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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