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Synthesis, spectral characterization and larvicidal activity of acridin-1(2*H*)-one analogues

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ABSTRACT

Acridin-1(2*H*)-one analogue of 7-chloro-3,4-dihydro-9-phenyl-2-[(pyridine-2yl) methylene] acridin-1(2*H*)-one, **5** was prepared by using 7-chloro-3,4-dihydro-9-phenylacridin-1(2*H*)-one, **3** and picolinaldehyde, **4** in the presence of KOH at room temperature. These compounds were characterized by analytical and spectral analyses. The purpose of the present study was to assess the efficacy of larvicidal and repellent activity of synthesized 7-chloro-3,4-dihydro-9-phenyl-acridin-1(2*H*)-one analogues such as compounds **3** and **5** against the early fourth instar larvae of filariasis vector, *Culex quinquefasciatus* and Japanese encephalitis vector, *Culex gelidus* (Diptera: Culicidae). The compound exhibited high larvicidal effects at 50 mg/L against both the mosquitoes with LC_{50} values of 25.02 mg/L ($r^2 = 0.998$) and 26.40 mg/L ($r^2 = 0.988$) against *C. quinquefasciatus* and *C. gelidus*, respectively. The 7-chloro-3,4-dihydro-9-phenyl-acridin-1(2*H*)-one analogues that are reported for the first time to our best of knowledge can be better explored for the control of mosquito population. This is an ideal ecofriendly approach for the control of Japanese encephalitis vectors, *C. quinquefasciatus* and *C. gelidus*.

Introduction

The *Culex quinquefasciatus* is the most widely distributed mosquito in India, mainly found in urban and suburban areas. Synthetic chemical larvicides continue to be applied for controlling mosquitoes in most parts of the world. But many of these chemicals are toxic to human, plant and animal life and resistance can be problematic in maintaining control, especially with organophosphate and pyrethroid larvicides [1]. The *C. quinquefasciatus*, a vector of lymphatic filariasis, India, is endemic for lymphatic filariasis caused by *Wuchereria bancrofti* [2]. Lymphatic filariasis is a widely distributed tropical disease with around 120 million people infected worldwide. Japanese encephalitis (JE) is a disease caused by an arbovirus that is mainly transmitted by the bite of infected *Culex tritaeniorhynchus* mosquitoes. The annual incidence and mortality estimates for JE are 30,000 to 50,000 and 10,000, respectively, [3]. The methanol extracts of dried root powder of *Rhinacanthus nasutus* was tested against *A. aegypti* and *C.quinquefasciatus* larvae [4]. The petroleum ether (60–80 °C) extracts of the leaves of *Vitex negundo* were evaluated for larvicidal activity against larval stages of *C. tritaeniorhynchus* [5]. Despite variation in geographic distribution of the virus, mosquito vector species is relatively constant. The most important mosquito vector in Asia is *C. tritaeniorhynchus*, which breeds in stagnant waters like paddy fields or drainage ditches [6]. Other species are *Culex vishnui* (India), *C. gelidus* and *Culex fuscocephala* [7] (India, Malaysia and Thailand). The adults are mainly exophilic and often stay indoors before and after feeding on blood [8]. They are mainly zoophagous but also feed on man. *C. tritaeniorhynchus* can also act as a vector of filariasis.

Insecticide resistance is increasingly becoming a problem for malaria vector control programmes. Widespread use of the same insecticides in the agricultural sector has made the situation worse. Resistance may develop due to changes in the mosquito's enzyme systems, resulting in more rapid detoxification or sequestration of the insecticide, or due to mutations in the target site preventing the insecticide–target site interaction [9]. Insecticides that can be used in malaria control are increasingly becoming limited. *C. tritaeniorhynchus* was found susceptible to permethrin and resistant to DDT, dieldrin, fenitrothion and propoxur [10] and resistant to organophosphorous insecticides [11]. The aphid control measures have largely been depending on the use of chemical pesticides, including chlorinated hydrocarbons, organophosphates and carbamates [12], which cause resistant development in the target population [13].

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There are numerous biologically active fused heterocyclic rings. Among these acridone and its analogues are one such scaffold known to associate with several biological activities [14] such as amsacrine (cytotoxic and antiviral agents) [15], clomacran (tranquilizer), monometacrine (antidepressant), acridine carboxamide (antitumour) [16] and in natural products such as plakinidine A and B (anthelmintic) [17], dercitin (anticancer) [18]. Among the naturally occurring compounds, chalcones and their synthetic analogues possess good biological properties. They are also effective *in vivo* as cell proliferating inhibitors, anti-tumour promoting and chemo preventing agents. Since a number of clinically useful anticancer drugs have genotoxic effects due to interaction with the amino groups of nucleic acids, chalcones may be devoid of this important side effect [19]. Some chalcones are a very important class of compounds from a biological point of view, particularly as anti-oxidants, anti-inflammatory, pulmonary carcinogens inhibitors, antimalarial and anti-leishmanials [20]. Chalcones are key precursors in the synthesis of a large array of biologically important heterocycles. Thus, the synthesis of chalcones has generated a vast interest to organic/medicinal chemists.

Among the various methods solvent-free chemical reactions are gaining importance due to the advantages and environmentally friendly processes they offer, as compared to conventional reactions [21]. In recent years organic reactions in the solid state have been attracting the synthetic organic chemists because of their simplicity and synthetic value [22,23]. Therefore, these facts led us to investigate the neat reaction [24]. It has many advantages including, reduced pollution, low costs and simplicity in process and handling. In this report we have achieved 7-chloro-3,4-dihydro-9-phenyl-2-[(pyridine-2yl) methylene] acridin-1(2*H*)-one, **5** using green chemical approach. The efficacy of larvicidal and repellent activity of synthesized compounds **3** and **5** against the early fourth instar larvae of filariasis vector that is, *C. quinquefasciatus*, Japanese encephalitis vector, that is, *C. gelidus* is reported here.

Materials and methods

Chemicals and instruments

Solvents and reagents were commercially sourced and used without further purification. Thin layered chromatography (TLC) was performed on preparative plates of silica gel. Visualization was made with iodine chamber. Column chromatography was performed by using silica gel (60−120 mesh). Melting points were measured on Elchem Microprocessor based DT apparatus using an open capillary tube and are corrected with standard benzoic acid. The NMR spectra were recorded on a Bruker Advance III – 500 MHz spectrometer using TMS as internal standard (chemical shifts δ in ppm). Mass was recorded on Finnigan Mat 8230 Mass Spectrometer.

Chemistry

The synthetic strategy leading to the key precursor **1** and **2** and the target compounds **3** and **5** are illustrated in Fig. 1. The key intermediates, 7-chloro-3,4-dihydro-9-phenylacridin-1(2*H*)-one, **3** was prepared by using 2-amino-5-chlorobenzophenone, **1** with cyclohexane-1,3-dione, **2** in the presence of glacial CH_3COOH and Conc. H_2SO_4 at 150 °C for 6 h. The compound **5** was prepared by the reaction of compound **3** with picolinaldehyde, **4** in the presence of KOH.

*General procedure for the synthesis of 7-chloro-3,4-dihydro-9-phenyl-acridin-1(2H)-one analogues, **3**, **5***

*7-Chloro-3,4-dihydro-9-phenylacridin-1(2H)-one, **3***

In a round bottom flask, a mixture of 2-Amino-5-chloro benzophenone, **1** (0.01 mol), 1, 3-hexanedione, **2** (0.012 mol), glacial acetic acid (10 mL) and conc.H_2SO_4 (3–4 drops) were heated at 150 °C for 6 h. The completion of the reaction was monitored by TLC.

Then the reaction mixture was cooled at room temperature followed by this mixture was added to the crushed ice with drop by drop addition. The solid formed was allowed to stand for 30 min and filtered off to dryness. Then the compound was recrystallized using ethanol. The structure of the recrystallized compound **3** was confirmed by ^{1}H NMR ^{1}H NMR, ^{13}C NMR and Mass analysis.

*7-Chloro-3,4-dihydro-9-phenyl-2-[(pyridine-2yl) methylene] acridin-1(2H)-one, **5***

About 7-chloro-3,4-dihydro-9-phenylacridin-1(2*H*)-one, **3** (0.001 mol) and picolinaldehyde, **4** (0.001 mol) were taken in a mortar. KOH (5 pellet) was added to the mortar and grind well and leave it for 5 h at room temperature to get compound **5**. The completion of the reaction was monitored by checking TLC. Then the reaction mixture was cooled at room temperature followed by this mixture was added to the crushed ice with drop by drop

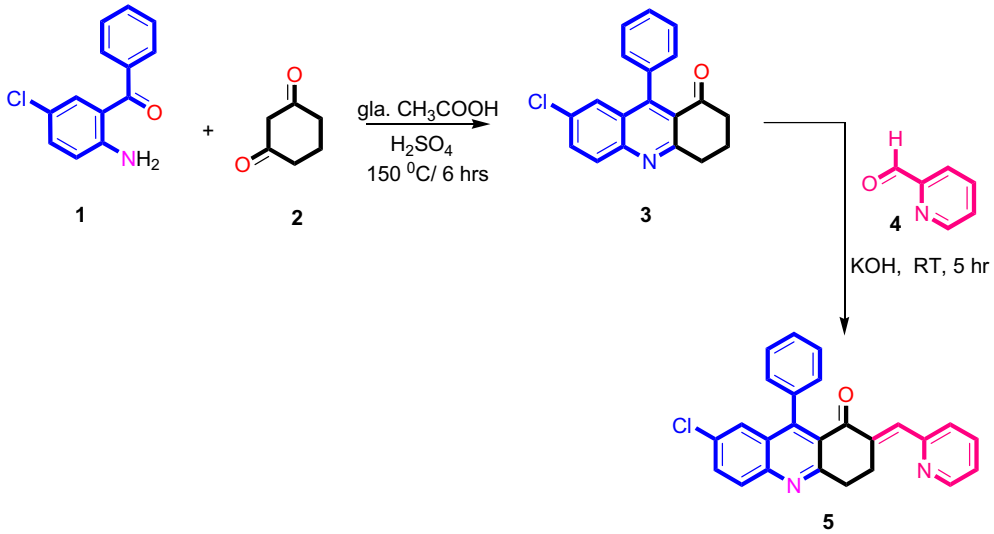

Fig. 1. Synthesis of 7-chloro-3,4-dihydro-9-phenyl-2-pyridine2yl)methylene]acridin-1(2*H*)-one, **5**.

addition. The solution was neutralized by the addition of dil. HCl. Then the precipitate was allowed to settle down and filtered off and dried. The product so obtained was purified by performing column chromatography using petroleum ether and ethylacetate in the ratio of 9:1. Purified compound was confirmed by ^{1}H NMR, ^{13}C NMR and Mass analysis.

Mosquito larvicidal assay

Insect rearing

The *C. quinquefasciatus* and *C. gelidus* larvae were collected from rice field and stagnant water area of Melvisharam (12° 56′23″ N, 79°14′23″ E) and identified in Zonal Entomological Research Centre, Vellore (12°55′48″ N, 79°7′48″ E), Tamil Nadu, to start the colony, and larvae were kept in plastic and enamel trays containing tap water. They were maintained and reared in the laboratory as per the reported method [25].

Larvicidal bioassay

During preliminary screening with the laboratory trial, the larvae of *C. quinquefasciatus* and *C. gelidus* were collected from the insect-rearing cage and identified in the Zonal Entomological Research Centre, Vellore. For the bioassay test, mosquito larvae were taken in five batches of 20 in 250 mL of water and Physicochemical data of synthesized compounds **3** and **5**. The control was set up with dechlorinated tap water. The number of dead larvae was counted after 24 h of exposure, and the percentage of mortality was reported from the average of five replicates. The experimental media in which 100% mortality of larvae occurs alone were selected for dose response bioassay.

Dose response bioassay

The synthesized compounds **3** and **5** toxicity test was performed by placing 20 mosquito larvae into 200 mL of sterilized double-distilled water with nanoparticles into a 250-mL beaker (Borosil). The synthesized compound solutions were diluted using double-distilled water as a solvent according to the desired concentrations (50 and 10 mg/L). Each test included a set of control group (distilled water) with five replicates for each individual concentration. The numbers of dead larvae were counted after 24 h of exposure *C. quinquefasciatus* and *C. gelidus*, the percentage mortality was reported from the average of five replicates. However, at the end of 24 h the selected test samples turned out to be equal in their toxic potential.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit were calculated [26]. Results with $p < 0.05$ were considered to be statistically significant.

Results

*Spectral data of compounds **3** and **5***

The nature of the compound **3** is yellow in colour with 81% yield, and the melting point is 185 °C. The spectral data of compound **3** as ^{1}H NMR ($CDCl_3$): δ (ppm) 2.24–2.31 (q, 2H, $-CH_2$), 2.72 (t, 2H, $-CH_2$), 3.38 (t, 2H, $-CH_2$), 7.16–7.19 (m, 2H), 7.43–7.44 (d, 1H), 7.52–7.54 (q, 3H), 7.69–7.72 (q, 1H), 8.01 (t, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 21.2, 34.4, 40.5, 124.4, 126.7, 127.9, 128.3, 130.0, 132.4, 132.6, 136.8, 146.9, 162.5, 197.6. HRMS is the further supported data for finding the formation of expected compound **3**, and we have observed the molecular ion peak around 307.0764. The fragmentation as follows 307.0764 (5%), 281.0728 (17%), 219.2697 (10%), 155.2955 (21%), 143.2400 (100%), 128.2381 (100%), 112.3339 (30%), 100.3460 (75%), 80.3947 (100%), 74.4690 (80%), 61.2416 (100%).

The melting point that we have observed for compound 5 is 191 °C with 92% yield. The NMR report of compound **5** is supported for the compound confirmation. ^{1}H NMR ($CDCl_3$): δ (ppm) 2.24–2.31 (q, 2H, $-CH_2$), 2.72 (t, 2H, $-CH_2$), 3.38 (t, 2H, $-CH_2$), 7.16–7.19 (m, 2H), 7.43–7.44 (d, 1H), 7.52–7.54 (q, 3H), 7.69–7.72 (q, 1H), 8.01 (t, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 25.4, 33.2, 122.9, 126.6, 127.5, 128.4, 132.4, 134.3, 136.4, 136.6, 139.1, 146.9, 149.5, 150.7, 155.0, 161.9, 187.9. Further this target molecule has been confirmed by

Table 1
Mosquito larvicidal activity of compounds **3** and **5** against the early fourth instar larvae of *C. quinquefasciatus* and *C. gelidus*.

Compounds	Species	Concentrations (mg/L)	% Mortality (mg/L) ± SD	95% Confidence interval for LC_{50} (LCL–UCL)[a] (mg/L)	Slope	r^2
	C. quinquefasciatus	50	62			
		40	44			
3		30	31	42.78	31	0.989
		20	16	37.5–48.78		
		10	07			
	C. gelidus	50	59			
		40	38			
		30	26	45.44	14	0.978
		20	14	42.1–48.95		
		10	04			
	C. quinquefasciatus	50	100			
		40	83			
5		30	52	25.02	34	0.998
		20	34	22.5–27.76		
		10	17			
	C. gelidus	50	100			
		40	79			
		30	56	26.40	56	0.988
		20	31	23.7–28.59		
		10	20			

[a] LCL, lower confidence level; UCL, upper confidence level.

High Resonance Mass Spectrometry. The calculated mass is 396.1029 but the actual mass that we have observed through HRMS is 396.1028. The fragmentation is as follows 396.1028 (37%), 302.2106 (13%), 351.0925 (13), 292.9968 (25%), 229.0848 (40%), 209.0697 (100%), 79.1620 (55%), 190.0011 (15%), 135.1195 (20%).

*Mosquito larvicidal activity of compounds **3** and **5***

In the present study, mosquito's larvae were exposed to varying concentrations of synthesized compounds **3** and **5** for 24 h. The larval percent mortality observed in green synthesis of synthetic compound **3** was 07, 16, 31, 44 and 62; 04, 14, 26, 38 and 59 at 50 mg/L against *C. quinquefasciatus* and *C. gelidus* respectively. Values of the efficacy of compound **3** were against *C. quinquefasciatus* (LC_{50} = 42.78 mg/L; r^2 = 0.989) and *C. gelidus* (LC_{50} = 45.44 mg/L; r^2 = 0.978). The larval percent mortality observed in green synthesis of synthetic compound **5** was 17, 34, 52, 83 and 100; 20, 31, 56, 79 and 100 at 50 mg/L against *C. quinquefasciatus* and *C. gelidus*, respectively. The maximum efficacy was observed in compound **5** against the larvae of *C. quinquefasciatus* (LC_{50} = 25.02 mg/L; r^2 = 0.998) and *C. gelidus* (LC_{50} = 26.40 mg/L; r^2 = 0.988), respectively. In the present study, the larvicidal activity results showed the highest mortality in compound **5** than **3**. The control (distilled water) showed nil mortality in the concurrent assay. The chi-square value was significant at $p \leqslant 0.05$ level. The complete mortality was observed for compounds **3** and **5** for larvae of *C. quinquefasciatus* and *C. gelidus* at 50 mg/L (Table 1).

Discussion

*Spectral data of compounds **3** and **5***

The ^{1}H NMR spectra of compound **3** show three peaks in aliphatic region such as three doublet at δ 2.72, 3.38 and one quartet at δ 2.24–2.31 corresponding to three $-CH_2$ protons. When we are comparing the ^{1}H NMR spectra of compound **3** with compound **5** there is a difference in the aliphatic range that is, disappearance of C_2 proton ($-CH_2$) which is the evidence for the formation of compound **5**. Further it was supported by ^{13}C NMR and HRMS.

*Larvicidal studies of compounds **3** and **5***

All the compounds **3** and **5** were screened for the efficacy of larvicidal and repellent activity against the early fourth instar larvae of filariasis vector (*C. quinquefasciatus*) and Japans encephalitis vector (*C. gelidus*) at 10–50 mg/mL. The compounds exhibited high larvicidal effects at 50 mg/L against both the mosquitoes with LC_{50} values of 25.02 mg/L (r^2 = 0.998) and 26.40 mg/L (r^2 = 0.998) against *C. quinquefasciatus* and *C. gelidus* respectively. To our best of knowledge there is no report in the literature for the control of mosquito population by using compounds **3** and **5**. This is an ideal eco-friendly approach for the control of filariasis vector, *C. quinquefasciatus* and Japanese encephalitis vector, *C. gelidus*.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2012.04.015.

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