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L-Proline as a Valuable Scaffold for the Synthesis of Novel Enantiopure Neonicotinoids Analogs

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ABSTRACT: In this research, six neonicotinoid analogs derived from L-proline were synthesized, characterized, and evaluated as insecticides against *Xyleborus affinis*. Most of the target compounds showed good to excellent insecticidal activity. To the best of our knowledge, this is the first report dealing with the use of enantiopure L-proline to get neonicotinoids. These results highlighted the compound **9** as an excellent candidate used as the lead chiral insecticide for future development. Additionally, molecular docking with the receptor and compound **9** was carried out to gain insight into its high activity when compared to dinotefuran. Finally, the neurotoxic evaluation of compound **9** showed lower toxicity than the classic neonicotinoid dinotefuran.

KEYWORDS: *neonicotinoids, nitroguanidines, chiral, enantiopure compound, insecticidal activity, docking*

INTRODUCTION

Chirality plays an important role in all living organisms and it is quite clear that diastereomer discrimination abounds in the domain of medicinal chemistry and pharmacology.[1] Nonetheless, most of the neonicotinoids used so far are nonchiral or administered as a racemic mixture.[2,3] Since the naturally occurring amino acids are the L-enantiomer, here, it is interesting to synthesize and evaluate a series of neonicotinoids that merge an L-amino acid such as L-proline, which is cheap and commercially available. On the other hand, the redbay ambrosia beetle, *Xyleborus glabratus*, is the vector of the laurel wilt disease fungal pathogen, *Raffaellea lauricola*. Since the vector's initial detection in the U.S.A. in the early of 2000s, laurel wilt has killed millions of redbay, *Persea borbonia* trees, and other members of the plant family Lauraceae.[4] In this context, avocado (*Persea Americana* Mill.) is the most important agricultural crop susceptible to laurel wilt.[5] As the disease continues to move to the south and west from its original focus, it has caused significant concern as Florida, California and other avocado-producing areas such as Mexico. In the absence of effective control measures, monetary losses caused by laurel wilt could eventually range up to 54 million in the U.S.A., and even greater loses might occur if the disease moves elsewhere.[6]

Management is currently focused on monitoring, sanitation, and direct control using contact or systemic insecticides.[7] Indeed, efficacious and cost-effective measures are urgently needed to protect avocado from laurel wilt. In this context, neonicotinoids are an important type of compounds with potent insecticidal activity. Since their introduction in the 1980s, these compounds have been established as the insecticides of choice for agricultural, animal health, and public health usages.[8,9] These are a type of insecticides that acts selectively on the central nervous system of the insect and can be efficient ligands for the nicotinic acetylcholine receptors (nAChRs) of insects.[10] However, with the increase of neonicotinoids used on the crop protection for a long time, the problems of cross-resistance[11] and bee toxicity[12] have received more attention, which calls for a new strategy of molecular design to find new leading compounds. The neuro-insecticides selectively act on the insect central nervous system (CNS) as an agonist of the postsynaptic nicotinic acetylcholine receptors (nAChRs) and present a higher selectivity when compared to the vertebrates.[13−15] The great attributes of the neonicotinoids are their novel mode of action, low mammalian toxicity, broad insecticidal spectrum, and good systemic properties.[16] Another important property of neonicotinoids is their environmental footprint which allows the replacement of the more toxic and nonselective organophosphorus, pyrethroid, and carbamate insecticides. The reported neonicotinoids so far can be classified according to the pharmacophore as *N*-nitroguanidines, (imidacloprid, thiamethoxam, clothianidin, and dinotefuran), nitromethylenes (nitenpyram) and *N*-cyanoamidines (acetamiprid and thiacloprid) (Figure 1).

All of these compounds are characterized by their high insecticidal activities against insects and relative safety toward

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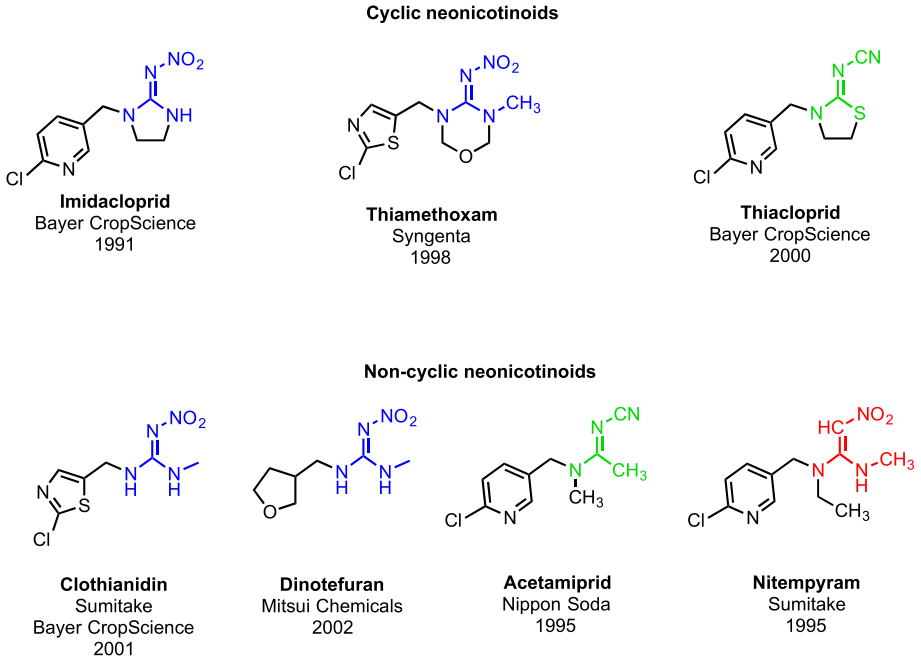

Figure 1. Classic neonicotinoids.

mammals and aquatic life.[17] Encouraged by this, we hereby introduce a new structure developing strategy, using dinotefuran as the lead compound, and a series of novel neonicotinoids derivatives were designed by introducing the enantiopure amino acid L-proline. Therefore, in search of improvement, the length of the carbon chain that connected the nitroguanidine and the L-proline was evaluated against the beetle *Xyleborus affinis*. Furthermore, the interactions between these new compounds and nAChR were also investigated by molecular docking. To the best of our knowledge, this is the first report that describes the incorporation of the enantiopure amino acid L-proline into the nitroguanidine core to get highly active insecticides against *Xyleborus affinis*.

MATERIALS AND METHODS

General Information. Nuclear magnetic resonance (^{1}H, ^{13}C, DEPTQ135) was recorded in a Bruker Avance III HD equipped with a BBO probe. The solvents used were $CDCl_3$ or DMSO-d_6, the chemical shifts (δ) were expressed in parts per million and coupling constants (J) in Hertz. The following abbreviations were used to explain NMR multiplicities: s = singlet, t = triplet, q = quartet, d = doublet, dd = double of doublets, ddd = double of doublet of doublets, dt = doublet of triplets, m = multiplet, and brs = broad signal. Spectra were processed using TopSpin 4.0.7 from Bruker BioSpin. Melting points were measured on a Stuart SMP10 apparatus using open glass capillaries and the values were uncorrected. Reactions were monitored by thin layer chromatography (TLC) performed on silica gel 60 F254 plates (Merck) and visualization was carried out with ammonium molybdate, 2,4-dinitrophenylhidrazine, phosphomolybdic acid, potassium permanganate or UV. High-resolution mass spectra (HRMS) were obtained in a Q-TOF mass spectrometer equipped with an electrospray ionization (ESI) interface Synapt G2-Si, Waters Inc. All of the mixture sensitive reactions were performed under nitrogen atmosphere. Anhydrous THF and Et_2O were dried with benzophenone and sodium. All other commercial reagents were purchased with Sigma-Aldrich and used without further additional purification.

Experimental Procedures. *Preparation of Compound* ***1***. First, using an ice-salt bath to keep the temperature between 0 and −5 °C, 30 mL of concentrated H_2SO_4 was slowly added to 14 mL of concentrated HNO_3 (warning: the temperature increases suddenly). Then 10 g (71.84 mmol) of *S*-methylisothiourea hemisulfate salt was added in one portion and the mixture was stirred for 6 h keeping the temperature at 0 °C. The mixture was poured in crushed ice and a white solid appeared, which was recovered by filtration, washed with distilled water and dried in high vacuum.

Data for Compound ***1***. Yield: 74%, white solid. Spectral data matched with previous reports,[18] the ^{1}H NMR data is provided here for convenience:. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.15 (brs, 2H), 2.40 (s, 3H).

Preparation of Compound ***3***. A 18.7 g sample of compound **2** (92.5 mmol) was added over 30 min at 0 °C to a stirred solution of 5 g (36.9 mmol) *S*-methylnitroisothiourea in 50 mL of pyridine and used as a solvent. After the mixture was stirred for 10 min, it was poured in crushed ice, diluted with 100 mL of concentrated HCl, and the white solid was collected by filtration; then the solid was crystallized in EtOH to obtain 6.8 g (65%) of white crystals which were suspended in 70 mL of acetonitrile and cooled at 0 °C. Afterward, 13.4 mL (0.83g, 26.9 mmol) of methylamine solution in methanol (2.0 M) was added over 1 h. The reaction was stirred for 30 min at room temperature, and then the solid phthalimide was discarded by filtration and the filtrate was evaporated. The residue had enough purity to use in the next reaction without further purification.

Data for Compound ***3***. Yield: 80%, white solid. Spectral data matched with previous reports;[18] however, the ^{1}H NMR data is provided here for convenience. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.83 (brs, 1H), 2.91 (brs, 3H), 2.44 (s, 3H).

Preparation of Compound ***5***. A 5.0 g (43.4 mmol) sample of of L-proline and 9.38 g of KOH (167.2 mmol) were dissolved in 2-propanol (30 mL); then 7.5 mL of BnCl (8.25 g, 65.17 mmol) was added dropwise for over 2 h at 40 °C, and the reaction was stirred 6 h at the same temperature. The mixture was acidified until pH of 5−6 with concentrated HCl and 15 mL of chloroform was added; this solution was stirred overnight at room temperature. The solid that formed was filtered and washed with plenty of DCM. The filtrate was evaporated under reduced pressure, the residue was solubilized with acetone, and the mixture was left to cool in an ice

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bath; a white solid appeared, and it was filtered and washed with cool acetone.

Data for Compound ***5***. Yield: 70%, white solid. Spectral data matched with previous reports;[19] the ^{1}H NMR data is provided here for convenience. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.51−7.36 (m, 5H), 4.31 (d, J = 12.8 Hz, 1H), 4.08 (d, J = 12.8 Hz, 1H), 3.87 (t, J = 7.8 Hz, 1H), 3.32−3.24 (m, 1H), 2.97 (q, J = 8.8 Hz, 1H), 2.35−2.22 (m, 1H), 2.00−1.89 (m, 2H), 1.85−1.71 (m, 1H).

Preparation of Compound ***6***. The solution of 6.4 g (31.18 mmol) *N*-benzyl-*S*-proline in 64 mL of THF was treated with 1.77 g (37.95 mmol) of $LiAlH_4$ at 0 °C. The mixture was stirred at room temperature for 12 h, and KOH (10% in water) was added until a white precipitate appeared. The mixture was filtered and the residue was washed with THF, then the filtrate was dried over Na_2SO_4 and evaporated. The crude was used without further purification.

Data for compound ***6***. Yield: 83%, slightly yellow oil. Spectral data matched with previous reports;[1] the ^{1}H NMR data is provided here for convenience. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.35−7.23 (m, 5H), 3.97 (d, J = 13.0 Hz, 1H), 3.66 (dd, J = 10.7, 3.4 Hz, 1H), 3.43 (dd, J = 10.8, 2.1 Hz, 1H), 3.35 (d, J = 13.0 Hz, 1H), 2.97 (ddd, J = 9.4, 6.2, 3.1 Hz, 1H), 2.79 (s, 1H), 2.77−2.70 (m, 1H), 2.29 (td, J = 9.4, 7.6 Hz, 1H), 1.94 (dq, J = 12.7, 8.8 Hz, 1H), 1.88−1.80 (m, 1H), 1.75−1.63 (m, 2H).

Preparation of Compound ***7***. A 1.37 g sample of triphenylphosphine (5.23 mmol) and 0.92 g (6.27 mmol) of phthalimide were dissolved in 15 mL of anhydrous THF; then a mixture of 1.0 g (5.23 mmol) of **6** in 4 mL of THF was added, and the reaction was stirred for 10 min. Then a solution of diethyl azodicarboxylate (40% wt in toluene) 2.27 mL (0.91 g, 5.23 mmol) was added dropwise, and the mixture was refluxed for 8 h. The reaction was allowed to cool at room temperature, and the solvents were evaporated under reduced pressure; the residue was dissolved in diethyl ether (50 mL) and stirred for 1 h after which a precipitate appeared. The precipitate was filtered and discarded, and the filtrate was evaporated under reduced pressure. The residue was passed through a short pad of silica (hexane/AcOEt = 7:3) in order to remove the excess of triphenylphosphine oxide; in this way, the reaction yielded 1.36 g (85%) of the corresponding intermediate as a colorless oil. This was dissolved in EtOH (40 mL) and 1.1 mL (0.56 g, 11.18 mmol) of hydrazine monohydrate (64 wt %) was added, and the reaction was refluxed for 2 h; after this time, HCl (6 mL of 1.0 M in water) was added and the heating was continued for 30 min. The reaction was filtered, and the filtrate was evaporated. The residue was dissolved in NaOH (10 mL of solution 1.0 M in water) and extracted with DCM (×3), then organic phases were combined, dried over Na_2SO_4, and evaporated under reduced pressure.

Data for Compound ***7***. Yield: 68%, colorless oil, spectral data matched with previous reports;[20] the ^{1}H NMR data is provided here for convenience. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.34−7.28 (m, 4H), 7.25−7.21 (m, 1H), 3.97 (d, J = 13.1 Hz, 1H), 3.30 (d, J = 13.1 Hz, 1H), 2.98−2.91 (m, 1H), 2.77 (dd, J = 12.9, 5.4 Hz, 1H), 2.71 (dd, J = 12.9, 3.4 Hz, 1H), 2.59−2.52 (m, 1H), 2.24−2.16 (m, 1H), 1.94−1.81 (m, 2H), 1.74−1.63 (m, 2H), 1.60−1.51 (m, 2H).

Preparation of Compound ***8***. A 1.0 g (5.25 mmol) sample of **7** and 0.94 g (6.30 mmol) of *S*-methyl-*N*-methylnitroisothiourea were dissolved in 20 mL of DCM and stirred at room temperature for 18 h. The crude was purified FCC (DCM/2-propanol = 9.5:0.5).

Data for Compound ***8***. Yield: 43%, colorless oil, $[\alpha]^{23.5}$= −78.4 (c 1, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6, 60 °C) δ: 8.76 (b, 1H), 7.87 (b, 1H), 7.33−7.20 (m, 5H), 3.98 (d, J = 13.2 Hz, 1H), 3.36 (d, J = 13.3 Hz, 1H), 3.34−3.29 (m, 1H), 3.23 (dt, J = 13.5, 4.3 Hz, 1H), 2.88−2.80 (m, 2H), 2.75 (d, J = 4.3 Hz, 3H), 2.23 (q, J = 8.4 Hz, 1H), 1.96−1.84 (m, 1H), 1.71−1.53 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d6, 60 °C) δ: 158.9, 139.8, 128.9, 128.5, 127.2, 62.0, 58.3, 54.0, 44.0, 28.5, 28.4, 23.1. HRMS (ESI): calculated for $C_{14}H_{22}N_5O_2^+$ [M + H]$^+$, 292.1768; found, 292.1773 (1.4).

Preparation of Compound ***9***. A 1.25 g (6.56 mmol) sample of **7** and 1.06 g (7.88 mmol) of *S*-methylnitroisothiourea were dissolved in 20 mL of DCM and stirred at room temperature for 18 h. The crude was purified using FCC (DCM/2-propanol = 9.5:0.5) and after that was recrystallized in DCM/hexane.

Data for Compound ***9***. Yield: 65%, crystalline white solid, mp 130−133 °C, $[\alpha]^{23.5}$ = −65.2 (c 1, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6, 60 °C) δ: 8.70 (b, 1H), 7.80 (s, 2H), 7.37−7.21 (m, 5H), 4.00 (d, J = 13.3 Hz, 1H), 3.41−3.30 (m, 2H), 3.27−3.22 (m, 1H), 2.87−2.77 (m, 2H), 2.23 (td, J = 9.2, 7.2 Hz, 1H), 1.91 (dt, J = 12.2, 8.2 Hz, 1H), 1.72−1.52 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6, 60 °C) δ: 160.5, 139.8, 129.0, 128.5, 127.2, 62.1, 58.4, 54.0, 43.8, 28.4, 23.0. HRMS (ESI): calculated for $C_{13}H_{20}N_5O_2^+$ [M + H]$^+$, 278.1612; found, 278.1617 (−2.9).

Preparation of Compound ***10***. A 4.1 mL sample of Et_3N (2.97 g, 29.35 mmol) was added in a single portion to a stirred solution of **7** (4.33 g (22.63 mmol) in 57 mL DCM); afterward, the mixture was cooled at 0 °C and 2.27 mL (3.37 g, 29.41 mmol) of methanesulfonyl chloride was slowly added, and the mixture was stirred for 2 h at 0 °C. Afterward, 15 mL of water was added, and the organic phase was separated; the aqueous phase was extracted with 30 mL of DCM, the organic extracts were washed with brine, dried over Na_2SO_4, filtered, and evaporated in a rotatory evaporator to obtain the crude mesylate. This was dissolved in 33 mL of DMSO, 4 Å molecular sieve (1.85 g), KI 0.75 g (11.43 mmol), and KCN 3.38g (51.9 mmol) were successively added, and the reaction was stirred during 4 h at 60 °C. The reaction was diluted with water and extracted with AcOEt; the organic phase was washed with aqueous saturated solution of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and evaporated with a rotatory evaporator. The residue was purified by FCC (hexane/AcOEt = 9:1).

Data for Compound ***10***. Yield: 57%, colorless oil, $[\alpha]^{23.5}$ = −81.5 (c 1, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.36−7.29 (m, 4H), 7.28−7.23 (m, 2H), 3.90 (d, J = 13.1 Hz, 1H), 3.47 (d, J = 13.0 Hz, 1H), 3.05−2.97 (m, 1H), 2.87−2.80 (m, 1H), 2.48−2.34 (m, 2H), 2.30 (td, J = 9.4, 7.0 Hz, 1H), 2.17−2.04 (m, 1H), 1.91−1.80 (m, 1H), 1.78−1.70 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 139.0, 128.6, 128.3, 127.1, 118.4, 59.7, 58.7, 54.4, 30.9, 23.4, 22.5. HRMS (ESI): calculated for $C_{13}H_{17}N_2^+$ [M + H]$^+$, 201.1391; found, 201.1394 (1.0). IR (ATR) υ_{max}: 2953, 2797, 2246, 1453 cm^{-1}.

Preparation of Compound ***11***. To a stirred solution of **10** (1.2 g (6 mmol)) in 20 mL of anhydrous diethyl ether was slowly added a suspension of $LiAlH_4$ (0.68g (17.97 mmol)) in 20 mL of diethyl ether at 0 °C. The mixture was stirred for 12 h at room temperature before the reaction was quenched with NaOH (10% in water); the suspension was filtered over Celite, the solid residue was washed with DCM, and then the filtrate was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was obtained with a sufficient purity thus purification was not needed.

Data for Compound ***11***. Yield: 82%, colorless oil, $[\alpha]^{23.3}$ = −81.5 (c 1, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.34−7.28 (m, 4H), 7.26−7.21 (m, 1H), 4.06 (d, J = 12.7 Hz, 1H), 3.16 (d, J = 12.7 Hz, 1H), 2.90 (ddd, J = 9.8, 7.4, 2.8 Hz, 1H), 2.84 (ddd, J = 12.2, 8.9, 5.7 Hz, 1H), 2.72 (ddd, J = 12.2, 8.7, 6.2 Hz, 1H), 2.42 (qd, J = 8.0, 3.4 Hz, 1H), 2.09 (td, J = 9.3, 8.1 Hz, 1H), 1.98−1.91 (m, 1H), 1.91−1.81 (m, 1H), 1.75−1.62 (m, 2H), 1.58−1.46 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 139.5, 129.0, 128.2, 126.8, 62.4, 58.7, 54.1, 39.5, 37.7, 30.2, 22.1. HRMS (ESI): calculated for $C_{13}H_{20}N_2Na^+$ [M + H]$^+$, 227.1524; found, 227.1525 (0.4)

Preparation of Compound ***12***. This compound was prepared following the same procedure described for **8**. In this case, 0.6 g (2.93 mmol) of **11** was reacted with 0.52 g (3.52 mmol) of *S*,-*N*-dimethylnitroisothiourea in DCM (15 mL). The crude was purified FCC (gradient from DCM/2-propanol = 9.5:0.5 to DCM/2-propanol = 8:2).

Data for Compound ***12***. Yield: 69%, colorless oil, $[\alpha]^{23.4}$= −60.6 (c 1, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.77 (s, 1H), 7.34−7.16 (m, 6H), 3.95 (d, J = 13.2 Hz, 1H), 3.36−3.17 (m, 3H), 2.87−2.71 (m, 4H), 2.55−2.49 (m, 1H), 2.20−2.06 (m, 1H), 1.98−1.80 (m, 2H), 1.69−1.55 (m, 3H), 1.54−1.45 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 158.2, 139.8, 129.2, 128.5, 127.1, 61.6, 58.2, 53.7, 38.8, 29.9, 28.6, 22.4. HRMS (ESI): calculated for $C_{14}H_{22}N_5O_2^+$ [M + H]$^+$, 306.1925; found, 306.1930 (−1.3).

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Preparation of Compound ***13***. This compound was prepared following the same procedure described for **9**. In this case, 0.25 g (1.22 mmol) of **11** was reacted with 0.19 g (1.46 mmol) of *S*-methylnitroisothiourea. The crude was purified using FCC (DCM/2-propanol/MeOH = 8.5:1.5:0.1).

Data for Compound ***13***. Yield: 56%, colorless oil, $[\alpha]^{23.5}$ = −63.4 (*c* 1, MeOH). ^{1}H NMR (500 MHz,$CDCl_3$) δ: 8.91 (s, 1H), 8.43 (s, 2H), 7.38−7.27 (m, 5H), 3.87 (d, *J* = 12.7 Hz, 1H), 3.46−3.20 (m, 3H), 2.97 (ddd, *J* = 10.8, 7.4, 3.7 Hz, 1H), 2.84−2.73 (m, 1H), 2.38 (q, *J* = 9.8, 9.2 Hz, 1H), 2.02−1.93 (m, 1H), 1.92−1.82 (m, 2H), 1.81−1.66 (m, 2H), 1.65−1.55 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.2, 138.0, 129.2, 128.6, 127.6, 62.0, 59.2, 53.5, 38.1, 31.5, 29.2, 22.5. HRMS (ESI) calculated for $C_{14}H_{22}N_5O_2^+$ $[M + H]^+$, 292.1768; found, 292.1773 (0).

Preparation of Compound ***14***. A sample of 2.48 g of $LiAlH_4$ (65.14 mmol) was suspended in 65 mL of THF; afterward 5.0 g (43.43 mmol) of *S*-proline was added at 0 °C. The mixture was refluxed for 2 h under nitrogen atmosphere. After cooling the reaction, a solution of KOH (10% in water) was added; next the mixture was filtered and the residue was refluxed with 50 mL of THF for 1 h and then filtered again. The organic extracts were combined, dried over Na_2SO_4, and evaporated under reduced pressure at ∼40 °C. The residue was used in the next reaction without further purification.

Data for Compound ***14***. Yield: 83%, brown oil. Spectral data matched with previous reports;[21] the ^{1}H NMR data is provided here for convenience. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.60−3.54 (m, 1H), 3.39−3.32 (m, 2H), 3.18−3.04 (m, 3H), 3.01−2.87 (m, 2H), 1.90−1.67 (m, 2H), 1.50−1.39 (m, 1H).

Preparation of Compound ***15***. A 6.2 mL sample of triethylamine (4.5 g, 44.48 mmol) was slowly added to a stirred solution of **14** (3.0 g (29.6 mmol)) in a 45 mL mixture of 1,4-dioxane/water 1:1, and the reaction was stirred for 15 min. Di-*tert*-butyldicarbonate (8.4 g (38.55 mmol)) in 10 mL of 1,4-dioxane was added dropwise at 0 °C. The reaction was stirred during 12 h. The reaction was diluted with AcOEt (100 mL) and the organic phase was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified using FCC (hexane/AcOEt = 1:1).

Data for Compound ***15***. Yield: 90%, colorless oil. Spectral data matched with previous reports;[21] the ^{1}H NMR data is provided here for convenience. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.85−4.77 (m, 1H), 4.02−3.94 (m, 1H), 3.69−3.55 (m, 2H), 3.52−3.42 (m, 1H), 3.36−3.27 (m, 1H), 2.07−1.97 (m, 1H), 1.80 (dh, *J* = 26.5, 6.5 Hz, 2H), 1.57−1.50 (m, 1H), 1.47 (s, 9H).

Preparation of Compound ***16***. A solution of **15** in 11 mL of DCM was added to a stirred solution of oxalyl chloride 0.71 mL (1.06g, 8.35 mmol) in 11 mL of DCM cooled at −78 °C, then a solution of DMSO 0.54 mL (0.59g, 7.55 mmol) in 11 mL of DCM was slowly added. After 5 min. The reaction was stirred 20 min and 4.84 mL of Et_3N (3.51g, 34.68 mmol) was added in a single portion; the mixture was stirred for another 10 min and then allowed to warm to room temperature. The mixture was diluted with additional DCM and afterward was washed with saturated aqueous solution of NH_4Cl and brine; the organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The crude was used in the next reaction without any purification.

Afterward, a stirred solution of triethyl phosphonoaceate (1.66 mL (1.87g, 8.36 mmol)) in anhydrous THF (10 mL) was added dropwise in a suspension of NaH (0.2 g (8.36 mmol)) in 5 mL of anhydrous THF at 0 °C; after the addition, the reaction was stirred at room temperature for 30 min, then the mixture was cooled at 0 °C and the crude *N*-Boc-*S*-prolinal was added dropwise in anhydrous THF (10 mL) to the mixture reaction. The reaction was stirred for 8 h at room temperature. MeOH (1 mL) was added to quench the reaction, solvents were evaporated under reduced pressure, and the residue was solubilized in AcOEt, washed with brine, dried over Na_2SO_4, filtered, and evaporated. The crude was purified by FCC (hexane/AcOEt = 7:3).

Data for Compound ***16***. Yield: 81%, slightly yellow oil, $[\alpha]^{23.5}$ = −28.9 (*c* 0.1, $CHCl_3$). Some signals are duplicated due to two rotamers that were observed under the conditions in which the spectrum was acquired, and the asterisk indicates the minor one. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.87−6.78 (m, 1H), 5.85−5.80 (m, 1H), 4.51* (brs, 1H), 4.22−4.14 (m, 2H), 3.46−3.36 (m, 2H), 2.13−2.08 (m, 1H), 1.9−1.83 (m, 2H), 1.8−1.75 (m, 1H), 1.46* (s, 9H), 1.42 (s, 9H), 1.31, 1.29 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5, 154.3*, 154.3, 148.5, 148.2*, 120.4, 120.0*, 79.7, 79.6*, 60.3, 60.0*, 57.8, 57.5*, 46.6*, 46.2, 31.7, 30.8*, 28.5*, 28.4, 23.5*, 22.9, 14.25. HRMS (ESI): calculated for $C_{14}H_{23}NNaO_4$ $[M + Na]^+$, 292.1524; found, 292.1525 (3.4 ppm).

*Preparation of Compound Int-***17**. In a round-bottom flask equipped with a magnetic stirrer was prepared a solution of **16** (1.73g (6.42 mmol)) in 20 mL of ethanol, and subsequently 173 mg of Pd(OH) (10% w/w) was added. Then the reaction was purged using vacuum and then refilled with a balloon of hydrogen; this heterogeneous solution was stirred for 2 h. Finally, the mixture was filtered over a small pad of Celite and silica, the solid was washed with DCM, and the filtrate was evaporated under reduced pressure.

Data for Compound ***Int-17****: Yield*. 95%, slightly yellow oil, $[\alpha]^{23.5}$ = −48.5 (*c* 0.1, $CHCl_3$). Some signals are duplicated due to two rotamers that were observed under the conditions in which the spectrum was acquired, the asterisk indicates the minor one. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.11 (q, *J* = 7.0 Hz, 2H), 3.88−3.73 (m, 1H), 3.48−3.24 (m, 2H), 2.28 (q, *J* = 8.0, 7.4 Hz, 2H), 2.08−1.75 (m, 4H), 1.73−1.56 (m, 2H), 1.45 (s, 9H), 1.24 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.7, 173.4*, 155.9*, 154.7, 79.3*, 78.9, 60.3, 56.6, 46.4*, 46.1, 31.5*, 31.2, 30.7, 29.9, 29.6*, 28.5, 28.4*, 24.8, 23.7*, 23.0. HRMS (ESI): calculated for $C_{14}H_{25}NNaO_4^+$ $[M + Na]^+$, 294.1681; found, 294.1684 (1.0 ppm).

Preparation of Compound ***17***. A 4.2 mL (0.18 g, 8.39 mmol) sample of a solution of 2.0 M $LiBH_4$ in THF was added dropwise to a stirred solution of **Int-17** (1.74 g (6.46 mmol)) in anhydrous THF (30 mL) at room temperature; the stirring was continuous for 12 h. Next, the reaction was quenched by the careful addition of 3 mL of MeOH, and volatiles were evaporated under reduced pressure; the residue was partitioned in AcOEt/water (3:1) (50 mL), the organic phase was separated, and the remaining aqueous phase was extracted with additional AcOEt. The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated. The residue was purified by FCC (Hexane/AcOEt = 1:1).

Data for Compound ***17***. Yield: 95%, colorless oil, $[\alpha]^{23.5}$ = −47.14 (*c* 0.1, $CHCl_3$). Some signals are duplicated due to two rotamers that were observed under the conditions in which the spectrum was acquired; the asterisk indicates the minor one. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.67 (brs, 2H), 3.44−3.23 (m, 2H), 2.01 (brs, 1H), 1.97−1.76 (m, 4H), 1.70−1.51 (m, 3H), 1.46 (s, 9H), 1.39 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 154.8, 79.1, 62.7*, 62.4, 57.0*, 56.6, 46.5, 46.1*, 30.8, 30.2, 29.5*, 29.1, 28.6, 23.7, 23.0*. HRMS (ESI): calculated for $C_{12}H_{23}NNaO_3^+$ $[M + Na]^+$, 252.1575; found, 252.1584 (3.2 ppm).

Preparation of Compound ***18***. The procedure used to synthesize **18** was identical to that described to obtain *N*-Boc-*S*-prolinal. In this case 1.39 g (6.06 mmol) of **17**, 0.56 mL (0.85 g, 6.66 mmol) of oxalyl chloride, 0.51 mL (0.57 g, 7.28 mmol mmol) of DMSO, and 4.22 mL (3.06 g, 30.24 mmol) were used to perform the reaction. The crude was purified in FCC (hexane/AcOEt = 8:2).

Data for Compound ***18***. Yield: 86%, colorless oil, $[\alpha]^{25.2}$ = −7.82 (*c* 0.1, $CHCl_3$). Some signals are duplicated due to two rotamers that were observed under the conditions in which the spectrum was acquired; the asterisk indicates the minor one. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.77 (s, 1H), 3.93−3.75 (m, 1H), 3.50−3.25 (m, 2H), 2.58−2.37 (m, 2H), 2.05−1.80 (m, 3H), 1.78−1.69 (m, 1H), 1.67−1.57 (m, 2H), 1.46 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 202.3, 201.8*, 155.1*, 154.9, 79.5, 79.2*, 56.4, 46.6, 46.2*, 40.9, 40.7*, 30.7, 30.4*, 30.2, 28.5, 28.4*, 27.0, 23.7, 23.01*, 14.4. HRMS (ESI): calculated for $C_{12}H_{21}NNaO_3^+$ $[M + Na]^+$ 250.1419, found 250.1422 (1.2 ppm).

Preparation of Compound ***19***. A 0.56 mL (0.57 g, 2.89 mmol) sample of *N*,*N*-dibenzylamine was added to a stirred solution of 0.6 g (2.63 mmol) of **18** and 0.48 mL of Et_3N (0.34 g, 3.36 mmol) in

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1,2-DCE (20 mL) at 0 °C, and then the mixture was stirred for 5 min. Afterward, 1.12 g (5.28 mmol) of sodium triacetoxyborohydride was added in one portion. After the mixture was stirred for 16 h at room temperature, saturated aqueous sodium bicarbonate solution was added, the organic phase was separated, and the aqueous phase was extracted with AcOEt. The organic extracts were combined, dried over Na_2SO_4, filtered, and evaporated. The residue was purified in FCC (hexane/AcOEt = 8.5:1.5).

Data for Compound ***19***. Yield: 93%, colorless oil, $[\alpha]^{25.4}$ = −32.36 (*c* 0.1, $CHCl_3$). Some signals are duplicated due to two rotamers that were observed under the conditions in which the spectrum was acquired; the asterisk indicates the minor one. ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 7.39−7.35 (m, 4H), 7.34−7.29 (m, 4H), 7.27−7.21 (m, 2H), 3.81−3.62 (m, 1H), 3.61−3.50 (m, 4H), 3.44−3.23 (m, 2H), 2.50−2.37 (m, 2H), 1.93−1.74 (m, 3H), 1.61−1.55 (brs, 2H), 1.54−1.39 (m, 11H), 1.34−1.21 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 154.6, 139.8, 128.7, 128.1, 126.7, 78.9, 58.2, 57.2, 53.6*, 53.4, 46.4*, 46.0, 32.5, 31.8*, 30.7, 29.9*, 28.5, 26.9*, 23.93, 23.09. HRMS (ESI): calculated for $C_{26}H_{37}N_2O_2^+$ $[M + H]^+$, 409.2855; found, 409.2866 (2.7 ppm).

Preparation of Compound ***20***. A 48 mg sample of Pd/C (10% w/w) was added to a solution of **19** (0.48 g (1.17 mmol)) in methanol (15 mL), and the heterogeneous mixture was stirred during 48 h under H_2 atmosphere. The mixture was filtered through a pad of Celite, the solid was washed with DCM, the filtrate was evaporated under reduced pressure to obtain the amine which was used in the next reaction without any further purification.

Data for Compound ***20***. Yield: 93%, colorless oil, $[\alpha]^{22}$ = −39.9 (*c* 0.1, $CHCl_3$). Some signals are duplicated due to two rotamers that were observed under the conditions in which the spectrum was acquired; the asterisk indicates the minor one. ^{1}H NMR (500 MHz, DMSO-d_6, 50 °C) *δ*: 3.69−3.59 (m, 1H), 3.30−3.23 (m, 1H), 3.23−3.13 (m, 1H), 2.66−2.55 (m, 2H), 1.94−1.69 (m, 3H), 1.60 (brs, 2H), 1.40 (s, 9H), 1.36 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6, 50 °C) *δ*: 154.0, 78.5, 57.0, 46.5, 41.4, 32.1*, 31.5, 30.7*, 29.9, 28.9, 28.7*, 28.6, 23.7, 23.0*. HRMS (ESI): calculated for $C_{12}H_{25}N_2O_2^+$ $[M + H]^+$, 229.1916; found, 229.1918 (0.9).

Preparation of Compound ***21***. This compound was prepared following the same procedure described for **9**. In this case 0.6 g (2.62 mmol) of **20** was reacted with 0.47 g (3.15 mmol) of *S*-methyl-*N*-methylnitroisothiourea in DCM (15 mL). The crude was purified in FCC (hexane/AcOEt = 6:4).

Data for Compound ***21***. Yield: 62%, colorless oil, $[\alpha]^{22.7}$ = +12.12 (*c* 0.1,$CHCl_3$). Some signals are duplicated due to the two rotamers that were observed under the conditions in which the spectrum was acquired; the asterisk indicates the minor one. ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 9.07 (brs, 1H), 7.25 (brs, 1H), 3.63 (brs, 1H), 3.30−3.11 (m, 4H), 2.77 (s, 2H), 1.95−1.69 (m, 3H), 1.60 (s, 2H), 1.53−1.42 (m, 2H), 1.39 (s, 9H), 1.34−1.20 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) *δ*: 159.5, 158.3, 79.7*, 79.5, 55.1, 46.5, 46.4*, 41.6, 41.3*,40.9, 31.2, 30.7*, 28.5, 28.4, 25.4, 23.5, 23.3*. HRMS (ESI): calculated for $C_{14}H_{27}N_5NaO_4^+$ $[M + Na]^+$, 352.1960; found, 352.1956 (−1.4).

Preparation of Compound ***23***. A 0.43 mL (0.47 g, 6.0 mmol) sample of acetyl chloride in DCM (1 mL) was added dropwise at 0 °C to a stirred solution of **21** (0.5 g (1.51 mmol)) in a blend of DCM/MeOH 2:1 (3 mL). The reaction was stirred for 2 h. Then, the solvents were evaporated under reduced pressure to obtain the intermediate hydrochloride (0.40 g (1.5 mmol)), which was dissolved in acetonitrile (20 mL). Next, tetrabutylammonium iodide (0.61 g (1.65 mmol)), potassium carbonate (0.52 g (3.76 mmol)), and benzyl bromide (0.18 mL (0.27 g, 1.57 mmol)) were added sequentially. After the reaction was refluxed during 8 h, the mixture was filtered and the remaining solid was washed with DCM and evaporated. The residue was purified using FCC (DCM/2-propanol/NH_4OH = 8:3:0.2).

Data for Compound ***23***. Yield: 54%, colorless oil, $[\alpha]^{24.5}$ = −39.96 (*c* 0.1,$CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 9.32 (br s, 1H), 7.38−7.27 (m, 5H), 6.43 (br s, 1H), 4.02 (d, *J* = 12.7 Hz, 1H), 3.48 (br s, 1H), 3.34 (d, *J* = 12.7 Hz, 1H), 3.31−3.21 (m, 2H), 3.03 (ddd, *J* = 10.4, 7.3, 3.2 Hz, 1H), 2.76 (d, *J* = 4.8 Hz, 3H), 2.72−2.64 (m, 1H), 2.32 (dd, *J* = 9.1 Hz, 1H), 2.04−1.95 (m, 1H), 1.83−1.53 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 158.3, 137.3, 129.5, 128.5, 127.7, 64.3, 59.0, 54.4, 41.9, 29.7, 29.5, 28.1, 25.0, 22.1. HRMS (ESI): calculated for $C_{16}H_{26}N_5O_2^+$ $[M + H]^+$, 320.2086; found, 320.2087 (0.9).

Preparation of Compound ***24***. A 0.44 g (3.29 mmol) sample of *S*-methylnitroisothiourea was added in one portion to a stirred solution of *N*-Boc-(*S*)-prolinamine 0.55 g (2.80 mmol) in DCM (20 mL) at room temperature. The reaction was stirred for 12 h, and the volatiles were evaporated under reduced pressure. Finally, the residue was purified using FCC (hexane/AcOEt = 4:6).

Data for Compound ***24***. Yield: 33%, white solid, $[\alpha]^{26}$ = −17.43 (*c* 1, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 9.18 (brs, 1H), 8.78 (brs, 2H), 3.84−3.73 (m, 1H), 3.44−3.26 (m, 3H), 3.23−3.13 (m, 1H), 2.11−2.02 (m, 1H), 1.98−1.82 (m, 3H), 1.47 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 159.3, 154.8, 79.6, 55.4, 45.5, 43.2, 28.4, 27.4, 22.5. HRMS (ESI): calculated for $C_{11}H_{21}N_5NaO_4^+$ $[M + Na]^+$, 310.1941; found, 310.1490 (−0.3).

Preparation of Compound ***25***. A 0.4 mL (0.44 g, 5.57 mmol) sample of acetyl chloride in DCM (1 mL) was added dropwise to a stirred solution of **24** (0.4 g (1.4 mmol)) in a blend of DCM/MeOH 2:1 (3 mL) at 0 °C. After the mixture was stirred for 2 h, the solvents were evaporated, and the residue was purified using FCC (AcOEt/MeOH = 8:2).

Data for Compound ***25***. Yield: 77%, yellow oil, $[\alpha]^{21}$ = 12.8 (*c* 1, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6, 50 °C) *δ*: 8.19 (brs, 4H), 3.71−3.62 (m, 1H), 3.57−3.45 (m, 2H), 3.24−3.11 (m, 2H), 2.12−2.01 (m, 1H), 2.00−1.81 (m, 2H), 1.64 (dq, *J* = 12.8, 8.1 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6, 50 °C) *δ*: 159.8, 58.5, 45.5, 42.2, 27.9, 23.5. HRMS (ESI): calculated for $C_6H_{14}N_5O_2^+$ $[M + H]^+$, 188.1147; found, 188.1151 (2.1).

Biological Assay. The insecticidal activities of title compounds against *Xyleborus affinis* were tested according to the previously reported procedure of the contact toxicity on filter paper.[20] A Whatman grade 42, ashless filter paper (5 cm diameter) was placed in a glass Petri dish (50 × 17 mm diameter). An aliquot of 0.25 mL of the solution of a 0.05 M compound in dimethyl sulfoxide/water (1:1) was applied uniformly to the filter paper disc; the mixture of solvents was used as the negative control, and the solution of dinotefuran at the same concentration was used as positive control. The solvent was allowed to distribute evenly for 5 min prior to the introduction of five adult insects into each dish. Since the boiling point of dimethyl sulfoxide and water are high enough to be volatilized at room temperature, it is not necessary to replenish the solvent mixture. According to statistical requirements, each treatment was replicated three times at 25 °C ± 1 °C with the organism grown in the laboratory. *Xyleborus affinis* were reared in an artificial media according to Biedermann et al.[22] with some modifications. Rearing media were maintained in a climatic chamber at 26 °C and 60% of RH in complete dark. Adults were obtained at 30 days after female inoculation by dissecting the media culture. Insect mortalities were recorded after 12 h. Insects were presumed dead if they remained immobile and did not respond to three probings with a blunt dissecting probe after a 5 min recovery period.

Docking Study. The high nAChR inhibitory activity of compound **9** was chosen to perform the ligand−protein interaction, and AutoDock 4.2 was used to carry out the molecular docking simulations. Because the amino acids forming the active pockets are both structurally and functionally consistent in the diverse nAChRs and AchBPs, the crystal structure of the *Aplysia californica* binding protein (aChBP) complex with the neonicotinoid imidacloprid (PDB: 3C79) was used as the template to construct the models.[23,24] The receptor was prepared for docking, and the lower energy conformations of each compound were optimized by the semi-empirical method PM3. The compound **9** and dinotefuran were flexibly docked automatically in the active site of nAChR. The structure was prepared using the UCSF-Chimera software by removing waters and cocrystallized ligands, and then adding Gasteiger charges and polar hydrogens. The search space in the

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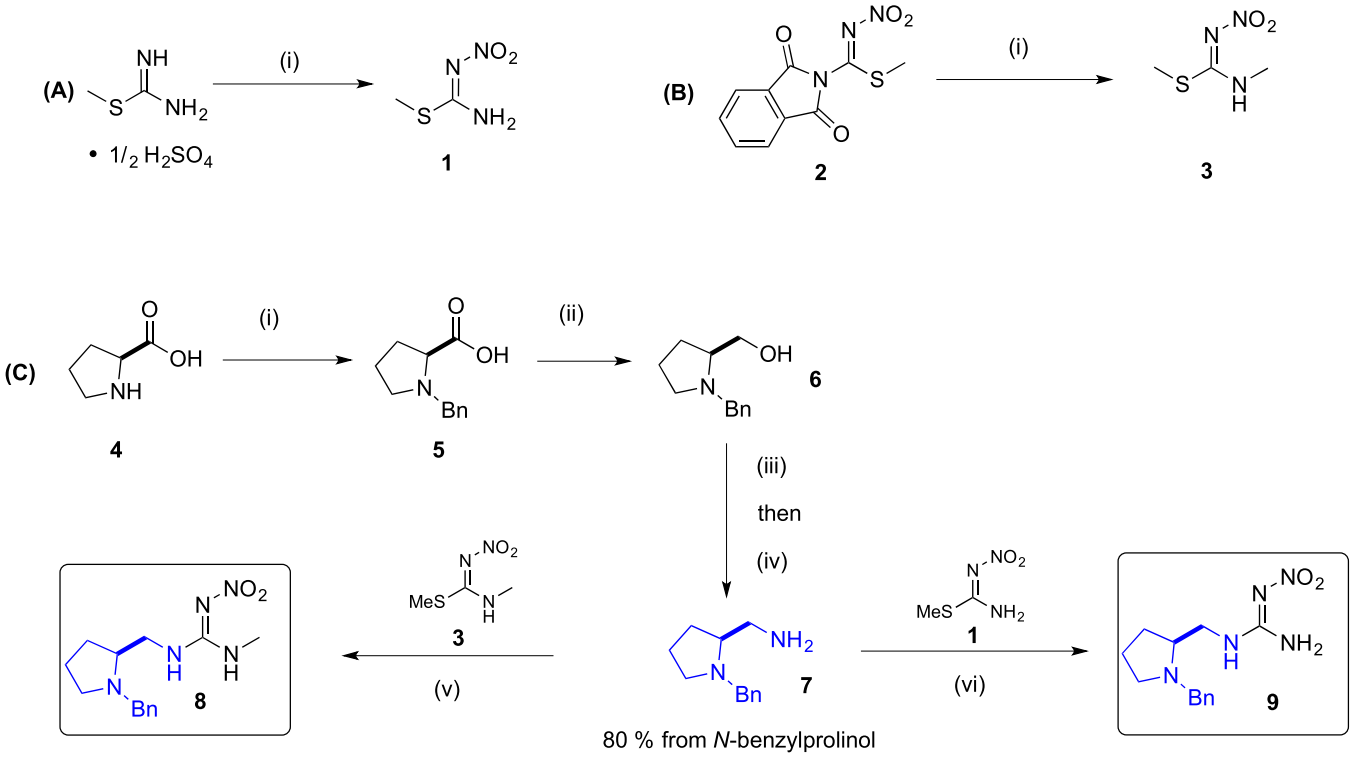
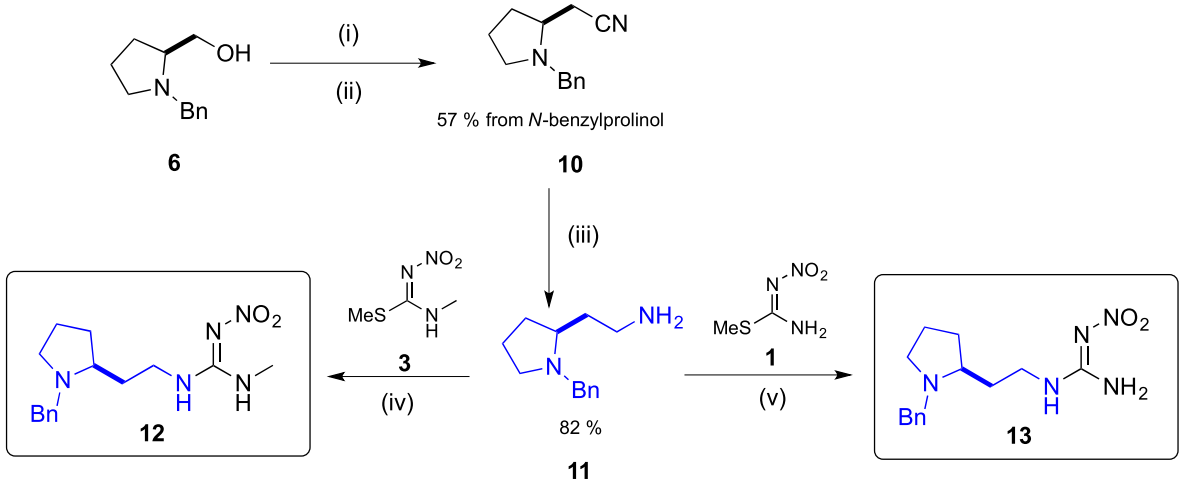
Scheme 1. Synthesis of Target Molecules 8 and 9[a]



[a](A) (i) HNO_3, H_2SO_4, 6 h, 0 °C. (B) CH_3NH_2 2 M, CH_3CN, 0 °C to r.t., 24 h. (C) (i) KOH, BnCl, *i*-PrOH, r.t., 40 °C, 6 h; (ii) $LiAlH_4$, THF, 0 °C to room temperature, 12 h; (iii) Phtalamide, PPh_3, DEAD, THF, reflux, 8 h; (iv) Hidrazine, EtOH, r.t., reflux, 2 h; (v) and (vi) CH_2Cl_2, r.t, 18 h.

Scheme 2. Synthesis of Compounds 12 and 13[a]



[a]Reagents and conditions: (i) MsCl, Et_3N, CH_2Cl_2, 0 °C, 2 h; (ii) NaCN, DMSO, 60 °C, 4 h; (iii) $LiAlH_4$, r.t., 12 h; (iv) and (v) CH_2Cl_2, r.t., 12 h.

protein (grid-box) was calculated using the AutoDockTools and AutoGrid softwares.

The molecular docking was performed on AutoDock4.2 software using 100 runs and 5 million energy evaluations; the remaining parameters were set as default.

For each cluster, the conformation with the lowest binding energy in the binding site was chosen for further analysis and comparison. AccelrysDS visualizer 2.5 [Accelrys Inc., San Diego, CA (2009)] was used for molecular modeling to determine their binding orientations and interactions.

Neurotoxic Effect. Female rats of the Wistar strain of 220−250 g of weight were used to test the neurotoxic effect of Dinotefuran and compound **9**. The guidelines of the Official Mexican Standard (NOM-062-ZOO-1999), as well as the protocol and norms of the international and universal bioethics committee were followed to care and use the laboratory animals. The animals were in an area with a regulated temperature of 25 ± 2 °C, a relative humidity of 50 ± 15%, and dark light cycles of 12 h each.

Animals were divided into three groups: The first group was given a 1000 mg/kg dose of dinotefuran as a positive control, the second group was given a 1000 mg/kg dose of compound **9**, and the third group was given 2 mL/kg of saline as a control. Studies were conducted by the EPA using a lethal dose 50 of double (LD50 = 2000 mg/kg).

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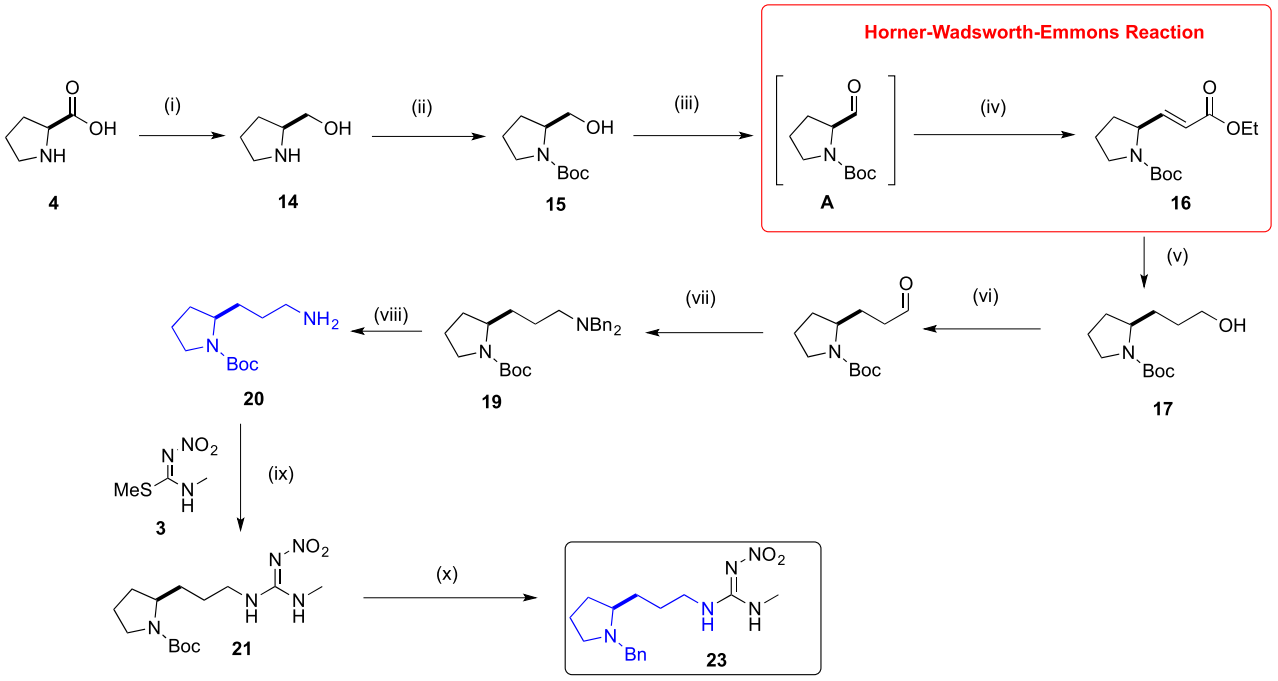
Scheme 3. Synthesis of Compounds 21 and 23[a]



[a]Reagents and conditions: (i) $LiAlH_4$, THF, reflux, 2 h; (ii) Boc_2O, Et_3N, 1,4-diox/H_2O, 12 h; (iii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 45 min; (iv) $(MeO)_2OPCH_2CO_2Et$, NaH, THF, 0 °C, 8 h; (v) H_2, Pd/C, EtOH, 2 h then $LiBH_4$, THF, r.t., 12 h; (vi) Dess-Martin periodinane, CH_2Cl_2; (vii) $NHBn_2$, $NaBH(OAc)_3$, DCE, r.t; (viii) H_2, Pd/C, EtOH, r.t., 12 h; (ix) CH_2Cl_2, r.t., 8 h; (x) AcCl, MeOH, 0 °C then K_2CO_3, TBAI, BnBr, CH_3CN.

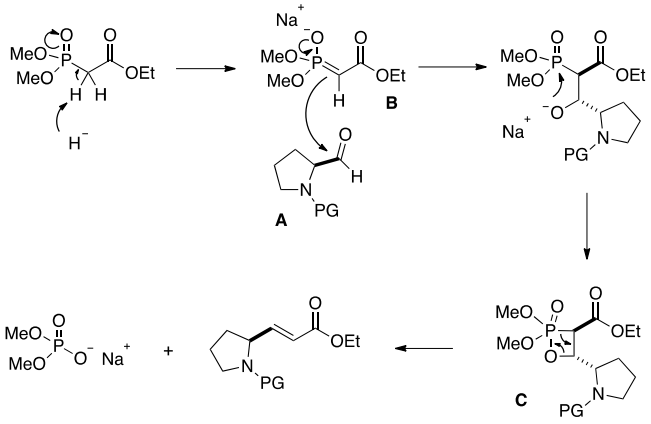
For animal training, the equipment was adjusted to a constant speed of 16 rpm. Animals were placed on the rotating rod for 10 min and the number of falls and the time in which they had the first fall were evaluated. This procedure was carried out during three consecutive days. On the fourth day, the training test was repeated to make sure the animals were not uncoordinated and healthy. Thereafter, the test compounds dinotefuran, compound **9**, and saline were orally administered. One hour after the injection, it was evaluated the number of falls and the time for the first fall. It was used with the same parameters of speed (16 rpm) and the time (10 min) to identify any alteration in the locomotive activity of the rat, which indicated a secondary effect in the central nervous system level. Results were expressed as the average ± standard error and analyzed by a one−way ANOVA followed by a Tukey to compare differences between groups.

RESULTS AND DISCUSSION

Synthesis of Derivatives. The synthesis of the target molecules **8** and **9** was shown in Scheme 1. First, compound **1** was prepared using a solution of HNO_3 and H_2SO_4 during 6 h at 0 °C, and then compound **3** was prepared using as a starting material the dioxoisoindoline **2** and a solution of methylamine in acetonitrile [see Scheme 1A,B]. Then, after the *N*-protection of the L-proline, this was reduced with lithium aluminum hydride to obtain the *N*-benzyl prolinol **6**. The Mitsunobu type reaction of **6**, followed by the reaction with hydrazine in ethanol delivers the diamine **7**. Finally, this diamine was coupled with the compounds **1** or **3** to obtain the nitroguanidine **9** and **8**, respectively.

The L-proline derivatives with two methylenes between the nitroguanidine and the pyrrolidine moiety were synthesized starting from the *N*-benzyl prolinol **6**; this alcohol was converted to the cyano derivative through an S_N2 reaction. Next, the reduction of the cyano compound **10** gave the diamine **11** in 82% yield (Scheme 2). Finally, this diamine was coupled with the compounds **1** or **3** to obtain the nitroguanidines **13** and **12**, respectively.

Scheme 4. Mechanism of the Horner−Wadsworth−Emmons Reaction



The synthesis of the derivatives with three methylenes is shown in Figure 4. Here, the L-proline was reduced to the amino alcohol **14**, followed by the protection of the nitrogen with Boc_2O to furnish the *N*-Boc protected amino alcohol **15**. Then, the alcohol was oxidized by a Swern reaction, which was followed by a Horner−Wadsworth−Emmons reaction to allow us to obtain the ethyl acrylate **16**. The compound **16** was reduced with lithium borohydride to obtain the alcohol **17**. Next, the oxidation of the alcohol **17** with Swern reaction, followed by reductive amination in the presence of dibenzyl amine, gave the diamine *N*-Boc protected **19**, which after catalytic hydrogenation provided the free diamine **20**.

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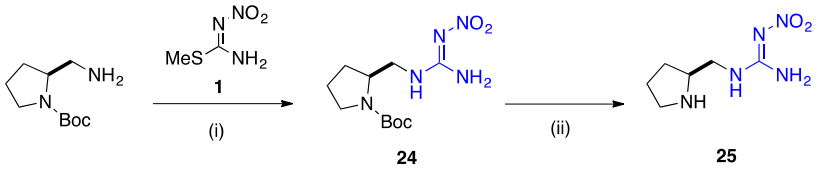
Scheme 5. Synthesis of Compound 25[a]



[a]Reagents and conditions: (i) DCM, r.t., 12 h; (ii) DCM/MeOH (2:1), AcCl, 2 h, 0 °C.

Table 1. Bioassay of L-Proline Neonicotinoids Derivatives

compound[a]	length carbon chain	mortality %[b]
dinotefuran	1	40
8	1	73
9	1	93
12	2	80
13	2	53
21	3	33
23	3	60
25	1	13

[a]A total of 0.25 mL of compound was used with a concentration 0.05 M. [b]The mortality was determined after 12 h.

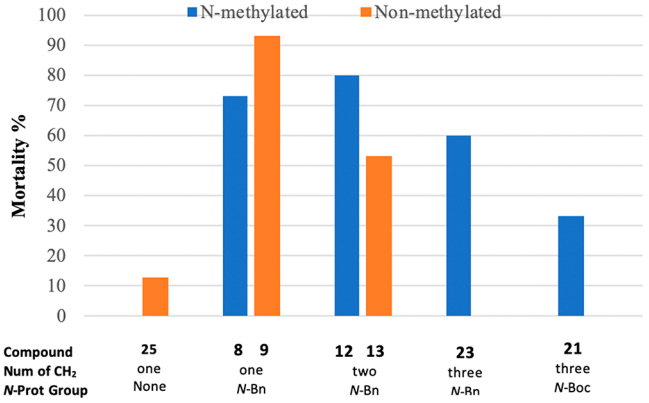


Figure 2. Effect of the length of carbons atoms on the insecticidal activity.

Scheme 6. Biological Activity of Opposite Enantiomers of α-Methylbenzylamine

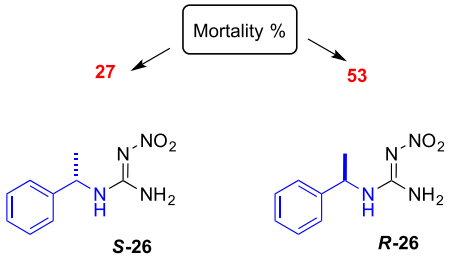


Table 2. Affinity and Inhibition Constant[a]

compound[a]	free energy (kcal/mol) "Score"	inhibition constant (K_i)
dinotefuran	−5.64	73.06 μM
9	−6.57	15.23 μM

[a]To perform the simulations, the parameters of 100 runs with 5 million evaluations were used for each molecular coupling experiment using a Lamarkian genetic algorithm.

Then, after the coupling with compound **3** the deprotection/protection reaction delivered the compounds **21** and **23**, respectively. Finally, compound **25** was synthesized in three steps starting from the 2-aminomethyl pyrrolidine (Scheme 3).

Most of the reactions showed in these synthetic procedures are nucleophilic substitutions, reduction, and oxidation reactions. Nonetheless, from the mechanistic point of view, the Horner−Wadsworth−-Emmons reaction is quite interesting. In Scheme 4, it is disclosed the mechanism reaction is similar to the mechanism of the Wittig reaction. Here, the stereochemistry is set by steric approach control, where the antiperiplanar approach of the carbanion **B** to the carbon of the carbonyl group of compound **A** is favored when the aldehydic hydrogen eclipses the bulky phosphoranyl moiety. As a result, the ester group is placed *syn* to the aldehyde pyrrolidine group, thus the alkene assumes *E*-orientation of these groups after rotation to form the oxaphosphetane **C**. Lastly, the resulting phosphate byproduct is readily separated from the desired products by simple washing with water.

Finally, Scheme 5 shows the synthesis of compounds **24** and **25**. As a starting material, diamine *N*-Boc was used to couple to compound **1**, then after the deprotection reaction compound **25** was obtained.

Insecticidal Activities. In general, all of the compounds showed from good to excellent insecticidal activity against *Xyleborus affinis*. In Table 1, it was shown that compounds **8** and **9** had an outstanding insecticidal activity of 73% and 93%, respectively, while the control dinotefuran had 40% mortality after 12 h. When the number of methylenes between the nitroguanidine and the pyrrolidine moiety is increased by two carbons, the insecticidal activity diminished to 80% and 53% for compounds **12** and **13**, respectively.

The bioassay of compounds **21** and **23** showed that the increased number of methylenes up to three lessen the insecticidal activity. Indeed, the percent mortality was reduced by up to 33%. The protecting group also played an important role in the insecticidal activity, and it was observed from Figure 2 that the *N*-benzyl group was indispensable to obtain high mortality. The use of Boc and free amine dramatically diminished the mortality percent up to 13%.

The analysis of nonmethylated compounds **9**, **13**, and **25** showed a negative impact on the insecticidal activity if the number of methylenes was increased. The *N*-methylated compounds had a similar behavior except for the compound **8** that had minor insecticidal activity compared to **12** (Figure 2). Finally, compound **9** without the *N*-benzyl moiety **25** was also studied; nonetheless, the bioassay showed 13% of mortality after 12 h.

As a part of our research program of biologically active and enantioenriched compounds, two experiments were run to demonstrate and gain insight into the importance of chirality

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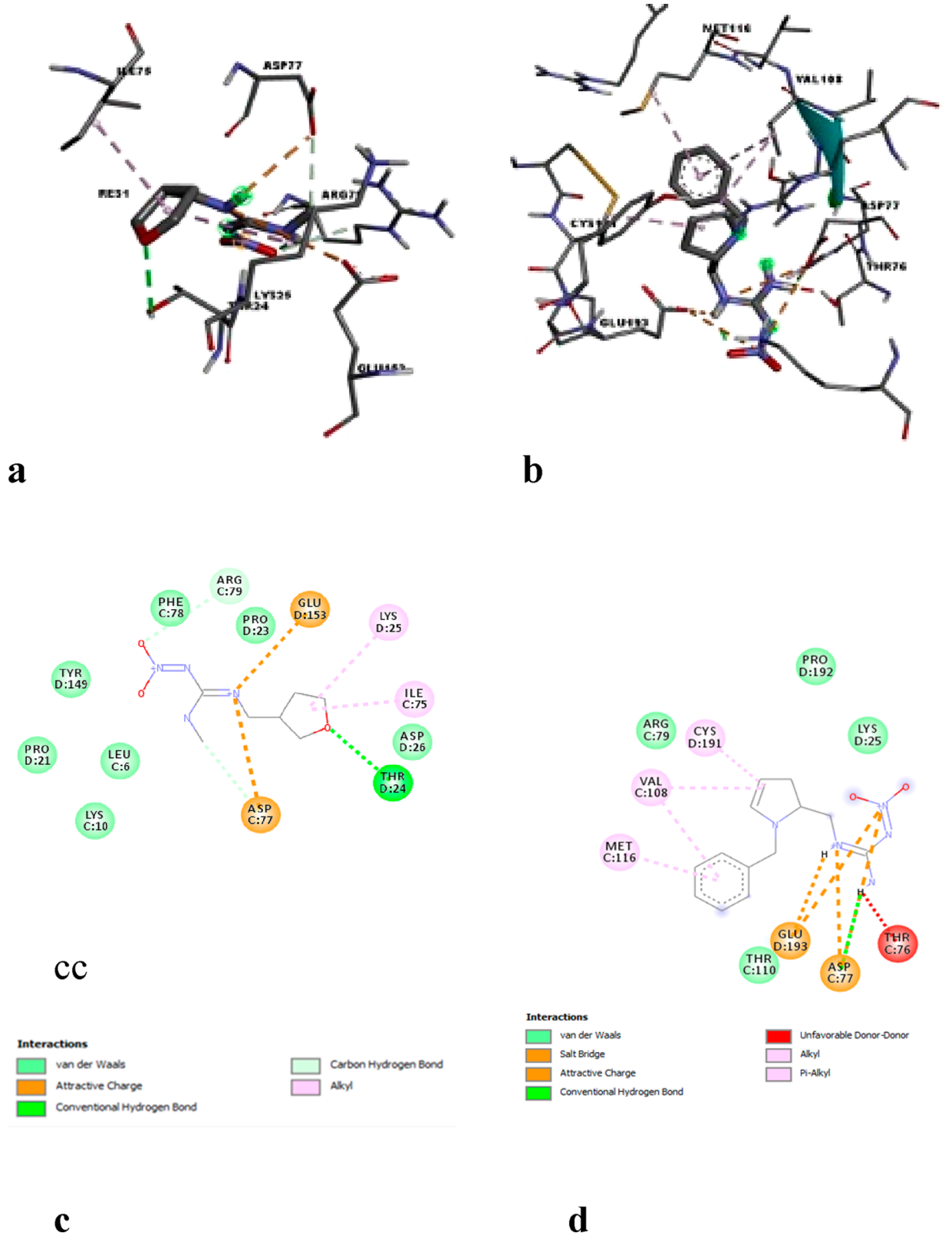

Figure 3. (a,c) The 2D and 3D representations of the interaction of dinotefuran with the residues of the search cavity in 3C79. (b,d) The 2D and 3D representations of the interaction of compound **9** with the residues of the search cavity in 3C79.

in the use of new neonicotinoids. Thus, the (*S*)-(−)- *α*-methylbenzylamine *S*-**26** and the (*R*)-(+)-*α*-methylbenzylamine *R*-**26** were used to synthesize opposite enantiomers of neonicotinoids. These two isomers are commercially available and cheap, and the synthesis to obtain the desired neonicotinoids is one-pot. The results are disclosed in Scheme 6. To our delight, the *R*-enantiomer had higher mortality than the *S*-enantiomer, 53 and 27, respectively.

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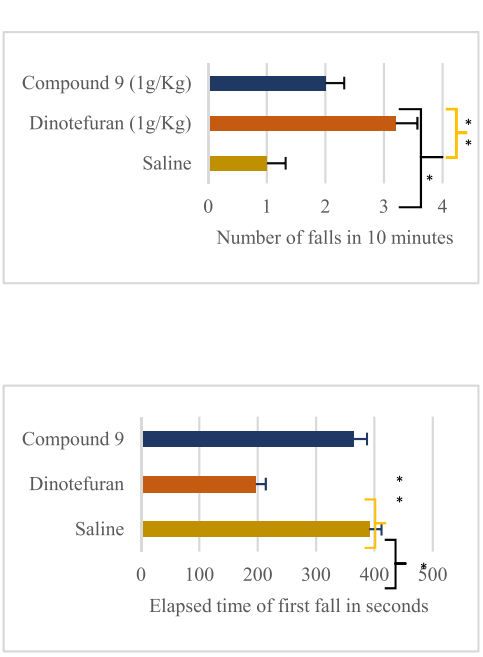

Figure 4. Neurotoxic evaluation of compound **9**. (A) Number of rat falls during the period of 10 min. (B) Elapsed time required for the first rat fall. Data are the mean ± SEM of six animals. * Significantly different from the saline group ($P \leq 0.001$) and ** significantly different between dinotefuran and compound **9** ($P \leq 0.001$) as determined by one-way ANOVA followed by the Tukey's test.

These results suggest that both the chirality and the absolute configuration of bioactive compounds play an essential role in the response produced in the insect.

Docking. The result obtained showed that the score of the compound **9** was −6.57 kcal/mol, whereas the dinotefuran was −5.64 kcal/mol; this suggests a better affinity with the receptor and to our delight this is in agreement with the experimental result (see Table 2, compound **9**). Another interesting result derived from docking was the inhibition constant, which was lower for compound **9** (15.23 uM) when compared to dinotefuran (73.06); these results suggest that compound **9** is an improved and more potent inhibitor since the concentration required to produce half-maximum inhibition is almost five times lower than dinotefuran (Table 2).

The 3D representation of the molecular recognition with the residues of the search cavity of the binding protein 3C79 (AChBP) with compound **9** and dinotefuran showed different sorts of interactions between them (see Figure 3). For instance, the dinotefuran showed interactions with the receptor in Phe78 and the oxygen of the nitro moiety, and the oxygen of the heterocyclic ring interacted with the Thr24 of the receptor. On the other hand, compound number **9** showed a higher degree of interactions between the receptor and nitroguanidine moiety of the neonicotinoid analog. Also, an interaction of the cation-π with the aromatic ring of compound **9** and the Met116 and Val108 was found, which plays an important role in neonicotinoid's insecticidal activity.[25]

Neurotoxic Effect. Albeit neonicotinoids have proven to be less toxic to the environment than others insecticidal compounds, some research had suggested the risk to the exposure of neonicotinoids to mammals and humans, mainly damaging their acetylcholine receptors.[26] In this regard, the neurotoxic effect of dinotefuran and compound **9** were analyzed using the RotaRod test with female rats of the Wistar strain. It was observed in Figure 4 that dinotefuran was more neurotoxic than compound **9**. The elapsed time required for the first fall and the number of falls was higher when the rats were administered with dinotefuran. These results suggest that compound number **9** is safer for mammalians than the commercially available dinotefuran.

In summary, a series of novel neonicotinoid's analogs were designed and synthesized by introducing the amino acid L-proline. To the best of our knowledge, this is the first report of neonicotinoids that incorporate an enantiopure L-amino acid. The bioassay results indicated that compound **9** had an excellent insecticidal activity. Hence, this sort of novel compound that emerged can be as a strategic model to develop new chiral and enantiopure neonicotinoids with high insecticidal activity and low toxicity to mammalians.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c05997.

Chemicals and instruments; data on 1H NMR, HRMS, optical rotation, and melting points of target compounds; complementary information on docking and neurotoxic effect. (PDF)

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Notes

The authors declare no competing financial interest.

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