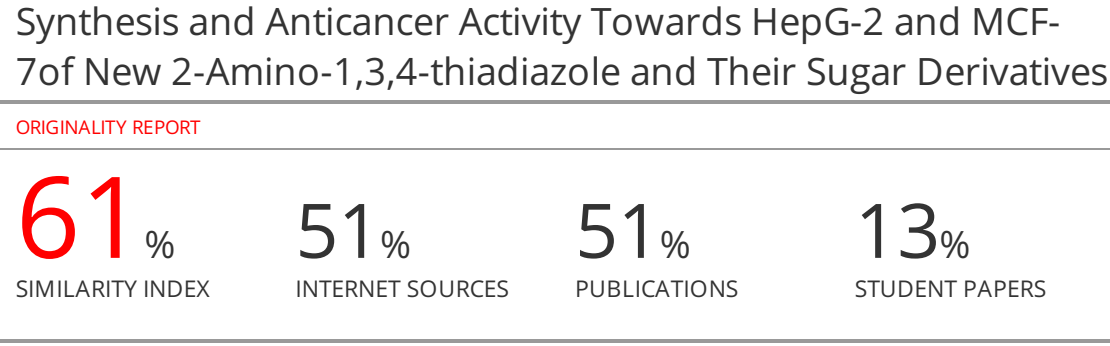
**Reviewer’s Comments**

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**Synthesis and Anticancer Activity Towards HepG-2 and MCF-7of New 2-Amino-1,3,4-thiadiazole and Their Sugar Derivatives**

**Abstract**:

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**),whichthen kept with conc. H2SO4 overnight to yield 1,3,4-thiadiazol-2-aminederivatives (**7**-**9**). Compounds (**10**-**18**) were yielded by reaction of compounds (**7**-**9**) 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 designated*in vitro* for their cytotoxicity activities on the HepG-2 and MCF-7 human cancer cell lines. Compounds **6** and **13** were found to be the more potent for their cytotoxicity activities on the two cancer cell lines.

**Key Words**: Thiosemicarbazide, 1,3,4-Thiadiazol-2-amine, Cytotoxicity, HepG-2, MCF-7

**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 morepotent and effective antimicrobial drugs.

The hybridization of the pharmacophores 1,3,4-Thiadiazole and 4-  
thiazolidinone in one molecular frame could show highly effective antiinflammatory 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 towardsCOX-2 over COX-1 enzyme due to their large volume which will not fit in the smaller COX-1 binding pocket [9]. To test these hypotheses, we have synthesized compounds 1, 3 and 6a.This compound incorporating both 4-thiazolidinone and 1,3,4-thiadiazole6a was compared of its building blocks; 1,3,4-thiadiazole 121 and4-thiazolidinone 122 in terms of molecular volume, potency and selectivity against both COX-2 and 15-LOX enzymes. Molecular volume was calculated using MOE program vsurf-D1 descriptor. The hybrid 123 was about two folds larger, three folds more potent, and two folds more selective than 121 against COX-2. The hybrid 123 also demonstrates better inhibitory activity against 15-LOX (about 1.5 folds more potent than 1). Based on these results, we designed a library of new compounds 6–9 to elaborate other structural features of the hybridmolecules. The arylidene moiety attached to the 4-thiazolidinone ring isvaried as a fine tuner to optimise both selectivity and potency against COX-2 and 15-LOX enzymes.

1,3,4-Thiadiazoles exhibit a broad spectrum of biological activities [10] such as antimicrobial, antiinflammatory, anticancer, antituberculosis, antiparasitic, anticonvulsants, antioxidant, herbicidal and insecticidal properties. Desaglybuzole 124 (antidiabetic), Acetazolamide125 (for glaucoma), Furidiazine126(antimicrobial) and Butazolamide 127 (diuretic) are commercially available 1,3,4-thiadiazole drugs.Inrecent 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. Therfore, we synthesized new 2-(*p*-Substituted-phenylglycyl)hydrazine-1-carbothioamide 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 designated*in vitro* for their cytotoxicity activities on the HepG-2 and MCF-7 human cancer cell lines.

**Materials and Methods:-**

**Experimental 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 25 ºC, unless otherwise stated. The NMR signals were referenced to TMS and the solvent shift ((CD3)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 (CH3OH) 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-carbothioamide (4-6)**

To a well stirredsuspension of thiosemicarbazide (10 mmol) in ethanol (5 mL), was added ethyl *p*-substituted-phenyl glycinate (**1**-**3**); namely, ethyl p-tolylglycinate, ethyl p-methoxyphenylglycinate or ethyl *p*-bromophenylglycinate, 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 (NH2), 3178 (NH), 1721 (C=O), 1609 (C=N); 1H NMR (DMSO-d6, 300 MHz): δ 2.1 (s, 3H, CH3), 4.5 (s, 2H, CH2), 5.73 (br.s, 2H, NH2), 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 (C10H14N4OS, 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-carbothioamide (5)**

Yield: 79%; m.p. 274-276 ºC. IR (KBr) cm-1, ύ: 3378-3264 (NH2), 3177 (NH), 1728 (C=O), 1620 (C=N); 1H NMR (DMSO-d6, 300 MHz): δ 3.9 (s, 3H, CH3), 4.61 (s, 2H, CH2), 5.75 (br.s, 2H, NH2), 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 (C10H14N4O2S, MWt: 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-carbothioamide (6)**

Yield: 79%; m.p. 275-277 ºC. IR (KBr) cm-1, ύ: 3380-3266 (NH2), 3181 (NH), 1730 (C=O), 1621 (C=N); 1H NMR (DMSO-d6, 300 MHz): δ 4.62 (s, 2H, CH2), 5.75 (br.s, 2H, NH2), 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 (C9H11BrN4OS, MWt: 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-thiadiazol-2-amine (7-9)**

A mixture of compounds (**4**-**6**) (0.05 mol) and conc. H2SO4 (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 (NH2, NH), 1620 (C=N); 1H NMR (DMSO-d6, 300 MHz): δ 2.6 (s, 3H, CH3), 4.62 (s, 2H, CH2), 5.20 (br.s, 2H, NH2), 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 (C10H12N4S, MWt: 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 (NH2, NH), 3050 (C-H), 1610 (C=N); 1H NMR (DMSO-d6, 300 MHz): δ 3.9 (s, 3H, CH3), 4.61 (s, 2H, CH2), 5.75 (br.s, 2H, NH2), 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 (C10H12N4OS, MWt: 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 (NH2, NH), 3065 (C-H), 1615 (C=N); 1H NMR (DMSO-d6, 300 MHz): δ 4.02 (s, 2H, CH2), 5.75 (br.s, 2H, NH2), 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 (C9H9BrN4S, MWt: 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*-methoxyphenylmino)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*-substitutedamino)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-d6, 300 MHz): δ 2.6 (s, 3H, CH3), 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, CH2), 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 (C16H22N4O5S, MWt: 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-d6, 300 MHz): δ 2.6 (s, 3H, CH3), 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, CH2), 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 (C16H22N4O5S, MWt: 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-d6, 300 MHz): δ 2.6 (s, 3H, CH3), 3.62-3.65 (m, 2H, H-5′,5``), 3.94-4.25 (m, 2H, H-4′,3′), 4.26 (m, 2H, CH2), 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 (C15H20N4O4S, MWt: 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-d6, 300 MHz): δ 3.31-3.37 (m, 2H, H-6′,6′′), 3.62-3.65 (m, 1H, H-5′), 3.81 (s, 3H, CH3), 3.94-4.25 (m, 2H, H-4′,3′), 4.32 (s, 2H, CH2), 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 (C16H22N4O6S, MWt: 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-d6, 300 MHz): δ 3.31-3.37 (m, 2H, H-6′,6′′), 3.62-3.65 (m, 1H, H-5′), 3.81 (s, 3H, CH3), 3.94-4.25 (m, 2H, H-4′,3′), 4.32 (s, 2H, CH2), 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 (C16H22N4O6S, MWt: 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. 281-283 ºC. IR (KBr) cm-1, ύ: 3460 (OH), 3225 (NH), 1681, 1610 (C=N); 1H NMR (DMSO-d6, 300 MHz): δ 3.62-3.65 (m, 2H, H-5′,5``), 3.80 (s, 3H, CH3), 3.94-4.25 (m, 2H, H-4′,3′), 4.26 (m, 2H, CH2), 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 (C15H20N4O5S, MWt: 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-d6, 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, CH2), 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 (C15H19BrN4O5S, MWt: 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-d6, 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, CH2), 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 (C15H19BrN4O5S, MWt: 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); 1H NMR (DMSO-d6, 300 MHz): 3.62-3.65 (m, 2H, H-5′,5``), 3.94-4.25 (m, 2H, H-4′,3′), 4.26 (m, 2H, CH2), 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 (C14H17BrN4O4S, MWt: 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*-substitutedamino)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,thendried, and recrystalized from ethyl acetate to give the acetylated products (**19**-**21**), respectively.

***N*-(Penta-*O*-acetyl-D-galactopyranosyl)-5-[(*p*-tolylamino)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 (C24H30N4O9S,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*-tolylamino)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 (C24H30N4O9S; 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*-bromophenylamino)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 (C20H23BrN4O7S, MWt: 543.39) 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 at 0ºC in CH2Cl2 (50 mL), then a solution of chloroacetyl chloride (0.83 ml, 11 mmol) in CH2Cl2 (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 CH2Cl2 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). 1H NMR (DMSO-d6, 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 (C12H13ClN4OS, MWt: 296.77) 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). 1H NMR (DMSO-d6, 300 MHz): δ 2.10 (s, 3H, CH3), 2.35 (s, 3H, CH3), 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: 262.09 (100.0%), 263.09 (13.0%), 264.08 (4.5%), 263.09 (1.5%); Elemental Analysis for (C12H14N4OS, MWt: 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;

**Biological Activity**

**Materials and Methods**

**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 37°C in a humidified atmosphere of 5% CO2.

**MTT cytotoxicity assay**

The cytotoxicity activities on the HepG-2, and MCF-7 human cancer cell lines were estimated, 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 (3 x 104 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. Allvalues were represented as mean ± SD. The IC50s were determined by the SPSS probit analysis software program (SPSS Inc., Chicago, IL).

**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**), respectively to give compounds (**4**-**6**), respectively,Compounds (**4**-**6**)were proved by their analytical and spectroscopic analyses. Their IR spectra showed absorption bands characterizing the stretching NH2groupsin the range 3380-3266 and NH groups in the range 3181-318 cm-1 in addition to C=O showed the absorption bands around 1730-1721. The 1H NMR spectra of the same compounds showed signals for D2O, exchangeable NH2 and NH groups at their specific regions. These compounds were then kept with conc. H2SO4 overnight to yield compounds (**7**-**9**) respectively. The IR spectra showed absorption bands characterizing the NH2 and NH groups in the range 3283-3228 cm-1 (c.f. Scheme 1& Experimental section). Also, 1H NMR spectra of the same compounds showed signals for D2O exchangeable NH2, NH at their specific regions which helped in proving their structure (*cf*. experimental section & Scheme 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 ethanolandacatalytic amount of acetic acid was added to the mixture and allowed to reflux to yield compounds (**10**-**18**), respectively. Their IR spectra showed the disappearance of the bands which charachterizes for NH2 and appearance of the strong and broad bands characterizing the poly-hydroxyl chain and NH groups in the range 3460-3225 cm-1.(*cf*. experimental section & Scheme 2)



Different derivatives of glycosides **10**, **11** and **18** in pyridine was added acetic anhydride and the obtained clear solution was stirred at room temperature to give the acetylated products (**19**-**21**), respectively. Their IR spectra showed an absorption band characterizing the poly NH groups around 3255-3225 cm-1, also, the strong broad bands of OH groups were disapeared and replaced by methyl groups.(*cf*. experimental section & Scheme 3)



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, was reacted with acetic anhydride. The IR spectra of compounds **22** and **23** showed two different bands, the band of NH group at 3230 in compound **22** while at 3235 cm-1in compound **23**, also, the a band of C=O group was at 1672 and 1681 cm-1 in the same compound respectively.(*cf*. experimental section & Scheme 4)



**Biological Evaluation**

**Cytotoxicity activity**

Six new compounds were designated*in vitro* for their cytotoxicity activitiesagainst the HepG-2 and MCF-7 human cancer cell lines through the employment of the MTT assay. The percentages of viable cells and their IC50 values were measured and weresubsequentelly; assessed with those of the control, Doxorubicin® (Figures1-2 and Table 1). The attained results revealed that all compounds presented dose-dependentcytotoxicity activities against both cell varieties (Figures 1-2). 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 1 and Table 1). Regarding breast cancer cells (MCF-7); Compounds **6**, **7**, **4,** and **13** were more potent;Compounds **1** and **16** had slightly less cytotoxic activity relative to the positive control (Figure 2 and Table 1).

Fig. **1**: Dose-dependent cytotoxicity data of the compounds against the HepG-2 human cancer type, according to the MTT assay after 48 h of exposure.



13 6 16 1 4 7

Fig. **2**: Dose dependent cytotoxicity data of the compounds on the MCF-7 human cancer type according to the MTT assay after 48 h of exposure.



13 6 16 1 4 7

Table **1**: The cytotoxic IC50 values of the compounds according to the MTT assay on the two human cell types.

|  |  |  |
| --- | --- | --- |
| **Compound** | **IC50 (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 |

**Conclusion**

New heterocyclic compounds were synthesized by reaction of compounds **1**, **2** or **3** with thiosemicarbazide to give compounds (**4**-**6**),whichthen kept with conc. H2SO4 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 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 cytotoxicity activities on the HepG-2 and MCF-7 human cancer cell lines where compounds **6** and **13** were found to be more potent for their cytotoxicity activities on the two cancer cell lines as compared with the reference drug **Doxorubicin**.

**Conclusion**

**Conflict of interest**

**Author’s Contribution**

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