



SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF PHENOTHIAZINE-3-SULFONATE DERIVATIVES

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ABSTRACT

Phenothiazine derivatives are biologically active and play a vital role in medicinal chemistry. The present report explains a simple and efficient synthesis of title compounds. The compounds are also screened for their biological activity and shown good results. Potassium 1-amino-10,10a-dihydro-4aH-phenothiazine-3-sulfonate (**1**) reacts with Benzaldehyde to form Potassium-1-(benzylideneamino)-10,10a-dihydro-4aH-phenothiazine-3-sulfonate (**2**), which undergoes N-alkylation to give potassium-1-(benzylideneamino)-10,10a-dihydro-10-((oxiran-2-yl)methyl)-4aH-phenothiazine-3-sulfonate (**3**). Compound **3** reacts with different amines to give Potassium-1-(benzylideneamino)-10, 10a-dihydro-10-(2-hydroxy-2-(pyrrolidin-1-yl)ethyl)-4aH-phenothiazine-3-sulfonate (**4a-c**). All the newly synthesized quinoxaline-benzohydrazide derivatives have been characterized by ¹H NMR, IR and mass spectroscopic analysis. The synthesized compounds have been screened for antibacterial activity. Most of the compounds show significant antibacterial activity.

Key words: Phenothiazine-3-sulfonate, Antibacterial activity.

INTRODUCTION

Reactive oxygen species (ROS) are major free radicals generated in many redox processes, which may induce oxidative damage to biomolecules, including carbohydrates, proteins, lipids, and DNA. Reactive oxygen species affect living cells, which mediate the pathogenesis of many chronic diseases, such as atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancers, and other degenerative diseases.

Phenothiazine was first prepared by Bernthsen in 1883 in the course of proof of structure studies on Lauth's violet and methylene blue. Since then it has played an important role in dye chemistry as the parent compound of the thiazine dyes¹. In the last twenty years

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phenothiazine and its derivatives have found numerous applications in other fields, and this has stimulated further research on these compounds. Phenothiazine was discovered to have insecticidal properties in 1934; further work demonstrated its usefulness as a urinary antiseptic and an anti-helminthic². Its derivatives have been particularly valuable in human medicine as antihistamines³⁻⁵, in the treatment of Parkinson's disease⁶, and as antiemetics⁷, to mention a few of their many applications.

They have also been successfully employed as antioxidants⁸. Meyer and Jacobsen have given an excellent summary of the chemistry of phenothiazine up to 1920 with particular emphasis on its relation to methylene blue⁹. Gilman's students¹⁰⁻¹² have reviewed the literature on phenothiazine in their doctoral dissertations. Metcalf has discussed the chemistry of phenothiazine in his monograph on insecticides.

Substituted phenothiazines have also attracted interest because of their optoelectrochemical and photophysical properties¹³⁻¹⁶. As the substituents on the phenothiazine rings have a great influence on their properties, efficient methods for the preparation of a diverse range of substituted phenothiazines are highly desirable.

Owing to the widespread applications, synthetic and biological activity evaluation of phenothiazine and their derivatives, we undertook synthesis of title compounds.

EXPERIMENTAL

Materials and methods

The solvents were purified according to standard procedures prior to use, and all commercial chemicals were used as received. ¹H NMR and ¹³C NMR spectra were recorded in Varian MR-400 MHz and 100 MHz instruments, respectively. Chemical shifts are reported in δ (ppm) downfield from tetramethylsilane (TMS) with reference to internal standard and the signals were reported as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), m (multiplet) and coupling constants in Hz. The mass spectra were recorded on Agilent ion trap mass spectrometer. Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR spectrometer.

Potassium-1-(benzylideneamino)-10,10a-dihydro-4aH-phenothiazine-3-sulfonate (2)

To a solution of Potassium 1-amino-10, 10a-dihydro-4aH-phenothiazine-3-sulfonate (**1**) (0.01 mole) in ethanol (60 mL), Benzaldehyde (0.01 mole) and a few drops of glacial acetic acid were added and the mixture was refluxed for 8 hrs. It was then cooled, concentrated and poured into crushed ice and filtered. The solid thus obtained was purified by recrystallization from ethanol.

^1H NMR (DMSO- d_6 , 400 MHz); δ = 10.80 (brs, 1H), 8.18 (s, 1H), 7.80 (d, 2H, J = 7.8 Hz), 7.60 (d, 2H, J = 8.0 Hz) 7.41 (t, 2H), 7.21-7.01 (m, 5H); ^{13}C NMR (DMSO- d_6 , 100 MHz); δ = 156.1, 144.2, 134.4, 130.4, 130.0, 129.5, 129, 128.0, 127.5, 127.0, 125.1, 124.8, 123.8, 120.0, 118.2, 116.0, 110.2, 108.0; Mass: m/z = 421.1 $[\text{M} + \text{H}]^+$.

Potassium-1-(benzylideneamino)-10,10a-dihydro-10-((oxiran-2-yl)methyl)-4aH-phenothiazine-3-sulfonate (3)

A round-bottomed flask was charged with $\text{Pd}_2(\text{dba})_3$ (5 mol%), (0.01 m mol), potassium-1-(benzylideneamino)-10,10a-dihydro-4aH-phenothiazine-3-sulfonateepoxide (2) (0.01 m mol), Cs_2CO_3 (1.5 m mol) and dry 1,4-dioxane (5 mL). The flask was flushed with argon for 5 min. The mixture was heated at reflux 4 h under magnetic stirring. After cooling down to RT, the reaction mixture was concentrated and the residue was purified by flash column chromatography on silica gel.

^1H NMR (DMSO- d_6 , 400 MHz); δ = 8.18 (s, 1H), 7.91 (t, 1H), 7.80 (m, 5H) 7.61 (s, 1H), 7.48 (m, 3H), 3.82 (m, 2H), 3.0 (m, 1H) 2.30 (m, 2H); ^{13}C NMR (DMSO- d_6 , 100 MHz); δ = 160.5, 148.4, 144.0, 141.6, 136.0, 135.7, 132.1, 127.8, 126.0, 125.2, 122.1, 120.0, 118.0, 116.1, 115.1, 62.0, 56.1, 45.2; Mass: m/z = 477.1 $[\text{M} + \text{H}]^+$.

Potassium-1-(benzylideneamino)-10,10a-dihydro-10-(2-hydroxy-2-(pyrrolidin-1-yl)ethyl)-4aH-phenothiazine-3-sulfonate (4a)

To a stirred solution of compound 4 (0.01 mol) and amine (0.01 mol) in ethanol (25 mL), AlCl_3 (50 mg) was added. The reaction mixture was stirred at room temperature for an 30-45 min. After completion of the reaction, as monitored by TLC, the reaction mixture was washed with diethyl ether (3×10 mL). The combined organic extracts were concentrated under reduced pressure and the resulting product was purified by column chromatography.

^1H NMR (DMSO- d_6 , 400 MHz) δ = 8.02 (s, 1H), 8.0 (s, 1H), 7.81 (d, 2H, J = 8.0 Hz), 7.58 (d, 2H, J = 8.0), 7.45 (m, 3H), 7.30 (t, 2H), 7.09 (t, 1H), 4.40 (d, 1H, J = 5.8 Hz) 4.20 (t, 1H, 3.65 (m, 4H), 2.90 (m, 2H), 2.80 (m, 2H) 1.81 (m, 4H). ^{13}C NMR (DMSO- d_6 , 100 MHz); δ = 165.1, 147.2, 166.1, 144.0, 142.1, 139.9, 139.6, 137.8, 137.4, 136.8, 135.1, 133.0, 132.0, 129.4, 129.0, 128.1, 125.0, 68.1, 62.0, 58.0, 56.1, 28.2.

Potassium-1-(benzylideneamino)-10, 10a-dihydro-10-(2-hydroxy-2-(morpholine-4-yl)ethyl)-4aH-phenothiazine-3-sulfonate (4b)

^1H NMR (DMSO- d_6 , 400 MHz); δ = 8.21 (s, 1H), 8.01 (s, 1H), 7.82 (d, 2H, J = 8.0) 7.58 (d, 2H, J = 8.0 Hz), 7.43 (m, 3H), 7.36 (t, 2H), 7.08 (t, 1H), 4.40 (d, 1H, J = 5.2 Hz),

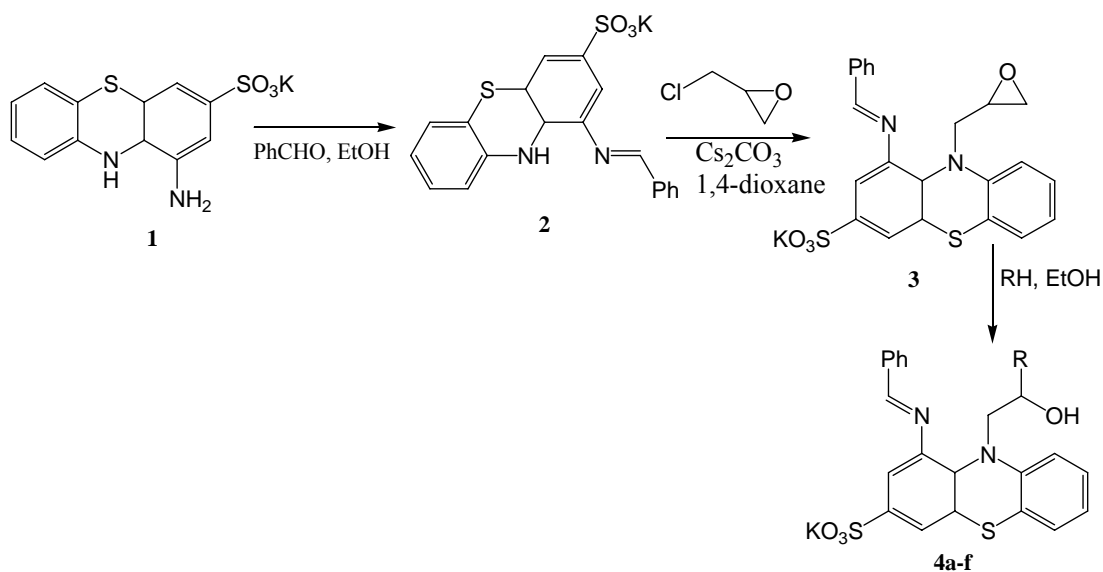
4.21 (t, 1H), 4.01 (t, 4H), 3.70 (t, 4H), 2.90 (m, 2H), 2.80 (m, 2H); ^{13}C NMR (DMSO- d_6 , 100 MHz); δ = 165.0, 147.1, 144.0, 142.4, 141.8, 138.6, 138.2, 134.6, 132.4, 131.6, 131.2, 129.4, 129.2, 126.1, 124.6, 124.2, 67.8, 64.1, 60.2, 58.2, 54.2.

Potassium-1-(benzylideneamino)-10,10a-dihydro-10-(2-hydroxy-2-(4-(pyrimidin-2-yl)-piperazine-1-yl)ethyl)-4aH-phenothiazine-3-sulfonate (4c)

^1H NMR (DMSO- d_6 , 400 MHz); δ = 8.20 (s, 1H), 8.02 (s, 1H), 7.80 (m, 5H), 7.62 (d, 2H, J = 8.0 Hz), 7.56 (m, 3H), 7.40 (t, 2H), 7.12 (t, 1H), 4.38 (d, 1H, J = 5.2 Hz), 4.20 (t, 1H), 3.72-3.68 (m, 8H), 2.92 (m, 2H), 2.82 (m, 2H); ^{13}C NMR (DMSO- d_6 , 400 MHz); δ = 164.8, 160.8, 147.2, 146.2, 144.3, 144.0, 142.2, 142.8, 139.2, 138.2, 134.8, 132.4, 132.0, 131.4, 129.6, 129.0, 126.2, 124.2, 124.0, 68.0, 61.2, 61.0, 58.4, 54.4.

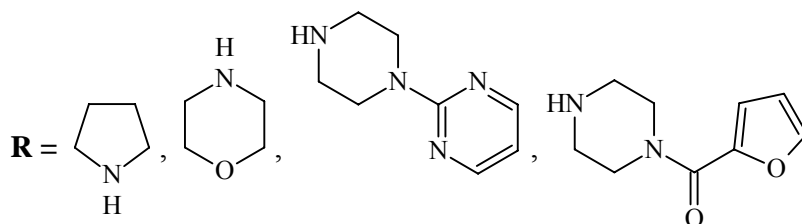
Potassium-1-(benzylideneamino)-10, 10a-dihydro-10-(2-hydroxy-2-(4-(furancarbo-2-yl)-piperazine-1-yl)ethyl)-4aH-phenothiazine-3-sulfonate (4d)

^1H NMR (DMSO- d_6 , 400 MHz); δ = 8.22 (s, 1H), 8.05 (s, 1H), 7.85 (m, 5H), 7.65 (d, 2H, J = 7.8 Hz), 7.62 (m, 3H), 7.42 (t, 2H), 7.14 (t, 1H), 4.39 (d, 1H, J = 5.2 Hz), 4.22 (t, 1H), 3.76-3.66 (m, 8H), 2.93 (m, 2H), 2.80 (m, 2H). ^{13}C NMR (DMSO- d_6 , 100 MHz); δ = 164.9, 161.2, 148.3, 146.1, 144.2, 144.0, 142.8, 142.0, 140.6, 139.3, 138.3, 134.9, 132.5, 132.1, 129.5, 129.0, 126.8, 124.2, 68.2, 61.6, 58.5, 54.6.



Cont...

Amines



Scheme 1

Antibacterial activity

Preparation of sample/test solution for antibacterial activity

The antibacterial activity testing of the selected cultures was carried out according to the method described by Raman. Each selective medium was inoculated with the microorganism suspended in nutrient broth. Once the agar was solidified, it was punched with the wells of 6 millimeters diameter and was filled with 25 μ L of the plants extract and some were kept as blanks (sterilized distilled water). Gentamycin sulfate were used as positive control was sterile distilled water. The plates were incubated at $35 \pm 2^\circ\text{C}$ for 24 hrs and the antimicrobial activity was observed and calculated.

Table 1: Antimicrobial activity of Phenothiazine-3-sulfonate derivatives

	4a	4b	4c	4d	4e	Tetracycline
<i>Gram-positive</i>						
<i>Bacillus subtilis</i>	11	8	9	7	4	20
<i>Bacillus cereus</i>	10	7	7	8	2	16
<i>S. aureus</i>	8	10	6	7	5	13
<i>Gram-negative</i>						
<i>E. coli</i>	9	13	7	10	5	12
<i>Proteous vulgaris</i>	14	11	10	8	3	14
<i>k. pneumonia</i>	6	7	9	7	4	11

The zone of inhibition were represented in mm

RESULTS AND DISCUSSION

The synthesis of the target compounds was carried out according to the representative **Scheme 1**.

All the compounds were screened for antibacterial activity and all the compounds have shown significant activity towards bacteria. Among all the derivatives compound **4a** and **4b** are more active toward *E. coli* and *Proteous vulgaris*.

Compounds which were synthesized, posses antibacterial activity. Among the compounds tested (5a-e) showed prominent antibacterial activity compared with that from other compound 5a and 5b noticed highest zone of inhibition where as other compounds are also showed significant zone of inhibitions against *protius vulgaris* (Table) according to the data obtained in the current study it has been understood that compounds from are more effective than the compounds. This might because of nitrogen. However some of the compounds have same functional group are also showed good activity. All the gram negative strains are more susceptible against tested compounds.

CONCLUSION

In conclusion, the present paper describes the synthesis and antibacterial activity of new Phenothiazine-3-sulfonate derivatives and was screened against four bacterial strains such *Bacillus subtilis*, *Bacillus cereus*, *s. aureus*, *Escherichia coli*, *Proteous vulgaris* and *k. pneumonia*. It is observed that within the series, maximum compounds exhibited moderate antibacterial activity.

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