Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/1195
DOI URL: http://dx.doi.org/10.21474/IJAR01/1195
ISSN NO. 2320-5407

RESEARCH ARTICLE

ANTIMICROBIAL AND ANTICANCER ACTIVITIES OF SYNTHESIZED AZO COMPOUNDS OF 2-AMINO BENZOTHIAZOLE .

Jasmin Sugantha Malar S[1] and Abbs Fen Reji TF[2].

1. Department of Chemistry and Research Centre, Women's Christian College, Nagercoil, Tamilnadu, India.
2. Department of Chemistry and Research Centre, Nesamony Memorial Christian College, Marthandam, Tamilnadu, India.

Manuscript Info

Manuscript History

Received: 12 June 2016
Final Accepted: 16 July 2016
Published: August 2016

Key words:-
Thiazole, Antimicrobial, Anticancer, Disc diffusion, Broth dilution.

Abstract

In this investigation, azo compounds were synthesized in excellent yields *via* the diazotization of substituted 2-amino benzothiazole followed by coupling with aromatic compounds like 1-naphthol, 2-naphthol, resorcinol, phenol and para nitro phenol. The newly synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR ,^{13}C NMR and mass spectral techniques, and have been tested *in vitro* against a number of microorganisms in order to assess their antimicrobial properties using disk diffusion method and the minimum inhibitory concentrations (MIC) by the broth micro dilution technique. Their anticancer activity against human breast cancer cell line (MCF7) were determined by MTT assay.

Introduction:-

Benzothiazole is a privileged bicyclic ring system. It contains a benzene ring fused to a thiazole ring. The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities like-antimicrobial (Javed S. A. *et. al.*, 2004, Bujdakova H. *et. al.*, 1994), antitubercular (Bhusari K. P., 2000), antitumour (Moustafa T. Gabr *et. al.*, 2014), antimalarial (Souvik Sarkar *et. al.*, 2016) , anticonvulsant (Alam M. *et. al.*, 2004), anthelmintic (Balaji P. N., *et. al.*, 2014), analgesic (Siddiqui N. *et. al.*,2004) and anti-inflammatory (Venkatesh P. *et. al.*, 2009).

Being a heterocyclic compound, benzothiazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. Its aromaticity makes it relatively stable, although as a heterocycle, it has reactive sites, which allow for functionalization. Benzothiazole is a colorless, slightly viscous liquid with a melting point of 2 °C, and a boiling point of 227-228 °C. The density of benzothiazole is 1.644 g/ml, and molecular mass is 139.19 g mol^{-1}. Benzothiazole has no household use. It is used in industry and research. A large number of therapeutic agents are synthesized with the help of benzothiazole nucleus. During recent years there have been some interesting developments in the biological activities of benzothiazole derivatives. These compounds have special significance in the field of medicinal chemistry due to their remarkable pharmacological potentialities.

Corresponding Author:- Dr. S. Jasmin Sugantha Malar
Address:- Department of Chemistry and Research Centre, Women's Christian College, Nagercoil, Tamilnadu, India.

Thus the important role displayed by benzothiazole and its derivatives for various therapeutic and biological activities prompted us to synthesize some azo compounds bearing benzothiazole moiety in order to achieve compounds having better therapeutic activities. In this present work, we have synthesized and characterized five azo compounds namely 2-[1-hydroxynaphthyl]- azobenzothiazole, 2-[2-hydroxynaphthyl]-azobenzothiazole, 2-[2,4-dihydroxyphenyl]-azobenzothiazole, 2-[4-hydroxyphenyl]-azobenzothiazole and 2-[2-nitro-5-hydroxyphenyl]-azobenzothiazole.

Experimental:-

All chemicals used were of analytical grade and were obtained from Merck, Nice and CDH. Melting points were determined in open capillary tubes and were uncorrected. The purities of the compounds were checked by thin layer chromatography (TLC) using silica gel plates (Merck) and chloroform : ethanol as a solvent system. The spots were developed in an iodine chamber and visualized under ultraviolet (UV) lamp. The IR spectra of azo compounds were recorded on Schimadzu FTIR spectrophotometer model 8400 S in KBr wafer, the NMR spectra were obtained on 400 MHz FT NMR spectrometer using $CDCl_3$ as solvent and reported relative to TMS as internal standard and mass spectrum was taken using mass spectrometer by LCQ technique.

Synthesis of azo compounds:-

Azo compounds were synthesized according to the method reported in literature(Vogel A.I., 1989).
There are two steps in the synthesis of azo compounds:

Diazotisation of 2-amino benzothiazole:-

A solution 2-amino benzothiazole (10 mmol) and 8 mL of 3 M HCl was heated gently, then water (10 mL) was added in order to dissolve the solid. The mixture was cooled to 0^{o} C in an ice bath with stirring. This solution was cooled to 0–5 ºC, and a freshly prepared solution of 1 M sodium nitrite (10 mL) was then added drop wise, maintaining the temperature below 5 º C. The solution was kept in an ice bath and used immediately in the next step.

Coupling with aromatic compounds:-

The aromatic compound taken (10 mmol) was dissolved in 10 mL of 2 M sodium hydroxide, and cooled to 0-5 ºC in an ice bath. This solution was then gradually added to the cooled benzene (or substituted) diazonium chloride solution. The resulting mixture was stirred at 0–5°C for at least 15 minutes until the crystallization is complete (giving a coloured solid). The pH of the solution was adjusted with dilute HCl or NaOH solutions (0.1 M) in order to induce precipitation. The resulting coloured precipitate was filtered, washed several times with cold water and was recrystallized from hot chloroform to yield azo compound. Azo compounds were synthesized according to following **scheme 1**.

Scheme -1

Step I.

Diazotisation of 2-amino benzothiazole.

The physical and analytical data obtained for the synthesised compounds are shown in **Table 1**.

Table 1:- Physical and Analytical data of substituted azo compounds of 2-amino benzothiazole.

Compd	R	M. P. (°C)	Colour	Yield [%]	Molecular formula	M. W
1	2-hydroxynaphthyl	75	Red	76	$C_{17}H_{11}N_3SO$	305.35
2	1-hydroxynaphthyl	106	Coffee brown	80	$C_{17}H_{11}N_3SO$	305.35
3	2,4-dihydroxyphenyl	81	Scarlet red	87	$C_{13}H_9N_3O_2S$	271.29
4	4-hydroxyphenyl	68	Brick red	93	$C_{13}H_9N_3OS$	255.30
5	2-nitro-5-hydroxyphenyl	68	Orange	78	$C_{13}H_8N_4O_3S$	300.29

Antimicrobial activity:-

The synthesized azo compounds were screened for the presence of antibacterial constituents against four strains of bacteria *i.e, Staphylococcus aureus*, *Klebsiellapneumoniae*, *E.Coli*, *Streptococcus,* and two species of fungi *i.e.,* against *Candida albicans* and *Candida glabrata* by disc diffusion method(Jhon Oakes *et. al.,* 1998, Fish L. L. *et. al.,* 1985, Abou-Zeid *et. al.,* 1969). The bacterial inhibition zone values are summarized in **table 2**. All the azo compounds showed remarkable activity against used microbes and results were compared with standard drugs(Amikacin, Fluconozole).

Table 2:- Antimicrobial activity of substituted azo compounds of 2-amino benzothiazole.

Compd	R	*Klebsiella pneumoniae (-)*	*E.coli (-bacilli)*	*Staphylococcus aureus (+cocci)*	*Streptococcus (+cocci)*	*Candida albicans*	*Candida glabrata*
1	2-hydroxynaphthyl	15	16	15	15	10	12
2	1-hydroxynaphthyl	14	13	15	12	14	10
3	2,4-dihydroxyphenyl	15	15	12	10	12	12
4	4-hydroxyphenyl	12	12	10	12	14	11
5	2-nitro-5-hydroxyphenyl	15	15	11	10	11	13
Std		20	22	23	20	16	15

Minimum Inhibitory Concentration (MIC):-

The minimal inhibitory concentration (MIC) was determined by broth dilution method(Tang H. A. *et. al.,* 2003).The MIC value was defined as the lowest concentration of compounds whose absorbance was comparable with the negative control wells (broth only, without inoculum). The MIC values are reported at **table 3**.

Table 3:- MIC value of substituted azo compounds of 2-amino benzothiazole.

Compd	R	*Klebsiella pneumonia (-)*	*E.coli (-bacilli)*	*Staphylococcus aureus (+cocci)*	*Streptococcus (+cocci)*	*Candida albicans*	*Candida glabrata*
1	2-hydroxynaphthyl	1400	1600	1600	1400	1600	1400
2	1-hydroxynaphthyl	1400	1600	1400	1600	1600	1600
3	2,4-dihydroxyphenyl	1400	1200	1200	1400	1200	1400
4	4-hydroxyphenyl	1400	1200	1400	1600	1600	1400
5	2-nitro-5-hydroxyphenyl	1200	1400	1600	1600	1400	1400

Anticancer activity:-

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37° C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week(Mosmann T., 1983, Monks A. *et.al.,* 1991).

The % cell inhibition was determined using the following formula.
% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC_{50} was determined using Graph Pad Prism software.

Results and discussion:-

In this study five azo compounds were synthesized by coupling of substituted 2-amino benzothiazole with different aromatic compounds. They were characterized by IR, UV, ^{1}H NMR, C^{13} NMR and mass spectrum.

Spectroscopic characterization of synthesized compounds:-

UV spectra:-

All the synthesized compounds show λmax in the region 350 – 370 nm, confirms the presence of N=N bond in these compounds.

IR spectra:-

The glance at the structure of azo compounds, one may expect the absorption bands due to N=N, -N-H, C-H=C-H and C-N vibrations in IR region. All the synthesized compounds showed absorption bands for different types of vibrations which were shown by azo compounds. This confirms the success of the synthesis**.**

Nuclear Magnetic Resonance Spectra:-

^{1}H NMR spectra:-

The ^{1}H NMR (300 MHz, DMSO-d_6) spectrum of compound I shows a singlet at δ 5.5 is assigned to hydroxyl hydrogen of 2-naphthol. The multiplet at δ 7.13 - 7.24 is assigned to H-3 of naphthyl ring and H-7 of benzothiazole ring. The multiplet at δ 7.28 – 7.37 is assigned to H-1 and H-6 of naphthyl ring. The singlet at δ 7.42 – 7.52 is due to H-7 of naphthyl ring and H-5, H-6 of benzothiazole ring. The doublet at δ7.6-7.67 has been attributed to H-4 and H-8 of naphthyl ring. The multiplet at δ 7.7 – 7.78 is attributed to H-5 of naphthyl ring.

^{13}C NMR spectra:-

The ^{13}C NMR (75 MHz, DMSO-d_6) spectrum of compound III has six peaks: three peaks due to three carbon each and two peaks due to one carbon each and one peak accounting two carbons. Thus the spectrum accounts for all the thirteen carbons respectively.

Mass spectra:-

The FAB MS shows a strong MH^+ peak at m/z 306 for compound II confirmed the molecular weight of the compound.

Antimicrobial activity:-

The synthesized azo compounds of benzothiazole showed bactericidal and fungal activity. All the compounds were found to exhibit moderate to good antifungal activity against both the tested bacteria and fungi.

Minimum inhibitory concentration (MIC):-

MIC is the lowest amount of drug at which, it is able to inhibit the growth of specified microorganism. MIC value of the synthesized azo compounds were calculated against four strains of bacteria *i.e, Staphylococcus aureus, Klebsiellapneumoniae, E.Coli, Streptococcus* and two species of fungi *i.e.*, against *Candida albicans* and *Candida glabrata* using broth micro dilution method. The MIC value for all the synthesized compounds were found to be between 1200-1600 μg/mL.

Anticancer activity:-

The synthesized azo compounds of benzothiazole were screened for their anticancer activity using the human breast cancer cell line (MCF 7) and it was observed that the compound I had shown prominent anticancer activity. IC_{50} value is 50.73 μM.

Acknowledgements:-
The authors are grateful to SAIF, Lucknow and CECRI, Karaikudi for providing necessary facilities during the tenure of this work.

Conclusion:-
Modifications on the benzothiazole nucleus have resulted in a large number of compounds having diverse pharmacological activities. The synthesis, structures and biological activities of benzothiazole derivatives have long been focused of research interest in the field of medicine, due to potential activities exhibited by them. The biological profiles of these new generations of benzothiazoles represent much progress with regards to older compounds. Looking into the medicinal importance of benzothiazole moiety, it was thought worthwhile to synthesize certain newer derivatives of benzothiazole and screen them for their biological activities.

References:-
1. Abou-Zeid, Abou-Zeid A., Shehata and Youssef, Indian J Pharm., **1969,** 31(3), 72.
2. Alam M. and Siddiqui N. Synthesis of new benzothiazole incorporated sulphonamides as potent aniconvulsants, Indian Journal of Heterocyclic Chemistry, **2004**, 13, 361-4.
3. Balaji P. N., Ranganayakulu D., Ramya Yadav K., Jayamma J., Reddy kumar S. and Sivaramaiah C., Anthelmintic and Anti-microbial activities of synthesized heterocyclic pyrazole and its derivatives from fluoro substituted hydrazino benzothiazole, International Journal of Pharm Tech Research, **2014**, 6(7), 1970-1975.
4. Bhusari K. P., Synthesis and antitubercular activity of some substituted 2-(4-amino phenylsulphonamido) benzothiazoles, Indian Journal Heterocyclic Chemistry **2000**, 9, 213-216.
5. Bujdakova H. and Muckova M., Antifungal activity of a new Benzothiazole derivative against *Candida in vitro* and *in vivo*. International Journal of Antimicrobial Agents, **1994,** 4, 303-8.
6. Fish L. L. and Crumbliss A.L., Inorganic Chemistry, 24, **1985**, 2198.
7. Javed S. A., Siddiqui N. and Drabu S., Synthesis and antibacterial activiy of some 2- [(4α- halophenyl) thioureido]-6-substituted benzothiazoles. Indian Journal of Heterocyclic Chemistry, **2004**, 13, 287-8.
8. Jhon Oakes and Gratton, Journal of Chemical Society, PerkinTrans, 2, **1998**, 2201- 2206.
9. Monks A., Scudiero D., Skehan P., Shoemaker R., Paull K., Vistica D., Hose C., Langley J., Cronise .P, Vaigro-Wolff A., Gray-Goodrich M., Campbell H., Mayo J. and Boyd , Journal of the National Cancer Institute, 83, **1991**, 757-766.
10. Mosmann T., Journal of Immunological Methods, 65, **1983**, 55-63.
11. Moustafa T. Gabr, Nadia S. El-Gohary, Eman R. El-Bendary and Mohamed M. El- Kerdawy Synthesis and *in vitro* antitumor activity of new series of benzothiazole and pyrimido[2,1-*b*]benzothiazole derivatives, European Journal of Chemistry, **2014**, 85, 576-592.
12. Siddiqui N, Alam M, Siddiqui AA. Synthesis and analgesic activity of some 2-[{4- (Alkyl thioureido) phenyl}sulphonamido]-6-substituted benzothiazoles, Asian Journal Chemistry, **2004**, 16, 1005- 8.
13. Souvik Sarkar, Asim A Siddiqui, Shubhra J Saha, Rudranil De, Somnath Mazumder, Chinmoy Banerjee, Mohd S Iqbal, Shiladitya Nag, Susanta Adhikari and Uday Bandyopadhyay, Antimalarial activity of small-molecule benzothiazole hydrazone, *Antimicrobial Agents Chemotherapy, **2016**, 60(7), 4217-28.*
14. Tang H. A., Wang L. F. and Yang R. D., Transition Metal Chemistry, 28(4), **2003**, 395- 398.
15. Venkatesh P. and Pandeya S.N., Synthesis, characterisation and anti-inflammatory activity of some 2-amino benzothiazole derivatives, International Journal of Chem Tech Research, **2009**, 1(4), 1354-1358.
16. Vogel A.I., Text book of Practical Organic Chemistry, 5 th ed ELBS and Longman, London, UK, **1989**, 946.