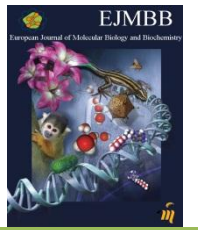
e - ISSN - 2348-2206
Print ISSN - 2348-2192

European Journal of Molecular Biology and Biochemistry

Journal homepage: www.mcmed.us/journal/ejmbb



SYNTHESIS, SPECTRAL EVALUATION AND BIOLOGICAL ASSESSMENT OF THE SUBSTITUTED 2-AMINOBENZENETHIOLS

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Article Info

Received 23/01/2015
Revised 16/02/2015
Accepted 19/03/2015

Key words:- 2-aminobenzenethiol, spectral properties, biological assessment.

ABSTRACT

The heterocyclic chemistry constitutes an important class of heterocycles. The substituted 2-aminobenzenethiol play an important role in modern heterocyclic chemistry because they are widely used as synthetic precursor of potential biological, pharmacological and industrially useful agents and compounds that are extensively used as tranquilizer, sedative, CNS depressant, antispasmodic, antiulcer, antibacterial, antifungal, antioxidant, antimicrobial, anticancer agents, antitumor, antituberculosis, antiproliferative analgesic, vasodilator etc.

INTRODUCTION

The compounds containing heteroatoms like nitrogen and sulfur are of immense importance not only biologically but also industrially. The majority of these synthesized products along with their biological assessment [1-5] are often produced due to the heterocyclic structures. The substituted compounds containing heteroatoms can offer a huge level of structural diversity and have proven to be broadly useful as therapeutic agents. Therefore, development of synthetic drugs having heteronuclei is also an incessant process in continuation of our research to build up new synthetic drugs. A minor change in nature and position of substituents leads to diverse effect in their biological activities. 2-aminobenzenethiol are of outmost significance due to its promising pharmacological activities such as antiemetics, antihistamines, tranquilizers, antipyretics, anti-inflammatory, analgesic, bactericides, fungicides, diuretics, and various pharmacological and biological properties.

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The substituted 2-aminobenzenethiol used as heterocyclic base for the synthesis of phenothiazines and benzothiazines. The arylamine (I) is converted into phenylthiourea by reaction with ammonium thiocyanate. The synthesized phenylthiourea cyclized into 2-amino-benzothiazoles (III) by bromination in chloroform then alkaline hydrolysis of substituted 2-aminobenzothiazoles followed by neutralization with glacial acetic acid yields 2-aminobenzenethiols [7-14] (IV) Scheme-1. The biologically active heterocyclic compounds were tested for their antimicrobial activities.

EXPERIMENTAL

Melting points were taken in open glass capillary tube using Gallenkamp melting point apparatus and are uncorrected. The purity of synthesized compound was checked by thin layer chromatography and visualized by UV light or in iodine chamber. The IR spectra were recorded in KBr on SHIMADZU 8400S FTIR spectrophotometer and wave no. is given in cm^{-1}. The ^{1}H and ^{13}C NMR were recorded on JEOL AL spectrometer in $CDCl_3$/DMSO-d_6 using TMS as an internal standard at 300.15 and 75.47 MHz, respectively and chemical shift

were measured in δ ppm. FAB (Fast Atom Bombarding) mass spectra were recorded on JEOL SX 102/DA 600 using Argon/Xenon gas. ^{19}F NMR spectra were recorded in $CDCl_3$ using CF_3COOH as standard compound. 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].

Preparation of substituted 2-aminobenzenethiols (IVa-b)

The substituted 2-aminobenzothiazole (0.1 mole) (IIIa-b), potassium hydroxide (5 times by weight of 2-aminobenzothiazole) and water (10 times by weight of 2-aminobenzothiazole) separately mixed in a beaker and added to round bottom flask (500 mL) containing substituted 2-aminobenzothiazole. It was refluxed until liberation of ammonia gas was finished (30-35 hours).The total contents present in round bottom flask were filtered and neutralized with glacial acetic acid with continuous stirring. Temperature was kept between 0-5°C (by adding ice). Otherwise a decomposed greenish mass is formed instead of 2-aminobenzenethiol. A yellowish precipitate obtained after the complete neutralization, which was extracted 2-3 times with solvent ether. A yellow solid mass was obtained on evaporation of ether layer and then it was recrystallized from ethanol. The physical and analytical data of substituted 2-aminobenzenethiols (IVa-b) are given below.

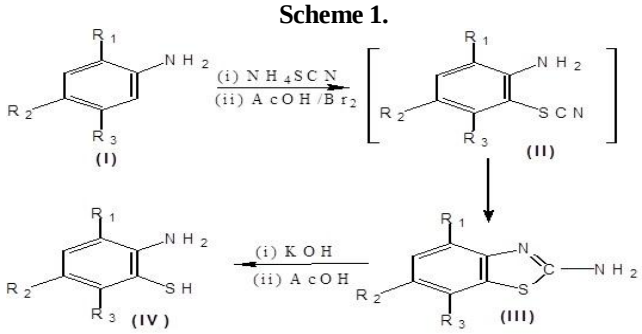
2-amino-5-fluorobenzothiazole (IIIa):

Black solid; m.p. : 83°C ; Yield :60%, IR (KBr) : *v* 3495-3385 (NH_2), 1068 (C-S), and 1025 (C–F) cm^{-1} ; ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 4.20 (s, 2H, NH_2), 7.52-6.93 (m, 3H, Ar-H), ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 118.2 (C–1), 140.2 (C-2), 118.6 (C-3), 110.1 (C-4**),** 150.2 (C-5), 115.6 (C-6); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -28.127 (s, 1F at C-5); MS (FAB) 10 kV, m/z (rel. int.) : 143 $[M]^+$ (100); "Anal. Calcd for C_6H_6FNS : C, 50.34; H, 4.19; N, 9.79; Found : C, 50.58; H, 4.24; N, 9.84".

2-Amino-6-fluoro-3-methylbenzenzenethiol (IVb):

Brown solid; m.p. : 93°C ; Yield : 68%, IR (KBr) : *v* 3485-3380 (NH_2), 1065 (C-S), and 1020 (C–F) cm^{-1} ; ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 3.85 (s, 2H, NH_2), 6.58-6.20 (m, 2H, Ar-H), ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 65.2 (C–2), 109.2 (C-4), 106.6 (C-5), 150.1 (C-6**),** 112.2 (C-7), 12.8 (CH_3 at C-4); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -29.127 (s, 1F at C-6); MS (FAB) 10 kV, m/z (rel. int.) : 168 $[M]^+$ (100); "Anal. Calcd for C_7H_8FNS : C, 53.50; H, 5.09; N, 8.91; Found : C, 53.76; H, 5.13; N, 8.97".

Scheme 1.



Scheme 2.

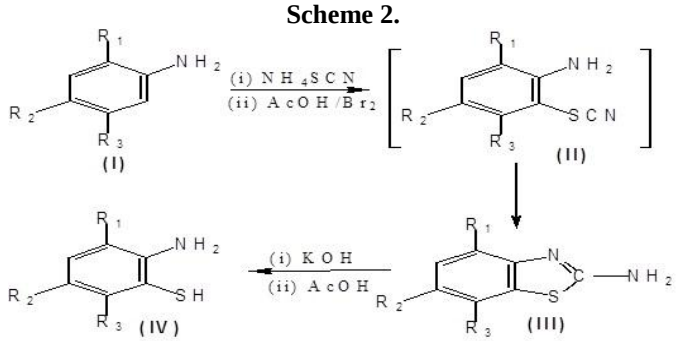


Table 1. Antimicrobial activity of synthesized compounds

	Minimal Inhibition Concentrations of bacterial strains (MIC) in µg/mL				Minimal Inhibition Concentrations of fungal Strains (MIC) in µg/mL			
Compounds	***Bacillus subtilus* MTCC 121**	***Staphylococcus aureus* ATCC 25917**	***E. coli* DH 5 alpha MTCC 1786**	***Pseudomonas aeruginosa* ATCC 6624**	**Aspergillus niger NCIM 27821**	***Penicillium funiculosum* NCIM 1174**	***Fusarium oxysporum* NCIM 1228**	***Trichoderma reesei* NCIM 992**
IIIa	30.6	52.5	52.4	54.1	32.1	61.3	68.2	50.1
IIIb	39.2	61.8	64.7	58.3	35.9	65.3	70.3	51.6
IVa	33.2	51.9	63.2	52.2	33.2	61.8	69.4	52.8
Ivb	42.8	66.5	69.4	60.1	37.6	68.9	72.7	53.3
Ampicillin sodium salt	42	61	64	56	-	-	-	-
Fluconazole	-	-	-	-	32	65	72	55

Antimicrobial Activity

Broth microdilution method is used to calculate the minimum inhibitory concentrations (MICs, μg mL^{-1}) of the chemical compounds assays as per NCCLS-1992 manual. Two Gram-negative (*Escherichia coli* DH5 alpha MTCC 1786 and *Pseudomonas aeruginosa* ATCC 6624) and two Gram-positive (*Bacillus subtilis* MTCC-121and *Staphylococcus aureus* ATCC 25917) bacteria were used as quality control strains. *Aspergillus niger* NCIM 27821, *Penicillium funiculosum* NCIM 1174, *Fusarium oxysporum* NCIM-1228 and *Trichoderma reesei* NCIM-992 were the reference strains for testing antifungal activities of the compounds. Ampicillin sodium salt and Fluconazole were used as standard antibacterial and antifungal drugs, respectively. DMSO is used for preparing solutions of test compounds and standard drugs. Each synthesized drug was diluted obtaining 1000 μg/mL concentration as a stock solution. Now for primary screening 500, 250 and 125 μg/mL concentrations of the synthesized drugs were taken. The synthesized drugs found active in this primary screening were further tested in a second set of dilution against all microorganisms in which the drugs were again diluted to obtain 100, 50, 25, 20, 15 μg/mL concentrations. The highest dilution showing at least 99% inhibition was taken as MIC. This means that the lowest concentration of each chemical compound in the tube with no growth (i.e. no turbidity) of inoculated bacteria/ fungi was recorded as minimal inhibitory concentration of that compound. Luria broth (Himedia) medium was used to carry out antibacterial activities of the bacterial strains while 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 h at 3.262 xg (150 rpm). After incubation, MIC values were recorded. The MICs of tested compounds in μg/mL against certain strains of bacteria and fungi are shown in Table 1.

RESULTS AND DISCUSSION

Chemistry

The substituted 2-aminobenzothiazole (0.1 mole) (IIIa-b), potassium hydroxide (5 times by weight of 2-aminobenzothiazole) and water (10 times by weight of 2-aminobenzothiazole) separately mixed in a beaker and added to round bottom flask (500 mL) containing substituted 2-aminobenzothiazole. It was refluxed until liberation of ammonia gas was finished (30-35 hours).The total contents present in round bottom flask were filtered and neutralized with glacial acetic acid with continuous stirring. Temperature was kept between 0-5°C (by adding ice). Otherwise a decomposed greenish mass is formed instead of 2-aminobenzenethiol. A yellowish precipitate obtained after the complete neutralization, which was extracted 2-3 times with solvent ether. 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.

ACKNOWLEDGMENTS

The authors are extremely thankful to the Department of Chemistry, University of Rajasthan, Jaipur for providing necessary facilities. The authors are also grateful to the Institute of Seminal Applied Sciences, Jaipur for providing assistance in carrying out anti-microbial assessment.

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