Facile synthesis of some novel 6-alkyl or aryl-7H-tetrazolo[5,1-b][1,3,4] thiadiazine

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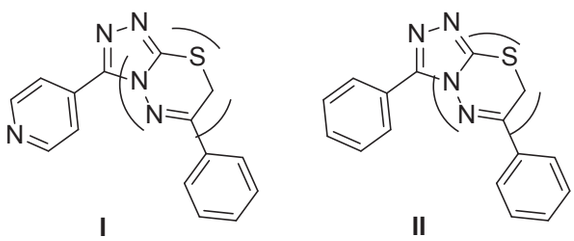
New *bis*-heterocyclic thiadiazine derivatives containing a tetrazole nucleus have been synthesised by simple, high yielding routes and characterised by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. The key step in the construction of the six-substituted 7H-tetrazolo[5,1-b][1,3,4]thiadiazine derivatives involved the cyclocondensation of sodium 1-amino-1H-tetrazole-5-thiolate with α-haloketones. The radical scavenging activity of the new tetrazolothiadiazines was also investigated.

Keywords: tetrazolo nucleus, thiadiazine, α-haloketones, heterocyclisation, radical scavenging activity

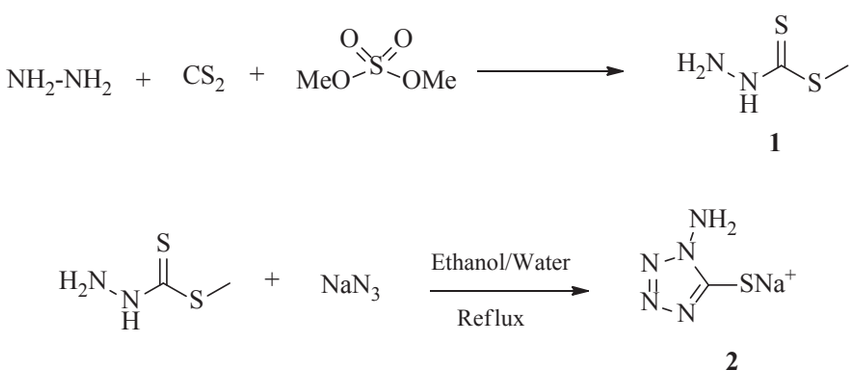
Thiadiazine derivatives are an important class of pharmacologically active compounds.[1–4] Syntheses of 1,3,4-thiadiazines have attracted widespread attention due to their diverse applications as antibacterial,[5,6] antimycobacterial,[7,8] antimycotic,[9,10] antifungal[11,12] and antidepressant agents.[13] For example, the fused heterocyclic compounds I and II (triazolothiadiazines) have been reported as novel analgesic and antibacterial compounds (Scheme 1).[14,15] In addition, compounds incorporating tetrazole rings have attracted attention due to their diverse pharmacological properties. For example angiotensin II receptor blockers, such as losartan[16] and candesartan, contain tetrazoles.[17] In view of the biological potential of the above pharmacophores and in continuation of our efforts to develop novel routes to heterocyclic derivatives of thiadiazine with potential biological activity, we considered the synthesis of novel 6-alkyl or aryl-7H-tetrazolo[*5,1*b][*1,3,4*] thiadiazines. In view of the biological importance of these heterocycles, we decided to synthesise the tetrazole thiadiazine compounds wherein the biologically active thiadiazine moiety is fused to potent tetrazole ring at the 4,5 positions. We now report the details of the synthesis of novel 6-alkyl or aryl-7H-tetrazolo[*5,1*-b][*1,3,4*]thiadiazines. Their ability at scavenging free radicals were measured by DPPH (2,2′-diphenyl-1-picrylhydrazyl) reduction spectrophotometric assay.



Scheme 1 Compounds reported as anti-inflammatory/analgesic agents.

Result and discussion

The amino and mercapto groups are nucleophilic centres for the synthesis of condensed heterocyclic rings. The required sodium 1-amino-*1*H-tetrazole-5-thiolate was prepared as previously reported in 80% yield (Scheme 2). We found that the one-pot cyclocondensation of the tetrazole with *α*-haloketones can be carried out in high yields by the reaction of sodium 1-amino-*1*H-tetrazole-5-thiolate with the α-halocarbonyl compounds (Scheme 3). The structures of all the products were confirmed by 1H NMR, ^{13}C NMR, FTIR and mass spectral data and elemental analysis. In their ^{1}H NMR spectra, the absence of the peaks corresponding to the NH_2 protons of compound **2** clearly confirmed the formation of tetrazolothiadiazine ring. In the ^{1}H NMR spectra of compounds **(4a–g)**, the signal due to the –S–CH_2– protons of tetrazolothiadiazine ring gave rise to a singlet peak at 3.7–4.7 ppm. The FT-IR spectra of these compounds lacked the absorption corresponding to the NH_2 of compound **2** and the carbonyl group of the α-halocarbonyl compounds confirmed the formation of the tetrazolothiadiazine ring. The mechanism must involve two straightforward steps, namely the nucleophilic substitution by the sulfur of the chlorine and cyclisation through direct attack of the amino group on the carbonyl group. First we report the details of the synthesis of novel 6-alkyl or aryl-7H-tetrazolo[*5,1*-b][*1,3,4*]thiadiazine.



Scheme 2 Synthesis of sodium 1-amino-1H-tetrazole-5-thiolate.

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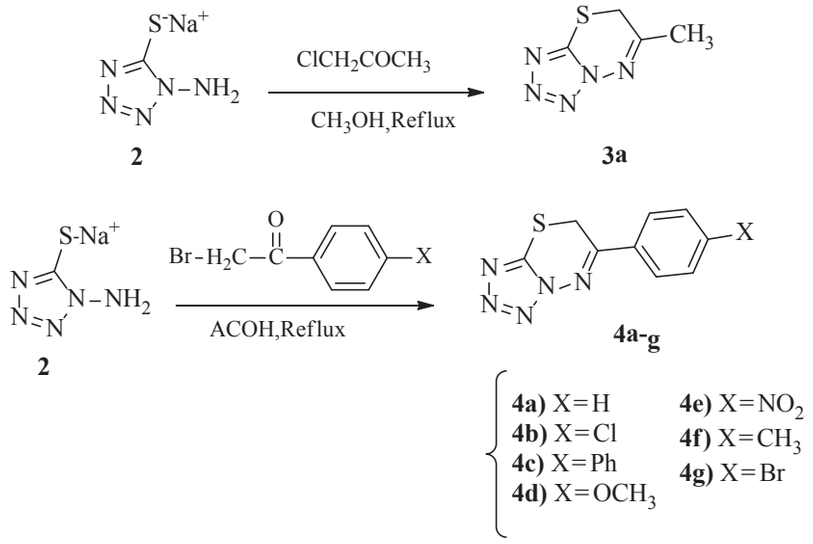

Scheme 3 Synthesis of 6-alkyl or aryl-*7*H-tetrazolo[*5,1*-b][*1,3,4*]thiadiazine.

Interest in the fused tetraazolo[*3,4-b*][*1,3,4*]thiadiazine systems is stimulated by their broad biological activity. In a simple and fast evaluation of their biological activity, we determined the radical scavenging activity of these compounds. Since free radicals are believed to play an important role in many pathological states, radical scavenging compounds have received increased attention in medicinal research. Recently, interest in the application of antioxidants to medical treatment has been stimulated by links between the development of human diseases and oxidative stress.[18] Free radicals play a role in the pathogenesis of chronic degenerative diseases including cancer, autoimmune, inflammatory, cardiovascular and neurodegenerative diseases and ageing. It is also known that oxidative stress can be induced by a wide range of environmental factors including UV stress, pathogen invasion, pesticide action and oxygen shortage. Consequently, synthetic and natural compounds with potential antioxidant activity have received increased attention in biological research, medicine and pharmacy. There are many ways to evaluate the free radical scavenging activity of tested compounds. One of the most widely used detection procedures, which facilitates analysis of various antioxidants, is based on 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) bleaching.[19] The tetrazolo compounds **4b** and **4e** showed good antioxidant activity.

Experimental

Melting points were recorded on an Electrothermal type 9100 melting point apparatus and are uncorrected. The IR spectra were obtained on an Avatar 370 FT-IR Thermo-Nicolet spectrometer. The ^{1}H NMR (100 MHz) spectra were recorded on a Bruker AC 400 spectrometer. The mass spectra were scanned on a Varian Mat CH-7 at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyser.

Synthesis of sodium 1-amino-1H-tetrazole-5-thiolate **(2)**

Methyl dithiocarbazate **1** (27.5 g, 225 mmol)[20] and sodium azide 97% (15.1 g, 225 mmol) were added to a mixture of ethanol (300 mL) and water (80 mL), then the mixture was refluxed for 17 h. After the reaction, the mixture was evaporated at 45 °C under reduced pressure to remove solvent. Then, ethanol (150 mL) was added to the residue, and the resultant precipitate was collected by filtration. The precipitate was washed with ethanol and then dried to give sodium 1-amino-1H-tetrazole-5-thiolate **2** (26.6 g, 85%) as a white powder; m.p. 88–90 °C; ^{1}H NMR (100 MHz, DMSO-d_6-*d*6): δ 10.1 (br. s, 2H, NH_2); IR (KBr disc): ν_{max} 3424, 3272, 1657, 1607 cm^{-1}. The product was also characterised by conversion to the free thiol by careful acidification with dilute H_2SO_4 to give 1-amino-1*H*-tetrazole-5-thiol, m.p. 160–161 °C (dec.).[21]

Synthesis of 6-methyl-7H-tetrazolo[5,1-b][1,3,4]thiadiazine **(3a)**

A mixture of **2** (1 mmol) and chloroacetone (1 mmol) in methanol (5 mL) was refluxed for about 4 h. The progress of the reaction was monitored by TLC using chloroform: methanol (20 : 1). After the completion of the reaction, the mixture was concentrated and the resultant precipitates were collected by filtration. The precipitate was recrystallised from ethanol to give crystals; m.p. 92–94 °C, yield 90%; IR (KBr) ν_{max} (cm^{-1}): 1624 (C=N), 1266 (C–S). ^{1}H NMR (100 MHz, $CDCl_3$, 25 °C, ppm) δ 3.72 (s, 2H, CH_2), 2.48 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 158.01, 142.49, 26.46, 24.00. EI-MS *m/z*: 156. Anal. calcd for $C_4H_5N_5S$: C, 30.96; H, 3.25; N, 45.13; S, 20.66; found: C, 31.23; H, 3.37; N, 46.45; S, 20.34%.

Synthesis of substituted-6-phenyl-[1,2,4]tetrazolo-[5,1-b][1,3,4] thiadiazines **(4a–g);** *general procedure*

A mixture of **2** (1 mmol) and the substituted phenacyl bromide (1 mmol) in acetic acid (5 mL) was refluxed for about 4 h. The progress of the reaction was monitored by TLC using chloroform: methanol (20 : 1). After the completion of the reaction, the mixture was concentrated and the resultant precipitates were collected by filtration. The precipitate was recrystallised from ethanol to give the crystalline products.

6-Phenyl-7H-tetrazolo[5,1-b][1,3,4]thiadiazine **(4a)**: M.p. 165–167 °C; yield 90%; IR (KBr) ν_{max} (cm^{-1}): 1589 (C=N), 1269 (C–S). ^{1}H NMR (DMSO-d_6): δ (ppm), 8.02 (dd, 2H, J_1 = 8 Hz, J_2 = 1.2 Hz), 7.67 (tt, 1H, J_1 = 7.2 Hz, J_2 = 1.6 Hz), 7.59 (dt, 2H, J_1 = 8 Hz, J_2 = 1.6 Hz), 4.17 (s, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ154.31, 142.96, 133.13, 132.30, 129.38, 127.83, 23.93. EI-MS *m/z*: 217. Anal. calcd for $C_9H_7N_5S$: C, 49.76; H, 3.25; N, 32.24; S, 14.76; found: C, 49.95; H, 3.33; N, 32.83; S, 14.64%.

6-(4-Chlorophenyl)-7H-tetrazolo[5,1-b][1,3,4]thiadiazine **(4b):** M.p. 176–178 °C; yield 85%; IR (KBr) ν_{max} (cm^{-1}): 1655 (C=N), 1270 (C–S). ^{1}H NMR (DMSO-d_6): δ (ppm), 7.80 (d, 2H, *J* = 8.8 Hz), 7.99 (d, 2H, *J* = 8.8 Hz), 4.60 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ156.44, 144.53, 132.72, 132.09, 130.037, 127.26, 24.08. EI-MS *m/z*: 251. Anal. calcd for $C_9H_6ClN_5S$: C, 42.95; H, 2.40; N, 27.82; S, 12.74; found: C, 43.26; H, 2.51; N, 28.14; S, 12.49%.

6-([1,1′-Biphenyl]-4-yl)-7H-tetrazolo[5,1-b][1,3,4]thiadiazine **(4c)**: M.p. 201–203 °C; yield 78%; IR (KBr ν_{max} (cm^{-1}): 1650 (C=N), 1265 (C–S). ^{1}H NMR (DMSO-d_6): δ (ppm) 8.10 (d, 2H, *J* = 8.2 Hz), 7.8

Table 1 DPPH bleaching IC_{50} of the synthetic compounds

Compound name	IC_{50} (μM) ± SD
3a	283.8 ± 13.9
4a	87.2 ± 4.3
4b	32.2 ± 2.4
4c	> 1000
4d	114.5 ± 4.8
4e	31.9 ± 11.1
4f	98.7 ± 4.1
4g	528.2 ± 12
4-MNPB	96.3 ± 3.9
Ascorbic acid	22.5 ± 1.81

(d, 2H, J = 8.2 Hz), 7.68 (dd, 2H, J_1 = 7.2 Hz, J_2 = 1.2 Hz), 7.52 (dt, 2H, J_1 = 7.2 Hz, J_2 = 1.2 Hz), 7.45 (tt, H, J_1 = 7.2 Hz, J_2 = 1.2 Hz), 4.18 (s, 2H, CH_2). EI-MS *m/z*: 293. Anal. calcd for $C_{15}H_{11}N_5S$: C, 61.42; H, 3.78, N, 23.87; S, 10.93; found: C, 61.63; H, 3.87; N, 24.26; S, 10.81%.

6-(4-Methoxyphenyl)-7H-tetrazolo[5,1-b][1,3,4]thiadiazine **(4d):** M.p. 150–152 °C; yield 85%; IR (KBr) ν_{max} (cm^{-1}): 1687 (C=N), 1264 (C–S). ^{1}H NMR (DMSO-d_6/$CDCl_3$): δ (ppm) 3.93 (s, 3H, OCH_3), 7.03 (d, 2H, J = 8.8 Hz), 7.97 (d, 2H, J = 8.8 Hz), 4.09 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ163.41, 155.44, 143.68, 130.20, 124.59, 114.67, 55.73, 23.91. EI-MS *m/z*: 247. Anal. calcd for $C_{10}H_9N_5OS$: C, 48.57; H, 3.67; N, 28.32; S, 12.97; found: C, 48.95; H, 3.77; N, 28.66; S, 12.61%.

6-(4-Nitrophenyl)-7H-tetrazolo[5,1-b][1,3,4]thiadiazine **(4e):** M.p. 200–202 °C; yield 90%; IR (KBr) ν_{max} (cm^{-1}): 1645 (C=N), 1270 (C–S). ^{1}H NMR (DMSO-d_6): δ (ppm) 7.98 (d, 2H, J = 9.2 Hz), 8.10 (d, 2H, J = 9.2 Hz), 4.702 (s, 2H, CH_2). EI-MS *m/z*: 262. Anal. calcd for $C_9H_6N_6O_2S$: C, 41.22; H, 2.31; N, 32.05; S, 12.23; found: C, 41.40; H, 2.41; N, 32.53; S, 12.10%.

6-(p-Tolyl)-7H-tetrazolo[5,1-b][1,3,4]thiadiazine **(4f):** M.p. 146–148 °C; yield 70%; IR (KBr) ν_{max} (cm^{-1}): 1630 (C=N), 1260 (C–S). ^{1}H NMR (DMSO-d_6): δ (ppm) 2.09 ($3H_s$,CH_3), 6.95 (d, 2H, J = 7.6 Hz), 7.13 (d, 2H, J = 7.6 Hz), 4.41 (s, 2H, CH_2). EI-MS *m/z*: 231. Anal. calcd for $C_{10}H_9N_5S$: C, 51.93; H, 3.92; N, 30.28; S, 13.86; found: C, 52.12; H, 4.08; N, 30.69; S, 13.71%.

6-(4-Bromophenyl)-7H-tetrazolo[5,1-b][1,3,4]thiadiazine **(4g)**: M.p. 183–185 °C; yield 80%; IR (KBr) ν_{max} (cm^{-1}): 1640 (C=N), 1272 (C–S). ^{1}H NMR (DMSO-d_6-d6): δ (ppm) 7.50–8.00 (m, 4H), 4.45 (s, 2H, CH_2). EI-MS *m/z*: 296. Anal. calcd for $C_9H_6BrN_5S$: C, 36.50; H, 2.04; N, 23.65; S, 10.83; found: C, 36.69; H, 2.12; N, 24.16; S, 10.70%.

Determination of DPPH bleaching

The radical scavenging activity of the new series of tetrazolo thiadiazine was also determined. The ability of scavenging free radicals was measured by DPPH reduction spectrophotometric assay. 25 μM solution of DPPH in absolute ethanol was prepared. This solution was added to an equal volume of the solution of the test compounds (dissolved in ethanol) to obtain the desired concentration. Ethanol was used as control solution. After 30 min at room temperature, the absorbance was recorded at 517 nm and compared to NDGA (nordihydroguaiaretic acid). DPPH bleaching IC_{50} of the synthetic compounds is shown in Table 1. Our results were compared with ascorbic acid and 4-MNPB (pyrimido[4,5-b][1,4] benzothiazine)[22]. Tetrazolothiadiazines **4b** and **4e** showed good antioxidant activity.

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