Antibactericidal Effect of Some Substituted-1, 2, 4-Triazole Derivatives

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Summary: The syntheses of a series of some substituted-1, 2, 4-triazole derivatives are described. The reaction of sodium salt of α-sulphonated of stearic acid hydrazide **(1)** with carbon disulphide in alcoholic KOH yielded the corresponding sodium 1-(4-amino-5-mercapto-4H-[1, 2, 4]-triazol-3yl)-heptadecane-1-sulfonate **(2)**. The reaction of compound **(2)** with urea, chloroacetyl chloride, chloroacetaldehyde and phenacyl bromides yielded the substituted 1, 2, 4-triazole derivatives **(3)**, **(4)**, **(5)** and **(6)** respectively. All the synthesized compounds were characterized by IR, PMR, Mass spectral data and elemental analyses. All newly synthesized compounds have been assayed for their antibacterial activities against *S. aureus*, *E. coli*, *B. subtilis and P. aeruginosa*.

Introduction

The chemistry of heterocyclic compounds continues to be an active field in the organic chemistry. Triazole-derivatives have occupied a unique position in heterocyclic chemistry due to their biological activities [1-4].

1, 2, 4-Triazoles as antibacterial agents can be grouped according to the mode of action, i.e. the ability to inhibit the synthesis of the cell wall, cell membrane, proteins and nucleic acids of bacteria. The syntheses of 1, 2, 4-triazoles has also attracted wide spread attention due to the diverse agricultural and industrial activities, including anti-inflammatory, analgesic, antitumoral, anticonvulsant and tranquilizing activities shown by these compounds [5, 6]. In view of these observations and in continuation of our earlier work [7-29] on the syntheses of some 1, 2, 4- and 1, 2, 3- triazole derivatives, we now report the syntheses of some substituted 1, 2, 4-triazole derivatives and their antibacterial activities, derived from sodium 1-(4-amino-5-mercapto-4H-[1, 2, 4]-triazol-3yl)-heptadecane-1-sulfonate **(2)**.

Results and Discussion

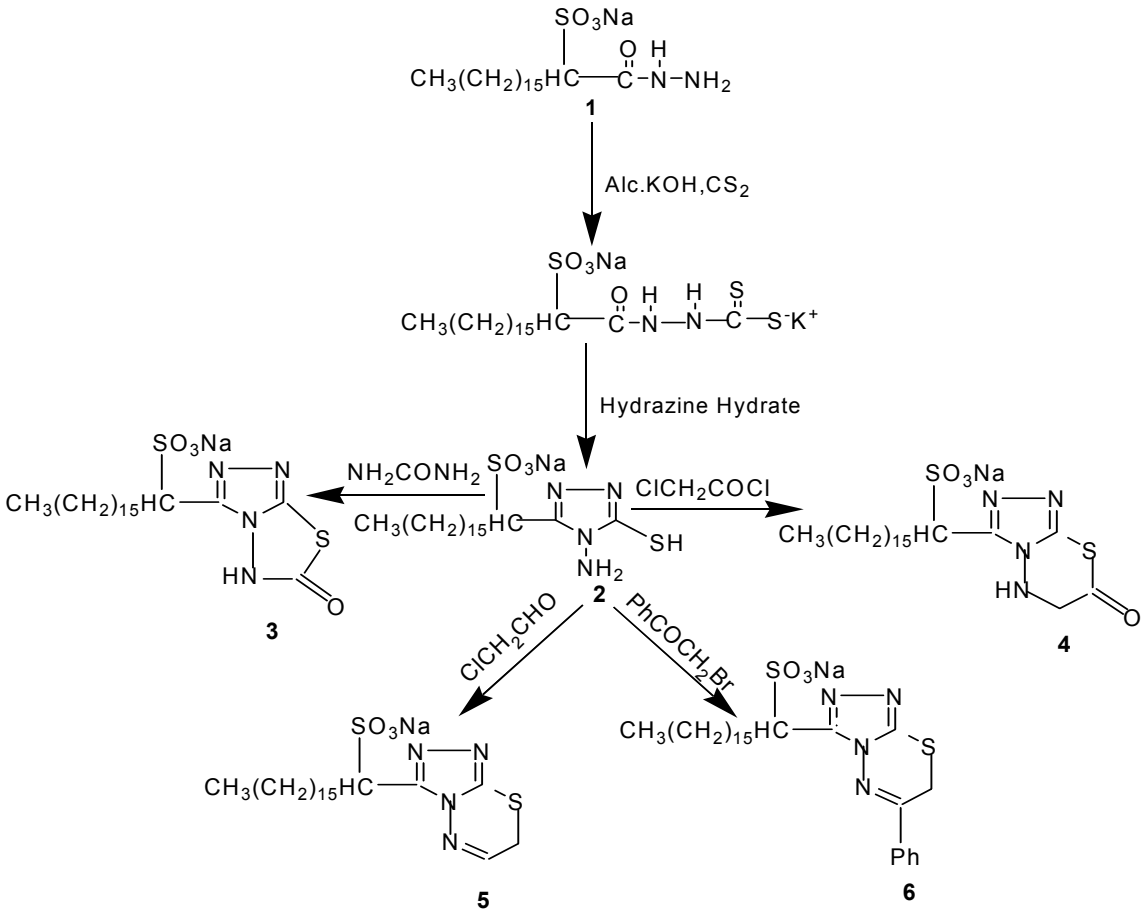
In the present study, the reaction of sodium salt of α-sulphonated of stearic acid hydrazide **(1)** with carbon disulphide in alcoholic KOH yielded the potassium salt of the corresponding dithiocarbazinate [30] in quantitative yield. The potassium salts upon reaction with hydrazine hydrate (99%) yielded sodium 1-(4-amino-5-mercapto-4H-[1, 2, 4] triazol-3yl) heptadecane-1-sulfonate **(2)**, which is required as a starting material. The reaction of compound **(2)** with urea in 5% NaOH afforded compound **(3)**. Compounds **4**, **5** and **6** were obtained through the reaction of triazole **2** with chloroacetyl chloride, chloroacetaldehyde and phenacyl bromide respectively **(**Scheme-1**)**.

All the synthesized compounds were characterized by IR, PMR, Mass spectral data and elemental analyses. All newly synthesized compounds have been assayed for their antibacterial activity against *S. aureus*, *E. coli*, *B. subtilis and P. aeruginosa*.

The IR spectrum of the compound **2** showed C=N characteristic absorption band at 1598 cm^{-1}. The PMR spectrum of **2** exhibits a singlet characteristic signal at δ 3.05 (1H, s, SH) and mass spectra of **2** showed molecular ion peaks at m/z 452 (M^+) in confirmity with the assigned molecular formulae.

The IR spectrum of the compound **3** showed C=N characteristic absorption band at 1599 cm^{-1}. The PMR spectrum of **3** exhibits triplet characteristic signal at δ 4.23 (1H, t, $CH\text{-}SO_3Na$) and a multiplet at 1.27-1.32 (30H, m, CH_2) and mass spectra of **3** showed molecular ion peaks at m/z 492(M^+) in confirmity with the assigned molecular formulae.

The IR spectrum of the compound **4** showed C=N characteristic absorption band at 1588 cm^{-1}. The PMR spectrum of **4** exhibits triplet characteristic signal at δ 4.23 (1H, t, $CH\text{-}SO_3Na$) and mass spectra of **4** showed molecular ion peaks at m/z 495(M^+) in confirmity with the assigned molecular formulae.



Scheme 1

The IR spectrum of the compound **5** showed C=N characteristic absorption band at 1605 cm^{-1}. The PMR spectrum of **5** exhibits triplet characteristic signal at δ 4.21 (1H, t, CH-SO_3Na) and a doublet at 3.36 (2H, d, CH_2) and mass spectra of **5** showed molecular ion peaks at m/z 481(M^+) in confirmity with the assigned molecular formulae.

The IR spectrum of the compound **6** showed C=N characteristic absorption band at 1590 cm^{-1}. The PMR spectrum of **6** exhibits triplet characteristic signal at δ 4.26 (1H, t, CH-SO_3Na) and mass spectra of **6** showed molecular ion peaks at m/z 499(M^+) in confirmity with the assigned molecular formulae.

These compounds were tested against various bacterial strains and the details are provided in the experimental section. These compounds were tested against various bacterial strains and the details are provided in the experimental section. All synthesized compounds showed reasonable activity against *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa* but the control of ampicillin is more active than all of the synthesized compounds.

Experimental

Melting and boiling points were determined on a Gallen Kamp apparatus in open capillaries and are uncorrected. IR spectra (KBr in cm^{-1}) were recorded on a Jasco FT-IR 5300 spectrophotometer and proton magnetic resonance (PMR) spectra (DMSO-d6) on a Varian EM-390 spectrometer using TMS as an internal standard (chemical shift in δ ppm). Mass spectra were recorded on a Jeol JMS-D 300Mass spectrometer operating at 70eV.The purity of the compounds was confirmed by TLC using silica gel G and purified by column chromatography. For TLC, Merck silica gel 60G plate was used. For column chromatography, Merck silica gel 60 (0.063-0.200mm) was used. The necessary chemicals were obtained from Merck and Fluka. All compounds showed satisfactory elemental analyses.

Sodium 1-(4-amino-5-mercapto-4H-[1, 2, 4]-triazol-3yl)-heptadecane-1-sulfonate (2)

Acid hydrazide **1** (0.01 mol) was reacted with carbon disulphide (0.01 mol) in the presence of alcoholic KOH (80 mL), the reaction mixture was diluted with ether (20 mL) and refluxed for a period of 12 hours. The hydrazine hydrate (99%, 0.01 mol) was gradually added to the potassium salt (0.01 mol) dissolved in water (30 mL) with the stirring and the mixture was refluxed for 05 hours, hydrogen sulphide evolved. After cooling, the yellow precipitate was filtered and dried. The dried product was washed with 30 mL of ethanol to afford the compound **(2)** and was used directly for the next step without further purification (yield 82%), m.p. 95 ^{0}C. Anal.Calc. for $C_{19}H_{37}N_4NaO_3S_2$, C, 50.22; H, 8.20; N, 15.27 %; Found C, 51.08; H,8.15; N, 15.22% ; IR (KBr) : 3325(NH), 2925-2845 (CH), 2370 (SH) and 1598 cm^{-1} (C=N); PMR: δ 1.01 (3H, t, CH_3, *J*=7.0), 1.27-1.32(30H, m, CH_2), 3.05 (1H, s, SH), and 4.22 ppm(1H, t, CH-SO_3Na); MS: m/z 456 (M^+) other peaks observed at 331, 271, 231, 191, 171, 93,73, 63 and 52.

Sodium 1-(6-oxo-5, 6-dihydro-[1, 2, 4]-triazolo-[3, 4-b][1, 3, 4]-thiadiazol-3yl)- heptadecane-1-sulfonate (3)

A suspension of compound **2** (0.01 mol), urea (0.01 mol) and 5% sodium hydroxide (30 mL) was heated under reflux for 5 hours. After cooling, the mixture was poured in to a beaker containing 100mL of ice-water and the precipitate was filtered and the filtrate was acidified with dilute HCl and dried. The dried product was recrystallized from an ethanol to give the desired compound **3** (yield 80%), m.p. 75 ^{0}C. Anal.Calc. for $C_{20}H_{35}N_4NaO_4S_2$, C, 50.12; H, 7.13; N, 14.37 %; Found C, 50.31; H,7.17; N, 14.31% ; IR (KBr) : 3225(NH), 2920-2849 (CH), 1676 (C=O) and 1599 cm^{-1} (C=N); PMR: δ 1.00 (3H, t, CH_3, *J*=7.1), 1.27-1.32 (30H, m, CH_2) and 4.23 ppm (1H, t, CH-SO_3Na, *J*=3.6); MS: m/z 482 (M^+) other peaks observed at 321, 267, 244, 192, 171, 97,77, 52 and 43.

Sodium 1-(7-oxo-6, 7-dihydro-5H-[1, 2, 4]-triazolo-[3, 4-b][1, 3, 4]-thiadiazol-3-yl)-heptadecane-1-sulfonate (4)

A suspension of compound **2** (0.01 mol), chloroacetyl chloride (0.01 mol) and dry dioxane (30 mL) was heated under reflux for 12 hours. After cooling, the mixture was poured in to a beaker containing 100mL of ice-water and the precipitate was filtered and dried. The dried product was recrystallized from benzene to give the desired compound **4** (yield 72%), m.p. 62 ^{0}C. Anal.Calc. for $C_{21}H_{37}N_4NaO_4S_2$, C, 51.78; H, 7.55; N, 13.68 %; Found C, 51.90; H,7.80; N, 13.71% ; IR (KBr) : 3339 (NH), 1675(C=O) and 1588 cm^{-1} (C=N); PMR: δ 1.01 (3H, t, CH_3, *J*=7.3), 1.25-1.34 (30H, m, CH_2), 3.70 (2H, s, CH_2, *J*=6.0) and 4.23 ppm (1H, t, CH-SO_3Na, *J*=4.7); MS: m/z 495 (M^+) other peaks observed at 321, 261, 251, 195, 172, 92,72, 57 and 45.

Sodium 1-(7H-[1, 2, 4]-triazol-[3, 4-b] [1, 3, 4]-thiadiazin-3-yl)-heptadecane-1-sulfonate (5)

A suspension of compound **2** (0.01 mol), chloroacetaldehyde (0.01 mol) and conc. HCl (5 mL) in dry ethanol (60 mL) was heated under reflux for 5 hours. After cooling, the mixture was poured in to a beaker containing 100mL of ice-water and the precipitate was filtered and dried. The dried product was recrystallized from ethanol to give the desired compound **5** (yield 75%), m.p. 69 ^{0}C. Anal.Calc. for $C_{21}H_{37}N_4NaO_3S_2$, C, 52.40; H, 7.81; N, 14.62 %; Found C, 52.70; H,7.86; N, 14.63% ; IR (KBr) : 2918-2848 (CH) and 1605 cm^{-1} (C=N); PMR: δ 1.00 (3H, t, CH_3, *J*=7.0), 1.28-1.34 (30H, m, CH_2), 3.36 (2H, d, CH_2, *J*=4.5) and 4.21 ppm (1H, t, CH-SO_3Na, *J*=4.8); MS: m/z 480 (M^+) other peaks observed at 311, 265, 244, 198, 175, 99,79, 54 and 47.

Sodium 1-(6-phenyl-7H-[1, 2, 4]-triazolo-[3, 4-b][1, 3, 4]-thiadiazin-3-yl)-heptadecane-1-sulfonate (6)

A suspension of compound **2** (0.01 mol), anhydrous sodium acetate (0.01 mol) and phenacyl bromide (0.01 mol) was heated under reflux for 2 hours 30 minutes and then allowed to cool. After concentration at 25 ^{0}C under reduced pressure, the crude solid was washed with water, filtered and dried. The dried product was recrystallized from an ethanol to give the desired compound **6** (yield 80%), m.p. 80^{0}C. Anal.Calc. for $C_{27}H_{41}N_4NaO_3S_2$, C, 58.35; H, 7.41; N, 12.56 %; Found C, 58.31; H, 7.38; N, 12.79%; IR (KBr) : 2905-2852 (CH), 1590 (C=N) and 1350 cm^{-1} (C=S); PMR: δ 1.02 (3H, t, CH_3, *J*=7.1), 1.28-1.33 (30H, m, CH_2), 4.26 (1H, t, CH-SO_3Na, *J*=4.3) and 6.31-7.81 ppm (5H, m, Ar-H); MS: m/z 555 (M^+) other peaks observed at 277, 236, 168, 134, 92, 72, 62, 53 and 44.

Antibacterial Activity

The antibacterial activity of five compounds **(2**, **3**, **4**, **5** & **6)**, was investigated by employing the filter paper disc method [31-34]. Representative organisms selected for evaluation of antibacterial activity were *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa*. The antibacterial activity of each of the

compounds was evaluated in triplicate at 100 µg $mL^{-1}$and 10 µg mL^{-1} concentrations. The compounds were tested as a solution or suspension in DMF (99.80 % anhydrous). An important and useful control drug Ampicillin was also tested under similar conditions, with view to compare the results.

Ampicillin is a beta-lactam antibiotic [35] that has been used extensively to treat bacterial infections since 1961. Ampicillin is able to penetrate Gram-positive and some Gram-negative bacteria [36-37]. Ampicillin acts as a competitive inhibitor of transpeptidase enzymes. As a powder ampicillin is white with a slight yellow cast and is soluble in water.

The result indicates that all of the synthesized compounds showed moderate to strong activity against these bacterial strains **(**Table- 1**)**. All compounds showed good activity against *S. aureus*, *E. coli*, *B. subtilis and P. aeruginosa* at 100 µg mL^{-1} concentration and decreased at 10 µg mL^{-1} concentrations. From the above observation it is clear that the synthesized compounds have remarkable activity and that the tested compounds **(2**, **3**, **4**, **5** & **6)** were highly active towards the selected pathogens.

Table-1: Evaluation of antibacterial activity of the compounds 2, 3, 4, 5 & 6.

	Average zone of Inhibition/mm							
Compd.	***S. aureus***		***E. coli***		***B. subtilis***		***P.aeruginosa***	
	100 µg mL^{-1}	**10 µg mL^{-1}**	**100 µg mL^{-1}**	**10 µg mL^{-1}**	**100 µg mL^{-1}**	**10 µg mL^{-1}**	**100 µg mL^{-1}**	**10 µg mL^{-1}**
2	**24**	**19**	**23**	**18**	**22**	**18**	**21**	**19**
3	**21**	**18**	**20**	**17**	**20**	**16**	**21**	**18**
4	**20**	**18**	**21**	**17**	**20**	**16**	**19**	**16**
5	**24**	**19**	**22**	**18**	**23**	**18**	**22**	**19**
6	**23**	**19**	**22**	**17**	**22**	**19**	**22**	**18**
Standard (Ampicillin)	**28**	**22**	**26**	**20**	**24**	**20**	**24**	**20**
Control	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**

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