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Aminoanthraquinones as Novel Ligands at the Polyamine Binding Site on the *N*-Methyl-D-aspartate Receptor Complex

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Abstract—As part of a drug discovery program using high-throughput radioligand-binding assays, aminoanthraquinones were identified as potential modulators of *N*-methyl-D-aspartate (NMDA) receptor function. Aminoanthraquinones may represent a novel class of polyamine binding site ligands with a unique pharmacophore and may facilitate the rational design of novel NMDA-receptor modulators. © 2000 Elsevier Science Ltd. All rights reserved.

The *N*-methyl-D-aspartate (NMDA) receptors are ligand-gated ion channels endogenously activated by glutamate. Activation of NMDA receptors opens ion channels, allowing Ca^{2+}, Na^{+}, and K^{+} influx, which results in cell depolarization. Activation of the NMDA receptors may lead to hyperexcitability. The hyperexcitability associated with NMDA receptors has been linked to a number of disease states, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and depression. In addition, this hyperexcitability appears to play a major role in cell death and neurodegeneration associated with hypoxia-reperfusion injury, head trauma, epilepsy, and alcoholism.[1–9] In light of these findings, the development of agents that attenuate NMDA-receptor channel opening is an important research challenge.

The NMDA-receptor complex is regulated by a number of exogenous and endogenous ligands. Separate regulatory sites have been identified for the agonist glutamic acid, the co-agonist glycine, the allosteric modulator Zn^{2+}, agents that bind within the channel, such as Mg^{2+}, phencyclidine, ketamine and dizocilpine (MK-801), redox agents that regulate channel opening and multiple sites for polyamine (spermidine [SPD] and spermine [SPM]) binding. A number of these modulatory sites on the NMDA-receptor complex may offer targets for novel drug design. The therapeutic potential of antagonists that directly block channel opening, or that bind within the channel, is limited due to their low affinity, abuse potential, and neurotoxicity.[10] In contrast, the polyamine recognition sites are modulatory in nature. Antagonists at these sites attenuate channel opening with minimal toxicity and little abuse potential. As a result, polyamine binding site antagonists may hold the most promise for the discovery and development of NMDA-receptor modulators.

The endogenous amines, SPD and SPM, modulate the NMDA-receptor with a complex, bi-phasic action. For example, low concentrations of SPD and SPM (3–100 μM) enhance the binding of use-dependent channel blockers such as [^{3}H]-MK-801, while at higher concentrations (>100 μM), these polyamines inhibit [^{3}H]-MK-801 binding.[11–14] At low concentrations of polyamines, glutamate-induced neurotoxicity is attenuated, but at higher concentrations the effects of glutamate are enhanced.[15] These data suggest that polyamines modulate the NMDA-receptor through at least two distinct mechanisms.

There are at least three independent polyamine-binding sites on the NMDA-receptor that might offer potential targets for the development of selective pharmacologic agents.[16] Existing polyamine binding domain antagonists, such as ifenprodil, are not highly selective and exhibit relatively low receptor affinities.[17] The identification of new agents that are selective for specific polyamine sites on the NMDA-receptor complex might provide the basis for the development of novel therapeutic drugs. Such agents would be invaluable as

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receptor probes for elucidating the role of these sites in normal and pathological neurological states. This study was conducted in order to evaluate the binding displacement properties of a number of amino-9,10-anthraquinones. These compounds were selected in order to determine the effect of atomic spacing and relative amino juxtaposition on [^{3}H]-SPD and [^{3}H]-MK-801 binding. The [^{3}H]-SPD and [^{3}H]-MK-801 displacement by amino-9,10-anthraquinones reported here suggests that these agents may have the potential to modulate NMDA activity by interacting at the polyamine binding site.

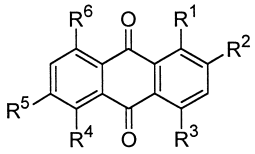
The high-throughput, 96-well microtiter plate assay for [^{3}H]-SPD binding is based upon a procedure described previously by Mantione and co-workers.[18] Measurement of [^{3}H]-MK-801 displacement, a measure of inhibition of channel opening, is based upon the [^{3}H]-MK-801 binding assay described by Yoneda and co-workers.[19] All of the aminoanthraquinones analyzed in this study were obtained from commercial sources. Serial concentrations of each compound were analyzed in triplicate for their capacity to displace [^{3}H]-SPD and [^{3}H]-MK-801. Where appropriate, quench corrections were made using a standard nitromethane curve. As a result of the screening of over 200 aromatic and aliphatic amino compounds, we identified aminoanthraquinones as having [^{3}H]-SPD and [^{3}H]-MK-801 displacing properties in rat brain homogenate. The [^{3}H]-SPD binding data represent displacement of [^{3}H]-SPD binding at all polyamine binding sites in the homogenate, and not just those on the NMDA-receptor complex. The [^{3}H]-MK-801 assay does not differentiate between direct and allosteric [^{3}H]-MK-801 displacement. The [^{3}H]-SPD and [^{3}H]-MK-801 binding data taken together, however, suggest that these compounds are most likely interacting with the polyamine binding site on the NMDA-receptor complex. The structure–activity data are summarized in Table 1.

It is evident that only one amino moiety, in either the 1 or 2 position, is required for activity. This is a somewhat surprising result, since the endogenous ligands, SPD and SPM, each contain multiple amino moieties. 2-Aminoanthraquinone (**1**) was the most potent compound analyzed in this study, with an IC_{50} of 116±15 μM in the [^{3}H]-SPD displacement assay. Ifenprodil, a prototypical polyamine site antagonist, had an IC_{50} of approximately 500 μM in the same [^{3}H]-SPD displacement assay (data not shown). These data suggest that 2-aminoanthraquinone may be altering NMDA channel opening, since it also inhibited [^{3}H]-MK-801 binding (IC_{50}: 119±30 μM). While not as potent as 2-aminoanthraquinone, 1-aminoanthraquinone (**4**) was also effective at displacing both [^{3}H]-SPD and [^{3}H]-MK-801 binding (IC_{50}: 218±44 μM and 140±46 μM, respectively).

The relative juxtaposition of electron-donating substituents was found to be an important determinant of ligand displacement activity. When an amino moiety occupies the 1 or 2 position, electron-donating substituents (i.e., amino or hydroxyl) at the 4 and 5 positions of the aminoanthraquinone molecule have no effect on either [^{3}H]-SPD or [^{3}H]-MK-801 binding. Compounds **2**, **3**, **5**, and **6** all retained the capacity to displace [^{3}H]-SPD and inhibit [^{3}H]-MK-801 binding. In contrast, substitution with an amino or a hydroxyl at positions 6 or 8 (**8**, **9**, and **10**) significantly curtailed the interaction with these binding sites; their capacity to displace [^{3}H]-SPD and [^{3}H]-MK-801 was reduced by as much as 10-fold. The capacity of certain aminoanthraquinones to displace [^{3}H]-SPD and significantly alter [^{3}H]-MK-801 binding, suggests that this class of compounds are acting as ligands at the NMDA polyamine binding site.

Since concurrent in vivo assessment of the neurological impairment induced by active compounds is a vital component of drug discovery, an initial evaluation of the CNS activity of these compounds was conducted in mice ($n = 24$ per compound) using the rotorod test, positional sense test, and gait and stance test.[20]

Table 1. Aminoanthraquinones evaluated for their ability to displace [^{3}H]-SPD or [^{3}H]-MK-801 from their respective NMDA binding sites. Data for each compound represent the mean IC_{50} (μM) extrapolated from plots of percent displacement as a function of concentration



Compound	R^1	R^2	R^3	R^4	R^5	R^6	SPD Displacement IC_{50} (μM)±SEM	MK-801 Inhibition IC_{50} (μM)±SEM
1	H	NH_2	H	H	H	H	116±15	119±30
2	NH_2	H	OH	H	H	H	179±30	158±58
3	NH_2	H	NH_2	NH_2	H	H	184±22	232±64
4	NH_2	H	H	H	H	H	218±44	140±46
5	NH_2	H	H	NH_2	H	H	338±58	323±91
6	NH_2	H	NH_2	H	H	H	412±38	567±72
7	NH_2	NH_2	H	H	H	H	602±139	414±106
8	NH_2	H	NH_2	NH_2	H	NH_2	>1000	>1000
9	H	NH_2	H	H	NH_2	H	>1000	>1000
10	NH_2	H	OH	OH	H	NH_2	>1000	>1000
11	H	H	H	H	H	H	>1000	>1000

Compounds **2**–**7**, which were among the most active anthraquinones tested, did not induce neurological impairment at any dose tested, as measured by the rotorod test in mice after intraperitoneal administration. Similarly, intraperitoneal administration of compounds **2**–**7** did not result in any behavioral impairment as measured by the positional sense test and the gait and stance test. Compounds **1**, **8**, and **10** exerted minimal CNS impairment in mice; notably, this activity was observed only after administration of relatively high doses of these compounds (300 mg/kg).

The discovery that this class of compounds may modulate NMDA-receptor activity has potential value in understanding the role of polyamine activation of the NMDA-receptor. Additionally, this discovery will facilitate the rational design of therapeutically useful NMDA-receptor modulators. Furthermore, initial preclinical studies suggest that these compounds may have low acute neurotoxicity. Ongoing studies are currently focusing on the design and development of agents of this structural class that may have therapeutic potential for the treatment of a number of NMDA-mediated pathologies.

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