Available online on 15.05.2022 at *http://ujpr.org*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)
Open access to Pharmaceutical Research

Volume 7, Issue 2, 2022

RESEARCH ARTICLE

SYNTHESIS AND ANTICANCER ACTIVITY TOWARDS HEPG-2 AND MCF-7 OF NEW 2-AMINO-1,3,4-THIADIAZOLE AND THEIR SUGAR DERIVATIVES

Samy A. El Assaly[1], Nagwan S. El Bakary[2], Mohammed T. Abdel Aal[3], Wael A. El-Sayed[4,5], Ibrahim F. Nassar[*6], Hanem M. Awad[7]

*[*1]Natural and Microbial Products Chemistry Department, National Research Center (NRC), Dokki, Giza, Egypt.*
[2,3]Chemistry Department, Faculty of Science, Menoufia University, Shebin El-Kom, Egypt.
[4]Natural and Microbial Products Chemistry Department, National Research Center (NRC), Dokki, Giza, Egypt. [4]Department of chemistry, College of Science, Qassim University, KSA.
[5]Photochemistry Department, National Research Centre, El-Behouth St, Dokki, Cairo, Egypt.
[6]Faculty of Specific Education, Ain Shams University (ASU), 365 Ramsis street, Abassia, Cairo, Egypt.
[7]Tanning Materials and Leather Technology Department, National Research Centre, Dokki, Cairo, Egypt.

ABSTRACT

Background: In recent papers, it was found that 1,3,4-oxadiazole, 1,3,4-thiadiazoleand 1,2,4-triazole pharmacophores are present in several drugs, tiodazosin and nesapidil (antihypertensive), raltegravir (antiretroviral), Furamizole, cefazolin and ceftezole (antibiotics), acetazolamide and methazolamide (carbonic anhydrase inhibitors), sulfamethizole (antibacterial), fluconazole, ravuconazole, voriconazole, itraconazole, posaconazole, and tebuconazole (antifungal).

Methods: Thiosemicarbazide was reacted with ethyl p-substituted-phenyl glycinate; namely, ethyl p-tolylglycinate (1), ethyl p-methoxyphenylglycinate (2) or ethyl p-bromophenylglycinate (3), respectively to give compounds 4-6, which then kept with conc. H_2SO_4 overnight to yield 1,3,4-thiadiazol-2-amine derivatives 7-9. Compounds 10-18 were yielded by reaction of compounds 7-9 with D-sugars namely, D-galactose, D-glucose and/ or D-xylose in ethanol and catalytic amount of acetic acid. Compounds (10-18) were then acetylated with acetic anhydride to form compounds (19-21). Finely compound 7 was reacted with chloroacetyl chloride and/or acetic anhydride to afford compounds 22 and/or 23 respectively.

Results: Six compounds were evaluated in vitro for their cytotoxic activity on the HepG-2 and MCF-7 human cancer cell lines.

Conclusion: Among the tested compounds, compounds 6 and 13 were found to be the more potent for their cytotoxic activity on the two cancer cell lines.

Keywords: 1,3,4-Thiadiazol-2-amine, Cytotoxicity, HepG-2, MCF-7, Thiosemicarbazide.

Article Info: Received 15 February 2022; Revised 12 March; Accepted 30 April, Available online 15 May 2022

Cite this article-
Nassar IF, El Bakary NS, Abdel Aal MT, El-Sayed WA. Synthesis and anticancer activity towards HepG-2 and MCF-7of new 2-amino-1,3,4-thiadiazole and their sugar derivatives. Universal Journal of Pharmaceutical Research 2022; 7(2):74-80.

DOI: *https://doi.org/10.22270/ujpr.v7i2.755*

Address for Correspondence:
Dr. Ibrahim F. Nassar, Faculty of Specific Education, Ain Shams University (ASU), 365 Ramsis street, Abassia, Cairo, Egypt. Tel- +20 100 139 6728; E-mail: *Dr.Ibrahim.Nassar@sedu.asu.edu.eg*

INTRODUCTION

1,3,4-oxadiazole, 1,3,4-thiadiazoleand 1,2,4-triazole pharmacophores are present in several drugs viz., tiodazosin and nesapidil (antihypertensive), raltegravir (antiretroviral), Furamizole, cefazolin and ceftezole (antibiotics)[1], acetazolamide and methazolamide (carbonic anhydrase inhibitors), sulfamethizole (antibacterial)[2], fluconazole, ravuconazole, voriconazole, itraconazole, posaconazole, and tebuconazole (antifungal)[3-8]. It is also observed that in response to antimicrobial resistance, medicinal chemists have intended to concentrate their efforts on the development of more potent and effective antimicrobial drugs. The hybridization of the pharmacophores 1,3,4-Thiadiazole and 4-thiazolidinone in one molecular frame could show highly effective anti-inflammatory with broad spectrum and minimum side effects. Combining both scaffolds was expected to inhibit both COX-2 (1, 3, 4-thiadiazole), LOX (4-thiazolidinone) and provide better selectivity towards COX-2 over COX-1 enzyme due to their large volume which will not fit in the smaller COX-1 binding pocket[9].

1,3,4-Thiadiazoles exhibit a broad spectrum of biological activity[10] such as antimicrobial, anti-

inflammatory, anticancer, antituberculosis, antiparasitic, anticonvulsants, antioxidant, herbicidal and insecticidal properties. Desaglybuzole 124 (antidiabetic), Acetazolamide 125 (for glaucoma), Furidiazine 126(antimicrobial) and Butazolamide 127 (diuretic) are commercially available 1,3,4-thiadiazole drugs.In recent years, we were put in a project aiming for the development of a series of novel anticancer agents[11-23] which contributed in publishing some effective papers in this order. Therefore, we synthesized new 2-(*p*-Substituted-phenylglycyl)hydrazine-1-carbo-thioamide derivatives which were cyclized to 1,3,4-thiadiazole-2-amine derivatives and then were reacted with *D*-sugars namely, *D*-galactose, *D*-glucose or *D*-xylose in ethanol and catalytic amount of acetic acid. Compounds (**10-18**) were then acetylated with acetic anhydride to form compounds (**19-21**). Finely, compound **7** was reacted with chloroacetyl chloride and/or acetic anhydride to afford compounds **22** and/or **23** respectively. Six compounds were evaluated *in vitro* for their cytotoxicity activity on the HepG-2 and MCF-7 human cancer cell lines.

MATERIALS AND METHODS

All the fine chemicals are purchased from the sigma Aldrish company and the pure solvents are purchased from El Gomhoria chemical company, Cairo, Egypt. The spectroscopic analyses are performed at the Microanalytical Center, Cairo university, Cairo, Egypt. The biological Activity of the new compounds were performed at the biological activity center, Al Azher University, Nasr City, Cairo, Egypt.

Experiments for Chemistry part.

General Procedures

TLC was performed using aluminum plates pre-coated with silica gel 60 or 60 F254 (Merck) and visualized by iodine or UV light (254 nm). Melting points were determined on a Böetius PHMK (VebAnalytik Dresden) apparatus. The NMR spectra were recorded on a Varian Gemini 300 and Bruker DRX 400 spectrometer at 25ºC, unless otherwise stated. The NMR signals were referenced to TMS and the solvent shift ($(CD_3)_2SO$ δ H 2.50 and δ C 39.5). Coupling constants are given in Hz and without sign. The IR-spectra were recorded (KBr) on a Jasco FT/IR-410 instrument; the UV−VIS spectra were recorded (CH_3OH) on a M40 Karl Zeiss Jena instrument. Mass spectrometry was carried out on a Varian FINNIGAN MAT 212 instrument and the elemental analysis on the Perkin Elmer 240 instrument.

2-(*p*-Substituted-phenylglycyl)hydrazine-1-carbo-thioamide (4-6)

To a well stirred suspension of thiosemicarbazide (10 mmol) in ethanol (5 mL), was added ethyl *p*-substituted-phenyl glycinate (**1-3**); namely, ethyl p-tolyl glycinate, ethyl p-methoxyphenyl glycinate or ethyl *p*-bromophenyl glycinate, respectively. The reaction mixture was refluxed for 4 hrs, and then the solvent was reduced under vacuum. The remaining residue was left to cool at room temperature and the precipitated solid was filtered, dried, and crystallized form ethanol to give compounds (**4-6**), respectively.

2-(*p*-tolylglycyl)hydrazine-1-carbothioamide (4)

Yield: 79%; m.p. 275-277 ºC. IR (KBr) cm^{-1}, ύ: 3375-3265 (NH_2), 3178 (NH), 1721 (C=O), 1609 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 2.1 (s, 3H, CH_3), 4.5 (s, 2H, CH_2), 5.73 (br.s, 2H, NH_2), 6.46 (d, 2H, Ar-H), 6.9 (d, 2H, Ar-H), 7.19 (br.s, 1H, NH), 7.55 (br.s, 1H, NH), 8.63 (s, 1H, NH). m/z: 238.09 (100.0%), 239.09 (10.8%), 240.08 (4.5%), 239.09 (1.5%); Elemental Analysis for ($C_{10}H_{14}N_4OS$, M. Wt: 238.31) Calcd. C, 50.40; H, 5.92; N, 23.51; S, 13.46; Found: C, 50.45;H, 5.89; N, 23.50; S, 13.49.

2-(*p*-methoxyphenylglycyl)hydrazine-1-carbothioa-mide (5)

Yield: 79%; m.p. 274-276 ºC. IR (KBr) cm^{-1}, ύ: 3378-3264 (NH_2), 3177 (NH), 1728 (C=O), 1620 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 3.9 (s, 3H, CH_3), 4.61 (s, 2H, CH_2), 5.75 (br.s, 2H, NH_2), 6.5 (d, 2H, Ar-H), 6.94 (d, 2H, Ar-H), 7.20 (br.s, 1H, NH), 7.56 (br.s, 1H, NH), 8.65 (s, 1H, NH). m/z: 254.08 (100.0%), 255.09 (10.8%), 256.08 (4.5%), 255.08 (1.5%); Elemental Analysis for ($C_{10}H_{14}N_4O_2S$, M.Wt: 254.31) Cacd: C, 47.23; H, 5.55; N, 22.03; S, 12.61; Found: C, 47.43; H, 5.60; N, 22.0; S, 12.66.

2-(*p*-bromophenylglycyl)hydrazine-1-carbothioa-mide (6)

Yield: 79%; m.p. 275-277 ºC. IR (KBr) cm^{-1}, ύ: 3380-3266 (NH_2), 3181 (NH), 1730 (C=O), 1621 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 4.62 (s, 2H, CH_2), 5.75 (br.s, 2H, NH_2), 6.55 (d, 2H, Ar-H), 6.95 (d, 2H, Ar-H), 7.25 (br.s, 1H, NH), 7.59 (br.s, 1H, NH), 8.69 (s, 1H, NH). MS m/z: 303 (M^+, 70%). m/z: 301.98 (100.0%), 303.98 (97.3%), 302.99 (9.7%), 304.99 (9.5%), 303.98 (4.5%), 305.98 (4.4%), 302.98 (1.5%), 304.98 (1.4%); Elemental Analysis for ($C_9H_{11}BrN_4OS$, M.Wt: 303.18) Calcd: C, 35.66; H, 3.66; Br, 26.36; N, 18.48; S, 10.57; Found: C, 35.45; H, 3.76; Br, 26.46; N, 18.55; S, 10.45.

5-[(*p*-Substituted-phenylimino)methyl]-1,3,4-thiad-iazol-2-amine (7-9)

A mixture of compounds (**4-6**) (0.05 mol) and conc. H_2SO_4 (20 mL) was kept overnight at room temperature, then poured into cold water, neutralized with liquid ammonia, and filtered. The product that obtained was recrystallized from ethanol–water (1:1) to give compounds (**7-9**).

5-[(*p*-tolylamino)methyl]-1,3,4-thiadiazol-2-amine (7)

Yield: 74%; m.p. 270-272 ºC. IR (KBr) cm^{-1}, ύ: 3400-3283 (NH_2, NH), 1620 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 2.6 (s, 3H, CH_3), 4.62 (s, 2H, CH_2), 5.20 (br.s, 2H, NH_2), 6.98 (d, 1H, Ar-H), 7.11 (d, 1H, Ar-H), 7.24 (d, 1H, Ar-H), 7.76 (d, 1H, Ar-H), 13.17 (s, 1H, NH); MS m/z: 220 (M^+, 70%). Elemental Analysis for ($C_{10}H_{12}N_4S$, M.Wt: 220.29) Calcd: C, 54.52; H, 5.49; N, 25.43; S, 14.55; Found: C, 54.56; H, 5.45; N, 25.50; S, 14.40.

5-[(*p*-methoxyphenylmino)methyl]-1,3,4-thiadiazol-2-amine (8)

Yield: 74%; m.p. 269-271 ºC. IR (KBr) cm^{-1}, ύ: 3350, 3228 (NH_2, NH), 3050 (C-H), 1610 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 3.9 (s, 3H, CH_3), 4.61 (s, 2H, CH_2), 5.75 (br.s, 2H, NH_2), 6.5 (d, 2H, Ar-H), 6.94 (d, 2H, Ar-H), 7.56 (br.s, 1H, NH), MS m/z: 236 (M^+,

70%). Elemental Analysis for ($C_{10}H_{12}N_4OS$, M.Wt: 236.29) Calcd: C, 50.83; H, 5.12; N, 23.71; S, 13.57; Found: C, 50.89; H, 5.23; N, 23.71; S, 13.47.

5-[(*p*-bromophenylamino)methyl]-1,3,4-thiadiazol-2-amine (9)

Yield: 74%; m.p. 270-272 ºC. IR (KBr) cm^{-1}, ύ: 3350, 3230 (NH_2, NH), 3065 (C-H), 1615 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 4.02 (s, 2H, CH_2), 5.75 (br.s, 2H, NH_2), 6.55 (d, 2H, Ar-H), 6.95 (d, 2H, Ar-H), 7.25 (br.s, 1H, NH); MS m/z: 284 (M^+, 1.90%), 285 (M^+, 7.63%). Elemental Analysis for ($C_9H_9BrN_4S$, M.Wt: 285.16) :Calcd: C, 37.91; H, 3.18; Br, 28.02; N, 19.65; S, 11.24; Found: C, 37.87; H, 3.23; N, 19.70; S, 11.24.

***N*-(*D*-Galactopyranosyl)-5-[(*p*-subistitutedamino)methyl]-1,3,4-thiadiazol-2-amine (10-18)**

A mixture of 5-[(*p*-tolylamino)methyl]-1,3,4-thiadiazol -2-amine (**7**), 5-[(*p*-methoxy phenylmino) methyl]-1,3, 4-thiadiazol-2-amine (**8**), 5-[(*p*-bromophenylamino) methyl]-1,3,4-thiadiazol-2-amine (**9**) (0.01 mol), *d*-galactose, *d*-glucose or *d*-xylose (0.011 mol) in ethanol (30 mL), and a catalytic amount of acetic acid (3 drops) were heated at reflux temperature for 4 hrs. The formed precipitate was filtered on hot, washed with water several times, dried, and recrystallized from ethanol to give compounds (**10-18**), respectively.

***N*-(*D*-sugarpyranosyl)-5-[(*p*-substituted amino) methyl]-1,3,4-thiadiazol-2-amine (10)**

Yield: 88%; m.p. 266-268 ºC. IR (KBr) cm^{-1}, ύ: 3460 (OH), 3225 (NH), 1681, 1610 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 2.6 (s, 3H, CH_3), 3.31-3.37 (m, 2H, H-6′,6″), 3.62-3.65 (m, 1H, H-5′), 3.94-4.25 (m, 2H, H-4′,3′), 4.32 (s, 2H, CH_2), 4.41 (m, 1H, OH), 4.77-4.86 (m, 2H, OH and H-2′), 4.98-5.24 (m, 2H, 2OH), 5.80 (d, 1H, *J* = 8.2 Hz, H-1′), 6.46 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 9.94-10.02 (br.s, 2H, 2NH ex.); Elemental Analysis for ($C_{16}H_{22}N_4O_5S$, M.Wt: 382.44) Calcd: C, 50.25; H, 5.80; N, 14.65; S, 8.38; Found: C, 50.45; H, 5.86; N, 14.45; S, 8.34.

***N*-(*D*-Glucopyranosyl)-5-[(*p*-tolylamino)methyl]-1,3,4-thiadiazol-2-amine (11)**

Yield: 63%; m.p. 249-251 ºC. IR (KBr) cm^{-1}, ύ: 3460 (OH), 3225 (NH), 1681, 1610 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 2.6 (s, 3H, CH_3), 3.31-3.37 (m, 2H, H-6′,6″), 3.62-3.65 (m, 1H, H-5′), 3.94-4.25 (m, 2H, H-4′,3′), 4.32 (s, 2H, CH_2), 4.41-4.49 (m, 2H, 2OH), 4.77-4.86 (m, 2H, OH and H-2′), 4.98-5.04 (m, 1H, OH), 5.82 (d, 1H, *J* = 8.2 Hz, H-1′), 6.46 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 9.94-10.02 (br.s, 2H, 2NH ex.); Elemental Analysis for ($C_{16}H_{22}N_4O_5S$, M.Wt: 382.44) Calcd: C, 50.25; H, 5.80; N, 14.65; S, 8.38; Found: C, 50.34; H, 5.87; N, 14.55; S, 8.40.

***N*-(*D*-Xylopyranosyl)-5-[(*p*-tolylamino)methyl]-1,3,4-thiadiazol-2-amine (12)**

Yield: 68%; m.p. 246-248 ºC. IR (KBr) cm^{-1}, ύ: 3460 (OH), 3225 (NH), 1681, 1610 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 2.6 (s, 3H, CH_3), 3.62-3.65 (m, 2H, H-5′,5``), 3.94-4.25 (m, 2H, H-4′,3′), 4.26 (m, 2H, CH_2), 4.41-4.49 (m, 2H, 2OH), 4.77-4.86 (m, 2H, OH and H-2′), 5.49 (d, 1H, *J* = 8.2 Hz, H-1′), 6.46 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 9.94-10.02 (br.s, 2H, 2NH ex.); Elemental Analysis for ($C_{15}H_{20}N_4O_4S$, M.Wt: 352.41) Calcd: C, 51.12; H, 5.72; N, 15.90; S, 9.10; Found: C, 51.22; H, 5.66; N, 15.90; S, 9.40.

***N*-(*D*-Galactopyranosyl)-5-[(*p*-methoxyphenylamino)methyl]-1,3,4-thiadiazol-2-amine (13)**

Yield: 62%; m.p. 222-224ºC. IR (KBr) cm^{-1}, ύ: 3460 (OH), 3225 (NH), 1681, 1610 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 3.31-3.37 (m, 2H, H-6′,6″), 3.62-3.65 (m, 1H, H-5′), 3.81 (s, 3H, CH_3), 3.94-4.25 (m, 2H, H-4′,3′), 4.32 (s, 2H, CH_2), 4.41 (m, 1H, OH), 4.77-4.86 (m, 2H, OH and H-2′), 4.98-5.24 (m, 2H, 2OH), 5.80 (d, 1H, *J* = 8.2 Hz, H-1′), 6.46 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 9.94-10.02 (br.s, 2H, 2NH Elemental Analysis for ($C_{16}H_{22}N_4O_6S$, M. Wt: 398.43) Calcd: C, 48.23; H, 5.57; N, 14.06; S, 8.05; Found: C, 48.33; H, 5.52; N, 14.0; S, 8.0.

***N*-(*D*-Glucopyranosyl)-5-[(*p*-methoxyphenylmino) methyl]-1,3,4-thiadiazol-2-amine (14)**

Yield: 68%; m.p. 251-253 ºC. IR (KBr) cm^{-1}, ύ: 3460 (OH), 3225 (NH), 1681, 1610 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 3.31-3.37 (m, 2H, H-6′,6″), 3.62-3.65 (m, 1H, H-5′), 3.81 (s, 3H, CH_3), 3.94-4.25 (m, 2H, H-4′,3′), 4.32 (s, 2H, CH_2), 4.41-4.49 (m, 2H, 2OH), 4.77-4.86 (m, 2H, OH and H-2′), 4.98-5.04 (m, 1H, OH), 5.82 (d, 1H, *J* = 8.2 Hz, H-1′), 6.46 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 9.94-10.02 (br.s, 2H, 2NH ex.); m/z: 398.13 (100.0%), 399.13 (17.3%); Elemental Analysis for ($C_{16}H_{22}N_4O_6S$, M.Wt: 398.43) Calcd: C, 48.23; H, 5.57; N, 14.06; S, 8.05; Found: C, 48.33; H, 5.45; N, 14.0; S, 8.12.

***N*-(*D*-Xylopyranosyl)-5-[(*p*-methoxyphenylamino) methyl]-1,3,4-thiadiazol-2-amine (15)**

Yield: 79%; m.p. 281-283ºC. IR (KBr) cm^{-1}, ύ: 3460 (OH), 3225 (NH), 1681, 1610 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 3.62-3.65 (m, 2H, H-5′,5``), 3.80 (s, 3H, CH_3), 3.94-4.25 (m, 2H, H-4′,3′), 4.26 (m, 2H, CH_2), 4.41-4.49 (m, 2H, 2OH), 4.77-4.86 (m, 2H, OH and H-2′), 5.49 (d, 1H, *J* = 8.2 Hz, H-1′), 6.46 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 9.94-10.02 (br.s, 2H, 2NH ex.); Elemental Analysis for ($C_{15}H_{20}N_4O_5S$, M.Wt: 368.41) Calcd: C, 48.90; H, 5.47; N, 15.21; S, 8.70; Found: C, 48.89; H, 5.50; N, 15.27; S, 8.77.

***N*-(*D*-Galactopyranosyl)-5-[(*p*-bromophenylamino) methyl]-1,3,4-thiadiazol-2-amine (16)**

Yield: 74%; m.p. 266-268 ºC. IR (KBr) cm^{-1}, ύ: 3460 (OH), 3225 (NH), 1681, 1610 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 3.31-3.37 (m, 2H, H-6′,6″), 3.62-3.65 (m, 1H, H-5′), 3.94-4.25 (m, 2H, H-4′,3′), 4.32 (s, 2H, CH_2), 4.41 (m, 1H, OH), 4.77-4.86 (m, 2H, OH and H-2′), 4.98-5.24 (m, 2H, 2OH), 5.80 (d, 1H, *J* = 8.2 Hz, H-1′), 6.46 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 9.94-10.02 (br.s, 2H, 2NH ex.); Elemental Analysis for ($C_{15}H_{19}BrN_4O_5S$, M.Wt: 447.30) Calcd: C, 40.28; H, 4.28; N, 12.53; S, 7.17; Found: C, 40.35; H, 4.14; N, 12.45; S, 7.23.

***N*-(*D*-Glucopyranosyl)-5-[(*p*-bromophenylamino) methyl]-1,3,4-thiadiazol-2-amine (17)**

Yield: 89%; m.p. 270-272 ºC. IR (KBr) cm^{-1}, ύ: 3460 (OH), 3225 (NH), 1681, 1610 (C=N); ^1H NMR (DMSO-d_6, 300 MHz): δ 3.31-3.37 (m, 2H, H-6′,6″), 3.62-3.65 (m, 1H, H-5′), 3.94-4.25 (m, 2H, H-4′,3′), 4.32 (s, 2H, CH_2), 4.41-4.49 (m, 2H, 2OH), 4.77-4.86 (m, 2H, OH and H-2′), 4.98-5.04 (m, 1H, OH), 5.82 (d, 1H, *J* = 8.2 Hz, H-1′), 6.46 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 9.94-10.02 (br.s, 2H, 2NH ex.); Elemental Analysis for ($C_{15}H_{19}BrN_4O_5S$, M.Wt: 447.30) Calcd:

C, 40.28; H, 4.28; N, 12.53; S, 7.17; Found: C, 40.34; H, 4.14; N, 12.50; S, 7.19.

***N*-(*D*-Xylopyranosyl)-5-[(*p*-bromophenylamino) methyl]-1,3,4-thiadiazol-2-amine (18)**

Yield: 77%; m.p. 275-277 ºC. IR (KBr) cm^{-1}, ύ: 3460 (OH), 3225 (NH), 1681, 1610 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz): 3.62-3.65 (m, 2H, H-5′,5``), 3.94-4.25 (m, 2H, H-4′,3′), 4.26 (m, 2H, CH_2), 4.41-4.49 (m, 2H, 2OH), 4.77-4.86 (m, 2H, OH and H-2′), 5.49 (d, 1H, *J* = 8.2 Hz, H-1′), 6.46 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 9.94-10.02 (br.s, 2H, 2NH ex.); Elemental Analysis for ($C_{14}H_{17}BrN_4O_4S$, M. Wt: 417.28) Calcd: C, 40.30; H, 4.11; Br, 19.15; N, 13.43; S, 7.68; Found: C, 40.40; H, 4.31; Br, 19.12; N, 13.41; S, 7.66.

***N*-(Tetra-*O*-acetyl-D-sugerpyranosyl)-5-[(*p*-substit-utedamino)methyl]-1,3,4-thiadiazol-2-amine (19-21)**

To a solution of glycosides **10**, **11** and **18** (1 mmol) in pyridine (15 mL) was added acetic anhydride (5 mmol) and the obtained clear solution was stirred at room temperature for 10 hrs. The reaction mixture was poured onto crushed ice, and the product that separated out was filtered off, washed with sodium hydrogen carbonate, water, then dried and recrystalized from ethyl acetate to give the acetylated products (**19-21**), respectively.

***N*-(Penta-*O*-acetyl-D-galactopyranosyl)-5-[(*p*-tolyl-amino)methyl]-1,3,4-thiadiazol-2-amine (19)**

Yield: 80%; m.p. 256-258 ºC. IR (KBr) cm^{-1}, ύ: 3225 (NH), 1748 (C=O), 1610 (C=N). m/z: 550.17 (100.0%), 551.18 (26.0%), 552.17 (4.5%), 552.18 (3.2%), 552.18 (1.8%), 551.17 (1.5%), 553.17 (1.2%). Elemental Analysis for ($C_{24}H_{30}N_4O_9S$, M. Wt: 550.58) Calcd: C, 52.36; H, 5.49; N, 10.18; S, 5.82. Found; C, 52.26; H, 5.42; N, 10.18; S, 5.80

***N*-(Penta-*O*-acetyl-D-glucopyranosyl)-5-[(*p*-tolyla-mino)methyl]-1,3,4-thiadiazol-2-amine (20)**

Yield: 89%; m.p. 270-272 ºC. IR (KBr) cm^{-1}, ύ: 3255 (NH), 1748 (C=O), 1608 (C=N)

m/z: 550.17 (100.0%), 551.18 (26.0%), 552.17 (4.5%); Elemental Analysis for ($C_{24}H_{30}N4O_9S$; 550.58) Calcd: C, 52.36; H, 5.49; N, 10.18; S, 5.82; Found: C, 52.23; H, 5.50; N, 10.22; S, 5.82.

***N*-(Tetra-*O*-acetyl-D-xylopyranosyl)-5-[(*p*-bromo-phenylamino)methyl]-1,3,4-thiadiazol-2-amine (21)**

Yield: 84%; m.p. 270-272 ºC. IR (KBr) cm^{-1}, ύ: 3225 (NH), 1751 (C=O), 1612 (C=N). m/z: 542.05 (100.0%), 544.05 (97.3%), 543.05 (21.6%), Elemental Analysis for ($C_{20}H_{23}BrN_4O_7S$, M.Wt: 543.39) Calcd: C, 44.21; H, 4.27; Br, 14.70; N, 10.31; S, 5.90; Found: C, 44.11; H, 4.34; Br, 14.70; N, 10.23 S, 5.95.

2-Chloro-*N*-(5-[(*p*-tolylamino)methyl]-1,3,4-thiadiazol-2-yl)acetamide (22)

To a round bottomed flask, was added compound **17** (10 mmol) and triethylamine (13 mmol). The mixture was stirred in CH_2Cl_2 (50 mL) at 0ºC, then a solution of chloroacetyl chloride (0.83 ml, 11 mmol) in CH_2Cl_2 (10 mL) was added to the mixture slowly. The reaction mixture was warmed at room temperature and stirred for 1 h. After completion of the reaction, the mixture was diluted with CH_2Cl_2 and was mixed with saturated NaCl. The organic layer was dried over anhydrous sodium sulfate, the solvent was removed under reduced pressure, and the remaining solid was washed with cold ethanol to afford compound **22**. Recrystalized from ethyl alcohol. Yield: 77%; m.p 245-247 ºC. IR (KBr) cm^{-1}, ύ: 3230 (NH), 1672 (C=O), 1610 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.35 (s, 3H, CH3), 4.22 (s, 2H, CH2) 4.33 (s, 2H, CH2), 6.45 (d, 2H, 2CH), 7.10 (d, 2H, 2CH), 7.35 (s, 1H, NH ex.), 12.50(s, 1H, NH ex.); m/z: 296.05 (100.0%), 298.05 (32.0%), 297.05 (13.0%); Elemental Analysis for ($C_{12}H_{13}ClN_4OS$, M.Wt: 296.77) Calcd: C, 48.57; H, 4.42; Cl, 11.95; N, 18.88; S, 10.80; Found: C, 48.59; H, 4.36; Cl, 11.99; N, 18.88; S, 10.76.

***N*-(5-[(*p*-Tolylamino)methyl]-1,3,4-thiadiazol-2-yl) acetamide (23)**

To a stirred heterogeneous suspension of the amine **7** (1 mmol) in water (5 mL) was added HCl 6N (in the volume range of 240-400 µL) until the solution became homogeneous (pH ≈ 1.5). The resulting homogenous solution was cooled in an ice bath. To this was then added anhydride (1-1.5 mmol) followed by solid sodium bicarbonate (185-300 mg) until there was no further effervescence or pH of the mixture became ca 5.5. The precipitate product was filtered, washed with water (2 × 1 mL), and dried to give compound (**23**). Recrystalized from chloroform. Yield: 80%; m.p. 266-268 ºC. IR (KBr) cm^{-1}, ύ: 3235 (NH), 1681 (C=O), 1612 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.10 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.33 (s, 2H, CH_2), 6.45 (d, 2H, 2CH), 7.10 (d, 2H, 2CH), 7.35 (s, 1H, NH ex.), 12.50(s, 1H, NH ex.); m/z: 262.09 (100.0%), 263.09 (13.0%), 264.08 (4.5%), 263.09 (1.5%); Elemental Analysis for ($C_{12}H_{14}N_4OS$, M.Wt: 262.33) Calcd: C, 54.94; H, 5.38; N, 21.36; S, 12.2; Found: C, 54.64; H, 5.42; N, 21.26; S, 12.02.

Cytotoxic Activity

Cell culture conditions

The cells of human liver carcinoma (HepG-2), and human breast adenocarcinoma (MCF-7) were purchased from the American Type Culture Collection (Rockville, MD). All cells were maintained in a DMEM medium, which was supplemented with 10% of heat-inactivated fetal bovine serum (FBS), 100U/ml of each of penicillin and streptomycin. The cells were grown at 37ºC in a humidified atmosphere of 5% CO_2.

MTT cytotoxicity assay

The cytotoxicity activity of the new compounds on the HepG-2, and MCF-7 human cancer cell lines were evaluated, employing the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which was grounded on the reduction of the tetrazolium salt by the mitochondrial dehydrogenases in viable cells[24-26]. The cells were dispensed in a 96 well sterile microplate ($3x10^4$ cells/well), followed by their incubation at 37ºC with a series of different concentrations of 10 µl of each compound or Doxorubicin® (positive control, in DMSO) for 48 h in serum free medium prior to the MTT assay. Subsequently, the media were carefully removed, 40 µL of MTT (2.5 mg/mL) were added to each well, and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 µL of DMSO. The absorbance was measured at 570 nm applying a SpectraMax® Paradigm® Multi-

Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells relative to the untreated control cells. All experiments were conducted in triplicate and were repeated on three different days. All values were represented as mean ±SD. The IC_{50}s were determined by the SPSS probit analysis software program (SPSS Inc., Chicago, IL).

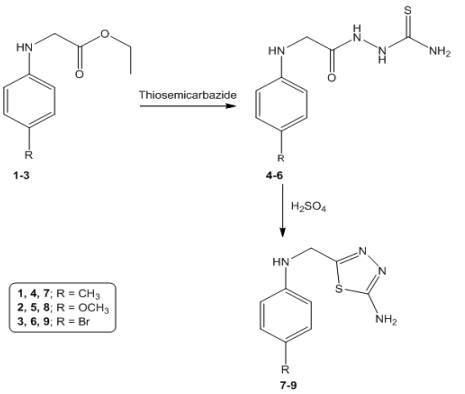

Figure 1: Synthesis of compound 4-9.

RESULTS AND DISCUSSION

Thiosemicarbazide was reacted with ethyl *p*-substituted-phenyl glycinate; namely, ethyl *p*-tolylglycinate (**1**), ethyl *p*-methoxyphenylglycinate (**2**) or ethyl *p*-bromophenylglycinate (**3**), to give compounds (**4-6**), respectively. Composition and structure of compounds (**4-6**) were proved by their elemental and spectroscopic analyses. Their IR spectra showed absorption bands characterizing the stretching NH_2 groups in the range 3380-3266 and NH groups in the range 3181-318 cm^{-1} in addition to C=O which showed the absorption bands around 1730-1721 cm^{-1}. The ^1H NMR spectra of the same compounds inferred signals for D_2O exchangeable NH_2 and NH groups at their specific regions. These compounds were then kept with conc. H_2SO_4 overnight to form compounds (**7-9**) respectively. The IR spectra of compounds (**7-9**) showed absorption bands characterizing the NH_2 and NH groups in the range 3283-3228 cm^{-1}.Also, ^1H NMR spectra of these compounds inferred signals for D_2O exchangeable NH_2, NH groups at their specific regions which helped to prove the structure of such compounds (Figure 1).

On the other hand, a mixture of 5-[(p-substituted amino)methyl]-1,3,4-thiadiazol-2-amine derivatives (**7-9**) and *D*-galactose, *D*-glucose or *D*-xylose in ethanol and acatalytic amount of acetic acid was added to the mixture and refluxed to yield compounds (**10-18**), respectively. Their IR spectra showed the disappearance of the bands which characterizes for NH_2 and appearance of the strong and broad bands characterizing the poly-hydroxyl chain and NH groups in the range 3460-3225 cm^{-1}(Figure 2). The acetylated derivatives **19-21** were produced by reacting the glycoside derivatives **10**, **11** and **18** in pyridine with acetic anhydride and the obtained clear solution was stirred at room temperature. Composition and structure of compounds **19-21** were proved by their elemental and spectroscopic analyses.

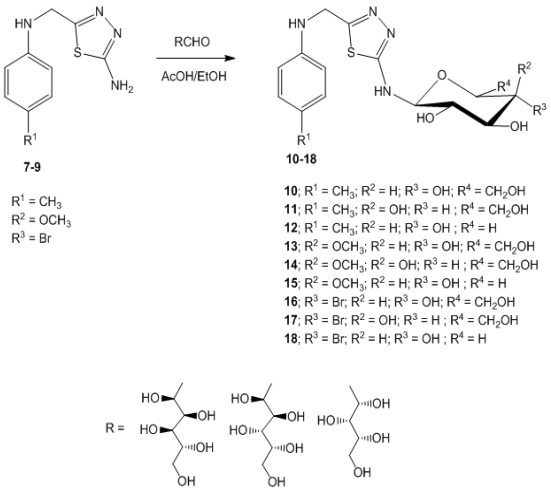

Figure 2: Synthesis of compounds 10-18.

Their IR spectra inferred absorption bands characterizing the poly NH groups around 3255-3225 cm^{-1}.Also, the strong broad bands of OH groups were disappeared and replaced by methyl groups (Figure 3).

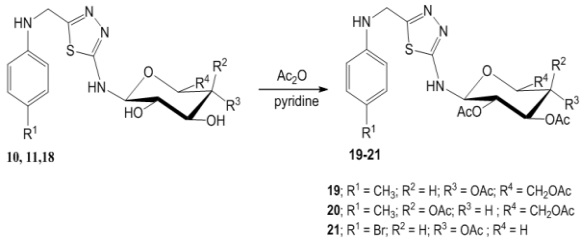

Figure 3: Synthesis of compounds 19-21.

2-Chloro-*N*-(5-[(*p*-tolylamino)methyl]-1,3,4-thiadiazol -2-yl)acetamide (**22**) was produced when compound **7** was reacted with chloroacetyl chloride.While,*N*-(5-[(*p*-Tolylamino)methyl]-1,3,4-thiadiazol-2-yl)acetamide (**23**) was produced when the same compound **7** was reacted with acetic anhydride.

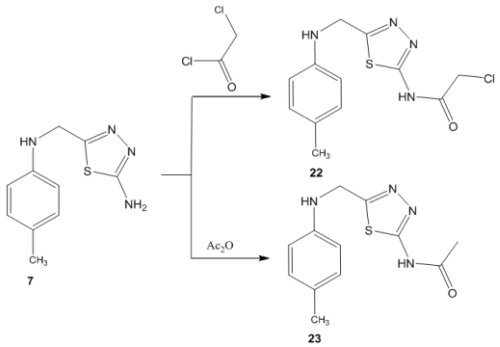

Figure 4: Synthesis of compounds 22 and 23.

The IR spectra of compounds **22** and **23** inferred two different bands, the band of NH group at 3230 cm^{-1} in compound **22** while at 3235 cm^{-1}in compound **23**, also, the a band of C=O group was at 1672 and 1681 cm^{-1} in the same compound respectively (Figure 4).

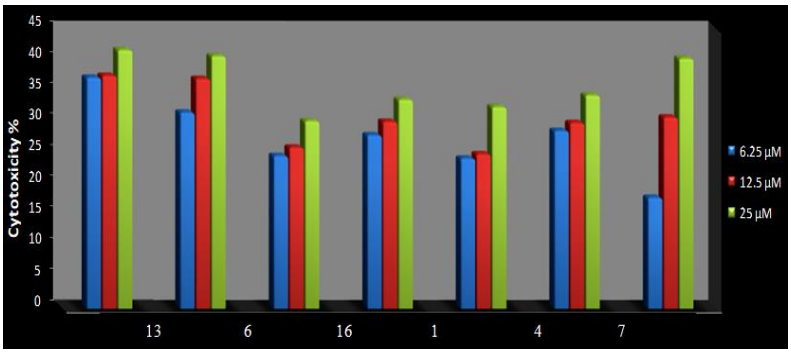

Figure 5: Dose-dependent cytotoxicity data of the compounds against the HepG-2 human cancer type, according to the MTT assay after 48 h of exposure.

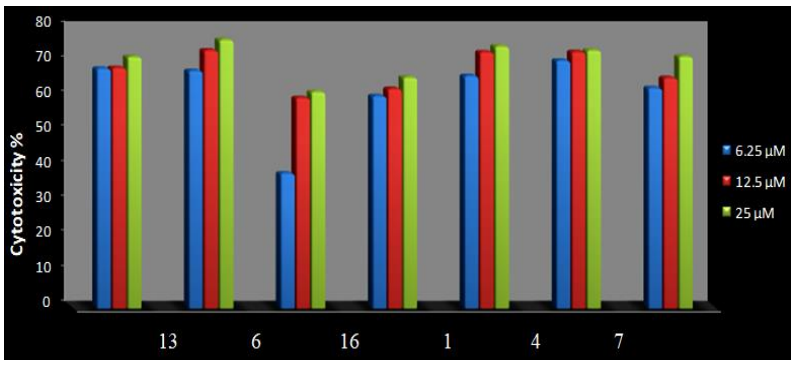

Figure 6: Dose dependent cytotoxicity data of the compounds on the MCF-7 human cancer type according to the MTT assay after 48 h of exposure.

Cytotoxicity activity

Six of the new compounds were evaluated *in vitro* for their cytotoxic activity against the HepG-2 and MCF-7 human cancer cell lines through the employment of the MTT assay. The percentages of viable cells and their IC_{50} values were measured and were subsequentelly assessed with those of the control, Doxorubicin® (Figure 5, Figure 6 and Table 1).

Table 1: The cytotoxic IC_{50} values of the compounds according to the MTT assay on the two human cell types.

Compound	IC_{50} (µM) ±SD	
	HepG-2	MCF-7
1	29.7±2.9	12.2±1.5
4	32±3.1	9.4±0.8
6	26.3±2.8	9.1±0.6
7	29.5±2.6	9.1±0.5
13	24.9±2.5	10.2±1.3
16	32.1±3.1	15.3±1.7
Doxorubicin	28.5±1.9	10.3±0.8

The results revealed that, all compounds presented dose-dependent cytotoxic activity against both cell varieties (Figure 5, Figure 6). The constructed deduction from these outcomes is that in assessment with the positive control doxorubosin, compounds**13** and **6** were more potent; compounds **7** and **1** displayed comparable cytotoxic activity; compounds **4** and **16** had slightly less activity relative to the positive control, regarding human liver cancer (HepG-2) (Figure 5 and Table 1).

Regarding to breast cancer cells (MCF-7); compounds **6**, **7**, **4,** and **13** were more potent, and compounds **1** and **16** had slightly less cytotoxic activity relative to the positive control (Figure 6 and Table 1).

CONCLUSION

New heterocyclic compounds were synthesized by reaction of compounds **1**, **2** and/or **3** with thio-semicarbazide to give compounds **4-6**,whichthen kept with conc. H_2SO_4 overnight to yield derivatives **7-9**, then compounds **10-18** were also yielded by reaction of compounds **7-9** with *D-sugars* namely, *D*-galactose, *D-Glucose* or *D*-xylose in ethanol and a catalytic amount of acetic acid. Compounds **10-18** were then acetylated with acetic anhydride to form compounds **19-21**. Finely, compound **7** was reacted with chloroacetyl chloride and/or acetic anhydride to afford compounds **22** and/or **23** respectively. Six new derivative compounds were designated *in vitro* for their cytotoxic activity on the HepG-2 and MCF-7 human cancer cell lines where compounds **6** and **13** were found to be more potent for their cytotoxic activity on the two cancer cell lines as compared with the reference drug Doxorubicin.

CONFLICT OF INTEREST

The authors stated that they do not have any conflict of interest.

AUTHOR'S CONTRIBUTIONS

All the authors contributed in experimental and interpreting the results of the work. Every one writes the section that he works on it.

REFERENCES

1. Gnanasekaran KK, Nammalwar B, Murie M, Bunce RA, Efficient synthesis of 1,3,4-oxadiazoles promoted by NH_4Cl. Tetrahedron Lett 2014, 55, 6776-6778. *https://doi.org/10.1016/j.tetlet.2014.10.028*
2. Singh SJ, Rajamanickam S, Gogoi A, Patel B K, Synthesis of 2-amino-substituted-1, 3, 4-thiadiazoles via 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) mediated intra-molecular C–S bond formation in thiosemicarbazones Tetrahedron Lett., 2016, 57, 1044-1047. *https://doi.org/10.1016/j.tetlet.2016.01.083*
3. Tatar E, Karakus S, Kucukguzel SG, *et al*. Design, synthesis, and molecular docking studies of a conjugated Thiadiazole – Thiourea Scaffold as antituberculosis agents biol. Pharm Bull 2016, 39, 502-515. *https://doi.org/10.1248/bpb.b15-00698*
4. Tsukuda Y, Shiratori M, Watanabe H, *et al*. Modeling, synthesis and biological activity of novel antifungal agents. Bioorg Med Chem Lett 1998; 8: 1819-1894. *http://dx.doi.org/10.4103/2231-4040.161515*
5. Roberts J, Schock K, Marino S, Andriole VT. Efficacies of two new antifungal agents, the Triazole Ravuconazole and the Echinocandin LY-303366, in an experimental model of invasive *Aspergillosis Antimicrob*. Agents Chemother 2000; 44, 3381-3388. *http://dx.doi.org/10.1128/aac.44.12.3381-3388.2000*
6. Espinel-Ingroff A. In vitro activity of the new triazole voriconazole (UK-109,496) against opportunistic filamentous and dimorphic fungi and common and emerging yeast pathogens. J Clin Microbiol 1998; 36: 198-202. *http://dx.doi.org/10.1128/jcm.36.1.198-202.1998*
7. Krakovsky EMDJ, Rybak MJ. The triazole antifungal agents: A review of itraconazole and fluconazole. Pharmacother 1990; 10: 146-149. *http://dx.doi.org/10.1358/dot.2015.51.12.2421058*
8. Pfaller MA, Messer S, Jones R N. Activity of a new triazole, Sch 56592, compared with those of four other antifungal agents tested against clinical isolates of *Candida* spp. and *Saccharomyces cerevisiae*. Antimicrob Agents Chemother 1997; 41: 1120-1124. *http://dx.doi.org/10.1128/AAC.41.2.233*
9. Blobaum AL, Marnett LJ. Structural and functional basis of cyclooxygenase inhibition. J Med Chem 2007; 50: 1425-1441. *http://dx.doi.org/10.1021/jm0613166*
10. Shrivastava K, Purohit S, Singhal S. Studies on nitrogen and sulphur containing heterocyclic compound: 1, 3, 4-thiadiazole. Asian J Biomed Pharm Sci 2013; 3: 6.
11. El-Sayed WA, Nassar IF, Abdel-Rahman AA. Synthesis and anti-tumor activity of new [1,2,4] triazine and [1,2,4] triazolo[4,3-b] [1,2,4] triazine derivatives and their Thioglycoside and Acyclic C-nucleoside Analogs. J Heterocycl Chem 2011; 48: 135-143. *http://dx.doi.org/10.1002/jhet.522*
12. Nassar IF. Synthesis and anti-tumor activity of new substituted Mercapto-1,2,4-Triazine derivatives, their Thioglycosides and Acyclic S-Glycoside analogs. J Heterocycl Chem 2013; 50: 129-134. *http://dx.doi.org/10.1002/jhet.1022*
13. Nassar IF, Atta-Allah SR, Elgazwy ASH.,A convenient synthesis and molecular modeling study of Novel Pyrazolo[3,4-d]pyrimidine and Pyrazole derivatives as anti-tumor agents. J Enzym Inhib Med Chem 2015; 30: 396-405. *http://dx.doi.org/10.3109/14756366.2014.940936*
14. Abou El saoud YMH, El Gazwy ASSH, Nassar IF, Ismail NSM, Abdel Sattar NA. Qualitative Structure Activity Relationship (QSAR) of new organic compounds synthesized by stille cross coupling reaction with antitumor activity. WO 2015; 2015127941 A1.
15. Abu-Dief AM, Nassar IF, Elsayed WH. Magnetic $NiFe_2O_4$ nanoparticles: efficient, heterogeneous and reusable catalyst for synthesis of acetylferrocene chalcones and their anti-tumour activity. Appl Organometal Chem 2016; 30; 917-239. *http://dx.doi.org/10.1002/aoc.3521*
16. Nassar IF, El Farargy AF, Abdelrazek FM, Ismail NSM. Synthesis and anticancer evaluation of novel Pyrazole, Pyrazolo[3,4-d] Pyrimidine and their glycoside derivatives. Nucl Nucleot Nucl Acids 2017; 36(4), 275-291. *http://dx.doi.org/10.1080/15257770.2016.1276290*
17. Nassar IF, El Farargy AF, Abdelrazek FM. Synthesis and anti-cancer activity of some new fused pyrazoles and their glycoside derivatives. J Heterocyclic Chem 2018; 55: 1709-1719. *http://dx.doi.org/10.1002/jhet.3208*
18. Nassar IF, Att-Allah SR, Hemdan MM. Utility of Thiophene-2-carbonyl isothiocyanate as a synthon of 1,2,4- Triazole, 1,3.4-Oxadiazole and 1,3.4-Thiadiazole derivatives with evaluation of their antitumor and antimicrobial activities. Phosph Sulph Silic 2018; 193(10): 630-636. *http://dx.doi.org/10.1080/10426507.2018.1487435*
19. Nassar IF, El-Sayed WA, Ragab TIM, Shalaby ASG, Mehany ABM. Design, synthesis of novel pyridine and pyrimidine sugar compounds as antagonists targeting the erα via structure-based virtual screening. Mini Rev Med Chem 2019; 19: 395-409. *http://dx.doi.org/10.2174/1389557518666180820125210*
20. Kassem AF, Nassar IF, Abdel-Aal MT, Awad HM, El-Sayed WA. Synthesis and anticancer activity of new ((Furan-2-yl)-1,3,4-thiadiazolyl)-1,3,4-oxadiazole acyclic sugar derivatives. Chem Pharm Bull 2019; 67: 888-598. *http://dx.doi.org/10.1248/cpb.c19-00280*
21. Nassar IF, El Farargy AF, Abdelrazek FM, Hamza Z. Synthesis of new Uracil derivatives and their sugar hydrazones with potent antimicrobial, antioxidant and anticancer activities. Nucl Nucleot Nucl Acids 2020; 39(7): 991-1010.*http://dx.doi.org/10.1080/15257770.2020.1736300*
22. Atta-Allah S R, Ismail N SM, Nassar IF. Synthesis, design and anti-inflammatory activity of novel 5-(Indol-3- yl) thiazolidinone derivatives as COX-2 inhibitors. Lett Drug Des Disc 2021; 18(6): 525-541. *http://dx.doi.org/10.2174/1570180817999201123164201*
23. Abdel-Rahman AA-H, Shaban AKF, Nassar IF, *et al*. Discovery of new pyrazolopyridine, furopyridine, and pyridine derivatives as CDK2 inhibitors: Design, synthesis, docking studies, and anti-proliferative activity. Molec 2021; 26(13). *http://dx.doi.org/10.3390/molecules26133923*
24. Nassar IF, NagwanEB, Abd El-Aal, MT, El-Sayed WA. Synthesis, reactions and anticancer evaluation of new Pyrimidine-2(1H)-thione derivatives. Res J Chem Env 2021; 25 (2): 113-124.
25. Alminderej F M, Elganzory HH, El-Bayaa M N, Awad HM, El-Sayed WA. Synthesis and cytotoxic activity of new 1,3,4-Thiadiazole Thioglycosides and 1,2,3-Triazolyl-1,3,4-Thiadiazole N-glycosides. Molecules 2019; 24, 3738. *http://dx.doi.org/10.3390/molecules24203738*
26. Haiba ME, Al-Abdullah ES, Ahmed NS, Ghabbour, HA, Awad HM. Efficient and easy synthesis of new Benzo[H]Chromene and Benzo[h]Quinoline derivatives as a new class of cytotoxic agents. J Mol Struct 2019; 1195(5): 702-711. *http://dx.doi.org/10.1155/2020/8649745*