Synthesis and Antimicrobial Evaluation of some novel Schiff bases derived from Benzothiazole derivative.

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Abstract : In an attempt to find out a new class of antimicrobial agents new series of Benzylidine-2- imino-1,3-benzothiazole and other related products containing benzothiazole moiety were prepared. The synthesis was carried out by condensation of different aromatic aldehydes with benzothiazole derivative using different procedures. The synthesised compounds were characterized and evaluated for antimicrobial activities.
The newly synthesized compounds were tested against representatives of gram-positive and gram- negative bacteria (staphylococcus aureus and Basillus subtilis.) by agar diffusion method using Ampicillin as control. The result indicates that the compounds possessed a broad spectrum of activity against the tested microorganisms. Structures of the newly synthesized compounds were established by physical and spectroscopic analysis.

Keywords : Benzothiazole moiety, aromatic aldehydes, antimicrobial activity.

1. Introduction :

The literature survey reveals that the benzothiazole ring is present in various marine or terrestrial natural compounds which shows useful biological activities. [11-15] The benzothiazole derivatives gained more importance and interest due to their anticancer [18] anticonvulsant[20] antitumor [22] antiviral [23] antibacterial[24]antimicrobial[25] and fungicidal activities [26]. They are also used as anti-allergic[27], anti-inflammatory [28] and anthelmintic [29] agents and as appetite depressant [31] and photographic sensitizers[33]. A detail literature survey indicates that benzothiazole ring system have a unique position in the designing and synthesis of novel biological active agents with remarkable analgesic and anti-inflammatory activities[36]

The preliminary study of the structure- activity relationship reveals that electronic factors in benzothiazole ring has a great effect on a antimicrobial activity of these compounds. The presence of amino group at 2- position increases the basicity of benzothiazole. Lone pair of electrons on azomethine nitrogen is not involved in aromatic sextet and is thus available for protonation. The presence of an amino group leads to the development of new properties that are associated with the manifestation of basicity and in addition to this, change the properties of the benzothiazole ring . The structure can be represented by tautomerism of imino – enamine tupe as bellow.

This tautomerism explains the greater reactivity and lower stability with respect to electrophilic reagents.
In general chemical properties of 2-aminobenzothiazles are determined by the thiazole ring, the benzene ring condensed with it and the amino group.

In view of the above mentioned facts and in continuation of our interest in the synthesis of heterocycles containing benzothiazole moiety , to identify new candidates that may be value in designing new, potent, selective and less toxic antimicrobial agents, we thought to design a new series using 2-amino derivative of benzothiazole.

We report herein the synthesis and antimicrobial evaluation of some novel structure hybrids incorporating both benzothiazole moiety and different aromatic aldehydes through imine linkages at 2-position of benzothiazole.

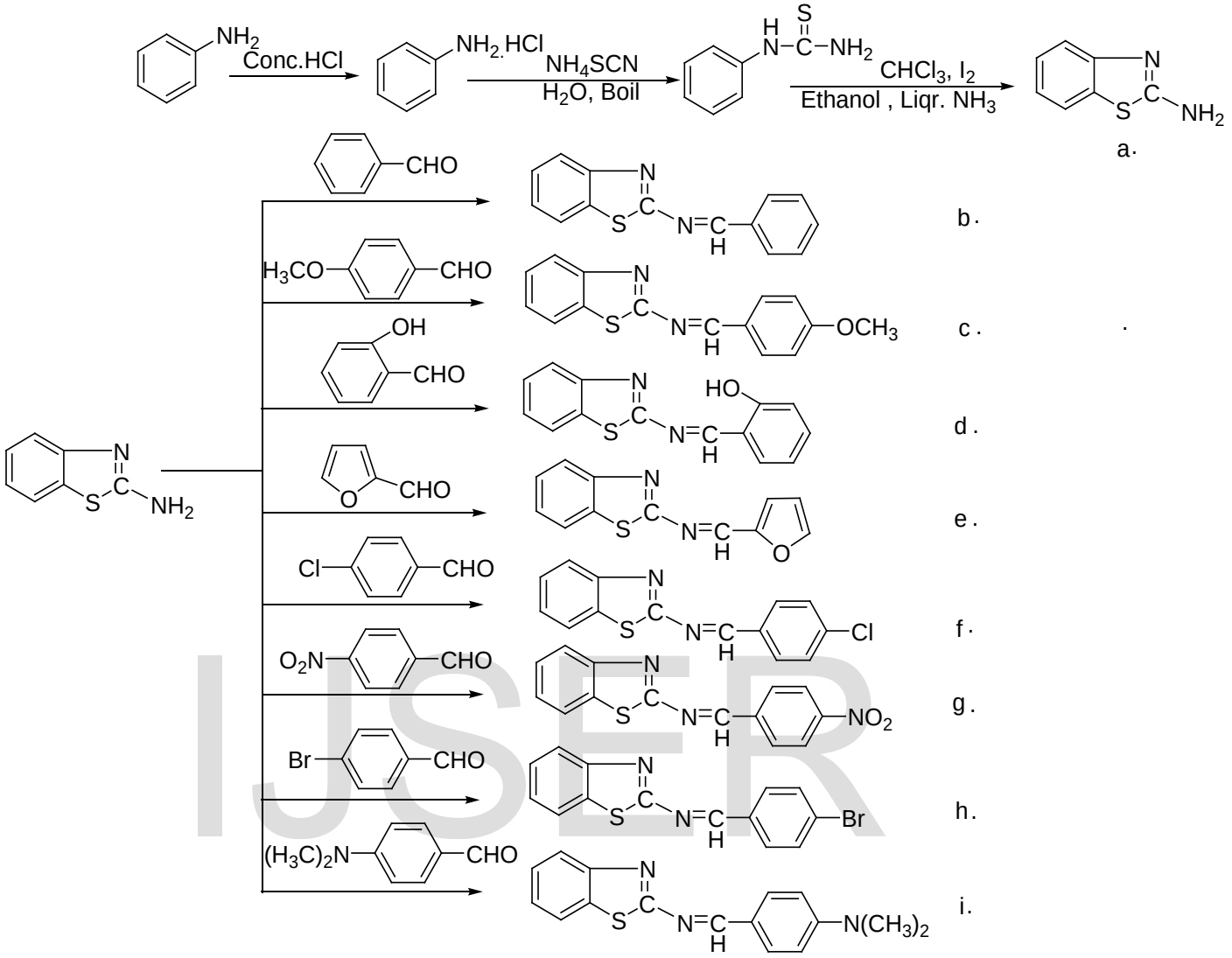
This was thought and suggested in an attempt to investigate the influence of structure variation on the anticipated biological activities, hoping to add some synthetic strategies and biological significance to the target molecules. The substitution pattern of benzothiazole ring was carefully selected so as to confer different electronic environment to the molecules.

2. Chemistry

The synthetic strategies adopted for the synthesis of the intermediates and target compounds are depicted in scheme-I. In scheme I the starting compound(a), 2-amino-1,3-benzothiazole was prepared by the cyclization of phenylthiourea in presence of I_2. The product obtained was recrystallized from 50 % alcohol and vacuum dried. The test compound had constant in agreement with the reported literature values. The structures confirmed by mass, IR, NMR, etc.

The general method which is employed to prepare schiffs bases (b- i) is outlined in scheme –I. Desired schiffs bases were prepared by condensing 2-amino-1,3-benzothiazole with selected aromatic aldehydes. The nucleophilic addition of $-NH_2$ group of hetero to aromatic aldehydes is not so straight forward and easier due to the presence of azomethine Nitrogen in heterocyclic ring. So different conditions were used to synthesis the target compounds eg. Azeotropic distillation method, using dehydrating agents, use of catalysts, etc. All compounds (b-i) were purified by recrystallization using absolute ethanol in order to avoid hydrolysis at varying rates of the compounds. The chemical structures of (a-i) were supported on the basis of LC-MS analysis and spectral data.

Scheme-I



3. Result and discussion :

The structures of newly described compounds (a-i) were confirmed by FT-IR ,^{1}H-NMR, LC-MS spectroscopic methods. Physical, chemical, IR, NMR, Mass relevant data of the compounds are reported in experimental section(4) separately. The experimental data obtained for each target molecule matches significantly with the reported data in literature and calculated.

The newly synthesized target compounds were evaluated for their in vitro bacterial activity against as example of Gram-positive bacteria (staphylococcus aureus and Basillus subtilis.) . Agar diffusion method was for the determination of the preliminary antibacterial activity. Ampicillin was used as reference drug. The result were recorded for each tested compounds as the average diameter of inhibition zones (IZ) of bacterial growth around the disks in cm. The minimum inhibitory concentration(MIC) measurement was determined for compounds showed significant growth inhibition zones

using serial dilution method. The MIC and inhibition diameters values are recorded in Table - 3.1

Table-3.1. Minimum Inhibitory Conecentration of Compound (b-i) ; zone inhibition diameter in (cm)

Compound	Basillus subtilis			Staphylococcus aureus		
	Concentration (µg / ml)			Concentration (µg / ml)		
	50	100	200	50	100	200
b	2.0	2.6	2.7	1.8	1.8	1.7
c	2.0	2.7	2.7	1.7	1.8	1.7
d	2.0	2.6	2.6	1.8	1.9	1.8
e	2.0	2.6	2.7	1.7	1.7	1.8
f	2.0	2.5	2.7	1.8	1.6	1.7
g	2.7	3.4	3.8	1.5	1.6	1.8
h	2.7	3.4	3.8	1.5	1.6	1.9
i	2.7	3.4	3.9	1.9	1.8	1.9

In general observation it is found that among all the compounds prepared, compound(i) bearing dimethylamino group showed the highest antibacterial activity followed by compound (g). Surprisingly compound(h) bearing bromo group showed appreciable activity against basillus subtillis and moderate activity against staphylococcus aureus.

So the result reveals that the benzothiazole moiety having imine linkages with aromatic nuclei having electron releasing group at preferably p-position might be interesting enough for further investigation on potential antimicrobial effects.

4. Experimental

All melting points were measured on a Gallenkamp electrothermal melting point apparatus. IR spectra were recorded for KBr disc on a FT-IR BRUKER ALPHA-200455. ^{1}H NMR spectra were measured on a Bruker AC 300 (300 MHz) in CDCl3 or DMSO-d6 as solvent, using TMS as an internal standard, and chemical shifts are expressed as δ ppm. LC- Mass spectra were determined on Finnigan Incos 500 (70 ev).

The progress of the reaction was monitored by Thin layer chromatography with F254 silica gel precoated sheets (MERCK) using pet. ether / ethyl acetate 55/45 as eluent; uv light was used for detection. Solvents unless otherwise specified , were of analytical reagent grade or of the highest quality commercially available.

Benzothiazole derivative and series of Benzylidine-2-imino-1,3-benzothiazole were prepared by the following procedures.

4.1 General procedure for the synthesis of 2-amino-1,3-benzothiazole ***(a)***

Aniline was treated with conc. HCl to get water soluble salt of aniline .

The mixture of aniline hydrochloride salt and ammonium thiocyanate solution in water refluxed till turbidity was found and then cooled to room temperature and poured in ice cold water. White crystals of phenylthiourea obtained was filtered, washed and vacuum dried. The phenylthiourea and iodine in chloroform was stirred for four hours at lower temperature. After completion of reaction , ethanol was added to the reaction mixture and

then basified with liquor ammonia. A solid product of compound(a) obtained was filtered , washed, recrystallised from 50% ethanol and vacuum dried.
Gray color crystals; yield 78 % m.p. 126-128 ^{0}C ,
Mole formula,$C_7H_6N_2S$ Mol. mass. 150
Mass [M/Z (rel. Int)] , M^+150 expected 150.
IR-KBr ν (cm $^{-1}$). 3399-3274 (1^{0}amine N-H str) , 3037 (ArC-H Str), 1590 (C=N thiazole), 1646-1648 (ArC=C str.)
^{1}H NMR,300MHz, $CDCl_3$ Ar-NH_2 δ 5.8(s), Ar-H δ 7-7.7(m).

4.2 *Synthesis of Benzylidine-2- imino-1,3-benzothiazole* ***(b)***

To a solution of compound **a**.(0.5 g, 33mmol) and pinch of anhydrous $ZnCl_2$ in ethanol(20 mL) added dropwise benzaldehyde(0.45ml, 37mmol) in ethanol(5mL) at boiling temperature. The reaction mixture was refluxed for 5hrs and then after completion of reaction, maximum ethanol was distilled out. The buff colour precipitate was formed after cooling, filtered, washed,recrystallised from absolute alcohol and dried to give compound (**b**)
Buff colour crystals; yield 56%; mp 122-124 ^{0}C, mol. formula $C_{14}H_{10}N_2S$, mol. mass 238
Mass [M/Z (rel. Int)] , M^+238,
IR-KBr ν (cm $^{-1}$). 2942,1283, 1063 (Azomethine -CH=N- str), 1586.90(C=N str thiazole,) 3073.18(ArC-H str.)
^{1}H NMR,(300MHz, $CDCl_3$, δ ppm) 10.0 (s ,1H,azomethine –CH=N-) 7.2-8.0 (m, 10H, aromatic C-H).

4.3 *Synthesis of p- methoxybenzylidine-2- imino-1,3-benzothiazole* ***(c)***

To a solution of compound **a**.(0.5 g, 33mmol) and 2-3 drops of glacial acetic acid in ethanol(20 mL) added dropwise p-anisaldehyde(0.6mL, 44mmol) in ethanol(5mL) at boiling temperature over a period of 25 min. The reaction mixture was refluxed for 4hrs and then after completion of reaction, maximum ethanol was distilled out. The pale yellow precipitate was formed after cooling, filtered, washed,recrystallised from absolute alcohol and dried to give compound (**c**)
Pale yellow crystals; yield 62%; mp 180-182 ^{0}C, mol. formula $C_{15}H_{12}N_2SO$, mol. mass 268
Mass [M/Z (rel.Int)] , M^+268 . IR-KBr ν (cm $^{-1}$). 2945, 1252.65,1019.99 (Azomethine -CH=N- str), 1594.63 (C=N str thiazole,), 3184 (ArC-H str.), 3298.35(OCH_3)
^{1}H NMR,(300MHz, $CDCl_3$, δ ppm) 9.9 (s ,1H,azomethine –CH=N-), 3.9 (s ,3H, OCH_3), 7.-7.3 (m, 4H, aromatic C-H), 7.5 (d, 2H, ArC-H), 7.9 (d, 2H, ArC-H),

4.4 *Synthesis of o-hydroxybenzylidine-2- imino-1,3-benzothiazole* ***(d)***

To a solution of compound **a**.(0.5 g, 33mmol) and 2-3 drops of glacial acetic acid in ethanol(20 mL) added dropwise salicyldehyde(0.5mL, 40mmol) in ethanol(5mL) at boiling temperature over a period of 20 min. The reaction mixture was refluxed for 3hrs and then after completion of reaction, maximum ethanol was distilled out. The yellow precipitate was formed after cooling, filtered, washed ,recrystallised from absolute alcohol and dried to give compound (**d**)
Yellow crystals; yield 58%; mp 144-146 ^{0}C, mol. formula $C_{14}H_{10}N_2SO$, mol. mass 254
Mass [M/Z (rel.Int)] , M^+254.8
IR-KBr ν (cm $^{-1}$). 2920,1282.35,1064.40 (Azomethine -CH=N-str), 1607.48 (C=N str thiazole,), 3020 (ArC-H str.), 3693.75(ArOH)
^{1}H NMR,(300MHz, $CDCl_3$, δ ppm) 9.4 (s ,1H,azomethine –CH=N-), 12.2 (s, 1H,PhO-H), 7.-8.0 (m, 8H ArC-H)

4.5 *Synthesis of furfurylidine-2- imino-1,3-benzothiazole* ***(e)***

To a solution of compound **a**.(0.5 g, 33mmol) in ethanol(20 mol) added drop wise a solution of furfuraldehyde (0.4mL, 41mmol) and 2-3 drops of glacial acetic acid in ethanol(5mL) at boiling temperature over a period of 25 min. Toluene(25 mL) was added to reaction mixture after 1 hr ,water formed was removed continuously by azeotropic distillation method. The reaction mixture was refluxed for 4hrs and then after completion of reaction, maximum ethanol and toluene was distilled out. The dark gray precipitate was formed after cooling, filtered, washed ,recrystallised from absolute alcohol and dried to give compound (**e**)
Dark gray crystals; yield 65%; mp 190-192 ^{0}C,
mol. formula $C_{12}H_8N_2SO$, mol. mass 228
Mass [M/Z (rel.Int)] , M^+228.
IR-KBr ν (cm $^{-1}$). 2942, 1286, 1098 (Azomethine -CH=N- str), 1520.00 (C=N str thiazole,), 3061 (ArC-H str.), 3187.09 (C-H str furan)
^{1}H NMR,(300MHz, $CDCl_3$, δ ppm) 8.1 (s ,1H,azomethine –CH=N-), 7.5.-7.7 (m, 4H ArC-H);
7.6 (d, 1H,OC-H), 7.5 (d, 1H, fuC-H), 7.2(t, 1H, fuC-H).

4.6 *Synthesis of p-chlorobenzylidine-2- imino-1,3-benzothiazole* ***(f)***

To a solution of compound **a**.(0.5 g, 33mmol) and pinch of anhydrous $ZnCl_2$ in ethanol(20 mL) added dropwise p-chlorobenzaldehyde(0.6 g, 42mmol) in ethanol(5mL) at reflux temperature. The reaction mixture was refluxed for 7hrs and then after completion of reaction, maximum ethanol was distilled out. The buff colour precipitate was formed after cooling, filtered, washed , recrystallised from absolute alcohol and dried
to give compound (**f**)

Buff colour crystals; yield 70 %; mp 130-132 ^{0}C, mol. formula $C_{14}H_{10}N_2S$, mol. mass 273
Mass [M/Z (rel.Int)] , M^+272.57.
IR-KBr ν (cm $^{-1}$). 2954.2, 1289.01, 1089 (Azomethine -CH=N- str), 1524.72 (C=N str thiazole,),
3069.40 (ArC-H str.), 749.97(Ar.C-Cl str)
^{1}H NMR,(300MHz, $CDCl_3$, δ ppm) 10.0 (s ,1H,azomethine –CH=N-) 8.2 (dd, 4H, ArC-H), 7.5 (m, 4H ArC-H)

4.7 *Synthesis of p-nitrobenzylidine-2- imino-1,3-benzothiazole* ***(g)***

To a solution of compound **a**.(0.5 g, 33mmol) in ethanol(20 mL) added drop wise a solution of p-nitrobenzaldehyde (0.648g, 42mmol) and a few drops of glacial acetic acid in ethanol(5mL) at boiling temperature over a period of 20 min. The reaction mixture was refluxed for 8hrs and then after completion of reaction, maximum ethanol was distilled out. The yellow precipitate was formed after cooling, filtered, washed , recrystallised from absolute alcohol and dried

to give compound (**g**)
Yellow crystals; yield 65%; mp 145-147 ^{0}C, mol. formula $C_{14}H_9N_3SO_2$, mol. mass 283
Mass [M/Z (rel.Int)] , M^+283 IR-KBr ν (cm $^{-1}$). 2948, 1248, 1062 (Azomethine -CH=N- str), 1612.16 (C=N str thiazole,),
3185.35 (ArC-H str.), 1345.39(Ar-NO_2 str.)
^{1}H NMR,(300MHz, $CDCl_3$, δ ppm) 9.4 (s ,1H,azomethine –CH=N-), 12.2 (s, 1H,PhO-H), 7.-8.0 (m, 8H ArC-H)

4.8 *Synthesis of p-bromobenzylidine-2- imino-1,3-benzothiazole* ***(h)***

To a solution of p-bromobenzaldehyde(0.793 g, 42mmol) and pinch of anhydrous $ZnCl_2$ in ethanol(20 mL) added drop wise a solution of compound **a**.(0.5 g, 33mmol) in ethanol(5mL) at reflux temperature. The reaction mixture was refluxed for 7hrs and then after completion of reaction, maximum ethanol was distilled out. The pale green colour precipitate was obtained after cooling, filtered, washed , recrystallised from absolute alcohol and dried to give

compound (**h**)
Pale green crystals; yield 60%; mp 190-192 ^{0}C, mol. formula $C_{14}H_9N_2SBr$, mol. mass 317
Mass [M/Z (rel.Int)] , M^+317.IR-KBr ν (cm $^{-1}$). 2969, 1281, 1063 (Azomethine -CH=N- str), 1524.00 (C=N str thiazole,), 3085 (ArC-H str.), 668.05 (Ar.C-Br str.)
^{1}H NMR,(300MHz, $CDCl_3$, δ ppm) 10.0 (s ,1H,azomethine –CH=N-) 8.0 (dd, 2H, ArC-H),
7.9 (dd, 2H, ArC-H), 7.7 (m, 4H ArC-H)

4.9 *Synthesis of p-N,N-dimethylaminobenzylidine-2- imino-1,3-benzothiazole* ***(i)***

To a solution of p-N,N-dimethylaminobenzaldehyde (0.640 g, 42mmol) and a few drops of glacial acetic acid in ethanol(20 mL) added drop wise a solution of compound **a**.(0.5 g, 33mmol) in ethanol(5mL) at reflux temperature over a period of 20 min. The reaction mixture was refluxed for 3.5hrs and then after completion of reaction, maximum ethanol was distilled out.
The orange precipitate was formed after cooling; filtered, washed, recrystallised from absolute alcohol and dried

to give compound (**i**)
Orange crystals; yield 70%; mp 206-208 ^{0}C, mol. formula $C_{16}H_{15}N_3S$, mol. mass 281
Mass [M/Z (rel.Int)] , M^+282.2 IR-KBr ν (cm $^{-1}$). 2950, 1255.61, 1010 (Azomethine -CH=N- str), 1577.78 (C=N str thiazole,), 3000 (ArC-H str.), 2866.52-N(CH_3)$_2$, 2966.48(CH_3)
^{1}H NMR,(300MHz, $CDCl_3$, δ ppm) 8.9 (s ,1H,azomethine –CH=N-) (s, 6H, -N(CH_3)$_2$), 7.8 (d, 2H, ArC-H),
7.9 (d, 2H, ArC-H), 7.2-7.6 (m, 4H ArC-H)

5. Conclusion :

The method employed in preparation of benzothiazole moiety and its derivative (b-i) gives excellent practical yield and high purity with simple method.
A series of Benzylidine-2- imino-1,3-benzothiazole have been synthesized and screened for their antimicrobial activity. The compounds having electron donating groups on aromatic nucleus shows better biological activity. The result suggested that among the compounds tested, compound (i0 has exhibited higher activity. It can be inferred from the above results that the new synthesized compounds possessing dimethyl amino group exhibit better antibacterial activity.

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