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DESIGN, SYNTHESIS AND EVALUATION OF CHLOROTHIAZIDE DERIVATIVES

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ABSTRACT

Hydrochlorothiazide and chlorthiazide are high potent diuretics used in treatment of hypertension. In recent research trends shows that chlorthiazide derivatives are used in osteoporosis and also has antimicrobial activity against various bacterial species, In this view a series of Chlorthiazide Derivatives were designed, synthesized six compounds by condensing chloroacetyl chloride with chlorthiazide by using ethanol as a solvent refluxed at 60c temperature formed compound is taken as intermediate further refluxed and condensed with Aromatic amines there by formation of substituted chlorthiazide derivatives, (Ctd-1, Ctd-2, Ctd-3, Ctd-4, Ctd-5, Ctd-6) and confirmed by the physical and chemical properties, IR, NMR spectra's. Screened for Antibacterial activity.

KEYWORDS

Antibacterial, Chlothiazide and Chloroacetylchloride.

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INTRODUCTION

One of the most common diseases in the developed world is Arterial hypertension. Due to the major cardiovascular risk factors there is an occurrence of various cardiac deceases like coronary heart disease, heart failure, stroke, and chronic kidney disease. Most of the people worldwide suffered with blood pressure and increased up to 60% reported by World Health Organization according to these report 7.1 million deaths per year by Arterial hypertension[1].

Hydrochlorothiazide is abbreviated as HCTZ. Hydrochlorothiazide is a common diuretic used for hypertension. HCTZ affects the distal renal tubular mechanism of electrolyte reabsorption for diuretic efficiency and increases excretion of sodium and chlorine in approximately the same amounts. The mechanism of the above anti-hypertension drug is not known but may be related to the excretion and distribution of body sodium. The production of HCTZ is with the reaction of 5-chloro-2, 4-disulfamylaniline (DSA) and formaldehyde in alkaline solution[1].

The impurity and degradation profiles of a drug substances are critical to its safety assessment and the optimization of the manufacturing process[1].

This drug belongs to thiazide class of diuretics. It helps to reduce the blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. The main site of action in the nephron appears on an electro neutral Na+ Cl- co transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide includes a natriuresis and concomitant water loss. Thiazides diuretics improved the reabsorption of calcium in site where it is unrelated to sodium transport. On other mechanisms HCTZ is believed to lower peripheral vascular resistance[2].

This drug sometime used for hypercalciuria, Dent's disease and Meniere's disease. For diabetes insipidus, the effect of thiazide diuretics is presumably mediated by a hypovolemia induced increase in proximal sodium and water reabsorption, thereby diminishing water delivery to the ADH-sensitive sites in the collecting tubules and reducing the urine output[2].

Thiazides are also used in the treatment of osteoporosis. Thiazides decrease mineral bone loss by promoting calcium retention in the kidney and by directly stimulating osteoblast differentiation and bone mineral formation[3].

Hydrochlorothiazide, it's IUPAC name is 6-chloro-1, 1-dioxo-3, 4- dihydro-2H-1, 2, 4-benzotiadiazine-7-sulfonamide. It is white crystalline solid of melting point of 273c.

Hydrochlorothiazide is stable and incompatible with strong oxidizing agents and it is insoluble in water. It's molecular formula is C7H8ClN3O4S2 and the molecular weight is 297.74, and it's monatomic mass is 296.7. The molecular structure is as below in figure[3].

These compounds are weakly acidic

1. H atom at N-2 is the most acidic due to the electron-withdrawing effects of the neighboring sulfone group.
2. Sulfonamide group at C-7 provides an additional point of acidity in molecule but is less acidic than N-2 proton. A sulfamoyl group removal or replace will decrees the diuretic activity.
3. Due to the possible formation of a water-soluble sodium salt it can be used for I.V. dosing.
4. An electron-withdrawing group is essential at position 6.
5. The diuretic activity is enhanced by substitution at position 3.
6. Substitution of 6-Cl by 6-CF3 alter duration of action without any potency changes.
7. Saturation of thiadiazine ring to give 3, 4-dihydro derivative and replacement replace or removal of sulfonamide group at position C-7 yields compounds with little or no diuretic activity.

Mechanism of action

Thiazides diuretics antagonize Na+—Cl– symport in the luminal membrane of the epithelial cells in the distal convoluted tubule. So thiazides are restrict NaCl reabsorption in the distal convoluted tubule and in the proximal tubule. Thiazides diuretics improves the Ca++ reabsorption in the distal convoluted tubule by restricting Na+ entry and thus enhancing the activity of Na+ —Ca++ exchanger in the basolateral membrane of epithelial cells.

The Sulfomyl derivatives are most important moieties has been widely used as building block for pharmaceutical agents as well as biologically active compounds. Thiazole and thiazine skeletons are the core structure of many natural and synthesized products, which shows diverse pharmacological properties as exemplified by antimicrobial,

insecticidal, and anti-inflammatory activities. These are widely used in agricultural field. Various novel benzothiazole compounds are synthesized as potential antimicrobial and antiparasitic agents. Benzisothiazolin-3-one is having fungicidal activity with good sterilization and anticorrosion activities. Benzothiazinone derivatives have capable of control effect on bacterial blight of tomato. Thiazole compounds are also reported as herbicide safener for alachlor on grain sorghum. Many bioactive compounds have been discovered by combining active subunits of known active molecules.

For example, the new fungicide fluopyram was designed by splicing active groups of fluopicolide and flutolanil A variety of 1-aryl-4-hydroxy-1Hpyrrol- 2(5H)-one derivatives with antibacterial activity bearing 1, 3, 4-oxadiazole moiety were also designed via placing the natural substructure 1-aryl-4-hydroxy-1Hpyrrol- 2(5H)-one at the two positions of 1, 3, 4-oxadiazole through a sulfide bridging group. Many successful cases have been reported in recent years[4].

In continuation of our previous investigations on the synthesis of nitrogen-containing heterocyclic compounds, a series of substituted thiazide/thiazole compounds were designed based on active subunit combination and bioisosteric replacement; the sulfur and nitrogen-containing heterocycle were retained with modification on the sulfonylurea functional groups. The synthesis of novel substituted thiazide/thiazole compounds was synthesized via cyclization acylation and carbamylation without any expensive reagent or catalyst. The bioactivity determination was carried out on maize from the injury of chlorsulfuron[4].

Sulfonamide was the first antimicrobial drug that its chemical moiety is also present in other medications which are not antimicrobials, including thiazide diuretics (including hydrochlorothiazide), loop diuretics (including furosemide), some COX-2 inhibitors (e.g. celecoxib) and also utilized in the treatment of inflammatory bowel disease (e.g. Sulfasalazine). Recently, sulfa drugs were introduced as protease inhibitors; therefore, they can be used as anticancer, anti-inflammatory and antiviral agents. Some of sulfonamide derivatives with photodynamic activities used against nasopharyngeal carcinoma cells and their anti-tumor and anti-angiogenesis activities were shown in a dose dependent manner. In a study to find new anti-tumor agents, a series of sulfonamide hydroxamic acids and anilides have been synthesized and evaluated as histone deacetylase (HDAC) inhibitors which can induce hyper-acetylation of histones in human cancer cells. Bouchain *et al.* Showed that synthesized sulfonamides selectively inhibit proliferation, blocks the cell cycle and induce apoptosis in human cancer cells but not in normal cells. E7070, [N-(3-Chloro-7-indolyl)-1, 4-benzenedisulfonamide] is a novel sulfonamide anticancer agent currently in phase II clinical development for the treatment of solid tumors. This compound, indisulam, strongly inhibits carbonic anhydrase, a critical enzyme involved in many physiological processes and whose association with cancer became obvious in the last period, is a cell-cycle inhibitor that arrests the cell cycle at the G1/S transition. In an attempt to find new antibacterial and anti-inflammatory compounds, a series of sulfonamides have been synthesized and their biological effects including antibacterial, anti-inflammatory and also cytotoxic properties were evaluated[5].

MATERIAL AND METHODS

Synthesis Work

General Planning

In the present work chlorthiazide has been chosen as starting material. This is broad class of chemical compounds with many important pharmacological properties it is widely used in various pharmaceutical preparations. The formation of final products was monitored by TLC. The completed products show significant colour under UV light. Structures of all synthesized compounds have been characterized by IR, NMR and Biological evaluation studies for anti-bacterial, have been done.

Synthesis of intermediate from chlorthiazide and chloro acetyl chloride

Take equimolar quantity of chlorthaizide and chloro acetyl chloride and placed in 250ml round bottom

flask. Add ethanol as a solvent and reflux for 6 hours at 60°C. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of solid precipitate of intermediate. Washed, filter, dried at room temperature. Weigh the dried product and calculate the percentage yield and determine the melting point.

Synthesis of final compounds from Intermediate and Aromatic amines

Take equimolar quantity of intermediate and aromatic amines placed in a round bottom flask. Add ethanol refluxed for 6 hours at 60°C. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of final compound.

Synthesis of [4-(2-(6-chloro-1, 1-dioxido-7-sulfamoyl-2H- benzo [1, 2, 4] thiadiazin-2-yl] acetamide) benzoic acid (CPD-1)

Take equimolar quantity of chlorthaizide and chloro acetyl chloride and placed in 250ml round bottom flask. Add ethanol as a solvent and reflux for 6 hours at 60°C .ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of solid precipitate of intermediate. Washed, filter, dried at room temperature. Weigh the dried product and calculate the percentage yield and determine the melting point.

Take equimolar quantity of intermediate and Para amino benzoic acid placed in a round bottom flask. Add ethanol refluxed for 6 hours at 60°C. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of final compound (CPD-1).

Synthesis of 2-(6-chloro-1, 1-dioxido-7-sulfamoyl-2H-benzo [1, 2, 4] thiaziazin-2-yl)-N, N-diphenyl acetamide [Final product] - (CPD-2)

Take equimolar quantity of chlorthaizide and chloro acetyl chloride and placed in 250ml round bottom flask. Add ethanol as a solvent and reflux for 6 hours at 60°C. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of solid precipitate of intermediate. Washed, filter, dried at room temperature. Weigh the dried product and calculate the percentage yield and determine the melting point.

Take equimolar quantity of intermediate and Diphenyl amine placed in a round bottom flask. Add ethanol refluxed for 6 hours at 60°C. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of final compound (CPD-2).

Synthesis of 2-(6-chloro-1, 1-dioxido-7-sulfamoyl-2H-benzo [1, 2, 4] thiadiazin 2-yl)-N-(4-sulfamoylphenyl) acetamide-CPD-3)

Take equimolar quantity of chlorthaizide and chloro acetyl chloride and placed in 250ml round bottom flask .Add ethanol as a solvent and reflux for 6 hours at 60°C. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of solid precipitate of intermediate. Washed and filter, dried at room temperature. Weigh the dried product and calculate the percentage yield and determine the melting point.

Take equimolar quantity of intermediate and sulphanilamide placed in a round bottom flask. Add ethanol refluxed for 6 hours at 60°C. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of final compound (CPD-3).

Synthesis of 2-(6-chloro-1, 1-dioxido-7-sulfamoyl-2H-benzo [1, 2, 4] thiadiazin-2-yl)-N, N-diethylacetamide - (CPD-4)

Take equimolar quantity of chlorthaizide and chloro acetyl chloride and placed in 250 ml round bottom flask. Add ethanol as a solvent and reflux for 6 hours at 60°C. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of solid precipitate of intermediate. Washed and filter, dried at room temperature. Weigh the dried product and calculate the percentage yield and determine the melting point.

Take equimolar quantity of intermediate and Diethyl amine placed in a round bottom flask. Add ethanol refluxed for 6 hours at 60°C. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of final compound (CPD-4).

Synthesis of 2-(6-chloro-1, 1dioxido-7-sulfamoyl-2H-benzo[e] [1, 2, 4] thiadiazin-2-yl)-N-formayl-N-methylacetamide-CPD-5)

Take equimolar quantity of chlorthaizide and chloro acetyl chloride and placed in 250ml round bottom flask .Add ethanol as a solvent and reflux for 6 hours at 60ºC. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of solid precipitate of intermediate. Washed, filter, dried at room temperature. Weigh the dried product and calculate the percentage yield and determine the melting point.

Take equimolar quantity of intermediate and N-N dimethyl formamide placed in a round bottom flask. Add ethanol refluxe for 6 hours at 60ºC. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of final compound (CPD-5).

Synthesis of N-acetyl-2-(6-chloro-1, 1-dioxido-7-sulfamoyl-2H-benzo [1, 2, 4] thiadiazin-2-yl)-N-phenylacetamide - (CPD-6)

Take equimolar quantity of chlorthaizide and chloro acetyl chloride and placed in 250ml round bottom flask. Add ethanol as a solvent and reflux for 6 hours at 60ºC. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of solid precipitate of intermediate. Washed and filter, dried at room temperature. Weigh the dried product and calculate the percentage yield and determine the melting point.

Take equimolar quantity of intermediate and Acetanilide placed in a round bottom flask. Add ethanol refluxed for 6 hours at 60ºC. Ensure the completion of reaction by TLC. Resulting reaction mixture is poured into ice cold water there by formation of final compound (CPD-6).

Biological activity method

Antibacterial screening

The microbial assay is a comparison of inhibition of growth of micro-organisms by measured concentrations of test compounds with that obtained by known concentration of a standard antibiotic. The microbial assay methods employed are turbidometric (tube-dilution) method and cylinder plate (cup-plate) method while the turbidometric method inhibition of growth of microbial culture in a uniform solution of antibiotic in a fluid medium is measured. It is compared with the synthesized compounds. Here, the growth of microbes will be measured. In cylinder plate method mainly based on diffusion of antibiotic from a vertical cylinder through a solid agar medium in a Petri plate to an extent. The growth of added microorganisms is inhibited in a zone around the cylinder containing solution of the Standard antibiotics. The cup-plate method is simple and measurement of inhibition of microorganisms is also easy. So we have used the cup-plate method for antibacterial evaluation of the test compounds. The media was prepared from nutrient agar 2%, peptone 1%, beef extract 1%, sodium chloride 0.5%, and distilled water up to 100ml. All the ingredients were weighed and added to water. The above solution is heated on water bath for one hour until it becomes clear. This nutrient media was sterilized by autoclave. The antibacterial activity was measured against *Bacillus subtillis* (MTCC-212), Staphylococcus aureus (MTCC-737) were used as Gram-positive bacteria, *Escherichia coli* (NCLM-2066) were used as Gram-negative bacteria and *Candida albicans* (MTCC-227) was used as fungi for this study. Master culture is made on agar slant of the above nutrient media and placed in refrigerator. The working culture was prepared from it by weekly transferred in nutrient agar medium[6,7].

Preparation of inoculums

In aseptic conditions from the master culture transferred small amount of culture to 15ml of sterile normal saline (0.9% NaCl solution) thoroughly mix and used for the antibacterial activity. About 0.5mL of inoculum was added to the sterilized Petri dish and melted agar cooled to 45°C was added, mixed gently, and allowed to solidify. Take Watmann filter paper disk and keep in each plate which is soaked in test drug solutions. The solution was allowed to diffuse for a period of 90 min. Petri dishes was incubated at 37°C for 24 h and then measure the zone of inhibition.

Preparation of test solution

Specified quantity of the compound was weighed and dissolved in 5ML of DMSO and further dilution was made to get the concentration of 500, 1000, and 1500µg/ML. In the same way the standard drugs Ampicillin, Cephalexin, and Miconazole was dissolved in definite quantity of water to obtain the concentration of 500, 1000, and 1500µg/ml each. The images of zone of inhibition are given in Figure No.1, 2 and 3, the results are shown in Table No.5 and the histogram of antibacterial and antifungal activity is given in.

EXPERIMENTAL WORK

All the chemicals used for the synthesis of title com- pounds were procured from Santhiram College of Pharmacy and S. D. Fine Chem. Ltd., Mumbai, Finar Chemicals Ltd., Ahmedabad, Loba Chemie Pvt. Ltd, Mumbai.

The scheme of synthesis is given and the chemicals were used without further purification. All the melting points were determined in open capillaries and are uncorrected. Thin layer chromatography was performed on microscopic slides ($2 \times 7.5 cm^2$) coated with silica- Gel-G and spots were visualized under UV light. IR spectra of all the compounds were recorded in KBr on FT-IR 8400 Shimadzu spectrophotometer using KBr. The ^1H NMR was recorded on Bruker advanced–II NMR-400 MHz instruments (SAIF-IITM) using DMSO and Chloroform as solvent and tetramethyl silane as internal standard, chemical shifts were expressed as δ values (ppm).

RESULTS AND DISCUSSION

All the title compounds were synthesized by coupling Aromatic amines with chloroacetyl chlorthiazide; Intermediate was synthesized by condensing chlorthiazide and chloroacetylchloride which are starting reagents. All the synthesized compounds were confirmed by I.R, NMR spectral studies.

In I.R wave numbers for synthesized compounds the wave numbers ranges C=C $1400cm^{-1}$ -$1600cm^{-1}$ for C-H $3000cm^{-1}$ – $3100cm^{-1}$ for C-Br 500 cm^{-1}-600 cm^{-1} for C-O $1000cm^{-1}$-$1300cm^{-1}$ for NH $3100cm^{-1}$ -$3500cm^{-1}$ for OH 3300- $2500cm^{-1}$.

The results for the synthesized NMR compound the Chemical shift ranges form7-9 for Aromatic -C-H-, 2.4-3 for Aliphatic -CH_2- , 8.1-8.6 for benzylidene imines=CH=, 4 for Methyl -CH_3.

The Anti-bacterial activity for gram positive Staphylococcus aureus is sensitive to compound (CTD-1) shows MIC at 0.4mg/ml. The Anti-bacterial activity for gram-positive Streptococcus mutant is sensitive to compounds (CTD-1 to 5) shows MIC at 0.4mg/ml. The Anti-bacterial activity for gram Negative E. coli is sensitive to compounds (CTD-1; 3; 5) shows MIC at 25mg/ml. The Antibacterial activity for gram Negative Pseudomonas is sensitive to compounds (CTD-2 and 4) shows MIC at 25mg/ml.

SUMMARY

Novel Chlorothiazide derivatives are synthesized, characterized by the IR, NMR spectra and screened for anti-bacterial activity by Agar diffusion method for antibacterial activity. Microbial assay method. The synthetic scheme of Chlorthiazide derivatives involves the reaction between chlorthiazide with chloroacetylchloride. The formed intermediate was further treated with aromatic amines resulting in novel chlorthiazide derivatives. IR spectroscopy is performed by using pressed pellet technique and it is useful in determining the important functional groups of the compound as a part of its structural identification, NMR spectra is performed for identifying type and number of protons of derivatives. All the spectral studies were consistent to original values. Biological screening was performed on bacteria among which compounds exhibited less activity than standard.

Table No.1: Physiochemical properties of Chlorthiazide derivatives

S.No	Code	Mol.formula	Mol.wt	M. p	Solubility	Colour	Rf
1	CTD- 1	$C_{16}H_{13}CN_4O_7S_2$	472.88	7.5	methanol	Reddish brown	0.9
2	CTD- 2	$C_{21}H_{17}CN_4O_5S_2$	504.97	9.1	methanol	Blue	0.7
3	CTD- 3	$C_{15}H_{14}CN_5O_7S_3$	507.95	8.2	methanol	Cream	0.8
4	CTD- 4	$C_{13}H_{17}ClN_4O_5S_2$	408.03	8.9	methanol	Cream	0.6
5	CTD- 5	$C_{11}H_{11}ClN_4O_6S_2$	394.81	7.8	methanol	Brown	0.8
6	CTD-6	$C_{17}H_{15}ClN_4O_6S_2$	470.91	9.2	methanol	Cream	0.9

Table No.2: Structural and IUPAC representation of Compounds

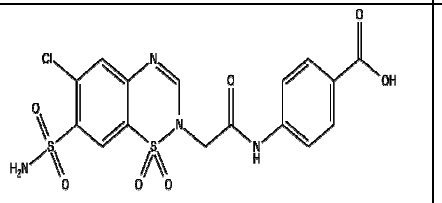
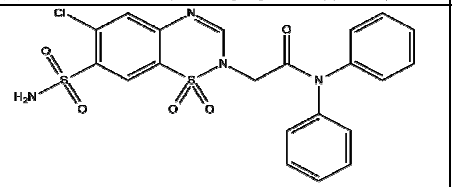
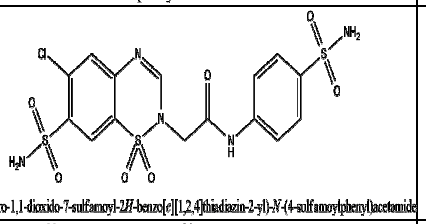
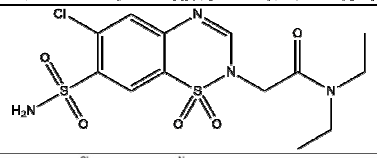
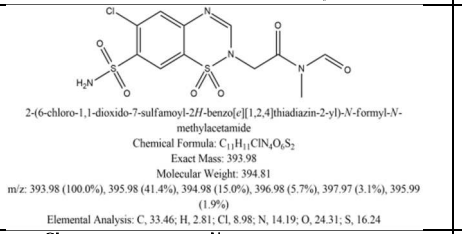
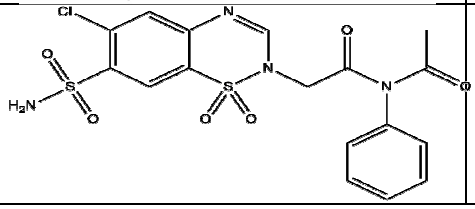
S.No	Code	Structure	IUPAC Name
1	CTD- 1	 4-(2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[1,2,4]thiadiazin-2-yl)acetamido)benzoic acid	[4-(2-(6-chloro-1, 1-dioxido-7-sulfamoyl-2H- benzo [1, 2, 4] thiadiazin-2-yl] acetamido) benzoic acid
2	CTD- 2	 2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[1,2,4]thiadiazin-2-yl)-*N*,*N*-diphenylacetamide	2-(6-chloro-1, 1-dioxido-7-sulfamoyl-2H-benzo[1, 2, 4] thiaziazin-2-yl)-N, N-diphenyl acetamide
3	CTD- 3	 2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[*e*][1,2,4]thiadiazin-2-yl)-*N*-(4-sulfamoylphenyl)acetamide	2-(6-chloro-1, 1-dioxido-7-sulfamoyl-2H-benzo[e] [1, 2, 4] thiadiazin 2-yl)-N-(4-sulfamoylphenyl) acetamide
4	CTD- 4		2-(6-chloro-1,1-dioxido-7-sulfamoyl-2H-benzo[e][1, 2, 4] thiadiazin-2-yl)-N, N-diethylacetamide
5	CTD- 5	 2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[*e*][1,2,4]thiadiazin-2-yl)-*N*-formyl-*N*-methylacetamide Chemical Formula: $C_{11}H_{11}ClN_4O_6S_2$ Exact Mass: 393.98 Molecular Weight: 394.81 m/z: 393.98 (100.0%), 395.98 (41.4%), 394.98 (15.0%), 396.98 (5.7%), 397.97 (3.1%), 395.99 (1.9%) Elemental Analysis: C, 33.46; H, 2.81; Cl, 8.98; N, 14.19; O, 24.31; S, 16.24	2-(6-chloro-1,1dioxido-7-sulfamoyl-2H-benzo[e] [1, 2, 4] thiadiazin-2-yl)-N-formayl-N-methylacetamide
6	CTD-6		N-acetyl-2-(6-chloro-1, 1-dioxido-7-sulfamoyl-2H-benzo(e) [1, 2, 4] thiadiazin-2-yl)-N-phenylacetamide

Table No.3: IR Spectra interpretation of Compounds

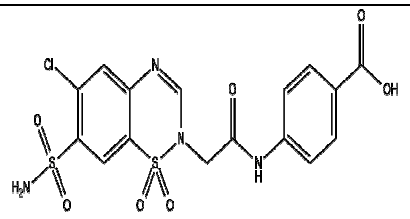
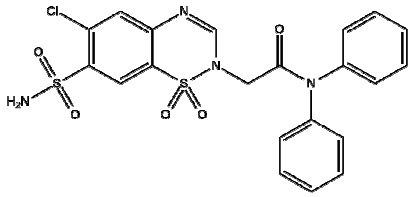
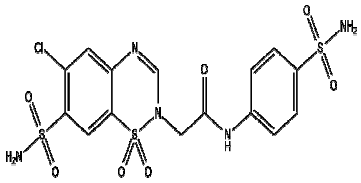
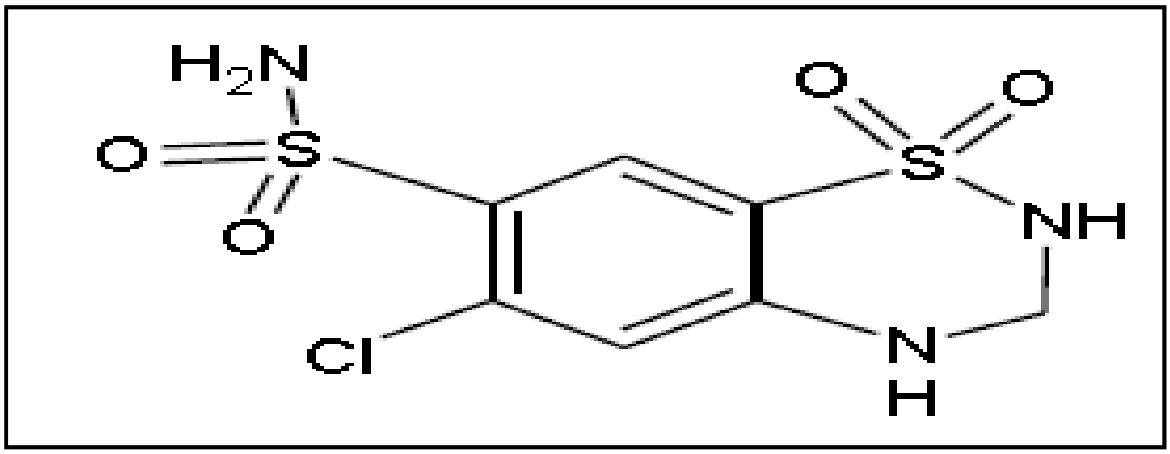
S.No	Compound	Structure	IR PEAKS IN cm^{-1}
1	CTD- 1	 4-(2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[1,2,4]thiadiazin-2-yl)acetamido)benzoic acid	C-H- 2925.37 NH- 1541.12 S=O- 1636.16 NH_2- 3372.74 C-cl- 669.97
2	CTD- 2	 2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[1,2,4]thiadiazin-2-yl)-*N*,*N*-diphenylacetamide	C-H- 2924.35 NH- 1548.31 s=O- 1630.83 C-cl- 669.43 NH2- 3457.05
3	CTD- 3	 2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[e][1,2,4]thiadiazin-2-yl)-*N*-(4-sulfamoylphenyl)acetamide	C-H- 3016.14 NH_2- 3375.10 NH- 1543.76 CH- 2925.67 s=O- 1626.88 C-cl- 670.23

Table No.4: NMR Spectra's Interpretation of Compounds

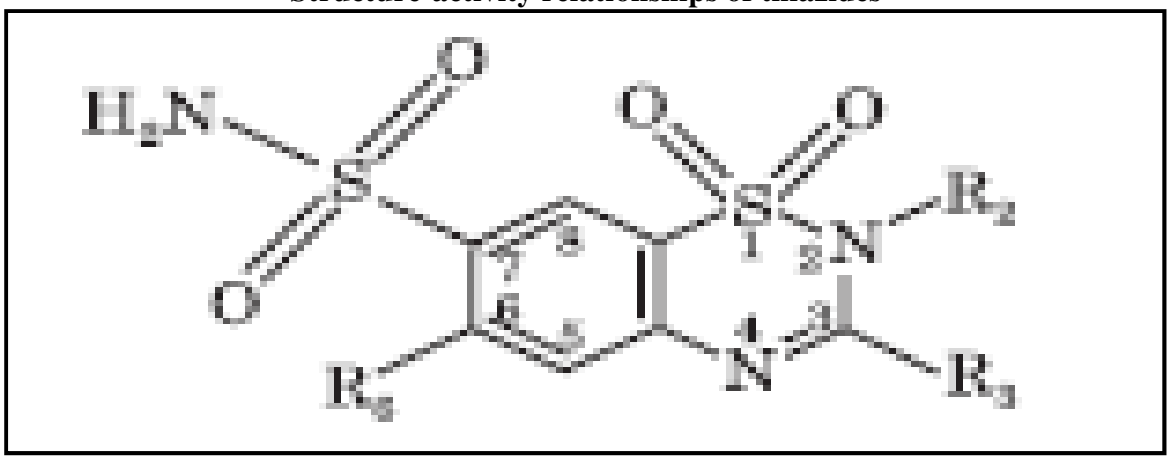
S.No	Compound	Type of proton	No. of protons
1	CPD- 1	Aromatic -CH-	7
		Aliphatic -CH2-	3
		Methyl –CH3	1
		Amine-NH-	2
2	CPD- 2	Aromatic -CH-	7
		Aliphatic -CH2-	3
		Methyl -CH3	1
		Amine-NH-	2
3	CPD- 3	Aromatic -CH-	6
		Aliphatic -CH2-	3
		Methyl -CH3	2
		Amine-NH-	2
4	CPD- 4	Aromatic -CH-	6
		Aliphatic -CH2-	3
		Methyl -CH3	1
		Amine-NH-	2
5	CPD- 5	Aromatic -CH-	6
		Aliphatic -CH2-	3
		Methyl -CH3	1
		Amine-NH-	2

Table No.5: Anti-bacterial activity

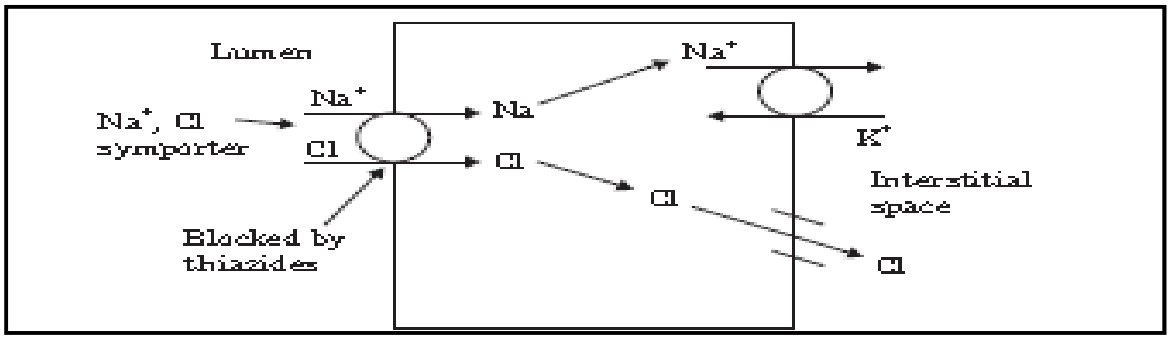
S.No	Compound code/name	Concentration (mg/ml)	Zone of Inhibition (mm)			
			B. subtilis	*S. aureus*	*E. coli*	*C. albicans*
1	CTD-1	0.5	2	1	-	2
		1.0	4	3	2	4
		1.5	6	5	4	7
2	CTD-2	0.5	-	-	-	-
		1.0	2	2	3	3
		1.5	3	3	5	6
3	CTD-3	0.5	5	5	-	-
		1.0	7	7	2	3
		1.5	8	8	3	5
4	CTD-4	0.5	3	3	1	3
		1.0	5	5	3	4
		1.5	6	6	4	7
5	CTD-5	0.5	-	-	-	2
		1.0	3	3	-	5
		1.5	5	5	3	7



Structure-activity relationships of thiazides



Drug	Structure
Chlorothiazide	$R_2 - H, R_3 - H, R_6 - Cl$
Hydrochlorothaizide	$R_2 - H, R_3 - H, R_6 - Cl$ (Saturated between C_3 and N_4)
Hydroflumethiazide	$R_2 - H, R_3 - H, R_6 - CF_3$ (Saturated between C_3 and N_4)
Bendroflumethiazide	$R_2 - H, R_3 - CH_2$–Ph, $R_6 - CF_3$ (Saturated between C_3 and N_4)



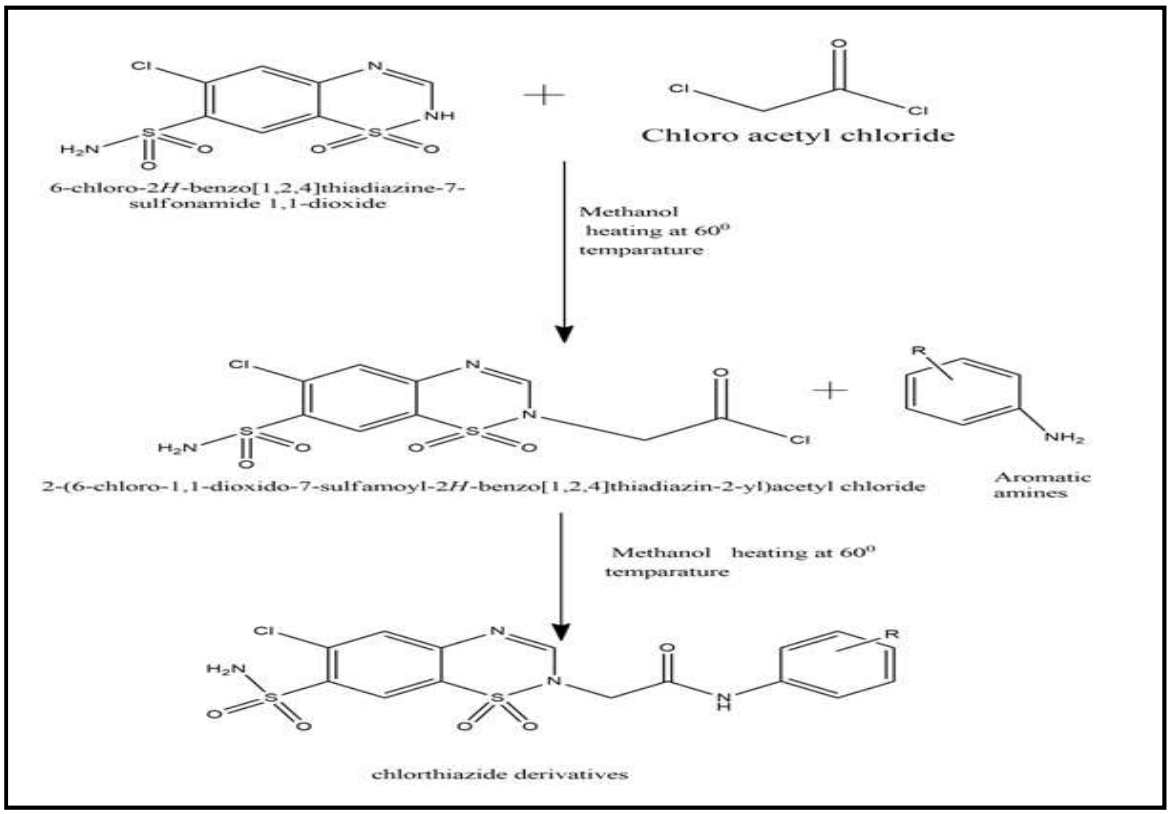


Figure No.1: Scheme of the synthesis

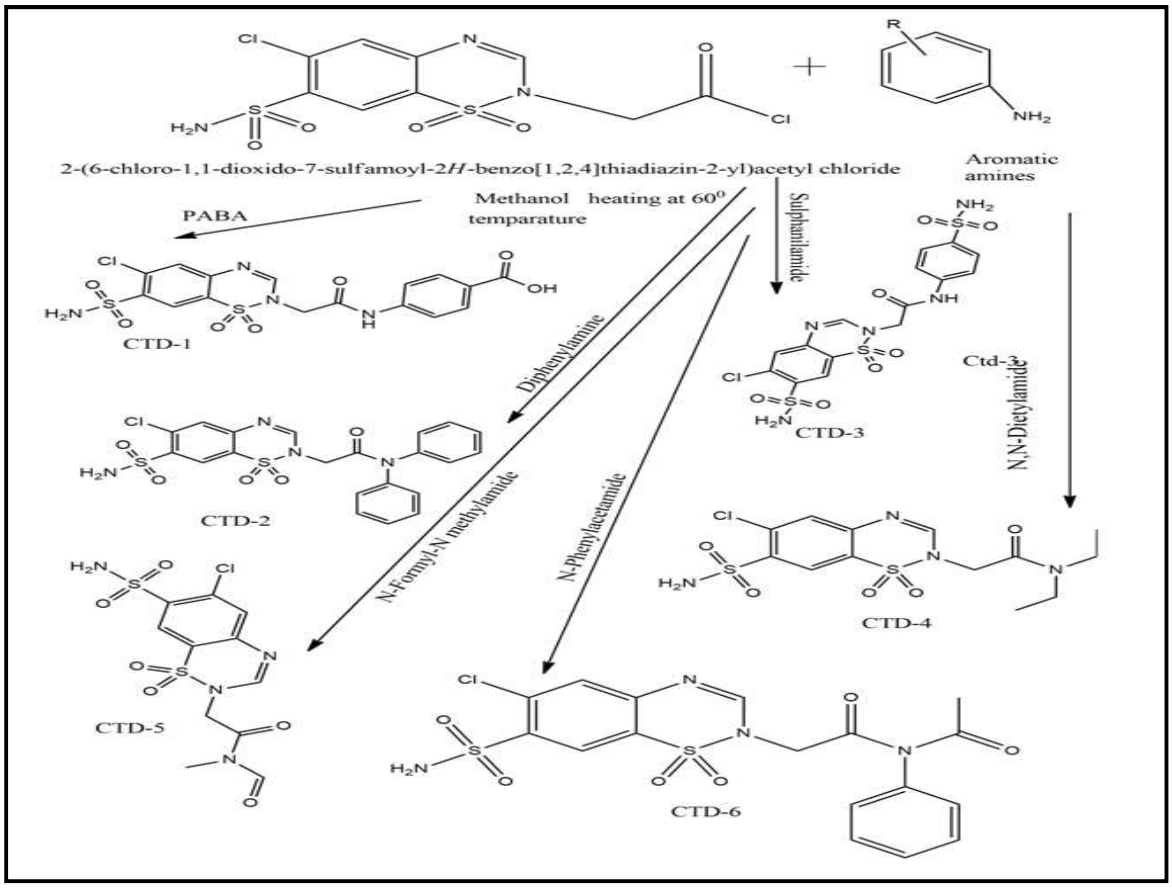
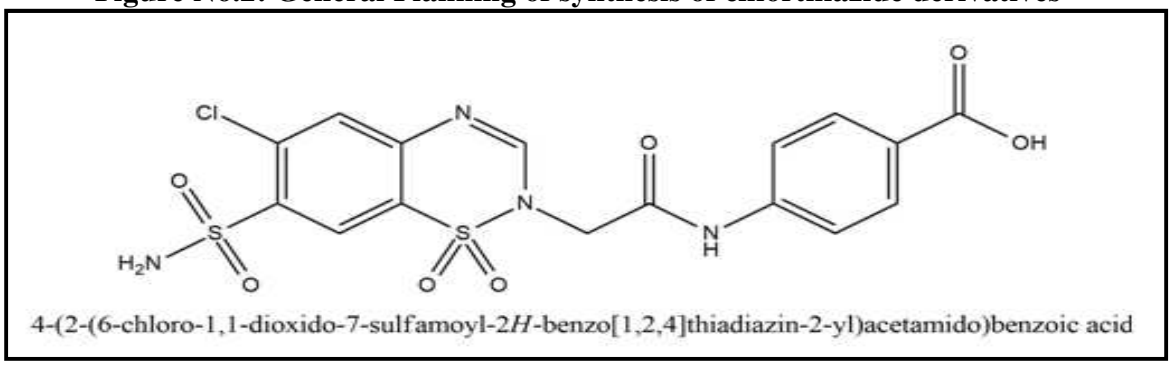
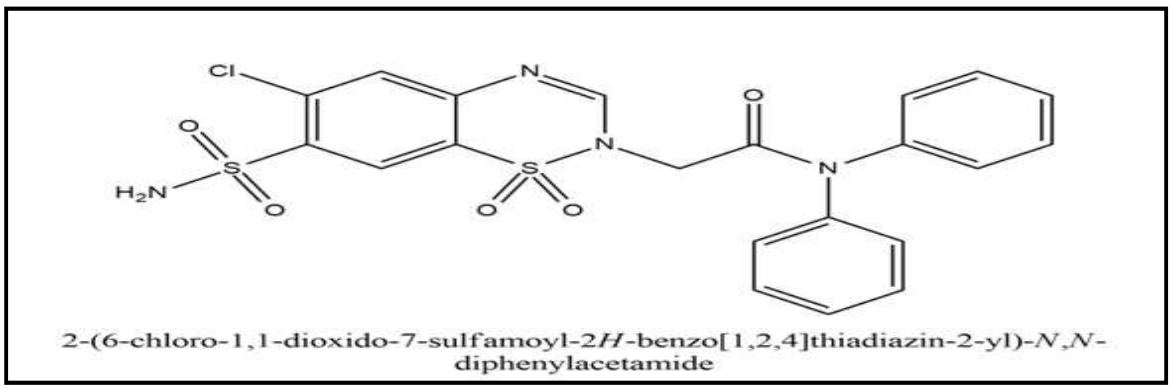


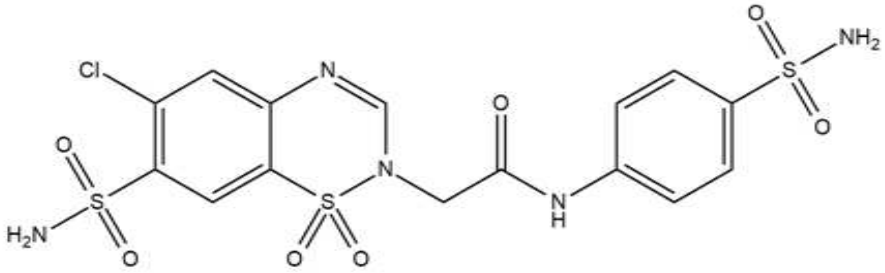
Figure No.2: General Planning of synthesis of chlorthiazide derivatives



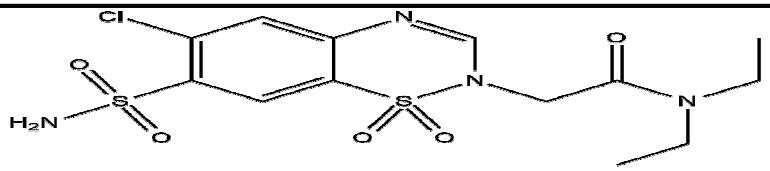
4-(2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[1,2,4]thiadiazin-2-yl)acetamido)benzoic acid



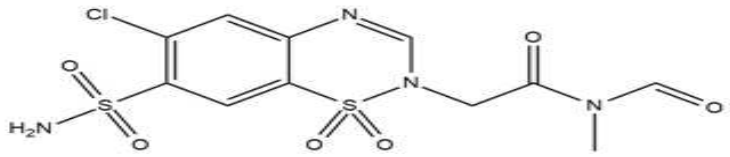
2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[1,2,4]thiadiazin-2-yl)-*N*,*N*-diphenylacetamide



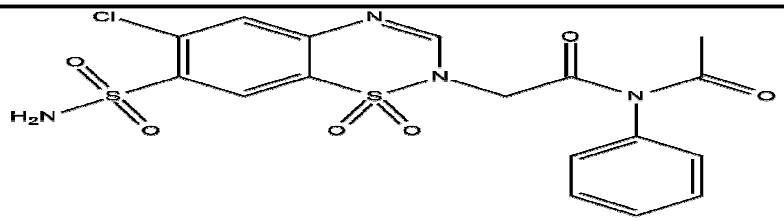
2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[*e*][1,2,4]thiadiazin-2-yl)-*N*-(4-sulfamoylphenyl)acetamide



2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[*e*][1,2,4]thiadiazin-2-yl)-*N*,*N*-diethylacetamide
Chemical Formula: $C_{13}H_{17}ClN_4O_5S_2$
Exact Mass: 408.03
Molecular Weight: 408.88
m/z: 408.03 (100.0%), 410.03 (41.2%), 409.04 (14.4%), 411.03 (7.1%), 412.03 (3.5%), 409.03 (3.1%), 410.04 (2.2%)
Elemental Analysis: C, 38.19; H, 4.19; Cl, 8.67; N, 13.70; O, 19.56; S, 15.68



2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[*e*][1,2,4]thiadiazin-2-yl)-*N*-formyl-*N*-methylacetamide
Chemical Formula: $C_{11}H_{11}ClN_4O_6S_2$
Exact Mass: 393.98
Molecular Weight: 394.81
m/z: 393.98 (100.0%), 395.98 (41.4%), 394.98 (15.0%), 396.98 (5.7%), 397.97 (3.1%), 395.99 (1.9%)
Elemental Analysis: C, 33.46; H, 2.81; Cl, 8.98; N, 14.19; O, 24.31; S, 16.24



N-acetyl-2-(6-chloro-1,1-dioxido-7-sulfamoyl-2*H*-benzo[*e*][1,2,4]thiadiazin-2-yl)-*N*-phenylacetamide
Chemical Formula: $C_{17}H_{15}ClN_4O_6S_2$
Exact Mass: 470.01
Molecular Weight: 470.91
m/z: 470.01 (100.0%), 472.01 (41.6%), 471.02 (18.8%), 473.01 (8.7%), 474.00 (3.1%), 471.01 (3.1%), 472.02 (2.9%)
Elemental Analysis: C, 43.36; H, 3.21; Cl, 7.53; N, 11.90; O, 20.39; S, 13.62

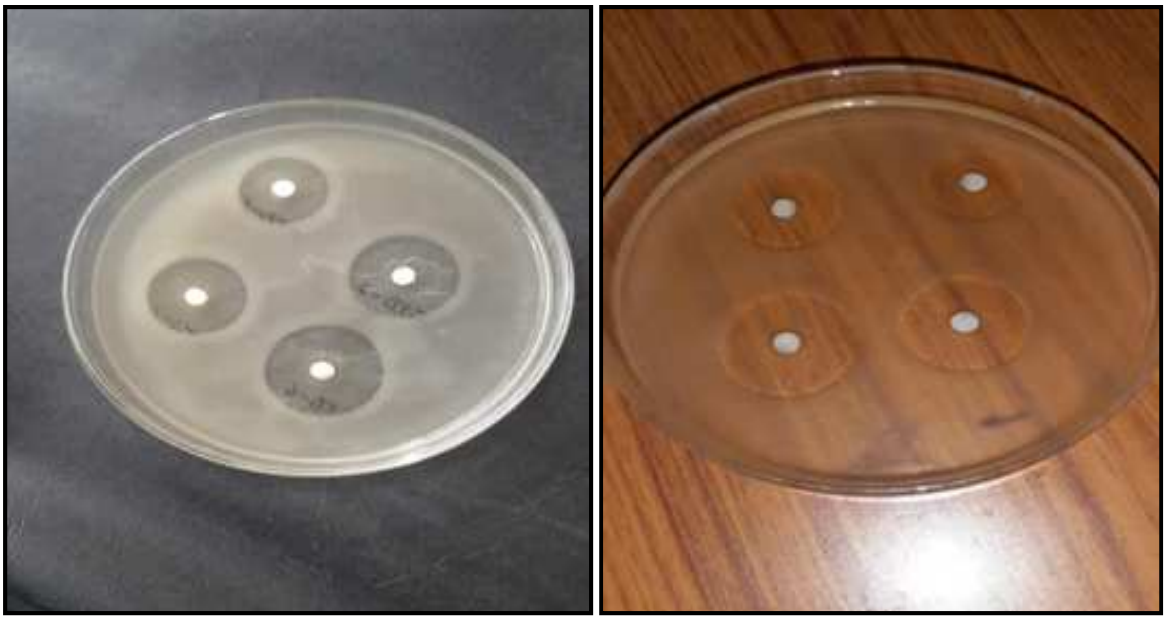


Figure No.3: Zone of inhibition of synthesized compounds against *bacteria salmonella*

CONCLUSION

The six chlorthiazide derivatives were synthesized in a facile procedure. All the compounds synthesized were characterized by IR, NMR spectra and best reports were obtained which are matching with derivatives. These compounds showed reserved anti-bacterial activity towards Gram positive and Gram negative bacteria. All six compounds showed potent antibacterial activity. Out of six compounds three compounds show potent anti-bacterial at 1.6µg/ml which is more potent against anti-microbial than standard drugs. Therefore anti-bacterial activity appears to be promising to develop therapeutically useful new molecules.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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