# SYNTHESIS AND CHARACTERISATION OF NEW CHROMANO PYRIMIDINES AND STUDY OF ANTIMICROBIAL ACTIVITY

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**ABSTRACT**: The title compounds, (five new chromano pyrimidines) were synthesized by condensation of chalcones with guanidine hydrochloride in the presence of potassium t- butoxide and t-butanol.

The synthesis involves following steps- Starting with the acylation of Resorcinol using anhydrous $ZnCl_2$ and glacial acetic acid, which afforded Resacetophenone **1**. Resacetophenone was then subjected to nuclear prenylation using isoprene in the presence of polyphosphoric acid and xylene, to form 7-hydroxy-6-acetyl 2,2'-dimethyl Chroman **2**. Condensation of Chroman with various substituted benzaldehydes in the presence of alcoholic KOH furnished different chalcones **3-7**. Finally, Chalcones were condensed with guanidine hydrochloride in the presence of potassium t- butoxide and t-butanol and the title compounds **8-12** were obtained. Compounds thus obtained were characterized by various spectroscopic techniques to confirm their structures. The title compounds were tested at different concentrations for their antibacterial activity against Gram-positive bacteria *Bacillus subtillis and Bacillus pumilus* and Gram-negative bacteria *Escherichia coli* and *Proteus vulgaris*. Antifungal activity of the title compounds was tested against *Rhizopus oryzae* and *Aspergillus niger* at different concentrations. Minimum inhibitory concentration was also determined.

**Keywords**: Chromano Pyrimidines, Synthesis, Characterization, Antibacterial & Antifungal, Activity.

**INTRODUCTION**: Heterocyclic compounds play a vital role in biological systems and in industry. Many compounds contain heterocyclic rings like vitamins, antibiotics, amino acids, plants pigments, nucleic acids, drugs etc. Most important usage is in pharmaceutical industries [1] because they are present in various medicines.

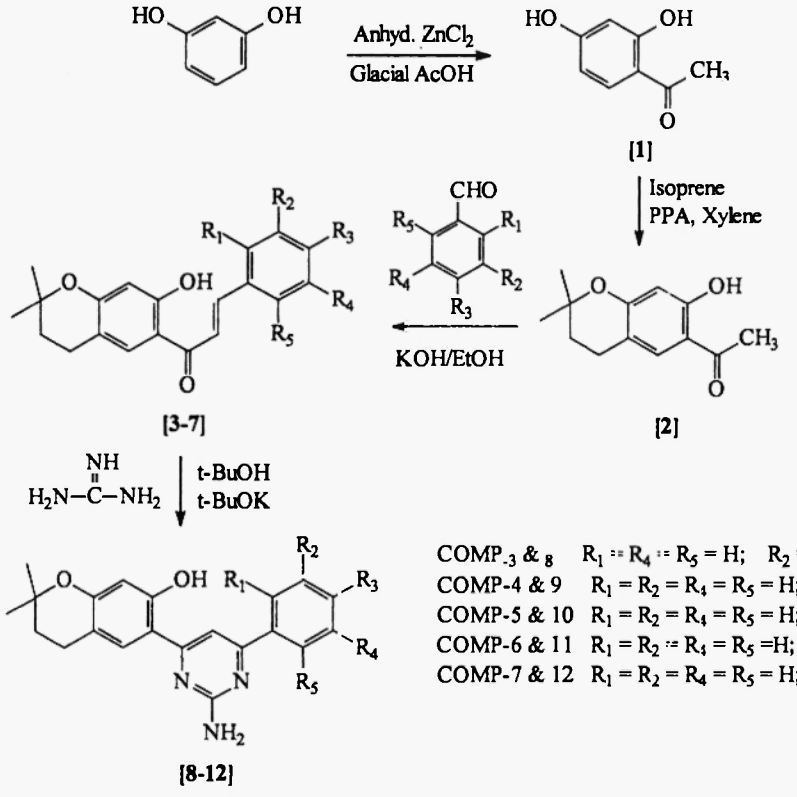
Literature survey reveals that Pyrimidines exhibit a wide spectrum of physiological activities. Bacimethrin (5-hydroxy methyl- 2-methoxy pyrimidine-4-amine), isolated from *Bacillus megatherium* is active against several yeasts and bacteria [2]. Amicetin, bacimetin and plicacetin, derivatives of cytosine are active against acid fast, gram-positive bacteria [2]. The wide spectrum antibiotics phleomycin, bleomycin and related families are having antineoplastic and antitumor activities. The 5-substituted pyrimidines are particularly important because of their significant utility in diverse pharmacological studies [3]. Most drugs in the pyrimidine series fall in four categories: the barbiturates, the sulphonamides, the antimicrobial and the antitumor agents. Sulphadiazine and sulphamerazine are used in bacterial chemotherapy. Other pyrimidine sulphonamides currently in clinical use include sulphamethoxy diazine, sulphamethoxine and sulphametomidine. These sulphonamides are used in acute urinary tract infections, cerebrospinal meningitis or to the numerous patients sensitive to penicillin [2, 4]. Trimethoprim is widely used in combination with sulphamethoxazole (commercial names Septrin, Bactrim) as general systemic antibacterial agent against a whole variety of Gram-positive and Gram-negative organisms [5]. Hydroxy and alkoxy pyrimidines are antiallergics and anti-inflammatory [6] Most commonly known pyrimidines used in antitumor therapy are Methotrexate [7] and 5- Fluorouracil.

As is evident from the above information, pyrimidines form important classes of compounds with a wide variety of pharmaceutical applications. 2,2-dimethyl Chromene ring occurs frequently in natural products. How ever 2,2-dimethyl Chromans occur rarely in nature but are useful degradation products for the structure elucidation of a large number of

natural products. They have been reported to show marked physiological activities [8 - 10]. Keeping this in view, five new chromano pyrimidines **8-12** were synthesized and their antimicrobial activity was studied.

In this paper, we report the synthesis of chromano pyrimidines from chalcones employing the 3+3 route or CCC, NCN route in which the NCN component is guanidine and CCC component is chalcone (α,β-unsaturated carbonyl compound) **[SCHEME].**

**SCHEME**




COMP-3 & 8 $R_1 = R_4 = R_5 = H$; $R_2 = R_3 = -(OCH_3)_2$
COMP-4 & 9 $R_1 = R_2 = R_4 = R_5 = H$; $R_3 = -N(CH_3)_2$
COMP-5 & 10 $R_1 = R_2 = R_4 = R_5 = H$; $R_3 = Cl$
COMP-6 & 11 $R_1 = R_2 = R_4 = R_5 = H$; $R_3 = -OCH_3$
COMP-7 & 12 $R_1 = R_2 = R_4 = R_5 = H$; $R_3 = -CN$

**The five different chalcones 3-7 were synthesized by condensing chroman 2 with various substituted Benzaldehydes in the presence of alcoholic KOH [11]. They are:**

7-Hydroxy-6- (3′,4′-dimethoxy) cinnamoyl,3,4 –dihydro-2,2-dimethyl-2H-benzo (1,2b) Pyran **3**
7-Hydroxy-6-(4′,N,N′-dimethyl amino)cinnamoyl,3,4–dihydro-2,2-dimethyl-2H- benzo (1,2b) Pyran 4
7-Hydroxy-6- (4′-chloro) cinnamoyl, 3,4 –dihydro-2,2-dimethyl-2H-benzo (1,2b) Pyran **5**
7-Hydroxy-6- (4′-methoxy) cinnamoyl, 3,4 –dihydro-2,2-dimethyl-2H-benzo (1,2b) Pyran 6
7-Hydroxy-6- (4′-cyano) cinnamoyl, 3,4 –dihydro-2,2-dimethyl-2H-benzo (1,2b) Pyran 7

Chroman **2** (0.01 mol) was condensed with various substituted Benzaldehydes (0.01 mol) in the presence of EtOH and aqueous KOH and stirred for 48 hours at room temperature. The mixture on acidification with 1:1 HCl and extraction with Ethyl acetate followed by purification by column chromatography furnished chalcones **3-7**. Chroman **2** was condensed with Veratraldehyde to give compound **3**, with 4-N, N'dimethyl amino benzaldehyde to give compound 4, with 4-Chlorobenzaldehyde to give compound **5**, with Anisaldehyde to give compound 6 and finally, with Cyano benzaldehyde to give compound 7. Purity of Chalcones obtained, was checked by H.P.L.C, purity was above 99%.

The chalcones **3-7** thus obtained, were condensed with guanidine hydrochloride [12] in alkaline medium viz., in potassium tertiary butoxide in the presence of t-butanol at reflux temperatures which after usual work up and purification by column chromatography, furnished the title compounds **8-12**. They were further crystallized from methanol, to furnish crystalline products. The compounds showed purity > 99% as evidenced by H.P.L.C, data is presented in **Table - 1**.

The five new chromano pyrimidines synthesized are –

2-amino-4- (3′,4′-dimethoxy phenyl)-6-(2″,2″-dimethyl,7″-hydroxy chroman) pyrimidine **8**

2-amino-4- (4′-N,N-dimethyl amino phenyl)-6-(2″,2″-dimethyl,7″-hydroxy chroman) pyrimidine 9
2-amino- 4-(4′-chloro phenyl)-6-(2″,2″-dimethyl,7″-hydroxy chroman) pyrimidine 10
2-amino-4- (4′-methoxy phenyl)-6-(2″,2″-dimethyl,7″-hydroxy chroman) pyrimidine 11
2-amino-4- (4′-cyano phenyl)-6-(2″,2″-dimethyl,7″-hydroxy chroman) pyrimidine 12

The pyrimidine derivatives on purification and crystallization formed as bright yellow coloured crystalline solids. The IR spectra showed the absorption at 1650 $cm^{-1}$and 1590 $cm^{-1}$ characteristic [13] of the C=N and C=C stretch of the pyrimidine system and exhibited two sharp peaks at 3360 and 3200 $cm^{-1}$ indicating the symmetric and asymmetric NH stretchings of the amino group. A small shoulder like peak at 3360 $cm^{-1}$ indicated the hydrogen bonded N-H stretch [14]. They also exhibited the characteristic out of plane CH bendings [15] at 1020 and 820 $cm^{-1}$.

The $^1H$ NMR spectra showed characteristic $C_5$ - H as a singlet at around δ 7.7.

The physical and H.P.L.C data are tabulated in table 1 and $^1H$ N.M.R spectral characteristics of the above new chromano pyrimidines are presented in the table 2.

**TABLE-1: PHYSICAL CHARACTERISTICS ‹OF 2-AMINO-4-SUBSTITUTED PHENYL-6- (2″,2″-DIMETHYL,7″-HYDROXY CHROMAN) PYRIMIDINES 8-12**

| Comp.No | Molecular formula | Yield % | $R_f$ values @ | Melting points (in °C) | H.P.LC PURITY* | Retention time (in minutes) |
|---|---|---|---|---|---|---|
| 8 | $C_{22}H_{25}O_4N_3$ | 61% | 0.6 | 177 | 100% | 3.845 |
| 9 | $C_{22}H_{26}O_2N_4$ | 60% | 0.5 | 216 | 99.7% | 4.214 |
| 10 | $C_{20}H_{20}O_2NCl$ | 67% | 0.5 | 235 | 100% | 3.842 |
| 11 | $C_{21}H_{23}O_3N_3$ | 63% | 0.4 | 163 | 100% | 3.558 |
| 12 | $C_{21}H_{20}O_2N_4$ | 50% | 0.4 | 142 | 100% | 4.172 |

@ HEXANE – ETHYL ACETATE

* H.P.L.C. EXPERIMENTAL CONDITIONS: Mobile phase – Acetonitrile.
Column- Silica gel
Injected Quantity- 10ul
Flow rate-1.0 ml/min.
Detector: UV detector
$\lambda_{max}$ 254 nm

**TABLE-2: $^1H$ NMR DATA* OF 2-AMINO-4-SUBSTITUTED PHENYL –6-(2′,2″-DIMETHYL,7″HYDROXY CHROMAN) PYRIMIDINES 8 – 12**

| PROTON | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|
| 2″a | 1.42(s, CH3) | 1.19(s, CH3) | 1.31(s, CH3) | 1.20 (s,$CH_3$) | 1.25(s, CH3) |
| 2″b | 1.53(s, CH3) | 1.31(s, CH3) | 1.4(s, CH3) | 1.42(s, CH3) | 1.43(s, CH3) |
| 3″ | 1.94 (t, 3″ –$CH_2$) | 1.9(t, 3″ –$CH_2$) | 1.82 (t, 3″ –$CH_2$) | 1.85 (t, 3″ –$CH_2$) | 1.8 (t, 3″ –$CH_2$) |
| 4″ | 2.8 (t, 2H, 4″-$CH_2$) | 2.8 (t, 2H,4″-$CH_2$) | 2.7(t, 2H, 4″-$CH_2$) | 2.78(t, 2H 4″-$CH_2$) | 2.73(t, 2H, 4″-$CH_2$) |
| 5″ | 7.5(s, 1H) | 7.3 (s, 1H) | 7.2(s, 1H) | 7.8(s, 1H) | 7.3(s, 1H) |
| 8″ | 6.3(s, 1H) | 6.25(s, 1H) | 6.8(s, 1H) | 7.2(s, 1H) | 6.8(s, 1H) |
| 5 | 7.7(s, 1H) | 7.5(s, 1H) | 7.5(s, 1H) | 7.55(s, 1H) | 7.8(s, 1H) |
| Aromatic protons | 7.7-8.0 (m, 4H) | 8.3 (br) | 7.5-8.1 (m, 4H) | 7.5- 8.1 (m,4H) | 7.7-8.0 (m, 4H) |
| - $OCH_3$ -$OCH_3$ | 3.9(s–$OCH_3$) 3.8(s–$OCH_3$) | - | - | 3.9(s–$OCH_3$) | - |
| -N-Methyl | - | 3.65 | - | - | - |
| -$NH_2$ | - | 5.1(br s) | - | - | - |

* $CDCl_3$/ TMS

## EXPERIMENTAL

Melting points are uncorrected. IR spectra were recorded on Perkin-Elmer 841 Infra red spectrophotometer or Thermo Nicolet FT IR spectrophotometer. $^1$H NMR spectra were taken on Perkin- Elmer R-32, 90 MHz or JEOL-JNM Ex-90, 90MHz in $CDCl_3$ using TMS as internal reference. All the solvents were of analytical grade and were distilled before use. The H.P.L.C was recorded using Shimadzu LC 6A with Shimpack silica gel column.

## GENERAL SYNTHESIS OF CHROMANO PYRIMIDINES

Chalcones **3-7** were condensed with Guanidine hydrochloride in the presence of tertiary butanol and Potassium tertiary butoxide, and refluxed on water bath for 4 hours. After usual work up, Pyrimidines **8-12** [SCHEME] were obtained as bright yellow solids, which were further, purified by column chromatography and crystallized from methanol. The purity was checked by H.P.L.C. and the compounds were characterized using various spectroscopic techniques like NMR, IR etc.

## ANTIMICROBIAL ACTIVITY

The title compounds **8-12** were tested for their antibacterial activity against Gram-positive bacteria *Bacillus subtilis* and *Bacillus pumilus* and Gram-negative bacteria *Escherichia coli* and *Proteus vulgaris*, at concentrations of 5,10,20,50,100,200, 300µg/ml. The cultures of *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli* and *Proteus vulgaris* grown over night at 37°C were used for testing the antibacterial activity which was checked employing cup plate method [16]. Nutrient agar medium (Himedia, India) was dissolved in water and pH was adjusted to 7.0.This was then distributed in 20ml quantity in boiling tubes, which were then plugged tightly with non absorbent cotton and sterilized. The bacterial culture (50µl) was then added aseptically to the agar medium maintained at 45°C, mixed well and poured immediately in sterilized petriplates. After hardening, cups of 8mm diameter each were cut into agar and 50µl test solutions of varying concentrations were placed in these cups. The plates were incubated at 37°C for 24 hours and the diameter of inhibition zone was measured in mms. Solvent DMSO alone was kept as control, which did not have any inhibition zone. The activity was compared with standard antibiotic Benzyl Penicillin. The comparable antibacterial activities of the title compounds are presented in the tables-3 & 4.

**Table-3: Antibacterial Activity Inhibition zones (in mm)**

| Compounds Concentration (µg/ml) | Gram-Positive Bacteria | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Bacillus subtilis*(in mm) | | | | | | | *Bacillus pumilus* (in mm) | | | | | | |
| | 5 | 10 | 20 | 50 | 100 | 200 | 300 | 5 | 10 | 20 | 50 | 100 | 200 | 300 |
| 8 | 11 | 13 | 14 | 16 | 18 | 20 | 21 | 10 | 12 | 13 | 15 | 17 | 19 | 20 |
| 9 | 13 | 15 | 17 | 18 | 21 | 25 | 30 | 9 | 10 | 12 | 14 | 15 | 17 | 19 |
| 10 | 13 | 14 | 16 | 18 | 22 | 23 | 28 | - | 14 | 12 | 18 | 18 | 19 | 21 |
| 11 | 16 | 15 | 16 | 17 | 18 | 20 | 22 | 11 | 13 | 13 | 18 | 19 | 21 | 23 |
| 12 | 15 | 15 | 17 | 18 | 19 | 20 | 21 | - | 9 | 11 | 11 | 12 | 14 | 18 |
| Benzyl Penicillin | - | 26 | - | 25 | 29 | 16 | - | - | 24 | - | 14 | 16 | 22 | - |

**Table-4: Antibacterial Activity Inhibition zones (in mm)**

| Compounds Concentration (µg/ml) | Gram-Negative Bacteria | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Escherichia coli* (in mm) | | | | | | | *Proteus vulgaris* (in mm). | | | | | | |
| | 5 | 10 | 20 | 50 | 100 | 200 | 300 | 5 | 10 | 20 | 50 | 100 | 200 | 300 |
| 8 | - | 12 | 14 | 16 | 24 | 39 | 40 | 11 | 11 | 12 | 13 | 14 | 16 | 17 |
| 9 | - | 13 | 15 | 26 | 30 | 41 | 50 | 12 | 13 | 13 | 14 | 16 | 17 | 19 |
| 10 | - | 11 | 14 | 16 | 20 | 40 | 46 | 9 | 9 | 11 | 12 | 13 | 15 | 16 |
| 11 | 18 | 25 | 28 | 34 | 35 | 52 | 53 | 13 | 16 | 16 | 18 | 19 | 20 | 21 |
| 12 | 12 | 14 | 16 | 20 | 24 | 48 | 50 | 10 | 12 | 13 | 13 | 14 | 17 | 19 |
| Benzyl Penicillin | - | 34 | - | 40 | 43 | 40 | - | - | 20 | - | 22 | 25 | 20 | - |

Antifungal activity of the compounds was tested against *Rhizopus oryzae* and *Aspergellius niger* using cup plate method [16]. Potato dextrose agar (Himedia, India) was dissolved in water and pH was adjusted to 5.6. This was then distributed 20ml each in boiling tubes which were plugged tightly with non-absorbent cotton and sterilized. To this 50µl of fungal spore suspension was added and thoroughly mixed with 20 ml medium aseptically and poured in to petriplates. When agar solidified, cups of 8mm diameter were made on each of the seeded plates. These cups were filled with 50µl of test samples of varying concentrations. The petriplates were incubated at room temperature for 2 days. The inhibition zones produced by test compounds were compared with inhibition zones produced by pure ketoconazole used as standard. The results are tabulated in table-5.

**Table- 5: Antifungal Activity Inhibition Zones (in mm)**

| Compounds Concentration (µg/ml) | *Rhizopus oryzae* | | | | | | *Aspergillus niger* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 20 | 50 | 100 | 200 | 5 | 10 | 20 | 50 | 100 | 200 |
| 8 | 9 | 11 | 13 | 15 | 17 | 19 | 12 | 13 | 14 | 16 | 18 | 19 |
| 9 | 10 | 12 | 13 | 15 | 16 | 17 | 11 | 13 | 15 | 16 | 17 | 19 |
| 10 | 11 | 14 | 17 | 17 | 18 | 19 | 10 | 12 | 14 | 15 | 16 | 17 |
| 11 | 12 | 14 | 17 | 18 | 21 | 22 | 14 | 16 | 18 | 20 | 23 | 24 |
| 12 | 10 | 11 | 13 | 16 | 17 | 18 | 11 | 12 | 14 | 15 | 17 | 18 |
| Ketoconazole | - | 15 | - | 18 | 21 | - | - | 13 | - | 16 | 22 | - |

Solvent DMSO did not have inhibition zone against all the six organisms.

**DISCUSSION:** The results show that all the five title compounds **8-12** showed remarkable antibacterial activity against all the four organisms. The minimum inhibition concentration (MIC) was 5µg/ml against *B.subtilis* and *P.vulgaris* in all the five compounds; MIC was 5µg/ml against *B.pumilus* for compounds **8, 9** and **11** and in case of *E.coli* MIC was 5µg/ml in compounds **11** and **12**. MIC was 10µg/ml against *E.coli* for compounds **8, 9** and **10** and compounds **10** and **12** against *B.pumilus* respectively. On the whole compound 11 showed remarkable activity in all the organisms. It showed inhibition zones comparable to that of benzyl penicillin at 200µg/ml and 300µg/ml concentrations. All the five title compounds showed significant activity against *E.coli*.

Compounds **8, 9, 10, 11** and **12** showed remarkable anti fungal activity where in the MIC was 5µg/ml for all the compounds. Compound **11** showed greater antifungal activity than the other four compounds both against *R oryzae* and *A niger*.

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