Journal of Applied Pharmaceutical Science Vol. 5 (05), pp. 101-105, May, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50519
ISSN 2231-3354

Environmental biotoxicity screening of some pyrrole and 1,4-dihydropyridine heterocyclic derivatives

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ARTICLE INFO

Article history:
Received on: 24/02/2015
Revised on: 24/03/2015
Accepted on: 19/04/2015
Available online: 27/05/2015

Key words:
Pyrrole derivative, 1,4-dihydropyridine derivative, Brine shrimp cytotoxicity, Ichthyotoxic screening, Larvicidal activity, Nematicidial activity.

ABSTRACT

Synthesis of pyrrole **1-3** and 1,4-dihydropyridine derivatives **4-6** were prepared from condensation method and synthesized compounds were screened for environmental biotoxicity such as Brine shrimp cytotoxicity, Ichthyotoxic, Larvicidal and Nematicidial activities. Among the compounds **3** and **6** shows that highly toxic (LD_{50}: 8.72 and 12.30 µg/mL) against Brain shrimp cytotoxic screening and compound **3** and **6** was highly toxicity (LD_{50} : 5.01 and 7.75 µg/mL) against Antifeedant screening (ichthyotoxic profile). The compounds **3** and **6** was highly active (LD_{50}: 12.88 µg/mL, and 14.79 µg/mL) against Larvicidal activity and compound **3** and **6** was highly active (LD_{50} : 8.20 µg/mL, and 7.43 µg/mL) against Nematicidal activity.

INTRODUCTION

The nematicide and larvicidal use is slate for reduction due to environmental problems, human and animal health concerns. For example, effective nematicides such as dibromochloropropane (DBCP) and ethylenedibromide (EDB) have been withdrawn from the market due to their deleterious effects on humans and the environment. According to World Health Organization (WHO), the one of strategies is to destroy their vectors or intermediate hosts. The best method is control of mosquito larvae using insecticides (Sun *et al*., 2010; Talontsi *et al*., 2011) such as organo-phosphates, natural products and heterocyclic types. It is an urgent need to develop new insecticides which are more environmentally safe and also biodegradable molecules. The pyrrole and 1,4-dihydropyridine derivatives have received considerable attention of synthetic and biological important such as anticoagulant (Idhayadhulla *et al*., 2012), anticonvulsant (Idhayadhulla *et al*. 2012) particularly 1,4-dihydropyridine with thiosemicarbazide also biological importance such as anticonvulsant (Surendra Kumar *et al*., 2010), anticoagulant (Surendra Kumar *et al*., 2011b), anticancer (Surendra Kumar *et al*., 2011c). Our Previous work Brine shrimp cytotoxicity, Larvicidal, Nematicidial and Antifeedant activity were screening by using natural and marine based natural products (Manilal *et al*., 2011; Manilal *et al*., 2009), although first time we are focused by drug molecules screened for Brine shrimp cytotoxicity, Larvicidal, Nematicidial and Antifeedant (Deepa *et al*.,2010) activities. Insecticidal, nematicidal and acaricidal activities of pyrrole compounds were previously reported in (United States Patent 7186722 and Patent 7, 783/739, October 28, 1991). Synthesis and Larvicidal activities, antifungal activities of novel Chlorantraniliprole derivatives and their target in the Ryanodine Receptor reported in (Qichao *et al*., 2015; Xudong *et al*., 2007; Drissa *et al*., 2011). Insecticidal, nematicidal and acaricidal activities of pyrrole compounds were previously reported in (United States Patent 7186722 and Patent 7, 783 / 739, October 28, 1991).

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Synthesis and Larvicidal activities, antifungal activities of novel Chlorantraniliprole derivatives and their target in the Ryanodine Receptor reported in (Qichao *et al*., 2015; Xudong *et al*.,2007; Drissa *et al.*, 2011). In our present work, we are report here the synthesis of pyrrole and 1,4-dihyropyridine derivatives and their biotoxicity screening against Brine shrimp, ichthyotoxic toxicity larvicidal, and nematicidial activity screening.

MATERIALS AND METHODS

Cytotoxic activity

The cytotoxic activity of the newly synthesized test compounds **1-6** was conducted according to the methodology described elsewhere (Manilal *et al*., 2009), The freshly hatched free-swimming nauplii of *Artemia salina* (Linnaeus) (*Artemia salina*, Sanders Great Salt Lake Brine Shrimp Company L.C., U.S.A.) was used as the test organism. The assay system was prepared with 2 mL of filtered seawater containing chosen concentration of synthesized chemicals (10, 20, 30 40 µg/mL) in cavity blocks (embryo cup).

Parallel vehicle control (using 2 % methanol) and negative control (without vehicle) wells were also kept. In each cavity blocks, 20 nauplii were transferred and the setup was allowed to remain for 24 h, under constant illumination. After 24 h, the dead nauplii were counted with a hand lens. Based on the percent mortality, the LD_{50} of the test compounds was determined using probit scale (Wardlaw, 1985).

Ichthyotoxicity

The icthyotoxic activity of synthesized test compounds1-6 was carried out following the methodology of (Manilal *et al*., 2010). Briefly, five fingerlings of *Oreochromis mossambicus* (Peters) (1.5±1 cm) were introduced in each experimental and control glass bowls containing 1,000 mL of freshwater dissolved with chosen concentrations (10, 20, 30 and 40 µg/mL) of synthesized compounds 1-6. Immediate reflex changes and mortality were observed continuously for six hours at 1h interval for the next 12 h. After 24 h of exposure, the number dead and live fishes were counted.

Larvicidal activity

The assessment of larvicidal activity of synthesized test compounds 1-6 was tested against the urban mosquitoes *C. quinquefasciatus* using standard bioassay protocol (Manilal et al., 2011). Egg rafts of mosquito were obtained from drainage system. Eggs were reared under standard insectary conditions at ambient temperature (29±3°C), relative humidity 80±5%, 12:12 light: dark photoperiod and fed with ground shrimp feed daily. Larval development was monitored for seven days. The second and third stage larvae were collected at the tip of a pasture pipette and placed in cotton bud to remove excess water and transferred gently to the test vial (10 / vial) by tapping. The larval mortality was observed using various concentrations of synthesized compounds1-6(10,20,30,40 µg/mL).

Nematicidial activity

For the determination of nematicidal activity, juveniles of *Meloidogyne javanica* were used as test organism (Manilal *et al*., 2009). Assay system was prepared with 2 ml Milli Q water containing different concentrations (10, 20, 30 and 40 µg/mL) of synthesized test compounds 1-6 in glass tubes.Ten juveniles of M.javanica were transferred in test, positive (with 2% methanol) and negative(without vehicle) control tubes. Mortality was observed under a zoom stereomicroscope after 24 h of exposure.

Acute toxicity of synthesized compounds can be determined by the calculation of LD_{50}, i.e., the dose that will kill 50% of animals of a particular species. The LD_{50} method and calculation is described from the literature of (Miller and Tainter *et al.*,1944).

STRUCTURE ACTIVITY RELATIONSHIPS (SAR)

From the results of biotoxicity screening of pyrrole and 1,4-dihydropyridine derivatives, the following structure activity relationships figure.1 can be derived. The compounds **1-4** show that 100 % mortality at concentration 40 µg/mL. The compound **6** containing sulphur group and it shows that 100% mortality at concentration 30µg/mL against Brain shrimp cytotoxic.

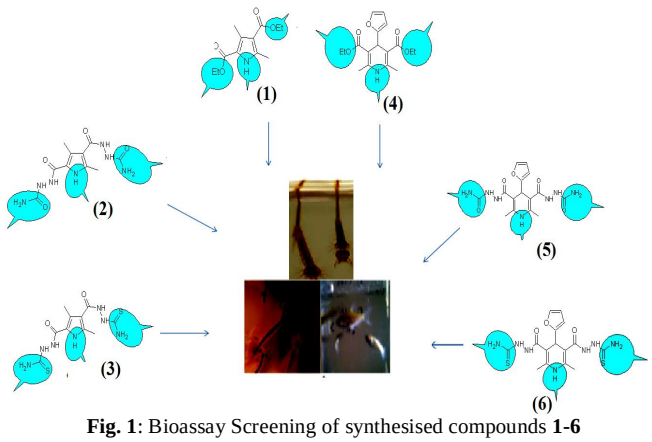
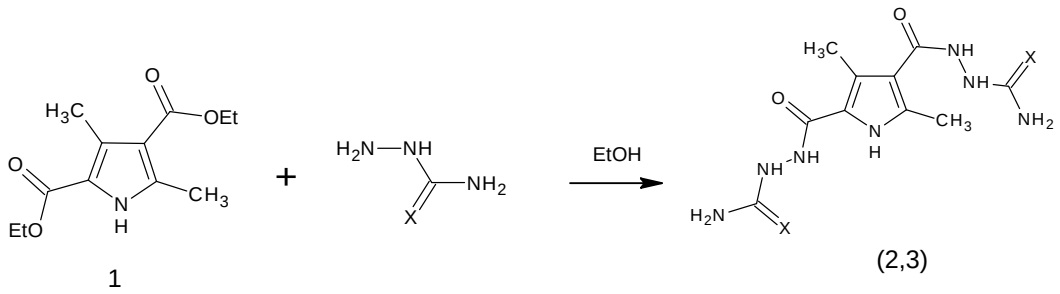


Fig. 1: Bioassay Screening of synthesised compounds **1-6**

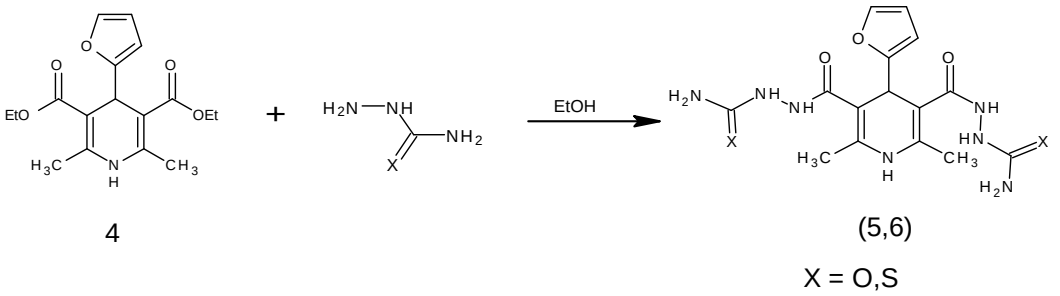
The compound **1, 2, 4** shows that 100 % mortality at concentration 40 µg/mL. The compounds **3,6** containing -NH- and methyl group and it show that 100 % mortality at concentration 30 µg/mL against Larvicidal activity.

The compounds **1, 2, 3, 4** and **6** show that 100 % mortality at concentration 40 µg/mL. The compounds **3, 6** containing -NH- and methyl group and it show that 100 % mortality at concentration 30 µg/mL against Nematicidal activity.

The compounds **1, 2, 4** and **5** exhibit 100 % mortality at concentration 40µg/mL with in 5-6 h where as the compounds **3, 6** shows that 100 % mortality at concentration 30µg/mL against Antifeedant activity. Therefore the compounds **3, 6** containing sulphur groups exhibit high toxic compared with compounds **1, 2, 4** and **5**.



Scheme 1. Synthetic route of the compound **(2,3)**



Scheme 2. Synthetic route of the compound (**5** and **6**).

Table 1: Physiochemical properties of the compounds (**1- 6)**

Compd. No	Mp(° C)	M.W	Yield (%)	MF	Elemental Analysis Calculated (Found) %			
					C	H	N	S
1	127	239.27	71	$C_{18}H_{17}NO_4$	60.24(60.28)	7.16(7.13)	5.85(5.81)	-
2	115	297.12	89	$C_{10}H_{15}N_7O_4$	40.40(40.45)	5.09(5.09)	32.98(32.98)	-
3	95	329.40	85	$C_{10}H_{15}N_7O_2S_2$	36.46(36.40)	4.59(4.51)	29.77(29.72)	19.47(19.41)
4	158	319.35	91	$C_{17}H_{21}NO_5$	63.94(63.91)	6.63(6.69)	4.39(4.41)	-
5	180	377.35	86	$C_{15}H_{19}N_7O_5$	47.74(47.80)	5.08(5.12)	25.98(25.99)	-
6	214	409.48	88	$C_{12}H_{19}N_7O_3S_2$	44.00(44.06)	4.68(4.74)	23.94(23.98)	15.66(15.71)

RESULTS AND DISCUSSION

Synthesis

We are reported previously above synthesized title compounds in international journal of biological chemistry (Idhayadhulla, *et al.*, 2013), it is representing in Scheme 1. The characterization of synthesized compounds are summarized in Table1.

Bio-toxicity screening

Brain shrimp cytotoxic activity

Brain shrimp cytotoxic activity was screened for compounds **1-6**. The test was carried out by room temperature and measured the toxicity influence of the compounds. The compounds **1, 2, 4** and **5** were reduced to 100% mortality at (40 µg/mL). The compounds **3, 6** have very low LD_{50} value compared with other compounds. The compound **1-6** shows that LD_{50} values 19.96, 21.79, 8.72, 25.87, 26.22 and 12.30µg/mL respectively. The values are summarized in Table 2. Brain shrimp cytotoxic activity of the compounds **1-6** shows in figure 2 at concentration (10-40) µg /mL.

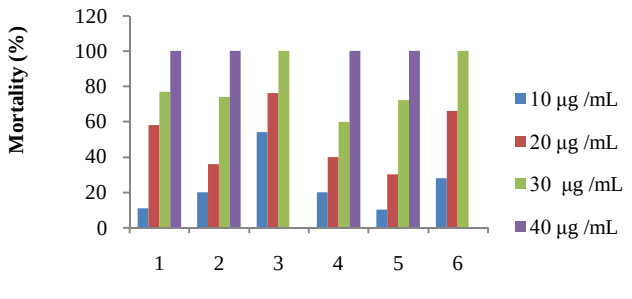


Fig. 2: Brine shrimp cytotoxic activity of compounds (**1-6**).

Table 2: Brine shrimp cytotoxic activity for synthesized compounds **(1-6).**

Comp No	Mortality (%)Room temp Concentration(µg /mL)				LD_{50}
	10	20	30	40	LD_{50}(µg/mL)
1	11 ± 3.2	58 ± 3.8	77 ± 3.9	100 ± 0.0	19.96
2	20 ± 2.2	36 ± 4.4	74 ± 3.8	100 ± 0.0	21.79
3	54 ± 2.1	76 ± 3.2	100 ± 0.0	-	8.72
4	20 ± 3.2	40 ± 3.8	60 ± 3.9	100 ± 0.0	25.87
5	10 ± 3.2	30 ± 3.7	72 ± 3.8	100 ± 0.0	26.22
6	28 ± 3.1	66 ± 2.2	100 ± 0.0	-	12.30

Ichthyotoxicity

Antifeedant activity (Ichthyotoxicity profile) was screened against compounds **1-6**, the activity was measured death percentage at 6h. The compounds **1, 2, 4** and **5** were found to be 100% mortality within 4-5h at concentration (40µg/mL) and their LD_{50} value (14.45, 15.13, 13.05, and 12.91 µg/mL). The compounds **3, 6** was found 100% mortality within 3-4h at concentration (30µg/mL) when their LD_{50} value 5.01, 7.75 (µg/mL) the values are summarized the Table 3. Antifeedant activity of compounds **1-6** shows in figure 3 at concentration (10-40)µg/mL). Figure 3 shows that Ichthyotoxicity profile (Oreochromis mossambicus) fingerlings of the compounds **1-6**.

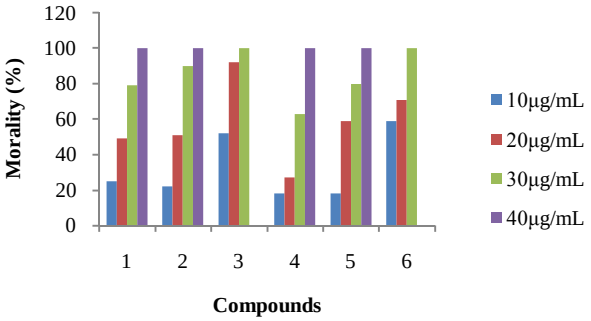


Fig. 3: Antifeedant activity of the compounds (**1-6**).

Table 3: Antifeedant activity of synthesized compounds **(1- 6**) (Ichthyotoxicity profile, Oreochromis mossambicus fingerlings)

Comp. No	Mortality (%), Room temp Concentration (µg/mL)				Time(h) of death 100(%)	LD_{50}(µg/mL)
	10	20	30	40		
1	25 ± 4.8	49 ± 3.7	79 ± 8.2	100 ± 0.0	5	14.45
2	22 ± 3.7	51 ± 2.3	90 ± 2.5	100 ± 0.0	6	15.13
3	52 ± 4.1	92 ± 2.3	100 ± 0.0	-	3	5.01
4	18 ± 1.2	27 ± 5.7	63 ± 4.2	100 ± 0.0	5	13.05
5	18 ± 2.1	59 ± 7.3	80 ± 2.0	100 ± 0.0	4	12.91
6	59 ± 2.2	71 ± 3.5	100 ± 0.0	-	2	7.75

Value were the means of three replicates ± SD.

Larvicidal activity

Larvicidal activity was screened for compounds **1-6** at 24h suggested that second instar larvae and test was carried out by room temperature. The compounds **1**, **2, 4** and **5** are produced 100% mortality at (40µg/mL) and the compound **3, 6** was found 100% mortality at concentration (30µg/mL). Compounds **(1-6)** have lethal effect and killed 50% of second instars larvicidal when their LD_{50} value 15.48, 12.80, 10.88, 13.00, 18.34 and 11.79 µg/mL respectively. The compound 3, 6 has very highly toxic LD_{50} value when compared with compounds **1, 2, 3, 4** and **6**. The values are summarized in Table 4. Larvicidal activity variation of compounds **1-6** shows in figure 4 at concentration (10-40) µg /mL.

Nematicidal activity

Nematicidal activity was screened for compounds **1-6**. The test was carried out by room temperature and measured the toxicity influence of the compounds. The compounds **1**, **2, 4** and **5** had produced 100% mortality at (40 µg/mL), lethal effect and killed 50% of Nematicidal when their LD_{50} value was 16.21, 16.22, 8.20, 12.89, 18.62 and 7.43 µg/mL respectively. The compounds **3, 6** was highly toxic LD_{50} value compared with compounds **1, 2, 4** and **5** the values are summarized in Table 5. Nematicidal activity variation of compounds **1-6** shows in figure 5 at concentration (10-40 µg /mL).

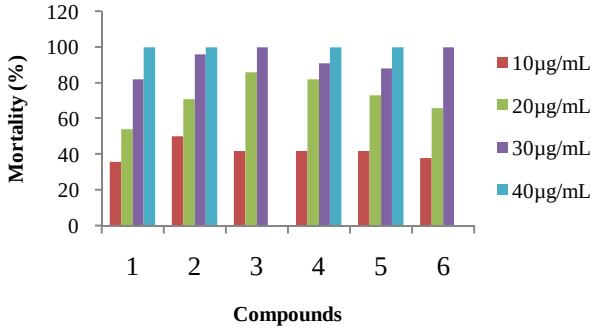


Fig. 4: Larvicidal activity of compounds (1-6).

Table 4: Larvicidal profile of compound **(1- 6)** on second instar larvae of Culex sp.

Comp. No.	Mortality (%)Room temp Concentration(µg				LD_{50}(µg /mL)
	10	**20**	**30**	**40**	
1	36 ± 4.1	54 ± 40	82 ± 4.4	100 ± 0.0	15.48
2	50 ± 4.8	71 ± 4.2	96 ± 0.0	100 ± 0.0	12.80
3	42 ± 4.0	86 ± 2.5	100 ± 0.0	-	10.88
4	42 ± 4.1	82 ± 4.0	91 ± 0.0	100 ± 0.0	13.00
5	42 ± 4.8	73 ± 4.2	88 ± 0.0	100 ± 0.0	18.34
6	38 ± 1.9	66 ± 4.8	100 ± 0.0	-	11.79

Value were the means of three replicates ± SD.

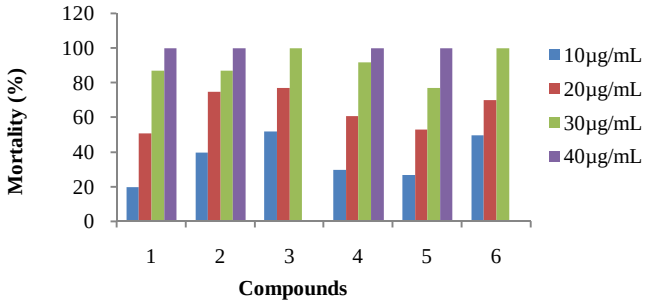


Fig. 5: Nematicidal activity of the compounds (**1-6**).

Table 5: Nematicidal activity of synthesized compounds **(1- 6**).

Comp No	Mortality (%)Room temp Concentration(µg /mL)				LD_{50} (µg /mL)
	10	**20**	**30**	**40**	
1	20 ± 3.8	51 ± 3.1	87 ± 3.2	100 ± 0.0	16.21
2	40 ± 4.1	75 ± 4.8	87 ± 4.9	100 ± 0.0	16.22
3	52 ± 3.7	77 ± 2.8	100 ± 0.0	-	8.20
4	30 ± 4.8	61 ± 4.1	92 ± 4.2	100 ± 0.0	12.89
5	27 ± 4.8	53 ± 4.1	77 ± 4.2	100 ± 0.0	18.62
6	50 ± 4.1	70 ± 4.8	100 ± 0.0	-	7.43

Value were the means of three replicates ± SD.

CONCLUSION

The synthesized compounds **1-6** were screening the environmental biotoxicity of Brine shrimp cytotoxicity, Ichthyotoxicity profile and insecticidal activity of Larvicidal, Nematicidal activity**.** The compounds **3**, **6** was highly toxic against all bioassays against Brain shrimp cytotoxic, Antifeedant, Larvicidal and Nematicidal screening. Such observation was consistent with the results about the sulfur containing heterocyclic compounds **3** and **6** are highly toxic and good activity against all bioassays. These findings demonstrate that the environmental biotoxicity represent a new template for future studies.

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How to cite this article:

Surendra Kumar, Akbar Idhayadhulla, Aseer Manilal, Behailu Merdekios. Environmental biotoxicity screening of some pyrrole and 1,4-dihydropyridine heterocyclic derivatives. J App Pharm Sci, 2015; 5 (05): 101-105.