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EXCITATORY AMINO ACIDS: 6-PHOSPHONOMETHYLTETRAHYDRO-4-PYRIMIDINECARBOXYLIC ACIDS AND THEIR ACYCLIC ANALOGUES ARE COMPETITIVE N-METHYL-D-ASPARTIC ACID RECEPTOR ANTAGONISTS.

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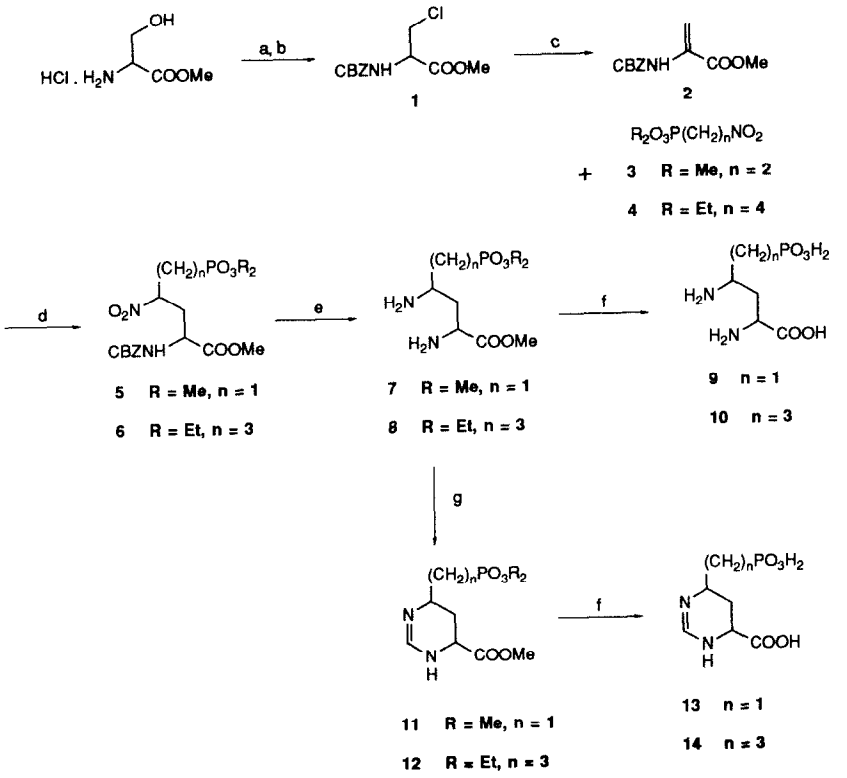
Abstract. 6-Phosphonoalkyltetrahydro-4-pyrimidinecarboxylic acids and their acyclic equivalents were determined to be competitive N-methyl-D-aspartic acid (NMDA) receptor antagonists using a [^{3}H]-CPP binding assay. Results suggest that internal hydrogen bonding interactions may exert either a positive or negative influence on NMDA receptor affinity depending on whether an appropriate receptor active configuration is stabilized.

Changes in homeostasis following a neuronal insult, such as increased glutamate release, can result in excitotoxicity and ultimately in neurodegeneration and cell death.[2] Excitatory amino acid neurotransmitter pathways may be important in the etiology and progression of a number of neurological disease states including epilepsy,[3] cerebral ischemia and stroke,[4] Alzheimer's Disease,[5] Huntington's Disease,[6] and others, including AIDS-induced neurodegeneration.[7] Antagonists of excitatory amino acid pathways are being studied to determine whether neuroprotective effects can be predictably obtained and separated from the potential adverse effects that may result by interference with such critical neuronal systems.

Excitatory amino acid neurotransmitters are known to stimulate a number of subtypes of receptors that are classified as N-methyl-D-aspartate receptors (NMDA) and non-NMDA receptors (kainate receptors, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, quisqualate receptors, (1S,2R)-1-amino-1,2-cyclopentanedicarboxylic acid (ACPD) receptors).[8] The NMDA receptor has been studied intensely for a decade now but until recently details of the molecular biology of the receptor have been lagging. However, cloning of the NMDA receptor[9] and/or a major glutamate-binding subunit of the NMDA receptor complex[10] are now published. Previous information about the NMDA receptor complex was discerned from traditional biochemical and physiological methods. Kinetic analysis of whole cell voltage clamp recordings has suggested a model of the NMDA receptor that requires the stoichiometry of two molecules of glutamate and two molecules of glycine to bind to the NMDA receptor for activation of ion channel gating.[11] The hypothesized 2:2 stoichiometry of glutamate:glycine supports a model of multiple conformations of a single NMDA receptor subtype.[12] It has been suggested that 2-amino-5-phosphonopentanoic acid (AP5)-like antagonists, such as cis-4-(phosphonomethyl)-2-piperidinecarboxylic acid (CGS 19755), and 2-amino-7-phosphonoheptanoic acid (AP7)-like antagonists, such as 4-(3-phosphonopropyl)2-piperazinecarboxylic acid (CPP), may have different binding domains. Different activation states of the enzyme complex defined by the pattern of glutamate/glycine binding in a single class of NMDA receptor may present partially overlapping recognition sites for the phosphonic acid antagonists. Alternatively, there may be two (or more) independent classes of NMDA receptor subtypes.[13-15]

Several potent competitive NMDA antagonists have been studied to date.[16-20] These have been useful for SAR and molecular modeling, as pharmacological tools for understanding the NMDA receptor and some (CGS 19755, CPP-ene, NPC 12626, for example) are being examined in clinical trials. Novel antagonists of the NMDA receptor system are still being designed because anticonvulsant and neuroprotective properties in vitro and in vivo have been demonstrated. Competitive NMDA receptor antagonists that are described in the literature generally have an α-amino acid group and a terminal phosphonic acid functionality. The influence of conformational restrictions on binding kinetics was recently discussed.[21] In order to further characterize the SAR of competitive NMDA antagonists, we have synthesized a novel class of inhibitors with a tetrahydropyrimidine template, and determined their affinity to NMDA receptors using [^{3}H]-CPP binding in rat brain membranes.[22] Acyclic derivatives were also examined to describe intramolecular interactions which can either contribute to receptor binding affinity or destabilize the required configuration.

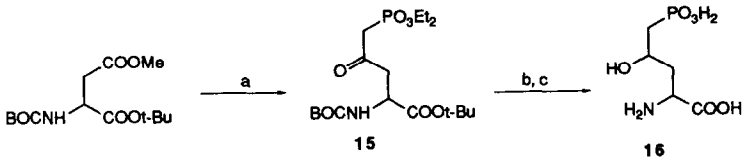


Scheme 1. a. PCl_5 (1.1 eq), AcCl, 0°C to RT, 2.5 h (74%); b. CBZCl (1.2 eq), $NaHCO_3$, CH_2Cl_2/H_2O, 0°C to RT, 1.5 h (97%); c. CH_3ONa, 0°C, 30 min (94%); d. **3** or **4**, KF-Al_2O_3, THF, RT, 24 h (64% for **5**, 37 % for **6**); e. 10% Pd-C, conc. HCl, CH_3OH; Silica Gel Chromatography (EtOAc:HCOOH:H_2O = 4:1:1; 65% for **7**, 55% for **8**); f. 6 N HCl, 110°C, 15 h (75% for **9**, 70% for **10**, 72% for **13**, 75% for **14**); g. $AcOCH(OCH_3)_2$, DMF, RT, 24 h (71% for **11**, 64% for **12**).

The syntheses of the 6-phosphonoalkyl-4-carboxytetrahydropyrimidines **11** and **12**, as well as the diamino derivatives **9** and **10**, are described in Scheme 1. Conversion of serine methyl ester into the α,β-unsaturated amino acid derivative **2** required three steps: replacement of the hydroxyl group with chlorine, protection of the amino group with benzyl chloroformate to give N-benzyloxycarbonyl chloroalanine methyl ester **1**, and dehydrohalogenation of the chlorine atom with sodium methoxide treatment to afford **2**. Compound **2** was then coupled with the nitrophosphonate derivatives **3**[23] and **4**[24] via a Michael reaction to give the γ-nitro amino acid derivatives **5** and **6**, respectively. The most suitable catalyst for this reaction proved to be KF-Al_2O_3,[25] which gave only desired monoalkylation. Hydrogenation of the nitro intermediates **5** and **6** gave the diamino esters **7** and **8**, which were hydrolyzed in refluxing 6 N HCl to provide **9** and **10**.

Cyclization of the diamino functionalities of **7** and **8** provided the tetrahydropyrimidine rings of **11** and **12**. Initial efforts to cyclize the tetrahydropyrimidine rings with either trimethylorthoformate or (dimethoxy)methylene acetate employing the crude hydrogenation products gave only traces of the desired material. When purified **9** and **10** (silica gel chromatography with EtOAc:HCOOH:H_2O = 4:1:1 as the eluant) were treated with (dimethoxy)methylene acetate in DMF, the cyclization went cleanly to afford **11** and **12**. Hydrolysis in refluxing 6 N HCl gave the desired acids **13** and **14**.

The 4-hydroxy AP5 analog **16** was synthesized from N-BOC γ-methyl tert-Butyl aspartic ester. Displacement of the methyl ester of **16** with lithium diethyl methylphosphonate in THF at -78°C gave the 4-oxo-5-phosphononorvaline ester **15**.[26] Reduction of the ketone followed by hydrolysis gave compound **16** (Scheme 2).



Scheme 2. a. $(EtO)_2POCH_2Li$ (2.4 eq), THF, -78°C, 30 min (42%); b. $NaBH_4$ (1.2 eq), CH_3OH, 0°C, 30 min (91%); c. 6 N HCl, 110°C, 15 h (78%).

Compounds in this study were designed to examine structural features which may affect affinity at the NMDA receptor. The 6-phosphonoalkyl-4-carboxytetrahydropyrimidines are structurally related to the reference agents CPP and CGS 19755 in that they have the carboxylic acid group constrained within a 6-membered ring, and a terminal phosphonic acid group. However, in this series the α-amino group is connected electronically to the other ring nitrogen (N-1), which may affect the pKa of the α-amino group (N-3) and bonding interactions at the receptor binding site. Whether these properties would enhance or diminish receptor affinity could not be predicted a priori.

The tetrahydropyrimidine derivatives **13** and **14** have chiral centers at C-4 and C-6, and the diastereomers (7:3 mixture by NMR) were not separable by silica gel chromatography.[27] Based on the reference agents CPP and CGS 19755, we expected that the phosphonomethyl derivative **13** would have similar affinity to that of the phosphonopropyl derivative **14**. However, as can be seen in Figure 1, **13** (IC_{50} = 5.95 μM) is significantly less potent than **14** (IC_{50} = 0.42 μM). In a previous study with the phosphonomethylpiperazine derivative related to CPP, we saw a similar decrease in affinity.[28] One explanation for the decreased affinity in the case of **13** is that an internal hydrogen bond interaction between a hydroxyl of

the phosphonate group and N-1 of the pyrimidine can form a six member ring that stabilizes a receptor inactive configuration. This type of interaction apparently is minimized when the side chain separating the phosphonate and the ring system is lengthened to three carbons, and the difference in potency between **14** and **13** reflects the strength of a hydrogen bond interaction. The amidine functionality in the tetrahydropyrimidine ring system is slightly deleterious to receptor binding affinity relative to the piperazine ring system, due either to a change in basicity associated with electron withdrawing resonance of the conjugated imine,[29] or perhaps to a change in ring conformation induced by the internal double bond. Although the reasons for the reduction in potency relative to CPP are unclear, **14** is still a potent NMDA antagonist and is quite active against glutamate-induced calcium influx in cultured neuronal cells[30] (data not shown).

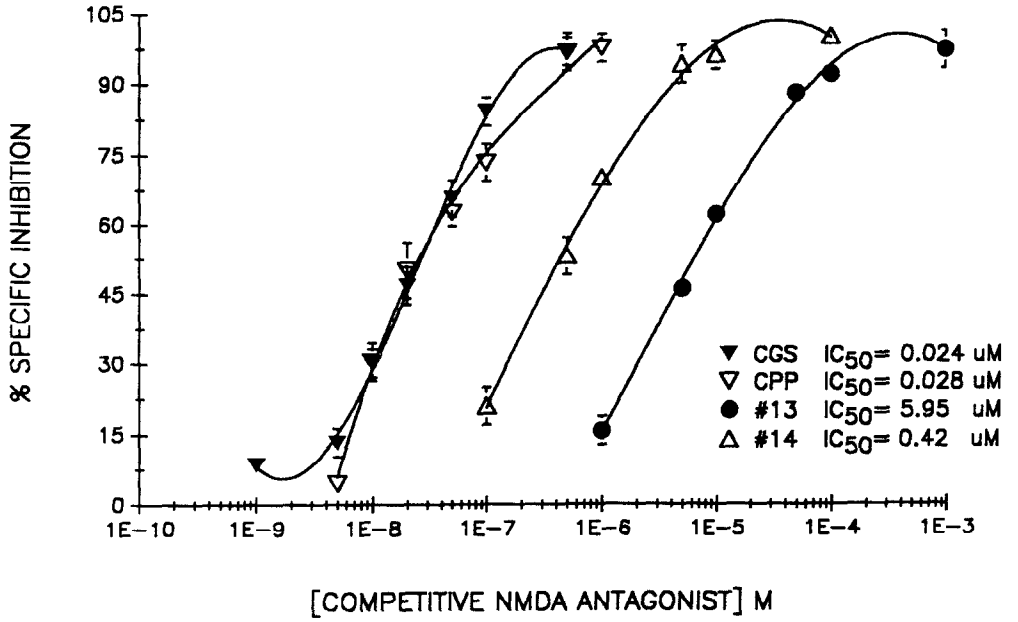


Figure 1. Displacement of [^{3}H]-CPP binding by CPP, CGS 19755 and the tetrahydropyrimidines (**13** and **14**). IC_{50} values represent determinations using means of four to fourteen individual data points taken from three to five independent experiments. Binding to NMDA receptors in rat brain membranes were carried out using 10 nM [^{3}H]-CPP as the radioligand and 100 μM glutamate to define non-specific binding according to previously described methods.[22]

Those compounds which provide a rigid template for the key interaction of the α-amino acid and the terminal phosphonic acid group have been shown to provide an entropic advantage over those without constraints. This is typified by the potent reference agents, CGS 19755, which is constrained in a piperidine ring system,[16] and (E)-2-amino-4-methyl-5-phosphono-3-pentenoate, which is constrained by its double bond.[18] (R)-4-Oxo-5-phosphononorvaline (MDL 100453, K_i = 0.11 μM) was recently shown to have improved affinity relative to its parent, AP5 (K_i = 0.17 μM).[26] This was explained by suggesting that enolization of the ketone provides an olefinic functionality to enhance NMDA receptor affinity in analogues of AP5 and CPP.[18,19] Data shown in Figure 2 suggest that compounds that are able to form an internal hydrogen bond

coupling the α-amino group to C-4 can provide a substantial enhancement in NMDA receptor affinity. This concept suggests that a hydroxyl functionality at C-4 may form an internal hydrogen bond with the α-amino group to give a stabilized 6-membered ring system in a receptor-preferred configuration. The 4-hydroxy AP5 derivative was prepared to examine whether this is a feasible explanation since it no longer contains the enolic bond that would provide structural rigidity. Binding results in membranes (IC_{50} = 0.13 μM) demonstrate that **16** has affinity comparable to the keto analogue, MDL 100453. In contrast, although the diamino derivatives **9** and **10** potentially have the ability to form similar hydrogen bonding interactions, they are more likely to exist as diammonium species that prefer a configuration with the diamines extended away from one another because of charge interactions. The weak affinity of **9** (IC_{50} = 1.44) and **10** (IC_{50} = 2.27) support this explanation. A previous result from our laboratory with the sulfinyl AP5 derivative **17** (IC_{50} = 0.24 μM) provides additional support for this hypothesis.[31] The sulfoxide oxygen may form a stable 6-membered ring hydrogen bond interaction with the α-amino group, thus stabilizing a configuration similar to that seen in cyclic analogs. Both **16** and **17** are significantly more active than the acyclic diamines **9** and **10**, have approximately the same activity as the pyrimidine derivative **14**, and have configurations that may be stabilized by an internal hydrogen bond. Our results also suggest that differences in binding between the AP5- and AP7-like antagonists are more likely due to properties of the analogues rather than having to invoke multiple NMDA receptors.[32]

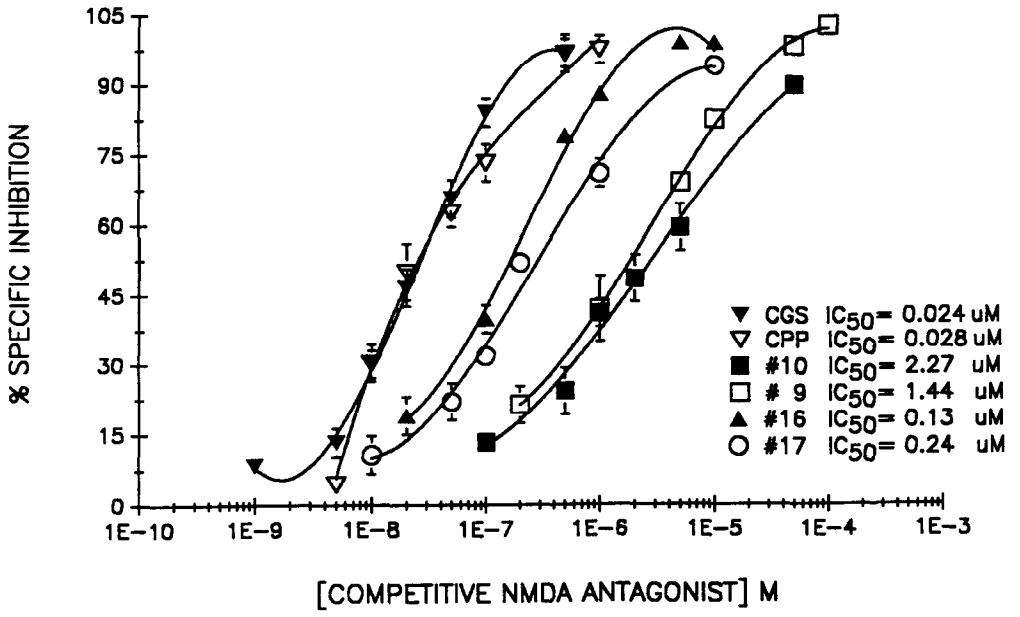


Figure 2. Displacement of [^{3}H]-CPP binding by CPP, CGS 19755, and the acyclic derivatives (**9**, **10**, **16** and **17**). IC_{50} values represent determinations using means of four to fourteen individual data points taken from three to five independent experiments. Each data point was acquired in the presence of 10 nM [^{3}H]-CPP. Nonspecific binding was determined in the presence of 100 μM glutamate.

References and Notes

1. J. T. Drummond, Department of Biochemistry, University of Michigan, Ann Arbor, MI.
2. For recent reviews see: Johnson, G.; Bigge, C. F. Ann. Rep. Med. Chem.,; Bristol, J., Ed.; Academic Press: New York, NY, **1991**, pp. 11-22; Olney, J. W. Ann. Rev. Pharmacol. Toxicol. **1990**, 30, 47-71.
3. Dingledine, R.; McBain, C. J.; McNamara, J. O. Trends in Pharmacol. Sci. **1990**, 11, 334.
4. Scatton, B.; Frost, J.; George, P.; Carter, C.; Benavides, J. Current Patents, **1991**, 523-45; McCulloch, J. Arzneim.-Forsch. Drug Res. **1991**, 41 (I), 319-24.
5. Collingridge, G. L.; Sanger, W. Trends in Pharmacol. Sci. **1990**, 11, 290.
6. Young, A. B.; Greenamyre, J. T.; Hollingsworth, Z.; Albin, R.; D'Amato, C.; Shoulson, I.; Penny, J. B. Science **1990**, 241, 981.
7. Jansen, K. L. R.; Faull, R. L. M.; Dragunow, M.; Synek, B. L. Neuroscience **1990**, 39, 613-628.
8. Monaghan, D. T.; Bridges, R. C.; Cotman, C. W. Ann. Rev. Pharmacol. Toxicol. **1989**, 29, 365.
9. Moriyoshi, K.; Masu, M.; Ishii, T.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. Nature **1991**, 354, 31-37.
10. Kumar, K. N.; Tilakaratne, N.; Johnson, P. S.; Allen, A. E.; Michaelis, E. K. Nature **1991**, 354, 70-73.
11. Benveniste, M.; Mayer, M. L. Biophys. J. **1991**, 59, 560-73.
12. Mayer, M.L.; Vyklicky, L.; Sernagorn, E. Drug Dev. Res. **1989**, 17, 263-80.
13. Yoneda, Y.; Ogita, K. Neurosci. Res. **1991**, 10 (1), 1-33.
14. Wood, P. L.; Rao, T. S.; Iyengar, S.; Lanthorn, T.; Monahan, J.; Cordi, A.; Sun, E.; Vazquez, M ; Gray, N.; Contreras, P. Neurochem. Res. **1990**, 15, 217-30.
15. Reynolds, I. J.; Miller, R. J. Mol. Pharmacol. **1988**, 33, 581-4.
16. Hutchison, A. J.; Williams, M.; Angst, C.; et al. J. Med. Chem. **1989**, 32, 2171-8.
17. Ornstein, P. L.; Schaus, J. M.; Chambers, J. W.; et al. J. Med. Chem. **1989**, 32, 827-33.
18. a) Fagg, G. E.; Pozza, M. F.; Olpe, H.-R.; et al. Br. J. Pharmacol. **1989**, 97, 582P; b) Fagg, G. E.; Olpe, H.-R.; Pozza, M. F.; et al. Br. J. Pharmacol. **1990**, 99, 791-7.
19. Aebischer, B.; Frey, P.; Haerter, H.-P.; Herrling, P. L.; Mueller, W.; Olverman, W. J.; Watkins, J. C. Helv. Chim. ACTA **1989**, 72, 1043-51.
20. Ferkany, J. W.; Kyle, D. J.; Willets, J.; Rzeszotarski, W. J.; Guzewska, M. E.; Ellenberger, S. R.; Jones, S. M.; Sacaan, A. I.; Snell, L. D.; et al. J. Pharmacol. Exp. Ther. **1989**, 250 (1), 100-9.
21. Benveniste, M.; Mayer, M. L. Br. J. Pharmacol. **1991**, 104, 207-21.
22. Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, M. J. Pharmacol. Exper. Ther. **1987**, 240, 778-84.
23. Dimethyl 2-nitroethylphosphonate (3) was prepared by reaction of nitroethylene with trimethylphosphite in the presence of acetic acid. Acetic acid was found to suppress the polymerization which was a problem in this reaction.
24. Diethyl 4-nitrobutylphosphonate (4) was prepared from 4-bromobutylphosphonate by reaction with NaI, followed by $AgNO_2$.
25. Bergbreiter, D. E.; Lanonde, J. J. J. Org. Chem. **1987**, 52, 1601-03.
26. Whitten, J. P.; Baron, B. M.; Muench, D.; Miller, F.; White, H. S.; McDonald, I. A. J. Med. Chem. **1990**, 33, 2961-63.
27. The diastereomers of **13** and **14** have not been separated and so the enantiomers were not studied independently. It may be that differences in affinity are related to the cis/trans relationship of the carboxylate and alkylphosphonate side chain.
28. Hays, S. J.; Bigge, C. F.; Novak, P. M.; Drummond, J. T.; Johnson, G.; Bobovski, T. P.; Brahce, L. J.; Coughenour, L. L. J. Med. Chem. **1990**, 33, 2916-24.
29. Amidines are generally more basic than amines. The basicity of N-3 could be increased or decreased relative to N-1 of CGS 19755, depending on which N provides the best stabilization of the charge in the pyrimidine ring.
30. Marcoux, F. W.; Goodrich, J. E.; Probert, A. W., Jr.; Dominick, M. A. Sigma and Phencyclidine-like Compounds as Molecular Probes in Biology, Domino, E. F., Kamenka, J.-M., Eds.; NPP Books: Ann Arbor, MI, 1988; pp 735-46.
31. Wedler, F. C.; Farrington, G. K.; Kumar, A.; Bigge, C. F.; Ortwine, D. F.; Johnson, G. Life Sci. Adv. Neurochem. **1991**, in press.
32. This is also supported by our recent NMDA receptor modeling. Ortwine, D. F.; Bigge, C. F.; Humblet, C.; Johnson, G.; Malone, T. QSAR: Rational Approaches to the Design of Bioactive Compounds; Silipo, C. and Vittoria, A; Eds.; Elsevier, **1991**, v. 16, 485-88.