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Synthesis of iminosaccharides of 2-aminobenzothiazole : A comparative study of conventional and green chemical routes

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Abstract: In this study, iminosaccharides of 2-aminobenzothiazole were synthesized using three different methods *i.e.* conventional synthesis, microwave assisted synthesis using ethanol as a solvent and microwave assisted solvent free synthesis using HY Zeolite as a solid support. Comparison of all mentioned methods has been presented, which clearly indicates that solvent free synthesis of iminosaccharides of 2-aminobenzothiazole in microwave afforded better yield in short time via eco friendly route as compared to other two methods. Iminosaccharides of 2-aminobenzothiazole was synthesized by the reaction of 2-aminobenzothiazole (**1**) with various hexoses (**2 a-e**). Structures of all the synthesized products have been elucidated on the basis of their elemental and spectral analysis.

Keywords: Iminosaccharides, 2-Aminobenzothiazole, Microwave irradiation, HY zeolite, Green synthesis.

Introduction

"Better things for better living through chemistry" has always been the motto of chemists to improve the general quality of life but there are some adverse outcomes due to all these endeavors undertaken by the scientists all over the world. That is why there is an increasing local and global concern for environmental pollution. This offers incentive to explore new greener route for chemical synthesis. Hence, efforts to minimize the major disadvantages of conventional synthesis, i.e. pollution, led to the advent of green chemistry. Microwave assisted organic synthesis (MAOS) is an important part of green chemistry. In the recent year MAOS has emerged as a new tool in organic synthesis [1-

4]. The salient features of these high yield protocols are the enhanced reaction rates, greater selectivity and the experimental ease of manipulation.

Solvent creates environmental pollution in traditional methods and microwave-assisted synthesis also. But, if the reaction is carried out in a solvent-free condition by using clays, silica, zeolite or any other solid support, then it will be a green chemical method. Such reactions, not only take care of the environment but these are having easier work up and time saving also [5-8]. Therefore, a microwave-assisted synthesis on solid support avoids the use of solvent and it provides a clean and efficient technology giving higher yields in relatively in short time period and it is economically viable also.

Iminosaccharide are five or six member ring sugar analogues where a nitrogen atom has replaced the oxygen atom in ring of the structure. In other words, iminosaccharide are small organic molecules, which mimic monosaccharides but contain a nitrogen atom in place of the endocyclic oxygen. Iminosaccharide are important class of biologically compounds and play a vital role in medicinal chemistry. Iminosaccharide derivatives exert antiviral effects against several human viral pathogens including HIV, HBV, Dengue and Japanese Encephalitis viruses [9]. Iminosaccharides show anti-diabetic and anti-cancer activities with glycosidase and β-glycosidase inhibitory properties and work as therapeutic agents [10-16].

In view of the role of iminosaccharides in medicinal chemistry and urgent need of its green synthesis, it was planned to synthesis iminosaccharides of 2-aminobenzothiazole using three different methods including conventional and green synthesis and represents a comparative study for all these methods.

Results and Discussion

The structures of the final products were established on the basis of their analytical and spectral data. Disappearance of two bands at 3400 and 3260 cm^{-1} due to $-NH_2$ stretching (symmetric and asymmetric) and appearance of bands in the region of 3300-3450 cm^{-1} for OH stretching and 1620-1640 cm^{-1} corresponding to C=N stretching confirms the assigned structure of iminosaccharides. In ^1H NMR spectrum the absence of signals at δ 5.81-5.88 due to proton of NH_2 and presence of a doublet for imino protons (CH=N-) at δ 8.50 and a singlet for OH proton at δ 5.01 favors the formation of final product **(3 a-e)**. The signals of proton of sugar chain were congregated with the solvent

absorption in a broad signal at δ 4.49-3.60. Further, mass spectrum also supported the structure of iminosaccharides.

Reaction of 2-aminobenzothiazole (**1**) with various hexoses (**2 a-e**) in ethanol using acetic acid as a catalyst afforded iminosaccharides (Schiff bases) with azomethine linkage **(3 a-e)**. In this conventional method, 10-15 hours of refluxing is required. When the same reactions were carried out in microwave, the reaction time was reduced from hours to minutes and the yield of products was also increased. But in these processes ethanol and acetic acid is still to be used, which is not eco-friendly. Therefore, iminosugars were synthesized using zeolite as a solid support under microwave irradiation *i.e.* under solvent free conditions. As a result, solvent was eliminated from the synthesis and reaction time was also found to be reduced and the yield of product was also increased. A comparison of time required for synthesis of product and yield of products in conventional method and microwave methods (with and without using NaY zeolite) has been reported in **Table 1**.

Table 1: Physical data of synthesized compounds

Compounds	**R**	**Mol. Formula**	**Mol. Weight**	**M.P. (°C)**	**Yield[a] (%) [Time] (hr.)**	**Yield[b] (%) [Time] (min.)**	**Yield[c] (%) [Time] (min.)**
3a	D-Glucose	$C_{13}H_{16}N_2O_5S$	312	255-258	87 [12-14]	88 [17]	93 [14]
3b	D - Galactose	$C_{13}H_{16}N_2O_5S$	312	270-274	72 [12-13]	75 [16]	92 [10]
3c	D- Allose	$C_{13}H_{16}N_2O_5S$	312	292-296	68 [12-14]	75 [17]	92 [15]
3d	D-mannose	$C_{13}H_{16}N_2O_5S$	312	267-271	83 [13-15]	88 [18]	95 [12]
3e	D-Altrose	$C_{13}H_{16}N_2O_5S$	312	249-252	84 [13-16]	90 [20]	97 [15]

a = Conventional synthesis, b = Microwave assisted synthesis using solvent, c = microwave assisted solvent free synthesis using zeolite as a solid support

The compounds have duly characterized on the basis of IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis.

Compound **3a:** IR (KBr): 3430, 3329 (OH str.), 3085 (CH str., Ar-H), 2940, 2880 (CH str.), 1590 (cyclic C=N str.), 1638 (open C=N str.), 1530, 1460, and 1423 (C=C str.) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.50 (d, 1H, CH=N), 7.76 – 7.10 (m, 9H, Ar-H), 5.01 (s, 1H, OH), 4.49–3.60 (sugar proton); ^{13}CNMR: 68.34, 70.12, 72.32, 73.21, 74.21, 117.54, 118.43, 121.11, 122.78, 135.92, 156.11, 168.54, 170.05; Mass (m/z): 312 $[M]^{+\cdot}$, 178 [M-C_7H_4NS], 161 [M-$C_5H_{11}O_5$], 164 [M-$C_9H_6N_2S$], 151 [M-$C_8H_5N_2S$], 148 [M-$C_6H_{12}O_5$], 134 [M-$C_6H_{12}O_5N$], 66 [M-$C_7H_{12}NO_5S$]; Analytical data calculated / Found: C - 49.99 / 49.95, H - 5.16 / 5.18, N - 8.97 / 8.99

3b: IR (KBr): 3428, 3320 (OH str.), 3088 (CH str., Ar-H),2942, 2882 (CH str.),1596 (cyclic C=N str.), 1636 (open C=N str.), 1533, 1464,1428 (C=C str.); ^{1}HNMR (400 MHz, DMSO-d_6)**:** 8.59 (d, 1H, CH=N),7.78 – 6.97 (m, 9H, Ar-H),5.11 (s, 1H, OH),4.56 – 3.39 (sugar proton): ^{13}CNMR:67.12, 70.12, 71.65,72.34,73.89, 118.23, 119.02, 121.11, 123.55, 136.23, 158.10, 169.62, 171.90: Analytical data calculated / Found: C- 49.99 / 49.94, H- 5.16 / 5.21, N- 8.97 / 8.99

3c: 3424 (OH str.), 3078, 3310 (CH str., Ar-H),2944, 2889 (CH str.), 1594 (cyclic C=N str.), 1630 (open C=N str.), 1540, 1462, 1412 (C=C str.); ^{1}HNMR (400 MHz, DMSO-d_6)**:**8.59 (d, 1H, CH=N), 7.78 – 6.97 (m, 9H, Ar-H), 5.11 (s, 1H, OH),4.56 – 3.39 (sugar proton): ^{13}CNMR:68.2 72.11, 74.25, 74.81, 75.21, 120.12, 120.56, 121.56,122.90, 139.11, 160.16, 170.78, 174.67. Analytical data calculated / Found: C- 49.99 / 49.98, H- 5.16 / 5.19, N-8.97 / 9.00

3d: 3426, 3312 (OH str.), 3087 (CH str., Ar-H),2945, 2886 (CH str.), 1593 (cyclic C=N str.), 1633 (open C=N str.), 1533, 1455, 1418 (C=C str.),^{1}HNMR (400 MHz, DMSO-d_6)**;** 8.54 (d, 1H, CH=N), 7.75 – 7.16 (m, 9H, Ar-H), 5.09 (s, 1H, OH), 4.53 – 3.62 (sugar proton) ^{13}CNMR: 69.11, 73.34, 73.88, 74.12, 75.21, 120.11, 121.56, 121.89,122.03, 138.22, 158.21, 168.34, 172.69. Analytical data calculated / Found: C- 49.99 / 49.96, H- 5.16 / 5.20, N-8.97 / 9.01

3e: 3428, 3334 (OH str.), 3082 (CH str., Ar-H), 2943, 2877 (CH str.), 1600 (cyclic C=N str.), 1636 (open C=N str.),1528, 1461, 1424 (C=C str.), ^{1}HNMR (400 MHz, DMSO-d_6);

8.60 (d, 1H, CH=N), 7.70 – 7.21 (m, 9H, Ar-H),5.12 (s, 1H, OH), 4.44 – 3.63 (sugar proton),^{13}CNMR:69.21,70.29.70.91,71.45,71.78,119.01,120.56,120.94,121.11,139.62,159.56, 169.23,172.34. Analytical data calculated / Found: C- 49.99 / 50.01, H-5.16 / 5.21, N-8.97 / 8.92.

Antimicrobial Activity:

Five synthesized compounds (**3a-e)** were *in vitro* screened for their antibacterial and antifungal activity using 100 ppm concentration in DMF by cup and well method [17]. The micro-organisms used as antibacterial are *Escherichia coli, Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and the fungal strains *Candida albicans* and *Aspergillus fumigatus* were used. The activity is presented as zone of inhibition in mm and compared with activity of controls C_1 (for antibacterial activity C_1= Ciprofloxacin and for antifungal activity C_1= Amphotericin B) giving activity index value (**Table 2**).

All the compounds have shown moderate activity against *P. aeruginosa* where as strong activity against *B. subtilis, P. mirabilis* and *E. coli*. Activity index value against *B. subtilis, P.mirabilis* and *E. coli* was more than one for all the synthesized compounds. It was observed that all the compounds show stronger activity then the standard used against fungal strain *Candida albicans* and *Aspergillus fumigatus.*

It may be concluded from the activity study that compounds **3c** and **3d** were found to be the strongest amongst all synthesized compounds. Compounds have more comprehensive fungus-inhibiting properties than that of the bacteria. Even two folds antifungal activity was observed than standards.

Table 2: Antimicrobial activity of the synthesized compounds on 100 ppm (3a-e).

Zone of Growth Inhibition (mm) (activity index)

Compd. No.	Antibacterial Activity				Antifungal Activity	
	E. coli	*B. subtilis*	*P. mirabilis*	*P. aeruginosa*	*C. albicans*	*A. fumigatus*
3a	23 (1.43)	20 (1.17)	18 (1.12)	19 (1.05)	26 (1.52)	27 (2.70)
3b	24 (1.50)	22 (1.29)	17 (1.06)	18 (1.00)	27 (1.58)	28 (2.80)
3c	25 (1.56)	23 (1.35)	19 (1.18)	20 (1.11)	29 (1.70)	29 (2.90)
3d	21 (1.31)	21 (1.23)	17 (1.06)	17 (0.94)	28 (1.64)	26 (2.60)
3e	21 (1.31)	18 (1.05)	19 (1.18)	17 (0.94)	26 (1.52)	27 (2.70)
C_1	16	17	16	18	17	10

(Activity index) = Inhibition zone of compound/Inhibition zone of the standard drug.

For antibacterial activity: C_1 = Ciprofloxacin

For antifungal activity: C_1 = Amphotericin B

Conclusion

It is clear from **Table 1** that yield of final products is highest for microwave assisted synthesis using zeolite and lowest for conventional synthesis. Moreover, time required for synthesis is lowest for solvent free conditions in microwave. Thus, synthesis of reported product in microwave using zeolite as a solid support and catalyst is an efficient and environmental benign route.

Experimental

All compounds and reagents were commercially available and were used without further purification. All the commercial reagents and compounds were purchased from Sigma Aldrich, Spectrochem, Himedia and Merck. All reactions were carried out in a domestic microwave oven (Videocon, Model No.–VH19SWWM-MM2). Melting points were determined in open capillaries and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) using TLC plate purchased from Merck,

using ethyl acetate: n-hexane (2:8) as eluent and visualization was accomplished by iodine vapors. The IR spectra of compounds were recorded using KBr discs on FTIR RX1 Perkin Elmer Spectrophotometer. The Nuclear Magnetic Resonance spectra (NMR) were recorded on Bruker Advance II 400 spectrometer with DMSO–d_6 as a solvent using TMS as an internal standard. The FAB mass spectra were recorded on JEOL SX-102/DA-6000 mass spectrometer. Elemental analysis was conducted using a Perkin Elmer CHN analyzer.

Conventional synthesis of iminosaccharides of 2-aminobenzothiazole (3 a-e)

2-aminobenzothiazole (**1** 0.01 mol) and various hexoses (**2 a-e** 0.02 mol) were dissolved in ethanol and a few drops of acetic acid were added to it. Then the reaction mixture was refluxed for 15-25 hours. After cooling, the product separated was washed with H_2O and extracted with ethyl acetate. The combined organic layers were dried under anhydrous Na_2SO_4. The solvent was evaporated by vacuum distillation and the crude product was dried and purified by recrystallization from ethanol.

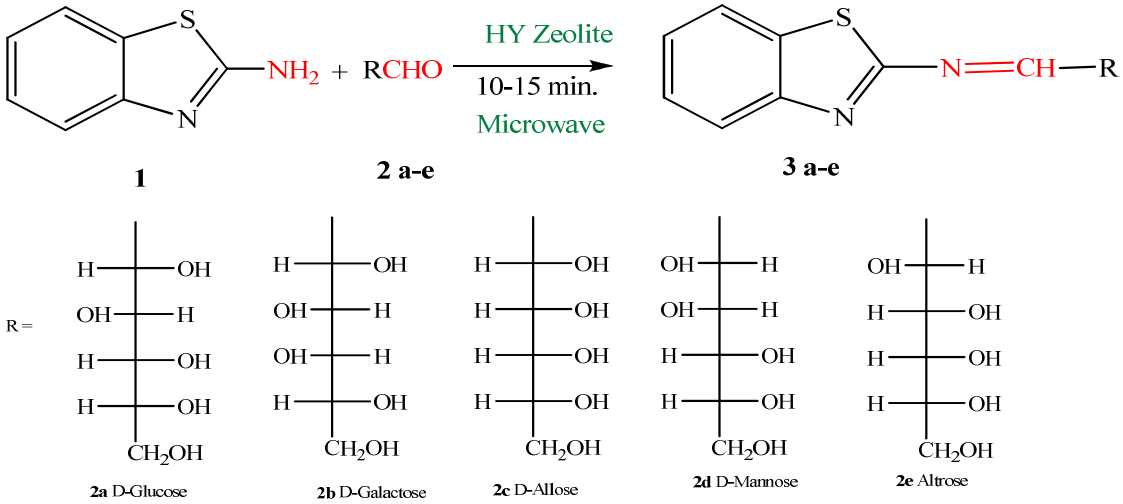
Microwave assisted synthesis of iminosaccharides of 2-aminobenzothiazole (3 a-e)

2-aminobenzothiazole (**1**, 0.01 mol) and various hexoses (**2 a-e**, 0.02 mol) were dissolved in ethanol and a few drops of acetic acid were added to it. The reaction mixture was transferred in an Erlenmeyer flask and irradiated under microwave irradiation for 10 min with a time interval of 30 seconds. After cooling, the product separated was washed with H_2O and extracted with ethyl acetate. The combined organic layers were dried under anhydrous Na_2SO_4. The solvent was evaporated by vacuum distillation and the crude product was dried and purified by recrystallization from ethanol.

Microwave assisted solvent free synthesis of iminosaccharides of 2-aminobenzothiazole using HY Zeolite as a solid support and catalyst (3 a-e)

2-aminobenzothiazole (**1**, 0.01 mol), various hexoses (**2**, a-e 0.02 mol) and HY zeolite (4.0 g) were mixed in pestle and mortar and irradiated under microwave irradiation for 10-15 min. The product was separated from zeolite by stirring it with ethanol for 15-20 min two to three times and evaporating the solvent by vacuum distillation. Product was purified by recrystallization from ethanol. Zeolite was reused after washing with ethanol and drying in air overnight.

The syntheses of compounds (**3 a-e**) are shown in Scheme 1 given below.



Scheme - 1

Acknowledgements

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Reply to Referee

To,
Dr. Daryoush Zareyee
Managing Editor
Iran JOC

Subject: Submission of revised manuscript.

Dear Dr. Zareyee,

We are thankful to you for sending us the referee's comments for our paper. The title of the paper is little modified as "**Synthesis of iminosaccharides of 2-aminobenzothiazole : A comparative study of conventional and green chemical routes**". We have given the answers of referee's comments below.

Answers to referee's comments are given below:

1) Paragraph two in introduction part have been changed and 4 references [5-8] have been quoted.

2) The scheme 1 and Table 2 have properly placed at the appropriate places.

3) Name of the journals has been italicized.

4) Typographical errors in the data analysis and the experimental part have been corrected.

Some questions that should be answered:

5) Zeolite provides a solid support to the reactants by keeping these adsorbed on the surface close to each other, so that on microwave irradiation, the desired reaction takes place.

6) Without zeolite the reactants required solvent ethanol and the yields are reported in Table 1 as Yield[b]. In the absence of ethanol solvent and zeolite, no reaction takes place.

7) Zeolite can be reused 5-6 times without loosing its activity as a solid support, but after this, a slight decrease in the yield of the product has been observed.

8) Quantity of zeolite used was 4.0 g. It was decided on the basis of some experiments performed based on amount of zeolite used. This amount gave optimum yield and therefore it was used in the further investigations.

9) No reaction was observed in the absence of microwaves and in presence of zeolite.

We hope that you will find the revised paper suitable now for publication in your esteemed journal "Iran JOC". Kindly inform us, if there is any more query.

Thanking you.

Yours sincerely,
Dr. Manish K. Rawal and Dr. Nasir Hussain
Department of Chemistry
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Annexure – VII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Title of the project: "Conventional and Microwave Assisted Solvent Free Synthesis of Some Benzothiazole Derivatives and Their Comparison"

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR:

Dr. Manish Kumar Rawal, Department of Chemistry, Vidya Bhawan Rural Institute, Badgaon Road, Udaipur – 313001 (Raj.)

3. NAME AND ADDRESS OF THE INSTITUTION

Vidya Bhawan Rural Institute, Badgaon Road, Udaipur (Raj.), Pin- 313001

4. UGC APPROVAL LETTER NO. AND DATE :

F. No.:MS-50/303013/12-13/CRO Dated 31 March 2013

5. DATE OF IMPLEMENTATION: 14 April 2013

6. TENURE OF THE PROJECT: 18 months

7. TOTAL GRANT ALLOCATED: Rs.1, 00,000.00

8. TOTAL GRANT RECEIVED: Rs. 67500.00

9. FINAL EXPENDITURE: Rs. 90808.00

10. TITLE OF THE PROJECT:

"Conventional and microwave assisted solvent free synthesis of some benzothiazole derivatives and their comparison"

11. OBJECTIVES OF THE PROJECT:

Since a large number of natural products and target drug compounds contain heterocyclic core; thus, heterocyclic are of paramount importance to medicinal and agricultural chemists. The growth of chemistry has been closely associated with the development of such new bio-active molecules, which have been synthesized using newer and greener routes. There is an increased interest in technologies and concepts that facilitates more rapid synthesis. One such high speed technology is Microwave Assisted Organic Synthesis (MAOS). Microwave assisted synthesis allows chemists to work faster, generate higher yields of products and increase the purity with environment friendly process. The efficiency of microwave flash heating in dramatically reducing reactions times (from days and hours to minutes and second) is just one of the many advantages medicinal importance. Microwave assisted synthesis of benzothiazole derivatives of biological and have been reported with so many advantages, but still there are rooms to investigate this field and to develop greener routes to synthesize other useful compounds using microwaves. Therefore, it has been planned to synthesize some benzothiazole derivatives under microwave irradiation using solvent free conditions. The conventional syntheses of heterocyclic compounds have also been compared with microwave assisted synthesis of same compounds.

12. WHETHER OBJECTIVES WERE ACHIEVED

The objectives of project were achieved. As per the scheduled plan of work total of eleven substituted benzothiazoles have been synthesized using solvent free conditions under microwave irradiation. Out of them six diverse aminobenzothiazole were synthesized using NaY zeolite as a solid support in microwave and remaining five

iminosaccharides of 2-aminobenzothiazole were synthesized over HY zeolite under microwave irradiation.

13. ACHIEVEMENTS FROM THE PROJECT

The proposed diverse benzothiazoles were successfully synthesized through microwave method under solvent free conditions. This process provides a green chemical route for the synthesis of diverse benzothiazole in short period of time with better yield of the products.

Iminosaccharides of 2-aminobenzothiazole were synthesized using three different methods *i.e.* Conventional synthesis, microwave assisted synthesis using ethanol as a solvent and microwave assisted solvent free synthesis using HY Zeolite as a solid support. Comparison of all mentioned methods was also presented which clearly indicate that solvent free synthesis of iminosaccharides of 2-aminobenzothiazole in microwave afforded better yield in short time via eco friendly route as compared to remaining two methods.

14. SUMMARY OF THE FINDINGS: Separate sheet attached

15. CONTRIBUTION TO THE SOCIETY

Benzothiazole and its derivatives are of particular interest within the realm of medicinal chemistry. Substituted benzothiazoles possesses antitumor, antimicrobial, vasodilator, antitubercular, antifungal, analgesics, anti HIV and CNS activities. Similarly substituted 2-aminobenzothiazoles possess antimicrobial and various other pharmacological activities like diuretic, antiulcer, antihistamine and anticancer properties. 2-aminobenzothiazoles are also used as an intermediate in the synthesis of antibiotics, sulfa drugs and some dyes.

Iminosaccharide are important class of biologically compounds and play a vital role in medicinal chemistsry. Iminosaccharide derivatives exert antiviral effects against several human viral pathogens including HIV, HBV, Dengue and Japanese Encephalitis viruses. Iminosaccharides show anti-diabetic and anti cancer activities with glycosidase and β-glycosidase inhibitory properties and act as therapeutic agents.

It is clear from above discussion that benzothiazole and its iminosaccharides are very useful compounds in medicinal chemistry and other fields. Several methods for the synthesis of these compounds were reported in literature. It is clear from literature survey that most of the methods for synthesis of 2-aminobenzothiazoles are having restrictions *i.e.* use of acids, use of costly starting material and use of solvents. Moreover these methods are not ecofriendly and create environmental pollution. Therefore, there exists a broad scope for development of such new methods for synthesis of 2-aminobenzthiozoles which can eliminate or minimize the above mentioned drawbacks. One of the best ways to achieve this target is use of microwave assisted synthesis instead of conventional heating. Microwave assisted synthesis is ecofriendly method as it offers a rapid and efficient synthesis. Moreover, the combination of solid support with microwave assisted synthesis controls the requirement of solvents. Avoiding organic solvents during the reactions leads to a clean, efficient and economical technology as safety is largely increased, experimental process is considerably simplified, cost is reduced and pollution due to waste disposal of solvent can be avoided. Thus solvent free synthesis under microwave irradiation provides a green chemical approach for the synthesis of 2-aminobenzothiazoles and its iminosaccharides. We have synthesized total eleven substituted benzothiazoles using solvent free conditions under microwave irradiation. Out

of them six diverse aminobenzothiazole were synthesized using NaY zeolite as a solid support in microwave and remaining five iminosaccharides of 2-aminobenzothiazole were synthesized over HY zeolite under microwave irradiation.

Out of three methods as mentioned earlier, the microwave based methods have been found to give better yields in lesser time than conventional methods. Further elaboration of experimental details leads to the conclusion that solvent free synthesis using HY zeolite presents better option than microwave assisted synthesis using solvent. The reason lies in the fact that the pollutant solvent which is also having other handling difficulties is also removed. Moreover the microwave assisted methods offer an ecofriendly approach to the organic synthesis.

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT:

No

17. NO. OF PUBLICATIONS OUT OF THE PROJECT:

Two papers were communicated for publication, one of which has been accepted and details are as under:

(I) **Journal Name**: Iranian Journal of Organic Chemistry (**Accepted and attached)**
Title of Manuscript: "Comparative study and synthesis of iminosaccharides of 2-aminobenzothiazole: Conventional Method v/s Green route"

Abstract: In this study iminosaccharides of 2-aminobenzothiazole were synthesized using three different methods *i.e.* Conventional synthesis, microwave assisted synthesis using ethanol as a solvent and microwave assisted solvent free synthesis using HY Zeolite as a solid support. Comparison of all mentioned methods was also presented which clearly indicate that solvent free synthesis of iminosaccharides of 2-aminobenzothiazole in microwave afforded better yield in short time via eco friendly

route as compared to remaining two methods. Iminosaccharides of 2-aminobenzothiazole was synthesized by the reaction of 2-aminobenzothiazole (1) with various hexoses (2 a-e). Structure of all the synthesized products have been elucidated on the basis of their elemental and spectral analysis.

(II) Title: "Green synthesis and characterization of diverse 2-aminobenzothiazoles over Nay zeolite"

Abstract: In this study a one-pot strategy for the synthesis of 2-aminobenzothiazole in microwave using dichloroiodate is developed. This green chemical approach is based on the direct reaction of substituted aniline (1a-f) and potassium thiocyanate (2) in the presence of sodium dichloroiodate using NaY zeolite as a solid support under microwave irradiation. Uses of NaY zeolite as a solid support and $NaICl_2$ as a catalyst provide an efficient and green chemical route for synthesis in short time. The synthesized compounds have been characterized on the basis of their elemental and spectral analysis.

(Dr. T. P. Sharma)	**(Dr. Saba Khan)**	**(Dr. Manish Kumar Rawal)**
Principal	**Co Investigator**	**Principal Investigator**

UNIVERSITY GRANTS COMMISSION
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ज्ञान-विज्ञान विमुक्तये

SUMMARY

MINOR RESEARCH PROJECT

Conventional and microwave assisted solvent free synthesis of some benzothiazole derivatives and their comparison

Department of Chemistry

Vidya Bhawan Rural Institute, Udaipur

Principal Investigator

Dr. Manish Kumar Rawal

Co Investigator

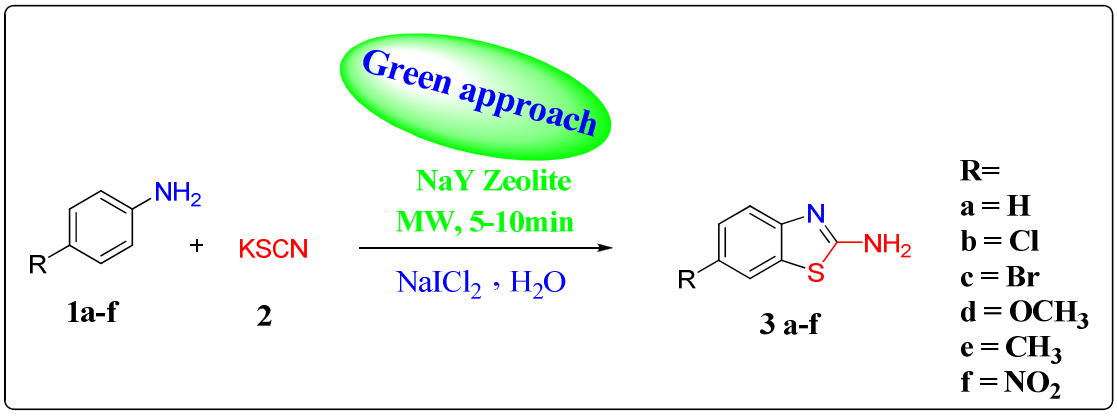
Dr. Saba Khan

14. SUMMARY OF THE FINDINGS

We have synthesized total 11 derivatives of benzothiazole using conventional method and microwave assisted synthesis (using solvent and solvent free conditions). Summary of complete work are described below under two titles A and B.

(A) Synthesis of diverse 2-aminobenzothiazoles over Nay zeolite

In this study a one-pot strategy for the synthesis of 2-aminobenzothiazole in microwave using dichloroiodate is developed. This green chemical approach is based on the direct reaction of substituted aniline (1a-f) and potassium thiocyanate (2) in the presence of sodium dichloroiodate using NaY zeolite as a solid support under microwave irradiation. Uses of NaY zeolite as a solid support and $NaICl_2$ as a catalyst provide an efficient and green chemical route for synthesis in short time. The synthesized compounds have been characterized on the basis of their elemental and spectral analysis.



Method for the Synthesis of 4-substituted-1,3-benzothiazol-2-amine (3 a-f)

Substituted aniline (1, 0.1 mol) and potassium thiocyanate (2, 0.5 mol) was dissolved in water and NaY zeolite (5.0 g) was added to it. The slurry formed was kept in an oven at 600 ^{o}C for 1 hour. After cooling, $NaICl_2$ (2 M) was added to it and mixed together in a

mortar. The reaction mixture was transferred in an Erlenmeyer flask with loose funnel cap and irradiated under microwave irradiation for 5-10 min with a time interval of 20 seconds. After cooling, the product was separated with methanol and the catalyst was filtered off. The crude was washed successively with 10% aqueous solution of $Na_2S_2O_3$, 10% aqueous solution of $NaHCO_3$ and finally with H_2O. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated by vacuum distillation and the crude product was dried and purified by recrystallization from methanol. NaY zeolite was reused after washing with ethanol and drying in air overnight.

Results and discussion

Conventional methods for the synthesis of compounds **3a-f** require 2-3 steps and a suitable solvent. Use of $NaICl_2$ for C-S bond formation can eliminate the extra steps and provide us one step synthesis for 2-aminobenzothiazole. But still it required 2-7 hours of refluxing and stirring. Thus the same reaction was carried out in microwave using DMSO: H_2O as a solvent. In this process the reaction time was reduced from hours to minutes but DMSO is still to being used, which is not eco -friendly. Therefore compounds **3a-f** were synthesized using NaY zeolite as a solid support under microwave irradiation. As a result solvent was eliminated from the synthesis and reaction time was also found to be reduced. In general microwave assisted synthesis under solvent free conditions using $NaICl_2$ provides one step green chemical route for the synthesis of 2-aminobenzothiazole.

Reaction between substituted aniline **(1 a-f)** and potassium thiocyanate **(2)** in the presence of $NaICl_2$ over NaY zeolite in microwave afforded 6-substituted-2-aminobenzothiazoles **3 a-f (scheme)**. Formation of these compounds **(3a-f)** was

confirmed on the basis of their analytical and spectral data. Physical, analytical and spectral data of all the synthesized compounds are presented in **table 1, table 2** and **table 3** respectively.

Table 1: Physical data of synthesized compounds

Compd.	R	M. P. (^{0}C)	Yield (%)	Mol. Formula	Mol. Weight
3a	H	125	88	$C_7H_6N_2S$	150
3b	Cl	195	93	$C_7H_5ClN_2S$	184
3c	Br	215	92	$C_7H_5BrN_2S$	229
3d	OCH_3	164	95	$C_8H_8N_2OS$	180
3e	CH_3	133	94	$C_8H_8N_2S$	164
3f	NO_2	245	93	$C_7H_5N_3O_2S$	195

Table 2: Analytical data of synthesized compounds

Compd.	Calculated / Found (%)		
	C	**H**	**N**
3a	55.97 / 55.99	4.03 / 4.05	18.65 / 18.63
3b	45.53 / 45.56	2.73 / 2.75	15.17 / 15.14
3c	36.70 / 36.74	2.20 / 2.22	12.33 / 12.34
3d	53.31 / 53.33	4.47 / 4.45	15.54 / 15.51
3e	58.51 / 58.54	4.91 / 4.88	17.06 / 17.05
3f	43.07 / 43.04	2.58 / 2.59	21.53 / 21.54

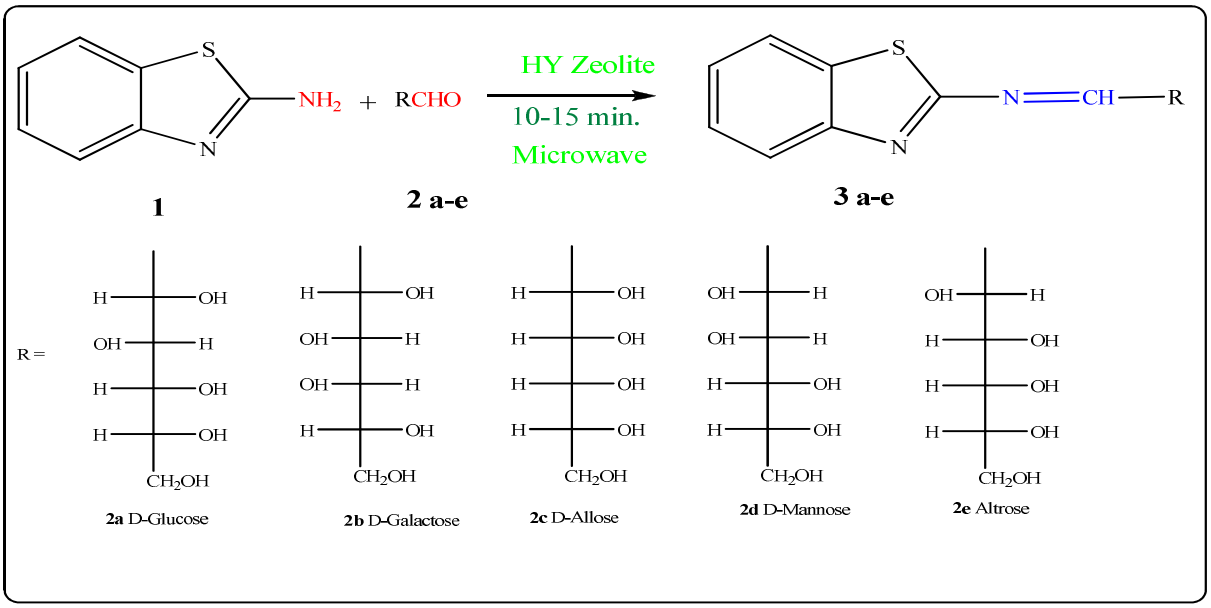
Table 3: Spectral data of synthesized compounds

Compd.	IR (cm^{-1})	^{1}H NMR (δ)	^{13}C NMR
3a	3400 and 3260 (NH_2 str.), 3046 (C-H str., aromatic), 1640 (C=N str.), 1520 and 1438 (C=C str. Ar)	7.71-7.48 (m, 4 H, Ar-H) and 5.88-5.81 (s, 2H, NH_2)	116.2, 119.7, 122.2, 123.4, 129.1, 151.2 and 164.1
3b	3454 and 3258 (NH_2 str.), 3076 (C-H str., aromatic), 1630 (C=N str.), 1525 and 1440 (C=C str. Ar) and 755 (C-Cl str.)	7.50-7.28 (m, 3 H, Ar-H) and 5.35-5.27 (s, 2H, NH_2)	116.5, 119.4, 123.1, 127.9, 130.2, 150.1 and 164.4
3c	3450 and 3255 (NH_2 str.), 3077 (C-H str., aromatic), 1640 (C=N str.) 1534 and 1439 (C=C str. Ar)	7.71-7.48 (m, 3 H, Ar-H) and 5.88-5.81 (s, 2H, NH_2)	115.9, 117.4, 122.7, 126.4, 151.1 and 163.2
3d	3375 and 3280 (NH_2 str.), 3084 (C-H str., aromatic), 1630 (C=N str.), 1541 and 1452 (C=C str. Ar)	7.50-7.11 (m, 3 H, Ar-H), 5.09-5.05 (s, 2H, NH_2) and 3.80 (s, 3H, $-OCH_3$)	56.1, 105.6, 113.9, 119.9, 132.9, 146.4, 155.9 and 164.3
3e	3370 and 3281 (NH_2 str.), 3081 (C-H str., aromatic), 1635 (C=N str.), 1530 and	7.60-7.31 (m, 3 H, Ar-H), 5.72-5.43 (s, 2H, NH_2) and 2.48 (s,	20.7, 115.8, 119.4, 125.1, 129.7, 132.7, 149.2 and 166.2

	1451 (C=C str. Ar)	3H, CH_3),	
3f	3450 and 3290 (NH_2 str.), 3041 (C-H str., aromatic), 1649 (C=N str.), 1556 and 1480 (C=C str. Ar), 1525 and 1323 (NO_2 str.)	8.48-8.10 (m, 3 H, Ar-H) and 5.87-5.82 (s, 2H, NH_2)	115.9, 118.8, 121.6, 130.2, 143.1, 152.9 and 166.4

(B) Comparative study and synthesis of iminosaccharides of 2-aminobenzothiazole:

In this study iminosaccharides of 2-aminobenzothiazole were synthesized using three different methods *i.e.* Conventional synthesis, microwave assisted synthesis using ethanol as a solvent and microwave assisted solvent free synthesis using HY Zeolite as a solid support. Comparison of all mentioned methods was also presented which clearly indicate that solvent free synthesis of iminosaccharides of 2-aminobenzothiazole in microwave afforded better yield in short time via eco friendly route as compared to remaining two methods. Iminosaccharides of 2-aminobenzothiazole was synthesized by the reaction of 2-aminobenzothiazole (1) with various hexoses (2 a-e). Structure of all the synthesized products have been elucidated on the basis of their elemental and spectral analysis.



METHODS FOR SYNTHESIS:

[1] Conventional method for the Synthesis of iminosaccharides of 2-aminobenzothiazole (3 a-e)

2-aminobenzothiazole (**1** 0.01 mol) and various hexoses (**2 a-e** 0.02 mol) were dissolved in ethanol and a few drops of acetic acid were added to it. Then the reaction mixture was refluxed for 15-25 hours. After cooling, the product separated was washed with H_2O and extracted with ethyl acetate. The combined organic layers were dried under anhydrous Na_2SO_4. The solvent was evaporated by vacuum distillation and the crude product was dried and purified by recrystallization from ethanol.

[2] Microwave assisted synthesis of iminosaccharides of 2-aminobenzothiazole(3 a-e)

2-aminobenzothiazole (**1**, 0.01 mol) and various hexoses (**2 a-e**, 0.02 mol) were dissolved in ethanol and a few drops of acetic acid were added to it. The reaction mixture was transferred in an Erlenmeyer flask and irradiated under microwave irradiation for 10

min with a time interval of 30 seconds. After cooling, the product separated was washed with H_2O and extracted with ethyl acetate. The combined organic layers were dried under anhydrous Na_2SO_4. The solvent was evaporated by vacuum distillation and the crude product was dried and purified by recrystallization from ethanol.

[3] Microwave assisted solvent free synthesis of iminosaccharides of 2-aminobenzothiazole using HY Zeolite as a solid support and catalyst (3 a-e)

2-aminobenzothiazole (**1**, 0.01 mol), various hexoses (**2**, a-e 0.02 mol) and HY zeolite were mixed in pestle and mortar and irradiated under microwave irradiation for 10-15 min. The product was separated from zeolite by stirring it with ethanol for 15-20 min two to three times and evaporating the solvent by vacuum distillation. Product was purified by recrystallization from ethanol. Zeolite was reused after washing with ethanol and drying in air overnight.

Results and Discussion

The structures of the final products were established on the basis of their analytical and spectral data. Disappearance of two bands at 3400 and 3260 cm^{-1} due to -NH_2 stretching (symmetric and asymmetric) and appearance of bands in the region of 3400-3450 cm^{-1} for OH stretching and 1620-1640 cm^{-1} corresponding to C=N stretching confirms the assigned structure of iminosaccharides. In ^1H NMR spectrum the absence of signals at δ 5.81-5.88 due to proton of NH_2 and presence of a doublet for imino protons (CH=N-) at δ 8.50 and a singlet for OH proton at δ 5.01 favors the formation of final product **(3 a-e)**. The signals of proton of sugar chain were congregated with the solvent

absorption in a broad signal at δ 4.49-3.60. Further, mass spectrum also supported the structure of iminosaccharides.

Reaction of 2-aminobenzothiazole (**1**) with various hexoses (**2 a-e**) in ethanol using acetic acid as a catalyst afforded iminosaccharides (Schiff bases) with azomethine linkage **(3 a-e)**. In this conventional method 10-15 hours of refluxing is required. When the same reactions were carried out in microwave the reaction time was reduced from hours to minutes and the yield of products was also increased. But in these processes ethanol and acetic acid is still to be used, which is not eco -friendly. Therefore iminosugars was synthesized using zeolite as a solid support under microwave irradiadion *i.e.* under solvent free conditions. As a result solvent was eliminated from the synthesis and reaction time was also found to be reduced and the yield of product also increased. A comparison of time required for synthesis of product and yield of products in conventional method and microwave methods (with and without using NaY zeolite) has been reported in **table 4**.

It is clear from the **table 4** that yield of final products is highest for microwave assisted synthesis using zeolite and lowest for conventional synthesis. Moreover, time required for synthesis is lowest for solvent free conditions in microwave. Thus, synthesis of reported product in microwave using zeolite as a solid support and catalyst is an efficient and environmental benign route.

Table: 4 Physical data of synthesized compounds

Compounds	R	Mol. Formula	Mol. Weight	M.P. (°C)	Yield[a] (%) [Time] (hr.)	Yield[b] (%) [Time] (min.)	Yield[c] (%) [Time] (min.)
3a	D-Glucose	$C_{13}H_{16}N_2O_5S$	312	255-258	87 [12-14]	88 [17]	93 [14]
3b	D - Galactose	$C_{13}H_{16}N_2O_5S$	312	270-274	72 [12-13]	75 [16]	92 [10]
3c	D- Allose	$C_{13}H_{16}N_2O_5S$	312	292-296	68 [12-14]	75 [17]	92 [15]
3d	D-mannose	$C_{13}H_{16}N_2O_5S$	312	267-271	83 [13-15]	88 [18]	95 [12]
3e	D-Altrose	$C_{13}H_{16}N_2O_5S$	312	249-252	84 [13-16]	90 [20]	97 [15]

a = Conventional synthesis, b = Microwave assisted synthesis using solvent, c = microwave assisted solvent free synthesis using zeolite as a solid support

Antimicrobial Activity:

Five synthesized compounds (**3a-e)** were *in vitro* screened for their antibacterial and antifungal activity using 100 ppm concentration in DMF by cup and well method. The micro-organisms used as antibacterial are *Escherichia coli, Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and the fungal strains *Candida albicans* and *Aspergillus fumigatus* were used. The activity is presented as zone of inhibition in mm and compared with activity of controls C_1 (for antibacterial activity C_1= Ciprofloxacin and for antifungal activity C_1= Amphotericin B) giving activity index value (**Table 5**).

All the compounds have shown moderate activity against *P. aeruginosa* where as strong activity against *B. subtilis, P. mirabilis* and *E. coli*. Activity index value against *B. subtilis, P.mirabilis* and *E. coli* was more than one for all the synthesized compounds. It was observed that all the compounds show stronger activity then the standard used against fungal strain *Candida albicans* and *Aspergillus fumigatus.*

It may be concluded from the activity study that compounds **3c** and **3d** were found to be the strongest amongst all synthesized compounds. Compounds have more

comprehensive fungus-inhibiting properties than that of the bacteria. Even two folds antifungal activity was observed than standards.

Table 5: Antimicrobial activity of the synthesized compounds on 100 ppm (3a-e).

Zone of Growth Inhibition (mm) (activity index)

Compd. No.	Antibacterial Activity				Antifungal Activity	
	E. coli	*B. subtilis*	*P. mirabilis*	*P. aeruginosa*	*C. albicans*	*A. fumigatus*
3a	23 (1.43)	20 (1.17)	18 (1.12)	19 (1.05)	26 (1.52)	27 (2.70)
3b	24 (1.50)	22 (1.29)	17 (1.06)	18 (1.00)	27 (1.58)	28 (2.80)
3c	25 (1.56)	23 (1.35)	19 (1.18)	20 (1.11)	29 (1.70)	29 (2.90)
3d	21 (1.31)	21 (1.23)	17 (1.06)	17 (0.94)	28 (1.64)	26 (2.60)
3e	21 (1.31)	18 (1.05)	19 (1.18)	17 (0.94)	26 (1.52)	27 (2.70)
C_1	16	17	16	18	17	10

(Activity index) = Inhibition zone of compound/Inhibition zone of the standard drug.
For antibacterial activity: C_1 = Ciprofloxacin
For antifungal activity: C_1 = Amphotericin B

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