Synthesis Characterization and Antimicrobial Activity of N-Nitroso-2, 6-Diphenylpiperidin-4-One Semicarbazone

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ABSTRACT
Among the family of heterocyclic compounds, The nitrogen containing six membered heterocycle, the piperidine structural was the most dominant and very prevalent element in nature and often found to be naturally occurring bioactive compounds such as alkaldoids[1]. Piperidin-3- one derivatives are used as precursors for the synthesis of antimalarial agents Febrifugine and Isofebrifugine. Piperidin-4-ones mostly display varied and potent biological properties such as Antiviral, Antitumour, Analgesic, Antimicrobial, Fungicidal, Herbicidal, Insecticidal, Antihistaminic, Anti-inflammatory and Anticancer. CNS stimulant and recent reports suggest that compounds containing piperidin-4-one moiety elicit excellent activity when aromatic substitutions are present at 2- and/or 6-positions. In this work, the compound N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone has been prepared and analyzed. The product showed positive nitrogen test (Lassign test), 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 CHN analysis FT-IR, and ¹H NMR Spectral data and the compounds were screened for their antimicrobial activity against gram positive bacteria Bacillus subtilis, Staphylococcus aureus and Gram-negative bacteria Escherichia coli by using Ciprofloxacin as standard and fungi Candida albicans by using Cetramazole as standard. The compound exhibited significant activities against all the tested bacterial and fungal strains.

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using PE SCIEX API 4000 mass spectrometer. Microanalyses were performed on VarioMicro V2.2.0 CHN analyser.

Experimental Method

i). 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) was heated in a boiling water bath maintaining the temperature 50 - 55°C with stirring until the colour of the solution changed to deep red orange. The solution immediately cooled in ice water, after cooling ether (100 ml) was added to it, the ether insoluble bispidine (2,4,6,8-tetra phenyl 1-3,7-diazabicyclo(3.3.1)nonan-9-one, (m.p 235-236°C) was filtered off and 5ml of conc HCl was added to the filtrate. The precipitated 2,6-diphenylpiperidin-4-one hydrochloride was collected by filtration and washed with 3:1 mixture of ether and ethanol. The hydrochloride (m.p 215-216°C) obtained was dispersed in minimum amount of acetone and ammonia solution was added drop wise to it until a clear solution was obtained. The clear solution was poured into cold water (500ml) and solid obtained was filtered and dried. The solid obtained was recrystallized using ethanol (yield 25%) melting point 103-104°C [6].

Scheme 1

ii). Synthesis of N-nitroso-2,6-diphenylpiperidin-4-one

Dissolve 0.01g of 2,6-diphenylpiperidin-4-one in the ethanol-water mixture (60ml + 40ml) and add 1.0 ml of concentrated HCl to this solution, the solution heated and stirred at 49-55°C. Sodium nitrite dissolved in 25 ml of ethanol: water mixture (60ml + 40ml) and add 1.0 ml of water mixture (10ml+15ml) and take this solution in an addition funnel. This solution was added in drops over a period of 1.5 hours while the mixture was stirred at 50-60°C. After the addition was completed stirring continued for another 4 hours. To this reaction mixture about 75ml of ether was added. The product soluble in ether was separated by using a separation funnel. The separated ether was allowed for evaporation. The solid obtained is recrystallized from ethanol [9].

Scheme 2

iii). Synthesis of N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone

The compound N-nitroso-2,6-diphenylpiperidin-4-one semi carbazone is prepared by dissolving N-Nitroso-2,6-diphenylpiperidin-4-one in 10 ml of methanol and heated over a water bath at (50-60)°C. Dissolved 1.0g of semi carbazide hydro chloride in 3 ml of ammonia solution drop by drop, an equal amount of methanol was also added to it (1:1) ratio. This solution was added to the above reaction mixture in three portions at an interval of 30 minutes. After the addition was completed the heating and stirring are continued at another five hours. Then the reaction mixture was poured into ice cold water with shaking. The pure solid separate are filtered washed with water and dried and purified through short column, m. p 150°C [10].

Scheme 3

iv). Antibacterial activity by disc diffusion method

Nutrient agar plates were prepared under steriled conditions and incubated overnight to detect contamination. About 0.2ml of working stock culture was transferred into separate nutrient agar plates and spread thoroughly using a glass spreader. Whatmann No.1 discs (6 mm in diameter) were impregnated in the test compounds dissolved in DMSO (200 mg/ml) for about half an hour. Commercially available drug disc (Ciprofloxacin) was used as positive reference standard. Negative controls were also prepared by impregnating the disc of same size in DMSO solvent. The discs were placed on the inoculated agar plates and incubated at 37 ± 1°C for about 18-24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism.

v). Antifungal activity by disc diffusion method

Sabouraud’s dextrose agar (SDA) medium was used for the growth of fungi and testing was done in Sabouraud’s dextrose broth (SDB) medium [15]. The subculture and the viable count were carried out by the same procedure used for in antibacterial studies except the temperature, which should be maintained at 28 ± 1°C for about 72 h. Similarly for disc diffusion method, the petridishes were incubated at 28 ± 1°C for about 72 h [17]. The same concentration of the test compound, solvent (DMSO) and Cetramazole (standard) prepared previously were used for the antifungal studies.

vi). Minimum Inhibitory Concentration (MIC)

The lowest concentration of the test compounds which caused apparently the inhibition of growth of organism, was taken as the minimum inhibitory concentration (MIC). The minimum inhibitory concentration was recorded by visual observation after 24 h (bacteria) and 72-96 h (fungi) of incubation. The sterile distilled water and DMSO did not show any inhibition.

Results and discussion

The compound N-nitroso-2,6-diphenylpiperidin-4-one semi carbazone has been reported in this work. When analyzed the products showed positive nitrogen test (Lassign test), a single spot in TLC and sharp melting point for the purity of the compound. The product expected should have the following structure based upon stoichimetry of the reaction (Scheme - 1). The structure was further confirmed from the CHN analysis FT-IR, and 1H NMR Spectral data.

i). CHN Analysis

From this data the empirical formula of N-nitroso-2,6-diphenylpiperidin-4-one has been arrived The CHN analysis report is given in Table-I.
The presence of high hydrophobic content and δ values are made on the basis of literature values.

The molecular formula obtained as C16H10N2O2. From this molecular formula the double bond equivalence has been calculated.

\[
\text{DBE} = \frac{C + \frac{1}{2}H + \frac{1}{2}N}{2} = \frac{16 + \frac{1}{2}10 + \frac{1}{2}2}{2} = 12
\]

The DBE value 12 accounts the structure given in Fig. 1 for two benzene ring, one cyclic ring, one C=N, one N=O, and one C=O group. The above structure is further confirmed from the IR and H1 NMR spectra.

ii). IR Spectral analysis

The important IR data are collected from the spectrum (fig. 2) obtained are given in the Table-II. Assignments of the frequencies were made on the basis of the literature values [11]. The formation of the compound N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone was realized by the analysis of IR data.

iii). H1-NMR Spectral analysis

To give further support for the structure of the compound, the important H1 NMR data are collected from the spectrum (Fig.3) obtained and are given in Table-III. Assignment of the chemical shift (δ ) values are made on the basis of literature values [11].

![Fig 3. 1H NMR data of -nitroso-2,6-diphenylpiperidin-4-one semicarbazone](image)

The 'H-NMR data show that the aromatic proton absorbs in the range δ 7.2-7.4 since the compound in (fig.1) contains two phenyl group. The benzylic protons at C2 and C6 absorb down field at 8 2.7 and 2.8 respectively. The methyl proton at C3 and C5 protons absorb at δ 3.4 and 3.5 respectively. The secondary amine (-NH group) of semicarbazide moiety absorb at δ 8.1 presents as a sharp singlet. The primary amine (-NH2 group) of semicarbazide moiety absorbs at δ10.1 as a sharp singlet. Based on the CHN analysis, IR and 1H NMR spectral data the structure of the compound was assigned is given in fig. 1.

iv). In vitro Antimicrobial assay

The N-nitroso-2,6-diaryl piperidin-4-ones and its semicarbazone derivatives were screened in vitro for their potency against bacterial strains such as, B. subtilis, S.aureus and Escherichia coli and fungal strains such as C. albicans and A. flavus[13]. The in vitro activities of the test compounds were studied using agar plates containing Sabourauds dextrose broth for fungi and in Nutrient broth for bacteria. The test compounds were tested against each microbial species. The antibacterial and antifungal potencies of the test compound were compared with ciprofloxacin (bacteria) and cetramazole (fungi). The antimicrobial inhibitions of test compound are expressed as the area of zone of inhibition and summarized in Table IV.

The compound N-nitroso-2,6-diphenylpiperidin-4-ones semi carbazone display very good antifungal and moderate antibacterial activity. This marked antifungal and antibacterial activity may be due to the presence of high hydrophobic content of this family of compounds and the piperidine ring system. The compound containing the piperidin-4-one segment are more active against bacteria as compared to that of semicarbazone segment, presumptively due to the strong interaction of the later with the agar medium, which hinders their diffusion in agar medium.

Conclusion

The N-nitroso-2,6-diaryl piperidin-4-ones and its semicarbazone was characterized by CHN analysis, IR and 1H NMR. The spectral data supported and confirmed the formation of semicarbazone derivative. All the compound was screened for their antimicrobial activity and they exhibited excellent activity against B.subtilis, S.aureus and C.albicans.
References


Table 1. CHN analysis of compound N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone

<table>
<thead>
<tr>
<th>C %</th>
<th>H%</th>
<th>N%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental value 64.07</td>
<td>5.60</td>
<td>20.74</td>
</tr>
<tr>
<td>Theoretical value 64.09</td>
<td>5.63</td>
<td>20.77</td>
</tr>
</tbody>
</table>

Table 2. IR Data of compound N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone

<table>
<thead>
<tr>
<th>Group</th>
<th>Stretching frequency (cm⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>primary amide</td>
<td>3450</td>
</tr>
<tr>
<td>Aromatic C-H</td>
<td>2927</td>
</tr>
<tr>
<td>Cyclic C-H</td>
<td>2857</td>
</tr>
<tr>
<td>ON</td>
<td>1563</td>
</tr>
<tr>
<td>C=O</td>
<td>1637</td>
</tr>
<tr>
<td>N-N=N(Nitroso)</td>
<td>1452</td>
</tr>
<tr>
<td>Secondary amide</td>
<td>1418</td>
</tr>
</tbody>
</table>

Table 3. ¹H-NMR data of the compound N-nitroso-2,6-diphenylpiperidin-4-one semicarbazone

<table>
<thead>
<tr>
<th>Chemical shift(δ)</th>
<th>Nature of peak</th>
<th>No. of Protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2-7.4</td>
<td>Multiplet</td>
<td>10</td>
<td>Aromatic proton</td>
</tr>
<tr>
<td>5.5</td>
<td>Triplet</td>
<td>1</td>
<td>Benzyllic proton at C-2</td>
</tr>
<tr>
<td>5.6</td>
<td>Triplet</td>
<td>1</td>
<td>Benzyllic proton at C-6</td>
</tr>
<tr>
<td>3.4</td>
<td>Multiplet</td>
<td>2</td>
<td>Methylene proton at C-3</td>
</tr>
<tr>
<td>3.5</td>
<td>Multiplet</td>
<td>2</td>
<td>Methylene proton at C-5</td>
</tr>
<tr>
<td>10.1</td>
<td>Singlet</td>
<td>2</td>
<td>–C=N=N−</td>
</tr>
<tr>
<td>8.1</td>
<td>Singlet</td>
<td>1</td>
<td>–C=N=N−</td>
</tr>
</tbody>
</table>

Table 4. Antimicrobial activity of the synthesized compound

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration in DMF (μg/well)</th>
<th>Antibacterial activity Zone of inhibition in mm</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Compound</td>
<td>600</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>600</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Cetramazole</td>
<td>600</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Periasamy Selvakumar, Sathiah Thennarasu, and Asit Baran Mandal

“Synthesis of Novel Pyridopyridazin-3(2H)-one Derivatives and Evaluation of Their Cytotoxic Activity against MCF-7 Cells” ISRN Medicinal Chemistry Volume 2014 (2014), Article ID 410716, 7 pages
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