Chapter 6
Spectral Studies

SYNTHESIS OF CERTAIN SCHIFF'S BASES OF CYANO PYRANS, CARBOETHOXY PYRANS AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai

In partial fulfilment of the award of degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

Submitted by

BIJOSH.K

Under the guidance of

Prof. M. FRANCIS SALESHIER, M.Pharm.,
Department of Pharmaceutical Chemistry

MARCH - 2009

COLLEGE OF PHARMACY

SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES

Coimbatore - 641 044.

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Certificate

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Prof. M. FRANCIS SALESHIER, M. Pharm.,
Head of the Department,
Department of Pharmaceutical Chemistry,
College of Pharmacy, SRIPMS,
Coimbatore – 44.

Place: Coimbatore
Date:

Certificate

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Dr. T.K. Ravi, M.Pharm., Ph.D., FAGE.,
Principal,
College of Pharmacy, SRIPMS,
Coimbatore – 44.
Place: Coimbatore

Date:

Certificate

This is to certify that the Antimicrobial studies which was a part of the dissertation entitled **"SYNTHESIS OF CERTAIN SCHIFF'S BASES OF CYANO PYRANS, CARBOETHOXY PYRANS AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES"** was carried out by **BIJOSH.K** in the Department of Pharmaceutical Biotechnology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai under my supervision and co-guidance to my fullest satisfaction.

Prof. S. Krishnan, M. Pharm., (Ph.D).,
Head of the Department,
Dept. of Pharmaceutical Biotechnology,
College of Pharmacy, SRIPMS,
Coimbatore – 44.

Place: Coimbatore

Date:

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*I sincerely thank **Prof. S. Krishnan, M. Pharm., (Ph.D).,** Head of the Department of Pharmaceutical Biotechnology, who provided the latest information and valuable suggestion for completing my microbiological studies.*

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*My special thanks to **Library Staffs** of SRIPMS and Bharathiyar University, Coimbatore for providing the library facilities during the literature survey.*

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*I wish to thank my **classmates** and all my friends **Jiji, Knolin, Lavanya, Manju, Manjula, Mugeer, Mahalakshmi, Shakila Banu, Subageetha, Santhana Krishnan, Munavir, Nishad, Arun, Prabith, Mithun** and **My roommates** for their help and encouragement during the course of my study.*

*I wish to thank **Mrs. Mini Nair and Mr. Niranjan, Saraswathi Computer Centr**e for their help in bringing this manuscript in a neat manner.*

*Above all I humbly submit my dissertation work into the hands of **Almighty**, who is the source of all wisdom and knowledge for the successful completion of this dissertation work.*

Bijosh.K

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INTRODUCTION

ANTIBACTERIAL AGENTS[1-5]

Drugs used for treating infectious disease are called antibiotics, anti-infectious agents, antimicrobial or chemotherapeutic agents. The first three-antibiotics, anti-infectious agents and antimicrobial drugs are generally used to describe the drug used for treating infectious disease, while the term chemotherapeutic drugs is more associated with drugs used for treating cancer.

Antibiotics are the compounds produced by microorganisms and that are able to kill or inhibit the growth of bacteria and other microorganisms. This definition makes a specific distinction between antimicrobial drugs produced by microorganisms and completely synthetic product. Today the word antibiotic is used quite often for specifying antimicrobial drugs in general.

CLASSIFICATION

1.	**Beta-lactam antibiotics**	**:**	Penicillins, Cephalosporins, Monabactams, Carbapenems
2.	**Aminoglycosides**	**:**	Streptomycin, Gentamicin
3.	**Macrolide antibiotics**	**:**	Erythromycin, Roxithromycin
4.	**Glycopeptide antibiotis**	**:**	Vancomycin, Teicoplanin
5.	**Polypeptide antibiotics**	**:**	Polymyxin-B, Bacitracin

6.	**Lincomycins**	**:**	Clindamycin.
7.	**Tetracyclines**	**:**	Oxytetracycline, Doxycycline.
8.	**Nitrobenzene derivatives**	**:**	Chloramphenicol
9.	**Sulfonamide and related drugs:**		Sulfadiazine, Dapsone.
10.	**Diaminopyrimidines**	**:**	Trimethoprim, Pyrimethamine
11.	**Quinolones**	**:**	Nalidixic acid, Ciprofloxacin
12.	**Nitrofuran derivatives**	**:**	Nitrofurantoin, Furazolidone

ANTIFUNGAL AGENTS

Antifungal agents are the drugs used for superficial and systemic fungal infection.

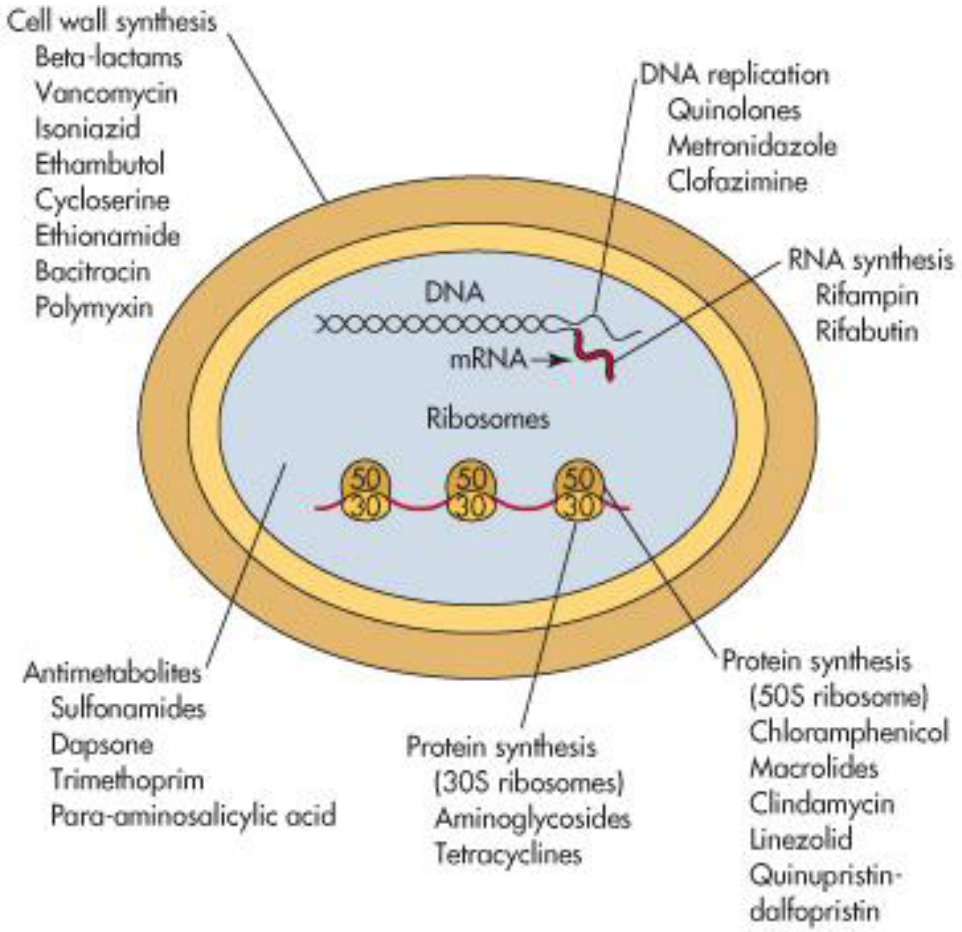
Classification

Antifungal agents are classified as follows

1.	***Antibiotics***		
	(a) Polyenes	:	Amphotericin-B, Nystatin
	(b) Heterocyclic benzofuran	:	Griseofulvin
2.	***Antimetabolites***	*:*	Flucytosine
3.	***Azoles***		
	(a) Imidazoles:		
	Topical	:	Clotrimazole, Miconazole
	Systemic	:	Ketoconazole
	(b) Triazoles	:	Fluconazole
4.	***Allylamine***	*:*	Terbinafine
5.	***Other topical agents***	:	Benzoic acid, Tolnaftate
6.	***New antifungal agents***	:	Echinocandins, Sordarin

derivatives.

BASIC MECHANISMS OF ANTIBIOTICS



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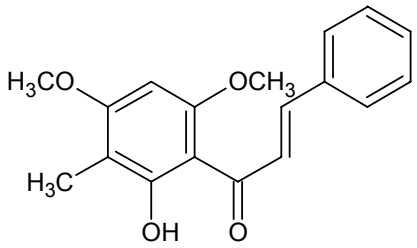
CHALCONES [6]

Chalcone is an aromatic ketone that forms the central core for a variety of important biological compounds. They show antibacterial, antifungal, antitumor, anti-inflammatory properties. Some chalcones demonstrated the ability to block voltage-dependent potassium channels. They are also intermediates for the biosynthesis of flavonoids, which are the substances widespread in the plants with an array of biological activities. Chalcones are the intermediates for the synthesis of pyrans[7], pyrazolines[8,9], 1,5-benzodiazepine[10], 1,5-benzothiazepine[11].

NATURALLY ISOLATED CHALCONES:

1) Aurentiacin[12]:

Isolated from *Pityrogramma triangularis* var. *pallida*

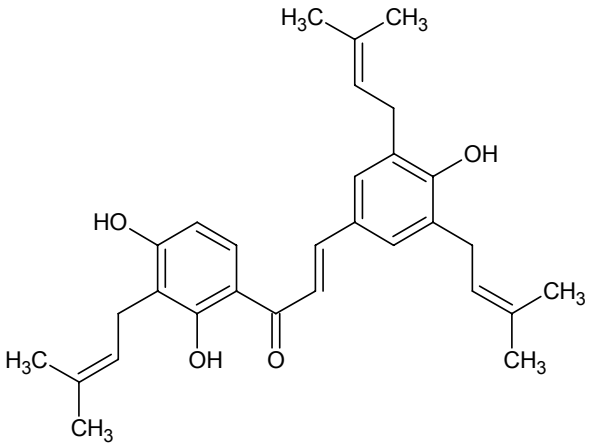


Aurentiacin

2) Sophoradin[13]:

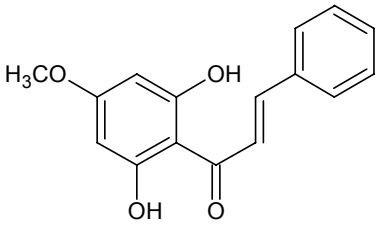
It is an isoprenyl chalcone isolated from a Chinese drug, Guang-dou-gen or Shan-dou-gen, which is the root of *Sophora subprostrata* (Leguminosae). The isolated compound showed anti-ulcer effect on both

Shay's pylorus-ligated rats and water immersed and restraint stress rats.



3) 2',6'-dihydroxy-4'-methoxychalcone[14]

This compound was isolated from inflorescences of *Piper aduncum* belongs to the family Piperaceae which showed a significant *in vitro* activity against promastigotes and amastigotes of *Leishmania amazonensis.*



2',6'-dihydroxy-4'-methoxychalcone

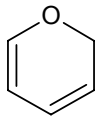
4) Licochalcone A[15]

It is an oxygenated chalcone isolated from the roots of Chinese licorice plant, which inhibited the growth of both *Leishmania major* and

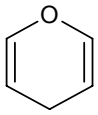
Leishmania donovani promastigotes and amastigotes.

PYRANS[16]

In chemistry, a pyran is a six membered heterocyclic ring consisting of five carbon atom and one oxygen atom containing two double bonds. The molecular formula is C_5H_6O. There are two isomers of pyran that differ by the location of double bonds. In 2*H*-pyran, the saturated carbon is at position 2, whereas in 4*H*-pyran, the saturated carbon is at position 4. Although the pyran themselves have little significance in chemistry, a variety of their derivatives are important biological molecules. 4*H*-Pyran easily disproportionate to the corresponding dihydropyran and pyrylium ion, which is easily hydrolyzed in aqueous medium.



2*H*-pyran



4*H*-pyran

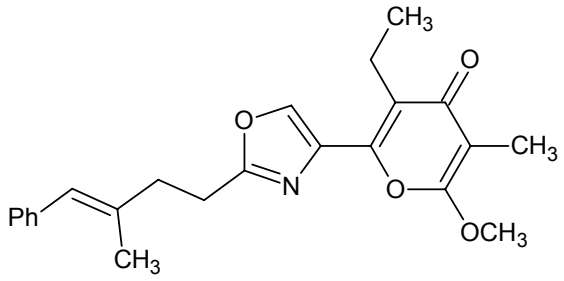
Six membered heterocyclic compounds containing oxygen atom such as 4H-pyran-4-one (γ-pyrone) constitute an important class of biologically active natural and synthetic product. A large number of natural product containing γ-pyrone unit has been isolated which shows anti-HIV, anti-tumor, radical scavenging activity, antifungal, Inhibitor of bone resorption, antibacterial, cytotoxic activity. In the light of these results a number of aromatic and heteroaromatic derivatives of pyran with cabonitrile or carboethoxy group has been prepared and screened for their

possible antibacterial and antifungal activities.

NATURAL PRODUCTS CONTAINING PYRAN MOIETY:

1) Phenoxan[17]:

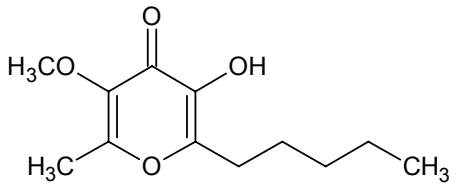
It is a naturally occurring heterocyclic compound, isolated from a soil microorganism and discovered to have Anti-HIV activity.



Phenoxan

2) Allixin[18,19]:

It is one of the phytoalexins, first isolated from garlic, *Allium sativum* L. It has anti-tumor and radical scavenging effect.

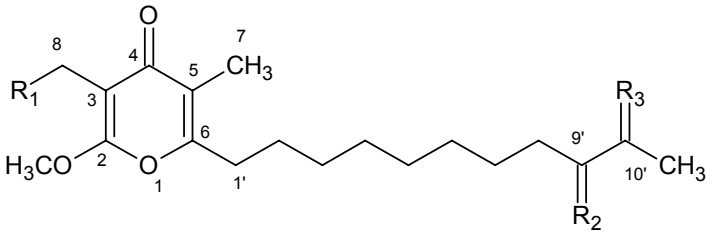


Allixin

3) 9'-oxopodopyrone & 10'-oxopodopyrone[20]:

Isolated from leaves of *Gonystylus keithii*. This γ-pyrone markedly inhibited the bovine parathyroid hormone induced Ca release from neonatal mouse calvaria *in vitro*. It is the first time γ-pyrone showed inhibitory effects on bone resorption, and these compounds may be seed

compound of new drug for osteoporosis.

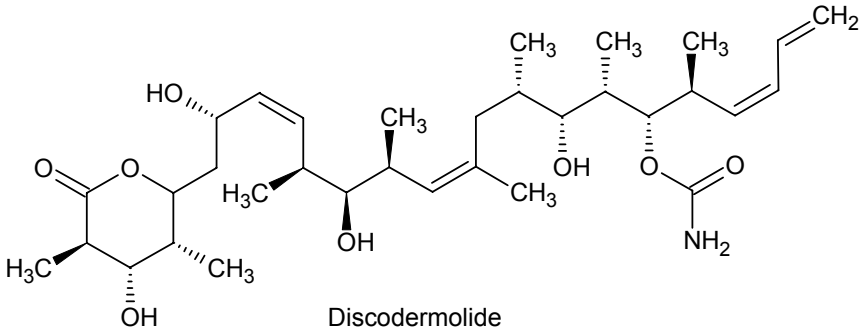


9'-oxopdopyrone R_1,R_3= H, R_2 = O

10'-oxopdopyrone R_1,R_2= H, R_3=O

4) Discodermolide[21]:

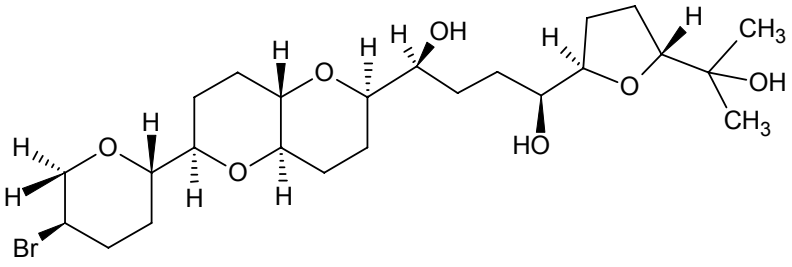
It is a marine natural product isolated from sponge *Discodermia dissoluta*, reported to inhibit the proliferation of T cells and exhibit immunosuppressive activity.



Discodermolide

5) Thyrsiferol[22]:

It is isolated from marine red algae of the genus *Laurencia thyrsifera* was found to have cytotoxic, anti-viral and anti-tumor activity.



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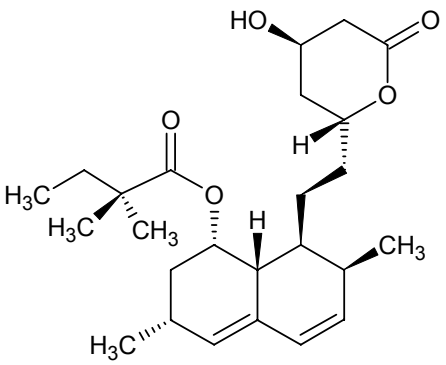
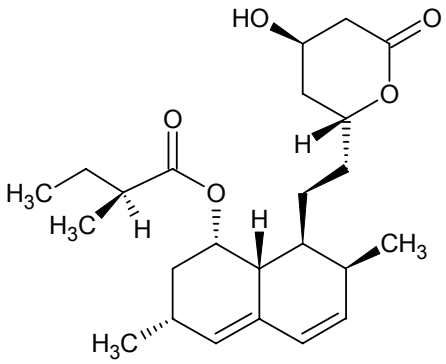
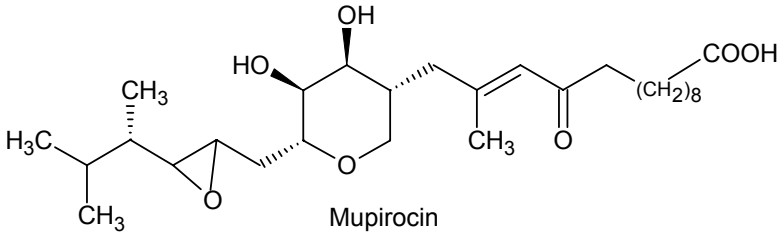
MEDICINALLY USEFUL DRUGS WITH PYRAN MOIETY[23]:



Simvastatin



Lovastatin



Mupirocin

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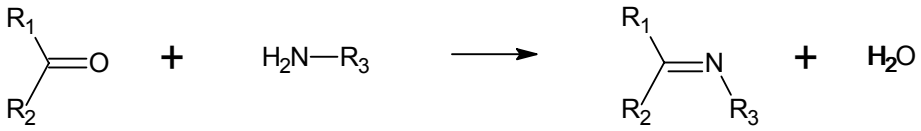
SCHIFF'S BASE[24]

A Schiff's base is a functional group that contains a carbon-nitrogen double bond in which the nitrogen atom connected to an aryl or alkyl group but not hydrogen. Schiff's base are of general formula $R_1R_2C=N-R_3$, where R_3, is an aryl or alkyl group that makes the Schiff's base a stable imine. A Schiff's base synthesized from an aromatic amine and a carbonyl compound by nucleophilic addition leads to form a hemiaminal, followed by a dehydration to generate an imine. Due to their diverse reactivity, imines are common substrates in a wide variety of transformations.



R_1 , R_2 = H, alkyl, aryl
R_3 = alkyl, aryl

However the equilibrium in this reaction usually lies in favour of the free carbonyl group and amine, so that azeotropic distillation or use of dehydrating agent such as molecular sieves are required to push the reaction in favor of imine formation. Addition reaction of carbonyl compound with primary amines give imines that are stable under inert atmosphere but In the presence of oxygen or water, such imines quite readily hydrolyze or oligomerize. However, with an aryl group or certain stabilizing alkyl substituents on nitrogen, the imine formed is stable to oxygen and water is called a Schiff's base.

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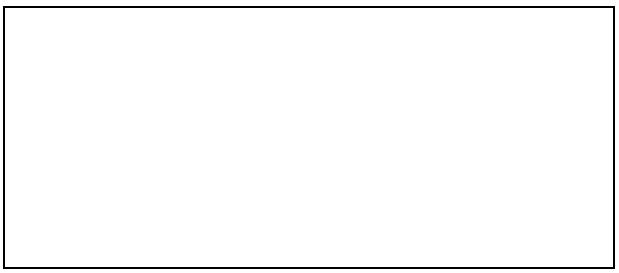
LITERATURE REVIEW

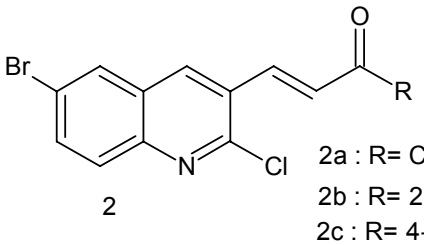
CHALCONES

Chalcones with antimicrobial activity

1) **Popat *et al.*,[25] (2004)** synthesized various chalcones of 1-aryl-3-*m*-chlorophenyl-2-propene-1-one. The synthesized compounds were shown antibacterial activity against *Escherichia coli, Proteus vulgaris, Bacillus megaterium, Staphylococcus aureus.* The above compounds also showed antifungal activity against *Aspergillus niger*.



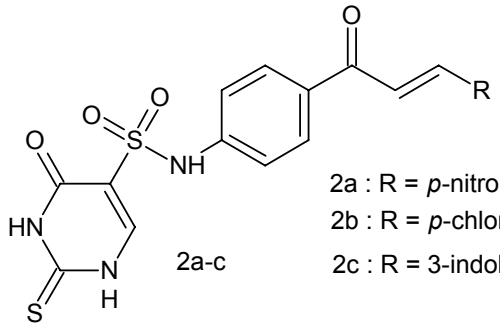
2) **Patel *et al.*,[26] (2003)** synthesized 3-(2'-chloro-6'-bromoquinolin-3'-yl)-1-aryl-2-propen-1-one derivatives. The synthesized compounds were shown antibacterial activity against *Escherichia coli, Proteus vulgaris, Bacillus megaterium, Staphylococcus aureus* and antifungal activity against *Aspergillus niger*.



2

2a : R= C_6H
2b : R= 2-Br-C
2c : R= 4-C
2d : R= 4-Cl-C

3) **Fathalla *et al.*,[27] (2005)** synthesized 2-Thiouracil-5-sulphonic acid - N-(4-(3-substituted-2-propen-1-oxo) phenyl) amide derivatives. The synthesized compounds were shown *invitro* antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and antifungal activity against

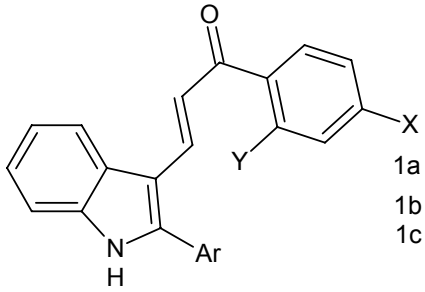


2a-c

2a : R = *p*-nitrophenyl
2b : R = *p*-chlorophenyl
2c : R = 3-indolyl

Microsporium canis, Sporotrichum schenkii.

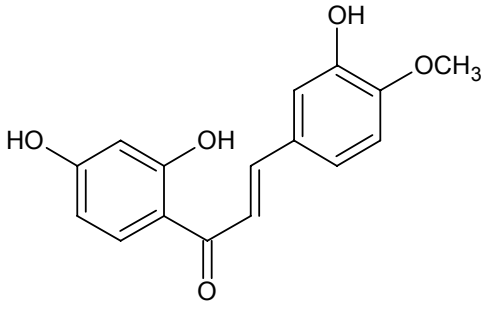
4) **Dandia *et al.*,[28] (1993)** synthesized 2-aryl-3-(3-aryl-3-oxoprop-1-enyl)indole derivatives. The synthesized compounds were shown antibacterial activity against *Escherichia coli* and antifungal activity against *Rhizoctonia solani.*



1

1a : Ar= C_6H_5
1b : Ar= C_6H_5, X= F , Y= CH
1c : Ar= C_6H_5

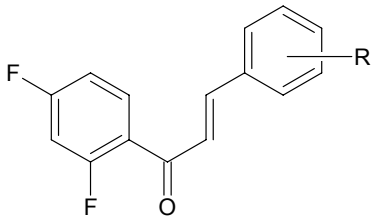
5) **Mostahar *et al.*,[29] (2006)** synthesized (E)-1-(2',4'-dihydroxyphenyl)-3-(3''-hydroxy-4-methoxyphenyl)prop-2-en-1-one. The synthesized compound was shown antibacterial activity against *Bacillus megaterium, Streptococcus-β-haemolyticus, Escherichia coli, and Klebsiella species* and also found antifungal activity against *Aspergillus niger, Aspergillus fumigatus*.



Chalcones with anti-inflammatory activity

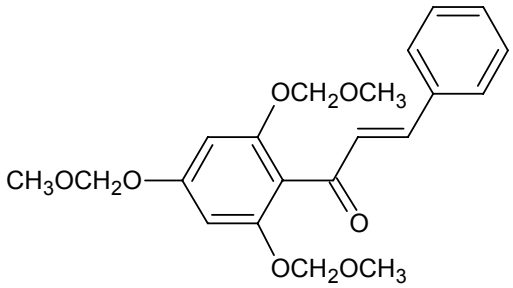
6) **Jadhav *et al.*,[30] (2007)** synthesized 2',4'-difluorinated chalcones.

The synthesized compounds were shown anti-inflammatory activity.



R = 4-OCH_3, 3-Nitro, or 3,4,5-Trimethoxy

7) **Jin *et al.*,[31] (2007)** synthesized 2',4',6'-tris(methoxymethoxy) chalcone derivative. The synthesized compound has potent anti-inflammatory activity via heme oxygenase dependent pathway.



Chalcones having cytotoxic activity

8) **Mostahar *et al.*,[32] (2006)** synthesized (E)-1-(2',4'-dihydroxyphenyl)-3-(3''-hydroxy-4''-methoxyphenyl)prop-2-en-1-one. The synthesized compound was shown cytotoxic activity which is compared with standard Bleomycin and Gallic acid.

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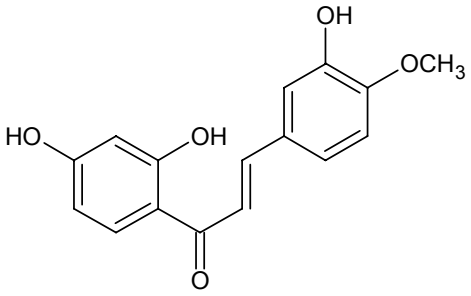
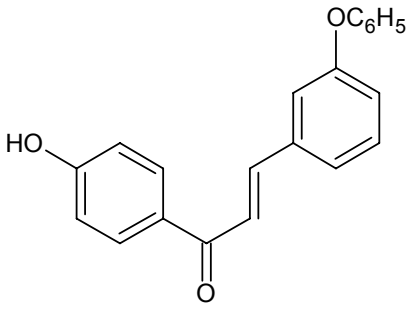


Chalcones having insecticidal activity

9) **Mudaliar *et al.*,[33] (1995)** synthesized 1-(4'-hydroxyphenyl) -3- (3''-phenoxy) phenyl-2-propene-1-one. The synthesized compound was shown insecticidal activity on house fly adult. The efficacy of this compound was compared with standard insecticide, Fenvalerate.



Chalcone with antioxidant activity

10) **Kim *et al.*,[34] (2008)** synthesized a series of dihydroxylated chalcone derivatives with diverse substitution on phenyl ring B and the *para*-substituents on phenyl ring A were prepared and their radical scavenging activities were evaluated by simple DPPH test.

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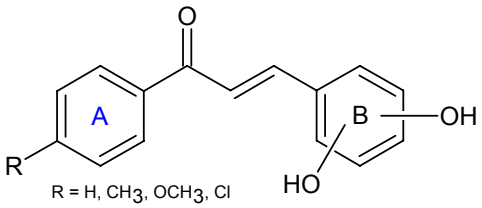
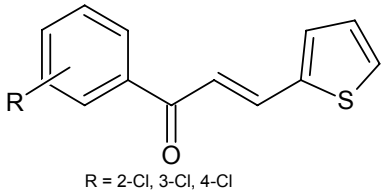


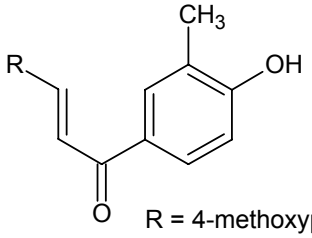
Chalcone with antiparasitic activity:

11) **Laliberte *et al.*,[35] (1968)** synthesized some new heterocyclic chalcone analogues. The synthesized compounds were shown antiparasitic activity.



Chalcones with antimycobacterial activity

12) **Yar *et al.*,[36] (2007)** synthesized 1-(4-hydroxy-3-methylphenyl)-3-[(substituted)phenyl]-2-propen-1-ones. The synthesized compounds were shown antimycobacterial activity.



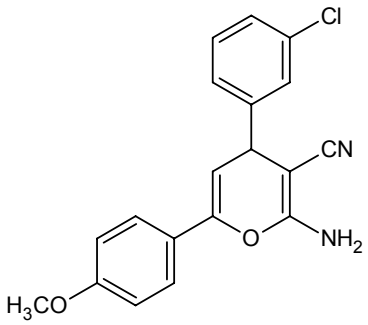
R = 4-methoxyphenyl
R = 4-Chlorophenyl
R = 4-Fluorophenyl

Chapter 6
Spectral Studies

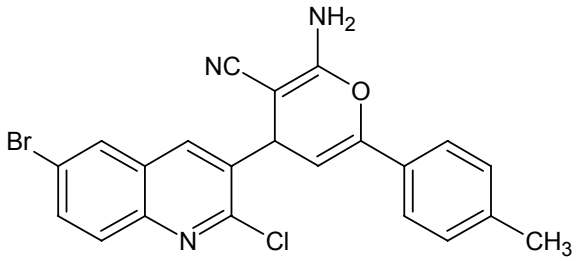
CYANOPYRANS

Cyanopyran with antimicrobial activity

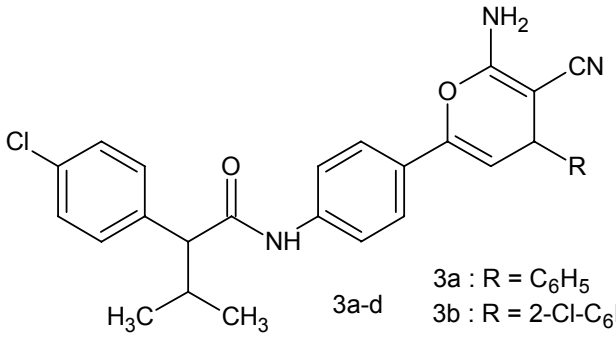
1) **Popat *et al.*,[25] (2004)** were synthesized 2-amino-3-cyano-4-(3''-chlorophenyl)-6-(4-methoxyphenyl)-pyran. The synthesized compound was shown antibacterial activity against *Escherichia coli, Proteus vulgaris, Bacillus megaterium, Staphylococcus aureus* and antifungal activity against *Aspergillus niger.*



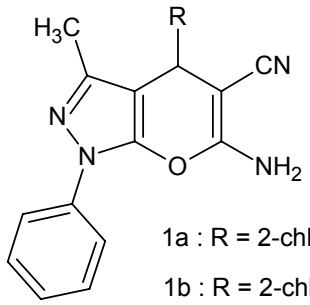
2) **Patel *et al.*,[26] (2003)** synthesized 2-amino-3-cyano-4-(2'-chloro-6'-bromoquinolin-3'-yl)-)-6-(*p*-tolyl)-4H-pyran. The synthesized compound was shown antibacterial activity against *Escherichia coli, Proteus vulgaris, Bacillus megaterium, Staphylococcus aureus* and antifungal activity against *Aspergillus niger.*



3) **Kanjariya *et al.*,[37] (2004)** synthesized 6-(α- isopropyl-*p*-chlorophenyl-acetamidophenyl)-4-aryl-2-amino-3-cyanopyran. 3(a-d). All the compounds were shown antibacterial activity against *Escherichia coli, Proteus vulgaris, Bacillus megaterium, Staphylococcus aureus* and antifungal activity against *Aspergillus niger.*



4) **Nakum *et al.*,[38] (2002)** synthesized 2-amino-3-cyano-4-(2'-chloro substituted quinolin-3'-yl/aryl)-5-methyl-7-phenylpyrazolo (5,6-d)-4H-pyrans (1a-b). The Synthesized compounds were shown antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus pumilus and Candida albicans.*



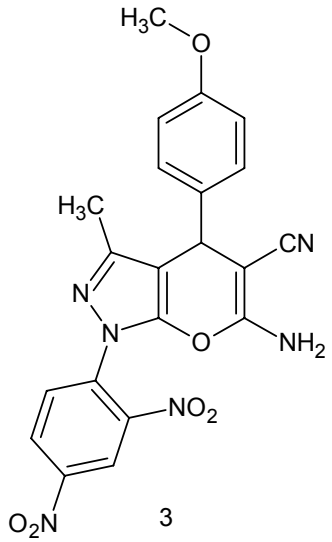
1a-c

1a : R = 2-chloro-7-methyl-3-quinolinyl

1b : R = 2-chloro-6-methyl-3-quinolinyl

1c : R = 2-chloro-6-methoxy-3-quinolinyl

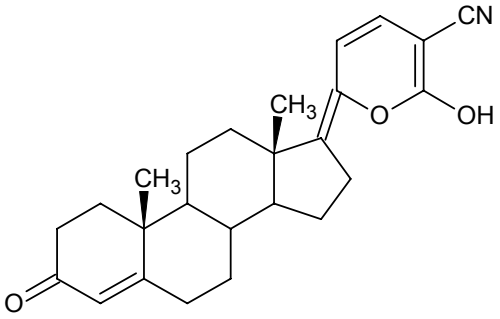
5) **Ei-assiery *et al.*,[39] (2004)** synthesized 6-amino-1-(2,4-dinitrophenyl)-4-(4-methoxyphenyl)-3-methyl-1,4- dihydropyrano 2,3-c]pyrazole-5-carbonitrile (3) which showed antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Serratia marcescens, Proteus mirabilis* and antifungal activity *against Aspergillus fungytus.*



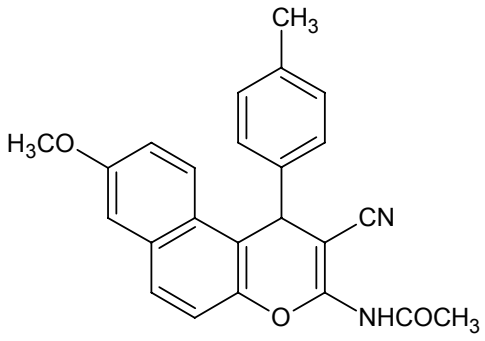
3

6) **Mohareb *et al.*,[40] (2008)** synthesized 17-(2-hydroxy-3-cyanopyran-

6-yl)androst-4-en-3-one (6b). The synthesized compound showed antibacterial activity against *Bacillus cereus, Bacillus subtilis and antifungal activity against Candida albicans.*



7) **Fathy *et al.*,[41] (2004)** synthesized 2-acetylamino-7-methoxy-4-(*p*-tolyl)-4H-naptho[2,1-b]pyrane-3-carbonitrile (4a). The synthesized compound was shown antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Serratia marcescens, Proteus mirabilis, and Escherichia coli.*

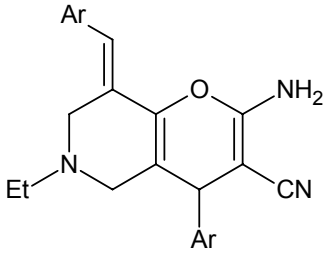


4a

Cyanopyrans with anticancer activity

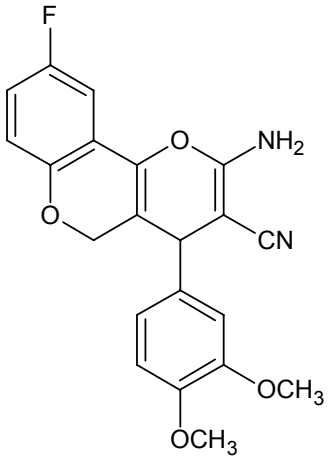
8) **Hammam *et al.*,[42] (2001)** synthesized 2-Amino-4-aryl-8-arylmethylene-

6-ethyl-5,6,7,8-tetrahydropyrano[3,2-c]pyridine- 3- carbonitrile which showed anticancer activity.



Ar = 4-Cl-C_6H_4, 4-Br-C_6H_4

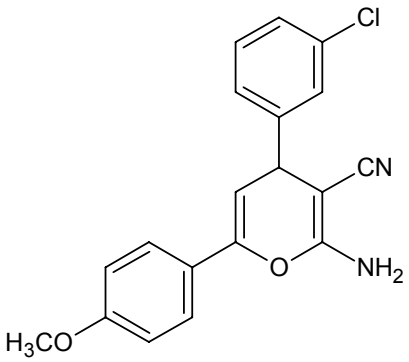
9) **Hammam *et al.,*[43] (2005)** synthesized 2-Amino-4-aryl-9-fluoro-4,5-dihydrobenzo[b]pyrano[4,3-b]pyrane-3-carbonitrile. The synthesized compound was shown anticancer activity.



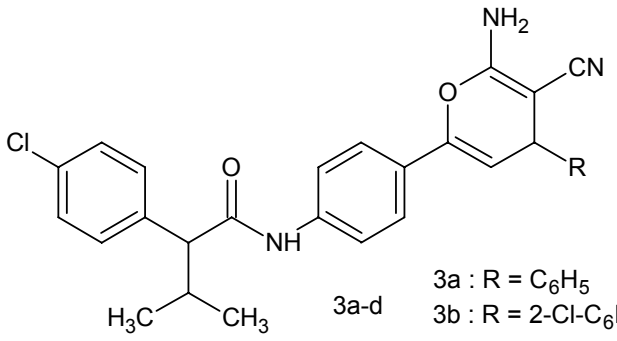
Cyanopyrans with antimycobacterial activity

10) **Popat *et al.,*[25] (2004)** synthesized 2-amino-3-cyano-4-(3"-

chlorophenyl)-6-(4-methoxyphenyl)-pyran. The synthesized compound showed antimycobacterial activity.



11) **Kanjariya *et al.*,[26] (2004)** synthesized 6-(α- isopropyl-*p*-chlorophenylacetamidophenyl)-4-aryl-2-amino-3-cyanopyrans 3(a-d). All the compounds were shown antimycobacterial activity.



3a-d

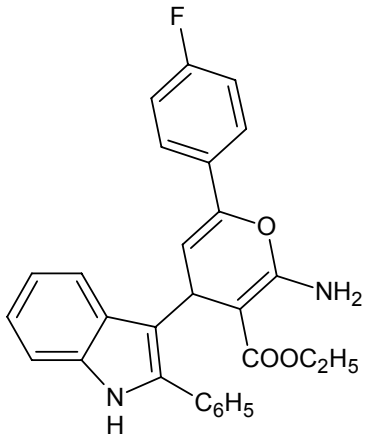
3a : R = C_6H_5
3b : R = 2-Cl-C_6H_4-
3d : R = 2,6-$(Cl)_2$-C_6H_3-

CARBOETHOXY PYRANS

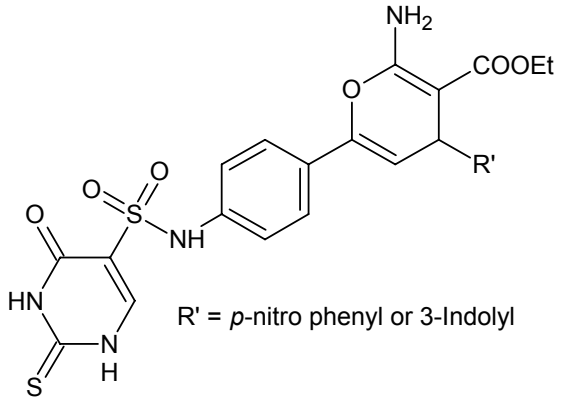
Pyrans with Antibacterial Activity

12) **Dandia *et al.*,[28] (1993)** synthesized 3-[6-amino-5-carboethoxy-2-(4-

fluorophenyl)-pyran-4-yl]-2-phenyl indole. The synthesized compound was shown antibacterial activity against *Staphylococcus albus*, *Escherichia coli* and antifungal activity against *Rhizoctonia solani.*



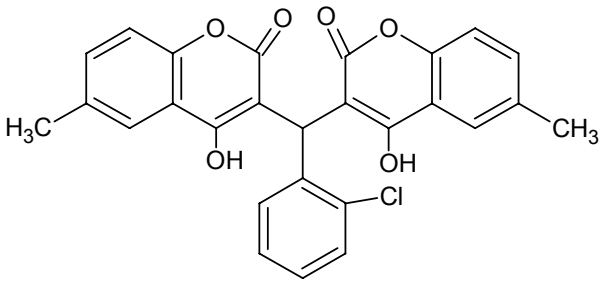
13) **Fathalla *et al.,*[27] (2005)** synthesized 2-Thiouracil-5-sulphonic acid N-(4-(6-amino-5-carboethoxy-4-substituted pyrano)phenyl) amide derivatives. The synthesized compounds were shown antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus.*.



R' = *p*-nitro phenyl or 3-Indolyl

Pyran with HIV-1 and HIV-2 inhibitors

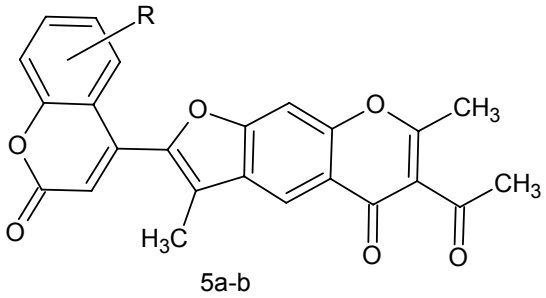
14) **Chavda *et al.*,[44] (2002)** synthesized monomethylated dimeric benzopyrans. Among the synthesized compounds 3,3'-(2-chlorobenzylidene)-bis-(6-methyl-4-hydroxy coumarin) (3d) showed activity against HIV1 and HIV 2.



3d

Pyran with antiinflammatory activity

15) **Ghate *et al.*,[45] (2005)** synthesized 6-Acetyl-3, 7-dimethyl-2-(coumarin-4'-yl)furo[3,2-g]chromen-5-one. The synthesized compounds were shown excellent anti-inflammatory activity by carageenan induced edema model in rats using phenylbutazone as standard.

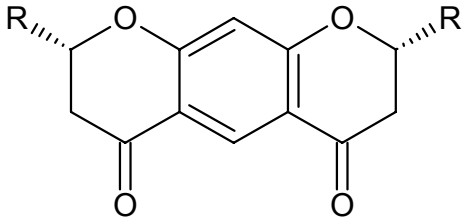


5a-b

5a = R= 6-CH_3
5b = R= 7-CH_3

Pyran with anifeedant activity

16) **Reddy *et al.*,[46] (1995)** synthesized 2,8-disubstituted-2,3,7,8-tetrahydro-4,6-dioxo-4H,6H-benzo[1,2-b;5,4-b']dipyran. The synthesized compound was shown antifeedant activity.



R = 4-Cl-C_6H_4

Pyran with antiallergic activity

17) **Shahrisa *et al*., [47] (2000)** synthesized 6-(2-Furyl)-2-(tetrazol-5-yl)-4H-pyranone which showed antiallergic activity.

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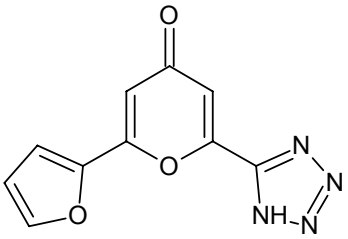
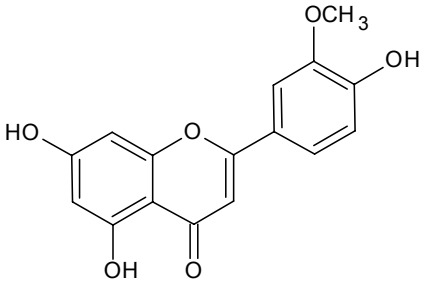


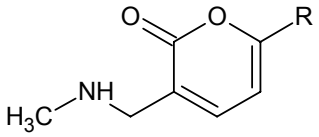
Pyran with antioxidant activity

18) **Shetgiri *et al.,*[48] (2003)** synthesized 4', 5, 7-trihydroxy-3'-methoxyflavanone which showed excellent antioxidant activity.



Pyran with acetylcholinesterase inhibitor activity

19) **Jiang *et al.,*[49] (2005)** synthesized 3-N-aminomethyl-6-substituted phenyl-2H-pyran-2-one . The synthesized compound was shown selective potent acetylcholinesterase inhibitor activity.



R = 2-Br-C_6H_4, 3,4- $(OCH_3)_2$-C_6H_3

SCHIIF'S BASES

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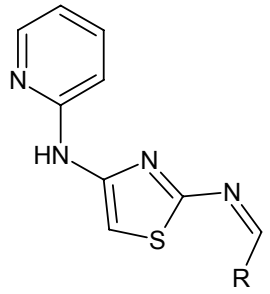
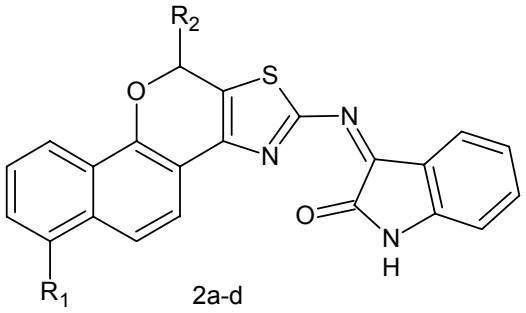
Schiff's base with antimicrobial activity

1) **Singh *et al.*,[50] (2006)** synthesized 2-(2'-*o*-hydroxy arylideneimino-1'-3'-thiazol-4'yl) aminopyridine which showed antibacterial activity against *Staphylococcus aureus, Escherichia coli* and antifungal activity against *Candida albicans, Candida krusei, Candida glabrata, Aspergillus fumigatus.*



R = O-hydroxyphenyl

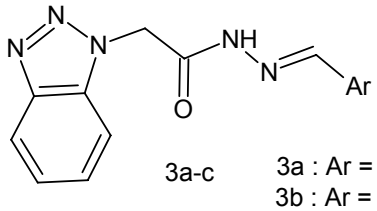
2) **Suryavanshi *et al.*,[51] (2006)** synthesized Naptho[1,2-b]pyrano[3,4-d]thiazol-8-yl(3-imino-2-oxo)-1H-indole as an intermediates for the synthesis of 2-azetidinone and thiazolidinone derivatives, the synthesized Schiff's bases were shown antibacterial activity against *Staphylococcus aureus, Escherichia coli,.*



2a-d

2a : R_1 = H , R_2 = H
2b : R_1 = H , R_2 = Br
2c : R_1 = H , R_2 = OH

3) **Asati *et al.*,[52] (2006)** synthesized Arylidene acetohydrazido benzotriazoles as an intermediates for the synthesis of thiazolidinone derivatives. The synthesized imino compounds were shown antibacterial activity against *Bacillus subtilis, Escherichia coli* and antifungal activity against *Aspergillus niger, Candida albicans, Candida panical.*

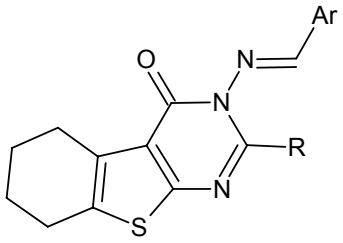


3a-c

3a : Ar = 4-CH_3 C_6H_4
3b : Ar = 2-CH_3 C_6H_4
3c : Ar = 3-CH_3 C_6H_4

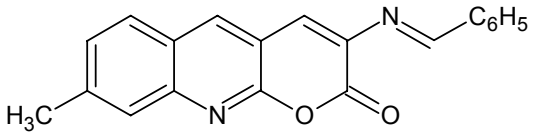
4) **Narayana *et al.*,[53] (2006)** were synthesized 2-Methyl/ethyl-3-

{[(aryl)methyl-ene]amino}-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-ones. The synthesized intermediates were shown antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and antifungal activity against *Aspergillus fumigatus, Aspergillus flavus*.

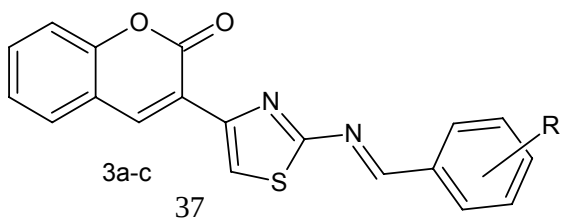


R = $-CH_3$ or $-C_2H_5$
Ar = 4-CH_3 or 4-OCH_3 or 3-OCH_3

5) **Rajendran *et al.*,[54] (2002)** synthesized Schiff's base of 8-Methyl-3-amino-2H-pyrano[2,3,-b]quinolin-2-one. The synthesized compound was shown antifungal activity against *Aspergillus niger, Fusarium species*.



6) **Naik *et al.*,[55] (2006)** synthesized 2-N-(substituted benzylidene)imino-4-(coumarin-3-yl)thiazoles (3a-c).the synthesized Schiff's bases were active against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*.



3a-c

3a : R = 3-OCH_3
3b : R = 3,4,5-$(OCH_3)_3$
3c : R = 4-$N(CH_3)_3$

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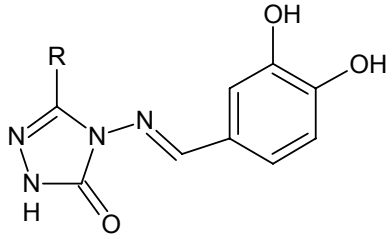
Schiff's base with antioxidant activity

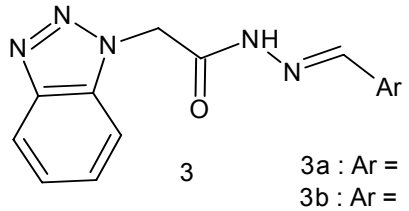
7) **Yuksek *et al.*,[56] (2006)** synthesized 3-Alkyl(aryl)-4-(3,4-dihydroxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones.The synthesized compounds were shown antioxidant activity.



R = $-CH_3$, $-C_2H_5$, $-CH_2-C_6H_4-Cl$

Schiff's base with analgesic activity:

8) **Asati *et al.*,[52] (2006)** synthesized Arylidene acetohydrazido benzotriazoles as an intermediates for the synthesis of thiazolidinone derivatives. The synthesized imino compounds were shown analgesic activity in albino rats by Eddy and Leimbach method.



3

3a : Ar = 4-CH_3 C
3b : Ar = 2-CH_3 C
3c : Ar = 3-CH_3 C

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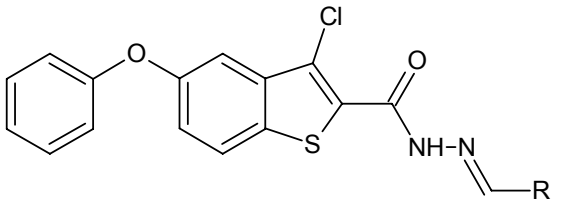
Spectral Studies

Schiff's Base with Antitubercular Activity

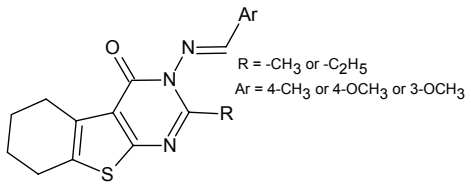
9) **Vasoya *et al.*,[57] (2005)** synthesized- 2-[(4'-Hydroxy-3'-methoxyphenyl)-hydrazinocarbonyl]-3-chloro-5-phenoxybenzo[b]-thiophenes (2e). The synthesized compounds showed antitubercular activity.



R = N-$(CH_3)_2$-C_6H_4 or 4'-Cl-C_6H_4 or 4'OH,3'-OCH_3-C_6H_3

Schiff's base with anti-inflammatory activity

10) **Narayana *et al.*,[58] (2006)** synthesized 2-Methyl/ethyl-3-{[(aryl)methyl-ene]amino}-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one. The synthesized intermediates were shown excellent anti-inflammatory activity.



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CHEMISTRY

CHALCONES

General Reactions

Nucleophilic addition reaction[59]

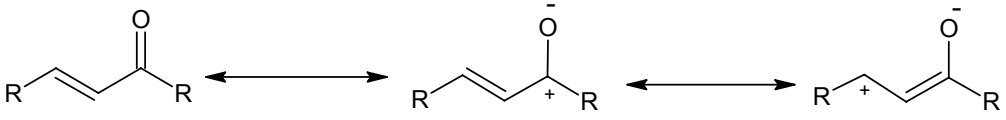
From the resonance structure of an α, β-unsarurated carbonyl compounds, nucleophilic addition reaction takes place by simple addition or conjugate addition.

Simple Addition:

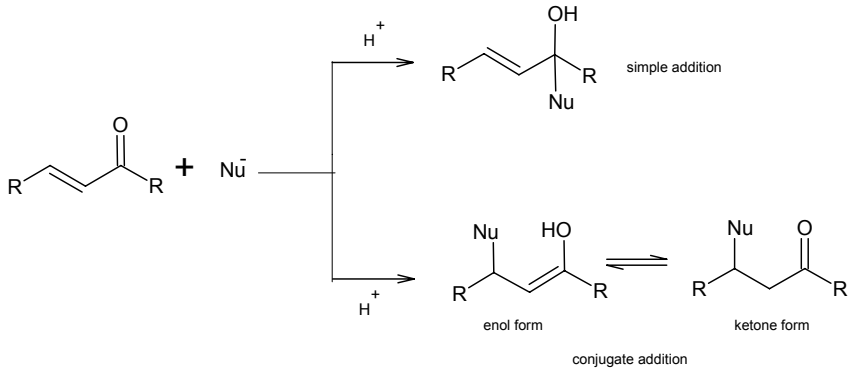
That is one in which nucleophile add across the double bond of the carbonyl group.

Conjugate Addition

That is one in which nucleophile add on the β-carbon atom.



Resonance structure of chalcone



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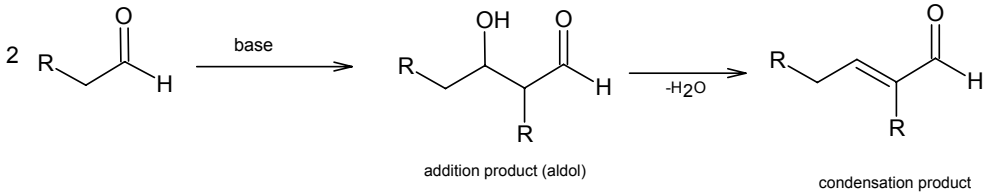
Almost all nucleophilic reagents that add at carbonyl carbon of the aldehyde or ketone, is also capable of adding at β-carbon of an α,β-unsaturated carbonyl compound. In many instance conjugate addition is the major reaction path.

Mechanism of Chalcone Formation

ALDOL CONDENSATION[60,61]



Aldol condensation is a powerful way of making carbon carbon bonds. Two aldehyde or ketone react to form initial addition product which is a β-hydroxyaldehyde, but these substances usually eliminate water to form α,β-unsaturated carbonyl compounds.

Aldol condensation can occur between

- Two identical or different aldehydes.
- Two identical or different ketones.
- An aldehyde and a ketone.

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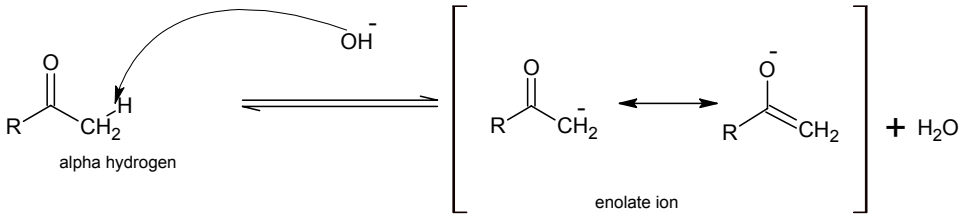
CLAISEN-SCHMIDT REACTION

It is the condensation of aromatic aldehyde having no α-hydrogen with aromatic ketones or ester having α-hydrogen, in the presence of 10% alkali solution to give α,β-unsaturated aldehyde or ketone is known as CLAISEN-SCHMIDT REACTION.

MECHANISM

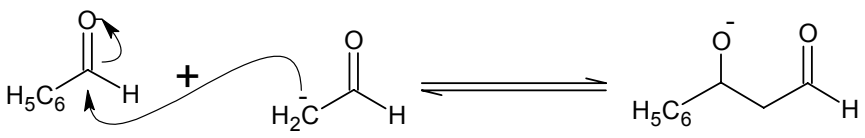
Step-1:

A strong base abstract the acidic α-hydrogen atom from the carbonyl compound to give resonance stabilized enolate ion.



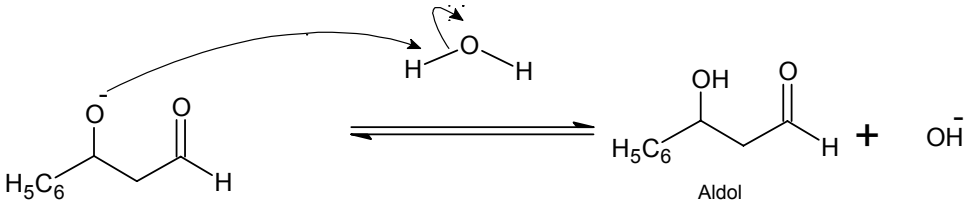
Step-2:

The resulting anion can react with the carbonyl carbon of another molecule through a nucleophilic addition reaction to produce an intermediate alkoxide.



Step-3:

The intermediate alkoxide deprotonate water molecule to produce a hydroxide ion and a compound that contains both a carbonyl moiety and –OH group called an aldol.



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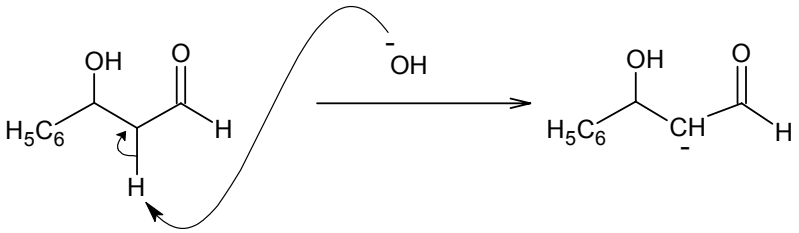
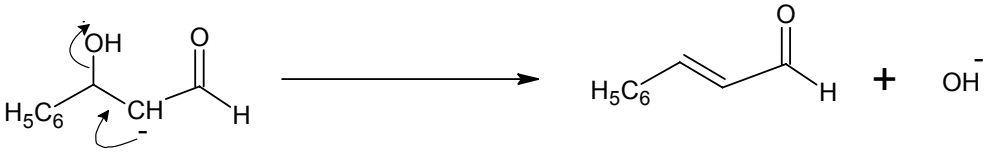
Step-4:

Hydroxide ion functions as a base and removes an acidic α-hdrogen giving the reactive enolate.



Step-5

The electrons associated with the negative charge of the enolate are used to form the C=C and displace the leaving group, regenerating hydroxide ion giving the conjugated aldehyde.



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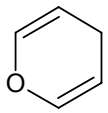
PYRANS[62,63]

Six membered oxygen heterocycles constitute a group of compounds which occurs widely throughout the plant kingdom

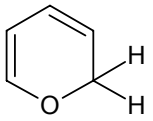
Structure : 

Molecular formula : C_5H_6O

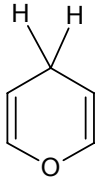
Molecular weight : 82.10

Here the main discussion is about 4H-pyran.

Six membered oxygen heterocycles constitute a group of compounds which occur widely throughout the plant kingdom. According to the position of unsaturated carbon atom, the pyran heterocycles can be classified as 2H-pyran and 4H-pyran.



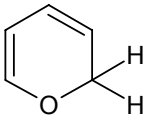
2H-pyran



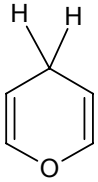
4H-pyran

NOMENCLATURE

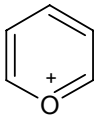
All the compounds are discussed based on three molecules: 2H-pyran, 4H- pyran, and the pyrylium cation.



2H-pyran



4H-pyran



pyrylium cation

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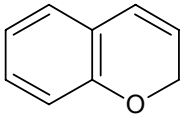
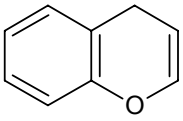
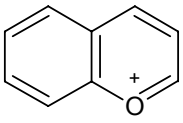
Names which have been used for the benzologue of 2H-pyran includes: 2H-1-benzo-pyran, benzo-α-pyran, chrom-3-ene and 2H-chromene. A similar situation exists for corresponding derivatives of 4H-pyran. The benzologue of pyrylium cation is known both as benzopyrylium and chromylium.



2H-chromene
(2H-1-benzopyran)



4H-chromene
(4H-1-benzopyran)



1-benzopyrylium

GENERAL REACTIONS

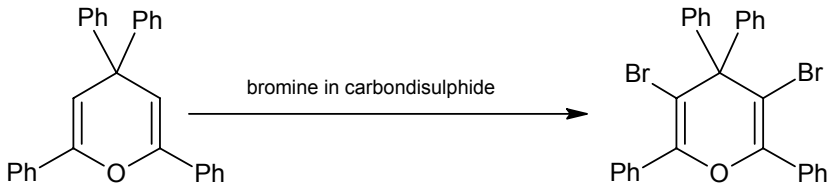
Substitution reaction

a) Electrophilic substitution reaction:

Electrophilic substitution reaction usually takes place at position 3rd and/or 5th position where a relatively high π-electron density is located in all pyran like heterocycles.

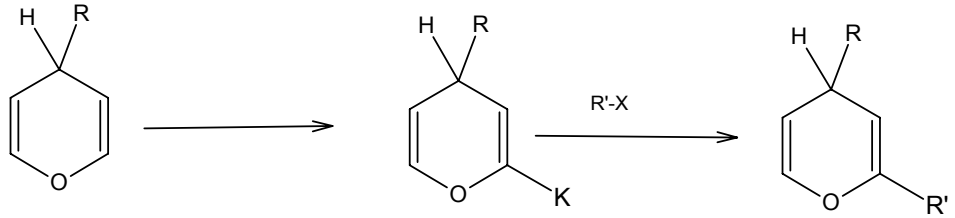
Bromination

2,4,4,6-tetraphenyl-4H-pyran easily gave 3,5-dibromo derivative in the presence of bromine in carbon disulphide.



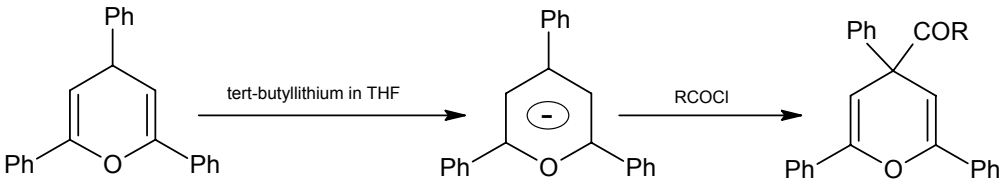
Alkylation, Acylation and silylation

Unsubstituted 4H-pyran and its 4-methyl derivatives were converted with a butyllithium potassium tert-butoxide mixture to corresponding 4H-pyran-2-yl-potassium intermediates capable of alkylation or silylation to 2-substituted-4H-pyran.



R = H,Me R' = MeI

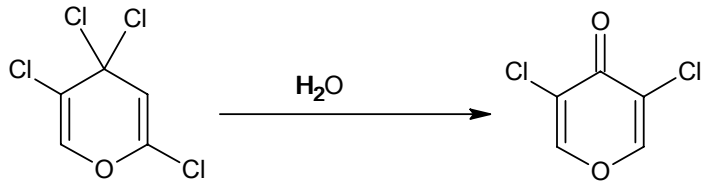
4H-pyran in the presence of sodium amide in liquid ammonia or with tert-butyllithium in tetrahydrofuran, an anionic species has been formed which was easily alkylated or acylated with methyl iodide,benzyl chloride exclusively at 4th position.



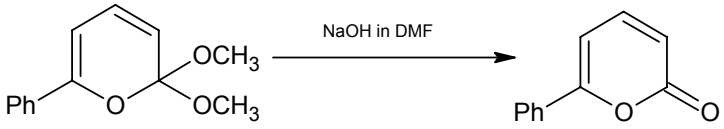
b) Nucleophilic substitution reaction

Hydrolysis

2,4,4,5-tetrachloro-4H-pyran easily hydrolyzed with water to get 2,3-dichloro-4-pyrone.

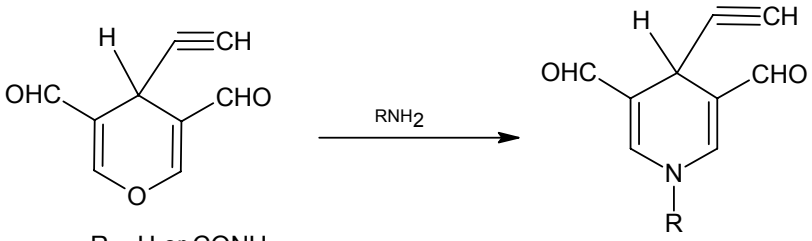


2,2-dimethoxy-6-phenyl-2H-pyran with sodium hydroxide in DMF gives 6-phenyl-2-pyrone.



c) Conversion to other heterocycles

Conversion to 1,4-dihydropyridines:

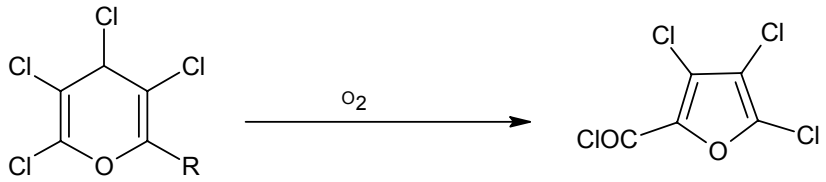


R = H or $CONH_2$,

A large number of 1,4-dihydropyridines were prepared from 3,5-diformyl-4-ethynyl-4H-pyran in the presence of primary amines, ammonium acetate, urea.

Ring contraction:

The oxidation of 2-substituted 3,4,5,6-tetrachloro-2H-pyran with molecular oxygen at 20^{o}C was accompanied by contraction of six membered ring to 2-furylcarbonyl chloride.



Ring expansion

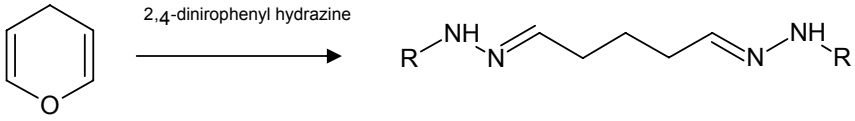
Decomposition of 2-azido-2H-pyran generates seven membered heterocyclic systems.

R = H or Ph

d) Ring opening reaction

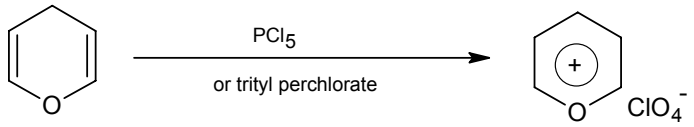
Reaction of 4H-pyran with Amines, Hydrazine, Hydroxylamines:

Unsubstituted 4H-pyran with 2,4-dinitrophenyl hydrazine afford identical bishydrazones.



e) Oxidation

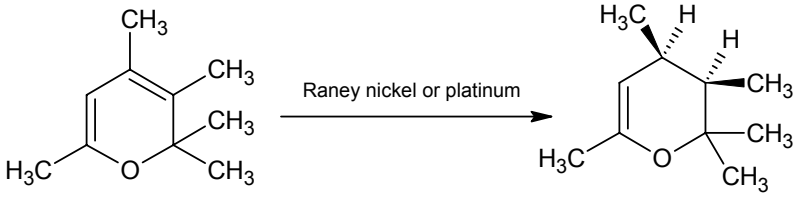
Pyran with oxidizing agents in acidic medium leads to corresponding pyrylium salts. Unsubstituted 4H-pyran as well as its methyl derivatives react with trityl perchlorate or with phosphrous pentachloride to give pyrylium salts.

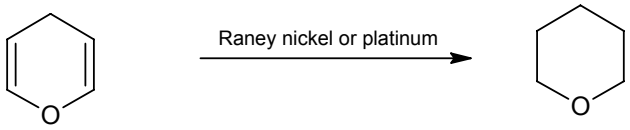


f) Reduction

Partial hydrogenation of corresponding 2H-pyran to give dihydropyran derivative

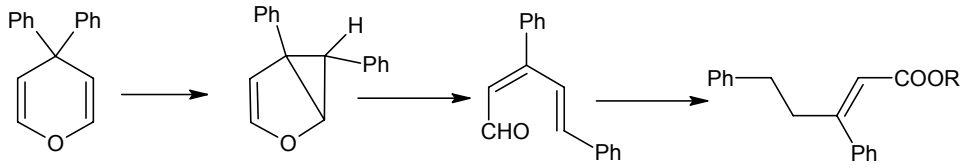
Hydrogenation of unsubstituted 4H-pyran with the help of Palladium or Adam platinum catalyst gives tetrahydropyran derivatives.





g) Photochemical reaction

Photolysis of 4,4-diphenyl-4H-pyran in tert-butyl alcohol gave a mixture of unsaturated aldehyde and other compounds.



R = H, t-but

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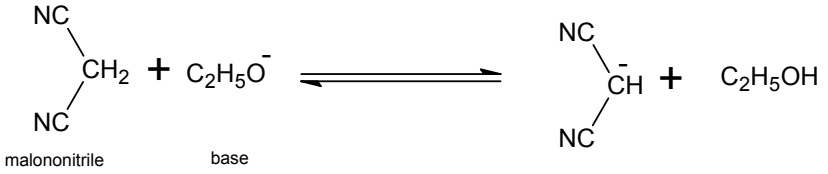
MECHANISM INVOLVED IN THE SYNTHESIS OF CYANOPYRAN[64-69]

Synthesis of cyanopyran has been carried out by Michael reaction of chalcone followed by an intramolecular cyclization.

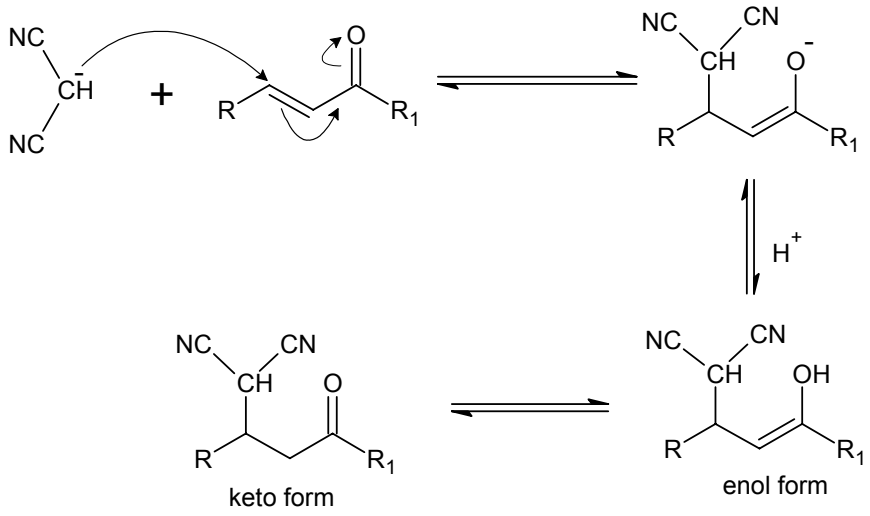
Step-1:

Base facilitates production of anion from malononitrile.(Base: Sodium ethoxide, pyridine, piperidine)



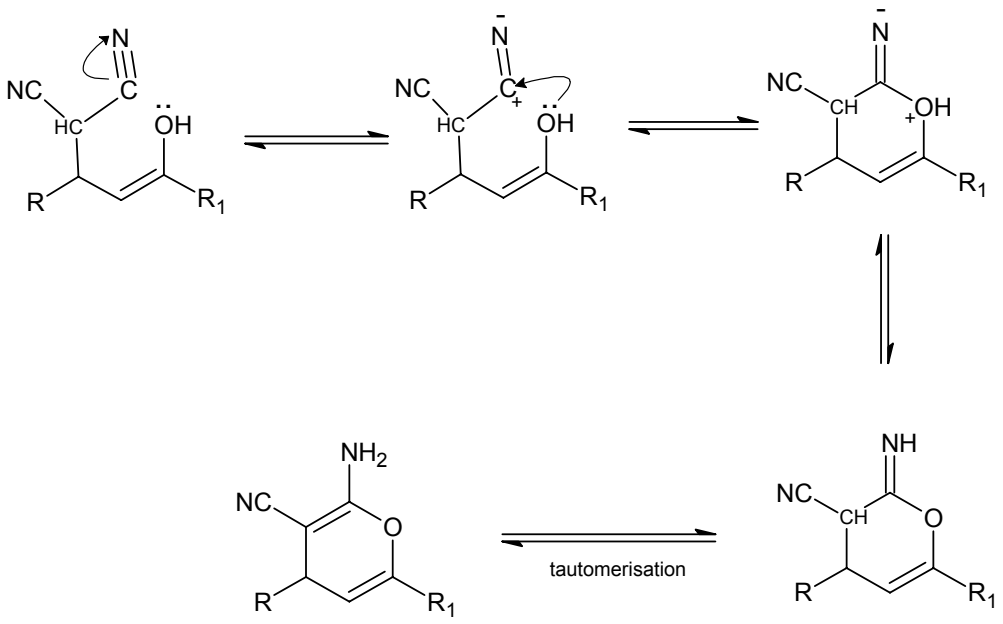
Step-2:

Conjugate addition of anion to the α,β-unsaturated carbonyl compound, followed by acceptance of a proton from alcohol to give an enol form which finally tautomerises to keto form.



Step-3

Enolic form of the Michael adduct undergoes nucleophilic intramolecular addition of hydroxy group to cyano group to form cyclic intermediate, which tautomerises to form 2-amino-3-cyano-4,6-disubstituted pyran.



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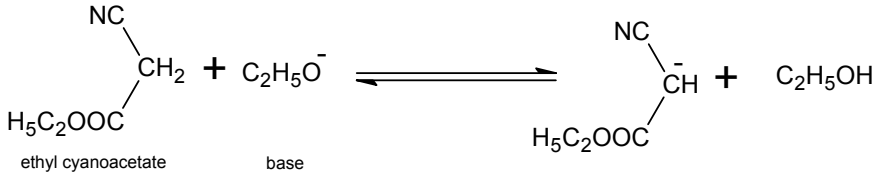
MECHANISM INVOLVED IN THE SYNTHESIS OF CARBOETHOXY PYRAN:

Synthesis of carboethoxypyran has been carried out by Michael reaction of chalcone followed by an intramolecular cyclization.

Step-1

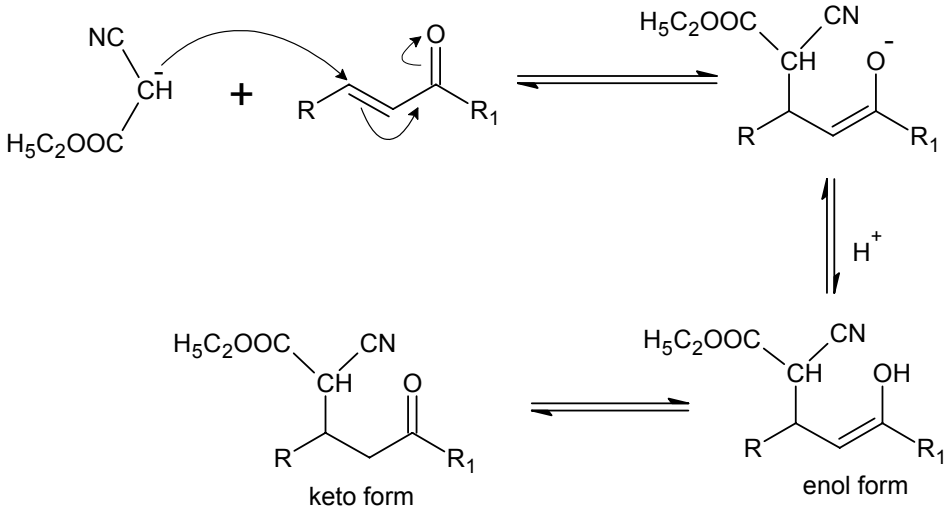
Base facilitates production of anion from ethyl cyanoacetate.

(Base: Sodium ethoxide, pyridine, piperidine)



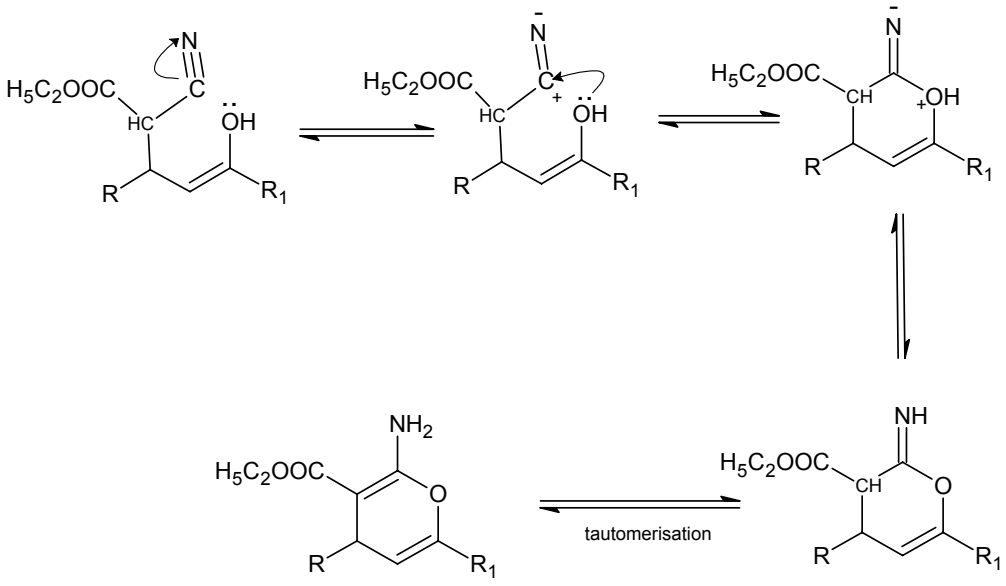
Step-2

Conjugate addition of anion to the α,β-unsaturated carbonyl compound, followed by acceptance of a proton from alcohol to give an enol form which finally tautomerises to keto form.



Step-3

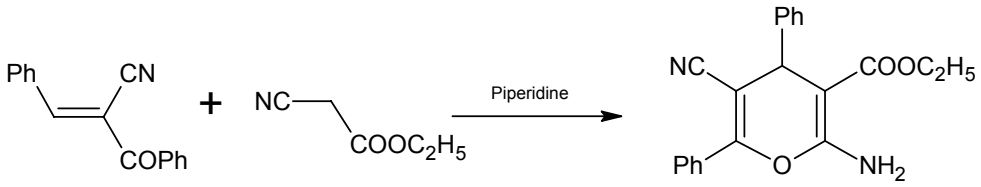
Enolic form of the Michael adduct undergoes nucleophilic intramolecular addition of hydroxy group to cyano group to form cyclic intermediate, which tautomerises to form 2-amino-3-carboethoxy-4,6-disubstituted pyran.



SYNTHESIS OF PYRANS

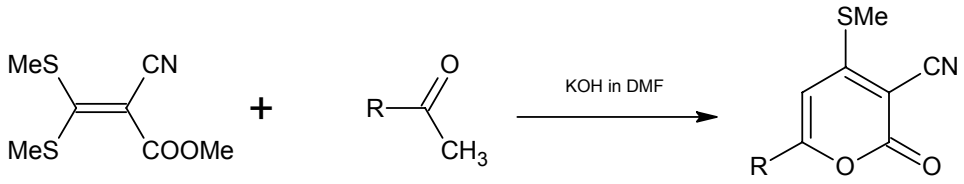
1) From α-cyanochalcone[69]:

The reaction of ethyl cyanoacetate with α-cyanochalcone in the presence of a base piperidine leads to the formation of *β*-enaminoester via Michael addition followed by cyclization to give pyrans



2) From Ketene dithioacetal[70]

The synthesis of 2H-pyran-2-one derivatives by the reaction of various type of acetyl compounds with ketene dithioacetal (methyl-2-cyano-3,3-bis(methylthio)acrylate in the presence of powdered potassium hydroxide in N,N-dimethyl formamide.



3) From Acrylonitrile derivatives (α,β-unsaturated nitrile)[71]

The reaction of α,β-unsaturated nitrile with ethyl acetoacetate in ethanol in the presence of piperidine give corresponding polyfunctional pyran derivatives.

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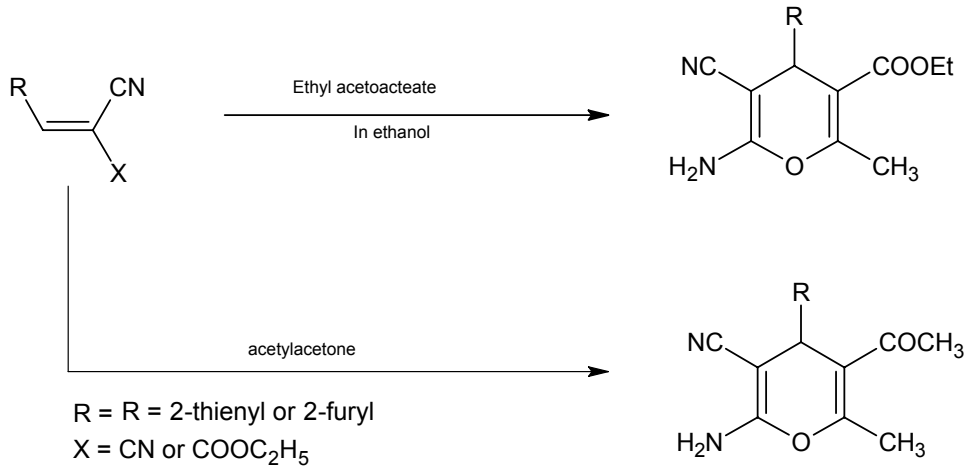
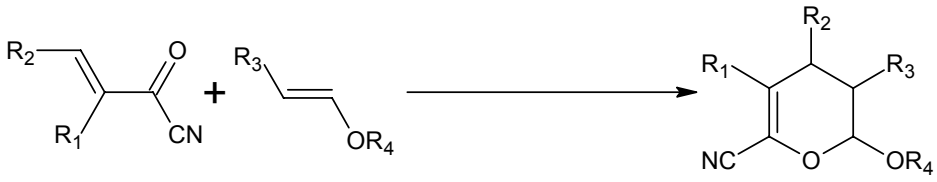


R = R = 2-thienyl or 2-furyl

X = CN or $COOC_2H_5$

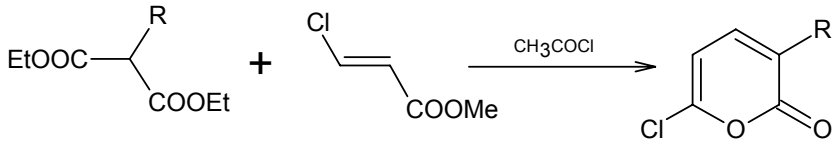
4) From crotonyl cyanide[72]:

The reaction of crotonyl cyanide with ethyl vinyl ether or butyl vinyl ether at room temperature give 2-alkoxy-3,4-dihydro-2H-pyran-6-carbonitrile in quantitative yield.



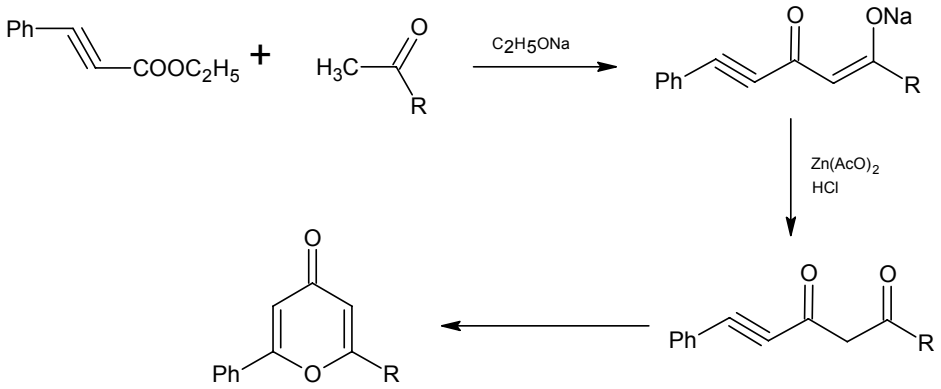
5) From Diethyl malonate[73]:

The appropriately substituted diethyl malonate is heated with methyl cis -2-chloroacrylate in tetrahydrofuran. The resulting triester were saponified , acidified, decarboxylated to give substituted pentenedioic acid followed by reflux with acetyl chloride to get 3-alkyl-6-chloro-2-pyrone.



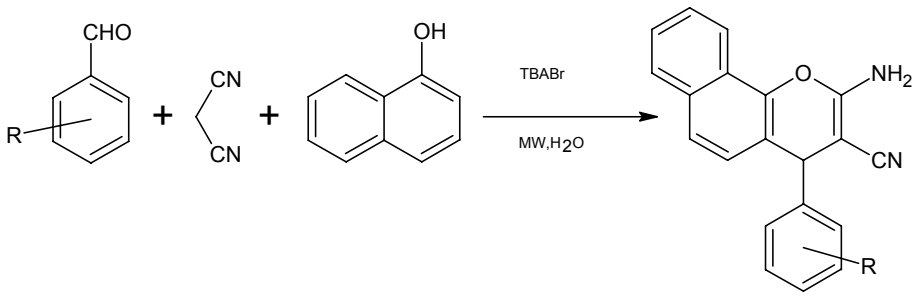
6) From ethyl phenylpropiolate[74]:

The reaction between ethyl phenylpropiolate and suitable ketone in 1:1 molar ratio in dry ether at 0^0C using sodium ethoxide as a base, then the reaction mixture was treated with zinc acetate and hydrochloric acid to form acetylenic β-diketones, which on heating underwent cyclization to give 4H-pyran-4-one.



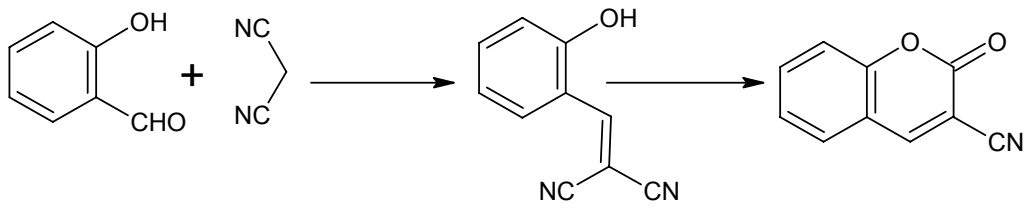
7) From α-napthol or β-napthol[75]:

One pot condensation of aryl aldehyde, α-napthol or β-napthol and malononitrile in the presence of catalytic amount of tetrabutylammoniumbromide heated under Microwave oven.



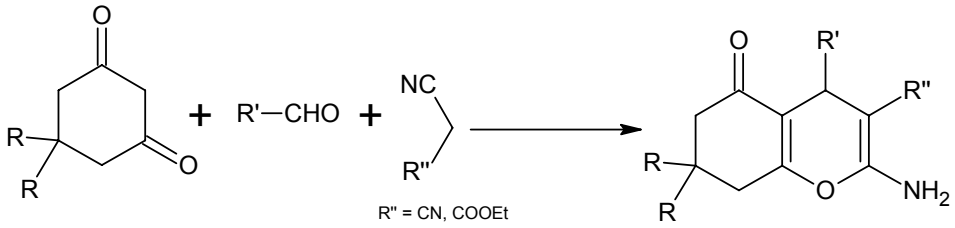
8) From salicyladehyde[77]:

The reaction of salicyladehyde with different active methylene compounds gives arylidene intermediate by Knoevenagel condensation, followed by cyclization to give substituted benzopyran.

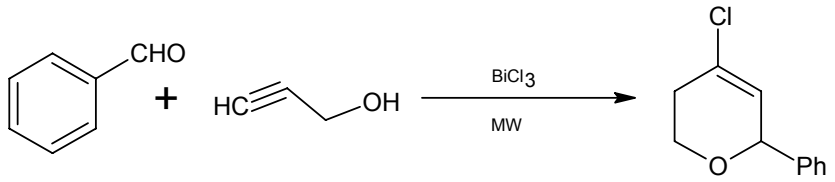


9) From 1,3-cyclohexadione[78]

A mixture of an aldehyde, 1,3-cyclohexadione derivative and an active methylene compound was stirred at room temperature in the presence of an ionic liquid 1-butyl-3-methylimidazolium hydroxide for a certain period of time to give benzo[b]pyran derivatives.



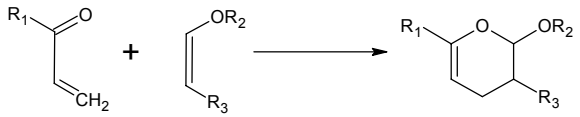
10) From Homopropargylic alcohol[79]



Various aldehydes or ketones were reacted with homopropargylic alcohol in the presence of bismuth chloride under microwave irradiation will give 4-chloro-5,6-dihydro-2H-pyran.

11) Cycloaddition reaction[80]

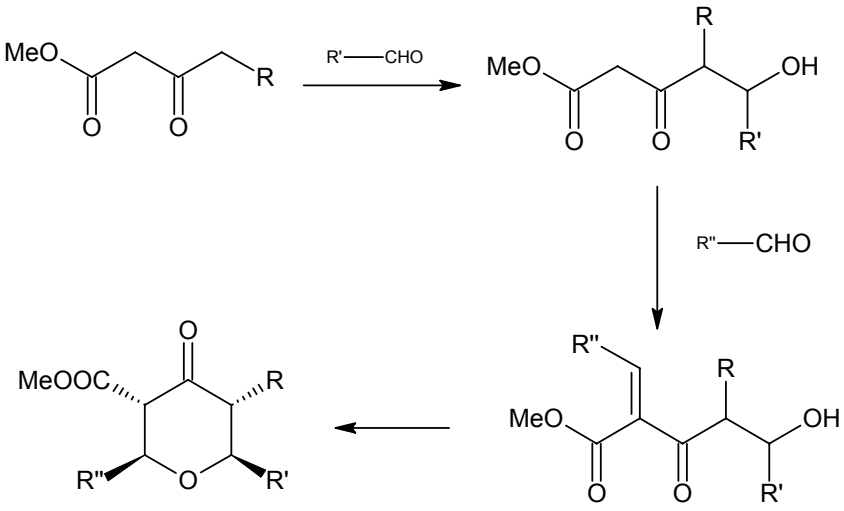
2-Methoxy-6-methyl-3,4-dihydro-2H-pyran synthesized by (4+2) cycloadditon reaction from corresponding vinyl ketones and alkyl vinyl ether.



$R_1 = CH_3$, $R_2 = CH_3$, $R_3 = H$

12) Maitlan-Japp reaction[81]

Condensation of two different aldehyde and a derivative of a β-ketoester in the presence of a lewis acid to form tetrahydropyran-4-one in excellent yield.



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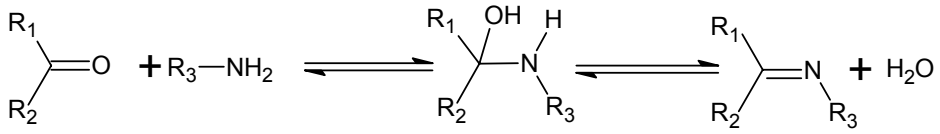
SCHIFF'S BASE[82]

GENERAL METHODS FOR THE FORMATION OF C=N BONDS:

a) Amine carbonyl condensations:

The classic method for the introduction of the carbon nitrogen double bond into the molecule involves the condensation of aldehydes and ketones with a variety of amino compounds (amines, hydroxylamines, hydrazines) followed by elimination of elements of water to give corresponding azomethines.

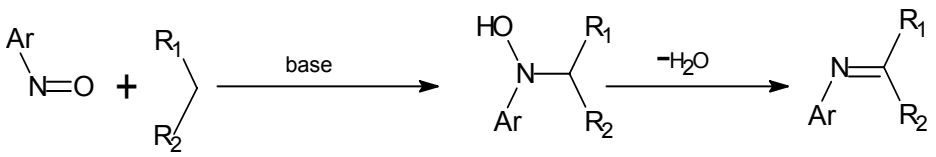


R_1,R_2 = H, alkyl, aryl

R_3 = alkyl, aryl, OH,OR,NHR

b) Condensation reaction involving active methylene compounds:

Aromatic nitroso compounds undergo base catalyzed condensation with active methylene compounds to give intermediate adducts (hydroxylamine derivatives) which can be dehydrated to azomethines.



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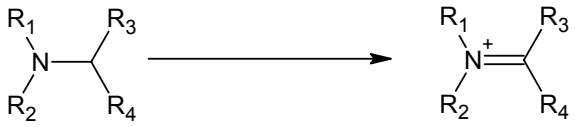
c) Dehydrogenation (oxidation) of amino compounds to azomethines:

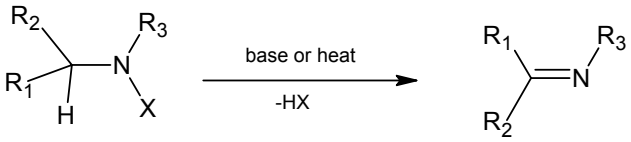
The dehydrogenation of primary or secondary alkylamines over nickel, platinum, chromium catalysts or in contact with sulphur, or selenium gives acceptable yields of corresponding azomethines.

Reagent used: Hydride transfer reagents (diazonium fluoroborates, trityl perchlorate)



d) Elimination reaction leading to azomethines:

Thermal and base catalyzed elimination of substrates derived by electrophilic attack (halogenations, nitrosation, nitration, sulphonation) at the nitrogen atom of primary and secondary alkylamines.



GENERAL REACTION

a) Reaction with electrophiles

Electrophiles attack predominantly at nitrogen atom in the azomethines.

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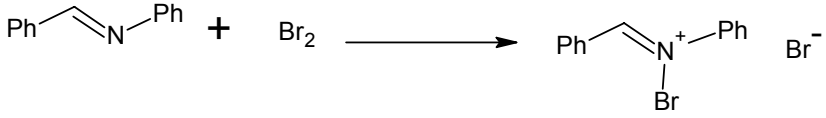
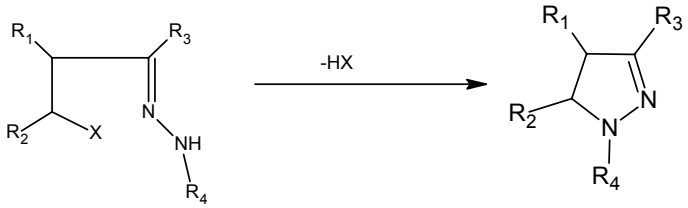
Halogenation

Halogens are reported to add in a 1,2-fashion to the carbon-nitrogen double bond in N-arylaldimines. The product of the reaction of benzylidene aniline with bromine in carbon tetrachloride is formulated as an N-bromoiminium bromide.



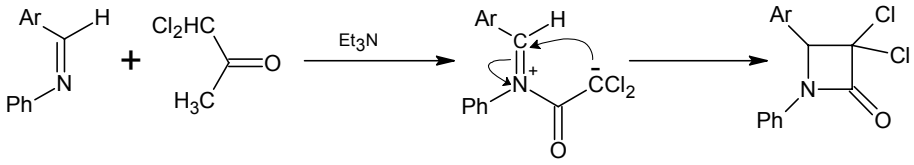
Alkylation:

Direct alkylation of N-alkyl aldimines and ketimines occurs at the nitrogen atom to give corresponding iminium salt. N-alkylation of N-monosubstituted hydrazones has been applied intramolecularly providing a general route to pyrazolines.



Acylation:

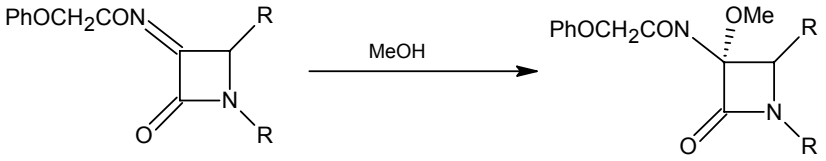
Acylation of schiff's base by acid anhydrides, acid chlorides, and acyl cyanides is initiated by attack at the nitrogen atom and leads to net addition of acylating agent to the carbon nitrogen double bond.



b) Reaction with nucleophiles

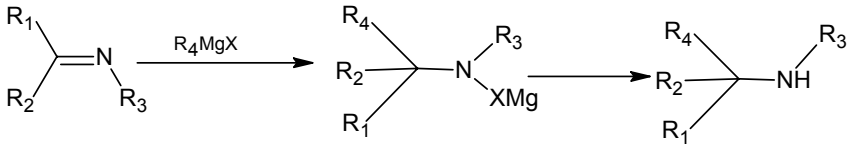
Nucleophilic reagents attack azomethines at imidyl carbon atom.

1) Alkoxide adds to schiff's base giving corresponding α-alkoxyamino compounds.



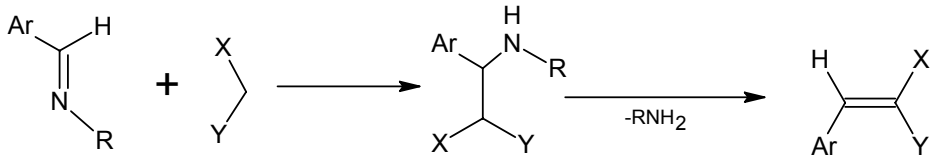
2) Addition of hydrogen cyanide to schiff's base occur readily and provides a viable route to α-amino nitriles, which can inturn be used as the precursors for the synthesis of α-aminoacids.

3) Reaction with Grignard reagents: Schiff's base lacking hydrogen atoms α to the carbon nitrogen double bond react with Grignard reagents to give adducts which on hydrolytic workup afford secondary amine in excellent yield.



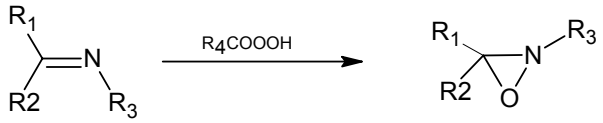
4) Reaction with active methylene compounds: Schiff's base react readily with active methylene compounds to give adducts which tend to eliminate the elements of an amine affording the

corresponding alkene.



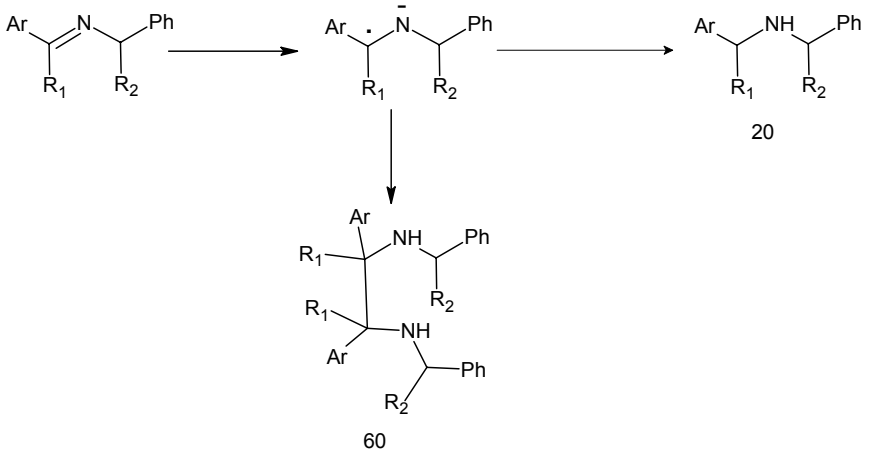
c) Oxidation

Oxidation of schiff's base with a peroxy acid results in cleavage of carbon nitrogen bond to give a carbonyl compound and a nitroso compound. On the other hand oxidation using peroxy acid at low temperature (0^{o}c) affords an excellent synthetic route to oxaziridines.



d) Reduction

Alkali metals in inert solvents such as ether or toluene tend to promote reductive dimerization by a radical coupling mechanism to afford diamino compound as a major product.



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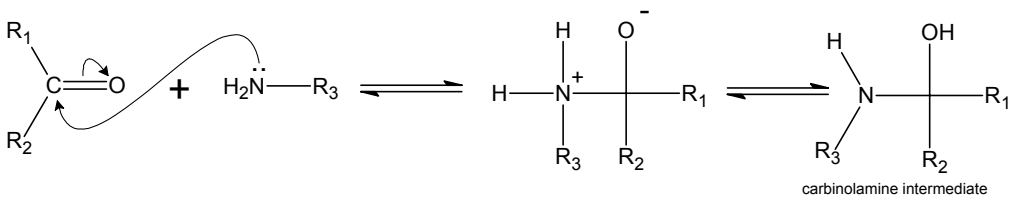
Metal proton reagents (Sodium, Sodium amalgam, Magnesium, Aluminum in ethanol etc.) smoothly reduce Schiff's base to corresponding amines.

MECHANISM

Imines are prepared by a reaction between a carbonyl compound and a primary amine. If the imine contains a hydrogen atom, it is unstable and usually cannot be isolated. However when the imine contains aromatic group on the nitrogen, the resulting imine is stable and can be isolated. The products are called schiff's bases.

Step-1:



carbinolamine intermediate

Nucleophilc addition of the amine to the carbonyl compound followed by transfer of a proton from nitrogen to oxygen leads to the formation of tetrahedral carbinolamine intermediate.

Step-2:

Elimination of water to gives the corresponding azomethines.

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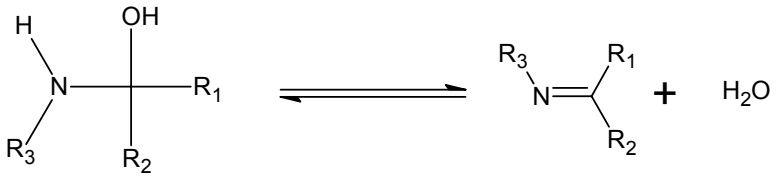

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PURPOSE OF WORK

Antimicrobial agents are the most commonly used and misused drugs. The main consequence of the widespread use of antimicrobial agents leads to the emergence of antibiotic resistant pathogens. This situation fueling an ever increasing needs for new drugs.

OBJECTIVES

1. To synthesize new derivatives and evaluating their possible antimicrobial activity.
2. To study the minimum inhibitory concentration of the highly active compounds.
3. To synthesis highly potent, less toxic compounds.
4. To remove untoward side effects and to prevent development of resistance by infectious microorganisms.

AIM

From the literature survey, six membered heterocyclic compounds containing one oxygen atom such as 4H-pyran or 4H-pyrone constitute an important class of various natural and synthetic products which possess antibacterial, antifungal, antioxidant, anti-HIV and anti-tumor activity.

From the literature review, it was found that various heterocyclic rings and substituted phenyl groups in the pyran ring shows various biological activities. It was also reveals that schiff's bases show different biological activities. In the light of these observations, the purpose of work

is to synthesize cyano pyrans, carboethoxy pyrans and it's Schiff's bases through appropriate synthetic routes and to screen their biological activity.

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EXPERIMENTAL WORK

1. MATERIALS AND METHODS

Chemicals used

Substituted acetophenone (*p*-nitro acetophenone, *p*-chloro acetophenone, *p*-fluoro acetophenone, *p*-methyl acetophenone, *p*-methoxy acetophenone), Sodium Hydroxide, Glacial Acetic Acid, Furfuraldehyde, p-dimethyl amino benzaldehyde, methanol, dioxan, pyridine.

All the chemicals were procured from Hi-media, Sigma Aldrich, S.D.Fine Chemicals Ltd, and Loba Chemicals Pvt. Ltd. All the compounds procured were purified and dried using standard methods before use.

Apparatus used

Beakers (100 and 250ml), RBF, reflux condenser, guard tube, test tubes, conical flask, glass rods, mechanical stirrer, TLC plates, pipettes and heating mantle.

Analytical work

Melting points were determined by using melting point apparatus MR-VIS, Visual Melting Range Apparatus, LABINDIA and were uncorrected.

Reactions were monitored by thin layer chromatography (TLC) on a precoated silica gel G plates using Iodine vapour as visualizing agent.

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Purity of the compounds were recorded on JASCO V-530 UV/VIS spectrophotometer in the Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore-641 044.

IR spectra were recorded on JASCO FTIR-420 Series, Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore-641 044.

PMR spectra were recorded on BRUKER FT-NMR, Shastra University, Thanjur.

Mass spectra were recorded on Shimadzu, LCMS 2010 EV from the Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore-641 044.

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2. SYNTHETIC WORK

SCHEME-1

Step 1: Synthesis of various chalcone derivatives[25]

An ethanolic solution of 0.01 mol aldehyde and 0.01 mol of *p*-substituted acetophenone in the presence of catalytic amount of 30% KOH was stirred for 24 hrs. The resulting solution was then poured over crushed ice, the separated solid was filtered and recrystallized. The purity of the compound was established by getting a single spot on TLC plates. The melting points were determined and uncorrected.

Step 2: Synthesis of cyanopyran derivatives[25]

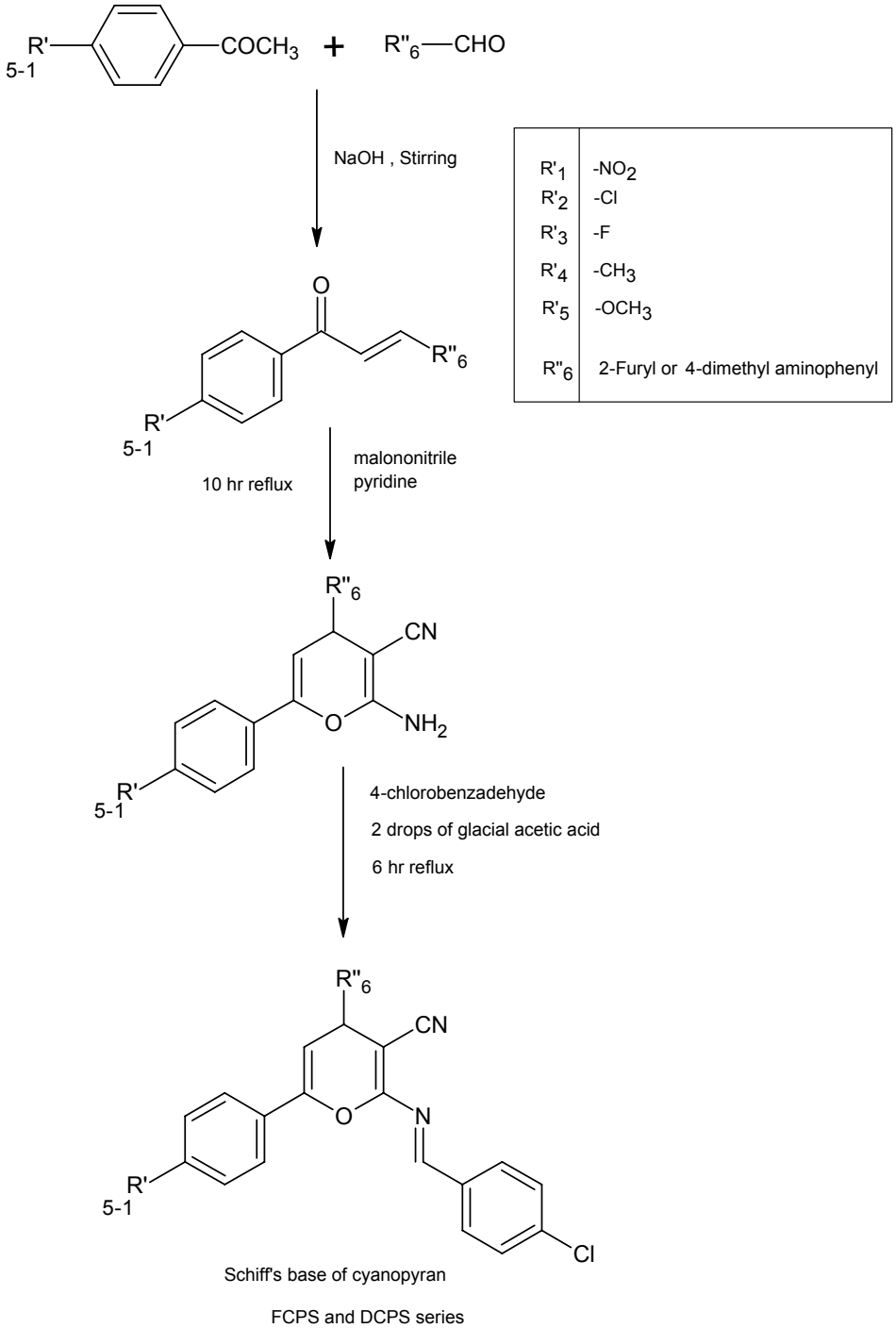
A mixture of above prepared chalcone (0.01 mol) and malononitrile (0.01 mol) dissolved in pyridine (20 ml) was heated under reflux for 10 hrs in an oil bath. The reaction mixture was cooled and poured over crushed ice. The product was isolated and recrystallized. The purity of the compound was established by getting a single spot on TLC plates. The melting points were determined and uncorrected.

Step 3: Synthesis of Schiff's base[83]

The Equimolar mixture of aldehyde and cyanopyran in dioxan was heated under reflux for 6 hrs in presence of few drops of glacial acetic acid. The reaction mixture was cooled and poured in crushed ice. The product was filtered and recrystallized. The purity of the compound was

established by getting a single spot on TLC plates. The melting points were determined and uncorrected.

SCHEME-1


R'
5-1
COCH3
+
R"6—CHO
NaOH , Stirring
R'1 -NO2
R'2 -Cl
R'3 -F
R'4 -CH3
R'5 -OCH3
R"6 2-Furyl or 4-dimethyl aminophenyl
O
R"6
R'
5-1
10 hr reflux
malononitrile
pyridine
R"6
CN
O
NH2
R'
5-1
4-chlorobenzadehyde
2 drops of glacial acetic acid
6 hr reflux
R"6
CN
O
N
R'
5-1
Cl
Schiff's base of cyanopyran
FCPS and DCPS series

SCHEME-2

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Step 1: Synthesis of various chalcone derivatives[25]

An ethanolic solution of 0.01 mol aldehyde and 0.01 mol of *p*-substituted acetophenone in the presence of catalytic amount of 30% KOH was stirred for 24 hrs. The resulting solution was then poured over crushed ice, the separated solid was filtered and recrystallized. The purity of the compound was established by getting a single spot on TLC plates. The melting points were determined and uncorrected

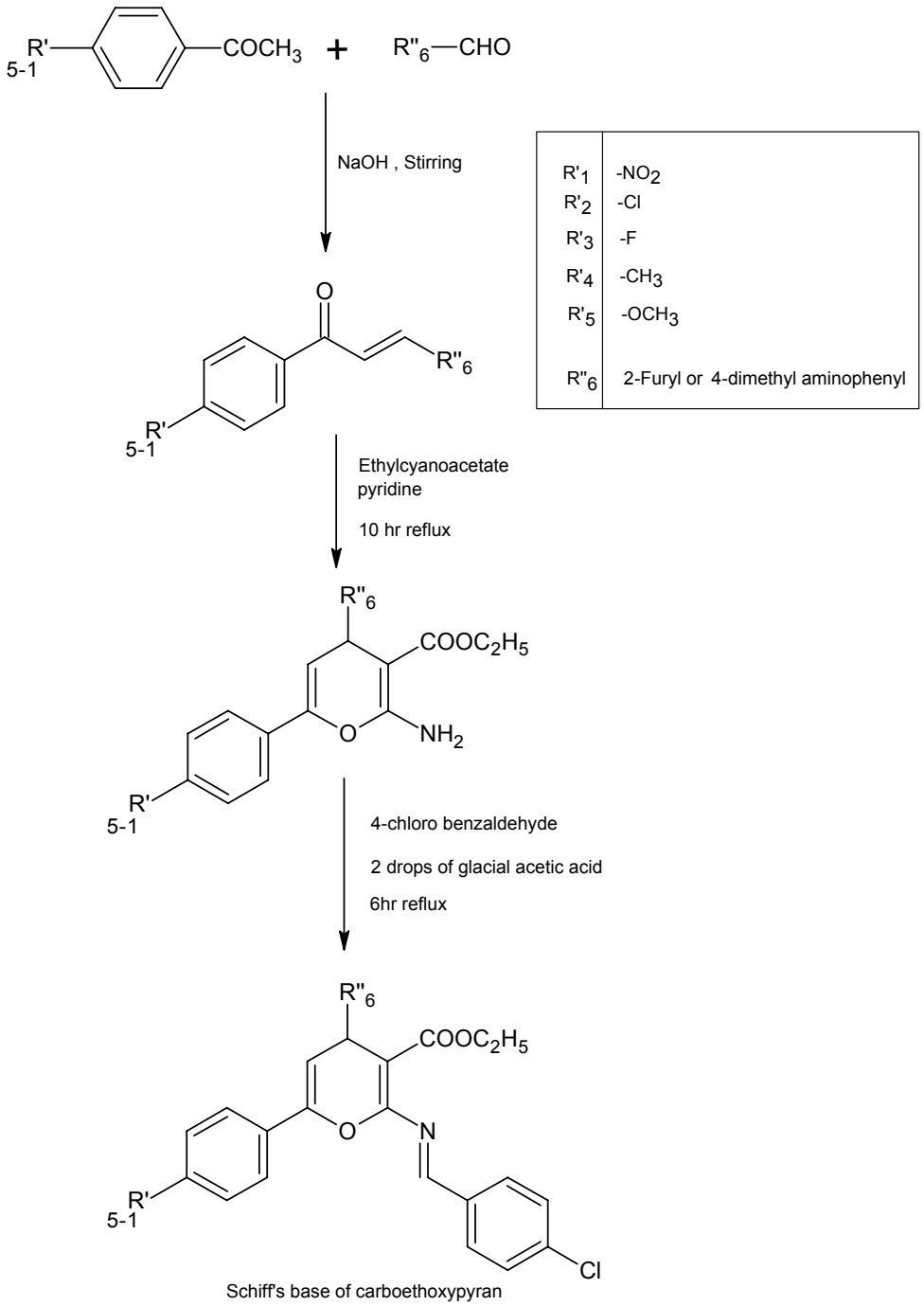
Step 2: Synthesis of carboethoxypyran derivatives[25,27]

A mixture of above prepared chalcone (0.01 mol) and ethylcyanoacetate (0.01 mole) dissolved in pyridine (20 ml) was heated under reflux for 10 hrs in an oil bath. The reaction mixture was cooled and poured over crushed ice. The product was isolated and recrystallized. The purity of the compound was established by getting a single spot on TLC plates. The melting points were determined and uncorrected.

Step 3: Synthesis of Schiff's base[83]

The Equimolar mixture of aldehyde and cyanopyran in dioxan was heated under reflux for 6 hrs in presence of few drops of glacial acetic acid. The reaction mixture was cooled and poured in crushed ice. The product was filtered and recrystallized. The purity of the compound was established by getting a single spot on TLC plates. The melting points were determined and uncorrected.

SCHEME-2



Schiff's base of carboethoxypyran
FPS and DPS series

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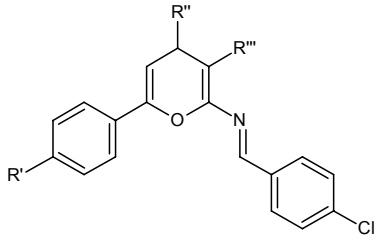
Table: 1 PHYSICAL DATA OF NEWLY SYNTHESIZED COMPOUNDS



Compound code	R'	R''	R'''	Molecular weight	Molecular formula	% yield	R_f value	Melting point
FCP_1S	$-NO_2$	2-furyl	-CN	431.827	$C_{23}H_{14}ClN_3O_4$	65%	0.6	180-182
DCP_1S	$-NO_2$			484.933	$C_{27}H_{21}ClN_4O_3$	75%	0.66	140-142
FCP_3S	-F			404.320	$C_{23}H_{14}ClN_2O_2$	53%	0.63	240-245
DCP_2S	-Cl			474.381	$C_{27}H_{21}Cl_2N_3O$	81%	0.68	162-163
FCP_4S	$-CH_3$			400.856	$C_{24}H_{17}ClN_3O_2$	47%	0.67	165-167
DCP_3S	-F			457.928	$C_{27}H_{21}ClFN_3O$	72%	0.63	168-170
DCP_4S	$-CH_3$			453.962	$C_{28}H_{24}ClN_3O$	58%	0.74	189-192
FCP_5S	$-OCH_3$			416.856	$C_{24}H_{17}ClN_2O_3$	63%	0.72	178-179
DCP_5S	$-OCH_3$	4-Dimeyl amino phenyl		469.962	$C_{28}H_{24}ClFN_3O_2$	59%	0.57	171-172
DP_1S	$-NO_2$		$-COOC_2H_5$	531.986	$C_{29}H_{26}ClN_3O_5$	67%	0.65	110-113
DP_2S	-Cl			521.434	$C_{29}H_{26}Cl_2N_2O_3$	73%	0.75	122-125
DP_3S	-F			504.40	$C_{29}H_{26}ClFN_2O_3$	57%	0.56	130-133
DP_4S	$-CH_3$			501.015	$C_{30}H_{29}ClN_2O_3$	49%	0.62	120-124
DP_5S	-OC			517.015	$C_{30}H_{29}ClFN_2O_4$	63%	0.69	119-120

	H_3							

Recrystallization solvent: methanol except FCP_1S (Dioxan + Methanol)

Solvent system for TLC: Ethyl acetate: n-hexane (6:4) or n-hexane : Dichloromethane (3:7)

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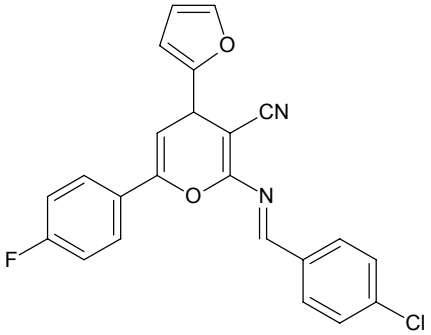
SPECTRAL STUDIES OF THE SYNTHESIZED COMPOUNDS

The structure of the synthesized cyanopyran and carboethoxy pyran derivatives were established on the basis of UV, IR, NMR, Mass spectral data. The purity of cyanopyran and carboethoxy pyran derivatives was established by single spot on TLC plate.

SPECTRAL DATA OF FCP_3S

Compound code : FCP_3S

Structure :



Chemical Name	:	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-fluorophenyl)-4-(furan-2-yl)-4*H*-pyran-3-carbonitrile
Molecular formula	:	$C_{23}H_{14}ClN_2O_2$
Molecular weight	:	404.32
Percentage yield	:	53%
Melting point	:	240-241 ^oC
R_f Value	:	0.63

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1) UV/ Visible spectrum:

λ-max : 386 mn
Solvent used : Methanol

2) IR SPECTRAL DATA [84,85]: (KBr pellet method)

Table: 2 IR Spectral Data of FCP_3S

Sl. No	Peak Number	Types of vibration	Frequency (cm^{-1})
1	22	C≡N Stretching	2213.88
2	23	C=N, C=C Stretching	1604.28
3	28	C–O–C Stretching	1154.19
4	17	Aromatic CH Stretching	3116.42

3) PMR Spectral data [25,26,85,86]

The proton magnetic resonance spectrum of the compound FCP_3S was in full agreement with its molecular formula with regard to proton count and chemical shift.

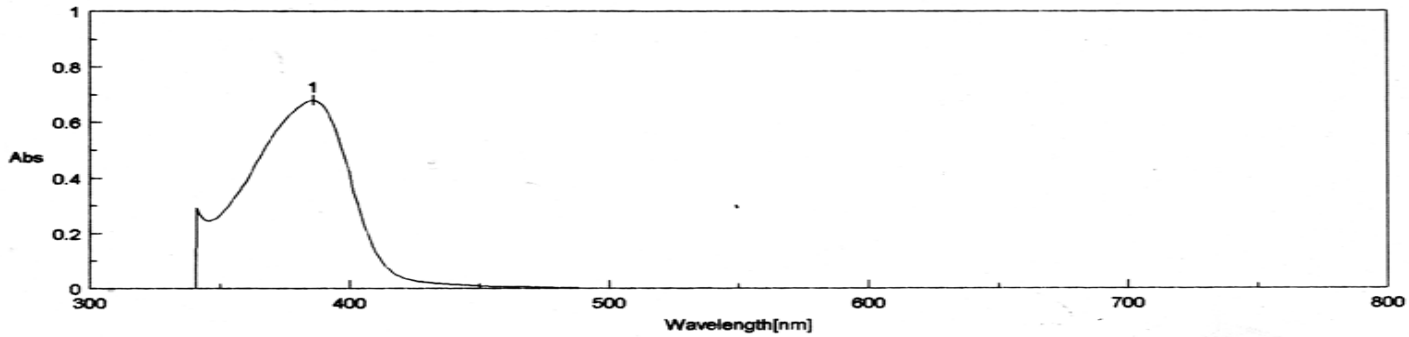
Table: 3 PMR Spectral Data of FCP_3S

Sl no	δ- Value	Types of proton	Number of protons
1	2.7	–CH	1
2	6.9	N=CH	1
3	7.1-8.1	Aromatic & Heteromatic protons	12

4) Mass spectrum

Mass spectrum of the compound FCP_3S was full agreement with its molecular weight.

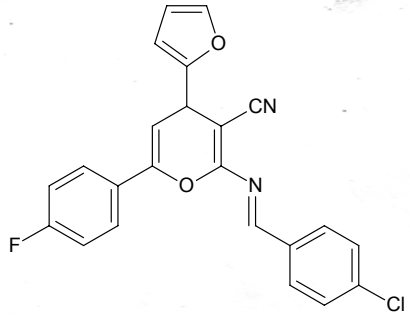
UV Spectrum of FCP_3S



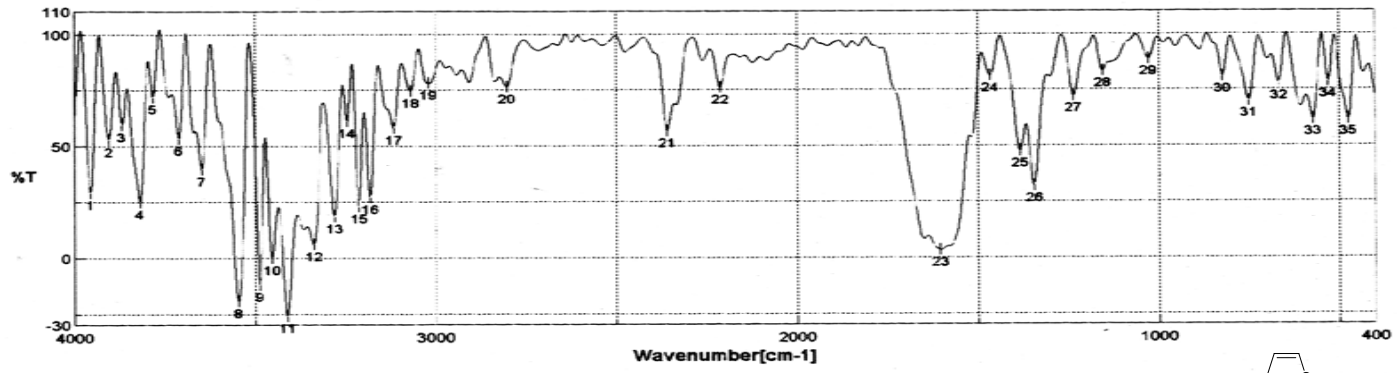
Date	11/27/2008 9:50AM
Model	V-530
Serial No.	B107260512
Band width	2.0 nm
Response	Medium
Measurement range	800 - 200 nm
Data pitch	1nm
Scanning speed	200nm/min
Sample ID	4
No. of cycle	1
Sample name	
Operator	pharm analysis
Comment	

File name FCP 3 S

1: 386, 0.67871

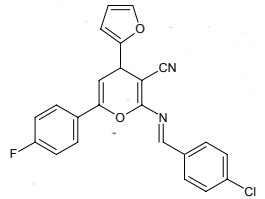


IR Spectrum of FCP_3S



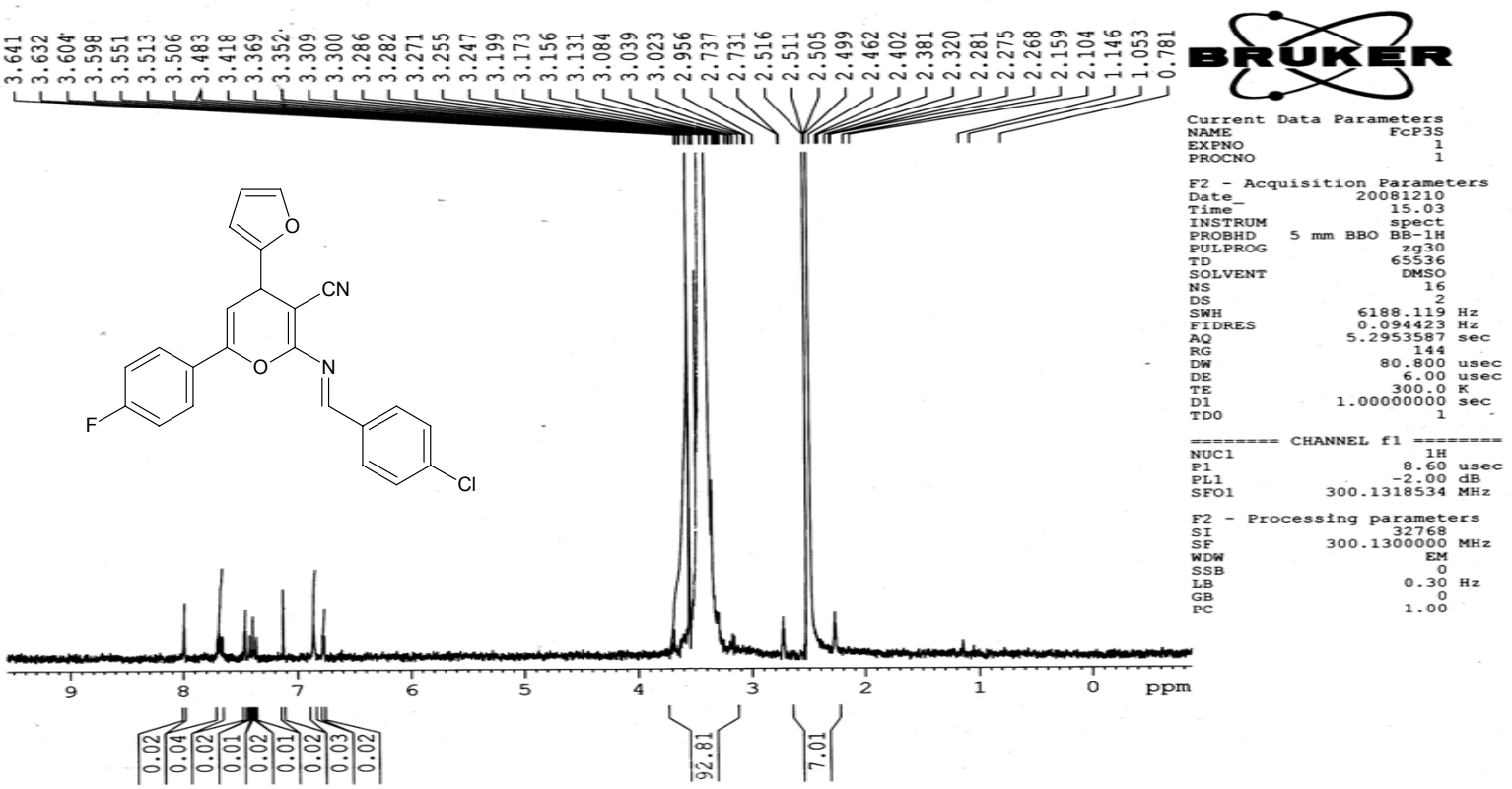
Accumulation	40
Zero Filling	ON
Gain	16
Date/Time	11/19/2008 10:51AM
Operator	C.Geetha
File Name	FCP 3S m
Sample Name	FCP 3S
Comment	

Resolution	4 cm-1
Apodization	Cosine
Scanning Speed	2 mm/sec
Update	12/10/2008 4:03PM



No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3956.25	29.3339	2	3905.15	52.9315	3	3868.5	59.5967	4	3818.36	25.2402	5	3783.65	72.0179
6	3712.3	53.3438	7	3648.66	39.5821	8	3546.45	-19.0751	9	3487.63	-11.8422	10	3453.88	0.00983699
11	3412.42	-25.8959	12	3339.14	6.33365	13	3282.25	18.5688	14	3245.61	51.0491	15	3213.79	23.0459
16	3181.97	27.2076	17	3116.4	58.0094	18	3069.16	74.5004	19	3020.94	78.0427	20	2803.99	76.4241
21	2358.52	56.6791	22	2213.88	76.0233	23	1604.48	3.30046	24	1465.63	81.1762	25	1383.68	47.6032
26	1345.11	32.1768	27	1235.18	72.2438	28	1154.19	83.2981	29	1026.91	88.749	30	821.527	80.8316
31	749.209	69.9936	32	666.285	77.7668	33	571.79	61.664	34	529.364	78.4379	35	474.403	61.5559

PMR Spectrum of FCP_3S



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MASS SPECTRUM OF FCP_3S

Report(Report Editor) Status:Temporary

DEPARTMENT OF PHARMACEUTICAL ANALYSIS
College of Pharmacy, SRIPMS, Coimbatore

Sample Information @D:\Shimadzu MS Data\Methods\chemistry 2009\chemistry 35.lcd

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Dilution Factor	: 1
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Vial#	: 17
Injection Volume	: 10
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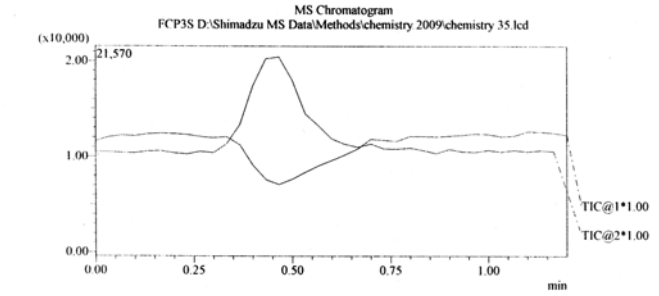
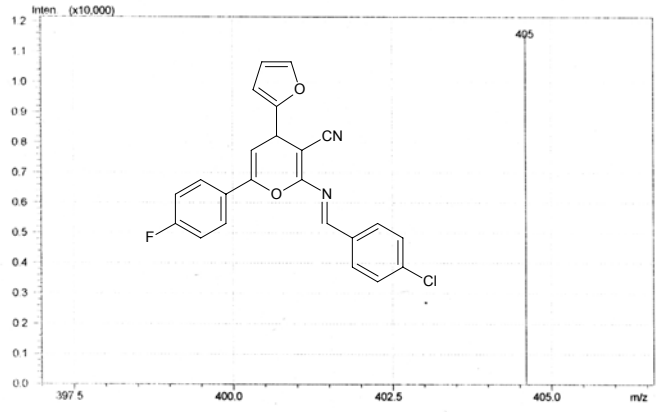


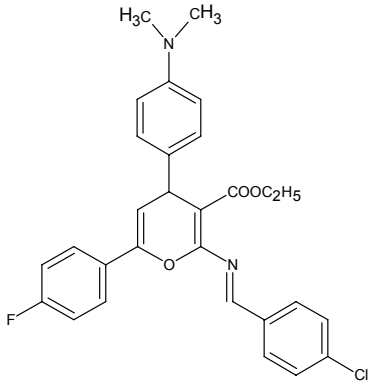


Chapter 6
Spectral Studies

SPECTRAL DATA OF DP_3S

Compound code : DP_3S

Structure :



Chemical Name : 2-{[(*E*)-(4

Molecular formula : $C_{29}H_{26}Cl$

Molecular weight : 504.24

Percentage yield : 57%

Melting point : 130-133 ^{o}C

R_f Value : 0.56

1) UV/ Visible spectrum:

λ-max : 421 nm

Solvent used : Methanol

2) IR SPECTRAL DATA[84,85]: (KBr pellet method)

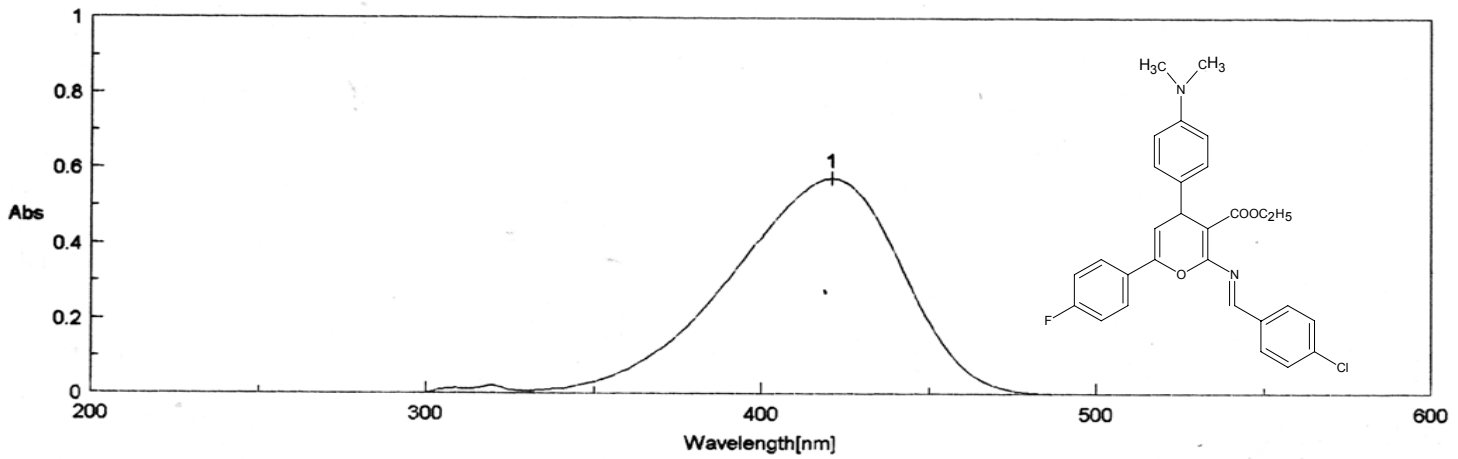
Table: 4 IR Spectral Data of DP_3S

Sl no	Peak Number	Types of vibration	Frequency (cm^{-1})
1	20	C=N, C=C, C=O Stretching	1588.09
2	27	C–O–C Stretching	1087.66
3	25	C–O Stretching in ester	1227.47
4	15	Aromatic CH stretching	3195.47

3) Mass spectrum

Mass spectrum of the compound DP_3S was full agreement with its molecular weight.

UV SPECTRUM OF DP_3S



Date	11/22/2008 0:10PM	File name	DP3S
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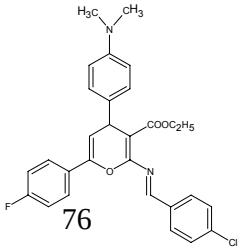
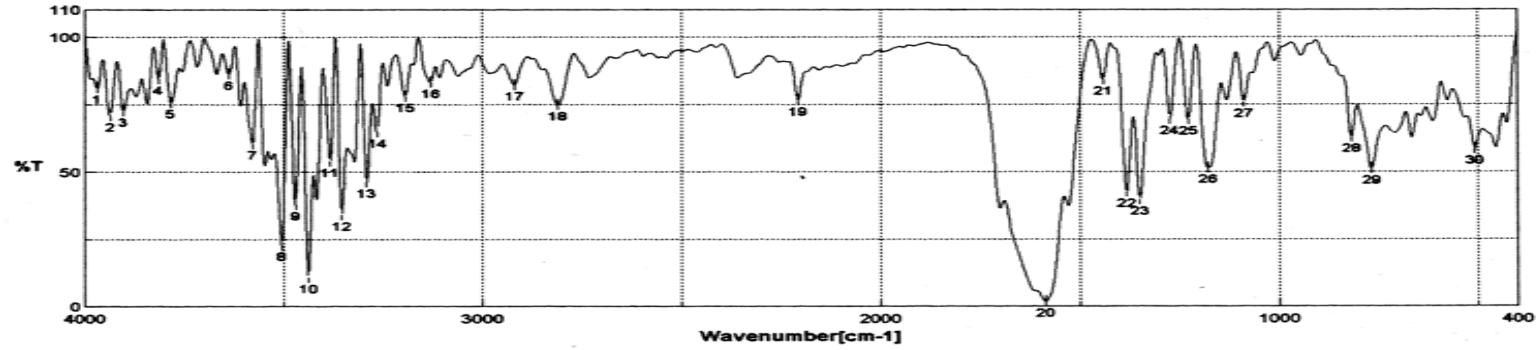
Response	Medium		
Measurement range	800 - 200 nm		
Data pitch	1nm		
Scanning speed	200nm/min		
Sample ID	19		
No. of cycle	1		
Sample name			
Operator	pharm analysis		
Comment			

IR SPECTRUM OF DP_3S





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Zero Filling	ON	Apodization	Cosine
Gain	16	Scanning Speed	2 mm/sec
Date/Time	12/15/2008 11:41AM	Update	1/9/2009 3:04PM
Operator	C.Geetha		
File Name	Memory#2		
Sample Name	DP 3s		
Comment			

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3968.78	81.2375	2	3936.97	70.9703	3	3902.25	72.8797	4	3814.51	84.5312	5	3782.69	75.8079
6	3637.09	86.3635	7	3578.27	60.5445	8	3504.99	22.9903	9	3470.28	38.0262	10	3438.48	10.9309
11	3384.46	54.0982	12	3355.53	34.1005	13	3293.82	46.2984	14	3266.82	64.7765	15	3195.47	78.0647
16	3131.83	83.3421	17	2920.66	82.295	18	2811.7	75.0011	19	2209.06	76.8941	20	1588.09	1.96523
21	1442.49	84.3377	22	1383.68	42.7172	23	1349.93	39.9508	24	1274.72	69.7327	25	1227.47	69.4941
26	1177.33	51.5553	27	1087.66	76.1321	28	817.67	63.0505	29	767.53	51.2688	30	508.151	58.587

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MASS SPECTRUM OF DP_3S

DEPARTMENT OF PHARMACEUTICAL ANALYSIS
College of Pharmacy, SRIPMS, Coimbatore

Sample Information @D:\Shimadzu MS Data\Methods\chemistry 2009\chemistry 37.lcd

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Dilution Factor	: 1
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Vial#	: 19
Injection Volume	: 10
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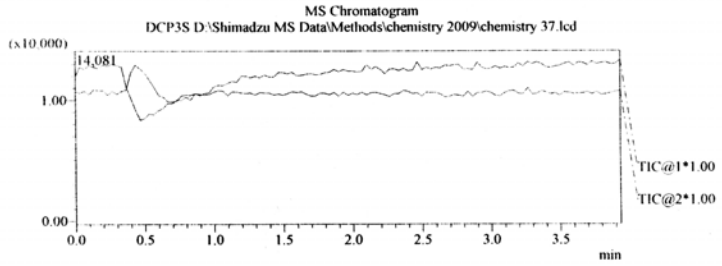

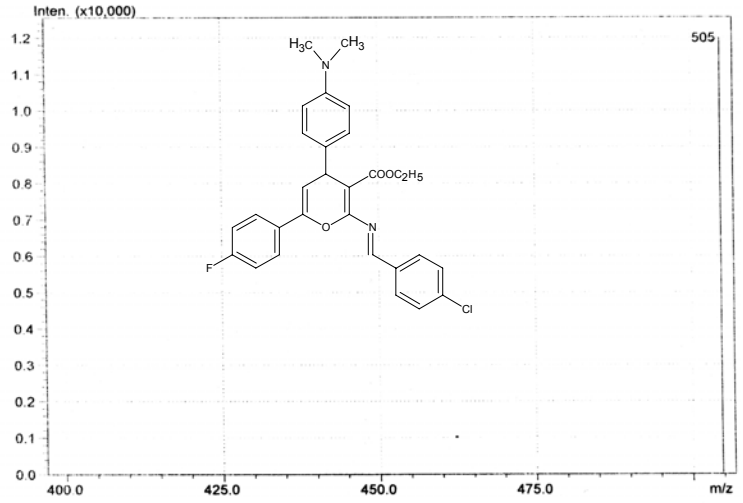

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ANTIMICROBIAL SCREENING [87-89]

APPARATUS AND CHEMICALS REQUIRED

Sterile discs	:	Hi Media
Standard discs of drugs	:	Hi Media
Sterile swab	:	Hi Media
Non- absorbent cotton	:	Rama Raju Surgical cotton Ltd.
Conical flask (250 ml)	:	Borosil
Test Tubes	:	Borosil
Petri dishes	:	SD Fine - Chem Ltd.
Micropipettes	:	VARI pipettes (Hi- Tab Lab)
Hot air oven	:	Technico Equipments
Autoclave	:	Universal Autoclave
Laminar Flow Unit	:	CLEAN AIR Instruments Inc.
Incubator	:	Technico Incubator
Micro tips	:	Tarsons

The antibacterial and antifungal screening were carried out in the Pharmaceutical Biotechnology Laboratory, College of Pharmacy, SRIPMS, Coimbatore.

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SCREENING FOR ANTIBACTERIAL ACTIVITY

Media : Mueller- Hinton agar

Mueller Hinton broth gelled by the addition of 2% agar (bacteriological grade).

Ingredients

Casein enzymic hydrolysate	:	17.5 gm/Ltr
Beef infusion	:	300gm/ Ltr
Soluble starch	:	1.5 gm/ Ltr
Final pH at 25^0C	:	7.4 ± 0.2

Preparation

The ingredients were dissolved in distilled water with the aid of heat and pH was adjusted to 7.2 - 7.6 using alkali or dilute acid.

Sterilization

15-20ml of Mueller Hinton agar was transferred to test tubes and sealed with non-absorbent cotton. It was then autoclaved at a pressure of 15 psi (121^oc) for not less than15 minutes.

Organisms used

Staphylococcus aureus NCIM 5021, *Bacillus subtilis* NCIM 2010 *Proteus vulgaris* NCIM 2027 and *Pseudomonas aeruginosa* NCIM 5029 were collected from the National Chemical Laboratory, Pune and stored in the Pharmaceutical Biotechnology Laboratory, College of Pharmacy,

SRIPMS, Coimbatore-44. The strains were confirmed for their purity and identity by Gram's staining method and by their characteristic biochemical reactions. The selected strains were preserved by sub culturing them periodically on nutrient agar slants and storing them under frozen conditions. For the study, fresh 24 hr broth cultures were used after standardization of the culture.

Working conditions

The entire work was done using horizontal laminar flow hood so as to provide aseptic conditions. Before commencement of the work air sampling was carried out using a sterile nutrient agar plate and exposing it to the environment inside the hood. After incubation it was checked for the growth of microorganism and absence of growth confirmed aseptic working conditions.

Preparation of Inoculum

The inoculum for the experiment was prepared fresh in Mueller Hinton broth from preserved frozen slants. It was incubated at 37 ^{o}C for 18-24 hrs and used after standardization.

Drugs used : Schiff's bases of Cyano pyrans, Carboethoxy pyrans

Standard used : Ciprofloxacin (5 mcg /disc)

Vehicle used : N,N, Dimethyl formamide

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ANTIBACTERIAL SCREENING BY KIRBY-BAUER METHOD

Mueller Hinton agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37^{o}C before inoculation. The organism was inoculated in the plates prepared earlier, by dipping a sterile swab in the previously standardized inoculum, removing the excess of inoculum by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times, rotating the plates through an angle of 60^{o} after each application. Finally the swab was pressed round the edge of the agar surface. It was allowed to dry at room temperature, with the lid closed. The sterile disc containing test drugs, standard and blank were placed on the previously inoculated surface of the Mueller Hinton agar plate and it was kept in the refrigerator for one hour to facilitate uniform diffusion of the drug. Plates were prepared in triplicate and they were then incubated for 18-24 hrs. Observations were made for zone of inhibition around the drugs and compared with that of standard. All the compounds synthesized were tested for antibacterial activity against gram-positive and gram-negative bacteria.

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Table: 5 QUANTITATIVE SCREENING OF THE TEST COMPOUNDS FOR ANTIBACTERIAL ACTIVITY AGAINST GRAM POSITIVE ORGANISMS

Sl. No.	Compound Code	Diameter of Zone of Inhibition in mm			
		Staphylococcus aureus NCIM 5021		*Bacillus subtilis* NCIM 2010	
		1000mcg/disc	500mcgg/disc	1000mcg/disc	500mcg/disc
1	FCP_1S	20	16	17	13
2	*FCP_3S		21		20
3	FCP_4S	21	15	19	15
4	FCP_5S	17	13	17	14
5	DCP_1S	18	15	15	13
6	DCP_2S	17	14	20	16
7	*DCP_3S		22		26
8	*DCP_4S		17		19
9	*DCP_5S		22		17
10	DP_1S	22	15	19	16
11	*DP_2S		18		23
12	DP_3S	19	14	21	16
13	DP_4S	17	14	19	15
14	DP_5S	16	13	16	14
15	Blank (DMF)	-	-	-	-
16	Standard Ciprofloxacin (5 mcg/disc)	36		36	

(-) indicates no zone of inhibition

Zone diameter: 18 and above- Sensitive, 12-17-Moderately sensitive, <12-Resistant

* Compounds were screened only at concentration of 500 mcg/ disc.

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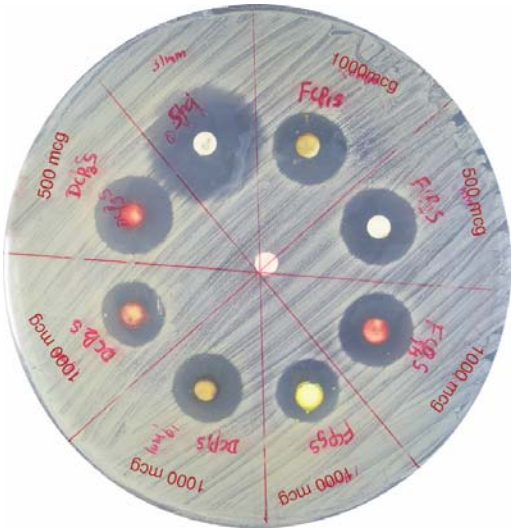
SCREENING OF SYNTHESIZED COMPOUNDS FOR ACTIVITY AGAINST GRAM POSITIVE ORGANISMS

Zone of inhibition of the sensitive compounds against *Staphylococcus aureus* NCIM 5021

Concentration used : **1000 mcg /disc and 500 mcg/disc**

Standard disc : **Ciprofloxacin 5 mcg /disc**

Solvent used : **N,N-Dimethyl formamide**



Chapter 6
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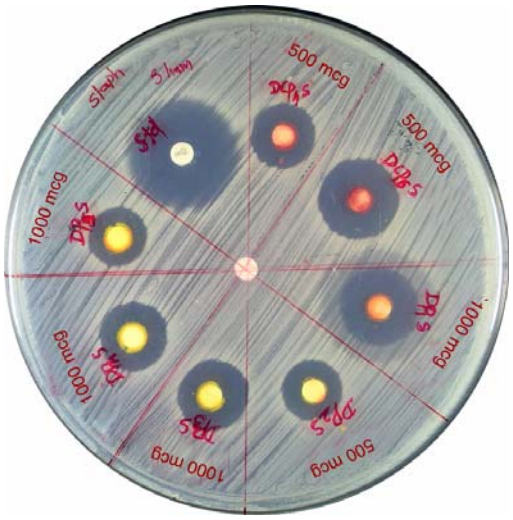

SCREENING OF SYNTHESIZED COMPOUNDS FOR ACTIVITY AGAINST GRAM POSITIVE ORGANISMS

Zone of inhibition of the sensitive compounds against *Basillus subtilis* NCIM 2010

Concentration used	**: 1000 mcg/disc and 500 mcg/disc**
Standard disc	**: Ciprofloxacin 5 mcg/disc**
Solvent used	**: N,N-Dimethyl formamide**

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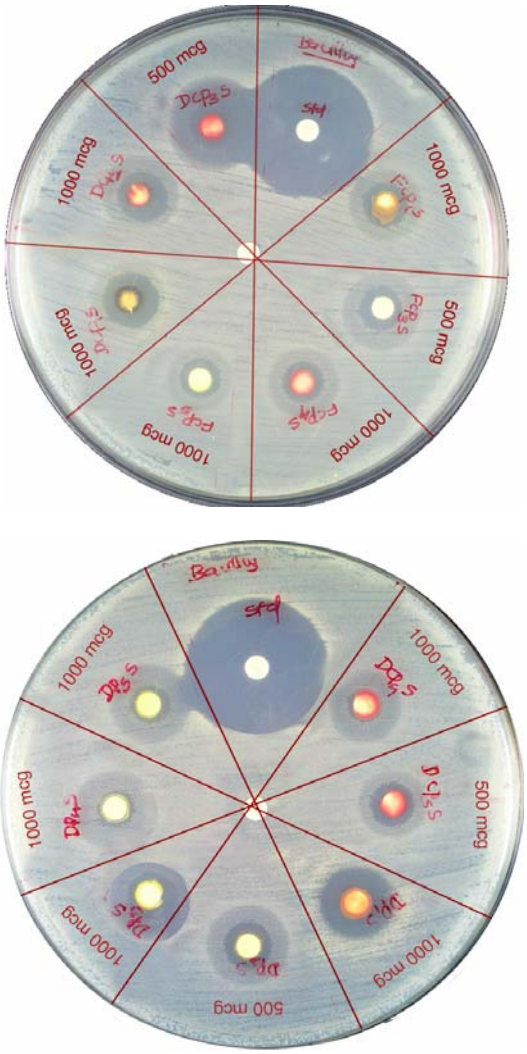

Table: 6 QUANTITATIVE SCREENING OF THE TEST COMPOUNDS FOR ANTIBACTERIAL ACTIVITY AGAINST GRAM NEGATIVE ORGANISMS

S. No.	Compound Code*	Diameter of Zone of Inhibition in mm			
		Proteus vulgaris NCIM 2027		*Pseudomoas aeruginosa* NCIM 5029	
		1000mcg/disc	500mcg/disc	1000mcg/disc	500mcg/disc
1	FCP1S		-	13mm	-

2	*FCP_3S	-	-	-	-
3	FCP_4S	-	-	-	-
4	FCP_5S	-	-	-	-
5	DCP_1S	-	-	-	-
6	DCP_2S	-	-	-	-
7	*DCP_3S	-	-	-	-
8	*DCP_4S	-	-	-	-
9	*DCP_5S	-	-	-	-
10	DP_1S	-	-	-	-
11	*DP_2S	-	-	-	-
12	DP_3S	<11mm	-		-
13	DP_4S	-	-	-	-
14	DP_5S	-	--	-	-
15	Blank (DMF)	-	-	-	-
16	Standard Ciprofloxacin (5 mcg/disc)	38		23	

(-) indicates no zone of inhibition

Zone diameter: 18 and above- Sensitive, 12-17-Moderately sensitive, <12-

Resistant

***** Compounds were screened only at concentration of 500 mcg/ disc

SCREENING OF SYNTHESIZED COMPOUNDS FOR ACTIVITY AGAINST GRAM NEGATIVE ORGANISMS

Zone of inhibition of the sensitive compounds against *Pseudomoas aeruginosa* NCIM 5029

Concentration used : 1000 mcg/disc and 500 mcg/disc

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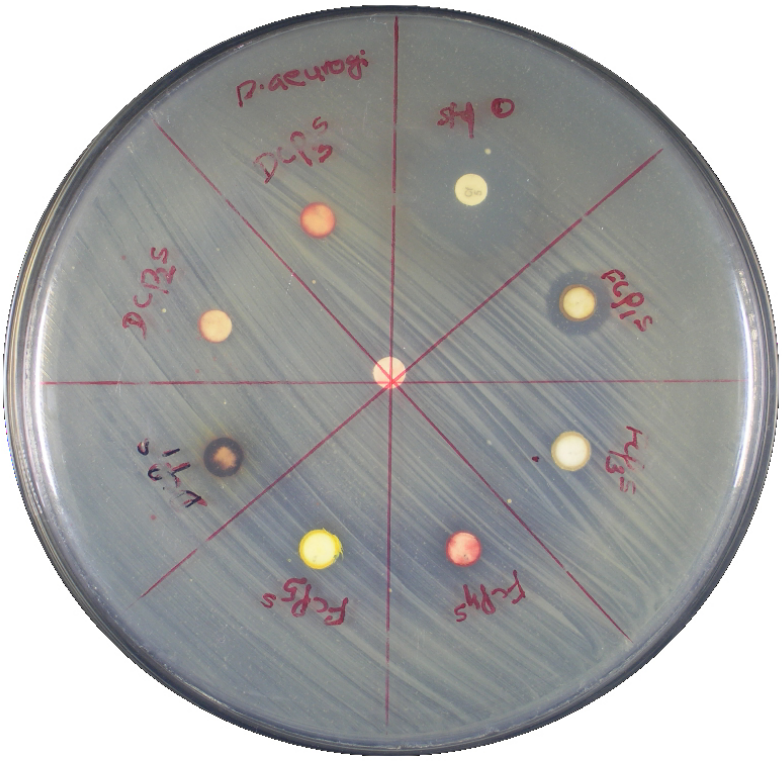
Standard disc : **Ciprofloxacin 5 mcg/disc**

Solvent used : **N,N-Dimethyl formamide**



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DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

Compound with good activity were selected for the determination of minimum inhibitory concentration. Muller Hinton broth is used as the media for bacterial screening.

Preparation of inoculums

The inoculum for the experiment was prepared fresh in the media from preserved frozen slant culture. It was kept incubated at 37^{o}C for 24 hrs and used for the study after dilution to give 1: 10 or 1:100 dilutions.

Standardization of inoculum

After overnight incubation, the test organism was diluted to 1:100. Standard drop of 0.01 ml was used for the determination of minimum inhibitory concentration.

In Vitro determination of Minimum Inhibitory Concentration

It was carried out by two fold serial dilution technique.

Procedure

- Test tubes were numbered as 1-9 and 1 ml of Muller Hinton broth was added to each tubes. They were autoclaved at a pressure of 15 psi at 121^{o}C temperature not less than 15 mins.

- 1ml of the diluted stock solution (1000 mcg/disc) was added to the first test tube and serially transfer 1 ml upto the 8th test tube to obtain the quantities indicated.
- From the 8th test tube, 1 ml was discarded.
- The 9th test tube was used as the control.
- Diluted broth culture of the test organism (0.01ml) was added to all the test tubes including the control with a standard micropipette. Mixed gently and incubated at 37°C for 16-18 hrs.
- Readings were observed.
- The above procedure was carried out in duplicate.
- The MIC was interpreted as the highest dilution of the test compound, which showed clear fluid with no development of turbidity.

Table: 7

Tube number	1	2	3	4	5	6	7	8	Control
Media (ml)	1	1	1	1	1	1	1	1	1
Drug in serial dilution (ml)	1	1	1	1	1	1	1	1	-
Discard (ml)	-	-	-	-	-	-	-	1	-
Culture	Add 0.01 ml to each tube, Mix gently, kept in incubator for 24 hrs								

Concentration (mcg/ml)	1024	512	256	128	64	32	16	8	-

Media used for bacterial screening: Muller Hinton broth

IN VITRO MIC DETERMINATION OF COMPOUNDS AGAINST *Staphylococcus aureus* NCIM 5021

The in vitro MIC determination of FCP_3S, DCP_3S, DCP_5S was carried out by two fold serial dilution technique against staphylococcus aureus NCIM 5021. The concentration range was fixed from 1024 to 8 mcg/ml. The results were tabulated below

Table: 8

Compound code	Concentration (mcg/ml)								
	1024	512	256	128	64	32	16	8	MIC values (mcg/ml)
FCP_3S	-	-	-	-	+	+	+	+	128
DCP_3S	-	-	-	-	-	+	+	+	64
DCP_5S	-	-	-	-	-	+	+	+	64
Control	-	-	-	-	-	-	-	-	
Blank	+	+	+	+	+	+	+	+	

(+) indicates turbidity

(-) indicates clear

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IN VITRO MIC DETERMINATION OF COMPOUNDS AGAINST *Bacillus subtilis* NCIM 2010

The in vitro MIC determination of FCP_3S, DCP_3S, DP_2S was carried out by two fold serial dilution technique against staphylococcus aureus NCIM 5021. The concentration range was fixed from 1024 to 8 mcg/ml. The results were tabulated below.

Table: 9

Compoud code	Concentration (mcg/ml)								
	1024	**512**	**256**	**128**	**64**	**32**	**16**	**8**	**MIC values (mcg/ml)**
FCP_3S	-	-	-	-	+	+	+	+	128
DCP_3S	-	-	-	-	-	-	-	+	16
DP_2S	-	-	-	-	-	+	+	+	64
Control	-	-	-	-	-	-	-	-	
Blank	+	+	+	+	+	+	+	+	

(+) indicates turbidity

(-) indicates clear

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SCREENING FOR ANTIFUNGAL ACTIVITY

MATERIALS AND METHODS:

Media **:** Sabouraud Dextrose Agar

Ingredients

Mycological peptone	:	10 gm
Dextrose	:	40 gm
Agar	:	15 gm
Final pH at 25^0C	:	5.4 ± 0.2

Water to make 1000 ml

Preparation

65 gm of Sabouraud dextrose agar was suspended in 1000 ml of distilled water and boiled to dissolve the medium completely.

Sterilization

15-30 ml of Sabouraud dextrose agar was transferred to comical flask and sealed. It was then autoclaved at a pressure of 15 psi (121^oc) for not less than15 minutes.

Organism used

Candida albicans NCIM 3100 and *Aspergillus niger* NCIM 545 were procured from National Chemical Laboratory, Pune and stored in the Pharmaceutical Biotechnology Laboratory, College of Pharmacy, SRIPMS, Coimbatore.

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Working conditions

The entire work was done using horizontal laminar flow hood so as to provide aseptic conditions. Before commencement of the work air sampling was carried out using a sterile nutrient agar plate and exposing it to the environment inside the hood. After incubation it was checked for the growth of microorganism and absence of growth confirmed aseptic working conditions.

Preparation of inoculum

The inoculum for the experiment was prepared fresh in Sabouraud Dextrose broth from preserved frozen slants. It was incubated at 25^{o}C for 24-48 hrs and used after standardization.

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ANTIFUNGAL SCREENING

Sabouraud dextrose agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 25^{o}C just before inoculation.

The organisms (*Candida albicans* NCIM 3100 and *Aspergillus niger* NCIM 545*)* were inoculated in the plates prepared earlier by dipping sterile swab in the inoculum, removing the excess of inoculum by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking a swab all over the surface of the medium three times, rotating the plates through the angle of 60^{o} after each application. Finally the swab was pressed round the edges of the agar surface. It was left to dry at room temperature with the lid closed. Sterile discs containing the test, standard and blank were placed in the petridish aseptically.

Plates were prepared in triplicate and they were incubated at 25^{o}C for 24-48 hrs, after placing them in the refrigerator for one hour to facilitate uniform diffusion. Observations were made for the zone of inhibition around the discs and compared with that of Fluconazole, the standard. All the compounds were tested for antifungal activity.

Drugs used	:	Schiff's bases of carboethoxy pyrans and cyano pyrans
Standard used	:	Fluconazole (10mcg/disc)
Vehicle used	:	N,N-Dimethyl formamide

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Table: 10 QUANTITATIVE SCREENING OF THE TEST COMPOUNDS FOR ANTIFUNGAL ACTIVITY

Sl. No.	Compound Code*	Diameter of Zone of Inhibition in mm			
		Candida albicans NCIM 3100		*Aspergillus niger* NCIM 545	
		1000mcg/disc	500mcg/disc	1000mcg/disc	500mcgg/disc
1	FCP1S		-	-	-
2	*FCP_3S	-	-	-	-
3	FCP_4S	-	-	-	-
4	FCP_5S	-	-	-	-
5	DCP_1S	-	-	-	-
6	DCP_2S	-	-	-	-
7	*DCP_3S	-	-	-	-
8	*DCP_4S	-	-	-	-
9	*DCP_5S	-	-	-	-
10	DP_1S	-	-	-	-
11	*DP_2S	-	-	-	-
12	DP_3S	-	-	-	-
13	DP_4S	-	-	-	-
14	DP_5S	-	--	-	-
15	Blank (DMF)	-	-	-	-
16	Standard (Fluconazole 10 mcg/disc	26		28	

(-) indicates no zone of inhibition

Zone diameter: 18 and above- Sensitive, 12-17-Moderately sensitive, <12-

Resistant

* Compounds were screened only at concentration of 500 mcg/ disc

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RESULTS AND DISCUSSION

SYNTHESIS

All the newly synthesized compounds were evaluated for their physical (Melting point and TLC) and spectral (IR, PMR, Mass) data.

ANTIBACTERIAL ACTIVITY

All the newly synthesized compounds were screened for their antibacterial activity against both gram negative and gram positive organisms by Agar diffusion method (Kirby-Bauer method).

Gram positive organisms screened

Staphylococcus aureus NCIM 5021

Bacillus subtilis NCIM 2010

Gram negative organisms screened

Pseudomonas aeruginosa NCIM 5029

Proteus vulgaris NCIM 2027

Concentrations of 1000 mcg/disc, 500 mcg/disc were used for the test compounds and results are compared with standard drug Ciprofloxacin at 10 mcg/disc concentration. Dimethyl formamide was used as the solvent. The results were interpreted as per Kirby-Bauer method (18 mm & above: Sensitive, 12-17 mm: Moderately sensitive, <12 mm: resistant).

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Activity against Gram positive Organism

Organism: *Staphylococcus aureus* NCIM 5021

- Among the newly synthesized compounds FCP_1S, FCP_4S, DCP_1S, DP_1S, DP_3S, were found to be sensitive at concentration of 1000 mcg/disc.
- The compounds FCP_3S, DCP_3S, DCP_5S were found to be sensitive at concentration of 500 mcg/disc. All the other compounds were moderately sensitive.
- MIC values of the compounds FCP_3S, DCP_3S, DCP_5S were determined as follows,

Compound Code	MIC Value
FCP_3S	128 mcg/disc
DCP_3S	64 mcg/disc
DCP_5S	64 mcg/disc

Organism: *Bacillus subtilis* NCIM 2010

- Among the newly synthesized compounds FCP_4S, DCP_2S, DP_1S, DP_3S, DP_4S, were found to be sensitive at concentration of 1000 mcg/disc.
- The synthesized compounds FCP_3S, DCP_3S, DCP_4S, DP_2S were found to be sensitive at concentration of 500 mcg/disc, and all the other compounds were moderately sensitive.

- MIC values of the compounds FCP_3S, DCP_3S, DP_2S were determined as follows:-

Compound Code	MIC Value
FCP_3S	128 mcg/disc
DCP_3S	16 mcg/disc
DP_2S	64 mcg/disc

Activity against Gram negative Organism

- The compound FCP_1S was found to be moderately sensitive against *pseudomonas aeruginosa* NCIM 5209. All the other compounds were inactive against pseudomonas *aeruginosa* NCIM 5209 and *proteus vulgaris* NCIM 2027.

ANTIFUNGAL ACTIVITY

All the synthesized compounds were screened for antifugal activity against *Candida albicans* NCIM 3100 and *Aspergillus niger* NCIM 545 by Agar diffusion method (Kirby-Bauer method) using Fluconazole (25 mcg/disc) as standard and Dimethyl formamide as solvent.

- All the newly synthesized compounds found to be inactive against *Candida albicans* NCIM 3100, *Aspergillus niger* NCIM 545.

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SUMMARY AND CONCLUSION

SUMMARY

In the present work 14 Schiff's bases of carboethoxy pyrans and cyano pyrans were synthesized in two schemes.

SCHEME-I

This scheme involved the synthesis of 9 Schiff's bases of cyano pyrans.

STEP-I

This step involved the formation of chalcones from aldehydes and ketones in the presence of a base.

STEP-II

This step involved the formation of cyano pyrans from chalcones in the presence of pyridine, malononitrile.

STEP-III

In this step, Schiff's bases prepared from cyano pyrans and aldehyde in dioxan.

SCHEME-II

This scheme involved the synthesis of 5 Schiff's bases of carboethoxy pyrans.

STEP-I

This step involved the formation of chalcone from aldehyde and ketones in the presence of a base.

STEP-II

This step involved the formation of carboethoxy pyran from chalcone in the presence of pyridine, ethylcyanoacetate.

STEP-III

In this step, Schiff's base prepared from carboethoxy pyran and aldehyde in dioxan.

SPECTRAL STUDIES

The structure of the synthesized compounds has been established on the basis of UV, IR, PMR and Mass spectral data.

SCREENING FOR BIOLOGICAL ACTIVITY

Activity against Gram positive Organism:

Organism: *Staphylococcus aureus*

- Among the newly synthesized compounds FCP_1S, FCP_4S, DCP_1S, DP_1S, DP_3S were found to be sensitive at concentration of 1000 mcg/disc.
- The compounds FCP_3S, DCP_3S, DCP_5S were found to be sensitive at concentration of 500 mcg/disc. All the other compounds were moderately sensitive.

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Organism: *Bacillus subtilis*

- Among the newly synthesized compounds FCP_4S DCP_2S, DP_1S, DP_3S, DP_4S, were found to be sensitive at 1000 mcg/disc.

- The synthesized compounds FCP_3S, DCP_3S, DCP_4S, DP_2S were found to be sensitive at concentration of 500 mcg/disc. All the other compounds were moderately sensitive.

Activity against Gram negative Organism

- The compound FCP_1S was found to be moderately sensitive against *pseudomonas aeruginosa*. All the other compounds were not active against *Pseudomonas aeruginosa* and *proteus vulgaris.*

ANTIFUNGAL ACTIVITY

- All the newly synthesized compounds were found to be inactive against *Candida albicans*, *Aspergillus niger*.

CONCLUSION

- All the newly synthesized compounds were found to be sensitive, moderately sensitive against gram positive organisms.
- Among the newly synthesized compounds FCP_1S, FCP_4S, DCP_1S, DP_1S, DP_3S were found to be sensitive at concentration of 1000 mcg/disc against *staphylococcus aureus.*

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- The compounds FCP_3S, DCP_3S, DCP_5S were found to be sensitive at concentration of 500 mcg/disc against *Staphylococcus aureus*. All the other compounds were moderately sensitive.
- Among the newly synthesized compounds FCP_4S DCP_2S, DP_1S, DP_3S, DP_4S, were found to be sensitive at 1000 mcg/disc against *Bacillus subtilis*.

- The synthesized compounds FCP_3S, DCP_3S, DCP_4S, DP_2S were found to be sensitive at concentration of 500 mcg/disc against Bacillus subtilis. All the other compounds were moderately sensitive.
- The compound FCP_1S were found to be moderately sensitive against *pseudomonas aeruginosa*. All the other compounds were not active against *Pseudomonas aeruginosa* and *proteus vulgaris.*
- All the newly synthesized compounds were not active against *Candida albicans, Aspergillus niger.*

From the above observation, it was concluded that various derivatives of carboethoxy pyran and cyano pyran are one of the milestone in the research area. In the present work, all the compounds were active against gram positive bacteria. This work may encourage to do further studies on pyran moiety.

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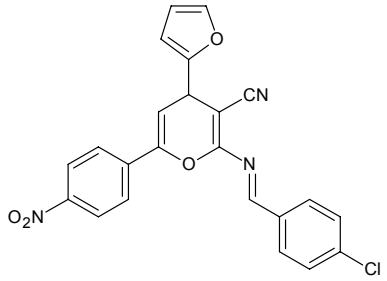
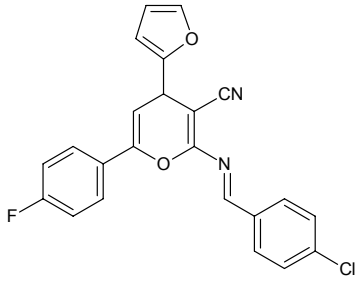
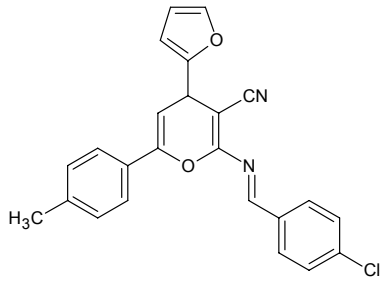
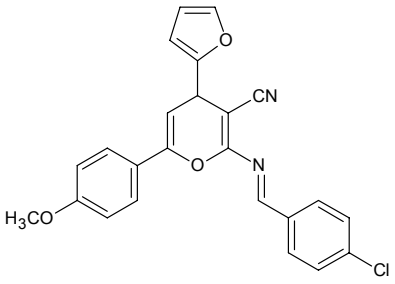
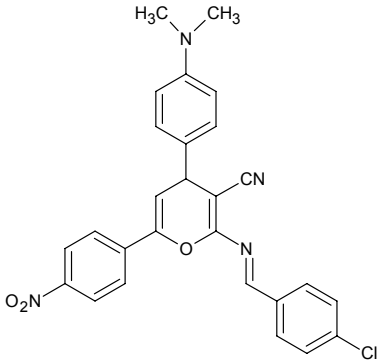
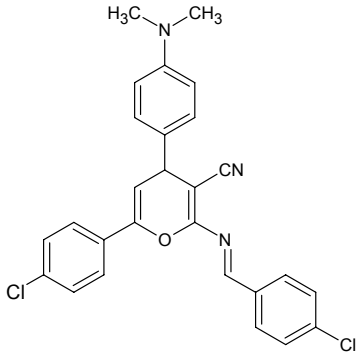
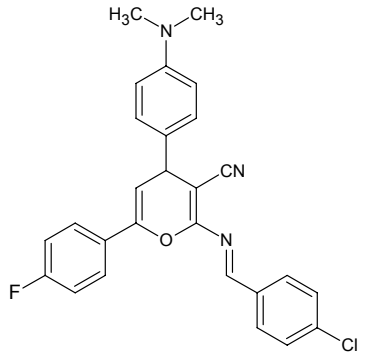
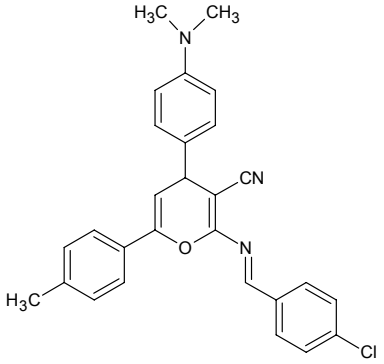
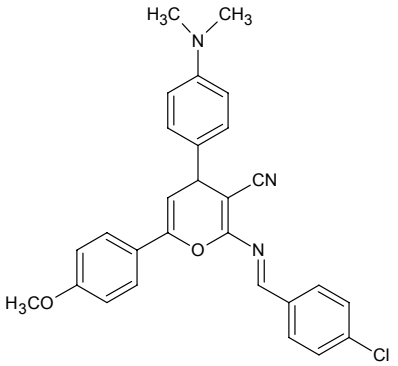
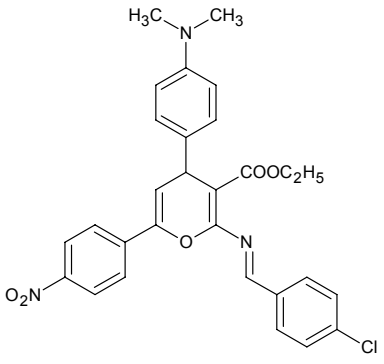
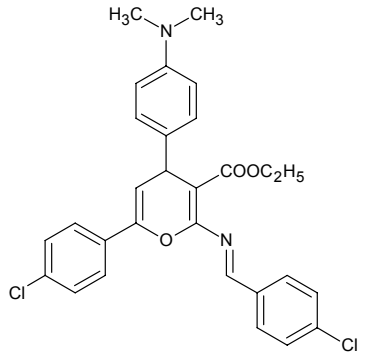
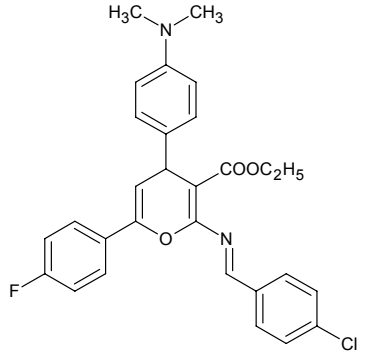
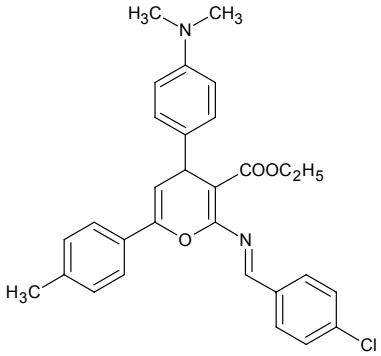
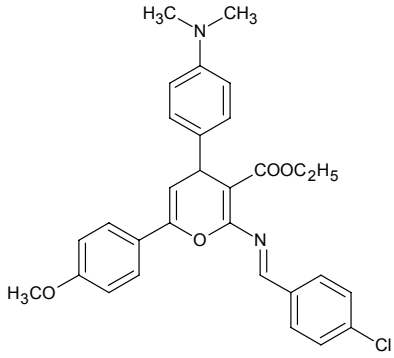
LIST OF NEWLY SYNTHESIZED COMPOUNDS

Compound code	Chemical name	Structure
FCP_1S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-nitrophenyl-)-4-(furan-2-yl)-4*H*-pyran-3-carbonitrile	
FCP_3S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-fluorophenyl)-4-(furan-2-yl)-4*H*-pyran-3-carbonitrile	
FCP_4S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-methylphenyl-)-4-(furan-2-yl)-4*H*-pyran-3-carbonitrile	

FCP_5S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-methoxyphenyl)-)-4-(furan-2-yl)-4*H*-pyran-3-carbonitrile	
DCP_1S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-nitrophenyl)-4-(4-dimethyl aminophenyl)- 4*H*-pyran-3-carbonitrile	
DCP_2S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-chlorophenyl)-4-(4-dimethyl aminophenyl)- 4*H*-pyran-3-carbonitrile	

DCP_3S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-fluorophenyl)-4-(4-dimethyl aminophenyl)- 4*H*-pyran-3-carbonitrile	
DCP_4S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-methylphenyl)-4-(4-dimethyl aminophenyl)- 4*H*-pyran-3-carbonitrile	
DCP_5S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-methoxyphenyl)-4-(4-dimethyl aminophenyl)- 4*H*-pyran-3-carbonitrile	
DP_1S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-nitrophenyl)-4-(4-dimethyl aminophenyl)- 4*H*-pyran-3-carboxylate	

DP_2S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-chlorophenyl)-4-(4-dimethyl aminophenyl)- 4*H*-pyran-3-carboxylate	
DP_3S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-fluorophenyl)-4-(4-dimethyl aminophenyl)- 4*H*-pyran-3-carboxylate	
DP_4S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-methylphenyl)-4-(4-dimethyl aminophenyl)- 4*H*-pyran-3-carboxylate	
DP_5S	2-{[(*E*)-(4-chlorophenyl)methylidene]amino}-6-(4-methoxyphenyl)-4-(4-dimethyl aminophenyl)- 4*H*-pyran-3-carboxylate	

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