Dual-Target–Directed Drugs that Block Monoamine Oxidase B and Adenosine A_{2A} Receptors for Parkinson's Disease

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Summary: Inadequacies of the current pharmacotherapies to treat Parkinson's disease (PD) have prompted efforts to identify novel drug targets. The adenosine A_{2A} receptor is one such target. Antagonists of this receptor (A_{2A} antagonists) are considered promising agents for the symptomatic treatment of PD. Evidence suggests that A_{2A} antagonists may also have neuroprotective properties that may prevent the development of the dyskinesia that often complicates levodopa treatment. Because the therapeutic benefits of A_{2A} antagonists are additive to that of dopamine replacement therapy, it may be possible to reduce the dose of the dopaminergic drugs and therefore the occurrence of side effects. Inhibitors of monoamine oxidase (MAO)-B also are considered useful tools for the treatment of PD. When used in combination with levodopa, inhibitors of MAO-B may enhance the elevation of dopamine levels after levodopa treatment, particularly when used in early stages of the disease when dopamine production may not be so severely compromised. Furthermore, MAO-B inhibitors may also possess neuroprotective properties in part by reducing the damaging effect of dopamine turnover in the brain. These effects of MAO-B inhibitors are especially relevant when considering that the brain shows an age-related increase in MAO-B activity. Based on these observations, dual-target-directed drugs, compounds that inhibit MAO-B and antagonize A_{2A} receptors, may have value in the management of PD. This review summarizes recent efforts to develop such dual-acting drugs using caffeine as the lead compound. **Key Words:** Parkinson's disease, monoamine oxidase B, adenosine A_{2A} receptor, dual-target-directed drug, caffeine.

INTRODUCTION

Strategies for the treatment of Parkinson's disease (PD) are currently focused on restoring the function of dopamine in the striatum of the brain.[1] The deficiency of dopaminergic innervation in the Parkinsonian striatum arises as a consequence of the degeneration of dopaminergic nigrostriatal neurons and the depletion of dopamine stores.[2] Replenishing these depleted stores with levodopa, the immediate metabolic precursor of dopamine, and mimicking dopamine-mediated neurotransmission with dopamine agonists have become the foundation of current PD therapy.[1,3] Dopamine replacement therapies offer effective relief of the motor deficits associated with PD, especially in the early stages of the disease. Dopamine replacement therapy, however, does not slow the underlying neurodegenerative processes and, as the disease advances, further neuronal loss occurs and drug efficacy is gradually lost.[4] This is accompanied by a progression of the symptoms.

To maintain an adequate therapeutic effect, dosages of the dopaminergic drugs have to be increased. However, the tolerated dosage of levodopa is limited by the numerous adverse effects associated with long-term therapy, such as the development of abnormal involuntary movements (also termed dyskinesia) and behavioral disturbances.[5,6] Therefore drugs that delay the initiation of levodopa therapy and/or allow for the reduction of levodopa dose are an important component of PD therapy.[7,8] These drugs are generally divided into two categories: 1) those that provide symptomatic relief of PD symptoms that are additive to the effects of levodopa and 2) those

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that may delay or halt the underlying degeneration of the dopaminergic neurons.

Among the adjunctive drugs to levodopa are inhibitors of the enzyme monoamine oxidase B (MAO-B).[9] Since the oxidative deamination reaction catalyzed by MAO-A and MAO-B appears to be a major catabolic pathway of dopamine in the striatum,[10–12] inhibition of these enzymes in the brain may slow the depletion of dopamine stores and elevate the levels of endogenous dopamine, and dopamine produced from exogenously administered levodopa.[12,13] Furthermore, inhibitors of the MAOs may also exert a neuroprotective effect by decreasing the production of potentially hazardous by-products of dopamine metabolism in the brain.[14] Considering that MAO-B activity exhibits an age-related increase in the human brain,[15–17] whereas MAO-A activity remains constant,[18] inhibition of this enzyme is especially relevant in PD.

Antagonists of the adenosine A_{2A} receptor (A_{2A} antagonists) are another class of promising anti-Parkinsonian agents and a leading candidate class for the nondopaminergic treatment of symptomatic PD.[19–21] A_{2A} antagonists may also possess neuroprotective properties and may prevent the development of dyskinesia that is usually associated with levodopa treatment.[22,23] (*E*)-8-(3-Chlorostyryl)caffeine (CSC) (**1**) (FIG. 1), a well known A_{2A} antagonist,[24,25] has been shown to be a potent, reversible inhibitor of MAO-B.[26–28] This finding has raised the possibility of designing dual-target-directed drugs that may provide enhanced symptomatic relief and that may also slow the progression of PD by protecting against further neurodegeneration.

A_{2A} RECEPTORS IN THE CNS

Adenosine, an endogenous purine nucleoside that is present in all mammalian tissues, exerts a variety of physiological effects. Four adenosine receptor subtypes have been cloned and characterized: A_1, A_{2A}, A_{2B} and A_3. All adenosine receptors are members of the G-protein-coupled receptor family and have seven transmembrane domains. Adenosine-mediated intracellular signaling occurs via the increase or decrease of intracellular cyclic adenosine monophosphate (cAMP) levels with A_1 and A_3 receptors inhibiting adenylate cyclase, whereas A_{2A} and A_{2B} receptors stimulate adenylate cyclase activity. In contrast to the A_{2B} and A_3 receptors, the A_1 and A_{2A} receptor subtypes bind adenosine with high affinity and are highly expressed in the brain.[29] Whereas A_1 receptors are widely distributed in the brain,[30] A_{2A} receptors are highly enriched in the striatum and are almost exclusively located on the gamma-aminobutyric acid (GABA)ergic striatopallidal neurons where they are colocalized with the dopamine D_2 receptors.[31–33] A_{2A} and D_2 receptors have been suggested to interact with each other; A_{2A} receptor stimulation has been shown to exert a functional antagonistic effect on D_2 receptors. For example, A_{2A} agonists decrease the binding affinity of D_2

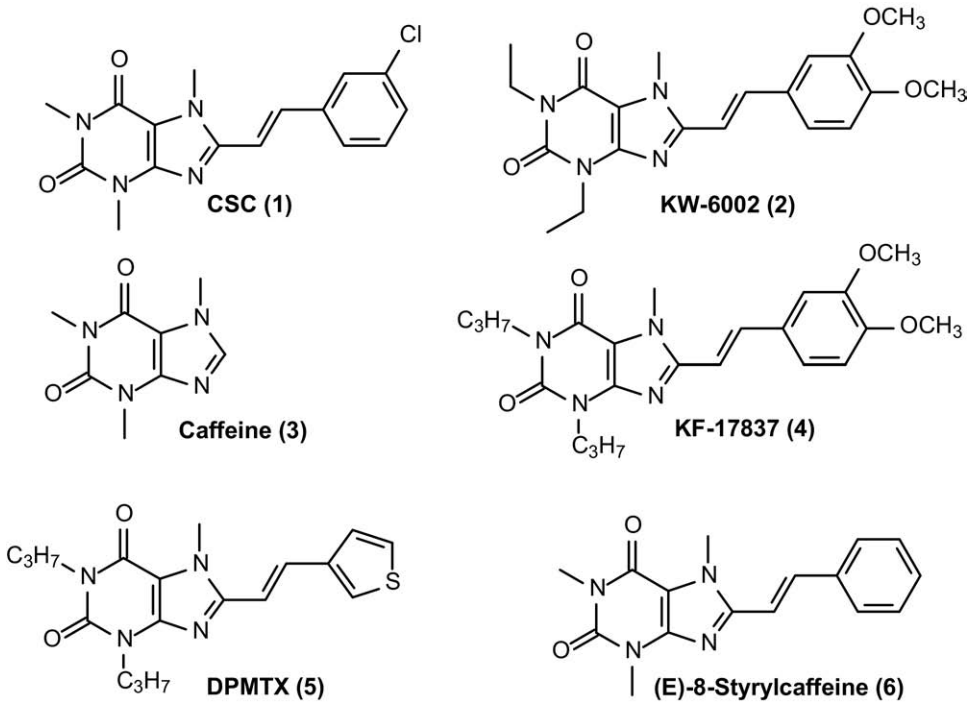


FIG. 1. The chemical structures of caffeine and caffeine derived A_{2A} antagonists and monoamine oxidase (MAO)-B inhibitors. CSC = (*E*)-8-(3-Chlorostyryl)caffeine; DPMTX = (*E*)-1,3-dipropyl-7-methyl-8-[2-(3-thienyl)ethenyl)]xanthine.

agonists for the D_2 receptor in the striatum and also reduces G-protein coupling activity of the D_2 receptor.[34,35] The antagonism of the D_2 receptors by A_{2A} receptors may also occur at the second messenger level or further down the signal transduction pathway, because both receptors couple to adenylate cyclase and other messenger systems.[19] The antagonistic relationship between these receptors and the distinctive CNS distribution of the A_{2A} receptor provide the basis for targeting the A_{2A} receptor as a potential nondopaminergic treatment strategy for PD. Blockade of the A_{2A} receptor in striatopallidal neurons potentiates D_2 receptor-mediated neurotransmission, and therefore reduces the effects of striatal dopamine depletion in PD.[20,36] Accordingly, antagonism of the A_{2A} receptor partially restores motor activity in animal models of PD.[37] A_{2A} receptor antagonism also may exert anti-Parkinsonian effects that are independent of an interaction with D_2 receptors, because the motor stimulation afforded by A_{2A} antagonists is still present in D_2 knock-out mice.[38]

A_{2A} ANTAGONISTS IN THE SYMPTOMATIC TREATMENT OF PD

As mentioned in the previous section, the observation that A_{2A} receptor stimulation counters the effects of D_2 receptors in GABAergic striatopallidal neurons[34] suggests that antagonists of the A_{2A} receptor may reduce the effects of striatal dopamine depletion in PD and possibly potentiate the motor actions of levodopa and dopamine agonists.[20] In accordance with this view, A_{2A} antagonists were found to enhance the motor activity of levodopa and dopamine agonists in 6-hydroxydopamine-lesioned rats,[39,40] whereas agonists of the A_{2A} receptor displayed the opposite effect.[41] In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD, A_{2A} antagonists have been shown to reverse the MPTP-induced Parkinsonism in monkeys[37,42] and to potentiate the levodopa-induced restoration of motor activity.[43,44] A_{2A} antagonists also consistently enhance basal locomotor activity in unlesioned rodents,[45] as well as in animals rendered cataleptic with the dopamine receptor antagonist haloperidol.[46]

These observations have led to clinical trials in PD patients with the caffeine-derived A_{2A} antagonist (*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KW-6002) (**2**).[47,48] KW-6002 was shown to potentiate the symptomatic benefits conferred by a reduced dose of levodopa in relatively advanced PD and to produce motor enhancement that was comparable with that of an optimal levodopa dose.[49] Recently, a double-blind, randomized, multicenter clinical trial confirmed the motor benefit of KW-6002.[50] Furthermore, KW-6002 prolonged the therapeutic action of a full dose of levodopa. KW-6002 also significantly potentiated the antitremorgenic effect of levodopa.[47] This antitremorgenic potential is in agreement with preclinical data demonstrating that A_{2A} antagonists countered the tremulous jaw movements in rats treated with the acetyl cholinesterase inhibitor tacrine.[51] Taken together, these findings suggest that antagonism of the A_{2A} receptor may be a valuable strategy in the symptomatic management of PD, especially as an adjunct to levodopa therapy. Because the therapeutic benefits of A_{2A} antagonists are an additive to those of dopamine replacement therapy, it may be possible to reduce the dose of dopamine replacement drugs and the occurrence of side effects (Table 1).

Table 1. *Summary of the Anti-Parkinsonian Effects of A_{2A} Antagonists*

Antisymptomatic
1. Enhance the motor restorative effects of levodopa[43,49]
2. Prolong the therapeutic action of levodopa[49]
3. Potentiate the antitremorgenic effect of levodopa[47]
4. Enhance locomotion in animals rendered cataleptic with haloperidol[46]
Neuroprotective
1. Caffeine consumption is associated with a reduced risk of developing PD[52–54]
2. Protect against MPTP-induced neurotoxicity in mice[56,57]
3. Protect against 6-hydroxydopamine-induced neurotoxicity in rats[57–59]
Antidyskinetic
1. Prevent the development of apomorphine-induced dyskinesia in MPTP-lesioned monkeys[23]
2. Potentiate motor benefits of levodopa without potentiation of dyskinesia[47]

MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD = Parkinson's disease.

A_{2A} ANTAGONISTS AND NEUROPROTECTION

Currently the approved anti-Parkinsonian drugs are aimed at relieving the symptoms of PD, but none have been found to delay the underlying neurodegenerative processes. Although the development of symptomatic treatment strategies remains an important goal, neuroprotective drugs would be of greater value in the long-term treatment of PD.[4] Pharmacological and epidemiological evidence have suggested that antagonism of adenosine receptors, specifically the A_{2A} receptor subtype, may protect against dopaminergic neuronal degeneration in PD and animal models of PD.[19,22] For example, the consumption of caffeine (**3**) and caffeinated coffee have been shown to correlate with a reduced risk of developing PD in men,[52–54] as well as women who have not taken postmenopausal estrogens.[55] Depending on the amount of coffee consumed, the risk of developing PD was greater than five-fold lower among coffee

drinkers.[53] Because the reduced incidence of PD did not correlate with the consumption of decaffeinated coffee, caffeine was suggested to be responsible for the protective effect.[52] This was further substantiated by the finding that caffeine protects mice against the nigrostriatal degenerative effects of the Parkinsonian-inducing agent MPTP.[56] This effect of caffeine, a nonselective A_1/A_{2A} antagonist, is probably related to blockade of A_{2A} receptors, because a variety of selective A_{2A} antagonists, and not A_1 antagonists, were also shown to attenuate the neurotoxic action of MPTP.[56,57] The protective effect of A_{2A} receptor blockade is also applicable to other models of PD because caffeine[58] and selective A_{2A} antagonists[57,59] have been shown to reduce dopaminergic neuronal cell loss induced by 6-hydroxydopamine in rats. These observations suggest that the lowered risk of developing PD, conferred by coffee consumption, is dependent on the antagonistic action of caffeine at A_{2A} receptors. Because A_{2A} receptor blockade is associated with a neuroprotective effect, the adenosine A_{2A} receptor may be considered a promising target for the long-term treatment of PD.[19] The mechanism by which caffeine and A_{2A} antagonists protect against the neurodegenerative processes associated with PD and neurotoxins is not clear at present. One possible mechanism may involve the reduction of glutamate release by A_{2A} antagonists, and therefore the reduction of a possible excitotoxic component of the neurodegenerative process.[19,22]

A_{2A} ANTAGONISTS AND DYSKINESIA

As mentioned in the introduction, levodopa and dopamine agonists provide effective symptomatic relief of the motor deficits in the early stages of PD.[3] Long-term levodopa and dopamine agonist therapy, however, are associated with the development of dyskinesia.[6] Because the dyskinesia can be as disruptive as the primary symptoms of PD, the development of adjunct therapy that suppresses or prevents levodopa-induced dyskinesia is of interest. Laboratory evidence suggests that A_{2A} antagonists may exhibit antidyskinetic effects in primate models.[37,60–62] In MPTP-lesioned monkeys, the A_{2A} antagonist KW-6002 has been shown to prevent the development of dyskinesia that was induced by the D_1/D_2 agonist apomorphine.[23] Dyskinesia is observed only after the discontinuation of KW-6002 treatment. This apparent antidyskinetic effect of A_{2A} antagonists is especially relevant in the light of the observation that the therapeutic benefits of A_{2A} antagonists are additive to those of levodopa and dopamine agonists, and it may therefore be possible to reduce the dose of the dopaminergic drugs and the severity of dyskinesia.[37,39,40,42] In agreement with this view, the results of clinical trials demonstrated that KW-6002 potentiated the motor benefits of a reduced dose of levodopa and at the same time produced only approximately half the amount of dyskinesia that were observed with an optimal dose of levodopa.[47] It should be noted that the antidyskinetic effect of KW-6002 remains unclear at this point. Although several animal studies support an antidyskinetic effect, a recent clinical trial has shown an increased incidence of dyskinesia.[50]

MAO-B IN THE CNS

Based on the nature of the cofactor, amine oxidases may be divided into two groups. The first group, the semicarbazide sensitive quinoprotein amine oxidases, possesses a quinone cofactor derived from a tyrosine residue and involves a cupric ion-dependent redox process.[63] The second group is the flavin adenine dinucleotide-containing amine oxidases, which include MAO-A, MAO-B and polyamine oxidases.[63] MAO-A and MAO-B are located on the outer mitochondrial membrane and, in contrast to polyamine oxidases, the FAD cofactors are covalently attached to the enzymes via a thio ether linkage between the side chain of a cysteinyl residue and the C8α-position of the flavin adenine dinucleotide.[64] In both MAO-A and MAO-B this cysteine is part of the conserved pentapeptide Ser-Gly-Gly-Cys-Tyr. MAO-A and MAO-B, that are products of different genes located on the X chromosome, consist of 527 and 520 amino acids, respectively, and have approximately 70% amino acid sequence identity. These data, together with identical exon-intron organization, suggest that the two isoforms are derived from a common ancestral gene.[65,66]

Due to their role in the catabolism of monoamine neurotransmitters in the CNS and peripheral tissues, MAO-A and MAO-B are of considerable pharmacological interest.[67] MAO-A preferentially catalyzes the oxidative deamination of serotonin and is irreversibly inhibited by low concentrations of clorgyline. MAO-B preferentially catalyzes the deamination of the false neurotransmitters benzylamine and β-phenylethylamine and is irreversibly inhibited by low concentrations of (*R*)-deprenyl.[67,68] Both isoforms catalyze the oxidation of dopamine, epinephrine, and norepinephrine.[14,67] Although MAO activity is present in most mammalian tissues, the two isoforms are expressed in a tissue-selective manner. For example, MAO-B is the main form in human liver tissue,[69] whereas MAO-A is the main form in human placental[70] and gut tissues.[71] Both isoforms are present in the human brain, although they are differently distributed[72] with MAO-B present in higher concentrations.[18,73] In subhuman primates, MAO-B also has been shown to be the dominant isoform in the brain.[74,75] Of particular interest is the observation that MAO-B is the prevalent form in the human basal ganglia.[11,73] Immunohistochemical studies have shown that MAO-B is predominantly located in the glial cells and serotonergic neurons,

whereas MAO-A is the predominantly located in catecholaminergic neurons.[72,76]

MAO-B INHIBITORS IN THE SYMPTOMATIC TREATMENT OF PD

Both MAO-A and MAO-B are important targets for the development of new drugs.[67] Because the MAO-B isoform appears to be predominantly responsible for dopamine metabolism in the human basal ganglia,[10,11] inhibitors of MAO-B have been used in the therapy of PD. Furthermore, MAO-B activity, as well as density,[15,16,73] has been shown to increase with age in most brain regions, including the basal ganglia. Because MAO-B is located in the glial cells, this increased activity may be attributed to an age-associated glial cell proliferation.[72,76,77] In contrast, MAO-A activity remains constant with age.[18] Considering that PD occurs predominantly in the elderly, inhibition of MAO-B in the brain may conserve the depleted supply of dopamine in the Parkinsonian brain and prolong the activity of dopamine derived from its metabolic precursor levodopa.[78] For example, MAO-B inhibitors have been shown to enhance the elevation of dopamine levels in the striatum of primates treated with levodopa.[12,13] This elevation was accompanied by a reduction in the oxidative metabolism of dopamine.[12] Accordingly, MAO-B inhibitors are currently recommended as adjunctive therapy in PD patients treated with levodopa.[9] In early PD, treatment with MAO-B inhibitors such as (*R*)-depenyl allows for a reduction in levodopa and dopamine agonist doses and delays the emergence of disabilities that require the initiation of levodopa therapy (Table 2).[79,80] It should be noted that both MAO-A and MAO-B contribute to oxidation of dopamine in the primate brain. Even though MAO-A activity is much lower than MAO-B activity in the striatum, the extent by which the MAO-A selective inactivator (i.e., clorgyline) enhances the elevation of dopamine levels in the striatum of primates treated with levodopa, it is similar to that obtained with (*R*)-deprenyl.[12] Therefore, it has been suggested that MAO-A/B mixed inhibitors may be more efficacious in the inhibition of dopamine metabolism and the treatment of PD.[14]

Table 2. *Summary of the Anti-Parkinsonian Effects of MAO-B Inhibitors*

Antisymptomatic
1. Enhance the elevation of dopamine levels in primates treated with levodopa[12,13]
2. Allow for the reduction of levodopa and dopamine agonist dose[79,80]
3. Delay the emergence of disability that require levodopa treatment[79,80]
4. Enhance the motor restorative effects of dopamine agonists[81]
Neuroprotective
1. (*R*)-Deprenyl and rasagiline may slow the progression of the symptoms of PD[83,84]
2. Protect against the neurotoxic effects of MPTP[94]

MAO = monoamine oxidase; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

In the United States, two irreversible MAO-B inhibitors, (*R*)-deprenyl and rasagiline, are approved as monotherapy or adjunctive therapy for the symptomatic treatment of PD.[9] The reversible inhibitor safinamide, currently in phase III development, has shown significant improvement of motor scores when co-administered with dopamine agonist drugs.[81] Another reversible MAO-B inhibitor, lazabemide, was shown to delay the need for levodopa in early untreated PD. The benefits conferred by lazabemide were similar to those observed after one year of (*R*)-deprenyl treatment.[82]

MAO-B INHIBITORS AND NEUROPROTECTION

An important goal of PD therapy is the preservation of the dopaminergic nigrostriatal neurons by protecting against underlying neurodegenerative processes. Although no current treatment has demonstrated a neuroprotective effect, clinical and preclinical results suggest that MAO-B inhibitors may delay disease progression in early PD.[9] For example, in a seven-year study, (*R*)-deprenyl, in combination with levodopa or as monotherapy in PD patients, has been shown to slow disease progression compared to placebo-treated counterparts.[83] Similarly, the results of a one-year trial with rasagiline as monotherapy in PD patients were compatible with a neuroprotective effect in addition to symptomatic benefits.[84]

The neuroprotective effect of MAO-B inhibitors may be explained, in part, by considering the metabolic byproducts generated by the action of MAO-B on monoamines. In the catalytic cycle of MAO-B, one mole each of an iminiumyl intermediate that is hydrolyzed to the aldehyde product and H_2O_2 are produced for each mole of monoamine substrate oxidized (FIG. 2). These catabolic products may be neurotoxic if not rapidly inactivated by centrally located aldehyde dehydrogenase (ADH)[85–87] and glutathione peroxidase,[88] respectively. When H_2O_2 accumulates it may react in the Fenton reaction with ferrous ion to generate the highly reactive hydroxyl radical.[14] The hydroxyl radical damages virtually all types of biomolecules including proteins, DNA, lipids, carbohydrates, and amino acids. The reduction of the toxic metabolic byproducts of MAO-B is especially relevant in PD when considering the age-dependent increase in brain MAO-B activity and iron content. Furthermore, the levels of glutathione, the electron donor for the reduction of H_2O_2 by glutathione peroxidase, may be lowered in the Parkinsonian brain.[89] Furthermore, the expression of ADH was found to be reduced in the

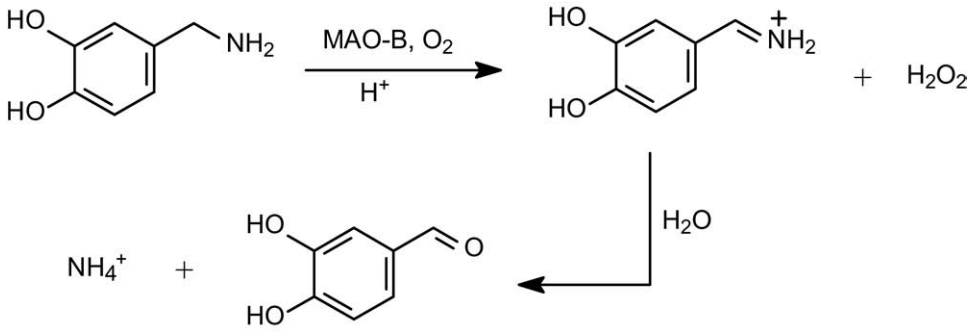


FIG. 2. The monoamine oxidase (MAO)-B catalyzed oxidation of dopamine to yield one mole each of the iminiumyl intermediate (that is hydrolyzed to the aldehyde product), H_2O_2, and NH_4^+.

substantia nigra of PD patients.[90] This suggests that a deficiency of ADH activity in the CNS could allow for the accumulation of the toxic aldehyde species generated by the action of MAO-B on monoamines. Therefore, inhibitors of MAO-B may exert a neuroprotective effect by stoichiometrically decreasing aminyl-derived aldehyde and H_2O_2 production in the brain.

A second, more theoretical rationale for the use of MAO-B inhibitors as neuroprotective agents arises from the fact that MAO-B has been implicated in neurodegenerative processes resulting from exposure to xenobiotic amines. The first step of the bioactivation of the Parkinsonian-inducing pro-neurotoxin MPTP is catalyzed by MAO-B.[91] The ultimate product, 1-methyl-4-phenylpyridinium (MPP^+), is a mitochondrial toxin that causes selective degeneration of nigrostriatal dopaminergic neurons in humans and experimental animals.[92,93] Inhibitors of MAO-B protect against the neurotoxic effects of MPTP, an effect that is almost certainly linked to the blockade of the metabolic bioactivation of MPTP.[94] The finding that a small organic molecule induces Parkinsonism has raised the possibility of the existence of an endogenous or environmental toxin that may contribute to the etiology of PD.[95] Should such a putative neurotoxin require bioactivation by MAO-B, inhibitors may protect against idiopathic PD. To date no endogenous or environmental MPTP-like neurotoxin that is activated by MAO-B has been shown to contribute to the etiology of PD.

It should be noted that the neuroprotective effects conferred by propargyl-derived MAO-B inhibitors may involve unknown pathways that are independent of MAO-B inhibition. Laboratory evidence suggests that antiapoptotic[96–98] and antioxidant[99,100] mechanisms may contribute to the neuroprotective properties of (*R*)-deprenyl and rasagiline.

METHYLXANTHINES AS A_{2A} ANTAGONISTS

The natural methylxanthinyl derivative, caffeine, is arguably the world's most widely consumed psychoactive dietary component.[101] Although caffeine exhibits only moderate A_{2A} antagonism properties with virtually no selectivity between the A_1 and A_{2A} receptor subtypes,[25] it provided a template for the development of the first selective A_{2A} receptor antagonists. Structure-activity relationship studies have demonstrated that substitution of the xanthine ring at C-8 with a diverse set of functional groups yields compounds endowed with more potent and selective adenosine-receptor antagonistic properties than caffeine.[102] For example, 8-cyclopentyl-1,3-dipropylxanthine is a potent and selective A_1 antagonist and is used as a reference antagonist in pharmacological and biochemical studies.[103] Of particular importance was the discovery that substitution at C-8 with a styryl functional group yielded potent and selective A_{2A} antagonists.[24,46,102,103] Therefore, a large portion of the of reported A_{2A} antagonists are 1,3-dimethyl, 1,3-diethyl or 1,3-dipropyl substituted xanthinyl analogues bearing an (*E*)-8-styryl moiety modified on the phenyl ring.

Of the (*E*)-8-styrylcaffeinyl-derived A_{2A} antagonists, KW-6002 is of particular importance because, as mentioned previously, this compound is undergoing clinical trials as a novel, nondopaminergic agent for the treatment of PD.[42,104] KW-6002, as derived from the prototype (*E*)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KF-17837) (**4**),[105] demonstrated superior bioavailability after oral administration.[46] KF-17837 is also reported to ameliorate motor deficits in experimental animals by virtue of antagonizing A_{2A} receptors.[106] Another xanthinyl derivative, CSC is commercially available and is used extensively as a reference A_{2A} antagonist in pharmacological studies.[24,25] Replacement of the styryl phenyl ring with a heterocyclic system as in the case of (*E*)-1,3-dipropyl-7-methyl-8-[2-(3-thienyl)ethenyl)]xanthine (DPMTX) (**5**) also produces a series of potent and selective A_{2A} antagonists.[107] Structure-activity relationship studies have shown that alkylation at N-7 of the xanthine and the *trans* geometry about the styryl double bond are structural features that are important for potent A_{2A} antagonism (Table 3).[104]

Table 3. *Binding Constants for the Antagonism of A_{2A} Receptors and the Inhibition of MAO-B by Caffeine Derivatives*

	K_i (nM)	
	A_{2A} Receptor	MAO-B
Caffeine (**3**)	22000[25]	3.6 mM
CSC (**1**)	36,[25] 54,[24] 30.2[110]	80.6[110]
KW-6002 (**2**)	2.2,[46] 4.46[110]	28000[27]
(*E*)-8-Styrylcaffeine (**6**)	94[24]	2864[28]
Structure **7**	104[110]	42.1[110]
Structure **8**	153[110]	148.6[110]
Structure **9**	2.74[110]	Weak inhibitor[110]

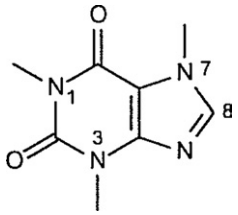
CSC = (*E*)-8-(3-Chlorostyryl)caffeine; MAO = monoamine oxidase.

METHYLXANTHINES AS DUAL-TARGET-DIRECTED DRUGS

Among the A_{2A} antagonists that have been demonstrated to protect animals against the neurotoxic effects of MPTP, is CSC. As part of an investigation to determine the mechanism by which CSC protects mice against the neurotoxic effects of MPTP, it was discovered that, in addition to being a potent and selective A_{2A} antagonist, CSC also acted as a highly potent, competitive, and reversible inhibitor of MAO-B.[26] This finding has raised the possibility of designing dual-target-directed drugs that block at both A_{2A} receptors and at MAO-B. To determine the structural requirements necessary for methylxanthines to act as MAO-B inhibitors, various substituted methylxanthines were evaluated as MAO-B inhibitors.[27,28,108,109] Analogous to what has been observed with A_{2A} antagonists, substitution of the caffeine ring at C-8 with a variety of groups yielded compounds endowed with more potent MAO-B inhibition activities than caffeine. Also, analogous to A_{2A} antagonists, the styryl side chain was found to be especially efficient in enhancing the MAO-B inhibition potency of caffeine-derived inhibitors. For example, CSC inhibited baboon liver MAO-B with a K_i value of 80.6 nM, approximately 45,000 times more potent than caffeine (K_i = 3.6 mM).[110] The K_i value for the inhibition of MAO-B by CSC is comparable to that reported for the antagonism of A_{2A} receptors (K_i = 36–54 nM).[24,25] Structure-activity relationship studies further revealed that an electron-deficient styryl side chain was more effective in enhancing MAO-B inhibition potency and that the *trans* geometry about the styryl double bond is a requirement for MAO-B inhibition.[27,28] Saturation of the styryl double bond has a negative effect on inhibition potency.[27] This supports the observation that many MAO-B inhibitors contain planar conjugated heterocyclic systems. Of significance was the finding that ethyl substitution at positions 1 and 3 of the caffeinyl ring has a negative effect on the potency of MAO-B inhibition compared to methyl substitution.[27,110] This represents a limitation in the development of caffeine-derived, dual-target-directed drugs because, in general, 1,3-diethyl substitution of the caffeine ring leads to enhanced A_{2A} antagonism.[25,46,110] Although 1, 3, and 7 methyl substitution is probably optimal for the design of xanthine-based reversible MAO-B inhibitors, ethyl or propyl functional groups at C-1 and C-3 are optimal for A_{2A} antagonism. Accordingly, the potent A_{2A} antagonist KW-6002 was found to be a relatively weak MAO-B inhibitor with a K_i value of 28 μM.[27] As for A_{2A} antagonists,[24] alkylation at N-7 of the xanthine ring is also a requirement for potent MAO-B inhibition (Table 4).[27]

The potency of MAO-B inhibition by (*E*)-8-styrylcaffeinyl analogues may possibly be explained by inspecting the crystal structure of human recombinant MAO-B.[111–113] The active site of the enzyme consists of an entrance connected to the substrate cavity. Relatively large inhibitors, such as the reversible inhibitor 1,4-diphenyl-2-butene, exhibit a dual-binding mode that involves traversing both the entrance and substrate cavities.[112] (*E*)-8-Styrylcaffeines probably exhibit a similar mode of binding with the caffeinyl ring located in the substrate cavity of the active site, whereas the styryl substituent extends into the entrance cavity.[28,114] Without the styryl side chain, caffeine is expected to bind to either the substrate or the entrance cavity leaving the other cavity unoccupied. Therefore, the dual binding

Table 4. *Summary of the Known Structural Requirements of Caffeinyl Analogues to Act as A_{2A} Antagonists and MAO-B Inhibitors*



Structural features enhancing both A_{2A} antagonism and MAO-B inhibition
1. Styryl substitution at C-8 of caffeine
2. Phenylbutadienyl substitution at C-8 of caffeine
3. *Trans* geometry about the styryl double bond
4. Methylation at N-7 of the xanthinyl ring

Structural features optimal for A_{2A} antagonism but not MAO-B inhibition
1. Diethyl or dipropyl substitution at C-1 and C-3 of the xanthinyl ring

Structural features optimal for MAO-B inhibition but not A_{2A} antagonism
1. Dimethyl substitution at C-1 and C-3 of the xanthinyl ring
2. Electron deficient styryl ring

MAO = monoamine oxidase.

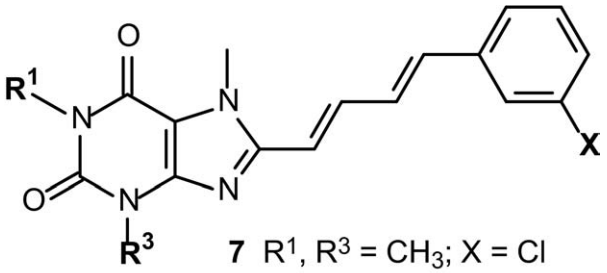


FIG. 3. The chemical structures of (*E*,*E*)-8-(4-phenylbutadien-1-yl) caffeine analogues **7–9**.

mode of the styryl side chain possibly enhances the interactions between the inhibitor and the active site amino acid residues and hence the binding affinity. The finding that caffeine is a weak MAO-B inhibitor is in support of this hypothesis.

In a recent study it was shown that substitution with the phenylbutadienyl side chain at C-8 may be more effective than the styryl side chain in promoting binding between the active site of MAO-B and caffeine (FIG. 3).[110] For example, (*E*,*E*)-8-[4-(3-chlorophenyl)butadien-1-yl]caffeine (**7**) (K_i = 42.1 nM) was approximately 1.9 times more potent as an MAO-B inhibitor than CSC (K_i = 80.6 nM), whereas (*E*,*E*)-8-(4-phenylbutadien-1-yl)caffeine (**8**) (K_i = 148.6 nM) was almost 20 times more potent than the corresponding unsubstituted (*E*)-8-styrylcaffeine (**6**) (K_i = 2864 nM).[28] The finding that (*E*,*E*)-8-(4-phenylbutadien-1-yl) caffeine analogues also act as potent A_{2A} antagonists suggest that they may be promising lead compounds for the development of dual-target-directed drugs. However, the observation that 1,3-diethyl substitution decreases MAO-inhibitory properties while being required for potent A_{2A} antagonism also applies to (*E*,*E*)-8-(4-phenylbutadien-1-yl) caffeines (compare structure **8** with **9**).

CONCLUSIONS

Because of the multifactorial nature of PD, several molecular drug targets and treatment strategies have been pursued.[3] Some therapies provide relief of PD symptoms, whereas other therapies are aimed at protecting against the underlying neurodegenerative processes. Therapies that act at multiple targets and provide both symptomatic and neuroprotective benefits, in principle, may be more effective in treating complex neurodegenerative diseases such as PD.[115] Because A_{2A} antagonists and MAO-B inhibitors potentiate the motor restorative effects of levodopa by acting at different targets, the combination of these two activities in a single drug may be particularly advantageous as an adjunct to levodopa therapy. The involvement of the A_{2A} receptor and MAO-B in neuroprotection suggests that dual-target-directed drugs also may exhibit enhanced neuroprotective properties. Although a number of methylxanthines have been shown to act as A_{2A} antagonists and MAO-B inhibitors, optimizing the structures for dual action remains a challenge because modifications that lead to enhanced A_{2A} antagonism frequently have the opposite effect on MAO-B inhibition and vice versa.

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