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RESEARCH ARTICLE

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

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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.

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).

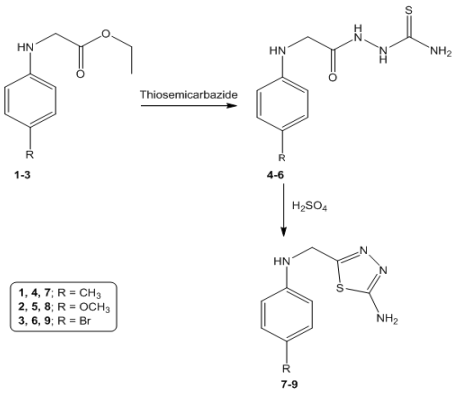

Figure 1: Synthesis of compound 4-9.

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.

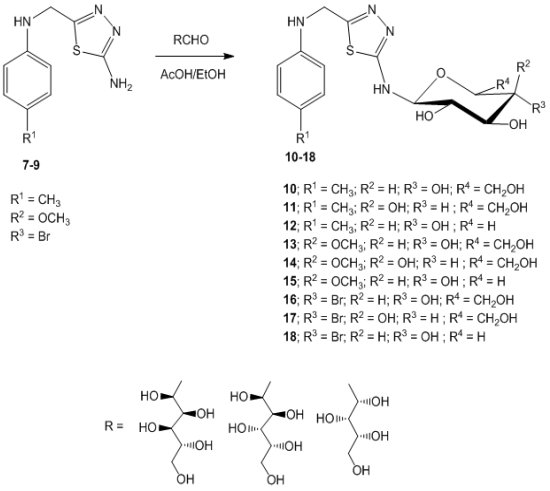

Figure 2: Synthesis of compounds 10-18.

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).

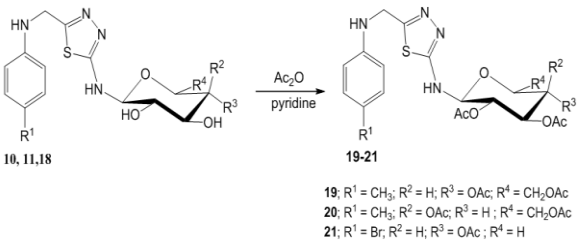

Figure 3: Synthesis of compounds 19-21.

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.

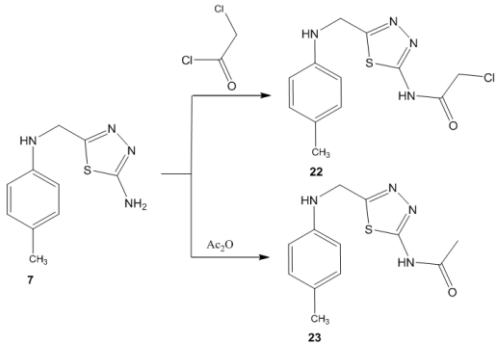

Figure 4: Synthesis of compounds 22 and 23.

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).

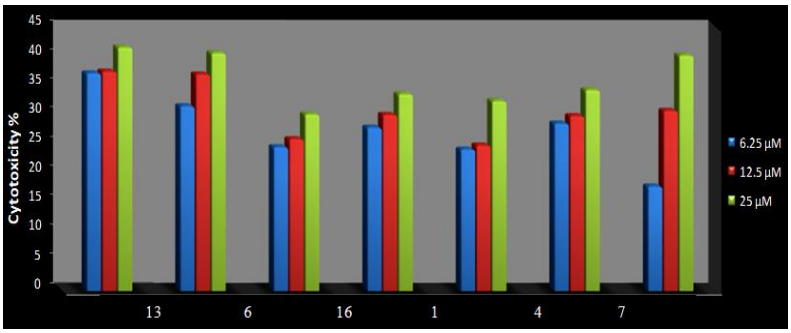

Figure 5: Dose-dependent cytotoxicity data of the compounds against the HepG-2 human cancer type, according to the MTT assay after 48 h of exposure.

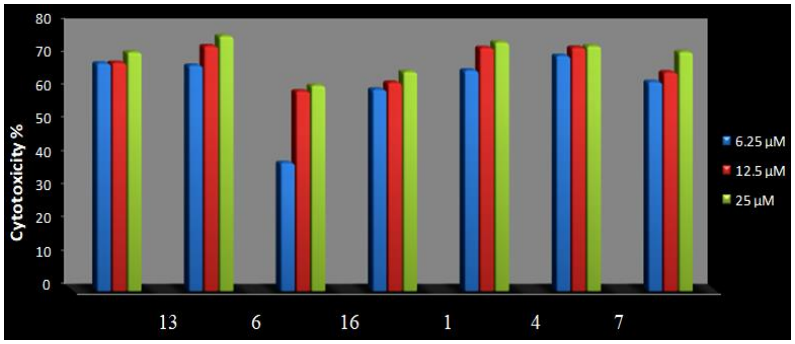

Figure 6: Dose dependent cytotoxicity data of the compounds on the MCF-7 human cancer type according to the MTT assay after 48 h of exposure.

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).

CONCLUSIONS

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.

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AUTHOR'S CONTRIBUTIONS

Nassar IF: writing original draft, conceptualization. **El Bakary NS:** methodology, formal analysis, conceptualization. **Abdel Aal MT:** data curation, investigation. **El-Sayed WA:** editing, data interpretation. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

None to declare.

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