Syntheses of chalcones and 2-aminopyrimidines and their evaluation as monoamine oxidase inhibitors and as adenosine receptor antagonists

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ABSTRACT

Title

Syntheses of chalcones and 2-aminopyrimidines and their evaluation as monoamine oxidase inhibitors and as adenosine receptor antagonists

Keywords

2-Aminopyrimidines, adenosine A_{2A} antagonists, chalcones, monoamine oxidase inhibitors.

Background and rationale

Parkinson's disease is a neurodegenerative disorder characterised by reduced levels of dopamine in the brain. The cause of Parkinson's disease is still unknown; however several theories pertaining to the etiology exist. Current treatment mainly aims at dopamine replacement, with agents such as levodopa and dopamine agonists that provide patients with symptomatic relief. This relief is unfortunately only temporary as the progression of the disease is not halted. Furthermore, these therapies are associated with a range of side effects and novel approaches to the treatment are thus urgently required. Adenosine A_{2A} receptor antagonists recently emerged as a promising non-dopaminergic alternative, not only as symptomatic treatment, but also as potential neuroprotective therapy.

Adenosine A_{2A} receptors are co-localised with dopamine D_2 receptors in the striatum and other nuclei of the basal ganglia. Adenosine A_{2A} stimulation decreases the affinity of dopamine for the D_2 receptor, and increase cyclic AMP (cAMP) levels. The stimulation of dopamine D_2 receptors, in contrast, decreases cAMP levels and therefore these receptors (A_{2A} and D_2), act in an opposing manner. Adenosine A_{2A} antagonism will thus have similar effects as dopamine D_2 agonism and will reduce the postsynaptic effects of dopamine depletion to give symptomatic relief. There are also several mechanisms where by adenosine A_{2A} antagonists may be neuroprotective, for example by preventing glutamate excitotoxicity, that may cause damage to dopaminergic neurons. A number of adenosine A_{2A} antagonists have already reached clinical trials and promising results were obtained, especially when combined with levodopa. Consequently, A_{2A} antagonists are realistic prospects that have therapeutic potential in diseases with dopaminergic hypofunction, like Parkinson's disease. Many of the current A_{2A} antagonists contain an amino-substituted heterocyclic scaffold, such as an aminopyrimidine. The primary aim of this study was the design, synthesis and evaluation of 2-aminopyrimidine derivatives as adenosine A_{2A} receptor antagonists.

Monoamine oxidase B (MAO-B) inhibitors are also promising candidates for the symptomatic treatment of Parkinson's disease, since MAO-B is the enzyme primarily responsible for the

catabolism of dopamine in the brain. Irreversible inhibitors of MAO-B, such as selegeline and rasagiline, have been used clinically for the treatment of Parkinson's disease. This type of inhibition comes with certain disadvantages as it may take up to several weeks after termination of treatment for the enzyme activity to recover. Reversible inhibitors in contrast will have much better safety profiles seeing that they will not inactivate the enzyme permanently and allow for competition with the substrate.

When dopamine is oxidized by MAO, toxic metabolic by-products, such as hydrogen peroxide (H_2O_2) forms, and this is believed to be a possible cause of Parkinson's disease. MAO-B inhibitors will therefore not only provide symptomatic relief but may also alter the progression of the disease by preventing the formation of these byproducts. Promising MAO-B inhibitory activities have been reported for chalcones, and since the intermediates obtained in the synthesis of aminopyrimidines in this study are chalcones, a secondary aim of this study was the screening of selected chalcone intermediates as inhibitors of MAO–B.

Results

Design and synthesis: A series of 2-aminopyrimidines were designed using known active structures and literature pharmacophores. A molecular modelling study (Discovery Studio 3.1, Accelrys) was further done to investigate the feasibility of these compounds as potential adenosine A_{2A} antagonists. All of the designed aminopyrimidines were successfully docked in the binding site of the adenosine A_{2A} receptor. Binding orientations and observed interactions with important residues in the active site were similar to those observed for known A_{2A} antagonists. It was therefore concluded that these compounds may be potential A_{2A} antagonists and the designed compounds were thus synthesised. Structures were primarily confirmed with nuclear magnetic resonance spectroscopy and mass spectrometry.

MAO-B inhibition studies: Selected chalcones were evaluated using a fluorometric assay and kynuramine as substrate. The compounds were potent and selective inhibitors of the MAO-B enzyme with IC_{50} values ranging between 0.49-7.67 µM. (2*E*)-3-(3-Chlorophenyl)-1-(5-methyl-2-furyl)prop-2-en-1-one (**1c**) was the most potent compound with an IC_{50} value of 0.49 µM and was approximately 60 times more selective towards MAO-B than MAO-A. Some preliminary structure activity relationships were derived, for example, phenyl substitution with an electron withdrawing chlorine group generally resulted in better activity than substitution with electron donating methoxy groups. Further investigation of structure activity relationships are however required as a very small series of chalcones were screened.

Reversibility studies and mode of inhibition: A dilution assay was used to determine whether compound (**1c**) binds reversibly or irreversibly to the MAO-B enzyme. This was done by

measuring the recovery of enzymatic activity after a large dilution of the enzyme-inhibitor complex. The results from the reversibility studies showed that the inhibition of the most potent compound (**1c**) is reversible as the catalytic activities are recovered to approximately 80% and 50% respectively, compared to the control measured in the absence of an inhibitor. For the mode of inhibition, sets of Lineweaver–Burk plots were constructed. The Lineweaver-Burk plots intersected on the y-axis which indicates that compound **1c** is a competitive inhibitor of the MAO-B enzyme.

In vitro adenosine A_{2A} assays: Radioligand binding assays were used to determine the affinity of the synthesised 2-aminopyrimidines for the adenosine A_{2A} receptor. This assay was performed with the radioligand [^{3}H]NECA in the presence of N6-cyclopentyladenosine (CPA). Compounds **2a** - **2h** showed moderate to weak affinity in the assay, while promising affinities were observed for compounds **2j** - **2n,** which all exhibited K_i values below 55 nM. The compound with the highest affinity was 4-(5-methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**2m**) with a K_i value of 5.76 nM, which is comparable to the K_i value of 2.10 nM obtained for the known amino-substituted heterocyclic adenosine A_{2A} antagonist, ZM 241385. The higher affinities of compounds (**2j** – **2n**) could, at least in part, be explained by the molecular modellling studies. In the docking experiments an additional hydrogen bond interaction was observed between the amide carbonyl and tyrosine 271 indicating that this structural feature is a major contributing factor to the improved affinity observed for these derivatives.

In vivo adenosine A_{2A} assays: The haloperidol induced catalepsy assay was used to determine whether the two compounds with the highest affinity for the adenosine A_{2A} receptor (**2m** and **2k**) are antagonists of the A_{2A} receptor. These compounds caused a statistically significant reduction in catalepsy, which clearly illustrate that they are adenosine A_{2A} antagonists.

The objectives of this study as set out were thus successfully realised and promising results were obtained. During this study, several novel 2-aminopyrimidines and chalcones were synthesised, and the respective adenosine A_{2A} antagonistic and monoamine oxidase inhibitory activities for all of the screened compounds were determined for the first time.

OPSOMMING

Titel

Sintese van chalkone en 2-aminopirimidiene en hulle evaluering as monoamienoksidaseremmers en adenosienreseptor-antagoniste.

Kernwoorde

2-Aminopirimidiene, adenosien A_{2A} antagonisme, chalkone, monoamienoksidaseremmers

Agtergrond en motivering

Parkinson se siekte is 'n neurodegeneratiewe versteuring wat gekenmerk word deur verlaagde dopamienvlakke in die brein. Die oorsaak van Parkinson se siekte is nog onbekend maar daar is egter verskeie teorieë aangaande die etiologie. Huidige behandeling is hoofsaaklik gemik op dopamienvervanging, met middels soos levodopa en dopamien-agoniste, wat simptomatiese verligting aan pasiënte verskaf. Hierdie verligting is ongelukkig net tydelik omdat die verloop van die siekte nie gekeer word nie. Verder gaan hierdie terapieë hand aan hand met 'n verskeidenheid newe-effekte en nuwe benaderings tot die behandeling is dus dringend nodig. Adenosien A_{2A}-reseptorantagoniste het onlangs na vore gekom as 'n belowende, nie-dopaminergiese alternatief, nie net as simptomatiese behandeling nie, maar ook as potensiële neurobeskermende terapie.

Adenosien A_{2A}-reseptore kom saam met dopamien D_2 reseptore in die striatum en ander kerne van die basale ganglia voor. Adenosien A_{2A}-stimulasie verminder die affiniteit van dopamien vir die D_2-reseptor, en verhoog sikliese AMP (cAMP) vlakke. Die stimulasie van dopamine D_2-reseptore, in teenstelling, verminder cAMP vlakke en daarom funksioneer hierdie twee reseptors (A_{2A} en D_2) dus op 'n teenoorgestelde wyse. Adenosien-A_{2A} antagonisme sal dus dieselfde effek as D_2-agoniste hê en die postsinaptiese gevolge van dopamienuitputting verminder om simptomatiese verligting te gee. Daar is ook verskeie meganismes waarvolgens adenosien A_{2A}-antagoniste moontlik neurobeskermend kan wees, byvoorbeeld deur die voorkoming van glutamaateksitotoksisiteit, wat dopamienneurone kan beskadig. Verskeie adenosine A_{2A}-antagoniste is reeds aan kliniese proewe onderwerp en belowende resultate is verkry, veral ten opsigte van kombinasiebehandeling met levodopa. Gevolglik is daar 'n definitiewe moontlikheid dat A_{2A}-antagoniste, in siektes met dopaminergiese hipofunksie, soos Parkinson se siekte, terapeutiese potensiaal sal hê. Baie van die huidige A_{2A}-antagoniste bevat 'n amien-gesubsidieerde heterosikliese kern, soos 'n aminopirimidien. Die primêre doel van hierdie studie was om 2-aminopirimidienderivate te ontwerp, te sintetiseer en te evalueer as adenosien A_{2A}-reseptorantagoniste.

Monoamienoksidase B- (MAO-B-) remmers is ook belowende kandidate vir die simptomatiese behandeling van Parkinson se siekte, aangesien MAO-B die ensiem is wat primêr verantwoordelik is vir die katabolisme van dopamien in die brein. Onomkeerbare remmers van MAO-B, soos selegelien en rasagilien, word klinies gebruik vir die behandeling van Parkinson se siekte. Onomkeerbare inhibisie word geassosieer met sekere nadele, aangesien dit 'n paar weke na die beëindiging van die behandeling kan neem vir die ensiemaktiwiteit om te herstel. In teenstelling daarmee sal omkeerbare remmers oor veel beter veiligheidsprofiele beskik omrede hulle nie die ensiem permanent inaktiveer nie en omdat daar kompetisie tussen hulle en die substraat moontlik is.

Wanneer dopamien deur MAO geoksideer word, word toksiese metaboliese byprodukte, soos waterstofperoksied (H_2O_2) gevorm, en daar word geglo dat hierdie byprodukte 'n moontlike oorsaak van Parkinson se siekte kan wees. MAO-B-remmers sal dus nie net simptomatiese verligting verskaf nie, maar kan moontlik ook die progressie van die siekte stop deur die vorming van hierdie byprodukte te voorkom. Belowende MAO-B-inhiberende aktiwiteite is vir chalkone aangemeld, en aangesien hierdie tipe verbinding verkry word tydens die sintese van aminopirimidiene, is die sekondêre doel van die studie om geselekteerde chalkoon-intermediêre te evalueer as remmers van MAO-B.

Resultate

Ontwerp en sintese: Deur bekende strukture met goeie aktiwiteit en farmakofore wat uit die literatuur bekend is in aanmerking te neem, is 'n reeks 2-aminopirimidiene ontwerp. ‘n Molekulêre modelleringstudie (Discovery Studio 3.1, Accelrys) is uitgevoer om te ondersoek of hierdie verbindings gepas as adenosien A_{2A}-antagoniste optree. Al die ontwerpte aminopirimidiene het suksesvol in die bindingsetel gepas en bindingsoriëntasies sowel as interaksies met belangrike aminosure in die aktiewe setel was soortgelyk aan dié vir bekende A_{2A}-antagoniste. Daar is dus tot die gevolgtrekking gekom dat hierdie verbindings, potensiële-A_{2A} antagoniste mag wees en daar is voortgegaan met die sintese van die verbindings. Strukture is hoofsaaklik met kernmagnetiese resonans-spektroskopie en massaspektrometrie bevestig.

MAO-B inhibisiestudies: Geselekteerde chalkone is met behulp van 'n fluorometriese tegniek geëvalueer, waar kinuramien die substraat was. Relatiewe goeie aktiwiteit en selektiwiteit is vir die MAO-B-ensiem verkry, met IC_{50}-waardes wat gewissel het tussen 0.49-7.67 µM. (2*E*)-3-(3-(chloorfeniel)-1-(5-metiel-2-furiel)prop-2-en-1-oon (**1c**) was gevind as die mees potente verbinding met 'n IC_{50}-waarde van 0.49 µM en dit was ongeveer 60 keer meer selektief vir MAO-B as vir MAO-A. ’n Paar voorlopige struktuuraktiwiteitsverwanskappe is afgelei, bv., fenielsubstitusie met ‘n elektrononttrekkende chloorgroep het in die algemeen tot beter

aktiwiteit gelei as substitusie met die elektronskenkende metoksigroep. Die posisie van substitusie het ook blykbaar 'n geringe uitwerking op die aktiwiteit, maar dit moet verder ondersoek word. Verdere ondersoek na die struktuuraktiwiteitsverwantskappe is egter nodig aangesien ‘n baie klein reeks chalkone getoets is.

Omkeerbaarheidstudies en meganisme van remming: 'n Verdunningstoets is gebruik om te bepaal of verbinding **1c** omkeerbaar of onomkeerbaar aan die MAO-B-ensiem bind. Dit is uitgevoer deur die herstel van ensimatiese aktiwiteit na 'n groot verdunning van die ensiem-remmerkompleks te meet. Die resultate van die omkeerbaarheidstudies het getoon dat die inhibisie van die mees potente verbinding (**1c**) omkeerbaar is, aangesien die katalitiese aktiwiteite tot ongeveer 80% en 50% onderskeidelik, in vergeleke met die kontrolegroep, herwin is. Vir die wyse van inhibisie, is Lineweaver-Burk-grafieke opgestel. Die Lineweaver-Burk-kurwes sny die y-as, wat daarop dui dat verbinding **1c** 'n kompeterende inhibeerder van die MAO-B-ensiem is.

In vitro adenosien A_{2A}-studies: Radioligandbinding-studies is gebruik om die affiniteit van die gesintetiseerde 2-aminopirimidiene vir die adenosien A_{2A}-reseptor te bepaal. Hierdie studie is met die radioligand, [^{3}H]NECA, in die teenwoordigheid van N^6-siklopentieladenosien (CPA) uitgevoer. Verbindings **2a** - **2h** het matige tot swak affiniteit in hierdie studie gelewer, terwyl belowende affiniteite vir verbindings **2j** - **2n** waargeneem is, met K_i-waardes laer as 55 nM. Die verbinding met die beste affiniteit was 4-(5-metielfuraan-2-iel)-6-[3-(piperidien-1-karboniel)feniel]pirimidien-2-amien (**2m**) met 'n K_i-waarde van 5.75 nM, wat vergelykbaar is met die K_i-waarde van 2.10 nM vir die bekende amino-gesubstitueerde heterosikliese adenosien A_{2A}-antagonis, ZM 241385. Die beter affiniteit van verbindings (**2j** – **2n**) kan, ten minste ten dele, verduidelik word deur molekulêre moduleringstudies. In die passingstudie is die teenwoordigheid van ’n addisionele waterstofbindingsinteraksie opgemerk tussen die amiedkarbonielgroep en tirosien 271 wat aanduidend is dat hierdie struktuureienskap ’n kardinale rol in die beter affiniteit van hierdie derivate speel.

In vivo adenosien A_{2A}-studies: Die haloperidol-geïnduseerde katalepsietoets is gebruik om te bepaal of die twee verbindings met die hoogste affiniteite (**2m** en **2k**), antagoniste van die A_{2A}-reseptor is. Albei verbindings het katalepsie statisties beduidend verminder, wat dus dui dat hulle wel adenosien A_{2A}-antagoniste is.

Die doelwitte van hierdie studie, soos uiteengesit, is dus suksesvol bereik en belowende resultate is verkry. Gedurende hierdie studie is verskeie nuwe aminopirimidiene en chalkone gesintetiseer en die adenosien A_{2A}-antagoniste en monoamienoksidase-inhiberende aktiwiteite is vir die eerste maal vir al hierdie verbindings bepaal.

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ABBREVIATIONS

6-OHDA	6-hydroxydopamine
ACB	nucleus accumbens
ADME	absorption, distribution, metabolism and excretion
AM	extended amygdale
APCI	atmospheric-pressure chemical ionisation
Asn	asparagine
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
CB	cerebellum
CC	cingulated cortex
$CDCl_3$	deuterochloroform
CDI	1,1'-carbonydiimidazole
CNS	central nervous system
COMT	catechol-o-methyltransferase
COX-2	cyclooxygenase-2
CP	caudate putamen
CPM	counts per minute
CPA	N^6-cyclopentyladenosine
DA	dopamine
DMF	dimethylformamide
DMSO	deuterated dimethylsulfoxide
DS	Discovery Studio
EI-HRMS	electron impact high resolution mass spectrometry
FAD	flavin adenine dinucleotide
GABA	gamma-aminobutyric acid
Glu	glutamic acid
GP	globus pallidus
GPCR	G-protein-coupled receptor
Gpi	globus pallidus interna
Gpe	globus pallidus externa
HIP	hippocampus
His	histidine

HPLC	high performance liquid chromatography
HYP	hypothalamus
Ile	isoleucine
i.p.	intraperitoneally
LC	locus coeruleus
Leu	leucine
MAO	monoamine oxidase
MAPK	mitogen-activated protein kinase
Met	methionine
mGlu5	5 metabotropic glutamate
MPP^+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSN	medium spiny neurons
NMR	nuclear magnetic resonance
[^{3}H]NECA	[^{3}H]5'-N-ethylcarboxamide-adenosine
NC	neocortex
OB	olfactory bulb
OT	olfactory tubercle
PD	Parkinson’s disease
PDB	protein data bank
Phe	phenylalanine
PGE2	prostaglandin E2
Pro	proline
ROS	reactive oxygen species
SD	standard deviation
SEP	septum
Ser	serine
SET	single electron transfer
SNc	substantia nigra pars compacta
SNr	substantia nigra pars-reticula
STN	subthalamic nucleus
STR	striatum
THA	thalamus
TLC	thin layer chromatography
Trp	tryptophan
Tyr	tyrosine
VC	visual cortex

NMR:

δ	delta scale used to indicate chemical shift
J	coupling constant
br d	broad doublet
br s	broad singlet
br t	broad triplet
d	doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
m	multiplet
p	pentet/quintet
ppm	parts per million
q	quartet
s	singlet
t	triplet

Kinetics:

[E]	enzyme concentration
ES	enzyme-substrate complex
[I]	inhibitor concentration
[P]	product concentration
[S]	substrate concentration
K_d	equilibrium dissociation constant
K_m	Michaelis-Menten constant
L	radioligand [^{3}H]NECA concentration
V_i	initial reaction velocity
V_{max}	maximum value of V_i