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1-Benzyloxy-4,5-dihydro-1*H*-imidazol-2-yl-amines, a Novel Class of NR1/2B Subtype Selective NMDA Receptor Antagonists

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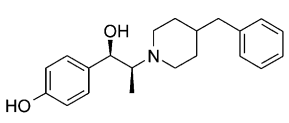
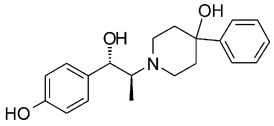
Abstract—Screening of the Roche compound depository led to the identification of (1-benzyloxy-4,5-dihydro-1*H*-imidazol-2-yl)-butyl amine **4**, a structurally novel NR1/2B subtype selective NMDA receptor antagonist. The structure–activity relationships developed in this series resulted in the discovery of a novel class of potent and selective NMDA receptor blockers displaying activity in vivo.

Excessive activation of *N*-methyl-D-aspartate (NMDA) receptors and resulting calcium overload of neurons is thought to be a key contributor to neuronal cell death following acute cerebral ischaemia. In this context NMDA receptor antagonists have been demonstrated to be potent neuroprotective agents in animal models of stroke.[1,2] Native NMDA receptors are present as complexes containing an NR1 subunit together with one or more of the four NR2 subunits (NR2A-D).[3,4] During the last decade, a number of NR1/2B subtype selective blockers[5] have been described such as ifenprodil **1**,[6] CP-101,606 **2**,[7] and others.[8–13] These compounds display neuroprotective effect in vivo without inducing the side effects associated with non-selective receptor antagonists[14–17] via a state-dependent block of NMDA receptors.[18] This makes NR1/2B subtype selective blockers potentially attractive drugs for the treatment of neurodegenerative disorders such as stroke,[2] brain trauma,[2] pain[17,19] and Parkinson's disease.[20]

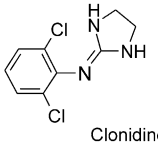


(rac)-Ifenprodil (**1**)

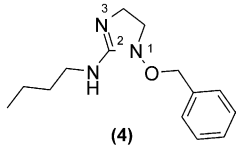


CP-101,606 (**2**)

The majority of NR1/2B subtype selective NMDA receptor antagonists described so far are structurally related to ifenprodil **1**. The pharmacophore for this series consist of a basic nitrogen linked by spacers to two aromatic groups, one bearing a phenolic-OH group or a bioisostere.[21] As part of our CNS discovery program, we set out to develop potent, NR1/2B subtype selective blockers structurally distinct from ifenprodil **1**. To achieve this, screening of the Roche compound depository was performed employing tritiated Ro-25-6981 as radioligand in a competition-binding assay.[22] This screening campaign led to the identification of a novel structural class of NMDA antagonists exemplified by (1-benzyloxy-4,5-dihydro-1*H*-imidazol-2-yl)-butyl-amine[23,24] **4** as the most active representative with a K_i of 60 nM.



Clonidine (**3**)



(**4**)

Chemistry

Compound **4** is related to the marketed hypotensive α_2 adrenergic agonist clonidine[25] **3**, interestingly however, such 2-amino-alkyl imidazoline derivative were found to be devoid of this undesired activity. Using **4** as a lead compound we attempted to develop new, potent, sub-

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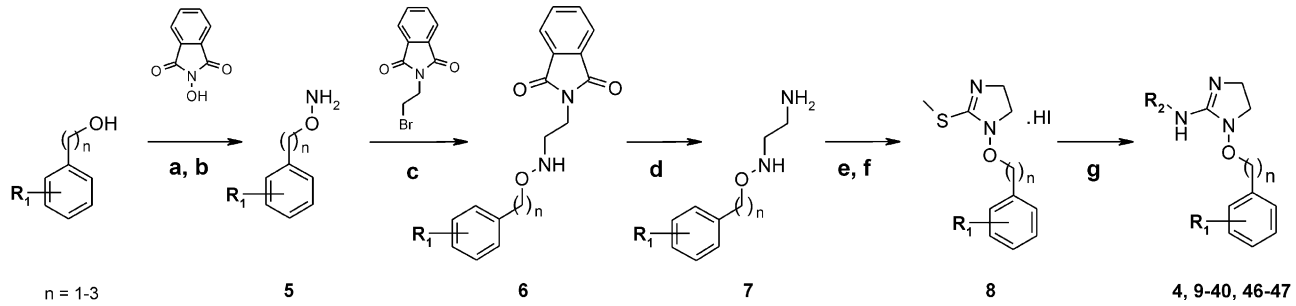


Scheme 1. (a) PPh_3, DEAD, THF, 0–50 °C, 45–85%; (b) $N_2H_4.H_2O$, EtOH, 78 °C, 80%; (c) DMF, $NaHCO_3$, 80 °C, 20–70%; (d) $N_2H_4.H_2O$, EtOH, 78 °C 60–90%; (e) CS_2, EtOAc, 60 °C, 50–95%; (f) MeI, Me_2CO, rt, 100%; (g) R_2NH_2 (3 equiv), EtOH, 78 °C, 10–90%.

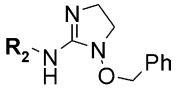
type selective NMDA receptor antagonists as alternatives to the known chemotypes, for detailed pharmacological evaluation. The main synthetic scheme for the preparation of various 1-benzyloxy-4,5-dihydro-1*H*-imadazol-2-yl-amines is outlined in Scheme 1. *O*-Benzyl-hydroxylamines **5** were prepared by coupling benzyl alcohols with *N*-hydroxyphthalimide under Mitsunobu conditions[26] in good yield, followed by release of the phthalimide group with hydrazine monohydrate. Primary amines **7** were then obtained by *N*-alkylation of **5** with bromoethyl-phthalimide affording imides **6** which were once again deprotected with hydrazine hydrate. Amines **7** were then converted to the corresponding cyclic thioureas with carbon disulfide and then activated by *S*-alkylation using methyl iodide affording cyclic isothiouronium salts **8**. The final products **4**, **9**–**40**, **46**–**47** were obtained in satisfactory yield and good purity by heating salts **8** with an excess of amine under reflux in ethanol for several h followed by extractive work-up, chromatography and trituration of the free-base from ether with ethanolic HCl resulting in isolation of the corresponding hydrochloride salts. Although primary amines reacted smoothly, secondary amines failed under the same conditions.

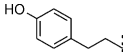
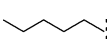
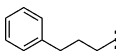
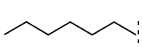
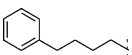
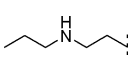
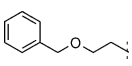
Results and Discussion

Our first objective was to define the key structural elements required for activity (SAR). For synthetic reasons the benzyloxy moiety was kept constant initially and the 2-amino substituent was varied (Table 1) in order to identify the optimal aliphatic side-chain length. Since *N*-aryl substitution is known to potentiate α-adrenergic activity[27] these were not explored further. Of the aliphatic derivatives the *N*-pentyl derivative **14** was rapidly found to be optimal with a K_i of 26 nM. Longer derivatives **15**, shorter alkyl chains **4**, **9**, **12**, **13** as well as bulky derivatives **10** and those containing a polar group **11**, **16** all displayed a sharp loss in affinity toward the NMDA receptor. In particular, branched derivatives **17**, **18** show a steep drop-off in affinity with increasing steric bulk, this is also evident by comparing entries **10** and **17**. In contrast, aryl-alkyl derivatives with varying spacers displayed a relatively flat SAR. Polar atoms in the linker **25** were found to be detrimental, whilst a terminal phenolic moiety **21** slightly enhances affinity. The most active analogue in this series is the benzyl derivative **19** (K_i 130 nM), in contrast, the polar 3-pyridine congener **20** is significantly less active (K_i 380 nM). Next, exploration around the benzyl substituent was performed, by keeping the *N*-pentylamine substituent fixed at C2 on the imidazoline ring (Table 2). Variations at the *ortho*, *meta* and *para* positions were explored with electron withdrawing, electron donating and small lipophilic residues to determine the optimal electronic and steric requirements for activity.

In the *ortho* position small electron withdrawing lipophilic substituent such as fluorine **26** are preferred, (K_i 15 nM), whereas more bulky electron donating substituents such as methoxy are detrimental for binding affinity **29** (K_i 280 nM). In the *meta* position the same trend holds **30** > **31** ≫ **32** ∼ **33**. Interestingly the nitro group, although a strong sigma-acceptor and relatively

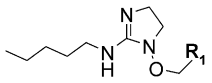
Table 1. NMDA affinities of compounds **4**, **9**–**25**, variation of R_2

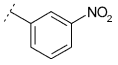
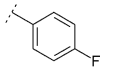
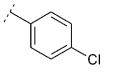
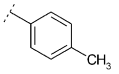
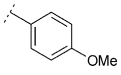
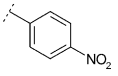
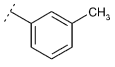
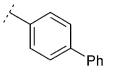
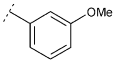


Compd	R_2	K_i [μM][a] NMDA[b]	Compd	R_2	K_i [μM][a] NMDA[b]
9		1.5	**17**		0.26
10		5.6	**18**		21
11		2.25	**19**		0.13
12		1.1	**20**		0.38
13		0.75	**21**		0.38
4		0.06	**22**		1.1
14		0.026	**23**		0.75
15		0.075	**24**		0.38
16		12.8	**25**		1.5

[a]Binding affinities are quoted as K_i values and are the geometric mean of at least two experiments with <20% variance.
[b]Displacement of [^{3}H]-25-6981.[22]

Table 2. Affinities of compounds **14**, **26–40**, variation of Aryl R_1



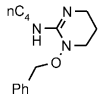
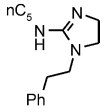
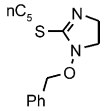
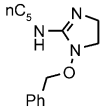
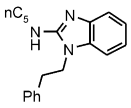
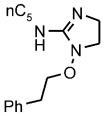
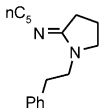
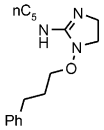
Compd	R_1	K_i [μM][a] NMDA[b]	Compd	R_1	K_i [μM][a] NMDA[b]
14		0.026	**33**		0.53
26		0.015	**34**		0.10
27		0.038	**35**		0.17
28		0.034	**36**		0.23
29		0.280	**37**		0.11
30		0.023	**38**		1.1
31		0.038	**39**		1.1
32		0.560	**40**		0.056

[a,b]See Table 1.

small in size, also only possesses weak affinity suggesting the benzyl moiety occupies a strict size limited pocket. The *para* position is the most sensitive to substitution effects, even fluorine **34** has a detrimental influence compared to hydrogen. Interestingly the 4-methoxy group in **37** has identical activity to fluorine in **34** which stands in contrast to the *ortho* and *meta* positions. Accordingly the 4-nitro **38** and 4-phenyl **39** analogues were as expected only found to be weakly active, confirming that bulky groups are not accommodated in this position. Finally, the electron-rich thiophene analogue **40** is well tolerated, but slightly less active than the phenyl counterpart **14**.

Surprisingly, in contrast to the results obtained from exploration of space around the 4-position of the benzyl group, extending the benzylether linker in **14** to the phenyl-ethyl derivative **46** provides a substantial improvement in affinity, affording in the most potent analogue identified in this study with K_i 7.5 nM. However, the chain extended phenyl-propyl ether analogue **47** suffers a substantially loss in activity (K_i 980 nM), in accord with SAR expectations, indicating that a three-carbon tether is clearly too long to be accommodated by the NMDA receptor in this ligand binding site. Structural modifications were directed next toward the core imidazoline ring system in order to determine the importance of basicity, the hetero-atoms as well as to investigate the steric and orientational requirements (Table 3). Modest enlargement of the imidazoline ring in **4** to the tetrahydropyrimidine homologue **41** causes more than ten-fold loss in activity. This is likely due to the larger steric demand of a 6-membered ring than the small changes in substituent exit vector or enhanced basicity. Similarly, the apparently conservative replacement of the N1-oxygen in **14** by a methylene group in **45** significantly reduces the affinity by almost a log unit, suggesting either conformation and/or basicity play an important role. Since **42**, **44**, **45** are all isosteric to **14** and protonated a physiological pH, the reason for the drop in activity of **45** compared to **14** is likely due to a conformational change. Since by removal of the O-atom acceptor an intramolecular hydrogen-bond from the C2-NH to O which could fix the orientation of both the N- and O-substituents is probably lost. In the case of

Table 3. Activities of imidazoline and tether variations **14**, **41–47**

Compd	Structure	K_i [μM][a] NMDA[b]	pK_a[c]	Compd	Structure	K_i [μM][a] NMDA[b]	pK_a[c]
41		0.75	>12	**45**		0.23	>12
42		6.0	8.5	**14**		0.026	10.1
43		33	7.4	**46**		0.0075	9.9
44		6.4	>11	**47**		0.98	10.0*

[a,b]See Table 1; * calculated pK_a value.
[c]pK_a values determined using the potentiometric method.[28]

Table 4. selectivity profile of compounds **14** and **46**

Compd	K_i [μM][a] NMDA[b]	K_i [μM] α1[c]	K_i [μM] α2[d]	IC_{50} [μM] NR2B[e]	IC_{50} [μM] NR2A[e]	K^+ channel% effect[f] at 10μM
14	0.026	5.5	30	0.21	10	48
46	0.0075	4.9	10	<0.1	4.7	63

[a,b]See Table 1.
[c][^{3}H]-prazosin displacement.[30]
[d][^{3}H]-RX821002 displacement.[31]
[e]Electro-physiology on Xenopus oocytes expressing recombinant rat NMDA receptor subunits, either NR2B or NR2A together with NR1C.[32] Geometric mean values of 3–6 experiments.
[f]K^+ current was evoked by depolarisation to 40 mV in whole-cell patch-clamp experiments on cultured rat cortical neurones. Arithmetic mean values of 3–5 experiments.

entry **42** where the C2 NH group has been replaced by a sulfur atom the loss in affinity is dramatic, less so the change in basicity of **14** (pK_a 10.1) to **42** (pK_a 8.5) indicating the requirement for an H-bond donor at C2. Entry **44** also lends supports to this conclusion, as replacement of the 3-nitrogen atom by a methylene group removes the NH, resulting in loss of activity. Here the pK_a is very similar to that of **45** and **14** but there is a 20-fold loss in affinity for the NMDA receptor strongly suggesting a mandatory requirement for a hydrogen bond donor at the C2-position. Attempts to prepare the imidazole counterpart of the imidazoline were unfortunately all unsuccessful. However, the benzimidazole analogue **43** could be prepared and despite adequate basicity for protonation at physiological pH (pK_a 7.4) NMDA receptor activity was lost completely, probably as a result of the unfavorable additional steric bulk of the annulated benzimidazole ring system.

The most active compounds identified from these optimization studies **14** and **46** were further profiled in order to determine the potential side-effect liabilities and to assess the selectivity (Table 4). Gratifyingly, compounds **14** and **46** were found to possess good separation of activity towards α1- and α2-adrenergic receptors and were highly selective for NMDA receptors containing the NR2B subunit (co-expressed with NR1C). Since basic CNS active compounds can possess various undesired ion channel activities these analogues were additionally tested for their effect on voltage-activated K^+ channels. At a high concentration (10 μM) both compounds inhibited the K^+ current in cultured neurons, but the IC_{50} was 50 times above that for the NMDA-receptor (NR2B+NR1C). Finally, compounds **14** and **46** were also tested in vivo in the standard sound induced audiogenic seizures assay[29] after *i.p.* administration and both derivatives were found to be active, below 20 mg/kg indicating adequate penetration into the brain.

Conclusions

Starting from the novel imidazoline **4**, identified via HTS, an SAR was established and the key requirements for activity determined. This led to the development of highly active and selective NMDA NR1A/2B sub-type derivatives **14** and **46**, which were active in vivo and devoid of significant α-adrenergic and K^+ channel activity. These compounds represent a novel class of NMDA receptor antagonists unrelated to the classical ifenprodil chemotypes **1** and **2**, justifying further pharmacological characterization, and with potential utility as neuroprotective agents.

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References and Notes

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