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Dihydropiperazine Neonicotinoid Compounds. Synthesis and Insecticidal Activity

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Syntheses of various isomeric dihydropiperazines can be approached successfully by taking advantage of the regioselective monothionation of their respective diones. Preparation of the precursor unsymmetrical *N*-substituted piperazinediones from readily available diamines is key to this selectivity. The dihydropiperazine ring system, as exemplified in 1-[(6-chloropyridin-3-yl)methyl]-4-methyl-3-oxopiperazin-2-ylidenecyanamide **(4)** and 1-[(2-chloro-1,3-thiazol-5-yl)methyl]-4-methyl-3-oxopiperazin-2-ylidenecyanamide (**25**), has been shown to be a suitable bioisosteric replacement for the imidazolidine ring system contained in neonicotinoid compounds. However, placement of the cyanoimino electron-withdrawing group further removed from the pyridine ring, as in 4-[(6-chloropyridin-3-yl)methyl]-3-oxopiperazin-2-ylidenecyanamide (**3a)**, or relocation of the carbonyl group, as in 1-[(6-chloropyridin-3-yl)methyl]-4-methyl-5-oxopiperazin-2-ylidenecyanamide (**5**), results in significantly decreased bioisosterism. The dihydropiperazine ring system of **4** and **25** also lends a degree of rigidity to the molecule that is not offered by the inactive acyclic counterpart 2-[(6-chloropyridin-3-yl)-methyl-(methyl)amino]-2-(cyanoimino)-*N*,*N*-dimethylacetamide (**6**). A pharmacophore model is proposed that qualitatively explains the results on the basis of good overlap of the key pharmacophore elements of **4** and imidacloprid (**1**); the less active regioisomers of **4** (**3a**, **5**, and **6**) feature a smaller degree of overlap.

KEYWORDS: Dihydropiperazine; neonicotinoid; synthesis; molecular modeling; pharmacophore; nicotinic acetylcholine receptor; bioisosterism; cotton aphid

INTRODUCTION

Since the advent of imidacloprid (**1**) 15 years ago (*1*−*3*), the search for new neonicotinoid insecticides has been an intense and competitive effort on the part of several research groups within the agrochemical industry (*4*−*10*). Neonicotinoid compounds, a term proposed by Yamamoto to describe this class of chemistry (*11*), are potent insecticides that primarily control sap-feeding pests and are relatively safe toward mammals, making this class of chemistry particularly attractive. This selectivity arises, in part, from a lower affinity for the mammalian nicotinic acetylcholine receptor (nAChR) than for the insect receptor (*12*, *13*). This particular mode of action is not well represented in the arsenal of insect control agents available to the grower and thus is quite suitable for use in integrated pest management practices (*14*).

A major emphasis in this search has involved the modification of the substructure containing the electron-withdrawing group C = X. In the case of **1** and **2** this substructure is the imidazolidine ring. A variety of replacements for this ring have been found to be suitable, including five- and six-membered saturated and unsaturated systems (*2*, *15*−*17*) and fused rings (*3*, *4*) as well as acyclic examples (*18*). We report herein that the dihydropiperazine ring is also bioisosteric (*19*) with these examples. Compounds **3a** and **4** feature dihydropiperazine rings in which a carbonyl group has been inserted into the imidazolidine ring of **2** at points a and b, respectively (**Figure 1**). These compounds, which have not been previously reported, were found to bind to the nAChR of housefly head membrane (*Musca domestica*) and afforded control of sap-feeding insects such as cotton aphid (CA, *Aphis gossypii*). This paper describes the syntheses and biological activities of a number of dihydropiperazine derivatives targeted to assess the potential of this ring system for use in neonicotinoid compounds.

EXPERIMENTAL PROCEDURES

Chemistry. Melting points are uncorrected. All reagents purchased were used without further purification. Solvents were dried using 3 Å molecular sieves. Chromatography was performed using 230−400 mesh ASTM silica gel 60 from EM Science, Darmstadt, Germany. Proton NMR spectra were obtained on a Varian Gemini 300 spectrometer using deuteriochloroform as solvent unless otherwise indicated and are reported in parts per million (δ) downfield from tetramethylsilane as internal reference. Infrared spectra were obtained on a Bio-Rad spectrophotometer using potassium bromide pellets or as neat oils and are reported as wavenumbers (cm^{-1}). Mass spectra were obtained on a

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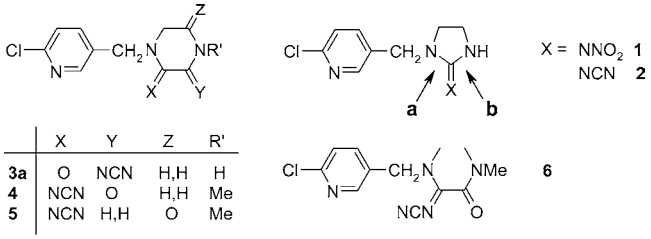


	X	Y	Z	R'
3a	O	NCN	H,H	H
4	NCN	O	H,H	Me
5	NCN	H,H	O	Me

Figure 1.

Hewlett-Packard model 5989A mass spectrometer using the electron impact-direct insertion probe (EI-DIP), chemical ionization (CI), fast atom bombardment (FAB), chemical desorption (CD), or electrospray (ES) technique and are reported as *m*/*z*. Microanalyses were performed by Midwest Microlab of Indianapolis, IN.

1-[(6-Chloropyridin-3-yl)methyl]piperazine-2,3-dione (8). A solution of 925 mg (4.98 mmol) of the diamine **7** and 730 mg (4.99 mmol) of diethyloxalate in 30 mL of ethanol was heated under reflux for 20 h, then cooled to room temperature, and concentrated to a residue, which was recrystallized from ethanol to afford 448 mg (38%) of **8**: mp 170.5−173.5 °C; ^{1}H NMR δ 8.33 (d, 1H, J = 2.4 Hz), 7.70 (dd, 1H, J = 8.3 Hz and J = 2.4 Hz), 7.51 (br s, 1H), 7.35 (d, 1H, J = 8.4 Hz), 4.69 (s, 2H), 3.52 (br s, 4H); IR (KBr) cm^{-1} 3538, 3205, 1696, 1671; MS (EI-DIP), *m*/*z* 241 ([M + 2]$^+$, 11), 239 (M$^+$, 29), 126 (100), 70 (83).

Anal. Calcd for $C_{10}H_{10}ClN_3O_2$: C, 50.11; H, 4.20; N, 17.53. Found: C, 50.22; H, 4.27; N, 17.35.

1-[(6-Chloropyridin-3-yl)methyl]-3-thioxopiperazin-2-one (9). A mixture of 2.65 g (11.1 mmol) of the dione **8** and 1.23 g (5.53 mmol) of phosphorus pentasulfide in 30 mL of dry pyridine was heated at 80 °C for 3 h, then cooled, and added to 350 mL of ice water. The solution was then extracted twice with dichloromethane, and the combined extracts were dried ($MgSO_4$). Concentration gave 2.47 g (87%) of **9** as a pale yellow powder: ^{1}H NMR δ 8.68 (br s, 1H), 8.35 (d, 1H, J = 2.8 Hz), 7.75 (dd, 1H, J = 8.0 Hz and J = 2.5 Hz), 7.35 (d, 1H, J = 8.4 Hz), 4.69 (s, 2H), 3.51−3.62 (m, 4H); IR (KBr) cm^{-1} 3145, 1670; MS (EI-DIP), *m*/*z* 257 ([M + 2]$^+$, 28), 255 (M$^+$, 68), 227 (17), 194 (42), 126 (100), 86 (64), 70 (61). A portion was recrystallized from ethanol, mp 161.5−162.5 °C, to give the following analytical data.

Anal. Calcd for $C_{10}H_{10}ClN_3OS$: C, 46.96; H, 3.94; N, 16.43; S, 12.54. Found: C, 47.01; H, 4.01; N, 16.33; S, 12.41.

1-[(6-Chloropyridin-3-yl)methyl]-3-(methylthio)-5,6-dihydropyrazin-2(1*H*)-one (10a). To a mixture of 100 mg (2.50 mmol) of 60% sodium hydride/mineral oil dispersion in 3 mL of dry dimethylformamide was added dropwise at 0 °C a solution of 570 mg (2.23 mmol) of the thione **9** in 6 mL of dimethylformamide. The mixture was allowed to warm to room temperature over 1 h, then cooled in an ice bath, and treated with 0.14 mL (319 mg, 2.25 mmol) of methyl iodide in one portion. The contents were stirred at room temperature for 2 h and were added to 90 mL of ice water. The aqueous mixture was extracted twice with dichloromethane, and the combined extracts were dried ($MgSO_4$). Concentration gave 680 mg of an oil, which was chromatographed on silica gel using gradient elution beginning with dichloromethane and proceeding to 4:1 dichloromethane/ethyl acetate, affording 369 mg (62%) of **10a** as a light yellow solid: mp 83−84 °C; ^{1}H NMR δ 8.33 (d, 1H, J = 2.6 Hz), 7.68 (dd, 1H, J = 8.2 Hz and J = 2.4 Hz), 7.33 (d, 1H, J = 8.4 Hz), 4.61 (s, 2H), 3.79 (t, 2H, J = 6.4 Hz), 3.38 (t, 2H, J = 6.4 Hz), 2.28 (s, 3H); MS (ESI), *m*/*z* 271 ([M + H + 2]$^+$, 37), 269 ([M + H]$^+$, 100).

Anal. Calcd for $C_{11}H_{12}ClN_3OS$: C, 48.97; H, 4.48; N, 15.58. Found: C, 48.88; H, 4.54; N, 15.35.

4-[(6-Chloropyridin-3-yl)methyl]-3-oxopiperazin-2-ylidenecyanamide (3a). A solution of 96.0 mg (0.356 mmol) of the thioimidate **10a** and 166 mg (3.95 mmol) of cyanamide in 2.5 mL of ethanol was heated at 50−60 °C for 3 h and then allowed to cool and stand overnight. The precipitate was collected and washed with cold ethanol to afford 75 mg (80%) of **3a** as white crystals: mp 185−187 °C; ^{1}H NMR (DMSO-d_6) δ 9.86 (br s, 1H), 8.41 (d, 1H, J = 2.4 Hz), 7.84 (dd, 1H, J = 8.3 Hz and J = 2.4 Hz), 7.52 (d, 1H, J = 8.3 Hz), 4.63 (s, 2H), 3.57 (m, 2H), 3.42 (m, 2H); IR (KBr) cm^{-1} 3215, 2182, 1676, 1645, 1621; MS (EI-DIP), *m*/*z* 265 ([M + 2]$^+$, 19), 263 (M$^+$, 55), 238 (35), 220 (35), 126 (100).

Anal. Calcd for $C_{11}H_{10}ClN_5O$: C, 50.10; H, 3.82; N, 26.56. Found: C, 50.16; H, 3.73; N, 26.38.

1-[(6-Chloropyridin-3-yl)methyl]piperazine-2,3-dione 3-*O*-Methyloxime (3b). To a mixture of 0.080 g (0.96 mmol) of methoxylamine hydrochloride in 1.0 mL of absolute ethanol was added in one portion a solution of 106 mg (0.393 mmol) of the thioimidate **10a** in 2 mL of absolute ethanol. The solution was stirred at room temperature for 4 h and then added to 25 mL of ice water. The pH was adjusted from 3 to 7 with solid sodium bicarbonate, and then the solution was extracted once with dichloromethane. The extract was dried ($MgSO_4$) and concentrated to give 85 mg of an oil, which was chromatographed on silica gel using the following gradient: dichloromethane and progressing to 95:5 dichloromethane/methanol. Combining like fractions afforded 85 mg (72%) of **3b** as a glass containing 11% w/w of dichloromethane, which could not be removed under high vacuum: ^{1}H NMR δ 8.31 (d, 1H, J = 2.2 Hz), 7.72 (dd, 1H, J = 8.0 Hz and J = 2.5 Hz), 7.32 (d, 1H, J = 8.1 Hz), 5.37 (br s, 1H), 4.68 (s, 2H), 3.97 (s, 3H), 3.45−3.36 (m, 4H); IR (neat) cm^{-1} 2942, 1680, 1616; MS (EI-DIP), *m*/*z* 270 ([M + 2]$^+$, 5), 268 (M$^+$, 13), 237 (30), 173 (100), 126 (100). Additional drying at high temperature and high vacuum resulted in the following analytical data.

Anal. Calcd for $C_{11}H_{13}ClN_4O_2$: C, 49.17; H, 4.87; N, 20.85. Found: C, 48.62; H, 4.85; N, 20.10.

1-[(6-Chloropyridin-3-yl)methyl]piperazine-2,3-dione 3-Oxime Hydrochloride (3c). To a mixture of 147 mg (2.11 mmol) of hydroxylamine hydrochloride in 2 mL of ethanol was added 142 mg (0.526 mmol) of the thioimidate **10a** followed by 2 mL of ethanol. Within minutes a solution existed, and within 1 h a precipitate was present. After stirring overnight at room temperature, the precipitate was collected and air-dried to afford 83.5 mg (54%) of **3c** as an off-white powder: mp 198−199 °C; ^{1}H NMR (DMSO-d_6) δ 11.38 (br s, 1H), 9.24 (br s, 1H), 8.42 (d, 1H, J = 2.5 Hz), 7.85 (dd, 1H, J = 8.0 Hz and J = 2.6 Hz), 7.54 (d, 1H, J = 8.0 Hz), 4.67 (s, 2H), 3.59 (m, 2H), 3.47 (m, 2H); IR (KBr) cm^{-1} 2949, 2762, 1667; MS (EI-DIP), *m*/*z* 256 ([M + 2 − HCl]$^+$, 1), 254 ([M − HCl]$^+$, 4), 238 (54), 155 (45), 126 (100).

Anal. Calcd for $C_{10}H_{12}Cl_2N_4O_2$: C, 41.20; H, 4.18; N, 19.08. Found: C, 41.19; H, 4.25; N, 19.10.

1-[(6-Chloropyridin-3-yl)methyl]piperazine-2,3-dione 3-Thiosemicarbazone (3d). A mixture of 504 mg (1.87 mmol) of the thioimidate **10a** and 170 mg (1.87 mmol) of thiosemicarbazide in 10 mL of dry ethanol was heated at 50−60 °C for 4 h. Upon cooling, the precipitate was collected to afford 513 mg (88%) of **3d** as a light yellow solid: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 9.83 (s, 1H), 8.37 (d, 1H, J = 2.4 Hz), 8.12 (br s, 1H), 7.85 (dd, 1H, J = 8.3 Hz and J = 2.4 Hz), 7.51 (d, 1H, J = 8.4 Hz), 7.48 (s, 1H), 7.31 (br s, 1H), 4.63 (s, 2H), 3.50 (m, 2H), 3.33 (m, 2H); IR (KBr) cm^{-1} 3416, 3274, 1614; MS (EI-DIP), *m*/*z* 240 ([M + 2 − NCSNH$_2$]$^+$, 23), 238 ([M − NCSNH$_2$]$^+$, 74), 155 (100).

Anal. Calcd for $C_{11}H_{13}ClN_6OS$: C, 42.24; H, 4.19; N, 26.87. Found: C, 42.27; H, 4.14; N, 26.60.

***N*-{4-[(6-Chloropyridin-3-yl)methyl]-3-oxopiperazin-2-ylidene}-1,1,1-trifluoromethanesulfonamide (3e).** A solution of 204 mg (0.756 mmol) of the thioimidate **10a** and 510 mg (3.42 mmol) of trifluoromethanesulfonamide in 2 mL of tetrahydrofuran was heated at 50−55 °C for 10 h and then allowed to cool. The solution was concentrated to a residue, which was chromatographed on silica gel and eluted with dichloromethane followed by dichloromethane/ethyl acetate mixtures to afford 50 mg (18%) of **3e**: mp 142−152 °C; ^{1}H NMR δ 9.98 (br s, 1H), 8.34 (d, 1H, J = 3.1 Hz), 7.74 (dd, 1H, J = 8.2 Hz and J = 2.6 Hz), 7.37 (d, 1H, J = 8.4 Hz), 4.69 (s, 2H), 3.63−3.70 (m, 4H); IR (neat) cm^{-1} 3288, 1688, 1635; MS (EI-DIP), *m*/*z* 372 ([M + 2]$^+$, 0.6), 370 (M$^+$, 2), 269 (18), 237 (100).

Anal. Calcd for $C_{11}H_{10}ClF_3N_4O_3$: C, 35.63; H, 2.72; N, 15.11. Found: C, 35.84; H, 2.77; N, 14.93.

1-[(6-Chloropyridin-3-yl)methyl]-3-ethoxy-5,6-dihydropyrazin-2(1*H*)-one (10b). A solution of 177 mg (0.656 mmol) of the thioimidate **10a** and 97 mg (0.65 mmol) of trifluoromethanesulfonamide in 20 mL of absolute ethanol was stirred at room temperature for 2 h and then

under reflux for 10 h. Upon cooling, the solution was concentrated in vacuo to give a residue, which was chromatographed on silica gel eluting with dichloromethane and then 9:1 dichloromethane/methanol to afford 135 mg (77%) of **10b**: mp 100−107 °C; ^{1}H NMR δ 8.33 (d, 1H, J = 2.1 Hz), 7.70 (dd, 1H, J = 8.3 Hz and J = 2.4 Hz), 7.34 (d, 1H, J = 8.4 Hz), 4.63 (s, 2H), 4.18 (q, 2H, J = 7.1 Hz), 3.63 (m, 2H), 3.39 (m, 2H), 1.38 (t, 3H, J = 7.1 Hz); MS (EI), m/z 269 ([M + 2]$^+$, 8), 267 (M$^+$, 26), 239 (17), 126 (100). GC-MS analysis revealed an impurity at greater retention time accounting for 6% of the total signal from the flame ionization detector and resulted in an erroneous elemental analysis. The impurity was not identified.

1-Methylpiperazine-2,3-dione (11). A solution of 1.46 g (10.0 mmol) of diethyloxalate and 0.741 g (10.0 mmol) of *N*-methylethylenediamine in 6 mL of absolute ethanol was heated under reflux for 20 h and then concentrated to a residue, which was recrystallized from 4 mL of ethanol to afford 0.686 g (54%) of **11**: mp 153.5−156.5 °C [lit. 158 °C (*i*-PrOH) (*20*)]; ^{1}H NMR δ 8.03 (br s, 1H), 3.47 (br s, 4H), 3.11 (s, 3H); IR (KBr) cm^{-1} 3231, 1697, 1669; MS (EI-DIP), m/z 128 (M$^+$, 28), 100 (100).

Anal. Calcd for $C_5H_8N_2O_2$: C, 46.87; H, 6.29; N, 21.87. Found: C, 46.81; H, 6.24; N, 21.94.

1-Methyl-3-thioxopiperazin-2-one (12). To a mixture of 4.90 g (11.8 mmol) of 97% Lawesson's reagent in 140 mL of dry toluene was added at 0 °C 3.01 g (23.5 mmol) of the dione **11**. The contents were mechanically stirred at 45−50 °C for 1.5 h and then at room temperature for 3 h. The mixture was filtered to afford 5.7 g of a solid, which was dissolved in 200 mL of dichloromethane. The solution was treated with silica gel, and the solvent was removed in vacuo to afford a dry flowable solid, which was placed at the top of a column of silica gel. Elution with dichloromethane followed by gradually increasing the polarity to 95:5 dichloromethane/methanol gave 2.8 g (83%) of **12** as a yellow solid containing a trace amount of Lawesson's reagent byproducts as evidenced by proton NMR. Recrystallization of a small amount (320 mg) from methanol afforded 202 mg of **12** as bright yellow crystals: mp 215−216 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.06 (br s, 1H), 3.55 (m, 2H), 3.52 (m, 2H), 2.95 (s, 3H); IR (KBr) cm^{-1} 3172, 1662, 1535; MS (EI-DIP), m/z 144 (M$^+$, 100), 69 (63).

Anal. Calcd for $C_5H_8N_2OS$: C, 41.64; H, 5.59; N, 19.43; S, 22.24. Found: C, 41.87; H, 5.72; N, 19.71; S, 21.87.

5,6-Dihydro-1-methyl-3-methylthiopiperazin-2-one (13). To a mixture of 1.96 g (13.6 mmol) of the thione **12** in 25 mL of dry acetonitrile was added at 0 °C 0.888 mL (2.02 g, 14.3 mmol) of methyl iodide followed by 1.88 g (13.6 mmol) of anhydrous potassium carbonate. The contents were allowed to warm to room temperature and stir overnight. Methyl iodide (0.089 mL) was again added, and after 6 h, the mixture was filtered and the filtrate concentrated to a residue, which was triturated under 50 mL of dichloromethane and filtered again. The filtrate was diluted with dichloromethane and dried over $MgSO_4$. Concentration gave 1.8 g of a yellow oil, which was chromatographed on silica gel eluting with dichloromethane/ethyl acetate mixtures to give 1.38 g (64%) of **13** as a solid: mp 38.5−40.5 °C; ^{1}H NMR δ 3.81 (t, 2H, J = 6.5 Hz), 3.42 (t, 2H, J = 6.5 Hz), 3.04 (s, 3H), 2.25 (s, 3H); IR (KBr) cm^{-1} 1666, 1597; MS (EI-DIP), m/z 158 (M$^+$, 90), 143 (65), 115 (30), 87 (50), 72 (100).

Anal. Calcd for $C_6H_{10}N_2OS$: C, 45.54; H, 6.37; N, 17.71. Found: C, 45.32; H, 6.49; N, 17.57.

5,6-Dihydro-1-methyl-3-cyanoiminopiperazin-2-one (14). A mixture of 1.27 g (8.03 mmol) of the thioimidate **13** and 1.32 g (31.4 mmol) of cyanamide in 10 mL of absolute ethanol was heated at 58−60 °C for 4 h and then allowed to cool. The precipitate was collected and recrystallized from methanol to afford 570 mg (47%) of **14** as white crystals: mp 202−4 °C (dec); ^{1}H NMR (DMSO-d_6) δ 9.79 (br s, 1H), 3.55 (m, 2H), 3.42 (m, 2H), 2.96 (s, 3H); IR (KBr) cm^{-1} 3198, 2194, 2163, 1624; MS (EI-DIP), m/z 152 (M$^+$, 100).

Anal. Calcd for $C_6H_8N_4O$: C, 47.36; H, 5.30; N, 36.82. Found: C, 47.11; H, 5.30; N, 36.42.

1-[(6-Chloropyridin-3-yl)methyl]-4-methyl-3-oxopiperazin-2-ylidenecyanamide (4) and (6-Chloropyridin-3-yl)methyl(4-methyl-3-oxo-3,4,5,6-tetrahydropyrazin-2-yl)cyanamide (15). To a mixture of 347 mg (8.67 mmol) of 60% sodium hydride/mineral oil dispersion in 9 mL of dry tetrahydrofuran was added in one portion 1.08 g (7.10 mmol) of the piperazinone **14**. The mixture was warmed to 41 °C, whereupon hydrogen gas evolution began to be observable. After heating at 41−48 °C until deprotonation was complete, the mixture was cooled to 20 °C and treated dropwise with 1.21 g (7.47 mmol) of 2-chloro-5-chloromethylpyridine in 3 mL of tetrahydrofuran. This was immediately followed by dropwise addition of 6 mL of dry dimethylformamide, and then the contents were heated at 45−50 °C for 7 h. Upon cooling, the mixture was concentrated in vacuo to remove the volatiles. The residue was diluted with heptane and concentrated again. This was repeated once more with heptane. The residue was then triturated under 100 mL of dichloromethane. The solid was filtered and the filtrate concentrated to give 2.63 g of a solid, which was recrystallized from 55 mL of methanol to afford 856 mg (43%) of **4** as white needles: mp 189−190.5 °C; ^{1}H NMR (DMSO-d_6) δ 8.41 (d, 1H, J = 2.4 Hz), 7.85 (dd, 1H, J = 2.6 Hz and J = 8.2 Hz), 7.53 (d, 1H, J = 8.2 Hz), 4.74 (s, 2H), 3.67 (m, 2H), 3.56 (m, 2H), 2.96 (s, 3H); IR (KBr) cm^{-1} 2185, 1680; MS (EI-DIP), m/z 277 (M$^+$, 14), 126 (100).

Anal. Calcd for $C_{12}H_{12}ClN_5O$: C, 51.89; H, 4.36; N, 25.22. Found: C, 52.01; H, 4.37; N, 25.17.

The mother liquor of **4** was concentrated to a residue, which was dissolved in dichloromethane, filtered, and chromatographed on silica gel using ethyl acetate as eluant to give 139 mg (7%) of **15** as a solid: mp 113−116 °C; ^{1}H NMR δ 8.45 (d, 1H, J = 2.4 Hz), 7.79 (dd, 1H, J = 2.4 Hz and J = 8.4 Hz), 7.35 (d, 1H, J = 8.7 Hz), 4.80 (s, 2H), 3.74 (m, 2H), 3.46 (m, 2H), 3.09 (s, 3H); IR (KBr) cm^{-1} 2229, 1679, 1634; MS (EI-DIP), m/z 279 ([M + 2]$^+$, 7), 277 (M$^+$, 21), 236 (27), 126 (100).

Anal. Calcd for $C_{12}H_{12}ClN_5O$: C, 51.89; H, 4.36; N, 25.22. Found: C, 52.01; H, 4.42; N, 24.97.

1-Methylpiperazin-2,5-dione (16). A solution of 9.74 g (66.6 mmol) of glycylsarcosine in 60 mL of ethylene glycol was heated at reflux for 40 min. The ethylene glycol was removed in vacuo to afford a solid that was recrystallized from 2-propanol to afford 7.02 g (82%) of **17**: mp 135.5−139 °C [lit. 141−143 °C (*i*-PrOH) (*21*)]; ^{1}H NMR δ 6.76 (br s, 1H), 4.03 (s, 2H), 3.98 (s, 2H), 3.00 (s, 3H); IR (KBr) cm^{-1} 3235, 1687, 1658; MS (EI-DIP), m/z 129 ([M + H]$^+$, 100).

Anal. Calcd for $C_5H_8N_2O_2$: C, 46.87; H, 6.29; N, 21.87. Found: C, 47.04; H, 6.29; N, 21.80.

1-Methyl-5-thioxopiperazin-2-one (17) and 1-Methylpiperazin-2,5-dithione (18). A mixture of 460 mg (3.58 mmol) of **16** and 748 mg (1.79 mmol) of 97% Lawesson's reagent in 22 mL of dry toluene was heated at 55−60 °C for 1 h, cooled to room temperature, and filtered to afford 434 mg of a white solid, which was an equimolar mixture of **17** and **18** as evidenced by proton NMR and GC-MS analysis. The mixture was dissolved in 250 mL of dichloromethane and treated with silica gel. After removal of the solvent, the dry powder was placed at the head of a column of silica gel and eluted with a gradient beginning with dichloromethane and proceeding to 95:5 dichloromethane/methanol. First to be eluted was 150 mg (26%) of the dithione **18**: mp 214−218 °C (dec); ^{1}H NMR (DMSO-d_6) δ 10.81 (br s, 1H), 4.56 (s, 2H), 4.31 (s, 2H), 3.33 (s, 3H); IR (KBr) cm^{-1} 1598, 1556; MS (EI-DIP), m/z 160 (M$^+$, 100), 58 (72).

Anal. Calcd for $C_5H_8N_2S_2$: C, 37.47; H, 5.03; N, 17.48; S, 40.02. Found: C, 37.52; H, 5.01; N, 17.38; S, 40.00.

The monothione **17** (160 mg) was eluted shortly thereafter. It was found to be contaminated with **18**. Recrystallization from methanol gave 70 mg (14%): mp 153.5−155.5 °C; ^{1}H NMR (DMSO-d_6) δ 10.61 (br s, 1H), 4.35 (s, 2H), 3.84 (s, 2H), 2.83 (s, 3H); IR (KBr) cm^{-1} 1685, 1650; MS (EI-DIP), m/z 144 (M$^+$, 100), 58 (31).

Anal. Calcd for $C_5H_8N_2OS$: C, 41.64; H, 5.59; N, 19.43; S, 22.24. Found: C, 41.35; H, 5.59; N, 19.07; S, 21.84.

3,6-Dihydro-1-methyl-5-methylthiopiperazin-2-one (19). A mixture of 1.9 g (10.2 mmol) of a 50 wt % mixture of the monothione/dithione (**17**/**18**), 1.82 g (13.2 mmol) of potassium carbonate, and 1.24 mL (2.83 g, 19.9 mmol) of methyl iodide in 25 mL of dry acetonitrile was stirred at room temperature overnight. The mixture was filtered and the filtrate concentrated to dryness. The residue was chromatographed on silica gel using dichloromethane as eluant and progressing to 94:6 dichloromethane/methanol. A yield of 1.5 g (65%) of **19** was obtained as a liquid: ^{1}H NMR δ 4.28 (t, 2H, J = 2.9 Hz), 4.02 (t, 2H,

J = 2.9 Hz), 2.99 (s, 3H), 2.36 (s, 3H); MS (EI-DIP), *m*/*z* 158 (M^+, 100), 143 (7), 125 (43), 72 (31), 57 (43).

3,6-Dihydro-1-methyl-5-cyanoiminopiperazin-2-one (20). A solution of 870 mg (5.5 mmol) of the thioimidate **19** and 870 mg (21 mmol) of cyanamide in 6 mL of ethanol was heated at 60−70 °C for 3 h. Removal of volatiles in vacuo at 40−45 °C gave a residue that was chromatographed on silica gel eluting with 95:5 dichloromethane/methanol to afford 640 mg of **20** contaminated with cyanamide. This material was recrystallized from methanol (9 mL) to give 434 mg (52%) of pure **20**: mp 188−192 °C (dec); ^{1}H NMR (DMSO-d_6) δ 9.62 (br s, 1H), 4.33 (br s, 2H), 3.84 (s, 2H), 2.87 (s, 3H); MS (EI-DIP), *m*/*z* 152 (M^+, 100).

Anal. Calcd for $C_6H_8N_4O$: C, 47.36; H, 5.30; N, 36.82. Found: C, 47.37; H, 5.31; N, 36.59.

1-(2-Chloropyridin-5-yl)methyl-3,6-dihydro-4-methyl-2-cyanoiminopiperazin-5-one (5). To a solution of 2 mL of dry tetrahydrofuran and 2 mL of dry dimethylformamide cooled in an ice bath was added in one portion 100 mg (2.50 mmol) of 60% sodium hydride/mineral oil dispersion. This was followed by portionwise addition of 383 mg (2.50 mmol) of the amidine **21**. The mixture was heated to 60 °C, then allowed to cool to room temperature, and treated dropwise via syringe with a solution of 405 mg (2.50 mmol) of 2-chloro-5-chloromethylpyridine in 2 mL of dry tetrahydrofuran. After stirring at room temperature for 1 h, the contents were heated at 40 °C for 8 h. The mixture was cooled to room temperature, diluted with heptane, and concentrated in vacuo to remove as much of the dimethylformamide as possible. The resulting oil was chromatographed on silica gel eluting with 96:4 dichloromethane/methanol to afford 590 mg (85%) of **5**: mp 123−125 °C; ^{1}H NMR δ 8.38 (d, 1H, J = 2.5 Hz), 7.71 (dd, 1H, J = 2.6 Hz and J = 8.1 Hz), 7.38 (d, 1H, J = 8.4 Hz), 4.72 (s, 2H), 4.55 (s, 2H), 4.00 (s, 2H), 3.05 (s, 3H); IR (KBr) cm^{-1} 2181, 1670; MS (EI-DIP), *m*/*z* 279 ($[M + 2]^+$, 8), 277 (M^+, 23), 126 (100).

Anal. Calcd for $C_{12}H_{12}ClN_5O$: C, 51.89; H, 4.36; N, 25.22. Found: C, 51.66; H, 4.35; N, 24.94.

***N*-Methyl-2-methylamino-2-thioxoacetamide (21).** To a mixture cooled in ice of 4.95 g (37.2 mmol) of ethyl thiooxamate in 5 mL of dry tetrahydrofuran was added over a 1−2 min period 41 mL (82 mmol) of a 2.0 M solution of methylamine in tetrahydrofuran. The contents were then heated at 40 °C for 1 h and then stirred at room temperature for 2 h. The solution was concentrated to dryness to afford 4.8 g (98%) of **21** as a solid. Recrystallization of 167 mg of **21** from methanol afforded 100 mg: mp 129−131 °C [lit. 122−4 °C (*22*)]; ^{1}H NMR δ 9.61 (br s, 1H), 8.21 (br s, 1H), 3.25 (d, 3H, J = 5.2 Hz), 2.94 (d, 3H, J = 4.8 Hz); IR (KBr) cm^{-1} 3303, 3247, 1678; MS (EI-DIP), *m*/*z* 133 ($[M + H]^+$,1), 65 (100).

Anal. Calcd for $C_4H_8N_2OS$: C, 36.34; H, 6.10; N, 21.20. Found: C, 36.53; H, 6.13; N, 21.03.

2-Methylimino-2-methylthio-*N*,*N*-dimethylacetamide (22). To a suspension of 2.4 g (0.060 mol) of 60% sodium hydride/mineral oil dispersion in 20 mL of dry tetrahydrofuran was added at 15−20 °C 3.56 g (0.0269 mol) of the thioacetamide **21** through a solid addition funnel over a 20 min period. The contents were allowed to stir at room temperature for 1 h, then cooled to 15 °C, and treated with 4.3 mL (0.069 mol) of methyl iodide in 4 mL of tetrahydrofuran dropwise via syringe over a 10−15 min period. The contents were stirred at room temperature for 3 h and then concentrated to a solid residue, which was triturated under 250 mL of ethyl ether. The mixture was filtered, and the filtrate was dried over sodium sulfate. Concentration gave 4.67 g of a liquid, which was chromatographed on silica gel using ethyl acetate as eluant to afford 2.66 g (62%) of **22** as a mixture of syn and anti isomers: ^{1}H NMR (data for predominant isomer given) δ 3.26 (s, 3H), 3.03 (s, 6H), 2.40 (s, 3H); IR (KBr) cm^{-1} 1660, 1624; MS (EI-DIP), *m*/*z* 160 (M^+, 6), 145 (10), 113 (42), 88 (90), 72 (100).

Anal. Calcd for $C_6H_{12}N_2OS$: C, 44.97; H, 7.55; N, 17.48. Found: C, 44.62; H, 7.46; N, 17.40.

2-Cyanoimino-2-methylamino-*N*,*N*-dimethylacetamide (23). A solution of 1.61 g (10.0 mmol) of **22** and 670 mg (16 mmol) of cyanamide in 4 mL of absolute ethanol was heated at 90 °C for 10 h. The ethanol was removed in vacuo, the residue was triturated under dichloromethane and filtered, and the filtrate was dried over sodium sulfate. Concentration gave 1.4 g of a residue, which was chromatographed on silica gel using 98:2 dichloromethane/methanol as eluant and proceeding to 93:7 over a 1 h period to afford 740 mg (48%) of **23**. A small amount was triturated under ethyl ether to give an analytical sample of a white solid: mp 87−88 °C; ^{1}H NMR δ 7.39 (br s, 1H), 3.12 (s, 3H), 3.04 (s, 3H), 2.99 (d, 3H, J = 4.5 Hz); IR (KBr) cm^{-1} 3313, 2195, 1667, 1614; MS (EI-DIP), *m*/*z* 155 ($[M + H]^+$, 5), 154 (M^+, 4), 126 (19), 72 (100).

Anal. Calcd for $C_6H_{10}N_4O$: C, 46.74; H, 6.54; N, 36.34. Found: C, 46.85; H, 6.47; N, 36.02.

2-[(6-Chloropyridin-3-yl)methylmethylamino-2-cyanoimino]-*N*,*N*-dimethylacetamide (6). To a suspension of 230 mg (5.76 mmol) of 60% sodium hydride/mineral oil in 3 mL of dry dimethylformamide cooled in an ice bath was added dropwise via syringe a solution of 740 mg (4.80 mmol) of the amidine **23** in 5 mL of dry tetrahydrofuran. After 15 min, a solution of 855 mg (5.28 mmol) of 2-chloro-5-chloromethylpyridine in 5 mL of tetrahydrofuran was added dropwise via syringe. This was followed by the addition of 3 mL of dimethylformamide. The contents were stirred overnight at room temperature and then concentrated in vacuo to remove most of the solvent. The residue was triturated under 100 mL of dichloromethane and then filtered, and the filtrate was dried over sodium sulfate. Concentration gave 1.75 g of an oil, which was chromatographed on silica gel eluting with 98:2 dichloromethane/methanol for 1 h and proceeding to 95:5 over a 0.5 h period to afford 990 mg (74%) of **7**: mp 141−144 °C; ^{1}H NMR (DMSO-d_6) δ 8.41 (d, 1H, J = 1.8 Hz), 7.82 (dd, 1H, J = 8.2 Hz and J = 2.6 Hz), 7.58 (d, 1H, J = 8.3 Hz), 4.73 (m, 2H), 3.05 (s, 3H), 2.97 (s, 3H), 2.94 (s, 3H); IR (KBr) cm^{-1} 2183, 1657; MS (EI-DIP), *m*/*z* 280 ($[M + H]^+$, 9), 279 (M^+, 6), 208 (38), 126 (48), 72 (100).

Anal. Calcd for $C_{12}H_{14}ClN_5O$: C, 51.52; H, 5.04; N, 25.04. Found: C, 51.37; H, 5.00; N, 24.84.

***N*-[(Tetrahydrofuran-3-yl)methyl]-5,6-dihydro-4-methyl-2-cyanoiminopiperazin-3-one (24).** To a suspension of 120 mg (3.0 mmol) of 60% sodium hydride/mineral oil dispersion in 3 mL of tetrahydrofuran was added in one portion 387 mg (2.54 mmol) of **14**. The mixture was heated at 40−45 °C for 1 h and was then cooled to room temperature. It was then treated dropwise via syringe with a solution of 458 mg (2.54 mmol) of tetrahydrofuran-3-ylmethyl sulfonate (*23*) in 3 mL of tetrahydrofuran followed by 3 mL of dimethylformamide. The contents were then heated to 90−100 °C while a stream of nitrogen removed most of the tetrahydrofuran. After 10h the mixture was allowed to cool and was concentrated to a residue which was triturated under dichloromethane to give 320 mg of a yellow solid. The filtrate was concentrated to a residue which was chromatographed on silica gel (230−400 mesh) using 95/5 dichloromethane/methanol as eluant to give 200 mg (33%) of **30**, mp 170−6 °C; ^{1}H NMR δ 3.45−3.96 (m, 10H), 3.13 (s, 3H), 2.74 (m, 1H), 2.07 (m, 1H), 1.66 (m, 1H); IR (KBr) *cm*$^{-1}$ 2166, 1676; MS (ES^+) *m*/*z* 237 ($[M+1]^+$, 11).

Anal. Calcd. for $C_{11}H_{16}N_4O_2$: C, 55.91; H, 6.83; N, 23.71. Found: C, 55.75; H, 6.77; N, 23.60.

***N*-(2-Chlorothiazol-5-yl)methyl-5,6-dihydro-4-methyl-2-cyanoiminopiperazin-3-one (25).** To a suspension of 145 mg (3.62 mmol) of 60% sodium hydride/mineral oil dispersion in 4 mL of dry tetrahydrofuran was added 460 mg (3.02 mmol) of **14** in one portion. The mixture was heated at 40 °C for 0.5 h, cooled in ice, and then treated with a solution of 592 mg (3.24 mmol) of 92% 2-chloro-5-chloromethylthiazole (*24*) in 3 mL of tetrahydrofuran followed by 3 mL of dry dimethylformamide. The contents were heated at 40−45 °C for 18 h, cooled to room temperature, then diluted with heptane, and concentrated to a solid residue, which was triturated under dichloromethane. The mixture was filtered, and the filtrate was dried over sodium sulfate. Concentration gave a residue that was chromatographed on silica gel (230−400 mesh) using dichloromethane as eluant and progressing in a gradient manner to 97:3 dichloromethane/methanol to give 400 mg (44%) of **31**: mp 201−204 °C; ^{1}H NMR δ 7.53 (s, 1H), 4.82 (s, 2H), 3.69 (m, 2H), 3.59 (m, 2H), 3.11 (s, 3H); IR (KBr) cm^{-1} 2176, 1679; MS (EI-DIP), *m*/*z* 285 ($[M + 2]^+$, 11), 283 (M^+, 27), 248 (24), 190 (29), 132 (100).

Anal. Calcd for $C_{10}H_{10}ClN_5OS$: C, 42.33; H, 3.55; N, 24.68. Found: C, 42.25; H, 3.39; N, 24.36.

Binding to Nicotinic Acetylcholine Receptor. Adult houseflies (*Musca domestica*) were first frozen with liquid nitrogen and then shaken to separate the heads from the bodies. Heads were recovered by sieving. Housefly head homogenate was prepared by first placing the heads in ice-cold (4 °C) buffer containing 0.2 M sucrose, 1 mM ethylenediaminetetraacetic acid, 10 mM Trizma-HCl, and 0.1 mM phenylmethanesulfonyl fluoride and then homogenizing in a blender. The resulting homogenate was then filtered through cheesecloth, and the effluent was centrifuged at 1000*g* (4 °C) for 15 min. The supernatant was then centrifuged again at 35000*g* (4 °C) for 20 min. The supernatant from the first high-speed spin was discarded, and the remaining pellet was resuspended in the aforementioned buffer and was then centrifuged a second time at 35000*g* for 20 min. The supernatant was again discarded, and the remaining pellet was resuspended in 50 mM Tris-HCl at pH 7.4. Aliquots of head membrane homogenate were stored at −80 °C.

When needed, aliquots of protein were thawed and resuspended in binding buffer containing 120 mM NaCl and 50 mM Trizma-HCl, pH 7.4. Experiments were performed in 96-well polypropylene microtiter plates (Beckman Coulter, Fullerton, CA). [^{3}H]Imidacloprid was synthesized by Amersham Pharmacia Biotech (Piscataway, NJ; 30.1 mCi/mmol) and was stored at −80 °C in ethanol. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

For all experiments, [^{3}H]imidacloprid was diluted to a final test concentration of 2 nM. Test compounds were first dissolved at high concentrations in DMSO and then diluted to the appropriate test concentrations. The highest concentration of DMSO, 0.25%, had no demonstrable effect on the binding of [^{3}H]imidacloprid. Tissue, test compounds, and radioactivity were combined and incubated at room temperature (22 °C) for 1 h. The assay was terminated by filtration through a GF/C glass fiber filter, rinsing with ice-cold binding buffer, and employing a TomTec automated harvester (TomTec, Hamden, CT). Bound radioactivity was counted on a Wallac 1205 Betaplate liquid scintillation counter (Wallac, Inc., Gaithersburg, MD). Nonspecific binding was defined by binding in the presence of 10^{-4} M nicotine. Each compound was run three times in triplicate, except where noted.

Biology. *Cotton Aphid (CA; Aphis gossypii).* Summer crookneck squash seedlings (*Cucurbita pepo*) at the expanded cotyledon stage were pruned to a single cotyledon. All stages of cotton aphid were transferred to the seedlings by placing infested leaf sections on seedlings 16−24 h prior to the application of the test material. As the infested sections dry out, the aphids move to the succulent plant material. The dried out leaf sections were removed, and plants were examined for good infestation prior to application of experimental compounds

The experimental compounds were dissolved at 1 mg/mL in 90:10 acetone/alcohol and then diluted in water containing 0.05% Tween 20. Serial dilutions were made to yield solutions of 50, 5.0, and 0.50 ppm.

Application was made with a hand-held Devilbiss, air-brush sprayer. The squash cotyledons were sprayed on both the upper and lower sides of the cotyledon until runoff, and then all plants within the treatment were sprayed evenly until the remaining spray solution was completely used. Each rate had four replications (plants). Controls consisted of eight plants treated with diluent prepared with a blank stock solution only.

Tests were held in a holding room for 72 h at approximately 74 °F, 40% relative humidity, and 24 h photoperiod prior to grading. Tests were graded by assessing the live aphid count (all nonwinged stages) on the underside of each cotyledon using a dissecting binocular microscope. Live count results were used to calculate percent control using Abbott's correction formula for solvent blank controls.

Molecular Modeling. Overlays of molecular models were constructed in Sybyl 6.6 (Tripos, St. Louis, MO) using the active analogue method (*25*, *26*). The molecules were minimized using the Tripos force field (*27*) and Gästeiger−Hückel charges (*28*, *29*). The minimization parameters used were as follows: method = powell, termination criteria = energy, minimum energy change = 0.00001, maximum iterations = 1000000, dielectric function = distance, dielectric constant = 2.0. Default values were used for all other variable parameters. FlexS 1.5 (*34*) was used for rigid overlays of minimized structures.

Table 1. Binding Affinity to the nAChR of Housefly Head Membrane and Activity on Cotton Aphid

compound	K_i (nM) ± SEM[a]	50 ppm	5 ppm	0.5 ppm
2	17[b]	+++	+++	nt
3a (R = CN)	441 ± 106	+++	+	−
3b (R = OMe)	1530 ± 159	+++	++	+
3c (R = OH·HCl)	3340 ± 886	++	+	−
3d (R = $NHCSNH_2$)	3919 ± 754	−	−	nt
3e (R = SO_2CF_3)	593 ± 243	+++	+++	−
4	6 ± 3.3	+++	nt	+++[c]
5	>10000	++	−	nt
6	3833 ± 1623	+	−	nt
8	400 ± 101	+++	+++	+
9	1777 ± 226	+++	+	+
15	>10000	−	−	nt
24	517 ± 223	−	−	nt
25	4 ± 1.4	+++	nt	++[c]

+++ = 90–100% mortality; ++ = 70–89% mortality; + = 50–69% mortality; − = <50% mortality. [a] Standard error of measurement with $n \geq 3$, [b] $n = 1$, [c] at 0.78 ppm; nt = not tested.

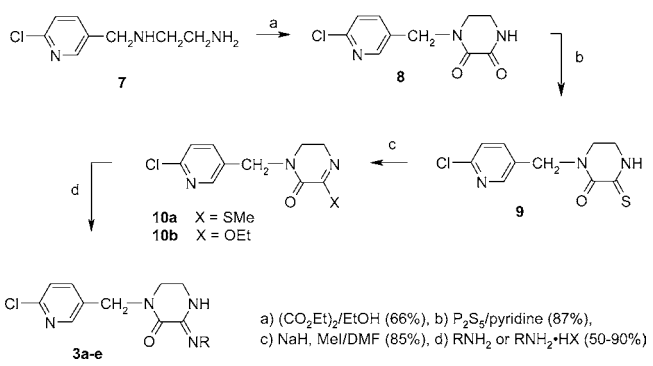
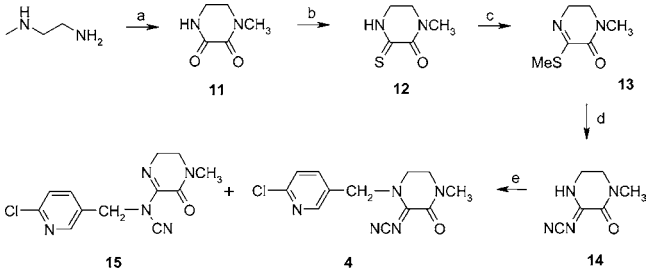


Figure 2.

RESULTS AND DISCUSSION

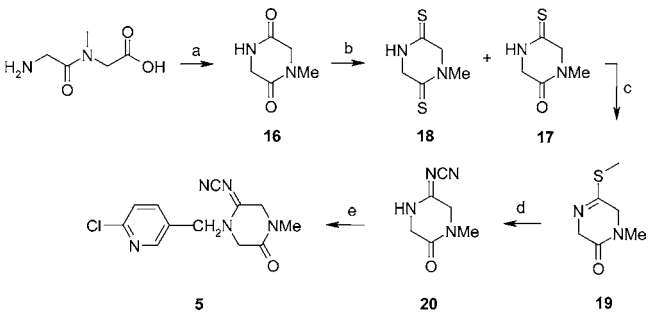
Chemistry. The key methodology used in the syntheses of compounds **3**−**6** involved discrimination between carbonyl groups by selectively converting one of them to a thiocarbonyl. In the route used to synthesize **3a**−**e** (**Table 1**), the carbonyl groups of the dihydro-2,3-piperazinedione system were differentiated by preparing the *N*-alkylated derivative **8** from the diamine **7** (*30*) and diethyloxalate (**Figure 2**). The C-3 carbonyl was then selectively converted to the thiocarbonyl using phosphorus pentasulfide to give **9**. The thioimidate **10a** was obtained under strongly basic conditions using sodium hydride and was found to be stable toward silica gel chromatography, although the crude thioimidate was used with success in the final reaction, preparation of the amidines **3**. Amidines **3a**−**d** were prepared in ethanol; however, solvolysis of **10a** occurred in the presence of trifluoromethanesulfonamide to give the ethyl imidate **10b**. This is not a surprising result considering the weak nucleophilicity of trifluoromethanesulfonamide and its greater acidity compared to the other amines used. The desired amidine **3e** was obtained in low yield when the reaction was carried out in tetrahydrofuran.

The positions occupied by cyanoimino and carbonyl in **3a** are switched in the structure of **4**. *N*-Methyldihydropiperazine-2,3-dione (**11**) was prepared by the condensation of *N*-methylethylenediamine and diethyloxalate (**Figure 3**). Conversion to the 3-thione **12** was found to be more convenient using Lawesson's reagent, and then sulfur was methylated under mildly basic conditions. Alkylation of **14** with 5-chloromethyl-2-chloropyridine (*31*) proceeded to give both regioisomers **4** and **15** in a 10:1 ratio with the desired isomer **4** predominating.



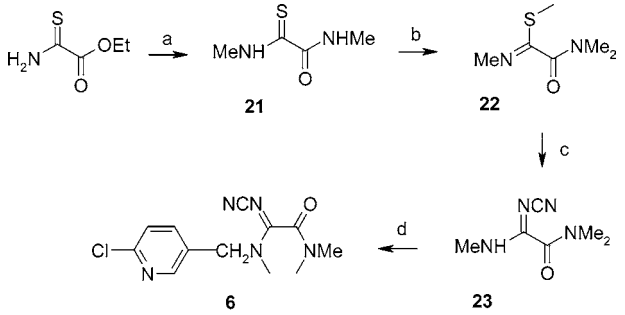
a) $(EtO_2C)_2$/EtOH (53%), b) Lawesson's/PhMe (88%), c) MeI, K_2CO_3/MeCN (64%), d) NH_2CN/EtOH (47%), e) 5-Chloromethyl-2-chloropyridine, NaH/THF/DMF (51%),

Figure 3.



a) Δ /$HOCH_2CH_2OH$ (82%), b) Lawesson's/PhMe, c) MeI, K_2CO_3/MeCN, d) NH_2CN/EtOH (52%), e) NaH, 5-chloromethyl-2-chloropyridine/THF, DMF (85%)

Figure 4.



a) $MeNH_2$/THF (98%), b) NaH, MeI/THF (62%), c) NH_2CN/EtOH d) NaH, 5-chloromethyl-2-chloropyridine/THF, DMF (61%)

Figure 5.

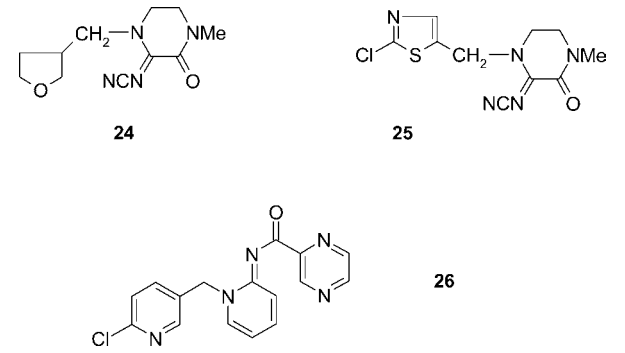


Figure 6.

The amidine carbons in **3a** and **4** (C-3 and C-2, respectively) are both flanked by carbonyl groups, which may impart undesired reactivity to the amidine moiety such as susceptibility to hydrolysis. The dihydropiperazine **5** was targeted with this in mind as it features the carbonyl group now at C-5 of the ring. Its synthesis, outlined in **Figure 4**, begins with the cyclodehydration of glycylsarcosine under refluxing ethylene glycol to give the required dihydropiperazinedione **16** in good yield (*21*, *32*). Treatment with half of an equivalent of Lawesson's reagent resulted in unavoidable formation of the dithione **18** along with an equal amount of the desired **17**. Careful monitoring of this reaction, which was carried out in toluene, revealed that no reaction occurred at 40 °C, but at 50 °C both products were observed to have been formed. Chromatographic separation of **17** and **18** was problematic, so it was decided to treat the mixture to the methylation conditions. This afforded the desired *S*-methylated product **19**, which was easily purified by silica gel chromatography. Conversion to the amidine **20** with cyanamide was then followed by alkylation, which gave **5** as the only detectable isomer. Its structure was established through selective INEPT carbon-13 NMR experiments.

Because it is known that acyclic analogues of **1** and **2** retain efficacy associated with the imidazolidines, it seemed prudent to prepare the acyclic example **6** to determine the effect on biological activity of unhindered rotation about the C2−C3 bond. **Figure 5** outlines the synthesis of **6** starting from commercially available ethyl thiooxamate. When treated with aqueous methylamine at room temperature, the thiooxamate yielded two products in addition to the desired **21** identified by GC-MS as *N*,*N*′-dimethyloxamide and the *N*-methylamidine of the starting thiooxamate. It was found that these byproducts could be completely suppressed by using methylamine in tetrahydrofuran under anhydrous conditions. Compound **21** was then *N*,*S*-dimethylated to give the thioimidate **22**, which was considerably less reactive toward cyanamide than the thioimidates **10a**, **13**, and **19**. Alkylation of **23** under the standard conditions gave **6**, the proton NMR spectrum of which in deuteriochloroform exhibits restricted rotation within the molecule.

In addition to the 2-chloropyridinyl moiety of **4**, 3-tetrahydrofuranylmethyl and a thiazolylmethyl were also introduced at N1 of the dihydropiperazine **14** to give **24** and **25** (**Figure 6**) using the same methodology as described above for the synthesis of **4**.

Biology. Compounds were first evaluated for their ability to bind to the nAChR of housefly head membrane (*M. domestica*) followed by an in vivo assay in which percent mortality of cotton aphid (CA; *A. gossypii*) on squash was observed at 50, 5, and 0.5 parts per million (ppm). The results of these tests are compiled in **Table 1**. Compounds **4** and **25** exhibit the highest affinity for the nAChR, that is, the lowest K_i values, and were found to be the most active in vivo on CA. Compound **4** demonstrated broad-spectrum activity affording control of sweet potato whitefly (WF), brown planthopper (BPH), and green leafhopper (GLH) (data not shown). Compounds with higher K_i values were invariably less active on CA than **4** and **25**; however, the K_i value was not always a good predictor of in vivo activity within this set. For example, **24** performs more poorly in vivo at 50 ppm than anticipated on the basis of its in vitro result similar to those for **3a**, **3e**, and **8**, which gave excellent control of CA. Neither metabolism nor distribution studies were conducted to shed light on anomalies such as this, although there was no evidence to suggest that any compound within **Table 1** was chemically unstable under the in vitro experimental conditions.

Compounds **3a** and **5**, piperazinone regioisomers of **4**, are seen to possess 2−4 orders of magnitude less affinity, respectively, for the receptor, and this is reflected in lower efficacy on CA. Compound **6**, which mimics the piperazinone substitution pattern of **4** but is acyclic, was found to perform poorly in both in vitro and in vivo assays.

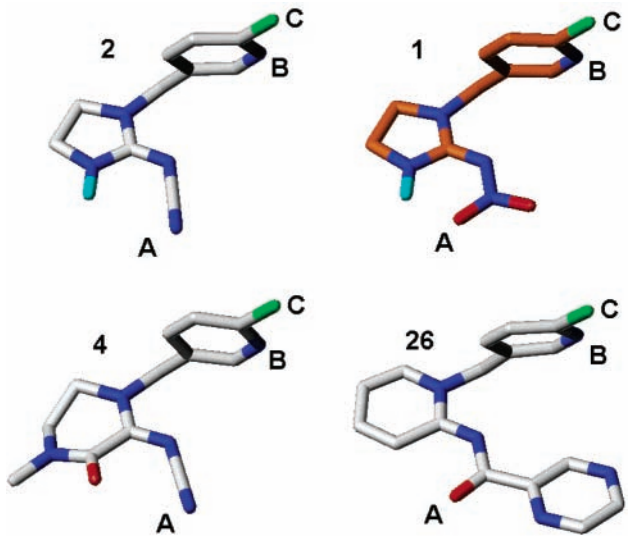


Figure 7. Four compounds used to define the pharmacophore model (**1**, **2**, **4**, and **26**).

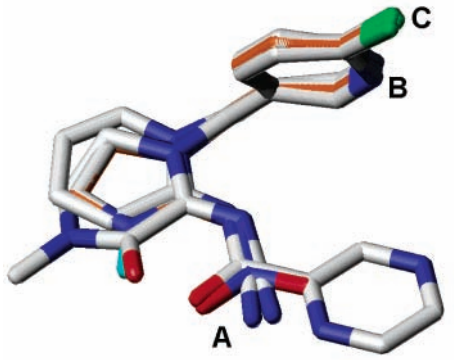


Figure 8. Overlay of **1** (orange), **2**, **4**, and **26**.

Molecular Modeling. In an effort to understand the results of the in vitro and in vivo testing in a qualitative manner, compound **4** and the reference compounds **1**, **2**, and **26** (*10*, **Figure 6**), all potent binders in the imidacloprid housefly head binding assay (**1**, ~0.1 nM; **2**, 17 nM; **4**, 6 nM; and **26**, 0.4 nM), were first modeled and their minimized conformations overlaid to define our pharmacophore model. The procedure involved building **2** in a conformation similar to its reported crystal structure (*33*), minimizing it, and then doing a rigid overlay of **1** and **4** onto **2** using FlexS 1.5 (*34*). The minimized structure of **26** was also overlaid on **1** using FlexS. **Figure 7** shows these minimized structures of the four compounds. All possess two hydrogen bond acceptors (labeled A and B) and a chlorine atom on the pyridine ring (labeled C), which we consider key pharmacophore elements, and overlap well as illustrated in **Figure 8**. This pharmacophore model differs from the previous one published by Okazawa and co-workers (*35*, *36*). In their model, the chloropyridinyl ring is in an extended conformation pointing away from the nitroimino or cyanoimino hydrogen bond accepting groups. In our model, the chloropyridinyl ring is in a folded conformation pointing toward the nitroimino or cyanoimino hydrogen bond accepting groups. Compound **26** fits well in the folded shape but cannot adopt the extended conformation needed to fit the Okazawa model. Therefore, we feel the folded shape explains our current binding data better than the extended model. The two active analogues, **2** and **4** (see **Figure 9**, **4** in orange), show a high degree of overlap of their NCN and chloropyridinyl groups and a small

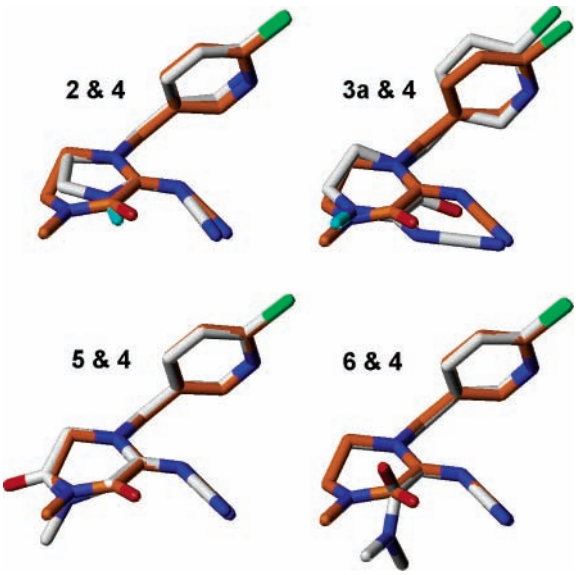


Figure 9. Overlays of **2**, **3a**, **5**, and **6** with **4** (orange).

degree of variation in the overlap of their five- and six-membered rings, respectively. Both of these molecules demonstrate tight binding to the housefly head nicotinic receptor as well as high efficacy on CA. Note that the NH of **2** is equivalent in position to the carbonyl group of **4** rather than the *N*-methyl group. This suggests that the NH/carbonyl position does not provide a significant degree of binding to the receptor because opposite charges are represented by these two functionalities. Compounds **3a**, **5**, and **6**, which exhibit decreased binding to the receptor with consequential loss of mortality observed against CA, each possess features or changes in geometries that reduce overlap of the pharmacophore elements with those of **4** (**Figure 9**). Only the terminal nitrogen atom of the cyanoimino group of **3a** overlaps with the cyanoimino group of **4**, and overlap of their chloropyridinyl groups has also been reduced. Although the chloropyridinyl and cyanoimino moieties of **5** and **4** superimpose well, the carbonyl oxygen of **5** occupies a region of space not encroached upon by **4** and may well be responsible for the observed lack of activity. The acyclic analogue **6** features a carbamoyl group which cannot adopt coplanarity with its cyanoimino group, an arrangement that would require 20 kcal/mol. This results in very poor overlap of the oxygen atoms of the carbamoyl groups of **4** and **6** and places the *N*,*N*-dimethylamino group of the carbamoyl functionality of **6** into a region of molecular space not impinged upon by **4** or the three reference compounds.

Conclusions. Syntheses of various isomeric dihydropiperazines can be approached successfully by taking advantage of the regioselective monothionation of their respective diones. Preparation of the precursor unsymmetrical *N*-substituted piperazinediones from readily available diamines is key to this selectivity.

The dihydropiperazine ring system, as exemplified in **4** and **25**, has been shown to be a suitable bioisosteric replacement for the imidazolidine ring system contained in neonicotinoid compounds such as **2**. However, placement of the cyanoimino electron-withdrawing group further removed from the pyridine ring, as in **3a**, or relocation of the carbonyl group, as in **5**, results in significantly deceased bioisosterism. The dihydropiperazine ring system of **4** and **25** also lends a degree of rigidity to the molecule that is not offered by the inactive acyclic counterpart **6**. The pharmacophore model proposed here qualitatively

explains our results and is different from the previously published model.

ABBREVIATIONS USED

1, 2-chloro-5-({2-[oxido(oxo)hydrazono]imidazolidin-1-yl}-methyl)pyridine; **2**, 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-ylidenecyanamide; **26**, *N*-[(2*E*)-1-[(6-chloro-3-pyridinyl)-methyl]-2(1*H*)-pyridinylidene]-2-pyrazinecarboxamide.

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