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Antiviral Activity of Novel Quinoxaline Derivative Against Herpes Simplex Virus Type 1, Human Cytomegalovirus, and Varicella-Zoster

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ABSTRACT

Quinoxalines are nitrogen-containing heterocyclic compounds that are both widely recognized and significant. They form the ring complexity that consists of a pyrazine ring and a benzene ring. Distinctly substituted quinoxalines and related compounds, which are incorporated with an abundance of functional groups, constitute significant biological substances. A substantial quantity of studying has been focused towards this class. An assortment of innovative 5H-indolo-(2,3-b) quinoxaline derivatives that have a possibility to act as antiviral drugs. Plaque-reduction technique was implemented to evaluate the antiviral and cytotoxicity of a number of the synthesized compounds. The investigation employed the following methods: adjusting the virus by successive dilutions in the culture medium and assaying it in three separate batches on Vero the monolayers in each well of microtiter trays. The EC50, or the compound concentration necessary for minimizing virus-induced cytopathicity or viral plaque development by 50%, was used to express antiviral activity. Compound b demonstrated promising antiviral activity, while the majority of the compounds investigated exhibited cytotoxicity at a concentration of 160 ug/ml. The current study offered a comprehensive understanding of the synthesis of quinoxaline and its biological properties, as well as its antiviral properties against varicella-zoster virus, human cytomegalovirus, and herpes simplex virus type 1.

Keywords : Antiviral Activity, Cytotoxicity, Herpes Simplex Virus Type 1, Human Cytomegalovirus, And Varicella-Zoster

I. INTRODUCTION

Heterocyclic compound synthesis has been for years a fascinating area of research [1]. Quinoxalines are a renowned and significant N-containing heterocyclic compound that is composed of a ring combination between a pyrazine ring and a benzene ring, which is why they are referred to as benzopyrazine [2].

Quinoxaline derivatives are a significant category of heterocyclic compounds where several atoms of carbon of the naphthalene ring are swapped by N [3]. Some of other people, that they can be employed to combat infections such as leishmania, tuberculosis, tumors, cancer, melancholy, and neurologic disorders [4]. All of these activities are facilitated by the quinoxaline structural nucleus. The quinoxaline structure functions as a prerequisite for the creation of a vast array of new compounds that are suitable for a variety of uses [5].

These substances are significant for their biological properties [6]. In particular, they are employed as anti-tumor, antifungal, antiviral, antibacterial, anti-inflammatory in nature anti-tubercular, anticonvulsant, anti-malarial, antileishanial, and trypanocidal compounds [7].

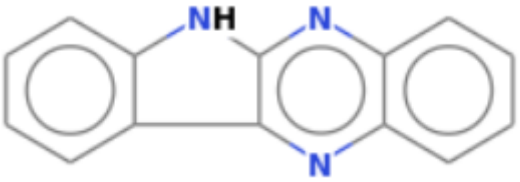
The Alphaherpesviridae subgroup is home to the linear dsDNA virus known as Herpes simplex virus type 1 (HSV-1). HSV-1 is chiefly liable for the establishment of both initial and recurring vesicular lesions in the orolabial and genital mucosa [8]. The progression of infection by HSV is restricted by antiviral therapy [9]. Risk factors for orolabial herpes involve anything that involves contact with the saliva of a person with the infection, such as sharing drinkware or cosmetics or engaging in mouth-to-mouth contact. In the event of orolabial inflammation caused by HSV-1 in the juvenile people, thumb suckling and nail gnawing are indicators of risk for herpetic whitlow [10]. The antiviral capacity of herpes simplex virus type 1 is described in this study using a quinoxaline derivative.

The primary form of varicella is caused by the Varicella-zoster virus (VZV), an essential part of the Herpesviridae family, while zoster is caused by the virus as a recurrence [11]. Varicella and zoster are both illnesses that are prominent on a global scale and may result in substantial consequences. Nevertheless, a wide range of testing techniques are available for detecting VZV infections. Antiviral treatment of VZV infections and their avoidance through vaccination or passive immunoprophylaxis are well-established in medical care, compared with numerous other viral infections. The current manuscript offers a comprehensive summary of the fundamental understanding of the antiviral activity of VZV infections [12].

Human cytomegalovirus (HCMV) has the potential to induce severe end-organ disease and propagate at a high level if the body's defenses is compromised [13]. There is significant progress being made in the comprehension of the history and pathophysiology of HCMV infection and disease in the immunocompromised host [14, 15]. This study's objective is to evaluate the novel compounds that are influencing the design of randomized controlled trials of new antiviral pharmaceuticals and vaccines that are currently being evaluated.

II. MATERIAL AND METHOD

2.1 Synthesis of indolo[2,3-b] quinoxalines derivatives



On a 0.10 molar scale, indolo[2,3b] quinoxaline and 2,3dimethyl-6Hindolo[2,3b]quinoxaline analogues were made using Schunck's and Marchewski's approach. The compounds were added to a solution of 1-chloro-2-dimethylaminoethane hydrochloride, K2CO3, in acetone and refluxed for 24 hours. The reaction mixture was then poured into water, and the solid that resulted was isolated by filtration.

2.2 Antiviral and cytotoxicity screening of herpes simplex virus type 1

Synthesis - Compounds were dispersed in 50 ml of DMSO then aliquoted into sterilized growth medium. Both combinations was sub-diluted in sterilized media and used to evaluate samples at 2–200 ug/ml in micotiter plate wells. To create virus and cell culture

H. simplex type I (HS-I) stock, Vero cells were treated with 1 virion per 10 cells and grown for 3 days. The temperature was -80°C. To make working stocks, the virus was serially diluted in the incubator and quantified in triplicate on Vero single layers in each microtiter tray well. Virus suspensions with 30 plaques per well were kept at 4°C until needed. Dulbeccois altered Eagle's medium with 10% (v/v) calf serum and 60 lg/ml streptomycin sulfate was used to cultivate Vero African green monkey kidney cells. A humidified environment with 15% (v/v) CO2 maintained the cells at 37°C.

Unless otherwise stated, supplied all medium components. Medium containing 1% (v/v) calf serum was used to maintain vero stocks at 34°C. For virus titration or antiviral screening, Vero cells were suspended in medium after trypsin-EDTA treatment, calculated with a hemocytometer, reduced in medium with 10% calf serum to 2 9 104 cells per 200 ml culture, divided into each well of a microtiter tray, & culturing until confluent.

2.2 Antiviral screening procedures of herpes simplex virus type 1

The microtiter packaging containing confluent monolayer cultures of Vero cells had been reversed. The medium was agitated and replenished with numerous dilutions of sterile preparations of samples in triple in 100 ll medium. The tittered virus was then added to 100 ll medium comprising 10% (v/v) calf serum in each well. Aphidicolin was implemented as a positive control. The final array of wells in each tray was designated for controls that hadn't been subjected to compounds or virus treatment. The containers were inverted onto a pad of tissue paper after being cultured for 66 hours. For a minimum of 20 minutes, the remaining cells were fixated with 3.7% (v/v) formaldehyde in saline after being meticulously cleansed with medium. The cells that were fixed were visually inspected after being cleansed with water. Confluent, essentially unaltered monolayers of labeled Vero cells treated with HS-1 are used to identify antiviral activity. Additionally, the concentration that resulted in an about fifty percent loss of the monolayer surrounding the lesions triggered by HSV-1 was employed for calculating cytotoxicity.

2.3 Antiviral activity of varicella-zoster virus and human cytomegalovirus

The components were tested against “Varicella zoster virus (VZV)” and Human cyto megalo virus (HCMV) strains. The viruses' cytopathicity or plaque formation was suppressed in HEL and human fibroblast cells. Growth in a solution of different test chemicals was followed by the distribution of 100 CCID 50 of virus or 20plaque forming units (PFU) into combination cell cultures in microtiter 96-well plates. CCID 50 is the viral dosage needed to infect 50% of cell cultures. Once viral cytopathicity or plaque development was complete, control virus-infected cell cultures without test compounds were collected. The chemical concentration that reduced virus-induced cytopathicity or plaque by 50% was employed to demonstrate antiviral efficacy. The minimum cytotoxic concentration (MCC) or chemical concentration that caused a microscopically discernible cell morphology change was used to show cytotoxicity of test substances.

III.RESULT AND DISCUSSIONS

Table 1 : Antiviral activity of Quinoxaline derivative against Herpes simplex virus type 1

S.NO.	COMPOUND	ANTIVIRAL PLAQUE REDUCTION (%)	MIC (UG/ML)	CYTOTOXICITY 50CD (UG/ML)
1	a	--	--	--
2	b	25	20	200
3	c	<26	80	160
4	d	<26	80	160
5	e	<26	80	160
6	Aphidicolin	100	5	20

The 5H-indolo-(2,3b) quinoxaline derivatives were tested for possible antiviral activity as well as cytotoxicity toward Herpes simplex virus cultured on Vero African monkey kidney cells using a revised plaque-reduction assay. Table 1 summarizes the antiviral and cytotoxicity information related to these compounds. The 5H-indolo-(2,3b) quinoxaline chemicals exhibited the highest antiviral activity, with b demonstrating a decrease of 26% in the number of plaques at a concentration of 20 mg/ml with cytotoxicity 200ug/ml. Conversely, the remaining ten compounds at high levels decreased the total amount of plaques by just under 26%. Additionally, compound a exhibited the least cytotoxicity of all the compounds that were evaluated. We can infer that the antiviral properties may be attributed to the thiourea moiety, which underscores the significance of the selected moieties in the creation of antiviral activity (Table 1). The majority of the unbiased 5H-indolo[2,3-b]quinoxaline derivatives exhibited encouraging cytotoxic characteristics against all of the examined compounds except a compound, despite the presence of a variety of alkyl amino side chains.

Table 2 Antiviral activity and cytotoxicity against varicella-zoster virus (VZV) in HEL cell cultures and HCMV in fibroblast cells.

S.No.	Compound	Antiviral Plaque reduction (EC50 uM) a	Cytotoxicity (uM)	Antiviral Plaque reduction (EC50 uM) b	Cytotoxicity (uM)
		VZV strain (OKA)		HCMV	
1	a	31.29	58.26	20	20
2	b	100	>100	20	20
3	c	31.29	36.45	>20	20
4	d	>20	100	14.22	>100
5	e	>20	100	27.30	>100
6	Acyclovir	0.49	>440	5	>300

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU). [b] Minimum cytotoxic concentration that causes a microscopically detectable alternation of cell morphology.

In the current study the synthesized compounds exhibited only a limited amount of antiviral properties against the varicella-zoster virus, which encompassed both VZV strains (OKA). The antiviral effect of compound b was most effective against the herpes simplex virus type 1, but it was substantially less compared to that of the standard chemicals' acyclovir. The quinoxaline derivatives exhibited cytotoxicity against all three viruses: herpes simplex virus type 1, human cytomegalovirus, as well varicella-zoster. The cytotoxicity value of Acyclovir against varicella-zoster virus is >440, while it is >300 against human cytomegalovirus.

The broad range of biological activity exhibited by quinoxaline derivatives has piqued the interest of researchers. The transcription of herpes simplex viruses of types 1, cytomegalovirus, varicella zoster, and shingles viruses were inhibited by quinoxalines, as reported in article by Pereira et al. (2015) [14].

The discovery of possible antiviral medications with a quinoxaline nucleus has received a lot of attention. The fact that quinoxalines have different antiviral characteristics suggests that each one's effectiveness depends on particular substitution sequences. The antiherpes virus activity of many al 6H-indolo-(2,3-b) quinoxalines was evaluated by Shibinskaya et al. [2015]. The most effective molecule was 2,3-dimethyl(dimethylaminoethyl)5H-indolo-(2,3-b)quinoxaline. Additional research was done on this compound's antiviral effects and mechanism of action. Depending on the cell type and viral number employed in the study, the chemical reduced the reproduction of varicella-zoster virus, cytomegalovirus, and herpes simplex virus type 1 in cultured tissue at dosages ranging from 1 to 5 μM. Usually, the initial CMV transmission is harmless and

asymptomatic. However, in immunocompromised people, including transplant recipients, a CMV infection can have serious repercussions and harm organs like the liver, lungs, or eyes [11]. Cellular toxicity was seen at a concentration of 10 to 30 μM, but antiviral activity was seen in human bladder cancer and human embryonic lung fibroblast cell lines at doses 3 to 15 times lower than those that induced cellular toxicity. At high doses (about 300 μM), indolo quinoxalines were shown to inactivate virions; at lower quantities (around 3 μM), they decreased the synthesis of viral DNA and protein. One methylene group's extension or reduction, which depended on the alkyl chain's dimension, reduced the antiherpesvirus activity.

IV.CONCLUSION

The antiviral effect of quinoxaline chemicals was demonstrated against varicella-zoster, cytomegalovirus, and herpes simplex virus type 1. The maximal activity was demonstrated against the varicella-zoster virus, as opposed to one of the two viruses, human cytomegalovirus and herpes simplex virus type 1.The quinoxaline derivatives inhibited the growth of herpes simplex virus type 1, human cytomegalovirus, and varicella-zoster. The majority of the unbiased 5H-indolo[2,3b]quinoxaline derivatives exhibited encouraging cytotoxic characteristics against all of the examined organisms, despite the presence of a variety of alkyl amino side chains.

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