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Synthesis and antibacterial activity of some novel fluorobenzothiazole derivatives

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ABSTRACT

*A series of 2,2'-(2-(2-benzylidenehydrazinyl)-6-fluorobenzo[d]thiazol-7-ylazanediyl) diethanol derivatives **(4a-e)** were synthesized in order to examine their in vitro antimicrobial activities against Staphylococcus aureus, Bacillus subtilis Gram-positive and Escherichia coli, Pseudomonas aeruginosa Gram-negative bacteria. 4-fluoro-3-chloroaniline was treated with potassium thiocyanate in presence of bromine in glacial acetic acid to get 2-amino-7-chloro-6-fluoro benzothiazole **(1)**, which was further treated with hydrazine hydrate and concentrated HCl to get of 2-amino-7-chloro-6-fluoro-benzothiazole **(2)**. Then Schiff's base **(3a-e)** is prepared by reaction of fluoro-benzothiazole with different substituted aromatic aldehydes. The final targeted compounds **(4a-e)** were synthesized by substituting seventh chlorine of compound **3a-e** by diethanolamine in presence of triethylamine. All the compounds were characterized by melting point, TLC and the chemical structures of the compounds were elucidated by IR, ^1H NMR, mass spectroscopy. Among the series, compounds **4b, 4d** and **4e** exhibited good antibacterial activity profile as compared with the standard. In summary, preliminary results indicate that some of the newly synthesized title compounds exhibited promising antibacterial activities and they warrant more consideration as prospective antimicrobials.*

Keywords: 2-aminobenzothiazole, Schiff's base, antibacterial activity.

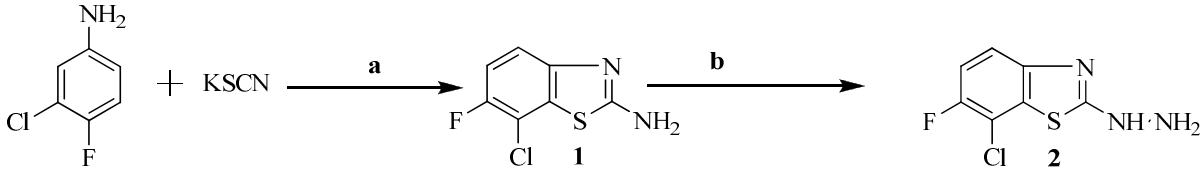
INTRODUCTION

The benzothiazole ring is present in various marine or terrestrial natural compounds, which have useful biological activities [1–4]. Benzothiazole and its bioisosteres; benzoxazole and benzimidazole, were studied for their antitumor, antiviral and antimicrobial activities [5–9]. In the last few years, it was reported that the 2 and 5-substituted benzothiazole, benzoxazole and benzimidazole derivatives had antimicrobial activities against some Gram-positive, Gram negative bacteria and the yeast *Candida albicans*, and these compounds provided a wide variety of *in vitro* antimicrobial effects especially against the enterobacter *Pseudomonas aeruginosa* and the yeast *C. albicans* [10–16].

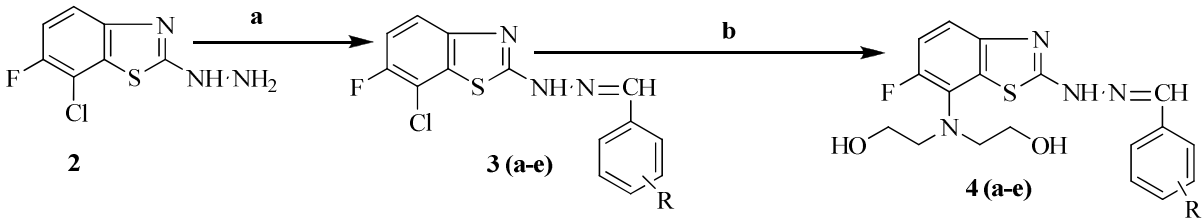
In view of these observations, some novel 2,2'-(2-(2-benzylidenehydrazinyl)-6-fluorobenzo[d]thiazol-7-ylazanediyl) diethanol derivatives **(4a-e)** have been synthesized by reacting 2-(2-substituted benzylidenehydrazinyl)-7-chloro-6-fluorobenzo[d]thiazole **(3a-e)** with diethanolamine, in order to examine their *in vitro* antimicrobial activities against *Staphylococcus aureus, Bacillus subtilis* Gram-positive and *Escherichia coli, Pseudomonas aeruginosa* Gram-negative bacteria in comparison with standard drug ampicillin.

MATERIALS AND METHODS

The melting points were determined with an electrothermal melting point apparatus and are uncorrected. Infrared spectra (KBr disc) were performed on FTIR-8400 Shimadzu and the frequencies were expressed in cm^{-1}. ^1H NMR spectra were recorded on Bruker-Avance 400 MHz instrument with TMS (0 ppm) as an internal standard; the chemical shifts (δ) are reported in ppm and coupling constants (J) are given in Hertz (Hz). Signal multiplicities are represented by s (singlet), d (doublet), t (triplet) and m (multiplet). Mass spectra were recorded on ESI-MS, Thermo, Finnigan LCQ deca xp max. Elemental analyses were performed on Perkin-Elmer 2400. The purity of the compounds was checked on Merck precoated silica gel 60 F-254. Column chromatography was performed using P.D. fine chem. silica gel (100-200 mesh). Yields were not optimized. All the solvents and reagents were used without further purification.



Scheme 1. **Reagents and conditions**: (a) Br_2, CH_3COOH, 5 ˚C, NH_3; (b) $NH_2.NH_2.H_2O$/ HCl, Ethylene glycol, reflux 2h.



R = 3a, 4a = 4-OCH_3, 3b, 4b = 3,4,5-OCH_3, 3c, 4c = 2-OH, 3d, 4d = 4-OH, 3e, 4e =4-OH-3-OCH_3

Scheme 2. Reagents and conditions: (a) Substituted aromatic aldehydes, 2-3 drops of glacial acetic acid, benzene, reflux 12 h; (b), Diethanolamine, triethylamine, ethanol, reflux 4 h.

Synthesis of 7-chloro-6-fluorobenzo[d]thiazol-2-amine (1)

To a stirred mixture of glacial acetic acid (40 ml) precooled at 5 ˚C were added 40 g (0.4123 mol) potassium thiocyanate and 7.25 g (0.0498 mol) of 4-fluoro-3-chloro aniline. To that 6 ml of bromine in 24 ml of glacial acetic acid was added slowly at such a rate that the temperature should not rise beyond 5 ˚C, for a period of 2 h. Stirring was continued for an additional 2 h at the same temperature and at room temperature for 10 h. It was allowed to stand overnight, add 30 ml of water and heated at 85 ˚C, filtered hot. The filtrate was cooled and neutralized with ammonia solution to pH 6. A light yellow precipitate was collected, washed with water and recrystallized by toluene.

Yield 76%; slight yellowish crystalline; mp 180-182 ˚C; IR (υ_{max}, cm^{-1}, KBr): 3480 (N-H), 1199 (C-F), 681 (C-Cl); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 4.21 (s, 2H, NH_2), 7.62-7.60 (d, 1H, J = 7.22, ArH), 7.70- 7.68 (d, 1H, ArH, J = 8.59 Hz); ESI-MS, m/z: 201.98 $[M]^+$, 203.86 $[M+2]^+$; Anal. calcd. for $C_7H_4ClFN_2S$: C, 41.49; H, 1.99; N, 13.82. Found: C, 41.51; H, 2.03; N, 13.81.

Synthesis of 7-chloro-6-fluoro-2-hydrazinylbenzo[d]thiazole (2)

10 ml of conc. HCl was added drop-wise with stirring to 10 ml (0.3 mol) hydrazine hydrate at 5-10 ˚C followed by ethylene glycol 40 ml. To the above solution 2.025 g (0.01 mol) of compound **(1)** in portion was added and the resulting mixture was refluxed for 2 h. On cooling solid separated out, was filtered and washed with water, dried and recrystallized by ethanol.

Yield 66%, mp 217-219 ˚C, light brown needle shaped crystals; IR (υ_{max}, cm^{-1}, KBr): 3380 (N-H), 1200 (C-F), 683 (C-Cl); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 5.09 (s, 2H, NH_2), 7.42-7.41 (d, 1H, ArH, J = 6.64 Hz), 7.81-7.79 (d, 1H, ArH, J = 9.24 Hz), 9.20 (s, 1H, NH); ESI-MS, m/z: 217.02 $[M]^+$, 217.05 $[M+2]^+$; Anal. calcd. for $C_7H_5ClFN_3S$: C, 38.63; H, 2.32; N, 19.31. Found: C, 38.61; H, 2.34, N, 19.31.

Synthesis of 2-(2-substituted benzylidenehydrazinyl)-7-chloro-6-fluorobenzo[d]thiazole (3a-e)

To a mixture of compound (**2**) 1.08 g (0.005 mol) and different substituted aromatic aldehydes (0.005 mol) in benzene with 2-3 drops of glacial acetic acid was refluxed for 12 h (reaction was monitored by TLC). On cooling, solid separated out, was filtered and recrystallized by benzene.

7-chloro-6-fluoro-2-(2-(4-methoxybenzylidene)hydrazinyl)benzo[d]thiazole (3a)

Yield 78%, mp 237-238 ˚C, yellowish crystals; IR (υ_{max}, cm^{-1}, KBr): 3373 (N-H), 1250 & 1050 (C-O-C),1194 (C-F), 693 (C-Cl); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 3.02 (s, 3H, OCH_3), 4.56 (s, 1H, -N=CH-), 7.39-7.37 (m, 4H, ArH), 7.47-7.46 (d, 1H, ArH, J = 6.84 Hz), 7.87-7.86 (d, 1H, ArH, J = 9.42 Hz), 10.01 (s, 1H, NH); ESI-MS, m/z: 336.48 $[M]^+$, 338.07 $[M+2]^+$.

7-chloro-6-fluoro-2-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl)benzo[d]thiazole (3b)

Yield 72%, mp 264-266 ˚C, reddish brown crystals; IR (υ_{max}, cm^{-1}, KBr): 3337 (N-H), 1247 & 1033 (C-O-C), 1208 (C-F), 707 (C-Cl); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 3.14 (s, 9H, OCH_3), 4.64 (s, 1H, -N=CH-), 7.43-7.41 (s, 2H, ArH), 7.49-7.48 (d, 1H, ArH, J = 8.36 Hz), 7.88-7.87 (d, 1H, ArH, J = 9.24 Hz), 10.08 (s, 1H, NH); ESI-MS, m/z: 396.68 $[M]^+$, 398.26 $[M+2]^+$.

2-((2-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)hydrazono)methyl)phenol (3c)

Yield 57%, mp 213-215 ˚C, reddish crystals; IR (υ_{max}, cm^{-1}, KBr): 3415 (OH), 3362 (N-H), 1185 (C-F), 694 (C-Cl); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 4.09 (s, 1H, OH), 4.59 (s, 1H, -N=CH-), 7.38-7.36 (m, 4H, ArH), 7.45-7.43 (d, 1H, ArH, J = 8.39 Hz), 7.82-7.80 (d, 1H, ArH, J = 9.21 Hz), 9.99 (s, 1H, NH); ESI-MS, m/z: 322.42 $[M]^+$, 324.13 $[M+2]^+$.

4-((2-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)hydrazono)methyl)phenol (3d)

Yield 79%, mp 252-254 ˚C, dark reddish crystals; IR (υ_{max}, cm^{-1}, KBr): 3402 (OH), 3364 (N-H), 1181 (C-F), 698 (C-Cl); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 4.03 (s, 1H, OH), 4.59 (s, 1H, -N=CH-), 7.37-7.35 (m, 4H, ArH), 7.44-7.42 (d, 1H, ArH, J = 8.34 Hz), 7.83-7.82 (d, 1H, ArH, J = 9.2 Hz), 9.97 (s, 1H, NH); ESI-MS, m/z: 322.12 $[M]^+$, 324.03 $[M+2]^+$.

4-((2-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)hydrazono)methyl)-2-methoxyphenol (3e)

Yield 82%, mp 242-244 ˚C, Yellowish crystals; IR (υ_{max}, cm^{-1}, KBr): 3415 (OH), 3352 (N-H), 1275 & 1030 (C-O-C), 1204 (C-F), 691 (C-Cl); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 3.07 (s, 3H, OCH_3), 4.11 (s, 1H, OH), 4.63 (s, 1H, -N=CH-), 7.40-7.38 (m, 3H, ArH), 7.46-7.45 (d,

1H, ArH, J = 8.28 Hz), 7.81-7.80 (d, 1H, ArH, J = 9.24 Hz), 9.99 (s, 1H, NH); ESI-MS, m/z: 352.23 $[M]^+$, 354.53 $[M+2]^+$.

Synthesis of 2,2'-(2-(2-substituted benzylidenehydrazinyl)-6-fluorobenzo[d]thiazol-7-ylazanediyl) diethanol (4a-e)

To a mixture of compound **(3a-f)** (0.005 mol), diethanolamine 0.479 mL (0.005 mol) and triethylamine in ethanol was refluxed for 4 hours. On cooling, solid separated out, was filtered and recrystallized by DMF-water mixture.

2,2'-(6-fluoro-2-(2-(4-methoxybenzylidene)hydrazinyl)benzo[d]thiazol-7-ylazanediyl) diethanol (4a)

Yield 82%, mp 292-294 ˚C, ash coloured powder; IR (υ_{max}, cm^{-1}, KBr): 3412 (OH), 3363 (N-H), 1270 & 1039 (C-O-C), 1209 (C-F); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 3.07 (s, 3H, OCH_3), 3.16-3.14 (t, 4H, CH_2), 3.85-3.83 (t, 4H, CH_2), 3.91 (s, 2H, OH), 4.69 (s, 1H, N=CH-), 7.32-7.30 (m, 4H, ArH), 7.49-7.47 (d, 1H, ArH, J = 8.21 Hz), 7.85-7.83 (d, 1H, ArH, J = 9.19 Hz), 9.94 (s, 1H, NH); ESI-MS, m/z: 405.43 $[M]^+$, 407.11 $[M+2]^+$.

2,2'-(6-fluoro-2-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl)benzo[d]thiazol-7-ylazanediyl) diethanol (4b)

Yield 70%, mp 301-303 ˚C, white powder; IR (υ_{max}, cm^{-1}, KBr): 3416 (OH), 3337 (N-H), 1249 & 1041 (C-O-C), 1189 (C-F); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 3.17 (s, 9H, OCH_3), 3.19-3.17 (t, 4H, CH_2), 3.88-3.85 (t, 4H, CH_2), 4.09 (s, 2H, OH), 4.71 (s, 1H, -N=CH-), 7.45-7.43 (s, 2H, ArH), 7.51-7.49 (d, 1H, ArH, J = 8.18 Hz), 7.86-7.84 (d, 1H, ArH, J = 9.22 Hz), 10.11 (s, 1H, NH); ESI-MS, m/z: 465.38 $[M]^+$, 467.12 $[M+2]^+$.

2,2'-(6-fluoro-2-(2-(2-hydroxybenzylidene)hydrazinyl)benzo[d]thiazol-7-ylazanediyl) diethanol (4c)

Yield 78%, mp 242-244 ˚C, buff coloured powder; IR (υ_{max}, cm^{-1}, KBr): 3403 (OH), 3348 (N-H), 1191 (C-F); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 3.14-3.12 (t, 4H, CH_2), 3.91-3.89 (t, 4H, CH_2), 4.16 (s, 3H, OH), 4.75 (s, 1H, -N=CH-), 7.39-7.37 (m, 4H, ArH), 7.48-7.46 (d, 1H, ArH, J = 8.43 Hz), 7.86-7.84 (d, 1H, ArH, J = 9.26 Hz), 10.04 (s, 1H, NH); ESI-MS, m/z: 391.34 $[M]^+$, 392.93 $[M+2]^+$.

2,2'-(6-fluoro-2-(2-(4-hydroxybenzylidene)hydrazinyl)benzo[d]thiazol-7-ylazanediyl) diethanol (4d)

Yield 84%, mp 276-278 ˚C, off white powder; IR (υ_{max}, cm^{-1}, KBr): 3404 (OH), 3346 (N-H), 1193 (C-F); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 3.14-3.11 (t, 4H, CH_2), 3.92-3.90 (t, 4H, CH_2), 4.08 (s, 3H, OH), 4.66 (s, 1H, -N=CH-), 7.32-7.30 (m, 4H, ArH), 7.46-7.43 (d, 1H, ArH, J = 8.37 Hz), 7.87-7.84 (d, 1H, ArH, J = 9.29 Hz), 10.01 (s, 1H, NH); ESI-MS, m/z: 391.34 $[M]^+$, 392.93 $[M+2]^+$.

2,2'-(6-fluoro-2-(2-(4-hydroxy-3-methoxybenzylidene)hydrazinyl)benzo[d]thiazol-7-ylazanediyl) diethanol (4e)

Yield 77%, mp 288-290 ˚C, buff coloured powder; IR (υ_{max}, cm^{-1}, KBr): 3411 (OH), 3386 (N-H), 1255 & 1033 (C-O-C), 1186 (C-F); ^{1}H NMR (400MHz, DMSO-d_6), δ (ppm): 3.09 (s, 3H, OCH_3), 3.16-3.13 (t, 4H, CH_2), 3.90-3.87 (t, 4H, CH_2), 4.15 (s, 3H, OH), 4.69 (s, 1H, -N=CH-), 7.34-7.31 (m, 3H, ArH), 7.44-7.42 (d, 1H, ArH, J = 8.18 Hz), 7.86-7.84 (d, 1H, ArH, J = 9.21 Hz), 9.95 (s, 1H, NH); ESI-MS, m/z: 421.11 $[M]^+$, 422.89 $[M+2]^+$.

Antibacterial activity

Medium

The solid media Müller–Hinton agar (MHA; beef infusion 300 g/L, casein acid hydrolysate 17.5 g/L, starch 1.5 g/L, agar 17 g/L, and distilled water 1000 mL, adjusted to pH = 7.4) was used for the antibacterial activity.

Test microorganisms

Two Gram-positive bacteria namely, *Staphylococcus aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633) and two Gram-negative bacteria namely, *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) were used for the antibacterial activity.

Minimum inhibitory concentration [17]

The *in vitro* antibacterial activity of the newly synthesized ten compounds **(3a-e** and **4a-e)** was evaluated using the conventional agar-dilution method [18]. Twofold serial dilutions of the compounds and reference drug (ampicillin) were prepared in MHA. Drugs (10.0 mg) were dissolved in DMSO (1 mL) and the solution was diluted with distilled water (9 mL). Further progressive double dilution with melted MHA was performed to obtain the required concentrations of 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.05 µg/mL. The bacterial inoculates were prepared by suspending 24 h old bacterial colonies from MHA media in 0.85% saline. Inoculates were adjusted to 0.5 McFarland Standard (1.5×10^8 CFU/mL) [22]. The suspensions were then diluted in 0.85% saline to give 10^7 CFU/mL. Petri dishes were spot-inoculated with 1 µL of each prepared bacterial suspension (10^4 CFU/spot) and incubated at 37 ˚C for 24 h. At the end of the incubation period, MIC was determined, which is the lowest concentration of the test compound that resulted in no visible growth on the plate. A control test was also performed with test medium supplemented with DMSO at the same dilutions as used in the experiment in order to ensure that the solvent had no influence on bacterial growth.

Table 1. *In vitro* antibacterial activity of newly synthesized compounds against selected strains (MIC in µg/mL).

Compound	Gram-positive organisms		Gram-negative organisms	
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
3a	64	128	64	>128
3b	32	32	64	64
3c	64	128	128	>128
3d	32	32	64	32
3e	32	64	16	32
4a	8	16	8	8
4b	4	2	1	2
4c	32	16	32	64
4d	8	4	2	4
4e	4	4	1	2
Ampicillin	0.5	0.5	0.5	0.5

RESULTS AND DISCUSSION

The preparation of final compounds **4a-e** was accomplished by synthetic sequence illustrated in **Scheme 1** and **2**. The compound **1** was synthesized from 4-fluoro-3-chloro aniline and potassium thiocyanate with bromine in acetic acid. The presence of two doublet at δ 7.6 to 7.7 ppm and a primary amine peak at δ 4.21 ppm in NMR spectra indicates the formation of basic ring. This was treated with hydrazine hydrate and conc. HCl in the presence of ethylene glycol yielded

compound **2**. Two singlet peak one at δ 9.2 ppm for a secondary amine and another at δ 5.09 for primary amine indicate the formation of hydrazino derivative. Which was further treated with different substituted aromatic aldehydes to get different Schiff's bases **3a-e**. The presence of extra aromatic peak and disappearance of primary amine peak confirmed the formation of compound **3a-e.** The synthesis of target compounds **4a-e**, were carried out by refluxing **3a-e** with diethanolamine in alcohol with catalytic amount of triethylamine. Presence of two triplets each accounting four protons at δ 3.11-3.16, 3.83-3.91 and the presence of a singlet peak at δ 3.91-4.16 for hydroxyl group accounting two protons indicates the formation of target compounds and all the compounds structure were further confirmed by mass spectra.

All the newly synthesized compounds **3a-e** were evaluated for their *in vitro* antibacterial activity against two Gram-positive bacterial strains namely, *Staphylococcus aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633) and two Gram-negative bacterial strains namely, *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) using the conventional agar-dilution method. Ampicillin was used as the reference standard. The results of the *in vitro* antibacterial activity screening of the novel series of 2,2'-(2-(2-benzylidenehydrazinyl)-6-fluorobenzo[d]thiazol-7-ylazanediyl) diethanol derivatives **(4a-e)** and 2-(2-benzylidenehydrazinyl)-7-chloro-6-fluorobenzo[d]thiazole derivatives **(3a-e)** are summarized in Table 1. Among the series tested, three compounds **(4b, 4d** and **4e**) exhibited excellent antibacterial activity against both Gram-positive and Gram-negative bacteria while compounds 4a showed moderate antibacterial activity against the tested organisms. However, all other compounds in the series were found to have less or poor activity against both Gram-positive and Gram-negative bacteria as compared to the standard. Minimum inhibitory concentration (MIC) was recorded as the lowest concentration of a compound that inhibits the growth of the tested microorganisms. In comparing the MIC values with the standard ampicillin (MIC = 0.5 µg/mL), compounds **4b, 4d,** and **4e** exhibit the most potent *in vitro* antibacterial activity against all evaluated organisms. Especially compounds **4b** and **4e** (MIC = 1 to 4 µg/mL) and **4d** (MIC = 2 to 8 µg/mL) showed high antibacterial activity while compound **4a** (MIC = 8 to 16 µg/mL) showed respectable antibacterial activity. A brief investigation of the structure-activity relationship (SAR) revealed that the compounds with substitution of seventh chlorine with diethanolamine contributed to a better antibacterial activity. Further, the presence of a 3,4,5-trimethoxy (3,4,5-OCH_3), 4-hydroxy-3-methoxy (4-OH-3-CH_3) and 4-hydroxy (4-OH) group on the phenyl ring of benzothiazole nucleus influenced the antibacterial activity. It is interesting to note that the introduction of a hydroxyl group at fourth position have good antibacterial activity **(4d)** compared to introduction at second position **(4c)** of the aromatic ring.

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