*Bioorganic & Medicinal Chemistry Letters*, Vol.3, No.1, pp. 55-60, 1993
Printed in Great Britain

0960-894X/93 $5.00 + .00


# SYNTHESIS AND ACTIVITY OF SPIROISOINDOLINES AS NOVEL NONCOMPETITIVE NMDA ANTAGONISTS

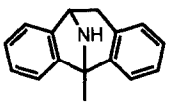
Thomas M. Bare*, Clyde W. Draper, Charles D. McLaren, Linda M. Pullan, Jitendra Patel and Jitendra B. Patel
ICI Pharmaceuticals Group, A Business Unit of ICI Americas Inc., Wilmington, DE 19897

**ABSTRACT:** A series of novel spiroisoindolines was designed and synthesized as potential noncompetitive NMDA antagonists. Affinities of these compounds for the noncompetitive NMDA binding site were determined using [$^3$H]TCP and found to possess $IC_{50}$s ranging from 0.065 to 17μM. In vivo testing of 2'-methylspiro-[4,5,6,7-tetrahydrobenzothiophene-4,1'-(1,3-dihydroisoindole)] (**43**) showed it to antagonize NMDA-induced convulsions, to be neuroprotective in a gerbil model of ischemia, and not to generalize to MK-801 in a drug discrimination paradigm.

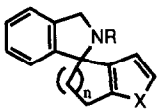
The excitatory amino acids (EAAs) aspartate and glutamate are now generally considered to be the major neurotransmitters in the mammalian central nervous system (CNS).[1] Excessive and/or prolonged stimulation of EAA receptors may be associated with the pathology of a number of neurodegenerative disorders, such as stroke, epilepsy, Huntington's chorea, and Alzheimer's and Parkinson's disease.[2] EAA antagonists, therefore, might be of therapeutic use by preventing overstimulation of these receptors and subsequent neurodegeneration.

Of the three distinct types of EAA receptors, the N-methyl-D-aspartate (NMDA) receptor has been best characterized. This receptor contains at least four binding sites that gate an ion channel which is permeable primarily to calcium and sodium cations.[1] The NMDA antagonist phencyclidine (PCP) is thought to bind to a site within the ion channel and, consequently, to cause a noncompetitive inhibition of the receptor.[3] MK-801 (**1**, dizocilpine) is a dibenzocycloheptenimine which interacts at the noncompetitive (PCP) site of the NMDA receptor.[3b] This compound is a potent anticonvulsant agent and has been shown to exert a neuroprotective effect in animal stroke models when administered as long as 24 hours after onset of an ischemic event.[4]

Using the conformationally rigid MK-801 (**1**) as a reference molecule, we designed and subsequently synthesized a series of semirigid spiroisoindolines **2**, key structural features of which overlap with corresponding elements of MK-801 with varying degrees depending on the size (n = 1, 2, or 3) of the spirobenzo- or spirothieno-cycloalkane ring. The spiroisoindolines were screened for their ability to bind to the noncompetitive NMDA site using tritiated N-(1-[2-thienyl]cyclohexyl)piperidine ([$^3$H]TCP) and selected analogs were then evaluated in a number of in vivo and in vitro screens used to characterize classical noncompetitive NMDA antagonists.




**1**



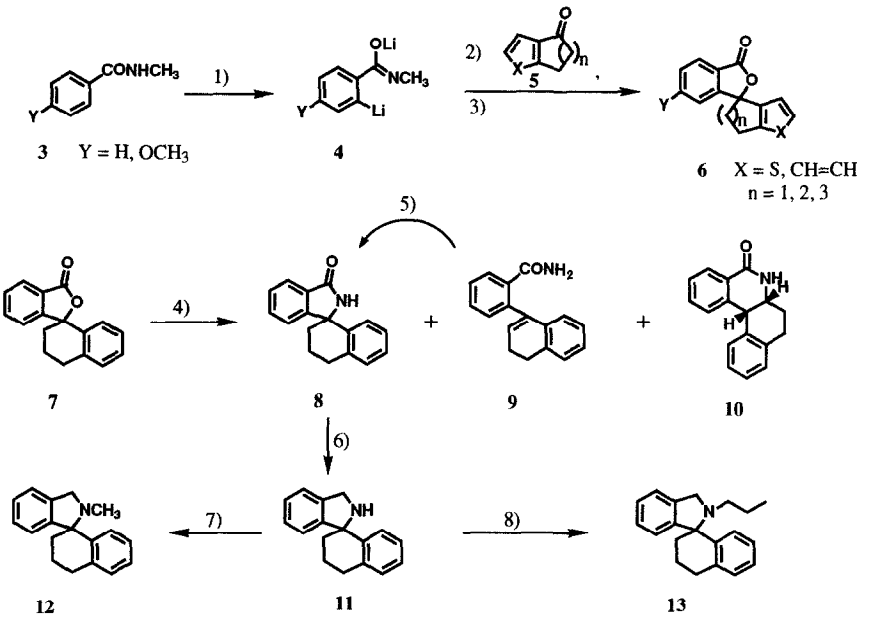

**2**

X = CH=CH, S
R = H, alkyl

Two synthetic routes to the spiroisoindolines were investigated and employed. The key intermediates for both routes were the spirolactones **6** obtained by reaction of an ortho metallated amide[5] **4** and the appropriate benzo- or thienocycloalkanone **5** (**Scheme I**). The first route is illustrated (**Scheme I**) using the spirolactone **7** obtained from

**4** (Y = H) and 1-tetralone. Heating **7** with concentrated ammonium hydroxide, ammonium chloride and ammonia gas in ethanol in a pressure vessel at 240 °C provided a 17% yield of the six-membered ring lactam **10** and a 53% combined yield of a mixture of the spirolactam **8** and olefin amide **9**. This latter mixture, in which **9** was the main component, was refluxed for 3 hr with anhydrous trifluoroacetic acid (TFA) whereupon the olefin amide **9** was converted to lactam **8**. Reduction of **8** with lithium aluminum hydride provided a good yield of the spiroisoindoline **11** isolated as its hydrochloride salt. N-methylation of **11** to provide **12** was accomplished using Eschweiler-Clark conditions. Alkylation of **11** using iodopropane and potassium carbonate in dimethylformamide gave the N-propyl analog **13** in good yield.

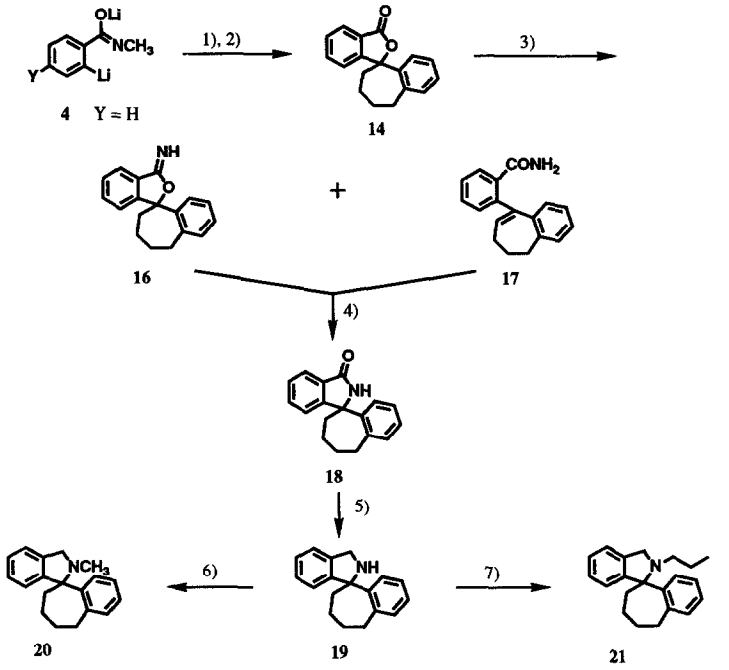
**Scheme I**[a]




[a] (1) n-BuLi, THF/hexane; (2) **5**/THF/-78 °C --> 0 °C; (3) $H_2O$, then reflux; (4) $NH_4OH$, $NH_4Cl$, $NH_3$, EtOH, 240 °C; (5) TFA/reflux; (6) $LiAlH_4$/THF/reflux; (7) $HCO_2H/CH_2O/H_2O$; (8) $K_2CO_3$/n-PrI/DMF

An alternate, more efficient route (**Scheme II**), to the desired spiroisoindolines involved reaction of the spirolactones with methylchloroaluminum amide (**15**) prepared from trimethylaluminum and ammonium chloride.[6] For example, the spirolactone **14** obtained from benzosuberone and **4** (Y = H) provided a 97% yield of an approximate 3:1 mixture of the iminoether **16** and olefin amide **17**, respectively, when heated with methylchloroaluminum amide at 50 °C in toluene for 17 hr. Since preliminary experiments indicated that both **16** and **17** were individually converted to the lactam **18** in hot TFA, the crude reaction mixture was refluxed with TFA to provide the desired spirolactam **18** in 96% yield Reduction of this lactam to the spiroisoindoline **19** and subsequent alkylation to form **20** and **21** were accomplished using reaction conditions described previously.

**Scheme II**[a]



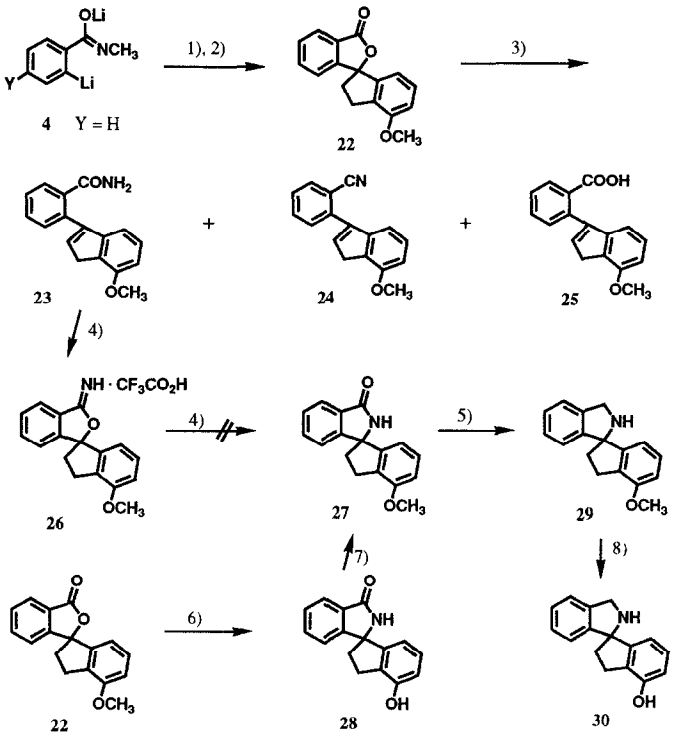

[a] (1) benzosuberone/THF/-78 °C --> 0 °C; (2) $H_2O$, then reflux; (3) $CH_3AlClNH_2$(**15**)/$PhCH_3$/50 °C; (4) TFA/reflux; (5) $LiAlH_4$/THF/reflux; (6) $HCO_2H/CH_2O/H_2O$; (7) $K_2CO_3$/n-PrI/DMF

The spirolactones obtained by reaction of dimetalated N-methylbenzamides with indanones (**Scheme III**) did not react with methylchloroaluminum amide in the "normal" mode as described above. For example, on reaction of lactone **22** with **15**, the products isolated included the olefin nitrile **24** and the olefin acid **25** in addition to the olefin amide **23**. While **23** formed the cyclic iminoether **26** in TFA, no rearrangement to the spirolactam **27** was observed, even under prolonged refluxing with TFA. Consequently, lactone **22** was heated with ammonium hydroxide in a pressure vessel to provide a modest yield of the lactam **28** in which the methoxy group had been cleaved to the phenol. Methylation of the phenol **28** followed by lithium aluminum hydride reduction of the resulting lactam **27** provided the methoxy spiroisoindoline **29**. Boron tribromide cleavage of the methoxy substituent gave the phenolic compound **30**.

The spiroisoindolines were evaluated for their ability to interact at the noncompetitive site of the NMDA receptor using [$^3$H]TCP according to the procedure of Vignon et al..[7] The results are shown in **Tables 1** and **2**. It appears that the benzocyclopentyl compounds (**31**, **32** and **33**) have comparable or better potency than the corresponding benzocyclohexyl (**11**, **12** and **13**) or benzocycloheptyl (**19**, **20** and **21**) analogs. The most potent compound synthesized was the N-methyl substituted benzocyclopentyl spiro analog **32** ($IC_{50}$ [$^3$H]TCP = 65 nM). Within a homologous series (**2**, n = 1, 2 or 3), a compound with a phenyl hydroxyl group substituted meta to the "benzyl amine" moiety of the isoindoline ring was always more potent than the parent or corresponding methoxy substituted compound. This situation is similar to that observed with PCP and MK-801 analogs where meta-hydroxy aryl substituted compounds

are more potent than the parent compounds.[8] Of the thienylcyclohexyl spiro compounds (**Table 2**), the N-methyl analog **43** had the greatest affinity ($IC_{50}$ [$^3$H]TCP = 490 nM) for the noncompetitive receptor binding site.

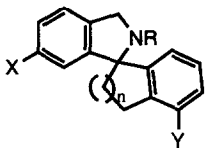
## Scheme III[a]




[a](1) 6-methoxy-1-indanone/THF/-78 °C; (2) $H_2O$, then reflux; (3) $CH_3AlClNH_2$ (**15**)/$PhCH_3$/50 °C; (4) TFA/reflux; (5) $LiAlH_4$/THF/reflux; (6) $NH_4OH$/280 °C; (7) $K_2CO_3/CH_3I$/DMF; (8) $BBr_3/CH_2Cl_2$

Additional pharmacological testing showed that below 100 μM, no interactions of the NMDA competitive ([$^3$H]CPP)[9] site, the strychnine-sensitive ([$^3$H]strychnine)[10] and -insensitive ([$^3$H]glycine)[11] glycine sites with 2'-methylspiro[4,5,6,7-tetrahydrobenzothiophene-4,1'-(1,3-dihydroisoindole)] (**43**) were observed, also, in rat cortical cell cultures[11], compound **43** attenuated NMDA-induced cytotoxicity (68% protection at 100 μM). Preliminary in vivo studies (mice) showed that convulsions induced by intracerebral administration of NMDA[12] (0 4 μg/mouse) were antagonized by compound **43** with an $ED_{50}$ = 32 mg/kg, ip; additionally, MK-801 demonstrated activity in this test ($ED_{50}$ = 0 3 mg/kg, ip) Also, in the gerbil, ischemia-induced cell death in the CA1 region of the hippocampus[13] was significantly attenuated (84% reduction in CA1 damage) by administration of **43** (60 mg/kg, ip, administered 45 min pre and 5 min post ischemic event); MK-801 demonstrated significant protection (42%) in this paradigm at 1 mg/kg, ip. Thus, although relatively weak in comparison with MK-801, compound **43**, like classical noncompetitive NMDA antagonists, exhibits anticonvulsant as well as neuroprotective properties

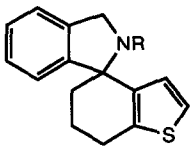
**TABLE 1. Structure, Synthetic Methods and [$^3$H]TCP Binding for Spiroisoindolines**




| Compounds | Synthetic Method[a] | X | Y | n | R | $IC_{50}$ [$^3$H]TCP μM[d] |
|---|---|---|---|---|---|---|
| **31**[b] | I | H | H | 1 | H | 0.98 |
| **32**[c] | I | H | H | 1 | $CH_3$ | 0.065 |
| **33**[c] | I | H | H | 1 | n-$C_3H_7$ | 0.44 |
| **29** | III | H | $OCH_3$ | 1 | H | 5.0 |
| **30** | III | H | OH | 1 | H | 0.73 |
| **11** | I | H | H | 2 | H | 0.89 |
| **12**[c] | I | H | H | 2 | $CH_3$ | 1.5 |
| **13**[c] | I | H | H | 2 | n-$C_3H_7$ | 1.6 |
| **34** | II | $OCH_3$ | H | 2 | H | 4.0 |
| **35** | II | OH | H | 2 | H | 0.24 |
| **36**[c] | II | $OCH_3$ | H | 2 | $CH_3$ | 0.79 |
| **37**[c] | II | OH | H | 2 | $CH_3$ | 0.20 |
| **19** | II | H | H | 3 | H | 1.7 |
| **20**[c] | II | H | H | 3 | $CH_3$ | 1.7 |
| **21**[c] | II | H | H | 3 | n-$C_3H_7$ | 17.0 |
| **38** | II | $OCH_3$ | H | 3 | H | 4.8 |
| **39**[c] | II | $OCH_3$ | H | 3 | $CH_3$ | 2.7 |
| **40**[c] | II | $OCH_3$ | H | 3 | n-$C_3H_7$ | 17.0 |
| **41** | II | OH | H | 3 | $CH_3$ | 0.16 |
| **MK-801** | | | | | | 0.007 |

a Refers to **Scheme** number.
b Isolated as HCl salt unless indicated otherwise.
c Oxalate salt.
d The numbers are the fitted $IC_{50}$ values determined from a logit-log of three concentrations each in triplicate. MK-801 has an average $IC_{50}$ of 5.7+/-0.8 nM. The 95% confidence limits for the $IC_{50}$ values are approximately three fold the stated value. When multiple determinations have been run, the standard error of the mean has been about 20% of the mean.

**TABLE 2. Structure, Synthetic Method and [$^3$H]TCP Binding for Thienospiroisoindolines**




| Compound | Synthetic Method[a] | R | $IC_{50}$ [$^3$H]TCP μM[b] |
|---|---|---|---|
| **42**[c] | II | H | 0.60 |
| **43**[d] | II | $CH_3$ | 0.49 |
| **44**[d] | II | n-$C_3H_7$ | 1.5 |

a Refers to **Scheme** number
b The numbers are the fitted $IC_{50}$ values determined from a logit-log of three concentrations each in triplicate. MK-801 has an average $IC_{50}$ of 5.7+/-0.8 nM. The 95% confidence limits for the $IC_{50}$ values are approximately three fold the stated value. When multiple determinations have been run, the standard error of the mean has been about 20% of the mean
c HCl salt
d Oxalate salt

However, in contrast to other spiroisoindolines from this series (for example, compound **31** as a representative member) and other classical noncompetitive NMDA antagonists (including MK-801) which generalize (at doses of 30 mg/kg, ip and 0.3 mg/kg,[14] ip for **31** and MK-801, respectively) to the cue produced by PCP or MK-801 in the drug discrimination paradigm,[15] compound **43** (30 mg/kg, ip) did not elicit a generalized response in MK-801 trained rats. Additionally, in mice **43** (up to 80 mg/kg, ip) did not produce effects such as body rolling, ataxia and hyperlocomotion which were observed after administration of PCP-like compounds such as MK-801 (0.5-1.0 mg/kg, ip) and **31** (20-40 mg/kg, ip). These results suggest that **43** may have less of the psychostimulant liabilities characteristically associated with noncompetitive NMDA receptor antagonists.

In summary, a series of spiroisoindolines, designed as semirigid analogs of MK-801, were synthesized and screened for their ability to interact at the noncompetitive site of the NMDA receptor ionophore complex. The compounds were found to be noncompetitive NMDA antagonists with varying degrees of potency. Further comparative in vivo testing of compound **43** demonstrated that it was unique in the series. In vivo, compound **43**, like other members of this series, antagonized NMDA-induced convulsions and was neuroprotective in the gerbil ischemia test. However, in contrast to other noncompetitive NMDA antagonists and to other spiroisoindolines described in this paper, **43**, when administered at doses demonstrating antagonism of NMDA -induced convulsions, did not generalize to MK-801 in a drug discrimination paradigm. This data suggests that appropriate structural modifications within a chemical series of NMDA antagonists may be useful in separating the psychotomimetic side effects associated with classical noncompetitive NMDA antagonists from the anticonvulsant and/or antiischemic properties of these compounds.

## REFERENCES


1. (a) Johnson, G. *Annu. Rep. Med. Chem.* **1989**, 24, 41. (b) Honore, T. *Med. Res. Rev.* **1989**, 9, 1.
2. Choi, D. W. *Neuron* **1988**, 1, 623.
3. (a) Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. *Ann. Rev. Pharmacol. Toxicol.* **1989**, 29, 365.
   (b) Wong, E.H.F.; Kemp, J.A.; Priestly, T.; Knight, A.R.; Woodruff, G.N.; Iverson, L.L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, 83, 7104.
4. Gill, R.; Foster, A. C.; Woodruff, G. N. *Neurosci.* **1988**, 25, 847.
5. (a) Mao, C.L.; Barnish, I.T.; Hauser, C.R. *J. Het. Chem.* **1969**, 6, 475
   (b) Bailey, D.M.; DeGrazia, C.G. *J. Org. Chem.* **1970**, 35, 4093.
6. Levin, J. I.; Turos, E.; Weinreb, S. M *Syn. Commun.* **1982**, 12, 989.
7. Vignon, J.; Chicheportiche, R.; Chicheportiche, M.; Damenka, J. -M.; Geneste, P.; Lazdunski, M. *Brain Res.* **1983**, 280,194.
8. (a) Lazdunski, M. In: *Phencyclidine and Related Arylcyclohexylamines: Present and Future Applications*; Kamenka, J. M.; Domino, E. F.; Geneste, P., Eds., NPP Books: Ann Arbor, MI, **1983**, 83.
   (b) Kamenka, J. M.; Chiche, B.; Goudal, R.; Geneste, P.; Vignon, J.; Vincent, J. P ; Lazdunski, M. *J. Med. Chem.* **1982**, 25, 431.
   (c) Thompson, W.J. et al. *J. Med. Chem.* **1990**, 33, 789.
9. Pullan, L.M.; Powel, R.J.;Stumpo, R.J.; Britt, M.; Klicka, A.B., Meiners, B.A.; Salama, A.I. *Eur. J. Pharmacol. Mol. Pharmacol. Sect.* **1990**, 189, 237.
10. Carlos, G.; Marvizon, G.; Vazquez, J; Calvo, M.G.; Mayor, F. Jr.; Gomez, A.R.; Valdivieso, F.; Benavides, J. *Mol. Pharmacol.* **1986**, 30, 590.
11. Pullan, L.M.; Britt, M.; Chapdelaine, M.J.; Keith, R.A.; LaMonte, D.; Mangano, T.; Patel, J.; Powel, R.J.; Stumpo, R.J.; Warwick, P.J.; Zinkand, W.C.; Salama, A.I. *J. Neurochem.* **1990**, 55, 1346.
12. Singh, L.; Oles, R.J.; Tricklebank, M.D. *Brit. J. Pharmacol.* **1990**, 99, 285.
13. Boast, C.; Gerhardt, S.C.; Pastor, G.; Lehmann, J.; Etienne, P.E.; Liebman, J.M. *Brain Res.* **1988**, 442, 345.
14. Tricklebank, M.D.; Singh, L.; Oles, R.J.; Wong, E.H.F.; Iversen, S.D. *European J. Pharmacol.* **1987**, 141, 497.
15. Willetts, J.; Balster, R.L. *Eur. J. Pharmacol.* **1988**, 146, 167.