International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.4, No.2, pp 553-557, April-June 2012

Synthesis and Characterization of new Chromano N-Phenyl Pyrazoles and their Bioassay against Vibriospecies

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Abstract: A series of five new chromano pyrazoles were synthesized by condensation of poly substituted chalcones with phenyl hydrazine hydrochloride in presence of pyridine and ethanol. The compounds were characterized by various spectroscopic techniques. The title compounds were tested at different concentrations for their bioassay against various Vibrio species *viz) Vibrio alginolyticus, Vibrio fluvialis, Vibrio parahaemolyaticus.*
Keywords: Chromano pyrazoles, Synthesis, Characterization, and Vibrio bioassay.

INTRODUCTION:

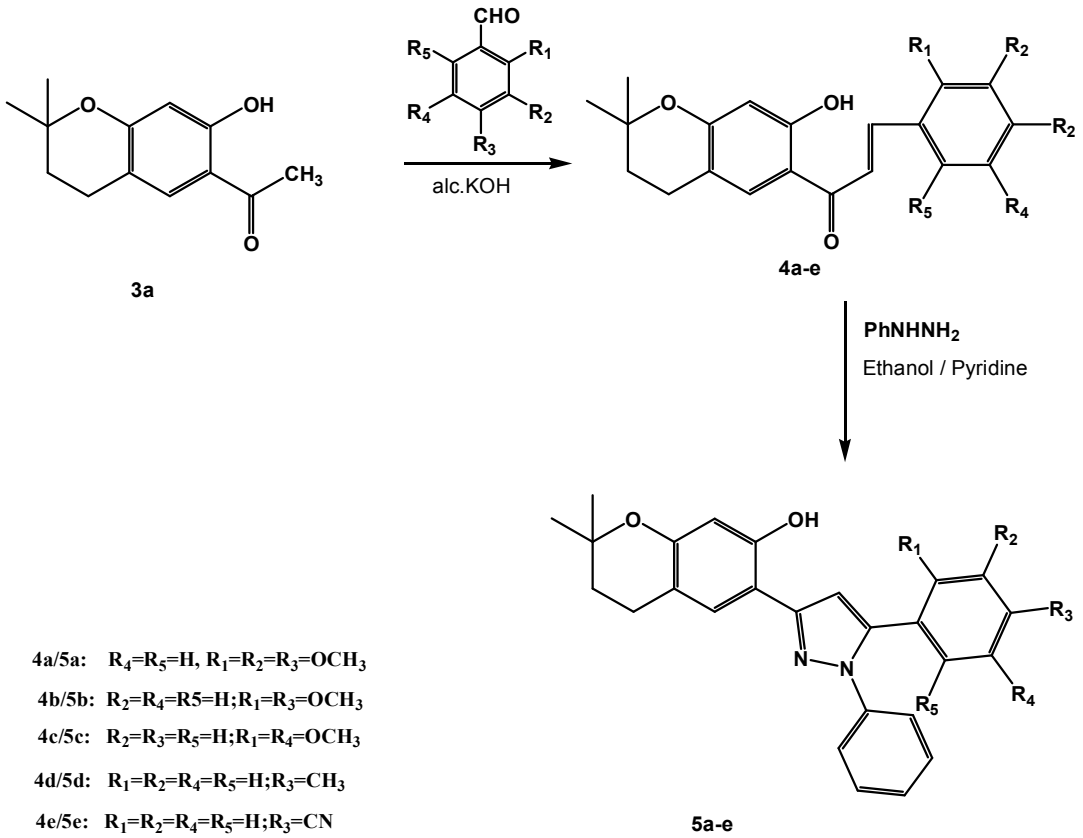
The pyrazole ring is the basic moiety for a number of dyes and drugs. Pyrazole containing compounds have many practical applications in the field of medicine [1] and their biological activities [2] are well documented. A literature survey revealed that substituted pyrazoles are potent and selective inhibitors as antilepedimic [3], anti-inflammatory [4], anti hypertensive and analgesic. And also pyrazoles have vital role in the field of agriculture as insecticides and herbicides [5].
In continuation of previous work [6-7], we focused on the development of synthesis of poly functional substituted heterocyclic compounds as bioactive molecules. The synthesis of 1-phenyl 1-aryl– 6-(2", 2" - dimethyl-7" hydroxy chromano) pyrazoles [5a-e] were achieved and reported here in scheme-1. All the synthesized compounds were evaluated for their antibacterial activity. The biological activities of the synthesized compounds were compared with the standard drug Tetracycline.

METERIALS AND METHOD

Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded using KBr on a Shimadzu IR Affinity spectrophotometer. ^{1}HNMR was recorded on Jeol-FT-NMR-90MHz spectrophotometer in $CDCl_3$ using TMS as internal reference. All the solvents were of analytical grade and were distilled before use. The HPLC was recorded using Shimadzu LC 6A with Shimpack silica gel column. Mass spectra were recorded on a Varian Atlas CH-7 mass spectrometer.

Synthesis of resacetophenone (2) [7]

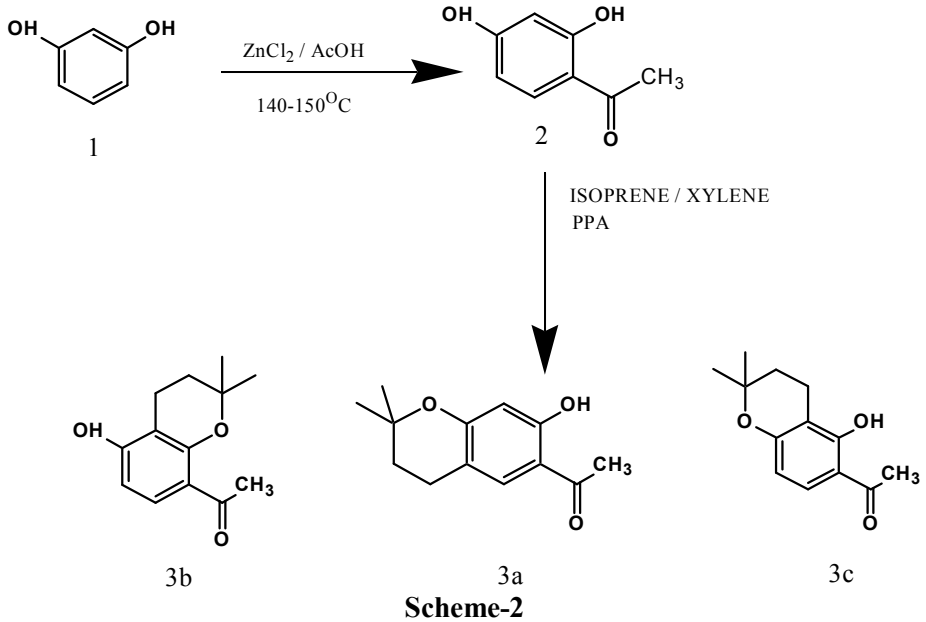
Resorcinol (1) (0.02 moles) was treated with freshly fused $ZnCl_2$ (0.026 moles) dissolved in glacial acetic acid (30 ml) while heating. It was refluxed to 140 – 150 ˚C for about 6-8 hrs. The reaction mixture was treated with 1:1 HCl then freezes to 0˚C. Red crystals were filtered and dried. Recrystallised resacetophenone m.p. 145˚C was noted.



Scheme-1

Synthesis of chroman (3a)

Resacetophenone (0.007moles) was prenylated by adding isoprene (0.015moles) in 5 ml xylene slowly. Freshly prepared 2 ml poly phosphoric acid was added while stirring at room temp. After completion, the reaction mixture was treated with chloroform and washed with 5% $NaHCO_3$.The chloroform layer was separated and removed under reduced pressure. The gummy residue was subjected to isolate individual components by column chromatography. The three isomers were identified as 3a, 3b, 3c with the spectral analysis.



Scheme-2

Synthesis of chromano chalcones(4a-e):

Chroman (3a) (0.01 mol) was condensed with various substituted benzaldehydes (0.01 mol) in the presence of ethanol and aq. KOH and stirred for 8 hours at room temperature. The reaction mixture was acidified with 1:1 HC1 and extracted with ethyl acetate. The crude product thus obtained was purified over column chromatography and purity of the chalcones (4a-e) was checked by HPLC and it was above 99%.

***7-hydroxy-6-(2',3',4'-trimethoxy)cinnamoyl,3,4-dihydro-2,2-dimethyl-2H benz (1,2b) pyran (4a)*:** IR (KBr, cm^{-1}) : 2950, 2850, 1640, 1600, 1460, 1290, 1170, 1020, ^{1}H NMR : δ 1.48 (s, 6H),1.96 (t, 2H), 2.55 (s, 3H) ,6.3 (s, ArH),7.4 (s, Ar-H), 13.1 (s, OH)MS: .m/z 398(M^+)

***7-hydroxy-6- (2',4'-dimethoxy) cinnamoyl,3,4 - dihydro-2,2-dimethyl-2H-benz (1,2b) pyran (4b)*:**IR (KBr,cm^{-1}) : 2800, 1640, 1600, 1580, ^{1}HNMR : δ 1.48 (s, 6H),1.96 (t, 2H), 2.55 (s, 3H),6.2-6.3 (m, Ar-H), 7.38 (dd, CH), 7.4 (s, Ar-H), 8.17 (dd, CH), 13.1(s-OH)MS: .m/z 348(M^+)

***7-hydroxy-6- (2',5'-dimethoxy) cinnamoyl,3,4-dihydro-2,2-dimethyl-2H-benz (1,2b) pyran (4c)*:** IR (KBr, cm^{-1}) : 3430, 2950, 1640, 1600, 1460, 1400, 1290, 1260, 1020, ^{1}HNMR : δ 1.48 (s, 6H),1.86 (t, 2H), 2.55 (s, 3H), 6.4-6.3 (m, Ar-H), 7.27 (dd,CH), 7.4 (s, Ar-H), 7.80 (dd, CH), 13.1(s, OH). MS: m/z 348 (M^+)

***7-hydroxy-6-(4'-methyl)cinnamoyl,3,4-dihydro-2,2-dimethyl-2H-benz(1,2b) Pyran (4d)*:** IR (KBr, cm^{-1}) : 3056, 2930, 1650, 1600, 1105, ^{1}HNMR : δ 1.4 (s, 6H), 2.5(t , 2H), 1.8 (t, 2H), 2.35 (s, Ar-CH_3), 6.38 (s, Ar-H), 7.01-7.39(m, Ar-H),12.2 (s, OH) MS: m/z 322(M^+).

***7-hydroxy-6-(4'-cyano)cinnamoyl,3,4-dihydro-2,2-dimethyl-2H-benz(1,2b)pyran (4e)*:** IR (KBr, cm^{-1}) : 2229, 1651, 1610, 1118, ^{1}HNMR : δ 1.48 (s, 6H), 1.96 (t, 2H), 2.55 (s, 3H), 6.2-6.3 (m, Ar-H), 7.38 (dd, CH), 7.4 (s, Ar-H), 8.17(dd, CH), 13.1(s, OH). MS: m/z 344(M^+).

Synthesis of chromano N-phenyl pyrazoles(5a-e):

The chalcones (4a-e) thus obtained were condensed with phenyl hydrazine hydrochloride in ethanol and catalytic amount of pyridine. After usual work up and purification by column chromatography, the title compounds (5a-e) were furnished. They were further recrystallized from methanol to obtain bright brownish colored products. The obtained products purity was checked by HPLC, it was above 95%. Their physical characteristics were evaluated and presented in Table – 1.

***1-phenyl – 2 - (2', 3', 4'-trimethoxy phenyl)-6-(2",2"-dimethyl,7"-hydroxy chromano) pyrazole (5a)*:** IR (KBr, cm^{-1}) : 3122, 2398, 1540, 1260, 1160, 1020, ^{1}H NMR : δ 1.42 (s, 6H), 1.76 (t, 3H), 2.7 (t, 3H), 3.95 (s, 3xOCH_3), 7.0 (s, 4H), 6.8-7.7 (m, Ar-H), 7.0 (s, pyrazole 1H), MS : m/z 486 ($M^{+)}$

***1-phenyl –2-(2',4'-dimethoxy phenyl)-6-(2",2"-dimethyl,7"-hydroxy chromano) pyrazole (5b)*:** IR (KBr, cm^{-1}) : 3012, 2980, 1614, 1585, 1020, ^{1}HNMR: δ 1.35 (s, 6H), 1.7 (t, 2H), 2.7 (t, 3H), 3.9 (s, OCH_3), 4.1 (s, OCH_3), 6.6.-7.0 (m, Ar-H), 7.2 (s, 4H), 13.2 (s, OH), MS : m/z 456 (M^+)

***1-phenyl– 2-(2',5'-dimethoxy phenyl)-6-(2",2"-dimethyl,7"-hydroxy chromano) pyrazole (5c)*:** IR (KBr, cm^{-1}) : 3356 , 2972, 1548, 1502, ^{1}HNMR : δ 1.35 (s, 6H), 1.7 (t, 2H), 2.7 (t, 2H), 3.8 (d, 2xOCH_3), 6.0-7.2(m, Ar-H), 7.2 (s, 4H), 12.28 (s, OH), MS : m/z 456 (M^+)

***1-pheny –2 -(4'-methyl phenyl)-6-(2",2"-dimethyl,7"-hydroxy chromano) pyrazole (5d)*:** IR(KBr, cm^{-1}) : 3010 , 2943, 2852, 1540,^{1}HNMR : δ 1.33(s,6 CH_3),1.70 (t, 2H), 2.3(s, Ar-CH_3), 2.5 (t, 2H), 7.0 (s, 4H), 7.24 (m, Ar-H), 11.5 (s, OH), MS : m/z 410 (M^+)

***1-phenyl –2 -(4'-cyano phenyl)-6-(2",2"-dimethyl,7"-hydroxy chromano) pyrazole (*5e)*:** IR (KBr, cm^{-1}) : 3032, 2274, 1534, 1612, 1021,^{1}HNMR : δ 1. 33 (s, 6H), 1.86 (t, 2H), 2.64 (t, 2H) , 6.29 (s, Ar-H), 7.3 (m – ArH), 7.4 (s, 4H), , 12.2(s, OH), MS : m/z 421(M^+).

Table-1: Physical Characteristics of chromano pyrazoles (5a-e)

Compound No.	Molecular Formula	M .P. (˚C)	Colour	Rf Value	Elemental analysis % Observed (Calcd)		
					C	H	N
5a	$C_{29}H_{28}N_2O_5$	195	Bright orange	6.0	71.6 (71.65)	5.76 (5.75)	5.76 (5.70)
5b	$C_{28}H_{28}N_2O_4$	164	Brown	5.1	73.68 (93.60)	6.14 (6.10)	6.14 (6.10)
5c	$C_{28}H_{28}N_2O_4$	190	Brown	5.0	73.68 (73.60)	6.14 (6.10)	6.14 (6.10)
5d	$C_{27}H_{26}N_2O_2$	130	yellowish Orange	8.0	81.95 (81.90)	6.82 (6.88)	6.82 (6.88)
5e	$C_{27}H_{23}N_3O_2$	198	Dark brown	3.0	76.95 (77.00)	6.17 (6.15)	6.65 (6.60)

Table – 2: Antimicrobial zone of inhibition in mm.

Compounds ..	*Vibrio alginolyticus* in (mm)Conc µg/ml				*Vibrio fluvialis* in (mm) Conc. µg/ml				*Vibrio parahaemolyticus* in (mm) Conc.µg/ml			
	40	50	100	200	40	50	100	200	40	50	100	200
4a	11	11	11	12	-	11	12	12	-	-	-	12
4b	11	11	11	12	-	11	11	12	-	-	-	11
4c	10	11	11	12	-	11	11	12	-	-	-	11
4d	-	-	-	11	-	-	-	-	-	-	-	-
4e	10	11	12	12	-	12	12	14	-	11	12	14
5a	15	16	21	25	14	16	20	22	15	15	18	22
5b	14	16	22	26	15	15	20	20	15	18	18	22
5c	14	16	22	26	15	15	20	20	15	18	18	22
5d	10	14	16	20	13	18	18	18	15	15	18	18
5e	15	18	20	28	17	19	20	25	12	15	18	20
Tetra Cyclin	20	22	28	30	20	25	25	30	20	22	25	28

Results and discussion

N-phenyl pyrazoles were synthesized by treating chalcones with five different aryl aldehydes. All the chalcones were yellow solids and showed strong IR band at 1650-1690 representing unsaturated carbonyl group. The condensation product was achieved within six hours of stirring. While chalcones were refluxed with phenyl hydrazine in methanol it consumed 10-15hours. In the Ipyrazole IR bandat 1650-1690 was absent in the pyrazole molecules, since α-β unsaturated carbonyl is cyclized to pyrazole ring. The ring proton was predominant around 7.02 in ^{1}HNMR. Further C=N, C-N strechings at 1540, 1090 cm^{-1} in IR confirms N phenyl pyrazoles .The mass spectra for the pyrazoles showed corresponding molecular ion peaks.

The poly functionally substituted pyrazole showed comparatively low yields due to heavy substitution yet showed strong activity when they were tested against different vibrio species. Among the five pyrazoles, cyano substituted pyrazole **5e** showed typical cyanalmin activity .The presence of methoxy group on **5a, 5b, 5c** have showed more activity[8] when compared to methyl substituted pyrazole **5d.** The present data confirms N-phenyl pyrazoles do have vital role in controlling the pathogenic bacteria reported first time.

Bioassay of N-phenyl pyrazoles

The title compounds were tested for their antibacterial activity against vibrio species at different concentrations. Shrimp cultivation is the important aqua culture activities in Asia and South America but it has faced severe problems, virus and bacterial infections particularly luminous vibriosis was a major problem for shrimp cultivation. The causative agents for vibriosis were due to *Vibrio harvevi, Vibrio parahaemolyaticus* and others. *Vibrio harvevi* was a serious pathogen for a wide range of marine animals and other aquaculture [9-11] synthesis. Antibiotics such as tetracycline, chloramphenical, oxolinic acid have been commonly used to control shrimp diseases. The title compounds were now screened for their control levels were compared with the standard drug

tetracyclin. Each v*ibrio s*pecies after isolation was grown over TCBSA (Thio sulphate Citrate Bile salts Sucrose Agar medium). Since *vibrio* species grow over specific medium. The sub cultures *of Vibrio alignolyticus, Vibrio fluvailis, Vibrio parahaemolyticus were grown overnight at* 37°C. The cup plate method was employed for the studies. The subculture was inoculated in sterile medium which were placed on pre autoclaved Petri dishes. The Petri dishes were batch wise autoclaved while the medium was prepared. TCBSA medium 8.9 gm taken in 100 ml of water boiled on hot water bath at above 100°C for 45 min. Agar medium cooled to 40°C and carefully poured into the sterile Petri dishes. The depth of the medium should be optimum such that well size 8 mm, when the TCBSA medium set hard. Four wells of 8 mm size were made at equidistance with help of borer on the well set TCBSA medium. About 50 µl test solution of (4a-e) and (5a-e) in various concentrations were placed in these cups. Compounds were taken in DMSO. DMSO has practically no activity against these *vibrio* species. The plates were incubated at 37°C for 24 hrs. The activity was compared with standard drug tetracycline .The results were listed and tabulated in Table -2.

Acknowledgements:

We acknowledge with thanks to the following: Dr.B.Shankar Rao MAPPL LABS, Nellore and Mrs.T.P.Suhasini, Mr.B.S.Diwakar, Dept.of Organic Chemistry, Andhra University.

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