Oriental Journal of Chemistry Vol. **25**(2), 329-334 (2009)

Synthesis of 2-amino-4-(2^1-nitro)phenyl-6(2^{11}, 2^{11}-dimethyl, 7^{11}-hydroxy chroman) pyrimidine and study of their antimicrobial activity

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(Received: March 10, 2009; Accepted: April 13, 2009)

ABSTRACT

Resorcinol (1) was acylated using acetic acid in the presence of fused $ZnCl_2$ at 140-150^{0}C for 15 min with stirring. The reaction mixture is left for 1hr and then 100ml of 1:1 HCl was added to break the zinc chloride complex and within 5 minutes precipitation commenced. The precipitate was washed with very dilute HCl and water. A red precipitate was obtained; which was crystallized from 20% HCl to give resacetophenone (II) needles and characterized by comparing its spectral data (IR, NMR) with those reported in literture m.p. 145°C.

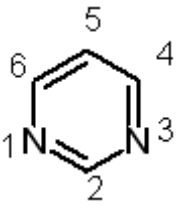
Ahluwalia et al[1] reported the synthesis of 2, 2-dimethyl chromans in very good yield by condensing polyphenol derivates with isoprene in presence of orthophosphoric acid as catalyst. In our attempts to modify the reaction conditions, PPA was found to be a better condensing agent compared to orthophosphoric acid to result in a more or less homogenous reaction mixture leading to the cyclic chroman derivatives in very good yields.[2] A solution of Isopene in xylene was added dropwise during 8 hours to a mixture of resacetophenone and PPA in xylene with constant stirring at 30-35°C. Stirring was continued for further 1 hour. The reaction mixture was taken into reduced pressure to give a pale yellow gummy material. This on column chromatography over silica gel yielded the chroman in hexane / ethyl acetate cluates (96:4) and the unreacted resactophenone from hexane / ethyl acetate eluates. The resacetophenone (II) on nuclear prenylation[2,3] with isoprene in the presence of PPA at room temperature resulted the formation of 2, 2-dimethyl-6-acetyl-7-hydroxy chroman (III) in 63% yield. (III) crystallized as colourless needles. The chroman (III) on condensation with substituted and unsubstituted benzaldehydes in the presence of 30% alcoholic alkali[4] at room temperature resulted the formation of chalcone derivatives in good yield (85%). The thin layer chromatography (TLC) of these chalcones showed characteristic colours with methanol – sulphuric acid (9:1) as a spraying reagent. They also exhibited the characteristic colour test with antimony trichloride[5]. With the above procedure, compound (IV) was synthesized.

Key words: Synthesis, pyrimidine derivatives antimicrobial activity.

INTRODUCTION

Chemistry of Pyrimidines

Pyrimidine can be regarded as a cyclic amine. Pyrimidine[1] can also known as m-diazone (or) 1,3-diazine. It is the parent substance of large group of heterocyclic compounds & plays a vital role in many biological process, as found in nucleic acids, several vitamins, co-enzymes and purines.



Scheme 1

Pyrimidine[1] is the most important member of all the diazines as this ring occurs widely in living organisms. Purines, Uric acid, alkoxan, barbuturic acid and a mixture of antimalarial and antibacterial also contain the Pyrimidine ring. Substances containing unfused Pyrimidine rings occur in free state as in Uracil , Thymine , Orotic acid , Cytosine, the glycosides, Vitamin B (thiamine), amicetin and various others.

The chemistry[6,7] of Pyrimidine has been widely studied. Pyrimidine was first isolated by Gabriel and Colman in 1899. Since Pyrimidine is symmetrical about the line passing C_2 and C_5, the positions of C_4 and C_6 are equivalent and so are N-1 and N-3. When a hydroxyl or amino group is present at the 2, 4 or 6 position than they are tautomeric with oxo and imino respectively.

Pyridine itself is not found in nature but substituted pyrimidines and compounds containing the Pyrimidine ring are widely distributed in nature [9,10] Derivatives of barbituric acid , widely used in medicines, for ex., veronal, pentothiol, luminol are used as hyphotics while is used as anaesthetic.

Three Pyrimidines are of considerable biological important because of their relation to the nucleic acids viz., uracil, thymine, cytosine. The purine ring system obtained from the fussion of pyrimidine and Imidazole nucleic also is important because certain of its derivatives, in particular adenine and guanidine which are building blocks of RNA & DNA.A variety of natural product such as alkaloids also contain pyrimidine ring system, these include Hypoxanthine, Xanthine which occur in tea and caffeine and Theeophylise are the constituents of tea leaves. Theobromine is found in cocoa beans.

EXPERIMENTAL

Step I : Preparation of Resacetophenone(II)

Freshly fused 33 g of $ZnCl_2$ was dissolved in 32 ml of acetic acid while heating and when all the $znCl_2$ is almost dissolved 22 g of resorcinol was added and heated to 140-150^{0}C for 15min^{-1} with stirring. After that this is left for 1 hr and then 100ml of 1:1 HCl was added to break the zinc chloride complex. Within 5 minutes precipitation commenced when the mixture came to room temperature. It is cooled to 5^{0}C and then filtered. The precipitate is washed with very dilute HCl and water. A red precipitate obtained was crystallized from 20% HCl to give resaetophenone needles.

m.p.	:	145^{0}C.
R_f (TLC)	:	0.4 (Hex: EtOAc, 8:2)
Molecular formula	:	$C_8H_8O_3$
Required (%)	:	C, 63.15; H, 5.263; O, 31.578
Found (%)	:	C, 63.1; H, 5.3; O, 31.6
IR (V_{max}^{Nujol})	:	3309 cm^{-1} (-OH str); 1591 cm^{-1} (C=O str.)
^{1}H NMR ($CDCl_3$/TMS)	:	δ 2.8 (s), 6.25 (s), 6.8 (d), 8 (d), 11.45 (s, 2-OH).

Step II : Nuclear prenylation of resacetophenone (III)

A solution of isoprene (1.5ml, 0.015 mol) in xylene (5 ml) was added dropwise during 8 hr to a mixture of resacetophenone (1.41g, 0.0072 mol) and PPA (2ml) in xylene (3ml) with constant stirring at 30-35^{0}C. Stirring was continued for further 14 hrs. The reaction mixture was taken in chloroform (100ml) and the chloroform solution was washed with $NaHCO_3$ (5%, 3x60 ml) water; dried over $MgSO_4$ and removed under reduced pressure to give gummy material. This on column chromatography over silica gel yielded the chroman on elution with hexane / EtOAc (96:4).

m.p.	:	92°C.
R_f	:	0.8 (Hex: EtOAc, 8.6:1.4)
Molecular formula	:	$C_{13}H_{16}O_3$
Required (%)	:	C, 70.90; H, 7.272; O, 21.818
Found (%)	:	C, 70.85 H, 7.3; O, 21.90
IR (V_{max}^{Nujol})	:	3350 cm^{-1} (OH str); 1680 cm^{-1} (C=O str.)
^{1}H NMR ($CDCl_3$/TMS)	:	δ 1.3 (s, gem-dimethyl); d 1.8 (t, 2H, J = 7 Hz), d 2.7 (t, 2H, J = 7 Hz); 5-H d 6.3 (s, 1H); d 7.5 (s, 1H); δ 2.5 (s, 3H); d 13.1 (s, 1H).

Step III: Synthesis of 7-hydroxy-6(2'-nitro) cinnamoyl, 3,4-dihydro 2,2-dimethyl-2H benzo (1,2b) Pyran(IV)

2.2-diemthy 1-6-acetyl-7-hydroxy chroman (III) on condensation with 2-nitro benzaldehyde in the presence of 30% alcoholic alkali at room temperature resulted the formatin of chalcone (IV) in good yield (85%); crystallized from hexane / EtoAc as yellow needles**.** molecular formula $C_{20}H_{19}O_4N$; m.p: 145^{0}C, and the purity of the compound (IV) was checked by HPLC.

Column	:	Shim-pack CLC-Sil
Mobile phase	:	CH_3CN
Flow rate	:	1 ml/min
Detector	:	UV (254nm)
Injected quantity	:	5ml
R_1(TLC)	:	0.53 (8.2)
Purity (%)	:	97.4%

Data of HPLC Chromatogram of 7-Hydroxy – 6-2 (2'Nitro) Cinnamoyl, 3,4-Dihydro 2,2-Dimethyl-2h Benzo (1,2b) Pyran (IV)

S. No.	Reten. Time (Min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05 (min)
1.	3.077	1.467	.267	0.6	0.8	0.11
2.	3.217	4.692	0.595	2.0	1.8	0.13
3.	3.977	224.750	31.688	97.3	97.4	0.11
	Total:	230.909	32.550	100.0	100.0	

M.P	:	145^{0}C
Rf(TLC)	:	0.53 (8.2)
Molecular formula	:	$C_{20}H_{19}O_4N$
^{1}H NMR ($CDCl_3$, TMS)	:	δ 1.35 & 1.4 (s-gem dimethyl) δ 1.80 (t2H, methylene protons) δ 2.8 (t2H, methylene protons) δ 7.25 (s, 5H δ 6.4 (s. 8H) δ 7.63, 8.05 (d, a-H, b-H) δ 7.45 (m), d 7.6 (m), (phenolic protons)
IR V_{max} cm^{-1}	:	1720 cm^{-1} (C=O) str), 1611cm^{-1} (a, b-unsaturated carbonyl carbon); 1157cm^{-1} (C-O str), 1455 $cm,^{-1}$, (C=C str) 3253 cm^{-1}, (O-H str).

Table 1: Physical characteristics of 7-hydroxy-6-(2'-nitro) cinnamoyl 1,3,4-dihydro 2,2-dimethyl-2H benzo (1, 2b) pyran (IV)

Compound No.	m.p.	Yield (°C)	R_f^0 (%)	Colour developed onTLC	Molecular formula
IV	145^{0}C	85%	0.53(8:2)	Pink	$C_{20}H_{19}O_4N$

a = Hexane: EtoAc

The physical and spectral characteristics of chalcone (iv) were given in tables 1 and ii

The chalcone (IV) which was synthesized have now been taken for the preparation of corresponding new Pyrimidine derivative. The condensation above chalcone with guanidine hydrochloride[11] in alkaline medium viz., in potassium tertiary butoxide in presence of t-butanol at reflux temperatures resulted the formation of corresponding Pyrimidine derivative.

Table 2: ^{1}HNMR data of 7-hydroxy-6-(2'-nitro) cinnamoyl 3,4-dihydro 2, 2-dimethy 1-2H benzo (1, 2b) pyran (IV)*

Proton No.	V
2a	1.35 (s, CH_3)
2b	1.4 (s, CH_3)
3	1.80 (t, J=9 Hz, CH_2)
4	2.8 (t, J=9 Hz, CH_2)
5	7.25 (s, 1H)
8	6.4 (s, 1H)
a-H & b-H	7.63 & 8.05 (d, 2H)
Aromatic Protons	7.45 (s, 5H)

*$CDCl_3$ / TMS

Step IV: Synthesis of 2-amino-4-(2^1-nitro) phenyl-6(2^{11}, 2^{11} -dimethyl, 7^{11} – hydroxyl chroman) pyrimidine (V).

The condensation of 7-hydroxy-6-(2,-nitro) cinnamoyl, 3,4-dihydro2,2-dimethyl-2H benzo (1-2b) pyran (IV) with guanidine hydrochloride in the presence of potassium t-butoxide in t-butoxide in t-butanol was refluxed on water bath for 4 hrs. the solvent was evaporated and the residue dissolved in water and neutralized with dil HCl, where upon a bright yellow solid separated out, which was filtered and crystallized from chloroform / methanol**.** The purity of the compound checked by HPLC.

Column	:	Shim-pack CLC-Sil
Mobile phase	:	CH_3CN
Flow rate	:	1 ml/min
Detector	:	UV (254nm)
Injected Quantity	:	5ml
M.P.	:	210^{0}C
R_f (TLC)	:	0.74 (6:4)
Purity	:	99.4%
IR V_{max} cm^{-1}	:	3346cm^{-1}, 3184cm^{-1} (N-Hstr), 1668cm^{-1}, 1614 cm^{-1}, 1456 cm^{-1}, (C=C str) 1378 cm^{-1}, 1167 cm^{-1}, (C-O str).

The ^{1}HNMR spectrum of compound (V) showed the characteristic C_5-H proton at d7.42. Gem – dimethyl group resonates at d1.3 and d1.4 as singlets and methylene protons 3^{11}-CH_2 and 4^{11}-CH_2 at d1.80 and d 2.78 besides other protons confirming its structure (V).

Table 3: Physical characteristics of 2-amino -4-(2^1-nitro)phenyl-6(2^{11}, 2^{11}-dimethyl, 7^{11}-hydroxy chroman) pyrimidine (V)

Compound No.	m.p. (°C)	Yield (%)	R_f^0	Molecular formula
IV	210^{0}C	65%	0.74	$C_{21}H_{20}O_4N_4$

a = Hexane: EtoAc

Table 4: ^{1}HNMR data of 2-amino-4-(2^1-nitro)phenyl-6(2^{11}, 2^{11}-dimethyl, 7^{11}-hydroxy chroman) pyrimidine (V)

Proton No.	V
2^{11}a	1.3 (s, CH_3)
2^{11}b	1.4 (s, CH_3)
3^{11}	1.80 (t, J=9 Hz, CH_2)
4^{11}	2.78 (t, J=9 Hz, CH_2)
5^{11}	7 (s, 1H)
8^{11}	6.4 (s, 1H)
5	7.42 (d, 2H)
Aromatic Protons	7.8 (s, 5H)

*$CDCl_3$ / TMS

Based upon the above spectral data the compound V was confirmed as 2-amino-4-(2^1-nitro) phyenyl 6(2^{11}, 2^{11}-dimethyl, 7^{11}-hydroxy chroman) pyrimidine (V).

The physical and spectral data was tabulated below in Table III and Table IV.

Antimicrobial activity of the synthesized compound V

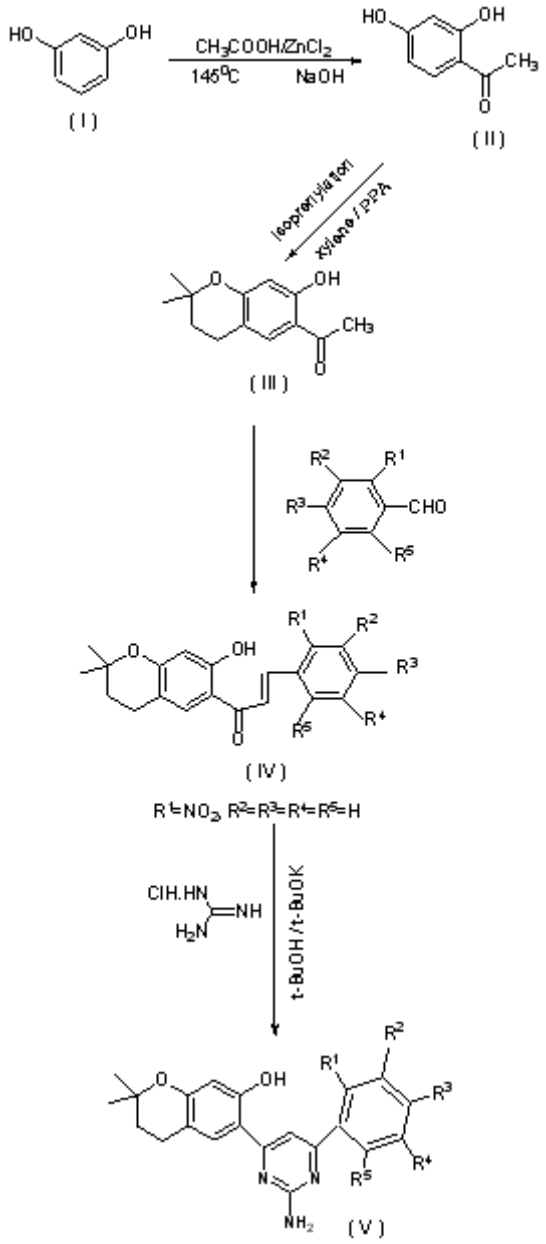
In continuation of our studies in the Department of Organic Chemistry FDW for potential active compounds, it is proposed to synthesize this compound V in pure for our study in antibacterial form property. There is first report of this type of investigation.

The compound V was recrystallized from the ethanol and the purity is rechecked by HPLC > 99.5% (Sol Sys. : Acetonitrile).

The antibacterial studies were investigated in the Department of Pharmaceutical Sciences,

Table 5: Antibacterial activity

Compound	Gram Positive Bacteria	Inhibition zone (cm)
V	B.s.	1.1



Scheme 2

Andhra University in the Laboratory of Prof. P. Ellaiah, Micro Organism Culture were obtained from Microbial Culture Center, Delhi.

The following microorganisms were taken for Invitro screening.

Gram Positive Bacteria

Bacillius subtiliis

Gram Negative Bacteria

E-Coli [Escherichia coli].

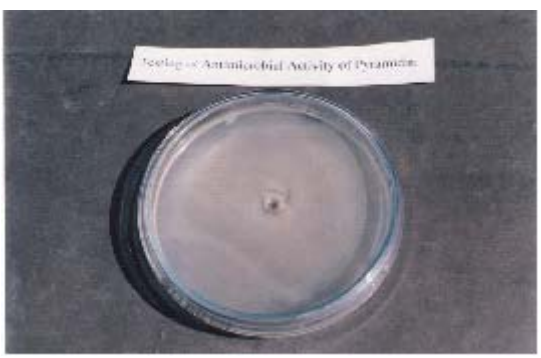
To test microbial activity of synthesized compound; different methods are available in the literature. Most common is "Cup Plate Method".

Methodology

- Prepare nutrient agar medium & pour in the plates aseptically.
- After cooling, these were inoculated with 24hr culture of B. subtiliis & spread throughout the agar surface.
- Make a well on the agar surface with the help of a barer. Add 100ml of different concentrations (50mg, 100mg & 200mg) in the wells to labeled petri plates.
- Allow the plates to remain in room temperature for diffusion of the compound.
- Next, place the petri plates in the incubator.
- Over after 24hrs.

Photographs were taken after 18hrs of incubations at concentrate of 100ml for the newly synthesized compound V. The data is recorded below.

MIC minimum inhibitory concentration of the synthesize compound V by cup plate method.



RESULTS AND DISCUSSION

Antimicrobial activity was observed on the plate at 200mg concentration and the data is presented in Table 5.

The above data is confirms that the compound V possess anti bacterial properties. Further studies are in progress

We initially proposed that pyrimidines may exhibit biological activity. Hence we synthesized this new compound and examined the antibacterial activity and it is observed that Compound V is exhibiting antibacterial activity. This is the first report of the synthesis of Compound V.

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