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N-Nitroso-2, 6- Diphenylpiperidin-4-One Semicarbazone Synthesis, Characterization, Antioxidant Activity and Molecular Docking with its Binding Site.

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Abstract: *Piperidines are very important compounds because of their presence in numerous alkaloids, pharmaceuticals, agrochemical and as synthetic intermediates. Biologically active alkaloids of the substituted piperidine ring system have been targeted for their total or partial synthesis. Synthetic efforts were also made fro modifications of pethidine and morphine like compounds, which led to the synthesis of new opiate having agonist as well as antagonistic activities. In many families of opiate narcotic analgesics small variations in chemical structures have changed the extent to which an analogue exhibits agonist and antagonist activity During the course of present work several alkyl/acyl/sulfonyl/benzoyl-aryl derivatives of 4-methylpiperidine were synthesized in order to evaluate their biological effects. The resulting derivatives were screened for brine shrimp lethality (cytotoxic) phytotoxic antibacterial, antifungal, insecticidal, urease inhibition and analgesic activities. N-nitroso-2,6- diphenylpiperidin-4-one semicarbazone has been prepared and analyzed. The product showed a single spot in TLC and sharp melting point for the purity of the compound. The structure of the compound was further confirmed from the FT-IR, and the compound has being screened for its antioxidant activity against ascorbic acid as standard. The compound exhibited significant % inhibition activities against ascorbic acid. Finally the compound was docked in Arguslab and the compound bind with 5hmh protein which works for cell trafficking, DNA repair etc.*

Keywords: *N-nitroso-2,6- diphenylpiperidin-4-one semicarbazone, antioxidant activity , docking.*

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I. Introduction

Heterocyclic chemistry is a very important branch of organic chemistry accounting for nearly one-third of modern publications. In fact two thirds of organic compounds are heterocyclic compounds. Heterocyclic compounds constitute the largest and most varied family of organic chemistry, after all every carboxylic compound i.e. any group of organic compound that contain carbon in all ring atom; regardless the structure and functionally may converted into a heterocyclic analogue by replacing one or more of the ring carbon atom with different elements. The oxygen, nitrogen and sulfur (the most common heterocyclic element), are the numerous replacement of the ring carbon. The best known of the simple heterocyclic compounds are pyridine, pyrrole, furan, and thiophene. A molecule of pyridine contains a ring of six atoms—five carbon atoms and one nitrogen atom. Pyrrole, furan, and thiophene molecules each contain five member rings, composed of four atoms of carbon and one atom of nitrogen, oxygen, or sulfur, respectively.

Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals.

Endogenous antioxidants are enzymes, like superoxide dismutase, catalase, glutathione peroxidase or non enzymatic compounds, such as uric acid, bilirubin, albumin, metallothioneins. When endogenous factors cannot ensure a rigorous control and a complete protection of the organism against the reactive oxygen species, the need for exogenous antioxidants arises, as nutritional supplements or pharmaceutical products, which contain as active principle an antioxidant compound. Amongst the most important exogenous antioxidants, vitamin E, vitamin C, β-carotene, vitamin E, flavonoids, mineral are well known.

Exogenous antioxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but can also be synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene, gallates, etc .

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs.

Mechanism of Action of Antioxidants

LMWAs (low molecular weight antioxidants) are small molecules that frequently infiltrate cells, accumulate (at high concentrations) in specific compartments associated with oxidative damage, and then are regenerated by the cell. In human tissues, cellular LMWAs are obtained from various sources. Glutathione (GSH), nicotinamide adenine dinucleotide (reduced form), and carnosine are synthesized by the cells; uric acid (UA) and bilirubin are waste products of cellular metabolism; ascorbic acid (AA), tocopherols and polyphenols are antioxidants obtained from the diet.

Among these LMWAs, a considerable attention was focused on ascorbic acid (AA), known for its reductive properties and for its use on a wide scale as an antioxidant agent in foods and drinks; it is also important for therapeutic purposes and biological metabolism.

Ascorbic acid is an antioxidant with therapeutic properties, which plays an important role in activating the immune response, in wound healing, in osteogenesis, in detoxifying the organism, in iron absorption, in collagen biosynthesis, in preventing the clotting of blood vessels, and in many other metabolic processes.

Vitamin C can be easily oxidized, its degradation being accelerated by heat, light and the presence of heavy metal cations. Thus, due to its content variation, vitamin C represents an important quality indicator of foodstuffs and contributes to the antioxidant properties of food.

Special attention has been dedicated to the study of antioxidant action mechanism. The excess free radicals circulating in the body oxidize the low density lipoproteins (LDL), making them potentially lethal; the excess free radicals can also accelerate aging processes and have been linked to other very serious pathologies, such as brain stroke, diabetes mellitus, rheumatoid arthritis, Parkinson's disease, Alzheimer's disease and cancer. Physiologically, the oxygenated free radicals are among the most important radical species. Reactive oxygen species (ROS) comprise species with a strong oxidizing tendency, both of a radical nature (the superoxide radical, the hydroxyl radical) and a non-radical nature (ozone, hydrogen peroxide).

A number of chemical and physical phenomena can initiate oxidation, which proceeds continuously in the presence of (a) suitable substrate(s), until a blocking defence mechanism occurs . Target substances include oxygen, polyunsaturated fatty acids, phospholipids, cholesterol and DNA.

The essential features of oxidation via a free radical-mediated chain reaction are initiation, propagation, branching and termination steps. The process may be initiated by the action of external agents such as heat, light or ionizing radiation or by chemical initiation involving metal ions or metalloproteins.

The synthetic as well as natural piperidine display strong antioxidant activity due to its ability to inhibit or quench free radicals (hydroxy and ROS). The medicinal value of piperine is very huge due to its antioxidant, antiplatelet, anti-inflammatory, antihypertensive, hepatoprotective, antithyroid, antitumor, and antiasthmatic activities. Various phenolic amides extracted from pepper, which exhibited promising antioxidant properties in FTC and TBA assays. All the phenolic amides showed good antioxidant activity than α-tocopherol at 0.01% concentration.

The molecular docking method determines interaction between ligand and target molecule. It predicts binding affinity of ligand to form a stable complex with protein by finding preferred orientation of minimum free binding energy. This interaction involves many types of non-covalent interactions such as hydrogen bond, ionic bond, hydrophobic and Van der Waals. Molecular docking study can be possible in between protein-protein, protein-ligand and protein-nucleotid. Multiple steps of molecular docking method consist of preparation of 3-D structure of proteins, preparation of ligands, estimation of binding energy of protein-ligand complex and analysis of results.

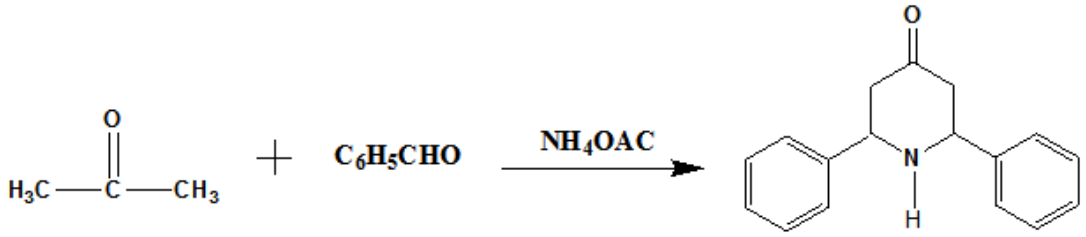
II. Materials And Methods

All the solvents used were of spectral grade. The melting points of the compounds were measured in open capillarie. The FT IR spectra were obtained on Bruker.The reactions were followed on precoated TLC plates (Silica gel G) visualizing the spots in iodine chamber.

EXPERIMENTAL METHOD

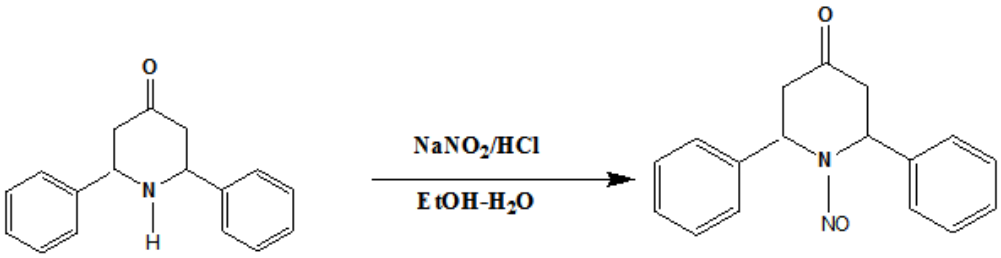
Scheme 1: Synthesis of 2, 6-diphenylpiperidin-4-one

A mixture of acetone (0.1 mol) and benzaldehyde (0.2 mol) and anhydrous ammonium acetate (0.05 mol) will be heating in a boiling water bath maintaining the temperature 50-55°C with stirring until the colour of the solution will change to deep red orange. The solution immediately cool in ice water, after cooling ether (100 ml) was add to it, the ether insoluble bispidine (2,4,6,8-tetra phenyl-3,7-diazabicyclo (3.3.1) nonan-9-one, m.p (235-236°C) will filter off and 5 ml conc. HCl will add to the filtrate. The precipitated 2,6-diphenylpiperidine-4-one hydrochloride will collect by filtration and wash with 3:1 mixture of ether and ethanol. The hydrochloride (m.p 215-216°C) obtain will dispersed in minimum amount of acetone and ammonia solution will add drop wise to it until a clear solution was obtain. The clear solution will pour into cold water (500 ml) and solid obtain will filter and dry. The solid obtain will recrystallize using ethanol (yield 25%) melting point 101-105°C.



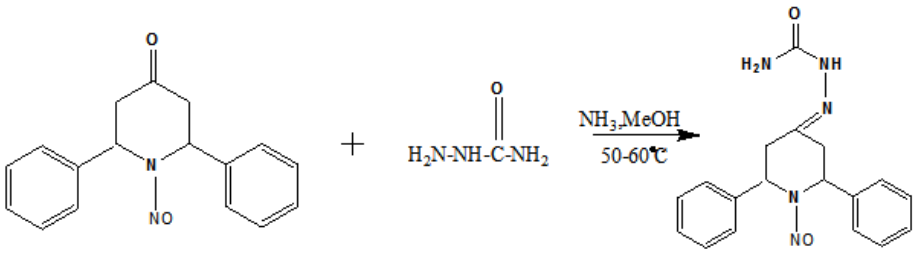
Scheme 2: Synthesis of N-nitroso-2, 6-diphenylpiperidin-4-one

0.0l g of 2,6-diphenylpiperidin-4-one dissolve in the ethanol-water mixture (60 ml+40 ml) and add 1.0 ml of concentrated HCl to this solution, the solution heat and stirre at 49-55°C. Sodium nitrite dissolve in 25 ml of ethanol: water mixture (10 ml+15 ml) and take this solution in an addition funnel. This solution will add in drops over a period of 1.5 hours while the mixtures will stir at 50-60°C. After the addition, stirring continued for another 4 hours. To this reaction mixture about 75 ml of ether will add. The product soluble in ether was separate by using a separation funnel. The separated ether will allow for evaporation. The solid obtain will recrystallize from ethanol.



Scheme 3: Synthesis of N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone

The compound N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone will prepare by dissolving N-Nitroso-2,6-dipheny lpiperidin-4-one in 10 ml of methanol and heat over a water bath at (50-60)°C. 1.0 g of semicarbazide hydrocholoride will dissolve in 3 ml of ammonia solution drop by drop, an equal amount of methanol will also add to it in 1:1 ratio. This solution will add to the above reaction mixture in three portions at an interval of 30 minutes. After the addition, heating and stirring are continuing to another five hours. Then the reaction mixture will pour into ice cold water with shaking. The pure solid separated will filter wash with water and dry and purify through short column, m. p 151°C.



Antioxidant Activity By DPPH Method

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported. Briefly, an 50 µg different concentration (25,50,100,200 & 400µg/mL) of the N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone in methanol were added to 5ml of 100 µM solution of DPPH in methanol .The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = {(A0 –A1)/A0)*100}

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of samples and reference.

Antioxidant Activity Phosphomolybdenum Assay (Pm)

Total antioxidant activity was estimated by phosphomolybdenum Assay. The total antioxidant assay based on reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V).Molybdate Reagent Solution was prepared by 1ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added in 20 ml of distilled water and made up volume to 50 ml by adding distilled water. N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone in differentconcentration ranging from 25, 50, 100, 200, 400 µl were added to eachtest tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95◦C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm.Ascorbic acid was used as positive reference standard.

%inhibition= (1- Absorbance of sample/absorbance of control) * 100

Docking of N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone

Chem Draw Ultra 8.0:It is used to design 2D structure of molecules.

Chem3D Ultra 8.0: It is used to design 3D structure of molecules.

Arguslab: It is used for docking study of the designed molecules

III. Result And Discussion

The compound N-nitroso-2,6-diphenylpiperidin-4-onesemicarbazone has been reported in this work. a single spot in TLC and sharp melting point for the purity of the compound. The structure was further confirmed from the FT-IR. And docking was done with Arguslab with the ligand 5hmh which is ligase inhibitor and it confirm the antioxidant activity.

IR spectral analysis

The important IR data are collected from the spectrum (Figure 1, 2 3) obtained are given in the Table 1 . Assignments of the frequencies were made on the basis of the literature values. The formation of the compound N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone was realized by the analysis of IR data.The IR spectrum of the compound N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone shows a sharp band at 1641 cm-1 which corresponds to C=0 stretching frequency of amide. The literature value of carbonyl group was found to be 1640 cm-1. The band at 1370 cm-1 shows the presence of C=N group. This confirms the formation of the product. The presence of a band at 3357cm-1 is a proof for the presence of N-H of primary amide group. The stretching frequency at 2952 cm-1 corresponds to aromatic C-H stretching frequency. The literature value for aromatic C-H is found to be 3100-3000 cm-1. The stretching frequency at 2841 cm-1 corresponds to cyclic C-H stretching of pyridine ring system, the literature value for such system is 3100-3000 cm-1 The band at stretching frequency 1451 cm-1 corresponds to N-N=0 group.

Antioxidant Activity By DPPH Method

The anti oxidant activity was found to be potent as compared to standard of ascorbic acid. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. The collected data of % inhibition and IC 50 was calculated as shown in table 2 and figure 4.

Antioxidant Activity Phosphomolybdenum Assay (Pm)

The antioxidant activity of the compound was found to reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V). The % inhibition and IC 50 was calculated as shown in figure 5 and table 3.

Docking of N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone

The compound was docked with different protein like 5itq, 1ktf, 5hmh. But compound was docked with 5hmh which shows binding site with 50 GLY i.e. an amino acid and its binding energy is -10.4532 kcal/mol as shown in figure 6. 5hmh is ligase/ligase inhibitor. Ligases regulates diverse areas such as cell trafficking, DNA repair, and signaling and is of profound importance in cell biology. Ligases are also key players in cell cycle control, mediating the degradation of cyclins, as well as cyclin dependent kinase inhibitor proteins. So it can be assume that the compound is having antioxidant property by binding with 5hmh protein.

IV. Conclusion

The compound N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone was synthesized the characterized IR spectra was done. It confirms the formation of semicarbazone derivative.The antioxidant property was found to be potent and binding site for anti oxidant property was found.
Melting point was determined at open capillaries. The reaction was confirmed on TLC plate and visualizing the spot in iodine chamber.

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CONFLICT OF INTEREST: There is no conflict of interest associated with this work.

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