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Synthesis & evaluation of antibacterial activity of some pyrimidine derivatives

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Abstract
A series of 6-aryl-5-cyano-2-thiouracil derivatives 2a-2e was synthesized by the reaction of ethyl cyanoacetate with thiourea and aldehydes , namely 4-chlorobenzaldehyde to give Compounds 6-(4-chlorophenyl)-4-oxo-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carbonitrile , compound 1 were chlorinated to give the chloro compounds 2 that is 4-chloro-6-(4-chlorophenyl)-2-thioxo-1, 2dihydropyrimidine-5-carbonitrile. , then condensation of compound 2 via different reagents such as P-Bromoaniline, p-Nitrpaniline , hydrazine hydrate, 2,4-dinitroaniline, and anthranillic acid to give compounds 2a-2e respectively. All the synthesized compound was purified by successive recrystallization .the purity of compound was checked by performing TLC. For the TLC chloroform, methanol used as an mobile phase in the proportion of (9:1), and for visualization of the spot iodine vapor is used. All structures of the new compounds were elucidated by their spectral data such as IR, NMR. The antibacterial activity of all of these compounds was studied .The synthesized compounds are evaluated for antibacterial activity against gram positive and gram negative bacteria , staphylococcus aureus, bacillus subtilis, is gram positive bacteria and *E. coli* , *Salmonella typhi* is gram positive bacteria are used to evaluation of antibacterial activity.

Keywords: derivatives, antibacterial activity, gram positive, salmonella typhi

Introduction
Pyrimidine is 6 – membered heterocyclic ring compound containing nitrogen and carbon. Pyrimidine are base of RNA and DNA. Pyrimidine shows significant activity such as anti-tumor, antimicrobial, antifungal, antiviral activity.

Scheme

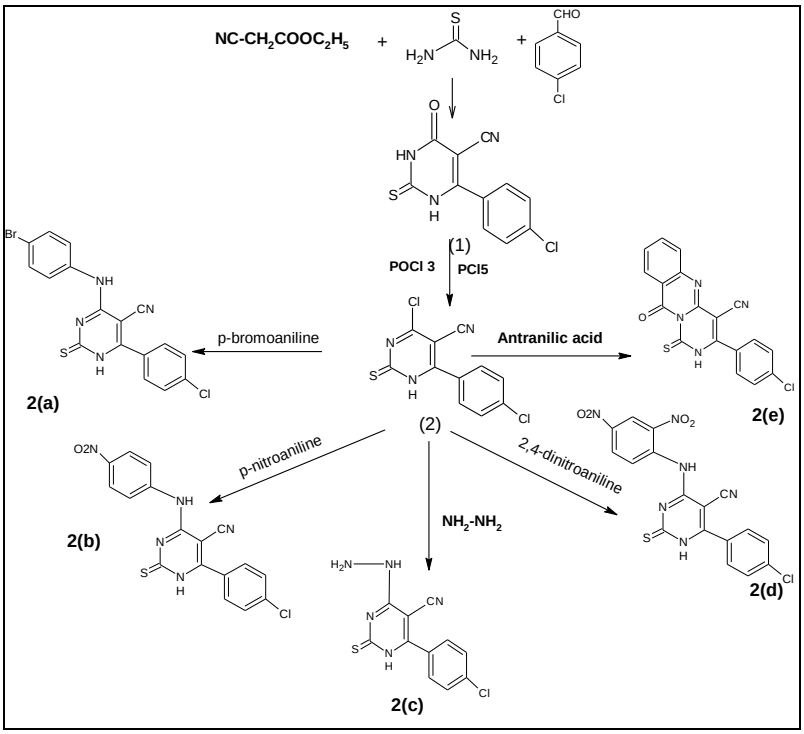

Fig 1

Materials and Methods

All the chemicals and solvent were procured from commercial source. The reagent was purchased from Research lab, sigma Aldrich, lobachemiepvt. Ltd., Himedia laboratories. Melting points of all synthesized compounds was determined by open capillary method and are uncorrected. Thin layer chromatography was used to assess the course of reaction and the purity of the intermediate and the final compounds were confirmed by applying a single spot on TLC plate silica gel G by using chloroform / methanol (9:1) used as mobile phase .TLC plates were visualized using iodine chamber the IR spectra were recorded using KBR disc on Jasco FTIR-410.

Experimental procedure [6, 7]

Procedure for preparation of Compounds 1

A mixture of thiourea (0.76 gm, 0.01 mol), ethyl cyanoacetate (1.13 gm, 1.07 ml, 0.01 mole) and aldehydes (0.01 mol), anhydrous potassium carbonate (o.o1 mol) in absolute ethanol (25 ml) was heated under reflux for 12 hr. The reaction mixture was allowed to cool and formed precipitate was filtered. The residue was triturate with water and neutralized with acetic acid. The precipitate was filtered, washed twice with water, dried and crystallize from ethanol to give compounds 1 6-(4-chlorophenyl)-4-oxo-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carbonitrile. 1 M.p-258-260 oc , yield 84.31 % , molecular formula C13H6N30SCl ,molecular wt- 263.28. IR-1340-1015 (C-N), 1440, 1606 (C=C aromatic), 1686 (C=O), 2231(CN) & 3300(N-H Sec).

Procedure for preparation of Compounds 2

A mixture of 1 (0.01 mol) and phosphorus pentachloride (0.01 mol) in phosphorus oxychloride (20 ml) was heated on a steam bath for 4 hr and the reaction mixture poured gradually on to crashed ice. The precipitate was filtered off, dried then crystallized from the proper solvent to give compounds 2.

4-chloro-6-(4-chlorophenyl)-2-thioxo-1, 2dihydropyrimidine-5-carbonitrile .2

m.p- 138-144oc , yield 73.02 %, molecular formula C11H5N3S Cl2 , molecular wt- 281.73 .IR -1330-1076 (C-N) , 1476,1403 (C=C Ar), 1691(C=N), 2231(CN),3300 (Sec N-H).

Procedure for preparation of compound 2a

A mixture of compound 2 (0.01 mol) and 4-bromoaniline (0.01 mol) in isopropyl alcohol (30ml) was refluxed for 18 hr. The reaction was monitored by TLC. The reaction mixture was then refrigerated overnight. The product obtained was filtered, dried.

4-(4-bromophenylamino)-6(4-chlorophenyl)-2-thioxo-1,2-dihyropyrimidine-5-carbonitrile (2a)

m.p- 308-310^{oc}, yield 30.52 %, molecular formula , $C_{17}H_{10}N_4SBrCl$ molecular wt- 406 .IR -2242 (C-N) , 1488 (C=C Ar), 1507 (c-c Ar) , 796 (C-Cl), 1092 (C=S).

Procedure for preparation of compound 2b

A mixture of compound 2 (0.01 mol) and 4-nitroaniline (0.01 mol) in isopropyl alcohol (30ml) was refluxed for 18 hr. The reaction was monitored by TLC. The reaction mixture was then refrigerated overnight. The product obtained was filtered, dried.

4-(4-Nitrophenylamino)-6(4-chlorophenyl)-2-thioxo-1, 2-dihyropyrimidine-5-carbonitrile

m.p- 308-310^{oc}, yield 24.63 %, molecular formula, $C_{17}H_{10}N_5SCl$ molecular wt- 382.3 .IR -2210 (C-N) , 1494 (C=C Ar), 1541 (c-c Ar) , 750 (C-Cl), 1110 (C=S), 1500 (Ar-NO2)

Procedure for preparation of Compounds 2c

A mixture of 2, (0.01 mol) and hydrazine hydrate (0.01 mol) in methanol (10 ml) was stirring for 17 hr. The precipitate was filtered off, dried then crystallized from the proper solvent to give compounds 2c,

4-hydrazino-6-(4-chlorophenyl)-2-thioxo-1, 2-dihydropyrimidine-5-carbonitril. (2c)

M. p.160-164 OC, yield 46.55 , Molecular formula C11H8N5Cl Molecular weight 277 .IR 1486-1592(C=C Ar) , 1092 (C=S) 587 (C-Cl), 2924 (C-H Ar) , 2250 (CN). H1 NHR chemical shift in DMSO 7.1-7.7(4H,Ar-H), 11.8(1-H,NH, D2O Exchangeable)

Procedure for preparation of compound 2d

A mixture of compound 2 (0.01 mol) and 2, 4-dinitroaniline (0.01 mol) in isopropyl alcohol (30ml) was refluxed for 18 hr. The reaction was monitored by TLC. The reaction mixture was then refrigerated overnight. The product obtained was filtered, dried.

4-(2-,4-dinitrophenylamino)-6-(4-chlorophenyl)-2-thioxo1,2dihydropyrimidine-5-carbonitrile.

(2d)m.p- 162-164^{oc} , yield 52.38%, molecular formula $C_{17}H_9N_6O_4SCl$, , molecular wt- 427 .IR -2242 (C-N) , 1475(C=C Ar), 1508 (c-c Ar) , 650 (C-Cl), 1127 (C=S), 1396 (Ar-NO2) ,1647(C=N)

Procedure for preparation of compound 2e

Mixture of compound 2 (0.01 mol) ,and anthranillic acid (1.37 gm , 0.01 mole) was reflux in butanol (50ml) for 12 hr , cooled ,filtered , dried and recrystallized from DMF / water to gives compound 2e.

10-oxo-3-(4-chlorophenyl)-1-thioxo-2,10-dihydro-1-H-pyrimido-(6-1-b)-quanazoline-4-carbonitrile .

m.p- 242-246^{oc}, yield 14 %, molecular formula $C_{18}H_9N_4OSCl$, , molecular wt- 364 .IR -2211 (C-N) , 1475(C=C Ar), 1507 (c-c Ar) , 797 (C-Cl), 1065 (C=S), ,1652(C=N)

Antibacterial activity [8]

All the newly synthesized compounds have been done in vitro for their antibacterial activity against gram-negative and gram- positive bacteria. Gram positive bacteria are staphylococcus aureus and bacillus substillis& gram negative bacteria are E- coli and salmonella typhi at a concentration of 100µg/ml by paper disc diffusion method. DMSO was used as a control solvent. Under similar conditions ciprofloxacin was used as a standard drug for comparison.

Preparation of bacterial suspension

Bacterial suspension was made in sterile isotonic solution of sodium chloride.

Procedure

1. Plates wash and sterile at 1210c for 1 hr in autoclave
2. Poured 30 ml of sterile nutrient agar in Petri plate and allowed to solidify
3. Suspension of micro organism is spread on solidify agar by using spreader.
4. Test sample and standard paper disc placed on media.
5. Plate was kept in refrigerated at 40 0c for 15 min to allow diffusion
6. Plates incubate at 370c for 24 hr.
7. Zone of inhibition measured using scale in mm.

Formula and procedure for preparation of media

Table 1: Media for gram positive bacteria --- nutrient agar media

Sr No.	Ingredients	Quantity
1	Beef extract	10 gm
2	Peptone	10 gm
3	NaCl	5 gm
4	Distilled water	1000 ml
5	Agar	20 gm

Final pH (at 25°C) 7

Directions: Suspend beef exact, peptone, NaCl in 1000 ml distilled water, stirred until complete dissolution. After dissolution add agar and Heat to boiling to dissolve the medium completely, and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring.

Table 2: Media for gram negative bacteria – Mac-conkey agar medium

Sr No.	Ingredients	Quantity
1	Peptone	20 gm
2	lactone	10 gm
3	Sodium chloride	5 gm
4	Bile salt	5 gm
5	Neutral red solution	10 ml
6	Distilled water to make	1000 ml
7	Crystal violet	1 mg
8	agar	20 gm
	Final P^H	7 to 7.4

Directions: Suspend 50 grams in 1000 ml distilled water. Heat to boil for dissolve medium completely. Sterilize by autoclaving at 15 lbs pressure for 15 minutes. Cool to 50°C.

Result of antibacterial activity

Table 3: Inhibition zone in mm.

	Compound no.	Organisms			
		Gram positive		Gram negative	
		Staphylococcus aureus	Bacillus substillis	E-coli	Salmonella typhi
1	2a	11	10	10	13
2	2b	11	12	12	12
3	2c	9	11	11	12
4	2d	12	10	9	9
5	2e	12	10	11	14
6	Standard (ciprofloxacine)	18	19	20	22
7	Control (DMSO)	-	-	-	-

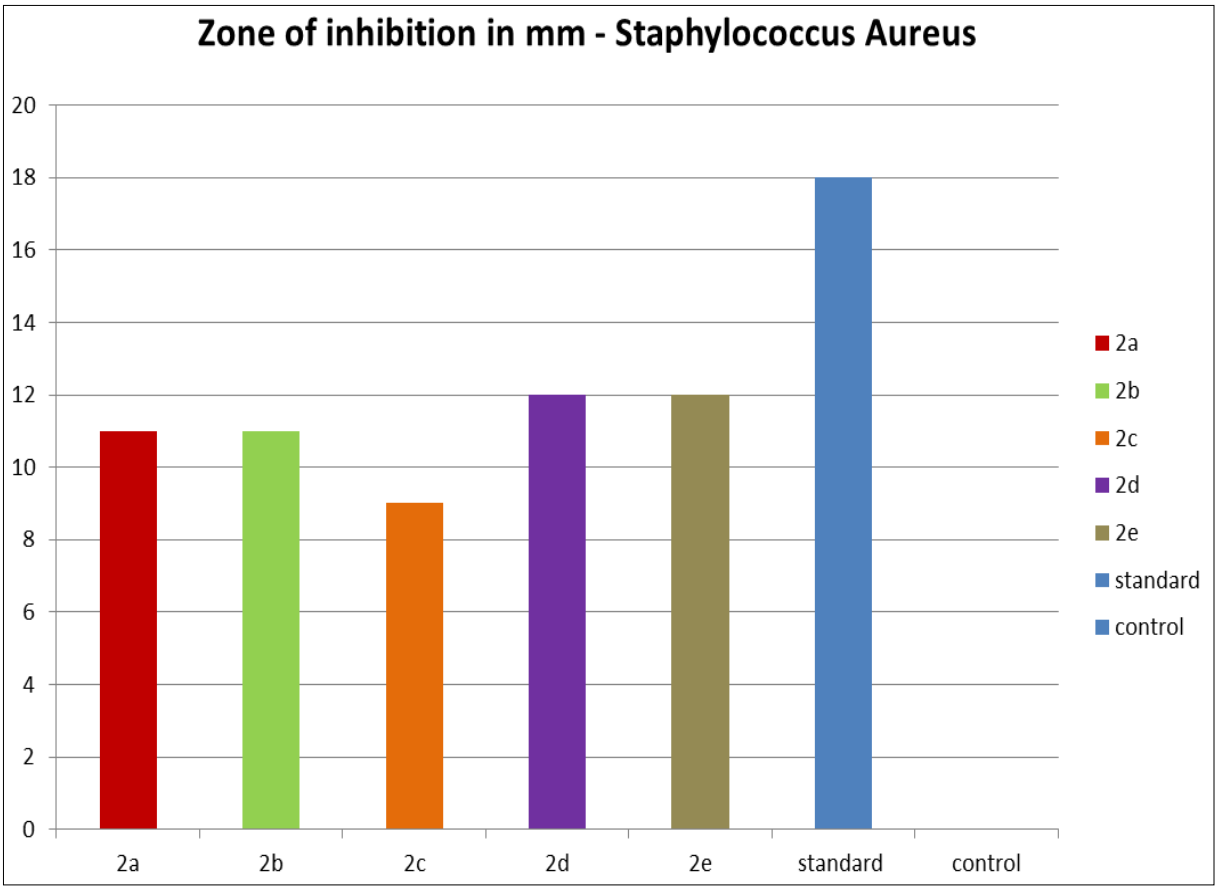

Fig 2: Zone of inhibition against staphylococcus aureus

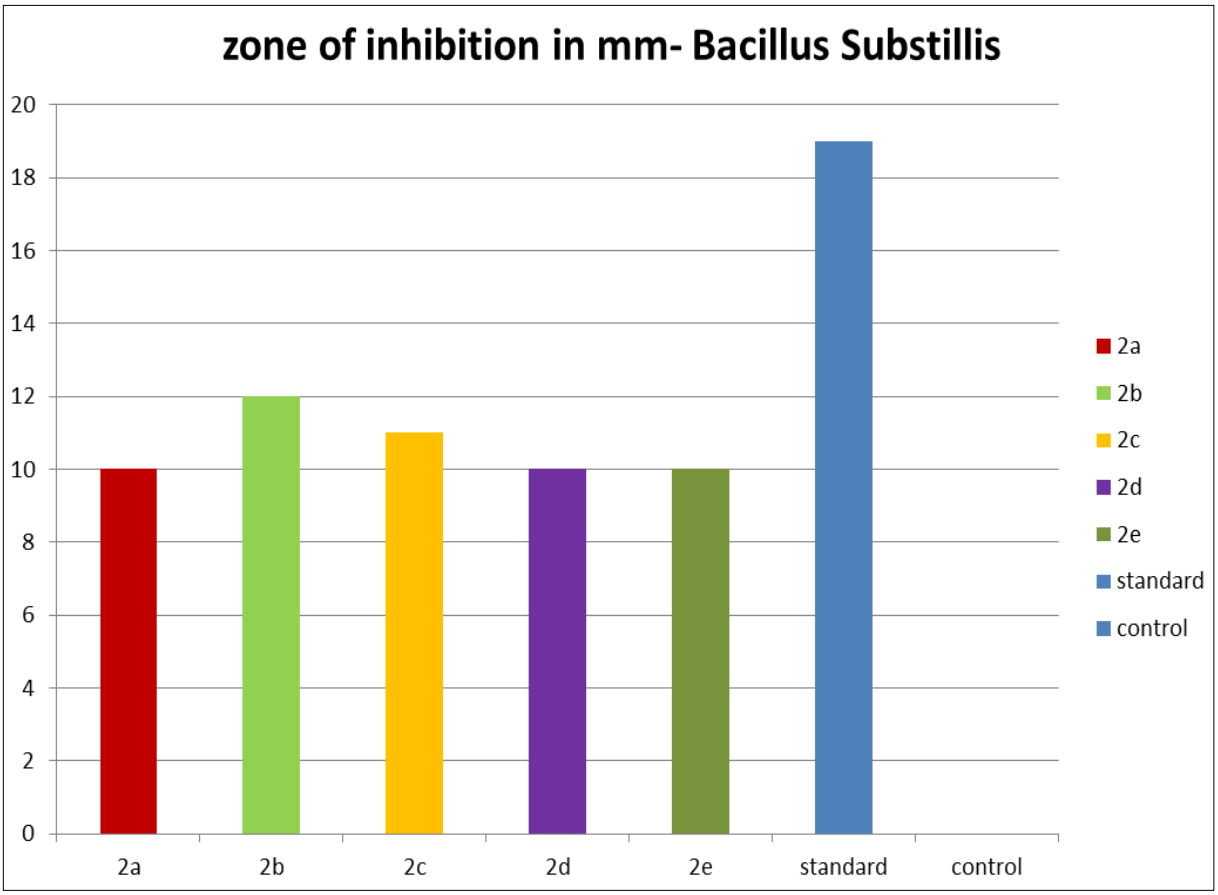

Fig 3: Zone of inhibition against bacillus substillis

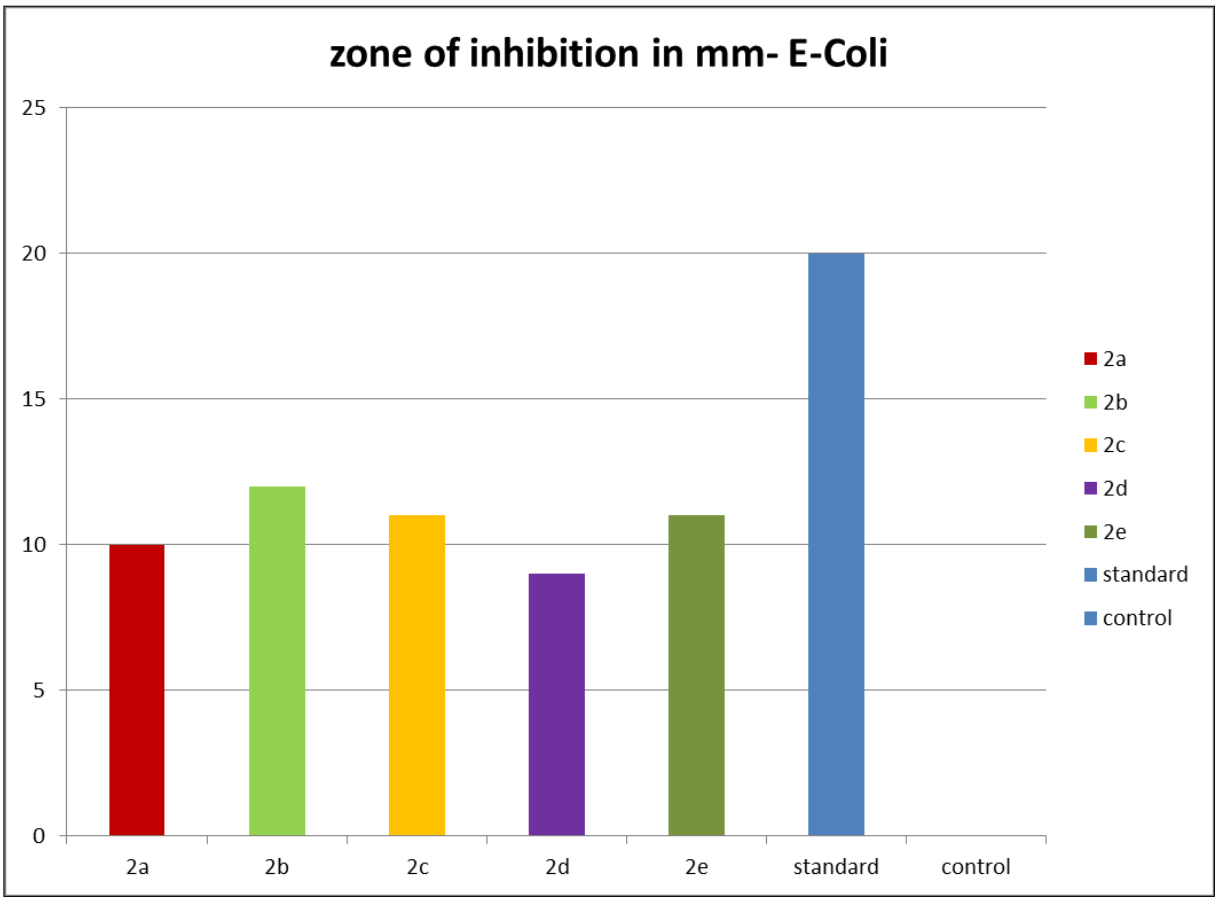

Fig 4: Zone of inhibition against E-Coli.

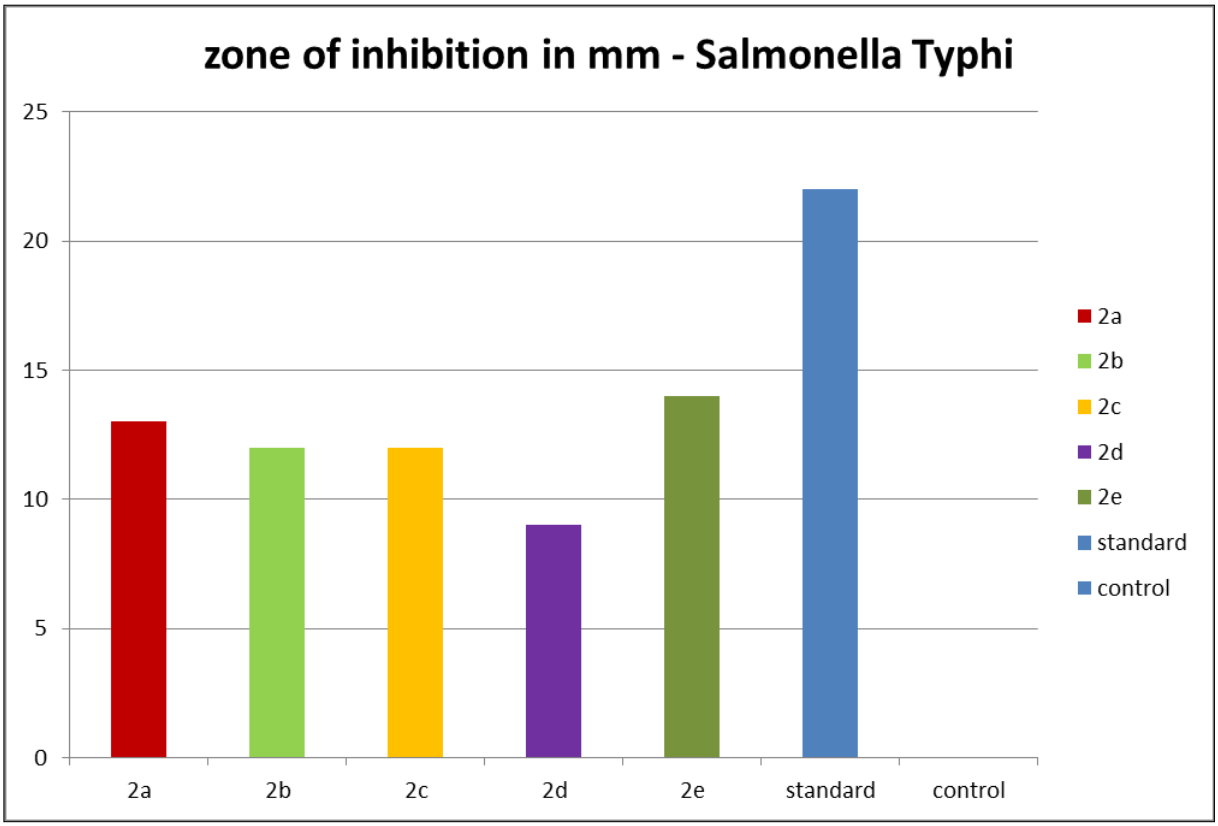

Fig 5: Zone of inhibition against salmonella typhi

Result and Discussion

Thiourea and ethyl cyanoacetate reacted with the aldehydes (4-chlorobenzaldehyde) to give 6-(4-chlorophenyl) -4-oxo-2-thioxo-)-1, 2, 3, 4-tetrahydropyrimidine-5-carbonitrile1. A compound 4-chloro-2-thioxo-6(4-

chlorophenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carbonitrile (2), could be Prepared through the reaction of 6-(4-chlorophenyl)- 4-oxo-2-thioxo-)-1, 2, 3, 4 tetrahydropyrimidine-5-carbonitrile (1) with phosphorus pentachloride and phosphorus oxy chloride. Compound 2 also reacted with P-Bromoaniline, p-Nitrpaniline, hydrazine hydrate, 2,4-dinitroaniline, and anthranillic acid to give compounds 2a-3e respectively.

Conclusion

The proposed pyrimidine derivatives were synthesized successfully as per the planning. All the compounds were evaluated for antibacterial activity. All the synthesized compounds were found to have activity against both gram positive and gram negative micro-organisms. Result reveled that comp no. 2a and 2b shows highest antibacterial activity on salmonella typhi. 2d and 2e shows moderate activity against staphylococcus aureus.

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