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Improving the antidepressant action and the bioavailability of sertraline by co-crystallization with coumarin 3-carboxylate. Structural determination

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ABSTRACT

To improve the antidepressant action of sertraline a new salt with coumarin-3-carboxylate anion (SerH-CCA) has been synthesized by two different methods and characterized by FTIR spectroscopy and structural determinations by X-ray diffraction methods. The new salt is stabilized by strong intermolecular H-bonds involving the protonated amine group of SerH and the deprotonated carboxylate group of CCA. These findings can be correlated with the interpretation of the infrared spectrum. The salt, sertraline (SerHCl) and the sodium salt of coumarin-3-carboxylate (NaCCA) were orally administered male Wistar rats (10 mg/kg, based on sertraline). Rats were evaluated in separate groups by means of the forced swimming (FST). SerH-CCA produced antidepressant effects in a magnitude that exceeded SerHCl individual effects. None of these treatments affected activity levels by the open field OFT tests. We have also determined that the ion pair also improve the binding to bovine serum albumin (BSA) of the drug but retain its antimicrobial activity. It is reasonable to conclude that the replacement of chloride anion by a large organic anion in sertraline strengthens the pharmacological action of the native drug, binding to BSA with higher activity and retaining the antimicrobial activity of the antidepressant compound.

1. Introduction

Many cases of depression can be related to changes in the neurochemistry of three monoamine neurotransmitters that are derivatives of aminoacids, namely serotonin, norepinephrine and dopamine. Sertraline (Ser, (1S, 4S)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphathalen-1-amine) is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class that acts as a reuptake inhibitor by blocking the action of the serotonin transporter. Increased concentrations of serotonin are then produced and a serotonergic neurotransmission is facilitated [1]. It has been determined that organic acids have the ability to improve the bioavailability of sertraline hydrochloride. Therefore, ionic pairs with malate, citrate, adipate, L-aspartate, tartrate and L-glutamate anions have been prepared to deliver a solution of drug. Salts of sertraline with acetate, L-lactate and L-apartate [2] showed higher solubility in water and improved the bioavailability and the stability as compared with pure sertraline.

In addition, a lot of coumarins from natural sources have been identified from natural sources, especially from green plants. Coumarins (known as 2H-chromen-2-one1,2-benzopyrone) and their derivatives have stimulated interesting research in biology and

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medicine, due to their antibiotic, antioxidant, anticancer [3,4] and antidepressant properties [5].

For these reasons we have undertaken the development of pharmacodynamics hybrids, possessing the characteristics of both a typical antidepressant substance and an antioxidant compound. The ionic pair containing the cationic form of sertraline (SerH) with the anion of coumarin 3-carboxylic acid (HCCA, 2-*oxo*-2H-1-benzopyran-3-carboxylic acid), coumarin 3-carboxylate (CCA^-) has been characterized by FTIR spectroscopy and its solid structure determined by X-ray diffraction methods.

It has been shown that sertraline has also antibacterial and antifungal activities and has augmented the antibacterial activities of antibiotics [6]. Then, another reason to synthezise the ion pair SerH-CCA was to determine the probable improvement of the antimicrobial activity of SerHCl.

Serum albumin acts as a transport protein carrying large organic anions, such as fatty acids and many drugs. One of the most important factors affecting the distribution and the free, active concentration of many administered drugs is the binding affinity to serum albumin. The interaction of sertraline with human serum albumin with the formation of 1:1 complex and significant binding affinity has recently been reported [7]. Previous data on the pharmacokinetics of sertraline showed that the compound is able to bind to plasma protein in a 98% and the maximum concentrations occur in an estimated mean time between 6 and 8 h in humans [8]. It was then interesting to measure the interaction and potential transport of CCA and SerH-CCA to determine a probable improvement of the bioavailability of the new ion pair. An increase of the binding constant has been found for the binding of the ion pair to BSA with respect to sertraline-BSA for 2 and 8 h of incubation, showing a better behavior of the transport in biological systems.

The development of animal models of anxiety and stress has helped to identify the pharmacological mechanisms and potential clinical effects of several drugs. Animal models of anxiety are based on conflict situations that can generate opposite motivational states induced by approach-avoidance situations. The antidepressant activity of the new compound has been tested on male Wistar rats and compared with the activity of sertraline hydrochloride and the sodium coumarin 3-carboxylate salt. The antidepressant activity of the salt with improvement of the bioactivity of the SSRI pharmacotherapeutic agent sertraline has been determined i*n vivo.*

2. Material and methods

2.1. Materials

Sertraline hydrochloride (SerHCl) and coumarin 3-carboxylic acid were obtained from Sigma Chemical Company (St. Louis, MO) and used as supplied. All the solvents used were from analytical grade. 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.

2.2. Preparative

Sertraline coumarin 3-carboxylate was prepared from basic sertraline and coumarin 3-carboxylic acid. In a first step, 1 mmol sertraline hydrochloride was dissolved in 20 mL of boiling water. A solution of 1 M sodium hydroxide was added up to a final pH value of 7 and the mixture was stirred during 30 min. The white precipitate was cooled to room temperature and the centrifuged to yield basic sertraline. This solid was washed several times with water to remove chloride ions. A solution of basic sertraline was prepared by dissolving 1 mmol of the compound in 20 mL of warm ethanol. Coumarin 3-carboxylic acid, HCCA, was separately dissolved in 20 mL ethanol. This solution was added in small portions to the solution of sertraline under constant stirring. The reaction mixture was then allowed to cool to room temperature and filtered. Single crystals of SerH-CCA suitable for structural X-ray diffraction work were obtained by slow solvent evaporation of the solution.

Alternatively, sertraline coumarin 3-carboxylate can also be prepared directly from sertraline hydrochloride, without isolation of the free base of sertraline. In a first step the sodium salt of CCA (NaCCA) was prepared. HCCA, 2 mmol was dissolved in 40 mL of boiling water, and an equivalent quantity of NaOH was then added. The yellow solution was cooled to room temperature and the white precipitate of NaCCA was filtered out in vacuum and washed with acetone. This solid (2 mmol) was dissolved in a boiling mixture of 40 mL of ethanol and 20 mL of water, and then mixed with a solution of sertraline hydrochloride (2 mmol in 40 mL) of boiling ethanol. Single crystals of SerH-CCA have been obtained by slow evaporation of the solution.

FTIR spectrophotometry (see below) confirms that the same compound has been obtained with both preparative methods.

2.3. X-ray diffraction data

The measurements were performed on an Oxford Xcalibur Gemini, Eos CCD diffractometer with graphite-monochromated CuKα (λ = 1.54184 Å) radiation. X-ray diffraction intensities were collected (ω scans with ϑ and κ-offsets), integrated and scaled with CrysAlisPro1 [9] suite of programs. The unit cell parameters were obtained by least-squares refinement (based on the angular settings for all collected reflections with intensities larger than seven times the standard deviation of measurement errors) using CrysAlisPro. Data were corrected empirically for absorption employing the multi-scan method implemented in CrysAlisPro. The structure was solved by direct methods with SHELXS-97 of the SHELX suite of programs [10] and the corresponding molecular model developed by alternated cycles of Fourier methods and full-matrix least-squares refinement with SHELXL-97 of the same package [10].

Most hydrogen atoms were located in a difference Fourier map phased on the heavier atoms. However, all but the amine hydrogen atoms were positioned on stereo-chemical basis and refined with the riding model. The methyl H-positions were optimized by treating them as a rigid group which was allowed to rotate during the refinement around the N-C bonds such as to maximize the residual electron density at the calculated positions. As a consequence the $-CH_3$ group converged to a staggered conformation. The amine H-atoms were refined at their found position with isotropic displacement parameters and the N-H bond distances restrained to a target value of 0.86(1) Å. Crystal data, data collection procedure, structure determination methods and refinement results are summarized in Table 1. Crystallographic structural data have been deposited at the Cambridge Crystallographic Data Centre (CCDC). Any request to the CCDC for this material should quote the full literature citation and the reference number CCDC 991981.

2.4. Biological experiments

2.4.1. Antimicrobial assays

The antibacterial and antifungal profile of the ionic pair and its components (sertraline and coumarin 3-carboxylic acid) have been studied against bacterial and fungal strains by the agar dilution method. Control strains included five bacterial strains: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis ATCC 1263* and *Enterococcus faecalis* ATCC 29212 and seven strains of Candida namely: *Candida parapsilosis* ATCC 22019, *Candida albicans* ATCC

Table 1
Crystal data and structure refinement results for SerH-CCA.

Empirical formula	$C_{27}H_{22}Cl_2NO_4$
Formula weight	496.36
Temperature	295(2) K
Wavelength	1.54184 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 11.4045(6) Å b = 8.1812(5) Å c = 12.7394(5) Å β = 91.792(4)
Volume	1188.0(1) $Å^3$
Z, density (calculated)	2, 1.388 Mg/m^3
Absorption coefficient	2.746 mm^{-1}
F(000)	516
Crystal size	0.278 × 0.134 × 0.042 mm^3
ϑ-range for data collection	3.47–70.99°
Index ranges	$-13 \le h \le 12$, $-9 \le k \le 9$, $-15 \le l \le 15$
Reflections collected	4964
Independent reflections	3314 [R(int) = 0.0289]
Observed reflections [I > 2σ(I)]	2651
Completeness to ϑ = 70.99°	99.6%
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3314/3/316
Goodness-of-fit on F^2	1.040
Final R indices[a] [I > 2σ(I)]	R1 = 0.0731, w*R*2 = 0.2067
R indices (all data)	R1 = 0.0853, w*R*2 = 0.2298
Absolute structure parameter	0.04(4)
Largest diff. peak and hole	1.047 and −0.363 e.$Å^{-3}$

[a] $R_1 = \Sigma||F_o|-|F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(|F_o|^2-|F_c|^2)^2/\Sigma w(|F_o|^2)^2]^{1/2}$.

10231, and clinical isolates of *Candida tropicalis, Candida krusei, Candida glabrata, Candida parapsilosis* and *Candida albicans*. Mueller Hinton Broth (MHB) or Mueller Hinton Agar (MHA) have been used for the cultivation/assay medium for all strains. The inocula of bacterial and fungal strains were prepared from 18 h-old broth cultures. A McFarland 0.5 suspension was prepared for each isolate (~10^8 colony forming units (CFU) per mL, CFU mL^{-1}). The fungal suspension (~10^8 CFU per mL) was directly inoculated onto the agar surface, whereas the bacterial suspension was 1:10 diluted prior to inoculation (~10^7 CFU per mL). The minimum inhibitory concentration (MIC) was determined by the agar dilution method. The compounds were dissolved in 50% aqueous dimethylsulfoxide (DMSO) to a final concentration of 15 mg mL^{-1}. Serial two-fold water dilutions were prepared from the stock solution of the antimicrobial agents to give concentrations ranging from 14.65 to 15,000 μg mL^{-1}. All solutions were sterilized by filtration before use. Then, 0.5 mL of each dilution of antimicrobial solution were added to 4.5 mL of melted MHA and poured into a square (45 × 15 mm) plate. The final concentrations ranging in the MHA were from 1.46 to 1500 μg mL^{-1}. An agar plate without antibacterial agent was established as a sterility and organism growth controls. The plates were prepared and used on the same day. After cooling and drying, the plates were inoculated with each microbial suspension. The inoculum of 2 μL of the germ suspensions were streaked onto the plates and incubated aerobically at 37° for 24 h and 48 h for bacteria and fungi, respectively. Each MIC experiment was repeated three times. Inhibition of microbial growth in the plates containing tested solutions was judged by comparison with growth in control plates. The MIC was defined as the lowest dilution of the complex that inhibited the visible growth of the tested organism. A single colony or a faint haze caused by the inoculum was considered to be no growth.

2.4.2. Bovine serum albumin (BSA) interaction

BSA was dissolved in Tris-HCl (0.1 M, pH 7.4) buffer to attain a final concentration of 6 μM. Sertraline hydrochloride, coumarin 3-carboxylic acid and SerH-CCA were added dropwise to the BSA solution and left to rest to ensure the formation of homogeneous solutions with concentrations ranging from 2 to 100 μM. The fluorescence intensity was measured (excitation at 280 nm and emission at 348 nm) at 37 °C. To ensure an effective interaction of the compounds with the protein and based on previous pharmacokinetic studies, two different incubation times were selected (2 h and 8 h) [8]. For each sample and concentration, three independent replicates were performed. The measurements were carried out on a Perkin-Elmer LS-50B luminescence spectrometer (Beaconsfield, England) equipped with a pulsed xenon lamp (half peak height < 10 ls, 60 Hz), an R928 photomultiplier tube and a computer working with FLWinLab software. Both excitation and emission slits were set at 10 nm throughout this study.

2.5. Pharmacological experiments

2.5.1. Animals

Experiments were carried out on male Wistar rats weighing 200–310 g. The animals were maintained on a 12 h light (08:00–20:00 h) −12 h dark cycle, with free access to food and water, except during testing. They were housed in groups of four, in individual polyethylene cages (55 × 38 × 30 cm). Rat weights were recorded before and at the end of the experiments. Animals were used only in one experiment. 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 and AVMA Guidelines for the Euthanasia of Animals, 2013 Ed. The study obtained the clearance from the Ethics Committee for the care and use of Laboratory Animals of the Universidad Nacional de La Rioja, Argentina.

2.5.2. Drugs and treatment

Sertraline hydrochloride; the sodium salt of coumarin 3-carboxylate, NaCCA and the salt SerH-CCA were orally administered in a dose of 10 mg/kg of sertraline per kg of the animal and SerH-CCA and NaCCA in the equivalent quantities because it was proved that sertraline produced the most robust effect in the force swimming test under similar experimental conditions [11]. All of the control rats received injections of saline solution (0.9% NaCl). Rats were treated with the saline solution of sertraline hydrochloride, sodium salt of coumarin 3-carboxylic acid or a suspension of the ion pair once a day.

The rats were randomly divided into four groups and conducted the following treatments: Group 1: saline (control group), Group 2: SerHCl (sertraline hydrochloride), Group 3: SerH-CCA and Group 4: NaCCA.

2.5.3. Forced swimming test (FST)

In this study, we used the forced swimming test, a well-accepted model to test the antidepressant-like action of drugs [12] and to identify in rats treatments with antidepressant efficacy in humans [13]. Stress is a well-known risk factor in the development of depression. The forced swimming test employs forced swimming stimuli as stressor to generate a behavior characterized by increased immobility time.

Swimming sessions were conducted by placing rats in individual Plexiglas cylinders (46 cm tall × 20 cm in diameter), filled with water (23–25 °C) up to 30 cm from bottom. All swimming sessions were carried out between 12.00 and 18.00 h.

In our protocol, two sessions were conducted: an initial 15 min pre-test on day 1 followed by a 5 min test on day 15. Drugs treatment began on day 1 after the pre-test session and it was administered from day 1–14 [14]. At the end of both swimming sessions, rats were removed from the cylinders, dried with towels, placed in

heated cages for 15 min, allowed to rest and recover, and then returned to their home cages. The cylinders were emptied and cleaned between rats. Each animal was assigned randomly to a treatment, and was only employed for one pre-test/test session.

2.5.4. Behavioral scoring

For behavioral sampling, rats were rated at 5 s intervals throughout the duration of the forced swimming session. At each 5 s interval, the predominant behavior was assigned to one of 3 categories: (1) immobility: floating in the water without struggling, and making only those movements necessary to keep the head above the water; (2) swimming: making active swimming motions, more than necessary to merely keep the head above water (i.e., moving around in the cylinder); and (3) climbing: making active movements with forepaws in and out of the water, usually directed against the walls. Scores for each behavior were expressed as total behavioral counts per 5-min session [14].

2.5.5. Open field test (OFT)

This test was carried out to determine whether different treatments that were effective in the forced swimming test had non-specific effects on locomotor activity in rats exposed previously to FST. Until day 14, these studies were conducted exactly as the forced swimming test studies: all rats underwent the first day of the forced swimming test but instead of re-testing in the forced swimming test on day 15, animals were subjected to an open field session. All animals were placed gently in the centre of the open field arena, owed to explore freely and its locomotion was measured by the number of squares entered with all four paws (counts), during a period of 5 min. The apparatus for the open field test consisted of a black, square open field (60 cm by 60 cm) with the floor divided in squares (15 × 15 cm) by means of white lines. Testing was performed between 12.00 and 18.00 h, illuminated with a 75 W electric bulb, hung 75 cm above it, in a quiet room. During all the experiments the laboratory room was dark. After each animal was removed, the open field was carefully cleaned with a damp cloth. The behavior was scored by an observer who was unaware of the experimental procedures previously performed on the animals and the results were expressed as mean ± S.E.M.

3. Results and discussion

3.1. Crystallographic structural results and discussion

Fig. 1 shows an ORTEP [15] drawing of the salt and the corresponding intra-molecular bond distances and angles are given in Table 2. The pharmaceutical sertraline, is found in the solid as the cationic moiety protonated at its amino group, namely (1*S*,4*S*)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydro naphthalen-1-amine (for short, SerH). The anionic counterpart is coumarin 3-carboxylic acid, which appears deprotonated at its carboxylic group, namely 2-Oxo-2*H*-1-benzopyran-3-carboxylate (CCA).

Because extended molecular orbital π-delocalization, the phenyl and dichlorophenyl groups of SerH were planar (*rms* deviation of non-H atoms from the best least-square plane of 0.008 and 0.023 Å, respectively). The planes in turn subtended a dihedral angle of 73.7(1)° with each other. Because of the same reason, the fused rings of CCA were planar (*rms* deviation of atoms from the best least-square plane of 0.0362 Å). The carboxylate group departed slightly from the plane (angled at 12.9 (7)°). Intra-molecular bond distances and angles within SerH in SerH-CCA agreed with corresponding values reported for sertraline hydrochloride polymorphs and for other SerH salts [2,16–18].

Although the conformation of SerH has closely been related to the one reported in Refs. [16], however, it differed significantly

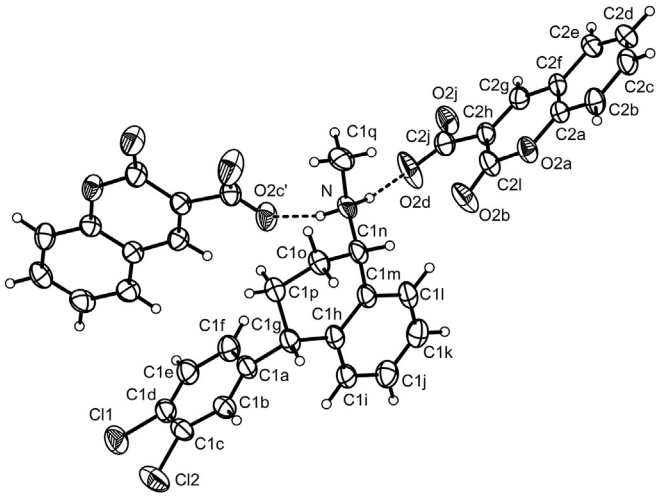

Fig. 1. Drawing of SerH-CCA showing the labeling of the non-H atoms and their displacement ellipsoids at the 30% probability level. The inter-molecular H-bonds are indicated by dashed lines. The partially labeled CCA molecule is related to the labeled one through the symmetry operation -x, y-1/2, 2-z.

when compared with the other compounds. This can be traced to relatively unhindered rotational freedom of SerH around the C-C σ-bond linking the dichlorophenyl and the naphthalene rings and also around the C(naph)-N σ-bond. As expected, the mayor differences in bond lengths of SerH as compared with neutral sertraline [19], showed up as a lengthening of C(naph)-N and N-CH_3 bond distances (of 0.021 Å and 0.059 Å, respectively) upon protonation at the amino group.

Bond distances and angles within the CCA anion accorded with reported values in other coumarin salts, only differing with these salts in the dihedral angle subtended by the −COO group with the fused ring plane [20]. As expected, the main differences in bond distances of CCA when compared with neutral coumarin [21] occurred due to the bond structure change at the carboxylic group upon deprotonation from formally C═O double and C-OH single bonds [lengths of 1.214 and 1.330 Å] in neutral coumarin to two localized C-O σ-bond plus a delocalized π-bond in the $-COO^-$ carboxylate group of CCA (C-O bond distances of 1.219 and 1.224 Å].

As shown in Fig. 1, the crystalline salt has further been stabilized by strong and linear intermolecular H-bonds involving the SerH amine > NH_2^+ as the donor group and as acceptors the carboxylate $-COO^-$ oxygen atoms from two neighboring, symmetry related, CCA ions [N … O bond distances of 2.712(9) and 2.759(6) A, and corresponding N-H … O bond angles of 170(7) and 161(7)°]. The N-H … O bonding occurred near along the oxygen lone-pair orbitals.

3.2. FTIR spectroscopy

Infrared spectroscopy is a powerful tool for the identification of the functional groups in a molecule and the determination of their modifications when the molecule is structurally modified. The FTIR absorption spectra of the potassium salt of coumarin 3-carboxylate, sertraline hydrochloride and the SerH-CCA salt are shown in Fig. 2. Selected FTIR band frequencies and assignments are given in Table 3. The assignments have been performed considering previous interpretations of SerHCl [22] and of coumarin 3-carboxylic acid [23] vibration spectra.

As expected, the FTIR spectrum of the single crystal of SerH-CCA salt included the contribution of both spectra of CCA and SerH constituents. The main bands due to SerH in the ionic pair were those that entail the NH_2 vibration modes, mainly located

Table 2
Bond lengths [Å] and angles [°] for SerH-CCA.

C(1A)-C(1B)	1.354(8)	C(1E)-C(1D)-Cl(1)	119.0(5)
C(1A)-C(1F)	1.411(9)	C(1D)-C(1E)-C(1F)	119.8(6)
C(1A)-C(1G)	1.508(7)	C(1A)-C(1F)-C(1E)	119.8(6)
C(1B)-C(1C)	1.447(8)	C(1A)-C(1G)-C(1H)	112.4(5)
C(1C)-C(1D)	1.35(1)	C(1A)-C(1G)-C(1P)	111.4(5)
C(1C)-Cl(2)	1.725(6)	C(1H)-C(1G)-C(1P)	110.8(5)
C(1D)-C(1E)	1.338(9)	C(1I)-C(1H)-C(1M)	118.1(5)
C(1D)-Cl(1)	1.745(6)	C(1I)-C(1H)-C(1G)	119.9(5)
C(1E)-C(1F)	1.419(8)	C(1M)-C(1H)-C(1G)	122.0(6)
C(1G)-C(1H)	1.515(9)	C(1J)-C(1I)-C(1H)	121.8(6)
C(1G)-C(1P)	1.523(8)	C(1K)-C(1J)-C(1I)	119.5(7)
C(1H)-C(1I)	1.394(8)	C(1J)-C(1K)-C(1L)	120.6(7)
C(1H)-C(1M)	1.400(7)	C(1K)-C(1L)-C(1M)	121.0(6)
C(1I)-C(1J)	1.38(1)	C(1L)-C(1M)-C(1H)	119.0(6)
C(1J)-C(1K)	1.37(1)	C(1L)-C(1M)-C(1N)	118.6(5)
C(1K)-C(1L)	1.36(1)	C(1H)-C(1M)-C(1N)	122.4(6)
C(1L)-C(1M)	1.41(1)	N-C(1N)-C(1M)	108.5(5)
C(1M)-C(1N)	1.517(9)	N-C(1N)-C(1O)	113.2(5)
C(1N)-N	1.490(8)	C(1M)-C(1N)-C(1O)	112.5(5)
C(1N)-C(1O)	1.52(1)	C(1P)-C(1O)-C(1N)	111.6(6)
C(1O)-C(1P)	1.517(7)	C(1O)-C(1P)-C(1G)	110.9(5)
C(1Q)-N	1.513(9)	C(1N)-N-C(1Q)	115.3(6)
C(2A)-O(2A)	1.360(6)	O(2A)-C(2A)-C(2B)	117.8(5)
C(2A)-C(2B)	1.377(8)	O(2A)-C(2A)-C(2F)	121.0(4)
C(2A)-C(2F)	1.394(8)	C(2B)-C(2A)-C(2F)	121.2(5)
C(2B)-C(2C)	1.386(9)	C(2C)-C(2B)-C(2A)	119.3(6)
C(2C)-C(2D)	1.35(1)	C(2D)-C(2C)-C(2B)	120.0(6)
C(2D)-C(2E)	1.377(9)	C(2C)-C(2D)-C(2E)	121.9(6)
C(2E)-C(2F)	1.407(7)	C(2D)-C(2E)-C(2F)	119.2(6)
C(2F)-C(2G)	1.430(7)	C(2A)-C(2F)-C(2E)	118.2(5)
C(2G)-C(2H)	1.333(8)	C(2A)-C(2F)-C(2G)	117.2(5)
C(2H)-C(2I)	1.465(9)	C(2E)-C(2F)-C(2G)	124.6(5)
C(2H)-C(2J)	1.533(7)	C(2H)-C(2G)-C(2F)	122.6(5)
C(2I)-O(2B)	1.190(7)	C(2G)-C(2H)-C(2I)	120.1(5)
C(2I)-O(2A)	1.398(6)	C(2G)-C(2H)-C(2J)	119.1(5)
C(2J)-O(2D)	1.218(8)	C(2I)-C(2H)-C(2J)	120.9(5)
C(2J)-O(2C)	1.225(8)	O(2B)-C(2I)-O(2A)	115.5(5)
		O(2B)-C(2I)-C(2H)	128.5(5)
C(1B)-C(1A)-C(1F)	118.9(6)	O(2A)-C(2I)-C(2H)	116.0(5)
C(1B)-C(1A)-C(1G)	122.3(6)	O(2D)-C(2J)-O(2C)	124.8(5)
C(1F)-C(1A)-C(1G)	118.8(5)	O(2D)-C(2J)-C(2H)	118.6(6)
C(1A)-C(1B)-C(1C)	120.0(6)	O(2C)-C(2J)-C(2H)	116.3(5)
C(1D)-C(1C)-C(1B)	119.4(5)	C(2A)-O(2A)-C(2I)	123.0(4)
C(1D)-C(1C)-Cl(2)	124.1(5)		
C(1B)-C(1C)-Cl(2)	116.5(5)		
C(1C)-C(1D)-C(1E)	122.0(6)		
C(1C)-C(1D)-Cl(1)	118.9(5)		

at 1640 cm^{-1}, 1467 cm^{-1}, 1337 cm^{-1} and 1136 cm^{-1}. As it was mentioned in the precedent section, the acidic carboxylic group (COOH) of CCA deprotonated upon formation of the new salt, and generated the carboxylate (COO^-) anion. From the FTIR spectra it can be seen that the carbonyl stretching bands were located at the same position for both CCA and SerH-CCA but the carboxylate symmetric and anti-symmetric stretching modes were red-shifted in the salt by *ca.* 30 cm^{-1}. As shown above, the crystal salt ~~is~~ was stabilized by strong intermolecular (Ser) $>NH_2^+ \cdots {}^-OOC$-(CCA) bonds that weaken the COO^- bond order with the concomitant decrease in the stretching frequencies with respect to the potassium salt. The H-bond formation was also observed in the FTIR spectra of both SerHCl and the crystal salt in the range 3200–2600 cm^{-1} as a broad and strong band (Fig. 2). For SerHCl the hydrogen bonding originated in the NH—Cl group produced a shift of the N-H stretching frequency to lower values [22], because the chloride anion removed electron density from the N-H bond. The same perturbation on the N-H bond has been produced by the carboxylate anion of coumarin 3-carboxylate due to the formation of the NH—OOC-(CCA) hydrogen-bond.

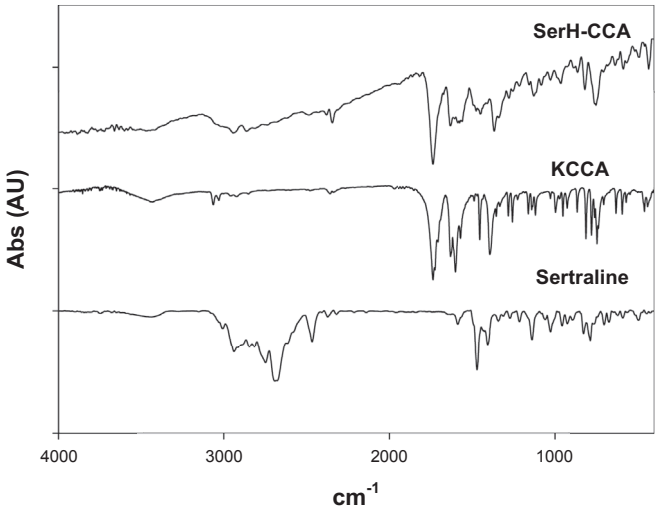

Fig. 2. FTIR spectra for sertraline hydrochloride (SerHCl), the potassium salt of coumarin 3-carboxylic acid (KCCA) and the ionic pair SerH-CCA.

3.3. Antimicrobial activity

The common use of antimicrobial agents in the therapy of infectious diseases has been an unprecedented event because the healing and control of infections allow the reduction of adult mortality. The activity of an antimicrobial agent against bacteria primarily depends on its ability to penetrate the bacterial cell wall, a necessary step for access to its site of action. Such capacity is related to their physico-chemical nature and the barrier in the membrane. For instance, the cell walls of Gram-positive and Gram-negative bacteria are not identical, being the former more permeable to antibiotics than the latter. Exploring antimicrobial activities will increase the use and application of the psychotropic drugs in clinical approaches. Considering that both sertraline and coumarins displayed antimicrobial activities, we tested if the new compound SerH-CCA was able to present and/or improve this behavior. The results are shown in Table 4.

The antibacterial activity results significant when the MIC values are of 100 μg mL^{-1} or less. When the MIC values are in the 100–500 μg mL^{-1} range a moderate activity is described and an inactive behavior is referred when MIC values results higher than 1000 μg mL^{-1} [24]. Despite coumarins displayed antimicrobial activities, coumarin 3-carboxylic acid (HCCA) did not show a deleterious behavior on the strains. This behavior has previously been reported but it has been determined that the antimicrobial activity of CCA resulted improved upon metal coordination especially with the silver cation, Ag(I) [25]. This result was the reason to hipotesise that the behavior of CCA could be improved by salt formation with sertraline, for which an antimicrobial behavior has been described [26,27]. Our results showed that the ionic pair SerH-CCA retained the antimicrobial activity of the antidepressant drug improving the CCA activity like in the silver-CCA coordination complex. This improvement resulted more effective with Gram (+) bacteria than Gram (−), the latter being generally more resistant because they bear a double membrane and provide greater resistance to drugs and antibiotics.

3.4. Interaction with BSA

In these studies it has been considered that fluorescence quenching refers to any process, which decreases the fluorescence intensity of a sample. A variety of molecular interactions

Table 3
Selected FTIR wavenumbers (cm^{-1}) of KCCA, SerHCl and SerH-CCA.

Tentative assignment	KCCA	SerHCl	Tentative assignment	SerH-CCA
ν C═O	1732 (vs)			1732 vs
carbonyl	1721 (sh)			1702 sh
	1704 (sh)			
		1640 *m*	NH_2 scissors	**1640 sh**
ν C═C + ν CCH	1626 (s)	1626 *m*	ν C═C (PhCl)	1628 *m*,
				1608 *m*,
ν_{as} COO^-	1599 (vs)			1558 *m*
ν C═C + δ CCH	1569 (m)	1583 *m*	ν C═C (Ph)	1585 *m*
	1616 (vs) 1569 (s)	1515 w	(PhCl),CH_2 scissors	1573 *m*
			δ_{as} CH_3	
δ CH cycle	1451 *m*	**1467 vs**	ωCH_2, δ_s CH_3	**1473 *m***
		1433 s	ωNH_2, δ CH cycle	1448 *m*
ν_s COO^-	1390 (s)			1365 s
		1337 *m*	ρ CH_3, τ NH_2, δ CH cycle	**1337 *m***
			τ CH_2	
δ CCH + ν(C-O_{lac})	1158 *m*	**1136 s**	δCCH ip (PhCl, Ph), δCCH cycle,	1156 w
δ CCH	1137 *m*		τ CH_2, τNH_2	**1136 sh**
	1116 *m*			1125 *m*
				1115 sh

Bold: modes involving the NH_2 vibrations; s, strong; m, medium; w, weak; ν, stretching; δ, bending; ρ, rocking; τ, torsion.

Table 4
Minimum inhibitory concentrations (MICs) of SerHCl, HCCA and SerH-CCA for bacterial and fungal strains. MIC values in μg mL^{-1}.

	SerHCl	HCCA	SerH-CCA
E. coli ATCC 35218	24	>1500	94
P. aeruginosa ATCC 27853	750	1500	750
E. faecalis ATCC 29212	24	1500	48
S. aureus ATCC 25923	24	1500	48
S. epidermidis ATCC 1263	24	1500	48
C. albicans	6	>1500	24
C. albicans ATCC 10231	12	>1500	24
C. parapsilosis	12	>1500	24
C. parapsilosis ATCC 22019	6	>1500	24
C. krusei	3	>1500	12
C. glabrata	3	>1500	12
C. tropicalis	6	>1500	24

can result in quenching, including excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation and collisional quenching [28]. The studies concerning the interaction and potential transport with bovine serum albumin were made with coumarin carboxylic acid and the salt SerH-CCA measuring the florescence intensity of the BSA before and after addition of these compounds. If the intrinsic fluorescence of BSA resulted quenched in the presence of the compounds, it was an indication that some type of interactions was produced between them. When a 6 μM BSA solution has been excited at 280 nm, the fluorescence peak occurred at 348 nm (see Fig. 3A), and the intensity varied when binding to the different compounds. From Fig 3A the fluorescence quenching upon the addition of different concentrations of the studied compounds can be observed. The type of interaction between the compounds and the albumin can be inferred from this Figure. The emissions of the fluorescence spectrum of BSA lead to a blue-shift if the indole group of tryptophan was buried in the protein (more hydrophobic environment). If the emission was red-shifted, there was an unfolding of the protein and a less hydrophobic environment was expected. In this experiment, the compounds caused a progressive reduction of the fluorescence intensity producing a bathochromic shift (red shift) in the maximum emission wavelength of BSA. This modification has been attributed to an increase in the polarity of the protein environment. There was a net change in the polarity around the tryptophan residue and an alteration in peptide strand of BSA molecules in which an electrostatic type of interaction could be suggested [29].

These data have been compared with the effects previously observed for sertraline hydrochloride. Clinical pharmacokinetics of sertraline is well established. It is known that is highly bound, approximately 98% to plasma proteins and that it is slowly absorbed, reaching after at 4–8 h at the maximum concentrations in plasma [30]. Considering the previous data, the experiments were carried out at 37 °C (310 K) and in an average time of 8 h to ensure the formation of the complexes with albumin. The determinations were also performed after 2 h for the sake of comparison. A concentration range of 2–100 μM was used because at higher concentrations other effects (intrinsec fluorescence of the ligands) that are outside the scope of the discussion are produced.

The Stern Volmer Equation (1) has been used for the analysis of the interaction and the determination of the Stern Volmer constant (K_{sv}) and the apparent binding constant (K_b) and number of binding sites (n) were calculated with Equation (2). F_0 and F represent the fluorescence intensities before and after the addition of the quencher.

$$F_0/F = 1 + K_{sv}[Q] \quad (1)$$

$$\log[(F_0 - F)/F] = \log K_b + n \log[Q] \quad (2)$$

Fig. 3 B and C show the plots performed on the basis of the quenching of fluorescence of albumin for HCCA and SerH-CCA in the measured concentration range. In a given concentration range, the F_0/F versus [Q] curve (Stern-Volmer curve) should be linear if the quenching type is either static or dynamic. If the quenching type is a combined one (both static and dynamic), the Stern-Volmer plot presents an upward curvature [31]. A good linearity in the F_0/F vs [Q] plots can be observed from Fig. 3. B.

Taking into account the well known relationship between the quenching rate constant of the biomolecule K_q and the dynamic quenching constant K_{sv} ($K_q = K_{sv}/\tau_0$, where τ_0 is the average lifetime of the biomolecule without quencher), and considering that the fluorescence lifetime of the biopolymer is 10^{-8} s, K_q can be calculated using this equation. The calculated K_q values have resulted 3 orders of magnitude greater than the maximum diffusion collision quenching rate constant (2.0×10^{10} L mol^{-1} s^{-1}). In general, the constants K_{sv} from static quenching are higher than

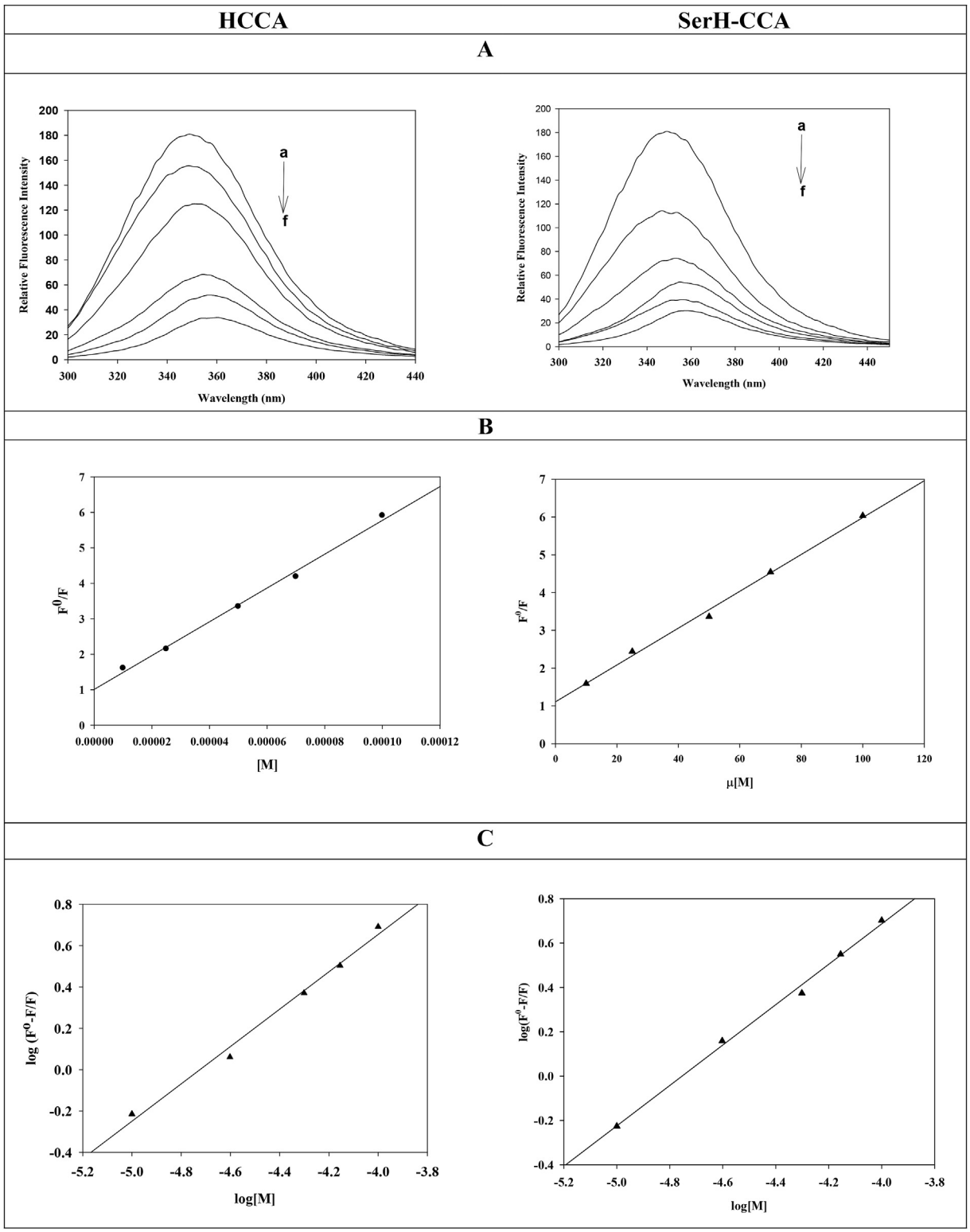

Fig. 3. A. Fluorescence spectra, BSA concentration 6 μM and concentrations of the different compounds: (a) 0, (b) 10 μM (c) 25 μM, (d) 50 μM, (e) 70 μM and (f) 100 μM. **B**. Stern Volmer plots. **C**. log [(F_0-F)/F]. vs log [Q] (T = 310 K, pH 7.4, k_{exc} = 280 nm, k_{em} = 348 nm, incubation time = 2 h). HCCA-BSA (left column) and SerH-CCA (right column).

those from collisional quenching. A higher value is considered as static quenching with complex formation and a lower value is an indication of a collisional quenching [32]. The calculated values are depicted in Table 5. From these data it can be inferred that the fluorescence quenching processes on BSA by these compounds may be mainly governed by a static quenching mechanism. This implies that the quenching has been originated from the formation of a compound with BSA.

Table 5
Stern Volmer quenching K_{SV}, bimolecular quenching K_q and apparent binding K_b constants and number of binding sites for the systems SerHCl-BSA, HCCA-BSA and SerH-CCA-BSA at 310 K, pH = 7.4.

System	K_{sv} (M^{-1}) ± DS	K_q (M^{-1} s^{-1})	K_b (M^{-1}) ± SD	n ± SD	R^2
Incubation time 2 h					
HCCA-BSA	$4.76 \times 10^4 \pm 0.05$	$4.76 \times 10^{12} \pm 0.05$	$1.86 \times 10^4 \pm 0.19$	0.90 ± 0.01	0.996
SerHCCA- BSA	$4.79 \times 10^4 \pm 0.04$	$4.79 \times 10^{12} \pm 0.04$	$2.29 \times 10^4 \pm 0.20$	0.91 ± 0.05	0.997
SerHCl- BSA	$2.37 \times 10^3 \pm 0.03$	$2.37 \times 10^{11} \pm 0.03$	40.7 ± 1.10	0.45 ± 0.01	0.995
Incubation time 8 h					
HCCA- BSA	$4.53 \times 10^4 \pm 0.10$	$4.53 \times 10^{12} \pm 0.10$	$3.80 \times 10^7 \pm 0.21$	1.73 ± 0.03	0.999
SerHCCA- BSA	$4.05 \times 10^4 \pm 0.08$	$4.05 \times 10^{12} \pm 0.08$	$1.09 \times 10^4 \pm 0.17$	0.85 ± 0.05	0.998
SerHCl- BSA	$2.03 \times 10^3 \pm 0.02$	$2.03 \times 10^{11} \pm 0.02$	$4.27 \times 10^3 \pm 0.07$	1.08 ± 0.03	0.992

SD: Standard deviation; R: correlation coefficient.

The values of the association constants of these generally reversible processes are in the range 10^4-10^6 M^{-1}. The calculated values of K_b (Table 5) indicated that HCCA and SerH-CCA could be transported in a reversible manner by BSA [33].

The binding constant for sertraline was in the same order of magnitude than the recently reported value obtained using human serum albumin (HSA) [7]. It could be noted that K_b increased for an incubation time of 8 h assuming the presence of at least one binding site with the protein during the time of interaction. This fact is in accordance with *in vivo* studies of sertraline relative to the assimilation time [8]. In addition, these K_{sv} values have resulted in the same order of magnitude than those for other antidepressant drugs [34–36]. Moreover, it can be seen that a higher incubation time improved the interaction of the system HCCA-BSA being the binding constant higher (see Table 5). There were no significant differences in the behavior of the system SerHCCA-BSA upon increasing of the incubation time. The same trend has been observed for the number of binding sites. At an incubation time of 2 h, the binding of SerH to BSA is weak, and the calculated number of binding sites was lower than one. For SerHCCA the number of binding sites to BSA is near one and the binding constant remained at the same value at 2 and 8 h incubation time. In conclusion, the replacement of the chloride anion of sertraline by the higher anion coumarin 3-carboxylate, substantially improved the capacity of sertraline to bind albumin particularly at lower incubation times. The investigation of the binding interaction between drugs and serum albumin is very important in pharmacology because drug interactions with the protein are responsible of their biological distribution. In this report it was determined that sertraline hydrochloride quenched the intrinsic fluorescence of BSA (like for HSA [7]), binding to the protein with high efficiency at 8 h of incubation time. Furthermore, it can be remarked that the replacement of the anion in sertraline hydrochloride by carboxylic acids not only can improve the solubilities and stabilities of the drug [3,37], but it also can generate a better transport and bioavailability of the drug in the biological systems through electrostatic interactions.

3.5. Antidepressant activity

Forced swimming test: The forced swimming test is one of the most widely used tests of antidepressant actions and is used to infer “depression-like” behavior. According to the reported data, sertraline hydrochloride reduced immobility and increased swimming behavior hence indicating an antidepressant effect. Administration of NaCCA had no significant effect on FST (Fig. 4). However, the treatment of SerH-CCA decreased immobility and increased swimming behavior in a magnitude that exceeded SerH individual effects.

Open field test (OFT): To rule out that the antidepressant effects observed in the FST were non-specific, the OFT test has been used. The effects of different treatments on spontaneous locomotor activity in rats are shown in Fig. 5. None of these treatments affected activity levels, according to the total number of squares crossed by the animal during the first 5 min of the test. These results confirmed the specificity of the FST results because they were not attributable to the locomotor activity.

In the present study, it can be demonstrated that the antioxidant substance coumarin-3-carboxylate sodium salt resulted inactive in the forced swimming test, however there was an increase in the

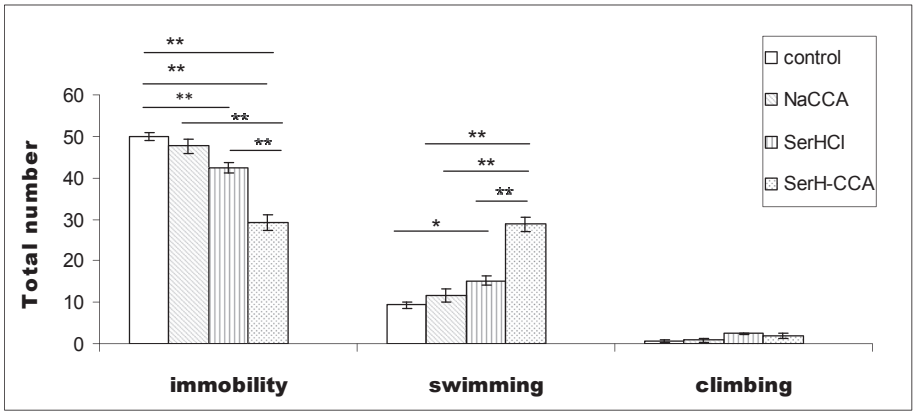

Fig. 4. Comparison of the behavioral effects produced in the forced swimming test FST for the control (saline solution) and for sodium coumarin 3-carboxylate (NaCCA), sertraline hydrochloride (SerHCl) and the ion pair sertraline coumarin 3-carboxylate (SerH-CCA). Values represent mean (±S.E.M.) counts of immobility, swimming and climbing behaviors when sampled every 5 s during the 5 min test period. $^*P < 0.05$, $^{**}P < 0.01$, n = 6 rats per group. Data were analyzed by two-way ANOVA followed by Tukey's test for multiple comparisons.

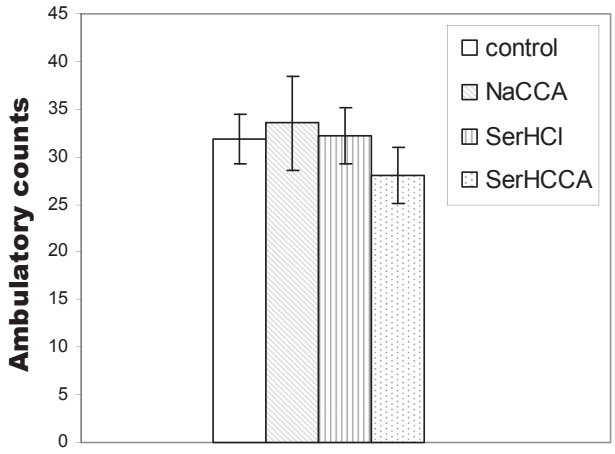

Fig. 5. Comparison of the behavioral effects produced in the open field test (OFT) for the control (saline solution) and for sodium coumarin 3-carboxylate (NaCCA), sertraline hydrochloride (SerHCl) and the ion pair sertraline coumarin 3-carboxylate (SerH-CCA). Values represent mean (±S.E.M.) counts of immobility, swimming and climbing behaviors when sampled every 5 s during the 5 min test period. $^{*}P < 0.05$, $^{**}P < 0.01$, n = 6 rats per group. Data were analyzed by two-way ANOVA followed by Tukey's test for multiple comparisons.

antidepressant activity of sertraline when given in combination to CCA in the ion pair form. The behavioral outcome of the antidepressant action by the new salt has then been demonstrated by a decrease in immobility time and increased swimming behavior in the forced swimming test in the tested groups. These results can suggest that the antidepressant like effect of CCA is detected only with simultaneous administration of monoamine reuptake inhibitors. Besides, the marked synergistic effect of the enhancement of the activity of sertraline hydrochloride resulted in a high probability that the new salt can be evaluated as a new antidepressant drug.

4. Conclusion

Various strategies have been widely investigated to enhance the bioavailability and transport of pharmaceutical compounds. One of them is to synthezise crystalline solid formulations with different physicochemical properties such as salt formation with different ions that increase the solubilities. To further increase the bioactivity of the pharmaceutical sertraline, its salt with a biologically active compound, namely coumarin 3-carboxylate, was prepared. X-ray diffraction methods and FTIR spectroscopy allowed the determination of the structure of the ion pair SerH-CCA and of the presence of strong H-bonds between the amino (SerH cation) and the carboxylate (CCA anion) that stabilized the lattice. The behavioral effects produced in the forced swimming test on male Wistar rats confirmed that the new ion pair possesses antidepressant properties not inferior to those of sertraline, and it is reasonable to conclude that this new class of drug strengthens the action of the native drug. Although the antimicrobial effect is similar to that of sertraline, this new ion pair has a higher binding constant to BSA and could therefore change the transport, disposition and pharmacological effects of the commercial drug.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cbi.2016.03.010.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.cbi.2016.03.010

Supplementary Information Available

Tables of fractional coordinates and equivalent isotropic displacement parameters of the non-H atoms (Table S3), atomic anisotropic displacement parameters (Table S4), hydrogen atoms positions (Table S5), and H-bond distances and angles (Table S6). X-ray crystallographic information files (CIF) is available for the salt. CIF files are also available from the Cambridge Crystallographic Data Center (CCDC), deposition number 991981.

References

[1] D. Chancellor, Nat. Rev. Drug Discov 10 (2011) 809–810.
[2] E.M. Tanya Am, W.J. Curatolo, H.L. Friedman, R.M. Chanker, S.M. Herbig, D.T. Friesen, J.B. West, Sertraline salts and sustained-release dosage forms of sertraline. Patent: WO 1999001121 A1.
[3] I. Kostova, Curr. Med. Chem. Anti-Cancer Agents 5 (2005) 29–46.
[4] I.K. Georgieva, T. Mihaylov, N. Trendafilova, J. Inorg. Biochem. 135 (2014) 100–112.
[5] K.V. Sashidhara, R.K. Modukuri, S. Singh, K.B. Rao, G.A. Teja, S. Gupta, S. Shukla, Bioorg. Med. Chem. Lett. 25 (2014) 337–341.
[6] M. Ayaz, F. Subhan, J. Ahmed, A. Khan, F. Ullah, I. Ullah, G. Ali, N. Syed, S. Hussain, J. Biol. Res. 22 (2015) 1–8.
[7] M. Shahlaei, B. Rahimi, A. Nowroozi, M.R. Ashrafi-Kooshk, K. Sadrjavadi, R. Khodarahmi, Chem. Biol. Int. 242 (2015) 235–246.
[8] R.A. Ronfeld, L.M. Tremaine, K.D. Wilner, Clin. Pharmacokin 32 (1997) 22–30.
[9] CrysAlisPro, Oxford Diffraction Ltd., version 1.171.33.48 (release 15-09-2009 CrysAlis171.NET).
[10] G.M. Sheldrick, Acta Crystallogr. A. 46 (1990) 467–473.
[11] S. Bilge, A. Bozkurt, D.B. Bas, E. Aksoz, E. Savli, F. Ilkaya, Y. Kesim, Pharmacol. Rep. 60 (2008) 872–879.
[12] R.D. Porsolt, G. Anton, N. Blavet, M. Jalfre, Eur. J. Pharmacol. 47 (1978) 379–391.
[13] P. Willner, Psychopharmacol. (Berl.) 83 (1984) 1–16.
[14] J.P. Rénéric, M. Bouvard, L. Stinus, Behav. Brain Res. 136 (2002) 521–532.
[15] C.K. Johnson, ORTEP-II. A Fortran Thermal-Ellipsoid Plot Program, Report ORNL-5318, Oak Ridge National Laboratory, Tennessee, USA, 1976.
[16] K. Ravikumar, B. Sridhar, M.N. Bhanu, Acta Crystallogr. Sect. E Struct. Rep. Online 62 (2006) o565–o567.
[17] F. Caruso, A. Besmer, M. Rossi, Acta Crystallogr. Sect. C Cryst Struct Commun C 55 (1999) 1712–1714.
[18] O. Almarsson, M.B. Hickey, M.L. Peterson, S.L. Morissette, S. Soukasene, C. McNulty, M. Tawa, J.M. MacPhee, J.F. Remenar, Cryst. Growth Des. 3 (2003) 927–933.
[19] Q. He, S. Rohani, J. Zhu, H. Gomaa, Cryst Growth Des. 10 (2010) 1633–1645.
[20] A.D. Yan, G.O. Delori, B. Lloyd, T. Patel, G. Friic, M. Day, D.K. Buar, W. Jones, J. Lu, M. Wei, D.G. Evans, X. Duan, Cryst. Eng. Comm. 14 (2012) 5121–5123.
[21] J.A.K. Howard, M.F. Mahon, P.R. Raithby, H.A. Sparkes, Acta Crystallogr. Sect.C: Struct Sci 65 (2009) 230–237.
[22] G.E. Escudero, V. Ferraresi Curotto, C.H. Laino, R. Pis Diez, P.A.M. Williams, E.G. Ferrer, Spectrochim. Acta Part A 104 (2013) 271–279.
[23] B.S. Creaven, M. Devereux, I. Georgieva, D. Karcza, M. McCann, N. Trendafilova, M. Walsh, Spectrochim Acta Part A 84 (2011) 275–285.
[24] A.A. Aliero, A.D. Ibrahim, in: Y. Kumar (Ed.), Salmonella: A Diversified Superbug, In Tech, Open Access Company, Croatia, Europe, 2012, pp. 1–26. Chap. 4.
[25] B.S. Creaven, D.A. Egan, K. Kavanagh, M. Andy Noble, B. Thati, M. Walsh, Inorg. Chim. Acta 359 (2006) 3976–3984.
[26] J.L. Muñoz-Bellido, S. Muñoz-Criado, J.A. García-Rodríguez, Int. J. Antimicrob. Agents 14 (2000) 177–180.
[27] C. Lass-Flörl, M. Ledochowski, D. Fuchs, C. Speth, L. Kacani, M.P. Dierich, A. Fuchs, R. Würzner, FEMS Immunol, Med. Microbiol. 35 (2003) 11–15.
[28] A. Varlan, M. Hillebrand, Molecules 15 (2010) 3905–3919.
[29] S. Cao, X. Jiang, J. Chen, J. Inorg. Biochem 104 (2010) 146–152.

[30] C.L. DeVane, H.L. Liston, J.S. Markowitz, Clin. Pharmacokinet 41 (2002) 1247–1266.
[31] H. Zhang, Y. Wang, Z. Fei, L. Wu, Q. Zhou, Dyes Pigment 78 (2008) 239–247.
[32] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Second ed., Plenum Press, New York (, 1999.
[33] N. Barbero, E. Barni, C. Barolo, P. Quagliotto, G. Viscardi, L. Napione, S. Pavan, F. Bussolino, Dyes Pigments 80 (2009) 307–313.
[34] U. Katrahalli, S. Jaldappagari, S.S. Kalanur, J. Lumin. 130 (2010) 211–216.
[35] J. Lin, Y. Liu, M. Chen, H. Huang, L. Song, J. Lumin. 146 (2014) 114–122.
[36] X. Liu, Y. Du, Eur. J. Med. Chem. 45 (2010) 4043–4049.
[37] Q. He, S. Rohani, J. Zhu, H. Goma, Chirality 24 (2012) 119–128.