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Some 1,2,4-triazole derivatives: Synthesis and Antiviral Evaluation

Aymn E. Rashad[1,2*], Ahmed H. Shamroukh[1,3], Abeer H. El-Sayed[4], Jehane A. Micky[5], Nermin A. Marsok[5] and Farouk M. E. Abdel-Megeid[1]

[1]*Photochemistry Department, Chemical Industries Research Division, National Research Centre, Dokki, 12622, Giza, Egypt*
[2]*Current address: Chemistry Department, Faculty of Science and Humanities, Huraiymla, Shaqra University, (KSA)*
[3]*Current address: Chemistry Department, Faculty of Science, Hail University, (KSA)*
[4]*College of Science and Arts at Alasyah, Qassim University, (KSA)*
[5]*Chemistry Department, Faculty of Science, Al-Azhar University, Egypt*

ABSTRACT

The starting material 2-(5-phenyl-3H-1,2,4-triazol-3-ylsulfanyl)acetohydrazide (1) was used as a precursor for preparation of some novel substituted 1,2,4-triazole derivatives 2-6. Also, some acyclic C-nucleosides 7a,e were prepared by treating compound 1 with some aldoses. Moreover, treatment of compound 7b with acetic anhydride gave the acetylated product 8b. Antiviral bioassays were carried out to test compounds 2–5, 7a, 7b, 7d, 7e, and 8b. The CC_{50} and IC_{50} were determined in addition to the selectivity index (SI) for tested compounds (Tables 1-3). It was obvious that, most of the tested compounds have significant antiviral activity among of these compounds the sugar hydrazone derivatives 7a, 7b and 7d have the highest antiviral activity against HBV virus in comparing with the anti-influenza drug Zanamivir. While, all of the tested compounds have no antiviral activity against herpetic viral type -1 and type-2 in comparison with reference antiviral drug Acyclovir.

Keywords: Triazoles; aldoses, C-nucleosides, antiviral activity.

INTRODUCTION

In the last decade, our research group interested in a research program on heterocyclic compounds which may serve as leads for designing novel chemotherapeutic agents, we were particularly interested in azoles, azines, fused azoloazines and their nucleosides. We interested in different methods of preparations, reactions as well as studying their pharmacological applications [1-5].

Moreover, triazoles and their related fused heterocycles are of considerable chemical and pharmaceutical utility and many of their derivatives were reported to possess antiviral [6], antimicrobial [7], anti-inflammatory [8], anticancer [9], and antioxidant [10] activities.

In addition, the synthesis of C-nucleosides were shown to exhibit prominent and versatile biological activities [11,12], and many of their derivatives have been prepared recently as potential antimicrobial [13] and antiviral agents [14]. So, many reports [15,16] appeared dealing with this class of nucleosides.

Based on the above mentioned research results, the goal of this study is not only to synthesize some novel substituted triazoles and their corresponding C-nucleosides but also to obtain new compounds which are expected to possess notable pharmacological applications.

MATERIALS AND METHODS

1. Chemistry

All melting points are uncorrected and measured using Electro-Thermal IA 9100 apparatus (Shimadzu, Japan). Infrared spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). ^{1}H NMR and ^{13}C NMR spectra were determined on a Jeol-Ex-400 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shifts were expressed as part per million; (δ values, ppm) against TMS as internal reference. Mass spectra were recorded on VG 2AM-3F mass spectrometer (Thermo Electron Corporation, USA). Microanalyses were operated using Mario El Mentar apparatus, Organic Microanalysis Unit, and the results were within the accepted range (± 0.20) of the calculated values. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-precoated aluminum sheets (Type 60 F254; Merck, Darmstadt, Germany). The above spectral data were made at central labs of faculty of science, Saudi Arabia, and the Regional Centre for Mycology and Biotechnology, Egypt. Compound **1** was prepared according to reported methods, compound **1** (m.p 148-151 ˚C and *lit.* m.p 152-155˚C [17]).

*General procedure for the Synthesis of **2-6***

A mixture of compound **1** (1 mmol) and 3,4-dimethoxy- benzaldehyde, 3,5-dichlorobenzaldehyde, 3-(4-(dimethylamino)phenyl)-acrylaldehyde, 2,4-dihydroxybenzaldehyde, 2-hydroxynaphthalene-1-carbaldehyde (1 mmol) in absolute ethanol (30 mL) was refluxed for 3-7 h. The reaction mixture was cooled and the solid product was filtered off, dried, and recrystallized from appropriate solvents to give compounds **2-6**, respectively.

*2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-(2-(3,4-dimethoxyphenyl)ethyliden)- acetohydrazide (**2**).*

White crystals; (Yield 70 %); m.p. 185-187 ^{o}C; IR (KBr) υ cm^{-1}: 1675.26 (C=N triazole), 1774.11 (C=O), 2800.03 (OCH_3), 3421.60 (NH aliphatic); ^{1}H NMR (DMSO-d_6) δ ppm: 3.36 (s, 2H, SCH_2), 3.46 (s, 6H, $2OCH_3$), 4.27 (s, 1H, CH triazole), 7.02-7.94 (m, 8H, Ar-H), 8.11 (s, H, CH aliphatic), 11.66 (s, 1H, NH aliphatic); ^{13}C NMR (DMSO-d_6) δ ppm: 38.1(aliphatic SCH_2), 56.6 ($2OCH_3$), 84.1 (triazole SCH), 125.1-138.2 (Ar-C), 145.3 (CH aliphatic), 165.1 (triazole), 173.4 (C=O amide); Anal. calcd. for $C_{19}H_{19}N_5O_3S$ (397. 45) (%): C, 57.42; H, 4.82; N, 17.62; S, 8.07. Found (%): C, 57.65; H, 4.65; N, 17.81; S, 7.87.

*2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-(2-(3,5-dichlorophenyl) ethylidene)- acetohydrazide (**3**).*

White crystals; (Yield 65 %); m.p. 202-204 ^{o}C; IR (KBr) υ cm^{-1}: 1664.61 (C=N triazole), 1774.21 (C=O), 2367.72 (CH aliphatic), 3422.30 (NH aliphatic); ^{1}H NMR (DMSO-d_6) δ ppm: 3.36 (s, 2H, SCH_2), 4.70 (s, 1H, CH triazole), 7.03-7.95 (m, 8H, Ar-H), 8.20 (s, H, CH aliphatic), 11.70 (s, 1H, NH aliphatic); ^{13}C NMR (DMSO-d_6) δ ppm: 39.5 (aliphatic SCH_2), 85.3 (triazole SCH), 124.3-131.2 (Ar-C), 153.1 (CH aliphatic), 168 (triazole), 174.4 (C=O amide); Anal. calcd. for $C_{17}H_{13}Cl_2N_5OS$ (406. 29) (%): C, 50.26; H, 3.23; N, 17.24; S, 7.89. Found (%): C, 50.47; H, 3.55; N, 17.69; S, 7.52.

*2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-(4-(4-(dimethylamino)phenyl)but-3-enyliden)acetohydrazide (**4**).*

Brown crystals; (Yield 75 %); m.p. 199- 201 ^{o}C; IR (KBr) υ cm^{-1}: 160.48 (C=N triazole), 1710.71 (C=O), 2902.71 (NH aliphatic); ^{1}H NMR (DMSO-d_6) δ ppm: 2.51 (s, 2H, CH_3), 3.36 (s, 2H, SCH_2), 4.26(s , 1H , CH triazole), 6.69-7.91(m, 9H, Ar-H), 7.93(s , H , CH aliphatic), 11.56(s, 1H, NH aliphatic); ^{13}C NMR (DMSO-d_6) δ ppm: 40.6 (CH_3), 39.5 (aliphatic SCH_2), 82.5 (triazol SCH), 112.3 (CH aliphatic), 124.0-130.2 (Ar-C), 168.4 (triazol), 173.7 (C=O amide); Anal. calcd. for $C_{19}H_{20}N_6OS$ (380. 47) (%): C, 59.98; H, 5.30; N, 22.09; S, 8.43. Found (%): C, 59.71; H, 5.66; N, 22.21; S, 8.21.

*2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-(2-(2,4-dihydroxyphenyl)ethyliden)- acetohydrazide (**5**).*

White crystals; (Yield 77 %); m.p. 189- 191 ^{o}C; IR (KBr) υ cm^{-1}: 1629.56 (C=N triazole), 1774.03 (C=O), 3421.81 (NH aliphatic), 3568.18 (2OH broad band); ^{1}H NMR (DMSO-d_6) δ ppm: 3.33 (s, 2H, SCH_2), 4.26 (s, 1H , CH triazole), 6.33 (s , 2H, 2OH), 7.33- 7.69 (m, 8H, Ar-H), 8.21 (s, H, CH aliphatic), 11.86 (s , 1H, NH aliphatic); ^{13}C NMR (DMSO-d_6) δ ppm: 39.1(aliphatic SCH_2), 80.1 (triazol SCH), 124.1-130.2 (Ar-C), 148.1 (CH aliphatic), 169.2 (triazol), 170.2 (C=O amide); Anal. calcd. for $C_{17}H_{15}N_5O_3S$ (369. 4) (%): C, 55.27; H, 4.09; N, 18.86; S, 8.68. Found (%): C, 55.49; H, 4.31; N, 18.67; S, 8.48.

*2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-(2-(2-hydroxynaphthalen-1-yl)ethyliden)- acetohydrazide (**6**).*

White crystals; (Yield 76 %); m.p. 212- 214 ^{o}C; IR (KBr) υ cm^{-1}: 1686.02 (C=N triazole), 1773.66 (C=O), 3420.21 (NH aliphatic), 3568.11 (OH); ^{1}H NMR (DMSO-d_6) δ ppm: 3.34 (s, 2H , SCH_2), 4.37 (s, 1H , CH triazole), 4.81 (s , 1H , OH), 7.21 - 7.94 (m, 5H, Ar-H), 7.21 - 8.78 (m, 6H, naphthaline), 9.23 (s , H , CH aliphatic), 12.25 (s, 1H, NH aliphatic); ^{13}C NMR (DMSO-d_6) δ ppm: 36.4 (aliphatic SCH_2), 109.1 (triazol SCH), 119.9-133.1 (Ar-C), 124.7-160.1 (naphthaline), 147.1 (CH aliphatic), 167.3 (triazole), 174.2 (C=O amide); Anal. calcd. for $C_{21}H_{17}N_5O_2S$ (403. 46) (%): C, 62.52; H, 4.25; N, 17.36; S, 7.95. Found (%): C, 62.34; H, 4.51; N, 17.57; S, 7.71.

*General procedure for the Synthesis of **7a-e**.*
A mixture of compound **1** (1 mmol) and D-glucose, D-galactose, D-mannose, L-arabinose or D-Xylose (3 mmol) in ethanol (30 mL) and a catalytic amount of glacial acetic acid were refluxed on a hot water bath for 3-5 h (reaction progress was monitored by TLC). The reaction mixture was cooled and the precipitate formed was filtered off, washed with cold ethanol, dried, and crystallized from appropriate solvents to give compounds **7a-e**, respectively.

*2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-(3,4,5,6)-3,4,5,6,7-pentahydroxyheptyliden) acetohydrazide (**7a**).*
White crystals; (Yield 65 %); m.p. 251- 253 ^{o}C; IR (KBr) υ cm^{-1}: 1595.84 (C=N triazol), 1675.82 (C=O), 3400.13 (NH aliphatic , OH glucose broad band); ^{1}H NMR (DMSO-d$_6$) δ ppm: 1.00 (s, NCH aliphatic), 3.22-4.51 (m, CHOH, CH$_2$OH glucose), 3.51 (s, 2H , SCH$_2$), 3.82-4.32 (m, CH$_2$, glucose), 4.21 (s, 1H, CH triazole), 4.81 (m, CH, glucose), 7.28-8.20 (m, 5H, Ar-H), 9.80 (s, 1H, NH aliphatic); ^{13}C NMR (DMSO-d$_6$) δ ppm: 39.00 (aliphatic SCH$_2$), 62.21, 70.09, 73.98, 75.52, 82.13 (glucose), 90.34 (triazole SCH), 122.51-137.53 (Ar-C), 157.31 (C=N aliphatic), 160.41 (triazole), 165.11 (C=O amide); Anal. calcd. for $C_{16}H_{20}N_5O_6S$ (410.00) (%): C, 46.82; H, 4.91; N, 17.06; S, 7.81. Found (%): C, 47.05; H, 4.66; N, 16.86; S, 8.02.

*2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-(3,4,,6)-3,4,5,6,7-pentahydroxyheptyliden)acetohydrazide (**7b**).*
White crystals; (Yield 60 %); m.p. 249- 251 ^{o}C; IR (KBr) υ cm^{-1}: 1639.57 (C=N triazole), 1677.84 (C=O), 3267.80 (NH aliphatic), 3428.34 (OH glactose); ^{1}H NMR (DMSO-d$_6$) δ ppm: 2.7 (s, NCH aliphatic), 3.54-4.62 (m, CHOH, CH$_2$OH galactose), 3.80 (s, 2H, SCH$_2$), 4.20- 4.82 (m, 1H, CH$_2$OH galactose), 4.23 (s, 1H, CH triazole), 7.80 - 8.20 (m, 5H, Ar-H), 11.80 (s, 1H, NH aliphatic); ^{13}C NMR (DMSO-d$_6$) δ ppm: 38.2 (aliphatic SCH$_2$), 63.2, 70.7, 76.1, 78.9, 84.3 (galactose), 92.1 (triazole SCH), 122.1-136.8 (Ar-C), 160.2 (C=N), 162.3 (C, triazole), 172.4 (C=O amide); Anal. calcd. for $C_{16}H_{20}N_5O_6S$ (410.00) (%): C, 46.82; H, 4.91; N, 17.06; S, 7.81. Found (%): C, 47.04; H, 4.67; N, 16.84; S, 8.04.

*2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-((3,4,5,6)-3,4,5,6,7-pentahydroxyheptyliden)acetohydrazide (**7c**).*
Bright white crystals; (Yield 71 %); m.p. 246- 248 ^{o}C; IR (KBr) υ cm^{-1}: 1693.50 (C=N triazole), 1750.00 (C=O), 3228.82 (NH aliphatic), 3456.44 (OH mannose, broad band); ^{1}H NMR (DMSO-d$_6$) δ ppm: δ 1.3 (s, NCH aliphatic) , 2.7-4.18 (m, CHOH, CH$_2$OH D-mannose) , 3.3 (s, 2H, SCH$_2$), 4.15(s , 1H , CH triazole), 4.20- 4.82 (m, 3H, CH, CH$_2$OH mannose), 7.28 - 8.20 (m, 5H, Ar-H), 11.80 (s , 1H, NH aliphatic); ^{13}C NMR (DMSO-d$_6$) δ ppm: 38.2 (aliphatic SCH$_2$), 57.3, 70.5, 73.1, 77.1, 79.5 (mannose), 90.5 (triazole SCH), 122.2-138.5 (Ar-C), 158.4 (C=N), 164.7 (triazole), 170.2 (C=O amide); MS m/z (%):410.41 (M^{+}, 1.27%); Anal. calcd. for $C_{16}H_{20}N_5O_6S$ (410.00) (%): C, 46.82; H, 4.91; N, 17.06; S, 7.81. Found (%): C, 47.06; H, 4.68; N, 16.86; S, 8.01.

2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-(3,4,5)-3,4,5,6-tetrahydroxyhexyliden) -acetohydrazide (7d).
White crystals; (Yield 68 %); m.p. 244- 246 ^{o}C; IR (KBr) υ cm^{-1}: 1678.76 (C=N triazole), 1734.95 (C=O), 2944.71 (CH aliphatic), 3084.33 (CH aromatic), 3327.67 (NH aliphatic, OH arabinose); ^{1}H NMR (DMSO-d$_6$) δ ppm: δ 1.6 (s, NCH aliphatic), 3.00- 4.56 (m, CHOH, CH$_2$OH , L-arabinose) , 3.71 (s, 2H, SCH$_2$), 3.89 (s, 1H, CH triazole), 4.65-5.23 (m, CH$_2$OH, CHOH, L-arabinose), 7.80 - 8.34 (m, 5H, Ar-H), 8.32 (s, 1H, NH aliphatic); ^{13}C NMR (DMSO-d$_6$) δ ppm: 39.1 (aliphatic SCH$_2$), 57.1, 72.4, 75.1, 78.9 (L-arabinose), 93.5 (triazol SCH), 123.2-136.8 (Ar-C), 153.4 (C=N), 164.3 (triazole), 174.9 (C=O amide); Anal. calcd. for $C_{15}H_{19}N_5O_5S$ (381.41) (%): C, 47.24; H, 5.02; N, 18.36; S, 8.41. Found (%): C, 47.45; H, 4.86; N, 18.56; S, 8.19.

2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-(3,4,5)-3,4,5,6-tetrahydroxyhexyliden)- acetohydrazide (7e).
White crystals; (Yield 60 %); m.p. 240-242 ^{o}C; IR (KBr) υ cm^{-1}: 1654.15 (C=N triazole), 1684.54 (C=O), 3263.82 (NH aliphatic, OH D-Xylose); ^{1}H NMR (DMSO-d$_6$) δ ppm: δ 1.88 (s, NCH aliphatic), 2.09-4.88 (m, CHOH, CH$_2$OH D- Xylose), 3.20 (s , 2H , SCH$_2$), 3.80 (s, 1H, CH triazole), 4.30-9.23 (m, CHOH, CH$_2$ OH, D- Xylose), 7.40-7.80 (m, 5H, Ar-H), 11.4 (s, 1H, NH aliphatic); ^{13}C NMR (DMSO-d$_6$) δ ppm: 36.2 (aliphatic SCH$_2$), 58.4, 64.1, 70.2, 78.6, 88.1, 95.1 (triazol SCH), 122.1-137.5 (Ar-C), 150.9 (C=N), 164.2 (triazole), 178.2 (C=O amide); Anal. calcd. for $C_{15}H_{19}N_5O_5S$ (381.41) (%): C, 47.24; H, 5.02; N, 18.36; S, 8.41. Found (%): C, 47.43; H, 4.83; N, 18.55; S, 8.22.

2-(5-Phenyl-3H-1,2,4-triazol-3-ylsulfanyl)-N-(3,4,5,6)-3,4,5,6,7-pentaactylheptyliden)- acetohydrazide (8b).
A solution of the compound **7b** (1 mmol) in a mixture of acetic anhydride / pyridine (20 ml, 1:1) were stirred overnight at room temperature, poured into ice-water (25 mL). The mixture was then extracted from chloroform several times (150 mL), after the removal of chloroform under reduced pressure, the precipitate was collected, dried, and crystallized from methanol to give compound **8b** as brown crystals, yield 40 %, m.p. 231- 233 ^{o}C. IR (KBr) υ cm^{-1}: 1646.89 (C=N triazole), 1702.27 (C=O), 2961.57 (NH aliphatic), 3409.28 (OH glactose); ^{1}H NMR (DMSO-d$_6$) δ ppm: δ 2.1 (s, CH$_2$, galactose) , 2.27-2.46 (m, OAc), 3.54-4.62 (m, CHOH, CH$_2$OH galactose) , 3.80 (s, 2H , SCH$_2$), 4.20- 4.82 (m , 1H , CH$_2$ OH galactose), 4.23 (s, 1H, CH triazole), 7.30 - 7.82 (m, 5H, Ar-H), 11.55 (s, 1H, NH aliphatic); ^{13}C NMR (DMSO-d$_6$) δ ppm: 22.5 (CH$_2$ galactose), 38.1 (aliphatic SCH$_2$), 62.7, 68.2, 70.12,

74.3, 78.1 (galactose), 92.2 (triazole SCH), 122.2-138.3 (Ar-C), 156.2 (C=N), 163.4 (triazole), 169.6 (C=O amide), 174 (C=O OAc); Anal. calcd. for $C_{26}H_{27}N_5O_9S$ (585.59) (%): C, 53.33; H, 4.65; N, 11.96; S, 5.48. Found (%): C, 53.01; H, 4.93; N, 12.18; S, 5.67.

2. Antiviral activity

2.1 Methodology

Evaluation of the antiviral activity using cytopathic effect inhibition assay [18], this assay was selected to show specific inhibition of a biologic function, that is, a cytopathic effect in susceptible mammalian cells.

In brief, monolayers of 10000 vero cells adhering at the wells in a 96 – well microtiter plate were incubated for 24h at 37 ^oC in humidified incubator with 5 % CO_2.

The plates were washed with fresh DMEM and challenged with 10^4 doses of hepatitis B virus, HSV-1, and HSV-2 virus, and the cultures were simultaneously treated with two-fold serial dilutions of the tested compounds, starting from 1000 µg /m L and going up to about 2 µg /mL (1000, 500, 250, 125, 62.5,……, 1.95 µg /mL) in a fresh maintenance medium, following this, they were incubated at 37 ^oC for 48 h. Infection controls, as well as an untreated vero cell concentration of the tested compounds. Every 24 h, an observation was made under the inverted microscope until the virus in the control wells showed complete viral – induced cytopathic effect. Antiviral activity was determined by the inhibition of the cytopathic effect compared to a control that is the protection offered by the tested compounds to the cells was scored.

Three independent experiments were assessed, each containing four replicates per treatment Adefovir, which is clinically used for the treatment of hepatitis B viral disease, Acyclovir which is clinically used for the treatment of herpetic viral type-1 and 2 disease and Cidofovir, was used as positive control in this assay system.

After the incubation period, the media was aspirated, and then the cells were stained with a 1% crystal violet solution for 30 min., thereafter, all excess stain was removed by rinsing the plates with tap water.

The plates were allowed to dry , and then glacial acetic acid (30 %) was added to all wells and mixed thoroughly, the absorbance of the plates was measured after gentle shaking on a microplate reader (TECAN, inc.) at 590 nm. The viral inhibition rate was calculated as follows:

[(ODtv – ODcv) / (ODcd – Odcv)] × 100, ODtv, ODcv, and ODcd indicate the absorbance of the tested compounds with virus infected cells, the absorbance of the virus control and the absorbance of the cell control, respectively.

From these data, the dose that inhibited viral infection by 50 % (IC_{50}) was estimated with respect to virus control from the graphic plots, using STATA modeling software. IC_{50} was determined directly from the curve obtained by plotting the inhibition of the virus yield against the concentration of the samples. The selectivity index (SI) was calculated from the ratio of CC_{50} to IC_{50} in order to determine whether each compound had sufficient antiviral activity that exceeded its level of toxicity [19]. This index is referred to as a therapeutic index and it was also used to determine whether a compound warranted further study. Compounds that have SI value of 2 or more were considered to be active.

2.2 Cytotoxicity evaluation using viability assay

The vetro cell lines in the cytotoxicity assay were seeded in 96 -well plates at a cell concentration of 1×10^4 cells per well in 100 µ L of the growth medium.

Fresh medium containing different concentrations of the tested samples was added after 24 h of seeding. Serial two - fold dilutions of the tested compounds were added confluent cell monolayers dispensed into 96- well, flat – bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette .
The micrtiter plates were incubated at 37 ^{o} C in a humidified incubator with 5 % CO_2 for a period of 48 h.

Three wells were used for each concentration of the tested samples. Control cells were incubated without test samples and with or without DMSO. The small percentage of DMSO present in the wells (maximal 0.1 %) was not found to affect the experiment.

After the end of the incubation period, the viable cell yield was determined by a colorimetric method. In brief, the media were aspirated, and the crystal violet solution (1%) was added to each well for at least 30 min. Then stain was

removed, and the plates were rinsed using tap water until all excess stain was removed, Glacial acetic acid (30%) was then added to all wells and mixed thoroughly.

Absorbance of the glacial acetic acid solution in each well was then measured at 590 nm using a microplate reader (Sunrise, Inc, USA).

The absorbance was proportional to the number of surviving cells in the culture plate.

All the results were corrected for background absorbance detected in wells without added stain.

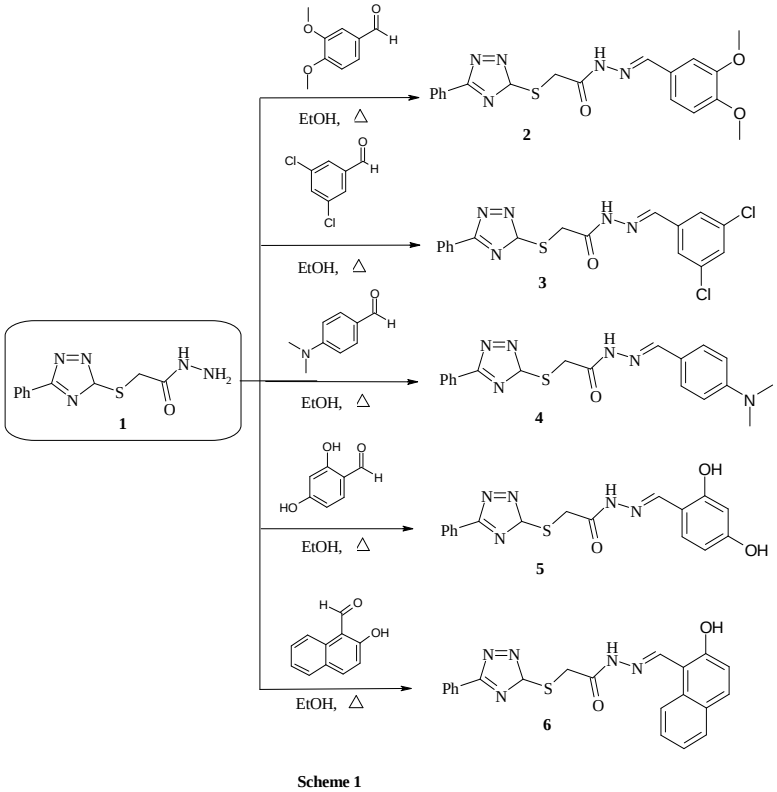
Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate and the cell cytotoxic effect of each tested compound was calculated.

RESULTS AND DISCUSSION

2.1. Chemistry

In this work, the starting material 2-(5-phenyl-3*H*-1,2,4-triazol-3-ylsulfanyl)acetohydrazide **1** was prepared in good yield according to the reported procedure [17], and treated with 3,4-dimethoxy-benzaldehyde to afford 1-(1*H*-indol-3-yl)ethylidene)-2-(5-phenyl-3*H*-1,2,4-triazol-3-ylsulfanyl)-acetohydrazide **2**.

The structure of compound **2** was supported by elemental analysis and spectral data, especially the absence of the NH_2 group in IR spectrum. Also, the ^{1}H NMR spectrum of compound **2** revealed signals at 3.46 ppm characteristic for $2OCH_3$ and at 8.11 ppm for CH aliphatic. Moreover, the ^{13}C NMR spectrum of the mentioned compound showed signals at: δ, ppm 56.6 ($2OCH_3$), 145.3 (CH aliphatic) (cf. Experimental).

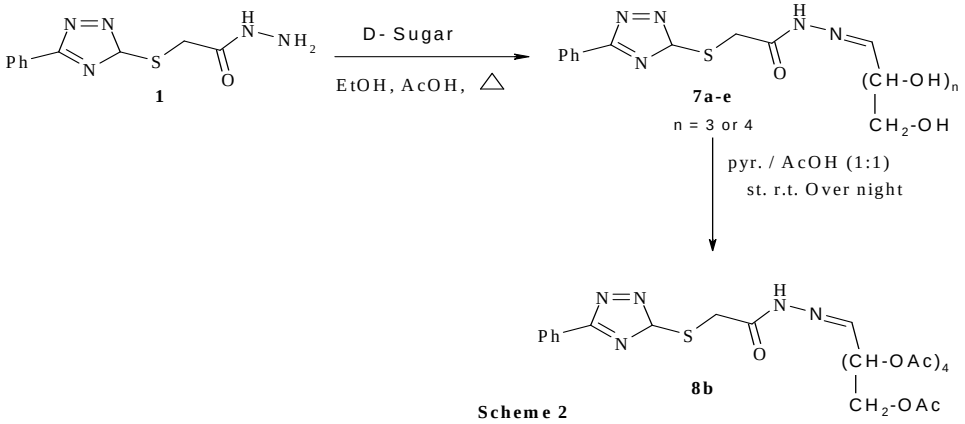


Scheme 1

Similarly, treatment of compound **1** with 3,5-dichlorobenzaldehyde, 3-(4-(dimethylamino)phenyl)acrylaldehyde, 2-hydroxynaphthalene-1-carbaldehyde or 2,4-dihydroxybenzaldehyde, in ethanol, afforded compounds **3-6,** respectively. Like that of compound **2**, the structure of compounds **3-6** was confirmed by elemental analysis and the spectral data. The IR and ^{1}H NMR spectra for these compounds revealed the absence of the NH_2 group and the presence of signals characteristic for OH groups in compounds **5** and **6**. Meanwhile, the ^{13}C NMR spectra of the mentioned compounds showed signals characteristic for CH aliphatic as well as substituted phenyl in compounds **3, 4**, **6** and naphthyl signals in compound **5** (cf. Experimental).

On the other hand, nucleoside analogues as chemotherapeutic agents have been comprehensively described in the literature [9–14], and many of these analogues were synthesized recently as antiviral agents [20–22]. In particular, C-nucleosides were reported to be of great value from the biological point of view [12-14], and many of their derivatives have been synthesized and were tested also against herpes simplex viruses [14, 16, 23].

In this investigation, the hydrazone derivatives **7a,e** were prepared by reacting compound **1** with some monosacharides: namely, D-glucose, D-galactose, D-mannose, L-arabinose or D-Xylose in absolute ethanol and catalytic amounts of glacial acetic acid. The latter compounds revealed absorption bands for (OH+NH), and (C=N) in IR spectra and their ^{1} H NMR spectra showed the presence of the sugar protons, NH, and azo-methine (CH=N) (cf. Experimental). Acetylation of the hydrazone derivative **7b** with acetic anhydride at room temperature gave the O-acetylated sugar derivative **8b**. The IR spectrum of compound **8b** revealed the absence of hydroxyl groups and showed absorption bands due to NH and C=O groups. Its ^{1} H NMR spectrum showed the presence of OAc groups and one exchangeable NH, while the ^{13}C NMR spectrum revealed the presence of acetoxy groups (cf. Experimental).



Scheme 2

2.2. Biological activity

Antiviral bioassays.

Antiviral bioassays were carried out to tested compounds **2–5**, **7a**, **7b**, **7d**, **7e** and **8b**. The CC_{50} and IC_{50} were determined in addition to the selectivity index (SI) for tested compounds (Tables 1-3). It was obvious that, most of the tested compounds have significant antiviral activity among of these compounds the sugar hydrazone derivatives **7a**, **7b** and **7d** have the highest antiviral activity against HBV virus in comparing with the anti-influenza drug Zanamivir.

While, all of the tested compounds have no antiviral activity against herpetic viral type -1 and type-2 in comparison with reference antiviral drug (Acyclovir).

Table 1: Antiviral activity against HBV virus of the prepared compounds by determination of both CC_{50} and IC_{50}

Compound	CC_{50} (µg/ml)	IC_{50} (µg/mL) HBV	Selectivity index
2	95.55	98.51±0.11	0.97
5	127.60	87.4±0.23	1.46
7a	255.45	80.87 ± 0.780	3.16
7b	208.97	78.86± 0.88	2.65
7d	179.86	87.74±0.31	2.05
Adefovir	540	305	1.77

CC_{50} is 50% cytotoxicity concentration, IC_{50} is 50% inhibitory concentration, Selectivity index (SI = CC_{50}/IC_{50}), d No SI can be obtained.

Table 2: Antiviral activity against herpetic viral type -1 of the prepared compounds by determination of both CC_{50} and IC_{50}

Compound	CC_{50} (µg/ml)	IC_{50} (µg/mL) Adenovirus	Selectivity index
3	95.55	>100	D
4	180.41	>100	D
7e	208.97	>100	D
8b	179.86	>100	D
Acyclovir	130	2.1	61.9

CC_{50} is 50% cytotoxicity concentration, IC_{50} is 50% inhibitory concentration, Selectivity index (SI = CC_{50}/IC_{50}), d No SI can be obtained.

Table 3: Ativiral activity against herpetic viral type- 2 of the prepared compounds by determination of both CC_{50} and IC_{50}

Compound	CC_{50} (µg/ml)	IC_{50} (µg/mL)	Selectivity index
		Adenovirus	
3	315.11	>100	D
4	251.03	>100	D
7e	203.75	>100	D
8b	100.02	>100	D
Acyclovir	130	2.9	44.82

CC_{50} is 50% cytotoxicity concentration, IC_{50} is 50% inhibitory concentration, Selectivity index (SI = CC_{50}/IC_{50}), and No SI can be obtained.

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