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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL INVESTIGATION OF SOME BENZOTHIAZOLE DERIVATIVES

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ABSTRACT

A series of some novel benzothiazole derivatives were synthesized and evaluated for anti-inflammatory, antioxidant and antimicrobial activity. The structures of the compounds were confirmed by spectral data. The anti-inflammatory, antioxidant and antimicrobial activities were determined by trypsin-induced hydrolysis of BSA, DPPH free radical scavenging assay, agar diffusion method respectively.

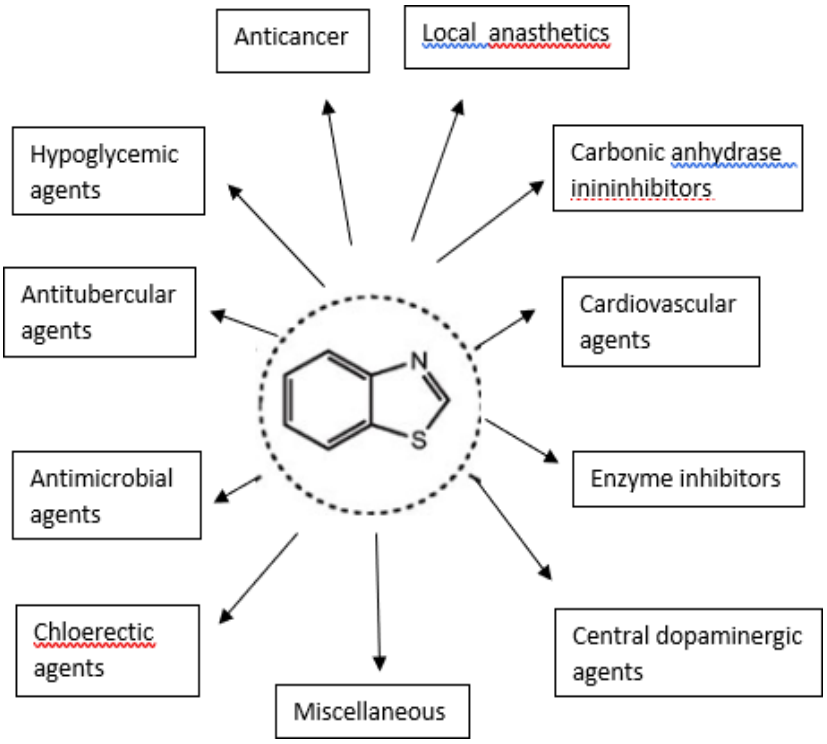
KEYWORDS: Benzothiazoles, synthesis, anti-inflammatory, antioxidant, antimicrobial.

1. INTRODUCTION

Benzothiazole finds an important place in heterocyclic chemistry, it occurs in many marine as well as natural plant products and is extensively used as reactant or intermediate.[1-7] It comprises a novel class of theraupeutic compounds shown to exert a wide range of molecular design, remarkable optical, liquid, electronic properties and biological activity such as antitumour, antimicrobial, anti-inflammatory, antioxidant, anticonvulsant etc. Various benzo-thiazoles such as 2-aryl benzothiazoles received much attention due to unique structure and its uses as radioactive amyloid imagining agents.[8-13]

Heterocycles are also useful compounds because of their synthetic utility as synthetic intermediate, protecting group, chiral auxillaries, organic catalysts in organic synthesis etc.[14] Therfore, new methods for synthesis of heterocycles have been paid too much attention. The alkaloids have a major group of naturaly occurring heterocyclic comounds. Alkaloids such as Ergotamine (indole based) and Cinchonine(quinolone based) exhibited antimigraine antimalarial activities respectively, they contain basic N-atoms. A Triazole based alkaloid Posaconazole has also been used as antifungal drug.[15]

The Aim of the present study is to synthesize some benzothiazole derivatives from 2-(4-aminophenyl)benzothiazol-5-ol as starting compound in which NH_2 and endocylic N functions are suitably situated to enable reaction with common electrophillic agents to form a variety of fused heterocyclic derivatives and to evaluate them for their activities.[16]



Typically, multi-component reactions (MCRs) are an powerful implement which attract much more attention in synthetic organic reactions with varied range of complexity where the initial materials are easily available. In MCRs the protection- deprotection steps are non-existent and environmentally benign.

The advantages of MCRs include shorter reaction times, lower costs, high atom economy, avoidance of time consuming, expensive purification process and energy saving.[17]

The benzothiazole is connected to the inclusion of the -S-C=N group in its molecular edge as toxophoric unit. Moreover, they have enhanced lipid solubility with hydrophilicity and they are easily metabolized by routine biochemical reactions and are noncarcinogenic in nature. Dithioate derivatives produce another class of sulfur-containing compounds that have been known as herbicides and fungicides.[18]

2. EXPERIMENTAL

2.1 MATERIALS AND METHODS

2.1.1 Preparation of the parent compound 2-(4-aminophenyl) benzothiazol-5-ol (1)[19]

It was synthesized in four steps as follows:

(i) Preparation of [(4-hydroxyphenyl)-4-azaneyl) (4-nitrophen) methanone (a)

To a solution of p-amino phenol and p- nitrobenzoyl chloride, pyridine (40 ml) was added followed by the addition of toluene (30ml) then the mixture was refluxed for 5hrs. The product obtained was recrystallized from alcohol. Yield:60%, m.p-150^{0}C.

(ii) Preparation of N-(4-hydroxyphenyl)-4-nitrobenzothioamide (b)

To an ethanolic solution of compound (a), Lawesson's reagent (0.6 molar eq) was added. The mixture was heated for 2hrs after which it was dried and recrystallised from alcohol.

Yield:65%, m.p-160^{0}C.

(iii) Preparation of 2-(4-nitrophenyl)benzothiazol-6-ol (c)

To a benzene solution of compound (b),0.5 ml ethanol and 1ml NaOH was added. The solution was cooled in an ice bath and freshly prepared aqueous potassium ferricynide(2-3 molar equivalent) was added. The reaction mixture was stirred at room temperature. Then the mixture was neutralized with 1M HCl. The organic layer was removed under vaccum and residue was washed and recrystallised from alcohol.

Yield:50 %, m.p-190^{0}C

(iv) Preparation of 2-(4-aminophenyl) benzothiazol-5-ol (1)

To an ethanolic solution of compound (c), 10 ml water,4 g iron powder and 7g ammonium chloride was added. The mixture was stirred at 85^{0}C for one hr. cooled at room temp. then filtered and washed with water and recrystallised from alcohol.

Yield:65%,m.p-140^{0}C.

2.1.2 Preparation of 2-chloro-N-(4-(5-hydroxy benzothiazol-2-yl) phenyl) acetamide 2(a)[20]

Compound 1 was refluxed with chloroacetyl chloride in the presence of potassium carbonate and chloroform to yield compound 2(a)

Yield-60% m.p-140^{0}C

2.1.3 Preparation of 2-(diphenylamino)-N –(4-(5-hydroxy benzothiazol-2-yl)phenyl) acetamide 2(b)

The condensation of 2(a) with secondry amine in absolute alcohol to give the final compound 2(b) which was separated and recrystallized by alcohol. Yield-80% m.p-196^{o}C.

2.1.4 Preparation of 3-(4-(5-hydroxybenzothiazol-2-yl)phenyl)-2-iminothiazolidin-4-one 3(a)[21]

Compound 1 was refluxed with NH_4SCN in presence of absolute ethanol to yield 3(a). Yield-70% m.p-150^{0}C

2.1.5Preparation of 5-butylidene-3-(4-(5-hydroxybenzothiazol-2-yl)phenyl)-2 iminothiazolidin-4-one 3(b)

3(a) and benzaldehyde were added to a solution of anhydrous sodium acetate in glacial acetic acid. The mixture was heated at 100^{0}C for 8hrs. Cooled to room temperature and poured into ice water.The solid 3(b) obtained was filtered, washed with water, dried and recrystallized from ethanol.

Yield-75% m.p-148^{0}C

2.1.6Preparation of 2-(4-((4-chlorobenzylidene)amino)phenyl)benzothiazol-5-ol (4)[22]

Compound 1 was refluxed on water bath with p-chloro benzaldehyde and 2-3 drops of glacial acetic acid in methanol for about 5 hrs. The solid obtained was recrystallised from methanol and chloroform.

Yield-60% m.p-70^{o}C

2.2 BIOLOGICAL INVESTIGATION

2.2.1Antiinflammatory Activity

The anti-proteolytic activityof the 2-(4-((4-chlorobenzylidene)amino)phenyl)benzothiazol-5-ol**(4)** and 5-butylidene-3-(4-(5-hydroxybenzothiazol-2-yl)phenyl)-2 iminothiazolidin-4-one **3(b)** was evaluated by trypsin-induced hydrolysis of BSA. Different amounts (200 to 1000 µg) of test samples were added to trypsin (100 U) and incubated for 15 min. Bovine serum albumin (1%, in 0.1M Phosphate buffer, pH 7.4) was added and further incubated for 20 min at 37. The reaction was terminated by addition of 3 ml of 5% TCA (Trichloro acetic acid). The reaction mixture was centrifuged at 5000 rpm for 5 min and the absorbance of clear supernatant liquid was recorded at 280 nm. The enzyme activity with no test sample was taken as control (100%

activity). The enzyme activity of trypsin in the presence of test samples was reported as percent relative activity.

2.2.2 Antioxidant Activity

The antioxidant activity of compound 2-(diphenylamino)-N–(4-(5-hydroxy benzothiazol-2-yl)phenyl)acetamide **2(b)** and 5-butylidene-3-(4-(5-hydroxybenzothiazol-2-yl)phenyl)-2 iminothiazolidin-4-one **3(b)** was analyzed using DPPH free radical scavenging assay method. Different concentrations of compounds (250, 500, 750,1000, 1250µg in 100µl) were taken in test tubes an d 2.9 ml of DPPH solution(2.5mg/100ml) was added. The reaction mixture was incubated for 30 min in dark and absorbance at 520 nm was recorded. The radical scavenging activity of the tested sample was calculated using the formula

$$\%\text{Antioxidant activity} = [(Ac - As)/Ac] \times 100$$

Where Ac and As are the absorbances of the control and sample, respectively. The control was prepared by adding 10µl of methanol in place of the sample.

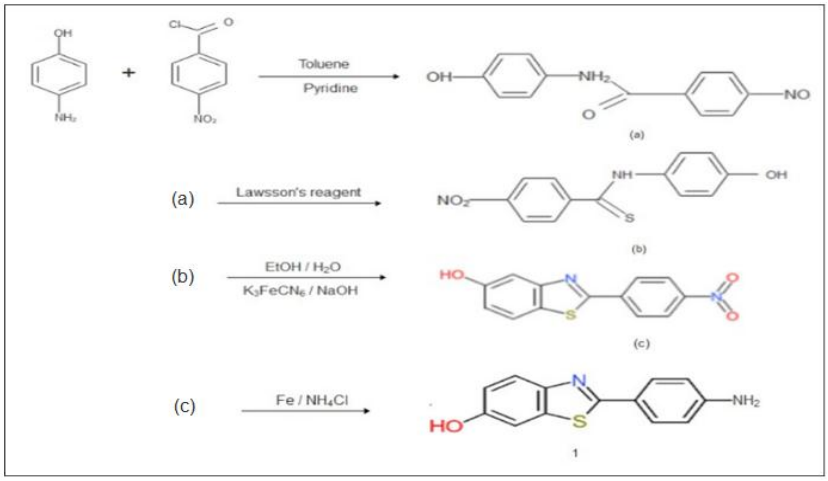
2.2.3 Antimicrobial Activity

The antibacterial activity of all the compounds was studied by agar diffusion technique using Salmonella typhi, Escherichia coli, Micrococcus luteus, Bacillus licheniformis (gram negative)and Staphococcus aureus, Bacillus subtilis, S.pyrogenes, Proteusvulgaris(gram positive)bacteria, All the compounds were dissolved in DMSO at the concentration of 100ug/ml for testing antibacterial activity. The compound diffused into the medium and produced concentration gradient, after the incubation period, the zone of inhibition was measured in mm.

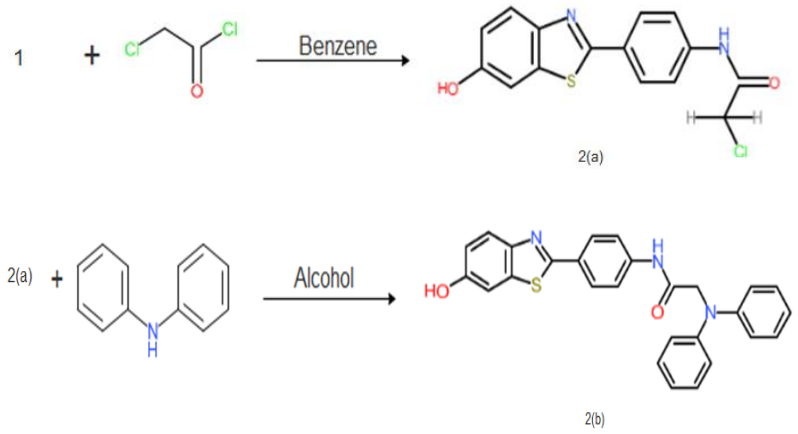
3. RESULTS AND DISCUSSIONS

The parent benzothiazole derivative (1) was synthesized by Jacobson method using Lawesson's reagent. The product obtained was used for the synthesis of other benzothiazole derivatives.

3.1 Synthesis of 2-(4-aminophenyl) benzothiazole-5-ol (1)

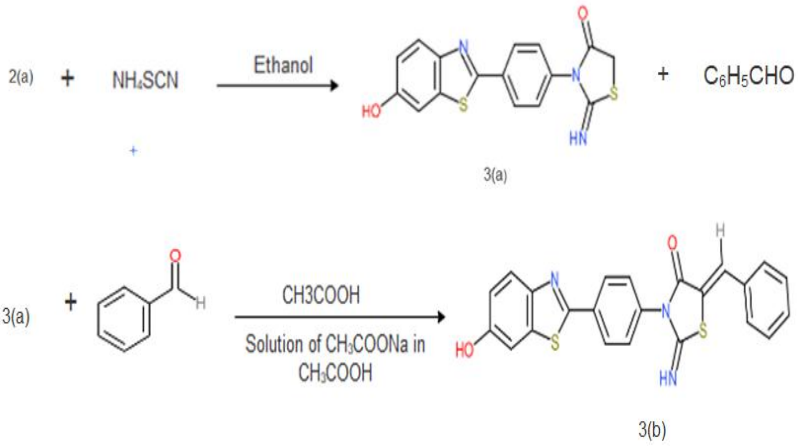


3.2 Synthesis of 2-(diphenylamino)-N-(4-(5-hydroxybenzothiazol-2-yl) acetamide 2(b)



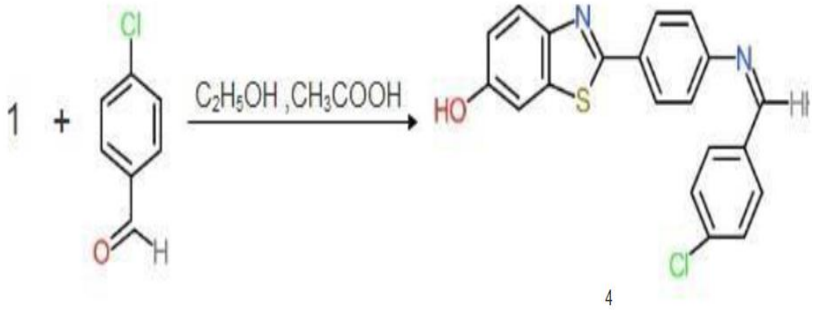
Scheme 1.

3.3 Synthesis of 5-butylidene-3-(4-(5-hydroxybenzothiazol-2-yl)phenyl)-2-iminothiazolidin-4-one 3(b)



Scheme 2.

3.4 Synthesis of 2-(4-((4-chlorobenzylidene)amino)phenyl)benzothiazol-5-ol (4)



Scheme: 3.

3.5 The Spectral data of the synthesized compounds are as followes

- **Compound** 2**(a)**

IR(cm^{-1})-3479(O-H)3387(N-H)3055(C-H)1659(C=O),^{1}HNMR(DMSO)δppm-2.55(CH_2), 6.5157,6.5235,6.7582,6.7803,7.2-8.2(Ar-H)^{13}CNMR(DMSO)δppm-143.40,129.10,119.57, 118.67,40.13,39.08

- **Compound 2(b)**

IR(cm^{-1})-3380.69(O-H)3128.80(C-H)1696.51(C=O)1137.92(C-N),^{1}HNMR(DMSO)δppm-6.5555(OH),7.484,7.5035,7.5460,7.6134,7.6297,7.6509,7.6653,7.6954,7.7169,7.6452,7.6343 ,7.5681,8.1645,8.1931,8.2781(Ar-H),^{13}CNMR(DMSO)δppm 143.40, 131.10, 119.57, 116.67, 40.13, 39.08,39.50

- **Compound 3(a)**

IR(cm^{-1})-3271(N-H)3040(O-H),1695(C=O),^{1}HNMR(DMSO)δppm-7.4-7.9(Ar-H) ^{13}CNMR(DMSO)δppm-170.64,132.47,131.47,129.43,120.4,39.34

- **Compound 3(b)**

IR(cm^{-1})-3320(N-H)3060(O-H)1639(C=N),^{1}HNMR(DMSO)δppm-2.51(CH_2)7.4-7.9(Ar-H),^{13}CNMR(DMSO)δppm-167.64,132.47,131.47,132.47129.43,120.4,39.97,39.34

- **Compound 4**

IR(cm^{-1})-3486.69(O-H), 1673.27(C=N),1540.32(C=C), 777.93(C-Cl), ^{1}HNMR (DMSO) δppm-2.5469(CH),7.62(OH),6.7952,6.7734(C=C-H),7.4569 ,7.5764, 7.5981, 8.2430, 8.2635, 8.3046(Ar-H), ^{13}CNMR(DMSO)δppm 134.61, 129.07, 127.65, 125.86, 122.41, 118.66,108.65.

Some of these synthesized compounds were tested for antiinflammatory activity and antioxidant ativity and all were tested for antimicrobial activity and it was shown in Table-1,2 and 3 respectively.

3.6 Antioxidant Activity

The DPPH solution has a deep purple color, with a strong absoption at 517 nm, and turns to yellow in the presence of antioxidants, which neutralizes the free radicals by pairing the DPPH odd electron with a hydrogen atom or by electron donation. Reduction of DPPH absorption at 517 nm represents the capacity of antioxidants to scavenge free radical.

The inhibitory effects of different concentrations of synthesized compounds 2(b) and 3(b)on DPPH radical are presented in Table-1 Chart-1 antioxidant activity.

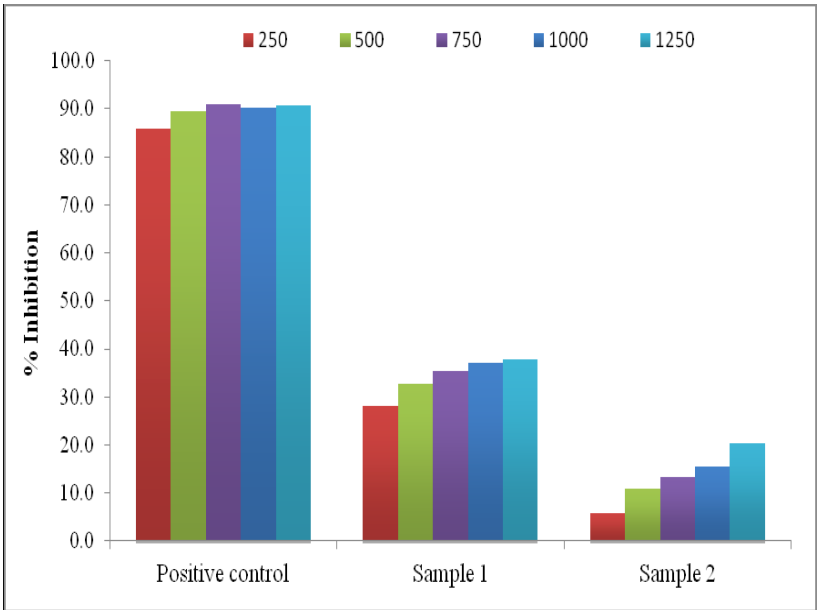


Chart 1-Antioxidant activity.

Table-1 % Inhibition of 2(b) and 3(b) and Ascorbic acid (positive control).

Concentration(ug/ml)	Positive control	2(b)	3(b)
250	85.9	28.1	5.96
500	89.4	32.8	11.20
750	91.0	35.3	12.65
1000	90.1	37.1	16.01
1250	90.6	37.8	20.75

The Chart- 1 and Table -1 showes that compound 2(b) has moderate antioxidant activity at all concentration with respect to positive control.

3.7 Anti-inflammatory Activity

Table-2 Relative activity of (4) and (3b)at different concentration with respect to control.

Concentration(ug)	Control	(4)	(3b)
200	100	123.553	106.732
400	100	143.595	100.923
600	100	159.733	105.568
800	100	204.991	97.942
1000	100	255.511	93.143

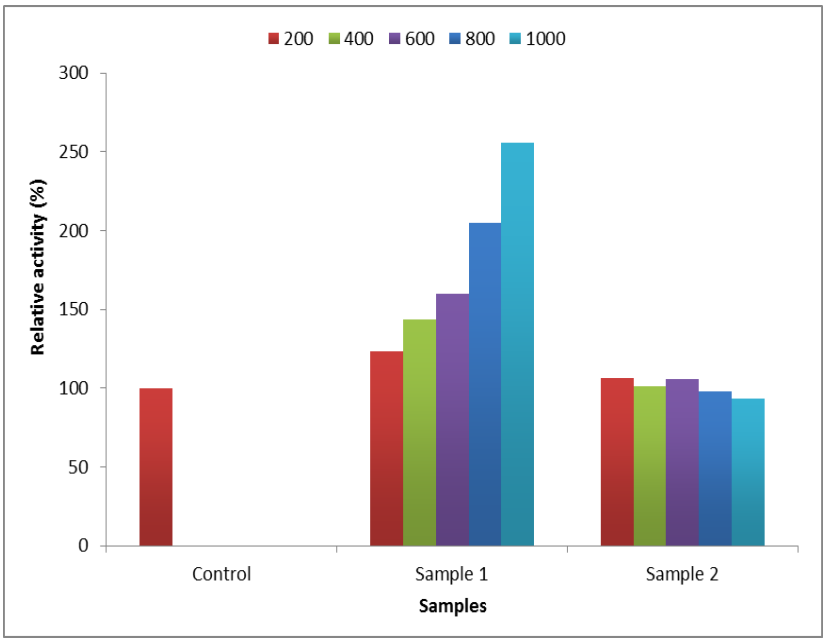


Chart 2: Antiinflammatory activity.

The Table-3 and chart-2 indicate that the compounds **(4)** showes the good anti-inflammatory activity by inhibiting protein denaturation and it increases with increasing the concentrations.

3.8 Antimicrobial Activity

Table-3: Antimicrobial activity.

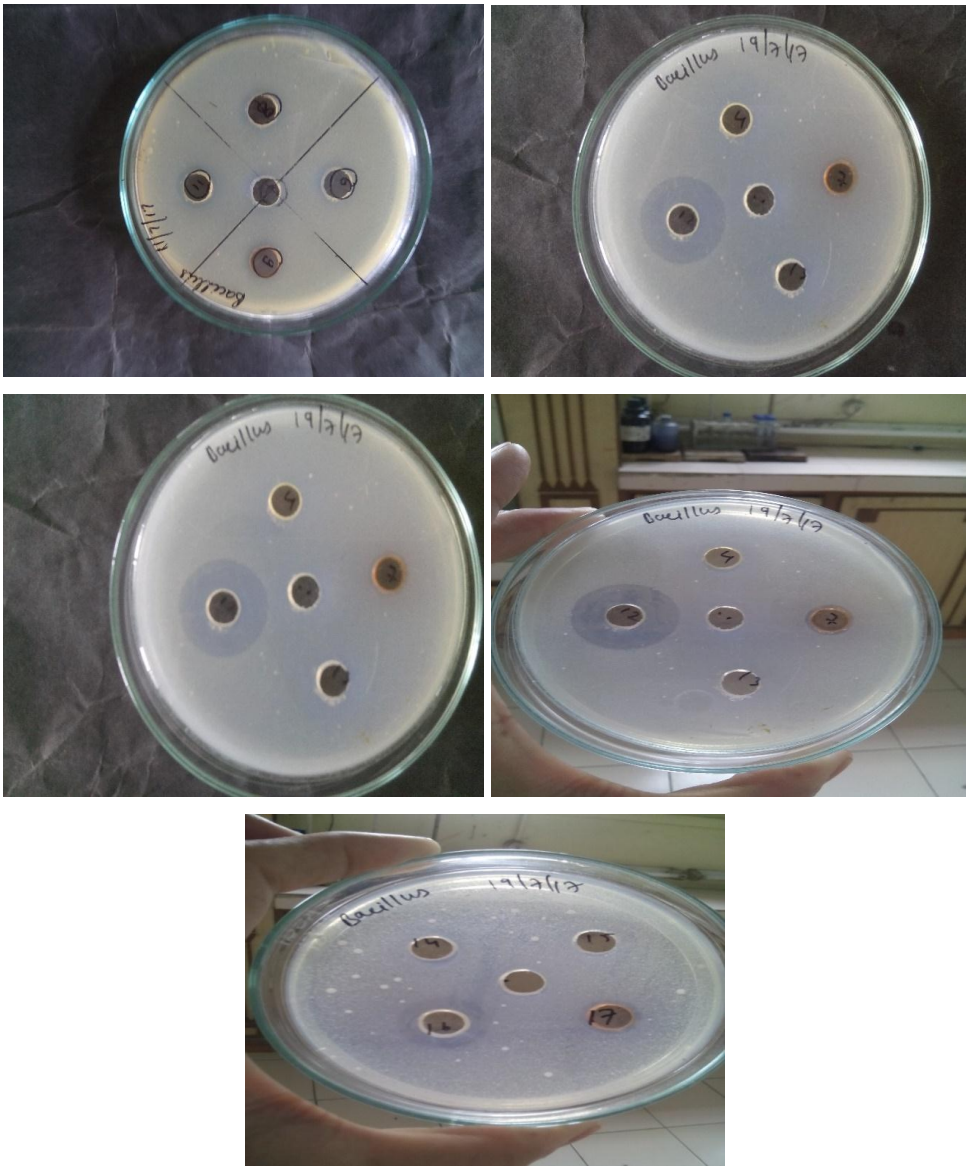
Gram -ve bacteria

Compounds	Salmonella typhi	Escherichia coli	Micrococcus luteus	Bacillus licheniformis
2(a)	26	22	20	19
2(b)	20	15	20	26
3(a)	18	15	22	16
3(b)	18	19	23	16
4	19	15	16	14
chloramphenicol	28	23	30	26
DMSO	--	--	--	--

Gram +ve bacteria

Compounds	Staphococcus aureus	Bacillus subtilis	S.pyrogenes	Proteus vulgaris
2(a)	18	14	15	14
2(b)	18	17	19	15
3(a)	26	27	20	16
3(b)	26	23	22	17
4	26	28	22	14
chloramphenicol	28	30	24	22
DMSO	--	--	--	--

All the synthesized compound are broad spectrum because they are active against both gram positive and gram negative bacteria. The result of antibacterial activity show that the compounds have moderate to good antibacterial activity. However compound 2(b) was found to be the most potent.



4. CONCLUSSION

In the present study compound 1 has been used as a template for the synthesis of some new benzothiazole derivatives(2a,b; 3a,b; and 4). All the synthesized compound were tested for biological activities. Out of these compound **2(b**) shows moderate antioxidant activity, compound **4** shows good anti-inflammatory activity and rest of the compounds show very low antioxidant and anti-inflammatory activity. All the synthesized compounds show good antimicrobial activity.

5. ACKNOWLEDGMENT

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