Mosquito-Larvicidal, Anti-Bacterial and Anti-Fungal Properties of Novel 2, 4-Disubstituted-[1, 3] -Thiazoles

ABSTRACT

A series of novel hydrazones of 2, 4-disubstituted-[1, 3] thiazole (5) were prepared by the reaction of thiosemicarbazone (3) with different substituted phenacyl halides (4) in the presence of dimethylformamide as a solvent. The requisite thiosemicarbazone (3) was synthesized by the treatment of thiosemicarbazide (1) with 2, 4-dichloro-5-fluroacetophenone (2) in presence of catalytic amount of mineral acid. All the novel compounds were confirmed and characterized by elemental analysis, FT-IR, 1H-NMR, 13C-NMR and Mass spectra and were subjected to anti-bacterial, anti-fungal activity studies and also screened for mosquito-larvicidal activity studies against two pathogenic vecotors namely: Anopheles stephenesis (malaria vector) and C. tritaeniothoruchs (Japanese encephalitis). Among the novel 2, 4-disubstituted [1, 3]-thiazoles (5a-5o), the compounds 2-{2-[1-(2, 4-dichloro-5-fluorophenyl)ethylidene]hydrazinyl}-4-(4-fluorophenyl)[1, 3]-thiazole (5j) and 2-[2-{1-(2, 4-dichloro-5-fluorophenyl)ethylidene]hydrazinyl]-4-(4-nitrophenyl)[1, 3]-thiazole (5m) exhibit anti-bacterial and anti-fungal properties. Though the mosquito-larvicidal activity is low among the novel derivatives, yet, 4-(4-bromomethyl)-2-[2-{1-(2, 4-dichloro-5-fluorophenyl)ethylidene]hydrazinyl]-4-[4-(nitrophenyl)][1, 3]-thiazole (5i) and 2-[2-{1-(2, 4-dichloro-5-fluorophenyl)ethylidene]hydrazinyl]-4-[4-(fluorophenyl)][1, 3]-thiazole may be recommended as mosquito larvicidal agents for further studies.

1. Introduction

The chemistry of hydrazones of [1, 3] thiazole bearing aryl moiety analogues has been an interesting field of study for a long period of time. The voluminous literature available beench that hydrazones of [1, 3] thiazole possess excellent biological properties. In addition to these, the Chemistry of carbon-nitrogen double bond of hydrazones is the backbone of condensation reaction in benzo-fused N-heterocycles [Rashed et al., 1990]. Hydrazones contain azomethine (—NH—N=C-) group, which constitute the important class of compounds for newer drug development [Rollas and Kucukguzel, 2007]. Hence, researchers have synthesized numerous hydrazones of [1, 3]-thiazole and evaluated their biological activities. Moreover, the hydrazones of thiazole are used for the treatment of cardiac toxicity [Mean and Moccelo, 1994], fungicidal [Gindher et al., 2001; Wilson et al., 2001], anti-HIV infection [Hantzsch et al., 1987; Bhattacharya et al., 2005; Bell et al., 1995] and alzheimer diseases [Dasdon et al., 1945; King and Halavacck, 1950; Heldebrant and Jessop, 2003; Chandrashekar et al., 2002]. They are found to be associated with diverse pharmacological activities such as Lipoxigenase Inhibitors and anti-Inflammatory agents [Geronikaki et al., 2007; Sharma et al., 2009; Salgan-Goks en et al., 2006; Haviv et al., 2006], anti-cancer [Bijev, 2006], anti-bacterial [Tsui and Ishikawa, 1994], analgesic [Argyropoulou et al., 2009; Lima et al., 2000], anti-tubercular [Trautman and L. Longe, 1948], anti-malarial [Walcourt et al., 2004], central nervous system (CNS) stimulate [Surray, 1949], anti-tumor [Cocero et al., 1999; Gu et al., 1999; Jiang and Gu, 2000], anti-filarial [Kumar et al., 1993], anti-convulsant [Dimmock et al., 2004], anti-platelet [Silva et al., 2004], herbicidal and insecticidal [Metzger, 1984], antihypertensive [Patt et al., 1992], anti-inflammatory products [Haviv et al., 1988; Lednicer et al., 1990; Todeschini et al., 1998; Radhwan et al., 2007; Almasirad et al., 2005; Murineddu et al., 2001] and so on. Prompted by the enormous potential activities of [1, 3] -thiazole and their hydrazone derivatives, a plan was drawn to synthesize hydrazones of 2, 4-disubstituted-[1, 3] thiazoles and screen them for anti-bacterial, anti-fungal and mosquito-larvicidal properties.

2. Pharmacology:

The novel synthesized compounds (5a – 5o) were evaluated for anti-bacterial, anti-fungal and mosquito-larvicidal activity studies.

2.1 Anti-bacterial activity

All the synthesized compounds were evaluated for their anti-bacterial properties by Disc diffusion method [Bauer et al., 1966; Vardar-Unlu et al., 2003]. The microorganisms used in study of anti-bacterial properties were collected from Institute of Microbial Technology (IMTECH), Chandigarh, India. Two Gram +ve bacteria namely Staphylococcus aureus MTCC-7443 and Bacillus subtilis MTCC-441; two Gram -ve bacteria namely Escherichia coli MTCC-725, Aeremons hydrophila MTCC-1739 were used.

The bacterial strains were inoculated on Nutrient Agar (NA) and incubated for 24h at 37°C. Sterile empty discs (6mm diameter) (Himedia Laboratories Pvt. Ltd. Mumbai). The test compounds were dissolved in 5mL of DMSO taken as the solvent; from the stock solution 100μL of respective compound in the selected concentration (500μg/disc) was loaded on the disc individually and aseptically, dried and were used for screening the anti-bacterial assay.

Sterile discs were saturated with 100μL of the test solution, dried under laminar air flow and placed on the Nutrient Agar (NA) plate for bacteria, which was inoculated with a lawn...
of the test microorganisms. Plates were incubated at 37°C, for 18 to 24h for bacteria. The compounds that produced distinct circular zones of inhibition around the discs and the diameters of clear zones were determined and used as an indication of anti-bacterial activity. Streptomycin, an antibiotic drug at a dose of 10μg/disc was used as the reference standard.

2.2 Anti fungal activity
All the synthesized compounds were evaluated for their anti-fungal properties by Disc diffusion method [Bauer et al., 1966; Vardar-Unlu et al., 2003]. The microorganisms used for the study were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. Four fungal species namely, Aspergillus niger MTCC-281, Aspergillus flavus MTCC-871, Candida albicans MTCC 183 and Alternaria alternata MTCC -149 were used.

The fungal strains were inoculated on Potato Dextrose Agar (PDA) for 48h at 27°C, then suspended in saline solution (0.85 and adjusted to yield approximately 1.0x10^8.1.0x10^9 cfu/ml by using spectrophotometer (25% transmittance at 530nm) as per the guidelines given in Indian Pharmacopoeia (2007).

Sterile emulsions (6mm diameter) were purchased from Hi-media Laboratories Pvt. Ltd. Mumbai. 50μg of each test compound was dissolved in 5ml of DMSO (Dimethyl Sulphoxide) to prepare the stock solution. From the stock solution 100μL of respective each test compound was loaded on the disc individually and aseptically, dried under laminar air flow and placed on the Potato Dextrose Agar (PDA) plate, which had been inoculated with a lawn of the test microorganisms. Plates were incubated at 27°C for 48h for fungi. Discs treated with 100 μL DMSO were used as negative controls. Commercial anti-fungal drug such Nystatin was used (10 μg/mL) as reference standard for anti-fungal activities. Distinct circular area around the discs representing the zone of inhibition was measured to determine the potentiality of anti-fungal activities.

2.3 Mosquito-Larvicidal activity
All the synthesized compounds were tested for their mosquito-larvicidal activity using two pathogenic vectors namely; Anopheles stephensi (malaria vector) and C. tritaeniorhynchus (Japanese encephalitis) as per the standard WHO guidelines [WHO, 1981]. In 500mL beakers containing 250mL of water and 25 numbers of late III or early IV instar mosquito larvae for various concentrations of the extracts. A negative control was kept with each set of experiment and mortality was recorded after 24h of exposure. Malathion, the commercial insecticide (Hindustan Insecticides Ltd, New Delhi, India) was used as the reference standard. Experiments were performed in triplicates for each sample. Median lethal concentration (LC50) with 95% confidence limit (CI) was calculated using Abbott’s formula and Log probit analysis [Raymond et al., 1993] and results are expressed as mg/mL. Relative potency was determined for comparison with the reference standard using the formula.

3. Results and Discussion
3.1 Chemistry
A thiosemicabazon (3) was synthesized by the treatment of 2, 4-dichloro-5-fluoracetophenone (2) with thiosemicabazole (1) in presence of catalytic amount of a mineral acid and ethanol as solvent (Scheme 1). Condensation of thiosemi-carbazone (3) with different substituted phenacyl halides (4) in presence of dimethylformamide for 12h at reflux temperature furnished hydrazones of 2, 4-disubstituted-1, [3] -thia- zole (5) (Scheme 2). All the compounds were characterized on the basis of elemental analysis, IR, 1H-NMR, 13C-NMR and Mass spectra. All the novel synthesized 2-[2-(1-2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl]-4-(aryl)-[1, 3] thiazoles (5a - 5o), were procured from IMTECH, Chandigarh. The fungus, A. alternate did not show very good response against any of the the tested compounds. However, based on the zone of inhibition values, (5m) (12.8mm), (5c) (12.5mm) and (5j) (12.2mm) compounds can be considered to possess moderate activity against A. alternate. Overall, among the tested compounds, (5j) and (5c) were found to be more effective against the fungus, C. albicans against which they showed relatively higher activity. (5c) and (5j) compounds are more effective against A. niger. The fungus, A. flavus is more sensitive to (5j) and (5c). The fungus, P. aeruginosa was also found to be sensitive to (5a) and (5c). The fungus, A. alternata did not show any response against any of the tested compounds. However, overall, among the tested compounds, (5j) may be considered as an anti-fungal drug candidate for further studies.

3.2 Pharmacological Screening
Anti-bacterial Activity
All the novel 2, 4- disubstituted-[1, 3]-thiazoles (5a- 5o) (Table 2, Figure 1) exhibited anti bacterial activity at the tested concentrations either in Gram +ve or Gram –ve or in both bacterial species under study. Among the tested compounds, (5m) showed the maximum activity against S. aureus, followed by (5i) and (5j) with zone of inhibition values 26.7, 23.6 and 20.6 mm respectively. Subtilis was found to be more sensitive to (5m) (18.9mm), (5j) (18.6) and (5c) (17.6mm) as indicated by the relatively maximum values for zone of inhibition. With reference to Gram -ve bacteria, (5j) was more effective against P. aeruginosa (19.2 mm) and K. pneumonia (17.5mm). P. aeruginosa was also found to be sensitive to (5a) with the zone of inhibition 18.2mm. Overall, (5m) compound is more effective against Gram +ve bacteria and (5j) is more effective against Gram -ve bacteria. Considering antibacterial activity against both Gram (+ve and –ve) bacteria, (5j) exhibited relatively good activity with the zone of inhibition values ranging from 17.5 to 20.6mm. Therefore, 2-[2-(1-2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl]-4-(4-fluoro-phenyl)-[1, 3]-thiazole (5j) can be regarded as an agent with broad spectrum anti-bacterial activity, and recommended as drug candidate for further studies.

Anti-fungal Activity
All the tested 2, 4- disubstituted-[1, 3] thiazoles (5a- 5o)(Table 3, Figure 2) exhibited a little or more fungicidal activity depending upon the type of fungi. Among all the tested compounds, (5j) and (5c) were found to be more effective against the fungus, C. albicans against which they showed relatively higher activity. (5c) and (5j) compounds are more effective against A. niger. The fungus, A. flavus is more sensitive to (5j) and (5c). The fungicide, Mancozeb showed very good response against any of the tested compounds. However, overall, among the tested compounds, (5j) may be considered as an anti-fungal drug candidate for further studies.

Mosquito-Larvicidal Activity
Novel 2, 4-disubstituted-[1, 3] thiazoles (5a - 5o)( Table 4, Figure 3) exhibited mosquito- larvicidal activity against two pathogenic vectors with LC50 values ranging between 99.79 and 151 μg/mL respectively. B. subtilis was found to be sensitive to (5m) (18.9mm), (5j) (18.6) and (5c) (17.6mm) as indicated by the relatively maximum values for zone of inhibition more than 12 against all the tested fungal species. Thus, 2-[2-(1-2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl]-4-(4-fluoro-phenyl)-[1, 3]-thiazole (5j) may be considered as an anti-fungal drug candidate for further toxicity and clinical studies.
Heterocyclic compounds with these moieties have been reported to exhibit insecticidal/mosquito-larvicidal activity.

4. Experimental Section
The chemicals used in the work were of standard quality and obtained from Sigma Aldrich (India), Alfaaesar (U. K.), S. D. Fine (Mumbai) and Hi-media (Mumbai). The novel synthesized 2, 4- disubstituted 1, 3- thiazoles (5a - 5o) were confirmed by the spectral data. Melting points of novel compounds were determined in open capillary tubes and are uncorrected. The purity of synthesized compounds was checked by TLC observing single spot on Merck silica gel 60 F254 coated alumina plates. The IR spectra (cm⁻¹) were recorded on a Shimadzu FTIR S77 Infrared spectrophotometer in KBr pellets. The 1H-NMR and 13C-NMR spectra were recorded on a Brucker AMX-400(400 MHz) spectrometer using recorded using CDCl₃-d₆/DMSO-d₆ as solvent and TMS as the internal standard. All the chemical shift values are reported in δ scale downfield from TMS. The splitting patterns are designated as follows; s, singlet; d, doublet; t, triplet; m, multiplet. The Mass spectra were recorded on Perkin-Elmer 018444 – Y, Triple Quadrupole LC/MS Spectrometer. The molecular ion peaks (m/z) (Scheme 2)

4.1. General procedure for the Synthesis of Schiff base (thiosemicarbazone): 2-[1-(2, 4-dichloro-5-fluorophenyl) ethyldiene] hydrazine carboxyhomeide (3)
The synthesis of a Schiff base (thiosemicarbazone) (3) was carried out on a Elementar Vario EL III analyzer. Heterocyclic compounds with these moieties have been reported to exhibit insecticidal/mosquito-larvicidal activity. An equimolar quantities of thiosemicarbazide (1) (0.01 mol) and 2, 4-dichloro-5-fluorophenacyl bromide/chloride (0.001 mol) (4) in dimethylformamide (20 mL) was refluxed for 12h. The progress of the reaction was monitored by TLC at regular intervals. The reaction mixture was cooled, poured into crushed ice. The solid thiosemicarbazone (3) was separated was washed with ice cold water (100 mL) and dried. The product obtained was recrystallized from ethylacetate (Scheme 1).

4.2. General procedure for the Syntheses of 2-[2-[1-(2, 4-dichloro-5-fluorophenyl) ethyldiene] hydrazinyl]-4-(aryl)-1, 3-thiazoles (5a - 5o)
An equimolar quantities of thiosemicarbazone (3) (0.001 mol) and different substituted phenacyl bromide/chloride (0.001 mol) (4) in dimethylformamide (20 mL) was refluxed for 12h. The progress was monitored by TLC at regular intervals. The excess of solvent was distilled off and the solid that separated was collected by filtration, dried and recrystallized from ethanol/chloroform to yield the resultant analytical samples (5a – 5o) (Scheme 2).
Table 3: Anti-fungal activity: Anti-fungal activity of 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(aryl)-[1, 3]-thiazoles (5a-5o) and Nystatin (Reference standard)

| Tested compounds | C. tritaeniorhynchus | A. flavus | C. albicans | A. alternata |
|------------------|---------------------|----------|-------------|-------------|
| Diameter of zone of inhibition (mm±SD) | | | | |
| 5a               | 8.2±0.78            | 0        | 11.6±1.09   | 0           |
| 5b               | 0                   | 9.5±0.76 | 12.0±0.87   | 8.5±0.69    |
| 5c               | 16.25±1.25          | 14.65±1.08 | 17.1±1.25   | 12.5±1.45   |
| 5d               | 12.0±1.31           | 12.8±0.86 | 13.5±0.91   | 10.2±1.06   |
| 5e               | 12.2±0.83           | 11.1±0.79 | 12.3±0.69   | 0           |
| 5f               | 11.6±0.89           | 12.2±1.56 | 14.1±1.43   | 9.28±0.78   |
| 5g               | 12.0±0.81           | 12.0±1.06 | 14.0±1.24   | 10.2±1.03   |
| 5h               | 10.6±0.87           | 9.6±0.69  | 13.6±0.89   | 9.6±0.76    |
| 5i               | 8.4±0.74            | 11.0±0.89 | 10.3±1.03   | 0           |
| 5j               | 16.0±1.53           | 15.2±2.04 | 17.0±1.54   | 12.2±1.09   |
| 5k               | 0                   | 11.0±0.89 | 11.4±0.86   | 0           |
| 5l               | 11.4±0.81           | 0        | 10.0±0.48   | 0           |
| 5m               | 14.2±1.56           | 13.2±1.26 | 15.6±1.08   | 12.8±1.21   |
| 5n               | 0                   | 8.2±0.76  | 13.8±1.34   | 0           |
| 5o               | 0                   | 10.4±1.34 | 0           | 0           |
| 5h Streptomycin   | 31.5±0.84           | 33.6±0.78 | 26.3±1.08   | 22.4±0.79   |

*Mean values (n = 3)

Table 4: Mosquito-Larvicidal Activity: Mosquito-Larvicidal activity of novel 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(aryl)-[1, 3]-thiazoles (5a-5o) against C. tritaeniorhynchus and A. stephensi

| Tested compounds | C. tritaeniorhynchus | A. stephensi |
|------------------|---------------------|-------------|
| LC50 (µg/mL)     | 95% CI              | *Relative potency | LC50 (µg/mL) | 95% CI | *Relative potency |
| 5a               | 141.78              | 123.98-159.88 | 0.06 | 136.75 | 119.8-153.65 | 0.076 |
| 5b               | 144.92              | 128.62-160.82 | 0.06 | 138.43 | 123.23-153.93 | 0.085 |
| 5c               | 143.68              | 127.48-160.08 | 0.06 | 125.95 | 111.65-140.05 | 0.083 |
| 5d               | 151.23              | 134.13-169.03 | 0.06 | 147.92 | 132.62-163.62 | 0.070 |
| 5e               | 136.7                | 121.55-152.45 | 0.07 | 124.38 | 111.78-137.28 | 0.084 |
| 5f               | 141.28              | 121.98-161.08 | 0.06 | 146.75 | 131.65-161.65 | 0.071 |
| 5g               | 125.46              | 110.76-140.36 | 0.07 | 130.25 | 116.15-144.65 | 0.08 |
| 5h               | 104.34              | 90.14-118.84 | 0.09 | 108.72 | 99.52-118.22 | 0.096 |
| 5i               | 99.79               | 88.19-111.69 | 0.09 | 103.42 | 90.72-116.32 | 0.100 |
| 5j               | 103.25              | 90.95-115.15 | 0.07 | 100.32 | 89.12-111.72 | 0.104 |
| 5k               | 138.72              | 123.92-153.52 | 0.06 | 125.99 | 112.19-139.19 | 0.083 |
| 5n               | 113.42              | 99.82-127.22 | 0.08 | 123.89 | 109.79-138.79 | 0.084 |
| 5m               | 125.48              | 108.88-142.49 | 0.07 | 107.90 | 96.7-119.3 | 0.097 |
| 5n Malathion      | 8.9                 | 7.1-10.8     | 1.0 | 10.42 | 8.32-12.52 | 1.0 |

*Mean values (n = 3)

CI – Confidence Interval
*Relative potency - LC50 standard / LC50 tested substance
Malathion – Reference standard

Figure 1: Results of Anti-bacterial Activity (Zone of Inhibition) of 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(aryl)-[1, 3]-thiazoles (5a-5o) and Streptomycin (Reference standard).

Figure 2. Results of Anti-fungal Activity of 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(aryl)-[1, 3]-thiazoles (5a-5o) and Nystatin (Reference standard).
4.3. The Analyses of the samples
All the synthesized novel compounds were analyzed spectroscopic techniques and their results are interpreted as below.

4.3.1. The IR, 1H-NMR, 13C-NMR, mass spectra and elemental analyses of 2, 4-disubstituted [1, 3]-thiazoles (5a-5o) are mentioned below:

4.3.2. The IR, 1H-NMR, 13C-NMR, mass spectra and elemental analyses of synthesized Schiff base (thiosemicarbazone) (3) IR (KBr, cm⁻¹): 3344 and 3279 (-NH₂ sym and asym stretch), 1545 and 1480 (C=C), 1014 (Ar-F), 785 and 735 (Ar-Cl). 1H-NMR (CDCl₃-d, δ, ppm): 2.21 (3H, s, -CH₃), 6.87 (1H, s, thiazole ring), 7.14 (1H, d, J=6.4 Hz, 4-carboxyphenyl), 7.27 (2H, dd, J=9 Hz, 4-Cl₄-5-F-phenyl), 7.41 (1H, d, J=16 Hz, 4-chlorophenyl), 7.46 (2H, J=8 Hz, 4-methylphenyl), 8.51 (1H, s, -NH). 13C-NMR: 17.85 (-CH₃), 58.52 (-OCH₃), 104.26 (thiazole-C-5), 147.2 (C=N stretch), 1588 (azomethine), 1561 and 1472 (C=C), 1057 (Ar-C atoms) and 179.78 (-C=S atom). LC-MS, [M⁺], (m/z): 405.3/407.3; Anal. Cald for C₁₈H₁₁Cl₂FN₄S: C, 53.32; H, 2.75; N, 13.81. m. p. 248-250°C; Yield: 71%

4.3.2.5. 4-(4-chlorophenyl)-2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene]-hydrazinyl}-[1, 3]-thiazole (5f) IR (KBr, cm⁻¹): 3344 and 3279 (-NH₂ sym and asym stretch), 1545 and 1480 (C=C), 1014 (Ar-F), 785 and 735 (Ar-Cl). 1H-NMR (CDCl₃-d, δ, ppm): 2.23 (3H, s, -CH₃), 6.87 (1H, s, thiazole ring), 7.23 (1H, d, J=9 Hz, 2-4-Cl₄-5-F-phenyl), 7.39 (1H, d, J=6.8 Hz, 2-4-Cl₄-5-F-phenyl), 7.47 (2H, dd, J=9 Hz, 4-benzonitrile), 7.61 (2H, J=8 Hz, 4-chloroaniline), 7.69 (2H, dd, J=6.8 Hz, 4-chlorobenzonitrile), 8.53 (1H, s, -NH). 13C-NMR: 16.97 (-CH₃), 58.52 (-OCH₃), 104.26 (thiazole-C-5), 148.52 (azomethine), 155.02 and 164.69 (thiazole ring), 125.50 and 130.62 (4Cl atoms of 4-methylphenyl), 127.81 and 144.91 (2Cl atom of 4-methylphenyl), 118.05, 118.37, 121.80, 131.85 and 133.70 (Ar-C atoms). LC-MS, [M⁺], (m/z): 395/397; Anal. Cald for C₁₈H₁₁Cl₂FN₄S: C, 53.34; H, 2.75; N, 13.82. m. p. 248-250°C; Yield: 71%

4.3.2.6. 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene]-hydrazinyl}-4-(4-chlorophenyl)ethylidene] [1, 3]-thiazole (5e) IR (KBr, cm⁻¹): 3344 and 3279 (-NH₂ sym and asym stretch), 1545 and 1480 (C=C), 1014 (Ar-F), 785 and 735 (Ar-Cl). 1H-NMR (CDCl₃-d, δ, ppm): 2.21 (3H, s, -CH₃), 6.87 (1H, s, thiazole ring), 7.14 (1H, d, J=6.4 Hz, 4-carboxyphenyl), 7.27 (2H, dd, J=9 Hz, 4-Cl₄-5-F-phenyl), 7.41 (1H, d, J=16 Hz, 4-chlorophenyl), 7.46 (2H, J=8 Hz, 4-methylphenyl), 8.51 (1H, s, -NH). 13C-NMR: 17.85 (-CH₃), 58.52 (-OCH₃), 104.26 (thiazole-C-5), 147.2 (C=N stretch), 1588 (azomethine), 1561 and 1472 (C=C), 1057 (Ar-C atoms) and 179.78 (-C=S atom). LC-MS, [M⁺], (m/z): 405.3/407.3; Anal. Cald for C₁₈H₁₁Cl₂FN₄S: C, 53.34; H, 2.75; N, 13.82. m. p. 248-250°C; Yield: 71%

Figure 3. Mosquito-Larvicidal Activity of Hydrozones of 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene]hydrazinyl}-4-(aryl)-[1, 3]-thiazoles (5a-5o) against two pathogenic vectors C. tritaeniorhynchus and A. stephensi.
4.3.2.7. 2-[2-(1, 4-dichloro-5-fluorophenyl)ethylidene] hydrazinyl]-4-(3, 4-dichlorophenyl) thiazole (5i).

IR (KBr, cm⁻¹): 3281 (-NH), 3045 (Ar-H), 2970 (C-CH₃), 1578 (N, 9.36. Found: C, 45.44; H, 2.26; N, 9.35. m. p. 184-186°C. Yield: 70%

4.3.3.8. 4-(4-bromophenyl)-2-{2-[1-(2, 4-dichloro-5-fluoro-phenyl) ethylidene] hydrazinyl}-4-(4-fluorophenyl) – [1, 3]-thiazole (5j).

IR (KBr, cm⁻¹): 3199 (C=O), 3006 (Ar-H), 2972 (C-H), 1600 (amine), 1560 and 1471 (C=O, 1010 (Ar-F), 777 and 717 (Ar-Cl), 667(Ar-Br). 1 H-NMR (CDCl₃, δ ppm): 2.30 (3H, s, -CH₃), 7.48 (1H, s, thiazole ring), 7.51 (1H, d, J=9 Hz, 2, 4-Cl₂-5-F-phenyl), 7.51 (1H, d, J=9 Hz, 2, 4-Cl₂-5-F-phenyl), 7.79 (1H, d, J=9 Hz, 2, 4-Cl₂-5-F-phenyl), 8.08 (H, s, 3-Cl₂phenyl), 11.42 (1H, s, -NH). 13C-NMR: 17.92 (CH₂), 106.00 (thiazole-C-S), 144.96 (azomethine), 157.14 and 164.99 (thiazole ring), 130.81 and 131.37 (4 C atoms of 3-bromophenyl). 118.22, 116.27, 124.13, 127.10, 128.17, 130.08, and 139.29 (Ar-C-atoms). LC-MS, [M⁺], (m/z): 450.2/452.2; Anal. Cald for C₁₇H₁₀Cl₄FN₃S: C, 45.46; H, 2.24; N, 9.36. Found: C, 45.45; H, 2.27; N, 9.34. m. p. 176-180°C. Yield: 72%

4.3.2.9. 4-(4-bromophenyl)-2-{2-[1-(2, 4-dichloro-5-fluoro-phenyl) ethylidene] hydrazinyl}-4-(2, 4-difluorophenyl) thiazole (5k).

IR (KBr, cm⁻¹): 3234 (NH), 3044(Ar-H), 2974(C=O), 1600 (azomethine), 1560 and 1471 (C=O, 1010 (Ar-F), 777 and 717 (Ar-Cl), 667(Ar-Br). 1 H-NMR (CDCl₃, δ ppm): 2.26 (3H, s, -CH₃), 6.90 (1H, s, thiazole ring), 7.28 (1H, d, J=9 Hz, 2, 4-Cl₂-5-F-phenyl), 7.46 (1H, d, J=9 Hz, 2, 4-Cl₂-5-F-phenyl), 7.51 (2H, d, J=8 Hz, 4-bromophenyl), 7.66 (2H, d, J=8 Hz, 4-bromophenyl), 8.91 (1H, s, -NH). 13C-NMR: 16.75 (CH₂), 101.16 (thiazole-C-S), 150.55 (azomethine), 150.02 and 168.69 (thiazole ring), 127.50 and 131.62 (4 C atoms of 4-bromophenyl). 118.05, 118.37, 128.12, 127.71, 130.67, 132.5 (3Cl₂phenyl), 139.49 (Ar-Cl). LC-MS, [M⁺], (m/z): 459.9; Anal. Cald for C₁₃H₁₀Cl₂F₄N₃S: C, 44.47; H, 2.41; N, 9.15. Found: C, 44.45; H, 2.44; N, 9.14. m. p. 126-128°C. Yield: 77%

4.3.2.10. 2-[2-(1, 4-dichloro-5-fluorophenyl)ethylidene] hydrazinyl]-4-(4-fluorophenyl) – [1, 3]-thiazole (5l).

IR (KBr, cm⁻¹): 3214 (-NH), 3023(Ar-H), 2968 (C=O), 1598 (azomethine), 1560 and 1489 (C=O, 1028 and 1087 (Ar-F), 777 and 722 (Ar-Cl), 667(Ar-Br). 1 H-NMR (CDCl₃, δ ppm): 2.27 (3H, s, -CH₃), 6.83 (1H, s, thiazole ring), 7.25 (1H, d, J=9 Hz, 2, 4-Cl₂-5-F-phenyl), 7.41 (1H, d, J=6.4 Hz, 2, 4-Cl₂-5-F-phenyl), 7.76 (4H, m, 4-fluorophenyl), 8.86 (1H, s, -NH). 13C-NMR: 16.95 (-CH₂), 105.16 (thiazole-C-S), 147.52 (azomethine), 154.02 and 163.39 (thiazole ring), 128.62, 135.45 127.81 and 144.91 (C atoms of 4-fluorophenyl) 110.05, 118.13, 121.82, 130.67, 131.85 and 133.70 (Ar-C-atoms). LC-MS, [M⁺], (m/z): 398/400; Anal. Cald. For C₁₃H₁₀Cl₂F₄N₃S: C, 44.79; H, 2.78; N, 10.54. Found: C, 51.25; H, 2.80; N, 10.54. m. p. 152-154°C. Yield: 69%
4.3.2.15. 4-(biphenyl-4-yl)-2-(2-[1-(2, 4-dichloro-5-fluoro-phenyl) ethylidene] hydrazinyl}-[1, 3] -thiazole (5o).

IR (KBr, 5. Conclusion

Novel 2, 4-disubstituted 1, 3- thiazoles (5a - 5o) exhibited mosquito- larvicidal activity against two pathogenic vectors with LC50 values ranging between 99.79 and 151 µg/mL against C. tritaeniorhynchus, while for A. stephensi, between 100.32 and 147.92 µg/mL. Among the tested compounds (5i) and (5j) were more potential against both the types of mosquito larvae. Among the new thiazole derivatives synthesized and tested in the present study, 4-(4-bromomethyl)-2-[2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(4-nitrophenyl) ethylidene] hydrazinyl}-4-(4-nitrophenyl) - 1, 3-thiazole (5i) and 2-[2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-1, 3-thiazole (5j) are most potent and may be recommended as mosquito larvicidal agents for further studies.

All the tested compounds of 2, 4-disubstituted 1, 3-thiazoles (5a - 5o) possess antibacterial activity in in vitro test system but with different sensitivity. 2-[2-[1-(2, 4-dichloro-5-fluoro-phenyl) ethylidene] hydrazinyl}-4-(4-fluorophenyl)-1, 3-thiazole (5j) is found to possess broad spectrum antibacterial activity against Gram +ve and Gram –ve species. In addition to the anti-bacterial properties the compounds (5a - 5o) exhibit a little or more fungicidal activity depending upon the type of fungus. The compound (5m) is most potent among all the novel derivatives and hence, 2-[2-[1-(2,4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl} may be considered as anti-fungal drug candidates for further toxicity and clinical studies.

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