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E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Synthesis and spectral evaluation of novel bioactive substituted 2-Aminobenzenethiols

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Abstract

The investigation of substituted 2-Aminobenzenethiols has steadily flourished because they exhibit numerous pharmaceutical and analytical applications. Due to the pharmaceutical properties of 2-Aminobenzenethiols, these compouns have been used for various clinical purposes like sedative, antihelmintics, antiinflammatory, antimalarials, antibacterial, anticonvulsants and pesticides. 2-Aminobenzenethiols have been extensively used in analytical chemistry as a redox indicators as well as antiosidants for dyes and lubricants. These compounds also used as reagents in spectrophotometry.

Keywords: 2-Aminobenzenethiols, Spectral Properties, Biological Evaluation.

Introduction

Heterocyclic compounds possess promising medicinal activities and used as tranquilizers, antihistamines, diuretics, analgesics, neuroleptics, sedatives, antiinflammatories, antivirals, anesthetic, tuberculostatic, CNS depressants, anticancer, antidepressants, antipyretics, antiparkinson drugs, antibacterial and antifungal etc. A slight change in the substitution pattern of heterocyclic nucleus causes a remarkable difference in their biological activities. So it has been considered worthwhile to extend our efforts to synthesize 2-aminobenzenethiols as a precursor to obtain the drugs of improved therapeutic effects with minimum side effects.

The substituted 2-aminobenzenethiol used as base template for the synthesis of phenothiazines and benzothiazines. First step involves the conversion of arylamine into phenylthioureas (IIa-c) by treating with ammonium thiocyanate (NH_4SCN). In the second step 2-aminobenzothiazoles (IIIa-c) are produced by cyclisation of phenylthioureas in presence of bromine in chloroform. Finally the alkaline hydrolysis of substituted 2-aminobenzothiazoles followed by neutralization with glacial acetic acid yield 2-aminobenzenethiols (IVa-c) (Scheme1). The biologically active heterocyclic compounds were tested for their antimicrobial activities.

Experimental

Elemental analyses, melting point determinations and different spectroscopic techniques like IR, ^{1}H NMR, ^{13}C NMR show the authenticity of the compounds. Melting points were determined in open glass capillary tubes using gallen kamp melting point apparatus and were uncorrected. The purity of the synthesized compounds were checked by thin layer chromatography using silica gel “G” as adsorbent in various non-aqueous solvent systems, visualizing these by UV light or Iodine chamber. IR spectra were recorded in KBr on SHIMADZU 8400 S FT IR spectrometer. The ^{1}H NMR spectra on JEOL AL-300 FT NMR spectrometer at (300 MHz) in DMSO-d_6 using TMS (Tetra Methyl Silane) as an internal standard (chemical shifts are measured in δ ppm). 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.*

Synthesis of 2-aminobenzothiazole in two steps

Preparation of substituted phenylthiourea (IIa-c)

Substituted aniline (**Ia-c**) (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. (Scheme-1)

Preparation of substituted 2-aminobenzothiazole (IIIa-c)

0.1 Mole synthesized substituted phenylthiourea (**IIa-c**) 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°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. (Scheme-1)

Synthesis of substituted 2-aminobenzenethiol (IVa-c)

A mixture of substituted 2-aminobenzothiazole (IIIa-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°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). (Scheme-1)

The physical and analytical data of substituted 2-aminobenzenethiols **(IVa-c)** are given below.

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

Pale yellow solid; m.p. : 109°C ; Yield :42%, IR (KBr) : *v* 3480-3360 (NH_2), 2610 (S-H) and 790 (C–Cl) cm–1; ^{1}H NMR spectral data (300.15 MHz, Me_2SO-d6, δ ppm from TMS) : δ 4.55 (S, 2H, NH_2), 7.84 (S, 1H, Ar-H), 1.89 (s, 1H, S-H), ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 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 $[M]^+$ (100); "Anal. Calcd for $C_6H_4Cl_3NS$: 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°C ; Yield : 52%, IR (KBr) : *v* 3430-3320 (NH_2), 2540 (S-H) and 580 (C–Br) cm^{-1}; ^{1}H NMR spectral data (300.15 MHz, Me_2SO-d6, δ ppm from TMS) : δ 4.24 (S, 2H, NH_2), 7.34-6.94 (M, 3H, Ar-H), 1.54 (S, 1H, S-H), ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 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 $[M]^+$ (100); "Anal. Calcd for C_6H_6BrNS : C, 35.29; H, 2.94; N, 6.86; Found : C, 35.84; H, 3.21; N, 7.03".

(IVc) 2-Amino-5-bromobenzenethiol

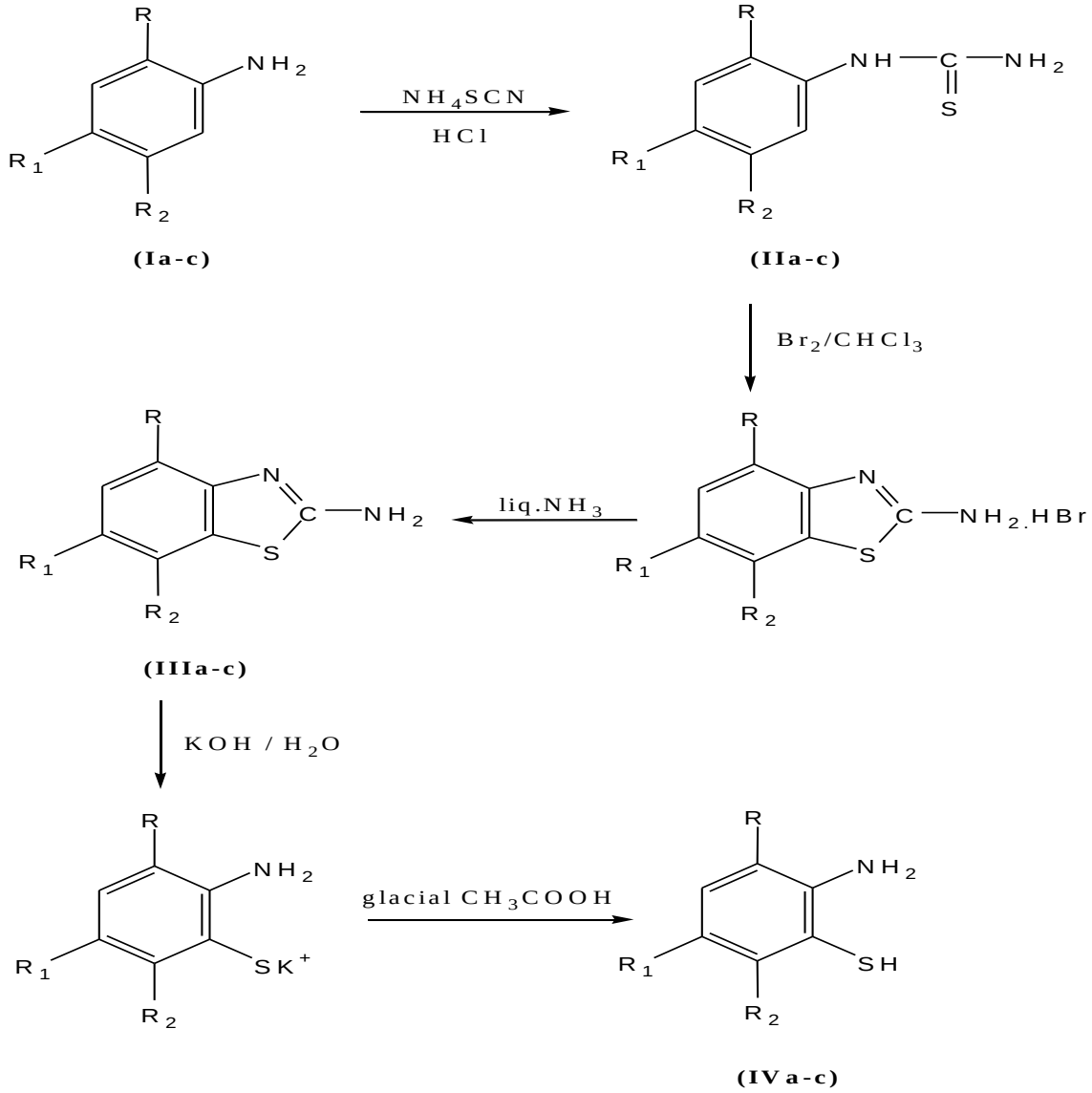
Yellowish solid; m.p. : 105°C ; Yield : 54%, IR (KBr) : *v* 3350-3280 (NH_2), 2380 (S-H) and 1250-1040 (C–O-C) cm^{-1}; ^{1}H NMR spectral data (300.15 MHz, Me_2SO-d6, δ ppm from TMS) : δ 4.09 (S, 2H, NH_2), 7.11-6.63 (m, 3H, Ar-H), 1.38 (S, 1H, S-H), ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 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 (OCH_3 at C-5); MS (FAB) 10 kV, m/z (rel. int.) : 155 $[M]^+$ (100); "Anal. Calcd for C_7H_9NOS: C, 54.19; H, 5.81; N, 9.03; Found : C, 54.91; H, 5.17; N, 9.24".

Biological evaluation (Antimicrobial activity) of synthesized compounds

The objective of this study was to determine antimicrobial activity (antibacterial and antifungal) of synthesized substituted 2-aminobenzenethiol **(IVa-c)**. Thus for determining antibacterial activity, varied range of heterocyclic compounds were tested against *Escherichia coli* (gram negative) MTCC 2939, *Bacillus subtilis* (gram positive) MTCC 441 and *Fusarium oxysporum* MTCC 1755, *Aspergillus niger* MTCC 281 used for determining antifungal activity of the synthesized compounds.

Synthesized compounds were assessed for their antimicrobial activity (antibacterial and antifungal) using agar well diffusion method. Streptomycin was used as standard antibacterial drug and Ketoconazole was used as standard antifungal drugs. As per NCCLS-1992 manual, Minimum Inhibitory Concentrations (MICs, μg ml^{-1}) of synthesized compounds assays were carried out by broth microdilution method. Stock solution of 1000 μg/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 micro-organisms. These drugs were also diluted to obtain 100, 50, 25, 20, 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 hrs 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.



Scheme-1

Where,
R = H, Cl
R_1 = Cl, Br, OCH_3
R_2 = H, Cl

Table 1
Minimum inhibitory concentrations (μg ml^{-1}) of synthesized substituted 2-aminobenzenethiols (a-c)

Compound No.	Compound			MICs of bacterial strains in μg/ml		MICs of fungal strains in μg/ml	
	R	R_1	R_2	*Escherichia coli* MTCC 2939	*Bacillus subtilis* MTCC 441	*Fusarium oxysporum* MTCC 1755	*Aspergillus niger* MTCC 281
IVa	Cl	Cl	Cl	117	95	110	95
IVb	H	OCH_3	H	124	110	125	48
IVc	H	Br	H	81	95	88	62
Streptomycin				68	46	-	-
Ketoconazole				-	-	74	38

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

Result and Discussion

In summary, this article reflects upto date and comprehensive coverage of biomedical aspects and convenient methods of synthesis of substituted 2-aminobenzenethiols **(a-c)**. Elemental analysis and spectroscopic data completely support the proposed structures for the synthesized compounds. The MIC values of antibacterial and antifungal screening revealed that excellent antibacterial and antifungal activities against all the four selected strains of bacteria and fungi respectively were exhibited by compounds.

The paper showed that a slight change in substitution pattern affects the biological activity tremendously. By comparing different compounds we can get an idea of drug designing so that we can design better substituted 2-aminobenzenethiols **(a-c)** templates which may have potential to be used as a new class of antibacterial and antifungal drugs. The motive of our research is to extend the area of research by synthesizing new and better templates of 10*H*-phenothiazines and screening them as potential antibacterial and antifungal drugs but further biomedical research is required.

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