Screening of some synthetic fused heterocyclic pyrimidines for anti-avian influenza virus (H5N1) activity

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ABSTRACT

Thirteen chemical compounds belonging to the fused heterocyclic pyrimidines were synthesized and screened in order to assay their antiviral activity against the avian influenza virus (H5N1) A/Chicken/Qalubiya/1/2006(H5N1) by plaque reduction assay on MDCK cells. All tested compounds were non-toxic on the cells. Ten out of thirteen compounds (10, 13, 9, 12, 1, 11, 7, 8, 3 and 4) showed moderate percentage of inhibition respectively at concentration 20 μg/ml while the remaining three compounds (5, 2 and 6) showed weak inhibitory activity respectively at same concentration.

Key words: Anti, avian influenza virus, H5N1 – pyrimidine derivatives.

INTRODUCTION

Avian influenza A/H5N1 presents one of the major hazards to mankind in the 21st century. Influenza virus A/H5N1 possesses a number of characteristics of the ideal future pandemic pathogen such as the following: the potential for the infection of the greatest number of animal species (compared to other known influenza strains), high mortality rate (30-70%) as well as the continuous evolution towards the increase of virulence, the ability for human transmission and the resistance to the existing therapeutic agents. World Health Organization (WHO) has identified avian influenza A/H5N1 as a disease with pandemic potential, which represents a health risk to the world population as a whole (Tapper, 2006). Periodically, completely novel antigenic subtypes of influenza viruses have been introduced in the human population, causing large-scale global outbreaks with high death tolls (Basler and Aguilar, 2008). Although vaccination provides the primary protection against influenza virus infections but the continuous and unpredictable antigenic variation in the influenza viruses has made vaccine strains relatively or totally ineffective beside they must be selected annually. Therefore, vaccine production may not satisfy the need during an influenza pandemic (Gong et al., 2007). The use of antiviral drugs against influenza virus could therefore represent a first line of defense against a new pandemic, allowing the control of the infection until sufficient quantities of a suitable vaccine can be produced (Sugrue et al., 2008). Two classes of these antiviral drugs, adamantanes and neuraminidase (NA) inhibitors, are currently prescribed for the prophylaxis and treatment of influenza infections (Hsieh and Hsu, 2007). Adamantanes (amantadine and rimantadine) target the proton channel formed by the viral M2 protein whereas two NA inhibitors, orally bioavailable oseltamivir and inhaled zanamivir, are the only drugs currently recommended for the
treatment of influenza virus infection. These drugs have been proven to be useful for reducing clinical symptoms, but their utility has been limited by side effects and high rate of emergence of resistant viral strains (Gubareva et al., 1998; Kiso et al., 2004 and Le et al., 2005). Therefore the need for identifying new alternative anti-influenza drugs becomes a matter of certainty and an urgent global need in the face of new pandemic strains. The objective of this study was to identify novel anti-influenza virus using heterocyclic pyrimidine compounds because an interest is aroused to the biologically active pyrimidine and fused heterocyclic pyrimidine nucleosides as potent antiviral agents (Cristescu and Czobor, 1998; Rashad and Ali, 2006).

MATERIALS AND METHODS

I) Chemistry:

A) Tested compounds: All chemical compounds under this investigation were kindly synthesized by Prof. Dr. Aymn Elsayed Rashad, Professor of Photochemistry, Photochemistry Department, National Research Centre and Prof. Dr. Klaus Banert Professor of Organic chemistry, Chemnitz University of Technology, Germany.

Compound number one: 1-(6-p-Tolyl-pyridazin-3-yl)-1, 5-dihydro-pyrazolo[3,4-d]-pyrimidin-4-one was prepared by Shamroukh et al., (2005). M.p. 383–4°C. IR spectrum (KBr, ν cm⁻¹): 3102 (NH), and 1700 (CO); ¹H NMR spectrum (DMSO-d₆, δ ppm): 2.41 (s, 3H, p-CH₃-tolyl), 7.40 (d, 2H, J = 7.8 Hz, H₃, H₅-tolyl), 8.11 (d, 2H, J = 7.5 Hz, H₂, H₆-tolyl), 8.23 (s, 1H, H₃-pyrazole), 8.30 (d, 1H, J = 9.3 Hz, H₅-pyridazine), 8.45–8.48 (m, 2H, H₄-pyridazine and H₆-pyrimidine) and 12.51 (brs, 1H, NH, exchangeable with D₂O). MS, m/z (%): 304 (M⁺, 100). Analysis for C₁₆H₁₂N₆O (304.31): required C, 63.15; H, 3.97; N, 27.62; found C, 63.20; H, 4.05; N, 27.48.

Compound number two: 5-Amino-1-(6-p-tolyl-pyridazin-3-yl)-1H-pyrazole-4-carboxylic acid amide was prepared by Shamroukh et al., (2005). M.p. 215–217°C. IR (KBr, ν cm⁻¹): 3390-3250 (NH₂), 2218 (C≡N). ¹H NMR (DMSO-d₆, δ ppm): 2.40 (s, 3H, p-CH₃-tolyl), 7.10 (brs, 2H, NH₂, exchangeable with D₂O), 7.40 (d, 2H, J = 7.8 Hz, H₃, H₅-tolyl), 7.63 (s, 2H, NH₂, exchangeable with D₂O), 8.05–8.07 (m, 3H, H₂, H₆-tolyl and H₃-pyrazole), 8.17 (d, 1H, J = 9.3 Hz, H₅-pyridazine), 8.40 (d, 1H, J = 9.3 Hz, H₄-pyridazine); MS, m/z (%): 294 (M⁺, 100). Analysis for C₁₅H₁₄N₆O (294.32): required C, 61.22; H, 4.79; N, 28.55; found C, 61.12; H, 4.58; N, 28.75.


Compound number four: 2-Aminoindeno[2,1-b]thiophene-3-carbonitrile was prepared by Hegab et al., (2007). IR; ν₂₂₈₀, 2200 (NH₂), 2210 (CN). ¹H NMR (DMSO-d₆, δ ppm): 3.75 (s, 2H, CH₂), 4.80 (s, 2H, NH₂, D₂O exchangeable), 7.20–7.60 (m, 3H, Ar-H), 7.75 (d, J = 6.00 Hz, 1H, Ar-H). MS: m/z 212 (M⁺, 100), 196 (1.71), 184 (4.20).

Compound number five: 5,6,10-Trihydronaphtho[1′,2′:4,5]thieno[2,3-d]pyrimidin-11-one was prepared by Rashad et al., (2005). M.p. 268-270°C. Calcd. for C₁₄H₁₀N₂OS (254.31): C, 66.12; H, 3.96; N, 11.02; S, 12.61. Found: C, 66.20; H, 3.80; N, 10.83; S, 12.40. IR (KBr): ν NH cm⁻¹ 3100, ν CO 1625 cm⁻¹. ¹H NMR (DMSO-d₆, δ ppm): 2.80–3.10 (m, 4H, 2CH₂), 7.20-7.50 (m, 3H, Ar-H), 10.83 (d, 1H, C=O). 5.60 (d, J = 10.45 Hz, 1H, Ar-H), 8.10 (s, 1H, NH, exchangeable with D₂O). MS, m/z (%): 254 (M⁺, 100), 226 (5.67).

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1H-NMR (CDCl3, δ ppm): 2.86-2.97 (m, 4H, 2CH2), 3.37 (s, 3H, OCH3), 3.54 (t, J=7Hz, 2H, CH2O), 3.69 (t, J=7.5 Hz, 2H, CH2N), 7.20-7.40 (m, 3H, Ar-H), 7.80 (d, J=8 Hz, 1H, Ar-H), 8.25 (s, 1H, C3-H); 13C-NMR (CDCl3, δ ppm): 25.56 (C-5), 29.78 (C-6), 29.78 (OCH3), 58.89 (CH2O), 71.21 (CH2N), 125.94-128.02 (Ar-C), 130.97 (C-11a), 135.94 (C-11b), 141.24 (C-6a),151.34 (C-7a), 162.98 (C-9), 166.22 (C-11); calculated for C17H16N2OS2 (328.46): C, 62.17; H, 4.91; N, 8.53; S, 19.52. Found: C, 62.10; H, 4.85; N, 8.63; S, 19.60.

Compound number seven: 1,10-Dihydronapheno[1',2':4,5]thieno[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5-one was prepared by Rashad et al. (2010). M.p. 250-252 °C. IR spectrum (KBr, ν, cm−1): 3343 (NH), 1643 (CO); 1H NMR spectrum (CDCl3, δ ppm): 3.36 (s, 2H, CH2), 6.90 (s, 1H, C3-H), 7.24-7.54 (m, 4H, Ar-H), 9.85 (s, 1H, NH, D2O exchangeable); 13C NMR (DMSO-d6, δ ppm): 36.58 (C-10), 119.45-164.32 (Ar-C), 170.50 (C-O). MS, m/z (%): 280 (M+, 100). Anal. calcd. for C14H12N2O5: C, 59.99; H, 2.88; N, 19.99; S, 11.44. Found: C, 60.03; H, 2.95; N, 19.89; S, 11.38.

Compound number eight: 11-Amino-5,6-dihydronaphtho[1',2':4,5]thieno[2,3-d]pyrimidine-9(8H)-thione was prepared by Abdel-Megeid et al. (1998). M.p. 150-2°C; Calcd. for C13H12N2S2 (286.78): C, 63.04; H, 3.85; N, 9.80; S, 11.20. Found: C, 63.80; H, 3.83; N, 9.40; S, 11.20. IR (KBr, ν, cm−1): 1055 (C-Cl); 1H NMR (DMSO-d6, δ ppm): 2.7 (s, 3H, CH3), 2.83 (m, 4H, CH2CH2), 7.2-7.4 (m, 3H, Ar-H); MS, m/z (%): 288 (M+, 33Cl, 38.4), 286 (M+, 35Cl, 100), 271 (1.47), 251 (7.25), 249 (9.28), 209 (9.51), 164 (7.65), 139 (4.86).

Compound number nine: 11-Hydrazino-9-methyl-5,6-dihydronaphtho[1',2':4,5]thieno[2,3-d]pyrimidine was prepared by Abdel-Megeid et al. (1998). M.p. 182-4°C; IR (KBr, ν, cm−1): 3400-3440 (NH2), 3200 (NH); 1H NMR (CDCl3, δ ppm): 2.6 (s, 3H, CH3), 2.8-3.0 (m, 4H, CH2CH2), 4.2 (s, 2H, NH2, D2O exchangeable), 6.45 (s, H, NH, D2O exchangeable), 7.2-7.5 (m, 4H, Ar-H); MS, m/z (%): 282 (M+, 100), 264 (38.24), 224 (58.99), 209 (6.62), 164 (8.22), 139 (8.9).

Compound number ten: 11-Chloro-9-methyl-5,6-dihydronaphtho[1',2':4,5]thieno[2,3-d]pyrimidine was prepared by Abdel-Megeid et al., (1998). M.p. 150-2°C; Calcd. for C13H12ClN2S2 (286.78): C, 63.04; H, 3.85; N, 9.80; S, 11.20. Found: C, 63.80; H, 3.83; N, 9.40; S, 11.20. IR (KBr, ν, cm−1): 1055 (C-Cl); 1H NMR (DMSO-d6, δ ppm): 2.7 (s, 3H, CH3), 2.8-3.0 (m, 4H, CH2CH2), 7.2-7.4 (m, 3H, Ar-H); MS, m/z (%): 288 (M+, 37Cl, 38.4), 286 (M+, 35Cl, 100), 271 (1.47), 251 (7.25), 249 (9.28), 209 (9.51), 164 (7.65), 139 (4.86).

Compound number eleven: 5-Amino-1-(9-methyl-5,6-dihydronaphtho[1',2':4,5]thieno[2,3-d]pyrimidin-11-yl)-1H-pyrazole-4-carboxylic acid methyl ester was prepared by Rashad et al., (2009). M.p. 139-141 °C. IR ν 3464, 3354 (NH2), 1685 (CO) cm−1. 1H NMR (DMSO-d6, δ ppm): 33 (t, J = 6.9 Hz, 3H, CH3), 2.81 (s, 3H, CH3), 2.95-3.07 (m, 4H, CH2CH2), 4.28 (q, J = 7.5 Hz, 2H, CH2), 6.43 (d, J = 8 Hz, 1H, Ar-H), 6.81 (s, 2H, NH2, D2O exchangeable), 6.90-7.35 (m, 4H, 3Ar-H and C3-H). 13C NMR (DMSO-d6, δ ppm): 14.4 (CH3), 25.1 (CH3), 25.2 (C-5'), 29.3 (C-6'), 59.8 (OCH2), 96.4 (C-4), 125.6-164.3 (Ar-C), 172 (CO). MS, m/z (%): 405 (M+, 100). Anal. calcd. for C21H19N2O3S: C, 62.20; H, 4.72; N, 17.27; S, 7.91. Found: C, 62.30; H, 4.65; N, 17.33; S, 7.81.

Compound number twelve: 5-Amino-1-(9-methyl-5,6-dihydronaphtho[1',2':4,5]thieno[2,3-d]pyrimidin-11-yl)-3-methylsulfanyl-1H-pyrazole-4-carbonitrile was prepared by Rashad et al., (2009). M.p. 124-126 °C. IR ν 3460, 3346 (NH2), 2215 (CN) cm−1. 1H NMR (DMSO-d6, δ ppm): 1.58 (s, 3H, SCH3), 2.80 (s, 3H, CH3), 2.90-3.05 (m, 4H, 2CH2), 6.50 (d, J = 8 Hz, 1H, Ar-H), 6.60 (s, 2H, NH2, D2O exchangeable), 7.00-7.25 (m, 3H, Ar-H). 13C NMR (DMSO-d6, δ ppm): 11.6 (CH3), 25.1 (CH3), 25.3 (C-5'), 29.3 (C-6'), 113.0 (C-4), 115.5 (CN), 126.0-172.3 (Ar-C). MS, m/z (%): 404.09 (M+, 100). Anal. calcd. for C20H17N3O2S: C, 59.38; H, 3.99; N, 20.78; S, 15.85. Found: C, 59.29; H, 4.05; N, 20.71; S, 15.92.

Compound number thirteen: 1-(5, 6-Dihydronaphtho[10, 20-4, 5]thieno[2, 3-d]pyrimidin-11-yl)-1H-pyrazolo[3, 4-d]pyrimidin-4(3H)-one was prepared by Rashad...
et al., (2009). M.p. 265–267°C. IR (KBr) nmax: cm⁻¹: 3200 (NH), 1674 (C=O), 1587 (C=N). ¹H NMR (DMSO-d₆, δ ppm): δ: 2.90–3.00 (m, 4H, C₅₀-CH₂ C₆₀-CH₂), 7.20–7.30 (m, 4H, 3Ar-H C₃-H), 7.40–7.50 (m, 2H, Ar-H NH, D₂O exchangeable), 8.40 (s, 1H, C₆-H), 9.20 (s, 1H, C₉-H). ¹³C NMR (DMSO-d₆, δ ppm): δ: 23.50 (C-50), 29.30 (C-60), 119,122,125.20, 126, 126.50, 126.90, 132, 138, 140.30, 158.40, 167.10 (sp² carbon atoms), 165 (C=O). MS m/z (%): 372 (M⁺, 27%), 344 (M++ CO, 10%), 316 (16%), 292 (42%). Anal. calcd. for C₁₉H₁₂N₆OS (372.41): C 61.28, H 3.25, N 22.57, S 8.61. Found: C 61.05, H 3.37, N 22.41, S 8.57.

2- Antiviral Bioassays

Cells: Madin-Darby Canine Kidney (MDCK) cells were maintained at the Environmental Virology Laboratory at the National Research Center. Madin-Darby canine kidney (MDCK) cells were kindly provided by Dr. Richard Webby, St. Jude Children’s Research Hospital, Department of Virology and Molecular Biology, USA, as a confluent sheet in 75 cm² tissue culture flask. The cell lines was propagated till confluence for several passages, harvested in aliquots and stored in liquid nitrogen till being used. MDCK cells are recommended by WHO as the preferred cell line for culturing influenza viruses.

Virus: The highly pathogenic avian influenza virus (HPAI) H5N1 used in this study was isolated from the infected chickens in Egypt in 2006 (characterized at immunologic and molecular levels) A/Chicken/Qalubiya/1/2006(H5N1) and was kindly provided by Prof. Dr. M. A. Ali professor of virology, National Research Center. All viral manipulations were performed under the appropriate biosafety level 3 laminar air flow isolator at Virology Lab., Department of water pollution, NRC.

Cell culture propagation: The MDCK cells were grown to confluence in complete growth medium in 75 cm² culture flasks (Greiner bio-one GmbH, Germany). The medium was discarded and then cells were treated with trypsin-verse for dissociation of the cell monolayer. Cells were incubated for 0.5–5 min at 37°C and detachment of the cells was carefully observed. Cells were spun down at 2500 g for 2 min, resuspended in 1 ml complete medium. The exact cell count and viability was recorded by mixing a 50 µl aliquot of the cells with equal volume of trypan blue (5 gm/L; BiochromKG, Berlin, Germany) using a hemocytometer (Right Line; Sigma, Deisenhofen, Germany). According to the recorded count certain volume of the resuspended cells was used to prepare fresh cultures in plates or flasks.

Viral titer determination by plaque assay: Monolayer MDCK cells (6 × 10⁵ cells/well) were washed once with Dulbecco’s phosphate-buffered saline (DPBS), and infected with a serially diluted viral suspension. After adsorption for 1 h at 37°C, the viral suspension was replaced with over layer medium, (DMEM with penicillin [100 U/mL], streptomycin [100 µg/mL], L-glutamine [2 mM], and nonessential amino acid mixture [0.1 mM]) containing 2.5 µg/mL trypsin and 0.3 % agarose). After incubation for 2-3 days at 37°C under 5 % CO₂, the cells were fixed with 10 % formaldehyde and then stained with 1 % crystal violet. The titer of the virus was expressed in plaque-forming units (PFU) per milliliter.

Preparation of synthetic compounds for bioassay: Tested compounds were dissolved as 10 mg each in 1 ml of 10 % Dimethyl Sulfoxide (DMSO) in deionized water (900 µL de-ionized water and 100 µL DMSO). Decontamination was carried out by adding 1 % antibiotic-antimycotic mixture (10,000 U penicillin G sodium, 10,000 µg streptomycin sulfate, and 250 µg amphotericin B, PAA Laboratories GmbH, Austria) and the compounds were incubated at 37°C for 30 min then stored at -20°C. Sterility test was performed out in nutrient agar to ensure the sterility of the prepared compounds.

Cytotoxicity Assay: Cell toxicity was carried out to determine the cell culture safe doses of the dissolved compounds and performed by Cell morphology technique (Aquino et al., 1989) which was made in 96 well plates: In order to determine the safe dose of the tested compounds that doesn’t harm the (MDCK) cells, different concentrations from each compound ranging from 10 to 100 µg / ml.
were applied to cells. Each concentration was tested for cytotoxicity by observing any morphologically changes in the cultured cells microscopically after 24 hours incubation. **Antiviral Screening:** Plaque reduction assay is the most widely accepted method for determining the % inhibition of virus as a result of being subjected to a given material (Tebas et al., 1995). A 12 well plate was cultivated with the MDCK cells (10^5 cell/ml) and incubated for 24 hours at 37°C. Virus was diluted to give 10^3 PFU/ml final concentration and mixed with the safe concentrations of each compound as mentioned previously and incubated for 1 hour at 37°C. Growth medium was removed from the multi-well plate and virus-compound mixture was inoculated in each well. After 1 hour contact time at 37°C for virus adsorption, the inoculum was aspirated and 1 ml of cell-specific 2x medium 2 % agarose was overlayed the cell sheet. The plates were left to solidify at room temperature and incubated for 2-3 days at 37°C until the development of the viral plaques. Formalin was added for two hours for fixation then plates were stained with crystal violet staining solution. Control virus and cells were treated identically without addition of compounds. Viral plaques were counted and the percentage of virus reduction was calculated through the following equation: % inhibition = viral count (untreated) – viral count (treated)/ viral count (untreated) x 100.

**RESULTS AND DISCUSSION**

The result in Fig. 1 showed that the dilution 10^{-3} of H5N1 virus was the most suitable dilution which gave 80 – 100 PFU/ml. So, this dilution (10^{-3} of virus) will be applied in subsequent experiments. Also the results of cytotoxicity test indicated that all tested concentrations starting from 10 to 100 µg/ml for each compound were safe on MDCK cells and didn't show any toxicity on MDCK cells.

![Fig. A: Plaque infectivity titration of H5N1 virus on MDCK cells](image)

The lowest concentrations 10 & 20 µg/ml for each compound was preferably chosen to be used in plaque reduction assay since the commercially available anti-H5N1 therapeutic agents are effective at low concentration. This result agreed with several searches in that field for example, toxicity test using neutral red dye uptake method (Smee et. al, 2002), using 0.011 % final concentration of the dye for 2 h for the compound 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene) amino]-N-(4,6-dimethyl-2-pyrimidin-2-yl)benzenesulpho-namide and its derivatives showed that no toxicity was apparent to the uninfected stationary MDCK cell monolayers at the
higher (100 μg/ml) concentration (Selvam et al., 2006). A series of pyrazinecarboxamide derivatives were found to inhibit the influenza viral RNA-dependent RNA polymerase without cytotoxicity to mammalian cells (Furuta et al., 2009). The cytotoxicity test using MTT assay (Mossman, 1983) for some novel fused thiophene and thienopyrimidine derivatives showed that they were safe on MDCK cells (Rashad et al., 2010). However, the acyclic nucleosides in the 5-alkynyl- and 6-alkylfuro[2,3-d]pyrimidine series showed cellular toxicity against PBM, CEM and VERO cells, probably by inhibiting cell DNA synthesis (Amblard et al., 2005).

Plaque reduction assay was applied to get the percentage of virus inhibition as a result of treatment with certain compound. The results in Table 1 showed that 10 out of 13 compounds showed moderate percentage of inhibition at concentration 20 μg/ml. These compounds were number 10, 13, 9, 12, 1, 11, 7, 8, 3 and 4 giving 66.6, 66.6, 60.0, 60.0, 58.3, 55.0, 53.3, 53.3, 50.0 and 50.0 % of inhibition, respectively. The remaining 3 compounds showed weak inhibitory activity at same concentration. These compounds were number 5, 2 and 6 giving 35, 33.3 and 33.3 % of inhibition, respectively. Generally, at conc. 10 μg/ml the percentage inhibition was slightly reduced for most tested compounds comparing with 20 μg/ml but this reduction was non significance.

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Several reports also have been demonstrated the effectiveness of synthetic fused heterocyclic pyrimidine compounds in inhibiting influenza viruses. For example, The 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene) amino]-N-(4,6-dimethyl-2-pyrimidin-2-yl) benzenesulphonamide and its derivatives showed antiviral potencies (90% inhibition) against influenza A (H1N1, H3N2, and H5N1) and B viruses in MDCK cell culture (Selvam et al., 2006). The novel 4-((3-(2-amino-4-hydroxy-6-methyl-5-pyrimidinyl) propyl) amino) phenyl-1-chloro-3-buten-2-one showed the ability to inhibit the avian influenza H5N1 virus (Lee et al., 2009). Some derivatives containing dihydronaphtho, naphtho [2, 1-b] thiophene and thieno [2, 3-d] pyrimidine ring systems showed promising antiviral activity against H5N1 virus (Rashad et al., 2010). Pyrazole compound BPR1P0034 showed potent and selective anti-influenza virus activity (Shih et al., 2010). However, some newly synthesized indenothienopyrimidine derivatives didn’t show any antiviral activity against H5N1 virus (Shamrokh et al., 2010) also the novel 4-Amino-2- (benzylthio) -6-(4-methoxyphenyl) pyrimidine -5-carbonitrile didn’t possess any antiviral activity against H5N1 virus (Mahmoud et al., 2011) while the antiviral activity of the novel acyclic nucleosides in the 5-alkyny1- and 6-alkylfuro [2, 3-d] pyrimidine series. Bioorganic & Medicinal Chemistry 13: 1239–1248.


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Screening of some synthetic fused heterocyclic pyrimidines for anti-avian influenza

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ARABIC SUMMARY

فحص بعض المركبات المختلطة من المشتقات الحلقاتية للبيريميدينات كمضاد لفيروس أنفلونزا الطيور (إتش 5 إن 1)

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ثلاثة عشر مركب كيميائي ينتموا إلى المركبات الحلقاتية للبيريميدينات تم تحضير وتحليلهم باستخدام تقنية الرنين المغناطيسي النووي. وعمل اختبار سمية المركبات الكيميائية المستخدمة في هذه الدراسة لتحديد التركيزات الآمنة على الخلايا ونظامها والتأثير المتناقض للمركبات المختلطة لكل المركبات المختبرية على الخلايا MDCK ولا يمكن لهم أي تأثير سام حتى إذا استخدمت بتكرارها العالية. وقطر انتهاز البصع الفيروسية لمعروفه نسبة تثبيط الفيروس نتيجة تعرضه للمركبات الكيميائية المختلفة أن المركبات (10, 13, 9, 12, 11, 7, 3 و 4) لهم قدرة تثبيطية ملحوظة على الفيروس حيث بلغت من 50 إلى 66.6 % عند التركيز 20 ميكروجرام /مليлитرو بينما الثلاث مركبات المتبقية (2 و 6) كانت لهم قدرة تثبيطية ضعيفة على الفيروس عند نفس التركيز.