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ARTICLE

Design and Synthesis of Novel Insecticides Based on the Serotonergic Ligand 1-[(4-Aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine (PAPP)†

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1-[(4-Aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine (PAPP) is a 5-HT_{1A} agonist and was reported to display high affinity for serotonin (5-HT) receptor from the parasitic nematode *Haemonchus contortus*. The present investigation explored the possibility of using PAPP as a lead compound of new insecticides with novel mode of action. On the basis of the PAPP scaffold, a series of 1-arylmethyl-4-[(trifluoromethyl)pyridin-2-yl]piperazine derivatives were designed, synthesized, and evaluated for biological activities against the armyworm *Pseudaletia separata* (Walker). Bioassays showed that most of the target compounds displayed certain growth-inhibiting activities or larvicidal activities against armyworm. The quantitative structure−activity relationship (QSAR) for growth-inhibiting activities was also analyzed and established.

KEYWORDS: PAPP; 1-arylmethyl-4-[(trifluoromethyl)pyridin-2-yl]piperazine; larvicidal activity; growth inhibiting activity; QSAR

INTRODUCTION

As one of the main pest management tools, insecticides play a very important role in modern agriculture. However, the frequent and wide application of traditional insecticides has brought about an inevitable problem, namely, the occurrence and development of resistance of pest species. Therefore, it is critically necessary to discover new insecticidal candidates with novel modes of action.

In invertebrate species, serotonin [5-hydroxytrytryptamine (5-HT)] as a significant monoamine neurotransmitter is widely distributed throughout the central and peripheral nervous systems and involved in a number of physiological processes such as feeding, procreation, and development (*1*−*7*). Some 5-HT receptors (5-HTRs) have already been cloned from *Drosophila*, molluscs, and nematodes. It is interesting that most of them show relatively low affinities with serotonin with the exception of the 5-HT_{1Hc} receptor from the parasitic nematode *Haemonchus contortus*. The 5-HT_{1Hc} receptor displayed nanomolar binding affinity for serotonin and serotonergic ligands. Such a unique pharmacological profile was distinct from the other known invertebrate and mammalian 5-HT receptors (*8*, *9*).

It has been found that many N^1-substituted N^4-arylpiperazine derivatives, for example, PAPP, BMY7378, and NAN-190 (**Table 1**), showed high affinities and good selectivity for 5-HTRs with the incorporation of the appropriate N^1- and N^4-substituent groups (*9*−*12*). Among those notable serotonergic ligands, PAPP, a N^1-arylated alkyl N^4-arylpiperazine derivative, exhibited the highest affinity with 5-HT_{1Hc} receptor as shown in **Table 1** (*9*) according to Smith et al.'s testing results. Thus, it might be reasonable to regard PAPP as a potential lead compound for discovering insecticidal candidates with a novel mode of action.

To validate the hypothesis, the structural motif of PAPP was shared involving the introduction of appropriate N^1- and N^4-substituent groups for agrochemicals by the following two ways. First, owing to the successful application in neonicotinoids, arylmethyl groups might be adopted as profitable N^1-substituent groups. Second, due to the unique bioactivities in the agrochemicals (i.e., herbicides fluazifog-P-butyl and haloxyfog and insecticides fluazuron and chlorfluazuron), the trifluoromethylpyridine ring might be a good candidate as the N^4-substituent group. Therefore, a series of 1-arylmethyl-4-[(trifluoromethyl)pyridin-2-yl]piperazine derivatives (**Scheme 1**) were designed, synthesized (**Scheme 2**), and bioassayed. It was found that most of the target compounds exhibited certain growth-inhibiting activities or larvicidal activities against the armyworm *Pseudaletia separata* (Walker). This result verified that PAPP could become a competent lead compound for the development of new insecticides.

MATERIALS AND METHODS

Synthesis. All chemicals or reagents were purchased from standard commercial suppliers. Analytical thin-layer chromatography (TLC) was carried out on precoated silica gel (SiO_2) plates (60 F_{254}). Melting points were determined on a Büchi B540 (Büchi Labortechnik AG, Flawil, Switzerland) and are uncorrected. The infrared spectra were recorded on a Nicolet 470 IR Fourier transform spectrometer in KBr pellets. ^{1}H NMR spectra were recorded on a Bruker WP-500SY (500 MHz) spectrometer in $CDCl_3$ with TMS as an internal standard. High-resolution mass spectra were obtained on a MicroMass GCT CA 055 spectrometer.

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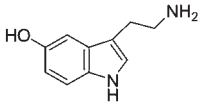
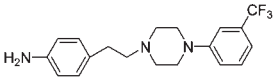
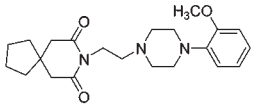
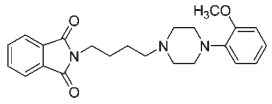
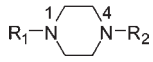
Table 1. Affinities of N^1-Substituted N^4-Arylpiperazine Derivatives Binding to the Cloned 5-HT_{1Hc} Receptor of the Parasitic Nematode *Haemonchus contortus* Reported by Smith et al. (*9*)

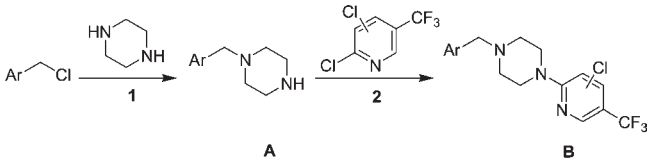
Compounds	Chemical Structures	Ki [a] (nM)
Serotonin		0.8
PAPP		0.4
BMY7378		3.6
NAN-190		7.7

[a] K_i values (nM) for [^{3}H]-serotonin binding to Sf9 cell membranes expressing the Parasitic nematode *Haemonchus contortus* 5-HT_{1Hc} receptor.

Scheme 1. Structure of Target Compounds



Scheme 2. Synthesis of Target Compounds **B**



1) TEA , EtOH, 55 °C; 2) K_2CO_3, CH_3CN

General Synthetic Procedure for the Target Compounds. To a warm (oil bath temperature ca. 50 °C) solution of piperazine (4.0 mmol) and triethylamine (3.0 mmol) in EtOH (30 mL) was added dropwise aryl chloromethane (2.0 mmol) in ethanol (15 mL), and then the reaction mixture was allowed to stir at 55 °C for 12 h. After the reaction finished, the solvent was removed under vacuum. The oily residue was treated with water (15 mL) and extracted with CH_2Cl_2 (4 × 15 mL). The combined extracts were washed with water, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel with CH_2Cl_2/EtOH (10:1 v/v) to obtain 1-(arylmethyl)piperazine (**A**) in 80−86% yield.

Equimolar amounts (2.0 mmol) of 1-(arylmethyl)piperazine (**A**), anhydrous K_2CO_3, and 2,3-dichloro-5-(trifluoromethyl)pyridine (or 6-chloro-3-(trifluoromethyl)pyridine) in MeCN (20 mL) were stirred at 55 °C for 10 h. After cooling, the reaction mixture was treated with water (15 mL) and extracted with EtOAc (4 × 15 mL). The combined extracts were washed with water (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel with petroleum ether/EtOAc (4:1 v/v) to yield the desired product (**B**).

When 2,6-dichloro-3-(trifluoromethyl)pyridine was used, the reaction mixture was stirred at 45 °C for 10 h. The 6-substituted products as the major reaction products were formed along with minor 2-substituted impurities, which could be removed later by column chromatography.

Compounds $\mathbf{C_1}$ and $\mathbf{C_2}$ were synthesized in a similar way.

1-(4-Chlorobenzyl)-4-[5-(trifluoromethyl)pyridin-2-yl]piperazine (***B1***): yield, 75%; white solid; mp, 91−92 °C; IR (KBr) υ 2797, 1610, 1507, 1300, 1109, 815 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.52−2.54 (m, 4H, pip-H), 3.53 (s, 2H, CH_2), 3.64−3.66 (m, 4H, pip-H), 6.62 (d, J = 9.01 Hz, 1H, Py-H), 7.30−7.34 (m, 4H, Ar−H), 7.61−7.62 (m, 1H, Py-H), 8.39 (s, 1H, Py-H); EI-MS, m/z (%) 355 (6, M^+), 209 (4), 193 (16), 180 (34), 175 (37), 168 (8), 125 (100), 56 (10). HRMS (EI+) calcd for $C_{17}H_{17}ClF_3N_3$, 355.1063; found, 355.1064.

1-[(6-Chloropyridin-3-yl)methyl]-4-[5-(trifluoromethyl)pyridin-2-yl]piperazine (***B2***): yield, 73%; yellow solid; mp, 88−89 °C; IR (KBr) υ 2806, 1606, 1500, 1374, 1118, 817 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.53−2.55 (m, 4H, pip-H), 3.54 (s, 2H, CH_2), 3.65−3.66 (m, 4H, pip-H), 6.64 (d, J = 9.02 Hz, 1H, Py-H), 7.33−7.34 (m, 1H, Py-H), 7.62 (dd, J_1 = 2.25 Hz, J_2 = 2.24 Hz, 1H, Py-H), 7.70 (dd, J_1 = 2.25 Hz, J_2 = 2.29 Hz, 1H, Py-H), 8.34 (s, 1H, Py-H), 8.38 (s, 1H, Py-H); EI-MS, m/z (%) 356 (12, M^+), 337 (7), 230 (10), 194 (28), 175 (100), 147 (25), 126 (67), 56 (23). HRMS (EI+) calcd for $C_{16}H_{16}ClF_3N_4$, 356.1016; found, 356.1037.

1-[(2-Chlorothiazol-5-yl)methyl]-4-[5-(trifluoromethyl)pyridin-2-yl]piperazine (***B3***): yield, 72%; yellow solid; mp, 61−62 °C; IR (KBr) υ 2813, 1613, 1414, 1328, 1119, 811 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.59−2.61 (m, 4H, pip-H), 3.65−3.67 (m, 4H, pip-H), 371 (s, 2H, CH_2), 6.63 (d, J = 9.02 Hz, 1H, Py-H), 7.38 (s, 1H, thiazol-H), 7.63 (dd, J_1 = 2.24 Hz, J_2 = 2.25 Hz, 1H, Py-H), 8.39 (s, 1H, Py-H); EI-MS, m/z (%) 362 (12, M^+), 327 (14), 230 (7), 201 (8), 187 (21), 175 (100), 165 (14), 131 (31), 56 (10). HRMS calcd for $C_{14}H_{14}ClF_3N_4S$, 362.0580; found, 362.0583.

1-(2-Chlorobenzyl)-4-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B4***): yield, 72%; white solid; mp, 7071 °C; IR (KBr) υ 2823, 1606, 1329, 1117, 776 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.67−2.69 (m, 4H, pip-H), 3.55−3.57 (m, 4H, pip-H), 3.70 (s, 2H, CH_2), 7.23−7.25 (m, 2H, Ar−H), 7.37 (d, J = 7.65 Hz, 1H, Ar−H), 7.50−7.51 (m, 1H, Ar−H), 7.74 (d, J = 1.71 Hz, 1H, Py-H), 8.39 (s, 1H, Py-H); EI-MS, m/z (%) 389 (M^+), 221 (5), 193 (11), 180 (100), 168 (18), 125 (67), 56 (7). HRMS (EI+) calcd for $C_{17}H_{16}Cl_2F_3N_3$, 389.0673; found, 389.0682.

1-(4-Chlorobenzyl)-4-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B5***): yield, 72%; yellow oil; IR (KBr) υ 2823, 1606, 1325, 1126, 750 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.59−2.61 (m, 4H, pip-H), 3.54−3.56 (m, 6H, CH_2, pip-H), 7.29−7.32 (m, 4H, Ar−H), 7.74 (d, J = 1.50 Hz, 1H, Py-H), 8.38 (s, 1H, Py-H); EI-MS, m/z (%) 389 (6, M^+), 340 (4), 209 (11), 193 (14), 180 (56), 168 (10), 125 (100), 56 (13). HRMS (EI+) calcd for $C_{17}H_{16}Cl_2F_3N_3$, 389.0673; found, 389.0665.

1-(2,6-Difluorobenzyl)-4-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B6***): yield, 75%; yellow solid; mp, 43−45 °C; IR (KBr) υ 2844, 1611, 1314, 1134, 785 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.67−2.69 (m, 4H, pip-H), 3.53−3.55 (m, 4H, pip-H), 3.79 (s, 2H, CH_2), 6.91−6.93 (m, 2H, Ar−H), 7.26−7.27 (m, 1H, Ar−H),7.72 (d, J = 2.00 Hz, 1H, Py-H), 8.37 (s, 1H, Py-H); EI-MS, m/z (%) 391 (18, M^+), 372 (12), 221 (12), 195 (33), 182 (100), 170 (25), 127 (74). HRMS (EI+) calcd for $C_{17}H_{15}ClF_5N_3$, 391.0875; found, 391.0861.

1-(Pyridin-3-ylmethyl)-4-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B7***): yield, 69%; yellow oil; IR (KBr) υ 2792, 1597, 1324, 1123, 760 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.62−2.64 (m, 4H, pip-H), 3.54−3.56 (m, 4H, pip-H), 3.60 (s, 2H, CH_2), 7.28−7.29 (m, 1H, Py-H), 7.73−7.75 (m, 2H, Py-H), 8.39 (s, 1H, Py-H), 8.54 (d, J = 4.00 Hz, 1H, Py-H), 8.58 (s, 1H, Py-H), 8.54 (d, J = 4.00 Hz, 1H, Py-H), 8.58 (s, 1H, Py-H). EI-MS, m/z (%) 356 (4, M^+), 209 (10), 180 (7), 160 (15), 147 (100), 135 (10), 93 (42), 56 (11);. HRMS (EI+) calcd for $C_{16}H_{16}ClF_3N_4$, 356.1016; found, 356.1021.

1-(6-Chloropyridin-3-ylmethyl)-4-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B8***): yield, 76%; yellow oil; IR (KBr) υ 2803, 1597, 1449, 1310, 1128, 749 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.59−2.61 (m, 4H, pip-H), 3.52−3.54 (m, 4H, pip-H), 3.56 (s, 2H, CH_2), 7.32 (d, J = 8.10 Hz, 1H, Py-H), 7.70 (d, J = 7.17 Hz, 1H, Py-H), 7.75 (d, J = 1.84 Hz, 1H, Py-H), 8.35 (d, J = 1.95 Hz, 1H, Py-H), 8.39 (s, 1H, Py-H); EI-MS, m/z (%) 390 (7, M^+), 221 (13), 209 (58), 194 (47), 181 (100), 126 (89), 56 (13). HRMS (EI+) calcd for $C_{16}H_{15}Cl_2F_3N_4$, 390.0626; found, 390.0628.

1-[(2-Chlorothiazol-5-yl)methyl]-4-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B9***): yield, 72%; yellow oil; IR (KBr) υ 2823, 1597, 1316, 1126, 750 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.64−2.66 (m, 4H, pip-H), 3.52−3.54 (m, 4H, pip-H), 3.72 (s, 2H, CH_2), 7.38 (s, 1H, thiazol-H), 7.74−7.75 (m, 1H, Py-H), 8.39 (s, 1H, Py-H); EI-MS, m/z (%) 396 (7, M^+),

361 (24), 223 (29), 209 (76), 187 (53), 165 (33), 145 (16), 131 (100), 56 (21). HRMS (EI+) calcd for $C_{14}H_{13}Cl_2F_3N_4S$, 396.0190; found, 396.0200.

1-(4-Chlorobenzyl)-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{10}$**): yield, 46%; yellow oil; IR (KBr) υ 2808, 1601, 1329, 1129, 805 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.51−2.53 (m, 4H, pip-H), 3.54 (s, 2H, CH_2), 3.64−3.66 (m, 4H, pip-H), 6.47 (d, J = 8.80 Hz, 1H, Py-H), 7.29−7.32 (m, 4H, Ar−H), 7.67 (d, J = 8.80 Hz, 1H, Py-H); EI-MS, m/z (%) 389 (54, M^+), 370 (11), 264 (17), 223 (18), 209 (25), 180 (100), 168 (39), 125 (83). HRMS (EI+) calcd for $C_{17}H_{16}Cl_2F_3N_3$, 389.0673; found, 389.0673.

1-(2,3-Dichlorobenzyl)-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{11}$**): yield, 47%; yellow oil; IR (KBr) υ 2823, 1617, 1319, 1113, 769 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.61−2.63 (m, 4H, pip-H), 3.65−3.69 (m, 6H, CH_2, pip-H), 6.48 (d, J = 8.40 Hz, 1H, Py-H), 7.22 (m, 1H, Ar−H), 7.41−7.42 (m, 2H, Ar−H), 7.68 (d, J = 8.00 Hz, 1H, Py-H); EI-MS, m/z (%) 423 (22, M^+), 404 (21), 388 (19), 264 (14), 243 (29), 214 (100), 158 (79), 123 (8). HRMS (EI+) calcd for $C_{17}H_{15}Cl_3F_3N_3$. 423.0284; found. 423.0288.

1-[(6-Chloropyridin-3-yl)methyl]-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{12}$**): yield, 46%; yellow solid; mp, 98−99 °C; IR (KBr) υ 2810, 1611, 1315, 1123, 750 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.52−2.54 (m, 4H, pip-H), 3.54 (s, 2H, CH_2), 3.64−3.65 (m, 4H, pip-H), 6.50 (d, J = 8.79 Hz, 1H, Py-H), 7.33 (d, J = 8.08 Hz, 1H, Py-H), 7.66−7.68 (m, 2H, Py-H), 8.34 (d, J = 2.17 Hz, 1H, Py-H); EI-MS, m/z (%) 390 (14, M^+), 221 (10), 209 (41), 194 (11), 181 (100), 169 (12), 126 (72), 56 (10). HRMS (EI+) calcd for $C_{16}H_{15}Cl_2F_3N_4$, 390.0626; found, 390.0633.

1-[(2-Chlorothiazol-5-yl)methyl]-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{13}$**): yield, 46%; yellow oil; IR (KBr) υ 2806, 1593, 1316, 1126, 778 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.57−2.59 (m, 4H, pip-H), 3.63−3.65 (m, 4H, pip-H), 3.70 (s, 2H, CH_2), 6.47 (d, J = 8.66 Hz, 1H, Py-H), 7.38 (s, 1H, thiazol-H), 7.68 (d, J = 8.66 Hz, 1H, Py-H); EI-MS, m/z (%) 396 (12, M^+), 361 (37), 223 (40), 209 (82), 187 (61), 175 (24), 165 (11), 153 (15), 145 (15), 131 (100), 56 (37). HRMS (EI+) calcd for $C_{14}H_{13}Cl_2F_3N_4S$, 396.0190; found, 396.0196.

1-(2,6-Difluorobenzyl)-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{14}$**): yield, 44%; white solid; mp, 65.0−65.9 °C; IR (KBr) υ 2886, 1613, 1314, 1122, 791 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.62−2.64 (m, 4H, pip-H), 3.67−3.69 (m, 4H, pip-H), 3.83 (s, 2H, CH_2), 6.45 (d, J = 8.80 Hz, 1H, Py-H), 6.94−6.95 (m, 2H, Ar−H), 7.29 (m, 1H, Ar−H), 7.66 (d, J = 8.80 Hz, 1H, Py-H); EI-MS, m/z (%) 391 (19, M^+), 264 (13), 221 (10), 182 (100), 127 (76), 56 (2). HRMS (EI+) calcd for $C_{17}H_{15}ClF_5N_3$, 391.0875; found, 391.0870.

1-(4-Iodobenzyl)-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{15}$**): yield, 45%; white solid; mp, 78.8−79.7 °C; IR (KBr) υ 2813, 1593, 1321, 1129, 791 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.52−2.54 (m, 4H, pip-H), 3.52 (s, 2H, CH_2), 3.64−3.66 (m, 4H, pip-H), 6.47 (d, J = 8.80 Hz, 1H, Py-H), 7.11−7.13 (m, 2H, Ar−H), 7.69 (m, 3H, Ar−H, Py-H); EI-MS, m/z (%) 481 (23, M^+), 285 (11), 272 (87), 260 (31), 216 (100), 209 (8). HRMS (EI+) calcd for $C_{17}H_{16}ClF_3IN_3$, 481.0030; found, 481.0031.

1-(4-tert-Butylbenzyl)-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{16}$**): yield, 45%; white solid; mp, 111.5−112.1 °C; IR (KBr) υ 2806, 1600, 1321, 1113, 797 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.34 (s, 9H, $C(CH_3)_3$), 2.55−2.57 (m, 4H, pip-H), 3.57 (s, 2H, CH_2), 3.66−3.68 (m, 4H, pip-H), 6.46 (d, J = 9.20 Hz, 1H, Py-H), 7.28−7.29 (m, 2H, Ar−H), 7.37−7.38 (m, 2H, Ar−H), 7.66 (d, J = 9.20 Hz, 1H, Py-H); EI-MS, m/z (%) 411 (27, M^+), 264 (30), 202 (50), 190 (19), 147 (100), 117 (13), 56 (4). HRMS (EI+) calcd for $C_{21}H_{25}ClF_3N_3$, 411.1689; found, 411.1689.

1-(2-Chlorobenzyl)-4-[6-chloro-3-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{17}$**): yield, 24%; yellow oil; IR (KBr) υ 2826, 1587, 1302, 1103, 759 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.64−2.66 (m, 4H, pip-H), 3.44−3.46 (m, 4H, pip-H), 3.69 (s, 2H, CH_2), 6.88 (d, J = 7.98 Hz, 1H, Py-H), 7.22−7.23 (m, 2H, Ar−H), 7.36 (m, 1H, Ar−H), 7.53 (m, 1H, Ar−H), 7.74 (d, J = 8.07 Hz, 1H, Py-H); EI-MS, m/z (%) 389 (12, M^+), 209 (12), 180 (86), 168 (28), 125 (100), 56 (19). HRMS (EI+) calcd for $C_{17}H_{16}Cl_2F_3N_3$, 389.0673; found, 389.0700.

1-(2-Chloro-6-fluorobenzyl)-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{18}$**):yield, 46%; yellow oil; IR (KBr) υ 2849, 1596, 1324, 1124, 780 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.65−2.67 (m, 4H, pip-H), 3.66−3.69 (m, 4H, pip-H), 3.79 (s, 2H, CH_2), 6.45 (d, J = 8.80 Hz, 1H, Py-H), 7.02 (m, 1H, Ar−H), 7.23−7.25 (m, 2H, Ar−H), 7.65 (d, J = 8.80 Hz, 1H, Py-H); EI-MS, m/z (%) 407 (18, M^+), 264 (11), 221 (14), 198 (100), 186 (44), 143 (82), 42 (6). HRMS (EI+) calcd for $C_{17}H_{15}Cl_2F_4N_3$, 407.0579; found, 407.0579.

1-(3-Chlorobenzyl)-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{19}$**): yield, 47%; yellow oil; IR (KBr) υ 2823, 1611, 1308, 1134, 780 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.54−2.56 (m, 4H, pip-H), 3.55 (s, 2H, CH_2), 3.66−3.68 (m, 4H, pip-H), 6.47 (d, J = 8.80 Hz, 1H, Py-H), 7.26−7.28 (m, 3H, Ar−H), 7.39 (s, 1H, Ar−H), 7.67 (d, J = 8.80 Hz, 1H, Py-H); EI-MS, m/z (%) 389 (29, M^+), 264 (13), 221 (16), 209 (22), 180 (100), 168 (34), 125 (63), 42 (6). HRMS (EI+) calcd for $C_{17}H_{16}Cl_2F_3N_3$, 389.0673; found, 389.0673.

1-(2-Chlorobenzyl)-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{20}$**): yield, 51%; yellow oil; IR (KBr) υ 2806, 1593, 1113, 750 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.59−2.61 (m, 4H, pip-H), 3.65−3.67 (m, 6H, CH_2, pip-H), 6.46 (d, J = 8.78 Hz, 1H, Py-H), 7.23−7.25 (m, 2H, Ar−H), 7.36−7.37 (m, 1H, Ar−H), 7.50 (d, J = 7.23 Hz, 1H, Ar−H), 7.66 (d, J = 8.81 Hz, 1H, Py-H); EI-MS, m/z (%) 389 (M^+), 209 (12), 180 (86), 168 (28), 125 (100), 69 (10), 56 (19). HRMS (EI+) calcd for $C_{17}H_{16}Cl_2F_3N_3$, 389.0673; found, 389.0700.

1-(2,4-Dichlorobenzyl)-4-[6-chloro-5-(trifluoromethyl)pyridin-2-yl]piperazine (***B*$_{21}$**): yield, 56%; yellow oil; IR (KBr) υ 2833, 1606, 1328, 1135, 804 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.58−2.60 (m, 4H, pip-H), 3.64−3.68 (m, 6H, CH_2, pip-H), 6.48 (d, J = 8.80 Hz, 1H, Py-H), 7.26 (m, 1H, Ar−H), 7.41−7.43 (m, 2H, Ar−H), 7.68 (d, J = 8.80 Hz, 1H, Py-H); EI-MS, m/z (%) 423 (18, M^+), 264 (31), 214 (98), 202 (36), 158 (100), 123 (6), 56 (5). HRMS calcd for $C_{17}H_{15}Cl_3F_3N_3$, 423.0284; found, 423.0285.

1-[(6-Chloropyridin-3-yl)methyl]-4-phenylpiperazine (***C*$_1$**): yield, 87%; white solid; mp, 135−136 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 2.63−2.65 (m, 4H, pip-H), 3.43−3.45 (m, 4H, pip-H), 3.69 (s, 2H, CH_2), 6.88 (d, J = 7.98 Hz, 1H, Py-H), 7.21−7.23 (m, 2H, Ar−H), 7.34−7.36 (m, 2H, Ar−H), 7.53 (m, 1H, Ar−H), 7.74 (d, J = 8.07 Hz, 1H, Py-H); EI-MS, m/z (%) 287 (100, M^+), 181 (16), 161 (52), 154 (42), 126 (64), 106 (68), 77 (44), 56 (52). HRMS (EI+) calcd for $C_{17}H_{16}Cl_2F_3N_3$, 287.1189; found, 287.1189.

1-[(6-Chloropyridin-3-yl)methyl]-4-[2,6-dinitro-4-(trifluoromethyl)phenyl]piperazine (***C*$_2$**): yield, 79%; yellow solid; mp, 162−164 °C; IR (KBr) υ (cm^{-1}) 1540, 1321, 1156 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.54−2.56 (m, 4H, pip-H), 3.14−3.16 (m, 4H, pip-H), 3.55 (s, 2H, CH_2), 7.34 (d, J = 8.07 Hz, 1H, Py-H), 7.68 (s, 1H, Py-H), 8.03 (s, 2H, Ar−H), 8.32 (s, 1H, Py-H); EI-MS, m/z (%) 445 (1, M^+), 291 (5), 231 (7), 126 (100), 90 (8), 42 (11). HRMS (EI+) calcd for $C_{17}H_{15}ClF_3N_5O_4$, 445.0765; found, 445.0746.

Biological Assay. Larvae of the armyworm, *P. separata* (Walker), were reared continuously on freshly cut maize seedling under standard laboratory conditions (25 °C, 60% relative humidity, and a 16:8 h light/dark photoperiod). All compounds were dissolved in acetone and diluted with water containing Triton X-100 (0.1 mg L^{-1}) to 500 and 250 mg L^{-1} in concentration for bioassay. Fresh leaves were dipped in diluted solutions of the chemicals for 5 s, and excess fluid was removed. Water containing Triton X-100 (0.1 mg L^{-1}) and acetone was used as control.

Effects on larval growth were assessed using second-instar larvae of the armyworm. After weighing, each batch of second-instar larvae of the armyworm (n = 10) was released on leaves. Controls were treated similarly. Each treatment was replicated three times. Larvae were reweighed after 120 h for every batch to count the growth inhibition. Mortality was assessed 72 h after treatment.

RESULTS AND DISCUSSION

Biological Activity. The biological activities against armyworm, *P. separata* (Walker), of the target compounds are summarized in **Table 2**. Most of the compounds **B$_1$**−**B$_{17}$** showed certain growth-inhibiting activities. Through careful observation, the treated larvae were found to decrease feeding compared to the control ones. It was also found that the bodies of the treated larvae were thinner and shorter than those of control ones, and the growth development of treated larvae lagged behind that of the control larvae. Generally, the symptoms induced by the target compounds were similar to those described for typical insect

Table 2. Biological Activities *I* (%) of Compounds $\mathbf{B_1}-\mathbf{B_{21}}$ against Armyworm

compd	R_1	R_2	*I* (%)	
$\mathbf{B_1}$	4-chlorobenzyl	5-(trifluoromethyl)pyridin-2-yl	76.9[a]	
$\mathbf{B_2}$	(6-chloropyridin-3-yl)methyl	5-(trifluoromethyl)pyridin-2-yl	73.7[a]	
$\mathbf{B_3}$	(2-chlorothiazol-5-yl)methyl	5-(trifluoromethyl)pyridin-2-yl	48.4[a]	
$\mathbf{B_4}$	2-chlorobenzyl	3-chloro-5-(trifluoromethyl)pyridin-2-yl	78.6[a]	
$\mathbf{B_5}$	4-chlorobenzyl	3-chloro-5-(trifluoromethyl)pyridin-2-yl	55.5[a]	
$\mathbf{B_6}$	2,6-difluorobenzyl	3-chloro-5-(trifluoromethyl)pyridin-2-yl	36.6[a]	
$\mathbf{B_7}$	(pyridin-3-yl)methyl	3-chloro-5-(trifluoromethyl)pyridin-2-yl	78.6[a]	
$\mathbf{B_8}$	(6-chloropyridin-3-yl)methyl	3-chloro-5-(trifluoromethyl)pyridin-2-yl	83.5[a]	
$\mathbf{B_9}$	(2-chlorothiazol-5-yl)methyl	3-chloro-5-(trifluoromethyl)pyridin-2-yl	88.6[a]	
$\mathbf{B_{10}}$	4-chlorobenzyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	84.1[a]	
$\mathbf{B_{11}}$	2,3-dichlorobenzyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	51.2[a]	
$\mathbf{B_{12}}$	(6-chloropyridin-3-yl)methyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	25.4[a]	
$\mathbf{B_{13}}$	(2-chlorothiazol-5-yl)methyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	89.5[a]	
$\mathbf{B_{14}}$	2,6-difluorobenzyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	22.2[a]	
$\mathbf{B_{15}}$	4-iodobenzyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	13.7[a]	
$\mathbf{B_{16}}$	4-*tert*-butylbenzyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	30.0[a]	
$\mathbf{B_{17}}$	2-chlorobenzyl	6-chloro-3-(trifluoromethyl)pyridin-2-yl	74.8[a]	
$\mathbf{B_{18}}$	2-chloro-6-fluorobenzyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	27.5[b]	
$\mathbf{B_{19}}$	3-chlorobenzyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	100[b]	71.3[c]
$\mathbf{B_{20}}$	2-chlorobenzyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	93.3[b]	76.6[c]
$\mathbf{B_{21}}$	2,4-dichlorobenzyl	6-chloro-5-(trifluoromethyl)pyridin-2-yl	100[b]	76.6[c]
$\mathbf{C_1}$	(6-chloropyridin-3-yl)methyl	phenyl	0[a]	
$\mathbf{C_2}$	(6-chloropyridin-3-yl)methyl	2,6-dinitro-4-(trifluoromethyl)phenyl	14.9[a]	

[a] Growth inhibiting activities at 500 mg L^{-1}, $I\% = 1 - T/D$ (D = the increased weight of the control larvae during 120 h; T = the increased weight of the treated larvae during 120 h). [b] Mortalities of the armyworm at 500 mg L^{-1} (*I*%) during 72 h. [c] Mortalities of the armyworm at 250 mg L^{-1} (*I*%) during 72 h.

Figure 1. Symptoms of poisoned armyworm induced by $\mathbf{B_{21}}$ at 500 mg L^{-1}.

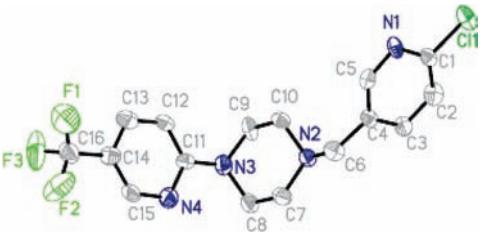


Figure 2. Crystal structure of compound $\mathbf{B_2}$.

growth regulators. Interestingly, compounds $\mathbf{B_{18}}-\mathbf{B_{21}}$ exhibited distinctly different bioactivities from compounds $\mathbf{B_1}-\mathbf{B_{17}}$. They caused the armyworm's death within 72 h at 500 and 250 mg L^{-1}. It was found that the larvae could hardly move after eating the leaves containing the test substances ($\mathbf{B_{18}}-\mathbf{B_{21}}$), and the bodies remained spasmodic until the test larvae died as shown in **Figure 1**. The causation of the bioactivity difference between $\mathbf{B_{18}}-\mathbf{B_{21}}$ and $\mathbf{B_1}-\mathbf{B_{17}}$ is still under investigation.

Effect of N^4-Aryl Group on Activities. To verify the effect of an N^4-aryl group on activities, compounds $\mathbf{C_1}$ and $\mathbf{C_2}$ were prepared by replacing the trifluoromethylpyridinyl group on N^4-substitutents of $\mathbf{B_2}$ with substituted phenyl groups. The bioassays showed that such replacement did weaken the growth-inhibiting activity, which demonstrated that a suitable N^4-substitutent, such as the trifluoromethylpyridinyl group, was significant for a compound to remain effective against the insect.

Quantitative Structure−Activity Relationship (QSAR). The relationship between growth-inhibiting activities and the structures of compounds $\mathbf{B_1}-\mathbf{B_{17}}$ was analyzed by a QSAR (Hansch) approach against physicochemical parameters. The computational work was accessed from the Drug Discovery Workbench (DDW) module of Cerius[2] (version 4.8). The structures were minimized on the basis of the crystal structure of $\mathbf{B_2}$ (**Figure 2**; the crystallographic data have been deposited at the Cambridge Crystallographic Data Centre in supplementary publication CCDC-658617. Copies of the data can be obtained, free of charge, at http://www.ccdc.cam.ac.uk/data_request/cif.

The data matrix was analyzed by the partial least-squares (PLS) method. The quality of each of the regression models was evaluated using the square of the correlation coefficient (r^2), cross-validation squared correlation coefficient (XVr^2), and predicted sum of squares (PRESS). The cross-validation squared correlation coefficient XVr^2 was used to measure the predictive ability of the equation (*13*, *14*).

To find out which parameters are more important to activity, several equations were obtained according to the different combinations of the parameters. The most significant combination of physicochemical parameters affecting activity were log *P*(o/w), PEOE_VSA-1, and vsa_hyd, which were calculated by MOE (*15*, *16*) presented in **Table 3**. The growth inhibiting activity data (% *I*) at 500 mg L^{-1} were converted to $\log\{I/[(100 - I) \times MW]\}$ (*17*) as a dependent parameter, X.

$$X = \log\{I/[(100 - I) \times MW]\}$$

The physical parameters log *P*(o/w), PEOE_VSA-1, and vsa_hyd are the independent variables of the regression, and

Table 3. Experimental Activities, Calculated Activities, and Physicochemical Parameters of Compounds B_1−B_{17}

compd	I%	X[a]	calcd[b]	log P(o/w)[c]	PEOE_VSA-1[d]	vsa_hyd[e]
B_1	76.9	−2.027	−2.4145	3.83276	54.091755	299.9382
B_2	73.7	−2.104	−2.02767	3.00476	41.836849	284.101
B_3	48.4	−2.587	−1.87392	2.69176	33.766548	274.2206
B_4	78.6	−2.025	−2.16922	4.45776	95.928604	318.2314
B_5	55.5	−2.494	−2.41951	4.45976	83.673698	318.2314
B_6	36.6	−2.894	−2.99858	4.16976	41.836849	310.1802
B_7	78.6	−1.986	−2.06364	2.63476	41.836849	293.1117
B_8	83.5	−1.887	−2.03269	3.63176	71.4188	302.3942
B_9	88.6	−1.707	−1.87893	3.31876	63.348495	292.5139
B_{10}	84.1	−1.868	−1.99634	4.00076	71.4188	293.3834
B_{11}	51.2	−2.607	−2.37305	5.41676	113.25565	327.5138
B_{12}	25.4	−1.992	−2.02729	3.00376	41.836849	284.101
B_{13}	89.5	−1.667	−1.84258	3.68776	63.348495	283.5031
B_{14}	22.2	−3.137	−2.96223	4.53876	41.836849	301.1694
B_{15}	13.7	−3.481	−3.53444	5.42676	54.091755	325.1491
B_{16}	30.0	−2.982	−2.81454	5.73576	134.84459	365.5528
B_{17}	74.8	−2.117	−2.13287	4.82676	95.928604	309.2206

[a] $X = \log\{I/[(100 - I) \times MW]\}$ (I% = growth-inhibiting activities at 500 mg L^{-1}; MW = molecular weight). [b] The calculated activities. [c] Log P(o/w) = octanol/water partition coefficient. [d] PEOE_VSA-1 = van der Waals surface area of atomic partial charge $-0.10 \le q \le -0.05$. [e] vsa_hyd = approximate van der Waals surface area of hydrophobic atoms.

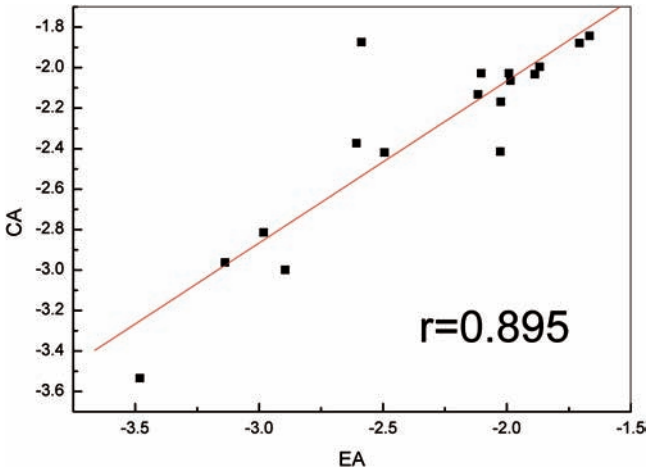


Figure 3. Relationship between the experimental activities (EA) and the calculated activities (CA).

X is the dependent variable.

$$X = -0.61955 + 0.01530(\mathrm{PEOE_VSA\text{-}1}) - 0.67611 \log P(\mathrm{o/w}) \quad (1)$$

$n = 17$, $r = 0.825$, $r^2 = 0.681$, $XVr^2 = 0.500$, and RMSE = 0.295.

Equation **1** is a divariant model with comparatively lesser r^2 and XVr^2 values.

$$X = 3.94200 + 0.02036(\mathrm{PEOE_VSA\text{-}1}) - 0.38769 \log P(\mathrm{o/w}) - 0.01991(\mathrm{vsa_hyd}) \quad (2)$$

$n = 17$, $r = 0.895$, $r^2 = 0.801$, $XVr^2 = 0.663$, and RMSE = 0.233.

As shown in **Figure 3**, higher values of r^2 and XVr^2 were observed with eq **2** in which X was correlated with log P(o/w), PEOE_VSA-1, and vsa_hyd. Therefore, eq **2** explained the activities of these compounds better than eq **1** and revealed that lower log P(o/w) and vsa_hyd values and higher PEOE_VSA-1 values could result in better bioactivities. In addition, log P(o/w) was found to be the most important factor for the activities.

Lower log P(o/w) suggested that the decrease of lipophilic property would strengthen the growth-inhibiting activity. This result suggested the introduction of a hydrophilic factor, such as the use of heterocyclic rings including oxygen and/or nitrogen might benefit the bioactivity of compounds. In agreement with the lower log P(o/w), lower vsa_hyd values meant the decrease of surface area of hydrophobic atoms in the molecule. A higher PEOE_VSA-1 value was associated with the increase of electronegative atoms in the molecule. Thus, the application of an electronegative atom or group (i.e., chlorine, fluorine, and trifluoromethyl group) would be encouraged for better bioactivity.

In conclusion, a series of 1-arylmethyl-4-[(trifluoromethyl)-pyridin-2-yl]piperazine compounds derived from PAPP were synthesized, and their biological activities against armyworm were evaluated. By the preliminary bioassay, most of the target compounds exhibited certain growth-inhibiting activities or larvicidal activities against armyworm, *P. separata* (Walker). The results indicated that PAPP could become a lead compound for insecticides with novel modes of action. QSAR demonstrated that log P(o/w), PEOE_VSA-1, and vsa_hyd were the critical parameters for growth-inhibiting activities of target compounds. Further structural modification and biological evaluation are currently under investigation and will be reported in due course.

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