Synthesis of new derivatives of pyrimido[5,4-e][1,2,4]triazolo[3,4-b] [1,3,4]thiadiazine and their enzyme inhibitory activity assessment on soybean 15-lipoxygenase

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The synthesis of new derivatives of pyrimido[5,4-e][1,2,4]triazolo[3,4-b][1,3,4]thiadiazine is described. These derivatives have a wide range of medicinal applications. Their inhibitory activity against the enzyme 15-lipoxygenase was also investigated.

Keywords: pyrimidotriazolothiadiazines, triazoles, annulated pyrimidines, 15-lipoxygenase inhibitor, heterocyclisation

Pyrimidine derivatives and heterocyclic annulated pyrimidines continue to attract great interest because of their wide variety of interesting biological activities, such as anticancer, antibacterial, antiviral, and anti-inflammatory activities. Moreover, triazoles and especially fused triazoles are also an important class of heterocyclic compounds with antifungal, bactericidal, anxiolytic, anticonvulsant and antidepressant activities.

Numerous methods for the synthesis of 1,2,4-triazoles have been reported, which includes utilising toxic reagents such as phosphorus oxychloride, lead tetraacetate, and bromine, as well as other oxidative reagents such as chloramine-T, iodobenzene diacetate, iron(III) chloride, and CuCl₂. The synthesis of 1,2,4-triazoles by an electrochemical method has also been reported. Some triazolothiadiazines have also been reported which possess a broad spectrum of biological activities. Keeping this in mind, and due to our recent studies on the enzyme inhibitory activity of pyrimido[5,4-e][1,2,4]triazolo[3,4-b][1,3,4]thiadiazines against 15-lipoxygenase (15-LO), we considered the synthesis of pyrimidotriazolothia-

diazine compounds wherein the biologically active pyrimidine moiety is fused to a potent triazole[3,4-b][1,3,4]thiadiazine ring across the 6,7-positions.

We now describe the synthesis of some new derivatives of tricyclic 3,6-dimethyl-5H-pyrimido[5,4-e][1,2,4]triazolo[3,4-b][1,3,4]thiadiazines 5a–f and their enzyme inhibitory activity towards 15-LO.

Results and discussion

5-Bromo-2,4-dichloro-6-methylpyrimidine 1 was prepared according to our previously published method. Treatment of compound 1 with 1-amino-2-mercapto-5-methyl-1,2,4-triazole 2 which was prepared from the reaction of hydrazine hydrate with CS₂ followed by reaction with acetic acid according to the published procedure afforded the intermediate 3. The facility with which substitution of the C-4 chlorine atom in compound 1 occurs by nucleophilic attack of the sulfur function in the triazole 2 had been established previously using similar conditions. Subsequent reaction of compound 3 with various secondary amines led to the selective replacement of the chlorine atom at the 2-position of the pyrimidine ring and gave the corresponding dithioureido-sulfide intermediates 4a–f. The latter compounds subsequently underwent an intramolecular S₈Ar reaction in the presence of NaNH₄ in boiling acetonitrile to give the desired tricyclic 3,6-dimethyl-5H-pyrimido[5,4-e][1,2,4]triazolo[3,4-b][1,3,4]thiadiazines 5a–f (Scheme 1).

The structural assignment of compounds 5a–f is based upon spectroscopic and microanalytical data. For example, the 1H NMR spectrum of 5d showed two singlet peaks at δ 2.44 and 2.50 ppm belonging to methyl groups of the triazole and pyrimidine moieties, respectively. The multiplet signals in the range of δ 3.44–3.63 ppm corresponded to the morpholine ring proton signals. The spectrum of the precursor 4d showed the NH signals at δ 4.92 ppm which was removed on adding D₂O. However, the 1H NMR spectrum of the cycloised product 5d did not show this signal and instead an exchangeable broad singlet peak at δ 7.72 ppm confirmed that heterocyclisation to 5d had occurred. The IR spectrum was devoid of the NH absorption bands at ν 3313 and 3137 cm⁻¹ of the precursor, but an absorption band at ν 3324 cm⁻¹ demonstrated the existence of the NH group in product 5d. The mass spectrum of 5d showed a molecular ion signal at m/z 304 (M⁺) corresponding to the molecular formula C₁₂H₁₀N₄S₂O₃.

The inhibitory property of compounds 4a–f and 5a–f on 15-LO was assessed according to our previously reported procedure. The compounds showed very low inhibitory activity.

Pyrimido[5,4-e][1,2,4]triazolo[3,4-b][1,3,4]thiadiazine with the results of our previous work.

Experimental

Melting points were recorded on an Electrothermal type 9100 melting point apparatus and are uncorrected. The IR spectra were obtained on a Varian FT-IR Thermo-Nicolet spectrometer. The 1H NMR (100 MHz) spectra were recorded on a Bruker AC 100 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 microanayser.
3-(5-Bromo-2-chloro-6-methylpyrimidin-4-ylthio)-5-methyl-4H-1,2,4-triazol-4-amine (3): To a stirred solution of 5-bromo-2,4-dichloro-6-methylpyrimidine (1) (2.44 g, 10 mmol) and Et$_3$N (1.6 mL, 13 mmol) in CH$_3$CN (25 mL), a solution of 1-amino-2-mercapto-5-methyl-1,3,4-triazole (2) (1.31 g, 10 mmol) in CH$_3$CN (30 mL) was added dropwise over 30 min. The solution was stirred vigorously until the white precipitate is appeared. Stirring was then continued at room temperature for an extra 30 minutes and the resulting solid was filtered and washed with warm water. Yield 95%, m.p. 207–208 °C, 8.29%.

The appropriate secondary amine (12 mmol) was added to a stirred mixture of compound (3) (3.35 g, 10 mmol) in ethanol (30 mL), and the solution was heated under reflux for 6 h. After cooling the solution, water (20 mL) was added and the resulting solid was filtered and recrystallised from ethanol.

Synthesis of compounds (4a–f): general procedure
The appropriate secondary amine (12 mmol) was added to a stirred mixture of compound (3) (3.35 g, 10 mmol) in ethanol (30 mL), and the solution was heated under reflux for 6 h. After cooling the solution, water (20 mL) was added and the resulting solid was filtered and recrystallised from ethanol.

3-(5-Bromo-6-methyl-4-(pyrrolidin-1-yl)pyrimidin-4-ylthio)-5-methyl-4H-1,2,4-triazol-4-amine (4a): Yield 78%, m.p. 221–222 °C, 1H NMR (CDCl$_3$) δ 1.88 (m, 4H, 2CH$_2$), 2.43 (s, 3H, CH$_3$), 2.52 (s, 3H, CH$_3$), 3.11–3.32 (m, 4H, 2CH$_2$N), 4.89 (s, 2H, NH$_2$, D$_2$O exchangeable); IR (KBr disc) ν 3284, 3170, 2970, 1564, 770 cm$^{-1}$; MS (m/z) 383 (M$^+$) 385 (M$^+$ + 2). Anal. Calcd for C$_{13}$H$_{18}$BrN$_7$S: C, 40.63; H, 4.37; N, 24.57; S, 7.98%.

A mixture of each of compounds (3a–f) (10 mmol), NaN$_3$ (30 mmol) in dry acetonitrile (50 mL) was heated under reflux for about 5h the progress of the reaction was monitored by TLC using chloroform: methanol (9:1). The mixture was cooled and the solvent was removed under reduced pressure. Then, a solution of acetic acid (1 mL) in water (20 mL) was added to the residue and the resulting precipitant was filtered off and recrystallised from ethanol.

3,6-Dimethyl-8-(pyrrolidin-1-yl)-5H-pyrimido[5,4-c][1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5a): Yield 60%, m.p. 280–282 °C,
3.6-Dimethyl-8-(4-piperidin-1-yl)-1H-pyrimido[5,4-e][1,2,4]-triazolo[3,4-b][1,3,4]thiadiazine (5b): Yield 70%, m.p. 220–222 °C. H NMR (CDCl3) δ 1.81–1.95 (m, 4 H, 2CH2), 2.39 (s, 3H, CH3), 2.46 (s, 3H, CH3), 3.35 (t, 4H, 2CH2N), 2.72 (s, 3H, NH, D2O exchangeable). IR (KBr disc) ν 3292, 1591 cm⁻¹; MS (m/e) 303 (M⁺), Anal. Calcd for C13H18N8S: C, 49.04; H, 5.70; N, 35.19; S, 8.43. Yield 65%, m.p. 250–253 °C. H NMR (CDCl3) δ 2.36 (s, 3H, CH3), 2.45 (s, 3H, CH3), 3.52–3.59 (m, 4H, CH2N), 3.63–3.73 (m, 4H, CH2N), 7.72 (br s, 1H, NH, D2O exchangeable). IR (KBr disc) ν 3323, 2961, 1617 cm⁻¹; MS (m/e) 305 (M⁺). Anal. Calcd for C18H20N8S: C, 56.82; H, 5.30; N, 29.45; S, 8.43.

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