A Convenient Synthesis and Biological Activities of Novel 6-Aryl-3-(1,2,3,4-tetrahydroxybutan-1-yl)-7*H*-1,2,4-triazolo-[3,4-*b*][1,3,4]thiadiazines

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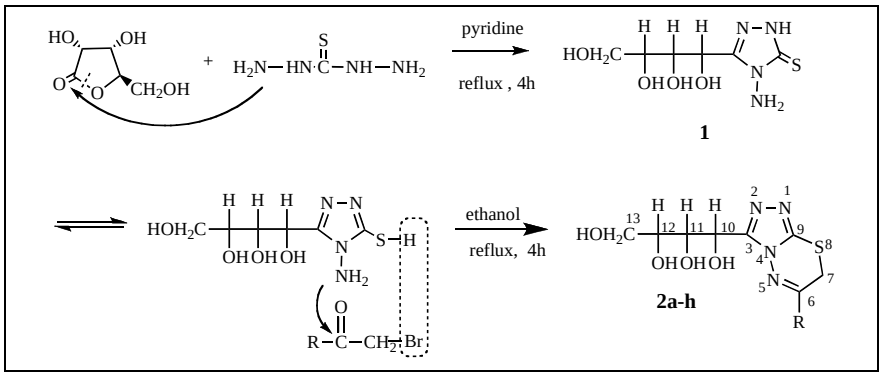
Received March 29, 2007



A series of novel 6-aryl-3-(1,2,3,4-tetrahydroxybutanol-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines were easily synthesized in high yields by means of the reactions of 4-amino-5-(1,2,3,4-tetrahydroxybutyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**1**) with substituted ω-bromoacetophenones or ω-chloroacetophenone. Nearly all of the title compounds possess plant growth-promoting activities.

J. Heterocyclic Chem., **45**, 987 (2008).

INTRODUCTION

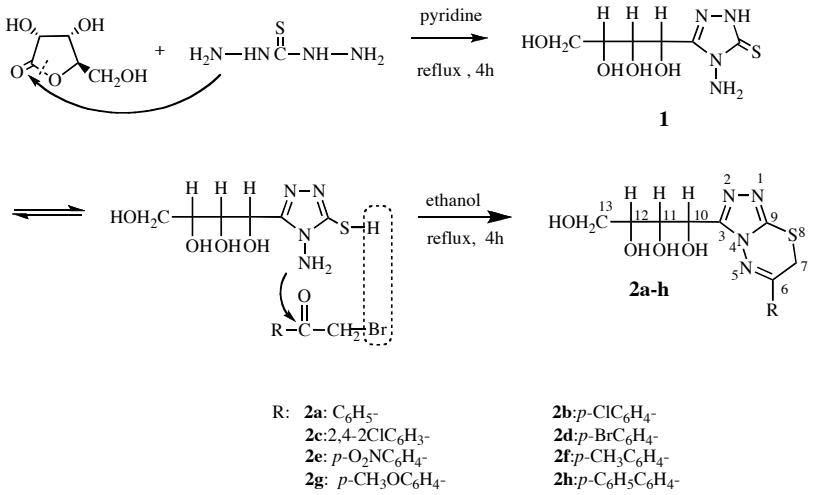
1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines are condensed heterocyclic compounds having a wide range of applications. A literature survey showed that this kind of compounds combined the properties of triazoles and thiadiazines [1,2]; thereby some of them showed wide spectrum of biological activities, such as antimicrobial, antibacterial, antifungal, anti-inflammatory, diuretic, anthelmintic, analgesic and antiparasitic [3]. They can also be used as plant growth inhibitors [4-8]. Our researches have been devoted for several years to the synthesis of a series of novel compounds - 7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines [9-12]. However, we noticed that, besides our research in this respect, almost all of the substitutions at 3-, 6- and 7-positions in this kind of compounds, which are currently available are alkyl or aryl groups [13-16]. It is disadvantageous to use these compounds as medicament because of their poor water-solubility. Considering that the D-ribonic acid residues play a special role in the body, we have synthesized a series of new compounds attaching alditolyl residues at the 3-position of 7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines, which may improve their transportation and absorption in biological systems.

The structures of all products have been characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and MS. The synthetic route to the compounds is shown in Scheme 1.

RESULTS AND DISCUSSION

In spite of R- with electron-withdrawing group or electron-donating group, the desired products were obtained in good yields, so the generality of the reaction is excellent. When **2b-e** having electron-withdrawing group were used, the rate of reactions was much faster than that in the case of R- with electron-donating group, which can be observed by the rate of the appearance of precipitation in the reaction system. The reason is that the electron-withdrawing group can increase the positive polarity of the carbonyl carbon, which is favorable for the attack of amino-group. Nevertheless, we intended to synthesize 6-(4-fluorophenyl)-3-(1,2,3,4-tetrahydroxybutan-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine and 6-(2,4-difluor-ophenyl)-3-(1,2,3,4-tetrahy-droxybutan-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine when we mixed compound **1** with 2-bromo-4'-fuoroacetophenone or 2-chloro-2', 4'-difuoroacetophenone in the same manner as we did in preparing **2a-h**. Unluckily we didn't succeed though we had tried several times and at different pH values. It is still a question to be solved. The solubility of the newly synthesized compounds, **2a-2h**, is much greater than that of the compounds which we prepared in our previous research [9-12] (see Table 1). The title compounds have been investigated for their biological activities on regulating the growth of wheat and radish

Scheme 1



the synthetic route to the title compounds

with reference of sterilized distilled water. After treating with culture solution of 10 μg/mL and 100 μg/mL of the title compounds **2a-h** for 5 days, the growth regulating percentage has been calculated. The data of biological activity test are presented in Table 2. The results indicated that among the tested compounds, almost all the newly prepared compounds showed moderate to good promoting effect on the growth of the stalk and the radicel of the wheat and radish at a mass concentration of 10 μg /mL and 100 μg /mL. However, it is interesting that **2b** showed a small amount of inhibiting effect on the growth of the radicel of the radish at 100 μg/mL and **2e** showed the same case, but at 10 μg /mL. Therefore the structure and activity relationship is worth studying further.

IR Spectra of the Title Compounds. The absence of NH_2, S─H and C═S absorption bands in the IR spectra has confirmed that the title compounds **2** were obtained *via* cyclocondensation. The stretching vibration peaks of OH group are at 3200─3600 cm^{-1}. The C─H stretching vibration peaks of CH_2 group are at 2920─2980 cm^{-1}. The

Table 1

The Water-Solubility of the Title Compounds.

Compounds	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**
Solubility in H_2O at 20°C(mg/100mL)	102	240	110	135	35	255	260	66

Table 2

Effect of compounds **2a-h** on the plant growth-regulating of wheat and radish.[a]

Compounds	Concentrations (μg/mL)	Radish		Wheat	
		Stalk	Radicel	Stalk	Radicel
2a	100	+++	+++	++	++
	10	++	+++++	+++	+++
2b	100	++	**	++	++
	10	+++	+++	++++	*
2c	100	++	++	++	+
	10	+	++	++	++
2d	100	+++	++	++	++
	10	++	+++	++	++
2e	100	++	+++	++	++
	10	++	***	++	++
2f	100	++	++++	++	++
	10	++	+++	++	++
2g	100	++	+++++	++	++
	10	+++	+++	+++	++
2h	100	++	++	++	+++
	10	+++	+++	++	++

a: " + " represents promotion rate. + : <10%; ++ : 10-30%; +++ : 30-50%; ++++ : 50-70%; +++++ : 70-90%; ++++++: >90%.
" * " represents inhibition rate. * : <10%; ** : 10-30%; *** : 30-50%.

characteristic stretching vibrations of the rings of the products are at 1570─1600 cm^{-1} (C=N), 1440─1520 cm^{-1}(N=C─S). The bending vibrations of C─S─C are in the region 680─700 cm^{-1}.

^{1}H NMR of the Title Compounds. In the ^{1}H NMR spectrum of the intermediate 4-amino-5-(1,2,3,4-tetrahydroxybutyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **1**, there are absorptions at δ 13.55 (s, 1H, N─H), 5.51 (s, 2H, -NH_2). In all the title compounds **2**, the above two absorptions have disappeared and the observation of additional resonances assigned to the SCH_2 (δ 4.4─4.6 ppm), which also confirmed the ring-closure. We note that, in almost all title compounds (except **2c**), there are broad and single absorption peaks at δ5.1─6.3 ppm assigned to the O─H. The C_{10}─H absorption peaks in the title compounds all show clear double peaks.

^{13}C NMR of the Title Compounds. The intermediate 4-amino-5-(1,2,3,4-tetrahydroxybutyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione exhibited absorption peaks at δ 165.1, 152.1 due to N─C=N, N─C=S respectively, at δ 72.8, 72.7, 66.1, 62.5 due to the four carbon atoms of the D-ribonic acid residues. The title compounds showed absorptions at δ 154─163, 154─157, 141─149 due to N─C=N, N─C=S and Ph─C=N group respectively. The δ values (114─143) of the C─H of the phenyl group are different because of the phenyl group with different substituted groups. The chemical shift values (δ 26.0─23.0) of SCH_2 are decreasing in the order of Ar = 2, 4-dichlorophenyl (25.95), 4-nitrophenyl, phenyl, 4-chlorophenyl, 4-bromophenyl, 4-diphenyl, 4-methylphenyl, 4-methoxylphenyl (22.94).

We are grateful for financial support from the Natural Science Foundation of Zhejiang Province, China (Project No. M203149).

EXPERIMENTAL

Mps (uncorrected) were taken on an XT-4 melting point apparatus; IR spectra were determined on a Nicolet 670FT-IR using the smart OMNI-Sampler in the range 4000─400 cm^{-1}; ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Avance-300 NMR spectrometer respectively in DMSO-d_6 solution using TMS as an internal reference; MS spectra were recorded on an Agilent 1100 LC/MS. The contents of carbon, hydrogen and nitrogen were determined on a Flash-1112 series elemental analyzer.

4-Amino-5-(1,2,3,4-tetrahydroxybutyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (1). To a solution of D-(+)-ribonic γ-lactone (1.24 g, 8 mmol) dissolved in pyridine was added thiocarbohydrazide (0.89 g, 8 mmol), which was prepared by means of the method from literature [17]. The mixture was refluxed for 4 h under stirring. After concentration under reduced pressure, the crude product was recrystallized from alcohol to afford compound **1** as white powder, 1.69 g (89%), mp 156-158°C (from ethanol). IR(cm^{-1}): 3347 (OH), 2940 (CH_2), 1603 (C=N), 1507 (N=C─S). ^{1}H NMR (DMSO-d_6, 300MHz, ppm): 13.55 (s, 1H, N─H, disappear upon D_2O treatment), 5.69 (d, 1H, *J*=5.8Hz, CH_2OH-CHOH-CHOH-C*H*OH-), 5.51 (s, 2H, -NH_2, disappear upon D_2O treatment), 4.90 (d, 1H, O─H, *J*=6.1Hz, disappear upon D_2O treatment), 4.83-4.79 (m, 1H, O─H, disappear upon D_2O treatment), 4.69 (d, 1H, O─H, *J*=4.9Hz, disappear upon D_2O treatment), 4.46-4.44(m, 1H, ─OH, disappear upon D_2O treatment), 3.94─3.95 (m, 1H, CH_2OH-CHOH-C*H*OH-), 3.57─3.61 (m, 2H, C*H_2*OH-), 3.42─3.48 (m, 1H, CH_2OH-C*H*OH-). ^{13}C NMR (DMSO-d_6, 75MHz, ppm): 165.13, 152.05, 72.83, 72.73, 66.11, 62.45. MS-ESI: *m/z* (relative intensity, %): 237 [M^++1](100), 219 (2.0), 181 (1.5), 159 (3.0). Anal. Calcd for $C_6H_{12}N_4O_4S$: C, 30.50; H, 5.12; N, 23.72. Found: C, 30.28; H, 5.18; N, 23.46.

General Method for the Preparation of 6-ar-yl-3-(1,2, 3,4-tetrahydroxybutan-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines 2a-h. A mixture of **1** (1.0 mmol) and substituted ω-bromoacetophenones or ω-chloroacetophenone in ethanol (20 mL) was refluxed for 4h under stirring. The solid obtained on cooling was filtered, washed with cold water, air dried and recrystallized from C_2H_5OH to give compounds **2a-h**.

6-Phenyl-3-(1, 2, 3, 4-tetrahydroxybutan-1-yl)-7*H*-1, 2, 4-triazolo[3, 4-*b*][1, 3, 4]thiadiazine (2a). This compound was prepared from **1** and ω-chloroacetophenone and obtained as pale yellow powder (0.27 g, 80%), mp 139─141°C (from ethanol). IR(cm^{-1}): 3293 (OH), 2920 (CH_2), 1590 (C=N), 1445 (N=C─S) , 693 (C─S─C). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): 7.57─8.07 (m, 5H, Ar-H), 5.87 (br, 4H, O─H, disappear upon D_2O treatment), 5.15 (d, 1H, *J*=6.4Hz, CH_2OH-CHOH-CHOH-C*H*OH-), 4.41─4.54 (m, 2H, SCH_2), 4.09─4.13 (m, 1H, CH_2OH-CHOH-C*H*OH-), 3.57─3.69 (m, 2H, C*H_2*OH-), 3.45─3.51 (m, 1H, CH_2OH-C*H*OH-). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 156.30, 154.51, 141.59, 133.38, 132.37, 129.21, 127.89, 72.83, 72.54, 66.42, 62.74, 23.12. MS-ESI: *m/z* (relative intensity, %): 337 [M^++1](100), 246 (3.0), 217(2.0). Anal. Calcd for $C_{14}H_{16}N_4O_4S$: C, 49.99; H, 4.79; N, 16.66. Found: C, 49.72; H, 4.66; N, 16.87.

6-(4-Chlorophenyl)-3-(1,2,3,4-tetrahydroxy-butan-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine (2b). This compound was prepared from **1** and 2-bromo-4'-chloroacetophenone and obtained as pale yellow powder (0.33 g, 89%), mp 185─189°C (from ethanol). IR(cm^{-1}): 3505 (OH), 2978 (CH_2), 1587 (C=N), 1519 (N=C─S) , 698 (C─S─C). ^{1}H NMR (DMSO-d_6, 300MHz, ppm): 7.64-8.10 (m, 4H, Ar-H), 6.36 (br, 4H, O─H, disappear upon D_2O treatment), 5.16 (d, 1H, *J*=6.2Hz, CH_2OH-CHOH-CHOH-C*H*OH-), 4.39-4.55 (m, 2H, SCH_2), 4.06-4.10 (m, 1H, ─CH_2OH-CHOH-C*H*OH-), 3.58-3.66 (m, 2H, C*H_2*OH-), 3.48-3.50 (m, 1H, CH_2OH-C*H*OH-). ^{13}C NMR (DMSO-d_6, 75MHz, ppm): 155.51, 154.51, 141.66, 137.33, 132.15, 129.70, 129.32, 72.84, 72.34, 66.35, 62.72, 23.05. MS-ESI: *m/z* (relative intensity, %): 373 [M^++2] (35), 371 [M^+](100), 323 (0.5). Anal. Calcd for $C_{14}H_{15}ClN_4O_4S$: C, 45.35; H, 4.08; N, 15.11. Found: C, 45.50; H, 4.15; N, 14.92.

6-(2,4-Dichlorophenyl)-3-(1,2,3,4-tetrahydroxybutan-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine (2c). This compound was prepared from **1** and 2,2',4'-trichloro-acetophenone and obtained as white powder (0.32 g, 80%), mp 180-182°C (from ethanol). IR(cm^{-1}): 3240 (OH), 2930 (CH_2), 1581 (C=N), 1466 (N=C─S), 702 (C─S─C). ^{1}H NMR (DMSO-d_6, 300MHz, ppm): 7.11─7.87 (m, 3H, Ar-H), 5.72 (br, 1H, O─H, disappear upon D_2O treatment), 4.94 (d, 1H, *J*=8.1Hz, CH_2OH-CHOH-CHOH-C*H*OH-), 4.68(br, 3H, O─H, disappear upon D_2O treatment), 4.23(s, 2H, SCH_2), 4.02-4.05 (m, 1H, CH_2OH-CHOH-C*H*OH-), 3.58-3.66 (m, 2H, C*H_2*OH-), 3.46-3.49 (m, 1H, CH_2OH-C*H*OH).

^{13}C NMR (DMSO-d_6, 75MHz, ppm): 154.61, 154.41, 140.31, 136.33, 133.63, 132.73, 132.51, 129.89, 128.14, 73.20, 72.89, 65.95, 62.63, 25.95. MS-ESI: *m/z* (relative intensity, %): 407 [M^++2](66), 405 [M^+](100), 371 (2.0). Anal. Calcd for $C_{14}H_{14}Cl_2N_4O_4S$: C, 41.49; H, 3.48; N, 13.83. Found: C, 41.26; H, 3.32; N, 14.02.

6-(4-Bromophenyl)-3-(1,2,3,4-tetrahydroxy-butan-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine (2d). This compound was prepared from **1** and 2,4'-dibromoacetophenone and obtained as pale yellow powder (0.37g, 90%). mp 195-200°C (from ethanol). IR(cm^{-1}): 3514 (OH), 2979 (CH_2), 1584 (C=N), 1520 (N=C─S), 696 (C─S─C). ^{1}H NMR (DMSO-d_6, 300MHz, ppm): 7.81-8.02 (m, 4H, Ar-H), 6.20 (br, 4H, O─H, disappear upon D_2O treatment), 5.19 (d, 1H, *J*=5.7Hz, CH_2OH-CHOH-CHOH-C*H*OH-), 4.41-4.57 (m, 2H, SCH_2), 4.06-4.10 (m, 1H, CH_2OH-CHOH-C*H*OH-), 3.57-3.66 (m, 2H, C*H*$_2$OH-), 3.44-3.50 (m, 1H, CH_2OH-C*H*OH-). ^{13}C NMR (DMSO-d_6, 75MHz, ppm): 156.00, 154.53, 141.91, 132.42, 132.28, 129.88, 126.42, 72.81, 72.20, 66.41, 62.73, 23.05. MS-ESI: *m/z* (relative intensity, %): 417 [M^++2](100), 415 [M^+](100), 414(1.0). Anal. Calcd for $C_{14}H_{15}BrN_4O_4S$: C, 40.49; H, 3.64; N, 13.49. Found: C, 40.26; H, 3.28; N, 13.77.

6-(4-Nitrophenyl)-3-(1,2,3,4-tetrahydroxy-butan-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine (2e). This compound was prepared from **1** and 2-bromo-4'-nitroacetophenone and obtained as yellow powder (0.35 g, 92%), mp 199-200°C (from ethanol). IR(cm^{-1}): 3361 (OH), 2960 (CH_2), 1578 (C=N), 1522 (N=C─S), 686 (C─S─C). ^{1}H NMR (DMSO-d_6, 300MHz, ppm): 8.23-8.43 (m, 4H, Ar-H), 5.77 (br, 4H, O─H, disappear upon D_2O treatment), 5.15 (d, 1H, *J*=6.2Hz, CH_2OH-CHOH-CHOH-C*H*OH-), 4.47-4.61 (m, 2H, SCH_2), 4.07-4.11 (m, 1H, CH_2OH-CHOH-C*H*OH-), 3.60-3.66 (m, 2H, C*H*$_2$OH-), 3.45-3.50 (m, 1H, CH_2OH-C*H*OH-). ^{13}C NMR (DMSO-d_6, 75MHz, ppm): 154.59, 154.39, 149.30, 141.27, 139.12, 129.13, 124.04, 72.68, 72.31, 66.17, 62.53, 23.12. MS-ESI: *m/z* (relative intensity, %): 382[M^++1](100), 352 (7.0), 352 (6.0), 262 (2.0). Anal. Calcd for $C_{14}H_{15}N_5O_6S$: C, 44.09; H, 3.96; N, 18.36. Found: C, 44.26; H, 4.08; N, 18.02.

6-(4-Methylphenyl)-3-(1,2,3,4-tetrahydroxy-butan-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine (2f). This compound was prepared from **1** and 2-bromo-4'-methyl-acetophenone and obtained as pale yellow crystal (0.27 g, 77%), mp 172-174°C (from ethanol). IR(cm^{-1}): 3257 (OH), 2970 (CH_2), 1595 (C=N), 1519 (N=C─S), 694 (C─S─C). ^{1}H NMR (DMSO-d_6, 300MHz, ppm): 7.41-8.00 (m, 4H, Ar-H), 6.16 (br, 4H, O─H, disappear upon D_2O treatment), 5.22 (d, 1H, *J*=5.4Hz, CH_2OH-CHOH-CHOH-C*H*OH-), 4.41-4.59 (m, 2H, SCH_2), 4.07-4.11 (m, 1H, ─CH_2OH-CHOH-C*H*OH-), 3.60-3.67 (m, 2H, C*H*$_2$OH-), 3.44-3.50 (m, 1H, CH_2OH-C*H*OH-), 2.41(s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 75MHz, ppm): 157.36, 154.43, 143.10, 142.44, 130.22, 129.84, 128.02, 72.73, 71.95, 66.53, 62.75, 23.10, 21.23. MS-ESI: *m/z* (relative intensity, %): 351[M^++1](100), 246 (1.0). Anal. Calcd for $C_{15}H_{18}N_4O_4S$: C, 51.42; H, 5.18; N, 15.99. Found: C, 51.20; H, 5.06; N, 16.22.

6-(4-Methoxylphenyl)-3-(1,2,3,4-tetrahydroxybutan-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine (2g). This compound was prepared from **1** and 2-bromo-4'-methoxy-acetophenone and obtained as pale yellow powder (0.27 g, 74%), mp 186-188°C (from ethanol). IR(cm^{-1}): 3318 (OH), 2920 (CH_2), 1586 (C=N), 1514 (N=C─S), 698 (C─S─C). ^{1}H NMR (DMSO-d_6, 300MHz, ppm): 6.99─8.07 (m, 4H, Ar-H), 5.64 (br, 4H, O─H, disappear upon D_2O treatment), 5.21 (d, 1H, *J*=5.4Hz, CH_2OH-CHOH-CHOH-C*H*OH-), 4.38-4.56 (m, 2H, SCH_2), 4.07-4.11 (m, 1H, ─CH_2OH-CHOH-C*H*OH-), 3.87(s, 3H, OCH_3), 3.57-3.65 (m, 2H, C*H*$_2$OH-), 3.44-3.49 (m, 1H, CH_2OH-C*H*OH-). ^{13}C NMR (DMSO-d_6, 75MHz, ppm): 162.94, 156.88, 154.29, 142.38, 130.05, 125.08, 114.75, 72.74, 71.96, 66.53, 62.76, 55.82, 22.94. MS-ESI: m/z (relative intensity, %): 367[M^++1](100), 247 (2.0). Anal. Calcd for $C_{15}H_{18}N_4O_5S$: C, 49.17; H, 4.95; N, 15.29. Found: C, 49.38; H, 5.05; N, 15.56.

6-(4-Diphenyl)-3-(1,2,3,4-tetrahydroxybutan-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine (2h). This compound was prepared from **1** and 4-phenylphenacyl bromid and obtained as pale yellow crystal (0.36 g, 87%), mp 192-194°C (from ethanol). IR(cm^{-1}): 3323 (OH), 2930 (CH_2), 1595 (C=N), 1519 (N=C─S), 702 (C─S─C). ^{1}H NMR (DMSO-d_6, 300MHz, ppm): 7.41-8.16 (m, 9H, Ar-H), 5.09 (br, 4H, O─H, disappear upon D_2O treatment), 5.14 (d, 1H, *J*=6.8Hz, CH_2OH-CHOH-CHOH-C*H*OH-), 4.40-4.55 (m, 2H, SCH_2), 4.10-4.15 (m, 1H, CH_2OH-CHOH-C*H*OH-), 3.61-3.72 (m, 2H, C*H*$_2$OH-), 3.46-3.52 (m, 1H, CH_2OH-C*H*OH-). ^{13}C NMR (DMSO-d_6, 75MHz, ppm): 155.42, 154.46, 143.70, 141.30, 138.89, 132.31, 129.25, 128.49, 128.29, 127.31, 127.05, 72.94, 72.66, 66.28, 62.74, 23.03. MS-ESI: *m/z* (relative intensity, %): 413[M^++1](100), 293 (2.0), 279(1.0). Anal. calcd. (%) for $C_{20}H_{20}N_4O_4S$: C, 58.24; H, 4.89; N, 13.58. Found: C, 58.02; H, 4.78; N, 13.76.

Biological Evaluation.

Effect of compounds 2a-h on the vegetative growth of wheat and radish plant. Seeds were rinsed with sterilized distilled water four times. All subsequent manipulations were carried out under a horizontal laminar flow. Twenty seeds of each species were chosen and individually placed in culture dishes of 9cm diameter containing two pieces of filter paper and 5 mL solution of the tested compounds **2a-h** (10 μg /mL and 100 μg/mL, respectively), and were incubated in a growth chamber at 25°C, with a 16h/8h photoperiod. The set of controls with sterilized distilled water were simultaneously prepared. Plant lengths were recorded on the 5th day for the treated plants and for the set controls. Experiments were run in duplicate. The equations of the growth regulating percentage (the stalk and the radicel of the wheat and radish) are: {the average of sample length (cm) – the average of the controls (cm)}/the average of the controls (cm)*100%.

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