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Short communication

Evaluation of antibacterial activity of 3,5-dicyano-4,6-diaryl-4-ethoxycarbonyl-piperid-2-ones

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1. Introduction

The importance of heterocyclic chemistry in biological system, as well as in chemotherapy is now well established [1]. Substituted lactams constitute central and important area of synthetic organic, medicinal and pharmacological chemistry [2,3]. Serving as attractive scaffolds for drug design and conferring drug-like characteristics into numerous structural motifs, lactams, pyridones and related heterocyclic systems are finding increasing applications in organic and medicinal chemistry. The backbone structure of these rigidified peptidomimetics maintain or restrict biologically relevant dihedral angle, conformational and stereochemical information derived from a parent peptide array [4] Figs. 1 and 2.

Naturally occurring monosaccharides-like alkaloids containing a piperidine moiety and their analogues such as nojiromycin-δ-lactams are significant. Since they are potent and selective glycosidase inhibitors [5,6] and thus, are expected to have potential chemotherapeutic utilities such as antidiabetic [7], novel anticancer [8,9] and anti-HIV agents [10]. Prompted by the wide variety of biological applications of δ-lactams, it was considered worthwhile to prepare the new substituted δ-lactams in a simple and efficient manner with a hope to get more biological potency.

Though there are number of drugs such as ampicillin, cotrimoxazole etc. which exhibit antibacterial activity, the preparation of these drugs involves multi steps and needs expensive chemicals and sophisticated reaction conditions. With this in view, we thought of synthesising new compounds starting from inexpensive reagents in a simple procedure and successfully carried out the reaction to get the title compounds [11]. This paper describes the screening of antibacterial activity of various synthesised 3,5-dicyano-4,6-diaryl-4-ethoxycarbonyl-piperid-2-ones (**1**) against bacterium *Escherichia coli* and *Bacillus cirroflagellosus* were described.

2. Experimental

A series of 3,5-dicyano-4,6-diaryl-4-ethoxycarbonyl-piperid-2-ones (**1**) [11] prepared from aromatic simple imines and ethyl cyanoacetate were screened for their antibacterial activities against Gram negative bacterium *E. coli*, Gram positive

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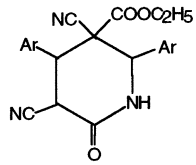
bacterium *B. cirroflagellosus*. Results of such studies are discussed in this paper.



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1 a) Ar = C_6H_5; b) Ar = 4-$H_3COC_6H_4$; c) Ar = 4-$(H_3CO)_2C_6H_3$; d) Ar = 4-$(H_3CO)_3C_6H_2$; e) Ar = 4-ClC_6H_4.

2.1. Evaluation of antibacterial activity

2.1.1. Principle

Methods used for analysing compounds for antibacterial activity are varied. In this experiment, the analysis was carried out by the paper disc diffusion assay [12,13], which is a simple and rapid method. The entire surface of the selective agar is inoculated with the bacteria and sterile filter paper discs of uniform size and thickness containing known amounts of the compounds is placed on the seeded agar. The compound diffuses into the agar and prevents the growth of the bacteria, indicated by a clearing zone around the disc if the compound does possess bactereocidal or bactereostatic effect. In most studies inhibition zones are compared with those determined for antibiotics.

3. Method

Briefly a lawn of the organism (*E. coli*) was prepared by spreading 50 ml of overnight cultures (conc. 10^6–10^7 CFU/ml) onto agar set in petri dishes. LB Agar medium was used for the bacteria, made by adding 1.5 g of agar, 1 g of NaCl, 1 g of tryptone and 0.5 g of yeast extract to 100 ml of water (pH of the medium: 7.4).

Sterile filter paper discs (measuring 6 cm in diameter) containing the required concentration of the compounds are placed at equal distances on the agar plate. Control experiments were performed where only equivalent volumes of the solvents without the added test compounds were applied to the paper discs. Ampicillin at a concentration of 2 μg was used as the positive control. Plain dry discs were used as negative controls. The plates were incubated at 37 °C for 24 h to check clearing zones around the discs.

The relative percentage inhibition with respect to standard was calculated by using the following formula.

Relative percentage inhibition of the test sample

$$= \frac{100 \times (a-b)}{(c-b)}$$

Where, a, total area of inhibition of the test sample; b, total area of inhibition of the solvent; c, total area of inhibition of the standard drug. The total area of the inhibition was calculated by using area $= \pi r^2$ where, $r =$ radius of zone of inhibition.

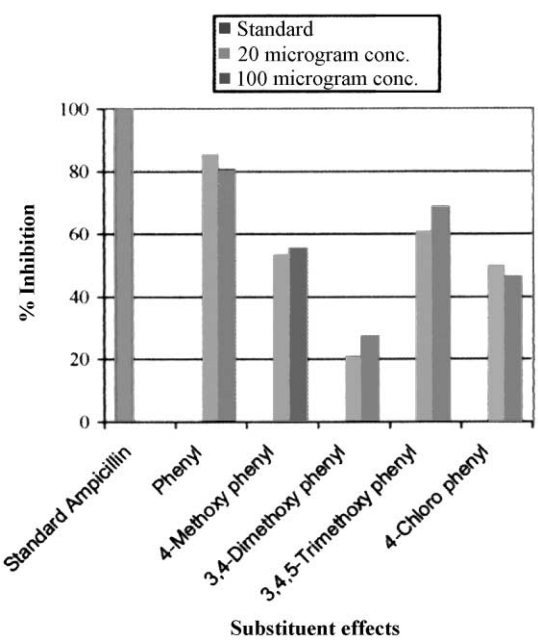

Fig. 1. Antibacterial activity of synthesised compounds against *E. coli*.

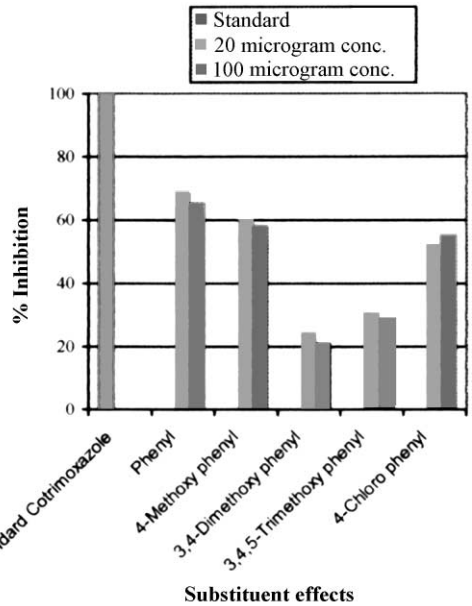

Fig. 2. Antibacterial activity of synthesised compounds against *B. ciroflagellosus*.

4. Results and discussion

E .coli cells were found to be resistant to the synthesised compounds (**1**) at the 20 and 100 μg concentrations. Inhibition zones were observed around the point where the test samples were placed on the discs. The distance (r) between the point of placement of the test sample and inhibition zone was measured, from this, the total area of inhibition was calculated using the equation, area $= \pi r^2$. Similarly, the total areas of the zone of inhibition were calculated for the standard drug and the solvent used. The relative percentage of inhibition with respect to the standard drug was calculated using the equation as above, by subtracting the area of zone of inhibition of the solvent (since the solvent also shows some degree of antibacterial activity) from the area of zone of inhibition of the test sample. The same procedure was used with *B. cirroflagellosus*.

One of the possible modes of action could be that, the strained lactam of 2-piperdone (**1**) act to acylate a critical cell constituent on the N–H, SH or OH function, thereby blocking the function of such cell constituent. Chemically, the acylation process would be favoured by the removal of the lactam ring. Biochemically the acylation could destroy the activity of an essential cell constituent [14,15]. Another possibility is that the synthetic product (**1**) may act on a cell constituent not by the covalent bond but by a non-covalent combination [16].

The compound **1a** having no substituents on the aromatic ring showed remarkable activity against *E. coli* and *B. cirroflagellosus*, which may be due to the electron withdrawing effect of the benzene ring on lactam ring, which facilitates the nucleophillic attack of the cell constituent at CO or NH sites of the lactam ring. The compound **1b–d** having electron releasing methoxy groups as substituents on the aromatic rings showed promising activity against both the bacterium. The relatively less activity of these compounds **1b–d** with respect to **1a** is due to the fact that these electron-donating groups reduces the positive charge on the carbonyl carbon atom through inductive effect, thereby reducing the rate of the nucleophillic attack of the cell constituent to an extent. **1e** having chloro substituent at *para* position of the aromatic rings has showed considerable bacterial activity, which may be due to anomalous behaviour of chlorine atom as it behaves both as electron-withdrawing and electron-donating group depending upon the situation which facilitates the nucleophillic attack of the cell constituent.

5. Conclusion

It is interesting and significant to note from the data in Table 1 that all the synthesised new products 3,5-dicyano-4,6-diaryl-4-ethoxycarbonyl-piperid-2-ones (**1**) have showed some degree of antibacterial activity against the bacterium *E. coli* and *B. cirroflagellosus*. Though the synthesised compounds exhibit relatively less bacterial activity compared with that of standard drugs ampicillin or cotrimoxazole, it is noteworthy to observe that these drugs exhibits more bacterial activity, but the preparation of these drugs requires multi step process, which in turn is highly expensive, while the lactams (**1**) [11] reported in this paper can be

Table 1
Antibacterial activity of δ-lactams

Compound	*E. coli*				*B. cirroflagellosus*			
	Area of zone of inhibition (mm^2)		Relative percentage inhibition		Area of zone of inhibition (mm^2)		Relative percentage inhibition	
	Concentration		Concentration		Concentration		Concentration	
	20 μg	100 μg	20 μg	100 μg	20 μg	100 μg	20 μg	100 μg
1a	183.8	179.0	85.5	80.7	268.8	260.1	68.5	65.2
1b	147.4	149.5	53.5	55.4	246.0	240.5	60.0	57.9
1c	109.3	116.8	20.9	27.4	149.5	141.0	24.0	20.9
1d	156.1	165.1	61.0	68.7	165.1	160.6	30.2	28.5
1e	143.0	138.9	49.9	46.2	224.3	232.3	51.9	54.9
Standard	201.6[a]				353.3[b]			
Solvent	84.9[c]				84.9[c]			

[a] Ampicillin.
[b] Cotrimoxazole.
[c] Ethanol.

prepared in one step starting from inexpensive imines and ethyl cyanoacetate in a simple procedure. From this observation, it is concluded that with a slight modification on the lactam ring, a medicinal chemists or pharmacists can replace the existing expensive drugs with this type of lactams for the treatment of antibacterial activity.

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References

[1] K. Ajay Kumar, K.M. Lokanatha Rai, K.B. Umesha, Ind. J. Heterocycl. Chem. 10 (2000) 79 Previous paper.
[2] C.M. Marson, A. Fallah, Tetrahedron Lett. 35 (1994) 293.
[3] G. Lowe, β-Lactam antibiotics, in: D.H.R. Barton, W.D. Ollis (Eds.), Comprehensive Organic Chemistry, vol. 5, Pergomon press, Oxford, 1979, p. 289.
[4] N.K. Minami, J.E. Reiner, J.E. Semple, Bioorg. Med. Chem. Lett. 9 (1999) 2625.
[5] Y. Nishimura, J. Synth. Org. Chem. Jpn. 49 (1991) 846.
[6] A.B. Hughes, A. Rudge, Natural Prod. Rep. 11 (1994) 135.
[7] P.B. Anzeveno, L.J. Creemer, J.K. Daniel, C.-H. King, P.S. Liu, J. Org. Chem. 54 (1989) 2529.
[8] C.-H. Wong, D.P. Dumas, Y. Ichikawa, K. Koseki, S.J. Danishefsky, B.W. Westone, L.B. Lowe, J. Am. Chem. Soc. 114 (1992) 7321.
[9] T. Tsuruoka, H. Fukuyasu, M. Ishi, T. Usui, S. Shibahara, S. Inouye, J. Antibiot. 49 (1996) 155.
[10] R.A. Gruters, J.J. Neefjer, M. Tersmette, R.E.Y. de Goeds, A. Tulp, H.G. Huisman, F. Miedema, H.L. Ploegh, Nature 74 (1987) 330.
[11] K. Ajay Kumar, K.M. Lokanatha Rai, A. Hassner, J. Org. Chem., (2001), communicated.
[12] W.H. Seeky, P.J. Van Denmark, Microbes in Action, W.H. Freeman, SanFransisco, 1972.
[13] S. Chand, I. Lusunzi, D.A. Veal, L.R. Williams, Rapid screening of the antimicrobial activity of extracts and natural products, J. Antibiot. 47 (11) (2001) 1295.
[14] K.M. Lokanatha Rai, C. Anjanamurthy, Curr. Sci. 55 (1986) 702.
[15] J.A. Montgomery, T.P. Johnson, Y.F. Shearz, in: A. Berger (Ed.), Medicinal Chemistry Part I, Wiley, New York, 1970, p. 680.
[16] R.E. Jordon, N. Hewitt, W. Lewis, H. Kagen, C. Frazblau, Biochemistry 13 (1974) 3497.