Pharmacological and insect antifeedant activities of some 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides

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ABSTRACT

A series containing twelve titled compounds were synthesized by solvent-free method and their purities were examined by literature procedure. These compounds were subjected to study the pharmacological effects such as antibacterial, antifungal and antioxidant activities and the insect antifeedant activities using Bauer-Kirby disc diffusion method with their bacterial and fungal strains, DPPH radical scavenging and leaf disc bio-assay method with 4th instar larvae Achoea janata L.

Keywords: Halo aryl 4,5-dihydro-1H-pyrazole-1-carbothioamides; Antibacterial activity, Antifungal activity, Antioxidant activity, insect antifeedant activity

1. INTRODUCTION

Generally, pyrazoline and their derivatives possess important biological activities due to the presence of C=N, N-N and other polar functional groups attached the pyrazoline moiety [1-2]. The important biological activities of pyrazolines are anti-bacterial [3], antifungal [4], anti-depressants [5], anticonvulsant [6], anti-inflammatory [7], anti-tumour [8], anaesthetic [9], analgesic [10], anti-cancer [11] MAO-B inhibitors [12], steroidal, nitric oxide synthase inhibitor, anti-viral and cannabinoid CBI receptor antagonists [7]. Compounds containing unsaturation and halogen atoms are possess insect antifeedant activities [13]. The compounds possess hydroxy and methoxy groups have shown antioxidant activities [14]. Numerous solvent assisted or solvent-free methods with or without catalysts are available in the literature for synthesis of these pyrazoline derivatives such as, Lewis acids, bases and their salts [11,15,16], CH3COOH/ CH3COONa [1], NaOH/ EtOH [15,17,18], KOH/EtOH [19, 20], neat reaction in ethanol [1,12] and basic alumina/K2CO3 [21,22], Fly-ash:H2SO4 [23], SiO2-H3PO4 [24], Preheated fly-ash [25], fly-ash:PTS [26], Fly-ash:H2O [27] and SoCl2/Ether [28]. The starting materials for the reactions are carbonyl compounds, chalcones with hydrazine derivatives. Many pyrazoline derivatives are visible such as 4,5-dihydro-1H-pyrazoles, pyrazoline carbothioamides, pyrazoline carboxamides and N-acetyl pyrazoline derivatives.

In these derivatives, the formation of pyrazoline ring was confirmed by infrared and NMR spectra. From infrared spectra the cyclic C=N and N-N are the characteristics stretches (v, cm⁻¹) [29]. The protons of the pyrazoline rings are existed in the trans- positions gave
doublet of doublets and this is confirmed by \(^1\)H NMR spectra. The Hammett spectral correlation study was reported in the literature [30].

The infrared stretches, \(^1\)H and \(^{13}\)C chemical shifts data of the pyrazolines were correlated with Hammett equation using substituent constants, F and R parameters applying single and multiple correlation equations [31]. From the results of statistical analysis, the effects of substituents on the spectral frequencies were studied. Ranganathan et al have studied the synthesis, spectral correlations and antimicrobial activities of some 3-(5-chlorothiophen-2-yl)-4,5-dihydro-5-(substituted phenyl)-1\(\text{H}\)pyrazoline derivatives [28].

Thirunarayanan and Sekar have studied the solvent-free synthesis and spectral correlations of some 1-acetyl pyrazolines [32]. Within the view there is no report available for the study of pharmacological and insect antifeedant activities of some pyrazoline carbothioamides in past in the literature. Therefore the author have taken efforts for studying the pharmacological effects such as antibacterial, antifungal and antioxidant activities and the insect antifeedant activities using Bauer-Kirby [33] disc diffusion method with their bacterial and fungal strains, DPPH[34] radical scavenging and leaf disc bio-assay method[35] with 4\(^{th}\) instar larvae Achoea janata L.

2. EXPERIMENTAL

2.1. Synthesis of pyrazoline thiocarbamides

The titled pyrazoline thiocarbamides were synthesized and their purities were examines by literature method [36]. The general structure of the pyrazoline thiocarbamides was shown in Fig. 1.

![Fig. 1. The general structure of 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1\(\text{H}\)-pyrazole-1-carbothioamides.](image-url)
2. 2. Antimicrobial activity

The antimicrobial activities of the prepared 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides were evaluated by measuring the zone of inhibition of the compounds against the indicated bacterial and fungal strains. Two Gram-positive pathogenic strains (Staphylococcus aureus, Enterococcus faecalis) and four Gram-negative strains (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus vulgaris) were chosen.

The disc diffusion technique followed the Bauer-Kirby [33] method, at a concentration of 250 µg/mL with ampicillin and streptomycin used as the standard drugs. For the study of antifungal activities of all pyrazoline thiocarboamides with Candida albicans the disc diffusion technique was followed, while the two other strains (Penicillium sp. and Aspergillus niger), the dilution method [33] was used. The drug dilution was 50 µg/mL. Griseofulvin was used as the standard drug.

2. 2. 1. Measurement of antibacterial sensitivity

The antibacterial sensitivity assay was performed using the Bauer-Kirby [33] disc diffusion technique. In each Petri plate about 0.5 mL of the bacterial test sample was spread uniformly over solidified Mueller-Hinton agar using a sterile glass spreader.

Then 5 mm discs made from Whatman No. 1 filter paper were saturated with the potential inhibitor solution and placed on the medium using sterile forceps. The plates were incubated for 24 h at 37 °C upside down to prevent the collection of water droplets over the medium.

After 24 h, the plates were examined and the diameter values of the zone of inhibition were measured. Triplicate results were recorded.

2. 2. 2. Measurement of antifungal sensitivity

Antifungal sensitivity was determined by using the Bauer-Kirby [33] disc diffusion technique. The PDA medium was prepared and sterilized as above and added to the Petri plate containing 1 mL of the fungal species. The plate was rotated clockwise and counter clockwise for uniform spreading. The discs were impregnated with the test solution, prepared by dissolving 15 mg of the 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides in 1 mL of DMSO solvent.

The medium was allowed to solidify and incubate for 24 h. The plates were examined and the diameter of the zone of inhibition was measured. Triplicate results were recorded.

2. 3. Antioxidant activity

The antioxidant activities of the synthesized 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides were evaluated by the DPPH radical scavenging technique [34]. Acetate (0.1 mol/L) was prepared by dissolving 1.64 g of sodium acetate in 15 mL of water and 150 µL of acetic acid. The final volume was adjusted to 20 mL by adding water. DPPH solution (0.2 mmol) was prepared by dissolving 3.9 g of DPPH in 50 mL of ethanol, and α-tocopherol solution was prepared by adding 1 mg to 10 mL of ethanol. A series of test tubes was arranged with 1.0 mL of buffer solution mixed with 0.5 mL of DPPH solution. A series of concentrations of synthesized 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides with α-tocopherol (1 µg in 1 mL of ethanol) was added to each tube and mixed.
After 30 min at room temperature the absorbance of each solution was measured by UV spectrophotometry at 517 nm. A mixture of buffer solution and ethanol was used as the reference for the spectrophotometer. A graph was plotted with the weight of the 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides versus absorption and IC\textsubscript{50} values were determined. The antioxidant activity was expressed in terms of IC\textsubscript{50} (µg/mL, concentration required to inhibit DPPH radical formation by 50 %). α-Tocopherol was used as a positive control. The radical scavenging activity was calculated as DPPH radical scavenging activity (% of inhibition) =

\[
\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100
\]

2. 4. Measurement of Insect antifeedant activity

Generally organic compounds which are having carbonyl, unsaturation and halogen substitutions, they possess insect antifeedant activity. Therefore, the author wishes to examine the insect antifeedant activity of these 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides and found to be active as insect antifeedants.

This test was performed with a 4\textsuperscript{th} instar larva Achoea janata L against castor semilooper, were reared as described on the leaves of castor, Ricinus communis in the laboratory at the temperature range of 26 °C ±1 °C and a relative humidity of 75-85 %.

The leaf – disc bioassay method was used against the 4\textsuperscript{th} instar larvae to measure the antifeedant activity [36]. The 4\textsuperscript{th} instar larvae were selected for testing because the larvae at this stage feed very voraciously.

Castor leaf discs of a diameter of 1.85 cm were punched and intact with the petioles. The synthesized aryl 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides were dissolved in acetone at a concentration of 200 ppm dipped for 5 minutes. The leaf discs were air-dried and placed in one litre beaker containing little water in order to facilitate translocation of water. Therefore, the leaf discs remain fresh throughout the duration of the rest, 4\textsuperscript{th} instar larvae of the test insect, which had been preserved on the leaf discs of all 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides and allowed to feed on them for 24 h. The area of the leaf disc consumed were measured by Dethler’s method [37]. The observed antifeedant activity of 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides was presented in Tables 2 and 3.

3. RESULTS AND DISCUSSION

3. 1. Antibacterial sensitivity assay

The disc-diffusion technique was followed using the Bauer-Kirby [33] method, at a concentration of 250 µg/mL, with ampicillin and streptomycin used as the standard drugs. The measured antibacterial activities of all 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides and allowed to feed on them for 24 h. The area of the leaf disc consumed were measured by Dethler’s method [37]. The observed antifeedant activity of 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides is presented in Table 1. Compounds 2 and 3 showed the maximum zone of inhibition against Escherichia coli, at 20-24 mm, compared to other pyrazoline thiocarbamides such as 5, 6, 9 and 10.
These latter compounds are moderately active, with 13-19 mm zones of inhibition. Carbothioamides 7, 8, 11 and 12 were active with an 8-12 mm of zone of inhibition.

The compounds 1 and 4 were inactive. The pyrazoline thiocarbamides 2, 3 and 6 were found to be effective against *S. aureus* strain with 20-24 mm of zones of inhibition. Compounds 5 and 10 are moderately active with 13-19 mm of zones of inhibition. The thioamides 4, 11 and 12 were moderately active with an 8-12 mm zone of inhibition. Compounds 1, 7, 8 and 9 were inactive against *S. aureus*.

The pyrazoline thiocarboamide derivatives 3 and 5 were shown to be more active against *Pseudomonas*, with greater than a 20 mm zone of inhibition, while the other derivatives showed zones of inhibition between 12-19 mm. Compound 4 and 6 are inactive against the *Pseudomonas aeruginosa* strain. Pyrazoline thiocarbamides 2, 3, 6 and 9 were more effective against the *Klebsiella pneumoniae* strain with 20-24 mm zones of inhibition, while the thioamides 5 and 10 showed moderate activity with a 13-19 mm zone of inhibition. The parent compound 1, 11 and 12 were active with an 8–12 mm zone of inhibition. Compounds 4, 7 and 8 were inactive against the *P. aeruginosa* species.

The 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides 5 and 9 were active when they were screened with *Phaseolus vulgaris* with 20-24 mm zones of inhibition and compounds 1-4 and 10 were moderately active with 13-19 mm zones of inhibition. Pyrazoline thiocarboamides 6 and 8 were ineffective against the *P. vulgaris* strain.

The 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides 3 and 6 showed greater activity against *Enterococcus faecalis*, with 20-24 mm zones of inhibition. Compounds 1, 4, 5, 7 and 10 were moderately active with 13-19 mm zones of inhibition. The pyrazoline carbothioamides derivatives 2, 8, 11 and 12 were active with 8-12 mm zones of inhibition. The compound 9 was inactive when it was screened against *E. faecalis*.

3. 2. Antifungal sensitivity assay

The observed antifungal activities of all prepared 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides are presented in Table 1. The study of antifungal activities of all 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides against *Candida albicans* showed that compounds 3, 9 and 10 are most effective, with 20 mm zones of inhibition at 250 µg/mL per disc, while the pyrazoline thiocarboamides 2, 5, 6, 11 and 12 are moderately active with 1319 mm zones of inhibition and compound 1 and 7 was active with an 8-12 mm zone of inhibition. The compounds 4 and 8 are inactive against *C. albicans*.

Thiocarboamide derivatives 4, 9 and 10 are more effective against *Penicillium* species relative to compounds 1, 2 and 7. The pyrazoline thiocarboamides 5, 6 and 8 were inactive against the *Penicillium sp*. fungal strain.

The zone of inhibition of pyrazoline thiocarboamides 3, 6 and 10 were most effective against *Aspergillus niger* relative to compounds 1, 2, 4, 8 and 9. The compounds 4, 11 and 12 shows two fungal colonies and compound 7 showed little to no effectiveness with any fungal strain. The presence of a chloro, methoxy and nitro substituents appear to be responsible for the antimicrobial activities of pyrazoline thiocarboamides.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
<th>Antioxidant activity (DPPH radical scavenging)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(E. \text{ coli})</td>
<td>(S. \text{ aures})</td>
<td>(P. \text{ aeruginosa})</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>++</td>
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<td>++</td>
<td>++</td>
</tr>
<tr>
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</tr>
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<td>–</td>
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</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>12</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>

Table 1. Antibacterial, antifungal and antioxidant activities of 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides.
3. 3. Antioxidant activity

The antifungal activities of the 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides were measured using the DPPH radical scavenging [34] method. The observed antioxidant activities of pyrazoline thiocarboamides are presented in Table 1. From the Table 1, the hydroxy- and methoxy-substituted carbothioamide derivatives (compounds 11, 4, 12 and 8) showed significant antioxidant activity. The other compounds including the parent showed lesser antioxidant activity.

3. 4. Insect antifeedant activity

The observed antifeedant activity of 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides was presented in Table 2, and the Table 2 reveals that compound 3 3-(2,4-dichloro-5-fluorophenyl)-5-(4-chloro phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides was found to reflect satisfactory antifeedant. This test is performed with the insects which ate only two-leaf disc soaked under the solution of this compound. Compound 2 showed enough antifeedant activity but lesser than 3. Further compound 3 was subjected to measure the antifeedant activity at different 50, 100, 150 ppm concentrations and the observation reveals that as the concentrations decreased, the activity also decreased. It is observed from the results in Table 3 and that the compound 3 3-(2,4-dichloro-5-fluorophenyl)-5-(4-chloro phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides showed an appreciable antifeedant activity at 150 ppm concentration.

Table 2. The insect antifeedant activities of the 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides.

<table>
<thead>
<tr>
<th>Entry</th>
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<th>6-8 pm</th>
<th>8-10 pm</th>
<th>10-12 pm</th>
<th>12-6 am</th>
<th>6-8 am</th>
<th>8 am – 12Nn</th>
<th>12 Nn – 4pm</th>
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</tr>
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</tr>
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</tr>
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</tr>
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</table>

Number of leaf discs consumed by the insect (Values are mean + SE of five).
Table 3. Antifeedant activity of compound 3 [3-(2,4-dichloro-5-fluorophenyl)-5-(4-chloro phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide] showed an appreciable antifeedant activity at 3 different concentrations.

<table>
<thead>
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<th>ppm</th>
<th>4-6 pm</th>
<th>6-8 pm</th>
<th>8-10 pm</th>
<th>10-12 pm</th>
<th>12-6 am</th>
<th>6-8 am</th>
<th>8 am – 12 Nn</th>
<th>12 Nn – 4 pm</th>
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<td>0</td>
<td>0</td>
<td>0.5</td>
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</tbody>
</table>

Number of leaf discs consumed by the insect (Values are mean + SE of five).

4. CONCLUSIONS

A series of aryl 3-(2,4-dichloro-5-fluorophenyl)-5-(substituted phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamides have been synthesized by solvent-free synthetic method and their purities were examined from the data published earlier in literature. The antimicrobial activities of the pyrazoline thiocarboamides have been evaluated using Bauer-Kirby methods. The compounds 2, 3, 5, 6 and 9 show significant antibacterial activity against bacterial strains with 20-24 mm of zones of inhibition. Compounds 1, 4, 5, 7, 9 and 10 active against 13-10 mm of zone of inhibition against the bacterial strains.

The pyrazoline derivatives 1, 2, 4, 7, 8, 11 and 12 active against the bacterial strains with 20-24 mm zones of inhibition. The pyrazoline thiocarboamides derivatives 3, 4, 6, 9 and 10 more active against fungal strains with 20-24 mm of zone of inhibition. The compounds 1, 2, 4-9, 11 and 12 were shows one fungal colonies against fungal strains. The thiocarboamides derivatives 1, 2, 5, 7, 11 and 12 shows two fungal colonies against 8-12 mm of zone of inhibition against the fungal strains.

The chloro-, methoxy- and nitro- substituents of the pyrazoline thiocarboamides have good antimicrobial activities. The antioxidant activities of the thiocarboamides derivatives were measured by a DPPH radical scavenging method; the compounds containing hydroxy and methoxy substituents showed antioxidant activity. Compound 3 shows good insect antifeedant activity against the 4th instar larvae Achoea Janata L with castor leaf disc bio-assay method.

References


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