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Short Communication

IN-VITRO MICROBIOLOGICAL EVALUATION OF 5-ETHOXYCARBONYL-4-ARYL-6-METHYL-3, 4-DIHYDROPYRIMIDIN-2(1H)-ONES

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Abstract: Six 5-ethoxycarbonyl-4-aryl- -6-methyl-3,4-dihydropyrimidin-2(1H)-ones **4a–g** are screened for their antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and antifungal activity against *Candida albicans, Aspergillus flavus, Rhizopus* and *Mucor*. Ciprofloxacin is used for the standard for antibacterial and Amphotericin B is used for the standard for antifungal studies. Compounds **4b**, **4e** and **4f** exhibited excellent *in vitro* antibacterial activity against all the tested organisms. Where as, the same set of compounds exerted potent *in vitro* antifungal activity against *Candida albicans, Aspergillus flavus* and *Rhizopus.*

Key words3,4-Dihydropyrimidinones; antibacterial activity, antifungal activity, Ciprofloxacin, Amphotericin B

INTRODUCTION

In the past decade, dihydropyrimidinones (DHPMs) and their derivatives have attracted considerable interest because they exhibit promising activities as calcium channel blockers, α-1a-antagonists and neuropeptide Y (NPY) antagonists.[1] Furthermore, several bioactive isolated marine alkaloids were also found to contain the 2-amino-1,4-dihydropyrimidinone-5-carboxylate core.[2] Most notably among them are the batzalladine alkaloids, which have been found to be potent HIV-gp-120-CD4 inhibitors.[3] Dihydropyrimidin-2(1H)-ones are pharmacologically active as antioxidant agents[4] .

The aim of this study was to evaluate the biological activities of dihydropyrimidin-2(1H)-ones **4a–4f**. The results of the antibacterial and antifungal activities are discussed in this paper.

To percept structure-activity relationship well, numberings of the target compound is shown below **Fig. 1**.

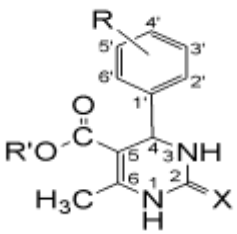

Figure 1

MATERIALS AND METHODS

The compounds were synthesized by the literature procedure [5]. The *in vitro* antimicrobial activities of the compounds were tested in Sabouraud's dextrose broth (SDB, Hi-media, Mumbai) for fungi and nutrient broth (NB, Hi-media, Mumbai) for bacteria by the twofold serial dilution method[6].

The test compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain 1 mg/ml stock solutions. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1 °C while fungal spores from 24 h to 7-day-old Sabouraud's agar slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by the plating technique and adjusted in the range of 10^4–10^5 cfu/ml. The final inoculum size was 10^5 cfu/ml for the antibacterial assay and 1.1–1.5 × 10^2 cfu/ml for the antifungal assay. Testing was performed at 7.4 ± 0.2. Exactly 0.2 ml of the solution of test compound was added to 1.8 ml of seeded broth to form the first dilution. One ml of this was diluted with a further 1 ml of the seeded broth to give the second dilution and so on until six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control and likewise solvent controls were also run simultaneously. The tubes were incubated in biochemical oxygen demand (BOD) incubators at 37 ± 1 °C for bacteria and 28 ± 1 °C for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 h (for bacteria) and 72–96 h

(for fungi) of incubation. Ciprofloxacin was used as a standard for the bacterial study while Amphotericin B was used as a standard for the fungal study.

RESULT AND DISCUSSION

Physical data of the compounds **4a-4f** are given in the **Table 1**.

Table 1: Physical data of compounds **4a**–**4f**

Compound	R	R′	X	Reaction time (h)	Yield (%)[a]	m.p. (°C)
4a	H	C_2H_5	O	2	98	202–203
4b	4-Cl	C_2H_5	O	2.30	92	212–213
4c	4-CH_3	C_2H_5	O	2	90	216–217
4d	4-OCH_3	C_2H_5	O	3	90	201–202
4e	4-NO_2	C_2H_5	O	3	84	206–207
4f	4-F	C_2H_5	O	2	88	183–184

All the bacterial strains namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and fungal strains namely *Candida albicans*, *Aspergillus flavus*, *Rhizopus* and *Mucor* were obtained from Faculty of Medicine, Annamalai University, Annamalainagar, Tamil Nadu, India.

In vitro antibacterial and antifungal activity

Compounds **4a–4f** were tested for their antibacterial activity *in vitro* against *Staphylococcus aureus*, *Escherichai coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Ciprofloxacin was used as standard drug. Minimum inhibitory concentration (MIC) in µg/ml values shown in **Table 2**.

Table 2: *In vitro* antibacterial activities (MIC) values for compounds **4a**–**4f**

Compound	Minimum inhibitory concentration (MIC) in µg/ml				
	Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Salmonella typhi*
4a	200	–	200	200	100
4b	25	50	25	12.50	12.50
4c	100	200	200	100	50
4d	50	100	100	50	50
4e	3.13	6.25	6.25	3.13	3.13
4f	6.25	12.50	12.50	3.13	6.25
Ciprofloxacin	25	50	50	25	25

"–"No inhibition even at a high concentration of 200 µg/ml

All the 3,4-dihydropyrimidin-2(1H)-ones **4a–4f** exerted potent antibacterial activity *in vitro* against the tested bacterial strains. Moreover, compounds **4b**, **4e** and **4f exerted** excellent antibacterial activities against *S. aureus, E. coli, K. pneumoniae, P. aeruginosa* and *S. typhi*.

The *in vitro* antifungal activity of the synthesized compounds **4a–4f** was studied against the fungal strains viz., *C. albicans*, *A. flavus*, *Rhizopus* and *Mucor*. Amphotericin B was used as a standard drug. Minimum inhibitory concentration (MIC) in µg/ml values is shown in **Table 3.**

Table 3: *In vitro* antifungal activities (MIC) values for compounds **4a–4f**

Compound	Minimum inhibitory concentration (MIC) in µg/ml			
	Candida albicans	*Aspergillus flavus*	*Rhizopus*	*Mucor*
4a	100	200	100	–
4b	25	25	12.50	50
4c	100	100	100	200
4d	50	50	100	100
4e	3.13	3.13	3.13	12.50
4f	3.13	6.25	6.25	12.50
Amphotericin B	50	25	50	25

"–" No inhibition even at a high concentration of 200 µg/ml

Compounds **4b**, **4e** and **4f** exhibited excellent antifungal activities against all the tested fungal strains except *Mucor*.

CONCLUSION

Results of this study show that compounds **4b**, **4e** and **4f** which contain chloro, nitro and fluoro moieties exerted excellent antimicrobial activities against the tested organisms. Further development of this group of 3,4-dihydropyrimidinones may lead to compounds with better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to come to blows bacterial and fungal infections.

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