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RESUMO

O aparecimento na última década de Novas Substâncias Psicoativas (NSP), cada vez em maior número, tem levantado uma série de desafios para a sociedade em geral e em particular para a comunidade científica. O número de novas substâncias psicoativas que surgem a cada ano tem aumentado sucessivamente. De facto, relatórios publicados por diversas organizações, nomeadamente o *European Monitoring Centre for Drugs and Drug Addiction* (EMCDDA) e a Europol, referem que em 2013 foram notificadas na Europa 81 novas substâncias, um número muito superior às reportadas em 2012 (72) e em 2011 (49). Assim, torna-se urgente e emergente o desenvolvimento de novas tecnologias de deteção e análise adequadas ao controlo destas NSP, especialmente porque estes produtos são comercializados recorrentemente em misturas de duas ou mais substâncias psicoativas. As metodologias mais usadas no controlo analítico envolvem o uso de técnicas cromatográficas acopladas a diversos tipos de detetores. No entanto, os métodos eletroquímicos têm sido considerados uma ferramenta muito útil tanto na deteção como na quantificação de substâncias psicoativas, dos seus precursores e metabolitos. Na realidade, o desenvolvimento e utilização de sensores eletroquímicos poderão possibilitar a deteção e monitorização dos constituintes de uma amostra de forma rápida, fidedigna e pouco dispendiosa.

Neste contexto, o projecto desenvolvido foi focado na síntese de NPS, nomeadamente derivados de piperazinas e feniletilaminas e de seus intermediários e no seu estudo eletroquímico. Os compostos foram sintetizados com rendimentos médios a elevados e caracterizados por RMN de ^{1}H, ^{13}C e DEPT. O estudo eletroquímico foi realizado usando as técnicas de voltametria de impulso diferencial e cíclica e pelos resultados obtidos demonstra-se que estes métodos são adequados ao estudo do perfil redox das substâncias psicoativas. Os resultados obtidos permitirão num futuro próximo estabelecer processos analíticos adequados para a monitorização das NSP.

PALAVRAS-CHAVE: Novas Substâncias Psicoativas (NPS), Piperazinas, Feniletilaminas, Eletroquímica, Voltametria.

ABSTRACT

The emergence in the last decade of an increasing number of New Psychoactive Substances (NPS) has raised a number of challenges to our society and particularly to the scientific community. The number of new psychoactive substances detected each year has been increasing successively. In fact, reports published by various organizations, including the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and the Europol, informed that in 2013, 81 new substances were detected in Europe, a higher number than those reported in 2012 (72) and in 2011 (49). It is urgent to develop new technologies to facilitate the detection and analysis of these NSP and its appropriate control, especially since these products are recurrently sold in mixtures of two or more psychoactive substances. The methods most commonly used in analytical tests involve the use of chromatographic techniques coupled to various types of detectors. Electrochemical methods have proven to be a very useful tool both in the detection and quantification of psychoactive substances, their precursors and metabolites. Indeed, the development and use of electrochemical sensors allows the detection and monitoring of constituents in a sample quickly, reliably and inexpensively. Therefore, the main objective of this project was to synthesize NPS, namely phenethylamines and piperazines derivatives and its intermediates and perform electrochemical studies of the synthesized substances.

The compounds were synthesized with medium to high yields and characterized by ^{1}H NMR and ^{13}C and DEPT. The electrochemical studies were conducted using differential pulse voltammetry and cyclic voltammetry and the results obtained showed that these methods are adequate for the investigation of the oxidation profile of psychoactive substances. The achieved results will allow establishing in the near future analytical standards to monitor NPS.

KEYWORDS: New Psychoactive Substances (NPS), Piperazines, Phenethylamines, Electrochemistry, Voltammetry.

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LIST OF ABREVIATIONS

δ	Chemical shift
^{13}C NMR	Carbon-13 nuclear magnetic resonance spectroscopy
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy
DMSO	Dimethyl sulfoxide
NMR	Nuclear magnetic resonance
DEPT	Distortionless enhancement by polarization transfer
dd	Doublet of doublets
d	Doublet
J	Coupling constant
m	Multiplet
s	Singlet
t	Triplet
TLC	Thin-layer chromatography
TMS	Tetramethylsilane
EMCDDA	European monitoring centre for drugs and drug addiction
NPS	New psychoactive substance
HPLC	High-performance liquid chromatography
GC	Gas chromathography
LSD	Lysergic acid diethylamine
DMT	Dimethyltryptamine
MDMA	3,4-methylenedioxymethamphetamine
PCP	1-(1-phenylcyclohexyl)piperidine)
MS-EI	Mass spectrometry-electron impact
CV	Cyclic voltammetry
DA	Dopamine
5-HT	5-Hidroxytryptamine
5-MeO-αMT	5-methoxy-*α*-methyltryptamine
CNS	Central nervous system
p-FPP	*para*-Fluorophenylpiperazine
m-CPP	*meta*-Chlorophenylpiperazine
DOB	4-bromo-2,5-dimethoxy-*α*-methyl-*β*-phenethylamine
2C-H	2,5-dimethoxy-*β*-phenethylamine
2C-B	4-bromo-2,5-dimethoxy-*β*-phenethylamine
UV	Ultraviolet
DCM	Dichloromethane

1. Introduction

1.1 Historical overview

The quest and use of psychoactive substances[1] by human societies as a mean to reach alterated states of consciousness is an ancient practice. Early collector tribes, in the search for nourishment, tried all kinds of plants and fungi. Some of them were foods, containing essential nutrients, others cured their illnesses or eventually killed them, acting as medicines or poisons. A few had strange effects in their body and mind, increasing alertness and physical activity, inducing relaxation and dissociative states or altering their perception of reality [1, 2]. Nowadays it is known that these plants contained psychoactive compounds. Depending on the effects these compounds have in the central nervous system (CNS) they can be classified as stimulants, depressants and/or hallucinogens [3].

Due to the properties and effects of these plants and funguses, some were added to the diet and rituals of primitive cultures. They were used as medicines, as physical and mental enhancers to activities as hunting or heavy work, or in divine ceremonies and rites of passage. There is an estimated number of 4000 plants with psychoactive properties, and about 60 of them have been in constant use, somewhere in the world, throughout history [4]. One of these examples is the long history of traditional use of Kratom (*Mitragyna speciosa)* in Southeast Asia, producing effects of relaxing euphoria mirroring that of opiates. Malay and Thai natives use Kratom, for its opium-like effects, to enhance tolerance towards hard work in extreme heat [5].

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[1] **Notes on the terminology**: In this work the term “psychoactive substance” is emphasized over “drug” or “drug of abuse” because of the popular confusion surrounding exactly what is a “drug” or what is “use” or “abuse”. Many researchers don’t identify licit substances as drugs, as caffeine, alcohol or even nicotine. This can be considered an arbitrary and inaccurate distinction. The concept “abuse” is commonly conceived as any situation which became excessive, dangerous or unacceptable. However, the perception of abuse of a psychoactive substance is highly subjective, it’s a not standardized concept meaning different things to different researchers. To some, it’s the use of any psychoactive substance, licit or illicit, under any circumstances; to others is the use of illicit substances or any drug only for nonmedical purposes. Abuse is currently used in some scientific literature as pejorative adjective for any psychoactive substance consumption that is not social or legally approved. Since 2006 the World Health Organization recommends the use of other terminology than “drug of abuse”.

Since ancient times, the use of Ma Huang (*Ephedra sinica Stapf* and other species of *Ephedra*), a low shrub that grows in China, India and Europe, has been known for their stimulant effect. The psychoactive content of Ma Huang is ephedrine and pseudoephedrine, a phenylethylamine type of substances, which possess amphetamine-like effects. The origins of Chinese medicine are lost in legend, but historians agree that Chinese physicians began prescribing ephedra tea for colds, asthma and hay fever around 3,000 b.C. Recently it was also found ephedra specimes in a 60,000 year old burial site in Iraq, along with seven other medicinal plants [6-8]. Other plants such Iboga (*Tabernanthe iboga*) (Figure 1), an hallucinogenic plant from which the alkaloid Ibogaine (10-methoxybogamine) can be extracted, have a medicinal and ceremonial use in West Central Africa. The ritual of eating Iboga's roots has been a sacrament in Bwiti religion for several centuries, and was likely practiced among Pygmies in much earlier times, with the aim of keeping social cohesion through a social binding experience, a connection with their ancestrals throughout time and the binding of their community on the basis of a shared experience of a distinctive consciousness and belief system [9-11].

Figure 1. Iboga plant (*Tabernanthe iboga*). Adapted from [1].

The major part of psychoactive compound's history was written over their use with religious and medicinal proposes. However, the recreational use, with the aim of enhancing performance and enjoyment, always made part of that history [12].

In our society, alcohol has acquired over time a rich and diverse set of symbolic contexts in which it may be used, whether the aim is celebratory, consolatory, or gastronomic. The fermentation of sugar in ethanol is one of the earliest organic reactions that men learned to carry out and the history of man-made ethanol is very long [13]. Drinking alcoholic beverages has become a social ritual and many people lost sight of the fact that ethanol is an effective, although presenting low potency, psychoactive substance.

Other psychoactive substances or preparations have been used recreationally for centuries. Since 1500 b.C, every year in Eleusis (ancient Greece), a harvesting festival took place, where initiation ceremonies were held. There, initiates, priests and other participants drank a psychoactive preparation as part of the rite of passage. This preparation named *Kykeon*, was mainly made of water, barley and naturally occurring flavouring substances as mint [14]. Although some mysteries and scientific discussion remain[2] about the psychoactive content of the drink, it is still one of the first records of recreational drug use. Alciabiades, a noble Athenian, was convicted of profanation in 415 b.C. for having the Eleusinian sacrament in his home and using it for the entertainment of his friends [12, 15]. Early recreational use of other psychoactive materials as tobacco [16], coffee [17], cocoa [18], coca [19] and opium [20] is also well documented. The Age of Discoveries in the 15th century had a major role in the establishment of these psychoactive drugs as a commodity, and their exchange and trade all over the world.

It can be said that there is a trend in the human society history of searching for more refined and potent substances, either in the form of plant extracts or actually new synthetic entities. When distillation techniques were applied to fermented beverages it was possible to obtain spirits - beverages with a much higher alcohol content, with more than 40% ABV (alcohol by volume), than beer or wine that had only 6-15% ABV. An increase in alcoholism-related problems was notorious with the introduction of spirits in the 16th century society's daily life [21]. With the impetus of industrial revolution in late 18th century, phyto and synthetic chemistry suffer great advances that led to the isolation of morphine from the opium poppy and the isolation of cocaine from coca leaves. Despite humans had a long history of non-problematic use of coca leaves and opium, after these refined and pure forms appeared it can be argued that some problems related with problematic and uncontrolled use might have increased [22].

More potent and concentrated forms of a substance, along with more effective consumption routes, can present more risks to the users related with overdosing. In fact, at higher doses the same substance can present a totally different spectrum of effects and risks. At the same time, is well known by psychopharmacologists and anthropologists that the effects and the impact of the exposure to a psychoactive substance is not only dependent of the chemical and pharmacological properties of the substance, but also of the environment (*e.g.* social and cultural context) and the subject (metabolism, social background, experience expectations, etc.) [2, 22].

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[2] Some authors speculate that the barley used in the preparation of the drink was parasitized by ergot (*Claviceps purpurea*) and that the psychoactive properties of the fungus's alkaloids ergonovine and methylergonovine, structurally related with LSD, triggered the intense experiences reported [13]. Other theories have been discussed [15].

With the synthesis of *d*-amphetamine in the late 18th century, the synthesis of mescaline (3,4,5 – trimethoxyphenethylamine) in 1919 and DMT (dimethyltriptamine) in 1931 and semi-synthesis of LSD (lysergic acid diethylamide) in 1938, the core of western synthetic psychoactive substances was established. Most of the synthetic substances that fuelled the beat generation of the 50’s, the psychedelic revolution of the 60’s or the rave scene in the 90’s are derived from amphetamines (MDMA), phenethylamines (mescaline), ergolines (LSD) or tryptamines (DMT) (Figure 2). New substances sporadically appear, but only a comparatively small number has emerged on the drug markets and diffused to broader section of the population [23].

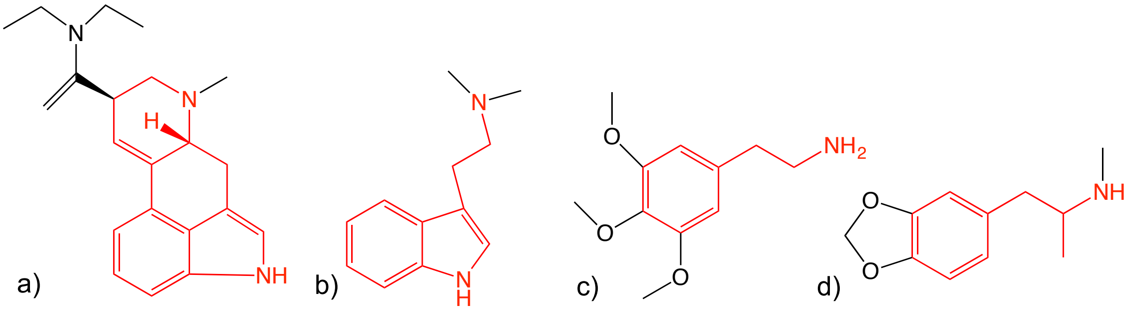


Figure 2. Four classes of psychoactive substances. a) LSD with the ergoline scaffold outlined in red; b) DMT with the tryptamine scaffold outlined in red; c) Mescaline with the phenethylamine scaffold outlined in red; d) MDMA with the amphetamine scaffold outlined in red.

In 90’s two important publications trigged a change in the field of new psychoactive substances (NPS). PiHKAL – Phenethylamines I Have Known and Loved (1991) and TiHKAL - The continuation (1997), published by Alexander Shulgin, medicinal chemist and pharmacologist, and his wife Ann Shulgin, where the doses, the effects and the synthesis of more than 200 phenethylamine derivatives are described [24, 25]. In the years that follow these publications, many new substances appearing in the illicit market were substituted one-ring phenethylamines which have been described in PiHKAL. The compilation of this information along with the rising of internet access and the growing of online communities in late 90’s, enabled and fostered the free exchange of knowledge about new substances, their effects, basic laboratory techniques, *do it yourself* lab equipment and specially how to synthesize new psychoactive substances [26].

1.2 New Psychoactive Substances: *Catch me if you can*

> *"(...) it is likely that the future drugs of abuse will be synthetics rather than plant products. They will be synthesized from readily available chemicals, may be derivatives of pharmaceuticals, will be very potent, and often very selective in their action. In addition, they will be marketed very cleverly. The "Designer Drug" problem may become an international problem. A single gram of any very potent drug like 3-methylfentanyl could be synthesized at one location, transported to distribution sites worldwide, and then formulated (cut) into many thousand, perhaps a million, doses. Preventing the distribution of such small amounts of the pure drug will be exceedingly difficult."*
>
> **Gary Henderson, 1988 [27]**

In 1988 Gary Henderson, one of the first researchers using the term *designer drugs*, made an interesting exercise of futurology drawing an accurate picture of the future of psychoactive substances and their market [27]. At that moment, the term *designer drugs* was pointing towards analogs of compounds with proven pharmacological activity that were manufactured by underground chemists for sale in the streets in an effort to circumvent the law. The "cat and mouse" game, the search for new chemical entities with the ability to bypass the law and the creation of new laws able to control the new substances, is not new. In fact, it happened with the consecutive efforts to control the esters of morphine in Opium International Conventions between 1910-1930, creating simultaneously pressure over underground chemists to prepare different and more potent opioid derivatives [23, 28].

The European Monitoring Center for Drugs and Drug Addiction (EMCDDA) defined NPS as 'a new narcotic or psychotropic drug, in pure form or in preparation, that is not controlled by the United Nations drug conventions, but which may pose a public health threat comparable to that posed by substances listed in these conventions [29]. The EMCDDA states that in 2013 the number of NPS detected by the national focal points of European countries was 81. A high number specially when compared with the 14 NPS found in 2005. These numbers rise to 41 in 2010, 49 in

2011 and 74 in 2012. It is evident that in recent years a rising number of NPS are appearing in the market belonging to increasingly broader chemical and pharmacological families (Figure 3) [30]. A high number of new cannabinoid receptor agonists (collectively referred to as synthetic cannabinoids) [31], cathinones [32], piperazines [33] and more recently highly potent *N*-benzyl-phenethylamines [34] have been detected circulating in the market. As Henderson predicted, these psychoactive products are subjected to aggressive marketing, with colourful packages and appealing brand names, are more potent and are always presented as legal substitutes for known substances such as cannabis, MDMA or LSD. Nowadays, anyone with access to the internet can easily buy legal new psychoactive substances, the so-called 'legal highs', in online markets [35]. Actually, in recent years a new kind of online market has emerged, established in the *deep web* (a non-indexed part of world wide web) and working with bitcoins (digital currency), where anyone can anonymously buy legal and scheduled psychoactive substances [36]. With a reference to the ancient trade route in Asia one of these online markets it's called *Silk Road 2.0*. Again, governments are working in legislative responses to control the emergence of these compounds [29].

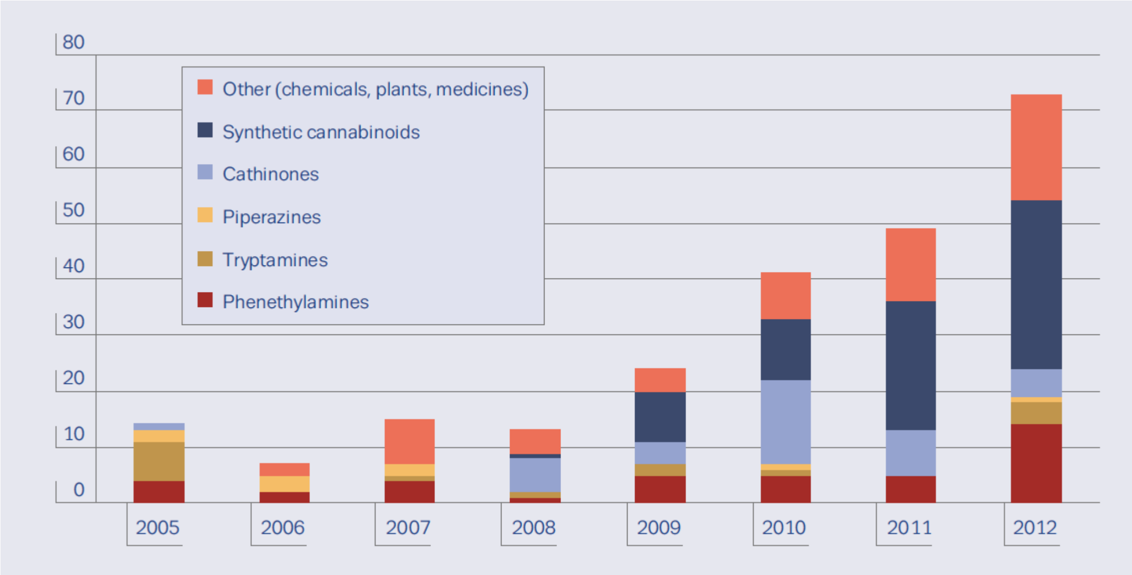


Figure 3. Number of New Psychoactive Substances in Europe from 2005 to 2012. Adapted from [29].

Every new psychoactive substance circulating in the market poses big challenges to harm reduction technicians, forensic chemists, pharmacologists, medical staff and policymakers. The largely unknown pharma and toxicological effects, potency or routes of administration can pose serious risks to the users. Using a new substance, legal or not, for which complete pharmacological data does not exists always presents a higher risk than being exposed to an extensive studied one. Based on this fact, a huge debate is taking place on how to regulate the NPS market, adopting new approaches that might reduce the drive to produce new and possibly more dangerous, substances [37, 38].

Although some of the substances had been created to evade drug legislation, others such as phenylpiperazine or *N*-benzylphenethylamine derivatives came from legitimate research, as potential therapeutic agents. Some researchers already expressed their concern about the use of information to prepare and commercialize untested compounds [39]. At the same time there are some are worries that an important an unfortunate outcome of the legislative control on psychoactive substances might be to make research into their mechanisms of action and potential therapeutic uses (e.g. depression or post-traumatic stress disorder) difficult and in many cases impossible [40].

It is a fact that New Psychoactive Substances present themselves as a public health issue, therefore it is urgent to develop more efficient ways to identify these substances and increase the level of knowledge available about them [23, 29, 30].

2. The Project

2.1 Relevance, context and aim

Since 2008 I've been colaborating with CHECK!N (APDES), a non-govermental organization that works with young people that use psychoactive substances. Part of my work in this organization was to establish and develop a drug checking service where users can have their psychoactive substances (pills, LSD blotters, etc.) chemicaly analysed, and at the same time accessing professional counseling. Among other things, drug checking allows the detection of toxic adulterants present in the user's samples, but at the same time gives the possibility of monitoring the illegal market and study new trends in drug consumption. The work with this organization gave me the oportunity of closely follow the NPS phenomenon and all the relevant changes in the drug market, legal and ilegal, since it's beginning in the Portuguese context [41].

The need for synthetic and analytical chemistry income in this area is evident. The emergence of an increasing number of new substances created a necessity of analytical standards and the development of easy, fast and low-cost techniques that allow rapid and efficient identification of these new compounds. Although the time between the detection of a new compound in the market and its identification has been decreasing, thanks to dissemination systems like the *Early Warning System* of European Union [29], huge difficulties still arise to researchers to acquire certified reference materials, mainly because the lack of licenses or commercial supliers. Targeted organic synthesis has been sugested to support analytical chemists, with several advantages namely: i) the compound may not be commercially available; ii) independence from commercial suppliers (taking into account the high cost of reference materials); iii) ability to observe impurities related to synthesis, which can be relevant to characterize a new emerging psychoactive product and give some insight into future retrosynthetic studies; iv) synthesis of intermediates, analogs and derivatives of currently emerging substances allows the sharing of highly updated data with the scientific, the law enforcement or the clinical communities [23]. At the same time the preparation of these compounds can create a basis for further studies beyond analytical characterization such as metabolism and pharmacological/toxicological research.

Electrochemical methods have been shown to be advantageous technique to study psychoactive substances [42-44]. A real time *in situ* detection and identification system is highly desirable for the fast and efficient monitoring of NPS. Progress in the area of analytical chemistry is aimed at bringing data close to the field to overcome several limitations, as the lag time of identification, difficulties in drug sample collection and transport due to legislative restrictions, etc. A vast number of electrochemical devices for *in situ* analysis have already been developed and tested, especially for environmental monitoring [45, 46]. Recent progresses in screen printed and carbon nanotube modified electrodes have showed the possibility of miniaturization and portability, sensitivity and selectivity, wide linear range, minimal power requirement and cost effective instrumentation [45, 47]. In order to monitor of psychoactive substances, preliminary studies of the electroactivity of psychoactive compounds for further research and development are required.

Therefore the main aim of the current project is:

i) The preparation and fully spectroscopic characterization of reference materials of psychoactive substances, their intermediates, derivatives and/or analogs;

ii) The study of the electrochemical behaviour of synthesized compounds.

Table 1. List of compounds under study in this project.

Number	Name
Compound 1	2,5-dimethoxy-*β*-nitrostyrene
Compound 2	2,5-dimethoxy-*β*-methyl-*β*-nitrostyrene
Compound 3	3,4,5-trimethoxy-*β*-nitrostyrene
Compound 4	2,3,4-trimethoxy-*β*-nitrostyrene
Compound 5	2,5-dimethoxy-*β*-phenethylamine
Compound 6	3,4,5-trimethoxy-*β*-phenethylamine
Compound 7	2,5-dimethoxy-*α*-methyl-*β*-phenethylamine
Compound 8	4-bromo-2,5-dimethoxy-*β*-phenethylamine
Compound 9	4-bromo-2,5-dimethoxy-*α*-methyl-*β*-phenethylamine
Compound 10	2,5-dimethoxy-*N*-(2-methoxybenzyl)-*β*-phenethylamine
Compound 11	*p*-fluorphenylpiperazine
Compound 12	*m*-chlorophenylpiperazine
Compound 13*	*o*-chloprophenylpiperazine
Compound 14*	*p*-chlorophenylpiperazine
Compound 15*	phenylpiperazine

*Commercial compound

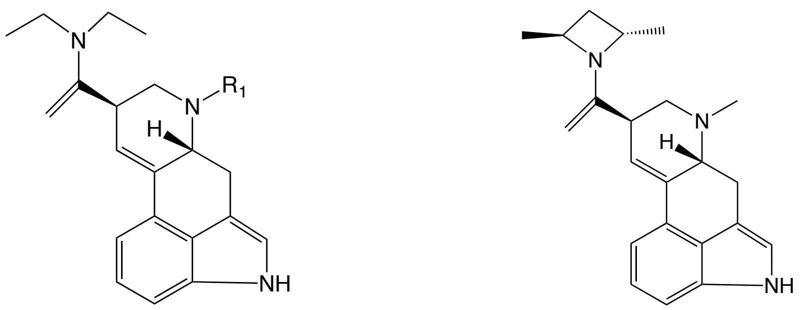
2.2 Preparation of analytical standards of psychoactive substances

2.2.1 Background

As previously referred, the number of chemical families of psychoactive substances circulating in the illegal market has increased substantially in the last decade [29]. Most of the new synthetic substances detected belong to the chemical families of phenethylamines, cathinones, piperazines and synthetic cannabinoids. Substances based on the structure of tryptamines, ergolines and arylcyclohexylamines were also found circulating [48, 49]. It is worthwile to mention that synthetic cannabinoids, due to its unique chemistry and pharmacology features were considered out of the scope of this work, therefore will not be addressed.

Ergolines

The modern and systematic study of new psychoactive chemical entities began when in 1943 Albert Hofmann discovered the psychoactive effects of lysergic acid diethylamide (LSD, Figure 4a), an ergoline derivative [38]. In the same period the neurotransmitter serotonin was being investigated and, due to the similarity in their chemical structure, it was suggested that the effect of LSD was a result of some kind of interference with serotonin neurotransmission in the brain [50]. In the present we know that LSD is an agonist of the serotonin receptor 2A (5-HT$_{2A}$) among other neuroreceptors [51]. In the following years, Hofmann and his team prepared other ergoline derivatives that presented psychoactive properties. An example was the analogue series where the *N*-methyl group was substituted by saturated and unsaturated alkyl chains (Figure 4b) [52]. In recent years the diethylamide moiety was rigidified into a dimethylazetidide ring giving a potent 5-HT$_{2A}$ agonist called LSZ (Figure 4c) [53]. These and others LSD analogues have been detected in the illegal market [54].



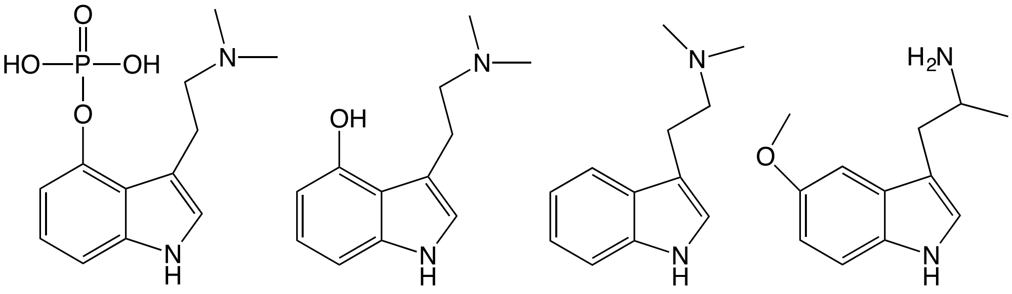
	R_1	
a) LSD	CH_3	(Hofmann 1943)
b) AL-LAD	CH_2-HC=CH	(Hofmann 1985)

c) LSZ
(Nichols 2002)

Figure 4. Chemical structures of ergoline derivatives: LSD-analogs.

Tryptamines

The chemistry of tryptamines also crossed Hofmann's path when he devoted some of his research to study the chemistry behind the psychoactive effects of psilocybe mushrooms, traditionally used in some remote areas of Mexico [55]. The psychoactive compound present in these particular mushrooms was called psilocybin (Figure 5a), a substituted tryptamine that is a prodrug, converted into the pharmacologically active substance psilocin (Figure 5b) after *in vivo* dephosphorylation by phosphatases [56]. Some years before, Stephen Szara already had solved the mystery behind the psychoactive snuff powder that Haitian natives used in ceremonies, synthesizing, studying it's pharmacology and comparing the properties of *N,N*-dimethyltryptamine (DMT, Figure 5c) and 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) with the compounds extracted from the Haitian mixture. In the following years Szara and co-workers prepared and assayed a series of DMT homologues [57]. Some years later Alexander Shulgin and collaborators started working with tryptamine derivatives, describing the effects of 5-methoxy-*α*-methyl-tryptamine (5-MeO-αMT, Figure 5d), a compound that results from minor modifications of the chemical structure of serotonin (Figure 5e). As it is known, the highly polar free hydroxyl group in 5-position prohibits the penetration of serotonin in the CNS when administered peripherally, as well as the exposed amino group is immediately removed by monoamine oxidase enzyme. With the added methoxyl group in the 5-position the compound is able to cross the blood-brain barrier, and the addition on an adjacent α-methyl group removes the liability of enzymatic attack [58]. Shulgin explored a vast number of new tryptamines in subsequent years [25] and some of them have been detected circulating in the market [49].

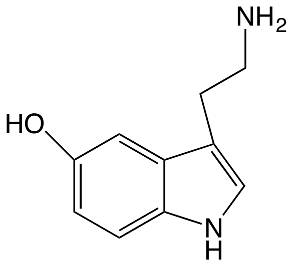


a) Psilocybin (Hofmann 1959)

b) Psilocin (Hofmann 1959)

c) DMT (Szara 1956)

d) 5-MeO-αMT (Pietri 1958)

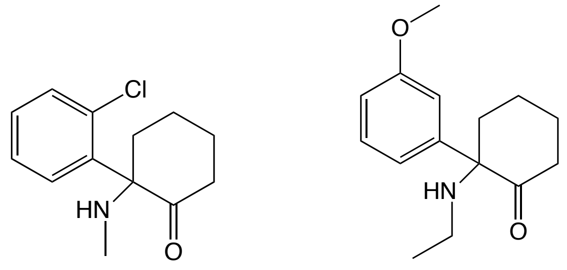


e) Serotonin

Figure 5. Chemical structures of tryptamine derivatives.

Arylcyclohexylamines

Dissociative anaesthetic is the medical term used to describe the effect of ketamine-like substances. Ketamine (Figure 6a) is an uncompetitive *N*-methyl-D-aspartate receptor (NMDAR) antagonist [48]. The first arylcyclohexylamine with anaesthetic properties was discovered, 1-(1-phenylcyclohexyl)piperidine (PCP), by Victor Maddox by serendipity. While investigating the reaction of Grignard reagents with nitriles, and unaware of the early studies on Bruylants reaction (the substitution of aminonitriles by Grignard reagents), 1-piperidinocyclohexanecarbonitrile (PCC) was treated with the phenylmagnesium bromide reagent and underwent substitution with the Grignard rather than the expected addition to the cyano group, thus yielding PCP (Figure 7) [48, 59].

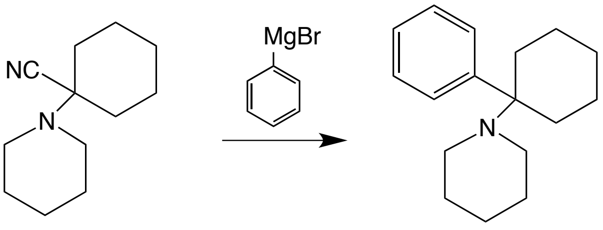


a) Ketamine
(Stevens 1962)

b) MXE
(Anonymous 2006)

Figure 6. Chemical structures of arylcyclohexylamines.

During that period a plethora of arylcyclohexilamines was prepared and evaluated in Parke-Davis with the aim of developing and enhancing their anesthetic properties, including ketamine in 1962. Ketamine and a few of its analogues became popular outside of the their medical applications and were detected circulating in the street market since 1960, but since the late 1990's new arylcyclohexilamines derivatives appeared, in the illegal market, as is the case of 2-(3-methoxyphenyl)-2-(ethylamino)cyclohexanone (MXE) (Figure 6b). The recipe to prepare MXE appeared in an online forum in 2006 and soon it was detected circulating in the market [60, 61].



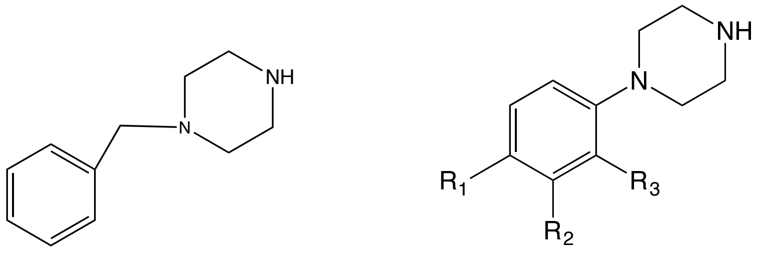
PCC

PCP
(Maddox 1956)

Figure 7. Grignard's reaction to prepare PCC from PCP.

Piperazines

Piperazine itself does not appear to have psychoactive properties, however a considerable number of piperazine derivatives, as benzylpiperazine (BZP) (Figure 8a), have been used recreationally by its stimulant effect [62]. BZP was prepared for the first time in 1944 at Burroughs Wellcome & Co., but its first mention as a potential recreational drug only appears decades later in PiHKAL [24, 63]. The backbone of these derivatives is a piperazine moiety attached to a phenyl or benzyl group. This backbone has been extensively used in pharmaceutical research, since some drugs can be metabolized in compounds that are now recognised as recreational drugs, like m-chlorophenylpiperazine (mCPP, Figure 8c) and p-fluorphenylpiperazine (pFPP, Figure 8e) that are active metabolites of the antidepressant Trazodone [64] and the hypnosedative Niaprazine [65], respectively. mCPP was patented in 1966 as an appetite suppressant and the action of its isomers, o-chlorophenylpiperazine (Figure 8b) and p-chlorophenilpiperazine (Figure 8d), was also described [66]. Phenyl and benzyl piperazine derivatives have been detected circulating in the market, sold as *party pills,* sometimes in combination with other substances [33, 62].



a) BZP
(Baltzly 1944)

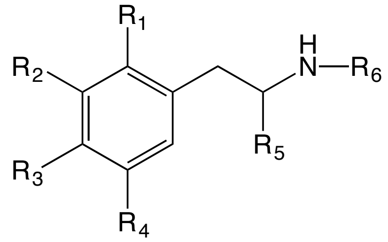
	R$_1$	R$_2$	R$_3$	
b) oCPP	H	H	Cl	(Moser 1966)
c) mCPP	H	Cl	H	(Moser 1966)
d) pCPP	Cl	H	H	(Moser 1966)
e) pFPP	F	H	H	

Figure 8. Chemical structures of piperazine derivatives.

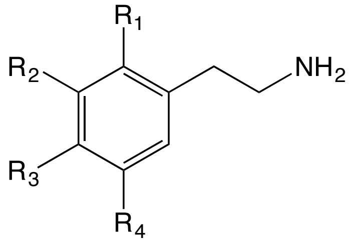
β-Phenethylamines

Mescaline (Figure 9e) has been known as the main responsible for the psychoactive effect of the peyote cactus since its isolation and characterization by Heffter [67], however Spath was the first to describe the synthetic process of this phenethylamine in 1919 [68]. Three decades before, in 1887, Edeleano had published the method of preparation of *α*-methyl-*β*-phenethylamine, known today as amphetamine, from ephedrine [69]. Later in 1913 a worker in Merck prepared the 3,4-methylenedioxy-*N*-methylamphetamine (MDMA, Figure 9a) as an intermediate of methylhydrastinin, a blood clotting agent [70].

The systematic preparation of bioactive analogues of phenethylamines only began in the 1950's, with the synthesis of 3,4,5-trimethoxyamphetamine (TMA, Figure 9b), the α-methyl analogue of mescaline by Hey [71]. Alexander Shulgin became interested in these compounds and during the next decades he explored and published the synthesis and pharmacology of analogues of *β*-phenethylamines. Small changes at the 4-position in the aromatic ring can modify both the quantitative and qualitative psychoactive effects that are produced. In general, the potency increases as the nature of the group in the 4-position varies from H < OR < SR < R < X (R= alkyl; X=halogen) [72]. Also, the extension of the carbon chain from two carbons to three, from a *β*-phenethylamine to *α*-methylphenethylamine scaffold, also appears to protect the nitrogen from metabolic removal and augment lipofilicity, leading to an effectively increase on the potency of the psychoactive effect [72, 73]. Based on the mescaline structure and its analogues, as 2,5-dimethoxy-*β*-phenethylamine (2C-H, Figure 9f), a series of new compounds was prepared and pharmacologically evaluated by Shulgin and co-workers. They reported sulphur analogs as the 4-methylthio-2,5-dimethoxyphenethylamine (2C-T, Figure 9h) [74], the bromine analogue 4-bromo-2,5-dimethoxyphenethylamine (2C-B, Figure 9g) [75] as well its amphetamine analogue, 4-bromo-2,5-dimethoxyamphetamine (DOB, Figure 9d) [76], and 4-alkyl analogues as 2,5-dimethoxy-4-methylamphetamine (DOM, Figure 9c) [77]. In the last decades different analogues of phenethylamines have been detected in street samples [30, 78, 79].



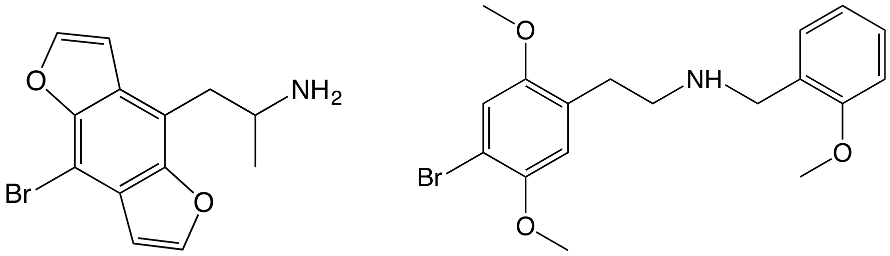
	R_1	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	
a) MDMA	H	O-CH_2-O		H	CH_3	CH_3	(Kollisch 1912)
b) TMA	H	OCH_3	OCH_3	OCH_3	H	H	(Hey 1947)
c) DOM	OCH_3	H	CH_3	OCH_3	H	H	(Shulgin 1963)
d) DOB	OCH_3	H	Br	OCH_3	H	H	(Shulgin 1967)



	R_1	**R_2**	**R_3**	**R_4**	
e) Mescaline	H	OCH_3	OCH_3	OCH_3	(Spath 1919)
f) 2C-H	OCH_3	H	H	OCH_3	(Buck 1932)
g) 2C-B	OCH_3	H	Br	OCH_3	(Shulgin 1974)
h) 2C-T	OCH_3	H	SCH_3	OCH_3	(Nichols 1967)

Figure 9. Chemical structures of *β*-phenethylamine and *α*-methyl-*β*-phenethylamine derivatives.

In recent years new phenethylamine derivatives have been synthesized, studied and later found their way to the market as recreational psychoactive substances. Compounds with constrained methoxy groups, as the benzodifuran derivative of DOB (Bromo Dragon-FLY, Figure 10a), were prepared by Parker and co-workers in 1998. These constrained compounds have been classified as the most potent phenethylamine derivatives known at this time [80]. In 2003 Hein added a 2-methoxy group to phenethylamine scaffold like 2C-B and boosted it's activity by more than two orders, creating the *N*-benzylphenethylamine family (Figure 10b) [81]. These substances and their derivatives already have been detected in the market and some reports of severe intoxication and fatalities related with the overdose with these potent 5-HT$_{2A}$ agonists have been recently published [34, 82, 83].



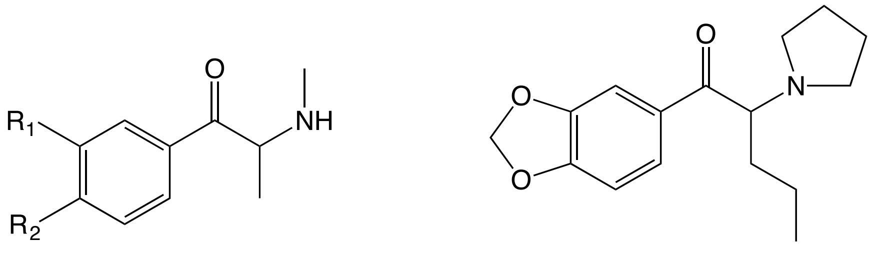
a) Bromo Dragon-FLY
(Parker 1998)

b) 25B-NBOMe
(Hein 2003)

Figure 10. Chemical structures of benzodifuran and *N*-benzylated phenethylamines.

Cathinones

The latest version of sympathomimetic compounds to emerge as recreational psychoactive substances was the synthetic cathinone derivatives. Cathinone occurs naturally in Khat, (*Catha Edulis Forsk*), an evergreen shrub growing wild or cultivated in a region extending from Southern Africa to the Arabian Peninsula. Cathinone and *α*-methyl-*β*-phenethylamine are chemical analogues and cathinone is chemically classified as *β*-ketones due to the carbonyl group in that position. This ketone group increases the polarity of the molecule, making it more hydrophilic and less able to cross the blood brain barrier and produce central stimulant effects [84]. Higher doses are required to produce equivalent response, compared with their relative amphetamines [85]. In 1928 methcathinone (Figure 11a), the cathinone's analogue of methamphetamine, was prepared by the first time by Hyde and co-workers [86]. In the next year the synthesis of its 4-methyl analogue, 4-methyl-*N*-methylcathinone (mephedrone, Figure 11b), was published [87] and in 1969 a patent for another analogue of methcathinone, 3,4-methylenedioxypyrovalerone (MDPV, Figure 11d), was submited [88]. However, the illegal market of cathinones was limited to methcathinone until the end of the last century, and essentially restricted to former Soviet Union [89]. In 1996 Shulgin and colleagues patented the 3,4-methylenedioxy analogue of methcathinone as an antidepressant and antiparkinsonic agent, naming it methylone (Figure 11c), but no pharmaceutical interest rose about its properties [90]. A vast number of new cathinone derivatives became popular and were sold without control in some specialized shops, mainly online, during the first decade of 2000's, as it was the case of mephedrone and MDPV [32, 91].



	R_1	**R_2**	
a) Methcathinone	H	H	(Hyde 1928)
b) Mephedrone	H	CH_3	(Sánchez 1929)
c) Methylone	$O\text{-}CH_2\text{-}O$		(Shulgin 1996)

d) MDPV
(Koppe 1969)

Figure 11. Chemical structures of cathinone derivatives.

The selection of the target compounds as well as the planning and organization of their synthetic routes was the first step of this current project. This selection was based on their relevance in the actual drug market, along with reagents availability and the needs for forensic electrochemical data. The synthetic routes were chosen based on the number of reaction steps, control of parallel side reactions, reagents and instrumentation available for the synthesis and purification and the overall cost of the methodology.

The aim of this project relied on the synthesis of compounds of the following chemical families:

i) phenethylamines, some relevant analogs and their main synthetic intermediates;

ii) piperazines and some relevant analogs.

2.2.2 Synthesis of *β*-phenethylamine derivatives and intermediates

α-methyl-*β*-phenethylamine and *β*-phenethylamine derivatives

Phenethylamines comprises a benzene ring attached to a amine group via an ethyl side chain. Phenethylamine derivatives fulfill an important biological function in the living world and are importante intermediates for many pharmacological active compounds [92]. There is a vast number of synthetic routes in the literature to prepare *α*-methyl-*β*-phenethylamines[3] [93], although few routes allow the preparation of both *α*-methyl-*β*-phenethylamine and *β*-phenethylamine derivatives. Of the reported methods, the most important routes to obtain these amine derivatives are via nitrostyrene (Figure 12, pathway a) and via nitrile (Figure 12, pathway b) and both of these routes start from the benzaldehyde with the corresponding substitution pattern. The synthetic route via nitrile intermediate is suited to the large scale prodution and some optimization have been done [94]. The cyanide compounds are obtained by reducing the aldehyde with sodium borohydride [95] or using copper chromite in a current of hydrogen [96] arising an alcohol. The obtained alcohol reacts in dry benzene with thionyl chloride and later with sodium cyanide in a ketone solvent to yield the target compound, a nitrile derivative [95]. To obtain the amine derivative, hydrogenation of the nitrile over a skeletal of a nickel catalyst (Raney Nickel) with alcohol and ammonia have been described to be used in industrial processes with good yields [97].

The route for preparation of substituted *β*-phenethylamines via nitrostyrene intermediate is shorter, avoids working with sodium cyanide and allows the preparation of both *β*-phenethylamines and its *α*-methyl derivatives.

Catalytic hydrogenation of *β*-nitrostyrenes over palladium on charcoal at low temperature has been described with good yields, however these methods are not convenient because the reactions have to be carried out in strong acidic solutions, with tedious treatments and severe reaction conditions (*e.g.* 50-80 ºC under 30-100 atm) [98, 99]. The use of $LiAlH_4$ in large scale productions is dangerous, although in the context of this small scale work few amounts of this reducting agent are needed. After

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[3] **Note on nomenclature:** To improve the overall clarity of the text it was decided to follow the usual naming found in specialized literature. In phenethylamines ($PhCH_2\underline{C}H_2NH_2$) the considered alpha-carbon (Cα) is the one attached to the amine group; in nitrostyrenes ($Ph\underline{C}H{=}CHNO_2$), the Cα is the one attached to the phenyl group.

the nitroaldolic condensation, the nitrostyrene derivatives were submited to a simultaneous reduction of the double bond and nitro group by $LiAlH_4$ under anydrous conditions and inert atmosphere. The specific experimental conditions used will be described in the experimental section.

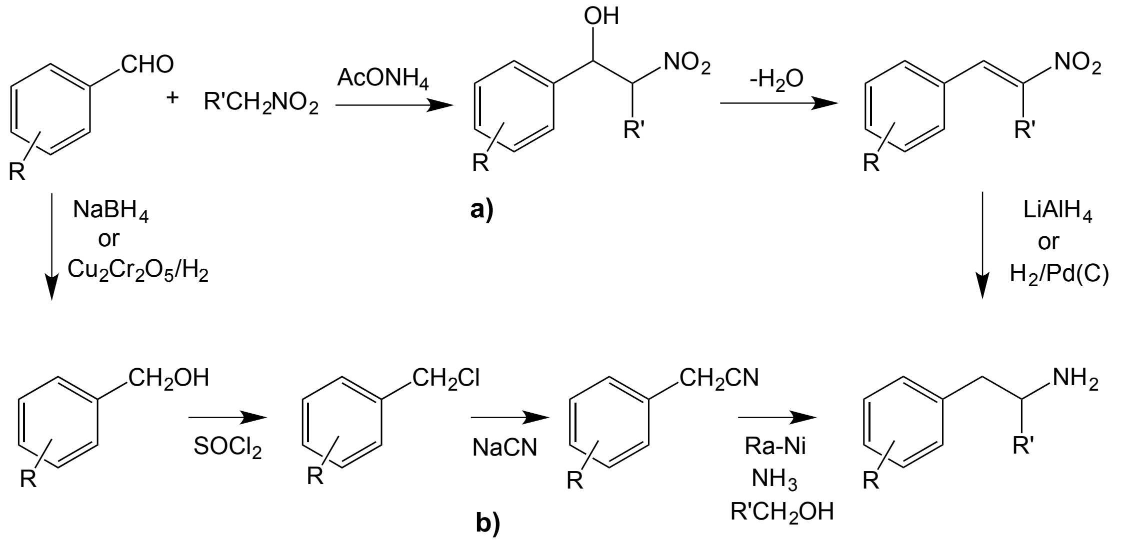


Figure 12. Two synthetic strategies for obtaining *β*-phenethylamine derivatives (R'=H: *β*-phenethylamines; R'=CH_3: *α*-methyl-*β*-phenethylamine).

2.2.3 Synthesis of *β*-nitrostyrene and *β*-methyl-*β*-nitrostyrene derivatives

Nitroalkenes or nitroolefins combine the functionality of their constituent parts, an alkene and a nitro group (NO_2). These compounds which are mainly based on a *β*-nitrostyrene moiety are important raw materials with several applications in organic synthesis. These conjugated nitroolefins work as activated alkenes that can be used as starting materials to obtain other nitroalkenes, amines, hydroxylamines, aldoximes, ketoximes, or aldoximes [100].

The *β*-nitrostyrene derivatives can be obtained by direct nitration from styrene [101]. However, the most common synthetic route is a nitroaldolic condensation between benzaldehyde, with the substitution pattern of the required final compound, and the adequate nitroalkene. In the first step, this route involves a Henry reaction between the carbonyl compound and the nitroalkane in basic conditions, where a nucelophilic attack of the carbanion of the nitroalkene to the carbonyl occurs, stabilized by the electron withdrawing group. This step yields a *β*-nitroalcohol, which, after dehydration, forms the intended *β*-nitrostyrene (Figure 12a). The formed intermediate is difficult to isolate, especially when aromatic aldehydes are used [102]. The nitroaldolic condensation is a classic synthetic reaction that implies the formation of a carbon-carbon bond. This reaction occurs easily in the presence of basic catalysis, with organic or inorganic nature, as primary and tertiary amines, alkaline metal hydroxides, carbonates and alkoxides, among others [102, 103]. Ammonium acetate and buffer solution of ammonium acetate in acetic acid are the most common catalysts used in the Henry reaction [104]. Other strategies as the use of microwaves has been proposed to minimize heating at high temperatures [103]. Unwanted parallel reactions can occur along with the aldolic condensation, as the *Cannizzaro* reaction or polymerization can accur simultaneously to the formation of the nitroalkenes [105]. These parallel reactions can be minimized, or even avoided, with the use of the proper catalyst [100].

This condensation reaction proceeds in a non-aqueous environment because nitrostyrenes are highly insoluble in water. The final compounds are usually very coloured, from light yellow to intense orange, due to the existence of a pronounced electronic delocalization through the double bond and the aromatic ring (highly conjugated system). The *β*-nitrostyrene derivatives show a predominance of the *E* isomer (trans) [106].

In the present work the synthethic route used to prepare the *β*-nitrostyrene and *β*-methyl-*β*-nitrostyrene derivatives was the Henry's reaction. Accordingly, the benzaldehyde with the corresponding substitution pattern was condensed with nitromethane, for the *β*-nitrostyrene, or nitroethane, for the *β*-methyl-*β*-nitrostyrene. The nitroalkanes themselves were used as solvents and were heated in reflux, using ammonium acetate as catalyst.

2.2.4 Synthesis of halogenated *β*-phenelethylamine derivatives

Electrophilic aromatic substitution reaction is one of the most widely used and extensively studied class of organic reactions. The reactivity towards substitution is affected by the substituents in the aromatic substrate, the nature of the electrophile and the polarity of the solvent. Usually electrophilic substitution requires a Lewis acid catalyst such aluminium trichloride or iron [107]. However, the halogenation of some benzene derivatives only requires a strong polar solvent, such acetic acid [108]. Methoxy and alkylamine groups are, respectively, strong and medium activating groups and ortho, para directors in the benzene ring. Taking in account with the indutive and ressonance effects and the position of the substituents, the 4-position in the aromatic ring is highly activated. These conditions turn the halogenation possible by using only a highly polar solvent, such acetic acid, without the presence of a catalyst. In adition, some work done in the halogenation of phenyalkylamines indicate that amines have been found to catalyse halogenation of activated aromatic species, and this has been attributed to the formation, *in situ*, of *N*-haloamines [109]. The brominations in the present work were performed by the mixture of elemental bromine to the corresponding amines in acetic acid. The specific conditions will be described in the experimental section.

2.2.5 Synthesis of *N*-benzylated *β*-phenethylamine derivatives

Reductive amination is one of the simplest ways of preparing secondary amines. The reductive amination can be performed in a direct (Figure 13a) and in an indirect (Figure 13b) pathway. In an indirect reductive amination the primary amine is condensated with an aldehyde or ketone to form an imine or Schiff base. Then, the imine is reduced, often with a complex hydride reducing agent, yielding the secondary amine [110]. In the direct reductive amination mild borohydride reagents, such $NaBH_3CN$ or $NaBH(OAc)_3$ are used and usually the borohydride is mixed together with the aldehyde and the amine to reduce the iminium intermediate without reducing the aldehyde or ketone. Catalytic hydrogenation also can be used to reduce the imine [111].

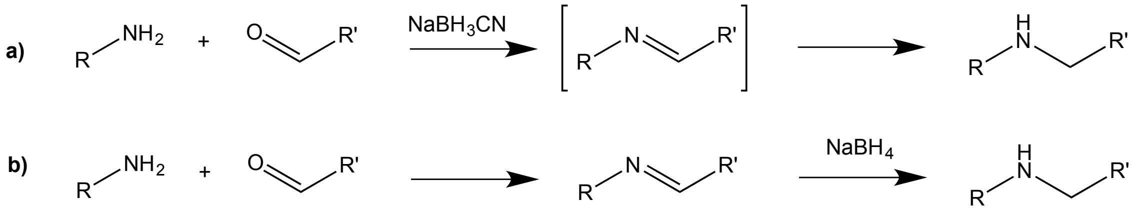


Figure 13. Scheme of a) direct and b) indirect reductive amination.

Other route to prepare secondary amines is via formation of a secondary amide. The secondary amide is formed from carboxylic acids [112], esters [113] or anhydrides [114] and then converted to a secondary amine with a strong reducing agent as $LiAlH_4$ [115], AlH_3 [116] or diisobutylaluminium hydride (DIBAL) [117].

For the purpose of this work the reductive amination was the first choice reaction. Comparing with via amide intermediate, the conditions are milder, which is an advantage because not all substrates are compatible with these conditions. Also, the starting materials for reductive amination are easily prepared or are commercially available besides the work-up of the reduction of amides can be tedious and somehow messy. The indirect reductive amination is a better choice when primary amines or aldehydes are used to avoid dialkylation.

2.2.6 Synthesis of phenylpiperazine derivatives

Phenylpiperazine is a simple chemical compound featuring a phenyl group bound to a piperazine ring. Phenylpiperazine and its derivatives are an important class of CNS drug discovery process and can be used as building blocks for the preparation of several bioactive compounds with distinct pharmacological activities [118]. A number of methods have been explored to prepare complex phenylpiperazine derivatives [63, 119, 120]. However, the most easy and effective synthetic route to synthesize mono-subtituted phenylpiperazines is the convertion of substituted anilines into phenylpiperazines, by reaction with a nitrogen mustard-type reagent, the bis(2-chloroethyl)amine (Figure 14). Some variations of this route have been applied, as the use of the bis(2-chloroethyl)amine in alkoxycarbonyl-protected form [121] or in its HCl salt form [122]. The use of the more reactive counterpart, the bis(2-bromoethyl)amine [122] or the use iodide salt as a catalyst in the reaction was also studied [123].

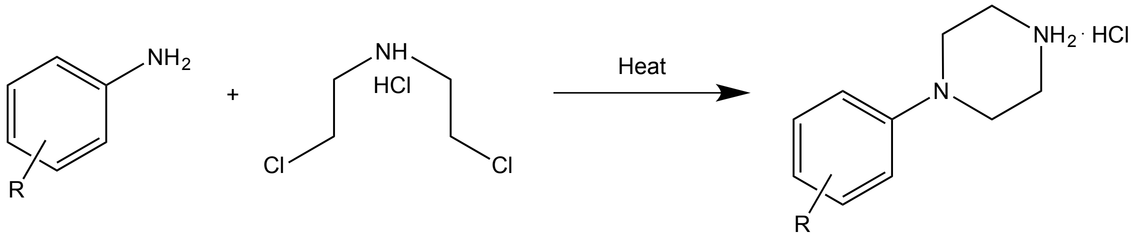


Figure 14. Synthesis of phenypiperazine derivatives.

2.3 Electrochemical analysis: voltammetry

2.3.1 Background

As published by a relevant number of research groups, a range of laboratory-based chromatographic techniques, specially gas chromatography/ mass spectrometry (GC-MS) and liquid cromatography/mass spectometry (LC-MS) have been employed in the analysis of new psychoactive substances [124-131] (Table 2).

Table 2. GC-MS and LC-MS-based analytical methods for the analysis of NPS.

Target analyte	Analytical Method	Detection	Matrix	Ref.
2C-B, 2C-H, 2C-I, 2C-T-7, 2C-T-2, 4-MTA, DOB, DOET, DOI, DOM	GC-MS	EI, SIM	Urine	[124]
AMT, DMT, DPT, 5-MeO-DIPT, 2C-B, 2C-T-7	GC-MS	EI, SIM	Urine, Blood	[125]
TFMPP, mCPP, MeOPP	GC-MS	EI, SIM	Hair	[126]
2,5-DMA, 2C-B, 2C-D, 2C-H, 2C-I, 2C-P, 2C-T-2, 2C-T-4, 2C-T-7, TMA, 3,4-DMA, 4-MTA, 5-MeO-DMT, AMT, BZP, DiPT, DMT, DOB, DOET, DOM, DPT, mCPP, MDBP, MDDMA, MeOPP, MiPT, PMA, PMMA, TFMPP, TMA-6	LC-MS/MS	ESI+, MRM	Plasma	[127]
BZP, TFMPP, 3-HO-BZP, 4-HOBZP, 4-HO-TFMPP	LC-MS/MS	ESI+, SIM	Plasma, Urine	[128]
DMT, NMT, 5-MeO-DMT, bufotenine	LC-MS/MS	ESI+, MRM	Urine	[129]

Abbreviations: EI, Electron Ionisation; SIM, selected ion monitoring; ESI, electronspray ionization; MRM, multiple reaction monitoring; 2C-I, 2,5-dimethoxy-4-iodophenethylamine; 2C-T-7, 2,5-dimethoxy-4-propylthiophenethylamine; 2C-T-2, 2,5-dimethoxy-4-ethylthiophenethylamine; 4-MTA, 4-methylthioamphetamine; DOET, 2,5-dimethoxy-4-ethylamphetamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; DPT, *N*,*N*-dipropyltryptamine; 5-MeO-DIPT, 5-methoxy-diisopropyltryptamine; 2,5-DMA, 2,5-dimethoxyamphetamine; 2C-D, 2,5-dimethoxy-4-methylphenethylamine; 2C-P, 2,5-dimethoxy-4-(n)-propylphenethylamine; 2C-T-4, 2,5-dimethoxy-4-isopropylthiophenethylamine; TMA, 3,4,5-trimethoxyamphetamine; 3,4-DMA, 3,4-dimethoxyamphetamine; DiPT, diisopropyltryptamine; MDBP, 3,4-methylenedioxybenzylpiperazine; MDDMA, 3,4-methylenedioxy-*N*,*N*-dimethylamphetamine; MeOPP, para-Methoxyphenylpiperazine; MiPT, methyl-isopropyltryptamine; PMA, para-Methoxyamphetamine; PMMA, para-methoxy-*N*-methylamphetamine; TFMPP, 3-Trifluoromethylphenylpiperazine; TMA-6, 2,4,6-trimethoxyamphetamine; 3-HO-BZP, 3-hidroxy-benzylpiperazine; 4-HO-BZP, 4-hidroxy-benzylpiperazine 4-HO-TFMPP, 4-hidroxy-3-trifluoromethylphenylpiperazine.

These techniques are mainly directed for laboratory-based and not for *in situ* analysis (*e.g.* parties where the substances are used, emergency rooms, etc.). Electrochemistry has been shown as an important area and electrochemical studies of bioactive compounds are important to understand the redox profile of the substances [42, 43, 47]. As already described is this text, the possibility of building electrochemical sensors able to perform analysis of psychoactive substances *in situ* is of huge interest. However, regarding psychoactive substances there are only a few studies about electrochemistry, mostly about electrooxidation and reduction of derivatives of *α*-methyl-*β*-phenethylamines [132-134], cathinones [135] and intermediates as *β*-methyl-*β*-nitrostyrenes [136, 137]. Therefore, at this moment, it can be considered a neglected field. Besides the importance of redox data for areas such forensic chemistry, helping in the development of new forensic assays, is also noteworthy its interest in toxicologic studies, helping to reveal metabolic pathways [42, 44].

Electrochemical techniques are important tools in fundamental studies of oxidation and reduction of organic and inorganic compounds. Among these, polarographic and voltammetric techniques are the principal ones [138]. Their wide application is related with the comparatively non-expensive instrumentation, very good selectivity and rapid analysis time. When compared with other techniques, as chromatography, the effect of interferents (*e.g.* other substances) found is low [42, 138]. Actually, voltammetric techniques main advantages are: (i) the direct measurement on the sample, without previous separation or purification steps; (ii) the speed of the analysis linked to low-cost instruments and materials; (iii) high selectivity even in the presence of chemical interferents.

Voltammetric experiments are carried out in simple electrochemical cells that consist in multi-phase systems in which electrical energy is used to bring about a chemical change (electrolysis) in the species of the cell. The electrochemical cell consists in three conductors (electrodes), immersed in an electrolytic conductor (ionic solution) containing the substance of analytical interest (analyte). The application of a voltage or current from an external source to the electrodes produces an electrical response from the analyte in the cell in solution. The nature of this response may be used [139].

2.3.2 Cyclic voltammetry

Cyclic voltammetry (CV) is perhaps the most versatile analytical technique for the study of electroactive species. Some researchers call it the electrochemical spectroscopy [140]. The effectiveness of cyclic voltammetry comes from its ability to study the redox behaviour over a potential wide range. The resulting voltammogram is analogous to a conventional spectrum in that it conveys information as a function of an energy scan. In cyclic voltammetry, starting from an initial potencial (E_i), a potential sweep in the form of staircase is applied to the working electrode (Figura 15a). After reaching the end (E_f), the potential is reversed and it returns to the initial potential (E_i). The most important parameter in CV is the scan rate, controlling the timescale of voltammetric experiment. The voltammogram is the instrumental output of voltammetry, and the cyclic voltammogram is usually a current-potential curve. The main features of this voltammogram are the cathodic and anodic peak potentials, the cathodic and anodic peak currents, and the half-peak potential (Figura 15b) [138, 141].

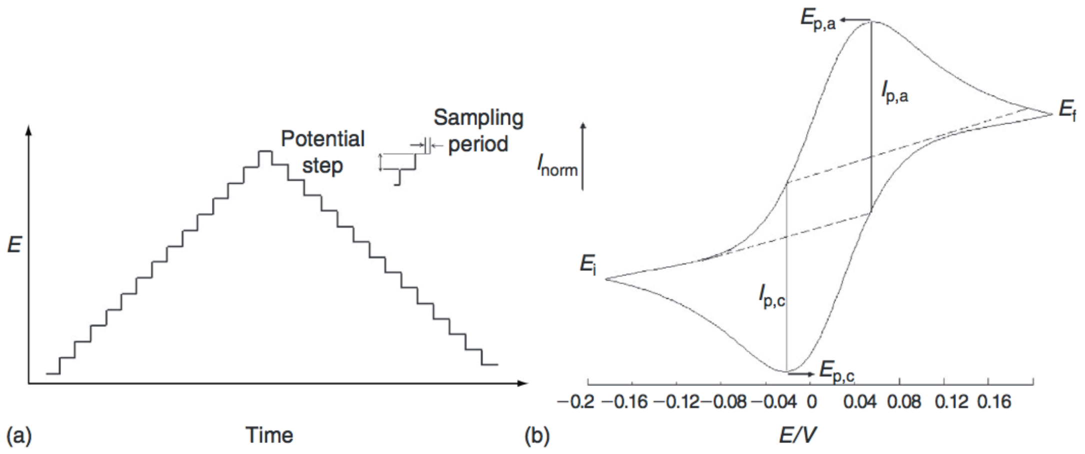


Figure 15. (a) Staircase potencial used in cyclic voltammetry and (b) a cyclic voltammogram simulated for one-electron reversible charge transfer.: $E_{p,c,}$ cathodic peak potential; $E_{p,a,}$ anodic peak potential; E_i, initial potential; $E_{f,}$ switching potential, $I_{p,c,}$ cathodic peak current; $I_{p,a}$, anodic peak current. Adapted from [141].

2.3.3 Differential pulse voltammetry

The potential applied between electrodes to initiate electron exchange causes charging and discharging of the electrical double layer in the interface electrode-electrolyte. This charge is called non-faradaic or capacitive current, and interferes with measurements. To minimize it, and increase sensitivity, pulse techniques have been developed. The capacitive current decays more with time than faradaic current in diffusion-controlled electrode reactions, therefore sampling currents in the end of applied pulses turn possible to get negligible capacitive currents. This is the theory behind normal pulse voltammetry, where potential pulses with constant width and increased amplitudes are applied and the current measured at the end of each pulse.

When a series of periodical constant pulses of potencial are superimposed to a linear scanning, a consistent enhancement of signal is achieved (Figure 16). If the intensity of current is measured as the difference just before and at the end of the pulse, the interference of capacitive current is lower than in normal pulse voltammetry [138, 141].

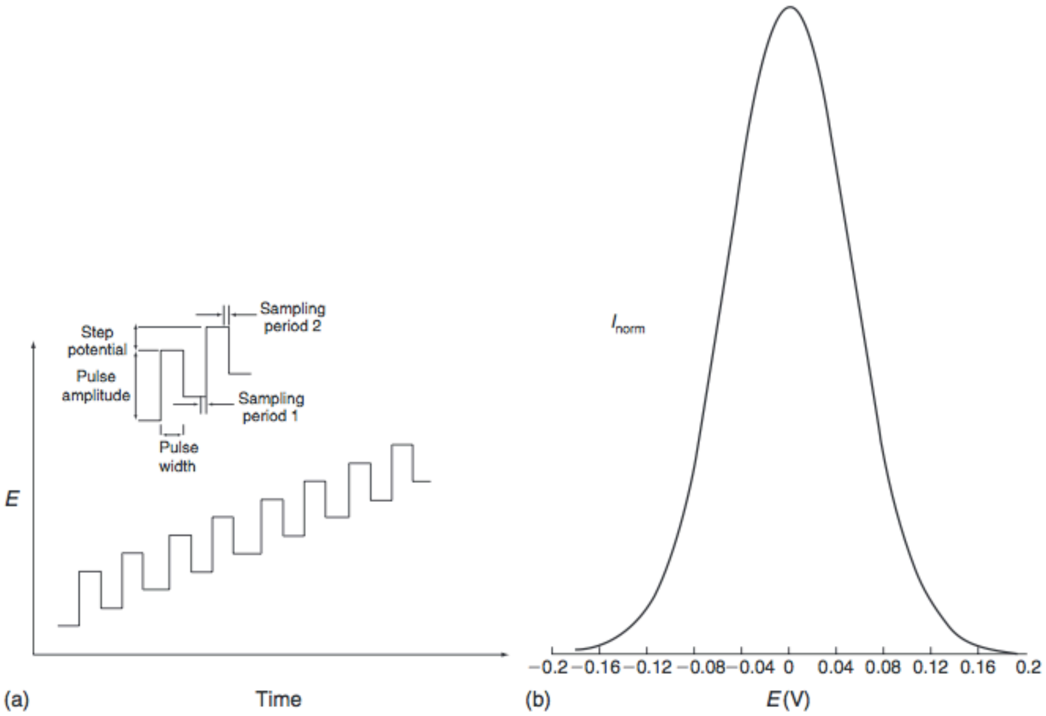


Figure 16. (a) The potential form used in differential pulse voltammetry and (b) an example of a differential pulse voltammogram. Adapted from [141].

3. Experimental

3.1 Materials and Methods

3.1.1 Reagents and Solvents

The solvents and reagents used were *pro analysis* grade and were acquired from *Merck*, *Sigma-Aldrich*, *Carlo Erba*, *Alfa Aesar* and *Fluka*. The argon was of the highest grade available. All reagents were used without additional further purification.

3.1.2 Apparatus and Analytical Conditions

All reactions were monitored by Thin-Layer Chromatography (TLC) performed on 0.2 mm *Merck* aluminium sheets precoated with silica gel 60 F254.

For analytical control of the purity of the final compounds and follow-up of the reactions different eluent systems were used and specified for each synthesis, achiving optimal resolution of the components. Chromatograms were visualized under a *Vilber Lourmat* ultra-violet (UV) light with the wavelenght of 254 and/or 365 nm.

The reagents and all other compounds were weighed in a *Kern ABJ-NM/ABS-N* scale, the solvents were evaporated in a rotary vacuum evaporator *Buchi Rotavapor R-210* and the compounds were dried in a vacuum oven *Mti Corporation*. *SONICA Ultrasonic Cleaner* was used for the sonication of some of the synthesized compounds.

Short-path vacuum distillation was performed on a *Büchi Glass Oven B-585*. Advanced automated flash purification from *Biotage Isolera™ Prime* (silica gel Merck 0.040-0.063 mm) was also used in the isolation of some of compounds.

^{1}H and ^{13}C NMR data were recorded, at room temperature, on a *Bruker Avance III 400* located in Centro de Materiais da Universidade do Porto (CEMUP, Porto, Portugal) operating at 400 MHz and 101 MHz, respectively, and processed using *MestreNova* software. Dimethyl sulfoxide-*d6* was used as solvent unless specified in spectroscopic data. Assignments were also made from DEPT (distortionless enhancement by polarization transfer) experiments. In the ^{1}H-NMR spectra the chemical shifts values are reported (expressed in δ, ppm) relative to tetramethylsilane (TMS), along with multiplicity of the signal, number of protons and coupling constants (expressed in Hertz (Hz)). In ^{13}C-NMR spectra the chemical shifts values are reported, expressed in δ (ppm), with the DEPT spectra values appearing underlined.

Mass spectra were obtained by electron impact mass spectrometry (EI-MS) using a Hewlett-Packard 5888A. The data are reported as m/z (% of relative intensity of the most important fragments). The melting points of all synthesized compounds were measured using a Stuart Scientific from Bibby Scientific and are uncorrected.

Voltammetric experiments were performed at room temperature using *Metrohm Autolab PGSTAT 12* potentiostat/galvanostat. The measurements were carried out in one-compartment glass electrochemical cell equipped with three-electrode system of a bare or modified glassy carbon working electrode *GCE* (d= 2 mm), a platinum wire counter electrode and an Ag/AgCl (saturated KCl) reference electrode. To voltammeric measurements universal buffer solutions were used with a pH range between 1 and 12 (Table 3). A pH-meter *Crison* with glass electrode was used for preparation of these buffer solutions.

Stock solutions of the tested compounds (10 mM) were prepared by dissolving the appropriate amount in ethanol. The voltammetric working solutions were prepared, in the electrochemical cell, to get a final concentration of 0.1 mM. The pH 7.3 supporting electrolyte was prepared by diluting 6.2 mL of 0.2 M dipotassium hydrogen phosphate and 43.8 mL of 0.2 M potassium dihydrogen phosphate to 100 mL.

Conditions used in differential pulse voltammetry and cyclic voltammetry assignments are spectified the results section.

Table 3. Buffer solutions used in voltammetric studies

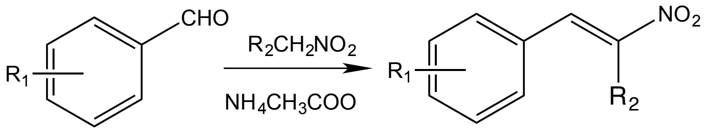
Buffer Solutions *			**pH**
KCl 0.2M (25.0 mL)	+	HCl 0.2 M (42.5 mL)	1.2
KCl 0.M (25.0 mL)	+	HCl 0.2 M (6.5 mL)	2.0
CH_3COONa 0.2M (3.7 mL)	+	CH_3COOH 0.2M (46.3 mL)	3.4
CH_3COONa 0.2M (13.2 mL)	+	CH_3COOH 0.2M (36.8 mL)	4.3
CH_3COONa 0.2M (41.2 mL)	+	CH_3COOH 0.2M (8.8 mL)	5.4
Na_2HPO_4 0.2M (6.2 mL)	+	NaH_2PO_4 0.2 M (43.8 mL)	6.1
Na_2HPO_4 0.2M (30.5 mL)	+	NaH_2PO_4 0.2 M (19.5 mL)	6.9
Na_2HPO_4 0.2M (47.4 mL)	+	NaH_2PO_4 0.2 M (2.6 mL)	8.0
$Na_2B_4O_7.10H_2O$ 0.2M (50.0 mL)	+	NaOH 0.2 M (0.2 mL)	9.2
NH_4Cl 0.2 M (7.0 mL)	+	NH_3 0.2 M (8.0 mL)	9.2
$Na_2B_4O_7.10H_2O$ 0.2M (50.0 mL)	+	NaOH 0.2M (18.3 mL)	10.0
NH_4Cl 0.2M (2.0 mL)	+	NH_3 0.2M (8 mL)	10.2
Na_2HPO_4 0.2M (50.0 mL)	+	NaOH 0.2M (4.1 mL)	11.0
KCl 0.2M (25.0 mL)	+	NaOH (6.0 mL)	12.1

* (dilluition to 100 mL)

3.2 Synthetic Procedures

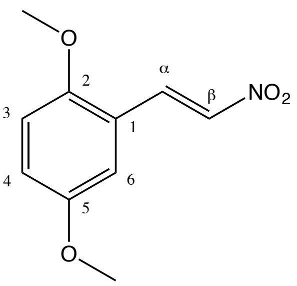
3.2.1 Synthesis of *β*-nitrostyrenes

General Procedure



The synthetic methods used to obtain β-nitrostyrenes and β-methyl-β-nitrostyrenes was adapted from previously described literature [24, 134]. Briefly a solution of benzaldehyde with the aromatic substitution pattern of the target compound (*ca.* 40 mmol) in nitromethane or nitroethane (*ca.* 20 mL) was treated with ammonium acetate (*ca.* 10 mmol) and refluxed for 4 to 8 h. After cooling the reaction mixtures to room temperature, the solvent was partially evaporated, diluted with diethyl ether, and washed twice with 100 mL of water. The organic layer was dried over anhydrous sodium sulphate ($NaSO_4$), filtered and concentrated. The obtained residues were recrystalized from hot isopropyl alcohol, diethylether/petroleum benzene (40-60º) or methanol/H_2O and yellow to red crystals were obtained.

3.2.1.1 Synthesis of 2,5-dimethoxy-*β*-nitrostyrene (compound 1)



In a three-necked round bottom flask, 2,5-dimethoxybenzaldehyde (4.26 g, 26.0 mmol) and ammonium acetate (0.42 g, 5.5 mmol) were dissolved in nitromethane (15 mL). The reaction was refluxed for 7h and monitored by TLC (diethyl ether/petroleum benzene (5:5)). After cooling the reaction to room temperature, a dense brown solid formed and after filtration and washing with water (75 mL) to remove remaining nitromethane and ammonium acetate, the solid was left to dry in the vaccum oven for several days. The crystalization from isopropyl alcohol yielded a dark yellow/orange solid (5.06 g).

Yield: 93%

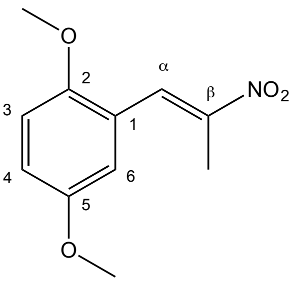
^{1}H NMR (400 MHz, DMSO) δ= 3.76 (3H, s, 5-OCH_3), 3.88 (3H, s, 2-OCH_3), 7.09-7.15 (2H, m, H3 and H4), 7.42 (1H, d, *J* = 2.4 Hz, H6), 8.19 (2H, s, Hα and Hβ);

^{13}C NMR (101 MHz, DMSO) δ= 56.2 (5-OCH_3), 56.8 (2-OCH_3), 113.7 (C6), 115.4 (C4), 119.2 (C1), 120.7 (C3), 134.7 (Cβ), 138.8 (Cα), 153.6 (C2), 153.9 (C5);

MS (EI) m/z (%): 209 ($M^{\cdot+}$, 100), 178 (10), 162 (48), 148 (47), 147 (36), 133 (44), 105 (17), 98 (16), 91 (19), 84 (14), 77 (32).

Melting Point: 113-115 ºC

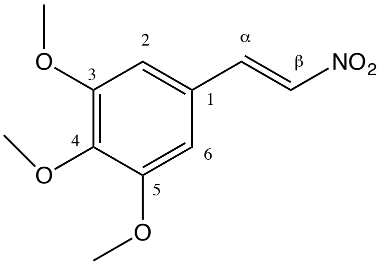
3.2.1.2 Synthesis of 2,5-dimethoxy-*β*-methyl-*β*-nitrostyrene (compound 2)



In a three-necked round bottom flask, 2,5-dimethoxybenzaldehyde (2.18 g, 17.0 mmol) and ammonium acetate (0.19 g, 2.1 mmol) were dissolved in nitroethane (15 mL). The reaction was refluxed for 7h and monitored by TLC (diethyl ether/petroleum benzene (5:5)). After cooling the reaction to room temperature, a dense brown solid formed and after filtration and washing with water (75 mL) to remove remaining nitromethane and ammonium acetate, the solid was left to dry in the vaccum oven for several days. Crystalization from isopropyl alcohol yield a dark yellow solid (2.77 g, η =77%).

After TLC and melting point control against an analytical standard that was previously prepared by the research group of Medicinal Chemistry of Centro de Investigação em Química da Universidade do Porto, the compound was used without further spectroscopic characterization.

3.2.1.3 Synthesis of 3,4,5-trimethoxy-*β*-nitrostyrene (compound 3)



A mixture of 3,4,5-trimethoxybenzaldehyde (1.37 g, 7.0 mmol) and ammonium acetate (0.078 g, 1.0 mmol) was dissolved in nitromethane (15 mL). It is important to mention that in this particular synthesis the %mol of ammonium acetate relative to the benzaldehyde was kept low (13 %mol), since higher amounts (>20%mol) seems to provide extensive side reactions. The temperature was then raised to 100 ºC for 8h. After, the volatiles were removed by vaccum distillation and the obtained brown oil was dissolved in a small portion of diethyl ether that was washed with water (3 × 75 mL). The organic layer was dried over $NaSO_4$, filtered and evaporated. The remaining oil was dissolved in small quantity of methanol and the addition of cold water revealed intense orange crystals (1.22 g). The TLC system used to monitor the reaction was diethyl ether/petroleum benzene (5:5).

Yield: 73%

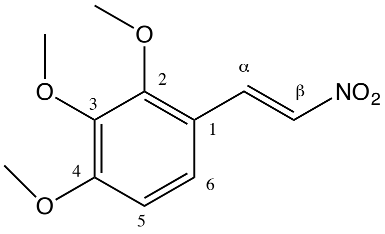
^{1}H NMR (400 MHz, DMSO) δ= 3.73 (3H, s, 4-OCH_3), 3.83 (6H, s, 3-OCH_3 and 5-OCH_3), 7.25 (2H, s, H2 and H6), 8.06 (1H, d, *J* = 13.5 Hz, Hα), 8.28 (1H, d, *J* = 13.5 Hz, Hβ);

^{13}C NMR (101 MHz, DMSO) δ= 56.5 (4-OCH_3), 56.7 (3-OCH_3), 57.0 (2-OCH_3), 97.8 (C5), 109.9 (C1), 113.4 (C6), 135.1 (Cβ), 135.7 (Cα), 143.6 (C3), 154.9 (C2), 156.0 (C4);

MS (EI) m/z (%): 239 ($M^{\cdot+}$, 100), 224 (18), 192 (66), 178 (23), 177 (27), 163 (24), 149 (25), 135 (19), 107 (17), 92 (16), 77 (18).

Melting Point: 128-130 ºC

3.2.1.4 Synthesis of 2,3,4-trimethoxy-*β*-nitrostyrene (compound 4)



A mixture of 2,3,4-trimethoxybenzaldehyde (3.49 g, 17.8 mmol) and 0.14 g (1.8 mmol) of ammonium acetate was dissolved in nitromethane (20 mL). The solution was heated for 8h. The nitromethane was removed by vaccum distillation giving a red orange oil. The obtained oil was dissolved in a small portion of diethyl ether that was washed with water (3 × 100 mL). The organic layer was dried over $NaSO_4$, filtered and evaporated. The crystalization from methanol/H_2O yielded a red solid (3.59 g). The TLC system used to monitor the reaction was diethyl ether/petroleum ether (5:5).

Yield: 86%

^{1}H NMR (400 MHz, DMSO) δ = 3.76 (3H, s, 2-OCH_3), 3.89 (3H, s, 3-OCH_3), 3.94 (3H, s, 4-OCH_3), 6.77 (1H, s, H6), 7.38 (1H, s, H5), 8.11 (1H d, *J* = 13.5 Hz, Hα), 8.21 (1H, d, *J* = 13.5 Hz, Hβ);

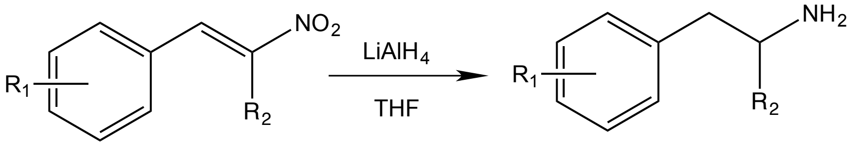
^{13}C NMR (101 MHz, DMSO) δ= <u>56.7</u> (2C, 3-OCH_3 and 5-OCH_3), <u>60.7</u> (4-OCH_3), <u>108.2</u> (2C, C2 and C6), 126.1 (C1), <u>137.9</u> (Cβ), <u>140.2</u> (Cα), 141.3 (C4), 153.6 (2C, C3 and C5);

MS (EI) m/z (%): 239 ($M^{\cdot+}$, 100), 224 (8), 192 (36), 178 (13), 177 (46), 163 (14), 149 (28), 135 (13), 134 (14), 119 (13), 92 (19), 77 (19).

Melting Point: 118-120 ºC

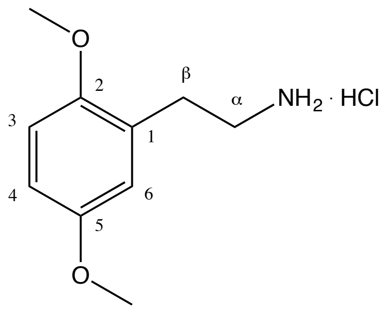
3.2.2 Synthesis of *β*-phenethylamines and *α*-methyl-*β*-phenethylamines

General Procedure



A solution of the appropriate *β*-nitrostyrene or *β*-methyl-*β*-nitrostyrene (*ca.* 8.0 mmol) in anhydrous tetrahydrofuran (THF) (*ca.* 30 mL) was added dropwise to a stirred suspension of lithium aluminum hydride ($LiAlH_4$) (*ca.* 20 mmol) also in anhydrous THF (*ca.* 50 mL) under argon. The reaction mixture was brought and maintained in reflux for 16 to 36h. After cool down to room temperature the excess $LiAlH_4$ was destroyed with a small portion of ice/water. Additional THF was required for easy stirring and more water was needed to the complete conversion of the aluminum salts to a loose, filtrable solid residue. The inorganic residue was then removed by vacuum filtration over celite, the solvent dried under anhydrous Na_2SO_4, evaporated, and the residue diluted with diethyl ether and extracted with HCl 2M solution (3 × 100 mL). The acidic extract was alkalinized with a 4M NaOH solution and extracted with diethyl ether (3 × 100 mL). The organic layer was further dried over anhydrous $NaSO_4$, filtered and concentrated to yield light brown oily residues. The residue was purified by short path distillation giving a colourless/pale yellow oil that was dissolved in isopropyl alcohol, treated with 2M HCl ethereal solution and crystalized from diethyl ether.

3.2.2.1 Synthesis of 2,5-dimethoxy-*β*-phenethylamine (compound 5)



To a suspension of $LiAlH_4$ (0.97 g, 25.6 mmol) in anhydrous THF (50 mL) a solution of 2,5-dimethoxy-*β*-nitrostyrene (1.07 g, 5.1 mmol) in anhydrous THF (30 mL) was added dropwise. The reaction mixture was refluxed under argon and acquired an intense dark red colour that faded away after 2.5h reacting. The reaction was quenched with ice/water after 14h boiling. The inorganic residue was removed by vacuum filtration, the solvent dried under anhydrous Na_2SO_4, evaporated, and the residue diluted with diethyl ether and then extracted with a HCl 2M solution (3 × 100 mL). The acidic extract was alkalinized with a 4M NaOH solution and extracted with diethyl ether (3 × 75 mL). The organic solvent was dried over anhydrous Na_2SO_4, filtered and concentrated to yield a residue of brown oil (0.94 g). The residue was distilled at 110-120ºC / 0.260 Torr giving a light yellow oil. The HCl salt was prepared according to the general procedure and a yellow powder was obtained (0.86 g).

Yield: 78%

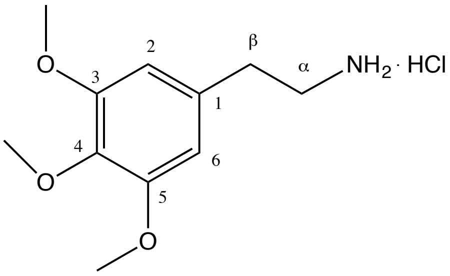
^{1}H NMR (400 MHz, DMSO) δ= 2.81-2.85 (2H, m, Hα), 2.96 (2H, t, *J*= 7.2 Hz, Hβ), 3.70 (3H, s, 2-OCH_3); 3.75 (3H, s, 5-OCH_3), 6.78-6.82 (2H, m, H4 and H6), 6.92 (1H, d, *J* = 8.5 Hz, H3), 7.93 (3H, *br*s, NH_3^+);

^{13}C NMR (101 MHz, DMSO) δ: $\underline{29.5}$ (Cβ), $\underline{40.0}$ (Cα), $\underline{56.7}$ (5-OCH_3), $\underline{57.1}$ (2-OCH_3), $\underline{113.2}$ (C3), $\underline{113.6}$ (C4), $\underline{117.8}$ (C6), 127.5 (C1), 152.6 (C2), 154.4 (C5);

MS (EI) m/z (%): 181 ($M^{\cdot+}$, 36), 152 (100), 137 (62), 121 (36), 108 (10), 91 (20), 77 (24).

Melting Point: 137-139 ºC

3.2.2.2 Synthesis of 3,4,5-trimethoxy-*β*-phenethylamine (compound 6)



To a suspension of $LiAlH_4$ (1.62 g, 43.0 mmol) in anhydrous THF (50 mL) a solution of 3,4,5-trimethoxy-*β*-nitrostyrene (1.81 g, 7.6 mmol) in anhydrous THF (30 mL) was added dropwise and the reaction mixture was refluxed under argon. The reaction was quenched with ice/water after 36h boiling. The inorganic residue was removed by vacuum filtration, the solvent dried under anhydrous Na_2SO_4, evaporated, and the residue diluted with diethyl ether and then extracted with a HCl 2M solution (3 × 100 mL). The acidic extract was alkalinized with a 4M NaOH solution and extracted with diethyl ether (3 × 75 mL). The organic solvent was dried over anhydrous Na_2SO_4, filtered and concentrated to yield a residue of a dark brown oil (1.23 g). The residue was distilled at 130-150ºC / 0.220 Torr giving a yellow oil. The HCl salt was prepared according to the general procedure and a white powder was obtained (0.79 g).

Yield: 42%

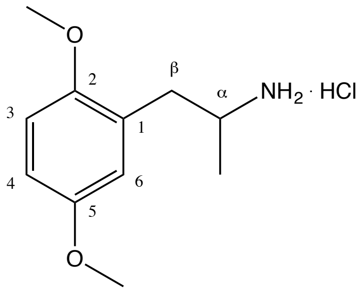
^{1}H NMR δ= 2.80 (2H, t, *J*=7.6 Hz, Hβ), 3.03 (2H, m, Hα), 3.64 (3H, s, 4-OCH_3), 3.77 (6H, s, 3-OCH_3 and 5-OCH_3), 6.57 (2H, s, H2 and H6), 7.76 (3H, *br*s, NH_3^+);

^{13}C NMR (101 MHz, DMSO) δ: 33.7 (Cβ), 40.5 (Cα), 56.3 (2C, 3-OCH_3 and 5-OCH_3), 60.4 (4-OCH_3), 106.5 (2C, C2 and C6), 133.3 (C1), 136.7 (C4), 153.4 (2C, C3 and C5);

MS (EI) m/z (%): 211 ($M^{\cdot+}$, 48), 194 (18), 182 (100), 181 (71), 167 (69), 151 (25), 136 (18), 121 (10), 95 (10), 80 (18), 77 (12);

Melting Point: In aquisition

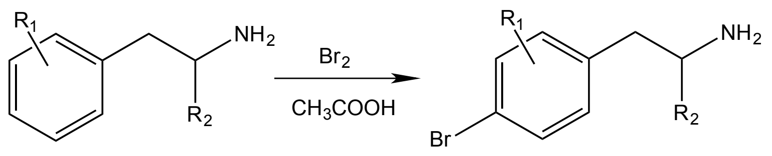
3.2.2.3 Synthesis of 2,5-dimethoxy-*α*-methyl-*β*-phenethylamine (compound 7)



To a suspension of $LiAlH_4$ (1.87 g, 49.0 mmol) in anhydrous THF (50 mL) a solution of 2,5-dimethoxy-*β*-methyl-*β*-nitrostyrene (2.01 g, 7.6 mmol) in anhydrous THF (30 mL) was added dropwise and the reaction mixture was refluxed under argon. The reaction was quenched with ice/water after 18h boiling. The inorganic residue was removed by vacuum filtration, the solvent dried under anhydrous Na_2SO_4, evaporated, and the residue diluted with diethyl ether and then extracted with a HCl 2M solution (3 × 100 mL). The acidic extract was alkalinized with a 4M NaOH solution and extracted with diethyl ether (3 × 100 mL). The organic solvent was dried over anhydrous Na_2SO_4, filtered and concentrated to yield a residue of a dark brown oil (1.65 g). The residue was distilled at 135-150ºC / 0.270 Torr giving a yellow oil. The HCl salt was prepared according to the general procedure and a white powder was obtained (1.07 g, η=61%).

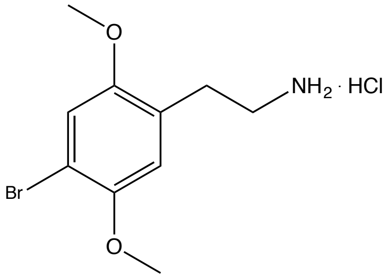
After TLC and melting point control against an analytical standard that was previously prepared by the research group of Medicinal Chemistry of Centro de Investigação em Química da Universidade do Porto, the compound was used without further spectroscopic characterization.

3.2.3 Synthesis of halogenated *β*-phenethylamines



The procedure to halogenate *β*-phenethylamine derivatives was adapted from a previously described procedure [24]. To a solution of the substituted *β*-phenethylamine (*ca.* 3.0 mmol) in acetic acid (*ca.* 15 mL), elemental Bromine (Br_2) (*ca.* 3.5 mmol) was added under vigorous stirring. In a short period a solid spontaneously was formed as the hydrobromide of the expected compound. A solution KOH 4M was added until basic pH was reached and the solution extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent removed. The dark brown residue was destilled recurring to a short path destillator yielding a colourless oils. The residue was treated with 2M HCl ethereal solution and corresponding the salt prepared.

3.2.3.1 Synthesis of 4-bromo-2,5-dimethoxy-*β*-phenethylamine (compound 8)



To a solution of 2,5-dimethoxy-*β*-phenethylamine (0.65 g, 3.0 mmol) in acetic acid (15 mL), elemental bromine (0.180 mL, 3.5 mmol) was added under vigorous stirring. In 5 min an insoluble dark yellow solid was formed. The hydrobromide of the require compound was obtained. A solution of KOH 4M was added until basic pH and solution extracted with CH_2Cl_2 (3 × 75 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent removed. The dark brown residue was destilled at 130ºC - 160ºC at 0.215 Torr yielding a pale yellow oil. The oil residue was after treated with 2M HCl ethereal solution, further diethyl ether was added until a persistent turvation was produced and a white solid was formed (0.15 g).

Yield: 17%

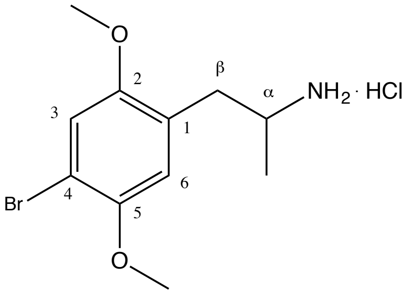
^{1}H NMR (DMSO, 400 MHz): δ= 2.83 (2H, t, *J*=7.5 Hz, Hβ), 2.98 (s, 2H, t, *J*=7.5 Hz, Hα), 3.77 (3H, s, 2-OCH_3), 3.80 (3H, s, 5-OCH_3), 7.00 (1H, s, H6), 7.21 (1H, s, H3), 7.81 (3H, *br*s, NH_3^+);

^{13}C NMR (101 MHz, DMSO) δ: 27.9 (Cβ), 38.4 (Cα), 56.2 (5-OCH_3), 56.6 (2-OCH_3), 108.9 (C4), 115.5 (2C, C3 and C6), 125.5 (C1), 149.4 (C5), 151.6 (C2);

MS (EI) m/z (%): 261 ($M^{\cdot+}$+2, 25), 259 ($M^{\cdot+}$, 26), 232 (97), 230 (100), 217 (23), 215 (24), 201 (13), 199 (13), 180 (10), 152 (10), 134 (13), 121 (13), 105 (18), 92 (13), 91 (17), 77 (35).

Melting Point: 228-231 ºC

3.2.3.2 Synthesis of 4-bromo-2,5-dimethoxy-*α*-methyl-*β*-phenethylamine (compound 9)



To a solution of 2,5-dimethoxy-*α*-methyl-*β*-phenethylamine (1.00 g, 4.3 mmol) in acetic acid (15 mL), elemental bromine (0.25 mL, 4.8 mmol) was added under vigorous stirring. In 10 min an insoluble yellow solid was formed. The hydrobromide of the require compound was obtained. A solution of KOH 4M was added until basic pH and solution extracted with CH_2Cl_2 (3 × 75 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent removed. The dark brown residue was destilled at 120ºC - 135ºC at 0.250 Torr yielding a pale yellow oil. The oil was after treated with 2M HCl ethereal solution, further diethyl ether was added until a persistent turvation was produced and white solid was formed (0.28 g).

Yield: 21%

^{1}H NMR (DMSO, 400 MHz): δ= 1.12 (3H, d, *J*=6.5 Hz, α-CH_3), 2.75 (2H, d, *J*=7.9 Hz, Hβ), 2.86-2.90 (1H, m, Hα), 3.76 (3H, s, 2-OCH_3), 3.79 (3H, s, 5-OCH_3), 7.01 (1H, s, H6), 7.20 (1H, s, H3), 8.01 (3H, *br*s, NH_3^+);

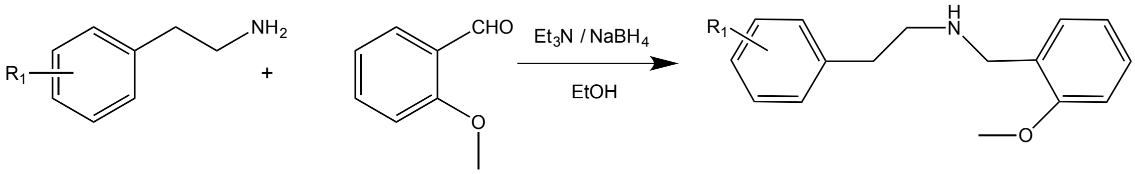
^{13}C NMR (101 MHz, DMSO) δ: 17.9 (α-CH_3), 34.6 (Cβ), 46.6 (Cα), 56.1 (5-OCH_3), 56.5 (2-OCH_3), 108.9 (C4), 115.7 (2C, C3 and C6), 124.9 (C1), 149.2 (C5), 151.6 (C2);

MS (EI) m/z (%): 275 ($M^{\cdot+}$2, 6), 273 ($M^{\cdot+}$, 6), 232 (97), 230 (100), 217 (13), 215 (14), 201 (16), 199 (16), 152 (38), 134 (30), 121 (25), 105 (24), 98 (49), 92 (18), 91 (26), 77 (57), 57 (59).

Melting Point: 201-204 ºC

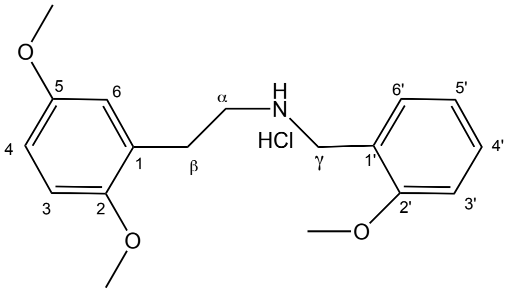
3.2.4 Synthesis of *N*-Benzylated *β*-henethylamines

General Procedure



This procedure was adapted from the literature [142]. To a suspension of *β*-phenethylamine hydrochloride (*ca.* 1.0 mmol) and the aldehyde (*ca.* 1.1 mmol) in EtOH (*ca.* 10 mL), triethylamine (*ca.* 1.0 mmol) was added. The mixture was stirred and the formation of the imine was monitored by TLC. After $NaBH_4$ (*ca.* 2.0 mmol) was added to the reaction it was stirred for aditional 60 min. The volatiles were removed under reduced pressure. The residue was dissolved in ethyl acetate (AcOEt, 25 mL) and water (25 mL), the organic layer was colected and washed twice with water. The organic layer was dried over $NaSO_4$, filtered and evaporated yielding an amber oil. Advanced automated flash purification from *Biotage Isolera™ Prime* was used for further purification. The remaining oils were treated with 2M HCl ethereal solution and the hydrochloride salt after recrystalization with EtOH.

3.2.4.1 Synthesis of 2,5-dimethoxy-*N*-(2-methoxybenzyl)-*β*-phenethylamine (compound 10)



To a suspension of 2,5-dimethoxy-*β*-phenethylamine hydrochloride (0.22 g, 1.0 mmol) and the 2-methoxybenzaldehyde (0.15 g, 1.1 mmol) in EtOH (10 mL), triethylamine (0.14 mL, 1.0 mmol) was added. The mixture was stirred and the formation of the imine was monitored by TLC (DCM/MeOH/NH_3 (9.0:0.9:0.1)). After $NaBH_4$ (0.080 g, 2.0 mmol) was added to the reaction it was stirred for aditional 60 min. The volatiles were removed under reduced pressure. The residue was dissolved in ethyl acetate (AcOEt, 25 mL) and water (25 mL), the organic layer was colected and washed twice with water. The organic layer was dried over anhydrous $NaSO_4$, filtered and evaporated yielding an amber oil. An automated flash purification device was used for further purification. The remaining oils were treated with 2M HCl ethereal solution and the hydrochloride salt was prepared as described in general procedure, yielding a white solid (0.26 g).

Yield: 77%

^{1}H NMR (DMSO, 400 MHz): δ= 2.66 (4H, s, Hβ and Hα), 3.66 (2H, s, Hγ), 3.67 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 6.71 (1H, d, *J*=2.2 Hz, H6), 6.84 (1H, dd, *J*=7.4 and 2.2 Hz, H4), 6.88 (1H, dd, *J*=7.4 and 1.0 Hz, H3'), 6.92-6.94 (2H, m, H3 and H6'), 7.19 (1H, ddd, *J*=7.6, 7.4 and 1.7 Hz, H4'), 7.25 (1H, dd, *J*=7.6 and 1.7 Hz, H6'), 7.35-7.37 (2H, m, NH_2^+);

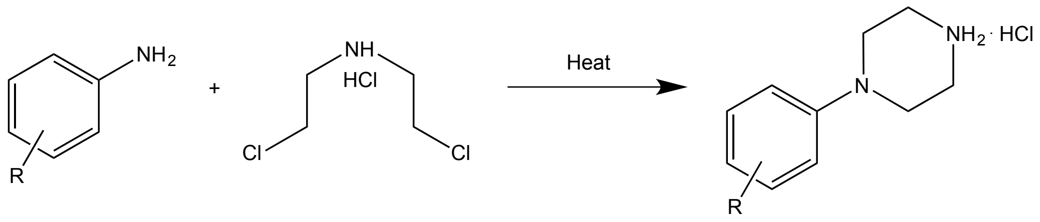
^{13}C NMR (101 MHz, DMSO) δ: 30.9 (Cα), 48.0 (Cγ), 49.3 (Cβ), 55.6, 55.7, 56.2 (3C, 3×OCH_3), 110.8 (C3'), 111.6 (C3), 112.0 (C4), 120.4 (C5'), 128.1 (C4'), 129.0 (C1'), 129.3 (C6'), 130.0 (C1), 151.8 (C2), 153.4 (C5), 157.4 (C2');

MS (EI) m/z (%): 300 ($M^{\cdot+}$, 3), 150 (71), 121 (100), 91 (37), 77 (4).

Melting Point: 114-116 ºC

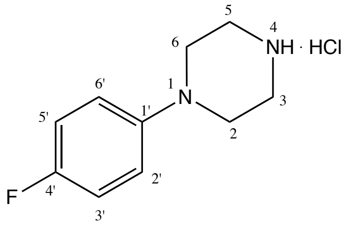
3.2.5 Synthesis of phenylpiperazine derivatives

General Procedure



After several attempts to prepare phenylpiperazines derivatives the best results rose from a high temperature solvent-free route adapted from the literature [143]. A mixture of aniline with the proper substituion pattern (*ca.* 5.0 mmol) and bis(2-chloroethyl)amine hydrochloride (*ca.* 5.5 mmol) was heated at 165-170 ºC for 4 to 7 hours. When the reaction was stopped by letting the reaction cool down to room temperature, the glass-like mixture was treated with acetone (*ca.* 5 mL) and placed on a ultrasonic bath at 45 ºC for 4 to 5 h. The solvent was then removed, the solids dissolved in diethyl ether (50 mL) and washed with aqueous NaOH 3M (3 × 50mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and treated with 2M HCl ethereal solution to afford the hydrochloride salt. The phenylpiperazine salts were then cristalized from diethyl ether to provide analytically pure compounds.

3.2.5.1 Synthesis of *p*-fluorophenylpiperazine (compound 11)



A mixture of 4-fluoroaniline (0.45 mL, 4.8 mmol) and bis(2-chloroethyl)amine hydrochloride (1.02 g, 5.7 mmol) was heated at 165 ºC. Slow evolution of hydrogen chloride was observed during the first hours. After 5 h the reaction was stopped by letting the reaction cool down to room temperature, the glass-like mixture was treated with acetone (5 mL) and placed on a ultrasonic bath at 45 ºC for 3 h. The solvent was then removed, the solids dissolved in diethyl ether (50 mL) and washed with aqueous NaOH 3M (3 × 50mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and treated with 2M HCl ethereal solution to afford the hydrochloride salt. The salt was then recrystalized from diethyl ether to provide analytically pure compounds (0.301 g). The TLC system used to monitor the reaction was $MeOH/NH_3$ (9.9:0.1).

Yield: 27%

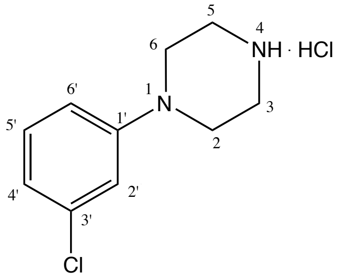
^{1}H NMR (DMSO, 400 MHz): δ= 3.16-3.23 (4H, m, H5 and H3), 3.28-3.34 (4H, m, H2 and H6), 6.98-7.05 (2H, m, H2' and H6'), 7.06-7.13 (2H, m, H3' and H5'), 9.49 (s, 2H, NH_2^+);

^{13}C NMR (101 MHz, DMSO) δ: $\underline{42.5}$ (2C, C3 and C5), $\underline{46.1}$ (2C, C2 and C6), $\underline{115.4}$ (2C, C3' and C5', J_{CF}=22.2), $\underline{117.8}$ (2C, C2' and C6', J_{CF}=7.1), 146.8 (C1', J_{CF}=2.0), 156.4 (C4', J_{CF}=244.4);

MS (EI) m/z (%): 180 ($M^{\cdot+}$, 68), 161 (1), 150 (4), 139 (24), 138 (100), 123 (58), 122 (66), 109 (18), 95 (80), 75 (36), 56 (58).

Melting Point: 193-196 ºC

3.2.5.2 Synthesis of *m*-chlorophenylpiperazine (compound 12)



A mixture of 3-chloroaniline (0.55 mL, 5.2 mmol) and bis(2-chloroethyl)amine hydrochloride (1.09 g, 6.1 mmol) was heated at 170 ºC (the solid melted at arround 140 ºC). Slow evolution of hydrogen chloride was observed during the first hour. After 2 h a thin white powder began to accumulate in the top of the reaction vessel. TLC and NMR analysis confirmed this powder as the final expected compound, which sublimed due to high temperatures and condensed in the cold top of the vessel. After 4 h the reaction was stopped by letting the reaction cool down to room temperature, the glass-like mixture was treated with acetone (5 mL) and placed on a ultrasonic bath at 45 ºC for 3 h. The solvent was then removed, the solids dissolved in diethyl ether (50 mL) and washed with aqueous NaOH 3M (3 × 50mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and treated with 2M HCl ethereal solution to afford the hydrochloride salt. The salt was then recristalized from diethyl ether to provide analytically pure compounds (0.591 g). The TLC system used to monitor the reaction was $MeOH/NH_3$ (9.9:0.1).

Yield: 49%

^{1}H NMR (DMSO, 400 MHz): δ= 3.11-3.23 (4H, m, H5 and H3), 3.40-3.47 (4H, m, H2 and H6), 6.87 (1H, dd, *J*=7.8 and 1.6 Hz, H4’), 6.95 (1H, dd, *J*=7.8 and 2.4, H6’), 7.03 (1H, t, *J*=8.1 Hz, H2’), 7.29 (1H, t, *J*=7.0 Hz, H5’), 9.49 (s, 2H, NH_2^+);

^{13}C NMR (101 MHz, DMSO) δ: 42.7 (2C, C3 and C5), 45.3 (2C, C2 and C6), 114.7 (C2’), 115.8 (C6’), 119.7 (C4’), 131.1 (C5’), 134.3 (C3’), 151.7 (C1’);

MS (EI) m/z (%): 196 ($M^{\cdot+}$, 1), 154 (1), 141 (1), 129 (82), 128 (25), 127 (97), 100 (18), 94 (23), 92 (100), 65 (77).

Melting Point: 208-210 ºC

4. Results and discussion

4.1 Synthesis of analytical standards of psychoactive substances

In the current project phenylpiperazine derivatives and *β*-phenethylamine relevant derivatives and intermediates were prepared. The overall yields of each synthetic procedure performed are resumed in Table 4. All the compounds were obtained with reasonable to good yields.

Table 4. List of the compounds and yields of synthesized compounds.

Compound	Name	η (%)
1	2,5-dimethoxy-*β*-nitrostyrene	93
2	2,5-dimethoxy-*β*-methyl-*β*-nitrostyrene	77
3	3,4,5-trimethoxy-*β*-nitrostyrene	73
4	2,3,4-trimethoxy-*β*-nitrostyrene	86
5	2,5-dimethoxy-*β*-phenethylamine	78
6	3,4,5-trimethoxy-*β*-phenethylamine	42
7	2,5-dimethoxy-*α*-methyl-*β*-phenethylamine	61
8	4-bromo-2,5-dimethoxy-*β*-phenethylamine	17
9	4-bromo-2,5-dimethoxy-*α*-methyl-*β*-phenethylamine	21
10	2,5-dimethoxy-*N*-(2-methoxybenzyl)-*β*-phenethylamine	77
11	*p*-fluorphenylpiperazine	27
12	*m*-chlorophenylpiperazine	49

Synthesis of *β*-nitrostyrene derivatives

The *β*-nitrostyrene derivatives prepared, 2,5-dimethoxynitrostyrene, 2,5-dimethoxy-*β*-methylnitrostyrene and 3,4,5-trimethoxynitrostyrene (compounds 1, 2, 3 and 4, respectively), were obtained by Henry reaction in good yields (from 77 to 93 %). The modification of the reaction conditions (decrease of the quantity of the catalyst added) to prepare compound 3 led to a slight increase in the yield, by reducing side reactions. The proposed mechanism for the Henry reaction is depicted in Figure 17.

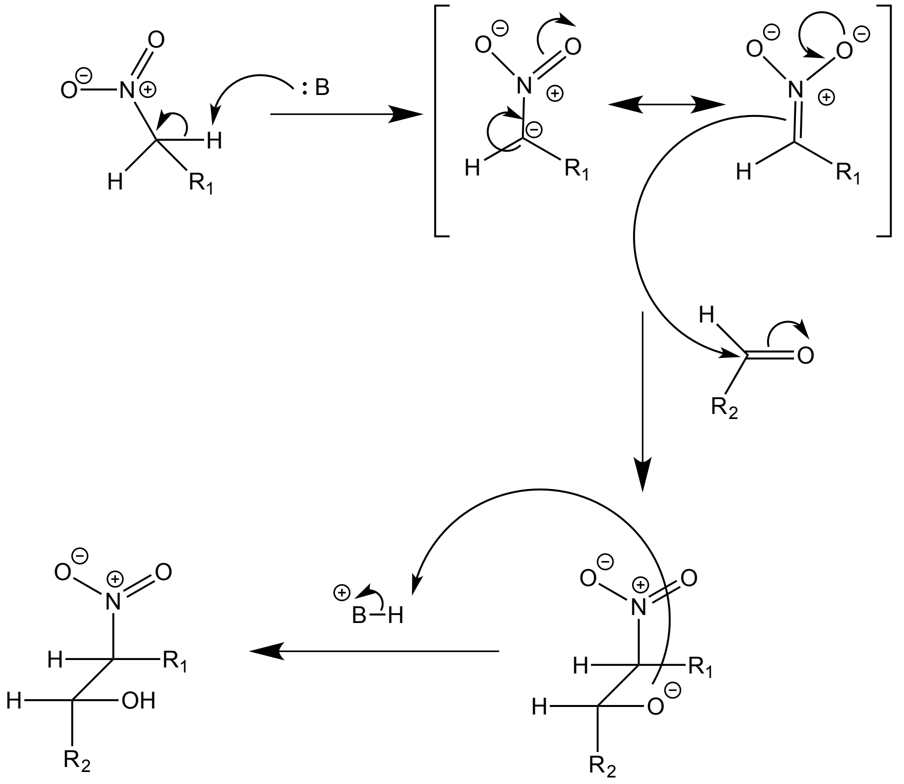


Figure 17. Proposed mechanism for the Henry reaction to obtain the *β*-nitroalcohol (R_1=H, *β*-phenethylamine; R_1=CH_3, *α*-methyl-*β*-phenethylamine; R_2= substituted phenyl group).

The reaction begins with the deprotonation of the nitroalkane (nitromethane or nitroethane) at Cα position by the base (ammonium acetate), forming a resonance-stabilized anion. After, a *β*-nitro alkoxide is formed by the alkylation of the nitroalkane by the substituted benzaldehyde. The protonation of the alkoxide by the previously protonated base yielded a *β*-nitroalcohol. The dehydration of the alcohol, promoted by heating the reaction, yields the conjugated nitrostyrene.

Synthesis of *β*-phenethylamine derivatives

The *β*-phenethylamine derivatives (compounds 5, 6 and 7) were obtained with yields from 48 to 78%. These compounds were synthesized by reducing simultaneously the double bond and the nitro group from the respective *β*-nitrostyrene or *β*-methyl-*β*-nitrostyrene. The increase of the added quantities of the reducing agent ($LiAlH_4$), compared to previous described methods, slightly improved the yield of the reactions. The purification step by short path distillation resulted in high purity products, although there is a possibility of some additional mass loss during this process.

Halogenation of *β*-phenethylamine derivatives

The aromatic brominated compounds, 9 and 10, were obtained after halogenation with elemental bromine in acetic acid of compounds 5 and 7 with yields of 17 and 21 %, respectively. The proposed mechanism for this bromination is depicted in Figure 18.

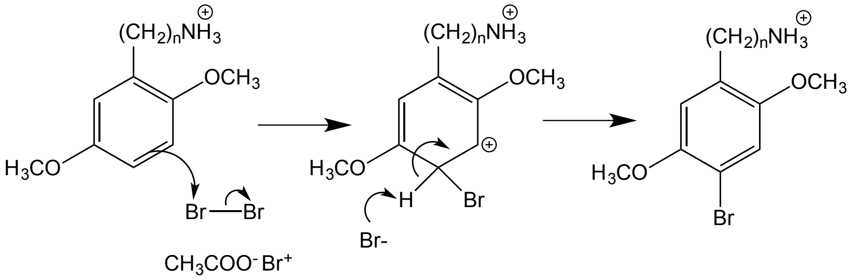


Figure 18. Proposed mechanism for the bromination of the phenylalkylamines.

This aromatic electrophilic substitution usually requires harsh conditions as a Lewis acid catalyst (*e.g.* $FeBr_3$). However, in the present case the aromatic ring is activated for aromatic electrophilic substitution as it has methoxy group that are activating substituents (indutive and mesomeric) to *ortho* and *para* position. These catalysts-free conditions are possible considering the extra polarization of bromine in polar solvents, such as acetic acid. The low yields obtained in the two final products are possible due to some loss during purification process by short path destillation and/or side reaction.

N-benzylation of phenethylamine derivatives

For the preparation of *N*-benzylated amines an indirect reductive amination was conducted with a yield of about 77%. The proposed mechanism for this reaction is schematized in Figure 19b. First, triethylamine is added to amine salt to obtain its basic form (Figure 19a). Then, the amine compound reacts with the carbonyl group to form a hemiaminal species that subsequently lose one molecule of water to form an imine (Figure 19b, Step 1). The reducing agent, sodium borohydride, is then added to yield the required *N*-benzylamine derivative (Figure 19b, Step 2).

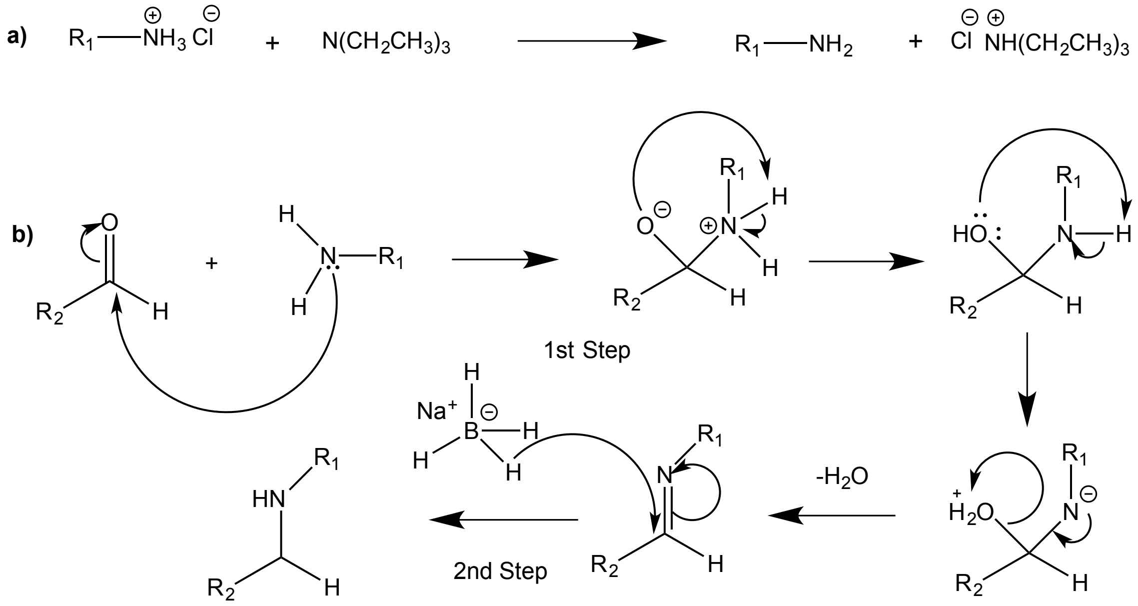


Figure 19. a) Reaction of amine hydrochloride with triethylamine; b) General two-step mechanism proposed for indirect reductive amination (R_1 = alkylphenyl; R_2 = Ph-2-OCH_3).

Synthesis of phenylpiperazine derivatives

For the phenylpiperazine derivatives (compounds 11 and 12) the yields obtained were 27 and 49%, respectively. In Figure 20 is pictured the proposed mechanism for this SN_2 reaction step.

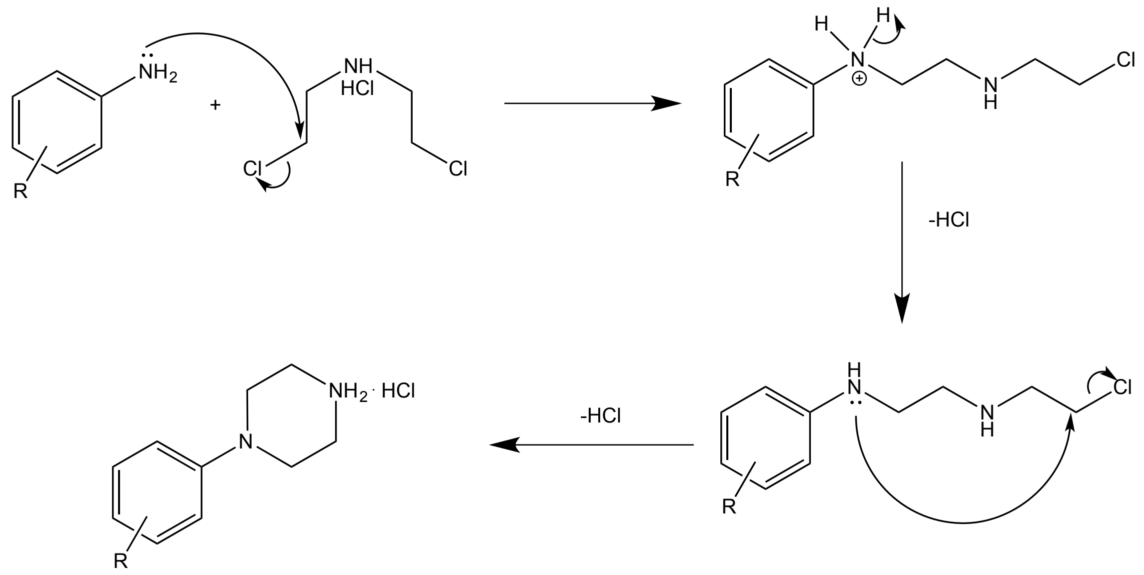


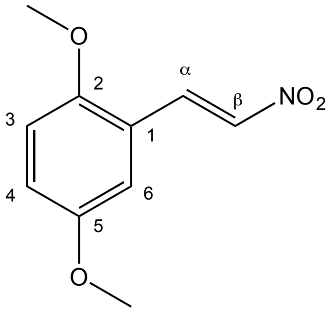
Figure 20. Proposed mechanism for the synthesis of phenylpiperazine derivatives.

Although anilines are poor nucleophiles, when bis(2-chloroethyl)amine and substituted aniline are mixed and exposed to high temperatures, an intramolecular SN_2 nucleophilic substitution is promoted, even in solvent-free conditions. The use of a high boiling point solvent could improve the yield of these reactions.

4.2 Structural elucidation of synthesized compounds

The ^{1}H NMR data of the synthesized compounds is summarized in tables 5 to 10.

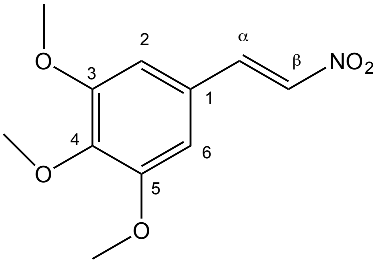
Table 5. ^{1}H NMR data of 2,5-dimethoxynitrostyrene (compound 1).

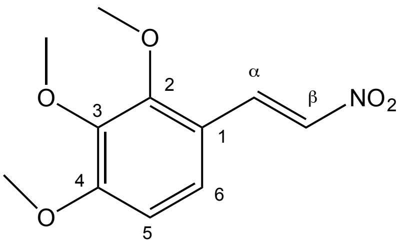


Compound 1

	δ (ppm)	Integ.	Mult.	*J* (Hz)
H3	**7.15-7.09**	**2**	**m**	**-**
H4	**7.15-7.09**	**2**	**m**	**-**
H6	**7.42**	**1**	**d**	**2.4**
Hα / Hβ	**8.19**	**2**	**s**	**-**
2-OCH_3	**3.88**	**3**	**s**	**-**
5-OCH_3	**3.76**	**3**	**s**	**-**

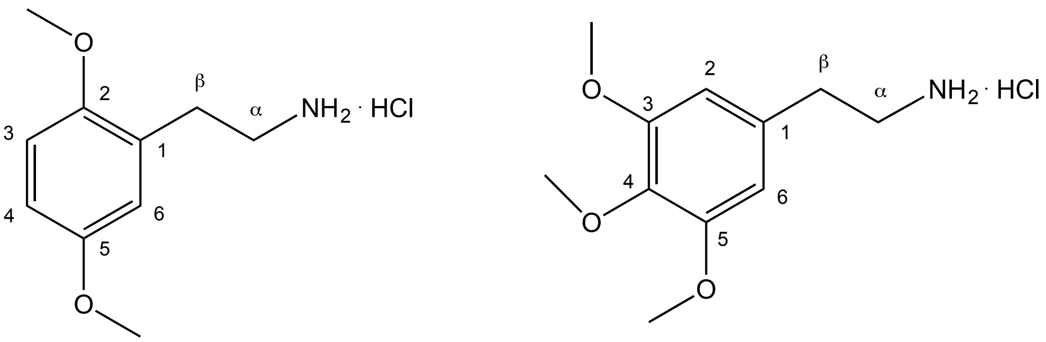
Table 6. ^{1}H NMR data of trimethoxynitrostyrenes (compounds 3 and 4).





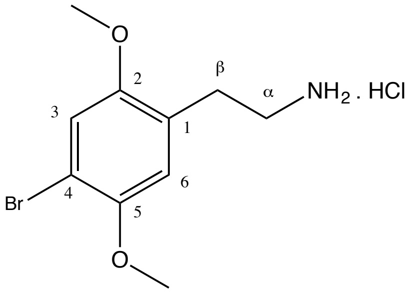
	Compound 3				Compound 4			
	δ (ppm)	**Integ.**	**Mult.**	***J* (Hz)**	**δ (ppm)**	**Integ.**	**Mult.**	***J* (Hz)**
H2	7.25	2	s	-		-	-	-
H5	-	-	-	-	7.38	1	s	-
H6	7.25	2	s	-	6.77	1	s	-
Hα	8.06	1	d	13.5	8.11	1	d	13.5
Hβ	8.28	1	d	13.5	8.21	1	d	13.5
2-OCH_3	-	-	-	-	3.76	3	s	-
3-OCH_3	3.83	6	s	-	3.89	3	s	-
4-OCH_3	3.73	3	s	-	3.94	3	s	-
5-OCH_3	3.83	6	s	-	-	-	-	-

Table 7. ^1H NMR data of phenethylamine derivatives (compounds 5 and 6).

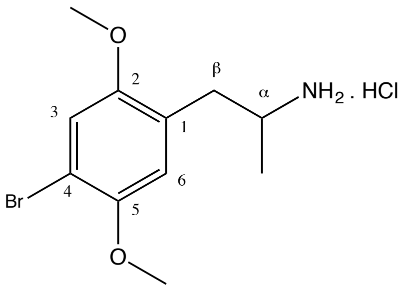


	Compound 5				**Compound 6**			
	δ (ppm)	**Integ.**	**Mult.**	***J* (Hz)**	**δ (ppm)**	**Integ.**	**Mult.**	***J* (Hz)**
H2	-	-	-	-	6.57	2	s	-
H3	6.92	1	d	8.5	-	-	-	-
H4	6.82-6.78	2	m	-	-	-	-	-
H6	6.82-6.78	2	m	-	6.57	2	s	-
Hα	2.85-2.81	2	m	-	3.09-3.03	2	m	-
Hβ	2.96	2	t	7.2	2.80	2	t	7.6
2-OCH_3	3.70	3	s	-	-	-	-	-
3-OCH_3	-	-	-	-	3.77	6	s	-
4-OCH_3	-	-	-	-	3.64	3	s	-
5-OCH_3	3.75	3	s	-	3.77	6	s	-
NH_3^+	7.93	3	br s	-	7.76	3	br s	-

Table 8. ^{1}H NMR data of halogenated phenethylamines (compounds 8 and 9).



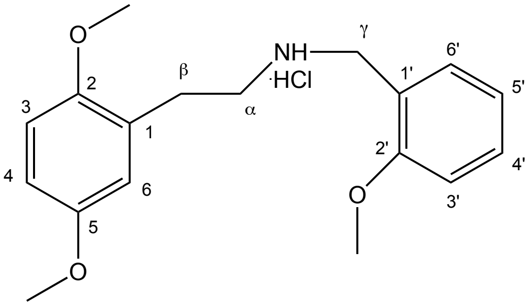
Compound 8



Compound 9

	δ (ppm)	Integ.	Mult.	*J* (Hz)	δ (ppm)	Integ.	Mult.	*J* (Hz)
H3	7.21	1	s	-	7.20	1	s	-
H6	7.00	1	s	-	7.01	1	s	-
Hα	2.98	2	t	7.5	2.90-2.86	1	m	-
Hβ	2.83	2	t	7.5	2.75	2	d	7.9
2-OCH$_3$	3.77	3	s	-	3.76	3	s	-
5-OCH$_3$	3.80	3	s	-	3.79	3	s	-
α-CH$_3$	-	-	-	-	1.12	3	d	6.5
NH$_3^+$	7.81	3	br s	-	8.01	3	br s	-

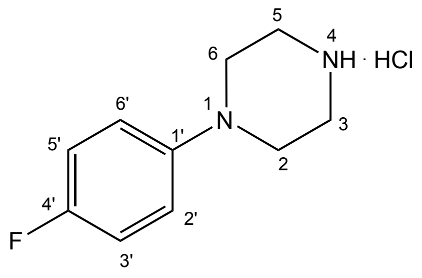
Table 9. ^{1}H NMR data of 2,5-dimethoxy-*N*-(2-methoxybenzyl)-*β*-phenethylamine (compound 10).



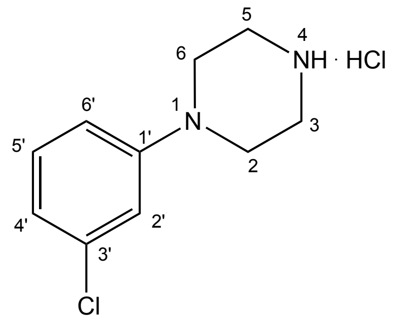
Compound 10

	δ (ppm)	Integ.	Mult.	*J* (Hz)
H3	6.94-6.92	2	m	-
H4	6.84	1	dd	7.4; 2.2
H6	6.71	1	d	2.2
H3'	6.88	1	dd	7.4; 1.0
H4'	7.19	1	ddd	7.6; 7.4; 1.7
H5'	6.94-6.92	2	m	-
H6'	7.25	1	dd	7.6; 1.7
Hα	2.66	4	s	-
Hβ	2.66	4	s	-
Hγ	3.66	2	s	-
OCH_3	3.74	3	s	-
OCH_3	3.69	3	s	-
OCH_3	3.67	3	s	-
NH_2^+	7.37-7.35	2	m	-

Table 10. ^{1}H NMR data of compounds 11 and 12.



Compound 11

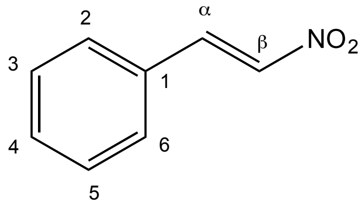


Compound 12

	δ (ppm)	Integ.	Mult.	*J* (Hz)	δ (ppm)	Integ.	Mult.	*J* (Hz)
H2	3.34-3.28	4	m	-	3.47-3.40	4	m	-
H3	3.23-3.16	4	m	-	3.23-3.11	4	m	-
H5	3.23-3.16	4	m	-	3.23-3.11	4	m	-
H6	3.34-3.28	4	m	-	3.47-3.40	4	m	-
H2’	7.05-6.98	2	m	-	7.03	1	t	8.1
H3’	7.13-7.06	2	m	-	-	-	-	-
H4’	-	-	-	-	6.87	1	dd	7.8; 1.8
H5’	7.13-7.06	2	m	-	7.29	1	t	7.0
H6’	7.05-6.98	2	m	-	6.95	1	dd	8.4; 2.4

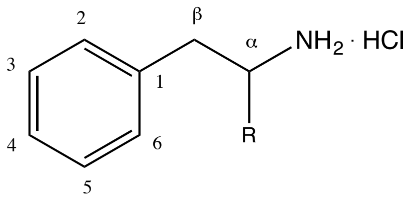
The ^{13}C NMR data of the synthesized compounds is summarized in tables 11 to 14.

Table 11. ^{13}C NMR data of compounds 1,3 and 4 .



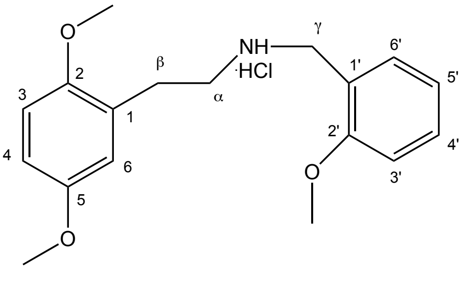
	Compound 1	**Compound 3**	**Compound 4**
	δ (ppm)	**δ (ppm)**	**δ (ppm)**
C1	119.2	126.1	109.9
C2	153.6	108.2	154.9
C3	120.7	153.6	143.6
C4	115.4	141.3	156.0
C5	153.9	153.6	97.8
C6	113.7	108.2	113.4
Cα	138.8	140.2	135.7
Cβ	134.7	137.9	135.1
2-OCH_3	56.8	—	57.0
3-OCH_3	—	56.7	56.7
4-OCH_3	—	60.7	56.5
5-OCH_3	56.2	56.7	—

Table 12. ^{13}**C NMR data of compounds 5, 6, 8 and 9.**



	Compound 5	Compound 6	Compound 8	Compound 9
	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)
C1	127.5	133.3	125.5	124.9
C2	152.6	106.5	151.6	151.6
C3	113.2	153.4	115.5	115.7
C4	113.6	136,7	108.9	108.9
C5	154.4	153.4	149.4	149.2
C6	117.8	106.5	115.5	115.7
Cα	40.0	40.5	38.4	46.6
Cβ	29.5	33.7	27.9	34.6
2-OCH$_3$	57.1	—	56.6	56.5
3-OCH$_3$	—	56.3	—	—
4-OCH$_3$	—	60.4	—	—
5-OCH$_3$	56.7	56.3	56.2	56.1
αCH$_3$	—	—	—	17.9

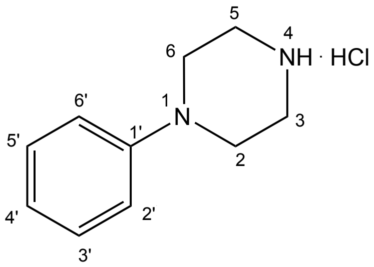
Table 13. ^{13}C NMR data of compounds 10.



Compound 10

	δ (ppm)
C1	130.0
C2	151.8
C3	111.6
C4	112.0
C5	153.4
C6	116.8
C1'	129.0
C2'	157.4
C3'	110.8
C4'	128.1
C5'	120.4
C6'	129.3
Cα	30.9
Cβ	49.3
Cγ	48.0
3×OCH$_3$	55.6 55.7 56.2

Table 14. ^{13}C NMR data of compounds 11 and 12



	Compound 11	Compound 12
	δ (ppm); J (Hz)	**δ (ppm)**
C1’	146.8; J_{CF}=2.0	151.7
C2’	117.8; J_{CF}=7.1	114.7
C3’	115.4; J_{CF}=22.2	134.3
C4’	156.4; J_{CF}=244.4	119.7
C5’	115.4; J_{CF}=22.2	131.1
C6’	117.8; J_{CF}=7.1	115.8
C2/C6	46.1	45.3
C3/C5	42.5	42.7

Some regards on NMR (^{1}H and ^{13}C) and EI-MS analysis can be pointed out in order to achieve a thorough spectroscopic characterization of the final compounds and their synthetic intermediates, namely:

i. The synthesis of β-nitrostyrene derivatives, with the *E* (*trans*) configuration, was successfully accomplished. When a characteristic outline of the ^{1}H NMR signals is present, is possible to assign a doublet that displays a coupling constant of around 13.5 Hz, representative of a *E* diastereomer with a strong electron withdrawing group (nitro group). In this chemical environment, the *trans* coupling constant tends to decrease to values lower than 14 Hz.

ii. Whenever it was possible to properly allocate all the doublets, triplets and/or doublets of doublets that correspond to the aromatic protons, the accurate assignment of the chemical shifts and the multiplicity pattern was described. Therefore, the substitution pattern of the aromatic ring can be clearly recognized: the monosubstituted phenyl ring in *ortho* (compound 10), *meta* (compound 11) and *para* positions (compound 12); the disubstituted ring in positions 2 and 5 (compounds 1, 5 and 10); and the trisubstituted benzene with a pattern of 3,4,5 (compounds 3 and 6), 2,3,4 (compound 4) and 2,4,5 (compounds 8 and 9) are easily determined. It is worthwhile to mention that in the aromatic region of the ^{1}H NMR spectra sometimes the corresponding signals tend to overlap. The number of aromatic and aliphatic carbons was also clearly assigned by the analysis of ^{13}C NMR spectra; the data that was outlined from the DEPT analysis allows the recognition the nature of the substituted carbons (mainly in the aromatic area of the spectra).

iii. In compounds 8 and 9, the loss of an aromatic proton confirms that the aromatic electrophilic substitution with a bromine atom, in *para* position to the aliphatic side chain, was successfully accomplished. This conclusion was reinforced by the analysis of the ^{13}C NMR spectra, since the carbon bearing the bromine atom is located at down field values compared to the other aromatic carbons.

iv. The side chain of the β-phenethylamine derivatives, either linear or branched, was distinctly assigned with the accurate integration of the signals in the aliphatic part of the spectra. Additionally, the typical coupling constants of the doublets and the triplets (usually between 7 and 8 Hz) were observed in ^{1}H NMR spectra.

v. The number of methoxy groups was also clearly observed and attributed both in ^{1}H and ^{13}C NMR spectra, with signals that are characteristic of this functional group (between 3.6 and 3.9 ppm in ^{1}H NMR and between 56 and 60 ppm in ^{13}C NMR).

vi. The cyclization of the compounds containing the pyperazine ring was undoubtedly achieved, since the correct number of aliphatic protons and carbons in the spectra was straightforwardly noticed.

vii. From the analysis of the peak of the amine protons in ^{1}H NMR spectra, although quite erratic along the chemical shift axis, it was possible to confirm de protonation of this group, thus identifying the preparation of the hydrochloride salt.

viii. Compound 11, bearing a fluorine atom, presents a specific ^{13}C NMR spectrum; in fact, the unique splitting pattern of the carbon peaks, with very typical coupling constants (J_{CF}) along the aromatic ring, is clearly observed. The J_{CF} values decrease with the distance to the carbon that displays the halogen atom.

ix. The compounds containing an amine group display a weak (sometimes completely absent) molecular ion peak. The base peaks are the fragment that corresponds to a typical α-cleavage (to the amine group) in the side chain of the aromatic ring.

x. In halogen-containing compounds (and the resulting mass fragments), the peaks can also be readily identified in the mass spectra. In fact, the typical isotopic features of the presence of chlorine and bromine are obvious. Compounds and fragments displaying a bromine atom (compounds 8 and 9) show a characteristic pattern of M and M+2 peaks of almost equal intensity; on the other hand, compound 12, bearing a chlorine, have M and M+2 peaks of about 3:1 relative intensity.

4.3 Electrochemistry

In this work, electrochemical behaviour of phenylpiperazine and phenethylamine derivatives, along with their nitrostyrene intermediates, were studied using differencial pulse and cyclic voltammetry.

4.3.1 Electrochemical studies of phenylpiperazine derivatives

The electrochemical profile of the synthesized *para*-fluorphenylpiperazine (compound 11) and *meta*-chlorophenylpiperazine (compound 12) was studied along with the commercially available isomers and analogues: phenylpiperazine (compound 15) and *ortho* and *para*-chlorophenylpiperazine (compounds 13 and 14, respectively) (Figure 21 and Table 15).

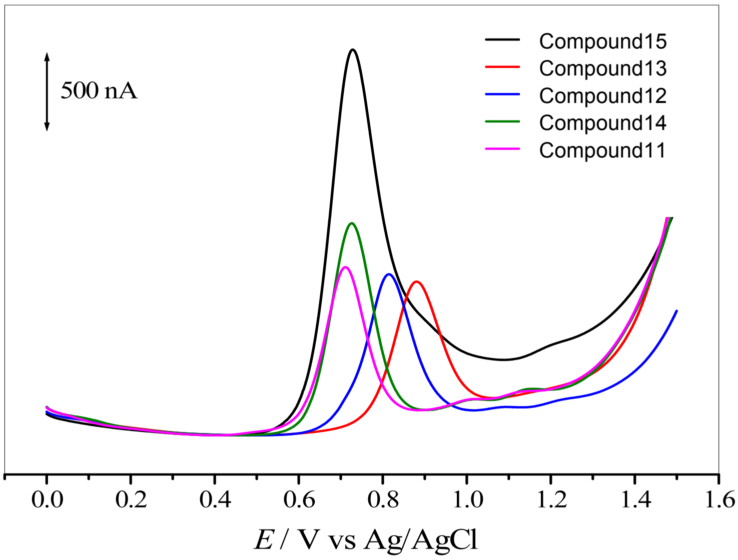


Figure 21. Differential pulse voltammograms for 100 μM solutions in phosphate buffer pH 7 of compounds 11-15. Scan rate: 5 mV s^{-1}.

Table 15. Potencial peaks of phenylpiperazine derivatives at physiological pH.

Compound	E_p / V
11	0.710
12	0.814
13	0.879
14	0.726
15	0.728

Using differential pulse voltammetry, one well-defined anodic peak can be observed at physiological pH for the phenylpiperazine derivatives. This single peak is probably related with an oxidation process occurring at the nitrogen of the piperazine ring bounded to the phenyl group (important to notice that the other nitrogen atom is protonated at this specific pH, Figure 22). Computational studies showed that the nitrogen of the piperazine ring attached to phenyl group is more negatively charged and thus is more easily oxidated compared to the other nitrogen atom (Figure 23a) [144]. The peaks observed for phenylpiperazine (compound 15), *para*-chlorophenylpiperazine (compound 14) and *para*-fluorphenylpiperazine (compound 11) are almost of the same potential, E_p = +0.728, E_p = +0.726 and E_p = +0.710, respectively. The presence of electron withdrawing groups of halogen type in *para*-position to piperazine ring does not influence noticeably its oxidation potential. However, when comparing the E_p of *para*-chlorophenylpiperazine with the E_p of its *meta* and *ortho* isomers (compound 12 and 13, respectively*)* a slight increase of the peak potential is observed, E_p = +0.814 and E_p = +0.879, respectively. As the oxidation process occurs at the nitrogen of the piperazine ring bounded to the phenyl group, the presence of electron-withdrawing substituents in the *para*-position seems to have a slight effect (not disturb or facilitate) on the removal of the electron. On the opposite, the presence of chlorine at *ortho* and *meta* positions tends to make the oxidative process more difficult. Since the oxidation mechanism probably involves the aromatic delocalization/stabilization of the unpaired electron, it is possible to point out that the observed differences are related to the nature and position of the halosubstituents, and to an inherent competition between their resonance and inductive effects. Halogen substituents inductively withdraw electron density and are donors by resonance effect. Thus it seems that the intermediates can be more stabilized in *para* position by a resonance contribution (although a balance of inductive effects due to the different halogen electronegativity is felt (*e.g.* chlorine vs fluorine)). In *ortho* position the effect is probably the same, but the inductive and steric effects are more prevalent and the stabilization of the generated intermediate in the electrochemical process is therefore more difficult (Figure 23a and b). The proposed mechanism for this oxidation reaction is schematized in Figure 22 and the stabilization structures in Figure 23.

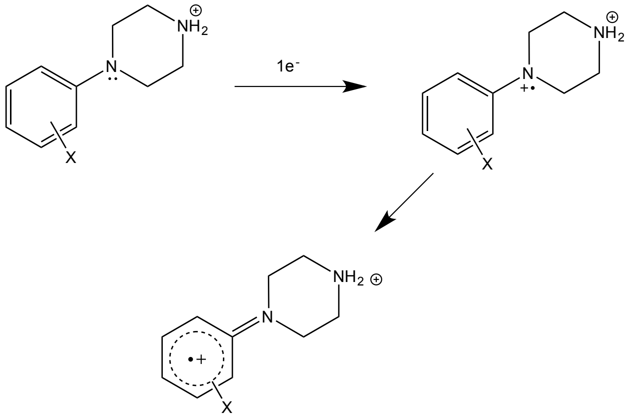


Figure 22. Proposed mechanism for oxidation of phenylpiperazine derivatives (X=Halogen). Adapted from [144].

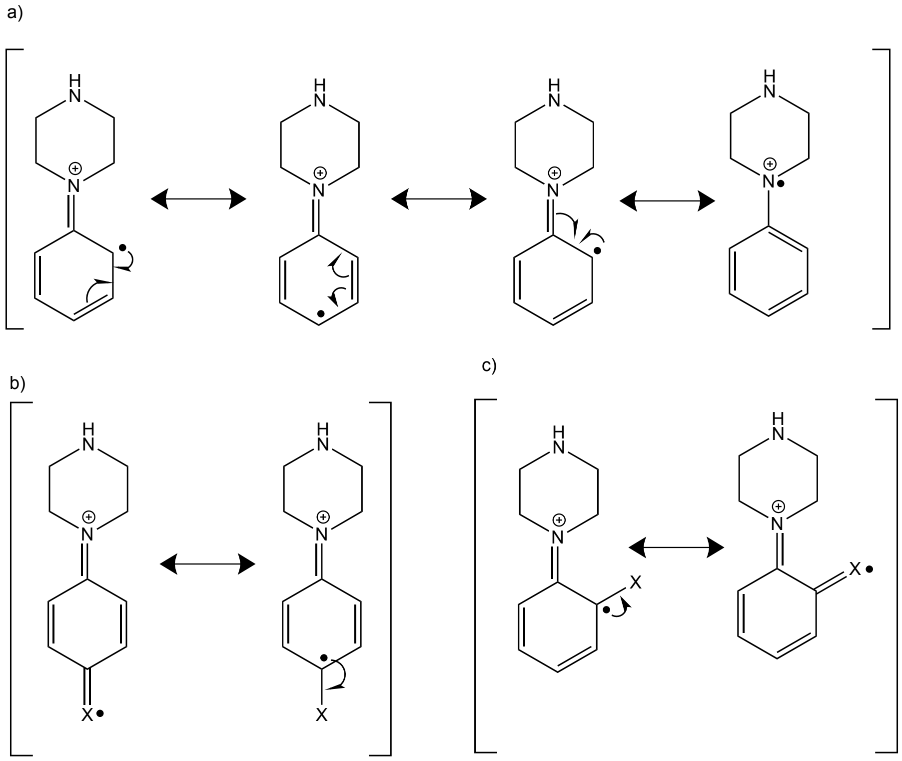


Figure 23. a) Proposed resonance structures that stabilize the radical intermediate formed on the oxidation process of phenylpiperazine(X=Halogen); Extra resonance structures that stabilize halosubstituted phenylpiperazine in b) *para* position and c) *ortho* position.

Differential pulse voltammograms for these phenylpiperazine derivatives were obtained in electrolytic solutions of pH ranging 1 to 12, and some variation of oxidation potential was detected (Figure 24 and 25). In these assays the *E*p values shifted to more negative potentials with the increase of pH. These findings sugest that at low pH, with consequent protonation of the amine group, the mechanistic pathway through the radical cation formation is affected. At these pH values, the protonated amine can probably stabilize the formed cation.

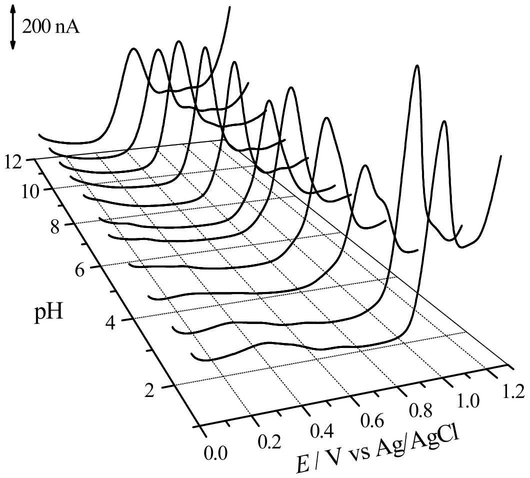


Figure 24. 3D representation of differential pulse voltammograms of solutions of 100 μM of *m*-chlorophenylpiperazine (compound 12) vs pH. Scan rate: 5 mV s^{-1}.

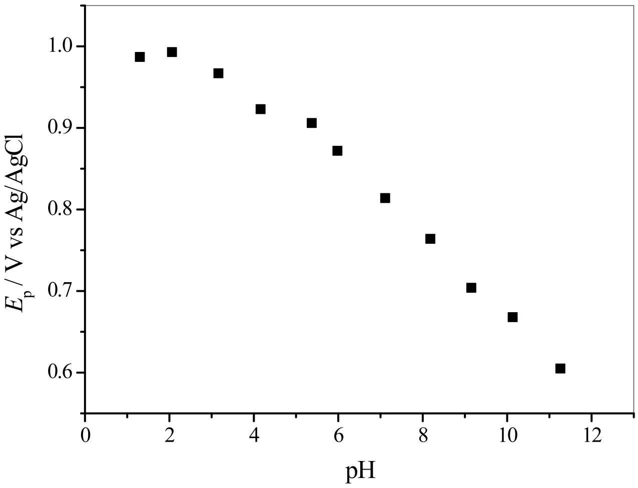


Figure 25. Representation plot of E_p vs. pH from differential pulse voltammograms of solutions of 100 μM of *m*-chlorophenylpiperazine (compound 12). Scan rate: 5 mV s^{-1}.

Cyclic voltammetry was also used to study the electrochemistry of the *m*-chlorophenylpiperazine (Figure 26). The well-defined and irreversible peak observed for the mCPP is ascribed to the oxidation of the nitrogen atom.

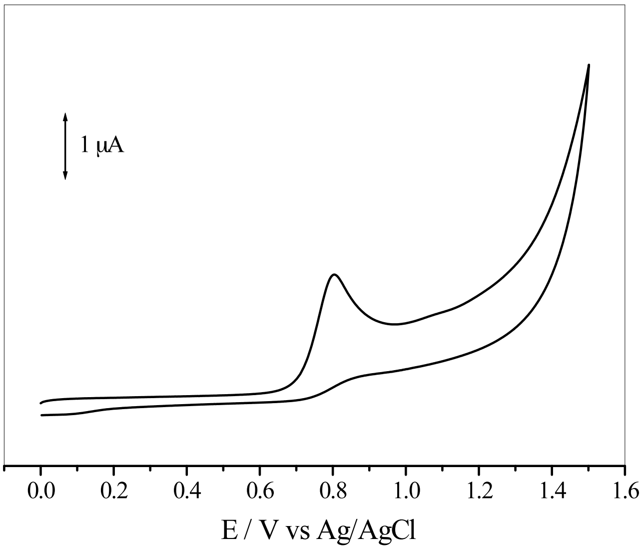


Figure 26. Cyclic voltammograms for 100 μM solutions of *m*-chlorophenylpiperazine (compound 12) in phosphate buffer pH 7. Scan rate: 5 mV s^{-1}.

In resume, it was concluded that the presence of a halogen substituent in *para* postion does not affect the oxidation potencial of the aromatic ring of phenylpiperazine derivatives, although this potencial is increased when the substituent is in *meta* or *ortho* positions. The oxidation is pH dependent and irreversible.

4.3.2 Electrochemical studies of *β*-phenethylamine derivatives and their intermediates

β-Phenethylamine derivatives

The anodic oxidation of phenethylamine derivatives was also studied. Voltammograms of 3,4,5-trimethoxy-*β*-phenethylamine (compound 6), 2,5-dimethoxy-*β*-phenethylamine (compound 5) and its brominated analogues, 4-bromo-2,5-

dimethoxy-*β*-phenethylamine (compound 8) and 4-bromo-2,5-dimethoxy-*α*-methyl-*β*-phenethylamine (compound 9) were obtained (Figure 27, Table 16).

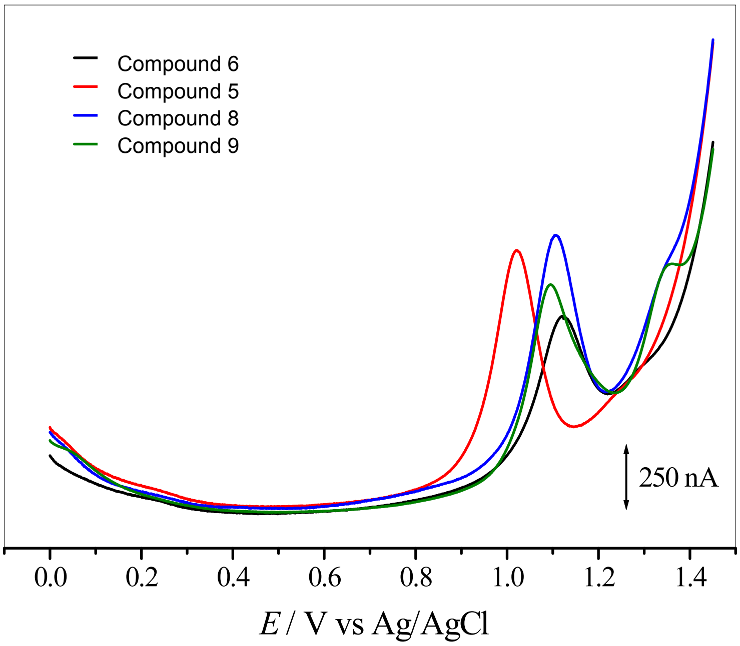


Figure 27. Differential pulse voltammograms for 100 μM solutions of compounds 5, 6, 8 and 9. Scan rate: 5 mV s^{-1} in phosphate buffer pH 7.

Table 16. Potencial peaks of phenethylamine derivatives at physiological pH.

Compound	**E_p / V**
5	1.020
6	1.118
8	1.103
9	1.092; 1.339

For 2,5-dimethoxyphenethylamine and its brominated derivative (compound 5 and 8, respectively) the values of the single peaks obtained are E_p=+1.020 V and E_p= +1.103 V, respectively. The single waves observed for these compounds are probably due to the removal of an electron from the β-carbon (Cβ) (Figure 28).

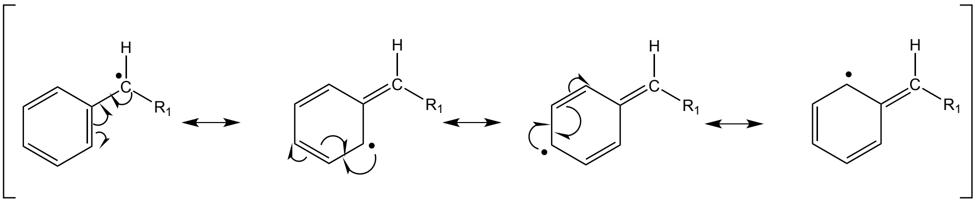


Figure 28. Proposed resonance structures that stabilize the radical intermediate formed on the oxidation process of phenethylamines.

As it was proposed for phenylpiperazine derivatives with nitrogen, Cβ is prone to facile oxidation as compared to the other carbon atoms. Considering the mesomeric effect in the aromatic system, substituents at *ortho* and *para* positions are more able to affect stabilization of the intermediate radical. The effect of the presence of an electron withdrawing halogen-type substituent in C4 position (*para*) (*e.g.* compound 8) is noticeable by the increase of the potential needed to oxidize Cβ. Although halogen atoms have non-bonding valence electron pairs that participate in p-π conjugation, their strong inductive effects predominate. As occurs in the phenylpiperazine oxidation process, electron withdrawing substituents remove electron density of the aromatic ring system making the removal of an electron more difficult [42, 145]. The methoxy substituents are electron donor groups, which facilitate oxidation of Cβ, and at the same time they can probably stabilize the formed radicals when located in *para* and *ortho* positions.

The application of potencial over a 4-bromo-2,5-dimethoxy-*α*-methyl-*β*-phenethylamine (compound 9) solution revealed two peaks, the first at E_p= +1.092 V and the second at E_p= +1.339V. As the electronic environment of the ring is similar to compound 8, an identical potential to remove one electron of Cβ was expected. However, possibly the electron donor methyl group attached to the C*α* affects slightly this value, decreasing the required potential to oxidize Cβ (first peak). Oxidation potentials of phenethylamines are usually lower than the related *α*-methyl-*β*-phenethylamines [145]. Probably the electron donor methyl group attached to C*α* also afects the oxidation potential needed to oxidize the amine group, a possible cause for the appearence of the second peak at E_p= +1.339 V.

The electrochemical study of 3,4,5-trimethoxyphenethylamine (compound 6) revealed one anodic peak at E_p= + 1,118 V. The electronegative oxygen atom of the methoxy groups helps to stabilize the cation formed and the substitution pattern influences their relative abilities to reinforce conjugative stabilization of the cation radical. The mesomeric effect is probably reinforced by the methoxy substituents in *ortho* (compound 5) and in *para* position (compound 6). The proximity of the methoxy substituents in the aromatic ring of the Cβ facilitates its oxidation.

The effect of pH on the oxidation potential of 3,4,5-trimethoxyphenethylamine was also evaluated (Figure 29 and 30). Differential pulse voltammograms wer obtained in electrolytic solutions of pH ranging 1 to 12 to study the effect of pH on this phenethylamine derivative oxidation profile. The peak values of Ep did not shifted substantialy with pH variation. This sugests that the decrease of pH of the medium, and the consequent protonation of the amine group do not influence the mechanistic pathway of oxidation.

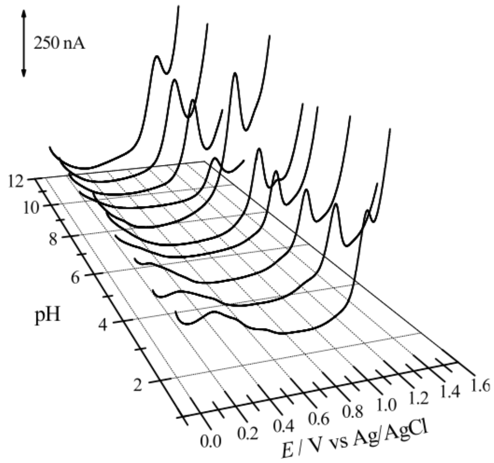


Figure 29. 3D representation of differential pulse voltammograms of solutions of 100 μM 3,4,5-trimethoxyphenethylamine (compound 6) vs pH. Scan rate: 5 mV s^{-1}.

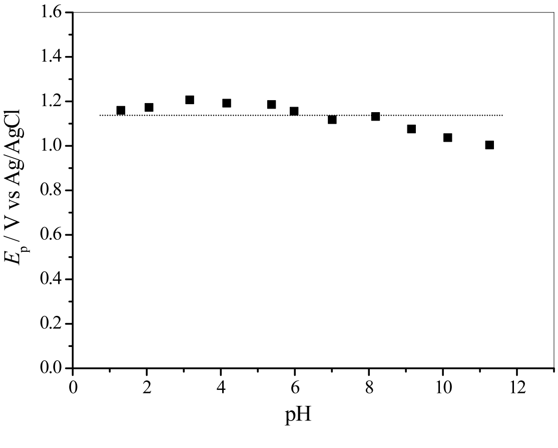


Figure 30. Representation plot of E_p vs. pH from differential pulse voltammograms of solutions of 100 μM 3,4,5-trimethoxyphenethylamine (compound 6). Scan rate: 5 mV s^{-1}.

Cyclic voltammetry was also used to study the electrochemistry of 3,4,5-trimethoxyphenethylamine (Figure 31). The peak observed is, as mentioned before, problably related to the oxidation of the β-carbon. The shape of the voltammogram denotes the irreversibility of the reaction.

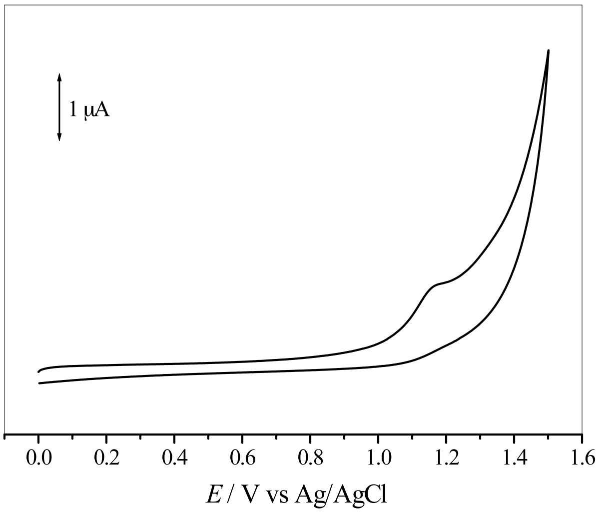


Figure 31 Cyclic voltammograms for 100 μM solutions of 3,4,5-trimethoxyphenethylamine (compound 6) in phosphate buffer pH 7. Scan rate: 20 mV s^{-1}.

β-Nitrostyrene derivatives

The oxidation of the phenethylamine intermediates (*β*-nitrostyrenes) was studied by differential voltammetry at physiologic pH (Figure 32, Table 17). Voltammograms of 2,5-dimethoxynitrostyrene (compound 1), 3,4,5-trimethoxynitrostyrene (compound 3) and 2,3,4-trimethoxynitrostyrene (compound 4) were obtained.

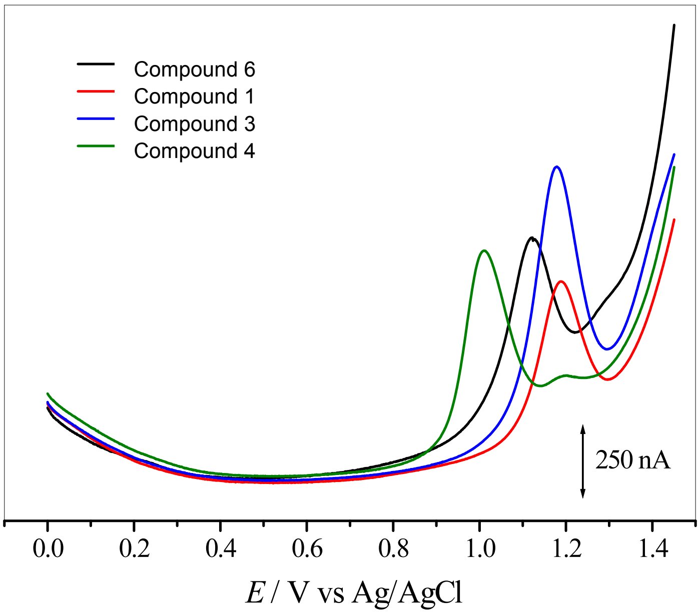


Figure 32. Differential pulse voltammograms for 100 µM solutions in phosphate buffer pH 7 of compounds 1, 3, 4 and 6. Scan rate: 5 mV s^{-1}.

Table 17. Potencial peaks of nitrostyrene derivatives at physiological pH.

Compound	E_p / V
1	1.187
3	1.176
4	1.009

Cyclic voltammograms for nitrostyrene intermediates were recorded. All compounds produced a well-defined anodic peak at physiological pH. The oxidation potentials of 2,5-dimethoxynitrostyrene, 3,4,5-trimethoxynitrostyrene and its 2,3,4-trimethoxy analogue were E_p= +1.187V, E_p= +1.176V and E_p= +1.009V, respectively. The mesomeric and inductive effect of methoxy substituents is patent in this assignment. The presence of methoxy groups in the vicinity of Cα probably reduces the oxidation potential compared to compounds with methoxy groups in more remote positions. Also, it is noticeable that mesomeric effect is predominant when methoxy groups are in *ortho* and *para* positions. The existence of two methoxy groups in these positions (*e.g.* compound 4 with one methoxy group in *para* and other in *ortho* position) reduces drastically its oxidation potential value relatively to compounds with only one methoxy group in this positions (*e.g.* compound 3 with one methoxy group in *para* position).

The oxidation values of nitrostyrenes are usually higher than its phenethylamine analogues [132]. This can be observed by comparison of 3,4,5-trimethoxynitrostyrene and 2,5-dimethoxynitrostyrene oxidation values to the ones obtained with the corresponding phenethylamine (compounds 6 and 5, respectively). This difference is probably due to the negative mesomeric and inductive effects of the nitro group, by oposition of the positive mesomeric effect of the amine group.

5. Conclusions and future perspectives

The proposed aims for this project were achieved since it was possible to synthesize 10 final compounds and intermediates. The compounds were fully characterized by NMR (^{1}H, and ^{13}C, DEPT) and EI-MS. To the fully assignment of all the carbons and protons, bidimensional NMR, as COSY and HSQC, should be used in the future. The compounds were prepared with reasonable to good yields, although some synthetic procedures could be improved, as the case of phenylpiperazine derivatives.

Currently, *N*-benzyl derivatives are of the utmost importance in the psychoactive market, and seems reasonable to predict that other *N*-benzlylated compounds of actually known substances (*e.g.* cathinone and piperazine derivatives) will probably be used recreationally in the future. Therefore their synthesis and characterization would be of most interest for forensic science.

The electrochemical behaviour of 11 compounds, 7 synthesized and 4 commercially available, was also studied. Differential and pulse voltammetry proved to be adequate methods for the study of oxidation of phenylpiperazine and *β*-phenethylamine, their derivatives and intermediates. The conjugation of the gathered results along with computational data would help to reveal oxidation mechanisms and understand the effect of the addition of different groups in psychoactive substance's moiety.

6. References

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