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Microwave-assisted synthesis, structural activity relationship and biological activity of some new quinoxaline Schiff base derivatives as highly potent spirochete bactericidal agents

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ABSTRACT

The main aim of this work was to synthesise a new (E)-3-(4-or3-Aminophenylimino) quinoxaline-2(3*H*)-one oxime Schiff base derivatives and evaluate the anti-leptospiral activity against *Leptospira icterohaemorrhagiae*. The mentioned derivatives were prepared by performing microwave-assisted condensation reactions of (E)-3-(4-or3-Aminophenyl imino)quinoxaline-2(3*H*)-one and aromatic aldehydes. The structures of these interesting compounds were characterised by FT-IR, ^{1}H NMR and mass spectroscopy. Furthermore, these compounds were screened for spirocidal activity against *Leptospira icterohaemorrhagiae* by using *in vitro* and *in vivo* method. The anti-leptospiral activity result reveals that most of the compounds were exhibiting considerable activity against *Leptospira icterohaemorrhagiae*. Compound **6c** demonstrated remarkable activity at low concentration against the *Leptospira icterohaemorrhagiae* as compared to the standard drugs.

1. Introduction

Leptospirosis is an acute anthropo-zoonotic infection (transmitted from animal to animal and man) that occurs in many parts of the world, but most frequently in tropical and subtropical regions (Sambasiva et al., 2003). It is caused by spirochete bacteria of the genus *Leptospira* (Evangelista and Coburn, 2010). The bacteria spread mainly through the urine of infected animals (Rats, moles and mice) which can get into water or soil and survive there for weeks to months. It also transmitted through the semen of the infected animals (Sumanta et al., 2015). These microbes can enter the body through the skin or mucous membrane, especially if the skin is broken from a cut or scratch, swallowing contaminated water, splashing of contaminated water into the nose or eyes (Ko et al., 2009). Some of the research studies suggested that it is an occupational disease occurs worldwide, but is most common in China, Vietnam, Japan and Korea. This disease usually affects people living with health hazards in the workplace, such as sewer worker, slaughterhouse worker, veterinarian, surfers, swimmers and farmers etc., (Rafizah et al., 2013). Some of the symptoms of leptospirosis are high fever, headache, chills, joint pain, muscle aches, diarrhoea, jaundice and vomiting (Daher et al., 2010). In India, leptospirosis has been known to be endemic since in the early period of the 20th century and emerging health issues over the next three to four decades. It has been diagnosed by enzyme-linked immunosorbent assay (ELISA), microscopic agglutination test (MAT), polymerase chain reaction (PCR), serological test and dark field microscopy (DFM) (Musso and Scola, 2013). The microbial growth has been controlled by using the medication such as doxycycline, penicillin, ampicillin, amoxicillin and third generation cephalosporin (Jessica et al., 2006; Edwards, 1959) etc., There is a requirement of highly potent molecule to control this disease effectively.

Quinoxalines and their derivatives are nitrogen based six-membered heterocyclic compounds used in food and pharmaceutical industry (Soleymani et al., 2012). They also called as benzopyrine and widely employed in many pharmaceutical applications such as anti-inflammatory (Burguete et al., 2011), anti-oxidant (Hossain et al., 2012), anti-viral (Vieira et al., 2014), anti-bacterial (Shen et al., 2016), anti-malarial (Shekhar et al., 2014), anti-cancer (Alinezhad et al., 2013), anti-depressant (Vadhat and Baghery, 2013; Galal et al., 2011), anti-protozoal (Marella et al., 2013), hypolipidemic (Singh et al., 2011), anti-HIV (Ali et al., 2007), anti-convulsant (Elhelby et al., 2011) and anti-leptospiral agents (Natarajan et al., 2013). It's also found in many antibiotic as a part of the molecular structure such as actinoleutin,

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echinomycin and levomycin etc., In the present study, aforesaid benefits of quinoxalines heterocyclic ring promoted us to prepare a bunch of novel (E)-3-(4-or3-Aminophenylimino) quinoxalin-2 (3*H*)-one oxime Schiff base derivatives and evaluate the activity against spirochete bacteria by *in vitro* and *in vivo* method. The report stated that most of the prepared analogues were exhibiting excellent activity against spirochete bacteria. The compound structures were characterised by FT-IR, ^{1}H NMR, mass spectroscopy and elemental analysis.

2. Materials and methods

2.1. Chemistry

The melting points of synthetic analogues were recorded by open capillary tube method and are uncorrected. Functional group present in the prepared analogues was confirmed by using Fourier transform infrared spectroscopy (FT-IR) between the ranges from 4000 cm^{-1} to 400 cm^{-1}. The number of protons present in the compound was calculated by Bruker ^{1}H NMR spectroscopy from chemical shift (δ), molecular weight of the compound was determined by the Shimadzu mass spectrometer and element analysis was performed on Perkin Elmer 2400 CHN elemental analyser.

2.1.1. Preparation of quinoxaline-2,3-dione: ***[1]***

A mixture of benzene-1,2-diamine (1.08 g) and oxalic acid (5 g) was stirred occasionally for 10 min and irradiated in a domestic microwave oven at an emitted power of 400 W (5 power) for 3 min under solvent free condition. The resulting hot solution was poured into a crushed ice. The precipitated solid was separated by vacuum filter, washed with a small portion of cold water, dried and recrystallized from ethanol (Gris et al., 2008; Puratchikody et al., 2011).

2.1.2. Preparation of 3-(Hydroxyimino) quinoxaline-2(3H)-one: ***[2]***

A mixture of quinoxaline-2,3-dione (1.89 g) and hydroxylamine hydrochloride (1.08 g) was added to a beaker contained pyridine (5 mL). Then the mixture was irradiated in a domestic microwave oven at an emitted power of 400 W (5 power) for 5 min and poured carefully into ice-cold water with constant stirring. The precipitated product was separated by vacuum filter, washed with a small portion of cold water, dried and recrystallized from ethanol (Hajipour et al., 2010).

2.1.3. Preparation of (E)-3-(4-or3-Aminophenylimino) quinoxaline-2 (3H)-one oxime: ***[3,4]***

An equimolar mixture of 3-(Hydroxyimino)quinoxaline-2(3*H*)-one (0.01 mol) and benzene-1,4-diamine (0.01 mol)/benzene-1,3-diamine (0.01 mol) was dissolved in 10 mL of ethanol and add a few drops of glacial acetic acid. The mixture was irradiated in a domestic microwave oven at an emitted power of 400 W (5 power) for 2 min. The resulting solution was allowed to stand for 5 min at room temperature. A precipitate thus obtained was filtered, washed thoroughly with a small portion of cold water, dried and recrystallized from ethanol (Puratchikody et al., 2013).

2.1.4. General procedure for different Schiff base preparation: ***[5a-f & 6a-f]***

Compound (E)-3-(4-Aminophenylimino)-3,4-dihydroquinoxaline-2(3*H*)-one oxime (0.1 mol)/(E)-3-(3-Aminophenylimino)-3,4-dihydroquinoxaline-2(3*H*)-one oxime (0.1 mol) was dissolved in 10 mL of ethanol and a few drops of glacial acetic acid. The required aldehyde was added to the above mixture and stirred with the aid of magnetic stirrer for 30 min and kept in a microwave oven at an emitted of 400 W for 45 s. The resulting hot solution was transferred into ice-cold water, which led to the separation of solid products. The separated product was filtered, washed, dried and recrystallized from hot ethanol (Fig. 1 & Table 1) (Kedy et al., 2015)

2.2. X-ray crystallography

X-ray crystallography is a tool used to investigate the three-dimensional picture of the atomic and molecular structure of a crystal by using X-ray light, which has wavelengths of 1 Ã (10^{-8}cm). The beam of X-ray strikes a crystal and causes the diffraction of light into specific directions, fed into the computer and using a mathematical equation to calculate the position of every atom in the crystallised molecule.

2.3. Biological evaluation

2.3.1. Preparation of culture media

The survival, growth and motility of bacteria have been assessed in a suitable culture medium (Ellinghausen-McCul lough-Johnson-Harris medium). Leptospiral EMJH culture media were prepared by mixing of monopotassium phosphate (0.3 g), disodium phosphate (1 g), sodium chloride (1 g), Ammonium chloride (0.25 g), Thiamine (0.005 g) etc., (Zacarias et al., 2008).

2.3.2. Preparation of culture

To determine the anti-leptospiral activity of synthetic analogues, an appropriate culture was required. It has been prepared by mixing of *Leptospira icterohaemorrhagiae* strain (Icter No: 1) with EMJH medium (Gonçalves et al., 2010).

2.3.3. In vitro leptospiral activity

The stock solutions were prepared from test compounds using dimethyl sulfoxide (DMSO). In addition to that serial dilutions are made from each compound (10^{-1} to 10^{-5}) by using mentioned stock solutions. The micro dilution procedure was adopted in each test compound and achieved the concentration of 5.0×10^5 CFU/mL in 96 well plates against spirochete bacterial culture. The plates were incubated at room temperature for 7 days. Finally, the motility of the spirochetes was observed under dark field microscopy (Bhatia et al., 2015). Here, DMSO and Penicillin G were acting as a control and standard respectively.

2.3.4. In vivo leptospiral activity

Selected compounds from the *in vitro* evaluation were utilised to perform the *in vivo* anti-leptospiral study. The whole experimental protocol was approved by the institutional animal ethics committee at GIET School of pharmacy, Rajahmundry, India (GSP/PY/02/2016). To test the anti-leptospiral activity, Wistar rats of either sex with an average weight of 200–225 g was selected, divided into nine groups and maintained each group containing six animals (standard, test and control). These animals were placed into different cages and provided well-ventilated, temperature controlled (30 ± 1 °C) lab conditions during the entire period of study (8 days). On day 0, all the experimental animals were screened for the absence of earlier leptospirosis from urine sample using dark field microscopy. On day 1, Group I was treated with daily 1 mL of 0.1% carboxymethyl cellulose as considered as normal control. The remaining groups of animals were infected by administering 1×10^7 org/mL of *Leptospira icterohaemorrhagiae* intraperitoneally. Group II animals were also given daily 1 mL of 0.1% carboxymethyl cellulose (infected control). The test compounds **(5b, 5c, 5e, 6b, 6c** and **6e)** were given daily to group III to group VIII containing animals with the dose of 250 mg/kg body weight. Penicillin G administered to group IX animals and had been treated as a standard. Administration of test and the standard drug was done by the oral route.

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