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SYNTHESIS AND EVALUATION OF NOVEL BENZOTHIAZOLE DERIVATIVES FOR ITS ANTI-ARTHRITIS ACTIVITY

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ABSTRACT

It is an inflammatory form of arthritis that can affect areas outside the joints.[1] It is chronic inflammatory illness most commonly caused by genetic predisposition and environmental factors, especially cigarette smoking. Benzothiazoles are heterocyclic dicyclic molecules with a benzene atom connected to a ring with nitrogen and sulfur atoms. The melting point of benzothiazole is 2ºC, and the boiling point is 227-228ºC. Benzothiazole has a molecular mass of 135.19 g/mol and a density of 1.24g/ml. In the home, benzothiazole serves no purpose. It has practical and academic applications. This research was based on the synthesis and evaluation of novel benzothiazole derivatives for its anti-arthritis activity. Novel derivatives of benzothiazoles were synthesized using substituted aniline. They were determined for physical properties, i.e., melting point was determined as 3ºC, 4ºC, 3ºC, 2ºC, 3ºC and 2ºC for the benzothiazole derivatives C1, C2, C3, C4, C5 and C6, respectively. TLC stands for thin layer chromatography and is used in synthetic chemistry to infer the production of a molecule based on its Rf value, which varies depending on the compound. Rf value was obtained as 0.67, 0.72, 0.79, 0.68, 0.73 and 0.79 of C1, C2, C3, C4, C5 and C6, respectively. While determining antirheumatic arthritis effect, different derivatives of benzothiazole were evaluated for rheumatoid factor. In conclusion, C1 demonstrated rheumatoid factor as 4.68±0.12, 8.83±0.19, and 6.17±0.16 in group 1, 2, and 3, respectively. Fellow researchers will evaluate the mode of action that how benzothiazole derivatives treat and prevent the progression of rheumatoid arthritis.

KEYWORDS: Benzothiazole, anti-arthritis, RA, TLC and FTIR.

INTRODUCTION

It is an inflammatory form of arthritis that can affect areas outside the joints.[1] It is chronic inflammatory illness most commonly caused by genetic predisposition and environmental factors, especially cigarette smoking.[2,3] Small peripheral joints are usually affected first, but if left untreated, it can extend to proximal joints and become symmetric.[4] Degeneration of joints occurs when inflammation wears away at cartilage and causes bone to erode into the joint socket. Symptoms of early RA often appear within the first six months of the disease's onset, while those of established RA typically appear after the disease has been present for longer than six months. Untreated RA leads to a rise immortality disability.[5]

Heritability estimates for rheumatoid arthritis range from 40% to 65% in seropositive individuals and 20% in seronegative cases.[6-10] Cigarette smoking is a basic etiology of RA.[11-14] Other environmental factors may also have a part in the development of RA, which is why it's important to consider all of your options. Silica, asbestos, textile dust, and P. gingivalis are all examples.[15,16] This shows that an autoimmune inflammatory response in the joints is triggered by external exposure to different antigens in areas of the host far from the joints. The lungs, oral cavity, and digestive system all qualify as far-flung organs or regions.[17] Alterations to the make-up and activity of the gut microbiome have also been linked to RA. Rheumatoid arthritis patients have less variety in their gut microbiome than healthy people do because of the disease (a phenomenon known as "dysbiosis"). Faecalibacterium, Collinsella, Eggerthalla, and Actinobacteria are among the genera that have seen an uptick. Collinsella causes alterations in gut mucosal permeability and has been linked to a worsening of rheumatoid arthritis.[18-20]

The rate of occurrence is significantly lower in East Asia and Africa than it is in Central and South America.[21] In US & other western nations of northern Europe, the annual incidence of RA is approx. 40/1 lakh people.[22] Epidemiological studies show that women, in comparison to males, are more likely to get RA, with a 3.6% lifetime risk of RA compared to a 1.7% risk in men.[23] The greatest incidence of RA occurs in those aged 65 to 80 years old. An analysis of 60 population-based studies found an annualised prevalence of 0.51 percent for RA between 1955 and 2015.[24]

Benzothiazole

Benzothiazoles are heterocyclic dicyclic molecules with a benzene atom connected to a ring with nitrogen and sulfur atoms.[25] It has several medicinal uses, including as an analgesic[26],

anti-inflammatory[27], anti-diabetic[28], and cancer preventative.[29] Numerous marine and terrestrial compounds with useful biological characteristics contain benzothiazoles. Benzothiazole is used to treat a wide range of conditions, including cancer, central muscle relaxants, and neurological disorders.[30]

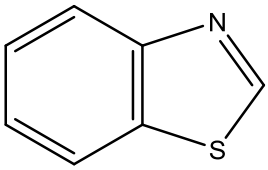


Fig. 1: Structure of Benzothiazole.

IUPAC name: 1,3-Benzothiazole

Molecular formula: C_7H_5NS

It is used in the synthesis of numerous medicinal medicines. The biological activities of benzothiazole derivatives have undergone some fascinating changes in recent years. These molecules are of especial importance in the field of medicinal chemistry. The development of novel benzothiazoles is facilitated by the ease with which their biological qualities as a drug carrier can be obtained.[31] Because it is a heterocyclic molecule, benzothiazole is used as a building block in the synthesis of more complex compounds with desirable biological properties. It is relatively stable due to its aromaticity, but it can be functionalized because it is a heterocycle with reactive sites. The melting point of benzothiazole is 2ºC, and the boiling point is 227-228ºC. Benzothiazole has a molecular mass of 135.19 g/mol and a density of 1.24g/ml. In the home, benzothiazole serves no purpose. It has practical and academic applications.[32]

MATERIALS AND METHODOLOGY

Experimental Requirements

A. Benzothiazole, Freund's adjuvant, distilled water, indomethacin (API), paraffin and ethanol.

B. Digital weighing balance, round bottom flask, condenser, clinical thermometer, plethysmograph, and digital pH meter.

Synthesis of novel derivatives of benzothiazole

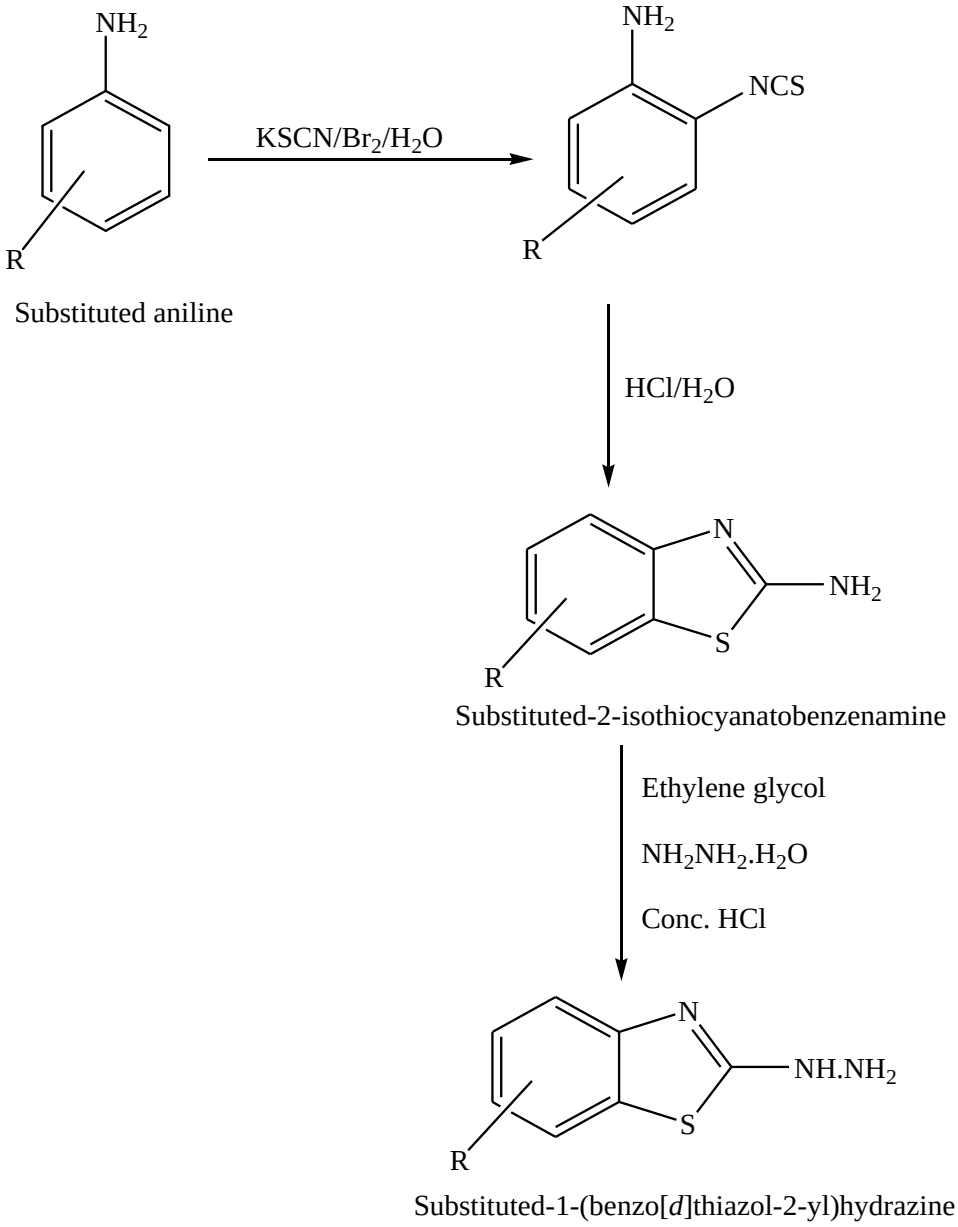


Fig. 2: Scheme for synthesis of benzothiazole derivatives.

The above scheme was followed in the synthesis of benzothiazole derivatives in which substituted aniline reacts with potassium thiocyanate to develop substituted isothiocyanate interpermeate I that further made reacted with HCl to obtain the Intermediate II that later received the final derivative.

Procedure of synthesis (C1)

The compound aniline was used as precursor in synthesis of compound C1. Aniline was mixed with potassium thiocyanate in bromine water. Upon reaction, 2-isothiocyanatobenzenamine was developed as Intermediate I that was further reacted with

hydrochloric acid to obtain the benzo[d]thiazol-2-amine (Intermediate II). The benzo[d]thiazol-2-amine was mixed in hydrazine monohydrate mixed with ethylene glycol in the presence of conc. HCL. After reaction was cooled and washed with cold water a yellowish precipitate was found.

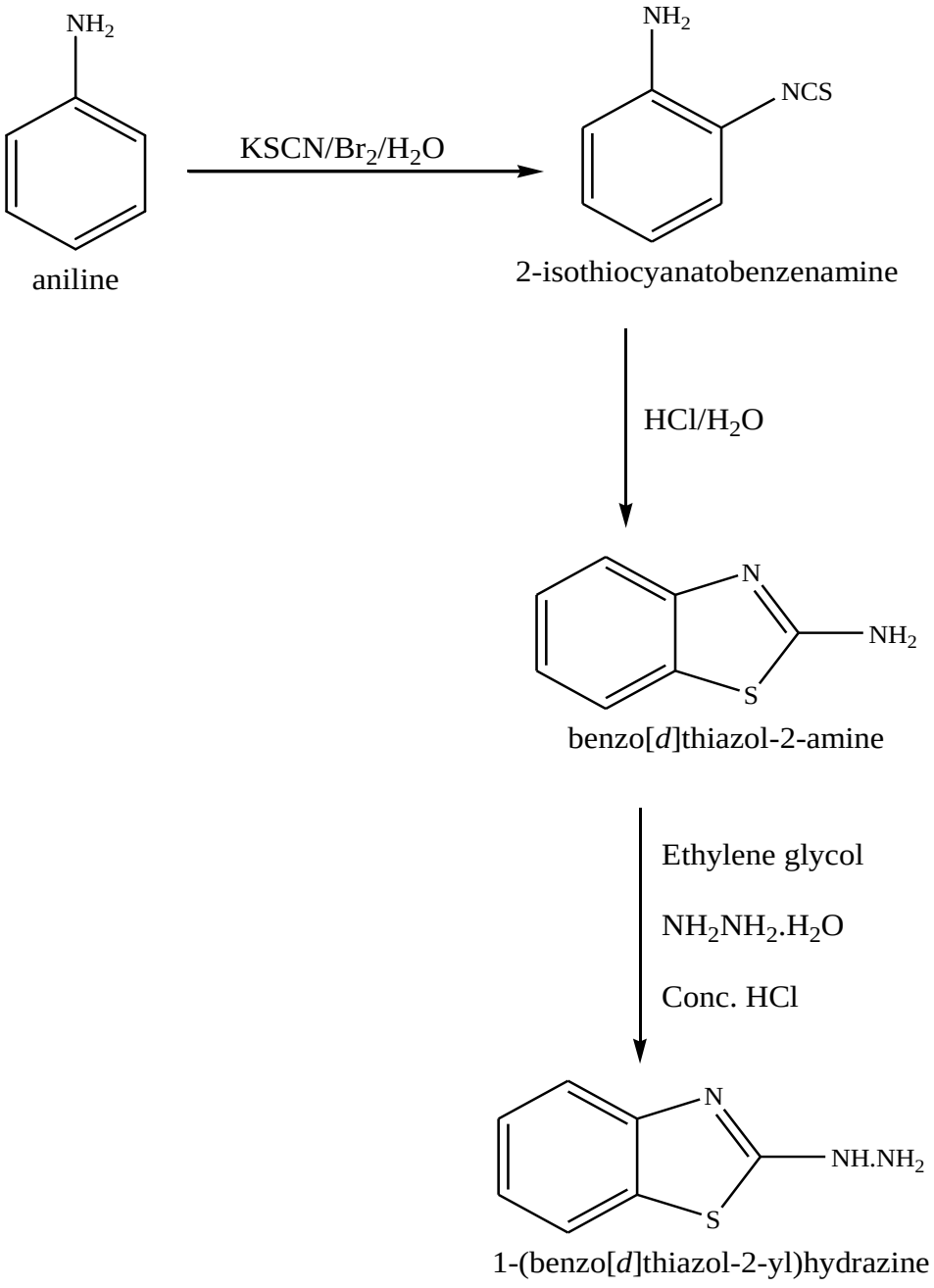


Fig. 3: Synthesis of benzothiazole derivative (C1).

Procedure of synthesis (C2)

In this procedure, ppotassium thiocyanate made reacted with substituted aniline in the presence under ambient environment. The produced Intermediate I was acidified with

hydrochloric acid to obtain substituted-2-isothiocyanatobenzenamine as Intermediate II. The Intermediate II was reacted with ethylene glycol, and hydrazine monohydrate in presence of conc. hydrochloric acid thus a yellowish precipitate of substituted-1-(benzo[d]thiazol-2yl-hydrazine.

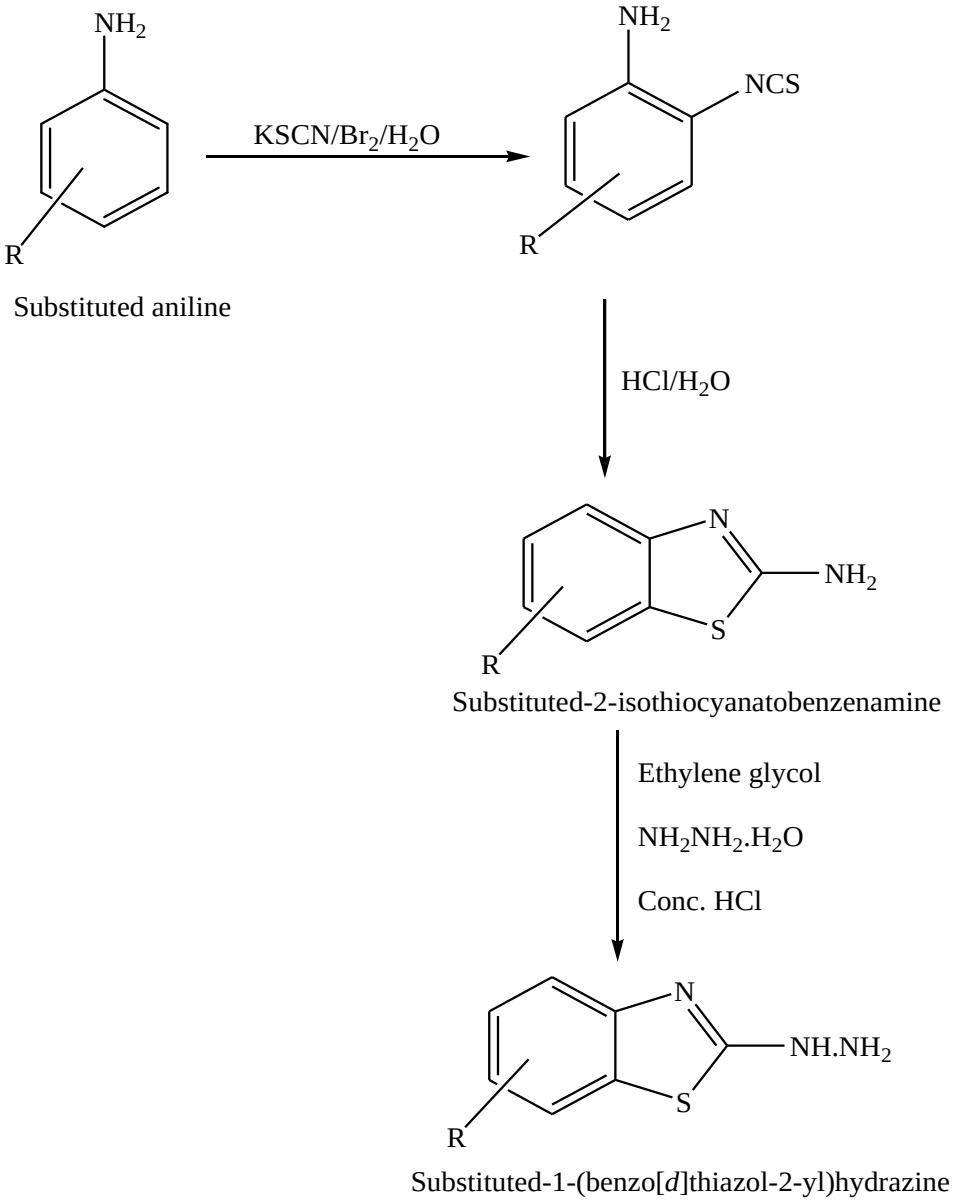


Fig. 4: Synthesis of benzothiazole derivative (C2).

Procedure of synthesis (C3)

In this process, benzothiazole was synthesized by the 4-chlorobenzenamine (reagent/precursor). Upon reaction of 6-chlorobeno[d]thiazol-e-amine (Intermediate II) with ethylene glycol, and hydrazine monohydrate in acidic environment (conc. HCl) a pale precipitate of 1-(6-chlorobenzo[d]thiazol-e-yl)hydrazine.

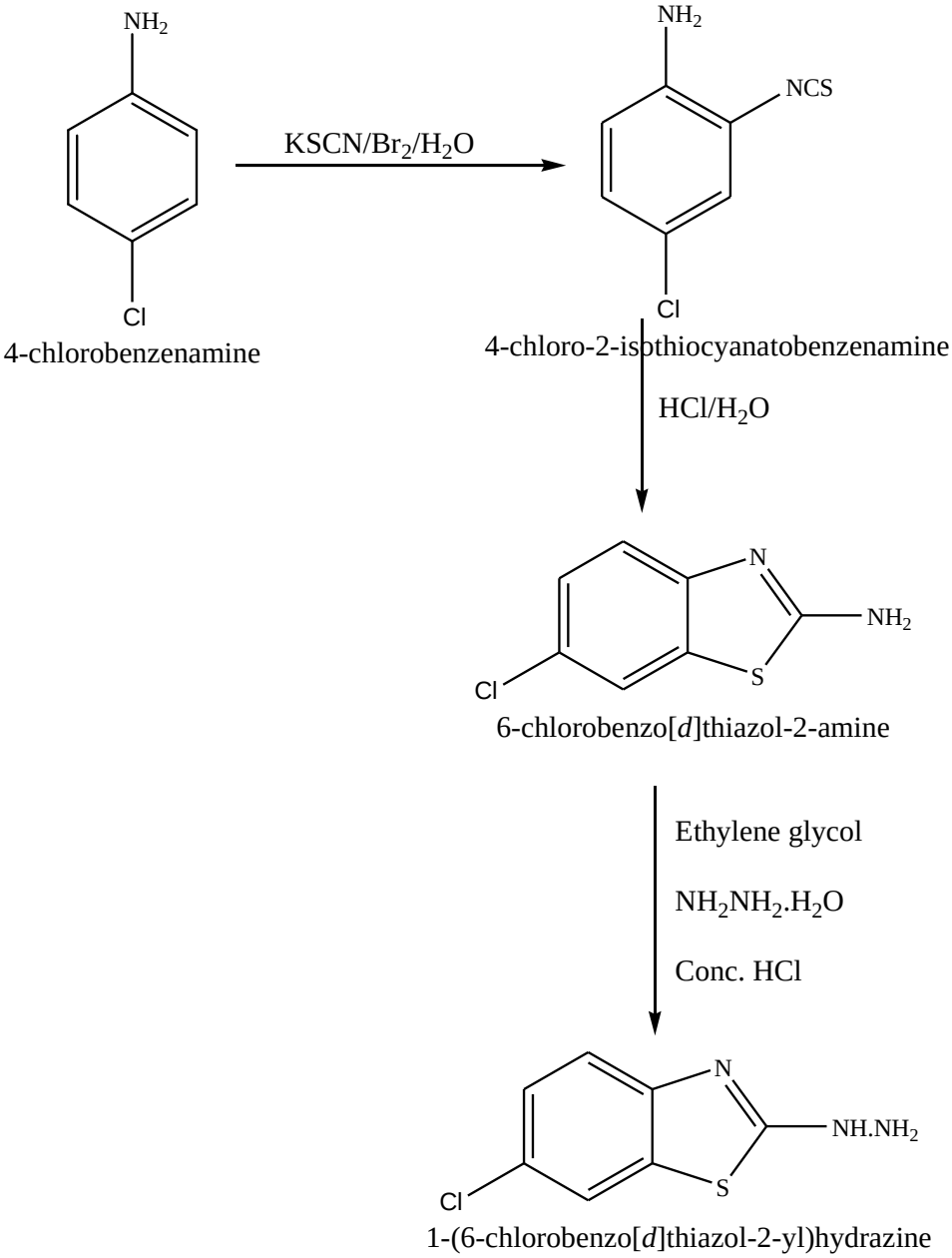


Fig. 5: Synthesis of benzothiazole derivative (C3).

Procedure of synthesis (C4)

4-bromobenzamine was used as precursor moiety for the development of compound C4. In this 6-bromobenzo[d]thiazol-2-amine was made reacted with ethylene glycol, hydrazine monohydrate in acidic environment (conc. HCl) then 1-(6-bromobenzo[d]thiazol-2-yl)hydrazine.

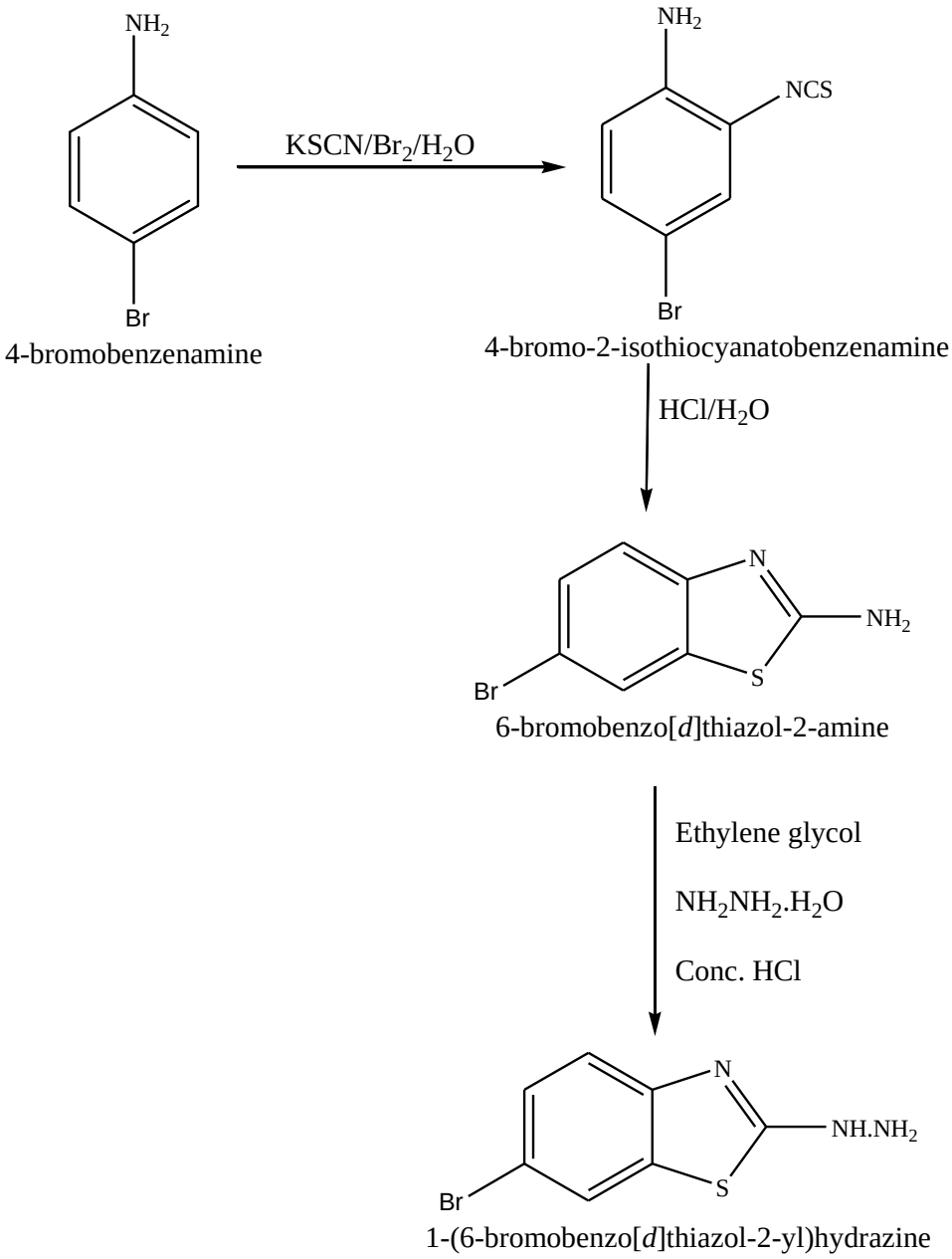


Fig. 6: Synthesis of benzothiazole derivative (C4).

Procedure of synthesis (C5)

4-aminophenol was kept as starting material for the synthesis of 2-hydrazinyl benzo[thiazole-6-ol in the presence of concentrated HCl and hydrazine monohydrate.

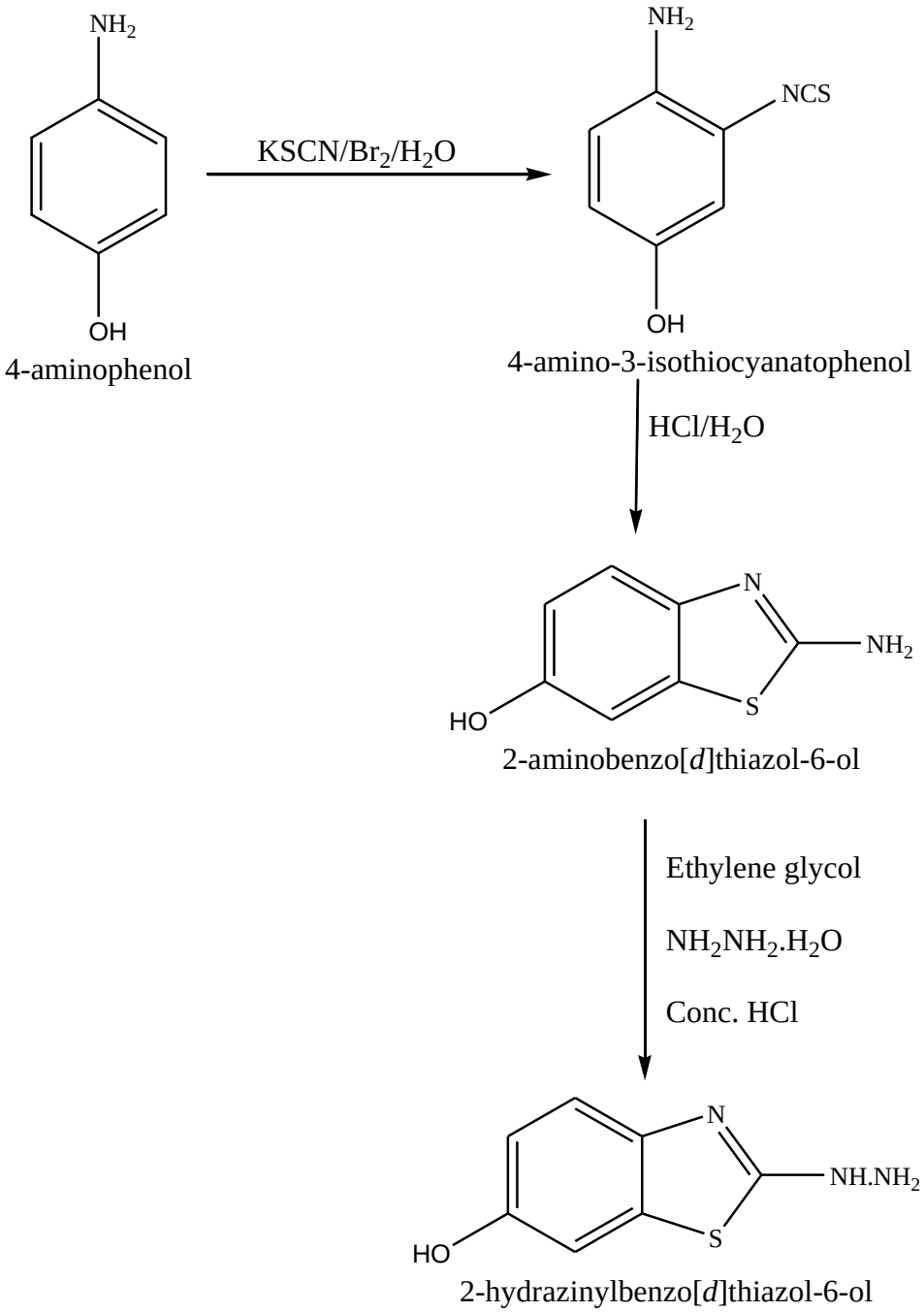


Fig. 7: Synthesis of benzothiazole derivative (C5).

Procedure of synthesis (C6)

In this procedure, 4-methoxybenzamine produced the 1-(6-methoxybenzo[d]thiazol-2yl)hydrazine in the ambient environment made by ethylene glycol, presence of concentrated HCl and hydrazine monohydrate after synthesizing the intermediate I & II.

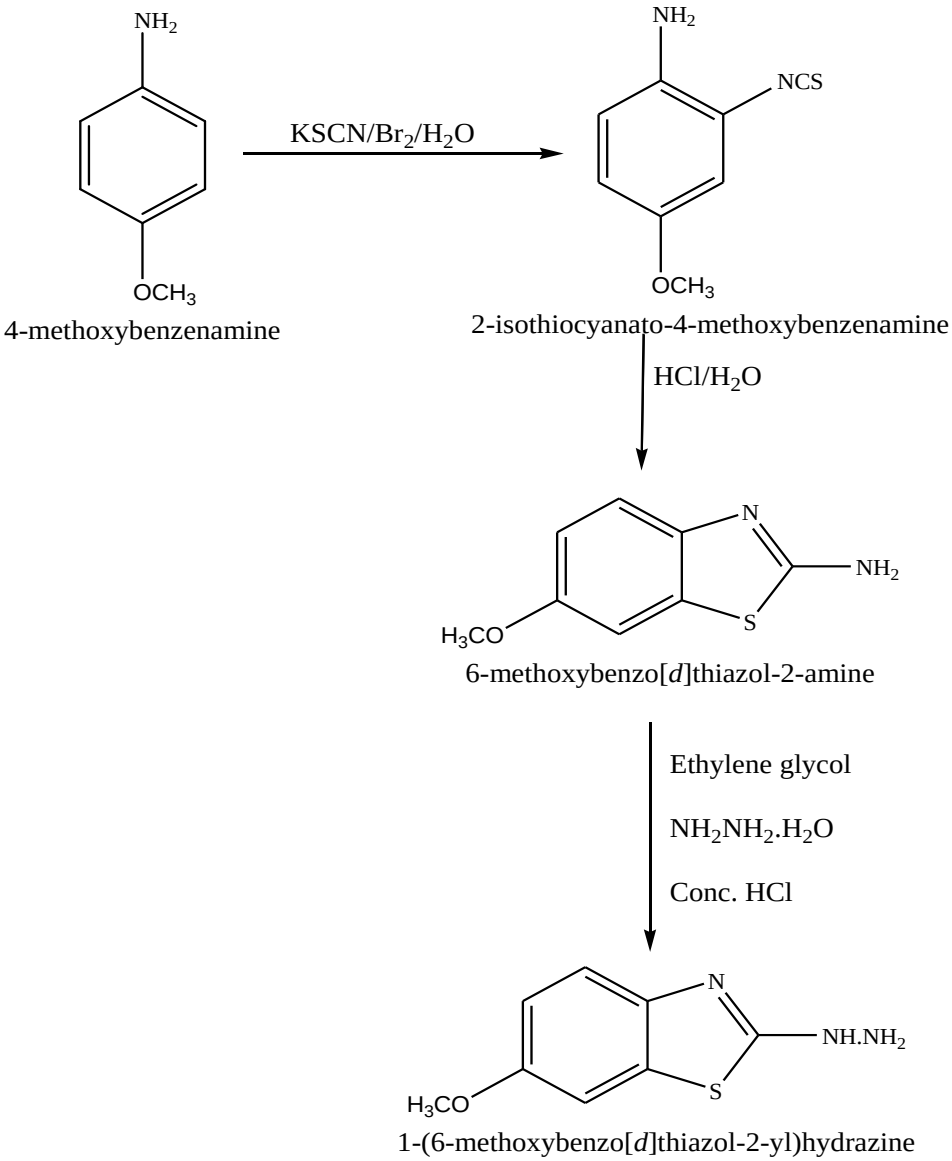


Fig. 8: Synthesis of benzothiazole derivative (C6).

IDENTIFICATION OF PHYSICAL PROPERTIES

Melting point determination

Melting point determination: Thiel's melting point tube was used to determine the melting point of an organic compound (capillary tube method). The most important and straightforward means of distinguishing one compound from another is to determine its melting point.

Thin Layer Chromatography (Rf value)

TLC stands for thin layer chromatography and is used in synthetic chemistry to infer the production of a molecule based on its Rf value, which varies depending on the compound. It also aids in confirming the reaction's progress.

Infrared Spectroscopy

Infrared spectroscopy (IR) is one of the most essential methods for determining different functional groups and probable chemical structures. The main benefit of IR over other techniques is that it easily produces fingerprints (1300-650 cm-1) of molecules' structure (functional group, associating with one other). There are no two compounds with the same fingerprint region. This method is based on the molecular vibration of the chemical, which causes each bond to vibrate at a particular frequency, which corresponds to the IR frequency. As a result, IR spectra of each bond was created. On a Jasco V410, FTIR spectra were obtained in KBr powder.

NMR Spectroscopy

By exposing a substance to two magnetic forces, one fixed and the other fluctuating at a radio frequency, the interaction between matter and electromagnetic forces can be seen. The sample detects energy at a certain combination of fields, and absorption is detected as a change in single developed by a radio frequency detector and amplifier. The magnetic dipolar character of a spinning nucleus can be linked to this absorption energy. Nuclear Magnetic Resonance is the name for this technology. This method is beneficial for determining the molecule's structure. A Bruker Ultraspec 500MHz/ AMX400MHz spectrometer was used to measure 1H- NMR spectra in CDCl3 and d6-DMSO.

Animal preparation

Animal House, Advance Institute of Biotech & Paramedical Sciences, Kanpur didprovide rats (either sex) weighing 130-160g. The rats are kept in ideal conditions, with a room temperature of 25°C and a light/dark cycle of 12 hours. Free water and food were provided on a consistent schedule, and the relative humidity was maintained at 50%. Until an hour before the trial, the rats are fasting but have unrestricted access to water (Bhajoni et al., 2016).

Experimental design

All the rats were divided in diverse 4 groups (n=6)-

Group 1: rats administered vehicle daily for 21 days.

Group 2: rats administered Freund’s adjuvant (1%) intradermally for 21 days.

Group 3: rats administered Freund’s adjuvant (1%) + indomethacin (10mg/kg) for 21 days.

Group 4: rats administered Freund’s adjuvant (1%) + all the novel benzothiazole derivatives (200mg/kg) for 21 days.

Evaluation parameters

i. Freund's adjuvant induced arthritis

The progression of the disease and the effects of the drugs on complete Freund's adjuvant induced arthritis in rats were analyzed by measuring the volume displacement by plethysmograph in the swollen hind paw on day 0, 4, 8, 14, and 21 to confirm the reduction in arthritis as the disease progressed. with the left paw served as a control. Simultaneously, indomethacin as standard was given intraperitoneally. Both hind paws are diagnosed, just above the ankle joint and recorded for the volume of inflammation.[33]

ii. Rheumatoid factor (RF) test

The presence of rheumatoid factor (RF) can be determined by analysing blood samples. The immune system produces proteins called rheumatoid factors. Antibodies are proteins made by your immune system that seek out and destroy disease-causing bacteria and viruses. However, rheumatoid factors are actually antibodies that can sometimes cause harm to the body by attacking healthy cells and tissues. This is a classic symptom of an autoimmune disease. Rheumatoid factors are only found in the blood of some people. And there are some perfectly healthy persons who have them. Rheumatoid factors (RF) are typically low, but high RF levels are associated with autoimmune disorders and other health issues.

RESULTS AND DISCUSSION

Synthesized derivatives

Novel benzothiazole derivatives (C1-C6) were developed by above declared scheme. The procedure was followed as conventional procedures for the benzothiazole synthesis, already mentioned in materials and methods section. After synthesis, all the derivatives undergone evaluation of physical parameters in terms of % yield, melting point and molecular weight.

Identification of physical properties

Melting point determination

Melting point was determined as 3ºC, 4ºC, 3ºC, 2ºC, 3ºC and 2ºC for the benzothiazole derivatives C1, C2, C3, C4, C5 and C6, respectively.

Thin Layer Chromatography (Rf value)

TLC stands for thin layer chromatography and is used in synthetic chemistry to infer the production of a molecule based on its Rf value, which varies depending on the compound. Rf

value was obtained as 0.67, 0.72, 0.79, 0.68, 0.73 and 0.79 of C1, C2, C3, C4, C5 and C6, respectively.

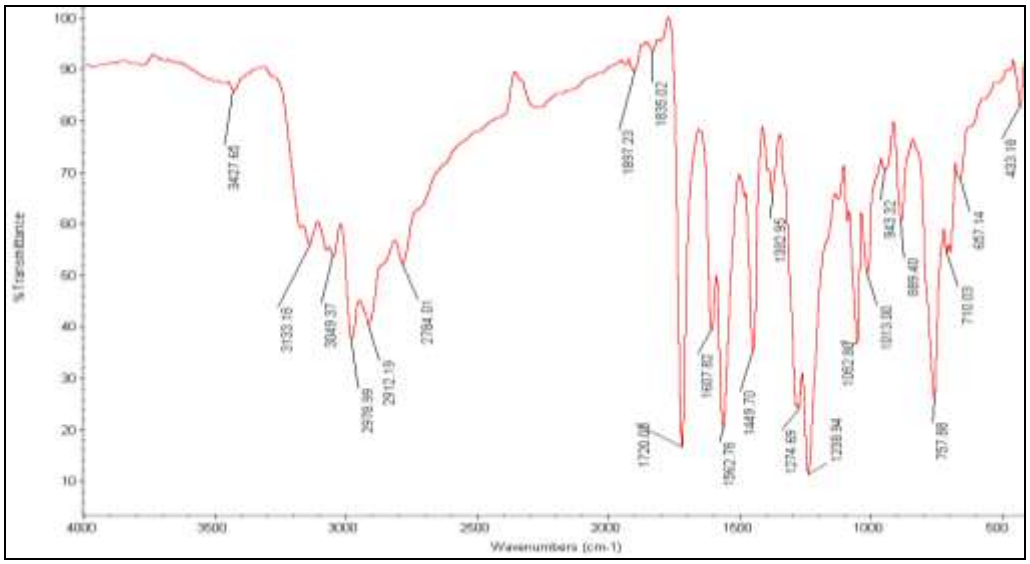
All the synthesized derivatives of benzothiazole were tested for their physical properties. Various profiles i. e., percentage yield, melting point, molecular weight and functional groups attached with were tested. C5 and C6 were demonstrated for its highest % yield as 73.18% and 74.26. Lowest % yield was seen in C4 as 68.35%. The highest melting point was found in compound C4 as 6ºC. Highest melting point indicates about the strongest density of the compound. Molecular weight was also found significant in the analogues of benzothiazole developed. Molecular weight was found as 164.22, 186.18 and 136.61 for C4, C5 and C6 respectively. Maximum Rf was seen in C-6 as 0.79. The following table summarized physical properties of all the compounds.

Table 1: Physical properties of synthesized benzothiazole derivatives.

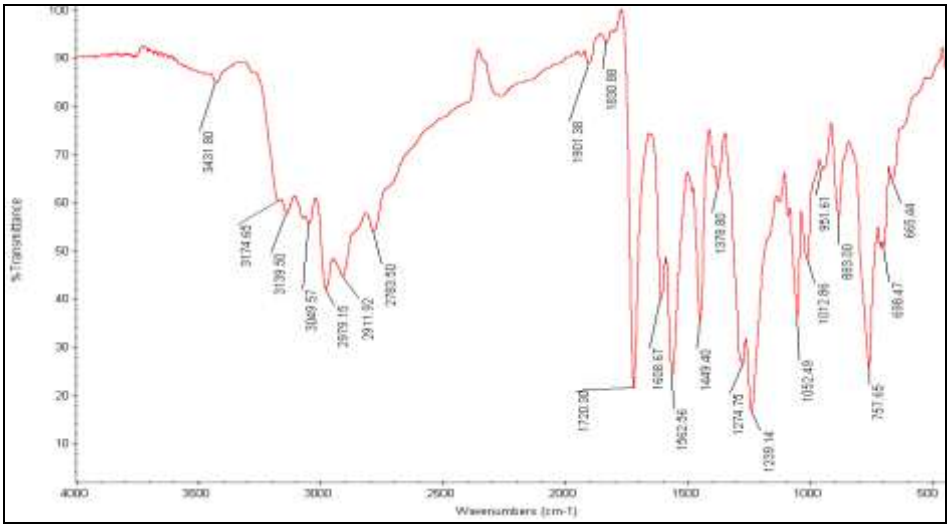
Compound	Yield (%)	Rf Value	Melting point	Molecular weight
C1	69.12	0.67	3ºC	147.42
C2	67.20	0.72	4ºC	139.18
C3	71.18	0.79	3ºC	155.19
C4	68.35	0.68	6ºC	164.22
C5	73.18	0.73	3ºC	186.18
C6	74.26	0.79	2ºC	136.61

Infrared Spectroscopy

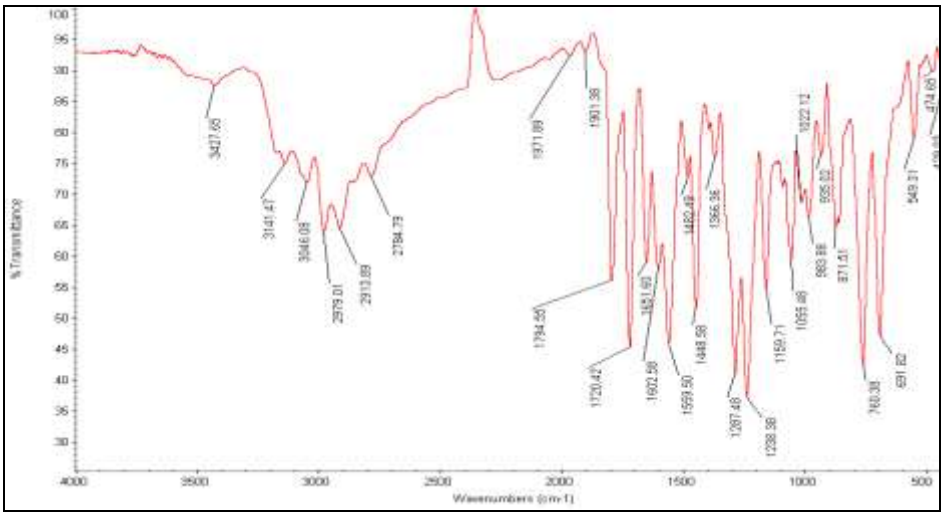
Compound from C1-C6 were analyzed for infrared spectroscopy and these spectra confirmed for the physical characteristics of benzothiazole analogues.



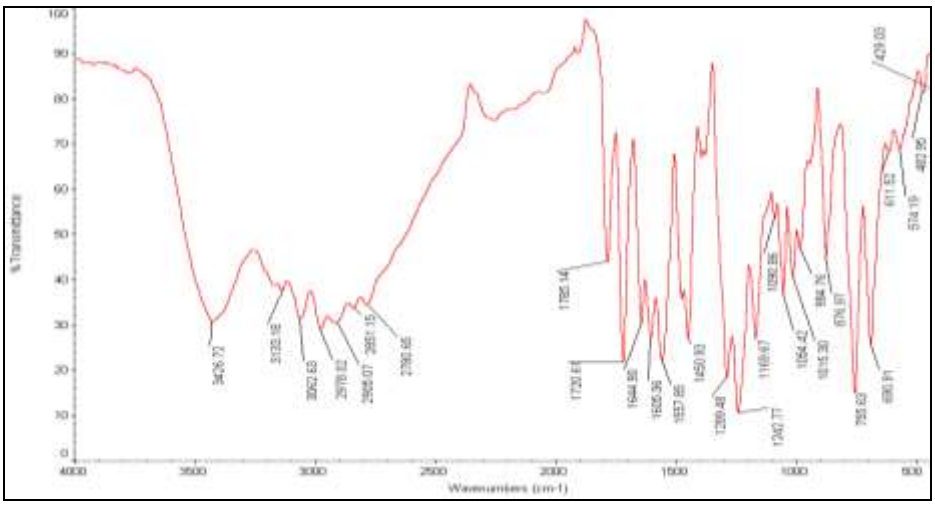
IR Spectra of C1.



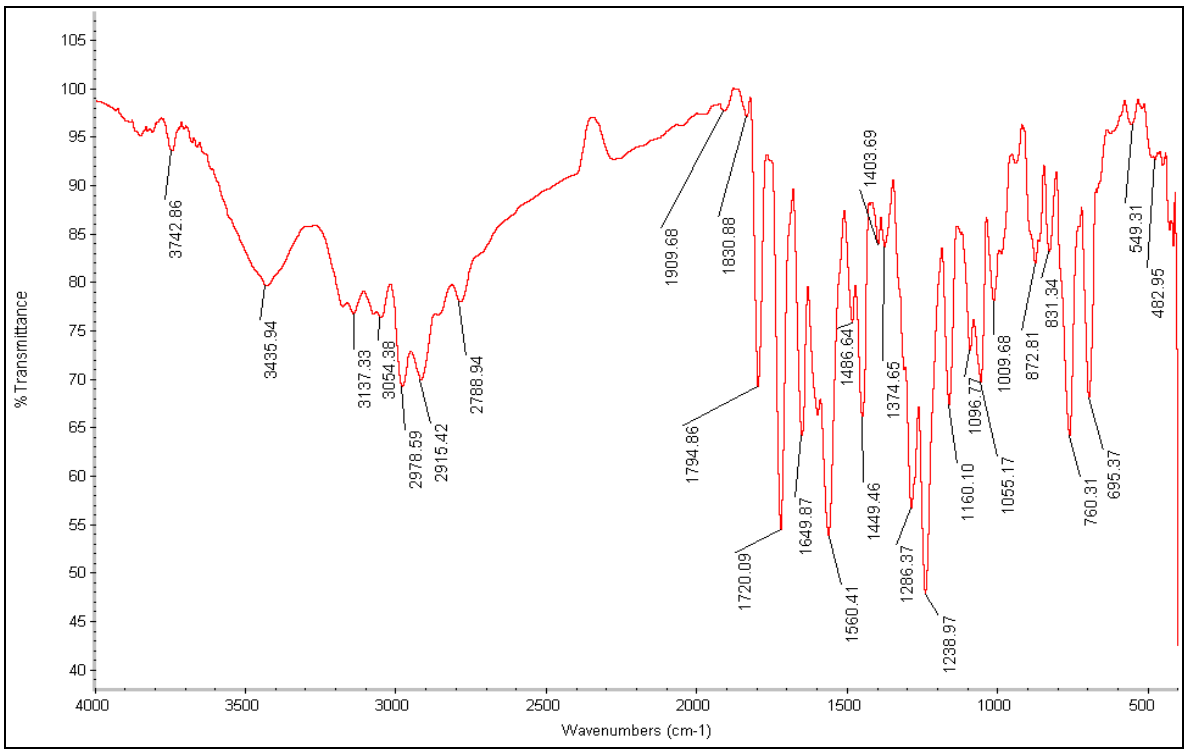
IR Spectrum of C2.



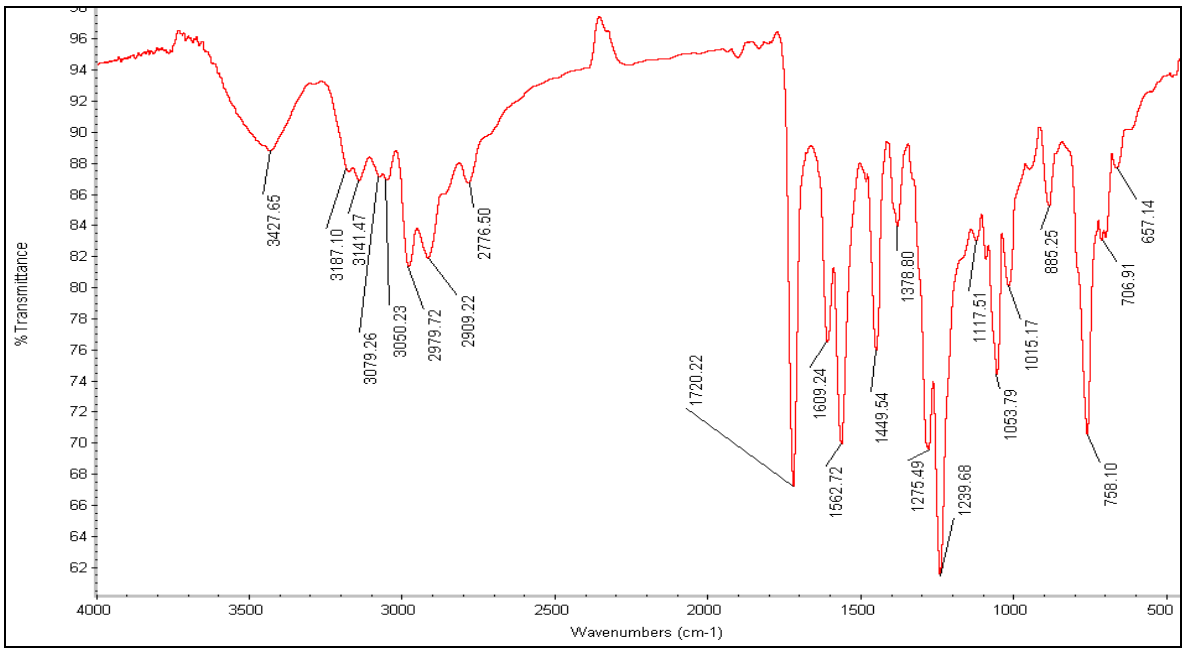
IR Spectrum of C3.



IR Spectrum of C4



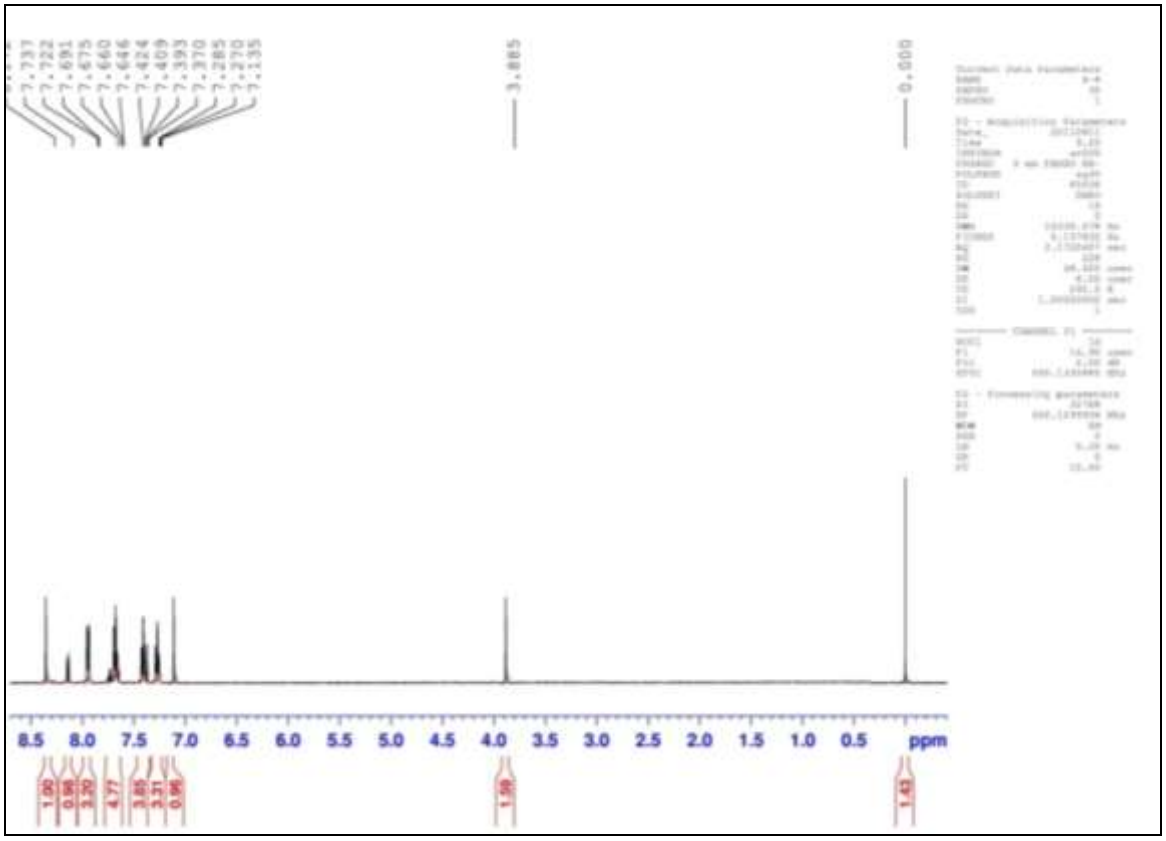
IR Spectrum of C5



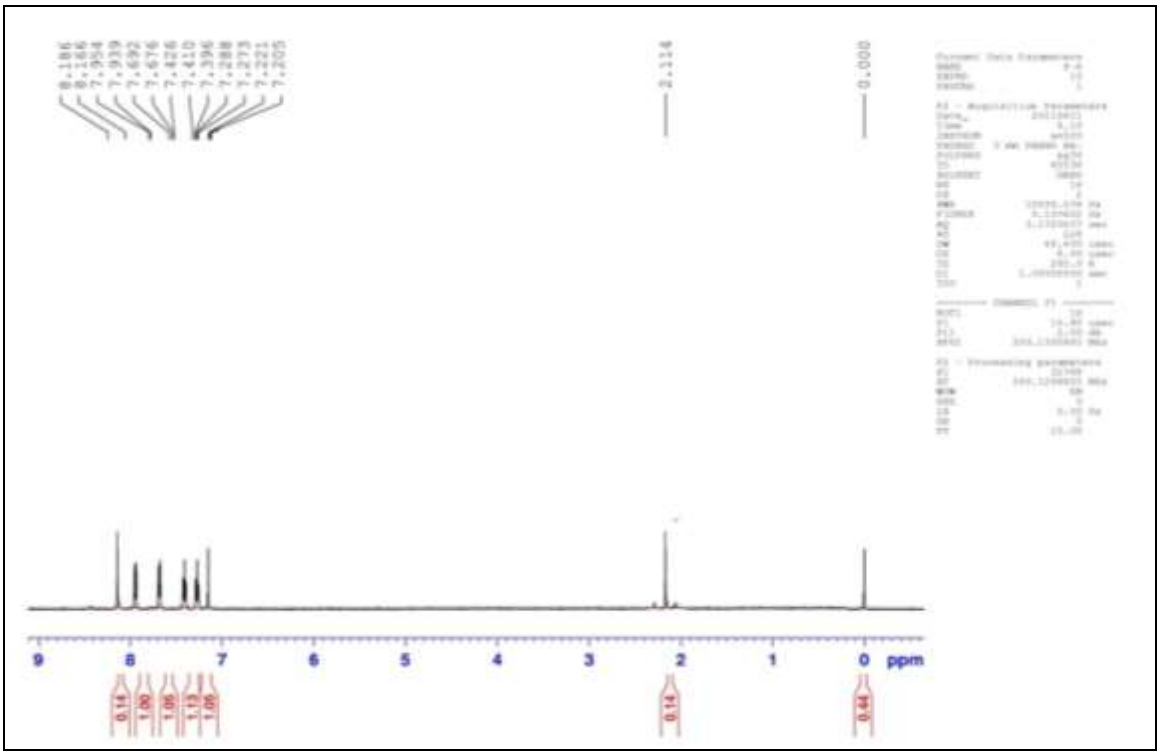
IR Spectrum of C6

NMR spectroscopy

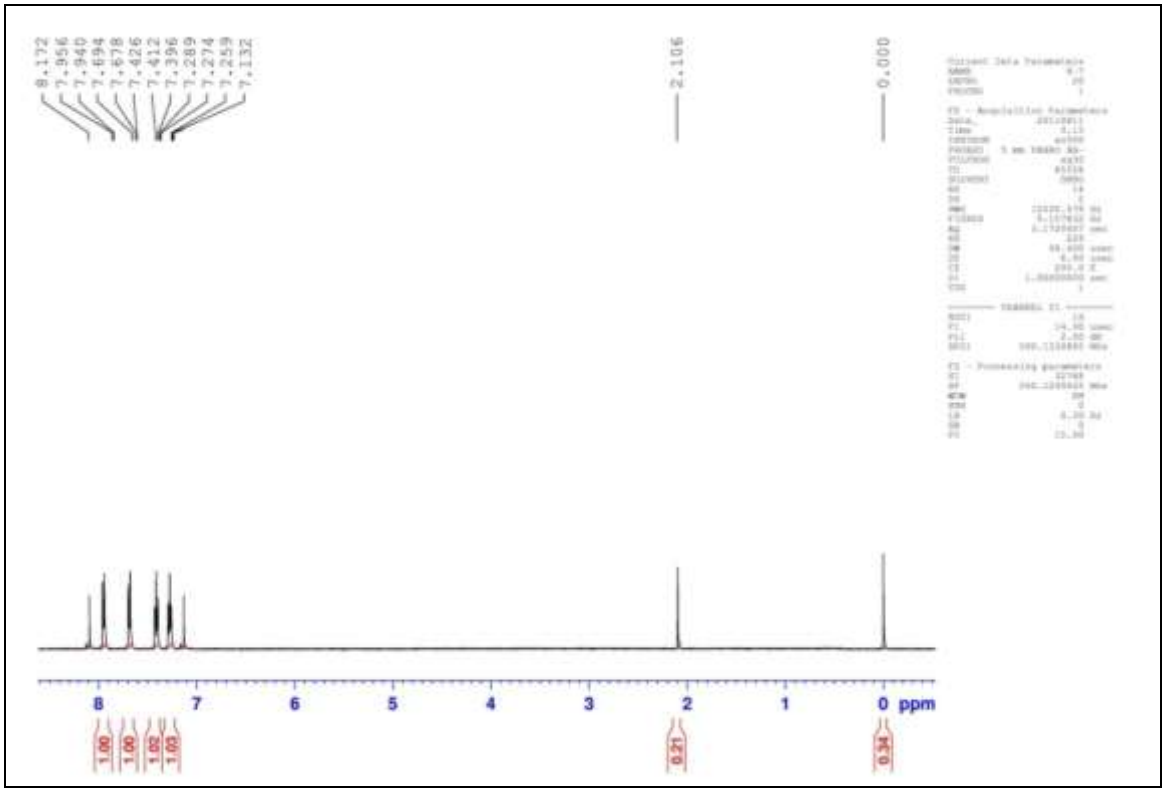
It showed a near spectra to parent molecule- benzothiazole when analyzed. Therefore, it confirmed that the derivatives have almost similar molecular structures.



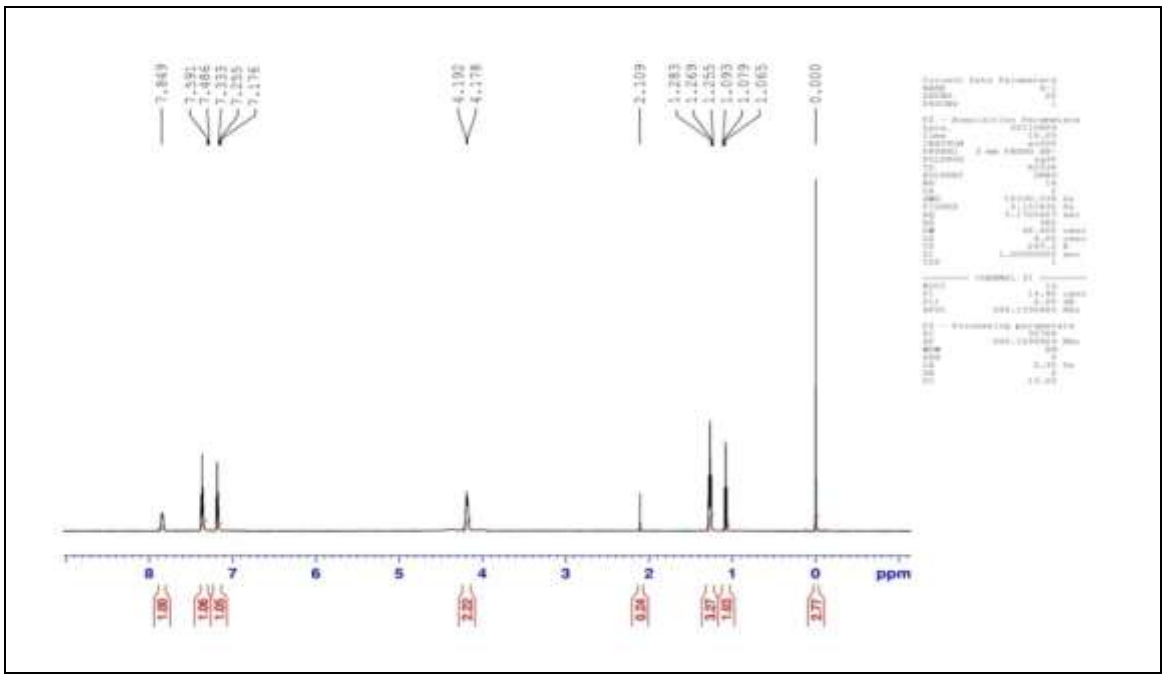
NMR Spectrum of C1



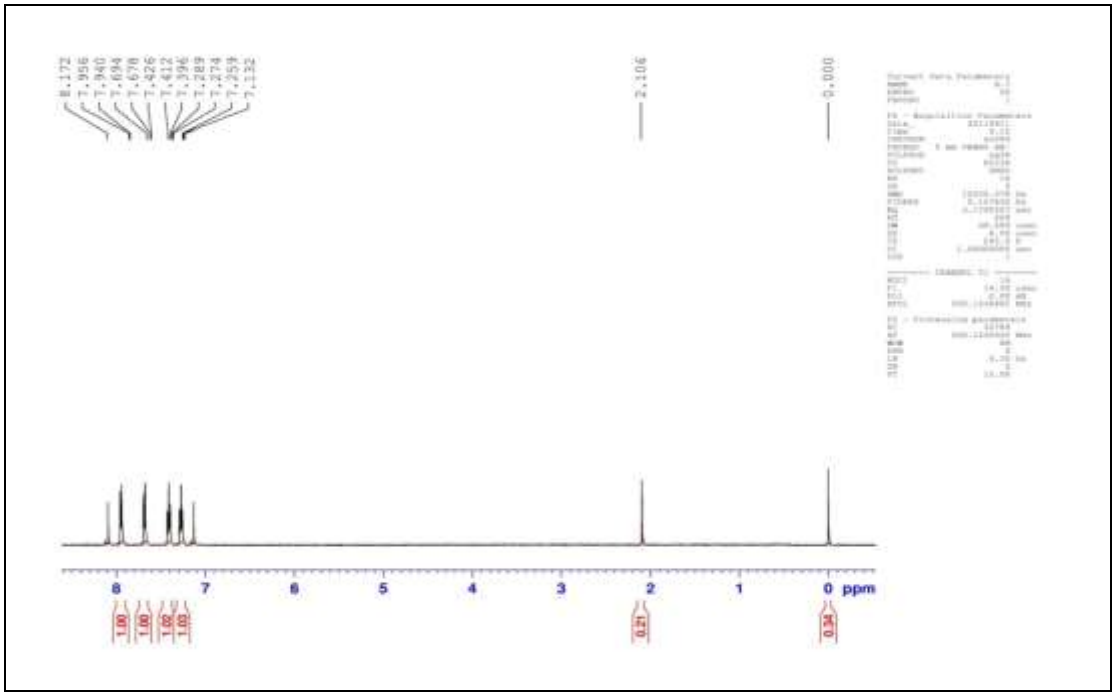
NMR Spectrum of C2



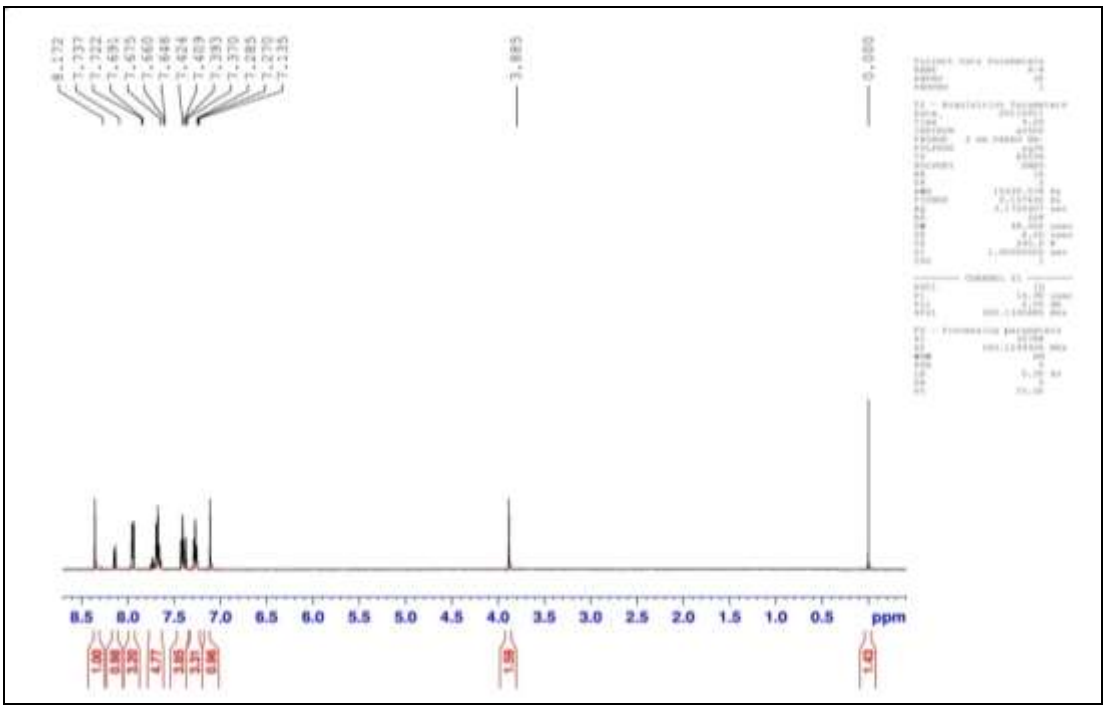
NMR Spectrum of C3



NMR Spectrum of C4



NMR Spectrum of C5



NMR Spectrum of C6

Evaluation Anti-rheumatic arthritis effect

Freund's adjuvant induced arthritis

All the benzothiazole derivatives were evaluated for anti-rheumatic arthritis effect by using Freund's adjuvant (1%) induced paw edema model. In this method, Freund's adjuvant (1%)

was given to group 2 in the dose of 0.1 ml for 21 days on daily basis. After completion of treatment time, they all were evaluated after 30, 60, 120 and 180 minutes for reduction in paw edema. Indomethacin was given in the dose of 10mg/kg b. w. of rats.

Compound C1, C2, C3, C4, C5 and C6 showed the volume of left hind paw as 2.11±0.27**, 2.29±0.28**, 2.32±0.04***, 2.41±0.02***, 2.37±0.20*** and 2.28±0.11***respectively, whereas indomethacin treated group showed 1.93±0.27*** and negative control group 2.86±0.28*, after 30 min of treatment. It showed that all the synthesized compounds exhibited significantly anti-rheumatoid arthritis potential when compared with standard and control group.

Table 2: Volume of left hind paw in control, std. and benzothiazole derivatives.

Compounds	Dose (mg/kg)	Volume of left hind paw (Mean± SEM)			
		30 min	60 min	120 min	180min
Vehicle	2ml	1.16±0.34*	1.23±0.10	1.14±0.32	1.26±0.14
Freund's adjuvant (1%)	0.1 ml	2.86±0.28*	3.45±0.02***	4.21±0.20**	4.77±0.07**
Indomethacin + Freund's adjuvant (1%)	200	1.93±0.27***	2.29±0.26**	2.81±0.23**	3.42±0.09**
Freund's adjuvant (1%) + C1	200	2.11±0.27**	2.69±0.12**	3.28±0.29**	3.91±0.14***
Freund's adjuvant (1%) + C2	200	2.29±0.28**	2.32±0.14**	3.46±0.29***	3.76±0.06**
Freund's adjuvant (1%) + C3	200	2.32±0.04***	2.36±0.03***	3.38±0.04**	3.92±0.04**
Freund's adjuvant (1%) + C4	200	2.41±0.02***	2.62±0.08**	3.68±0.18**	3.73±0.37**
Freund's adjuvant (1%) + C5	200	2.37±0.20***	2.48±0.29**	3.58±0.17**	3.67±0.27**
Freund's adjuvant (1%) + C6	200	2.28±0.11***	2.82±0.17***	3.62±0.02***	3.86±0.30**

Level of significance= *

When compared to the control group (n=6), experimental values were significantly different at the ≤P0.05 level.

Rheumatoid factors test

While determining antirheumatic arthritis effect, different derivatives of benzothiazole were evaluated for rheumatoid factor. C1demonstratedrheumatoid factor as 4.68±0.12, 8.83±0.19, and 6.17±0.16 in group 1, 2, and 3, respectively.

In Group 4, rheumatoid factor was noted as 7.45±0.25, 7.49±0.18, 6.10±0.39, 7.67±0.14, 8.63±0.22 and 7.09±0.29 in C1, C2, C3, C4, C5 and C6, respectively.

Table 3: Rheumatoid factor of derivatives in treated rats.

Compounds	Dose (mg/kg)	Rheumatoid factor
Vehicle	2ml	4.68±0.12
Freund's adjuvant (1%)	0.1 ml	8.83±0.19
Indomethacin + Freund's adjuvant (1%)	200	6.17±0.16
Freund's adjuvant (1%) + C1	200	7.45±0.25
Freund's adjuvant (1%) + C2	200	7.49±0.18
Freund's adjuvant (1%) + C3	200	6.10±0.39
Freund's adjuvant (1%) + C4	200	7.67±0.14
Freund's adjuvant (1%) + C5	200	8.63±0.22
Freund's adjuvant (1%) + C6	200	7.09±0.29

Level of significance= *

When compared to the control group (n=6), experimental values were significantly different at the ≤P0.05 level.

CONCLUSION

Joint deterioration and permanent incapacity are two of the long-term effects of rheumatoid arthritis, a chronic, inflammatory illness. In order to prevent permanent harm and the loss of vital body functions, early diagnosis and intervention are crucial. By stating the goals and then executing the protocols to reach and assess them, treat-to-target recommendations[34] can help guide the treating physician's decisions. Better treatment results can be achieved, for example, if patients are referred to specialists sooner rather than later. New insights into disease pathways made possible by developments in molecular medicine have allowed for the development of more potent therapies. By tailoring care to everyone, we can find effective treatments for patients more quickly and reduce the risk of their disease worsening while we test them. Researchers are also using gene array analysis to predict which patients will develop severe RA. Significant advancements in the treatment and management of RA are expected soon.

It might be helpful in the treatment of rheumatoid arthritis (RA) which is an auto-immune disorder and severely affecting a large population worldwide. It will be cost-effective with easy availability to mankind. Fellow researchers will evaluate the mode of action that how benzothiazole derivatives treat and prevent the progression of rheumatoid arthritis.

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CONFLICT OF INTEREST

Authors have confirmed for none conflict of interest.

REFERENCES

1. Klareskog L, Ronnelid J, Saevarsdottir S, Padyukov L, Alfredsson L. The importance of differences; On environment and its interactions with genes and immunity in the causation of rheumatoid arthritis. J Intern Med., 2020; 287(5): 514-533.
2. Smolen J S, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet, Oct 22, 2016; 388(10055): 2023-2038.
3. Bullock J, Rizvi SA A, Saleh A M, Ahmed SS, Do DP, Ansari RA, Ahmed J. Rheumatoid Arthritis: A Brief Overview of the Treatment. Med PrincPract., 2018; 27(6): 501-507.
4. Sparks JA. Rheumatoid Arthritis. Ann Intern Med., Jan 01, 2019; 170(1): 1-16.
5. Pincus T, O Dell JR, Kremer JM. Combination therapy with multiple disease-modifying antirheumatic drugs in rheumatoid arthritis: a preventive strategy. Ann Intern Med., Nov 16, 1999; 131(10): 768-74.
6. Kłodzinski L, Wisłowska M. Comorbidities in rheumatic arthritis. Reumatologia, 2018; 56(4): 228-233.
7. Gregersen P K, Silver J, Winchester R J. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum., 1987; 30(11): 1205-13.
8. Weyand C M, Hicok K C, Conn D L, Goronzy J J. The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis. Ann Intern Med., 1992; 117(10): 801-6.
9. Du Teil Espina M, Gabarrini G, Harmsen HJM, Westra J, van Winkelhoff AJ, van Dijl JM. Talk to your gut: the oral-gut microbiome axis and its immunomodulatory role in the etiology of rheumatoid arthritis. FEMS Microbiol Rev., Jan 01, 2019; 43(1): 1-18.

10. Wu H, Liao W, Li Q, Long H, Yin H, Zhao M, Chan V, Lau CS, Lu Q. Pathogenic role of tissue-resident memory T cells in autoimmune diseases. Autoimmun Rev., Sep, 2018; 17(9): 906-911.
11. Okada Y, Eyre S, Suzuki A, Kochi Y, Yamamoto K. Genetics of rheumatoid arthritis: 2018 status. Ann Rheum Dis., Apr, 2019; 78(4): 446-453.
12. Dedmon LE. The genetics of rheumatoid arthritis. Rheumatology (Oxford), Oct 01, 2020; 59(10): 2661-2670.
13. Padyukov L. Genetics of rheumatoid arthritis. Semin Immunopathol, Jan, 2022; 44(1): 47-62.
14. Ciccacci C, Conigliaro P, Perricone C, Rufini S, Triggianese P, Politi C, Novelli G, Perricone R, Borgiani P. Polymorphisms in STAT-4, IL-10, PSORS1C1, PTPN2 and MIR146A genes are associated differently with prognostic factors in Italian patients affected by rheumatoid arthritis. Clin Exp Immunol, Nov, 2016; 186(2): 157-163.
15. Stanford SM, Maestre MF, Campbell AM, Bartok B, Kiosses WB, Boyle DL, Arnett HA, Mustelin T, Firestein GS, Bottini N. Protein tyrosine phosphatase expression profile of rheumatoid arthritis fibroblast-like synoviocytes: a novel role of SH2 domain-containing phosphatase 2 as a modulator of invasion and survival. Arthritis Rheum, May, 2013; 65(5): 1171-80.
16. Stolt P, Bengtsson C, Nordmark B, Lindblad S, Lundberg I, Klareskog L, Alfredsson L., EIRA study group. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population-based case-control study, using incident cases. Ann Rheum Dis., Sep, 2003; 62(9): 835-41.
17. Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. Arthritis Rheum, Oct, 2004; 50(10): 3085-92.
18. Linn-Rasker SP, van der Helm-van Mil AH, van Gaalen FA, Kloppenburg M, de Vries RR, le Cessie S, Breedveld FC, Toes RE, Huizinga TW. Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles. Ann Rheum Dis., Mar, 2006; 65(3): 366-71.
19. Klareskog L, Stolt P, Lundberg K, Källberg H, Bengtsson C, Grunewald J, Rönnelid J, Harris HE, Ulfgren AK, Rantapää-Dahlqvist S, Eklund A, Padyukov L, Alfredsson L. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum, Jan, 2006; 54(1): 38-46.

20. Lundstrom E, Källberg H, Alfredsson L, Klareskog L, Padyukov L. Gene-environment interaction between the DRB1 shared epitope and smoking in the risk of anti-citrullinated protein antibody-positive rheumatoid arthritis: all alleles are important. Arthritis Rheum, Jun, 2009; 60(6): 1597-603.
21. Derksen VFAM, Huizinga TWJ, van der Woude D. The role of autoantibodies in the pathophysiology of rheumatoid arthritis. Semin Immunopathol, Jun, 2017; 39(4): 437-446.
22. Cross M, Smith E, Hoy D, Carmona L, Wolfe F, Vos T, Williams B, Gabriel S, Lassere M, Johns N, Buchbinder R, Woolf A, March L. The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis., Jul, 2014; 73(7): 1316-22.
23. Myasoedova E, Crowson CS, Kremers HM, Therneau TM, Gabriel SE. Is the incidence of rheumatoid arthritis rising?: results from Olmsted County, Minnesota, 1955-2007. Arthritis Rheum, Jun, 2010; 62(6): 1576-82.
24. Crowson CS, Matteson EL, Myasoedova E, Michet CJ, Ernste FC, Warrington KJ, Davis JM, Hunder GG, Therneau TM, Gabriel SE. The lifetime risk of adult-onset rheumatoid arthritis and other inflammatory autoimmune rheumatic diseases. Arthritis Rheum., Mar, 2011; 63(3): 633-9.
25. Ong CK, Lirk P, Tan CH, Seymour RA. An evidence-based update on nonsteroidal anti-inflammatory drugs. *Clin Med Res.*, Mar, 2007; 5((1)): 19–34.
26. Whittle SL, Colebatch AN, Buchbinder R, Edwards CJ, Adams K, Englbrecht M, et al. Multinational evidence-based recommendations for pain management by pharmacotherapy in inflammatory arthritis: integrating systematic literature research and expert opinion of a broad panel of rheumatologists in the 3e Initiative. *Rheumatology (Oxford),* Aug, 2012; 51((8)): 1416–25.
27. Tian H, Cronstein BN. Understanding the mechanisms of action of methotrexate: implications for the treatment of rheumatoid arthritis. *Bull NYU Hosp Jt Dis.*, 2007; 65((3)): 168–73.
28. Volin MV, Harlow LA, Woods JM, Campbell PL, Amin MA, Tokuhira M, et al. Treatment with sulfasalazine or sulfapyridine, but not 5-aminosalicyclic acid, inhibits basic fibroblast growth factor-induced endothelial cell chemotaxis. *Arthritis Rheum.*, Sep, 1999; 42(9): 1927–35.
29. Rein P, Mueller RB. Treatment with Biologicals in Rheumatoid Arthritis: an Overview. *RheumatolTher.*, Dec, 2017; 4(2): 247–61.

30. Rosman Z, Shoenfeld Y, Zandman-Goddard G. Biologic therapy for autoimmune diseases: an update. *BMC Med.*, Apr, 2013; 11(1): 88.
31. Louie GH, Ward MM. Changes in the rates of joint surgery among patients with rheumatoid arthritis in California, 1983-2007. *Ann Rheum Dis.*, May, 2010; 69(5): 868–71.
32. Escott-Stump S. *Nutrition and Diagnosis-Related Care.* Philadelphia: Lippincott Williams & Wilkins, 2011.
33. Pandey S. Various Techniques for The Evaluation of Anti Arthritic Activity In Animal Models. J Adv Pharm Technol Res., Apr-Jun, 2010; 1(2): 164–171.
34. Cho Y, T. R. Ioerger, and J. C. Sacchettini, "Discovery of novel nitrobenzothiazole inhibitors for *Mycobacterium tuberculosis* ATP phosphoribosyl transferase (HisG) through virtual screening," *Journal of Medicinal Chemistry*, 2008; 51(19): 5984–5992.