Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

Pharmacophore
an International Journal
ISSN 2229-5402

SYNTHESIS AND BIOLOGICAL SCREENING OF NOVEL PYRAZOLES AND THEIR PRECURSORS AS POTENTIAL ANTIVIRAL AGENTS

Mosaad S. Mohamed[1], Yasser M. Zohny[1*], Waleed. M. El-Senousy[2], Asmaa M. Abou El-Elaa[1]

1. ***Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Helwan University, Ain-Helwan, Helwan, Cairo 11795, Egypt.***
2. ***National Research Center, (NRC), Dokki, Cairo, Egypt. Virology Lab., Water Pollution Research Department, Cairo, Egypt.***

ARTICLE INFO

Received:
29th Oct 2017
Received in revised form:
27th Jan 2018
Accepted:
02th Feb2018
Available online:
28th Feb 2018

Keywords: *Hydrazone, Hydrazonopyrazole, Pyrazole, Arylazopyrazole, Anti-HCV.*

ABSTRACT

Some novel substituted pyrazoles 4_{a-t} were synthesized from hydrazone synthons 3_{a-x} after reaction with hydrazine hydrate. The hydrazones 3_{a-x} were afforded through coupling of their corresponding diazonium salts with ethyl cyanoacetate, malononitrile, acetyl acetone and ethyl acetoacetate, respectively. The antiviral activity of such compounds was evaluated against a wide set of viruses; adenovirus type 7, rotavirus, HSV-1 and HCV viruses in different cell lines. Amongst the tested compounds, 3_b, 3_h, 3_p, 3_w, 4_g, 4_q and 4_r showed significant antiviral activity. Compounds 4_g and 4_r particularly exhibited the highest activity of all the tested title compounds against all the study viruses. On the other hand, compounds 3b, 3h, 3p, 4g, 4_q and 4_r showed significant anti-HSV-1 activity. Hydrazones 3_b and 3_h showed moderate anti-HCV activity. Pyrazoles 4_q and 4_r showed moderate activity against adenovirus type-7. On the contrary, the title pyrazoles 4_{a-t} showed no significant anti-HCV activity and the title hydrazones 3_{a-x} had no significant antiviral activity against adenovirus type-7.

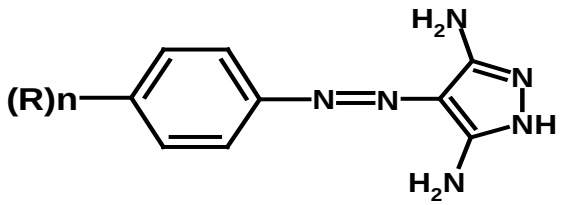
To Cite This Article: Mosaad S. Mohamed, Yasser M. Zohny, Waleed. M. El-Senousy, Asmaa M. Abou El-Elaa, (2018), "Synthesis and Biological Screening of Novel Pyrazoles and their Precursors as Potential Antiviral Agents", ***Pharmacophore, 9(1),*** *126-139.*

Introduction

Viral infections are considered as one of the principal threats to human life and health worldwide. Treatment of such viral infections still represents a rich field of scientific research because of the viruses' mutability that gives new drug-resistant strains. According to a study carried out in 2013, Egypt has the highest prevalence of hepatitis C virus (HCV) in the world, estimated nationally at 14.7% [1]. This unparalleled level of exposure to this infection appears to reflect a national level epidemic. Thus, HCV infection and its complications are among the leading public health challenges in Egypt as they impair the quality of life and may even be fatal to the immunocompromised and elder population. On the other hand, Gastroenteric viruses like rotavirus and adenovirus still cause outbreaks in Egypt as well, despite the fact that these viruses are controlled in many countries [2].

The presented work is a continuation of our efforts [3] to provide new Gastroenteric antiviral agents and new anti-HCV active compounds. It aims to synthesize arylazopyrazoles from hydrazone synthons 3_{a-x} (Scheme 2) which are structurally related to the compounds previously prepared by [4] (Diagram 1).

Corresponding Author: Yasser M. Zohny, Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Helwan University, Ain-Helwan, Helwan, Cairo 11795, Egypt. Email: dryasserzohny @ hotmail.com



R= 2-OH, 4-OH , 2-NO_2

Diagram 1. Arylazopyrazoles previously prepared by Petr Cankar et al. (2013)

Hydrazone synthons which were used to obtain arylazopyrazoles are considered as a class of biologically active compounds with wide therapeutic activities. They act as antimicrobial, anti-inflammatory, and anticancer agents [5-7]. Recently, Arylhydrazones, in particular, have drawn attention as antiviral leads that showed promising antiviral activities against yellow fever virus (YFV), herpes simplex virus (HSV-1) and HIV-1 [8-11]. On the other hand, the target compounds; 4-arylazopyrazoles have the pyrazole nucleus which show diverse biological activities such as antimicrobial, anti-inflammatory, analgesic, anticancer, and antiviral activities [6, 12-15]. More specifically, 4-arylazo-2,5-diamino-pyrazole derivatives were proven to be useful for the inhibition of cyclin-dependent kinases (CDKs), and thus have applications as antimitotic and apoptotic drugs, particularly anticancer and/or antiviral drugs. Such compounds exert their antiviral activity through inhibition of cell proliferation via inhibition of cellular CDK activity to extent impending viral replication.
4-Arylazo-3,5-diamino-pyrazole derivatives which were reported by [4] had high potency against human cytomegalovirus (HCMV), herpes simplex virus type 1 (HSV-l), human immunodeficiency virus type 1 (HIV-1), and varicella Zoster virus (VZV) [4]. The 4-arylazo-3,5-diamino-pyrazole derivatives showed potency against DNA viruses as they had the ability to inhibit CDK2, CDK7, CDK8 or CDK9 through interacting with the enzyme's ATP binding site [4]. The aforementioned patent research by [4] showed that the 4-substitutedphenylazo-3,5-diaminopyrazoles with small polar groups such as nitro and hydroxy substituted phenyl derivatives showed the highest activity. As an example, the 4-hydoxyphenyl derivative showed IC_{50} of 3.6 and 2.5 against CDK1 and CDK2 respectively. On the other hand, 2-hydroxyphenyl derivative showed IC_{50} of 2.1 and 3.5 against CDK1 and CDK2 respectively, but for the nitro derivatives, the ortho substitution showed higher activity than meta and para substitution. The o-nitrophenyl derivative showed IC_{50} of 2.6 and 6.1 against CDK1 and CDK2, respectively.
In the current work, we aimed to synthesize arylazopyrazoles bearing different substituents on the aryl ring, and have different groups on the C3 and C5 positions on the pyrazole ring. The target compounds and their arylhydrazones' precursors were tested against gastroenteric viruses (adenovirus type 7, rotavirus wa strain, HSV-1 virus) and HCV 4a genome

Materials and Methods

Chemistry

All starting materials and reagents were purchased from SigmaAldrich, BDH, Loba and Fluka used without further purification. All melting points were uncorrected and measured using Electrothermal IA 9100 apparatus (Shimadzu, Japan). The progress of the reactions was monitored by TLC using TLC plates precoated with UV fluorescent silica gel Merck 60 F 254 nm that was visualized using UV lamp. The solvent system used in TLC was chloroform: ethanol [4.5: 0.5] IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA), Faculty of Science, Cairo University, 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, Elmentar apparatus (Shimadzu, Japan), and Organic Microanalysis Unit, Faculty of Science, Cairo University, Cairo, Egypt.

General procedure for synthesis of hydrazones 3_{a-x}:

Primary aromatic and heterocyclic amines (0.01 mol) were dissolved in a mixture of concentrated HCl (4 ml) and water (2 ml) stirred, and then cooled to 0°C in an ice bath. A cold aqueous solution of sodium nitrite (0.01 mol) was then added. The obtained diazonium salt solution was filtered onto a cooled mixture of sodium acetate (5g) and active methylene compound 2_{a-d} (0.01 mol) in ethanol 95% (10 ml). The reaction mixture was stirred for 20 min. The resulting solid was collected by filtration, washed with water (2x 5 ml) and recrystallized from ethanol [16, 17].

Ethyl-2-cyano-2-[(p-methoxyphenyl) hydrazono] acetate 3a; Yellow crystals, yield 73%, m.p. 114-115 °C [18].

Ethyl-2-cyano-2-[(2-chlorophenyl) hydrazono] acetate 3b; Yellow crystals, yield 70%, m.p. 145°C [19].

Ethyl-2-cyano-2-[(3-chlorophenyl) hydrazono] acetate 3c; Yellow crystals, yield 73.5%, m.p. 93°C [20].

Ethyl-2-cyano-2-[(4-bromophenyl) hydrazono] acetate 3d; Yellow crystals; yield 75%, m.p. 153°C [21].

Ethyl-2-cyano-2-[(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl) hydrazono] acetate 3e; Orange crystals, yield 87.4 %, m.p. 164°C [22].

2-[(p-methoxyphenyl) hydrazono] malononitrile 3f; Orange crystals, yield 73%, m.p. 144°C [21, 22].

2-[(2-chlorophenyl) hydrazono] malononitrile 3g; Yellow crystals, yield 95%, m. p101°C [23].

2-[(3-chlorophenyl) hydrazono] malononitrile 3h; Yellow crystals, yield 97%, m. p165.4-166 [24].

2-[(4-bromophenyl) hydrazono] malononitrile 3i; Yellow crystals, yield 93.3%, m.p. 176°C [25].

2-[4-(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl) hydrazono]-malononitrile 3j; Orange crystals, yield 90%, m.p. 140°C [26].
3-[(p-methoxyphenyl) hydrazono] pentane-2,4-dione 3k; Yellow crystals, yield 73%, m.p. 110°C [27].
3-[(2-chlorophenyl) hydrazono] pentane-2,4-dione 3l; Yellow crystals, yield 70%, m.p. 124°C [28].
3-[(3-chlorophenyl) hydrazono] pentane-2,4-dione 3m; Yellow crystals, yield 73.5%, m.p. 95°C [29].
3-[(4-chlorophenyl) hydrazono] pentane-2,4-dione 3n; Yellow crystals, yield 76%, m.p 127 °C [30, 31].
3-[(4-bromophenyl) hydrazono] pentane-2,4-dione 3o; Yellow crystals, yield 75%, m.p. 145°C [32].
3-[4-(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl) hydrazono]-pentane-2,4-dione 3p; Orange crystals, yield 87.4%, m.p. 179°C [33].
3-[(α-napthyl) hydrazono] pentane-2,4-dione 3q; Yellow crystals, yield 80 %, m.p. 114 °C [30].
Ethyl-2-[(p-methoxyphenyl) hydrazono]-3-oxobutanoate 3r; Red crystals, yield 77%, m.p. 120°C [34].
Ethyl-2-[(2-chlorophenyl) hydrazono]-3-oxobutanoate 3s; Yellow crystals, yield 77%, m.p. 81°C [35].
Ethyl-2-[(3-chlorophenyl) hydrazono]-3-oxobutanoate 3t; Orange crystals, yield 73%, m.p 86-88°C [36].
Ethyl-2-[(4-chlorophenyl) hydrazono]-3-oxobutanoate 3u; Yellow crystals, yield 80 %, m.p 93 °C [37].
Ethyl-2-[(4-bromophenyl) hydrazono]-3-oxobutanoate 3v; Yellow crystals, yield 65%, m.p 70°C [28].
Ethyl-2-[(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl) hydrazono]-3-oxobutanoate 3w; Yellow crystals, yield 71%, m.p 180°C [38].
Ethyl-2-[α-(naphthyl) hydrazono]-3-oxobutanoate 3x: Yellow crystals, yield 58 %, m.p 57 °C [39].

General procedure for synthesis of azopyrazoles 4_{a-t}:

Solution of hydrazones 3 (0.01 mol) and hydrazine hydrate (0.02 mol) in 40 ml (toluene for compounds 3_{a-j} or ethanol for compounds 3_{k-t}) was heated under reflux with stirring for 4-5 h. For compounds 3_{a-j}, the formed solid in toluene was collected by filtration, boiled with hexane and then filtered off while it was hot. For compounds 3_{k-t}, ethanol was evaporated to dryness then the formedsolid mass was boiled with hexane and filtered off while it was hot without further purification [16, 17].

3-Amino-4-[(4-methoxyphenyl) diazenyl]-1,2-dihydropyrazol-5-one 4a; Dark brown crystals (from hexane), yield 72.9%, m.p 260°C, IR (KBr) ν (cm^{-1}): 3432 (NH_2),3341 (NH),3210 (NH),1640 (C=O),1554 (NH bending). MS (EI) m/z (%): molecular ion peak at 233 ($M^{+\bullet}$,57.02). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 1.77 (s, 1H, NH, D_2O exchangable), 3.74 (s, 3H, OCH_3), 5.69 (s, 1H, NH, D_2O exchangable), 6.9-7.4 (dd, 4H, Ar-H), 8.5 (s, 1H, NH_2, D_2O exchangable), Anal.Calcd. For $C_{10}H_{11}N_5O_2$; C, 51.50; H, 4.75; N, 30.03; Found: C, 51.41; H, 4.64; N, 30.11.

3-Amino-4-[(2-chlorophenyl)diazenyl]-1,2-dihydropyrazol-5-one 4b; Reddish brown crystals (from hexane), yield 88.4%, m.p 240 °C, IR (KBr) ν (cm^{-1}): 3458 (NH_2), 3290 (NH), 3178 (NH),1640 (C=O). MS (EI) m/z (%): molecular ion peak at: 236.95 ($M^{+\bullet}$, 100), 238.99 ($M+2^{+\bullet}$, 35.9). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 1.9 (s, 1H, NH, D_2O exchangable), 5.9 (s, 2H, NH_2, D_2O exchangable), 7.1-7.7 (m, 4H, Ar-H), 10.4 (s, 1H, NH, D_2O exchangable), Anal.Calcd. For $C_9H_8ClN_5O$:C, 45.49; H, 3.39; N, 29.47; Found: C,45.29; H, 3.59; N, 29.55.

3-Amino-4-[(3-chlorophenyl)diazenyl]-1,2-dihydropyrazol-5-one 4c; Reddish brown crystals (from hexane), yield 85%, m.p 255 °C, IR (KBr) ν (cm^{-1}): 3386 (NH_2), 3326 (NH) , 3187 (NH),1635 (C=O),1596 (NH bending), MS (EI) m/z (%): molecular ion peak at: 237.05 ($M^{+\bullet}$, 80.05), 239.05 ($M+2^{+\bullet}$, 25.9).^{1}HNMR (DMSO-d6, 300MHz) δ ppm:1.89(s, 1H, NH, D_2O exchangable), 5.9 (s, 2H, NH_2, D_2O exchangable), 7.1-7.7 (m, 4H, Ar-H), 10.5 (s, 1H, NH, D_2O exchangable), Anal.Calcd. For $C_9H_8ClN_5O$: C, 45.49; H, 3.39; N, 29.47; Found; C, 45.52; H, 3.53; N, 29.51.

3-Amino-4-[(4-bromophenyl)diazenyl]-1,2-dihydropyrazol-5-one 4d;Reddish brown crystals (from hexane), yield 71.4%, m.p 230 °C (d), 261 °C, IR (KBr) ν (cm^{-1}): 3364 (NH_2), 3300 (NH), 3178 (NH), 1641(C=O). MS (EI) m/z (%): bmolecular ion peak at: 280.97 ($M^{+\bullet}$, 93.31), 282.93 ($M+2^{+\bullet}$,100). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 1.89 (s, 1H, NH, D_2O exchangable), 5.86 (s, 2H, NH_2, D_2O exchangable), 7.5 (dd, 4H, Ar-H), 10.5(s, 1H, NH, D_2O exchangable), Anal.Calcd. For $C_9H_8BrN_5O$: C, 38.32; H, 2.86; N, 24.83. Found: C, 38.55; H, 2.87; N, 24.98.

3-Amino-4-[(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl)diazenyl]-1,2dihydropyrazol-5-one 4e; Brown crystals (from hexane), yield 87.4%, m.p 275 °C, IR (KBr) ν (cm^{-1}): 3431 (NH_2), 3341 (NH), 3210 (NH),1640 (C=O), 1554 (NH bending),MS (EI) m/z (%):molecular ion peak at 313.1 ($M^{+\bullet}$,1.38). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm:1.81 (s, 1H, NH, D_2O exchangable), 3.2 (s, 3H, CH_3), 3.4 (s, 3H, CH_3), 5.98 (s, 2H, NH_2, D_2O exchangable), 7.42-7.67 (m, 5H, Ar-H), 8.21 (s, 1H, NH, D_2O exchangable). Anal.Calcd. For $C_{14}H_{15}N_7O_2$: C, 53.67; H, 4.83; N, 31.29. Found: 53.77; H, 4.78; N, 31.42.

3,5-Diamino-4-[(4-methoxyphenyl)diazenyl]-1H-pyrazole 4f; Brown crystals (from hexane), yield 81.17%, m.p 255-257 °C, IR (KBr) ν (cm^{-1}): 3457 (NH_2), 3396 (NH_2), 3274 (NH), 2952 (CH aliphatic), 1611 (NH bending),MS (EI) m/z (%): molecular ion peak at 232 ($M^{+\bullet}$,61.37). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 3.79 (s, 3H, CH_3), 5.9 (s, 4H, two NH_2, D_2O exchangable), 6.96, 7.65 (dd, 4H, Ar-H), 10.48 (s, 1H, NH, D_2O exchangable) [40].

3,5-Diamino-4-[(2-chlorophenyl) diazenyl]-1H-pyrazole 4g; Brown crystals (from hexane), yield 71.58%, m.p 218-220°C [41].

3,5-Diamino-4-[(3-chlorophenyl)diazenyl]-1H-pyrazole 4h; Brown crystals (from hexane), yield 73.8%, m.p 242-244°C,IR (KBr) ν (cm^{-1}): 3436 (NH_2), 3391 (NH_2), 3288 (NH), 1512 (NH bending). MS (EI) m/z (%): molecular ion peak at 236 ($M^{+\bullet}$, 75.23), 238.1 ($M+2^{+\bullet}$, 23.67).^{1}HNMR (DMSO-d6, 300MHz) δ ppm:6.1 (s, 2H, NH_2, D_2O exchangable), 6.5 (s, 2H, NH_2,

D_2O exchangable), 7.26-7.85 (m, 4H, Ar-H), 8.48 (s, 1H, NH, D_2O exchangable). Anal.Calcd. For $C_9H_9ClN_6$: C, 45.68; H, 3.83; N, 35.51. Found: C, 45.88; H, 3.8; N, 35.44.

3,5-Diamino-4-[(4-bromophenyl)diazenyl]-1H-pyrazole 4i; Brown crystals (from hexane), yield 72.46%, m.p 240°C, IR (KBr) ν (cm^{-1}): 3412 (NH_2), 3396 (NH_2), 3287 (NH), 3187 (CH aromatic), 1608 (NH bending). MS (EI) m/z (%): molecular ion peak at: 280.05 ($M^{+\bullet}$, 0.06), 282 ($M+2^{+\bullet}$, 0.06). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm:5.95 (s, 4H, two NH_2, D_2O exchangable), 6.93, 7.69, 7.85 (dd, 4H, Ar-H), 8.51 (s, 1H, NH, D_2O exchangable). Anal.Calcd. for $C_9H_9BrN_6$: C, 38.45; H, 3.23; N, 29.90. Found: C, 38.05; H, 3.43; N, 30.12.

3,5-Diamino-4-[(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl) diazenyl]-1H-pyrazole 4j; Brown crystals (from hexane), yield 88.66%, m.p 320-330°C, ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 1.93 (s, 3H, CH_3), 3.39 (s, 3H, N-CH_3), 5.95 (s, 2H, NH_2, D_2O exchangable), 6.4 (s, 2H, NH_2, D_2O exchangable), 7.37-7.59 (m, 5H, Ar-H), 8.66 (s, 1H, NH, D_2O exchangable) [42].

4-[(4-methoxyphenyl)diazenyl]-1H-3,5-dimethylpyrazole 4k; Brown crystals (from hexane), yield 78%, m.p 178 °C, IR (KBr) ν (cm^{-1}): 3313 (NH), 2917 (CH aliphatic), 1622 (C-O).MS (EI) m/z (%):molecular ion peak at 230.05 ($M^{+\bullet}$, 25.9), ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 1.93 (s, 6H, two CH_3), 3.79 (s, 3H, O-CH_3), 6.47-6.59 (dd, 4H, Ar-H),8.56 (s, 1H, NH, D_2O exchangable). Anal.Calcd. for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33; Found C, 62.39; H, 6.53; N, 24.03.

4-[(2-chlorophenyl)diazenyl]-1H-3,5-dimethylpyrazole 4l; Brown crystals (from hexane), yield 74.5%, m.p 210°C, IR (KBr) ν (cm^{-1}): 3422 (NH). MS (EI) m/z (%): molecular ion peak at 234.1 ($M^{+\bullet}$, 10.75), 236 ($M+2^{+\bullet}$, 4.30).^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 1.986 (s, 6H, two CH_3), 7.06-7.56 (m, 4H, Ar-H) 8.69 (s, 1H, NH, D_2O exchangable). Anal.Calcd for $C_{11}H_{11}ClN_4$, C, 56.30; H, 4.72; N, 23.87 Found: C, 56.40; H, 4.52; N, 23.9.

4-[(3-chlorophenyl)diazenyl]-1H-3,5-dimethylpyrazole 4m; Brown crystals (from hexane), yield 81%, m.p 190 °C, IR (KBr) ν (cm^{-1}): 3429 (NH), 2921 (CH aliphatic). MS (EI) m/z (%): molecular ion peak at 234.2($M^{+\bullet}$, 0.45),236.15 ($M+2^{+\bullet}$, 0.52). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 2.0 (s, 6H, two CH_3), 7.16-7.87 (m, 4H, Ar-H) 8.48 (s, 1H, NH, D_2O exchangable). Anal.Calcd for $C_{11}H_{11}ClN_4$: C, 56.30; H, 4.72; N, 23.87. Found: C, 56; H, 4.89; N, 23.8.

4-[(4-bromophenyl)diazenyl]-1H-3,5-dimethylpyrazole 4n; Brown crystals (from hexane), yield 85%, m.p 230°C, IR (KBr) ν (cm^{-1}): 3346 (NH), 1606 (NH bending), MS (EI) m/z (%):molecular ion peak at 278 ($M^{+\bullet}$, 19.2),280.11($M+2^{+\bullet}$, 19.11). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 2.14 (s, 6H, two CH_3), 7.15, 7.77 (dd, 4H, Ar-H) 8.67 (s, 1H, NH, D_2O exchangable). Anal.Calcd for$C_{11}H_{11}BrN_4$: C, 47.33; H, 3.97; N, 20.07. Found: C, 47.43; H, 4; N, 20.02.

4-[(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl)diazenyl]-1H-3,5-dimethylpyrazole4o; Dark red crystals (from hexane), yield 87%, m.p 185 °C, IR (KBr) ν (cm^{-1}): 3427 (NH), 1644 (NH bending), MS (EI) m/z (%): molecular ion peak at 310.30 ($M^{+\bullet}$, 17.5).^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 1.66 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.8 (s, 3H, N-CH_3), 7.0-7.7 (m, 4H, Ar-H), 8.75 (s, 1H, NH, D_2O exchangable) Anal.Calcd for $C_{16}H_{18}N_6O$: C, 61.92; H, 5.85; N, 27.08; Found: 62.02; H, 5.95; N, 27.03 [43].

4-[(4-methoxyphenyl)diazenyl]-1H-3-methylpyrazol-5-one 4p; Red crystals (from hexane), yield 85 %, m.p 190 °C, IR (KBr) ν (cm^{-1}): 3745 (NH), 3428 (NH), 1664 (C=O), MS (EI) m/z (%):molecular ion peak at 232.1 ($M^{+\bullet}$, 42.91).^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 2.0 (s , 3H , CH_3), 2.38 (s, 1H , NH, D_2O exchangable), 3.72 (s, 3H , O-CH_3), 6.96-7.44 (dd, 4H, Ar-H), 11.47 (s, 1H, NH, D_2O exchangable).Anal.Calcd for $C_{11}H_{12}N_4O_2$: C, 42.73; H, 3.23; N, 19.93; Found: C, 42.83; H, 3.33; N, 20.1.

4-[(2-chlorophenyl)diazenyl]-1H-3-methylpyrazol-5-one 4q;Orange crystals (from hexane), yield 79 %, m.p 300 °C, IR (KBr) ν (cm^{-1}): 3413 (NH), 1664(C=O). MS (EI) m/z (%): molecular ion peak at 236.17 ($M^{+\bullet}$, 100), 238.17($M+2^{+\bullet}$, 36.25). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 2.2 (s, 3H, CH_3), 2.5 (s, 1H, NH, D_2O exchangable), 7.0-7.7 (m, 4H, Ar-H), 10.79 (s, 1H, NH, D_2O exchangable). Anal.Calcd for$C_{10}H_9ClN_4O$: C, 50.7; H, 3.7; N, 23.8; Found: C, 50.75; H, 3.83; N, 23.67.

4-[(3-chlorophenyl)diazenyl]-1H-3-methylpyrazol-5-one 4r; Orange-Marron crystals (from hexane), yield **87** %, m.p 280 °C, IR (KBr) ν (cm^{-1}): 3285 (NH), 3134 (NH), 1665 (C=O). MS (EI) m/z (%): molecular ion peak at 236.16 ($M^{+\bullet}$, 100), 238.1 ($M+2^{+\bullet}$, 36.21). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm:2.11 (s, 3H, CH_3), 2.45 (s, 1H, NH, D_2O exchangable), 7.16-7.58 (m, 4H, Ar-H), 11.57 (s, 1H, NH, D_2O exchangable). Anal.Calcd for$C_{10}H_9ClN_4O$: C, 50.75; H, 3.83; N, 23.67 Found: C, 50.69; H, 3.94; N, 23.88.

4-[(4-bromophenyl)diazenyl]-3-methyl-1H-pyrazol-5-one 4s; Dark red crystals (from hexane), yield 84%, m.p 152 °C,IR (KBr) ν (cm^{-1}): 3423 (NH), 3242 (NH), 2924 (CH aliphatic), 1665 (C=O). MS (EI) m/z (%): molecular ion peak at 280.02 ($M^{+\bullet}$, 50.12), 282 ($M+2^{+\bullet}$ 49.3). ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 1.96 (s, 3H, CH_3), 2.25 (s, 1H, NH, D_2O exchangable), 7.07-7.58 (dd, 4H, Ar-H), 8.25 (s, 1H, NH, D_2O exchangable). Anal.Calcd for $C_{10}H_9BrN_4O$: C, 42.73; H, 3.23; N, 19.93. Found: C, 42.43; H, 3.43; N, 19.98.

4-[(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl)diazenyl]-3-methyl-1H-pyrazol-5-one 4t; Dark red crystals (from hexane), yield 84 %, m.p 228 °C, IR (KBr) ν (cm^{-1}): 3423 (NH), 3241.75 (NH), 2924 (CH aliphatic), 1664 (C=O). MS (EI) m/z (%): molecular ion peak at312.03 ($M^{+\bullet}$, 0.21), ^{1}HNMR (DMSO-d6, 300MHz) δ ppm: 2.01 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.42 (s, 1H, NH, D_2O exchangable), 2.66 (s, 3H, N-CH_3), 6.99-7.98 (m, 5H, Ar-H), 8.25 (s, 1H, NH, D_2O exchangable). Anal.Calcd for $C_{15}H_{16}N_6O_2$: C, 57.68; H, 5.16; N, 26.91. Found: C, 57.73; H, 5.26; N, 26.96.

Antiviral Activity:

Cytotoxicity assay on MA 104, Hep-2, Vero, and Huh 7.5 cell lines:

All tested title compounds (100mg) were dissolved separately in 10ml of DMSO. Cell monolayers Hep2, MA104, Vero (obtained from The Holding Company for Biological Products & Vaccines VACSERA, Egypt) and Huh 7.5 (obtained from the lab. of Prof.Dr. Charles Rice, The Rockefeller University, USA) were trypsinized, washed with culture medium and plated in a 96-well flat-bottomed plate with $5x10^3$ cells per well for both cell lines, and incubated for 24h. Each of the testedtitle compounds DMSO solution was diluted (Greiner-Bio one, Germany) (10 fold dilutions of decontaminated samples with 12µl of 100x of Antibiotic, antimycotic mixture was added to 500µl of each sample) and were added to the appropriate incubated cell wells, then the plates were incubated for a further 48h at 37°C in a humidified incubator with 5% CO_2. After incubation, the supernatants were removed from the wells, and cell viability was evaluated using microscopical examination (inverted light microscopy), trypan blue and the antiviral colorimetric assay (MTT) technique [44-46]. The results were obtained from triplicate assays of each concentration of the tested title compounds. The percentage of cytotoxicity was calculated as: [(A-B)/A] x100, where A and B are the optical density (OD) at 492nm of untreated and treated cells, respectively.

Cell morphology evaluation by inverted light microscopy [45]**:**

MA 104, Hep-2, Vero, and Huh 7.5 cell lines ($2x10^5$ cells/ml) were prepared in 96-well tissue culture plates (Corning, US). After incubation at 37ºC for 24h in a humidified 5% CO_2 atmosphere, the cell monolayers were confluent. Then, the medium was removed from each well and replenished with 100µl of bifold dilutions of each of the tested title compounds prepared in Dulbecco´s Modified Eagle Medium (DMEM) (Gibco- BRL (GIBCO). For cell controls, 100µl of DMEM without the addition of any of the tested compound was added to each cell. All the prepared cultures were incubated at 37ºC in a humidified 5% CO_2 atmosphere for 72h. Cell morphology was observed daily for microscopically detectable morphological alterations, such as loss of confluence, cell rounding,shrinking, cytoplasm granulation and vacuolization with the concomitant scoring of morphological changes.

Cell viability test by Trypan blue dye exclusion method [44]**:**

MA 104, Hep-2, Vero, and Huh 7.5 cell lines ($2x10^5$ cells/ml) were grown in 12-well tissue culture plates **(Corning, US)**. After an incubation period of 24h at 37ºC in a humidified 5% CO_2 atmosphere, the media were removed from each well. Then, cell cultures were replenished with 100µl per well of each of the bifolded dilutions of each one of the tested title compounds. All the cell cultures were incubated at 37ºC in a humidified 5% CO_2 atmosphere for 72 h. After incubation, the media were removed, cells were trypsinized and an equal volume of 0.4% (w/v) trypan blue dye aqueous solution was added to the cell suspension. Viable cells were counted under the phase contrast microscope.

Determination of the effect of the title compounds against Adenovirus type 7, and Herpes Simplex Virus 1 (HSV 1) using the viral plaque assay [47]**:**

A set of ten folds' dilutions of each of adenovirus type 7 and HSV-1 were prepared. Another two sets of (100µl) of nontoxic doses of each of the tested title compounds; one set were mixed with (100µl) of ten-fold dilutions of adenovirus type 7. The other set was mixed with ten-fold dilutions of HSV-1. The four sets were incubated for 30 min at 37ºC. Then, (100µl) of each of the incubated mixtures were incuolated in 12-well plates in HEP-2, and Vero cell lines for adenovirus type 7 and HSV-1, respectively. The cell cultures were incubated for 1 hat 37 ºC in a 5% CO_2–water vapor atmosphere,the plates were only rocked intermittently to help adsorption and avoid cell drying. After adsorption, each well was treated with 1 ml of media (DMEM) and 1 ml (1 %) agarose, and the plates were incubated at 37 ºC in a 5% CO_2–water vapor atmosphere for the appropriate incubation period. Then, the cells were stained with 0.4 % crystal violet after formalin fixation, and the number of plaques was counted. The viral titers were then calculated and expressed as plaque-forming units per milliliter (pfu/ml)**.**

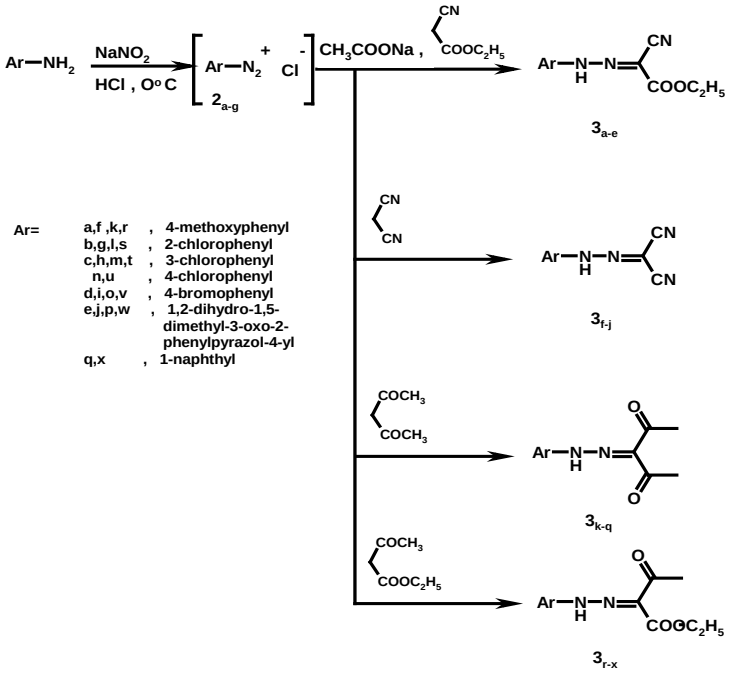
Determination of antiviral effect of the title compounds against Hepatitis C virus genotype 4a replicon (ED-43/SG-Feo (VYG) replicon) [48]**:**

ED-43/SG-Feo (VYG) replicon of HCV genotype 4a (obtained from the Lab. of Prof.Dr. Charles Rice, the Rockefeller University, USA) was treated with the non-toxic doses of each of the tested title compounds. According to Saeed et al. (2013), HCV RNA was quantified in the Huh 7.5 infected cells treated with tested title compounds using qRT-PCR (Taqman probe kit, Qiagen) and according to the manufacturer's instructions to show a dose-dependent decrease in subgenomic RNA copies [48].

Results and Discussion

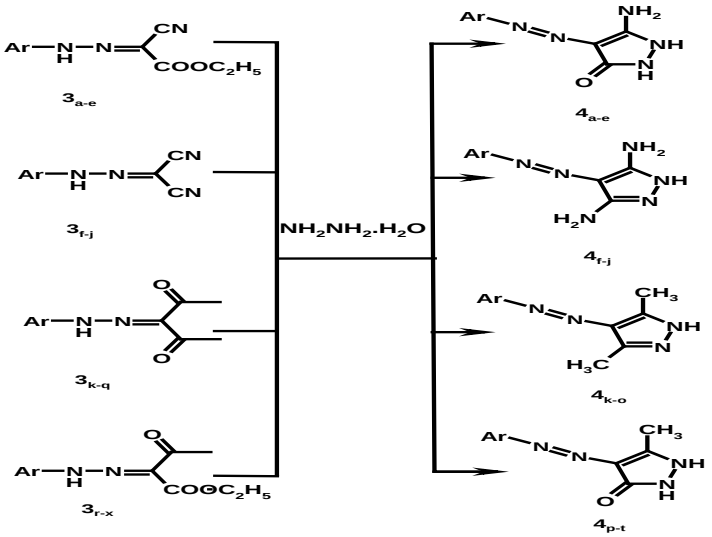
Chemistry

Arylhydrazones 3_{a-x} were prepared as reported in the literature [16, 17, 49] via diazotization of the appropriate primary aromatic amine. Coupling of the afforded aryl diazonium salts 2_{a-g} with active methylene compounds; ethylcyanoacetate, malononitrile, acetylacetone and ethylacetoacetate in sodium acetate buffered medium yielded ethyl-2-aryl-1-cyanohydrazonoacetates 3_{a-e}, 2-arylhydrazonomalononitriles 3_{f-j}, 2-aryldiacetylmethylenehydrazones 3_{k-q} and ethyl-1-acetyl-2-arylhydrazonoacetates 3_{r-x} respectively (Scheme 1).



Scheme 1: Hydrazone synthesis

The cyclization of arylhydrazones 3_{a-x} into the corresponding pyrazole derivatives 4_{a-t} was achieved by heating the arylhydrazones 3_{a-x} under reflux with hydrazine hydrate for 4h [34, 49, 50] (Scheme 2).



Scheme 1: Arylazopyrazoles and arylazopyrazolones syntheses

In part of this initiative, the structures of the prepared pyrazole analogues 4_{a-e} were established by microanalyses and spectral data. Thus, the IR spectra of these compounds revealed stretching absorption bands at ν cm^{-1} 3454-3364, 3341-3289, 3209-3178 corresponding to NH_2, NH. Additionally, it has been witnessed that the IR spectra showed neither cyano nor ester function (characteristic for 3_{a-e}), but amide absorption band at ν cm^{-1} 1641-1635. The ^{1}HNMR spectra of these compounds showed signals at δ ppm 1.77-1.89, 5.69-5.98 and 7.9-10.56 corresponding to NH, NH_2 and amidic NH respectively. The chemical shifts of these exchangeable protons were confirmed by D_2O experiment. The ^{1}HNMR spectrum of the pyrazole derivative 4_a exhibited at δ ppm 3.74 (s, 3H, OCH_3), 6.90, 7.45 (dd, 4H, p-disubstituted phenyl), while that of 4_e showed at δ ppm 3.2 and 3.4 two signals (s, 6H, 2 CH_3) characteristic for the two methyls of antipyrine moiety. The mass spectrum of compound 4_a displayed the molecular ion at m/z 233 (57%), while the mass spectra of the chloro analogues $4_{b,}$ 4_c showed a parent molecular ion at m/z 236.99 (100%), 238.99 (35.9%) and 237 (80.5%), 239 (25.9%) corresponding to $M^{+\bullet}$and $M+2^{\urcorner+\bullet}$, respectively.

As it has been communicated and acknowledged for the novel compunds $4_{h,\ i}$, the IR spectra of these compounds revealed stretching absorption bands at ν cm^{-1} 3412- 3391, 3288-3287 corresponding to NH_2, NH. Additionally, it has been witnessed

that the IR spectra showed neither cyano nor ester function. The ^{1}HNMR spectra of these compounds showed signals at δ ppm 5.94-6.10, 8.48-8.51 corresponding to theNH_2 and NH protons, respectively. The mass spectrum of the compound 4_h revealed its molecular ion peak at m/z 236.09 (75.23%) and 238.10 (23.67%) corresponding to $M^{+\bullet}$and $M+2^{+\bullet}$ respectively. Moreover, the mass spectrum of 4_i divulged the molecular ion peak at m/z 280.05 (0.06%), 282 (0.06%) corresponding to $M^{+\bullet}$and $M+2^{+\bullet}$ respectively.

It's clearly evident that, the IR spectra of compounds 4_{k-o} showed no absorption bands for ketonic carbonyls, but the NH stretching absorption bands at ν cm^{-1} 3429-3312. Their ^{1}HNMR spectra showed singlets due to NH protons at δ ppm 8.56-8.69. In addition, two singlets appeared at δ ppm 1.89-2.14 (6H) corresponding to the two methyl groups. In compound 4_k, the methoxy group protons appeared at δ ppm 3.79 as a singlet (3H) while the p-disubstituted phenyl ring protons appeared as two doublets at δ ppm 6.59, 6.74, respectively. The mass spectra of the chloropyrazole 4_l and its bromoanalogue 4_n exhibited the molecular ion peaks at m/z 234.10 (10.75%), 236 (4.3%) and 278 (19.2%), 280 (19.1%) corresponding to $M^{+\bullet}$and $M+2^{+\bullet}$ respectively.

Accordingly, the IR spectra for compounds 4_{p-t} revealed stretching absorption bands at ν cm^{-1} 3428-3185 and 1664-1665 corresponding to NH_2 and amide carbonyls, respectively. ^{1}HNMR spectra showed signals corresponding to the methyl, the exchangeable and the aromatic protons at the expected δ values. For instance, compound 4_p exhibited a singlet at δ ppm 3.72 (3H, OCH_3), and two doublets at δ ppm 6.96, 7.44 (4H, p-disubstituted phenyl), and 4_t revealed two singlets at δ ppm 2.26 and 2.66 corresponding to the two methyls of antipyrinemoiety. The mass spectra of compounds 4_{p-t} were in accordance with the expected m/z values. Thus, mass spectrum of compound 4_p showed the parent molecular ion at m/z 232 (42.9%), while the spectra of the chloro analogues $4_{q, r}$ and the bromo analogue 4_s exhibited the parent molecular ions at m/z 236 (100%), 238 (36.25%) and 280 (50%), 282 (49.3) corresponding to $M^{+\bullet}$and $M+2^{+\bullet}$, respectively.

Biological Results and Discussion:

- **Determination of the title compounds non-toxic doses**

The antiviral activities of the synthesized title compounds were evaluated against a wide set of viruses in different cell lines using trypan blue dye exclusion method [44] and the viral plaque assay [51]. The antiviral activities were scanned against rotavirus Wa strain, adenovirus type 7, and Herpes Simplex Virus 1 (HSV 1), respectively. The antiviral activity against hepatitis C virus was also evaluated against hepatitis C virus genotype 4a replicon (ED-43/ 3G - Feo (VYG) replicon). MA104, HEP-2, and Vero cell monolayers were used in the study. At least five dilutions were used for each of the tested title compounds and the results were obtained from the triplicate assay for each concentration. The percentage of cytotoxicity was calculated as [(A-B)/A] x 100, where A and B are the optical densities (OD) at 492 nm of treated and untreated cells respectively.

Table 1. Nontoxic doses of tested title compounds on different cell lines for hydrazones 3_{a-x}.

3	Nontoxic doses on MA104 cell line (µg/ml)	Nontoxic doses on Hep2 cell line (µg/ml)	Nontoxic doses on Vero cell line (µg/ml)	Nontoxic doses on Huh 7.5 cell line (µg/ml)
a	70	80	80	70
b	70	70	70	70
c	70	70	70	70
d	70	70	70	60
e	70	70	70	60
f	70	70	70	60
g	60	60	60	50
h	70	70	70	70
i	60	70	70	60
j	60	60	60	60
k	70	80	70	70
l	70	80	70	70
m	60	60	60	60
n	60	70	70	60
p	70	70	70	70
q	70	80	80	70
r	70	80	80	80
w	70	80	70	60

Table 2. Nontoxic doses of tested title compounds on different cell lines for pyrazoles 4_{a-t}.

4	Nontoxic doses on MA104 cell line (µg/ml)	Nontoxic doses on Hep2 cell line (µg/ml)	Nontoxic doses on Vero cell line (µg/ml)	Nontoxic doses on Huh 7.5 cell line (µg/ml)
a	80	90	90	80
b	60	60	60	60

c	80	80	80	80
d	80	80	80	80
e	70	80	80	70
f	80	80	80	80
g	60	60	60	60
h	70	80	70	70
i	70	70	80	70
j	70	80	80	70
l	70	80	80	70
p	60	70	70	60
q	60	70	70	60
r	70	70	70	70

- **Statistical analyses of the viral inhibition activities**

Statistical analyses for the percent inhibition of viral titers achieved by each of the title compounds' non-toxic doses against each of the study viruses were conducted. In our study, only percent inhibition values of viral titres above 50% were considered significant. As shown in figure 2, compounds 3_b, 3_h, 4_g and 4_r showed significant activity against rotavirus Wa strainof viral titers percent inhibition values of 66.7 %, 63.3 %, 70 % and 76.6 %, respectively (Diagram 2).

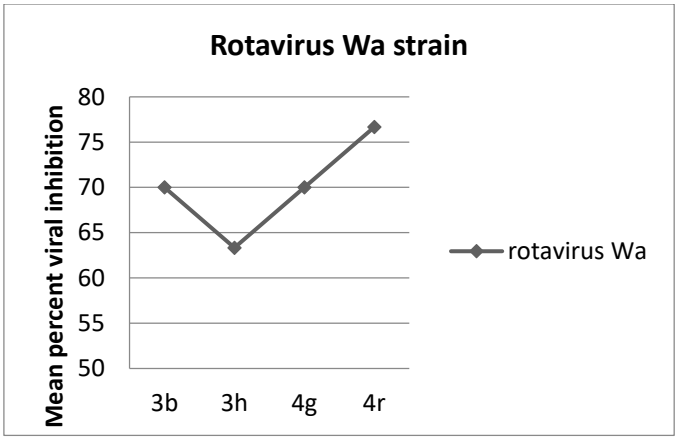


Diagram 2. The percentage inhibition of rotavirus wa strain titers caused by compounds 3_b, 3_h, 4_g and 4_r.

Compounds 4_q and 4_r showed the highest activity against adenovirus type 7 among the title pyrazoles of viral titers percent inhibition values of 50% and 53.3%, respectively. But, none of the tested hydrazones showed significant activity against adenovirus type 7 (Diagram 3).

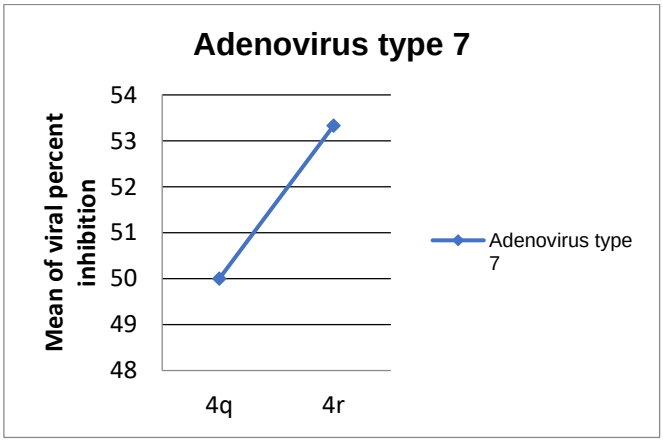


Diagram 3. The percentage inhibition of adenovirus type 7 titers caused by compounds 4_q and 4_r.

On the contrary, only the hydrazones 3_b and 3_h showed significant activity against Hep C 4a viral genome where both showed viral titers percent inhibition values of 53.3% but none of the pyrazoles were significantly active (Diagram 4).

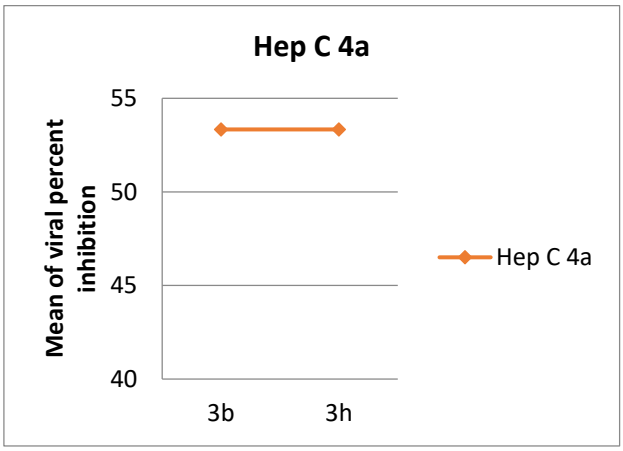
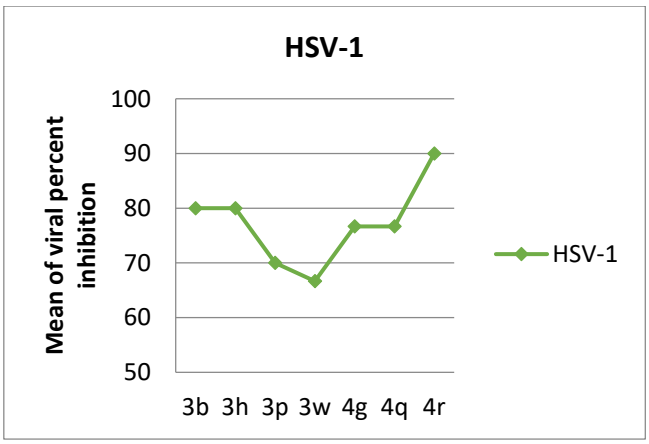


Diagram 4. The percentage inhibition of Hep C 4a titers caused by compounds 3_b and 3_h

Most of the title compounds showed promising activities against HSV-1 of viral titers percent inhibition values of 80%, 80%, 70%, 66.7%, 76.7%, 70% and 90% of the compounds 3_b, 3_h, 3_p, 3_w, 4_g, 4_q and 4_r, respectively (Diagram 5).



Duagram 5. The percent inhibition of HSV-1 viral titers caused by compounds 3_b, 3_h, 3_p, 3_w,4_g, 4_q and 4_r

Compounds 4q, 3h, 4g, 4r showed the highest activity towards HSV-1 virus of IC_{50} values of 10, 20, 20, 30 µg/ml, repectively.

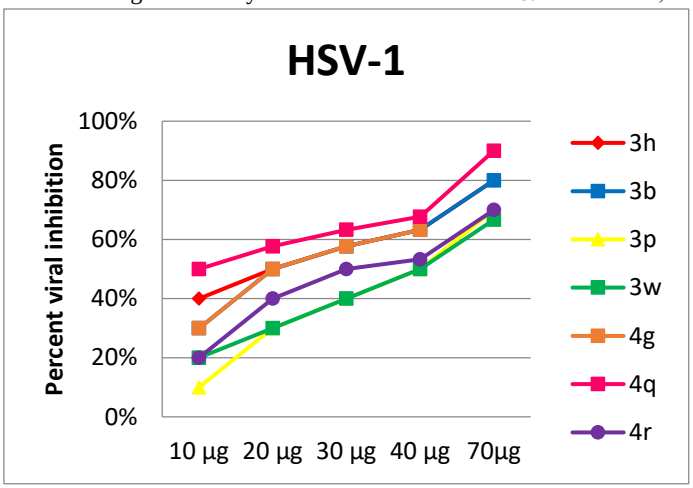


Diagram 6. determination of IC_{50} of the study compounds against HSV-1

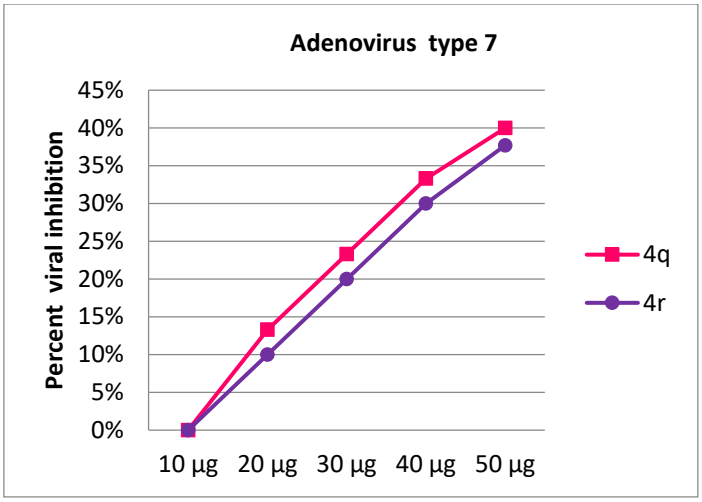


Diagram 7. determination of IC_{50} of the study compounds against Adenovirus type 7

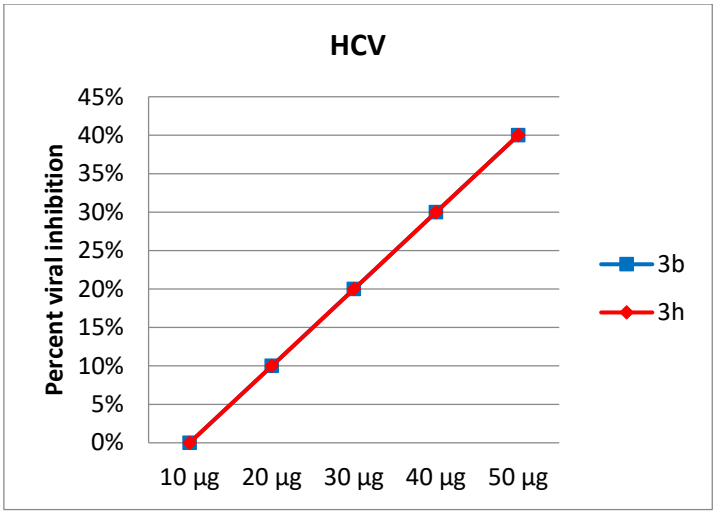


Diagram 8. determination of IC_{50} of the study compounds against HCV virus

On the other hand, compounds 4g & 4r showed the highest activity toward Rotavirus Wa strain of IC_{50} values of 30 µg/ml**.**

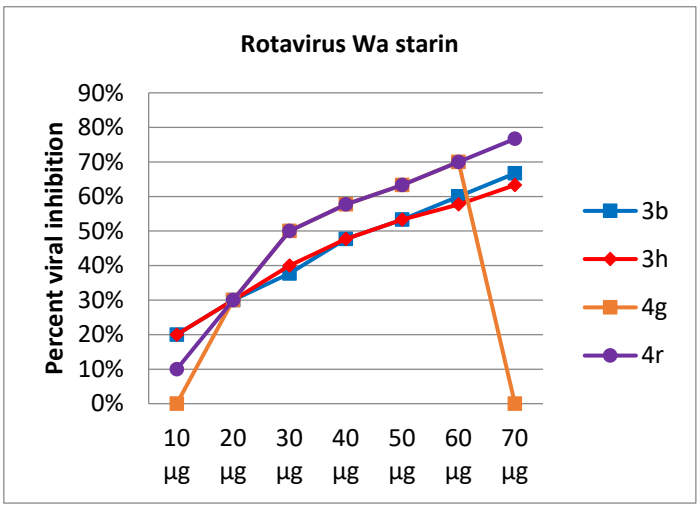


Diagram 9. determination of IC_{50} of the study compounds against Rotavirus Wa virus

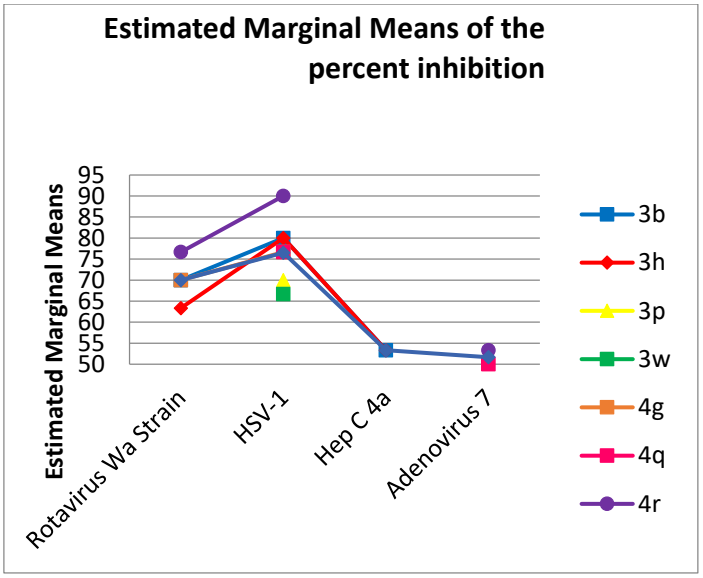


Diagram 10. The estimated marginal means of percentage viral titers inhibition at the non-toxic doses of the whole study.

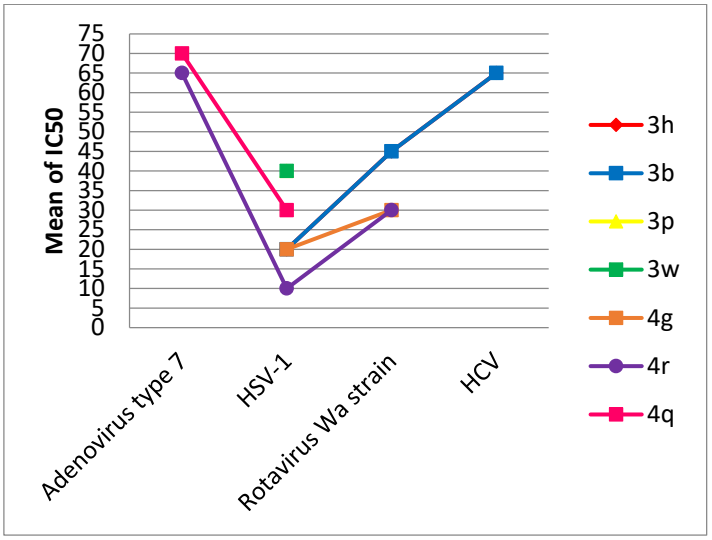


Diagram 11. The IC_{50} (µg) of the active compounds against the study viruses

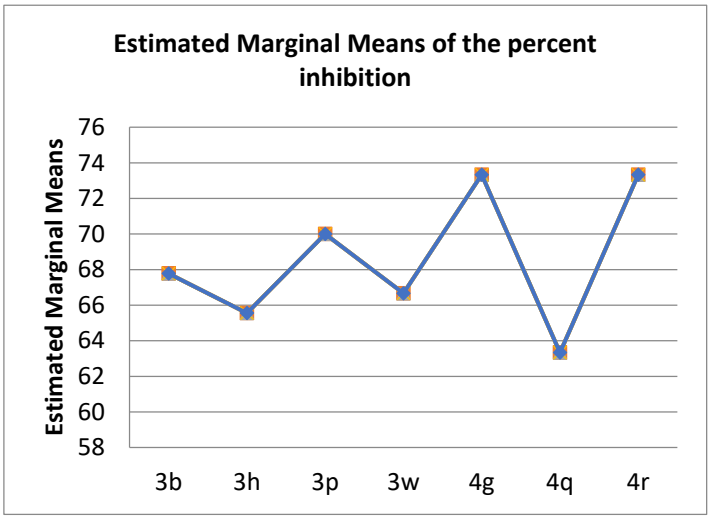


Diagram 12. Compounds 4_g, 4_r showed the highest activity of all the tested title compounds against all the study viruses

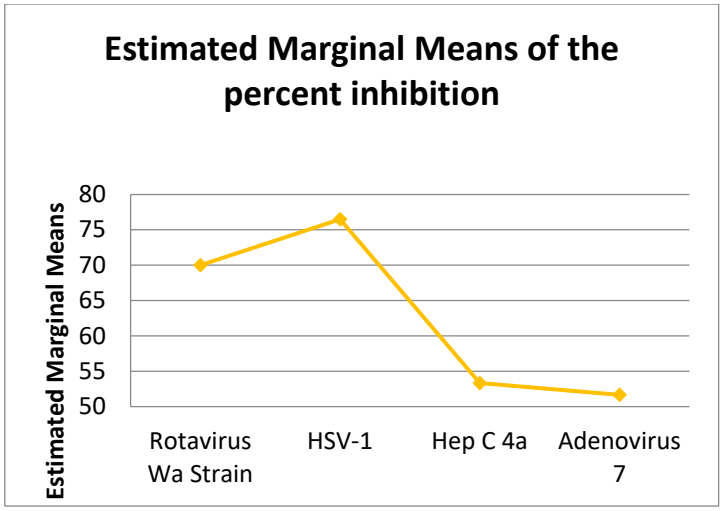


Diagram 13. It shows that all of the compounds mentioned in our study are the most active towards HSV-1 virus followed by rotavirus Wa strain

- **Structure-activity relationship**

As shown in Figures 10-13, the tested compounds showed inhibition activities against the DNA viruses HSV-1 virus and rotavirus wa strain. However, as indicated in Figure 12, only o-, m-chlorophenyl 3_b, 3_h, 4_g, 4_q and 4_r and antipyrinyl 3_p, 3_w derivatives of hydrazone and pyrazole showed significant antiviral activity. On the other hand, the bromo, methoxy, p-chlorophenyl analogues and 3,5-dimetheylpyrazoles were inactive against the tested viral species. The chloropyrazoles 4g, 4_r exhibited the highest antiviral activity of estimated marginal means of 73.333. While the antipyrinylhydrazone analogues 3_p and 3_w showed relatively lower antiviral activity of estimated marginal means of 70.000 and 66.667. Although m-chlorophenylpyrazole 4_r showed non-significant higher activity than o-chlorophenylpyrazole analogue 4_q of activity estimated marginal mean of 65.000 and 63.333, respectively. The results revealed that o-chlorophenyl analogues 3_b, 4_g and 4_q of estimated marginal means of 67.778, 73.333 and 63.333 respectively exhibited relatively higher antiviral activity than m-chlorophenyl analogue 3_h of estimated marginal means of 65.556, respectively (Diagram 12). Generally, these results indicated that pyrazole derivatives suitably substituted by aryl or antipyrinyl moieties could be promising scaffold as antiviral agents**.**

Conclusion

Compounds 4r, 3b, 3h, 4q showed the highest activity towards HSV-1 virus of IC_{50} values of 10, 20, 20, 30 µg/ml, respectively. On the other hand, compounds 4g & 4r showed the highest activity toward Rotavirus Wa strain of IC_{50} values of 30 µg/ml.

References

1. Mohamoud Y, Mumtaz G, Abu-Raddad L (2012) The epidemiology of hepatitis C virus in Egypt: a systematic review. Int J Infect Dis 16: e98–e99. doi: 10.1016/j.ijid.2012.05.228.
2. El-Senousy WM, Barakat AB, Ghanem HE, Kamel M a. (2013) Molecular epidemiology of human adenoviruses and rotaviruses as candidate viral indicators in the Egyptian sewage and water samples. World Appl Sci J 27:1235–1247. doi: 10.5829/idosi.wasj.2013.27.10.81200.
3. Mohamed MS, Abd El-Hameed RH, Sayed AI, Soror SH (2015) Novel antiviral compounds against gastroenteric viral infections. Arch Pharm (Weinheim) 348:194–205. doi: 10.1002/ardp.201400387.
4. Petr Cankar O (CZ); I, Frysova M (CZ); V, Krystof O (CZ); RL, et al (2013) 4-Arylazo-3, S-Diamino-Pyrazole Compounds and Use Thereof. United States Pat.
5. Govindasami T, Pandey A, Palanivelu N, Pandey A (2011) Synthesis, Characterization and Antibacterial Activity of Biologically Important Vanillin Related Hydrazone Derivatives. 2011:71–77. doi: 10.4236/ijoc.2011.13012.
6. Hamdy NA, Abdel-Aziz HA, Kamel GM, Fakhr IMI (2013) Convenient synthesis, anti-inflammatory, analgesic and ulcerogenic activites of some new bis-hydrazones and pyrazole derivatives. Acta Pol Pharm - Drug Res 70:469–480.
7. Wagnat W, Nahed N (2013) Synthesis and anti-tumor evaluation of novel hydrazide and hydrazide-hydrazone derivatives. Acta Pharm 63:45–57. doi: 10.2478/v10007-acph-2013-0004.
8. Abdel-Aal MT, El-Sayed WA, El-Kosy SM, El-Ashry ESH (2008) Synthesis and antiviral evaluation of novel 5-(N-arylaminomethyl-1,3,4- oxadiazol-2-yl) hydrazines and their sugars, 1,2,4-triazoles, tetrazoles and pyrazolyl derivatives. Arch Pharm (Weinheim) 341:307–313. doi: 10.1002/ardp.200700154.

9. Nalan Terzioglu; Nilgün Karali; Aysel Gürsoy; Christophe Pannecouque; Pieter Leysen; Jan Paeshuyse; Johan Neyts; Erik De Clercq (2006) Synthesis and primary antiviral activity evaluation of 3-hydrazono-5-nitro-2-indolinone derivatives. Arch Org Chem 2006:109–118. doi: http://dx.doi.org/10.3998/ark.5550190.0007.113.
10. Abdel-Aziza HA, Abdel-Wahab BF, Badria FA (2010) Stereoselective Synthesis and Antiviral Activity of (1 E ,2 Z ,3 E)-1-(Piperidin-1-yl)-1-(arylhydrazono)-2- [(benzoyl/benzothiazol- 2-oyl) hydrazono]-4-(aryl) but-3-enes. Arch Pharm (Weinheim) 343:152–159. doi: 10.1002/ardp.200900195.
11. Tatar E, Küçükgüzel İ, Daelemans D, et al (2013) Some Hydrazones of 2-Aroylamino-3-methylbutanohydrazide: Synthesis, Molecular Modeling Studies, and Identification as Stereoselective Inhibitors of HIV-1. Arch Pharm (Weinheim) 346:140–153. doi: 10.1002/ardp.201200311.
12. Pevarello P, Brasca MG, Amici R, et al (2004) 3-Aminopyrazole Inhibitors of CDK2 / Cyclin A as Antitumor Agents. 1. Lead Finding. J Med Chem 3367–3380.
13. Ahasan NB, Islam R (2007) Cytotoxicity study of pyrazole derivatives. 81–87.
14. El-Sabbagh OI, Baraka MM, Ibrahim SM, et al (2009) Synthesis and antiviral activity of new pyrazole and thiazole derivatives. Eur J Med Chem 44:3746–53. doi: 10.1016/j.ejmech.2009.03.038
15. El-sayed WA, Flefel EM, Morsy EMH (2012) Anticancer and antimicrobial activities of some synthesized pyrazole and triazole derivatives. 4:23–32.
16. Atalla AA, Gaber AM, Al-wahab KAA (2000) Studies on aminopyrazoles: antibacterial activity of some novel pyrazolo [1, 5- a] pyrimidines containing sulfonamido moieties. Farm 55:596–602.
17. Azab ME, Youssef MM, El-bordany EA (2013) Synthesis and Antibacterial Evaluation of Novel Heterocyclic Compounds Containing a Sulfonamido Moiety. 832–844. doi: 10.3390/molecules18010832.
18. Karcı F, Demirçalı A (2007) Synthesis of disazo pyrazolo[1,5-a] pyrimidines. Dye Pigment 74:288–297. doi: 10.1016/j.dyepig.2006.02.007.
19. Draber W. KHB (1972) Quantitative Structure-Activity Studies of Hydrazones, Uncouplers of Oxidative Phosphorylation. Z Naturforsch 27:159.
20. Leonard NJ, Boyd SN, Herbrandson HF (1947) Cinnolines. III. Attempted Syntheses of the Cinnoline Nucleus 1. J Org Chem 12:47–56. doi: 10.1021/jo01165a007.
21. Dubenko R. G. and Pel'kis P. S. (1971) Investigations in the Field of Arylhydrazones of Substituted Glyoxylic Acids (IV). J Org Chem USSR (English Transl 1:1269.
22. Aly H, El-Gazzar M (2012) Novel Pyrazole Derivatives as Anticancer and Radiosensitizing Agents. Arzneimittelforschung 62:105–112. doi: 10.1055/s-0031-1297252.
23. Deeb A, Yassin F, Ouf N, Shehta W (2010) Pyridazine derivatives and related compounds. 23*. Synthesis of 3-substituted pyrazolo[3,4-c] pyridazines and their application as disperse dyes. Chem Heterocycl Compd 46:212–222. doi: 10.1007/s10593-010-0494-1.
24. Roger W. Adder and Donald B Wright, i'n P (1964) United States Patent 3157569. 3–7.
25. Amer AM, El-Bermaui MA, Ahmed AFS, Soliman SM (1999) On the Chemistry of Cinnoline I. Synthesis and Reactions of (4-Amino-cinnolin-3-yl)-p-tolyl-methanones. Monatshefte für Chemie / Chem Mon 130:1409–1418. doi: 10.1007/PL00010201.
26. Fadda AA, Etman HA, El-Seidy MY, Elattar KM (2012) Utility of Enaminonitriles in Heterocyclic Synthesis: Synthesis of Some New Pyrazole, Pyridine, and Pyrimidine Derivatives. J Heterocycl Chem 49:774–781. doi: 10.1002/jhet.855.
27. Mustroph H, Potocnak J, Grossmann N (1984) Untersuchungen zum photochemischen Ausbleichverhalten von tautomeriefähigen Azofarbstoffen. VI. Zur photochemischen Oxydation von Phenylazo-acetylacetonen und Phenylazopyrazolonen durch Singulettsauerstoff. J Prakt Chemie 326:979–984. doi: 10.1002/prac.19843260617.
28. Nagarajua V., Srinivasulua R. (2011) Synthesis, characterization and antibacterial screening of new pyrazole and pyrazoline-5-one derivatives.
29. Sethukumar A, Arul Prakasam B (2010) Spectral and cyclic voltammetric studies on some intramolecularly hydrogen bonded arylhydrazones: Crystal and molecular structure of 2-(2-(3-nitrophenyl) hydrazono)-5,5-dimethylcyclohexane-1,3-dione. J Mol Struct 963:250–257. doi: 10.1016/j.molstruc.2009.11.001.
30. Ojha S (1979) J Indian Chem Soc 56:1233–1236.
31. Sahu V, Sharma P, Kumar A (2012) Synthesis and QSAR modeling 1-[3-methyl-2-(aryldiazenyl)-2H-aziren-2-yl] ethanones as potential antibacterial agents. Med Chem Res 22:2476–2485. doi: 10.1007/s00044-012-0242-4
32. Mitchell A, Nonhebel DC (1979) Spectroscopic studies of tautomeric systems—III. Tetrahedron 35:2013–2019. doi: 10.1016/S0040-4020(01)88971-7.
33. Ravindran R (2004) Synthesis and characterisation of iron(III) complexes of 1,2-dihydro-1-phenyl-2,3-dimethyl-4-[2',4'-pentanedione-3'- hydrazono]pyrazol-5-one.
34. Karcı F, Demirçalı A (2007) Synthesis of disazo pyrazolo[1,5-a] pyrimidines. Dye Pigment 74:288–297. doi: 10.1016/j.dyepig.2006.02.007.

35. Kjellin C (1897) Ueber die Einwirkung von Brom auf β-Benzylhydroxylamine und die Constitution der Bisnitrosylbenzyle. Berichte der Dtsch Chem Gesellschaft 30:1969–1971. doi: 10.1002/cber.189703002155.
36. Shetty S, Kalluraya B, Peethambar SK, Telkar SB (2013) Type II diabetes-related enzyme inhibition and molecular modeling study of a novel series of pyrazolone derivatives. Med Chem Res 23:2834–2846. doi: 10.1007/s00044-013-0846-3.
37. Makisumi Y (1961) Synthesis of Potential Anticancer Agents. IX. Condensation of some 2-, 5-, and 7-Methyl-s-triazolo [2, 3-α] pyrimidines with Benzaldehyde. Chem Pharm Bull 9:883–889. doi: 10.1007/s13398-014-0173-7.2
38. Emerson E, Sagal J (1948) The Condensation of Aminoantipyrine. VII. Hydrolysis in The Presence of Nitrous Acid (1) 1. J Org Chem 13:535–537. doi: 10.1021/jo01162a009.
39. Ojha, A. C.; Jain R (1982). Renu Polish J Chem 56:1553–1557.
40. Elfahham HA, Elgemeie GEH, Ibraheim YR, Elnagdi MH (1988) Studies on 3,5-diaminopyrazoles: New routes for the synthesis of new pyrazoloazines and pyrazoloazoles. Liebigs Ann der Chemie 1988:819–822. doi: 10.1002/jlac.198819880820.
41. Elgemeie GH, El-Ezbawy SR, Ali HA (2001) Reactions of Chlorocarbonyl Isocyanate with 5-Aminopyrazoles and Active Methylene Nitriles: A Novel Synthesis of Pyrazolo[1,5-a]-1,3,5-Triazines and Barbiturates. Synth Commun 31:3459–3467. doi: 10.1081/SCC-100106205.
42. Krystof V, Cankar P, Frysová I, et al (2006) 4-arylazo-3,5-diamino-1H-pyrazole CDK inhibitors: SAR study, crystal structure in complex with CDK2, selectivity, and cellular effects. J Med Chem 49:6500–9. doi: 10.1021/jm0605740.
43. Farghaly AM, El-Khwass SM, Khalil MA, et al (1981) Some novel pyrazolone derivatives as anti-inflammatory agents. Pharmazie 36:93–5.
44. Walum E, Strenberg K JD (1990) Understanding Cell Toxicology: Principles and Pratice. Ellis Howood, NewYork, pp 97–111.
45. Simões CMO, Amoros M, Girre L (1999) Mechanism of antiviral activity of triterpenoid saponins. Phyther. Res. 13:323–328.
46. Mosmann T (1983) Rapid Colorimetric Assay for Cellular Growth and Survival. J. Immunol. Methods 65:55–63.
47. Schmidtke M, Knorre C, Blei L, et al (1998) Penetration and Antiviral Activity of Coxsackievirus B3 (Cvb3)-Specific Phosphorothioate Oligodeoxynucleotides (Ps-Odn). Nucleosides and Nucleotides 17:1557–1566. doi: 10.1080/07328319808004686.
48. Saeed M, Gondeau C, Hmwe S, et al (2013) Replication of hepatitis C virus genotype 3a in cultured cells. Gastroenterology 144:56–58. e7. doi: 10.1053/j.gastro.2012.09.017.
49. Al-Etaibi AM, Al-Awadi N a, El-Apasery M a, Ibrahim MR (2011) Synthesis of some novel pyrazolo[1,5-a] pyrimidine derivatives and their application as disperse dyes. Molecules 16:5182–5193. doi: 10.3390/molecules16065182.
50. Hassanien AA, Amr AE, Ghozlan SAS (2000) Utility of (p -Sulfonamidophenyl) azomalononitrile, and Ethyl Acetoacetate in Synthesis of Fused Azole and Azines Derivatives II. J Chinese Chem Soc 47:1273–1278. doi: 10.1002/jccs.200000176.
51. Vlietinck AJ VB DA (1998) Leads for antivirals from tradicional medicines. 333–344.