be safely used for the preparation of solid dispersions of celecoxib and improve its dissolution and bioavailability on oral administration.

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Antimicrobial Activity and Structure-Activity Relationship of Acyclic Nucleosides

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Various acylonucleoside analogs were synthesized and evaluated for antibacterial and antifungal activity. All the acyclic nucleosides exhibited good antifungal activity compared to the standard drug clotrimazole. However, none of the compounds possess broad-spectrum antibacterial activity. The structure-activity relationships of acyclic nucleosides were studied in order to develop the most potential antifungal agent for preclinical evaluation.

Acyclic substituted pyrimidines (acyclic nucleosides or acyclonucleosides)[1] are the sugar modified nucleoside analogs possessing biological and pharmacological activities. This group of compounds has gained increasing importance through their biological activities, particularly in the field of antiviral chemotherapy[2]. A number of acyclonucleoside analogs are used clinically for the treatment of herpes simplex virus type-1 (HSV-1), type-2 (HSV-2), vericella zoster (VZV) and HIV-infections[3,4]. It has been found that acyclonucleosides are monophosphorylated by viral thymidine kinase to the monophosphates and subsequently converted into diphosphates and then to the corresponding triphosphates by cellular enzymes. The triphosphate forms are then recognized by viral reverse transcriptase as substrate and the corresponding nucleoside triphosphates are incorporated into growing DNA chains, which lead to DNA chain termination[5]. Besides antiviral activity, acylonucleosides are also used as antitumor agents[6-8]. In addition, various acyclonucleosides are potent inhibitor of certain enzymes, such as uridine phosphorylase, thymidine phosphorylase, which are involved in the catabolism of clinically useful nucleosides. Chu and coworkers[9] synthesized 5-benzylacyclouridine and 5-benzyloxybenzylacyclouridine (Fig. 1, I and II) and were found to be good inhibitors of uridine phosphorylase. Goudgaon *et al.*[10] synthesized phenylselenenyl- and phenylthio-substituted pyrimidines (Fig. 1, III and IV) as potent inhibitors of dihydrouracil dehydrogenase and uridine phophorylase. Some of the pyrimidine compounds like 5-fluorouracil is used clinically for the treatment of various types of cancer. Hitchings *et al.* showed substituted pyrimidines possessed marked antimalarial[11] and antileukemic[12] properties. Hence the synthesis and biologi-

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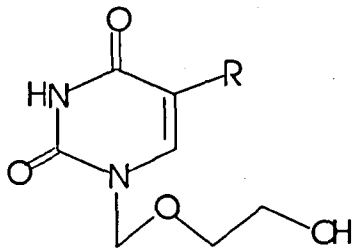

Fig. 1: Structure of some novel acyclonucleosides.

The substitutions are, for I- R=$CH_2C_6H_5$, for II- R=$CH_2C_6H_4OCH_2C_6H_5$, for III- R=SePh and for IV-R=SPh.

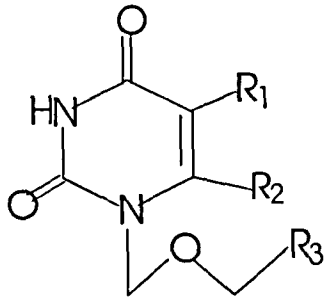

Fig. 2: Structure of acyclonucleosides.

The substitutions are, for 1- R_1=CH_3, R_2=H, R_3=CH_2OH, for 2- R_1=Cl, R_2=H, R_3=CH_2OH, for 3- R_1=Br, R_2=H, R_3=CH_2OH, for 4- R_1=H, R_2=SePh, R_3=CH_2OH, for 5- R_1=Cl, R_2=SePh, R_3=CH_2OH, for 6- R_1=CH_3, R_2=H, R_3=CH_2OAc, for 7- R_1=Cl, R_2=H, R_3=CH_2OAc, for 8- R_1= F, R_2=H, R_3=CH_3, for 9- R_1= SePh, R_2=H, R_3=CH_3, for 10- R_1=H, R_2= SePh, R_3=CH_3 for 11- R_1=CH_2CH_3, R_2=H, R_3=CH_3, for 12- R_1=CH_3, R_2=H, R_3=CH_3, for 13-R_1=CH_2CH_3, R_2=SePh, R_3 =CH_3, and for 14-R_1=F, R_2=SePh, R_3=CH_3.

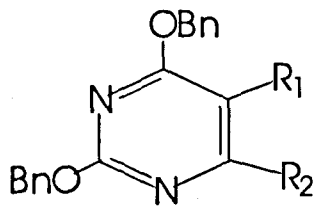

Fig. 3: Structure of benzyl protected substituted pyrimidines.

The substitutions are, for 15- R_1=SPh, R_2=H, for 16.- R_1=H, R_2=SePh, for 17- R_1=H, R_2= SPh and for 18- R_1=SePh, R_2=H.

cal evaluation of benzyl protected and C_5 and C_6 substituted pyrimidines is desirable to determine the potential antimicrobial activity of acyclic nucleosides. Based on the potential usefulness of the acyclic nucleosides and substituted pyrimidines in chemotherapy, we report here the random screening for *in vitro* antimicrobial activity of acyclonucleosides.

The acyclic nucleosides were synthesized according to the synthetic methods described earlier by our group[10,13] (Figs. 2, 3 and 4). The antimicrobial activity was carried out against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus*, and fungi, *Aspergillus niger* and *Aspergillus flavus* by cup-plate method[14]. Aseptic conditions were maintained throughout the experiment.

Antibacterial screening required nutrient agar media and it was prepared by dissolving bacteriological peptone (5 g), dextrose (1 g), NaCl (5 g) in 1 l distilled water. pH was adjusted to 7-7.4 by using 40% aqueous NaOH solution. To this solution 20 g of agar was added. Then it was sterilized for 30 min at 15 lbs pressure. The organisms used in the present study were obtained from the laboratory stocks. Twenty four hours before the day of testing, the above mentioned 4 bacteria were subcultured separately into 25 ml of sterilized nutrient broth, which was prepared in the same manner as that of nutrient media but without agar. Inoculated subcultured broths were kept at room temperature for 24 h. Test solutions of all the compounds and gentamycin (+ ve control) were prepared in distilled dimethyl formamide (DMF) at the concentration of 100 µg/ml and 50 µg/ml. After sterilization of the nutrient agar media, it was allowed to cool to 45°. Subculture of bacterial strain was inoculated into nutrient agar media. Inoculated media was poured into the petriplates and allowed to solidify. With the help of 6 mm sterile cork borer the cups were punched and scooped out the set agar. The cups were then filled individually with 0.1 ml of the test solution, gentamycin and solvent control. The plates were allowed to stay for 24 h at 37° to know the bacterial growth inhibition, developed around the hole was measured for the particular test solution with particular organism.

Potato dextrose agar (PDA) was used for the antifungal screening. To 500 ml distilled water, 250 g peeled potatoes were added in a conical flask and boiled. After boiling, supernatant i.e., extract was saved and 20 g of dextrose was added to it. In another conical flask 20 g of agar was added containing 250 ml of distilled water and was boiled to dis-

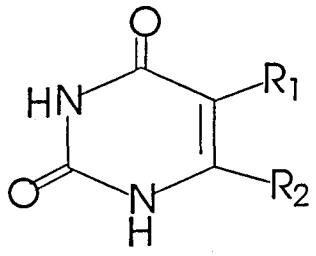

Fig. 4: Structure of substituted uracils.

The substitutions are, for 19- R_1=H, R_2=SePh and for 20- R_1=SePh, R_2=H.

solve agar completely. Then contents of both the flask were mixed and measured. Required amount of distilled water was added to make 1 l and then sterilized at 15 lbs pressure. One day prior to the testing, inoculation of the fungi were made separately into 25 ml of sterile distilled water and kept at room temperature. Test solutions, clotrimazole (+ ve control) were prepared at the concentration of 100 µg/ml in distilled DMF. Before pouring the sterilized media into the petriplates, streptomycin was added to it in order to prevent the bacterial contamination. Then media was poured into the petriplates and allowed to solidify the media. Fungal subculture was inoculated on the solidified media. With the help of 6 mm sterile cork borer, the cups were punched and scooped out the set agar. The cups were filled separately with test solution, clotrimazole and solvent control. The plates were allowed to stay for 24 h at room temperature and zone of inhibition was measured in mm.

In the present study, we have evaluated various substituted pyrimidines for antimicrobial activities. None of the tested compounds exhibited bacterial growth inhibition against Gram – ve bacteria, *P. aeruginosa, K. pneumoniae and E. coli.* However, all the compounds showed activity against Gram + ve bacterium, *S. aureus* (Table 1) at the concentrations of 100 µg/ml and 50 µg/ml. Hydrophobic property of the molecule increases the antibacterial activity. Com-

TABLE 1: ANTIBACTERIAL ACTIVITY OF ACYCLONUCEOSIDES.

Compd. No.	Dose (µg/ml)	zone of inhibition (mm)	Dose (µg/ml)	Zone of inhibition (mm)
		S. aureus		*S. aureus*
1	100	20	50	16
2	"	12	"	9
3	"	16	"	10
4	"	12	"	8
5	"	20	"	12
6	"	21	"	17
7	"	18	"	13
8	"	15	"	10
9	"	17	"	13
10	"	16	"	11
11	"	20	"	14
12	"	20	"	13
13	"	22	"	15
14	"	20	"	16
Gentamycin	"	27	"	20

All the synthesized acyclonucleosides were evaluated for antibacterial activity against *S. aureus* at the concentrations of 100 µg/ml and 50 µg/ml

pounds with CH_3 or $-CH_2OAc$ instead of $-CH_2OH$ at R_3 position showed good antibacterial activity than later. Compounds 1 and 2 showed less activity than compounds 6 and 7, respectively, and also compound 10 exhibited good activity than 4. Acyclopyrimidines with substitution at both C_5 and C_6 positions exhibited greater activity than acyclopyrimidines with C_5 substitution alone, for e.g., 5 and 14 showed better activities than 2 and 8, respectively. Presence of -SePh, $-CH_2CH_3$, or $-CH_3$ groups except halogen at C_5 position of the acycloprimidine increases the antibacterial activity of the compound, i.e., compound 8 showed less activity than 9, 11 and 12. Similarly, compounds 2 and 3 showed less activity than 1 and compound 6 exhibited more activity than 7. From Table 2 it is clear that C_5 and C_6 substituted pyrimidines possessed more or less similar antibacterial activity against all four bacteria. Benzyl protected pyrimidine with C_5- SePh substitution showed good activity than C_5 SPh substituted pyrimidine. It has been observed that C_6-SPh substituted pyrimidines showed more activity than pyrimidine with C_5-SPh, for e.g., compound 17 exhibited more activity than 15. Compounds without benzyloxy group at C_2 and C_4 positions (19 and 20) did not show any activity against *P. aeruginosa*. Various substituted pyrimidines were evaluated for antifungal activity against *A. niger* and *A. flavus* (Table 3). In the case of *A. niger*, compounds 3 and 7 showed greater antifungal activity than the clotrimazole. Compounds 1, 2, 4, 11 and 13 exhibited comparable activity to the standard drug clotrimazole, and remaining compounds (5, 6, 8, 9, 10, 12 and 14) possessed moderate activity. However, compounds 1, 2, 3, 4, 7, 9,.12 and 22 displayed good activity against *A. flavus* and other compounds (5, 6, 8, 10, 11 and 14) have less activity compared to clotrimazole. Among R_3-CH_3 substituted acyclonucleosides, the substitution at C_5 and C_6 of the pyrimidine ring increases the antifungal activity. Compound 13 has greater antifungal activity than 10. Similarly, compound 14 exhibited better activity than 8. The presence of $-SePh$, $-CH_2CH_3$, and $-CH_3$ (9, 11 and 12) instead of halogen at C_5 (8) of acyclopyrimidine increases the antifungal activity. This indicates that the presence of less hydrophilic group is necessary for the enhancement of antifungal property. However, in compounds bearing R_3-CH_2OH and CH_2OAc, the presence of electron with-drawing group at C_5 position increased the antifungal activity e.g., compound 1 showed less activity than 2 (except activity against *A. niger*) and 3. Similarly, compound 7 has more activity than 6. Acyclonucleosides with R_3-CH_2OH were more active than acylonuleosides with R_3-CH_2OAc and CH_3 for e.g., compounds 1 and 4 showed more activity than compounds 6 and 10 respectively. Except compound 7, which has more activity than 2 in case of *A. niger*. Table 3 also revealed that among C_5 and C_6 substituted pyrimidines, compound 15 possessed good activity against *A. niger*.

In conclusion, the results of antimicrobial screening reveal that acyclonucleosides exhibited good antifungal activity and moderate activity against *S. aureus*. For the good antibacterial property these compounds should have C_5 and C_6 substitutions and they should be substituted with $-SePh$, $-CH_2CH_3$ and $-CH_3$ groups. For good antifungal activity, acyclonucleosides bearing R_3-CH_2OH should have electron with-drawing group at C_5 position and acyclonucleosides with

TABLE 2: ANTIBACTERIAL ACTIVITY OF SUBSTITUTED PYRIMIDINES.

Compd No.	Dose (μg/ml)	Zone of inhibition (mm)			
		P. aeruginosa	*K. pneumonia*	*E. coli*	*S. aureus*
15	100	15	13	0	13
16	"	15	11	17	18
17	"	15	17	12	18
18	"	18	12	12	15
19	"	0	19	16	15
20	"	0	16	12	17
Gentamycin	"	25	25	25	25

All the synthesized substituted pyrimidines were evaluated for antibacterial activity against *P. aeruginosa, K. pneumonia, E. coli and S. aureus* at the concentration of 100 μg/ml.

TABLE 3: ANTIFUNGAL ACTIVITY OF SUBSTITUTED PYRIMIDINES.

Compound No.	Dose (µg/ml)	Diameter of zone of inhibition (mm)	
		A. niger	*A. flavus*
1	100	26	20
2	"	25	35
3	"	30	23
4	"	26	20
5	"	20	15
6	"	22	15
7	"	30	20
8	"	15	14
9	"	15	20
10	"	22	12
11	"	27	17
12	"	20	20
13	"	25	22
14	"	22	15
15	"	35	22
16	"	18	22
17	"	25	23
Clotrimazole	"	25	40

All the synthesized substituted pyrimidines were evaluated for antifungal activity against *A. niger* and *A. flavus* at the concentration of 100 µg/ml.

R_3-CH_3 should be substituted with electron donating or hydrophobic groups.

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