Selective Mono-Acylation of Piperazine Derivatives with

*Pseudomonas Stutzeri* lipase (PSL)

A thesis submitted to the University of Manchester for the degree of Organic Chemistry Mphil degree

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In the modern pharmaceutical industry, N-acylation reactions are one of the most frequently used chemical reactions. The chemical synthesis of amide bonds has some limitations, such as by-products that are harmful to the environment, which can also affect the yield, and the need for either harsh reaction conditions or stoichiometric amounts of complex coupling agents. To overcome these problems, we have investigated the use of some specific enzymes as biocatalysts for amidations. Biocatalysts have the advantage of specificity and more benign reaction conditions. In this project Pseudomonas Stutzeri Lipase (PSL) was used for the selective acylation of piperazines with esters as the acyl donors. A panel of amines and esters was screened to explore the substrate specificity of the enzyme.
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**Abbreviations**

PSL: *Pseudomonas Stutzeri* Lipase
DCC: N,N’-Dicyclohexylcarbodiimide
MTBE: Methyl tert-Butyl Ether
Molsieve 4Å: Molecular Sieve 4Å
$^1$H NMR: Hydrogen Nuclear Magnetic Resonance
$^{13}$C NMR: Carbon Nuclear Magnetic Resonance
HPLC: High Pressure Liquid Chromatography
DCM: Dichloromethane
GSK: GlaxoSmithKline
DMSO: Dimethylsulfoxide
THF: Tetrahydrofuran
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1. Introduction

The piperazine nucleus has been classified as a privileged motif by the pharmaceutical industry and is present in a large number of active pharmaceutical ingredients. In 2005 the American Chemical Society Green Chemistry Institute Pharmaceutical Roundtable has determined amide formation as one of the most utilized and problematic synthesis for the modern pharmaceutical industry.¹

Pharmaceutical companies such as GlaxoSmithKline, Pfizer and AstraZeneca have generated significant economic income from a range of drugs which contain amide bonds. Therefore, there is significant research activity in the efficient, environmentally friendly and selective synthesis of amides.

1.1. Amide synthesis

There are many methods for chemical amide bond generation (Figure 1). A common method is using N,N’-dicyclohexylcarbodiimide (DDC) or thionyl chloride to convert a carboxylic acid into an active carbonyl, such as an anhydride or an acid chloride followed by substitution of the leaving group with the amine. The coupling agent used depends upon the type of carbonyl required.²

![Figure 1. General synthesis of amides from esters or carboxylic acids](image)

The mechanism for forming an acid chloride using thionyl chloride is shown in Figure 2.
This scheme is applicable to both primary and secondary amines. The amine acts as the nucleophile which attacks the activated carbon atom. A bulky tertiary amine is used to deprotonate the quaternary ammonium intermediate, taking away the unfavorable positive charge and making it more stable.

1.2. Enzyme

‘Biocatalysis’ generally refers to the use of an enzyme to catalyse a reaction, eliminating the need for stoichiometric activating agents. This method can provide novel synthetic paths which may have higher rates of reactions. Extensive research has been carried out into this area as an alternate, eco-friendlier technique to create a range of different chemical products.

In enzyme catalyzed addition reaction, Michael addition reaction is the most studied category. In this reaction Michael donor contains an amine group and nucleophilic group, based on this enzyme have the ability of catalyzing amidation. (Figure 3).

Apart from hydrolases, bacteria isomerase 4-OT and YwhB in Na₂HPO₄ (pH 6.8) buffer solution can
catalyze the reaction of water. The study found the enzyme can active carbonyl group of acrylates to let the reaction happen (Figure 4).7

![Diagram of Michael addition to intermediate acceptor](image)

**Figure 4** Michael addition to intermediate acceptor

Some acylases not only catalyze Michael addition reactions, but also catalyze Markovnikov addition. For example, Penicillin G acylase can effectively catalyze the Markovnikov addition of allopurinol and vinyl esters (Figure 5).8

![Diagram of Markovnikov addition to different chain lengths](image)

**Figure 5** Markovnikov addition to different chain lengths

Cytochrome P450 enzymes (CYPs or P450s) are known as drug degradation. The reductive scission of molecular oxygen of P450 attracted an atom into the substrate, at same time water was produced by the second oxygen atom. Almost every P450 is deriving electrons from the pyridine cofactors NADH or NADPH (Figure 6).
Although aqueous solution is the best media for natural enzymes media, but aqueous solutions cause lots side effects due to insoluble compounds and unfavorable for thermodynamic equilibrium processes. Fortunately, in a large number of studies proved that the activity of the enzyme can be active in the organic solvent with little or no water. While using organic solvents have enhancement of enzyme selectivity, it is easy to see enzyme shows lower activity and stability in non-aqueous organic solvents. Also there are some disadvantages have been reported, such as costly procedural requirements, inactivation of enzymes due to irreversible changes, lack of stability and dispensability.

Ionic liquids are also known as molten salts which are organic salts with very low melting points. Enzymes generally exhibit higher activity when using ionic liquids as reaction media. Ionic liquids have demonstrated the advantage in some particular enzymes. Under this media, showing higher product yield than organic solvents. It is because the polarity of ionic liquids which led to increased substrate solubility and more favorable structural adaptability of lipase. Supercritical fluids are also used

(a) Three-protein systems: P450 and reductase can either be soluble or membrane-bound.
(b) Two-protein

Figure 6 P450 catalyzed reactions general steps.
in enzymes reactions, these media are most suitable for increasing mass transfer rates, overcoming the limit of diffusion and enhancing solubility of substrates. The solubility of substrates can be controlled by changing temperature and pressure which provides a prefect reaction condition for reaction.\textsuperscript{13}

Enzymes have been applied to a number of reactions, however, surprisingly little has been reported on biocatalytic amide synthesis from esters and amines. One class of enzymes that have been successfully employed are lipases and esterases.\textsuperscript{13} Due to their stable structures, they withstand organic solvents but also can be used in aqueous solutions. An important feature of lipase reactivity is that they work directly with the ester without the necessity of hydrolyzing it to a carboxylic acid or using an activating agent to transform the carbonyl into an anhydride or acid chloride; this reduces the need for toxic chemicals.\textsuperscript{14} Therefore, biocatalysts are of great interest to the pharmaceutical industry, since they increase atom economy whilst maintaining mild reaction conditions.

1.3. Lipases

Lipases are a specific class of esterases produced by secretion from Candida albicans. In biological systems and nature, they are used for the hydrolysis of large triacylglycerols and therefore only with little hydrophobic region.\textsuperscript{15}

The application of lipases is very broad, ranging from paper manufacture to use in detergents for the breakdown of fats from food on clothing to smaller, more water soluble compounds.\textsuperscript{16} The property that makes them extremely useful is their wide substrate scope where the active site can accommodate a variety of compounds such as esters, amines and alcohols as well as showing good regio- and enatioselectivity.\textsuperscript{17}

Lipase active sites are characteristic for their triad, a three amino acid sequence, of Ser-Asp-His.\textsuperscript{18} These residues collaborate with one another and, most commonly, hydrolyse fats. Interactions with fats indicate that this class of enzymes favours the catalysis of more hydrophobic moieties. An attractive quality of lipases is that they can be recycled and used again with other compounds.\textsuperscript{19} As we know the mechanism of the enzyme catalysed reaction is a prerequisite for a prediction of enantioselectivity by means of molecular modelling. But the lipase catalysed reactions is little bit different from enzyme catalysed reaction. There are two transition states which for the acylation of the active serine of the enzyme and the deacylation of the intermediate serine ester. Lipases typically have an \textit{\alpha}/\textit{\beta}-hydrolase
fold which is mostly parallel with β-sheets, flanked on both sides by α-helices. The catalytic triad, the oxyanion hole, and sites capable of binding and orientation of the substrates are the active sites. The nucleophilic serine residue is usually found is hydrophobic which makes it suitable to contain triglycerides and other hydrophobic substrates. (Figure 7).²⁰

There are three kinds of lipase reaction medium systems²¹: first is non-polar organic solvent (Micro aqueous medium system). Under this system large amounts of water have been substituted with non-polar organic solvent and the solid lipase, suspended in the organic phase, however, there still essential water in order to maintain the catalytic activity of the enzyme (generally less than 2% moisture content). The second is aqueous single-phase system. In this system, an organic solvent and water form a homogeneous single-phase solution system in which lipase, substrates and products can be dissolved. The third is an aqueous two-phase / multi-phase system. This system consists of an aqueous phase containing the dissolved lipases and a non-polar organic solvent (high lipid solubility). It is important
to know, however that no matter what system is chosen, all the reaction media contain an amount of organic solvent and water. They all have a significant impact on the catalytic reaction.

1.4. *Pseudomonas Stutzeri* Lipases (PSL)

*Candida albicans* secrete a specific class of enzymes which are called lipases. In biological systems, lipases are used for hydrolysis of large triacylglycerides. Lipases are widely used in paper manufacture, as detergents and in clothing production. The broad range of applications shows lipases can coexist with a variety of compounds such as esters, amines and alcohols.\(^{21}\) Compared to chemical catalysts the advantage of lipases is they can be recycled and reused for further reactions. In addition, the active site of lipases confers high selectivity to the catalytic reaction.

The lipase used in this project (*Pseudomonas Stutzeri* lipase) is relatively new in regards to amide production and has gained recent interest.\(^{23}\) So far, amide synthesis with PSL is still relatively unexplored. Previous studies have used PSL as biocatalyst by simply mixing the substrates with it in tubes set at temperature of 50–52°C and leaving the reaction overnight.\(^{23}\) In terms of reaction conditions and efficiency the results looked very promising.

1.5. Synthetic routes to mono-acylated piperazines

One of the most frequently used route to monoacylated pipeazines starts from commercially available mono Boc-protected parent compound (Figure 8). The Boc-group is stable in the presence of most nucleophiles and bases\(^{24}\), and can be removed at the end of the synthesis using trifluoroacetic acid in dichloromethane.

![Figure 8 Monoacylated piperazines synthesis from Boc-protected intermediate](image)

This strategy clearly has several drawbacks: the need for stoichiometric activation agents and Boc-
protection lead to poor atom economy, and furthermore by-products are produced as well as the desired amide. This can lead to issues regarding product purification. In addition, most strategies would require hydrolysis of the ester before coupling,

And finally, amide synthesis is an exothermic process, and on industrial scale it may cause significant efforts and costs to control the heat released.

Thus there is strong demand to develop new synthetic methods to avoid the necessity for stoichiometric, possibly toxic, activating agents leading to poor atom economy. One solution to these problem is the use of enzymes as biocatalysts to promote the acylation of amines with esters without the need for any other reagents with potentially high selectivity.

1.6. Aims and objectives

There are two lipases which have been identified for amide synthesis: PSL and Novozyme 435. While there is significant literature regarding Novozyme 435, including extensive directed evolution (to enhance the substrate scope), relatively limited research has been performed using PSL. However, in a recent study on the reaction of PSL with a limited substrate panel, PSL outperformed Novozyme 435, especially in regards to affinity for secondary amines to make tertiary amides.

This project is focused on the reaction shown in Figure 9. The aim was to investigate the scope of PSL for monoacylation of piperazine, avoiding diacylation side products.

![Figure 9 Enzyme-catalysed acylation of piperazine](image)

Secondly, the aim was to explore the substrate scope of the reaction by screening a range of esters and amines for mono-acylation.
2. Results and discussion

In the first instance, the substrate scope of PSL with respect to a panel of amines and esters was explored. Seven esters (Figure 10) and two amines (Figure 11) were chosen to determine which combination would give the best conversions and which ester was most suitable for PSL.

![Figure 10 Amines used as substrates](image1)

![Figure 11 Esters used as substrates](image2)

All the reactions were performed under the same conditions (Temperature: 50°C; Stirring speed: over 600 rpm) and concentration (Concentration ratio: ester to amine = 1:1), for 48 hours, then analyzing the product was analyzed by NMR spectroscopy.

2.1. Reactions of esters with 2-methylpiperazine

Methyl 2-pyrazinecarboxylate (A)

It was generally found that the NMR spectra were complicated due to the fact that the amide bonds in
the products such as 1A could exist as two rotamers. It was therefore very difficult to assign any coupling constants in the piperazine ring system (Figure 12 to Figure 14).

Figure 12 1A’H-NMR
The $^1$H spectrum and COSY of 1A showed the following chemical shifts: the proton from C-CH$_3$(H13) shows the signal at $\delta = 3.78$ ppm, the proton from N-H group shows the signal at $\delta = 1.69$ ppm. Because there is a pyrazine ring, and the pyrazine ring is affected by the carbonyl group, the chemical shift of pyrazine ring is higher than 8.5 lower than 9.0, based on this the doublet signal at $\delta = 8.49$ ppm is H1, the singlet signal at $\delta = 8.86$ ppm is proton (H5), the triplet signal at $\delta = 8.57$ ppm suggests the attribution of the proton (H2). The doublet signal at $\delta = 1.09$ ppm was assigned to the -CH$_3$ group. The chemical shift of the piperazine ring hydrogens are affected by carbonyl group and methyl group so the signals are around 2.5 to 3.5, the multiplet signal at $\delta = 2.92$ ppm belongs to the rest six protons (H10, H11 and H14).
Compared to the starting materials, the $^{13}$C spectrum of the product showed the following chemical shifts: the signal of C7 is shifted from 165.31 ppm to 165.08 ppm, the C4 is shifted from 151.67 ppm to 145.25 ppm, the C2 is shifted from 144.20 ppm to 142.60 ppm, C5 is shifted from 142.13 ppm to 145.58 ppm and C1 is shifted from 141.60 ppm to 145.58 ppm; the C15 is shifted from 18.93 ppm to 19.49 ppm, C13 is shifted from 51.03 ppm to 54.29 ppm, C14 is shifted from 54.50 ppm to 51.25 ppm, C11 is shifted from 44.85 ppm to 42.80 ppm, C10 is shifted from 46.41 ppm to 50.74 ppm. Reaction process can be speculated as following Figure 19.

![Figure 14 1A $^{13}$C-NMR](image)

![Figure 15 1A reaction formula](image)
Methyl picolinate (B)

Due to the fact that the amide bonds in the products such as 1B could exist as two rotamers, it was very difficult to assign any coupling constants from the starting materials obstructing in NMR spectroscopies. (Figure 16 to Figure 18).
The $^1$H spectrum and COSY spectrum of 1B showed the following chemical shift: compare with starting materials the proton from C-CH$_3$ (H13) shows the signal at $\delta =$ 3.77 ppm, the proton from N-H (H12) group shows the signal at $\delta =$ 1.88 ppm. The protons of the pyridine ring are affected by carbonyl group so the chemical shifts of the pyridine ring are around 7.0 ppm to 8.60 ppm, due to the incomplete reaction the spectrum has too many impurities, so the coupling also has changed, based on this the doublet signal at $\delta =$ 8.52 ppm is H2, the multiplet signal at $\delta =$ 7.73 ppm is proton (H5), the multiplet signal at $\delta =$ 7.55 ppm is the proton (H6), the multiplet signal at $\delta =$ 7.27 ppm is the proton (H1). The doublet signal at $\delta =$1.09 ppm was assigned to the -CH$_3$ group. The chemical shift of porton of the piperazine ring are affected by the carbonyl group and methyl group so the signal is around 2.60 ppm to 3.40 ppm, the multiplet signal at $\delta =$ 2.89 ppm assigned to the six protons (H10, H11 and H14).
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shifts: the signal of C7 is shifted from 166.75 ppm to 166.27 ppm, the C4 is shifted from 150.10 ppm to 153.08 ppm, the C2 is shifted from 151.55 ppm to 148.60 ppm, C6 is shifted from 137.27 ppm to 135.84 ppm, C1 is shifted from 127.78 ppm to 125.76 ppm and C5 is shifted from 126.48 ppm to 123.16 ppm; the C15 is shifted from 18.93 ppm to 18.38 ppm, C13 is shifted from 51.03 ppm to 53.21 ppm, C14 is shifted from 54.50 ppm to 49.45 ppm, C11 is shifted from 44.85 ppm to 44.34 ppm, C10 is shifted from 46.41 ppm to 49.96 ppm.

Reaction process can be speculated as following Figure 23.

Figure 19 1B reaction formula
Methyl nicotinate (C)

The nmr spectra were complicated, because the amide bonds in products such as 1C could exist as two rotamers. Consequently, it is very difficult to assign any coupling constants in the piperazine ring system (Figure 20 to Figure 22).

Figure 20 1C $^1$H-NMR
The $^1$H spectrum and COSY spectrum of 1C showed the following chemical shifts: compare with starting material the two doublet signals at $\delta = 3.49$ ppm and $\delta = 1.69$ ppm respectively suggested the attribution of the protons each connected with the C-CH$_3$ and N-H. The chemical shift of protons of the pyridine ring is around 7.0 to 9.0. Based on this the doublet signal at $\delta = 8.60$ ppm corresponds to the two protons H1 and H3. The multiplet signal at $\delta = 7.67$ ppm is proton (H5), the doublet signal at $\delta = 7.30$ ppm suggested the attribution of the proton (H6). The multiplet signal at $\delta = 1.09$ ppm was assigned to the -CH$_3$ group. The chemical shift of the piperazine ring is around 2.5 to 3.5, so the multiplet signal at $\delta = 2.77$ ppm corresponds to the six protons (H10, H11 and H14).
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shift: the signal of C7 is shifted from 164.67 ppm to 165.72 ppm, the C1 is shifted from 153.27 ppm to 148.83 ppm, the C3 is shifted from 152.51 ppm to 146.05 ppm, C4 is shifted from 127.02 ppm to 133.13 ppm, C5 is shifted from 139.28 ppm to 129.88 ppm and C6 is shifted from 122.11 ppm to 121.59 ppm; the C15 is shifted from 18.93 ppm to 18.14 ppm, C13 is shifted from 51.03 ppm to 49.90 ppm, C14 is shifted from 54.50 ppm to 52.11 ppm, C11 is shifted from 44.85 ppm to 45.42 ppm, C10 is shifted from 46.41 ppm to 44.44 ppm.

Reaction process can be speculated as following Figure 27.

![Reaction formula](image)
Methyl benzoate (D)

It was generally found that the nmr spectra were complicated due to the fact that the amide bond in products such as 1D can exist as two rotamers. It was therefore very difficult to assign any coupling constants in the piperazine ring system (Figure 24 to Figure 26).

![Figure 24 1D 1H-NMR](image-url)
The \(^1\)H spectrum and COSY spectrum of **1D** showed the following chemical shifts: the proton of N-H (H12) group shows the signal at \(\delta = 1.18\) ppm and the protons from methyl group (H15) are a multiplet signal at \(\delta = 0.92\) ppm. The benzene ring is affected by carbonyl group and so the chemical shifts are higher than for a normal benzene ring, being around 7.0 ppm to 8.0 ppm. Based on this the doublet signal at \(\delta = 7.97\) ppm corresponds to H3 and H5, the triplet signal at \(\delta = 7.49\) ppm is proton (H1), the triplet signal at \(\delta = 7.37\) ppm is the proton (H2 and H6). The doublet signal at \(\delta = 0.92\) ppm was assigned to the -CH\(_3\) group. The chemical shift of the piperazine ring hydrogen are affected by the carbonyl group and methyl group so the signals are around 2.50 ppm to 3.00 ppm, the multiplet signal at \(\delta = 2.76\) ppm belongs to the rest seven protons (H13, H10, H11 and H14).
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shift: the signal of C7 is shifted from 167.27 ppm to 168.64 ppm, the C4 is shifted from 130.28 ppm to 131.26 ppm, the C2 is shifted from 128.54 ppm to 127.94 ppm, C6 is shifted from 128.54 ppm to 127.94 ppm, C1 is shifted from 133.62 ppm to 128.51 ppm, C3 is shifted from 129.91 ppm to 128.54 ppm and C5 is shifted from 129.91 ppm to 126.77 ppm; the C15 is shifted from 18.93 ppm to 18.40 ppm, C13 is shifted from 51.03 ppm to 52.36 ppm, C14 is shifted from 54.50 ppm to 50.16 ppm, C11 is shifted from 44.85 ppm to 45.68 ppm, C10 is shifted from 46.41 ppm to 50.46 ppm.

Reaction process can be speculated as following Figure 31.
Methyl isonicotinate (E)

Due to the fact that the amide bond in products such as 1E can exist as two rotamers, the nmr spectra were complicated and it was very difficult to assign any coupling constants in the piperazine ring system (Figure 28 to Figure 30).

Figure 28 1E ¹H-NMR
The $^1$H spectrum and COSY spectrum of 1E showed the following chemical shifts: the two multiplet signals at $\delta=1.02$ ppm suggested proton from methyl group (H15), and singlet signal at $\delta=1.91$ ppm suggested the protons from N-H (H12). Because there is a pyridine ring, but it is different from ester B and C, the chemical shift of pyridine ring is around 7.00 ppm to 8.75 ppm. Based on this the doublet signal at $\delta=8.63$ ppm are two protons (H2 and H6), the multiplet signal at $\delta=7.21$ ppm corresponds to H3 and H5. The multiplet signal at $\delta=4.49$ ppm suggested the attribution of the proton (H13). The chemical shift of protons of the piperazine ring is around 2.50 ppm to 3.50 ppm, the multiplet signal at $\delta=2.96$ ppm corresponds to the six protons (H10, H11 and H14).
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shift: the signal of C7 is shifted from 167.27 to 167.60 ppm, the C2 is shifted from 150.23 ppm to 150.60 ppm, the C6 is shifted from 150.23 ppm to 150.31 ppm, C4 is shifted from 143.75 ppm to 143.59 ppm, C3 is shifted from 117.21 ppm to 121.19 ppm; the C15 is shifted from 18.93 ppm to 19.20 ppm, C13 is shifted from 51.03 ppm to 48.98 ppm, C14 is shifted from 54.50 ppm to 47.94 ppm, C11 is shifted from 44.85 ppm to 42.38 ppm, C10 is shifted from 46.41 ppm to 46.30 ppm.

Reaction process can be speculated as following Figure 35.
Methyl 3-phenylpropionate (F)

The nmr spectra were complicated, because the amide bond in products such as 1F can exist as two rotamers. So it is very difficult to assign any coupling constants in the piperazine ring system (Figure 32 to Figure 34).

Figure 32 1F 'H-NMR
The $^1$H spectrum and COSY spectrum of $\textbf{1F}$ showed the following chemical shifts: the two doublet signals at $\delta = 1.12$ ppm and $\delta = 1.00$ ppm suggested the attribution of the protons each connected with the C-CH$_3$ and N-H. The chemical shift of benzene ring is around 7.0 to 7.5, based on this the multiplet signal at $\delta = 7.20$ ppm is five protons in benzene ring (H1, H2, H3, H4 and H6). The multiplet signal at $\delta = 2.78$ ppm suggested the attribution of the rest eleven protons of the product. Lots of impurity peaks showed in this spectrum which are residual materials.
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shift: the signal of C9 is shifted from 173.30 ppm to 170.54 ppm, the C5 is shifted from 140.87 ppm to 141.28 ppm, the C1 and C3 is shifted from 128.91 ppm to 128.51 ppm, C4 and C6 is shifted from 128.43 ppm to 126.46 ppm, C2 is shifted from 126.57 ppm to 126.18 ppm, the C7 is shifted from 31.50 ppm to 19.54 ppm and the C8 is shifted from 40.34 ppm to 20.06 ppm; the C17 is shifted from 18.93 ppm to 19.41 ppm, C15 is shifted from 51.03 ppm to 51.83 ppm, C16 is shifted from 54.50 ppm to 52.92 ppm, C13 is shifted from 44.85 ppm to 42.03 ppm, C12 is shifted from 46.41 ppm to 50.92 ppm. Reaction process can be speculated as following Figure 39.

![Figure 34 1F $^{13}$C-NMR](image)

![Figure 35 1F reaction formula](image)
2.2. Reactions of esters with 2-phenylpiperazine

Methyl 2-pyrazinecarboxylate (A)

It was generally found that the nmr spectra were complicated due to the fact that the amide bond in products such as 2A can exist as two rotamers. It was therefore very difficult to assign any coupling constants in the piperazine ring system (Figure 36 to Figure 38).
The $^1$H spectrum and COSY of 2A showed the following chemical shifts: the proton from benzene ring shows the signal at $\delta = 7.27$ ppm, the proton from N-H group shows the signal at $\delta = 1.95$ ppm. Because there is a pyrazine ring, and the pyrazine ring is affected by carbonyl group so the chemical shift of pyrazine ring is higher than 8.5 lower than 9.0, based on this the doublet signal at $\delta = 8.87$ ppm is H5, the doublet signal at $\delta = 8.54$ ppm is H1, the multiplet signal at $\delta = 8.47$ ppm suggested the attribution of the proton (H2). The chemical shift of the piperazine ring hydrogens are affect by carbonyl group and methyl group so the signals are around 2.5 to 3.5, the multiplet signal at $\delta = 2.92$ ppm belongs to the rest six protons (H10, H11 and H14).
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shift: the signal of C7 is shifted from 165.31 ppm to 164.15 ppm, the C4 is shifted from 151.67 ppm to 148.48 ppm, the C2 is shifted from 144.20 ppm to 144.56 ppm, C5 is shifted from 142.13 ppm to 141.60 ppm and C1 is shifted from 141.60 ppm to 144.59 ppm; the C15 is shifted from 142.37 ppm to 144.27 ppm, C13 is shifted from 59.86 ppm to 60.05 ppm, C14 is shifted from 51.94 ppm to 48.58 ppm, C11 is shifted from 44.49 ppm to 44.93 ppm, C10 is shifted from 46.41 ppm to 45.00 ppm, C16 and C20 is shifted from 127.90 ppm to 127.63 ppm, C17 and C19 is shifted from 128.17 ppm to 127.38 ppm, C18 is shifted from 126.22 ppm to 126.39 ppm.

Reaction process can be speculated as following Figure 47.
Methyl picolinate (B)

Due to the fact that the amide bond in products such as 2B can exist as two rotamers, the nmr spectra were complicated and it was very difficult to assign any coupling constants in the piperazine ring system (Figure 40 to Figure 42).
The $^1$H spectrum and COSY spectrum of 2B showed the following chemical shift: compare with starting materials the proton from N-H (H12) group shows the signal at $\delta = 2.02$ ppm, the proton (H13) shows the multiplet signal at $\delta = 4.67$ ppm. The chemical shift of benzene ring which connected with piperazine ring is around 7.0 ppm to 7.50 ppm which signal at $\delta = 7.24$ ppm. The protons of the pyridine ring are affected by carbonyl group so the chemical shifts of the pyridine ring are around 7.0 ppm to 8.60 ppm, due to the incomplete reaction the spectrum has too many impurities, so the coupling also has changed, based on this the doublet signal at $\delta = 7.39$ ppm is H1, the multiplet signal at $\delta = 8.52$ ppm is proton (H2), the doublet signal at $\delta = 7.72$ ppm is the proton (H5), the doublet of doublets signal at $\delta = 7.57$ ppm is the proton (H6). The multiplet signal at $\delta = 3.81$ ppm suggested two protons (H14), the multiplet signal at $\delta = 2.89$ ppm assigned to the four protons (H10 and H11).
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shifts: the signal of C7 is shifted from 166.75 ppm to 166.58 ppm, the C4 is shifted from 150.10 ppm to 140.11 ppm, the C2 is shifted from 151.55 ppm to 147.36 ppm, C6 is shifted from 137.27 ppm to 136.07 ppm, C1 is shifted from 127.78 ppm to 128.45 ppm and C5 is shifted from 126.48 ppm to 122.77 ppm; the C15 is shifted from 142.37 ppm to 139.98 ppm, C13 is shifted from 59.86 ppm to 59.17 ppm, C14 is shifted from 51.94 ppm to 53.54 ppm, C11 is shifted from 44.49 ppm to 44.96 ppm, C10 is shifted from 46.41 ppm to 45.70 ppm, C16 and C20 is shifted from 127.90 ppm to 127.56 ppm, C17 and C19 is shifted from 128.17 ppm to 127.56 ppm, C18 is shifted from 126.22 ppm to 125.92 ppm.

Reaction process can be speculated as following Figure 51.
Methyl nicotinate (C)

The nmr spectra were complicated, it is because the amide bond in products such as 2C can exist as two rotamers. So it is very difficult to assign any coupling constants in the piperazine ring system (Figure 44 to Figure 46).
The $^1$H spectrum and COSY spectrum of 2C showed the following chemical shifts: compare with starting material the singlet signals at $\delta = 4.63$ ppm respectively suggested the attribution of the protons H13, and the singlet signals at $\delta = 2.09$ ppm connected with nitrogen. The chemical shift of protons of the pyridine ring is around 7.0 to 9.0. Based on this the multiplet signal at $\delta = 9.16$ ppm corresponds to the proton H3, the multiplet signal at $\delta = 8.23$ ppm is H5, the doublet of doublets signals at $\delta = 8.23$ ppm suggested the attribution of the proton (H1), the multiplet signal at $\delta = 7.31$ ppm suggested the attribution of the benzene ring and proton (H6). The chemical shift of the piperazine ring is around 2.5 to 3.5, the multiplet signal at $\delta = 3.02$ ppm corresponds to the six protons (H10, H11 and H14).
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shift: the signal of C7 is shifted from 164.67 ppm to 165.67 ppm, the C1 is shifted from 153.27 ppm to 148.72 ppm, the C3 is shifted from 152.51 ppm to 148.72 ppm, C4 is shifted from 127.02 ppm to 133.00 ppm, C5 is shifted from 139.28 ppm to 135.01 ppm and C6 is shifted from 122.11 ppm to 124.88 ppm; the C15 is shifted from 142.37 ppm to 139.96 ppm, C13 is shifted from 59.86 ppm to 59.24 ppm, C14 is shifted from 51.94 ppm to 51.30 ppm, C11 is shifted from 44.49 ppm to 43.42 ppm, C10 is shifted from 46.41 ppm to 44.98 ppm, C16 and C20 is shifted from 127.90 ppm to 126.62 ppm, C17 and C19 is shifted from 128.17 ppm to 126.43 ppm, C18 is shifted from 126.22 ppm to 125.57 ppm.

Reaction process can be speculated as following Figure 55.
Methyl benzoate (D)

It was generally found that the nmr spectra were complicated due to the fact that the amide bonds in the products such as 2D could exist as two rotamers. It was therefore very difficult to assign any coupling constants in the piperazine ring system (Figure 48 to Figure 50).
The $^1$H spectrum and COSY of 2D showed the following chemical shifts: the proton connect with nitrogen shows the signal at $\delta = 1.11$ ppm. Because there are two benzene rings, chemical shift of benzene ring is around 7.00 ppm to 8.00 ppm, based on this the multiplet signal at $\delta = 7.24$ ppm suggested two protons (H2 and H6), the multiplet signal at $\delta = 7.48$ ppm assigned to the proton H1, the signal of H3 and H5 is multiplet signal which showed at 7.98 ppm. The multiplet signal at $\delta = 4.65$ ppm is H13; the multiplet signal at $\delta = 2.81$ ppm corresponds to the four protons (H14 and H10) and the singlet signal at $\delta = 2.63$ ppm assigned to the two protons (H11).
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shift: the signal of C7 is shifted from 167.27 ppm to 169.37 ppm, the C4 is shifted from 130.28 ppm to 131.88 ppm, the C2 is shifted from 128.54 ppm to 127.58 ppm, C6 is shifted from 128.54 ppm to 127.58 ppm, C1 is shifted from 133.62 ppm to 128.66 ppm, C3 is shifted from 129.91 ppm to 127.33 ppm, C5 is shifted from 129.91 ppm to 127.33 ppm; the C15 is shifted from 142.37 ppm to 141.57 ppm, C13 is shifted from 59.86 ppm to 60.83 ppm, C14 is shifted from 51.94 ppm to 53.04 ppm, C11 is shifted from 44.49 ppm to 44.90 ppm, C10 is shifted from 46.41 ppm to 46.61 ppm, C16 and C20 is shifted from 127.90 ppm to 127.48 ppm, C17 and C19 is shifted from 128.17 ppm to 127.40 ppm, C18 is shifted from 126.22 ppm to 126.43 ppm.

Reaction process can be speculated as following Figure 59.
Methyl isonicotinate (E)

Due to the fact that the amide bond in products such as 2E can exist as two rotamers, the nmr spectra were complicated and it was very difficult to assign any coupling constants in the piperazine ring system (Figure 52 to Figure 54).
The $^1$H spectrum and COSY spectrum of 2E showed the following chemical shifts: the singlet signal at $\delta=1.18$ ppm suggested the protons from N-H (H12). The chemical shift of benzene ring is around 7.00 ppm to 7.50 ppm, so the multiplet signal at $\delta=7.29$ ppm suggested the attribution of the proton from benzene ring. The doublet of doublets signals at $\delta=8.70$ ppm corresponds to the two protons (H2 and H6), the doublet of doublets signals at $\delta=7.77$ ppm are two protons (H3 and H5). The piperazine ring is affected by carbonyl group and benzene ring is around 2.50 ppm to 3.20 ppm. Based on this the multiplet signal at $\delta=3.02$ ppm corresponds to the four protons (H10 and H14). The singlet signal at $\delta=4.62$ ppm assigned to the proton (H13), the doublet of doublets signals at $\delta=7.77$ ppm is H11.
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shift: the signal of C7 is shifted from 167.27 to 166.88 ppm, the C2 is shifted from 150.23 ppm to 149.58 ppm, the C6 is shifted from 150.23 ppm to 149.58 ppm, C4 is shifted from 143.75 ppm to 139.74 ppm, C3 is shifted from 117.21 ppm to 121.83 ppm, C5 is shifted from 117.21 ppm to 121.83 ppm; the C15 is shifted from 142.37 ppm to 139.24 ppm, C13 is shifted from 59.86 ppm to 59.17 ppm, C14 is shifted from 51.94 ppm to 48.14 ppm, C11 is shifted from 44.99 ppm to 44.83 ppm, C10 is shifted from 46.41 ppm to 45.61 ppm, C16 and C20 is shifted from 127.90 ppm to 127.71 ppm, C17 and C19 is shifted from 128.17 ppm to 127.66 ppm, C18 is shifted from 126.22 ppm to 125.82 ppm.

Reaction process can be speculated as following Figure 63.
Methyl 3-phenylpropionate (F)

The nmr spectra were complicated, it is because the amide bond in products such as 2F can exist as two rotamers. So it is very difficult to assign any coupling constants in the piperazine ring system (Figure 56 to Figure 58).

![Figure 56 2F 1H-NMR](image)
The $^1$H spectrum and COSY spectrum of 2F showed the following chemical shifts: the multiplet signal at $\delta = 7.23$ ppm suggested the attribution of the protons from two benzene ring. The singlet signal at $\delta = 1.18$ ppm suggested the proton connected with nitrogen. Proton H15 has coupling with H16, so the multiplet signal at $\delta = 3.63$ ppm is H15 and one proton from H16 which is close to H15. The doublet of doublets signals at $\delta = 3.41$ ppm suggested the rest proton from H16 and two protons from H12. The multiplet signal at $\delta = 2.87$ ppm corresponds to the four protons (H13 and H7), the multiplet signal at $\delta = 2.51$ ppm suggested the protons H8.
Compared to the starting materials, the $^{13}$C spectrum of this product showed the following chemical shift: the signal of C9 is shifted from 173.30 ppm to 168.81 ppm, the C5 is shifted from 140.87 ppm to 138.96 ppm, the C1 and C3 is shifted from 128.91 ppm to 126.80 ppm, C4 and C6 is shifted from 128.43 ppm to 126.73 ppm, C2 is shifted from 126.57 ppm to 126.39 ppm, the C7 is shifted from 31.50 ppm to 29.06 ppm and the C8 is shifted from 40.34 ppm to 33.11 ppm; the C17 is shifted from 142.37 ppm to 139.34 ppm, C15 is shifted from 59.86 ppm to 58.24 ppm, C16 is shifted from 51.94 ppm to 51.18 ppm, C13 is shifted from 44.49 ppm to 44.46 ppm, C12 is shifted from 46.41 ppm to 46.92 ppm, C18 and C22 is shifted from 127.90 ppm to 126.67 ppm, C19 and C21 is shifted from 128.17 ppm to 126.63 ppm, C20 is shifted from 126.22 ppm to 126.27 ppm.

Reaction process can be speculated as following Figure 67.
3. Conclusion

*Pseudomonas Stutzeri Lipase* (PSL) was successfully used as a biocatalyst for amide synthesis directly from methyl esters generating amides such as **1A**, **1B**, **1C**, **1D**, **1F**, **2A**, **2B**, **2C**, **2D**, **2E**. PSL show activity towards simple esters (**A**, **B**, **C**, **D** and **E**) and amines (**1** and **2**) and is more effective when interacting with secondary amines. The natural function of lipases is to interact with fats, hydrolysing them to smaller units of fatty acids and glycerol, showing that the enzyme has a higher affinity for hydrophobic substances. That is why methyl-3-phenylpropionate (**F**) is a better substrate for PSL than methyl benzoate (**D**) due to it being more hydrophobic.

When compared the reactivity of the same ester (**A**, **B**, **C** and **D**) with different amides, the ester showed more activity when using 2-phenylpiperazine (**2**). However, when the ester was methyl benzoate (**D**), the yield was lower with 2-phenylpiperazine and however more side products than using 2-methylpiperazine.

The results have shown that PSL can be used to catalyse the formation of amides from piperazine derivatives and a range of methyl esters. These reactions have never been reported before and allow for direct amide bond formation under mild reaction conditions with no need for hydrolysis of the ester and subsequent activation.

When the crude reaction products were analysed, many of the nmr spectra showed starting materials or side products, often the diacylated side-products. The complex NMR spectra show the need to improve process conditions. Due to low concentrations of reactants it is important to find a suitable extraction method. Problems encountered were low solubility of substrates in MTBE, hence requiring alternative solvents and need for high temperature resulting in reduced enzyme activity.

Interestingly, in many cases (**1A**, **1B**, **1C**, **1D**, **2A**, **2B**, **2C**, **2D** and **2E**) mono-acylation was predominant, which would lead to useful acylpiperazine intermediates. In addition, with substituted piperazines only one regioisomeric product (**1A**, **1B**, **1C**, **1D**, **2A**, **2B**, **2C** and **2D**) was observed. The best ester substrates for PSL were found to be methyl benzoate (**D**), methyl nicotinate (**C**), methyl picolinate (**B**) and methyl 2-pyrazinecarboxylate (**A**). These esters not only reacted well with all three amines, but also showed good regioselectivity with PSL.
Future work might involve increasing the concentrations of reaction components to drive reactions to completion and facilitate extraction. Reaction optimization will hopefully generate pure products for full characterization.

4. Experimental Section

4.1. Materials

Molecular Sieve 4Å (Sigma-Aldrich), *Pseudomonas Stutzeri Lipase* (Meito Sangyo Co. LTD, Japan)

4.2. Chemicals

Amine: piperazine (anhydrous, ≥99.0%, Sigma-Aldrich), 2-methylpiperazine (95%, Sigma-Aldrich), 2-phenylpiperazine (96%, Sigma-Aldrich)

Esters: methyl benzoate (99%, Sigma-Aldrich), methyl picolinate (99%, Sigma-Aldrich), methyl isonicotinate (98%, Sigma-Aldrich), methyl nicotinate (≥99%, Sigma-Aldrich), methyl 2-fluorobenzoate (97%, Sigma-Aldrich), methyl 3-phenylpropanoate (≥99%, Sigma-Aldrich), methyl cinnamate (analytical reference material, Sigma-Aldrich), methyl 2-pyrazinecarboxylate (97%, Sigma-Aldrich)

Solution: *tert*-Butyl methyl ether (99.8%, Sigma-Aldrich), d-chloroform (99.96%, Sigma-Aldrich)

4.3. Synthetic and purification

3.3.1 Synthetic method

The ester (2 mmol) was dissolved to make a 0.1M solution in 10 mL of MTBE. The amine solution (2 mmol) was made in a similar way. Molecular Sieves 4Å (100 mg) and PSL (60 mg), were added to 2 mL tubes. The two solutions (ester/amine: 50/50) were added into the tubes and the reaction mixture was heated at 50°C, for 48 hours. The reaction mixture was then centrifuged for 15 minutes at 4°C, 4200 rpm. The supermatant was collected and evaporated on a rotary evaporator to obtain the product amide.
3.3.2 Purification

After the reaction, the reaction mixture was centrifuged in an Eppendorf 5415 R centrifuge at 2400 RPM 4°C for 15 minutes and the supernatant was transferred to a clean tube, the residue was washed with MTBE several times, Combined organics fractions were evaporated under low pressure, and the resulting crude residue dissolved in CDCl$_3$ for $^1$H NMR and $^{13}$C NMR analysis.

4.4. Analytical methods

$^1$H NMR and $^{13}$C NMR spectroscopy was used to test experimental samples.  
$^1$H NMR: Product was dissolved in CDCl$_3$ and measured on a 400 MHz spectrometer.  
$^{13}$C NMR: The products were dissolved in either CDCl$_3$ or DMSO-d6 and NMR spectroscopy was carried out using a 100 MHz spectrometer.

2-methylpiperazine (1)

\[
\begin{array}{c}
\text{N} \\
\text{H} \\
\text{Me} \\
\text{N}
\end{array}
\]

$^1$H NMR (400 MHz, Chloroform) $\delta$ 2.88 – 2.58 (m, 6H), 2.48 (s, 1H), 1.11 (d, $J = 2.8$ Hz, 4H), 0.99 (s, 1H).  
$^{13}$C NMR (100 MHz, Chloroform) $\delta$ 54.50 (s), 51.03 (s), 46.41 (s), 44.85 (s), 18.93 (s).

2-phenylpiperazine (2)

\[
\begin{array}{c}
\text{N} \\
\text{Ph} \\
\text{H}
\end{array}
\]

$^1$H NMR (400 MHz, Chloroform) $\delta$ 7.32 (t, $J = 18.0$ Hz, 5H), 3.82 (s, 1H), 3.21 – 2.53 (m, 6H), 1.26 (d, $J = 29.6$ Hz, 2H).  
$^{13}$C NMR (100 MHz, Chloroform) $\delta$ 142.37 (s), 128.17 (s), 127.90 (s), 126.22 (s), 59.86 (s), 51.94 (s), 46.41 (s), 44.99 (s).

methyl 2-pyrazinecarboxylate (A)
$^1$H NMR (400 MHz, Chloroform) δ 9.93 (s, 1H), 9.13 (s, 1H), 8.99 (s, 1H), 3.81 (s, 3H).

$^{13}$C NMR (100 MHz, Chloroform) δ 165.31 (s), 151.67 (s), 144.20 (s), 142.13 (s), 141.60 (s), 52.13 (s).

methyl picolinate (B)

$^1$H NMR (400 MHz, Chloroform) δ 8.89 (s, 1H), 8.34 (s, 1H), 7.98 (s, 2H), 3.90 (s, 3H).

$^{13}$C NMR (100 MHz, Chloroform) δ 166.75 (s), 151.55 (s), 150.10 (s), 137.27 (s), 127.78 (s), 126.48 (s), 52.13 (s).

methyl nicotinate (C)

$^1$H NMR (400 MHz, Chloroform) δ 9.06 (s, 1H), 8.80 (s, 1H), 8.17 (s, 1H), 7.46 (s, 1H), 3.90 (s, 3H).

$^{13}$C NMR (100 MHz, Chloroform) δ 164.67 (s), 153.27 (s), 152.51 (s), 139.28 (s), 127.02 (s), 122.11 (s), 52.13 (s).

methyl benzoate (D)

$^1$H NMR (400 MHz, Chloroform) δ 8.04 (s, 2H), 7.66 (s, 1H), 7.53 (s, 2H), 3.90 (s, 3H).

$^{13}$C NMR (100 MHz, Chloroform) δ 167.27 (s), 133.62 (s), 130.28 (s), 129.91 (s), 128.54 (s), 52.13 (s).

methyl isonicotinate (E)
methyl 3-phenylpropanoate (F)

1H NMR (400 MHz, Chloroform) δ 7.35 – 7.09 (m, 5H), 3.61 (s, 3H), 2.85 (s, 2H), 2.53 (s, 2H).
13C NMR (100 MHz, Chloroform) δ 173.30 (s), 140.87 (s), 128.91 (s), 128.43 (s), 126.57 (s), 51.87 (s), 32.83 (s), 30.21 (s).

methyl cinnamate (G)

1H NMR (400 MHz, Chloroform) δ 7.90 – 7.41 (m, 6H), 6.31 (s, 1H), 3.77 (s, 3H).
13C NMR (100 MHz, Chloroform) δ 168.23 (s), 144.27 (s), 136.15 (s), 128.70 (d, J = 13.1 Hz), 128.05 (s), 116.07 (s), 52.02 (s).

(3-methylpiperazin-1-yl)(pyrazin-2-yl)methanone (IA)

1H NMR (400 MHz, CDCl₃) δ 8.86 (s, 1H), 8.57 (t, J = 2.7 Hz, 1H), 8.49 (ddd, J = 4.0, 2.5, 1.6 Hz, 1H), 3.78 (ddt, J = 16.6, 8.3, 4.7 Hz, 1H), 3.14 – 2.72 (m, 6H), 1.90 (s, 1H), 1.18 (s, 3H).
13C NMR (100 MHz, CDCl₃) δ 165.08 (s), 145.58 (s), 145.25 (s), 142.60 (s), 54.29 (s), 51.25 (s), 50.74 (s), 42.80 (s), 19.49 (s).

(3-methylpiperazin-1-yl)(pyridin-2-yl)methanone (IB)
$^1$H NMR (400 MHz, CDCl$_3$) δ 8.52 (d, $J = 0.8$ Hz, 1H), 7.73 (tt, $J = 7.7$, 1.8 Hz, 1H), 7.55 (dt, $J = 7.8$, 1.1 Hz, 1H), 7.30 – 7.23 (m, 1H), 3.82 – 3.70 (m, 1H), 3.16 – 2.61 (m, 6H), 1.88 (s, 1H), 1.13 – 1.03 (m, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 166.27 (s), 153.08 (s), 148.60 (s), 135.84 (s), 125.76 (s), 123.16 (s), 53.21 (s), 49.96 (s), 49.45 (s), 44.34 (s), 18.38 (s).

(3-methylpiperazin-1-yl)(pyridin-3-yl)methanone (1C)

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.60 (dd, $J = 4.7$, 1.5 Hz, 2H), 7.75 – 7.60 (m, 1H), 7.32 (ddd, $J = 12.2$, 5.3, 2.9 Hz, 1H), 3.57 – 3.40 (m, 1H), 3.11 – 2.35 (m, 6H), 1.69 (s, 1H), 1.28 – 0.96 (m, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 165.72 (s), 148.83 (s), 146.05 (s), 133.13 (s), 129.88 (s), 121.59 (s), 52.11 (s), 49.90 (s), 45.42 (s), 44.44 (s), 18.14 (s).

(3-methylpiperazin-1-yl)(phenyl)methanone (1D)

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.97 (d, $J = 8.2$ Hz, 2H), 7.49 (t, $J = 7.4$ Hz, 1H), 7.37 (t, $J = 7.7$ Hz, 2H), 2.92 – 2.55 (m, 7H), 1.18 (s, 1H), 0.92 (t, $J = 8.8$ Hz, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 168.64 (s), 131.26 (s), 128.51 (s), 127.94 (d, $J = 3.2$ Hz), 126.77 (d, $J = 12.6$ Hz), 52.36 (s), 50.46 (s), 50.16 (s), 45.68 (s), 18.40 (s).

(3-methylpiperazin-1-yl)(pyridin-4-yl)methanone (1E)
$^1$H NMR (400 MHz, CDCl$_3$) δ 8.63 (d, $J = 4.5$ Hz, 2H), 7.23 – 7.19 (m, 2H), 4.57 – 4.42 (m, 1H), 3.46 – 2.55 (m, 6H), 1.91 (s, 1H), 1.14 – 0.90 (m, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 167.60 (s), 150.60 (s), 150.31 (s), 143.59 (s), 122.83 (s), 121.19 (s), 48.98 (s), 47.94 (s), 46.30 (s), 19.20 (s).

1-(3-methylpiperazin-1-yl)-3-phenylpropan-1-one (1F)

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.20 (d, $J = 4.1$ Hz, 5H), 2.78 (ddd, $J = 25.2$, 11.7, 2.7 Hz, 11H), 1.12 (s, 1H), 1.00 (s, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 170.54 (s), 141.28 (s), 128.51 (s), 128.46 (s), 126.18 (s), 52.92 (s), 51.83 (s), 50.92 (s), 42.03 (s), 20.06 (s), 19.54 (s), 19.41 (s).

(E)-1-(3-methylpiperazin-1-yl)-3-phenylprop-2-en-1-one (1G)

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.47 – 7.44 (m, 2H), 7.33 – 7.24 (m, 4H), 6.81 (d, $J = 15.4$ Hz, 1H), 3.97 – 3.79 (m, 1H), 3.07 – 2.58 (m, 6H), 1.62 (s, 1H), 1.04 (d, $J = 6.0$ Hz, 3H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 164.36 (s), 143.85 (s), 134.32 (s), 127.77 (s), 127.05 (s), 116.78 (s), 53.03 (s), 50.80 (s), 46.34 (s), 45.36 (s), 19.04 (s).

(3-phenylpiperazin-1-yl)(pyrazin-2-yl)methanone (2A)

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.87 (dd, $J = 8.9$, 1.4 Hz, 1H), 8.54 (dd, $J = 15.2$, 2.5 Hz, 1H), 8.50 –
8.42 (m, 1H), 7.32 – 7.22 (m, 5H), 4.72 – 4.61 (m, 1H), 3.14 – 2.73 (m, 6H), 1.95 (s, 1H).
$^{13}$C NMR (100 MHz, CDCl$_3$) δ 164.15 (s), 148.48 (s), 144.59 (s), 144.56 (s), 144.27 (s), 141.60 (s), 127.63 (s), 127.38 (s), 126.39 (s), 60.05 (s), 48.58 (s), 45.00 (s), 44.93 (s).

(3-phenylpiperazin-1-yl)(pyridin-2-yl)methanone (2B)

![Chemical Structure](image)

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.58 – 8.44 (m, 1H), 7.72 (d, $J = 7.7$ Hz, 1H), 7.57 (dd, $J = 12.5, 7.8$ Hz, 1H), 7.39 (d, $J = 7.3$ Hz, 1H), 7.29 – 7.18 (m, 5H), 4.74 – 4.55 (m, 1H), 3.95 – 3.67 (m, 2H), 3.12 – 2.63 (m, 4H), 2.02 (s, 1H).
$^{13}$C NMR (100 MHz, CDCl$_3$) δ 166.58 (s), 147.36 (s), 140.11 (s), 139.98 (s), 136.07 (s), 127.56 (s), 125.92 (s), 123.45 (s), 122.77 (s), 59.17 (s), 53.54 (s), 45.70 (s), 44.96 (s).

(3-phenylpiperazin-1-yl)(pyridin-3-yl)methanone (2C)

![Chemical Structure](image)

$^1$H NMR (400 MHz, CDCl$_3$) δ 9.20 – 9.13 (m, 1H), 8.70 (dd, $J = 4.9, 1.7$ Hz, 1H), 8.25 – 8.19 (m, 1H), 7.33 – 7.29 (m, 6H), 4.63 (s, 1H), 3.07 – 2.98 (m, 6H), 2.09 (s, 1H).
$^{13}$C NMR (100 MHz, CDCl$_3$) δ 165.67 (s), 148.72 (s), 139.96 (s), 135.01 (s), 133.00 (s), 126.62 (s), 126.43 (s), 125.57 (s), 124.88 (s), 59.24 (s), 51.30 (s), 44.98 (s), 43.42 (s).

phenyl(3-phenylpiperazin-1-yl)methanone (2D)

![Chemical Structure](image)

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.02 – 7.94 (m, 2H), 7.50 – 7.45 (m, 1H), 7.27 – 7.23 (m, 9H), 4.65 (s, 1H), 2.84 – 2.78 (m, 4H), 2.63 (d, $J = 1.7$ Hz, 2H), 1.11 (s, 1H).
$^{13}$C NMR (100 MHz, CDCl$_3$) δ 169.37 (s), 141.57 (s), 131.88 (s), 128.66 (s), 127.58 (s), 127.48 (s), 53
(3-phenylpiperazin-1-yl)(pyridin-4-yl) methanone (2E)

1H NMR (400 MHz, CDCl₃) δ 8.70 (dd, J = 4.4, 1.6 Hz, 2H), 7.77 (dd, J = 4.4, 1.6 Hz, 2H), 7.31 – 7.27 (m, 5H), 4.62 (s, 1H), 3.05 – 3.00 (m, 4H), 2.78 (dd, J = 12.6, 3.5 Hz, 2H), 1.18 (s, 1H).

13C NMR (100 MHz, CDCl₃) δ 166.68 (s), 149.58 (s), 139.74 (s), 139.24 (s), 127.71 (s), 127.66 (s), 125.82 (s), 121.83 (s), 59.17 (s), 48.14 (s), 45.61 (s), 44.83 (s).

3-phenyl-1-(3-phenylpiperazin-1-yl) propan-1-one (2F)

1H NMR (400 MHz, CDCl₃) δ 7.27 – 7.22 (m, 10H), 3.68 – 3.59 (m, 2H), 3.41 (dd, J = 10.7, 3.0 Hz, 3H), 2.90 – 2.85 (m, 4H), 2.54 – 2.50 (m, 2H), 1.18 (s, 1H).

13C NMR (100 MHz, CDCl₃) δ 168.81 (s), 139.34 (s), 138.96 (s), 126.80 (s), 126.73 (s), 126.67 (s), 126.63 (s), 126.39 (s), 126.27 (s), 58.24 (s), 51.18 (s), 46.92 (s), 44.46 (s), 33.11 (s), 29.06 (s).

(E)-3-phenyl-1-(3-phenylpiperazin-1-yl) prop-2-en-1-one (2G)

1H NMR (400 MHz, CDCl₃) δ 7.48 – 7.36 (m, 5H), 7.36 – 7.30 (m, 6H), 7.24 – 7.22 (m, 1H), 4.03 – 3.87 (m, 1H), 3.67 (dd, J = 10.2, 2.8 Hz, 2H), 3.16 – 2.90 (m, 4H), 1.16 (d, J = 12.1 Hz, 1H).

13C NMR (100 MHz, CDCl₃) δ 165.48 (s), 144.89 (s), 134.39 (s), 130.32 (s), 128.91 (s), 128.81 (s), 128.43 (s), 128.09 (s), 127.78 (s), 126.90 (s), 117.81 (s), 62.00 (s), 51.72 (s), 47.78 (s), 46.05 (s).
5. Acknowledgements

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References


