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“SYNTHESIS AND ANTI-BACTERIAL ACTIVITY OF SOME NOVEL PYRAZOLIDINEDIONE SUBSTITUTED DERIVATIVES OF 2-QUINOLONES”

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ABSTRACT

Although a number of drugs are available in the market, thrust for discovery of new antimicrobial drugs with better pharmacokinetic profile, and lesser toxicity has become necessary in the field of medicinal chemistry due to fast development of microbial resistance towards the existing molecules. The 2(1H) quinolinone, often referred to as carbostyril moiety, is an important structural unit present in many biologically active molecules. Quinolone antibiotics have gained wide acceptance for the treatment of various bacterial infections. Their mode of action is believed to involve inhibition of bacterial DNA gyrase, an enzyme essential for DNA replication. Many natural and synthetic products containing heterocyclic rings such as pyrazoles, pyrazolones, and isoxazolidines and pyrazolidinediones were reported to possess varied pharmacological activities. Research on 2-quinolone and pyrazolidine 3,5-dione and their synthetic analogs have revealed to possess various pharmacological activities along with wide range of antimicrobial activity. It is our interest to synthesize some new 2-quinolones containing pyrazolidinedione moiety and evaluate their antimicrobial activity.

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INTRODUCTION

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Most of this activity is directed to new natural or synthetic organic compounds.

The process of establishing a new pharmaceutical is exceedingly complex and involves the talents of people from a variety of disciplines, including chemistry, biochemistry, molecular biology, physiology, Pharmacology, pharmaceutics and medicine. Medicinal chemistry, itself, is concerned mainly with the organic, analytical, and bio chemical aspects of this process, but the chemist must interact productively with those in other disciplines. Thus, medicinal chemistry occupies a strategic position at the interface of chemistry and biology.

To provide an understanding of the principles of medicinal chemistry, it is necessary to consider the physicochemical properties required to develop new pharmacologically active compounds, their mechanism of action and the drug metabolism including possible biological activities of the metabolites. The relation between stereochemistry of a compound and its activity cannot be ignored.

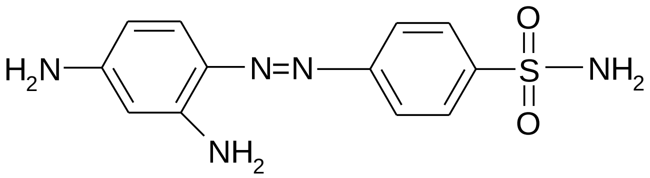
Modern drug design requires a good understanding of how the drug is transported in to the body, distributed throughout the body compartments, metabolically altered by the liver and other organs, and excreted from the patient along with the structural characteristics of the receptor. Acid – base chemistry is used to aid in formulation and biodistribution. Structural attributes and substituent patterns responsible for optimum pharmacological activity need to be considered.

Antibiotics and Antimicrobials:

The history of work on the prevention of bacterial infection can be traced back to the 19th century when Joseph Lister (in 1867) introduced antiseptic principles in surgery and posttraumatic injury. He used phenol (carbolic acid) as a wash for the hands, as a spray on an incision site and on bandages applied to wounds. Lister's principles caused a dramatic decrease in the incidence of postsurgical infections.

A round 1881 and continuing to 1900, microbiologist, Paul Ehrlich, a disciple of Robert Koch, began work with a set of antimicrobial dyes and antiparasitic organic arsenicals. His goal was to develop compounds that retained antimicrobial activity at the expense of toxicity to the human host, at the time that Ehrlich began his experiments; there were only a few compounds that could be used in treating infectious diseases[1].

In 1935 a red dye called prontosil(1) was found to be effective against streptococcal infections in vivo. prontosil was eventually recognized as prodrug for a new class of antimicrobial agents. The sulpha drugs (sulphonamides). The discovery of these drugs was a real breakthrough since they represented the first drugs to be effective against bacterial infections carried in the blood stream. They were the only effective drugs until penicillin became available in the early 1940.



PRONTOSIL
(1)

Although penicillin was discovered in 1928, it was not until 1940, the effective means of isolating it were developed by Flory and Chain. Society was then rewarded with a drug which revolutionized the fight against bacterial infection and proved even more effective than the sulphonamides.

In 1944, the antibiotic streptomycin was discovered from a systematic search of soil organisms. After the Second World War, the effort continued to find other novel antibiotics. This led to the discovery of the peptide antibiotics (e.g.Bacitracin; 1945, chloramphenicol (1947 The tetracycline antibiotics (e.g.chlortetracycline1948), them acrolide antibiotics (e.g.Erythromycin;1952), the cyclic peptide antibiotics (e.g.cycloserine;1955), and, in 1955, the first example of second major group of beta lactum antibiotics, cephalosporin C[2].

In 1942, Waksman proposed the widely cited definition that "an antibiotic is a substance produced by micro organisms, which has the capacity of inhibiting the growth and even of destroying other micro organisms" [1].

Quinolone-Antibacterials:

The quinolones comprise a series of synthetic anti bacterial agents patterned after nalidixic acid, a naphthyridine derivative introduced for the treatment of Urinary tract infections in 1963.

Isosteric heterocyclic groupings in this class include the quinolones (e.g.Norfloxacin, Ciprofloxacin, and lomefloxacin), the naphthyridines (e.g.Nalidixic acid andEnoxacin), and the Cinnolines(e.g.Cinoxacin). Until recently the clinical usefulness of the quinolones has been largely confined to the treatment of Urinary tract infections. For the treatment of Urinary tract infections, good oral absorption, activity against common gram-negative urinary pathogens, and comparatively higher urinary (compared with plasma and tissue) concentrations are the key useful properties. As a result of extensive structure-activity investigations leading to compounds with enhanced potency, extended spectrum of activity, and improved absorption and distribution properties, the class as evolved to the point that certain newer members are useful for the treatment of a variety of serious systemic infections. In fact, these more potent analogues are sometimes classified separately (from the urinary tract-specific agents) as the fluoroquinolones, because all members of the group have a common 6-fluoro substituent.

Quinolones are relatively late arrivals on the antibacterial scene, but are proving to be very useful therapeutic agents. They are particularly useful in the treatment of urinary tract infections and also for treatment of infections resistant to the more established antibacterial agents [2].

Mechanism of Action:

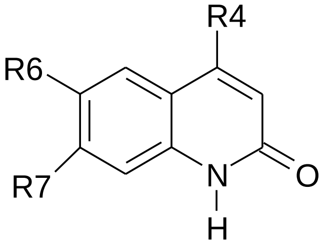
Quinolone antibacterials act by inhibition of bacterial topoisomerase II (DNA gyrase) and probably topoisomerase IV in gram-positive species, thus inhibiting tertiary negative super coiling of bacterial DNA. This effect, perhaps associated with binding of quinolones to a DNA-gyrase complex, is rapidly bactericidal. The minimum bactericidal concentration is usually only two to four times the minimum inhibitory concentration (MIC) and a prolonged post antibiotic effect is produced at concentrations exceeding the minimum inhibitory concentration (MIC) [3].

TABLE 1. CLASSIFICATION OF QUINOLONES.

First generation Quinolones:	e.g. Oxolinicacid, Cinoxacin, Paramedicacid, Pipermidic acid, Flemiquine.
Second generation Quinolones:	e.g. Norfloxacin, Pefloxacin, Enoxacin, Fleroxacin, Lomefloxacin, Ciprofloxacin, Ofloxacin, Rufloxacin.
Third generation Quinolones:	e.g. Grepafloxacin, Sparfloxacin, Gatifloxacin, Levofloxacin, Pazufloxacin, Trovofloxacin, Tosufloxacin, Clinafloxacin, Temafloxacin, Balofloxacin.

2-Quinolones:

General Structure:



(2)

Properties:

Carbostyril, 2-(1H)-quinolinone, 2-quinolinol, mp 199-200 °C, it is very sparingly soluble in water, soluble in alcohol, ether, dil. Hcl and it forms easily hydrolysed sodium and potassium salts [4].

The 2-quinolone (carbostyril) skeleton is an important structural moiety present in a large number of alkaloids. and in biologically active compounds. Some of them exhibit, for example, antioxidative activity, Nitric oxide production inhibitory activity and cytotoxicity against human tumor cell lines. Some of them are angiotensinII receptor antagonists, glycine NMDA receptor antagonists, endothelin receptor antagonists, antiplatelet agents and antitumor agents[5].

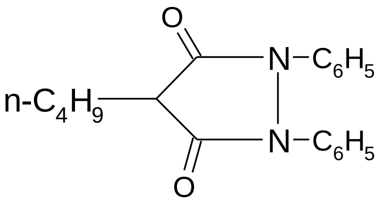
2-Quinolones are also valuable intermediates in organic synthesis. Some quinolin-2(1H)-ones have been used as intermediates in the synthesis of HIV-I reverse transcriptase inhibitors. 5-HT3 receptor antagonist or AMPA/kainate antagonist [6].

Carbostyrils offer the advantage of greater chemical and thermal stability[7]. Carbostyrils also represent a group of naturally occurring lactams which are potential antimicrobial, analgesic, anti-inflammatory, anticancer, and anti-HIV agents[8].

Pyrazolidinediones:

The discovery of the valuable therapeutic properties of 4-n-butyl-1, 2diphenylpyrazolidine-3,5-dione (butazolidine, phenylbutazone, butadione) (3)promoted the development of the chemistry of this group of compounds. thus, whereas a total of about 25 papers appeared in the 60 years after the synthesis of the first pyrazolidine-3,5-diones, in the last decade, and mainly during the last 5 years, no fewer than 300 papers and patents dealing with this group of substances have been published, not counting the hundreds of papers connected with the study of butadione itself.

As of now, various methods of synthesizing pyrazolidine-3,5-diones have been developed and many of their properties have been studied. The following is a brief survey of the published material on the chemistry of pyrazolidine-3,5-diones and their applications.



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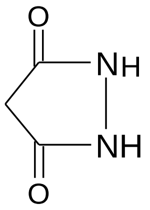

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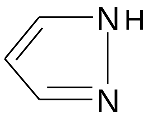

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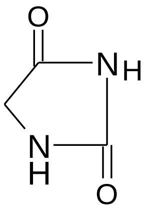

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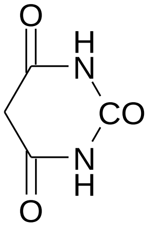
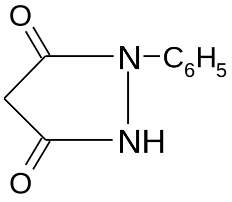


(7)

Pyrazolidine-3,5-dione(4) is the 3,5-dioxo- derivative of completely hydrogenated pyrazole(5). It is isomeric with hydantoin(6) and is structurally related to barbituric acid(7), and also to pyrazolones.



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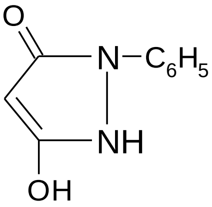

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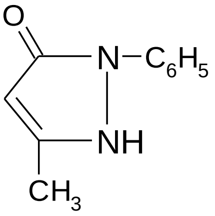


(10)

Thus, the enol form (9) of 1-phenylpyrazolidine-3,5-dione(8) differs from 3-methyl-1-phenylpyrazol-5-one(10) only in having a hydroxyl group in place if the methyl. The conversion of pyrazolidine-3,5-dione to pyrazolones and pyrazoles was described in 1898.

For partially substituted pyrazolidine-3,5-diones,electrophillic substitution of the hydrogen atoms at C4,and to a lesser extent of those at the nitrogen atoms, is the most characteristic reaction. Depending on the nature of the electrophillic reagent and on the number and type of the substituents already present, substitution takes place at the C or N atoms; a completely substituted compound may be formed.

METHODOLOGY

Chemicals and Reagents

The chemicals and reagents used in the present project were of AR and LR grade, procured from Aldrich, Hi-media, Loba, Merck, Ranbaxy, Qualigens and S.D– Fine Chem Ltd. Agar – Agar, Glacial acetic acid, Aniline, Hydrazine hydrate, Benzocaine, Hydrochloric acid, Beef extract, Iodine, Chloroform, Liquid paraffin, Ciprofloxacin, Methanol, Conc. Hydrochloric acid, Para aminobenzoic acid, Conc. Sulfuric acid, Para Nitro aniline, Dextrose, Peptone, Dioxane, Potassium Bromide, Diethyl ether, Sodium bi-carbonate, Diethyl malonate, Sodium chloride, Ethyl acetoacetate, Silicagel G, Ethanol and Toluene.

Analytical Techniques

Physical data:

Melting points of the synthesized compounds were taken in open capillary tubes using thiel’s apparatus and are uncorrected.

Thin Layer Chromatography (TLC):

Purity of the compounds was checked by thin layer chromatography using silica gel G as stationary phase and Toluene and ethanol (95:5) as mobile phase. The resolved components were visualized as brown spots by using iodine chamber.

Instrumentation:

The techniques employed for the characterization of the synthesized compounds were IR and Mass spectral analysis.

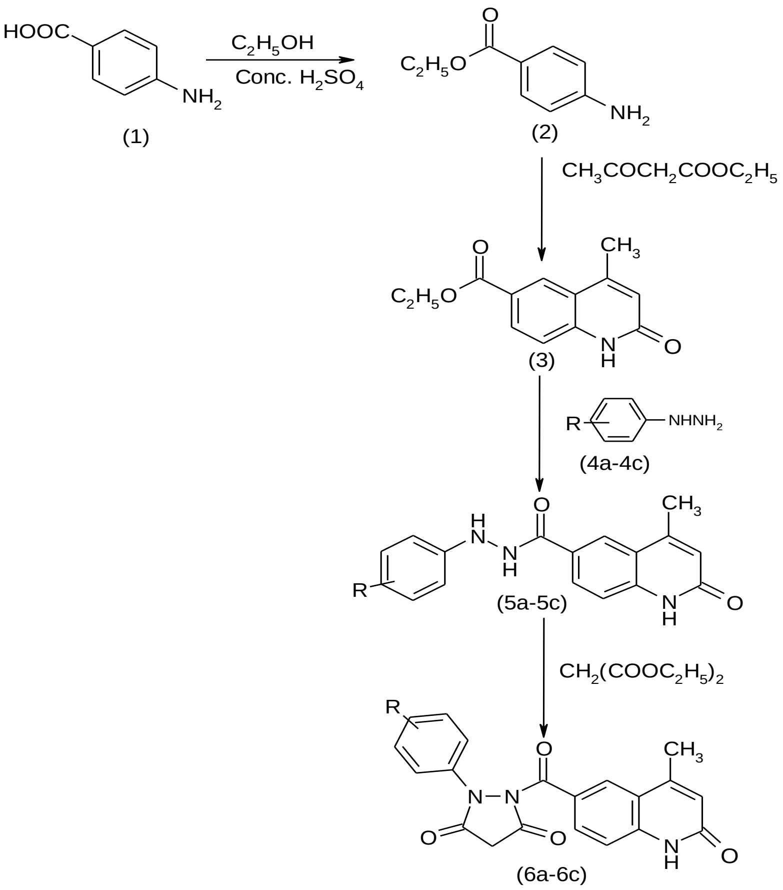
Infrared spectra:

The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (model: 8400S, Shimadzu) in the range of 400-4000 using diffuse reflectance system and values of ν_{max} are reported in cm^{-1}.

Mass spectra:

Mass spectra were recorded on Mass spectrophotometer (model: LCMS- 2010 A, Shimadzu)

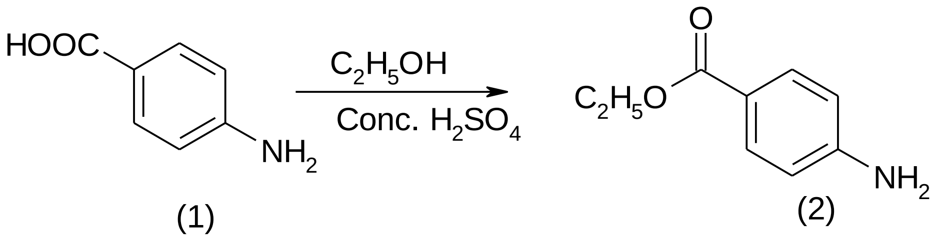
SCHEME FOR SYNTHETIC METHODLGY:



S/No.	COMPOUND	R
1	6A	H
2	6B	4-NO_2
3	6C	4-Cl

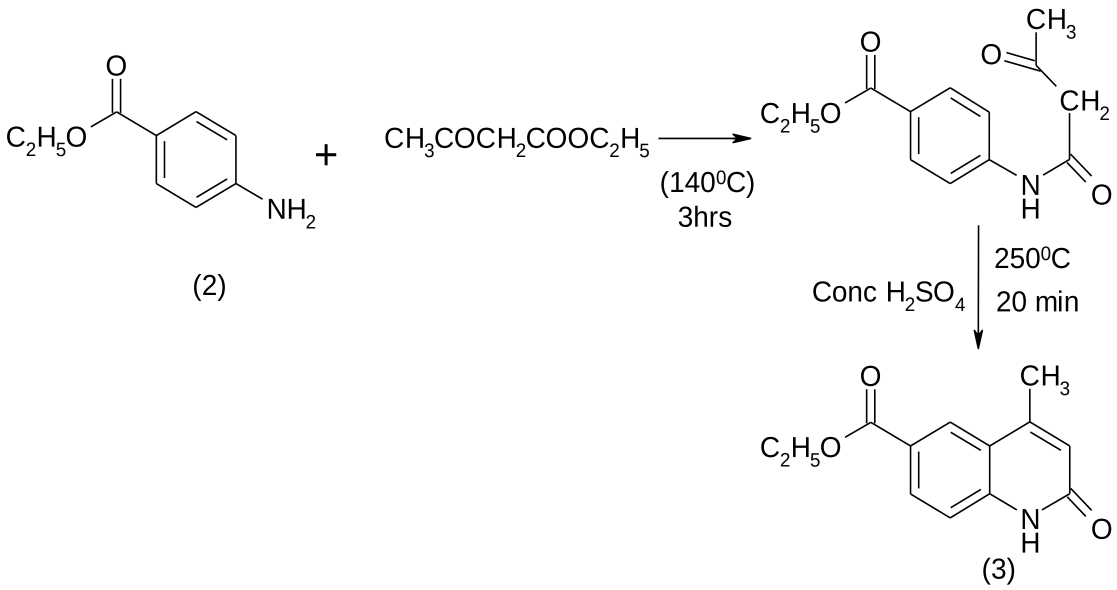
SYNTHETIC WORK:-

Synthesis of Ethyl p-aminobenzoate [2][54]:-



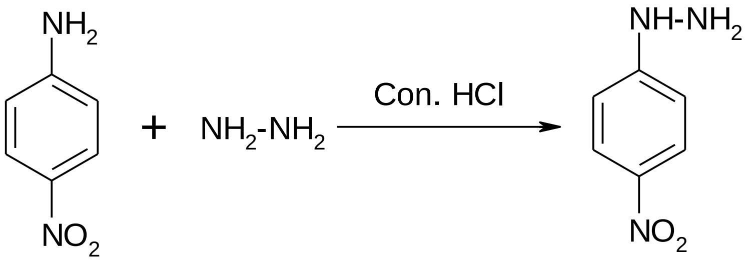
P-amino benzoic acid (6g, 0.046 mol) was dissolved in 75ml of ethanol and was heated on a sand bath until the entire solid dissolved. Cooled to room temperature and conc. Sulfuric acid (12.5ml) was added. An air condenser was attached and refluxed for 60-70 min. A large amount of precipitate was formed when sulfuric acid was added, but dissolved during refluxation. Then the mixture was allowed to cool at room temperature and a solution of sodium bicarbonate (10 %) was added to neutralize the excess sulfuric acid. As pH increased, white precipitate of ethyl p-amino benzoate (benzocaine) was produced. The product was collected by vacuum filtration. It was dried in open container and purified from ethanol. The yield of the product, m.p.87-88^{0}C was 72.22%
Molecular Formula ($C_9H_{11}O_2N$), Molecular Weight (165), Melting Point (87-88^{0}C), Theoretical Yield (7.2g), Practical Yield (5.2g), Percentage Yield (72.22%), TLC System (Toluene: Ethanol(95:5)) and Rf Value (0.93).

Synthesis of 4-methyl-2-oxo-1,2 dihydro-quinoline-6-carboxylic acid ethyl ester[3][13,55,]:-



A mixture of ethyl p-amino benzoate (2) (12g, 0.07 mol) and ethyl acetoacetate (13ml, 0.1 mol) in Dioxane (25ml) was heated at 140˚C for 3hrs in an oil bath, cooled at room temperature and conc. Sulfuric acid (20ml) was added. Again the mixture was heated at 250˚C in an oil bath for 20 min, cooled and the mixture was poured in 500ml of ice-cold water with constant stirring. Separated solid was filtered, dried, and recrystallized from ethanol. The yield of the product, m.p. 195-197⁰C was 53.57%
Molecular Formula ($C_{13}H_{13}O_3N$), Molecular Weight (231.23), Melting Point (195-197⁰C), Theoretical Yield (8.4g), Practical Yield (4.5g), Percentage Yield (53.57%), TLC System (Toluene: Ethanol(95:5)) and Rf Value (0.74).

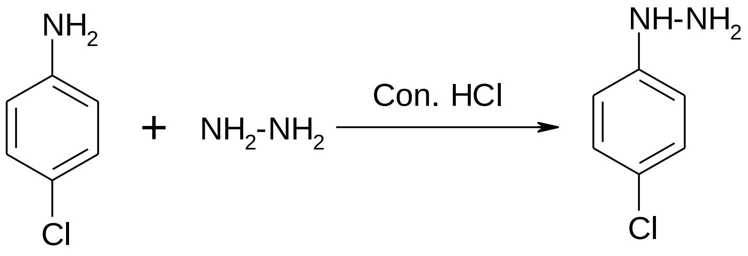
Preparation of phenyl hydrazines[56]:-
synthesis of 4-nitrophenylhydrazine [4b]:-



(4b)

To a solution of hydrazine hydrate (10ml) and conc. Hydrochloric acid(20ml) 4-Nitroaniline (12g, 0.087mol) followed by ethylene glycol (10ml) were added. The mixture was refluxed for 2 hours, cooled to room temperature. The Separated solid was filtered, dried and recrystallised from ethanol. The yield of the product, m.p. 132-133⁰C was 74.43%
Molecular Formula ($C_6H_7O_2N_3$, Molecular Weight (153), Melting Point (132-133⁰C), Theoretical Yield (13.30g), Practical Yield (9.9g), Percentage Yield (74.43%), TLC System (Toluene: Ethanol(95:5)) and Rf Value (0.55).

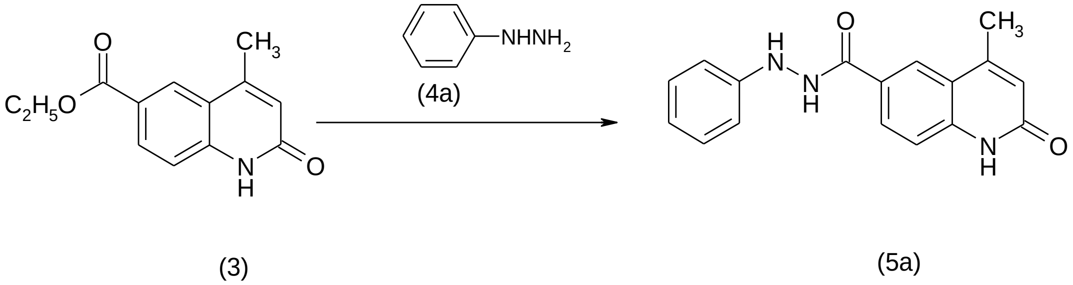
synthesis of 4-chloro phenyl hydrazine [4c]:-



(4c)

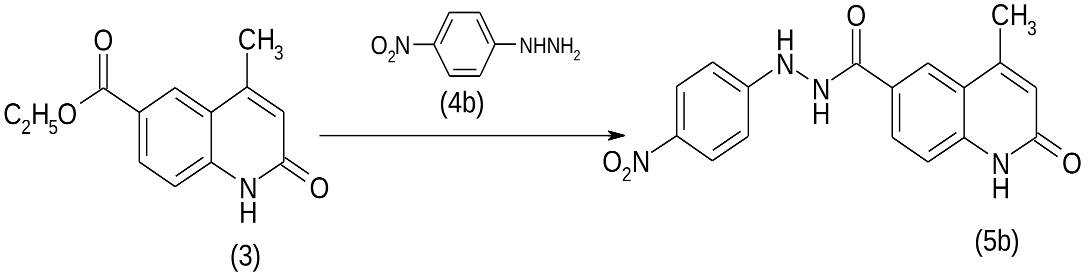
To a solution of hydrazine hydrate (10ml) and conc. Hydrochloric acid (20ml) 4-chloroaniline (12g, 0.094mol) followed by ethylene glycol (10ml) were added. The mixture was refluxed for 2 hours, cooled to room temperature. The Separated solid was filtered, dried and recrystallised from ethanol. The yield of the product, m.p. 117-119^0C was 76.80%
Molecular Formula ($C_6H_7N_2Cl$), Molecular Weight (142.23), Melting Point (117-119^0C), Theoretical Yield, (13.41g), Practical Yield (10.3g), Percentage Yield (76.80%), TLC System (Toluene: Ethanol(95:5)) and Rf Value(0.63).

Preparation of phenylhydrazides[57]:-
Synthesis of 4-methyl-2-oxo-1,2 dihydro quinoline-6carboxylic acid N-phenylhydrazide. [5a]



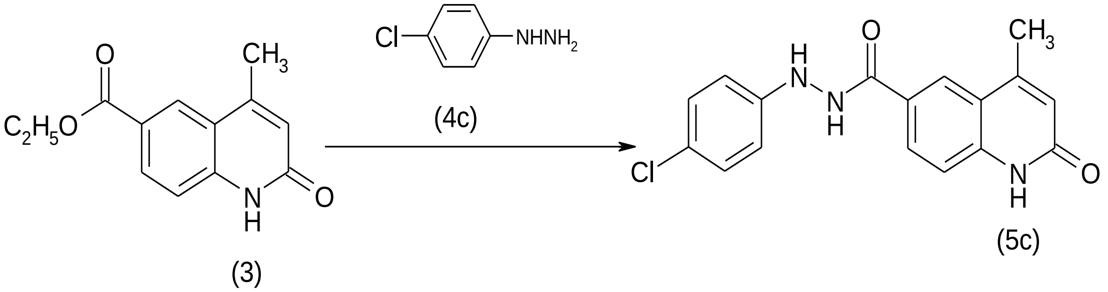
To a suspension of 4-methyl-2-oxo-1,2 dihydro-quinoline-6-carboxylic acid ethyl ester [3] (6g, 0.026mol) in methanol (10ml) phenyl hydrazine solution [4a] (6ml, 0.05 mol) in ethanol(10ml) was added at room temperature. After stirring, the mixture was diluted with ethanol (20ml) and refluxed for 1hr, cooled to room temperature, filtered and washed the solid with diethyl ether (20 ml) and purified from ethanol. The yield of the product, m.p. 276-277^OC was 60.52%
Molecular Formula ($C_{17}H_{15}O_2N_3$), Molecular weight (293.31), Melting point (276-277^OC), Theoretical Yield (7.6g), Practical Yield (4.6g), Percentage Yield (60.52%), TLC System (Toluene: Ethanol(95:5) and Rf Value (0.27).

Synthesis of 4-methyl-2-oxo-1,2 dihydro quinoline-6carboxylic acid N'-(4-nitrophenyl)-hydrazide:[5b]



To a suspension of 4-methyl-2-oxo-1,2 dihydro-quinoline-6-carboxylic acid ethyl ester [3] (6g, 0.026mol) in methanol (10ml) 4-nitro phenyl hydrazine[4b] (4.5g, 0.03 mol) in ethanol (10ml) was added at room temperature. After stirring, the mixture was diluted with ethanol (20ml) and refluxed for 1hr, cooled to room temperature, filtered and washed the solid with diethyl ether (20 ml) and purified from ethanol. The yield of the product, m.p. 273-274°C was 64.75%
Molecular Formula ($C_{17}H_{14}O_4N_4$), Molecular Weight (338.30),Melting Point (273-274°C), Theoretical Yield (12.2g), Practical Yield (7.9g), Percentage Yield (64.75%), TLC System (Toluene : Ethanol (95 : 5)) and R_f Value (0.47).

Synthesis of 4-methyl-2-oxo-1,2 dihydro quinoline-6carboxylic acid N'-(4-chlorophenyl)-hydrazide:[5c]

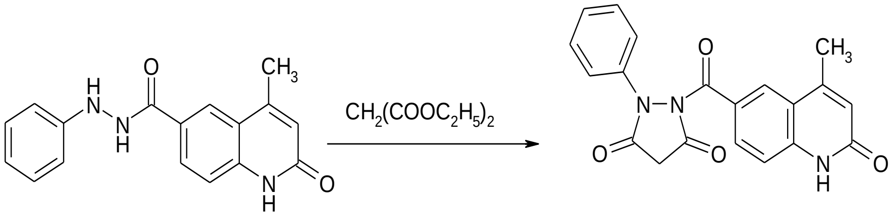


To a suspension of 4-methyl-2-oxo-1,2 dihydro-quinoline-6-carboxylic acid ethyl ester [3] (6g, 0.026mol) in methanol (10ml) 4-chloro phenyl hydrazine[4c] (4g, 0.028 mol) in ethanol (10ml) was added at room temperature. After stirring, the mixture was diluted with ethanol (20ml) and refluxed for 1hr, cooled to room temperature, filtered and washed the solid with diethyl ether (20 ml) and purified from ethanol. The yield of the product, m.p. 282-283°C was 66.44%

Molecular Formula ($C_{17}H_{13}O_2N_3Cl$), Molecular Weight (327.76), Melting Point (282-283°C), Theoretical Yield (11.89g), Practical Yield (7.9g), Percentage Yield (66.44%), TLC System (Toluene : Ethanol (95:5)) and R_f Value (0.25).

Preparation of pyrazolidinedione derivatives[49]:

Synthesis of 1-(4-methyl-2-oxo-1,2 dihydro quinoline-6-carbonyl)-2-Phenyl-pyrazolidine-3, 5-dione:[6a]

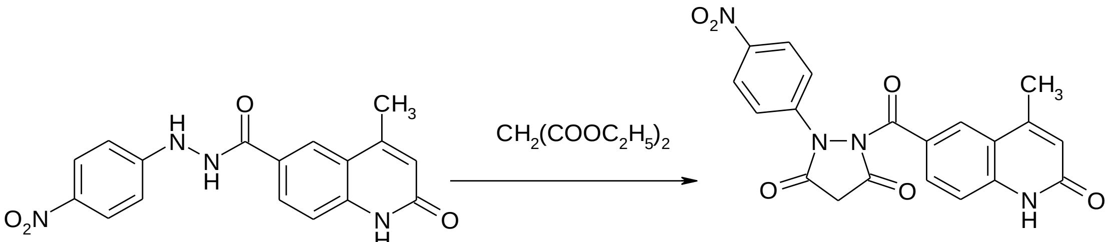


(5a) (6a)

To a mixture of 4-methyl-2-oxo-1,2 dihydroquinoline-6 carboxylic acid-N'-phenyl hydrazide (6g, 0.02mol) (5a) and diethyl malonate (3ml, 0.015 mol) ethanol (90ml) and acetic acid (1ml) were added and refluxed for 5hrs. The reaction mixture was left in open dish for 2-3 hrs. The solid precipitate formed was filtered, dried and recrystallized from ethanol. The yield of the product, m.p. 235-237°C was 52.77%

Molecular Formula ($C_{20}H_{15}O_4N_3$), Molecular Weight (361.52), Melting Point (235-237°C), Theoretical Yield (3.6g), Practical Yield (1.9g), Percentage Yield (52.77%), TLC System (Toluene : Ethanol (95:5)) and R_f Value (0.17)

Synthesis of 1-(4-methyl-2-oxo-1, 2-dihydro quinoline-6-carbonyl)-2-(4-nitrophenyl)-pyrazolidine-3, 5-dione:[6b]

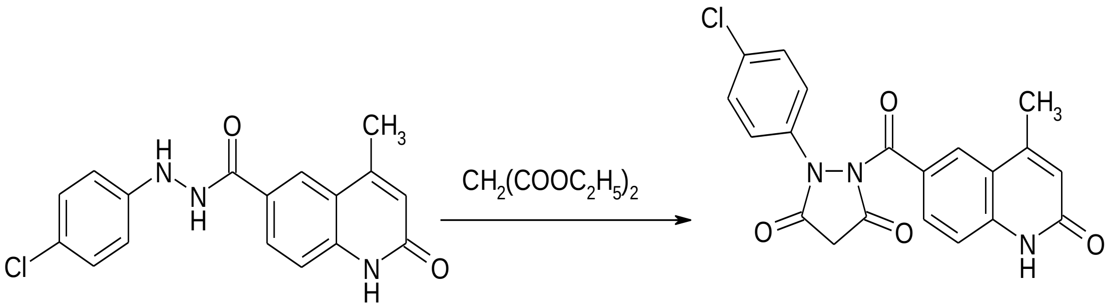


(5b) (6b)

To a mixture of 4-methyl-2-oxo-1,2 dihydro quinoline-6carboxylic acid N-(4-nitrophenyl)-N phenylhydrazide (5b) (6g, 0.017) and diethyl malonate (4ml, 0.02mol) ethanol (90ml) and acetic acid (1ml) were added and refluxed for 5hrs. The reaction mixture was left in open dish for 2-3 hrs. The solid precipitate formed was filtered and dried and purified from ethanol. The yield of the product, m.p. 247-249°C was 41.66%

Molecular Formula ($C_{20}H_{14}O_6N_4$), Molecular Weight (406.35), Melting Point (247-249°C), Theoretical Yield, (3.6g), Practical Yield (1.5g), Percentage Yield ((41.66%), TLC System Toluene : Ethanol (95 : 5) and R_f Value (0.36).

Synthesis of 1-(4-methyl-2-oxo-1,2-dihydro quinoline-6-carbonyl)-2-(4-chlorophenyl)-pyrazolidine-3, 5-dione. [6c]

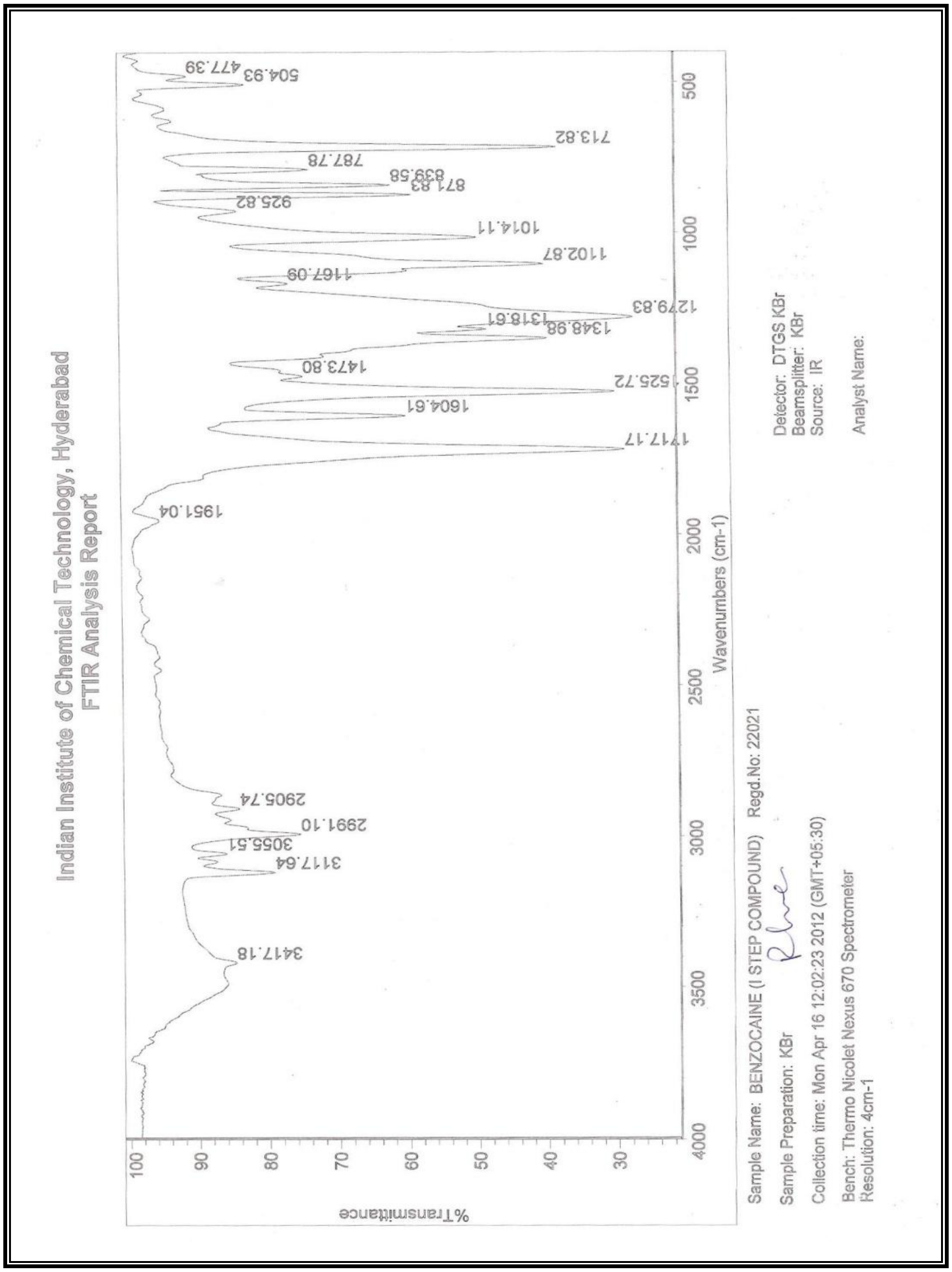


(5c) (6c)

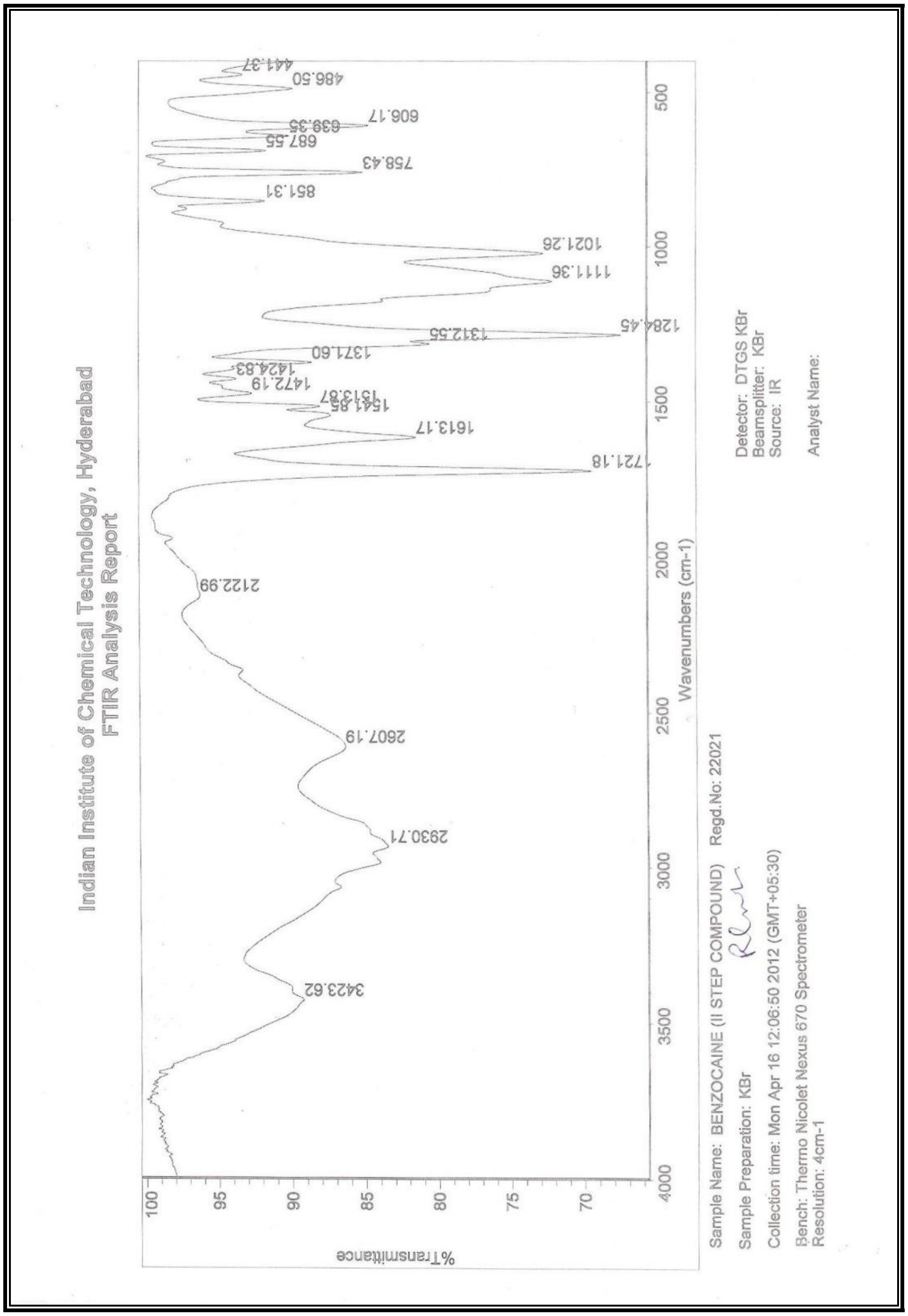
To a mixture of 4-methyl-2-oxo-1,2 dihydro quinoline-6carboxylic acid N'-(4-chlorophenyl)-hydrazide (5c) (6g, 0.018) and diethyl malonate (4ml, 0.02mol) ethanol (90ml) and acetic acid (1ml) were added and refluxed for 5hrs. The reaction mixture was left in open dish for 2-3 hrs. The solid precipitate formed was filtered, dried and recrystallized from ethanol. The yield of the product, m.p. 251-252°C was 44.44%

Molecular Formula ($C_{20}H_{14}O_2N_3Cl$), Molecular Weight (395.58), Melting Point (251-252°C), Theoretical Yield (3.6g), Practical Yield (1.6g), Percentage Yield (44.44%), TLC System (Toluene : Ethanol (95:5)) and R_f Value (0.32).

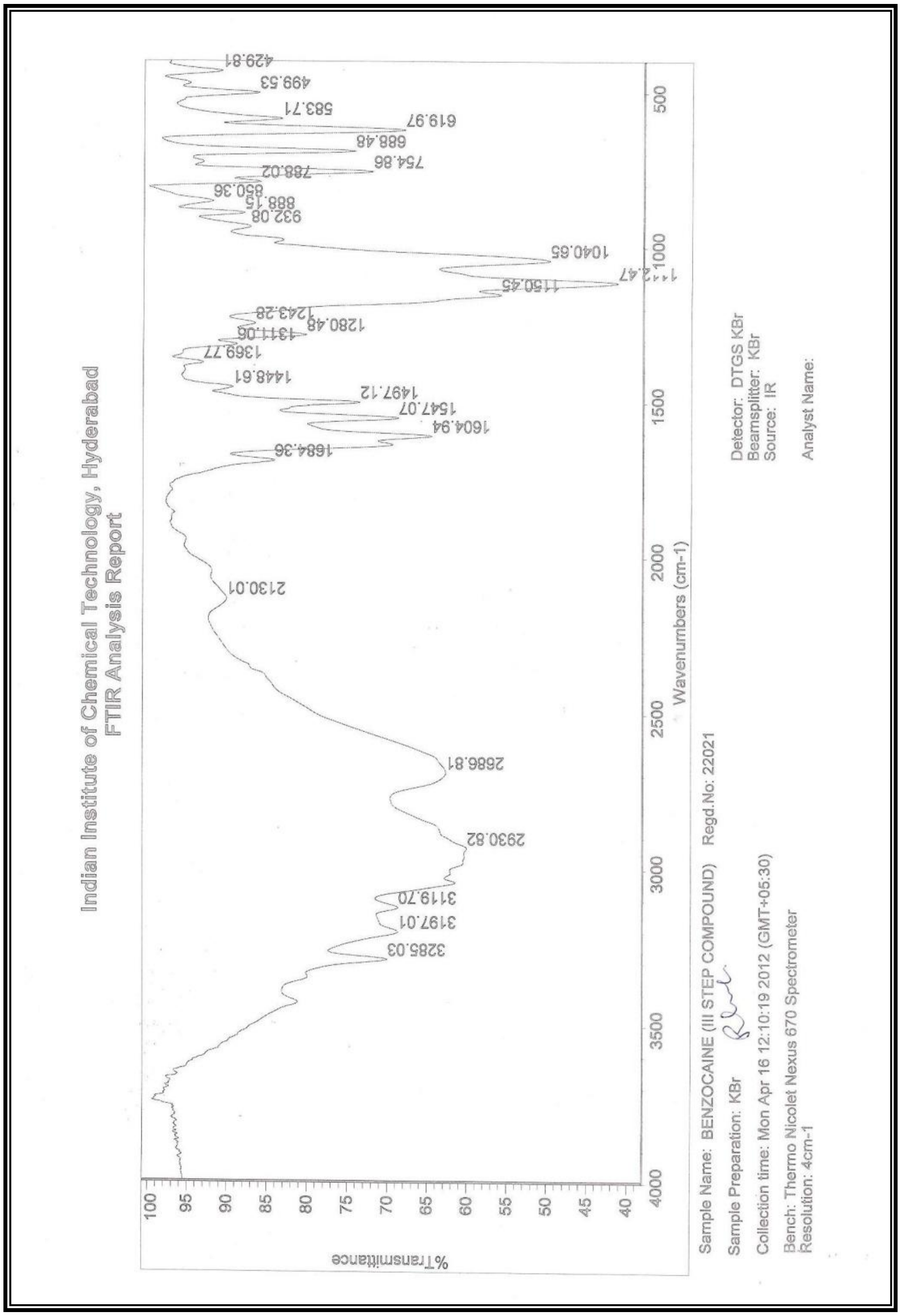
C. Spectra



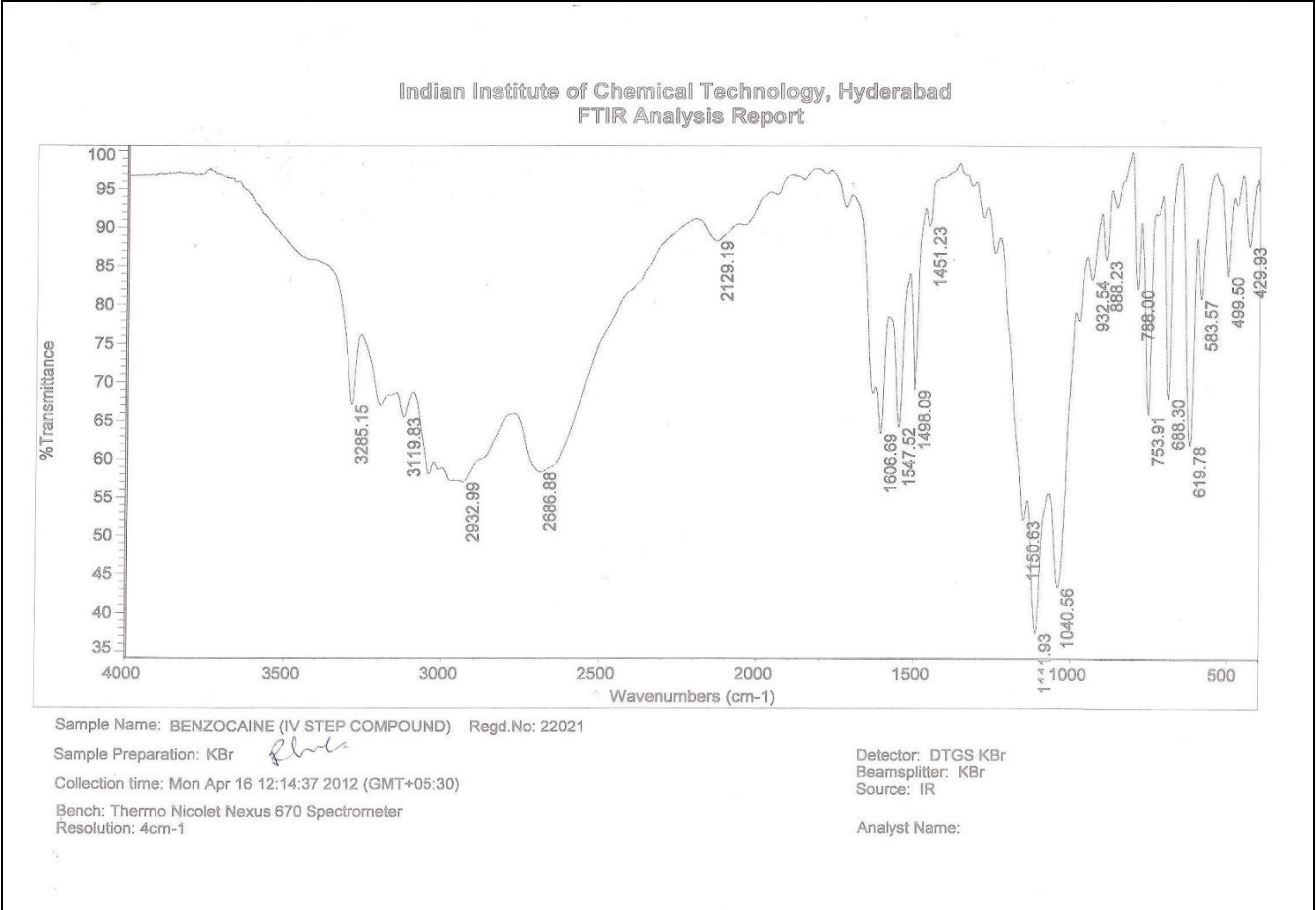
IR Spectrum of [2]



: IR Spectrum of [3]



IR Spectrum of [5a]



Indian Institute of Chemical Technology, Hyderabad
FTIR Analysis Report

Sample Name: BENZOCAINE (IV STEP COMPOUND) Regd.No: 22021

Sample Preparation: KBr

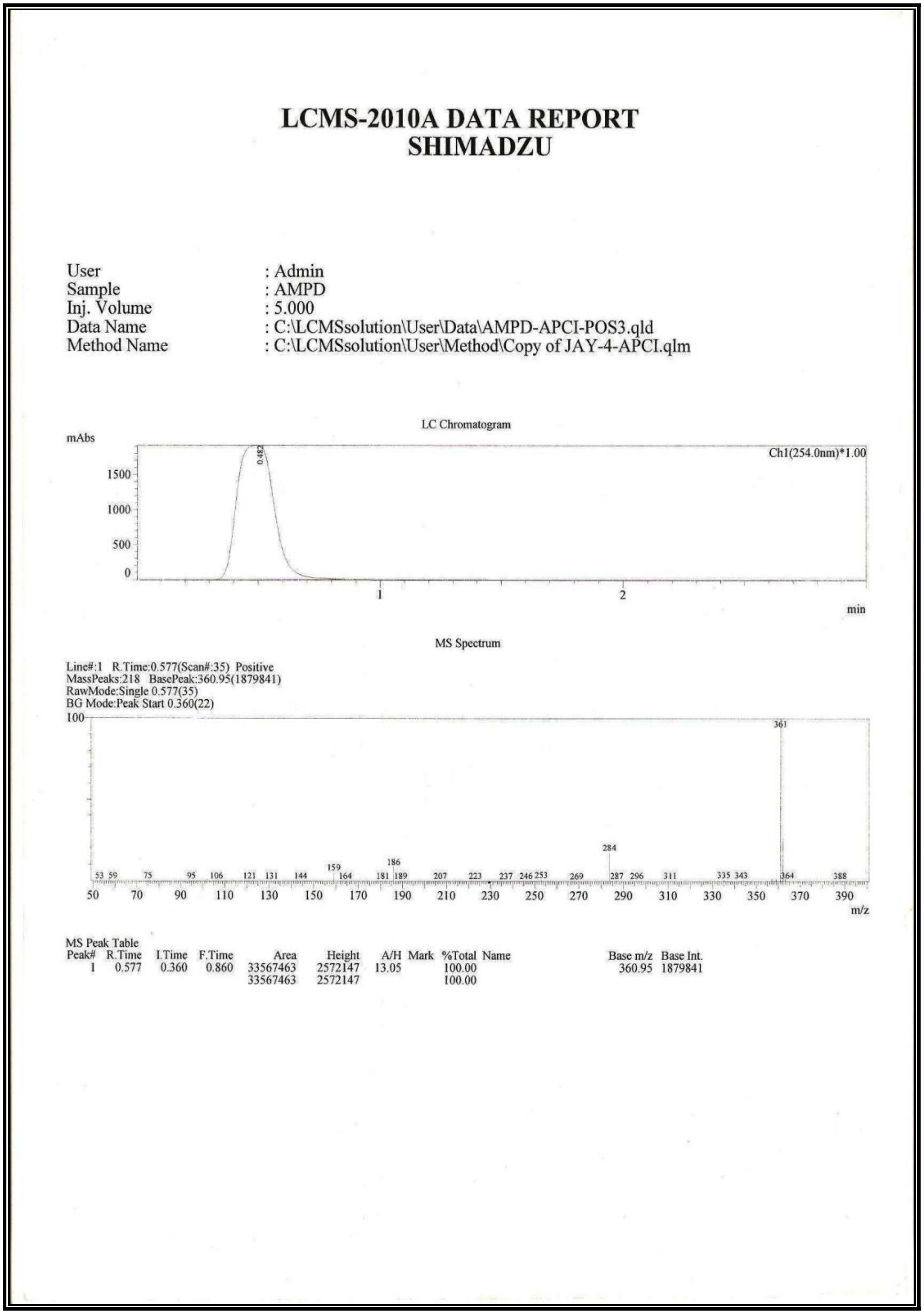
Collection time: Mon Apr 16 12:14:37 2012 (GMT+05:30)

Bench: Thermo Nicolet Nexus 670 Spectrometer
Resolution: 4cm-1

Detector: DTGS KBr
Beamsplitter: KBr
Source: IR

Analyst Name:

IR Spectrum of [6a]



LCMS-2010A DATA REPORT
SHIMADZU

User	: Admin
Sample	: AMPD
Inj. Volume	: 5.000
Data Name	: C:\LCMSsolution\User\Data\AMPD-APCI-POS3.qld
Method Name	: C:\LCMSsolution\User\Method\Copy of JAY-4-APCI.qlm

LC Chromatogram

MS Spectrum

Line#:1 R.Time:0.577(Scan#:35) Positive
MassPeaks:218 BasePeak:360.95(1879841)
RawMode:Single 0.577(35)
BG Mode:Peak Start 0.360(22)

MS Peak Table

Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Mark	%Total	Name	Base m/z	Base Int.
1	0.577	0.360	0.860	33567463	2572147	13.05		100.00		360.95	1879841
				33567463	2572147			100.00			

Mass Spectrum of 1-(4-methyl-2-oxo-1, 2-dihydro quinoline-6- carbonyl)-2-Phenyl-pyrazolidine-3, 5-dione:[6a]

MICROBIOLOGICAL SCREENING [59,60]

Antimicrobial evaluation can be done by following methods:

- Tube dilution method
- Agar diffusion method

a) Cup-plate method
b) Disc diffusion method

Tube dilution method:

In the tube dilution method, the dilutions of the antimicrobial agents prepared in the nutrient medium such that the concentration of the drug covers its clinical significant range. An equal volume of broth containing 10^5 to 10^6 bacteria per ml is added to each tube and to a control tube that contains no antimicrobial agent. The tubes are examined for visible turbidity after overnight incubation.

This method is used for determining antimicrobial susceptibility in liquid media, however it is cumbersome and has given way in the clinical laboratory to micro-dilution tests and automated testing procedures. This method is used to determine the minimum inhibitory concentration of antimicrobial agent.

Agar diffusion method:

In this agar diffusion method agar plates are prepared by pouring nutrient agar media, each of them is inoculated with a particular micro-organism, like gram positive and gram negative bacteria.

Cup-plate method:

After agar solidifies, cups are made in the nutrient agar. The antimicrobial test drugs are placed in the cups. The drug diffuses through the agar around the cup. The plates are incubated at a temp of 37ºC for 24hrs for bacteria and at 25ºC for 48hrs for fungi. The antimicrobial substances inhibit the growth of micro-organism and produce a clear zone of inhibition. The diameter of this zone can be measured and is a measure of the degree of activity of the antimicrobial substances.

Disc diffusion method:

The single disc method involves the use of one disc of either a high or low antibiotic concentration, determining the relative sensitivity of the organism to the drug which requires interpretation of zone sizes. In double disc method, the interpretation is simpler. Here, both high and low strength discs are applied for each antibiotic. The organism is reported sensitive if a clear zone appears around both the discs. If the zone appears around the high concentration alone, the organism is considered sensitive to the drug at that conc. only.

Screening of antibacterial activity by agar diffusion method:

In our current study, the antibacterial activity was carried out by the agar diffusion method. Here the responses of the organisms to the synthesized compounds were measured and compared with the responses of the standard drugs. The standard reference drugs used in the antibacterial screening was Ciprofloxacin.

Micro-organisms:

The test organisms were procured from Shadan institute of medical sciences. All those organisms were clinical isolates.

- *Bacillus subtilis* (gram positive spore forming bacteria)
- *Escherichia coli* (gram negative bacteria)

Sterilization of equipments required:

Petridishes, cork borer, beakers, glass syringes and test tubes were sterilized by dry heat sterilization at 160ºC for 1hr in hot air oven.

Preparation of sample solutions:

Each sample compound was dissolved in DMSO to make the concentrations of 1000µg/ml.

Standards used Ciprofloxacin – 30mcg

Composition of Nutrient Agar media:

Beef extract	:	3g
Peptone	:	5g
NaCl	:	5g
Agar	:	15g
Water	:	1000ml

Preparation of Nutrient Agar media:

The preparation of the bacteriological media involves the following steps:-

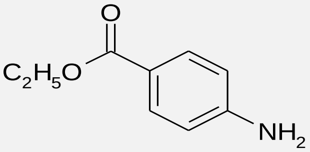
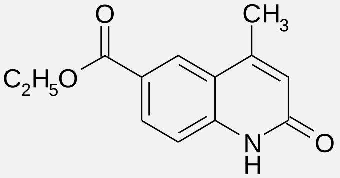
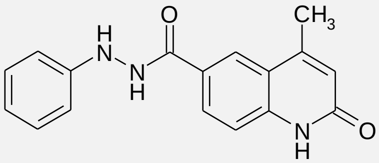
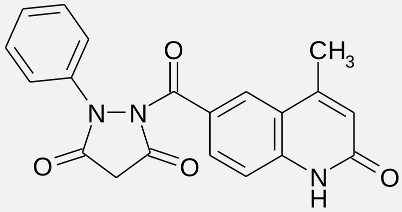
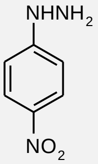
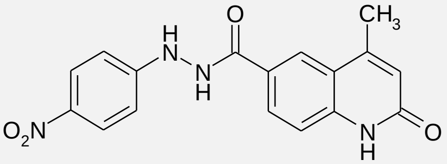
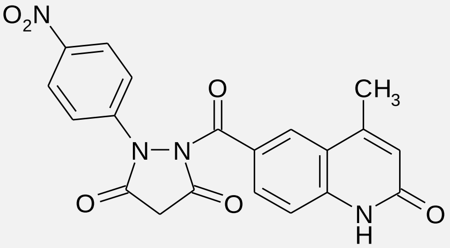
- Each ingredient was taken in 1000ml beaker and dissolved in 1000ml of distilled water by boiling.
- The pH of the medium was determined with a pH meter and adjusted to 5.8.
- The media so prepared was sterilized by autoclaving at a temp. of 121ºC for 15mins or till the pressure reached up to 15 lbs.

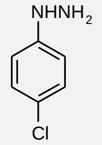
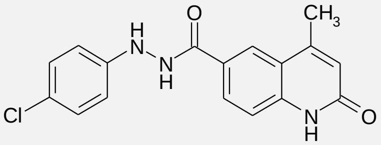
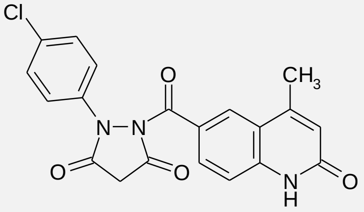
Preparation of Agar plates:

The media was cooled to 45º-46ºC and inoculated with suspension of organisms. It was mixed well and 200ml of inoculated media was transferred into each of the plate (for each organism) and was kept at room temperature until the agar medium was completely solidified. Bores were made using cork borer. The bores were filled with 0.1ml of solution of test drug and control solution. The sterile discs of standard reference drugs were placed on to the surface of agar. The petridishes were kept for 2hrs to allow the drug to diffuse into the agar media. A sterile atmosphere was being maintained during the entire process by carrying out the work under Laminar Air Flow bench. All the plates were incubated for 24hrs at 37ºC. At the end of incubation period, diameter of the zones of inhibition were measured and recorded.

RESULTS

Physical Data of Synthesized Compounds.

Sl. No.	Compound	Structure	Chemical Name	Mol. Formula	Mol. Wt. (g)	m.p (ºC)	% Yield
01	2		Ethyl p-amino benzoate	$C_9H_{11}O_2N$	165	82-84	72.22
02	3		4-methyl-2-oxo-1,2 dihydro-quinoline-6 carboxylic acid ethyl ester	$C_{13}H_{13}O_3N$	231.23	195-197	53.57
03	5a		4-methyl-2-oxo-1,2 dihydroquinoline-6carboxylic acid N’-phenylhydrazide	$C_{17}H_{15}O_2N_3$	293.31	276-277	60.52
04	6a		1-(4-methyl-2-oxo-1,2-dihydro quinoline-6-carbonyl)-2-Phenyl-pyrazolidine-3, 5-dione	$C_{20}H_{15}O_4N_3$	361.52	225-277	52.77
08	4C		4-nitrophenylhydrazine	$C_6H_7N_3O_2$	153	132-133	74.43
09	5C		4-methyl-2-oxo-1,2 dihydro quinoline-6carboxylic acid N’-(4-nitrophenyl)-hydrazide	$C_{17}H_{14}O_4N_4$	338.30	273-274	64.75
10	6C		1-(4-methyl-2-oxo-1, 2-dihydro quinoline-6-carbonyl)-2-(4-nitrophenyl)-pyrazolidine-3, 5-dione	$C_{20}H_{14}O_6N_4$	406.35	247-249	41.66

13	4E		4-chlorophenylhydrazine	$C_6H_7N_2Cl$	142.23	117-119	76.80
14	5E		4-methyl-2-oxo-1,2 dihydro quinoline-6carboxylic acid N'-(4-chlorophenyl)-hydrazide	$C_{17}H_{13}O_2N_3Cl$	327.76	282-283	66.44
15	6E		1-(4-methyl-2-oxo-1, 2-dihydro quinoline-6-carbonyl)-2-(4-chlorophenyl)-pyrazolidine-3, 5-dione	$C_{20}H_{14}O_2N_3Cl$	395.58	251-252	44.44

TLC characterization of Synthesized Compounds
Stationary phase : silica gel G
Mobile phase : Toluene : Aq. Ethanol (95:5)
Visualization : Iodine Chamber

Table-IV: Spectral Data of Synthesized Compounds [90]

Sr. No.	Compound	R_f
01	2	0.95
02	3	0.74
04	5a	0.30
05	6a	0.19

Table-(IV.A) IR Spectral Data.

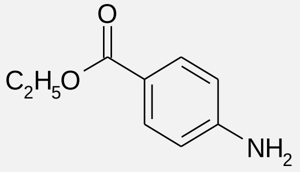
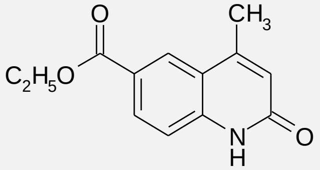
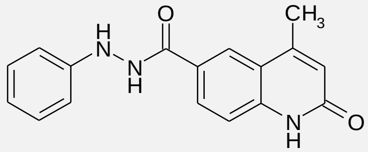
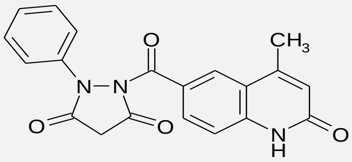
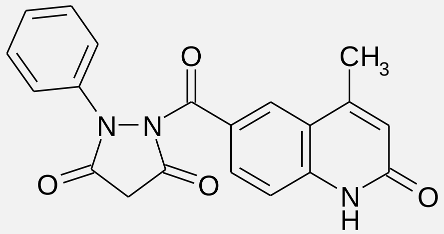
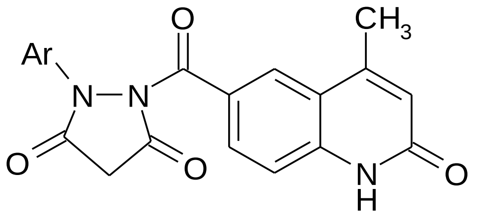
Fig. No.	Compound	Structure	IR Spectral Data ν_{max} (cm^{-1})
1	2		3285 (N-H),3061(Ar ,C-H), 1311(C-O),1684 (C=O), 2930(aliphatic C-H Stretching)
2	3		3423 (N-H), 3060 (Ar, C-H), 2930 (aliphatic C-H Stretching) 1312(C-O),1721 (C=O), 1613 (>C=O, quinolone)
3	5a		3417 (N-H), 3055 (Ar, C-H), 2991(aliphatic C-H Stretching) 1717 (C=O), 1525,1279 (CONH) 1604 (>C=O, quinolone)
4	6a		3285 (N-H), 3062 (Ar, C-H), 2932, (aliphatic C-H Stretching), 1720 (C=O), 1606 (>C=O, quinolone), 1689 (>C=O, pyrazolidinedione)

Table-(IV.B) Mass Spectral Data.

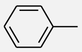
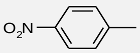
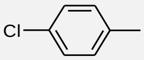
Fig. No.	Compound	Structure/ Mol. Wt. (Calculated)	Mol. Wt. (Observed)
6	6a		361 (M$^+$), 362 (M+1) and Other peaks at 284,186,159

ANTIMICROBIAL ACTIVITY:

RESULTS OF ANTIBACTERIAL ACTIVITY

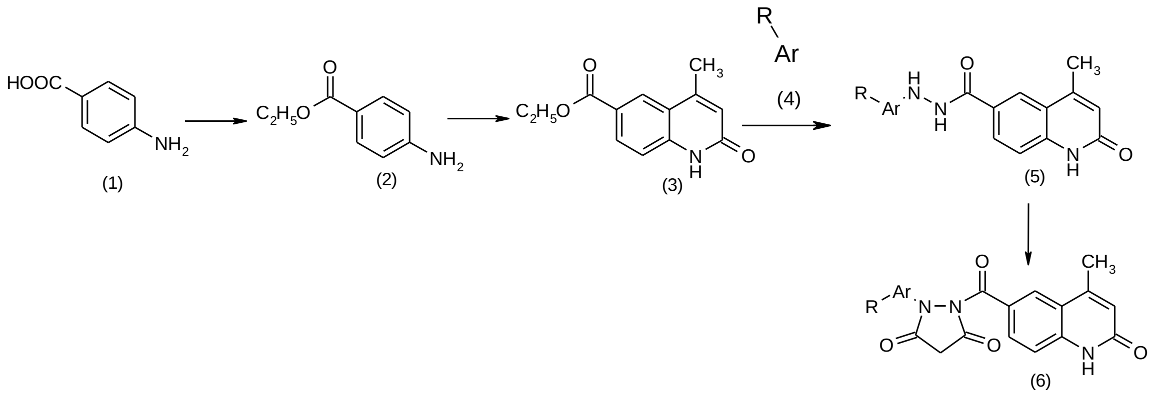


(6a-6c)

Compounds	Ar	Zone of Inhibition (mm)	
		B.substilis	E.coli
6a		12	17
6b		11	8
6c		7	8
Ciprofloxacin	……………..	28	34
Control (DMF)	……………..	NI	NI

NOTE: - Average Zone diameter of triplicates in mm.
NI : - No inhibition

DISCUSSION
The structures of new compounds prepared during present investigation have been established by their m.p and spectral studies. In the following section the characterisation of some selected compounds has been discussed.



Synthesized compounds (6a-6c).

S/No.	COMPOUND	R
1	6a	H
2	6b	4-Cl
3	6c	4-NO_2

The compound Ethyl p-amino benzoate [2] was prepared by esterification of PABA[1]. The melting point of PABA is 187-189ºC. The melting point of the ethyl p-amino benzoate is 82-84ºC. The difference in melting point clearly indicates the formation.

The compound 4-methyl-2-oxo-1,2 dihydro quinoline-6-carboxylic acid ethyl ester [3] is prepared from ethyl p-amino benzoate by Conrad-Limpach reaction. The IR spectrum shows carbonyl group (>C=O of 2-quinolone) at 1613 cm^{-1} and a sharp peak at 3423 cm^{-1} corresponding to N-H stretching of 2^o amine which clearly indicate formation of the compound [3].

The compound 4-methyl-2-oxo-1,2-dihydro quinoline-6-carboxylicacid-N-Phenyl hydrazide [5a] is prepared from phenyl hydrazine [4a]. The IR spectrum of [5a] shows V_{max} 1604cm^{-1} and 1240cm^{-1} due to 2-quinolone moiety, (-CONH-) and 1716cm^{-1} due to C=O stretch band of the hydrazide.

The compound 1-(4-methyl-2-oxo-1,2-dihydro quinoline-6-carbonyl)-2-phenyl-pyrazolidine-3,5-done [6a] was prepared from [5a] with diethyl malonate. The IR spectrum of [6a] clearly indicate carbonyl group (>C=O of pyrazolidine-3, 5-dione) at 1689cm-1.

The formation of [6a] has also been confirmed by its Mass spectra. The presence of. The molecular ion peak of [6a] has been observed at 361 in the mass spectrum is in good agreement with calculated molecular weight.

Biological Activity

Antimicrobial activity:

Antibacterial activity:

The antibacterial activity of newly synthesized 2-quinolone derivatives has been evaluated against Gram positive *Bacillus subtilis, and Gram negative Escherichia coli* by agar diffusion method. The standard used are ciprofloxacin.

Bacillus subtilis (Gram positive)

The zone of inhibition exhibited by 100µg of the compound 1-(4-methyl-2-oxo-1, 2-dihydroquinoline-6-carbonyl)-2-phenyl-pyrazolidine-3,5-dione[6a] against *Bacillus subtilis* (14mm) but is much less than the inhibition shown by 30µg of ciprofloxacin (36mm).

All compounds have shown antibacterial activity against Gram positive bacteria *Bacillus subtilis.*

The order of the antibacterial activity against gram positive organisms for the synthesized compounds is as follows.

Bacillus subtilis

6b (11mm) > 6A (12mm) > 6C (7mm)

Escherichia coli (Gram negative)

The zone of inhibition exhibited by 100µg of the compound 1-(4-methyl-2-oxo-1, 2- dihydro quinoline-6-carbonyl)-2-phenyl-pyrazolidine-3,5-dinone [6a]. against *Escherichia coli* is much less than the inhibition shown by 30µg of ciprofloxacin (34mm).

All compounds have shown antibacterial activity against Gram negative bacteria, *Escherichia coli and Shigella.*

The order of the antibacterial activity activity against gram negative organisms for the synthesized compounds is as follows.

Escherichia coli

6a(17mm) >6b&6C (8mm).

CONCLUSION

The main objectives of the present study are to synthesize, purify, characterize and evaluate the biological activity of 1-(4-methyl-2-oxo-1, 2-dihydro quinoline-6-carbonyl)-2-Phenyl-pyrazolidine-3, 5-dione and its different derivatives.

PABA on esterification gave benzocaine (Ethyl p-aminobenzoate) [2] which then undergoes Conrad-Limpach reaction to get 4-methyl-2-oxo-1,2 dihydro-quinoline-6-carboxylic acid ethyl ester [3]. This compound was further treated with different substituted phenylhydrazines[4a-4c] to get corresponding N'substituted phenylhydrazides of 4-methyl-2-oxo-1,2 dihydro quinoline-6carboxylic acid [5a-5c]. They were further treated with diethylmalonate to get 1-(4-methyl-2-oxo-1, 2-dihydro quinoline-6-carbonyl)-2-(Substituted Phenyl)-pyrazolidine-3, 5-diones. [6a-6c]

a. The yield of the products ranged from 40-60%.
b. The purity of the compounds was checked by TLC.
c. The functional groups in the title compounds are indicated by their IR spectra.
d. The structures of these representative samples are finally confirmed by their Mass spectra.
e. All the synthesized final compounds [6c-6c] were screened for.

Antibacterial activity against gram-positive and gram-negative microorganisms using Ciprofloxacin as standard references.

From this study it may be concluded that some of the derivatives of 1-(4-methyl-2-oxo-1, 2-dihydro quinoline-6-carbonyl)-2-Phenyl-pyrazolidine-3,5-dione show reasonable antimicrobial activity. With these encouraging results, all the synthesized compounds can be further explored for structural modification and detailed microbiological investigations to arrive at possibly newer potent antimicrobial agents.

LIST OF ABBREVIATIONS USED

ABBREVIATIONS	FOR
Ar	Aryl
Conc.	Concentrated
cm	Centimeter
°C	Degree centigrade
DMSO	Dimethyl suphoxide
PABA	P-Aminobenzoicacid
G	Gram
µg/ml	Microgram per millilitre
hr	Hours
IR	Infra red
MeO	Methoxy
ml	millilitre
m	Multiplet
N	Normal
M^+	Molecular ion
Me	Methyl
min	Minutes
mol	Mole
mm	Millimeter
mol.formula	Molecular formula
Mol.wt	Molecular weight
m.p	Melting point
MS	Mass spectroscopy
nm	Nanometer
Std	Standard
TLC	Thin layer chromatography
Rf	Retention factor
%	Percent
λ_{max}	Absoption maxima
ν_{max}	Transmittance
µg	Microgram
Ph	Phenyl

ACKNOWLEDGMENT

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