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Synthesis and Biological Studies of Schiff Bases from Substituted Amino Benzothiazole on *In-Vitro* Anti-Inflammatory and Anti-Microbial Activities.

Balaji PN*, KP Priyanka, P Sai Sahithi, Reddy Prasanna K, R Lathamani, and Aswini M

Department of Pharmaceutical Chemistry, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati. 517503. Andhra Pradesh, India.

ABSTRACT

The Aim of this research work is to develop some new chemical compounds which can show theprominent action on inflammation caused by COX enzymes in body and disease causes from microbes. So the compounds fused heterocyclic ring contains N, S and O with other ring components shows desired pharmacological and biological activities are prepared. A serious of substituted Schiff bases i.e. N-[(Z)-phenylmethylidene]-1,3-benzothiazol-2-amine were synthesized from fluoro and nitro substituted Benzothiazol-2-amine with different aryl aldehydes under conventional method. The structure for compounds has been determined by Physical and spectral data like M.P, IR by KBrmethod,^{1}H-NM & Mass Spectroscopy. All the compounds are evaluated for their in-vitroanti-inflammatory and anti-microbial activity by standard methods.

Keywords: Benzothiazol-2-amine, Schiff bases, protein denaturation,anti-inflammatory and anti-microbial activity.

*Correspondence author

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INTRODUCTION

The Chemistry of heterocyclic compounds has been an interesting field for the study of long time for Developments of various types of drugs representfor most important advances in the therapeutics for cure and control of the serious infections or in prevention and treatment of infectious complications. 2-aminobenzothiazole were intensively studied as the scaffold in one of privileged structure in medicinal chemistry and reported cytotoxic on cancer cells and other numerous biological activities such as antimicrobial, anthelmintic, anti-diabetic activities and so on[1-7]. It must be emphasized that combination of 2-aminobenzothiazole with other heterocyclic is a well known approach to design new drug molecules, which allows achieving new pharmacological profile with toxicity lowering.

Compounds containing azomethine group (-CH=N-) is known as Schiff bases. Day by day Schiff bases are more frequently applied for the betterment of human welfare. The importance of the Schiff base is due its versatile nature. Literature survey shows that many Schiff bases exhibit biological activities such as antifungal, antibacterial, anthelmintic, antitumor, anti-inflammatory, and Anti-convulsant[8-12]. From the above two discussion we have planned to synthesize the heterocyclic compound which connects the fluoro & nitro substituted benzothiazole with different aryl aldehydes byazomethine bridge to produce more useful products. The structure for synthesized compounds was characterized by physical and spectroscopic data's like M.P, TLC, IR,^{1}H-NMR and Fab-mass spectroscopy[13-14]. All the synthesized compounds are screened for in-vitro anti-inflammatory activity by using inhibition of albumin denaturation technique, which was studied according to Muzushima and Kabayashi with slight modification. [15-17] and antimicrobial activity by the disc diffusion method by measuring diameter of zone of inhibition in mm[18-21]. This was carried over G+ve*Staphylococcus aureus* organism and for G-ve*Escherichia coli* organism for anti-bacterial activity. Antifungal activity was performed over *Candida albicans* and *Aspergillus flavus* the potency of activity was compared with a know standard drugs.

EXPERIMENTAL

All the chemicals are used in the synthesis are obtain from S.D. fine &Merckchemicals. the melting point for the compounds were determined by open capillary method which are incorrect, the synthesized compounds are characterized and identified by FT-IR by KBr method using ANALYTICAL TECHNOLOGIES FT-IR spectrophotometer 2202. Some selected compounds were subjected to ^{1}H-NMR spectra data were recorded on Bruker 400 MHZ in $CDCl_3$ using TMS as an internal standard and FAB- Mass for structural confirmation, all the compounds are screened for *in-vitro*anti-inflammatory and anti-microbial activity, the results are shown in the table.

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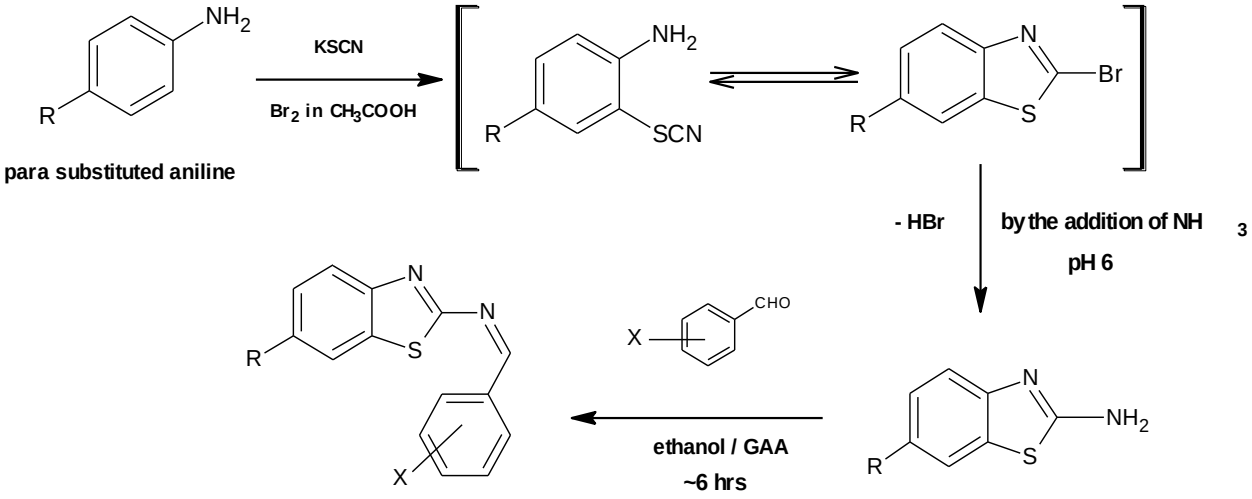
SYNTHETIC SCHEME



R = F and NO_2 X = p - Cl , p - OCH_3 , p - $N(CH_3)_2$, Vanilline

EXPERIMENTAL WORK

Synthesis of Substituted 2-aminobenzthiazole

The glacial acetic acid (20 ml) was taken in RB flask and cooled to 5 ^{0}C and Potassium thiocyanate (8gm, 0.08M) and substituted anilines (0.1M) was added to it with constant stirring. To this the Bromine in glacial acetic acid (1.6ml in 6ml) was added drop wise during stirring after completion of bromine addition the solution was stirred for 3 hours at 10^0 C and later the stirring was continued for 10 more hours at room temperature. The mixture was allowed to stand for overnight during which a orange colored precipitate was settled, and to this 10ml of distilled water was added and the mixture was heated on water bath at 85^0 C. Filter in hot condition, the orange residue was placed in to RBF later by adding 10ml of glacial acetic acid heated again for 85^0 C on water bath and filtered. The filtrate was combined and cooled and neutralized with 96% ammonia solution to pH 6 which produces a dark yellow precipitate this was filtered dried and subjected to recrystalization with ethanol. **M.p** = 220±2 ^{o}C (for Fluoro substitution) and 175±2 ^{o}C (for Nitro substitution).

Synthesis of Schiff-base from Substituted 2-aminobenzothiazole

An equimolar mixture of substituted 2-amino benzothiazole (0.01M) with different substituted aldehydes (0.01M) is taken in to 250ml round bottomed flask containing 30ml of ethanol and condensed. To this reacting mixture 2-3 drops of glacial acetic acid/conc. sulphuric acid is added and the condensation was continued for 6 hours. Later the mixture was cooled and poured into a crushed ice. The solid was separated, this separated solid was filtered dried and recrystallized by absolute alcohol.
The reaction is monitored by TLC and all the compounds are characterized by physical and spectral data as shown below table no 1.

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Table 1: Characteristic Analytical Data for Synthesized Compounds

S.No	C.C	MOLECULAR FORMULA	M.Wt	% YIELD	M.P ^{0}C	R_f Value *	CALCULATED %				
							C	H	N	O	S
1	BS-1	$C_{15}H_{11}N_3O_4S$	329.33	69	137	0.82	54.7	3.37	12.76	19.43	9.74
2	BS-2	$C_{15}H_{12}FN_2O_2S$	302.32	77	90	0.67	59.59	3.67	9.27	10.58	10.61
3	BS-3	$C_{14}H_9ClFN_2S$	272.75	72	190	0.77	57.83	2.77	9.64	_	11.03
4	BS-4	$C_{16}H_{14}N_4O_2S$	382.38	78	163	0.70	58.88	4.32	17.17	9.80	9.82
5	BS-5	$C_{15}H_{11}N_3O_3S$	313.33	64	230	0.85	57.50	3.57	13.4	15.20	10.23

* **n-Hexane : Ethyl acetate (6:4)**

Spectral Data for the Synthesized Compounds

BS-1.*2-methoxy-4-{(Z)-[(6-nitro-1,3-benzothiazol-2-yl)imino]methyl}phenol*

IR (KBr) cm-1: 1545 (Ar-C=C), 3110(Ar-C-H), 1630 (C=N, Schiff bases), 1330 (C-NO_2), 620 (C-S), 1300(C=N, thiazol). ^{1}H-NMR ($CDCl_3$, δ ppm) 2.49 (s, 3H, -OCH_3), 9.2 (s,1H, -OH), 7.98 (s, 1H, N=CH), 7.42-7.78 (m, 6H,Ar-H).

BS-2.*4-{(Z)-[(6-fluoro-1,3-benzothiazol-2-yl)imino]methyl}-2-methoxyphenol*

IR (KBr) cm-1: 1550 (Ar-C=C), 3080(Ar-C-H), 1600 (C=N, Schiff bases),1100 (C-F), 690 (C-S), 1340(C=N, thiazol).^{1}H-NMR ($CDCl_3$, δ ppm) 3.42 (s, 3H, -OCH_3), 9.54 (s, 1H, -OH), 7.72 (s, 1H, N=CH), 7.18-7.47 (m, 6H, Ar-H). M/z 301

BS-3.*N-[(Z)-(4-chlorophenyl)methylidene]-6-fluoro-1,3-benzothiazol-2-amine*

IR (KBr) cm-1: 1540 (Ar-C=C), 3065(Ar-C-H), 1610 (C=N, Schiff bases),1320 (C-NO_2), 605 (C-S), 1300(C=N, thiazol).

BS-4.*N-{(Z)-[4-(dimethylamino)phenyl]methylidene}-6-nitro-1,3-benzothiazol-2-amine*

IR (KBr) cm-1: 1520 (Ar-C=C), 3120(Ar-C-H), 1640 (C=N, Schiff bases), 1270 (C-F), 630 (C-S), 1310(C=N, thiazol).^{1}H-NMR ($CDCl_3$, δ ppm) 7.92 (s, 1H, N=CH), 7.56-7.89 (m, 7H, Ar-H), 2.56 (s, 6H, N(CH_3).M/z 382.

BS-5.*N-[(Z)-(4-methoxyphenyl)methylidene]-6-nitro-1,3-benzothiazol-2-amine*

IR (KBr) cm-1: 1510 (Ar-C=C), 3090(Ar-C-H), 1650 (C=N, Schiff bases), 1340 (C-NO_2), 695 (C-S), 1360(C=N, thiazol).

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BIOLOGICAL ACTIVITY

Anti-inflammatory activity (*in-vitro model*)

Many *in vitro* assays, each based on a specific biochemical or cellular mechanism, have been developed for the initial screening of the anti-inflammatory compounds. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins as an *in vitro* screening model for anti-inflammatory compounds. The synthesized compounds are screened for anti-inflammatory activity by using inhibition of albumin denaturation technique, which was studied according to Muzushima and Kabayashi with slight modification.

The standard drug and test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.0%. Test solution (1 ml) containing different conc. of drugs was mixed with 1 ml of 1% mM albumin solution in phosphate buffer and incubated at 27 ± 1^0 C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at $60^0\pm 1^0$C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible SpectrophotometerAnalytica). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The ibuprofen was used as standard drug. Results are tabulated in table no 2.

$$\% \text{ of inhibition} = Vt - \left(\frac{1}{Vc} \times 100\right)$$

Where, Vt = mean absorbance value of test group.
Vc = mean absorbance value of control group.

Table 2

S.No	Sample	Mean absorbance value ± SEM	% Inhibition of denaturation
1.	Control	0.0800	-
2.	Ibuprofen	0.155 ± 0.0080	93.75
3.	BS-1	0.131 ± 0.0070	63.75
4.	BS-2	0.128 ± 0.0065	60.01
5.	BS-3	0.136 ± 0.0035	70.00
6.	BS-4	0.142 ± 0.0050	78.25
7.	BS-5	0.132 ± 0.0073	72.12

Antimicrobial activity

All synthesized compounds were screened for antibacterial and antifungal activity by cup platemethod from the standard procedure; the two concentrations are taken i.e. 50 & 100 µg/ml over a different bacterial strains and fungal strains as shown in table. The values obtained are compared with the values produced from the standard drugs like Ampicillin for bacterial and Griseofulvin for fungal, the dimethyl formamide (DMF) was used as control for

all the strains. Some of the compounds show significant property compared with the standard and other shows moderate. This will be shown in the table no 3 and 4.

Table 3: Antibacterial activity of synthesized compounds

S. No.	Compound code	*Mean zone of inhibition in (mm)*			
		Staphylococcus aureus (G+Ve)		*Escherichia coli* (G-ve)	
		50 μg	100 μg	50 μg	100 μg
1.	Ampicillin	20	22	18	21
2.	BS-1	17	20	14	16
3.	BS-2	13	15	14	17
4.	BS-3	14	16	15	19
5.	BS-4	15	17	14	16
6.	BS-5	14	18	15	18
7.	Control (DMF)	-	-	-	-

Table 4: Antifungal activity of synthesized compounds

S. No.	**Compound Code.**	***Mean zone of inhibition in (mm)***			
		Candida albicans		***Aspergillus flavus***	
		50 μg	**100 μg**	**50 μg**	**100 μg**
1	Griseofulvin	18	21	19	23
2	BS-1	17	19	14	16
3	BS-2	16	17	17	20
4	BS-3	14	17	16	18
5	BS-4	13	15	15	18
6	BS-5	17	19	15	17
7	Control (DMF)	-	-	-	-

RESULT AND DISCUSSION

The desired Schiff bases are obtained by treating fluoro and nitro substituted Benzothiazol-2-amine with different aromaticaldehydes in ethanol (GAA as catalytic) media by the conventional methods, and the reaction was monitored by TLC on regular intervals of time and the characterization done by physical and spectral data. All the above synthesized compounds are evaluated for in-vitro anti-inflammatory activity according to Muzushima and Kabayashi with slight modification and antimicrobial (zone of inhibition) by cup plate method with50 μg & 100 μg concentrations. From the above obtainedresults the compound BS-4 show potent and other compounds 5>3>1>2 shows significant to moderate anti-inflammatory activity compared with STD Ibuprofen value. And the anti-microbial (for bacteria & fungi) activity all the compounds shows prominent activity by compared with Ampicillin standard drug for bacteria and Griseofulvin standard drug for fungi.

CONCLUSION

The Structure for synthesized compounds are identified by physical and spectral analysis and compounds shows promising activity for in-vitro anti-inflammatory and

antimicrobial activity, based upon the above results the compounds were further investigated for other activities.

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