Available online at http://s-epub.in/ojs/index.php/TPHRES/

THE PHARMA RESEARCH

An International Journal of Pharmacy Research

Published on: 15-09-2013 **ISSN: 0975-8216**

IC Value: 4.36

SCREENING OF INVITRO ANTI-INFLAMMATORY ACTIVITY OF SOME NEWLY SYNTHESIZED PYRIMIDO [2, 1-B] [1, 3] BENZOTHIAZOLE AND ITS SUBSTITUTED DERIVATIVES

Mukesh Yadav

Affiliation:

Pharmacology Department

ABSTRACT

A series of pyrimido[2,1-b][1,3]benzothiazole derivatives were synthesized by reaction of 2-amino benzothiazole, aromatic aldehyde and active methylene compound in ethanol by conventional, as well as, microwave irradiation methods. The microwave irradiation technique gives better yield in shorter reaction time. Different heterocyclic compounds are made to synthesize by large number of efforts and their derivatives were found to possess antitumor, antidiabetic, antimicrobial, anticonvulsant and anthelmintic activities. The small and simple benzothiazole nucleus and its derivatives possess various diverse biological properties. These activities are also possessed by its substituted derivatives as well. Literature revealed that benzothiazole derivatives may serve as an important model on as potent anti-inflammatory agent. All the synthesized compounds were screened for their anti-inflammatory activity in-vitro.Most of the derivatives showed enhanced anti-inflammatory activity as compared to the standard drug. So, benzothiazole derivatives can serve as future therapeutic leads for the discovery of anti-inflammatory drugs.

Keywords: Pyrimido[2,1-b][1,3]benzothiazole, NSAID's, Anti-inflammatory Activity.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been available to small animal practitioners for many years, but their use has remained relatively uncommon. Recently, new discoveries about inflammatory mediators and their interactions in the inflammatory cascade, as well as new data on the biochemical mediators associated with osteoarthritis, have led to increased use of NSAIDs. The arrival of NSAIDs with better defined safety and efficacy profiles for dogs

has also dramatically increased their use. NSAIDs are known to provide analgesia, anti-inflammatory and antipyretic capabilities, yet the exact mechanisms of action for this group of drugs are still being elucidated. The classic explanation of their anti-inflammatory mode of action is inhibition of the cyclooxygenase (COX) enzymes. These enzymes are active in the metabolism of arachidonic acid. Furthermore, certain NSAIDs may have selectivity in their actions against these isoenzymes of cyclooxygenase. Likewise, conventional thinking states that NSAIDs act peripherally to provide analgesia. However, recent data also support a central mechanism of action for pain modulation, which may account for a significant portion of the therapeutic benefits they provide when treating osteoarthritis (OA). With these new insights, this article focuses on products used in the management of osteoarthritis. [1]

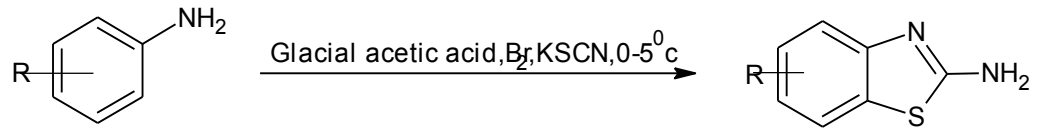
The chemistry and biological study of heterocyclic compounds has been an interesting field for a long time in medicinal chemistry. A number of heterocyclic derivatives containing nitrogen and sulphur atom serve as a unique and versatile scaffolds for experimental drug design. Benzothiazole is a heterocyclic compound, weak base, having varied biological activities and still of great scientific interest now a days. They are widely found in bioorganic and medicinal chemistry with application in drug discovery. Benzothiazole is one of the most important heterocyclic that has received overwhelming response owing to its diversified molecular design and remarkable optical and electronic properties. [2] Benzothiazole consists of thiazole ring fused with benzene ring and possesses multiple applications. The survey of literature related to benzothiazole reveals the presence of this bicyclic ring system in various amine or terrestrial natural compounds, which have useful biological properties. In recent years heterocyclic compounds analogues and derivatives have attracted strong interest due to their biological and pharmacological properties. Benzothiazole derivatives possess a wide spectrum of biological applications such as antitumor, antimicrobial, schictosomicidal, anti-inflammatory, anticonvulsants, Antidiabetic, antipsychotic and diuretic etc. [3]

EXPERIMENTAL SECTIONS

Step I-General synthesis of 1, 3-bennzothiazole-2-amine [4,5,6]

To glacial acetic acid (20ml) cooled below room Temperature were added 8gm (0.08mol) of Potassium thiocyanate and (0.01 mol) Substituted aniline. The mixture was placed in a Water bath and stirred with magnetic stirrer while 1.6ml of bromine in 6ml of glacial acetic acid was added from a dropping funnel at such a rate that the temperature never rises beyond room Temperature. After all the bromine was added (105 min.), the solution was stirred for 2 hours Below room temperature and at room Temperature for 10 hours, it was then allowed to Stand overnight, during which

period an orange Precipitate settle at the bottom, water (6ml) was Added quickly and slurry was heated at 85°C and Filtered hot. The orange residue was placed in a Reaction flask and treated with 10ml of glacial Acetic acid heated again to 85°C and filtered hot. The combined filtrate was cooled and neutralized by ammonia solution to pH 6, precipitate was collected and recrystallized.

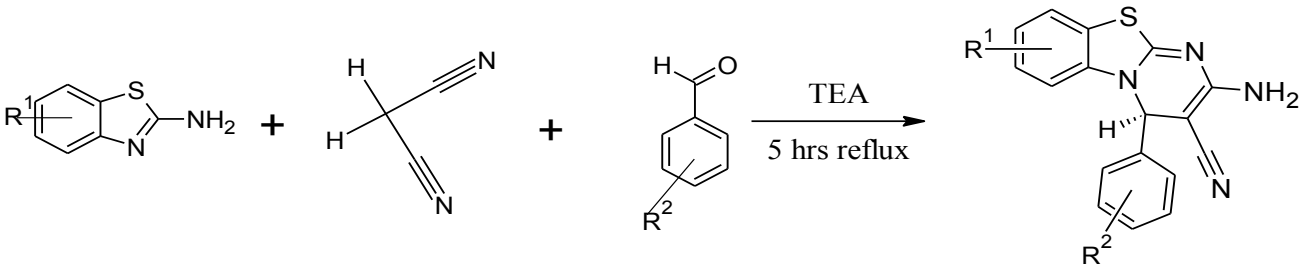


Substituted aniline

1,3-bennzothiazole-2-amine

Step II- A solution of substituted benzthiazole, malononitrile and substituted aldehyde in ethanol (25ml) add 4-5 drops of TEA. Placed in MW oven and irradiated for 4 min at 640 W. The reaction mixture was cooled at room temperature to give a solid mass which crystallized from ethanol.

FIGURE: - Reaction Scheme



1,3-Benzothiazol-2-amine

Propanedinitrile

Benzaldehyde

4-Bhenyl-4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-2-amine

Table 1: Physical constant of Synthesized compounds

Comp. ID	Substituents		M.p. (0C)	Synthesis Method								
				Reaction time & Rf value						%Yield		
	R1	R2		Conventional		MW		US		Conv.	MW	US
1a	Cl	NO_2	98-100	5hrs	0.72	5 min.	0.71	4hrs	0.72	72.20	91.65	87.43
1b	NO_2	Br	158-160	5 hrs	0.64	5 min.	0.63	4hrs	0.63	70.54	90.00	85.76
1c	Br	F	98-100	5 hrs	0.68	5 min.	0.66	4hrs	0.68	70.42	92.73	86.22
1d	NO_2	3,4,5-OCH_3	136-138	5hrs	0.64	5 min.	0.64	4hrs	0.64	68.56	88.55	85.54
1e	NO2	OH	150-152	5hrs	0.60	5 min.	0.62	4hrs	0.62	65.56	88.45	81.56
1f	OCH_3	3,4,5-OCH_3	140-142	5hrs	0.74	5 min.	0.72	4hrs	0.72	70.21	87.56	75.25
1g	Cl	Cl	158-160	5hrs	0.66	5 min.	0.68	-	-	55.25	74.45	-
1h	Cl	3,4,5-OCH_3	150-152	5hrs	0.66	5 min.	0.68	-	-	70.56	81.25	-
1i	Cl	Cl	158-160	5hrs	0.74	5 min.	0.72	-	-	68.12	81.56	-
1j	3-Cl,4-F	Br	142-144	5hrs	0.74	5 min.	0.70	-	-	58.25	77.12	-
1k	Br	Cl	158-160	5hrs	0.70	5 min.	0.72	-	-	74.12	90.12	-

R_f value was determined in benzene: ethyl acetate (8:2)

SPECTRAL DATA

(1a)4(*R*)2-amino-8-chloro-4-(3-nitrophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile

% Yield -91.65; Mp.98-100 (0C) IR :(KBr/cm-1)3381 (-NH str); 3183 (Ar-C-H); 2227 (CN str); 1678 (C=N); 1452(NO_2); 687(C-Cl); 1H NMR DMSO- δ (ppm) 7.0(m, 8H-Ar-H), 3.3 (s, 2H -NH_2), 2.0 (1H-Py-H) Mass (TOF MS ES) m/z: 383.81 (M+) Anal. Calcd. For $C_{17}H_{10}ClN_5O_2S$;C, 52.80; H, 2.75; N, 8.43; O, 18.37; S, 8.42.

(1b)4(*R*)2-amino-4-(4-bromophenyl)-8-nitro-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile

% Yield -90.00; Mp.158-160 (0C) IR :(KBr/cm-1); 3417(-NH str); 3032 (Ar-C-H); 2306 (CN); 1648 (C=N); 1481 (NO_2) ; 1H NMR DMSO- δ (ppm) 6.8-8.2(m, 8H-Ar-H), 3.0 (s, 2H -NH_2), 2.0 (1H-Py-H) Mass (TOF MS ES) m/z: 428.26

(M+) Anal. Calcd. For $C_{17}H_{10}BrN_5O_2S$; C, 46.84; H, 2.45; N, 52.16; O, 7.49; S, 7.51.

(1c)4(*R*)2-amino-8-bromo-4-(4-fluorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile

% Yield -92.73; Mp.98-100 (0C) IR :(KBr/cm-1); 3381(-NH str); 3035 (Ar-C-H); 2227 (CN); 1627 (C=N); 1222(C-F);1H NMR DMSO- δ (ppm) 7.1m, 8H-Ar-H), 2.9 (s, 2H -NH_2), 2.0 (1H-Py-H) Mass (TOF MS ES) m/z: 401.25 (M+) Anal. Calcd. For $C_{17}H_{10}BrFN_4S$; C, 49.99; H, 2.92; N, 13.99; S, 7.91.

(1d)4(*R*)2-amino-8-nitro-4-(3,4,5-trimethoxyphenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile

% Yield-88.55; Mp.136-138 (0C) IR :(KBr/cm-1); 3453(-NH str); 2979 (Ar-C-H); 2217 (CN); 1640 (C=N); 1500 (NO_2); 1H NMR DMSO- δ (ppm) 6.6-8.2(m, 8H-Ar-H), 2.8 (s, 2H -NH_2), 2.0 (1H-Py-H) Mass (TOF MS ES) m/z: 439.44 (M+) Anal. Calcd. For $C_{20}H_{17}N_5O_5S$; C, 53.81; H, 3.99; N, 16.01; O, 18.90; S, 7.29.

(1e)4(*R*)2-amino-4-(4-hydroxyphenyl)-8-nitro-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile

% Yield -88.45; Mp.150-152 (0C) IR :(KBr/cm-1); 3457(-NH str); 3076 (Ar-C-H); 2221 (CN); 1649 (C=N); 1493 (NO_2) 1H NMR DMSO- δ (ppm) 6.7-8.3(m, 8H-Ar-H), 2.34 (s, 2H -NH_2), 2.0 (1H-Py-H) Mass (TOF MS ES) m/z: 365.36 (M+) Anal. Calcd. For $C_{17}H_{11}N_5O_3S$; C, 54.56; H, 3.19; N, 19.89; O, 13.88; S, 8.48.

(1f)4(*R*)2-amino-7-methoxy-4-(3,4,5-trimethoxyphenyl)-4*H*-pyrimido[2,1*b*][1,3]benzothiazole-3-carbonitrile

% Yield -87.56; Mp.140-142 (0C) IR :(KBr/cm-1); 3452(-NH str); 3018 (Ar-C-H); 2218 (CN); 1642 (C=N); 1H NMR DMSO- δ (ppm) 6.5-8.0(m, 8H-Ar-H), 3.5 (s, 2H -NH_2), 2.0 (1H-Py-H) Mass (TOF MS ES) m/z: 424.47 (M+) Anal. Calcd. For $C_{21}H_{20}N_4O_4S$; C, 7.52; H, 4.74; N, 13.22; O, 15.10; S, 59.39.

(1g)4(*R*)2-amino-7-chloro-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile

% Yield -74.85; Mp.158-160 (0C) IR :(KBr/cm-1); 3448 (-NH str); 3033 (Ar-C-H); 2224 (CN); 1648 (C=N); 704 (C-Cl); 1H NMR DMSO- δ (ppm) 6.8-8.5 (m, 8H-Ar-H), 3.35 (s, 2H -NH_2), 2.0 (1H-Py-H) Mass (TOF MS ES) m/z: 373.25 (M+) Anal. Calcd. For $C_{17}H_{10}Cl_2N_4S$; C, 8.55; H, 2.69; N, 15.05; S, 54.71.

(1h)4(*R*)2-amino-8-chloro-4-(3,4,5-trimethoxyphenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile

% Yield -81.25; Mp.150-152 (0C) IR :(KBr/cm-1); 3452 (-NH str); 3030 (Ar-C-H); 2220 (CN); 1645 (C=N); 690 (C-Cl); 1H NMR DMSO- δ (ppm) 6.6-8.4(m, 8H-Ar-H), 3.34 (s, 2H -NH_2), 2.0 (1H-Py-H) Mass (TOF MS ES) m/z: 428.89 (M+); Anal. Calcd. For $C_{20}H_{17}ClN_4O_3S$; C, 7.47; H, 4.01; N, 13.01; O, 11.14; S, 56.11.

(1i)4(*R*)2-amino-6-chloro-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile

% Yield -81.56; Mp.158-160 (0C) IR :(KBr/cm-1); 3454 (-NH str); 3034 (Ar-C-H); 2305 (CN); 1641 (C=N); 705 (C-Cl) 1H NMR DMSO- δ

(ppm) 7.0(m, 8H-Ar-H), 3.2 (s, 2H -NH_2), 2.1 (1H-Py-H) ;Mass (TOF MS ES) m/z: 373.25 (M+); Anal. Calcd. For $C_{17}H_{10}Cl_2N_4S$; C, 54.71; H, 2.69; N, 8.55; S, 15.05;

(1j)4(*R*)2-amino-4-(4-bromophenyl)-7-chloro-8-fluoro-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile.

% Yield -77.12; Mp.142-144 (0C) IR :(KBr/cm-1); 3381 (-NH str); 3183 (Ar-C-H); 2227 (CN); 1678 (C=N); 1H NMR DMSO- δ (ppm) 7.0(m, 8H-Ar-H), 3.23 (s, 2H -NH_2), 2.1 (1H-Py-H) Mass (TOF MS ES) m/z: 335.70 (M+); Anal. Calcd. For $C_{17}H_9BrClFN_4S$; C, 46.86; H, 2.07; N, 12.88; S, 7.35.

(1k)4(*R*)2-amino-8-bromo-4-(4-chlorophenyl)-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carbonitrile.

% Yield -90.12; Mp.158-160 (0C) IR :(KBr/cm-1); 3387(-NH str); 3035 (Ar-C-H); 2227 (CN); 1627 (C=N); 687 (C-Cl); 1H NMR DMSO- δ (ppm) 7.5(m, 8H-Ar-H), 3.64 (s, 2H -NH_2), 2.0 (1H-Py-H) Mass (TOF MS ES) m/z: 417.71 (M+); Anal. Calcd. For C, 48.91; H, 7.62; N, 13.42; S, 2.43;

Table No.2 Elemental Analysis

Code	Calculated % of Elements					Found % of Elements				
	C	H	N	O	S	C	H	N	O	S
1a	53.20	2.63	18.25	8.34	8.35	52.80	2.75	18.37	8.42	8.43
1b	47.68	2.35	16.35	7.47	7.49	46.84	2.45	16.52	7.49	7.51
1c	50.89	2.51	13.96	-	7.99	49.99	2.92	13.99	-	7.91
1d	54.66	3.90	15.94	18.20	7.30	53.81	3.99	16.01	18.90	7.29
1e	55.88	3.03	19.17	13.14	8.78	54.56	3.19	19.89	13.88	8.48
1f	59.42	4.72	13.20	15.08	7.55	59.39	4.74	13.22	15.10	7.52
1g	54.70	2.70	15.01	-	8.59	54.71	2.69	15.05	-	8.55
1h	56.01	4.00	13.06	11.19	7.48	56.11	4.01	13.01	11.14	7.47
1i	54.70	2.70	15.01	-	8.59	54.71	2.69	15.05	-	8.55
1j	46.86	2.08	12.86	-	7.36	46.86	2.07	12.88	-	7.35
1k	48.88	2.41	13.41	-	7.68	48.91	2.43	13.42	-	7.62

***IN-VITRO* ANTI INFLAMMATORY ACTIVITY: [7, 8,9,10]**

The synthesized compounds were screened fo r anti-inflammatory activityusing inhibition of albumin denaturation technique .The standar d drug and test compounds were dissolved in minimum quantity of dimethyl formamide (D MF) and dilute with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solut ion was less than 2.5%.Test solution (1ml) con

taining different concentrations of drug was m ixed with 1 ml of 1mM albumin solution in ph osphate buffer andIncubated at 27° + 1° C in B OD incubator for 15 min. Denaturation was in duced by keeping thereaction mixture at 60° + 1° C in water bath for 10 min.After cooling, th e turbidity was measured at 660 nm (UV-Visibl eSpectrophotometer). Percentage of inhibition of denaturation was calculated from control where nodrug was added. Each experi ment was done in triplicate and average is tak en.

The Diclofenac was used as standard drug.

The percentage inhibition of denaturation was calculated by using following formula.

% of Inhibition = 100 X {Vt / Vc – 1}

Where, Vt = Mean absorbance of test sample.

Vc = Mean absorbance of control

RESULT: The compounds were synthesized under available laboratory conditions and were confirmed by physicochemical and spectral data.

Table 3: Screening of *invitro* anti inflammatory activity

Compounds	**Absorbance value (Mean+SE)**	**Inhibition of denaturation(in%)**
Control	0.097 + 0.009	**-**
1a	0.158 + 0.004	63.93
1b	0.117+ 0.004	21.40
1c	0.147+ 0.003	51.01
1d	0.137 + 0.002	41.80
1e	0.171 + 0.002	72.81
1f	0.163 + 0.002	67.02
1g	0.120 +0.001	22.46
1h	**0.175+ 0.004**	**78.93**
1i	**0.171 + 0.003**	**75.80**
Diclofenac	0.191 +0.002	92.86

CONCLUSION

In the research work carried out under the title, "**Screening of *invitro* anti-inflammatory activity of some newly synthesized pyrimido[2,1-*b*][1,3]benzothiazole and its substituted derivatives**" it can be concluded that the substituted derivatives pyrimido [2,1-b] benzothiazole have proven to be effective anti-inflammatory drug candidates. Most of the derivatives showed enhanced anti-inflammatory activity as compared to the standard drug Diclofenac sodium. Compounds **1h and 1i** have excellent activity, as compared to the standard drug. **Chloro and methoxy**

substituted derivatives showed enhanced antiinflammatory activity. So, these types of derivatives of Benzothiazole can serve as future therapeutic leads for the discovery of anti-inflammatory drugs. It can be concluded that this class of compounds certainly holds great promise towards good active leads in medicinal chemistry.

ACKNOWLEDGEMENT

We express sincere gratitude to Principal, Amrutvahini College of pharmacy, Sangamner for providing laboratory facilities. Director, SAIF Punjab University, Chandigarh for spectral analysis.

REFERENCES

1. Budsberg, S (1999), "Current and future trends in the use of NSAIDs for the treatment of osteoarthritis", *Waltham Focus*, Vol. 9(2), 26-31.

2. Yadav,P and Senthilkumar, G (2011), "Benzothiazole: Different Methods of Synthesis and Diverse Biological Activities", *Int. J. Pharm. Drug Res.,* Vol. 3(1), 01-07.

3. Khokra, S; Arora, K; Mehta, H; Aggarwal, A and Yadav M (2011), "Common methods to synthesize Benzothiazole derivatives and their medicinal significance: A review", Int*. J. Pharm. Res.,* Vol. 2(6), 1356-1377.

4. P S Yadav, Devprakash, Senthilkumar G P. Benzothiazole: Different Methods of Synthesis and Diverse Biological Activities; International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(1): 01-07.

5. Gupta S, Ajmera N, Gautam N, Sharma R, Gauatam D. Novel synthesis and biological activity study of pyrimido[2,1-*b*]benzothiazoles. Ind J Chem. 2009; 48B:853-858.

6. Kumbhare RM, Ingle VN. Synthesis of novel benzothiozole and benzisoxazole functionalized unsymmetrical alkanes and study oftheir antimicrobial activity. Ind J Chem. 2009; 48B:996-1000.

7. Venkatesh, P and Pandeya, S (2009), "Synthesis, characterization and anti-inflammatory activity of some 2-amino Benzothiazole derivatives", *Int. J. Chem. Tech. Res.*, Vol. 1(4), 1354-1358.

8. Gelias & M.N. A.Rao,Inhibition of Albumin Denaturation and Antiinflammatory Activity of Dehydrozingerone and Its Analogs, Indian.J.Exp.Biol, vol.26, July1998.

9. Gajraj Sharma, E.Jayachandran, G.M.Sreenivasa, Synthesis of Bioactive Molecule FluoroBenzothiazole Comprising Quinazolylinyl Oxadiazole Derivative For Biological and PharmacologicalScreening ,Ultra Chemistry vol.4(1),103:114, 2008.

10. LAD Williams, A Connar, L Latore etal, The invitro Anti-denaturation Effects Induced by Natu

ral Products and Non-steroidal Compounds in Heat Treated (Immunogenic)Bovine Serum Albumin is Proposed as a Screening Assay for the Detection of Anti-inflammatory Compounds', without the use of Animals ,in the early Stages of the Drug Discovery Process, West Indian Med J.57(4):327,2008.