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4-Amino pyrimidine derivatives: design, synthesis, antiviral, and antibacterial investigation

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ABSTRACT

Numerous novel 4-Amino-pyrimidine-5-carbonitrile, 1,3-thiazine, and 1,3,4-thiadiazole derivatives were produced using 2-pyridin-4-ylmethylene) hydrazine-1-carboximidamide as the main starting material. In vitro tests were used to evaluate antiviral and antibacterial qualities. The synthesised compounds were characterised by elemental analysis, FT-IR, 1H NMR, and LC-MS spectral investigations.

Keywords: *pyrimidine; P2X3 receptor; antiviral; antimicrobial.*

1. INTRODUCTION

Finding and creating new medications is greatly aided by the chemistry of heterocyclic molecules. Numerous pyrimidine scaffolds have been created and produced as potential medications with a range of pharmacological applications. (1) .

Heterocyclic compounds aid in the creation of cutting-edge materials in the field of materials science, including polymers, dyes, and organic semiconductors. Electronics, photonics, and nanotechnology can all advance as a result of their distinct electrical qualities, which can be customized for particular uses.

Agrochemistry, organic, bioorganic, and medicinal chemistry are just a few of the fields of chemistry that use pyrimidine derivatives. Furthermore, living plants and animals, including humans, contain these substances.(2).

Necessity of discovering and developing an entirely new category of antimicrobial substances that work against harmful bacteria that are growing tolerant to the antibiotics used in the current regime stems from the growing health issues. (3). One major medical issue is the growing resistance of human infections to modern antibiotic treatments. (4). The 20th century saw the development and widespread distribution of vaccinations against bacterial toxins and numerous other acute viral diseases. There has been significant advancement in this sector since the discovery of multiple synthetic antiviral, antibacterial, and antifungal drugs. (5)

Heterocyclic drugs, pyrimidines gives or accepts hydrogen. (6) . Drugs comprising pyrimidines include pharmacological effects such as eradicating bacteria, strengthening the immune system, combating thyroid issues and tuberculosis, lowering inflammation, and thwarting cancer, viruses, and malaria parasites. (7). Given that potent antibiotics can reduce the risk of fatal illnesses and increase mortality rates worldwide (8) . Bioactive compounds Many natural products contain pyrimidines, and their derivatives have been synthesised for use in medicine. (9) . Angina pectoris and hypertension can be treated with biologically active 1,4-dihydropyrimidines. (10). One intriguing direction for additional medical study and development is thioxopyrimidines. (11) .

All things considered, pyrimidine is an essential topic of research in organic chemistry and molecular biology due to its structural simplicity and the biological significance of its derivatives.

2. EXPERIMENTAL

Uncorrected melting points were measured using a digital Electrothermal 9100 device (Kleinfeld, Gehrden, Germany). The Varian PerkinElmer 1430 ratio-recording infrared spectrophotometer (Nicolet, Madison, WI, USA) was used to record the Fourier transform infrared spectra (KBr). Using tetramethylsilane as an internal standard, the Varian Mercury VX-300 NMR

spectrometer (Varian Inc., Palo Alto, CA, USA) recorded the 1H and 13C NMR spectra (400 MHz for 1H NMR, 100 MHz for 13C NMR). Chemical shifts are reported as δ values. TLC (Merck, Darmstadt, Germany) was used to track the reaction's progress using UV-precoated sheets, fluorescent silica gel, 60 F254 (Merck), and spots were visible when exposed to iodine vapour or radiation from the UV.

2-(Pyridin-4-ylmethylene)hydrazine-1-carboximidamide (3)

The reaction mixture was maintained in an ice bath whilst aminoquanidine hydrochloride (0.01 mol) was agitated into a solution of pyridine-4-carboxaldehyde (0.01 mol) in ethanol (20 mL). Nitric acid was added gently to the previous mixture five drops at a time. For two more hours, there was continuous stirring. Ethanol was filtered, dried, and crystallised to remove the precipitate after diluting the reaction mixture with water.

Mp = 243 oC. IR (KBr): 3224 cm-1 (NH2), 1634 cm-1 (C=N), 1H NMR (DMSO-D6): δ = 6.50 (broad singlet, 2H, NH2), 7.93 (s, 1H, CH=N), 8.33 (d, 2H, J = 6.40 Hz, Hpyridine), 8.84 (d, 2H, J = 7.20 Hz, Hpyridine), 12.97 (s, 1H, NH), 13C NMR (DMSO-d6): δ = 87.82, 122.8, 133.8, 160.6, 165.6 (sp2-carbons).

4-Amino-6-(4-chlorophenyl)-2-(2-(pyridin-4 ylmethylene) hydrazinyl) pyrimidine-5-carbonitrile (9)

For six hours, a combination of piperidine (5 drops), ethanol (25 mL), benzylidine (6 mol), and carboximidamide 3 (0.01 mol) was cooked under reflux. The precipitate that was produced was gathered using filtering, dried, and crystallised using ethanol.

IR (KBr): 3334 cm-1 (NH2), 2224 cm-1 (C≡N), 1631 cm-1 (C=N). 1H NMR (DMSO-D6): δ = 2.99 (broad singlet, 2H, NH2), 4.64 (s, 1H, CH=N), 7.69 (d, 2H, Haryl), 7.91(d, 2H, Haryl), 8.91 (d, 2H, Hpyridine), 8.69 (d, 2H, Hpyridine), 12.15 (s, 1H, NH), 13C NMR (DMSO-d6): δ = 82.25, 112.0, 123.0, 128.5, 130.0, 132.1, 134.3, 138.2, 144.1, 150.5, 160.1, 166.4 (sp2-carbons).

4-Amino-6-(4-fluorophenyl)-2-(2-(pyridin-4-ylmethylene) hydrazinyl) pyrimidine-5-carbonitrile (10)

For six hours, a combination of piperidine (5 drops), ethanol (25 mL), 4-fluorobenzylidine 6 (0.01 mol), and carboximidamide 3 (0.01 mol) was heated under reflux. The precipitate that was produced was gathered using filtering, dried, and crystallised using ethanol.IR (KBr): 3335, 3102 cm-1 (NH2), 2230 cm-1 (C≡N), 1629 cm-1 (C=N). 1H NMR (DMSO-D6): δ = 3.60 (broad singlet, 2H, NH2), 4.45 (s, 1H, CH=N), 7.44 (d, 2H, Haryl), 7.49 (d, 2H, Haryl), 8.01 (d, 2H, Hpyridine), 8.70 (d, 2H, Hpyridine), 12.01 (s, 1H, NH), 13C NMR (DMSO-d6): δ = 81.17, 112.0, 123.0, 128.0, 130.7, 133.2, 141.9, 144.2, 148.9, 150.4, 160.1, 166.1 (sp2-carbons).

3. MICROBIOLOGICAL ANALYSIS

Agar plates were coated with 100 µl of the microbial suspension, which matched the broth in which they were kept. Each organism that may be pathogenic should have isolated colonies chosen from primary agar plates and subjected to the disc diffusion procedure to determine its susceptibility. For 48 hours, plates containing filamentous fungi (like Aspergillus flavus), Gramme (+) bacteria (like Staphylococcus aureus and Bacillus cereus), Gramme (-) bacteria (like Escherichia coli and Pseudomonas aeruginosa), and yeast (like Candida albicans) were incubated at 25°C. Next, millimetres were used to measure the inhibition zones' sizes. (12).

Filter discs impregnated with 10 µl of solvent (distilled water, chloroform, or DMSO) were employed as a negative control, whereas standard discs containing the antibacterial and antifungal agents ampicillin and amphotericin B were tilized as positive controls for antimicrobial activity. For 48 hours, plates containing filamentous fungi (like Aspergillus flavus), Gramme (+) bacteria (like Staphylococcus aureus and Bacillus cereus), Gramme (-) bacteria (like Escherichia coli and Pseudomonas aeruginosa), and yeast (like Candida albicans) were incubated at 25°C. Next, millimetres were used to measure the inhibition zones' sizes.

4. ANTIVIRAL ASSAYS

The antiviral activity of the new compounds 5–24 was evaluated against the vaccinia virus, herpes simplex virus type 2 (G), and herpes simplex virus type 1 (KOS) using the cytopathicity (CPE) assay.Stock solutions of the test compounds were prepared in DMSO at a concentration of 10 mg/ml. One CCID50 is the 50% infective dose for cell culture, and 100 CCID50 of the virus were applied to confluent cells in 96-well plates. (13). The virus was eliminated and repeated dilutions of the compounds were added following a 2-hour adsorption period at 37 °C. After that, the cultures were maintained at 37°C for three days until the infected and untreated viral control cultures had complete CPE. Table 2 displays the findings as the 50% concentration that is effective (EC50). The compound's 50% effective antiviral concentration, or EC50, is the quantity needed to prevent 50% of virus-infected cells from becoming cytopathogenic. The symbol ">" is used to indicate the highest concentration at which the compounds were tested and found to be inactive.

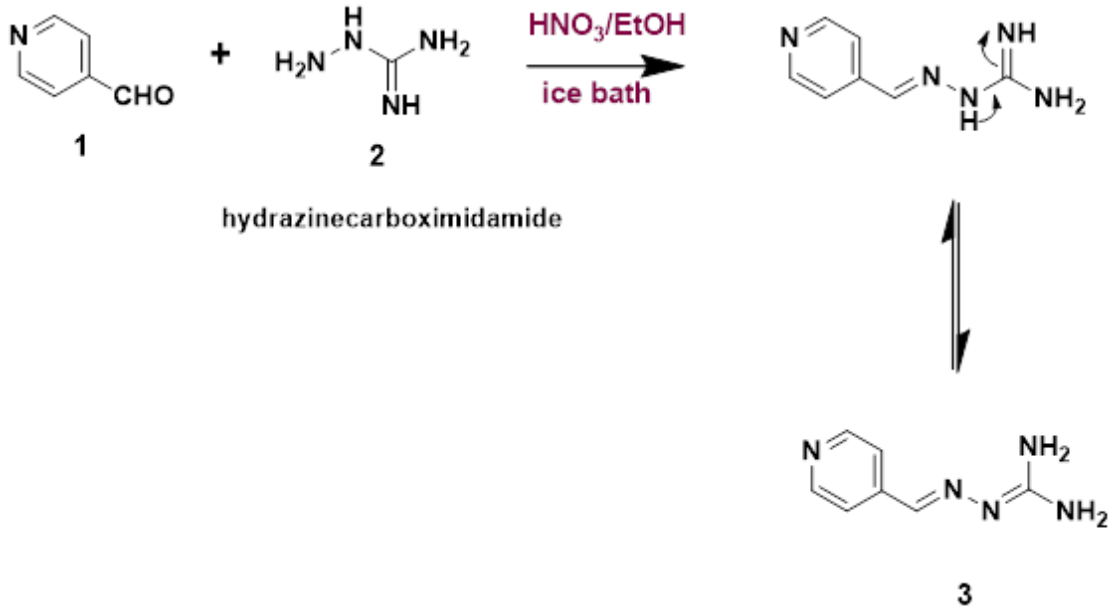
5. RESULTS AND DISCUSSION

A large and varied family of organic molecules known as heterocyclic compounds is made up of atoms from at least two different elements arranged in a ring configuration. These compounds usually contain carbon atoms in the ring along with one or more heteroatoms, such as nitrogen, oxygen, or sulphur. Because of the distinct structural and electrical characteristics that these heteroatoms bestow, heterocyclic compounds are highly significant in a variety of scientific and industrial domains (14).

Heterocyclic compounds are significant because of their adaptability and variety of uses in many different fields. Their structural variety and functional adaptability are what keep research, medicine, and technology moving forward. Pyrimidine is an aromatic heterocyclic organic compound characterized by a six-membered ring structure composed of four carbon atoms and two nitrogen atoms at positions 1 and 3. It belongs to a class of compounds known as diazines, which include other heterocyclic compounds like pyrazine and pyridazine. Pyrimidine itself is a colorless solid with a distinct odor and serves as a fundamental structure in various biological molecules (15).

Pyrimidine derivatives are essential parts of nucleic acids in the field of biochemistry. Pyrimidine derivative bases, cytosine, thymine, and uracil, are all necessary for the proper construction and operation of DNA and RNA. In DNA and RNA, cytosine pairs with guanine, whereas thymine pairs with adenine, and uracil pairs with adenine in RNA. Genetic information must be stored and transferred via these pairings [González & Guzmán, 2008], [Singh, et al, 2014].

A number of compounds with biological activity can be synthesised using pyridine-4-carbaldehyde as a heterocyclic key. Therefore, in the presence of a few drops of nitric acid and at a low temperature, it can react with a source of nitrogen nucleophile, such as hydrazinecarboximidamide, to produce the equivalent 2-(Pyridin-4-ylmethylene)hydrazine-1-carboximidamide 3 in good yield (Scheme 1). Condensation reaction facilitates a smooth reaction progression as showen in the scheme 1. The structure of compound 3 was supported from spectral analysis. For example, its IR show 3224 cm-1 (NH2), 1634 cm-1 (C=N) and 1H NMR have signals at 6.50 (broad singlet, 2H, NH2), 7.93 (s, 1H, CH=N), 8.33 (d, 2H, J = 6.40 Hz, Hpyridine), 8.84 (d, 2H, J = 7.20 Hz, Hpyridine), 12.97 (s, 1H, NH). On the other hands the 13C NMR (DMSO-d6): δ = 87.82, 122.8, 133.8, 160.6, and 165.6 for sp2-carbons. Hydrazone 3 contains two primary nitrogen centers that play an importance key intermediate in the synthesis of several biologically active heterocyclic compounds. The two nitrogen represent as two nucleophile centers to form the desired heterocyclic scaffolds.



Scheme 1: Synthesis of 2-(pyridin-4-ylmethylene) hydrazine-1-carboximidamide 3.

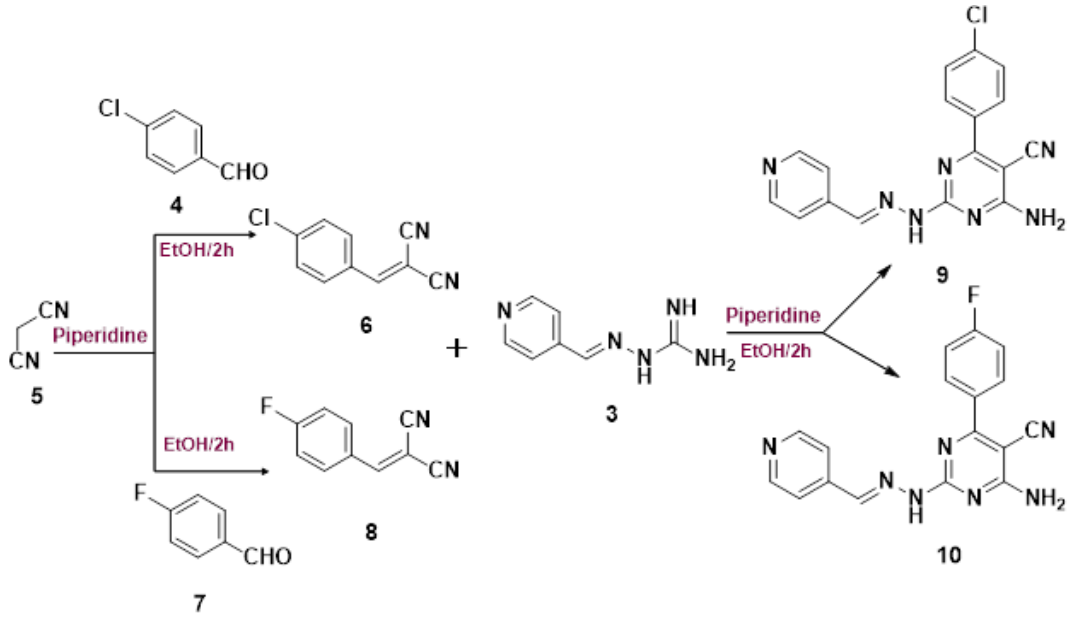
Condensation of 4-chlorobenzaldehyde with malononitrile in the presence of mild base such as piperidine in ethanol at room temperature for two hours afforded the corresponding 2-(4-Chlorobenzylidene) malononitrile **6** in good yield (Scheme 3).

In the same manner, 2-(4-fluorobenzylidene) malononitrile **8** was obtained via reaction of 4-fluorobenzaldehyde with malononitrile (Scheme 2).

[3+3]-Cycloaddition of 2-(pyridin-4-ylmethylene) hydrazine-1-carboximidamide 3 with 2-(4-chlorobenzylidene) malononitrile in the presence of piperidine as a basic catalyst in ethanol afforded the corresponding pyrimidine derivative **9**,

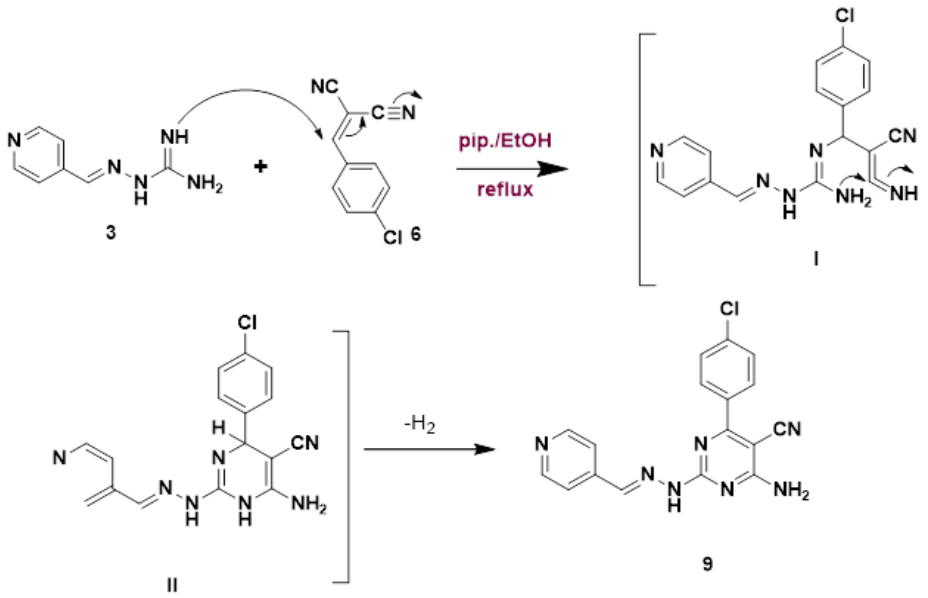
namely 4-amino-6-(4-chlorophenyl)-2-(2-(pyridin-4-ylmethylene) hydrazinyl) pyrimidine-5-carbonitrile **(9)** (Scheme 2).

The IR of pyrimidine **9** assigned bands at 3334 cm^{-1} (NH_2), 2224 cm^{-1} (C≡N), 1631 cm^{-1} (C=N). ^{1}H NMR (DMSO-D6): δ = 2.99 (broad singlet, 2H, NH_2), 4.64 (s, 1H, CH=N), 7.69 (d, 2H, H_{aryl}), 7.91(d, 2H, H_{aryl}), 8.91 (d, 2H, $H_{pyridine}$), 8.69 (d, 2H, $H_{pyridine}$), 12.15 (s, 1H, NH), ^{13}C NMR (DMSO-d_6): δ = 82.25, 112.0, 123.0, 128.5, 130.0, 132.1, 134.3, 138.2, 144.1, 150.5, 160.1, 166.4 (sp^2-carbons).



Scheme 2: Synthesis of 4-amino-6-pyrimidine-5-carbonitrile derivatives.

Mechanistically, the formation of pyrimidine **9** proceed via aza-Michael addition of amidine derivative **3** to benzylidine **6** affording intermediate **I**, the latter adduct is subjected to intramolecular cyclization and dehydrogenation process affording the final product **9** (Scheme 3).

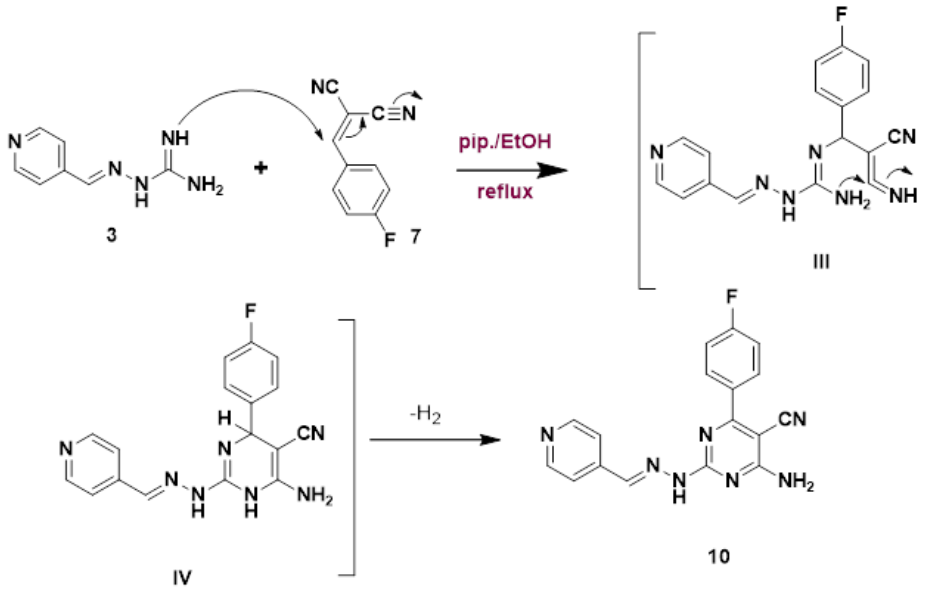


Scheme 3: Mechanistic route for formation of compound 9.

In the same way, 4-amino-6-(4-chlorophenyl)-2-(2-(pyridin-4-ylmethylene) hydrazinyl) pyrimidine-5-carbonitrile **(10)** was obtained *via* [3+3]-Cycloaddition of 2-(pyridin-4-ylmethylene) hydrazine-1-carboximidamide **3** with 2-(4-fluorobenzylidene) malononitrile in the presence of piperidine as a basic catalyst in ethanol (Scheme 2).

The IR of pyrimidine **10** confirm its structure through the appearance of bands at 3335, 3102 cm^{-1} (NH_2), 2230 cm^{-1} (C≡N), 1629 cm^{-1} (C=N). ^{1}H NMR (DMSO-D6): δ = 3.60 (broad singlet, 2H, NH_2), 4.45 (s, 1H, CH=N), 7.44 (d, 2H, H_{aryl}), 7.49 (d, 2H, H_{aryl}), 8.01 (d, 2H, $H_{pyridine}$), 8.70 (d, 2H, $H_{pyridine}$), 12.01 (s, 1H, NH), ^{13}C NMR (DMSO-d_6): δ = 81.17, 112.0, 123.0, 128.0, 130.7, 133.2, 141.9, 144.2, 148.9, 150.4, 160.1, 166.1 (sp^2-carbons).

Mechanistically, the formation of pyrimidine **10** proceed *via* aza-Michael addition of amidine derivative **3** to benzylidine **7** affording intermediate **III**, the latter adduct is subjected to intramolecular cyclization and dehydrogenation process affording the final product **10** (Scheme 4).



Scheme 4: Mechanistic route for formation of compound 10.

Anti-microbial Activity

Against ampicillin (β- lactamase sensitive antibiotic) new synthesised compounds were comparable; however moieties of hetero cyclic ring increased the broad-spectrum anti-microbial activities. Compound **10**showed most potent antimicrobial activity towards all stains.

On other hand, compound **9** showed moderate potent antimicrobial activity towards all stains. 4-Amino-6-(4-chlorophenyl)-2-(2-(pyridin-4-ylmethylene)hydrazinyl)pyrimidine-5-carbonitrile **(9)** , although **t**he structure is similar to compound **10**, but with a chlorophenyl group which might slightly lower its activity compared to the fluorophenyl group **(*16*)**. Also, Finally, 2-(Pyridin-4-ylmethylene)hydrazine-1-carboximidamide **(3)** showed less activity while hydrazine derivatives can have antimicrobial properties, this compound has the simplest structure among the listed ones, potentially limiting its overall activity compared to the more complex and functionally diverse compounds above. **(Table 1)**

Table (1): Microbiological analysis results.

Sample	**Inhibition zone diameter (mm/mg sample) ± SD**					
	Bacterial species				**Fungal species**	
	***B. cereus* (G^+)**	***S. aureus* (G^+)**	***E. coli* (G^-)**	***P. aeruginosa* (G^-)**	***A. flavus***	***C. albicans***
ATCC	14578	6528	8738	9026	9642	10230
Standard	--	--	25 ± 0.0	27 ± 0.0	--	--
	28 ± 0.0	27 ± 0.0	--	--	--	--
	--	--	--	--	17	21 ± 0.0

Control: DMSO	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
3	7 ± 0.10	5.67 ± 1.15	7.67 ± 1.11	6.7 ± 0.10	6 ± 0.21	5.2 ± 0.12
9	21.14 ± 1.18	19.17 ± 1.11	18.11 ± 1.15	15 ± 0.01	15 ± 0.10	15 ± 0.31
10	22.13 ± 0.17	22.11 ± 1.15	21.11 ±1.15	21.17 ± 0.19	20.11 ±1.11	20 ± 1.01

Antiviral activity

Using the cytopathicity (CPE) assay, novel drugs were evaluated against a wide panel of viruses in various cell cultures.and the reference antiviral drug (brivudin) was used to compare their activities. HEL cell culture was used to evaluate compounds against vaccinia virus [VV], herpes simplex virus type 2 (G) [HSV-2G], and herpes simplex virus type 1 (KOS) [HSV-1 KOS] (Table 2). **(*17*)**

Having this group there could increase its activities. Since its EC_{50} values fell between 2 and 4 mg/ml, the substituent demonstrated moderate activity. The remaining drugs showed the same level of action against all viruses in HEL cell culture, with an EC_{50} of ¼ 20 mg/ml. (Table 2).

Table (2): Antiviral analysis results.

Compounds	**EC50[b] (mg/ml)**		
	Herpes simplex virus-1(KOS)	**Herpes simplex virus-2 (G)**	**Vaccinia virus**
3	>20	>20	>20
9	>20	>20	>20
10	>20	>20	>20
Brivudin	**>250**	**> 0.08**	**>126**

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