International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 01-11-2021, Accepted: 17-11-2021, Published: 02-12-2021
Volume 6, Issue 6, 2021, Page No. 133-141

Synthesis, insecticidal assessments of some new heterocyclic compounds containing azobenzene moiety against the phytophagous pest, Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.)

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Abstract

2-Cyano-*N*-(4-(phenyl diazenyl) phenyl) acetamide was a key intermediate for the synthesis of innovative heterocyclic compounds such as thiazoles, thiophenes, and pyrazoles incorporating azobenzene moiety. The structures of the newly synthesized compounds were elucidated by IR, MS and ^{1}H NMR spectral analysis. Toxicological aspects of the demonstrative compounds of the synthesized products against the cotton leafworm, *Spodoptera littoralis* under laboratory conditions were investigated. Regarding the determined LC$_{50}$ and LC$_{90}$ values, azobenzenes bearing amide moiety 16, 17, 6a, 8a and 7 showed the most potent toxic effects with LC$_{50}$ values of 24.995, 31.291, 45.048, 64.739 and 82.248 ppm, respectively, and toxicity index being 53.75, 42.94, 29.83, 20.76 and 16.34%, respectively, comparing with the commercial already known, carbamate insecticide, lannate 90% SP which was the most effective compound (the lowest LC$_{50}$ value, 13.435 ppm, toxicity index, 100%).

Keywords: cyanoacetamide; azobenzene; heterocyclic; insecticidal activity, cotton leafworm; *spodoptera littoralis*

Introduction

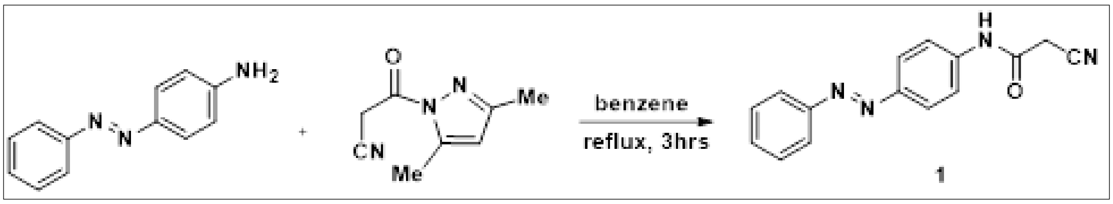
Cyanoacetamides showed their potency as reactants or intermediates of reactions. That was because of the carbonyl and the cyano functions which were helpful to get expanded varities of heteocyclic compounds. Synthesis of them could be done by certain economical methods depending on the substitution of heteryl or aryl amines with alkyl cyanoacetates using different reaction conditions to produce cyanoacetamide derivatives [1]. Available cyanoacetyl pyrazole was synthesized firstly by Ried *et al* [2], then it was used efficiently to get *N*-alkyl and *N*-aryl cyanoacetamides. Polyfunctional cyanoacetamides having both electrophilic and nucleophilic characteristics which had been utilized to outline diverse heterocyclic moiety with various ring sizes.

So, cyanoacetamides showed their efficacy to synthesize three-membered rings [3], five and six membered rings [4-8]. Moiety of the azobenzene (AZB) showed its carcinogenic properties clearly through formulated alanine and its metabolites in experimental animals. Such compounds were known to cause some haemolytic anemia [9] and haematological parameters beside their various effects including carcinogenicity that correlated with invivo genotoxicity in rodents.

Results and Discussion

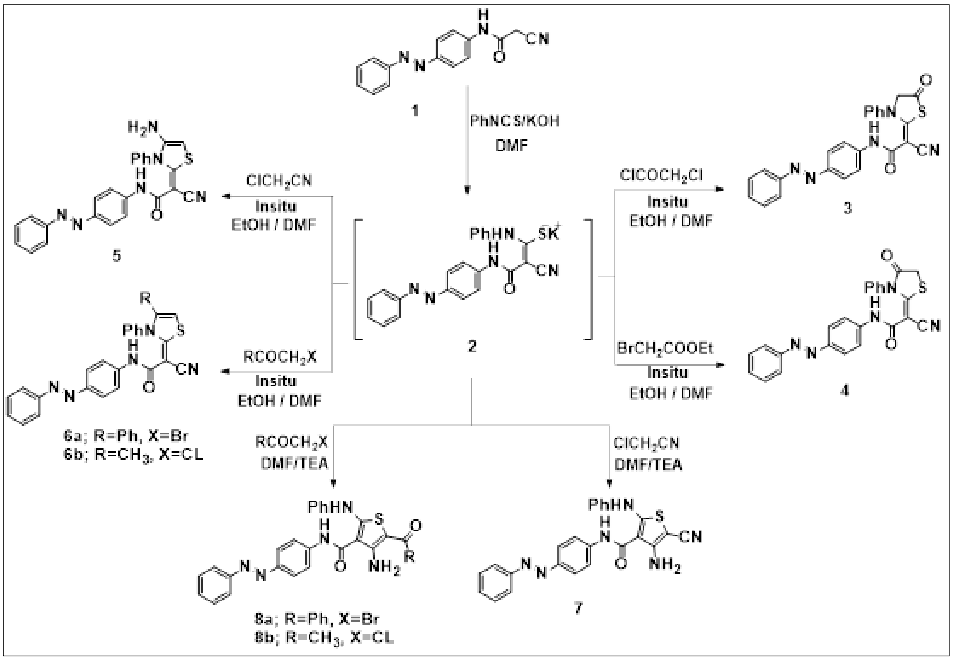
Chemistry

The synthetic strategies adopted for the synthesis of the intermediate and target compounds are depicted in schemes (1-4). The starting compound 2-cyano-N-(4-(phenyl diazenyl) phenyl) acetamide (1) was prepared by cyano acetylation of 4-amino azobenzene with 1-cyano acetyl-3,5-dimethyl pyrazole in dry benzene (Scheme 1).



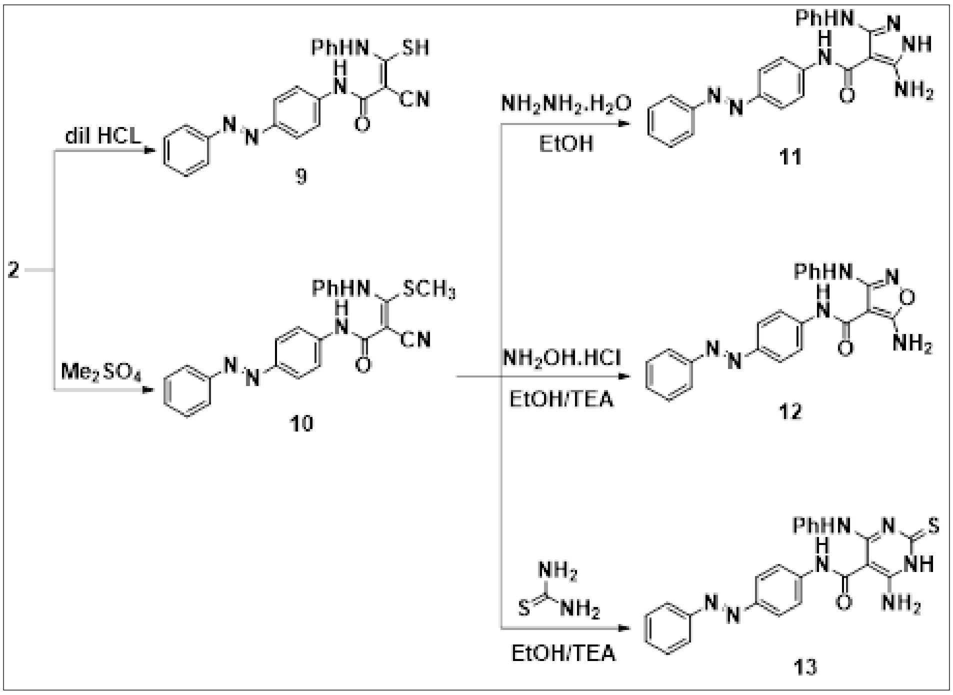
Scheme 1

The active methylene group in the cyanoacetamide derivative 1 was readily added to phenyl isothiocyanate in DMF in the presence of an equimolar ratio of potassium hydroxide yielding the non-isolable intermediate potassium sulphide salt 2 which underwent *in situ* heterocyclization up on treatment with α-halocarbonyl compounds such as chloroacetyl chloride, ethyl bromoacetate, phenacyl bromide and chloroacetone to afford the corresponding thiazole derivatives 3, 4, 5, 6a and 6b, respectively. The assignment of these structures was based on the elemental analysis and spectral data. The IR spectra displayed absorption bands assignable to cyano function and broad peak for NH group. The IR spectrum of 3 displayed stretching frequencies at 3403, 2191, 1733 and 1696 cm^{-1} for NH, cyano group and two carbonyl groups, respectively. Its ^{1}H NMR spectrum (DMSO-d_6) showed the appearance of certain signals such as, firstly, a singlet signal for methylene protons at δ_H 4.04 ppm, then multiplet signals for aromatic protons at δ_H 7.28-7.96 ppm and finally, a singlet signal at δ_H 9.80 ppm for NH proton. The mass spectrum revealed a molecular ion peak at m/z 439 corresponding to the molecular formula $C_{24}H_{17}N_5O_2S$. The structure of the isolated product 4 was elucidated on the basis of its spectral data (IR and MS). In addition, the intermediate potassium sulphide salt 2 underwent *in situ* heterocyclization up on treatment with chloroacetonitrile by stirring in Ethanol–DMF (1:1) affording the thiazole derivative 5. The assignment of structure 5 was based on both elemental and spectral analyses. The IR spectrum displayed absorptions at 3350 and 3314cm^{-1} assignable to the newly formed amino group. Cyano absorption appeared at 2191 cm^{-1} in addition to a strong absorption at 1664 cm^{-1} for amidic carbonyl function. In addition, the mass spectrum of the thiazole structure 5 showed a molecular ion peak at m/z 438 corresponding to the molecular formula $C_{24}H_{18}N_6OS$. Furthermore, the intermediate potassium sulphide salt 2 underwent *in situ* heterocyclization up on treatment with phenacyl bromide by stirring in Ethanol–DMF (1:1) affording the structure 6a which was established on the basis of spectral data. The IR spectrum revealed an absorption band at 3287 cm^{-1} for NH group, a sharp band at 2203 cm^{-1} for cyano function and a strong sharp band at 1639 cm^{-1} for amidic carbonyl group. Its ^{1}H NMR spectrum (DMSO-d_6) revealed the presence of a singlet signal at δ_H 7.11 ppm assignable for the thiazole ring proton, multiplet signals for aromatic protons at δ_H 7.13-7.94 ppm and a singlet signal at δ_H 10.29 ppm for NH proton. Its mass spectrum revealed a molecular ion peak at m/z 499 corresponding to the molecular formula $C_{30}H_{21}N_5OS$. Moreover, the assignment of 6b was based on both elemental analysis and spectral data. The IR spectrum displayed absorptions at 3386, 2180 and 1668 cm^{-1} for NH, cyano and amidic carbonyl groups, respectively. The ^{1}H NMR spectrum (DMSO-d_6) revealed the presence of three singlet signals at δ_H 1.33, 6.97 and 9.34 ppm assignable for the methyl protons, methine proton and NH proton, respectively. Also, multiplet signals were detected for aromatic protons at δ_H 7.41-7.86 ppm. Its mass spectrum revealed a molecular ion peak at m/z 437 corresponding to the molecular formula $C_{25}H_{19}N_5OS$. On the other hand, it has been found that the *in situ* reaction of 2 with chloroacetonitrile, phenacylbromide and chloroaceton in refluxing conditions in DMF in the presence of catalytic amounts of TEA afforded thiophene derivatives 7, 8a and 8b, respectively. Compound 7 was also confirmed by spectral analyses. The IR spectrum displayed absorptions at 3403, 3333 cm^{-1} for NH_2 group and 3351 cm^{-1} for NH, cyano and amidic carbonyl groups appeared at 2197 and 1660 cm^{-1}. Its mass spectrum revealed a molecular ion peak at m/z 438 corresponding to the molecular formula $C_{24}H_{18}N_6OS$. Moreover, the IR spectrum of thiophene derivative 8a showed absorption bands at 3401 and 3338 cm^{-1} for NH_2 group, 3285, 2185, 1689 and 1648 cm^{-1} for NH, cyano, carbonyl and amidic carbonyl, respectively. The ^{1}H NMR spectrum (DMSO-d_6) of 8a confirmed the presence of multiplet signals for aromatic protons at δ_H 7.31-8.04 ppm and two singlet signals at δ_H 10.29 and 11.1 ppm assignable for two NH protons. Alongside, its mass spectrum revealed the molecular ion peak at m/z 517 corresponding to the molecular formula $C_{30}H_{23}N_5O_2S$. Furthermore, structure 8b was consisted on the basis of spectral data. The IR spectrum detected absorption bands at 3438 and 3422 cm^{-1} for NH_2 group, 3352 cm^{-1} for NH and sharp bands at 1720 and 1650 cm^{-1} for carbonyl and amidic carbonyl functions. The ^{1}H NMR spectrum (DMSO-d_6) of 8b showed the presence of a singlet signal at δ_H 2.39 ppm assignable for the methyl protons, multiple signals for aromatic protons at δ_H 7.39-7.95 ppm and singlet signal at δ_H 9.83, 10.20ppm assignable to NH protons. Its mass spectrum detected the molecular ion peak at *m/z* 455 corresponding to the molecular formula $C_{25}H_{21}N_5O_2S$.



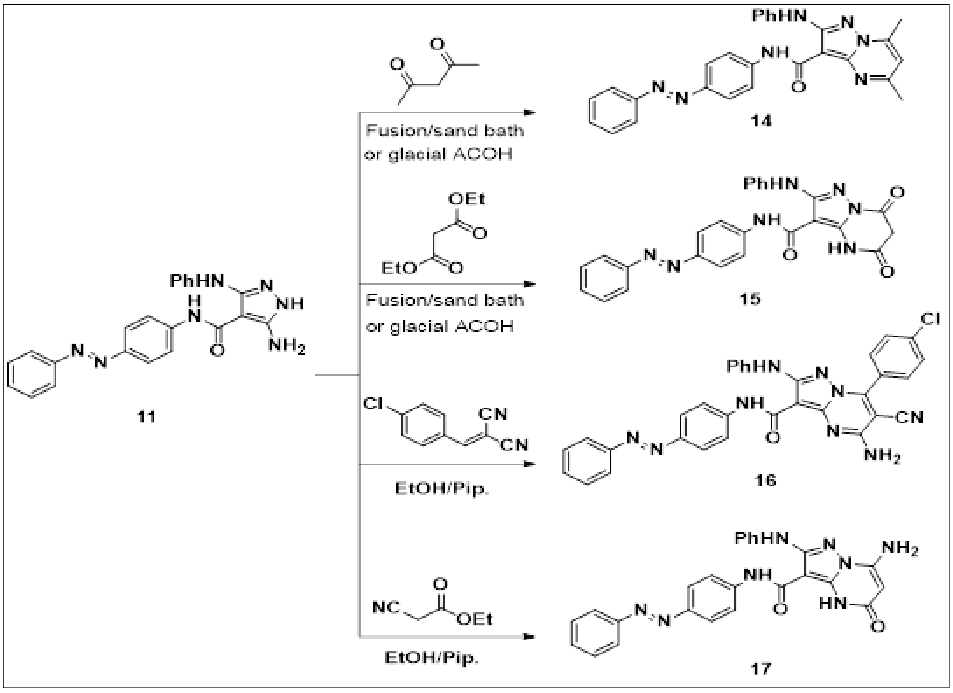
Scheme 2

Acidification of intermediate 2 with hydrochloric acid afforded compound 9 which was confirmed by IR and mass spectroscopy. When intermediate potassium sulphide salt 2 reacted with dimethyl sulphate, they afforded (*E*)-2-cyano-3-(methylthio)-3-(phenylamino)-*N*-(4-(*E*)-phenyldiazenyl) phenyl) acrylamide (10). The IR spectrum showed absorption bands at 3313, 3255 cm-1 for two NH stretching, a band at 2199 cm-1 for cyano group, and a strong absorption band for amidic carbonyl group at 1636 cm-1. Its 1H NMR spectrum (DMSO-*d*6) displayed a strong singlet signal at δ_H 2.29 ppm for methyl protons and a multiplet signals for aromatic protons at δ_H 7.21-7.86 ppm and two sharp singlet signals at δ_H 9.92 ppm, δ_H 11.55 ppm appeared for NH-Ph, NHCO protons, respectively. The mass spectrum showed a molecular ion peak at m/z 413 corresponding to the molecular formula $C_{23}H_{19}N_5OS$. Compound 10 was utilized as a starting material for the preparation of a wide variety of fused hetero compounds by reaction with bifunctional nucleophilic reagents. Heating 10 with hydrazine hydrate afforded pyrazole derivative 11 while refluxing 10 with hydroxyl amine hydrochloride and TEA in ethanol afforded isoxazole derivative 12. Finally, refluxing 10 with thiourea in ethanol afforded pyrimidine derivative 13. The IR spectrum of 11 displayed stretching bands at 3446 and 3362 cm-1 for the formed amino group and two absorption bands for two NH groups at 3291 and 3255 cm-1 while carbonyl absorption appeared at 1640 cm-1. Its ^{1}H NMR spectrum (DMSO-d_6) displayed a sharp signal at δ 6.13 ppm assignable to amino protons, three sharp signals for the three NH protons at δ_H 8.55, 9.11 and 11.39 ppm; while aromatic protons appeared at δ_H 7.21-7.89 ppm. The mass spectrum showed the molecular ion peak at *m/z* 397 corresponding to a molecular formula $C_{22}H_{19}N_7O$. The IR spectrum of 12 displayed stretching bands at 3448 and 3355 cm-1 for the formed amino group and two absorption bands for two NH groups at 3328 and 3273 cm-1 in addition to a carbonyl absorption at 1658 cm-1. Its mass spectrum demonstrated the molecular ion peak at *m/z* 398 corresponding to the molecular formula $C_{22}H_{18}N_6O_2$. The IR spectrum of 13 displayed stretching bands at 3478 and 3375 cm-1 for the formed amino group and two absorption bands for two NH groups at 3330 and 3279 cm-1 in addition to a carbonyl absorption at 1635 cm-1. The mass spectrum showed the molecular ion peak at *m/z* 441 compatible with the molecular formula $C_{23}H_{19}N_7OS$. The structure of aminopyrazole 11 was a versatile reagent that had been extensively used as a synthetic starting material for the synthesis of several polysubstituted fused pyrazoles of a potential biological activity [10]. It was of interest to study the reactivity of 5-amino pyrazole 11 towards a variety of chemical reagents [11].



Scheme 3

Cyclocondensation of compound 11 with either acetyl acetone or diethyl malonate in boiling acetic acid or fusion in sand bath produced in each case a single product, as evidenced by TLC. The reaction product can be formulated as pyrazolo[1,5-a] pyrimidine derivatives 14 and 15, respectively. Evidence for assigned structures was provided by spectroscopic data. For example, the IR spectrum of compound 14 showed the appearance of an absorption band at 1661 cm^{-1} corresponding to the amidic carbonyl group. The mass spectrum showed a molecular ion peak at *m/z* 461 corresponding to its molecular formula $C_{27}H_{23}N_7O$. The formation of compound 15 is most likely to proceed via elimination of ethanol and intramolecular cyclization. Thus, the IR spectrum of 15 displayed the presence of absorption bands at 3442 and 3468 cm^{-1} corresponding to two NH groups and 1658, 1675 and 1740 for three amidic carbonyl functions. The mass spectrum showed a molecular ion peak at *m/z* 465 corresponding to its molecular formula $C_{25}H_{19}N_7O_3$. Furthermore, the reactivity of aminopyrazole 11 towards benzylidene malononitrile was also investigated as an alternative route to obtain pyrazolo[1,5-a] pyrimidine derivative. Thus, the reaction of 11 with *p*-chlorobenzilidine malononitril in ethanol containing a catalytic amount of pipridine afforded a product for structure 16. The structure of the compound 16 was identified on the basis of spectral data and elemental analysis. The IR spectrum showed an absorption bands at 3443, 3410 (NH_2), 2215 (CN), 1639 (CO). The mass spectrum showed the molecular ion peak at MS, *m/z* 583.Moreover, heating 11 with ethyl cyanoacetate in ethanol and a catalytic amount of piperidine furnished pyrazolo[1,5-a] pyrimidine derivative 17 in a reasonable yield. Elemental analysis and spectral data were in agreement with the formation of pyrazolo[1,5-a] pyrimidine 17. The IR spectrum of 17 displayed stretching bands at 3435and 3342 cm-1 for amino group and three absorption bands for three NH groups at 3322, 3291 and 3277 cm-1, while carbonyl absorptions appeared at 1646 cm-1. Moreover, the mass spectrum showed a molecular ion peak at *m/z* 464 corresponding to the molecular formula $C_{25}H_{20}N_8O_2$.



Scheme 4

Insecticidal activity

Toxicity test for the cotton leafworm, *Spodoptera littoralis*

In this study, a laboratory bioassay was conducted to evaluate the insecticidal efficacy of twelve innovatively synthesized tested compounds as toxic agents towards the susceptible laboratory strain of the 2nd larval instar of the momentous phytophagous pest, the Egyptian cotton leafworm, *S. littoralis* (Boisd.), in Table 1. The bio assessment results publicized that, all tested compounds possess strong insecticidal activity after 3 days of treatment with (LC_{50}) values that ranged from 203.241 to 24.995 ppm. The efficacy of the different tested compounds was measured by comparing the newly synthesized compounds (based on LC_{50} value) with the already known, carbamate insecticide, lannate 90% SP which was the most effective compound (the lowest LC_{50} value, 13.435, toxicity index, 100%). Regarding the determined LC_{50} and LC_{90} values, 16, 17, 6a, 8a and 7 showed the most potent toxic effects with LC_{50} values of 24.995, 31.291, 45.048, 64.739 and 82.248 ppm, respectively, and toxicity index being 53.75, 42.94, 29.83, 20.76 and 16.34%, respectively. It is interesting to note that the insecticidal activities of the tested compounds against the 2nd instar larvae of *S. littoralis* (Boisd.) after 3 days of treatment obey the following smooth order: 16>17>6a>8a>7>5>4>6b>8b>12>13>1.

Table 1: Susceptibility of the 2nd instar larvae of the laboratory strain of cotton leafworm, *S. littoralis* (Boisd.) to the the newly synthesized compounds as insecticidal agents after 3 days of treatment

Tested compounds	LC_{50} (ppm) and confidence limits at 95%	LC_{90} (ppm) and confidence limits at 95%	Slope	Toxicity index % at LC_{50} value
Lannate 90% SP	13.435 11.545 15.342	34.975 28.579 47.652	3.084+/- 0.391	100
16	24.995 15.460 32.867	85.777 65.423 137.411	2.393+/- 0.460	53.75
17	31.291 19.210 42.588	164.285 118.377 281.652	1.780+/- 0.295	42.94
6a	45.048 31.262 58.693	231.570 164.083 407.394	1.803+/- 0.275	29.83
8a	64.739 47.269 83.098	370.370 262.120 629.870	1.692+/- 0.223	20.76
7	82.248 64.155 102.106	377.698 277.548 594.019	1.936+/- 0.231	16.34
5	96.628 76.389 118.603	443.683 335.221 656.024	1.936+/- 0.208	13.91

4	124.304 101.133 150.145	510.230 389.794 741.761	2.090+/- 0.217	10.81
6b	129.948 103.236 160.695	678.782 486.653 1105.436	1.785+/- 0.203	10.34
8b	135.603 108.087 168.182	706.673 496.932 1201.669	1.788+/- 0.213	9.91
12	167.180 133.085 208.640	722.080 492.727 1410.179	2.017+/- 0.311	8.04
13	169.932 130.164 212.512	948.653 630.158 1928.279	1.711+/- 0.259	7.91
1	203.241 163.156 251.325	958.453 657.216 1803.152	1.903+/- 0.271	6.61

Note: Toxicity index is defined as the ratio of the most effective compound's LC_{50} value to the other tested compound's LC_{50} value multiplying by 100.

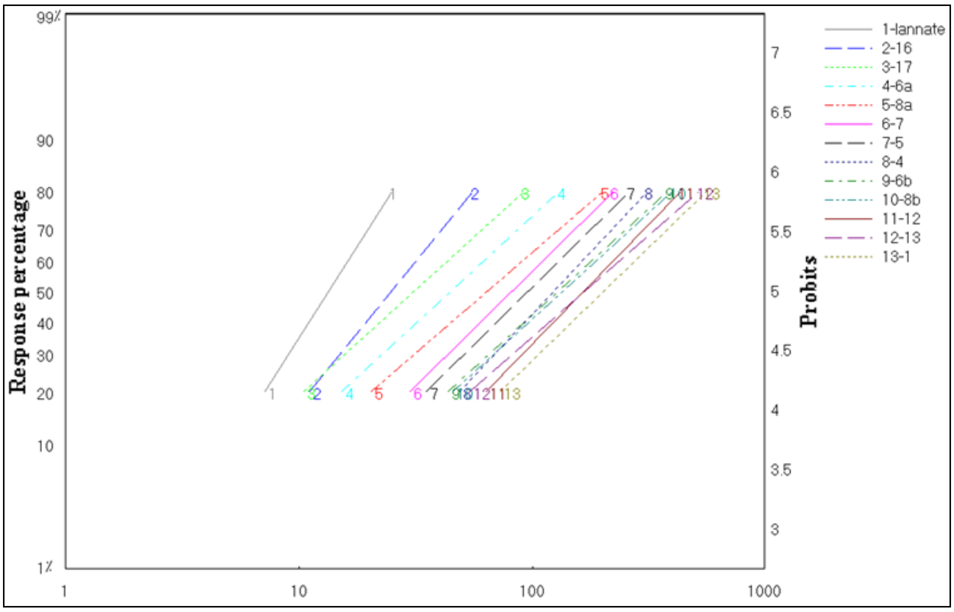


Fig 1: Probit regression lines for the new heterocyclic compounds (µg/ml) compared with Lannate 90% SP. against the laboratory strain of cotton leafworm, S. littoralis (Boisd.).

Experimental

All melting points (uncorrected) were in degree centigrade and determined on Gallenkamp electric melting point apparatus. The IR spectra were recorded (KBr) on a Mattson 5000 FTIR spectrophotometer at Micro analytical Unit, Faculty of Science, Mansoura University. The ^{1}H NMR spectra were measured on Bruker WP 400 MHz in DMSO-*d*6 as solvent, using TMS as an internal reference at Microanalytical Unit, Faculty of Pharmacy, Beni Sewif University. Mass spectra were recorded on Finnegan MAT 212 instrument at Micro analytical Unit, faculty of Science, Cairo University and the Regional Center for Mycology and Biotechnology at Al-Azhar University.

Synthesis of (Z)-2-Cyano-N-(4-(Phenyldiazenyl) Phenyl) acetamide (1)

A solution of *p*-amino azobenzene (1.97 g, 0.01 mol) in dry benzene (20 ml) was added to a solution of 1-cyano-acetyl-3,5- dimethylpyrazole (1.63 g, 0.01 mol) in the same solvent and the mixture was heated under reflux for 3 hrs. After cooling, the solid portion was isolated and recrystallized from dry ethanol to give 1; golden yellow crystals;Yield (90%); mp189-190℃; IR (KBr)vmax.cm-1: 3280 (NH), 2256 (CN), 1669 (CO); ^{1}H NMR (DMSO-d6): δH 3.97 (s, 2H, CH2), 7.56-7.93 (m, 9H, Ar-H), 10.66 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d6): δ_C ppm 24.9,119.3,123.0, 124.0,125.7, 129.0, 130.9, 140.7, 150.9, 152.7, 168.2. MS: m/z 264 (M+, 31.8), 159 (66), 77 (100), 65 (19.93), 51 (25). Anal. Calcd. For C15H12N4O, (264): C 68.16; H 4.56; N 21.18 %. Found: C 68.17; H 4.58; N 21.20%.

General procedure for the synthesis of thiazole derivatives 3, 4, 5, 6 and 7

To a stirred solution of non-isolable salt **2**, (0.167 ml, 0.001 mol) in DMF (20 ml), the appropriate α-halocarbonyl compounds such as chloroacetyle chloride, ethylbromoacetat, chloroacetonitrile, chloroacetone, and phenacyl bromide (0.001 mol) in ethanol (20ml) were added and continued stirring for 12 hrs. The reaction mixture was poured on ice water. The formed solid product was collected by filtration, washed with ethanol and recrystallized from a mixture of ETOH/DMF (2:1) to afford the corresponding thiazole derivatives 3, 4, 5, 6 and 7.

Synthesis of (E)-2-cyano-2-(5-oxo-3-phenylthiazolidin-2-ylidene)-N-(4-((E)-phenyldiazenyl) phenyl) acetamide (3)

yellow powder; Yield(65%); mp. 220-222℃; IR (KBr) vmax.cm^{-1}: 1733 (C=O), 1696 (amidic C=O), 2191 (CN), 3403 (NH); ^{1}H NMR (DMSO-d_6): δ_H 4.04 (s, 2H, CH_2), 7.28-7.96 (m, 14H, Ar-H), 9.80 (s, 1H, NH); MS: *m/z* 439, M^+; 90.09 (21.01); 71.07(23.00). Anal.Calcd.for ($C_{24}H_{17}N_5O_2S$), (439) C, 65.59; H, 3.90; N, 15.94%. Found. C, 65.58; H, 3.91; N, 15.96%.

Synthesis of (E)-2-cyano-2-(4-oxo-3-phenylthiazolidin-2-ylidene)-N-(4-((E)-phenyldiazenyl) phenyl) acetamide (4*)*

Yellow powder;Yield (60%); mp. 225-230℃; IR (KBr) νmax.cm^{-1}: 1696(CO), 2204 (CN), 3307(NH); ^{1}H NMR (DMSO-d6): δ_H 4.16 (s, 2H, CH2), 7.32-8.41(m, 14H, Ar-H), 10.12 (s,1H, *N*HCO). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 32.6, 69.3, 114.6, 119.5, 123.0, 128.0, 129.0, 130.9, 139.1, 150.9, 163.8, 167.5, 178.6. MS: *m/z* 439 (M^+, 3.91), 350.07 (7.98), 257.14 (14.07), 239.99 (100.00), 118.08 (46.73), 71.07(23).Anal. calcd for($C_{24}H_{17}N_5O_2S$), (439), C, 65.59; H, 3.90; N, 15.94%. Found C, 65.58; H, 3.91; N, 15.95%.

Synthesis of (E)-2-(4-amino-3-phenylthiazol-2(3H)-ylidene)-2-cyano-N-(4-((E) phenyldiazeny l)phenyl) acetamide (5*)*

Orange powder ; Yield (50%); mp. 220-225 ℃; IR (KBr) *νmax.*cm-1: 1664 (amidic carbonyl), 2191(CN), 3314, 3350 (NH_2);); ^{1}H NMR(DMSO-d_6): δ_H 5.18 (s,2H, NH_2), 5.66 (s, 1H, olefinic H-thiazole ring), 7.2-8.41(m, 14H, Ar-H), 10.12 (s, 1H, *N*HCO). MS: *m/z*, 440 (M^++1, 0.88), 439 (M^+, 3.14), 334.08 (5.75), 215.05 (11), 197.13 (19.49), 120.08 (25.48), 92.09 (62.74), 77.08 (100), 51.08 (19.96). Anal. Calcd. for ($C_{24}H_{18}N_6OS$). C, 65.74; H, 4.14; N, 19.17%. Found: C, 65.73; H, 4.16; N, 19.19%.

Synthesis of (2E)-2-cyano -2-(3, 5,-diphenylthiazol-2(3H)-ylidene)-N-(4-(phenyldiazenyyl) phenyl) acetamide (6a)

Orange powder; Yield (80%); mp. 220-225℃; IR (KBr) *νmax.*cm-1: 3287(NH), 2203(CN), 1639 (CO); ^{1}H NMR(DMSO-d_6): δ_H 7.11 (s,1H, thiazole H-4), 7.13-7.94 (m, 19H, Ar-H), 10.29 (s, 1H, *N*HCO).MS: *m/z*, 500 (M^++1, 0.52), 499 (M^+, 0.71), 494.45 (2.30), 367.30 (10.40), 339.28 (12.80), 319.04 (28.71), 313.27 (42.94), 294.11 (23.99), 265.26 (15.49), 239.23 (22.32), 236.22 (41.81), 105.04 (100).Anal.Calcd.for ($C_{30}H_{21}N_5OS$).(499). C, 72.12; H, 4.24; N, 14.02%. Found. C, 72.55; H, 4.16; N, 14.19%.

Synthesis of (E)-2-cyano-2-(4-methyl-3-phenylthiazol-2(3H)-ylidene)-N-(4-((E)-phenyldiazenyl) phenyl) acetamide (6b)

Pale yellow powder;Yield (88%);mp. 230-235℃; IR (KBr) νmax.cm^{-1}: 3386 (NH), 1668 (CO) 2180(CN) ; ^{1}H NMR (DMSO-d_6): δ_H 1.33 (s, 3H, CH_3), 6.97 (s,1H, thiazole H-4), 7.31-7.86 (m, 14H, Ar-H), 9.34 (s,1H, *N*HCO).MS: *m/z* 437 (M^+, 13), 425(25.09), 363 (55.01), 332.07 (77.60), 317 (65.00), 241(100), 117.1 (11.00), 59 (45.30). Anal.Calcd. for $C_{25}H_{19}N_5OS$; C, 68.63; H, 4.38; N, 16.01%. Found. C, 68.43; H, 4.27; N, 15.85%.

General procedure for synthesis of thiophene derivatives 7, 8a and 8b

To a solution of intermediate 2 in DMF (10ml), the appropriate α-halo compound such as chloroacetonitrile, α-halocarbonyl compounds such as phenacyl bromide and chloroacetone (0.001 mol) and few drops of triethylamine were added. The reaction mixture was refluxed for 3hrs, and then allowed to cool. The reaction mixture was poured onto ice water (50ml). The solid product that formed was collected by filtration, dried and recrystallized from ethanol to afford the corresponding thiophene derivatives 7, 8a and 8b

Synthesis of (E)-4-amino-5-cyano-2-(phenylamino)-N-(4-(phenyldiazenyl) phenyl) thiophene-3-carboxamide (7)

Pale brown powder; Yield (55%); mp.250-255℃; IR (KBr) νmax.cm^{-1}: 3213 (NH), 33351, 3333 (NH_2), 2197 (CN), 1660 (amidic carbonyl). ^{1}H NMR (DMSO-d_6): δ_H 5.82 (s, 2H, NH_2), 7.02-8.41(m. 14H, Ar-H), 10.24 (s, 1H, NH). MS: *m/z*, 440 (M^++1, 0.53), 439 (M^+, 1.49), 405.19 (6.57), 340.18 (30.21), 300.17 (90.60), 239.24 (29.42), 236.19 (33.41), 208.15 (13.43), 135.11 (27.19), 95.11 (53.26), 79.08 (86.25), 69.10 (98.24), 57.09 (100), 41.07(70.70) Anal.calcd. For ($C_{24}H_{18}N_6OS$), (440). C, 65.74; H, 4.14; N, 19.17%. Found: C, 65.73; H, 4.16; N, 19.19%.

Synthesis of (E)-4-amino-5-benzoyl-2-(phenyl amino)-N-(4-(phenyldiazenyl) phenyl) thiophene-3-carboxamide (8a)

Deep brown powder; Yield (85%); mp. 245-250 ºC; IR (KBr) νmax.cm^{-1}:3401, 3338 (NH_2), 1648, 1689 (CO) 2185(CN) and 3285(NH); 1H NMR (DMSO-*d*6): 7.35-8.04 (m, 19H, ArH), 10.295 (s, 1H, NH), 11.1 (s, 1H, *N*HCO). MS: *m/z*, 518 (M^++1, 6.00) 517.16 (M^+, 5.34), 448.38 (8.05), 389.24 (25.34), 367.21 (16.39), 215.09 (31.90), 105.07 (40.45), 91.09 (50.70), 84.08 (100), 70.10 (49.42). Anal. Calcd. For($C_{30}H_{23}N_5O_2S$). C, 69.61; H, 4.48; N, 13.53%. Found. C, 69.56; H, 4.27; N, 13.42%.

Synthesis of (E)-5-acetyl-4-amino-2-(phenylamino)-N-(4-(phenyldiazenyl) phenyl) thiophene-3-carboxamide (8b)

Brown powder; Yield (88%); mp. 230-235 ℃; IR (KBr) νmax.cm^{-1}: 3438, 3422(NH_2) and 3352 (NH), 1650 (amidic CO), 1720 (CO). ^{1}H NMR (DMSO-d_6): δ_H 2.39 (s, 3H, CH_3), 7.39-7.95 (m, 14H, Ar-H), 9.83 (s, 1H, NHCO); 10.20 (s, 1H, NH)). MS: *m/z* 455 (M^+, 43), 437 (29), 405 (50), 363 (62), 347 (50), 113 (35), 59 (100). Anal.Calcd. for ($C_{25}H_{21}N_5O_2S$),(455). C, 65.92; H, 4.65; N, 15.37%. Found. C, 65.84; H, 4.27; N, 15.42%.

Synthesis of (E)-2-cyano-3-mercapto-3-(phenylamino)-N-(4-((E)-phenyldiazenyl) phenyl) acrylamide (9)

A solution of intermediate **2** in DMF (20ml) was poured onto ice cold water (100ml) containing few drops of HCL (0.1 N). The solid product that separated was filtered, washed with water and crystallized from ETOH to give **9**. Yellow crystals; yield 82%; mp. 150-155℃; IR (KBr) νmax.cm^{-1}: 3403 (NH), 2194 (CN), 1638 (CO).). ^{1}H NMR (DMSO-d_6): δ_H 6.69-8.41 (m. 14H, Ar-H), 10.12(s, 1H, *N*HCO), 10.74(s, 1H, NH). MS: *m/z* 399 (M^+, 15.3), 381.33 (10.29), 332.43 (13.51), 261.14 (30.09), 173.1 (32.97), 153.12 (22.23), 97.09 (100), 57.07 (90.30), 43.07 (64.88). Anal.Calcd. for ($C_{22}H_{17}N_5OS$). (399). C, 66.15; H, 4.29; N, 17.53%. Found, C, 66.12; H, 4.28; N, 17.50%.

Synthesis of (E)-2-cyano-3-(methylthio)-3-(phenylamino)-N-(4-((E)-phenyldiazenyl) phenyl) acrylamide (10)

To a solution of compound 2 (4.38g, 0.01 mol) in DMF (20ml), add dimethyl sulfate was added (1.26 ml, 0.01 mol) and stirred for 12 hr at room temp. The precipitated product that formed was collected by filtration, washed with petroleum ether (40-60), dried and crystallized from a mixture of DMF/ETOH (1:1) to give 10. Yellow crystals; Yield (62%); mp.150-155℃; IR (KBr) νmax.cm^{-1}: 3313, 3255 (NH), 2199 (CN), 1636 (CO). ^{1}H NMR (DMSO-d_6): δ_H 2.29 (s, 3H, CH_3), 7.21-7.86 (m, 14H, ArH), 9.92 (s, 1H, *N*HPh), 11.55 (s, 1H.*N*HCO). MS: *m/z*(%), 414 (M^++1, 50.00), 413 (M^+, 70.63), 308.09 (16.11), 217.03 (60.73), 197.12 (69.77), 169.05 (28.08),1 20.05 (56.99), 92.08 (38.32), 77.06 (100).Anal.Calcd. for ($C_{23}H_{19}N_5OS$).(413). C, 66.81; H, 4.63; N, 16.94%. Found, C, 66.78; H, 4.28; N, 16.85%.

Synthesis of (E)-5-amino-3-(phenylamino)-N-(4-(phenyldiazenyl) phenyl)-1H-pyrazole-4-carboxamide (11)

A mixture of 10 (0.3 g, 0.001 mol) and hydrazine hydrate 98% (1ml, 0.001 mol) was refluxed in ethanol (10ml) for 3hrs, and then allowed to cool. The precipitate that formed was collected by filtration, dried and recrystallized from a mixture of DMF/ETOH (1:1) to give compound 11.yellow needles crystals; Yield (47%) ; mp. 175-180 ̊c; IR; (KBr)νmax.cm^{-1} 3446 (NH); 3291, 3255; 3446, 3362 (NH_2), 1640 (amidic CO). ^{1}H NMR (DMSO-d_6): δ_H 2.02 (s, 3H, CH_3), 7.21-7.89 (m, 14H, ArH), 6.13 (s, 2H, NH_2),8.55 (s,1H, NH), 9.11 (s, 1H, NH), 11.55 (s, 1H, *N*HCO).). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C ppm 84.7, 117.9, 123.0, 124.6, 129.3, 130.9, 140.3, 140.9, 150.9, 152.8, 164.9. MS: *m/z* (%), 398 (M^++1, 8.22) 397.14 (M+, 35.84), 201.11 (92.38), 200.09 (100), 197.11 (46.34), 174.12 (25.25), 120.10 (51.34), 92.11 (84.42), 77.09 (84.42). Anal.Calcd for ($C_{22}H_{19}N_7O$). (397). C, 66.49; H, 4.82; N, 24.67%. Found C, 66.43; H, 4.76; N, 24.64%.

Synthesis of (E)-5-amino-3-(phenylamino)-N-(4-(phenyldiazenyl) phenyl) isoxazole-4-carboxamide (12)

A mixture of 10 (0.3 g, 0.001 mol) and hydroxyl amine hydrochloride (0.07 g, 0.001mol), was heated in refluxed ethanol (10 ml) with a catalytic amount of triethy lamine (5-8 drops and then left to cool. The reaction mixture was poured on to ice coled water (50 ml). The resulting solid was filtered off, dried well and recrystallized from ETOH/DMF (1:3) to yield compound 12; yellow crystals; Yield(45%); mp. 200-205 ̊c; IR; (KBr)νmax.cm^{-1} 3328,3273 (NH);3448, 3355(NH_2); 1658 (CO).). ^{1}H NMR (DMSO-d_6): δ_H 6.2(s, 2H, NH_2), 7.02-8.41 (m, 14H, Ar-H), 8.82 (s, 1H, NH), 10.22(s, 1H, *N*HCO). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C ppm 78.6, 117.8, 122.8, 124.8 129.0, 130.9, 140.6, 140.9, 150.7, 150.9, 152.8, 164.9, 168.8 MS: *m/z*, 399 (M^++1, 0.79), 398 (M+, 15.06), 345.02 (26.05), 256.05 (51.72), 119.07 (100), 77.09 (52.33).Anal. Calcd for ($C_{22}H_{18}N_6O_2$). (398). C, 66.32; H, 4.55; N, 21.09%. Found C, 66.35; H, 4.53; N, 21.23%.

Synthesis of (E)-6-amino-4-(phenylamino)-N-(4-(phenyldiazenyl) phenyl)-2-thioxo-1,2-dihydropyrimidine-5-carboxamide (13)

To a solution of 10 (0.3g, 0.001 mol), thiourea (0.076g, 0.001mol), and a catalytic amount of TEA (5-8) drops were added and refluxed in ethanol (10ml) for 3 hrs. Then, the reaction was left to cool. The reaction mixture was poured onto ice water. The precipitate was collected, filtered, dried and recrystallized from EtOH/few drops of DMF to yield 13; yellow crystals;Yield(50%); mp. 200-210˚c; IR; (KBr) νmax.cm^{-1}; 3330,3279 (NH); 3478, 3375 (NH_2), 1635 (NHCO).). ^{1}H NMR (DMSO-d_6): δ_H 6.49(s, 2H, NH_2), 6.69-8.41(m, 14H, Ar-H), 10.74 (s, 1H, NH), 11.07 (s, 1H, *N*HCO), 13.03 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C ppm 75.9, 119.5, 121.8, 123.2 124.6, 129.3, 130.8, 139.3, 150.9, 158.8, 163.0, 180.6. MS: *m/z* 441 (M^+, 15.96), 411.21 (14.84), 367.15 (17.24), 308.12 (100); 170.35 (54.96), 121.24 (81.85), 47.02 (20.81). Anal. Calcd for ($C_{23}H_{19}N_7OS$). (441). C, 62.57; H, 4.34; N, 22.21%. Found C, 66.35; H, 4.51; N, 22.34%.

General procedure for the reaction of 3, 5-aminopyrazole 11 with 1,3–dicarbonyl compounds 14 and 15

To a solution of compound 11(0.3g, 0.001mol), the appropriate 1,3-dicarbonyl compounds [such as acetyl acetone and diethyl malonate] (0.001 mol) were added in in glacial acetic acid (25ml). The reaction mixture was refluxed for 3 hrs, or fusion in sand bath, and then poured onto crushed ice; the precipitate that formed was collected by filtration, washed with ethanol, dried and recrystallized from a mixture of DMF/ETOH (1:1) to give compounds 14 and 15.

Synthesis of (E)-5,7-dimethyl-2-(phenylamino)-N-(4-(phenyldiazenyl)phenyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (14)

Orange crystals; Yield(55%); mp. 230-235̊c; IR; (KBr) νmax.cm^{-1}: 3444 (NH); 1661 (CO). ^{1}H NMR (DMSO-d_6): δ_H 2.42(s, 3H, CH3), 2.73(s,3H, CH3), 7.06 (s,1H, olefinic H), 7.02-.8.41(m, 14H, Ar-H), 10.22 (s,1H, *N*HCO), 10.54 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C ppm 13.6, 17.8, 24.7, 97.5, 101.9, 108.7, 117.9, 119.0, 122.6, 129.7, 130.6 137.8, 140.9, 143.7, 145.8, 152.6, 164.7. MS: *m/z* 461.53 (M^+, 1.28), 445.11 (10.75), 415.24 (21.80), 338.09 (15.77), 265.17 (61.69), 246.23 (22.81), 227.2 (58.59), 180.16 (21.36), 84.12 (30.05), 51.06 (46.28), 43.07 (100). Anal.Calcd for ($C_{27}H_{23}N_7O$). C, 70.27; H, 5.02; N, 21.06%. Found, C, 70.25; H, 5.11; N, 21.13%.

Synthesis of (E)-5,7-dioxo-2-(phenylamino)-N-(4-(phenyldiazenyl) phenyl)-4,5,6,7-tetrahydropyrazolo[1,5-a] pyrimidine-3-carboxamide (15)

Deep orange crystals;Yield (50%) ;mp. 240-245̊c; IR; (KBr) νmax.cm^{-1}: 3468,3442 (NH), 1740 (CO), 1658,1675and 1740 (CO). ^{1}H NMR (DMSO-d_6): δ_H 3.29 (s, 2H, CH_2), 7.02-8.41(m, 14H, Ar-H), 10.22 (s,1H, *N*HCO), 10.54 (s,1H, NH), 11.26(s,1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C ppm 35.7, 85.4, 117.8, 122.6, 124.6, 129.2, 130.9, 140.1, 141.6, 147.1, 150.9, 152.8, 164.5, 166.2, 168.4. MS: m/z 465, (M+, 14.99), 395.39 (8.97), 381.19 (17.36), 339.19 (10.09), 319.14 (22.19), 307.24 (18.17), 301.34 (21.61), 270.15 (25.83), 228.23 (24.73), 208.22 (30.32), 175.11 (42.47), 151.13 (26.90), 8.07 (100), 52.06 (55.09). Anal. Calcd. for ($C_{25}H_{19}N_7O_3$).(465). C, 64.51; H, 4.11; N, 21.06%. Found, C, 64.49; H, 42.2; N,21.16%.

General procedure for the reaction of 3,5-amino pyrazole 11 with 2-(4-chlorobenzylidene) malononitrile and ethyl cyano acetate 16 and 17

To a solution of compound 11 (0.3gm 0.001 mol), the appropriate arylidene [namely 2-(4-chlorobenzylidene) malononitrile (0.189 g, 0.001 mol) and ethylcyanoacetate (0.113 ml, 0.001 mol)] containing a catalytic amount of pipridine were added in in ethanol (25 ml). The reaction mixture was refluxed for 3 hrs to afford compounds 16 and 17, respectively.

Synthesis of (E)-5-amino-7-(4-chlorophenyl)-6-cyano-2-(phenylamino)-N-(4-(phenyldiazenyl) phenyl) pyrazolo[1,5-a] pyrimidine-3-carboxamide (16)

Brown powder; Yield (55%); mp. 255-260℃; IR (KBr) νmax.cm^{-1}: 3443, 3410 (NH_2), 3333 (NH), 2215 (CN), 1639 (CO). ^{1}H NMR (DMSO-d_6): δ_H 7.02-8.41(m, 18H, Ar-H), 7.51 (s, 2H, NH_2), 10.22 (s, 1H, *N*HCO), 10.54 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C ppm 87.9, 97.6, 115.8, 117.9, 122.5, 124.5, 128.7, 129.0, 130.8, 134.3, 140.5, 147.0, 150.4, 152.6, 152.8, 164.6, 169.8. MS: *m/z* 583.14 (M^+, 2.55), 423.14 (40.24), 359.17 (71.64), 347.09 (23.75), 254.15 (74.08), 163.11 (100), 135.12(45.68), 80.08 (21.61), 55.09 (36.31). Anal. Calcd. for ($C_{32}H_{22}ClN_9O$). C, 65.81; H, 3.80; N, 21.58%. Found, C, 65.77; H, 3.83; N, 21.52%.

Synthesis of (E)-7-amino-5-oxo-2-(phenylamino)-N-(4-(phenyldiazenyl) phenyl)-4,5-dihydropyrazolo[1,5-a] pyrimidine-3-carboxamide (17)

Orange crystals; Yield(50%); mp. 250-255℃; IR (KBr)νmax.cm^{-1}: 3435 and 3342 (NH_2), 3322,3291,3277 (NH); 1646 (*N*HCO). ^{1}H NMR (DMSO-d_6): δ_H 6.22 (s, 1H, olefinic H), 6.81 (s, 2H, NH_2), 7.02-8.41(m, 14H, Ar-H), 10.22 (s, 1H, *N*HCO), 10.54(s, 1H, NH), 11.46(s, 1H, NHCO). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C ppm 80.7, 85.6, 117.8, 123.0, 129.1 130.9, 140.4, 147.3, 150.9, 156.6, 162.5, 164.7. MS: *m/z* 464.29 (M^+, 4.37), 423 (40.24), 359.17 (71.64), 318.10 (90.06), 254.15 (74.08), 163.11 (100), 135.12 (45.68), 107.10 (20.69), 80.08 (21.61), 55.09 (36.31). Anal. Calcd. for ($C_{25}H_{20}N_8O_2$). C, 64.65; H, 4.34 N, 21.58%. Found, C, 65.77; H, 4.31; N, 21.52%.

Laboratory Bioassay

Cotton leaf worm (*Spodoptera littoralis*, Family; Lepidoptera)

A laboratory susceptible strain of cotton leafworm *S. littoralis* (Boisd.) was kept up in an incubator under controlled conditions of 25 ± 1°C and 70 ± 5% RH, in cotton leafworm research department building, plant protection research institute, ARC, and without any exposure to chemical still the time of treatment. Larval jars were supplied daily with fresh Castor leaves, *Ricinus communis* L., as a source of food which was provided daily. The adult were kept separately and mated on the third day of emergence in clean jars, adults were fed on 10% honey solution, fresh green leaves of Dafla, *Nerium oleander* (L.) were provided for egg laying as depicted by El-Defrawi *et al.* (1964) [12].

Pesticides:

Lannate 90% SP was obtained from Central Agricultural Pesticides Laboratory (CAPL) in Dokki, Giza, Egypt (Figure 4).

Toxicological studies

The experiments were carried out using the leaf dip technique as described by Sadek (2003) [13]. Six concentrations of each compound were formulated as emulsions in solvent, and 0.1% Triton X-100 was used as a surfactant. The emulsions were used immediately after preparation. For larvicidal action, fresh castor bean [14], were left in the shade to dry before being offered to the larvae. The larvae were allowed to feed on the treated leaves for 48 hours and then changed to untreated leaves. Four replicates of 10 larvae each were used for each concentration in addition to the control. Control (check) tests were carried out using the same technique. Castor bean leaves were dipped in a solution of 0.1% Triton X-100 and solvent at the same ratio used in the synthesized compound tests. Larval mortality counts were calculated at 1, 2 and 3 days after exposure period. Mortality was corrected according to Abbott's formula, and then subjected to probit analysis. The toxicity lines (LC-p lines) were drawn on log concentration–probit paper and statistically analyzed according to Finney's method [15], to obtain the LC_{50} and LC_{90} values of different tested compounds in order to determine the most effective one. In addition, we were also assessed slope values of the tested compounds. Furthermore, the efficacy of the distinctive compounds was estimated by comparing the tested compounds with the most effective compound using the following equation:

Toxicity index=LC_{50} of the most effective compound/LC_{50} of the tested compound x 100, according to Sun [16].

Conclusion

In this present work, it was thought worthwhile to build up some innovative bioactive polyfunctionallized substituted heterocyclic compounds incorporating a azobenzene moiety of potential insecticidal efficacy against the cotton leafworm, *Spodoptera littoralis* under laboratory conditions. Regarding the determined LC_{50} and LC_{90} values, azobenzenes bearing amide moiety 16, 17, 6a, 8a and 7 exhibited the ultimate powerful toxic effects with LC_{50} values of 24.995, 31.291, 45.048, 64.739 and 82.248 ppm, respectively, and toxicity index being 53.75, 42.94, 29.83, 20.76 and 16.34%, respectively, comparing with the commercial already known, carbamate insecticide, lannate 90% SP which was the most effective compound (the lowest LC50 value, 13.435 ppm, toxicity index, 100%).

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