrOH, reflux.

BPH and GLH, along with the combined contact/systemic activity data for the compounds versus CPH. It is apparent from the data in Table 2 that substitution on the benzo-ring (Y-group) lowers the insecticidal activity of compounds **1**. Except for cases where Y = 9-F (**1q**, **1ac**, **1ao**, and **1ap**) or 9-Cl (**1s**), all examined substitution patterns for Y (other than H) gave compounds that

Table 1.



2

Compd	Y	R^1	R^4	%Yield	mp°C
2a	H	H	CH_3	76	136–138
2b	5-Cl	H	CH_3	88	157 (dec)
2c	3,5-di-Cl	H	CH_3	95	141–145
2d	3,5-di-Br	H	CH_3	97	149–151
2e	5-NO_2	H	CH_3	96	86–90
2h	H	H	C_2H_5	48	114–116
2m	5-OCF^3	H	CH_3	77	115–117
2n	H	CH_3	C_2H_5	15	93–94
2o	3-CH_3	H	CH_3	83	141–143
2p	3,5-di-t-Bu	H	CH_3	94	151–155
2s	4-Cl	H	CH_3	84	125–130
2t	5- CH_3	H	CH_3	89	130–133
2v	3-F	H	CH_3	93	161–162
2x	3,4-di-O CH_3	H	CH_3	82	142–144
2aa	6-OCH_3	H	CH_3	77	136–137
2ab	4,6-di-Cl	H	CH_3	85	187–190
2ac	4-F	H	CH_3	86	126–130
2aj	H	H	H	31	n.d
2ak	H	C_2H_5	CH_3	45	n.d.
2al	H	CH_3	H	14	n.d.
2an	H	C_2H_5	H	11	n.d.
2aq	H	CH_3	CH_3	32	n.d.

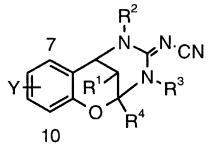
nd, not determined.

were inactive at the highest test concentrations (100 ppm for BPH and GLH, 250 ppm for CPH).

Compounds where X = CH_2 (in Table 3) were somewhat more active than analogous X = O compounds (Table 2).

For compounds **1** where R^1 = Me, exo- orientation of the bridgehead methyl group (relative to the guanidine moiety) gave higher activity than endo- (see e.g., **1aq** vs **1ar**, **1au** vs **1at** and **1az** vs **1ay**). Exo-methyl substitution on the bridgehead carbon gave higher activity than no

Table 2. Compounds **1** (X = O)



1 (X = O)

Compd	Y	R^1	R^2	R^3	R^4	% Yield[a]	Mp (°C)	Insecticidal activity[g] BPH	GLH	CPH
1a	H	H	H	H	CH_3	63	271–273[h]	++	++	+++
1b	8-Cl	H	H	H	CH_3	32	264–266	–	–	–
1c	8,10-di-Cl	H	H	H	CH_3	19	288–290	–	–	–
1d	8,10-di-Br	H	H	H	CH_3	16	284 (dec)	–	–	–
1e	8-NO_2	H	H	H	CH_3	18	>225	–	–	–
1f	H	H	$C(O)CH_3$	H	CH_3	73[c]	219–221	++	++	+++
1g	H	H	CH_3	H	CH_3	40[c]	>220	++	++	+
1h	H	H	H	H	C_2H_5	36	240–245	++	+	+
1i	H	H	SO_2CH_3	H	CH_3	13[c]	238–240	–	–	–
1j	H	H	H	H	Ph	85	253–256	–	–	–
1k	H	H	CO_2CH_3	H	CH_3	12[c]	199 (dec)	++	++	++
1l	H	H	CH_3	CH_3	CH_3	15[c]	187 (dec)	++	++	++
1m	8-OCF_3	H	H	H	CH_3	48	288–289	–	–	–
1n	H	CH_3[b]	H	H	C_2H_5	10	249–252	–	–	–
1o	10-CH_3	H	H	H	CH_3	46	279 (dec)	–	–	–
1p	8,10-di-*t*-Bu	H	H	H	CH_3	28	320–321	–	–	–
1q	9-F	H	$C(O)CH_3$	H	CH_3	6[d]	207–210	–	–	–
1r	H	H	CH_2Ph	H	CH_3	80[c]	>225	–	–	–
1s	9-Cl	H	H	H	CH_3	50	>225	+	+	–
1t	8-CH_3	H	H	H	CH_3	58	>225	–	–	–
1u	H	H	C_2H_5	H	CH_3	16[c]	>225	++	+	++
1v	10-F	H	H	H	CH_3	43	>225	–	–	–
1w	H	H	2-CH_2-pyridyl	H	CH_3	75[c]	>225	+	–	–
1x	9,10-di-OCH_3	H	H	H	CH_3	12	>225	–	–	–
1y	H	H	allyl	H	CH_3	38[c]	208–210	+	–	++
1z	H	H	CH_2SiMe_3	H	CH_3	14[c]	186–189	+	–	+
1aa	7-OCH_3	H	H	H	CH_3	46	>225	–	–	–
1ab	7,9-di-Cl	H	H	H	CH_3	59	280–282	–	–	–
1ac	9-F	H	H	H	CH_3	60	>225	++	++	++
1ad	H	H	CH_2CO_2Me	H	CH_3	13[c]	>225	+	–	+
1ae	H	H	propargyl	H	CH_3	60[c]	204 (dec)	++	+	+
1af	H	H	$C(O)CH_2CH_3$	H	CH_3	60[c]	196–198	++	++	++
1ag	H	H	C(O)*n*-Pr	H	CH_3	72[c]	198–199	++	+	–
1ah	H	H	C(O)*i*-Pr	H	CH_3	21[c]	206–209	++	+	+
1ai	8-NH_2	H	H	H	CH_3	55[e]	234–235	–	–	–
1aj	H	H	H	H	H	5	205–210	+	++	–
1ak	H	Et[b]	H	H	CH_3	3	219–221	–	–	–
1al	H	CH_3[b]	H	H	H	14	Oil	+	++	++
1am	H	H	$C(O)CF_3$	H	CH_3	2[c]	Oil	–	–	–
1an	H	Et[b]	H	H	H	7	249–252	–	–	–
1ao	9-F	H	CH_3	H	CH_3	10[d]	>225	nt	nt	+
1ap	9-F	H	CO_2CH_3	H	CH_3	14[d]	209–211	nt	nt	+
1aq	H	exo-CH_3[f]	H	H	CH_3	nd	248–9	+++	++	+++
1ar	H	endo-CH_3[f]	H	H	CH_3	nd	279–280	+	–	+

[a]Yields of chromatographically homogeneous products after purification.
[b]Product isolated as a mixture of exo- and endo-isomers.
[c]Yield starting from **1a**.
[d]Yield starting from **1ac**.
[e]Yield starting from **1e**.
[f]Relative to the guanidine-containing ring.
[g]Insecticidal activities refer to the following approximate LC80 values: BPH or GLH: '–' = >100ppm, '+' = <100ppm, '++' = <10ppm, '+++' = <1ppm, '++++' = <0.1ppm; CPH: '–' = >250ppm, '+' = <250ppm, '++' = <50ppm, '+++' = < 10ppm, '++++' = <2.5 ppm.
[h]From ref 8a.

substitution (see e.g., **1aq** vs **1a**, **1al** vs **1aj** and **1au** vs **1as**). Ethyl bridgehead substitution decreased actitivity (see **1ak** vs **1a** and **1an** vs **1aj**).

In general, compounds **1** with R^2 = Me, COR or CO_2R had about the same level of activity as the unsubstituted compounds. Allyl, propargyl as well as larger alkyl substituents (i.e., larger than ethyl) gave compounds that were less active, while benzyl, trifluoroacetyl and methanesulfonyl substitution resulted in a loss of activity. In the case of benzoxadiazocines (X = O), the only compound prepared with R^3 substitution, **1l**, had about the same level of activity as the protio-analogue **1g**. For the benzodiazocines (X = CH_2), R^3 substitution resulted in a decrease in activity (see e.g., **1ax** vs **1as** and **1aw** vs **1as**).

Methyl substitution at R^4 was preferred for maximum insecticidal activity for **1** where X = O. When R^4 was either H or Et, activity decreased while Ph substitution at R^4 resulted in inactive compounds. For compounds **1** where X = CH_2, substitutents R^4 = H or CH_3 gave comparable activity.

Reversible AChE inhibition analogous to huperzine A

The bridged-bicyclic cyanoguanidines **1** inhibit insect AChE in a reversible manner, similar to huperzine A.[13,14] Time course AChE inhibition studies using enzyme from southern corn rootworm (SCRW) (*Diabrotica undecimpunctata*) (Fig. 4) or GLH (Fig. 5) demonstrate the contrasting inhibition mechanisms exhibited by **1a** versus paraoxon. When AChE from either insect was incubated for 120 min with a concentration of paraoxon which normally inhibits AChE by 50% after a 30 min exposure, enzyme inhibition continued to increase and approached 100%, typical of an irreversible inactivation mechanism. Comparable incubations of AChE with **1a** resulted in enzyme inhibition remaining constant at about 50% regardless of incubation time. This is consistent with an equilibrium (reversible) inhibition mechanism. Similar results were observed for **1a** with AChE from SCRW (Fig. 4) and GLH (Fig. 5), indicating a similar mechanism of inhibition occurs in each insect.

Since AChE is a soluble enzyme, reversibility of enzyme inhibition by **1a** was further demonstrated through the use of molecular size exclusion chromatography (Fig. 6). If inhibition is a reversible phenomenon, then molecular size chromatography should separate a small molecule inhibitor from the large AChE enzyme. To test this experimentally, SCRW AChE was pre-incubated

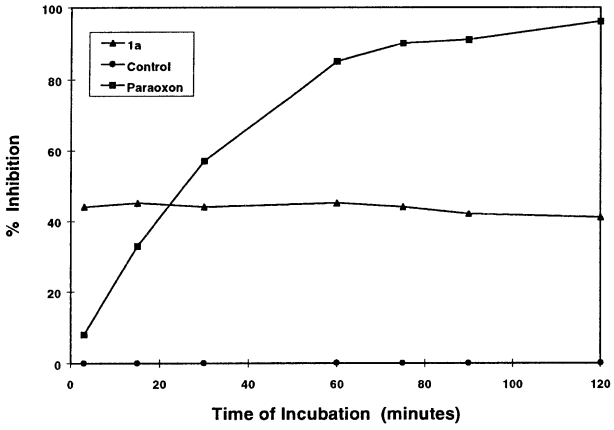
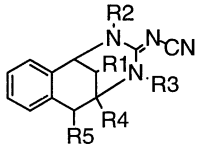


Figure 4. Time-course of inhibition of AChE from Southern corn rootworm (*Diabrotica undecimpunctata*) by **1a** or paraoxon. AChE from Southern corn rootworm was incubated for up to 120 min with 2 μM **1a** or 300 nM paraoxon. The concentrations chosen were approximately equal to the I_{50} measured for each compound in dose–response experiments. The control AChE preparation was incubated similarly, except for the lack of exposure to either compound. Each data point represents the mean of triplicate determinations. The SEM was typically less than 5%.

Table 3. Compounds **1** (X = CHR_5)



1 (X = CHR^5)

Cmpd	R^1	R^2	R^3	R^4	R^5	Mp°C	Insecticidal activity[a]		
							BPH	GLH	CPH
1as	H	H	H	H	H	250	+	+ + +	+ +
1at	endo-CH_3[b]	H	H	H	H	254–256	+	+ +	–
1au	exo-CH_3 [b]	H	H	H	H	286–287	+ +	+ + + +	+ + + +
1av	H	C(O)CH_3	H	H	H	218–220	nt	nt	+
1aw	H	H	C(O)CH_3	H	H	225–227	–	+ +	+
1ax	H	H	H	H	CH_3	234–238	nt	nt	–
1ay	H	H	H	CH_3	H	272–273	+ + +	+ +	+ + +
1az	exo-CH_3[b]	H	H	CH_3	H	250–255	nt	nt	+ + + +

[a]Insecticidal activities refer to the following approximate LC80 values: BPH or GLH: '–' = > 100 ppm, '+' = < 100 ppm, '+ +' = < 10 ppm, '+ + +' = < 1 ppm, '+ + + +' = < 0.1 ppm; CPH: '–' = > 250 ppm, '+' = < 250 ppm, '+ +' = < 50 ppm, '+ + +' = < 10 ppm, '+ + + +' = < 2.5 ppm, nt = not tested.
[b]Relative to the guanidine-containing ring.

for 30 min at room temperature with either paraoxon or **1a** (at 1 or 10 μM, respectively), or DMSO (solvent control). The enzyme-inhibitor and enzyme-solvent control mixtures were loaded onto identical Sephadex G-25-300 columns and eluted with buffer as indicated. Fractions were collected, aliquots added to assay tubes and the standard AChE assay was run without any further incubation. The aliquots of the column fractions thus served as the only potential source of AChE present in the assay tubes. Therefore, detection of enzyme activity is an indication of non-inhibited AChE being present in the column fraction. The reversible nature of the AChE inhibition by **1a** was evident by the recovery of approximately equal amounts of enzyme activity from all fractions off the columns containing **1a**-treated AChE and the DMSO-treated AchE (see Fig. 6). In contrast, the paraoxon-treated AChE appeared to be irreversibly inactivated, as less than 20% of the control level of activity was recovered from the column.

Modeling studies

Molecular modeling studies were carried out to investigate the structural similarity between Huperzine A and compound **1a**. Both molecules are fairly rigid and share a common shape of bridged bicyclic structures. To investigate the potential binding mode of **1a**, manual docking studies were performed using the AchE–HupA crystal[15] structure complex. Huperzine A was removed from the AchE–HupA crystal structure complex and replaced with a model of **1a**. Two potential binding modes were determined and the results are presented in Figure 7a and b in terms of the overlap with bound Huperzine-A. The three main factors used to guide the docking process were overall shape complementarity, establishing polar interactions with residues Tyr 130 and Glu 199 and forming hydrophobic contacts with Phe 330, 331, 290. The two binding modes presented differ in regard to the orientation of the cyanoguanidine. In Figure 7a, the cyano nitrogen of **1a** is postulated to occupy the region of space that is occupied by water 618 in the AChE–HupA crystal structure complex (*Z*-configuration). In Figure 7b, the cyano group is rotated approximately 180° about the double bond, placing the cyano nitrogen in the region of space occupied by the oxygen atom of the Huperzine pyridone (*E*-configuaration).

In these figures, good overlap can be seen between the bicyclic ring systems. In addition, the nitrogen of the cyanoguanidine moiety of **1a** lines up nicely with the nitrogen of the pyridone ring of Huperzine A. These modeling studies combined with the similarity in profile of enzyme inhibition for the two molecules suggest that they may share the same binding site in the enzyme. In addition these overlaps imply that the benzene ring and oxygen atoms of compound **1a** may not be essential features and that substitution on the bridging methylene of compound **1a** may be desirable.

In fact, it is interesting to note that some of the most active analogues **1** (as AChE inhibitors or versus insects) contain substitution on the bridging methylene group (R^1 other than H). (See, for example, compound **1aq** in Table 2 and **1au** and **1az** in Table 3.) It is interesting that when $R^1 = Et$, insecticidal activity decreased (see **1ak** and **1an** in Table 2). Since Huperzine A contains an ethylidine substituent at the bridgehead position, we would like to investigate the activities of structures **1** that also contain a bridgehead ethylidene substituent (synthesis efforts towards these analogues are currently underway).

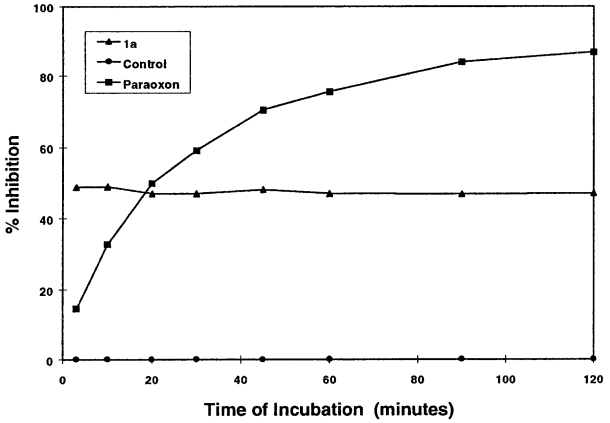


Figure 5. Time–course inhibition of AChE from Green leafhopper (*Nephotettix cincticeps*) by **1a** and paraoxon. AChE from Green leafhopper was incubated for up to 120 min with 3 μM **1a** or 1 μM paraoxon. The concentrations chosen were approximately equal to the I_{50} measured for each compound in dose–response experiments. The control AChE preparation was incubated similarly, except for the lack of exposure to either compound. Each data point represents the mean of triplicate determinations. The SEM was typically less than 5%.

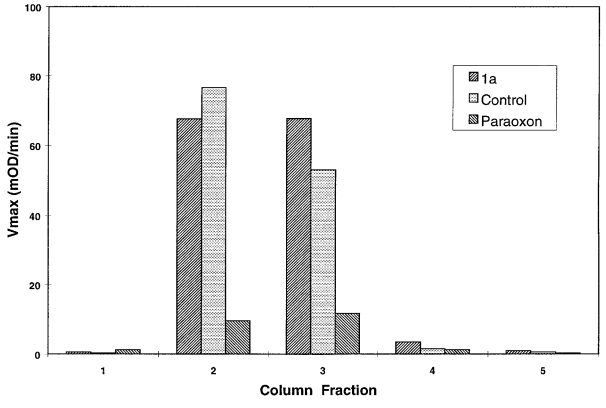


Figure 6. Molecular size-exclusion chromatography of AChE from Southern corn rootworm (*Diabrotica undecimpunctata*) treated with **1a** or paraoxon. Identical Sephadex G-25-300 columns were poured with bed volumes of approximately 10 mL. The columns were equilibrated with 0.1 M sodium phosphate, pH 7.4. Next, a 3 mL aliquot of AChE was mixed with **1a** (10 μM final concentration), paraoxon (1 μM final concentration) or dimethyl sulfoxide (solvent control). The enzyme-inhibitor mixture was incubated for 30 min at 23 °C. Following incubation, 2 mL of each mixture was loaded onto separate columns and eluted with sodium phosphate buffer. Five 2 mL fractions were collected with 20 μL aliquots taken from each fraction and assayed in the standard AChE assay. AChE assay conditions were identical to that in Methods except: no additional solvent or compound was added to the well, 190 μL of buffer was used, the microplate was not incubated prior to ATC/DTNB addition.

Conclusions

Tricyclic benzodiazocines **1** are a novel class of insect control compounds which display activity towards a variety of economically-important rice hopper species. The structure–activity relationships of substituents around the tricyclic nucleus of **1** was limited to either hydrogen or methyl groups for R^1, R^4, and R^5 for highest insect activity. Benzodiazocines **1** ($X=CH_2$) were somewhat more insecticidally active than analogous benzoxadiazocines **1** ($X=O$). In general, higher insecticidal activities of compounds **1** corresponded to lower IC_{50} levels in AChE assays, indicating cholinesterase inhibition as the primary mode of insecticidal action. The compounds **1** were found to inhibit AChE in a reversible manner, analogous to structurally-related Huperzine A. Molecular overlays of **1a** with Huperzine A show significant homology between the two structures. It is not currently known whether compounds **1** function pharmacologically as cognition enhancers in mammals, analogous to Huperzine A.

Experimental

General methods

^1H NMR spectra were recorded using a Varian Unity Plus300-NB spectrometer (300 MHz) in the solvent indicated. Coupling constants are reported in hertz. Spectral data for minor isomers in a product mixture are indicated in parentheses. IR spectra were recorded using a Perkin-Elmer 1600 FTIR instrument. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse NJ, USA. Thin-layer chromatography (TLC) was carried out using Whattman silica gel 60A plates. Silica gel (flash) chromatography was performed using EM Science silica gel 60 (230–400 mesh). All reactions were carried out under a positive pressure of nitrogen using reagent grade or anhydrous solvents as received.

Procedure A: preparation of compounds 2. The preparation of 4-(2-hydroxy-phenyl)-but-3-en-2-one (**2a**) has been previously described[8] and is representative. Aqueous sodium hydroxide solution (1.0 N, 18.0 mL, 18.0 mmol) was added to a solution of salicylaldehyde (2.0 g, 16.4 mmol) and acetone (12 mL, 164 mmol) at room temperature (slight exotherm to 27 °C). The resulting yellow solution was stirred at room temperature for 14 h, at which time the resulting dark brown mixture was acidified by dropwise addition of aqueous HCl (1.0 N, 23 mL, 23 mmol) at <10 °C. The resulting yellow solid was isolated by filtration and dried under vacuum to give 2.0 g of **2a** (76% yield). ^1H NMR (DMSO-d_6) δ 2.30 (s, 3H), 6.81 (distorted d, 1H, $J=5.7$ Hz), 6.85 (m, 1H), 6.92 (d, 1H, $J=8.4$ Hz), 7.24 (td, 1H, $J=8.1$ 2.4, Hz), 7.60 (d, 1H, $J=8.1$ Hz), 7.78 (d, 1H, $J=16.2$ Hz), 10.3 (s, 1H).

Using the appropriately substituted salicylaldehydes, the following compounds were similarly prepared.

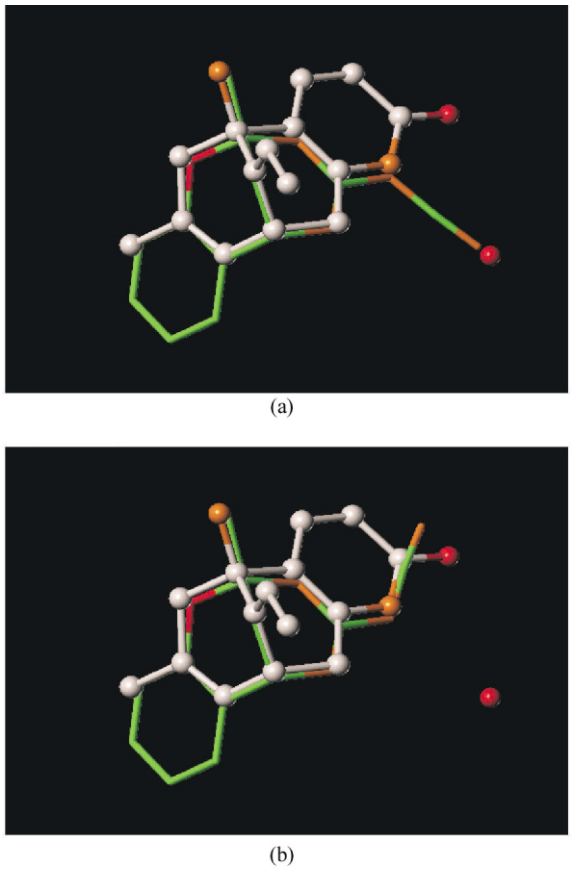


Figure 7. (a) Overlap of Compound **1a** (green) and Huperzine A (silver) with Z-configuration assumed for double bond. (b) Overlap of Compound **1a** (green) and Huperzine A (silver) with E-configuration assumed for double bond.

4-(5-Chloro-2-hydroxy-phenyl)-but-3-en-2-one (2b). ^1H NMR (DMSO-d_6) δ 2.30 (s, 3H), 6.90 (d, 1H, $J=9.3$ Hz), 6.94 (s, 1H), 7.26 (dd, 1H, $J=9.3$, 2.2, Hz), 7.67 (d, 1H, $J=4.5$ Hz), 7.70 (d, 1H, $J=9.3$ Hz), 10.54 (s, 1H).

4-(3,5-Dichloro-2-hydroxy-phenyl)-but-3-en-2-one (2c). ^1H NMR (DMSO-d_6) δ 2.33 (s, 3H), 6.93 (d, 1H, $J=16.5$ Hz), 7.58 (d, 1H, $J=2.3$ Hz), 7.74 (d, 1H, $J=2.6$ Hz), 7.75 (d, 1H, $J=16.5$, Hz), 10.39 (br s, 1H); IR (KBr pellet) 3220, 1660, 1648, 1623, 1462, 1253, 980 cm^{-1}. Anal. calcd for $C_{10}H_8Cl_2O_2$: C, 51.98; H, 3.49. Found: C, 51.78; H, 3.46.

4-(3,5-Dibromo-2-hydroxy-phenyl)-but-3-en-2-one (2d). ^1H NMR (DMSO-d_6) δ 2.32 (s, 3H), 6.90 (d, 1H, $J=16.5$ Hz), 7.76 (d, 1H, $J=16.5$ Hz), 7.80 (d, 1H, $J=2.3$, Hz), 7.88 (d, 1H, $J=2.3$, Hz), 10.25 (br s, 1H). Anal. calcd for $C_{10}H_8Br_2O_2$: C, 37.54: H, 2.52. Found: C, 37.61; H, 2.48.

4-(2-Hydroxy-5-nitro-phenyl)-but-3-en-2-one (2e). ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H), 7.05 (d, 1H, $J=16.5$, Hz), 7.10 (d, 1H, $J=9.6$ Hz), 7.74 (d, 1H, $J=16.5$ Hz), 8.15 (dd, 1H, $J=7.5$, 2.9 Hz), 8.51 (d, 1H, $J=2.9$ Hz), 11.9 (br s, 1H); IR (KBr pellet) 3076, 1637, 1607, 1519, 1496, 1345, 1306, 981 cm^{-1}. Anal. calcd for $C_{10}H_9NO_4$: C, 57.97; H, 4.38; N, 6.76. Found: C, 57.64; H, 4.33; N, 6.55.

1-(2-Hydroxy-phenyl)-pent-1-en-3-one (2h). Prepared following Procedure A using 2-butanone in place of acetone. ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 3H, J=7.2 Hz), 2.75 (q, 2H, J=7.2 Hz), 6.89 (m, 2H), 6.99 (d, 1H, J=16.5 Hz), 7.26 (d(apparent)t, 1H, J=7.8, 1.2, Hz), 7.48 (d, 1H, J=7.8 Hz), 7.91 (d, 1H, J=16.5 Hz).

4-(2-Hydroxy-5-trifluoromethoxy-phenyl)-but-3-en-2-one (2m). ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H), 6.95 (d, 1H, J=16.0 Hz), 6.99 (d, 1H, J=9.0 Hz), 7.25 (dd, 1H, J=9.0, 2.5 Hz), 7.65 (d, 1H, J=2.5 Hz), 7.72 (d, 1H, J=16.0 Hz); IR (KBr pellet) 3020, 1638, 1429, 1261, 1212, 979 cm^{-1}. Anal. calcd for $C_{11}H_9F_3O_3$: C, 53.67; H, 3.68. Found: C, 53.83; 3.57.

1-(2-Hydroxy-phenyl)-2-methyl-pent-1-en-3-one (2n). Prepared following Procedure A using 3-pentanone in place of acetone. ^{1}H NMR ($CDCl_3$) δ 1.18 (t, 3H, J=7.5 Hz), 1.98 (d, 3H, J=1.5 Hz), 2.86 (q, 2H, J=7.5 Hz), 5.08 (s, 1H), 6.88 (d, 1H, J=7.8, Hz), 6.97 ((apparent)t, 1H, J=6.6 Hz), 7.25 (m, 2H), 7.63 (s, 1H).

4-(2-Hydroxy-3-methyl-phenyl)-but-3-en-2-one (2o). ^{1}H NMR (DMSO-d_6) δ 2.20 (s, 3H), 2.33 (s, 3H), 6.70-6.85 (m, 2H), 7.15 (d, 1H, J=6.0 Hz), 7.48 (d, 1H, J=6.0 Hz), 7.92 (distorted d, 1H, J=16.0 Hz).

4-(3,5-Di-*tert*-butyl-2-hydroxy-phenyl)-but-3-en-2-one (2p). ^{1}H NMR (DMSO-d_6) δ 1.27 (s, 9H), 1.38 (s, 9H), 2.35 (s, 3H), 6.65 (d, 1H, J=16.5, Hz), 7.29 (d, 1H, J=2.2, Hz), 7.43 (d, 1H, J=2.2, Hz), 7.97 (d, 1H, J=16.5, Hz), 9.07 (s, 1H); IR (KBr pellet) 3327, 2962, 1628, 1271, 1224, 978 cm^{-1}. Anal. calcd for $C_{18}H_{26}O_2$: C, 78.79; H, 9.55. Found: C, 78.49; H, 9.46.

4-(4-Chloro-2-hydroxy-phenyl)-but-3-en-2-one (2s). ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 6.81–6.96 (m, 3H), 7.60–7.75 (m, 2H), 10.75 (s, 1H).

4-(2-Hydroxy-5-methyl-phenyl)-but-3-en-2-one (2t). ^{1}H NMR (DMSO-d_6) δ 2.21 (s, 3H), 2.29 (s, 3H), 6.80 (d, 1H, J=16.5, Hz), 6.81 (d, 1H, J=8.4, Hz), 7.05 (dd, 1H, J=8.4, 2.0 Hz), 7.41 (s, 1H), 7.74 (d, 1H, J=16.5 Hz), 9.98 (s, 1H). Anal. calcd for $C_{11}H_{12}O_2$: C, 74.98; H, 6.86. Found: C, 75.04; H, 6.95.

4-(3-Fluoro-2-hydroxy-phenyl)-but-3-en-2-one (2v). ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H), 6.75–6.90 (m, 2H), 7.24 (br t, 1H, J=9.6 Hz), 7.46 (d, 1H, J=7.8 Hz), 7.79 (d, 1H, J=16.5 Hz), 10.4 (s, 1H). Anal. calcd for $C_{10}H_9FO_2$: C, 66.67; H, 5.03. Found C, 66.41; H, 5.02.

4-(2-Hydroxy-3,4-dimethoxy-phenyl)-but-3-en-2-one (2x). ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H), 3.69 (s, 3H), 3.83 (s, 3H), 6.60 (d, 1H, J=8.7 Hz), 6.74 (d, 1H, J=16.2 Hz), 7.36 (d, 1H, J=8.7 Hz), 7.70 (d, 1H, J=16.2 Hz), 9.65 (s, 1H).

4-(2-Hydroxy-6-methoxy-phenyl)-but-3-en-2-one (2aa). ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H), 3.83 (s, 3H), 6.54 ((apparent) t, 2H, J=8.4 Hz), 7.11 (d, 1H, J=16.5 Hz), 7.18 (t, 1H, J=8.4 Hz), 7.86 (d, 1H, J=16.5 Hz), 10.35 (s, 1H). Anal. calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.25; H, 6.16.

4-(2,4-Dichloro-6-hydroxy-phenyl)-but-3-en-2-one (2ab). ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H), 6.97 ((apparent)s, 1H), 7.16 ((apparent)s, 1H), 7.18 (d, 1H, J=16.5 Hz), 7.69 (d, 1H, J=16.5 Hz).

4-(4-Fluoro-2-hydroxy-phenyl)-but-3-en-2-one (2ac). ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 6.60–6.75 (m, 2H), 6.81 (d, 1H, J=16.5 Hz), 7.60–7.70 (m, 1H), 7.72 (d, 1H, J=16.5 Hz), 10.70 (s, 1H). Anal. calcd for $C_{10}H_9FO_2$: C, 66.67; H, 5.03. Found: C, 66.68; H, 4.97.

3-(2-Hydroxy-phenyl)-propenal (2aj). A mixture of 2.5 g (11.4 mmol) of 2-iodophenol, 1.41 mL (17.0 mmol) of acrolein, 2.39 g (28.4 mmol) of sodium hydrogencarbonate, 3.16 g (11.4 mmol) of tetrabutylammonium chloride hydrate and 26 mg (0.11 mmol) of palladium(II) acetate in 45 mL of DMF was heated at 60 °C for 16 h. After cooling to room temperature the mixture was poured into water and extracted with ethyl acetate. The organic layers was separated and then dried (sodium sulfate). The solvent was removed with a rotary evaporator. The residue was purified by MPLC (15–20% ethyl acetate in hexanes as eluant) to afford 0.66 g (31%) of the title compound as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 6.88 (dd, 1H, J=8.1, 1.2 Hz), 6.98 (d(apparent)t, 1H, J=7.5, 1.2 Hz), 7.00 (dd, 1H, J=15.9, 7.8 Hz), 7.31 (d(apparent)t, 1H, J=7.8, 1.8 Hz), 7.51 (dd, 1H, J=7.5, 1.8 Hz), 9.68 (d, 1H, J=7.8 Hz).

Compounds **2ak**, **2al**, **2an**, and **2aq** were prepared by a similar procedure.

3-(2-Hydroxy-benzylidene)-pentan-2-one (2ak). Prepared using 3-methylene pentan-2-one: ^{1}H NMR ($CDCl_3$) δ 1.06 (t, 3H, J=7.5 Hz), 2.46 (q, 2H, J=7.5 Hz), 2.47 (s, 3H), 5.26 (s, 1H), 6.88 (d, 1H, J=8.4 Hz), 6.97 ((apparent)t, 1H, J=7.5 Hz), 7.26 (m, 2H), 7.59 (s, 1H).

3-(2-Hydroxy-phenyl)-2-methyl-propenal (2al). Prepared using methacrolein: ^{1}H NMR ($CDCl_3$) δ 2.04 (d, 3H, J=1.5 Hz), 6.91 (d, 1H, J=8.1 Hz), 6.99 ((apparent)t, 1H, J=8.1 Hz), 7.28 (d(apparent)t, 1H, J=7.8, 1.5 Hz), 7.45 (dd, 1H, J=7.8, 1.5 Hz), 7.64 (s, 3H).

2-(2-Hydroxy-benzylidene)-butyraldehyde (2an). Prepared using 2-methylene butyraldehyde: ^{1}H NMR ($CDCl_3$) δ 1.12 (t, 3H, J=7.5 Hz), 2.50 (q, 2H, J=7.5 Hz), 5.14, (s, 1H), 6.86 (d, 1H, J=7.5 Hz), 7.00 ((apparent)t, 1H, J=7.5 Hz), 7.28 ((apparent)t, 1H, J=8.4 Hz), 7.41 (d, 1H, J=8.4 Hz), 7.50 (s, 1H), 9.60 (s, 1H).

4-(2-Hydroxy-phenyl)-3-methyl-but-3-en-2-one (2aq). Prepared using 3-methylene butan-2-one: ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.48 (s, 3H), 4.92 (s, 1H), 6.83 (d, 1H, J=5.1 Hz), 6.98 ((apparent)t, 1H, J=4.5 Hz), 7.29 (m, 2H), 7.63 (brs, 1H).

Procedure B: preparation of compounds 1. The preparation of 9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$] trideca-2,4,6-trien-11-ylidene cyanamide (**1a**) has been previously described[8] and is representative. Piperidine (2.5 mL) was added to a mixture of **2a** (12.4 g, 76.3 mmol), cyanamide (9.6 g, 229 mmol), and 1,2-dimethoxyethane (150 mL) at room temperature. The resulting bright orange mixture was heated at reflux for 6 h, cooled to room temperature, and filtered. The solid product was washed with 1,2 dimethoxyethane and dried to give 8.16 g of **1a** as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.68 (s, 3H), 2.15 (br d, 2H, J = 3.0 Hz), 4.47–4.52 (m, 1H), 6.81 (d, 1H, J = 7.2 Hz), 6.92 (t, 1H, J = 7.2 Hz).

The following compounds were prepared by use of Procedure B using the appropriate substrates **2**.

4-Chloro-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]-trideca-2,4,6-trien-11-ylidene-cyanamide (1b). ^{1}H NMR (DMSO-d_6) δ 1.68 (s, 3H), 2.16 (m, 2H), 4.52 (br d, 1H), 6.86 (d, 1H, J = 8.7 Hz), 7.20–7.35 (m, 2H), 8.63 (br s, 1H), 8.76 (s, 1H); IR (KBr pellet) 3195, 2190, 1650, 1551, 1473, 1173, 712 cm^{-1}. Anal. calcd for $C_{12}H_{11}ClN_4O$: C, 54.87; H, 4.22; N, 21.33. Found: C, 54.62; H, 4.14; N, 21.26.

4,6-Dichloro-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6-trien-11-ylidene-cyanamide (1c). ^{1}H NMR (DMSO-d_6) δ 1.74 (s, 3H), 2.22 (m, 2H), 4.60 (m, 1H), 7.28 (d, 1H, J = 2.4 Hz), 7.54 (d, 1H, J = 2.4 Hz), 8.72 (s, 1H), 8.90 (s, 1H). Anal. calcd for $C_{12}H_{10}Cl_2N_4O$: C, 48.51; H, 3.39; N, 18.85. Found: C, 47.73; H, 3.25; N, 18.56.

4,6-Dibromo-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6-trien-11-ylidene-cyanamide (1d). ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 3H), 2.21 (m, 2H), 4.58 (distorted q, 1H), 7.43 (d, 1H, J = 2.1 Hz), 7.74 (d, 1H, J = 2.1 Hz), 8.70 (br s, 1H), 8.91 (s, 1H). Anal. calcd for $C_{12}H_{10}Br_2N_4O$: C, 37.34; H, 2.61; N, 14.51. Found: C, 37.31; H, 2.49; N, 14.38.

9-Methyl-4-nitro-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6,11-tetraen-11-yl-cyanamide (1e). ^{1}H NMR (DMSO-d_6) δ 1.74 (s, 3H), 2.25 (m, 2H), 4.70 (m, 1H), 7.05 (d, 1H, J = 9.0 Hz), 8.10 (dd, 1H, J = 9.0, 2.4 Hz), 8.24 (d, 1H, J = 2.4 Hz), 8.75 (br s, 1H), 8.96 (s, 1H).

9-Ethyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6,11-tetraen-11-yl-cyanamide (1h). ^{1}H NMR (DMSO-d_6) δ 0.95 (t, 3H, J = 7.5 Hz), 1.99 (m, 2H,), 2.08 (d, 2H, J = 3.0 Hz), 4.51 (br s, 1H), 6.82 (d, 1H, J = 7.5 Hz), 6.89 ((apparent)t, 1H, J = 7.5 Hz). 7.24 (m, 2H), 8.55 (br s, 1H), 8.66 (br s, 1H); IR (KBr): 3216, 2184, 1618 cm^{-1}.

9-Phenyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6,11-tetraen-11-yl-cyanamide (1j). Prepared from commercially available 2-hydroxychalcone using Procedure B: ^{1}H NMR (DMSO-d_6) δ 2.32 (m, 2H), 4.56 (br s, 1H), 7.01 (m, 2H), 7.31 (m, 2H), 7.46 (m, 3H), 7.65 (d, 2H, J = 8.4 Hz), 8.57 (br s, 1H), 9.00 (br s, 1H).

9-Methyl-4-trifluoromethoxy-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6-trien-11-ylidene-cyanamide (1m). ^{1}H NMR (DMSO-d_6) δ 1.69 (s, 3H), 2.18 (distorted d, 2H), 4.55 (br s, 1H), 6.93 (d, 1H, J = 8.7 Hz), 7.15–7.25 (overlapping s and d, 2H total), 8.65 (br s, 1H), 8.79 (s, 1H); IR (KBr pellet) 3187, 2183, 1639, 1250 cm^{-1}.

9-Ethyl-13-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]-trideca-2,4,6-trien-11-ylidene-cyanamide (1n). ^{1}H NMR (DMSO-d_6) δ 0.97 (m, 6H), 1.73 (dt, 1H, J = 7.5, 7.2 Hz), 2.02 (dt, 1H, J = 7.5, 7.2 Hz), 2.28 (d, 1H, J = 6.3 Hz), 4.21 (br s, 1H), 6.83 (d, 1H, J = 8.1 Hz), 6.92 ((apparent)t, 1H, J = 7.5 Hz), 7.20 (m, 2H), 8.45 (br s, 1H), 8.58 (br s, 1H).

6,9-Dimethyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6-trien-11-ylidene-cyanamide (1o). ^{1}H NMR (DMSO-d_6) δ 1.70 (s, 3H), 2.10 (s, 3H), 2.13 ((apparent)d, 2H), 4.47 (br s, 1H), 6.82 (t, 1H, J = 7.5 Hz), 7.07 ((apparent)d, 2H), 8.63 (br s, 2H). Anal. calcd for $C_{13}H_{14}N_4O$: C, 64.45; H, 5.82; N, 23.12. Found: C, 63.92; H, 5.80; N, 22.94.

4,6-Di-*tert*-butyl-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6-trien-11-ylidene-cyanamide (1p). ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H), 1.33 (s, 9H), 1.72 (s, 3H), 2.10 (m, 2H), 4.44 (m, 1H), 7.12 (d, 1H, J = 2.4 Hz), 7.16 (d, 1H, J = 2.4 Hz), 8.59 (br d, 1H), 8.71 (s, 1H); IR (KBr pellet) 3222, 2954, 2175, 1630, 1162 cm^{-1}. Anal. calcd for $C_{20}H_{28}N_4O$: C, 70.56; H, 8.29; N, 14.46. Found: C, 68.94; H, 8.40; N, 15.28.

5-Chloro-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]-trideca-2,4,6-trien-11-ylidene-cyanamide (1s). ^{1}H NMR (DMSO-d_6) δ 1.68 (s, 3H), 2.16 (m, 2H), 4.52 (br s, 1H), 6.92 (d, 1H, J = 2.1 Hz), 7.00 (dd, 1H, J = 8.1, 2.1 Hz), 7.26 (d, 1H, J = 8.1 Hz), 8.70 (br s, 1H), 8.78 (s, 1H).

4,9-Dimethyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6-trien-11-ylidene-cyanamide (1t). ^{1}H NMR (DMSO-d_6) δ 1.66 (s, 3H), 2.11 ((apparent)d, 2H), 2.23 (s, 3H), 4.42 (m, 1H), 6.71 (d, 1H, J = 7.8 Hz), 6.98–7.05 (m, 2H), 8.61 (br s, 2H).

6-Fluoro-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]-trideca-2,4,6-trien-11-ylidene-cyanamide (1v). Compound **1v** was purified by silica gel chromatography using a gradient of 5–25% EtOH in dichloromethane as the eluant. ^{1}H NMR (DMSO-d_6) δ 1.72 (s, 3H), 2.21 (m, 2H), 4.56 (br s, 1H), 6.88–6.98 (m, 1H), 7.05–7.20 (m, 2H), 8.74 (d, 1H), 8.79 (s, 1H).

5,6-Dimethoxy-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6,11-tetraen-11-yl-cyanamide (1x). ^{1}H NMR (DMSO-d_6) δ 1.70 (s, 3H), 2.12 (m, 2H), 3.63 (s, 3H), 3.75 (s, 3H), 4.42 (m, 1H), 6.63 (d, 1H, J = 8.4 Hz), 6.92 (d, 1H, J = 8.4 Hz), 8.62 (m, 1H), 8.73 (s, 1H).

3-Methoxy-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.$0^{2,7}$]trideca-2,4,6-trien-11-ylidene-cyanamide (1aa). ^{1}H NMR (DMSO-d_6) δ 1.68 (s, 3H), 2.04 (m,

2H), 3.82 (s, 3H), 4.74 (br s, 1H), 6.43 (d, 1H, $J=8.2$), 6.57 (d, 1H, $J=8.2$ Hz), 7.17 (t, 1H $J=8.2$ Hz), 8.55 (s, 1H), 8.66 (br s, 1H).

3,5 - Dichloro - 9 - methyl - 8 - oxa - 10,12 - diaza - tricyclo[7.3.1.0^{2,7}]trideca - 2,4,6 - trien - 11 - ylidene - cyanamide (1ab). ^{1}H NMR (DMSO-d_6) δ 1.71 (s, 3H), 2.17 (m, 2H), 4.79 (br s, 1H), 6.99 (d, 1H, $J=2.1$ Hz), 7.21 (d, 1H, $J=2.1$ Hz), 8.80 (s, 1H), 9.01 (br s, 1H). Anal. calcd for $C_{12}H_{10}Cl_2N_4O$: C, 48.51; H, 3.39; N, 18.85. Found: C, 48.10; H, 3.30; N, 18.70.

5-Fluoro-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]-trideca-2,4,6-trien-11-ylidene-cyanamide (1ac). ^{1}H NMR (DMSO-d_6) δ 1.68 (s, 3H), 2.15 (m, 2H), 4.52 (m 1H), 6.70 (dd, 1H, $J=8.0$, 1.5 Hz), 6.78 (dt, 1H, $J=8.0$, 1.5 Hz), 7.27 ((apparent)t, 1H, $J=8.0$ Hz), 8.67 (m, 1H), 8.75 (s, 1H).

8-Oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6-trien-11 - ylidene - cyanamide (1aj). ^{1}H NMR ($CDCl_3$) δ 2.22 (d(apparent)t, 1H, $J=13.2$, 2.7 Hz), 2.35 (d(apparent)t, 1H. $J=13.2$, 2.7 Hz), 4.55 (br s, 1H), 4.63 (brs, 1H), 6.76 (br, 1H), 6.90 (d, 1H, $J=8.4$ Hz), 6.96 ((apparent)t, 1H, $J=7.2$ Hz), 7.22 (m, 2H), 7.39 (br s, 1H).

13-Ethyl-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]-trideca - 2,4,6 - trien - 11 - ylidene - cyanamide (1ak). ^{1}H NMR (DMSO-d_6) δ 0.99 (t, 3H, $J=7.2$ Hz), 1.59 (s, 3H), 2.02 (m, 2H), 4.37 (br s, 1H), 6.80 (d, 1H, $J=7.8$ Hz). 6.92 ((apparent)t, 1H, $J=7.5$ Hz), 7.22 (m, 2H), 8.52 (br s, 1H), 8.57 (br s, 1H).

13-Methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6 - trien - 11 - ylidene - cyanamide (1al). ^{1}H NMR ($CDCl_3$) δ 1.18 (d, 3H, $J=6.9$ Hz), [1.06 (d, 3H, $J=6.9$ Hz)], 2.47 (m, 1H), [2.40 (m, 1H)], 4.23 (m, 1H), [4.19 (m, 1H)], 5.23 (m, 1H), [5.26 (m, 1H)], 6.67 (br s, 1H), 6.89 (d, 1H, $J=7.8$ Hz), 6.95 ((apparent)t, 1H $J=7.5$ Hz), 7.22 (m, 2H), 7.33 (br s, 1H).

13-Ethyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6 - trien - 11 - ylidene - cyanamide (1an). ^{1}H NMR ($CDCl_3$) δ 1.06 (t, 3H, $J=7.5$ Hz), 2.20 (dt, 1H, $J=7.5$, 6.1 Hz), 4.35 (m, 1H), 5.31 (m, 1H), 6.75 (br s, 1H), 6.89 (d, 1H, $J=8.1$ Hz), 6.95 ((apparent)t, 1H $J=7.5$ Hz), 7.24 (m, 2H), 7.42 (br s, 1H).

Reaction of **2aq** with cyanamide and piperidine as described for **1a** gave a mixture of endo- and exo- isomers that were separated by silica gel chromatography. The faster eluting isomer was the *endo*-compound: ***rel*-(1*R*,9*R*,13*S*)-9,13-dimethyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6-trien-11-ylidene-cyanamide (1ar)**. ^{1}H NMR (DMSO-d_6) δ 1.02, (d, 3H, $J=6.6$ Hz), 1.58 (s, 3H), 2.24 (m, 1H) 4.20 (m, 1H), 6.80 (d, 1H, $J=8.7$ Hz), 6.91 ((apparent)t, 1H $J=7.5$ Hz), 7.20 (m, 2H), 8.56 (br s, 2H). Further elution gave the exo-isomer: ***rel*-(1*R*,9*R*,13*R*)-9,13-dimethyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6-trien-11-ylidene-cyanamide (1aq)**. ^{1}H NMR (DMSO-d_6) δ 0.84, (d, 3H, $J=6.9$ Hz), 1.58 (s, 3H), 2.18 (dq, 1H, $J=6.9$, 2.7 Hz) 4.18 (dd, 1H, $J=4.5$, 2.7 Hz), 6.81 (d, 1H, $J=8.1$ Hz), 6.95 ((apparent)t, 1H $J=7.5$ Hz), 7.24 (m, 2H), 8.60 (br s, 1H) 8.71 (br s, 1H).

12-Acetyl-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]-trideca-2,4,6-trien-11-ylidene-cyanamide (1f). Sodium hydride (60% in oil, 74 mg, 1.8 mmol) was added to a solution of **1a** (420 mg, 1.8 mmol) and DMF (11 mL) with stirring at 0–5 °C (CAUTION: hydrogen evolution). The resulting mixture was stirred at 0 °C for 1 h and was then treated with acetic anhydride (0.26 mL, 2.8 mmol). The resulting mixture was stirred at room temperature for 14 h and was then cooled to 0 °C and quenched with excess acetic acid (0.5 mL). The resulting mixture was diluted with water, causing the formation of a white precipitate. The solid was isolated by filtration, washed with water, suspended in acetonitrile and concentrated on the rotovap to give 360 mg (73%) of **1f** as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.80 (s, 3H), 2.22 (dd, 1H, $J=13.8$, 3.3 Hz), 2.38 (dd, 1H, $J=13.8$, 2.7 Hz), 2.48 (s, 3H), 5.78 (distorted s, 1H), 6.87 (d, 1H, $J=8.1$ Hz), 6.96 (t, 1H, $J=7.5$ Hz), 7.2–7.3 (m, 2H), 9.35 (s, 1H); IR (KBr pellet) 2185, 1707, 1621, 1470, 1235, 754 cm^{-1}. Anal. calcd for $C_{14}H_{14}N_4O_2$: C, 62.21; H, 5.22; N, 20.73. Found: C, 61.18; H, 5.35; N, 19.47. ^{1}H NMR NOE experiments showed NOE enhancement between signals at δ 1.80 and 9.35, indicating proximity between the C9 methyl substituent and the N10 proton. Therefore, acylation occurred preferrentially at the N12 nitrogen.

9,12-Dimethyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca - 2,4,6 - trien - 11 - ylidene - cyanamide (1g). Sodium hydride (60% in mineral oil, 74 mg, 1.8 mmol) was added to a solution of **1a** (420 mg, 1.8 mmol) and DMF (11 mL) at 0 °C. After 1 h, iodomethane (0.34 mL, 5.5 mmol) was added at <10 °C (slightly exothermic). The resulting mixture was stirred at room temperature for 2 days and was then quenched by carefully adding water (30 mL). The resulting solid product was isolated by filtration, washed with water, and dried in vacuo to give **1g** as a white solid (180 mg, 40% yield). ^{1}H NMR (DMSO-d_6) δ 1.76 (s, 3H, C–CH_3), 2.19 (dd, 1H, $J=13.3$, 2.7 Hz), 2.30 (dd, 1H, $J=13.4$, 2.9 Hz), 3.00 (s, 3H, N–CH_3), 4.56 (distorted s, 1H, angular C–H), 6.86 (d, 1H, $J=8.2$ Hz), 6.93 (t, 1H, $J=7.5$ Hz), 7.25 (t, 1H, $J=7.5$ Hz), 7.34 (d, 1H, $J=7.6$ Hz), 8.40 (s, 1H, N–H). ^{1}H NMR NOE experiments showed NOE enhancement between signals at δ 3.00 and 4.56 (indicating proximity between the N12 methyl and the C1 methine proton) and enhancement between signals at δ 1.76 and 8.40 (indicating proximity between the C9 methyl and the N10 proton). Thus, methylation occurred preferentially at the N12 nitrogen.

12-Methanesulfonyl-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca - 2,4,6 - trien - 11 - ylidene - cyanamide (1i). Sodium hydride (60% in oil, 73 mg, 1.8 mmol) was added to a solution of **1a** (414 mg, 1.8 mmol) and DMF (11 mL) with ice-water bath cooling (CAUTION: hydrogen evolution). After stirring at 5 °C for 30 min, methane sulfonic anhydride (347 mg, 2.0 mmol) was added (slightly exothermic to 10 °C). The resulting yellow solution was stirred at room temperature for 16 h

and was then cooled to 5 °C and treated with an additional portion of methane sulfonic anhydride (150 mg). After stirring an additional 16 h at room temperature, the reaction was diluted with water (30 mL) and the resulting green suspension was filtered. The tan solid product was washed with water and triturated with hexanes to give 75 mg (13%) of **1i** as a tan solid. ^{1}H NMR (DMSO-d_6) δ 1.81 (s, 3H), 2.37 (dd, 1H, J = 13.6, 2.9 Hz), 2.42 (dd, 1H, J = 13.5, 2.9 Hz), 3.32 (s, 3H), 3.47 (s, 3H), 5.62 ((apparent)t, 1H), 6.92 (d, 1H, J = 8.1 Hz), 7.02 (t, 1H, J = 7.5 Hz), 7.28–7.35 (m, 2H), 9.62 (s, 1H).

11 - Cyanoimino - 9 - methyl - 8 - oxa - 10,12 - diaza - tricyclo[7.3.1.0^{2,7}]trideca - 2,4,6 - triene - 12 - carboxylic acid methyl ester (1k). Sodium hydride (60% in mineral oil, 70 mg, 1.8 mmol) was added to a solution of **1a** (400 mg, 1.8 mmol) and DMF (11 mL) at 5 °C (CAUTION: hydrogen evolution). The resulting nearly homogeneous mixture was stirred at 5 °C for 30 min and was then treated with dimethyl pyrocarbonate (0.21 mL, 1.9 mmol). The resulting solution was stirred at room temperature for 14 h and was then quenched by carefully adding water (30 mL). The solid product was collected by filtration and was purified by chromatography on silica gel using 20% EtOAc in hexanes as the eluant to give 48 mg (12%) of **1k** as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.74 (s, 3H), 2.25 (dd, 1H, J = 13.9, 3.4 Hz), 2.42 (dd, 1H, J = 14.0, 2.7 Hz), 3.86 (s, 3H), 5.45 ((apparent)t, 1H, J = 2.9 Hz), 6.88 (d, 1H, J = 7.9 Hz), 7.02 (t, 1H, J = 7.6 Hz), 7.31 (t, 1H, J = 7.6 Hz), 7.38 (dd, 1H, J = 7.6, 1.4 Hz), 9.42 (s, 1H).

9,10,12-Trimethyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]-trideca-2,4,6-trien-11-ylidene cyanamide (1l). Sodium hydride (180 mg, 4.4 mmol) was added to a solution of **1a** (400 mg, 1.8 mmol) and DMF (11 mL) at 5 °C (CAUTION: hydrogen evolution). After stirring for 30 min at 5 °C, iodomethane (0.55 mL, 8.8 mmol) was added. The resulting thick yellow suspension was stirred at room temperature for 14 h and was then treated with an additional portion of iodomethane (0.11 mL) at 5 °C. After stirring 14 h at room temperature, the mixture was cooled to 5 °C and was then carefully quenched by the addition of water (50 mL). The crude product was isolated by filtration and was then purified by silica gel chromatography using a gradient of 25–33% EtOAc in hexanes as the solvent. After initial elution of **1g** (R_f 0.79, silica gel TLC, 100% EtOAc), **1l** was obtained as a white solid (67 mg, 15%). R_f (silica gel TLC, 100% EtOAc) = 0.47; ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 3H), 2.24 (dd, 1H, J = 13.5, 3.1 Hz), 2.42 (dd, 1H, J = 13.7, 2.5 Hz), 3.00 (s, 3H), 3.26 (s, 3H), 4.54 ((apparent)t, 1H, J = 2.9 Hz), 6.88 (d, 1H, J = 8.0 Hz), 6.99 (t, 1H, J = 7.5 Hz), 7.27 (t, 1H, J = 7.8 Hz), 7.40 (dd, 1H, J = 7.6, 1.4 Hz).

12-Acetyl-5-fluoro-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6-trien-11-ylidene-cyanamide (1q). Compound **1q** was prepared using a method analogous to that described for the preparation of compound **1f** (using **1ac** in place of **1a**). After silica gel chromatography using a gradient of 20–60% ethyl acetate in hexanes as eluant, **1q** was isolated in 6% yield as a solid. R_f (silica gel TLC, 50% EtOAc in hexanes) = 0.47; ^{1}H NMR (DMSO-d_6) δ 1.80 (s, 3H), 2.25 (dd, 1H, J = 14.1, 3.2 Hz), 2.39 (dd, 1H, J = 13.7, 2.6 Hz), 2.48 (s, 3H), 5.76 (t, 1H, J = 2.9 Hz), 6.77–6.85 (m, 2H), 7.34 (t, 1H, J = 7.7 Hz), 9.42 (s, 1H).

12-Benzyl-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]-trideca-2,4,6-trien-11-ylidene-cyanamide (1r). Compound **1r** was prepared in 80% yield using a procedure analogous to that described for the preparation of **1g** (using benzyl bromide in place of iodomethane). ^{1}H NMR (DMSO-d_6) δ 1.80 (s, 3H), 2.20 (d, 1H, J = 13.6 Hz), 2.27 (dd, 1H, J = 13.7, 2.9 Hz), 4.45 (d, 1H, J = 15.5 Hz), 4.53 (m, 1H), 4.94 (d, 1H), 6.86 ((apparent)d, 2H, J = 7.7 Hz), 7.1–7.4 (m, > 7H), 8.59 (s, 1H).

12-Ethyl-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]-trideca-2,4,6-trien-11-ylidene-cyanamide (1u). Compound **1u** was prepared in 16% yield (after trituration with acetonitrile) using a procedure analogous to that described for the preparation of **1g** (using iodoethane instead of iodomethane). ^{1}H NMR (DMSO-d_6) δ 1.13 (t, 3H, J = 7.0 Hz), 1.76 (s, 3H), 2.20 (m 2H), 3.25–3.40 (m, 1H, overlaps H_2O signal), 3.62–3.72 (m, 1H), 4.67 (s, 1H), 6.85 (d, 1H, J = 8.1 Hz), 6.92 (t, 1H, J = 7.4 Hz), 7.24 (t, 1H, J = 7.4 Hz), 7.40 (d, 1H, J = 7.4 Hz), 8.37 (s, 1H).

9-Methyl-12-pyridin-2-ylmethyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6-trien-11-ylidene-cyanamide (1w). Sodium hydride (154 mg of a 60% suspension in mineral, 3.85 mmol) was added to a solution of **1a** (400 mg, 1.75 mmol), 2-picolyl chloride hydrochloride salt (316 mg, 1.9 mmol) and DMF (11 mL) at 0 °C (slightly exothermic). The resulting suspension was stirred at room temperature for 2 days and was then cooled to 0 °C and quenched by carefully adding water (30 mL). The solid product was isolated by filtration, dried on the frit, washed with hexanes and dried again to give **1w** as a light pink solid (420 mg, 75% yield). ^{1}H NMR (DMSO-d_6) δ 1.81 (s, 3H), 2.22 (br d, 1H, J = 4.8 Hz), 2.44 (dd, 1H, J = 13.4, 3.2 Hz), 4.61 (d, 1H, J = 16.2 Hz), 4.67 (m, 1H), 4.98 (d, 1H, J = 16.2 Hz), 6.82–6.90 (m, 2H), 7.20–7.35 (m, 4H), 7.78 (dt, 1H, J = 7.6, 1.9 Hz), 8.57 (d, 1H, J = 4.8 Hz), 8.61 (s, 1H).

12-Allyl-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]-trideca-2,4,6-trien-11-ylidene-cyanamide (1y). Compound **1y** was prepared in 38% yield using a procedure analogous to that described for the preparation of **1g** (using allyl bromide in place of iodomethane): ^{1}H NMR (DMSO-d_6) δ 1.78 (s, 3H), 2.20 (dd, 1H, J = 13.4, 2.4 Hz), 2.28 (dd, 1H, J = 13.4, 2.9 Hz), 3.85 (dd, 1H, J = 16.1, 6.2 Hz), 4.35 (dd, 1H, J = 15.9, 4.9 Hz), 4.54 (s, 1H), 5.21 (d, 1H, J = 10.3 Hz), 5.30 (s, 1H), 5.73–5.85 (m, 1H), 6.84 (d, 1H, J = 8.0 Hz), 6.92 (t, 1H, J = 7.5 Hz), 7.24 (t, 1H, J = 7.8 Hz), 7.35 (d, 1H, J = 7.6 Hz), 8.50 (s, 1H).

9-Methyl-12-trimethylsilanylmethyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6-trien-11-ylidene-cyanamide (1z). Compound **1z** was prepared in 14% yield

using a procedure analogous to that described for the preparation of **1g** (using iodomethyl trimethylsilane in place of iodomethane): ^{1}H NMR (DMSO-d_6) δ 0.04 (s, 9H), 1.77 (s, 3H), 2.25 (m, 2H), 2.84 (d, 1H, J = 15.0 Hz), 3.16 (d, 1H, J = 15.0 Hz), 4.46 (m, 1H), 6.86 (d, 1H, J = 8.1 Hz), 6.92 (t, 1H), 7.25 (dt, 1H, J = 7.6, 1.8 Hz), 7.44 (dd, 1H, J = 7.7, 1.4 Hz), 8.28 (s, 1H).

(11 - Cyanoimino - 9 - methyl - 8 - oxa - 10,12 - diaza - tricyclo[7.3.1.0^{2,7}]trideca-2,4,6-trien-12-yl)-acetic acid methyl ester (1ad). Prepared in 13% yield using a procedure analogous to that described for the preparation of **1g** (using methyl bromoacetate in place of iodomethane). Pure product was isolated after trituration with hot methanol. ^{1}H NMR (DMSO-d_6) δ 1.80 (s, 3H), 2.24 (br d, 1H, J = 13.7 Hz), 2.32 (dd, 1H, J = 13.5, 3.0 Hz), 3.66 (s, 3H), 4.28 (d, 1H, J = 17.7 Hz), 4.39 (d, 1H, J = 17.7 Hz), 4.71 (br t, 1H, J = 2.6 Hz), 6.85 (d, 1H, J = 6.9 Hz), 6.91 (t, 1H, J = 6.9 Hz), 7.22 ((apparent)t, 1H, J = 7.8 Hz), 7.41 ((apparent)d, 1H, J = 7.8 Hz), 8.72 (s, 1H).

9 - Methyl - 12 - prop - 2 - ynyl - 8 - oxa - 10,12 - diaza - tricyclo[7.3.1.0^{2,7}]trideca - 2,4,6 - trien - 11 - ylidene - cyanamide (1ae). Prepared in 60% yield using a procedure analogous to that described for the preparation of **1g** (using propargyl chloride in place of iodomethane). ^{1}H NMR (DMSO-d_6) δ 1.25 (br s, 1H), 1.77 (s, 3H), 2.25 (m, 2H), 4.16 (dd, 1H, J = 17.7, 2.5 Hz), 4.47 (dd, 1H, J = 17.7, 2.5 Hz), 4.77 (br t, 1H), 6.83 (d, 1H, J = 7.4 Hz), 6.92 (t, 1H, J = 6.6 Hz), 7.24 (t, 1H, J = 6.6 Hz), 7.42 (d, 1H, J = 6.6 Hz), 8.67 (s, 1H).

9 - Methyl - 12 - propionyl - 8 - oxa - 10,12 - diaza - tricyclo[7.3.1.0^{2,7}]trideca - 2,4,6 - trien - 11 - ylidene - cyanamide (1af). Prepared in 60% yield using a procedure analogous to that described for the preparation of **1f** (using propionic anhydride in place of acetic anhydride). ^{1}H NMR (DMSO-d_6) δ 1.07 (t, 3H, J = 6.9 Hz), 1.80 (s, 3H), 2.22 (dd, 1H, J = 13.5, 3.6 Hz), 2.35 (dd, 1H, J = 13.5, 2.6 Hz), 2.70–2.85 (m, 1H), 2.95-3.08 (m, 1H), 5.77 (br t, 1H, J = 3.0 Hz), 6.90 (d, 1H, J = 7.5 Hz), 6.96 (t, 1H, J = 7.5 Hz), 7.24 (br t, 1H, J = 7.5 Hz), 7.31 (d, 1H, J = 7.5 Hz), 9.31 (s, 1H).

12 - Butyryl - 9 - methyl - 8 - oxa - 10,12 - diaza - tricyclo[7.3.1.0^{2,7}]trideca - 2,4,6 - trien - 11 - ylidene - cyanamide (1ag). Prepared in 72% yield using a procedure analogous to that described for the preparation of **1f** (using *n*-butyric anhydride in place of acetic anhydride). ^{1}H NMR (DMSO-d_6) δ 0.90 (t, 3H, J = 7.2 Hz), 1.5–1.7 (m, 2H), 1.80 (s, 3H), 2.23 (dd, 1H, J = 14.4, 3.0 Hz), 2.35 (dd, 1H, J = 14.4, 2.4 Hz), 2.72–2.85 (m, 1H), 2.90–3.02 (m, 1H), 5.75 (br t, 1H, J = 3.0 Hz), 6.88 (d, 1H, J = 7.8 Hz), 6.96 (t, 1H, J = 7.8 Hz), 7.22–7.37 (m, 2H), 9.34 (br s, 1H).

12 - Isobutyryl - 9 - methyl - 8 - oxa - 10,12 - diaza - tricyclo[7.3.1.0^{2,7}]trideca - 2,4,6 - trien - 11 - ylidene - cyanamide (1ah). Prepared in 21% yield using a procedure analogous to that described for the preparation of **1f** (using isobutyric anhydride in place of acetic anhydride). ^{1}H NMR (DMSO-d_6) δ 1.11 (m, 6H), 1.81 (s, 3H), 2.24 (dd, 1H, 14.1, 3.3), 2.36 (dd, 1H, J = 14.1, 2.4 Hz), 3.67 (m, 1H), 5.59 (t, 1H, J = 3.0 Hz), 6.89 (d, 1H, J = 7.4 Hz), 6.97 (td, 1H, J = 7.4, 1.5 Hz), 7.27 (td, 1H, J = 6.9, 1.5 Hz), 7.38 (dd, 1H, J = 6.9, 1.5 Hz), 9.38 (s, 1H).

4-Amino-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]-trideca-2,4,6-trien-11-ylidene-cyanamide (1ai). 10% Palladium on carbon (56 mg) was added to a nitrogen-degassed solution of **1e** (56 mg, 0.20 mmol) and methanol (11 mL). The resulting mixture was degassed under hydrogen and stirred under 1 atmosphere of hydrogen (balloon) for 1.5 h. The resulting mixture was degassed under nitrogen and filtered through Celite. The filter caked was washed with methanol and the combined filtrates were concentrated to give crude product. Silica gel chromatography using 10% ethanol in methylene chloride gave **1ai** as a solid (29 mg, 55% yield). ^{1}H NMR (DMSO-d_6) δ 1.61 (s, 3H), 2.02 (m, 2H), 4.26 (m, 1H), 4.75 (br s, 2H), 6.4–6.5 (m, 3H), 8.43 (s, 1H), 8.60 (m, 1H).

9-Methyl-12-(2,2,2-trifluoro-acetyl)-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca-2(7),3,5-trien-11-ylidene-cyanamide (1am). To a nearly homogeneous solution of **1a** (300 mg, 1.3 mmol) and THF (15 mL) was added trifluoroacetic anhydride (0.61 mL, 7.9 mmol) at room temperature. After 6 h, the reaction mixture was heated at reflux. After 1.5 h at reflux, the reaction mixture was cooled to room temperature and concentrated. Silica gel chromatography of the residue using 20% EtOAc in hexanes as the eluant gave **1am** as an oil (6 mg, 2% yield). R_f (silica gel TLC, 50% EtOAc in hexanes) = 0.76; ^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H), 2.22 (dd, 1H, J = 13.8, 2.9 Hz), 2.52 (dt, 1H, J = 13.9, 2.1 Hz), 6.15 (t, 1H, J = 2.9 Hz), 6.74 (br s, 1H), 6.91 (d, 1H, J = 8.3 Hz), 6.99 (t, 1H, J = 7.6 Hz), 7.31 (t, 1H, J = 7.7 Hz), 7.61 (dd, 1H, J = 7.6, 1.4 Hz); MS (CI) m/e 325 (M + 1, 100%).

5 - Fluoro - 9,12 - dimethyl - 8 - oxa - 10,12 - diaza - tricyclo[7.3.1.0^{2,7}]trideca - 2,4,6 - trien - 11 - ylidene - cyanamide (1ao). Compound **1ao** was prepared using a procedure analogous to that described for the preparation of **1g** (using **1ac** in place of **1a**). After trituration of the crude product with methanol, **1ao** was isolated as a white solid in 10% yield. ^{1}H NMR ($CDCl_3$) δ 1.81 (s, 3H), 2.25 (dt, 1H, J = 12.9, 2.2 Hz), 2.33 (dd, 1H, J = 13.2, 3.2 Hz), 3.09 (s, 3H), 4.29 (t, 1H, J = 3.1 Hz), 6.18 (br s, 1H), 6.6–6.7 (m, 2H), 7.11 (dd, 1H, J = 8.3, 6.0 Hz).

11-Cyanoimino-5-fluoro-9-methyl-8-oxa-10,12-diaza-tricyclo[7.3.1.0^{2,7}]trideca - 2,4,6 - triene - 12 - carboxylic acid methyl ester (1ap). Compound **1ap** was prepared using a method that was analogous to that described for the preparation of **1f** (using dimethyl pyrocarbonate in place of acetic anhydride and using **1ac** in place of **1a**). After quenching the reaction with water, the resulting mixture was extracted with ethyl acetate (3×) and the combined organics were washed with water, dried over $MgSO_4$, and concentrated. Silica gel chromatography of the residue using 50% EtOAc in hexanes gave **1ap** as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.74 (s, 3H), 2.26 (br d, 1H, J = 14.1 Hz), 2.47 (dd, 1H, J = 14.8, 3.0 Hz),

3.86 (s, 3H), 5.45 (t, 1H, $J=3.0$ Hz), 6.90–6.78 (m, 2H), 7.42 (dd, 1H, $J=8.5$, 6.5 Hz), 9.51 (s, 1H).

1,2,5,6-Tetrahydro-4*H*-1,5-methano-benzo[*e*][1,3]diazocin-3-ylidene-cyanamide (1as). 1.29 g (7.93 mmol) of *cis* 1,2,3,4-tetrahydro-naphthalene-1,3-diamine was dissolved in 70 mL of 2-propanol. 1.89 g (7.93 mmol) of diphenyl cyanocarbonimidate was added and the mixture was refluxed for 4 h. After cooling, 0.93 g (57%) of the product was filtered off as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.95 (d, 1H, $J=12.3$ Hz), 2.04 (d, 1H, $J=12.3$ Hz), 2.87 (d, 1H, $J=17.4$ Hz), 3.01 (dd, 1H, $J=12.3$, 3.6 Hz), 3.94 (s, 1H), 4.39 (d, 1H, $J=3.6$ Hz), 7.20 (m, 4H), 7.83 (s, 1H), 8.16 (s, 1H); IR (KBr): 3257, 2161, 1630 cm^{-1}. Anal. calcd for $C_{12}H_{12}N_4$: C, 67.91; H, 5.70; N, 26.40. Found: C, 67.82; H, 5.69; N, 26.54.

***rel*-(1*R*,5*R*,11*R*)-11-Methyl-1,2,5,6-tetrahydro-4*H*-1,5-methano-benzo[*e*][1,3]diazocin-3-ylidene-cyanamide (1at) and *rel*-(1*R*,5*R*,11*S*)-11-methyl-1,2,5,6-tetrahydro-4*H*-1,5-methano-benzo[*e*][1,3]diazocin-3-ylidene-cyanamide (1au).** Compounds **1at** and **1au** were prepared starting from 2-benzyl-3-methyl-succinic acid 1-methyl ester[16] following the same general Scheme. The diastereomers were separated at the lactam stage. **1at** (endo-isomer): ^{1}H NMR (DMSO-d_6) δ 1.01 (d, 3H, $J=5.1$ Hz), 2.13 (m, 1H), 2.90 (dd, 1H, $J=13.6$, 0.9 Hz), 3.05 (dd, 1H, $J=13.6$, 3.0 Hz), 3.56 (m, 1H), 4.08 (m, 1H), 7.22 (m, 4H), 7.74 (s, 1H), 8.08 (m, 1H). **1au** (exo-isomer): ^{1}H NMR (DMSO-d_6) δ 0.88 (d, 3H, $J=6.9$ Hz), 2.51 (m, 1H), 2.90 (d, 1H, $J=18.0$ Hz), 3.05 (dd, 1H, $J=18.0$, 4.2 Hz), 3.69 (m, 1H), 4.08 (m, 1H), 7.21 (m, 4H), 7.84 (s, 1H), 8.15 (m, 1H). The stereochemical assignments of **1at** and **1au** were made by a NOE difference experiment. Irradiation of the proton at C-11 (δ 2.13) of **1at** gave rise to enhancement of the signal at δ 3.05 (one of the benzylic protons at C-6). Irradiation of the methyl group of **1au** gave rise to enhancement of the signal at δ 3.05.

2-Acetyl-1,2,5,6-tetrahydro-4*H*-1,5-methano-benzo[*e*][1,3]diazocin-3-ylidene-cyanamide (1av) and 4-Acetyl-1,2,5,6-tetrahydro-4*H*-1,5-methano-benzo[*e*][1,3]diazocin-3-ylidene-cyanamide and (1aw). To a solution of 0.10 g (0.47 mmol) of compound **1as** in 5 mL of DMF at 0 °C was added 19 mg (0.47 mmol) of sodium hydride (60% dispersion in mineral oil). After 1 h 0.070 mL (0.71 mmol) of acetic anhydride was added and the reaction was stirred overnight at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was separated and dried (sodium sulfate). The solvent was removed with a rotary evaporator. The residue was purified by MPLC (40–50% ethyl acetate in hexanes as eluent). Eluting first was compound **1aw** (13 mg, 11% yield) and then compound **1av** (26 mg, 22% yield). **1av**: ^{1}H NMR ($CDCl_3$) δ 2.20 (m, 2H), 2.60 (s, 3H), 3.07 (m, 2H), 4.19 (m, 1H), 6.07 ((apparent(t), 1H, $J=3.0$ Hz), 7.20 (m, 4H), 7.51 (d, 1H, $J=7.2$ Hz). **1aw**: ^{1}H NMR ($CDCl_3$) δ 2.24 (m, 2H), 2.60 (s, 3H), 3.11 (m, 2H), 4.57 (m, 1H), 5.19 (m, 1H), 7.14 (d, 1H, $J=7.5$ Hz), 7.28 (m, 4H).

5-Methyl-1,2,5,6-tetrahydro-4*H*-1,5-methano-benzo[*e*][1,3]diazocin-3-ylidene-cyanamide (1ax). Compound **1ax** was prepared from 2-benzyl-2-methyl-succinic acid[17] following the same general Scheme. ^{1}H NMR (DMSO-d_6) δ 1.40 (s, 3H), 1.86 (d, 1H, $J=12.6$ Hz), 2.06 (d, 1H, $J=12.6$ Hz), 2.91 (s, 2H), 4.44 (br s 1H,), 7.21 (m, 4H), 7.60 (br s, 1H), 8.12 (brs, 1H).

***rel*-(1*R*,5*S*,6*R*)-6-Methyl-1,2,5,6-tetrahydro-4*H*-1,5-methano-benzo[*e*][1,3]diazocin-3-ylidene-cyanamide (1ay).** Compound **1ay** was prepared from 2-(1-phenyl-ethyl)-succinic acid 1-ethyl ester, which was prepared from 2-(1-phenyl-ethylidene)-succinic acid 1-ethyl ester[18] by hydrogenation. ^{1}H NMR (DMSO-d_6) δ 1.17 (d, 3H, $J=5.4$ Hz), 1.83 (d(apparent)t, 1H, $J=9.9$, 2.4 Hz), 2.20 (d, 1H, $J=9.9$ Hz), 2.99 (q, 1H, $J=5.4$ Hz), 3.63 (m, 1H), 4.35 (m, 1H), 7.22 (m, 4H), 7.89 (br s, 1H), 8.15 (br s, 1H).

***rel*-(1*R*,5*R*,11*S*)-5,11-Dimethyl-1,2,5,6-tetrahydro-4*H*-1,5-methano-benzo[*e*][1,3]diazocin-3-ylidene-cyanamide (1az).** Compound **1az** was prepared from 2-benzyl-2,3-dimethyl-succinic acid which was obtained by hydrolysis of 2,3-dicyano-2,3-dimethyl-4-phenyl-butyric acid ethyl ester.[19] ^{1}H NMR (DMSO-d_6) δ 0.84 (d, 3H, $J=7.2$ Hz), 1.33 (s, 3H), 1.97 (q, 1H, $J=7.2$ Hz), 2.82 (s, 2H), 4.14 (m, 1H), 7.22 (m, 4H), 7.56 (br s, 1H), 8.16 (br s, 1H).

Compound **1az** was isolated as predominantly a single isomer. The stereochemistry at C-11 was assigned by similarity of the shift of the methyl group to that of compound **1au**.

Biological Assays

Corn planthopper test

Test unit. The test unit consists of a plastic cup containing 126±4 g of sterilized, non-fertilized sassafras (sandy loam) soil. One pre-germinated Pioneer variety 3394 corn seed was placed in a 1 inch depression in the soil and covered. The test unit was watered with 15 mL of distilled water and placed in a closed plexiglas box inside a greenhouse operating 24 °C and 36% relative humidity for 4 days at which time it was ready for test. A snug fitting test unit lid with a small opening at the top was placed on all test units prior to test.

Compound application. Test compounds were formulated at 250 ppm in 20% acetone/80% water containing 500 ppm Ortho X-77 surfactant. For example, 2.5 mg of compound **1** was formulated in 10 mL solution. Compounds were applied through the opening in the test unit lid with an atomizer sprayer fitted with a Model 17690-1/8JJAU nozzle and a spray set-up consisting of a J2850 Fluid Cap and J70 Air Cap (Spray Sytems, Inc.). The sprayer was operated at 12–13psi. For each compound, two test units were sprayed with a total of 2 mL each of test solution. After spraying, test units were placed in a ventilated enclosure for 10–15 min to dry. 50 and 10 ppm solutions of **1** were prepared by serial dilutions using 20% acetone: 80% water containing 500 ppm Ortho X-77 surfactant.

Insect infesting/evaluation. After drying, a thin layer of white quartz sand wea poured onto the soil of each test unit to aid in the evaluation of live and dead insects at the conclusion of the test. Each unit was infested with a minimum of 15 nymphs of the corn planthopper, *Peregrinus maidis*, which were approximately 21 days old. Infested test units were held in a growth chamber operating at 22 °C and 50% relative humidity with a 16:8 light/dark photoperiod. Insect mortality was evaluated at 6 days post-infestation. Moribund insects were counted as dead. A compound **1** was considered 'active' when the observed insect mortality was 80% or greater.

Green leafhopper soil/systemic test

Test unit. Rice seedlings were raised in a nursery box. Five rice seedlings (1.5 leaf stage, about 10 cm tall) were transplanted into a $\frac{1}{2}$ oz. Plastic cup containing Kumiai brown artificial soil.

Compound application. Seven mL of a 100 ppm solution of the test compound in 2.5:97.5 acetone/water (prepared by dissolving, for example, 5 mg compound **1** in 1.25 mL acetone and then diluting the resulting solution with 48.75 mL water) was drenched onto the soil of each test. There were four replications per test. After application, the test units were covered and held in a growth chamber at 27 °C and 65% relative humidity for 24 h. 10 and 1.0 ppm solutions of **1** were prepared via serial dilutions using 2.5:97.5 acetone/water.

Insect infesting/evaluation. After drying, the test units were placed in conical shaped test units and the surface of the soil was covered with 2–3 mm of quartz sand to aid in the evaluation of live and dead insects at the conclusion of the test. Approximately eight to ten green leafhopper nymphs (*Nephotettix cincticeps*, 3rd–4th instar) were infested into each test unit using an aspirator. The test units were then held in a growth chamber at 27 °C and 65% relative humidity. Insect mortality was evaluated at 6 days post-infestation. Insects that cannot walk are classified as dead. A test compound **1** was considered to be 'active' when the observed insect mortality was 80% or greater.

Brown planthopper soil/systemic test. Test was carried out in a manner identical to that described above for green leafhopper except using brown planthopper nymphs (*Nilaparvata lugens*).

AChE assays

Preparation of insect AChE extracts. Using a teflon/glass tissue homogenizer, Southern corn rootworm (SCRW, *Diabrotica undecimpunctata*) larvae were homogenized in ice-cold 0.1 mM sodium phosphate buffer (approx. 17 mL buffer/g SCRW). The homogenate was then centrifuged at 0 °C for 10 min at 5000 rpm. An aliquot of the supernatant was tested in the AChE assay (see below), omitting the inhibitor, and the V_{max} was determined. The remaining supernatant was diluted with sufficient buffer to give a mixture having a V_{max} value of approx. 150 mOD/min. The resulting partially-purified AChE extract was stored in 1.5 mL aliquots at −80 °C until ready for use. AChE extracts for cornplanthopper (CPH) and green leafhopper (GLH) were prepared similarly.

AChE inhibition assay. The procedure used was a variation of the colorimetric assay developed by Ellman et al.[20] The assay was run in 96-well plate format using a Molecular Devices Thermo-max plate reader. Assay components were as follows: 100 mM sodium phosphate, pH 7.4 was used as buffer, 75 mM acetylthiocholine iodine (ATC) in buffer was used as substrate, 10 mM 5,5-dithio-bis(2-nitrobenzoic acid + 17.8 mM sodium bicarbonate) (DTNB) in buffer was used as the colorimetric reagent, 2-methoxy ethanol was used as solvent.

The assay was run in a final volume of 225 μL. To 185 μL of assay buffer in microplate wells was added 5 μL of 5 mM paraoxon in 2-methoxy ethanol or 5 μL of test compound in 2-methoxy ethanol at concentrations ranging from 1 nM to 100 μM final concentration in the assay. The assay was initiated by addition of 20 μL of partially-purified insect AChE (see above) and the mixture was allowed to incubate for 30 min at room temperature. After the first 25 min of incubation, a 1:1 mixture of ATC/DTNB was prepared. At 30 min, 15 μL of this substrate plus colorimetric reagent mixture was added to all wells and the microplate put in the plate reader. The change in OD 405 nm was followed for 5 min (with a read interval of 9 s). V_{max} (maximal δOD/min) was determined using SoftMax Pro software (Molecular Devices). % Inhibition was then calculated versus solvent controls and IC_{50} values were calculated. Triplicate determinations were made for each concentration of test compound.

Acknowledgements

We gratefully acknowlege Professor Jan Svetlik of Comenius University, Czech. Republic, for supplying us with our initial sample of **1a**. We would also like to thank John Groce, Gina Blankenship and Jim Doughty for assistance in obtaining ^{1}H NMR and mass spectral analyses.

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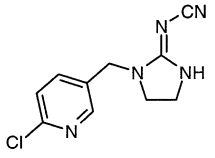

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