Regioselective Synthesis, Spectroscopic Characterization, and Computational Chemical Study of Spiro[indoline-3,4′-Pyrazolo[3,4-b] Pyridine Derivatives as Agrochemical Agents

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ABSTRACT

Herein, new four-component domino reactions are described and utilized to synthesize spiro[indoline-3,4′-pyrazolo[3,4-b]pyridine derivatives via one-pot reaction. Different hydrazine derivatives such as hydrazine hydrate, methyl hydrazine, and phenylhydrazine were allowed to react with active methylene precursors (CH₂CN) as malononitrile, ethyl cyanoacetate, isatin, and 1,3-dicarbonyl compounds in the presence of (+)-camphor-10-sulfonic acid (CSA) as the acid catalyst under microwave condition using water as the solvent. Two and three fused heterocyclic rings were obtained in a single synthetic operation with a facile work-up and less waste generation due to the absence of extraction and purification steps. Structural features of the obtained compounds were confirmed by utilizing elemental analyses, mass spectrometry, ¹H-NMR, and ¹³C-NMR microanalyses. Insecticidal assessments for some of the synthesized pyrazole derivatives against the cotton leafworm Spodoptera littoralis were conducted. In addition, fungicidal activities against Rhizoctonia solani and Fusarium solani soil-borne fungi were also performed. The tested compounds showed poor insecticidal activity compared with the standard insecticide chlorpyrifos ethyl. Meanwhile, they showed remarkable fungicidal activity against tested pathogens. Analysis of the fungicidal study indicated that R. solani fungus was found to be more sensitive to the tested compounds than F. solani fungus. Some pyrazole derivatives particularly possessed close or even higher fungicidal activities than the standard fungicide Pencycuron (Monceren 25%WP). The density functional theory was then applied to explore the structural and electronic characteristics of these materials.

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1. Introduction

Multicomponent reactions (MCRs), particularly those performed in aqueous media, have become an increasingly useful tool for the synthesis of chemically and biologically important compounds because of their convergence, atom economy, and green chemistry point of view. These reactions which involve at least three different simple substrates are powerful means for expedient building up of molecular complexity and diversity through the facile formation of several new covalent bonds in a one-pot transformation. This strategy is considered as a close approach to the concept of the ideal synthesis and particularly well adapted for combinatorial synthesis. The search for alternative reaction media to replace volatile, flammable, and often toxic organic solvents, commonly employed in organic synthesis is considered as an urgent demand for the development of green chemical processes. From both environmental and economic points of view, water has emerged as the medium of choice to perform organic reactions. Water is one of the most environmentally acceptable, safest, and abundant solvents. In general, water enables facile work-up protocols since most organic compounds are lipophilic and readily segregated from aqueous media. In addition, many organic reactions take place “on water,” i.e. the reactants initially ameliorated in water, exhibit important rate enhancements. Furthermore, water as a reaction medium enables novel solvation and assembly processes conferring unique selectivity and reactivity.

Fused pyrazole moieties are considered as an interesting family of heterocycles due to their multiple applications such as pharmaceuticals, herbicides, insecticides, and fungicides. For example, pyrazolopyridine derivatives exhibit various remarkable pharmacological properties. Interestingly, pyrazolo[3,4-b]quinoline derivatives also display relevant bioactivities. Pyridopyrimidine motif, a well-known pharmacophore in drug design has a wide range of biological activities. Spiro-oxindole substructures, present in many alkaloids are well-known for their interesting structural and biological properties. Due to all the aforementioned facts, the development of an aqueous facile one-step strategy to synthesize biologically active pyrazole derivatives is considered an urgent demand. Evaluations of their diverse pesticidal activities were also conducted.

2. Result and discussion

2.1. Chemistry

In a one-pot sequential synthesis of a target product, each reaction has to proceed in an excellent yield, in which the generation of byproducts and side-products is minimized as much as possible.

Scheme 1. Synthesis of Spiro-heterocycles 4-27.
As the number of reaction steps increases, byproducts and side-products accumulate more and more affecting the following reaction yields. Subsequent reactions, thus, have to proceed in the presence of these accumulated byproducts and side-products. In this article, one-pot, four-component procedure was emanated from the reaction of hydrazine derivatives with active acetonitrile derivatives, 1,3-dicarbonyl compounds, and isatin in water for the construction of spiro-heterocycles (Scheme 1) via adducts, comprising spiro-oxindole, pyrazole, pyrazolopyridine, pyrazolopyridopyrimidine, and pyrazoloquinoline substructures. Thus, it is important to not only select suitable reactions with minimal byproducts but also to optimize the reaction conditions by using density functional theory (DFT) methods to suppress the generation of undesired side-products. The four-components; hydrazine derivatives, active acetonitrile, 1,3-dicarbonyl compounds, and isatin in the presence of 1 equivalent of p-toluensulfonic acid (p-TSA) as a catalyst were examined under microwave condition for 25 min, to afford compound in 80% yield (Scheme 1). Interestingly, when (+)-camphor-10-sulfonic acid (CSA) was used instead, 0.5 equivalents of acid were sufficient for reflux to achieve completion in 1 h, affording the product in a good yield of 92%. It is pertinent to note that CSA, a mild, inexpensive, readily available Brønsted acid, has recently emerged as a powerful catalyst for organic transformations in an aqueous medium. The structure of the spiro-heterocycles is in full agreement with elemental analyses, 1H, and 13C spectroscopic data, as illustrated below for a representative example (compound 5).

In the 1H-NMR spectrum of 5, hydrogens of the two methyl groups of the pyrazolopyridine moiety appeared as singlet signals at 2.49 and 3.04 ppm. The relevant 13C-NMR signals appeared at C-8 at 11.4 ppm, C-7 at 21.1 ppm, and Spiro C-6 at 58.9 ppm. Three singlet peaks at 5.78, 9.70,
and 10.34 ppm were attributed due to the presence of NH groups in dihydropyridine, pyrazolo, and oxindole, respectively. The NMR spectroscopic data of the known compounds, 9, 10, 11, and 12 reported in the present work agree well with the reported literature. A plausible mechanism for the formation of the spiro-heterocycles is proposed in Scheme 2. The sequence of reactions is, presumably triggered by the formation of 3-methyl-1-phenylpyrazol-5-one from the acid-catalyzed reaction of phenyl hydrazine > methyl hydrazine > hydrazine hydrate as nucleophiles toward ethyl cyanoacetate. The intermediate of pyrazolone upon reaction with isatin afforded the adducts in turn, which have been isolated as the sole reaction product in some of the reactions carried out during the optimization studies. DFT reveals the nucleophilicity index of the hydrazine derivatives as it discloses the electrophilicity index of the ethyl cyanoacetate > malononitrile > Isatin > β-diketone precursors (see more in DFT study). This compound, upon reaction with the starting 1,3-diketones under acidic conditions presumably furnishes intermediate, which subsequently underwent annulation leading to the final spiro-heterocycles via an intermediate. HOMO energies

| Nucleophiles          | Electrophiles          |
|-----------------------|------------------------|
| Hydrazine             | malononitrile          |
| Methyl hydrazine      | Ethylcyanoacetate      |
| Phenylhydrazine       | thiobarbituric         |
|                       | benzoylacetone         |
|                       | benzoylacetophenone    |
|                       | 3-pyridinoyl acetone   |
|                       | Isatin                 |
|                       | 3-pyridinoyl acetone   |
|                       | benzoyleacyonacetone   |
|                       | thiobarbituric         |
|                       | Isatin                 |
|                       | benzoyleacyonacetone   |
|                       | Ethylcyanoacetate      |
|                       | malononitrile          |

Figure 1. Outline the \( E_{\text{HOMO-LUMO}} \) of nucleophiles toward \( E_{\text{HOMO-LUMO}} \) of the electrophiles.
values of the aminopyrazolespiro products 4-27 were lower than that of hydrazine derivatives. As anticipated, the competitive reaction of the aminospiro products 4-27 with hydrazine derivatives toward LUMO values of ethyl cyanoacetate and malononitrile took place. The involvement of pyrazolone in this reaction is supported by the fact that when hydrazine, methyl hydrazine, and phenylhydrazine (1 mmol) were allowed to react ethyl cyanoacetate (1 mmol), CSA (0.5 mmol), and water (10 mL) for 20 min at 100 °C under microwave reaction condition to afford pyrazolone in 98% yield.31

Furthermore, the reaction of aminopyrazolone is more preferred than aminospiro products 4-27 toward isatin and β-diketone precursors in the presence of CSA under microwave followed by reflux conditions for 1 h afforded the corresponding spiroproducts 4-27 in near quantitative (96%) yield. The greater efficacy of CSA (pKₐ 1.2) compared with p-toluene sulfonic acid (pKₐ 0.7) is explicable based on the higher acidity of the former.32

Pyrazole and oxindole systems as vital building blocks for constructions of more sophisticated scaffolds with outstanding biological potent targets led to attempts to isolate those adducts as the sole reaction products were done in some of the reactions. This goal was achieved by optimizing the conditions to establish a new three-component method for the synthesis of 3-(4-pyrazolyl) oxindoles utilizing 0.5 equivalents of CSA as the acid catalyst. The results obtained from the reaction between phenylhydrazine, ethyl cyanoacetate, and isatin in water at 100 °C for 10 min are summarized in Scheme 2. Interestingly, these conditions are highly appreciated since they led to an excellent yield of pyrazole oxy indoline adduct 4.

2.2. DFT study

DFT study explains the increasing electrophilicity on the carbonyl in both ethyl cyanoacetate and cyano of the malononitrile moieties than that of 1,3 diketones precursors, thiobarbituric, or isatin despite their low E_LUMO values than former ones.

It is well known that high E_HOMO is likely to indicate a strong tendency of the molecule to donate electrons. The low values of the energy gap (ΔE = E_LUMO − E_HOMO) will render good inhibition efficiencies because the energy needed to remove an electron from the last occupied orbital will be low.36,37 DFT is based on quantum chemical computation which outlines the structure optimization of the intermediate that reacted to afford the desired product. Figure 1 confirmed high separately of HOMO-LUMO level of hydrazine derivatives nucleophiles and (XCH₂CN) electrophiles are supported to the authors to explain the hydrazine nucleophiles will prefer to attack the carbonyl or cyano of XCH₂CN moieties to form pyrazole intermediate before attack isatin followed by 1,3-dicarbonyl to afford the target of spiro derivatives 4-27. So, in the present reaction of the phenylhydrazine HOMO (−5.750 eV) prefer to react with carbon electrophiles, e.g. malononitrile, the LUMO energy (3.58 eV) rather than LUMO of benzoyl acetone LUMO (−5.186 eV) although lower values of the energy gap (ΔE = E_LUMO − E_HOMO) between benzoyl acetone and phenylhydrazine due to the high charge density of the 1,3-dicarbonyl precursors and high values of the dipole moments of the malononitrile (μ = 9.4) and ethyl cyanoacetate (μ = 6.157) inspire the good matching and approaching to LUMO energy of the electrophilic site of malononitrile to form the corresponding pyrazole intermediate (I) via addition reaction followed by cyclization (Scheme 2).

So, DFT simulation helped us to know why the MCRs of hydrazine derivatives with XCH₂CN, isatin, and 1,3-dicarbonyl precursors yielded newly heterocyclic compounds 4-27. The structures of 4-27 have been supported by full spectral analysis and micro-analytical data. Quantum chemical parameters calculation uses the DFT method for the calculations of the newly synthesized compounds (Table 1). The dipole moment, hardness, softness, and surface area (nm²) for newly spiro-pyrazole derivatives carrying hydrophobic groups were agreed with an excellent explanation for the synthesized compounds and their insecticidal efficiency. Also, the Ionization potential (I, eV),
transferred electrons, and charge density distribution ($\Delta N$) indicate the greater value pyrazole derivatives $5, 9, 12, 14,$ and $17$ have the maximum transfer of electron, and hence, the greater tendency of adsorption and inhibition for the cancer cell. The optimization structures of the synthesized pyrazole $4-27$ as outlined in Figure 2–4 (See more in the Supporting Information).

From DFT optimization of pyrazole intermediate (I), it is confirmed that the nucleophilicity index of the carbon (C5) was more nucleophilic than the nitrogen atom of the amino group (N6) than that of hydrazine derivatives. The DFT calculation was in good agreement with the reactivity of the electrophilic site of isatin (lower LUMO) that will be more approached by the higher nucleophilic site of the C5 (higher HOMO) of the pyrazole intermediate (I) to the more electrophilic center (C9) in the isatin moiety (Figure 2).

### 2.3. Biological activity assays

Egyptian cotton worm, *Spodoptera littoralis*, is one of the most serious phytophagous insect pests on cotton as well as most field crops and vegetables in Egypt.$^{38}$ Both *Rhizoctonia. solani* and *Fusarium solani* are plant pathogenic fungi that cause significant establishment and yield losses to several important food crops globally.$^{39}$ As a result, the second objective of this study is to evaluate the insecticidal and fungicidal activities of some selected examples of the prepared compounds against such destructive pests searching for new and locally prepared active ingredients that can be used to fight these pests. Data in Table 2 shows the insecticidal activity of the selected pyrazole derivatives against the fourth of the Egyptian cotton leafworm. It is clear that mortality slightly increases as concentrations of tested compounds increases while mortality caused by the standard insecticide, chlorpyrifos-ethyl, follows the same trend with a very high rate. At 500 ($\mu$g/larva) treatment, standard insecticide causes 100% mortality while that of the tested compounds ranges between 0.0% and 13.3% only. Also, LC$_{50}$ value of the most active derivative, compound 14, is 3993.3 $\mu$g/larva as compared with the standard insecticide whose LC$_{50}$ value is 44.4 $\mu$g/larva. This means compound 14 has 1.2% toxicity as compared with chlorpyrifos-ethyl. In other words, standard insecticide has 89.9-fold activity of compound 14. All these facts reflect the poor insecticidal activity of the synthesized pyrazoles against the target pest.

Twelve selected spiropyrazole derivatives were evaluated for their *in vitro* antifungal activity against *Rhizoctonia solani* and *Fusarium solani* soil-borne fungi. Inhibition zones and the other toxicity parameters of both tested compounds and standard fungicide pencycuron (Monceren 25%WP) are listed in Tables 2 and 3. Tested compounds showed varied antifungal activity against two pathogenic fungi. As compared with the standard fungicide, pyrazole derivative 12 is the

| Compound | $\zeta$: Absolute electronegativity (eV) | $\mu$: Dipole moment (Debye) | $\eta$: Global hardness (eV) | $S$: Global softness (eV)$^{-1}$ | $\chi$: Global electrophilicity index (eV) |
|----------|-----------------------------------|----------------------------|--------------------------|----------------------------|-------------------------------------|
| 1        | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 2        | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 3        | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 4        | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 5        | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 6        | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 7        | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 8        | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 9        | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 10       | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 11       | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 12       | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 13       | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |
| 14       | 0.00                              | 3.99                       | 1.510                    | 0.662                      | 10.788                              |

Table 1. Global reactivity indices and energy level distribution of frontier orbitals.
most potent one against *R. solani* fungus as their toxicity index and EC50 values are 100.0, 206.5 ppm and 89.5, 230.8 ppm for pyrazole derivative 12 and pencycuron, respectively, that means compound 12 has 1.12-fold the activity of the standard fungicide. Compounds 13 and 14 showed moderate activity as their toxicity indices are 70.8 and 83.5, respectively, as compared with pencycuron. The fungicidal potentiality of studied spiropyrazoles against *F. solani* fungus is

![Figure 2](image1.png)

*Figure 2.* Outline the separated charge density both of HOMO (pyrazole intermediate) and LUMO (Isatin moiety).

![Figure 3](image2.png)

*Figure 3.* Outline the regioselective Imino Spiroproduct (4) than amino isomer.

\[
E = 64.3 \text{ Kcal/mol} \\
\Delta E = 5.17 \text{ eV} = 119.22 \text{ Kcal/mol}
\]

\[
E = 61.9 \text{Kcal/mol} \\
\Delta E = 4.33 \text{ eV} = 99.85 \text{ Kcal/mol}
\]
Figure 4. Outline the regioselective amino spiroproduct (10) than Imino isomer.

Table 2. Insecticidal activity of some selected synthesized compounds against the fourth instar larvae of the susceptible strain of cotton leafworm *Spodoptera littoralis*.

| Compd No. | Mortality % after 24 h | LC$_{50}$ (μg/larva) | T.I* | The slope of the toxicity line |
|-----------|------------------------|-----------------------|------|-------------------------------|
| Conc. (μg/larva) | 1000 | 500 | 250 | 125 | 65 |
| 4 | 13.3 | 10.0 | 6.7 | 0.0 | 0.0 | 7352.0 | 0.60 | 1.17 |
| 6 | 10.0 | 6.7 | 0.0 | 0.0 | 0.0 | 5380.3 | 0.83 | 1.64 |
| 10 | 10.0 | 6.7 | 0.0 | 0.0 | 0.0 | 5654.5 | 0.78 | 1.55 |
| 14 | 16.7 | 13.3 | 6.7 | 0.0 | 0.0 | 3993.3 | 1.2 | 1.44 |
| 17 | 6.7 | 3.3 | 0.0 | 0.0 | 0.0 | 9095.6 | 0.49 | 1.55 |
| 18 | 13.3 | 13.3 | 10.0 | 6.7 | 0.0 | 17545.5 | 0.25 | 0.78 |
| 19 | 10.0 | 6.7 | 3.3 | 0.0 | 0.0 | 9477.0 | 0.47 | 1.27 |
| 20 | 6.7 | 0.0 | 0.0 | 0.0 | 0.0 | 7385.2 | 0.60 | 1.79 |
| 23 | 10.0 | 10.0 | 3.3 | 0.0 | 0.0 | 9497.0 | 0.47 | 1.17 |
| Chlorpyrifos-ethyl | 100.0 | 100.0 | 96.7 | 83.3 | 66.7 | 44.4 | 100.0 | 2.37 |

*Toxicity index is the % of the activity of the tested compounds to the most potent one whose T.I is taken as 100.
Table 3. Inhibition rates of the synthesized compounds against *R. Solani* fungus and *F. Solani* fungus.

| Compd No. | % of inhibitions | EC₅₀ (ppm) | T.I* | The slope of the toxicity line |
|-----------|------------------|------------|------|-----------------------------|
| Conc. (ppm) | 500 | 400 | 300 | 200 | 100 | 500 | 400 | 300 | 200 | 100 | 500 | 400 | 300 | 200 | 100 |
| 4 | 40.6 | 21.7 | 10.0 | 4.7 | 0.0 | 609.8 | 33.9 | 3.78 |
| 6 | 21.5 | 15.7 | 8.9 | 1.7 | 0.0 | 789.4 | 26.2 | 3.59 |
| 10 | 58.9 | 51.1 | 27.0 | 9.0 | 0.0 | 418.8 | 49.1 | 4.16 |
| 12 | 81.3 | 70.7 | 52.7 | 41.4 | 33.6 | 206.5 | 100.0 | 2.01 |
| 13 | 76.2 | 68.9 | 43.3 | 21.8 | 13.8 | 391.8 | 70.8 | 3.04 |
| 14 | 77.7 | 59.8 | 50.9 | 41.8 | 27.9 | 274.3 | 81.5 | 3.95 |
| 15 | 54.8 | 49.1 | 36.4 | 17.8 | 8.0 | 433.4 | 47.7 | 3.35 |
| 17 | 33.2 | 21.9 | 10.9 | 0.0 | 0.0 | 609.1 | 34.0 | 4.44 |
| 18 | 39.0 | 31.9 | 20.0 | 5.5 | 0.0 | 570.0 | 36.2 | 3.38 |
| 19 | 23.9 | 18.7 | 10.0 | 0.0 | 0.0 | 730.7 | 28.3 | 3.72 |
| 20 | 37.3 | 14.5 | 10.9 | 0.0 | 0.0 | 603.6 | 34.2 | 4.76 |
| 23 | 67.3 | 44.8 | 30.9 | 20.8 | 0.0 | 397.0 | 52.0 | 3.54 |
| Pencycuron | 85.2 | 74.5 | 62.3 | 43.2 | 15.9 | 230.8 | 89.5 | 2.75 |

Pencycuron

Inhibition rates of the synthesized compounds against *F. Solani* fungus

| Compd No. | % of inhibitions | EC₅₀ (ppm) | T.I* | The slope of the toxicity line |
|-----------|------------------|------------|------|-----------------------------|
| Conc. (ppm) | 500 | 400 | 300 | 200 | 100 | 500 | 400 | 300 | 200 | 100 | 500 | 400 | 300 | 200 | 100 |
| 4 | 30.6 | 22.7 | 10.0 | 0.0 | 0.0 | 647.6 | 74.2 | 3.97 |
| 6 | 41.8 | 26.4 | 11.8 | 0.0 | 0.0 | 539.8 | 89.0 | 5.04 |
| 10 | 15.4 | 10.0 | 0.0 | 0.0 | 0.0 | 757.3 | 63.5 | 5.27 |
| 12 | 45.4 | 38.2 | 23.2 | 11.8 | 0.0 | 517.5 | 92.9 | 3.12 |
| 13 | 43.6 | 32.9 | 7.9 | 0.0 | 0.0 | 509.3 | 94.4 | 5.92 |
| 14 | 31.9 | 27.0 | 11.5 | 0.0 | 0.0 | 600.5 | 80.0 | 4.32 |
| 15 | 38.2 | 22.5 | 13.1 | 0.0 | 0.0 | 573.7 | 83.8 | 4.62 |
| 17 | 25.5 | 21.1 | 17.6 | 8.9 | 0.0 | 894.3 | 53.7 | 2.25 |
| 18 | 36.4 | 22.8 | 13.3 | 0.0 | 0.0 | 585.7 | 82.1 | 4.45 |
| 19 | 19.7 | 12.9 | 6.4 | 0.0 | 0.0 | 823.4 | 58.4 | 3.73 |
| 20 | 28.8 | 23.1 | 12.3 | 0.0 | 0.0 | 649.4 | 74.0 | 3.95 |
| 23 | 46.7 | 41.8 | 33.1 | 18.8 | 0.0 | 480.6 | 100.0 | 2.76 |
| Pencycuron | 51.1 | 42.6 | 32.7 | 20.6 | 7.3 | 490.7 | 97.9 | 2.10 |

*Toxicity index is the % of the activity of the tested compounds to the most potent one whose T.I is taken as 100.

shown in Table 3. It can be seen that *F. solani* is less sensitive to the tested compounds than *R. solani* fungus as 500 ppm treatment showed ranges of inhibition percentages from 23.9% to 85.2% and 19.7% to 51.1% for *R. solani* and *F. solani* fungi, respectively. Compound 23 was found to be a more toxic derivative as its toxicity index is very close to that of the standard fungicide. Toxicity indexes for the pencycuron and pyrazole derivative 23 are 97.9 and 100.0, respectively. The good fungicidal activity of derivative 12 and 23 against tested fungi may be due to the substituent 2- nicotinoyl at position 5' of the pyrazolopyridine ring system. 40, 41

2.4. DFT calculation of the synthesized spiro indoline reveals the insecticidal activity

Moreover, quantum chemical parameters calculations using the DFT method used for the calculations of the synthesized compounds are in good agreement with the antibacterial activity. Theoretical DFT parameters for aminopyrazole-iminopyrazole carrying hydrophobic groups showed an excellent efficiency as insecticidal agents. Also, the Ionization potential (1 eV), transferred electrons, and charge density distribution (ΔN) indicate the greater value of spiropyrazole derivatives 10, 12, 13, and 23 have the maximum transfer of electron and the hence greater tendency of adsorption and inhibition for the insect. HOMO of the imine form of the azahydrazide I precursor of the spiropyrido-indoline moiety 13 was −5.70 eV while HOMO of the amino pyrazole II was −7.34 eV. So, the competitive isomerization on the azahydrazide-thiol III (HOMO −2.24 eV) becomes more preferred to isomerized with thiol of azahydrazide although the steric hindrance that causes the non-planarity structure of 13III (Scheme 3). HOMO-LUMO
conduction band of the compound 13 lies between valence bands of the acetylcholine insect which made it more vulnerable for ease inhibition. From the DFT study, the conduction band of the spiropyrazole derivatives 10, 12, 13, and 23 were from side to side the valence band of the acetyl cholinase of the Spodoptera littoralis, Rhizoctonia solani, and Fusarium solani along with matching together the hydrogen bonds.

3. Conclusions

Herein, new four-component domino reactions for the synthesis of spiro[indoline-3, 7'-pyrazolo[3,4-b]pyridines containing up to five rings in good to excellent yields from the reactions of phenylhydrazine, 3-aminocrotononitrile, isatin, and 1,3-dicarbonyl compounds in the presence of CSA in an aqueous medium have been described. This protocol is endowed with prominent advantages such as convergence, short reaction time, excellent yields, easy operation, and broad scope of applicability. Furthermore, it is an environmentally friendly strategy since it does not require the using of metal-containing catalysts and using water as the reaction medium. In addition, purification is done by simple filtration which means avoiding the use of organic solvents at any point of the experimental procedure. This study also demonstrates that the selectivity of the reactions can be controlled by a judicious choice of the catalyst, leading to the development of a three-component synthesis of 3-(5-amino-4-pyrazolyl) oxindoles. The biological assays demonstrate that synthesized spiropyrazoles possessed poor insecticidal activity against Egyptian cotton leafworm, Spodoptera littoralis, but they showed good fungicidal activity against both R. solani and F. solani soil-borne fungi. Ongoing researches within our group will reveal more development as potent agents for this kind of structural feature in pharmaceutical and agricultural fields.

4. Experimental

4.1. Chemical synthesis

4.1.1. General

Melting points were measured in open capillary tubes and are uncorrected. The $^1$H NMR and $^{13}$C NMR, spectra were recorded on a Bruker (Avance) 300 MHz NMR instrument using TMS as internal standard and DMSO-$d_6$ as solvent. Standard Bruker software was used throughout. Chemical shifts are given in parts per million (d scale) and the coupling constants are given in Hertz. Silica gel-G plates (Merck) were used for TLC analysis with a mixture of petroleum ether (60–80°C) and ethyl acetate as eluent. Elemental analyses were performed on a Perkin Elmer 2400 Series II Elemental CHN analyzer.

4.1.2. General procedure for the synthesis of spiro[indoline-3,4'-pyrazolo[3,4-b] pyridines (4-27)

A mixture of hydrazine derivatives (1 mmol), malononitrile/ethyl cyanoacetate (1 mmol), and CSA (0.5 mmol) in water (10 mL) was added to isatin (1 mmol) and a suitable cyclic 1,3-
dicarboxyl compound (1 mmol). The reaction mixture was heated under microwave condition at 100°C followed by if need to reflux for 1–2 h. After completion of the reaction monitored by TLC, the reaction mixture was cooled to room temperature, the precipitate filtered off, and washed with methanol to obtain the pure spiro-heterocycles 4–27 as yellow solids. Spectroscopic data for all these compounds are given below.

**4.1.2.1. 5’-Benzoyl-3’-imino-6’-methyl-3a’,4’-dihydrospiro[indoline-3,7’-pyrazolo[4,3-b]pyridine]-2 (2H)-one (4).** Isolated as a yellow solid, yield: 88%, mp 202–204°C (recrystallization from EtOH); ¹H-NMR (300 MHz, DMSO-d₆) 2.69 (s, 3H, CH₃), 5.84 (d, 1H, CH), 6.42 (s,1H, NH), 7.41–7.52 (m, 9H, ArH), 9.77 (s,1H, NH), 10.48 (bs, 2H, 2NH); ¹³C NMR (75 MHz, DMSO-d₆) 11.4, 47.1, 58.9, 101.7, 108.6, 109.3, 121.6, 123.4, 123.6, 127.4, 129.6, 136.6, 137.1, 137.9, 141.8, 145.1, 155.1, 179.8, 193.8. Anal. Calcd for C₂₅H₁₆N₅O₂ (371) m/z: 371.12 (100.0%), 373.13 (27.7%).

**4.1.2.2. 5’-Benzoyl-3’-imino-6’-dimethyl-2’,3’,3a’,4’-tetrahydrospiro[indoline-3,7’-pyrazolo[4,3-b]pyridin]-2-one (5).** Isolated as a yellow solid, yield: 82%, mp 240–242°C (recrystallization from EtOH/DMF mixture); ¹H-NMR (300 MHz, DMSO-d₆) 2.49 (s, 3H, CH₃), 3.04 (s, 3H, CH₃), 4.86 (d, 1H, CH), 5.93 (s,1H, NH), 7.22–7.69 (m, 9H, ArH), 9.43 (s,1H, NH), 10.21 (s, 1H, NH); ¹³C NMR (75 MHz, DMSO-d₆) 11.4, 21.1, 48.1, 56.9, 121.7, 128.6, 129.3, 131.6, 133.4, 134.6, 137.4, 139.3, 139.6, 140.1, 140.9, 141.8, 145.1, 155.1, 166.8, 196.8. Anal. Calcd for C₂₂H₁₈N₅O₂ (385) m/z: 447.15 (100.0%), 449.16 (23.8%), 450.16 (2.7%), 449.15 (1.5%), 450.16 (1.4%) Element Anal.: %C, 68.56; H, 4.18; N, 16.16; Found: C, 68.05; H, 4.47; N, 17.85.

**4.1.2.3. 5’-Benzoyl-3’-imino-6’-phenyl-2’,3’,3a’,4’-tetrahydrospiro[indoline-3,7’-pyrazolo[4,3-b]pyridin]-2-one (6).** Isolated as a yellow solid, yield: 78%, mp 264–271°C (recrystallization from EtOH/DMF mixture); ¹H-NMR (300 MHz, DMSO-d₆) 7.68 (m, 14H, ArH), 9.77 (s, 1H, NH), 10.37 (s, 1H, NH); ¹³C NMR (75 MHz, DMSO-d₆) 7.82 (m, 14H, ArH), 9.47 (s, 1H, NH), 10.12 (s, 1H, NH); Anal. Calcd for C₂₇H₂₀N₅O₂ (447) m/z: 447.15 (100.0%), 449.16 (29.2%), 450.16 (2.7%), 449.15 (1.5%), 450.16 (1.4%) Analytical data: %C, 68.56; H, 4.70; N, 18.17; Found: C, 68.05; H, 4.47; N, 17.85. E = 63.19 Kcal/mol ΔE = 4.82 eV = 111.15 Kcal/mol

**4.1.2.4. 5’-Benzoyl-3’-imino-6’-phenyl-2’,3’,3a’,4’-tetrahydrospiro[indoline-3,7’-pyrazolo[4,3-b]pyridin]-2-one (7).** Isolated as a yellow solid, yield: 85%, mp 222–224°C (recrystallization from EtOH/Diox mixture); ¹H-NMR (300 MHz, DMSO-d₆) 6.72 (s, 1H, NH), 8.99 (s,1H, NH), 10.17 (s, 2H, 2NH); ¹³C NMR (75 MHz, DMSO-d₆) 11.6, 47.1, 57.9, 121.7, 128.6, 129.3, 131.6, 133.4, 134.6, 137.4, 139.3, 139.6, 140.1, 140.9, 141.8, 145.1, 155.1, 166.8, 196.8. Anal. Calcd for C₂₇H₂₀N₅O₂ (447) m/z: 477.15 (100.0%), 499.16 (29.2%), 500.16 (2.7%), 499.15 (1.5%), 500.16 (1.4%) Analytical data: %C, 72.47; H, 4.50; N, 15.65; Found: C, 72.12; H, 4.31; N, 15.24. E = 67.28 Kcal/mol ΔE = 3.98 eV = 91.78 Kcal/mol

**4.1.2.5. 5’-Benzoyl-3’-imino-2’-methyl-6’-phenyl-2’,3’,3a’,4’-tetrahydrospiro[indoline-3,7’-pyrazolo[4,3-b]pyridin]-2-one (8).** Isolated as a yellow solid, yield: 88%, mp 202–204°C (recrystallization from EtOH/DMF mixture); ¹H-NMR (300 MHz, DMSO-d₆) 6.23 (s, 1H, NH), 7.21–7.72 (m, 14H, ArH), 9.77 (s,1H, NH), 10.37 (s, 1H, NH); ¹³C NMR (75 MHz, DMSO-d₆) 21.4, 47.1, 58.3, 121.7, 123.6, 125.3, 125.7, 129.1, 129.4, 129.6, 132.6, 134.1, 137.9, 141.8, 145.1, 155.1, 167.8, 193.8. Anal. Calcd for C₂₇H₂₀N₅O₂ (447) m/z: 477.15...
(100.0%), 449.16 (29.2%), 450.16 (2.7%), 449.15 (1.5%), 450.16 (1.4%). Elemental Anal: %C, 72.47; H, 4.50; N, 15.65; Found: C, 72.05; H, 4.27; N, 15.28.

4.1.2.6. 5'-Benzoyl-3'-imo-2',6'-diphenyl-2',3',3a',4'-tetrahydrosipro[indoline-3,7'-pyrazolo[4,3-b]pyridin]-2-one (9). Isolated as a yellow solid, yield: 84%, mp 254–256°C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d6) 5.46 (d, 1H, CH), 6.17 (s,1H, NH), 7.11–7.82 (m, 19H, ArH), 9.62 (s,1H, NH), 10.31 (s,1H, NH); 13C NMR (75 MHz, DMSO-d6) 45.1, 56.9, 111.7, 118.6, 119.3, 121.6, 123.4, 123.6, 127.4, 129.6, 136.6, 137.1, 137.9, 141.8, 145.1, 155.1, 169.8, 193.8. Anal. Calcd for C32H23N2O2 (509) m/z: 510.17 (100.0%), 511.17 (34.6%), 512.18 (5.8%), 511.17 (1.5%). Elemental Anal: %C, 75.43; H, 4.34; N, 13.74; Found: C, 75.02; H, 4.01; N, 13.58.

4.1.2.7. 3'-Imino-6'-methyl-5'-nicotinoyl-2',3',3a',4'-tetrahydrosipro[indoline-3,7'-pyrazolo[4,3-b]pyridin]-2-one (10). Isolated as a yellow solid, yield: 90%, mp 198–200°C (recrystallization from EtOH); 1H-NMR (300 MHz, DMSO-d6) 2.69 (s, 3H, CH3), 3.65 (s,1H, NH), 5.83 (d, H, CH), 6.76 (s, 2H, NH2), 7.41–7.52 (m, 8H, ArH), 10.37 (s, 1H, NHsia); 13C NMR (75 MHz, DMSO-d6) 12.4, 47.1, 58.9, 106.7, 108.6, 111.3, 121.6, 123.4, 123.6, 127.4, 129.6, 136.6, 137.1, 137.9, 141.8, 145.1, 155.1, 168.8, 193.8. Anal. Calcd for C20H15N6O2 (372) m/z: 373.12 (100.0%), 374.12 (21.6%), 375.12 (2.2%), 374.11 (1.8%). Elemental Anal: %C, 64.51; H, 4.05; N, 22.57; Found: C, 64.15; H, 3.84; N, 22.37.

4.1.2.8. 3'-Imino-6'-dimethyl-5'-nicotinoyl-2',3',3a',4'-tetrahydrosipro[indoline-3,7'-pyrazolo[4,3-b]pyridin]-2-one (11). Isolated as a yellow solid, yield: 82%, mp 222–224°C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d6) 2.63 (s, 3H, CH3), 2.85 (s, 3H, CH3), 5.23 (d, 1H, CH), 6.52 (s,1H, NH),7.41–7.52 (m, 8H, ArH), 9.69 (s,1H, NH), 10.11 (s,1H, NH); 13C NMR (75 MHz, DMSO-d6) 11.4, 21.1, 47.1, 58.4, 113.7, 117.6, 119.3, 121.6, 123.4, 125.6, 127.4, 129.6, 136.6, 137.1, 137.9, 141.8, 145.1, 155.1, 179.8, 193.8. Anal. Calcd for C20H17N6O2 Exact Mass: (386) m/z: 386.13 (100.0%), 387.14 (22.7%), 389.14 (2.5%), 388.13 (1.8%). Elemental Anal: %C, 65.27; H, 4.42; N, 21.75; Found: C, 64.89; H, 4.27; N, 21.52.

4.1.2.9. 3'-Imino-6'-methyl-5'-nicotinoyl-2'-phenyl-2',3',3a',4'-tetrahydrosipro[indoline-3,7'-pyrazolo[4,3-b]pyridin]-2-one (12). Isolated as a yellow solid, yield: 80%, mp 248–250°C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d6) 2.69 (s, 3H, CH3), 5.35 (d, 1H, CH), 5.97 (s,1H, NH), 7.22–7.75 (m, 13H, ArH), 9.43 (s,1H, NH),10.37 (s, 1H, NH); 13C NMR (75 MHz, DMSO-d6) 11.4, 46.1, 58.5, 111.2, 118.3, 120.3, 121.6, 123.4, 127.6, 128.4, 129.6, 134.3, 135.6, 137.9, 139.3, 141.8, 145.1, 155.1, 167.8, 196.2. Anal. Calcd for C26H18N2O2 (448) m/z: 448.15 (100.0%), 449.15 (28.1%), 450.16 (3.8%), 450.15 (1.8%). Elemental Anal: %C, 69.63; H, 4.26; N, 18.74; Found: C, 69.20; H, 4.00; N, 18.48.

4.1.2.10. 3'-Imino-7'-thioxo-3',3a',4',6',7',8'-hexahydrosipro[indoline-3,9'-pyrazolo[3',4':5,6]pyrido[3,2-d]pyrimidine]-2,5'(2'H)-dione (13). Isolated as a yellow solid, yield: 82%, mp 126–128°C (recrystallization from EtOH); 1H-NMR (300 MHz, DMSO-d6) 4.98 (d, 1H, CH), 6.77 (s,1H, NH),7.41–7.52 (m, 4H, ArH), 9.33 (bs,2H, 2NH), 10.28 (bs,2H, 2NH), 12.25 (s,1H, 1NH); 13C NMR (75 MHz, DMSO-d6) 57.1, 58.6, 111.7, 117.6, 121.6, 123.4, 126.6, 127.4, 129.6, 137.1, 137.9, 141.8, 155.1, 165.1, 177.8. Anal. Calcd for C13H10N7O2S (353) m/z: 353.05 (100.0%), 355.06 (16.2%), 356.05 (4.5%), 355.05 (2.2%), 356.06 (1.2%). Elemental Anal: %C, 50.99; H, 2.84; N, 27.75; S, 9.07; Found: C, 50.58; H, 2.62; N, 27.49; S 8.83.

4.1.2.11. 3'-Imino-2'-methyl-7'-thioxo-3',3a',4',6',7',8'-hexahydrosipro[indoline-3,9'-pyrazolo[3',4':5,6]pyrido[3,2-d]pyrimidine]-2,5'(2'H)-dione (14). Isolated as a yellow solid, yield: 88%,
4.1.2.12. 3'-Imino-2'-phenyl-7'-thioxo-3',3a',4',6',7',8'-hexahydrospiro[indole-3',9'-pyrazolo-lo[3',4';5,6']pyrido[3,2-d]pyrimidine]-2',5'(2'H)-dione (15). Isolated as a yellow solid, yield: 88%, mp 192–194°C (recrystallization from EtOH); 1H-NMR (300 MHz, DMSO-d$_6$) 2.69 (s, 3H, CH$_3$), 5.90 (d, 1H, CH), 6.87 (s,1H, NH), 7.77–7.95 (m, 9H, ArH), 10.64 (bs, 2H, 2NH); 13C NMR (75 MHz, DMSO-d$_6$) 11.6, 47.6, 58.7, 111.2, 118.1, 118.5, 121.6, 123.4, 125.6, 127.4, 129.6, 132.6, 134.1, 137.9, 141.8, 145.1, 156.1, 166.8, 195.8. Anal. Calcd for C$_{21}$H$_{14}$N$_7$O$_2$S (429) m/z: 372.12 (100.0%), 373.13 (22.7%), 374.13 (2.5%), 373.12 (1.5%). Elemental Anal.: %C, 67.73; H, 4.33; N, 15.05; Found: C, 67.55; H, 4.17; N, 14.78.

4.1.2.13. 5'-Benzoyl-6'-methyl-3'a,4'-dihydrospiro[indole-3,7'-pyrazolo[4,3-b]pyridine]-2,3'(2'H)-dione (16). Isolated as a yellow solid, yield: 88%, mp 216–218°C (recrystallization from EtOH); 1H-NMR (300 MHz, DMSO-d$_6$) 2.69 (s, 3H, CH$_3$), 5.90 (d, 1H, CH), 6.87 (s,1H, NH), 7.77–7.95 (m, 9H, ArH), 10.64 (bs, 2H, 2NH); 13C NMR (75 MHz, DMSO-d$_6$) 11.6, 47.6, 58.7, 111.2, 118.1, 118.5, 121.6, 123.4, 125.6, 127.4, 129.6, 132.6, 134.1, 137.9, 141.8, 145.1, 156.1, 166.8, 195.8. Anal. Calcd for C$_{21}$H$_{14}$N$_7$O$_2$S (429) m/z: 372.12 (100.0%), 373.13 (22.7%), 374.13 (2.5%), 373.12 (1.5%). Elemental Anal.: %C, 67.73; H, 4.33; N, 15.05; Found: C, 67.55; H, 4.17; N, 14.78.

4.1.2.14. 5'-Benzoyl-2',6'-dimethyl-3'a,4'-dihydrospiro[indole-3,7'-pyrazolo[4,3-b]pyridine]-2,3'(2'H)-dione (17). Isolated as a yellow solid, yield: 85%, mp 236–238°C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d$_6$) 2.69 (s, 3H, CH$_3$), 3.14 (s, 3H, CH$_3$), 5.90 (d, 1H, CH), 6.87 (s,1H, NH), 7.77–7.95 (m, 9H, ArH), 10.61 (s, 1H, NH); 13C NMR (75 MHz, DMSO-d$_6$) 10.2, 27.7, 57.1, 58.6, 107.7, 118.6, 123.3, 125.6, 126.4, 128.4, 129.6, 135.6, 136.1, 137.9, 141.8, 145.1, 155.1, 167.8, 193.8. Anal. Calcd for C$_{22}$H$_{16}$N$_4$O$_3$ (372) m/z: 386.14 (100.0%), 387.14 (23.8%), 388.14 (2.7%), 387.13 (1.5%). Elemental Anal.: % C, 68.38; H, 4.70; N, 14.50; Found: C, 68.05; H, 4.47; N, 14.28.

4.1.2.15. 5'-Benzoyl-6'-methyl-2'-phenyl-3'a,4'-dihydrospiro[indole-3,7'-pyrazolo-[4,3-b]pyri dine]-2,3'(2'H)-dione (18). Isolated as a yellow solid, yield: 88%, mp 202–204°C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d$_6$) 2.69 (s, 3H, CH$_3$), 5.90 (d, 1H, CH), 6.54 (s,1H, CH), 7.77–7.95 (m, 14H, ArH), 10.36 (s, 1H, NH); 13C NMR (75 MHz, DMSO-d$_6$) 11.4, 57.1, 57.9, 113.7, 118.6, 119.3, 121.6, 123.4, 123.6, 127.4, 129.6, 136.6, 137.1, 137.9, 141.8, 145.1, 155.1, 168.8, 193.8. Anal. Calcd for C$_{22}$H$_{20}$N$_4$O$_3$ (448) m/z: 448.15 (100.0%), 449.16 (29.2%), 450.16 (2.7%), 449.15 (1.5%), 450.16 (1.4%) Elemental Anal: %C, 72.31; H, 4.50; N, 12.49; Found: C, 72.02; H, 4.31; N, 12.24.

4.1.2.16. 5'-Benzoyl-6'-phenyl-3'a,4'-dihydrospiro[indole-3,7'-pyrazolo-[4,3-b]pyridine]-2,3'(2'H)-dione (19). Isolated as a yellow solid, yield: 82%, mp 258–260°C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d$_6$) 5.49 (d, 1H, CH), 6.77 (s,1H, NH), 7.41–7.52 (m, 14H, ArH), 10.30 (bs, 2H, 2NH); 13C NMR (75 MHz, DMSO-d$_6$) 51.7, 58.9, 111.3, 118.2, 119.6, 121.6, 123.4, 123.6, 127.4, 129.6, 136.6, 137.1, 137.9, 141.8, 145.1, 155.1, 166.2, 169.8, 192.9. Anal. Calcd for C$_{26}$H$_{14}$N$_4$O$_3$ (434) m/z: 434.14 (100.0%), 435.14 (28.1%), 436.14 (2.7%), 435.13 (1.5%), 436.14 (1.1%) Elemental Anal: C, 71.88; H, 4.18; N, 12.90; Found: C, 71.59; H, 3.87; N, 12.68.
4.1.2.17. 5′-5′-Benzoyl-2′-methyl-6′-phenyl-3′a,4′-dihydrospiro[indoline-3,7′-pyrazolo-[4,3-b]pyridine]-2,3′(2′H)-dione (20). Isolated as a yellow solid, yield: 74%, mp 232–234 °C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d6) 2.99 (s, 3H, CH3), 5.27 (d, 1H, CH), 5.97 (s,1H, NH),7.31–7.82 (m, 14H, ArH), 10.27 (s, 1H, NH); 13C NMR (75 MHz, DMSO-d6) 27.7, 56.1, 58.8, 110.7, 116.6, 118.3, 121.6, 123.4, 125.6, 127.4, 129.6, 134.6, 135.1, 137.9, 141.8, 145.1, 155.1, 166.8, 196.8. Anal. Calcd for C27H20N4O3 (448) m/z: 448.15 (100.0%), 449.16 (29.2%), 450.16 (2.7%), 449.15 (1.5%), 450.16 (1.4%). Elemental Anal: %C, 72.31; H, 4.50; N, 12.49; Found: C, 72.05; H, 4.27; N, 12.28.

4.1.2.18. 5′-Benzoyl-2′,6′-diphenyl-3′a,4′-dihydrospiro[indoline-3,7′-pyrazolo-[4,3-b]pyridine]-2,3′(2′H)-dione (21). Isolated as a yellow solid, yield: 80%, mp 240–242 °C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d6) 5.86 (d, 1H, CH), 6.52 (s,1H, NH), 7.12–7.64 (m, 14H, ArH), 10.43 (s,1H, NH); 13C NMR (75 MHz, DMSO-d6) 56.1, 58.9, 111.7, 118.6, 119.3, 121.6, 123.4, 125.6, 127.4, 128.4, 129.6, 134.6, 136.1, 137.9, 141.8, 145.1, 155.1, 166.4, 193.8. Anal. Calcd for C32H22N4O3 (510) m/z: 510.17 (100.0%), 511.17 (34.6%), 512.18 (5.8%), 511.17 (1.5%). Elemental Anal: %C, 75.28; H, 4.34; N, 10.97; Found: C, 75.02; H, 4.01; N, 10.64.

4.1.2.19. 6′-Methyl-5′-nicotinoyl-3′a,4′-dihydrospiro[indoline-3,7′-pyrazolo-[4,3-b]pyridine]-2,3′(2′H)-dione (22). Isolated as a yellow solid, yield: 78%, mp 250–252 °C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d6) 2.96 (s, 3H, CH3), 5.83 (d, 1H, CH), 5.77 (s,1H, NH),7.41–7.52 (m, 8H, ArH), 9.97 (s,1H, NH), 10.37 (s, 1H, NH); 13C NMR (75 MHz, DMSO-d6) 16.4, 57.1, 58.9, 109.7, 112.6, 118.3, 121.6, 123.4, 126.6, 127.4, 129.6, 135.6, 136.6, 137.9, 141.8, 145.1, 155.1, 165.8, 191.9. Anal. Calcd for C20H15N5O3 (373) m/z: 373.12 (100.0%), 374.12 (21.6%), 375.12 (2.2%), 374.11 (1.8%). Elemental Anal: %C, 64.34; H, 4.05; N, 18.76; Found: C, 64.05; H, 3.84; N, 18.49.

4.1.2.20. 2′,6′-Dimethyl-5′-nicotinoyl-3′a,4′-dihydrospiro[indoline-3,7′-pyrazolo-[4,3-b]pyridine]-2,3′(2′H)-dione (23). Isolated as a yellow solid, yield: 80%, mp 236–238 °C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d6) 2.74 (s, 3H, CH3), 2.98 (s, 3H, CH3), 5.50 (d, 1H, CH), 5.87 (s,1H, NH),7.30–7.72 (m, 8H, ArH), 10.24 (s, 1H, NH); 13C NMR (75 MHz, DMSO-d6) 16.4, 25.1, 57.5, 58.6, 108.7, 115.6, 119.3, 121.6, 123.4, 126.6, 127.4, 129.6, 135.6, 136.1, 137.9, 141.8, 145.1, 155.1, 168.8, 194.8. Anal. Calcd for C21H17N5O3 Exact Mass: (387) m/z: 387.13 (100.0%), 388.14 (22.7%), 389.14 (2.5%), 388.13 (1.8%). Elemental Anal: %C, 65.11; H, 4.42; N, 18.08; Found: C, 64.89; H, 4.27; N, 17.83.

4.1.2.21. 6′-Methyl-5′-nicotinoyl-2′-phenyl-3′a,4′-dihydrospiro[indoline-3,7′-pyrazolo-[4,3-b]pyridine]-2,3′(2′H)-dione (24). Isolated as a yellow solid, yield: 78%, mp 222–224 °C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d6) 2.58 (s, 3H, CH3), 5.59 (d, 1H, CH), 6.11 (s,1H, NH), 7.23–7.67 (m, 8H, ArH), 10.28 (s, 1H, NH); 13C NMR (75 MHz, DMSO-d6) 15.4, 55.1, 57.9, 111.7, 118.6, 119.3, 121.6, 123.4, 124.6, 127.4, 129.6, 134.6, 137.1, 137.9, 141.8, 145.1, 155.1, 169.8, 195.4. Anal. Calcd for C23H19N3O3 (449) m/z: 449.15 (100.0%), 450.15 (28.1%), 451.16 (3.8%), 450.15 (1.8%). Elemental Anal: %C, 69.48; H, 4.26; N, 15.58; Found: C, 69.20; H, 4.00; N, 15.24.

4.1.2.22. 7′-Thioxo-2′,3′a,4′,6′,7′,8′-hexahydrospiro[indoline-3,9′-pyrazolo-[3′,4′:5,6]-pyrido[3,2-d]pyrimidine]-2,3′,5′-trione (25). Isolated as a yellow solid, yield: 70%, mp 136–138 °C (recrystallization from EtOH/DMF mixture); 1H-NMR (300 MHz, DMSO-d6) 5.69 (d, 1H, CH), 5.77 (s,1H, NH),7.41–7.52 (m, 4H, ArH), 9.60 (bs, 2H, 2NH), 10.56 (s, 1H, 1NH), 12.75 (s,1H, NH); 13C NMR (75 MHz, DMSO-d6) 57.1, 58.9, 121.6, 123.4, 123.6, 127.4, 129.6, 131.6, 134.1, 137.9, 141.8, 145.1, 165.1, 175.8. Anal. Calcd for C31H10N3O3S (354) m/z: 354.05 (100.0%), 355.06 (16.2%),
356.05 (4.5%), 355.05 (2.2%), 356.06 (1.2%). Elemental Anal: %C, 50.84; H, 2.84; N, 23.72; S, 9.05; Found: C, 50.58; H, 2.62; N, 23.49; S, 8.83.

4.1.2.23. \textit{2'-Methyl-7'-thioxo-2',3a',4',6',7',8'-hexahydrospiro[indoline-3,9'-pyrazolo[3',4':5,6]pyrido[3,2-d]pyrimidine]-2,3',5'-trione} (26). Isolated as a yellow solid, yield: 88%, mp 202–204°C (recrystallization from EtOH/DMF mixture); $^1$H-NMR (300 MHz, DMSO-$d_6$) 2.87 (s, 3H, CH$_3$), 5.53 (d, 1H, CH), 5.54 (s, 1H, NH), 7.37–7.49 (m, 4H, ArH), 8.83 (bs, 2H, 2NH), 12.77 (s, 1H, NH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) 23.3, 57.3, 58.5, 122.6, 123.4, 125.4, 125.6, 127.4, 129.6, 133.6, 135.1, 137.9, 141.8, 145.1, 166.6, 176.1. Anal. Calcd for C$_{16}$H$_{12}$N$_6$O$_3$S (368) m/z: 368.07 (100.0%), 369.07 (17.3%), 370.06 (4.5%), 369.07 (2.2%), 370.08 (1.4%). Elemental Anal: %C, 52.17; H, 3.28; N, 22.81; Found: C, 51.92; H, 3.02; N, 22.63; S, 8.46.

4.1.2.24. \textit{2'-Phenyl-7'-thioxo-2',3a',4',6',7',8'-hexahydrospiro[indoline-3,9'-pyrazolo[3',4':5,6]pyrido[3,2-d]pyrimidine]-2,3',5'-trione} (27). Isolated as a yellow solid, yield: 78%, mp 226–228°C (recrystallization from EtOH/DMF mixture); $^1$H-NMR (300 MHz, DMSO-$d_6$) 5.70 (d, 1H, CH), 6.17 (s, 1H, NH), 7.31–7.79 (m, 9H, ArH), 8.57 (s, 2H, 2NH), 12.54 (s, 1H, NH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) 57.1, 58.9, 121.7, 124.6, 125.3, 126.6, 127.4, 128.4, 129.6, 134.6, 135.1, 137.9, 141.8, 145.1, 165.1, 175.8. Anal. Calcd for C$_{21}$H$_{14}$N$_6$O$_3$S (430) m/z: 430.08 (100.0%), 431.09 (22.7%), 432.08 (4.5%), 432.09 (2.5%), 431.08 (2.2%), 433.08 (1.0%). Elemental Anal: %C, 58.60; H, 3.28; N, 19.52; S, 7.45; Found: C, 58.34; H, 3.00; N, 19.24; S, 7.21.

4.2. Biological assay

4.2.1. Evaluation of the insecticidal activity

A topical application bioassay procedure is used in this section.$^{42}$ Fourth-instar larvae of a susceptible strain of cotton leafworm, \textit{Spodoptera littoralis}, at an average weight of 38–40 mg/larva were selected from a laboratory colony reared on an artificial diet under controlled conditions at 25 ± 2°C, 70 ± 5% RH, and 16 h light photoperiod. An appropriate amount of each tested compound and standard insecticide was dissolved in DMSO as a solvent to prepare a series of concentrations of 1000, 500, 250, 125, and 65 l/g/larva. Half a L of each tested solution was applied to the larval second thorax using a Micro-applicator fitted with a 1-mL glass syringe having a curved, blunt 20-gauge hypodermic needle. A solvent solution only was applied topically as a control. For each compound five serial concentrations of tested compounds and standard insecticide were carried out with three replicates, using 10 larvae in each replicate. Larvae were fed by clean and untreated castor leaves. All treatments were incubated at 26 ± 2°C, and 65 ± 5% RH until the recording of the results. The mortality data were recorded 24 h after treatment. The larva was considered dead if no movement was detected when it was touched with a small brush. The LC$_{50}$, slope of toxicity line, toxicity indexes, were determined by a computerized analysis program (LDP Line program). Data obtained are illustrated in Table 1.

4.2.2. Evaluation of the fungicidal activity

The food poison technique is applied to evaluate the inhibition potentiality of the investigated compounds to the mycelial linear growth of the tested fungi.$^{43}$ Potato-dextrose agar (PDA) was used as food in the molten stage. Fifty milliliters of the aforementioned medium were poured into 150 mL conical flasks and autoclaved at 121°C for 20 min. Three drops of 25% lactic acid were added to prevent bacterial contamination. Dilutions for each of the tested compounds were carried out (v/v) by dissolving appropriate amounts of each compound in 10 mL DMSO. Equal volumes of DMSO containing diluted compounds were added to sterile molten (40°C) PDA to get a series of concentrations of 500, 400, 300, 200, and 100 ppm for each compound in PDA. A
zero (0) concentration treatment was prepared for each fungus, which contains an equivalent volume of solvent only, and used as control. Compounds-amended PDA was dispensed aseptically into 9 cm diameter Petri dishes. Plugs of mycelium (4 mm diameter) were cut from the margins of actively growing cultures of the F. Solani and R. Solani fungi and placed in the center of compound-amended and unamended PDA plates with 3 replicate plates for each fungus. All plates were incubated at 25 ± 1 °C. Colony diameter (in millimeters) was measured after complete growth of the control (5–7 days). Percentages of mycelial growth inhibition were calculated from the formula: Mycelial growth inhibition = [(DC – DT)/DC] × 100.44 DC and DT are average diameters of the fungal colony of control and treatment, respectively. The estimated effective concentration (EC50) values, which give 50% inhibition of fungi radial growth, toxicity index (T.I), and slopes of toxicity lines for each compound under investigation were calculated by using the LDP line program and tabulated in Tables 2 and 3.

4.2.3. Computational methods
DFT studies were carried out for the synthesized compounds using Materials Studio 6.0 (MS 6.0) software from Accelrys, Inc. DMol3 module was used to perform the DFT calculations using Perdew and Wang LDA exchange-correlation functional and DND basis set. The calculated parameters involved the electron density, dipole moment and Frontier molecular orbitals, and the molecular surface area. Frontier molecular orbitals include the highest occupied molecular orbitals (HOMOs) and the lowest unoccupied molecular orbitals (LUMOs).45

Disclosure statement
No potential conflict of interest was reported by the authors.

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