ISSN: 2357-0547 (Print)
ISSN: 2357-0539 (Online)

Research Article / JAPR / Sec. B
Abd El-Hameed et al., 2021, 5 (1), 202-210

Journal of Advanced Pharmacy Research

Section B: Pharmaceutical Analytical & Organic Chemistry, Medicinal & Biochemistry

Synthesis of Heterocyclic and Non-heterocyclic Compounds Derived from Novel 2-Furanones and Evaluation of their Anti-viral Activity

Rania Helmy Abd El-Hameed, Hend Medhat El-Shanbaky* , Mosaad Sayed Mohamed

Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt

***Corresponding author:** Hend Medhat El-Shanbaky, Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt. Tel. +20225541601
Email address: h1990shanbaky@yahoo.com*

Submitted on: 14-11-2020; Revised on: 08-12-2020; Accepted on: 09-12-2020

To cite this article: Synthesis of Heterocyclic and Non-heterocyclic Compounds Derived from Novel 2-Furanones and Evaluation of their Anti-viral Activity. *J. Adv. Pharm. Res.* **2021**, *5* (1), 202-210. DOI: 10.21608/aprh.2020.49788.1120

ABSTRACT

Objective: The aim of the present study is to synthesize novel 2-furanones due to their biological activities and also their ability to be converted to several biologically active heterocyclic and non-heterocyclic compounds. **Methods:** Two novel 2-furanone derivatives were synthesized and used to prepare fourteen heterocyclic and non-heterocyclic compounds. Structures of all the newly synthesized compounds were confirmed by elemental and spectral analysis including Mass, IR and ^{1}H-NMR spectroscopy. Evaluation of antiviral activity of selected examples of the obtained compounds was performed using two gastroenteric viruses: adenovirus and rotavirus**. Results:** Two pyridazinone derivatives namely; 3-(3,4-Dichlorophenyl)-5-[(2-methoxypheny)l-methyl]-1H-pyridazin-6-one (**7a**) and 3-(3,4-Dichlorophenyl)-5-[(4-flurophenyl)l-methyl]-1H-pyridazin-6-one (**7b**) showed high activities against rotavirus. **Conclusions:** Compounds **7a** and **7b** can be considered as promising anti-rotaviral agents needing further investigations and clinical studies.

Keywords: *Furanone; Hydrazide; Pyridazinone; Rotavirus; Adenovirus*

INTRODUCTION

Viruses are from the prime causes of diseases worldwide; viral gastroenteritis is a serious viral infection which affects millions of individuals around the world, most of them are children. The viral gastroenteritis constitutes around 21–40% of infectious diarrhea cases in developed countries [1,2]. The infection may occur due to different viruses, for example, coxsackievirus, adenovirus, and rotavirus. Rotavirus is the leading cause of severe gastroenteritis in the pediatric population worldwide [3] adenovirus is the second most common virus causing gastroenteritis in young children,[4] and coxsackievirus is considered the least dangerous cause of gastroenteritis among the three viruses [5]. In spite of the fact that in the recent years, various anti-viral agents have been produced, there is a need to create new and safe antiviral drugs.

2-Furanones; well-known heterocyclic derivatives; had attracted a great attention during the last decade due to facile ring opening and conversion to other heterocycles; pyrrolones, pyridazinones, pyrazoles and oxadizoles. These heterocycles acquired an obvious medicinal interest as antimicrobial [6-8], anti-inflammatory [8-16], antimycobacterial [18,19] and anti-cancer agents [20-22]

Literature is also enriched with furanone derivatives and other heterocyclic and non-heterocyclic compounds derived from them having antiviral activities [23-25], as shown in **Figure 1**; 2-furanone derivative **A** showed remarkable activity comparable to that of zanamivir [23], and furanone derivative **B** which is reported as potent anti-adenoviral agent. [26] Both hydrazide **C** and

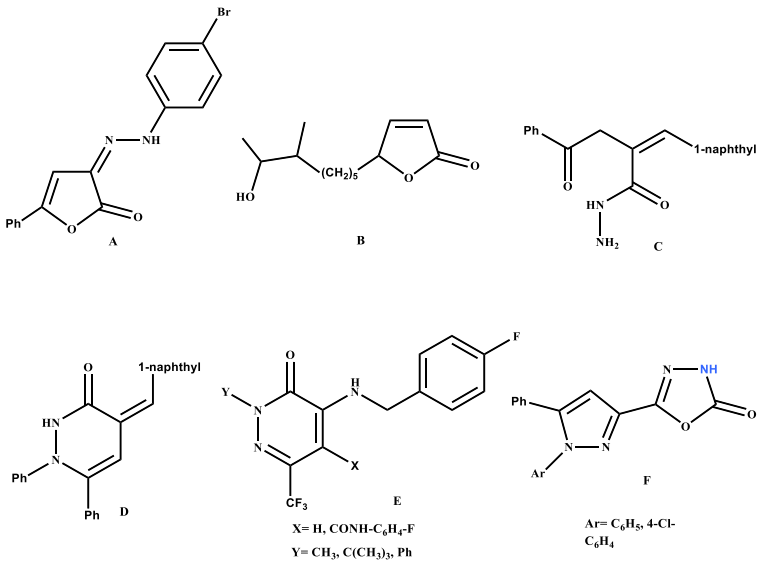


Figure 1. Reported potent antiviral derivatives.

pyridazinone **D** were reported to have promising antiviral activities against anti-avian influenza virus H5N1 virus. [27] Fluorinated pyridazinone **C** were reported as multi-functional antiviral agents comparable to Ribavirin. [28] Oxazizole derivatives **D** were reported as promising antiviral agents comparable to that of acyclovir. [15]

The previously mentioned antiviral activity stimulates our attention to continue our previous work [29] to synthesize new antiviral agent against gastroenteric viral infection.

MATERIAL AND METHODS

Synthesis of lead compounds

All commercial chemicals used as starting materials and reagents in this study were purchased from Merck (Darmstadt, Germany) and were of reagent grade. All melting points were 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 (USA), Faculty of Science, Cairo University, Cairo, Egypt. ^{1}H-NMR spectra were determined on a Varian Mercury (300 MHz) spectrometer (Varian UK) and chemical shifts were expressed as ppm against TMS as internal reference (The Main Chemical warfare Laboratories, Almaza, Cairo, Egypt). Mass spectra were recorded on 70 eV (EI Ms-QP 1000 EX, Shimadzu, Japan), Faculty of Science, Cairo University, Cairo, Egypt. Microanalyses were operated using Vario, Elementar apparatus (Shimadzu, Japan), Organic Microanalysis Unit, Faculty of Science, Cairo University, Cairo, Egypt. Column Chromatography was performed on (Merck) Silica gel 60 (particle size 0.06-0.20 mm). All the listed compounds are new except compound **1** was previously reported [30].

General procedure for the synthesis of compounds 2a,b:

A mixture of compound **1** (0.03 mol) and equimolar amount of aromatic aldehyde was refluxed in acetic anhydride (15 mL) with triethylamine (3- 4 drops) for 4 h. After completion of reaction, the product was filtered, washed with ethanol and recrystallized from ethanol to obtain compounds **2 a,b.**

5-(3,4-Dichlorophenyl)3-[(2-methoxyphenyl)methylene]-furan-2-one (2a)

Yield: 52%; m.p.: 218-220 °C; IR (KBr) υ (cm^{-1}): 1753 (C=O); MS (EI) m/z: 350 (M+4, 4.35%), 348 (M+2, 29.8%), 346 (M$^+$, 44.87%), 173 (benzoyl fragment, 100%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 7.0-8.1 (m, 9H, Ar-H+ CH methylene); Anal. Calcd. for $C_{18}H_{12}Cl_2O_3$ (347.19): C, 62.27; H, 3.48%. Found: C, 62.57; H, 3.15%.

5-(3,4-Dichlorophenyl)3-[(4-flurophenyl)methylene]-furan-2-one (2b)

Yield: 34%; m.p.: 240-242 °C; IR (KBr) υ (cm^{-1}): 1752 (C=O); MS (EI) m/z: 338 (M+4, 3.9%), 336 (M+2, 27.66%), 334 (M$^+$, 41.69%), 173 (benzoyl, 100%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 7.3-8.1 (m, 9H, Ar-H+ CH methylene); Anal. Calcd. for $C_{17}H_9Cl_2FO_2$ (335.13): C, 60.92; H, 2.71%. Found: C, 60.72; H, 2.64%.

General procedure for the synthesis of compounds 3a,b

A solution of the furanone derivatives **2a,b** (0.01 mol) and ammonium acetate (7.7g, 0.1 mol) in acetic acid (10 mL) was refluxed for 3h. The reaction mixture was left to cool at room temperature and the product obtained was filtered off, recrystallized from ethanol to give compounds **3a,b**

5-(3,4-Dichlorophenyl)-3-[(2-methoxyphenyl)methylene]-1H-pyrrol-2-one (3a)

Yield: 30%; m.p.: > 300 °C; IR (KBr) υ (cm^{-1}): 3314 (NH), 1769 (C=O); MS (EI) m/z: 349 (M+4, 11.9%), 347 (M+2, 67.2%), 345 (M^+, 100%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.9 (s, 3H, OCH_3), 6.9-8.2 (m, 9H, Ar-H+ CH methylene), 10.7 (s, 1H, NH, D_2O-exchangeable); Anal. Calcd. for $C_{18}H_{13}Cl_2NO_2$ (346.20): C, 62.45; H, 3.79; N, 4.05%. Found: C, 62.31; H, 3.37; N, 4.36%.

5-(3,4-Dichlorophenyl)-3-[(4-flurophenyl)methylene]-1H-pyrrol-2-one (3b)

Yield: 35%; m.p.: > 300 °C; IR (KBr) υ (cm^{-1}): 3698 (NH), 1698 (C=O); MS (EI) m/z: 337 (M+4, 12.8%), 335 (M+2, 64.18%), 333 (M^+, 100%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 7.0-8.18 (m, 10H, Ar-H+ CH methylene), 10.5 (s, 1H, NH, D_2O-exchangeable); Anal. Calcd. for $C_{17}H_{10}Cl_2FNO$ (334.17): C, 61.10; H, 3.02; N, 4.19%. Found: C, 61.42; H, 3.24; N, 4.54%.

General procedure for the synthesis of compounds 4a-c

To a solution of the furanone **2a,b** (0.01 mol) in absolute ethanol (20 mL), benzylamine (1.07 mL, 0.01 mol) was added and the reaction mixture was refluxed for 5 h. The product was filtered off, washed with ethanol and finally recrystallized from a suitable solvent to give the amides **4a,b.**

N-Benzyl-2-[(2-methoxyphenyl)methylene]-4-(3,4-dichlorophenyl)-4-oxo-butanamide (4a)

Yield: 45%; m.p.: 250-252 °C; IR (KBr) υ (cm^{-1}): 3275 (NH), 1771, 1742 (2C=O); MS (EI) m/z: 453 (M^+, 3.1%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.7 (s, 2H, CH_2-CO), 3.9 (s, 3H, OCH_3), 4.2 (dd, 2H, CH_2-NH), 7 (s, 1H, NH, D_2O-exchangeable), 6.9-7.6 (m, 13H, Ar-H + CH methylene); Anal. Calcd. for $C_{25}H_{21}Cl_2NO_3$ (454.32): C, 66.09; H, 4.66; N, 3.08%. Found: C, 66.25; H, 4.37; N, 3.42%.

N-Benzyl-2-[(4-flurophenyl)methylene]-4-(3,4-dichlorophenyl)-4-oxo-butanamide (4b)

Yield: 30%; m.p.: 174-176 °C; IR (KBr) υ (cm^{-1}): 3245 (NH), 1678, 1642 (2C=O); MS (EI) m/z: 441 (M^+, 2.32%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.3 (s, 2H, CH_2-CO), 4.2 (dd, 2H, CH_2-NH), 7.0-8.0 (m, 14H, Ar-H + CH methylene + NH, D_2O-exchangable); Anal. Calcd. for $C_{24}H_{18}Cl_2FNO_2$ (442.29): C, 65.17; H, 4.10; N, 3.17%. Found: C, 65.59; H, 4.32; N, 3.47%.

General procedure for the synthesis of compounds 5a-c

A solution of the furanone **2a,b** (0.01 mol) and phenyl hydrazine 3ml in Na ethoxide (10ml) was refluxed for 3h. The product obtained was filtered, washed with water and recrystallized from ethanol to give compounds **5a,b**.

6-(3,4-dichlorophenyl)-4-[(2-methoxyphenyl) methylene]-1-phenyl-1,4-dihydropyridazin-3(2H)-one (5a)

Yield: 80%; m.p.: >300°C; IR (KBr) υ (cm^{-1}): 3311 (NH), 1727 (C=O); MS (EI) m/z: 436 (M^+, 0.14%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 6.8-8.5 (m, 15H, Ar-H + CH methylene + NH-D_2O exchangeable); Anal. Calcd. for $C_{24}H_{18}Cl_2N_2O_2$ (437.30): C, 65.92; H, 4.15; N, 6.41%. Found: C, 65.42; H, 4.28; N, 6.34%.

6-(3,4-dichlorophenyl)-4-[(4-flurophenyl)methylene]-1-phenyl-1,4-dihydropyridazin-3(2H)-one (5b)

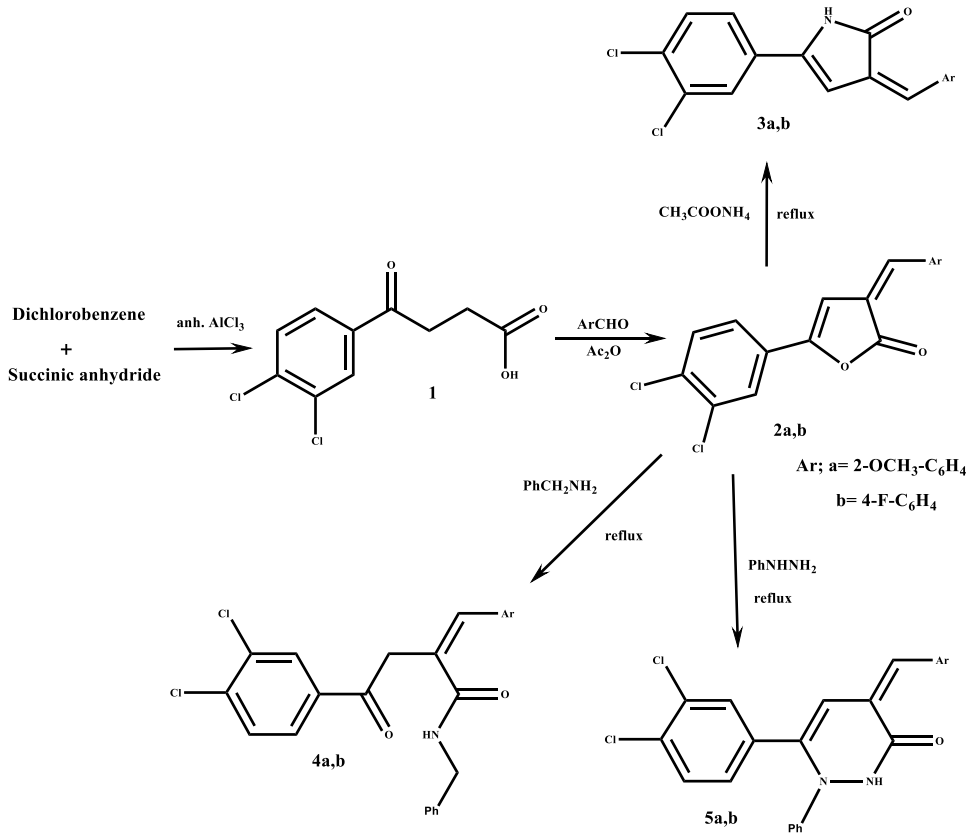
Yield: 69%; m.p.: >300°C; IR (KBr) υ (cm^{-1}): 3333 (NH), 1725 (C=O); MS (EI) m/z: 424 (M^+, 0.5%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 6.7-8.5 (m, 15H, Ar-H + CH methylene + NH-D_2O exchangeable); Anal. Calcd. for $C_{23}H_{15}Cl_2FN_2O$ (425.27): C, 64.96; H, 3.56; N, 6.59%. Found: C, 64.64; H, 3.21; N, 6.37%.

General procedure for the synthesis of compounds 6a,b

To a solution of the furanones **2a,b** (0.01 mol) in absolute ethanol (20 mL), hydrazine hydrate (3.5 mL, 0.11 mol) was added. The reaction mixture was left at room temperature with occasional shaking until complete dissolving and poured onto ice water. The product obtained **6a,b** was filtered off, washed with hexane.

4-(3,4-Dichlorophenyl)-2-[(2-methoxyphenyl) methylene]-4-oxo-butanehydrazide (6a)

Yield: 42%; m.p.: 138-140 °C; IR (KBr) υ (cm^{-1}): 3317 (NH), 3213 (NH_2), 1672,1650 (2 C=O); MS (EI) m/z: 380 (M+2, 1.9%), 378 (M^+, 3.24%), 362 (M+2 – H_2O, 54.51%), 360 (M – H_2O, 84.59%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.1 (s, 2H, CH_2), 3.8 (s, 3H, OCH_3), 4.4 (s, 2H, NH_2, D_2O-exchangeable), 6.7 (s, 1H, NHCO, D_2O-exchangeable), 6.9-7.6 (m, 8H, Ar-H + CH-methylene); Anal. Calcd. for $C_{18}H_{16}Cl_2N_2O_3$ (379.23): C, 57.01; H, 4.25; N, 7.39%. Found: C, 57.22; H, 4.15; N, 7.58%.



Scheme A. Synthesis of compounds 2a,b - 5a,b.

4-(3,4-Dichlorophenyl)-2-[(4-flurophenyl)methylene]-4-oxo-butanehydrazide (6b)

Yield: 70%; m.p.: 180-182 °C; IR (KBr) υ (cm^{-1}): 3279 (NH), 3250 (NH_2), 1692,1656 (2 C=O); MS (EI) m/z: 368 (M+2, 1.79%), 366 (M^+, 2.67%), 337 (M+2 – $NHNH_2$, 39%), 335 (M-$NHNH_2$, 62%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.2 (s, 2H, CH_2), 4.4 (s, 2H, NH_2, D_2O-exchangeable), 6.8 (s, 1H, NHCO, D_2O-exchangeable), 7.1-7.6 (m, 8H, Ar-H + CH-methylene); Anal. Calcd. for $C_{17}H_{13}Cl_2FN_2O_2$ (367.20): C, 55.61; H, 3.57; N, 7.63%. Found: C, 55.82; H, 3.21; N, 7.42%.

General procedure for the synthesis of compounds 7a,b

Method 1: A solution of hydrazides **6a,b** (0.01 mol) in HCl/AcOH (1:3) was refluxed for 3 h. The solid that separated after concentration and cooling was re-crystallized from ethanol to obtain compounds **7a,b.**

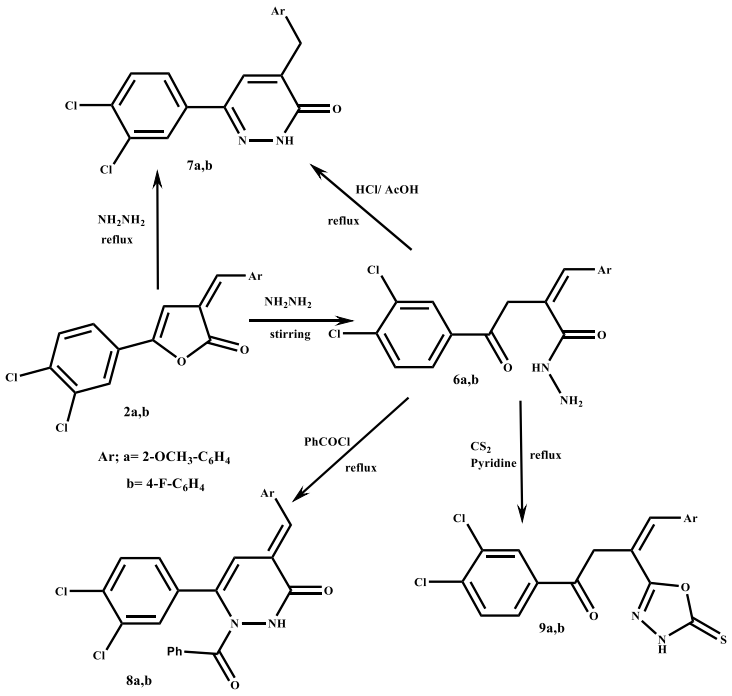
Method 2: To a solution of the furanones **2a,b** (0.01 mol) in absolute ethanol (20 mL), hydrazine hydrate (3.5 mL, 0.11 mol) was added. The reaction mixture was refluxed for 4 h, then cooled and poured onto ice water. The product obtained **7a,b** was filtered off, washed with hexane.

3-(3,4-Dichlorophenyl)-5-[(2-methoxypheny)l-methyl]-1H-pyridazin-6-one (7a)

Yield: (1^{st} method: 69%, 2^{nd} method: 65%); m.p.: 208-210 °C; IR (KBr) υ (cm^{-1}): 3218 (NH), 1649 (C=O); MS (EI) m/z: 364 (M+4, 7.8%), 362 (M+2, 45.67%), 360 (M^+, 70.36%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.71 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 6.8-7.9 (m, 8H, Ar-H), 13.2 (s, 1H, NH-pyridazinone, D_2O-exchangeable); Anal. Calcd. for $C_{18}H_{14}Cl_2N_2O_2$(361.22): C, 59.85; H, 3.91; N, 7.76%. Found: C, 59.74; H, 3.52; N, 7.42%.

3-(3,4-Dichlorophenyl)-5-[(4-fluropheny)l-methyl]-1H-pyridazin-6-one (7b)

Yield: (1^{st} method: 44%, 2^{nd} method: 40%); m.p.: 212-214 °C; IR (KBr) υ (cm^{-1}): 3187 (NH), 1647 (C=O); MS (EI) m/z: 352 (M+4, 12.2%), 350 (M+2, 65.76%), 348 (M^+, 100%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.8 (s, 2H, CH_2), 7.0-8.1 (m, 8H, Ar-H), 13.27 (s, 1H, NH-pyridazinone, D_2O-exchangeable); Anal. Calcd. for $C_{17}H_{11}Cl_2FN_2O$(349.18): C, 58.48; H, 3.18; N, 8.02%. Found: C, 58.56; H, 3.25; N, 8.42%.



Scheme B. Synthesis of compounds 6a,b - 9a,b.

General procedure for the synthesis of compounds 8a,b

To a solution of the hydrazides **6a,b** (0.01 mol) in dry benzene (20 mL), benzoyl chloride (1.4 mL, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h. The solvent was evaporated, and the solid obtained was washed thoroughly with ethanol, drained, and recrystallized from the suitable solvent to give compounds **8a,b.**

2-benzoyl-5-[(2-methoxyphenyl)methylene]-3-(3,4-dichlorophenyl)-1H-pyridazin-6-one (8a)

Yield: 35%; m.p.: 177-179 °C; IR (KBr) υ (cm^{-1}): 3217 (NH), 1790,1694 (2 C=O); MS (EI) m/z: 465 (M^+, 5%), 105 (benzoyl, 100%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 6.9-8 (m, 14H, Ar-H + CH methylene), 11.2 (s, 1H, NH, D_2O-exchangeable); Anal. Calcd. for $C_{25}H_{18}Cl_2N_2O_3$ (465.32): C, 64.25; H, 4.31; N, 5.99%. Found: C, 64.51; H, 4.16; N, 5.64%.

2-benzoyl-5-[(4-flurophenyl)methylene]-3-(3,4-dichlorophenyl)-1H-pyridazin-6-one (8b)

Yield: 36%; m.p.: 266-268 °C; IR (KBr) υ (cm^{-1}): 3186 (NH), 1710,1672 (2 C=O); MS (EI) m/z: 454 (M+2, 4.25%), 452 (M^+, 6.25%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 7-8.2(m, 14H, Ar-H+ CH methylene), 11.2(s, 1H, NH, D_2O-exchangeable); Anal. Calcd. for $C_{24}H_{15}Cl_2FN_2O_2$ (453.28): C, 63.59; H, 3.34; N, 6.18%. Found: C, 63.24; H, 3.17; N, 6.55%.

General procedure for the synthesis of compounds 9a,b

A solution of hydrazides **6a,b** (0.01 mol) and carbon disulphide (3ml) in pyridine (10ml) was refluxed for 3h. The reaction mixture was left to cool at room temperature and poured onto ice water; the product obtained was filtered, washed with water and recrystallized from ethanol to give compounds **9a,b**

1-(3,4-dichlorophenyl)-4-(2-methoxyphenyl)-3-(2-thioxo-4,5-dihydro-1,3,4-oxadiazol-5-yl)but-3-en-1-one (9a)

Yield: 45%; m.p.: 136-138 °C; IR (KBr) υ (cm^{-1}): 3203 (NH), 1651 (C=O), 1244 (C=S); MS (EI) m/z: 420 (M^+, 1.47%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.7 (s, 3H, OCH_3), 3.8 (s, 2H, CH_2), 6.8-8.7(m, 8H, Ar-H+ CH methylene), 13.2(s, 1H, NH, D_2O-exchangeable); Anal. Calcd. for $C_{19}H_{14}Cl_2N_2O_3S$ (421.23): C, 54.17; H, 3.35; N, 6.65%. Found: C, 54.25; H, 3.24; N, 6.17%.

1-(3,4-dichlorophenyl)-4-(4-flurophenyl)-3-(2-thioxo-4,5-dihydro-1,3,4-oxadiazol-5-yl)but-3-en-1-one (9b)

Yield: 50%; m.p.: 191-193 °C; IR (KBr) υ (cm^{-1}): 3284 (NH), 1771 (C=O), 1224 (C=S); MS (EI) m/z: 408 (M^+, 0.15%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.8 (s, 2H, CH_2), 7.09-8.09 (m, 8H, Ar-H+ CH methylene), 13.3(s, 1H, NH, D_2O-exchangeable); Anal. Calcd. for $C_{18}H_{11}Cl_2FN_2O_2S$ (409.19): C, 52.83; H, 2.71; N, 6.85%. Found: C, 52.54; H, 2.75; N, 6.34%.

Biological evaluation of anti-viral activity

Cytotoxicity assay

All samples (100 mg) were dissolved in 500 µL of ethanol or acetic acid. Cell monolayers Hep2 and MA104 (obtained from The Holding Company for Biological Products & Vaccines VACSERA, Egypt) were trypsinized, washed with culture medium and plated in a 96-well flat bottomed plate with 5 X 10^3 cells per well for both cell lines. After 24 h incubation, each diluted (Greiner-Bio-One, Germany) tested material (10 fold dilutions of decontaminated samples which 12 µL of 100x of antibiotic, antimycotic mixture was added to 500 µL of each sample) was added to the appropriate wells and the plates were incubated for further 48 h at 37^{O}C in a humidified incubator with 5% CO2. The supernatants were removed from the wells and cell viability was evaluated using microscopical examination, trypan blue and the MTT technique [31-33]. The results are obtained from triplicate assays with at least 5 extract concentrations. The percentage of cytotoxicity is calculated as [(A-B)/A] X 100, where A and B are the OD492 of untreated and of treated cells, respectively.

Antiviral test

Non-toxic dose of each tested compound was used in the *in vitro* antiviral screening method was used to estimate the inhibition of the cytopathic effect (CPE) of the pure compound on MA104 and HEP-2 cell monolayers infected with rotavirus Wa strain with initial titre 1 X 10^6 PFU/mL (ATCC VR2018) and adenovirus type 7 with initial titre 1 X 10^7 PFU/mL (obtained by Dr. Ali Fahmy, VACSERA, EGYPT) using the endpoint titration technique (EPTT). [34] Confluent monolayers of MA104 and HEP-2 cells were grown in 96-well microtiter plates, which were infected with serial tenfold dilutions of rotavirus Wa strain and adenovirus type 7 suspensions, respectively. The viruses were allowed to adsorb for 60 min at 37^{O}C. Then, serial twofold dilutions of the test compounds in maintenance medium, supplemented with 2% serum and antibiotic, were added. The plates were incubated at 37^{O}C, and the viral cytopathic effect was recorded by light microscopy after 2 to 8 days. Virus suspensions are characterized by their virus titres, which are expressed as the smallest amount of virus capable of producing a reaction in the host cells. The antiviral activity is expressed as a reduction factor (RF), being the ratio of the viral titres in the virus control and in the presence of the maximal non-toxic dose of test substance.

MTT assay (antiviral colorimetric assay):

Both MA104 and Hep2 cell monolayers were grown in 96-well microtiter plates. Dilutions of the extracts, prepared as described above for the EPTT assay, were added 1 h before viral infection. Ten infectious doses of virus were added to each well and incubated at 37^{O}C in humidified 5% CO2 atmosphere for 48 h. Controls consisted of untreated infected, treated uninfected and untreated uninfected cells. Cell viability was evaluated by the MTT colorimetric technique. [33] Briefly, the supernatants were removed from the wells and 28 µL of an MTT (Sigma) solution (2 mg/mL in PBS) was added to each well. The plates were incubated for 1.5 h at 37^{O}C, and 130 µL of DMSO was added to the wells to dissolve the MTT crystals. The plates were placed on a shaker for 15 min and the optical density was determined at 492 nm (OD492) on a multiwell spectrophotometer. The 50% cytotoxic concentration (CC_{50}) of the test extract is defined as the concentration that reduces the OD492 of treated uninfected cells to 50% of that of untreated uninfected cells. The 50% antiviral effective concentration, i.e., 50% inhibitory concentration of the viral effect (IC_{50}) is expressed as the concentration that reduces the absorbance of infected cells to 50% when compared to infected cells and control cells. The percent protection is calculated as [(A-B)/C-B)] X 100, where A, B and C are the OD492 of treated infected, untreated infected, and untreated uninfected cells, respectively.

Data analysis

CC_{50} and IC_{50} for each compound were obtained from dose-effect-curves. The CC_{50} and IC_{50} are the average of four assays with 5 concentrations within the inhibitory range of the compound. The therapeutic index (i.e., selective index) is defined as CC_{50}/IC_{50}.

RESULTS AND DISCUSSION

Chemistry

4-Oxo-butanoic acid derivative **1** was synthesized as reported before [29] and it is used to prepare 2-furanone derivatives **2a,b** by reaction with aromatic aldehydes in acetic anhydride following modified Perkin reaction conditions. [7, 11, 12, 18, 19, 24] as revealed in **scheme A**.

2-Furanone derivatives **2a,b** is useful as starting materials in the synthesis of several heterocyclic and non-heterocyclic derivatives. When they were reacted, separately, with ammonium acetate; pyrrol-2-one derivatives **3a,b** were produced. 4-Oxo-butanamides **4a,b** were synthesized by refluxing 2-furanones **2a,b** with benzyl amine and 1-phenyl-pyridazinone **5a,b** were also prepared from 2-furanones by refluxing with phenyl hydrazine according to the reported procedure [19,21]

Pyridazinone derivatives **7a,b** can be prepared by stirring of 2-furanone derivatives **2a,b** with hydrazine hydrate to obtain hydrazide derivatives **6a,b** which can be cyclized by refluxing in HCl/AcOH to obtain the desired pyridazinone derivatives **7a,b**. They can be

Table 1. CC50 values of the tested compounds on Hep-2 and MA-104 cell lines

Compounds	CC50 for Hep-2 cell line (mM)	CC50 for MA-104 cell line (mM)
2a	**166**	**80.22**
2b	**40.76**	**112**
6a	**2.66**	**138**
6b	**3.18**	**77.3**
7a	**48.8**	**44**
7b	**5.6**	**162**

prepared directly by refluxing 2-furanone derivatives **2a,b** with hydrazine hydrate.

Hydrazide derivatives **6a,b** were refluxed with benzoyl chloride to afford 2-benzoyl-pyridazinone derivatives **8a,b.**

Finally, oxadiazol-thione derivatives **9a,b** were prepared via heating under reflux hydrazide derivatives **6a,b** with carbon disulfide in pyridine [6, 7, 21, 24, 35] as revealed in **scheme B**.

Biological results

Cytotoxicity

Cytotoxicity testing of any chemotherapeutic agent (including antiviral compounds) is a very important step in its biological evaluation, as it should determine that neither acute nor long-term toxicity should occur to the host. Cytotoxicity of the selected compounds compounds was examined on two different cell lines: Hep-2, and MA-104. Table 1 shows the CC50 for all the tested compounds. The CC50 was determined using two different techniques: by counting the number of viable cells and by examining the effect on cell morphology.

Antiviral assay results

The non-toxic doses of the compounds were tested against adenovirus type 7 and rotavirus Wa strain. Considerable antiviral activity was observed with some of the tested compounds against adenovirus type 7 and rotavirus Wa strain with different degrees of activity and specificity. **Figure 2** showed the Rotavirus % reduction by the tested compounds, the viral reduction % for the most potent compounds **7b** and **7a** reached 84% and 79% respectively, while compounds **6a** and **6b** showed moderate activity with viral reduction 53% and 47% respectively.

Figure 3 showed the Adenovirus % reduction by the tested compounds, compounds **2b, 6b, 7a** and **7b** showed only lower activities against adenovirus with % viral reduction ranging from 25.5 to 38%.

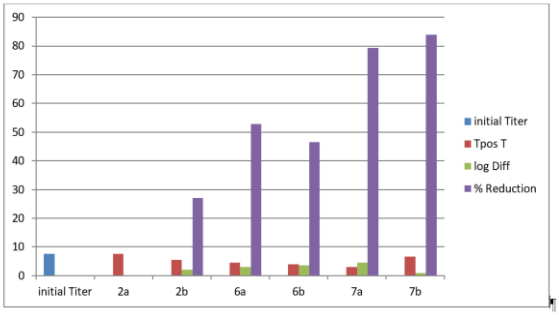


Figure 2. Rotavirus % reduction by the tested compounds 2a,b, 6a,b and 7a,b

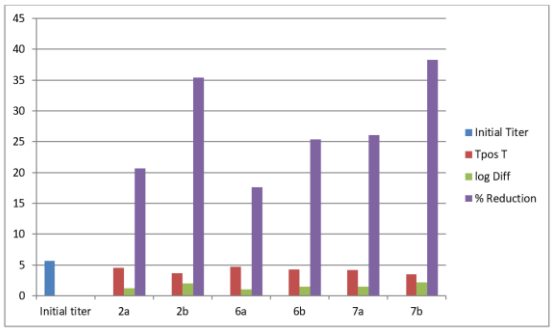


Figure 3. Adenovirus % reduction by the tested compounds 2a,b, 6a,b and 7a,b

To explain structureactivity relationship (SAR) of the tested compounds from the results; it was revealed that two newly synthesized furanone derivatives are inactive or with low activity against both rotavirus and adenovirus. Upon preparation of the hydrazides, they acquired moderate activity against rotavirus and low activity against against adenovirus.

Pyridazinone derivatives, in consistent with what was reported before, acquired high antiviral activity, herein with specificity against rotavirus over adenovirus. It is also noticed the superiority of the fluorinated derivative over the methoxy substituted derivative. **Figure 4**

CONCLUSION

In conclusion, as presented in this study, novel 2-furanone derivatives were synthesized and used them to prepare novel hydrazides, 2-pyrrolone, 2-pyridazinone and oxadiazole derivatives. 6 of the synthesized compounds were investigated for their anti-viral activity against gastroentericviruses rotavirus and adenovirus; the biological results revealed that pyridazinones **7a** and **7b** showed promising activity against rotavirus. Thus, these compounds might be promising anti-viral candidates and there is a need for more studies and structural optimization to improve their activity.

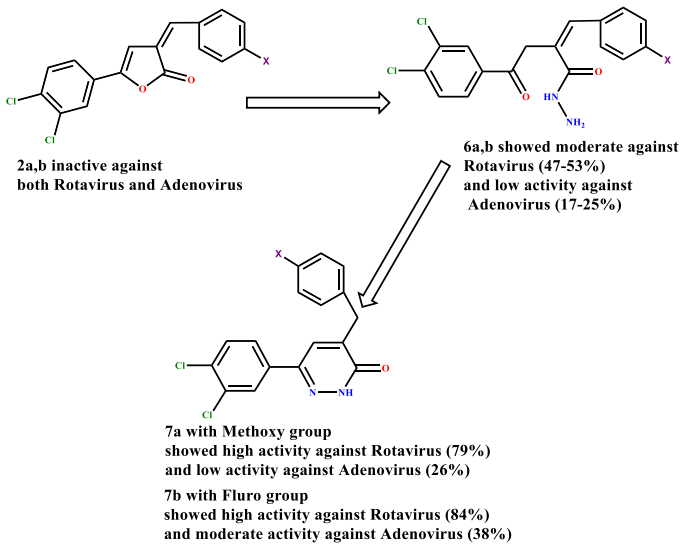


Figure 4. SAR of the tested compounds against both rotavirus and adenovirus

Acknowledgment

Authors are grateful to Science and Technology Development Fund (STDF) for financial support of this research.

Conflict of interest

Authors would like to declare that there are no relationships or interests that could have direct or potential influence or impart bias on the work.

REFERENCES

1. Eckardt, A.J.; Baumgart, D.C.; Viral gastroenteritis in adults. *Recent Pat. Antiinfect. Drug Discov.* **2011**, *6*, 54–63.
2. Khoury, H.; Ogilvie, I.; El Khoury, A.C.; Duan, Y.; Goetghebeur, M.M.; Burden of rotavirus gastroenteritis in the Middle Eastern and North African pediatric population. *BMC Infect. Dis.* 2011, *11*, 9–1.
3. Widdowson, M.; Steele, D.; Vojdani, J.; Wecker, J., & Parashar, U.; Global Rotavirus Surveillance: Determining the Need and Measuring the Impact of Rotavirus Vaccines. *The Journal of Infectious Diseases.* **2009**, *200*, 1-8
4. Khanna, R. Lakhanpaul; M. Burman-Roy, S.; *BMJ*. **2009**, *22*, 1350–1359.
5. Romero, J.R.; Pediatric group B coxsackievirus infections. *Curr. Top. Microbiol. Immunol*. **2008,** *323*, 223–239.
6. Abou-Elmagd, W. S. I.; El-Ziaty, A. K.; Abdalha, A. A.; Ring transformation and antimicrobial activity of indolyl-substituted 2(3H)-furanones. *Heterocycl. Commun*. **2015**, *21* (3), 179-185.
7. Ahmad, A.; Husain, A.; Khan, S. A.; Mujeeb, M.; Bhandari, A.; Design, synthesis, molecular properties and antimicrobial activities of some novel 2(3H) pyrrolone derivatives. *J. Saudi Chem. Soc*.. **2015,** *19*, 340–346.
8. Husain, A.; Khan, M.S.Y.; Hasan, S.M.; Alam, M.M.; Synthesis, reactions and biological activity of 2-Arylidene-4-(4-phenoxy-phenyl)but-3-en-4-olides . *Eur. J. Med. Chem.* **2005**, *40*, 1394–1404.
9. Husain, A.; Alam, M.M.; and Siddiqui, N.; Synthesis, reactions and biological activity of 3-arylidene-5-(4-methylphenyl)-2(3H)-furanones. *J. Serb. Chem. Soc.* **2009**, *74* (2), 103–115
10. Alam, M.M.; Husain, A.; Hasan, S.M.; Anwer, T.; Synthesis and pharmacological evaluation of 2(3H)-furanones and 2(3H)-pyrrolones, combining analgesic and anti-inflammatory properties with reduced gastrointestinal toxicity and lipid peroxidation. *Eur. J. Med. Chem.* **2009**, *44* (6), 2636–2642.
11. Khokra, S. L.; Chetan, J.; Kaushik , P.; Alam M.M.; Zaman, M.S.; Ahmad, A.; Alam S.; Husain, A.; Quinoline based furanones and their nitrogen analogues: Docking, synthesis and biologicalevaluation. *Saudi Pharm. J.* **2016**, *24*, 705–717.
12. Alam, M. M.; Sarkar, D. P.; Husain, A.; Marella, A.; Shaquiquazzaman, M.; Akhter, M.; Shaharyar, M.; Alam O.; and Azam, F.; Synthesis of quinoline-attached furan-2(3H)-ones having anti-inflammatory and antibacterial properties with reduced gastro-intestinal toxicity and lipid peroxidation. *J. Serb. Chem. Soc.* **2011**, *76* (12) 1617–1626.

13. Ahmed , E. M.; Kassab , A. E.; El-Malah, A. A.; Hassan, M.S.A.; Synthesis and biological evaluation of pyridazinone derivatives as selective COX-2 inhibitors and potential anti-inflammatory agents. *Eur J. Med. Chem.* **2019,** *1*(171), 25-37.
14. Takaya, M.; Sato, M.; Studies on pyridazinone derivatives. XVI. Analgesic-antiinflammatory activities of 3(2H)-pyridazinone derivatives. *J. Pharm. Soc. Japan* **1994** , *114* (2), 94-110.
15. Khan, M. S. Y.; Husain, A.; Syntheses and reactions of some new 2-arylidene-4-(biphenyl-4-yl)-but-3-en-4-olides with a study of their biological activity. *Pharmazie.* **2002**, *57*, 448-452.
16. Abouzid, K.; Bekhit, S. A.; Novel anti-inflammatory agents based on pyridazinone scaffold; design, synthesis and in vivo activity. *Bioorg. Med. Chem.* **2008**, *16*, 5547–555.
17. Khan, M. S. Y.; Husain, A.; Sharma, S.; Studies on butenolides: 2-Arylidene-4-(substituted aryl)but-3-ene-4-olides- synthesis, reactions and biological activity. *Indian J. Chem.* **2002**, *41*, 2160-2171
18. Husain, A.; Ahmad, A.; Bhandari, A.; Ram, V.; synthesis and antitubercular activity of pyridazinone derivatives. *J. Chil. Chem. Soc.* **2011**, 56 (3), 778-780.
19. Husain, A.; Alam, M. M.; Hasan S. M.; Yar, M. S.; 2(3H)-furanones and 2(3H)-pyrrolones: synthesis and antimycobacterial evaluation. *Acta Poloniae Pharmaceutica. Drug Res.*. **2009**, *66* (2) 173-180.
20. Abou-Elmagd, W. S. I.; Hashem, A. I.; Synthesis and antitumor activity evaluation of some novel fused and spiro heterocycles derived from a 2(3H)-furanone derivative. *J. Heterocycl. Chem*. **2016**, *53*, 202-208 .
21. Abou-Elmagd, W.S.I.; EL-Ziaty, A.K.; El-Zahar, M.I.; Ramadan, S.K.; Hashem, A.I.; Synthesis and Antitumor Activity Evaluation of Some N-Heterocycles Derived From Pyrazolyl-Substituted 2(3H)-furanone. *Synthetic Communi.* **2016**, *46* (14), 1197-1208.
22. Wu, Y. Cao, L.; Mei, W.; Wu, H.; Luo, S.; Zhan, H.; Wang, Z.; Bis-2(5H)-furanone derivatives as new anticancer agents: Design, synthesis, biological evaluation, and mechanism studies. *Chem. Biol. Drug Des.* **2018**, *92* , 1232–1240.
23. Flefel, E. M.; Abdel-Mageid, R. E.; Tantawy, W.A.; Ali M.A.; Amr, A. E.; Heterocyclic compounds based on 3-(4-bromophenyl)azo-5-phenyl-2(3H)-furanone: Anti-avian influenza virus (H5N1) activity. *Acta Pharm.* **2012,** *62*, 593–606.
24. Hashem, A. I.; Youssef, A. S.A.; Kandeel, K. A.; Abou-Elmagd, W. S.I.; Conversion of some 2(3H)-furanones bearing a pyrazolyl group into other heterocyclic systems with a study of their antiviral activity. *Eur. J. Med. Chem.* **2007,** *42*, 934-939.
25. Sayed, H. H.; Hashem, A. I.; Yousif1, N. M.; El-Sayed, W. A.; Conversion of 3-Arylazo-5-phenyl-2(3H)-furanones into Other Heterocycles of Anticipated Biological Activity. *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 315 – 319.
26. Strand, M.; Carlsson, M.; Uvell, H.; Islam, K.; Edlund, K.; Cullman, I. ; Altermark, B.; Mei, Y.; Elofsson, M.; Willassen, N.; Wadell, G.; Almqvist, F.; Isolation and Characterization of Anti-Adenoviral Secondary Metabolites from Marine Actinobacteria. *Mar. Drugs.* **2014**, *12*, 799-821
27. Flefel, E. M.; Tantawy, W. A.; Abdel-Mageid, R. E.; Amr, A. E.; Nadeem, R.; Synthesis and anti-viral activities of some 3-(naphthalen-1-ylmethylene)-5-phenylfuran-2(3H)-one candidates. *Res. Chem. Intermed.* **2014**, *40*, 1365–1381.
28. Wang, Z.; Wang, M.; Yao, X.; Li, Y.; Tan, J.; Wang, L.; Qiao, W.; Geng, Y.; Liu, Y.; Wang, Q.; design, synthesis and anti-viral activity of novel pyridazines. *Eur. J. med. Chem.* **2012**, *54*, 33-41.
29. Mohamed, M.S.; Abd El-Hameed, R. H.; Sayed, A. I.; Soror, S. H.; Novel Antiviral Compounds against Gastroenteric Viral Infections. *Arch. Pharm. Chem. Life Sci.* **2015**, *348*, 1–12.
30. Soliman A.Y.; Attia I.A.; Salem M.A.; Soliman E.A.; Gaber A.M.; synthesis and reactions of 2-[α-(3,4-disubstitutedphenacyl)-*p*-substitutedstyryl]-4H-3,1-benzoxazin-4-ones. *Chinese J. Chem.* **1996**, *14*(5), 437-446.
31. Simoes C.; Amoros M.; Girre L.; Mechanism of antiviral activity of triterpenoid saponins. *Phytother. Res.***1999**, *13* (4), 323-328 .
32. Walum E.; Strenberg K.; Jenssen D.; *Understanding Cell Toxicology: Principles and Pratice.*, Ellis Howood, NewYork **1990**.
33. Mosmann T.; Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods.* **1983**, *65* (1), 55-63
34. Vlietinck A.J.; Van Hoof L.; TottÈ J.; Lasure A.; Berghe D.V.; Rwangabo P.C.; Mvukiyumwami J.; Further evaluation of Rwandan medicinal plant extracts for their antimicrobial and antiviral activities. *J. Ethnopharmacol*. **2002**, *79*, 155-163.
35. Hashem, A. I.; Abou-Elmagd W. S. I.; Abd-Elaziz, A.; Synthesis and reactions of some 2(3H)- and 2(5H)-furanone derivatives: a comparative study. *Eur. Chem. Bull.* **2014**, *3* (11), 1064-1068.