Synthesis of Some Thiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-ones as Potential Platelet Aggregation Inhibitors

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Twenty new compounds having 2-methyl-6-benzylidenethiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-one (**2a-d**) and 2-methyl-6-(α-aminobenzyl)thiazolo-[3,2-*b*]-1,2,4-triazole-5-ol (**5-20**) structures were synthesized. Their structures were confirmed by elemental and spectroscopic analysis and their platelet aggregation inhibitory activities were investigated.

Synthese einiger Thiazolo[3,2-*b*]-1,2,4-triazol-5(6*H*)-one als potentielle Plättchenaggregations-Hemmer

Zwanzig neue Derivate des 2-Methyl-6-benzylidenethiazolo[3,2-*b*]-1,2,4-triazole-5(6*H*)-ones (**2a-d**) und des 2-Methyl-6-(α-aminobenzyl)thiazolo-[3,2-*b*]-1,2,4-triazole-5-ols (**5-20**) wurden synthetisiert. Deren Struktur wurde durch Elementaranalyse und spektroskopische Daten bestimmt. Die Thrombocytenaggregationshemmung wurde getestet.

A number of reports indicate that compounds containing the 1,2,4-triazole unit possess antiinflammatory[1,2], sedative, smooth muscle relaxation[3,4], anticonvulsant[5], diuretic[6], antituberculosis[7], and platelet aggregation inhibitory[8] activities.

After 1980, 2-[(4-phenyl-5-aryl-4*H*-1,2,4-triazole-3-yl)thio]acetic acid and their esters[9,10], 2-propionic acid and their esters[10] have been shown to possess analgesic and antiinflammatory activities. In view of these results, we synthesized a set of related structures with 6-benzylidenethiazolo[3,2-*b*]-1,2,4-triazol-5(6*H*)-one and 6-(α-aminobenzyl)thiazolo[3,2-*b*]-1,2,4-triazol-5-ol (Schemes 1 and 2).

Condensation of 3-methyl-5-mercapto-1,2,4-triazole (**1**) with benzaldehyde and chloroacetic acid, respectively, in the presence of acetic acid and acetic anhydride gave the 2-methyl-6-benzylidenethiazolo[3,2-*b*]-1,2,4-triazol-5(6*H*)-ones **2a-d**[11,12].

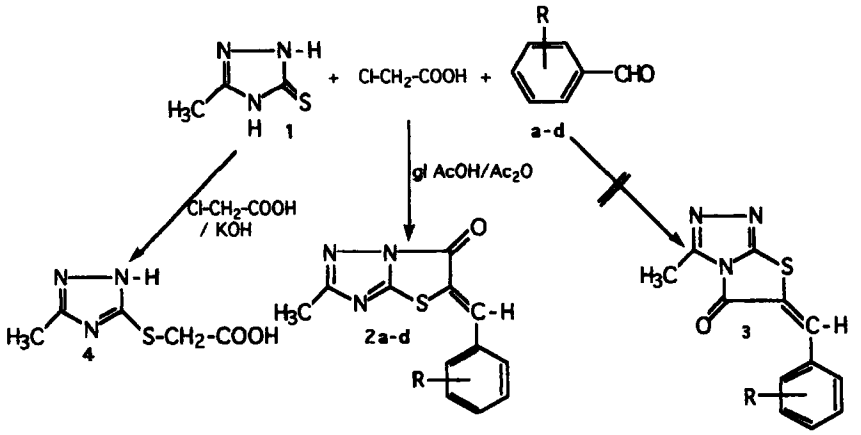
Michael addition of cyclic sec. amines to **2** then gave 6-(α-aminobenzyl)thiazolo[3,2-*b*]-1,2,4-triazol-5-ols (Scheme 2)[13-16].

Results and Discussion

Formation of **2** from compound **1** under similar conditions was described. On the other hand, compound **3** can also be obtained depending on the reaction conditions (Scheme 1)[17].

The IR spectra of **2** or **3** show a peak at 1720-1740 cm^{-1} (lactam carbonyl)[11,12], but they are not of much help in deciding between **2** or **3**.

Structural assignment for the cyclization product as **2**, not as **3** was achieved by comparing the chemical shift of the methyl protons of the cyclized product **2** with those of the noncyclic compound **4**. In compound **4**, CH_3 resonates at $\delta = 2.3$ ppm. If the structure were **3**, the methyl signal would resonate at a much lower field (0.55 ppm) due to the deshielding effect of the carbonyl group[18]. On the other hand, when the structure is **2**, the methyl protons resonate almost at the same position as the methyl protons of compound **4**. The methyl singlet of comp. **2** showed up at 2.35 ppm whereas the methyl singlet of comp. **4** appeared at 2.3 ppm. This indicates that the methyl signal at 2.35 ppm belongs to comp. **2**.



R : a: H ; **b:** 4-CH_3 ; **c:** 4-OCH_3 ; **d:** 3-NO_2

Scheme 1

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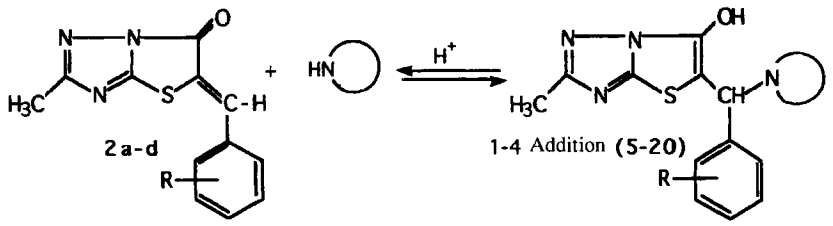
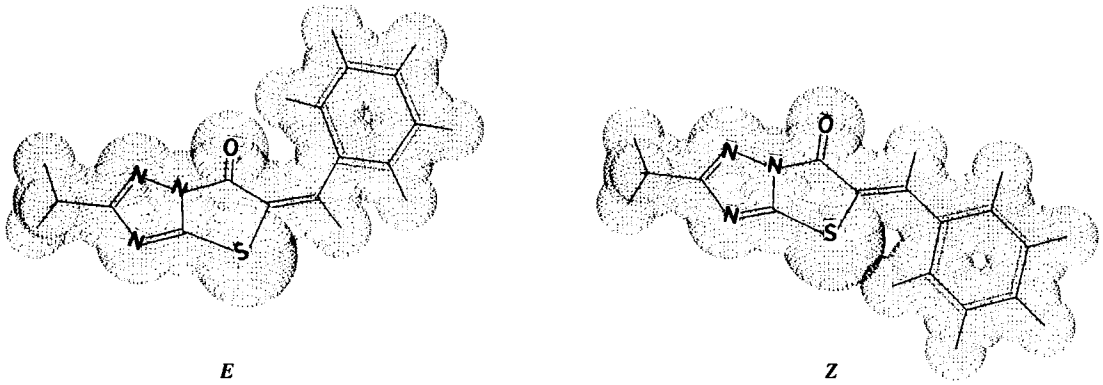


Scheme 2



E *Z*

Figure 1

Since in TLC studies compounds **2a-d** showed only one spot and the benzylidene-H of compounds **2a-d** resonate as a singlet at 8.1 ppm, it was decided that one geometric isomer only was obtained. A molecular modeling study was carried out with Hyper Chem version 3 (Polak-Ribiere PM 3). This study proved that the *E* and *Z* configurations are not energetically similar (For *E* = 41.26 kcal/mol the gradient is 0.0096 and for *Z* = 43.91 kcal/mol the gradient is 0.0096). It is seen that the *E* isomer has less energy than the *Z* isomer (Fig. 1). These results indicated that *E*-configuration of compounds **2a-d** were obtained. X-ray studies cannot be performed easily because of the presence of the sulphur atom.

Michael type addition of cyclic sec. amines (pyrrolidine, morpholine, piperidine, *N*-methylpiperazine) to **2** gave 2-methyl-6-(α-aminobenzyl)thiazolo[3,2-*b*]-1,2,4-triazol-5-ols **5-20**. The IR-spectra of **5-20** show an absorption at 3150 cm^{-1} (O-H of enol form) instead of a peak at 1720 cm^{-1} (lactam carbonyl). This is supported by ^{1}H-NMR results (enolic OH peak at 13.9 ppm and no benzylic protons at 4 - 6.5 ppm). These spectral data indicate that 1,4-addition products are obtained by *Michael* addition (Scheme 2).

We tried to prepare HCl- or H_2SO_4-salts of compounds **5-20** in order to increase their water solubility. All attempts, however, were unsuccessful due to *retro-Michael* reaction.

The antiaggregating activity was assayed on human platelets stimulated by collagen, ADP, U 46619, and thrombin. Tables 1 and 2 show the results obtained with each compound. ASA (Acetylsalicylic acid), tested under identical conditions, was used as a standard. It would appear from these results that the 2-methyl-6-benzylidenethiazolo[3,2-

Tab. 1: Antiplatelet activity of compounds **2a-d**

Agonist	Collagen 10^{-6} g/l		ADP $2{\cdot}10^{-6}$ M		U 46619 $5{\cdot}10^{-7}$		Thrombin
Comp. No. (mg/ml)	**0.1**	**0.01**	**0.1**	**0.01**	**0.1**	**0.01**	**0.1**
2a	51*	8	11	3	0	0	9
2b	23	4	4	0	4	5	16
2c	55	23	33	4	0	0	0
2d	47	18	8	0	0	0	0
ASA	86	40	13	0	0	0	10

*: % Inhibition

Tab. 2: Antiplatelet activity of compounds **5-2**

Agonist	Collagen 10^{-6} g/l			ADP $2 \cdot 10^{-6}$ M		U 46619 $5 \cdot 10^{-7}$ M			Thrombin
Comp. No. (mg/ml)	0.1	0.0316	0.01	0.1	0.01	0.1	0.0316	0.01	0.1
5	42*	3	0	8	0	36	2	0	18
6	24	7	0	5	0	19	6	0	6
7	41	5	0	2	0	12	2	0	11
8	38	4	0	3	0	37	7	0	6
9	16	2	0	2	0	6	0	0	14
10	22	2	0	5	0	14	5	0	24
11	37	5	0	5	0	18	1	0	24
12	27	3	0	3	0	9	10	0	18
13	49	4	0	9	0	12	3	0	15
14	44	2	0	7	0	13	4	0	6
15	47	6	0	10	0	10	1	0	17
16	38	7	0	1	0	28	7	0	14
17	52	6	0	14	0	51	15	0	36
18	49	4	0	20	0	43	6	0	29
19	67	27	0	18	0	35	11	0	26
20	60	12	0	2	0	53	4	0	28

*: % Inhibition

b]-1,2,4-triazol-5(6*H*)-ones **2a-d** were less active than ASA against aggregation induced by ADP and collagen at 0.1 mg/ml. The *Michael* products **5-20** showed anti-aggregating activity like ASA when the aggregation was induced by ADP and collagen. On the other hand, the same compounds were more active than ASA when aggregation was induced by U 46619 and thrombine. Additional pharmacological work will be required to confirm these results.

Experimental Part

Chemistry

Melting points: Capillaries, Thomas Hoover Unimeet apparatus, not corrected.- Elemental analyses: Hewlett Packard 185 CHN analyser.- IR spectra: Perkin Elmer 457 IR spectrometer.- ^{1}H-NMR spectra: Bruker FT-80 MHz spectrometer; $CDCl_3$ and [D_6]DMSO chemical shifts (δ) in ppm.- Mass spectra (70 eV): Zentrale Analytiker Fakultät Chemie und Pharmazie, Regensburg (Germany).- All chemicals were from Aldrich Chemical Company.

Procedure A

2-methyl-6-benzylidenethiazolo[3,2-b]-1,2,4-triazol-5(6H)-ones (**2**)

A mixture of 4 mmol of 3-methyl-5-mercapto-1,2,4-triazole (**1**), 6 mmol chloroacetic acid, 0.8 g of sodium acetate, 4 mmol benzaldehydes **a-d**, 6 ml of acetic anhydride and 8 ml of acetic acid was refluxed for 4 h. The mixture was poured into ice-water. The precipitate was separated and dissolved in dichloromethane. The org. layer was washed using 6% $NaHCO_3$ and brine, then the org. layer was evaporated under reduced pressure (Scheme 1).

Procedure B

2-Methyl-6-(α-aminobenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ols

0.5 mmol of 2-methyl-6-benzylidenethiazolo[3,2-*b*]-1,2,4-triazol-5(6*H*)-ones **2a-d** and 0.7 mmol of the corresponding amine were mixed in dry THF at room temp. The precipitated solid was crystallized by using an appropriate solvent (Scheme 2).

2-Methyl-6-benzylidenethiazolo[3,2-b]-1,2,4-triazol-5(6H)-one (**2a**)

Procedure A.- Yield 57%.- M.p. 204-205°C.- $C_{12}H_9N_3OS$ (243.3) Calcd. C 59.3 H 3.7 N 17.3 Found C 59.6 H 3.8 N 17.4.- IR (KBr): 1740 cm^{-1} (C=O).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.5 (s; 3H, CH_3-C=N), 6.9-7.3 (m; 5H, Ph), 8.1 (s; 1H, Ph-CH=).

2-Methyl-6-(4-methylbenzylidene)thiazolo[3,2-b]-1,2,4-triazol-5(6H)-one (**2b**)

Procedure A.- Yield 97%.- M.p. 205-206°C.- $C_{13}H_{11}N_3OS$ (257.3) Calcd. C 60.7 H 4.3 N 16.3 Found C 60.9 H 4.5 N 16.7.- IR (KBr): 1745 cm^{-1} (C=O).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.4 (s; 3H, CH_3-Ph), 2.5 (s; 3H, CH_3-C=N), 7.1-7.5 (m; 4H, Ph), 8.1 (s; 1H, Ph-CH=).

2-Methyl-6-(4-methoxybenzylidene)thiazolo[3,2-b]-1,2,4-triazol-5(6H)-one (**2c**)

Procedure A.- Yield 79%.- M.p. 208-209°C.- $C_{13}H_{11}N_3O_2S$ (273.3) Calcd. C 57.1 H 4.1 N 15.4 Found C 57.8 H 4.1 N 15.6.- IR (KBr): 1720 cm^{-1} (C=O).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.5 (s; 3H, CH_3-C=N), 3.8 (s;

3H, CH_3O-Ph), 7.0 (d; J = 8.7 Hz, 2H, Ph), 7.6 (d, J = 8.7 Hz, 2H, Ph), 8.1 (s; 1H, Ph-CH=).

2-Methyl-6-(3-nitrobenzylidene)thiazolo[3,2-b]-1,2,4-triazol-5(6H)-one (**2d**)

Procedure **A**.- Yield 76%.- M.p. 227-229°C.- $C_{12}H_8N_4O_3S$ (288.3) Calcd. C 50.0 H 2.8 N 19.4 Found C 50.1 H 2.8 N 19.5.- IR (KBr): 1720 cm^{-1} (C=O).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.5 (s; 3H, CH_3-C=N), 7.7-8.5 (m; 4H, Ph), 8.1 (s; 1H, Ph-CH=).

(∓)-2-Methyl-6-(α-N-pyrrolidinobenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**5**)

Procedure **B**.- Yield 83%.- M.p. 187-189°C.- $C_{16}H_{18}N_4OS$ (314.4) Calcd. C 61.1 H 5.8 N 17.8 Found C 60.9 H 5.8 N 17.9.- IR (KBr): 3120 cm^{-1} (O-H).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 1.6-2.0 (m; 4H, pyrr. 3-H_2 and 4-H_2), 2.3 (s; 3H, CH_3-C=N), 3.0-3.3 (m; 2H, pyrr. 2-H_2 or 5-H_2), 3.5-3.9 (m; 2H, pyrr. 5-H_2 or 2-H_2), 6.9 (s; 1H, Ph-CH-N), 7.3-7.7 (m, 5H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 314 (12; $M^{+\cdot}$), 281 (6), 245 (9), 244 (9), 243 (31), 115 (12), 70 (40), 44 (100).

(∓)-2-Methyl-6-(α-N-morpholinobenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**6**)

Procedure **B**.- Yield 69%.- M.p. 176-177°C.- $C_{16}H_{18}N_4O_2S$ (330.4) Calcd. C 58.2 H 5.5 N 17.0 Found C 58.0 H 5.5 N 17.1.- IR (KBr): 3120 cm^{-1} (O-H).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 2.3 (s; 3H, CH_3-C=N), 2.8-3.8 (m; 8H, morph.), 6.9 (s; 1H, Ph-CH-N), 7.3-7.7 (m; 5H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 331 (4), 330 (15; $M^{+\cdot}$), 297 (8), 245 (9), 244 (16), 243 (50), 115 (19), 86 (16), 43 (100).

(∓)-2-Methyl-6-[α-(N'-methyl-N-piperazino)benzyl]thiazolo[3,2-b]-1,2,4-triazol-5-ol (**7**)

Procedure **B**.- Yield 62%.- M.p. 193-195°C.- $C_{17}H_{21}N_5OS$ (343.5) Calcd. C 59.4 H 6.2 N 20.4 Found C 59.5 H 6.2 N 20.1.- IR (KBr): 3120 cm^{-1} (OH).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 2.2 (s; 3H, CH_3-N), 2.3 (s; 3H, CH_3-C=N), 3.7-4.3 (m; 8H, pip.), 6.9 (s; 1H, Ph-CH-N), 7.3-7.7 (m; 5H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 344 (3), 343 (16; $M^{+\cdot}$), 310 (3), 245 (8), 244 (49), 243 (17), 115 (13), 99 (32), 70 (100).

(∓)-2-Methyl-6-(α-N-piperidinobenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**8**)

Procedure **B**.- Yield 82%.- M.p. 170-173°C.- $C_{17}H_{20}N_4OS$ (328.4) Calcd. C 62.2 H 6.1 N 17.1 Found C 62.3 H 6.4 N 17.0.- IR (KBr): 3100 cm^{-1} (OH).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 1.2-1.8 (m; 6H, pip. 3-H_2, 4-H_2, 5-H_2), 2.3 (s; 3H, CH_3-C=N), 2.9-3.8 (m; 4H, pip. 2-H_2, 6-H_2), 6.9 (s; 1H, Ph-CH-N), 7.3-7.7 (m; 5H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 329 (2), 328 (7; $M^{+\cdot}$), 295 (10), 245 (6), 244 (16), 243 (70), 115 (19), 84 (100), 43 (97).

(∓)-2-Methyl-6-(α-N-pyrrolidino-4-methylbenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**9**)

Procedure **B**.- Yield 79%.- M.p. 195-198°C.- $C_{17}H_{20}N_4OS$ (328.4) Calcd. C 62.2 H 6.1 N 17.1 Found 61.9 H 6.3 N 16.7.- IR (KBr): 3120 cm^{-1} (OH).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 1.6-1.9 (m; 4H, pyrro. 3-H_2 and 4-H_2), 2.25 (s; 3H, CH_3-Ph), 2.3 (s; 3H, CH_3-C=N), 3.0-3.3 (m; 2H, pyrr. 2-H_2 or 5-H_2), 3.6-3.8 (m; 2H, pyrr. 5-H_2 or 2-H_2), 6.9 (s; 1H, Ph-CH-N), 7.2 (d; J = 8.4 Hz, 2H, Ph), 7.5 (d; J = 8.4 Hz, 2H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 329 (10), 328 (45; $M^{+\cdot}$), 259 (28), 258 (24), 257 (39), 214 (91), 115 (72), 70 (100), 43 (51).

(∓)-2-Methyl-6-(α-N-morpholino-4-methylbenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**10**)

Procedure B.- Yield 64%.- M.p. 162-165°C.- $C_{17}H_{20}N_4O_2S$ (344.4) Calcd. C 59.3 H 5.8 N 16.3 Found C 59.5 H 5.8 N 16.1.- IR (KBr): 3120 cm^{-1} (OH).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 2.3 (s; 6H, CH_3-C=N and CH_3-Ph), 3.4-3.7 (m; 8H, morph.), 6.9 (s; 1H, Ph-CH-N), 7.2 (d; J = 8.7 Hz, 2H, Ph), 7.5 (d; J = 8.7 Hz, 2H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 344 (4; $M^{+\cdot}$), 311 (2), 259 (10), 258 (28), 257 (100), 229 (17), 115 (35), 85 (35).

(∓)-2-Methyl-6-[α-(N'-methyl-N-piperazino)-4-methylbenzyl]thiazolo[3,2-b]-1,2,4-triazol-5-ol (**11**)

Procedure **B**.- Yield 66%.- M.p. 173-174°C.- $C_{18}H_{23}N_5OS$ (357.5) Calcd. C 60.5 H 6.5 N 19.6 Found C 60.1 H 6.6 N 19.4.- IR (KBr): 3140 cm^{-1} (OH).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 2.2 (s; 3H, CH_3-N), 2.3 (s; 6H, CH_3-C=N and CH_3-Ph), 3.0-3.8 (m; 8H, pip.), 6.9 (s; 1H, Ph-CH-N), 7.2 (d; J = 8.4 Hz, 2H, Ph), 7.5 (d; J = 8.4 Hz, 2H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 357 (3; $M^{+\cdot}$), 259 (8), 258 (27), 257 (100), 242 (13), 115 (22), 100 (36), 58 (88).

(∓)-2-Methyl-6-(α-N-piperidino-4-methylbenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**12**)

Procedure **B**.- Yield 77%.- M.p. 187-189°C.-$C_{18}H_{22}N_4OS$ (342.5) Calcd. C 63.1 H 6.5 N 16.4 Found C 62.9 H 6.4 N 16.5. -IR (KBr): 3120 cm^{-1} (OH).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 1.2-1.7 (m, 6H, pip. 3-H_2, 4-H_2, 5-H_2), 2.3 (s; 6H, CH_3-C=N and CH_3-Ph), 3.0-3.7 (m, 4H, pip. 2-H_2, 6-H_2), 6.8 (s; 1H, Ph-CH-N), 7.2 (d; J = 8.6 Hz, 2H, Ph), 7.5 (d; J = 8.6 Hz, 2H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 342 (17; $M^{+\cdot}$), 309 (28), 259 (15), 258 (15), 257 (21), 228 (98), 115 (40), 84 (100).

(∓)-2-Methyl-6-(α-N-pyrrolidino-4-methoxybenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**13**)

Procedure **B**.- Yield 92%.- M.p. 159-161°C.- $C_{17}H_{20}N_4O_2S$ (344.4) Calcd. C 59.3 H 5.8 N 16.3 Found C 59.0 H 6.1 N 16.1 .- IR (KBr): 3120 cm^{-1} (OH).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 1.7-2.0 (m; 4H, pyrro. 3-H_2 and 4-H_2), 2.3 (s; 3H, CH_3-C=N), 3.1-3.5 (m; 4H, pyrr. 2-H_2 and 5-H_2), 3.8 (s; 3H, CH_3O-Ph), 7.0 (m; 3H, Ph-CH-N and Ph), 7.6 (d; J = 8.7 Hz, 2H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 344 (2; $M^{+\cdot}$), 275 (7), 274 (17), 273 (100), 230 (12), 71 (25), 70 (16).

(∓)-2-Methyl-6-(α--N-morpholino-4-methoxybenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**14**)

Procedure **B**.- Yield 83%.- M.p. 158-160°C.- $C_{17}H_{20}N_4O_3S$ (360.4) Calcd. C 56.6 H 5.6 N 15.5 Found C 56.4 H 5.4 N 15.9.- IR (KBr): 3140 cm^{-1} (OH).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 2.3 (s; 3H, CH_3-C=N), 3.2-3.6 (m; 8H, morph.), 3.8 (s; 3H, CH_3O-Ph), 7.0 (m; 3H, Ph-CH-N and Ph), 7.6 (d; J = 7.7 Hz, 2H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 360 (2; $M^{+\cdot}$), 275 (7), 274 (17), 273 (100), 245 (10), 87 (60), 57 (8).

(∓)-2-Methyl-6-[α-(N'-methyl-N-piperazino)-4-methoxybenzyl]thiazolo[3,2-b]-1,2,4-triazol-5-ol (**15**)

Procedure **B**.- Yield 66%.- M.p. 166-167°C.- $C_{18}H_{23}N_5O_2S$ (373.5) Calcd. C 57.9 H 6.2 N 18.7 Found C 57.5 H 6.0 N 18.5.- IR (KBr): 3140 cm^{-1} (OH).- ^{1}H-NMR ([D_6]DMSO): δ (ppm) = 2.2 (s; 3H, CH_3-N), 2.3 (s; 3H, CH_3-C=N), 3.4-3.7 (m; 8H, pip.), 3.8 (s; 3H, CH_3O-Ph), 6.9 (s; 1H, Ph-CH-N), 7.0 (d; J = 10.0 Hz, 2H, Ph), 7.6 (d; J = 10.0 Hz, 2H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 373 (3; $M^{+\cdot}$), 275 (7), 274 (19), 273 (100), 159 (31), 100 (32), 58 (83).

(∓)-2-Methyl-6-(α-N-piperidino-4-methoxybenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**16**)

Procedure **B**.- Yield 67%.- M.p. 129-130°C.- $C_{18}H_{22}N_4O_2S$ (358.5) Calcd. C 60.3 H 6.2 N 15.6 Found C 60.0 H 6.4 N 15.5.- IR (KBr): 3120 cm^{-1} (OH).- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.3-1.7 (m, 6H, pip. 3-H$_2$, 4-H$_2$, 5-H$_2$), 2.3 (s; 3H, CH$_3$-C=N), 3.1-3.7 (m; 4H, pip. 2-H$_2$, 6-H$_2$), 3.8 (s; 3H, CH$_3$O-Ph), 6.8 (s; 1H, Ph-CH-N), 7.0 (d; J = 8.8 Hz, 2H, Ph), 7.6 (d; J = 8.8 Hz, 2H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 358 (2; $M^{+\cdot}$), 275 (7), 274 (17), 273 (100), 159 (35), 85 (31), 84 (52).

(∓)-2-Methyl-6-(α-N-pyrrolidino-3-nitrobenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**17**)

Procedure **B**.- Yield 81%.- M.p. 191-193°C.- $C_{16}H_{17}N_5O_3S$ (359.4) Calcd. C 53.5 H 4.8 N 19.5 Found C 53.9 H 4.8 N 19.3.- IR (KBr): 3100 cm^{-1} (OH).- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.6-1.9 (m; 4H, pyrr. 3-H$_2$ and 4-H$_2$), 2.3 (s; 3H, CH$_3$-C=N), 3.0-3.5 (m; 2H, pyrr. 2-H$_2$ or 5-H$_2$), 3.6-3.8 (m; 2H, pyrr. 5-H$_2$ or 2-H$_2$), 7.1 (s; 1H, Ph-CH-N), 7.6-8.5 (m; 4H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 359 (21; $M^{+\cdot}$), 290 (8), 289 (19), 288 (100), 115 (23), 71 (23), 70 (53), 43 (57).

(∓)-2-Methyl-6-(α-N-morpholino-3-nitrobenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**18**)

Procedure **B**.- Yield 59%.- M.p. 194-195°C.- $C_{16}H_{17}N_5O_4S$ (375.4) Calcd. C 51.2 H 4.6 N 18.7 Found C 50.9 H 4.4 N 18.3.- IR (KBr): 3120 cm^{-1} (OH).- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 2.3 (s; 3H, CH$_3$-C=N), 3.1-4.0 (m; 8H, morph.), 7.1 (s; 1H, Ph-CH-N), 7.6-8.6 (m; 4H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 375 (4; $M^{+\cdot}$), 290 (6), 289 (16), 288 (100), 115 (9), 87 (23), 57 (34).

(∓)-2-Methyl-6-[α-(N'-methyl-N-piperazino)-3-nitrobenzyl]thiazolo [3,2-b]-1,2,4-triazol-5-ol (**19**)

Procedure **B**.- Yield 68%.- M.p. 180°C (dec.).- $C_{17}H_{20}N_6O_3S$ (388.5) Calcd. C 52.6 H 5.2 N 21.6 Found C 52.2 H 5.4 N 21.8.- IR (KBr): 3120 cm^{-1} (OH).- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 2.2 (s; 3H, CH$_3$-N), 2.3 (s; 3H, CH$_3$-C=N), 3.0-3.8 (m; 8H, pip.), 7.0 (s; 1H, Ph-CH-N), 7.5-8.5 (m; 4H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 388 (5; $M^{+\cdot}$), 290 (7), 289 (16), 288 (100), 115 (23), 100 (31), 58 (42).

(∓)-2-Methyl-6-(α-N-piperidino-3-nitrobenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ol (**20**)

Procedure **B**.- Yield 86%.- M.p. 190-192°C. $C_{17}H_{19}N_5O_3S$ (373.4) Calcd. C 54.7 H 5.1 N 18.7 Found C 55.0 H 5.1 N 18.7.- IR (KBr): 3150 cm^{-1} (OH).- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.3-1.7 (m; 6H, pip. 3-H$_2$, 4-H$_2$, 5-H$_2$), 2.3 (s; 3H, CH$_3$-C=N), 3.1-3.7 (m; 4H, pip. 2-H$_2$, 6-H$_2$), 7.0 (s; 1H, Ph-CH-N), 7.6-8.5 (m; 4H, Ph), 13.9 (broad; 1H, OH).- MS m/z (%): 373 (5; $M^{+\cdot}$), 290 (8), 289 (17), 288 (100), 115 (10), 85 (39), 84 (68), 56 (21).

Screening on Platelet Aggregation Inhibitory Activity in vitro

Blood was withdrawn from the brachial vein of 4 healthy volunteers who had taken no medication for at least one week. The blood was collected into trisodium citrate solution (9 vol. of blood plus 1 vol. of 0.13 M trisodium citrate). Platelet rich plasma (PRP) was prepared by centrifugation of citrated blood at 210 x g for 10 min at room temp. Platelet poor plasma (PPP) was obtained by centrifugation at 3600 x g for 20 min. PRP and PPP were stored at room temp. in tightly capped polyethylene tubes for not more than 3 h. The final platelet concentration in PRP was measured with a cell counter (Mölab 8201 TA) and adjusted to 200 000/µl by diluting the PRP with PPP.

10 mg of the compounds were dissolved in 1 ml of DMSO except compounds **2a-d** which were solved in 1 ml DMSO and 100 µl N HCl and were introduced into the PRP under agitation at 37°C. After incubation, the inducing agent was added to the plasma medium. Platelet aggregation in PRP in response to various agonists was measured turbidimetrically at 37°C in a dual channel aggregometer (ELVI 840). All measurements were done in duplicate.

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