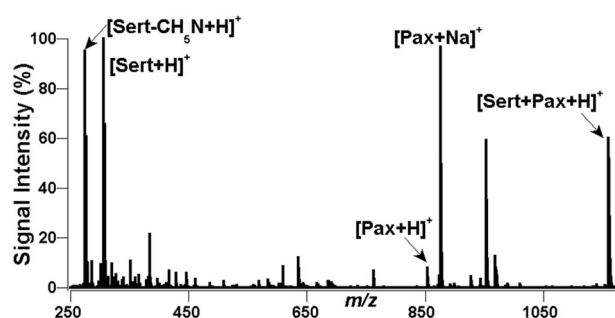


RESEARCH ARTICLE

An In Vitro Study of Aromatic Stacking of Drug Molecules

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Abstract. In this paper, drug–drug chemical interactions between two different aromatic compounds were studied by mass spectrometry. Specifically, we examined non-covalent complexes (NCX) between paclitaxel, a chemotherapeutic compound, and medications widely used in palliative care for depression, psychosis, and anxiety. It is unknown whether psychotropic medications directly interact with paclitaxel. Here, we use a simple and rapid electrospray ionization mass spec-

trometry in vitro assay, which has been predictive in the case of neuropeptides, to measure the relative strength of non-covalent interactions. This chemical interaction is most likely due to the overlap of aromatic rings of π -orbitals between paclitaxel and five commonly used medications: diazepam, clonazepam, sertraline, fluoxetine, and haloperidol. Molecular modeling illustrates that differences in the stability of the NCXs are likely due to the distance between the aromatic rings present in both the paclitaxel and antidepressant medications.

Keywords: Non-covalent interactions, Drug–drug interactions, π -Stacking

Received: 30 November 2018/Revised: 13 February 2019/Accepted: 17 February 2019/Published Online: 4 April 2019

Introduction

Thousands of drug–drug interactions (DDI) have been documented, while most have no adverse effects the threat of reduced efficacy of the drug treatment and negative health consequences make research of DDIs a high priority [1]. Major mechanisms of DDIs included chemical, pharmacodynamic, and pharmacokinetic [2, 3]. Chemical DDIs can be studied in vitro, compared to pharmacodynamic and pharmacokinetic DDIs that have to be studied in the more complex in vivo environment. Mass spectrometry is well-suited to study direct chemical interactions between compounds, especially non-covalent interactions [4, 5]. Furthermore, mass spectrometry has been used to measure the rate of formation and stability in the gas phase for the non-covalent complexes (NCX) formed by these interactions [6, 7]. Several studies have also been conducted on the formation of NCXs with drug compounds and other biomolecules, such as nucleic acids, lipids, etc. [8–14].

Palliative care in oncology often involves the use of multiple medications to aid in managing the various forms of anxiety and depression that coincide in patients dealing with cancer and its treatment(s) [15–17]. Thus, DDIs remain a critical concern of patients, caregivers, and drug manufacturers as there can be losses in efficacy and other adverse side effects. Paclitaxel (Taxol®) [18], a highly aromatic drug molecule used for cancer treatment, is a natural product isolated from the bark of the pacific yew (*Taxus brevifolia* Nutt) with activity against both leukemias [19] and solid tumors [20, 21]. Horwitz et al. [22] showed that the binding of paclitaxel to polymerized tubulin stabilizes it against disassembly, resulting in mitosis inhibition. Similarly, many drugs used to treat psychiatric disorders such as depression contain aromatic structures. For example, selective serotonin reuptake inhibitors like sertraline and fluoxetine [23], positive allosteric modulators of gamma-aminobutyric acid (GABA) receptor complex like clonazepam and diazepam [24], and dopamine receptor antagonists (D2) like haloperidol [25] all contain aromatic rings. In one recent study [26],

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researchers investigated the possible interaction between tamoxifen, another aromatic anti-cancer drug, with antidepressants in regard to increase risk of subsequent breast cancer in women. Statistics show that no increased risk was observed.

Non-covalent interactions between aromatic compounds, known as aromatic (π - π) stacking, is due to the overlap of π -orbital of the two aromatic electron clouds [27, 28]. Several studies have used mass spectrometry to investigate the formation of NCXs by aromatic stacking [29–32]. Direct chemical interaction via π - π stacking is very likely to occur between paclitaxel and antidepressant drugs. This type of direct chemical interaction could play a role in the bioavailability of these medications in vivo.

In the present work, we used electrospray ionization mass spectrometry (ESI-MS) to demonstrate the formation of NCXs of paclitaxel with five commonly used antidepressants (diazepam, clonazepam, sertraline, fluoxetine, and haloperidol). Additional ESI-MS/MS experiments were conducted to probe the effect that chemical structure has upon the stability and formation rate of these complexes in the gas phase.

Material and Methods

Electrospray Ionization Mass Spectrometry

Initial studies were conducted to investigate the ability of paclitaxel to form NCXs by π - π stacking in the gas phase with five common antidepressants: clonazepam, diazepam, fluoxetine, haloperidol, and sertraline. An Orbitrap-Velos mass spectrometer (Thermo Fisher, San Jose, CA) with a static nanospray source was used in positive ion mode for electrospray analysis. Spray voltages between 1.3–1.5 kV were employed, and 2- μ m nanospray tips (New Objective, Woburn, MA) were used. The resolving power of the instrument was set at 60,000 FWHM for both MS (identification of ionized unbound drugs and NCX) and MS² experiments (which exclusively dissociates the NCX). For MS experiments, one microscan at 500 ms was used to determine intensities of the NCX, as well as the free monomer drugs. For MS² experiments, a microscan time of 1000 ms was used in higher energy collision dissociation (HCD) mode with collision energies (CE) between 2 and 16 eV and a selection mass window of five daltons. The intensity of each NCX ion, as a function of its collision energy, gave a reasonable measure of the relative gas phase binding strength of paclitaxel and each aromatic compound.

Sample Preparation

The drug compounds: clonazepam (Clon) (C1277, Sigma-Aldrich, St. Louis, MO), diazepam (Diaz) (D0899, Sigma-Aldrich), fluoxetine (Fluox) (F132, Sigma-Aldrich), haloperidol (Hal) (H1512, Sigma-Aldrich), sertraline (Sert) (S6319,

Sigma-Aldrich), and paclitaxel (Pax) (sold under the brand name Taxol. 580,555, EMD Chemicals, Gibbstown, NJ) were used without further purification. Stock solutions were prepared as follows: clonazepam at 8 nmol/ μ L in 80% ethanol/20% acetone, diazepam at 10 nmol/ μ L in ethanol, fluoxetine at 10 nmol/ μ L in ethanol, haloperidol at 5 nmol/ μ L in methanol, sertraline at 5 nmol/ μ L in 50% ethanol/50% methanol, and paclitaxel at 2.5 nmol/ μ L in methanol. For mass analysis, the stock solutions were diluted in 100% methanol.

Molecular Modeling

Molecules were modeled with Spartan 10 (Wavefunction, Inc., Irvine, CA). For all molecules, the equilibrium geometry for the ground state in vacuum was calculated. Clonazepam, diazepam, fluoxetine, haloperidol, and sertraline were processed using Restricted Hartree-Fock with the basis set 3-21G*, while paclitaxel was processed using semi-empirical with the basis set PM3.

Results

Initial studies were conducted to investigate the ability of paclitaxel to form non-covalent complexes by π - π stacking in the gas phase with five common antidepressants.

To analyze the formation of NCXs between paclitaxel and aromatic antipsychiatric medications, mass spectra of clonazepam, diazepam, fluoxetine, haloperidol, and sertraline with paclitaxel were recorded in positive ion mode (Figure 1). Each mass spectrum was an average of 50 MS scans, and a model of the antidepressant aromatic structure used was included. We observed in Figure 1a–e, a singly charged NCX ion for each psychiatric compound with paclitaxel.

To confirm the assignments of the interaction between these aromatic compounds in Figure 1, we fragmented the peaks representing the NCXs formed by paclitaxel and each aromatic psychiatric compound (Figure 2), to see if the fragmentation of the complex would yield the individual drug compounds. MSⁿ analysis was conducted on each [NCX+H]⁺ ion. Each production spectrum yielded mass peaks (Figure 2a–e) that confirmed the NCXs assignments and showed that the singly charged NCX ion dissociated into the monomeric drug ions. In positive ion mode, the abundance of each monomeric drug ion varied mainly depending upon their proton affinity. A model of paclitaxel structure is seen in Figure 2a.

To compare the relative affinity of each aromatic antidepressant to form a NCX with paclitaxel, we performed an ESI-MS assay on a mixture of the five aromatic antidepressant compounds with paclitaxel. The mixture consisted of paclitaxel at 100 pmol/ μ L and clonazepam, diazepam, fluoxetine, haloperidol, and sertraline at 5 pmol/ μ L. Figure 3 shows an average mass spectrum of this mixture consisting of 500 MS scans. All five antidepressants formed singly charged NCX with paclitaxel, as expected, but the abundance of each NCX varied greatly. To compare NCX formation for each antidepressant,

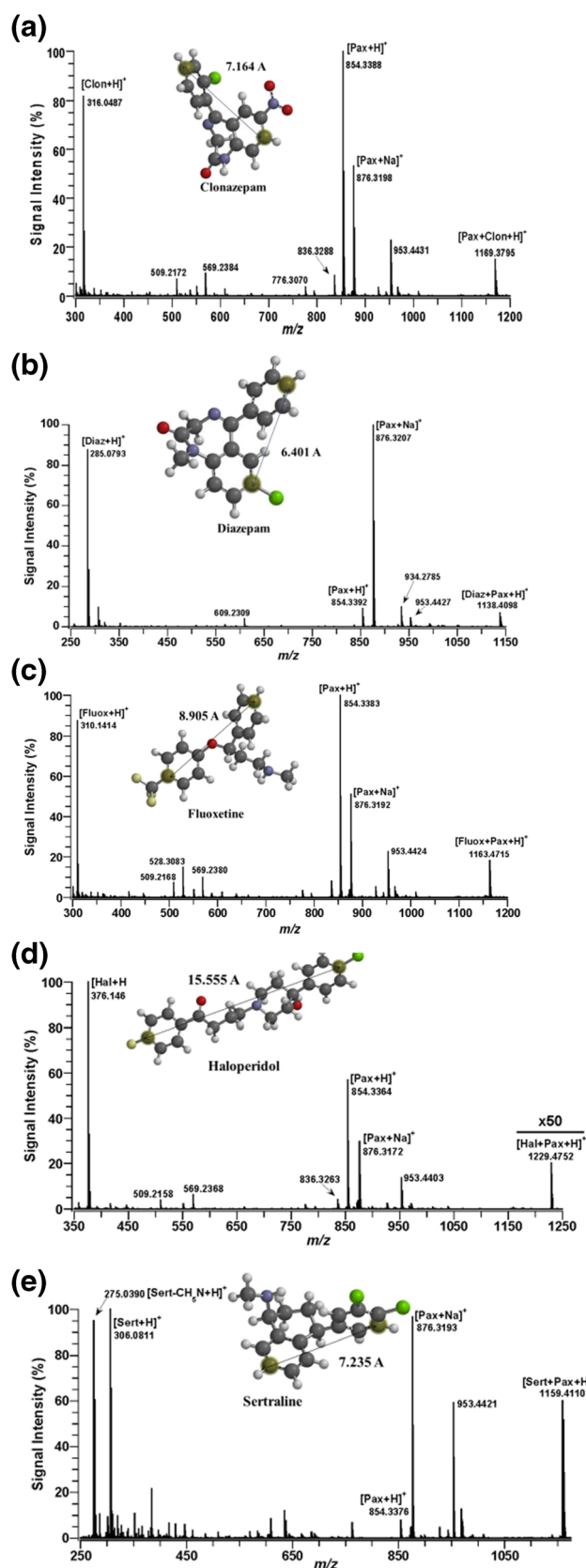


Figure 1. Mass spectra of 100 pmol/ μ L paclitaxel with 10 pmol/ μ L of (a) clonazepam, (b) diazepam, (c) fluoxetine, (d) haloperidol, and (e) sertraline. Structure of compound used is inserted above each spectrum

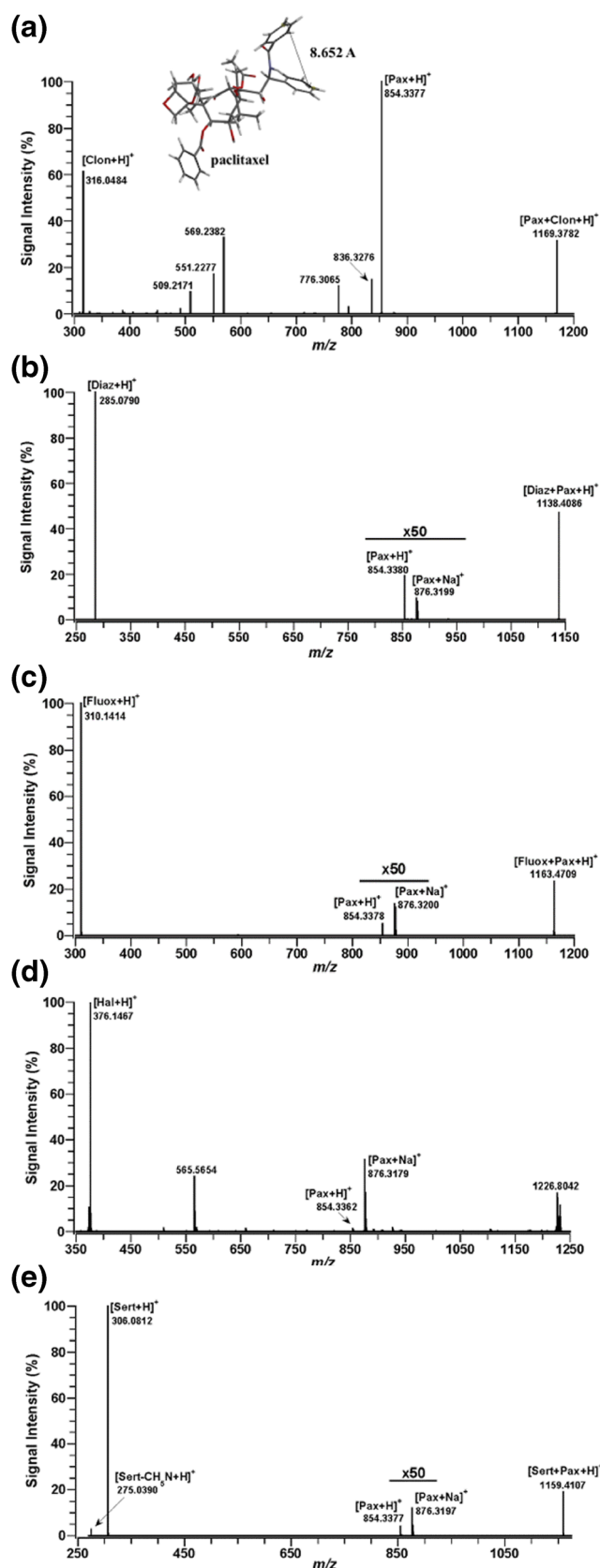


Figure 2. Product-ion spectra of the NCX^+ from a mixture of paclitaxel with (a) clonazepam at $\text{CE} = 8$ eV, (b) diazepam at $\text{CE} = 6$ eV, (c) fluoxetine at $\text{CE} = 10$ eV, (d) haloperidol at $\text{CE} = 10$ eV, and (e) sertraline at $\text{CE} = 10$ eV

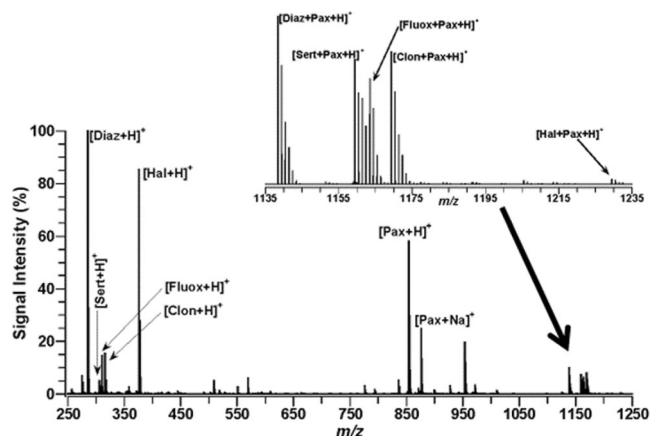


Figure 3. MS analysis of 100 pmol/uL paclitaxel and 5 pmol/μL of haloperidol, diazepam, clonazepam, sertraline, and fluoxetine

the ratio of the NCX peak to the corresponding antidepressant peak $[M+H]^+$ was used for comparison of the data recorded in Figure 3, to normalize the data in case of difference in proton affinity among the five antidepressants tested. In the case of sertraline, the $[M-CH_3N+H]^+$ peak was also included in the calculation. These results are listed in Table 1 and have the following affinities to form NCXs with paclitaxel from least to most: haloperidol < diazepam < fluoxetine < clonazepam < sertraline.

Additional experiments were conducted to evaluate the stability of the NCXs formed between paclitaxel and clonazepam, diazepam, fluoxetine, and sertraline (Figure 4). Haloperidol was not tested due to very low abundance of NCX ions and low stability of the NCX ion in the gas phase. In these experiments, the singly charged NCX ions were fragmented with collision energies between 2 and 16 eV in HCD mode. The relative ion intensities of each complex ion were plotted as a function of collision energy, using the average of 50 scans (Figure 4). The ion intensities of each complex were normalized by dividing by the total ion current in each spectra. Clonazepam and diazepam NCXs with paclitaxel were the least stable, while fluoxetine and sertraline NCXs with paclitaxel were the most stable.

Discussion

Molecular modeling was conducted on each of the drugs used in this study to compare the MS results with their

Table 1. Ratio of NCX mass peak/antidepressant ions and the distance between rings in the drugs used

Antidepressant	NCX ion/ antidepressant ion	Half-wave collision energy (eV)	Distance between aromatic rings (Å)
Haloperidol	0.0036	NA	15.555
Diazepam	0.099	4.5	6.401
Fluoxetine	0.43	6.5	8.905
Clonazepam	0.51	5	7.164
Sertraline	0.62	6.8	7.235

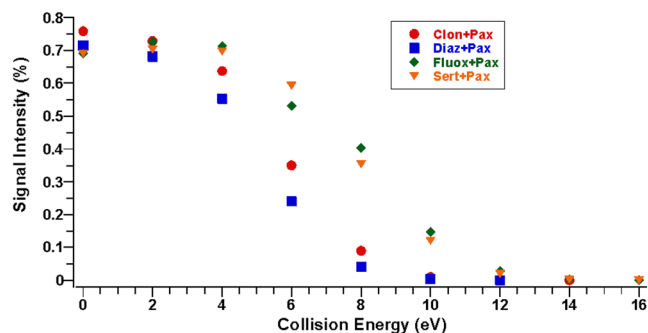


Figure 4. Stability of NCXs of paclitaxel with clonazepam, diazepam, fluoxetine, haloperidol, and sertraline at varying collision energies

structures (shown as inserts in Figure 1a–e). The models illustrate the ground state equilibrium geometry for (a) clonazepam, (b) diazepam, (c) fluoxetine, (d) haloperidol, (e) sertraline, and paclitaxel structure (seen in Figure 2a). Since π - π stacking results in the formation of NCXs between paclitaxel and the aromatic antidepressant compounds, comparing the distance between the aromatic rings in each compound might explain the MS results, i.e., the closer the value of the aromatic rings' distance in each compound to the rings in paclitaxel, the higher the affinity of the compounds to interact, and the more stable the NCX will be. Haloperidol was the least interactive in terms of NCX formation, most likely due to its linear structure compared to the more compact structure of the other antidepressants. Furthermore, the distance between its two aromatic groups is 1.8 times larger compared to paclitaxel, thus making the π - π stacking less efficient. Diazepam was the second least interactive, in terms of NCX formation, and its structure was the most compact of the compounds tested, in terms of the separation between the aromatic groups. The distance between diazepam's two aromatic groups was approximately 75% of the distance of the aromatic groups in paclitaxel. For stability, fluoxetine formed the most stable NCXs with paclitaxel and, based on its structure, the distance between its aromatic groups was the closest to paclitaxel of the compounds tested. The distance between fluoxetine's aromatic rings was calculated to be 8.905 Å, while the distance between paclitaxel's aromatic rings was calculated to be 8.652 Å. As these distances were the closest, it explains why fluoxetine and paclitaxel were more likely to form NCXs. The data also points to the importance of the distance between two aromatic rings in each molecule for the formation of stable π -stacking between two compounds.

Mass spectrometric analysis of solutions of paclitaxel and antidepressant drugs showed the presence of non-covalent interactions. The relative affinity of each drug–drug interaction, as measured by MS/MS experiments, fits well with our hypothesis that the interatomic distances of the aromatic rings of the antidepressant drugs and the molecular structure of paclitaxel suggest aromatic stacking are the driving force for the formation of non-covalent complexes.

Acknowledgements

The authors acknowledge Dr. Lorenzo Leggio, Dr. Michelle Leff, Dr. Gail Seabold, and Dr. J. Albert Schultz for their comments and Dr. Martha Vestling for her critical insight. This work was supported by the Intramural Research Program of the National Institute on Drug Abuse, NIH. The authors declare that they have no competing interests.

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