The synthesis and biological activities of 6-aryl-3-(D-glucoheptonic-hexitol-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines

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A series of novel 6-aryl-3-(D-glucoheptonic-hexitol-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazines (**2a–2j**) have been synthesised in high yield by means of the reaction of 4-amino-5-mercapto-3-(D-glucoheptonic-hexitol-1-yl)-1,2,4-triazole (**1**) with substituted ω-brom- oacetophenones. The structures of the compounds were determined by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and MS. Most possessed high plant growth-regulating activities.

Keywords: glucoheptonic acid residue, 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines, 1,2,4-triazolo-5-thione, D-glucoheptonic-hexitol-1-yl, synthesis

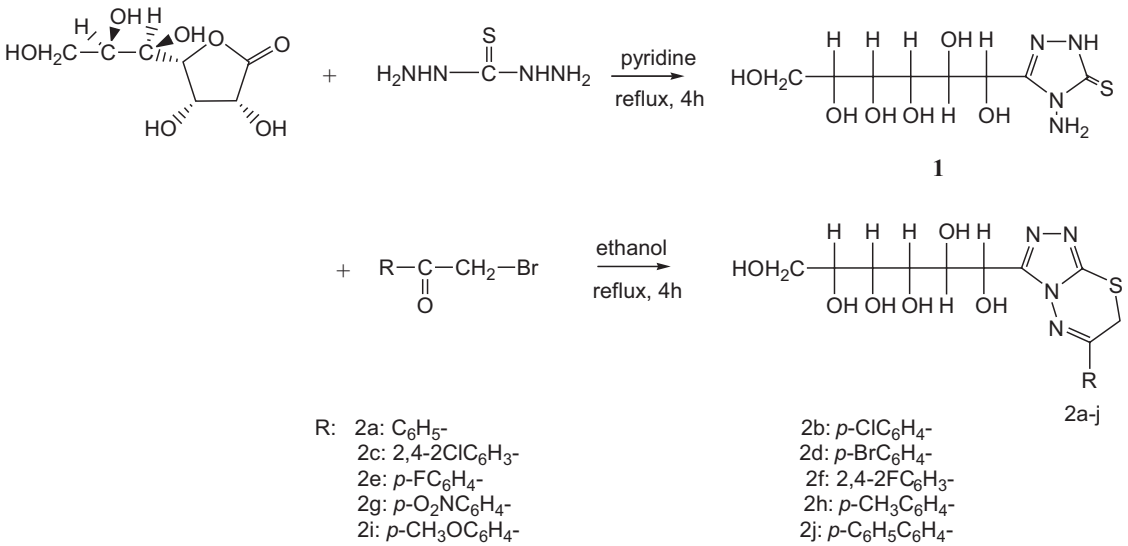
3,6-Disubstituted-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines are important because of their antimicrobial, antibacterial, antifungal, anti-inflammatory, diuretic, anthelmintic, analgesic and antiparasitic biological activity.[1,2] In our previous paper, we have reported the synthesis of some compounds containing the thiadiazine ring, which possessed moderate regulating effects on the growth of mung bean sprouts.[1] Recently, progress has been made[3-7] into combining the 1,2,4-triazole nucleus with *N*-bridged heterocycles.[3–7]

Nonetheless, almost all of the substituents at the 3- and 6-positions in these compounds which are currently available are alkyl or aryl groups. It is unsatisfactory to use these compounds as medicament due to their poor water-solubility. Hence attaching the D-hexitol-1-yl group to 7*H*-1,2,4-triazolo [3,4-*b*][1,3,4]thiadiazines at the 3-position could improve their absorption in biological systems. It was our objective to prepare the title compounds.

All the products have been characterised by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and MS. The DEPT spectra of **2a–j** all clearly show two secondary carbon atoms existing in each synthesised compound. The signals at δ 63.4, δ 22.8–25.9 were attributes to OCH_2, SCH_2 residues respectively. The synthetic route of the title compounds is shown in Scheme 1.

We note that, in spite of R- being either an electron-withdrawing group or an electron-donating group, the desired products were obtained in very good yields, so the generality of the reaction is excellent.

The newly synthesised compounds, **2a–2j**, were tested for their solubility in water. As we expected, the data showed that the water-solubility of the title compounds was greater than that of our previously prepared 3, 6-disubstituted-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (see Table 1). The title compounds have been investigated for their biological activities in regulating the growth of wheat and radish with reference of sterilised distilled water. After the growth regulating percentage has been calculated using solution of 10μg/ml and 100μg/ml of the title compounds **2a–2j** for 5 days, the biological activity data is presented in Table 2. The results indicated that among the tested compounds, almost all the newly prepared compounds showed a moderate to good inhibiting effect on the growth of the stalk and the radicel of the wheat and radish at a concentration of 10μg/ml and 100μg/ml. However, it is interesting that **2b**, **2h** and **2g** show promoting effect on the growth of the stalk and the radicel of the radish at lower concentration, 10μg/ml. Hence the structure: activity relationships are worth studying further.



Scheme 1

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Table 1 The water-solubility of the title compounds

Compounds	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j
Solubility in H_2O at 20°C(mg/100 ml)	12.0	13.3	2.0	12.5	15.4	12.5	20.0	2.2	20.0	2.0

Experimental

Carbon, hydrogen and nitrogen analyses were determined on a Flash-1112 series elemental analyser. Rotations were measured on a Rudolph AuTo Pol IV automatic polarimeter. IR spectra were recorded on a Nicolet 670FT-IR using the smart OMNI-Sampler in the range 4000–400 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-300 NMR spectrometer in DMSO-d_6 solution using TMS as an internal reference. MS spectra were recorded on an Agilent 1100 LC/MS. Melting points were determined on an XT-4 melting point apparatus and were uncorrected. All chemicals and solvents used were of AR grade.

*Synthesis of 4-Amino-3-(D-glucoheptonic-hexitol-1-yl)-1H-1, 2, 4-triazole-5-thione (**1**): general procedure*

To a solution of α-D-glucoheptonic-γ-lactone (4.16 g, 20 mmol) dissolved in pyridine was added thiocarbohydrazide (2.12 g, 20 mmol), which have been prepared by literature methods.[8] The mixture was refluxed for 4 h with stirring. After concentration under reduced pressure, the crude product was recrystallised from alcohol to afford compound (**1**): White powder, yield% 80%. M.p. 153–155°C (from ethanol). $[\alpha]_D$ = +34.5°(*c* 0.104, CH_3OH). IR(cm^{-1}): 3470 (OH), 2925 (CH_2), 1620 (C=N), 1502 (N=C–S). ^1H NMR (DMSO-d_6, 300 MHz, ppm): 13.54 (s, 1H, N–H), 5.46 (s, 2H, -NH_2), 4.05–3.99 (m, 6H, O–H), 3.42–3.38 (m, 7H, –OCH). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 165.3, 152.8, 74.1, 74.1, 71.7, 67.2, 65.0, 63.4. MS-ESI (*m/z*): 297(M^+ + 1), 279, 278, 241, 205, 181, 159. Elemental anal. calcd. (%) for $C_8H_{16}N_4O_6S$: C, 32.4; H, 5.4; N, 18.9. Found(%): C, 32.3; H, 5.5; N, 18.7.

Synthesisof6-aryl-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo [3,4-b][1,3,4]thiadiazines (**2**): *general procedure*

A mixture of 4-amino-3-(D-glucoheptonic-hexitol-1-yl)-1*H*-1, 2, 4-triazole-5-thione (1.7 mmol) and *ω*-bromoacetophenones (1.7 mmol) in ethanol (20 ml) was stirred under reflux for 4 h. The solid obtained on cooling was filtered, washed with cold water, air dried and recrystallised from ethanol to give the pure products (**2a–2j**).

6-Phenyl-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine (**2a**): White powder, yield% 91%. M.p. 196–198°C (from ethanol). $[\alpha]_D$ = –8.3°(*c* 0.096, CH_3OH). IR (cm^{-1}): 3494 (OH), 2920 (CH_2), 1590 (C=N), 1466 (N=C–S), 700 (C–S–C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): 8.04–7.59 (m, 5H, Ar–H), 5.04–4.22 (m, 6H, O–H), 4.35 (s, 2H, SCH_2), 4.07–3.96 (m, 7H, –OCH). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 154.8, 154.3, 140.2, 133.8, 131.9, 129.1, 127.6, 74.3, 74.0, 71.8, 67.4, 64.7, 63.4, 23.0. MS-ESI (*m/z*): 397(M^+ + 1), 379, 363, 333, 295, 279, 247, 217. Elemental anal. calcd. for $C_{16}H_{20}N_4O_6S$: C, 48.5; H, 5.1; N, 14.1. Found: C, 48.6; H, 5.1; N, 14.4%.

6-(4-Chlorophenyl)-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine (**2b**): White powder, yield% 89%. M.p. 223–225°C (from ethanol). $[\alpha]_D$ = –18.1°(*c* 0.110, CH_3OH). IR(cm^{-1}): 3492 (OH), 2910 (CH_2), 1592 (C=N), 1470 (N=C–S), 690 (C–S–C). ^1H NMR(DMSO-d_6, 300 MHz, ppm): 8.05–7.65(m, 4H, Ar–H), 5.02–4.35 (m, 6H, O–H), 4.33 (s, 2H, SCH_2), 4.28–3.24(m, 7H, –OCH). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 154.8, 153.2, 140.1, 136.8, 132.7, 129.4, 129.2, 74.2, 73.8, 71.8, 67.5, 64.8, 63.4, 22.9. MS-ESI (*m/z,* relative intensity, %): 433 (M^+ + 2) (35), 432 (M^+ + 1) (18), 431(M^+) (100), 413, 279, 251, 237, 204. Elemental anal. calcd. for $C_{16}H_{19}ClN_4O_6S$: C, 44.6; H, 4.4; N, 13.0. Found: C, 44.8; H, 4.5; N, 13.2%.

6-(2,4-Dichlorophenyl)-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine(**2c**): White powder, yield% 90%. M.p. 213–215°C (from ethanol). $[\alpha]_D$ = –4.536°(*c* 0.088, CH_3OH). IR (cm^{-1}): 3421 (OH), 2950 (CH_2), 1589 (C=N), 1470 (N=C–S), 695 (C–S–C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): 7.88–7.63 (m, 3H, Ar–H), 4.94–4.27 (m, 6H, O–H), 4.26 (s, 2H, SCH_2), 4.17–3.38 (m, 7H, –OCH). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 154.9, 154.3, 140.2, 136.3, 133.7, 132.7, 132.5, 129.9, 128.2, 74.2, 74.1, 71.7, 67.2, 64.6, 63.4, 25.9. MS-ESI (*m/z*, relative intensity, %): 467 (M^+ + 2) (67), 466 (M^+ + 1) (19), 465 (M^+) (100), 431, 285, 279, 204, 181. Elemental anal. calcd. for $C_{16}H_{18}Cl_2N_4O_6S$: C, 41.3; H, 3.9; N, 12.0. Found: C, 41.2; H, 3.85; N, 12.2%.

6-(4-Bromophenyl)-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine (**2d**): Pale yellow powder, yield% 87%. M.p. 225–227°C (from ethanol). $[\alpha]_D$ = –11.1°(*c* 0.108, CH_3OH). IR (cm^{-1}): 3490 (OH), 2950 (CH_2), 1587 (C=N), 1469 (N=C–S), 690 (C–S–C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): 7.98–7.79 (m, 4H, Ar–H), 5.02–4.98 (m, 1H, O–H), 4.54–4.30 (m, 5H, O–H), 4.36 (s, 2H, SCH_2), 4.24–3.39 (m, 7H, –OCH). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 154.8, 153.4, 140.1, 133.0, 132.2, 129.6, 125.7, 74.2, 73.8, 71.8, 67.5, 64.8, 63.4, 22.8. MS-ESI (*m/z*, relative intensity, %): 477 (M^+ + 2) (100), 476 (M^+ + 1) (19), 475 (M^+) (100), 459, 325, 297, 279, 237, 204. Elemental anal. calcd. (%) for $C_{16}H_{19}BrN_4O_6S$: C, 40.4; H, 4.0; N, 11.8. Found: C, 40.3; H, 4.1; N, 12.0%.

6-(4-Fluorophenyl)-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine(**2e**): White powder, yield% 86%. M.p. 203–205°C (from ethanol). $[\alpha]_D$ = –14.0°(*c* 0.114, CH_3OH).

Table 2 Effect of compounds **2a–2j** 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	+++	++++	*****	****
2i	100	****	****	***	+
	10	*****	****	****	**
2j	100	**	**	****	***
	10	**	***	*****	***

Inhibition rate: * <10%; ** 10–30%; *** 30–50%; **** 50–70%; ***** 70–90%; ****** >90%.
Promotion rate. + <10%; ++ 10–30%; +++ 30–50%; ++++ 50–70%; +++++ 70–90%; ++++++ >90%.

IR (cm^{-1}): 3490 (OH), 2956 (CH_2), 1597 (C=N), 1463 (N=C–S), 698 (C–S–C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): 8.12–7.40 (m, 4H, Ar–H), 5.03–4.30 (m, 6H, O–H), 4.37 (s, 2H, SCH_2), 4.24–3.41 (m, 7H, –OCH). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 166.0, 162.7, 154.8, 153.3, 140.1, 130.3, 130.2, 116.4, 116.1, 74.2, 73.9, 71.8, 67.5, 64.8, 63.4, 23.0. MS-ESI (*m/z*): 415 (M^+ + 1), 397, 379, 341, 326, 301. Elemental anal. calcd. for $C_{16}H_{19}FN_4O_6S$: C, 46.4; H, 4.6; N, 13.5. Found: C, 46.3; H, 4.6; N, 13.2%.

*6-(2, 4-Difluorophenyl)-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo[3,4-b] [1,3,4]thiad*iazine(**2f**): White powder, yield% 89%. M.p. 189–190°C (from ethanol). $[\alpha]_D$ = –11.7°(*c* 0.102, CH_3OH). IR (cm^{-1}): 3464 (OH), 2910 (CH_2), 1613 (C=N), 1470 (N=C–S), 690 (C–S–C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): 7.91–7.32 (m, 3H, Ar–H), 4.48–4.16 (m, 6H, O–H), 4.37 (s, 2H, SCH_2), 4.03–3.26 (m, 7H, –OCH). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 165.9, 162.7, 162.5, 154.9, 151.3, 140.1, 132.1, 132.0, 119.8, 119.7, 112.9, 112.6, 105.7, 105.4, 105.0, 74.3, 74.0, 71.7, 67.3, 64.7, 63.4, 25.1. MS-ESI (*m/z*): 433 (M^+ + 1), 341, 283, 281, 279, 254, 253, 156. Elemental anal. calcd. for $C_{16}H_{18}F_2N_4O_6S$: C, 44.4; H, 4.2; N, 13.0. Found: C, 44.3; H, 4.3; N, 12.7%.

6-(4-Nitrophenyl)-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine (**2g**): Pale yellow powder, yield% 91%. M.p. 230–232°C (from ethanol). $[\alpha]_D$ = –46.7°(*c* 0.108, CH_3OH). IR (cm^{-1}): 3313(OH), 2984 (CH_2), 1528 (C=N), 1471 (N=C–S), 677 (C–S–C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): 8.43–8.22 (m, 4H, Ar–H), 5.07–4.22 (m, 6H, O–H), 4.46 (s, 2H, SCH_2), 4.05–3.24 (m, 9H, –OCH). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 155.0, 152.6, 149.2, 140.1, 139.8, 129.0, 124.2, 74.1, 73.8, 71.9, 67.5, 64.9, 63.4, 23.1. MS-ESI (*m/z*): 442 (M^+ + 1), 413, 412, 411, 262, 232. Elemental anal. calcd. for $C_{16}H_{19}N_5O_8S$: C, 43.5; H, 4.3; N, 15.9. Found: C, 43.4; H, 4.3; N, 15.6%.

6-(4-Methylphenyl)-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine (**2h**): White powder, yield% 87%. M.p. 230–231°C (from ethanol). $[\alpha]_D$ = –12.7°(*c* 0.094, CH_3OH). IR (cm^{-1}): 3488 (OH), 2958 (CH_2), 1570 (C=N), 1471 (N=C–S), 682 (C–S–C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): 7.94–7.38 (m, 4H, Ar–H), 5.02–4.22 (m, 6H, O–H), 4.35 (s, 2H, SCH_2), 4.06–3.40 (m, 7H, –OCH), 2.40 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 154.7, 154.2, 142.1, 140.2, 131.0, 129.8, 127.6, 74.3, 74.0, 71.8, 67.4, 64.7, 63.4, 22.9, 21.2. MS-ESI (*m/z*): 411(M^+ + 1), 393, 287, 261, 259, 232, 231, 204. Elemental anal. calcd. for $C_{17}H_{22}N_4O_6S$: C, 49.75; H, 5.4; N, 13.65. Found: C, 49.6; H, 5.5; N, 13.4%.

6-(4-Methoxylphenyl)-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine (**2i**): White powder, yield% 85%. M.p. 210–212°C (from ethanol). $[\alpha]_D$ = –13.6°(*c* 0.088, CH_3OH). IR (cm^{-1}): 3337 (OH), 2920 (CH_2), 1606 (C=N), 1468 (N=C–S), 700 (C–S–C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): 8.02–7.99 (m, 2H, Ar–H), 7.15-7.12 (m, 2H, Ar–H), 5.00 (m, 1H, O–H), 4.34–4.31 (m, 5H, O–H), 4.30 (s, 2H, SCH_2), 4.25–4.07(m, 2H, –OCH), 3.86 (s, 3H, OCH_3), 3.59–3.55(m, 5H, –OCH). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm): 162.3, 154.6, 153.9, 140.2, 129.5, 126.0, 114.6, 74.3, 74.0, 71.8, 67.4, 64.7, 63.4, 55.7, 22.8. MS-ESI (*m/z*): 427(M^+ + 1), 396, 296, 277, 247, 228, 150. Elemental anal. calcd. for $C_{17}H_{22}N_4O_7S$: C, 47.9; H, 5.2; N, 13.1. Found: C, 47.7; H, 5.3; N, 13.35%.

6-(4-Diphenyl)-3-(D-glucoheptonic-hexitol-1-yl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazine (**2j**): Pale yellow powder, yield% 89%. M.p. 205–207°C (from ethanol). $[\alpha]_D$ = –12.5°(*c* 0.096, CH_3OH). IR (cm^{-1}): 3270 (OH), 2915 (CH2), 1635 (C=N), 1472 (N=C–S), 698 (C–S–C). 1H NMR (DMSO-d6, 300 MHz, ppm): 8.13–8.11 (m, 2H, Ar–H), 7.91–7.89 (m, 2H, Ar–H), 7.80–7.78 (m, 2H, Ar–H), 7.55–7.52 (m, 2H, Ar–H), 7.50–7.41 (m, 1H, Ar–H), 5.06 (s, 1H, O–H), 4.52–4.28(m, 5H, O–H), 4.42 (s, 2H, SCH2), 4.25–3.42 (m, 7H, –OCH). 13C NMR (DMSO-d6, 75 MHz, ppm): 154.8, 153.9, 143.3, 140.2, 139.0, 132.7, 129.2, 128.4, 128.3, 127.3, 127.0, 74.3, 74.0, 71.8, 67.4, 64.8, 63.4, 23.0. MS-ESI (*m/z*): 473(M^+ + 1), 472(M^+), 455, 427, 397, 321, 293, 279. Elemental anal. calcd. for $C_{22}H_{24}N_4O_6S$: C, 55.9; H, 5.1; N, 11.9. Found: C, 55.8; H, 5.2; N, 11.65%.

Biological evaluation

Effect of compounds **2a–2j** *on the vegetative growth of wheat and radish plant*: Seeds were rinsed with sterilised 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 9 cm diameter containing two pieces of filter paper and 5 ml solution of the tested compounds **2a–2j** (10μg/ml and 100μg/ml, respectively), and were incubated in a growth chamber at 25°C, with a 16 h/8 h photoperiod. The set of controls with sterilised distilled water were prepared simultaneously. Plant length were recorded on the 5th day both 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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