Synthesis of 7-Chloro-2,3-dihydro-2-[1-(pyridinyl)alkyl]-pyridazino[4,5-b]quinoline-1,4,10(5H)-triones as NMDA Glycine-Site Antagonists

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Abstract—Several members of the 7-chloro-2,3-dihydro-2-[1-(pyridinyl)alkyl]-pyridazino[4,5-b]quinoline-1,4,10(5H)-triones (2) have been identified as being potent and selective NMDA glycine-site antagonists. Increasing size of the alkyl substituent on the alpha-carbon led to a progressive decrease in binding affinity. Some of these analogues possess improved drug-like properties such as cellular permeability, solubility and oral absorption.
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N-Methyl-D-aspartate (NMDA) antagonists which act at the allosteric glycine co-agonist site are believed to have potential therapeutic value in treating a range of disorders including stroke, Parkinson's disease, depression, and neuropathic pain.[1] It has been recognized that a major hurdle in designing efficacious NMDA glycine-site antagonists is overcoming poor physical properties such as solubility, cell permeability, plasma protein binding and brain penetration.[2] In this respect, development of orally active glycine-site antagonists has been difficult.

In previous work, it has been shown that 7-chloro-2,3-dihydro-2-(phenyl)-pyridazino[4,5-b]quinoline-1,4,10(5H)-triones (PQT's) are potent NMDA glycine-site antagonists.[3] We sought to improve on the physical properties of these compounds while preserving the potency and selectivity for the NMDA-glycine site. Compound 1 (K_i = 115 nM vs [³H] MDL 105,519) was initially identified as a potent NMDA-glycine site antagonist, however poor drug-like properties (e.g., aqueous solubility less than 0.1 mg/mL and oral bioavailability

< 5%) became a large concern. Extensive formulation of 1 still did not lead to improvements in oral absorption (Fig. 1).

Compound 1 was then used as the starting point to incorporate chemical modifications to try and improve the physical properties of these compounds while maintaining reasonable affinity (e.g., < 250 nM) at the NMDA glycine site. Based on previous knowledge from the N-phenyl substituted PQT's, it was known that solubility was often improved when the planar geometry of these compounds was distorted. We followed the same strategy to improve the physical properties of 1, in this case employing alkyl-substitution on the α-carbon to give analogues such as 2.

1 R = H
Aq. Solubility = 0.05 mg/mL
Oral Bioavail. (rat) ~ 5%
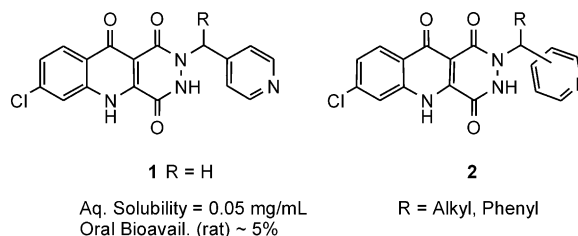
2
R = Alkyl, Phenyl

Figure 1.

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The synthesis of these analogues is shown below in Scheme 1. Commercially available pyridyl ketones **3** were converted to *tert*-butoxy hydrazones **4** in the presence of catalytic acid.[4] Alternatively, non-commercially available ketones were prepared according to the procedure of Sheldrake which employs alkylation of a ketal carbon and subsequent deprotection.[5] The hydrazones were then reduced using catalytic hydrogenation to give the hydrazines **5**. Hydrazines **5** were coupled with acid amide **6**[6] using carbodiimides to give the intermediate hydrazine amides **7**. The final cyclization was accomplished with methanesulfonic acid yielding **2a–t** as the methanesulfonic acid salts.

We were also interested in the pyridyl *N*-oxide derivatives of these compounds. In theory, these compounds may possess better water solubility than the parent compounds. The *N*-oxide analogue of **1** is readily prepared by dissolving **1** in methanolic choline hydroxide solution, followed by the addition of *m*-CPBA to give **8a**, or correspondingly **8b** Scheme 2.

Chiral analogues of **2** were prepared with greater than 98% enantiomeric purity. The chiral acetate **11** served as a good starting point and was prepared by enyzmatic resolution of 1-(2-pyridyl)ethanol **9** using Novozyme-435.[7] The acetate was then hydrolyzed and converted to mesylate **12**. The mesylate was displaced with *t*-butylcarbazate to give the desired chiral hydrazine (*S*)-**13**. Although the yield was low, the enantioselectivity was
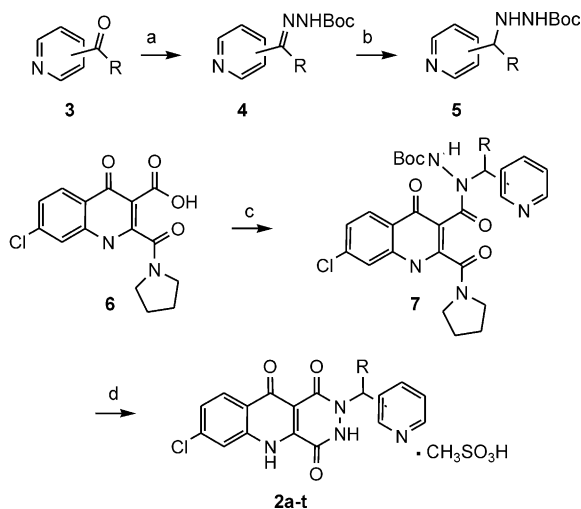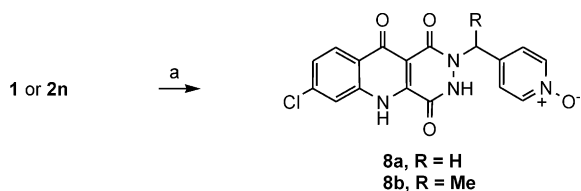
quite high.[8] The low yield was a result of the difficulty of separating the final material from excess *t*-butyl carbazate. Unfortunately, when the amount of *t*-butyl carbazate was reduced from five equivalents to one equivalent, the enantioselectivity dropped off rather precipitously. Using only one equivalent led to nearly racemic material. These results suggest a competitive S_N1 pathway can occur when the concentration of carbazate is low. As an alternative, the racemic hydrazine can be separated by preparatory chiral HPLC. Coupling of the chiral hydrazines was accomplished using EDCI in refluxing CH_2Cl_2 as outlined in Scheme 1 to give rise to **2c** (Table 1). The opposite enantiomer (*R*)-**13** was prepared in an analogous fashion to give **2d** beginning with the (*S*)-alcohol **10** also obtained from the chiral resolution described above (Scheme 3).

Biological and physical data of these compounds are shown in Table 1. From the data, it is apparent that affinity at the glycine site decreased with increasing substitution on the α-carbon.[9] In this regard, the chiral analogues **2c** and **2d** represent an interesting comparison. The (*S*)-isomer **2c** was reasonably potent, whereas the (*R*)-isomer was absent of activity. The pyridyl *N*-oxides **8a** and **8b** were less potent that the unoxidized analogues (**1** and **2n**) with a 5- to 10-fold decrease in binding affinity being observed.

Despite the drop-off in binding affinity, the drug-like physical properties of these α-branched compounds appeared to improve over the unsubstituted counterparts. For example, all of the unsubstituted analogues (R = H for **2a**, **2k**, **1**, **8a**) possessed poor Caco-2 permeabilities (A > B < 10×10^{-7}). In the 2- and 4-pyridyl series, substitution of alkyl groups on the α-carbon resulted in improved Caco-2 permeability. In these two series small aliphatic substitution (e.g., R = Me) led in some cases to 30-fold improvements in Caco-2 permeabilities (e.g., **2c**, **2n**) with only 2- to 3-fold loss in binding activity. The Caco-2 improvements were not seen in the 3-pyridyl series when R = Me, but did improve with R = Et (**2l** vs **2m**). However, the ethyl substituted compound was devoid of binding activity. These improvements in cellular permeability may be due to the increase of hydrophobicity at the α-carbon. The *N*-oxide **8a** showed little change in Caco-2 permeability over the parent compound **1**.

In general, solubilities of the substituted analogues were also improved from the lead compound **1**. The improved solubility may be due to distortion of the planar conformation, resulting in potentially weaker crystal packing forces. In some cases, this can be supported by a corresponding lowering of melting points between the α-alkyl substituted and unsubstituted analogues.[10] For example, compound **1** has a melting point range between 277 and 278 °C as the methanesulfonate salt. The α-methyl analogue **2n** has a melting point range between 245 and 247 °C for the same salt form. Similar observations are seen with **2a** and **2k** and their corresponding α-alkyl analogues.

Scheme 1. Reagents and conditions: (a) *t*-BocNHNH₂, THF, HCl (cat.), 60–95%; (b) 10% Pd/C, MeOH, H₂, 40 psi, 18 h, 35–98%; (c) **5**, CMC or EDCI, THF or CH_2Cl_2, DMAP (cat.), 15–78%; (d) CH_3SO_3H, THF, rt, 30–85%.

Scheme 2. Reagents and conditions: (a) choline hydroxide, MeOH; *m*-CPBA, 20%.

It is also apparent that the improvement in drug-like physical properties paralleled an improvement in oral

Table 1. Glycine site affinity, cellular permeability, solubility and oral bioavailability

2a–t
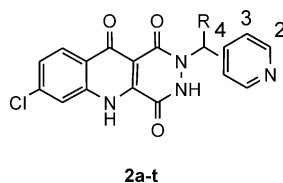
Compd[a]	Pyridyl position	R	[³H] MDL 105519 $K_i \pm$ SD (nM)[b]	Caco-2[c] (A > B) $\times 10^{-7}$	Sol (mg/mL)[d]	F (%)[e]
1	4	H	115 ± 58	2	0.05	5 ± 5
2a	2	H	146 ± 30	3	0.28	—
2b	2	Me	221 ± 81	21	>0.32	42 ± 16
2c	2	Me (S)	207 ± 80	91	>0.32	50 ± 8
2d	2	Me (R)	>10,000	130	>0.33	48 ± 17
2e	2	Et	2360 ± 499	79	>0.20	—
2f	2	*i*-Pr	2170 ± 1146	495	NT	—
2g	2	*n*-Bu	>10,000	160	>0.22	—
2h	2	*i*-Bu	>10,000	91	>0.13	—
2i	2	*c*-prop	996 ± 343	98	0.49	—
2j	2	*c*-pent	>10,000	56	>0.17	—
2k	3	H	99 ± 9	2	0.17	~12[f]
2l	3	Me	272 ± 60	8	>0.27	—
2m	3	Et	>10,000	50	>0.25	—
2n	4	Me	248 ± 70	58	>0.29	30 ± 8
2o	4	Et	1940 ± 408	110	>0.20	17 ± 7
2p	4	*c*-prop	751 ± 319	63	>0.19	—
2q	4	*n*-Pr	4340 ± 2767	160	>0.22	—
2r	4	*n*-Bu	5570 ± 1312	94	0.07	—
2s	4	*i*-Bu	5230 ± 2380	110	>0.17	—
2t	4	Ph	>10,000	40	0.13	—
8a[g]	4	H	439 ± 107	5	>0.16	—
8b[g]	4	Me	2420 ± 530	NT[h]	NT[h]	—

[a]All new compounds were fully characterized by ¹H NMR spectroscopy, MS, and CHN.
[b]Experiments run in duplicate. Displacement of [³H] MDL 105,519 in rat brain membranes. Experiments run in duplicate. Average K_i is reported.
[c]Caco-2 measurements were done with Caco-2 monolayers. Samples were taken from the apical and basolateral side and quantified by LC/MS/MS.
[d]Aqueous solubility of methanesulfonate salt at pH = 7.4.
[e]Percent rat oral bioavailability as the methanesulfonate salt. Male Sprague–Dawley rats were dosed orally (10 mg/kg, ASV suspension or meglumine solution) and intravenously (10 mg/kg). Plasma concentrations were measured until the drug was not quantifiable.
[f]High interanimal variability. Estimated bioavailability is reported.
[g]Pyridyl *N*-oxides.
[h]NT, not tested.
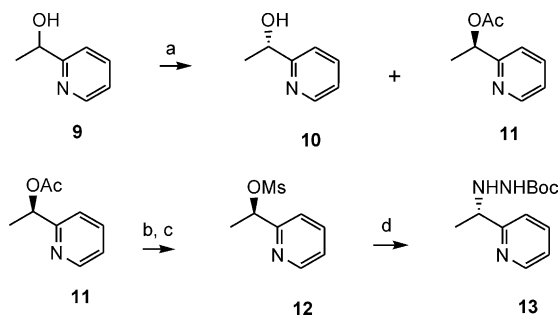
Scheme 3. Reagents and conditions: (a) Novozyme-435, Et₂O, vinyl acetate, 5 h, 4 Å sieves, rt; (b) K₂CO₃, MeOH, 98%; (c) MsCl or Ms₂O, DIEA, CH₂Cl₂ or toluene, 70%; (d) *t*-BocNHNH₂ (5 equiv), DIEA, DMF, 110 °C, 6 h, 30%, >98% ee.

The NMDA glycine-site antagonists presented here were designed to have improved solubility and cellular penetration over the parent compound **1**. The introduction of alkyl groups into the α-position led to such improvements. However, a narrow window of glycine binding site affinity exists for these analogues. It was determined that only a methyl group was tolerated without substantial loss in binding activity. The chiral analogue **2c** represents the best lead from this series. Although this compound had a 2- to 3-fold loss in binding activity, the gain of 10-fold improvement in oral bioavailability makes it a superior compound for future in vivo studies.

absorption. For the cases studied, the unsubstituted compounds **1** and **2k** possessed the lowest rat oral bioavailabilities. In contrast, the α-substituted analogues **2b**, **2c**, **2d**, **2n** and **2o** had the best oral bioavailabilities. It should also be noted that smaller interanimal variability was seen with the α-branched compounds as compared to **1** and **2k**.

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