ANTI-MICROBIAL EVALUATION OF 4-OXY/THIO SUBSTITUTED-1*H*-PYRAZOL-5(4*H*)-ONES

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Summary

A series of 4-Oxy/thio substituted-1*H*-pyrazol-5(4*H*)-ones **1-15** were subjected into antimicrobial evaluation and the compounds **1-7**.found to have good inhibition against bacterial and fungal strains tested.

Keywords*:* 4-Oxy/thio substituted-1*H*-pyrazol-5(4*H*)-ones; anti bacterial activity; fungicidal activity.

Introduction

Among the family of heterocycles, nitrogen containing heterocycles especially pyrazoles is an important class of heterocycles and its derivatives are reported to have the broad spectrum of biological activities such as antiinflammatory [1-2], herbicidal [3] and antiviral [4-5]. Pyrazole derivatives are extensively studied and used as antimicrobial agents [6-11]. Pyrazolones have been used extensively in pharmaceutical industry due to their numerous applications as analgesic, antipyretic, antiarthritic, uricosuric, antiinflammatory and antiphlogistic properties [10-15]. Some of the aryloxy pyrazolone derivatives are useful in the treatment of a variety of disorders caused by Human Immunodeficiency Virus (HIV) and other genetic ailments caused by retroviruses such as Acquired Immune Deficiency Syndrome (AIDS). As part of our on-going research aiming the synthesis of new antimicrobial compounds, new derivatives of pyrazol-5(4*H*)-ones have been synthesised and reported in literature by us with modified methodology [16-18]. In continuation of our earlier work here in we report the antimicrobial evaluation of some of these 4-Oxy/thio substituted-1*H*-pyrazol-5(4*H*)-ones.

Materials and methods

Chemistry

The 4-Oxy/thio substituted-1*H*-pyrazol-5(4*H*)-ones **1-15** were synthesised using the method available in literature [18].

Anti bacterial activity

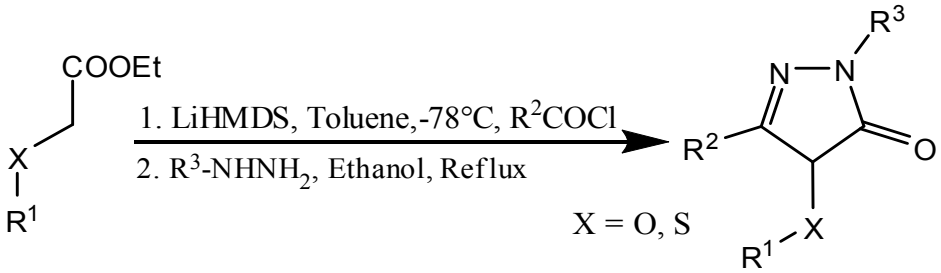
We have investigated newly synthesized pyrazolones for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATTC-27853) and *Klebsiella pneumonia* (recultured) bacterial strains by the disc diffusion method [19-20]. The discs measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 discs were dispensed to each screw-capped bottle and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using DMF. One mL containing 100 times the amount of chemical in each disc was added to each bottle, which contains 100 discs. The discs of each concentration were placed in triplicate in a nutrient agar medium separately seeded with fresh bacteria. The incubation was carried out at 37 °C for 24 h. Solvent and growth controls were kept, the zones of inhibition and minimum inhibitory concentrations (MIC) noted and compared with the standard ciprofloxin.

Antifungal activity

Newly synthesized pyrazolones were screened for their antifungal activity against *Aspergillus lavus* (NCIM No. 524), *Aspergillus fumigates* (NCIM No. 902), *Candida albicans* (NCIM No. 3100), *Penicillium marneffei* (Recultured) and *Trichophyton mentagrophytes* (Recultured) in DMSO by the serial plate dilution method [21-23]. Sabourands agar media was prepared by dissolving peptone (1g), D-glucose (4g) and agar (2g) in distilled water (100 mL) and the pH was adjusted to 5.7. Normal saline was used to make a suspension of spores of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL of saline to get a suspension of corresponding species. Agar media of 20 mL was poured into each Petri dish. An excess of suspension was decanted and the plates were dried by placing them in an incubator at 37 °C for 1h. Using an agar, punch wells were made on these seeded agar plates, and 10-50 µg/mL of the test compounds in DMSO were added into each labelled well. A control was also prepared for plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37 °C for 3-4 days. Antifungal activity was determined by measuring the inhibition zone and compared with the standard Ciclopiroxolamine.

Results and Discussion

A series of 4-oxy/thiosubstituted-1*H*-pyrazol-5(4*H*)-ones **1-15** were synthesised (Scheme 1), using the procedure already reported by us in literature [16,18] and then subjected to the anti-microbial evaluation. The results of antibacterial and anti-fungal activities were given Table-2 and Table-3 respectively.



Scheme 1 Synthesis of pyrazolones by cross-Claisen condensation

Table-1 Various 4-oxy/thiosubstituted-1*H*-pyrazol-5(4*H*)-ones prepared.

Product	X	R^1	R^2	R^3
1	O	-Ph	$-CH_3$	-H
2	O	$-CH_3$	-4Cl-Ph	-H
3	O	$-CH_2$-3Br-Ph	$-CH_2CH_3$	-H
4	O	$-4OCH_3$-Ph	$-CH_2CH_3$	-H
5	O	-4CN-Ph	$-CH_3$	-H
6	S	-Ph	$-(CH_2)_4CH_3$	-H
7	S	$-CH_2$Ph	-Cyclopropyl	-H
8	S	-4Cl-Ph	-Ph	-H
9	S	-Ph	-2Cl-5-pyridyl	-H
10	O	-Ph	$-CH_2CH_3$	$-CH_3$
11	O	-Ph	-Isobutyl	-4F-Ph
12	S	-Ph	$-CH_3$	-4F-Ph
13	O	-Ph	$-CH_2CH_3$	$-CH_2CF_3$
14	O	-Ph	$-CH_3$	-4F-Ph
15	S	-Ph	$-(CH_2)_4CH_3$	-2-pyridyl

Antibacterial activity

The antibacterial activity results shown in Table-2 reveal that most of them showed the good antibacterial activity. Among the compounds, **1-7,** were showed good inhibition towards all the four bacteria tested. Compound **9** was active against *S. Aureus, E. Coli* and *Klebsiella.* Compound **15** was active against *Klebsiella* but either moderately or very less active against all the rest of the bacterial strains tested. Compound **10** is not active against any of the tested bacterial strains. The structure activity relationship studies revealed that the compounds with aliphatic substituent at C-3 and free NH at N-1 (**1-7**) are very much active. The compounds with aryl substituent at C-3 and nitrogen substituted pyrazolones comparatively less active than **1-7**. The compound 15 with 2-pyridyl substituent at N-1 also exhibited very good activity against all the bacterial strains tested. Since almost all these compounds are effective against these organisms, there is a need for further studies.

Table-2 Antibacterial activities of the newly synthesized compounds (Zone of inhibition in mm, MIC in μg/mL given in parenthesis)

Compound No.	*S. aureus*	*E. coli*	*P. aeruginosa*	*Klebsiella*
1	22(6.25)	23(6.25)	22(6.25)	21(6.25)
2	19(6.25)	16(6.25)	14(6.25)	14(6.25)
3	17(6.25)	16(6.25)	19(6.25)	21(6.25)
4	20(6.25)	15(6.25)	18(6.25)	22(6.25)
5	19(6.25)	18(6.25)	16(6.25)	16(6.25)
6	20(6.25)	21(6.25)	<10(50)	<10(50)
7	18(6.25)	21(6.25)	22(6.25)	18(6.25)
8	<10(50)	<10(50)	<10(50)	<10(50)
9	24(12.5)	22(12.5)	<10(50)	25(12.5)
11	<10(50)	<10(50)	<10(50)	<10(50)
12	<10(50)	<10(50)	<10(50)	<10(50)
13	<10(50)	<10(50)	<10(50)	<10(50)
14	<10(50)	<10(50)	<10(50)	<10(50)
15	16(6.25)	14(25)	18(6.25)	20(6.25)
Ciprofloxacin	23(6.25)	32(6.25)	28(6.25)	24(6.25)

Antifungal activity

Table-3 Antifungal activities of the newly synthesized compounds (Zone of inhibition in mm, MIC in μg/mL given in parenthesis)

Compound No.	*Trichophyton*	*Penicillium*	*A. flavus*	*A. fumigates*
1	20(6.25)	21(6.25)	18(6.25)	19(6.25)
2	19(6.25)	17(6.25)	17(6.25)	14(6.25)
3	16(6.25)	18(6.25)	17(6.25)	20(6.25)
4	21(6.25)	16(6.25)	15(6.25)	22(6.25)
5	19(6.25)	18(6.25)	16(6.25)	16(6.25)
6	20(6.25)	21(6.25)	18(6.25)	19(6.25)
7	17(6.25)	22(6.25)	18(6.25)	17(6.25)
8	<10(50)	<10(50)	<10(50)	<10(50)
9	22(12.5)	20(12.5)	<10(50)	23(12.5)
10	<10(50)	<10(50)	<10(50)	<10(50)
11	<10(50)	<10(50)	<10(50)	<10(50)
12	<10(50)	<10(50)	<10(50)	<10(50)
13	<10(50)	<10(50)	<10(50)	<10(50)
14	<10(50)	<10(50)	<10(50)	<10(50)
15	26(6.25)	15(6.25)	18(6.25)	25(6.25)
Ciclopiroxolamine	27(3.125)	23(6.25)	27(3.125)	26(6.25)

The results of antifungal activity were given in Table-3 and compared with the standard Ciclopiroxolamine. Most of the synthesized pyrazolones have been found to show good activity against all the fungi tested. Particularly compounds **1-7, 15** were active against all the above fungi tested. Compound **9** has exhibited the moderate inhibition. Compounds **8, 10-14** have shown poor activity against all the fungi. The structure activity relationship studies based on the antifungal screening results, revealed that aliphatic substituent at C-3 with free NH at N-1 exhibited effective inhibition against these fungi. Free NH, or pyridyl substitution at N-1 increases the activity of the compounds but phenyl, or substituted phenyl at N-1 decreases the activity.

All the newly synthesized compounds were tested for their biological activities and most of the compounds showed either good or moderate activity against all the bacteria and fungi tested. Compounds **1-7** and **15** were showed the maximum activity against all the bacteria and fungi tested.

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