CHAPTER 4
BIOLOGICAL EVALUATION: APOPTOSIS & MONOAMINE OXIDASE B

The development of multifunctional compounds to treat the symptoms produced by neurodegenerative diseases and to possibly alter the underlying pathological processes that give rise to these diseases represents a challenge for medicinal chemistry. The compounds synthesised in this study were tested for anti-apoptotic and MAO-B inhibition activity. By halting the apoptotic cascade these compounds may eliminate the neuronal breakdown process and stop or slow the progression of neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD). Should these compounds also inhibit MAO-B activity, they may be effective in treating several of the symptoms produced by these diseases.

1 Apoptosis detection - DePsipher™ assay

1.1 Introduction

Apoptosis is a complex process that can be induced by many different factors, which in turn act through various cell death signalling pathways. There is an abundance of data suggesting that mitochondria play a critical role in apoptosis by releasing cytochrome c, and other proteins that are essential for the activation of pro-caspase-9 and the execution of apoptosis. Some suggest that mitochondria are the primary triggers of cell death, while others suggest that they act more as facilitators rather than essential role players in the cell death process. Either way the mitochondrion plays an important role in the execution and completion of the apoptotic process (Leist & Jaattela, 2001).

Cellular energy produced during oxidation reactions in the mitochondrial respiratory chain, is stored as a negative electrochemical gradient across the mitochondrial membrane, called delta-psi or $\Delta\Psi m$ that enables the cell to drive the synthesis of ATP. The mitochondrial membrane of healthy cells is referred to as being polarised, and disruption of $\Delta\Psi m$ has been shown to be one of the first intracellular changes following the onset of apoptosis. The so-called collapse of the $\Delta\Psi m$ results in a depolarised $\Delta\Psi m$, which causes the release of cytochrome c, making depolarisation a possible prerequisite for apoptosis to take place (LeMasters *et al.*, 1998; Facompre *et al.*, 2000).

1.2 Principle by which DePsipher™ works

DePsipher™ utilises a lipophilic cation (5,5´,6,6´-tetrachloro-1,1´,3,3´-tetraethyl-benzimidazolyl-carbocyaniniodide), which can be used as a mitochondrial activity marker to evaluate the viability of a cell population, detecting early apoptosis, and evaluating the effect of drugs on the cell population. The cation aggregates upon membrane polarisation forming an orange-red fluorescent compound. If the potential is disturbed, the dye can not access the transmembrane space and remains or reverts to its green monomeric form. Thus, healthy cells present both the polymeric (orange-red) and monomeric (green) form of the cation, with the monomeric form residing in the cytoplasm and the aggregated form in the transmembrane space. It can be concluded that in comparison with healthy cells, wherein apoptosis has not been induced, the ratio of orange-red fluorescence to green fluorescence of apoptotic cells will be lower. The fluorescence can be observed and measured by flow cytometry with the red aggregates having absorption/emission maxima of 585/590 nm, and the green monomers absorption/emission maxima of 510/527 nm.

1.3 Biological material

1.3.1 Neuroblastoma cells used – SK-N-BE(2)

In the current study the SK-N-BE(2) cell line was used to evaluate the anti-apoptotic activity of the synthesised compounds.

The SK-N-BE(2) neuroblastoma cell line was established in November of 1972 from a bone marrow biopsy taken from a child with disseminated neuroblastoma, after repeated courses of chemotherapy and radiotherapy. They have a reported saturation density greater than 1 x 10^6 cells/cm^2, and the morphology of the cells varies with some cells having long processes and others being epithelioid like. The cells aggregate, form clumps and float. They grow as a mixture of floating (suspension) and adherent cells (Biedler & Splengler, 1976a, 1976b; Biedler *et al.*, 1978; Barnes *et al.*, 1981).

1.3.2 Cell cultivation

The cells were cultivated in serum-rich growing medium, consisting of F12 nutrient (F12) supplemented with 10% foetal bovine serum (FBS), 1% penicillin/streptomycin (Pen/Strep) (10 000 units/100 mL stock) and 0.1% fungizone (FZ) (2.5 µg/mL units stock). They were incubated at 37 °C in a 5% CO_2 and 95% O_2 humidified atmosphere. The cells duplicated every 30-48 hours and when a confluency of 80% was reached, the adherent cells were detached from the flask bottom by means of trypsination. To ensure complete detachment of

the cells, they were incubated with trypsine for approximately 10 minutes. After trypsination, the cells were seeded in new flasks at a density of no less than 1/6 confluency.

1.3.3 Induction of apoptosis

In the current study trophic factor deprivation/serum starvation was used to induce apoptosis in cells. With growth factors being essential to the normal development and survival of neuronal cells, a shortage thereof would cause neurons to compete with one another for neurotrophic factors, with the unsuccessful ones dying. With no serum (neurotrophic factors) available all the cells will die in a given time. The serum deprived medium consisted of F12, Pen/Strep and FZ in the same ratio as mentioned above (see section 1.3.2).

1.3.4 Preparation of cells to be analysed by flow cytometry

1.3.4.1 Cells used to evaluate anti-apoptotic activity of compounds

Test compounds (**2** – **7**) and positive control

After the cells reached 80% confluency, the serum rich medium was replaced with serum deprived medium. The cells were then incubated under normal conditions (see section 1.3.2) for a period of 24 hours, which was sufficient time for apoptosis to be induced. Examining the cells under the microscope after this period of time, it was clear that their morphology had changed. None of the cells were any longer attached to the bottom of the flask, and their shape had also changed from the sprouting appearance to a spherical shape, being indicative of unhealthy cells.

After being incubated in the serum deprived medium for the indicated time, the medium was removed using centrifugation. The cells were then seeded in 24-well plates, with each well containing a concentration of 1×10^6 cells per 2 mL of serum deprived medium containing the test compounds, which were dissolved in dimethyl sulfoxide (DMSO). Final concentrations of samples to be analysed contained 1.25% (v/v) DMSO. The cells were then incubated under normal conditions for a period of 36 hours before being analysed.

The test compounds (**2**,**3**,**4a**,**4b**,**5a**,**5b**,**6**,**7**) were all tested in triplicate at three different concentrations (1 mM, 100 µM and 10 µM).

The positive control, selegiline, was tested in triplicate at two concentrations (100 µM and 10 µM). Included into the research was NGP 1-01 which was also tested in triplicate at only one concentration (1 mM).

1.3.4.2 Cells used for control experiments

In this study four control experiments were included.

Control experiment 1

After the cells reached 80% confluency, the serum rich medium was replaced with *serum deprived medium*. The cells were then incubated under normal conditions for a period of 24 hours. After being incubated in the *serum deprived medium*, the medium was removed using centrifugation. The cells were subsequently seeded in 24-well plates, with each well containing a concentration of 1 x 10^6 cells per 2 mL of *serum deprived medium* and dimethyl sulfoxide (DMSO) (1.25% (v/v)). After this the cells were incubated under normal conditions (37 °C in a 5% CO_2 and 95% O_2 humidified atmosphere) for a period of 36 hours before being analysed.

Control experiment 1 was included to evaluate the viability status of the cells, in the absence of test compound. The viability data will be compared to the data generated with the test compounds in order to determine if the test compounds attenuated the progression of the apoptotic process. With this control experiment the effect of dimethyl sulfoxide (DMSO) was also evaluated.

Control experiment 2

After the cells reached 80% confluency, the serum rich medium was replaced with *serum deprived medium*. The cells were then incubated under normal conditions for a period of 24 hours. After being incubated in the *serum deprived medium*, the medium was removed using centrifugation. The cells were subsequently seeded in 24-well plates, with each well containing a concentration of 1 x 10^6 cells per 2 mL of *serum deprived medium* with no DMSO content. The cells were incubated under normal conditions for a period of 36 hours before being analysed.

Control experiment 2 will be used to evaluate the effect of DMSO on the viability of the cells and on the progression of the apoptotic process.

Control experiment 3

After the cells reached 80% confluency, the serum rich medium was replaced with *serum deprived medium*. The cells were subsequently incubated under normal conditions for a period of 24 hours. After being incubated in the *serum deprived medium*, the medium was removed using centrifugation. The cells were then seeded in 24-well plates, with each well

containing a concentration of 1 x 10^6 cells per 2 mL of fresh *serum rich medium* with no DMSO present. After this the cells were incubated under normal conditions for a period of 36 hours before being analysed.

Since the re-introduction of serum rich medium is expected to significantly attenuate the apoptotic process, this control experiment will be used to estimate the potencies of the test compounds as anti-apoptotic agents.

Control experiment 4

After the cells reached 80% confluency, the serum rich medium was replaced with *fresh serum rich medium*. The cells were then incubated under normal conditions for a period of 24 hours. After being incubated in the *serum rich medium*, the medium was removed using centrifugation. The cells were subsequently seeded in 24-well plates, with each well containing a concentration of 1 x 10^6 cells per 2 mL of *serum rich medium* with no DMSO present. The cells were incubated under normal conditions for a period of 36 hours before being analysed.

Control experiment 4 was included to analyse and determine the viability status of the cells, when no apoptosis had been induced, and conditions had been favourable to the end. It was also included to ensure that the cells analysed were indeed healthy before inducing apoptosis.

1.4 Assay procedure

The cells of each sample were harvested consecutively as follows: After incubating the cells for 36 hours, the medium was removed from the cells using centrifugation. The cells that had adhered to the bottom of the flasks were removed by trypsination. The trypsine/cell mixture was added to the cells from which the medium had been removed. The trypsine was removed from the cells by centrifugation at 500 x g for five minutes at room temperature. The cells of each sample were then resuspended in 1 mL of diluted DePsipher solution, consisting of 1 µL DePsipher dye and 1 mL of pre-warmed F12 medium. The cells were incubated for 20 minutes at 37 °C and 5% CO_2. The samples were washed twice in PBS, and centrifuged at 500 x g between each wash. The cells were subsequently resuspended in 1 mL PBS and immediately taken to be analysed on the flow cytometer. The samples were kept shielded from light until analysis, since the DePsipher™ agent is light sensitive.

1.5 Data analysis

Analysis of the samples were performed on a BD FacsCalibur® flow cytometer [Becton Dickinson, San Jose (USA)], equipped with a 15 mV 488 nm, air-cooled argon-ion laser. Cells were gated in a forward scatter/side scatter plot to exclude debris. Green and red fluorescence were detected in the corresponding FL-1 and FL-2 photomultipliers *through* 530 nm (FITC) or 585 nm (PE/PI) bandpass filters respectively. In generating the data, the flow cytometer was set to analyse 50 000 events/cells.

Figure 4.1 contains a representative pseudo-colour graph of every sample, which was generated using FlowJo®, based on the data of the flow cytometry. On the x-axis the FL-1 (green) fluorescence intensity is plotted, and on the y-axis the FL-2 (red) fluorescence intensity is plotted. Both intensities are measured in MFI (mean fluorescence intensity). Each spot is representative of a single cell analysed with the flow cytometer. The graphs are divided into four separate quadrants, each containing a population of cells, which exhibit the same characteristics. In the upper left quadrant (Q1) lies all the cells which emit only red fluorescence, whereas the cells which emit only green fluorescence can be found in the lower right quadrant (Q4). The cells which emit both wavelengths of fluorescence can be found in the upper right quadrant (Q2), and the cells emitting neither of the two in the lower left quadrant (Q3).

The following can be concluded by taking note of the explanations of the different quadrants as well as the characteristics of cells in each of these quadrants: As healthy cells contain both the monomeric and polymeric form of the cation dye, and would thus have intensity in both the FL1 and FL2 channels used to analyse the samples, the MFI values of these cells will lie in Q2 (see control experiment 4). The cells with MFI values in Q1 have only red fluorescence, which might be due to low concentrations of the dye inside the cell, indicating that all the monomers were polymerised. All these cells can thus also be said to be healthy, together with these found in Q2. Cells wherein apoptosis have been induced only contain the monomeric form of the dye, and will not colour red at all, and will thus have MFI values in Q4. The cells lying in Q3 have not been dyed successfully or intensively enough, and have thus not coloured either red or green.

From the pseudo-colour graphs it is clear that there was a difference in the number of anti-apoptotic/healthy cells present in the different samples. Control experiment 1 had the least MFI values in Q1+Q2, and control experiment 4 the most. The MFI values of the other samples were either between the values of control experiments 1 and 4, or just above that of control experiment 4.

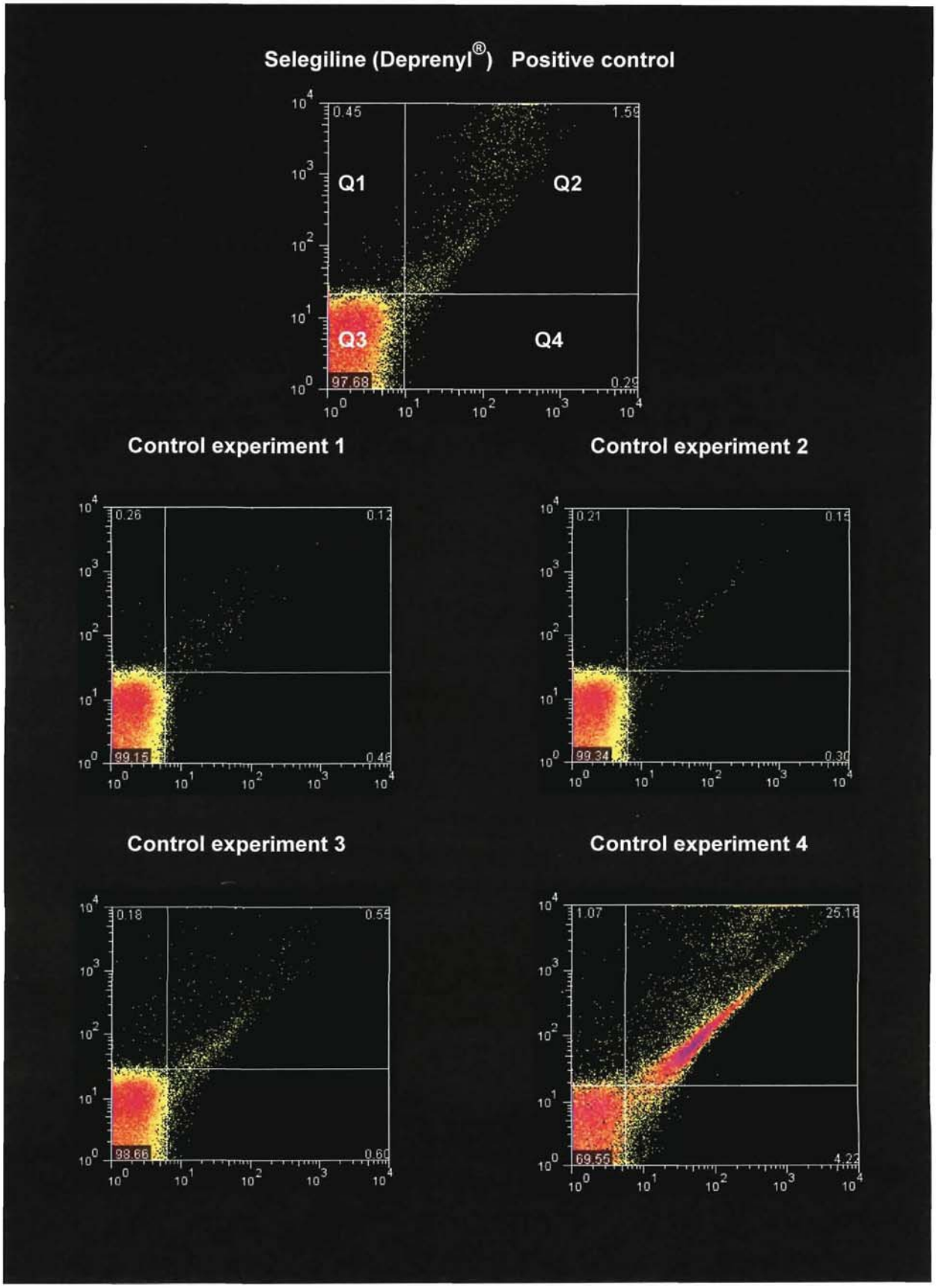


Figure 4.1: Pseudo-colour graphs of samples analysed

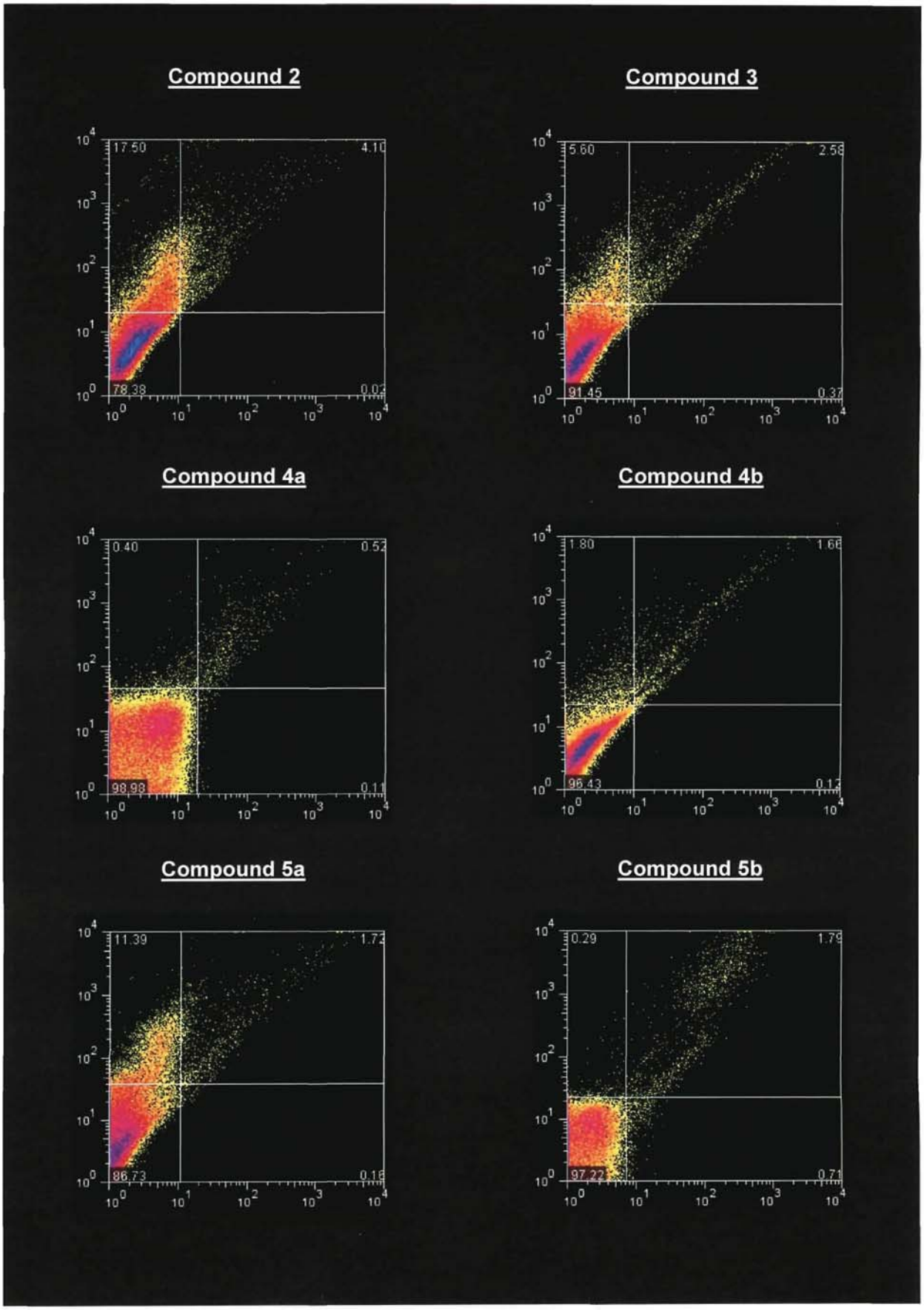


Figure 4.1: Pseudo-colour graphs of samples analysed (continued)

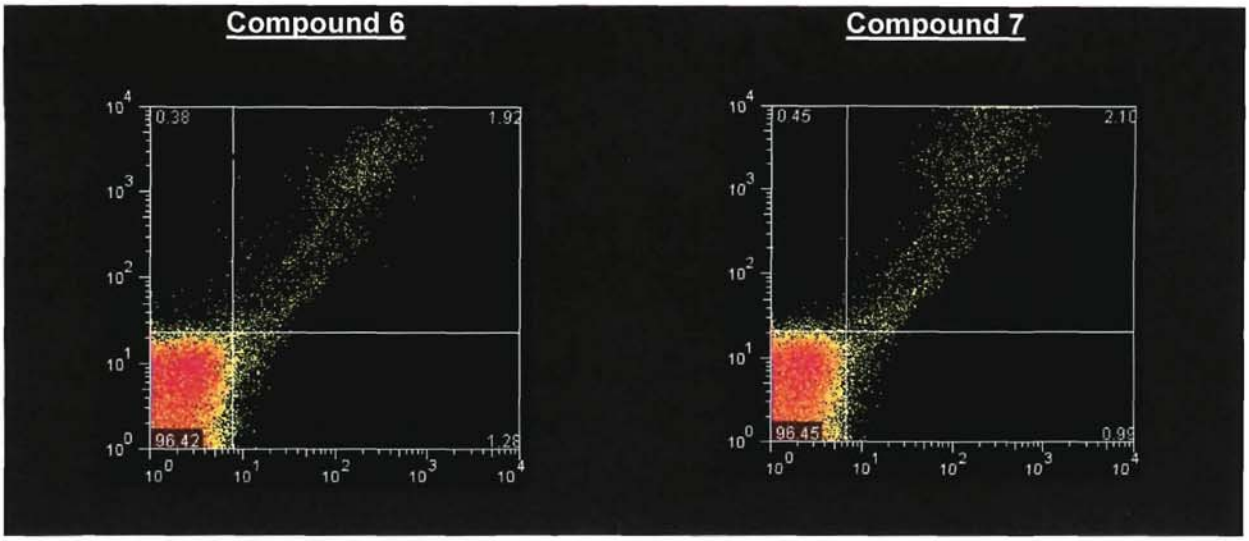


Figure 4.1: Pseudo-colour graphs of samples analysed (continued)

1.5.1 Results

The data generated by these flow cytometry experiments were used to determine if the test compounds have anti-apoptotic properties on cultured cells. The results are graphically summarised in a histogram (fig. 4.2). The percentage values used in plotting the histrogram (fig. 4.2) were representative of the percentage of the coloured cells which were still viable and non-apoptotic. The values utilised to quanitify cell viability were determined by the flow cytometer. Equation 4.1 was used to calculate the percentage of cells which were still viable in the samples analised. In determinining this value, the amount of cells in quadrant 1 (Q_1), quadrant 2 (Q_2) and quadrant 3 (Q_3) and the total amount of events analised (Q_{total}) were utilised.

$$\% \text{ Viable cells} = \frac{(Q_1 + Q_2)}{(Q_{total} - Q_3)} \times 100 \qquad \text{Eq. 4.1}$$

The results confirmed that a significant percentage of the cells had indeed coloured successfully with the dye. The percentage of the coloured cells that had fluorescence intensity in the FL2 channel, gave a quantitative indication of the number of healthy cells present in each sample, wherein apoptosis had not progressed or been induced.

In control experiment 1, 41.62% of the cells were apoptotic, with only 58.38% remaining healthy. This indicates that the method of apoptosis induction had indeed been successful. Comparing the values of control experiments 1 and 2, it is clear that even though the final dimethyl sulfoxide (DMSO) content in the samples was only 1.25%, the DMSO had a negative effect on the health of the cells. In control experiment 2 which did not contain any

DMSO, there was 7.19% more cells that were healthy. It can thus be said that the serum deprivation was not the only factor inducing apoptosis, but also the DMSO which served as solvent for the test compounds.

It has been shown in literature (Bar-Am *et al.*, 2005) that the anti-apoptotic effect of selegiline is dependent upon the propargylamine moiety in its structure. This anti-apoptotic activity is evident from the histogram. The positive control had 12-19% more cells that were healthy and wherein apoptosis had either not been induced or reached the level of mitochondrial depolarisation, compared to control experiment 1.

When compared to control experiment 1, compounds **2**, **3**, **4a**, **4b**, **NGP 1-01**, **5a**, **5b**, **6** and **7** all had significant anti-apoptotic activity. The anti-apoptotic activity of **2**, **3**, **4a**, **4b**, **5a**, **5b** and **6** were all extremely significant ($p < 0.001$), with that of **NGP 1-01** and compound **7** being significant ($p < 0.05$).

1.6 Discussion

In this study the contribution of several functional groups to the anti-apoptotic activity of propargylamine were evaluated. These groups included the following: a terminal acetylene group (**2**), an acetylene group between two non-polar groups (**3**), and a propargylamine connected to a cage compound (**4 – 7**). Besides the synthesised compounds an additional compound, **NGP 1-01** (Van der Schyf *et al.*, 1986), a well known calcium channel antagonist, was also evaluated for anti-apoptotic activity, as abnormally increased calcium levels acts as a trigger of apoptosis.

Even though the anti-apoptotic activity of **NGP 1-01** was significant, the activity of all the synthesised compounds, were higher. This might be due to **NGP 1-01** only inhibiting one of the triggers of apoptosis (calcium increase), whereas the synthesised compounds possibly inhibit the apoptotic cascade, directly or indirectly, at more than one point.

The activity of compounds **2** and **3** reveal that the acetylene group indeed contributes to anti-apoptotic activity, be it a terminal acetylene group (**2**) or an acetylene group between two non-polar groups (**3**). Whether this activity is solely due to the acetylene group, or the acetylene group in conjunction with a nearby electronegative atom/group, needs to be investigated further.

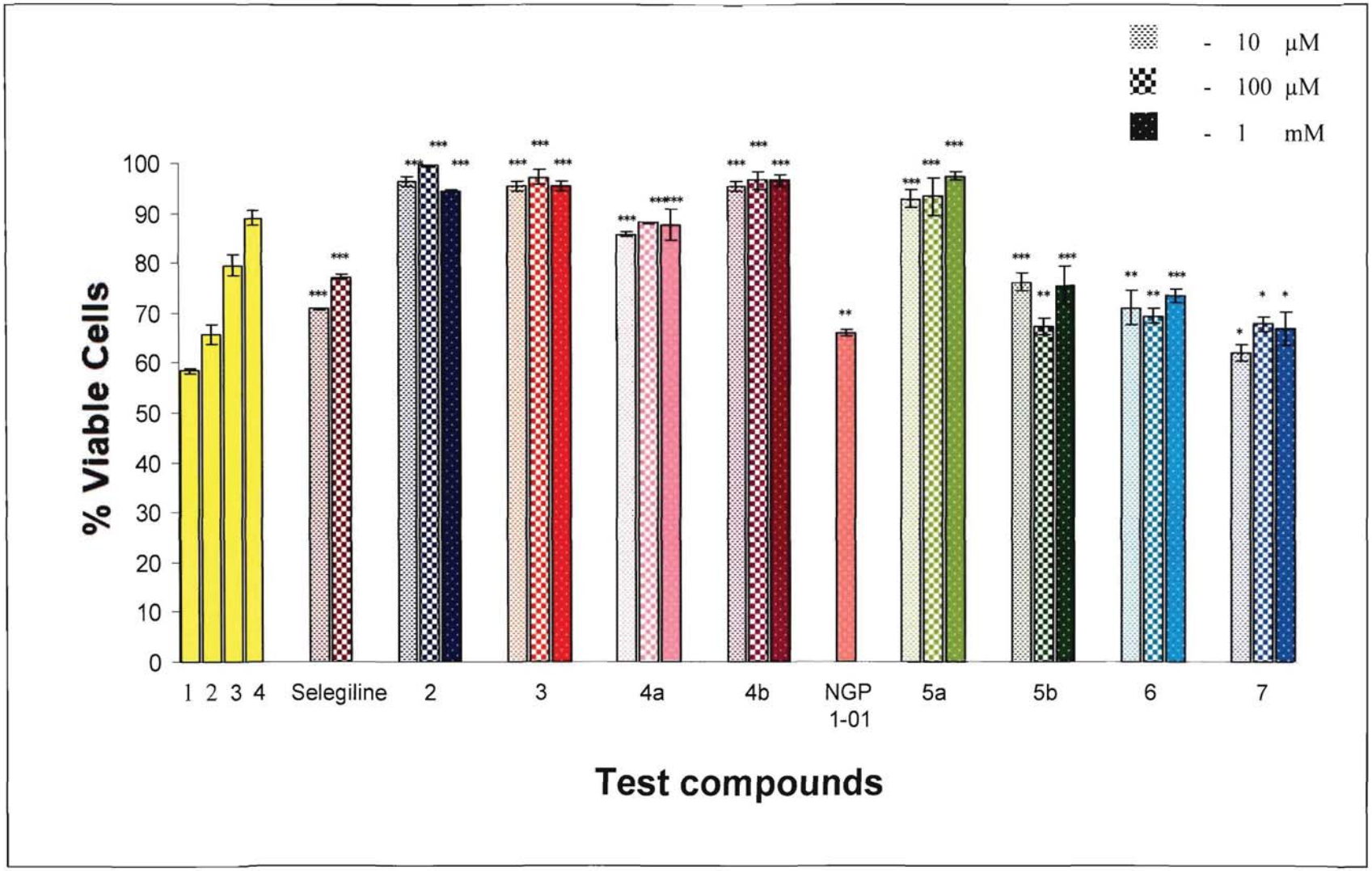


Figure 4.2: Anti-apoptotic activity of compounds (*** indicate significance with $p < 0.05$: * $p < 0.01$: ** $p < 0.001$: ***)

When comparing the anti-apoptotic activity of compounds **4a**, **4b**, **5a**, **5b**, it is clear that in this case it did not matter how the propargylamine moiety was connected to the polycyclic cage compound. Both the oxa- and aza-compounds had comparable activity. In the case of the oxa-compounds (**4a**, **4b**), the methylated cage had increased the activity. With the aza-compounds (**5a**, **5b**), this was not observed. With the activity of the oxa- and the aza-compounds being very similar, the conclusion can be made that both the *secondary and* tertiary propargylamine has anti-apoptotic activity.

Compounds **6** and **7** had significantly lower activities than that of compounds **2 – 5a**. With both these compounds (**6** and **7**) solubility problems were experienced at higher concentrations, which may explain the observed lower anti-apoptotic action. When looking at the structures of these two compounds, and comparing them to those of compounds **4a - 5b**, they are different in having an additional non-polar group besides the single acetylene group. This might make the compounds unfavourable for interaction with targets in the apoptotic pathway.

Comparing the percentage healthy cells of the samples treated with the test compounds **2**, **3**, **4b** and **5a**, with that of the control experiment 4, which is representative of healthy cells in a favourable environment, it is interesting to note that these samples had more healthy cells than that of control experiment 4. This may be attributed to the compounds inhibiting even baseline apoptotic processes. The percentage of the cells which were found to be apoptotic in control experiment 4 may be, amongst others, attributed to protocol handling of the cells during change of the medium. These processes are not favourable for the cells, and may also possibly be a trigger of apoptosis.

2 Monoamine oxidase B inhibition assay

2.1 Introduction

Monoamine oxidase B (MAO-B) is an enzyme that plays a crucial role in the oxidative deamination reactions of certain monoamine neurotransmitters, which perform fundamental roles in the normal functionality and wellbeing of the human central nervous system (CNS). When its activity increases, MAO-B causes an augmentation in the levels of oxidative stress in the CNS. The hydrogen peroxide (H_2O_2) produced during amine oxidation, may interact with free iron to form highly reactive hydroxyl radicals that can damage nucleic acids, proteins, and membrane lipids leading to neuronal degeneration (Nicotra *et al.*, 2004). Inhibition of MAO-B will lead to a decrease in oxidative stress and ultimately a decrease in neuronal degeneration. Thus it can be concluded that MAO-B inhibition is beneficial as a

neuroprotective strategy, and can play an important role in the treatment and prevention of neurodegenerative disorders.

Besides its protective role in neurodegenerative diseases, such as PD and AD, MAO-B inhibition also has therapeutic potential in the treatment of the symptoms caused by the pathology of these diseases. In Parkinson's disease elevated levels of MAO-B results in increased catabolism of dopamine, which ultimately leads to reduced concentrations of dopamine compared to normal physiological levels. This dopamine depletion contributes to the symptoms of Parkinson's disease. In Alzheimer's disease MAO-B activity may also be increased. This may contribute to neuronal degeneration which ultimately leads to the loss of memory and concentration (Wells *et al.*, 2003). Incorporating the therapeutic value of MAO-B inhibitors in the design of multifunctional neuroprotective agents, would thus be a rational decision.

2.2 Preparation of biological material

The mitochondrial fraction of baboon liver tissue, as source of the MAO-B enzyme, was isolated as described previously by Salach and Wyler (Salach & Wyler, 1987), and stored at - 70 °C. Following addition of an equal volume of sodium phosphate buffer (100 mM, pH 7.4) containing glycerol (50%, w/v) to the mitochondrial isolate, the protein concentration was determined by the method of Bradford using bovine serum albumin as reference standard (Bradford, 1976). In the current study, MMTP (K_m = 68.3 ± 1.60 μM for baboon liver MAO-B) served as substrate for the inhibition studies.

2.3 Assay procedure

The enzymatic reactions were prepared in sodium phosphate buffer (100 mM, pH 7.4) and contained MMTP (50 μM), the mitochondrial isolate (0.15 mg protein/mL) and various concentrations of the test compounds, spanning at least three orders of magnitude (0.3 - 300 μM). The concentration of the test compounds used in this study were 0.3 μM, 1 μM, 3 μM, 10 μM, 30 μM, 100 μM and 300 μM. The final volume of the incubations was 500 μL. The stock solutions of the inhibitors were prepared in dimethyl sulfoxide (DMSO) and were added to the incubation mixtures to yield a final DMSO concentration of 4% (v/v). DMSO concentrations higher than 4% are reported to inhibit MAO-B (Gnerre *et al.*, 2000). The reactions were incubated at 37 °C for 10 minutes and then terminated by the addition of 10 μL perchloric acid (70%). The samples were centrifuged at 16,000 g for 10 minutes, and the concentrations of the MAO-B generated product, $MMDP^+$ (Inoue *et al.*, 1999), were measured spectrophotometrically at 420 nm (ϵ = 25,000 M^{-1}) in the supernatant fractions.

2.4 Data analysis

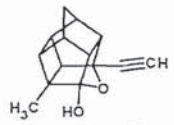
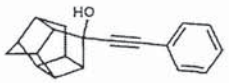
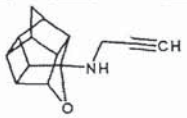
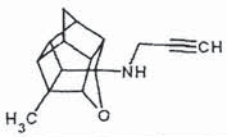
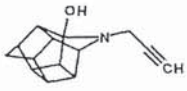
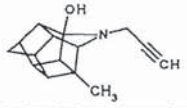
Due to the low enzyme inhibition activity of the test compounds, equation 4.2 was used to calculate the inhibition potencies of the compounds. These potencies are expressed as percentage inhibition (% inh) of the enzyme. In determining this value, the concentration of $MMDP^+$ produced in the absence of a test compound (C_0) and the concentration of $MMDP^+$ produced by MAO-B in the presence of a maximal concentration of the test compound (C_{300}) were utilised. All determinations were conducted in duplicate, yielding the percentage inhibition as an average of the two sets of data.

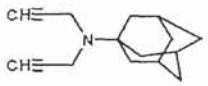
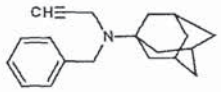
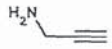
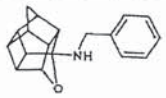
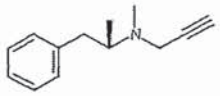
$$\% \text{ inh} = 100 - \left[\frac{C_{300} \times 100}{C_0} \right] \qquad \text{Eq. 4.2}$$

2.4.1 Results

In the following table a summary of the percentage inhibition of MAO-B by the test compounds is given.

Table 4.2: Monoamine oxidase B inhibition activity of synthesised compounds

Compound	Structure	% Inhibition at 300 μM and SEM
QRB 2		Inactive
QRB 3		73.32 ± 0.80
QRB 4a		00.00 ± 0.81
QRB 4b		11.67 ± 3.02
QRB 5a		5.83 ± 2.37
QRB 5b		10.83 ± 6.25

QRB 6		14.00 ± 0.39
QRB 7		20.13 ± 0.65
PA		19.93 ± 0.93
NGP 1-01		14.57 ± 4.46
Selegiline		93.33 ± 0.88

2.5 Discussion

The synthesised compounds were evaluated for MAO-B inhibition activity, with the aim of producing compounds with dual activity: anti-apoptotic and MAO-B inhibition activity. The only compound which had significant MAO-B inhibition activity, when compared to that of selegiline, was compound **3**. The other compounds either had no activity or exhibited little inhibition. Even though the activity of the compounds is low, valuable conclusions could still be made.

Comparing the MAO-B inhibition potencies of **4a** with **4b** and **5a** with **5b**, it can be concluded that in both cases the methyl group on the polycyclic cage, appeared to increase MAO-B inhibition activity of the compounds. This can be due to the methyl group enhancing the interaction between the compound and the non-polar regions of the enzyme. It is important to note the large standard deviations of these compounds, which emphasises need for further investigation considering the contribution of a methyl group to MAO-B inhibition. Another interesting observation is that the aza compounds (**5a** and **5b**) are slightly more favourable then the oxa-compounds (**4a** and **4b**) as MAO-B inhibitors, possibly because the hydroxyl group may act as a hydrogen bond donor within the enzyme active site. For comparison **NGP 1-01** was also evaluated as a MAO-B inhibitor. The activity of **NGP 1-01** was higher than that of compound **4a**. This indicates that the phenyl moiety of **NGP 1-01** is more effective than the propargyl moiety of compound **4a** in stabilising ligand-enzyme complexes. This trend was also observed for the other members of the synthetic series. Those containing a phenyl side chain (**3, 7**, **NGP 1-01** and **selegiline**), were consistently better inhibitors than the other compounds in the series. This trend may be attributed to the

planar character of the phenyl ring. It has been reported that planar compounds frequently act as potent inhibitors of MAO-B (Medvedev *et al.*, 1995; Petzer *et al.*, 2009). It can be concluded that the phenyl moiety, makes a ligand more favourable for interaction with the enzyme.

Compound **3** contains both a hydroxyl, and phenyl moiety, which is most probably the reason why this compound has activity much higher than that of the other synthesised compounds, making it a possible lead compound for further investigation.

3 Concluding remarks

The compounds synthesised had exceptionally good anti-apoptotic activity, but were not potent MAO-B inhibitors. The only compound exhibiting promising MAO-B inhibiting activity was compound **3**. This compound may be considered as promising as a multifunctional neuroprotective agent, due to its significant anti-apoptotic and MAO-B inhibition activity.