

Full Paper

Syntheses and Biological Activities of 6-Aryl-3-(3-hydroxy-propyl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines

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Abstract: A series of 6-aryl-3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b][1,3,4]-thiadiazines were synthesized by the reaction of 4-amino-3-(3-hydroxypropyl)-5-mercapto-1,2,4-triazole (**1**) with substituted ω -haloacetophenones. Their structures were confirmed by elemental analysis, IR, ¹H-NMR, and ¹³C-NMR. Tests of plant growth regulating effects showed that the title compounds display remarkable inhibitory activities on the growth of radish and wheat.

Keywords: Hydroxyl groups, 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine, NMR, plant growth regulation.

Introduction

Triazoles and their heterocyclic derivatives represent an interesting class of compounds which possess a wide range of biological activities, such as analgesic, anthelmintic, antitubercular, plant

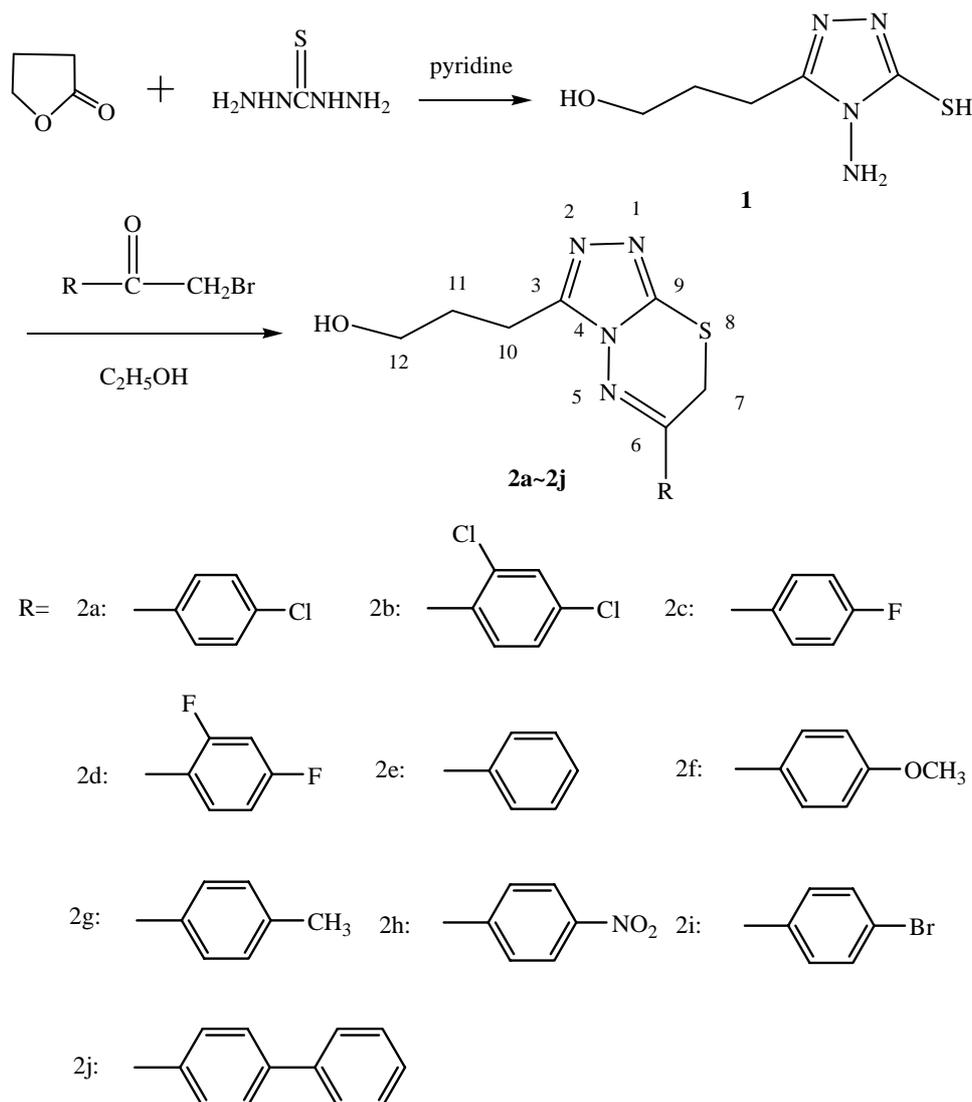
growth regulating, antiviral, antifungal and anticancer properties [1-6]. Attachment of an aliphatic side chain containing a hydroxyl group to the 3-position of the fused heterocycle could bring about changes in its solubility; moreover, the -OH group can be transformed into many other useful functional groups, allowing preparation of numerous novel compounds. With all these considerations in mind, we have synthesized a series of 6-aryl-3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines, then confirmed their structures by elemental analysis, IR, ^1H - and ^{13}C -NMR, and determined the plant growth regulating effects of all the title compounds and the key intermediate **1**.

Results and Discussion

Synthetic route and improvement of synthetic methods

The synthetic route used is shown in Scheme 1. After preparing 4-amino-3-(3-hydroxypropyl)-5-mercapto-1,2,4-triazole (**1**), the 3,6-disubstituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines **2a~2j** were obtained through the reaction with the appropriate substituted phenacyl bromide (or chloride).

Scheme 1. Synthesis of the title compounds.



The rates of ring-closure were closely related to the structures of the substituents R. When R contained electron-withdrawing groups, the rates of ring-closure were increased because the electron-cloud densities of the carbonyl carbons were lowered.

The syntheses of thiocarbohydrazide and 4-amino-3-(3-hydroxypropyl)-5-mercapto-1,2,4-triazole (**1**) were carried out according to the literature methods [7,8], with some improvements. First, if the reaction temperature of hydrazine hydrate and carbon disulphide was slowly elevated, formation of a yellow-green side product (m.p.:138~140°C) was increased, so we quickly raised the reaction temperature to 100~110°C after carrying out the reaction at room temperature for half an hour in order to maximize the yield of thiocarbohydrazide. Second, some water was added to the mixture when γ -butyrolactone was reacted with thiocarbohydrazide in pyridine. This water improved the solubility of the reactants and resulted in an improved yield of compound **1**. Finally, the solubility of the triazole intermediate in hot water differed obviously from that in cold water, so the crude product was recrystallized from water.

IR spectra of the title compounds

The absence of N-H, S-H and C=O absorptions in the IR spectra confirms that the cyclocondensation to form the title compounds has taken place. The characteristic stretching vibrations of the products are at 3405-3446 cm^{-1} (O-H, strong), 1635-1597 cm^{-1} (C=N), 1636, 1600, 1578, 1522, 1507, 1471 cm^{-1} (aromatic ring skeleton vibrations). The C-S-C bending vibrations are in the region around 670 cm^{-1} . The stretching vibration peaks of CH_2 are observed at about 2920 cm^{-1} .

$^1\text{H-NMR}$ spectra of the title compounds

In the $^1\text{H-NMR}$ spectra of triazolothiadiazines **2a~2j**, the characteristic downfield signal at δ 13.40 attributed to the -N=C-SH (-NH-C=S of the tautomer) in compound **1** is absent, as is a sharp signal at δ 5.49 attributable to the N-NH₂ group in the parent triazole. In the spectra of the title compounds, the signal at δ 1.80 is attributed to the CH_2 of the hydroxypropyl group, and that at δ 3.60 is attributed to the O-H group. Resonance peaks at δ 7.13~8.42 are attributed to the aromatic ring protons. As a result of the overall action of inductive effect and magnetic anisotropic effect of the aromatic rings, the chemical shift of the SCH_2 group in the thiadiazine moiety appears downfield at δ 4.26~4.57.

$^{13}\text{C-NMR}$ spectra of the title compounds

The $^{13}\text{C-NMR}$ spectra of compound **2h**, for instance, exhibits the expected 11 absorption peaks, *i.e.* δ 22.92, 123.95, 128.69, 139.47, 139.79, 148.99, 152.81, 153.73, 20.73 and 29.46 ppm, which are assigned to $\text{CH}_2\text{-CH}_2$ and one at δ 59.76, which is attributed to the O- CH_2 of the hydroxypropyl group. Because of the existence of carbon-nitrogen double bonds, the chemical shifts of the three carbon atoms in the triazole ring appear at δ 148.99, 152.81, 153.73, which conforms approximately with the values reported in the literature [9]. All the benzene carbons of the title compounds exhibit chemical shifts between 105.07~153.82, whereas the peaks with δ values near 23 ppm are attributed to the S- CH_2 in the thiadiazine rings.

Biological Activity

The effects of the title compounds **1**, **2a~2j** on sprouting of wheat and radish seeds have been investigated. After treating with solutions of 100 µg/mL and 10 µg/mL of the title compounds for 7 days, the germination percentages have been determined, and from the difference in length between stems and radicles of seedlings treated with the title compounds and those treated with distilled water, the plant growth regulating activities have also been calculated. A positive result represents a growth increase, whereas a negative result implies an inhibition:

$$\text{effect} = \frac{\text{the length of sample's stem/radicle} - \text{the reference's length}}{\text{the length of reference's stem/radicle}} \times 100\%$$

The results are shown in Table 1. From the data it is apparent that the newly synthesized compounds **2a~2j** show a more pronounced inhibiting effect than 4-amino-3-(3-hydroxypropyl)-5-mercapto-1,2,4-triazole (**1**) on the growth of wheat and radish.

Table 1. Plant growth regulating activities of compounds **1**, **2a~2j** (germination percentage % / effect (on stem) % / effect (on root) %).

Compd.	Radish		Wheat	
	10 ppm	100 ppm	10 ppm	100 ppm
1	80/25.6/22.8	85/9.7/-12.9	70/-20.6 /4.7	55/-23.8/-34.1
2a	20/-43.2/-50.9	-100%	81.3/-68/-68.9	-100%
2b	-100%	-100%	85/-94.7/-89.5	-100%
2c	20/-42.3/-73.8	-100%	94.7/-74.6/-71.9	-100%
2d	35/-51.4/-45.6	-100%	83.3/-65.9/-64.3	-100%
2e	5/-100/-91.3	-100%	83.3/-91.3/-84.7	-100%
2f	10/-52.7/-84.7	-100%	84.2/-87.9/-80.7	-100%
2g	10/-38.5/-66.2	-100%	100/-92/-87.7	-100%
2h	60/-36.3/-42.1	-100%	75/-35.4/-44.5	95/-96.6/-96.1
2i	20/-68.5/-72.7	-100%	94.7/-84.3/-77.8	-100%
2j	25/-57.7/-74.2	-100%	77.8/-87.9/-83.7	-100%
Reference	85/0/0	85/0/0	100/0/0	100/0/0

Experimental

General

All melting points were determined on an XT-4A apparatus. The IR spectra (KBr disks) were recorded on a Bruker Quinox 55 spectrophotometer. The ¹H-NMR and ¹³C-NMR spectra were measured at 25°C on a Bruker Advance 300 spectrometer operating at 300 and 75 MHz, respectively (DMSO-*d*₆ solutions using TMS as internal reference). Elemental analyses were carried out with a Flash-EA 1112 elemental analyzer. All the reagents used were AR grade.

Preparation of 4-amino-3-(3-hydroxypropyl)-3-(3-hydroxypropyl)-5-mercapto-1,2,4-triazole (1)

CS₂ (13 mL, 0.22 mol) was added dropwise under stirring to a mixture of 85% hydrazine hydrate (24 mL) and water (75 mL), then stirring was continued for 30 minutes at room temperature. The reaction temperature was then rapidly raised to 100~110 °. After refluxing for 2h, the reaction mixture was cooled in an ice-bath and filtered. The residue was washed with ethanol and recrystallized from water to afford pure thiocarbohydrazide crystals, mp: 171~172 °C. The thiocarbohydrazide thus prepared (1 mmol) was then added to a solution of γ -butyrolactone (1 mmol) in pyridine (20 mL) and water (2 mL), and the mixture was refluxed for 6 h. After removing the solvent, the white solid formed was recrystallized from water to give compound **1** in 76.6% yield; m.p.: 125~126 °C; Anal. Calc. (%) for C₅H₁₀N₄OS: C, 34.47; H, 5.79; N, 32.16. Found: C, 34.25; H, 5.70; N, 31.99; IR: 3390, 3309, 3189, 3096, 3044, 2925, 2775, 1615, 1570, 1508, 1326, 1056, 963 cm⁻¹; ¹H-NMR δ ppm: 1.73-3.45 (m, 6H, C-H), 4.53 (s, 1H, OH), 5.49 (s, 2H, NH₂), 13.40 (s, 1H, NH); ¹³C-NMR δ ppm: 21.28 (CH₂), 28.88 (Ar-CH₂), 59.94 (C-OH), 152.31 (C=N), 165.80 (C=S).

General method for the preparation of 6-aryl-3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines 2a~2j

The appropriate ω -haloacetophenone (1 mmol) was added to a solution of compound **1** (1 mmol) in 75% C₂H₅OH (20 mL). The mixture was refluxed for 4 h. The solid obtained on cooling was filtered, dried and recrystallized from 75% C₂H₅OH to give the title compounds. All the title compounds can be dissolved in ethanol. The physical and spectral data of the title compounds **2a~2j** are given in Tables 2~4.

Table 2. Physical data of compounds 2a~2j.

Compd.	Molecular formula	Colour	Yield/%	M.p./°C	Anal./% Found (calc.)		
					C	H	N
2a	C ₁₃ H ₁₃ ClN ₄ OS	white	76.5	134~136	50.28(50.57)	4.09(4.24)	18.43(18.14)
2b	C ₁₃ H ₁₂ Cl ₂ N ₄ OS	yellow	80.2	119~121	45.31(45.49)	3.61(3.52)	16.05(16.32)
2c	C ₁₃ H ₁₃ FN ₄ OS	white	59.6	163~165	53.67(53.41)	4.70(4.48)	19.44(19.17)
2d	C ₁₃ H ₁₂ F ₂ N ₄ OS	yellow	77.1	105~107	50.19(50.32)	3.66(3.90)	18.37(18.05)
2e	C ₁₃ H ₁₄ N ₄ OS	white	48.5	155~157	56.80(56.91)	5.36(5.14)	20.16(20.42)
2f	C ₁₄ H ₁₆ N ₄ O ₂ S	white	70.3	188~190	55.37(55.25)	5.18(5.30)	18.69(18.41)
2g	C ₁₄ H ₁₆ N ₄ OS	white	55.7	144~146	58.02(58.31)	5.72(5.59)	19.12(19.43)
2h	C ₁₃ H ₁₃ N ₅ O ₃ S	brown	74.1	163~165	48.64(48.89)	3.95(4.10)	22.25(21.93)
2i	C ₁₃ H ₁₃ BrN ₄ OS	white	66.9	156~158	43.97(44.20)	3.98(3.71)	15.68(15.86)
2j	C ₁₉ H ₁₈ N ₄ OS	yellow	72.3	175~177	65.42(65.12)	5.33(5.18)	15.84(15.99)

Table 3. IR data of compounds **2a~2j**.

Compd.	IR (ν/cm^{-1})
2a	3421 (OH), 2912 (CH_2), 1633 (C=N), 1462 (N=C-S), 626 (C-S-C).
2b	3436 (OH), 2921 (CH_2), 1635 (C=N), 1465 (N=C-S), 630 (C-S-C).
2c	3414 (OH), 2895 (CH_2), 1606 (C=N), 1497 (N=C-S), 629 (C-S-C)
2d	3417 (OH), 2975 (CH_2), 1620 (C=N), 1474 (N=C-S), 630 (C-S-C).
2e	3426 (OH), 2921 (CH_2), 1635 (C=N), 1447 (N=C-S), 688 (C-S-C).
2f	3446 (OH), 2979 (CH_2), 1597 (C=N), 1469 (N=C-S), 650 (C-S-C).
2g	3431 (OH), 2910 (CH_2), 1624 (C=N), 1462 (N=C-S), 635 (C-S-C).
2h	3405 (OH), 2929 (CH_2), 1620 (C=N), 1467 (N=C-S), 688 (C-S-C).
2i	3417 (OH), 2912 (CH_2), 1630 (C=N), 1460 (N=C-S), 624 (C-S-C).
2j	3417 (OH), 2902 (CH_2), 1632 (C=N), 1462 (N=C-S), 690 (C-S-C).

Table 4. NMR data of compounds **2a~2j**.

Compd.	$^1\text{H-NMR}$ (δ , ppm)	$^{13}\text{C-NMR}$ (δ , ppm)
2a	1.86~3.53 (m, 6H, C-H), 4.48 (s, 2H, S- CH_2), 7.66~8.14 (m, 4H, Ar-H)	20.77, 22.93, 29.17, 59.71, 129.19, 129.40, 132.00, 137.10, 141.28, 153.78, 155.26
2b	1.80~3.51 (m, 6H, C-H), 4.26 (s, 2H, S- CH_2), 4.53~4.56 (m, 1H, O-H), 7.61~7.86 (m, 3H, Ar-H)	20.82, 25.83, 29.52, 59.82, 127.99, 129.76, 132.25, 132.51, 133.45, 136.14, 140.00, 153.66, 154.56
2c	1.87~3.53 (m, 6H, C-H), 4.47 (s, 2H, S- CH_2), 7.41~8.14 (m, 4H, Ar-H)	20.75, 22.97, 29.27, 59.73, 116.38, 130.26, 141.20, 153.69, 155.28, 162.76, 166.09
2d	1.81~3.50 (m, 6H, C-H), 4.30 s, 2H, S- CH_2), 7.28~7.93 (m, 3H, Ar-H)	21.02, 25.25, 29.67, 60.03, 105.42, 112.63, 119.83, 132.15, 140.09, 151.84, 153.82, 162.70, 165.87
2e	1.86~3.53 (m, 6H, C-H), 4.49 (s, 2HS- CH_2), 7.56~8.06 (m, 5H, Ar-H)	20.98, 23.21, 29.35, 59.92, 127.79, 129.29, 132.46, 133.33, 141.68, 153.98, 156.61
2f	1.88~3.54 (m, 6H, C-H), 3.86 (s, 3H, CH_3), 4.49 (s, 2H, S- CH_2), 7.13~8.06 (m, 4H, Ar-H)	20.78, 22.69, 29.23, 55.57, 59.77, 114.52, 125.23, 129.48, 141.16, 153.50, 155.54, 162.49
2g	1.84~3.53 (m, 6H, C-H), 2.39 (s, 3H, CH_3), 4.36 (s, 2H, S- CH_2), 7.36~7.93 (m, 4H, Ar-H)	20.86, 21.00, 22.79, 29.58, 59.91, 127.32, 129.56, 130.76, 140.01, 142.00, 153.51, 154.45
2h	1.85~3.54 (m, 6H, C-H), 4.46 (s, 2H, S- CH_2), 8.24~8.42 (m, 4H, Ar-H)	20.73, 22.92, 29.46, 59.76, 123.95, 128.69, 139.47, 139.79, 148.99, 152.81, 153.73
2i	1.84~3.54 (m, 6H, C-H), 4.38 (s, 2H, S- CH_2), 4.54~4.57 (m, 1H, O-H), 7.77~7.96 (m, 4H, Ar-H)	20.75, 22.67, 29.48, 59.79, 125.51, 129.24, 131.95, 132.73, 139.83, 153.53, 153.58
2j	1.88~3.53 (m, 6H, C-H), 4.43 (s, 2H, S- CH_2), 4.59 (s, 1H, O-H), 7.43~8.12 (m, 9H, Ar-H)	20.89, 22.83, 29.62, 59.92, 126.84, 127.12, 128.01, 128.28, 129.06, 132.46, 138.80, 139.97, 143.21, 153.59, 154.14

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Sample Availability: Not available.