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Synthesis of new derivatives of 10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazines

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Abstract: New 10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazines were prepared and evaluated for inhibitory activity against soybean 15-lipoxygenase enzyme. These compounds were synthesized by the sequential treatment of 4-bromo-3,6-dichloropyridazine with 2-aminothiophenol and a secondary amine with the subsequent heterocyclization in the presence of sodium amide.

Keywords: 4-bromo-3,6-dichloropyridazine; 4-bromo-1,2-dihydropyridazine-3,6-dione; 3-bromomaleic anhydride; 10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazines; heterocyclization; 15-lipoxygenase inhibitor.

Introduction

As part of our studies in the field of fused benzothiazines as inhibitors of 15-lipooxygenase (15-LO) [1–3], it was of interest to make a series of 10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazines that are structurally homologous to pyrimido[4,5-*b*][1,4]benzothiazine, which is a potent 15-LO inhibitor. Recently, 15-LO has emerged as an attractive target for therapeutic intervention. 15-LO has been implicated in the progression of certain cancers and chronic obstructive pulmonary disease. Evidence for the inhibition of 15-LO in the treatment of vascular disease is, however, most compelling [4]. A perusal of the literature revealed that this family of heterocyclic compounds has not been investigated since the first report by Yoneda et al. in 1966 [5], when they described the synthesis of 3-chloro-10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazine from 3,5,6-trichloropyridazine and 2-aminothiophenol and explained the mechanism of the reaction using a molecular orbital method. In the present article, we wish to report on the synthesis of some new derivatives of 10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazines and give a brief account of their inhibitory activity against the soybean 15-LO enzyme.

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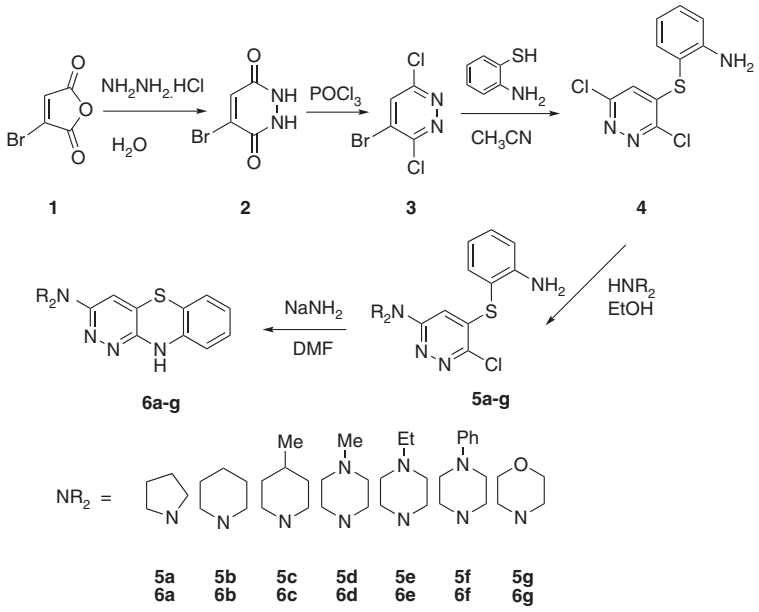
Results and discussion

The synthesis of 10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazines **6a–g** started from 3-bromomaleic anhydride (**1** in Scheme 1). Compound **1** was allowed to react with hydrazine hydrochloride to give 4-bromo-1,2-dihydropyridazine-3,6-dione (**2**) [6]. Chlorination of **2** with phosphoryl chloride yielded 4-bromo-3,6-dichloropyridazine (**3**) [6]. This compound was converted to 3-[(3,6-dichloropyridazine-4-yl)thio]analine (**4**) by selective displacement of the 4-bromine atom with 2-aminothiophenol in acetonitrile at room temperature [5]. The reaction time was reduced from 4 h to 20 min when 4-bromo-3,6-dichloropyridazine (**3**) instead of 3,4,6-trichloropyridazine was used. This modification resulted in an increased yield of **4** from 70% to 90% [5]. Then the key intermediate products, 2-{[3-chloro-6-(4-substituted-1-yl)pyridazine-4-yl]thio}anilines **5a–g** were obtained by the reaction of compound **4** with secondary amines in ethanol at 80°C [1]. Treatment of compounds **5a–g** with sodium amide in *N*,*N*-dimethylformamide furnished a host of 10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazines **6a–g** in good yields (39–70%). The structural assignments of compounds **4**, **5a–g**, and **6a–g** were based upon their spectral and microanalytical data.

The inhibitory property of compounds **6a–g** on 15-LO was assessed according to our previously reported procedure [1, 7]. The compounds show low inhibitory activity. A notable exception is compound **6d** with the inhibitory activity IC_{50} of 287 μm. By comparison with the activity of 4-methyl-2-(4-methylpiperazinyl)pyrimido[4,5-*b*]benzothiazine (4-MMPB, IC_{50}=20.7 μm) [1], one can come to the conclusion that replacement of the pyrimidine ring with the pyridazine moiety in the fused benzothiazine molecule



Scheme 1

has a profound diminishing effect on the enzyme inhibitory activity.

Conclusion

The sequential treatment of 4-bromo-3,6-dichloropyridazine with 2-aminothiophenol and then a secondary amine, followed by heterocyclization by treatment with $NaNH_2$ in DMF is a new and general route to 10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazines. The synthesized compounds are less active than 4-MMPB as inhibitors of the soybean 15-LO enzyme.

Experimental

Melting points were recorded on an Electrothermal 9100 melting point apparatus. The IR spectra were obtained in KBr pellets on an AVATAR 370 FT-IR Thermo Nicolet spectrometer. The ^{1}H NMR (100 MHz) spectra were recorded on a Bruker AC 100 spectrometer with TMS as internal reference. The mass spectra were scanned on a Varian Mat CH-7 instrument at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA 1112 instrument.

15-LO inhibitory assessment

SLO inhibitory assessment was performed by the Research Biochemistry Laboratory (Department of Laboratory Sciences, Mashhad University of Medical Science) using the previously published procedure [7].

Preparation of 3-[(3,6-dichloropyridazin-4-yl)thio] aniline (4)

To a solution of 4-bromo-3,6-dichloropyridazine (2.28 g, 20 mmol) and triethylamine (1.2 g, 12 mmol) in acetonitrile (20 mL), a solution of 2-aminothiophenol (1.25 g, 10 mmol) in acetonitrile (10 mL) was added dropwise with vigorous stirring. The mixture was stirred for 20 min at room temperature. The solvent was removed under reduced pressure, and the yellow residue was washed with water and then crystallized from ethanol: yield 90%; mp 153–155°C (lit. [5], mp 150°C); ^{1}H NMR ($CDCl_3$): δ 4.1 (br s, 2H, NH_2, D_2O exchangeable), 6.6 (s, 1H, H-5), 6.8–7.4 (m, 4H, Ar-H); IR: ν 3320 and 3387 cm^{-1} (NH_2); MS: *m/z* 270, 272 (M^+). Anal. Calcd for $C_{10}H_7N_3Cl_2S$: C, 44.13; H, 2.59; N, 15.44; S, 11.78. Found: C, 44.05; H, 2.43; N, 15.51; S, 11.77.

General procedure for the preparation of compounds 5a–g

A mixture of 3-[(3,6-dichloropyridazine-4-yl)thio]aniline (2.7 g, 10 mmol) and appropriate secondary amine (40 mmol) in ethanol (20 mL) was heated at 80°C for 10 h. The solvent was removed under reduced pressure, and the yellow residue was washed with water, crystallized from ethanol and water, and dried at 80°C to give **5a–g**.

2-{[3-Chloro-6-(pyrrolidin-1-yl)pyridazin-4-yl]thio}aniline (5a) This compound was obtained as a yellow powder in 55% yield; mp 185–187°C; ^{1}H NMR $CDCl_3$): δ 1.8 [t, 4H, *J* = 8.0 Hz, 2(CH_2-CH_2N)], 3.3 (t, 4H, *J* = 8.0 Hz, 2CH_2N), 4.3 (br s, 2H, NH_2, D_2O exchangeable), 5.7 (s, 1H, H-5), 6.7–7.5 (m, 4H, Ar-H); IR: ν 3322 and 3449 cm^{-1} (NH_2).

2-{[3-Chloro-6-(piperidin-1-yl)pyridazin-4-yl]thio}aniline (5b) This compound was obtained as a yellow powder in 48% yield; mp 199–200°C; ^{1}H NMR ($CDCl_3$): δ 1.4–1.7 (m, 6H, 3CH_2), 3.3 (t, 4H,

J = 8.0 Hz, $2CH_2$N-pyr), 4.3 (br s, 2H, NH_2, D_2O exchangeable), 6.0 (s, 1H, H-5), 6.7–7.5 (m, 4H, Ar-H); IR: ν 3403 and 3460 cm^{-1} (NH_2).

2-{[3-Chloro-6-(4-methylpiperidin-1-yl)pyridazin-4-yl]thio}aniline (5c) This compound was obtained as a yellow powder in 40% yield; mp 157–159°C; ^{1}H NMR ($CDCl_3$): δ 0.9 (d, *J* = 7.5 Hz, 3H, CH_3-CH), 1.1–1.7 (m, 5H, $2CH_2$ and CH), 2.8 (m, 4H, $2CH_2$N), 4.3 (br s, 2H, NH_2, D_2O exchangeable), 6.0 (s, 1H, H-5), 6.7–7.5 (m, 4H, Ar-H); IR: ν 3332 and 3417 cm^{-1} (NH_2).

2-{[3-Chloro-6-(4-methylpiperazin-1-yl)pyridazin-4-yl]thio}aniline (5d) This compound was obtained as a yellow powder in 76% yield; mp 165–167°C; ^{1}H NMR ($CDCl_3$): δ 2.3 (s, 3H, N-CH_3), 2.4 (t, 4H, *J* = 8.0 Hz, $2CH_2$N), 3.4 (t, 4H, *J* = 8.0 Hz, 2 CH_2N-pyr), 4.3 (br s, 2H, NH_2, D_2O exchangeable), 6.0 (s, 1H, H-5), 6.8–7.5 (m, 4H, Ar-H); IR: ν 3309 and 3366 cm^{-1} (NH_2); MS *m/z*: 335, 337 (M^+).

2-{[3-Chloro-6-(4-ethylpiperazin-1-yl)pyridazin-4-yl]thio}aniline (5e) This compound was obtained as a yellow powder in 76% yield; mp 206–208°C; ^{1}H NMR ($CDCl_3$): δ 1.1 (t, 3H, *J* = 8.0 Hz, CH_3-(CH_2N), 2.5 (m, 6H, 2(CH_2N)-CH_2), 3.3 (t, 4H, *J* = 8.0 Hz, $2CH_2$N-pyr), 4.3 (br s, 2H, NH_2, D_2O exchangeable), 6.0 (s, 1H, H-5), 6.7–7.5 (m, 4H, Ar-H); IR: ν 3313 and 3403 cm^{-1} (NH_2).

2-{[3-Chloro-6-(4-phenylpiperazin-1-yl)pyridazin-4-yl]thio}aniline (5f) This compound was obtained as a yellow powder in 75% yield; mp 176–178°C; ^{1}H NMR ($CDCl_3$): δ 3.3 (t, *J* = 8.0 Hz, 4H, $2CH_2$N-ph), 3.6 (t, *J* = 8.0 Hz, 4H, $2CH_2$N-pyr), 4.3 (br s, 2H, NH_2, D_2O exchangeable), 6.0 (s, 1H, H-5), 6.7–7.5 (m, 9H, Ar-H); IR: ν 3338 and 3444 cm^{-1} (NH_2).

2-{[3-Chloro-6-(4-morpholinopyridazin-4-yl]thio}aniline (5g) This compound was obtained as a yellow powder in 72% yield; mp 198–200°C; ^{1}H NMR ($CDCl_3$): δ 3.4 (t, *J* = 8.0 Hz, 4H, $2CH_2$-N(, 3.7 (t, *J* = 8.0 Hz, 4H, $2CH_2$-O(, 4.3 (br s, 2H, NH_2, D_2O exchangeable), 6.0 (s, 1H, H-5), 6.7–7.5 (m, 4H, Ar-H); IR: ν 3346 and 3439 cm^{-1} (NH_2). Anal. Calcd for $C_{14}H_{15}ClN_4OS$: C, 52.09; H, 4.68; N, 17.36; S, 9.93. Found: C, 52.10; H, 4.56; N, 17.09; S, 9.65.

General procedure for the conversion of (5a–g) to 10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazines (6a–g)

A mixture of compound **5a–g** (10 mmol) and $NaNH_2$ (30 mmol, 1.2 g) in DMF (20 mL) was heated at 80°C for 12 h. The solvent was removed under reduced pressure and a solution of acetic acid (0.7 g) in water (20 mL) was added to the residue. The solid material of **6a–g** was filtered off, washed with water, and crystallized from ethanol.

3-(Pyrrolidin-1-yl)-10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazine (6a) This compound was obtained as a dark green powder in 50% yield; mp 310°C (dec); ^{1}H NMR (DMSO-d_6): δ 1.95 [br, 4H, 2((CH_2)-CH_2N)], 3.4 (br, 4H, $2CH_2$N), 6.5 (s, 1H, H-5), 6.8–7.5 (m, 4H, Ar-H), 9.8 (br s, 1H, NH, D_2O exchangeable); IR: ν 3215 cm^{-1} (NH); MS: *m/z* 270 (M^+). Anal. Calcd for $C_{14}H_{14}N_4S$: C, 62.20; H, 5.22; N, 20.72; S, 11.86. Found: C, 62.55; H, 5.89; N, 20.59; S, 11.80.

3-(Piperidin-1-yl)-10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazine (6b) This compound was obtained as a dark green powder in 45% yield; mp 280°C (dec); ^{1}H NMR (DMSO-d_6): δ 1.4–1.7 (m, 6H, $3CH_2$), 3.3 (br, 4H, $2CH_2$N-pyr), 6.8–7.6 (m, 5H, H-5, Ar-H), 9.9 (br s, 2H, NH, D_2O exchangeable); IR : 3223 cm^{-1} (NH); MS: *m/z* 284 (M^+). Anal. Calcd for $C_{15}H_{16}N_4S$ (%): C, 63.35; H, 5.67; N, 19.70; S, 11.28. Found: C, 63.10; H, 5.96; N, 19.09; S, 10.99.

3-(4-Methylpiperidin-1-yl)-10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazine (6c) This compound was obtained as a brown powder in 39% yield; mp 225°C (dec);^{1}H NMR (DMSO-d_6): δ 0.8 (d, *J* = 7.5 Hz, 3H, CH_3-CH), 1.1–1.8 (m, 5H, $2CH_2$ and CH), 2.9 (m, 4H, 2(CH_2N), 6.5 (s, 1H, H-5), 6.7–7.3 (m, 4H, Ar-H), 8.6 (br s, 1H, NH, D_2O exchangeable); IR: ν 3211 cm^{-1} (NH); MS: *m/z* 298 (M^+). Anal. Calcd for $C_{16}H_{18}N_4S$: C, 64.40; H, 6.08; N, 18.78; S, 10.75. Found: C, 64.78; H, 6.14; N, 18.61; S, 10.53.

3-(4-Methylpiperazin-1-yl)-10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazine (6d) This compound was obtained as a gray powder in 60% yield; mp 270°C (dec); ^{1}H NMR (DMSO-d_6): δ 2.7 (s, 3H, N-CH_3), 3.2 (br, 4H, $2CH_2$N), 3.4 (br, 4H, $2CH_2$N-pyr), 6.8 (s, 1H, H-5), 7.0–7.8 (m, 4H, Ar-H) 9.4 (br s, 1H, NH, D_2O exchangeable); IR: ν 3269 cm^{-1} (NH); MS: *m/z* 299 (M^+). Anal. Calcd for $C_{15}H_{17}N_5S$: C, 60.18; H, 5.72; N, 23.39; S, 10.71. Found: C, 60.31; H, 5.62; N, 23.89; S, 10.33.

3-(4-Ethylpiperazin-1-yl)-10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazine (6e) This compound was obtained as a yellow powder in 70% yield; mp 282°C (dec); ^{1}H NMR (DMSO-d_6): δ 1.0 (t, *J* = 8.0 Hz, 3H, CH_3-CH_2N), 3.2 [br, 6H, 2(CH_2N)-CH_2], 3.6 (m, 4H, $2CH_2$N-pyr), 6.7–7.4 (m, 5H, H-5, Ar-H), 9.9 (br s, 1H, NH, D_2O exchangeable); IR: ν 3225 cm^{-1} (NH); MS: *m/z* 313 (M^+). Anal. Calcd for $C_{16}H_{19}N_5S$: C, 61.31; H, 6.11; N, 22.34; S, 10.23. Found: C, 61.14; H, 6.32; N, 22.63; S, 9.98.

3-(4-Phenylpiperazin-1-yl)-10*H*-benzo[*b*]pyridazino[3,4-*e*][1,4]thiazine (6f) This compound was obtained as a brown powder in 65% yield; mp 200°C (dec); ^{1}H NMR (DMSO-d_6): δ 3.3 (br, 8H, $2CH_2$N-ph, $2CH_2$N), 6.4 (s, 1H, H-5), 6.5–7.2 (br, 9H, Ar-H) 9.6 (br s, 1H, NH, D_2O exchangeable); IR: ν 3223 cm^{-1} (NH); MS: *m/z* 357 (M^+). Anal. Calcd for $C_{20}H_{19}N_5S$: C, 66.46; H, 5.30; N, 19.37; S, 8.87. Found: C, 66.21; H, 5.32; N, 19.43; S, 8.86.

4-(10*H*-Benzo[*b*]pyridazino[3,4-*e*][1,4]thiazine-3-yl)morpholine (6g) This compound was obtained as a dark yellow powder in 67% yield; mp 269°C (dec); ^{1}H NMR (DMSO-d_6): δ 3.2–3.7 [br, 8H, CH_2-(O,N)], 6.7–7.4 (m, 5H, H-5, Ar-H), 10.0 (br s, 1H, NH, D_2O exchangeable); IR: ν 3259 cm^{-1} (NH); MS: *m/z* 286 (M^+). Anal. Calcd for $C_{14}H_{14}N_4OS$: C, 58.72; H, 4.93; N, 19.57; S, 11.20. Found: C, 58.73; H, 4.86; N, 19.51; S, 11.25.

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