Anti-H5N1 Evaluation of Some Newly Synthesized Indenothienopyrimidine Derivatives

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Abstract: Some S-alkylated thieno[2,3-d]pyrimidine derivatives were synthesized via the reaction of 3,9-dihydroiden0 [1',2':4,5] thieno [2,3-d] Pyrimidine-4-thione (1) with different reagents. Moreover, the prepared products were tested for antiviral activity against H5N1 virus [A/chicken/Egypt/1:2006 (H5N1)] by determination of both EC50 and LD50. None of the tested compounds revealed promising activity in comparison to the anti-influenza drug, Zanamivir.

Key words: S- Alkylated · Indeno [1'2':4, 5] thieno [2, 3-d] pyrimidine · Anti-avian influenza virus (H5N1)

INTRODUCTION

During the spring of 2009, a pandemic influenza A (H5N1) virus merged and spread globally which is considered an avian disease. However, transmission between humans is very limited at present, but continued monitoring is required to identify any increase in viral adaptation to human hosts [1]. Therefore, scientists are working to find new ways to defeat virus that is increasingly resistant to drugs. It is well known that pyrimidine and fused thiene [2, 3-d] pyrimidine derivatives are of great biological interest, especially as antiviral [2, 3], antitumor [4, 5], antihypertensive [6], antihistaminic [7], neurotropic [8], analgesic [9-11], anti-inflammatory [12] and antimicrobial agents [13-15]. In connection with our research program for the synthesis of different fused heterocyclic compounds having antiviral activity [16-18], we describe here the synthesis of some new indeno [1'2':4, 5] thieno [2, 3-d] pyrimidine derivatives hoping to show promising antiviral activity.

MATERIALS AND METHODS

All melting points are uncorrected and measured using Electro-thermal IA 9100 apparatus, Shimadzu (Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Shimadzu), National Research Centre, Cairo, Egypt. 1H NMR and 13C NMR spectra were determined on a Jeol-Ex-300 NMR spectrometer and chemical shifts were expressed as part per million, ppm (δ values) against TMS as internal reference (National Research Centre, Cairo, Egypt). Mass spectra were recorded on EI + Q1 MSLMR UPLR, National Research Centre, Cairo, Egypt. Microanalyses were operated using Mario El Mentar apparatus, Organic Microanalysis Unit, National Research Centre, Cairo, Egypt. Column Chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm). Compound 1 was prepared according to a reported method [19].

4-Ethylsulfanyl-9H-indeno [1', 2':4, 5] thieno [2, 3-d] Pyrimidine (2): A solution of compound 1 (0.256g, 1 mmol) and sodium hydroxide (0.04g, 1 mmol) in ethanol (50ml) was treated with ethyl iodide (0.16ml, 1 mmol) and the reaction mixture was warmed on a steam bath at 70°C for 3h. The formed precipitate was filtered off, dried and recrystallized from ethanol to give compound 2. Yield 71%, mp 330-332°C. 1H NMR spectrum (DMSO-d6, δ ppm): 1.41 (t, 3H, J = 2.4 Hz, SCH3CH3), 3.44 (q, 2H, J = 2.4 Hz, SCH3CH3), 4.72 (s, 2H, CH2), 7.34 (m, 3H, Ar-H), 8.06 (d, J=8 Hz, 1H, Ar-H), 8.92 (s, 1H, C2-H). Anal. calcd. for C12H12N2S2 (284): C, 63.35; H, 4.25; N, 9.85; S, 22.55. Found: C, 63.11; H, 4.57; N, 9.44; S, 22.79.

4-Ethanesulfonfyl-9H-indeno [1', 2':4, 5] thieno [2, 3-d] Pyrimidine (3): A solution of compound 2 (0.286g, 1 mmol) in glacial acetic acid (25ml), hydrogen peroxide solution (1ml, 30%) was added dropwise with occasional shaking the reaction mixture was set aside at room

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temperature for 24 h. The formed precipitate was filtered off, dried and recrystallized from dioxane to give compound 3. Yield, 63%, mp 341-343°C. IR spectrum (KBr, ν cm⁻¹): 1338, 1148 (C=S-O); 1H NMR spectrum (DMSO-d₆, δ ppm): 1.54 (t, 3H, J=7 Hz, SCH₂CH₃), 4.13 (s, 2H, CH₂), 4.72 (q, 2H, J=7 Hz, SCH₂CH₃), 7.74-8.2 (m, 4H, Ar-H), 8.76 (s, 1H, C2-H); 13C NMR spectrum (DMSO-d₆, δ ppm): 14.5 (CH₃), 35.3 (C-9), 48 (SCH₂), 122.5-128.7 (Ar-C), 137.8 (C-4a), 140.4 (C-4b), 143.2 (C-9a), 144.8 (C-10a), 145.89 (C-2), 178.8 (C=O). Anal. calcld. for C₁₇H₉O₃N₄S₅ (316): C, 56.9; H, 3.82; N, 8.85; S, 20.27. Found: C, 56.55; H, 3.55; N, 8.93; S, 20.41.

4-(2,2-Dimethoxy-ethylsulfanyl)-9H-indeno [1‘,2‘:4,5] thieno [2,3-d] Pyrimidine (4): A solution of compound 1 (0.256 g, 1 mmol) and sodium hydroxide (0.04 g, 1 mmol) in ethanol (50 ml) was treated with chloroacetaldehyde dimethyl acetal (0.13 g, 1 mmol) and the reaction mixture was refluxed for 3 h. Then poured into water and the organic material was extracted with dimethyl ether, the solvent was removed under reduced pressure and chromatographed on a silica gel column with chloroform/petroleum ether (40-60) (9:1) as an eluent to give compound 4. Yield 62%, mp 339-341°C. 1H NMR spectrum (DMSO-d₆, δ ppm): 3.34-3.58 (m, 8H, 20CH₂, +SCH₂), 3.69 (s, 2H, CH₂), 4.71 (t, 1H, J=2.4 Hz, CH), 7.39 (m, 3H, Ar-H), 8.29 (d, 1H, J=7.2 Hz Ar-H), 8.49 (d, 1H, J=7 Hz, C2-H); 13C NMR spectrum (DMSO-d₆, δ ppm): 32.50 (C-9), 45.00 (SCH₂), 52.89 (2CH₂), 110.30 (CH), 122.5-128.7 (Ar-C), 134 (C-4a), 147.8 (C-4b), 140 (C-9a), 144.8 (C-10a), 155.8 (C-2), 160 (C=O). Anal. calcld. for C₁₇H₁₀O₃N₉S₅ (344): C, 59.28; H, 4.68; N, 8.13; S, 18.62. Found: C, 59.43; H, 4.85; N, 8.00; S, 18.48.

2-(9H-Indeno [1‘, 2‘:4, 5] thieno [2, 3-d] pyrimidin-4-ylsulfanyl)-ethanol (5): A solution of compound 1 (0.256 g, 1 mmol) and sodium hydroxide (0.04 g, 1 mmol) in ethanol (50 ml) was treated with 2-chloro ethanol (0.08 g, 1 mmol) and the reaction mixture was refluxed for 72 h. The formed precipitate was filtered off, dried and recrystallized from ethanol to give compound 5, yield 73%. mp 329-331°C. 1H NMR spectrum (DMSO-d₆, δ ppm): 3.3 (t, 2H, J=2.4 Hz, CH₂), 3.75 (t, 2H, J=2.4 Hz, CH₂), 5.20 (s, 1H, OH, exchangeable with D₂O), 4.09 (s, 2H, CH₂), 7.28-7.60 (m, 3H, Ar-H), 8.07 (d, J=7.2 Hz, 1H, Ar-H), 8.80 (s, 1H, C2-H); 13C NMR spectrum (DMSO-d₆, δ ppm): 32.5 (C-9), 36.5 (CH₃), 60.3 (CH₂), 122-129 (Ar-C), 136.5 (C-4a), 138 (C-4b), 146 (C-9a), 164.2 (C-10a), 152 (C2), 164 (C=O). Anal. calcld. for C₁₇H₁₀O₃N₉S₅ (340): C, 59.7; H, 4.03; N, 8.33; S, 21.35. Found: C, 59.65; H, 4.44; N, 9.12; S, 21.55.

4-Oxiranyl-methylsulfanyl-9H-indeno [1‘,2‘:4,5] thieno [2,3-d] Pyrimidine (6): A solution of compound 1 (0.256 g, 1 mmol) and sodium hydroxide (0.04 g, 1 mmol) in ethanol (50 ml) was treated with epichlorohydrine (0.09 g, 1 mmol) and the reaction was stirred at room temperature for 1 h. The formed precipitate was filtered off, dried and recrystallized from ethanol to give compound 9. Yield 70%, mp 333-335°C. IR spectrum (KBr, ν cm⁻¹): 3050 (CH), 1565 (C=N); 1H NMR spectrum (DMSO-d₆, δ ppm): 2.46 (d, 2H, J=7 Hz, SCH₂), 3.27-3.29 (m, 4H, 2CH₂), 3.98 (s, 1H, CH), 7.2-7.7 (m, 3H, Ar-H), 8.1 (d, J=2.5 Hz, 1H, Ar-H), 8.88 (s, 1H, C2-H); 13C NMR spectrum (DMSO-d₆, δ ppm): 33.46 (C-9), 36.46 (CH₃), 41.06 (CH₂), 60.40 (CH), 122.5-127.63 (Ar-C), 137 (C-4a), 138.2 (C-4b), 146.4 (C-9a), 146.8 (C-10a), 152.2 (C2), 163.25 (C=O). Anal. calcld. for C₁₇H₁₀O₃N₉S₅ (321): C, 61.5; H, 3.87; N, 8.97; S, 20.53. Found: C, 61.11; H, 3.54; N, 9.15; S, 20.83.

(9H-Indeno [1‘, 2‘:4, 5] thieno [2, 3-d] pyrimidin-4-ylsulfanyl)-acetic acid ethyl ester (7): A solution of compound 1 (0.256 g, 1 mmol) and sodium hydroxide (0.04 g, 1 mmol) in ethanol (10 ml) was treated with ethyl chloroacetate (0.13 mL, 1 mmol) and the reaction mixture was stirred at room temperature for 3 h. The formed precipitate was filtered off, dried and recrystallized from ethanol to give compound 7. Yield 87%, mp 237-238°C. IR spectrum (KBr, ν cm⁻¹): 1741 (C=O); 1H NMR spectrum (DMSO-d₆, δ ppm): 1.21 (t, 3H, J=7 Hz, OCH₂CH₃), 3.77 (s, 2H, CH₂), 4.17 (q, 2H, J=7 Hz, OCH₂CH₃), 4.33 (s, 2H, SCH₂), 7.32-7.63 (m, 3H, Ar-H), 8.6 (d, J=2.4 Hz, 1H, Ar-H), 8.8 (s, 1H, C2-H). Anal. calcld. for C₁₇H₁₀O₃N₉S₅ (342): C, 59.63; H, 4.12; N, 8.18; S, 18.73. Found: C, 59.63; H, 4.30; N, 8.01; S, 18.95.

(9H-Indeno [1‘, 2‘:4, 5] thieno [2, 3-d] pyrimidin-4-ylsulfanyl)-acetic acid (8): Method A: A solution of compound 1 (0.256 g, 1 mmol) and sodium hydroxide (0.12 g, 3 mmol) in ethanol (30 mL) was treated with ethyl chloroacetate (0.13 mL, 1 mmol) and the reaction mixture was refluxed for 20 min. The formed precipitate was filtered off, dissolved in water (30 mL) and acidified with hydrochloric acid (2 mL, 10%). The separated solid was filtered off, dried and recrystallized from dioxane to give compound 8 in 81% yield.

Method B: A mixture of compound 7 (0.15 g, 0.5 mmol) and sodium hydroxide (0.08 g, 2 mmol) in ethanol (30 mL) was refluxed for 15 min. The formed precipitate was filtered off,
dissolved in water (20 mL) and acidified with hydrochloric acid (1 mL, 10%). The separated solid was filtered off, dried and recrystallized from dioxane to give a compound identical in all aspects with compound 8 in 89% yield. M.p. 355-356°C. The obtained product was identified by mp and TLC in comparison with authentic samples from methods A and B. IR spectrum (KBr, v, cm⁻¹): 3425 (br, OH) and 1741 (C=O). ¹H NMR spectrum (DMSO-d₆, δ ppm): 4.15 (s, 2H, CH₂), 4.31 (s, 2H, SCH₂), 7.30-7.60 (m, 3H, Ar-H), 8.11 (d, 1H, J= 2.6 Hz, Ar-H), 8.60 (s, 1H, C₂-H), 13.00 (s, 1H, COOH, D₂O exchangeable). Anal. calcd. for C₁₅H₁₀N₂O₅S₂ (314): C, 57.31; H, 3.21; N, 8.91; S, 20.40. Found: C, 57.52; H, 3.08; N, 9.15; S, 20.25.

2-(9H-Indeno[1',2':4,5]thieno[2,3-d]pyrimidin-4-ylsulfanyl)-acetamide (9): To a solution of compound 7 (0.344g, 1 mmol) in ethanol (50mL), ammonium hydroxide solution (0.2 mL, 30%) was added and the reaction mixture was stirred at room temperature for 2h. The formed precipitate was filtered off, dried and recrystallized from ethanol to give compound 9. Yield 69%, mp 327-329°C. IR spectrum (KBr, v, cm⁻¹): 3402, 3368 (NH₂) and 1673 (C=O). ¹H NMR spectrum (DMSO-d₆, δ ppm): 4.15 (s, 2H, CH₂), 3.31 (t, 4H, SCH₂ + NH, D₂O exchangeable), 7.30-7.60 (m, 3H, Ar-H), 8.11 (d, 1H, J= 2.7 Hz, 1H, Ar-H), 8.70 (s, 1H, C₂-H). Anal. calcd. for C₁₅H₁₀N₂O₅S (370): C, 57.49; H, 3.54; N, 13.41; S, 20.46. Found C, 57.85; H, 3.88; N, 13.79; S, 20.21.

(9H-indeno [1', 2':4, 5]thieno [2, 3-d] pyrimidin-4-ylsulfanyl)-acetic acid hydrazide (10): To a solution of compound 7 (0.344g, 1 mmol) in ethanol (50mL), hydrazine monohydrate (0.6 mL, 99%) was added and the reaction mixture was heated on a water bath for 2h, after cooling the precipitated material was filtered off, washed with water, dried and recrystallized from ethanol to give compound 11. Yield 75%, mp 330-333°C. IR spectrum (KBr, v, cm⁻¹): 3422-3245 (NH₂, NH) and 1666 (C=O). ¹H NMR spectrum (DMSO-d₆, δ ppm): 4.15 (s, 2H, CH₂), 4.56 (s, 2H, SCH₂), 4.28 (br, 2H, NH, D₂O exchangeable), 7.3-7.64 (m, 3H, Ar-H), 8.6 (d, J= 8 Hz, Ar-H), 8.81 (s, 1H, C₂-H), 9.47 (s, 1H, NH, D₂O exchangeable). Anal. calcd. for C₁₅H₁₀N₂O₅S₂ (328): C, 54.86; H, 3.68; N, 17.06; S, 19.53. Found: C, 54.55; H, 3.98; N, 16.94; S, 19.77.

5-(9H-Indeno[1',2':4,5]thieno[2,3-d]pyrimidin-4-ylsulfanyl)methyl)-3H-[1,3,4]oxadiazole-2-thione (11): A solution of compound 10 (0.328g, 1 mmol) in (50mL) ethanol was added a solution of potassium hydroxide (0.084g, 1 mmol), followed by 5ml of carbon disulfide. The reaction mixture was refluxed for 12h. After removal of solvent under reduced pressure, the residual solid was dissolved in water (20 mL) and acidified with hydrochloric acid (2 mL, 10%). The separated solid was filtered off, dried and recrystallized from ethanol to give compound 11. Yield 76%, m.p. 330-335°C. IR spectrum (KBr, v, cm⁻¹): 3390 (NH) and 1368 (C=S). ¹H NMR spectrum (DMSO-d₆, δ ppm): 4.18 (s, 2H, SCH₂), 4.22 (s, 2H, CH₂), 7.27-7.76 (m, 3H, Ar-H), 8.38 (d, J= 2.3 Hz, Ar-H), 8.79 (s, 1H, C₂-H), 14.30 (s, 1H, NH, D₂O exchangeable). MS m/z (%): 370 (M⁺, 2), 256 (42), 118 (20). Anal. calcd. for C₁₅H₁₀N₂O₅S (370): C, 51.87; H, 2.72; N, 15.12; S, 25.97. Found: C, 51.55; H, 2.42; N, 15.55; S, 26.27.

RESULTS AND DISCUSSION

Chemistry: 3,9-Dihydroindeno [1', 2':4,5]thieno [2,3-d] pyrimidine-4-thione (1), as the key compound, was used for this study and for further syntheses of other fused heterocyclic compounds. It was previously synthesized by Hegab et al. [19] Ethylation of the sodium salt of compound 1 with ethyl iodide gave, 4-ethylsulfanyl-9H-indeno [1', 2':4,5]thieno [2, 3-d] Pyrimidine (2) (Scheme 1). Oxidation of the latter compound with hydrogen peroxide in acetic acid afforded the corresponding sulphone 3. The structures of the aforementioned compounds 2 & 3 were confirmed on the basis of their elemental and spectral data. Their ¹H NMR spectra showed triplet signals for the CH₃ at ð 1.41 & 1.52 ppm and quartet signals for the CH₃ protons at ð 3.44 & 4.72 ppm, respectively. (cf. Experimental). Similarly, treatment of compound 1 with chloroacetamide diethyl acetal in alcoholic sodium hydroxide yielded 4-(2,2-dimethoxy-ethylsulfanyl)-9H-indeno [1', 2':4,5] thieno [2, 3-d] pyrimidine (4) (Scheme 1). The proposed structure was confirmed by ¹H and ¹³C NMR spectral data. So, ¹³C NMR spectrum showed signals at ð 3.34-5.38 (m, 8H, 20CH₃ + S(CH₃)₂), 3.69 (s, 2H, CH₂), 6.71 (t, 1H, CH). In a similar manner, alkylation of compound 1 with 2-chloroethanol or epichlorohydrine in alcoholic sodium hydroxide gave 2-(9H-indeno [1', 2':4,5] thieno [2, 3-d] pyrimidin-4-ylsulfanyl)-ethanol (5) and 2-oximinoethylsulfanyl-9H-indeno [1', 2':4,5] thieno [2, 3-d] pyrimidine (6), respectively. The structures of the formed compounds were established by their elemental analysis and spectral data (¹H & ¹³C NMR). ¹H NMR spectrum of compound 5 showed signals at ð 3.3 (t, 2H, J= 7.5 Hz, CH₂), 3.75 (t, 2H, J= 7.5, CH₃), 5.20 (s, 1H, CH, exchangeable with D₂O).
While the $^1$H NMR spectrum of compound 6 showed signals at $\delta$ 2.46 (d, 2H, $J=7$ Hz, SCH$_2$), 3.27-3.29 (m, 4H, 2CH$_2$), 3.98 (m, 1H, CH), 7.2-7.6 (m, 3H, Ar-H), 8.1 (d, $J=7.2$ Hz, 1H, Ar-H), 8.88 (s, 1H, C2-H). Also, compound 1 was easily S-alkylated with ethyl chloroacetate in the presence of sodium hydroxide at room temperature, to give (9H-indeno [1',2':4,5] thieno [2,3-\textit{d}] pyrimidin-4-ylsulfanyl)-acetic acid ethyl ester (7). The $^1$H NMR
spectrum of compound 7 exhibited signals at δ 1.21 (t, 3H, J=7 Hz, OCH₂CH₃), 3.77 (s, 2H, CH₂), 4.17 (q, 2H, J=7 Hz, OCH₂CH₃), 4.33 (s, 2H, S-CH₃). On the other hand, upon carrying out the same reaction under reflux, the corresponding acid 8 was obtained presumably via formation of 7 and then hydrolysis of the ester group. Also, compound 8 could be prepared directly by refluxing compound 1 with chloroacetic acid in alkaline medium. The structure of compound 8 was confirmed by IR spectrum which revealed broad bands at 3425 cm⁻¹ (OH) and 1741 cm⁻¹ (C=O) of the carboxylic group. Moreover, the treatment of the ester 7 with ammonium hydroxide afforded the corresponding amide 9 (Scheme 1). Analytical and spectral data of this compound is in agreement with the assigned structure. ¹H NMR spectrum of compound 9 revealed the disappearance of ethyl group of compound 7. Many reports Shiba et al. [20] and Kamal El-Dean and Abdel-Moneam [21] have studied the chemical behavior of acid hydrazide towards different reagents in the hope of obtaining compounds for different application. Thus, the ester 7 was treated with hydrazine hydrate under reflux to yield the corresponding acid hydrazide 10. The structure of the product was confirmed on the basis of its elemental analysis and spectral data. ¹H NMR revealed the absence of the presence of signals for the NH₂ and NH groups at δ 4.28 & 9.47, respectively. 5-(9H Indeno [1',2':4,5] thieno [2,3-d]pyrimidin-4-ylsulfanyl)methyl)-3H-[1,3,4]oxadiazole-2-thione (11) was prepared following the procedure of Young and Wood [22], by heating the acid hydrazide 10 with carbon disulfide in the presence of sodium hydroxide. Inspection of the IR spectrum of the reaction product 11 revealed the absence of the C=O group and showed absorption bands characteristic for the NH and C=S groups and its ¹H NMR spectrum revealed signals for CH₂ and NH groups (cf. Experimental).

**Antiviral Screening**

**MTT Assay (Cytotoxicity Assay):** Samples were diluted with Dulbecco's Modified Eagle's Medium (DMEM) to the desired concentrations (10, 20, 30 and 40 μg / μl). Stock solutions of the test compounds were prepared in 10% DMSO in dH₂O. The cytotoxic activity of the extracts were tested in Madin Darby Canine kidney (MDCK) cells by using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method [23] with minor modification. Briefly, the cells were seeded in 96 well-plates (100 μl/well at a density of 3×10⁴ cells/ml) and incubated for 24 h at 37°C in 5% CO₂. After 24 h, cells were treated with various concentrations of the tested compounds in triplicates. After further 24 h, the supernatant was discarded and cell mono-layers were washed with sterile phosphate buffer saline (FBS) three times and MTT solution (20 μl of 5 mg/ml stock solution) was added to each well and incubated at 37°C for 4 h followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 μl of acidified isopropanol (0.04 M HCl in absolute isopropanol). Absorbance of formazan solutions were measured at λmax 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation:

\[
\text{Absorbance of cells without treatment - Absorbance of cells with treatment} \times 100 \%
\]

The plot of % cytotoxicity versus sample concentration was used to calculate the concentration which exhibited 50% cytotoxicity (LD₅₀).

**EC₅₀, LD₅₀ and Therapeutic Index:** The antiviral activity of the compounds was determined using cytopathogenicity (CPE) assay against avian influenza virus (H5N1). Stock solutions of the test compounds were prepared in DMEM at a concentration of 10 mg/ml. Cells grown to confluency in 96-well plates, were infected with 100 μl of stock virus. After an adsorption period of 2 h at 37°C, virus was removed and serial dilutions of the tested compounds were added, then maintenance DMEM with 2% FBS was added (100μl/well). The cultures were further incubated at 37°C for 3 days, until complete CPE was observed in the infected and untreated virus control. The determination of the anti-influenza virus activity of the tested compounds was based on virus-induced cytopathogenicity of H5N1-infected MDCK cells, measured at day 4 post virus infection by the MTT colorimetric method [24]. An absorbance of formazan was detected by a multi-well plate reader at 540 nm with 620 nm reference wavelength. The results were expressed as the 50% effective concentration (EC₅₀). The 50% effective antiviral concentration (EC₅₀) was defined as the compound concentration required for protecting 50% of the virus-infected cells against viral cytopathogenicity. The therapeutic index was calculated by dividing LD₅₀ on EC₅₀ according to Hayden et al. [25].

Table 1: Antiviral activity against H5N1 virus of the prepared compounds by determination of both EC₅₀ and LD₅₀

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<th>EC₅₀ (µg/ml)</th>
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**RESULTS**

Antiviral assay was carried out to test compounds 1-3 and 5-11 for antiviral activity. The test was performed to include the three possibilities for antiviral activity; virucidal effect, virus adsorption and effect on virus replication for H5N1. The obtained data showed that all the tested compounds are inferior in antiviral activity to Zanamivir.

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