

Synthesis of Pyrazolopyrimidine Derivatives and Its Antioxidants Activity

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Abstract:

The process of new drug development requires a great deal of time and resources. The theoretical studies have a fundamental role to minimize these factors because they show indications of potential drug applications. Several authors mention that it is not enough for a compound to present high biological activity and low toxicity to be tested as a drug; it is also necessary to meet the ADME pharmacokinetics parameters (absorption, distribution, metabolism and excretion), which determine the access and the concentration of the compound in the therapeutic target and its subsequent elimination by the organism. Many drug candidates can be discarded for presenting unfavorable pharmacokinetics. The ADME parameters can be verified by *in silico* studies based on calculated physico-chemical standards. These standards emphasize lipophilicity, water solubility, molecule size and flexibility.

Keywords: Pharmacokinetics, *In silico*, lipophilicity, Pyrazolopyrimidine derivatives.

1.1.INTRODUCTION:

Toxicity screening was performed for: Drug Induced Toxicity, Genomic Toxicity, Aquatic & Terrestrial Toxicity, Reproductive Toxicity, Environmental Factor. These toxicity values were adapted from literature support. For details please read the details from Cheng F *et al* 2012 (PubMed ID: 23092397). Toxicity may be due to the accumulation in a specific organ/ tissue (e.g. bosentan), the co-administration of other drugs affecting ADMET (absorption, distribution, metabolism, elimination and toxicity) Cmax reaching off target IC₅₀, or the high Cmax required for therapeutic effects. Assessing the relative drug efficacy and toxicity is important for medicinal

chemists, pharmacologists, pharmacists, physicians. As multiple treatment options are available for many diseases, relative toxicity assessment is necessary. Difficulty in direct clinical trial comparisons forces network meta-analyses for estimating the relative toxicity.

Therapeutic index (TI) assumes simplified linear relationships between receptor affinity, maximum unbound plasma drug concentration (C_{max}) and toxicity. But high TI does not guarantee safety. For drugs metabolized by cytochrome P450 (CYP450), estimating TI based on target potencies alone is insufficient.

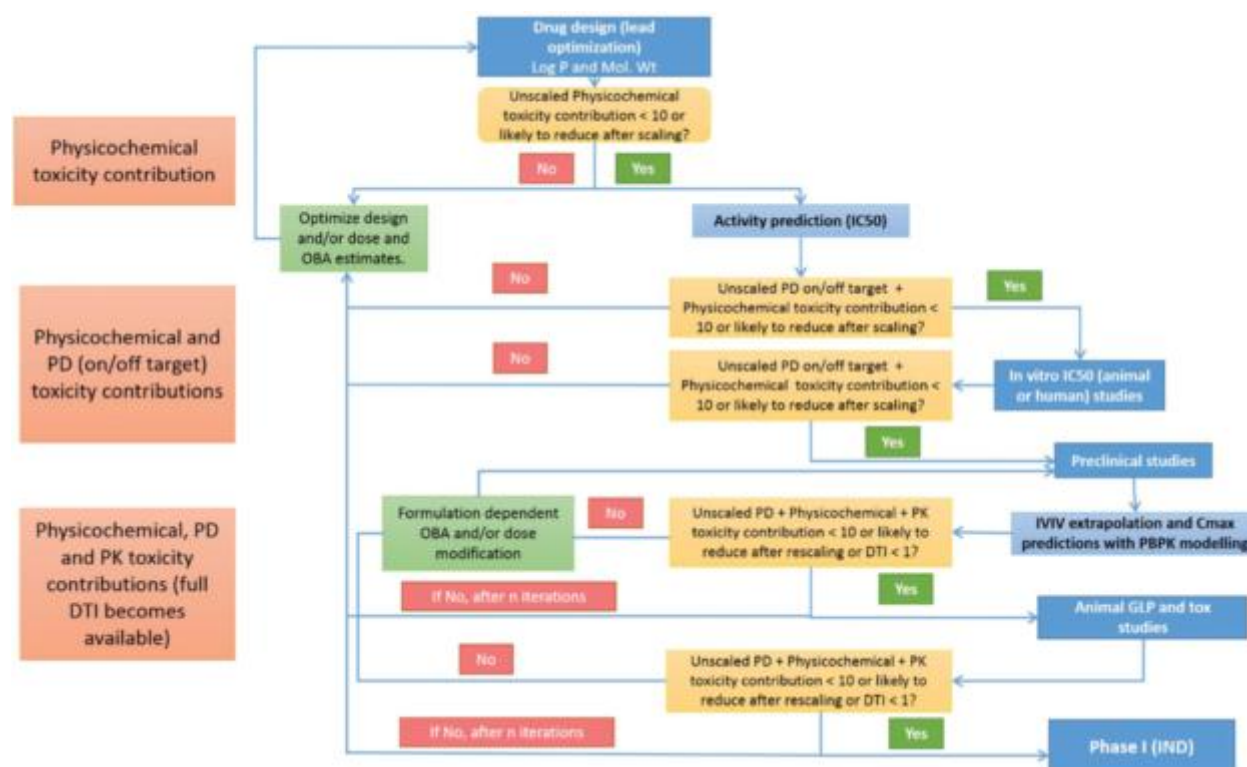


Figure 1.1
human health.[1]

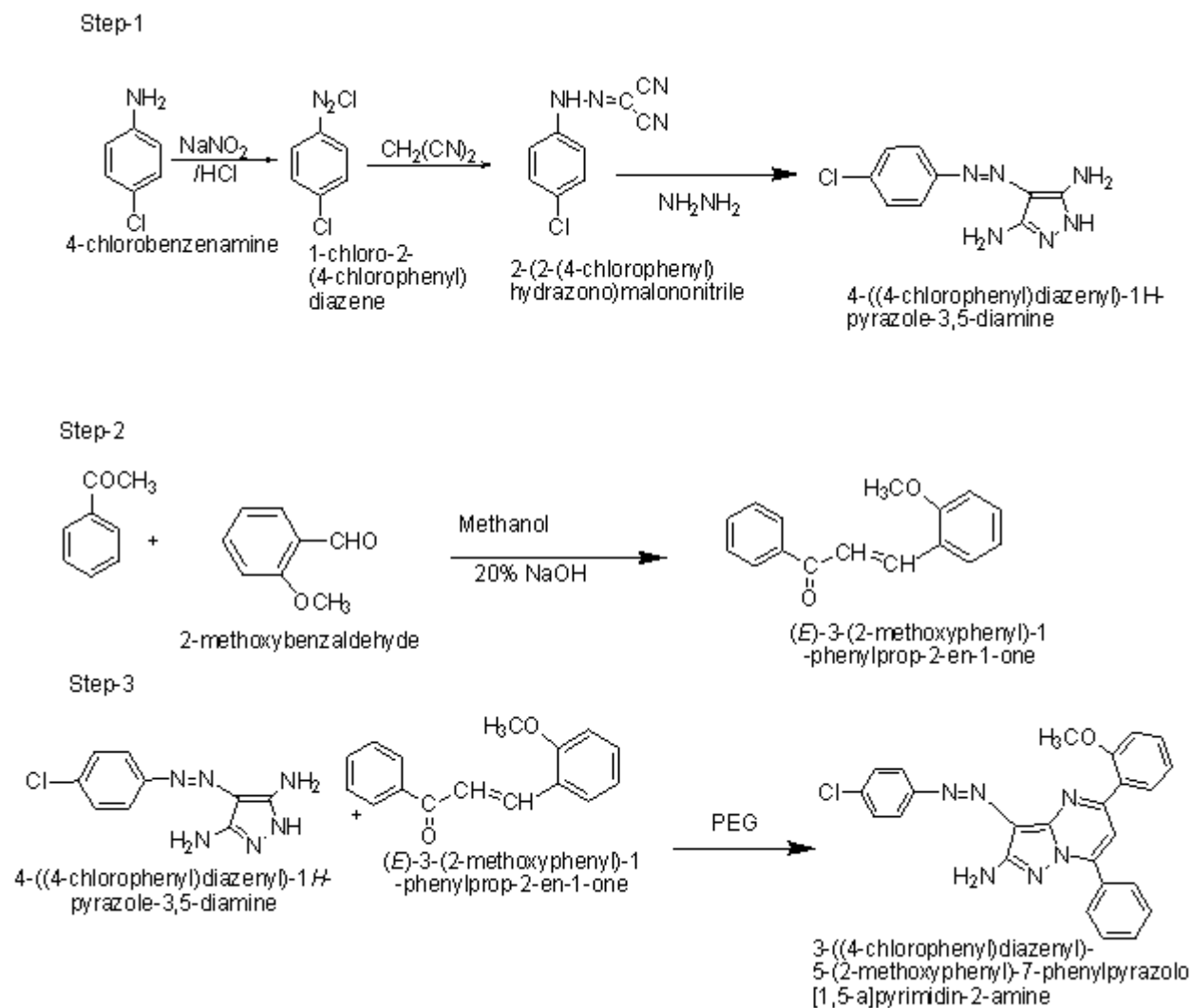
Additional preclinical studies, such as studies to qualify impurities and degradants in drug substance/product, are outlined in ICH Q3A and Q3B. Specific studies needed to qualify an impurity or degradant are generally not warranted before Phase III unless there are changes that result in a significant new impurity profile (eg, a new degradant formed by interactions between the components of the formulation, a new synthetic pathway). If a new degradant is formed by interactions between the components of the formulation, appropriate qualification studies should be conducted to support Phase II or later stages of development.

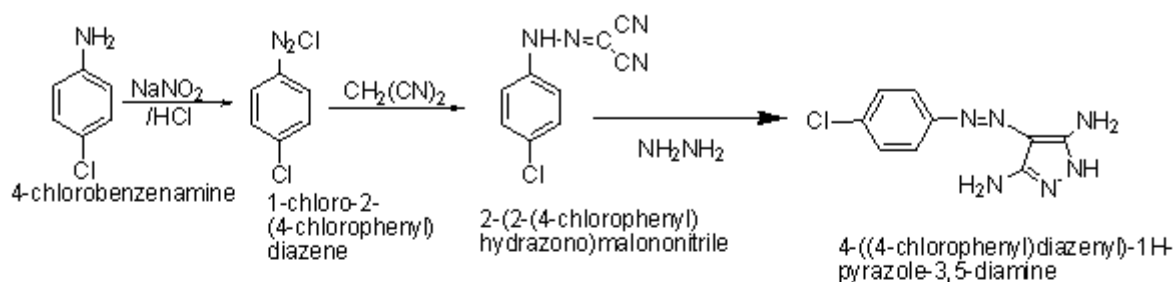
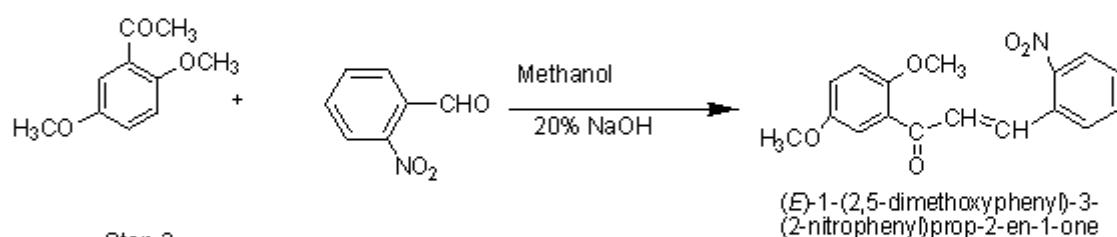
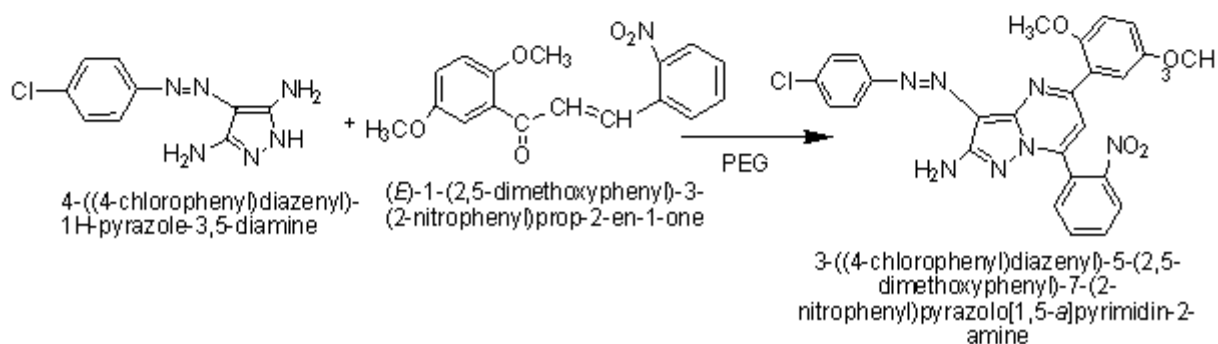
Chemical toxicity can be associated with many hazardous biological effects such as gene damage, carcinogenicity, or induction of lethal rodent or human diseases. It is important to evaluate the toxicity of all commercial chemicals, especially the high production volume (HPV)1 compounds as well as drugs or drug candidates, since these compounds could directly affect

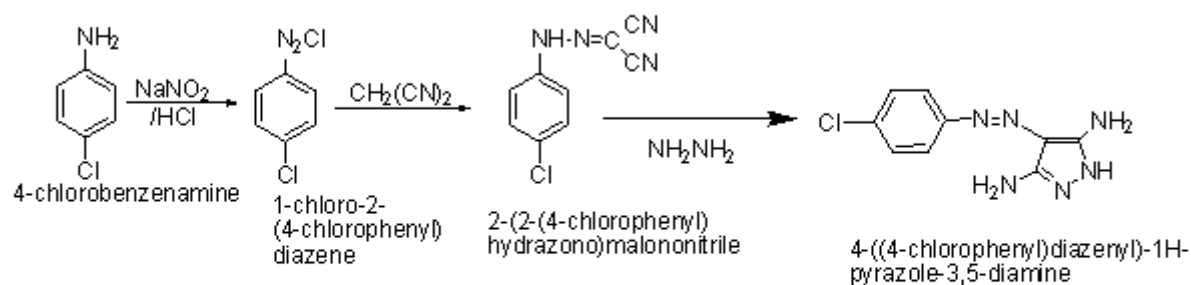
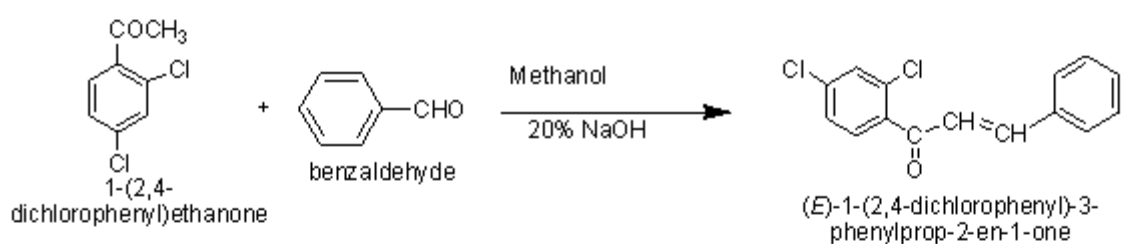
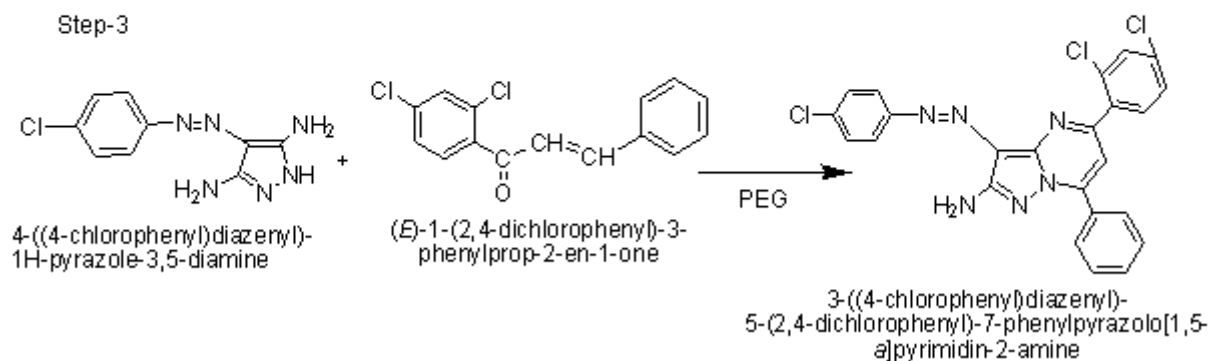
In silico study:

The process of new drug development requires a great deal of time and resources. The theoretical studies have a fundamental role to minimize these factors because they show indications of potential drug applications. Several authors mention that it is not enough for a compound to present high biological activity and low toxicity to be tested as a drug; it is also necessary to meet the ADME pharmacokinetics parameters (absorption, distribution, metabolism and excretion), which determine the access and the concentration of the compound in the therapeutic target and its subsequent elimination by the organism.

1.2. Synthesis

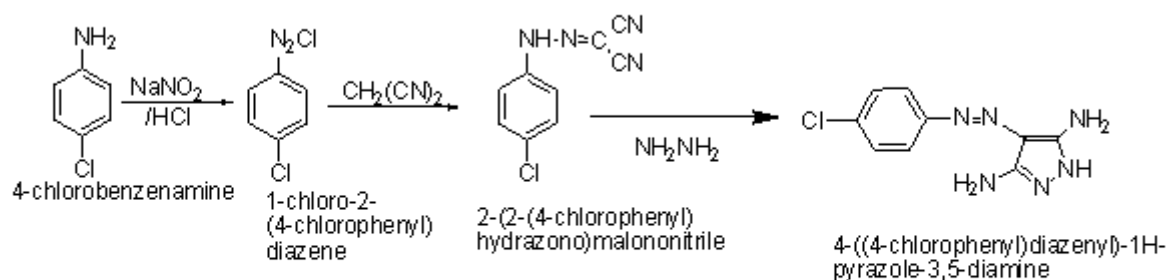
PP₁:

PP₂:**Step-1****Step-2****Step-3**

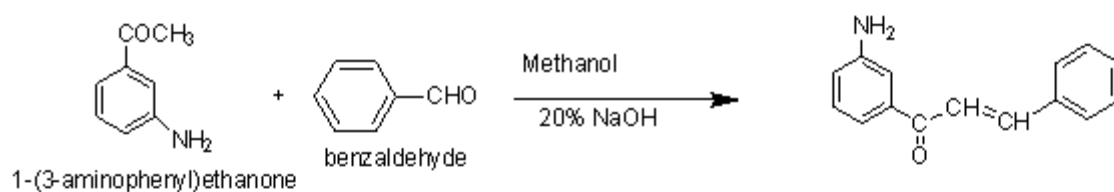
PP₃:**Step-1****Step-2****Step-3**

PP₄:

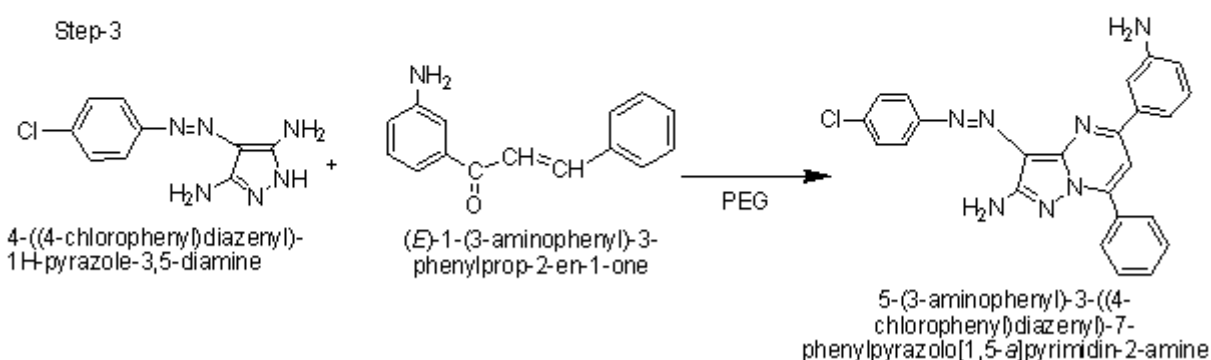
Step-1

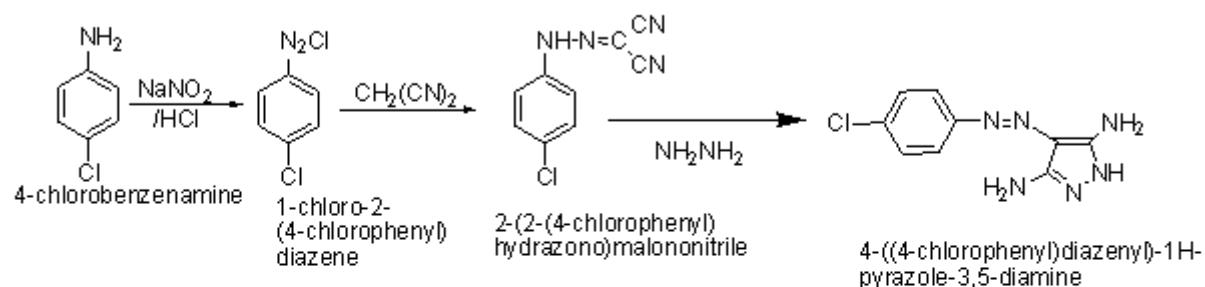
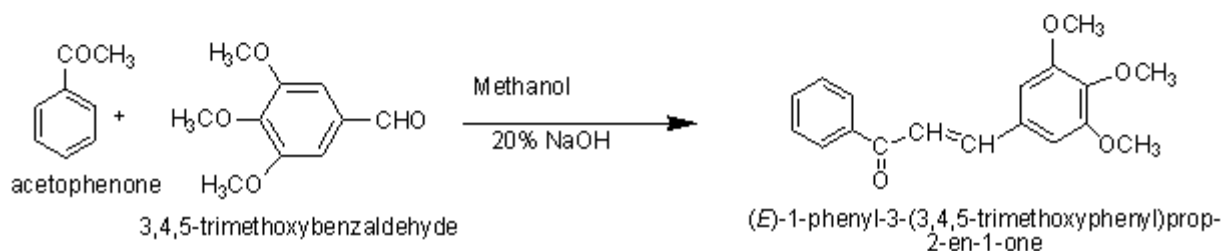
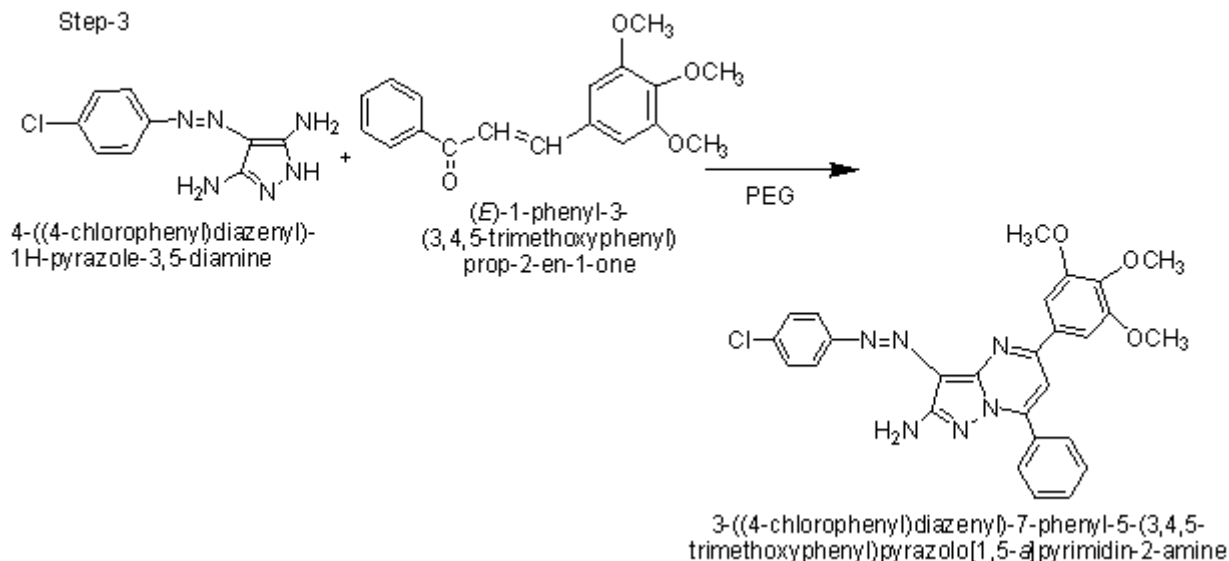


Step-2



Step-3



PP₅:**Step-1****Step-2****Step-3****1.3.Antioxidant activity:**

Oxidation is very crucial process of aerobic life system. As free radicals species with one or more unpaired electrons, are produced in normal or naturally or because of some biological dysfunction or pathological cell metabolism from xenobiotics or through

radiations. Electrons acceptors such as molecular oxygen react easily with free radicals to become harming radicals themselves [reactive oxygen species (ROS) and reactive nitrogen species (RNS)]. A variety of natural and synthetic substances are used by humans as antioxidant to capture and deactivate damaging ROS and RNS (Fig 1.1).

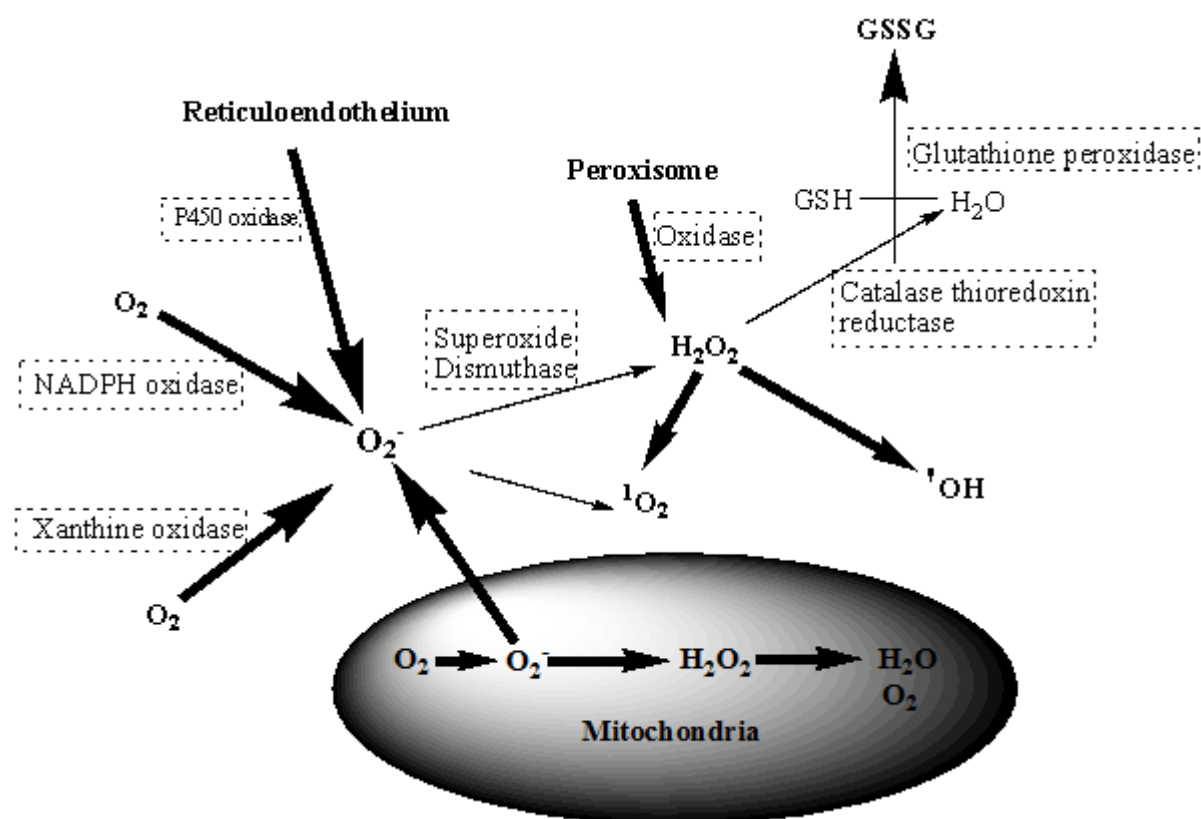


Figure: 1.2 Involvement of various enzymes in oxidation in body

Systemic hypoxia increases ROS generation and promotes leukocyte-endothelial adherence via reactive oxidant generation. Thus, antioxidants also prevent the increase in leukocyte-endothelial adhesive interactions observed in hypoxia.

Formation of ROS is characteristic of aerobic organism that normally defend themselves against highly reactive species such as superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and singlet oxygen (O_2), using enzymes like superoxide dimuthase, glutathione peroxidase and catalase and other naturally occurring antioxidants. This antioxidant defence system has co-evolved with aerobic metabolism to counteract oxidative damage from ROS and RNS. These antioxidant enzymes constitute a system that keeps ROS and RNS at low steady state in cell and tissue and prevent oxidative situations. As the compounds contains a high percentage of

nitrogen. Therefore it might have increased reducing power to scavenge free radicals, especially ROS and RNS [2].

1.4. Antioxidant agents:

Antioxidants are believe to quench free radicals. Free radicals are the atoms or the molecules with 'singlet', i.e. unpaired electron which makes them highly reactive.

An antioxidant is the compound which contains a highly labile hydrogen atom forming a radical, the radical formed should be stable and is not reactive so that it will not participate in the RS propagation step. Sterically hindered phenolic compounds satisfy most of the above-mentioned requirements.

Oxidative free radicals are generated by metabolic reations-create a chain reaction leading to membrane and other lipid peroxidation, DNA damage, etc. Thus,

reduction of the rate of these life-limiting metabolic processes by use of chemicals has been a subject of current research. [3]

1.5. Mechanism of action of antioxidant drugs

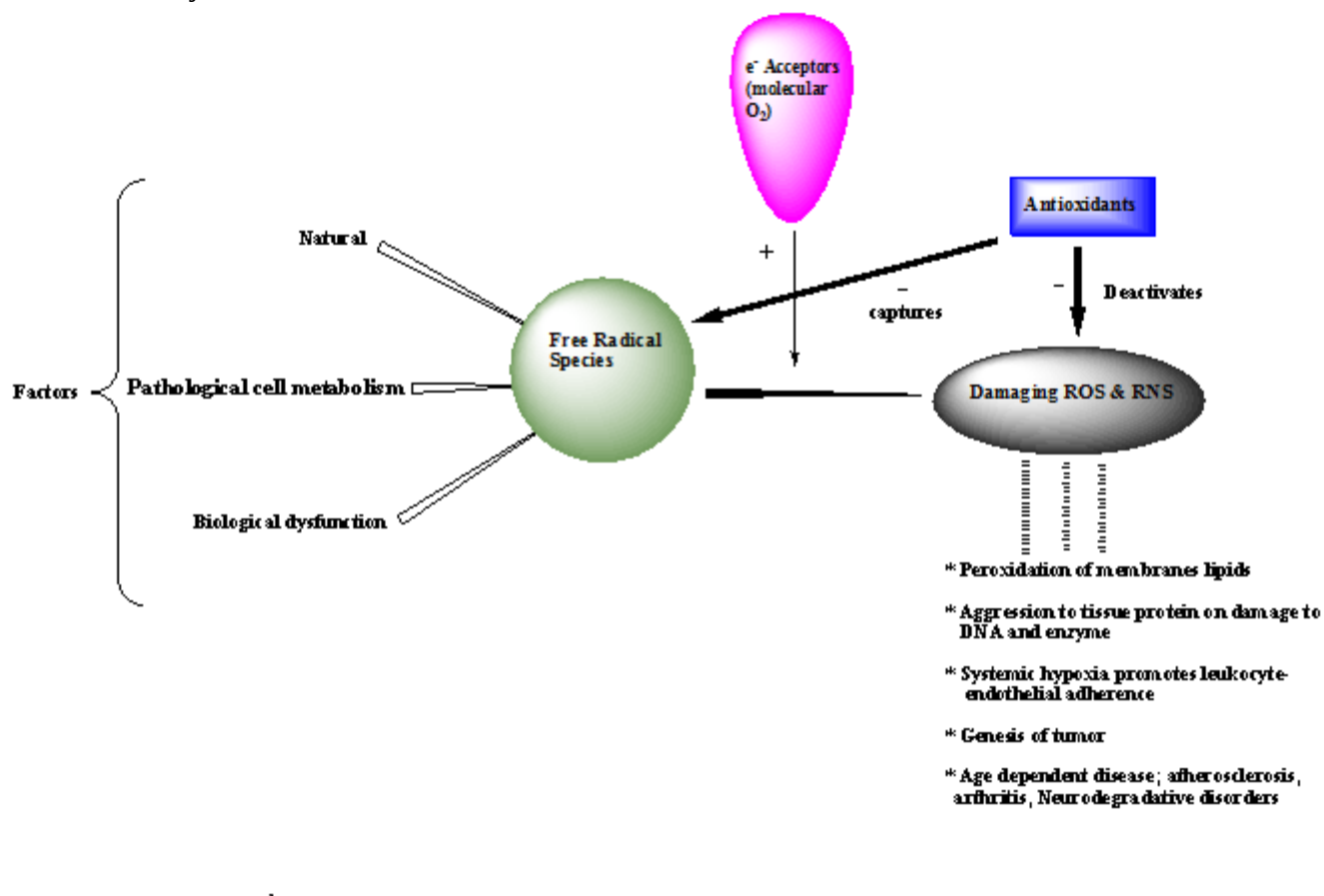


Figure1.3

The primary derivatives of oxygen, such as superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and singlet oxygen (O_2), play important role in mediating ROS-related effects. Short-lived reactive species generated in-situ and can react with non-radicals and produce chain reactions [4].

Excess of ROS has harmful effects like peroxidation of membranes lipids, aggression to tissue protein on damage to DNA and enzyme. Peroxidation of membrane-mediated effects of ROS are continuously generated in cells and exposed to an aerobic environment, and have been associated with genesis of tumor as well as age dependent disease

such as atherosclerosis, arthritis and neurodegradative disorders. The protection of an antioxidant can be explained by the capacity of that to scavenge free radicals, which are responsible for oxidative damage of lipids, proteins and nucleic acid [5].

We have contemplated research focusing on antioxidant pharmaceutically importance of pyrazole and synthesized a new series of pyrazolopyrimidine derivatives.

1.6. Methods for evaluation of antioxidant agents

For the study of antioxidant effects, the inhibition of lipid peroxidation, superoxide

generation and the activities of superoxide or hydroxyl radical scavenging are usually evaluated.

A) Antioxidant evaluation- *in vivo*

Treatment: Rats are weighed in beginning and end of experiments. Test drugs are administered for 3 days. Control rats are treated with the same volume of distilled water. Animals are decapitated for 24 hrs after last dose. The liver and kidney are rapidly removed, weighed and part thereof used for ascorbic acid determination. The rest of tissue were frozen for not more than 72hrs to await analysis of reduced glutathione (GSH) and lipid peroxide (LP) concentrations.

Biochemical determinations

GSH determination is performed by spectrophotometric method of **Sediac and Lindsay (1968)**. The lipid peroxidation is assayed by the method described by **Ramasay et al.**

1.7.Evaluations of enzyme activators and inhibitors agents:

One approach for the investigation and evaluation of biological activity is to identify enzyme activators or inhibitors.

The demonstration of that cyclic nucleotide are involved in many aspects of cellular activity and metabolism offer immense possibilities. Some diseases (such as nephrogenic diabetes insipidus, asthma, cancer, psoriasis, and obesity) and some circulatory disorders (platelet aggregartin, for example) seem to be released in abnormalities in the cyclic nucleotide system.

Therapeutic use of activators or inhibitors of this enzyme, which lies in cell membranes. There are two ways of increasing the concentrations of nucleotide 3',5'-monophosphate responsible for the biological; either by stimulating adenylate cyclase or by inhibiting phosphodiesterases,

the enzyme responsible for the hydrolysis of c-AMP into the nucleotide 5-monophosphate.

This can be exemplified by the several examples as follows.

Forskolin: Among the activators of adenylate cyclase, and apart from certain natural prostaglandins (PGI₂, and PGE₁), there is at present only specifically active substance available.

The pathological changes which are observed may be due to the above mentioned factors.

B) Antioxidant test- *in vitro*

To evaluate antioxidant effect of drug can be expressed by FeCl₃-Ascorbic induced non-enzymatic lipid-peroxidation, xanthine oxidase induced superoxide formation and free radical scavenging activity.

FeCl₃-Ascorbic acid stimulated lipid peroxidation:

The effects of test drug id determined by assay of MDA-TBA (Malondialdehyde-TrisHCl buffer & Ascorbic acid) level adduct at 532nm.

For the evaluation of antioxidant drugs there is usually a significant incresase in MDA level compared with that of normal control MDA-TBA adduct at 532nm.

The effect of anti-FeCl₃-Ascorbic acid and how it stimulate lipid peroxidation and its determination can be useful for antioxidant evaluation.

Nitric acid method: Inhibition of Xanthine oxidase:

Xanthine oxidase activity can be determined by xanthine oxidase system described by Chang *et at*, (1994).

For evaluation of the inhibition of superoxide generation, the activity of

xanthine oxidase towards xanthine as a substrate is assayed spectrophotometrically at 295nm (ascorbency of aric acid).

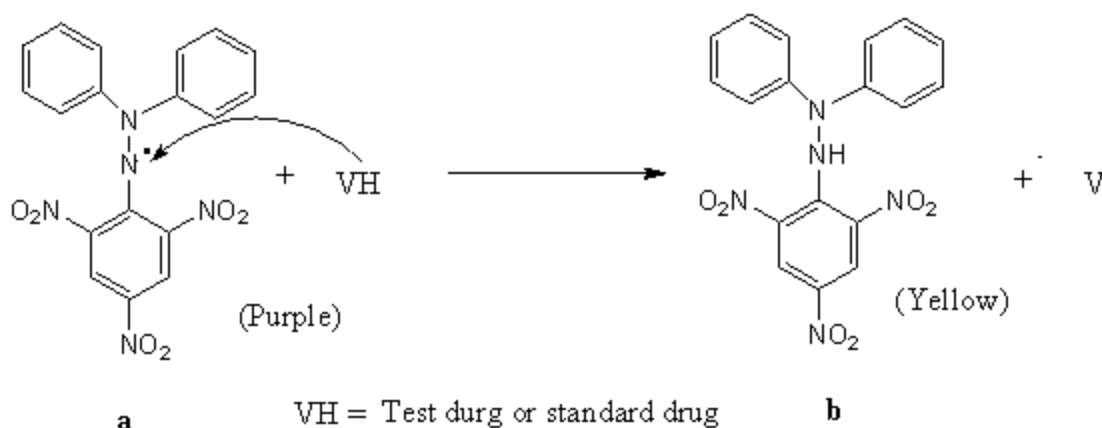
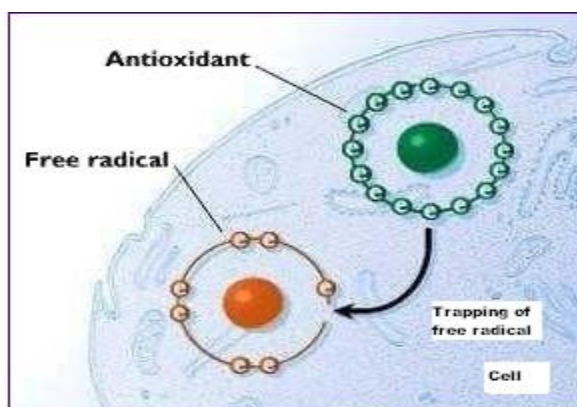
Enzyme assay for evaluation of Antioxidant Potentials from test drugs:

K_2HPO_4 at pH 7.0 homogenized with drug for few minues at 4 °C, aliquots of supernatant is used for enzyme activity measurements. SOD activity is determined based on the autoclatalytic transformation of epinephrine-adreno-chrome at pH 10.2 P-ase activity is measured using the method of Simon et al, (1974)

DDPH free radical scavenging method

Theory:

DPPH: 1,1-diphenyl-2-picrylhydrazyl radical is widely used to evaluate the free radical scavenging capacity of different antioxidants [6].



a; DPPH forming stable free radical at 517nm,

b; DPPH molecule forming stable diamagnetic spin paired molecule

Fig 1.4

Mechanism of action of antioxidant drugs via DPPH free radical scavenging method is based on either removal of one electron from DPPH unstable molecule or donation of one electron from antioxidant agent (molecules able to show paramagnetic characters in aqueous and alcoholic solution) to DPPH unstable molecule (Fig.1.4) [7,8].

The odd electron in DPPH molecule gives absorption maximum at 517nm, which disappeared after acceptance of an electron or hydrogen radical from an antioxidant to become a stable diamagnetic molecule of DPPH. Antiradical power of antioxidant by measurement of decrease in absorbance when DPPH free radical is scavenged by an antioxidant. Often standard drugs for comparison of antioxidant activity of test compounds are nordihydriguaiaretic acid (NGDA), butylated hydroxy toluene (BHT), caffeic acid, trolox and most commonly used are ascorbic acid [9].

Comparison between percentage scavenging DPPH free radical of standard drug (ascorbic acid) and different test compounds can be shown best through graphs (Fig.13-16). IC₅₀ values denotes the concentration of sample which is required to scavenge 50% of free radicals. A lower IC₅₀ (µg/mL) value indicates higher free radical scavenging activity.

2.1. Material and methods:

The antioxidant testing of the synthesized compounds was done by *In-vitro* DPPH free radical scavenging method.

2.2. Standard drug

Ascorbic acid 100, 200, 300, 400, 500 and 600 µg/ml

2.3. Solvent

Methanol

2.4. Experimental procedure:

Activity was performed by DPPH free radical scavenging method: [10]

- 1) Stock solutions (1000µg/mL) of all compounds were made and then further dilutions (100, 200,300,400,500,600 µg/mL) were prepared.
- 2) All dilutions were mixed with 1mL DPPH methanolic solution (0.1mM, 1.975 mg in 50 mL) and 1mL from each dilution.
- 3) Aliquots were allowed to incubate for 30min at RT and absorbance value were measured at $\lambda_{\max}$ 517nm in U.V. Spectrophotometer and converted to %scavenging activity. [11].

$$\% \text{ Scavenging} = \frac{\text{Absorbance of Control} - \text{Absorbance of test/std samples}}{\text{Absorbance of Control}} \times 100$$

Antioxidant activity (IC₅₀ value of synthesized compounds)

Table1

Compounds	Conc. (µg/ml)	Percentage Inhibition	IC ₅₀ (µg/ml)	Remarks
Std (Ascorbic acid)	100	42.58	56.97	
	200	69.13		
	300	73.92		
	400	82.16		
	500	85.16		
	600	87.03		
PP1	100	44.73	129.74	Highly active
	200	53.34		
	300	66.74		
	400	68.00		
	500	74.16		
	600	75.83		

PP2	100	41.00	246.93	Moderately active
	200	44.00		
	300	54.30		
	400	59.60		
	500	71.77		
	600	75.59		
PP3	100	24.16	431.45	Less active
	200	27.75		
	300	46.17		
	400	49.70		
	500	51.96		
	600	62.67		
PP4	100	12.20	593.19	Less active
	200	22.90		
	300	26.31		
	400	41.38		
	500	43.30		
	600	47.84		
PP5	100	8.30	683.65	Less active
	200	13.87		
	300	25.35		
	400	27.20		
	500	40.19		
	600	42.10		

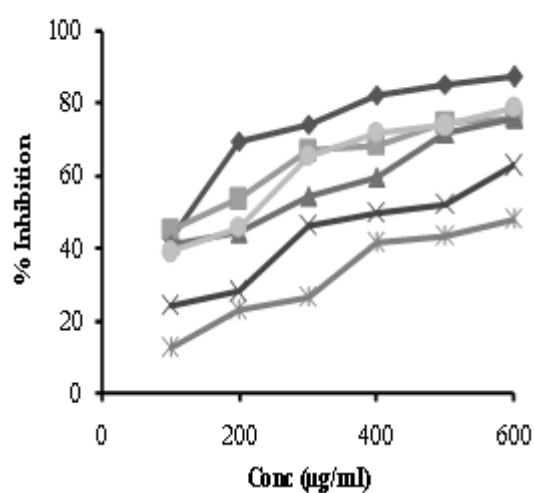


Figure: 1.5

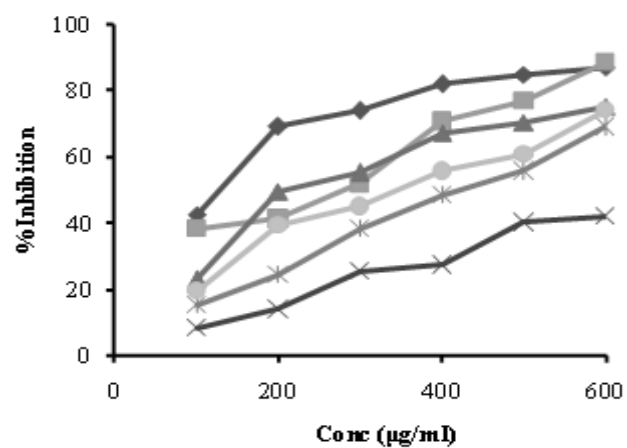


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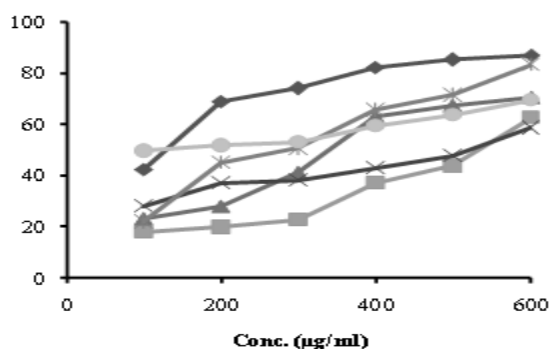


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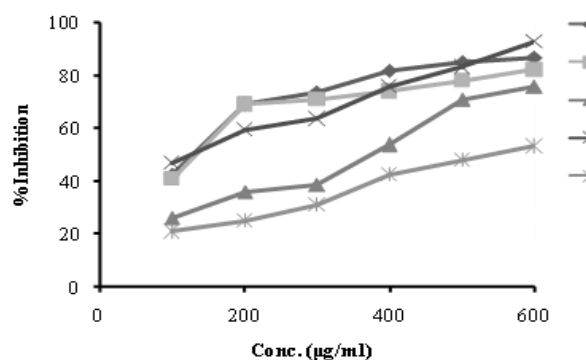
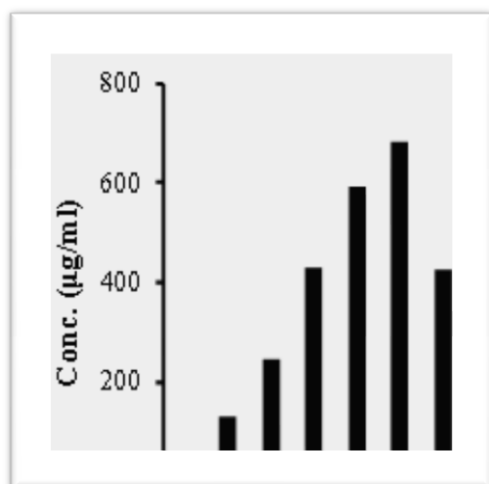


Figure: 1.8



Compounds

Figure 1.9

Result and Discussion:

This thesis deals primarily with the synthesis of heterocyclic compounds,

particular pyrazole derivatives. In recent years, considerable attention has been paid to their syntheses which undeniably play a significant choice of synthetic strategy and development of new classes of therapeutically active compounds. All the newly synthesized derivatives were tested *in-vivo* and *in-vitro* in order to evaluate their pharmacological activity. DPPH free radical scavenging method was used for antioxidant activity, results are shown in table1 Compound **PP1** was found to be highly active as its IC_{50} value (129 $\mu\text{g/ml}$) was closer to IC_{50} value of standard (56.97 $\mu\text{g/ml}$).

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