Bioorganic & Medicinal Chemistry Letters 19 (2009) 5132–5135

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of novel tricyclic benzo[4.5]cyclohepta[1.2]pyridine derivatives as NMDA/NR2B antagonists

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ARTICLE INFO

Article history:
Received 19 May 2009
Revised 25 June 2009
Accepted 2 July 2009
Available online 10 July 2009

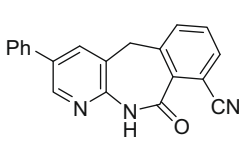
Keyword:
NR2B heterocycle

ABSTRACT

A novel series of annulated tricyclic compounds was synthesized and evaluated as NMDA/NR2B antagonists. Structure–activity development was directed towards in vitro optimization of NR2B activity and selectivity over the hERG K^+ channel. Preferred compounds were subsequently evaluated for selectivity in an α_1-adrenergic receptor binding counter-screen and a cell-based assay of NR2B activity.

In the central nervous system (CNS), the ligand-gated *N*-methyl-D-aspartate (NMDA) ion channel plays a critical role in the maintenance of normal synaptic activity and plasticity. Located in the postsynaptic membrane, NMDA ion channels are activated by strong membrane depolarization coupled with presynaptic release of the neurotransmitter glutamate. Opening allows for calcium and sodium ion flux into the cell.[1] Over-activation of the NMDA ion channel complex leads to cellular inundation by sodium and calcium ions and this phenomenon has been implicated in a number of disease states. Studies of NMDA antagonists in clinical trials revealed that non-specific blockade of the NMDA ion channel by agents such as ketamine (for pain) or memantine (Alzheimer's disease) were attended by deleterious side effects such as dysphoria and hallucination.[2] A subtype-specific NMDA ion channel antagonist could afford agents with an improved therapeutic index. NMDA ion channel subtypes are constructed of a heteromultimeric assembly of NR1 subunits (a–h) and at least one of four NR2 subunits (a–h). Expression of the NR2B subunit is delimited to the forebrain in mammals and has been linked to many neurological disorders including parkinsonism, cerebral ischemia, psychosis or neuropathic pain.[3] A small-molecule allosteric inhibitor of the NMDA/NR2B ion channel complex has the potential to give an improved therapeutic index over non-specific NMDA antagonists thereby allowing for the development of new treatment therapies for a number of neurological diseases.[4]

In the CNS, small-molecule annulated tricyclics are established primarily as antidepressant and antipsychotic agents.[5] The recent identification of a new class of tricyclic NR2B inhibitors[6] (**1**, NR2B K_i 19 nM, Fig. 1) inspired us to investigate a similar series of compounds possessing a central carbocyclic ring (**2**, Fig. 1).[7] Synthetic studies were initiated with the dual primary objectives of identifying compounds with optimized NR2B binding activity and minimized human ether-a-go-go (hERG)[8] K^+ channel activity. SAR in this series was developed by making modifications to the pendant substituents R_1 and R_2 as well as the central ring component of the tricycle. Selected compounds were further evaluated for both selectivity versus the α_1-adrenergic[9] receptor and for activity in a cell-based assay of NR2B antagonism. Herein we wish to report the development of an efficient, versatile synthesis of novel tricyclic compounds having intrinsic inhibitory activity versus the NR2B subtype of the NMDA ion channel complex.



1
NR2B K_i 19 nM
hERG IP 431 nM

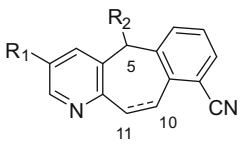


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Figure 1. Tricyclic lactam lead (**1**) and benzo[4.5]cyclohepta[1.2] pyridine target compounds (**2**).

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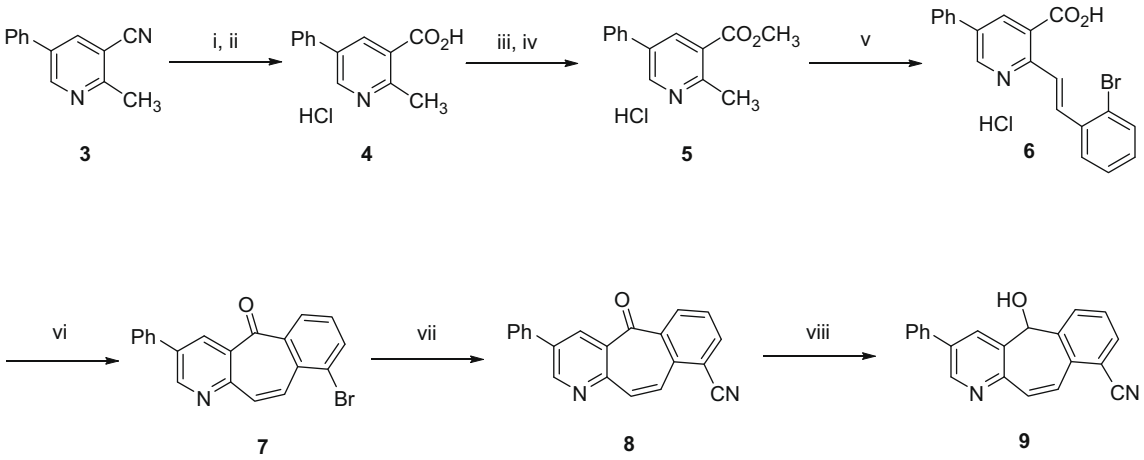
doi:10.1016/j.bmcl.2009.07.028



Scheme 1. Reagents and conditions: (i) 50% NaOH (aq), EtOH, reflux, 24 h; (ii) HCl (aq), 100%; (iii) $SOCl_2$, reflux, 0.75 h; (iv) MeOH, reflux, 0.5 h, 100%; (v) KO^{t}Bu, hexane, 0 °C, 0.75 h, 2-bromobenzaldehyde; (vi) polyphosphoric acid, 200 °C, 85%; (vii) CuCN, DMF, 120 °C, 24 h, 90%; (viii) $NaBH_4$, EtOH, reflux, 10 min, 77%.

Table 1
In vitro evaluation of compounds **8** and **9**[a]

Compound	NR2B binding K_i[b] (nM)	hERG IP[c] (nM)
8	1000	882
rac-**9**	2	5100
(+)-**9**	540	10,000
(−)-**9**	1	590

[a] Each entry represents the mean of three experiments.
[b] Inhibition of ^3H-[(*E*)-N1-(2-methoxybenzyl)-cinnamamidine] binding to hNR1a/NR2B receptors expressed in Ltk-cells.[14]
[c] Inhibition of MK-499 binding to hERG in HEK293 cells.[16]

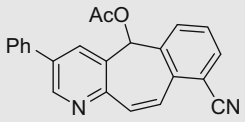
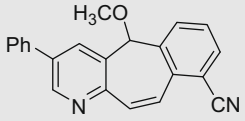
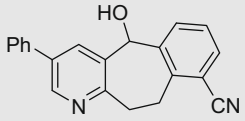
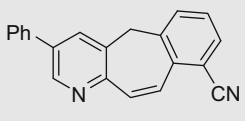
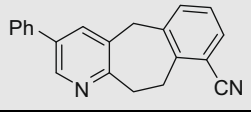
Initially, a classic synthetic approach[10] utilizing the known 3-cyano-2-methyl-5-phenylpyridine[11] (**3**) intermediate, was employed for the construction of the appropriately substituted tricyclic system (Scheme 1). Hydrolysis of the nitrile (**3**) followed by esterification of the resulting carboxylic acid (**4**) gave (**5**) in high yield.

Treatment of the 2,3,5-trisubstituted pyridine ester (**5**) with potassium-*t*-butoxide in hexane followed by reaction with 2-bromobenzaldehyde gave the Aldol product, **6** which was heated in polyphosphoric acid to afford the annulated tricyclic intermediate, **7**.

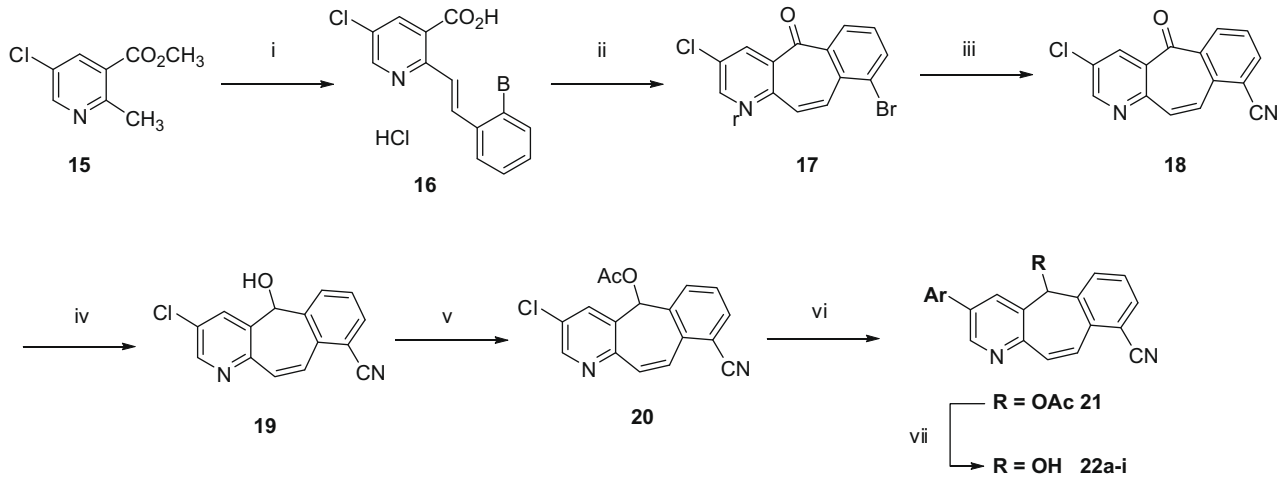
Application of the Rosenmund–von Braun aromatic cyanation reaction[12] gave a high yield of the aryl-nitrile **8**. Reduction of the ketone (**8**) with sodium borohydride gave the initial target compound, *rac*-**9**.[13]

Subsequent in vitro evaluation of *rac*-**9** showed it possessed excellent activity (K_i 2 nM) when tested in an assay for NR2B binding (Table 1).[14] Furthermore, only modest NR2B activity was observed in the ketone intermediate **8** (K_i 1000 nM), thus suggesting the importance of the pendant hydroxyl functionality for activity in this structural class. Resolution of *rac*-**9** was accomplished by

Table 2
Chemical modification of the central carbocycle of compound *rac*-**9** and resulting NR2B and hERG activities[a]

Reaction conditions	Yield (%)	Number	Product	NR2B binding K_i[b] (nM)	hERG IP[c] (nM)
HOAc, reflux, 24 h	41	**10**		10	653
60% NaH, CH_3I, MeCN, 15 min	71	**11**		4800	660
5% Pd on $BaSO_4$, H_2 (balloon), EtOH, 72 h	48	**12**		56	3564
Zn (2 equiv), HOAc, reflux, 24 h	26	**13**		2	65
Zn (16 equiv), HOAc, reflux, 48 h	55	**14**		77	154

[a] Each entry represents the mean of three experiments.
[b] Inhibition of ^3H-[(*E*)-N1-(2-methoxybenzyl)-cinnamamidine] binding to hNR1a/NR2B receptors expressed in Ltk-cells.[14]
[c] Inhibition of MK-499 binding to hERG in HEK293 cells.[16]



Scheme 2. Reagents and conditions: (i) KO^{t}Bu, THF, 0 °C, 0.75 h, 2-bromobenzaldehyde, 72%; (ii) polyphosphoric acid, 200 °C, 24 h, 82%; (iii) CuCN, DMSO, 150 °C, 6 h; (iv) $NaBH_4$, EtOH, reflux, 10 min, 87% (two steps); (v) Ac_2O, HOAc, reflux, 24 h, 58% (vi) $ArB(OH)_2$, $Pd_2(dba)_3$, Cs_2CO_3, Cy_3P, dioxane, 100 °C, 24 h, 53–88%; (vii) K_2CO_3 (aq), MeOH, 25 °C, 30 min, 48–93%.

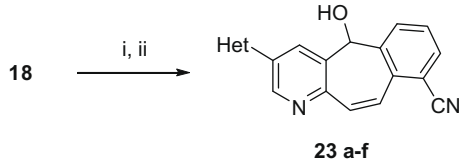
chiral preparative HPLC.[15] Unfortunately, NR2B and hERG[16] activities tracked together such that the enantiomer with the greatest NR2B activity, **(−)-9** (K_i 1 nM), was also the most potent hERG K^+ channel blocker (IP 590 nM). Additional synthetic modifications of *rac*-**9** were initiated to better define the SAR around the central ring in an effort to achieve better separation of NR2B and hERG activities.

The synthetic strategy outlined in Scheme 1 was useful in that it allowed for either derivitization of the pendant hydroxyl group or a step-wise reduction at carbons 5, 10 and 11 of the central ring (Fig. 1, Table 2). For instance compound *rac*-**9** was refluxed in acetic acid to give the acetyl ester **10**. Likewise the methyl ether (**11**) was prepared by submitting *rac*-**9** to Williamson etherification conditions. Selective reduction of the alkene in the presence of the benzylic hydroxyl on the central carbocycle was accomplished by hydrogenating *rac*-**9** in the presence of Rosenmund's catalyst (5% $Pd/BaSO_4$) to give the saturated hydroxycarbocycle derivative **12**. Reduction with zinc (2 equiv) in refluxing acetic acid gave the deoxygenated product, **13**. On extended refluxing with a large excess of zinc the fully reduced and deoxygenated carbocycle, **14** resulted.

Assessment of the acetyl ester (**10**) revealed a fivefold decrease in NR2B activity and a sevenfold *increase* in hERG activity versus *rac*-**9**. NR2B activity was even further diminished in the case of the methyl ether derivative **11**. Reduction of the alkene with retention of the pendant hydroxyl gave a derivative with reduced NR2B activity (**12**) while the deoxygenated derivative (**13**) showed NR2B activity comparable to *rac*-**9**. No advantage resulted when the central ring was both reduced and deoxygenated as in **14**. Examples **12**–**14** suggest the importance of the pendant hydroxyl for achieving separation between NR2B and hERG activities.

The synthesis summarized in Scheme 1 gave NR2B active compounds and allowed for limited SAR development of the central carbocycle as illustrated in Table 2. This route did however necessitate the early installation of the pendant aryl group in the 3-position of the pyridyl ring and this made subsequent development of SAR in this region of the molecule difficult. It was envisaged that a 3-halo substituent on the pyridine would allow for late-stage Suzuki[17] palladium mediated cross-couplings with boronic acids or amines thus affording synthetic products with both electronic and steric variation at the 3-pyridyl position of the tricyclic structure (Scheme 2).

Scheme 2 illustrates this alternative synthesis of annulated tricylics starting from the known 2,3,5-trisubstituted pyridine (**15**).[18] A key synthetic design feature concerned the utilization of a chlo-



Scheme 3. Reagents and conditions: (i) amine, $Pd_2(dba)_3$, *rac*-BINAP, NaO^{t}Bu, toluene, 100 °C, 24 h, 23–44%; (ii) $NaBH_4$, EtOH, reflux, 10 min, 48–60%.

Table 3
Aryl group variation of **22 a–i** and resulting NR2B and hERG activities[a]

Ar	Number	NR2B binding K_i[b] (nM)	hERG IP[c] (nM)
o-Tolyl	**22a**	6	1351
m-Tolyl	**22b**	4	1100
p-Tolyl	**22c**	10	1698
o-MeO-phenyl	**22d**	13	5455
m-MeO-phenyl	**22e**	9	1534
p-MeO-phenyl	**22f**	8	1612
o-Fluorophenyl	**22g**	16	4445
m-Fluorophenyl	**22h**	23	4968
p-Fluorophenyl	**22i**	3	512

[a] Each entry represents the mean of three experiments.
[b] Inhibition of ^3H-[(*E*)-N1-(2-methoxybenzyl)-cinnamamidine] binding to hNR1a/NR2B receptors expressed in Ltk-cells.[14]
[c] Inhibition of MK-499 binding to hERG in HEK293 cells.[16]

Table 4
Heterocycle analogs **23 a–f** and resulting in vitro activities[a]

Het	Number	NR2B binding K_i[b] (nM)	hERG IP[c] (nM)
Pyrrolidine	**23a**	720	15,500
Piperidine	*rac*-**23b**	43	10,000
Piperidine	(+)-**23b**	35,000	20,000
Piperidine	(−)-**23b**	17	20,000
Homopiperidine	**23c**	428	3000
Morpholine	**23d**	196	20,000
4-Me-piperidine	**23e**	83	5300
4-Me-piperazine	**23f**	>16,000	42,000

[a] Each entry represents the mean of three experiments.
[b] Inhibition of ^3H-[(*E*)-N1-(2-methoxybenzyl)-cinnamamidine] binding to hNR1a/NR2B receptors expressed in Ltk-cells.[14]
[c] Inhibition of MK-499 binding to hERG in HEK293 cells.[16]

rine atom as the appropriate halogen in the 5-position of the pyridine ring in **15**. This allowed the Rosenmund–von Braun cyanation reaction (**17**→**18**) to proceed with the bromide while retaining the heteroaryl-chloride for subsequent participation in cross-coupling reactions (**20**→**21**).

Data for the products obtained via this chemistry is illustrated in Table 3. Although this set of compounds possessed pendant aryl rings with diverse electronic and steric topology, testing did not reveal the emergence of any clear SAR trends regarding NR2B and hERG activities. There remained, however, a tendency for NR2B and hERG activities tracking together as in **22h** and **22i**.

A third synthetic iteration on the tricyclic motif involved the introduction of a weakly basic functionality into the 3-position of the fused pyridine heterocycle. Scheme 3 shows the two-step elaboration of intermediate **18** using Buchwald[19] amination chemistry to afford tertiary amine analogs (**23a–f**).

Derivatives possessing weakly basic cyclic amine in the 3-pyridyl position (Table 4) were not as active against NR2B as their aryl counterparts (Tables 1 and 3). Pyrrolidine (**23a**), homopiperidine (**23c**), morpholine (**23d**) and *N*-methylpiperazine (**23f**) derivatives exhibited poor activity when evaluated in the NR2B binding assay (K_i 196–16,000 nM). Modest NR2B activity was observed for the 4-methylpiperidine (**23e**, K_i 83 nM) derivative. The piperidine (*rac*-**23b**) analog, however, showed both good NR2B activity (K_i 43 nM) and an absence of hERG ion channel activity (IP 10,000 nM). Furthermore, chiral preparative HPLC resolution of *rac*-**23b** gave an active NR2B compound ((−)-**23b**, 17 nM) with greater than 1000-fold selectivity over hERG (IP 20,000 nM).

Two compounds, (−)-**9** and (−)-**23b**, were subsequently assessed in both an α_1-adrenergic receptor[20] counter-screen assay and a NR2B Ca^{2+} influx cell-based assay. Results for the α_1-adrenergic receptor assay ((−)-**9** K_i 5,000 nM, (−)-**23b** K_i > 21,000 nM) showed that the pendant piperidine analog had fourfold greater selectivity than the phenyl compound. Consistent with the NR2B binding assay results, comparison of (−)-**9** and (−)-**23b** in a NR2B Ca^{2+} influx cell-based assay[7] showed a threefold loss in activity ((−)-**9** IC_{50} 9 nM, (−)-**23b** IC_{50} 27 nM) for the piperidine derivative.

In summary, a new class of NMDA/NR2B inhibitors were discovered and their SAR investigated. Presence of a hydroxyl substituent on carbon-5 of the central ring and replacement of a pendant aryl with a piperidine heterocycle were necessary elements for achieving separation of NR2B and hERG activities. Compound (−)-**23b** possesses a balanced profile with good NR2B activity and selectivity over hERG and α_1-adrenergic receptors.

Acknowledgements

The authors wish to thank Jean-François Marcoux (Merck Process Research) and Garry R. Smith (Merck Research Laboratories) for providing scaled-up batches of synthetic intermediates and Joan S. Murphy (Merck Research Laboratories) for high resolution mass spectral analysis of all final compounds.

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