Synthesis of some Benzoylthiourea and Benzoylurea Derivatives as Insect Growth Regulators and Study of their Toxicity Impacts on Spodoptera littoralis (Boisd.)

1M.T.M.El-Wassimy, 2Safwat Aref A. Salam, 1Elsyad A. Allah, and 2Mohamed A. Gad

1Chemistry Department, Faculty of Science, Sohag University, Egypt.
2Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

ABSTRACT

Various benzoylthiourea and benzoylurea derivatives were synthesized. Compounds 2a-c were obtained by the reaction of 2, 4-dichlorobenzoyl isothiocyanate with primary aniline derivatives, Compounds 5d,e were obtained by the reaction of phenylurea with benzoyl chloride derivatives. The synthesized compounds were identified by IR, 1H NMR. Preliminary bioassays indicated that most of them showed moderate insecticidal activities against cotton leafworm Spodoptera littoralis (Boisd.) which study the susceptibility of laboratory of 2nd and 4th instars larvae of the cotton leafworm Spodoptera littoralis (Boisd.). Four concentration levels (500, 250, 125 and 65 ppm) were applied on the fresh plant food to the newly molted (2nd, 4th) instar nymphs. All results were obtained 24-48 hrs after feeding.

Key words: Benzoylthiourea derivatives, Benzoylurea derivatives, Spodoptera littoralis (Boisd.)

Introduction

Benzoylurea and benzoylthiourea insecticides act as powerful insect growth regulators (IGRs) which interfere with chitin synthesis in target pests and cause death. Benzoyl urea and benzoylthiourea insecticides have many attractive properties such as high selectivity, high biological activity, rapid degradation in soil and water and the acute low toxicity for animals, which make them suitable for inclusion in integrated pest management programs for crops (Bicchi et al., 1996). On the other hand, insect Growth Regulators (IGRs), also called third-generation insecticides, are pesticides that disrupt the normal activity of the endocrine or hormone system of insects, affecting the development, reproduction, or metamorphosis of the target insect. They have a much slower mode of action than synthetic chemical insecticides. IGRs include juvenile hormone (JH), mimic and chitin synthesis inhibitors (CSIs). CSIs, such as hexaflumuron, lufenuron and diflubenzuron, which inhibit the production of chitin, a major component of the insect exoskeleton. Insects treated with CSIs become unable to synthesize new cuticle, and therefore unable to successfully molt into the next stage. CSIs may be toxic to other arthropods, and IGR metabolites may have adverse effects on vertebrates due to their ability to bind to certain members of the nuclear hormone receptor family (Abbott, 1925; Bayoumi et al., 1998; Clarke and Jewess, 1990; Decombel et al., 2004). The benzoylurea constitute a class of the IGRs that interfere with insect growth and development by inhibiting chitin synthesis in insects (El-Defrawi et al., 1964; Rao and Subbaratnam, 2000). The cotton leafworm Spodoptera littoralis (Boisd.) is a major polyphagous key pest in Egypt. It is active all year round without hibernation period and attacking cotton as well as more than 29 hosts from other crops and vegetables (Raslan, 2002; Emam and Degheele, 1992; Guyer, and Neumann, 1988; Ishaaya et al., 1995; Ishaaya, and Swirski, 1970; Kiddis, et al., 1986; Laecke and Degheele, 1991; Ozmen, and Kilincer, 2002). The objective of this research was to evaluate the susceptibility laboratory of 2nd and 4th instars larvae of the cotton leafworm S. littoralis (Boisd.) to Dimilin analogue.

1-Dimiin

2- lufenuron

Corresponding Author: Mohamed Ahmed Gad, Plant Protection Research Institute, A. R. C., Dokki, Giza, Egypt
E-mail: samy_adjey@yahoo.com

404
Materials and Methods

All melting points are uncorrected and were determined by Kofeler melting point apparatus. IR (cm⁻¹) spectra were recorded (KBr disc) on a Shimadzu DR-8001 spectrophotometer. ¹H NMR (DMSO-d₆) spectra were recorded at 200 MHz on a Varian Gemini NMR spectrometer and also at 400 MHz, the chemical shift is expressed in δ value (ppm) using TMS as an internal reference. All NH groups were subjected to hydrogen/deuterium exchange test. Elemental analyses were carried out on a Perkin-Elmer 240°C Micro analyzer. The mass spectra were performed on Micro mass 7070E spectrometer using Direct Inlet and Shimadzu Qp-2010.

Chemical synthesis

The present work was conducted to prepare new derivatives of benzoylthiourea and benzoyl urea.

Synthesis of benzoylthiourea derivatives 2ac (General procedure):

Freshly prepared acid chloride (43 mmol) was added dropwise while stirring to an equimolecular amount of ammonium thiocyanate (3.2 g) in 20 ml dry acetone and refluxing for 3 hrs. A solution of amino compound (a: o-Anisidine, b: m-chloroaniline, c: 2,6-diaminopyridine) in the same solvent was added and the reaction solution was heated under reflux for 3 hours. The solution was poured on ice cubes. The resulting precipitate was collected by filtration, washed thoroughly and purified by crystallization from ethanol/dichloromethane mixture (1:1).

2,4-dichloro-N-[(2-methoxyphenyl)carbamothioyl]benzamide (2a):

White solid crystals (99% yield), mp. 142°C. IR (ν, cm⁻¹): 3396.02 (N-H), 3222.1 (N-H), 3026 (CHₑ₁₇), 1673.4 (C=O). ¹H NMR (DMSO-d₆), (δ ppm): 12.28 (s, 1H, N-Hₑₓch.), 11.5 (s, 1H, N-Hₑₓch.), 7.97-8.04 (m, 7H, Hₑₓch.), 3.4 (s, 3H, CH₃). ¹³CNMR (DMSO-d₆), (δ ppm): 178.08(C=O), 167.6(C=S), 152.3(C-OMe), 149.6(O-CH₃). 141.9(C-Cl, p-position), 146.0(C-Cl, p-position), 147.3(C-CH carbons at 134.2, 131.2, 129.9, 128.9, 128.0, 127.3. Anal. For C₁₇H₁₄N₂O₂S: 355.20 calcd/found: C:50.72/50.41, H:3.40/3.76 and N:7.89/7.44%.

2,4-dichloro-N-[(3-chlorophenyl)carbamothioyl]benzamide (2b):

White solid (99% yield), mp. 140°C. IR (ν, cm⁻¹): 3476.1(N-H), 3173.3 (N-H), 3047.6 (CHₑ₁₇), 1691 (C=O); ¹H NMR (DMSO-d₆), (δ ppm): 12.28 (s, 1H, N-Hₑₓch.), 11.5 (s, 1H, N-Hₑₓch.), 7.94-7.34 (m, 7H, Hₑₓch.). ¹³CNMR (DMSO-d₆), (δ ppm): 180.08(C=O), 177.6(C=S), 152.3(C-Cl, p-position), 149.6(C-Cl, p-position), 141.1(C-Cl, o-position), 146.0 (C-Cl, o-position), 141.3(C-CH carbons at 139.5, 138.8, 137.09, 132.3, 128.02, 119.9. Anal. For C₁₇H₁₂Cl₂N₂O₂S (359.6) calcd/found: C: 46.75/46.51, H: 2.52/2.76 and N: 7.47/7.34%.

N,N'-pyridine-2,6-diylicarbamothioyl)bis(2,4-dichlorobenzamide) (2c):

Yellow solid (98% yield), mp. 211°C; IR (ν, cm⁻¹): 3165.6 (N-H), 3017.5 (CHₑ₁₇), 1673 (C=O); ¹H NMR (DMSO-d₆), (δ ppm): 13.0 (s, 2H, N-Hₑₓch.), 12.09 (s, 2H, N-Hₑₓch.), 8.95-7.73 (m, 9H, Hₑₓch.) excha; ¹³CNMR (DMSO-d₆): 178.08(C=O), 167.6(C=S), 150.6(C-Cl, o-position), 137.4(C-Cl, p-position), 133.2(C-NH). 136.2(C-CH₂-CO, other aromatic C-H carbons at 131.9, 129.2, 128.6, 128.8, 121.8; Anal. For C₁₄H₁₀Cl₂N₂O₂S₂ (573.30) calcd/found: C: 44.00/44.10, H: 2.29/2.49 and N: 12.02/12.19%.

Synthesis of benzoylurea derivatives 5ac (general procedure):

o-Anisidine (10 mmole) dissolved in 100 ml dil. AcOH (10%) was added to 50 ml water solution of sodium cyanate (0.1mol) and stirred for 6 hours. The precipitated product was filtered off and crystallized from ethanol, 4-dioxane. A mixture of 1-(4-methoxyphenyl)urea (14mmole) and 2,4-dichlorobenzoyl chloride (2 mmole) was refluxed under dry conditions for 5 hours, cooled and treated several times with petroleum ether (80-100). The formed ppt was filtered off and crystallized from ethanol.

2,4-dichloro-N-[(2-methoxyphenyl)carbamoyl]benzamide (5a):

White solid (70% yield), mp. 125-127 °C; IR (ν, cm⁻¹): 3448 (OH), 3323 (NH), 2926.3 (CHₑ₁₇), 1662 (C=O). ¹H NMR (DMSO-d₆), (δ ppm): 9.79 (s, 1H, NHₑₓch.), 7.87-6.96 (m, 7H, Hₑₓch.;NHₑₓch.), 3.86 (s, 3H, CH₃). ¹³CNMR (DMSO-d₆), (δ ppm): 164.1(C=O), 151.03(C=O), 150.3(C-OMe), 149.6(O-CH₃), 148.6(C-Cl, o-position), 141.1(C-Cl, p-position), 148.0(C-CH₂-CO, other aromatic C-H carbons at 136.2, 134.27, 131.2, 129.9, 128.9, 128.0. Anal. For C₁₅H₁₀Cl₂N₂O₃ (339.17) calcd/found: C: 53.12/53.21, H:3.57/3.25 and N, 8.36/8.50%.
N-[(2-methoxyphenyl)carbamoyl]furan-2-carboxamide (5e):
White solid (73% yield), mp. 213 °C; IR (ν, cm⁻¹): 3408 (OH), 3223 (NH), 2926.3 (CH˺=˺), 1671 (C=O); HNMR (DMSO-d₆, δ ppm): 11.0 (s, 1H, NHexch.), 10.93(s, 1H, NHexch.), 8.02-6.76 (m, 7H, H₂=CH₃), 3.86 (s, 3H, CH₃). ¹³CNMR (DMSO-d₆, δ ppm): 158.91(C=O), 151.03(C=O), 145.2(C-O=Me), 139.9(O-C=H), 138.3(C-NH), 135.23(C=O) other aromatic C-H carbons at 132.2, 129.5, 128.7, 127.2, 122.8, 121.6. Anal. for C₁₃H₁₂N₂O₄ (260.21) calcd/found: C:60.12/60.21, H: 4.67/, 4.25 and N:10.76/10.56%.

Biological screening

A. toxicological studies:
The present work was conducted to study the susceptibility in laboratory of the cotton leafworm S. littoralis (Boisd.) to the benzoylthiourea and benzoylurea derivatives.

B. tested insect growth regulatory:
Five compounds were compared with the major dimilin 2, 4-diflubenzuron (1) which acts as insect growth regulator
1-2,6-difluoro- N-[(4-chlorophenyl)carbamoyl]benzamide (1):
2,2,4-dichloro-N-[(2-methoxyphenyl)carbamothioyl]benzamide (2a):
3-2,4-dichloro-N-[(3-chlorophenyl)carbamothioyl]benzamide (2b):
4- N,N′-(pyridine-2,6-diyldicarbamothioyl)bis(2,4-dichlorobenzamide) (2c):
5-2,4-dichloro-N-[(2-methoxyphenyl)carbamoyl]benzamide (5d):
N-[(2-methoxyphenyl)carbamoyl]furan-2-carboxamide (5e):

C. cotton leafworm strains:
Laboratory strain of the cotton leafworm S. littoralis (Boisd.) was obtained from assiut agricultural college as susceptible strain to carry out the present investigation.

Toxicity test: laboratory bioassay
A series of concentrations (in water and triton x-100) for each IGR were prepared as the active ingredients (a.i) based on ppm by diluting the commercial formulation. Castor-bean leaves were dipped for 30 seconds in each concentration then left to dry for one hour. The 2nd and 4th instars larvae of each tested strain were confined with treated leaves in glass jars covered with muslin for 24-48 hrs. Test also included a non treated control in which leaves were dipped in distilled water and triton x-100 (Blank). Treated leaves were then removed and fresh untreated leaves provided for three days. Three replicates (each of 10 larvae) were tested for each concentration. Daily inspection was carried out for all treatments and mortality percentages were recorded until for 3 day after treatment. The average of mortality percentage was corrected using Abbott’s formula (Ishaaya et al, 1995). The corrected mortality of each compound was statistically computed according to Finney, (1971). From which the corresponding concentration probit lines (LC-p lines) were estimated in addition to determine 50 and 90% mortalities, slope values of tested compounds were also estimated.

Results and Discussion

Synthesis.
The synthetic procedures for the title compounds are outlined in scheme 1 the intermediate compounds Wang et al., (2006) and (B) Clarire, et al., (2003) were prepared following the procedures reported previously. Structure of the synthesized compounds was elucidated according to the basis their spectral and elemental analyses. Toxicity test for the 2nd instar larvae of the cotton leafworm S. littoralis (Boisd.) as shown in Table (1) that compound 2a is the most effective IGR. 2a, Dimilin, 5a, 2b, 5c and 2c respectively shows the LC₅₀ values of 45.207, 46.84, 55.38, 82.64, 106.43 and 425.22ppm, respectively. However, LC₅₀ reached 788.857, 520.025, 506.42, 1151.52, 1536.6 and 4125.2 ppm, respectively. The toxicity index being 96.51, 100, 81.8, 54.8, 42.4 and 10.6% for 2a, Dimilin, 5a, 2b, 5c and 2c, respectively. In relation to the efficiency of tested IGRs against 4th instar larvae of the laboratory strain, as well 2a was the most effective IGR giving LC₅₀ value of 144.05 ppm followed by Dimilin, 5a, 2b, 5c, and 2c respectively. they were 1640.55, 2141.52, 3434.3, 4151.56 and 7347.4 ppm, respectively. The corresponding LC₅₀ reached 12592.756, 1451.342, 22019.071, 1573.332, 2527.096 and 1351.777 ppm, respectively. The toxicity index being 95.00, 100, 75.35, 54.07, 48.62 and 11.52% for Dimilin, 2a, 5a, 2b, 5c and 2c, (Based on LC₅₀ of Flufenoxuron 100.0%), respectively.
Scheme 1

Scheme 2

Table 1: Susceptibility of 2<sup>nd</sup> and 4<sup>th</sup> instars larvae of the laboratory strain of cotton leafworm, *Spodoptera littoralis* (Boisd.) to tested compounds

<table>
<thead>
<tr>
<th>Tested compounds</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; instar larvae</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; instar larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC&lt;sub&gt;50&lt;/sub&gt;(ppm) at 95%</td>
<td>Slope</td>
</tr>
<tr>
<td>Dimilin</td>
<td>46.842</td>
<td>1.295 ± 0.29</td>
</tr>
<tr>
<td>2a</td>
<td>45.207</td>
<td>1.208 ± 0.40</td>
</tr>
<tr>
<td>5d</td>
<td>55.38</td>
<td>1.07 ± 0.36</td>
</tr>
<tr>
<td>2b</td>
<td>82.646</td>
<td>0.1079 ± 0.36</td>
</tr>
<tr>
<td>5e</td>
<td>106.43</td>
<td>1.10 ± 0.36</td>
</tr>
<tr>
<td>2c</td>
<td>425.2</td>
<td>0.97 ± 0.35</td>
</tr>
</tbody>
</table>

Toxicity index = LC<sub>50</sub> of the most effective compound / LC<sub>50</sub> of the tested compound x 100
Fig.1: Insecticidal activities of Dimilin and compounds 2a,b,c and 5d,e against the 2nd larvae of S. littoralies (Bosid.) after 24 and 48 h of treatment.
Fig. 2: Insecticidal activities of Dimilin and compounds 2a,b,c and 5d,e against the 4th larvae of S. littoralis (Bosid.) after 24 and 48 h of treatment.
References


