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Synthesis and Biological Evaluation of Some Novel 3,5-Disubstituted-1,2,4-triazole Incorporated 2-Mercaptobenzothiazoles

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Abstract

Several 2-mercaptobenzothiazole derivatives **5a**–**i** containing 1,2,4-triazole moiety incorporating two additional substituents were synthesized. All the newly synthesized compounds were tested for *in vitro* activity against certain strains of bacteria such as *Enterococcus faecalis, Bacillus coagulans, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*. Compound **5a** showed significant activity against the Gram-negative bacteria *Escherichia coli*. Compounds **5a**–**i** were also screened for their antifungal activity against *Candida albicans* and compounds **5a**, **5b**, **5d** and **5g** displayed significant activity against this fungus. Some of these compounds were evaluated for their *in vivo* anti-inflammatory activity, acute toxicity and ulcerogenic actions. Tested compounds **5g** and **5h** showed significant anti-inflammatory activity and significant gastrointestinal protection compared to the standard drug diclofenac sodium. Molecular modeling studies of the synthesized compounds are presented.

Keywords: 2-Mercaptobenzothiazoles, 1,2,4-triazole, antimicrobial activity, anti-inflammatory activity, ulcerogenic effect.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs), one of the most commonly used class of medications to treat inflammation and pain, block two different cyclooxygenases (COX-1 and COX-2). COX-2, found in joint and muscle, contributes to the pain and inflammation.[1] Long term use of NSAIDs causes gastrointestinal (GI) disorders and renal toxicity[2,3] because they also block the COX-1 enzyme, which protects[4] the lining of the stomach from its acidic content. The introduction of the cyclooxygenase-2 (COX-2)-specific NSAIDs[5] in the late 1990s promised a revolution in NSAID therapy because of their much higher specificity for the COX-2 system, but unfortunately evidence of cardiovascular side effects including an increased risk of myocardial infarction began to emerge[6] causing these COX-2-specific NSAIDs to be withdrawn from the world market. Thus, the development of drugs with an effective anti-inflammatory profile, but with fewer side effects than NSAIDs, would be beneficial.

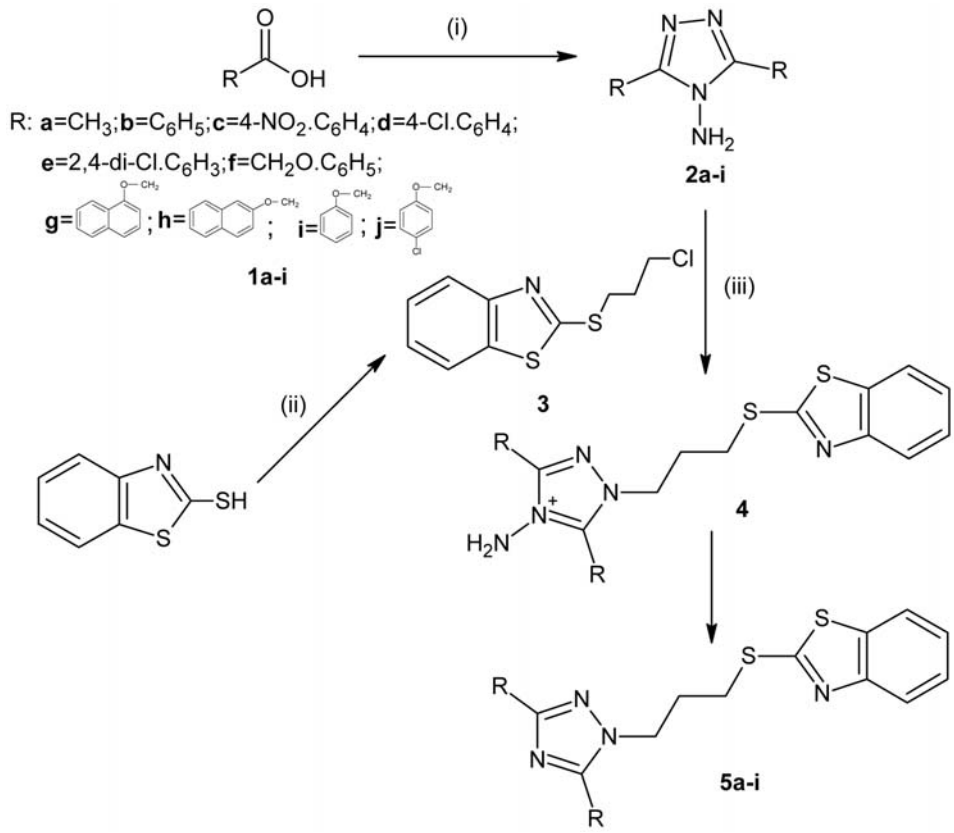
A literature survey revealed that compounds containing 1,2,4-triazole moiety possess a promising biological properties like antimicrobial[7,8] and anti-inflammatory.[9,10] In addition 2-mercaptobenzothiazoles are also known to posses antimicrobial[11,12] and anti-inflammatory activities.[13,14] Based on the above observations and results of our docking study it appeared of interest to link the benzothiazole nucleus at the second position to some 1,2,4-triazole ring system so as to bring them in the same framework; therefore attempting to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic effects that would increase the biological significance of the target molecules. In the present investigation we aimed to synthesize 2-{[3-(3,5-disubstituted-1*H*-1,2,4-triazol-1-yl)propyl]sulfanyl}-1,3-benzothiazoles **5a**–**i** to evaluate their antimicrobial, anti-inflammatory and ulcerogenic activities.

2. Results and Discussion

2. 1. Chemistry

Synthesis of the titled compounds **5a–i** was carried out as presented in the Scheme 1. Aryloxyacetic acids were prepared by reactions of appropriate phenols with chloroacetic acid in basic medium.[15] 4-Amino-3,5-disubstituted-1,2,4-triazoles[16] **2a–i** were synthesized by heating a mixture of the appropriate acid and hydrazine hydrate (85%) in an oil bath according to the procedure described in the literature. Intermediate 2-[(3-chloropropyl)sulfanyl]-1,3-benzothiazole (**3**) was synthesized[17] by stirring a mixture of 2-mercaptobenzothiazole and 1-bromo-3-chloropropane in dry toluene at 60 °C in the presence of powdered potassium carbonate. One-pot synthesis of the title compounds 2-{[3-(3,5-disubstituted-1*H*-1,2,4-triazol-1-yl)propyl]sulfanyl}-1,3-benzothiazoles **5a–i** via intermediate **4** was carried out by reactions[21] (described by Astleford *et al*. in 1989) of triazoles **2a–i** with compound **3** in refluxing isopropyl alcohol and subsequent treatment of the reaction mixture with conc. HCl, saturated sodium nitrite solution and saturated potassium carbonate solution.

Structures of the newly synthesized compounds were confirmed by the analytical and spectroscopic data. The infrared (IR) spectra of the synthesized compounds **5a–i** showed characteristic absorption bands in the range 1703–1637 cm^{-1} for C=N and 2935–2908 cm^{-1} for CH_2 stretching. The formation of the triazole ring in **5h** was supported by its proton magnetic resonance (^{1}H NMR) spectrum which showed three multiplet signals at δ 8.40–7.86, 7.57–7.41 and 7.27–6.98 ppm integrated for four, ten and four aromatic protons, respectively. Two triplet signals observed at δ 4.12 and 2.58 ppm were assigned to the OCH_2 and SCH_2 fragments, while a multiplet signal at δ 1.98–1.89 ppm was due to the CH_2 fragment of the propyl group linking the 2-mercaptobenzothiazole and 1,2,4-triazole rings. The OCH_2 fragments of 2-naphthoxymethyl groups present on the third and fifth position of the 1,2,4-triazole ring resonated as a singlet signal at δ 4.85 ppm. ^{13}C NMR spectra were recorded for compounds **5f** and **5h**. In the ^{13}C NMR spectrum of compound **5h** azomethine carbon of the benzothiazole ring exhibited a signal at δ 167.10 ppm, whereas 3-C and 5-C carbons of triazole nucleus were observed at δ 153.74 ppm. Three signals



Scheme 1. Synthetic route of 2-{[3-(3,5-disubstituted-1*H*-1,2,4-triazol-1-yl)propyl]sulfanyl}-1,3-benzothiazoles **5a-i**.

at δ 40.58, 31.28 and 21.82 ppm were assigned to the NCH_2, SCH_2 and CH_2 (propyl group) fragments, respectively. The signal at δ 66.98 ppm was assigned to the OCH_2 fragment of the 2-naphthoxymethyl group. Remaining carbon signals were observed at the expected chemical shift values. The mass spectrum of compound **5h** displayed **[**M^++2] peak at *m/z* 590 which is in agreement with the molecular formula $C_{34}H_{28}N_4O_2S_2$. A base peak was observed at *m/z* 94 due to the fragment $[C_6H_6N]^{+\cdot}$ and an intense peak at *m/z* 144 was assigned to the 2-naphthol fragment $[C_{10}H_7O]^{+\cdot}$, which is consistent with the structure of **5h**.

2. 2. Antimicrobial Activity

In vitro antimicrobial screening by the cup plate method[18] displayed moderate to weak inhibitory activity (inhibition zone 13–16 mm) of the tested compounds **5a**–**h** against Gram-positive bacterium *Enterococcus faecalis* whereas the rest of the compounds were found to be inactive against the same organism (Table 1). Tested compounds showed no activity against *Bacillus coagulans*. Compound **5a** having methyl group at the third and fifth position of the triazole ring showed significant inhibitory activity (inhibition zone 37 mm) against *Escherichia coli*, whereas the rest of the compounds showed a weak activity against the tested strains of Gram-negative bacteria. Compounds **5a**, **5b 5d** and **5f**–**h** exhibited significant inhibitory activity (inhibition zone 17–21 mm) against *Candida albicans*. In this regard compound **5a** having methyl group at the third and fifth position of the triazole ring showed maximum activity (inhibition zone 21 mm). From these result it is evident that substitution of small alkyl group at the third and fifth position of the triazole ring is optimal for activity against *E. coli* and *C. albicans*.

Table 1. Antimicrobial activity of 3,5-disubstituted-1,2,4-triazole incorporating 2-mercaptobenzothiazoles **5**.

Compound	Zone of inhibition (mm)[a,b]				
	E. f.	*B. c.*	*P. a.*	*E. c.*	*C. a.*
5a	–	–	11	37	21
5b	–	–	11	–	19
5c	–	–	11	–	13
5d	–	–	12	–	18
5e	16	–	12	–	16
5f	13	–	12	–	17
5g	14	–	12	–	18
5h	14	–	15	–	17
Ciprofloxacin	33	30	34	41	–
Ketoconazole	–	–	–	–	21
DMSO	–	–	–	–	–

Test compounds, ciprofloxacin and ketoconazole were tested at 100, 10 and 20 μg/mL concentration, respectively. [a] Average of three independent determinations. [b] – indicates no activity.
E. f.: Enterococcus faecalis; B. a.: Bacillus coagulans; P. a.: Pseudomonas aeruginosa; E. c.: Escherichia coli; C.a.: Candida albicans.

2. 3. Anti-inflammatory Activity

The anti-inflammatory activity results determined using the carrageenan induced paw oedema method[19] (described by Winter *et al.* in 1962) in rats are summarized in Table 2. From these results it is evident that the tested compounds showed moderate to weak activity (9.5–42.8% protection) at 0.5 and 1 h after carrageenan injection compared to the reference drug diclofenac sodium (29.9 and 49.3% at a dose of 20 mg/kg). At the second, third and fourth hour four compounds, namely **5f** and **5g**–**i** exhibited significant protection (51.1–67.4%) against carrageenan induced oedema. In this regard maximum activity (67.4% protection) was observed at the third hour for compound **5g** having 1-naphthyloxymethyl group at the third and fifth position of the triazole ring. It was observed that substitution with 1- or 2-naphthyloxymethyl groups (**5g** and **5h**) at the third and fifth position of the triazole ring enhances the activity, whereas the substitution with the methyl group resulted in a marked decrease in activity. Moreover, a marked decrease in anti-inflammatory activity was also observed when electron withdrawing NO_2 moiety was introduced in the phenyl rings (**5c**) attached to the third and fifth position of the 1,2,4-triazole ring.

2. 4. Ulcerogenic Effects

Synthesized compounds **5f**, **5g** and **5h** were tested for their ulcerogenic potential according to the method reported by Cioli *et al.*[20] The tested compounds showed low severity index (2.3 ± 0.3 to 3.5 ± 0.6) compared to the standard drug diclofenac sodium (severity index 4.4 ± 0.6). The maximum reduction in the ulcerogenic activity was found for compound **5h** (severity index 2.3 ± 0.3). The tested compounds **5f** and **5g** also exhibited better GI safety profile as compared to the standard drug diclofenac sodium (Table 3).

3. Experimental

3. 1. Chemistry

Reagents were of commercial grade and used as supplied. Melting points were determined in open glass capillaries and are uncorrected. The reaction progress and purity of the compounds were checked by thin-layer chromatography (TLC) on silica gel F_{254} plates from Merck. The IR spectra were recorded on KBr disks, using a Shimadzu 8400S FT-IR spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker AV-III 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C with DMSO-d_6 as the solvent. Chemical shifts are reported as δ ppm using the solvent as the internal standard. EI-MS were obtained on Jeol GC Mate II instrument. Elemental analyses (C, H, N) were carried out on a Flash EA 1112 series instrument and were within ±0.4% of calculated values.

Table 2. Anti-inflammatory activity of some selected 3,5-disubstituted-1,2,4-triazole incorporating 2-mercaptobenzothiazoles **5** by the carrageenan induced rat paw oedema method.

Compd.	Percent protection				
	30 min Mean % protection ±SEM	1 h Mean % protection ±SEM	2 h Mean % protection ±SEM	3 h Mean % protection ±SEM	4 h Mean % protection ±SEM
5a	10.4±0.8[ca]	28.3±0.7[b]	35.5±0.6[a]	37.2±0.8[a]	22.5±0.6[c]
5c	15.5±0.9[b]	34.1±0.9[b]	44.0±0.8[a]	46.3±0.6[b]	42.9±0.7[b]
5f	22.5±1.2[b]	42.8±0.7[b]	53.0±0.6[b]	62.3±0.5[a]	58.6±0.7[b]
5g	17.2±0.8[a]	37.7±0.5[a]	56.2±0.8[b]	67.4±1.1[b]	62.8±0.9[a]
5h	9.5±0.8[b]	32.0±1.1[a]	51.5±0.6[b]	62.4±0.5[b]	64.9±0.9[a]
5i	21.9±0.6[b]	38.3±0.9[b]	51.1±0.5[a]	59.6±0.8[b]	57.7±0.6[b]
Diclofenac sodium	29.9±1.2[c]	49.3±0.9[a]	61.1±1.2[b]	72.0±0.9[c]	71.1±1.2[b]

Test compounds **5** and diclofenac sodium were tested at 100 mg/kg and 20 mg/kg body weight, respectively. Result are expressed in mean ± SEM (n = 6). Significance levels: [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ compared with the respective control.

Table 3. Ulcerogenic effects of some selected 3,5-disubstituted-1,2,4-triazole incorporating 2-mercaptobenzothiazoles **5** by the Cioli's method.

Compound	Control 1% CMC	5f	5g	5h	Diclofenac sodium
Severity Index	0.23±0.9[b]	3.5±0.6[a]	2.7±0.8[c]	2.3±0.3[a]	4.4±0.6

[a] Test compounds **5** and diclofenac sodium were tested at 200 and 20 mg/kg body weight, respectively. Results are expressed in mean ± SEM (n = 6). Significance levels: [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ compared with the respective control.

Aryloxyacetic acids,[15] 4-amino-3,5-disubstituted-1,2,4-triazoles[16] and 2-[(3-chloropropyl)sulfanyl]-1,3-benzothiazole[17] were prepared as described in the literature.

General procedure for the synthesis of 2-{[3-(3,5-disubstituted-1*H*-1,2,4-triazol-1-yl)propyl]sulfanyl}-1,3-benzothiazoles 5a–i. A mixture of the appropriate 4-amino-3,5-disubstituted-1, 2, 4-triazole (10.5 mmol) and 3-chloroproplybenzothiazole (2.44 g, 10 mmol) in isopropyl alcohol (20 mL) was refluxed for 44–48 h. After completion of the reaction the excess solvent was removed under vacuum and 20 mL water was added to the reaction mixture. The content was cooled to 5 °C in an ice bath and conc. HCl (1.8 mL, 2.2 mmol) was added followed by a drop-wise addition of saturated aqueous sodium nitrite solution (3 mL, 11 mmol). The mixture was allowed to warm to the room temperature and then it was neutralized with saturated potassium carbonate solution. The separated solid was filtered, washed thoroughly with water, dried and recrystallized from an appropriate solvent to yield the title compounds **5a–i**.

2-{[3-(3,5-Dimethyl-1H-1,2,4-triazol-1-yl)propyl]sulfanyl}-1,3-benzothiazole (**5a**). Solvent of crystallization: acetone. Yield 55%, mp 118–120 °C. IR (KBr) ν: 3003, 2972, 2835, 1668, 756 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 7.63–7.17 (m, 4H, ArH), 4.21 (t, 2H, NCH_2), 2.58 (t, 2H, SCH_2), 2.12–2.01 (m, 2H, CH_2), 1.95 (s, 6H, 2×CH_3). MS: m/z 306 (M^++2), 274, 239, 225, 210, 190, 179, 166, 137, 121, 105, 91, 77. Anal. Calcd. for $C_{14}H_{16}N_4S_2$: C, 55.24; H, 5.30; N, 18.40. Found: C, 54.87; H, 5.27; N, 18.36.

2-{[3-(3,5-Diphenyl-1H-1,2,4-triazol-1-yl)propyl]sulfanyl}-1,3-benzothiazole (**5b**): Solvent of crystallization: acetone. Yield 62%, mp 188–190 °C. IR (KBr) ν: 3023, 2837, 1670, 1597, 742 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 8.34–7.79 (m, 4H, ArH), 7.63–7.39 (m, 10H, ArH), 4.01 (s, 2H, NCH_2), 2.75 (t, 2H, SCH_2), 2.02–1.96 (m, 2H, CH_2). MS: m/z 428 (M^+). Anal. Calcd. for $C_{24}H_{20}N_4S_2$: C, 67.26; H, 4.70; N, 13.07. Found: C, 67.31; H, 4.62; N, 13.12.

2-{[3,5-Bis(3,5-dinitrophenyl-1H-1,2,4-triazol-1-yl)propyl]sulfanyl}-1,3-benzothiazole (**5c**): Solvent of crystallization: acetone. Yield 50%, mp 130–132 °C. IR (KBr) ν: 3025, 2935, 1656, 1597, 1515, 1320, 750 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 8.37–7.87 (m, 6H, ArH),

7.74–7.48 (m, 4H, ArH), 4.34 (t, 2H, CH_2), 2.64 (t, 2H, SCH_2), 2.05–1.93 (m, 2H, CH_2). MS: *m/z* 609 (M^++1). Anal. Calcd. for $C_{24}H_{16}N_8O_8S_2$: C, 47.37; H, 2.65; N, 18.41. Found: C, 47.31; H, 2.58; N, 18.47.

2-({3-[3,5-Bis(4-chlorophenyl)-1H-1,2,4-triazol-1-yl]propyl}sulfanyl)-1,3-benzothiazole (**5d**): Solvent of crystallization: acetone. Yield 57%, mp 148–150 °C. IR (KBr) *v*: 3063, 2926, 1676, 1597, 1046, 738, 842 cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.23–7.85 (m, 4H, ArH), 7.68–6.98 (m, 8H, ArH), 3.98 (t, 2H, NCH_2), 2.51 (t, 2H, SCH_2), 1.99–1.91 (m, 2H, CH_2). MS: *m/z* 497 (M^+). Anal. Calcd. for $C_{24}H_{18}Cl_2N_4S_2$: C, 57.95; H, 3.65; N, 11.26. Found: C, 57.90; H, 3.58; N, 11.31.

2-({3-[3,5-Bis(2,4-dichlorophenyl)-1H-1,2,4-triazol-1-yl]propyl}sulfanyl)-1,3-benzothiazole (**5e**): Solvent of crystallization: acetone. Yield 65%, mp 154–156 °C. IR (KBr) *v*: 3063, 2935, 1663, 1597, 1034, 741 cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.34–7.78 (m, 6H, ArH), 7.64–7.42 (m, 4H, ArH), 4.12 (t, 2H, NCH_2), 2.63 (t, 2H, SCH_2), 2.08–2.01 (m, 2H, CH_2). MS: *m/z* 568 (M^++2). Anal. Calcd. for $C_{24}H_{16}Cl_4N_4S_2$: C, 50.90; H, 2.85; N, 9.89. Found: C, 50.82; H, 2.78; N, 9.92.

2-{[3-(3,5-Diphenoxymethyl-1H-1,2,4-triazol-1-yl)propyl]sulfanyl}-1,3-benzothiazole (**5f**):
Solvent of crystallization: chloroform and methanol (1:1). Yield 52%, mp 158–160 °C. IR (KBr) *v*: 3059, 2906, 1660, 1247, 1039, 748, 692, 735 cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.34–7.82 (m, 4H, ArH), 7.35–6.97 (m, 10H, ArH), 4.79 (s, 4H, 2×OCH_2), 3.94 (t, 2H, NCH_2), 2.54 (t, 2H, SCH_2), 2.07–1.99 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 167.1, 158.4, 152.0, 136.4, 129.9, 129.9, 127.0, 126.3, 125.1, 121.7, 121.1, 115.2, 66.4, 59.2, 41.2, 32.2, 23.3. MS: *m/z* 489 (M^++1). Anal. Calcd. for $C_{26}H_{24}N_4O_2S_2$: C, 63.91; H, 4.95; N, 11.47. Found: C, 63.89; H, 4.96; N, 11.50.

2-({3-[3,5-Bis(1-naphthoxymethyl)-1H-1,2,4-triazol-1-yl]propyl}sulfanyl)-1,3-benzothiazole (**5g**): Solvent of crystallization: chloroform and methanol (1:1). Yield 48%, mp 208 °C. IR (KBr) *v*: 3055, 2935, 1650, 1252, 1032, 736 cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.33–7.79 (m, 4H, ArH), 7.61–7.50 (m, 10H, ArH), 7.34–6.85 (m, 4H, ArH), 4.63 (s, 4H, 2×OCH_2), 4.25 (t, 2H, NCH_2), 2.57 (t, 2H, SCH_2), 2.11–2.02 (m, 2H, CH_2). MS: *m/z* 589 (M^++1). Anal. Calcd. for $C_{34}H_{28}N_4O_2S_2$: C, 69.36; H, 4.79; N, 9.52. Found: C, 69.33; H, 4.72; N, 9.49.

2-({3-[3,5-Bis(2-naphthoxymethyl)-1H-1,2,4-triazol-1-yl]propyl}sulfanyl)-1,3-benzothiazole (**5h**): Solvent of crystallization: chloroform and methanol (1:1). Yield 52%, mp 210 °C. IR (KBr) *v*: 3062, 2927, 1659, 1245, 1028, 738 cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.40–7.86 (m, 4H, ArH), 7.57–7.41 (m, 10H, ArH), 7.27–6.98 (m, 4H, ArH), 4.85 (s, 4H, 2×OCH_2), 4.12 (t, 2H, NCH_2), 2.58 (t, 2H, SCH_2), 1.98–1.89 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 167.1, 157.9, 153.7, 134.5, 127.8, 127.0, 126.8, 126.4, 125.7, 125.2, 123.6, 122.8, 122.6, 121.3, 121.2, 109.7, 106.2, 66.9, 40.5, 31.2, 21.8. MS: *m/z* 590 (M^++2), 456, 435, 407, 381, 280, 258, 231, 216, 198, 171, 157, 144, 129, 110, 94, 83, 77, 65. Anal. Calcd. for $C_{34}H_{28}N_4O_2S_2$: C, 69.36; H, 4.79; N, 9.52. Found: C, 69.34; H, 4.79; N, 9.48.

2-({3-[3,5-Bis(4-chlorophenoxymethyl)-1H-1,2,4-triazol-1-yl]propyl}sulfanyl)-1,3-benzothiazole (**5i**): Solvent of crystallization: ethanol and acetone (1:1). Yield 51%, mp 160 °C. IR (KBr) *v*: 3064, 2908, 1660, 1234, 1006, 742, 821 cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.37–7.78 (m, 4H, ArH), 7.52–6.76 (m, 8H, ArH), 4.67 (s, 4H, 2×OCH_2), 4.05 (t, 2H, NCH_2), 2.54 (t, 2H, SCH_2), 2.07–1.98 (m, 2H, CH_2). MS: *m/z* 559 (M^++2). Anal. Calcd. for $C_{26}H_{22}Cl_2N_4O_2S_2$: C, 56.01; H, 3.98; N, 10.05. Found: C, 55.97; H, 3.98; N, 10.11.

3. 2. Antimicrobial Activity

Antibacterial activity was evaluated on nutrient agar (Hi-media) plates (37 °C, 24 h) against *Enterococcus faecalis, Bacillus coagulans, Pseudomonas aeruginosa* and *Escherichia coli* by the cup plate method.[18] Test compounds were also evaluated[18] for their antifungal potential on Sabouraud dextrose agar (Hi-media) plates (26 °C, 48–72 h) against *Candida albicans.* Solutions of the test compounds, ciprofloxacin and ketoconazole were prepared in dimethylsulfoxide (DMSO) at the concentrations of 100, 10 and 20 μg/mL, respectively. The results (**Table 1**) were recorded as the average diameter of inhibition zones (three independent determinations) of bacterial or fungal growth around the disks and are given in mm.

3. 3. Pharmacological Activity

Animals were procured from the animal facility of the J. S. S. College of Pharmacy, Ootacamund, Tamil Nadu, India and were maintained in colony cages at 23±2 °C with relative humidity of 45–50% and under 12 h light and dark cycle. They were fed with the standard rat pellet diet (Hindustan Liver Ltd., Mumbai). Prior approval of the Local Animal Ethical Committee was obtained to carry out the experimental work on animals. The synthesized compounds **5a**, **5c**, **5f**, **5g**, **5h** and **5i** were evaluated for their acute toxicity and anti-inflammatory activities. Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Student's *t*-test to assess the statistical significance.

3. 3. 1. Acute Toxicity

Acute oral toxicity was performed for the synthesized compounds **5a**, **5c**, **5f**, **5g**, **5h** and **5i** following the Or-

ganization of Economic Cooperation and Development (OECD-423) guidelines (acute toxic class method). Swiss albino mice (n = 3) of either sex selected by random sampling were used for the study. The animals were fasted for 3–4 h with water *ad libitum,* after which the test compounds (suspension in 1% CMC) were administered orally at the doses of 50, 100, 250, 500 and 1000 mg/kg and the mice were observed for three days. No behavioral changes in animals were observed during the experiment and at the end hematological parameters were estimated and there was no observable change. In the present study, mortality was not observed even at 1000 mg/kg indicating that the compounds are nontoxic to animals.

3. 3. 2. Anti-inflammatory Activity

The acute anti-inflammatory activity results of the synthesized compounds was determined following the carrageenan induced paw oedema method[19] in Wistar albino rats (n = 6) of either sex (155–160 g). The animals were fasted for 24 h before the experiment with free access to water. The test compounds and diclofenac sodium were administered orally as suspension (1.0% w/v CMC solution). The control rats received appropriate volumes of CMC solution orally. Thirty minutes after administration of the test compounds, 0.1 mL carrageenan solution (1.0% w/v in sterile saline) was injected into the sub-plantar tissue of the right hind paw of each rat. The volume of the paw was measured at different time intervals of 0.5, 1, 2 and 3 h after the carrageenan injection by the means of plethysmometer (UGO Basile 7140, India). The percentage protection against inflammation was calculated by the following formula,

$$(Vc–Vt)/Vc \times 100 \quad (1)$$

where V_c is the oedema volume in control group and V_t is the oedema volume in groups treated with the test compounds. The anti-inflammatory activity results are summarized in Table 2.

3. 3. 3. Ulcerogenic Effects

The test compounds **5g** and **5i** were evaluated for their acute ulcerogenic effects according to the method of Cioli *et al.*[20] in Wistar albino rats (n = 6) of either sex. The test compounds and diclofenac sodium were administered orally as suspension in 1% carboxymethyl cellulose (CMC). Control group received appropriate volumes of 1% CMC. Food but not water was removed 24 h before administration of the test compounds. After compound treatment, the rats were fed with normal diet for 17 h and then sacrificed. Their stomachs were removed, cut out along the greater curvature and washed with distilled water and then gently cleaned by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. For each stomach the mucosal damage was assessed according to the following scoring systems: 0.5 redness; 1.0 spot ulcers; 1.5 haemorrhagic streaks; 2.0 ulcers >3 but ≤5; 3.0 ulcers >5. The mean score of each treated group minus the mean score of control group was regarded as the severity index (Table 3).

3. 3. 4. Docking studies of 2-{[3-(3,5-disubstituted-1*H*-1,2,4-triazol-1-yl)propyl]sulfanyl}-1,3-benzothiazoles

The type IV, cyclic AMP-specific phosphodiesterases (PDE4B)[22–24] is particularly abundant in immune and inflammatory cells, where an increase of cAMP leads to the inhibition of the synthesis and the release of pro-inflammatory mediators.[25] Due to their role in regulation of cell function, PDEs have become good clinical targets for the treatment of inflammation.[26] Benzothiazole nucleus linked to the small heterocyclic moiety[27] and triazole nucleus fused with the other heterocyclic system[28] is exploited as a PDE4 inhibitor. Combining some of the structural features of benzothiazole and triazole in a single molecule may lead to a new class of compounds which may be explored for the identification as novel PDE4B inhibitors. Prompted by this hypothesis we initially became interested in the evaluation of 2-{[3-(3,5-disubstituted-1*H*-1,2,4-triazol-1-yl)propyl]

Table 4. Docking study results of the 2-{[3-(3,5-disubstituted-1*H*-1,2,4-triazol-1-yl) propyl] sulfanyl}-1,3-benzothiazoles **5**.

Comp.	Glide score	Glide energy	E-1[a] (kcal/mol)	E-2[b] (kcal/mol)	E-3[c] (kcal/mol)	E-4[d] (kcal/mol)	RMSD
5h	–10.73	–57.67	–1.90	–7.63	–1.19	0	0.024
5i	–9.71	–55.08	–1.47	–7.35	–0.18	0	0.011
5g	–9.48	–51.49	–1.92	–7.85	0.08	0.21	0.011
5d	–8.80	–46.18	–1.47	–5.80	–0.29	0.21	0.021
5f	–8.61	–49.04	–1.22	–6.55	–0.24	0	0.028
5b	–8.37	–43.57	–1.87	–5.52	–0.17	0	0.033
5e	–8.16	–41.90	–1.82	–6.53	0.19	0	0.011
5c	–7.68	–52.85	–1.20	–5.51	–0.12	0	0.033
5a	–7.62	–33.59	–1.47	–4.21	–0.34	0.34	0.004

[a] Hydrophobic enclosure reward; [b] Lipophilic EvdW; [c] Electrostatic reward; [d] Rotational penalty

sulfanyl}-1,3-benzothiazoles (**5a–i**) for their potential affinities with respect to PDE4B through docking studies using the enzyme PDE4B co-crystallised with piclamilast[29] as the target. This complex was obtained from the RCSB protein data bank under the PDB code 1XM4.

The structures of the selected compounds **5a–i** are initially optimized using the Schrodinger Maestro version 9.2 software. The theoretical binding profile of each molecule was evaluated using Glide, version 5.7, Schrodinger, LLC, New York, NY, 2011 and the parameters such as the GLIDE scores, hydrophobic endurance reward, hydrophilic reward, RMSD, and penalties were obtained after docking of these molecules with PDE4B protein. The results are summarized in Table 4. The data clearly suggests that these molecules bind well with PDE4B. H-bonding interaction was observed in the case of compounds **5a–d**, **5f** and **5i**. In the case of the compound **5i** hydrogen bond interaction was observed between oxygen function of the one of the *p*-chlorophenoxymethyl residues and the magnesium metal ion mediated by a water molecule (Figure 1) whereas in compounds **5a** and **5d** hydrogen bonding was observed between the centre of the benzothiazole ring N(1) and the -NH_2 group of the Gln443 residue of the PDE4B protein (Figures 2 and 3) that is essential for nucleotide recognition and selectivity.[30] In compounds **5b** (phenyl) and **5c** (4-nitrophenyl) hydrogen bonding was observed between the thiol group present at the second position of the benzothiazole ring and the magnesium metal ion mediated by a water molecule (Figures 4 and 5). In both compounds a second hydrogen bond was also observed between the centre of the benzothiazole ring N(1) and the carbonyl group (C=O) of the Met347 residue, whereas in the compound **5f** hydrogen bonding was observed between oxygen function of one of the phenoxymethyl groups and a water molecule (Figure 6).

It is evident from the docking results (Table 4) that in compounds **5g** and **5h** one of the naphthyl rings is sandwiched in the hydrophobic clamp (hydrophobic enclosure reward –1.92 and –1.90 kcal/mol, respectively) (Figures 7 and 8) and the other naphthyl group is directed into the more capacious Q2 site adjacent to the methionine (Met431) at the entrance to the catalytic pocket, whereas in compounds **5b** and **5e**, phenyl or 2,4-dichlorophenyl groups are sandwiched by the hydrophobic clamp (hydrophobic enclosure reward –1.87 and –1.82 kcal/mol, respectively) (Figures 9 and 10). The remaining parts of

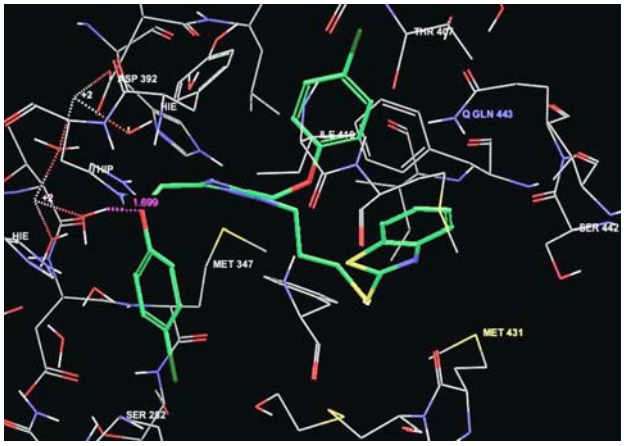


Figure 1. Docking of **5i** at the active site of PDE4B.

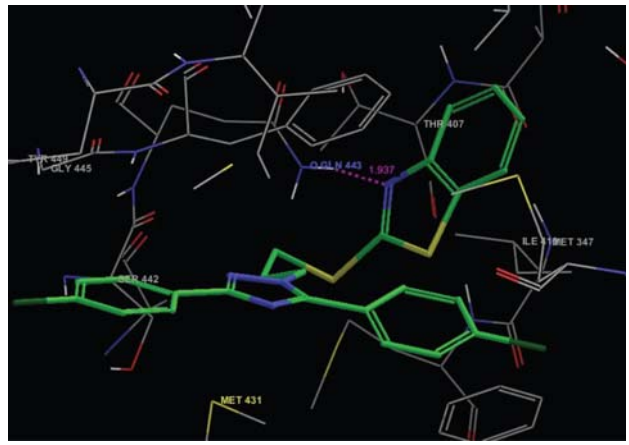


Figure 3. Docking of **5d** at the active site of PDE4B.

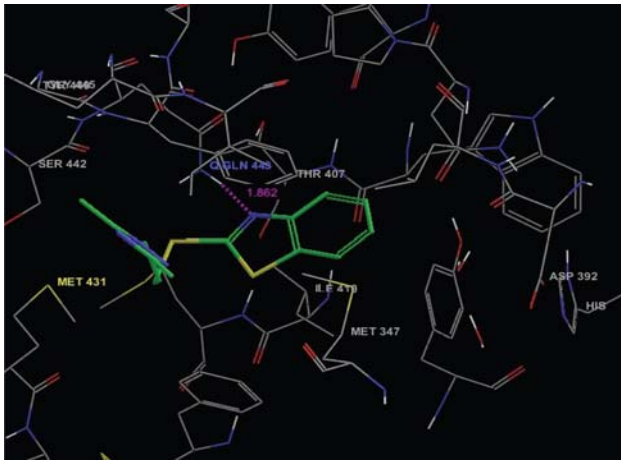


Figure 2. Docking of **5a** at the active site of PDE4B.

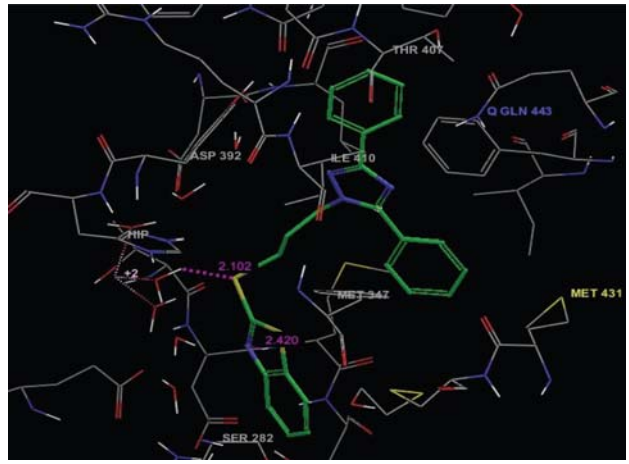


Figure 4. Docking of **5b** at the active site of PDE4B.

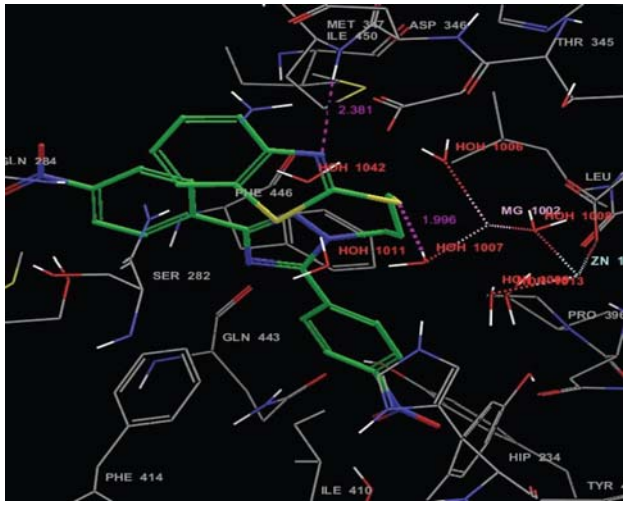


Figure 5. Docking of **5c** at the active site of PDE4B.

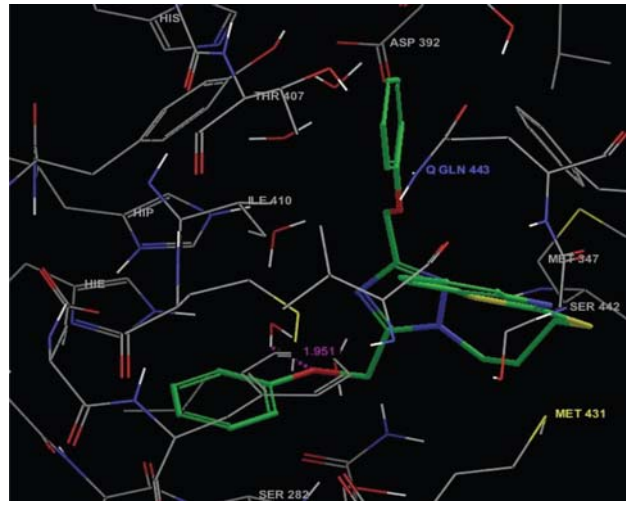


Figure 6. Docking of **5f** at the active site of PDE4B.

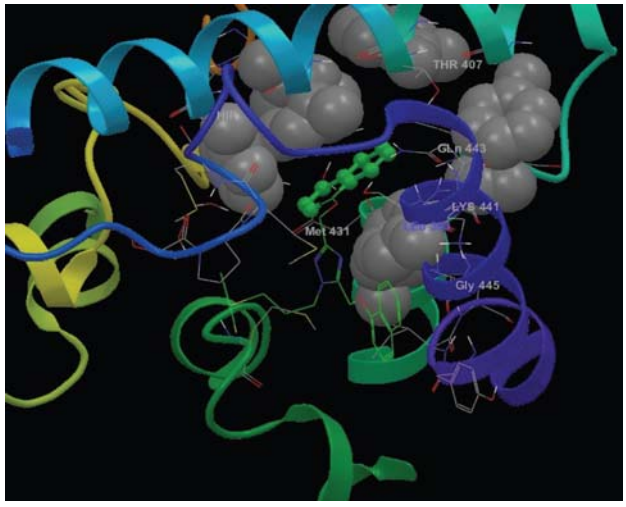


Figure 7. Docking of **5g** at the active site of PDE4B.

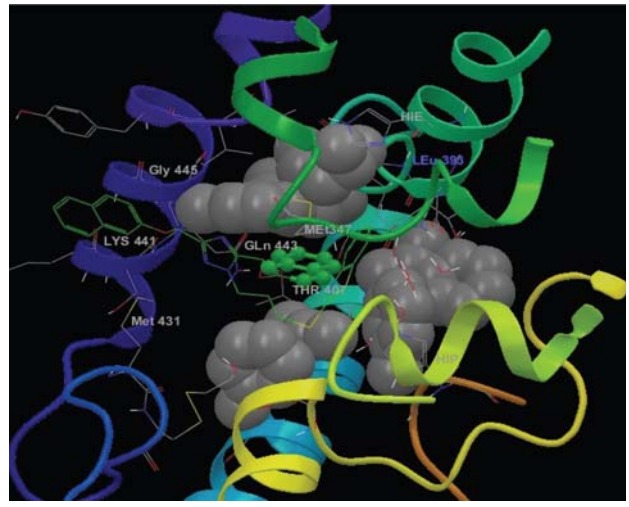


Figure 8. Docking of **5h** at the active site of PDE4B.

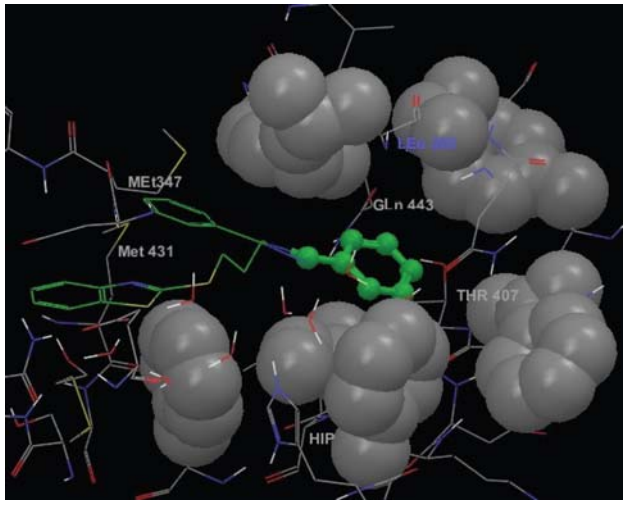


Figure 9. Docking of **5b** at the active site of PDE4B.

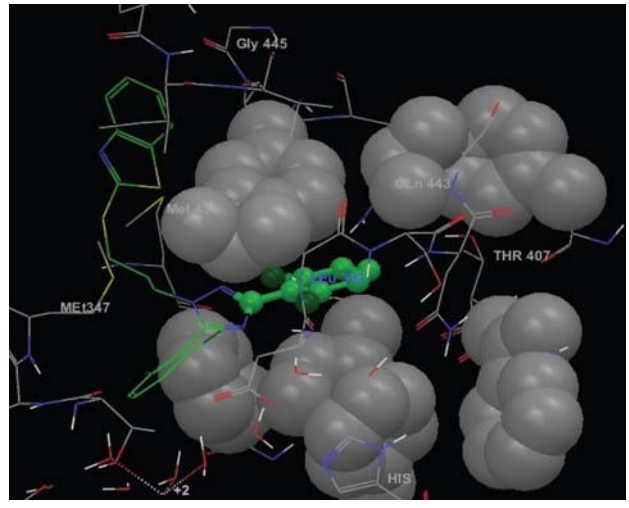


Figure 10. Docking of **5e** at the active site of PDE4B.

the molecules are shown to extend into the catalytic domain in close proximity to both the Zn^{2+} and Mg^{2+} cations. Such an orientation would block the approach of cAMP to the catalytic domain and forms the basis for inhibiting

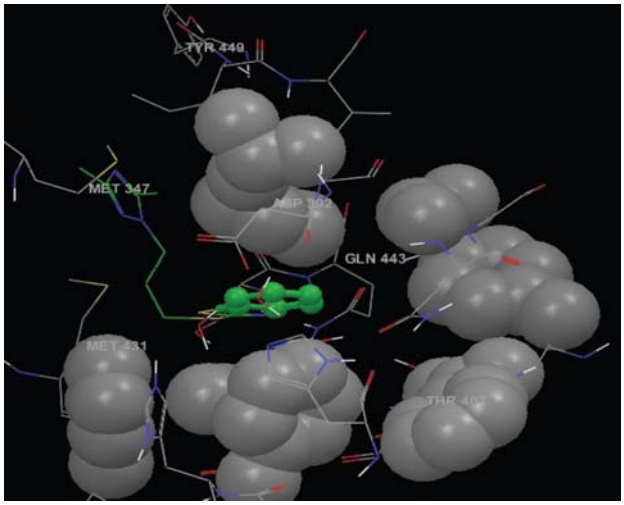


Figure 11. Docking of **5a** at the active site of PDE4B.

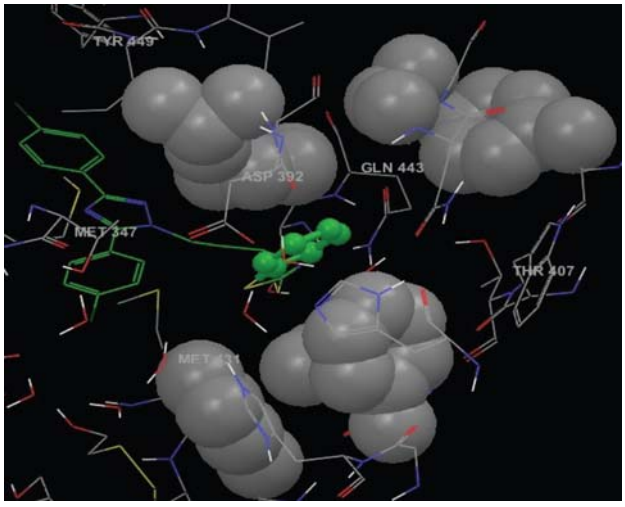


Figure 12. Docking of **5d** at the active site of PDE4B.

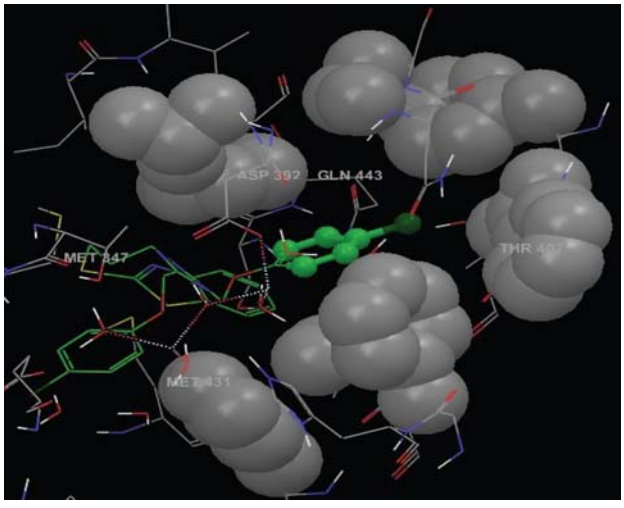


Figure 13. Docking of **5i** at the active site of PDE4B.

PDE4B. In the present investigation compound **5h**, having 2-naphthoxymethyl group at the third and fifth position of the 1,2,4-triazole ring, showed maximal Glide scores (–10.73 kcal/mol). This may be attributed to an improved fit of the ligand into the Q1 subpocket, as defined by Card *et al.*,[25] adjacent to the purine-scanning glutamine in the interior of the catalytic pocket.

In the compounds **5a** and **5d** benzothiazole ring (Figures 11 and 12) and in compound **5i** *p*-chlorophenoxymethyl group are sandwiched into the hydrophobic clamp (hydrophobic enclosure reward –1.47 kcal/mol, Figure 13). The remaining parts of the molecules are shown to extend into the catalytic domain in close proximity to both the Zn^{2+} and Mg^{2+} cations.

4. Conclusion

In the present investigation all the synthesized compounds **5** were found to be either weakly active or inactive against the tested strains of bacteria. Compound **5a** showed maximal inhibitory activity against *Candida albicans*. On the other hand, compounds **5g** and **5h** showed significant anti-inflammatory activity with significant reduction of gastrointestinal toxicity (severity index 2.7±0.8 and 2.3 ± 0.3, respectively) in an animal model and this correlates well with the docking study result (GLIDE scores –9.48 and –10.73 kcal/mol, respectively). Hence 2-({3-[3,5-bis(1/2-naphthoxymethyl)-1*H*-1,2,4-triazol-1-yl]propyl}sulfanyl)-1,3-benzothiazole (**5g** and **5h**) would represent a fruitful framework for the development of newer anti-inflammatory agents.

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Povzetek

Pripravili smo več 2-merkaptobenzotiazolnih derivatov **5a**–**i**, ki vsebujejo 1,2,4-triazolno enoto z vezanima dvema dodatnima substituentoma. Vse nove spojine smo *in vitro* testirali za morebitno aktivnosti proti določenim vrstam bakterij, kot so *Enterococcus faecalis, Bacillus coagulans, Pseudomonas aeruginosa, Escherichia coli* in *Candida albicans*. Spojina **5a** je pokazala opazno aktivnost proti Gram-negativni bakteriji *Escherichia coli*. Spojinam **5a**–**i** smo tudi določili morebitno delovanje proti glivam na primeru *Candida albicans*, spojine **5a**, **5b**, **5d** in **5g** so pokazale opazno aktivnost proti tej glivi. Za nekatere spojine smo tudi določili *in vivo* protivnetno aktivnost, akutno toksičnost in ulcerogeno aktivnost. Testirani spojini **5g** in **5h** sta pokazali pomembno protivnetno aktivnost in močno gastrointestinalno protekcijo v primerjavi s standardnim zdravilom diklofenak natrij. Predstavljene so tudi študije molekulskega modeliranja pripravljenih spojin.