Aminopyrimidine derivatives as adenosine antagonists

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Abstract

Title

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Keywords

2-Aminopyrimidines, dual adenosine A_1/A_{2A} antagonists, neuroprotection, Parkinson's disease

Aims of this project

The aim of this study was to design and synthesise novel 2-aminopyrimidine derivatives as potential adenosine A_1 and A_{2A} receptor antagonists.

Background and rationale

Parkinson's disease is the second most common neurodegenerative disorder (after Alzheimer's disease) and is characterised by the selective death of the dopaminergic neurons of the nigro-striatal pathway. Distinctive motor symptoms include bradykinesia, muscle rigidity and tremor, while non-motor symptoms, of which cognitive dysfunction is an example, also frequently occur. Current therapy provides symptomatic relief mainly by augmentation of dopaminergic signalling (levodopa, dopamine agonists, MAO and COMT enzyme inhibitors), but disease progression is not adequately addressed. New therapies that can prevent further neurodegeneration in addition to providing symptomatic relief are therefore urgently required.

Adenosine has an important function as neuromodulator in the central nervous system. The adenosine A_{2A} receptor in particular plays an essential role in the regulation of movement. This, coupled to the fact that it is uniquely distributed in the basal ganglia, contributes to its attractiveness as non-dopaminergic target in the treatment of movement disorders, such as Parkinson's disease. The efficacy of adenosine receptor antagonists has been illustrated in animal models of Parkinson's disease and several adenosine receptor antagonists have also reached clinical trials. The neuroprotective properties of adenosine A_{2A} receptor antagonists are further attributed to their ability to modulate neuro-inflammation and decrease the release of the excitatory neurotransmitter glutamate, which is implicated in neurotoxicity.

While adenosine A_1 receptor antagonism has a synergistic effect on the motor effects of adenosine A_{2A} receptor antagonism, it has the additional benefit of improving cognitive

dysfunction, a cardinal non-motor symptom of Parkinson's disease. Dual antagonism of adenosine A_1 and A_{2A} receptors therefore offers the potential of providing symptomatic relief as well as the neuroprotection so desperately needed in the clinical environment.

Amino substituted heterocyclic scaffolds, such as those containing the 2-aminopyrimidine motif, have been shown to exhibit good efficacy as dual adenosine receptor antagonists. Since the structure activity relationships of 2-aminopyrimidines have not been comprehensively explored, it is in this regard that this study aimed to make a contribution.

Results

Fourteen 2-aminopyrimidines were synthesised successfully over three steps, (although in low yields) and characterised by nuclear magnetic resonance and infrared spectroscopy, mass spectrometry, by determination of melting points and high performance liquid chromatography. Structure modifications explored included variation of the aromatic substituent on position 4, as well as variations in the substituents of the phenyl ring, present on position 6 of the pyrimidine ring.

Radioligand binding assays were performed to determine the affinities of the synthesised compounds for the adenosine A_1 and A_{2A} receptor subtypes. Several high dual affinity derivatives were identified during this study; the compound with the highest affinity was 4-(5-methylthiophen-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**39f**) with K_i values of 0.5 nM and 2.3 nM for the adenosine A_{2A} and adenosine A_1 receptors, respectively.

A few general structure activity relationships were derived, which included: The effect of the aromatic substituent (position 4) on A_{2A} affinity could be summarised (in order of declining affinity) as follows: 5-methylthiophene > phenyl > furan > pyridine > *p*-fluorophenyl > benzofuran. On the other hand, the effect of this substituent on A_1 receptor affinity could be summarised (in order of declining affinity) as follows: phenyl > 5-methylthiophene > *p*-fluorophenyl > benzofuran > pyridine. The affinities as exhibited by the methylthiophene derivatives **39f**, **39h – 39j**, further showed that while piperidine substitution (**39f**) resulted in optimal A_{2A} and A_1 affinity, pyrrolidine substitution (**39j**) was less favourable. Substitution at the 4' position of the phenyl ring, as well as thiazole substitution, generally resulted in poor adenosine A_1 and A_{2A} receptor affinity. However, 4-[2-amino-6-(5-methylfuran-2-yl)pyrimidin-4-yl]-*N*-(1,3-benzothiazol-2-yl)benzamide (**39l**) surprisingly demonstrated good affinity and selectivity for the adenosine A_1 receptor.

The results obtained during radioligand binding assays were rationalised by QSAR and molecular modelling (Discovery Studio 3.1, Accelrys) studies. The inverse relationship seen between log K_i (as indicator of affinity) and polar surface area, illustrated the importance of this physico-chemical property in the design of 2-aminopyrimidine A_{2A} antagonists. The results from the docking study further showed that the orientation adopted by derivatives in the binding cavity (and particular hydrogen bonding to Asn 253 and Glu 169) is of importance. Results from the MTT cell viability assay indicated that none of the high affinity derivatives had a significant effect on cell viability at 1 μM, a concentration much higher than their K_i values. However, incorporation of the furan, benzofuran and *p*-fluorophenyl groups as aromatic substituent and a pyrrolidine as amine substituent, presented liabilities.

Lastly, the haloperidol induced catalepsy assay (in rats) was used to give a preliminary indication of adenosine receptor antagonism or agonism. Compound **39f** failed to reverse catalepsy under standard conditions, but showed some reversal after an increased time period. Indications therefore exist that **39f** is an adenosine receptor antagonist that suffers from bioavailability issues. Compound (**39c**), 4-phenyl-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine which also demonstrated promising affinity in the radioligand binding assays however showed a statistically significant reduction in catalepsy, indicating adenosine A_{2A} receptor antagonism, and *in vivo* efficacy.

Highly potent, dual affinity aminopyrimidine derivatives with acceptable toxicity profiles were identified in this study, with compound **39c** demonstrating *in vivo* activity. The aim of designing and synthesising a promising dual adenosine A_1/A_{2A} receptor antagonist is therefore realised, with compound **39c** as the most favourable example.

Opsomming

Titel

Aminopirimidienderivate as adenosienantagoniste

Sleutelwoorde

2-Aminopirimidiene, dualistiese adenosien A_1/A_{2A}-antagoniste, neurobeskerming, Parkinson se siekte

Doel van die studie

Die doel van hierdie studie was om nuwe 2-aminopirimidienderivate as moontlike dualistiese adenosien A_1- en A_{2A}-reseptorantagoniste te ontwerp en te sintetiseer.

Agtergrond en motivering

Parkinson se siekte is die tweede algemeenste neurodegeneratiewe siekte (naas Alzheimer se siekte) en word gekenmerk deur die selektiewe afsterwing van die dopaminergiese neurone van die nigrostriatale senuweebaan. Kenmerkende motoriese simptome sluit bradikinesie, spierstyfheid en bewing in, terwyl nie-motoriese simptome, soos kognitiewe wanfunksie ook algemeen voorkom. Huidige terapie verskaf simptomatiese verligting hoofsaaklik deur die versterking van dopaminergiese seinoordrag (levodopa, dopamienagoniste, MAO- en KOMT-ensieminhibeerders), maar die verloop van die siekte word nie genoegsaam behandel nie. Nuwe terapieë, wat bo-en behalwe die verskaffing van simptomatiese verligting, ook verdere neurodegenerasie kan voorkom, is dus dringend nodig.

Adenosien verrig ‘n belangrike funksie as neuromoduleerder in die sentrale senuweestelsel. Die adenosien A_{2A} reseptor veral, speel ‘n belangrike rol in die regulering van beweging. Dit, en die feit dat die reseptor uniek in die basale ganglia verspreid is, dra by tot die gunstigheid daarvan as nie-dopaminergiese teiken in die behandeling van bewegingsafwykings, soos Parkinson se siekte. Die effektiwiteit van adenosienantagoniste as beide simptomatiese sowel as neurobeskermende middels is bewys in dieremodelle van Parkinson se siekte en verskeie adenosien reseptor antagoniste het ook kliniese proewe bereik. Die neurobeskermende eienskappe van adenosien A_{2A}-antagonisme word toegeskryf aan die vermoë van die middels om neuro-inflammasiete te moduleer en ‘n afname in die vrystelling van die eksitatoriese neuro-oordragstof glutamaat, wat geassosieer word met neurotoksisiteit, te bewerkstellig. Adenosien A_1-reseptorantagonisme het bo-en behalwe die

sinergisme van die motoriese effek van adenosien A_{2A}-antagonisme, ook die bykomende voordeel dat dit kognitiewe wanfunksie, wat ‘n belangrike nie-motoriese simptoom van Parkinson se siekte is, verlig. Dualistiese antagonisme van adenosien A_1 en A_{2A}-reseptore bied dus die moontlikheid om beide simptomatiese verligting en neurobeskerming, wat so dringend benodig word in die kliniese omgewing, te verskaf.

Die effektiwiteit van amiengesubstitueerde heterosikliese verbindings, soos die wat die 2-aminopirimidiengroep bevat, as dualistiese adenosienreseptorantagoniste, is reeds aangetoon. Aangesien die struktuuraktiwiteitverwantskappe vir die 2-aminopirimidiene nie voorheen volledig ondersoek is nie, is daar gepoog om in die verband ‘n bydrae te lewer tydens hierdie studie.

Resultate

Veertien 2-aminopirimidiene is suksesvol in drie stappe gesintetiseer (alhoewel lae opbrengste verkry is). Die gesintetiseerde verbindings is gekarakteriseer deur gebruik te maak van kernmagnetieseresonans- en infrarooi-spektroskopie, massa spektrometrie, smeltpuntbepalings en hoë drukvloeistofchromatografie. Strukturele veranderinge wat ondersoek is sluit variasie van die aromatiese substituent op posisie-4, sowel as veranderinge in die substitusie van die fenielring, teenwoordig op posisie-6 van die pirimidienring, in.

Die affiniteit van die gesintetiseerde verbindings vir die adenosien A_1- en A_{2A}-reseptore is met radioligandbindingstudies bepaal. Verskeie hoë-affiniteit derivate is gedurende hierdie studie geïdentifiseer. Die verbinding met die hoogste affiniteit was 4-(5-metieltiofeen-2-iel)-6-[3-(piperidien-1-karboniel)feniel]pirimidien-2-amien (**39f**) met K_i-waardes van 0.5 nM en 2.3 nM vir die adenosien A_{2A}- en A_1-reseptore, onderskeidelik.

Verskeie struktuuraktiwiteitsverwantskappe kon afgelei word, wat die volgende insluit: Die effek van aromatiese substitusie (in posisie 4) op affiniteit kan as volg opgesom word (in volgorde van afnemende affiniteit): 5-metieltiofeen > feniel > furaan > piridien > *p*-fluoorfeniel > bensofuraan. Aan die ander kant, kan die effek van aromatiese substitusie op affiniteit vir die A_1-reseptor as volg opgesom word (in volgorde van afnemende affiniteit): feniel > 5-metieltiofeen > *p*-fluoorfeniel > bensofuraan > piridien. Uit die studie van die metieltiofeenderivate **39f**, **39h – 39j**, het geblyk dat, terwyl piperidiensubstitusie (**39f**) optimale A_{2A} en A_1 affiniteit lewer, pirrolidien substitusie (**39j**) minder gunstig was. Substitusie op die 4' posisie (op die fenielring) sowel as substitusie met ‘n tiasoolgroep het oor die algemeen ‘n negatiewe impak op adenosien A_1 en A_{2A}-reseptoraffiniteit gehad. Goeie

affiniteit vir die adenosien A_1-reseptor is egter met 4-[2-amien-6-(5-metielfuraan-2-iel)pirimidien-4-iel]-*N*-(1,3-bensotiasol-2-iel)bensamied (**39l**) verkry.

Om die resultate van die radioligandbindingstudies te rasionaliseer, is kwantitatiewe struktuuraktiwiteitsverswantskap- en molekulêre modelleringstudies (Discovery Studio 3.1, Accelrys) gedoen. Die omgekeerde verwantskap tussen log K*i* (as aanduiding van affiniteit) en polêre oppervlakarea, het die belang van dié fisiese-chemiese eienskap tydens die ontwerp van 2-aminopirimidien-adenosien-A_{2A}-antagoniste beklemtoon. Die resultate van die passingstudie het verder aangetoon dat die die oriëntasie wat verbindings in die aktiewe setel aanneem (en veral die waterstofbindings met Asn 253 en Glu 169) van groot belang is.

Resultate verkry met die MTT-seltoksisteitstoets het aangedui dat geen van die mees aktiewe verbindings ‘n merkbare effek op seloorlewing by ‘n konsentrasie van 1 µM toon nie. Hierdie konsentrasie is baie hoër as die K_i-waardes van hierdie reeks verbindings. Daar is wel waargeneem dat die insluiting van ‘n furaan-, bensofuraan- en *p*-fluoorfenielgroep as aromatiese substituent en pirrolidien as amiensubstituent ‘n gevaar vir seltoksitieit kan inhou.

Laastens is die haloperidol-geïnduseerde katalepsiestudie (in rotte) gebruik om ‘n voorlopige aanduiding van adenosienreseptorantagonisme of –agonisme te gee. Verbinding **39f** het nie katalepsie verminder tydens die standaardtoets nie, maar geringe vermindering in katalepsie is waargeneem nadat die tyd van die standaardtoets verleng is. Dit blyk dus dat **39f** wel ‘n adenosienreseptorantagonis is, maar waarskynlik nie goeie breinbiobeskikbaarheid het nie. Verbinding (**39c**), 4-feniel-6-[3-(piperidien-1-karboniel)feniel]pirimidien-2-amien wat ook belowende affiniteit in die radioligandbindingstudies getoon het, het ‘n statistiese betekenisvolle verlaging in katalepsie teweeggebring. Hierdie verbinding is dus waarskynlik ‘n adenosien A_{2A}-reseptorantagonis met *in vivo* effektiwiteit.

Hoogs potente dualistiese 2-aminopirimidienderivate met aanvaarbare toksisteitsprofiele is gedurende hierdie studie geïdentifiseer, met verbinding **39c** wat *in vivo* aktiwiteit getoon het. Die doel van die studie, naamlik die ontwerp en sintese van moontlike adenosien A_1/A_{2A}-reseptorantagoniste is dus verwesenlik, met verbinding **39c** as die mees geskikte voorbeeld.

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List of Abbreviations

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[^{3}H]NECA	[^{3}H]5'N-ethylcarboxamide-adenosine
6-OHDA	6-hydroxydopamine
Abs	Absorbance
ADP	Adenosine-diphosphate
Asn	Asparagine
ATP	Adenosine- triphosphate
cAMP	Cyclic adenosine-monophosphate
$CDCl_3$	Deuterochloroform
CDI	1,1'-Carbonyldiimidazole
COMT	Catechol-*O*-methyl-transferase
COSY	Correlation spectroscopy
CPA	N^6-cyclopentyladenosine
CPM	Counts per minute
C-terminus	Cytosolic carboxy terminus
DAG	Diacylglycerol
DCM	Dichloromethane
DEPT	Distortionless enhancement by polarisation transfer
DMEM	Dulbecco's Modified Eagle Medium
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterodimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOPAC	3,4-Dihydroxyphenylacetic acid
ECL	Extracellular loops
EDAC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

FDA	Food and Drug Administrator USA
GABA	Gamma-amino butyric acid
GDNF	Glial cell derived neurotrophic factor
G_i	Inhibitory G-protein
Glu	Glutamic acid
GPCR	Guanine nucleotide-binding protein coupled receptor
G-protein	Guanine nucleotide-binding protein
G_s	Stimulatory G-protein
HMBC	Heteronuclear multiple bond correlation
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum correlation
IC_{50}	Half maximal inhibitory concentration
ICL	Intracellular loops
IP_3	Inositol triphosphate
IR	Infrared spectroscopy
K_d	Dissociation constant
K_i	Inhibition constant
Leu	Leucine
MAO	Monoamine oxidase
MAO-B	Monoamine oxidase isoform B
MeOH	Methanol
$MPDP^+$	1-Methyl-4-phenyl-2,3-dihydropyridinium ion
MPP	1-Methyl-4-phenyl-4-propionoxypiperidine
MPP^+	1-Methyl-4-phenylpyridinium ion
MPTP	1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NaH	Sodium hydride
NaOH	Sodium Hydroxide
NBS	*N*-Bromosuccinimide
NMDA	*N*-methyl-*D*-aspartate
NMR	Nuclear magnetic resonance
NSAIDs	Non-steroidal anti-inflammatory drugs
N-terminus	Amino-terminus
PBS	Phosphate-buffered saline
PDB	Protein Data Bank
Phe	Phenylalanine
QSAR	Quantitative structure activity relationship
ROS	Reactive oxygen species
RSMD	Root square mean deviation
RT	Room temperature
SNc	Substantia nigra pars compacta
TLC	Thin layer chromatography
Trp	Tryptophan

NMR:

δ	delta scale indicating 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
ppm	parts per million
q	quartet
s	singlet
t	triplet