ANTIMICROBIAL ACTIVITY OF 4{[2-{4-[(PHENOTHIAZINE 10YL-PHENYL-METHYL)-AMINO] 2, 3-DIHYDROBENZIMIDAZOL-1-YL) PHENYL-METHYL]AMINO}-BENZOIC ACID DERIVATIVES.

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Summary

Ten novel compounds have been synthesized. The in vitro screening was cerried out using selected microbes like B. subtilis (NCIM) 2063, Gram +ve), S. aureus(NCIM 2079, Gram +ve), E.coli (NCIM 2931, Gram -ve). The antimicrobial activity of novel synthesized compounds was evaluated by Disc- Diffusion method. Products IVc, IVf and IVi showed better, while IVb, IVd and IVh showed moderate and rest showed poor antimicrobial activity.

Key Words: Phenothiazine, B. subtilis, S. aureus, E.coli

Introduction

Since the 1940's the development of effective and safe drugs to deal with bacterial infections has revolutionised medical treatment and the morbidity and mortality from microbial disease have been dramatically reduced unfortunately the development of effective antibacterial drugs has been accompanied by the emergence of drug resistant organism.

- Antibiotic resistance in bacteria spreads at three levels.
- By transfer of bacteria between people.
- By transfer of resistance genes between bacteria (usually on plasmids).
- By transfer of resistance genes between genetic elements within bacteria on transposons.

Resistance to antibiotics:

- Resistance in bacterial populations can be spread from person to person by bacteria from bacterium by plasmids from plasmid to plasmid (or chromosome by transposons).
- Plasmids are extra chromosomal genetic elements that can replicate independently and can carry genes coding for resistance to antibiotics (γ genes).

- The main method of transfer of γ-genes from one bacterium to another is by conjugative plasmids which can cause the bacterium to make a connecting tube between bacteria through which the plasmid itself (and other plasmids) can pass.
- A less-common method of transfer is by transduction i.e. the transmission of an γ-gene carrying plasmid into a bacterium by a bacterial virus (phage).

  Transposons are stretches of DNA that can be transposed from one plasmid to another and also from plasmid to chromosome and vice versa. For example an γ-gene carrying transposon in one plamid codes for enzymes that cause integration of that plasmid into another plasmid followed by their seperation during this the transposon replicates so that both plasmids will then contain the γ-gene carrying transposon[1]. some penicillin resistant strains of *Staphylococcus aureus* (MIC_100 mg/L) are rendered susceptible to the antibiotic by concentrations of phenothiazine that have no effect on the bacterium , the initial resistance seen may be due to the presence of an efflux pump that is subject to inhibition by the phenothiazine[2].

Materials and Methods

This involves the measurement of the relative potency of the activity of the compounds by determining the amount requires producing the stipulated effect on a suitable organism under standard condition.

The *in vitro* screening was carried out using selected microbes like B. subtilis (NCIM 2063, Gram +ve), S. aureus (NCIM 2079, Gram positive), E. coli (NCIM 2931, Gram -ve).

Determination of Zone of Inhibition (DISC Diffusion Method)

The Disc Diffusion method of assay of drug potency is based on the measurement of the zone of microbial growth inhibition surroundings discs containing various concentrations of test compounds, which are placed on the surface of a solid nutrient previously inoculated with the culture of suitable microorganism. Inhibition produced by the test drug is compared with that produced by known concentration of reference standard.

Preparation of seeded broth

The strains of microorganisms obtained inoculated in conical flask containing 100ml of nutrient broth. These conical flasks were incubated at $37^0 \pm 2^0$C for 24hrs and were referred to as seeded broth.

Preparation of Culture media

The media used during the study was

- Nutrient agar medium
- Nutrient broth

The media was sterilized by autoclaved at 15lb/sq. inch pressure at 121 ± 1^0C for 20 minutes.

Procedure:

The Phenothiazine derivatives were examined for antimicrobial activity. The compound was dissolved in Dimethyl Formamide (DMF) 6% which was previously tested for antibacterical activity against all test and found to have no antimicrobial activity. Compound was dissolved in DMF (6%) which was dissolved tested for antibacterial activity and found to have no activity.

The compound was made solution of different concentration 50 µg/ml to 250 µg/ml and finally filter by using 0.45 µm Millipore filters. The sterile discs (Hi-Media, Mumbai) 6mm in diameter were impregnated with different concentrations of compound to prepare Discs with concentration of 50 µg/Disc to 250 µg/ml and placed in inoculated agar. The standard Disc (Hi-Media, Mumbai) of Ciprofloxacin was used as standard.

The controls were prepared using the same solvents employed to dissolve the compound. The inoculated plates with the test and standard Discs on them were incubated for

bacteria at $37^0C \pm 1^0c$ for 24 hrs and for fungal $25^0C \pm 1^0C$ for 24 hrs. The zone of inhibition of different compounds and standard drugs are recorded.

Determination of Minimum Inhibitory concentration of Phenothiazine derivatives:

Preparation of inoculums

On previously decontaminated laminar flow bench, inoculums was transferred by help of separate pre sterilized applicators into freshly prepared and sterilized nutrient broth and incubated for 24 hrs at 37 ± 2^0C.

Inoculation of Agar media

Agar media was prepared (as per I.P 1996) and sterilized, and inoculums of different microorganisms were spreaded over the surface of Petri discs, this same procedure was applied for all strains of microorganisms.

Preparation of test samples in different concentrations

Compound was dissolved in DMF 6% which was already checked for antimicrobial activity and was found no effect over any of the tested microorganisms, and prepared as 10 µg/ml to 50 µg/ml of concentrations.

Application of different test samples over the discs

All this procedure was carried out under aseptic conditions, maintained in sterilized room, which was already fumigated to decontaminate, Petri discs, which were already being spreaded with subcultures of different microorganisms, and the sterile discs (Hi-Media, Mumbai) 6mm in diameter were impregnated with different concentration (µg/ml) of test samples. The inoculated plates were incubated for bacteria $37^0C \pm 2^0C$ for 24 hrs and fungal $25^0C \pm 1^0C$ for 24 hrs[7-18].

Results and Discussion

Synthesised Compounds are;

1. 4{[2-{4-[(Phenothiazine 10yl-Phenyl-methyl)-amino]2,3-dihydrobenzimidazol-1-yl)Phenyl-methyl]amino}-benzoic acid. (IVa)

2. 4 {[(2-Chloro-Phenyl) -(2-{4-[(Phenothiazine 10yl-Phenyl-methyl)-amino]2,3-ihydrobenzimidazol-1-yl)Phenyl-methyl]amino}-benzoic acid. (IVb)

3. 4 {[(4-Chloro-Phenyl) -(2-{4-[(Phenothiazine 10yl-Phenyl-methyl)-amino]2,3-ihydrobenzimidazol-1-yl)Phenyl-methyl]amino}-benzoic acid. (IVc)

4. 4 {[(2-Hydroxy-Phenyl) -(2-{4-[(Phenothiazine 10yl-Phenyl-methyl)-amino]2,3-ihydrobenzimidazol-1-yl)Phenyl-methyl]amino}-benzoic acid. (IVd)

5. 4 {[(4-Hydroxy-Phenyl) -(2-{4-[(Phenothiazine 10yl-Phenyl-methyl)-amino]2,3-ihydrobenzimidazol-1-yl)Phenyl-methyl]amino}-benzoic acid. (IVe)

6. 4 {[(2-Nitro-Phenyl) -(2-{4-[(Phenothiazine 10yl-Phenyl-methyl)-amino]2,3-ihydrobenzimidazol-1-yl)Phenyl-methyl]amino}-benzoic acid. (IVf)

7. 4 {[(2-Methoxy-Phenyl) -(2-{4-[(Phenothiazine 10yl-Phenyl-methyl)-amino]2,3-ihydrobenzimidazol-1-yl)Phenyl-methyl]amino}-benzoic acid. (IVg)

8. 4 {[(4-Methoxy-Phenyl) -(2-{4-[(Phenothiazine 10yl-Phenyl-methyl)-amino]2,3-ihydrobenzimidazol-1-yl)Phenyl-methyl]amino}-benzoic acid. (IVh)

9. 4 {[(3-Hydroxy-Phenyl) -(2-{4-[(Phenothiazine 10yl-Phenyl-methyl)-amino]2,3-ihydrobenzimidazol-1-yl)Phenyl-methyl]amino}-benzoic acid. (IVi)

10. 4 {[(4-Hydroxy-2-Methoxy-Phenyl) -(2-{4-[(Phenothiazine 10yl-Phenyl-methyl)-amino]2,3-ihydrobenzimidazol-1-yl)Phenyl-methyl]amino}-benzoic acid. (IVj)

Compounds IVc, IVf, IVi shows good activity against *E.Coli* (gram negative) *B.subtilis*, and compound IVb, IVd, IVh shows moderate avtivity against *E.Coli*, *B.subtilis*, *S.aureus*. The assigned structure and Molecular formula of the newly synthesized compounds [VIa-j], were confirmed and supported by 1H-NMR,Mass, IR data and which was in full agreement with proposed structures. The compounds were screened *in vitro* for their antibacterial and antifungal potential by disc diffusion assay against selected pathogenic bacteria and human pathogenic fungi. The results of antibacterial and antifungal activities expressed in terms of Zone of inhibition are reported in Table [1, 2, 3] and MIC in Table [4]

Table no. 1 Antimicrobial activity against *E.Coli*

Sr.No	*Compounds*	*Diameter of Zone of Inhibition (mm)*				
		Different concentrations				
		50 µg	*100 µg*	*150 µg*	*200 µg*	*250 µg*
1.	IVa	*12*	*19*	*21*	*23*	*24*
2.	IVb	*10*	*14*	*19*	*19*	*19*
3.	IVc	*12*	*13*	*22*	*24*	*24*
4.	VId	*13*	*18*	*16*	*17*	*20*
5.	IVe	-	*6*	*9*	*11*	*13*
6.	IVf	*12*	*15*	*20*	*23*	*24*
7.	IVg	-	-	-	*10*	*11*
8.	IVh	*12*	*14*	*15*	*17*	*18*
9.	IV*i*	*13*	*14*	*16*	*21*	*24*
10.	IV*j*	-	-	-	-	*11*

Table no. 2 Antimicrobial activity against *S.aureus*

Sr.No	*Compounds*	*Diameter of Zone of Inhibition (mm)*				
		Different concentrations				
		50 µg	*100 µg*	*150 µg*	*200 µg*	*250 µg*
1.	IVa	*9*	*11*	*13*	*14*	*20*
2.	IVb	*11*	*17*	*15*	*16*	*18*
3.	IVc	*14*	*18*	*25*	*26*	*27*
4.	VId	*11*	*14*	*16*	*18*	*20*
5.	IVe	-	*7*	*11*	*13*	*15*
6.	IVf	*13*	*15*	*21*	*24*	*25*
7.	IVg	-	-	-	-	*12*
8.	IVh	*10*	*15*	*17*	*19*	*21*
9.	IV*i*	*12*	*15*	*22*	*23*	*25*
10.	IV*j*	-	-	-	-	*9*

Table no. 3 Antimicrobial activity against *B. substilis*

Sr.No	*Compounds*	*Diameter of Zone of Inhibition (mm)*				
		Different concentrations				
		50 µg	*100 µg*	*150 µg*	*200 µg*	*250 µg*
1.	IVa	14	15	17	18	19
2.	IVb	13	16	15	15	17
3.	IVc	14	11	21	24	25
4.	VId	10	15	16	17	21
5.	IVe	-	7	10	13	14
6.	IVf	13	14	21	21	24
7.	IVg	-	-	-	-	11
8.	IVh	11	16	16	18	21
9.	IV*i*	11	14	20	21	25
10.	IV*j*	-	-	-	-	8

Table no. 4: Minimum inhibitory concentration (MIC) of derivatives against *S. aureus*

Sr.No.	Compounds	MIC conc. µg/ml
1.	IVa	15
2.	IVb	10
3.	IVc	15
4.	VId	20
5.	IVe	10
6.	IVf	25
7.	IVg	15
8.	IVh	20
9.	IV*i*	15
10.	IV*j*	20

Conclusion

It is apparent that the Phenothiazine and structurally related compounds possessing three benzene rings are often endowed with potent antimicrobial action. Compounds IVc, IVf, IVi shows good activity against *E.Coli* (gram negative) *B.subtilis*, *S.aureus*. compound IVb, IVd, IVh shows moderate avtivity against *E.Coli*, *B.subtilis*, *S.aureus* .

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