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Original article

Cytotoxic, larvicidal, nematicidal, and antifeedant activities of piperidin-connected 2-thioxoimidazolidin-4-one derivatives

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ABSTRACT

The objective of this study was to investigate brine shrimp cytotoxicity, larvicidal, nematicidal, and antifeedant activities of novel piperidin-connected 2-thioxo-imidazolidin-4-one derivatives. The activities of target compounds were compared with some naturally occurring (−)-pinidinol, hydantocidin, and positive controls. Target compounds were synthesized via cyclocondensation method. The compounds were synthesized and then characterized by infrared spectroscopy, ^{1}H NMR, ^{13}C NMR, mass spectral, and elemental analyses. Brine shrimp cytotoxicity assay was investigated using freshly hatched, free-swimming nauplii of *Artemiasalina*. Larvicidal screening was performed against urban mosquito larvae (*Culex quinquefasciatus*). Nematicidal activity was evaluated using *juvenile* nematodes of *Meloidogyne javanica*. Regarding antifeedant activity, marine-acclimated *Oreochromis mossambicus* fingerlings were used. Compounds **3a-c** (piperidin-connected 2-thioxoimidazolidin-4-one) were found to be lethal to the second instar larvae of mosquito, which produced LD_{50} values of 1.37, 6.66, 6.51 μg/mL, compared to compounds (−) pinidinol and hyantocidin LD_{50} values of 18.28 and 22.11 μg/mL respectively. Compound **3a-c** was found to kill 100% of fish fingerlings within 6 h at 20 μg/mL, with LD_{50} values of 1.54, 1.79, 1.52 μg/mL, compared to compounds (−) pinidinol and hyantocidin with LD_{50} values of 10.21 and 21.05 μg/mL respectively. Compound **3c** with LD_{50} value of 1.57 μg/mL demonstrated high nematicidal activity compared to compound **3a**, **3b**, (−) Pinidinol and Hyantocidin LD_{50} values of 6.45, 2.42, 14.25, 26.30 μg/mL respectively. Therefore, the 2-thioxoimidazolidin-4-one with piperidin ring showed high potential cytotoxic, larvicidal, nematicidal, and antifeedent activities.

1. Introduction

Literature survey indicated that piperidine derivatives (Fig. 1) have been reported to possess significant pharmacological activities, such as analgesic, anti-inflammatory (Perumal et al., 2001) local anesthetic (Hagenbach et al., 1952), anti-cancer (Ilenna et al., 1985), and antimicrobial activities (Ramalingan et al., 2003). Specifically, substituted piperidine derivatives have been reported to possess effective biological and pharmacological activity (Casy et al., 1976). The imidazolidin-2,4-dione derivatives (Fig. 1) were also reported to exhibit potent activities, such as anticonvulsant (Marton Enisz et al., 1993), fungicidal (Metha et al., 1981), herbicidal (Hanessian et al., 1985), antitumor (Ahmed, 1998), anti-HIV (Comber et al., 1992), and hypolipidemic (Menendez et al., 1992) activities. α-Methyl multi-substituted piperidines ((−)-pinidinol) is a naturally occurring alkaloids, which have demonstrated interesting pharmacological properties (Strunz and Findlay, 1985). In addition, hydantocidin, isolated from *Streptomyces hygroscopicus* showed potent non-selective herbicidal activity (Nakajima et al., 1991).

The brine shrimp lethality test (BST) has been used as a simple and useful tool for toxicity screening (Carballo, 2002) of active plant extracts (Okoro et al., 2012) synthesized compounds, fungal toxins, heavy metals, and pesticides. It has been demonstrated that a positive relationship exists between brine shrimp lethality and human carcinomas. Thus, result of BST can also be extrapolated for cell line toxicity and antitumor activity (Andeson, 1991). In

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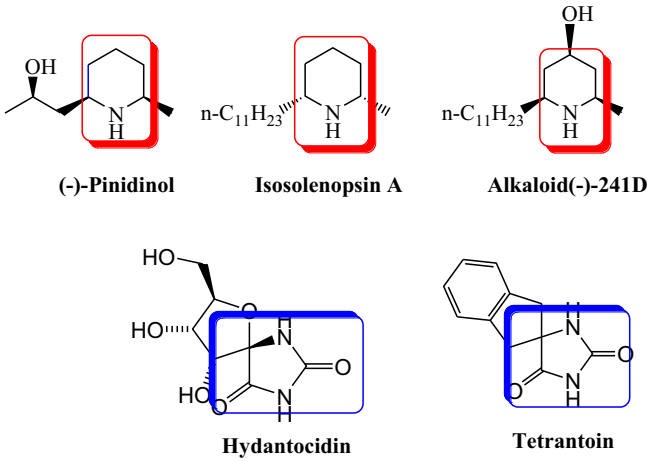


Fig. 1. Natural piperidine and imidazolidin-2,4-dione bioactive compounds.

addition, thymol, a natural monoterpene phenol derivative, is used as a rapidly degrading, non-persisting pesticide (Hu et al., 2008).

Mosquito larvae also are controlled with insecticides (Yang et al., 2002; Sun et al., 2010; Talontsi et al., 2011) and the best larvicides are natural products and heterocyclic compounds. For example, *N′-tert*-butyl-*NN′*-dibenzoylhydrazine (RH-5849) was reported as the first nonsteroidal ecdysone agonist in the mid-1980s (Wing et al., 1988). The mosquito borne diseases not only cause high levels of morbidity and mortality but also cause great economic loss and social chaos in the developing countries including costs of health care. Recent figures from the World Health Organization (WHO) evidenced that malaria accounts for at least 500 million infections and 3 million deaths annually. The prevalence of dengue fever has increased over the last 50 years, and over 2 billion people are under risk in more than 100 countries (Manilal et al., 2011). In this juncture, there is an urgent need to develop new insecticides that are more environmentally safe and specific against mosquitoes.

Plant-parasitic nematodes have been one of the most notorious plant pathogens worldwide (Pechacek et al., 1997; Ntalli et al., 2012). Thousands of crops and trees are susceptible, and the disease caused by phytonematodes results in huge agricultural losses annually (Chitwood, 2003). Levamisoleis used to treat parasitic-worm infections (Keiser et al., 2008). Plants attacked by nematodes show retarded growth and development, as well as loss in the quality and quantity of the harvest. Due to environmental problems, nematicides, such as dibromochloropropane (DBCP) and ethylenedibromide (EDB) were withdrawn from the market. Howbeit, some simple coumarins, furocoumarins, and dicoumarolums, display excellent nematicidal activity, and their skeletons have drawn interest for the development of efficient nematicides (Yang et al., 2002).

In order to reduce the environmental toxicity, pesticide residues, and nematode resistance, the development of new control substitutes are urgently needed (Seo et al., 2014). Natural products and their derivatives provide a promising source for the identification of modern pesticides (Chitwood, 2002). As an alternative to a large screening program for the identification of new active materials, a rational program of structural modification of known active compounds can be more efficient and beneficial.

Environmental concerns in research and industries are increasing with increasing pressure to reduce pollutants. This requires a new approach, which will minimize or eliminate the dispersion of harmful chemicals in the environment in a way that enhances industrial safety and meets the challenges of green chemistry. Monoterpenoid ketone (piperitone) that found in essential oils of many plants, shows various insecticidal, antifeedant, and repellent activities againstmany species of insects (Bowers et al., 1993). The antifeedant activity of piperitone was studiedin *Myzuspersicae*, and the biological consequences of structural modifications of piperitone (i.e., chlorinated, brominated, and iodinated lactone derivatives of piperitone) deterred the probing, feeding, and settling of this aphid (Grudniewska et al., 2011).

Considering these observations, in the present study, we synthesized series of bioactive piperidin-connected 2-thioxoimidazolidin-4-one derivatives, and screened brine shrimp cytotoxicity, larvicidal, nematicidal, and antifeedant activities.

2. Materials and methods

2.1. Chemicals and reagents

All chemical were purchased from the Merck, Sigma-Aldrich, and used without further purification. Solvent were dried and distilled prior to use. Merck pre-coated silica gel plates with a fluorescent indicator were used for analytical TLC. Flash column chromatography was performed using silica gel (Merck). Ethyl acetate – hexane was used as a eluting solvent for TLC and column chromatography. Melting points were recorded in open capillary tubes and were uncorrected. The FT-IR spectra (KBr) were recorded on a Shimadzu 8201pc (4000–400 cm^{-1}) spectrometer. The ^1H NMR (Proton Nuclear Magnetic Resonance Spectra) were obtained on a Bruker DRX-300 MHz and those of ^{13}C NMR (Carbon Nuclear Magnetic Resonance Spectra) were recorded on a Bruker DRX-75 MHz advance spectrometer. Chemical shifts of ^1H and ^{13}C Nuclear Magnetic Resonance spectra were expressed in ppm in downfield from tetramethylsilane. Mass spectrum (EI) was recorded on a Jeol JMS D-300 spectrometer operating at 70 eV. The elemental analysis (C, H, N, and S) were recorded using an Elemental analyzer model (Varian EL III) and agreed with the calculated values.

2.1.1. Synthesis of compounds (3a-c)

A reaction mixture was prepared by compound **2a** (0.1 mol), ethylchloroacetate (0.1 mol), and fused sodium acetate (0.03 mol) (4.1 g) in ethanol. The mixture was heated under reflux for 7 h. The reaction was complete as indicated by TLC (hexane -EtOAc, 4:1, v/v). After all starting material has been consumed, water (10 mL) was added and the solution was extracted with ethyl acetate (10 mL × 3). Evaporation of the solvent and purification by column chromatography on silica gel using hexane–ethyl acetateas same TLC eluent gave the pure product.

2.1.2. 3-(1,3-dimethyl-2,6-diphenylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one (***3a***)

Yellow color solid; mw 393; yield 81%; mp 136–139 °C; IR (kBr, cm^{-1}): 3022 (N-H), 3031 (Ar-H), 1750 (C=O), 1628 (C=N), 850 (Ar-H), 762 (C-N-C); ^1H NMR (DMSO d_6), δ(ppm): 11.55 (1H, s, N-H), 7.83–7.40 (10H, m, Ar-H), 3.94 (1H, d, 6-H, J = 7.2 Hz), 3.65 (1H, dd, 2-H, J = 7.2 Hz, J = 7.1 Hz), 3.60 (2H, s, CH_2N), 2.38 (1H, d, 3-H_{axial}, J = 3.2 Hz), 2.31 (1H, d, 5-H, J = 3.2 Hz), 1.92 (1H, d, 3-Heq, J = 4.3 Hz), 1.77 (3H, s, -NCH_3), 0.86 (3H, s, 5-CH_3); ^{13}C NMR (DMSO d_6), δ(ppm): 208.9 (1C, C=S), 191.0 (1C, C=O), 170.6 (1C, C=N), 142.7–127.0, (12C, Ph), 71.5 (1C, C2), 61.8 (1C, C6), 52.7 (1C, CH_2N), 40.9 (1C, N-$\underline{C}H_3$), 40.4 (1C, $\underline{C}$3-CH_3), 15.7 (1C, C3-$\underline{C}H_3$), 26.8 (1C, C5); EIMS m/z 393$[M]^+$ (21), 363 (100); Elemental analysis $C_{22}H_{24}N_4OS$: Expected: C, 67.32; H, 6.16; N, 14.27%; Found: C, 67.30; H, 6.15; N, 14.28%.

*2.1.3. 3-(2,6-bis(4-chlorophenyl)-1,3-dimethylpiperidin-4-ylideneamino)-2-thioxo imidazo lidin-4-one (**3b**)*

Yellow color solid; mw 461; yield (67%); mp 127–129 °C; IR (kBr, cm^{-1}): 3087 (N-H), 3025 (Ar-H), 1746 (C=O), 1683 (C=N), 860 (Ar-H), 830 (C-Cl), 732 (C-N-C); ^{1}H NMR (DMSO d_6), δ(ppm): 11.25 (1H, s, N-H), 7.43 (4H, d, Ar-H, J = 7.7 Hz), 7.14 (4H, d, Ar-H, J = 7.7 Hz), 3.80 (1H, d, 6-H, J = 3.1 Hz), 3.55 (1H, dd, 2-H, J = 2.1 Hz, J = 2.8 Hz) 3.36 (2H, s, CH_2N), 2.39 (1H, d, 3-H_{eq}, J = 2.1 Hz), 2.36 (1H, d, 3-H_{ax}, J = 2.8 Hz), 2.31 (1H, d, 5-H, J = 3.2 Hz), 1.89 (3H, s, -NCH_3), 0.56 (3H, s, 5-CH_3). ^{13}C NMR (DMSO d_6), δ (ppm): 209.3 (1C, C=S), 185.8 (1C, C=O), 156.2 (1C, C=N), 142.4–128.4 (10C, Ph), 131.8 (2C, C-Cl), 67.6 (1C, C2), 61.5 (1C, C6), 48.1 (1C, CH_2N), 41.2 (1C, N-CH_3), 36.2 (1C, C3-CH_3), 14.1 (1C, C3-CH_3), 23.1 (1C, C5); EIMS m/z 461[M]$^+$ (21), 433 (100); Elemental analysis $C_{22}H_{22}Cl_2N_4OS$: Expected: C, 57.27; H, 4.81; N, 12.14%; Found: C, 57.31; H, 4.82; N, 12.13%.

*2.1.4. 3-(2,6-bis(4-hydroxyphenyl)-1,3-dimethylpiperidin-4-ylideneamino)-2-thioxo imidazo lidin-4-one (**3c**)*

Yellow color solid; mw 425; Yield 87%; mp 145–148 °C; IR (kBr, cm^{-1}): 3048 (N-H), 1778 (C=O), 1642 (C=N), 1450 (OH), 821 (Ar-H), 718 (C-N-C); ^{1}H NMR (DMSO d_6), δ(ppm): 11.96 (2H, s, -OH), 10.89 (1H, s, N-H), 7.58 (4H, d, Ar-H, J = 7.5 Hz), 7.05 (4H, d, Ar-H, J = 7.5 Hz), 3.95 (1H, d, 6-H, J = 2.3 Hz), 3.68 (1H, dd, 2-H, J = 2.2 Hz, J = 2.4 Hz), 3.59 (2H, s, CH_2N), 2.41 (1H, d, 3-H_{eq}, J = 2.4 Hz), 2.37 (1H, d, 3-H_{ax}, J = 2.2 Hz), 2.31 (1H, d, 5-H, J = 2.3 Hz), 1.92 (3H, s, -NCH_3), 0.70 (3H, s, 5-CH_3); ^{13}C NMR (DMSO d_6), δ(ppm): 209.1 (1C, C=S), 172.2 (1C, C=O), 157.8 (1C, C=N), 132.5–116.2 (10C, Ph), 158.8 (2C, C-OH), 54.0 (1C, C2), 50.8 (1C, C6), 46.3 (1C, CH_2N), 40.2 (1C, N-CH_3), 34.8 (1C, C3-CH_3), 13.5 (1C, C3-CH_3), 23.7 (1C, C5); EIMS m/z 425[M]$^+$ (21), 396 (100); Elemental analysis $C_{22}H_{24}N_4O_3S$: Expected: C, 62.24; H, 5.70; N, 13.20; Found: C, 62.26; H, 5.71; N, 13.24%.

2.2. Biological screening

2.2.1. Brineshrimp cytotoxicity

The brine shrimp bioassay was performed according to a method described in our previous study (Manilal et al., 2009). Cytotoxicity of the newly synthesized compounds was determined using freshly hatched, free-swimming nauplii of *Artemiasalina*. The assay system was preparedusing 2 mL of filtered seawater containing the selected concentration of compound in a cavity block (embryo cup). Brine solution was prepared by dissolving 20 g of NaCl (non-iodized) in 1 L of tap water followed byfiltering. Brine shrimp (0.1 g/L) were taken in a beaker (2L) at room temperature, and the pH was adjusted to 8.5 using 0.1 M Na_2CO_3. Natural or artificial light (at night) was provided, and the liquid was oxygenated continuously using a bubble pump machine. Twenty nauplii were transferred to each treatment well, and the experiment continued for 24 h, under constant illumination. Positive control (thymol) and negative control (2% DMSO) wells were also used. Experimental conditions were standardized by preliminary experiments. After 24 h, dead nauplii were counted using a hand lens, and the percent mortality was calculated.

2.2.2. Larvicidal activity

Larvicidal screening was performed after Manilal et al., 2011. Synthesized compounds were tested against the urban mosquito larvae, *Culex quinquefasciatus*. Eggs of *C. quinquefasciatus* were obtained from the city drainage system. Eggs were placed in clean water and kept at room temperature for hatching. Larval development was monitored for 7 d. Second stage larvae were collected using a pasture pipette, placed in cotton to remove excess water, and transferred to test vials. Larval mortality was observed using increasing concentrations of synthesized compounds (10, 20, 30, and 40 μg/mL). The susceptibility or resistance of the mosquito larvae to the selected concentration of the synthesized compounds was determined with a standard protocol (WHO, 1981).

2.2.3. Nematicidal activity

Nematicidal activity was evaluated using juvenile nematodes of *Meloidogyne javanica* (Manilal et al., 2012). The assay system was prepared with 2 mL Milli-Q® water, containing different concentrations of compound (10, 20, 30, and 40 mg/mL) in glass tubes. Treated and control nematodes were held under the same conditions used for colony maintenance. Ten nematode juveniles were transferred intoeach tube. Positive (levamisole) and negative (2% DMSO) control tubes were included. Mortality was observed under a zoom stereomicroscope after 24 h of exposure. The mortality percentage was converted into probit scale to determine the LD_{50} values (Wardlaw, 1985).

2.2.4. Antifeedant activity

Fingerlings (1.5–2.0 cm) of marine acclimated *Oreochromis mossambicus* were used for evaluating antifeedant activity (Manilal et al., 2009). Ten fingerlings were introduced in experimental and control glass bowls, each containing one L of seawater and the selected concentration of compound. Immediate reflex changes and mortality were observed after Manilal et al. (2009). After 24 h of exposure, the number dead and live fish was counted.

2.2.5. Statistical analysis

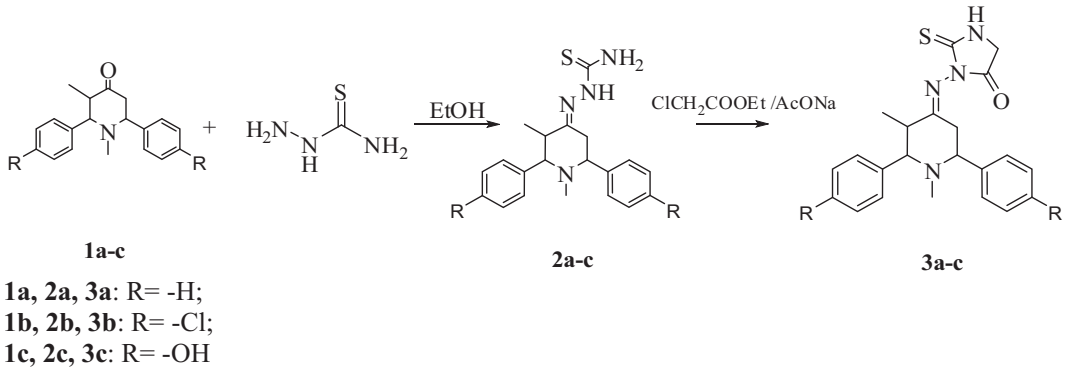
All the experiments were repeated three times to validate the findings statistically (Wardlaw, 1985). All the data are presented as mean ± standard deviation (S.D.). Mean values were compared among treatments and the control using one way analysis of variance (ANOVA) using SPSS at $P < 0.05$ levels.

3. Results and discussion

3.1. Chemistry

Compounds **1a-c** were synthesized according to a published method (Baliah et al., 1948). Compounds **2a-c** were prepared by methods used in previous reports (Balasubramanian et al., 2002; Venkateswarlu et al., 2005; Sampath et al., 2006). Imidazolidin-2,4-dione derivatives **3a-c** were prepared by cyclization of compounds **2a-c** with ethyl chloroacetate in the presence of fused sodium acetate, following the method previously described (Jamal Abdul Nasser et al., 2008). The compounds **2a-c** and **3a-c** were prepared according to the synthesis sequences illustrated in Scheme 1.

Physicochemical data of compounds **3a-c** are shown in the materials and methods section. The formation of all compounds was confirmed by IR spectroscopy, ^{1}H NMR, ^{13}C NMR, and elemental analyses. The IR spectrum of compound **3a** showed absorption bands at 3022–3087, 1628–1683, 1746–1778, and 718–762 cm^{-1}, corresponding to the NH, C=N, C=O, and C-N-C groups, respectively. Compounds **3a-c** showed a sharp singlet observed at δ 11.25–11.96, which confirmed the presence of the NH proton in imidazolidine-2,4-dione ring. The ^{13}C NMR spectra of compounds **3a-c** showed important peaks at δ 156.2–170.6, corresponding to C=N carbon, and δ 40.2–41.2, corresponding to N-CH_3 carbon. The above values are evident for formation of final target molecule. In addition, mass spectra showed that the molecular ion signals matched with the expected molecular weights of all synthesized compounds.



Scheme 1. Synthesis of 2-thio-imidazolidin-4-one derivatives **1a-c**, **2a-c**, and **3a-c**.

Table 1
Brine shrimp cytotoxic activity for synthesized compounds (**1a-c**, **2a-c**, and **3a-c**).

Comp. no.	Mortality (%) room temp				LD_{50} (μg/mL)
	Concentration (μg/mL)[a]				
	10	20	30	40	
1a	42.03 ± 1.86	54.43 ± 1.17	100 ± 0.0	–	17.66
1b	45.09 ± 1.27	86.28 ± 1. 36	100 ± 0.0	–	10.18
1c	39.48 ± 1.46	63.18 ± 1.20	100 ± 0.0	–	14.31
2a	43.04 ± 1.32	88.04 ± 1.59	100 ± 0.0	–	10.52
2b	62.37 ± 1.29	100 ± 0.0	–	–	6.84
2c	72.25 ± 1.30	100 ± 0.0	–	–	8.37
3a	70.28 ± 1.32	100 ± 0.0	–	–	0.99
3b	40.17 ± 1.22	100 ± 0.0	–	–	1.01
3c	68.98 ± 1.21	100 ± 0.0	–	–	2.81
(−)-Pinidinol	47.32 ± 1.09	56.36 ± 1.11	81.09 ± 0.22	91.21 ± 0.91	16.02
Hydantocidin	22.43 ± 1.17	42.09 ± 1.27	86.27 ± 1.19	100 ± 0.0	20.08
Thymol	51.08 ± 0.32	100 ± 0.0	–	–	9.11
DMSO	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0

Positive control: Thymol; Negative DMSO control.
[a] Values are the means of three replicates ± SD.

3.2. Biological activity

Compounds **1a-c** were showed lower degree of activities as compared to **3a-c**, measured in terms of brine shimp cytotoxicity (Table 1), lavicidal (Table 2), nematicidal (Table 3), and antifeedant (Table 4) activities. In particular, compounds **1a-c** became more active when converted to compounds **2a-c**. Toxicity subsequently diminished when compounds **2a-c** were converted to compounds **3a-c**. Compounds **3a-c** showed higher activity due to the presence of imidazolidin rings.

3.2.1. Brine shrimp cytotoxic activity

The mortality rate increased with increasing concentration of each sample. Brine shrimp cytotoxicity of compound **2b** was found to be lower. However, further modification into thio-imidazolidine ring of compound **3b**, cytotoxicity was drastically reduced. Compounds **1a-c** showed 100% mortality at 30–40 μg/mL. Compounds **3a-c** had lethal dose (LD_{50}) values of 0.99, 1.01, and 2.81 μg/mL, respectively. Compound **3c** was slightly less active as compared to compounds **3a** and **3b**. The values are summarized in Table 1.

A substance is considered cytotoxic if it inhibits vital metabolic processes or causes disorders in living organisms, resulting in perversion of behavior or death (Fatope, 1995).

The toxicity of compounds were compared with thymol, and thenatural products, (−)-pinidinol and hydantocidin. Thymol (5-methyl-2-iso-propylphenol) is a natural antimicrobial agent from thyme and thyme oil. It demonstrated significant antimicrobial activity against both gram-positive and gram-negative bacteria (Yu et al., 2010).

Consistent with the expected structure-activity relationships, the brine shrimp assay isconsidered a reliable indicator for the preliminary assessment of toxicity. Brine shrimp cytotoxicity testshave been used as bioassays for a variety of toxic substances (Meyer et al., 1982), and they can be extrapolated to assess cell-line toxicity and antitumor activity. Terpenes from seaweeds displayed wide spectra of cytotoxic and antitumor activities (Valls et al., 1995; Culioli et al., 2001). In the present study, compound **2b**, containing thiosemicarbazone and Cl-phenyl groups, exhibited lower brine shrimp cytotoxicity whereas compound **3b** showed highly active due to the imidazolidin ring bound to the piperidine ring. Particularly in this case, compound **3a** had the higher active compared to the other compounds due to the imidazolidin ring and phenol groups. The results revealed that compound **3a** could be utilized for the development of novel anticancer leads.

3.2.2. Larvicidal activity

Compound **2b** was low toxic due to the reaction of the piperdin-4-one ring with thiosemicarbazide. Compound **3b** exhibited higher toxicity than all other compounds such as **3a-3c 1a-c**, and **2a-c**. The higher rank of activity could be due to the presence of thio-imidazolidin ring. Compounds **3b** and **3c** showed highly active as compared to other compound at 24 h. Compounds **1a-c**, and **2a-c** produced 100% mortality at 30 μg/mL. Compounds **3a-c** displayed LD_{50} values of 1.37, 6.66, and 6.51 μg/mL, respectively. The values are summarized in Table 2.

Table 2
Larvicidal profile of compounds (**1a-c**, **2a-c**, and **3a-c**) on second instar larvae of *Culex quinquefasciatus*.

Comp. no	Mortality (%) room temp				LD_{50} (μg/mL)
	Concentration (μg/mL)[a]				
	10	20	30	40	
1a	30.44 ± 1.22	44.17 ± 1.15	57.17 ± 1.31	82.12 ± 1.08	27.43
1b	40.03 ± 1.37	50.05 ± 1.24	72.76 ± 1.20	90.09 ± 1.12	20.24
1c	39.12 ± 1.23	43.17 ± 1.20	58.18 ± 1.27	79.21 ± 1.21	24.89
2a	49.94 ± 1.18	64.98 ± 1.10	100 ± 0.0	–	11.77
2b	42.01 ± 1.43	69.03 ± 1.27	100 ± 0.0	–	15
2c	48.88 ± 1.57	60.17 ± 1.19	100 ± 0.0	–	12.56
3a	68.54 ± 1.41	100 ± 0.0	–	–	1.37
3b	66.28 ± 1.48	100 ± 0.0	–	–	6.66
3c	59.09 ± 1.40	100 ± 0.0	–	–	6.51
(−)-Pinidinol	40.14 ± 1.22	54.01 ± 0.35	70.10 ± 0.31	100 ± 0.0	18.28
Hydantocidin	33.12 ± 1.33	47.09 ± 0.21	54.44 ± 0.11	77.01 ± 1.22	22.11
Positive control	43.18 ± 0.32	56.76 ± 0.12	61.88 ± 1.12	100 ± 0.0	15.24
Negative control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0

Positive control: *N-tert*-butyl-*N,N′*-dibenzoylhydrazine; Negative control: DMSO.
[a] Values are the means of three replicates ±SD.

Table 3
Nematicidal activity of synthesized compounds (**1a-c**, **2a-c**, and **3a-c**).

Comp. no.	Mortality (%) room temp				LD_{50} (μg/mL)
	Concentration (μg/mL)[a]				
	10	20	30	40	
1a	39.33 ± 1.35	49.93 ± 1.29	84.07 ± 1.24	100 ± 0.0	21.85
1b	30.99 ± 1.16	47.88 ± 1.20	60.21 ± 1.17	100 ± 0.0	15.51
1c	36.55 ± 1.20	59.28 ± 1.31	64.17 ± 1. 20	100 ± 0.0	17.64
2a	48.09 ± 1.44	63.02 ± 1.30	81.03 ± 1.36	100 ± 0.0	11.78
2b	32.11 ± 1. 31	69.28 ± 1.16	100 ± 0.0	–	13.07
2c	48.23 ± 1.20	60.17 ± 1.31	100 ± 0.0	–	12.56
3a	78.02 ± 1.42	100 ± 0.0	–	–	6.45
3b	81.18 ± 1. 21	100 ± 0.0	–	–	2.42
3c	83.29 ± 1.19	100 ± 0.0	–	–	1.57
(−)-Pinidinol	40.99 ± 1.16	57.88 ± 1.20	80.21 ± 1.17	100 ± 0.0	14.25
Hydantocidin	34.19 ± 0.11	45.03 ± 1.01	59.19 ± 0.29	74.03 ± 0.17	26.30
Positive control	21.18 ± 0.32	49.80 ± 0.12	61.88 ± 1.12	78.93 ± 1.12	20.12
Negative control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0

Positive control: Levamisole.
Negative control: DMSO.
[a] Values are the means of three replicates ±SD.

Table 4
Antifeedant activities of compounds (**1a-c**, **2a-c** and **3a-c**) on *Oreochromis mossambicus* fingerlings.

Comp. no	Mortality (%) room temp				Time (h) of death	LD_{50} (μg/mL)
	Concentration (μg/mL)[a]					
	10	20	30	40		
1a	37.43 ± 1.30	42.32 ± 1.11	74.32 ± 1.19	100 ± 0.0	3	23.50
1b	26.43 ± 1. 13	47.01 ± 1.32	68.77 ± 1.26	100 ± 0.0	4	27.48
1c	31.01 ± 1. 20	43.31 ± 1.24	82.00 ± 1.39	100 ± 0.0	5	23.08
2a	48.32 ± 1.39	63.30 ± 1.01	76.98 ± 1.40	100 ± 0.0	4	12.13
2b	47.44 ± 1.47	74.66 ± 1.30	87.09 ± 2.8	100 ± 0.0	3	12.7
2c	41.32 ± 1.62	80.43 ± 1.21	91.09 ± 3.70	100 ± 0.0	2	12.49
3a	86.01 ± 1. 38	100 ± 0.0	–	–	6	1.54
3b	83.43 ± 1.41	100 ± 0.0	–	–	6	1.79
3c	85.30 ± 1. 37	100 ± 0.0	–	–	6	1.52
(−)-Pinidinol	49.88 ± 0. 10	68.32 ± 1.14	88.00 ± 1.20	100 ± 0.0	5	10.21
Hydantocidin	28.01 ± 0.82	46.11 ± 0.10	69.08 ± 0.12	80.5 ± 0.12	6	21.05
Positive control	28.18 ± 0.32	46.88 ± 0.12	69.88 ± 1.12	80.0 ± 0.0	6	21.12
Negative control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		0.0

Positive control: Piperitone; Negative control: DMSO.
[a] Values are the means of three replicates ±SD.

Larvicidal activity of compound **1b**, containing Cl-phenyl groups, was very low. However, the compound **3b** showed very high larvicidal activity compared to the other compounds due to its imidazolidin ring and phenol groups.

N-tert-butyl-*N,N′*-dibenzoylhydrazine (RH-5849) exhibited significant insecticidal activity against *Mythimna separata* and *Plutella xylostella* (Song et al., 2016). The LC_{50} of compound **3c** against *Culex quinquefasciatus* was 6.51 μg/mL, which was highly active

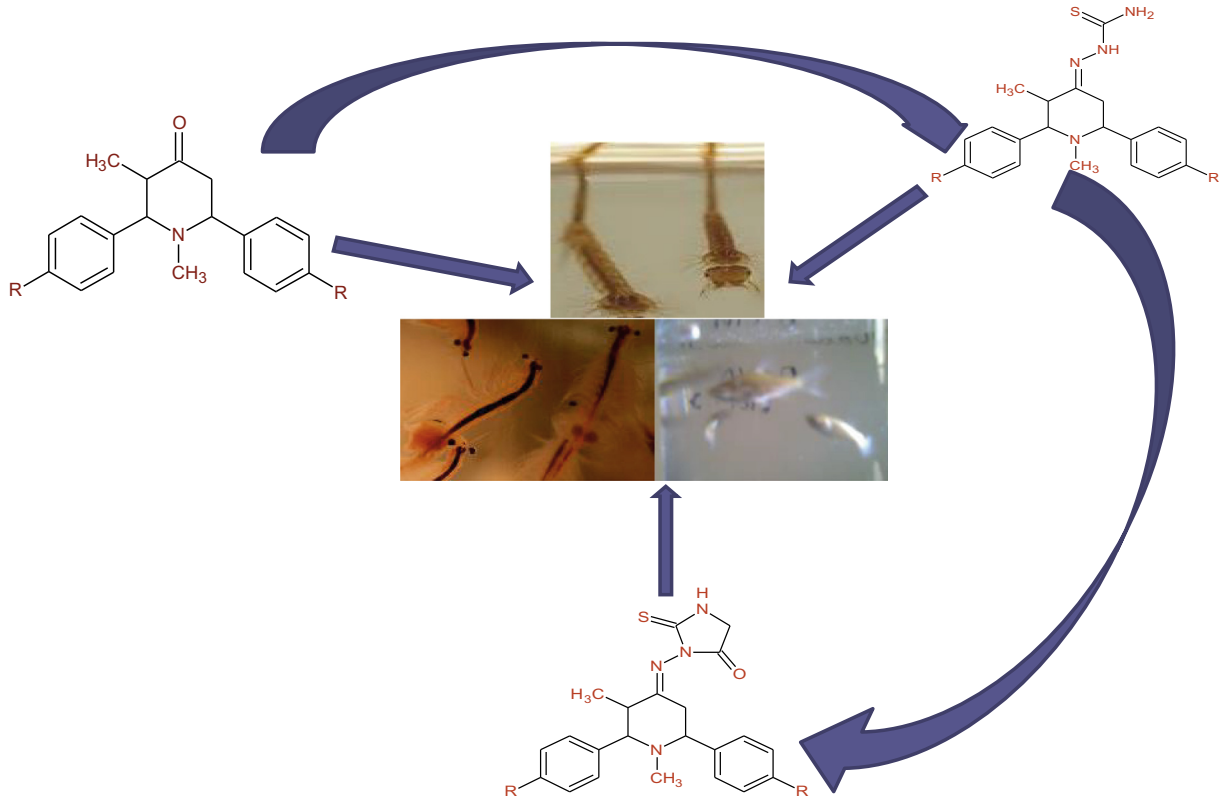


Fig. 2. Bioassay screening of synthesized compounds (**1a-c**, **2a-c**, and **3a-c**).

compared to *N′-tert*-butyl-*N*,*N′*-dibenzoylhydrazine (28.24 μg/mL), and also compared with natural product compounds such as (−)-pinidinol and hydantocidin. The LC_{50} value of compound **3a** against *Culex quinquefasciatus* was 1.37 μg/mL, comparable to that of RH-5849. However, compounds **2a-c** showed low activity against *C. quinquefasciatus*.

3.2.3. Nematicidal activity

Compounds **1a-c**, **2a-c**, and **3a-c** were screened for nematicidal activity. Compounds **3a-c** demonstrated highly active as compared with compounds **1a-c** and **2a-c**. Compounds **1a-c**, and **2a-c** produced 100% mortality at 30–40 μg/mL. Compounds **3b**, **3c** showed higher active than compound **3a**. The LD_{50} values of compounds **3a-c** were 6.45, 2.42, and 1.57 μg/mL, respectively. The values are summarized in Table 3.

Nematodes are tiny worms, and some are plant parasites. These plant parasitic nematodes play an important role in predisposition of the host plant to invasion by secondary pathogens (Jayasinghe et al., 2003). Chemical methods, combined with agriculturalpractices, have been the primary methods for nematode control (Thoden et al., 2009; Chitwoods et al., 2014; Giannakou et al., 2007; Kearn et al., 2014). To reduce environmental toxicity, pesticide residues, and nematode resistance, the development of new nematicides has become an urgent and challenging task (Seo et al., 2014). Natural products and their derivatives provide a promising source for the discovery of new pesticides (Chitwood, 2002).

As an alternative to large screening programs for the identification of novel active materials, structural modification of known compounds can be more efficient and equally useful. Nematicidal activity of compound **2a**, containing a thiosemicarbazone group, was low, but it was high for compound **3a** due to imidazole ring formation. Particularly in this case of compounds **3a-c** showed very high activity as compared to the other compounds due to the presence of the imidazole ring.

In this study, our synthesized compounds exhibited activities comparable to that of natural products with imidazolidin ring such as levamisole, (−)-pinidinol, and hydantocidin. According to a previous study (Wu et al., 2012), betaines of seaweed extracts can suppress the growth of nematodes. Terpenoid compounds in seaweeds are known to have nematicidal activity. Considering the emerging issues pertaining to the use of chemical pesticides, suitable alternative resources and ecofriendly perspectives are urgently required for sustainable agriculture. The present study reveals new insights into the development of ecofriendly biopesticides for the management of root-knot nematodes.

3.2.4. Antifeedant activity

Compounds **3a-c** showed high toxicity compared to other compounds, **1a-c** and **2a-c**. Compounds **1a-c** and **2a-c** produced 100% mortality at 40 μg/mL. Toxicity was measured as death percentage at 6 h. Compound **3a**, **3b**, and **3c** produced 100% mortality after 6 h of post exposure at 20 μg/mL with a corresponding LD_{50} value of 1.54 μg/mL, 1.79 μg/mL, and 1.52 μg/mL. The values are summarized in Table 4.

Synthetic analogues containing structural elements responsible for deterrent activity may play a role as antifeedants. Previous studies have indicated that active insect deterrents could be obtained by chemical transformation of natural products, such as monoterpenes in essential oils (Szczepanik et al., 2005). Thus, natural plant compounds may be applied as models or substrates for the synthesis of highly specific substances that inhibit the feeding of pests.

Antifeedant activity of compound **2b**, containing thiosemicarbazone and Cl-phenyl groups was low active, but it was much higher for compound **3b** due to imidazole ring formation. In partic-

ular, compound **3c** showed highly active compared to other compounds due to the imidazolidin ring and phenol groups. Therefore, the 2-thio-imidazolidin-4-one **(3a-c)** derivatives were considered as highly active compared tothe piperdine **(2a-c)** derivatives. The 2-thio-imidazolidin-4-onecompounds (**3a-c)** may be useful to develop novel pest deterrentsin the agricultural and food industries.

Many studies described that the chemical transformation of the piperitone molecule by the introduction of a lactone moiety resulted in good feeding deterrence against *Alphitobius diaperinus*. The number and type of halogen (Cl) substituent in the cyclohexane ring clearly affects the antifeedant potential of these monoterpenes (Argandona et al., 2002). Similarly, a chlorine atom enhances the insecticidal activity of ester derivatives of menthol against mosquitoes (Samarasekera et al., 2008). The introduction of a lactone moiety into a piperitone molecule dramatically changed its biological activity. Piperitone used as a positive control, it is derived from lactones (Grudniewska et al., 2013) and also all synthesized compounds were compared with natural product of (−)-pinidinol, and hydantocidin. Fig. 2 is a diagrammatic summary of our bio-toxicity analysis of all synthesized compounds **1a-c**, **2a-c**, and **3a-c**.

4. Conclusion

From the present study, it can be concluded that the piperidin-connected 2-thioxoimidazolidin-4-one showed highest and broadest spectra of bio-toxicities in brine shrimp cytotoxicity, antifeedant, nematicidal, and larvicidal bioassays in comparison to other compounds. Compound **3a** showed significant activity against mosquito larvae and compound **3c** showed significant nematicidal activity compared to the natural products and positive control. Based on the present findings, it could be envisaged that, these compounds might be a potential source for developing ecologically significant pesticides, and pharmaceuticals in future.

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