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ARTICLE

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Preparation of antidepressants for use in preclinical research

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Abstract

Obtaining drugs for use in basic and preclinical research has become increasingly difficult and in many instances is dependent upon the company's interest in the proposed research. In this paper, we describe a simple procedure for extracting the antidepressants sertraline, paroxetine, fluoxetine, venlafaxine, citalopram, escitalopram, duloxetine and atomoxetine from their readily available pharmaceutical preparations. With the exception of citalopram, escitalopram and duloxetine in which the free base was the final product, the hydrochloride salt forms of these drugs were prepared. This procedure provides these antidepressants in gram quantities in recovered yields ranging from 53% to 100% at over 99% purity and is in principle applicable to other drugs as well.

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Introduction

The availability of antidepressant drugs in gram or multi-gram quantities is necessary for basic investigations of their pharmacology, particularly in studies requiring large numbers of animals with long-term drug administration. Of the modern, non-tricyclic antidepressants that are monoamine transporter antagonists, only fluoxetine HCl is available for purchase; however, its current (May 2006) cost is $239 per 50 mg (www.SigmaRBI.com). No other selective serotonin reuptake inhibitor (SSRI) or serotonin/norepinephrine reuptake inhibitor (SNRI) are available except directly from the parent pharmaceutical companies. Upon written request, these compounds may or may not be provided directly to the investigator for use in discrete, approved animal experiments following review by the company. In contrast, the commercial pharmaceutical formulations of these same drugs are widely available, used routinely for clinical indications and are substantially less expensive on a dollar per gram basis. In an era of growing concern over the potential conflicts of interest between individual investigators and the pharmaceutical industry, lower cost alternatives and greater availability of these research tools are needed. Because of the large price differential and the refusal of requests to provide drugs for certain experiments, we became interested in using the commercial formulations of a number of antidepressants as a source for use in our animal research projects.

The primary obstacle to using the pharmaceutical formulations of these drugs is the large amount of vehicle and inert material which interfere with precise dosing and prevent dissolving the drugs in aqueous or other suitable media for injection or osmotic minipump administration. To obtain medications in a suitable form for our research programme, we developed an organic extraction method for obtaining paroxetine, sertraline, venlafaxine, fluoxetine, citalopram, escitalopram, duloxetine and atomoxetine from their commercially available drug formulations. This paper describes a simple method for extracting and reformulating these antidepressants as well as demonstrating their analytical purity.

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Materials and methods

All of the equipment, solvents and chemicals used for the drug extractions are available from commercial sources. Glassware as well as solvents for extraction and chromatography were obtained from VWR (West Chester, PA, USA). Chemicals were obtained from Aldrich Chemicals (Milwaukee, WI, USA). Thin layer chromatography (TLC) was performed using 250 μm layers of F-254 silica on aluminium plates obtained from Whatman (Clifton, NJ, USA). ^{1}H NMR spectra were recorded using a Varian spectrometer at 400 MHz. Elemental analyses were performed by Atlanta Microlabs Inc. (Norcross, GA, USA). All of the drugs used in the extraction were in their commercially available pharmaceutical formulations (branded and generic).

General procedure

The extractions and purifications should be performed in a functioning fume hood designed for handling volatile organic solvents. These procedures should be carried out only by those familiar with the safe handling of concentrated acids, bases and volatile organic solvents. In particular, diethyl ether is highly flammable and can build up pressure during extraction in separatory funnels. The techniques summarized here are covered in detail in many practical guides to organic chemistry (Vogel et al., 1996).

The pharmaceutical formulation of the drug is prepared for extraction by removing any readily separable packaging such as capsules. For some drugs, crushing the solid form such as tablets into a powder form using a mortar and pestle can decrease the time needed for drug extraction. This step may be particularly important if sustained release formulations are used. All of the antidepressants reported here are weak bases. To convert the salt of the drug in the pharmaceutical preparation into its free base form, the drug is added to aqueous sodium hydroxide (6 mM equivalents of sodium hydroxide per mmol of drug in double-deionized water) using ~75 ml of NaOH solution per gram of drug in an Erlenmeyer flask. An equal volume of diethyl ether is added to the flask, and the two phases are stirred vigorously until the solids are dissolved or pulverized into a fine powder. This stirring typically requires ~15–20 min, and the aqueous phase was pH 11 or higher at this step.

The stirring is then stopped, and the two phases are allowed to stand and separate. The organic phase is then decanted into a separatory funnel (500 ml or 1000 ml), and any accompanying aqueous phase is drained back into the Erlenmeyer flask. The aqueous phase is extracted with successive portions of organic solvent until there is minimal residual drug in the aqueous phase. During initial extractions, TLC analysis with UV detection of successive extract was performed to demonstrate adequate extraction of the drug into the organic phase. A total of 2–3 organic extractions removed the vast majority of the drugs prepared using this method.

The organic extracts containing the drug are combined in the separatory funnel and washed twice with equal volumes of a saturated aqueous sodium chloride solution to remove any residual water-soluble material. The organic phase is then placed in a clean Erlenmeyer flask, and the residual water is removed with anhydrous magnesium sulphate. This step is performed by adding portions (typically 2–3 g) of magnesium sulphate to the diethyl ether solution with stirring until the magnesium sulphate powder stops clumping. The magnesium sulphate is then removed through vacuum filtration using No. 2 Whatman filter paper (Florham Park, NJ, USA). The organic solvent is removed by rotary evaporation under reduced pressure to provide the free base, typically as an oil. Residual solvent is removed under a vacuum pump; gentle heating (~40–50 °C) may assist in removal of trace solvent. All drugs were stored in the dark. The purity of the crude extract was evaluated by proton NMR. For all the drugs described here, the proton NMR spectra revealed that the free bases at this point were suitable for conversion to their salt form or for direct use in animal studies.

For all of the drugs except citalopram, escitalopram and duloxetine, the free bases were readily converted into a solid form as their hydrochloride (HCl) salts. To prepare the HCl salt, the free base is dissolved in the minimum amount of ethanol needed to obtain a homogenous solution of the free base. If small amounts of particulate matter are present, the ethanol solution can be filtered through a small cotton plug in a Pasteur pipette pre-rinsed with ethanol. To the ethanolic solution of the free base on ice, 2 M equivalents of concentrated aqueous hydrochloric acid are added dropwise with stirring. Depending upon the drug, a crystalline solid or gel may form at this point. For some drugs (see details below), the crystalline solid can be isolated through vacuum filtration. In other cases, the solution can be concentrated with rotary evaporation under reduced pressure to provide the HCl salt of the drug in a solid form. Residual solvent can be removed with a vacuum pump.

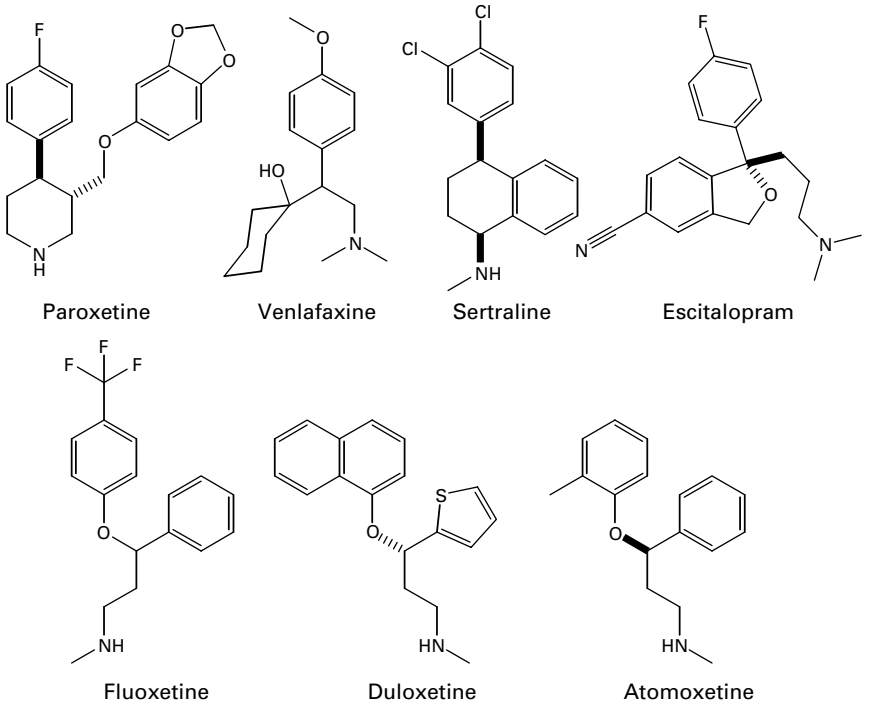

Figure 1. Antidepressants obtained from the extraction procedures.

The identity and purity of the final drug product was confirmed by proton NMR and elemental analysis. NMR was performed using deuterated chloroform for free bases or deuterated water or methanol for the salt form of the drugs. Drugs were considered pure and suitable for use in experiments if the proton NMR spectra corresponded to the spectra previously reported in the literature (Basappa et al., 2004; Kamal et al., 2002; Salsbury and Isbester, 2005) and the elemental analyses were within 0.4% of the theoretical values. Spectra for citalopram and duloxetine were not found in the scientific literature (United States and European patents only) but our proton NMR spectra matched the predicted spectrum for these compounds and are available upon request. The structures of the drugs isolated using this procedure are depicted in Figure 1.

Procedural details for specific drugs

Sertraline

Sertraline (Zoloft®, 25 or 50 mg tablets) preparation can be performed using the general procedure with formation of the HCl salt. The ^{1}H NMR ($CDCl_3$) can be found in Salsbury and Isbester (2005). Elemental analysis for sertraline HCl ($C_{17}H_{18}Cl_3N$): Calculated C: 59.58, H: 5.29, N: 4.09; Found C: 59.58, H: 5.26, N: 4.08.

Paroxetine

Paroxetine (Paxil® 20 mg or Paxil CR® 25 mg tablets) preparation is performed using the general procedure with formation of the HCl salt. The coloured film coat of the tablets was scraped off with a razor to avoid the need for flash column chromatography for purification of the free base extract. The HCl salt formation led to paroxetine HCl hemihydrate (2:1 molar ratio of paroxetine HCl to water). The ^{1}H NMR ($CDCl_3$) can be found in Salsbury and Isbester (2005). Elemental analysis of paroxetine HCl hemihydrate ($C_{38}H_{44}Cl_2F_2N_2O_7$): Calculated C: 60.88, H: 5.92, N: 3.74; Found C: 60.91, H: 5.98, N: 3.77.

Fluoxetine

Fluoxetine (generic fluoxetine 20 mg capsules) preparation is performed by emptying the drug from the capsules. To avoid formation of a viscous suspension during extraction, larger volumes of aqueous and diethyl ether phases were used than in the routine procedure (e.g. 2 g fluoxetine per 350 ml of aqueous sodium hydroxide and 350 ml of diethyl ether). The ^{1}H NMR ($CDCl_3$) can be found in Kamal et al. (2002). Elemental analysis for fluoxetine HCl ($C_{17}H_{19}ClF_3NO$): Calculated C: 59.05, H: 5.54, N: 4.05; Found C: 59.02, H: 5.62, N: 4.02.

Venlafaxine

Venlafaxine (Effexor XR® 75 mg capsules) was extracted using the general procedure with the exception that the saturated NaCl solution was not added to the original ether extract as this led to more difficult separation of the aqueous and organic fractions. Formation of the HCl salt with immediate isolation of the crystalline HCl salt via vacuum filtration leads to a more water soluble HCl salt form albeit at a reduced overall yield compared with letting the crystallization process proceed for several hours in which a polymorphic salt formed that was less soluble in aqueous solution. Both salt forms were soluble in polyethylene glycol 400:saline vehicles. The ^{1}H NMR ($CDCl_3$) can be found in Basappa et al. (2004). Elemental analysis of venlafaxine HCl ($C_{17}H_{28}ClNO_2$): Calculated C: 65.05, H: 8.99, N: 4.46; Found C: 65.00, H: 8.98, N: 4.44.

Escitalopram and citalopram

Escitalopram (Lexapro® 10 mg tablets) and citalopram (Celexa® 20 mg tablets) were extracted using the general procedure. Free base preparation lead to trace amounts of residual diethyl ether detectable by ^{1}H NMR. However, the percent contamination is estimated at <1% by elemental analysis and proton NMR. Elemental analysis for escitalopram free base ($C_{20}H_{21}FN_2O$): Calculated C: 74.05, H: 6.53, N: 8.64; Found C: 74.14, H: 6.73, N: 8.57. We were unable to obtain purified HCl salts using the simple procedure outlined above and used the free base preparations in our preclinical studies.

Duloxetine

Duloxetine (Cymbalta® 30 mg capsules) preparation is performed using the general procedure. The starting material is emptied from the capsules and ground before extraction. Duloxetine undergoes some decomposition under acidic conditions (Wheeler and Kuo, 1995) and this was noted during the attempted formation of the HCl salt. Duloxetine should be stored in the dark. The free base was pure by ^{1}H NMR ($CDCl_3$).

Atomoxetine

Atomoxetine (Strattera® 40 mg capsules) preparation is performed using the general procedure with formation of the HCl salt. The starting material is emptied from the capsules prior to extraction. The ^{1}H NMR ($CDCl_3$) can be found in Kamal et al. (2002). Elemental analysis of atomoxetine HCl ($C_{17}H_{22}ClNO$): Calculated C: 69.97, H: 7.60, N: 4.80; Found C: 69.64, H: 7.69, N: 4.85.

Results and discussion

The methods described here provide a means for obtaining analytically pure antidepressant drugs on a gram scale from inexpensive, commercially available pharmaceutical preparations. Combustion elemental analysis and proton NMR demonstrated that the final products were at least 99% pure. With the exception of citalopram, escitalopram and duloxetine, the HCl salt of the antidepressants was prepared. The drugs prepared using this method were suitable for use in many types of preclinical experiments. This method could be adapted to the extraction of other drugs not described here.

The initial step in the procedure is conversion of the pharmaceutical preparation (usually a salt) of the drug into the free base (deprotonated, non-ionized form) using sodium hydroxide. The organic extractions performed for each drug take advantage of the fact that the only significant component of pharmaceutical preparations of these drugs that are organic soluble under basic conditions are the drugs themselves. The inert vehicle and other components in the pharmaceutical formulations remain in the aqueous phase or are insoluble. The organic extracts containing the desired drug are washed with saturated aqueous sodium chloride to remove residual water-soluble contaminants. The small amount of residual water in the diethyl ether organic phase is removed with magnesium sulphate, and the organic solvent is then removed to provide the free base of the drug. For the drugs described here, the free bases were analytically pure at this point. It is possible that other drugs not described here could contain undesired organic-soluble material from their pharmaceutical formulations. These free bases could be purified using flash column chromatography which is routinely used in synthetic organic chemistry and is described in most laboratory organic chemistry texts.

In principle, the free bases can be used for experiments, but they are usually thick oils that are not soluble in water without acid or a buffer and are less easily handled than their solid HCl salt forms. The HCl salts were prepared in part because hydrochloric acid is a gas at room temperature allowing easy removal of the excess hydrochloric acid used in salt formation. Other salts (e.g. oxalate, hydrobromide, acetate) could be prepared from the free bases if desired.

The identity and purity of the products of these extractions were determined by proton NMR and combustion analysis. Proton NMR is a standard method for characterizing organic compounds, and each drug has its own unique spectrum. The NMR spectra of the extraction products were compared with the reported spectra in the literature to demonstrate identity. Proton NMR is also sensitive for detecting impurities that contain protons. Combustion elemental analysis is routinely used to determine the percentage of the molecular weight of a compound contributed by carbon (C), hydrogen (H) and nitrogen (N). The theoretical values for C, H and N are compared with the experimentally determined values, and the difference between the two values should be less than $\pm 0.4\%$. When this condition is met and the proton NMR spectrum demonstrates signals arising only from the drug, the product is considered pure. Correct elemental analysis is important for accurate drug dosing as it ensures that 1 g of product from extraction is 1 g of the drug and is not contaminated by other material. For example, venlafaxine HCl has a molecular formula of $C_{17}H_{28}ClNO_2$, and the theoretical percent of the mass of venlafaxine HCl due to carbon is 65.05. With an experimental value of 65.00, the estimated purity of the venlafaxine HCl is 99.92% (calculated from $65.00/65.05 \times 100\%$).

In the case of citalopram and escitalopram, the estimated purity based on proton NMR and elemental analysis is over 99% despite trace diethyl ether. It is extremely unlikely that the residual solvent contamination of escitalopram or citalopram has any pharmacological actions when used systemically although these compounds may not be appropriate for certain in-vitro studies such as determination of transporter- or receptor-binding affinites. Because diethyl ether and ethanol have protons, they can be readily detected by proton NMR. In the case of paroxetine, the HCl salt forms with a 2:1 ratio of molecules of paroxetine HCl to molecules of water, and this hemihydrate must be taken into account when calculating the theoretical percentages of C, H and N.

The simple organic extraction procedure described here provides an economical method to obtain antidepressant drugs suitable for use in preclinical research studies from their pharmaceutical preparations. The antidepressants sertraline, paroxetine, fluoxetine, venlafaxine, citalopram, escitalopram, duloxetine and atomoxetine were obtained in moderate to excellent yield (53–100%) in over 99% purity by elemental analysis and proton NMR. This method is expected to be readily applicable to obtaining a variety of other drugs from their pharmaceutical formulations.

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Statement of Interest

Zachary N. Stowe, M.D. is on the Speaker's Bureau of Eli Lilly, GSK, Pfizer and Wyeth. He is on the Advisory Board of Bristol–Myers Squibb and GSK. He provides Faculty/Speaker Training for GSK and Wyeth and has received research grants from Pfizer, GSK and Wyeth. Dr Owens has received research grants from Pfizer, GSK, Merck, Lundbeck, Cyberonics and Johnson & Johnson; he has consulted for Bristol–Myers Squibb, Sanofi-Aventis, Pfizer, Lundbeck, Sepracor, Forest Laboratories and Johnson & Johnson; and received speaker's honoraria from GSK.

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