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IN-VITRO ANTILEISHMANIAL AND ANTIGLYCATION EVALUATION OF BIPHENYL ANALOGUES

Qamar Ali*, Asad Gulzar, Abrar Hussain, Amina Asghar, Misbah Irshad, Ikram Ullah Bajwa
Division of Science and Technology, University of Education, Township Campus, Lahore, Pakistan
qamarhej@yahoo.com

***ABSTRACT**: A series of synthetic biphenyl analogues were synthesized and evaluated for in-vitro antileishmanial and antiglycation activities. In-vitro antileismanial bioassay was performed by using standard antilesishmanial compounds, amphotericin B and Pentamidine. Compounds 1-4 (57.2 - 91.89 ± 0.18 μM), were found to show moderate activities as compared to the standard pentamidine (IC_{50} = 5.09 ± 0.04 μM) and amphotericin B (IC_{50} (μg/mL) ± S.D. = 0.29 ± 0.05) respectively. Biphenyl synthetic analogues were also screened as antiglycation activity. Rutin was used as the standard inhibitor.*

Keywords: Biphenyl analogues, antileishmanial bioassay, Amphotericin B, Pentamidine, Antiglycation bioactivity, rutin

INTRODUCTION

Biphenyl analogues are an integral part of naturally occurring products [1, 2]. Biphenyl analogues present in natural products show bioactivities like male antifertility and antileukemic activity etc [3]. The biphenyl analogues are important motif in several drug intermediates and in drugs such as losartan and are used as active selective antagonists of nonpeptide angiotensin II type 1 (AT1) receptors for treatment of hypertension [4]. Due to ubiquitous bio-availability of biphenyl moiety, elegant synthetic methods have led to the synthesis of key intermediates [5, 6].

Leishmaniasis is a protozoan disease caused by species of the genus *Leishmania* affecting more than 12 million people in 88 countries in the world [7, 8]. The type of leishmaniasis depends on types parasite species and cellular systems of the patients. The control of this parasitic disease remains a serious problem owing to diversity in species of vectors, Leishmania species and diversity of animals [9]. Chemotherapy for leishmaniasis is costly, requires long-term treatment, showing high toxicities, growing clinical resistance and possibly resulting co-infectious leishmaniasis-AIDS [10-12].

Antiglycation agents may act by blocking carbonyl group on reducing sugars and Amadori products to inhibit AGEPs formation. Recently, drugs have been developed having ability to cleave AGEP cross-links resulting the reverse of diabetic complications. Compounds that are RAGEs (receptors for advance glycation endproducts) blockers have the ability to protect against diabetic complications [13]. The present studies are concerned with synthesis and evaluation of antileishmanial and antiglycation activities of biphenyl analogues.

MATERIAL AND METHODS

In order to investigate the difference in activity of synthesized biphenyl analogues, two different classes of compounds were synthesized from compound **1** and Fast blue B salt.

Synthesis of Biphenyl Analogues Based on [1, 1′-biphenyl] - 2, 2′-diol

The synthesis of biphenyl analogues derived from compound **1** was achieved through the route outlined in **Scheme 1**.

After dissolution of compound **1** in 25mL of acetone, 1.5g (10.21mmol) of K_2CO_3 was added. Then, 2.0mL (13.4mmol) of *tert*-butyl bromoacetate was added. After three hours, 1N HCl was added to quench the reaction. The required product was **2a** extracted with 30 mL of CH_2Cl_2 and purified through column chromatography giving 80% yield. Compound **2b** was obtained by treating **2a** with TFA for one hour, followed by work up with hexane and toluene giving 90% yield. Compound **2c** was obtained from **1** by refluxing with 1.0mL (13.4mmol) methyl chloroformate in the presence of acetone for thirty-six hours affording 80% yield. Compound **2d** was obtained by refluxing 1.3mL of dibromomethane (13.4mmol) with **1** affording 45% yield, while compound **2e** was obtained by treating the statistical amount of dibromomethane with **1** and K_2CO_3 in ethanol acting as solvent affording 50 % yield.

Synthesis of Biphenyl Analogues based on Fast Blue B Salt

The synthesis of biphenyl analogues **4a-b** was carried out from Fast Blue B salt (*o*-dianisidine bisdiazotated zinc double salt) through the route outlined in **Scheme 2**.

After dissolution of 28g (0.17mmol) of KI in 200mL of water, 10.00g (21mmol) of Fast Blue B salt was added. After stirring for twelve hours at room temperature, the crude extract was obtained by adding 30mL dichloromethane three times and was purified through column chromatography. The solvent system used for silica gel column was dichloromethane:hexane (1:4) resulted fine crystals of **4a** giving 70% yield. In addition to **4a**, fine crystals of **4b** were also obtained giving 25% yield.

The synthesized compounds were characterized by EIMS, and ^{1}H-NMR spectroscopic techniques.

1. Antileshamianl Evaluation of Synthesized Biphenyl Analogues

Antileishmanial evaluation was carried by growing *Leishmania major* in modified NNN biphasic medium. Elution of required *Leshamnia* promastigotes was carried out with RPMI 1640 medium having 10% foetal bovine serum (FBS). After extracting Parasites through centrifuging at 2000 rpm for a time period of ten minutes, dilution was carried out a final density of 10^6cells/mL.

In a 96-well micro liter plate, addition of 180μL and 100μL of medium was carried out in first row and other wells

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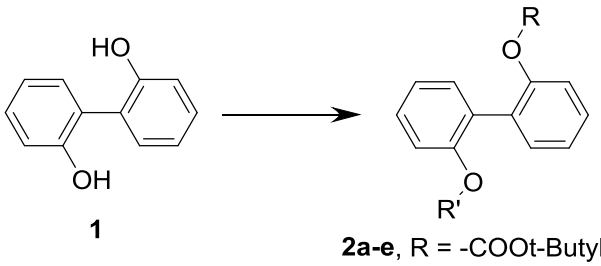
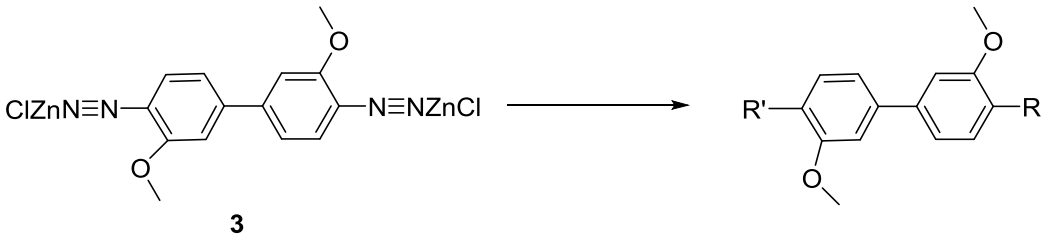
respectively. Then, experimental compound (20µL) was added in medium and serially diluted 100µL of parasite culture in all wells. One of the rows received Negative controls containing only medium while the other received



2a-e, R = -COOt-Butyl, -COOH, -COOMe, $-(CH_2)_3Br$, $-CH_2CH_2CH_2-$
R' = -COOt-Butyl, -COOH, -COOMe, $-(CH_2)_3Br$,$-CH_2CH_2CH_2-$

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



4a-b R = I. I
R' = I, H

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

Table - 1: Antileishmanial Bioassy of Biphenyl Analogues

Compound	IC_{50} (µg/mL) ± S.D.
2a	91.89 ± 0.71
2c	89.1 ± 1.52
2e	57.2 ± 1.4
4b	89.1 ± 1.52

Standard Drugs:
Amphotericin B (IC_{50} (µg/mL) ± S.D. = 0.29 ± 0.05)
Pentamidine (IC_{50} (µg/mL) ± S.D. = 5.09 ± 0.04)

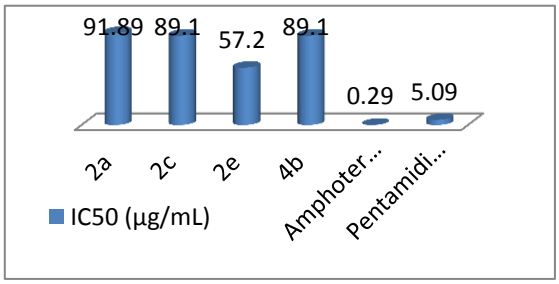


Figure - 1: Antileishmanial Bioassy

Table - 2: Antiglycation Activities of Biphenyl Analogues

Compound	Conc. (mM)	Inhibition (%)	IC_{50} ± SEM [µM]
2a		**- 9.6**	----------
2b		**46.1**	----------
2c		**30**	----------
2d	**1 mM**	**43**	----------
2e		**11**	----------
4a		**28.8**	----------
4b		**25**	---------

Standard Inhibitor:
Rutin was used as a positive control (IC_{50} = 294 ±1.50 µM)

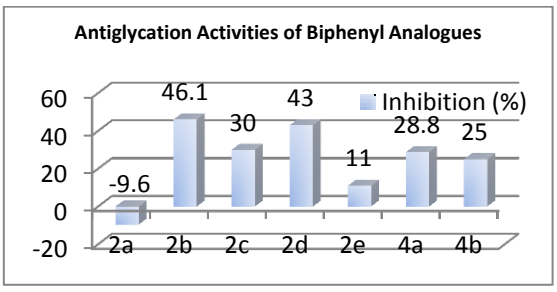


Figure - 2: Antiglycation Activities

positive control containing varying concentrations of standard *antilesishmanial* compounds, Pentamidine, and amphotericin B. After incubating for seventy-two hours at 21-22^{o}C, the culture was examined microscopically on Neubuer counting chamber and Software Ezfit 5.03 Perella Scientific was used for calculation of IC_{50} values of compounds.

Anti-glycation Activity

This assay is used to inhibit the Methyl Glyoxal mediated development of fluorescence of BSA. This assay was performed by using slightly modified Lee *et al* method. After incubation for nine days, specific fluorescence (excitation, 330nm; 440nm) for each compound was studied against sample blank on a microtitre plate spectrophotometer.

RESULTS AND DISCUSSION

In-vitro antileishmanial activity of compounds 1-4 showed IC_{50} values ranging between 57.2- 91.89μM. These values showed moderate antileishmanial activity as compared with standard, pentamidine (IC_{50} = 5.09 ± 0.04 μM), and amphotericin B (IC_{50} (µg/mL) ± S.D. = 0.29 ± 0.05) (Table - 1) respectively.

Antiglycation screening was carried out by incubating the compounds under study for nine days and developing specific fluorescence against the microtitre plate spectrophotometer. Rutin was used as the standard inhibitor with 82.5% inhibition at 3.0mM concentration. Inhibition (%) was calculated through the following formula:

Inhibition (%) = 1 - [(Fluorescence of test sample) / (Fluorscence of glycated)] × 100

Compounds 2a-e, and 4a-b showed very less antiglycation activity as compared to rutin taken as a positive control, while 2a was found to be inactive.

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