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Short Communication

Bioactivity of 6-Aryl-4,5-dihydropyridazin-3(*2H*)-ones Using Brine Shrimp (*Artemia salina*) Lethality Assay

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Abstract

Pyridazine is an important component of various biological active synthetic compounds which can leads to the development of novel and safe medicinal agents. In the present study, some 6-aryl-4,5-dihydropyridazin-3(2H)-one derivatives (**2a–2f**) were prepared from aroyl propionic acids (**1a-1f**). The final compounds (**2a–2f**) were evaluated for cyotoxicity activity by brine shrimp assay method. All the 6 compounds (**2a–2f**) were tested at dose level 5, 10, 20 (µg/ML) and potassium dichromate at dose level 10, 20, 30 (µg/ML) as reference drug. Compound **2c** and **2e** exhibited potent brine shrimp lethality with LC_{50} 1.023 and 1.20 µg respectively. Other compounds **2a**, **2b**, **2d**, and **2f** have also showed significant cytotoxicity with LC50 7.58, 6.76, 8.91 and 4.46 µg respectively. The present study supports that brine shrimp bioassay is simple reliable and convenient method for assessment of bioactivity of **2a-2f** and lends support for their uses in medicine.

Key words: Biologically active, Brine shrimp assay, cytotoxicity, pyridazine derivatives

1. Introduction

For new drug discovery research and for the synthesis of potent and selective synthetic anticancer compounds, brine shrimp lethality assay is considered to be a convenient and cheap tool for preliminary assessment of cytotoxicity, evan it could be implemented for toxicity study of fungi, heavy metals, pesticides and so many[1,2]. The *in vivo* lethality in a simple zoological organism, such as the brine shrimp lethality test, developed for Meyer *et al.*[2], might be used as a simple tool to guide screening and fractionation of physiologically active compounds, where one of the simplest biological responses to monitor is lethality, since there is only one criterion: either dead or alive. This general bioassay detects a broad range of biological activities and a diversity of chemical structures. One basic premise here is that toxicology is simply pharmacology at a higher dose, thus if we find toxic compounds, a lower, non-toxic, dose might elicit a useful, pharmacological, perturbation on a physiologic system. However, it has been demonstrated that shrimp lethality test correlates reasonably well with cytotoxic and other biological properties. Brine shrimp have been previously utilized in various bioassay systems. There have been many reports on the use of this animal for environmental studies, screening for natural toxins and as a general screening for bioactive substances[2-4]. It can also be further extanded for cell-line toxicity and anti tumor activity for that compound which emerge promising cytotoxicity towards brine shrimp larva[3]. Taxol [TM], a potent anticancer drug, obtained from *Taxus brevifolia* getting approval by FDA for the chemotherapy of ovarian, breast andnon-small-cell lung carcinomas was discovered by similar method[4].

Pyridazine is an important six member heterocyclic compound containing two nitrogen atoms at adjacent positions. A lot of research work on pyridazines has been done in the past. This nucleus gives out different derivatives with all

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different types of biological activities[5,6]. In recent years a substantial number of pyridazinones have been reported to possess antimicrobial[7], analgesic, antipyretics, anti-inflammatory[8,9], Antifeedant[10], antidiabetic[11], herbicidal[12], larvicidal[13], antiplatelet activities[14], anticancer[15,16], antitubercular[17], Antifungal[18], neurological disorders, antidepressant, anxiolytic, anticonvulsant[19,21], cardiotonic and antihypertensive agents[22,23], antiasthamaic, antiallergic, myocardial imaging agent, and other anticipated biological activities[24,25]. In particular, a large number of pyridazinone derivatives are well known as intermediates for drugs and agrochemicals. Some pyridazinone derivatives like indolidan, bemoradan, primobendan, levosimendan (antihypertensive), minaprine (antidepressant), emorfazone (antiinflammatory), and azanrinone (cardiotonic), already appeared in the clinical market[24-27]. During our literature survey, it is observed that some pyridazinone derivatives were possessed anticancer activity. Considering the 6-phenyl-3(2H)-pyridazinone residue as the pharmacophoric group for the activity, we have synthesized some 6-aryl pyridazinone derivatives and evaluated them for cytotoxic activity by brine shrimp assay method.

2. Material and Methods

2.1 General synthetic method of Aroyl propionic acids (1a-1f): After suspending anhydrous aluminum chloride (0.15 mol) in dry appropriate aryl compounds (50 mL) under anhydrous conditions, the mixture was refluxed on a water bath. Succinic anhydride (0.10 mol) was then added to the reaction mixture in small portions with continuous stirring. Stirring and heating were continued for 6 h. The reaction mixture was left overnight at room temperature and then made acidic by addition of an ice cold solution of concentrated hydrochloric acid (2.5% v/v). The mixture was concentrated to a small volume by heating on a water bath. The separated precipitate was filtered. It was purified by dissolving in 5% w/v sodium bicarbonate solution, followed by extraction with ether. The aqueous layer on acidification with dilute hydrochloric acid gave aroyl propionic acid [[6,17,21,27]]. It was crystallized from aqueous ethanol to give a compound. Spectral analysis of benzoyl propionic acid: IR (cm^{-1}) 3250 (OH), 1720 (C=O), 1H-NMR (δ, ppm): 2.59 (t, 2H, CH_2), 3.23 (t, 2H, CH_2), 7.53-7.62 (m, 3H, H-3-H-5'), 7.97 (d, 2H, H-2', H-6'), 12.17 (s, 1H, COOH).

All the remaining acids were synthesized by analogous procedure with minor modification in temperature of reaction and use of nitrobenzene as a solvent (Fig 1).

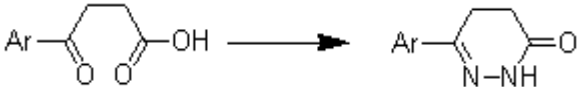
2.2 General Synthetic method of 6-Aryl-4,5-dihydropyridazin3-(2H)-ones (2a-2f):

To a solution of β-benzoyl propionic acid (1a) (0.1 mol) in methanol (30 mL), hydrazine hydrate (1mL) and sodium acetate (0.5 g) were added and the mixture was refluxed for 6 h. After completion of the reaction, methanol was distilled off and the content was poured into cold water. The solid that separated out was filtered and crystallized from methanol[6,17,21,27]. Spectral analysis of 6-phenyl-4,5-dihydropyridazin-3(2H)-one: IR (cm^{-1}): 3306 (NH), 1678 (C=O); ^{1}H-NMR (δ, ppm): 2.45 (t, 2H, CH_2), 2.93 (t, 2H, CH_2), 7.41(m, 3H, H-3'-H-5'), 7.74 (d, 2H, H-2', H-6'), 10.94 (s, 1H, CONH), MS (m/z): 174 (M+), 159, 147, 130, 115, 109.

1. *6-phenyl-4,5-dihydro pyridazin-3(2H)-one* ***(2a)***
2. *6-(4-methylphenyl-4,5-di hydropyridazin-3(2H)-one* ***(2b)***
3. *6-(4-ethylphenyl-4,5-di hydropyridazin-3(2H)-one* ***(2c)***
4. *6-(3,4-dimethylphenyl)-4,5-dihydropyridazin-3(2H)-one* ***(2d)***
5. *6-(2-naphthyl)-4,5-dihydro pyridazin-3(2H)-one* ***(2e)***
6. *6-(1,1'-biphenyl-4-yl)-4,5-dihydropyridazin-3(2H)-one* ***(2f)***

All the remaining acids were synthesized by analogous procedure with minor modification in temperature of reaction (Fig 1).

Figure 1. General Structure of synthesized compounds (1a-f & 2a-f)



Compounds (1a-1f) Compounds (2a-2f)

2.3 Culture, harvesting and Brine shrimp lethality bioassay: *Artemia salina* cysts were purchased from, Sahayamatha Salterns, Pvt , Ltd, 13A/1, pillaiyar koil street, Meenakshipurum, Tulicorin- 628002 and stored at -20^{0}c before use. *Arteimia salina* cysts (10 mg in 100ml) were incubated in artificial seawater under artificial light at 28^{0}c, pH 7-8, with gentle aeration[28]. After 24h incubation hatched nauplii were collected and further incubated for 24h in artificial seawater in the same condition. Hatched nauplii were than diluted to a concentration of 15-20 individuals per ML and transfer to 12–well

micro titrate plate. Three different concentration (5µg/ml, 10µg/ml, 20µg/ml) of drug suspensions were prepared by the use of 0.5% CMC (n=6) and transferred to same plate ,after 24 h incubation number of dead and living nauplii were counted by the help of a stereomicroscope. Percentage of mortality and LC_{50} (24h) was calculated by finney computer program[2].

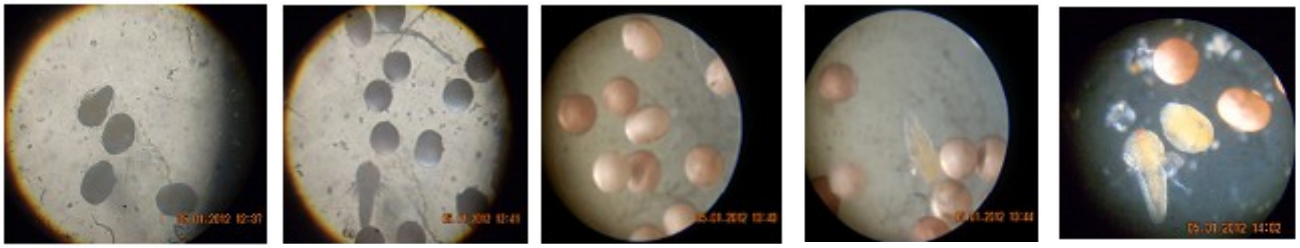
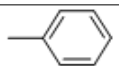
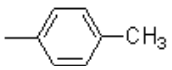
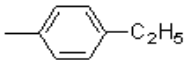
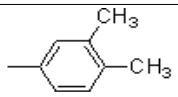
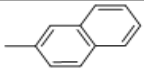
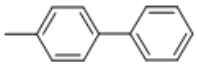


Figure 2. **Pictures of brime shrimp during experiment**

3. Result and discussion

Cytotoxicity of 6 synthetic compounds (**2a-2f**) were tested by using brine shrimp lethality assay method . All the compounds revealed significant cytotoxic activity. WHO classified crude and pure compound as toxic (LC_{50} value<1000µg/ml) and non toxic (LC_{50} value>1000 µg/ml) [29]. Hence result showed in the Table-1shown the potential cytotoxic effect above mentioned compound. All 6-aryl-4,5-dihydropyridazin-3(2H)-one derivatives (**2a–2f**) were prepared from aroyl propionic acids (**1a-1f**). The purity of the compounds was checked by thin layer chromatography. The structures of all synthetic compounds were characterized by IR spectra, NMR spectra and mass spectroscopy. All the 6 compounds (**2a-2f**) were tested at dose level 5, 10, 20 (µg/ML) and potassium dichromate at dose level 10, 20, 30 (µg/ML) as reference drug. Compound **2c** and **2e** exhibited potent brine shrimp lethality with LC50 1.023 and 1.20 µg respectively. Other compounds **2a**, **2b**, **2d**, and **2f** have also showed significant cytotoxicity with LC50 7.58, 6.76, 8.91 and 4.46 µg respectively. The final compounds (**2a–2f**) were showed good cytotoxic activity by brine shrimp lethality assay method (Fig 2). The order of activity was **2d**> **2a**> **2d**> **2f**> **2e**> **2c**. The present study supports that brine shrimp bioassay is simple reliable and convenient method for assessment of bioactivity of **2a-2f** and lends support for their use in medicine. The studied in this work showed significant lethality against brine shrimp, which has been successfully used as a simple biological test to guide the process of pyridazine compounds (**2a-2f**) in order to detect antitumour compounds.

Table 1: **Cytotoxic effect of different 6-aryl-4,5-dihydropyridazinones (2a-2f)**

Compounds	Ar	Conc tested (µg/ML)	LC_{50} (24h)
(2a)		5, 10, 20	7.58
(2b)		5, 10, 20	6.76
(2c)		5, 10, 20	1.023
(2d)		5, 10, 20	8.91
(2e)		5, 10, 20	1.20
(2f)		5, 10, 20	4.46
$K_2Cr_2O_3$a	----------	10,20,30	28.6

a= Potassium dichromate used as reference standard

4. Conclusion

An assessment of various literatures we can collect information about the different structure form of pyridazinone that has been utilized as a part of a large number of complex compounds & these compounds exhibits diversified pharmacological activities due to presence of pyridazine moieties [30-32]. As part of our program, we reported some pyridazinone compounds that containing the different substituent having interesting cytotoxic activity. In future we will also evaluate these compounds as anticancer agents by using tumour cell lines.

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