

s-Triazolo[3,4-b][1,3,4]thiadiazoles, s-Triazolo[3,4-b][1,3,4]thiadiazines and s-Triazolo[3',4':2,3]thiadiazino[5,6-b]quinoxaline Derivatives of Clubbed Triazole: Novel Pharmacophore as Dual Inhibitors

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N-[5-(4-Amino-5-mercapto-4H-[1,2,4]triazol-3-yl)-4-methyl-1,3-thiazol-2-yl]-acetamide (**1**) on condensation with chloroacetic acid, α -haloketone and benzoin furnished [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives (**2**), (**3**) and (**4**), respectively, while condensation with 2,3-dichloroquinoxaline, carbon disulphide, aromatic carboxylic acid and aromatic carboxaldehydes yielded the cyclic products, [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives (**5**), (**6**), (**7**), (**8**), respectively. The compounds have been characterized on the basis of elemental analysis and spectral data. The antibacterial and antiinflammatory activities of the compounds have been evaluated. Most of the compounds showed promising activities.

Key Words: Triazole, Thiazole, Antiinflammatory, Antibacterial.

INTRODUCTION

Identification of novel compounds which treat effectively both infectious and inflammatory states devoid of side effects associated remains a major challenge in biomedical research. Theazole antimicrobials may be regarded as a new class providing truly effective drugs those are reported to inhibit bacteria by blocking the biosynthesis of certain bacterial lipids and/or by additional mechanisms¹. These novel emerging major chemical groups as antimicrobials are triazole and thiazole derivatives²⁻⁶. Triazoles, in particular, substituted-1,2,4-triazoles and the open-chain thiosemicarbazide counterparts of 1,2,4-triazole, are among the various heterocycles that have received the most attention during the last two decades as potential antimicrobial agents⁷⁻¹⁰. Thiazole moiety has already been reported for its antimicrobial activity¹¹⁻¹⁴. Therefore, we have established a program¹⁵⁻²⁰ to discover agents that have a dual effect, as antiinflammatory-antimicrobial agents.

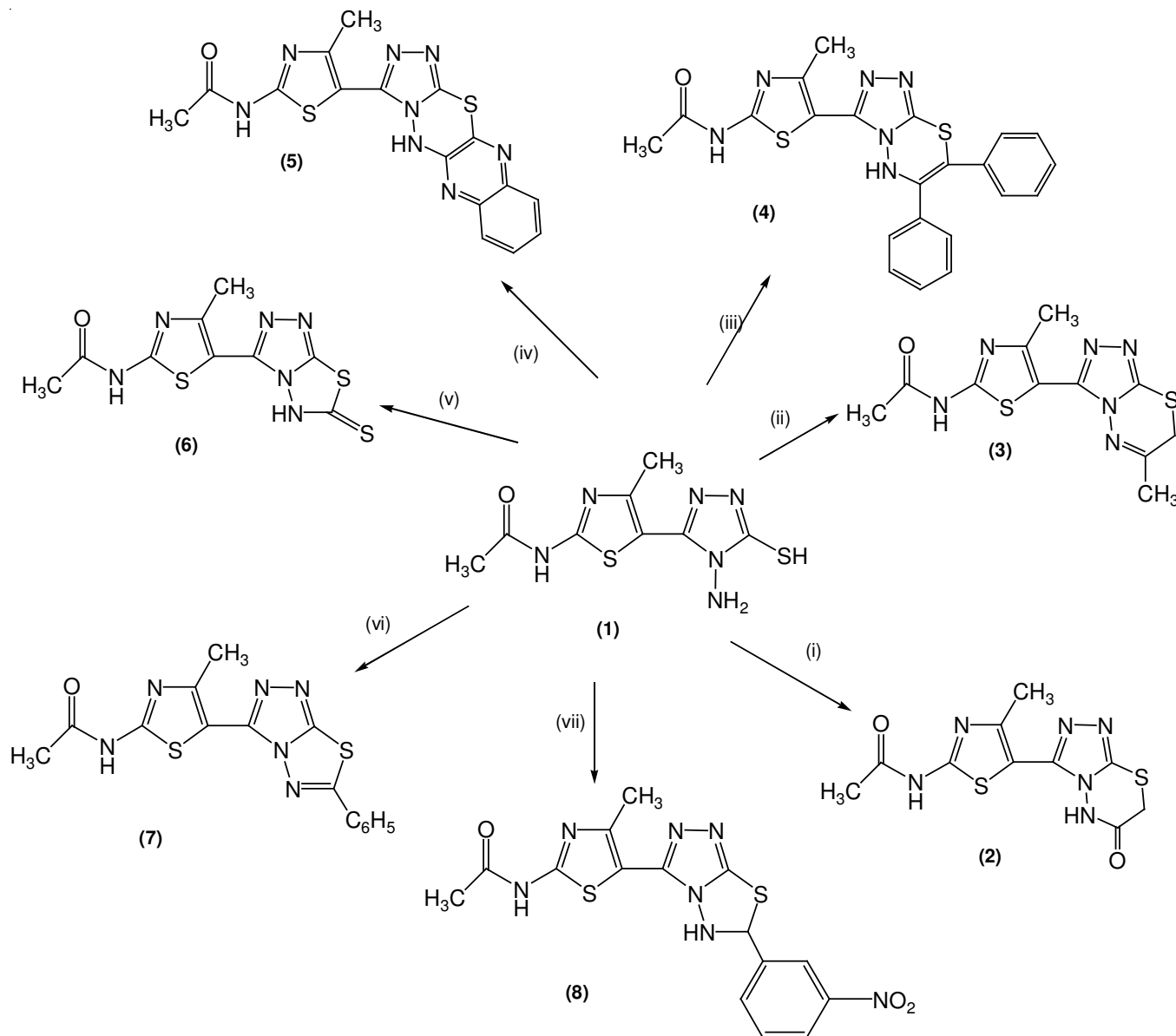
Microwave assisted reactions²¹ using dry media²² have attracted much interest because of the simplicity in operation, greater selectivity and rapid synthesis of variety of heterocyclic compounds²³. Keeping this in view, it appeared meaningful to develop rapid syntheses of title compounds under solvent free conditions using microwave irradiation. The synthesis entails the union of two biologically active nuclei, viz. triazole and

thiadiazole and also triazoles and thiadiazine. Earlier thiadiazoles and thiadiazines were synthesized in 6-7 h²⁴, while on solid support under microwave; the reaction was completed within 40-120 s with improved yield. Thus it was thought worthwhile to carry out synthesis by MORE method (**Scheme-I**).

EXPERIMENTAL

The melting points were recorded on electrothermal apparatus and are uncorrected. IR Spectra were recorded in KBr on a Perkin-Elmer-983; ¹H NMR spectra on a Bruker Avance 300 MHz instrument using CDCl₃ as solvent (chemical shifts in δ' ppm) using TMS as internal standard; mass spectra on a Finning LCQ mass spectrometer. Microwave irradiation was carried out in Raga Scientific Microwave Systems, Model RG31L at 2450 MHz. Elemental analyses were performed on a Heracus CHN-Rapid analyzer. The purity of the compounds was checked on silica gel coated Al plates (Merck).

N-[5-(4-Amino-5-mercapto-4H-[1,2,4]triazol-3-yl)-4-methyl-1,3-thiazol-2-yl]-acetamide (1**):** It was synthesized by reported method²⁵. Yield 89 %; m.p. 210-212 °C; ¹H NMR (CDCl₃): δ 2.12 (s, 2H, NH₂), 2.39 (s, 6H, CH₃), 5.92 (s, 1H, NH), 12.31 (s, 1H, SH); MS: m/z (%) = 270 (100), 237 (37), 168 (49), 128 (07); Anal. calcd. (%) for C₈H₁₀N₆OS₂: C, 35.54; H, 3.73; N, 31.09. Found (%): C, 35.71; H, 3.49; N, 31.24.



- (i) $\text{C}_6\text{H}_5\text{CHOHCOC}_6\text{H}_5, \text{NaOAc}$ (ii) $\text{CH}_3\text{COCH}_2\text{Br}, \text{K}_2\text{CO}_3$ (iii) $\text{C}_6\text{H}_5\text{CHOHCOC}_6\text{H}_5, \text{KOH}$
 (iv) 2,3-Dichloroquinoxaline, NaOAc (v) CS_2, KOH (vi) $\text{C}_6\text{H}_5\text{COOH}, \text{POCl}_3$ (vii) $2 \text{NO}_2\text{C}_6\text{H}_5$

Scheme-I

N-[4-Methyl-5-(5-oxo(4*H*,6*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazaperhydroin-3-yl)-1,3-thiazol-2-yl]acetamide (**2**): A solution of **1** (1 mmol), chloroacetic acid (1 mmol) and freshly prepared fused sodium acetate (1 mmol) was prepared. Acidic alumina (aluminium oxide, acidic, Brockmann I, ~150 mesh, 58 Å CAMAG 506-C-I, surface area 155 m²/g. pH = 6.0) 10 g was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath²⁶ and irradiated for 60 s at 145 °C. The mixture was cooled and then product was extracted with dry methanol and poured onto crushed ice. The solid thus separated was filtered, washed thoroughly with water and recrystallized from aqueous ethanol. Yield 93 %; m.p. 232-235 °C. ¹H NMR (CDCl_3): δ 2.13 (s, 6H, CH₃), 3.82 (s, 2H,

CH₂), 5.87 (s, 2H, NH); MS: *m/z* (%) = 310 (100), 274 (82), 261 (25), 207 (72), 154 (27), 115 (11); Anal. calcd. (%) for $\text{C}_{10}\text{H}_{10}\text{N}_6\text{O}_2\text{S}_2$: C, 38.70; H, 3.25; N, 27.08. Found (%): C, 38.54; H, 3.39; N, 27.26.

N-[4-Methyl-5-(5-methyl(6*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazin-3-yl)-1,3-thiazol-2-yl]acetamide (**3**): Solution of **1** (1 mmol) and *p*-bromophenacyl bromide (1 mmol) was added to acidic alumina at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 80 s at 168 °C. The mixture was cooled and then product was extracted with dry methanol and neutralized with aqueous potassium carbonate. The solid thus separated was filtered, washed thoroughly with water and recrystallized from ethanol. Yield 94 %; m.p. 229-231 °C; ¹H NMR (CDCl_3):

δ 1.1 (s, 3H, CH₃), 2.38 (s, 6H, CH₃), 3.14 (s, 2H, CH₂), 5.93 (s, 1H, NH); MS: *m/z* (%) = 308 (100), 286 (18), 251 (27), 197 (46), 163 (38); Anal. calcd. (%) for C₁₁H₁₂N₆O₂S₂: C, 42.84; H, 3.92; N, 27.25. Found (%): C, 42.67; H, 3.74; N, 27.51.

N-[5-(5,6-Diphenyl(4*H*-1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazin-3-yl))-4-methyl-1,3-thiazol-2-yl]acetamide (4): A solution of **1** (1 mmol) benzoin (1 mmol) and 2 N KOH solution was prepared. Acidic alumina was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40 s at 135 °C. The mixture was cooled and then product was extracted with acetone and was evaporated to dryness. The solid thus separated was washed thoroughly with water and recrystallized from ethanol. Yield 84 %; m.p. 242-245 °C; ¹H NMR (CDCl₃): δ 2.31 (s, 6H, CH₃), 5.73 (s, 2H, NH), 7.14-7.98 (m, 10H, ArH); MS: *m/z* (%) = 447 (100), 418 (26), 379 (73), 368 (21), 306 (52), 284 (74), 235 (39), 167 (47), 127 (24); Anal. calcd. (%) for C₂₂H₁₈N₆O₂S₂: C, 59.17; H, 4.06; N, 18.82. Found (%): C, 59.42; H, 4.17; N, 18.93.

N-(5-(4*H*-Quinoxalino[2,3-*e*]1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazin-3-yl))-4-methyl-1,3-thiazol-2-yl]acetamide (5): Solution of **1** (1 mmol), 2,3-dichloroquinoxaline (1 mmol) and fused sodium acetate (2 mmol) was added to acidic alumina at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 35 s at 180 °C. The mixture was cooled and then product was extracted with dry methanol, concentrated and cooled. The solid thus separated was filtered, washed thoroughly with water and recrystallized from ethanol. Yield 84 %; m.p. 242-245 °C; ¹H NMR (CDCl₃): δ 2.24 (s, 6H, CH₃), 5.54 (s, 2H, NH), 7.47-7.90 (m, 4H, ArH); MS: *m/z* (%) = 396 (100), 379 (53), 329 (48), 269 (92), 252 (64), 157 (22), 118 (43); Anal. calcd. (%) for C₁₆H₁₂N₈O₂S₂: C, 48.47; H, 3.05; N, 28.26. Found (%): C, 48.61; H, 3.34; N, 28.37.

N-[4-Methyl-5-(5-thioxo(4*H*-1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazolidin-3-yl))-1,3-thiazol-2-yl]acetamide (6): Carbon disulphide (1.5 mmol) was added dropwise with constant stirring to the solution of **1** (1 mmol) in methanolic KOH solution. Acidic alumina was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40 s at 180 °C. The mixture was cooled and then product was extracted with dry methanol, which was then poured onto ice and acidified with dil. HCl. The solid thus separated was filtered and recrystallized from aqueous ethanol. Yield 87 %; m.p. 221-224 °C; ¹H NMR (CDCl₃): δ 2.33 (s, 6H, CH₃), 5.95 (s, 2H, NH); MS: *m/z* (%) = 312 (100), 307 (27), 274 (67), 245 (73), 124 (76), 108 (16); Anal. calcd. (%) for C₉H₈N₆O₃S₃: C, 34.60; H, 2.58; N, 26.90. Found (%): C, 34.83; H, 2.71; N, 26.76.

N-[4-Methyl-5-(5-phenyl-(1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazolin-3-yl))-1,3-thiazol-2-yl]acetamide (7): A solution of **1** (1 mmol) and *p*-toluic acid (1 mmol) in POCl₃ (5 mL) was prepared. Acidic alumina was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40 s at 180 °C. The mixture was cooled and then poured onto ice and neutralized with aqueous potassium carbonate solution. The solid thus separated was filtered and recrystallized

from hexane. Yield 85 %; m.p. 217-221 °C; ¹H NMR (CDCl₃): δ 2.24 (s, 6H, CH₃), 5.78 (s, 1H, NH), 7.21-7.94 (m, 5H, ArH); MS: *m/z* (%) = 356 (100), 328 (58), 281 (63), 219 (48), 124 (72), 108 (24); Anal. calcd. (%) for C₁₅H₁₂N₆O₂S₂: C, 50.55; H, 3.39; N, 23.58. Found (%): C, 50.68; H, 3.53; N, 23.39.

N-{4-Methyl-5-[5-(3-nitrophenyl)(4*H*,5*H*-1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazolidin-3-yl)]-1,3-thiazol-2-yl]-acetamide (8): A solution of **1** (1 mmol) and *m*-nitrobenzaldehyde (1 mmol) was prepared. Acidic alumina was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40 s at 180 °C. The mixture was cooled and then product was extracted with dry toluene, concentrated and cooled. The solid thus separated was filtered and recrystallized from ethanol. Yield 85 %; m.p. 217-221 °C; ¹H NMR (CDCl₃): δ 2.37 (s, 6H, CH₃), 4.72 (d, 1H, CH), 5.58 (s, 1H, NH), 5.73 (d, 1H, NH), 7.43-7.87 (m, 4H, ArH); MS: *m/z* (%) = 403 (100), 384 (52), 349 (39), 274 (75), 237 (52), 176 (74), 143 (11); Anal. calcd. (%) for C₁₅H₁₃N₇O₃S₂: C, 44.66; H, 3.25; N, 24.30. Found (%): C, 44.84; H, 3.37; N, 24.46.

Pharmacology

Antiinflammatory activity²⁷: Wistar albino rats of either sex (180-200 g) were used for the antiinflammatory study. They were maintained under standard environmental conditions and were fed with standard pellet diet supplied by Hindustan Lever Ltd. Kolkata, India and water *ad libitum*.

$$\text{Antiinflammatory activity (\%)} = (1-D/C) \times 100$$

where, D- the % difference in paw volume after MCA was administered to the rats. C- The % difference in paw volume in control groups²⁸.

Antinociceptive effect²⁹: Wistar albino mice of both sex weighing between (20-25 g) were used for the antinociceptive study. They were maintained under standard environmental conditions and were fed with standard pellet diet supplied by Hindustan Lever Ltd. Kolkata, India and water *ad libitum*.

Analgesia was measured by the writhing assay using Wistar albino mice. Female mice were screened for writhing on day 1 by injecting intraperitoneally 0.2 mL of a 0.02 % aqueous solution of phenylquinone. They were kept on flat surface and the number of writhes of each mouse was recorded for 20 min. The mice showing significant (> 10) writhes were sorted out and used for analgesic assay on the following day. The mice consisting of 5 in each group and showing significant writhing were given orally a 50 mg/kg p.o. dose of the test compounds 15 min prior to phenylquinone challenge. Writhing was again recorded for each mouse in a group and a percentage protection was calculated using following formula:

$$\text{Protection} = 100 - \frac{\text{No. of writhing for treated mice}}{\text{No. of writhing for untreated mice}} \times 100$$

This was taken as per cent analgesic response and was averaged in each group of mice. Per cent of animals exhibiting analgesia was determined with each dose. All the compounds were screened for analgesic activity at 50 mg/kg po using ibuprofen as standard. The most active compounds were again retested at lower concentration for the same activity.

Antimicrobial activity: The compounds were screened for the antimicrobial activity against different microorganisms under the following conditions: Method: Well diffusion method³⁰; Medium: The nutrient agar medium; Solvent: Chloroform; Concentrations: 50 μ M and 100 μ M; Condition: 24 h at 24-28 °C; Standard: Antibiotic gentamycin.

The nutrient agar medium, 20 mL was poured into the sterile petri dishes. To the solidified plates, wells were made using a sterile cork borer 10 mm in diameter. The 24 h subcultured bacteria was inoculated in the petri-plates, with a sterile cotton swab dipped in the nutrient broth medium. After inoculating, the compounds were dissolved separately with the chloroform solvent and poured into the wells with varying concentrations ranging from 50 and 100 μ M using a micropipette. The plates were left over for 24 h at 24-28 °C. The antibiotic gentamycin was used as a standard for comparative study.

The percentage of inhibition was calculated by the formula
% Inhibition = Diameter of the inhibition zone \times 100

RESULTS AND DISCUSSION

Compounds **1** were synthesized as per the literature²⁵. Compounds **1**, adsorbed on acidic alumina (aluminium oxide, acidic, Brockmann I, ~150 mesh, 58 Å CAMAG 506-C-I, Surface area 155 m²/g. pH = 6.0), when treated with chloroacetic acid and sodium acetate it was transformed **2**, while on treatment with *p*-bromophenacyl bromide it produced **3**. Chemical transformation of **1** to **4** was achieved in very high yield on treatment with benzoin and 2 N KOH, a convergent synthesis. 2,3-Dichloroquinoxaline in presence of sodium acetate, when reacted with **1**, produced pure product of **5**. Carbon disulfide, when stirred with compound **1** in presence of methanolic KOH, then irradiated and on acidification gave **6**. A mixture of **1**, *p*-toluic acid and POCl₃, on microwave radiation gave **7** as a single product. A very unusual reaction has been observed, when *m*-nitrobenzaldehyde was made to react with compound **1** at very high voltage, for longer duration, which has been resulted in the formation of **8**. It was observed that there is remarkable loss of product (Yield 44 %) in the most of the steps in this conversion of **1** to different products when performed in conventional method, while reaction involving MORE method gave good yield (71-80 %). Compound **8** was not obtained when reaction was carried out by conventional method.

Pharmacology

Antiinflammatory activity and antinociceptive effect:

The results of the *in vitro* evaluation of antiinflammatory and antinociceptive activities are reported in Tables 1 and 2. During the preliminary screening compound was tested (Table-1) for its antiinflammatory and antinociceptive activities, which has exhibited 100 % inhibition at this concentration.

Thus, we have considered **1** as a lead molecule and subsequent structural modifications were carried out. As a first step towards lead optimization amino group was protected to the corresponding compounds **2** and **3** however, all of these modifications were resulted in a substantial decrease in activity. The next structural modification made was **4** but these changes were also resulted in a substantial loss of biological activity.

TABLE-1
ANTIINFLAMMATORY AND ANTINOCICEPTIVE
EFFECT OF SYNTHESIZED COMPOUNDS

Compd.	Antiinflammatory activity (%)	Antinociceptive effect (%)
1	50	25
2	100	50
3	100	50
4	NA	NA
5	50	25
6	50	50
7	50	25
8	50	25
Ibuprofen	75	50

Per cent inhibition. NA = Not active.

TABLE-2
RESULT OF SECOND LEVEL ANTIINFLAMMATORY
AND ANTINOCICEPTIVE ASSAYS

Compd.	Antiinflammatory activity (%)	Antinociceptive effect (%)
1	25	10
5	100	50
6	100	50
7	50	25
8	25	10
Ibuprofen	50	25

Compound **5** and **6** have shown excellent inhibition. Thus looking at the activity, it was decided to modify the structure some more. In order to optimize the bulky group component, two compounds **7** and **8** were synthesized and investigated, which revealed excellent activity. The results of the antiinflammatory and antinociceptive activities are quite interesting because all of these compounds have shown impressive percentage of inhibition. All the compounds that were active in the first level screening were then tested to determine potency in the lower dose secondary screening. More interestingly, compound **1**, **7** and **8** were the one, which have shown very good activity in secondary screening. Therefore, compounds **1**, **7** and **8** have been proven to be the most active.

Antimicrobial activity: From the antibacterial screening it was observed that all the compounds exhibited activity against all the organisms employed. Looking at the structure activity relationship, marked inhibition in bacteria was observed in the compounds **1**, **5**, **6**, **7** and **8** whereas **2**, **3** and **4** have shown moderate activity and others showed least activity (Table-3).

TABLE-3
ANTIBACTERIAL ACTIVITY OF THE
SYNTHESIZED COMPOUNDS

Compd.	Organisms			
	S.A.	P.A.	E.C.	S.T.
1	18	17	14	12
2	35	38	41	40
3	34	34	37	39
4	34	35	31	36
5	24	27	18	21
6	26	26	20	32
7	16	10	10	18
8	11	17	15	22
Gentamycin	22	22	20	16

S.A. = *Staphylococcus aureus*, P.A. = *Pseudomonas aeruginosa*, E.C. = *Escherichia coli*, S.T. = *Salmonella typhosa*

Conclusion

Screening of the *in vitro* antibacterial activity of this novel series has evidenced that derivatives with highly electronegative part at sulfhydryl group have emerged as new compounds endowed with antibacterial activity. Specifically compounds **1**, **7** and **8** probably due to their ability to increase the penetration in the bacterial cell have shown the best of all. Due to the better activity against the bacteria, compound **1**, **7** and **8** were the best choice for the preparations of new derivatives in order to improve its effectiveness on intracellular bacteria or in infected animal. Also, improvements are required to obtain new derivatives of these compounds, which able to achieve more effective antibacterial activity with lower toxicity to the mammalian cells.

There are two phases of carrageenan induced inflammatory reaction *viz.*, early or first phase and later or second phase. It has been proposed that early phase results from histamine, serotonin and bradykinin liberation, while later phase is associated with the release of prostaglandin. From the result, it is suggested that antioedematogenic effects of the compounds on carrageenan-induced oedema may be related to inhibition of inflammation mediator formation.

Finally it can be concluded that as the structure with similar skeleton showed promising dual inhibition, *i.e.* anti-inflammatory, antinociceptive and antibacterial activities, an ideal dual inhibitor with minimal toxicity and resistance to microbes with potent activity can be designed using above said compounds as lead molecules. The said dual inhibitor can be synthesized using MAOS so as to get the benefits of this novel technique.

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