Novel sulfanyl- and sulfinylcaffeine analogues as inhibitors of monoamine oxidase

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B.Pharm

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Abstract

Keywords

8-Sulfanylcaffeine analogues, 8-sulfinylcaffeine analogues, caffeine, monoamine oxidase inhibitors, Parkinson's disease.

Parkinson's disease (PD) is a neurodegenerative disorder, which is progressive in nature and usually associated with the elderly. It is the second most common age-related neurodegenerative disorder after Alzheimer's disease (AD). PD occurs when there is a dramatic loss of dopamine (DA) in the striatum, a substructure of the basal ganglia, of the brain due to the degeneration of the nigrostriatal pathway that contains the dopaminergic neurons. Motor symptoms of PD include bradykinesia, muscular rigidity and resting tremors. Non-motor symptoms include speech and sleep problems, hallucinations and depression. Diverse treatment options are available to treat the symptoms of PD, including levodopa (L-Dopa), DA agonists and monoamine oxidase B (MAO-B) inhibitors.

The MAOs are flavoproteins that are bound to the outer membrane of the mitochondria and catalyze the oxidative deamination of neurotransmitters such as serotonin (5-HT), noradrenaline (NA) and DA. Two isoforms occur, namely MAO-A and –B, which share a 70% sequence identity. MAO-A catalyzes the oxidation of 5-HT and MAO-B has a substrate specificity towards benzylamine and 2-phenylethylamine. DA, NA, adrenaline and tryptamine are oxidized by both forms. MAO-A plays an important role in depression while MAO-B plays an important role in PD. The two isoforms are not evenly distributed in the brain. Of particular relevance to PD is the observation that, in the basal ganglia, MAO-B is the predominant form and the oxidation of DA in this region is largely due to MAO-B activity. Also, with an increase in age, there is an up to fourfold increase in MAO-B activity in the brain. In the aged parkinsonian brain, MAO-B is therefore a major DA metabolizing enzyme and MAO-B inhibitors have an important role in the therapy of PD. MAO-B inhibitors may potentially reduce the metabolic destruction of DA and thereby provide relief from the symptoms of PD. MAO-B inhibitors may also exert a neuroprotective effect in PD. In the catalytic cycle of MAO-B, one mole each of an aldehyde, hydrogen peroxide and ammonia are formed for each mole of primary amine substrate oxidized. Ferrous iron, which is abundant in the basal ganglia, may react with the hydrogen peroxide to form hydroxyl radicals in the Fenton reaction. The hydroxyl radical damages virtually all types of biomolecules including proteins, DNA, lipids, carbohydrates and amino acids. The aldehyde, in

turn, may react with amino groups of proteins, and thus lead to cell injury. Inhibitors of MAO-B may reduce the MAO-catalyzed formation of hydrogen peroxide and aldehydes in the basal ganglia, and thus act as neuroprotective agents.

MAO-B inhibitors that are currently being used in the treatment of PD are selegiline and rasagiline. Both are irreversible inhibitors of MAO-B. While irreversible inhibitors of MAO have been used extensively as drugs, irreversible inhibition has a number of disadvantages. These include the loss of selectivity as a result of repeated drug administration and a slow and variable rate of enzyme recovery following termination of drug treatment. The turnover rate for the biosynthesis of MAO-B in the human brain may require as much as 40 days while with reversible inhibition, enzyme activity is recovered when the inhibitor is eliminated from the tissues. For these reasons the discovery of novel MAO-B inhibitors, which interact reversibly with the enzymes are of value in the therapy of PD.

The goal of this study was to design novel and reversible inhibitors of MAO-B, which may find application in the therapy of PD. In the current study, caffeine was used as scaffold for the design of new MAO inhibitors. Caffeine is reported to be a weak inhibitor of MAO-B, with an IC_{50} value of 5084 µM. Substitution at C-8 of the caffeine moiety, however, yields compounds with potent MAO-B inhibitory properties. Of particular importance to this study is a recent report that a series of 8-sulfanylcaffeine analogues acts as selective inhibitors of human MAO-B. Among the compounds examined, 8-[(phenylethyl)sulfanyl]caffeine was found to be a particularly potent MAO-B inhibitor with an IC_{50} value of 0.223 µM. In an attempt to further enhance the MAO-B inhibition potency of 8-[(phenylethyl)sulfanyl]caffeine, and possibly to discover highly potent MAO-B inhibitors, a series of five 8-[(phenylethyl)sulfanyl]caffeine analogues was synthesized and evaluated as inhibitors of human MAO-A and –B. For the purpose of this study 8-[(phenylethyl)sulfanyl]caffeine homologues containing C-3 alkyl (CF_3, CH_3 and OCH_3) and halogen (Cl and Br) substituents on the phenyl ring were considered. Furthermore, a series of two 8-sulfinylcaffeine analogues and one 8-sulfonylcaffeine were synthesized and their MAO inhibitory potencies were measured. The purpose with these compounds was to compare the MAO inhibitory properties of the 8-sulfinylcaffeine analogues and 8-sulfonylcaffeine with those of the 8-sulfanylcaffeine analogues. This study also investigates the MAO inhibition properties of three selected 8-[(phenylpropyl)sulfanyl]caffeine and two 8-(benzylsulfanyl)caffeine analogues.

Chemistry: The target 8-sulfanylcaffeine analogues were synthesized according to the literature procedure. 8-Chlorocaffeine was reacted with an appropriate mercaptan in the presence of

NaOH, to yield the target 8-sulfanylcaffeine analogues in yields of 6.4–50.7%. 8-Chlorocaffeine, in turn, was conveniently synthesized in high yield by reacting chlorine with caffeine in chloroform. In certain instances, the mercaptan starting materials were not commercially available and were thus synthesized according to the literature procedure by reacting an appropriate alkylbromide with thiourea. The resulting thiouronium salt was hydrolyzed in the presence of NaOH to yield the target mercaptan. The 8-sulfinylcaffeine analogues and 8-sulfonylcaffeine were synthesized by reacting the 8-sulfanylcaffeines with H_2O_2 in the presence of glacial acetic acid and acetic anhydride. The structures and the purities of the inhibitors were verified by NMR, MS and HPLC analyses.

MAO inhibition studies: The MAO inhibitory properties of the test compounds were examined using the recombinant human enzymes. The mixed MAO-A/B substrate, kynuramine, was employed as substrate for both enzymes and the inhibition potencies were expressed as the IC_{50} values.

The 8-[(phenylethyl)sulfanyl]caffeine analogues were found to be highly potent inhibitors of MAO-B. The IC_{50} values recorded for these homologues ranged from 0.017–0.125 µM, making them twofold to 13-fold more potent MAO-B inhibitors than the lead compound, 8-[(phenylethyl)sulfanyl]caffeine (IC_{50} = 0.223 µM). For comparison, the reversible MAO-B selective inhibitor, lazabemide, exhibits an IC_{50} value of 0.091 µM under the same conditions (unpublished data from our laboratory). Interestingly, both alkyl (CF_3, CH_3 and OCH_3) and halogen (Cl and Br) substitution lead to highly potent MAO-B inhibition. It may therefore be concluded that substitution on C-3 is a general strategy to enhance the MAO-B inhibition potency of 8-[(phenylethyl)sulfanyl]caffeine. The results of the MAO inhibitory studies with the 8-[(phenylpropyl)sulfanyl]caffeine analogues showed that these compounds are also inhibitors of MAO-B with IC_{50} values of 0.061–0.500 µM. Those homologues substituted with chlorine on the *para* and *meta* positions of the phenyl ring were found to be exceptionally potent inhibitors with IC_{50} values of 0.061 µM and 0.062 µM, respectively. For the series of 8-(benzylsulfanyl)caffeines, *meta* substitution with chlorine (IC_{50} = 0.227 µM) and bromine (IC_{50} = 0.199 µM) was also found to enhance the MAO-B inhibition potency of 8-(benzylsulfanyl)caffeine (IC_{50} = 1.86 µM). The results document that the 8-sulfinylcaffeines are also inhibitors of MAO-B with IC_{50} values of 11.8–131 µM. The 8-sulfonylcaffeine was also found to be a MAO-B inhibitor. Compared to the 8-sulfanylcaffeines, these homologues are, however, weaker inhibitors. It may, therefore, be concluded that 8-sulfinylcaffeines and 8-sulfonylcaffeines

are comparatively weak MAO-B inhibitors and less suited for the design of high potency MAO-B inhibitors.

The results also document that the 8-[(phenylethyl)sulfanyl]caffeines are relatively weak MAO-A inhibitors with IC_{50} values of 5.66–141 µM, with one homologue exhibiting no inhibition under the experimental conditions. As evident from the selectivity indices (SI values), the 8-[(phenylethyl)sulfanyl]caffeines were all selective inhibitors of the MAO-B isoform. Two compounds exhibited SI values in excess of 1000. Since these compounds are also highly potent MAO-B inhibitors, they represent suitable leads for the design of potent and selective MAO-B inhibitors. The 8-sulfinylcaffeines and 8-sulfonylcaffeine were found to be weak MAO-A inhibitors with IC_{50} values of 166–250 µM. The SI values demonstrate that these compounds are MAO-B selective inhibitors, although to a lesser degree than the 8-[(phenylethyl)sulfanyl]caffeines. The 8-[(phenylpropyl)sulfanyl]caffeines are also MAO-A inhibitors with IC_{50} values of 0.708–6.48 µM. It is noteworthy that these homologues are the most potent MAO-A inhibitors among the compounds evaluated in this study. In fact, one of the 8-[(phenylpropyl)sulfanyl]caffeines, 8-{[3-(4-chlorophenyl)propyl]sulfanyl}caffeine (IC_{50} = 0.708 µM), is the only compound with an IC_{50} value for the inhibition of MAO-A in the submicromolar range. The 8-[(phenylpropyl)sulfanyl]caffeines display, in general, lower degrees of selectivity for MAO-B than the corresponding 8-[(phenylethyl)sulfanyl]caffeines.

Reversibility studies: The reversibility of the interaction of a representative inhibitor, 8-{[2-(3-(trifluoromethyl)phenyl)ethyl]sulfanyl}caffeine, with MAO-B was investigated by evaluating the recovery of the enzymatic activity after dilution of the enzyme-inhibitor complex. For this purpose, MAO-B was preincubated with the test compound at concentrations of 10 × IC_{50} and 100 × IC_{50} for 30 min. The reactions were subsequently diluted 100-fold to 0.1 × IC_{50} and 1 × IC_{50}, respectively. The results show that, after dilution to 0.1 × IC_{50} and 1 × IC_{50}, the MAO-B catalytic activities are recovered to 35% and 22%, respectively, of the control value. For reversible enzyme inhibition, the enzyme activities are expected to recover to levels of approximately 90% and 50%, respectively, after 100-fold dilution of the preincubations containing inhibitor concentrations of 10 × IC_{50} and 100 × IC_{50}. After preincubation of MAO-B with the irreversible inhibitor (R)-deprenyl (at 10 × IC_{50}), and dilution of the resulting complex to 0.1 × IC_{50}, MAO-B activity is not recovered (3.0% of control). These data indicate that the test compound does indeed react reversibly with MAO-B but because enzyme activities are not recovered to the expected 90% and 50% respectively, it may suggest that the test compound possess a quasi-reversible or tight-binding component.

Hansch-type structure activity relationship studies: A limited Hansch-type QSAR study was performed for the inhibition of MAO by the 8-[(phenylethyl)sulfanyl]caffeines. For this purpose, five parameters were used to describe the physicochemical properties of the C-3 substituents on the phenyl rings of the inhibitors. The Van der Waals volume (V_w) and Taft steric parameter (E_s) served as descriptors of the bulkiness of the substituents, while the lipophilicities were described by the Hansch constant (π). The electronic properties were described by the classical Hammett constant (σ_m) and the Swain-Lupton constant (F). A one-parameter fit with the Taft steric parameter versus the inhibition potency ($\log IC_{50}$) yielded the best correlation with a correlation coefficient (R^2) of 0.912 and a statistical *F* value of 41.27 (F_{max} = 35). The positive sign of the E_s (+0.47) parameter coefficient indicated that the inhibition potencies of the 8-[(phenylethyl)sulfanyl]caffeines towards MAO-B may be enhanced by substitution with sterically large groups at C-3 of the phenyl rings of the inhibitors.

OPSOMMING

Kernwoorde

8-sulfanielkafeïen-analoë, 8-sulfinielkafeïen-analoë, kafeïen, monoamien oksidase inhibeerders, Parkinson se siekte.

Parkinson se siekte (PS) is ’n neurodegeneratiewe versteuring wat progressief van aard is en wat gewoonlik met bejaardes geassosieer word. Dit is die tweede mees algemene ouderdomsverwante neurodegeneratiewe versteuring naas Alzheimer se siekte (AS). PS kom voor wanneer daar ’n dramatiese verlies van dopamien (DA) in die striatum, wat ’n substruktuur van die basale ganglia is, plaasvind, as gevolg van die degenerasie van die nigrostriatale weg, wat dopaminergiese neurone bevat. Motoriese simptome van PS sluit in, bradikinese, spierrigiditeit en bewing tydens rus. Nie-motoriese simptome sluit in, spraak- en slaapprobleme, hallusinasies en depressie. Diverse behandelingsopsies is beskikbaar vir die behandeling van simptome wat gepaardgaan met PS. Dit sluit in behandeling met levodopa (L-Dopa), DA agoniste en monoamien oksidase B (MAO-B) inhibeerders.

Die MAO’e is flavoproteïene wat aan die buitenste membraan van die mitochondria gebind is en wat die oksidatiewe deaminasie van neuro-oordragstowwe, soos serotonien (5-HT), noradrenalien (NA) en DA kataliseer. Twee verskillende vorme van die ensiem, naamlik MAO-A en –B, wat ’n 70% ooreenkoms in aminosuurvolgorde vertoon, kom voor. MAO-A kataliseer die oksidasie van 5-HT en MAO-B toon substraatspesifisiteit teenoor bensielamien en 2-fenieletielamien. DA, NA, adrenalien en triptamien word deur beide vorme geoksideer. MAO-A speel ‘n belangrike rol in depressie terwyl MAO-B ‘n belangrike rol in PS speel. Die twee vorme van die ensiem is nie eweredig in die brein versprei nie. Ten opsigte van PS, is die waarneming dat MAO-B die oorheersende vorm in die basale ganglia is en dat oksidasie van DA in hierdie area grootliks aan die MAO-B-aktiwiteit toegeskryf kan word, uiters relevant. Met verhoogde ouderdom is daar tot soveel as ’n viervoudige toename van die MAO-B-aktiwiteit in die brein. In die verouderde Parkinson-brein is MAO-B dus die belangrikste DA-metaboliserende ensiem en derhalwe speel MAO-B-remmers ’n belangrike rol in PS-terapie. MAO-B-remmers kan potensieel die metaboliese afbraak van DA verminder en daarmee die simptome verlig wat gepaardgaan met PS. MAO-B-remmers kan ook ’n neurobeskermende effek hê wat PS betref. In die katalitiese siklus van MAO-B, word daar een mol elk van ’n aldehied en waterstofperoksied, sowel as ammoniak, tydens die oksidasie van een mol van ’n primêre

amiensubstraat gevorm. Tydens die Fenton-reaksie, mag Fe^{2+}, wat volop in die basale ganglia voorkom, met die waterstofperoksied reageer om hidroksielradikale te vorm. Die hidroksielradikale beskadig feitlik alle tipes biomolekules, insluitend, proteïne, DNS, lipiede, koolhidrate en aminosure. Die aldehied, op sy beurt, mag met die aminogroepe van proteïne reageer en dus tot selbeskadiging lei. Remmers van MAO-B kan die MAO-gekataliseerde vorming van waterstofperoksied en aldehiede in die basale ganglia verminder en dus as neurobeskermende verbindings optree.

MAO-B-remmers wat tans vir die behandeling van PS gebruik word, is selegilien en rasagiline. Beide is onomkeerbare remmers van MAO-B. Alhoewel onomkeerbare remmers van MAO al baie as geneesmiddels gebruik is, het hierdie tipe inhibisie talle nadele. Dit sluit in die verlies van selektiwiteit, as gevolg van herhaalde geneesmiddeltoediening en 'n stadige sowel as veranderlike koers van ensiemherstel na beëindiging van die geneesmiddelbehandeling. Die omsetsnelheid, vir die biosintese van MAO-B in die menslike brein, mag so lank as 40 dae neem, terwyl ensiemaktiwiteit, met omkeerbare inhibisie, herwin word sodra die remmer geëlimineer word uit die weefsel. Om hierdie redes is die ontdekking van nuwe, omkeerbare MAO-B-remmers van groot waarde in die terapie van PS.

Die doel van hierdie studie was om nuwe en omkeerbare remmers van MAO-B te ontwerp wat van toepassing kan wees in die terapie van PS. In die huidige studie is kafeïen gebruik as 'n uitgangsverbinding vir die ontwerp van nuwe MAO-remmers. Kafeïen is volgens die literatuur 'n swak remmer van MAO-B, met 'n IC_{50}-waarde van 5084 µM. Substitusie op C-8 van die kafeïenmolekule lewer verbindings met potente MAO-B-inhiberende eienskappe. Wat van besonderse belang vir hierdie studie is, is 'n onlangse verslag waarin 'n reeks 8-sulfanielkafeïen-analoë geïdentifiseer is as selektiewe remmers van menslike MAO-B. Onder hierdie verbindings wat ondersoek is, is gevind dat 8-[(fenieletiel)sulfaniel]kafeïen 'n besonderse kragtige remmer van MAO-B is met 'n IC_{50}-waarde van 0.223 µM. In 'n poging om die inhibisie teenoor MAO-B verder te versterk en om moontlik nuwe, hoogs potente remmers van MAO-B te ontdek, is 'n reeks van vyf 8-[(fenieletiel)sulfaniel]kafeïen-analoë gesintetiseer en as remmers van menslike MAO-A en -B geëvalueer. Vir die doel van hierdie studie, is 8-[(fenieletiel)sulfaniel]kafeïen-homoloë, met alkiel- (CF_3, CH_3 en OCH_3)- en halogeen- (Cl en Br)- substituente op die fenielring oorweeg. Verder is daar 'n reeks van twee 8-sulfinielkafeïen-analoë en een 8-sulfonielkafeïen gesintetiseer en as remmers van MAO geëvalueer. Die doel met hierdie verbindings was om die MAO-inhiberende eienskappe van die 8-sulfinielkafeïen-analoë en 8-sulfonielkafeïen met dié van die 8-sulfanielkafeïen-analoë te vergelyk. Hierdie studie ondersoek ook die inhiberende

eienskappe van drie geselekteerde 8-[(fenielpropiel)sulfaniel]kafeïen- en twee 8-(bensielsulfaniel)kafeïen-analoë teenoor MAO.

Chemie: Die teiken-8-sulfanielkafeïen-analoë is volgens die literatuurprosedure gesintetiseer. 8-Chlorokafeïen is met 'n toepaslike merkaptaan, in die teenwoordigheid van NaOH, gereageer om die teiken 8-sulfanielkafeïen-analoë te lewer met opbrengste van 6.40-50.67%. 8-Chlorokafeïen, op sy beurt, is met 'n hoë opbrengs gesintetiseer deur chloor met kafeïen, in chloroform te laat reageer. In sommige gevalle was die merkaptaan-aanvangsmateriaal nie kommersieel beskikbaar nie, en moes dit dus gesintetiseer word volgens die literatuurprosedure, deur 'n toepaslike alkielbromied met tioüreum te laat reageer. Die gevormde tioüroniumsout is in die teenwoordigheid van NaOH gehidroliseer om die teiken-merkaptaan te lewer. Die 8-sulfinielkafeïen-analoë en die 8-sulfonielkafeïen is gesintetiseer deur die 8-sulfanielkafeïne, met H_2O_2, in die teenwoordigheid van ysasynsuur en asynsuuranhidried te laat reageer. Die strukture en suiwerheid van die remmers is almal deur KMR-, MS- en HPLC-analise geverifieer.

MAO-inhibisiestudies: Die MAO-inhiberende eienskappe vir die remmers is ondersoek deur gebruik te maak van rekombinante menslike ensieme. Die gemengde MAO-A/B substraat, kinuramien, is as substraat vir beide ensieme gebruik en die inhibisiesterkte is as IC_{50}-waardes uitgedruk.

Dit is gevind dat die 8-[(fenieletiel)sulfaniel]kafeïen-analoë baie goeie MAO-B-remmers is. Die IC_{50}-waardes wat vir hierdie homoloë opgeteken is, het van 0.017-0.125 µM gewissel, wat aantoon dat hulle 2 keer tot dertien keer meer potente MAO-B-remmers is as die uitgangsverbinding, 8-[(fenieletiel]sulfaniel)kafeïen (IC_{50} = 0.223 µM). Ter vergelyking: die omkeerbare MAO-B selektiewe remmer, lasabemied, vertoon 'n IC_{50}-waarde van 0.091 µM onder dieselfde toestande (ongepubliseerde data vanuit ons laboratorium). Interessant genoeg, lei beide alkiel- (CF_3, CH_3 en OCH_3)- en halogeen- (Cl en Br)- substitusie tot hoogs potente MAO-B-remming. Dit kan dus afgelei word dat substitusie op C-3 'n algemene strategie is om die MAO-B-inhibisiesterkte van 8-[(fenieletiel]sulfaniel)kafeïen te verhoog. Die resultate van die MAO-inhiberende studies, met die 8-[(fenielpropiel)sulfaniel]kafeïen-analoë het getoon dat hierdie verbindings ook remmers van MAO-B is, met IC_{50}-waardes van 0.061-0.500 µM. Verbindings wat gesubstitueer is met chloor op die *para*- en *meta*-posisies van die fenielring, was besonder goeie remmers, met IC_{50}-waardes van 0.061 µM en 0.062 µM onderskeidelik. Vir die 8-(bensielsulfaniel)-kafeïenreeks, het *meta*-substitusie, met chloor (IC_{50} = 0.227 µM) en

broom (IC_{50} = 0.199 µM) sterker MAO-B-inhibisie getoon as 8-(bensielsulfaniel)kafeïen (IC_{50} = 1.86 µM). Die resultate dui daarop dat die 8-sulfinielkafeïene ook remmers van MAO-B is met IC_{50}-waardes van 11.8-131 µM. Die 8-sulfonielkafeïen was 'n swak remmer van MAO-B. Hierdie homoloë is egter swakker remmers as die 8-sulfanielkafeïene. Dit kan dus afgelei word dat die 8-sulfinielkafeïene relatiewe swak remmers van MAO-B is en minder geskik is vir die ontwerp van hoë sterkte MAO-B-remmers.

Die resultate toon ook dat die 8-[(fenieletiel)sulfaniel]kafeïene relatief swak MAO-A-remmers is met IC_{50}-waardes van 5.66-141 µM, met een homoloog wat geen inhibisie getoon het onder eksperimentele kondisies nie. Soos gesien kan word van die selektiwiteitsindekse (SI), was al die 8-[(fenieletiel)sulfaniel]kafeïne selektiewe remmers vir MAO-B. Twee verbindings het SI-waardes van meer as 1000 gehad. Aangesien hierdie verbindings ook hoogs potente MAO-B-remmers was, verteenwoordig hulle geskikte uitgangsverbindings vir die ontwerp van kragtige en selektiewe MAO-B-remmers. Daar is gevind dat die 8-sulfinielkafeïne en die 8-sulfonielkafeïne swak remmers van MAO-A is met IC_{50}-waardes van 166-250 µM. Die SI-waardes het getoon dat hul MAO-B-selektief is, maar in 'n mindere mate as die 8-[(fenieletiel)sulfaniel]kafeïne. Die 8-[(fenielpropiel)sulfaniel]kafeïne is ook remmers van MAO-A met IC_{50}-waardes van 0.708-6.48 µM. Dit is opmerklik dat hierdie homoloë die kragtigste MAO-A-remmers was tydens die evaluasie van hierdie studie. Trouens, een van die 8-[(fenielpropiel)sulfaniel]kafeïne, 8-{[3-(4-chlorofeniel)propiel]sulfaniel}kafeïen (IC_{50} = 0.708 µM), is die enigste verbinding met 'n IC_{50}-waarde vir MAO-A in die submikromolêre area. Die 8-[(fenielpropiel)sulfaniel]kafeïne vertoon, in die algemeen, laer selektiwiteit vir MAO-B as die ooreenstemmende 8-[(fenieletiel)sulfaniel]kafeïne.

Omkeerbaarheidstudies: Die omkeerbaarheid van die interaksie van 'n verteenwoordigende remmer, 8-{[2-(3-(trifluorometiel)feniel)etiel]sulfaniel}kafeïen, met MAO-B is ondersoek deur die evaluering van die ensimatiese herstel na verdunning van die ensiem-remmerkompleks. Vir hierdie doel is MAO-B met die toetsverbinding gepreïnkubeer by konsentrasies van 10 x IC_{50} en 100 x IC_{50} vir 30 min. Die reaksies is daarna tot 0.1 x IC_{50} en 1 x IC_{50} onderskeidelik verdun. Die resultate het getoon dat, na verdunning tot 0.1 x IC_{50} en 1 x IC_{50}, die MAO-B-katalitiese aktiwiteit herstel het tot 35% en 22% onderskeidelik van die kontrolewaarde. Vir omkeerbare ensieminhibisie, word daar verwag dat die ensiemaktiwiteite tot omtrent 90% en 50% onderskeidelik moet herstel, na 'n 100-voudige verdunning van die preïnkubasies met remmer-konsentrasies van 10 x IC_{50} en 100 x IC_{50}. Na preïnkubasie van MAO-B, met die onomkeerbare remmer, R-depreniel, (by 10 x IC_{50}), en die verdunning van die gevormde kompleks tot 0.1 x

IC_{50}, word MAO-B aktiwiteit nie herstel nie (3.0% van die kontrole). Hierdie data dui daarop dat die toetsverbinding wel omkeerbaar met MAO-B bind maar aangesien die ensiemaktiwiteite, na verdunning, nie na die verwagte 90% en 50% onderskeidelik herstel nie, kan dit daarop dui dat die toetsverbinding moontlik ‘n kwasi-omkeerbare of styfbindende komponent besit.

Hansch-tipe struktuuraktiwiteitsverwantskapstudies: ’n Beperkte Hansch-tipe QSAR-studie, vir die inhibisie van MAO-B deur die 8-[(fenieletiel)sulfaniel]kafeïen, is uitgevoer. Vir hierdie doel is vyf parameters gebruik om die fisies-chemiese eienskappe van die C-3-substituente op die fenielring van die remmers te beskryf. Die Van der Waals-volume (V_w) en die Taft steriese parameter (E_s) het gedien as beskrywers van die grootte van die substituente terwyl die lipofiliteit beskryf is deur die Hansch-konstante (π). Die elektroniese eienskappe is deur die klassieke Hammet-konstante (σ_m) en die Swain-Lupton-konstante (F) beskryf. ’n Enkel-parameterpassing, met die Taft steriese parameter teenoor die inhibisiesterkte ($\log IC_{50}$) het die beste korrelasie opgelewer met ’n korrelasiekoëffisiënt (R^2) van 0.912 en ’n statistiese F-waarde van 41.27 (F_{max} = 35). Die positiewe waarde van die E_s parameterkoëffisiënt (+0.47), dui daarop dat die inhibisiesterkte van die 8-[(fenieletiel)sulfaniel]kafeïne teenoor MAO-B versterk kan word deur substitusie met groot steriese groepe op C-3 van die fenielring.

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Abbreviations

4-HQ	- 4-Hydroxyquinoline
5-HT	- Serotonin or 5-hydroxytryptamine
AD	- Alzheimer’s disease
APCI	- Atmospheric-pressure chemical ionization
BBB	- Blood-brain barrier
CNS	- Central nervous system
COMT	- Catechol-O-methyl transferase
CSC	- (E)-8-(3-Chlorostyryl)caffeine
DA	- Dopamine
DAT	- Dopamine transporter
DMSO	- Dimethyl sulfoxide
DOPAC	- Dihydroxyphenylacetic acid
EI	- Electron ionization
FAD	- Flavin adenine dinucleotide
HPLC	- High performance liquid chromatography
HRMS	- High resolution mass spectra
L-Dopa	- Levodopa
LDL	- Low density lipoprotein
LRRK-2	- Leucine rich repeat kinase 2
MAO-A	- Monoamine oxidase A
MAO-B	- Monoamine oxidase B

MPPP	- 1-Methyl-4-phenyl-4-propionpiperidine
MPTP	- 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	- Mass spectra
NA	- Noradrenaline
NMR	- Nuclear magnetic resonance
PD	- Parkinson’s disease
SD	- Standard deviation
SET	- Single electron transfer
SI	- Selectivity index
SNpc	- Substantia nigra pars compacta