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In vitro anthelmintic and antimicrobial activity of synthesized fluoro & nitro substituted *N*-[(*Z*)-phenylmethylidene]-1,3-benzothiazol-2-amine and its derivatives

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ABSTRACT

The Intention of doing this research work is to develop some new chemical compounds which can show their action on both parasitic worms and microbes which causing disease. So 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 KBr method, ^{1}H-NMR & Mass spectroscopy. All the compounds are evaluated for their In-vitro anthelmintic and anti-microbial activity by standard methods.

Key words: Benzothiazol-2-amine, Schiff bases, anthelmintic and anti-microbial activity.

INTRODUCTION

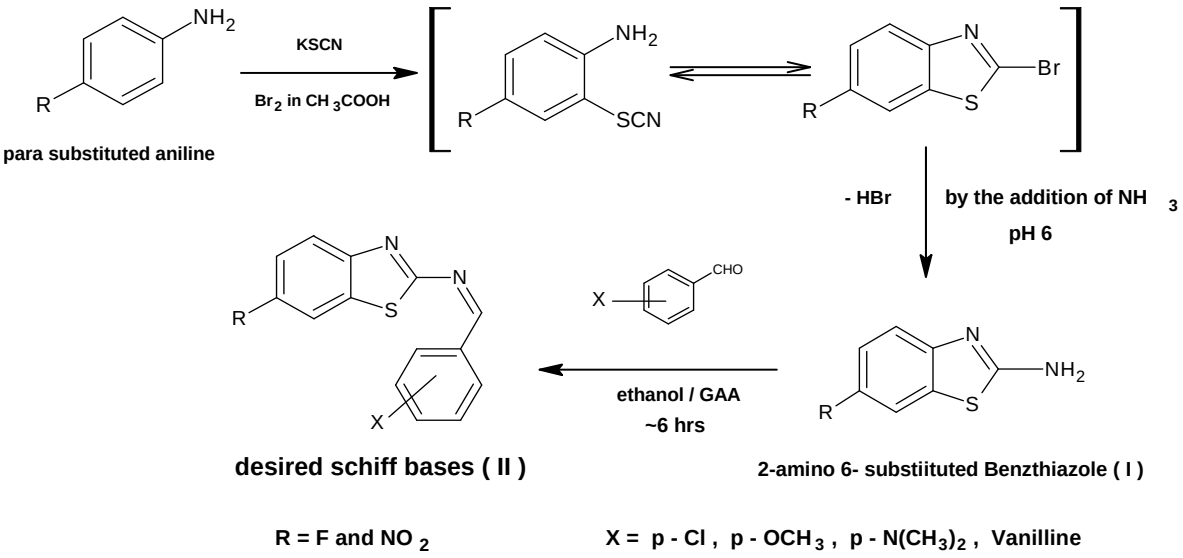
The developments of anthelmintic and antimicrobial drugs represent one of the most important advances in the therapeutics for cure and control of the serious infections or in prevention and treatment of infectious complications. 2-aminobenzothiazole were intensively studied as the scaffold in one of 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 by azomethine 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 & FAB-Mass [13-14]. All the synthesized compounds are screened for in-vitro anthelmintic by using Indian earth worm's *peritima postuma* by mathew *etal* standard procedure [15-16] and antimicrobial activity by the disc diffusion method by measuring diameter of zone of inhibition in mm [17-18]. The organism used here are G+ve *Bacillus cereus and Staphylococcus aureus* and for G-ve *Pseudomonas aeruginisa and Escherichia coli* for anti-bacterial activity. Antifungal activity was performed over organism *Candida albicans* and *Aspergillus flavus*, the potency of activity was compared with a known standard drugs.

MATERIALS AND METHODS

All the chemicals are used in the synthesis are obtain from S.D. fine & Merck chemicals. 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* anthelmintic and antimicrobial activity, the results are shown in the table.

Synthetic scheme



1. 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).

2. 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.

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).

Biological activity

Anthelmintic activity

The synthesized compounds evaluated for Anthelmintic activity by using Mathew *et al* method as standard by using Indian adult earthworms (*peritima postuma*). These earthworms (collected from the water logged areas of soil in and around Tiruchanoor, Tirupati) were washed with normal saline to remove all faecal materials. The earthworms in 4 - 5 cm in length and 0.1 - 0.2 cm in width were used for all experimental protocol because this earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study anthelmintic activity.

TABLE-2 Anthelmintic activity of synthesized compounds

S.NO	Compound code	% Concentration in w/v	*average time in minutes*	
			paralysis time	death time
1	CONTROL	0.9 %	-	-
2	ALBENDAZOLE	0.1 % 0.2 % 0.5 %	38 30 22	44 36 24
3	BS-1	0.1 % 0.2 % 0.5 %	26 34 12	42 38 14
4	BS-2	0.1 % 0.2 % 0.5 %	96 66 28	106 75 32
5	BS-3	0.1 % 0.2 % 0.5 %	65 76 54	71 78 57
6	BS-4	0.1 % 0.2 % 0.5 %	47 24 62	48 32 67
7	BS-5	0.1 % 0.2 % 0.5 %	20 46 10	24 53 13

Five earthworms of nearly equal size were placed in standard drug solution and test compounds solutions at room temperature. Normal saline used as control. The standard drug and test compounds were dissolved in minimum quantity of dimethyl sulfoxide (DMSO) and adjusted the volume up to 15 ml with normal saline solution to get the concentration of 0.1 % w/v, 0.2 % w/v and 0.5% w/v. Albendazole was used as a standard drug. The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive.

The mean lethal time for paralysis and death of the earthworms for different test compounds and standard drug are tabulated in table.-2

Antimicrobial activity

All synthesized compounds were screened for antibacterial and antifungal activity by cup plate method 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.

Table-3 Antibacterial activity of synthesized compounds

S. No.	Compound code	*Mean zone of inhibition in (mm)*			
		***Bacillus cereus* (G+ve)**		***Pseudomonas aeruginosa* (G-ve)**	
		50 µg	**100 µg**	**50 µg**	**100 µg**
1.	Ampicillin	18	22	21	24
2.	BS-1	15	18	17	21
3.	BS-2	17	19	16	20
4.	BS-3	16	20	17	19
5.	BS-4	15	19	13	17
6.	BS-5	17	18	15	20
7.	Control (DMF)	-	-	-	-

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)	-	-	-	-

RESULTS AND DISCUSSION

Schiff bases are been prepared by reacting the two different fluoro and nitro substituted Benzothiazol-2-amine with aryl aldehydes in ethanol (GAA as catalytic) media by conventional way, and the reaction was monitored by TLC on regular intervals and the characterization done by physical and spectral data. All the above synthesized compounds are evaluated for in-vitro anthelmintic and antimicrobial (by zone of inhibition) cup plate method with different concentrations. From the above activity report table will say the compound BS-5>1>4>2>3 shows prominent action over Indian earth worms *Peritima postuma* by comparing with Albendazole as standard drug values. And for anti-microbial activity all the compounds shows significant to moderate activity by compared with Ampicillin standard drug values for bacteria and Griseofulvin standard drug value for fungi.

CONCLUSION

The Structure for synthesized compounds are identified by physical and spectral analysis and compounds shows good activity for Anthelmintic and antimicrobial activity, based upon the above results the compounds were further screened for anti-tubercular activity are to be done.

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