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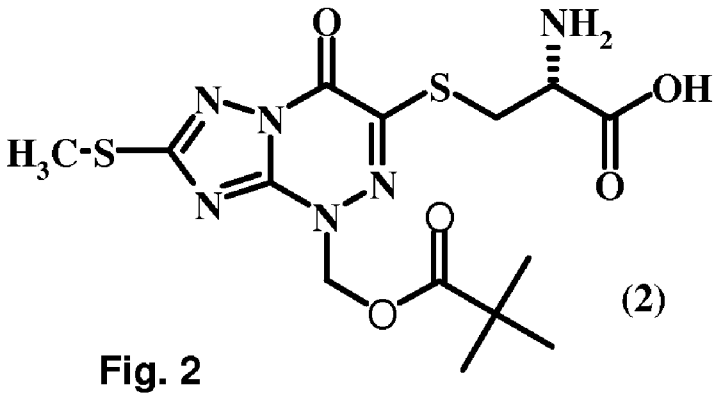
(54) **Title**: SMALL MOLECULE HAVING ANTIVIRAL PROPERTIES



(2)

Fig. 2

(57) **Abstract**: This patent disclosure describes a new scaffold for a small molecule having antiviral properties, molecule having antiviral properties with demonstrated low toxicity is disclosed.

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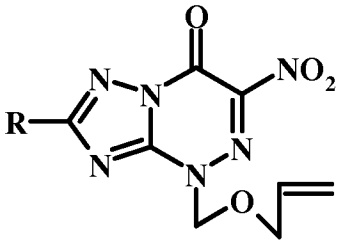
SMALL MOLECULE HAVING ANTIVIRAL PROPERTIES

Technical field to which the invention pertains.

The invention relates to biologically active compounds possessing antiviral properties for the treatment and prevention of viral infections of animals and humans. The invention can be used in hospitals, research laboratories, as well as livestock and poultry.

There is evidence of antiherpetic action (VL, Ruthenians, ON Chupakhin, E. H Ulomsky and other RF Patent 2345080 from 27.01.2009), as well as (ON Chupakhin, VL Rusinov, EN Ulomsky and other RF Patent 2376307 from 20.12.2009, the). Antiviral activity against influenza virus types A and B have R = H, CH3, SCH3 (V.L.Rusinov, E. N.Ulomsky, S. Deev. And other RF Patent 2340614 from 10.12.2008; VL Rusinov, ON Chupakhin, C . L. Deev, TS Shestakova, EN Ulomsky, LI Rusinov, OI Kiselev, EG Deeva Synthesis and antiviral activity of nucleoside analogues on the basis of 1,2,4-triazolo [3,2-c] [1,2,4] triazine-7 (4H)-ones Proceedings of the Academy of Sciences, Chemical Bulletin, 2010, № 1, p. 135-142.).

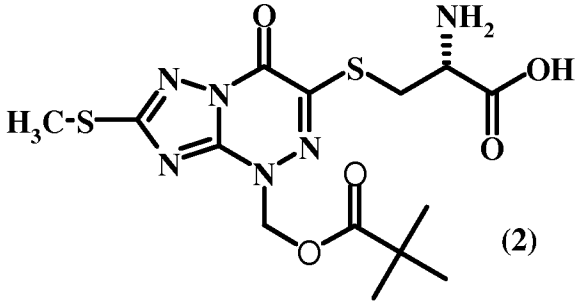
The closest in structure to the claimed compound from this series (1) can be regarded as a prototype. By using the compound (1) in a concentration of 40 ug / ml in in vitro experiments, the infectious titer of influenza A/H3N2 and influenza virus A/Gonkong/1/68 A/H5N1 A / Duck / Singapore R/F119-3/97 reduced by 0,5-3,0 lg. However, this compound at higher concentrations showed cytotoxicity. The concentration at which 50% of the cells die (CC50) is 80 ug / ml.



(1)

SUMMARY OF THE INVENTION

The invention is the small molecule **(2)** which has antiviral activity.



(2)

The small molecule (2) has demonstrated low toxicity.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a scaffold for a small molecule.

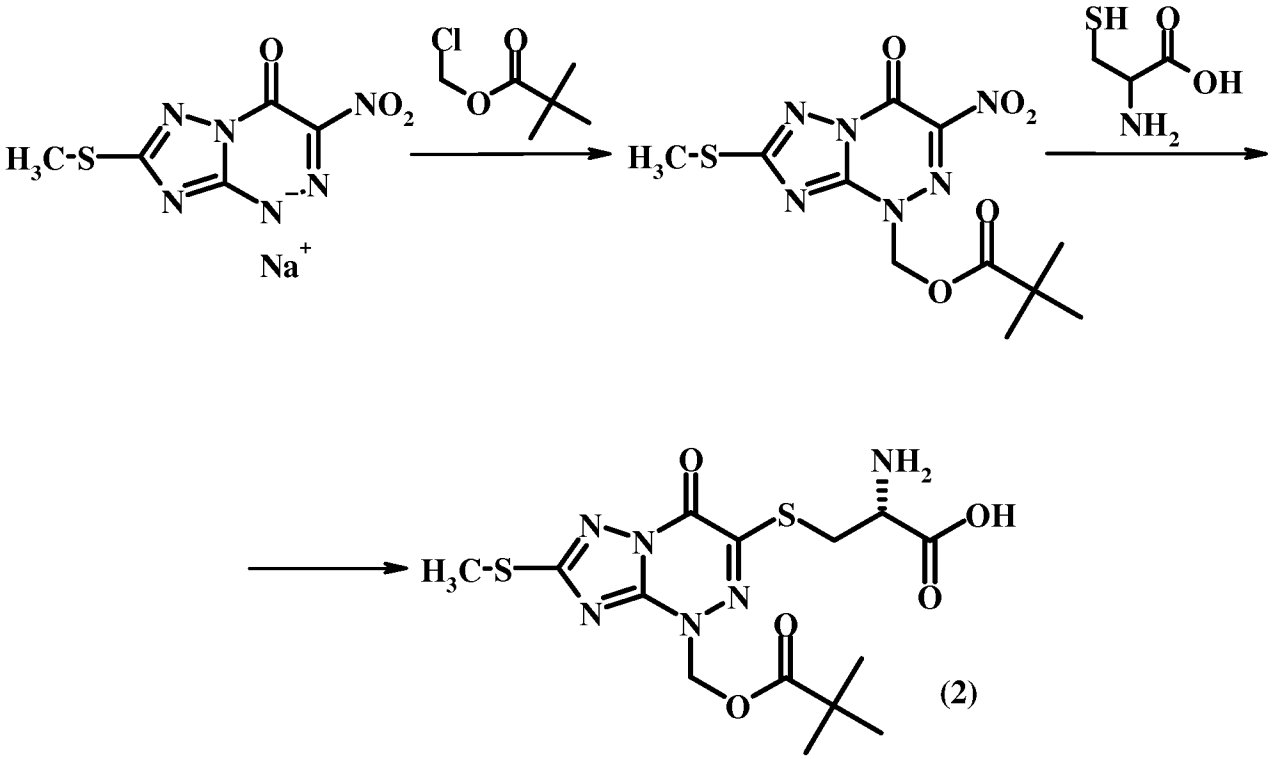
Fig. 2 shows a small molecule based on the scaffold of Fig. 1.

DETAILED DESCRIPTION OF THE INVENTION

Synthesis

Example **1.** Obtained from 0.05 mol of sodium salt of -1 ,2,4-triazolo [5,1-c] 1,2,4-triazine-7-one dihydrate action hlormetilpivalata with 0.05 mol using DMF as a solvent, a solution of one equivalent of cysteine in ethanol. After the reaction, the solvent was removed in vacuo and the resulting precipitate was purified by column chromatography (eluent - acetonitrile: water = 8:1).

The yield of compound (2) is 51%.



Chemical synthesis of the proposed scheme of compound (2) has the following physico-chemical characteristics: TPL = 204 OS, 1 H NMR spectrum in D2O δ, ppm: 5.18 (d, 1H, CH2), 5.13 (d, 1H, CH2) , 4.19 (m, 1H, CH), 3.91 (m, 1H, SSN2), 3.39 (m, 1H, SSN2), 2.69 (S, 3H, CH3), 1 18 (d, 9H, C (CH3) 3). Found: C - 40.56, H - 4,77, N - 20,32. Gross formula - C14H20N6O5S2. Calculated: C - 40.38, H - 4,84, N - 20,18%.

The compound produced from this synthesis is a pale yellow crystalline solid, soluble in water, methanol, dimethyl sulfoxide, insoluble in benzene, ether and most

other solvents. The compound is orally ingestible and show efficacy against viruses including the influenza virus.

Antiviral properties of the molecule of Fig. 2

Example 2 Toxicity and antiviral activity of compounds against influenza virus

cells. Used a one-day monolayer culture of epithelial cells MDCK (dog kidney)

Viruses. To assess the antiviral activity of the virus used the reference A / Puerto Rico/8/34, as well as pandemic influenza virus H1N1v A/Sankt-Peterburg/2/09 (similar to the so-called virus "swine flu» A/California/7/09).

The maximum tolerable concentrations of the compounds was determined by MTT test in cell culture MDCK.

Testing of toxicity was carried out as follows: weighed weigh weighing 5 mg in a sterile test tube 5 ml. and the diluted growth medium for the cells MDCK (α-MEM, Biolot, St. Petersburg) to a concentration of 1 mg / mL, thus obtaining a basic solution. More of the same medium made eight consecutive binary dilutions (500, 250, 125, 62.5, 31.25, 15.13, 7.56 and 3.78 mg / ml, respectively), which was used for toxicity testing. The experience set in the four parallels for each concentration. One-day cell culture MDCK, grown in 96-well plates (Costar), checked visually in an inverted microscope on the integrity of the monolayer. The plates were washed twice with medium without serum, and then have made the test compound in appropriate concentrations in a volume of 100 ml in each well. The plates were incubated for 72 h at 37 ° C in the presence of 5% CO2, and then recorded the results of the experiment visually assessing the integrity of the monolayer compared with control cells, and by the MTT (quantitatively evaluating the viability of the cells) using the tablet reader Hydex Chameleon. Statistical analysis was performed using the program Statistica 6.0.

Assessment of antiviral activity were carried out for two concentrations: maximum concentration (dilutions of the binary), which survived for 100% of a monolayer of cells, and a concentration equal to half the previous one. To assess the antiviral activity of the virus used the reference A / Puerto Rico/8/34. One-day cell culture MDCK, grown in 96-well plates (Costar), checked visually in an inverted microscope on the integrity of the monolayer. Next, prepare ten-fold dilution of virus on

maintenance growth medium with the addition of trypsin (from -1 to -6). Plates with a monolayer of cells were washed twice with medium without serum, after which the viral breeding have made the appropriate wells in a volume of 50 ml. Control wells filled with growth medium with an equal volume. The plates were incubated for 60 min at 37 ° C in the presence of 5% CO_2, and then washed with medium to remove unbound viral particles to cells. Continue to make the drug in the wells with virus dilutions in 100 ml of the appropriate concentration. Each concentration of test compound was placed in four parallels for each virus dilution. Control wells filled with growth medium in the same volume. Also, the wells were left to re-test toxicity used concentrations. The plates were incubated for 72 h at 37 ° C, and then recorded the results of the experiment visually assessing the integrity of the monolayer as compared to control cells and the degree of cytopathic effect of virus in cell culture, put hemagglutination reaction and MTT method was used to quantify cell viability using the tablet reader Hydex Chameleon.

Statistical analysis was performed using the program Statistica 6.0 [Borovikov VP, IP Borovikov Statistica. Statistical analysis and data processing in Windows. - M., 1997. - S. 33-34], using regression analysis [Rokitskii PF Biological statistics. - Minsk, 1967. - S. 155]. The results are presented using graphs derived from the linear regression equation, which has the general form $y = k + b * x$, where y is expressed by the constant k and the angular coefficient b, multiplied by the variable x. At the same time on the graph indicates the coefficient of determination, r2, and designated as expressing the spread of values around the regression line relative to the total spread of values. The closer the value of r2 to 1, the better the model explains the variability of the variables. The confidence interval for all the regression equations was equal to 95%.

Evaluation of toxicity of the claimed compounds

Evaluation of toxicity, as well as evaluation of the antiviral action of drugs produced by three methods:

1) monitoring and evaluation of the integrity of the monolayer cells under an inverted microscope. Implies a comparison of cell morphology of control wells with experienced and registration of changes under the influence of an agent (drug, virus, etc.). The change of morphology could include violating the integrity of the monolayer, changes in cell shape, expression of cytopathic effect in virus infection.

2) Registration in the presence of the virus haemagglutinin with a suspension of chicken erythrocytes (0.75%). The reaction of hemagglutination (RHA) to evaluate the qualitative presence of the virus in the sample.

3) The method of MTT. The method used in the evaluation of drug sensitivity, based on the ability of dehydrogenases of living cells to restore the form of colorless 3-4,5-dimethylthiazol-2-yl-2 ,5-difenilterarazola (MTT reagent) to blue crystalline farmazana soluble in DMSO or 96% alcohol. Saturation of color indicates the intensity of metabolic processes in cells, ie, the normal level of viability. The color intensity is recorded with a spectrophotometer and the absorbance values obtained can be used for statistical data processing.

The concentration of the drug during that kills 50% of a monolayer of cells in a test on the MDCK cells was 177 pg / ml. Drug concentration at which all the cells survive the monolayer was 62.5 micrograms / ml, which was used to test the antiviral activity of the drug.

In this test the viability of cells in the control was 0,571 ± 0,044. Thus, the absorbance value at which 50% of the surviving cell monolayer is 0.286. If you put this value on the y-axis graph, shown in Figure 1, drawn through a line parallel to the x-axis, and then from the point of intersection of this line with the schedule to the viability of the perpendicular to the x-axis, we obtain a value corresponding to the concentration of the claimed compounds which killed 50% of a monolayer of cells (see Table 2)

Evaluation of antiviral activity

The proposed connection of all three methods of assessment (concentration = 62.5 mg / ml) had a pronounced antiviral activity, reducing viral titers at 3 lg relative to the control of infectious virus activity.

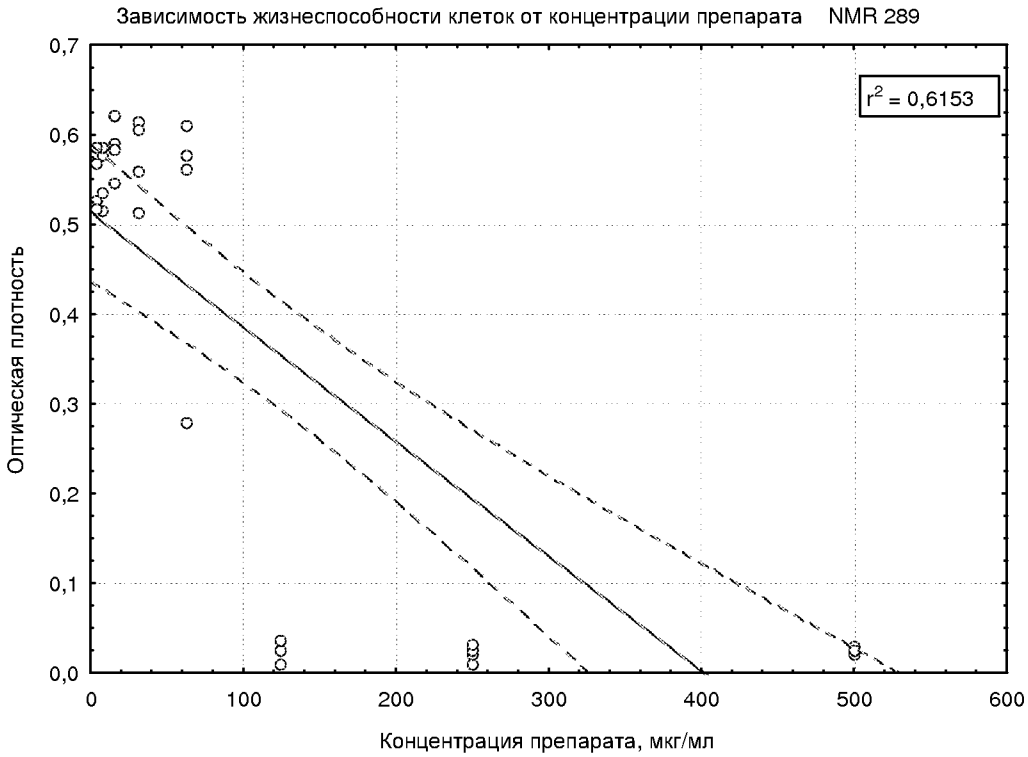


Table 1. Dependence of the viability of MDCK cells on the concentration of the claimed compounds.

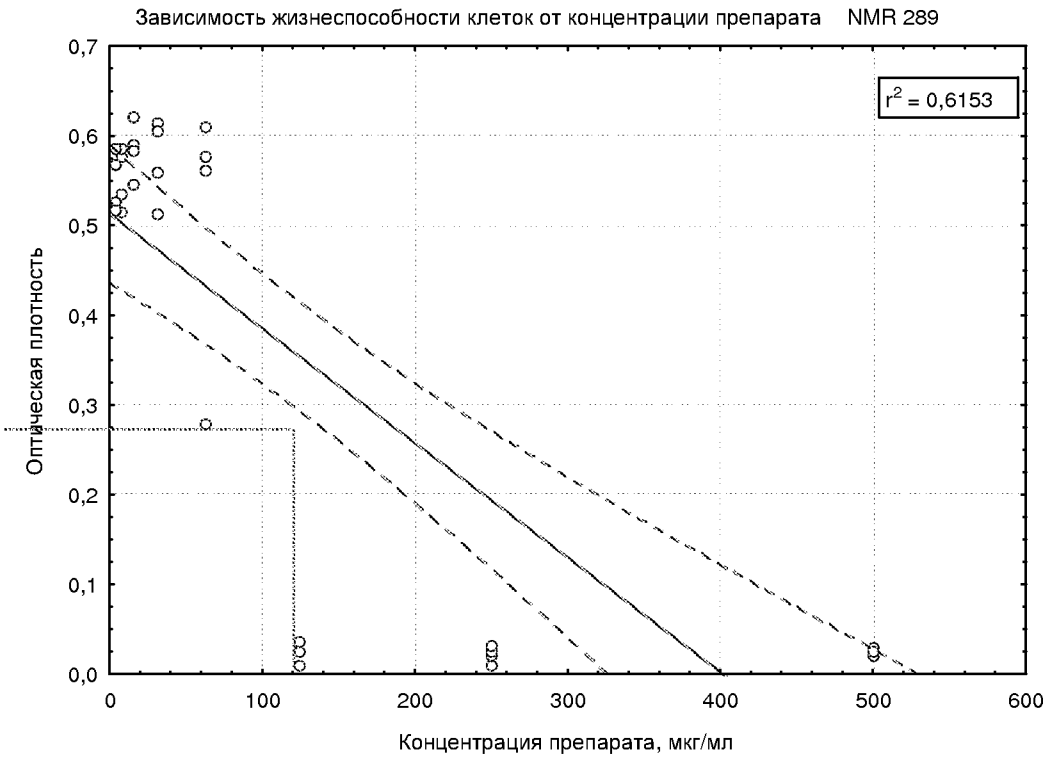


Table 2 Graphical calculation of the concentration of the claimed compounds in which the die 50% of the cell monolayer.

In constructing the graph corresponding to the action of the claimed compounds, the coefficient of determination r2 was equal to 0.86, which suggests that this model explains well the variation of the tested variables, namely, the effect of test compounds on cells infected with influenza virus. In constructing the perpendiculars through the points corresponding to an optical density at which 50% of surviving cells of the monolayer on the vertical axis and the intersection of the perpendicular to the line corresponding to the viability of cells in the drug, we have the following schedule as shown in Table 3.

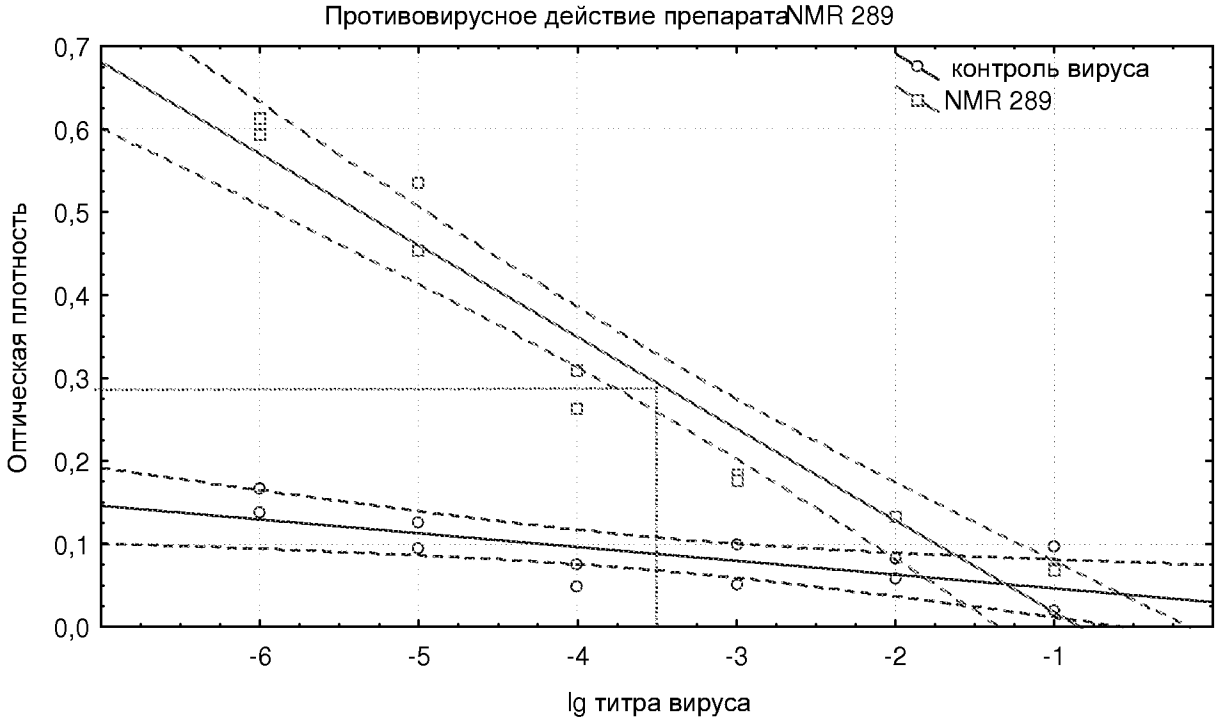


Table 3 The antiviral effect of the claimed compounds

From Table 3, it follows that the intersection point of the perpendicular to the x-axis corresponds to the dilution of the virus lg equal -3.5. Given the fact that the virus infects the drug in the absence of cell monolayer to a value equal to 10-6,5 (infectivity of the virus, clarified in a separate test for testing the biological properties of virus A/PR/8/34), we can say that the use of the drug under in vitro conditions reduces the activity of the infectious virus 3lg.

The data presented above show that the prototype compound (1) is more toxic than the claimed connection (for connection (1) = 80mkg/ml SC50 and SC50 to the claimed compounds = 177 ug

/ ml). Compound (1) are active against a pandemic influenza virus A/H1N1v strain - A/Sankt-Peterburg/2/09 (A/California/7/09-podobny). At the same time, the alleged connection (2) in much smaller concentrations, in comparison with toxic, has a pronounced antiviral activity, reducing the titers of virus A / Puerto Rico/8/34 virus and pandemic influenza H1N1v A/Sankt-Peterburg/2/09 (A/California/7/09-podobny) for 3,0 lg concentrations 62.5 and 125 ug / ml, respectively, then there is a more active antiviral compound than with a prototype.

CLAIMS

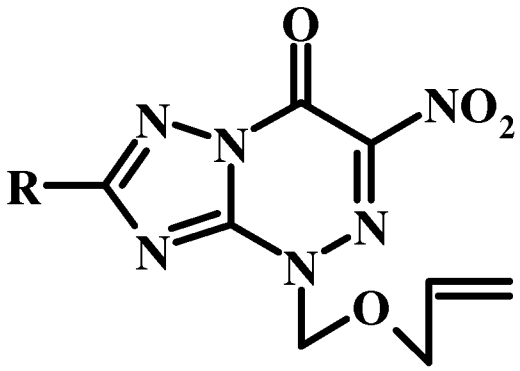
A compound with the following characteristics

1. A molecular scaffold as shown in Fig 1 .

2. A small molecule having antiviral properties as shown in Fig. 2.

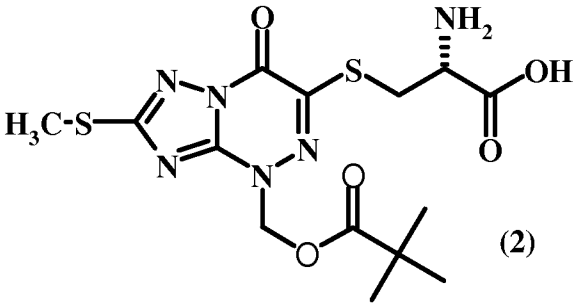
3. The small molecule of claim 2 in an orally ingestible form used to treat influenza infections in humans and animals.

4. The small molecule of claim 2 in an orally ingestible form used to treat viral infections.



(1)

Fig. 1



(2)

Fig. 2