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Exploration of Some Thieno[2,3-*b*]pyridines, Thieno[3,2-*d*]pyrimidinones, and Thieno[3,2-*d*][1,2,3]triazinones as Insecticidal Agents Against *Aonidiella aurantii*

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Abstract—Objective: *Aonidiella aurantii*, a highly polyphagous insect pest belonging to the Diaspididae family, is particularly harmful to citrus plants. This pest poses a significant threat to citrus production by infesting both fruits and foliage. In contrast, heterocycle-based compounds have proven to be essential in agrochemicals and pesticides, including fungicides, insecticides/acaricides, and herbicides. In light of this, our research group focused on the synthesis of the title compounds and the evaluation of their insecticidal activity against *Aonidiella aurantii*. **Methods:** Three main series of fused heterocyclic compounds, predominantly featuring the ethyl nicotinate scaffold as a substructure, were synthesized in our laboratory. Most of the synthesized compounds were evaluated for their insecticidal activity against the nymphs and adult females of *Aonidiella aurantii* using the guava leaf dipping technique. **Results and Discussion:** The reaction of ethyl 4-aryl-5-cyano-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylates (**Ia–Ib**) with *N*-aryl-2-chloroacetamides, carried out by refluxing in ethanol with a slight excess of anhydrous sodium carbonate, resulted in the formation of the corresponding 3-amino-4-aryl-2-[*N*-(aryl)carbamoyl]-5-ethoxycarbonyl-6-methylthieno[2,3-*b*]pyridines (**IIa–IIe**) in excellent yields. Upon boiling compounds (**IIb–IId**) with triethyl orthoformate in the presence of acetic anhydride, pyrido[3',2':4,5]thieno[3,2-d]pyrimidinones (**IIIb–IIId**) were obtained. Treatment of compounds (**IIa–IId**) with a concentrated solution of sodium nitrite in glacial acetic acid resulted in the formation of pyrido[3',2':4,5]thieno[3,2-*d*][1,2,3]triazinones (**IVa–IVd**). The structures of all compounds were confirmed through elemental and spectral analyses. **Conclusions:** Compounds containing the 4-chlorophenyl scaffold, such as (**Ib**) and (**IIId**), exhibited promising results against both nymphs and adult females of *Aonidiella aurantii*.

Keywords: thienopyridines, pyridothienopyrimidinones, pyridothieno[1,2,3]triazinones, insecticidal activity, *Aonidiella aurantii*, acetampirid

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INTRODUCTION

Aonidiella aurantii, a highly polyphagous insect pest belonging to the Diaspididae family, is characterized by its preference for citrus plants [1]. It is also known as the California red scale. With its long, piercing-sucking mouthparts, *Aonidiella aurantii* attacks every part of the tree above ground, including the fruits, leaves, branches, and twigs [2]. This leads to severe production losses as the sap from the plant tissue is extracted. The California red scale prefers fruit plants as its substrate, with leaves coming second, while branches and twigs are less preferred [3]. *Aonidiella aurantii* poses a significant threat to citrus production as it infests both fruits and foliage. Its consumption of leaves may turn them yellow, and severe infestations may result in dieback of stems and branches [4].

The pyridine ring is one of the most significant scaffolds in heterocyclic compounds due to its presence

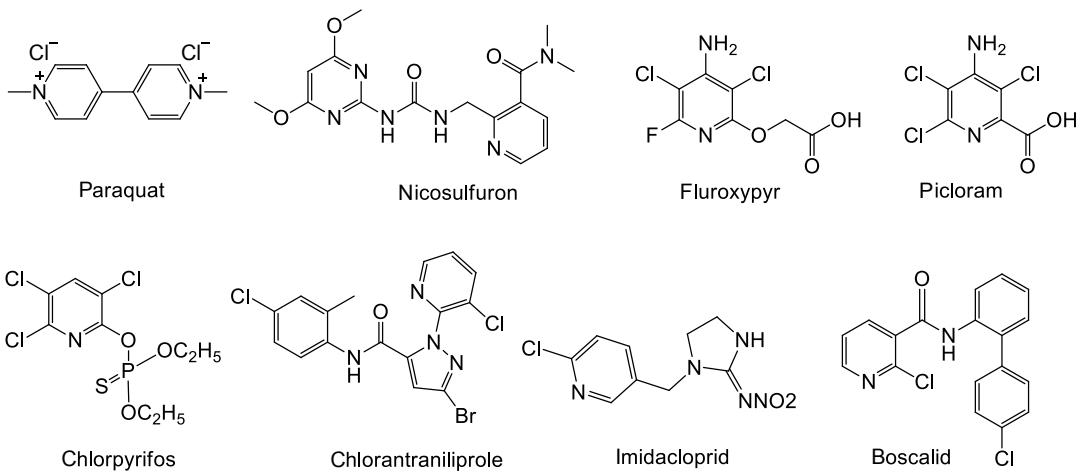


Fig. 1. The top eight pyridine-based commercial pesticides.

in several natural products [5, 6] and pharmaceuticals [7–10]. Pyridine derivatives are known to have versatile applications in industries such as agrochemicals [6, 10] and polymer production [11]. Other pyridine-based compounds are recognized for their antimicrobial [9], antiviral [9, 12, 13], anti-inflammatory [9, 14], anticancer [9, 15], and insecticidal activities [16–20]. The insecticidal activity of many thiophenes [21], and thieno[2,3-*b*]pyridines [16–18], has been well documented.

Pyridine-based compounds play a crucial role as agrochemicals or pesticides, including fungicides, insecticides/acaricides, and herbicides. According to the Phillips-McDougall report [22], there are eight crop protection products containing a pyridine moiety, as shown in Fig. 1, which were ranked among the top 45 products of 2013, with total sales of 5 billion USD, accounting for nearly 10% of the global agrochemical market. Notably, three pyridine-based products—chlorantraniliprole, imidacloprid, and paraquat—ranked no. 2, no. 5, and no. 7, with sales of 1240, 1070, and 905 million USD, respectively.

In contrast, pyridine-based compounds, compared to their benzenoid counterparts, exhibit a significant difference in hydrophobic constant (benzene 1.96 vs pyridine 0.65), which provides pyridine-based compounds with advantages such as higher bioactivity, lower toxicity, and enhanced systemic activity and/or excellent selectivity [23].

Pyridine-based neonicotinoids exhibit unique properties that make them highly effective against various insect pests while minimizing harm to non-target organisms. Their mode of action involves targeting the nervous systems of insects, specifically their nicotinic acetylcholine receptors (nAChRs). By binding to these receptors, neonicotinoids disrupt the normal transmission of nerve impulses, leading to paralysis and eventual death of the insects [24, 25].

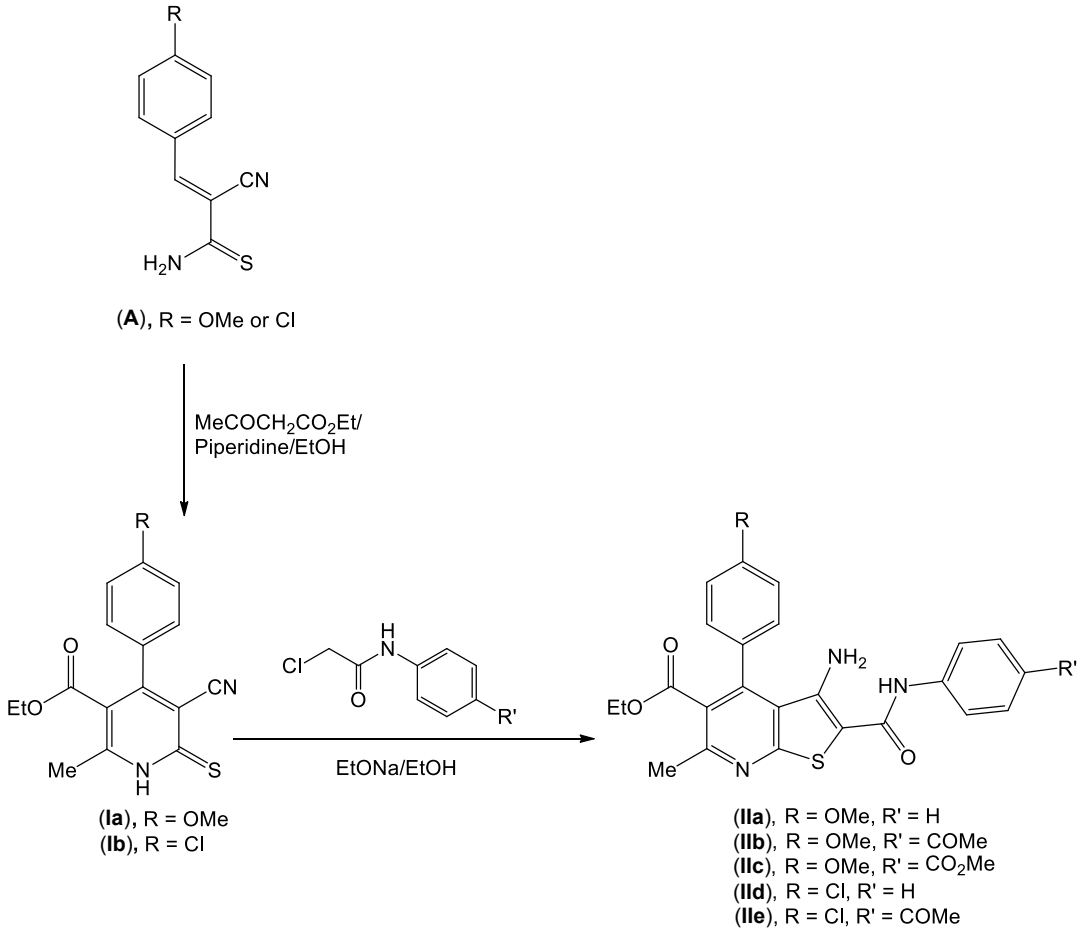
Considering the aforementioned findings, and as a continuation of our program aimed at synthesizing new pyridine compounds as insecticides [26–31], the current work was designed to explore several thieno-[2,3-*b*]pyridinones, pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidinones, and pyrido[3',2':4,5]thieno[3,2-*d*][1,2,3]-triazinones containing the ethyl nicotinate scaffold in their structures. These compounds are expected to be eco-friendly alternatives with higher insecticidal activity and lower toxicity to non-target organisms than currently available products. Additionally, they aim to overcome insect resistance to pesticides that have been frequently used over the years. The insecticidal activity of the title compounds against the nymphs and adult females of *Aonidiella aurantii* was evaluated in our laboratory, and the results obtained are presented below.

RESULTS AND DISCUSSION

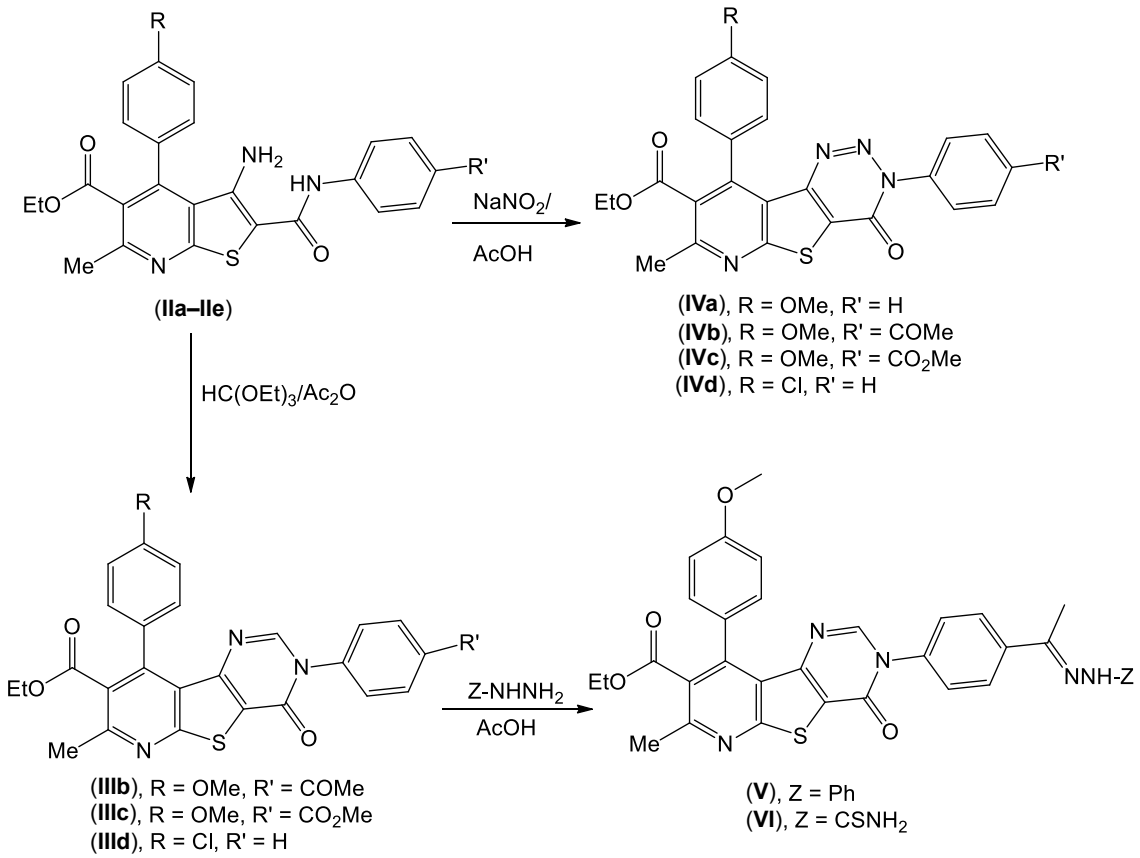
Chemistry

Ethyl nicotinate derivatives, ethyl 4-aryl-5-cyano-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylates (**Ia–Ib**), were synthesized by reacting benzylidene cyanothioacetamides (A) with ethyl acetoacetate in the presence of piperidine as a basic catalyst, according to the reported method [32]. The reaction of (**Ia–Ib**) with *N*-aryl-2-chloroacetamide, by heating in ethanol containing slightly excess molar amounts of anhydrous sodium carbonate for three hours, afforded the corresponding ethyl 3-amino-4-aryl-2-(*N*-aryl)carbamoyl-6-methylthieno[2,3-*b*]pyridine-5-carboxylates (**IIa–IIe**) in excellent yields (Scheme 1).

The latter compounds were used as key intermediates for synthesizing other target compounds. Thus, heating compounds (**IIb–IId**) with triethyl orthoformate in the presence of acetic anhydride resulted in the formation of the expected 3,9-diaryl-8-ethoxycarbonyl-7-methylpyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine-4(3*H*)-ones (**IIIb–IIId**) in very pure states. Diazotization of compounds (**IIa–IId**) in glacial acetic acid with a concentrated sodium nitrite solution resulted in diazotization followed by self-coupling, which afforded the promising compounds, 3,9-diaryl-8-ethoxycarbonyl-7-methylpyrido[3',2':4,5]thieno[3,2-*d*][1,2,3]triazine-4(3*H*)-ones (**IVa–IVd**). Condensation of acetyl compound (**IIIb**) with phenylhydrazine or thiosemicarbazide furnished the corresponding phenylhydrazone (**V**) or thiosemicarbazone (**VI**), respectively (Scheme 2).



Scheme 1. Synthesis of compounds (**Ia–Ib**) and (**IIa–IIe**).



Scheme 2. Synthesis of compounds (**IIIb–IIId**), (**IVa–IVd**), (**V**), and (**VI**).

Characterization

The structures of all new pyridines were elucidated and characterized by elemental analyses as well as spectral data (IR, ^{1}H NMR, and mass spectra). The characterization data of all compounds are consistent with their proposed structures [32] (cf. Experimental).

Insecticidal Activity

Compounds (**Ia–Ib**), (**IIb–IIe**), (**IIIb–IIId**), (**IVa–IVd**), (**V**), (**VI**), and acetamiprid were evaluated for their insecticidal activity toward the nymphs and adult females of *Aonidiella aurantii* using the guava leaf dipping technique in a laboratory setting [33–36]. The results obtained are summarized in Table 1 and Fig. 2. The results after 72 h of treatment revealed that: (i) all tested compounds exhibit insecticidal activity, ranging from weak to strong, towards the nymphs of *Aonidiella aurantii*, with LC_{50} values ranging from 2.441 to 0.202 mg/L; (ii) some compounds, such as (**Ib**), (**IIId**), and (**VI**), exhibit high activity against nymphs of *Aonidiella aurantii*, with LC_{50} values of 0.202, 0.331, and 0.345 mg/L, respectively, which are close to that of acetamiprid (LC_{50}, 187 mg/L); (iii) LC_{50} values of compounds (**Ia–Ib**), (**IIb–IIe**), (**IIIb–IIId**), (**IVa–IVd**), (**V**), (**VI**) towards nymphs of *Aonidiella aurantii* were 0.428, 0.202, 0.548, 0.646, 0.756, 0.642, 0.977, 0.798, 0.331, 2.441, 1.156, 1.463, 1.237, 1.332, and 0.345 mg/L, respectively, while the LC_{50} value of acetamiprid was 1.87 mg/L; (iv) all tested compounds exhibited insecticidal activity towards adult females of *Aonidiella aurantii*, ranging from weak to strong, with LC_{50} values ranging from 3.052 to 0.818 mg/L; (v) two compounds, (**Ib**) and (**IIId**), exhibited high activity against adult females of *Aonidiella aurantii*, with LC_{50} values of 0.818 and 0.973 mg/L, respectively, which are close to that of acetamiprid (LC_{50},

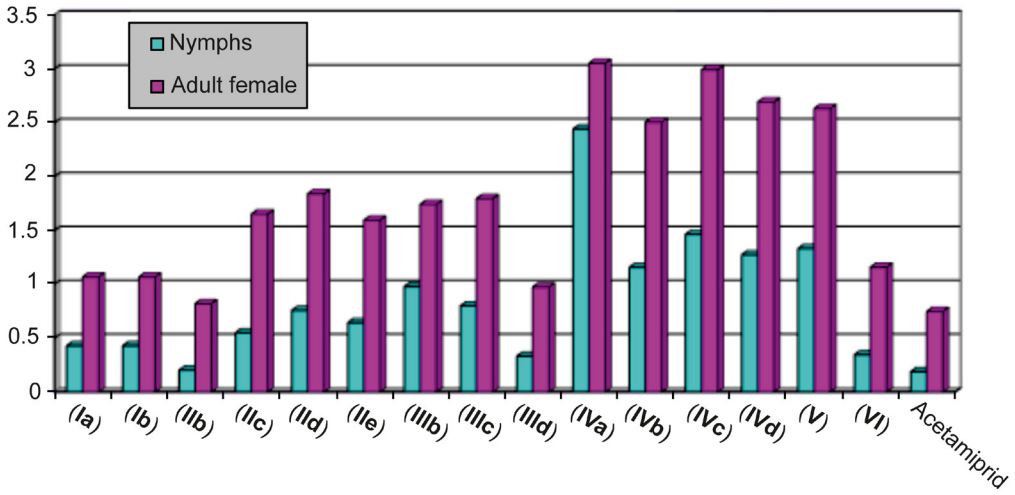


Fig. 2. Insecticidal activity of most synthesized compounds (**Ia–Ib**), (**IIb–IIe**), (**IIIb–IIId**), (**IVa–IVd**), (**V**), (**VI**), and acetamiprid towards the nymphs and adult females of *Aonidiella aurantia*.

0.746 mg/L); (vi) LC_{50} values of compounds (**Ia–Ib**), (**IIb–IIe**), (**IIIb–IIId**), (**IVa–IVd**), (**V**), (**VI**) towards adult females of *Aonidiella aurantii* were 1.067, 0.818, 1.652, 1.840, 1.595, 1.742, 1.907, 1.795, 0.973, 3.052, 2.505, 2.993, 2.693, 2.623, and 1.158 mg/L, respectively; (vii) all tested compounds exhibited stronger toxicological activity against nymphs of *Aonidiella aurantii* than against adult females, which is a logical result, as adults often have higher immunity; (viii) compounds (**Ib**) and (**IIId**), which showed the highest activity towards both nymphs and adult females of *Aonidiella aurantii*, contain the 4-chlorophenyl scaffold; this scaffold is a main constituent of the DDT insecticide (Table 1 and Fig. 2).

Table 1. Insecticidal effects of compounds (**Ia–Ib**), (**IIb–IIe**), (**IIIb–IIId**), (**IVa–IVd**), (**V**), (**VI**), and acetamiprid on nymphs and adult females of *Aonidiella aurantii* insects

	Nymphs				Adult females			
compound	LC_{50} (mg/L)	slope	χ^2	toxic ratio	LC_{50} (mg/L)	slope	χ^2	toxic ratio
(Ia)	0.428	0.472 ± 0.277	0.034	43.69	1.067	0.563 ± 0.289	0.046	69.91
(Ib)	0.202	0.376 ± 0.282	0.206	92.57	0.818	0.528 ± 0.273	0.282	91.19
(IIb)	0.548	0.497 ± 0.274	0.101	34.12	1.652	0.561 ± 0.265	0.366	45.15
(IIc)	0.646	0.497 ± 0.274	0.041	28.94	1.840	0.605 ± 0.268	0.133	40.54
(IId)	0.756	0.527 ± 0.273	0.036	24.73	1.595	0.683 ± 0.272	0.173	46.77
(IIe)	0.642	0.497 ± 0.274	0.041	29.12	1.742	0.605 ± 0.268	0.133	42.82
(IIIb)	0.977	0.505 ± 0.267	0.208	19.14	1.907	0.617 ± 0.271	0.252	39.11
(IIIc)	0.798	0.562 ± 0.273	0.046	23.43	1.795	0.682 ± 0.272	0.170	41.55
(IIId)	0.331	0.451 ± 0.292	0.015	56.49	0.973	0.580 ± 0.278	0.374	76.67
(IVa)	2.441	0.684 ± 0.283	0.392	6.58	3.052	0.661 ± 0.278	0.187	24.44
(IVb)	1.156	0.641 ± 0.268	0.249	16.17	2.505	0.614 ± 0.265	0.239	29.78
(IVc)	1.463	0.659 ± 0.276	0.326	12.78	2.993	0.658 ± 0.279	0.403	24.94
(IVd)	1.237	0.628 ± 0.273	0.281	15.11	2.693	0.698 ± 0.274	0.318	27.70
(V)	1.332	0.649 ± 0.278	0.213	14.03	2.623	0.623 ± 0.279	0.224	28.44
(VI)	0.345	0.443 ± 0.289	0.269	54.20	1.158	0.543 ± 0.283	0.566	64.42
Acetamiprid	0.187	0.401± 0.278	0.085	100	0.746	0.497 ± 0.274	0.101	100

EXPERIMENTAL

Melting points were determined on a Galan-Kamp apparatus and are reported as the temperature range. FT-IR spectra were recorded on a Pye-Unicam SP3-100 Spectrophotometer using the KBr disc technique (ν_{max}, cm^{-1}). NMR spectra were recorded on a Bruker spectrometer at 400 MHz for ^1H NMR, with chemical shifts reported in δ (ppm) and coupling constants (*J* values) in Hz, using $CDCl_3$ or DMSO-d_6 as solvents. MS spectra were recorded on a Jeol JMS-600 spectrometer. Elemental analyses for carbon, hydrogen, and nitrogen were performed using a Perkin Elmer 2400 LS Series CHN/O analyzer (Universiti Sains Malaysia, Minden, Malaysia). Acetamiprid and all other chemicals used in this work were purchased from Aldrich Chem. Co. and were used without further purification.

General procedure for synthesis of ethyl 5-cyano-4-(4-methoxyphenyl)-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylate (Ia) and ethyl 4-(4-chlorophenyl)-5-cyano-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylate (Ib). These compounds were synthesized according to the reported method [32].

General procedure for synthesis of ethyl 3-amino-4-aryl-2-(*N*-aryl)carbamoyl–6-methylthieno[2,3-*b*]pyridine-5-carboxylates (IIa–IIe). To a suspension of compound (**Ia**) or (**Ib**) (10 mmol) and the respective *N*-aryl-2-chloroacetamide (10 mmol) in 40 mL of ethanol, anhydrous sodium carbonate (1.30 g) was added. The resulting mixture was heated under reflux for 3 h. The yellow precipitate that formed upon cooling was collected by filtration, washed several times with water, dried in air, and then recrystallized from ethanol to give compounds (**IIa–IIe**).

Ethyl 3-amino-4-(4-methoxyphenyl)-6-methyl-2-(phenylcarbamoyl)thieno[2,3-*b*]pyridine-5-carboxylate (IIa). Yield: 92%. mp: 158–159°C. FT-IR (ν_{max}, cm^{-1}): 3500, 3300 (NH_2), 3200 (NH), 1720 (C=O, ester), 1660 (C=O, amide). ^1H NMR (400 MHz, $CDCl_3$), ppm: 8.70 (s, 1H, NH), 6.91–7.70 (m, 9H, ArH's), 5.80 (s, 2H, NH_2), 3.92–4.21(q, 2H, OCH_2), 3.79 (s, 3H, OCH_3), 2.62 (s, 3H, CH_3 at C-6), 0.80–1.11 (t, 3H, CH_3 of ester). $C_{25}H_{23}N_3O_4S$. Calculated, %: C, 65.06; H, 5.02; N, 9.10. Found, %: C, 65.17; H, 5.14; N, 9.00.

Ethyl 2-((4-acetylphenyl)carbamoyl)-3-amino-4-(4-methoxyphenyl)-6-methyl-thieno[2,3-b]pyridine-5-carboxylate (IIb). Yield: 93%. mp: 204–205°C. FT-IR (ν_{max}, cm^{-1}): 3500, 3300 (NH_2), 3200 (NH), 1720 (C=O, ester), 1680 (C=O, acetyl), 1660 (C=O, amide). ^1H NMR (400 MHz, $CDCl_3$), ppm: 9.02 (s, 1H, NH), 7.73–8.21 (dd, 4H, ArH's), 7.00–7.61 (dd, 4H, ArH's), 6.03 (s, 2H, NH_2), 4.01–4.39 (q, 2H, OCH_2), 4.00 (s, 3H, OCH_3), 2.79 (s, 3H, $COCH_3$), 2.61 (s, 3H, CH_3 at C-6), 1.02–1.31 (t, 3H, CH_3 of ester). $C_{27}H_{25}N_3O_5S$. Calculated, %: C, 64.40; H, 5.00; N, 8.34. Found, %: C, 64.27; H, 5.28; N, 8.11.

Ethyl 3-amino-2-((4-(methoxycarbonyl)phenyl)-carbamoyl)-4-(4-methoxy-phenyl)-6-methyl-thieno-[2,3-*b*]pyridine-5-carboxylate (IIc). Yield: 90%. mp: 210–211°C. FT-IR (ν_{max}, cm^{-1}): 3500, 3300 (NH_2), 3200 (NH), 1720 (C=O, ester), 1700 (C=O, ester), 1660 (C=O, amide). ^1H NMR (400 MHz, $CDCl_3$), ppm: 9.21 (s, 1H, NH), 7.49–8.10 (dd, 4H, ArH's), 6.92–7.50 (dd, 4H, ArH's), 5.99 (s, 2H, NH_2), 4.29–4.62 (q, 2H, OCH_2), 3.90 (s, 6H: OCH_3, CO_2CH_3), 2.59 (s, 3H, CH_3 at C-6), 0.98–1.20 (t, 3H, CH_3 of ester). $C_{27}H_{25}N_3O_6S$. Calculated, %: C, 62.41; H, 4.85; N, 8.09. Found, %: C, 62.50; H, 4.85; N, 8.01.

Ethyl 3-amino-4-(4-chlorophenyl)-6-methyl-2-(phenylcarbamoyl)thieno[2,3-*b*]pyridine-5-carboxylate (IId). Yield: 94%. mp: 175–176°C. FT-IR (ν_{max}, cm^{-1}): 3500, 3300 (NH_2), 3200 (NH), 1720 (C=O, ester), 1660 (C=O, amide). ^1H NMR (400 MHz, $CDCl_3$), ppm: 8.70 (s, 1H, NH), 7.01–7.69 (m, 9H, ArH's), 5.70 (s, 2H, NH_2), 3.80–4.29 (q, 2H, OCH_2), 2.58 (s, 3H, CH_3 at C-6), 0.69–1.02 (t, 3H, CH_3 of ester). $C_{24}H_{20}ClN_3O_3S$. Calculated, %: C, 61.86; H, 4.33; N, 9.02. Found, %: C, 61.77; H, 4.18; N, 9.24.

Ethyl 3-amino-4-(4-chlorophenyl)-6-methyl-2-((4-(prop-1-en-2-yl)phenyl)carbamoyl)thieno[2,3-*b*]-pyridine-5-carboxylate (IIe). Yield: 90%. mp: 239–240°C. FT-IR (ν_{max}, cm^{-1}): 3500, 3300 (NH_2), 3200 (NH), 1720 (C=O, ester), 1680 (C=O, acetyl), 1660 (C=O, amide). ^1H NMR (400 MHz, DMSO-d_6), ppm: 8.89

(s, 1H, NH), 7.31–8.00 (m, 8H, ArH's), 5.61 (s, 2H, NH2), 3.89–4.19 (q, 2H, OCH_2), 2.70 (s, 3H, $COCH_3$), 2.59 (s, 3H, CH_3 at C-6), 0.81–1.12 (t, 3H, CH_3 of ester). MS: 508 [*M* +, 100%]. $C_{26}H_{22}ClN_3O_4S$. Calculated, %: C, 61.47; H, 4.37; N, 8.27. Found, %: C, 61.26; H, 4.17; N, 8.31.

General procedure for synthesis of 3,9-diaryl-8-ethoxycarbonyl-7-methylpyrido[3′,2′:4,5]thieno-[3,2-*d*]pyrimidine-4(3*H*)-ones (IIIb–IIIe). A mixture of compounds (**IIb–IId**) (2 mmol) and triethyl orthoformate (1 mL) in acetic anhydride (5 mL) was refluxed at the boiling point for 4 h. The solid formed during heating was collected and recrystallized from an ethanol–chloroform mixture (3 : 1) to yield white needles of compounds (**IIIb–IIIe**).

Ethyl 3-(4-acetylphenyl)-9-(4-methoxyphenyl)-7-methyl-4-oxo-3,4-dihydropyrido[3′,2′:4,5]thieno-[3,2-*d*]pyrimidine-8-carboxylate (IIIb). Yield: 80%. mp: 185–186°C. FT-IR (ν_{max}, cm^{-1}): 1720 (C=O, ester), 1680 (C=O, acetyl), 1660 (C=O, pyrimidinone). ^1H NMR (400 MHz, $CDCl_3$), ppm: 8.18 (s, 1H, pyrimidine-H), 7.51–8.00 (dd, 4H, ArH's), 6.92–7.41 (dd, 4H, ArH's), 4.00–4.31 (q, 2H, OCH_2), 3.82 (s, 3H, OCH_3), 2.82 (s, 3H, $COCH_3$), 2.69 (s, 3H, CH_3 at C-7), 0.89–1.19 (t, 3H, CH_3 of ester). $C_{28}H_{23}N_3O_5S$. Calculated, %: C, 65.48; H, 4.51; N, 8.18. Found, %: C, 65.68; H, 4.54; N, 8.25.

Ethyl 3-(4-(methoxycarbonyl)phenyl)-9-(4-methoxyphenyl)-7-methyl-4-oxo-3,4-dihydro-pyrido-[3′,2′:4,5]thieno[3,2-*d*]pyrimidine-8-carboxylate (IIIc). Yield: 86%. mp: 180–181°C. FT-IR (ν_{max}, cm^{-1}): 1720 (C=O, ester), 1700 (C=O, ester), 1660 (C=O, pyrimidinone). ^1H NMR (400 MHz, $CDCl_3$), ppm: 8.18 (s, 1H, pyrimidine-H), 7.41–8.00 (dd,4H, ArH's), 6.89–7.48 (dd, 4H, ArH's), 4.31–4.61 (q, 2H, OCH_2), 3.90 (s, 6H: OCH_3, CO_2CH_3), 2.69 (s, 3H, CH_3 at C-7), 0.89–1.22 (t, 3H, CH_3 of ester). $C_{28}H_{23}N_3O_6S$. Calculated, %: C, 63.51; H, 4.38; N, 7.93. Found, %: C, 63.29; H, 4.42; N, 7.72.

Ethyl 9-(4-chlorophenyl)-7-methyl-4-oxo-3-phenyl-3,4-dihydropyrido[3′,2′: 4,5]thieno[3,2-*d*]pyrimidine-8-carboxylate (IIId). Yield: 87%. mp: 215–216°C. FT-IR (ν_{max}, cm^{-1}): 1720 (C=O, ester), 1660 (C=O, pyrimidinone). ^1H NMR (400 MHz, $CDCl_3$), ppm: 8.02 (s, 1H, pyrimidine-H), 7.18–7.58 (m, 9H, ArH's), 4.00–4.31(q, 2H, OCH_2), 2.78 (s, 3H, CH_3 at C-7), 1.02–1.29 (t, 3H, CH_3 of ester). $C_{25}H_{18}ClN_3O_3S$. Calculated, %: C, 63.09; H, 3.81; N, 8.83. Found, %: C, 63.35; H, 3.72; N, 8.91.

General procedure for synthesis of 3,9-diaryl-8-ethoxycarbonyl-7-methylpyrido[3′,2′:4,5]thieno-[3,2-*d*][1,2,3]triazine-4(3*H*)-ones (IVa–IVd). A 10% sodium nitrite solution (4 mL) was added dropwise to a solution of compound (**IIa–IId**) (2 mmol) in glacial acetic acid (15 mL) at 0°C with continuous stirring over 5 min. The mixture was then allowed to stand at room temperature for 30 min. The solid that precipitated upon dilution with water was collected by filtration and recrystallized from ethanol to yield white needles of compounds (**IVa–IVd**).

Ethyl 9-(4-methoxyphenyl)-7-methyl-4-oxo-3-phenyl-3,4-dihydropyrido[3′,2′:4,5]thieno [3,2-*d*]-[1,2,3]triazine-8-carboxylate (IVa). Yield: 82%. mp: 228–229°C. FT-IR (ν_{max}, cm^{-1}): 1720 (C=O, ester), 1660 (C=O, triazinone). ^1H NMR (400 MHz, $CDCl_3$), ppm: 6.90–7.68 (m, 9H, ArH's), 4.00–4.39 (q, 2H, OCH_2), 3.90 (s, 3H, OCH_3), 2.80 (s, 3H, CH_3 at C-6), 0.91–1.20 (t, 3H, CH_3 of ester). MS: 472 [*M* +, 100%]. $C_{25}H_{20}N_4O_4S$. Calculated, %: C, 63.55; H, 4.27; N, 11.86. Found, %: C, 63.78; H, 4.09; N, 12.06.

Ethyl 3-(4-acetylphenyl)-9-(4-methoxyphenyl)-7-methyl-4-oxo-3,4-dihydro-pyrido[3′,2′:4,5]-thieno[3,2-*d*][1,2,3]triazine-8-carboxylate (IVb). Yield: 82%. mp: 164–165°C. FT-IR (ν_{max}, cm^{-1}): 1720 (C=O, ester), 1680 (C=O, acetyl),1660 (C=O, triazinone). ^1H NMR (400 MHz, $CDCl_3$), ppm: 7.68–8.18 (dd, 4H, ArH's), 6.89–7.41 (dd, 4H, ArH's),), 4.00–4.30 (q, 2H, OCH_2), 3.78 (s, 3H, OCH_3), 2.80 (s, 3H, $COCH_3$), 2.69 (s, 3H, CH_3 at C-7), 0.90–1.20 (t, 3H, CH_3 of ester). $C_{27}H_{22}N_4O_5S$. Calculated, %: C, 63.02; H, 4.31; N, 10.89. Found, %: C, 63.31; H, 4.05; N, 10.66.

Ethyl 3-(4-(methoxycarbonyl)phenyl)-9-(4-methoxyphenyl)-7-methyl-4-oxo-3,4-dihydro-pyrido-[3′,2′:4,5]thieno[3,2-*d*][1,2,3]triazine-8-carboxylate

(IVc). Yield: 86%. mp: 173–174°C. FT-IR (ν_{max}, cm^{-1}): 1720 (C=O, ester), 1700 (C=O, ester), 1660 (C=O, triazinone). ^1H NMR (400 MHz, $CDCl_3$), ppm: 7.40–8.00 (dd, 4H, ArH's), 6.88–7.47 (dd, 4H, ArH's), 4.31–4.61 (q, 2H, OCH_2), 3.99 (s, 6H: OCH_3, CO_2CH_3), 2.81 (s, 3H, CH_3 at C-7), 1.00–1.30 (t, 3H, CH_3 of ester). $C_{27}H_{22}N_4O_6S$. Calculated, %: C, 61.12; H, 4.18; N, 10.56. Found, %: C, 61.00; H, 4.19; N, 10.32.

Ethyl 9-(4-chlorophenyl)-7-methyl-4-oxo-3-phenyl-3,4-dihydropyrido[3′,2′:4,5]thieno[3,2-*d*][1,2,3]-triazine-8-carboxylate (IVd). Yield: 81%. mp: 180–181°C. FT-IR (ν_{max}, cm^{-1}): 1720 (C=O, ester), 1660 (C=O, triazinone). ^1H NMR (400 MHz, $CDCl_3$), ppm: 7.11–7.60 (m, 9H, ArH's), 3.90–4.20 (q, 2H, OCH_2), 2.81 (s, 3H, CH_3 at C-7), 1.00–1.30 (t, 3H, CH_3 of ester). $C_{24}H_{17}ClN_4O_3S$. Calculated, %: C, 60.44; H, 3.59; N, 11.75. Found, %: C, 60.17; H, 3.38; N, 11.92.

General procedure for condensation of (IIIb) with phenylhydrazine or thiosemicarbazide. To a mixture of compound (**IIIb**) (1.03 g, 2 mmol) and either phenylhydrazine or thiosemicarbazide (2 mmol) in ethanol (20 mL), a few drops of acetic acid were added. The reaction mixture was refluxed for 2 h and then allowed to cool to room temperature. The precipitated solid was collected by filtration and recrystallized from an ethanol–chloroform mixture (3 : 1) to give white crystals of compound (**V**) or (**VI**), respectively.

Ethyl 9-(4-methoxyphenyl)-7-methyl-4-oxo-3-(4-(1-(2-phenylhydrazono)ethyl)phenyl)-3,4-dihydropyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine-8-carboxylate (V). Yield: 80%. mp: 265–266°C. FT-IR (ν_{max}, cm^{-1}): 3400 (NH), 1720 (C=O, ester), 1660 (C=O, pyrimidinone), 1600 (C=N). ^1H NMR (400 MHz, TFA), ppm: 8.22 (s, 1H, pyrimidine-H), 6.80–8.00 (m,15H, ArH's), 4.01–4.32 (q, 2H, OCH_2), 3.80 (s, 3H, OCH_3), 2.76 (s, 3H, CH_3 at C-7), 2.40 (s, 3H, CH_3C=N), 1.00–1.30 (t, 3H, CH_3 of ester). $C_{34}H_{29}N_5O_4S$. Calculated, %: C, 67.64; H, 4.84; N, 11.60. Found, %: C, 67.52; H, 4.91; N, 11.38.

Ethyl 3-(4-(1-(2-carbamothioylhydrazono)ethyl)-phenyl)-9-(4-methoxyphenyl)-7-methyl-4-oxo-3,4-dihydropyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine-8-carboxylate (VI). Yield: 84%. mp: 281–282°C. FT-IR (ν_{max}, cm^{-1}): 3500–3400 (NH_2, NH), 1720 (C=O, ester), 1660 (C=O, pyrimidinone), 1600 (C=N). ^1H NMR (400 MHz, DMSO-d_6), ppm: 8.18 (s, 1H, CH pyrimidine-H), 6.80–8.00 (m, 8H, ArH's), 5.49 (s, 2H, NH_2), 4.01–4.32 (q, 2H, OCH_2), 3.90 (s, 3H, OCH_3), 2.72 (s, 3H, CH_3C=N), 2.50 (s, 3H, CH_3 at C-6), 1.01–1.32 (t, 3H, CH_3 of ester). $C_{29}H_{26}N_6O_4S_2$. Calculated, %: C, 59.37; H, 4.47; N, 14.32. Found, %: C, 59.22; H, 4.18; N, 14.07.

Insect collection and rearing. The first batches of *Aonidiella aurantii* insects were collected in the last week of July 2024 from six distinct guava fields located in the Esna region, Luxor Governorate, Egypt. Both nymphs and adult females of *Aonidiella aurantii* were used to assess the toxicity of the target compounds in laboratory-based studies.

Toxicological activity assay. The toxicological efficacy of all target synthetic compounds was assessed using the guava leaf dipping technique in a laboratory setting [33–36] at the Agricultural Research Station, Plant Protection Department, El-Mattana, Esna District, Luxor Governorate, Egypt. Five concentrations of each chemical derivative, along with 0.11% Tween-20 as a surfactant, were prepared. For each concentration, twenty adult female *Aonidiella aurantii* insects and twenty nymphs were immersed for ten seconds (repeated three times). The treated insects were allowed to dry at 25°C for approximately 30 min, while the control group was submerged in water containing only Tween-20. The assays were conducted at room temperature with a relative humidity of 5%. The insects were subsequently transferred to glass jars filled with distilled water after the immersion period. Mortality was assessed at one, two, and three days following exposure. Dead and living insects were counted and recorded under a binocular microscope. Immobile *A. aurantii* individuals were considered dead. The corrected mortality percentages were calculated using the Abbott formula [37]:

$$\text{Corrected mortality percentage} = \left(\frac{(N_C) - (N_T)}{N_C}\right) \times 100,$$

where N_C is mortality percentage in control; N_T is mortality percentage in treatment.

The average of the death rates for the treatment across three time periods following application is known as the average general mortality percentage (GM) [38]:

$$GM = \frac{A}{B + C} \times 100,$$

where *GM* is the average general mortality (%); *A* is sum of the total corrected mortality of each application time (%); *B* is number of times after application; *C* is initial death time.

Statistical analysis. All data were recorded using the MSTATC Program software, followed by statistical analysis [39]. A Randomized Complete Block Design (RCBD) was employed, and the averages of the tested treatments were compared using the Least Significant Difference (LSD) test at a 5% probability level. The standard error between the various treatments was also determined. To evaluate the nonlinear correlations between the mortality percentages of *Aonidiella aurantii* at different stages (represented as the dependent variable *y*) and the concentrations of the tested treatments (acting as the independent factor *X*), the second-degree polynomial equation: $Y = a+b_1X + b_2X^2$ was used to represent this statistical relationship. The explained variance (eV %) was calculated, and the nonlinear regression method was applied to clarify the variability in mortality percentages due to varying concentrations. All data computations were performed using Microsoft Excel 2007. Probit analysis was applied to assess the LC_{50} and LC_{90} values to determine insecticidal efficiency [40], and the Ld-P Lines Program was used for this purpose. The toxicity or harmfulness index was determined using Sun's formulae [41].

CONCLUSIONS

In conclusion, we have successfully synthesized three series of fused heterocyclic compounds, including thienopyridines, pyridothienopyrimidinones, and pyridothienotriazinones, incorporating an ethyl nicotinate scaffold. These compounds were synthesized in high yields and in very pure form. They were characterized by elemental and spectral analyses. Most of the synthesized compounds were evaluated for their insecticidal activity against both the nymphs and adult females of *Aonidiella aurantii*, yielding promising results. Compounds (**Ib**) and (**IIId**) were found to be the most potent against both nymphs and adult females of *Aonidiella aurantii*, with activities nearly equivalent to that of acetamiprid, a reference insecticide. This enhanced activity may be attributed to the incorporation of the 4-chlorophenyl group in their structures.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies involving patients or animals as test objects.

Informed consent was not required for this article.

CONFLICT OF INTEREST

No conflicts of interest was declared by the authors.

AUTHOR CONTRIBUTION

The authors EAB and MAG—participated in the discussions. The authors FAT and RAE HM—contributed to article preparation. The authors MMSB and EK—synthesized and characterized of the compounds.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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