Synthesis, Characterization and Biological Studies of Some Bioactive Thiazolotriazole Derivatives

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The key precursor *rac*-2-(4-isobutylphenyl)ethyl-1,2,4-triazole-5-thione (**3**) was synthesized in good yield from Ibuprofen (**1**). One-pot three-component reactions of **3** with 5-aryl-furan-2-carboxaldehydes/substituted aromatic aldehydes and monochloroacetic acid in acetic acid in the presence of acetic anhydride and anhydrous sodium acetate afforded substituted thiazolo[3,2-*b*][1,2,4]triazole derivatives **4** and **5**. The structures of the newly synthesized compounds were elucidated by elemental analyses and spectral data. The compounds were tested for their *in-vitro* antimicrobial activities.

Key words: Ibuprofen, 1,2,4-Triazole-5-thione, Thiazolo[3,2-*b*][1,2,4]triazole, Antimicrobial Activity

Introduction

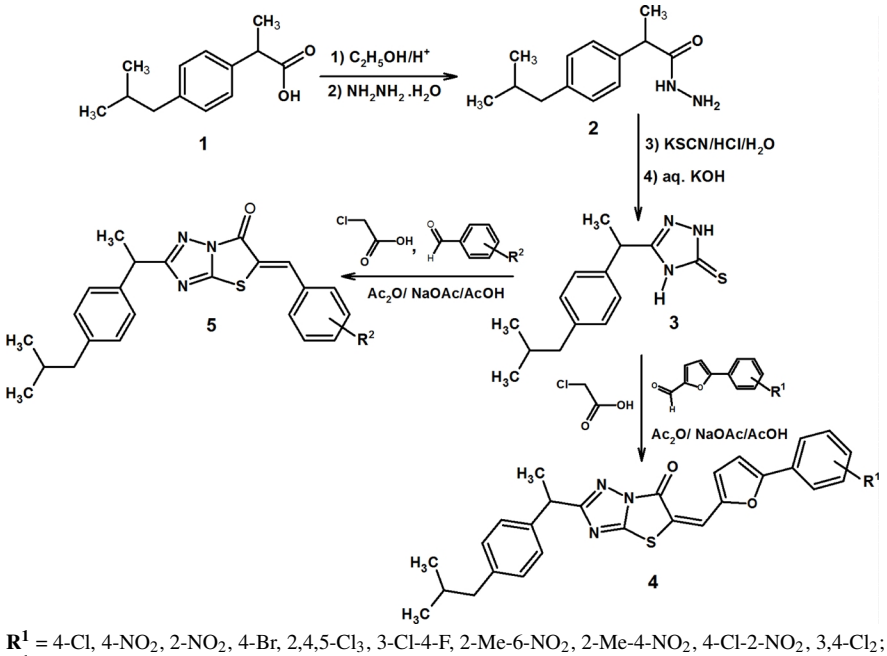
Non-steroidal anti-inflammatory drugs (NSAIDs) like Ibuprofen are widely used in the treatment of pain and inflammation, including osteoarthritis and rheumatoid arthritis [1 – 3]. The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis of arachidonic acid by inhibiting the cyclooxygenase enzyme (COXs) [4, 5]. Prolonged use of NSAIDs like ibuprofen has been associated with gastrointestinal (GI) complications ranging from stomach irritation to life-threatening GI ulceration bleeding and nephrotoxicity [6, 7]. Therefore, synthetic approaches based on chemical modifications of NSAIDs have been taken with the aim of improving their safety profile.

Substituted thiazoles have revealed anti-inflammatory, antimicrobial [8, 9], antitumor [10], anticonvulsant [11], analgesic [12], and anticancer [13] properties. 1,2,4-triazoles represent important pharmacophores and play a vital role as medicinal agents. 1,2,4-triazole derivatives due to their wide range of biological activities such as antimicrobial, anti-inflammatory [14], anticancer [15], antitubercular [16], and antimycotic [17] properties, have received considerable attention. The structural diversity and biological importance of 1,2,4-triazole-containing heterocycles have made them attractive targets for syntheses over many years. A large number of *N*-bridged heterocycles derived from 1,2,4-triazoles are important pharmacological agents, and a significant amount of research has been directed towards this class of compounds [18 – 20].

In the light of such observations, we herein report the synthesis of some fused bridgehead nitrogen heterocyclic systems, *viz.* substituted thiazolo[3,2-*b*]-[1,2,4]triazole derivatives incorporating the Ibuprofen moiety, and to screen them for antimicrobial activities.

Results and Discussion

The acid hydrazide *rac*-**2** was prepared by esterification of Ibuprofen followed by treatment with hydrazine hydrate in absolute alcohol [21, 22], and **2** converted into the corresponding thiosemicarbazide by treating it with potassium thiocyanate followed by cyclization with 5 % potassium hydroxide solution to yield 2-(4-isobutylphenyl)ethyl-1,2,4-triazole-5-thione **3** [15]. One-pot three-component condensation reactions [23 – 26] of **3** with monochloroacetic acid and 5-aryl-furan-2-carbaldehydes or substituted benzaldehydes in acetic acid in the presence of acetic anhydride and anhydrous sodium acetate afforded substituted thiazolo[3,2-



R^1 = 4-Cl, 4-NO_2, 2-NO_2, 4-Br, 2,4,5-Cl_3, 3-Cl-4-F, 2-Me-6-NO_2, 2-Me-4-NO_2, 4-Cl-2-NO_2, 3,4-Cl_2; **R**^{1} = 3,4-$(OCH_3)_2$, 3,4-$(OCH_3)_2$-5-NO_2, 4-OH, 3,5-F_2.

Comp.	4a	4b	4c	4d	4e	4f	4g
R^1	4-Cl	4-NO_2	2-NO_2	4-Br	2,4-Cl_2-5-F	3-Cl-4-F	2-Me-6-NO_2
R^2	–	–	–	–	–	–	–
Comp.	**4h**	**4i**	**4j**	**5a**	**5b**	**5c**	**5d**
R^1	2-Me-4-NO_2	2-NO_2-4-Cl	3,4-Cl_2	–	–	–	–
R^2	–	–	–	3,4-$(OMe)_2$	3,4-$(OMe)_2$-2-NO_2	4-OH	3,5-F_2

Scheme 1.

b][1,2,4]triazole derivatives **4** and **5** (Scheme 1). Substituted 5-aryl-furan-2-carbaldehydes were prepared through Meerwein reaction [23].

The structures of the newly synthesized compounds were confirmed by elemental analyses, IR, ^{1}H NMR and mass spectra. The data are presented in the Experimental Section. In the IR spectra of **3** the NH, C=S and C=N units displayed strong absorption bands in the regions around 3285, 1215, and 1608 cm^{-1}, respectively. The absorption bands associated with other functional groups appeared in the expected regions. ^{1}H NMR spectra of **3** showed a down-field D_2O-exchangeable broad singlet at δ = 12.64 ppm for its two tautomeric NH/SH protons. Further, an LC-MS spectrum of **3** showed the molecular ion peak at m/z = 262 which corresponds to its molecular formula $C_{14}H_{19}N_3S$.

The IR spectra of **4a**–**j** and **5a**–**d** displayed strong absorption bands in the regions 1725–1738 cm^{-1} (C=O) and 1598–1610 cm^{-1} (C=N), respectively. The absorption bands associated with other functional groups appeared in the expected regions. The absence of signals due to two protons of tautomeric NH/SH in the ^{1}H NMR spectra of **4a**–**j** and **5a**–**d** and the presence of a new singlet in the region δ = 7.81–8.34 ppm for the exocyclic methine proton of the thiazolidinone ring confirmed the transformation of **3** into the corresponding substituted thiazolo[3,2-*b*][1,2,4]-triazole derivatives **4a**–**j** and **5a**–**d**. Two distinct doublets observed for **4a**–**j** in their ^{1}H NMR spectra in the region δ = 6.82–7.00 ppm with J = 3.6 to 4.0 Hz (β-protons of the furan ring) also confirmed their structures. Further, in the ^{13}C NMR spectra, **4a**–**j** and **5a**–**d** showed their characteristic C=O carbon signal in the region δ = 176.82–176.98 ppm in addition to other characteristic signals of the remaining carbon atoms.

The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **4a**–**j** and **5a**–**d** exhibit moderate to good inhibition in DMSO. Compounds **4i** and **5c** showed comparatively good activity against all the bacterial strains, which can be attributed to the presence of pharmacologically active 4-chloro-2-nitroarylfuryl and 4-

hydroxy moieties attached to the thiazolotriazole ring. Compounds **4b**, **4c**, **4e**, **4j**, and **5b** showed moderate activity.

Compounds **4j** and **5c** showed comparatively good activity against all the fungal strains, which can be attributed to the presence of 3,4-dichloroarylfuryl and 4-hydroxy moieties attached to the thiazolotriazole ring. Compounds **4a**, **4b**, **4e**, **4f**, **4h**, **5a**, and **5b** exhibited moderate antifungal activity.

Conclusion

The present study reports the synthesis of thiazolotriazoles derived from Ibuprofen and the evaluation of their antimicrobial activities. The compounds exhibit moderate to good activity against all the pathogenic strains. Compound **5c** containing a 4-hydroxyphenyl group attached to the thiazolotriazole unit shows good antimicrobial activity.

Experimental Section

Instruments

The melting points were determined by an open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II-400 (400 MHz) spectrometer using TMS as an internal standard. Mass spectra were recorded on a Agilent Technology LC-mass spectrometer. Elemental analyses (CHNS) were performed on an CHNS Elementar Vario EL III. The progress of the reaction was monitored by thin-layer chromatography (TLC) on silica gel plates.

*rac-2-[4-Isobutylphenyl]propanehydrazide (**2**)*

A mixture of ethyl 2-(4-isobutylphenyl)propanoate (23.4 g, 0.1 mol) and hydrazine hydrate (9.3 mL, 0.2 mol) was refluxed in absolute alcohol (50 mL) for 8 h. The excess solvent was then distilled off under reduced pressure, and the concentrated solution was poured into ice cold water. The separated solid was filtered, washed and dried. The crude product was purified by recrystallization from ethanol.

M. p. 71 °C; yield 85 %. – IR (KBr, cm^{-1}): ν = 3449 (NH, NH_2), 3275 (NH_2), 2972 (C-H), 1695 (CO-NH) cm^{-1}. – ^{1}H NMR ($CDCl_3$): δ = 0.85 (d, J = 8 Hz, 6 H, 2× CH_3), 1.68 (d, J = 8 Hz, 3 H, CH_3), 1.80 – 1.88 (m, 1 H, CH), 2.45 (d, J = 4 Hz, 2 H, CH_2), 4.14 (q,J = 8 Hz, 1 H, CH), 4.0 (s, 2 H, NH_2), 7.5 (br s, 5 H, C_6H_4 and NH). – LC-MS: m/z = 220 [M]$^+$. – $C_{13}H_{20}N_2O$ (220.3): calcd. C 70.87, H 9.15, N 12.72; found C 70.85, H 9.13, N 12.70.

*rac-5[2-(4-Isobutyl)phenyl]ethyl-4H-1,2,4-trizole-3-thione (**3**)*

Propanehydrazide **2** (22 g, 0.1 mol) was dissolved in water (100 mL) containing concentrated hydrochloric acid (10 mL). Potassium thiocyanate (19.4 g, 0.2 mol) was added, and the mixture was warmed on a water bath for 5 h. The reaction mixture was cooled. The precipitated solid was filtered, dried and recrystallized from ethanol to get the aroyl thiosemicarbazide (m. p. 177 °C, yield 24.8 g (88 %)). A mixture of this aroyl thiosemicarbazide (27.9 g, 0.1 mol) and aqueous 5 % potassium hydroxide solution (100 mL) was refluxed 3 h. The reaction mixture was poured into crushed ice and acidified with dilute hydrochloric acid. The precipitate thus obtained was filtered, dried and recrystallized from ethanol.

M. p. 198 °C; yield 22.2 g (85 %). – IR (KBr, cm^{-1}): ν = 3100 (Ar-H), 2972 (C-H), 1215 (C=S) cm^{-1}. – ^{1}H NMR ($CDCl_3$): δ = 0.85 (d, J = 8 Hz, 6 H, 2× CH_3), 1.68 (d, J = 8 Hz, 3 H, CH_3), 1.80 – 1.88 (m, 1 H, CH), 2.45 (d, J = 4.0 Hz, 2 H, CH_2), 4.15 (q, J = 8.0 Hz, 1 H, CH), 697 (d, J = 8 Hz, 2 H, Ar-H), 706 (d, J = 8 Hz, 2 H, Ar-H), 12.64 (br-s, 2 H, NH/SH). – ^{13}C NMR ([D_6]DMSO): δ = 18.33, 18.78, 22.42, 30.28, 37.48, 126.13, 126.32, 128.25, 128.43, 136.20, 139.05, 161.05, 167.15. – LC-MS: m/z = 261 [M]$^+$. – $C_{14}H_{19}N_3S$ (261.3): calcd. C 64.31, H 7.33, N 16.08, S 12.27; found C 64.30, H 7.33, N 16.09, S 12.27.

*Procedure for the preparation of rac-2-(4-isobutylphenyl)-ethyl-5-[aryl/(5-arylfuran-2-yl)-methylidene]-[1,3]thiazolo[3,2-b][1,2,4]triazol-6(5H)-ones **4a** – **j** and **5a** – **d***

A mixture of the mercaptotriazole **3** (2.61 g, 0.01 mol), monochloroacetic acid (1.41 g, 0.015 mol), anhydrous sodium acetate (2 g, 0.024 mol), glacial acetic acid (20 mL), acetic anhydride (15 mL) and a 5-aryl-furan-2-carbaldehyde or substituted benzaldehyde (0.01 mol) was heated to reflux for 6 – 8 h. The reaction mixture was cooled and poured into crushed ice with vigorous stirring. The solid obtained was filtered, washed with water, dried and recrystallized from a mixture of ethanol and dimethyl formamide.

*rac-2-(4-Isobutylphenyl)ethyl-5-[(5-(4-chlorophenyl)furan-2-yl)methylidene]-[1,3]thiazolo[3,2-b][1,2,4]triazol-6(5H)-one (**4a**)*

M. p. 243 °C; yield 4.0 g (82 %). – IR (KBr, cm^{-1}): ν = 3028 (Ar-H), 2955 (C-H), 1726 (C=O), 162 (C=N), 839 (C-Cl). – ^{1}H NMR ([D_6]DMSO): δ = 0.88 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.52 (d, 3 H, CH_3, J = 7.6 Hz), 1.75 – 1.85 (m, 1 H, CH), 2.43 (d, 2 H, CH_2, J = 7.2 Hz), 4.31 – 4.33 (q, 1 H, CH, J = 7.2 Hz), 6.85 (d, 1 H, furan H, J = 4.0 Hz), 7.01 (d, 1 H, furan H, J = 4.0 Hz), 7.11 (d, 2 H, ArH, J = 8.4 Hz), 7.31 (d, 2 H, ArH, J = 8.4 Hz), 7.41 (d, 2 H, ArH, J = 8.0 Hz), 7.66 (d, 2 H, ArH, J = 8 Hz), 7.86 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D_6]DMSO): δ = 20.27, 22.84,

30.65, 40.58, 45.54, 109.88, 121.48, 123.16, 124.89, 126.48, 127.63, 127.79, 129.93, 136.01, 139.94, 140.96, 149.24, 156.75, 158.62, 160.79, 176.82. – LC-MS: m/z = 490 $[M]^+$, 492 $[M+2]^+$ in 3:1 ratio. – $C_{27}H_{24}N_3O_2ClS$ (489.12): calcd. C 66.18, H 4.94, N 8.58, S 6.54; found C 66.20, H 4.96, N 8.59, S 6.52.

*rac-2-(4-Isobutylphenyl)ethyl-5-[(5-(4-nitrophenyl)furan-2-yl)-methylidene]-[1,3]thiazolo[3,2-b][1,2,4]triazol-6(5H)-one (**4b**)*

M. p. 256 °C; yield 3.8 g (76 %). – IR (KBr, cm^{-1}): ν = 3102 (Ar-H), 2957 (C-H), 1729 (C=O), 1604 (C=N) – ^{1}H NMR ([D$_6$]DMSO): δ = 0.88 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.74 (d, 3 H, CH_3, J = 7.6 Hz), 1.79 – 1.86 (m, 1 H, CH), 2.43 (d, 2 H, CH_2, J = 7.2 Hz), 4.31 – 4.33 (q, 1 H, CH, J = 7.2 Hz), 6.86 (d, 1 H, furan H, J = 3.6 Hz), 7.02 (d, 1 H, furan H, J = 3.6 Hz), 7.10 (d, 2 H, ArH, J = 8.0 Hz), 7.28 (d, 2 H, ArH, J = 8.4 Hz), 7.50 (d, 2 H, ArH, J = 8.0 Hz), 7.64 (d, 2 H, ArH, J = 8.0 Hz), 7.87 (s, 1 H, exocyclic vinylic H) – ^{13}C NMR ([D$_6$]DMSO): δ = 20.28, 22.86, 30.66, 40.54, 45.58, 113.87, 122.38, 123.26, 124.769, 126.58, 127.52, 127.64, 129.62, 136.0, 139.94, 140.15, 145.20, 157.07, 159.46, 161.04, 176.92 – LC-MS: m/z = 501 $[M+1]^+$. – $C_{27}H_{24}N_4O_4S$ (500.5): calcd. C 64.78, H 4.83, N 1.19, S 6.41; found C 64.76, H 4.85, N 11.15, S 6.39.

*rac-2-(4-Isobutylphenyl)ethyl-5-[(5-(2-nitrophenyl)furan-2-yl)methylidene]-[1,3]thiazolo[3,2-b] [1,2,4]triazol-6(5H)-one (**4c**)*

M. p. 227 °C; yield 3.5 g (71 %). – IR (KBr, cm^{-1}): ν = 3082 (Ar-H), 2954 (C-H), 1731 (C=O), 161 (C=N). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.88 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.74 (d, 3 H, CH_3, J = 7.6 Hz), 1.79 – 1.86 (m, 1 H, CH), 2.43 (d, 2 H, CH_2, J = 7.2 Hz), 4.31 – 4.33 (q, 1 H, CH, J = 7.2 Hz), 6.86 (d, 1 H, furan H, J = 3.6 Hz), 7.02 (d, 1 H, furan H, J = 3.6 Hz), 7.10 (d, 2 H, ArH, J = 8.0 Hz), 7.28 (d, 2 H, ArH, J = 8.4 Hz), 7.5 – 7.8 (m, 4 H, ArH-NO_2), 7.87 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.28, 22.86, 30.66, 40.54, 45.58, 113.89, 122.48, 123.50, 124.69, 125.12, 125.76, 127.79, 129.85, 129.95, 130.63, 132.99, 137.70, 139.86, 140.97, 141.25, 148.17, 150.38, 153.95, 156.64, 160.90, 176.97 – LC-MS: m/z = 501 $[M+1]^+$. – $C_{27}H_{24}N_4O_4S$ (500.5): calcd. C 64.78, H 4.83, N 11.19, S 6.41; found C 64.77, H 4.85, N 11.16, S 6.39.

*rac-2-(4-Isobutylphenyl)ethyl-5-[(5-(4-bromophenyl)furan-2-yl)methylidene]-[1,3]thiazolo [3,2-b][1,2,4]triazol-6(5H)-one (**4d**)*

M. p. 233 °C; yield 4.1 g (78 %). – IR (KBr, cm^{-1}): ν = 3025 (Ar-H), 2954 (C-H), 1733 (C=O), 1611 (C=N), 881 (C-Br). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.88 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.76 (d, 3 H, CH_3, J = 7.2 Hz), 1.80 – 1.87 (m, 1 H, CH), 2.44 (d, 2 H, CH_2, J = 7.2 Hz), 4.30 – 4.35 (q, 1 H, CH, J = 7.2 Hz), 6.87 (d, 1 H, furan H, J = 3.6 Hz), 7.00 (d, 1 H, furan H, J = 3.6 Hz), 7.10 (d, 2 H, ArH, J = 5.6 Hz), 7.22 (d, 2 H, ArH, J = 5.6 Hz), 7.32 – 7.61 (m, 4 H, ArH-NO_2), 7.86 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.26, 22.84, 30.65, 40.58, 45.54, 109.10, 121.42, 123.12 124.74, 126.54, 127.72, 127.82, 129.96, 136.74, 140.02, 141.46, 149.84, 157.06, 159.64, 160.80, 176.86. – LC-MS: m/z = 534 $[M]^+$, 536 $[M+2]^+$ in 1:1 ratio. – $C_{27}H_{24}N_3O_2BrS$ (533.07): calcd. C 64.78, H 4.83, N 1.19, S 6.41; found C 64.77, H 4.85, N 11.16, S 6.39.

*rac-2-(4-Isobutylphenyl)ethyl-5-[(5-(2,4-dichloro-5-fluorophenyl)furan-2-yl)methylidene]-[1,3]thiazolo [3,2-b]-[1,2,4]triazol-6(5H)-one (**4e**)*

M. p. 258 °C; yield 4.4 g (79 %). – IR (KBr, cm^{-1}): ν = 3062 (Ar-H), 2986 (C-H), 1728 (C=O), 1599 (C=N). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.88 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.76 (d, 3 H, CH_3, J = 7.2 Hz), 1.80 – 1.87 (m, 1 H, CH), 2.44 (d, 2 H, CH_2, J = 7.2 Hz), 4.30 – 4.35 (q, 1 H, CH, J = 7.2 Hz), 6.87 (d, 1 H, furan H, J = 3.6 Hz), 7.00 (d, 1 H, furan H, J = 3.6 Hz), 7.10 (d, 2 H, ArH, J = 6.8 Hz), 7.22 (d, 2 H, ArH, J = 6.8 Hz), 7.41 (s, 1 H, ArH), 7.62 (s, 1 H, ArH), 7.89 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.32, 22.88, 30.68, 40.76, 45.60, 112.36, 122.67, 125.54, 125.96, 127.68, 128.24, 129.93, 140.14, 141.48, 151.62, 157.80, 159.84, 161.42, 176.96. – LC-MS: m/z = 542 $[M]^+$, 544 $[M+2]^+$, 546 $[M+4]^+$ in 9:6:1 ratio. – $C_{27}H_{22}N_3O_2Cl_2FS$ (541.07): calcd. C 59.78, H 4.09, N 7.75, S 5.91; found C 59.80, H 4.10, N 7.76, S 5.93.

*rac-2-(4-Isobutylphenyl)ethyl-5-[(5-(3-chloro-4-fluorophenyl)furan-2-yl)methylidene]-[1,3]thiazolo [3,2-b]-[1,2,4]triazol-6(5H)-one (**4f**)*

M. p. 249 °C; yield 4.3 g (85 %). – IR (KBr, cm^{-1}): ν = 3078 (Ar-H), 2983 (C-H), 1731 (C=O), 1599 (C=N). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.88 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.76 (d, 3 H, CH_3, J = 7.2 Hz), 1.80 – 1.87 (m, 1 H, CH), 2.44 (d, 2 H, CH_2, J = 7.2 Hz), 4.30 – 4.35 (q, 1 H, CH, J = 7.2 Hz), 6.87 (d, 1 H, furan H, J = 3.6 Hz), 7.00 (d, 1 H, furan H, J = 3.6 Hz), 7.08 (d, 2 H, ArH, J = 8.0 Hz), 7.12 (d, 2 H, ArH, J = 8 Hz), 7.35 – 7.58 (m, 3 H, ArH), 7.84 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.27, 22.84, 30.65, 40.58, 45.54, 109.91, 121.54, 123.32, 124.90, 126.72, 127.91, 129.64, 136.82, 139.98, 140.64, 149.02, 156.82, 158.94, 160.57, 176.83. – LC-MS: m/z = 508 $[M]^+$, 510 $[M+2]^+$ in 3:1 ratio. – $C_{27}H_{23}N_3O_2ClFS$ (507.11): calcd. C 63.84, H 4.56, N 8.27, S 6.31; found C 63.88, H 4.57, N 8.25, S 6.33.

*rac-2-(4-Isobutylphenyl)ethyl-5-[(5-(2-methyl-6-nitrophenyl)furan-2-yl)methylidene]-[1,3]thiazolo[3,2-b]-[1,2,4]triazol-6(5H)-one (**4g**)*

M. p. 239 °C; yield 4.4 g (87 %). – IR (KBr, cm^{-1}): ν = 3090 (Ar-H), 2978 (C-H), 1728 (C=O), 161 (C=N). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.88 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.36 (s, 3 H, CH_3), 1.76 (d, 3 H, CH_3, J = 7.2 Hz), 1.80 – 1.87 (m, 1 H, CH), 2.14 (s, 3 H, CH_3), 2.36 (d, 2 H, CH_2, J = 7.2 Hz), 2.61(s, 3 H, Ar-CH_3), 4.24 – 4.26 (q, 1 H, CH, J = 8.0 Hz), 6.86 (d, 1 H, furan H, J = 4.0 Hz), 7.02 – 7.05 (m, 3 H, 2 ArH and furan H), 7.23 (d, 2 H, ArH, J = 8.0 Hz), 7.41 (d, 2 H, ArH, J = 8.4 Hz), 7.87 (s, 1 H, exocyclic vinylic H), 8.08 (dd, 2 H, ArH, J = 8.4 Hz), 8.55 (d, 2 H, ArH, J = 8 Hz). – LC-MS: m/z = 514 (M^+). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.27, 20.68, 22.84, 30.68, 40.60, 45.60, 110.08, 121.26, 123.54, 124.90, 126.86, 127.08, 137.64, 139.74, 140.26, 149.68, 156.72, 159.68, 160.28, 176.88. – LC-MS: m/z = 515 $[M+1]^+$. – $C_{28}H_{26}N_4O_4S$ (514.16): calcd. C 65.35, H 5.09, N 10.89, S 6.23; found C 65.37, H 5.07, N 10.91, S 6.25.

*rac-2-(4-Isobutylphenyl)ethyl-5-[(5-(2-methyl-4-nitrophenyl)furan-2-yl)methylidene]-[1,3]thiazolo[3,2-b]-[1,2,4]triazol-6(5H)-one (**4h**)*

M. p. 263 °C; yield 3.7 g (72 %). – IR (KBr, cm^{-1}): ν = 3086 (Ar-H), 2985 (C-H), 1725 (C=O), 1602 (C=N). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.88 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.76(d, 3 H, CH_3, J = 7.2 Hz), 1.80 – 1.87 (m, 1 H, CH), 2.24 (s, 3 H, CH_3), 2.44 (d, 2 H, CH_2, J = 7.2 Hz), 4.30 – 4.35 (q, 1 H, CH, J = 7.2 Hz), 6.87 (d, 1 H, furan H, J = 3.6 Hz), 7.00 (d, 1 H, furan H, J = 3.6 Hz), 7.14 (d, 2 H, ArH, J = 8.0 Hz), 7.16 (d, 2 H, ArH, J = 8.0 Hz), 7.41 – 7.63 (m, 3 H, ArH), 7.86 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.27, 20.64, 22.84, 30.68, 40.60, 45.60, 110.14, 121.36, 123.60, 124.84, 126.53, 127.28, 137.56, 139.80, 140.32, 150.16, 156.78, 159.74, 160.72, 176.86. – LC-MS: m/z = 515 $[M+1]^+$. – $C_{28}H_{26}N_4O_4S$ (514.16): calcd. C 65.35, H 5.09, N 10.89, S 6.23; found C 65.36, H 5.10, N 10.91, S 6.25.

*rac-2-(4-Isobutylphenyl)ethyl-5-[(5-(4-chloro-2-nitrophenyl)furan-2-yl)methylidene]-[1,3]thiazolo[3,2-b]-[1,2,4]triazol-6(5H)-one (**4i**)*

M. p. 230 °C; yield 4.3 g (82 %). – IR (KBr, cm^{-1}): ν = 3072 (Ar-H), 2964 (C-H), 1727 (C=O), 1602 (C=N) – ^{1}H NMR ([D$_6$]DMSO): δ = 0.88 (d, 6 H, 2× CH_3,J = 6.4 Hz), 1.76 (d, 3 H, CH_3, J = 7.2 Hz), 1.80 – 1.87 (m, 1 H, CH), 2.44 (d, 2 H, CH_2, J = 7.2 Hz), 4.30 – 4.35 (q, 1 H, CH, J = 7.2 Hz), 6.87 (d, 1 H, furan H, J = 3.6 Hz), 7.00 (d, 1 H, furan H, J = 3.6 Hz), 7.14 (d, 2 H, ArH, J = 8.0 Hz), 7.16 (d, 2 H, ArH, J = 8.0 Hz), 7.41 – 7.63 (m, 3 H, ArH), 7.86 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.28, 22.86, 30.64, 40.60, 45.58, 110.20, 122.34, 123.95, 124.37, 126.84, 127.86, 137.06, 139.25, 140.50, 150.48, 156.84, 159.62, 160.92, 176.84. – LC-MS: m/z = 535 $[M]^+$, 537 $[M+2]^+$ in 3:1 ratio. – $C_{27}H_{23}N_4O_4ClS$ (534.11): calcd. C 60.61, H 4.33, N 10.47, S 5.99; found C 60.63, H 4.36, N 10.49, S 5.97.

*rac-2-(4-Isobutylphenyl)ethyl-5-[(5-(3,4-dichlorophenyl)-furan-2-yl)methylidene]-[1,3]thiazolo[3,2-b][1,2,4]triazol-6(5H)-one (**4j**)*

M. p. 265 °C; yield 3.9 g (76 %). – IR (KBr, cm^{-1}): ν = 3073 (Ar-H), 2963 (C-H), 1726 (C=O), 1605 (C=N). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.88 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.76 (d, 3 H, CH_3, J = 7.2 Hz), 1.80 – 1.87 (m, 1 H, CH), 2.44 (d, 2 H, CH_2,J = 7.2 Hz), 4.30 – 4.35 (q, 1 H, CH, J = 7.2 Hz), 6.87 (d, 1 H, furan H, J = 3.6 Hz), 7.00 (d, 1 H, furan H, J = 3.6 Hz), 7.14 (d, 2 H, ArH, J = 8.0 Hz), 7.16 (d, 2 H, ArH, J = 8.0 Hz), 7.41 – 7.63 (m, 3 H, ArH), 7.86 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.26, 22.84, 30.66, 40.61, 45.58, 110.24, 122.36, 124.42, 125.70, 126.80, 127.94, 137.74, 139.46, 140.84, 150.62, 156.92, 159.84, 160.28, 176.92. – LC-MS: m/z = 524 $[M]^+$, 526 $[M+2]^+$, 526 $[M+4]^+$ in 9:6:1 ratio. – $C_{27}H_{23}N_3O_2Cl_2S$ (523.08): calcd. C 61.83, H 4.42, N 8.01, S 6.11; found C 61.85, H 4.45, N 8.02, S 6.09.

*rac-2-(4-Isobutylphenyl)ethyl-5-(3,4-dimethoxybenzylidene)-[1,3]thiazolo[3,2-b][1,2,4]triazol-6(5H)-one (**5a**)*

M. p. 158 °C; yield 3.5 g (80 %). – IR (KBr, cm^{-1}): ν = 3048 (Ar-H), 2924 (C-H), 173 (C=O), 1600 (C=N). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.81 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.68 (d, 3 H, CH_3, J = 7.6 Hz), 1.71 – 1.79 (m, 1 H, CH), 2.36 (d, 2 H, CH_2, J = 7.2 Hz), 3.86 – 3.87 (2s, 6 H, 2× OC H 3), 4.11 – 4.17 (q, 1 H, CH, J = 7.2 Hz), 6.92 (d, 2 H, ArH, J = 8.4 Hz), 6.99 (d, 2 H, ArH, J = 8.4 Hz), 7.03 (d, 2 H, ArH, J = 8.0 Hz), 7.01 – 7.04 (dd, 2 H, ArH, J = 8.4 Hz), 7.22 (d, 2 H, ArH, J = 8.4 Hz), 8.05 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.31, 22.89, 30.65, 40.63, 45.54, 56.48, 56.65, 112.08, 113.57, 121.5, 125.83, 125.95, 127.74, 129.92, 139.90, 140.94, 141.94, 141.05, 150.04, 152.84, 156.97, 160.12, 176.84. – LC-MS: m/z = 449 $[M^+]$. – $C_{25}H_{27}N_3O_3S$ (449.56): calcd. C 66.79, H 6.05, N 9.35, S 7.13; found C 66.80, H 6.08, N 9.37, S 7.11.

*rac-2-(4-Isobutylphenyl)ethyl-5-(3,4-dimethoxy-5-nitrobenzylidene)-[1,3]thiazolo[3,2-b][1,2,4] triazol-6(5H)-one (**5b**)*

M. p. 165 °C; yield 3.8 g (78 %). – IR (KBr, cm^{-1}): ν = 3051 (Ar-H), 2966 (C-H), 1727 (C=O), 1602 (C=N). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.81 (d, 6 H, 2× CH_3,J = 6.4 Hz), 1.68 (d, 3 H, CH_3, J = 7.6 Hz), 1.71 – 1.79 (m,

Table 1. Antibacterial activity[a] of **4a – j** and **5a – d**.

Compound	MIC (μg mL^{-1}) and zone of inhibition (mm) in parentheses			
	S. aureus	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
4a	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
4b	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
4c	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
4d	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
4e	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
4f	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
4g	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
4h	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
4i	6.25 (20 – 26)	6.25 (16 – 20)	6.25 (16 – 20)	6.25 (16 – 20)
4j	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
5a	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
5b	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
5c	6.25 (20 – 26)	6.25 (16 – 20)	6.25 (16 – 20)	6.25 (16 – 20)
5d	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
Ampicillin (Standard)	1.56 (22 – 30)	6.25 (30 – 40)	6.25 (25 – 33)	6.25 (23 – 27)

[a] The MIC values were evaluated in the concentration range, 1.56 – 25 μg mL^{-1}.

Table 2. Antifungal activity[a] of **4a – j** and **5a – d**.

Compound	MIC (μg mL^{-1}) and zone of inhibition (mm) in parentheses			
	A. flavus	*A. fumigatus*	*P. marneffei*	*T. mentagrophytes*
4a	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
4b	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
4c	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
4d	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
4e	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
4f	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
4g	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
4h	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
4i	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
4j	6.25 (22 – 28)	6.25 (16 – 20)	6.25 (16 – 20)	6.25 (16 – 20)
5a	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
5b	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)	12.5 (11 – 15)
5c	6.25 (20 – 26)	6.25 (16 – 20)	6.25 (16 – 20)	6.25 (16 – 20)
5d	25 (< 10)	25 (< 10)	25 (< 10)	25 (< 10)
Itraconozole (Standard)	1.56 (22 – 30)	6.25 (30 – 40)	6.25 (25 – 33)	6.25 (23 – 27)

[a] The MIC values were evaluated in the concentration range, 1.56 – 25 μg mL^{-1}.

1 H, CH), 2.36 (d, 2 H, CH_2, J = 7.2 Hz), 3.86 – 3.87 (2s, 6 H, 2× OCH_3), 4.11 – 4.17 (q, 1 H, CH, J = 7.2 Hz), 6.96 (d, 2 H, ArH, J = 8.4 Hz), 7.00 (d, 2 H, ArH, J = 8.4 Hz), 7.4 – 7.7 (m, 2 H, ArH), 8.25 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.31, 22.88, 30.64, 40.63, 45.54, 56.55, 56.72, 113.23, 115.64, 122.06, 126.96, 128.08, 130.62, 140.68, 142.62, 142.42, 151.12, 153.32, 158.28, 161.26, 176.88. – LC-MS: m/z = 494 [M]$^+$. – $C_{25}H_{26}N_4O_5S$ (494.56): calcd. C 60.71, H 5.30, N 11.33, S 6.48; found C 60.73, H 5.32, N 11.36, S 6.51.

*rac-2-(4-Isobutylphenyl)ethyl-5-(4-hydroxy-5-nitrobenzylidene)-[1,3]thiazolo[3,2-b][1,2,4]triazol-6(5H)-one (**5c**)*

M. p. 135 °C; yield 3.3 g (82 %). – IR (KBr, cm^{-1}): ν = 3034 (Ar-H), 2957 (C-H), 1736 (C=O), 164 (C=N). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.81 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.68 (d, 3 H, CH_3, J = 7.6 Hz), 1.71 – 1.79 (m, 1 H, CH), 2.36 (d, 2 H, CH_2, J = 7.2 Hz), 4.11 – 4.17 (q, 1 H, CH, J = 7.2 Hz), 6.94 (d, 2 H, ArH, J = 8.4 Hz), 6.98 (d, 2 H, ArH, J = 8.4 Hz), 7.03 (d, 2 H, ArH, J = 8.0 Hz), 7.01 – 7.04 (dd, 2 H, ArH, J = 8.4 Hz), 7.22 (d, 2 H, ArH, J = 8.4 Hz), 8.92 (s, Ar-OH), 8.05 (s, 1 H, exocyclic vinylic H). – LC-MS: m/z = 406 [M$^+$+1]. – $C_{23}H_{23}N_3O_2S$ (405.51): calcd. C 68.12, H 5.72, N 10.36, S 7.91; found C 68.14, H 5.75, N 10.39, S 7.93.

*rac-2-(4-Isobutylphenyl)ethyl-5-(3,5-difluorobenzylidine)-[1,3]thiazolo[3,2-b][1,2,4]triazol-6(5H)-one (**5d**)*

M. p. 154 °C; yield 3.2 g (76 %). – IR (KBr, cm^{-1}): ν = 3074 (Ar-H), 2979 (C-H), 1728 (C=O), 161 (C=N). – ^{1}H NMR ([D$_6$]DMSO): δ = 0.81 (d, 6 H, 2× CH_3, J = 6.4 Hz), 1.68 (d, 3 H, CH_3, J = 7.6 Hz), 1.71 – 1.79 (m, 1 H, CH), 2.36 (d, 2 H, CH_2, J = 7.2 Hz), 4.11 – 4.17 (q, 1 H, CH, J = 7.2 Hz), 6.94 (d, 2 H, ArH, J = 8.4 Hz), 6.98 (d, 2 H, ArH, J = 8.4 Hz), 7.03 (d, 2 H, ArH, J = 8.0 Hz), 7.01 – 7.04 (dd, 2 H, ArH, J = 8.4 Hz), 7.22 (d, 2 H, ArH, J = 8.4 Hz), 8.05 (s, 1 H, exocyclic vinylic H). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.32, 22.82, 30.64, 40.65, 45.52, 112.25, 113.62, 121.47, 125.92, 125.92, 127.64, 139.76, 141.02, 149.88, 151.64, 156.06, 159.04, 176.86. – LC-MS: m/z = 426 [M+1]$^+$. – $C_{23}H_{21}N_3OF_2S$ (425.13): calcd. C 64.92, H 4.97, N 9.88, S 7.54; found C 64.94, H 4.99, N 9.89, S 7.57.

Antimicrobial activity

The newly synthesized compounds were screened for their antibacterial activity against *Staphylococcus aureus* (ATTC-25923), *Escherichia coli* (ATTC-25922), *Pseudomonas aeruginosa* (ATTC-27853), and *Klebsiella pneumoniae* (recultured) bacterial strains by the serial plate dilution method [27, 28]. Antibacterial activity was determined by measuring the diameter of the inhibition zone. The activity of each compound was compared with ampicillin as standard [29, 30]. The zone of inhibition was determined for **4a** – **j** and **5a** – **d**. The results are summarized in Table 1. The newly prepared compounds were also screened for their antifungal activity against *Aspergillus flavus* (NCIM No. 524), *Aspergillus fumigatus* (NCIM No. 902), *Penicillium marneffei* (recultured), and *Trichophyton mentagrophytes* (recultured) in DMSO by the serial plate dilution method [31, 32]. The antifungal activity was determined by measuring the diameter of the inhibition zone. The activity of each compound was compared with itraconozole as standard. Zones of inhibition were determined for **4a** – **n** and **5a** – **d**, and the results are summarized in Table 2.

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