Original Article

STUDIES ON ANTIVIRAL ACTIVITY OF 2,3-DIPHENYLQUINOXALINE

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Abstract
2,3-Diphenylquinoxaline (DPX) have been synthesized by condensation of benzil and O-phenylenediamine by microwave irradiation technique. Their chemical structure was assigned by means of spectral analysis (FT-IR, ^{1}H-NMR, MS). 2,3-diphenylquinoxaline (DPX) was tested for antiviral activity against Human immunodeficiency virus (HIV) -1 and 2 in Metallothionein (MT) - 4 cells and Hepatitis C virus (HCV) in Human Hepatoblastoma cells (Huh) -5-2 Cells. DPX active against replication of HIV-1(IIIB) in MT-4 cells.

Key words: Microwave Irradiation Technique, 2,3-Diphenylquinoxaline, Anti-HIV Activity.

Introduction
AIDS is a fatal pathogenic disease caused by Human Immunodeficiency Virus (HIV), a retrovirus. Recently much attention has been devoted to the development of vaccine and chemotherapeutic agents for the eradication of HIV/AIDS. Despite so much effort in the field of HIV, no effective vaccine is available till now to combat HIV/AIDS. The only available option is chemotherapy that can reduce the viral load and improve the quality of life of HIV/AIDS patients. The present is aimed at designing and synthesis of novel heterocyclic compounds, screening for their inhibitory activity against HIV and HCV is important co- viral infection in HIV/AIDS.

Quinoxaline derivatives are well known in the pharmaceutical industry and have been shown to possess a broad spectrum of biological activities including antiviral[1], antibacterial[2], anti-inflammatory[3], kinase inhibitors[4], anticancer[5] and anthelmintic agent[6]. A large number of quinoxaline have been synthesized and studied for wide range of antiviral activity, but the anti-HIV and HCV activity of quinoxaline relatively unexplored. Based on these findings, 2,3-diphenyquinoxaline were synthesized and screened for antiviral activity against HIV-1 & 2 in MT-4 cells and HCV in Huh-5-2 cells.

Experiment
Melting points were determined using open ended capillary tube method and are uncorrected. FT-IR recorded on Perkin Elmer–1605 series FT-IR in KBr disc. 1H NMR Spectra were recorded at 400MHz on Bruker FT-NMR spectrophotometer using TMS as internal standard.

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Mass spectra on a Varian Atlas CH-7 Mass Spectro-photometer at 70 eV. The figure-01 (scheme) that shows the synthesis of 2, 3-Diphenylquinoxaline.

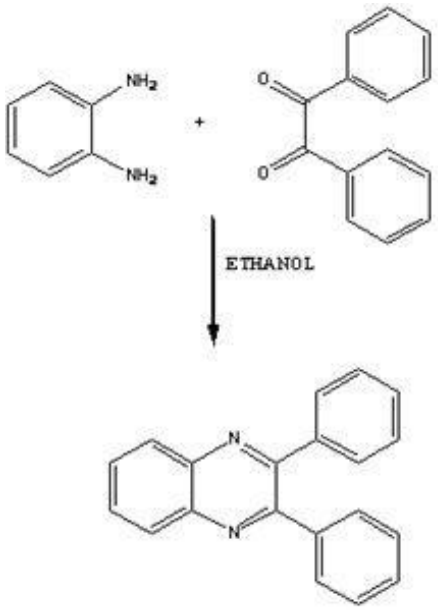

Figure-01
Scheme – Synthesis of 2,3-Diphenylquinoxaline

Synthesis of 2, 3-diphenylquinoxaline
Equimolar quantities (0.001) of o-phenylenediamine and benzil were dissolved in 10 ml warm ethanol. The reaction mixture was irradiated in an unmodified domestic oven at 80% intensity with 30s / cycle for 3 min and set aside. The resultant solid is washed with water, dried and recrystallized form ethanol. Yield 85 %; mp: 110° C;IR (KBr) 1620 (C=N), 1590 (C=C); H NMR (DMSO-D_6): 7.1-8.2 (m, 14H, Ar-H); El-MS (m/z): 282.12.

Anti-HIV activity

The compounds were tested for antiHIV activity against the replication of HIV-1(III_B) and HIV-2(ROD) in MT-4 cells[7]. The cells were grown and maintained in RPMI 1640 Medium supplemented with 10% heat–inactivated Fetal Calf Serum (FCS), 2mM- glutamine, 0.1% Sodium bicarbonate and 20μgm/ml gentamicin (culture medium). HIV-1 (HTLV-IIIB/LAI) strain and HIV-2 ($LAV\text{-}2_{ROD}$) strain were used in the experiment. The virus strains were propagated in MT-4 cells. Titer of virus stock was determined in MT-4 cells and the virus stock was stored at - 70°C until used.

Inhibitory effects of the compounds on HIV-1 and HIV-2 replications were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and were estimated by MTT assay. Briefly, 50 μl of HIV-1 and HIV-2 (100-300 $CCID_{50}$) was added to a flat-bottomed MT–4 cells were added at a final concentration of $6x10^5$ cells/ml. After 5th day of incubation, at 37°C the number of viable cells were determined by the 3 - (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. Cytotoxicity of the compounds for mock- infected MT-4 cells were assessed by the MTT method. Anti-HIV activity and cytotoxicity of standard AZT were also performed by a similar method in MT-4 cells. The antiHIV activity and cytotoxicity data are present in Table-01.

Anti-HCV activity

Replicon assay[8]: Huh-5-2 cells was cultured in RPMI medium (Products Manufactured Gibco) supplemented with 10% fetal calf serum, 2mML-glutamine (Life Technologies); 1x non essential amino acids (Life Technologies); 100 IU/ml penicillin and 100μg/ml streptomycin and 250 μg/ml G418 (Geneticin, Life Technologies). Cells were seeded at a density of 7000 cells per well in 96 well View Plate TM (Packard) in medium containing the same components as described above, except for G418. Cells were allowed to adhere and proliferate for 24 hr. At that time, culture medium was removed and serial dilutions of the test compounds were added in culture medium lacking G418. Interferon alfa 2a(500 IU) was included as a positive control. Plates were further incubated at 37^0C and 5% Co_2 for 72 hours. Replication of the HCV replicon in Huh-5 cells results in luciferase activity in the cells.

Luciferase activity is measured by adding 50 μl of 1 x Gloysis buffer (Promega) for 15 minutes of followed by 50 μl of the Steady-Glo Luciferase assay reagent (Promega). Luciferase activity is measured with luminometer and signal in each individual well in expressed as a percentage of the untreated cultures. Parallel cultures of Huh-5-2 cells, seeded at a density of 7000 cells/well of classical 96 well cell culture plates (Becton-Dickinson) are treated in a similar fashion except that no Glo-lysis buffer or Stady-Glo Luciferase reagent is added. Instead the density of the culture is measured by means of the MTS method (Promega). The antiHCV activity and cytotoxicity data are present in Table-02.

Table-01
Anti-HIV activity of 2,3-diphenylquinoxaline in MT-4 cells

Code	Strain	IC_{50} (μg/ml) [a]	CC_{50} (μg/ml) [b]	Max Prot (%)
DPX	III_B	3.84	15.27	59
	ROD	>15.4	15.4	0
AZT	III_B	0.0012	65.9	126
	ROD	0.00062	65.9	148

[a]50% effective concentration required to reduce virus induced cytopathicity by 50%.
[b]50% cytotoxic concentration required to reduce host cell viability by 50%.

Table-02
Anti-HCV activity of 2,3-diphenylquinoxaline in Huh-5-2 Cells

Concentration	Cell growth*	Viral RNA*
50 μg/ml	33	0
16.6	49	2
5.55	93	34
1.85	106	64
0.62	59	100
	CC50	**EC50**
	16	3

*(% of untreated control)
Interferon alfa-2b at 10,000 units/well reduced the signal in the viral RNA (luciferase) assay to background levels; without any cytostatic activity.

Results and Discussion

The reaction utilizes the microwave irradiation in an unmodified domestic microwave oven at 80% intensity with 30 s/cycle for 3min and set aside. The resultant solid was washed with dilute ethanol, dried, and recrystallized from ethanol. The yield was found to be 64–89%. Unlike conventional methods (duration-1 h), microwave-assisted reactions were very facile (2-3 min). The purity of the synthesized compounds was checked by TLC and the compounds of this study were identified by spectral data.

2,3-diphenylquinoxaline (DPX) was tested for antiviral activity against the replication of HIV-1 & 2 in MT-4 cells and anti-HIV activity is based on the inhibition of virus induced cytopathic effect in MT-4 cells. Cytotoxicity is also tested in uninfected MT-4 cell (Adult C type leukemic cells) and DPX active against replication of HIV-1 in acutely infected MT-4 cells with IC_{50} 3.84 μg/ml and cytotoxicity (CC_{50}) at the concentration of 15.27 μg/ml. DPX having 59% maximum protection against the HIV induced cytopathic effect in MT-4 cells and DPX clearly had some inhibitory activity against the *in vitro* HIV-1(IIIB) replication, whereas it was completely devoid of anti-HIV-2 (ROD) activity.

2,3-diphenylquinoxaline (DPX) was investigated for antiviral activity against Hepatitis C Virus (HCV) in Human Hepatoma cells (Huh-5-2 Cells) and Cytotoxicity is also tested in uninfected human hepatoma cell (Huh cells) by MTS assay. DPX inhibited the HCV RNA synthesis (IC_{50}) in sub genomic replicon replication assay at the concentration of 3µg/ml and cytotoxicity (CC_{50}) in mock infected Huh 5.2 cells was found to be 16µg/ml and Selectivity index (SI)=5. DPX had mild inhibitory activity against the HCV replication in human liver cells.

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