JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Studies in the Synthesis and Antimicrobial activity of 5-(1-Substituted Amino-2,4 Dithiobiureto) Aminoindole

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Abstract

A novel series of 5-(1-Substituted Amino-2,4 Dithiobiureto) Aminoindole [Va-c] is recently synthesized by the interaction of 5-(thiocarbamido)aminoindole with various substitutedisothiocynate [IVa], [IVb], [IVc] in presence of acetone medium, for creating a new and suitable route for the synthesis of 5-(1-Substituted Amino-2,4 Dithiobiureto) Aminoindole [Va-c] which increases the yield of product as well as maintain the purity of them by decreasing time duration of the reaction mentioned in the literature. The justification and determination of structures of synthesized molecules were done on the basis of elemental analysis, chemical characterizations, spectral data and antimicrobial activities were carried out on various microbes.

Key words: thiocarbamide, substituted isothiocynate, antimicrobial activity.

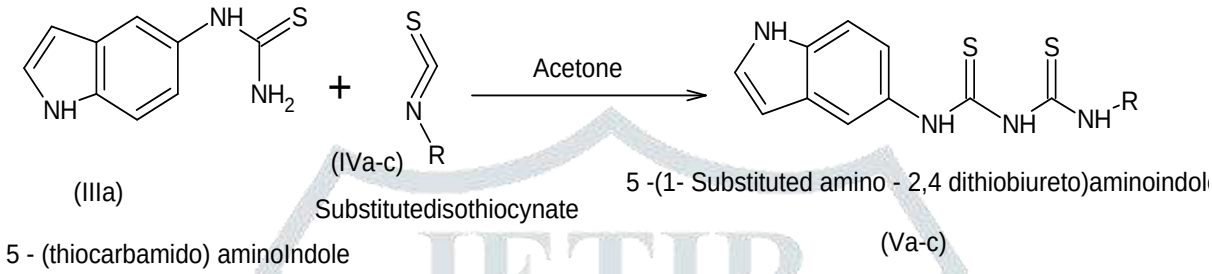
Introduction

The uses of indole nucleus containing heterocycles in the fields of medicinal, agricultural, pharmacological and biological sciences led to the creation of their own unique identity. Indoles shows antimicrobial, anti-inflammatory, anti-cancer and antioxidant properties[1-3].Organic chemistry revealed that 2,4-dithiobiureto nuclei contain heterocycles and heteroacycles with distinct effects because of their strong biological activities [4–15]. These 2,4-dithiobiurets can also be employed as good intermediates [16–19] in the synthesis of numerous heterocycles with notable biological activities. The biological uses of 2,4-dithiobiuret are altered by different substitutions on its nitrogen atom. Numerous 2,4-dithiobiurests were produced as part of a larger lab program to manufacture heteroacycles, and these were then converted into 5, 6, and 7 member heterocycles [20–23]. The synthetic uses of N-[7-(1-substituted)-2,4-dithiobiureto)-4-yl]-N,N-diethylpentane-1,4-diamine have been recently investigated in this lab[24]. Synthesis and characterization of hybrid nanocatalyst

(AgNPs-Fe2O3) for catalytic remediation of hazardous 2,4-dinitrophenol in the presence of sodium borohydridehave been sufficiently studied[25].

As a wider programme of this research laboratory, we have decided to synthesize a novel series of 5-(1-Substituted Amino-2,4 Dithiobiureto) Aminoindoleby interacting 5-(thiocarbamido)aminoindole with various substituted isothiocynate [IVa], [IVb], [IVc] in presence of acetone medium. Structure determination and identification of the synthesized product is done on the basis of chemical characteristics, elemental analysis and spectral data. The general reaction scheme is as shown in below,

Scheme-I



(IIIa)

5 - (thiocarbamido) aminoIndole

(IVa-c)

Substitutedisothiocynate

5 -(1- Substituted amino - 2,4 dithiobiureto)aminoindol

(Va-c)

Where R -phenyl, -*p*-tolyl, -*o*-nitrophenyl

Experimental

The melting point of the all-synthesized compounds was recorded using hot paraffin bath. The carbon and hydrogen analysis were carried out on Carlo-Ebra 1106 analyzer. Nitrogen estimation was carried out on Colman-N-analizer-29. IR spectra were recorded on Perkin Elmer Spectrometer in range 4000-400cm^{-1} in KBr pellets. The purity of compound was checked by TLC plate. All chemicals used were of AR-grade.

Synthesis of 5-(1-*p*-Tolylamino-2,4-dithiobiureto)aminoindole (Vb)

A reaction mixture of 5-(thiocarbamido) amino indole (IIIa) and *p*-tolyl isothiocynate in acetone medium was refluxed on water bath. During refluxing homogeneous reaction mixture was obtained and after 2 hrsfew orange shiny crystals were obtained in medium.

Similarly, 5-(1-*phenyl*amino-2,4-dithiobiureto) aminoindole (Va), 5-(1-*o*-nitrophenylamino-2,4-dithiobiureto) aminoindole (Vc)were synthesized by interacting 5-(thiocarbamido) amino indole (IIIa) with*phenyl*isothiocynate and *o*-nitrophenylisothiocynaterespectively.

Properties of (Vb): It is orange colourcrystalline solid, m.p. 198 °C. yield 82 %, R_f value was found to be 0.38, by using TLC aluminium plate.Gave Lassigne's positive test for nitrogen and sulphur.Product was desulphurized by alkaline plumbite solution indicating that sulphur is present in open chain. The elemental analysis showed all the expected elements in the Vb with their composition as carbon {(found 59.30 %) calculated 60.14%}, while next is nitrogen {(found 16.27 %calculated 16.35%)}, Sulphur is {(found 18.60 %calculated 18.94%)}.

FTIR Spectrum: Spectrumwas recorded using KBr-pellets and is reproduced asMBS-Vb-FTIR. Important absorption can be correlated as (cm^{-1}): N-H stretching at 3401.61cm^{-1} (3500-3000 cm^{-1}),Ar-H stretching at 2693.71cm^{-1} (3000-2500cm-^{1}), Ar-c=c stretching at 1610.63cm^{-1} (1600-1500cm^{-1}), C-N stretching

at 1091.76cm^{-1} (1200-1000cm)$^{-1}$, N-C=S stretching at 1461.14cm^{-1} (1550-1250cm^{-1}), NN>C=S stretching at 1411.95cm^{-1} (1400-1200cm-1), substitution at *p*-position of benzene ring 729.12cm^{-1} (770-690cm^{-1}).

PMR Spectrum: PMR spectrum of compound was recorded using DMSO-d_6 and denoted as MBS-Vb-PMR. This spectrum distinctly displayed signals at (δ ppm): signal at 7.3951-7.0013 ppm is due to Ar-H,signal at 10.98 ppm is due to pyrrole N-H, signal at 6.99 ppm is due to N-H in aromatic ring, signal at 2.51 to 2.50 isdue to CH3protons.

^{13}C Spectrum: ^{13}C spectrum of compound was recorded using DMSO-d_6 and denoted as MBS-Vb-^{13}C. This spectrum distinctly displayed signals due toC=S carbon at δ 183.69 ppm, Ar-C carbon at δ137.81-122.90 ppm, C-N at δ 39.75-39.08 ppm, -CH_3 carbons at δ20.72 ppm.

Properties of Va: It is shining ivory colour crystalline solid, Yield-94%, m.p.180 °C, R_f value was found to be 0.34, by using TLC aluminium plate.Gave Lassigne's positive test for nitrogen and sulphur.Product was desulphurized by alkaline plumbite solution indicating that sulphur is present in open chain. The elemental analysis showed all the expected elements in the Va with their composition as carbon {(found 58.87 %,) calculated 59.15%}, while next is nitrogen {(found 17.16 % calculated 17.35%)}, Sulphur is {(found 19.64 %calculated 19.94%)},M.F.: $C_{16}H_{14}N_4S_2$.

FTIR Spectrum: Spectrumwas recorded using KBr-pellets and is reproduced asMBS-Va-FTIR. Important absorption can be correlated as (cm^{-1}): N-H stretching at 3408.36cm^{-1} (3500-3000 cm^{-1}), Ar-H stretching at 2688.88cm^{-1} (3000-2500cm-^{1}), Ar-c=c stretching at 1611.59cm^{-1} (1600-1500cm^{-1}), C-N stretching at 1084.04cm^{-1} (1200-1000cm)$^{-1}$, N-C=S stretching at 1454.39cm^{-1} (1550-1250cm^{-1}), NN>C=S stretching at 1328.05cm^{-1} (1400-1200cm^{-1}).

PMR Spectrum: PMR spectrum of compound was recorded using DMSO-d_6 and denoted as MBS-Va-PMR. This spectrum distinctly displayed signals at (δ ppm): at 7.7226-6.4203 ppm is due to Ar-H, signal at 11.25 ppm is due to pyrrole -H, signal at 6.42 ppm is due to N-H in aromatic ring.

^{13}C Spectrum:^{13}C spectrum of compound was recorded using DMSO-d_6 and denoted as MBS-Va-^{13}C. This spectrum distinctly displayed signals due to C=S carbon at δ183.77 ppm,Ar-C carbon at δ134.46-122.11 ppm, signal due to C-N at δ 39.75-39.08 ppm, signal due to Ph-C at δ77.05 ppm.

Antimicrobial activities of 5-(1-*p*-Tolylamino-2,4-dithiobiureto) aminoindole (Vb),5-(1-*phenyl*amino-2,4-dithiobiureto) aminoindole (Va), 5-(1-*o*-nitrophenylamino-2,4-dithiobiureto) aminoindole (Vc)were investigated by disc diffusion method and MIC method on *S. aureus, S. Typhi, B.Cereus, Trichodema, A.Niger*and *E. coli.*

DiscDiffusionMethod

Every time, nutritional agar medium was made fresh and sterile. The process was conducted in an aseptic manner. Each item of glassware and equipment needed was sterilized. Melted medium (around 15-20 ml) was added to each sterile petri dish. Each petri plate received 0.05–0.1 ml (about 2-3 drops) of a freshly diluted culture of the organism under research that had been incubated for 24 hours. The agar plate was rotated on a level surface to fully combine the nutrition agar media and nutrient broth culture. The solidified state was maintained at room temperature.

Next, discs (6 mm in diameter) of sterile Whatman filter paper No. 1 that had been thoroughly moistened with the same concentration of each component were placed on the plate's surface. A 70% methanolmoistened disc was utilized as a control. After letting them spread throughout the medium, the plates were incubated for 24 hours at 37°C. The observation was made on the diameter of the inhibitory zones.The potato dextrose plate was the only instrument utilized in the identical process usedto assess antifungal activity.

MIC Method

The Serial Dilution Method was used as the procedure to find the MIC of different substances. One liter of distilled water was used to dissolve thirteen grams of dried medium to create nutrient broth. Each tube contained 5 milliliters of the medium, which had its pH adjusted to 7.4. For 20 minutes, all of the tubes were sterilized at 121°C.A final concentration of 1 x 10-2 M was obtained by dissolving the proper amount of the test chemical in 70% dioxane. Aseptically, different volumes of the aforementioned stock solution were introduced to the different nutritional broth tubes (i.e., 0.5, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0,…… 5.8, 6.0 ml). Each tube received a fresh 0.2 milliliter culture of the test bacteria. The test bacterium's inoculum size was changed to provide roughly 107 cFu. For twenty-four hours, each tube was incubated at 37°C. Five milliliters of the solvent and nutritional broth were added to an inoculation tube, which served as a control.Following a 24-hour incubation period, the MIC of each tube against the test bacterium was noted. The absence of visible turbidity in the tube containing the test substances at the greatest dilution was indicative of this. The following method was used to calculate the minimum inhibitory concentration (MIC) of different test chemicals against molds (fungus). This is how the potato dextrose broth was made. One liter of distilled water was mixed with two hundred grams of peeled potatoes. After 20 minutes of steaming, the capacity was reduced to one liter. To this was added 20 grams of dextrose. To achieve a final concentration of 1x10-2 M, the appropriate amount of test compounds was dissolved in a mixture of 70% dioxane. The potato dextrose broth tubes were aseptically filled with varying volumes of the aforementioned stock solution (i.e., 0.5, 1.0, 1.2,..., 6 ml). Each tube received an aseptic inoculation of a fresh fungal culture (0.2 ml of culture) for ninety-six hours, every tube was incubated at 28°C. All the tubes were checked for the MIC of the test compounds after 48 hours of incubation.

Table: 1 -Antibacterial and Antifungal Activity of Compounds

sample	***E. Coli***	***B. Cereus***	***S. Typhi***	***S. Aureus***	***Trichoderma***	***A. Niger***
Va	Active	Active	Active	Active	Active	Active
Vb	Active	Active	Active	Inactive	Inactive	Inactive
Vc	Active	Active	Active	Active	Active	Inactive

Table-2

Compound	*E. Coli*	*B. Cereus*	*S. Typhi*	*S. Aureus*	*Trichoderma*	*A. Niger*
Va	774	945	784	1149	1035	997
Vb	725	677	794	--	--	--
Vc	511	220	454	603	1372	853

Results and Discussion

The newly synthesized 5-(1-Substituted Amino-2,4 Dithiobiureto) Aminoindole showed good antimicrobial activities and Va and Vccompound showed the highest activity in MIC. All these compounds contain 2,4 dithiobiuretonucleus may be responsible for the higher activity.Va showed active against all the bacteria, Vc showed inactive against *A. Niger*. Vb was found to be inactive except *E. Coli, B. Cereus, S. Typhi* and *S. Aureus.*

Va, Vcare more active. This compound can be used as antimicrobial agent on *S. aureus, B. Cereus, S. Typhi, Trichodema*and *E. coli*.

Acknowledgment

Author is thankful to department of microbiology, Prof. Amol Nagrale, Shegaon for help on antimicrobial study. Also thankful to Dr. R. D. Isankar sir, Dr. D.T. Tayade sir and Dr P.V. Raut sir for their valuable guidance.

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