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Adamantane amine derivatives as dual acting NMDA receptor and voltage-gated calcium channel inhibitors for neuroprotection

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Abstract: The pathology of neurodegenerative disorders involves multiple pathways, and it is probably for this reason that targeting one particular step has only yielded limited results. The *N*-methyl-D-aspartate receptor (NMDAR) allows the influx of calcium ions for normal neuronal functioning. Overactivation of the NMDAR channel leads to excitotoxicity which can ultimately contribute to neuronal cell death. Calcium entry through voltage-gated calcium channels (VGCC) also contributes to this process. This paper describes a series of adamantane-derived compounds, structurally similar to NGP1-01, to act as dual channel inhibitors. By conjugating benzyl and phenylethyl moieties containing different functional groups (-H, -NO_2, -NH_2, -OCH_3) to the structure of amantadine, we were able to synthesise compounds that display both VGCC and NMDAR channel inhibition. Compounds **1**, **2**, **5** and **10** displayed significant inhibitory activity against both NMDAR channel (66.7 - 89.5 % at 100 µM) and VGCC (39.6 – 85.7 % at 100 µM). The results obtained indicate that these compounds hold potential as neuroprotective drug candidates.

Keywords: Amantadine, Neurodegeneration, NMDA Receptors, Voltage Gated Calcium Channels.

An important drug target for the treatment of neurodegenerative disorders is the ionotropic glutamate *N*-methyl-D-aspartate receptor (NMDAR) which is thought to play a role in multiple neurodegenerative disorders including Parkinson's disease (PD) and Alzheimer's disease (AD).[1] The NMDAR is unique in that it requires the binding of two agonists, namely glutamate and glycine, for activation. In its active state the channel allows the influx of sodium and calcium ions. Although calcium ions are important for cell growth, survival and physiological functioning, an excess is responsible for excitotoxicity, ultimately leading to neurodegeneration.[2] Blockers of NMDAR have been shown to have neuroprotective activity[3] and it is therefore a prime drug target for neurodegenerative diseases where excitotoxicity is involved.[4]

Unfortunately, NMDAR antagonists are associated with adverse central nervous system (CNS) side effects which include hallucinations.[4] These effects are especially seen with high-affinity NMDAR channel blockers such as phencyclidine (PCP) and dizocilpine (MK-801). MK-801 and PCP bind in a use-dependant manner to the MK-801/PCP binding site located in the inner pore of the NMDAR channel when it is activated and in its open state.[1] The amino-adamantane derivatives amantadine and memantine are low affinity uncompetitive NMDAR antagonists which also bind to the MK-801/PCP binding site and display a better side effect profile compared to MK-801 and PCP.[1,5-7] Both amantadine and memantine are FDA approved for PD and AD respectively and are safe and

effective treatment options. Amantadine expresses additional neurotherapeutic ability through the increment of extracellular dopamine levels *via* dopamine re-uptake inhibition and increased dopamine release,[8] which may be additionally useful in treatment of the motor symptoms of PD.[9] In addition to calcium overload associated with NMDAR, excessive calcium influx through voltage-gated calcium channels (VGCC) may also contribute to excitotoxicity and mitochondrial dysfunction.[10,11] Studies have shown that calcium channel blockers such as nimodipine have a synergistic effect when combined with NMDAR inhibitors in neuroprotective studies.[12,13]

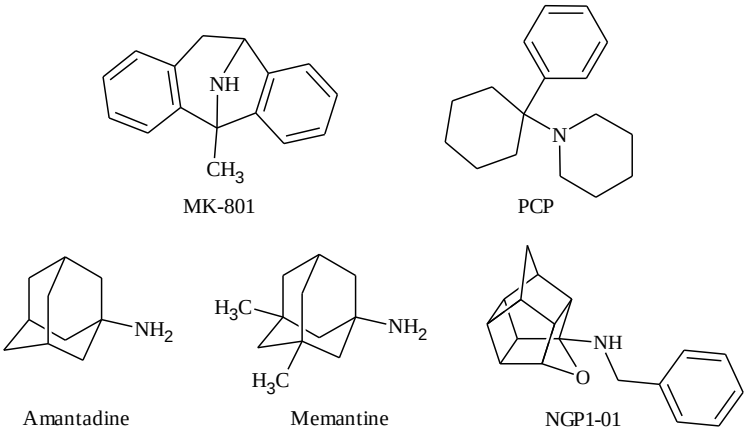
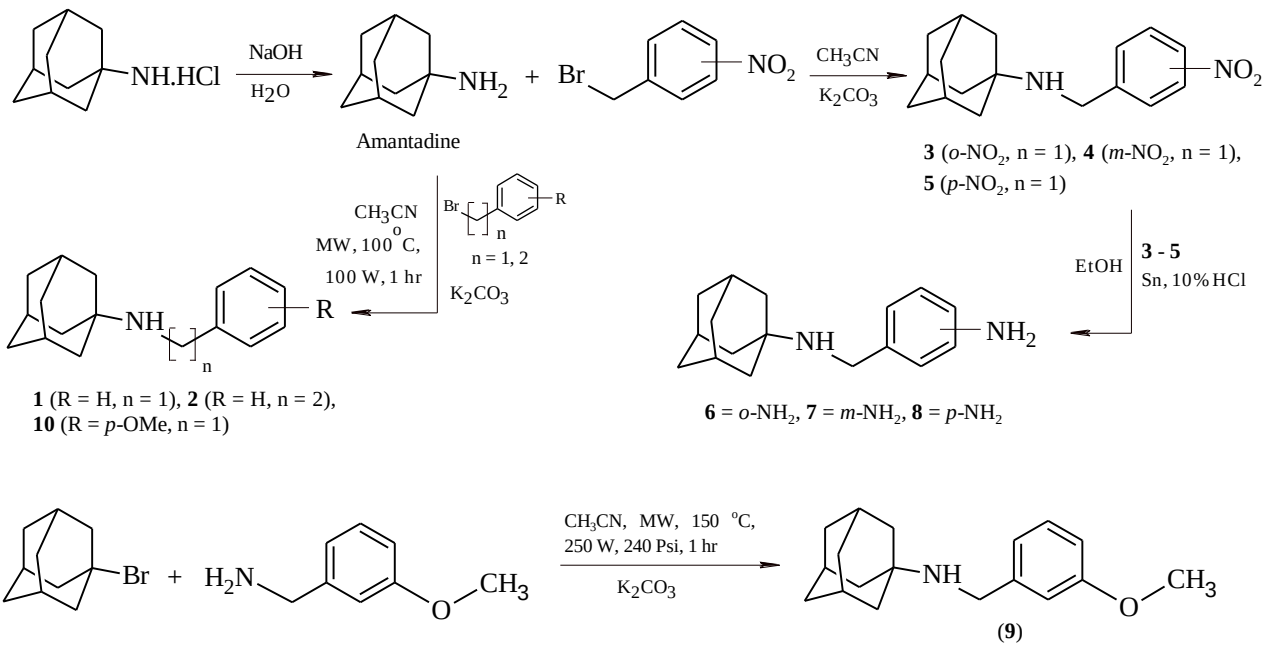


Figure 1*:* Structures of the high affinity NMDAR blockers MK-801 and PCP, and amantadine, memantine and NGP1-01.

A compound with multimodal activity, including NMDAR antagonism, calcium channel blocking activity and the ability to increase dopamine levels, could be a valuable treatment option for neurodegenerative disorders.[14] NGP1-01 is a multifunctional agent which blocks both the NMDAR as well as VGCC and also inhibits dopamine reuptake, with resulting neuroprotective activity shown *in vivo*.[15–18] Several (2-oxaadamant-1-yl)amines, structurally similar to NGP1-01 have also shown improved NMDAR activity when compared to amantadine.[19] Therefore the aim of this study was to synthesise compounds containing different aromatic moieties attached *via* the amine to the adamantane scaffold of amantadine. It was postulated that these compounds would display improved multifunctional neuroprotective effects compared to NGP1-01, especially NMDAR antagonism, because of the adamantane scaffold.

The target adamantane amine derivatives (**1**,[20] **3-8**, **10**) were synthesised by reacting amantadine with the respective benzylbromide moieties by means of S_N2 nucleophilic addition (Scheme 1). Compound **2**[21] was synthesised using 2-bromoethylbenzene to provide the ethyl linkage between the adamantane amine and the phenyl moiety as opposed to compound **1** which was synthesised using benzylbromide. Both these compounds were synthesised utilizing microwave irradiation, (MW, 100 °C, 100 W, 1 atm, 1 hour) in yields of 48 % and 47 %, respectively. In both these cases the reaction time was reduced from 12 hours to 1 hour and the yields significantly improved when

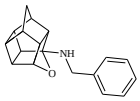
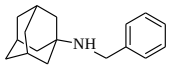
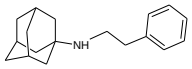
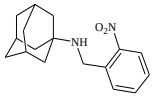
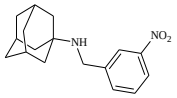
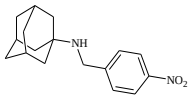
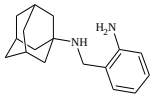
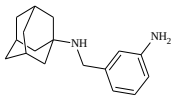
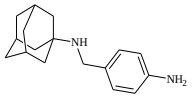
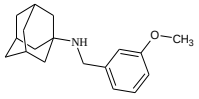
compared to the traditional method where only thermal heating was applied. The general synthetic route followed for the synthesis of compounds **3** – **5**[22] was initiated through the conjugation of a nitrobenzyl bromide to amantadine, followed by reduction of the nitro groups to the respective amines (**6** – **8**)[23] with tin powder over 10 % HCl in ethanol. The synthesis of compounds **9**[24] and 1**0**[25] and the corresponding *ortho*-methoxy derivative were attempted utilizing both conventional thermal heating as described for **3** - **5** and through MW irradiation. The conventional method was found to be ineffective as no or only trace amounts of the compounds formed after 24 hours of reflux (as per TLC). This might be because the methoxybenzyl bromide derivatives were not as reactive as the other corresponding benzylbromide moieties. Utilizing MW irradiation with conditions set at 100 °C, 100 W, 1 atm (open vessel) for 1 hour only compound **9** was synthesised in sufficient yield (18 %). Both the synthesis of compounds **10** and the corresponding *ortho*-methoxy derivative were attempted in the same manner as **9** however, both reactions did not proceed successfully. The MW irradiation conditions were increased up to 150 °C, 250 W, 240 psi (closed vessel) for 24 hours with still no compound formed in sufficient yield. A different strategy was employed where 1-bromo-adamantane and the *ortho*- or *para*-methoxybenzyl amine were used as starting reagents and conjugated *via* S_N2 nucleophilic substitution with MW irradiation (150 °C, 250 W, 240 psi). Utilizing this method we were able to synthesise compound **10** in an 25 % yield. The synthesis of the *ortho*-methoxy derivative using this method was unsuccessful. This could have resulted because of a chemical hindrance or deactivation of the reagent due to the methoxy substituent being in the *ortho* position of the benzyl moiety. All final compounds were confirmed by NMR, MS and IR.[20-25]

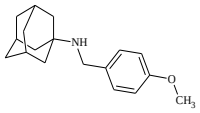


Scheme 1: General reaction schemes for the synthesis of compounds **1** - **10**.

All synthesized amantadine derivatives (**1** - **10**) were screened at 100 µM for their potential inhibitory activity on the VGCC and NMDAR channel exactly as previously described,[26] using the fluorescent ratiometric indicator, Fura-2 AM. Fresh synaptoneurosomes were prepared from rat brain homogenate[27] and incubated with Fura-2 AM. Thereafter the synthesized compounds were incubated for 30 minutes and a 140 mM KCl or a 100 µM NMDA/glycine solution, in the VGCC and NMDAR assay respectively, was added to depolarize the cell membranes or to stimulate calcium influx through the NMDAR channels. Calcium influx was then monitored based on the fluorescence intensity relative to that of a blank control (without inhibiting compound) over a 5 minute period. Two positive controls were included in the VGCC assay; nimodipine, a commercially available dihydropyridine calcium channel blocker and the prototype pentacycloundecane compound NGP1-01. Four positive controls, MK-801, NGP1-01, amantadine and memantine, were included in the NMDAR assay. Results are shown in table 1 and figure 2.

Table 1: Summary of effects the adamantane derivatives and related compounds on inhibition of NMDA/glycine induced calcium influx (at 100 µM) and KCl induced calcium influx inhibition (at 100 µM).

Test Compound	Structure	¥%NMDA activity	¥%VGCC activity
NGP1-01		22.2**	38.4**
(**1**)		66.7***	39.6***
(**2**)		89.5***	42.0***
(**3**)		60.6***	49.2***
(**4**)		36.6***	14.3*
(**5**)		74.8***	85.7***
(**6**)		31.3***	37.1***
(**7**)		48.3***	30.0***
(**8**)		50.4***	49.5***
(**9**)		70.2***	44.7***

(**10**)		79.2***	71.2***
Nimodipine	-	inactive	100***
MK-801	-	100***	inactive
Amantadine	-	84.6***	2.76
Memantine	-	92.5***	5.20

[¥]Control was taken as 0 % inhibition. Asterisks signifying statistical significance, (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$.

In the NMDAR experiments synaptoneurosomes incubated with test compounds **1** (66.7 %), **2** (89.5 %), **3** (60.6 %), **5** (74.8 %), **9** (70.2 %) and **10** (79.2 %) showed promising inhibitory activity of NMDA/glycine-mediated calcium influx (Table 1, Figure 3). Compounds **4** (47.2 %), **6** (31.3 %), **7** (48.3 %) and **8** (50.4 %) showed weaker inhibitory activity, although better than the reference compound NGP1-01 (22.2 %). Of the controls, MK-801 (100 %) was the most potent inhibitor followed by memantine (92.5 %) and amantadine (84.6 %). This was in agreement with previous studies.[9,14,15] MK-801 showed a complete blockage of NMDAR-mediated calcium entry.

Compound **2** showed the highest percentage NMDAR inhibition (89.5 %) of all synthesised compounds. An increase in chain length between the amantadine moiety and the aromatic group thus led to a significant increase ($p < 0.05$) in NMDAR inhibitory activity as can be seen when the activities of **1** (66.7 %) and **2** (89.5 %) are compared. Although both compounds are thought to fit into the channel in such a way as to allow free movement of the polycyclic adamantane structure to a certain degree, the ethyl linker is thought to enable the compound to penetrate deeper into the channel lumen thereby enabling more favourable interactions with the PCP-binding site. Geldenhuys and colleagues (2004, 2007) observed similar results in their work on NGP1-01 and its phenethyl derivative.[15,18] Further research will be conducted using radio-ligand and/or fluorescent ligand binding studies using techniques and compounds as described in one of our previous papers to confirm these observations.[28]

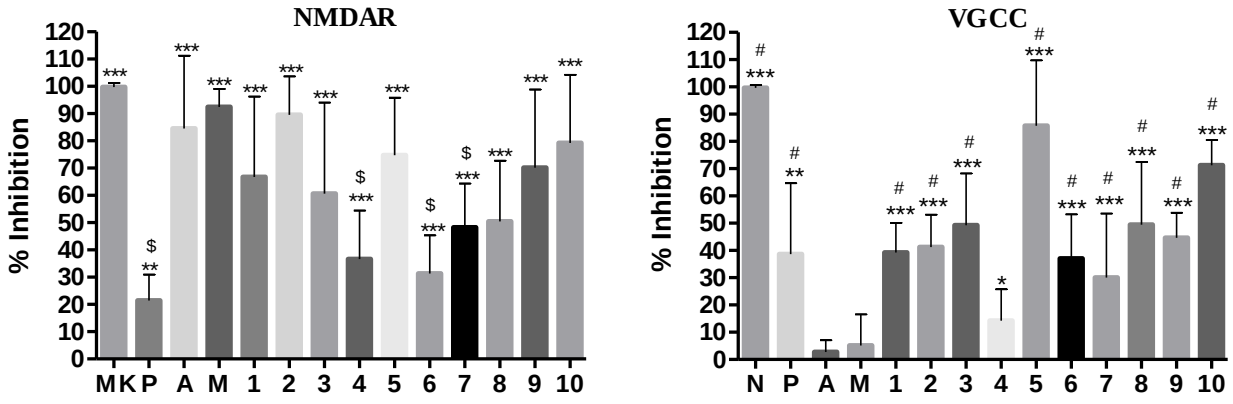


Figure 2: Screening of test compounds (100 μM, n = 9) for inhibition of NMDAR-mediated (left) and VGCC-mediated (right) calcium influx into murine synaptoneurosomes. Each bar represents mean percentage inhibition ± SEM. Abbreviations are: MK-801 (MK), NGP1-01 (P), amantadine (A),

memantine (M) and nimodipine (N). Statistical analysis was performed on raw data (t-test, Prism 4.03) with asterisks signifying significant inhibitory effect [(*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$] Compounds showing significant decrease in activity compared to amantadine are indicated by ($) $p < 0.05$, and compounds showing significant increase in inhibitory activity compared to amantadine are indicated by (#) $p < 0.05$.

Of the three nitro containing compounds, **4** showed the weakest, albeit still moderate, activity with 36.6 % inhibition. Compound **5** showed the highest activity with 74.8 % inhibition and the rank order that is followed for these compounds is *para>ortho>meta*. The good inhibitory activity of the nitro compounds may be as a result of the nitro group contributing to the *S*-nitrosylation of cysteine residues in the NMDAR channels, thus increasing NMDAR activity compared to **1** and NGP1-01.[29,30] The weaker activity of **3** (60.1 %) and **4** (36.6 %) compared to **5** (74.8 %)may be attributed to steric or conformational effects of the aromatic ring due to the nitro group being in the *ortho* or *meta* position. The nitro group in the *para* position in **5** may thus enable the compound to fit better at the site of interaction. The amine compounds (**6** - **8**) show weaker activity (31 - 51 %) than their respective nitro counterparts (**3** - **5**), as well as the methoxy compounds (**9** and **10**). This could indicate that the nitro group was involved in favourable binding interactions with NMDAR channels, and the reduction to the amines led to a decrease in activity. The methoxy derivatives **9** and **10** both showed good and improved inhibitory activity (70.2 % and 79.2 %, respectively) compared to **1** and NGP1-01. All the tested compounds showed better activity than NGP1-01 and none of them showed better NMDAR inhibitory activity than memantine. All the inhibitors and controls displayed statistically significant activity ($p < 0.05$), but very little or no improvement on the base structure, amantadine. Significant decreased activity, compared to amantadine (figure 3), was observed for compounds **4**, **6** and **7**.

For the VGCC assay, the controls that were tested included nimodipine (100 %), NGP1-01 (38.40 %), amantadine (2.76 %) and memantine (5.20 %). Of the polycyclic structures, NGP1-01 was the only control compound that displayed statistically significant ($p < 0.05$) inhibition of calcium influx *via* VGCC. Both amantadine and memantine showed little or no activity. In murine synaptoneurosomes incubated with the test compounds, **5** (85.7 %) and **10** (71.2 %) displayed promising inhibitory activity (Table 1) of calcium influx through VGCC. Both these compounds showed significantly better activity than NGP1-01. Compounds **1**-**3**, **6**-**8** and **9** showed moderate VGCC inhibitory ability within the same range as NGP1-01. All the test compounds, except **4**, showed a significant improvement on VGCC blocking activity when compared to amantadine (Figure 3).

The *para*-nitro compound (**5**, 85.7 %) displayed the best inhibition of calcium influx through VGCCs. The other nitro-derivatives compounds **3** (49.2 %) and especially **4** (14.3 %) showed

significantly weaker inhibitory activity ($p < 0.05$) than **5**. This may also be attributed to steric or conformational effects on the aromatic ring due to the nitro group being in the *ortho* or *meta* position. The good inhibitory activity of **5** may be as a result of the nitro group being in the *para* position, which enables the compound to fit better in the binding pocket at the site of interaction. Compound **2** (42.0 %) showed slightly, though not significantly, better activity than **1** (39.6 %) and the length of the linker does not exert the same effect as observed with the NMDAR assay. The amine compounds (**6** and **8**) show weaker activity (30 - 49.5 %) than their respective nitro counterparts (**3** and **5**). However, **7** (30.0 %) displayed better inhibitory activity than **4** (14.3 %) indicating that the smaller NH_2 moiety had a reduced effect on the possible binding conformation of these structures. The rank order for the amines is the same as that of the nitro compounds, *para*>*ortho*>*meta*. The *para*-methoxy derivative **10** (71.2 %) showed better inhibitory activity than the *meta*-methoxy compound (**9**, 44.7 %).

Test compounds **1**, **2**, **5** and **10** showed promising dual NMDAR and VGCC inhibitory activity. NGP1-01 also had significant inhibition on both NMDAR and VGCC calcium influx. Compounds **1** and **2** showed VGCC inhibition in the same range as NGP1-01, however their NMDAR channel inhibitory activity was significantly improved. This could largely be attributed to the incorporation of the amantadine moiety in **1** and **2**. The activity of **2** shows that a two carbon spacer provides improved dual channel blocking activity and future studies will include investigation of optimal chain length of the adamantane derivatives described in this study. Both compounds **1** and **2** may be better therapeutic options than NGP1-01 and amantadine or memantine because of this improved dual acting ability. The incorporation of the *para*-nitro and *para*-methoxy moieties on the benzyl function of **5** and **9** respectively, further improved this dual activity (Table 1). This is in agreement with previous studies done on similar pentacycloundecane derivates.[18,31] Amantadine and memantine are known NMDAR channel inhibitors, but have no VGCC activity. Therefore, the dual acting adamantane derivatives may enhance the neuroprotective effects known for amantadine and memantine by inhibiting two excitotoxic pathways simultaneously. Amantadine is also known to increase dopamine release and decrease dopamine reuptake in the CNS[8] and we expect that the new compounds could show similar activity. This needs to be confirmed in future studies.

Conclusion

We described the synthesis and biological evaluation of 10 adamantane amine compounds as potential dual VGCC blockers and NMDAR antagonists. Compounds **1**, **2**, **5** and **10** displayed significant inhibitory activity against both NMDAR- and VGCC calcium influx. These adamantane derived compounds are potentially better neurotherapeutic options than amantadine and memantine as they inhibit both NMDAR and VGCC-mediated calcium influx, whereas the base adamantane structures only inhibit NMDAR-mediated calcium influx. Additional assays on the influence of

these compounds on dopamine transmission and apoptosis, and in *in vivo* models will elaborate on the potential neuroprotective value.

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Notes and references

1. Dingledine, R,;Broges, K.; Bowie, D.; Traynelis, S.F. *Pharmacol Rev*. 1999, **51**, 7
2. Arundine, M., & Tymianski, M., *Cell Calcium*. 2013, **34**(4-5), 325.
3. Danysz, W.; Parsons, C. G. *Int J Geriatr Psychaitry*. 2003, **8**, S23.
4. Danysz, W.; Lanthorn, T.H.; *Amino Acids*. 2000, **19**, 131.
5. Parsons, C.G.; Danysz, W.; Quack, G. *Neuropharmacology*. 1999, **38**, 735.
6. Bornmann, J. Eur J Pharmacol. 1989, 166, 591.
7. Kornhuber, J.; Bornmann, J.; Retz, W.; hubers, M.; Riederer, P. *Eur J Pharmacol*. 1989, **166**, 589.
8. Mizoguci, K.; Yokoo, H.; Yoshida, M.; Tanaka, T.; Tanake, M. *Brain Res*. 1994, **662**, 255.
9. Danysz, W.; Parsons, C. G.; Kornhuber, J.; Schmidt, W. J.; Quack, G. *Neurosci Biobehav Rev*. 1997, **21**, 455.
10. Mattson, M. P. *Neuromol Med*. 2003, **3**, 65.
11. Cano-Abad, M. F.; Villarroya, M.; Garcia, A. G.; Gabilan, N. H.; Lopez, M. G. *J Biol Chem*. 2001, **276**, 39695.
12. Schurr, A. *Curr Drug Targets*. 2004, **5**, 603.
13. Stuiver, B.T.; Douma, B.R.; Bakker, R.; Nyakas, C.; Luiten, P.G. *Neurodegeneration*. 1996, **5**, 153.
14. Joubert, J.; Van Dyk, S.; Green, I.R.; Malan, S.F. *Bioorg. Med. Chem*. 2011, **19**, 3935-3944.
15. Geldenhuys, W. J.; Malan, S. F.; Bloomquist, J. R.; Van der Schyf. C. *J Bioorg Med Chem*. 2007, **5**, 1525.
16. Kiewert, C.; Hartmann, J.; Stoll, J.; Thekkumkara, T. J.; Van der Schyf., C. J.; Klein. *J Neurochem Res*. 2006, **31**, 395.
17. Mdzinarishvili, A.; Geldenhuys, W. J.; Abbruscato, T. J.; Bickel, U.; Klein, J.; Van der Schyf, C. J. *Neurosci Lett*. 2005, **383**, 49.
18. Geldenhuys, W. J.; Malan, S. F.; Murugesan, T.; Van der Schyf, C. J.; Bloomquist, J. R. *Bioorg Med Chem*. 2004, **12**, 1799.
19. Duque, M.D.; Camps, P.;Profire, L.; Montaner, S.; Vázquez, S.; Sureda, F.X.; Mallol, J.; López-Querol, M.; Naesens, L.; De Clercq, E.; Prathalingam, S.R.; Kelly, J.M. *Bioorg. Med. Chem. 2009, **17**, 3198.*
20. *Synthesis of **1***: Amantadine (11.4 mmol) and benzyl bromide (10.3 mmol) were added to a mixture of acetonitrile (10 ml) and K_2CO_3 (15.5 mmol). The reaction vial was placed in a CEM Discover Labmate (model number 908010) microwave reactor and reacted at 100 ^oC and 100 W for 60 min. After irradiation, the mixture was filtered to remove the K_2CO_3 and unreacted amantadine free base. The filtrate was collected and the excess solvent evaporated *in vacuo*. The reaction mixture was then acidified using 30 ml water which was made acidic with HCl until the pH was 3. This was followed by an extraction with DCM (3 x 30 ml). The combined aqueous layers were collected and made basic (pH = 12-14) using water saturated with NaOH and this was followed by a second extraction with DCM (3 x 30 ml). The combined organic layers were dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo* to yield *N*-benzyltricyclo[3.3.1.1^{3,7}]decan-1-amine (**1**) as a light yellow oil. $C_{17}H_{23}N$; Yield: 48%; mp: oil; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 7.4-7.1 (m, 5H), 3.8-3.6 (s, 2H), 2.2-2.0 (m, 3H), 1.8-1.4 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 141.4, 128.3, 128.3, 126.7, 50.9, 45.1, 42.8, 36.7, 29.6; MS (ESI-MS) *m/z*: 242.12 $(M+H)^+$, 134.97, 78.94; HR-ESI: calc. 241.1830, exp 241.1831; IR (ATR; cm^{-1}): 3025.9, 2900.9, 2845.9, 1603.7, 1451.3, 1146.4, 734.2, 694.6.
21. *Synthesis of **2***: Amantadine (3.3 mmol) and 2-bromoethylbenzene (3.3 mmol) were added to a mixture of acetonitrile (10 ml) and K_2CO_3 (4.5 mmol). The synthetic procedure from this point was the same as described for **1** which yielded *N*-(2-phenylethyl)tricyclo[3.3.1.1^{3,7}]decan-1-amine (**2**) as a white wax. *Physical data:* $C_{18}H_{25}N$; Yield: 47 %; mp: wax; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 7.4-7.1 (m, 5H), 3.6-3.5 (t, 2H), 3.1-3.0 (t, 2H), 1.9-1.5 (m, 3H), 1.4-1.1 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 138.9, 128.8, 128.6, 126.9, 50.00, 42.77, 41.63, 36.8, 35.4, 29.7; MS (ESI-MS) *m/z*: 256.15 $(M+H)^+$, 134.96, 78.95; HR-ESI$^+$: calc. 256.2065, exp. 256.2065; IR (ATR; cm^{-1}): 2912.2, 2891.9, 2802.6, 1603.4, 1455.2, 1362.8, 1075.0, 776.9, 695.8.

22. *Synthesis of **3-5***: Amantadine (11.4 mmol) and the appropriate nitrobenzyl bromide derivative (10.3 mmol) were added to a round-bottomed flask followed by acetonitrile (10 ml) and K_2CO_3 (15.5 mmol). This mixture was refluxed for 24 hours where after it was filtered. The filtrate was collected and the excess solvent evaporated *in vacuo*. The mixture was purified *via* flash chromatography to yield the title compounds (**3-5**) as yellow amorphous solids.

    *N-(2-nitrobenzyl)tricyclo[3.3.1.1^{3,7}]decan-1-amine (**3**)*: $C_{17}H_{22}N_2O_2$; Yield: 32 %; mp: 94 ^oC; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 7.9-7.8 (dd, J = 8.11 Hz, 1.37 Hz), 7.7-7.5 (m, 2H), 7.4-7.3 (m, 1H), 4.0-3.8 (s, 2H, CH_2), 2.2-1.9 (m, 3H), 1.8-1.4 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 149.6, 136.2, 133.3, 132.1, 127.8, 124.5, 51.1, 45.13, 42.4, 36.6, 29.5; MS (ESI-MS) *m/z*: 287.17 $(M+H)^+$, 214.02, 135.00, 79.89; HR-ESI$^+$: calc. 287.1754, exp 287.1756; IR (ATR; cm^{-1}): 2900.3, 2848.6, 1524.1, 1463.3, 1355.3, 1133.8, 779.4, 739.6.

    *N-(3-nitrobenzyl)tricyclo[3.3.1.1^{3,7}]decan-1-amine (**4**):* $C_{17}H_{22}N_2O_2$; Yield: 54 %; mp: 61 ^oC; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 8.3-8.2 (s, 1H), 8.1-8.0 (m, 1H), 7.7-7.6 (dd, 1H, J = 7.83 Hz, 1.64 Hz), 7.5-7.4 (m, 1H), 3.9-3.8 (s, 2H, CH_2), 2.2-2.0 (m, 3H), 1.8-1.4 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 144.3, 134.3, 129.1, 123.0, 121.7, 51.0, 45.1, 42.9, 36.7, 29.6; MS (ESI-MS) *m/z*: 287.17 $(M+H)^+$, 214.04, 135.00, 79.98; HR-ESI$^+$: calc. 287.1754, exp 287.1754; IR (ATR; cm^{-1}): 2898.3, 2847.1, 1524.5, 1463.3, 1359.3, 1133.5, 778.8, 715.8.

    *N-(4-nitrobenzyl)tricyclo[3.3.1.1^{3,7}]decan-1-amine (**5**):* $C_{17}H_{22}N_2O_2$; Yield: 57 %; mp: 94 ^oC; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 8.1-8.0 (d, 2H, J = 8.9 Hz), 7.6-7.4 (d, 2H, J = 8.9 Hz), 4.0-3.8 (s, 2H, CH_2), 2.2-2.0 (m, 3H), 1.8-1.4 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 149.9, 146.1, 128.7, 123.5, 51.0, 44.5, 42.9, 36.6, 29.8; MS (ESI-MS) *m/z*: 287.17 $(M+H)^+$, 214.00, 134.94, 78.95; HR-ESI$^+$: calc. 287.1754, exp 287.1754; IR (ATR; cm^{-1}): 2900.0, 2848.5, 1523.8, 1477.3, 1354.9, 1133.8, 779.5, 716.5.

23. *Synthesis of **6-8***: Previously prepared nitrobenzyltricyclo[3.3.1.1^{3,7}]decan-1-amines (**3**, **4** or **5**, 0.5 mmol) and tin powder (0.8 mmol) were added to a round-bottomed flask and set up under reflux conditions. This was followed by addition of 10 % hydrochloric acid (1.5 ml) down the condenser with continuous stirring, while elevating the temperature to reflux conditions. Ethanol (5 ml) was added as a solubilisation agent. Further HCl additions (2 x 2.3 ml) were made at 10 minute intervals. The reaction was refluxed overnight followed by an aqueous extraction with DCM (3 x 20 ml). The water phase was collected and alkalinised using a 40 % NaOH solution just past the turning point, and then extracted with DCM (3 x 20 ml). The combined organic fractions were washed with brine (2 x 15 ml), dried with anhydrous $MgSO_4$ and the solvent evaporated *in vacuo* to yield the products (**6-8**) as light brown amorphous solids.

    *N-(2-aminobenzyl)tricyclo[3.3.1.1^{3,7}]decan-1-amine (**6**)*: $C_{17}H_{24}N_2$; Yield: 70 %; mp: 71 ^oC; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 6.9-6.8 (m, 2H), 6.5-6.3 (m, 2H), 3.6-3.4 (s, 2H, CH_2), 2.1-1.8 (m, 3H), 1.8-1.4 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 146.9, 129.5, 128.0, 125.3, 117.7, 115.7, 50.5, 43.9, 42.7, 36.7, 29.5; MS (ESI-MS) *m/z*: 257.14 $(M+H)^+$, 105.94, 78.95; HR-ESI$^+$: calc. 257.2012, exp 257.2011; IR (ATR; cm^{-1}): 3402.2, 3282.5, 2903.1, 2849.3, 1616.9, 1494.4, 1357.8, 1072.3, 785.1, 710.0.

    *N-(3-aminobenzyl)tricyclo[3.3.1.1^{3,7}]decan-1-amine (**7**)*: $C_{17}H_{24}N_2$; Yield: 75 %; mp: 79 ^oC; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 6.9-6.7 (m, 1H), 6.5-6.2 (m, 3H), 3.6-3.4 (s, 2H, CH_2), 2.1-1.8 (m, 3H), 1.8-1.4 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 146.4, 142.7, 129.2, 118.4, 115.0, 113.5, 50.7, 45.0, 42.7, 36.7, 29.5; MS (ESI-MS) *m/z*: 257.13 $(M+H)^+$, 76.99; HR-ESI$^+$: calc. 257.2012, exp 257.2009; IR (ATR; cm^{-1}): 3399.9, 3174.3, 2897.4, 2845.9, 1603.7, 1493.6, 1356.6, 1343.0, 1067.8, 1038.0, 773.5, 708.4.

    *N-(4-aminobenzyl)tricyclo[3.3.1.1^{3,7}]decan-1-amine (**8**)*: $C_{17}H_{24}N_2$; Yield: 90 %; mp: 91 ^oC; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 6.9-6.8 (d, 2H, J = 8.8 Hz), 6.4-6.3 (d, 2H, J = 8.8 Hz), 3.6-3.4 (s, 2H, CH_2), 2.1-1.8 (m, 3H), 1.8-1.4 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 145.0, 129.3, 128.8, 115.1, 50.7, 44.5, 42.7, 36.7, 29.5; MS (ESI-MS) *m/z*: 257.15 $(M+H)^+$, 105.93, 78.95; HR-ESI$^+$: calc. 257.2012, exp 257.2010; IR (ATR; cm^{-1}): 3429.1, 3315.8, 2898.2, 2847.5, 1610.1, 1518.8, 1357.2, 1069.7, 1038.9, 761.6, 708.4.

24. *Synthesis of **9***: Amantadine (3.3 mmol) was added to a round-bottomed flask containing acetonitrile (10 ml), K_2CO_3 (4.5 mmol) and 3-methoxybenzylchloride (3.3 mmol). The synthetic procedure from this point was the same as described for **1** which yielded *N*-(3-methoxybenzyl)tricyclo[3.3.1.1^{3,7}]decan-1-amine (**9**) as a white powder. *Physical data:* $C_{18}H_{25}NO$; Yield: 18 %; mp: 124 ^oC; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 7.7-7.5 (m, 1H), 7.4-6.7 (m, 2H), 4.6-4.2 (bs, NH), 4.0-3.7 (2 x s, 5H), 2.1-1.8 (m, 3H), 1.8-1.4 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 162.5, 132.1, 129.7, 121.7, 110.6, 110.2, 55.2, 44.4, 42.5, 35.3, 29.2; MS (ESI-MS) *m/z*: 313.23, $[M+ACN+H]^+$ 151.98, 78.91; HR-ESI$^+$: calc. 272.2008, exp 272.2006; IR (ATR; cm^{-1}): 2934.6, 2864.2, 1586.1, 1454,8, 1265.0, 1174.7, 1075.2, 852.2.

25. *Synthesis of **10***: 1-Bromoadamantane (2.3 mmol) was added to the reaction vessel containing acetonitrile (3 ml), K_2CO_3 (3.5 mmol) and 4-methoxybenzylamine (2.3 mmol). The mixture was placed in the microwave reactor (240 Psi, 300 W, 150 ^oC, 60 min). After irradiation, the mixture was filtered by vacuum filtration to remove the K_2CO_3. The filtrate was collected and the excess solvent evaporated *in vacuo* and purified *via* flash chromatography to yield *N*-(4-methoxybenzyl)tricyclo[3.3.1.1^{3,7}]decan-1-amine (**10**) as a white powder. *Physical data:* $C_{18}H_{25}NO$; Yield: 25 %; mp: 225 ^oC; ^1H NMR (200 MHz, $CDCl_3$) δ_H: 7.3-7.1 (dd, 2H J = 6.62 Hz, 2.16 Hz), 6.9-6.7 (dd, 2H, J = 6.62 Hz, 2.16 Hz), 5.2-4.6 (bs, NH), 4.0-3.7 (2 x s, 5H), 2.2-1.8 (m, 3H), 1.8-1.4 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C: 158.5, 129.7, 128.4, 114.4, 55.2, 51.6, 45.2, 42.4, 35.5, 29.2; MS (ESI-MS) *m/z*:

313.23, $[M+ACN+H]^+$ 78.95; HR-ESI^+: calc. 272.2008, exp 272.2013; IR (ATR; cm^{-1}) : 2905.9, 2849.6, 1627.7, 1453.7, 1248.0, 1176.7, 1087.8, 813.0.

26. Zindo, F. T.; Barber, Q. R.; Joubert, J.; Bergh, J. J.; Petzer, J. P.; Malan, S.F.; *Eur J Med Chem*. 2014, **80**, 122.
27. Hollingsworth, E. B.; McNeal, E. T.; Burton, J. L.; Williams, R. J.; Daly, J. W.; Creveling, C. R. *J Neurosci*. 1985, 5, 2240.
28. Joubert, J.; Van Dyk, S.; Green, I.R.; Malan, S.F. *Eur. J. Med. Chem*. 2011, 46, 5010-5020.
29. Lemmer, H.J.R.; Joubert, J.; van Dyk, S.; van der Westhuizen, F.H.; Malan, S.F. *Med. Chem.* 2012, **8**, 361.
30. Lipton, S.A.; *Nat Res Drug Discov*. 2006, **5**, 160.
31. Liebenberg, W.; Van der Walt, JJ.; Van der Schyf, C.J. *Pharmazie*. 2000, 833.

GRAPHICAL ABSTRACT

Adamantane amine derivatives as dual acting NMDA receptor and voltage-gated calcium channel inhibitors for neuroprotection

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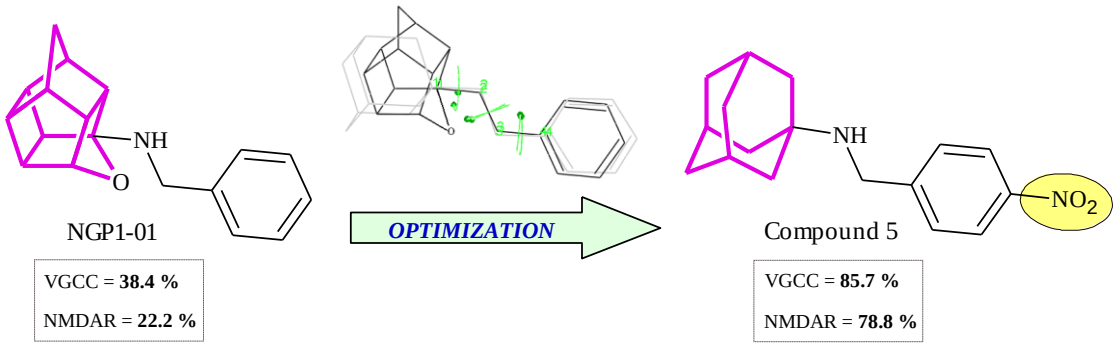

A series of adamantane-derived compounds, structurally similar to NGP1-01, were synthesised and showed significant dual NMDA receptor and VGCC inhibitory activities.