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Review Article

Multifunctional neuroprotective drugs for the treatment of cognitive and movement impairment disorders, including Alzheimer's and Parkinson's diseases

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Abstract

The concept of targeting multiple disease etiologies that lead to cognitive and movement impairment in neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease and stroke, is challenging the widely held assumption that "silver bullet" agents are superior to "dirty drugs" in the pharmacological treatment of these diseases. Accumulating evidence in the literature suggests that a drug with two or more mechanisms of action targeted at multiple etiologies of the same disease may offer greater therapeutic benefit in certain disorders than a drug that targets only one disease etiology. In addition, the use of a single such multifunctional drug possessing several pharmacologically active moieties may yield a more favorable side effect profile than a combination of several drugs that individually target the same disease etiologies. In this review, we offer a synopsis of therapeutic strategies and novel investigative drugs developed in our own and other laboratories that modulate multiple disease targets associated with cognitive and movement impairment disorders, including cognitive decline associated with Alzheimer's disease, Parkinson's disease with dementia and movement deficits in Parkinson's disease.

Introduction

Current therapeutic approaches for the treatment of cognitive deficits and movement disorders offer only limited and transient symptomatic benefit to patients. More importantly, these strategies do not attenuate neurodegeneration, the etiological process underlying many forms of cognitive loss, including Alzheimer's disease (AD) and stroke, and extrapyramidal symptoms. Recently, many clinicians and basic scientists have become convinced that targeting multiple etiologies of a disease simultaneously can be more beneficial than the currently accepted "silver bullet" approach to the treatment of a range of diseases, including cognitive deficit disorders (1-3).

In some cases already used in the clinic, multiple targeting is achieved simply by combining several drugs that independently act on different etiological targets of a disease or disease-causing organism. For example, such an approach is commonly used in AIDS therapy, where two HIV reverse transcriptase inhibitors and an HIV protease inhibitor are co-administered as a cocktail (reviewed in 4). Another example is the FDA's recent approval of the combination – in one preparation – of fluticasone, a corticosteroid, and salmeterol, a bronchodilator, to simultaneously treat the underlying inflammation and bronchoconstriction associated with asthma. However, the pharmaceutical combination of several drug molecules raises many challenges, not the least of which is the associated complexities of combining drug entities that have potentially differing bioavailability, pharmacokinetics and metabolism (5-7). Even worse, combinations may result in increased toxicity and side effects, and it is likely that unforeseen drug interactions may occur. In elderly patients – the population most at risk for AD (3) – such a burden may be life-threatening (8). It is therefore not surprising that research has focused increasingly on the

advantages associated with the design of single drug molecules that act on two or more specific etiological targets of a particular disease. In the short time since this approach has gained acceptance, such molecules have become known as dual-mechanism, bifunctional, multifunctional, multimechanistic, multimodal or hybrid drugs (1, 2, 9-11). Such multimechanistic drugs may be low-affinity ligands that modulate a variety of targets. In their "network approach," Csermely *et al.* propose studying the effects of drugs in the context of a network of relevant protein-protein interactions in order to improve drug design efforts (12).

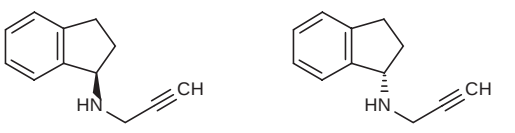
One disorder in which multimechanistic drugs may have application is AD. Generally considered to be a neurodegenerative disease, AD is associated with progressive loss of memory and cognition. It is the most common form of dementia, occurring in 60-70% of individuals over the age of 65 years (13). This loss of cognition is found to be more severe, both quantitatively and qualitatively, in dementia patients than in patients representative of a population undergoing a normal aging process (13). Due to their complex etiological profile, we consider AD and AD- and stroke-associated cognitive decline as disorders that will benefit from the development of multimechanistic drugs.

Very recently, Zlokovic (14) proposed a neurovascular hypothesis to explain, in part, the etiology of AD. This hypothesis suggests that faulty clearance of β-amyloid (Aβ) peptide (a definitive diagnostic marker in post-mortem AD brain) across the blood-brain barrier, combined with compromised angiogenesis and cerebrovascular senescence, could initiate a cascade of events that ultimately may lead to compromise of the blood-brain barrier, ischemia and neuronal dysfunction, injury and death. Such a hypothesis suggests that, in addition to the historically accepted etiologies of AD, Aβ clearance pathways also offer a multitude of therapeutic targets that may be seen as opportunities to apply multimechanistic drugs. These targets include (among others not specifically identified) induction of Aβ-degrading enzymes, activation of astrocytes, enhancement of the direct binding of Aβ to low-density lipoprotein receptor-related protein (LRP), lipoprotein receptor ligands or Aβ chaperones, and enhancement of LRP activity at the blood-brain barrier (14). Furthermore, the blocking of Aβ interaction with the receptor for advanced glycation end products (RAGE) at the blood-brain barrier may isolate the brain from the deleterious effects of the systemic pool of Aβ, which have been shown to be associated with neurovascular stress (14, 15). Agents that exhibit a dual mechanism, on the one hand through direct cytoprotection of stressed and ischemic brain endothelial cells and neurons, and on the other through systemic antiinflammatory and proangiogenic activity, could also protect the neurovasculature from the combined Aβ, hypoxic and excitotoxic injury seen in AD (14).

In this review, we will summarize new approaches whereby molecules have been either discovered or developed and synthesized expressly to target multiple disease etiologies in the treatment of AD and cognitive impairment. We will consider a few examples from the literature to illustrate our contention that drugs with multiple mechanisms of action may, in selected disease states, be better therapeutic agents than single-mechanism drugs. Some of the targeting approaches that will be discussed include, among others, the development of single molecular entities that combine activity as acetylcholinesterase (AChE) or cholinesterase (ChE) inhibitors, muscarinic cholinergic M_2 receptor antagonists, α_2-adrenoceptor agonists, monoamine oxidase (MAO) inhibitors and adenosine A_{2A} receptor antagonists; compounds with combined antioxidant and iron-chelating activity; NMDA receptor antagonists with anti-TNF-α effects and L-type calcium channel-blocking activity; nonsteroidal antiinflammatory drugs (NSAIDs) that stimulate the secretion of the nonamyloidogenic α-secretase form of soluble amyloid precursor protein (sAPPα); and glutamate carboxypeptidase II inhibitors that possess metabotropic glutamate $mGluR_3$-agonist activity. In this review, we have attempted to group therapeutically multifunctional compounds into two classes, *i.e.*, existing drugs that have been discovered or identified to possess two or more co-targeted therapeutic mechanisms of action, and drugs that have been specifically designed as such. We acknowledge that some overlap will exist within such a classification and have attempted to address this in the text.

Known drugs possessing two or more co-targeted therapeutic mechanisms of action

Rasagiline (**1**) is an antiparkinsonian drug with selective MAO-B-inhibitory activity (15). The (*S*)-isomer of this drug, TVP-1022 (**2**), demonstrates a MAO-inhibitory potency over 1,000 fold lower than that shown by rasagiline while retaining neuroprotective activity, indicating that the propargylamine moiety is responsible for the neuroprotective activity seen in both these compounds (16-19). The selectivity of rasagiline over TVP-1022 for MAO-B inhibition was demonstrated to be associated with the ability of rasagiline to enter the active-site gorge of MAO-B, while the entry of the (*S*)-isomer into this site is highly restricted. The neuroprotective activity of these compounds has been shown to be associated with the ability of propargylamine (18, 19) to protect mitochondrial viability via activation of Bcl-2 and protein kinase C (PKC) α and ε, and by downregulation of proapoptotic Fas, Bax, PKCδ and PKCγ (15). Additionally, these drugs induce the release of the soluble neuroprotective/neurotrophic form of amyloid precursor protein α (sAPPα) through a



Rasagiline (**1**) TVP-1022 (**2**)

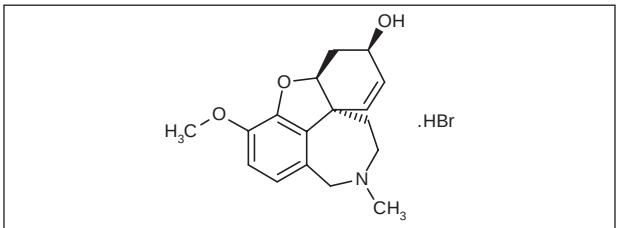
PKC/mitogen-activated protein (MAP)-mediated activation of α-secretase (10).

The nootropic or cognition-enhancing compound galantamine hydrobromide (Razadyne®; formerly Reminyl®; **3**) is a reversible, competitive AChE inhibitor approved in the U.S. in 2001 for the treatment of AD (20). In the context of cognitive disorders, galantamine (21) is a well-studied drug that was found to possess two mechanisms of action that may be useful in attenuating the symptoms of cognitive decline in AD: it selectively and competitively inhibits AChE (22), while simultaneously producing an allosteric modulation of nicotinic acetylcholine receptors (nAChR) (23). The primary activity of galantamine, therefore, is to increase the amount of acetylcholine in the cholinergic system (AD patients show reduced levels), thereby improving cognition (24, 25).

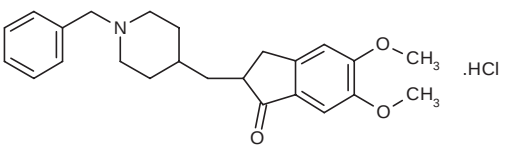
Other studies have suggested that galantamine may also have other mechanisms whereby it exerts a beneficial effect in AD patients. Akk and Steinbach (20) indicated that galantamine directly interacts (as a low-affinity agonist) with the nAChR at a binding site distinct (allosteric) from the site where nicotinic agonists such as acetylcholine, carbachol and choline bind. A downregulation of the glutamatergic NMDA receptors has also been observed in AD patients, and Moriguchi *et al.* (26) have shown that galantamine potentiates the activity of NMDA receptors. This dual mechanism of action on the cholinergic and glutamatergic systems in AD patients may be partly responsible for the improvement in cognition, memory and learning seen in patients treated with the drug. These observations provided the rationale for a phase II clinical trial of galantamine, where it was found that the drug significantly improved the core symptoms of AD as compared to placebo (27).

Donepezil hydrochloride (Aricept®; **4**) is another AChE inhibitor (33 nM) (28) that is used in the clinical management of AD. In addition to its AChE-inhibitory activity, donepezil is also a moderate NMDA receptor antagonist, binding to the dizocilpine (MK-801) binding site with an IC_{50} value of 135 ± 15.1 μM in synaptic membranes of rat cerebral cortex (29). It should be pointed out, however, that other studies reported contradictory findings that suggest that donepezil potentiates NMDA currents (24). Donepezil has also been shown to act as an antagonist at the muscarinic M_1 receptor (28).

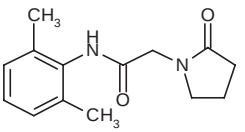
The nootropic compound nefiracetam (DM-9384; **5**) has been shown to have two mechanisms of action, both of which are thought to be beneficial clinically in AD patients (24). The drug was shown to potentiate both NMDA receptors and nAChR at nanomolar concentrations (24, 30-33). Nefiracetam potentiated α2β2-like acetylcholine currents in rat cortical neurons in primary culture, and it was seen to interact with the glycine binding site on the NMDA receptor, potentiating NMDA receptor currents. Modulation of the NMDA receptor by nefiracetam may have beneficial effects on cognition in AD patients. The NMDA receptor plays an important role in learning and memory, but it may also provoke neuronal cell death when overstimulated (excitotoxicity). This excessive stimulation allows Ca^{2+} to enter neuronal cells and triggers a series of events that lead to cell death (34). Blocking the NMDA receptor completely also leads to a series of side effects such as hallucinations and memory and cognitive deficits, which suggests that a modulator of the NMDA receptor may be more useful clinically and better tolerated (24, 34). Nefiracetam at nanomolar concentrations also potentiates α4β2-type neuronal nAChRs via G-coupled proteins, which may also contribute to its cognition-enhancing effects (35).



Galantamine hydrobromide (**3**)



Donepezil hydrochloride (**4**)



Nefiracetam (**5**)

Drugs specifically designed to possess two or more co-targeted therapeutic mechanisms of action

Choline/acetylcholinesterase inhibitors with additional co-targeted mechanisms

One of the hallmarks of AD is a deficiency in cholinergic neurotransmission. Treatment regimens have therefore been aimed at improving cholinergic function by inhibiting AChE, thereby increasing the amount of acetylcholine at the synapse (36). AD patients often also suffer from anxiety and depression, which are traditionally treated with serotonin transporter (SERT) inhibitors or selective serotonin reuptake inhibitors (SSRIs**)**. In contrast to other antidepressant drugs, SSRIs possess no or very limited anticholinergic action and therefore do not add to the likelihood of cholinergic-related side effects occurring when prescribed together with AChE inhibitors (36). Recently, Toda *et al.* (36) designed a series of dual-mechanism drugs (Fig. 1, Table I) with a view to developing improved pharmacological therapies for AD. These

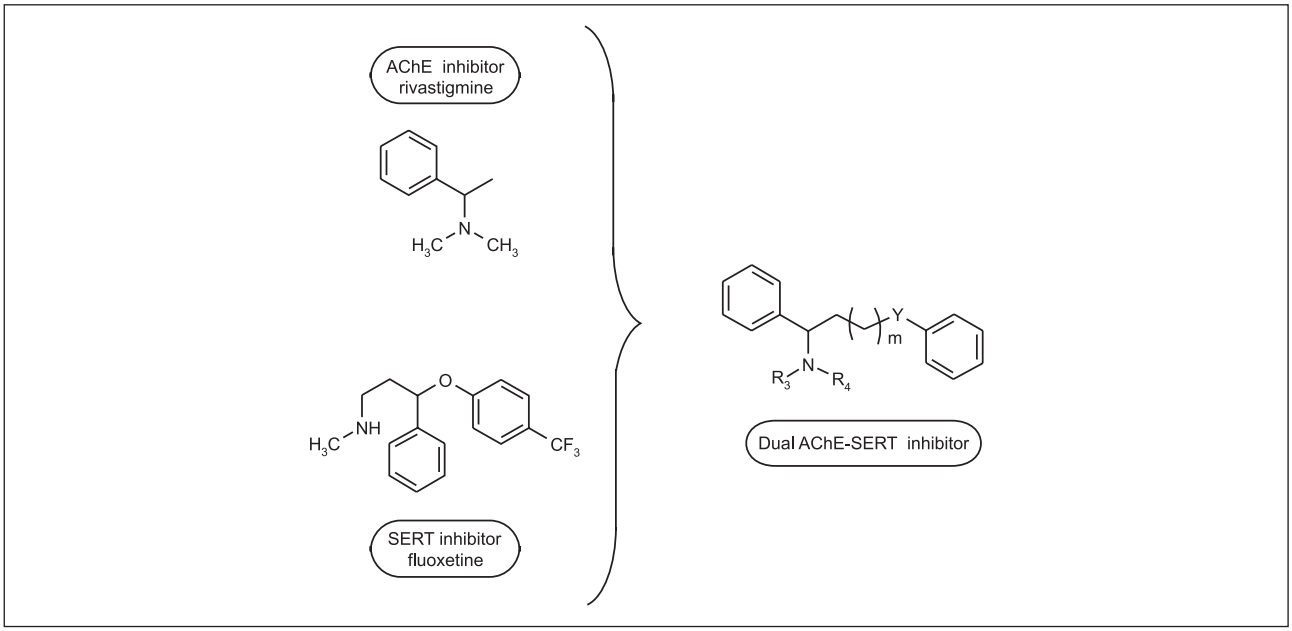


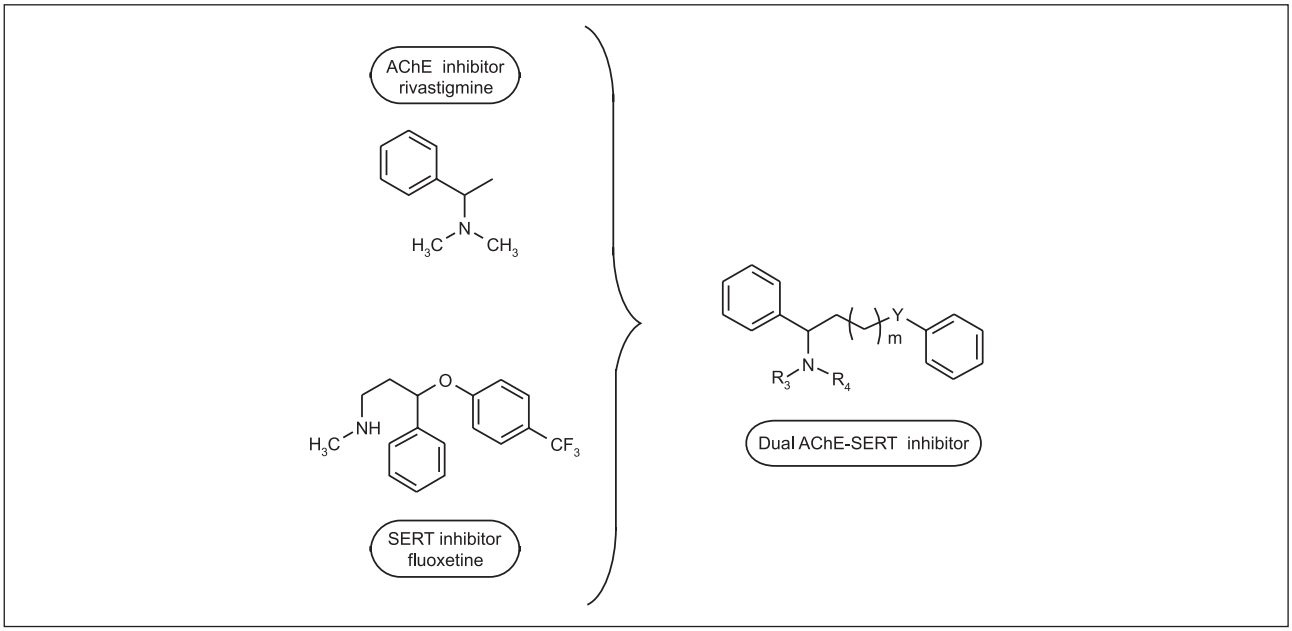
Fig. 1. Dual AChE/SERT inhibitors designed from the combination of rivastigmine and fluoxetine (36).

authors argued that a combination of AChE and SERT inhibition could result in a better therapeutic outcome when treating AD patients concomitantly suffering from depression. Using such drugs, adverse effects caused by excessive cholinergic stimulation could thereby be avoided. Earlier attempts to design dual AChE/SERT inhibitors resulted in the synthesis of compounds that showed an imbalance in their AChE- and SERT-inhibitory potencies (37). These early compounds were potent AChE inhibitors, but displayed only moderate SERT inhibition *in vitro*. Therefore, no antidepressant effect resulting from SERT inhibition could be discerned from the dominating AChE inhibition.

Figure 1 shows the design rationale used in these and subsequent studies. Rivastigmine, an acetylcholinesterase/butyrylcholinesterase inhibitor drug intended for the treatment of AD and parkinsonian dementia, and fluoxetine, a potent marketed SSRI, were chemically combined to form a series of new dual AChE/SERT inhibitors.

Structure-activity relationships were investigated with a focus on varying the substituents attached to one aromatic ring; modifying the carbamate group, the amine moiety and the length of the tether between the two aromatic rings; and substituting the ether oxygen with sulfur. From these studies, it was found that 4-nitro substitution resulted in promising dual activity in the compounds shown in Table I. Potent inhibition of both AChE (IC_{50} = 221 nM) and SERT (IC_{50} = 52 nM) was seen for compound **6a**. A migration of the dimethylcarbamate group from the *meta* (compound **6a**) to the *para* (compound **6b**) position on the aromatic ring resulted in more potent activity against both AChE (IC_{50} = 125 nM) and SERT (IC_{50} = 44 nM) as compared to compound **6a**. Removal of the dimethylcarbamate moiety by replacement with a hydroxyl group (compound **6c**) led to a loss of activity against both AChE and SERT, suggesting that the dimethylcarbamate moiety is essential for both activities. The presence of a secondary amine, such as the *N*-methylamine found in **6b**, was shown to be particularly favorable for dual inhibition, especially when compared to compound **6d**, which contains a tertiary amine and appears to lose all AChE-inhibitory activity (while retaining SERT inhibition at 44 nM). An increase in the tether length (m=2; compound **6e**) showed a virtual reversal of the selectivity profile found for compound **6d**, with **6e** showing greater activity against AChE (IC_{50} = 77 nM), but significantly decreased activity against SERT (IC_{50} > 1000 nM). A shortening of the tether length (compound **6f**) led to a significant decrease in activity against both AChE and SERT. These studies suggest m=1 to be the optimal tether length for dual inhibition, but that selectivity could effectively be altered by structural modification. Results obtained by replacing the ether oxygen with sulfur suggested that the ether oxygen was crucial to SERT but not to AChE inhibition (compare the activities of **6b** with those of **6g**). Since compound **6b** was found to be the best dual AChE/SERT inhibitor in the series, the compound (which is chiral) was subjected to stereochemical resolution. Racemic evaluation revealed the (*S*)-enantiomer to be the most potent dual inhibitor found in these studies.

Abe *et al.* (38) evaluated compound **6b** (RS-1259) in mice and rats for effects on cognitive performance. RS-1259 was orally active and led to an increase in acetylcholine and 5-HT in rat brain. Compared to donepezil, RS-1259 was weaker in increasing cholinergic transmission. However, the effect of RS-1259 on whole-brain consciousness was comparable to that produced by donepezil, indicating that both cholinergic and serotonergic systems may be involved in cognitive disorders.



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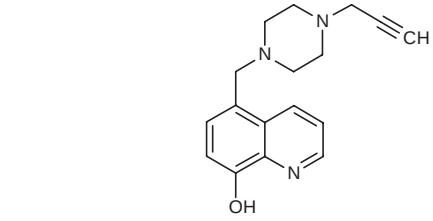
The apoptosis induced by $A\beta_{25-35}$ was also reduced in SH-SY5Y cells. In addition to its ability to inhibit AChE, ITH-4012 was also shown to act as a "calcium promoter", an activity that may lead to neuroprotection through the induction of antiapoptotic proteins. Somewhat paradoxically, some studies have shown that a mild and sustained elevation of the cytosolic calcium concentration may increase the survival of different neuronal cell types (44).

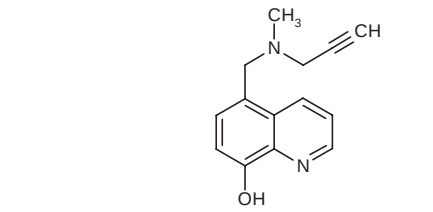
Iron chelators with radical-scavenging and brain-selective MAO-inhibitory activity

Degeneration of cholinergic cortical neurons and nigrostriatal dopaminergic neurons is the main pathological feature seen in the cognitive deficits observed in AD dementia and in dementia with Lewy bodies, as well as in the extrapyramidal symptoms associated with Parkinson's disease. Many subjects with AD, Parkinson's disease and dementia with Lewy bodies have dementia and depression that may result from the degeneration of cholinergic, noradrenergic and serotonergic neurons. In all three disorders, accumulation of iron is found inside amyloid plaques and neurofibrillary tangles, as well as inside some melanin-containing dopaminergic neurons (45). It has been suggested that iron accumulation may contribute to the oxidative stress-induced apoptosis reported in both diseases (16, 45). Such oxidative stress may result from increased glial MAO activity, which leads to an increase in hydrogen peroxide production that can generate reactive hydroxyl radicals through Fenton chemistry with intracellular iron. Iron chelators such as deferoxamine (Desferal®), clioquinol and VK-28 have been shown to have neuroprotective activity in animal models of AD and Parkinson's disease (45). Based on this observation, Zheng *et al.* (46) developed neuroprotective compounds with dual iron-chelating and MAO-B-inhibitory activity. These authors combined the antioxidant chelator moiety of an 8-hydroxyquinoline derivative of the neuroprotective brain-permeable iron chelator VK-28 with the propargylamine moiety found in compounds such as rasagiline and selegiline (Deprenyl). HLA-20 (**11**), in which the propargyl neuroprotective moiety is incorporated in the piperazino moiety, was identified as a potential lead compound for further studies. This compound has relatively poor selectivity for MAO-B, with an IC_{50} value of 110 μM vs. > 200 μM for MAO-A; however, it acts as an iron chelator and free radical scavenger with similar potency to deferoxamine and it is brain-permeable. Unlike HLA-20, the secondary amine M-30 (**12**) was found to be a highly potent MAO-A/B inhibitor *in vitro*, with brain selectivity for these enzymes *in vivo*. M-30 exhibits iron-chelating properties similar to those of deferoxamine and neuroprotective activities similar to those of rasagiline and ladostigil. In addition, M-30 behaves similarly to other propargylamine MAO inhibitors by acting as a suicide inhibitor after being identified and processed as a substrate by the enzyme (17, 46, 47).

In two mouse models of neurodegeneration, M-30 protected against MPTP- and kainate-induced neurotoxicity by virtue of both its MAO-A/B-inhibitory and iron-chelating/radical-scavenging properties.



HLA-20 (**11**)



M-30 (**12**)

The accumulation of iron at sites where neurons degenerate, both in Parkinson's disease and in AD, is thought to play a major role in the neurodegenerative process (45). The novel nontoxic lipophilic (and therefore brain-permeable) iron chelators VK-28 and M-30 (both of which possess the MAO-inhibitory and neuroprotective propargyl moiety of rasagiline) offer potential therapeutic benefits for these diseases. M-30 attenuates apoptotic events in SH-SY5Y neuroblastoma cells in a serum deprivation model via multiple protection mechanisms, including: 1) reduction of the proapoptotic proteins Bad and Bax; 2) reduction of apoptosis-associated Ser139-phosphorylated H2A.X; 3) induction of the antiapoptotic protein Bcl-2; and 4) inhibition of caspase-3 cleavage and activation. M-30 also promotes morphological changes, resulting in axonal growth-associated protein-43 (GAP-43), which is implicated in neuronal differentiation. In SH-SY5Y and CHO cells stably transfected with the APP "Swedish" mutation, M-30 markedly reduced cellular levels of holo-APP and β-*C*-terminal fragment (β-CTF) and caused a reduction in the levels of amyloidogenic Aβ peptide in the medium. In addition, nonamyloidogenic sAPPα levels in the cell medium and α-CTF levels in the cell lysate were also elevated. These results are consistent with the presence of an iron-responsive element (IRE) in the 5'-untranslated region (5'-UTR) of APP and demonstrate the effectiveness of M-30 in limiting holo-APP expression and Aβ peptide secretion. Therefore, the multifunctional properties of M-30 suggest that it may offer extraordinary potential as a drug for the treatment of AD (see Fig. 2) (48).

Antiinflammatory agents with antioxidant activities

Epidemiological studies indicate that the risk of AD may be decreased by the chronic use of NSAIDs (49-51). NSAIDs appear to inhibit the secretion of amyloidogenic

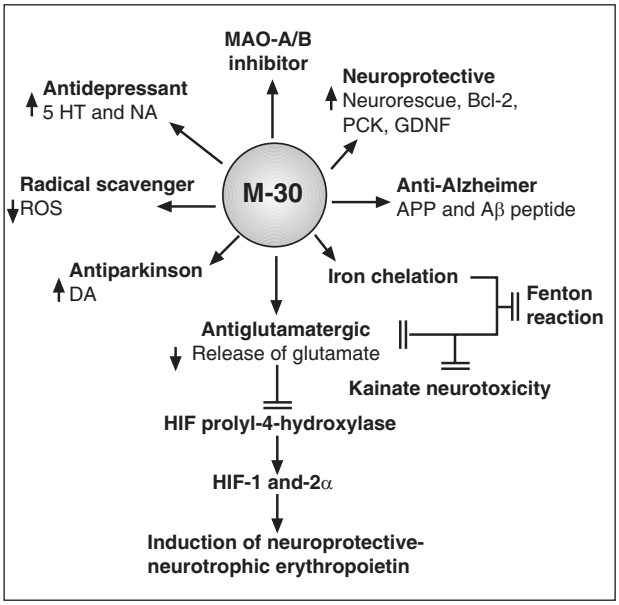
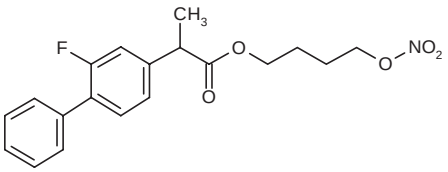


Fig. 2. Neuroprotective effects of M-30 (see text for discussion).

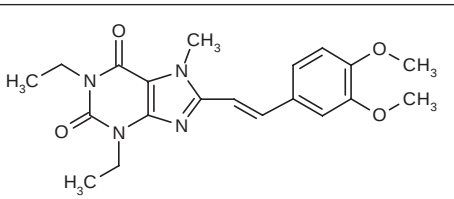
$A\beta_{42}$ in cultured cells of an AD model (51). $A\beta_{42}$ is the 42-residue isoform of the Aβ peptide that is believed to be one of the culprits responsible for AD pathogenesis. Among the various Aβ peptides, $A\beta_{42}$ is the isoform that initially deposits in the brain. Since NSAIDs have been shown to lower $A\beta_{42}$ levels, these drugs may confer protection against AD. The mechanism by which the NSAIDs lowered $A\beta_{42}$ levels was independent of their cyclooxygenase (COX)-inhibitory activity. Moreover, COX-2-selective inhibitors were shown to lack the ability to lower $A\beta_{42}$ levels. In addition, NSAIDs also stimulate the secretion of α-secretase, which favors the processing of the nonamyloidogenic soluble form of APP, sAPP (52).

Targeting the neuroinflammation associated with neurodegenerative diseases led to the development of compounds such as HCT-1026 (**13**) (53). This compound combines the antiinflammatory action of flurbiprofen, an NSAID, with the antioxidant activity of ferulic acid. HCT-1026 attenuated inflammation produced by the chronic infusion of lipopolysaccharide (LPS) in the ventricles of rats (54).

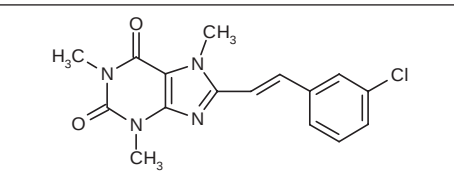
Multifunctionality in drugs that may have utility in the treatment or prevention of diseases associated with cognitive deficits is not only seen in compounds designed as such, but also in commonly prescribed drugs, including the popular cholesterol-lowering statin drugs. Epidemiological studies have shown that the dyslipidemic HMG-CoA reductase inhibitors may have utility in AD therapy (55-57). For example, Fassbender *et al.* (58) showed that the HMG-CoA reductase inhibitor simvastatin was able to reduce the levels of the Aβ peptides $A\beta_{42}$ and $A\beta_{40}$, both in primary hippocampal cells and in a guinea pig model. Additionally, statins have shown antiinflammatory activity that may be beneficial in AD therapy, probably in association with the inhibition of microglia-induced inflammation (59, 60).



HCT-1026 (**13**)



KW-6002 (**14**)



CSC (**15**)

Adenosine antagonists with concomitant MAO-inhibitory activity

In AD and Parkinson's disease, dual MAO-B inhibition and adenosine A_{2A} receptor blockade may be a novel therapeutic approach for preventing neuronal cell death. As detailed earlier, MAO-B plays a role in the catabolism of neurotransmitters such as dopamine, 5-HT and norepinephrine, and this leads to hydrogen peroxide formation, which contributes to oxidative stress and neuronal cell death (61). The increase in MAO-B levels observed in older patients (62) has led to a rationale supporting the use of drugs such as selegiline and lazabemide (63) in these patients, as well as to the design of drugs such as ladostigil (15). Caffeine, a nonselective adenosine receptor antagonist, is under some scrutiny as a potential drug for counteracting age-related cognitive decline. This research interest is supported by evidence that critical changes in adenosine-related neurotransmission occur with aging and may be counteracted by adenosine receptor antagonists (64-67). Caffeine, in fact, has been suggested to protect against Aβ neurotoxicity (65, 66). Furthermore, acute treatment with caffeine and the A_{2A} receptor antagonist ZM-241385 was recently found to reverse age-related olfactory deficits and memory decline in rats (67), clearly suggesting the involvement of A_{2A}, and not A_1, receptors in cognitive decline, and possibly in neurodegenerative processes. Based on this and other evidence in parkinsonian models, Petzer *et al.* (68) evaluated (*E*)-8-styrylxanthinyl-derived adenosine A_{2A} receptor antagonists for their inhibition of MAO-B. Included in these studies were KW-6002 (**14**), a potent A_{2A} receptor antagonist (K_i = 2.2 nM) that is undergoing clinical trials,

and (*E*)-8-(3-chlorostyryl)caffeine (CSC; **15**), which has been shown to be neuroprotective in the MPTP parkinsonian mouse model (69). All of the compounds tested showed MAO-B inhibition in the low micromolar to high nanomolar range, with K_i values of 21 and 0.1 μM for KW-6002 and CSC, respectively. These results suggest that the neuroprotective properties of KW-6002 and CSC may be partly due to MAO-B inhibition, acting in synergism with their A_{2A} antagonism (70).

NMDA antagonists that also act as calcium channel blockers

The divalent calcium cation plays an important role in neuronal cell death (34, 71-73). One of the receptors activated by glutamate, the NMDA receptor, is a major conduit for the influx of calcium ions into cells under excitotoxic conditions. The prevention of such an excessive influx of calcium (known as excitotoxicity) therefore remains a major strategy in the design of neuroprotective agents. Excess accumulation of calcium in neuronal cells rapidly leads to cell death through a variety of mechanisms, including activation of proteases, nucleases, phospholipases, nitric oxide synthase (NOS) and other degradative enzymes that lead not only to activation of death cascades but also to free radical formation (73). In AD, it is speculated that Aβ-activated microglia secrete tumor necrosis factor-α (TNF-α) and glutamate, which synergistically induce neuronal cell death (74) through a cascade initiated by the influx of calcium. NMDA receptor antagonists such as dizocilpine (MK-801) and memantine (see below) may have a dual mechanism for protecting neuronal cells, both by directly blocking the NMDA receptor and by attenuating TNF-α-induced potentiation of glutamate toxicity (75). Brain injury after an ischemic stroke also triggers a cascade of glutamate-associated excitotoxic events. Figure 3 shows a scheme of the events leading to cell death after an ischemic incident (72).

Cognitive impairment and dementia have both been reported to be increased after cerebral stroke, especially in the elderly (76), with up to 25% of stroke patients exhibiting signs and symptoms of dementia (77). Stroke is the third leading cause of death in the U.S. (72). A definite need therefore exists to design drugs that can protect or save neurons after an ischemic incident, especially because, to date, no effective treatment has been developed to prevent neuronal cells from dying during stroke conditions (71).

Several studies have shown that NMDA receptor antagonists such as dizocilpine (MK-801) and the polycyclic cage amine memantine display neuroprotective effects in experiments using ischemia paradigms in neurons (71, 78-82). An alternative pathway for calcium to enter neuronal cells is through voltage-gated ion channels, such as L-type calcium channels. Animal experiments with nimodipine have suggested that calcium channel antagonists may be neuroprotective in ischemia by antagonizing the influx of calcium into neuronal cells (71). The importance of calcium overload during cell death suggests that dual calcium channel and NMDA receptor antagonists might be useful as neuroprotective drugs in stroke and AD.

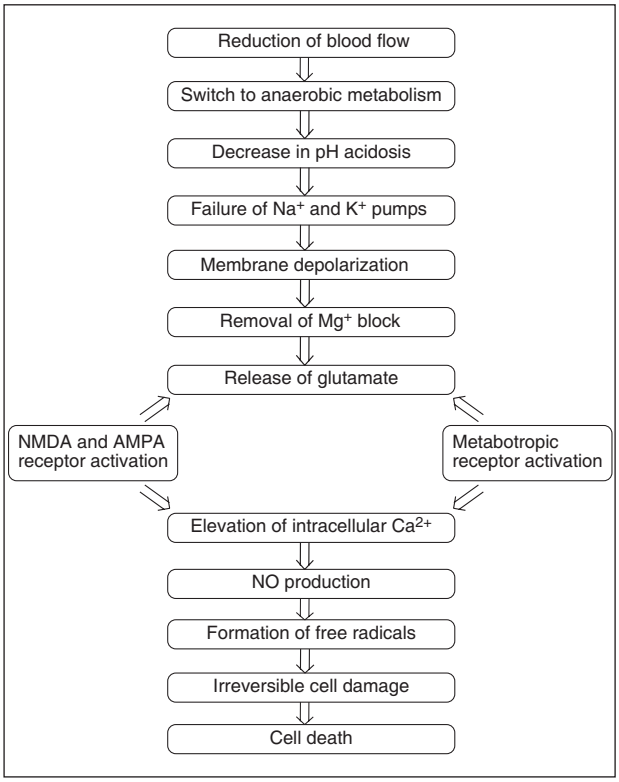
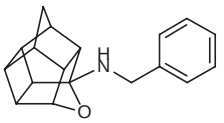
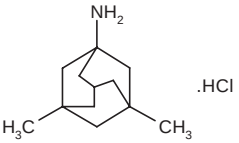


Fig. 3. Cascades that occur in neurons under conditions of acute ischemic stroke (72). Each step along the path represents a possible target for therapeutic intervention.

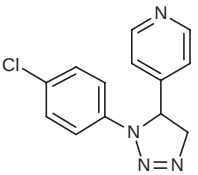
NGP1-01 (**16**) is a polycyclic cage amine derived from the reductive amination of benzylamine and Cookson's "bird cage" diketone (83, 84). Van der Schyf *et al.* (85) investigated the L-type calcium channel activity of NGP1-01 utilizing electrophysiological experiments in isolated guinea pig papillary muscle and sheep Purkinje fibers. The structural similarity of NGP1-01 to another polycyclic cage amine and NMDA receptor antagonist, memantine hydrochloride (**17**), led to the evaluation of NGP1-01 for potential NMDA receptor antagonism. Memantine is an uncompetitive NMDA receptor antagonist that is used clinically to treat AD (86-88). Its favorable fast on-off binding kinetics give this compound an improved side effect profile compared with other NMDA antagonists such as dizolcipine (89). NGP1-01 was also shown to be an uncompetitive NMDA antagonist in murine whole-brain synaptoneurosomes (unpublished data) and blocked NMDA-mediated $^{45}Ca^{2+}$ uptake with an IC_{50} value of 2.98 μM. This dual mechanism of modulating calcium entry into neuronal cells (Fig. 4) suggests that NGP1-01 may have utility as a neuroprotective agent in AD, stroke and other neurodegenerative diseases associated with cognitive decline (90). This possibility has recently been partially confirmed in *in vivo* studies using the middle cerebral artery occlusion (MCAO) mouse model of stroke,



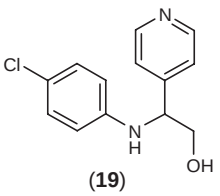
NGP1-01 (**16**)



Memantine hydrochloride (**17**)



ADD-17014 (**18**)



(**19**)

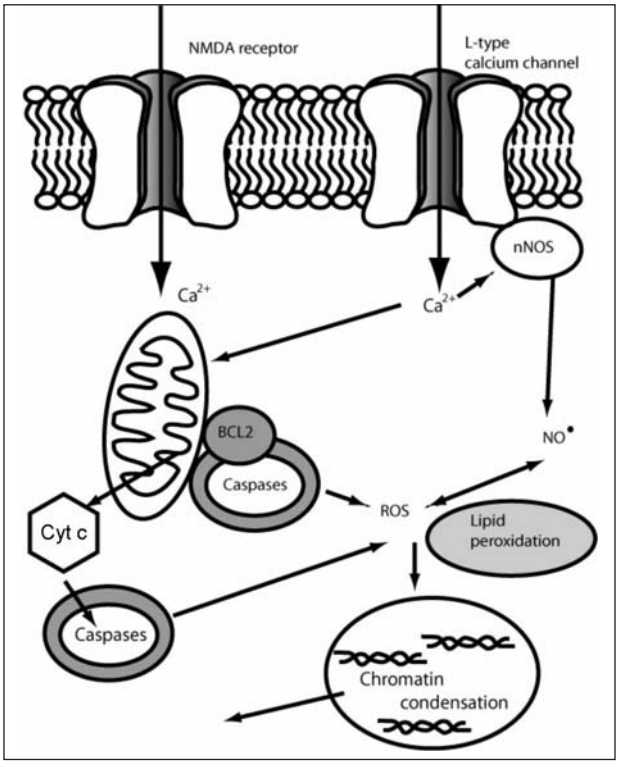


Fig. 4. Dual mechanism of neuroprotection proposed for the pentacycloundecylamine compound NGP1-01. NGP1-01 is an L-type calcium channel (85) and NMDA receptor (unpublished data) antagonist. By modulating Ca^{2+} entry during a cerebral ischemic incident, or in situations of chronic Ca^{2+} overload, neuronal cell death may be prevented.

wherein it was shown that NGP1-01, administered 30 min before MCAO, afforded substantial protection against cerebral ischemia-induced brain lesions, as well as against brain swelling measured 24 h after MCAO (91). A beneficial effect of NGP1-01 on cognitive function still remains to be proven in an animal model.

Glutamate antagonists that also act as glutamate release inhibitors

The Δ^2-1,2,3-triazoline anticonvulsant ADD-17014 (**18**) has been shown to modulate excitatory amino acid neurotransmission through a unique dual mechanism (92). The prodrug attenuates presynaptic glutamate release (83% at 100 μM), while its β-aminoalcohol metabolite (**19**) acts as an NMDA receptor antagonist by binding to the dizocilpine site (56% at 10 μM) located inside the NMDA receptor/ion channel. This compound may potentially be neuroprotective in stroke-induced cognitive deficits as a result of its antagonism of the NMDA receptor and its inhibition of glutamate release.

Nan *et al.* (93) synthesized novel compounds with dual mechanisms of action as inhibitors of glutamate carboxypeptidase II and agonists of metabotropic glutamate receptor ($mGluR_3$) activity (Fig. 5). These compounds may have utility in preventing neurodegenerative diseases associated with excessive activation of glutamate receptors, such as AD.

Designing an inhibitor of glutamate carboxypeptidase II would provide a tool for decreasing the amount of glutamate present, and *N*-acetyl-L-aspartyl-L-glutamate (NAAG) may act as a partial antagonist at some NMDA receptors. Agonist activity at the $mGluR_3$ may also be neuroprotective (94). Compound **20** exhibited good potency and selectivity as an $mGluR_3$ agonist, and it also inhibited glutamate carboxypeptidase II.

Multiple ion channel modulators

The polycyclic cage compound 6-benzylamino-3-hydroxyhexacyclo[6.5.0.$0^{3,7}$.$0^{4,12}$.$0^{5,10}$.$0^{9,13}$]tridecane (**21**) interacts with multiple channels (95, 96). Electrophysiological evaluation of compound **21** for calcium, potassium and sodium channel blockade in guinea pig cardiac papillary muscle indicated that this compound, at test concentrations of 10-50 μM, inhibited the ion currents for L-type calcium channels, sodium channels and the fast component of the delayed rectifier potassium channel. No effects were observed for the T-type calcium channel or for the inward rectifier and slow components of the delayed rectifier potassium channels. This compound could potentially have utility as a neuroprotective agent

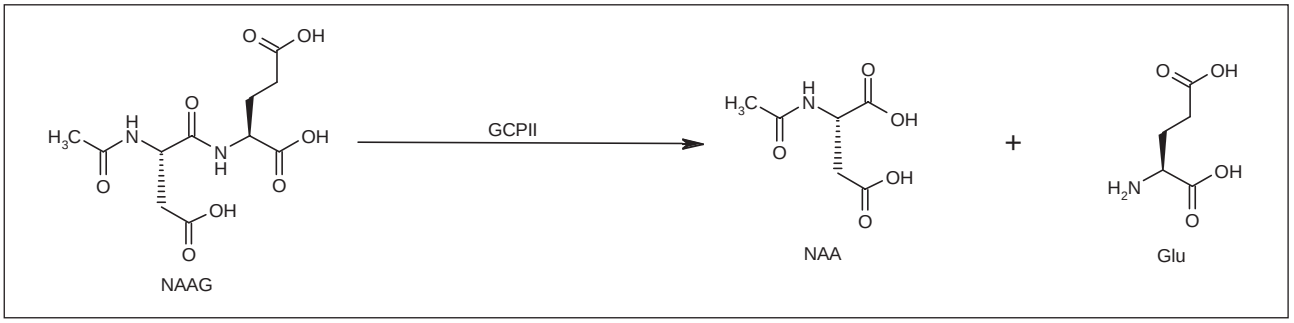
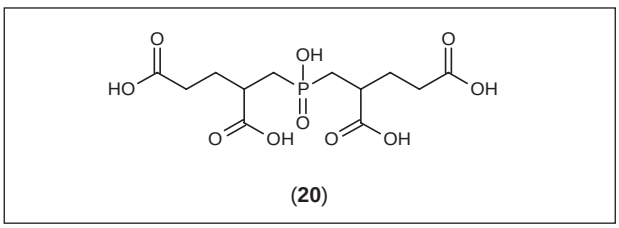
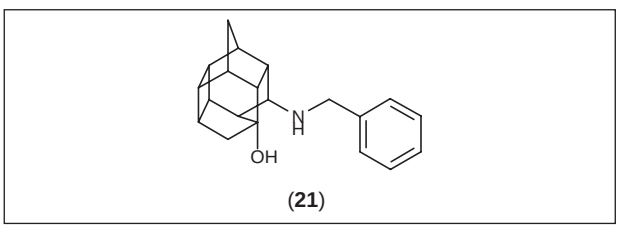


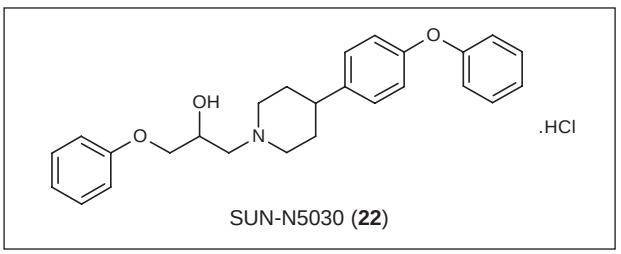
Fig. 5. Catabolism of *N*-acetyl-L-aspartyl-L-glutamate (NAAG) by glutamate carboxypeptidase II (GCPII; 93).



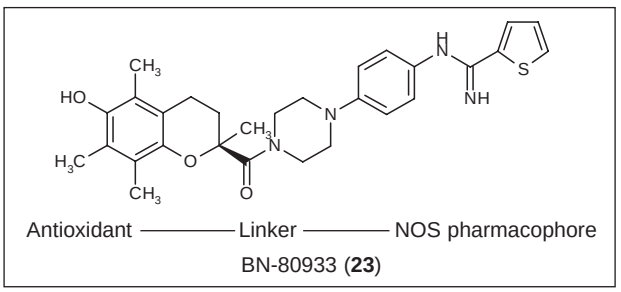
(**20**)



(**21**)

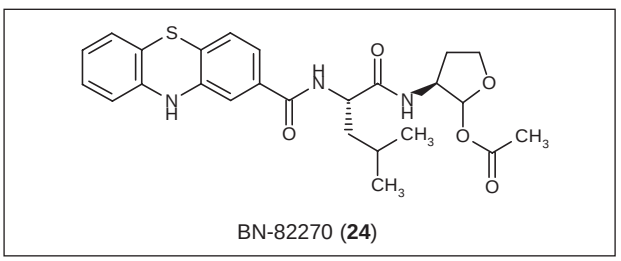


SUN-N5030 (**22**)



Antioxidant — Linker — NOS pharmacophore

BN-80933 (**23**)



BN-82270 (**24**)

due to its antagonism of both sodium and calcium channels (97), as well as of the fast component of the delayed rectifier potassium channel. The last mechanism is especially intriguing as a potential target for disorders associated with cognitive decline, since it is known that the globus pallidus plays a central role in the basal ganglia circuitry involved in cognitive and emotional functions (98). In the globus pallidus, the rapidly deactivating current is attributable to Kv3.1/3.2 channels (99), which may play a role in sustained high-frequency repetitive firing (98). Kv3.1-expressing neurons are also involved in the generation and maintenance of cortical fast gamma and slow delta oscillations, which are associated with behavior and cognition (100).

Yet another dual sodium and calcium channel blocker was synthesized and tested by Annoura *et al.* (101). SUN-N5030 (**22**) was shown to potently block neuronal sodium and calcium channels, and it exhibited neuroprotective activity in the transient MCAO rat stroke model.

Compounds with combined antioxidant and NOS-inhibitory activity

BN-80933 (**23**) was shown to inhibit both neuronal NOS and lipid peroxidation (102). This dual mechanism, obtained by combining NOS inhibition and antioxidant activity, may lead to better protection of neuronal cells during an ischemic event, resulting in a better outcome and a reduced risk of subsequent cognitive decline.

Simultaneous inhibition of calpain and lipid peroxidase

Auvin *et al.* (103) synthesized a group of compounds with dual calpain- and lipid peroxidase-inhibitory activity. Calpain is a Ca^{2+}-activated proteolytic enzyme. When intracellular Ca^{2+} increases, as in the case of excitotoxicity, calpain is activated, leading to the degradation of cytoskeletal and membrane proteins, an event that contributes to neuronal cell death (104). The most potent compound in this group was BN-82270 (**24**), which had IC_{50} values of 13.34 and 15.5 μM for calpain inhibition and cell death inhibition, respectively.

Conclusions

The treatment of cognitive decline and neurodegeneration associated with neurodegenerative diseases offers a vexing challenge, partly due to the complex pathology involved in the etiology of these diseases. For example, recent transcriptomic and proteomic analyses of the midbrain in the MPTP model of Parkinson's disease and of the substantia nigra pars compacta in parkinsonian brains have shown that the process of neurodegeneration is a cascade of molecular events, any one of which can initiate the process leading to neurological decline (105, 106). This process is considered to be similar to the domino effect, resulting in the fall of all the elements involved regardless of which element initiates the process. Similar results have been observed and reported in AD, Huntington's disease and amyotrophic lateral sclerosis (ALS), and the evidence obtained strongly suggests that these disorders share homologous processes of neurodegeneration. These findings may indicate why neuroprotection with single neuroprotective magic bullets has not been achieved in the clinic. The explanation may lie in the fact that many neurotoxic factors induced by gene mutations can initiate the various neurodegenerative disorders. Thus, preventing one process does not necessarily prevent the others from producing the neurotoxic action. Based on this reasoning, our hypothesis holds that multifunctional neuroprotective drugs with the ability to prevent several toxic cascades could be in a better position to prevent the neurodegenerative process. Here we have discussed two classes of multifunctional drugs. Some have been identified through serendipity to possess dual or multifunctional activity. This is not novel in itself, since most drugs have more than one pharmacological action, and activities initially considered to be "secondary" may become as prominent as the primary action for which the drug was first developed. However, the new approach involves the deliberate introduction of known pharmacological and neuroprotective moieties into a single pharmacophore in order to achieve several desired actions in a targeted manner. Such use of single drug molecules that act on more than one drug target in the etiological pathway of the disease may offer new strategies for the treatment of many diseases, including those associated with cognitive decline. The changeover from a "magic bullet" to a "magic shotgun" (1) approach may be key in future treatment regimens for neurodegenerative diseases and associated cognitive decline. Drug discovery endeavors will have to shift focus from the design of selective agents that address only one pathophysiological target, to agents that operate through multiple mechanisms designed to target the complexity of the disease state.

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