SYNTHESIS OF NOVEL NEONICOTINOIDS DERIVATIVES

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ABSTRACT

Neonicotinoid compounds have been used as insecticides since the 1990s to effectively control Hemipteran pests such as aphids, leafhoppers, whiteflies, and additionally, for their lethal activity and low toxicity in humans. The synthesis of novel neonicotinoids compounds was achieved using (S)-(-)-(1-methyl-2-pyrrolidinyl)methanol and (±)-2-(1-methyl-2-pyrrolidinyl)ethanol with nitrobenzoyl chlorides derivatives in diethyl ether at room temperature to concentrate in vacuum to subsequently neutralize (pH = 8) with 7.4% (w/v) isopropanol HCl solution to obtain hydrochloride salts. (S)-(1-methyl-2-pyrrolidinyl)methyl and (±)-2-(1-methyl-2-pyrrolidinyl)ethyl 2-nitrobenzoate salts was not possible to form.

Keywords: Neonicotinoids, nicotinic acetylcholine receptors, synthetic pesticides.

1. INTRODUCTION

Neonicotinoids (NEOs) are an emerging class of synthetic pesticides were discovered in the 1980s and they were used principally as insecticides since 1990s due to exert their neurotoxicity in the insects[1]–[4], acting as potent agonists of nicotinic acetylcholine receptors (nAChRs), which are widely distributed in the central nervous system (CNS) of these invertebrates[4],[5]. Since the advent of imidacloprid (IMI, commercial in 1991), neonicotinoid insecticides (Figure 1) quickly became important insecticides to effectively control Hemipteran pests such as aphids, leafhoppers, whiteflies, due to their unique lethal activity and low toxicity[6]. NEOs are fourth generation insecticides and, present four characteristics fragments, aromatic heterocyclic, a flexible chain, hydroheterocycle or guanidine/amidine, L-proline and an electron withdrawing group[7],[8]. NEOs are agonist for nAChRs in insects[9] and, mammalian receptors, being poor agonist for the last one and, besides these receptors are involved in the neuronal transmission, learning and memory[10],[11].

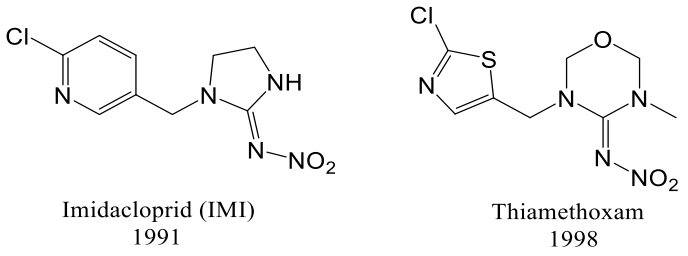

Figure 1. Insecticides targeting insect nAChRs.

Neuronal nAChRs are representative members of the Cys-Loop ligand-gated ion channels (LGICs) superfamily, protein complexes are composed of five subunits or subtypes [12]. Within these neuronal-type subunits, there are nine alpha subunits (α2 - α10) and three beta subunits (β2 - β4), where the conformation of the nAChRs is due to the combination of these subunits and, depending on the combination of the nAChRs can be homomeric or heteromeric[13],[14]. In the central nervous system, the most abundant subtypes are the $(\alpha4\beta2)_2\alpha4$, $(\alpha4\beta2)_2\beta2$ and α7 nAChRs[15]. The binding of an agonist ligand such as nicotine and acetylcholine (Figure 2) to the nAChRs induces the opening of the ion channel of the receptor, promoting the passage of cations through the lipid membrane of the protein complex[16]. On the other hand, when an antagonistic ligand such as mecamylamine (Figure 2) binds to these receptors, the ion channel does not open, preventing the flow of ions across the membrane[17]. The action of these ligands on the nAChRs is associated to different functions and disorders in the CNS, such as memory, learning, anxiety[17], ethanol consumption[18], depression and addiction to drugs of abuse such as nicotine (Figure 2).

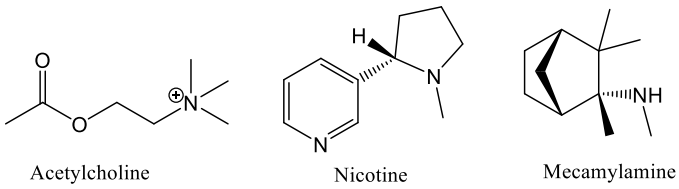

Figure 2. Chemical structure of classical agonists acetylcholine and nicotine, and chemical structure of antagonist mecamylamine.

In previous works, guided by the available information and the combination of the chemical structure of nicotine and acetylcholine, we designed and synthesized new NEOs derivatives with potential bioactivity on α4β2 nAChRs. In previous work, compound **(2)** showed poor antagonistic affinity on hα4β2 nAChR and moderate affinity on hα7 nAChR[13]. In this sense, according to these results and using compound **(2)** as a base, we decided to explore the synthesis of new NEOs compounds by orienting the $-NO_2$ group of the aromatic ring in different positions to study the substitution effect, to subsequently consider its wide use and increasing resistance from crop pests to insecticides[19],[20].

2. EXPERIMENTAL SECTION

2.1 Materials

All the reagents and solvents used for the synthesis of these compounds were obtained commercially and used without further purification. The nitro-benzoyl chloride derivates, (S)-(-)-(1-methyl-2-pyrrolidinyl)methanol and (±)-2-(1-methyl-2-pyrrolidinyl)ethanol obtained from (Ak Scientific Inc., CA, USA) and, dichloromethane, methanol, isopropyl alcohol obtained from (Merck Millipore, Santiago, Chile).

2.2 Characterization

Melting points were determined with a RY-1 Melting Point Tester. The IR spectra were recorded on a FT-IR IRSpirit Shimadzu and wavenumbers were reported in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using Bruker AMX 400 spectrometers at 400 MHz. Chemical shifts are reported relative to TMS (d= 0.00) or HDO (d= 4.79), $CDCl_3$ (d= 7,25) and coupling constants (J) are given in Hz. High resolution mass spectra (HRMS) were recorded using a Bruker compact QTOF MS with direct injection. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel pre-coated F_{254} Merck plates and, the compounds obtained were purified by column chromatography using CH_2Cl_2/CH_3OH (9:1) mixture as the mobile phase. Reagents and solvents utilized were commercially available and used without further purification.

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2.3 Synthesis

2.3.1 General Procedures for the Synthesis of (S)-(1-methyl-2-pyrrolidinyl)methyl Nitro-Benzoate Derivatives **(1-4)**.

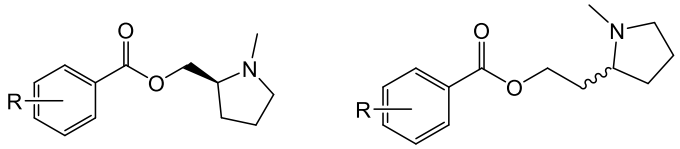
Nitro-Benzoyl chloride derivatives (3.7 mmol, 0.4–0.5 mL) were dissolved in dry diethyl ether (50 mL) and constantly stirred at room temperature. One equivalent of (S)-(-)-(1-methyl-2-pyrrolidinyl)methanol (3.7 mmol, 0.5 mL) was added drop by drop. The reaction mixture was kept at room temperature and stirred constantly for 24 hours. Then, the mixture was concentrated under vacuum to give a crude which was re-dissolved in water, adjusted to pH 8.0 and extracted with CH_2Cl_2 (3 X 20 mL). The hydrochloride salts were obtained from an isopropanol solution 7.4% HCl (w/v).

2.3.2 General Procedures for the Synthesis of (±)-2-(1-methyl-2-pyrrolidinyl)ethyl Nitro-Benzoate Derivates **(5-8)**.

Nitro-Benzoyl chloride derivatives (3.7 mmol, 0.4–0.5 mL) were dissolved in dry diethyl ether (50 mL) and constantly stirred at room temperature. One equivalent of (±)-2-(1-methyl-2-pyrrolidinyl)ethanol (3.7 mmol, 0.5 mL) was added drop by drop. The reaction mixture was kept at room temperature and stirred constantly for 24 hours. Then, the mixture was concentrated under vacuum to give a crude which was re-dissolved in water, adjusted to pH 8.0 and extracted with CH2Cl2 (3 X 20 mL). The hydrochloride salts were obtained from an isopropanol solution 7.4% HCl (w/v).

3. RESULTS AND DISCUSSION

The Nitro-arylpyrrolidine ester derivates **(1-8)** (Figure 3) they were synthesized from commercially available reagents: Nitro-benzoyl chloride derivates, (S)-(-)-(1-methyl-2-pyrrolidinyl)methanol and (±)-2-(1-methyl-2-pyrrolidinyl)ethanol.



(1) R= 2-NO_2
(2) R= 3-NO_2
(3) R= 4-NO_2
(4) R= 3,5-NO_2

(5) R= 2-NO_2
(6) R= 3-NO_2
(7) R= 4-NO_2
(8) R= 3,5-NO_2

Figure 3. Chemical structures of the 8 synthesized compounds used in this work.

For the synthesis of these compounds (Figure 3), the Nitro-benzoyl chloride derivates were reacted with (S)-(-)-(1-methyl-2-pyrrolidinyl)methanol or (±)-2-(1-methyl-2-pyrrolidinyl)ethanol to get the final NEOs **(1-8)** in 48-91% yields (Figure 4). Once the compounds were synthesized, the hydrochloride salts were formed from a 7.4% (w/v) isopropanol HCl solution. Compound **(2)** was synthesized and characterized in previous studies[13].

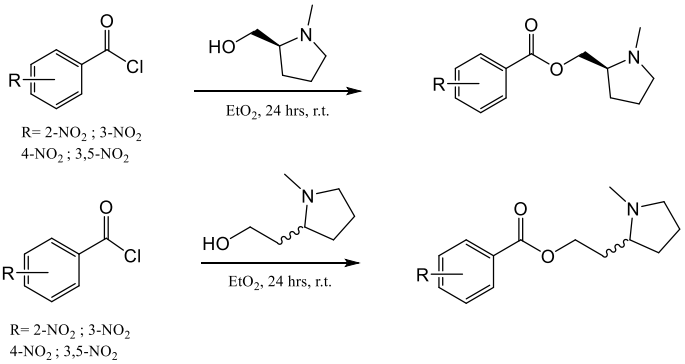


Figure 4. Scheme of synthesis of NEOs compounds **(1-8)**.

All the synthesized compounds **(1-8)**, which were fully characterized spectroscopically by FT-IR, ^{1}H-NMR, ^{13}C-NMR and HRMS, observing the following signs:

In ^{1}H-NMR, all the compounds **(1-8)** presented signals between δ 9.36 – 7.63 ppm (**Figures 1S-7S**) according to the presence of aromatic protons and, six signals corresponding to the 1-methylpyrrolidine fragment, where a singlet signal with an integration of three hydrogens between δ 3.12 – 1.95 ppm (**Figures 1S-7S**) corresponding to the -N(C**H$_3$**) of 1-methylpyrrolidine, two multiplets that integrate one hydrogen each one, appear between δ 4.89 – 4.23 ppm (**Figure 1S-7S**) corresponding to the -C**H$_2$**-N- of the heterocycle and three signal multiplets that integrate for four hydrogens between δ 2.65 – 1.57 ppm (**Figure 1S-7S**) -C**H$_2$**-C**H$_2$**- from the heterocycle fragment. On the other hand, in ^{13}C-NMR, all the compounds exhibited: a signal between δ 166.36 – 163.75 ppm (**Figure 8S-14S**), assignable to the carbonyl carbon of the ester group, aromatic carbon signals between 150.47 – 123.19 ppm (**Figure 8S-14S**) and, a signal of the carbon of N-**C**H$_3$ of 1-methylpyrrolidine between 40.36 – 39.09 ppm (**Figure 8S-14S**).

Additionally, in IR spectroscopy, all the compounds (**1-8**) showed: an aromatic C-H absorption stretching band between 3100 – 3048 cm^{-1} (**Figure 15S-21S**), an absorption stretching band of the carbonyl group of the ester between 1728 – 1723 cm^{-1} (**Figure 15S-21S**), a C-O absorption stretching band between 1274 – 1253 cm^{-1} and an absorption stretching band C-N between 1135 – 1098 cm^{-1} (**Figure 15S-21S**).

For high resolution mass spectroscopy, molar masses found by this technique of all compounds were compared with that calculated theoretically.

3.1 Chemistry

3.1.1 (S)-(1-methyl-2-pyrrolidinyl)methyl 2-NitroBenzoate (**1**):

Obtained as a brown oil yield 50.4%. Compound was prepared as described in general procedures (section 2.3.1) 2-NO_2Py ($C_{13}H_{16}N_2O_4$): IR (cm^{-1}) 3048, 2971, 1728, 1256, 1135, 722; ^{1}H-NMR (400 MHz, Chloroform-*d*) δ 7.89 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.74 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.70 – 7.58 (m, 2H), 4.36 (dd, *J* = 11.0, 4.9 Hz, 1H), 4.23 (dd, *J* = 11.0, 5.9 Hz, 1H), 3.10 – 3.00 (m, 1H), 2.59 – 2.51 (m, 1H), 2.39 (s, 3H), 2.32 – 2.21 (m, 1H), 2.04 – 1.92 (m, 1H), 1.84 – 1.71 (m, 2H), 1.70 – 1.57 (m, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 165.42, 148.30, 132.86, 131.71, 130.00, 127.74, 123.87, 68.47, 63.51, 57.52, 41.36, 28.57, 22.88. HRMS m/z calcd. for $C_{13}H_{16}N_2O_4$ (M+H), 265.1183; found, 265.1191.

3.1.2 (S)-(1-methyl-2-pyrrolidinyl)methyl 4-NitroBenzoate (**3**):

4-NO_2Py ($C_{13}H_{16}N_2O_4$) yield 59.7%; m.p. 112 – 115 °C; compound was prepared as described in general procedures (section 2.3.1): IR (cm^{-1}) 3065, 2956, 1724, 1253, 1098, 718; ^{1}H-NMR (400 MHz, Deuterium Oxide) δ 8.47 – 8.30 (m, 2H), 8.24 (d, J = 8.9 Hz, 2H), 4.89 – 4.81 (m, 1H), 4.71 – 4.55 (m, 1H), 4.08 – 3.93 (m, 1H), 3.85 – 3.78 (m, 1H), 3.37 – 3.26 (m, 1H), 3.10 (s, 3H), 2.56 – 2.42 (m, 1H), 2.35 – 2.22 (m, 1H), 2.18 – 2.04 (m, 2H). ^{13}C-NMR (101 MHz, D2O) δ 165.78, 150.65, 134.22, 130.83, 123.82, 67.40, 62.94, 57.28, 40.62, 26.15, 22.02. HRMS m/z calcd. for $C_{13}H_{16}N_2O_4$ (M+H), 265.1183; found, 265.1178.

3.1.3 (S)-(1-methyl-2-pyrrolidinyl)methyl 3,5-DinitroBenzoate (**4**):

3,5-NO_2Py ($C_{13}H_{15}N_3O_6$) yield 75.5%; m.p. 203 – 205 °C; compound was prepared as described in general procedures (section 2.3.1): IR (cm^{-1}) 3068, 2958, 1724, 1256, 1098, 720; ^{1}H-NMR (400 MHz, Deuterium Oxide) δ 9.36 (s, 1H), 9.22 (s, 2H), 4.92 (dd, *J* = 13.0, 3.1 Hz, 1H), 4.75 (d, *J* = 5.9 Hz, 1H), 4.08 – 3.94 (m, 1H), 3.91 – 3.78 (m, 1H), 3.38 – 3.28 (m, 1H), 3.12 (s, 3H), 2.56 – 2.44 (m, 1H), 2.36 – 2.23 (m, 1H), 2.22 – 2.09 (m, 2H). ^{13}C-NMR (101 MHz, D_2O) δ ^{13}C-NMR (101 MHz, D_2O) δ 163.75, 148.55, 132.22, 129.87, 123.47, 67.26, 63.13, 57.19, 40.35, 26.02, 25.71, 21.79, 21.75. HRMS m/z calcd. for $C_{13}H_{15}N_3O_6$ (M-H), 307.0825; found, 307.0804.

3.1.4 (±)-2-(1-methyl-2-pyrrolidinyl)ethyl 2-NitroBenzoate (**5**):

Obtained as a brown oil yield 60.0%. compound was prepared as described in general procedures (section 2.3.2) 2-NO_2EPy ($C_{14}H_{18}N_2O_4$): IR (cm^{-1}) 3100 – 3050, 2956, 1724, 1274 – 1249, 1099, 721; ^{1}H-NMR (400 MHz, Chloroform-*d*) δ 7.89 – 7.84 (m, 1H), 7.80 – 7.75 (m, 1H), 7.74 – 7.63 (m, 2H), 4.63 – 4.49 (m, 1H), 4.42 – 4.29 (m, 1H), 3.91 – 3.72 (m, 1H), 3.23 – 3.08 (m, 1H), 2.93 – 2.85

(m, 1H), 2.83 (s, 3H), 2.52 – 2.32 (m, 3H), 2.30 – 2.19 (m, 1H), 2.12 – 1.86 (m, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.96, 148.30, 133.18, 132.24, 130.31, 126.92, 123.78, 62.94, 56.04, 39.09, 29.45, 29.22, 21.45. HRMS m/z calcd. for $C_{14}H_{18}N_2O_4$ (M+H), 279.1339; found, 279.1357.

3.1.5 (±)-2-(1-methyl-2-pyrrolidinyl)ethyl 3-NitroBenzoate (**6**):

3-NO_2EPy ($C_{14}H_{18}N_2O_4$) yield 85.6%; m.p. 157 – 160 °C; compound was prepared as described in general procedures (section 2.3.2): IR (cm^{-1}) 3051, 2968, 1726, 1256, 1129, 722; ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.62 (t, *J* = 2.1 Hz, 1H), 8.38 (dd, *J* = 8.3, 2.3 Hz, 1H), 8.27 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 4.56 – 4.34 (m, 2H), 3.76 – 3.58 (m, 1H), 3.56 – 3.42 (m, 1H), 3.22 – 3.03 (m, 1H), 2.93 (s, 3H), 2.58 – 2.34 (m, 2H), 2.20 – 1.95 (m, 3H), 1.92 – 1.70 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 166.05, 147.84, 135.53, 130.78, 130.21, 128.08, 124.21, 67.01, 62.94, 56.17, 39.18, 29.41, 29.13, 21.18. HRMS m/z calcd. for $C_{14}H_{18}N_2O_4$ (M+H), 279.1339; found, 279.1337.

3.1.6 (±)-2-(1-methyl-2-pyrrolidinyl)ethyl 4-NitroBenzoate (**7**):

4-NO_2EPy ($C_{14}H_{18}N_2O_4$) yield 90.9%; m.p. 192 – 194 °C; compound was prepared as described in general procedures (section 2.3.2). IR (cm^{-1}) 3085, 2960, 1723, 1271, 1103, 718; ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.38 – 8.23 (m, 2H), 8.23 – 8.07 (m, 2H), 4.62 – 4.47 (m, 2H), 3.83 – 3.65 (m, 1H), 3.62 – 3.50 (m, 1H), 3.33 – 3.13 (m, 1H), 3.01 (s, 3H), 2.64 – 2.42 (m, 2H), 2.27 – 2.03 (m, 3H), 2.01 – 1.79 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 166.36, 150.47, 134.83, 130.63, 123.74, 67.03, 62.95, 56.16, 39.15, 29.40, 29.08, 21.17. HRMS m/z calcd. for $C_{14}H_{18}N_2O_4$ (M+H), 279.1339; found, 279.1358.

3.1.7 (±)-2-(1-methyl-2-pyrrolidinyl)ethyl 3,5-DinitroBenzoate (**8**):

3,5-NO_2EPy ($C_{14}H_{17}N_3O_6$) yield 59.4%; m.p. 115 – 117 °C; compound was prepared as described in general procedures (section 2.3.2). IR (cm^{-1}) 3085, 2965, 1728, 1271, 1098, 723; ^{1}H NMR (400 MHz, Deuterium Oxide) δ 9.34 (t, *J* = 2.1 Hz, 1H), 9.18 (d, *J* = 2.1 Hz, 2H), 4.68 – 4.61 (m, 2H), 3.78 – 3.74 (m, 1H), 3.64 – 3.55 (m, 1H), 3.29 – 3.23 (m, 1H), 3.02 (s, 3H), 2.65 – 2.53 (m, 2H), 2.24 – 2.11 (m, 3H), 2.00 – 1.91 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 164.19, 148.49, 132.82, 129.69, 123.19, 67.29, 66.89, 63.56, 58.23, 56.18, 39.19, 29.36, 29.12, 21.19. HRMS m/z calcd. for $C_{14}H_{17}N_3O_6$ (M+H), 324.1190; found, 324.1198.

CONCLUSIONS

The NEOs derivatives were synthesized using benzoyl acid chlorides nitro substituted and, (S)-(-)-(1-methyl-2-pyrrolidinyl)methanol or (±)-2-(1-methyl-2-pyrrolidinyl)ethanol, all these compounds were confirmed by confirmed by ^{1}H-NMR, ^{13}C-NMR and HRMS. Almost all compounds were able to transform in the respective chlorhydrate using a dissolution of 7.4% HCl w/v. All the chlorhydrate synthesized were soluble in water.

INTEREST CONFLICT

We declare no interest conflict in this work.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY INFORMATION

Supplementary information on the chemical characterization of all compounds by means of ^{1}H-NMR and ^{13}C-NMR is available in this section.

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