SYNTHESIS AND CHARACTERIZATION OF SOME DISTYRYL-DERIVATIVES FOR AGRICULTURAL USES

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Three distyryl-containing compounds, namely, 2-((cyanomethyl)thio)-4,6-distyrylnicotinonitrile (**2**), 3-amino-4,6-distyrylthieno[2,3-*b*]pyridine-2-carbonitrile (**3**) and 2-((2-cyanoethyl)thio)-4,6-distyrylnicotinonitrile (**4**) have been prepared and characterized by elemental and spectroscopic analyses. The three compounds contain the pyridine moiety and are considered neonicotinoids analogues. Because neonicotinoids were considered the most effective pesticides, the biological activity of the distyryl-containing compounds as potential insecticides against cowpea aphid, *Aphis craccivora* Koch was evaluated. The agricultural bioactivity results of these compounds showed that the insecticidal activity varied from good to moderate against cowpea aphid insects.

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Introduction

It is known that heterocyclic compounds, especially pyridine derivatives, are widely used and different important applicationsfor these compounds have been previously reported.[1-6] So, the chemists around the world focused on the synthesis and the applications of these compounds. Some neonicotinoids contain pyridine moiety in their structure. Different advantages of neonicotinoids insecticides such as their high efficacy with lack cross-resistance, low mammalian toxicity, a novel mode of action specific for (nAChRs) and protection of great range of crops, resulted in a large use of these compounds in the field of crop protection at the present time.[7-13]

Genotoxic effect, oxidative stress, DNA damage, and clastogenic effect are different results that were found when the exposure to imidacloprid as neonicotinoid insecticide was monitored in some modern researches after long-term exposure of rabbits to that insecticide.[14-17] So, in view of the above results, the work in this paper was planned to prepare some neonicotinoids analogues compounds and screening their toxicological activity as insecticides against cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae) hoping to be with a higher insecticidal activity and lower toxicity.

Experimental

Melting points of the prepared compounds were determined by the means of a Fisher-Johns apparatus. IR spectra and elemental analyses were determined by a Pye-Unicam SP3-100 spectrophotometer using the KBr disk technique and a Vario EL C, H, N, S analyzer, respectively. ^{1}H NMR, ^{13}C NMR, and DEPT 135 spectra measurement was accomplished via a Bruker 400 MHz spectrometer in the presence of TMS as an internal reference. Chemical shifts are given in δ (ppm). The purity of the synthesized compounds was checked by TLC. 3-Cyano-4,6-distyrylpyridin-2(1*H*)-thione (**1**) was prepared according to the reported method.[5] The neonicotinoid insecticide (acetamiprid, purity > 98 %) was purchased from Sigma-Aldrich (France). Batches of cowpea aphid were gathered from faba bean, *Vicia faba* L., growing on the fields of the experimental farm of Assiut University. Compounds **2-4** and acetamiprid were screened for their insecticidal activity against the gathered cowpea aphids.

2-((Cyanomethyl)thio)-4,6-distyrylnicotinonitrile (2).

A mixture of compound **(1)** (2 g, 0.006 mol), chloroacetonitrile (0.006 mol), and fused sodium acetate (0.6 g, 0.007 mol) in ethanol (25 mL) was heated under reflux for 30 min. The formed precipitate was collected and recrystallized from ethanol-dioxane mixture (1:2) as pale yellow crystals of compound **2**. Yield 89 %. m. p. 121-122 °C. IR (ν) (KBr): 3026 (C-H aromatic), 2978, 2931, 2848 (C-H aliphatic), 2246 (C≡N aliphatic), 2211 (C≡N conjugated), 1634 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.20-8.08 (m, 15H, 2CH=CH and Ar-H), 4.36 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 162.45, 159.37, 149.51, 137.68, 136.12, 135.56, 130.44, 129.90, 129.44, 128.07, 126.48, 118.10, 116.10, 115.19, 102.07, 16.39. DEPT 135 (DMSO-d_6, 100 MHz): δ = 136.12 (CH), 135.56 (CH), 130.44 (CH), 129.90 (CH), 129.43 (CH), 128.06 (CH), 126.48 (CH), 115.19 (CH), 16.39 (CH_2). Anal.Calcd. for $C_{24}H_{17}N_3S$: C, 75.96; H, 4.52; N, 11.07; S, 8.45. Found: C, 75.97; H, 4.49; N, 11.06; S, 8.47.

3-Amino-4,6-distyrylthieno[2,3-*b*]pyridine-2-carbonitrile (3)

In addition to the procedure reported before,[18] compound **(3)** was synthesized here by suspending of compound **(2)** (0.005 mol) in sodium ethoxide solution (0.5 g of sodium in

31 mL of absolute ethanol) and heating for 5 min under reflux. The formed product after cooling was collected and recrystallized from ethanol-dioxane mixture (1:2) as orange crystals of compound **3**. Yield 90 %.m. p. 156-157 °C. IR (ν) (KBr): 3383, 3314, 3199 (NH_2), 3019 (C-H aromatic), 2917 (C-H aliphatic), 2198 (C≡N) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ = 7.31-7.86 (m, 16H, 2CH=CH, NH of NH_2 and Ar-H), 6.18 (s, 1H, NH of NH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ= 160.43, 156.61, 151.90, 144.76, 144.31, 135.19, 129.35, 129.21, 128.11, 128.05, 127.79, 127.34, 123.00, 117.25, 115.87, 76.23. DEPT 135 (DMSO-d_6, 100 MHz): δ = 129.35 (CH), 129.20 (CH), 128.11 (CH), 128.05 (CH), 127.79 (CH), 127.34 (CH), 123.00 (CH), 117.24 (CH). The mass spectrum of compound **3** showed a molecular ion peak at m/z = 379.2 (M^+, 29.47 %) which is in agreement with its molecular formula ($C_{24}H_{17}N_3S$). Anal.Calcud. for $C_{24}H_{17}N_3S$: C, 75.96; H, 4.52; N, 11.07; S, 8.45. Found: C, 75.98; H, 4.47; N, 11.09; S, 8.46.

2-((2-Cyanoethyl)thio)-4,6-distyrylnicotinonitrile (4)

A mixture of compound **(2)** (2 g, 0.006 mol), acrylonitrile (0.006 mol) and fused sodium acetate (0.6 g, 0.007 mol) in ethanol (25 mL) was heated under reflux for 3 h. The formed precipitate was collected and recrystallized from ethanol-dioxane mixture (1:2) as yellow crystals of compound **4.** Yield 88 %. m. p. 158-159 °C. IR (ν) (KBr): 3024 (C-H aromatic), 2917, 2849 (C-H aliphatic), 2250 (C≡N aliphatic), 2211 (C≡N conjugated) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ = 7.19-7.92 (m, 15H, 2CH=CH and Ar-H), 3.51-3.54 (t, 2H, CH_2CN), 2.99-3.02 (t, 2H, SCH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 162.17, 157.40, 149.83, 138.63, 136.81, 135.67, 130.36, 129.82, 129.43, 128.05, 126.97, 119.63, 115.45, 114.72, 102.54, 25.98, 18.12. DEPT 135 (DMSO-d_6, 100 MHz): δ = 136.80 (CH), 135.67 (CH), 130.36 (CH), 129.82 (CH), 129.43 (CH), 128.05 (CH), 126.97 (CH), 114.71 (CH), 25.98 (CH_2), 18.12 (CH_2). Anal.Calcd. for $C_{25}H_{19}N_3S$: C, 76.31; H, 4.87; N, 10.68; S, 8.15. Found: C, 76.49; H, 4.86; N, 10.69; S, 8.17.

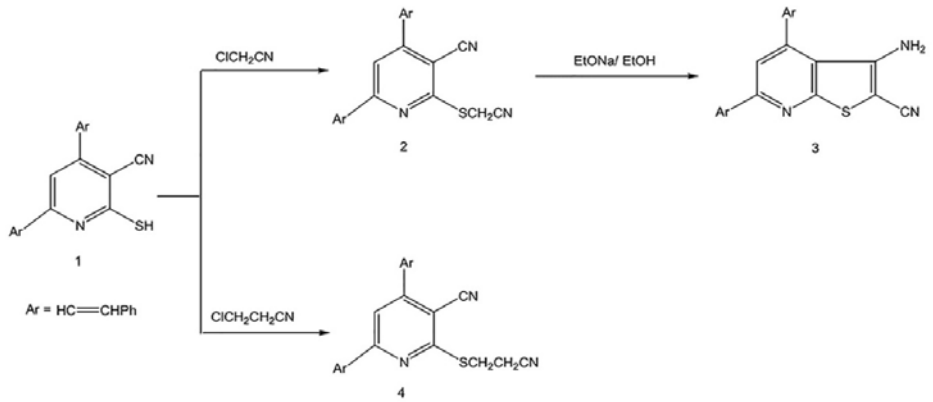
Laboratory bioassay

The insecticidal activity of the prepared compounds was evaluated via leaf dip bioassay method.[19] Laboratory screening data are reported here for the title compounds to find out the concentrations that are required to kill 50 % (LC_{50}) of the gathered insects. Number of six concentrations of each synthesized compound plus 0.1 % Triton X-100 (surfactant) was used. 20 adults and 20 nymphs of cowpea aphids, almost of the same size, were dipped 3 times in every concentration for ten seconds. The cowpea aphids were dried at RT for 0.5 h. Cowpea aphids control batches were also used. The aphids after drying were transferred to Petri dishes (9 centimeters diameter) and grasped for 24 and 48 h at photoperiod of 12:12 (light/ dark), 22 ± 2 °C and 60 ± 5 % relative humidity. Aphids mortality was counted after 24 and 48 h of test by means of a binocular microscope. The aphids that not capable of coordinating forward movement were considered dead. Toxicological activity check of the title compounds was repeated twice, and the results were corrected using Abbott's formula.[20] Slope values and median lethal concentrations (LC_{50}) of the prepared compounds were calculated through a probit regression analysis program and recorded in ppm.[21]

RESULTS AND DISCUSSION

The synthesis of the title compounds was started from **(1)** which was prepared according to the reported method.[5] Refluxing of compound **1** with chloroacetonitrile in ethanol containing slightly excess amounts of fused sodium acetate for 30 min resulted in the formation of 2-((cyanomethyl)thio)-4,6-distyrylnicotinonitrile **(2)**. The latter compound underwent intramolecular Thorpe-Ziegler cyclization upon refluxing in ethanol containing catalytic amounts of sodium ethoxide for 5 min to give the corresponding thienopyridine compound 3-amino-4,6-distyrylthieno[2,3-*b*]pyridine-2-carbonitrile **(3)** (Scheme 1).

Spectroscopic data and elemental analyses of compounds **2** and **3**were in agreement with their proposed structure. IR spectrum of compound **2** showed absorption bands at 2246 and 2211 cm^{-1} characteristic for (C≡N aliphatic) and (C≡N conjugated) groups. The absorption band of (C≡N conjugated) of compound **2** was disappeared when cyclised to give the thienopyridine **3** and was replaced by 3383 and 3199 cm^{-1} for NH_2. ^1H NMR spectrum (DMSO-d_6, 400 MHz) of compound **2** showed singlet signal at 4.36 for (CH_2) group. The signal of (CH_2) group of compound**2** in the ^1H NMR spectrum was disappeared when cyclised to give compound **3**.



Scheme 1. Synthesis of distryl compounds.

Table 1. Insecticidal activity of acetamiprid and compounds **2**-**4** against the cowpea aphid nymphs after 24 and 48 h of treatment.

	24 h after treatment			48 h after treatment		
Compd.	**Slope ± SE**	**LC_{50} (ppm)**	**Toxic ratio**	**Slope ± SE**	**LC_{50} (ppm)**	**Toxic ratio**
Acetamipri	0.34±0.02	0.045	1	0.42±0.03	0.006	1
2	0.44±0.03	0.064	0.703	0.48±0.03	0.013	0.462
3	0.38±0.03	0.097	0.464	0.49±0.04	0.018	0.333
4	0.41±0.03	0.052	0.865	0.48±0.03	0.010	0. 600

Table 2. Insecticidal activity of acetamiprid and compounds **2**-**4** against the cowpea aphid adults after 24 and 48 h of treatment.

	24 h after treatment			48 h after treatment		
Compd.	**Slope ± SE**	**LC_{50} (ppm)**	**Toxic ratio**	**Slope ± SE**	**LC_{50} (ppm)**	**Toxic ratio**
Acetamipri	0.24±0.02	0.225	1	0.32±0.03	0.023	1
2	0.36±0.02	0.322	0.699	0.39±0.03	0.035	0.657
3	0.39±0.03	0.754	0.298	0.45±0.03	0.049	0.469
4	0.36±0.02	0.282	0.798	0.41±0.03	0.034	0.676

DEPT 135 (DMSO-d_6, 100 MHz) spectrum of compound **2** showed characteristic signal at 16.39 for (CH_2) group. The signal of (CH_2) group of compound**2** in the DEPT 135 spectrum was disappeared when cyclised to give compound **3**. The mass spectrum of compound **3** showed a molecular ion peak at m/z = 379.2 (M^+, 29.47 %) which is in agreement with its molecular formula ($C_{24}H_{17}N_3S$).

Reflux of compound **1** with acrylonitrile in ethanol containing slightly excess amounts of fused sodium acetate for 3 h resulted in the formation of 2-((2-cyanoethyl)thio)-4,6-distyrylnicotinonitrile **(4)** (Scheme 1). The chemical structure of compound **4** was confirmed by elemental and spectral analyses.IR spectrum of compound **4** showed absorption bands at 2250 and 2211 cm^{-1} characteristic for (2C≡N) groups. ^1H NMR spectrum (DMSO-d_6, 400 MHz) of compound **4** showed two triplet signals at 3.51-3.54 and 2.99-3.02 for ($2CH_2$) groups. DEPT 135 (DMSO-d_6, 100 MHz) spectrum of compound **4** showed characteristic signals at 25.98 and 18.12 for ($2CH_2$) groups.

Insecticidal activity test for the cowpea aphid nymphs.

Compounds **2**, **3**, and **4** were investigated for their insecticidal activities against the nymphs of the collected aphids, and the results are presented in table 1. The insecticidal activity results indicated that, after 24 h of

treatment compounds **2**, **3**, and **4** exhibited high to low insecticidal activity against the cowpea aphid nymphs and the LC_{50} values ranged from 0.052 to 0.097 ppm, whereas the LC_{50} value of acetamiprid was 0.045 ppm. Whilst after 48 h of treatment, the insecticidal activity of compounds **2**, **3**, and **4** against cowpea aphid nymphs varied from strong to weak with LC_{50} values assorted from 0.01 to 0.018 ppm, but the LC_{50} value of acetamiprid was 0.006 ppm. These results indicate that compounds **2** and **4** have a high insecticidal activity close to that of acetamiprid insecticide against cowpea aphid nymphs after 24 and 48 h of test.

Insecticidal activity test for the adults of cowpea aphid

Compounds **2**, **3**, and **4** were investigated also for their insecticidal activity against the adults of the collected aphids, and the results are presented in table 2. From the results obtained after 24 h of insecticidal activity test, it was found that the compounds **2**, **3**, and **4** have strong to weak activity and LC_{50} values ranged from 0.282 to 0.754 ppm, while0.225 ppm was the LC_{50} value of acetamiprid. Compound **4** possess a high insecticidal activity, and its LC_{50} value is 0.282 ppm. After 48 h of the agricultural bioactivity test as insecticides, the insecticidal activity of compounds **2**, **3**, and **4** against cowpea aphid adults varied from high to low with LC_{50} values ranged from 0.034 to 0.049 ppm, whilst the LC_{50} value of acetamipridwas 0.023 ppm. Thus, compounds **2** and **4** showed a high insecticidal activity close to that of acetamiprid insecticide against cowpea aphid adults after 24 and 48 h of treatment.

Structure-action relationship

It is interesting to note that the insecticidal activity of the compound 2-((2-cyanoethyl)thio)-4,6-distyrylnicotinonitrile **(4)** with two cyano groups in its structure is more than that of the compound 2-((cyanomethyl)thio)-4,6-distyrylnicotinonitrile **(2)** that contains also two cyano groups in its structure, which may be due to the presence of the chain (CH_2CH_2CN) in compound **4** instead (CH_2CN) in compound **2**. The insecticidal activity of the opened form of compounds **2** is more than that of its closed form, the compound 3-amino-4,6-distyrylthieno[2,3-*b*]pyridine-2-carbonitrile **(3)**, which may be due to the presence of two cyano group in the former and one cyano group in the latter.

CONCLUSION

Three distyryl-containing compounds, which are considered neonicotinoid analogs, were prepared. The agricultural bioactivity as potential insecticides against

cowpea aphid, *Aphis craccivora* Koch for these compounds was investigated. The data obtained from this investigation proved that these compounds have insecticidal activities varied from good to moderateagainst cowpea aphids in comparison of acetamiprid insecticide as a reference.

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