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Research Article

Synthesis and Screening of Some New 2-Amino Substituted Benzothiazole Derivatives for Antifungal Activity

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ABSTRACT

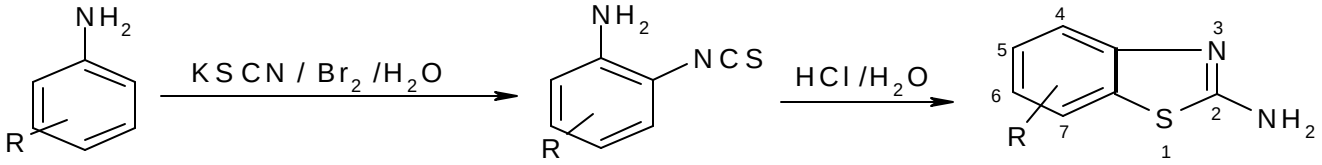
Some new 2-Amino substituted- benzothiazole ($\mathbf{A_{1\text{-}7}}$) were synthesized by treating with KSCN in presence of glacial acetic acid and with different Substituted aniline. Structures of the synthesized compounds were established on the basis of Melting Point, TLC, and IR spectral data. The anti-fungal activity of the synthesized compounds was evaluated by disc diffusion method.

Keywords: Benzothiazole, Synthesis, Aniline and Antifungal activity.

INTRODUCTION

A number of benzothiadiazoles showed selective antiproliferative activity, especially the phenyl-substituted benzothiazoles while condensed pyrimido benzothiazoles and benzothiazolo quinazolines exert antiviral activity. Substituted 2-(4-aminophenyl) benzothiazoles were developed and examined, *in vitro*, for their antiproliferative activity in ovarian, breast, renal and colon carcinoma human cell lines,imidazo benzothiazoles,as well as, polymerized benzothiazoles and other substituted benzothiazoles showed remarkable antitumor activity against malignant cell lines[1]. A series of potent and selective antitumor agents, mostly from substituted 2-(4-aminophenyl) benzothiazoles, were developed and comprised a novel class of antitumor active compounds, especially against sensitive breast tumor cell lines, *e.g*., MCF-7 and MDA 468 and extended to certain colon, lung, melanoma, renal, and ovarian tumor cell lines. Pyrimido benzothiazole and benzothiazolo quinoline derivatives, imidazo benzothiazoles as well as polymerized benzothiazoles showed remarkable anti-tumor activity [2] .

In recent year heterocyclic compounds analogues of benzothaizoles and derivatives have attracted strong interest due to their useful biological and pharmacological properties. Many of them shows antifungal, antibacterial, anticancer, anti-inflammatory, anticonvulsant and antiproliferative activities as well as inhibitory effects for thymidylate synthase and poly-(ADP-ribose) polymerase (PARP). Quinazolin-1-one derivatives are also found to be tranquilizer, antiallergic agent, an antiulcer agent, antiasthmatic agent [3], antifungal [4], [5], anti-inflammatory [6], analgesic [7], and anticancer[8], [9] activity. Benzothiazoles are also found to be antimicrobial [10], antifungal[11],[12], anticancer[13],[14] and anti-inflammatory[15],[16] activity. These biological data prompted us to synthesize some new benzothiazole derivatives.



Aniline 2-Amino Benzothiazole (A1-7)

Scheme 1

Experimental

Melting/boiling points of all the synthesized compounds

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Table 1. Characterization of the compounds (A_{1-7})

Code	Substituted Aniline(R)	Name of the compound	Molecular Formula	Molecular Wt.	% yield	M.P. ^{0}C	Rf	IR (KBr, cm^{-1})
A_1	P-COOH	2-amino 6-carboxylic benzothiazole	$C_8H_6N_2O_2S$	194.21	66.9	207	0.28	3454.29, 3269.35 (NH_2 stretching), 3087.47 (aromatic C-H stretching), 1632.11 (C=N)
A_2	4- Nitro	2-amino 6-Nitro benzothiazole	$C_7H_5N_3O_2S$	195.20	71.2	198	0.91	3444.09, 3344.86 (NH_2 stretching), 3228.16 (aromatic C-H stretching), 1635.60 (C=N)
A_3	3-Nitro	2-amino 7-Nitro benzothiazole	$C_7H_5N_3O_2S$	195.20	74.2	298	0.51	3386.22 (NH stretching), 3087.47 (aromatic C-H stretching), 1637.15 (C=N)
A_4	2-nitro	2-amino 4-Nitro benzothiazole	$C_7H_5N_3O_2S$	195.20	79.6	253	0.62	3480.96, 3365.85 (NH_2 stretching), 3091.60 (aromatic C-H stretching),1635.35 (C=N
A_5	4-Bromo	2-amino 6-Bromo benzothiazole	$C_7H_5BrN_2S$	229.10	66.9	203	0.28	3456.78, 3293.77 (NH_2 stretching), 3060.69 (aromatic C-H stretching), 1651.58 (C=N)
A_6	P-Chloro	2-amino 6-Chloro benzothiazole	$C_7H_5ClN_2S$	184.65	71.2	193	0.61	3456.45, 3293.54 (NH_2 stretching), 3037.36 (aromatic C-H stretching), 1651.58 (C=N).
A_7	2,4-di-Nitro	2-amino 4, 6-di Nitro benzothiazole	$C_7H_4N_4O_4S$	240.20	6 1.7	175	0.34	3517.22, 3458.06 (NH_2 stretching), 3081.02 (aromatic C-H stretching), 1648.07 (C=N).

Table 02: MIC range (µg/mL) of the title compounds for Antifungal Activity

Compound	*C. albicans*	*A. niger*	*A. flavus*
Title compounds (A_1 to A_7)	0.63 < 0.31	0.63 < 0.31	0.63 <0.31
Standard Drug (Clotrimazole)	0.16< 0.08	0.31 <0.16	0.31 <0.16

Table 03 Accurate MIC (µg/mL) of the title compounds for Antifungal Activity

Compound Code No.	*C. albicans*	*A. niger*	*A. flauus*
A_1	0.50	0.60	0.60
A_2	0.50	0.60	0.60
A_3	0.45	0.55	0.60
A_4	0.45	0.55	0.60
A_5	0.35	0.40	0.35
A_6	0.50	0.60	0.55
A_7	0.35	0.40	0.35
*Standard	0.10	0.30	0.30

*Clotrimazole

were determined by Thermonik Melting Point Boiling Point Determination Apparatus in open capillary tubes and uncorrected. The intermediate compounds synthesized were confirmed on the basis of their melting/boiling points reported in the literature and the functional group tests. All the solvents were used after purification, distillation and dried. Silica gel GF_{254} plates from e-Merck and Co., were used for TLC and spots located either by UV, dipping in potassium permanganate solution Column chromatography was performed on a neutral silica gel column (2.5 × 45 cm) using a suitable eluent. The IR spectra (KBr) were recorded on Nicolet impact-410FT using IR grade KBr dics. The physical data of the synthesized compounds were presented in Table 1.

Preparation of 2-amino benzothiazole

A solution of substituted aniline (0.085mol)in 95% acetic acid (50ml) was added to a solution of KSCN(0.308mol) in 95% acetic acid (100ml) .The mixture was cooled to 0°C & a solution of Br_2 (7.5ml) in acetic acid (30ml) was added slowly with stirring so that temp between 0&10°C. After addition was complete, the stirring was continued for 1hr at 5°C & then the mixture was poured into water .The solid was collected & re-crystallized from ethanol. The product (0.036mol) conc. HCl (27ml) and water (54ml) were refluxed for 2hr. The solution was cooled and the product was filtered off, washed with water & re-crystallized from ethanol to yield (mp-216 to 218°C).

The benzothiazole HCl salt was prepared from a suspension of 2-amino benzothiazole (0.25gm) in a dry toluene (25ml). The suspension was cooled to 0°C & saturated with dry HCl gas. After 5hr white solid ppt & was collected filter & washed with ethanol (Scheme 1).

Anti-fungal activity

All the newly synthesized compounds (A_{1-7}) were evaluated for *in vitro* antifungal activity against fungal strains such as *Candida albi cans, Aspergillus niger, Aspergillus flauus at* concentration 10 µg/mL by using serial dilution method[17] by using DMSO as solvent control. All experiments were performed in triplicate.

Experimental

Antifungal activity of the title compounds was carried out by

Preparation of Stock solution

Stock solutions of the test compounds and the standard drug having the concentration 10µg/mL were prepared in dimethyl sulfoxide (DMSO). Further dilutions were made from these stock solutions.

Preparation of Media

The following media were used for this study [18].

1. Sabouraud Liquid Medium (SLM)
2. Double Strength Sabouraud Liquid Medium (DSSLM)

The ingredients were mixed and boiled to affect the solutions. The pH was adjusted to 5.6 ± 0.2 after sterilization.

Sterilization

The sterilization of culture media, culture tubes, saline (NaC1, 0.9% w/v, in distilled water) and other materials was done by autoclaving at 15 lb/sq. inch pressure for 20 minutes.

Stock culture and inoculum

A loopful of fungal strain was transferred aseptically into the sterilized SLM and incubated at 25 ± 1°C for 48 h and 7 days respectively for *Candida albicans* and *Aspergillus* species *(A. niger* and *A.* flavus). This was taken as the stock culture. The fungal strain was subcultured by transferring aseptically a loopful of corresponding organism from the stock culture into the sterilized SLM and incubated as above. *C. albicans* culture was harvested by using sterilized saline solution and diluted suitably with the sterilized saline solution to get the spore count about 1 x 10^7 CFU/mL. Similarly *Aspergillus* species cultures were harvested with sterilized saline solution containing 0.05% w/v of polysorbate80.[19] and adjusted the spore count to about 1 x 10^7 CFU/mL with sterilized saline solution. An aliquot (0.1 mL) of this saline solution consisting of fungal strain was used for inoculation of the culture tubes.

Determination of the MIC range

The MIC range for the title compounds and standard drug are given in Table 02. The accurate MIC of all the title compounds and standard drug for a particular fungal strain was determined by making further dilutions between the observed MIC ranges.

Determination of accurate MIC of standard drug

Stock solution of the standard drug was diluted appropriately in DMSO to get the solutions having the following concentration ranges:

Standard drug solution	Conc. range (µg/mL)
Series A	0.64—0.30
Series B	0.34—0.14

The culture tubes were prepared and inoculated by adopting the similar procedure as used for the title compounds and culture tubes having the concentration ranges 0.32 - 0.15 µg/mL (against A. niger and A. flavus) and 0.17- 0.07 µg/mL (against C. albicans) were obtained from the above series of standard drug solutions A and B respectively. The results are shown in Table 03.

CONCLUSION

All the synthesized title compounds (A_1 — A_7) were evaluated for their antifungal activity as well and showed moderate activity with respect to standard drug. The MIC range was found to be 0.63-0.31 µg/mL for all title compounds. The title compounds showed no significant difference in their MIC values; however the compounds having the substituents like bromo or di-nitro exhibited more activity than rest of the title compounds. However, the activities of the tested compounds are much less than those of standard antifungal agent used.

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