Synthesis and anti H_5N_1 activities of some pyrazolyl substituted N-heterocycles

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Abstract: The acid hydrazide derivative **1** was utilized for the construction of pyridazine, pyridazinone, pyrazole, pyrazolone, pthalazinedione and schiff bases upon condensation with different carbonyl compounds. However, the reaction of Schiff base **13** with thioglycolic acid gives the thiazolidinone derivative **14**. The structures of the newly synthesized compounds were established on the basis of IR, ^{1}H-NMR, mass spectral data, and elemental analyses. The antiviral activities of the synthesized compounds against HPAI H_5N_1 were examined. Some of the tested compounds showed promising activities.

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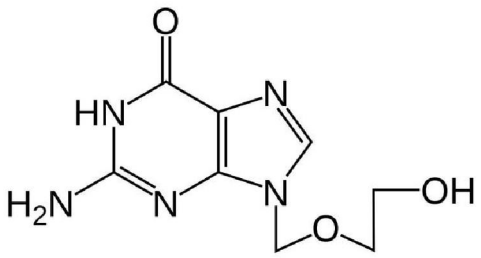
1. Introduction

Highly Pathogenic Avian influenza (HPAI) is a highly contagious disease of poultry caused by type A influenza H_5N_1 viruses of the family Orthomyxoviridae. [1] The rapid rate of spread and the high potential for genetic alterations of the virus has raised the specter of widespread human infection and the possibility of a pandemic.[2] World Health Organization reported that from 2003 tell 4 April 2016 in all worldwide there are 850 infection cases from which 449 were died. In Egypt the number of human infected cases from 2006 tell 4 April 2016 were 350 infection cases from which 116 were died.[3] Egypt is one of the few countries where H_5N_1 virus has become enzootic and is the only country with a high number of H_5N_1 outbreaks among poultry and cases among human.[4]

Nitrogen-containing heterocycles are widespread in nature, and their applications as pharmaceuticals and agrochemicals are important. [5-8] The utilization of 2(3*H*)-furanones for the construction of a wide variety of nitrogenous heterocyclic ring systems of synthetic and biological importance had been a subject of our research group concern. [9-23] There are various pyrazole derivatives are developed by linking N-heterocycles as a new scaffold of antitumor agents.[24,25]

The imidazolopyrimidinone derivative (*Acyclovir)* is used to treat infections caused by herpes viruses, such as genital herpes, cold sores, shingles, and chicken pox.

The role of antiviral is considered critical in preparedness for avian flu originated pandemic. Several at-risk nations have stored strategic stockpiles of antiviral especially *Oseltamivir* to be used at the face of HPAI H_5N_1 pandemic. However, resistance to *Oseltamivir* in the H_5N_1 subtypes in Vietnam and other human influenza A viruses, [1-4&26-30] has become a cause for worry as far as pandemic preparedness is concerned. Therefore, the search for alternative antivirals that can effectively inhibit H_5N_1 or other influenza A viruses, or act in synergy with available antivirals, is an urgent need of the hour. Several novel antiviral agents that may be effective against influenza virus, specifically the H5N1 avian flu virus, are currently under development.



Acyclovir

On the other hand vaccination in poultry flocks require at least three weeks for each vaccine to be effective. At this time, antiviral drugs are the only specific medical intervention that targets influenza and could prevent poultry infection till the applied vaccine(s) work efficiently. Drugs can be used to prevent influenza and, unlike vaccines, can also be used to treat cases that are identified.

On the bases of these observations, we aimed to synthesized pyrazolyl substituted N-heterocycles of anticipated biological activity and study their antiviral effect against HPAI H_5N_1.

2. Material and method

Biological activity

Cell line:

Continuous cell line of (MDCK) Madin-darby canine kidney cells were supplemented with minimum essential media (MEM) containing 10% heat inactivated fetal bovine serum, 100 IU/ml penciline, 100 µg/ml the cell were incubated at 37 ^{0}C in 5% CO_2 humidified atmosphere, The cells used at concentration of 2×10^5 /ml.

Virus:

HPAI H_5N_1 virus A/chicken/ EG/ 1575S/ 2015 (H_5N_1) obtained from Central laboratory for Evaluation of Veterinary Biologics (CLEVB), was used at a titer of 10^6 EID_{50}/ml.

Cytotoxicity assays

The maximum non-toxic concentration (MNTC) of each compound and the drug controls (amantadine hydrochloride) was determined based on cellular morphologic changes (Cytopathic effect (CPE)). [31] Tenfold serial dilutions of each compound stock were done beginning with 50 mg/ml then incubated in contact with confluent monolayer of MDCK cells in triplicate in 96-well plates for 4 days and the cells were observed under microscope every 24 h for visible CPE. The highest concentration of the compound without any CPE up to 4 days in all replicates was considered as the MNTC.

Virucidal activity:

Equal volumes of each compound at MNTC were added to the confluent monolayer of MDCK cells in 96- wells plate for 1 h. and then virus were mixed. The plate was incubated at 37°C and the wells were observed under an inverted microscope for virus induced CPE at 24, 48 and 72 h post-infection to record a score for each well on the basis of extent of CPE in the particular well (score 0 for 0% CPE, score 1 for 0–25% CPE, score 2 for 25–50% CPE, score 3 for 50–75% CPE and score 4 for 75–100% CPE). The cell control (uninfected untreated cells) and the virus control (infected but untreated) were kept in each plate throughout the test.

Antiviral Activity:

Each of two dilutions of the tested compounds, including the MNTC of each compound was inoculated into 5 embryonated chicken eggs of nine-day-old via the allantoic route. The inoculated compound/virus mixture volume was 0.2 ml/egg which prepared by suspending 0.1 mL of H_5N_1 in 0.1 mL of compound in DMSO. 0.2ml of H5N1 Virus diluted in saline solution without compounds was used as positive control. 0.2ml of saline solution was used as negative control. The eggs were incubated at 37 °C. Allantoic fluid from each egg was collected and tested by rapid hemagglutination test to detect H_5N_1 in the eggs.

Rapid Haemagglutination (HA) Test:

Haemagglutination activity of the allantoic fluids of inoculated eggs is measured by micro technique of Haemagglutination test. [32]

Experimental

Chemistry

Melting points were measured on a Gallen Kamp electric melting point apparatus. The infrared spectra were recorded using potassium bromide disks on FTIR Thermo Electron Nicolet 7600 (USA) infrared spectrometer at the Central laboratory of Faculty of science, Ain shams university. The ^{1}H-NMR spectra were run at 300 MHz on a GEMINI 300 BB NMR spectrometer using tetramethylsilane (TMS) as internal standard in deuterated dimethylsulphoxide (DMSO-d_6) at the main defense chemical laboratory. The mass spectra were recorded on a Shimadzu GC-MS QP- 1000EX mass spectrometer operating at 70 ev at the Microanalytical Center of Cairo university. The reactions and the purity of all the synthesized compounds were monitored by the thin layer chromatography using Merck Kieselgel 60 F_{254} aluminum backed plates. Spots visualization were carried out using a UV lamp.

General procedure for synthesis of the pyrazolyl pyridazine derivative (2)

To a solution of **1** (0.01 mol) and in ethanol/dioxane mixture (40 ml), acetonylacetone (0.01 mol) was added. The reaction mixture was refluxed for 7h. A solid product was precipitated while hot, filtered off and washed with ethanol, recrystallized from dioxane to give the pyrazolyl pyridazine derivative **2**.

1-(3,6-dimethylpyridazin-1(4H)-yl)2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-4-phenylbutane-1,4-dione (2).

Yellow crystals; m.p.: 190-192 ^{o}C, yield=70%, IR (KBr) (υ_{max}, cm^{-1}): 1695, 1654(C=O). ^{1}H-NMR (DMSO-d6): δH (ppm) δ 1.02 (s,3H, CH3), 2.48 (s, 3H, CH3), 3.37-3.42 (m,2H, CH_2, pyridazine), 3.92 (s, 2H, CH_2), 5.74-5.78 (m,1H, =CH, pyridazine), 7.12-8.32 (m,16H,ArH+ =CH), 9.21(s,1H, H pyrazolyl), 13.09 (br.s, 1H, NH, exchangeable) EIMS m/z (%): 500 ($M^{+\cdot}$, 5), 482 (42), 404 (100), 301(56), 257 (88), 202 (73), 154 (64), 104 (76), 77 (74), 51(34). Anal. Calcd. for $C_{32}H_{28}N_4O_2$ (500.22): C, 76.78; H, 5.64; N, 11.19. Found: C, 76.98; H, 5.80; N, 11.41.

General procedure for the reaction of (1) with acetyl acetone to give butane hydrazide derivative (3).

To a solution of **3** (0.01 mol) in ethanol (30 ml), acetic acid (1ml) acetylacetone (0.01 mol) was added. The reaction mixture was refluxed for 20 h., then left to cool at room temperature. The solid obtained was filtered off and washed with ethanol and recrystallized from ethanol/dioxane mixture to give the title compound **3**.

2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-4-oxo-N-(4-oxo-pentan-2-ylidene)-4-phenylbutane hydrazide(3)

Orange crystals; m.p.:202-204 ^{o}C, yield=53%. IR (KBr) (υ_{max}, cm^{-1}): 3129 (NH), 1712,1698, 1654(C=O). ^{1}H-NMR (DMSO-d6): δH (ppm) δ 1.89 (s,3H, CH3), 2.48 (s,3H, CH3), 3.55(s,2H, CH_2), 3.95 (s, 2H, CH_2), 7.26-8.06 (m, 16H, ArH+ =CH), 8.38 (s, 1H, H pyrazolyl), 13.09 br.s, 1H, NH, exchangeable). EIMS m/z (%): 504 (M^+, 3), 4 04 (100), 301(21), 257(25),77 (71),57(29). Anal. Calcd. For $C_{31}H_{28}N_4O_3$ (504.22): C, 73.79; H, 5.59; N, 11.10. Found: C, 73.42; H, 5.65; N, 11.53.

General procedure for the reaction of the acid hydrazide (1) with ethyl acetoacetate

To a solution of **1** (0.01 mol) in ethanol (30 ml), ethyl acetoacetate (0.01 mol) was added. The reaction mixture was refluxed for 6 h, cooled at room temperature. A white solid was formed, filtered off, washed with ethanol and then recrystallized from ethanol to give the ethyl butanoate derivative **5**.

Ethyl-3-(2-(2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-4-oxo-4-phenylbutanoyl)hydrazono)butanoate (5).

Orange crystals; m.p.:208-210^{o}C, (ethanol/dioxane) yield=45%. IR (KBr) (υ_{max}, cm^{-1}): 3298, 3204 (NH), 1734 (C=O ester), 1694, 1654 (C=O). ^{1}H-NMR (DMSO-d6): δH (ppm) 1.01 (t, 3H, $\underline{CH_3}CH_2OCO$, J=6.6Hz), 2.47 (s, 3H, CH_3), 3.54 (s, 2H, CH_2), 3.94 (s, 2H, CH_2), 3.41 (q, 2H, $CH_3\underline{CH_2}OCO$, J=6.9 Hz),7.26-8.06 (m, 16H, ArH+=CH), 8.40 (s, 1H, =CH, pyrazolyl), 13.16 (br.s, 1H, NH, exchangeable). EIMS m/z (%): 534 (M^+, 7), 470 (20), 404 (100), 389 (35), 301 (32), 257 (21), 154 (24), 104 (43), 77 (86), 51 (35). Anal. Calcd. for $C_{32}H_{30}N_4O_4$ (534.23): C, 71.89; H, 5.66; N, 10.48. Found: C, 71.97; H, 5.75; N, 10.23.

General procedure for synthesis of phenylpropanoate derivative (7).

An equimolar mixture of the acid hydrazide **1** and ethyl cinnamate was heated on sand bath at 180-190^{o}C for 2 h. The jelly product obtained was boiled with methanol, a yellow solid was separated out, filtered off while hot, washed with cold methanol and recrystallized from dioxane to give phenylpropanoate derivative **7**.

Ethyl-3-(2-(2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)4-oxo4-phenyl butanoyl)hydrazinyl)-3-phenylpropanoate(7)

Yellow crystals; m.p.:218-220 ^{o}C, yield=53%. IR (KBr) (υ_{max}, cm^{-1}): 3299, 3130 (NH), 1732, 1696, 1654 (C=O).). ^{1}H-NMR (DMSO-d6): δH (ppm) 1.04 (t, 3H, $\underline{CH_3}CH_2OCO$, J=6.6Hz), 2.97 (d, 2H, CH_2, J=7.3Hz), 3.40 (q, 2H, $CH_3\underline{CH_2}OCO$, J=6.9 Hz), 3.94 (s, 2H, CH_2), 4.20 (m, 1H, CH), 4.54 (br.s, 1H, NH, exchangeable) 6.96 (s, 1H, =CH), 7.15-8.07 (m, 20H, ArH), 8.39 (s, 1H, =CH, pyrazolyl), 13.09 (br.s, 1H, amide NH, exchangeable). EIMS m/z (%): 598 (M^+, 5), 486 (13), 404 (100), 390 (53), 301 (37), 257 (67), 154 (24), 105 (53), 77 (82), 51 (27). Anal. Calcd. for $C_{37}H_{34}N_4O_4$ (598.26): C, 74.23; H, 5.72; N, 9.36. Found: C, 74.57; H, 5.43; N, 9.63.

General method for the ring closure of the amide derivatives (3), (5) and (7) using HCl/AcOH mixture

A solution of **3**, **5 or 7** (1g) in a mixture of HCl/AcOH, 1:1 (30 ml), was heated under reflux for 1h and then left to cool. The solid obtained was filtered off, washed with water and recrystallized from ethanol to give the pyrazole derivatives **4**, **6** or **8** respectively.

1-(3,5-Dimethyl-1H-pyrazol-1-yl)-2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-4-phenyl butane-1,4-dione(4)

Yellow crystals; m.p.:189-192^{o}C, (ethanol) yield=50%. IR (KBr) (υ_{max}, cm^{-1}): 1699, 1659 (C=O). ^{1}H-NMR (DMSO-d6): δH (ppm) δ 2.89 (s,3H, CH3), 2.95 (s, 3H, CH3), 3.25 (s, 2H, CH_2), 6.93-8.11 (m, 17H, ArH+ =CH), 8.35(s,1H, H pyrazolyl). EIMS m/z (%): 486 (M^+, 7), 421 (34), 404 (100), 301 (27), 257 (34),77 (87), 57(54). Anal. Calcd. for $C_{31}H_{26}N_4O_2$ (486.21): C, 76.52; H, 5.39; N, 11.51. Found: C, 76.78; H, 5.63; N, 11.24.

2-((1,3-Diphenyl-1H-pyrazol-4-yl)methylene)-1-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylbutane-1,4-dione(6)

Yellow crystals; m.p.:195-197^{o}C, (ethanol) yield=53%. IR (KBr) (υ_{max}, cm^{-1}): 1714, 1678, 1654 (C=O). ^{1}H-NMR (DMSO-d6): δH (ppm) 2.06 (s, 3H, CH_3), 3.23 (s, 2H, CH_2), 3.56 (s, 2H, CH_2), 7.03-8.05 (m, 16H, ArH+=CH), 8.43 (s,1H, =CH, pyrazolyl). EIMS m/z (%): 488 (M^+,7), 448 (29), 404 (100), 389 (42), 301 (41), 257 (65), 154 (23), 104 (32), 77 (78), 51 (35). Anal. Calcd. for $C_{30}H_{24}N_4O_3$ (488.55):C, 73.76; H, 4.95; N, 11.47. Found: C, 73.37; H, 4.71; N, 11.13.

2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-1-(5-oxo-3-phenylpyridazin-1-yl)-4-phenylbutane-1,4-dione(8)

Yellow crystals; m.p.:198-199 ^{o}C, (ethanol) yield=34%. IR (KBr) (υ_{max}, cm^{-1}): 3232 (NH), 1710, 1695, 1654 (C=O)). ^{1}H-NMR (DMSO-d6): δH (ppm) 3.07 (m, 2H, CH_2), 3.43 (s, 2H, CH_2), 4.45 (m, 1H, CH), 5.78 (br.s,1H,NH, exchangeable) 6.87 (s,1H, =CH), 7.04-8.12 (m, 20H, ArH), 8.36 (s, 1H, =CH, pyrazolyl). EIMS m/z (%): 552 (M^+, 5), 448 (39), 404 (100), 390 (53), 301(37), 257(67), 154(24), 105 (53), 77 (82), 51 (27). Anal. Calcd. for $C_{35}H_{28}N_4O_3$ (552.63):C, 76.07; H, 5.11;N, 10.14. Found: C, 76.35; H, 5.39; N, 9.86.

General procedure for the reaction of (1) with diethyl malonate

To a solution of **1** (0.01 mol) in ethanol (30 ml), diethylmalonate (0.01 mol) was added. The reaction mixture was refluxed for 4 h, white crystals were formed while hot, filtered off, washed with cold ethanol and recrystallized from dimethylformamide (DMF) to give the pyrazolidine-3,5-dione derivative **9**.

1-(2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-4-oxo-4-phenylbutanoyl) pyrazolidine-3,5-dione (9).

m.p.:253-255°C, yield=30%. IR (KBr) (υ_{max}, cm^{-1}): 3306 (NH), 1775, 1725, 1695,1654(C=O)). ^{1}H-NMR (DMSO-d6): δH (ppm) 3.55 (s,2H,CH_2), 3.94 (s, 2H, CH_2), 4.97 (br.s, 1H, OH, lactim form, exchangeable) 6.66 (s, 1H, =CH), 7.15-8.40 (m, 15H, ArH), 9.14 (s, 1H, =CH, pyrazolyl), 13.12 (br.s, 1H, NH, exchangeable). EIMS m/z (%): 490M^+, 19), 448 (73), 404 (100), 391(34), 301(43), 257(67), 154(54), 105 (53), 77 (78),51(67). Anal. Calcd. for $C_{29}H_{22}N_4O_4$ (490.52):C, 71.01; H, 4.52;N, 11.42. Found: C, 71.36; H, 4.30; N, 11.83.

General procedure for the reaction of (1) with phthalic anhydride

A mixture of **1** (0.01mol) and phthalic anhydride (0.01mol) in dioxane (30 ml) in the presence of glacial acetic acid (1 ml) was refluxed for 6 h. The excess solvent was removed under reduced pressure, the deposited solid was filtered off, dried and then recrystallized from dioxane / DMF mixture to give the pthalazine-1,3-dione derivative **10**.

N-1,3-dioxoisoindolin-2-yl)-2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-4-oxo-4-phenyl butanamide(10)

Colorless crystals; m.p 312-314°C, yield 60%. IR (KBr) (υ_{max}, cm^{-1}): 3418, 3268 (NH), 1797, 1744 (coupling bands), 1723, 1687, 1656 (CO). ^{1}H-NMR (DMSO-d6): δH (ppm) 3.95 (s,2H,CH_2), 7.13 (s,1H, =CH), 7.23-8.18 (m, 19H, ArH), 9.29(s,1H, =CH, pyrazolyl), 13.10 (br.s, 1H, NH, exchangeable). EIMS m/z (%): 552(M^+,3), 534 (33), 404 (100), 390 (36), 331 (41), 301 (43), 271 (52), 257 (67), 169 (63), 105 (53), 77 (92), 51(37). Anal. Calcd. for $C_{34}H_{24}N_4O_4$ (552.59):C, 73.90; H, 4.38;N, 10.14. Found: C, 74.36; H, 4.67; N, 10.45.

General procedure for the reaction of (1) with n-bromononane

The acid hydrazide **1** (0.006 mol) was suspended in dry DMF (30 ml). Sodium hydride (60%, 0.24 g, 0.006 mol) was added. The mixture was stirred at room temperature for 20 minutes and then the n-bromononane (0.006 mol) was added. The reaction mixture was refluxed for 5 h. Then the mixture cooled to room temperature and the solvent was evaporated under vacuum. The residue was dissolved in dichloromethane (200 ml), and the organic phase was washed with water (2 x 50 ml). The organic layer was dried over sodium sulfate anhydrous and evaporated. The residue was purified by ethanol to give the pyridazinone derivative **11**.

4-((1,3-Diphenyl-1H-pyrazol-4-yl)methylene-1-nonyl-6-phenyl-1,4-dihydropyridazin-3(2H)-one (11)

Yellow crystals; m.p >320°C, yield 56%. IR (KBr) (υ_{max}, cm^{-1}): 3420 (NH), 1696 (CO). ^{1}H-NMR (DMSO-d6): δH (ppm) 0.81- 0.95 (t, 3H, CH3, J=6.2,6.6), 1.23–1.65 (m, 12H, 6CH2), 2.47-2.59 (m, 2H, CH2), 3.19-3.43 (t, 2H, N-CH2, J=6.7, 6.9), 6.99 (s, 1H, =CH, pyridazinone), 7.23(s, 1H, =CH), 7.39-8.08 (m, 15H, ArH), 8.89 (s, 1H, =CH, pyrazolyl), 13.11 (br.s, 1H, NH, exchangeable). EIMS m/z (%): 530(M^+,7), 389(41),277 (100), 263(67), 235(41), 77 (23), 51(54). Anal. Calcd. for $C_{35}H_{38}N_4O$ (530.72):C, 79.21; H, 7.22;N, 10.56. Found: C, 79.66; H, 7.67; N, 10.25.

General procedure for the reaction of (1) with benzil

A mixture of the hydrazide **1** (0.01 mol), benzil (0.01 mol) in dioxane (25ml) was heated under reflux for 6 h. Evaporation of excess solvent left a solid product, dried and then recrystallized from ethanol / dioxane mixture to give **12.**

2-((1,3-Diphenyl-1H-pyrazol-4-yl)methylene)-4-oxo-N′-(2-oxo-1,2-diphenylethylidene)-4-phenyl butane hydrazide (12)

Pale yellow powder; m.p: 215-217°C, yield 73%. IR (KBr) (υ_{max}, cm^{-1}): 3205, 3130 (NH), 1714, 1688, 1654 (CO). ^{1}H-NMR (DMSO-d6): δH (ppm) 3.94 (s, 2H, CH_2), 7.26-8.08 (m, 26H, ArH+=CH), 8.40 (s, 1H, =CH, pyrazolyl), 13.13 (br.s,1H,NH, exchangeable). MS, m/z (%): 614 (M^+, 0.6), 522 (52), 390 (85), 257 (84), 135 (65), 105 (69), 77 (100), 51(23). Anal. Calcd. For $C_{40}H_{30}N_4O_3$ (614.71): C, 78.16; H, 4.92; N, 9.11. Found: C, 78.53; H, 4.52; N, 9.34.

General procedure for the reaction of 1 with *p*-anisaldehyde.

To a solution of the acid hydrazide **1** (0.01 mol) in dioxane (30 ml), p-anisaldehyde (0.01 mol) was added. The reaction mixture was refluxed for 4 h., then left to cool at room temperature. The solid obtained was filtered off and washed with ethanol and recrystallized from dioxane to give the Schiff base product **13**.

3-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-1-((4-methoxybenzylidene)amino)-5-phenyl-1,3-dihydro-2H-pyrrol-2-one(13)

Orange crystals; m.p. 183-185°C, (dioxane) yield=94%. IR (KBr) (υ_{max}, cm^1) 1672 (C=O) 1613 (C=N). ^{1}H-NMR (DMSO-d_6): 3.52, 3.70 (2 s, 6H, 2OCH_3), 6.92-8.08 (m, 23H, 19ArH+ 2CH=+2pyrolone), 8.73, 8.94 (two s,2H, 2N=CH), 9.24, 9.34 (two s, 2H, 2 pyrazolyl). EIMS, *m/z* (%): 524 (M+2,84), 523 (M+1,60), 522(M^+,47), 404 (100), 389 (71), 360 (22), 301 (21), 245 (56), 104(38), 77(81), 51 (23).Anal. Calcd. For $C_{34}H_{26}N_4O_2$ (522.61):

C, 78.14; H, 5.01; N, 10.72. Found: C, 78.47; H, 5.45; N, 10.37%.

General procedure for reaction of (13) with thioglycolic acid.

To a solution of **13** (0.01 mol) in dry benzene (70 ml), thioglycolic acid (0.01 mol) was added. The reaction mixture was refluxed for 8 h. using water separator, then left to cool at room temperature. The solid obtained was filtered off and washed with dry benzene and recrystallized from benzene to give the thiazolidinone derivative **14**.

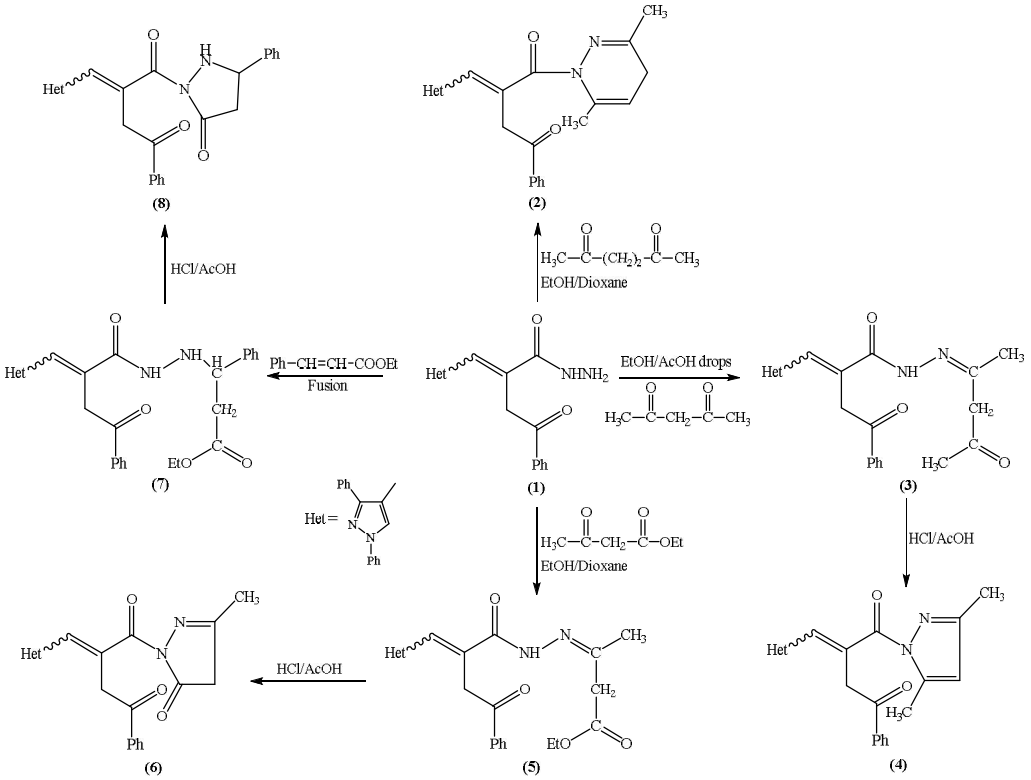
(3-(3-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)-2-oxo-5-phenyl-2,3-dihydro-1H-pyrrol-1-yl)-2-(4-methoxyphenyl)thiazolidin-4-one(14)

Yellow crystals; m.p. 195-197°C, yield=76%, IR (KBr) (υ_{max}, cm^{-1}): 3138 (NH), 1691, 1634, 1613 (C=O), ^{1}H-NMR (DMSO-d_6): δ 3.41-3.44 (m, 2H, CH_2), 3.78 (s, 3H, OCH_3), 6.97, 6.99 (two singlets, 2H, 2=CH), 7.19 (s, 1H, -CH-), 7.25-8.01 (m, 21H, 19ArH+2 pyrrolone), 9.33, 9.37 (two singlets, 2H, 2 pyrazolyl protons). EIMS, *m/z* (%): 596($M^{\cdot+}$, 2), 522 (95), 388 (91), 360 (22), 257 (45), 105 (76), 77 (100), 51 (27). Anal. Calcd. for $C_{36}H_{28}N_4O_3S$ (596.17): C, 72.46; H, 4.73; N, 9.39; S, 5.37. Found: C, 72.13; H, 4.25; N, 9.72; S, 5.59.

3. Results and discussion

Chemistry

The acid hydrazide derivative **1** was previously prepared by our research group upon hydrazinolysis of 3-(1,3-diphenylpyrazol-4-yl) methylene-5-phenyl-2(3*H*)-furanone.[13] Treatment of the acid hydrazide derivative **1** with acetonylacetone in refluxing ethanol afforded the pyridazine derivative **2**. The structure of compound **2** was substantiated from its analytical and spectral data. The IR spectrum showed the disappearance of NH_2 and NH bands. Further support for the structure of compound **2** was gained from ^{1}H-NMR and its mass spectrum that showed the correct molecular ion peaks beside some abundance peaks. (cf. experimental part). The acid hydrazone product **3** was obtained upon treatment the acid hydrazide derivative **1** with acetylacetone in refluxing ethanol/dioxane mixture. Ring closure of **3** by HCl/AcOH mixture gave the pyrazolyl derivative **4**. Treatment of an ethanolic solution of the acid hydrazide **1** with ethyl acetoacetate afforded the open chain adduct butanoate derivative **5**.



Scheme 1. Ring transformation of **1** into pyridazine, pyrazole, pyrazolone and pyridazinone derivatives **2, 4, 6** and **8** respectively.

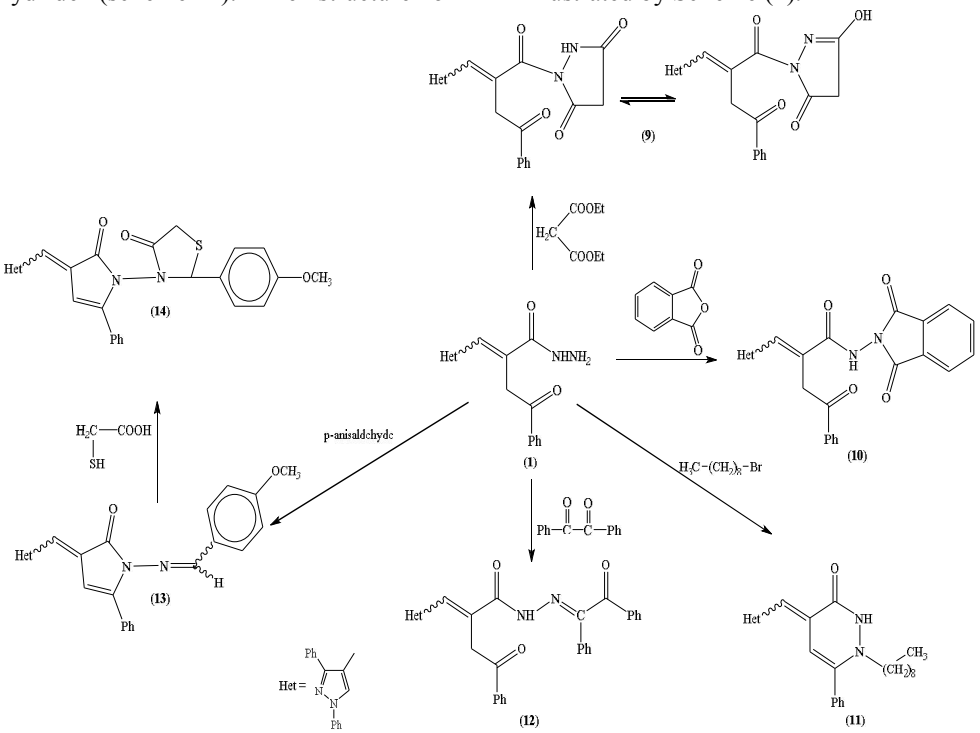
The IR spectrum of **5** exhibits bands corresponds to NH at 3204, ester and amide carbonyls at 1735 and1654 cm^{-1} respectively. Ring closure of **5** by HCl/AcOH mixture gave the pyrazolone derivative **6**. Fusion of **1** with ethylcinnamate afforded the *Michael* addition product **7**. The IR spectrum of **7** exhibits bands corresponds to NH at 3130, 3204, C=O (ester) at 1738 and C=O (amide) at 1653 cm^{-1}. Ring closure of **7** by HCl/AcOH mixture gave the pyrazolone derivative **8** (Scheme 1). Further evidence for the structure of compounds **3-8** were ascertained from their microanalytical and spectral data. (cf. experimental part).

Pyrazolidinone derivative **9** was obtained upon treatment of the acid hydrazide derivative **1** with diethylmalonate (scheme 2). Inspection of the IR and ^{1}H-NMR spectrum of compound **9** revealed its existence as enol tautomer. Thus, the IR didn't show the vibrational coupling of the carbonyl groups and the ^{1}H-NMR shows broad singlets for OH and amide NH groups at 4.96 and13.12 ppm respectively. (cf.experimental part).

N- phthalimide derivative **10** was obtained upon treatment of the acid hydrazide derivative **1** with phthalic anhydride (scheme 2). The structure of compound **10** was substantiated from its analytical and spectral data. The IR spectrum exhibits bands corresponds to the vibrational coupling of the carbonyl groups at 1744, 1724 cm^{-1}. Further support for the structure of compound **10** was gained from ^{1}H-NMR and its mass spectrum. (cf. experimental part).

On the other hand, when the acid hydrazide derivative **1** was treated with n-bromononane afforded the pyridazinone derivative **11**.

The Schiff bases were exhibited many biological activities such as antifungal, antibacterial, antitumor, anti-inflammatory and antipyretic activities [28-32]. Thus, the treatment of the acid hydrazide derivative **1** with benzil and/or p-anisaldehyde gave the Schiff bases aryl hydrazine derivatives **12** and **13** respectively. The hydrazone**13** which obtained in the previous step was treated with thioglycolic acid to give the thiazolidinone derivative **14**. The ^{1}H-NMR spectra of compounds **13** and **14** revealed the existence of keto-enol tautomers in equal ratios. The structures of all the products obtained from the foregoing reactions were illustrated from their analytical, as well as, spectral data (cf. the Experimental part). All the reactions investigated are illustrated by Scheme (2).



Scheme 2. Ring transformation of **1** into pyrazolone, phthalazindione, pyridazinone and thiazolidinone derivatives **9,10,11** and **14**respectively

Biological evaluation

In the present study, the MNTC varied from 50 µg/ml to 500µg/mL (Table 1). The concentrations above MNTC were toxic to the cells as evidenced by rounding, clumping and detachment from the well surface. The more Safe compounds are **1, 2, 9, 12**.

Concerning the efficacy, compounds numbers **2** and **9** show (IP > 95%) in tissue culture (Table 1).

Amantadine and Acyclovir used as reference drugs with the infected cells exhibited a moderate antiviral effect (IP < 5O %), (IP 50-90%) respectively. On the other hand 3 compounds shown inhibition of the replication of H5N1 virus in SPF ECE when compared with "Amantadine" (reference antiviral drug). These compounds are designated as **2, 9, 12**. Considering compounds **2** and **9** are the more potent, they prevent the virus replication in all the inoculated eggs at the two dilutions (50 and 500 µgm/ml). Finally, the outcome of these study is the possibility of using the new heterocyclic compounds in the production of effective antiviral drugs against HPAI H5N1 viruses.

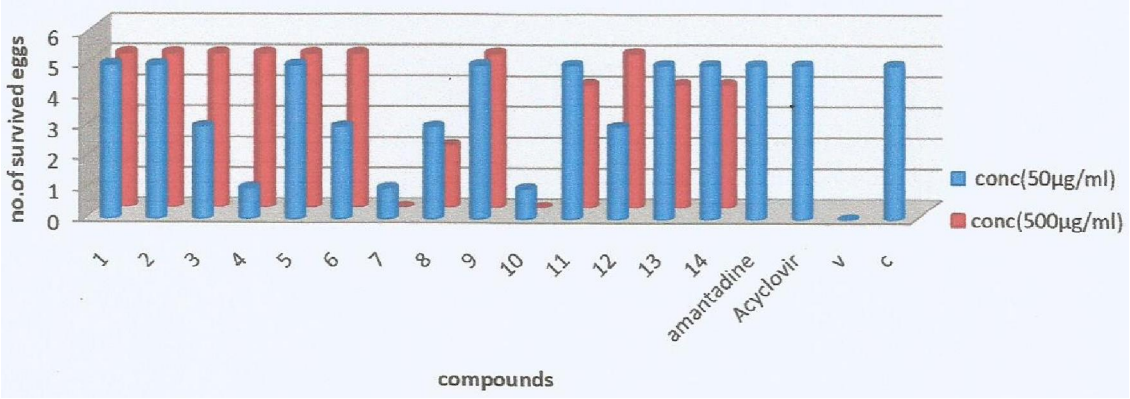


Fig.1 No. of survived eggs after one day from inoculation

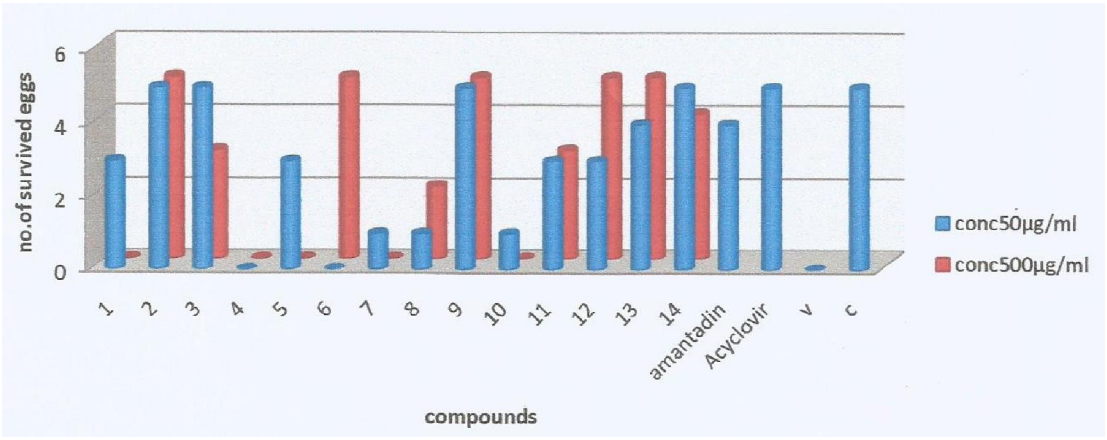


Fig.2. No. of survived eggs after two days from inoculation

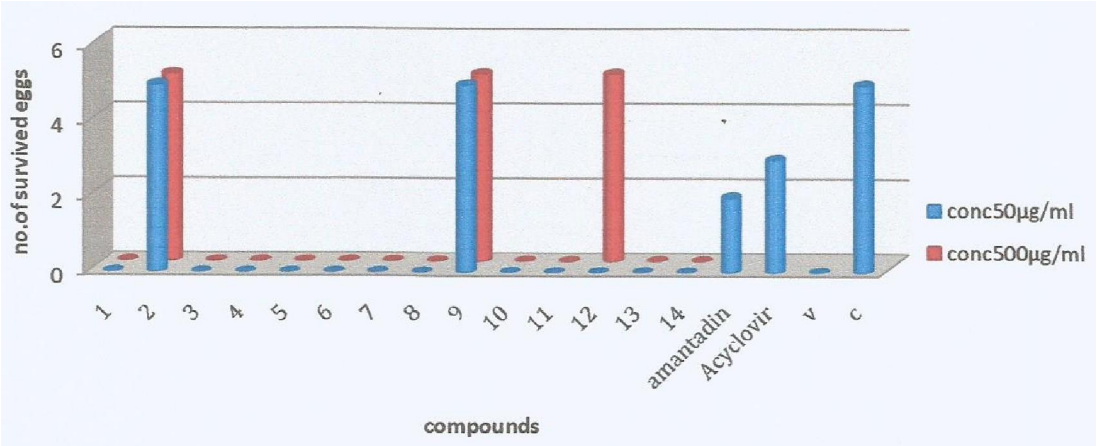


Fig.3. No. of survived eggs after three and four days from inoculation

Table (1). Maximum non toxic concentration of new synthesized compounds and cytopathic effect of H_5N_1

Compounds	$MNTC_{(\mu g/ml)}$	$TCID_{50}$(IP %)	CPE(score)
1	500	+	1
2	500	+++	0
3	500	-	4
4	500	+	3
5	500	+	2
6	500	+	2
7	500	+	3
8	500	-	3
9	500	+++	0
10	50	-	2
11	50	+	2
12	500	++	1
13	50	-	4
14	500	-	2
Amantadine	250	+	2
Acyclovir	500	++	1

MNTC: Maximum non toxic concentration $TCID_{50}$: 50 % Tissue culture infective doses
IP: Virus inhibition percentage, (-)= IP < 25%, (+) = IP 25-50%, (++) = IP 50-90%, (+++) =IP 90-95%
(0 for 0% CPE, 1 for 0–25% CPE, 2 for 25–50% CPE, 3 for 50–75% CPE and 4 for 75–100% CPE).

Table (2): Efficacy of new synthesized compounds on HPAI H_5N_1 Virus in ECE as measured by embryonic survival no.

		Hours post inoculation				
Compound NO.	Conc. µg/ml	24h No.of survival eggs	48h No.of survival eggs	72h No.of survival eggs	96h No.of survival eggs	+HA
1	50 500	5 5	3 0	0 0	0 0	+ve
2	50 500	5 5	5 5	5 5	5 5	-ve -ve
3	50 500	5 3	5 3	0 0	0 0	+ve
4	50 500	1 5	0 0	0 0	0 0	+ve
5	50 500	5 5	3 0	0 0	0 0	+ve
6	50 500	3 5	0 5	0 5	0 0	+ve -ve
7	50 500	1 0	1 0	0 0	0 0	+ve
8	50 500	3 2	1 2	0 0	0 0	+ve
9	50 500	5 5	5 5	5 5	5 5	-ve -ve
10	50 500	1 0	1 0	0 0	0 0	+ve
11	50 500	5 4	3 3	0 0	0 0	+ve
12	50 500	3 5	0 5	0 5	0 5	+ve -ve
13	50 500	5 4	3 3	1 0	0 0	+ve
14	50 500	4 5	4 5	0 2	0 0	+ve
Amantadin (25 µg/ml)		5	4	2	2	-ve
Acyclovir		5	5	4	3	-ve
Positive control (Virus only)		0	0	0	0	+ve
Negative control		5	5	5	5	-ve

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