

## SYNTHESIS AND SPECTRAL STUDIES IN THE CHEMISTRY OF 2-AMINOBENZENETHIOLS OF BIOLOGICAL SIGNIFICANCE

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### ABSTRACT

Heterocyclic Chemistry is the thrust area for a researcher. Various publications of research papers on biologically active 2-aminobenzenethiols in recent years have aroused our interest for the synthesis and biological activity (antioxidant and antimicrobial) of these heterocycles. This article reports synthesis of some new prospective bioactive 2-aminobenzenethiols, which have shown antimicrobial activity to a good level against some selected strains of fungi, Gram positive and Gram negative bacteria leading us to a possibly potent class of antimicrobial agents.

### INTRODUCTION

More than 90% of new drugs hold heterocycles and the interface between chemistry and biology, at which point so much new scientific insight, discovery and application taking place. The presence of heterocycles [1,2] in all kinds of organic compounds of attention in electronics, biology, optics, pharmacology, material sciences and so on is very well known. Between them, sulfur and nitrogen-containing heterocyclic compounds have maintained the interest of researchers through decades of historical development of organic synthesis [3]. Therapeutic use of 2-aminobenzenethiols [4] as sedatives, antihistaminics [5,6] and antipsychotics followed and continues to this day. 2-aminobenzenethiols derivatives also used for their cancer chemopreventive [7,8] effect such as calmodulin, neuroleptic [9] action connected with the dopaminergic [10,11] receptors blockade and protein kinase C inhibitory actions, anti-proliferative effect [12,13],

inhibition of P – glycoprotein transport function and reversion of multidrug resistance. In the acrylics industry, it is common practice to utilize 2-aminobenzenethiols to shortstop or terminate a runaway acrylic acid or monomer polymerization. The arylamine (I) is converted into phenylthiourea by reaction with ammonium thiocyanate. The synthesized phenylthiourea cyclized into 2-aminobenzothiazoles (III) by bromination in chloroform then alkaline hydrolysis of substituted 2-aminobenzothiazoles followed by neutralization with glacial acetic acid yields 2-aminobenzenethiols (IV) Scheme-1. The biologically active heterocyclic compounds were tested for their antimicrobial activities. To exhibit the potential of synthesized compounds as better antimicrobial agents [14] minimum inhibition concentration (MIC) against selected strains of fungi, Gram positive and Gram negative bacteria [15] belonging to Microbial Type Culture Collection (MTCC) was reported using agar well diffusion method.

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### Experimental

Purity of all the synthesized compounds were checked by thin layer chromatography (TLC) using silica



gel "G" as adsorbent in various non-aqueous solvent systems and visualization was accomplished by UV light or in an iodine chamber. All the melting points of synthesized compounds were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr on SHIMADZU 8400 S FT-IR spectrophotometer.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on JEOL AL-300 spectrometer at frequencies of (300.40 MHz and 75.45 MHz) respectively, using TMS as internal standard in  $\text{DMSO-d}_6$ . Mass spectra were recorded on WATERS (micromass MS technologies) Q-T by electron spray ionisation. The elemental analysis (C, H and N) were performed using vario-III analyser at CDRI Lucknow. The commercially available substituted arylamines were purchased from Sigma Aldrich and used without further purification and substituted 2-aminobenzenethiols were prepared according to method of R.R. Gupta *et al* [6].

### Synthesis of 2-aminobenzothiazole in two steps:

#### (i) Preparation of substituted phenylthiourea (II)

Substituted aniline (I) (0.1 mole), a mixture of concentrated hydrochloric acid (9 ml) and water (25 ml) were taken in a 250 ml R.B. flask, fitted with a reflux condenser and heated for half an hour, a solution of aniline hydrochloride was formed. It was then allowed to cool to room temperature and then 0.1 mole ammonium thiocyanate was added and refluxed for nearly 4-5 hrs. The solution was poured into crushed ice, solid separated out was filtered, washed with water, dried and crystallized from ethanol. (Scheme1).

#### (ii) Preparation of substituted 2-aminobenzothiazole (III)

0.1 Mole synthesized substituted phenylthiourea (II) was taken in a two necked R.B. flask (500 ml), and equipped with a mechanical stirrer. Bromine (0.1 mole) in chloroform (100 ml) was added drop wise with stirring to the reaction mixture over a period of 1 hr. and the temperature was maintained below  $5^\circ\text{C}$ . The reaction mixture continues stirred for a period of 4 hrs. Then the reaction mixture was refluxed until the evolution of hydrogen bromide vapors ceased (about 4 hrs). Dried solid was treated with sulfur dioxide water and filtered. Aqueous ammonia used for neutralization of filtrate and the precipitate obtained was filtered, washed with water and crystallized from ethanol (Scheme1).

#### Synthesis of substituted 2-aminobenzenethiol (IV)

A mixture of substituted 2-aminobenzothiazole (III a-c), potassium hydroxide (5 times by weight of 2-aminobenzothiazole) and water (10 times by weight of 2-aminobenzothiazole) were taken in a round bottom flask (250 ml). The mixture was refluxed until the liberation of ammonia gas stopped. The resulting mixture was filtered, and neutralized by acetic acid with continuous stirring (cold water used for dilution). Temperature of the solution kept below  $10^\circ\text{C}$  or else a decomposed greenish mass is

resulted instead of 2-aminobenzenethiol. A yellowish precipitate was obtained after complete neutralization followed by extraction (2-3 times) with solvent ether. A yellow solid was obtained on evaporation of ether and recrystallized from ethanol and 2-aminobenzenethiol was obtained finally (IVa-c). (Scheme1).

#### (IVa) 2-Amino-3,5,6-trichlorobenzenethiol

Pale yellow solid; m.p. :  $109^\circ\text{C}$  ; Yield :42%, IR (KBr) :  $\nu$  3480-3360 ( $\text{NH}_2$ ), 2610 (S-H) and 790 (C-Cl)  $\text{cm}^{-1}$  ;  $^1\text{H}$  NMR spectral data (300.15 MHz,  $\text{Me}_2\text{SO-d}_6$ ,  $\delta$  ppm from TMS) :  $\delta$  4.55 (s, 2H,  $\text{NH}_2$ ), 7.84 (s, 1H, Ar-H), 1.89 (s, 1H, S-H),  $^{13}\text{C}$  NMR (75.47 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm from TMS) :  $\delta$  120.2 (C-1), 146.2 (C-2), 118.6 (C-3), 126.1 (C-4), 122.2 (C-5), 131.6 (C-6); MS (FAB) 10 kV, m/z (rel. int.) : 228 [M]<sup>+</sup> (100); "Anal. Calcd for  $\text{C}_6\text{H}_4\text{Cl}_3\text{NS}$ : C, 31.51; H, 1.75; N, 6.13; Found: C, 31.04; H, 1.14; N, 6.60".

#### (IVb) 2-Amino-5-metoxhybenzenethiol

Brown solid; m.p. :  $114^\circ\text{C}$  ; Yield : 52%, IR (KBr) :  $\nu$  3430-3320 ( $\text{NH}_2$ ), 2540 (S-H) and 580 (C-Br)  $\text{cm}^{-1}$  ;  $^1\text{H}$  NMR spectral data (300.15 MHz,  $\text{Me}_2\text{SO-d}_6$ ,  $\delta$  ppm from TMS) :  $\delta$  4.24 (s, 2H,  $\text{NH}_2$ ), 7.34-6.94 (m, 3H, Ar-H), 1.54 (s, 1H, S-H),  $^{13}\text{C}$  NMR (75.47 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm from TMS) :  $\delta$  119.1 (C-1), 145.3 (C-2), 116.6 (C-3), 127.4 (C-4), 112.4 (C-5), 130.8 (C-6); MS (FAB) 10 kV, m/z (rel. int.) : 204 [M]<sup>+</sup> (100); "Anal. Calcd for  $\text{C}_6\text{H}_6\text{BrNS}$ : C, 35.29; H, 2.94; N, 6.86; Found : C, 35.84; H, 3.21; N, 7.03".

#### (IVc) 2-Amino-5-bromobenzenethiol

Yellowish solid; m.p. :  $105^\circ\text{C}$  ; Yield : 54%, IR (KBr) :  $\nu$  3350-3280 ( $\text{NH}_2$ ), 2380 (S-H) and 1250-1040 (C-O-C)  $\text{cm}^{-1}$  ;  $^1\text{H}$  NMR spectral data (300.15 MHz,  $\text{Me}_2\text{SO-d}_6$ ,  $\delta$  ppm from TMS) :  $\delta$  4.09 (s, 2H,  $\text{NH}_2$ ), 7.11-6.63 (m, 3H, Ar-H), 1.38 (s, 1H, S-H),  $^{13}\text{C}$  NMR (75.47 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm from TMS) :  $\delta$  118.4 (C-1), 136.9 (C-2), 114.5 (C-3), 115.1 (C-4), 149.2 (C-5), 112.6 (C-6), 55.3 ( $\text{OCH}_3$  at C-5); MS (FAB) 10 kV, m/z (rel. int.) : 155 [M]<sup>+</sup> (100); "Anal. Calcd for  $\text{C}_7\text{H}_9\text{NOS}$ : C, 54.19; H, 5.81; N, 9.03; Found : C, 54.91; H, 5.17; N, 9.24".

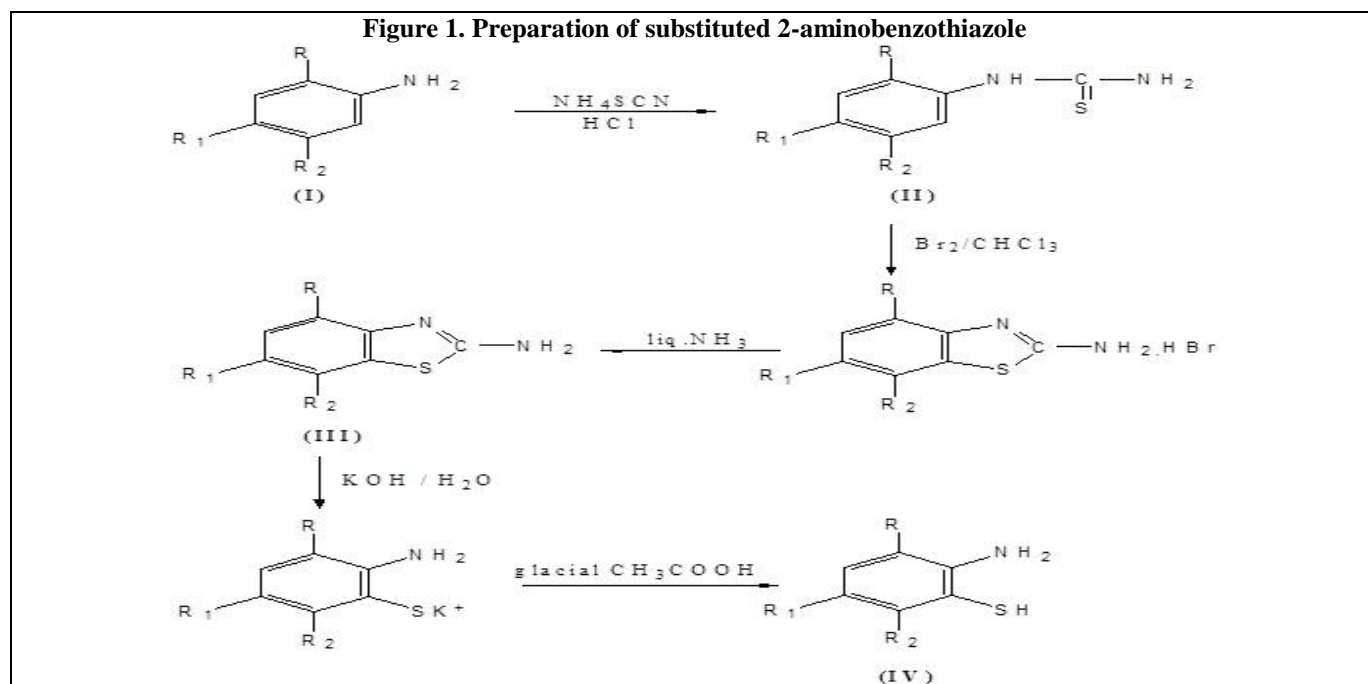
### Antimicrobial activity

Two Gram-negative (*Escherichia coli* MTCC 2939 and *Streptomyces griseus* MTCC 1998) and one Gram-positive (*Bacillus subtilis* MTCC-441) bacteria were used as quality control strains. *Fusarium oxysporum*, MTCC 1755, *Aspergillus niger* MTCC 281 and *Rhizopus stolonifer*, MTCC 2591 were the reference strains for testing antifungal activities of the compounds. Streptomycin and Ketoconazole were used as standard antibacterial and antifungal drugs, respectively. As per NCCLS-1992 manual, Minimum Inhibitory Concentrations (MICs,  $\mu\text{g ml}^{-1}$ ) of synthesized compounds assays were carried out by broth microdilution method. Stock solution of 1000  $\mu\text{g/ml}$  concentration for each synthesized



compound and standard drugs were prepared in DMSO. In primary screening, 500, 250 and 125 µg/ml concentrations of the synthesized drugs were taken. The synthesized drugs those found active in primary screening were further tested in a second set of dilution against all microorganisms. These drugs were also diluted to obtain 100, 50, 25, 15 µg/ml concentrations. The highest dilution showing at least 99% inhibition was taken as MIC which meant the lowest concentration of each chemical compound in the tube with no growth (i.e. no turbidity) of inoculated bacteria/ fungi was recorded as minimum inhibitory

concentration of that compound. Antibacterial activities of the bacterial strains were carried out in Luria broth (Himedia) medium and all fungi were cultivated in Sabouraud Dextrose Agar (Himedia) at pH 6.9 with an inoculum of  $10^8$  cfu/ml by the spectrophotometric method and an aliquot of 10 µl was added to each tube of the serial dilution and incubated on a rotary shaker at 37°C for 24 hours at 150 rpm. At the end of incubation period, MIC values were recorded. The MIC values of synthesized compounds in µg/ml against certain bacterial strain and fungal strain are shown in Table 1.



**Table 1. Minimum inhibitory concentrations ( $\mu\text{g ml}^{-1}$ ) of synthesized substituted 2-aminobenzenethiols (a-c)**

Compd. No.	Compound			Minimum Inhibitory Concentrations (MICs) of bacterial strains in $\mu\text{g/ml}$			Minimum Inhibitory Concentrations (MICs) of fungal strains in $\mu\text{g/ml}$		
	R	R <sub>1</sub>	R <sub>2</sub>	<i>E.coli</i> MTCC 2939	<i>Bacillus subtilis</i> MTCC 441	<i>Streptomyces griseus</i> MTCC 1998	<i>Fusarium oxysporum</i> MTCC 1755	<i>Aspergillus niger</i> MTCC 281	<i>Rhizopus stolonifer</i> MTCC 2591
IVa	Cl	Cl	Cl	114	90	92	102	85	77
IVb	H	OCH <sub>3</sub>	H	120	105	77	126	48	56
IVc	H	Br	H	78	88	52	79	54	68
Streptomycin				68	46	62	-	-	-
Ketoconazole				-	-	-	74	38	46

Streptomycin and Ketoconazole were used as standard antibacterial and antifungal drugs respectively.

## RESULT AND DISCUSSION

Synthesis of substituted 2-aminobenzothiazole involves two steps:

**Step-I:** involves the conversion of arylamine into phenylthioureas (II) by treating with ammonium thiocyanate (NH<sub>4</sub>SCN).

**Step-II:** The synthesized 2-aminobenzothiazoles (III) by cyclisation of phenylthioureas in presence of bromine in chloroform.

## CONCLUSION

Finally the alkaline hydrolysis of substituted 2-aminobenzothiazoles followed by neutralization with glacial acetic acid yields 2-aminobenzenethiols A yellow solid mass was obtained on evaporation of ether layer and then it was recrystallized from ethanol. The proposed structure of synthesized compounds is well supported by elemental analysis and spectral data.



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