BRINE-SHRIMP LETHALITY BIOASSAY AND ANTIBACTERIAL ACTIVITY OF BIPHENYL ANALOGUES

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ABSTRACT: *A series of biphenyl derivatives were synthesized and evaluated for brine shrimp lethal bioassay and antibacterial activities. The cytotoxic activity was assessed against the reference drug Etoposide. Compound **4a** (4, 4'-diiodo-3, 3'-dimethoxybiphenyl) exhibited excellent activity in Brine Shrimp Lethal Bioassay while compound **4b** (4-iodo-3, 3'-dimethoxybiphenyl biphenyl) showed moderate activity. Compound **4a** also showed low activity against B. subtilis. Other biphenyl derivatives showed moderate brine shrimp lethal bioassay but could not show any antibacterial activity to an appreciable extent.*

Keywords: Biphenyl analogues, brine shrimp lethal bioassay, etoposide, cytotoxic

1. INTRODUCTION

Biaryl structures are extensively found in many of the natural products including terpenes, alkaloids, flavonoids, lignan, and tannins etc [1, 2]. They have shown a wide diversity in their applications in biological systems. For example, vancomycin is found to be an integral part of many antibiotics: actinoidin A, complestatin, balhimyicn etc. Gossypol, a natural biaryl pigment found in cottonseed, has shown male antifertility activity. Among liganans, steganacin is a constituent of Steganotaenia araliacea possessing a significant antileukemic activity [3]. In addition, Losatan, an antihypertensive agent, has a biphenyl moiety acting as a spacer within the structure [4].

Heterobiaryls can be employed as ligands used in forming metal complexes that can act as bleaching agents, catalysts, and oxygen-binding molecules [5-7]. Atropisomeric biaryls have shown remarkable applications in agrochemical, pharmaceutical industries, and material sciences. They act as chiral ligands and have been used for development of a variety of catalysts used in transition metal-catalyzed asymmetric transformations [8-15]. Polyaryls such as poly-*p*-phenylene possess valuable conducting properties and are being used in making rechargeable batteries, solar cells, and a variety of other electrochemical devices [16-18].

In order to find out the cytotoxic and antibacterial activities of synthesized compounds, a variety of biphenyl derivatives having different moieties at 2, 2' and 3, 3' positions were synthesized.

2. MATERIAL AND METHODS

The synthesis of biphenyl derivatives was carried out from two different sources to investigate the difference in activity of synthesized compounds. Two different classes of compounds were synthesized from [1,1'-biphenyl]-2,2'-diol (compound **1**) and *o*-dianisidine bisdiazotated zinc double salt (compound **3**).

2.1 Synthesis of Biphenyl Analogues based on [1, 1'-biphenyl] - 2, 2'-diol

The synthesis of biphenyl analogues derived from **1** was achieved through the route outlined in **Scheme 1**. Compound **1** was dissolved in acetone (HPLC grade) and then K_2CO_3 was added .The reaction mixture was stirred for 30 minutes and then added *tert*-butyl bromoacetate. After 3 hours, 1N HCl was added to quech the reaction. The required product was **2a** extracted with CH_2Cl_2 three times and was purified through column chromatography giving 80% yield. Compound **2b** was obtained by treating **2a** with TFA for 1 hour and after work up with hexane and toluene. Compound **2c** was obtained from **1** by refluxing **1** and ethylacetate in the presence of acetone for 36 hours. Compound **2d** was obtained by treating dibromomethane (4 equivalents) with **1**, while compound **2e** was obtained by treating statistical amount of dibromomethane with **1** and K_2CO_3 in chloroform acting as solvent.

2.2. Synthesis of Biphenyl Analogues based on Fast Blue B salt

The synthesis of biphenyl analogues deriverd from *o*-dianisidine bisdiazotated zinc double salt (Fast Blue B salt) was achieved through the route outlined in **Scheme 2**. 28 g (0.17 mmol) of KI was dissolved in 200 mL of water. Then, added 10.00 g (21 mmol) of **3**. After stirring for 12 hours at room temperature, 30 mL of dichloromethane was added three times to extract the crude reaction mixture. The resulted crude mixture was purified through column chromatography. Solvent system used for silica gel column was dichloromethane:hexane (1:4) resulting fine crystals of **4a** giving 70 % yield. In addition to **4a**, fine crystals of **4b** were also obtained giving 25% yield.

3. BIOLOGICAL EVALUATION OF SYNTHESIZED BIPHENYL ANALOGUES

3.1 Brine-Shrimp Lethality Bioassay

Cytotoxic activity of synthesized biphenyl analogues was carried out by *Artemia salina* [19-21]. Test samples of different concentrations (10, 100, 1000 µg/mL) were prepared in DMSO and brine shrimps were hatched in sea water media at 27 ^oC for 2 days. Vials containing test samples, reference drug (Etoposide, LD_{50}= 7.465 µg/mL) were incubated at 27 ^oC for 24 hours, and LD_{50} values were determined to evaluate the cytotoxic effects of synthesized compounds by using the procedure of Meyer et al [22].

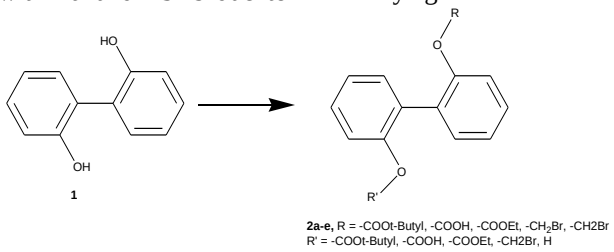
3.2 *In Vitro* Antibacterial Activity

In vitro antibacterial activity of synthesized biphenyl derivatives was carried out against bacterial strains including Escherichia *coli* (ATCC 25922), Bacillus *subtius* (ATCC 6633), Shigella *flexenari* (clinically isolated), staphylococcus *aureus* (ATCC 25923), Pseudomonas *aeruginosa* (ATCC 27853), and Salmonella *typhi* (ATCC 19430). Antibacterial activity of the synthesized compounds was compared with the reference antibacterial drug (imepinem) by using agar well diffusion method [23].

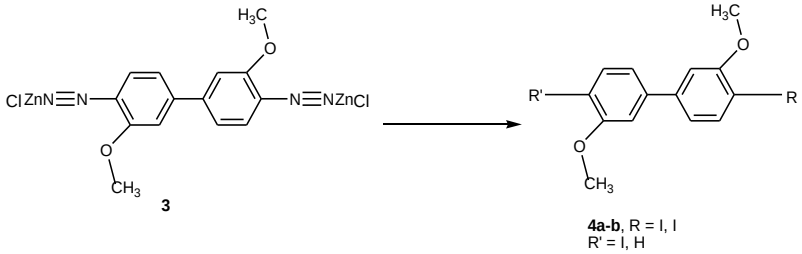
4. RESULTS AND DISCUSSION

Brine shrimp lethality assay correlates in most of cases with cytotoxic and antitumor properties. The LD_{50} value of the brine shrimp obtained from **4a** were found to be significant as compared to etoposide (LD_{50}= 7.465 µg/mL) used as a reference drug. This significant lethality (0.9 µg/mL) of **4a** to brine shrimp is an indicator of the presence of potent cytotoxic components which warrants further investigation. In addition to **4a**, **4b** (3.17 µg/mL) also showed moderate activity. The lethality associated with **4a** and **4b** is due to presence of iodine substituent at C-4 of aromatic rings of substituted biphenyl analogues.

In addition to brine shrimp lethal bioassay, in vitro antibacterial activities were also carried out for the synthesized biphenyl analogues. After incubation of plates at 37 ^{o}C for 14-19 h, the potency was determined by measuring the diameter of zones showing complete inhibition (mm). Most of the compounds showed inactive behavior against all bacterial strains except compound **4a** which showed low activity against B. *subtilis*.



Scheme - 1: Synthesis of [1, 1'-biphenyl] **- 2, 2'-diol analogues, 2a-e.**



Scheme - 2: **Synthesis of Fast Blue B salt analogues 4a-b**

Table-1: Brine shrimp lethality assay of Biphenyl Analogues

Assay	Etoposide (Reference) µg/mL	Compounds 2a	2b	2c	2d µg/mL	2e	4a	4b
Brine shrimp lethality assay	7.465	0	0	0	0	0	0.9	3.17

Table-2: In vitro Anti-bacterial Activity of Biphenyl Analogues Zone of Inhibition of the Tested Compound (mm)

Name of bacteria	Antibacterial Activity Compounds 2a	2b	2c	2d	2e	4a	4b
E. *coli*	0	0	0	0	0	0	0
B. *subtilis*	0	0	0	0	0	15	0
S. *flexinari*	0	0	0	0	0	0	0
S. *aureus*	0	0	0	0	0	0	0
P. *aeruginosa*	0	0	0	0	0	0	0
S. *typhi*	0	0	0	0	0	0	0

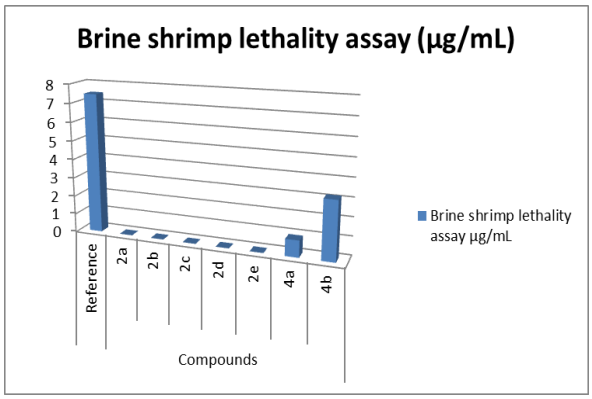


Figure-1: Brine shrimp lethality assay of Biphenyl Analogues

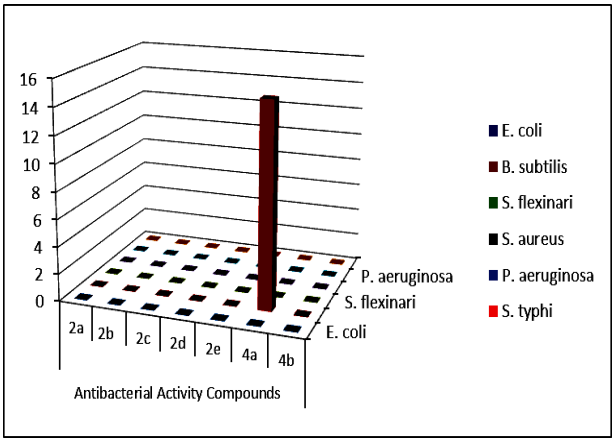


Figure-2: In vitro Anti-bacterial Activity of Biphenyl Analogues Zone of Inhibition of the Tested Compound (mm)

5. CONCLUSION

Synthesis of a series of seven compounds of biphenyl analogues **2(a-e)** and **4(a-b)** is illustrated in **Scheme 1** and **Scheme 2**. Biphenyl analogues were synthesized by using general synthetic route involving nucleophilic substitution reactions on functional biphenyl analogues. A detailed study on the biological usefulness including brine shrimp lethality assay, and antibacterial activities of some synthetic biphenyl analogues were carried out. Brine shrimp cytotoxicity assay serves as prescreen test for the identification of bioactive compounds to evaluate anticancer potential of any compound. Compound **4a** showed significant activity towards *Artemia salina* which means that this compound has strong potential for the anticancer screening. The biphenyl analogues were also evaluated for their antibacterial activities. Only compound **4a** showed low activity against B. *subtilis*.

6. ACKNOWLEDGEMENT

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