Table-2 shows cell divisions in the control were normal. Chromosome clumping at metaphase or late anaphase might have given rise to the pycnotic nuclei. Mitotic arrest was also observed in anaphase and early telophase. The appearance of pycnotic nuclei is suggestive of antimitotic arrest under the influence of antimitotic compounds after chromosomes had undergone prophasic condensation. The mechanism for antimitotic action of lupeol includes the possible inhibition of DNA replication prior to karyokinesis, depolymerization of DNA and nucleoproteins resulting in denatured chromosomes and the disassembly of microtubules resulting in spindle breakdown (8). It is interesting to note that cytological abnormalities such as pycnosis of nuclei, nuclear lesions, chromosome clumpings, tropokinesis, sticky bridges and stickiness have also been observed in onion root tip cells treated with adriamycin.

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Synthesis and Biological Activities of 1-[(3,4-dihydro-3-oxo-2h- 1,4-benzoxazin-2-yl) Acetyl]-3,4-disubstituted Pyrazoles and 3- Methyl-pyrazoliones

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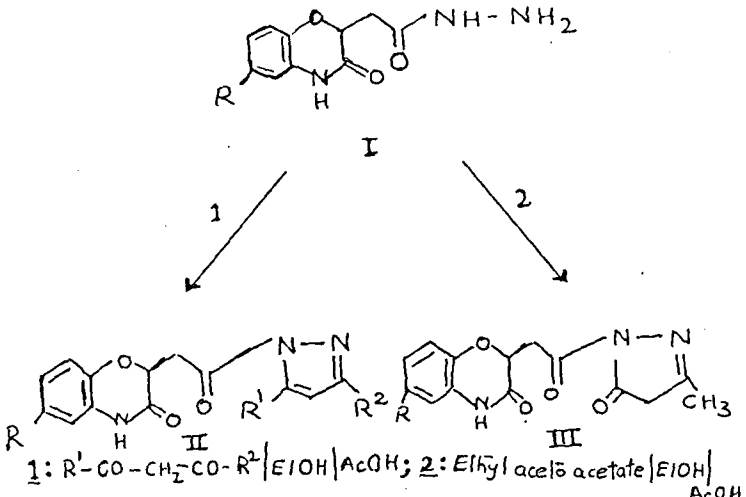
Some new 1-[(3,4-dihydro-3-oxo-2H-1, 4-benzoxazin-2yl) acetyl]-3, 4-disubstituted pyrazoles (II) and 1-[(3,4-dihydro - 3 - oxo - 2H - 1, 4 - benzoxazin - 2yl) - 3 - methyl pyrazolin - 5 - ones (III) have been synthesized and tested for their (II) antimicrobial activity. Some of the II exhibit a significant antimicrobial activity, compound IIa has been found to possess antihistaminic activity.

IN continuation of our work on benzoxazinone derivatives[1,2] and having synthesized the oxobenzoxazinyl-acetic acid hydriazides, it has been considered worthwhile to employ them as "synthons" for obtaining new oxo-benzoxazinyl pyrazolines and pyrazolones. In view of the known pharmacological and biological importance of the pyrazoline and chromone moieties it will be interesting to evaluate these new compounds for their possible biological properties.



The key intermediates for the synthesis of title compounds **Scheme-1**, (3,4-dihydro-3-oxo-2H-1, 4-benzoxazin-2-yl) acetic acid hydrazides (I) have been prepared from corresponding methyl esters[3].

Condensation of (3,4-Dihydro-3-oxo-2H-1,4-benzoxazin-2-yl) acetic acid hydrazides with 1,3-Bicarbonyl compounds:

Each of the oxo-benzoxazinyl acetic acid hydrazides (I) has been subjected to a condensation reaction with five different 1,3- bicarbonyl compounds in boiling alcohol containing a few drops of acetic acid to get crystalline solids. Relatively in good yields and high purity. The products have been purified by recrystallization and characterized as their respective 1-[(3, 4-dihydro-3- oxo-2H-1, 4-benzoxazin-2-yl) acetyl] - 3, 4-di- substituted pyrazoles (II) by their analytical and spectral properties.

For instance, an equimolar (0.01 mol) mixture (3,4-dihydro 3-oxo- 2H-1, 4-benzoxazin-2-yl) acetic acid hydrazide (I, R = H) and acetyl acetone in alcohol containing a few drops of acetic acid was refluxed on a steam-bath. Though a product started separating after 2 hrs, it was refluxed further for 1 hr. After removal of alcohol under reduced pressure, the residue was washed with benzene and purified by recrystallization from benzenechloroform (1:2) mixture. Its IR (KBr) spectrum showed absorption frequencies (in cm^{-1}) at: 3240 (-CO-NH), 1700 (cyclic C=O), 1680 (C=O), 1620 (C=N of pyrazole) and at 1230, 1120 (C-O-C. of benzoxazinone). PMR sepctrum (in $CDCL_3$, δ , ppm): 2.25 s, 3H, C_5-CH_3 of pyrazole), 2.55 (s, 3H, C_3-CH_3 of pyrazole, 3.8 (d, 2H, CH_2-Co), 5.2 (t, 1H, C_2-H of benzoxazinone), 6.0 (s, 1H, C_4-H of pyrazole), 6.8 - 7.3 (m, 4H, aromatic), and 9.8 (s, 1H, cyclic N-H, D_2O exchangeable). Mass spectrum of the compound recorded its molecular ion (M^+) at m/z 285 (35%) and showed characteristic fragmentation.

The remaining compounds (IIb - IIi) were prepared in a similar manner and the results are recorded in **Table 1.**

Condensation of (3,4-Dihydro-3-oxo-2H-1, 4-benzoxazin-2-yl) acetic acid hydrazides (I) with Ethyl acetoacetate:

Each of the two acid hydrazides (I) has been condensed with ethyl acetoacetate in alcohol by heating under reflux, in the presence of a catalytic amount of acetic acid. The products obtained have been pruified by recrystallization and characterized as their corresponding 1-[(3,4-dihydro-3-oxo-2H-1, 4 - benzoxazine-2yl) acetyl]-3-methyl-pyrazolin-5-ones (III) on the basis of their physical, analytical and spectral data.

For instace, (3,4-dihydro-3-oxo-2H-1, 4 - benzoxazin-2-yl)- acetic acid hydrazide (I, R=H) has been condensed with ethyl acetoacetate in equimolar ratio, by heating in alcohol (95%) containing a few drops of acetic acid for 4 hrs. The solvent was distilled-off under reduced pressure and the residue was washed with small portions of benzene to get a product. It was purified by recrystallization from dioxane to get a colourless crystalline solid, yield 78%, m.p. 265°. elemental analysis: Found: C, 58.46; H, 4.38; N, 14.60; $C_{14}H_{13}N_3O_4$ requires C, 58.53; H, 4.53, N, 14.63%. IR (Nujol, cm^{-1}): 3230 (N-H of benzoxazinone),1730 (C=O of pyrazolone), 1680 (C=O of benzoxazinone and CO-NH), 1610 (C=N of pyrazolone) and 1115, 1220 (C-O-C of benzoxazinone). PMR $CDCl_3$, $ ppm: 2.45 (s, 3H, C_3-CH_3 of pyrazolone), 3.5 (d, 2H, - CH_2CO-), 4.05 (s, 2H, CH_2-CO of pyrazolone), 4.8 (t, 1H, C_2- H of

Table 1. Physical and analytical Data of [(3, 4- Dihydro-3-oxo-2H-1, 4-benzoxazin-2-yl) acetyl]-3, 5-disubstituted-pyrazoles (III)

Compound No.	R	R^1	R^2	m.p. (°C)	Yield (%)	Mol.formula	Elemental annalysis % Found (Calod.)		
							C	H	N
IIIa	H	methyl	methyl	165	73	$C_{15}H_{15}N_3O_3$	63.22 (63.15)	5.29 (5.26)	14.81 (14.73)
IIIb	H	Phenyl	Phenyl	270	78	$C_{25}H_{19}N_3O_3$	73.32 (73.35)	4.63 (4.64)	10.23 (10.27)
IIIc	H	4-methyl-phenyl	Phenyl	260	82	$C_{26}H_{21}N_3O_3$	73.82 (73.76)	4.89 (4.96)	9.98 (9.93)
IIId	H	3-nitro-Phenyl	phenyl	120	84	$C_{25}H_{18}N_4O_5$	66.12 (66.08)	3.93 (3.96)	12.36 (12.33)
IIIe	H	4-chloro phenyl	phenyl	285	68	$C_{25}H_{18}ClN_3O_3$	67.59 (67.64)	4.02 (4.06)	9.49 (9.47)
IIIf	Cl	methyl	methyl	205	86	$C_{25}H_{18}ClN_3O_3$	56.28 (56.34)	4.26 (4.38)	13.01 (13.14)
IIIg	Cl	phenly	phenyl	300	79	$C_{25}H_{18}ClN_3O_3$	67.72 (67.64)	4.20 (4.05)	9.51 (9.47)
IIIh	Cl	4-methyl-phenyl	phenyl	124	80	$C_{26}H_{20}ClN_3O_3$	68.21 (68.19)	4.34 (4.37)	9.23 (9.18)
IIIi	Cl	4-chloro-phenyl	phenyl	300	74	$C_{25}H_{17}Cl_2N_3O_3$	62.72 (62.76)	3.48 (3.55)	8.75 (8.78)

Compound IIIa was recrystallized from benzene: chloroform (1:2) mixture
Compounds IIIb, IIId & IIIe from chloroform and IIIc, IIIf, IIIg, IIIh & IIIi from alcohol.

benzoxazinone), 6.6 - 7.2 (m, 4H, aromatic) and 10.2 (s, 1H, cyclic NH: D_2O exchangeable). Thus the compound could be characterized as 1-[(3,4-dihydro-3-oxo-2H-1, 4-benzoxazin 2-yl) acetyl]-3-methyl pyrazol-5-one (III, R=H).

Its mass spectrum has recorded the heaviest fragment at m/z 287 corresponding to its molecular ion and also characteristic fragmentation.

The other compound 1-[(6-chloro-3, 4-dihydro-3-oxo-2H-1, 4- benzoxazin-2-yl) acetyl]-3-methyl pyrazol-5-one (III, R=Cl) has also been prepared in a similar manner and purified by recrystallization from dioxane to get a white crystalline solid, yield 72%, m.p. 290°. Elemental analysis: Found : C, 52.30; H, 3.76, N, 13.12; $C_{14}H_{12}ClN_3O_4$ requires C, 52.25; H, 3.73; N, 13.06%.

Biological Screening: The new pyrozoles (II) were screened for their antibacterial and antifungal activities by standard methods. It is for the first time that an oxo-benzoxazinyl-pyrazole (IIa) was

evaluated as a model for its possible antihistaminic activity since such activity was reported for some pyrazolones from this laboratory[4].

The antibacterial and antifungal activity of the test compounds (II) was assayed at a concentration of 100 μ against two Gram-positive bacterial, two Gram-negative bacteria and four fungi employing the standard method[5].

It is interesting to note that the Gram(-)ve bacteria, E. Coli and P. Vulgaris were relatively more sensitive to the test compounds compared to the antibiotic streptomycin used as reference. Against the Gram (+)ve bacteria B. Subtilis, only compound IIi with a chloro-substituent in the benzo-moiety and 4- chlorophenyl and phenyl substituents at 3- and 5- positions, respectively of the pyrazole system was found to be equipotent to that of the standard benzylpencillin. It could also be seen that F. oxysporum, was relatively more sensitive to the test compounds than A. niger. compound IIe with a 4-chlorophenyl and phenyl groups as 3,5- substitutents, was found to be superior in its antifungal potency, while compounds IIb and IIi being next in the order.

Antihistaminic activity was determined by the isolated guinea-pig ileum method[6]. From a plot of dose and percent inhibition of histamine-induced contraction of the guinea-pig ileum, the ED_{50} values were calculated. The number of replications were four. The test results revealed that the representative compound IIa was a potent antihistamine as it caused 50% inhibition at a dose (ED_{50}) of 0.286 ± 0.6 mg.

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