Microwave and ultrasonic-assisted diastereoselective synthesis of highly functionalized Spiro pyrrolidine-2,3'-thieno[2,3-d]pyridazine with microbicidal and larvicidal properties supported by DFT simulation and molecular docking

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Abstract

A novel class of Spiro-heterocyclic derivatives (2-6) can be synthesized and assessed as ecofriendly microbicidal and insecticidal activities to establish a structure-activity relationship (SAR). The synthesis was taken out through green tools of a one-pot multicomponent reaction (MCR) of β-aroyl-acrylic acid and thioglycolic acid and β-(2-oxopyrrolidin-1-yl) ethanol. The one-pot MCR with ultrasonic and microwave-assisted irradiation was found to be a simple and efficient approach for preparing Spiro derivatives. Elemental analysis and spectroscopic data were used to describe the structures of all synthesized products. The regioselective formulation of Spiro-heterocyclic molecules is intriguing because these compounds have unique non-planar structures and a high potential for binding to biomaterials due to their inherent rigid chiral structure. The microbicidal and insecticidal pursuits of the compounds were investigated on ATCC coded test microorganisms and insect's 3 rd instar larvae with medicinal and economically valuable, Mythimna separate and Nilaparvata lugens , correspondingly compared to the standard pesticide chlorpyrifos, a pesticide with a similar mode of action. The preliminary testing demonstrated flourishing microbicidal capability to prevent the progress of threatening pathogenic bacteria besides blossoming insecticidal efficacy. Moreover, Molecular docking plus density function theory (DFT) simulations can be expended to validate the experimental results.

Introduction

In agricultural productivity, pesticides are essential. Farmers have used them in crop safeguarding to manage weeds and insects, and there have been reports of significant gains in agricultural goods and human being health [1], [2]. Herbivorous insects, like other insects, are exposed to the variability of microbial communities from their surroundings. Also, Insects and microorganisms have a variety of beneficial and destructive interactions. Insects hurt plants by consuming them and spreading plant diseases to injured regions. So, Insects could thus be classified as both pests and pathogens reservoirs or vectors [3]. Insecticide use is vital for the control of insect pests and insecticides resistance is one of the most important barriers facing the application of the traditional insecticide. Insects have created resistance to most chemical classes and new interactions for purpose in animal agriculture and public health with long-term exposure [4]–[6]. The systemic insecticides exert exclusive chemical, biological properties, and favorable safety profiles with relatively low risk for non-target organisms in the environment. They considered flexible in exposure methods with low application rate as well as excellent uptake and translocation in plants [7], [8]. Furthermore, neonicotinoids have a unique method of action on Niacinic acetylcholine receptors in insects that is selective, irreversible, and specific (nAChR) [9], [10]. Because the synthesized molecule functions similarly to those pesticides, it is expected to have great insecticidal effectiveness as well as a favorable safety profile. The 1,2-dichlorophenyl precursors in the produced Spiro compounds operate as a key plant hormone that governs many aspects of plant growth, development, and tolerance to biotic stress. Increases in oil percent, crude protein, total soluble sugars, and total free amino acid content have greatly increased the nutritive value and quality of grains [11], [12]. Highly convergent techniques for rapidly increasing the quantity of Spiro-heterocyclic compounds [13]–[17] and the products are created in a solo step (MCR), and the diversity may be easily obtained by modifying the reactive components that are carried out, resulting in higher harvests, briefer reaction times, and softer conditions under sonication. For the synthesis of bioactive spiro derivatives chemicals, ultrasonic and microwave-assisted reactions have become increasingly popular as clean, green, and ecologically friendly methods. In terms of waste minimization and energy conservation, they are an essential instrument for green chemistry [18]–[25]. Accelerated concern about the environment and safety has attracted global efforts to develop green eco-friendly procedures [26]–[28]. As an advantage, many organic reactions have been completed with greater yields, shorter reaction times, and milder conditions when water was used as the solvent instead of toxic organic solvents, attracting a lot of attention [29]–[32].

Experimental

Chemistry

Preparation of spirooxindoline Derivatives (Comp. 1)

Microwave-assisted of one-pot reaction of 4-(3,4-dichlorophenyl)-4-oxo-2-butenoic acid (10 mmol), thioglycolic acid(0.9 g, 10 mmol), and hydroxy pyrrolidone (10 mmol) in 10.0 ml aqueous methanol (1:3) was stirred at 60 ^{o}C. The solid precipitates were gathered by filtration and rinsed with cold methanol to provide the analytically pure product **1**. Enantio-separations of D/L-Spiro-oxindole **1** on HSA columns prepared by the SMCC method [33]. The HPLC conditions were as follows: sample concentration, 20μM Spiro-oxindole **1**, sample volume, 20μL, mobile phase, pH 7.0, 0.067M potassium phosphate buffer containing 5% 2-propanol and 1mM octanoic acid, flow rate for the SMCC HSA column, 1.5 ml/min, column size, 5 cm *4.6mm. Colorless solid, 76%, Mp.: 188–190°C.

Preparation of 2-(6'-(3,4-dichlorophenyl)-2-(-4'-oxo-3a',4'-dihydro-2'H-spiro[pyrrolidine-2,3'-thieno[2,3-c]furan]-1-yl)ethyl acetate (spiro Furanone Derivative 2).

Spiro compound **1** (0.01 mmol) and acetic anhydride (0.01 mmol) was sonicated smoothly fused for 20 min. The solid was separated, filtered off, dried, and recrystallized to afford (Comp. 2),Colorless solid, 72%, Mp.: 220–222°C. IR(KBr) v 3052, 1782, 1767 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6) δ 7.62-7.41 (dd, *J* = 8.7- 6.2 Hz Hz, 2H, ArH), 7.52 (s, 1H, ArH), 6.72 (s, 1H, CHFuro), 3.67 (t, *J* = 8.7 Hz, 2H, CH_2O), 2.96 (t, *J* = 8.4 Hz, 2H, NCH_2), 2.80–2.77 (d, *J* = 7.6 Hz, 2H, CH_2), 2.62 (s, 3H, CH_3), 2.50 (t, *J* = 7.7 Hz 2H, NCH_2Py), 2.11 (t, *J* = 7.7 Hz 2H, CH_2Pyrr), 1.95 (m, 2H, CH_2Pyrr), ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.59, 170.23, 161.36 , 141.26, 136.44, 132.08, 131.70, 130.95, 130.32 , 129.02, 128.55, 72.27, 68.20, 57.13, 55.35, 52.66, 44.40, 42.96, 39.33, anal. calcd for $C_{19}H_{19}Cl_2NO_{4S}$ (427): C 53.28, H 4.74, N 3.27, S 7.48, found: C 53.00, H 4.26, N 3.00, S 7.15.

Preparation of 7'-(3,4-dichlorophenyl)-1-(2-hydroxyethyl)-5'-methyl-5',6'-dihydro-2'H-spiro[pyrrolidine-2,3'-thieno[2,3-d]pyridazin]-4'(3a'H)-one (spiro Pyridazinone Derivative3).

Spiro furanone's reaction derivative **2** (0.01 mmol) and methyl hydrazine hydrate (0.01 mmol) was sonicated in butanol (5 ml) for 0.5h. The resulting mixture was concentrated, filtered off, dried, and recrystallized, to afford (Comp. 3), colorless solid, 74%, Mp.: 192–194°C.IR(KBr) v 3356, 3050, 1689 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6) δ 7.33 (d, *J* = 7.8 Hz, 1H, Ar), 7.24 (s, 1H, Ar), 7.16 (d, *J* = 8.4 Hz, 1H, Ar), 5.86 (s, 1H, OH), 3.67 (t, *J* = 8.4 Hz, 2H, CH_2O), 3.22–3.16 (dd, *J* = 9.2-6.4 Hz, 1H, CHCO), 3.14 (s, 3H, NCH_3), 3.08–3.04 (dd, *J* = 9.2, 7.6 Hz, 1H, CHPy), 2.92 (t, *J* = 8.4 Hz, 2H, NCH_2), 2.82–2.78 (d, *J* = 7.6 Hz, 2H, CH_2S), 2.41 (t, *J* = 7.7 Hz 2H, NCH_2Pyrr), 2.04 (t, *J* = 7.7 Hz 2H, CH_2Pyrr), 1.96 (m, 2H, CH_2Pyrr), ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.42, 163.41, 136.44, 132.08, 131.70, 130.95, 130.32, 129.02, 128.55, 72.27, 67.40, 57.13, 55.35, 52.66, 44.40, 42.96, 39.33, anal. calcd for $C_{18}H_{21}Cl_2N_3O_2S$ (413): C 52.18, H 5.11, N 10.14, S 7.74, found: C 51.95, H 4.86, N 9.94, N 7.50.

Preparation of Ethyl 2-(2-(7'-(3,4-dichlorophenyl)- 2-(5'-methyl-4'-oxo-3a',4',5',6'-tetrahydro-2'H-spiro[pyrrolidine-2,3'-thieno[2,3-d]pyridazin]-1-yl)ethoxy)acetate (Spiro Pyridazone Ester 4).

Admixture of pyridazinone derivative **3** (0.01 mmol) and ethyl chloroacetate (0.01 mmol) in dry dioxane (20 ml), in the dignity of potassium carbonate (3 g) was sonicated for 1h. The reaction mixture was concentrated, cooled, and poured into ice-cold water. The participated solid was filtered off, washed, dried, and recrystallized, to afford (Comp. 4), colorless solid, 65%(r.t.), Mp.: 202–204°C.IR(KBr) v 3424, 3050, 1742, 1691 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6) δ 7.23 (d, *J* = 8.4 Hz, 1H, Ar), 7.14 (s, 1H, Ar), 7.06 (d, *J* = 8.4 Hz, 1H, Ar), 4.95 (s, 2H, OCH_2), 4.02 (q, *J* = 7.8 Hz, 2H, $O\underline{CH_2}CH_3$), 3.64 (t, *J* = 8.4 Hz, 2H, CH_2O), 3.32–3.12 (d, *J* = 9.2 Hz, 1H, CHCO), 3.10 (s, 3H, NCH_3), 3.02–3.00 (dd, *J* = 9.2, 7.6 Hz, 1H, CH), 2.92 (t, *J* = 8.4 Hz, 2H, CH_2), 2.82–2.78(d, *J* = 7.6 Hz, 2H, CH_2S), 2.41 (t, *J* = 7.7 Hz 2H, NCH_2), 2.04 (t, *J* = 7.7 Hz 2H, CH_2), 1.96 (m, 2H, CH_2) 1.32 (t, *J* = 7.8 Hz, 3H, CH_3), ^{13}C NMR (125 MHz, DMSO) δ 195.09, 177.42, 162.26, 136.44, 132.08, 131.70 , 130.95, 130.32 , 129.02, 128.55, 72.27, 69.23, 56.54, 54.66 , 68.20, 57.13, 55.35, 51.21, 44.40, 42.96, 39.33, anal. calcd for $C_{22}H_{27}Cl_2N_3O_4S$ (499): C 52.80, H 5.44, N 8.40, S 6.41, found: C 52.54, H 5.25, N 8.13, N 6.17.

Preparation of -(2-(7'-(3,4-dichlorophenyl)-2-(5'-methyl-4'-oxo-3a',4',5',6'-tetrahydro-2'H-spiro[pyrrolidine-2,3'-thieno[2,3-d] pyridazin]-1-yl)ethoxy) acetohydrazide (Spiro Pyridazone Hydrazide 5).

Ester **4** (0.01 mmol) in ethanol (20 ml) was treated with hydrazine hydrate 98% (1.5ml,0.04 mmol) under 20 minutes of microwave irradiation. The solid that alienated after concentration and cooling were recrystallized, colorless solid, 72%, Mp.: 156–158°C.2IR(KBr) v 3341, 3186, 3050, 1690, 1665 cm^{-1}, ^1H NMR (300 MHz, DMSO) δ 9.61 (s, IH, NH), 7.23-7.06 (m, 3H, ArH), 5.38 (s, 2H, NH_2, exchangeable by D_2O), 4.95 (s, 2H, OCH_2), 3.64 (t, J = 8.4 Hz, 2H, CH_2O), 3.32–3.12 (d, J = 9.2 Hz, 1H, CHCO), 3.10 (s, 3H, NCH_3), 3.02–3.00 (dd, J = 9.2, 7.6 Hz, 1H, CH), 2.92 (t, J = 8.4 Hz, 2H, NCH_2), 2.82–2.78(bs, 2H, CH_2), 2.41 (t, J = 7.7 Hz 2H, NCH_2), 2.04 (t, J = 7.7 Hz 2H, CH_2), 1.96 (m, 2H, CH2Py), ^{13}C NMR (125 MHz, DMSO) δ 177.42-173.65, 161.26, 136.44, 132.08, 131.70, 130.95, 130.32, 129.02, 128.55, 72.27, 69.23, 54.66, 51.21, 44.40, 39.33, anal. calcd for $C_{20}H_{25}Cl_2N_5O_3S$ (485): C 49.39, H 5.18, N 14.40, N 6.59, found: C 49.15, H 4.85, N 14.12, S 6.33.

Preparation of N'-benzylidene-2-(2-(7'-(3,4-dichlorophenyl)-2-(-5'-methyl-4'-oxo-3a',4',5',6'-tetrahydro-2'H-spiro[pyrrolidine-2,3'-thieno[2,3-d]pyridazin]-1-yl)ethoxy)-N'-ethylideneacetohydrazide (Spiro Arylidene6).

A combination of hydrazide compound **5** (0.01 mmol) and aromatic aldehyde, namely benzaldehyde (0.01 mmol) in ethanol (20 ml) was sonicated for 0.5h. The solid that separated after cooling was filtered off, dried, and then crystallized from ethanol, colorless solid, 77%, m.p.: 174–176°C.IR(KBr) v 3370, 3050, 1695, 1669 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6) δ 10.65 (s, 1H, CHAr), 9.32 (s, 1H, 1-NH

exchangeable by D_2O), 7.43 -7.12 (m, 8H, ArH), 4.92 (s, 2H, OCH_2), 3.64 (t, *J* = 8.4 Hz, 2H, CH_2O), 3.32–3.12 (d, *J* = 9.2 Hz, 1H, CHCO), 3.10 (s, 3H, NCH_3), 3.02–3.00 (dd, *J* = 9.2, 7.6 Hz, 1H, CH), 2.92 (t, *J* = 8.4 Hz, 2H, NCH_2), 2.82–2.78(d, *J* = 7.6 Hz, 2H, CH_2S), 2.41 (t, *J* = 7.7 Hz 2H, NCH_2), 2.04 (t, *J* = 7.7 Hz 2H, CH_2), 1.96 (m, 2H, CH_2),anal. calcd for $C_{27}H_{29}Cl_2N_5O_3S$ (573): C 56.45, H 5.09, N 12.19, S 5.58, found: C 56.21, H 4.84, N 711.94, S 5.27.

Biological Tests:

Screening the Antimicrobial Pursuit & MIC of synthesized Spiro compounds:

Antagonistic pursuit of synthesized Spiro compounds 1-6 were evaluated against prokaryotic plus eukaryotic coded test organisms symbolized by Gram-Positive Bacteria including *Staphylococcus aureus* ATCC. 6538, *Bacillus subtilis* ATCC. 6633 and Gram-Negative bacteria including *Pseudomonas aeruginosa* ATCC. 9022 & *Escherichia coli* ATCC. 8739 Also, Unicellular Fungi *Candida Albicans* ATCC.10231 using agar well diffusion method. Mueller Hinton agar plates were seeded with an overnight test organism. One hindered microliter (100 µl) of synthesized Spiro compounds solution (400 µg ml^{-1}) was added for each well (0.8 mm) in a plate. To verify the minimum inhibitory concentration (MIC), different concentrations of synthesized Spiro compounds 1-6 solution were made by double fold dilution as the following (25,50,100,200,400 µg ml^{-1}). The plates were incubated at 37°C for 24 hrs. for bacteria and 25°C for 72 hrs. for unicellular fungi. After incubation, an inhibition zone was observed. The diameter of inhibition zones was measured in mm and the results were recorded [34], [35].

Evaluation of Larvicidal assay of Spiro compounds (1-6) against *Mythimna separata* and *Nilaparvata lugens* Larvae:

Insects rearing:

Laboratory reared insects of *Mythimna separata* colonies (paddy armyworm) and *Nilaparvata lugens* (brown planthopper) free from insecticides and pathogens obtained from Insect greenhouse of Pest control Analytical lab, Entom-Aysel Company, Cairo, Egypt were maintained starting from egg rafts. *M. separate* populations were reared in plates on a synthetic diet that included soybean wheat, yeast powder, casein, fresh maize leaf extract powder, cholesterol, sugar, agar, ascorbic acid, sorbic acid, methyl p-hydroxybenzoate, vitamin solution, and deionized water. Whereas collected population numbers of *Nilaparvata lugens* were reared in the laboratory on rice seedling (*Oryza sativa* L.) seedlings (7-10 days after germination) under controlled conditions, temperature 371 C and 70–80 percent relative humidity with a 16-8h (L/D) photoperiod. Bioassays were performed on third nymph instars from the second laboratory generation [36]–[38].

Larvicidal bioassay:

The larvicidal actions of the title compounds were done versus healthy late third instar larvae *Mythimna separata* tested by foliar application [39], [40]**.** Corn leaves were curved into the obtained solutions for 2–3 s. After air-drying, the soaked leaves were put into a culture dish with a piece of filter paper, followed by inoculation of [Ten] 3^{rd} -instar *M. separata* larvae per dish. Covered with gauze and kept in the observation room for normal cultivation at 24–27^{o}C. Mortality was assessed 48 h after treatment. The individuals who did not respond to the touch of the writing brush were recognized as dead.

While the insecticidal activities of the designated compounds against *Nilaparvata lugens* were investigated using a slightly modified version of the IRAC Susceptibility Test #05 [27], [39]. About ten rice seeds were sowed in cups that contain alluvial soil supplying nutrients. Bioassay was performed on seedling rice when it had four leaves. Agar powder was dissolved according to the manufacturer's instructions and allowed to cool to 37 degrees Celsius before being poured over the soil surface of the rice seedling cups. Rice plantlets were dipped into six doses of insecticide solution for 30 seconds each and incubated at room temperature until the seedling dried (> 15 minutes). [Ten] third instar nymphs were placed on the rice seedling, and the cup was encased with a plastic tube with a plastic sieve above. A test was repeated three times, and the results were averaged. *N. lugens* inhibition was tested on rice seedlings that had previously been inoculated with *N. lugens*. Following that, the resulting rice seedlings were kept. Chlorpyrifos has been used as a positive control.

Abbott's formula was used to calculate the mortality rate:

((treatment Mortality % - control Mortality %) / 100 - control mortality %) *100 [41].

Each test was repeated three times, with the results averaged. Under the same conditions, chlorpyrifos was tested as a control compound.

Calculation details

The DFT calculations were carried out using the DMmol3 module in Accelrys, Inc.'s Materials Studio 6.0 (MS6.0) software, with the Pardew and Wang LDA exchange-correlation functional and DND boundary condition. Frontier molecular orbitals, dipole moments, Fukui indices, as well as Mulliken atomic charges were all calculated parameters. For each molecule, the frontier molecular orbitals include the highest occupied orbital (HOMO) and the lowest unoccupied (LUMO) (LUMO). To demonstrate the adsorption of the research present on the surface of Pb (110) at the molecular level, a molecular dynamics (MD) simulation was performed using the citing Module and COMPASS forcefield in MS6.0 software [42].

Molecular docking simulations

The three-dimensional (3D) structures of the proven biologically active compounds structures of Spiro arylidene **6** and Chlorpyrifos (CPS) as a positive control was first drawn The docking process was carried out using the Lamarckian Genetic Algorithm (LGA) implanted in Auto Dock V4.2.6 apps (The Scripps Research Institute) including its graphical user interface (GUI) of Auto Dock Toolkit V1.5.6 for 100 interactions with a 6-31G (d, p) basis set, which describes all the employed structure atoms using Spartan V14 software (Wavefunction, Inc.).The geometrically optimized structures of the compounds under study were finally exported in PDB format. The previously labeled compounds were then labeled as ligands, and the PDB formatted files were transported into the Auto Dock Tools V1.5.6 apps for the next step. A blind docking approach was used to scan the entire protein surface for the most likely binding sites and modes of ligand-protein interaction to monitor all concentrations of active binding sites of DmAchE [43]. The binding energy scoring in Auto Dock comprises the summation of the Final Inter-Atomic Energy (FIA), Final Total Internal Energy (FTI), Torsional Free Energy (TF), and Unbound System's Energy (US), where the former one is composed of the energies of interactions such as H-bond (EHB), van der Waal forces (EVDW), electrostatic interactions (EELEC), de-solvation (EDESOLV) and ref-energies (EREF) [44].

Result And Discussion

Chemistry

This class of green chemistry is widely used nowadays and has become significant in combinatorial chemistry due to its process simplicity, mild conditions, atomic economy, and extension of the scope of substrates. Spiro-oxindoles showed remarkable applications of one-pot multicomponent reactions (MCRs) and microwave-assisted offers smooth reaction conditions, higher overall yield to obtain ample quantities of new products [42], [45]–[51]. As an advantage, this new approach was utilized when 4(3,4-dichlorophenyl)-4-oxo but-2-enoic acid is treated with thioglycolic acid and 2-oxo-pyrrolidine ethanol via MCR in ultrasonic and MW irradiation (W250 and T 150^{o}C) to afford Spiro-thiophene-pyrrolidine acid **1** that characterized multi-functionalization and bearing ArCO, OH, and COOHgroups that have microbicidal plus insecticidal activities [52], [53]. Sonication of compound **1** with acetic anhydride afforded the corresponding furanone **2**, supported by microanalytical data and ^{1}HNMR reveal 10.2 (s, 1H, COOH, exchangeable by D_2O), 6.85-6.80 (dd, *J* = 8.7- 6.2 Hz, 2H, ArH), 6.72 (d, 1H, ArH), 5.99 (s, 1H, CHCO), 4.17-4.13 (t, *J* = 8.7 Hz, 2H, CH_2O), 2.62-2.51 (t, *J* = 8.4 Hz, 2H, NCH_2), 2.49–2.44 (d, *J* = 7.6 Hz, 2H, CH_2), 2.41 (s, 3H, CH_3), 2.34 (t, *J* = 7.7 Hz 2H, NCH_2Py), 2.31 (t, *J* = 7.7 Hz 2H, CH_2Pyrr), 1.21-1.15 (m, 2H, CH_2Pyrr) in **Fig 6**. The reaction of the furanone that characterized by the activated lactonic group with a bifunctional nucleophile, when it was reacted with hydrazine hydrate afforded the corresponding pyridazinone **3,** typified by activated cyclic amide and hydrazide structures. Sonication of the pyridazinone **3** with ambient carbon electrophiles e.g. ethyl chloroacetate, the SN^2 mechanism (C-Cl) has enhanced vs tetrahedral mechanism (COOEt) in a weak basic medium e.g.potassium carbonate in the presence of as aprotic solvent e.g. acetone. The sonication afforded the corresponding bioactive ester **4** that characterized by carbonyl ester reveal in IR spectrum at 1742cm^{-1}and 1H-NMR reveal at chemical shift4.02 (q, *J* = 7.8 Hz, 2H, O$\underline{CH_2}CH_3$), **Formation** of the bioactive hydrazide structure **5**occurred, when the ester **4** was allowed to react with hydrazine hydrate. DFT and molecular docking are involved with the experimental results in which the extremely bioactive inherent Spiro structure contains the arylidene moiety **6**, it formed from the reaction of the hydrazide 5 with benzaldehyde in the presence of boiling ethanol under sonication reaction conditions **(Schemes 1-6).**

Antimicrobial Activity & MIC:

The antimicrobial properties of generated Spiro compounds (1-6) were determined using the zone of inhibition (mm), as shown in **Table (1) and Fig. (2)**, all synthesized Spiro compounds (1-6) had maximum activity against a variety of harmful microorganisms at Maximum conc. 400 g ml^{-1}. Hence, the maximum diameter of inhibition activities recorded for Spiro Arylidene (6) at Maximum conc. 400 µg ml^{-1} against *Staphylococcus aureus* of 22.33±0.88 mm, *Bacillus subtilis* of 29.27±0.33 mm, *Escherichia coli* of 22.67±0.33 mm, *Pseudomonas aeruginosa* of 19.33±0.33 mm & *Candida albicans* of 22.67±0.33 mm.

The antimicrobial action was also dose-dependent, therefore the MIC of produced Spiro compounds (1-6) for each Pathogenic organism was measured. Various concentrations of (25,50,100,200,400 g ml^{-1}) were tested to touch this goal. Henceforward, the synthesized Spiro compounds (2, 5& 6) exhibited the lowest MIC of 50 µg ml^{-1} against all pathogenic test organisms under investigation. Also synthesized Spiro compounds (4) exhibited MIC of 50 µg ml^{-1} against (*Bacillus subtilis, Escherichia coli* & *Candida albicans*) and MIC of 100 µg ml^{-1} against *Staphylococcus aureus* & *Pseudomonas aeruginosa*. While synthesized spiro compounds (1) exhibited MIC of 100 µg ml^{-1} only against *Pseudomonas aeruginosa* and MIC of 200 µg ml^{-1} against the rest microorganisms. Additionally, synthesized Spiro compound (3) exhibited MIC of 100 µg ml^{-1} against *Pseudomonas aeruginosa, Escherichia coli* & *Candida albicans,* and exhibited MIC of 200 µg ml^{-1} against *Bacillus subtilis* and MIC of 400 µg ml^{-1} against *Staphylococcus aureus.*

Our findings are consistent with those of Von Wintersdorff *et al.,* (2016) and Nazari *et al.,* (2017) [54], [55] they reported activities of generated Spiro compounds on abiotic surfaces have been recorded, including killing resistant bacteria strains and damaging plasmid and genomic DNA, which means limiting infection and gene transfer. In addition, Alves *et al.,* (2021) [56] showed that synthesized chiral spiro beta-lactams can lower HIV-1 coupled with Plasmodium infection. Moreover, (Ghorab *et al.,* 2016 and Alsayari *et al.,* 2021) [57], [58]observed the greatest inhibition zone diameter of selected novel spiro compound derivatives as potential antimicrobial and anticancer agents against some harmful test organisms.

Insecticidal activity

Compounds 1-6 were tested for insecticidal activity against healthy late third instar larvae of *Mythimna separata* and *Nilaparvata lugens* using the standard test [59], [60]with a minor variation. To obtain the required concentrations, the test analogs were disintegrated in DMF and serially mixed with water that included Triton X-80 (0.1 mg/L). The insects were reared at 25 (±1) °C and groups were transferred to glass Petri dishes. For statistical purposes, all experiments were carried out in three repetitions. The quantity and size of living insects in the positive control were compared to those in the negative control after 48 hours to determine mortality. The ratings were given on a scale of 0 to 100, with 0 indicating no activity and 100 signifying full eradication. The mortality rates were subjected to a probability analysis [61].The positive control was chlorpyrifos, and the negative control was water comprising Triton X-80 (0.1 mg/L). According to the results shown in Fig. 3 and Table 2, most of the title compounds had weak insecticidal activity against the 2 pests. Regrettably, some of the compounds demonstrated effective insecticidal activity. For example, compounds **2** and **6** exhibited 100% activities at 400 µg ml^{-1} and 50% ($IC_{50\ \%}$) activities at 100 and 50 µg ml^{-1} respectively against both *Mythimna separata* and *Nilaparvata lugens*. Meanwhile, the activities of compounds **2** and **6** against *Nilaparvata lugens* were like those of chlorpyrifos at 200 µg ml^{-1}, compounds **4** and **5** showed 50.0 and 63.3 % activity respectively against *Nilaparvata lugens* at 200 µg ml^{-1}. In addition, compounds **2** and **6** also showed good insecticidal activities, the mortalities of them against *Mythimna separata* were 100 % at (400 µg ml^{-1}) Conc., While the activity of Comp. **4** at 200 µg ml^{-1}concentration, against *Mythimna separata* was still 53.3%. Moreover, compounds **1** and **3** processed 56.6 % and 53.3% activities on *Mythimna separata* and 60 % and 56.6% on *Nilaparvata lugens* at 400 µg ml^{-1}, respectively.

The LC50 of compounds **2**, **4**, **5**, and **6** were investigated further, with the results listed in **(Table 3)**. The toxic ratio is the ratio of the LC_{50} value for baseline toxicity to the LC50 value for the chemicals.

Density functional theory(DFT) based characterization

Table 4 lists the molecular characteristics for the most effective compounds, as well as the fully optimized minimal energy geometrical configuration of the most potent insecticidal compounds, derived from the minimum inhibitory concentration LC_{50} for the most potent Spiro compounds **2**, **4**, **5**, and **6**. As a result, such compounds' significant insecticidal activity was supported by DFT-based LC50.

High E_{HOMO} is well recognized to suggest that molecules have a strong tendency to give electrons. Because the energy required to remove an electron from the final occupied orbital is low down, the energy gap ($E=E_{LUMO}-E_{HOMO}$) will result in strong inhibition efficiency [48], [50], [51]. The ΔE of a molecule is a measure of the hardness or softness of a molecule. Hard molecules are characterized by larger values of ΔE and vice versa. The lower the HOMO energy (more negative values) of the Spiro structure, the greater the trend of accepting electrons, according to the linear link between E_{HOMO} energy level and insecticidal activity. Increasing insecticidal efficiency is directly proportional to reducing E_{HOMO}, increasing E_{LUMO} values, and decreasing the energy gap (E). The polarizability of an electron cloud refers to its tendency to be distorted from its normal shape, the higher the polarizability, the more inhibitor molecules will leave the solvent bulk to be absorbed by a radical or oxidized surface to form a protective film, we can think of polarizability as a resultant of all intramolecular electron transfer interactions. The ease with which the electron can be distorted is aided by the greater volume.

Quantum calculations based on DFT can reveal the structural and electrical properties of organic compounds [39]. The energies of Frontier molecular orbitals are the values acquired from the DFT analysis performed for Spiro derivatives. The HOMOs indicate electrophilic attack sites, whereas the LUMOs represent nucleophilic assault sites [39]. The HOMO energy (E_{HOMO}) is a parameter that has a direct relationship with the ionization potential and indicates the susceptibility of organic molecules to electrophile assaults. Unlikely, the LUMO energy (E_{LUMO}) refers to electron affinity, and its value represents a molecule's vulnerability to a nucleophilic assault. To acquire these factors, the molecules must first be optimized in terms of geometry, and then these parameters must be determined. Figure 1 shows the HOMO and LUMO distributions. HOMOs are dispersed throughout the Spiro unit, whereas LUMOs are concentrated in the Spiro moiety, and this is true for all four compounds. As a result, in the study of electron transfer, Spiro moieties are the most active centers (either electron donation or acceptance). **Table 3** shows the obtained E_{HOMO} and E_{LUMO} of the produced compounds. The listed results indicate that the values of gap energy (ΔE), where $\Delta E = E_{LUMO} - E_{HOMO}$, follow the order: **Spiro ester 4 < Spiro hydrazide 5 < Spiro furanone 2 < Spiro arylidene 6**. ΔE is indicative of the reactivity of organic compounds towards the interaction with metal surfaces. Compounds having small ΔE values are generally referred to as soft compounds, while those having large values are called hard compounds. In general, soft compounds are more reactive towards the surface of insect interactions, being capable of donating electrons easily to insect surfaces [50]. Organic material must possess heteroatoms rich in non-bonded electrons (free lonely pairs) and/or aromatic rings with p-electrons to interact successfully with insect surfaces. During operation, the principal adsorption locations where electron transfer occurs between the additive and the microbes wall are heteroatoms and aromatic rings [51]. Apart from double bonds and aromatic rings containing p-electrons, the produced compounds under investigation are abundant in O and N atoms carrying free lone pairs of electrons. Because the energy required to remove an electron from the final occupied orbital (HOMO) of the Spiro compound is minimal and it is easy to donate electrons, low-gap-energy compounds often enable good interaction with the aglycone of the insect's cell wall. Furthermore, they require less energy to be excited and give energy, and they are more easily polarizable than hard compounds [47]. Ionization potential (I), electron affinity (A), absolute electronegativity (χ), and absolute chemical hardness (η) are some of the additional quantum chemical metrics that might assist forecast the molecular features of the Spiro structure. Spiro ester 4 Spiro hydrazide 5 < Spiro furanone 2 < Spiro arylidene 6. χ and η are two measures related to I and A, where $\chi = I+A/2$and $\eta = I-A/2$, I and A are calculated in turn, from EHOMO and ELUMO, where I = -EHOMO and A = -ELUMO.

For any two molecules in contact with each other, electrons will be partially transferred from the one of low value to that of higher value. For the studied compounds, $\chi$is generally lower than that of the aglycone of bacteria, which is 6 eV, and thus aglycone-Spiro interaction may proceed via electron transfer from the former to the latter. Because soft compounds are chosen for such interactions, Arylidene 6 is the best choice for interacting with its surface, as it has the maximum chemical softness (s), which is defined as the inverse of hardness ($s = 1/\eta$) [50].

The electrophilicity ω index is calculated using the following formula: $\omega = E2/\eta$. The electrophilicity ω index incorporates an electrophile's inclination to acquire an excessive amount of electron density, as determined by binding energy (E), and a molecule's reluctance to exchange electron density with the environment, as determined by. As a result, a good electrophile is a species with a high (E) value and a low (η). Furthermore, the calculation ΔN max = E/ η. gives the maximum number of electrons that an electrophile can obtain. As a result, Arylidene 6 and Furanone 2 are expected to be the most effective insecticides.

Molecular docking

In this framework, molecular docking simulations were used to investigate the potential activity of the synthesized furanone derivative products using the crystalline structure of native acetylcholinesterase (AchE) from *Drosophila Melanogaster* (PDB ID: 1QO9), and the results revealed that they have a high affinity for receptor binding sites, which agrees with experimental results from the tested insect's larvae. The most bioactive molecules of Spiro ester 4 < Spiro hydrazide 5 < Spiro furanone 2 < Spiro arylidene 6< Chlorpyrifos were docked in this study.

TO elucidate Insilco's reasoning for the bioassay investigations indicated above, in which the results were compared to chlorpyrifos, which served as a positive control. **Table 5** lists the docking parameters of Gibbs free binding energy (ΔG_b), anticipated inhibition constant (Ki), which is a universal measure that reflects the potency of the utilized enzyme inhibitor, as well as ligand Root Mean Square deviation (L-RMSD), H-bond count, and other - π interactions. [48], [52], [53], [61].

The manifested data displays that the synthesized compounds under study have a higher binding affinity than Chlorpyrifos, where all of the compounds had lower binding energy ranging from – 8.75 for compound 10 to – 7.75 for compound 13, compared to CPS, which had the lowest negatively charged value of – 4.32, implying that they will be able to compete for binding sites on the AchE substrate.

Conclusions

Here we reported a green synthetic route for important derivatives of Spiro pyrrolidine-2,3'-thieno[2,3-d] pyridazine bearing alcohol, ester, hydrazide, and arylidene moieties. Spiro derivatives **1, 2, 3, 4, 5,** and **6** are synthesized *via* greener methods, simple, novel, and eco-friendly synthetic protocols. A comparative study regarding the outcome yields and the time or reactions was done on microwave-assisted grinding strategies. Full structural elucidations for all synthesized compounds were based on elemental and spectroscopic analyses. Insecticidal evaluations were done on all the products. DFT-based bioassay supported by molecular docking of such compounds that have interesting functional groups e.g., Spirothiophene-pyrrolidine, carboxylic ester, hydroxyl, hydrazide structures, and arylidene moiety in one unit of Spiro structure enhanced the possibility of binding with arginine 265, which in turn favored the insecticidal activity. However, the structures of the produced compounds must be optimized, and structural modification and biological evaluation are now being carried out to fully exploit the potential of these derivatives with varied hydrophobic and hydrophilic substituents based on these discoveries. The promising compounds can be submitted to further industrial scale up as a future perspective based on the previous investigations.

Declarations

Ethics approval and consent to participate:

The competent ethical commission has given its approval to all of the studies. While Participation Consent: Not applicable.

Consent for publication:

Not Applicable

Availability of data and material:

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

Competing interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors Contributions:

The following authors made contributions to this work: Sameh A. Rizk provided the work concepts, methodology, interpreted the results, and prepared the manuscript, Nihad A. Rizk performed the synthetic work, interpreted the results, assisted in anti-insect results, and prepared the manuscript, and Abdullah M. Abdo participate in the synthetic work interpreted methodology, antibacterial, and anti-insect results, and collaborated in manuscript preparation. All authors read and approved the final manuscript.

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Tables

Table 1. Antimicrobial Activity of synthesized Spiro compounds (1-6) against some coded pathogenic microorganisms.

Comp. No.		Mean Diameter of inhibition zone (mm)				
		Gram +Ve Bacteria		Gram -Ve Bacteria		Unicellular Fungi
Conc. µg/ml		*Staphylococcus aureus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia Coli*	*Candida albicans*
1	25	0±0	0±0	0±0	0±0	0±0
	50	0±0	0±0	0±0	0±0	0±0
	100	0±0	0±0	10.33±0.33	0±0	0±0
	200	11.33±0.33	12.67±0.33	11.22±0.33	10.33±0.33	10.67±0.33
	400	12.33±0.88	13.27±0.33	11.31±0.33	12.33±0.33	11.56±0.33
2	25	0±0	0±0	0±0	0±0	0±0
	50	11±0.33	12.67±0.33	11.33±0.33	11.67±0.33	10.67±0.58
	100	14.67±0.33	16.33±0.33	14.67±0.33	13±0.58	13.33±0.33
	200	16.67±0.33	22.33±0.88	18.67±0.88	15.33±0.88	16.33±0.33
	400	20±0.33	28.33±0.33	21.67±0.33	18.67±0.33	17.33±0.33
3	25	0±0	0±0	0±0	0±0	0±0
	50	0±0	0±0	0±0	0±0	0±0
	100	0±0	0±0	10.33±0.33	10.33±0.33	10.67±0.33
	200	0±0	10.33±0.33	11.33±0.33	12.33±0.88	11.33±0.33
	400	11.67±0.33	14.67±0.33	12.67±0.33	13.67±0.88	12.33±0.33
4	25	0±0	0±0	0±0	0±0	0±0
	50	0±0	10.33±0.58	0±0	10.33±0.58	10.33±0.58
	100	11.67±0.33	12.33±0.33	13±0.33	13±0.33	13±0.33
	200	13.33±0.33	13.67±0.33	13.67±0.33	14.33±0.33	14.33±0.33
	400	15.33±0.88	16.67±0.33	14.33±0.33	15.67±0.33	15.67±0.33
5	25	0±0	0±0	0±0	0±0	0±0
	50	10.67±0.33	11.67±0.33	11.33±0.33	10.67±0.88	10.33±0.33
	100	13.33±0.33	16.33±0.33	14.67±0.33	11.33±0.88	12.58±0.33
	200	16.33±0.58	22.58±0.33	16.33±0.58	14.67±0.88	14.33±0.33
	400	18.33±0	25.33±0.58	19.67±0.33	16.33±0.33	15.33±0.33
6	25	0±0	0±0	0±0	0±0	0±0
	50	11.56±0.58	13.56±0.58	12.56±0.58	11.51±0.58	12.56±0.67
	100	15.67±0.33	16.67±0.58	16.67±0.33	15.87±0.33	16.67±0.88
	200	19.67±0.33	21.67±0.33	21.67±0.33	17.67±0.33	21.67±0.33
	400	22.33±0.88	29.27±0.33	22.67±0.33	19.33±0.33	22.67±0.33

, ± Value is the standard error value, shaded light gray colored cells represent MIC for synthesized spiro compounds (1-6), while shaded dark gray colored cells represent the maximum diameter of inhibition zone activities recorded against different pathogenic organisms.

Table 2. Insecticidal activity (%) of synthesized Spiro compounds (1-6) against 3rd instar larvae of *Mythimna separata* and *Nilaparvata lugens* after 48hrs. incubation

Comp. No.	Insecticidal activity (%) at different concentrations (µg ml^{-1})									
	Mythimna separata					*Nilaparvata lugens*				
	25	50	100	200	400	25	50	100	200	400
1	23.3±3.3	26.6±3.3	36.6±3.3	46.6±3.3	56.6±3.3	6.6±3.3	23.3±3.3	40±5.7	53.3±3.3	60±0
2	36.6±3.3	46.6±3.3	63.3±3.3	96.6±3.3	100±0	26.6±3.3	46.6±3.3	63.3±3.3	76.6±3.3	80±5.7
3	23.3±3.3	30.0±5.7	36.6±3.3	46.6±3.3	53.3±3.3	26.6±3.3	36.6±3.3	46.6±3.3	46.6±3.3	56.6±3.3
4	26.6±3.3	36.6±3.3	46.6±3.3	53.3±3.3	66.6±3.3	16.6±3.3	26.6±3.3	46.6±3.3	50±0	63.3±3.3
5	33.3±3.3	36.6±3.3	53.3±3.3	63.3±3.3	80±0	26.6±3.3	30±5.7	50±5.7	63.3±3.3	73.3±3.3
6	43.3±3.3	63.3±3.3	76.6±3.3	100±0	100±0	33.3±3.3	53.3±5.7	73.3±3.3	80±0	83.3±3.3
Chlorpyrifos	53.3±3.3	60±5.7	86.6±3.3	100±0	100±0	33.3±3.3	50±3.3	70±0	80±0	86.6±3.3

, control (dis. Water) mortality = 0 %

Table 3. LC_{50} values of **2, 4, 5, 6** and chlorpyrifos against *Mythimna separata* and *Nilaparvata lugens*, [a] toxic ratio

Insects	Compd.	LC_{50} (µg ml^{-1})	*y=a+bx*	[a] *Toxic ratio*
Mythimna separata	2	100	*y*=3.1852+1.0493*x*	0.98
	4	200	*y*=2.35527+1.59130*x*	0.99
	5	200	*y*=2.15892+1.2746*x*	0.99
	6	50	*y*=2.09929+1.7009*x*	0.98
	Chlorpyrifos	25	*y*=3.732+1.46*x*	0.98
Nilaparvata lugens	2	100	*y*=2.3643+1.5001*x*	0.97
	4	200	*y*=2.6981+1.1224*x*	0.98
	5	100	*y*=3.4268+0.8336*x*	0.98
	6	50	*y*=2.3290+1.4716*x*	0.97
	Chlorpyrifos	50	*y*=2.2280+1.3748*x*	0.97

Table 4: Quantum chemical parameters calculated for the studied compounds.

Comp. No.	E_{HOMO} (Ha)	E_{LUMO} (Ha)	ΔE (eV)	I (eV)	A (eV)	X (eV)	H (eV)	ω	ΔN	μ (Debye)
1	-0.1715	-0.1195	1.4131	4.667	3.254	3.960	0.707	11.1	2.151	4.2764
2	-0.1977	-0.1046	2.5357	5.382	2.846	4.114	1.267	6.675	1.137	3.9657
3	-0.1833	-0.1124	1.9281	4.989	3.061	4.025	0.964	8.402	1.542	3.8509
4	-0.1873	-0.106	2.2131	5.099	2.885	3.992	1.106	7.202	1.358	3.1413
5	-0.1858	-0.082	2.8238	5.057	2.233	3.645	1.412	4.706	1.188	4.9853
6	-0.1946	-0.0982	2.6237	5.2975	2.6737	3.9856	1.3118	6.0543	1.1488	8.7733

Table 5. The obtained docking parameters for selected active structures with AchE of *Drosophila Melanogaster*

Comp.	ΔGb (Kcal/mmol)	Ki (μM)	L-RMSD (Å)	H-Bond		π-interaction
				Sum	Bond length (Å)	
1	-8.59	0.505	2.178	-	-	5
2	-7.76	2.05	1.867	2	2.46 2.51	4
3	-8.75	0.387	1.112	1	2.13	4
4	-8.01	1.35	1.143	-	-	2
5	-7.96	1.46	2.409	1	1.96	2
6	-7.75	2.06	2.169	-	-	5
CPS	-4.32	679.90	1.963	1	2.23	5

* Carbon-Hydrogen bond (CH-O contact).

Figures

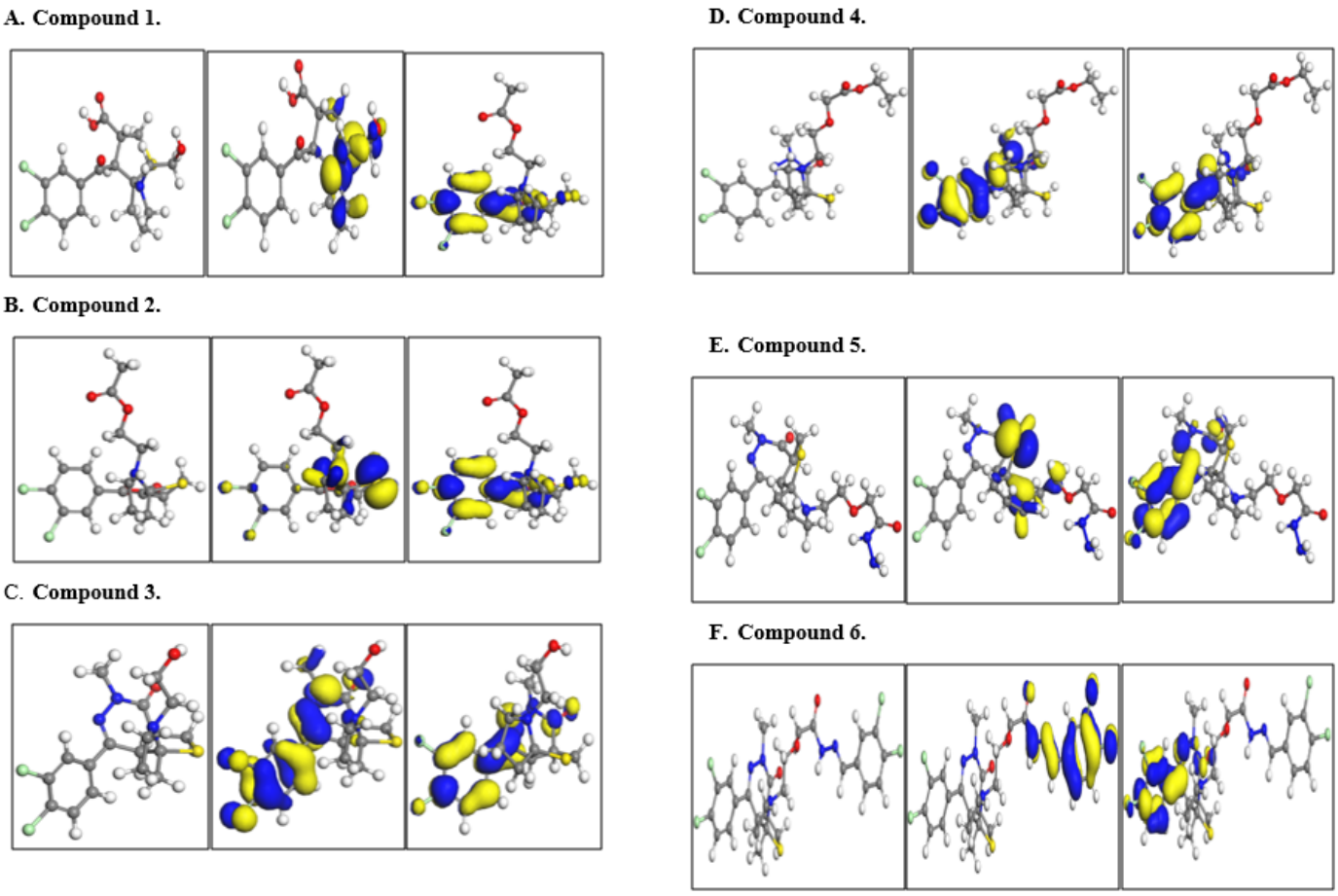

Figure 1

Chemical and optimized structures for the insecticidal agent:

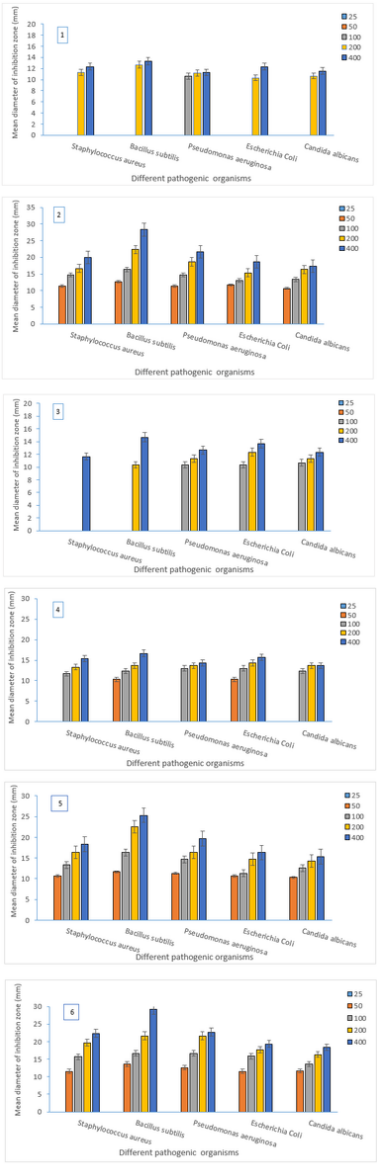

Figure 2

Outlines the Antimicrobial Activity of synthesized Spiro compounds (1-6) against some coded pathogenic microorganisms.

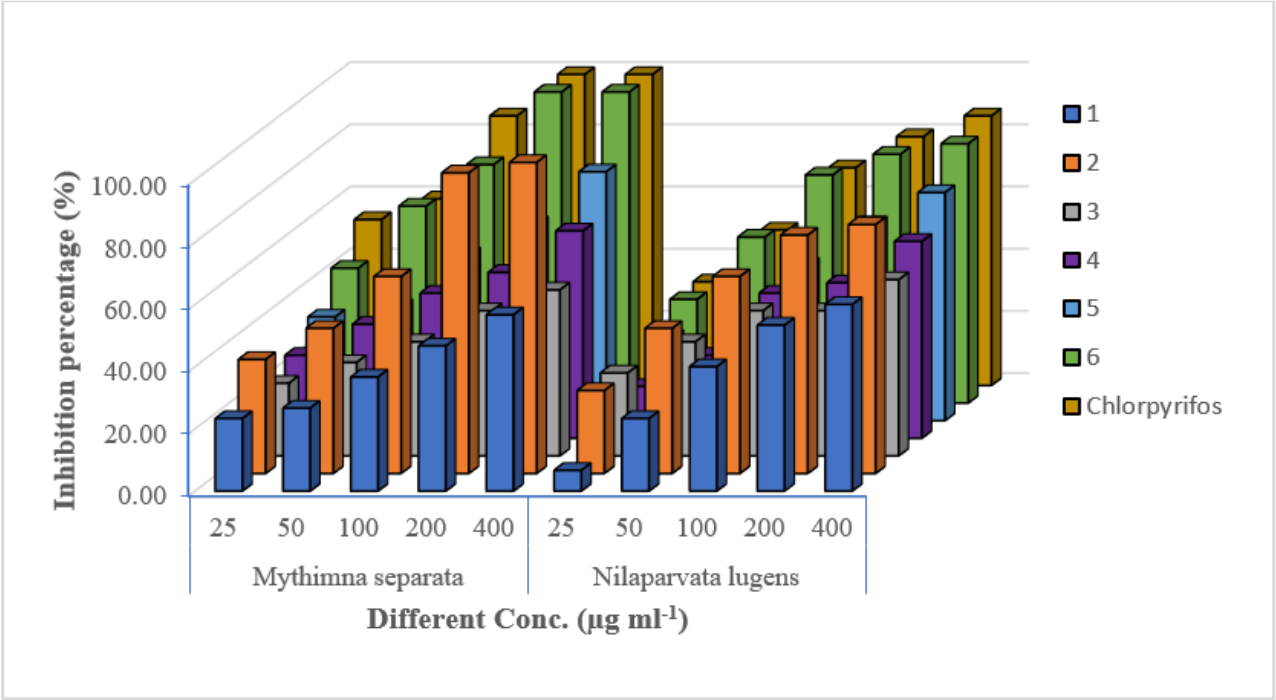

Figure 3

Insecticidal activity (%) of synthesized Spiro compounds (1-6) against 3rd instar larvae of *Mythimna separata* and *Nilaparvata lugens* after 48h incubation

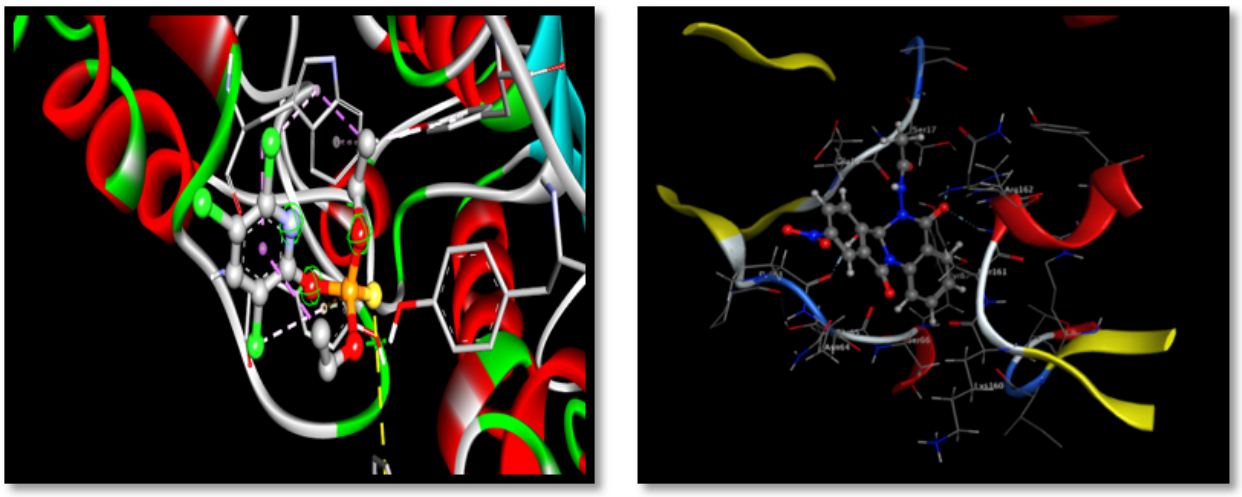

Figure 4

outline 3D of the Chlorpyrifos and most bioactive Spiro arylidene **6** Lowest binding energy 4.32

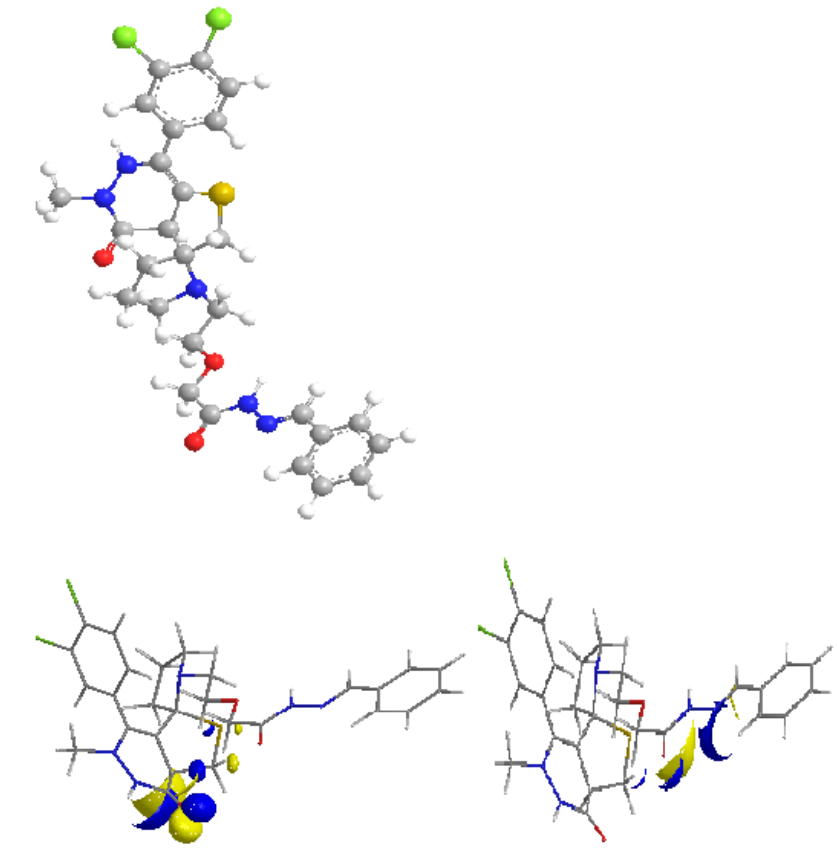

Figure 5

Optimized structure Surface configuration and active sites of the Spiro compound **6** (left), HOMO (middle), and LUMO (right) for most potent insecticidal compounds. Color index: White H, Grey C, Blue N, Red O, yellow S, and green Cl.

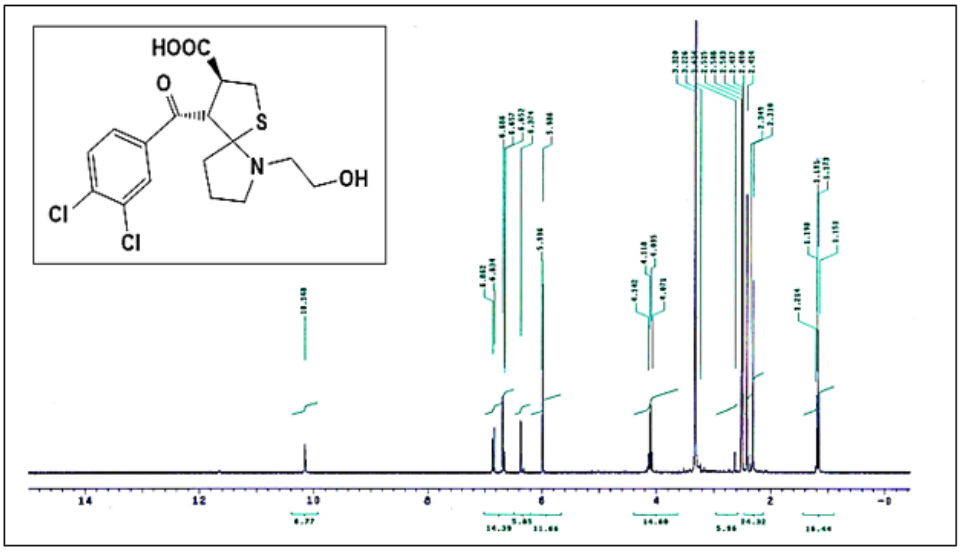

Figure 6

Outline 1H-NMR (DMSO) of the compound 1

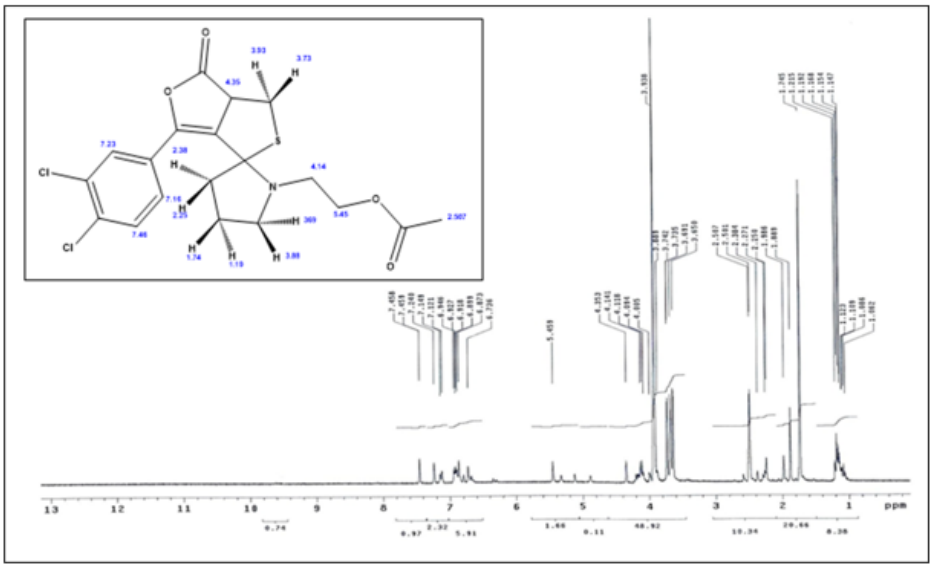

Figure 7

Outline ^{1}H-NMR (DMSO) of the compound 2

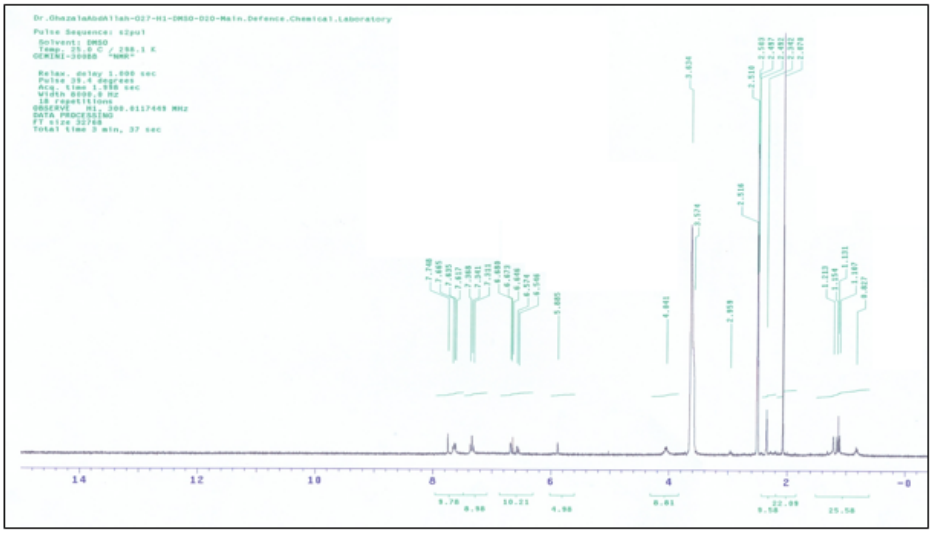

Figure 8

Outline ^{1}H-NMR (DMSO) of the compound 3

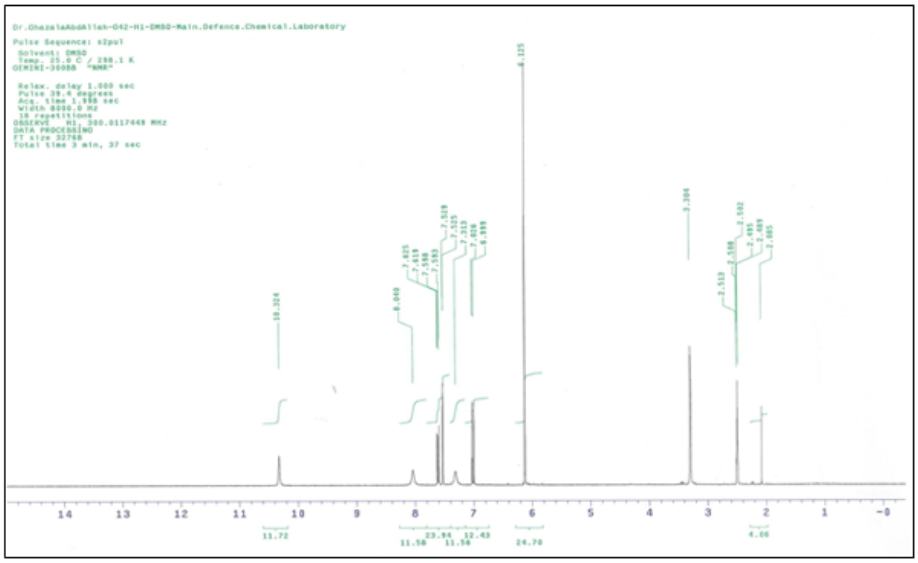

Figure 9

Outline ^{1}H-NMR (DMSO) of the compound 4

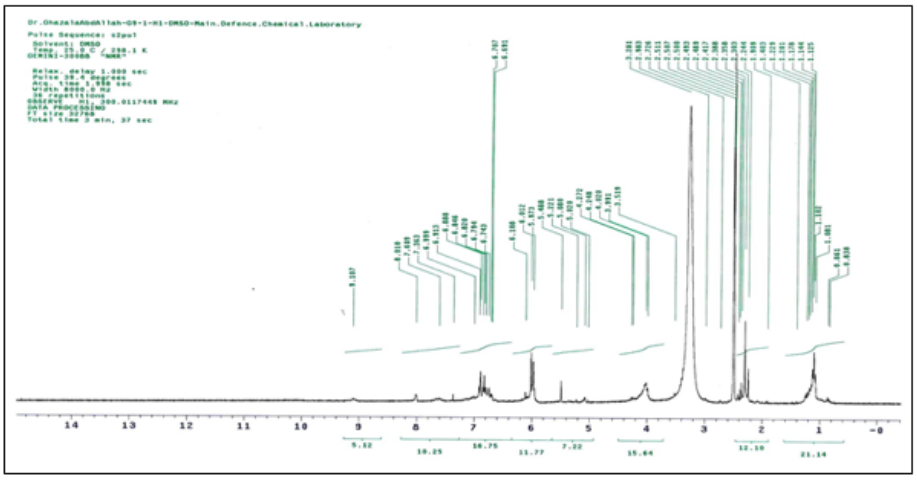

Figure 10

Outline ^{1}H-NMR (DMSO) of the compound 5

Supplementary Files

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