SYNTHESIS, CRYSTAL STRUCTURE AND ANTIOXIDANT POTENTIAL OF Di-(N-CINNAMYL) FLUOXETINE CHLORIDE

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ABSTRACT

A new derivative of Fluoxetine, N,N-dicinnamy1-N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxyl]butan-1-aminium chloride hydrate [Di-(N-Cinnamyl) Fluoxetine Chloride hydrate] was synthesized and characterized by single-crystal X-ray diffraction studies, elemental analyzer, thermogravimetric and FTIR spectroscopy analysis. The titled compound [C 46H 33CIF 4NO 9] crystallized in monoclinic, having unit cell parameters a = 19.155(14) Å, b = 9.193(5) Å, c = 18.596(13) Å and belongs to P2 1/c space group. The molecule shows disorder of F atoms and was modeled as two different orientations. The one-dimensional crystal packing features weak C-H···π interactions showing the formation of a chain along [001], provides stability to the crystal lattice. Antioxidant potential of Di-(N-Cinnamyl) Fluoxetine Chloride hydrate has been described and was found higher than Fluoxetine and N-Benzyl Fluoxetine.

Keywords: Fluoxetine, Derivative, Crystal structure, Antioxidant activity

1. INTRODUCTION

Fluoxetine (N,N-dimethyl-3-[4-(trifluoromethyl)phenoxyl]benzenepropanamine) (I) (Fig. 1) is being used worldwide in the treatment of major depression [1-4] as well as in the therapy of other syndromes, such as Panic fits, Bulimia nervosa and obsessive compulsive disorders [4-6]. Continuous Fluoxetine administration is reported to prevent recurrence of pulmonary arterial hypertension in rats [7]. However, recent studies have indicated that Fluoxetine has many additional effects like blockade of Na + and K + ion channels [8-10]. Antioxidant potential of this drug has been also a subject of interest, as it is known to integrate antioxidant and antidepressant properties [11]. It was suggested that Fluoxetine induces a drug-protective effect against the melanoma-induced oxidative changes (increased LP and decreased total glutathione (GSH)-level, as well as antioxidant enzyme properties [11]. It was suggested that Fluoxetine induces a drug-protective effect against the melanoma-induced oxidative changes (increased LP and decreased total glutathione (GSH)-level, as well as antioxidant enzyme activities) in the spleen. Fluoxetine dose-dependently reduced the amounts of free oxygen radicals (hydroxyl and superoxide anion radicals), generated in chemical systems [12]. Crystal structure of Fluoxetine has been reported as the hydrochloride, hydrochloride benzoic acid, hydrochloride succinic acid and hydrochloride fumaric acid [13-14]. Recently, we have reported synthesis and crystal structure of N-Benzyl Fluoxetine (II in Fig 1) [15]. In continuation of our ongoing studies, we herein report synthesis, crystal structure, thermal properties, antioxidant potential and metal chelation ability of Di-(N-Cinnamyl) Fluoxetine Chloride Hydrate (III) (Fig 1).

Figure 1: Chemical structures of Fluoxetine (I), N-Benzyl Fluoxetine (II) and Di-(N-Cinnamyl) Fluoxetine Chloride Hydrate (III).

2. EXPERIMENTAL

2.1 Materials and Measurements

All the chemicals used were of the highest purity grade available. Trolox (Hoffman-La Roche) (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), Diphenyl picryl hydrazyl (DPPH) was purchased from Aldrich Chemical Co., Gillingham, Dorset, UK. ABTS (2,2'-azinobis(3- ethylbenothiazoline-6-sulfonic acid) diazonium salt, potassium persulfate, BHA (butylated hydroxy anisole) were obtained from Fluka (UK) and HPLC grade ethanol from Rathburn Chemicals Ltd. (Walkerburn, Peebleshire, Scotland). Solvents were purified through distillation where necessary. Fluoxetine hydrochloride was kindly donated by Schazoo Laboratories, Lahore, Pakistan. Sodium hydride and cinnamyl chloride were obtained from Sigma Aldrich (Germany). The crystal determination was performed on a Bruker KAPA APEX II CCD diffractometer equipped with a graphite-mono chromatized MoKα radiation (λ =0.71073 Å). Elemental Analysis for %age determination of N, C and H were performed on Vario Micro Cube, Elementar, Germany. TGA analyses (25–600 °C) were recorded under an inert atmosphere (N 2) on a Hi-Res TGA 2950 TA at heating rate 10 °C/min. The IR spectrum of the compound was scanned through Thermo Nicolet FT-IR-200 (USA) using KBr pellets over the range 4000–400 cm⁻¹. Antioxidant studies were carried out using UV-1700 Pharma Spec UV-Visible Spectrophotometer, Shimadzu, Japan equipped with Peltier temperature controller.

2.2 Synthesis

A mixture of Fluoxetine hydrochloride 498 mg (1.45 mmol), sodium hydride 0.14 g (5.8 mmol) and N,N-dimethyl formamide (10 ml) was stirred at room temperature for 30 min, followed by the addition of cinnamyl chloride 407 µl (2.9 mmol). Stirring was continued for a period of 3 h and the contents were then poured over crushed ice. The precipitated product was isolated, washed and crystallized from methanol, giving off white Block-like crystals, suitable for X-ray analysis. IR ν

max

(C) were recorded under an inert atmosphere (N 2/ N 2) on a Hi-Res TGA 2950 TA at heating rate 10 °C/min. The IR spectrum of the compound was scanned through Thermo Nicolet FT-IR-200 (USA) using KBr pellets over the range 4000–400 cm⁻¹. Antioxidant studies were carried out using UV-1700 Pharma Spec UV-Visible Spectrophotometer, Shimadzu, Japan equipped with Peltier temperature controller.

2.3 Crystal structure determination

A white colored crystal suitable for data collection was mounted on glass fibers and data collection was performed on a Bruker SMART APEX II CCD diffractometer with graphite monochromated Mo Kα radiation at 296 K. The structures were solved by direct-methods using SHEXLXS-97 [16] from within the WINGX [17] suite of software. All non-hydrogen atoms were refined anisotropically. All H atoms attached to C atoms were refined using a riding model [C-H = 0.93Å and Uiso(H) = 1.2Ueq(C)]. The crystal of Di-(N-Cinnamyl) Fluoxetine Chloride hydrate has been described and was found higher than Fluoxetine and N-Benzyl Fluoxetine.
the mixture to stand in the dark, at room temperature for 12–16 h be use. Antioxidant activity of standard antioxidants and samples was determined by diluting the ABTS+ stock solution with PBS (Phosphate-buffered saline) of pH 7.4 to an absorbance of 0.70 (± 0.02) at 734 nm and equilibrated at 30 °C. An aliquot of 10 μL of standard solution was added to 2.99 mL of diluted ABTS+ solution (A = 0.700 ± 0.020), the absorbance was measured at 30 °C, with exactly 1 min intervals for total duration of 8 minutes. Solvent blanks were run in each assay for accurate readings. All determinations were carried out in triplicate. The percentage inhibition of absorbance at 734 nm was calculated by the following formula:

\[
I_{\%} = \left[ \frac{A_o - A_f}{A_o} \right] \times 100
\]

Where \( A_o \) and \( A_f \) are the absorbances of radical cation solution before and after the addition of sample/standard antioxidants, respectively. The percentage inhibition of ABTS+ at 734 nm is calculated and plotted as a function of concentration of standard antioxidant Trolox for the standard reference data and TEAC values were calculated for the samples.

2.4.3. 2, 2'-Diphenyl-1-picrylhydrazyl Radical Scavenging Capacity Assay (DPPH Assay)

In DPPH Assay 3 mL DPPH solution (25 mL/L) prepared in methanol was mixed with appropriate volumes of sample solutions. The absorption is recorded at 515 nm after every 5 minutes for a total duration of 30 min. The color of the solution faded upon reduction of DPPH free radical. The percentage inhibition of the DPPH was calculated.

\[
\%\text{DPPH inhibition} = \left[ \frac{[\text{DPPH}]_t - [\text{DPPH}]_{t'}}{[\text{DPPH}]_t} \right] \times 100
\]

Where \([\text{DPPH}]_t\) is the concentration of DPPH free radical without antioxidant samples and \([\text{DPPH}]_{t'}\) is the concentration of DPPH free radical after addition of antioxidant sample at time \( t \). A kinetic curve showing the scavenging of DPPH free radical in terms of decrease in absorbance at 515 nm as a function of time (min) was plotted for each sample and is compared with the standard antioxidants BHA and Trolox [21].

2.4.4. Ferric Reducing Antioxidant Power Assay (FRAP Assay)

The reducing capacity of Fluoxetine and its derivatives was measured using FRAP Assay. FRAP reagent contains 300 mMol acetate buffer of pH 3.6, 10 mMol TPTZ (2, 4, 6-tripyridyl-s-triazine), in 40 mMol HCl, 20 mmol/liter FeCl₃. Fresh working FRAP reagent was prepared as required by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml FeCl₃, 6H₂O solution, which was incubated at 37 °C throughout the monitoring period. An aliquot of sample (100 μl) was mixed with 300 μL of diluted water. Absorbance was taken at 593 nm after every minute for 4 minutes. Results were compared with standard curve of ferrous sulphate and FRAP values were calculated [22].

2.4.5. Metal Chelating Activity

The metal chelating activity was estimated depending on the decrease in the absorbance of the ferrous-ferrozine complex according to previously reported method. An aliquot of sample equal to 100 μL was added to 50 μL of ferrous sulphate solution (1 mM). The reaction was initiated by the addition of 200 μL ferrozine (5.0 mM), and then the total reaction volume was adjusted to 4 mL with ethanol. When the mixture had reached equilibrium (after 10 min), the absorbance at 562 nm was recorded. The control was prepared without the test compound [23]. Fe²⁺ chelating activity of the test compounds was calculated from the following formula and is compared with a positive control EDTA.

\[
\text{Chelating activity (\%)} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

3. RESULTS AND DISCUSSION

3.1. Crystal structure of C₁₁H₁₂ClF₅NO₃ (III)

The molecular structure and atom labeling scheme are shown in Fig. 3.
the phenyl rings C(1)-C(6) and C(9)-C(14) is 81.04 (2)°. The phenyl rings plane are approximately planar, with maximum deviation from the least-squares planes being 0.008 (5)Å for atom C(3), 0.011 (6)Å for atom C(12), 0.011 (6)Å for atom C(24) and 0.003 (8)Å for atom C(33). The water atom O (2) in the molecule at (x, y, z) acts as a hydrogen-bond donors (Table 3) to atoms Cl(1)i and Cl(1)ii so forming a centro symmetric R4222 centered at (1/2, 0, 0).
The one-dimensional crystal packing of III features weak C-H···π interactions. Fig. 4(a) shows the formation of a chain along [001] generated by the C-H···π interactions. Details of these interactions are given in Table 3.

Both the H atoms of water molecule are donated for hydrogen bond formation with two different Cl atoms. The H2A atom is donated to form an O-H···Cl (i=-1-x, -1+y, 1-z) hydrogen bond at a distance 2.40 Å and the D-H···A angle being 159.91°. The H2B atom is donated in a similar manner to form a hydrogen bond with another Cl (ii = x, -1+y, 1-z) atom at a distance of 2.362 Å with a D-H···A angle of 166.34°. In view of the van der waal’s radius of Cl atom, both these hydrogen bonds are at comparatively short distance and are significantly linear. Similar chlorine atom accepts H atoms from two different water molecules. The linkage of two Cl atoms with two water molecules and vice versa give way to a typical parallalelogram (Fig 4 a & b). In addition to these two hydrogen bonds, the Cl atom forms three comparatively weaker H bonds of C-H···Cl type with two neighboring molecules. One of them is H10 atom of a neighboring molecule with a symmetry ½-x, ½+y, ½-z and another with H26 atom of the same molecule with H···Cl distances of 2.804 Å and 2.769 Å respectively. H10-C1-H26 angle is 133.13°. Similarly the Cl atom forms a third C-H···Cl interaction with another neighboring molecule (symmetry –x, -y, -z) at a distance of 2.909 Å. The Cl atom thus binds three different symmetry related molecules and forms a trimer (Fig 5). There also exists a C-H···O hydrogen bond between the H18 atom and the O2 atom of water with a C-H-O distance of 2.614 Å and C-H-O angle 162.55°. This rich involvement of Cl atom and the water molecule in the formation of numerous intermolecular interactions play a decisive role in the molecular packing.

Figure 3. A view of the molecule of I, showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability.

Figure 4 (a): Part of the crystal structure of I, showing the formation of a chain along [001] generated by C-H···π interactions. For the sake of clarity, H and F atoms not involved in the motif shown have been omitted (b): A view of the molecular packing along b-axis highlighting the role of water molecule and the Cl atom in the molecular assembly through the formation of intermolecular interactions.

Figure 5: A molecular trimer formed through the formation of different interactions with a common Cl atom. Symmetry codes (i) x, y, z ; (ii) -x, ½+y, ½-z ; (iii) -x, -y, -z

Table 2. Selected Bond Lengths (Å) and Bond Angles (°) for Compound III.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Dist.</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(18)-N(1)</td>
<td>1.510 (7)</td>
<td>C(16)-N(1)-C(17)</td>
</tr>
<tr>
<td>C(16)-N(1)</td>
<td>1.503 (7)</td>
<td>C(16)-N(1)-C(18)</td>
</tr>
<tr>
<td>C(17)-N(1)</td>
<td>1.505 (7)</td>
<td>C(17)-N(1)-C(27)</td>
</tr>
<tr>
<td>C(8)-O(1)</td>
<td>1.430 (7)</td>
<td>C(1)-O(1)-C(8)</td>
</tr>
<tr>
<td>C(1)-O(1)</td>
<td>1.366 (7)</td>
<td>C(17)-N(1)-C(18)</td>
</tr>
<tr>
<td>C(27)-N(1)</td>
<td>1.523 (7)</td>
<td>C(16)-N(1)-C(27)</td>
</tr>
</tbody>
</table>

Table 3. Hydrogen Bonds for Compound III (Å and °).

<table>
<thead>
<tr>
<th>D-H···A</th>
<th>d(D-H)</th>
<th>d(H···A)</th>
<th>d(D···A)</th>
<th>&lt;(DHA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(2)-H(2A)···Cl(1)#1</td>
<td>0.84(2)</td>
<td>2.38(3)</td>
<td>3.219(7)</td>
<td>172(10)</td>
</tr>
<tr>
<td>O(2)-H(2B)···Cl(1)#2</td>
<td>0.84(2)</td>
<td>2.34(2)</td>
<td>3.178(6)</td>
<td>177(8)</td>
</tr>
<tr>
<td>C(6)-H(6)···Cg(4)</td>
<td>0.93</td>
<td>3.14</td>
<td>4.07(2)</td>
<td>178</td>
</tr>
<tr>
<td>C(22)-H(22)···Cg(1)#3</td>
<td>0.93</td>
<td>3.15</td>
<td>3.91(2)</td>
<td>141</td>
</tr>
<tr>
<td>C(31)-H(31)···Cg(1)#4</td>
<td>0.93</td>
<td>3.00</td>
<td>3.81(3)</td>
<td>147</td>
</tr>
</tbody>
</table>

Symmetry codes: #1: -x+1, -y+1, -z; #2: x, y-1, z; #3: -x+1, -y+1/2, -z+1/2; #4: x, -y+1/2, z-1/2
3.2 Thermal analysis
Thermal analysis of the titled compound has been studied using Differential Scanning Calorimeter/Thermo-gravimeter Analyzer (DSC/TGA) model SDT Q600 of TA Instruments, USA in the continuous nitrogen flow, with a ramp rate of 10°C per minute. The results of thermal analysis of the structure III are consistent with the proposed stoichiometry. The decomposition pattern of III is shown in Fig.6. At the first stage water is released, corresponding to the weight loss of about 2.8% (calculated value 3.1% for one lattice water molecules) between 45-160°C. The next total weight loss of 97.5% up to 288°C results from the successive release of Organic compound (calcd. 97.0%).

3.3 Antioxidant potential determination.
The overall results of antioxidant potential were summarized in Table 4 and the graphical presentation of results are shown in figure 7, 8, 9, 10. The results revealed that Cinnamyl fluoxetine (III) has a gigantic antioxidant potential than n-benzyl fluoxetine (II) and fluoxetine (I). Cinnamyl derivatives show a high antioxidant activity that is due to the presence of vinyl fragments [29]. Some natural cinamyl derivative i.e., Ferulic acid, Caffeic acid shows an excellent antioxidant activity [30]. Hence the addition of reactive cinamyl group to the parent Fluoxetine molecule is responsible for the towering antioxidant power of Cinnamyl fluoxetine.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TEAC value (mMol)</th>
<th>FRAP value (mg/L of FeSO_{4} equivalent)</th>
<th>%age bound iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample I</td>
<td>2.87±0.015</td>
<td>16.45±0.255</td>
<td>12.65±0.034</td>
</tr>
<tr>
<td>Sample II</td>
<td>3.25±0.195</td>
<td>18.54±0.675</td>
<td>23.86±0.245</td>
</tr>
<tr>
<td>Sample III</td>
<td>3.78±0.026</td>
<td>23.87±0.345</td>
<td>34.92±0.543</td>
</tr>
</tbody>
</table>

Data are mean ±SD (n = 3)

3.3.1 ABTS•+ decolorization assay
The basic principle underlying the ABTS•+ decolorization assay is that ABTS on reaction with K_{2}S_{2}O_{8} forms a greenish blue radical cation. Standard and sample antioxidants that are able to transfer an electron to ABTS radical cation scavenge the color of the solution proportionate to their amount. The extent of scavenging depends upon both the concentration of antioxidant and time duration for the reaction under analysis. The antioxidant activity of the Fluoxetine and its derivatives is determined by ABTS decolorization assay at pH 7.4 (PBS, 5 mM). Figure 7 shows the results of ABTS radical cation scavenging ability of Fluoxetine and its derivatives. The following trend is shown by Fluoxetine and its derivatives: Cinamyl Fluoxetine (III) >Benzyl Fluoxetine (II) >Fluoxetine (I).

3.3.2 DPPH Assay
DPPH is a commercially available stable free radical which has been widely used for estimating Free radical scavenging ability of pure antioxidants, herbs and other compounds [25-26]. Violet-colored DPPH radical, after accepting an electron or hydrogen atom from the antioxidant compounds, is converted into a colorless compound. A plot between % DPPH inhibition and the time after addition of neat or diluted stock solution of samples and of standard antioxidants i.e., Trolox and BHA is shown in Fig. 8. The radical scavenging ability was found to be in the decreasing order of Cinamyl Fluoxetine (III) >Benzyl Fluoxetine (II) >Fluoxetine (I).

3.3.3 FRAP Assay
The FRAP assay is rapid and simple approach to evaluate the reducing ability of antioxidants. Ferric reducing antioxidant potency of antioxidants employed Fe (III)-(TPTZ), Cl_{2} (pale yellow in color) as an oxidizing agent, When it came in contact with an antioxidant, it gets reduced to Fe (II)-(TPTZ), Cl_{2} (Blue in color) and absorbs at 593 nm (i.e., the colored complex is formed at a definitely acidic pH such as pH=3.6, much lower than the physiological pH) and insufficiently responsive to thiol-type (i.e., -SH containing) antioxidants [27] like glutathione [28]. The antioxidant activity of the Fluoxetine and its derivatives decreases in the order of Cinamyl Fluoxetine (III) >Benzyl Fluoxetine (II) >Fluoxetine (I) as shown in the table and Figure 9.
3.3.4 Metal Chelating Activity

The chelation of Fe\(^{2+}\) by Fluoxetine and its derivatives was estimated by the method of Dinis et al. (1994). Ferrozine can quantitatively form complex with Fe\(^{2+}\). However, in the presence of chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of color reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The transition metal ion, Fe\(^{2+}\) possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals. The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. Fluoxetine and its derivatives interfered with the formation of ferrous-ferrozine complex, suggesting that it has chelating activity and captures ferrous ion more efficiently than ferrozine. The antioxidant activity of the Fluoxetine and its derivatives decreases in the order of Cinamyl Fluoxetine (III) >Benzyl Fluoxetine (II) >Fluoxetine (I) (Fig. 10).

Figure 10: Metal Chelating activity of Fluoxetine and its derivatives.

4. SUPPLEMENTARY MATERIAL

CCDC No. 745879 contains the supplementary crystallographic data for compound III, can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

5. ACKNOWLEDGEMENT

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6. REFERENCES