3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones as potential larvicidal agents

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Abstract: The larvicidal effect of series of 3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones, **5a-e,** against *Chironomus tentans* Fabricius has been investigated. The results showed that tested compounds demonstrated strong larvicidal activity, and caused high percentage of mortality after 24 h at the doses of 40-100 μg/ml, especially in the case of 3-[(2-chloro-8-methyquinolin-3-yl)methyl]quinazolin-4(3*H*)-one, **5b,** that act as a promising larvicidal agent.

Keywords: Larvicidal activity, 3-[(2-chloroquinolin-3-yl)methyl]quinoline-4(3*H*)-ones, synthesis.

INTRODUCTION

Chironomidae family which include more than 5000 species are globally distributed, most abundant group of insects found in freshwater ecosystems (Thienemann *et al*., 1954). The aquatic midge (*Chironomus tentans* Fabricius), plays significant role in food web and environmental pollutants in the aquatic systems (Anderson *et al*., 2006). Chironomid larvae were widely used as fish food in Germany. Allergic diseases (Baur *et al*., 1982, Galindo et al., 1999) were observed in human beings, contamination of drinking water was also observed (Langton *et al*., 1988) due to the exposure of the larvae. They were considered as ecologically important invertebrates (Cranston *et al*., 1995) and are attractive animal model, popular in laboratory study of chemical-induced multiple biological level responses as they spend the longest period of their life cycle as larvae.

The present study was designed as short term experiments under controlled laboratory conditions, using *C. tentans* as a biological model system to demonstrate the percentage of mortality.

Numerous synthetic and natural organic compounds were investigated and proved to possess high larvicidal activity (Knowles *et al*., 1972; Begum *et al.,* 2011; Wang *et al.,* 2011) including, organophosphates (naled, chlorpyrifos parathion, mevinphos, azinphosmethyl), carbamates (fenothiocarb and aldicarb), nitrophenol derivatives (dinocap, binapacryl, dinobuton and dinitrocyclohexylphenol) and tectoquinone (Cheng *et al*., 2008), *p*-cymene (Kordali *et al*., 2008). However, only a small number of high level larvicidal activity with the safe environmental properties are required in agriculture (Kordali *et al*., 2008). As a part of our research work on synthesis of substituted quinolinones attached with biological properties (Roopan *et al.,* 2010a), we describe the synthesis and larvicidal activity of 3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones.

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MATERIALS AND METHODS

Chemicals and instruments

Solvents and reagents were commercially obtained from S.D. Fine chemicals and Aldrich Chemicals (India) sourced and used without further purification with the exception of THF, which was freshly distilled over sodium. Thin layer chromatography (TLC obtained from S.D. Fine, India) 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 taken on Elchem Microprocessor based DT apparatus in open capillary tubes and are corrected with benzoic acid. FTIR spectra were obtained on a Nucon Infrared spectrophotometer using KBr pellets. The NMR spectra were recorded on a Bruker Avance III - 500 MHz spectrometer using TMS as internal standard (chemical shifts δ in ppm). High resolution mass spectra (HRMS) were obtained using JEOL GC MATE II HRMS (EI) mass spectrometry.

Chironomus larvae

Chironomus tentans purchased from the Zoology specimen supplier, Vellore, Tamil Nadu, India, and maintained at 27°C.

Synthesis

The synthetic strategy leading to the key precursor and the target compounds are illustrated in **Scheme 1**. The key intermediates, 2-chloro-3-(chloromethyl)quinolines, **3a-e** were prepared from 2-chloro-quinoline-3-carbaldehydes, **1** (Khan *et al*., 2009, Roopan *et al*., 2010b) through (2-chloroquinolin-3-yl)methanol, **2** (Roopan *et al*., 2009). The compounds **5a–e** were prepared by *N*-alkylation of 4(3*H*)-quinazolinone, **4**. To a stirred solution of 4(3*H*)-quinazolinone, **4**, (1 mmol in 2ml of DMF) maintained at 110°C, KO^{t}Bu (1 mmol in 10 ml of THF) and 2-chloro-3-

(chloromethyl) quinolines, **3** (1 mmol) were added and continued the stirring until completion of the reaction (monitored by TLC). The structures of the purified compounds, **5** were determined by NMR and HRMS analyses (table 1).

Larvicidal bioassay

Newly molted, red head stage (0–24 h) last-instar larvae of *C. tentans* were selected and individually kept in 2-ml sterile aqueous concentrations in wells of sterile castor tissue culture plates. A minimum of five dilutions such as 20, 40, 60, 80, and 100 µg/ml of test compounds were prepared in distilled water from the stock 1 mg/ml in DMSO solution. Three replicates with 6 larvae were minimally tested for every concentration. Mortality counts were made after 24 h of treatment. Concentration–mortality curves were plotted and the LC_{50} values (µg/ml) were estimated by regression analysis. DMSO–water mixture was used as a control. For comparison commercial fluconazole (1 mg/ml) was used as the positive control. The dead larvae were counted after 24 h and percentage mortality is reported from the average of three replicates taken together. Larvicidal activity was evaluated 24 h after treatment. Larvae were considered to be dead if appendages did not move when prodded with a wooden dowel. The percentage of mortality was corrected for control mortality and effectiveness was reported as LC_{50} which represent the concentrations in µl with 50% larvae mortality in 24 h Fig. 1).

Statistical analysis

All results were expressed as mean ± SD (n = 3). The percentages of mortality were determined and transformed to descriptive statistics using Sigma plot 11.0. Mortality data collected after 24 h exposure in different dilutions of **5a-e** were used to determine the lethal concentration for 50% (LC_{50}) values of the respective species and reported in table 2.

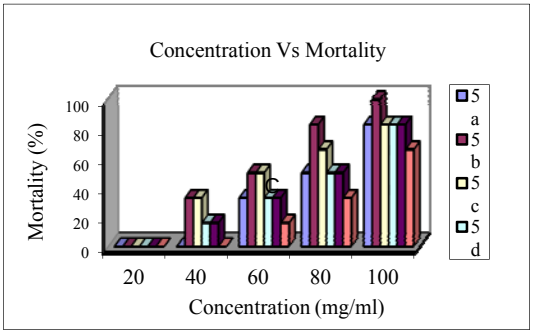
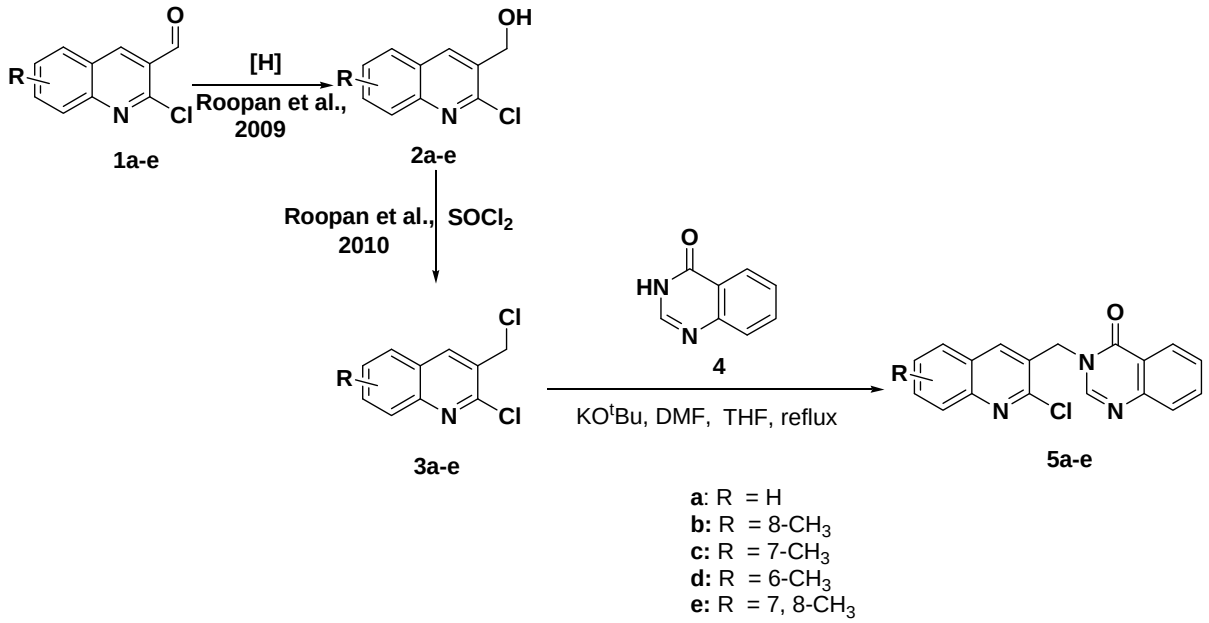


Fig. 1: Larvicidal activity of compounds **5a-e** against *C. tentans*

The results on larvicidal effect of 3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones, **5** are presented in table 2. Most of the tested compounds were moderately active against *C. tentans* at concentration of 40 µg/ml. However, increasing the concentration up to 100 µg/ml led to an increase in the larvicidal activity. Interestingly, the methyl substitution in C_8-position as in compound **5b** increased the larvicidal activity up to 100% mortality. The mortality result clearly indicates that 100 µg/ml of derivatives, **5a-e** showed high larvicidal activity against the *C. tentans* compared to standard Fluconazole. When we are introducing methyl group in the phenyl ring at 40(µg/ml)



Scheme 1: Synthesis of 3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones, **5a-e**

itself itshowing the property when comparted to the standard. Methyl group introduced in the 8th position (5d) will show higher property compared to 6th (5b) and 7th (5c) position. We introduced two methyl group in 7th and 8th position (5e) its showing same property of compound 5d. The results indicate that substitution in the quinoline ring of **5b** exhibited 83.33% mortality at 100 μg/ml. The methyl group when substituted in C_6 or C_7 position (**5c** and **5d**), for 40 μg/ml, moderate activity was observed respectively with 33.33 and 16.66 % mortality, however there was no difference in their activity at 100 μg/ml. The introduction of methyl group in the C_8-position (**5b**), interestingly showed 100 % mortality at 100 μg/ml. Based on this observation simultaneous introduction of methyl group in C_7 and C_8-position **5e** was done and the larvicidal effect showed no remarkable change when compared to **5c** and **5d**.

Table 1: Spectral characterization of 3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones, **5a-e**

Entry	M.p. (ºC)	Mol.formula	^{1}H NMR (δ ppm)	HRMS (M^+)
5a	188 [Roopan et al. 2011]	$C_{18}H_{12}ClN_3O$	IR (KBr pellets, cm^{-1}) ν: 1681, 1658; ^{1}H NMR: 8.57 (s, 1 H), 8.18 – 8.13 (t, 2 H), 8.02 – 7.94 (dd, J = 6.4, 7.9 Hz, 2 H), 7.89 – 7.84 (t, 1 H), 7.81 – 7.73 (dd, J = 7.0, 8.2 Hz, 2 H), 7.63 – 7.54 (m, 2 H), 5.39 (s, 2 H). ^{13}C NMR: 160.7, 148.9, 148.7, 148.5, 146.6, 137.6, 135.0, 131.3, 2 X128.5, 2 X 127.9, 127.8, 127.7, 127.3, 126.6, 122.2, 47.7.	321.6867
5b	178	$C_{19}H_{14}ClN_3O$	IR (KBr pellets, cm^{-1}) ν: 2920, 1675, 1609; ^{1}H NMR: 8.34 (s, 1 H), 8.32 (d, J = 8.0 Hz, 1 H), 8.12 (s, 1 H), 7.92 – 7.90 (d, J = 9.0 Hz, 1 H), 7.82 – 7.77 (m, 2 H), 7.59 – 7.54 (m, 3 H), 5.42 (s, 2 H), 2.52 (s, 3H). ^{13}C NMR: 161.2, 148.3, 148.0, 146.5, 145.9, 138.7, 137.6, 134.6, 133.3, 127.8, 127.7, 127.6, 127.0, 126.8, 2 X 126.6, 122.1, 47.7, 21.5.	335.8340
5c	197	$C_{19}H_{14}ClN_3O$	IR (KBr pellets, cm^{-1}) ν: 2924, 1668, 1611; ^{1}H NMR: 8.35 (s, 1 H), 8.34 – 8.32 (d, J = 8.0 Hz, 1H), 8.18 (s, 1H), 7.82 – 7.77 (m, 3H), 7.71 – 7.69 (d, J = 8.0 Hz, 1H), 7.57 – 7.54 (t, 1H), 7.41 – 7.40 (d, J = 8.0 Hz, 1H), 5.42 (s, 2 H), 2.56 (s, 3H). ^{13}C NMR: 161.2, 149.2, 148.0, 147.6, 146.5, 141.7, 139.1, 134.6, 133.3, 129.8, 127.7, 127.5, 127.2, 126.8, 125.8, 125.0, 122.1, 47.8, 21.9.	335.3824
5d	205	$C_{19}H_{14}ClN_3O$	IR (KBr pellets, cm^{-1}) ν: 2924, 1669, 1609; ^{1}H NMR: 8.34 (s, 1H), 8.32 (d, J = 8.0 Hz, 1 H), 8.12 (s, 1 H), 7.92 – 7.90 (d, J = 9.0 Hz, 1 H), 7.82 – 7.77 (m, 2 H), 7.59 – 7.53 (m, 3 H), 5.42 (s, 2 H), 2.51 (s, 3H). ^{13}C NMR: 161.2, 148.3, 148.0, 146.5, 145.9, 138.7, 137.6, 134.6, 133.3, 127.8, 127.7, 127.6, 127.0, 126.8, 2 X 126.6, 122.1, 47.8, 21.5.	335.5496
5e	192 [Roopan et al. 2011]	$C_{20}H_{16}ClN_3O$	IR (KBr pellets, cm^{-1}) ν: 1680, 1659; ^{1}H NMR: 8.34 – 8.33 (d, J = 5.5 Hz, 1 H), 8.31 (d, 1 H), 8.13 (s, 1 H), 7.81 – 7.75 (m, 2 H), 7.55 – 7.52 (t, 2 H), 7.38 – 7.36 (d, J = 8.5 Hz, 1 H), 5.41 (s, 2 H), 2.69 (s, 3 H), 2.49 (s, 3 H) ^{13}C NMR: 161.2, 148.1, 148.0, 146.5, 139.5, 139.1, 134.5, 133.8, 130.3, 127.6, 127.5, 2 X 126.8, 125.4, 125.2, 124.6, 122.1, 47.7, 20.7, 13.3.	349.4183

Table 2: Mortality of *C. tentans* induced by 3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones[a], **5a-e**

Treatment	Mortality (%)					LC_{50} (μg/ml)
	20(μg/ml)	40(μg/ml)	60(μg/ml)	80(μg/ml)	100(μg/ml)	
5a	-	-	33.33±0.19	50.00±0.11	83.33±0.16	80
5b	-	33.33±0.0	50.00±0.0	83.33±0.19	100±0.19	60
5c	-	33.33±0.0	50.00±0.0	66.66±0.0	83.33±0.0	60
5d	-	16.663±0.0	33.33±0.19	50.00±0.57	83.33±0.69	80
5e	-	16.663±0.0	33.33±0.19	50.00±0.57	83.33±0.69	80
[b]S	-	-	16.663±0.0	33.33±0.57	66.66±0.69	90

[a] Measurements are given as % mortality values±standard deviation. [b]S = Standard (fluconazole)

CONCLUSION

In conclusion, synthetic 3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones may be employed as potential larvicidal agent against *C. tentans*. The larvicidal effects of tested soumpounds, **5a-e**, were more effective in comparison with standard fluconazole. Presented results are promising and of economical and practical importance in an efficient control of *C. tentans* and could be improved in the near future.

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