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Sertraline and its iodine product: Experimental and theoretical vibrational studies Potential *in vitro* anti-thyroid activity of sertraline and iodine product toxicity with respect to male Wistar rats

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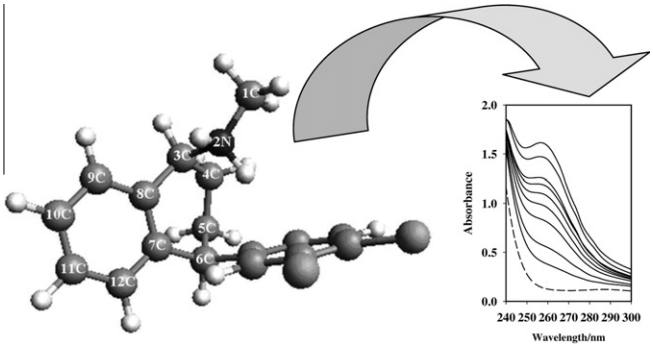
HIGHLIGHTS

- Vibrational characterization of sertraline and its iodine product.
- *In vitro* anti-thyroid activity of sertraline.
- Toxicity of the sertraline iodine product on male Wistar rats.

GRAPHICAL ABSTRACT

Complete vibrational characterization of the solid phase FT-IR spectra of sertraline hydrochloride and its sertraline–iodine product was presented. In addition, the evaluation of anti-thyroid activity of sertraline hydrochloride by using the Lang's method was performed together with toxic effect of the iodine product towards the male Wistar rats.



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ABSTRACT

Mayor depression, obsessive–compulsive panic, social anxiety disorders are common diseases that are usually treated with sertraline hydrochloride which is the active ingredient of the well known drugs as Zoloft and Lustral. In this work, we presented a more complete vibrational characterization of the solid phase FT-IR spectra of Sertraline hydrochloride and its sertraline–iodine product in which the conformational space of the molecules was investigated performing molecular dynamic simulations within an NVT ensemble. Geometrical, electronic and vibrational properties were calculated with the density functional theory. Comparison of the simulated spectra with the experimental spectra provides important information about the ability of the computational method to describe the vibrational modes of both molecules. In addition, for the first time we present the evaluation of anti-thyroid activity of sertraline hydrochloride by using the Lang's method. Also, with the aim to evaluate the antidepressant effect of its iodine product we demonstrated for this compound the toxic effect towards the male Wistar rats.

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Introduction

It is well known that sertraline ((1-S,cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthalene amine), the active ingredient in the antidepressant drug, is a selective serotonin re-uptake inhibitor that structurally differs from classic tricyclic antidepressants (TCAs). Sertraline has minimal side effects (insomnia, nervousness, nausea, diarrhea, etc.) contrasting with troublesome side effects associated to TCA [1–3]. For this reason, this medication became in last years in an excellent alternative medicine for treatment of depression having great commercial value. With the aim to produce other active forms used as antidepressant in humans, diverse crystals like polymorphs, solvates, hydrates and amorphous phases have been prepared, characterized and patented. High-throughput (HT) salt screening methodologies have been performed in order to prepare crystalline salt forms including acetic, citric, benzoic maleic, ethanesulfonic, malonic among others intending to improve sertraline activity. As an example, the sertraline hydrobromide contains four molecules of the salt in the unit in the noncentrosymmetric orthorhombic space group $P2_12_12_1$ being similar to that presented by sertraline hydrochloride ($SerH^+Cl^-$) salt but in opposition to chloride, bromide ion produces side effects like sedation and dermatological problems so limiting their therapeutic use [4]. Iodosertraline hydroiodide ($I\text{-}SerH^+I^-$) has been also prepared and characterized by mass spectrometry, thermal analyses and MO-calculations [5] but the information about its infrared spectrum was very scarce. One of the aims of this work is to obtain more complete details about vibrational characterization. Therefore, density functional theory calculations have been carried out in order to assign characteristic vibrational modes, especially those related to the presence of iodine in the structure.

We have also compared with the experimental FTIR spectrum of the sertraline hydrochloride, for the reason that this theoretical method has not been used when its vibrational spectrum was analyzed [6].

Other important objective of this investigation is to study the potential antidepressant effect for the iodine product; consequently, we used the forced swim test to investigate the antidepressant-like effect and measured its hydrochloride related compound in the same experimental conditions for comparative purposes. Another remarkable aspect of our interest is based on the fact that a variety of antidepressants has been recently proven to exhibit anti-thyroid activity due to the presence of electron-donating groups in their molecules. Previous studies have demonstrated that strong electron donor agents could produce the pharmacological activity *via* complexation of the molecular iodine in the thyroid gland or acting as inhibitor of thyroid peroxidase (TPO). In order to assess potential activity of sertraline hydrochloride towards the thyroid gland and also considering the viability of the formation of the iodine product, the chemical reactivity of this antidepressant compound with molecular iodine was investigated and the formation constant of its charge transfer complex was determined [7].

Materials and methods

Materials

Sertraline hydrochloride ($SerH^+Cl^-$) was obtained from Sigma Chemical Company (St. Louis, MO) and used as supplied. Iodosertraline hydroiodide (sertraline–iodine product, $I\text{-}SerH^+I^-$) salt was prepared and purified according to reported method by Zayed et al. [5]. All the solvent used was from analytical grade and purchased from Sigma.

FTIR spectra of powdered samples were measured with a Bruker IFS 66 FTIR-spectrophotometer from 4000 to 400 cm^{-1} in the form of pressed KBr pellets.

Electronic absorption spectra were recorded with a Hewlett-Packard 8453 diode-array spectrophotometer, using 1 cm quartz cells in the range (200–800 nm).

Computational details

The conformational space of the molecules was investigated using molecular dynamics simulations within an NVT ensemble. The AM1 semiempirical method [8] as implemented in the MOPAC program [9] was used as the force field in which nuclei move. Molecules were first heated from 0 to 600 K in 0.1 ps. The system temperature was then kept constant by coupling the system to a Berendsen thermostat [10] with a relaxation time of 0.5 ps. After an equilibration period of 20 ps, a 500-ps-long simulation was carried out saving the molecular coordinates every 25 ps. The time step for the simulations was 0.5 fs. The resulting geometries were then optimized using the AM1 method.

The geometry of the 20 conformers obtained according to the above methodology was further optimized using tools from the density functional theory [11–13] as implemented in the GAMESS-US program [14]. Calculations were accomplished using Zhao and Truhlar's hybrid meta-GGA functional M06-2X [15,16]. The 6-311G(d,p) basis set was used for all atoms. Numerical integration of exchange-correlation functional was performed on a grid containing 96 radial points and 590 angular points around each atom. That grid provided nuclear gradients that are accurate to about 0.00001 atomic units. The matrix of second derivatives of the energy with respect to the nuclear coordinates was calculated at the same level of theory. Then, the Hessian matrix was diagonalized and its eigenvalues were used to verify whether the optimized geometries were local minima or saddle points on the potential energy surface of the molecules.

All those conformers above the lowest-energy conformer up to 2 kcal mol^{-1} are included in the calculation of reported properties through a Maxwell–Boltzmann statistical average at 298.15 K according to

$$\langle A\rangle = \sum A_i \exp(-E_i/RT) \Big/ \sum \exp(-E_i/RT)$$

where E_i is the relative energy of the ith conformer and A_i is the given property. The arbitrary threshold of 2 kcal mol^{-1} assures that only those conformers contributing more than about 3% to every property are considered.

All figures were produced with the aid of the Gabedit program [17].

Forced swim test experiments

Animals

Experiments were carried out on male Wistar rats weighing 200–386 g. Rats were maintained on 8.64×10^4 s light (2.88×10^6–7.20×10^6 s)–8.64×10^6 s dark cycle with free access to food and water except during testing. Animals were employed only once in each test. All studies described were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institutes of Health, USA.

Drugs and treatment

Sertraline hydrochloride and sertraline–iodine product were administered intraperitoneally (i.p.) in a volume equivalent to 1 cm^3/kg. Rats were treated with either sertraline (as hydrochloride or iodine product) or saline (control rats) once a day. All of the control rats received injections of saline solution (0.9% NaCl).

In order to make valid comparisons with previously published data, the selected dose for both compounds were 10 mg/kg because it was proved that sertraline produced the most robust effect in the FST under similar experimental conditions [18]. Alternatively, doses of 5 mg/kg were used.

Forced swimming test (FST)

The procedure used was very similar to that described by Porsolt et al. [19], except that the water was 30 cm deep and pre-tests were performed 16 days before the test session. Porsolt et al. [19] noted that effects of pre-test sessions remained effective for as long as 2 weeks which was in agreement with our observations. The rats were randomly divided into three groups and conducted the following treatments: Group 1: saline (control group), Group 2: sertraline hydrochloride, and Group 3: sertraline–iodine product. Swimming sessions were conducted by placing rats in individual Plexiglas cylinders (46 cm tall × 20 cm in diameter) that had previously been filled with water (23–25 °C) up to 30 cm from bottom. All swimming sessions were carried out between 4.32×10^6 s and 6.48×10^6 s.

In vitro anti-thyroid activity

Iodine was obtained from Merck. It was bisublimed and was kept in dark in a desiccator containing P_2O_5. Spectroscopic grade solvent dichloroethane was used. Solutions of iodine and sertraline hydrochloride were prepared just before the beginning of experimentation by diluting gravimetrically prepared stock solutions in $C_2H_4Cl_2$ (dichloroethane). Iodine concentration was kept constant (4×10^{-4} mol L^{-1}), though the concentration of sertraline hydrochloride was varied between 1×10^{-4} mol L^{-1} and 1×10^{-3} mol L^{-1}. The reaction was carried out directly in the spectrophotometric cell by mixing 1.5 mL solutions each of sertraline hydrochloride (donor) and iodine (acceptor). Spectra were recorded immediately on double beam UV–Vis spectrophotometer. The temperature of the solutions was kept at 25° ± 1 °C during the measurements. Three independent replicates of each solution were measured. Formation constant (K_c) and the molar extinction coefficient were determined using Lang's method [20]. This method has been used to determine the formation constants of 1:1 stoichiometric complexes at the wavelength under analysis using:

$$[A_0][D_0]/d_c = ([A_0] + [D_0] - d_c/\varepsilon_c)/\varepsilon_c + 1/K_c\varepsilon_c \quad (1)$$

In which, d_c is the absorbance and ε_c is the molar extinction coefficient and K_c is the formation constant of the complex. Parameters were adjusted by using a program designed by our research group.

Eq. (1) can be re-written in the form:

$$Y = (1/\varepsilon_c)X + 1/K_c\varepsilon_c \quad (2)$$

where $Y = [A_0][D_0]/d_c$ and $X = [A_0] + [D_0] - d_c/\varepsilon_c$ this equation giving a straight line with slope $1/\varepsilon_c$ and Y-intercept $1/(K_c\varepsilon_c)$.

Iteration and linear regression method were used to solve Eq. (2). A suggested initial value of ε_c was dispensed and an X value was calculated. From the slope of the line, a new ε_c was determined and the process was successively repeated several times until the ε_c as well as K_c converged to particular values.

Results and discussion

Molecular geometries, analysis of the Infrared experimental spectra and theoretical spectrum simulations

No lower-energy conformers within 2 kcal mol^{-1} were found for sertraline hydrochloride ($SerH^+Cl^-$) or for iodosertraline hydroiodide ($I\text{-}SerH^+I^-$). Thus, all discussions are referred to the lowest-energy conformations of every species. Molecular schemes of $SerH^+Cl^-$ and $I\text{-}SerH^+I^-$ are shown in Fig. 1. The lowest-energy, optimized structures with the corresponding atom labels are also shown in the figure, whereas their geometric parameters are listed in Table 1. It is seen from the table that both bond distances bond angles were not much affected by the addition of iodine to C6. The (C5—C6—C13—C14) torsion angle, on the other hand, exhibited an important decrease of more than 50°.

The examined molecules have only one NH_2^+ group and consequently one symmetric and one asymmetric N—H stretching vibration, are predicted. As expected, the asymmetric stretching for the NH_2^+ group is located at a higher wavenumber than the symmetric stretching [21]. Usually, the ranges in which the frequencies related to these vibrational modes appear are 3420–3500 cm^{-1} (asymmetric NH_2^+ stretching) and 3340–3420 cm^{-1} (symmetric NH_2^+ stretching), respectively [22]. Calculated harmonic frequencies for those modes are located at 3477 cm^{-1} and 3361 cm^{-1} in $SerH^+Cl^-$, and at 3485 cm^{-1} and 3436 cm^{-1} in $I\text{-}SerH^+I^-$, see Table 2. As stated above, these modes were assigned to the asymmetric N—H stretching (higher frequency) and the symmetric N—H stretching (lower frequency) of the NH_2^+ group, in agreement to the expected value in the absence of H-bonding formation.

In contrast, salt formation (solid state) produced a considerable weakening of the normal NH stretching band. Hydrogen bonding originating in the N^+H—X-group disturbs NH stretching frequencies by removing electron density from the N—H bond, causing a shift to lower frequencies. Apparently this effect may be more marked in aromatic compounds in which several bands have been observed in 2800–2000 cm^{-1} range. This is a well-known behavior for amine salts. Several authors have studied the nature of hydrogen bonds being controversial in the vibrational assignments of the NH_2^+ stretching frequencies. Among others, Chenon and Sandorfy [23], Heacok and Marion [24], Balwin [25], Amourin da Costa et al. [26] and Giffin et al. [27] described that the stretching bands of protonated amino groups can be found between 2800 and 2400 cm^{-1} and some of them concluded that the hydrogen bonds of the amino hydrogen atoms (N^+H—X^-) caused a regular shift to lower wavenumbers with increasing anion size. On the basis of the theoretical data and taking into account the comparison with previous reported results, the experimental spectral bands at 2470 cm^{-1} and 2375 cm −1 for $SerH^+Cl^-$ and the doublet at 2451 cm^{-1} and 2434 cm^{-1} (higher frequency side) (Fig. 2A) and 2348 cm^{-1} (lower frequency side) for $I\text{-}SerH^+I^-$ (Fig. 2B) could be assigned to the asymmetric and symmetric stretching modes of the NH_2^+ group (data not shown in Table 2). On this basis, a shift to lower frequencies when the anion size increases from Cl^- to I^- can be observed (Fig. 2). The vibrational induced dipole of the NH_2^+ group induces a dipole in the anion which extent is proportional to the polarizability of the anion that increased with the electronic density from chlorine to iodine. The vibrational induced dipole of iodine then couples with the vibrational induced dipole of the NH_2^+ unit, shifting the stretching mode to lower frequency. Since the magnitude of the coupling is proportional to the polarizability of the anion, the frequency shift is lower for the iodine-containing amine salt than for the chlorine salt [28].

The vibrational NH_2^+ scissoring deformation appears in the 1650–1580 cm^{-1} region possibly coupled with the aromatic ring C=C stretching modes [7,28]. In fact, the calculated harmonic frequencies appeared at ca. 1636 and 1647 cm^{-1} while in the experimental spectrum they were observed at 1640 and 1645 cm^{-1} for $SerH^+Cl^-$ and for $I\text{-}SerH^+I^-$, respectively (Fig. 2). It can be observed that this band undergoes a shift to higher frequency in the sertraline iodine product. This behavior is in concordance with the expected one assuming that these movements are due to the

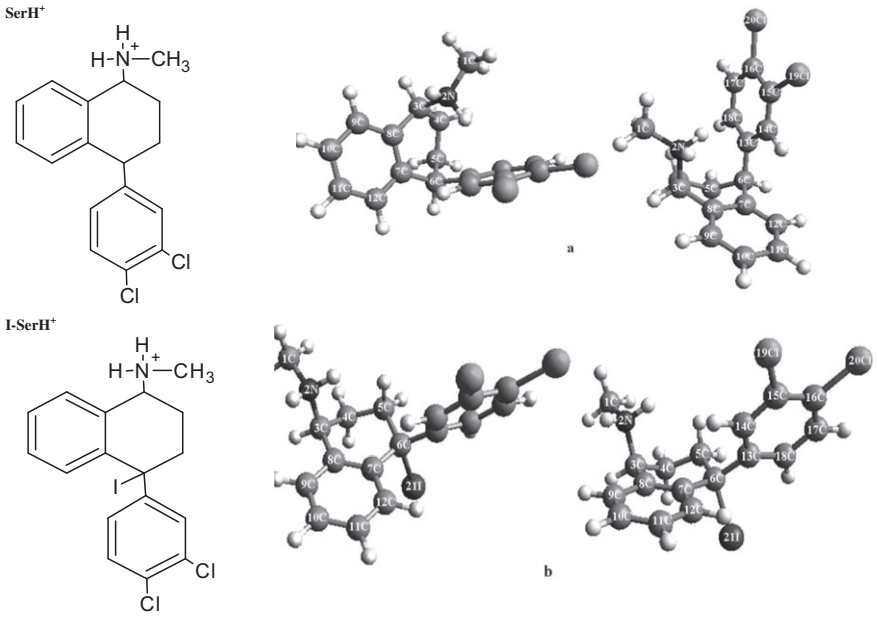

Fig. 1. Molecular schemes of the sertraline hydrochloride ($SerH^+Cl^-$) and sertraline–iodine product (Iodosertraline hydroiodide, I-$SerH^+I$) (left) and theoretical equilibrium geometries found for (a) ($SerH^+Cl^-$) and (b) I-$SerH^+I$ with atom numbering (right).

Table 1
Calculated geometrical parameters for $SerH^+Cl^-$ and I-$SerH^+I^-$. Equilibrium bond distances are indicated in Å. Selected bond angles and torsion angles are indicated in °. Numbers correspond to those in the figures.

	$SerH^+Cl^-$	I-$SerH^+I^-$
Bond distances (Å)		
C1—N2	1.49	1.49
N2—C3	1.54	1.54
C3—C4	1.54	1.52
C4—C5	1.54	1.52
C5—C6	1.54	1.54
C6—C7	1.51	1.52
C7—C8	1.40	1.40
C8—C9	1.39	1.40
C9—C10	1.39	1.38
C10—C11	1.39	1.39
C11—C12	1.39	1.38
C12—C7	1.39	1.40
C6—C13	1.53	1.52
C13—C14	1.40	1.39
C14—C15	1.39	1.39
C15—C16	1.40	1.39
C16—C17	1.39	1.39
C17—C18	1.39	1.38
C18—C13	1.40	1.39
C6—I21	–	2.23
$C—H_{average}$	1.09	1.09
$N—H_{average}$	1.03	1.02
$C—Cl_{average}$	1.72	1.73
Selected bond angles (°)		
C1—N2—C3	115.63	116.17
C4—C3—C8	114.86	113.09
C8—C9—C10	120.22	120.85
C7—C6—C13	112.07	113.63
C7—C6—I21	–	104.32
C14—C15—C16	119.96	120.03
C14—C15—Cl19	118.88	118.71
Selected torsion angles (°)		
C1—N2—C3—C4	71.47	67.36
C5—C6—C13—C14	149.74	93.48
C12—C7—C6—I21	–	73.71

increment in the *p* character in the lengthened of the XH bond which makes easier the stretching but more difficult the bending mode of this bond [22].

Additionally, at lower frequencies NH twisting and wagging vibrations of the NH_2^+ group in conjunction with other vibrational modes can be observed, having a good relationship between experimental and calculated frequencies (Table 2).

The identification of the C—N stretching vibrational mode is always problematic due to the possible overlapping with other bands in this region. Calculated frequencies for the C—N mode were obtained at ca. 1036 and 1024 cm^{-1}, allowing to assign the experimental stretching C—N bands at 1025 and 1028 cm^{-1} for $SerH^+Cl^-$ and I-$SerH^+I^-$, respectively.

It is well known that several vibrational modes are expected for the methyl group. This group gives rise to two C—H different modes, the symmetric and asymmetric stretches that typically fall in 2800–3000 cm^{-1} range and the asymmetric mode is expected to appear at higher frequency than the symmetric one. Calculated frequencies for asymmetric and symmetric C—H stretching modes fall in the range and expected order (3211 and 3108 cm^{-1} for $SerH^+Cl^-$ and 3202 cm^{-1} and 3109 for I-$SerH^+I^-$, respectively). In the experimental spectra only the asymmetric stretching mode could be observed as a shoulder in a sertraline iodine product (Table 2).

The C—H bonds can undergo bending vibrations and in this investigation these bands appeared in 1520–1400 cm^{-1} region. Taking into account the nature of the element to which the methyl group is joined, it is to be expected [22] that the methyl symmetric deformation mode for amine hydrochlorides appeared approximately at 1470 ± 5 cm^{-1}. This correlates very well with our assignments at 1467 and 1466 cm^{-1} (experimental) versus 1465 and 1462 cm^{-1} (theoretical) in the spectra of both $SerH^+Cl^-$ and I-$SerH^+I^-$, in which the two bands presented strong intensity. The CH_2 vibrational mode also contributed to this band in a minor proportion. At higher frequencies, bending vibrations of methyl and methylene groups are overlapped while at lower frequencies rocking vibrational mode for methyl group was also observed.

The C—H in-plane bending frequencies are typically located at ca. 1000–1300 cm^{-1} and the C—H out-of-plane bending vibrations at ca. 750–1000 cm^{-1} in the aromatic compounds. In our studies the in-plane bending vibrations were located between 1310 and 1050 cm^{-1} and the out-of-plane bending vibrations between 870

Table 2
Experimental and calculated vibrational wavenumbers (in cm^{-1}) of the infrared spectra of sertraline hydrochloride ($SerH^+Cl^-$) and sertraline–iodine product ($I\text{-}SerH^+I^-$). Tentative assignments are also given. Abbreviations: s, strong; m, medium; w, weak; sh, shoulder; vw, very weak.

$SerH^+Cl^-$ observed fundamentals (cm^{-1})	$SerH^+Cl^-$ calculated frequencies (cm^{-1})	Relative intensity	Tentative assignment	$I\text{-}SerH^+I^-$ observed fundamentals (cm^{-1})	$I\text{-}SerH^+I^-$ calculated frequencies (cm^{-1})	Relative intensity	Tentative assignment
	3477.588	1.645988	ν_{as} (NH_2^+)		3485.630	1.637130	ν_{as}NH (NH_2^+)
	3361.036	6.474864	ν_s (NH_2^+)		3436.370	0.752450	ν_sNH (NH_2^+)^2
3240 w	3235.060	0.022832	νCH (Ph—Cl)		3239.000	0.042490	νCH (PhCl)
	3220.853	0.024961	ν_{as}CH (PhCl)		3222.680	0.015490	νasCH (PhCl)
	3210.703	0.003422	ν_{as} (CH_3)	3201 sh	3202.240	0.004790	νas (CH_3)
3178 w	3189.295	0.062451	νCH (Ph)	3174 sh	3172.980	0.121640	νCH (Ph—Cl)
3154 w	3148.56	0.249631	ν_{as}CH (CH_2), νCH (cycle)				
	3134.931	0.079452	ν_{as}CH (CH_2)	3126 sh	3136.180	0.188960	νasCH (CH_2),
3076 w	3080.120	0.096218	νCH (cycle)		3113.540	0.147920	νCH (cycle)
	3107.867	0.084723	ν_s (CH_3)		3108.760	0.008290	νs (CH_3)
	1680.576	0.170565	νC═C (Ph)	1676 m	1676.840	0.053340	νC═C (Ph)
	1659.382	0.038256	νC═C (Ph, PhCl)		1662.100	0.088130	νC═C (Ph, PhCl)
1640 m	1636.625	0.593778	NH_2 scissors	1645 m	1647.280	0.887930	NH_2 scissors
1626 m	1619.768	0.245849	νC═C (PhCl)		1650.800	0.072340	νC═C (PhCl),
1583 m	1539.496	0.291458	νC═C (Ph)	1599 m	1522.360	2.438240	νC═C (PhCl)
1515 w	1521.819	1.622319	νC═C (PhCl), CH_2 scissors CH_3 asymmetric bend,	1517 m	1517.570	0.311450	νC═C (PhCl), CH_2 scissors CH_3 asymmetric bend,
1488sh	1509.426	0.229882	CH_3 asymmetric bend,	1489 m	1504.830	0.728590	CH_3 asymmetric bend,
1467 vw	1464.830	0.162601	CH_2 wagging, CH_3 symmetric bend	1466 s	1461.810	0.093670	CH_2 wagging, CH_3 symmetric bend
1433 m	1439.840	1.046371	NH_2 wagging, CH (cycle) bend CH_3 symmetric bend, CH_2 wagging	1447 s	1427.470	0.622850	NH_2 wagging, CH (cycle) bend CH_3 symmetric bend, CH_2 wagging
1423 m	1427.702	0.636848	CH_3 symmetric bend, δCCH ip (PhCl)	1416 sh	1425.520	1.619480	CH_3 symmetric bend, δCCH ip (PhCl)
893 m	890.7757	0.15424	δCCH op (PhCl, Ph), breath (Ph, PhCl) CH_3 rocking, CH_2 rocking, NH_2 rocking	896 m	896.060	0.423680	δCCH op (PhCl, Ph), breath (Ph, PhCl) CH_3 rocking, CH_2 rocking, NH_2 rocking
845 vw	858.5498	0.607433	δCCH op (PhCl, Ph), δCH cycle CH_3 rocking	876 sh	871.190	0.33260	δCCH op (PhCl, Ph), CH_3 rocking CH_2 rocking, NH_2 rocking
826 m	824.7017	0.077872	Breath (Ph), δCH cycle, CH_3 rocking CH_2 rocking	820 s	827.640	0.019180	Breath (Ph), δCH cycle, CH_3 rocking CH_2 rocking, NH_2 rocking
796 sh	814.479	0.079348	Breath (Ph), δCH cycle, CH_3 rocking CH_2 rocking	804 m	811.910	0.280820	Breath (Ph), δCH cycle, CH_3 rocking CH_2 rocking, NH_2 rocking
785 m	796.1824	0.808563	δCCH op (PhCl, Ph), CH_3 rocking CH_2 rocking	777 s	784.260	0.177090	Breath (Ph), CH_3 rocking, CH_2 rocking, NH_2 rocking
769 m	760.3515	0.542095	δCCH op (Ph, PhCl), CH_3 rocking CH_2 rocking	760 m	762.100	1.018710	δCCH op (Ph, PhCl), breath (Ph), CH_3 rocking CH_2 rocking, NH_2 rocking
743 w	742.8886	0.072814	δCCH op (Ph, PhCl), CH_3 rocking CH_2 rocking	743 m	731.770	0.175640	δCCH op (Ph, PhCl), breath (PhCl)
702 m	708.3854	0.076838	Breath (Ph, PhCl), CH_3 rocking CH_2 rocking	729 m	715.960	0.578870	δCCH op (Ph, PhCl), CH_3 rocking CH_2 rocking, NH_2 rocking
673 m	697.7009	0.32969	Breath (Ph), CH_2 rocking	673 m	699.090	0.367260	Breath (PhCl)
643 w	663.4005	0.166804	Breath (Ph), CH_3 rocking CH_2 rocking	634 w	633.010	0.297930	Breath (Ph), CH_2 rocking
620 m	634.8886	0.052174	Breath (Ph), CH_2 rocking				

(*continued on next page*)

Table 2 (*continued*)

SerH$^+$Cl$^-$ observed fundamentals (cm^{-1})	SerH$^+$Cl$^-$ calculated frequencies (cm^{-1})	Relative intensity	Tentative assignment	I-SerH$^+$I$^-$ observed fundamentals (cm^{-1})	I-SerH$^+$I$^-$ calculated frequencies (cm^{-1})	Relative intensity	Tentative assignment
589 m	590.0904	0.059073	Torsion and Breathing (Ph, PhCl, cycle)	617 m	616.220	0.161890	Torsion and breathing (Ph, PhCl, cycle)
563 w	579.0513	0.051966		558 w	564.980	0.018160	
534 w	544.8443	0.167831		526 w	526.300	0.369390	
514 m	511.9304	0.069119	Torsion and breathing (Ph, PhCl, cycle)	511 w	509.720	0.200080	Torsion and breathing (Ph, PhCl, cycle) νC—Cl
495 m	475.4928	0.016656	Torsion and breathing (Ph, PhCl, cycle) νC—Cl	487 m	474.370	0.011340	Torsion and breathing (Ph, PhCl, cycle) νC—Cl
				476 m	468.410	0.062460	Torsion and Breathing ring and Ph
445 w	455.8562	0.166197	Torsion and breathing (Ph, PhCl, cycle)	442 m	446.280	0.081480	Torsion and Breathing (Ph, PhCl, cycle) νC—I
426 w	423.2416	0.010425	Torsion and breathing (Ph, PhCl, cycle)	426 w	418.490	0.013730	Torsion and Breathing (Ph, PhCl, cycle)
1403 s	1396.279	0.142119	CH_3 symmetric bend, δCH cycle	1400 s	1397.530	1.797810	CH_3 symmetric bend, δCH cycle
				1387 s	1374.040	0.134550	CH_3 symmetric bend, δCH cycle
				1360 w	1366.360	0.172970	CH_3 symmetric bend, δCH cycle NH_2 wagging
1339 m	1343.336	0.083625	CH_3 rocking, NH_2 twisting, δCH cycle CH_2 twisting	1343 m	1345.620	0.274160	CH_3 rocking, NH_2 twisting, δCH cycle CH_2 twisting
1310 m	1297.727	0.228282	δCCH ip (PhCl, Ph), CH_2 twisting,	1285 m	1300.550	0.101220	δCCH ip (PhCl, Ph), CH_2 twisting,
1270 m	1274.131	0.212599	δCCH cycle	1267 m	1272.790	0.524880	δCCH cycle
1250 m	1258.731	0.107284		1251 m	1250.260	0.782840	
1248 w	1235.853	0.142985		1237 w	1233.280	0.341330	
1214 m	1217.275	0.220375	δCCH ip (PhCl, Ph), δCCH cycle CH_2 twisting, νCNC	1215 m	1224.860	0.149560	δCCH ip (PhCl, Ph), δCCH cycle CH_2 twisting, νCNC
				1202 m	1204.060	0.027430	δCCH ip (PhCl, Ph), δCCH cycle CH_2 twisting
				1171 w	1180.560	0.842210	δCCH ip (PhCl, Ph)
1136 s	1138.180	0.087837	δCCH ip (PhCl, Ph), δCCH cycle, CH_2 twisting, NH_2 twisting	1132 s	1134.070	0.163340	δCCH ip (PhCl, Ph), δCCH cycle, CH_2 twisting, NH_2 twisting
				1107 m	1106.700	0.160850	δCCH ip (PhCl, Ph), δCCH cycle CH_2 twisting
1081 w	1073.615	0.014896	δCCH ip (PhCl, Ph), δCCH cycle	1076 m	1075.450	0.041480	δCCH ip (PhCl, Ph), δCCH cycle,
1057 m	1058.431	0.875498	δCCH ip (PhCl, Ph), δCCH cycle	1052 m	1052.590	0.336240	δCCH ip (PhCl, Ph), δCCH cycle
1025 m	1036.345	0.365318	ν (C—N)	1028 s	1024.100	0.223770	ν (C—N)
1012 sh	1019.900	0.190899	δCCH ip (Ph), δCCC				
1002 sh	998.3846	0.008651	δCCH op (PhCl, Ph)	997 m	997.580	0.006180	δCCH op (PhCl, Ph)
				956 m	950.550	0.207630	δCCH op (PhCl, Ph), CH_3 rocking CH_2 rocking, NH_2 twisting
				924 m	917.160	0.05180	δCCH op (PhCl, Ph), CH_3 rocking, CH_2 rocking
				914 sh	913.260	0.030380	δCCH op (PhCl, Ph) CH_2 rocking

and 960 cm^{-1}, respectively, as expected. In general, the calculated C—H vibrations (stretching, in-plane and out-of-plane bending) are in good agreement with the experimentally accepted values (Table 2 and Fig. 3). As it has been stated, these bands are not appreciably affected by the nature of the substituent and for this reason the ones of the two phenyl groups of the molecule (one of them with chloride) are indistinguishable [29].

The ring stretching vibrations are representative of the aromatic ring. As usual, in substituted benzenes, the bands observed in the region 1400–1650 cm^{-1} are characteristic of the typical skeletal stretching modes of C=C bonds. In this work, the assigned skeletal vibrations for both the experimental spectra and theoretical harmonic frequencies were located in the 1500–1680 cm^{-1} region. Among the contribution of other vibrational modes, the theoreti-

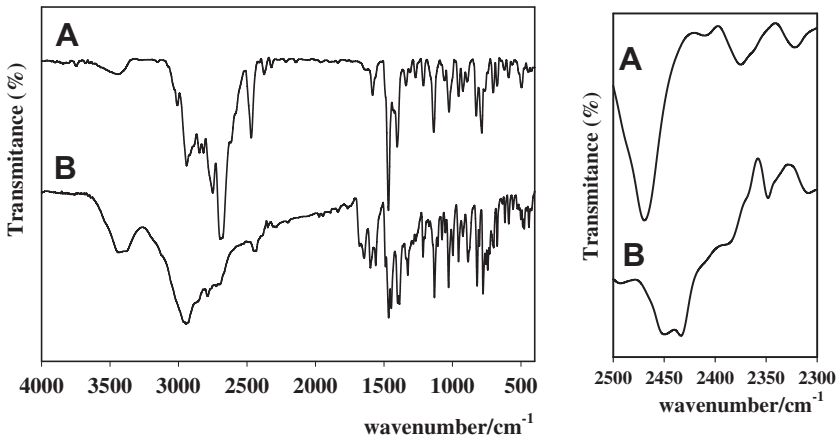

Fig. 2. Left side: Experimental FTIR spectra for sertraline hydrochloride ($SerH^+Cl^-$) (A) and sertraline–iodine product, $I\text{-}SerH^+I$ (B). Right side: expanded region (2500–2300 cm^{-1}) for (A) and (B) respectively.

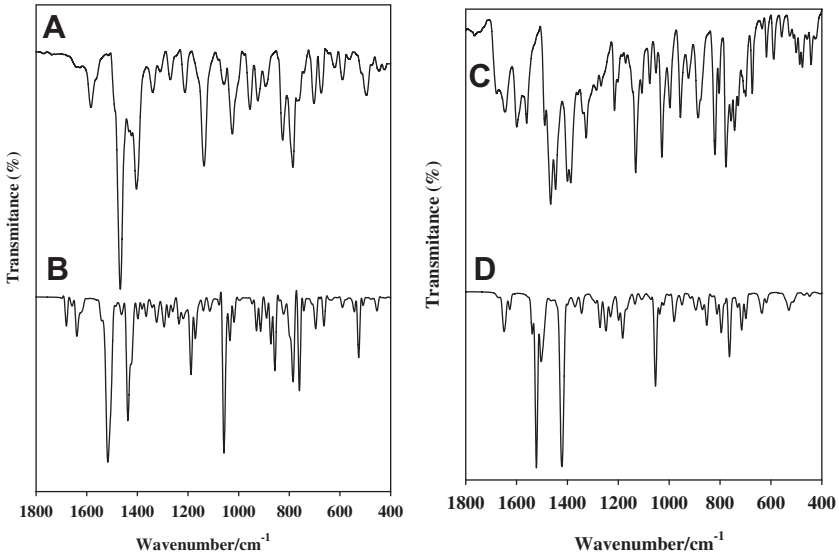

Fig. 3. Left side: Experimental (A) and theoretical (B) FTIR spectra for sertraline hydrochloride ($SerH^+Cl^-$). Right side: Experimental (C) and theoretical (D) FTIR spectra of sertraline–iodine product ($I\text{-}SerH^+I$).

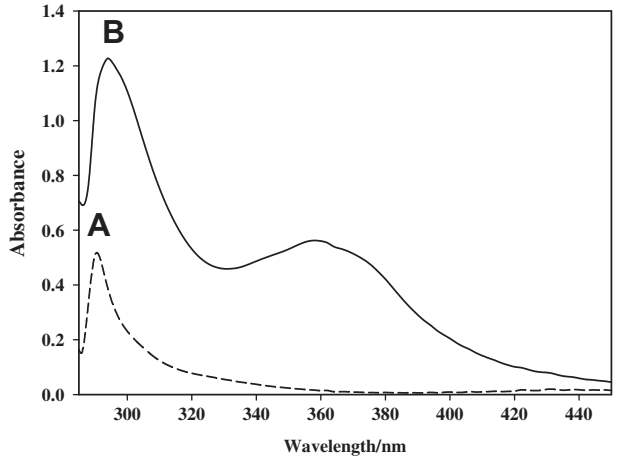

Fig. 4. Solution electronic absorption spectra in 200–450 nm region (dichloromethane): (A) sertraline hydrochloride ($SerH^+Cl^-$) and (B) sertraline–iodine product ($I\text{-}SerH^+I$).

cally calculated asymmetric stretching (CNC group) has been found to be consistent with the experimental value (Table 2).

One of the most relevant features of the computational analysis was the identification of C-halogen stretching vibration modes (Table 2). The values of the frequencies for the C—I bond stretching mode appears at lower frequencies than those of C—Cl bond as expected. This trend is in accordance with the higher atomic radii of iodine in comparison with chlorine that form a longer C—X bond and hence the band due to the stretching mode appeared at lower frequency.

It is worth to mention that in contrast to *ab initio* and density functional computations performed by Sagdinc et al. [6] for sertraline hydrochloride, in our results, a better concordance between experimental and theoretical data could be achieved (Table 2, Fig. 3). On the other hand, Zayed et al. [5] performed a vague analysis of the FTIR spectrum for sertraline iodine product in which the vibrational ranges for the main characteristic bands were given.

UV–Vis spectroscopy

Sertraline hydrochloride has a typical electronic absorption spectrum assumed for aryl halogen compounds and displays a

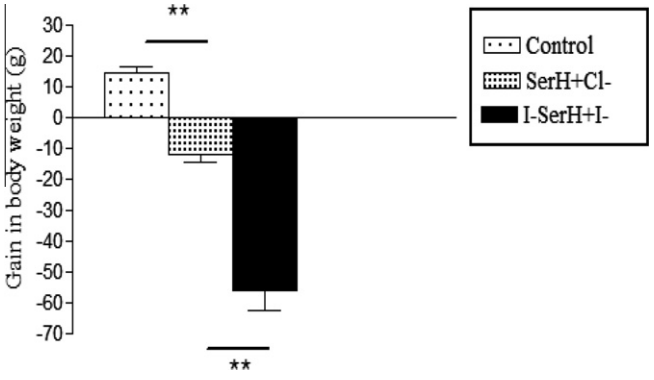

Fig. 5. Effect of sertraline hydrochloride ($SerH^+Cl^-$) and sertraline–iodine product ($I\text{-}SerH^+I$) on body weight of rats. Animals treated with sertraline–iodine product induced a body weight loss lower than that induced by sertraline [$P < 0.01$]. $^{**}P < 0.01$, 7–12 rats per group (Dose: 5 mg/kg). Data were analyzed with one-way ANOVA, followed by Tukey's test for multiple comparisons.

band at 290 nm in CH_2Cl_2 as a solvent (Fig. 4A). This band is known as secondary absorption band and shows a bathochromic shift with respect to benzene, as expected [30]. For sertraline iodine product, this band is located at 294 nm with a slight shift to the red in comparison with sertraline hydrochloride (Fig. 4B). Strikingly the iodine compound displays a new band at 360 nm probably arising from the presence of iodine in the molecule.

Forced swimming test (FST)

After performing the pre-test of FST, treatments were initiated in the three experimental groups. Between the 1st and 2nd day of treatment, the animals of Group 3 (sertraline–iodine) showed a clear central nervous system depression (decreased motor activity and loss of reflexes) including markedly body weight loss, leading to animal deterioration and death. All animals ($n = 5$) died between day 1 and day 2 of treatment, and the animals did not complete the planned treatment. Due to apparent toxicity of treatment, a lower dose (5 mg/kg) was employed. In this case all animals ($n = 5$) died between the 3rd and 4th day of treatment. This *short*-term *treatment* with sertraline–iodine induced a body weight loss lower than that induced by sertraline (Fig. 5). Our results demonstrate that sertraline–iodine produced dose-dependent toxicity.

Treatments could not be completed, and therefore, the antidepressant effect could not be evaluated in the FST test due to the manifestation of severe toxic effects by the sertraline–iodine compound at the doses tested (10 and 5 mg/kg). One possible explanation for the toxic effects may be the consequence of having used a dose of iodine-compound near the lethal dose 50 (LD50). LD50 values reported in the literature: (i) Oral rat LD50: 14 mg/kg (iodine crystals) [31], (ii) Oral rat LD50: 14 mg/kg (iodine tincture) [32], and (iii) Oral rat LD50: 4340 mg/kg (sodium iodide) [33].

In vitro anti-thyroid activity of sertraline hydrochloride

Anti-thyroid drugs are used to normalize thyroid function. A method able to determine if a substance has anti-thyroid activity was previously performed using sertraline hydrochloride in bulk powder and in pharmaceutical formulations by spectrophotometric determinations [34]. Furthermore, as previously reported data showed that nitrogen containing heterocyclic compounds were able to form charge transfer complexes with iodine [20], these compounds can be used to test the *in vitro* anti-thyroid activity.

The first step in thyroid hormone synthesis includes generation of an oxidized enzyme promoted by endogenously produced hydrogen peroxide. The oxidized enzyme reacts with iodide to form an "iodinating intermediate" (charge transfer complex). In the absence of an anti-thyroid drug, the intermediate reacts with specific tyrosine residues in thyroglobulin (T_g) to form mono-iodotyrosine (MIT) and di-iodotyrosine (DIT). In a subsequent intramolecular coupling of MIT and DIT, tri-iodothyronine (T_3) is produced, and the coupling of two DIT molecules forms thyroxine (T_4). An anti-thyroid drug can act as an alternative substrate for the iodinating intermediate, competing with thyroglobulin-linked tyrosine residue or interfering with thyroid peroxidase (TPO) enzyme by blocking its active Fe^{3+} [35]. In the past, several compounds have been tested with the aim to find a new class of anti-thyroid agents with minor cost and undesirable side effects including a series of antidepressants which have demonstrated *in vitro* anti-thyroid activity [36]. With the purpose of testing the potential anti-thyroid activity of sertraline hydrochloride, the iodine method was applied. Sertraline hydrochloride displayed no bands in the 245–600 nm range. When a solution of iodine was added to the antidepressant in the same solvent, the formation of complex led to the appearance of a new CT band located at 257 nm. As it is possible to observe in Fig. 6 (left side), the intensity of this band in-

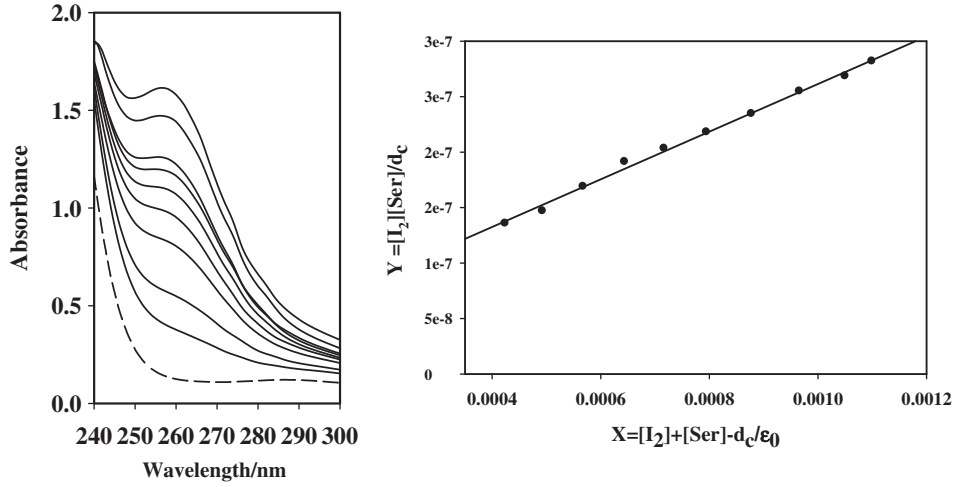

Fig. 6. Lang's method. Left side: electronic absorption spectra in the 240–300 nm region (25 °C, dichloroethane, $C_2H_4Cl_2$, 1 cm of optical path length). The CT band appears around 257 nm, when increasing sertraline hydrochloride concentration from 1×10^{-4} mol L^{-1} to 1×10^{-3} mol L^{-1}. In all cases the iodine concentration was constant at 4×10^{-4} mol L^{-1}. The reaction was carried out directly in the spectrophotometric cell by mixing 1.5 mL solutions each of sertraline hydrochloride (donor) and iodine (acceptor). Right side: Relationship between $[I_2][Ser]/d_c$ and $[I_2] + [Ser] - d_c/\varepsilon_c$ for sertraline–iodine complex under the same experimental conditions.

creased when increasing sertraline concentrations while the iodine concentration was constant any time in the experiment. The absorbance value for the band located at 257 nm was recorded for different concentrations of sertraline hydrochloride and constant concentration of iodine. For the sake of quantification a validate procedure was applied: the solution of the pure donor at the same concentration was located in the reference beam, and then spectra were obtained by subtraction of the iodine absorption. Data analysis was performed according to Lang's method (experimental, Section 4.4).

In Fig. 6 (right side), Eq. (3) was plotted. From this, the formation constant K_c value is 3277.48 L mol^{-1} being the molar coefficient of ε_c = 5358.39 L mol^{-1} cm^{-1}. Anti-thyroid activity can be expected from compounds whose formation constant of the iodinated complex K_c was larger than 100 L mol^{-1} [7]. For this reason, it is possible to assume a strong anti-thyroid action for sertraline. Anti-thyroid activities for other antidepressants were previously performed in dichloromethane and carbon tetrachloride as solvents. Although measurements were carried out in dichloromethane for sertraline, they seem to have an effect in the same order of magnitude than imipramine, desipramine as well as clomipramine [7].

Conclusions

In this work, we measured the experimental FTIR spectrum of sertraline hydrochloride and its iodine-product. The conformational space of the molecules was investigated using molecular dynamics simulations within an NVT ensemble. Their geometrical, electronic and vibrational properties were further calculated within the density functional theory.

A comparison of experimental and theoretical results provided a full description of the geometrical and vibrational properties of the iodine compound. One of the most relevant results was the assignment of the C-halogen stretching and the symmetric and asymmetric vibrational N—H modes of the NH_2^+ group in both compounds. Our results show a better concordance with the experimental FTIR of sertraline hydrochloride than the previously reported data. In addition, potential *in vitro* anti-thyroid activity was proved for sertraline hydrochloride demonstrating an effect in the same order of magnitude than imipramine, desipramine as well as clomipramine. Attempts to determine the antidepressant activity of the sertraline–iodine product failed because of the toxic effect that exerts this compound on the male Wistar rats.

Acknowledgements

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