SYNTHESIS OF NOVEL PYRIMIDO[5,4-*f*][1,2,4]TRIAZOLO[3,4-*b*][1,3,4]THIADIAZEPINES

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Abstract: Reaction of 4,6-dichloro-2-methylthiopyrimidine-5-carbaldehyde (**1**) with 3-substituted 4-amino-1,2,4-triazole-5-thiones (**2a-g**) at 50-60°C led to 7-chloro-9-methylthiopyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepines (**3a-g**) representing a new heterocyclic system. Performing of the reaction of **1** with 4-amino-1,2,4-triazole-5-thione (**2a**) at room temperature gave 7-chloro-9-methylthio-5,6-dihydropyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepin-6-ol (**5**), which with equimolar amount of sodium methoxide afforded 7-methoxy-9-methylthiopyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepine (**6**). Reaction of **5** with iodomethane in the presence of an excess of sodium methoxide led to 6,7-dimethoxy-5-methyl-9-methylthio-5,6-dihydropyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepine (**7**).

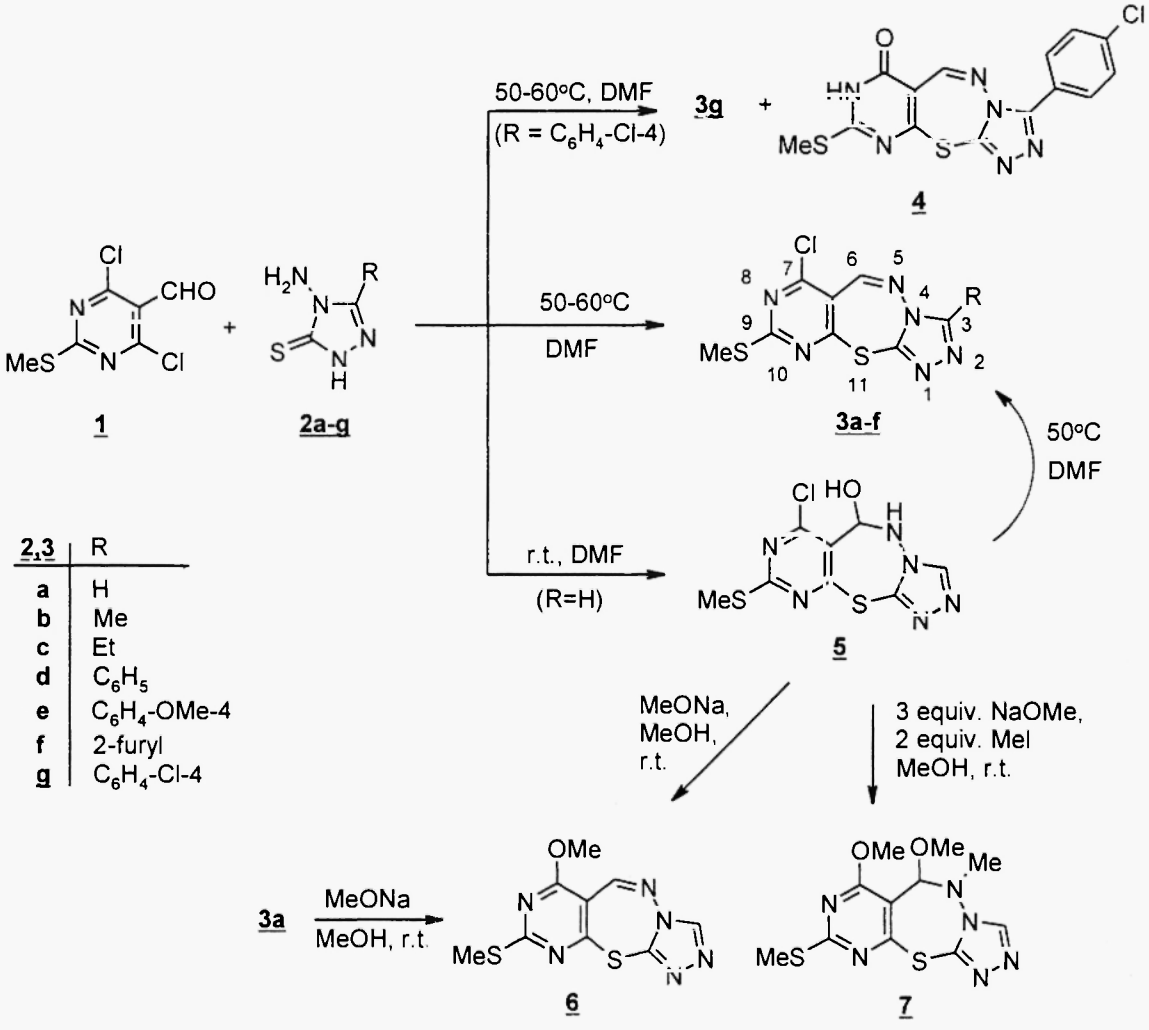
Introduction

Work in our laboratories has been recently concerned with the development of synthesis of new fused heterocyclic systems in order to search for new pharmocological or biologically active compounds. We have previously reported on the synthesis of linear annulated heterocycles containing the pyrimido[5,4-*e*][1,3]thiazine (1) and pyrimido[5,4-*f*][1,3,4]thiadiazepine (2,3) skeletons with potential pharmaceutical activity. The present work was undertaken to extend these investigations in order to prepare pyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepines for which alternate synthetic annulation methods are not readily available. The title heterosystem can be considered to some extent as analog of natural pyrrolo[2,1-*c*][1,4]benzodiazepine antitumor antibiotics which are well-known class of sequence-selective DNA binding agents (4,5). The cytotoxic and antitumor effects of these antibiotics are believed to arise from modification of DNA, which is caused by covalent binding of these compounds through azomethine bond of diazepine ring to the exocyclic N2 of guanine in the minor groove of duplex DNA (6-9). Recently some heterocyclic analogues of the natural pyrrolobenzodiazepines have been also synthesised (10). The designed by us pyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepines have similar structural features and therefore some DNA binding ability as well as pharmaceutical activity may be expected for them.

Results and Discussion

Compounds **3a-f** were synthesised by the cyclocondensation reaction between 4,6-dichloro-2-methylthiopyrimidine-5-carbaldehyde (**1**) and 3-substituted 4-amino-1,2,4-triazole-5-thiones (**2a-f**). The best yields were obtained by heating equimolar amounts of carbaldehyde **1** and triazoles **2a-f** in freshly distilled dimethylformamide at 50-60°C for 4-8 h (Scheme). Nevertheless, in the reaction of **1** with 4-amino-3-(4-chlorophenyl)-1,2,4-triazole-5-thione (**2g**) along with the expected **3g** a product of hydrolysis - 3-(4-chlorophenyl)-9-methylthio-7,8-dihydropyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepin-7-one (**4**) was formed.

Scheme



Performing the reaction of **1** with **2a** at room temperature allowed to isolate thiadiazepinol **5**, which appeared to be rather unstable and already during its crystallization partial elimination of water took place to give **3a**. Full dehydration was achieved by heating **5** at 50° in dimethylformamide for 3 h (Method B). A facile dehydration of compound **5** along with a substitution of the chlorine atom was also observed in its reaction

with an equimolar amount of sodium methoxide to give 7-methoxy derivative **6**. Compound **6** was alternatively synthesised by the reaction of **3a** with sodium methoxide. Treatment of **5** with two equivalents of iodomethane in the presence of an excess of sodium methoxide in methanol at room temperature afforded 6,7-dimethoxy-5-methyl-9-methylthio-5,6-dihydropyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepine (**7**). It should be noted, that the intermediate **5** was obtained only in the case of the unsubstituted system (R=H). Due to their lower stability the other 1,3,4-thiadiazepin-6-ols were not isolated.

The structures of all compounds **3-7** were substantiated by their elemental and spectral data. In the ^{1}H NMR spectra of **3a-q** a singlet at 8.30-8.88 ppm due to the resonance of C(6)-H along with a singlet at 2.58 – 2.77 ppm for the methylthio group and signals of substituents in position 3 of the heterosystem are observed. In the ^{13}C NMR spectra of the most soluble samples **3b** (solution in a mixture $CDCl_3$:CF_3COOH = 3:2) and **3c** (solution in $CDCl_3$) a signal of C(6) is observed at 144.4 and 150.8 ppm, respectively. The IR spectrum of **4** exhibited absorption at 3065 cm^{-1} and 1703 cm^{-1} which can be attributed to the absorption of the NH and CO groups of the pyrimidine ring. The mass spectra of **3q** and **4** showed the molecular ion peaks M^+ with m/z = 394 and 376, respectively. IR spectrum of **5** is characterised by the absorption of the amino and hydroxy groups at 3300 cm^{-1} and 3123 cm^{-1}, respectively. In the ^{1}H NMR spectrum of **5** there are three singlets at 2.76, 8.91 and 9.88 ppm corresponding to the methylthio group and to the protons at C(6) and C(3) of the heterosystem. It should be noted that the C(6)-H signal is observed in the unexpected region at 8.91 ppm. Nevertheless, the structure of thiadiazepinol **5** is unambiguously confirmed by its ^{13}C NMR spectrum, in which a signal of C(6) was found at 69.9 ppm. This is in agreement with the corresponding carbon chemical shift for compounds of the pyrrolo[2,1-*c*][1,4]benzdiazepine series (10,11). Moreover, in the same region at 71.3 ppm a signal of C(6) was also observed for compound **7**. Surprisingly, in the ^{1}H NMR spectrum of compound **7** the two methoxy groups showed a hide singlet at 4.11 ppm corresponding to six protons. In the ^{13}C NMR spectra of **7** the carbon atoms of the methoxy groups also resonated in close range (54.2 ppm and 55.6 ppm, respectively), indicating the similar shielding of substitutents in the positions 6 and 7 of the heterosystem. Both the IR and ^{1}H NMR spectra of **6** and **7** disclosed the absence of signals for the NH and HO groups.

Conclusion

In summary, cyclocondensation reaction between 4,6-dichloro-2-methylthiopyrimidine-5-carbaldehyde and 4-amino-1,2,4-triazole-5-thiones, the reported here, provides an easy access to the biologically important novel pyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepines.

Experimental

Melting points were determined in open capillaries and are uncorrected. IR spectra were run in Nujol mulls or in KBr discs on a Perkin-Elmer FT spectrophotometer Spectrum BX II. ^{1}H and ^{13}C NMR spectra were recorded with a Tesla BS 587A spectrometer (80 MHz) using tetramethylsilane as internal standard. Mass spectra were performed using direct insertion probe on a Kratos MS-30 spectrometer (70 eV). All

reactions and purity of the synthesised compounds were monitored by TLC using Silica gel 60 F_{254} aluminium plates (Merck). Visualization was accomplished by UV light.

4,6-Dichloro-2-methylthiopyrimidine-5-carbaldehyde (**1**) (12), 3-alkyl-4-amino-1,2,4-triazole-5-thiones (**2a-c**) (13) and 4-amino-3-aryl-1,2,4-triazole-5-thiones (**2d-g**) (14) were synthesised according to the described procedures.

3-Substituted 7-chloro-9-methylthiopyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepines (3a-f). *Typical procedure*. A mixture of **1** (1.11 g, 5 mmol), **2a-f** (5 mmol) and freshly distilled DMF (15 ml) was heated under stirring at 50-60°C for 4 h (in case R = aryl) or for 8 h (in case R = alkyl). After cooling to room temperature the precipitate was filtered off and recrystallised.

3a: Yield 41%, mp 169-170°C (from $CHCl_3$-octane). ^{1}H NMR (DMSO-d_6): 2.60 (3H, s, SCH_3), 8.59 (1H, s, C_6-H), 9.03 (1H, s, C_3-H). MS, m/z (%): 284 (100, M^+). Anal. Calcd. for $C_8H_5ClN_6S_2$: C 33.75; H 1.77; N 29.51. Found: C 33.72; H 1.79; N 29.45.

3b: Yield 49%, mp 180-182°C (from n-butanol). ^{1}H NMR ($CDCl_3$): 2.54 (3H, s, CH_3), 2.61 (3H, s, SCH_3), 8.32 (1H, s, C_6-H). ^{13}C NMR ($CDCl_3$:CF_3COOH=3:2): 11.6 (CH_3), 15.4 (SCH_3), 122.4 (C_{6a}), 144.4 (C_6), 155.8 (C_{11a}), 158.8 (C_7), 161.0 (C_{10a}), 168.9 (C_9), 178.8 (C_3). Anal. Calcd. for $C_9H_7ClN_6S_2$: C 36.18; H 2.36; N 28.13. Found: C 36.01; H 2.51; N 27.81.

3c: Yield 55%, mp 152-154°C (from heptane). ^{1}H NMR ($CDCl_3$): 1.38 (3H, t, J = 8 Hz, CH_3) 2.62 (3H, s, SCH_3), 2,91 (2H, q, J = 8 Hz, CH_2), 8.32 (1H, s, C_6-H). ^{13}C NMR ($CDCl_3$): 11.4 (CH_3), 15.2 (SCH_3), 19.1 (CH_2), 119.6 (C_{6a}), 141.9 (C_7 or C_{11a}), 150.8 (C_6), 158.2 (C_{11a} or C_7), 161.4 (C_{10a}), 166.7 (C_9), 177.4 (C_3). Anal. Calcd. for $C_{10}H_9ClN_6S_2$: C, 38.40; H, 2.90; N, 26.87. Found: C, 38.78; H, 2.84; N, 27.00.

3d: Yield 75%, mp 190-191°C (from ethanol). ^{1}H NMR ($CDCl_3$): 2.62 (3H, s, SCH_3), 7.51-7.99 (5H, m, Ar-H), 8.45 (1H, s, C_6-H). Anal. Calcd. for $C_{14}H_9ClN_6S_2$: C 46.60; H 2.51; N 23.29. Found: C 46.68; H 2.33; N 23.37.

3e: Yield 68%, mp 187-189°C (from ethyl acetate). ^{1}H NMR (DMSO-d_6): 2.63 (3H, s, SCH_3), 3.85 (3H, s, OCH_3), 7.13 (2H, d, J= 8 Hz, Ar-H), 7.98 (2H, d, J= 8 Hz, Ar-H), 8.78 (1H, s, C_6-H). Anal. Calcd. for $C_{15}H_{11}ClN_6OS_2$: C 46.09; H 2.84; N 21.50. Found: C 46.11; H 2.65; N 21.36.

3f: Yield 70%, mp 197-198°C (from chloroform). ^{1}H NMR (DMSO-d_6): 2.58 (3H, s, SCH_3), 6.61-7.12 (3H, m, furane-H), 8.65 (1H, s, C_6-H). Anal. Calcd. for $C_{12}H_7ClN_6OS_2$: C 41.09; H 2.01; N 23.96. Found: C 41.29; H 2.07; N 24.16.

7-Chloro-9-methylthiopyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepine (3a). *Method B.* A solution of **5** (0.5 g, 1.65 mmol) in freshly distilled DMF (10 ml) was heated at 50°C for 3 h and cooled to room temperature. The precipitate was filtered off and recrystallised to give 0.42 g (89%) of compound **3a**, mp 169-170 °C (from $CHCl_3$-octane).

7-Chloro-3-(4-chlorophenyl)-9-methylthiopyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*]-[1,3,4]thiadiazepine (3g) and 3-(4-Chlorophenyl)-9-methylthio-7,8-dihydropyrimido-[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazepin-7-one (4). A mixture of **1** (0.98 g, 4.4 mmol), **2g** (1.0 g, 4.4 mmol) and freshly distilled DMF (15 ml) was heated under stirring at 50-60°C for 5 h. After cooling to room temperature the precipitate was filtered off and recrystallised to give 0.71 g (41%) of compound **4**. Water (20 ml) was added to the filtrate, the precipitate was filtered off and recrystallised to give 0.68 g (39%) of compound **3g**.

3g: mp 186-187°C (from n-butanol). ^{1}H NMR (acetone-d_6): 2.77 (3H, s, SCH_3), 7.7 (2H, d, J = 8 Hz, Ar-H), 8.18 (d, 2H, J = 8,8 Hz, Ar-H), 8.88 (1H, s, C_6-H). MS, m/z (%): 394 (100, M^+), 361 (13), 313 (29). Anal. Calcd. for $C_{14}H_8Cl_2N_6S_2$: C 42.54; H 2.04; N 21.26. Found: C 42.36; H 2.12; N 20.91.

4: mp 254-256°C (from DMSO). IR: 3065 (NH), 1703 cm^{-1} (CO). ^{1}H NMR (DMSO-d_6): 2.66 (3H, s, SCH_3), 7.73 (2H, d, J = 8 Hz, Ar-H), 7.95 (2H, d, J = 9,6 Hz, Ar-H), 8.38 (1H, s, C_6-H). MS, m/z (%): 376 (100, M^+), 330 (12), 275 (28), 239 (46). Anal. Calcd. for $C_{14}H_9ClN_6OS_2$: C 44.62; H 2.41; N 22.30. Found: C 44.69; H 2.76; N 22.07.

7-Chloro-9-methylthio-5,6-dihydropyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepin-6-ol (5). A mixture of **1** (1.0 g, 4.5 mmol), **2a** (0.52 g, 4.5 mmol) and freshly distilled DMF (5 ml) was stirred at room temperature for 30 min. The precipitate was filtered off, washed with water and dried to give 0.56 g (41%) of the analytically pure compound **5**, which without further purification was used in the next steps. Mp 132-134°C. IR: 3300 (NH), 3123 cm^{-1} (OH). ^{1}H NMR (CF_3COOD): 2.76 (3H, s, SCH_3), 8.91 (1H, s, C_6-H), 9.88 (1H, s, C_3-H). ^{13}C NMR (DMSO-d_6:CF_3COOH=3:2): 14.3 (SCH_3), 69.9 (C_6), 119.2 (C_{6a}), 143.6 (C_7 or C_{11a}), 159.4 (C_{11a} or C_7), 162.3 (C_{10a}), 163.9 (C_3), 168.7 (C_9). Anal. Calcd. for $C_8H_7ClN_6OS_2$: C 31.74; H 2.33; N 27.76. Found: C 31.85; H 2.13; N 27.44.

7-Methoxy-9-methylthiopyrimido[5,4-*f*][1,2,4]triazolo[3,4-*d*][1,3,4]thiadiazepine (6). To a solution of sodium methoxide in methanol, prepared from sodium (0.013 g, 0.56 mmol) and methanol (5 ml), compound **5** (0.17 g, 0.56 mmol) was added portionwise. The suspension was stirred at room temperature for 4 h. Then the precipitate was filtered off, washed with water and recrystallised to give 0.06g (40%) of compound **6**, mp 170-172°C (from ethanol). ^{1}H NMR (DMSO-d_6): 2.6 (3H, s, SCH_3), 4.06 (3H, s, OCH_3), 8.38 (1H, s, C_6-H), 8.9 (1H, s, C_3-H). Anal. Calcd. for $C_9H_8N_6OS_2$: C 38.56; H 2.88; N 29.98. Found: C 38.66; H 2.89; N 29.85.

Compound **6** was also synthesised from **3a** using the above procedure. Yield 45%, mp 170-172°C (from ethanol).

6,7-Dimethoxy-5-methyl-9-methylthio-5,6-dihydropyrimido[5,4-*f*][1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazepine (7). A solution of sodium methoxide in methanol, prepared from sodium (0.046 g, 2 mmol) and anhydrous methanol (5 ml), was added dropwise to a solution of **5** (0.2 g, 0.66 mmol) in anhydrous methanol (10 ml). To a vigorously stirred solution iodomethane (0.182 g, 0.08 ml, 1.28 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. The precipitate was filtered off and recrystallized to give 0.1g (47%) of compound **7**, mp 174-175°C (from methanol). ^{1}H NMR ($CDCl_3$): 2.6 (3H, s, SCH_3), 2.76 (3H, s, NCH_3), 4.11 (6H, s, $2OCH_3$), 8.55 (1H, s, C_6-H), 8.68 (1H, s, C_3-H). ^{13}C NMR (DMSO-d_6:CF_3COOH=3:2): 14.2 (SCH_3), 40.1 (NCH_3), 54.2 (OCH_3), 55.6 (OCH_3), 71.3 (C_6), 109.2 (C_{6a}), 156.6 (C_7 or C_{11a}), 160.4 (C_{11a} or C_7), 163.9 (C_3). 168.3 (C_{10a}), 169.7 (C_9). Anal. Calcd. for $C_{11}H_{14}N_6O_2S_2$: C 40.48; H 4.32; N 25.75. Found: C 40.39; H 4.30; N 25.57.

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