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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antiinflammatory activity of certain benzothieno[3,2-*d*][1,2,4]triazolo[4,3-*b*] pyridazine derivatives

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Abstract 6-Bromo-4-chloro-1-hydrazinobenzothieno[2,3-*d*] pyridazine (**1**) was selected as the starting material for the synthesis of some novel fused benzothienotriazolopyridazine derivatives **2–16**. Thus, compound **1** was reacted with carbon disulfide, ethyl orthoformate, acetic anhydride, or 2-methoxybenzaldehyde followed by cyclization with bromine, to give the corresponding benzothienotriazolopyridazines **2–6**. Nucleophilic substitution of the 6-chloro with piperidine, *N*-methyl piperazine, hydrazine hydrate, or potassium hydroxide afforded 6-substituted benzothieno[3,2-*d*][1,2,4]triazolo[4,3-*b*] pyridazines **7–16**. The structures of the synthesized compounds were elucidated by elemental analysis and spectral data. All the newly synthesized compounds were subjected to evaluation for their antiinflammatory activity against carrageenan-induced paw edema at a dose of 10 mg/kg using indomethacin as the reference standard. Compounds **11** and **13** (6-hydrazinyl derivatives) significantly reduced the edema to 52.8 and 78.7%, respectively, as compared with indomethacin (50.5%).

Keywords Benzothieno[3,2-*d*][1,2,4]triazolo [4,3-*b*]pyridazines · Antiinflammatory activity

Introduction

Thienopyridazine derivatives have been reported to be physiologically and pharmacologically active and they gained much attention as important pharmacophore and privileged structure in medicinal chemistry encompassing a diverse range of biological activities including potent and selective phosphodiesterase IV inhibitor (Dal Piaz *et al.*, 1997), immunosuppressants (Bantick *et al.*, 1999), antiasthmatic (Yamaguchi *et al.*, 1995), antiinflammatory (Pieretti *et al.*, 2006), antibacterial, antifungal (Singh *et al.*, 2005), anticonvulsant (Fischer *et al.*, 1987), antispasmodic (Robev *et al.*, 1984), and antitumour activities (Dumas *et al.*, 2001, Wu, 2009). Previously, a number of preparative methods for benzothieno[3,2-*d*]pyridazines syntheses were elaborated, however, their pharmacolological properties were not investigated (Bezdrik *et al.*, 1908; Charles and Frederick 1945; Dore *et al.*, 1972a, b, c, 1973a, b; Guha and Mitra, 1966; Huntress and Hearon 1941; Kamal El-Dean *et al.*, 2004; Linstead *et al.*, 1937). We have recently developed a synthesis of the new building block 6-Bromo-4-chloro-1-hydrazinobenzothieno[2,3-*d*] pyridazine **1** through a series of steps and studied its application for the preparation of some fused tetrazolo- and triazinobenzothienopyridazine derivatives (Fig. 1) (Zaher *et al.*, 2009). Here, we communicate our new results on the application of **1** for the preparation of fused triazolobenzothienopyridazines bearing chloro moiety located at the 6-position. This electrophilic center made these synthons versatile building blocks for the further preparation of certain 6-substituted triazolobenzothienopyridazines of anticipated antiinflammatory activity.

Results and discussion

Chemistry

Our interest in developing synthetic approaches with a view to synthesize new 8-bromobenzothieno[3,2-*d*][1,2,4]

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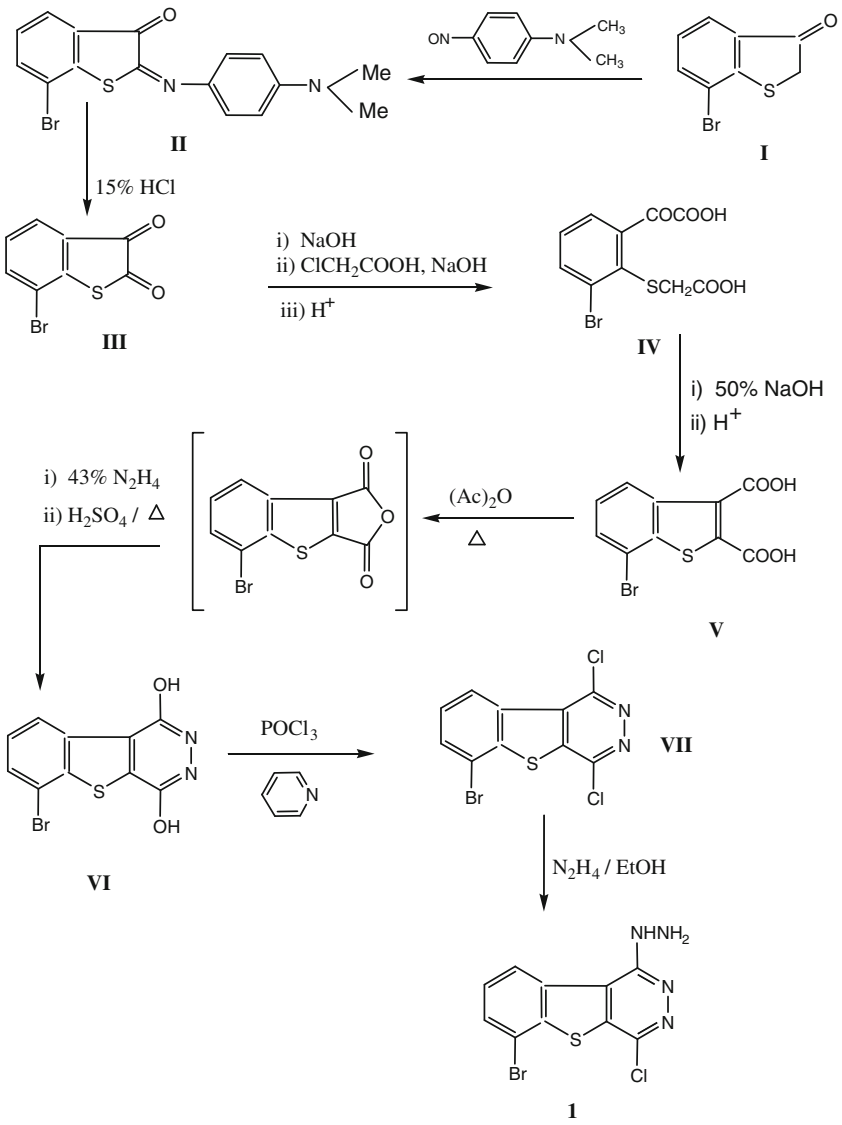


Fig. 1 Synthesis of 6-Bromo-4-chloro-1-hydrazinobenzothieno[2,3-*d*] pyridazine (**1**)

triazolo[4,3-*b*]pyridazine derivatives **2–16**, the 4-chloro-1-hydrazinobenzothieno[2,3-*d*] pyridazine **1** (Zaher *et al.*, 2009), was thus investigated as a good starting material for this purpose. 7-Bromo-2-(4-dimethylaminophenylimino) benzothiophen-3-one **II** (Zaher *et al.*, 2009) was produced through the condensation of 6-Bromobenzothiophene-3-one **I** (Guha and Mitra, 1966; Charles and Frederick 1945) with *p*-nitroso-*N,N*-dimethylaniline (Bezdrik *et al.*, 1908), acid hydrolysis of **II** gave 6-bromobenzothiophene 2,3-diones **III** (Zaher *et al.*, 2009). Reaction of compound **III** with chloroacetic acid in sodium hydroxide solution afforded *S*-(6-bromo-2-oxalophenyl)thioglycolic acid **IV** (Zaher *et al.*, 2009), and subsequent cyclization of **IV** with 50% aqueous sodium hydroxide provided 7-bromobenzothiophene-2,3-dicarboxylic acid **V** (Zaher *et al.*, 2009). The latter was refluxed with acetic anhydride to give the acid anhydride intermediate which was then warmed with hydrazine hydrate, then acidified with sulfuric acid to produce the target 6-bromo-1,2,3,4-tetrahydrobenzothieno[2,3-*d*] pyridazine 1,4-dione **VI** (Zaher *et al.*, 2009). Chlorination of the 1,4-dione **VI** with phosphorus oxychloride in pyridine afforded the 1,4-dichloro derivative **VII** (Zaher *et al.*, 2009). Hydrazinolysis of compound **VII** produced the 1-hydrazino derivative **1** (Zaher *et al.*, 2009).

Treatment of compound **1** with carbon disulfide, ethyl orthoformate, or acetic anhydride gave the 3-sulphanyl triazolo **2**, triazolo **3,** and 3-methyl triazolo **4** derivatives, respectively (Scheme 1). The IR spectra of compound **2** showed the characteristic C=S stretching absorption at δ 1,220 and 1,410 cm^{-1}, while the ^{1}H-NMR spectrum of compound **3** displayed the appearance of C_3H absorption at δ 9.79 ppm. Whereas the ^{1}H-NMR spectrum of compound **4** displayed a singlet signal at δ 1.22 ppm corresponding to CH_3. On the other hand, reaction of compound **1** with an

equimolar amount of 2-methoxybenzaldehyde in 0.1 N hydrochloric acid at 60°C afforded the derivative **5**, which was cyclized by bromine in acetic acid to give 3-(2-methoxyphenyl)8-bromo-6-chloro benzothieno[3,2-*d*][1,2,4]triazolo[4,3-*b*] pyridazine **6**. The ^{1}H-NMR spectra of compound **6** displayed the disappearance of N=CH absorption of the precursor **5.** The mass spectrum of compound **6** showed molecular ion at *m*/*z* 444 and M + 1 for ^{13}C at *m*/*z* 445. An intense peak at *m*/*z* 446 indicated the isotope peak for ^{37}Cl, ^{81}Br, or ^{34}S in addition to the presence of M + 2 + 1 for ^{13}C at *m*/*z* 447 and M + 4 indicating the isotopes ^{37}Cl, ^{81}Br, and/or ^{34}S. The molecular ions M + 4, M + 2, M^+ and the respective M + 3 and M + 1 lost ^{35}Cl and/or ^{37}Cl to give the corresponding peaks at 412, 411 (base beak), 410, and 409. The latter molecular ions lost N_2 to give peaks at *m*/*z* 384, 383, 482, and 381, respectively.

Attention was next turned to the nucleophilic substitution of the 6-chloro with piperidine, *N*-methyl piperazine, hydrazine hydrate, and potassium hydroxide to produce 6-substituted benzo thieno [3,2-*d*] [1,2,4] triazolo [4,3-*b*] pyridazines **7–16** (Scheme 2). The ^{1}H-NMR spectra of compounds **7–16** were consistent with the proposed structures. The mass spectrum of compound **8** showed molecular ion at *m*/*z* 402 and M + 1 for ^{13}C at *m*/*z* 403. Also, a peak at *m*/*z* 404 indicated the isotope peak for ^{81}Br or ^{34}S in addition to the presence of M + 2 + 1 for ^{13}C at *m*/*z* 405 and M + 4 at *m*/*z* 406 indicating the isotopes ^{81}Br and ^{34}S. The molecular ions M + 4, M + 2, M^+ and the respective M + 3 and M + 1 lost methylpiperazine, then either lost N_2 followed by the loss of HCN or the reverse, to give the corresponding peaks at 252, 251, 250, 249, and 248 ($C_{10}H_3BrN_3S$). In a similar way, the mass spectrum of compound **9** showed molecular ion peaks and the subsequent loss of piperidine (base peak), CH_3CN, and then N_2.

Antiinflammatory activity

All the newly synthesized compounds were subjected to preliminary testing for their antiinflammatory activity in comparison with the reference drug, indomethacin. The percentage reduction in the inflammation (i.e., reduction in the right hand paw volume of the animal) 3 h after administration of carrageenan was recorded. Only compounds **10**, **11, 13**, and **14** produced a reduction in edema volume compared with the control, compounds **11** and **13** (6-hydrazinyl derivatives) significantly reduced the edema to 52.8 and 78.7%, respectively. The percentage inhibition of edema produced by indomethacin was 50.5% (Fig. 2).

These results suggested that the incorporation of a triazole ring in the benzothienopyridazine system together with proper substitution, for example, 6-hydrazinyl provides a new promising scaffold with moderate antiinflammatory activity for further structural modification to enhance anti-inflammatory activity.

Experimental

Chemistry

Melting points were obtained on a Griffin apparatus and are uncorrected. Microanalyses for C, H, and N were carried out at the Microanalytical Center, Cairo University. IR spectra were recorded on a Shimadzu 435 spectrometer, using KBr disks. ^{1}H-NMR spectra were performed on a Joel NMR FXQ-200 MHz spectrometer, using TMS as the internal standard and DMSO-d_6 as solvent.

Mass spectra were recorded on a GCMP-QP1000 EX Mass spectrometer. Progress of the reactions were monitored by TLC using precoated aluminum sheet silica gel MERCK 60F 254 and were visualized by UV lamp.

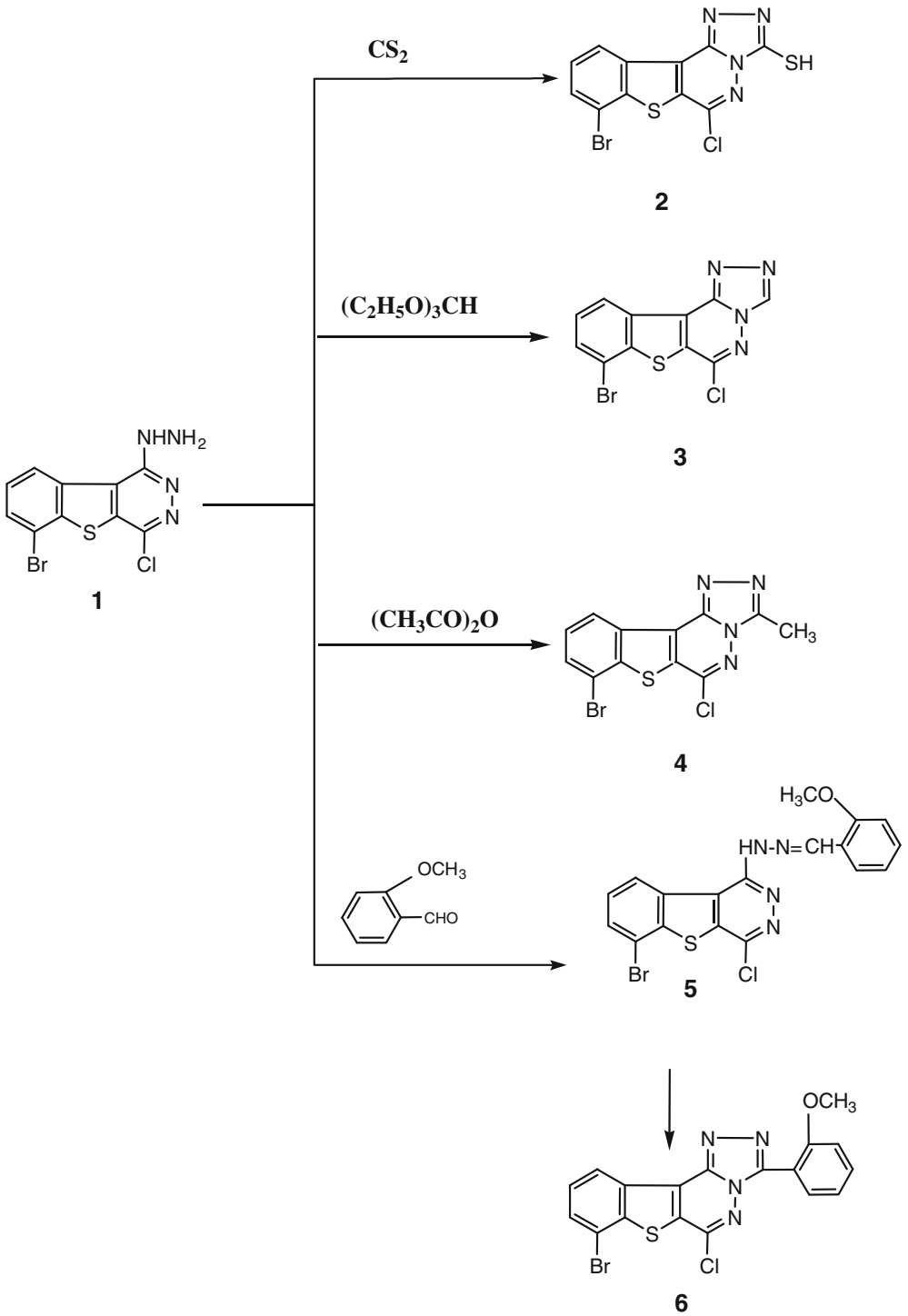
8-Bromo-6-chloro-3-sulphanyl benzothieno[3,2-*d*][1,2,4]triazolo[4,3-*b*] pyridazine (**2**)

To a suspension of 4-chloro-1-hydrazinobenzothieno[2,3-*d*]pyridazine **1** (0.46 g, 0.0014 mol) in absolute methanol (20 ml), potassium hydroxide (0.168 g, 0.003 mol) and carbon disulfide (2.28 g, 0.03 mol) were added. The reaction mixture was heated under reflux for 5 h, and then the solvent was distilled off under reduced pressure. The residue was dissolved in 10% aqueous potassium hydroxide (100 ml) and filtered. The filtrate was neutralized to litmus with 10% hydrochloric acid and the separated solid washed with water, dried, and crystallized from isopropanol.

Yield: 45.0%; mp: >300°C; IR (cm^{-1}): 3200–2500 (NH/SH), 1610 (C=N) and 1220, 1410 (C=S); ^{1}H-NMR: δ 7.62 (t, 1H, J = 7.6 Hz, $C_{10}H$), 7.92 (d, 1H, J = 7.6 Hz, C_9H), 8.63 (d, 1H, J = 7.6 Hz, $C_{11}H$), 14.70 (br s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{11}H_4BrClN_4S_2$ (371.65): C, 35.54; H, 1.08; N, 15.07. Found: C, 35.61; H, 1.11; N, 15.20.

8-Bromo-6-chlorobenzothieno[3,2-*d*][1,2,4] triazolo[4,3-*b*]pyridazine (**3**)

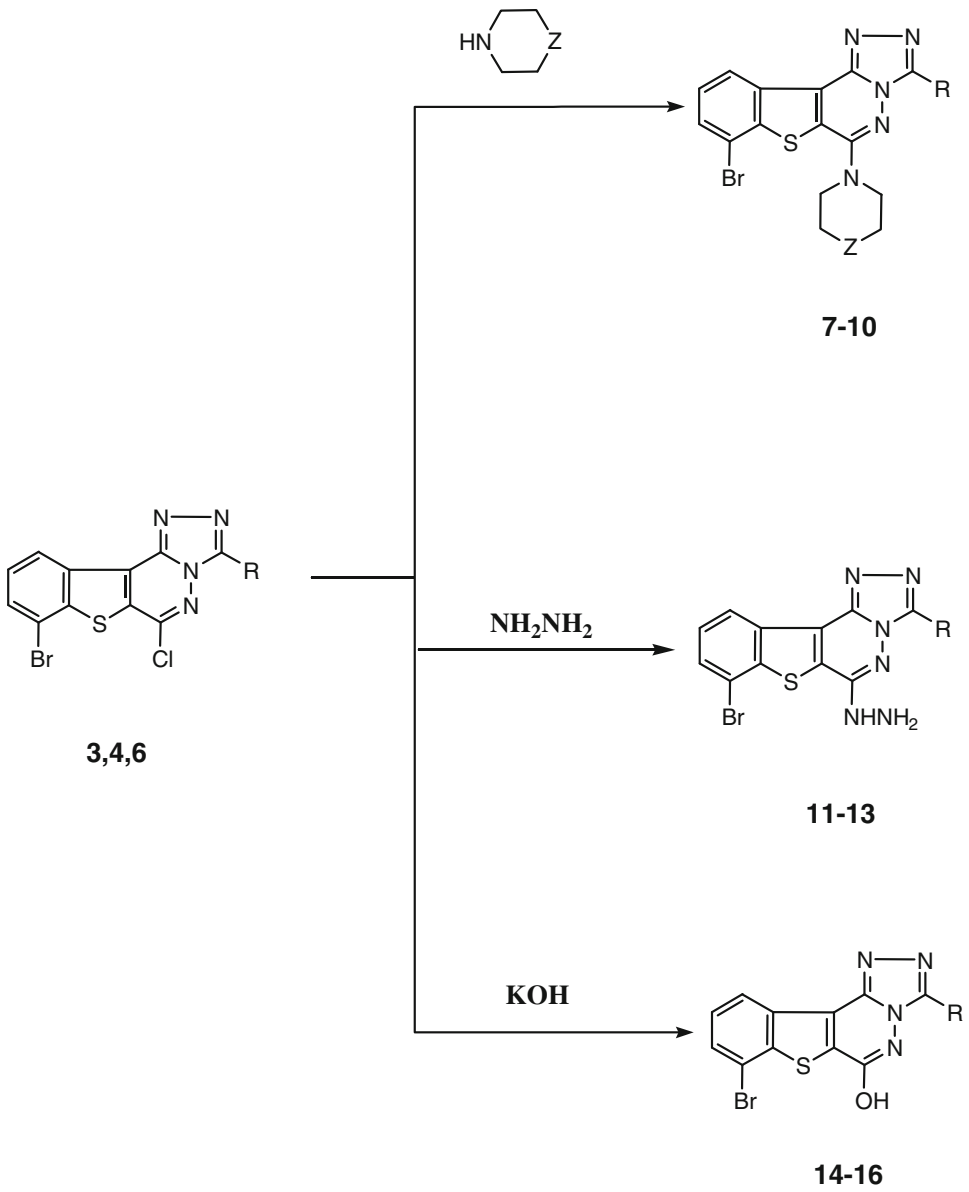
Compound **1** (0.46 g, 0.0014 mol) was heated under reflux with triethyl orthoformate (5 ml) for 3 h then cooled. The separated solid was filtered, washed with methanol, dried, and crystallized from isopropanol. Yield: 55.0%; mp: 240–242°C; IR (cm^{-1}): 1640 (C=N); ^{1}H-NMR: δ 7.55 (t, 1H, J = 7.8 Hz, $C_{10}H$), 7.86 (d, 1H, J = 7.8 Hz, C_9H), 8.60 (d, 1H, J = 7.8 Hz, $C_{11}H$), 9.79 (s, 1H, C_3H). Anal. Calcd. for $C_{11}H_4BrClN_4S$ (339.59): C, 38.90; H, 1.18; N, 16.49. Found: C, 39.20; H, 1.10; N, 16.60.



Scheme 1 Synthesis of compounds **2–6**

8-Bromo-6-chloro-3-methylbenzothieno[3,2-*d*] [1,2,4]triazolo[4,3-*b*] pyridazine (**4**)

A mixture of compound **1** (0.46 g, 0.0014 mol) and acetic anhydride (2 ml) was heated under reflux for 3 h. The excess acetic anhydride was distilled under diminished pressure and the residue was crystallized from isopropanol. Yield: 50.0%; mp: 234–236 °C; IR (cm^{-1}): 2900 (aliph H) and 1640 (C=N); ^{1}H-NMR: δ 1.19 (s, 3H, CH_3), 7.63 (m, 1H, $C_{10}H$), 7.92 (d, 1H, $J = 7.7$ Hz, C_9H), 8.67 (d, 1H, $J = 8.1$ Hz, $C_{11}H$). Anal. Calcd. for $C_{12}H_6BrClN_4S$ (353.62): C, 40.75; H, 1.71; N, 15.84. Found: C, 41.10; H, 1.65; N, 16.00.



3,11,14 R = H
4,12,15 R = CH_3
6,13,16 R = 2-OCH_3-C_6H_4

7 R = H, Z = CH_2
8 R = H, Z = N-CH_3
9 R = CH_3, Z = CH_2
10 R = 2-OCH_3-C_6H_4, Z = CH_2

Scheme 2 Synthesis of compounds **7–16**

8-Bromo-4-chloro-1-(2-methoxyphenyl hydrazino)benzothieno[2,3-*d*] pyridazine (**5**)

To a solution of compound **1** (0.46 g, 0.0014 mol) in 0.1 N hydrochloric acid (10 ml), 2-methoxybenzaldehyde (0.136 g, 0.001 mol) was added portionwise and the mixture was kept at 60°C with stirring for 10 min. The cold reaction mixture was neutralized to litmus with 10% aqueous sodium hydroxide solution and the precipitate was filtered, washed with water, and then crystallized from isopropanol.

Yield: 90.0%; mp: 205–207°C; IR (cm^{-1}): 3400 (NH), 2900 (aliph H) and 1640 (C=N); ^{1}H-NMR: δ 3.72 (s, 3H, OCH_3), 7.01–7.17 (m, 3H, 2ArH + NH), 7.51 (t, 1H, $C_{10}H$), 7.69–7.79 (m, 2H, ArH), 7.81 (d, 1H, $J = 7.6$ Hz, C_9H), 7.98 (d, 1H, $J = 7.6$ Hz, $C_{11}H$), 8.94 (s, 1H,

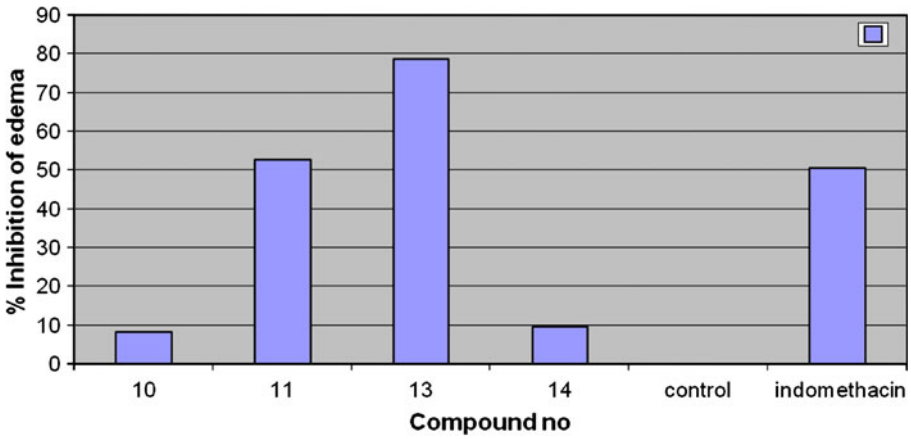


Fig. 2 Anti-inflammatory activity of compounds **10**, **11**, **13**, and **14** expressed as % Inhibition of edema

N = CH). Anal. Calcd. for $C_{18}H_{12}BrClN_4OS$ (447.73): C, 48.28; H, 2.70; N,12.51. Found: C, 48.40; H, 2.50; N, 12.60.

8-Bromo-3-(2-methoxyphenyl)-6-chlorobenzothieno[3,2-*d*][1,2,4]triazolo[4,3-*b*] pyridazine (**6**)

To a mixture of **5** (0.44 g, 0.001 mol), sodium acetate (0.25 g, 0.003 mol) and acetic acid (9 ml), bromine (0.16 g, 0.001 mol) in acetic acid (0.6 ml) was added dropwise while stirring at room temperature over a period of 1 h. The reaction mixture was then poured into cold water (50 ml), and the separated solid was filtered, washed with 10% aqueous sodium bisulfite solution (2 × 2 ml), then with water (20 ml), dried, and crystallized from isopropanol. Yield: 80.0%; mp: 250–252°C; IR (cm^{-1}): 1620 (C=N); ^{1}H-NMR: δ 3.84 (s, 3H, OCH_3), 7.01–7.21 (m, 2H, ArH), 7.79 (m, 1H, $C_{10}H$), 8.14 (m, 2H, ArH), 8.28 (m, 1H, C_9H), 8.88 (m, 1H, $C_{11}H$); MS *m/z*: 448 (M + 4, 17.55%), 447 (M + 3, 15.81%), 446 (M + 2, 44.22%), 445 (M + 1, 21.31%), 444 (M^+, 30.92%), 412 (M + 3-^{35}Cl, 23.23%), 411 (M + 4-^{37}Cl and M + 2-^{35}Cl, 100%), 410 (M + 3-^{37}Cl and M + 1-^{35}Cl, 22.04%), 409 (M + 2-^{37}Cl and M^+-^{35}Cl, 98.11%), 384, 383, 382, 381 ($C_{18}H_{10}BrN_2OS$, 29.94, 11.80, 35.20, 28.17%). Anal. Calcd. for $C_{18}H_{10}BrClN_4OS$ (445.71): C, 48.50; H, 2.26; N, 12.57. Found: C, 48.71; H, 2.30; N, 12.80.

General procedure for the preparation of compound **7–12**

To a suspension of the appropriate 8-bromo-4-chlorobenzothieno[3,2-*d*] [1,2,4]triazolo[4,3-*b*]pyridazine **3**, **4**, or **6**, (0.001 mol) in *n*-butanol (10 ml), the appropriate secondary amine (0.001 mol), and triethyl amine (2-3 drops) were added. The reaction mixture was heated under reflux for 4 h. After cooling the separated solid was filtered, washed with ethanol, and crystallized from the suitable solvent.

*8-Bromo-6-piperidin-1-ylbenzothieno[3,2-d][1,2,4]triazolo[4,3-b] pyridazine (**7**)*

Yield: 72.0%; mp: 218–220°C; IR (cm^{-1}): 2,900 (aliph H) and 1,590 (C=N); ^{1}H-NMR: δ 1.14–1.21 (m, 4H, piperidine $H_{3,5}$), 1.41–1.44 (m, 2H, piperidine H_4), 4.37–4.38 (m, 4H, piperidine $H_{2,6}$), 7.60–7.65 (m, 1H, $C_{10}H$), 7.92 (d, 1H, C_9H), 8.70 (d, 1H, $C_{11}H$), 8.81 (s, 1H, C_3H). Anal. Calcd. for $C_{16}H_{14}BrN_5S$ (388.28): C, 49.49; H, 3.63; N, 18.03. Found: C, 49.60; H, 3.60; N, 18.00.

*8-Bromo-6-(4-methylpiperazin-1-yl)benzothieno[3,2-d][1,2,4]triazolo[4,3-b] pyridazine (**8**)*

Yield: 79.0%; mp: 295–297°C; IR (cm^{-1}): 2,900 (aliph H) and 1,590 (C=N); MS *m/z*: 406 (M + 4, 0.48%), 405 (M + 3, 1.53%), 404 (M + 2, 6.66%), 403 (M + 1, 3.08%), 402 (M^+, 6.66%), 307, 306, 305, 304, 303 ($C_{11}H_4BrN_4S$ = M + 4, M + 3, M + 2, M + 1 and M^+-$C_5H_{11}N_2$, 0.42, 1.55, 1.83, 1.72, 1.37%), 280, 279, 278, 277, 276, 275 ($C_{11}H_4BrN_2S$ and/or $C_{10}H_3BrN_3S$=$C_{11}H_4BrN_4S$–N_2 or –HCN, 0.39, 0.78, 1.23, 1.41, 1.25, 0.97%), 252, 251, 250, 249, 248 ($C_{10}H_3BrNS$=$C_{11}H_4BrN_2S$–HCN and/or $C_{10}H_3BrN_3S$–N_2, 0.97,1.41, 1.18, 2.33. 0.80%), 70 (C_2NS, 100%). Anal. Calcd. for $C_{16}H_{15}BrN_6S$ (403.29): C, 47.65; H, 3.74; N, 20.83. Found: C, 47.50; H, 3.72; N, 21.00.

*8-Bromo-6-piperidin-1-yl-3-methylbenzothieno[3,2-d][1,2,4]triazolo[4,3-b] pyridazine (**9**)*

Yield: 82.0%; mp: 234–236°C; IR (cm^{-1}): 2,900 (aliph H) and 1,600 (C=N); ^{1}H-NMR: δ 1.23 (s, 1H, CH_3), 1.55–1.73 (m, 6H, piperidine $H_{3,4,5}$), 3.55–3.56 (m, 4H, piperidine $H_{2,6}$), 7.69 (t, 1H, J = 7.8 Hz, $C_{10}H$), 7.97 (d, 1H,

$J = 7.8$ Hz, C_9H), 8.80 (d, 1H, $J = 7.8$ Hz, $C_{11}H$); MS *m/z*: 405 (M + 4, 3.59%), 404 (M + 3, 12.15%), 403 (M + 2, 55.95%), 402 (M + 1, 18.74%), 401 (M^+, 53.97%), 321, 320, 319, 318, 316 ($C_{12}H_6BrN_4S$ = M + 4, M + 3, M + 2, M + 1 and M^+-$C_5H_{11}N$ or –$C_5H_{10}N$, 28.45, 45.54, 27.18, 41.77, 3.62%), 280, 279, 278, 277, 276 ($C_{10}H_3BrN_3S$=$C_{12}H_6BrN_4S$–CH_3CN, 3.33 4.01, 3.97, 4.49, 3.69%), 252, 251, 250, 250, 249, 248 ($C_{10}H_3BrNS$=$C_{10}H_3BrN_3S$–N_2, 4.27, 6.41, 6.16, 5.43, 2.55%), 85 ($C_5H_{11}N$, 12.98%) and 84 ($C_5H_{10}N$, 100%). Anal. Calcd. for $C_{17}H_{16}BrN_5S$ (402.30): C, 50.75; H, 4.00; N, 17.40. Found: C, 50.60; H, 4.10; N, 17.35.

*8-Bromo-3-(2-methoxyphenyl)-6-piperidin-1-yl benzothieno[3,2-d][1,2,4]triazolo[4,3-b] pyridazine (**10**)*

Yield: 65.0%; mp: 238–240°C; IR (cm^{-1}): 2900 (aliph H) and 1610 (C=N); ^{1}H-NMR: δ 1.22 (br s, 4H, piperidine $H_{3,5}$), 1.89 (br s, 2H, piperidine H_4), 3.82 (br s, 4H, piperidine $H_{2,6}$), 3.87 (s, 3H, OCH_3), 7.04–7.21 (m, 2H, ArH), 7.62–7.76 (m, 2H, ArH), 7.79 (m, 1H, $C_{10}H$), 8.26 (d, 1H, $J = 8.7$ Hz, C_9H), 8.88 (m, 1H, $C_{11}H$). Anal. Calcd. for $C_{23}H_{20}BrN_5OS$ (494.40): C, 55.87; H, 4.07; N, 14.16. Found: C, 55.90; H, 4.00; N, 14.00.

*8-Bromo-6-hydrazinobenzothieno[3,2-d] [1,2,4]triazolo[4,3-b] pyridazine (**11**)*

Yield: 82.0%; mp: 300°C; IR (cm^{-1}): 3400 (NH/NH_2) and 1610 (C=N); ^{1}H-NMR: δ 5.46 (br s, 3H, NH-NH_2, D_2O exchangeable), 7.55 (m, 1H, $C_{10}H$), 7.84 (m, 1H, C_9H), 8.65–8.72 (m, 2H, $C_{11}H$ and C_3H). Anal. Calcd. for $C_{11}H_7BrN_6S$ (335.17): C, 39.41; H, 2.10; N, 25.07. Found: C, 39.47; H, 2.30; N, 25.10.

*8-Bromo-6-hydrazino-3-methylbenzothieno[3,2-d] [1,2,4]triazolo[4,3-b] pyridazine (**12**)*

Yield: 69.0%; mp: 300°C; IR (cm^{-1}): 3400 (NH/NH_2), 2900 (aliph H), 1640 (C=N); ^{1}H-NMR: δ 1.75 (s, 3H, CH_3), 5.33 (br s, 3H, NH-NH_2, D_2O exchangeable), 7.56 (m, 1H, $C_{10}H$), 7.84 (m, 1H, C_9H), 8.69 (m, 1H, $C_{11}H$). Anal. Calcd. for $C_{12}H_9BrN_6S$ (349.20): C, 41.27; H, 2.59; N, 24.06. Found: C, 41.10; H, 2.60; N, 23.90.

*8-Bromo-3-(2-methoxyphenyl)-6-hydrazino benzothieno[3,2-d][1,2,4]triazolo[4,3-b] pyridazine (**13**)*

Yield: 77.0%; mp: 240–242°C; IR (cm^{-1}): 3450 (NH/NH_2) and 1590 (C=N); ^{1}H-NMR: δ 3.67 (br s, 3H, NH-NH_2, D_2O exchangeable), 3.88 (s, 3H, OCH_3), 7.14–7.26 (m, 2H, ArH), 7.63–7.67 (m, 2H, ArH),7.96 (m, 1H, $C_{10}H$), 8.48 (m, 1H, C_9H), 8.86 (m, 1H, $C_{11}H$). Anal. Calcd. for $C_{18}H_{13}BrN_6OS$ (441.30): C, 48.98; H, 2.96; N, 19.04. Found: C, 49.20; H, 2.93; N, 18.90.

*8-Bromo-6-hydroxybenzothieno[3,2-d][1,2,4]triazolo [4,3-b]pyridazine (**14**)*

Yield: 72.0%; mp: >300°C; IR (cm^{-1}): 3257–3361 (OH), 2850 (aliph H) and 1590 (C=N); ^{1}H-NMR: δ 7.59 (t, 1H, $J = 7.8$ Hz, $C_{10}H$), 7.82 (d, 1H, $J = 7.8$ Hz, C_9H), 8.62 (d, 1H, $J = 7.8$ Hz, $C_{11}H$), 8.73 (s, 1H, C_3H), 9.15 (s, 1H, OH, D_2O exchangeable). Anal. Calcd. for $C_{11}H_5BrN_4OS$ (321.14): C, 41.13; H, 1.56; N, 17.44. Found: C, 40.90; H, 1.56; N, 17.50.

*8-Bromo-6-hydroxy-3-methylbenzothieno [3,2-d][1,2,4]triazolo[4,3-b] pyridazine (**15**)*

Yield: 69.0%; mp: <300°C; IR (cm^{-1}): 3290-3400 (OH), 2950 (aliph H) and 1600 (C=N); ^{1}H-NMR: δ 1.18 (s, 3H, CH_3), 7.60 (m, 1H, $C_{10}H$), 7.89 (m, 1H, C_9H), 8.64 (m, 1H, $C_{11}H$), 12.19 (s, 1H, OH, D_2O exchangeable). Anal. Calcd. for $C_{12}H_7BrN_4OS$(335.17): C, 43.00; H, 2.10; N, 16.71 Found: C, 43.20; H, 2.20; N, 16.50.

*8-Bromo-3-(2-methoxyphenyl)-6-hydroxybenzothieno [3,2-d][1,2,4]triazolo[4,3-b] pyridazine (**16**)*

Yield: 70.0%; mp <300°C; IR (cm^{-1}): 3373–3404 (OH), 2927 (aliph H) and 1608 (C=N); ^{1}H-NMR: δ 3.84 (s, 3H, OCH_3), 4.68 (s, 1H, OH, D_2O exchangeable), 7.06–7.62 (m, 4H, ArH), 7.76 (m, 1H, $C_{10}H$), 8.38 (m, 1H, C_9H), 8.86 (m, 1H, $C_{11}H$). Anal. Calcd. for $C_{18}H_{11}BrN_4OS$ (411.27): C, 52.56; H, 2.69; N,13.70. Found: C, 52.49; H, 2.73; N, 13.70.

Antiinflammatory activity screening

The preliminary screening was performed applying the procedure of Winter *et al.* (1962) using groups of albino rats weighing 100–120 g each, 6 rats per group. The first group was injected with 0.05 ml of 1% carrageenan in the subplantar tissue of the right hind paw and served as untreated control. The positive control group was given 10 mg/kg indomethacin 1 h before carrageenan injection. The test compounds were suspended in 0.5% carboxymethylcellulose (CMC) and given to the rats orally at a dose of 10 mg/kg 1 h prior to carrageenan injection. In all groups, both hind limbs were dissected 4 h after carrageenan injection and weighed, and the difference in weight was calculated. The effect of the test compounds were compared with control and standard by ordinary one-way ANOVA (Table 1).

Table 1 Antiinflammatory activity of the tested compounds using acute carrageenan-induced paw edema in rats

Comp. no.	Increase in paw weight (mean ± SEM)	% Inhibition of edema
2	–	–
3	–	–
4	–	–
5	–	–
6	–	–
7	–	–
8	–	–
9	–	–
10	1.01 ± 0.008	8.4
11	0.52 ± 0.83*	52.8
12	–	–
13	0.24 ± 0.52*	78.7
14	0.99 ± 0.12	9.7
15	–	–
16	–	–
Control	1.11 ± 0.32	–
Indomethacin	0.01 ± 0.03*	50.5

* Significantly different from control group at $P < 0.05$ (one-way ANOVA followed by Tukey–Kramer multiple comparison test)

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