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RESEARCH ARTICLE

# Design, synthesis, characterization and biological studies of copper complex with biosensitive ligand

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## Abstract

A new biosensitive ligand (pyrazole derivative) was synthesized from acetophenone and benzaldehyde by Aldol condensation and then followed by condensation reaction with thiosemicarbazide under basic condition. The copper(II) complex was prepared and characterized by elemental, molar conductance, FTIR, $^{1}$H NMR and UV-Vis. spectroscopy. The electrochemical behavior of copper complex was studied and its salient features were discussed. It was concluded that the prepared copper complex may be mimic natural enzyme like SOD.

**Keywords**

Acetophenone

Benzaldehyde

FTIR

$^{1}$H NMR

UV-Vis. spectroscopy

## Introduction

Many heterocyclic compounds containing pyrazole moiety are biological significant and possessing a wide range of targetoriented bioactivities. The pyrazole derivatives make up the core structure of various boilogically active compounds. Molecules of many modern drugs, as well as of insect acaricides used in practice, contain the pyrazole ring as structural moiety [1-12]. Recently pyrazoles and its derivatives have been extremely useful intermediate for the synthesis of new biologically active compounds [13,14]. Pyrazole derivatives have attracted great atention due to wides pread applications in pharmaceutical [15] and agrochemical industries. The term pyrazole was given by Ludwig Knorr in 1883.

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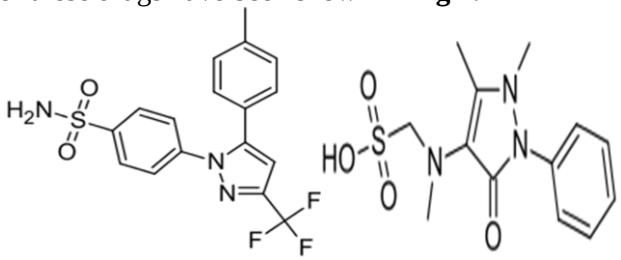
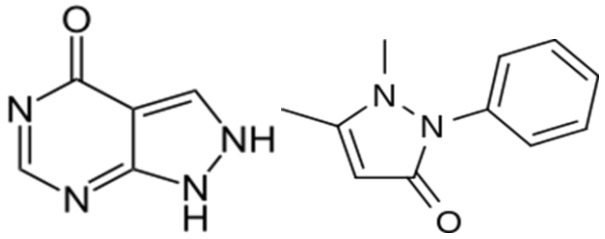
Pyrazole refers to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions. Being so composed and having pharmacological effects on humans, they are classified as alkaloids, although they are rare in nature. Pyrazoles are also the class of compounds that have the ring $C_3N_2$ with adjacent nitrogen centres. Notable drug that is a pyrazole is Celebrex. Derivatives of pyrazole are used for their analgesic, antiinflammatory, antipyretic, antiarrhythmic, tranquilizing, muscle relaxing, psychoanaleptic, anticonvulsant, monoamineoxidase inhibiting, antidiabetic and antibacterial activities. The pyrazole ring is present as the core in a variety of leading nonsteroidal antiinflammatory drugs and antihypertensive drugs. They have also found use as bifunctional ligands for metal catalysis, and in various building blocks for pharmaceutical and agricultural research [13-25]. Pyrazoles owing to

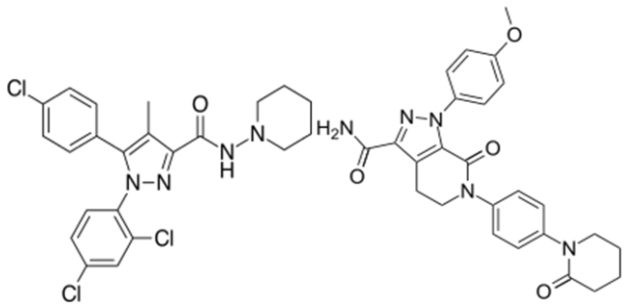
the presence of two neighbourhood nitrogen atoms, are also known as 1,2-diazoles. It has been the topic of medicinal research for the millions of researchers all over the world because of its large number of pharmacological activities. Some of the pyrazole possessing drugs like celecoxib, antipyrine, analgin, allopurinol, butazoline, phenylbutazone, oxyphenbutazone, novalgin, apixaban, pyrazofurin, ramifenazone, indisteron, fipronil, rimonabant and many more are already in market. Structure of some of these drugs have been shown in **Fig 1.**



Celecoxib Metamizole



Allopurinol Phenazone



Rimonabant Apixaban

**Fig 1** Pyrazole based drugs.

Phenylbutazone (**Fig 2**), a pyrazol derivative, is one of the oldest NSAID and it is used relatively little because of the agranulocytosis which it can elicit. There are other derivatives of pyrazole such as phenazone also called antipyrin and noraminopyrine also called noramidopyrine, noraminophenazone, metamizole and dipyrone. These two compounds are present in many proprietary formulations either alone or combined with other active principles.

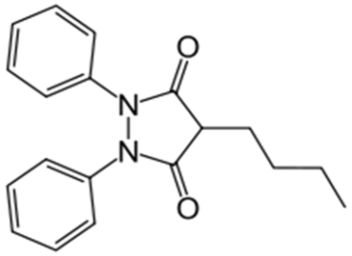


**Fig 2** Structure of phenylbutazone.

Pyrazole derivatives have a long history of application in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals. The Structure of chalone is shown in **Fig 3**.

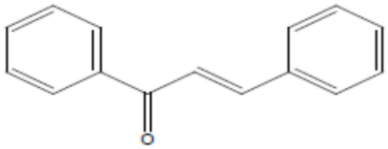


**Fig 3** Structure of chalcone.

Pyrazoles are important nitrogen containing 5-membered heterocyclic compounds and different methods have been produced for their synthesis. Pyrazole constitute interesting group of heterocycles because of their synthetic versatility and effective biological activities. In this work, novel pyrazole derivatives were synthesized via chalcone derivatives and evaluated for antibacterial and antifungal activity.

## Experimental

### Materials

All chemicals were obtained from Merck. 1,2-Dichloromethane, acetonitrile, acetone, methanol, N, N-dimethylformamide and dimethylsulphoxide were used of spectral grade.

### Preparation of supporting electrolyte

A clear solution of tetrabutylammonium bromide (25 mmol) in 20 ml water was mixed with 2.1 ml of 70% aqueous perchloric acid, tetrabutylammonium perchlorate got precipitated. It was filtered and washed with ethylacetate and dried. The sample were recrystallised from 3% aqueous perchloric acid.

### Preparation of chalcone

Aldol condensation of equimolar quantities of substituted acetophenone with appropriate quantity of substituted aromatic aldehyde in presence of aqueous

alcoholic alkali was used for formation of α, β – unsaturated ketones (i.e. chalcones). Equimolar portions of the substituted acetophenone (10 mmol) and substituted benzaldehyde (10 mmol) were dissolved in 15 mL of ethanol. The mixture was allowed to stirred for several minutes at 5-10 ºC. Aliquot of a 40% aqueous NaOH solution was then slowly added to the reaction flask. The reaction mixture was allowed to stirred at room temperature for approximately 4-6 h in ice bath. The crude product was washed first with cold water, neutrallized and precipitate formed was then collected and recrystallized from ethanol to give chalcone derivative.

**Preparation of pyrazole derivatives**

To mixture of chalcone derivative (10 mmol), thiosemicarbazide (10 mmol), and 10 % NaOH (0.025 mol, 10 ml) was refluxed in ethanol (25 ml) for 8-12 h. The resulting mixture was poured into ice-water and stirred. The crude product was washed with cold water and precipitate formed was collected and recrystallized from ethanol to give pyrazole derivative.

**Preparation of copper complex**

A hot ethanolic solution of pyrazole derivative (1 mmol) is refluxed with equimolar solution of the copper chloride for 4 h. The resulting solution was poured into crushed ice, cooled for 24 h. The precipitate was filtered and dried.

**Instruments**

Determination of CHN analysis was done by using Elementar Vario ELIII Carlo Erba. Molar conductance measurements of copper complexes were measured in DMSO solution using a coronation digital conductivity meter. The IR spectra of the ligands and their copper complexes were recorded on a Shimadzu FTIR IR Affinity$^{-1}$ using KBr disc in the range 4000-350 cm$^{-1.}$ The $^{1}$H NMR spectrum of the ligand was recorded using Bruker Avance II 400 MHz spectrometer. TMS is used as standard.

Electronic spectra were recorded in a Systronics 2201 Double beam UV-Vis., spectrometer within the range of 200-1100 nm region. The cyclic voltammogram of the copper complex was recorded by using CHI 604D electrochemical analyzer with three electrode system of glassy carbon as the working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as the reference electrode. Tetrabutylammonium perchlorate was used as supporting electrolyte.

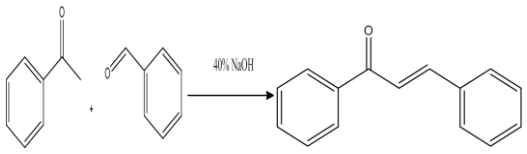
**SOD activity**

*In vitro* SOD activity was measured using alkaline DMSO as a source of superoxide radical ($O_2$) and nitroblue tetrazolium (NBT) as $O_2$scavenger. In general, 400 µl sample to be assayed was added to a solution containing 2.1 mL of 0.2 M potassium phosphate buffer (pH 8.6) and 1 mL of 56 µM NBT. The tubes were kept in ice for 15 min., and then 1.5 mL of alkaline DMSO solution was added while stirring. The absorbance was then monitored at 540 nm against a sample prepared under similar condition except that NaOH was absent in DMSO. A unit of SOD activity is the concentration of complex or enzyme, which causes 50% inhibition of alkaline DMSO mediated reduction of NBT.

## Results and discussion

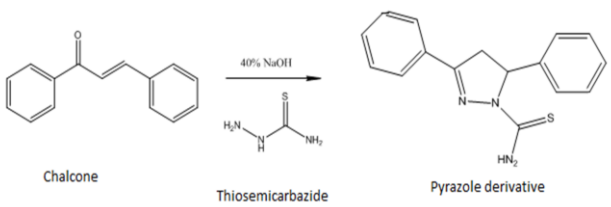
The systematic tailor made approach was adapted to synthesized pyrazole derivative (by the aldol condensation of acetophenone and 4-nitrobenzaldehyde in basic medium under reflux condition and followed by condensation reaction). The schematic representation is as follows:

**Stage-I**

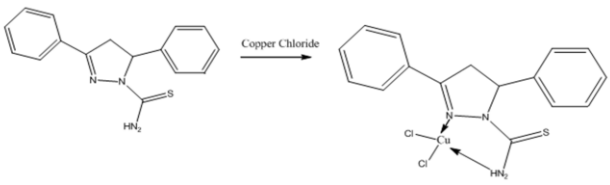



In the present work, benzaldehyde and acetophenone has been chosen to prepare pyrazole derivative. A bioactive ligand, 3,5-diphenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide have been synthesized by treating substituted (2*E*)-1,3-diphenylprop-2-en-1-one derivatives with thiosemicarbazide. The assignment of the structure was based on their correct elemental analyses and spectroscopic data.

**Stage-II**



**Stage-III**




Pyrazole derivative Metal complex

**5-(2-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide**

**IR KBr ν ($cm^{-1}$):** C=N str (1589.23), C=C Ar ring (1531.37, 1419.51, 1487.01, 1446.51), Ar-O-C str (1272.93), C-N str (1209.28), C=S str (1027.99).

**$^1$H NMR ($CDCl_3$) δ in ppm:** 7.1 ~ 7.6 (m, 5H, CH ring I), 6.8, 6.97, 7.02 & 7.1 (m, 5H, CH ring II), 3.4 (t, CH of pyrazole ring), 2.8 (d, $OCH_3$ of ring II), 2.2 (d, $CH_2$, pyrazole ring).

The elemental analyses agree well with a 1:1 metal to ligand stoichiometry for the copper complex. The metal complex is insoluble in water and other common organic solvents, but soluble in DMSO. The experimental conductivity valúes in DMSO solutions falls in the range of a non-electrolytic nature.

**IR spectra**

The IR spectrum of pyrazole and its copper complex was recorded. The IR spectrum of chalcone showed characteristic peaks of functional group C=O str between 1600-1700 $cm^{-1}$, Aromatic ring C=C str between 1450-1600 $cm^{-1}$. C-H def peaks were found in between 750-900 $cm^{-1}$. In the pyrazole derivative, the characteristic bands were observed in the spectra of 1250-1300, 1230-1250 and 1600-1650 $cm^{-1}$ for N-N=C, C-N and C=N stretching, respectively. Pyrazole ring posses a thiosemicarbazide skeleton and was formed because of appearance of peaks between 1000-1100, 1630-1700, 2950-300 $cm^{-1}$ for C=S stretching, $NH_2$ scissor, N=C-S stretching respectively.

**$^1$H-NMR spectrum**

Aryl proton on both the rings were found as multiplets in between δ 6.82 ~ 7.68 in most of the compounds, $OCH_3$ on aryl moieties was confirmed because of presence of singlet between δ 3.4 ~ 3.7. CH of pyrazole showed multiplet peaks in between δ 6.2 ~ 6.8. Proton attached with amine group on pyrazole ring was clarified by peaks in between δ 1.8 ~ 2.2.

**Electronic absorption spectrum**

The UV-Visible spectra of sulfathiazole and its complex in DMSO solution present absorption máxima attributable to the ligand. The band in the 250-270 nm is assigned to a $\pi$-$\pi$* transitions with in the organic molecule. An intraligand band at 290-300 nm is related to the $\pi$>$\pi$* transitions within the thiazole moiety. The band in the 360-380 nm región is ascribed to an intraligand transition of the $\pi$>$\pi$* type in accordance with the literature data. The fact that the bands due to sulphur atoms are not shifted suggests that this atom is not involved in coordination to metal ions.

**Cyclic voltammetry**

The electrochemical behaviour of copper complex **(Fig 4)** was recorded at a scan rate of 100 mV/s. The copper complex showed a cathodic peak at +1.0 V and anodic peak at -0.2 V which corresponds to Cu(II)/Cu(I) couple. The peak to peak separation of 1.2 V is due to quasi reversible in nature. Further, the cathodic peak was observed at -1.0 V which corresponds to ligand reduction. It is known that an adequate Cu(II)/Cu(I) redox potential for effective catalysis of superoxide radical must be required between −0.405 V for $O_2$/ $O_2^{\bullet-}$ and +0.645 V for $O_2^{\bullet-}$/$H_2O_2$ versus SCE (at pH 7) or between −0.762 and +0.29 V *versus* Ag/Ag $NO_3$/ ACN, respectively. The Cu(II)/Cu(I) redox couple is within this potential range; therefore, the copper complex may be expected to show SOD activity.

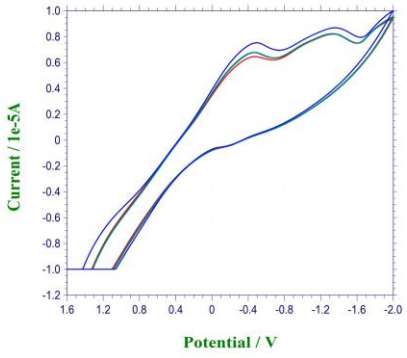
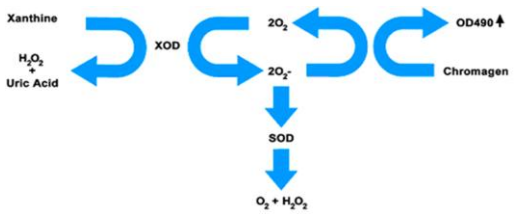



**Fig 4** Cyclic voltammogram of copper complex.

**SOD activity**

Metal complexes that can undergo such redox cycling are likely to function as superoxide scavengers as copper has been proven to be the active metal center in

the best studied SOD, many copper complexes have been synthesized and tested for SOD-like activity [17] and most of them appeared to be very efficient. Superoxide anions have a very short half life and, accordingly, they must be produced continuously. In this colorimetric based assay, superoxide ions are generated from the conversión of xanthine and oxygen to uric acid and hydrogen peroxide by xanthine oxidase. The superoxide anión then converts NBT to formazan, a colored product that absorbs light at 560 nm. SOD reduces the superoxide ion concentration and thereby lowers the rate of formazan formation. In the SOD like activity test, the metal complexes compete with NBT for oxidation of the generated superoxide ions. The more efficient the complex, the lower the concentration that corresponds to 50% inhibition of NBT reduction; this concentration is termed $IC_{J0}$ for comparative purposes. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The SOD assay measures all three types of SOD (Cu/ Zn, Mn, and FeSOD). The assay provides a simple, reproducible, and fast tool for assaying SOD activity in plasma, serum, erythrocyte lysates, tissue homogenates, and cell lysates.




Scheme of the SOD assay mechanism.

## Conclusions

The newly synthesized copper complex with biological active ligand (pyrazole). The structure of the ligand and its copper complex was investigated using spectral and analytical techniques. The distorted square planar geometry was assigned for copper complex on the basis of electronic spectrum. This complex may be mimics the active center of Cu/Zn superoxide dismutase. Its absorption spectrum showed a broad band above 600 nm as compared with the 670-680 nm band of the enzyme and also showed SOD activity.

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