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

KEYWORDS Mosquito-larvicidal activity, Anti-bacterial activity, Anti-fungal activity, Hydrazones of 1, 3-thiazole, Schiff base.

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ABSTRACT *A series of novel hydrazones of 2, 4-disubstituted-[1, 3] thiazole (5) were prepared by the reaction of thiosemicabazone (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 thiosemicabazide (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, ^{1}H-NMR, ^{13}C-NMR and Mass spectra and were subjected to anti-bacterial, anti-fungal activity studies and also screened for mosquito-larvicidal activitiy studies against two pathogenic vectors namely; Anopheles stephenesis (malaria vector) and C. tritaeniorhynchus (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-bromophenyl)-2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl} –[1, 3]-thiazole (5i) and 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(4-fluorophenyl)[1, 3]- thiazole (5j) 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 bears evidence 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 cardiotonic [Mean and Mocelo, 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 [Dadson 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 Lipoxygenase Inhibitors and anti-Inflammatory agents[Geronikaki et al., 2007; Sharma et al., 2009; Salgin-Goksen et al., 2004; Haviv et al., 2006], anti-cancer [Bijev, 2006], anti-bacterial [Tsuji 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[Cocco 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] - thiazoles 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, Aeromons 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 antifungal 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 on Potato Dextrose Agar (PDA) for 48h at 27°C, then suspended in saline solution (0.85 and adjusted to yield approximately $1.0x10^7$-$1.0x10^8$cfu/ml by using spectrophotometer (25% transmittance at 530nm) as per the guidelines given in Indian Pharmacopoeia (2007). Sterile empty discs (6mm diameter) were purchased from Himedia Laboratories Pvt. Ltd. Mumbai. 50mg 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 (LC_{50}) 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 thiosemicabazone (3) was synthesized by the treatment of 2, 4-dichloro-5-fluroacetophenone (2) with thiosemicabazide (1) in presence of catalytic amount of a mineral acid and ethanol as solvent (Scheme 1). Condensation of thiosemicarbazone (3) with different substituted phenacyl halides (4) in presence of dimethylformamide for 12h at reflux temperature furnished hydrazones of 2, 4-disubstituted-[1, 3] -thiazole (5) (Scheme 2). All the compounds were characterized on the basis of elemental analysis, IR, ^{1}H-NMR, ^{13}C-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 screened for their anti-bacterial, antifungal and mosquito-larvicidal studies. Characterization data of the novel compounds (5a- 5o) are tabulated in Table 1.

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) exhibited the maximum activity against S. aureus, followed by (5l) and (5j) with zone of inhibition values, 26.7, 23.6 and 20.6 mm respectively. B. 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 Gram -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-fluorophenyl)-[1, 3]-thiazole (5j) can be regarded as an agent with broad spectrum anti-bacterial activity, and recommended as drug candidate for further studies.

Antifungal 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 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 test compounds, (5j) can be considered as an agent with broad spectrum antifungal property showing the 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-fluorophenyl)-[1, 3]- thiazole (5j) may be considered as an anti-fungal drug candidate for further toxicity and clinical 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 LC_{50} 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. Comparatively (5h) also showed moderate activity against both the larvae. Results indicate that both C. tritaeniorhynchus and A. stephensi larvae are equally sensitive to the type of novel 2, 4-disubstituted [1, 3]- thiazoles. Relative potency determined comparing with the reference standard (malathion) indicate that the synthesized novel 2, 4-disubstituted- [1, 3]- thiazoles are not as potential as that of malathion, a commonly used commercial insecticidal/larvicidal agent. However, taken into consideration of various other factors such as persistence, half life, toxicity to non-target organisms, development of resistance, new agents need to be tested for their larvicidal activity to control disease vectors. In this context, among the new thiazole derivative in the present study, 4-(4-bromophenyl)-2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl} –[1, 3]-thiazole (5i) and 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(4-fluorophenyl)– [1, 3]- thiazole (5j) may be recommended as mosquito larvicidal agents for further studies. Studies on larvicidal activity against disease vectors, of thiazole derives are very scanty, but Venugopala et al., 2013 have reported the larvicidal activity of 2, 6-substituted benzo[d] thiazole and 2, 4-substituted benzo[d]thiazole analogues against Anopheles arabiensis. The presence of halogens, that is bromo group in (5i) and fluoro group in (5j) respectively, may be plausible reason for higher activity.

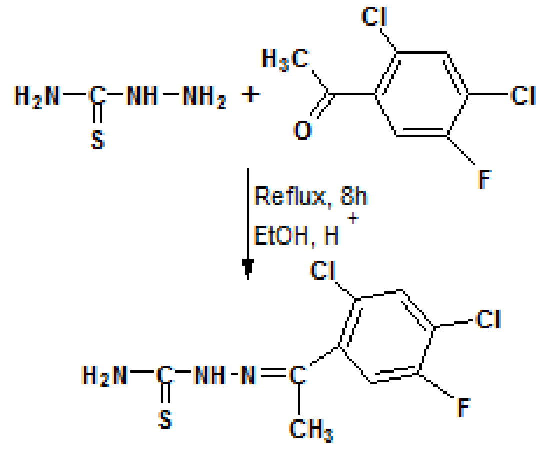
Heterocyclic compounds with these moieties have been reported to exhibit insecticidal/mosquito-larvicidal activity.

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 F_{254} coated alumina plates. The IR spectra (cm^{-1}) were recorded on a Shimadzu-FTIR 577 Infrared spectrophotometer in KBr pellets. The ^1H-NMR and ^{13}C-NMR spectra were recorded on a Brucker AMX-400(400 MHz) spectrometer using recorded using $CDCl_3$-d/ DMSO-d_6 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) were equivalent to the molecular weights of the newly synthesized compounds. The elemental analysis (C, H, N) was carried out on a Elementar Vario EL III analyzer.

4.1. General procedure for the Synthesis of Schiff base (thiosemicarbazone): 2-[1-(2, 4-dichloro-5-flurophenyl) ethylidene] hydrazine carbothioamide (3)

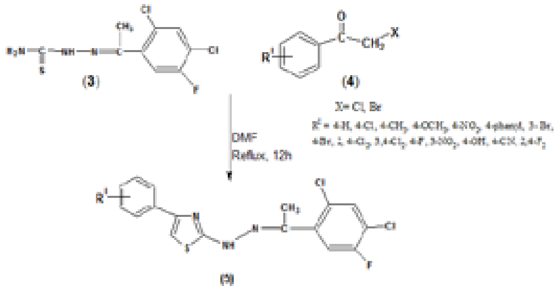
The synthesis of a Schiff base (thiosemicarbazone) (3) was done according to the procedure well described in the literature [Furniss et al., 2007; Bruice, 2006]. An equimolar quantities of thiosemicarbazide (1) (0.01 mol) and 2, 4-dichloro-5-fluoroacetophenone (2) (0.01 mol) in ethanol was refluxed for 8h in the presence of catalytic amount of mineral acid. 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) thus separated was washed with ice cool water (100 mL) and dried. The product obtained was recrystallized from ethylacetate (Scheme 1)



Scheme 1

4.2. General procedure for the Syntheses of 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] 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)



Scheme 2

Table 1 Characterization Data of 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(aryl)-[1, 3] thiazoles (5a- 5o)

Compound	R¹	m.p. (°C) (yield %)	Mol. Formula (Mol. wt)	% Composition, found (Cald) C	H	N
5a	4-H	86-88 (76)	$C_{17}H_{12}Cl_2FN_3S$ (380.27)	53.67 (53.69)	3.21 (3.18)	11.04 (11.05)
5b	4-CH_3	88-90 (78)	$C_{18}H_{14}Cl_2FN_3S$ (394.29)	54.82 (54.83)	3.60 (3.58)	10.65 (10.66)
5c	4-OCH_3	94-96 (75)	$C_{18}H_{14}Cl_2FN_3OS$ (410.29)	52.66 (52.69)	3.46 (3.44)	10.22 (10.24)
5d	4-CN	248-250 (71)	$C_{18}H_{11}Cl_2FN_4S$ (405.28)	53.32 (53.34)	2.75 (2.74)	13.81 (13.82)
5e	4-Cl	148-150 (72)	$C_{17}H_{11}Cl_3FN_3S$ (414.71)	49.21 (49.23)	2.69 (2.67)	10.12 (10.13)
5f	2,4-Cl_2	184-186 (70)	$C_{17}H_{10}Cl_4FN_3S$ (449.16)	45.44 (45.46)	2.26 (2.24)	9.35 (9.36)
5g	3,4-Cl_2	176-180 (72)	$C_{17}H_{10}Cl_4FN_3S$ (449.16)	45.45 (45.46)	2.25 (2.24)	9.35 (9.36)
5h	3-Br	114-116 (77)	$C_{17}H_{11}BrCl_2FN_3S$ (459.16)	44.46 (44.47)	2.44 (2.41)	9.14 (9.15)
5i	4-Br	126-128 (74)	$C_{17}H_{11}BrCl_2FN_3S$ (459.16)	44.45 (44.47)	2.43 (2.41)	9.13 (9.15)
5j	4-F	152-154 (69)	$C_{17}H_{11}Cl_2F_2N_3S$ (398.26)	51.25 (51.27)	2.80 (2.78)	10.54 (10.55)
5k	2,4-F_2	172-174 (75)	$C_{17}H_{10}Cl_2F_3N_3S$ (416.25)	49.03 (49.05)	2.44 (2.42)	10.08 (10.09)
5l	3-NO_2	212-214 (66)	$C_{17}H_{11}Cl_2FN_4O_2S$ (425.26)	47.99 (48.01)	2.63 (2.61)	13.15 (13.17)
5m	4-NO_2	234-236 (68)	$C_{17}H_{11}Cl_2FN_4O_2S$ (425.26)	47.99 (48.01)	2.62 (2.61)	13.16 (13.17)
5n	4-OH	174-176 (68)	$C_{17}H_{12}Cl_2FN_3OS$ (396.27)	51.10 (51.13)	3.07 (3.05)	10.59 (10.60)
5o	4-Ph	108-110 (71)	$C_{23}H_{16}Cl_2FN_3S$ (456.37)	60.51 (60.53)	3.54 (3.53)	9.19 (9.21)

Table 2: Anti-bacterial Activity:

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) against Gram +ve and Gram –ve Bacterial Species

Tested compounds (1mg/mL) / Bacterial species	Diameter of Zone of Inhibition (mm±SD)^A			
	Gram +ve Bacteria		Gram -ve Bacteria	
	S. aureus	B. subtilis	P. aeruginosa	K. pneumoniae
5a	-	-	18.2±0.59	11.2±0.42
5b	9.8±0.76	13.8±0.54	12.9±0.67	-

5c	21.9±0.83	17.6±0.67	14.9±0.73	12.4±0.34
5d	9.8±0.65	11.9±0.49	9.4±0.38	10.8±0.33
5e	10.6±0.71	12.6±0.57	10.4±0.45	11.9±0.45
5f	15.7±0.48	10.3±0.43	12.3±0.47	10.2±0.35
5g	14.7±0.67	13.2±0.49	15.9±0.55	11.7±0.57
5h	12.3±0.54	-	10.2±0.43	10.8±0.58
5i	11.4±0.53	10.9±0.53	-	-
5j	20.6±0.74	18.6±0.64	19.2±0.59	17.5±0.67
5k	12.9±0.69	11.3±0.39	10.5±0.44	-
5l	23.6±0.83	16.9±0.53	13.8±0.63	12.03±0.54
5m	26.7±0.75	18.9±0.74	16.7±0.58	13.3±0.46
5n	18.9±0.56	13.3±0.47	13.9±0.75	13.7±0.53
5o	12.9±0.62	-	13.3±0.63	10.2±0.42
[B] Streptomycin	31.5±0.84	33.6±0.78	26.3±1.08	22.4±0.79

[A]Mean values (n = 3)
[B] Reference standard (10 µg/disc) used as reference standard.
'0' Zone of inhibition < 7 mm indicating no sensitivity.

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 (1mg/mL) / fungal species	Diameter of zone of inhibition (mm±SD)[A]			
	A.niger	A. flavus	C. albicans	A.alternata
5a	8.2±0.78	0	11.6±1.09	0
5b	0	9.5±0.76	12.09±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	12.02±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	16.09±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
[C] Nystatin	21.8±1.65	18.6±1.79	21.0±2.07	16.5±1.36

[A]Mean values (n = 3)
[C] Reference standard (10 µg/disc) used as reference standard.
'0' Zone of inhibition < 7 mm indicating no sensitivity.

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
5l	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	104.32	90.72-118.12	0.09	120.25	109.05-131.75	0.087
5o	140.89	123.29-158.99	0.06	131.91	120.9-143.41	0.086
[D] Malathion	8.9	7.1-10.8	1.0	10.42	8.32-12.52	1.0

CI – Confidence Interval
*Relative potency - LC_{50} standard / LC_{50} tested substance
[D] 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).

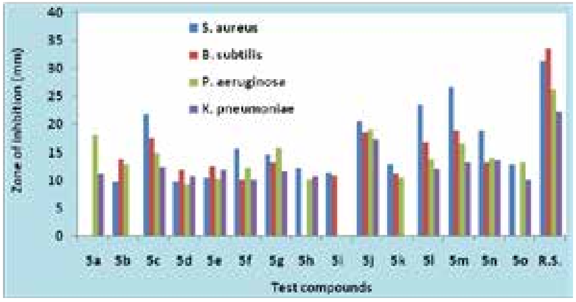


Figure 2. Results of Anti-fungal Activity of 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(aryl)-[1, 3]-thiazoles (5a- 5o) and Nystatin (Reference standard).

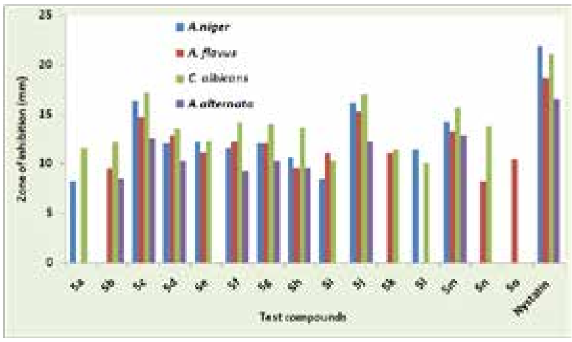
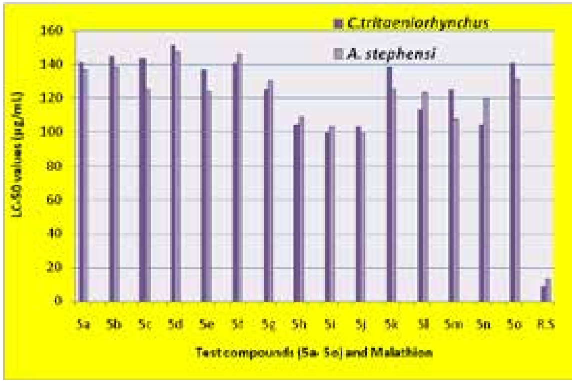


Figure 3. Mosquito-Larvicidal Activity of Hydrozones of 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(aryl)-[1, 3]-thiazoles (5a- 5o) against two pathogenic vectors C. tritaeniorhynchus and A. stephensi.



4.3. 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, ^{1}H- NMR, ^{13}C-NMR, mass spectra and elemental analyses of synthesized Schiff base (thiosemicarbazone) (3)

IR (KBr, cm^{-1}): 3344 and 3279 (-NH$_2$ sym and asym stretch), 3168(-NH), 3028 (Ar-H), 2945(C-CH$_3$), 1568(azomethine, -C=N stretch), 1545 and 1480 (C=C), 1012 (Ar-F), 765 and 721(Ar-Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.29 (3H, s, -CH$_3$), 6.42 (2H, s, - NH$_2$), 7.17 (1H, d, J= 8.8 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.49(1H, d, J=6.4 Hz, 2, 4-Cl$_2$-5-F-phenyl), 8.17 and 8.70 (1H, s, -NH), 10.00(1H, s, -SH). ^{13}C-NMR: 16.97 (-CH$_3$), 29.68 (-C-CH$_3$), 117. 92, 128.22, 129.03, 131.83 and 132.68 (Ar-C atoms) and 179.78 (-C=S atom). LC-MS, [M$^+$], (m/z): 280.0/282.2; Anal. Cald for $C_9H_8Cl_2FN_3S$: C, 53.69; H, 3.18; N, 11.05. Found: C, 53.67; H, 3.21; N, 11.04. m. p. 170-172°C, Yield: 84%

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

4. 3.2.1. 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-phenyl- [1, 3] -thiazole (5a)

IR (KBr, cm^{-1}): 3214 (-NH), 3026 (Ar-H), 2978 (C-CH$_3$), 1568 (azomethine), 1545 and 1480 (C=C), 1014 (Ar-F), 785 and 745 (Ar-Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.21 (s, 3H, -CH$_3$), 6.86 (s, 1H, thiazole ring), 7.1-7.3 (m, 5H, phenyl), 7.35 (d, 1H, J=9 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.39 (d, 1H, J=6.4 Hz, 2, 4-Cl$_2$-5-F-phenyl), 8.41 (s, 1H, -NH). ^{13}C-NMR: 17.05 (-CH$_3$), 105.18 (thiazole-C-5), 149.53 (azomethine), 154.05 and 163.64 (thiazole ring), 125.50 and 127.86 (4C- atoms phenyl ring), 129.86 and 145.92 (C-atoms phenyl), 118.25, 118.47, 121.92, 130.77, 131.89 and 133.90 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 380/382; Anal. Cald for $C_{17}H_{12}Cl_2FN_3S$: C, 53.69; H, 3.18; N, 11.05. Found: C, 53.67; H, 3.21; N, 11.04. m. p. 86-88°C, Yield: 76%

4.3.2.2 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(4-methyl phenyl)-[1, 3]-thiazole (5b)

IR (KBr, cm^{-1}): 3204 (-NH), 3023 (Ar-H), 2976 (C-CH$_3$), 1598 (azomethine), 1555 and 1470 (C=C), 1022 (Ar-F), 765 and 726 (Ar-Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.24 (s, 3H, -CH$_3$), 2.52 (s, 3H, Ar-CH$_3$), 6.89 (s, 1H, thiazole ring), 7.25 (d, 1H, J=9 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.41 (d, 1H, J=6.4 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.46 (d, 2H, J=8 Hz, 4-methylphenyl), 7.75 (d, 2H, J=8 Hz, 4-methylphenyl), 8.51(1H, s, -NH). ^{13}C-NMR: 16.95 (-CH$_3$), 22.45 (Ar-CH$_3$), 104.16 (thiazole-C-5), 148.52 (azomethine), 155.02 and 164.69 (thiazole ring), 125.50 and 130.62 (4C atoms of 4-methylphenyl), 127.81 and 144.91 (2C-atom of 4-methylphenyl), 118.05, 118.37, 121.82, 130.67, 131.85 and 133.70 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 395/397; Anal. Cald for $C_{18}H_{14}Cl_2FN_3S$: C, 54.83; H, 3.58; N, 10.66. Found: C, 54.82; H, 3.60; N, 10.65. m. p. 88-90°C, Yield: 78%

4.3.2.3. 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(4-methoxy phenyl) – [1, 3]-thiazole (5c)

IR (KBr, cm^{-1}): 3384 (-NH), 3021 (Ar-H), 2975 (C-CH$_3$), 1578 (azomethine), 1565 and 1480 (C=C), 1016 (Ar-F), 790 and 741 (Ar-Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.21 (s, 3H, -CH$_3$), 3.75 (s, 3H, Ar-OCH$_3$), 6.91 (s, 1H, thiazole ring), 6.77 (dd, 2H, J=6.8 Hz, 4-methoxylphenyl), 7.27 (dd, 2H, J=6.4 Hz, 4-methoxyphenyl), 7.25 (d, 1H, J=9 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.41 (d, 1H, J=6.8 Hz, 2, 4-Cl$_2$-5-F-phenyl), 8.53 (s,1H, -NH). ^{13}C-NMR: 16.97 (-CH$_3$), 58.52 (-OCH$_3$), 104.26 (thiazole-C-5), 148.52 (azomethine), 155.02 and 164.69 (thiazole ring), 125.50 and 130.62 (4C atoms of 4-methoxyphenyl), 127.81 and 163.91 (C-atom of 4-methoxyphenyl),118.05,118.37,121.82, 130.67, 131.85 and 133.70 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 395/397; Anal. Cald for $C_{18}H_{14}Cl_2FN_3OS$: C, 52.66; H, 3.44; N, 10.24. Found: C, 52.66; H, 3.46; N, 10.22. m. p. 94-96°C, Yield: 75%

4.3.2.4. 4-(2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}- [1, 3]-thiazol-4-yl) benzonitrile (5d)

IR (KBr, cm^{-1}): 3272 (-NH), 3013 (Ar-H), 2967 (C-CH$_3$), 2421 (-C-N stretch),1588 (azomethine), 1561(–C=N), 1557 and 1472 (C=C), 1012 (Ar-F), 787 and 735 (Ar-Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.23 (3H, s, -CH$_3$), 6.87 (1H, s, thiazole ring), 7.23 (1H, d, J=9 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.39 (1H, d, J=6.8 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.47 (2H, dd, J=8 Hz, 4-benzonitrile), 7.78 (2H, dd, J=8 Hz, 4-benzonitrile), 8.54 (1H, s, -NH). ^{13}C-NMR: 17.25 (-CH$_3$), 104.36 (thiazole-C-5), 148.72 (azomethine), 155.22 and 164.59 (thiazole ring), 125.70 and 130.82 (4C atoms of 4-benzonitrile), 165.72 and 144.91 (2C atoms of benzonitrile), 118.15, 118.47, 121.82, 130.77, 131.95 and 133.80 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 405.3/407.3; Anal. Cald for $C_{18}H_{11}Cl_2FN_4S$: C, 53.34; H, 2.74; N, 13.82. Found: 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 (5e)

IR (KBr, cm^{-1}): 3289 (-NH), 3025 (Ar-H), 2969 (C-CH$_3$), 1578 (azomethine), 1527 and 1395 (C=C), 1018 (Ar-F), 791, 775 and 746 (Ar-Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.35 (3H, s, -CH$_3$), 7.14 (1H, s, thiazole ring), 7.30 (1H, d, J=9.2 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.83(1H, d, J=7.81 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.86 (2H, dd, J=2 Hz, 4-chlorophenyl), 7.94 (2H, dd, J=2 Hz, 4-chlorophenyl), 8.51(1H, s, -NH). ^{13}C-NMR: 17.85 (-CH$_3$), 107.25 (thiazole-C-5), 143.98 (azomethine), 156.24 and 169.49 (thiazole ring), 130.49 and 131.47 (4C atoms of 4-chlorophenyl), 119.02, 119.26, 123.07, 125.34, 128.10, 128.17, 129.18 and 139.29 (Ar-C atoms). LC-MS, [M$^+$], (m/z): 415.7; Anal. Cald for $C_{17}H_{11}Cl_3FN_3S$: C, 49.23; H, 2.67; N, 10.13. Found: C, 49.21; H, 2.69; N, 10.12. m. p. 148-150°C, Yield: 71%

4.3.2.6. 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(2, 4- dichloro phenyl)– [1, 3]- thiazole (5f).

IR (KBr, cm^{-1}): 3279 (-NH), 3015 (Ar-H), 2970 (C-CH$_3$), 1568 (azomethine), 1537, 1475 (C=C), 1014 (Ar-F), 792, 767, 748 and 727 (Ar-Cl). 702 (Ar-F). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.36 (3H, s, -CH$_3$), 7.18 (1H, s, thiazole ring), 7.31(1H, d, J=9.2 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.84 (1H, d, J=7.81 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.5 (1H,s, 2, 4-dichlorophenyl), 7.34 and 7.36 (2H, dd, J=2.4 Hz, 2, 4-dichlorophenyl). 8.61 (1H, s, -NH). ^{13}C-NMR: 18.45 (-CH$_3$), 107.25 (thiazole-C-5), 143.98 (azomethine), 156.24 and 169.49 (thiazole ring), 119.02, 119.26, 123.07, 125.34, 128.10, 128.17, 129.18, 130.49, 130.88, 131.47, 132.57 and 139.29 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 450.2/452.2; Anal. Cald for $C_{17}H_{10}Cl_4FN_3S$: C, 45.46; H, 2.24; N, 9.36. Found: C, 45.44; H, 2.26; N, 9.35. m. p. 184-186°C, Yield: 70%

4.3.2.7. 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(3, 4-dichlorophenyl) – [1, 3]- thiazole (5g).

IR (KBr, cm^{-1}): 3281(-NH), 3045 (Ar-H), 2970 (C-CH$_3$), 1578 (azomethine), 1547 and 1485 (C=C), 1015 (Ar-F), 789, 769, 746 and 731(Ar-Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.32 (3H, s, -CH$_3$), 7.14 (1H, s, thiazole ring), 7.21 (1H, d, J=8 Hz 3, 4-dichlorophenyl), 7.25 (1H,d, J=2.4 Hz, 3, 4-dichlorophenyl). 7.70 (1H, d, J=2.4 Hz, 3, 4-dichlorophenyl). 7.51 (1H, d, J=2 Hz, meta coupling 3, 4-dichlorophenyl). 7.31 (1H, d, J=9.2 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.64 (1H, d, J=7.8 Hz, 2,4-Cl$_2$-5-F-phenyl), 8.48 (1H, s, -NH). ^{13}C-NMR: 18.47 (-CH$_3$), 107.28 (thiazole-C-5), 143.99 (azomethine), 156.29 and 169.69 (thiazole ring), 119.22, 119.46, 123.17, 125.54, 128.20, 128.37, 129.48, 130.29, 130.78, 131.57, 132.37 and 139.39 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 450.2/452.2; Anal. Cald for $C_{17}H_{10}Cl_4FN_3S$: 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%

4.3.2.8. 4-(3-bromophenyl)-2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}- [1, 3]- thiazole (5h).

IR (KBr, cm^{-1}): 3234 (-NH), 3014(Ar-H), 2974(C-CH$_3$), 1600 (azomethine), 1560 and 1471 (C=C), 1010 (Ar-F), 777 and 717 (Ar-Cl), 667(Ar-Br). ^{1}H-NMR (DMSO-d$_6$, δ, ppm): 2.30 (3H, s, -CH$_3$), 7.48 (1H, s, thiazole ring), 7.50 (1H, d, J=2Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.55 (1H, d, J=9.6 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.38 (1H, t, 3-bromophenyl), 7.87 (1H, d, J=3.2 Hz, 3-bromophenyl) and 7.89 (1H, d, J=2 Hz, 3-bromophenyl), 8.08 (H, s, 3-bromophenyl), 11.42 (1H, s, -NH). ^{13}C-NMR: 17.92 (-CH$_3$), 106.00 (thiazole-C-5), 144.96 (azomethine), 157.14 and 169.49 (thiazole ring), 130.81 and 131.37 (4 C atoms of 3-bromophenyl), 118.02, 118.26, 122.07, 124.37, 127.10, 128.17, 130.08, and 139.29 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 459.9/461.9; Anal. Cald for $C_{17}H_{11}BrCl_2FN_3S$: C, 44.47; H, 2.41; N, 9.15. Found: C, 44.46; H, 2.44; N, 9.14. m. p. 114-116°C, Yield: 77%

4.3.2.9. 4-(4-bromophenyl)-2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}- [1, 3]- thiazole (5i).

IR (KBr, cm^{-1}): 3234 (-NH), 3013(Ar-H), 2972 (C-CH$_3$), 1600 (azomethine), 1560 and 1471 (C=C), 1006 (Ar-F), 771 and 727 (Ar-Cl), 690 (Ar-Br). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.26 (3H, s, -CH$_3$), 6.90 (1H, s, thiazole ring), 7.28 (1H, d, J=9 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.46 (1H, d, J=6.8 Hz, 2, 4-Cl$_2$-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). ^{13}C-NMR: 16.75 (-CH$_3$), 102.16 (thiazole-C-5), 150.55 (azomethine), 158.02 and 168.69 (thiazole ring), 127.50 and 131.62 (4 C atoms of 4-bromophenyl), 118.05, 118.37, 121.82, 127.71, 130.67, 131.85, 133.70 and 144.94 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 459.9; Anal. Cald for $C_{17}H_{11}BrCl_2FN_3S$: C, 44.47; H, 2.41; N, 9.15. Found: C, 44.45; H, 2.43; N, 9.13. m. p. 126-128°C, Yield: 74%

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

IR (KBr, cm^{-1}): 3214 (-NH), 3023 (Ar-H), 2968 (C-CH$_3$), 1599 (azomethine), 1565 and 1489 (C=C), 1028 and 1007 (Ar-F), 772 and 729 (Ar-Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.27 (3H, s, -CH$_3$), 6.83 (1H, s, thiazole ring), 7.25 (1H, d, J=9 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.41 (1H, d, J=6.4 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.76 (4H, m, 4-fluorophenyl), 8.86 (1H, s, -NH). ^{13}C-NMR: 16.95 (-CH$_3$), 105.16 (thiazole-C-5), 147.52 (azomethine), 154.02 and 163.39 (thiazole ring), 128.62, 135.45 127.81 and 144.91(C atoms of 4-fluorophenyl) 118.05, 118.37, 121.82, 130.67, 131.85 and 133.70 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 398/400; Anal. Cald for $C_{17}H_{11}Cl_2F_2N_3S$: C, 51.27; H, 2.78; N, 10.54. Found: C, 51.25; H, 2.80; N, 10.54. m. p. 152-154°C, Yield: 69%

4.3.2.11. 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(2, 4-difluorophenyl) – [1, 3]-thiazole (5k).

IR (KBr, cm^{-1}): 3201(-NH), 3019 (Ar-H), 2971 (C-CH$_3$), 1597 (azomethine), 1563 and 1479 (C=C),1048, 1027 and 1009 (Ar-F), 770 and 725 (Ar- Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.31 (3H, s, -CH$_3$), 6.87 (1H, s, thiazole ring), 7.27 (1H, d, J=9 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.43 (1H, d, J=6.8 Hz, 2, 4-Cl$_2$-5-F-phenyl), 6.9-7.1 (2H, m, 2,4-difluorophenyl), 7.9- 8.0 (1H, t, 2, 4-difluorobenzaldehyde), 8.84 (1H, s, -NH). ^{13}C-NMR: 16.95 (-CH$_3$), 105.16 (thiazole-C-5), 147.52 (azomethine), 154.02 and 163.39 (thiazole ring), 118.05, 118.37, 121.82, 127.81, 128.62, 130.67, (C atoms of 2, 4-difluorophenyl), 131.85 133.70, 135.45, 137.81, 138.92 and 144.91 (Ar-C atoms). LC-MS, [M$^+$], (m/z): 417.2/419.2; Anal. Cald for $C_{17}H_{10}Cl_2F_3N_3S$: C, 49.05; H, 2.42; N, 10.09. Found: C, 49.03; H, 2.44; N, 10.08. m. p. 172-174°C. Yield: 70%

4.3.2.12. 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(3-nitrophenyl) – [1, 3]- thiazole (5l).

IR (KBr, cm^{-1}): 3278 (-NH), 3008 (Ar-H), 2977 (C-CH$_3$), 1598 (azomethine), 1572 and 1471 (NO$_2$ asymmetric and symmetric stretch) 1512 and 1436 (C=C), 1010 (Ar-F), 771 and 744 (Ar-Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.31 (3H, s, -CH$_3$), 7.64 (1H, s, thiazole ring), 7.56(1H, d, J=9.4 Hz, 2, 4-Cl$_2$-5-F-phenyl), 8.13 and 8.16 (1H, dd, J=2 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.71 (1H, t, 3-nitrophenyl), 7.87 (1H, d, J= 7.2Hz, 3-nitrophenyl), 8.30 (1H, d, J= 8Hz, 3-nitrophenyl), 8.71 (1H, s, 3-nitrophenyl), 11.51 (1H, s, -NH). ^{13}C-NMR: 17.91 (-CH$_3$), 107.05 and 144.98 (azomethine), 157.24 and 170.49 (thiazole ring), 130.89 and 131.47 (C atoms of 3-nitrophenyl), 119.02, 119.26, 123.07, 125.34, 128.10, 129.17, 130.08 and 139.29 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 425/427; Anal. Cald for $C_{17}H_{11}Cl_2FN_4O_2S$: C, 48.01; H, 2.61; N, 13.17. Found: C, 47.99; H, 2.62; N, 13.16. m. p. 212-214°C, Yield: 68%

4.3.2.13. 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(4-nitrophenyl) – [1, 3]- thiazole (5m).

IR (KBr, cm^{-1}): 3329 (-NH), 3020 (Ar-H), 2967 (C-CH$_3$), 1598 (azomethine), 1566 and 1471 (NO$_2$ asymmetric and symmetric stretch) 1517 and 1390 (C=C), 1012 (Ar-F), 763 and 717 (Ar-Cl). ^{1}H-NMR (CDCl$_3$-d, δ, ppm): 2.33 (3H, s, -CH$_3$), 7.13 (1H, s, thiazole ring), 7.29 (1H, d, J=9.2 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.84 (1H, d, J=7.84 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.95 (2H, dd, J=2 Hz, 4-nitrophenyl), 8.26 (2H, dd, J=2 Hz, 4-nitrophenyl), 8.71 (1H, s, -NH). ^{13}C-NMR: 17.91 (-CH$_3$), 107.05 (thiazole-C-5), 144.98 (azomethine), 157.24 and 170.49 (thiazole ring), 130.89 and 131.47 (4C atoms of 4-nitrophenyl), 119.02, 119.26, 123.07, 125.34, 128.10, 129.17, 130.08 and 139.29 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 425/427; Anal. Cald for $C_{17}H_{11}Cl_2FN_4O_2S$: C, 48.01; H, 2.61; N, 13.17. Found: C, 47.99; H, 2.62; N, 13.16. m. p. 234-236°C, Yield: 68%

4.3.2.14. 4-(2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}- [1, 3] -thiazol-4-yl) phenol (5n).

IR (KBr, cm^{-1}): 3580 (-OH), 3234(-NH), 3012 (Ar-H), 2971 (C-CH$_3$), 1600 (azomethine), 1560 and 1471 (C=C), 1014 (Ar-Br), 772 and 729 (Ar-Cl). ^{1}H-NMR (DMSO-d$_6$, δ, ppm): 2.29 (3H, s, -CH$_3$), 7.03 (1H, s, thiazole ring), 7.54 (1H, d, J=9.6 Hz, 2, 4-Cl$_2$-5-F-phenyl), 7.87 (1H, d, J=6.8 Hz, 2, 4-Cl$_2$-5-F-phenyl), 6.79 (2H,d, J=8.4 Hz, 4-hydroxyphenyl), 7.67 (2H,d, J=8 Hz, 4-hydroxyphenyl), 9.54 (1H, s, -NH), 11.34 (1H, s, -OH). ^{13}C-NMR: 15.95 (-CH$_3$), 104.16 (thiazole-C-5), 152.55 (azomethine), 156.02 and 169.69 (thiazole ring), 128.50 and 132.62 (4C atoms of 4-hyroxyphenyl), 119.15, 119.57, 123.82, 128.81, 131.77, 132.95, 134.70 and 145.94 (Ar-C-atoms). LC-MS, [M$^+$], (m/z): 396/398; Anal. Cald for $C_{17}H_{12}Cl_2FN_3OS$: C, 51.13; H, 3.05; N, 10.60. Found: C 51.10; H, 3.07; N, 10.59. m. p. 174-176°C, Yield: 68%

4.3.2.15. 4-(biphenyl-4-yl)-2-{2-[1-(2, 4-dichloro-5-fluorophenyl) 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 LC_{50} 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-bromophenyl)-2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl} -1, 3-thiazole (5i) and 2-{2-[1-(2, 4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(4-fluorophenyl) - 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-fluorophenyl) ethylidene] hydrazinyl}-4-(4-fluorophenyl)-1, 3-thiazole (5j) is found to possess broad spectrum anti-bacterial 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 fungi. The compound (5m) is most potent among all the novel derivatives and hence, 2-{2-[1-(2,4-dichloro-5-fluorophenyl) ethylidene] hydrazinyl}-4-(4-nitrophenyl)-1, 3-thiazole (5m) may be considered as anti-fungal drug candidates for further toxicity and clinical studies.

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