

Original Article

SYNTHESIS AND ANTIVIRAL ACTIVITY OF NOVEL ETHYL 2-(3-HETEROCYCLE-1H-INDOL-1-YL) ACETATE DERIVATIVES

ESLAM R EL-SAWY¹, HEBA M ABO-SALEM^{*1}, EMAN S ZARIE², HOWAIDA I ABD-ALLA¹, MOUNIR M EL-SAFY³, ADEL H MANDOUR¹

¹Chemistry Department of Natural Compounds, National Research Centre, Dokki 12311, Giza, Egypt, ²Functional Nanomaterials, Institute for Materials Science, Christian Albrechts University Kiel, Kaiserstrasse, Germany, ³Central Laboratory for Evaluation of Veterinary Biologics, Abbassia 13181, Cairo, Egypt
Email: hb_abosalem@yahoo.com

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ABSTRACT

Objective: Marek's disease (MD) is a widespread, herpesvirus-induced neoplastic disease in the domestic chicken that is caused by Marek's disease virus (MDV). Marek's disease virus (MDV) belongs to the alphaherpesvirus family such as Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2). Recently Bag and co-workers 2014 reported that, 7-methoxy-1-methyl-4, 9-dihydro-3H-pyrido [3, 4-b]indole (Harmaline) showed potent anti-HSV-1 activity against both wild type and clinical isolates of HSV-1. The present work aimed to synthesize some new heterocyclic systems incorporated to indole moiety starting from ethyl 2-(3-acetyl-1H-indol-1-yl)acetate (1) in order to evaluate their antiviral activity in a trail to explore potential antiviral agents against MDV to limit the disease course and losses.

Methods: Reaction of ethyl 2-(3-acetyl-1H-indol-1-yl) acetate (1) with semicarbazide hydrochloride yielded semicarbazone derivative 2. The oxidative cyclization of 2 using thionyl chloride and selenium dioxide afforded 1, 2, 3-thia and 1, 2, 3-selenadiazole derivatives 3 and 4, respectively. On the other hand, reaction of 1 with 4-chloro and 4-nitrobenzaldehydes under Claisen-Schmidt conditions gave α , β -unsaturated keto derivatives 5a, b. Cyclization of 5a, b using hydrazine hydrate, phenyl hydrazine, urea, thiourea or guanidine led to the formation of pyrazoles 6a, b, 7a, b, and pyrimidines derivatives 8a, b-10a, b, respectively. Condensation of 1 with phenyl hydrazine followed by Vilsmeier Haack formylation gave pyrazole-4-carboxaldehyde derivative 12. Reaction of aldehydic function group of 12 with different reagents led to the formation of pyrazol-5-ones 14-16, thiazolidinone 18, aziditine 19, 1, 6-diaminopyridine 21, triazolo(1, 5-a)pyridine 22 and pyrano(2, 3-c) pyrazole derivatives 23. The *in vitro* antiviral activity of the selected compounds 6a, b, 7a, b, 8a, b, 9a, b and 10a, b was studied against Marek's disease virus (MDV).

Results: Chicken embryo experiment showed that compounds 7b, 8b, 9b and 10a possessed significant antiviral activity with IC₅₀ ranged between 5 and 6 μ g/ml and substantial therapeutic indices (TI) of 80 and 83 were recorded. Cytotoxicity assay indicated that CC₅₀ of 7b, 8b, 9b and 10 were greater than 400 and 500 mg/ml.

Conclusion: Compounds 7b, 8b, 9b and 10a showed promising effect as anti-MDV infectivity application.

Keywords: Ethyl 2-(3-acetyl-1H-indol-1-yl) acetate, Pyrazole, Pyridine, Pyrimidine Marek's disease virus (MDV).

INTRODUCTION

Marek's disease (MD) is a widespread, herpesvirus-induced neoplastic disease in the domestic chicken that is caused by Marek's disease virus (MDV) [1]. Marek's disease virus (MDV) belongs to the alphaherpesvirus family such as Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2). Because MDV is ubiquitous and chickens acquire environmental infection early in life, they must be protected by vaccination in the hatchery. MD outbreaks may be associated with mortality immune suppression or excessive condemnation of carcasses can experience MD outbreaks at any age [2]. Recently Bag and co-workers 2014 reported that, 7-methoxy-1-methyl-4, 9-dihydro-3H-pyrido [3, 4-b] indole (Harmaline) showed potent anti-HSV-1 activity against both wild type and clinical isolates of HSV-1 [3]. On the other hand, indole and its derivatives are reported to show wide variety of pharmacological properties besides antiviral activity [4-6] such as anti-inflammatory [7, 8], anti-cancer [9, 10], antimicrobial [11, 12] and antioxidant [13, 14]. Also, literature revealed that pyrazole, pyridine and pyrimidine derivatives are known for their antiviral activities [15-23]. Based on the previous observations, the present work aimed to synthesize some new heterocyclic systems incorporated to indole moiety starting from ethyl 2-(3-acetyl-1H-indol-1-yl)acetate (1) and evaluating their antiviral activity as a trail to explore potential antiviral agents against MDV to limit the disease course and losses.

MATERIALS AND METHODS

General

Melting points were determined on digital melting point apparatus (Electrothermal 9100, Electrothermal Engineering Ltd, serial No.

8694, Rochford, United Kingdom) and are uncorrected. The micro analytical data were achieved on a Perkin-Elmer 2400 analyzer (Perkin-Elmer, 940 Winter Street, Waltham, Massachusetts 02451, USA) and were found within ± 0.4 % of the theoretical values. IR spectra were recorded on a Perkin-Elmer 1600 Fourier Transform Infrared Spectrophotometer (Perkin-Elmer). The NMR spectra were recorded on a Bruker Avance digital spectrometer (BRUKER, Germany) in DMSO-*d*₆, and chemical shifts (δ) are reported in ppm units relative to the standard tetramethylsilane (TMS). Mass spectra (EI) were recorded at 70eV with JEOL-JMS-AX500 mass spectrometer (JEOL Ltd. 1-2, Musashino 3-chome Akishima, Tokyo 196-8558, Japan). The chemicals and solvents were of commercial grad and used without further purification. Ethyl 2-(3-acetyl-1H-indol-1-yl)acetate (1), 2-cyanoacetic acid hydrazide, 3-amino-5-pyrazolone and 2'-acetyl-2-cyanoacetohydrazide were prepared as reported [24-27].

Synthesis

Ethyl 2-(3-(1-semicarbazidoethyl)-1H-indol-1-yl) acetate (2)

A mixture of ethyl 2-(3-acetyl-1H-indol-1-yl)acetate (1) (2.45 g, 10 m mol), semicarbazide hydrochloride (1.11 g, 10 m mol) and sodium acetate (0.86 g, 10 m mol) in absolute ethanol (20 ml) was refluxed for 2 h. The solid formed after cooling was collected by filtration, air dried and crystallized from absolute ethanol. Yield: 65%; MP: 200-202 °C; IR (KBr): 3380, 3210 (NH₂), 3180 (NH), 1682 and 1654 (C=O), 1604 (C=N), 1597 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.33 (t, 3H, CH₃), 2.23 (s, 3H, CH₃), 4.22 (q, 2H, CH₂), 5.26 (s, 2H, CH₂), 6.36 (s, 1H, NH), 7.12-7.46 (m, 3H, Ar-H), 7.45 (s, 1H, indolyl H-2), 8.24 (d, 1H, indolyl H-4), 9.12 ppm (s, 2H, NH₂); ¹³C NMR (125.7 MHz, DMSO-*d*₆): δ 15.0 and 47.5 (2CH₃), 61.4 (CH₂), 110.4-144.2 (Ar-C), 157.7 and 169.2 ppm (2C=O); Anal.

C₁₅H₁₈N₄O₃ (302.41): Calcd: C, 59.59; H, 6.00; N, 18.53; Found: C, 59.44; H, 5.98; N, 18.40.

Ethyl 2-(3-(1, 2, 3-thiadiazol-4-yl)-1H-indol-1-yl) acetate (3)

To compound **2** (0.3 g, 1 m mol), thionyl chloride (0.6 ml, 5 m mol, *d*=1.6) was added gradually at ice-bath temperature and then allowed to stand for 30 min. Chloroform (20 ml) was added and the reaction mixture was decomposed with the saturated solution of sodium carbonate. The organic layer was separated and dried over anhydrous sodium sulphate. After evaporation of all solvent, the obtained residue was crystallized from methanol-charcoal. Yield: 55%; MP: 60-62 °C; IR (KBr): 1654 (C=O), 1604 (N=N), 1577 cm⁻¹ (C=C); ¹H NMR (200 MHz, DMSO-*d*₆): δ 1.33 (t, 3H, CH₃), 4.33 (q, 2H, CH₂), 5.23 (s, 2H, CH₂), 6.99 (s, 1H, thiadiazolyl H-5), 7.22-8.22 (m, 4H, Ar-H), 8.44 ppm (s, 1H, indolyl H-2); ¹³C NMR (60 MHz, DMSO-*d*₆): δ 47.5 (CH₃), 61.4 (CH₂), 110.4-136.2 (Ar-C), 157.7 ppm (C=O); Anal. C₁₄H₁₃N₃O₂S (287.34): Calcd: C, 58.52; H, 4.56; N, 14.62; Found: C, 58.41; H, 4.35; N, 14.37.

Ethyl 2-(3-(1, 2, 3-selenadiazol-4-yl)-1H-indol-1-yl) acetate (4)

To a stirred and hot solution of compound **2** (0.3 g, 1 m mol) in glacial acetic acid (10 ml), powdered of selenium dioxide (0.14 g, 7 m mol) was added gradually. After complete addition, boiling and stirring were continued for another 1 h. The reaction mixture was filtered, then cooled and poured onto ice-water. The formed solid was collected, washed with water, air dried and crystallized from methanol-charcoal. Yield: 45%; MP: 97-100 °C; IR (KBr): 1666 (C=O), 1602 (N=N), 1578 cm⁻¹ (C=C); ¹H NMR (200 MHz, DMSO-*d*₆): δ 1.33 (t, 3H, CH₃), 4.34 (q, 2H, CH₂), 5.22 (s, 2H, CH₂), 5.58 (s, 1H, selenadiazolyl H-5), 7.32-7.66 (m, 3H, Ar-H), 8.12 (d, 1H, indolyl H-4), 8.46 ppm (s, 1H, indolyl H-2); ¹³C NMR (60 MHz, DMSO-*d*₆): δ 47.5 (CH₃), 61.4 (CH₂), 110.4-157.2 (Ar-C), 169.2 ppm (C=O); Anal. C₁₄H₁₃N₃O₂Se (334.23): Calcd: C, 50.31; H, 3.92; N, 12.57; Found: C, 50.21; H, 4.00; N, 12.32.

Synthesis of compounds 5a and 5b

A solution of compound **1** (2.45 g, 10 m mol), 4-chloro or 4-nitrobenzaldehydes (10 m mol) and aqueous potassium hydroxide solution (5 ml, 25 %) in ethanol (10 ml) was stirred at room temperature for 2 h, then left overnight in refrigerator. The solid that formed after neutralized with diluted hydrochloric acid (1:1) was collected by filtration, air dried and crystallized from absolute ethanol.

Ethyl 2-(3-((E)-3-(4-chlorophenyl) acryloyl)-1H-indol-1-yl) acetate (5a)

Yield: 80 %; MP: 178-180 °C; IR (KBr): 1705 (C=O), 1571 (C=C), 748 cm⁻¹ (Cl); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.20 (t, 3H, CH₃), 4.15 (q, 2H, CH₂), 5.84 (s, 2H, CH₂), 6.55 and 7.45 (2d, 2H, CH=CH), 7.07-8.00 (m, 8H, Ar-H), 8.43 ppm (s, 1H, indolyl H-2); MS (*m/z*): 367/369 [M⁺/M⁺+2]; Anal. C₂₁H₁₈ClN₃O₃ (367.83): Calcd: C, 68.57; H, 4.93; N, 3.81; Found: C, 68.42; H, 4.77; N, 3.65.

Ethyl 2-(3-((E)-3-(4-nitrophenyl) acryloyl)-1H-indol-1-yl) acetate (5b)

Yield: 78 %; MP: 260-262 °C; IR (KBr): 1715, 1688 (C=O), 1602 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.31 (t, 3H, CH₃), 4.21 (q, 2H, CH₂), 5.42 (s, 2H, CH₂), 7.26-8.04 (m, 8H, Ar-H), 8.50 and 8.67 (2d, 2H, CH=CH), 8.70 ppm (d, 1H, indolyl H-2); MS (*m/z*): 378 [M⁺]; Anal. C₂₁H₁₈N₂O₅ (378.38): Calcd: C, 66.66; H, 4.79; N, 7.40; Found: C, 66.51; H, 4.95; N, 7.24.

Synthesis of compounds 6a and 6b

A mixture of compound **5a** or **5b** (10 m mol) and hydrazine hydrate 99 % (0.5 ml, 10 m mol) in absolute ethanol (15 ml) and glacial acetic acid (0.5 ml) was refluxed for 2 h. The formed solid after cooling, was collected by filtration, air dried and crystallized from absolute ethanol.

Ethyl 2-(3-(5-(4-chlorophenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-1H-indol-1-yl) acetate (6a)

Yield: 35 %; MP: 232-234 °C; IR (KBr): 3322 (NH), 1678 (C=O), 1616 (C=N), 1577 (C=C), 748 cm⁻¹ (Cl); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 2.66 (dd, 1H, CH-pyrazolinyl), 4.25 (q, 2H, CH₂),

4.44 (dd, 1H, CH-pyrazolinyl), 5.21 (dd, 1H, CH-pyrazolinyl), 5.51 (s, 2H, CH₂), 7.21-8.21 (m, 8H, Ar-H), 8.24 (d, 1H, indolyl H-2), 8.97 ppm (s, 1H, NH); Anal. C₂₁H₂₀ClN₃O₂ (381.80): Calcd: C, 66.05; H, 5.28; N, 11.00; Found: C, 65.91; H, 5.02; N, 11.11.

Ethyl 2-(3-(5-(4-nitrophenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-1H-indol-1-yl) acetate (6b)

Yield: 30 %; MP: 188-190 °C; IR (KBr): 3300 (NH), 1645 (C=O), 1610 (C=N), 1578 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.24 (t, 3H, CH₃), 2.61 (dd, 1H, CH-pyrazolinyl), 4.15 (q, 2H, CH₂), 4.38 (dd, 1H, CH-pyrazolinyl), 5.12 (dd, 1H, CH-pyrazolinyl), 5.45 (s, 2H, CH₂), 7.17-8.25 (m, 8H, Ar-H), 8.52 (d, 1H, indolyl H-2), 9.75 ppm (s, 1H, NH); Anal. C₂₁H₂₀N₄O₄ (392.41): Calcd: C, 64.28; H, 5.14; N, 14.28; Found: C, 64.05; H, 5.01; N, 14.15.

Synthesis of compounds 7a and 7b

A mixture of compound **5a** or **5b** (10 m mol) and phenyl hydrazine (1.08 ml, 10 m mol) in absolute ethanol (15 ml) and glacial acetic acid (0.5 ml) was refluxed for 2 h. The formed solid after cooling, was collected by filtration, air dried and crystallized from absolute ethanol.

Ethyl 2-(3-(5-(4-chlorophenyl)-4, 5-dihydro-1-phenylpyrazol-3-yl)-1H-indol-1-yl) acetate (7a)

Yield: 40 %; MP: 147-149 °C; IR (KBr): 1678 (C=O), 1624 (C=N), 1578 (C=C), 748 cm⁻¹ (Cl); ¹H NMR (200 MHz, DMSO-*d*₆): δ 0.88 (t, 3H, CH₃), 1.19 (dd, 1H, CH of pyrazoline), 1.50 (dd, 1H, CH of pyrazoline), 3.29 (q, 2H, CH₂), 4.19 (dd, 1H, CH of pyrazoline), 5.29 (s, 2H, CH₂), 7.21-8.10 (m, 12H, Ar-H), 8.41 (d, 1H, indolyl H-4), 8.62 ppm (s, 1H, indolyl H-2); ¹³C NMR (60 MHz, DMSO-*d*₆): δ 25.9 (CH₃), 46.1, 61.4 and 62.4 (3CH₂), 62.9 (CH of pyrazoline), 110.3-139.9 (Ar-C), 169.1 ppm (C=O); Anal. C₂₇H₂₄ClN₃O₂ (457.95): Calcd: C, 70.81; H, 5.28; N, 9.18; Found: C, 70.67; H, 5.12; N, 9.04.

Ethyl 2-(3-(5-(4-nitrophenyl)-4, 5-dihydro-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (7b)

Yield: 32 %; MP: 87-89 °C; IR (KBr): 1678 (C=O), 1620 (C=N), 1577 cm⁻¹ (C=C); ¹H NMR (200 MHz, DMSO-*d*₆): δ 1.23 (t, 3H, CH₃), 2.22 (dd, 1H, CH of pyrazoline), 4.23 (m, 3H, CH of pyrazoline and CH₂), 5.19 (dd, 1H, CH of pyrazoline), 5.34 (s, 2H, CH₂), 6.77-8.42 (m, 12H, Ar-H), 8.61 (d, 1H, indolyl H-4), 9.12 ppm (s, 1H, indolyl H-2); Anal. C₂₇H₂₄N₄O₄ (468.50): Calcd: C, 69.22; H, 5.16; N, 11.96; Found: C, 69.12; H, 5.03; N, 12.01.

Synthesis of compounds 8a and 8b

A mixture of compound **5a** or **5b** (10 m mol) and urea (0.6 g, 10 m mol) in dry ethanol (10 ml) and glacial acetic acid (0.5 ml) was refluxed for 5 h. After cooling, the reaction mixture was poured onto ice-water (50 ml). The solid that formed was collected by filtration, air dried and crystallized from absolute ethanol.

Ethyl 2-(3-(6-(4-chlorophenyl)-2-oxo-1H-pyrimidin-4-yl)-1H-indol-1-yl)acetate (8a)

Yield: 56 %; MP: 102-104 °C; IR (KBr): 3320 (NH), 1670 (C=O), 1616 (C=N), 1554 (C=C), 750 cm⁻¹ (Cl); ¹H NMR (200 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 3.89 (s, 1H, NH), 4.29 (q, 2H, CH₂), 5.12 (s, 2H, CH₂), 5.23 (s, 1H, pyrimidinyl H-5), 7.16-8.43 (m, 8H, Ar-H), 8.69 ppm (s, 1H, indolyl H-2); Anal. C₂₂H₁₈ClN₃O₃ (407.85): Calcd: C, 64.79; H, 4.45; N, 10.30; Found: C, 64.61; H, 4.53; N, 10.18.

Ethyl 2-(3-(6-(4-nitrophenyl)-2-oxo-1H-pyrimidin-4-yl)-1H-indol-1-yl) acetate (8b)

Yield: 60 %; MP: 274-276 °C; IR (KBr): 3180 (NH), 1687 (C=O), 1645 (C=N), 1577 cm⁻¹ (C=C); ¹H NMR (200 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 4.23 (q, 2H, CH₂), 5.19 (s, 2H, CH₂), 5.30 (s, 1H, pyrimidinyl H-5), 7.19-8.42 (m, 9H, Ar-H and NH), 8.82 ppm (s, 1H, indolyl H-2); Anal. C₂₂H₁₈N₄O₅ (418.40): Calcd: C, 63.15; H, 4.34; N, 13.39; Found: C, 63.02; H, 4.23; N, 13.22.

Synthesis of compounds 9a and 9b

A mixture of compound **5a** or **5b** (10 m mol) and thiourea (0.76 g, 10 m mol) in dry ethanol (10 ml) and glacial acetic acid (0.5 ml) was refluxed for 5 h. After cooling, the reaction mixture was poured onto

ice-water (50 ml), and the solid that formed was collected by filtration, air dried and crystallized from absolute ethanol.

Ethyl 2-(3-(6-(4-chlorophenyl)-2-thioxo-1H-pyrimidin-4-yl)-1H-indol-1-yl)acetate (9a)

Yield: 61 %; MP: 170-172 °C; IR (KBr): 3228 (NH), 1670 (C=O), 1604 (C=N), 1567 (C=C), 1245 (C=S), 748 cm⁻¹ (Cl); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.42 (t, 3H, CH₃), 3.82 (q, 2H, CH₂), 4.45 (s, 1H, pyrimidinyl H-5), 5.29 (s, 2H, CH₂), 7.22-7.91 (m, 3H, Ar-H), 8.39 (d, 1H, indolyl H-4), 8.73 ppm (s, 1H, indolyl H-2); ¹³C NMR (125.7 MHz, DMSO-*d*₆): δ 10.8 (CH₃), 26.0 and 62.2 (CH₂), 62.9 (CH of pyrimidine), 111.2-130.4 (Ar-C), 139.1 (C=S), 170.1 ppm (C=O); Anal. C₂₂H₁₈ClN₃O₃S (423.92): Calcd: C, 62.33; H, 4.28; N, 9.91; Found: C, 62.17; H, 4.45; N, 10.02.

Ethyl 2-(3-(6-(4-nitrophenyl)-2-thioxo-1H-pyrimidin-4-yl)-1H-indol-1-yl)acetate (9b)

Yield: 60 %; MP: 209-211 °C; IR (KBr): 3200 (NH), 1645 (C=O), 1601 (C=N), 1577 cm⁻¹ (C=C); ¹H NMR (200 MHz, DMSO-*d*₆): δ 1.92 (t, 3H, CH₃), 4.32 (q, 2H, CH₂), 5.19 (s, 2H, CH₂), 5.32 (s, 1H, pyrimidinyl H-5), 7.29-8.40 (m, 9H, Ar-H and NH), 8.89 ppm (s, 1H, indolyl H-2); Anal. C₂₂H₁₈N₄O₅S (434.47): Calcd: C, 60.82; H, 4.18; N, 12.90; Found: C, 60.72; H, 4.04; N, 12.81.

Synthesis of compounds 10a and 10b

To a solution of compound **5a** or **5b** (10 m mol) in dry ethanol (15 ml), guanidine hydrochloride (0.96 g, 10 m mol) and anhydrous sodium acetate (0.82 g, 10 m mol) was added and the reaction mixture was refluxed for 3 h. The solid formed after cooling was collected by filtration, air dried and crystallized from absolute ethanol.

Ethyl 2-(3-(6-(4-chlorophenyl)-2-imino-1H-pyrimidin-4-yl)-1H-indol-1-yl)acetate (10a)

Yield: 60 %; MP: 104-106 °C; IR (KBr): 3200 (NH), 1667 (C=O), 1614 (C=N), 1591 (C=C), 751 cm⁻¹ (Cl); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 4.29 (q, 2H, CH₂), 5.21 (s, 2H, CH₂), 5.29 (s, 1H, pyrimidinyl H-5), 7.10-8.41 (m, 9H, Ar-H & NH), 8.39 (s, 1H, indolyl H-2), 9.99 ppm (s, 1H, NH); ¹³C NMR (125.7 MHz, DMSO-*d*₆): δ 14.0 (CH₃), 48.6 and 61.3 (CH₂), 110.8-158.3 (Ar-C), 183.1 (C=N), 192.6 ppm (C=O); Anal. C₂₂H₁₉ClN₄O₂ (406.89): Calcd: C, 64.94; H, 4.71; N, 13.77; Found: C, 64.78; H, 4.56; N, 13.58.

Ethyl 2-(3-(6-(4-nitrophenyl)-2-imino-1H-pyrimidin-4-yl)-1H-indol-1-yl)acetate (10b)

Yield: 65 %; MP: 243-245 °C; IR (KBr): 3220 (NH), 1675 (C=O), 1645 (C=N), 1577 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 4.22 (q, 2H, CH₂), 5.19 (s, 2H, CH₂), 5.30 (s, 1H, pyrimidinyl H-5), 7.1 (s, 1H, NH), 7.22-8.39 (m, 8H, Ar-H), 8.77 ppm (s, 1H, indolyl H-2); ¹³C NMR (125.7 MHz, DMSO-*d*₆): δ 31.1 (CH₃), 48.3 (CH₂), 111.3-148.1 (Ar-C), 170.1 (C=N), 183.1 ppm (C=O); Anal. C₂₂H₁₉N₅O₄ (417.42): Calcd: C, 63.30; H, 4.59; N, 16.78; Found: C, 63.18; H, 4.70; N, 16.82.

1-(1-(1-Ethylacetate-1H-indol-3-yl)ethylidene)-2-phenylhydrazine (11)

A mixture of compound **1** (2.45 g, 10 m mol) and phenyl hydrazine (1.08 ml, 10 m mol) in absolute ethanol (15 ml) and glacial acetic acid (0.5 ml) was refluxed for 3 h. After cooling, the reaction mixture was triturated with ice-water and the precipitate that formed was collected by filtration, air dried and crystallized from absolute ethanol. Yield: 60 %; MP: 82-84 °C; IR (KBr): 3218 (NH), 1645 (C=O), 1620 (C=N), 1577 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.23 (t, 3H, CH₃), 2.60 (s, 3H, CH₃), 4.21 (q, 2H, CH₂), 5.21 (s, 2H, CH₂), 6.51 (s, 1H, NH), 7.02-8.23 ppm (m, 10H, Ar-H); Anal. C₂₀H₂₁N₃O₂ (335.4): Calcd: C, 71.62; H, 6.31; N, 12.53; Found: C, 71.55; H, 6.22; N, 12.42.

Ethyl 2-(3-(4-formyl-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (12)

Vilsmeier-Haack reagent was prepared by an added phosphorus oxychloride (1.5 ml, 10 m mol) over a period of 0.5 h to a well stirred dimethylformamide (15 ml) at 0-5 °C. To this reagent, compound **11** (3.35 g, 10 m mol) in dimethylformamide (10 ml) was added

gradually at 0-5 °C while stirring. The reaction mixture was stirred for 15 h at room temperature, and then poured onto ice-water. The solid that formed was collected by filtration, air dried and crystallized from absolute ethanol. Yield: 55 %; MP: 140-142 °C; IR (KBr): 1701 and 1645 (C=O), 1601 (C=N), 1576 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 4.20 (q, 2H, CH₂), 5.32 (s, 2H, CH₂), 7.19-8.11 (m, 8H, Ar-H), 8.42 (d, 1H, indolyl H-4), 8.51 (s, 1H, indolyl H-2), 9.41 (s, 1H, pyrazolyl H-5), 10.21 ppm (s, 1H, CHO); ¹³C NMR (125.7 MHz, DMSO-*d*₆): δ 14.5 (CH₃), 47.7 and 61.5 (CH₂), 107.1-148.4 (Ar-C), 169.2 (C=O), 184.6 ppm (C=O of aldehyde); MS (*m/z*): 373 [M⁺]; Anal. C₂₂H₁₉N₃O₃ (373.4): Calcd: C, 70.76; H, 5.13; N, 11.25; Found: C, 70.65; H, 5.11; N, 11.11.

Ethyl 2-(3-(4-(2-cyanoacetylmino)methyl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (13)

A mixture of compound **12** (3.73 g, 10 m mol) and 2-cyanoacetic acid hydrazide (0.99 g, 10 m mol) in glacial acetic acid (20 ml) was stirred at room temperature for 2 h. The solid that formed was collected by filtration, air dried and crystallized from absolute ethanol. Yield: 77 %; MP: 216-218 °C; IR (KBr): 3218 (NH), 2190 (CN), 1665 (C=O), 1618 (C=N), 1578 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.32 (t, 3H, CH₃), 4.22 (q, 2H, CH₂), 5.10 (s, 2H, CH₂), 5.52 (s, 2H, CH₂), 7.01-8.02 (m, 9H, Ar-H), 8.21 (s, 1H, indolyl H-2), 8.75 (s, 1H, CH=N), 9.21 (s, 1H, pyrazolyl H-5), 10.11 ppm (s, 1H, NH); Anal. C₂₅H₂₂N₆O₃ (454.48): Calcd: C, 66.07; H, 4.88; N, 18.49; Found: C, 66.22; H, 5.01; N, 18.31.

Ethyl 2-(3-(4-(4-cyano-2, 3-dihydro-3-oxo-1H-pyrazol-5-yl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (14)

Compound **13** (0.93 g, 2 m mol) was heated under reflux in absolute ethanol (10 ml) containing triethylamine (0.5 ml) for 1 h. The reaction mixture was triturated with ice-water after cooling, and the precipitate that formed was collected by filtration, air dried and crystallized from absolute ethanol. Yield: 35 %; MP: 236 dec. °C; IR (KBr): 3118 and 3200 (NH), 2192 (CN), 1675 (C=O), 1623 (C=N), 1577 cm⁻¹ (C=C); ¹H NMR (200 MHz, DMSO-*d*₆): δ 1.41 (t, 3H, CH₃), 2.11 (s, 2H, 2NH), 4.61 (q, 2H, CH₂), 5.61 (s, 2H, CH₂), 7.10-8.41 (m, 10H, Ar-H), 8.99 ppm (s, 1H, pyrazolyl H-5); Anal. C₂₅H₂₀N₆O₃ (452.46): Calcd: C, 66.36; H, 4.46; N, 18.57; Found: C, 66.55; H, 4.32; N, 18.44.

Ethyl 2-(3-(4-(1-acetyl-4-cyano-2, 3-dihydro-3-oxo-1H-pyrazol-5-yl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (15)

A mixture of compound **12** (0.37 g, 1 m mol) and 2'-acetyl-2-cyanoacetohydrazide (0.141 g, 1 m mol) in absolute ethanol (15 ml) and triethylamine (1 ml) was refluxed for 3 h. The solid formed on hot was collected by filtration, washed with ethanol, air dried and crystallized from absolute ethanol. Yield: 45 %; MP: 199-201 °C; IR (KBr): 3220 (NH), 2220 (CN), 1645 (C=O), 1601 (C=N), 1577 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 1.81 (s, 3H, CH₃CO), 2.10 (s, 1H, NH), 3.89 (q, 2H, CH₂), 5.19 (s, 2H, CH₂), 7.01-8.40 (m, 9H, Ar-H), 8.83 (s, 1H, indolyl H-2), 8.99 ppm (s, 1H, pyrazolyl H-5); ¹³C NMR (125.7 MHz, DMSO-*d*₆): δ 20.9 and 21.5 (2 CH₃), 31.1 and 48.2 (2 CH₂), 117.6 (CN), 118.2-170.1 (Ar-C), 183.6 and 206.9 ppm (C=O); Anal. C₂₇H₂₂N₆O₄ (494.5): Calcd: C, 65.58; H, 4.48; N, 16.99; Found: C, 65.47; H, 4.31; N, 17.00.

Ethyl 2-(3-(4-(4, 5-dihydro-5-oxo-1H-pyrazol-3-ylimino)methyl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (16)

A mixture of **12** (0.37 g, 1 m mol) and 3-amino-5-pyrazolone (0.1 g, 1 m mol) in absolute ethanol (15 ml) and glacial acetic acid (0.3 ml) was refluxed for 3 h. The solid formed on hot was collected by filtration, washed with ethanol, air dried and crystallized from acetic acid. Yield: 56 %; MP: 196-198 °C; IR (KBr): 3200 (NH), 1677 (C=O), 1616 (C=N), 1577 cm⁻¹ (C=C); ¹H NMR (200 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 1.91 (s, 1H, NH), 2.19 (s, 2H, CH₂), 4.21 (q, 2H, CH₂), 4.91 (s, 2H, CH₂), 6.99 (s, 1H, pyrazolyl H-5), 7.11-8.31 (m, 9H, Ar-H), 8.72 (s, 1H, indolyl H-2), 10.30 ppm (s, 1H, CH=N); Anal. C₂₅H₂₂N₆O₃ (454.48): Calcd: C, 66.07; H, 4.88; N, 18.49; Found: C, 66.22; H, 4.75; N, 18.34.

Ethyl 2-(3-(4-(4-nitrophenylimino)methyl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (17)

A mixture of compound **12** (3.73 g, 10 m mol) and 4-nitroaniline (1.4 g, 10 m mol) in absolute ethanol (20 ml) and glacial acetic acid (0.3

ml) was refluxed for 4 h. The reaction mixture was poured onto water after cooling and the solid that formed was collected by filtration, air dried and crystallized from acetic acid-water. Yield: 60 %; MP: 116-118 °C; IR (KBr): 1678 (C=O), 1608 (C=N), 1577 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.31 (t, 3H, CH₃), 4.21 (q, 2H, CH₂), 5.21 (s, 2H, CH₂), 7.12-8.21 (m, 13H, Ar-H), 8.32 (s, 1H, indolyl H-2), 8.75 (s, 1H, CH=N), 9.12 ppm (s, 1H, pyrazolyl H-5); Anal. C₂₈H₂₃N₅O₄ (493.51): Calcd: C, 68.14; H, 4.70; N, 14.19; Found: C, 68.22; H, 4.79; N, 14.22.

Ethyl 2-(3-(4-(4-oxo-3-(4-nitrophenyl)-thiazolidin-2-yl)-1-phenyl-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate (18)

A solution of compound **17** (4.9 g, 10 m mol) and thioglycolic acid (0.92 g, 10 m mol) in dry 1, 4-dioxane (10 ml) was stirred for 4 h at room temperature, then anhydrous sodium sulphate (3 g) was added. The reaction mixture was refluxed for 6 h, and then filtered while hot. The solid that formed after cooling, was collected by filtration, air dried and crystallized from chloroform. Yield: 35 %; MP: 129-131 °C; IR (KBr): 1670 (C=O), 1614 (C=N), 1545 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 3.72 (s, 2H, CH₂ of thiazolidine), 4.20 (q, 2H, CH₂), 5.22 (s, 2H, CH₂), 5.92 (s, 1H, CH of thiazolidine), 7.10-7.67 (m, 11H, Ar-H), 8.09 (d, 1H, indolyl H-7), 8.41 (d, 1H, indolyl H-4), 8.60 (s, 1H, indolyl H-2), 9.43 ppm (s, 1H, pyrazolyl H-5); ¹³C NMR (125.7 MHz, DMSO-*d*₆): δ 14.5 (CH₃), 31.1 (OCH₂), 47.7 and 52.6 (2CH₂), 61.5 and 66.82 (CH), 107.1-169.7 (Ar-C), 184.6 and 206.9 ppm (2C=O); Anal. C₃₀H₂₅N₅O₅S (567.62): Calcd: C, 63.48; H, 4.44; N, 12.34; Found: C, 63.29; H, 4.22; N, 12.11.

Ethyl 2-(3-(4-(3-chloro-1-(4-nitrophenyl)-4-oxoazetidin-2-yl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (19)

A solution of chloroacetyl chloride (10 m mol) in dry 1, 4-dioxane (5 ml) and triethylamine (0.5 ml) was added to a solution of compound **17** (4.9 g, 10 m mol) in dry 1, 4-dioxane (10 ml) and the reaction mixture was refluxed for 13 h. After cooling, the reaction mixture was poured onto cold water (10 ml) and the solid that formed was collected by filtration, air dried and crystallized from absolute ethanol. Yield: 40 %; MP: 134-136 °C; IR (KBr): 1665 (C=O), 1620 (C=N), 1545 (C=C), 751 cm⁻¹ (Cl); ¹H NMR (600 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 3.60 and 3.26 (dd, 2H, 2CH azetidine), 4.20 (q, 2H, CH₂), 5.30 (s, 2H, CH₂), 7.22-7.69 (m, 11H, Ar-H), 8.10 (d, 1H, indolyl H-7), 8.49 (d, 1H, indolyl H-4), 8.60 (s, 1H, indolyl H-2), 9.40 ppm (s, 1H, pyrazolyl H-5); ¹³C NMR (150 MHz, DMSO-*d*₆): δ 14.00 (CH₃), 61.00 and 66.34 (CH₂) and CH), 106.7-147.9 (Ar-C), 168.8, 184.2 and 206.9 ppm (C=O); Anal. C₃₀H₂₄ClN₅O₅ (570.00): Calcd: C, 63.21; H, 4.24; N, 12.29; Found: C, 63.41; H, 4.11; N, 12.32.

Ethyl 2-(3-(4-(2, 2-dicyanovinyl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (20)

A solution of compound **12** (3.73 g, 10 m mol) and malononitrile (0.66 g, 10 m mol) in absolute ethanol (10 ml) containing piperidine (0.5 ml) was stirred for 30 min at room temperature. The precipitate that formed was collected by filtration, washed with absolute ethanol, air dried and crystallized from absolute ethanol. Yield: 65 %; MP: 219-221 °C; IR (KBr): 2190 (CN), 1645 (C=O), 1602 (C=N), 1576 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.31 (t, 3H, CH₃), 4.21 (q, 2H, CH₂), 5.21 (s, 2H, CH₂), 7.21-8.12 (m, 10H, Ar-H), 8.41 (s, 1H, CH=C), 8.42 ppm (s, 1H, indolyl H-2); Anal. C₂₅H₁₉N₅O₂ (421.45): Calcd: C, 71.25; H, 4.54; N, 16.62; Found: C, 71.11; H, 4.32; N, 16.54.

Ethyl 2-(3-(4-(1, 6-diamino-3, 5-dicyano-2-oxopyridin-4-yl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (21)

A mixture of compound **20** (0.84 g, 2 m mol) and 2-cyanoacetic acid hydrazide (0.198 g, 2 m mol) in absolute ethanol (20 ml) and piperidine (0.5 ml) was refluxed for 7 h. The solid that formed after cooling was collected by filtration, air dried and crystallized from absolute ethanol. Yield: 42 %; MP: 180-182 °C; IR (KBr): 3200, 3182 (NH₂), 2190 (CN), 1677 (C=O), 1607 (C=N), 1577 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 2.91 (s, 2H, NH₂), 4.21 (q, 2H, CH₂), 5.21 (s, 2H, CH₂), 7.01-8.21 (m, 9H, Ar-H), 8.21 (s, 1H, indolyl H-2), 8.99 (s, 1H, pyrazolyl H-5), 10.22 ppm (s, 2H, NH₂); Anal. C₂₈H₂₂N₈O₃ (518.53): Calcd: C, 64.86; H, 4.28; N, 21.61; Found: C, 64.75; H, 4.11; N, 21.52.

Ethyl 2-(3-(4-(2-methyl-5-oxo-6, 8-dicyano-1, 2, 3-triazolo(1, 5-a)pyridin-7-yl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (22)

A mixture of compound **20** (0.42 g, 1 m mol) and 2'-acetyl-2-cyanoacetohydrazide (0.141 g, 1 m mol) in dry ethanol (20 ml) was refluxed for 37 h. The solid that formed after cooling was collected by filtration, air dried and crystallized from absolute ethanol. Yield: 40 %; MP: 309 dec. °C; IR (KBr): 3300 (NH), 2209 (CN), 1675 (C=O), 1620 (C=N), 1598 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.21 (t, 3H, CH₃), 1.51 (s, 3H, CH₃), 4.21 (q, 2H, CH₂), 5.51 (s, 2H, CH₂), 6.66 (s, 2H, NH₂), 7.11-8.24 (m, 9H, Ar-H), 8.34 (s, 1H, indolyl H-2), 8.91 ppm (s, 1H, pyrazolyl H-5); Anal. C₃₀H₂₂N₈O₃ (542.55): Calcd: C, 66.41; H, 4.09; N, 20.65; Found: C, 66.31; H, 4.22; N, 20.45.

Ethyl 2-(3-(4-(3, 6-diamino-5-cyano-7H-pyrano(2, 3-c)pyrazol-4-yl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (23)

A mixture of compound **20** (0.42 g, 1 m mol) and 3-amino-5-pyrazolone (0.1 g, 1 m mol) in absolute ethanol (15 ml) and piperidine (0.5 ml) was refluxed for 3 h. The solid formed on hot was collected by filtration, washed with ethanol, air dried and crystallized from ethanol-water (10:1). Yield: 35 %; MP: 238-240 °C; IR (KBr): 3320, 3218 (NH₂), 2190 (CN), 1645 (C=O), 1611 (C=N), 1555 cm⁻¹ (C=C); ¹H NMR (500 MHz, DMSO-*d*₆): δ 1.29 (t, 3H, CH₃), 4.22 (q, 2H, CH₂), 5.51 (s, 2H, CH₂), 6.66 (s, 2H, NH₂), 7.00-8.02 (m, 9H, Ar-H), 8.12 (s, 1H, indolyl H-2), 8.91 (s, 1H, pyrazolyl H-5), 9.55 ppm (s, 2H, NH₂); Anal. C₂₈H₂₂N₈O₃ (518.53): Calcd: C, 64.86; H, 4.28; N, 21.61; Found: C, 64.75; H, 4.14; N, 21.54.

Antiviral evaluation

Virus

Herpes virus of turkeys (HVT) obtained from Hafez, Freie Universität, Berlin. The virus titre was 10^{6.5}TCID₅₀/ml (50% tissue culture infective dose).

Chicken embryo fibroblasts (CEF)

The cell lines were maintained and grown using 5-10 % newborn calf serum in Minimum Essential Medium (MEM) with Earl's salt base.

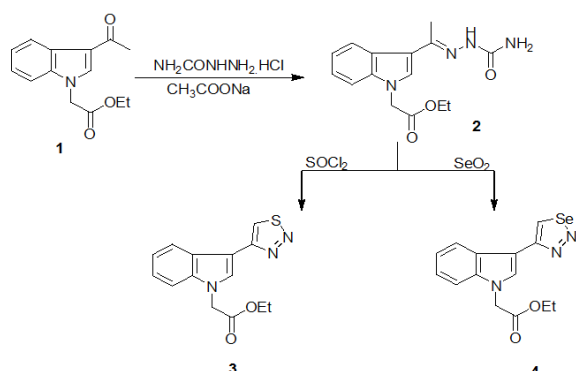
Evaluation of samples as inhibitory agents against MDV and their cytotoxicity

In vitro assay was performed in 24-well tissue culture plates following the procedure of Cox *et al.* [28]. Ten tissue culture plates of monolayers of CEF cell were divided as follows: five plates were infected with 5000 TCID₅₀/0.2 ml/well of virus and incubated for two days, while 2 wells were kept non-infected as control. After 2 d, the media was change and each sample at serial concentration of 5, 6, 7, 8, 9, 60, 70, 80, 500, 600, 700, 800, and 900 µg/ml was added separately, other three plates were infected with a mixture of 5000 TCID₅₀/0.2 ml/well of MDV and each sample (at the same serial concentration). The final three plates were inoculated with different concentrations of each sample. Two days later, the medium was changed. The procedure was repeated for the cell control, virus infectivity control and each sample, separately. The tested plates were incubated at 37 °C and 5 % CO₂ for seven days and examined for cytopathic changes. The procedure was carried out, separately for each sample. Cytotoxicity concentration fifty (CC₅₀) of each test compound was determined as the concentration of compounds that induced any deviation of the morphology than the normal control cells in 50 % of Vero cell monolayer's. Antiviral inhibitory concentration fifty (IC₅₀) of test compounds was assayed as the concentration of compounds that fully inhibited virus-cytopathic effect (100 TCID) in 50 % of monolayer's. Also, the therapeutic index (TI) of samples was expressed as CC₅₀/IC₅₀ [29].

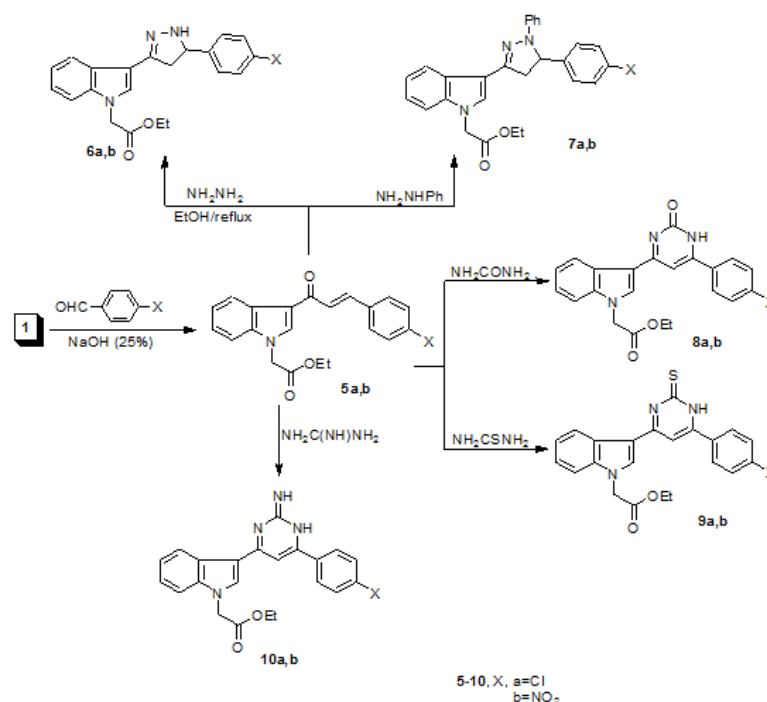
RESULTS AND DISCUSSION

The synthetic routes of the target compounds are outlined in Schemes 1, 2, 3 and 4. Condensation of ethyl 2-(3-acetyl-1H-indol-1-yl)acetate (1) with semicarbazide hydrochloride in the presence of sodium acetate under reflux in absolute ethanol yielded the corresponding semicarbazone derivative 2, Scheme 1. ¹H NMR spectrum of 2 revealed signals at δ 1.33 (t, CH₃), 2.23 (s, CH₃), 4.22 (q, CH₂), 5.26 (s, CH₂), 6.36 (s, NH exchangeable with D₂O), 7.12-

7.46 (m, 3H, Ar-H), 7.45 (s, indolyl H-2), 8.24 (d, indolyl H-4), 9.12 (s, NH₂ exchangeable with D₂O); Its ¹³C NMR revealed signals at δ 15.0 and 47.5 (2CH₃), 61.4 (CH₂), 110.4-144.2 (Ar-C), 157.7 and 169.2 (2C=O).



Scheme 1: Synthesis of 1, 2, 3-thiadiazole and 1, 2, 3-selenadiazole derivatives



Scheme 2: Synthesis of compounds 5a, b-10a, b

Acid catalyzed reaction of compound 1 with phenyl hydrazine afforded the corresponding hydrazone 11, which under Vilsmeier Haack formylation using 2.5 equivalent moles of Vilsmeier reagent (DMF/POCl₃) preformed double addition of reagent on methyl group to afford ultimately after hydrolysis, the cyclized pyrazole-4-carboxaldehyde derivative 12, Scheme 3. Compound 12 was confirmed based on its correct elemental analysis, as well as spectral data besides its chemical reaction with 2, 4-dinitrophenyl hydrazine, which gave the reddish product (hydrazone) with MP: 140-2 °C. The IR spectrum of compound 12 showed strong absorption bands at 1705 cm⁻¹ characteristic for the aldehydic group (C=O). Its ¹H NMR spectrum lacked the singlet signal of CH₃ protons of hydrazone 11 and revealed new singlet signal at 10.21 ppm for CHO proton besides characteristic singlet signal of pyrazolyl H-5 at 9.41 ppm. In addition, ¹³C NMR spectrum revealed new signal at 184.6 ppm due to the aldehydic group (C=O). Mass spectrum of 12 showed molecular ion peaks at *m/z*: 373 [M⁺].

Oxidative cyclization of compound 2 either with thionyl chloride or selenium dioxide led to the formation of ethyl 2-(3-(1, 2, 3-thiadiazol-4-yl)-1*H*-indol-1-yl)acetate (3) and ethyl 2-(3-(1, 2, 3-selenadiazol-4-yl)-1*H*-indol-1-yl)acetate (4), respectively, Scheme 1. ¹H NMR of compounds 3 and 4 lacked the presence of singlet signals at δ 2.23, 6.36 and 9.12 ppm of compound 2 and revealed new singlet signals at 8.44 and 5.58 ppm due to thiadiazolyl H-5 and selenadiazolyl H-5, respectively.

Reaction of 1 with 4-chloro and/or 4-nitrobenzaldehydes in ethanol and in the presence of aqueous potassium hydroxide 25 % at room temperature (Claisen-Schmidt reaction) furnished the corresponding α, β-unsaturated ketones 5a and 5b, respectively. Cyclocondensation the latter compounds either with hydrazine hydrate or phenyl hydrazine in the presence of glacial acetic acid as catalyst led to the formation of 4, 5-dihydro-1*H*-pyrazoles 6a, b and 4, 5-dihydro-1-phenylpyrazoles derivatives 7a, b, respectively, Scheme 2.

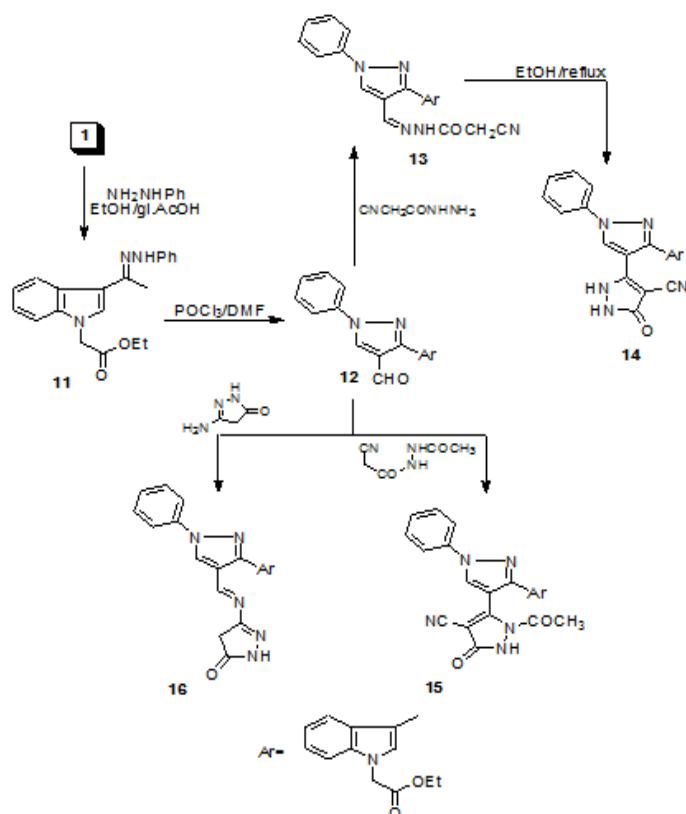
On the other hand, cyclocondensation of 5a and 5b either with urea or thiourea in the presence of glacial acetic acid as catalyst afforded 2-oxo-1*H*-pyrimidines 8a, b and 2-thioxo-1*H*-pyrimidines derivatives 9a, b, respectively. Also, cyclocondensation of 5a and 5b with guanidine hydrochloride in the presence of anhydrous sodium acetate yielded 2-imino-1*H*-pyrimidine derivatives 10a, b, Scheme 2.

Condensation of 12 with 2-cyanoacetic acid hydrazide in glacial acetic acid under stirring at room temperature led to the formation of hydrazone 13 which on heating in absolute ethanol containing triethylamine as a catalyst yielded 4-cyano-2, 3-dihydro-3-oxo-1*H*-pyrazole derivative 14. On the other hand, reaction of 12 with 2'-acetyl-2-cyanoaceto-hydrazide under reflux in absolute ethanol and in the presence of triethylamine as a catalyst afforded 1-acetyl-4-cyano-2, 3-dihydro-3-oxo-1*H*-pyrazole derivative 15, Scheme 3. Moreover, acid catalyzed reaction of 12 with 3-amino-5-pyrazolone under reflux in absolute ethanol gave ethyl 2-(3-(4-(4, 5-dihydro-5-oxo-1*H*-pyrazol-3-ylimino)methyl)-1-phenylpyrazol-3-yl)-1*H*-indol-1-yl)acetate (16), Scheme 3.

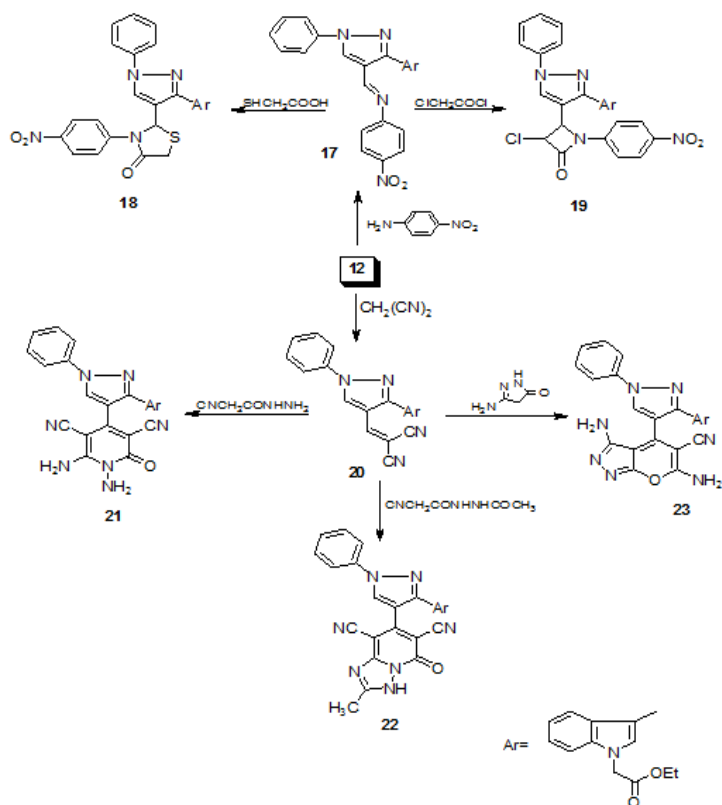
Acid catalyzed reaction of 12 with 4-nitroaniline yielded the corresponding Schiff base 17. Compound 17 reacted either with thioglycolic acid or chloroacetyl chloride in dry 1, 4-dioxane and gave 4-oxo-3-(4-nitrophenyl)thiazolidine 18 and 3-chloro-1-(4-

nitrophenyl)-4-oxo-azetidine derivatives 19, respectively, Scheme 4. On the other hand, base catalyzed reaction of 12 with malononitrile

afforded ethyl 2-(3-(4-(2, 2-dicyanovinyl)-1-phenylpyrazol-3-yl)-1H-indol-1-yl)acetate (20) Scheme 4.



Scheme 3: Synthesis of compounds 11-16



Scheme 4: Synthesis of compounds 17-23

Reaction of 20 with 2-cyanoacetic acid hydrazide in the presence of piperidine as catalyst yielded the cyclic 1, 6-diaminopyridine derivative 21, whereas, heating of 20 with 2'-acetyl-2-cyanoaceto-hydrazide under reflux in dry ethanol for 37 h led to the formation of fused system 1, 3, 4-triazolo(1, 5-*a*)pyridine derivative 22.

Finally, condensation of 20 with 3-amino-5-pyrazolone under reflux in absolute ethanol yielded the fused 3, 6-diamino-7*H*-pyrano (2, 3-*c*)pyrazole 23, Scheme 4.

In-vitro antiviral evaluation

The MDV infected CEF cell lines were used for evaluation of the *in vitro* antiviral effect of compounds 6a, b 7a, b 8a, b 9a, b and 10a, b. The results revealed that, compounds 7b, 8b, 9b as well as 10a showed effective antiviral activity with IC₅₀ range of 5-6 µg/ml and substantial therapeutic indices (TI) of 80 and 83 were recorded, table 1. Cytotoxicity assay indicated that CC₅₀ of 7b, 8b, 9b and 10 were greater than 400 and 500 mg/ml, table 1.

Table 1: CC₅₀ and IC₅₀ of tested compounds on CEF cell lines and their therapeutic indices (TI)

Compound No.	CC ₅₀	IC ₅₀	TI
6a	> 600	≤ 7	85
6b	> 600	≤ 7	85
7a	> 800	≤ 8	100
7b	> 500	≤ 6	83
8a	> 600	≤ 7	85
8b	> 400	≤ 5	80
9a	> 600	≤ 7	85
9b	> 500	≤ 6	83
10a	> 400	≤ 5	80
10b	> 600	≤ 7	85

CONCLUSION

A new series of 1, 2, 3-thiadiazole 3, 1, 2, 3-selenadiazole 4, pyrazoles 6a, b, 7a, b, pyrimidines 8a, b-10a, b, pyrazol-5-ones 14-16, thiazolidinone 18, aziditine 19, 1, 6-diaminopyridine 21, triazolo(1, 5-*a*)pyridine 22 and pyrano(2, 3-*c*)pyrazole derivatives 23, were prepared from ethyl 2-(3-acetyl-1*H*-indol-1-yl)acetate (1). The *in vitro* antiviral activity of compounds 6a, b 7a, b 8a, b 9a, b and 10a, b was studied against *Marek's disease virus (MDV)*. Compounds 7b, 8b, 9b and 10a showed promising effect as anti-MDV infectivity application.

ACKNOWLEDGMENT

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CONFLICT OF INTERESTS

Declare None

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