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Synthesis of some functionalized arylaminomethyl-1,2,4-triazoles, 1,3,4-oxa- and thiadiazoles

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Thiosemicarbazides undergo different cyclization reactions to give five membered heterocycles. The product of cyclization depends on the reagent used. This cyclization leads to the formation of 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole derivatives. The reaction of thioglycolyl hydrazide derivatives of the 1,2,4-triazole compounds was discussed. The activity against hepatitis B virus (HBV) has been tested.

1. Introduction

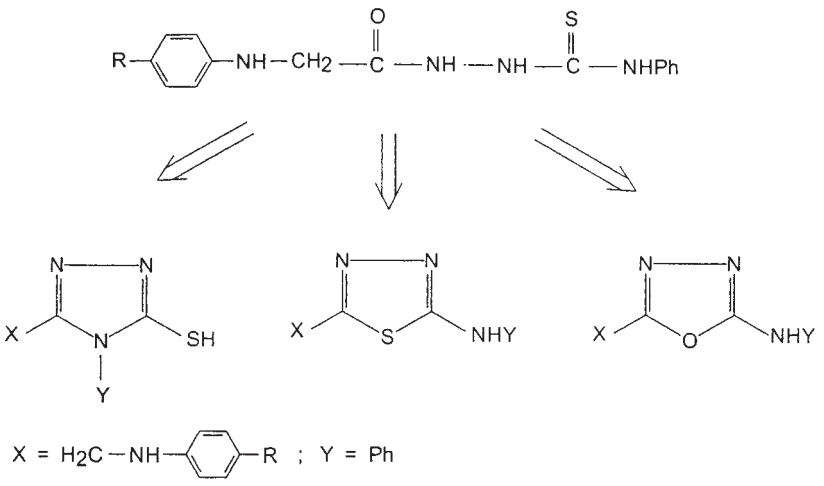
Substituted 1,2,4-triazoles, 1,3,4-oxadiazoles and thiadiazoles show sedative [1–3], antiinflammatory [4, 5] fungicidal [6–9], antibacterial [10, 11], antimicrobial [12], antihyperlipidemic [13], antitubercular [14] and insecticidal [15] properties.

The available literature [16–19] for the synthesis of 1,2,4-triazoles, 1,3,4-oxadiazoles and 1,3,4-thiadiazoles revealed that their retrosynthetic analysis leads to the conclusion that arylthiosemicarbazides can have ambident sites for cyclization which readily afford these heterocycles carrying various substituents as shown in Scheme 1. Thus, a library for those heterocycles can be designed following the combinatorial approach. In the present work these three types of heterocycles with Y = Ph whereas X is *N*-arylaminomethyl have been synthesized. The aryl group can possess various substituents.

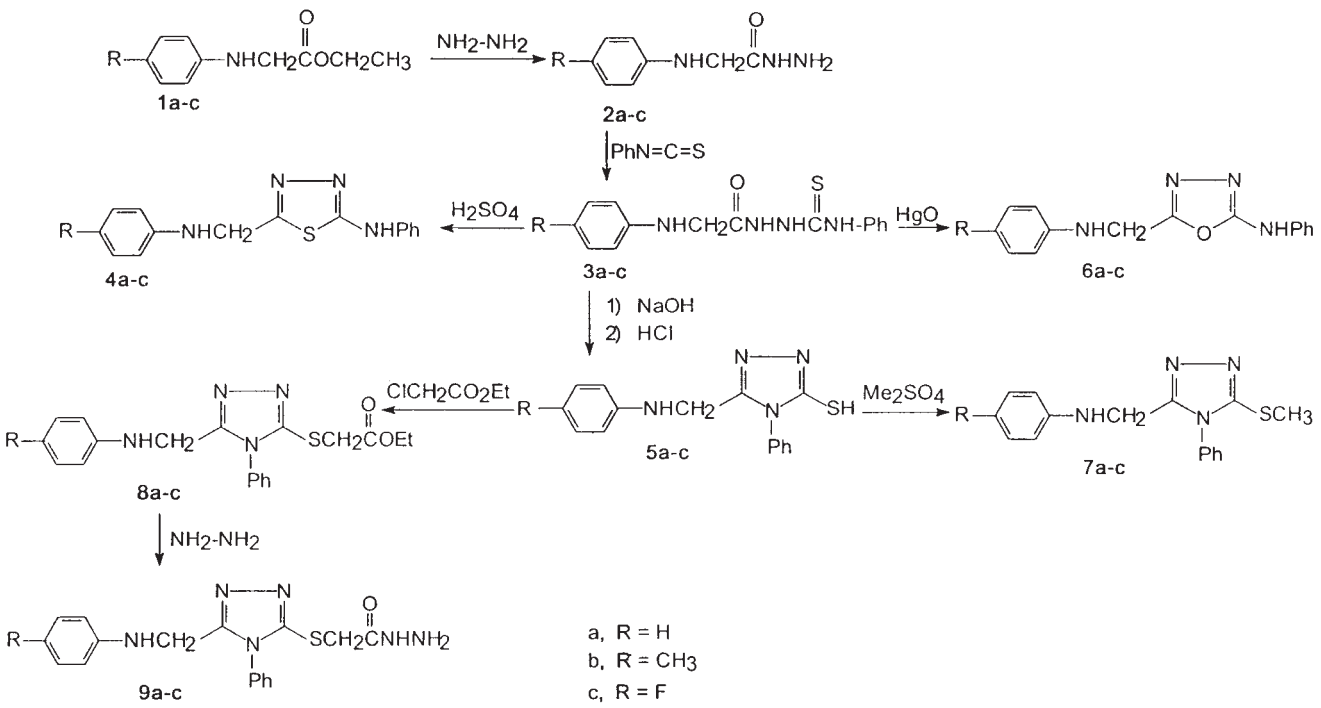
2. Investigations, results and discussion

The starting materials 1-(*N*-arylamino)acetyl-4-phenylthiosemicarbazides **3** were synthesized by treating *N*-arylglycine hydrazides **2** with phenylisothiocyanate [20]. The respective hydrazides **2** were obtained from ester **1** and hydrazine hydrate. Compounds **3a–c** show a charateristic carbonyl frequency at 1675–1690 cm^{-1}. When the thiosemicarbazides **3a–c** were treated with sulphuric acid, the 1,3,4-thiadiazoles **4a–c** were formed in 75–77% yield. The preference formation of the thiadiazole ring under such acidic conditions can be due to the loss of nucleophilicity of N-4 as a result of its protonation leading to a comparably increase in the nucleophilicity of the sulphur atom towards the attack of the carbonyl carbon. On the other hand, when the cyclization of **3a–c** was carried out under alkaline conditions, the nucleophilicity of N-4 is enhanced and leads to cyclization with the carbonyl carbon

Scheme 1



Scheme 2



atom to give the 1,2,4-triazoles **5a–c**. When the cyclization of **3a–c** was performed by mercuric oxide, the 1,3,4-oxadiazoles **6a–c** were formed. The mode of cyclization to **6** includes desulphurization by mercuric oxide which introduces the oxygen atom in the cyclization process.

The structure of the products **4–6** were established from their IR spectra where they show the absence of the C=O groups of their precursors. Their ^{1}H-NMR spectra agree with the assigned structures.

Methylation of **5** with dimethyl sulphate afforded crystalline products **7**, the analyses of which indicated that S-alkylation had taken place. Their ^{1}H-NMR spectra show a singlet at δ 2.7–2.8 ppm (SCH_3 group) and the absence of the SH signal.

Reaction of **5** with ethyl chloroacetate/OH^- gave the corresponding ethyl[(5-arylamino)methyl-4-phenyl-1,2,4-triazol-3-yl]thioglycolates **8**. Their IR spectra show a characteristic C=O absorption at 1735 cm^{-1}, and their ^{1}H-NMR spectra agree with the structures.

Treatment of **8** with hydrazine hydrate in ethanol gave the corresponding hydrazide derivatives **9** in 79–81% yields (Scheme 2). Their IR spectra show the characteristic band for N–CO at 1650–1690 cm^{-1}.

Condensation of the hydrazides **9a–c** with the monosaccharides D-mannose, D-galactose, and D-ribose gave the corresponding sugar hydrazones **10**, **11** and **12**, respectively (Schema 3). Their IR spectra show OH (3300–3450), NH (3100–3300), NCO (1690) cm^{-1}. The ^{1}H-NMR spectra are characterized by a doublet at δ 5.1–5.5 ppm corresponding to the proton at C-1 of the sugar. The protons of the sugar hydroxyls appear at δ 2.2–4.6 ppm. Elemental analyses of the synthesized

Scheme 3

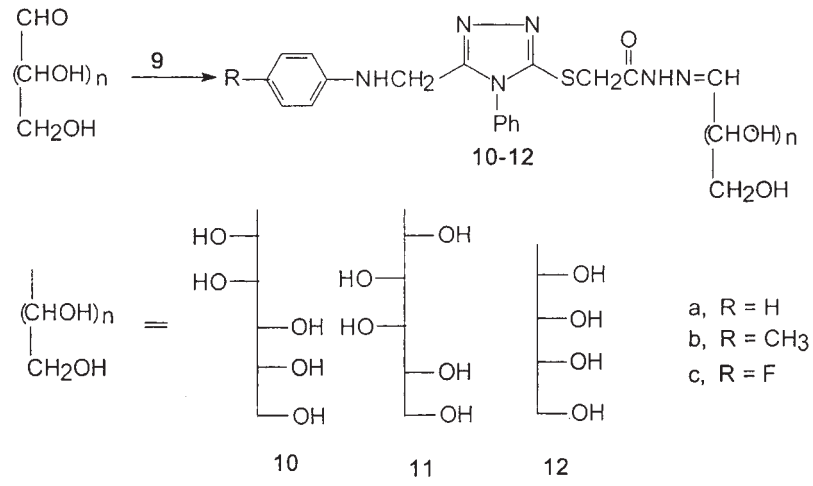


Table 1: Cytotoxic effect (CC_{50}), inhibitory concentration (IC_{50}) and selectivity index (SI) of selected compounds

Compd.	HBV DNA IC_{50} (μM)	Hep G2 2.2.15 CC_{50} (μM)	SI
Lamivudine	<0.1	>100	>1000
3a	11.4	>100	>8.7
3b	0.0	>100	>0.0
3c	0.0	>100	>0.0
4b	52.0	>100	>1.9
5a	8.0	>100	>12.5
5c	87.9	>100	>1.1
6a	72.2	>100	>1.4
6b	63.9	>100	>2.7
8a	31.6	>100	>3.2
8c	23.4	>100	>4.3
9a	0.0	>100	>0.0
9b	1.0	>100	>103.1
10a	0.0	>100	>0.0
10b	7.7	>100	>13.0
10c	23.3	>100	>4.3
11a	64.0	>100	>1.6
11b	0.0	>100	>0.0
11c	20.0	>100	>5.0
12a	0.0	>100	>0.0
12c	16.7	>100	>6.0

compounds are in agreement with the assigned structures.

The assignments of NH and OH groups in these compounds were determined by D_2O exchange.

The results of the viral sreening against HBV of selected compounds indicated that compound **9b** was the most effective one in this series against HBV with an effective concentration of 1.0 μM and a selectivity index >103.1 This compound has a low effective concentration as an antiviral agent and a low cytotoxic effect. Compounds **3a** and **10b** show moderate viral replication inhibition and moderate cytotoxicity with selectivity indexes >8.7 and >13.0, respectively. Compounds **8a**, **8c**, **10c**, **11c** and **12c** show very low inhibition and moderate cytotoxicity with selectivity indexes >3.2, >4.3, >4.3, >5.0 and >6.0. (Tables 1 and 2)

Table 2: Inhibition of HBV replication by selected compounds

Compd.	Concentration (μM)	HBV DNA in supernatant	Hep G2 viable cells
Lamivudine	1.0	0.25	1.00
3a	1.0	0.00	0.00
3b	1.0	0.31	0.38
3c	1.0	0.25	1.11
4b	1.0	0.35	0.22
5a	1.0	0.22	0.19
5c	1.0	0.41	0.38
6a	1.0	0.28	0.35
6b	1.0	0.05	0.11
8a	1.0	0.29	0.44
8c	1.0	0.19	0.33
9a	1.0	0.21	0.63
9b	1.0	0.94	0.83
10a	1.0	0.05	0.15
10b	1.0	0.00	0.00
10c	1.0	0.13	0.19
11a	1.0	0.00	0.00
11b	1.0	0.00	0.00
11c	1.0	0.00	0.00
12a	1.0	0.17	0.56
12c	1.0	0.00	0.00

Experimental

Melting points were determined with a Kofler block apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer model 1720 FTIR spectrometer as KBr discs. ^{1}H-NMR spectra were recorded with varian EM-390 (200 MHz) and Bruker AC-250 (250 MHz) spectrometers. The chemical shifts are expressed in δ (ppm), using tetramethylsilane as internal standard, coupling constants are given in Hz. Microanalyses were performed at the unit of microanalysis at Cairo University. Viral screening against HBV was conducted at the National Liver Institute, Menoufia University, Egypt.

3.1. 1-(N-Arylamino) acetyl-4-phenyl thiosemicarbazides 3a–c

To a solution of 2-arylaminoacetylhydrazide (**2a–c**) (0.1 mol) in ethanol (100 ml) phenylisothiocyanate (0.1 mol) was added. The reaction mixture was heated under reflux for 1 h. The product that separated on cooling was filtered, washed with EtOH and dried [10].

3.1.1. 4-Phenyl-1-(N-phenylamino)acetyl thiosemicarbazide (**3a**)

Yield: 85%; m.p. 161–163 °C; IR (cm^{-1}): 3200, 3100 (NH), 1195 (C=S), 1675 (C=O); ^{1}H-NMR (250 MHz, DMSO-d_6), (δ ppm): 3.8 (d, J = 2.5 Hz, 2 H, CH_2), 5.7 (bs, 1 H, NH), 6.6 (m, 2 H, Ar-H), 7.1 (t, 1 H, J = 8.5 Hz, Ar-H, 7.2 (t, 1 H, J = 8.5 Hz, Ar-H), 7.4–7.7 (m, 6 H, Ar-H), 9.5 (bs, 1 H, NH), 9.9 (bs, 1 H, NH), 10.2 (s, 1 H, NH).

3.1.2. 4-Phenyl-1-(N-p-tolylamino)acetyl thiosemicarbazide (**3b**)

Yield: 86%; m.p. 160–161 °C; IR (cm^{-1}): 3250–3200 (NH), 2985 (C–H), 1675 (C=O), 1195 (C=S); ^{1}H-NMR (250 MHz), DMSO-d_6), (δ ppm): 2.1 (s, 3 H, CH_3), 3.8 (d, J = 2.6 Hz, 2 H, CH_2), 5.8 (bs, 1 H, NH), 6.5 (d, 2 H, J = 8.5 Hz, PhH-2,6), 6.8 (d, 2 H, J = 8.5 Hz, PhH-3,5), 7.2–7.5 (m, 5 H, Ph), 9.5 (bs, 1 H, NH), 9.7 (bs, 1 H, NH), 10.2 (s, 1 H, NH).

3.1.3. 1-(N-p-Fluorophenylamino)acetyl-4-phenyl thiosemicarbazide (**3c**)

Yield: 86%; m.p. 158–159 °C; IR (cm^{-1}): 3200, 3210, 3100 (NH), 2995 (C–H), 1690 (C=O), 1195 (C=S); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 3.6 (s, 2 H, CH_2), 5.7 (bs, 1 H NH), 6.6 (d, J = 8.5 Hz, 2 H, PhH-2,6), 6.9 (t, 2 H, J = 8.5 Hz, PhH-3,5), 7.2–7.6 (m, 5 H, Ph), 9.7 (bs, 1 H, NH), 9.9 (bs, 1 H, NH), 10.2 (s, 1 H, NH).

3.2. 2-(N-Arylamino)methyl-5-(phenyl)amino-1,3,4-thiadiazoles 4a–c

A suspension of 1-(*N*-arylamino)acetyl-4-phenylthiosemicarbazide **3a–c** in cold conc. sulphuric acid (10 ml) was stirred until dissolution and then left at room temperatur for 2 h. The reaction mixture was poured onto crushed ice and the precipitated product was filtered, washed with water and recrystallized from ethanol.

3.2.1. 5-(Phenyl)amino-2-(N-phenylamino)methyl-1,3,4-thiadiazole (**4a**)

Yield: 75%; m.p. 193–195 °C; IR (cm^{-1}): 3300 (NH), 3110 (NH), 3050 (C–H), 1500 (C=N).

3.2.2. 5-Phenylamino-2-(N-p-tolylamino)methyl-1,3,4-thiadiazole (**4b**)

Yield: 76%; m.p. 194–195 °C; IR (cm^{-1}): 3200 (NH), 3110 (NH), 2290 (C–H), 1500 (C=N).

3.2.3 2-(N-p-Fluorophenylamino)methyl-5-phenylamino-1,3,4-thiadiazole (**4c**)

Yield: 77%; m.p. 195–197 °C; IR (cm^{-1}): 3300 (NH), 3200 (NH), 2290 (C–H).

3.3. 3-(N-Arylamino)methyl-5-mercapto-4-phenyl-1,2,4-triazoles 5a–c

A solution of 1-(*N*-arylamino)acetyl-4-phenylthiosemicarbazide **3a–c** (0.02 mol) in 2N NaOH (100 ml) was heated under reflux for 3 h. The reaction mixture was cooled and acidified with 2N HCl. The resulting precipitate was filtered, washed with ethanol and crystallized from ethanol.

3.3.1. 5-Mercapto-4-phenyl-3-(N-phenylamino)methyl-1,2,4-triazole (**5a**)

Yield: 78%; m.p. 190–191 °C; IR (cm^{-1}): 3110 (NH), 3050 (C–H), 2795 (SH); ^{1}H-NMR (250 MHz, DMSO-d_6), (δ ppm): 4.1 (s, 2 H, CH_2), 5.8 (bs, 1 H, NH), 6.5 (m, 2 H, Ar-H), 7.1 (t, 1 H, J = 8.5 Hz, Ar-H), 7.3–7.6 (m, 7 H, Ar-H), 13.8 (s, 1 H, SH).

3.3.2. 5-Mercapto-4-phenyl-3-(N-p-tolylamino)methyl-1,2,4-triazole (**5b**)

Yield: 79%; m.p. 192–193 °C; IR (cm^{-1}): 3350 (NH), 2980 (C–H), 2785 (SH); ^{1}H-NMR (250 MHz, DMSO-d_6), (δ ppm): 2.1 (s, 3 H, CH_3), 4.1 (s, 2 H, CH_2), 5.7 (bs, 1 H, NH), 6.4 (d, 2 H, J = 8.5 Hz, PhH-2,6), 6.8 (d, 2 H, J = 8.5 Hz, PhH-3,5), 7.3–7.7 (m, 5 H, Ph), 13.9 (s, 1 H, SH).

3.3.3. 3-(N-p-Fluorophenylamino)methyl-5-mercapto-4-phenyl-1,2,4-triazole (**5c**)

Yield: 77%; m.p. 191–193 °C; IR (cm^{-1}): 3150 (NH), 2950 (C–H), 2750 (C–H); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 4.2 (s, 2 H, CH_2), 5.8 (bs, 1 H, NH), 6.5 (t, 2 H, J = 8.5 Hz, PhH-2,6), 6.8 (t, 2 H, J = 8.5 Hz, PhH-3,5), 7.4–7.7 (m, 5 H, Ph), 13.8 (s, 1 H, SH).

3.4. 5-(N-Arylamino)methyl-2-(N-phenyl)amino-1,3,4-oxadiazoles 6a–c

Mercuric oxide (0.011 mol) was added to a methanolic solution of **3a–c** and the resulting mixture was refluxed for 3 h. The precipitated mercuric sulphide was filtered off and washed with hot methanol. The filtrate on cooling gave a precipitate which was recrystalized from ethanol.

3.4.1. 2-(N-Phenyl)amino-5-(N-phenylamino)methyl-1,3,4-oxadiazole (**6a**)

Yield: 77%; m.p. 197–199 °C; IR (cm^{-1}): 3250 (NH), 3150 (NH).

3.4.2. 5-(N-p-Tolylamino)methyl-2-(N-phenyl)amino-1,3,4-oxadiazole (**6b**)

Yield: 75%; m.p. 198–200 °C; IR (cm^{-1}): 3220 (NH), 3110 (NH).

3.4.3. 5-(N-p-Fluorophenylamino)methyl-2-(N-phenyl)amino-1,3,4-oxadiazole (**6c**)

Yield: 79%; m.p. 203–204 °C; IR (cm^{-1}): 3300 (NH), 3120 (NH).

3.5. 3-(N-Arylamino)methyl-5-methylmercapto-4-phenyl-1,2,4-triazoles 7a–c

A solution of **5a–c** (0.012 mol) in 10% KOH solution (7 ml) was stirred for 0.5 h. The reaction mixture was treated with dimethyl sulphate (0.014 mol) dropwise. The precipitated solid was filtered, washed with water and crystallized from ethanol.

3.5.1. 5-Methylmercapto-4-phenyl-3-(N-phenylamino)methyl-1,2,4-triazole (**7a**)

Yield: 79%; m.p. 137–138 °C; IR (cm^{-1}): 3250 (NH), 2990 (C–H), ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 2.5 (s, 3 H, SCH_3), 4.2 (s, 2 H, CH_2), 5.5 (bs, 1 H, NH), 6.4 (m, 2 H, Ar-H), 6.8 (m, 2 H, Ar-H), 7.4–7.6 (m, 6 H, Ar-H).

3.5.2. 5-Methylmercapto-4-phenyl-3-(N-p-tolylamino)methyl-4-phenyl-1,2,4-triazole (**7b**)

Yield: 74%; m.p. 138–139 °C; IR (cm^{-1}): 3200 (NH), 2995 (C–H), ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 2.2 (s, 3 H, CH_3), 2.5 (s, 3 H, SCH_3), 4.1 (d, J = 2.6 Hz, 2 H, CH_2), 5.8 (bs, 1 H, NH), 6.4 (d, 2 H, J = 8.5 Hz, PhH-2,6), 6.8 (d, 2 H, J = 8.5 Hz, PhH-3,5), 7.4–7.7 (m, 5 H, Ph).

3.5.3. 3-(N-p-Fluorophenylamino)methyl-5-methylmercapto-4-phenyl-1,2,4-triazole (**7c**)

Yield: 72%; m.p. 137.5–139 °C; IR (cm^{-1}): 3300 (NH), 2995 (C–H), ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 2.6 (s, 3 H, SCH_3), 4.1 (s, 2 H, CH_2), 5.8 (bs, 1 H, NH), 6.7 (t, 2 H, J = 8.5 Hz, PhH-2,6), 7.1 (t, 2 H, J = 8.6 Hz, PhH-3,5), 7.4 (m, 5 H, Ph).

3.6. Ethyl[(5-N-arylamino)methyl-4-phenyl-1,2,4-triazol)-3-yl]thioglycolates 8a–c

A solution of 3-(N-arylamino)methyl-5-mercapto-4-phenyl-1,2,4-triazole **5a–c** (0.01 mol) and NaOH (0.01 mol) in ethanol (30 ml) was heated under reflux for about 45 min. To the solution was added ethyl chloroacetate (0.01 mol) and the resulting mixture was heated for further about 4 h. The mixture was filtered, cooled and poured on crushed ice. The precipitate was filtered, washed with ethanol and crystallized from ethanol.

3.6.1. Ethyl[(4-phenyl-5-(N-phenylamino)methyl-1,2,4-triazol)-3-yl]thioglycolate (**8a**)

Yield: 68%; m.p. 168–169 °C; IR (cm^{-1}): 3385 (NH), 3050 (CH), 1735 (C=O); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 1.19 (t, 3 H, J = 6.9 Hz, CH_3), 3.3 (s, 2 H, CH_2), 4.5 (q, 2 H, J = 7.0 Hz, CH_2), 6.9 (t, 1 H, J = 8.5 Hz, Ar-H), 7.2 (t, 2 H, J = 8.5 Hz, Ar-H), 7.3–7.4 (m, 4 H, Ar-H), 7.5–7.7 (m, 3 H, Ar-H), 8.4 (s, 1 H, NH).

3.6.2. Ethyl[(4-phenyl-5-(N-p-tolylamino)methyl-1,2,4-triazol)-3-yl]thioglycolate (**8b**)

Yield: 70%; m.p. 169–171 °C; IR (cm^{-1}): 3390 (NH), 3050 (CH), 1735 (C=O).

3.6.3. Ethyl[(5-(N-p-fluorophenylamino)methyl-4-phenyl-1,2,4-triazol)-3-yl]-thioglycolate (**8c**)

Yield: 70%; m.p. 167–169 °C; IR (cm^{-1}): 3380 (NH), 1735 (C=O), 1600 (C=N); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 1.1 (t, 3 H, J = 7.0 Hz CH_3), 3.9 (s, 2 H, CH_2), 4.0 (q, 2 H, J = 7.1 Hz, CH_2), 6.8 (t, 2 H, J = 8.5 Hz, PhH-2,6), 7.3 (t, 2 H, J = 8.5 Hz, PhH-3,5), 7.4–7.6 (m, 5 H, Ph), 8.4 (s, 1 H, NH).

3.7. [5-(N-Arylamino)methyl-4-phenyl–1,2,4-triazol-3-yl-]thioglycolyl-hydrazides 9a–c

A mixture of ethyl[5-*N*-arylamino)methyl-4-phenyl-1,2,4-triazol)-3-yl]-thioglycolate **8a–c** (0.01 mol) and hydrazine hydrate (0.03 mol) in ethanol (30 ml) was heated under reflux for 3 h. The excess of ethanol was removed under reduced pressure and the resulting precipitate was filtered, washed with ethanol and crystallized from ethanol.

3.7.1. [4-Phenyl-5-(N-phenylamino)methyl-1,2,4-triazol-3-yl]thioglycolyl-hydrazide (**9a**)

Yield: 79%; m.p. 140–142 °C; IR (cm^{-1}): 3050 (C–H), 1680 (C=O); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 3.9 (s, 2 H, CH_2), 4.4 (s, 2 H, CH_2), 4.2 (bs, 2 H, NH_2), 5.8 (bs, 1 H, NH), 6.5 (t, 3 H, J = 8.5 Hz, Ar-H), 7.0 (t, 2 H, J = 8.5 Hz, Ar-H), 7.4–7.6 (m, 5 H, Ph), 9.8 (bs, 1 H, NH).

3.7.2. [4-Phenyl-5-(N-p-tolylamino)methyl-1,2,4-triazole-3-yl]thioglycolyl-hydrazide (**9b**)

Yield: 81%; m.p. 139–140 °C; IR (cm^{-1}): 3300–3315 (NH), 3050 (C–H), 1650 (C=O), 1550 (C=N).

3.7.3. [5-(N-p-Fluorophenylamino)methyl-4-phenyl-1,2,4-triazol-3-yl]-thioglycolylhydrazide (**9c**)

Yield: 80%; m.p. 140–142 °C; IR (cm^{-1}): 3310 (NH), 3210 (NH), 1690 (C=O); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 3.8 (s, 2 H, CH_2), 4.3 (s, 2 H, CH_2), 4.5 (bs, 2 H, NH_2), 5.8 (bs, 1 H, NH), 6.5 (dd, J = 5.7, 8.5 Hz, 2 H, PhH-2,6), 6.8 (dd, J = 5.7, 8.5 Hz, 2 H, PhH-3,5), 7.3–7.7 (m, 5 H, Ph), 9.3 (s, 1 H, NH).

3.8. Sugar[(5-N-arylamino)methyl-4-phenyl-1,2,4-triazol-3-yl]thioglycolyl-hydrazones 10–12

A solution of the sugar (0.01 mol) in water (3 ml) was treated with a solution of **9** (0.01 mol) in ethanol (100 ml) and few drops of glacial acetic acid. The mixture was boiled under reflux for 3 h. The excess ethanol was removed under reduced pressure and the residue was triturated with 15 ml diethyl ether and the product was filtered, washed with ether and crystallized from ethanol.

3.8.1. D-Mannose[4-phenyl-5-(N-phenylamino)methyl-1,2,4-triazol-3-yl-]-thioglycolylhydrazone (**10a**)

Yield: 70%; m.p. 99–101 °C; IR (cm^{-1}): 3460–3350 (OH), 3200 (NH), 2950 (C–H), 1690 (C=O), 1550 (C=N); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 3.7 (m, 3 H, 5′-H; 2 × 6′-H), 3.9 (m, 3 H, 2′-H; 3′-H; 4′-H), 4.2 (m, 3 H, 3 OH), 4.4 (bs, 2 H, 2 OH), 4.6 (bs, 4 H, 2 CH_2), 6.5 (m, 3 H, Ar-H), 7.0 (t, 2 H, J = 8.5 Hz, Ar-H), 7.4–7.6 (m, 5 H, Ph), 8.6 (bs, 1 H, NH).

3.8.2. D-Mannose[4-phenyl-5-(N-p-tolylamino)methyl-1,2,4-triazol-3-yl-]-thioglycolylhydrazone (**10b**)

Yield: 68%; m.p. 101–103 °C; IR (cm^{-1}): 3500–3350 (OH), 3200–3160 (NH), 3000 (C–O), 1675 (C=O); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 2.1 (s, 3 H, CH_3), 3.7 (m, 3 H, 5′-H; 2 × 6′-H), 3.9 (bs, 3 H, 2′-H; 3′-H; 4′-H), 4.2 (bs, 3 H, 3 OH), 4.4 (bs, 2 H, 2 OH), 4.6 (bs, 4 H, 2 CH_2), 5.5 (d, 1 H J = 2.5 Hz, CH), 6.4 (d, 2 H, J = 8.5 Hz, PhH-2,6), 6.8 (d, 2 H, J = 8.5 Hz, PhH-3,5), 7.3–7.7 (m, 5 H, Ph), 8.4 (bs, 1 H, NH).

3.8.3. D-Mannose[4-phenyl-5-(N-p-fluorophenylamino)methyl-1,2,4-triazol-3-yl-]thioglycolylhydrazone (**10c**)

Yield: 69%; m.p. 99–100 °C; IR (cm^{-1}): 3500–3340 (OH), 3180–3160 (NH), 1675 (C=O); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 3.6 (m, 3 H, 5′-H; 2 × 6′-H), 4.0 (bs, 3 H, 2′-H; 3′-H; 4′-H), 4.3 (bs, 3 H, 3 OH), 4.5 (bs, 2 H, 2 OH), 4.7 (bs, 4 H, 2 CH_2), 5.4 (d, 1 H J = 2.5 Hz, CH), 6.6 (t, 2 H, J = 8.5 Hz, PhH-2,6), 6.8 (t, 2 H, J = 8.5 Hz, PhH-3,5), 7.4–7.6 (m, 5 H, Ph), 8.6 (bs, 1 H, NH).

3.8.4. D-Galactose[4-phenyl-5-(N-phenylamino)methyl-1,2,4-triazol-3-yl]-thioglycolylhydrazone (**11a**)

Yield: 69%; m.p. 98–100 °C; IR (cm^{-1}): 3250–3200 (NH), 3450–3300 (OH), 2295 (C–H), 1685 (C=O); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 3.8 (m, 3 H, 5′-H; 2 × 6′-H), 4.1 (bs, 3 H, 2′-H; 3′-H; 4′-H), 4.3 (bs, 3 H,

3 OH), 4.4 (bs, 2 H, 2 OH), 4.6 (bs, 4 H, 2 CH_2), 5.1 (d, 1 H J = 2.5 Hz, CH), 6.5 (m, 2 H, Ar-H), 6.8 (t, 2 H, J = 8.5 Hz, Ar-H), 7.4–7.7 (m, 6 H, Ar-H), 8.9 (bs, 1 H, NH).

3.8.5. D-Galactose[4-phenyl-5-(N-p-tolylamino)methyl-1,2,4-triazol-3-yl] thioglycolylhydrazone (**11b**)

Yield: 68%; m.p. 96–98 °C; IR (cm^{-1}): 3350–3500 (OH), 3150–3200 (NH), 2940 (C–H), 1675 (C=O); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 2.1 (s, 3 H, CH_3), 3.9 (m, 3 H, 5′-H; 2 × 6′-H), 4.0 (m, 3 H, 2′-H; 3′-H; 4′-H), 4.2 (s, 2 H, CH_2), 4.3 (s, 2 H, CH_2), 4.4 (bs, 5 H, 5 OH), 6.5 (d, 2 H J = 8.5 Hz, PhH-2,6), 6.8 (d, 2 H, J = 8.5 Hz, PhH-3,5), 7.4–7.7 (m, 5 H, Ph), 8.5 (bs, 1 H, NH).

3.8.6. D-Galactose[4-phenyl-5-(N-p-fluorophenylamino)methyl-1,2,4-triazol-5-yl]thioglycolylhydrazone (**11c**)

Yield: 69%; m.p. 98–100 °C; IR (cm^{-1}): 3300–3450 (OH), 2290 (C–H), 1685 (C=O), 1550 (C=N) 3200–3250 (NH); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 3.9 (m, 3 H, 5′-H; 2 × 6′-H), 4.1 (m, 3 H, 2′-H; 3′-H; 4′-H), 4.3 (m, 5 H, 5 OH), 4.5 (bs, 4 H, 2 CH_2), 5.1 (d, 1 H J = 2.5 Hz, CH), 6.5 (dd, J = 5.7, 8.5 Hz, 2 H PhH-2,6), 6.8–7.0 (dd, J = 5.7, 8.5 Hz, 2 H, PhH-3,5), 7.2–7.7 (m, 5 H, Ph), 8.8 (bs, 1 H, NH).

3.8.7. D-Ribose[4-phenyl-5-(N-p-phenylamino)methyl-1,2,4-triazol-3-yl] thioglycolylhydrazone (**12a**)

Yield: 67%; m.p. 95–97 °C; IR (cm^{-1}): 3350–3400 (OH), 3200 (NH), 2950 (C–H), 1675 (C=O); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 3.1 (m, 5 H, 2′-H; 3′-H; 4′-H; 2 × 5′-H); 3.9 (bs, 4 H, 4 OH), 4.4 (bs, 4 H, 2 CH_2), 5.3 (d, 1 H J = 2.6 Hz, CH), 6.8 (m, 5 H, Ph), 7.8 (m, 5 H, Ph), 8.4 (bs, 1 H, NH).

3.8.8. D-Ribose[4-phenyl-5-(N-p-tolylamino)methyl-1,2,4-triazol-3-yl]thioglycolylhydrazone (**12b**)

Yield: 68%; m.p. 98–99 °C; IR (cm^{-1}): 3360, 3500 (OH), 2950 (C–H), 1690 (C=O) 3150–3200 (NH), 1550 (C=N); ^{1}H-NMR (200 MHz, DMSO-d_6), (δ ppm): 2.3 (s, 3 H, CH_3), 3.6 (m, 5 H, 2′-H; 3′-H; 4′-H; 2 × 5′-H); 4.2 (m, 4 H, 4 OH), 4.6 (bs, 4 H, 2 CH_2), 5.4 (d, 1 H, J = 2.5 Hz, CH), 6.5 (d, 2 H, J = 8.5 Hz, PhH-2,6), 6.8 (d, 2 H, J = 8.5 Hz, PhH-3,5), 7.4–7.6 (m, 5 H, Ph), 8.8 (bs, 1 H, NH).

3.8.9. D-Ribose[4-phenyl-5-(N-p-fluorophenylamino)methyl-1,2,4-triazol-3-yl]thioglycolylhydrazone (**12c**)

Yield: 69%; m.p. 94–96 °C; IR (cm^{-1}): 3350, 550 (OH), 2950 (C–H), 1690 (C=O) 3150–3200 (NH).

3.9. Biological activity studies

3.9.1. Preparation and culture of Hep G2 2.2.15 cells

The requiered cell line was made by transfection of Hep G2-cells with a plasmid containing multiple tandem copies of HBV genome (subtype ayw) [21]. The 2.2.15 cell line was maintained in RPMI-1640 (Glutamax) culture media containing 100 IU/ml nystatin + 380 µg/ml G418 (geneticin). The transfected Hep G2-2.2.15 cell line was kept in tissue culture flask at 37 °C + 5% CO_2. Subcultures were set up after a week by aspiration of the media from culture flask and washing the cells twice by PBS. A 10% versene/trypsin was added and the cells were incubated for 1 min at 37 °C. Lamivudine which is a potent selective inhibitor of HBV replication [22] was used as a standard for the comparative studies.

3.9.2. PCR-ELISA

The PCR reaction mixture contained 14 µL extracted supernatant, 4 mmol/L $MgCl_2$, 10 µmol/L DIG-11-dUTP, 190 µmol/L dTTP, 200 µmol/L dATP, dGTP, dGTP, 1,5 U Taq polymerase, 20 mmol/L HCL (pH 8.4), 50 mmol/L KCL, 1 µmol/L HCID-1 primer (5′GGA AAG AAG TCA GAA GGC A3′) and 1 µmol/L HCID-2 (5′TTG GGG GAG GAG ATT AGG TT3′), in total volume 50 µL. PCR reaction conditions were 32 cycles of 1 min at 94 °C, 30 s at 58 °C and 30 s at 72 °C + 3 s for each cycle in a thermal circler as discribed in literature [23].

3.9.3. Cytotoxicity assay

A colorimetric assay for living cells should use a colores substrate which is modified to a colored product by any living cell, but not by dead cells or tissue culture medium. 3-(3,5-Dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) is an attractive candidate for this purpose. The cytotoxic effect of the compounds was accessed by culturing the Hep G2-2.2.15 cells in the presence of compounds using a MTT-assay [24].

3.9.4 Calculation of IC_{50}, CC_{50} and SI

The 50% inhibitory concentration of antiviral drugs (IC_{50}) was determined by interpolation from the plots of amount of DNA copies versus antiviral drug concentrations. The 50% cytotoxic effect (CC_{50}) was calculated from the average viability of the cells with concentration of drugs. The selective index (SI) could be calculated as CC_{50}/IC_{50}.

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