

The Synthesis and Use of Certain Pyridine Derivatives
as Modulators of the G-protein Coupled Receptors
mGlu5 and P2Y₁₂

PETER BACH



UNIVERSITY OF GOTHENBURG

Department of Chemistry and Molecular Biology
University of Gothenburg
2012

DOCTORAL THESIS

Submitted for partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Chemistry

The Synthesis and Use of Certain Pyridine Derivatives as Modulators of the G-protein Coupled
Receptors mGlu5 and P2Y₁₂

PETER BACH

© Peter Bach

ISBN: 978-91-628-8579-3

<http://hdl.handle.net/2077/30683>

Department of Chemistry and Molecular Biology
SE-412 96 Göteborg
Sweden

Printed by Ineko AB
Källered, 2012

Abstract

The glutamatergic mGlu5 receptor and the purinergic P2Y₁₂ receptor are two important targets in the development of novel treatments of gastroesophageal reflux disease (GERD) and thrombosis, respectively.

Synthesis was developed to investigate the structure-activity relationships (SAR) of a novel series of 2-alkynylpyridine derivatives as mGluR5 antagonists. This led to the discovery of antagonists with potency in the low-nanomolar range. High microsomal metabolism, possibly due to high lipophilicity, remained an issue.

Further, SAR development for a series of ethyl 6-piperazinylnicotinates, featured by a urea linker, as antagonists of the P2Y₁₂ receptor showed the 3-ethoxycarbonyl substituent as central to binding. The low aqueous solubility was addressed by variation of the linker which led to the discovery of sulfonylureas as P2Y₁₂ antagonists. The chemical stability of the sulfonylurea compounds during prolonged storage in solution was found to be related to the sulfonyl urea linker and depended on the type of solvent and the substitution pattern of the sulfonyl urea functionality.

Synthesis was developed to facilitate the replacement of the 2-methyl substituent on pyridine with more electron donating substituents and of the 3-ethoxycarbonyl substituent with 5-ethyl-oxazoles. Both strategies led to compounds with higher metabolic stability, but also with lower potency.

Pair-wise comparison of compounds showed that a correctly positioned alkyl group, like in an ethyl ester or a 5-ethyl-oxazole, and a correctly positioned strong hydrogen bond acceptor both were required for binding.

Chemical design was used to study how the regioselectivity R_{sel} for the 2-position depended on the character of the 3-substituent in the reaction of 3-substituted 2,6-dichloropyridines with 1-methylpiperazine. It was found that R_{sel} depended on neither of the parameters PI, MR, or σ_p , but showed a statistically significant correlation with the Verloop steric parameter B1 (R^2 : 0.45, p = 0.006). This implied that 3-substituents that are bulky close to the pyridine ring directed the regioselectivity towards the 6-position.

With R^3 = -CO₂CH₃ a study of the solvent effect showed that R_{sel} could be predicted by the Kamlet-Taft equation: $R_{sel} = 1.28990 + 0.03992\alpha - 0.59417\beta - 0.46169\pi^*$ (R^2 = 0.95; p = 1.9×10^{-10}). The dependency on the solvatochromic β parameter meant that the 16:1 regioselectivity for the 2-isomer in DCM (β = 0.10) could be switched to a 2:1 selectivity for the 6-isomer in DMSO (β = 0.76).

Keywords: mGluR5, P2Y₁₂, gastroesophageal reflux disease (GERD), thrombosis, ethyl nicotinates, ureas, sulfonylureas, oxazoles, bioisosteres, regioselectivity, solvent effect.

List of Publications

The Thesis is based on the following papers that are referred to in the text by the Roman numerals I-VI:

- I Bach, P.; Nilsson, K.; Wållberg, A.; Bauer, U.; Hammerland, L. G.; Peterson, A.; Svensson, T.; Österlund, K.; Karis, D.; Boije, M; Wensbo, D. A New Series of Pyridinyl-alkynes as Antagonists of the Metabotropic Glutamate Receptor 5 (mGluR5). *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4792-4795.
- II Bach, P.; Nilsson, K.; Svensson, T.; Bauer, U.; Hammerland, L. G.; Peterson, A.; Wållberg, A.; Österlund, K.; Karis, D.; Boije, M; Wensbo, D. "Structure-activity Relationships for the Linker in a Series of Pyridinyl-alkynes that are Antagonists of the Metabotropic Glutamate Receptor 5 (mGluR5). *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4788-4791.
- III Bach, P. Boström, J.; Brickmann, K.; van Giezen, J. J. J.; Hovland, R.; Petersson, A. U.; Ray, A.; Zetterberg, F. A Novel Series of Piperazinyl-pyridine Ureas as Antagonists of the Purinergic P2Y₁₂ Receptor. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2877-2881.
- IV Bach, P.; Boström, J.; Brickmann, K.; van Giezen, J. J. J.; Groneberg, R. D.; Harvey, D. M.; O'Sullivan, M.; Zetterberg, F. Synthesis, Structure-Property Relationships and Pharmacokinetic Evaluation of Ethyl 6-Aminonicotinate Sulfonylureas as Antagonists of the P2Y₁₂ Receptor. *Manuscript*.
- V Bach, P.; Boström, J.; Brickmann, K.; Burgess, L. E.; Clarke, D.; Groneberg, R. D.; Harvey, D. M.; Groneberg, R. D.; Harvey, D. M.; Laird, E. R.; O'Sullivan, M.; Zetterberg, F. 5-Alkyl-1,3-oxazole Derivatives of 6-Amino-nicotinic Acids as Alkyl Ester Bioisosteres are Antagonists of the P2Y₁₂ Receptor. *Submitted*.
- VI Bach, P.; Marczyinke, M.; Giordanetto, F. Effects of the Pyridine 3-Substituent on the Regioselectivity in the Nucleophilic Aromatic Substitution Reaction of 3-Substituted 2,6-Dichloropyridines with 1-Methylpiperazine Studied by a Chemical Design Strategy. *Eur. J. Org. Chem.* *Accepted*.

Paper not included in the Thesis:

- VII Bach, P.; Isaac, M.; Slassi, A. Metabotropic Glutamate Receptor 5 Modulators and Their Potential Therapeutic Applications. *Expert Opin. Ther. Pat.* **2007**, *17*, 371-384.

Contribution report

Paper I

Participated in formulating the research problem
Major contribution to the experimental work
Contributed to interpretation of the results
Wrote the manuscript

Paper II

Participated in formulating the research problem
Major contribution to the experimental work
Contributed to interpretation of the results
Wrote the manuscript

Paper III

Participated in formulation of the research problem
Did parts of the experimental work
Contributed to interpretation of the results
Major contributions to writing of the manuscript

Paper IV

Participated in formulation of the research problem
Did parts of the experimental work
Contributed to interpretation of the results
Major contributions to writing of the manuscript

Paper V

Participated in formulation of the research problem
Did parts of the experimental work
Contributed to interpretation of the results
Major contributions to writing of the manuscript

Paper VI

Formulated the research problem
Did majority of the experimental work
Major contributions to interpretation of the results
Major contributions to writing of the manuscript

Abbreviations

Ac	acetyl
ADP	adenosine diphosphate
AMP	adenosine monophosphate
Ar	aryl
ATP	adenosine triphosphate
B3LYP	Becke, 3-parameter, Lee-Yang-Parr
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
Caco-2	adenocarcinoma cells from human colon
cAMP	cyclic adenosine monophosphate
CB ₁	cannabinoid receptor 1
CB ₂	cannabinoid receptor 2
CDI	<i>N,N'</i> -carbonyl diimidazole
cGMP	cyclic guanosine monophosphate
CL	clearance
CLint	intrinsic clearance
clogP	calculated logarithm of the partition coefficient between an octanol phase and an aqueous phase
CNS	central nervous system
COX-1	cyclooxygenase-1
Cpd	compound
CYP450	cytochrome P450
DABCO	1,4-diazabicyclo[2.2.2]octane
DAG	diacylglycerol
1,2-DCE	1,2-dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	<i>N,N</i> -diisopropylethylamine
DMA	dimethylacetamide
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DN	Gutmann's donor number
DNA	deoxyribonucleic acid
DPPPE	1,5-bis(diphenylphosphino)pentane
EDC	<i>N</i> -(3-dimethylaminopropyl)- <i>N'</i> -ethylcarbodiimide
ε	relative static permittivity
Et	ethyl

<i>F</i>	bioavailability (fraction)
FLIPR	fluorescent light imaging plate reader
FXS	fragile X syndrome
GABA	γ -amino butyric acid
GERD	gastro-(o)esophageal reflux disease
GP	glycoprotein
GPCR	G-protein coupled receptor
GTP γ S	guanosine 5'-O-[γ -thio]triphosphate
HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HBA	hydrogen bond acceptor
HLM	human liver microsomes
HMPA	hexamethylphosphoramide
HOBT	1-hydroxybenzotriazole
HRMS	high-resolution mass spectrometry
HTS	high-throughput screening
iGluR	ionotropic glutamate receptors
IP ₃	inositol-1,4,5-trisphosphate
<i>i</i> -Pr	iso-propyl
iv	intravenous
K-ATP	ATP-sensitive potassium channel
LC	liquid chromatography
LDA	lithium diisopropylamide
LES	lower (o)esophageal sphincter
LLE	lipophilic ligand efficiency
logD	logarithm of the distribution coefficient between an octanol phase and an aqueous phase at the pH indicated
Me	methyl
<i>m</i> -CPBA	meta-chloro-perbenzoic acid
mGluR	metabotropic glutamate receptor
MP	1-methylpiperazine
MPEP	2-methyl-6-(phenylethynyl)pyridine
MR	molar refractivity
Ms	methanesulfonyl (= mesyl)
MS	mass spectrometry
MTEP	3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine
μ	molecular dipolar momentum
NAM	negative allosteric modulator
MW	microwave oven, single node heating
NAD ⁺	nicotinamide adenine dinucleotide

NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced form of NADP
NOE	nuclear Overhauser effect
<i>n</i> -Pr	<i>n</i> -propyl
NSAID	non-steroid anti-inflammatory drug
PAR	protease-activated receptor
PDE3	platelet phosphodiesterase 3
PGI ₂	prostaglandin I ₂ (prostacyclin)
Ph	phenyl
PI	lipophilicity
π^*	dipolarity/polarizability
PIP ₂	phosphatidylinositol-4,5-bisphosphate
PKC	protein kinase C
PLC	phospholipase C
po	peroral
PPI	proton pump inhibitor
PRP	platelet-rich plasma
RLM	rat liver microsomes
RNA	ribonucleic acid
RPC	residual platelet count
R _{sel}	regioselectivity for the 2-position in 3-substituted 2,6-dichloropyridines
rt	room temperature
SAR	structure-activity relationship(s)
SEM	2-(trimethylsilyl)ethoxymethyl
sGC	soluble guanyl cyclase
S _N Ar	nucleophilic aromatic substitution
T3P	propylphosphonic anhydride
TBTU	O-(benzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium tetrafluoroborate
TEA	triethylamine
TEG	triethylene glycol
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLESR	transient lower esophageal sphincter relaxation
TM	transmembrane
TMEDA	<i>N,N,N',N'</i> -tetramethylethylenediamine
TP	TXA ₂ (thromboxane A ₂) receptor
Troc-Cl	2,2,2-trichloroethoxycarbonyl
TXA ₂	thromboxane A ₂
WPA	washed platelet assay

Table of Contents

1. Introduction	1
1.1 Use of pyridine derivatives as drug molecules	1
1.2 7-transmembrane G-protein coupled receptors as drug targets	3
2. Aims of the Thesis	5
3. The mGlu5 receptor	7
3.1 GERD and its connection to the mGluR5 receptor	7
3.2 Modulators of mGluR5 and their therapeutic potential	8
4. Identification and SAR of a new lead series of mGluR5 antagonists	11
4.1 Synthesis	11
4.2 Results and discussion of SAR	13
5. The role of platelets and purinergic receptors in primary hemostasis	17
5.1 Signalling events leading to platelet aggregation	18
5.2 Antagonists of the P2Y ₁₂ receptor	19
5.3 Other antagonists for the treatment of arterial thrombosis	21
6. Ethyl 6-amino nicotinate derivatives as P2Y₁₂ antagonists	23
6.1 Identification of a new lead series of ureas as P2Y ₁₂ antagonists	23
6.1.1 Synthesis	24
6.1.2 Results and discussion of SAR	25
6.2 Sulfonylureas as P2Y ₁₂ antagonists	27
6.2.1 Synthesis	27
6.2.2 Results and discussion of SAR	28
6.2.3 Stability of the sulfonylureas during prolonged storage in solution	31
6.3 Potential issue: microsomal stability of the nicotinic esters	33

6.3.1 Development of synthetic routes to facilitate variation of the pyridine 2-substituent	34
6.3.2 Development of synthetic routes to 5-ethyl-oxazoles	35
6.3.3 Results and discussion	37
6.4 Binding model of ethoxycarbonyl substituent and isosteres	41
6.5 Pharmacokinetic evaluation in vivo	42
7. Regioselectivity in the reaction of 3-substituted 2,6-dichloropyridines with 1- methylpiperazine	43
7.1 Synthesis	45
7.2 Results and discussion	48
8. Conclusions	53
Acknowledgments	55
Appendix	57
References and notes	59

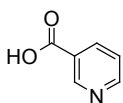
Papers I-VI

1. Introduction

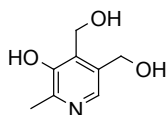
1.1 Use of pyridine derivatives as drug molecules

Pyridines represent a large group of compounds with applications as polymers, dyes, antioxidants, agrochemicals, and pharmaceuticals.¹ This overview will focus on the pyridines (excluding hydroypyridines) that are pharmaceuticals in frequent therapeutic use or with special structural features.

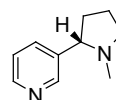
The pyridine structure is found in natural compounds like nicotinic acid (vitamin B3) and pyridoxine (vitamin B6). Nicotinic acid is required for the biosynthesis of the redox coenzyme nicotine adenine dinucleotide (NAD⁺), while pyridoxine is a coenzyme in transaminases.² Nicotinic acid has been in use for 50 years³ as a therapeutic agent to increase the relative levels of high-density lipoprotein and thereby reduce the risk of cardiovascular disease.⁴ Nicotine, whose toxicity has a defensive function in nature,⁵ is widely used for smoking cessation.



nicotinic acid
(vitamin B3)

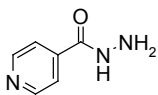


pyridoxine
(vitamin B6)

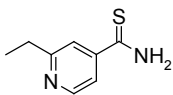


nicotine

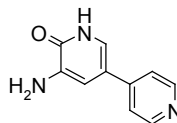
The pyridine moiety is also found in structurally simple drugs like isoniazid⁶ and ethionamide⁷ (both prodrugs for inhibitors of inter alia enoyl-acyl carrier protein reductase; tuberculosis), amrinone (phosphodiesterase 3 inhibitor; heart failure) and bupicomide (dopamin β -hydroxylase inhibitor; hypertension). Sulphapyridine (dihydropteroate synthetase inhibitor) was launched in 1938 and decreased the death rate in pneumococcal pneumonia from 25% to about 6%.⁸ It has since been replaced with other sulfonamide antibacterials.



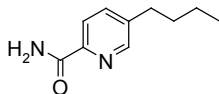
isoniazid



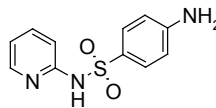
ethionamide



amrinone

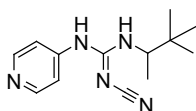


bupicomide

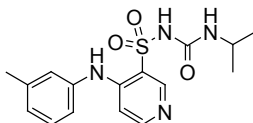


sulphapyridine

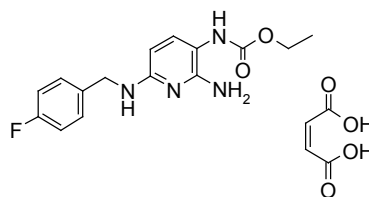
Pyridine derivatives used as blockers of ion channels include pinacidil (racemic, K-ATP activator; hypertension), torasemide (inhibitor of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ carrier system; diuretics), and flupirtine maleate ($\text{K}_{\text{v}7}$ activator; multiple sclerosis).



pinacidil

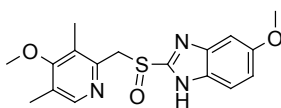


torasemide

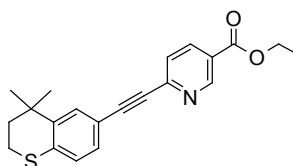


flupirtine maleate

Further examples of pyridines as drug molecules include omeprazole and its S-enantiomer esomeprazole (H^+/K^+ -ATPase inhibitors, also known as proton pump inhibitors (PPIs), peptic ulcer disease) and tazarotene (ornithine decarboxylase inhibitors; psoriasis).



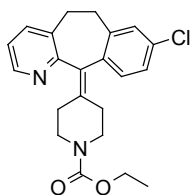
omeprazole



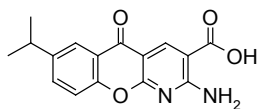
tazarotene

Many drug molecules contain a pyridine moiety as part of a more complex structure. Examples include loratadine, an antagonists of the histamine H1 receptor, that is widely used for the treatment of allergic rhinitis. Amlexanox (cysteinyl leukotriene receptor 1

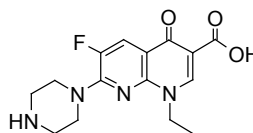
antagonist) is an antiinflammatory and antiallergic agent, while enoxacin (topoisomerase II inhibitor) belongs to the therapeutically important class of quinolones as antibacterials.



loratadine



amlexanox



enoxacin

1.2 7-Transmembrane G-protein coupled receptors as drug targets

7-Transmembrane (7TM or heptahelical) receptors⁹ are cell-membrane-bound receptors that are featured by a transmembrane domain of seven anti-parallel α -helices connected by extra- and intracellular loops. 7TM receptors play a central role in the communication to and between cells. The majority¹⁰ of 7TM receptors, of which >800 have been identified,^{11, 12} couple to G-proteins which elicits further intracellular signalling.

Of these, approximately 83 G-protein coupled receptors (GPCRs) have been successfully targeted with drugs (2009),¹³ and at least 27% of the drugs approved by the FDA (U.S. Food and Drug Administration) target a GPCR (2006).¹⁴

Both the mGlu5 and the P2Y₁₂ receptors belong to this therapeutically important class of targets.

2. Aims of the Thesis

- To investigate structure-activity relationships starting from high-throughput screening hits of antagonists of the mGluR5 and P2Y₁₂ receptors, respectively, in order to identify compounds with increased binding affinity and potency.
- To increase the aqueous solubility of compounds in the urea series of P2Y₁₂ receptor antagonists by structural modifications.
- To identify factors that influence the chemical stability of certain sulfonylurea compounds during prolonged storage in solution.
- To develop synthesis of ethyl nicotinate derivatives with increased microsomal stability of the 3-ethoxycarbonyl functionality.
- To develop synthesis of 5-ethyl-oxazoles to replace the 3-ethoxycarbonyl functionality of ethyl nicotinate derivatives in order to increase the microsomal stability of the compounds.
- To use chemical design in order to identify factors that govern the regioselectivity in the reaction of 3-substituted 2,6-dichloropyridines with 1-methylpiperazine.

3. The mGlu5 receptor

The neurotransmission of (S)-glutamate molecules is mediated through the G-protein-coupled metabotropic glutamate receptors (mGluR) and the voltage-gated ionotropic glutamate receptors (iGluR). Eight mGluR subtypes have been cloned. Based on their sequence homology, pharmacology, and preferred signal transduction pathways these have been divided into three groups of receptors: mGlu1 and mGlu5 form Group I, mGlu2 and mGlu3 form Group II, and mGlu4, mGlu6, mGlu7, and mGlu8 form Group III.

Particular to glutamate receptors is the large extracellular *N*-terminal domain (consisting of about 560 amino acid moieties) that contains the orthosteric binding site of the natural agonist (S)-glutamate. Binding of an agonist is described by the “venus fly trap” model¹⁵ (inspired by the insect capture mechanism of this plant) and leads to a conformational change in the 7TM domain that elicits the intracellular activation of the G-protein trimer and the subsequent signalling events.

The signalling of mGlu5 receptors is mainly mediated by G_q-proteins. This activates phospholipase C (PLC) that catalyses the hydrolysis of phosphatidyl-inositol-4,5-bisphosphate (PIP₂) to diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP₃), resulting in elevation of intracellular Ca²⁺ levels and activation of protein kinases C (PKC).¹⁶ There are at least ten isoforms of PKC, and activation of the classical PKCs (α , β I, β II, and γ) require that both DAG and Ca²⁺ be elevated. PKCs function by phosphorylating (and thereby activating) proteins that regulate very diverse cellular processes including downregulation of intracellular Ca²⁺ signalling, proliferation, differentiation, apoptosis, autophagy, and remodelling of the actin cytoskeleton.¹⁷

mGlu5 receptors are expressed extensively in humans, both in the CNS¹⁸ and in the periphery.¹⁹

3.1 GERD and its connection to the mGluR5 receptor

Gastroesophageal reflux disease (GERD)^{20, 21} is a condition where stomach content refluxes back into the esophagus (food pipe). The lower esophageal sphincter (LES) and the crural diaphragm are the major antireflux barriers. Reflux by transient LES relaxations (TLESRs)²² is a normal phenomenon for the venting of gas after meals, however in patients with GERD the TLESRs are more likely to be associated with acid reflux.²³ The LES relaxations are coordinated by the dorsal vagal complex of the hindbrain. This is composed of two parts, the nucleus tractus solitarius, that receives the

nerve signal (vagal afferent) from the LES, and the dorsal motor nucleus, from where the nerve signal (vagal efferent) for the LES to relaxate is submitted.²⁴

Typical symptoms of GERD are heartburn and regurgitation. However, GERD may lead to the development of more severe disease states like gastroesophagitis.²⁵ Current treatments²⁶ of GERD, in addition to changes in alimentary habits, include drugs that lower the acidity of the stomach content, including inorganic antacids (e.g. magnesium trisilicate), PPIs (e.g. omeprazole), and histamine H₂ inhibitors (e.g. ranitidine). However, even with the prevalent PPIs up to 50% of patients still experience GERD symptoms.²⁷ The GABA_B agonist baclofen and the, now withdrawn, CB₁ agonist rimonabant inhibit reflux, but both show (severe) side effects due to their CNS activity which limit their use.²⁸

The mGluR5 antagonist MPEP (Figure 1) was shown to effect a decrease in TLESRs in ferret²⁹ and dog.³⁰ The localization of the mGlu5, GABA_A, GABA_B, CB₁, and CB₂ receptors along the vago-vagal³¹ reflex pathway responsible for TLESRs was recently established in humans by immunohistochemical methods.³²

3.2 Modulators of mGluR5 and their therapeutic potential

Apart from treatment of GERD,³³ the potential therapeutic applications³⁴ of mGluR5 modulators cover a broad range of indications including pain,³⁵ smoking cessation,³⁶ and psychiatric/CNS disorders³⁷ like anxiety,³⁸ schizophrenia,³⁹ depression,⁴⁰ addiction,⁴¹ epilepsy,⁴² memory dysfunction,⁴³ and fragile X syndrome (FXS),⁴⁴ the latter being the most common single-gene cause of autism. The critical role of mGluR5 in the function of neural circuits, that are required for inhibitory learning mechanisms, that is extinction of previously acquired memories, makes mGluR5 a potential target in the treatment of e.g. post-traumatic stress syndrome.⁴⁵

Given the large number of mGluR5 modulators in the public domain, only a few key compounds from representative structural classes will be highlighted here, with emphasis on those compounds that have progressed into or failed clinical trials⁴⁶ since this area was reviewed by the author in 2007.⁴⁷

In 1999 the first subtype selective,⁴⁸ non-competitive mGluR5 antagonists, SIB-1757 and SIB-1893 (Figure 1), were reported.⁴⁹ These antagonists are structurally unrelated to (S)-glutamate and function as negative allosteric modulators (NAMs)⁵⁰ by binding to an allosteric site within the 7TM region. Allosteric binding means binding to a site different from the binding site of the natural ligand, also known as the orthosteric binding site. This can be an advantage when aiming for subtype selectivity, since the homology

between the mGluRs is lower outside the orthosteric binding site.⁵¹ Subsequent optimisation led to the discovery of 2-methyl-6-(phenylethynyl)pyridine (MPEP)⁵² and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP),⁵³ however their further development was hampered by low metabolic stability. Positron electron topography (PET) ligands,⁵⁴ that are based on the MPEP and MTEP core structures, have become tool compounds to study receptor occupancy and help guide dosing of mGluR5 modulators in CNS disorders.

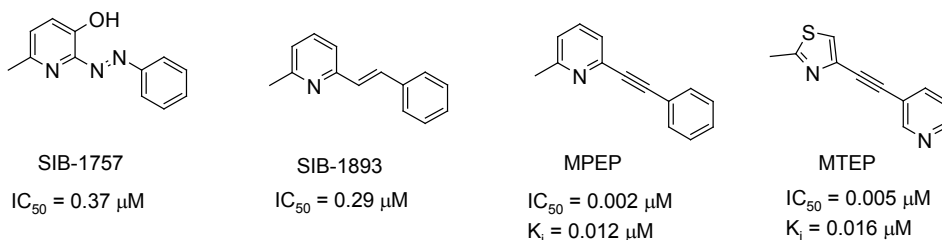


Figure 1. Early examples of subtype selective negative allosteric modulators of the mGlu5 receptor. IC₅₀: Ca²⁺ fluorescence assay (Fura 2-loaded cells); K_i: Displacement of [³H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine from rat cortical membranes.

In 2005 fenobam (Figure 2), a lead structure at McNeil Laboratories in the 1970's with a then unknown target, was reported⁵⁵ to be a potent, subtype-selective NAM of mGluR5. Soon after, SAR studies were published with fenobam as lead structure.⁵⁶ A phase II study of fenobam for the treatment of FXS was initiated by Neuropharm Ltd⁵⁷ (now Autism Therapeutics), however no progress has been reported.⁵⁸

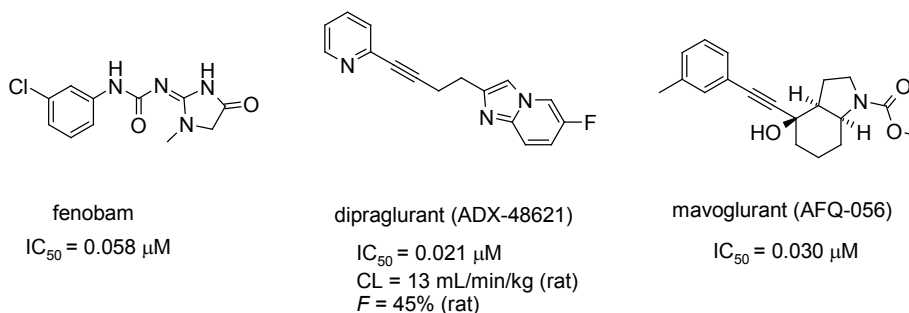


Figure 2. Examples of most advanced mGluR5 antagonists in clinical development. IC₅₀ values were determined in a Ca²⁺ flux assay.

Dipraglurant (ADX-48621) from Addex Therapeutics showed in vivo activity in different anxiety models in rat⁵⁰ and is currently in phase II for the treatment of dyskinesia in Parkinson's disease. Mavoglurant (AFQ-056)⁵⁹ is under active development by Novartis

for the treatment of FXS (phase II-III), Parkinson's disease (phase II), and Huntington's disease (phase II).

Also under active development, but with no structures in the public domain, are STX-107 (FXS, phase I, Seaside Therapeutics), RO-4917523 (depression and FXS, phase II, Roche), and RG-7090 (depression, phase II, Chugai). ADX-10059, which showed a significant reduction of reflux episodes in GERD in a proof-of-concept clinical study,⁶⁰ was discontinued after phase I in 2010, while AZD2066 (GERD, phase II, AstraZeneca) was discontinued in 2011. Our study of a novel series of mGluR5 antagonists is presented in the following.

4. Identification and SAR of a new lead series of mGluR5 antagonists (papers I/II)⁶¹

The 6-methyl-2-alkynylpyridine derivative (\pm)-**1** (Figure 3) was a quite potent mGluR5 antagonist (racemate, IC_{50} = 0.30 μ M, FLIPR assay) that resulted from an HTS (high-throughput screening) of the AstraZeneca compound collection. The compound with its two aryl groups joined by an alkyne containing linker had a clear structural resemblance to MPEP and MTEP. One feature that set compound (\pm)-**1** apart from MPEP and MTEP was the extended linker. The SAR of this hit structure was studied by varying both the two aryl groups and the methylenoxy (-CH₂O-) moiety of the linker.⁶²

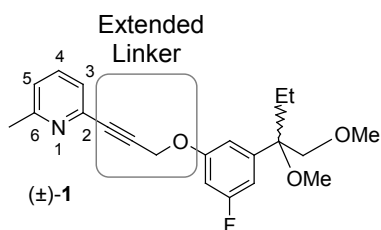
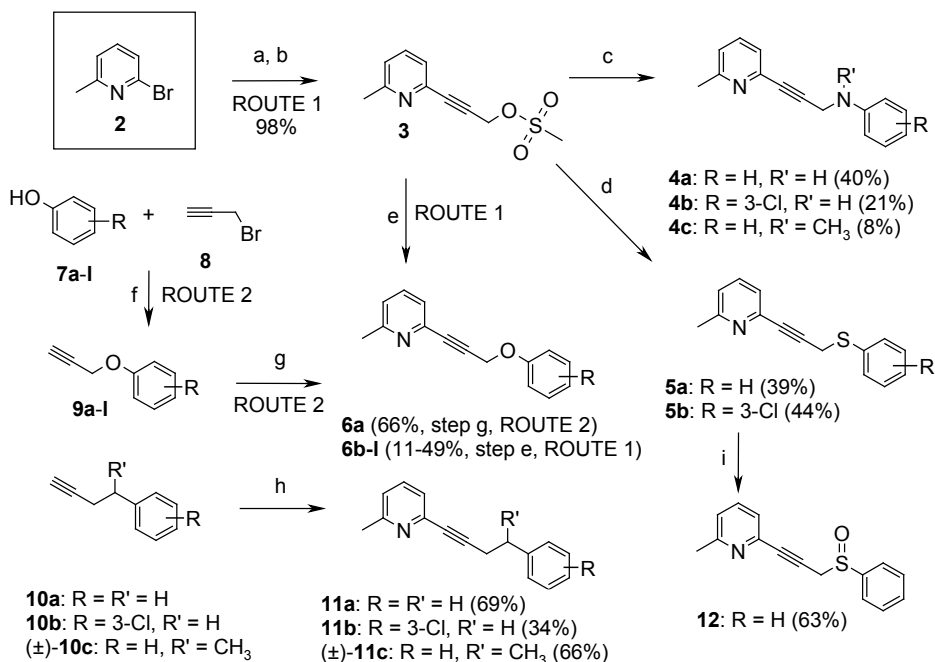


Figure 3. 2-Alkynyl-6-methylpyridine hit (\pm)-**1** from HTS (racemate, IC_{50} = 0.30 μ M, FLIPR) with the extended linker highlighted.

4.1 Synthesis

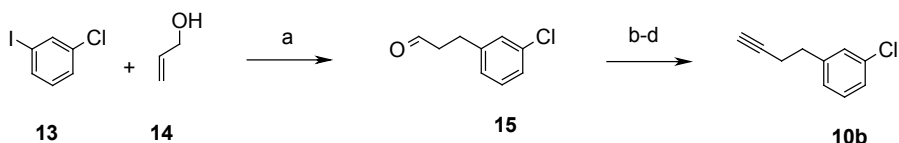
Sonogashira coupling⁶³ of 2-bromo-6-methylpyridine **2** (Scheme 1) with propargylic alcohol, followed by mesylation produced **3** that was treated with anilines or thiols to form amines **4a-c** and thioethers **5a-b**, respectively.⁶⁴ The ethers **6b-l** were prepared in a similar fashion by reaction with phenols (designated ROUTE 1) in 11-49% yields (see Table 1 for all ether variations). ROUTE 2, i.e. treatment of a phenol **7a-l** with propargylic bromide **8** to give terminal alkynes **9a-l**,⁶⁵ followed by Sonogashira coupling with **2**, was used for synthesis of **6a** (R = H),⁶⁶ scale-up of **6b-l** and for Sonogashira coupling of **9** with other heteroaryl halogenides than **2** to replace the 6-methylpyridine with alternative heterocycles.

The full-carbon analogs **11a-c** were prepared by Sonogashira coupling of the terminal alkynes **10a-c** with **2**. The sulfoxide **12** was obtained by simple oxidation of the thioether **5a** with *m*-CPBA. Further oxidation with *m*-CPBA to provide the sulfone was not possible due to the concomitant formation of the *N*-oxide.



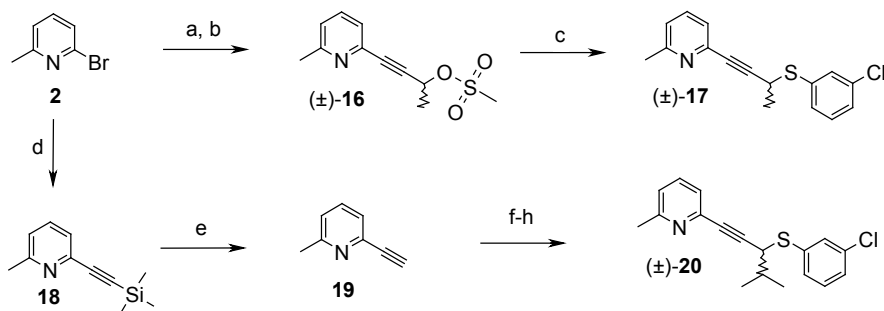
Scheme 1. Reagents and conditions: (a) $\text{HC} \equiv \text{CCH}_2\text{OH}$, $(\text{PPh}_3)_2\text{PdCl}_2$, CuI, TEA, 60 °C, 3.5 h (61%); (b) MsCl, TEA, DCM, -20 °C, 1 h (98%); (c) e.g. **4a**: PhNH_2 , TEA, rt, 1.5 h; (d) e.g. **5a**: PhSH , TEA, THF, rt, 1 h; (e) e.g. **6e**: 3-Cl-PhOH, K_2CO_3 , acetone, 60 °C, 5 h (40%); (f) e.g. **9a** (R = H): K_2CO_3 , acetone, 60 °C, 17 h (78%); (g) e.g. **6a** (R = H): $(\text{PPh}_3)_2\text{PdCl}_2$, CuI, TEA, 60 °C, 2 h; (h) e.g. **11b**: **2**, **10b**, $(\text{PPh}_3)_2\text{PdCl}_2$, CuI, TEA, 60 °C, 12 h; (i) *m*-CPBA, DCM, -50 °C to 0 °C, 1.5 h.

A method was developed to provide the terminal alkyne **10b** (Scheme 2), which contrary to **10a** and (±)-**10c** was not commercially available. Thus, coupling by the procedure of Jeffery⁶⁷ of 3-chloro-iodobenzene **13** with allyl alcohol **14** gave the aldehyde **15**. Conversion by the Corey-Fuchs⁶⁸ method to the corresponding 4,4-dibromoalkene, followed by elimination of HBr and halogen-lithium exchange⁶⁹ provided **10b**. The product was of high purity and no chromatography was needed in this sequence.



Scheme 2. Reagents and conditions: (a) $\text{Pd}(\text{OAc})_2$, $(n\text{-Bu})_4\text{NCl}$, NaHCO_3 , DMF, rt, 16 h, then 50 °C, 16 h (59%); (b) CBr_4 , PPh_3 , Zn, DCM, rt, 14 h (87%); (c) $\text{LiN}(\text{Si}(\text{CH}_3)_3)_2$, 1.5 equiv, THF, -78 °C, 0.5 h; (d) add *n*-BuLi, 2.5 equiv, -78 °C, 1 h, then rt, 1 h; quench with H_2O (96%).

Analogs with branching β to the phenyl group in the thioether sub-series were prepared as shown in Scheme 3. While (\pm)-**17** was prepared in analogy to **5a-b**, an alternative method was developed to attain (\pm)-**20**. This included a two-step conversion of **2** to the terminal alkyne **19**, followed by reaction with methylpropanal to give a secondary alcohol, which was mesylated in situ and treated with 3-chlorothiophenol. The low yield was ascribed to instability of the intermediary mesylate which would be prone to elimination to provide a trisubstituted, conjugated alkene.

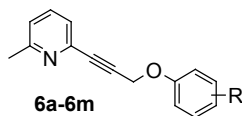


Scheme 3. Reagents and conditions: (a) $\text{HC}\equiv\text{CCH}(\text{CH}_3)\text{OH}$ (rac.), $(\text{PPh}_3)_2\text{PdCl}_2$, CuI , TEA , $60\text{ }^\circ\text{C}$, 4 h (67%); (b) MsCl , TEA , DCM , $-20\text{ }^\circ\text{C}$, 1 h (quant.); (c) 3-Cl-PhSH, TEA , rt , 16 h (34%); (d) $\text{HC}\equiv\text{C}(\text{Si}(\text{CH}_3)_3)$, $(\text{PPh}_3)_2\text{PdCl}_2$, CuI , TEA , $60\text{ }^\circ\text{C}$, 2 h then rt , 16 h (56%); (e) K_2CO_3 , DCM/MeOH , rt , 2 h (97%); (f) $\text{LiN}(\text{Si}(\text{CH}_3)_3)_2$, 2-methylpropanal, THF , $-78\text{ }^\circ\text{C}$ to rt ; (g) then add MsCl , TEA , DCM , rt , 3 h; (h) then add 3-Cl-PhSH, TEA , DCM , rt , 16 h (4%, 3 steps).

4.2 Results and discussion of SAR

Findings prior to this study from variation of the 6-methylpyridine moiety of compound **6i** (Table 1; $\text{IC}_{50} = 0.46\text{ }\mu\text{M}$) had shown that replacement of the 6-methyl substituent with a 6-hydrogen led to a six-fold lower potency in the FLIPR assay. The 5-methyl analog showed a 20-fold reduction in potency, while the 4-methyl and 3-methyl analogs had no activity. Replacement of the 6-methylpyridine with other heterocycles like pyrazine or 1,3-thiazole also led to inactive compounds. Thus, given the superiority of the 6-methylpyridines (which was also observed in MPEP analogs), this group was retained in further SAR studies.

Variation of the substitution pattern of the phenyl group (Table 1) was made for the propargylic ether sub-series. The remarkable lack of potency for the unsubstituted phenyl compound **6a**⁷⁰ compared to the substituted phenyls beared no resemblance in the MPEP series. The 2-position was unfavourable for substitution (**6b**) compared to the 3- and 4-position where lipophilic substituents like chlorine or methyl (**6c-f**) led to compounds with comparatively high potency (0.10-0.20 μM). The lower potency of *tert*-

Table 1. Effects on potency in the FLIPR assay of the propargylic ether sub-series by variation of the phenyl substituents (R).⁷¹

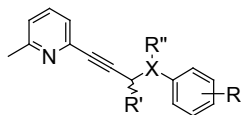
Cpd No	R	FLIPR ^a IC ₅₀ (μ M)	ACD logD ⁷²	LLE ^b	Cpd No	R	FLIPR IC ₅₀ (μ M)	ACD logD	LLE
6a	-H	>10 ^c	3.7	<1.3	6g	4- <i>tert</i> -Bu	0.45	5.4	0.9
6b	2-CH ₃	2.4	4.2	1.4	6h	3-NO ₂	0.42	3.7	2.7
6c	3-CH ₃	0.20	4.2	2.5	6i	4-OCH ₃	0.46	3.7	2.6
6d	4-CH ₃	0.11	4.2	2.5	6j	2,3-di-Cl	0.15	5.0	1.8
6e	3-Cl	0.10	4.5	2.5	6k	3,4-di-Cl	0.015	5.1	2.7
6f	4-Cl	0.10	4.4	2.6	6l	4-Cl, 3,5-di-CH ₃	0.015	5.3	2.5

^aG_q coupled signalling leads to mobilization of intracellular calcium. In the FLIPR assay the effect of an antagonist in suppressing the mobilization of intracellular calcium was measured by using the acetoxymethyl ester of the fluorescent calcium indicator fluo-3. ^bLLE = lipophilic ligand efficiency = pIC₅₀-ACDlogD. ^cMaximum concentration tested was 900 μ M.

butyl compound **6g** may be explained by steric reasons, by comparison with compound **6l** of similar lipophilicity.

The stability of compounds exposed to liver microsomes *in vitro* is used as a model for metabolic stability *in vivo*. Although few compounds were tested, it appeared that low *in vitro* stability in liver microsomes was an issue. This could be a consequence of the compounds having a comparatively high lipophilicity.⁷³ However, attempts to lower the lipophilicity failed; for instance led the introduction of substituents like nitro and methoxy (**6h/i**) in the 3- and 4-position, respectively, to lower potency and the replacement of the phenyl with 2-, 3-, or 4-pyridines gave inactive compounds (not shown). It was notable that both the replacement of lipophilic substituents (Cl, CH₃) in the 3- or 4-position with less lipophilic substituents (OCH₃, NO₂) and the introduction of additional lipophilic substituents (Cl, CH₃) as in **6k-l** left the Lipophilic Ligand Efficiency⁷⁴ (LLE; here defined as pIC₅₀-ACDlogD) unchanged, which indicated that lipophilicity is the driving force in the 3-, 4-, and 5-positions as provider of potency.

Replacement of the linker oxygen (Table 2) gave an increase in potency in the order N < O < S < C, as exemplified by the 3-Cl phenyl compounds **4b**, **6e**, **5b**, and **11b**, thus replacement of oxygen with carbon led to a 20-fold increase in potency (to 0.005 μ M; **11b** vs **6e**). While the introduction of a methyl substitution α to the phenyl group gave unchanged potency in the carbon sub-series ((\pm)-**11c** vs **11a**) a detrimental lowering of

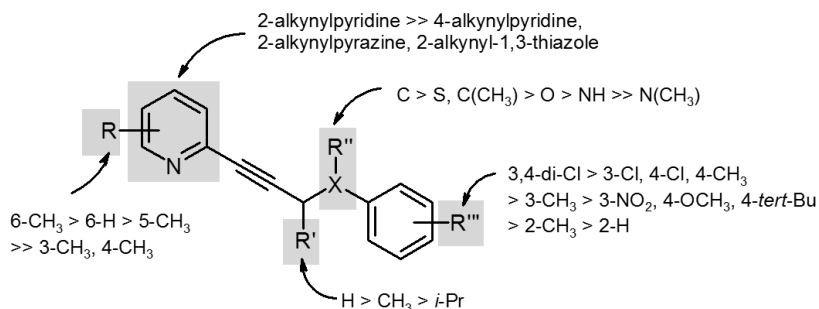
Table 2. Effects on potency by variation of both the linker (R' and X-R'') and the phenyl substituents (R).

Cpd No	R'	X-R''	R	Potency IC ₅₀ (μM) FLIPR	ACD logD	LLE ^a
4a	H	NH	H	0.22	3.1	3.6
4b	H	NH	3-Cl	0.17	4.3	2.5
4c	H	N(CH ₃)	H	6.7	4.5	0.67
5a	H	S	H	0.59	3.7	2.5
5b	H	S	3-Cl	0.043	4.9	2.5
6e	H	O	3-Cl	0.10	4.5	2.5
11a	H	C	H	0.078	4.1	3.0
11b	H	C	3-Cl	0.005	4.9	3.4
(±)- 11c	H	C(CH ₃)	H	0.059	4.6	2.6
12	H	S(=O)	H	>10 ^b	3.3	<1.7
(±)- 17	CH ₃	S	3-Cl	0.16	5.2	1.6
(±)- 20	<i>i</i> -Pr	S	3-Cl	0.46	6.1	0.23

^aLLE = lipophilic ligand efficiency = pIC₅₀-ACDlogD. ^bMaximum concentration tested was 900 μM.

potency from 0.22 μM to 6.7 μM was observed in the amine sub-series (**4a** vs **4c**). S-Oxidation of thioether **5a** gave sulfoxide **12** which showed no potency. Branching β to the phenyl group gave a 4-10 fold lowering of potency (from 0.043 μM) in the thioether sub-series (compare (±)-**17** and (±)-**20** with **5b**).⁷⁵

The SAR of the 2-alkynyl-pyridines are summarized in Figure 4 with the (usually made, seldomly challenged) assumption that the substituent effects are additive which requires that the ligands bind in a similar fashion.

**Figure 4.** Summary of SAR for the series of 2-alkynyl-pyridines as antagonists of the mGlu5 receptor.

The two lead compounds with highest potencies in the ether sub-series (**6e**) and the carbon sub-series (**11b**) were screened for metabolic stability in rat liver microsomes (RLM); both had high clearance ($CL_{int} > 100$ mL/min/mg). This was ascribed to the high lipophilicity (ACDlogD 4.5-4.9) of these compounds and prevented further development of the series. The compounds had acceptable levels of aqueous solubility,⁷⁶ for example 23 μ M for **11b** in buffer at pH7.4. Competitors successfully replaced the phenyl group in the carbon sub-series by a heterocycle which (combined with a left-side pyridine) resulted in the structurally similar Phase II-compound dipraglurant (Figure 2 above) which had lower lipophilicity (ACDlogD 3.3) and high metabolic stability in vivo.

Lead compound **11b** was tested in vivo in dog (N = 4) and showed a 31% reduction of TLESR at dose 3.9 μ mol/kg/h (1.0 mg/kg/h) given as an infusion. This was an encouraging result as a starting point for a lead compound in comparison to MPEP which in dog (N = 3) showed a 59% reduction of TLESR at the higher iv dose 8.7 μ mol/kg.³⁰

5. The role of platelets and purinergic receptors in primary hemostasis⁷⁷

Hemostasis⁷⁸ is a way for the human organism to repair the vasculature and prevent blood loss. Primary hemostasis ultimately leads to the formation of a platelet plug while secondary hemostasis results from the activation of the coagulation cascade that leads to formation of thrombin, which is the catalyst for the conversion of fibrinogen to the fibrin strands that stabilize the platelet plug.

Human platelets are subcellular fragments with a 2–5 μm diameter that are derived from the cytoplasm of megakaryocytes. Structural features of human platelets include granules (dense, lysosomal and α -granules; containing different cocktails of signalling molecules), mitochondria, a cytoskeleton, a surface-connected canalicular system and a dense tubular system, but no nucleus.⁷⁹ Platelets play a crucial role in hemostasis and thrombosis.

Activation of platelets can occur by vessel rupture, for example of an atherosclerotic plaque, whereby platelets become exposed to subendothelial proteins like collagen or von Willebrand factor.⁸⁰ This leads to adhesion of the platelets to the subendothelium, which is mediated by integrin and glucoprotein receptors, and to release of ADP and ATP from the platelets' dense granules and of fibrinogen, factor V, and P-selectin from their α -granules.⁸¹ These primary adherent platelets recruit further platelets from the blood stream and the aggregation is in part mediated by fibrinogen that links together transmembrane-bound glycoproteins on adjacent platelets.⁸²

Under high shear stress conditions (i.e. under high flow) the released ADP activates the purinergic P2Y₁ and P2Y₁₂ receptors. Activation of the G_q-coupled⁸³ P2Y₁ receptors leads to increased levels of PLC β , which catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to release inositol triphosphate (IP₃).⁸⁴ Binding of IP₃ to the IP₃ receptor mobilizes intracellular calcium ions, which together with an influx of calcium ions (through the P2X₁ ion channel) and actin polymerization results in a platelet shape-change from discoid to spherical.⁸⁵ Activation of the G_i-coupled P2Y₁₂ receptors inhibits adenylyl cyclase (AC) activity, thereby lowering the formation of cAMP.⁸⁶ The dual activation of P2Y₁ and P2Y₁₂, i.e. signalling via both the G_q and G_i pathways, which leads to increased Ca²⁺ signalling and decreased cAMP levels, respectively, is required for platelet aggregation.⁸⁷

The platelet P2Y₁ and P2Y₁₂ receptors belong to the P2 purinergic receptors,⁸⁸ that include two major families of receptors in humans; the G-protein coupled or metabotropic P2Y receptors and the ligand-gated ion channels or ionotropic P2X

receptors. Eight subtypes of P2Y receptors and seven subtypes of P2X receptors have been found in human. These include the P2Y receptors P2Y₁, P2Y₂, P2Y₄, P2Y₆, and P2Y₁₁₋₁₄ and the ion channels P2X₁₋₇.⁸⁹ Of these, P2Y₁, P2Y₁₂, P2Y₁₄ and P2X₁ are expressed on platelets. The cation channel P2X₁ is activated by ATP⁹⁰ (that is released from the dense granules). This leads to an influx of sodium and calcium ions, and thus to an amplification of the intracellular calcium signal. Signalling via P2X₁ is the fastest activation response of platelets, thus P2X₁ receptors play a priming role in platelet activation.⁹¹ The possible role of the P2Y₁₄ receptor in hemostasis is not clarified.⁹²

The purinergic signalling on platelets also include two of the four human adenosine GPCRs, namely the G_s-coupled A_{2A} and A_{2B} adenosine receptors, where binding of adenine has an anti-aggregatory effect.

It is remarkable how adenine and its derivatives cAMP, AMP, ADP, and ATP play central roles in both intercellular signalling, storage of energy (ATP) and storage/transmission of information (structural motifs of DNA/RNA).

5.1 Signalling events leading to platelet aggregation

For the initial activation of platelets to be followed by aggregation and formation of a platelet plug both a reduction in cAMP levels and prolonged calcium signalling are required.

High levels of cAMP make platelets remain in the quiescent state. This is achieved by adenosine, PGI₂ (prostacyclin), and NO (nitroxide) signalling.⁹³ Adenosine and PGI₂ bind to the adenosin receptor A_{2A} and the prostanoid TP receptor, respectively. Both these receptors are G_s coupled, thus their activation leads to increased adenylyl cyclase activity and consequently to raised levels of cAMP. NO activates sGC (soluble guanyl cyclase) which leads to increased cGMP levels. cGMP is an inhibitor of PDE3 (platelet phosphodiesterase 3), and since PDE3's function is to hydrolyze cAMP to AMP its inhibition also leads to increased levels of cAMP.

In addition to the purinergic signalling by P2Y₁ and P2Y₁₂ elicited by the released ADP, platelet activation by increased or sustained calcium concentration via the G_q/PLC-β pathway is also triggered by binding of thrombin to the protease-activated receptors⁹⁴ PAR1 and PAR4 and by binding of thromboxane A₂ (TXA₂) to the prostanoid TP receptor (= TXA₂ receptor)⁹⁵ (Figure 5). When platelets become exposed to ADP, the coordinated signaling⁹⁶ through integrin α_{11b}β₃ (mediated by exposure to the subendothelium) and P2Y₁/P2Y₁₂ leads to formation of TXA₂ from arachidonic acid by two oxidation steps

involving the enzymes cyclooxygenase 1 (COX-1) and TXA₂ synthase. The formed TXA₂ binds to the TP receptor, thereby further increasing the calcium signalling.

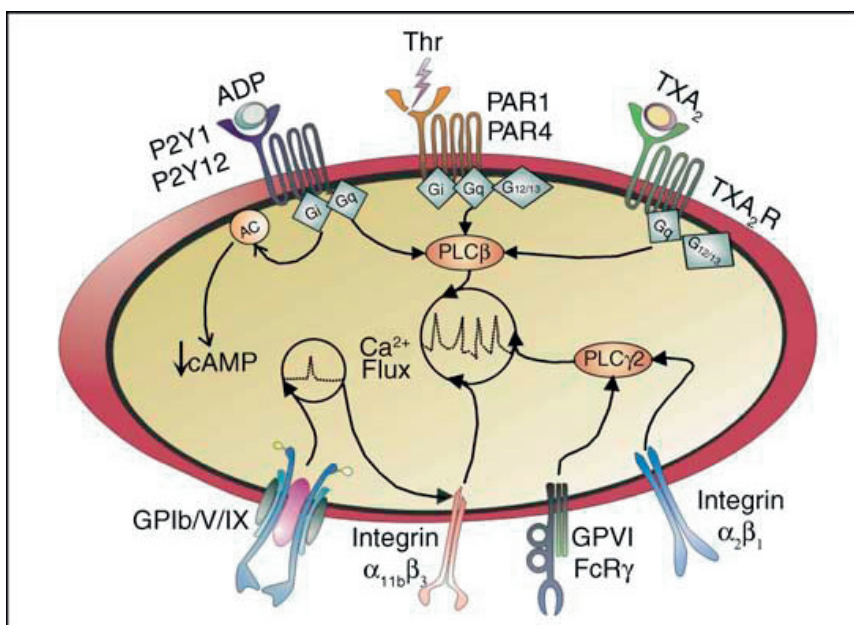


Figure 5. The cellular Ca²⁺ concentration is regulated by multiple input stimuli. The adhesion of platelets to the subendothelium (e.g. collagen and von Willebrand factor) and to other platelets is mediated by glycoprotein (GPIb/V/IX and GPVI/FcRγ) and integrin (α_{11b}β₃ and α₂β₁) receptors. Activation of the P2Y₁₂ receptor by ADP potentiates the effect of increased cellular Ca²⁺ concentration, thereby enhancing the platelet activation. © John Wiley and Sons. Reprinted with permission.

5.2 Antagonists of the P2Y₁₂ receptor

The central role of the P2Y₁₂ receptor in platelet function together with its restricted tissue distribution⁹⁷ (on platelets and in certain brain tissues,⁹⁸ glial and microglial cells and in vascular smooth muscular cells⁹⁹) makes it an attractive target for the development of novel antiplatelet therapies for the treatment of cardiovascular diseases like thrombosis.⁷⁹ Since such therapies also impair normal platelet function, a central concern is to improve the therapeutic window by separating antithrombotic effect and bleeding risk.

Ticlopidine (Figure 6) was the first oral 4,5,6,7-tetrahydrothieno[3,2,c]pyridine derivative (commonly termed a “thienopyridine”) for antithrombotic treatment when introduced in 1995, the target at that time being unidentified.⁹⁸ Due to side effects, ticlopidine has largely been replaced by clopidogrel,¹⁰⁰ prasugrel,¹⁰¹ and ticagrelor.

While the thienopyridines are pro-drugs that require metabolic activation to form the active compounds, which bind irreversibly to the P2Y₁₂ receptor, ticagrelor is a direct-acting, reversibly binding antagonist. Ticagrelor was developed from ATP as lead structure.¹⁰² Preclinical data¹⁰³ suggested that reversible binding¹⁰⁴ would not only lead to a faster off-set of effect, but also improve the separation of anti-thrombotic effect and bleeding risk. The clinical benefits of ticagrelor in comparison to clopidogrel were demonstrated in a phase III trial.¹⁰⁵

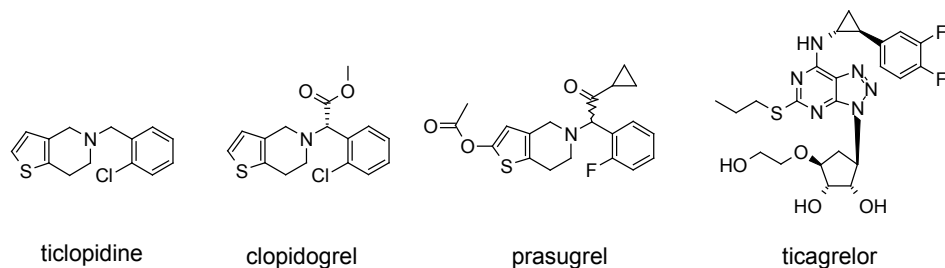


Figure 6. Examples of antagonists of the P2Y₁₂ receptor in therapeutic use. Due to side effects ticlopidine has been largely replaced by the other drugs.

Examples of other structural classes of P2Y₁₂ antagonists¹⁰⁶ are benzothiazolo[2,3-c]thiadiazines like **21**¹⁰⁷ and the sulfonylurea **22** (elinogrel potassium, Figure 7). The latter was abandoned in 2012 after an unsuccessful phase III trial.¹⁰⁸ Parlow et al. have reported several series of piperazinyl-glutamate-pyridines (e.g. **23**),¹⁰⁹ featured by the acidic side chain of the glutamic acid moiety. Other examples include anthroquinones (e.g. **24**),¹¹⁰ thienopyrimidines (e.g. **25**),¹¹¹ and series of adenosine analogs featured by one or more lipophilic substituents on the ribose moiety (e.g. **26**).¹¹²

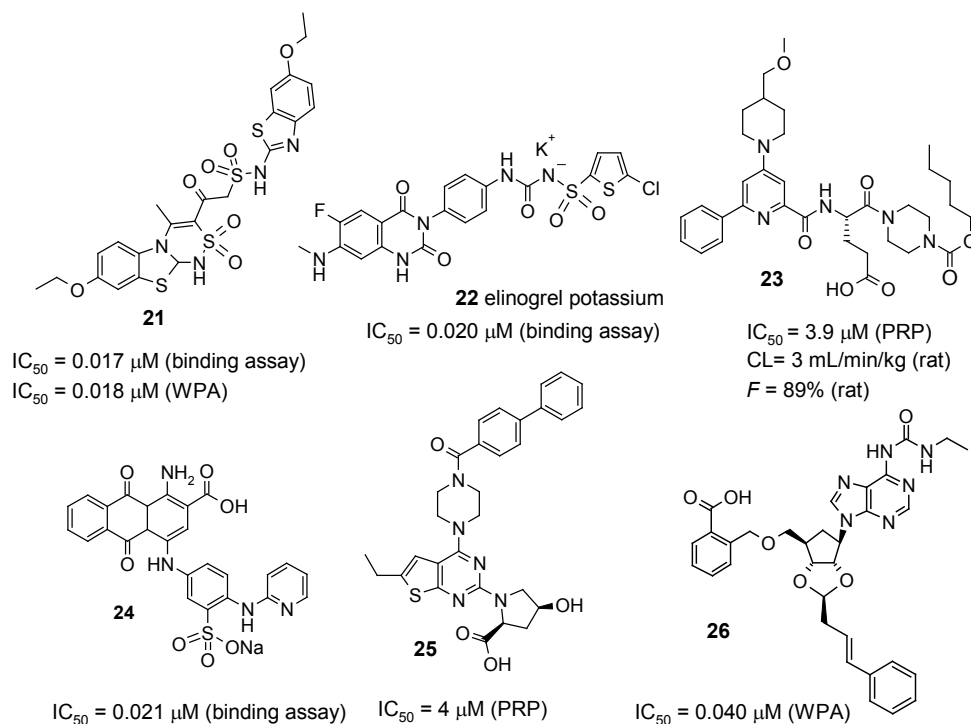


Figure 7. Examples of other structural classes of P2Y₁₂ antagonists. WPA = washed platelet assay, PRP = human platelet-rich plasma assay.

5.3 Other antagonists for the treatment of arterial thrombosis

Acute arterial thrombosis is accountable for most cases of myocardial infarction and ca. 80% of strokes, which collectively are the most common causes of death in the developed world.⁷⁹ Thrombi (= blood clots) that form in the arterial circulation are platelet-rich and are formed under high-shear stress conditions (i.e. under high flow) while thrombi in the venous circulation are fibrin-rich and are formed under low-shear stress conditions.¹¹³ This is reflected in the different ways in which thrombosis is treated since antiplatelet drugs are primarily effective in the arterial circulation and anticoagulants, i.e. drugs that target blood factors in the coagulation cascade, in the venous circulation. However, there is clinical evidence that the combination of antiplatelet and anticoagulant therapy is more effective than either treatment alone.¹¹⁴

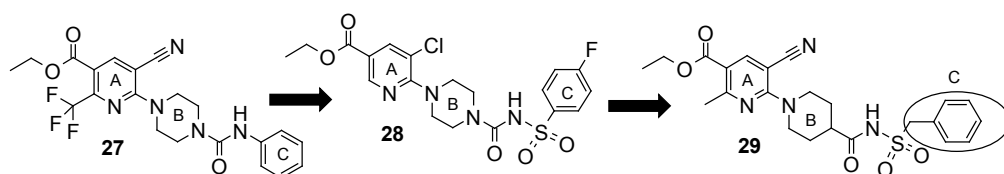
Apart from P2Y₁₂ antagonists, other antiplatelet agents are in current use. The weak COX-1 inhibitor, aspirin,¹¹⁵ is widely used for secondary prevention of cardiovascular

events (that is in patients with known cardiovascular disease). Abciximab, eptifibatide, and tirofiban are antagonists of the integrin $\alpha_{11b}\beta_3$ receptor. Dipyridamole is an adenosine reuptake inhibitor and thus increases the concentration of adenosine which has an anti-aggregatory effect by binding to the platelet A_{2A} and A_{2B} adenosine receptors. Cilostazol is a PDE3 inhibitor and thus increases the levels of cAMP (vide supra). No antagonists of the PAR1/PAR4 nor the TP (TXA_2) receptors have so far reached the market.

6. Ethyl 6-amino nicotinate derivatives as P2Y₁₂ antagonists¹¹⁶

We identified series of ethyl 6-amino nicotinate (Scheme 4), exemplified by the urea **27**, the sulfonylurea **28**, and the acyl sulfonamide **29**, as antagonists of the P2Y₁₂ receptor. These compounds shared the feature of being composed of chemically tractable building blocks, which meant that some SAR investigations could be made efficiently by using parallel synthesis.

In the following, the synthesis development, the SAR, and the improvements in properties (including potency, solubility, stability in solution, and metabolic stability) that resulted from specific variations of the linker, the type of central ring (B) and the substituents on the pyridine (A) and aryl/benzyl (C) rings, respectively, will be described for the urea and the sulfonyl urea series. The acyl sulfonamides will not be treated per se, but will be used as a reference series to describe the effects on e.g. metabolic stability by introduction of electron donating substituents in the pyridine 2-position and replacement of the 3-ethoxycarbonyl substituent with other functionalities.



Scheme 4. Progression in the development of ethyl nicotinate derivatives as P2Y₁₂ antagonists, exemplified by urea **27**, sulfonylurea **28**, and acyl sulfonamide **29**.

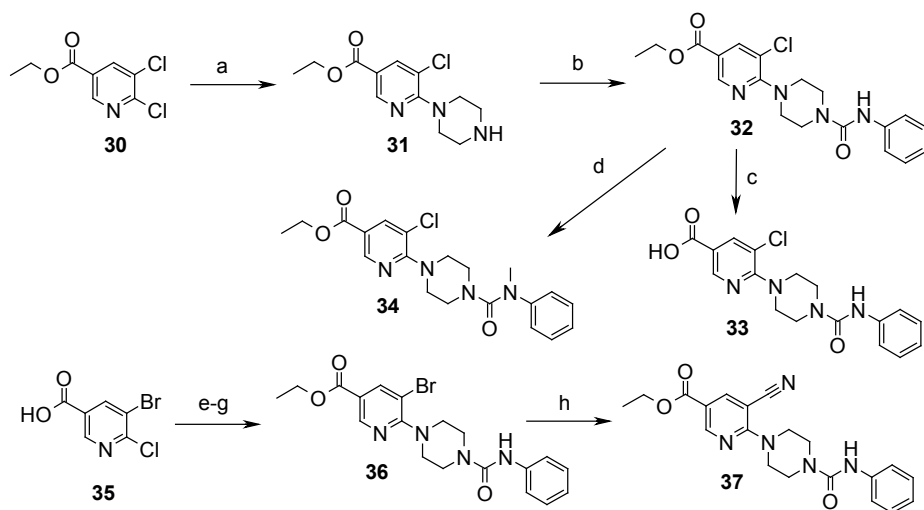
6.1 Identification of a new lead series of ureas as P2Y₁₂ antagonists (paper III)

The urea derivative **27** (Scheme 4; binding affinity: IC₅₀ = 0.33 μM, potency in GTPγS assay¹¹⁷: IC₅₀ = 0.68 μM) resulted from an HTS of the AstraZeneca compound collection against the human P2Y₁₂ receptor. Synthesis was developed to vary the substitution patterns of the A- and C-rings in order to study the SAR of the urea series.

6.1.1 Synthesis

Nucleophilic aromatic substitution (S_NAr) reaction of commercially available ethyl 5,6-dichloronicotinate **30** (Scheme 5) with piperazine produced **31**, which was treated with phenylisocyanate to provide **32**. Derivatization of **32** by hydrolysis of the ethyl ester under alkaline conditions gave nicotinic acid derivative **33** while simple *N*-methylation of the linker-nitrogen of **32** gave **34**.

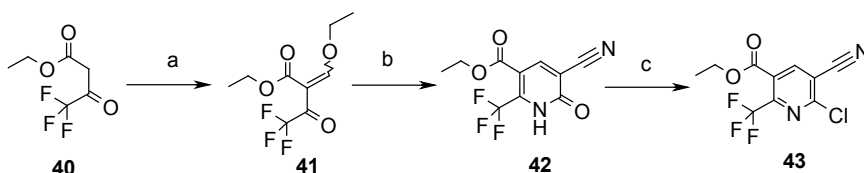
The nicotinic acid derivative **35** was esterified and the product was reacted in analogy to **30** to give **36** which was subjected to Pd-catalyzed cyanation¹¹⁸ to give **37**. Compounds **38** and **39** (Table 3) were prepared in analogy to **32**, starting from commercially available ethyl 6-chloronicotinate and 2-chloro-6-trifluoromethylnicotinonitrile, respectively.



Scheme 5. Reagents and conditions: (a) Piperazine, TEA, EtOH, MW, 120 °C, 10 min. (61%); (b) PhNCO, MeCN, rt, 20 h (94%); (c) LiOH, THF, rt, 16 h (91%); (d) NaH, MeI, DMF, 0 °C, 5 min. (38%); (e) H₂SO₄, EtOH, reflux, 5 h (78%); (f) piperazine, TEA, EtOH, MW, 120 °C, 10 min. (66%); (g) PhNCO, MeCN, rt, 16 h (95%); (h) KCN, Pd(OAc)₂, 1,5-bis(diphenylphosphino)pentane (DPPPE), TMEDA, toluene, 120 °C, 16 h (26%).

Ethyl 6-chloro-5-cyano-2-trifluoromethylnicotinate **43** (Scheme 6) was needed as starting material to make analogs of **27** with variations of the C-ring, prepared by consecutive treatment with piperazine and different isocyanates. Initially, methods like 5-carboxylation¹¹⁹ of the 5-anion of 2-chloro-6-trifluoromethylnicotinonitrile, followed by esterification were tested, however it soon became clear that a Hantzsch-type procedure¹²⁰ was the method-of-choice. Thus, reaction of the β -keto-ester **40** with

triethyl orthoformate produced **41** (as an *E/Z* mixture, however both isomers reacted in the next step). Condensation of **41** with 2-cyanoacetamide gave the pyridone **42** which was treated with oxalyl chloride to provide the 6-chloropyridine **43**. Although the Hantzsch-type of synthesis did not allow for variation of the pyridine 4-position (which could be interesting from a SAR perspective), it did allow for variation of the 2-position (vide infra).

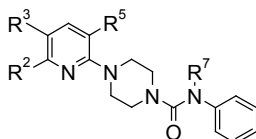


Scheme 6. Synthesis of ethyl 6-chloro-5-cyano-2-trifluoromethylnicotinate. Reagents and conditions: (a) $\text{HC}(\text{OEt})_3$, Ac_2O , $120\text{ }^\circ\text{C}$, 2 h, then $140\text{ }^\circ\text{C}$, 5 h (64% of *E/Z* mixture); (b) $\text{H}_2\text{NC}(\text{O})\text{CH}_2\text{CN}$, NaOEt , EtOH , rt, 16 h (83%); (c) $(\text{COCl})_2$, DMF (cat.), DCM , reflux, 14 h (91%).

6.1.2 Results and discussion of SAR

Initially it was investigated how the activity depended on the substitution pattern of the pyridine (A) ring. This was done by comparing compounds with identical B- and C-rings (Table 3). Replacement of the pyridine 2- CF_3 substituent of **27** with hydrogen (as in **37**) or of both the 2- CF_3 and 5-CN substituents with hydrogen (as in **38**) led to a 4-6 fold decrease in potency. The 5-Cl substituent could be replaced with 5-Br or 5-CN without affecting the affinity or potency (**36** and **37** vs **32**). Replacement of the 3-ethoxycarbonyl substituent of **27** with hydrogen led to an inactive compound (**39**).

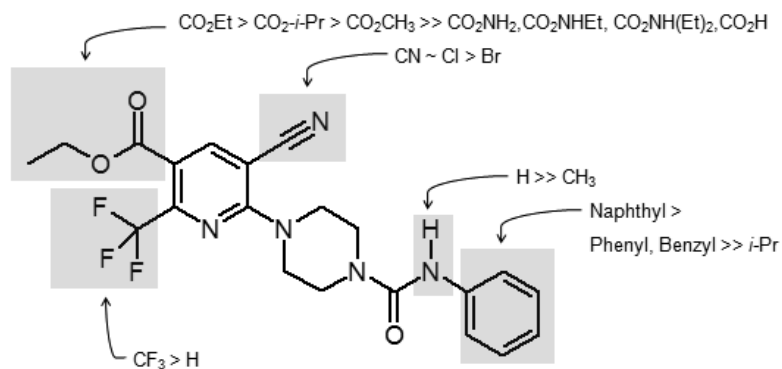
The importance of the 3-ethoxycarbonyl substituent was further underlined when noticing the lack of activity for the corresponding nicotinic acid derivative **33**. Replacement of the 3-ethoxycarbonyl with either 3-methoxycarbonyl or 3-isopropoxy-carbonyl lowered the potency 5-fold while replacement with structurally simple carboxamides (CONH_2 , CONHEt , $\text{CONH}(\text{Et})_2$) or methyl acylsulfonamide gave inactive compounds (not in table).¹²¹ *N*-Methylation of the urea linker of **32** (to give **34**) led to an 11-fold reduction in binding affinity and a 6-fold reduction in potency in the $\text{GTP}\gamma\text{S}$ assay.

Table 3. SAR of the urea series as function of the pyridine substituents R², R³, R⁵ and the urea substituent R⁷.^a

Cpd No	R ²	R ³	R ⁵	R ⁷	Binding IC ₅₀ (μM)	GTPγS IC ₅₀ (μM)
27	CF ₃	COOEt	CN	H	0.32	0.68
32	H	COOEt	Cl	H	0.88	1.7
33	H	COOH	Cl	H	>33 ^b	>33 ^b
34	H	COOEt	Cl	CH ₃	9.6	9.7
36	H	COOEt	Br	H	1.8	2.2
37	H	COOEt	CN	H	0.92	2.6
38	H	COOEt	H	H	4.1	3.8
39	CF ₃	H	CN	H	>33 ^b	>33 ^b

^aThe GTPγS functional assay is based on the principle that activation of P2Y₁₂ by an agonist (ADP) leads to conformational changes that favors binding of the receptor to a G-protein. This leads to formation of a agonist-receptor-G-protein ternary complex which induces GDP bound on the G-protein α-subunit to exchange with GTPγ³⁶S. The effect of an added antagonist can thus be determined by the fraction of unbound GTPγ³⁶S. ^bMaximum concentration tested was 33 μM.

A diverse¹²² compound library, based on the ethyl 6-piperazinylnicotinate scaffolds of compounds **27** and **32**, provided a set of 141 ureas with variation of the C-ring.¹²³ In brief, aromatic C-rings with high lipophilicity contributed significantly to increasing the potency. Of highest potencies were the 1-naphthyl (IC₅₀ = 0.10 μM) in the ethyl 5-chloro-6-piperazinylnicotinate sub-series and the 2-naphthyl (IC₅₀ = 0.083 μM) in the ethyl 5-cyano-6-piperazinylnicotinate sub-series. A summary of the SAR of the urea series is shown in Figure 8.

**Figure 8.** Summary of SAR for ethyl 6-piperazinylnicotinate urea derivatives as antagonists of the P2Y₁₂ receptor.

The solubility of the ureas was generally low; for example had compound **27** less than 0.1 μM solubility in aqueous buffer at pH 6.8. Lipophilicity ($\log D$)¹²⁴ data was available for 28 of the 41 compounds that had both binding affinity and GTP γ S potency lower than 1.0 μM ; their $\log D$ was in the high range $\approx 7 \log D \geq 4.9$. The high $\log D$ may be a reason for the low aqueous solubility and was a risk factor in the further development of the compounds into orally available drugs.¹²⁵

A selection of compounds were screened for in vitro clearance in rat liver microsomes (RLM) and human liver microsomes (HLM). All showed high clearance ($CL_{\text{int}} > 100 \mu\text{M}/\text{min}/\text{mg}$) which may also be related to the high lipophilicity of the compounds. Metabolite studies of compound **27** showed the corresponding carboxylic acid as the only metabolite.¹²⁶

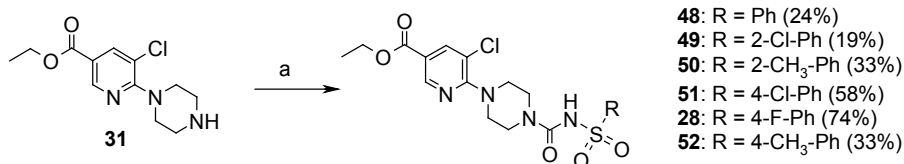
Attempts to solve the issues with low solubility and high lipophilicity led to the discovery of the sulfonylurea series while the issue with low microsomal stability was addressed by introduction of electron donating substituents in the pyridine 2-position and by replacement of the ethoxycarbonyl with other functionalities.

6.2 Sulfonylureas as P2Y₁₂ antagonists (paper IV)

The SAR of the linker region was investigated. By this strategy, sulfonylureas with increased solubility and potency compared to the matched-pairs ureas were discovered. Structural variations of the A-, B-, and C-rings were made to understand the scope of the sulfonyl ureas.

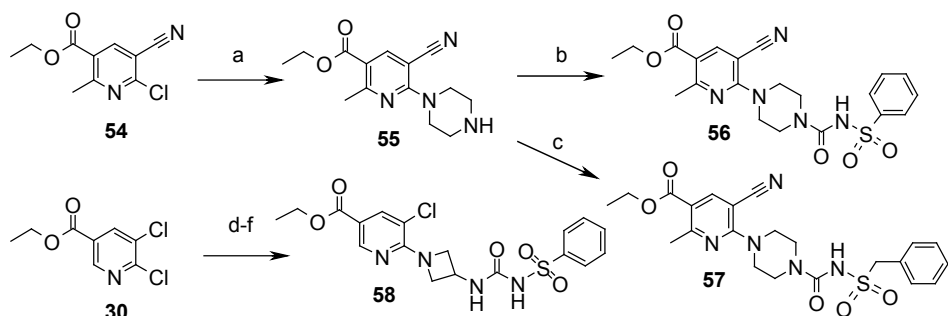
6.2.1 Synthesis

Compounds **44-47** (Table 4) with variation of the urea linker of **32** were prepared by treatment of the piperazinyropyridine **31** with commercially available iso(thio)cyanates. The sulfonylureas **28** and **48-52** were synthesized from **31** as exemplified in Scheme 7. Compound **53** (Table 5) was prepared from **43** by analogous procedures.



Scheme 7. Synthesis of sulfonamide derivatives. Reagents and conditions: (a) e.g. **51**: 4-Cl-PhSO₂NCO, THF, 0 °C to rt, 18 h.

Treatment of **54**¹²⁷ (Scheme 8) with piperazine gave **55**, which was reacted with benzenesulfonyl isocyanate to give **56**. Benzylsulfonamide was activated with CDI and then treated with **55** to give **57**. The sulfonylurea **58** was prepared in three steps from **30** by reaction with *tert*-butyl azetidin-3-ylcarbamate, followed by Boc-deprotection, and coupling of the formed amine with benzenesulfonyl isocyanate. Compounds **59** (Table 5) was prepared by a similar method.¹²⁸



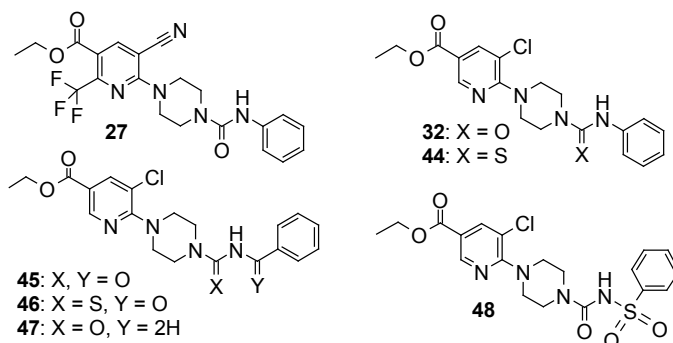
Scheme 8. Synthesis of sulfonamide derivatives. Reagents and conditions: (a) Piperazine, TEA, EtOH, MW, 160 °C, 25 min. (100%); (b) PhSO₂NCO, DCM, rt, 18 h (13%); (c) CDI, BnSO₂NH₂, DCE, rt, 16 h, then add **55**, DIPEA, rt, 16 h (58%); (d) *tert*-butyl azetidin-3-ylcarbamate, DIPEA, DMA, reflux, 16 h (50%); (e) HCl, DCM, rt, 16 h; (f) PhSO₂NCO, TEA, DCM, rt, 16 h (31%, 2 steps).

6.2.2 Results and discussion of SAR

Replacement of the urea linker in **32** (Table 4) with a thiourea linker (**44**) gave a significant lowering (from 0.88 to 12 μM) of the binding affinity. This could mean that a strong hydrogen bond acceptor like a urea¹²⁹ was needed in this position, however the acylurea/acylthiourea pair **45/46** showed the reverse effect. The replacement of one of the linker carbonyls of **45** with a methylene to give **47** resulted in higher binding affinity. Thus, from the SAR of these few compounds it appeared that one (and not two) strong hydrogen bond acceptors are required in the linker region. Considering that the diphosphate side chain of ADP (the natural agonist of the P2Y₁₂ receptor) was acidic and that sulfonylureas like **22** (Figure 7, vide supra) had been reported as P2Y₁₂ antagonists, an acidic linker was introduced.

This was done by lowering the pK_a of the urea linker of **32** with a sulfone moiety to give sulfonylureas like **48**; the linker being a carboxylic acid bioisostere.¹³⁰ The lead compound **48** showed an 8-fold higher GTPγS potency and a 2.5-fold higher aqueous solubility than the matched-pair urea **32**.

Table 4. ¹³¹ Binding affinity, potency, aqueous solubility, and lipophilicity of compounds leading from ureas **27** and **32** to sulfonylurea **48**.



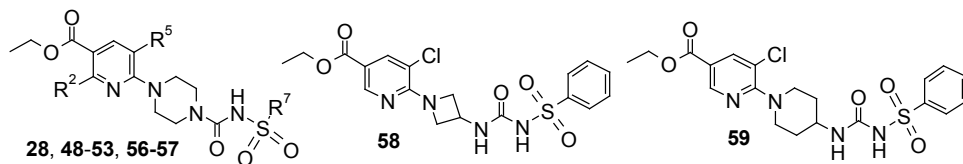
Cpd No	Binding IC ₅₀ (μM)	GTP _γ S IC ₅₀ (μM)	Solubility pH6.8 (μM)	logD ^a pH6.8	clogP
27	0.32	0.68	<0.1	4.0	4.0
32	0.88	1.7	40	-	3.3
44	12	3.7	-	-	4.1
45	9.9	5.3	-	-	3.0
46	2.4	0.99	-	-	4.2
47	2.4	1.7	-	-	3.5
48	0.18	0.22	100	-	3.3

^alogD was determined by a chromatographic method.

The higher solubility of **48** compared to **32** was likely due to its acidity ($pK_a = 3.8$) which made the compound negatively charged at the pH (6.8) where solubility was determined. Other urea-sulfonylurea matched-pairs showed the same trend.

Substitution in the phenyl 2-position with chlorine or methyl (**49** and **50**, Table 5) led to lower or similar binding affinity and GTP_γS potency compared to unsubstituted phenyl (**48**). A similar observation was made for substitution in the phenyl 4-position (**51**, **28**, **52**), irrespective of the character of the substituent (Cl, F, or CH₃). Like in the urea sub-series, the 2-CF₃/5-CN pyridines had higher or similar affinity and potency than the comparable 5-Cl pyridine derivatives (**53** vs **52**). The 2-CH₃/5-CN pyridine **56** had affinity and potency on level with the comparable 2-H/5-Cl pyridine **48** (both being benzene sulfonylureas).

Table 5. Effects on binding affinity, potency, lipophilicity (logD), aqueous solubility, permeability in Caco-2 monolayers in the apical (A) to basolateral (B) direction, and intrinsic clearance, CL_{int}, in rat liver microsomes (RLM) and human liver microsomes (HLM) by structural variations in the sulfonylurea series.^a



Cpd No	R ²	R ⁵	R ⁷	Binding IC ₅₀ (μM)	GTPγS IC ₅₀ (μM)	WPA IC ₅₀ (μM)	logD pH7.4	Solubility (μM) pH6.8	Caco-2 A to B (10 ⁻⁶ cm/s)	CL _{int} RLM	CL _{int} HLM
48	H	Cl	Ph	0.18	0.22	1.1	-	-	M (0.28)	H	H
49	H	Cl	2-Cl-Ph	0.87	0.99	1.1	0.6	-	-	-	-
50	H	Cl	2-CH ₃ -Ph	0.15	0.26	ND	0.7	-	-	H	H
51	H	Cl	4-Cl-Ph	0.52	0.38	-	1.3	-	H (1.2)	H	H
28	H	Cl	4-F-Ph	0.37	0.47	ND	0.6	-	M (0.54)	H	H
52	H	Cl	4-CH ₃ -Ph	0.30	0.30	0.19	0.9	87	H (2.5)	H	H
53	CF ₃	CN	4-CH ₃ -Ph	0.12	0.090	0.16	1.3	92	M (0.22)	H	H
56	CH ₃	CN	Ph	0.14	0.13	-	-	-	M (0.30)	-	-
57	CH ₃	CN	Bn	0.033	0.054	0.020	0.9	90	M (0.37)	H	L
58	-	-	-	0.43	0.38	.	0.8	-	H (38)	-	-
59	-	-	-	4.8	3.2	.	1.5	-	-	-	-

^a The washed-platelet assay measured the ability of the compounds to inhibit fibrinogen-induced aggregation of platelets. logD was determined by a shake-flask method. The Caco-2 assay measured the permeability of the compounds in the apical to the basolateral direction in Caco-2 cell monolayers and is an in vitro model of the absorption in vivo. Microsomal clearance (CL_{int}) in RLM and HLM has been reported as L/M/H = low, moderate or high by applying the following limits in RLM: high > 104 and low < 19 mL/min/kg and in HLM: high > 70 and low < 13 mL/min/kg.

The phenylsulfonylureas **58** and **59** had similar or lower potency than **48** while the benzylsulfonylurea **57** was the most promising in this series, showing a high potency in the WPA assay and being the only sulfonylurea with low clearance in HLM. Nonetheless, the high to moderate levels of intrinsic clearance, together with the moderate levels of permeability, observed for most compounds remained a general concern.

6.2.3 Stability of the sulfonylureas during prolonged storage in solution

It was observed that prolonged storage of solutions of some sulfonylureas in acetonitrile, DMSO or mixtures of acetonitrile and ammonium formate buffer (pH4) led to decomposition. To study this further, separate solutions of **51**, **52**, **58**, and **59** (Figure 9) in a nucleophilic solvent, ethanol (EtOH), and a less nucleophilic solvent, acetonitrile (MeCN), respectively, were stored during 21 days at room temperature and the decomposition was followed by LC/MS.¹³²

The piperazines **51** and **52** were the least stable, particularly in EtOH. During 21 days, the purity of **51** in EtOH decreased from 95% to 32% and in MeCN from 95% to 74%, while the purity of **52** in EtOH decreased from 89% to 9% and in MeCN from 89% to 80%. Notably, the azetidine **58** was stable in both EtOH and MeCN while the piperidine **59** was stable in MeCN and slightly unstable in EtOH, showing a decrease from 97% to 89% purity.

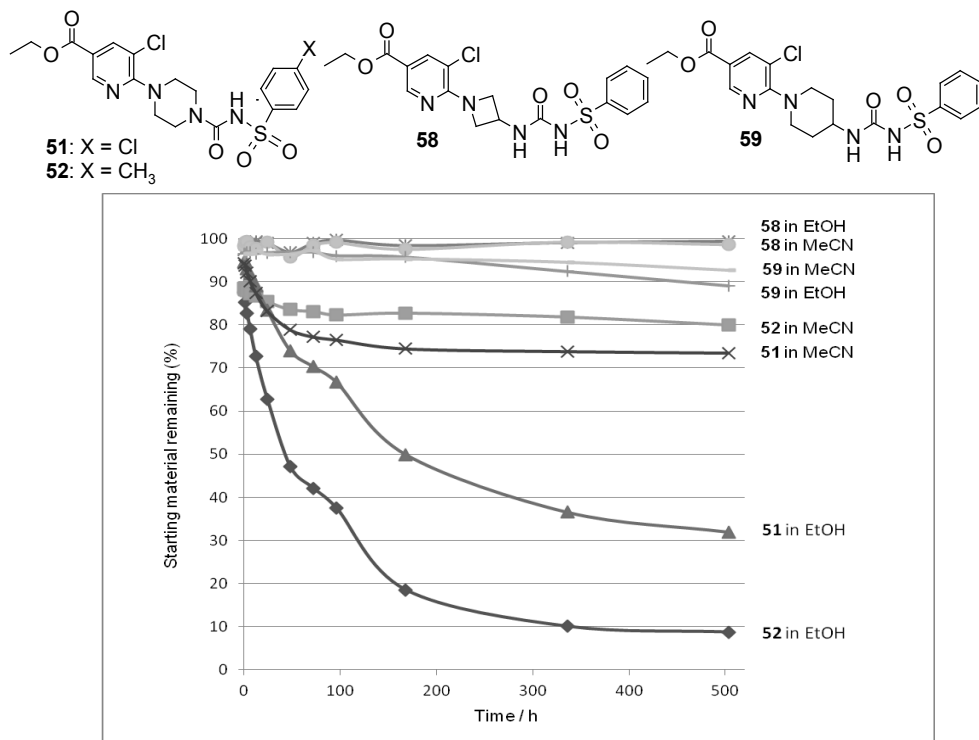


Figure 9. Stability of solutions of two piperazines, **51** and **52**, one azetidine, **58**, and one piperidine, **59**, in ethanol (EtOH) and acetonitrile (MeCN), respectively, during 21 days at room temperature.

Detected degradation products by LC-HRMS (mass detection limit < 200 D) were the piperazinyl-, azetidiny-, and piperidiny-pyridines, respectively, and, for solutions in EtOH, the ethyl sulfonyl carbamates. No degradation of the nicotinic esters was observed.

The lower stability of piperazines **51** and **52** compared to azetidine **58** and piperidine **59** mirrored the difference in stability for the pair of sulfonylureas **60** (tolbutamide) and **61** (Figure 10), as reported by Bottari et al.¹³³ Separately heating of **60** and **61** in EtOH at 80 °C for 1 h gave 59% recovery of **60**, while nothing remained of **61**. A similar result was obtained by Hutchby et al.¹³⁴ when heating the ureas **62** and **63** in MeOH at 70 °C; while **62** showed no conversion after 18 h, **63** was completely converted to methyl *N*-phenyl carbamate in less than 5 minutes. It thus seemed that the stability in solution of both ureas and sulfonylureas depended on their substitution pattern.

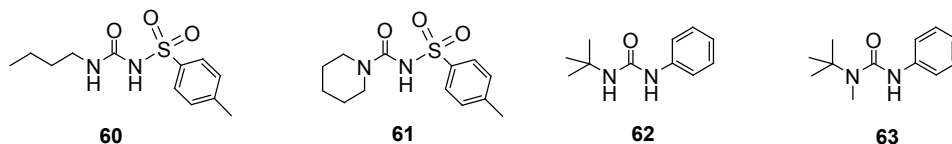


Figure 10. Literature examples of pairs of sulfonylureas and ureas whose stability in solution is dependent on the substitution pattern.

The ¹³C NMR chemical shifts (Table 6) of the linker carbonyls of the piperazines **51** and **52** were shifted downfield in both *d*₃-MeCN and *d*₆-EtOD compared to azetidine **58** and piperidine **59**. This likely inferred that **51** and **52** were (somewhat) better electrophiles,¹³⁵ however the small differences in chemical shift seemed unlikely to explain why **51** and **52** were much less stable.¹³⁶

Table 6. ¹³C NMR chemical shifts of the linker carbonyls in piperazines **51** and **52**, azetidine **58**, and piperidine **59**.¹³⁷

Cpd No	δ (linker-C) in <i>d</i> ₃ -MeCN	δ (linker-C) in <i>d</i> ₆ -EtOD
51	153.17	ND
52	156.03	158.17 ^a
58	151.82	154.99
59	151.27	152.85

^a The signal was broad which was likely due to the change in pH of the solution during partial decomposition.

The different electronic characters of the phenyl substituent of **51** (4-Cl, electron withdrawing) and **52** (4-CH₃, electron donating) makes **51** more acidic, and thus more easily deprotonated than **52**. Delocalization of the formed anion protects the carbonyl against nucleophilic attack by EtOH, in analogy to reports on sulfonylureas in aqueous solutions,¹³⁸ which may explain why **51** is more stable in EtOH than **52**.

6.3 Potential issue: microsomal stability of the nicotinic esters

Many of the nicotinic ester compounds showed high microsomal clearance in vitro (Table 5), and identification of metabolites by LC/MS showed the corresponding nicotinic acids to be the main metabolites. These had no or low P2Y₁₂ potencies in all three series of ureas, sulfonylureas and acyl sulfonamides. It was not clear whether the low in vitro metabolic stability would translate to the in vivo situation,¹³⁹ hence different strategies were pursued to stabilize or replace the ethyl ester functionality.

One strategy was to increase the electron density of the pyridine ring. It was assumed that the electron donating effect of the 6-amino substituent (azetidiny, piperidiny, or piperaziny) already provided stabilization of the ethyl ester and that additional stabilization could be attained by introduction of electron donating groups in the pyridine 2-position.

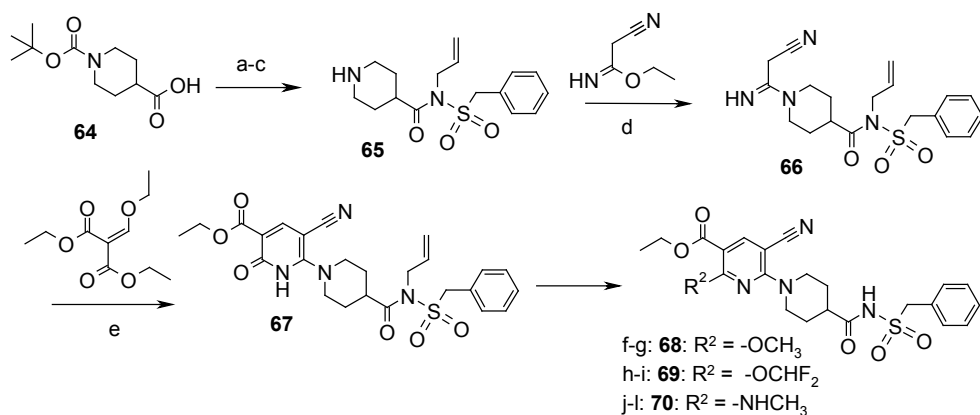
Further, it was investigated if the 3-ethoxycarbonyl (ethyl ester) functionality could be replaced by either branched esters,¹⁴⁰ non-cyclic isosteres like ether/amide/ketone or 5-membered heterocycles as non-classical isosteres.¹⁴¹

Synthesis of compounds with variation in the pyridine 2-position and of 5-ethyl-1,3-oxazole derivatives was developed by the author and is presented here. This was made by using the piperidiny-pyridine scaffold of the acyl sulfonamide series (exemplified by compound **29** in Scheme 4) that appeared from our later efforts to improve the properties of sulfonyl ureas. To give perspective, other compounds not synthesized by the author have been included in the discussion of the SAR and the binding model for the 3-ethoxycarbonyl region.

6.3.1 Development of synthetic routes to facilitate variation of the pyridine 2-substituent¹⁴²

A novel synthetic route (Scheme 9) was developed that included the use of a 2-pyridone intermediate which made a late derivatization of the pyridine 2-position possible.

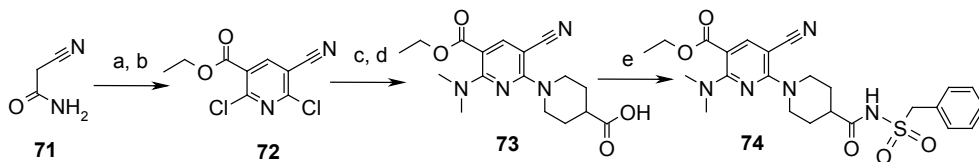
Commercially available **64** was converted in three steps into **65**. A protective group on the linker nitrogen was considered necessary to prevent side-reactions in subsequent steps. In addition, it made **65** easier to handle (extractable into an organic phase). An allyl protective group was chosen since its removal with Pd(0)/sulfonic acid was unlikely to jeopardize the ethyl ester.¹⁴³ However, this was a considered risk, since alkylation (and thus probably also allylation) of acyl sulfonamides make them prone to hydrolysis.¹⁴⁴ Step-wise condensation of **65** with ethyl 2-cyanoethanimidoate¹⁴⁵ and diethyl (ethoxymethylene)malonate produced the 2-pyridone **67**. Although yields were un-optimized, this presented a fast method to produce sufficient amounts of this pyridone for further derivatization.



Scheme 9. Reagents and conditions: (a) TBTU, TEA, THF, rt, 45 min., then add BnSO₂NH₂, LiCl, rt, 22 h (78%); (b) 3-bromoprop-1-ene, DIPEA, DMF, rt, 21 h; (c) TFA, DCM, rt, 4 h; (d) DIPEA, EtOH, 16 h; (e) rt, 18 h (13%, 4 steps); (f) MeI, Ag₂CO₃, DCM, DMSO, MW, 100 °C, 10 min. (68%); (g) Pd(PPh₃)₄, 4-CH₃PhSO₂Na, rt, 2 h (34%); (h) FSO₂CF₂COOH, MeCN, MW, 80-100 °C, 25 min.; (i) Pd(PPh₃)₄, 4-CH₃PhSO₂Na, DCM, rt, 20 h (22%, 2 steps); (j) Tf₂O, TEA, DCM, 0 °C, 1 h; (k) CH₃NH₂, THF, rt, 16 h, then add DIPEA, MW, 100-120 °C, 1 h; (l) 4-CH₃PhSO₂Na, Pd(PPh₃)₄, DCM, rt, 16 h (45%, 3 steps).

Treatment of **67** with methyl iodide¹⁴⁶ or fluorosulfonyldifluoroacetic acid,¹⁴⁷ followed by allyl deprotection produced **68** and **69**, respectively, while conversion into a triflate, followed by reaction with methylamine and allyl deprotection gave **70**. As an alternative procedure (Scheme 10), condensation of 2-cyanoacetamide **71** with diethyl (ethoxymethylene)malonate produced a 6-hydroxy-pyridin-2-one¹⁴⁸ that was reacted with oxalyl chloride to give ethyl 5-cyano-2,6-dichloronicotinate **72**. S_NAr with dimethylamine was regioselective for the pyridine 2-position. Subsequent treatment with

piperidine-4-carboxylic acid and coupling of the carboxylic acid functionality with benzylsulfonamide gave **74**.

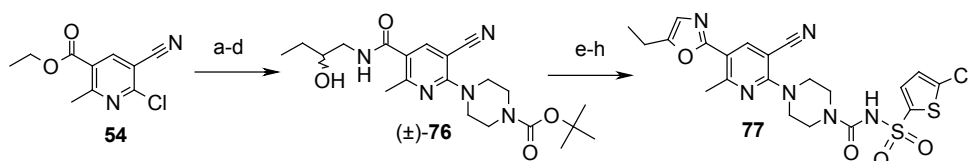


Scheme 10. Reagents and conditions: (a) NaOEt, EtOH, 80 °C, 20 min., add diethyl (ethoxymethylene)malonate, reflux, 16 h; (b) (COCl)₂, DMF, toluene, 85-100 °C, 20 h; (c) (CH₃)₂NH, MeCN, 0 °C → rt, 16 h; (d) piperidine-4-carboxylic acid, TEA, EtOH/water, MW, 120 °C, 20 min. (42%, 4 steps); (e) BnSO₂NH₂, TBTU, DIPEA, rt, 28 h (23%).

Assignment of regiochemistry was made by comparison with the regioisomer of **73** (obtained by treatment of **72** with the nucleophiles in opposite order). Only in the non-desired regioisomer is NOE observed between γ -protons (N-C-H) of the piperidine and methylene protons of the ethyl ester. 2-Aminopyridine **75** (Table 7) was prepared by similar procedures¹⁴⁹ which showed the same regioselectivity. The observed regioselectivity in the reaction of 2,6-dichloropyridines like **72** with an amine nucleophile gave inspiration to a more systematic study (vide infra, resulting in paper VI).

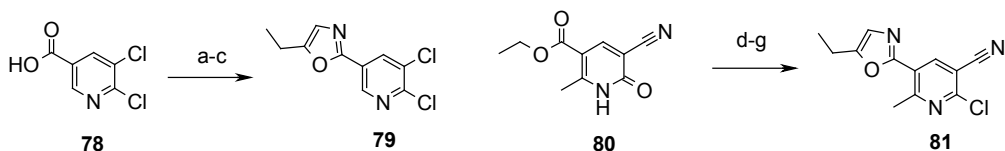
6.3.2 Development of synthetic routes to 5-ethyl-oxazoles (paper V)

The 5-ethyl-oxazole derivative **77** (Scheme 11) was prepared from **54** by an eight-step procedure. Thus, the ethyl ester functionality of **54** was hydrolyzed and the formed nicotinic acid derivative was coupled with (\pm)-1-aminobutan-2-ol to provide the 2-hydroxy amide (\pm)-**76**, which was oxidized by Swern oxidation to the corresponding 2-keto amide. While cyclization (Robinson-Gabriel oxazole synthesis) failed with PPh₃/I₂/TEA¹⁵⁰ and DBU/Pyridine/PPh₃/CCl₄,¹⁵¹ treatment with Cl₃CCOCl¹⁵² provided the cyclized material in 46% yield (POCl₃ was not tested, because of the acid-sensitive Boc-group).



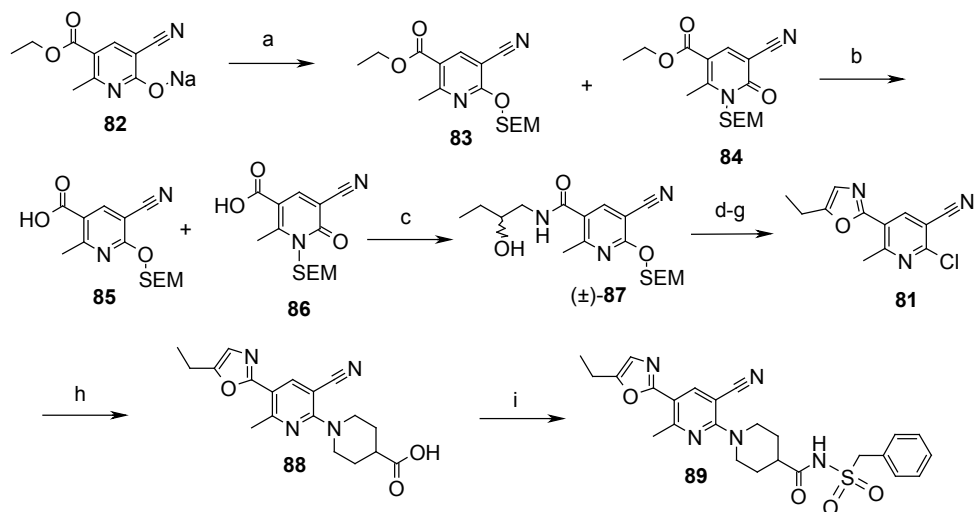
Scheme 11. Synthesis of 5-ethyl-oxazole derivative. Reagents and conditions: (a) piperazine, TEA, EtOH, MW, 170 °C, 20 min. (84%); (b) Boc₂O, K₂CO₃, THF/H₂O, rt, 16 h (94%); (c) LiOH, THF/H₂O, 60 °C, 5 h (75%); (d) EDC·HCl, HOBT, (\pm)-1-aminobutan-2-ol, DIPEA, rt, 14 h (96%); (e) (COCl)₂, DMSO, DCM, -78 °C, 5 min., add (\pm)-**76** in DCM, -78 °C, 30 min., add TEA, -78 °C to rt (96%); (f) pyridine, DMA, Cl₃CCOCl, DCM, 0 °C, 2 h, then rt, 16 h (46%); (g) TFA, DCM, rt, 40 min.; (h) 5-chlorothiophene-2-sulfonamide, CDI, DIPEA, DCM, rt to 50 °C, 18 h (37%, 2 steps).

Boc-deprotection with TFA followed by treatment with CDI-activated 5-chlorothiophene-2-sulfonamide finalized the synthesis of compound **77**. Similar procedures applied to **78** and **80** (Scheme 12) provided the 6-chloropyridines **79** and **81**, respectively. These were to be used as starting materials for exploration of alternative B- and C-rings.



Scheme 12. Synthesis of the 6-chloropyridine derivatives **79** and **81**. Reagents and conditions: (a) EDC, HOBT, (\pm)-1-aminobutan-2-ol, DCM, rt, 18 h; (b) (COCl)₂, DMSO, DCM, -78 °C, 10 min., add (\pm)-5,6-dichloro-*N*-(2-hydroxybutyl)nicotinamide in DCM/DMSO (3:1), -78 °C, 30 min., add TEA, -78 °C, 30 min., then rt, 3 h; (c) POCl₃, 90 °C, 30 min (38%, 3 steps); (d) LiOH, THF/H₂O, MW, 100 °C, 20 min. (98%); (e) POCl₃, 120 °C, 3 h; concentrate; add (\pm)-1-aminobutan-2-ol, TEA, THF, rt, 0 °C to rt, 5 h; (f) (COCl)₂, DMSO, DCM, -78 °C, <5 min., add (\pm)-6-chloro-5-cyano-*N*-(2-hydroxybutyl)-2-methylnicotinamide in DCM, -78 °C, 1 h, add TEA, -78 °C, 15 min., then rt, 15 min.; (g) POCl₃, 100 °C, 1.5 h (51%, 3 steps).

The ester hydrolysis product of **80** was difficult to handle due to low solubility in organic solvents and the sequence leading to **81** was difficult to reproduce. Thus, an alternative method (Scheme 13) was developed in which **82** (the sodium salt of **80**) was protected with a SEM-group to give the regioisomers **83** and **84** (94:6 ratio). The *O*-SEM regioisomer **83** was isolated by chromatography, however the subsequent treatment of **83** under alkaline condition to hydrolyze the ethyl ester led to migration of the SEM group such that the *O*-SEM and *N*-SEM regioisomers **85** and **86** were formed in a 1:1 ratio. The mixture was used in the subsequent amide coupling, in which only **85** reacted, to give (\pm)-**87**, while **86** was recovered from the reaction mixture. Swern oxidation of the secondary alcohol of (\pm)-**87**, followed by cyclization using Burgess' reagent,¹⁵³ SEM-deprotection with TFA, and treatment with oxalyl chloride provided the 6-chloropyridine building block **81**.¹⁵⁴ Compounds **79** and **81** were employed in synthetic procedures similar to those of the ethyl 6-chloronicotinate **30** and **54**, as exemplified with the two-step synthesis of **89** from **81** (Scheme 13).

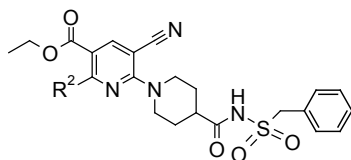


Scheme 13. Alternative route to the 6-chloropyridine building block **81** followed by synthesis of **89** in two steps. Reagents and conditions: (a) SEM-Cl, DIPEA, DCM, MW, 120 °C, 20 min. (58%, ratio **83/84** 96:4); (b) LiOH, THF/H₂O, rt, 4 h (96%, ratio **85/86** 1:1); (c) EDC·HCl, HOBT, (±)-1-aminobutan-2-ol, DIPEA, DCM, rt, 18 h (35%); (d) (COCl)₂, DMSO, DCM, -78 °C, <5 min., add (±)-**87** in DCM, -78 °C, 1 h, add TEA, -78 °C, 15 min., then rt, 15 min. (88%); (e) (CH₃CH₂)₃N⁺SO₂⁻CO₂CH₃ (= Burgess' reagent), THF, MW, 80 °C, 2 min. (67%); (f) TFA, DCM, 4 h (82%); (g) (COCl)₂, DCM, rt, 16 h, then 50 °C, 2 h (56%); (h) piperidine-4-carboxylic acid, EtOH, MW, 120 °C, 20 min. (100%); (i) BnSO₂NH₂, HATU, DIPEA, 18 h, then add EDC, HOBT, rt, 28 h, then add extra BnSO₂NH₂, 64 h (64%).

6.3.3 Results and discussion

As the only compounds that resulted from the variation of the pyridine 2-position, the 2-aminopyridines **70**, **74**, and **75** (Table 7) showed low to moderate intrinsic clearance in both RLM and HLM. Introduction of alkyl substituents on the 2-amino substituent of **75** had marked effects on the potency in the GTP_γS assay, giving a 200-fold increase (to 0.044 μM) by replacement with a 2-methylamino substituent (**70**) and a 27-fold increase with 2-dimethylamino substituent (**74**). This was contrasted by the potency pattern of the triad of compounds 2-methyl, 2-ethyl- and 2-*i*-propyl pyridine derivatives **29**, **90**, and **91**¹⁵⁵ that showed no differences in their potencies in the GTP_γS assay.

Compounds with high potency in the GTP_γS assay were also tested in the residual platelet count (RPC) assay.¹⁵⁶ This is a whole blood assay and is thus closer to the physiological conditions. Addition of the agonist ADP to whole blood induces platelet aggregation, however a number of platelets remain unaggregated, known as the residual platelets count. Co-addition of an antagonist leads to an increase in the residual platelets count, from which the potency of the antagonist is determined.

Table 7. Effects on binding affinity, potency, logD, permeability in Caco-2 monolayers, and intrinsic clearance CLint in RLM and HLM by variation of the pyridine 2-substituent.^a

Cpd No	R ²	$\sigma_p(R^2)^{157}$	Binding IC ₅₀ (μM)	GTPγS IC ₅₀ (μM)	RPC IC ₅₀ (μM)	LogD pH 6.8	Caco-2 A to B (10 ⁻⁶ cm/s)	CLint RLM (μl/min/mg)	CLint HLM (μl/min/mg)
29	-CH ₃	-0.17	0.011	0.025	3.2	2.8	H (18)	H	L
68	-OCH ₃	-0.27	0.005	0.008	0.56	1.9	M (0.30)	H	M
69	-OCHF ₂	-	0.009	0.016	0.48	2.9	L (<0.01)	-	H
70	-NHCH ₃	-0.70	0.013	0.044	12	3.3	-	L	M
74	-N(CH ₃) ₂	-0.83	0.32	0.31	-	2.6	-	L	M
75	-NH ₂	-0.66	2.5	8.9	-	-	L (0.12)	L	L
90	-CH ₂ CH ₃	-0.15	0.028	0.022	5.8	3.3	H (38)	H	H
91	- <i>i</i> -Pr	-0.15	0.061	0.041	5.9	3.9	H (31)	H	H

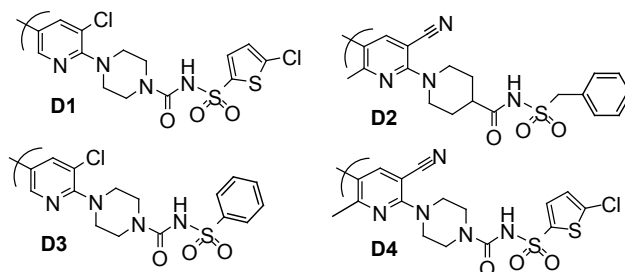
^a The RPC (residual platelet count) assay determines the number of single platelets that remain after induction of platelet aggregation in whole blood using ADP as agonist. Thus the effect of an antagonist is determined by an increase in the residual platelets count. Microsomal clearance (CLint) in RLM and HLM has been reported as L/M/H = low, moderate or high by applying the following limits in RLM: high > 104 and low < 19 mL/min/kg and in HLM: high > 70 and low < 13 mL/min/kg.

The comparatively high potency of the 2-aminopyridine derivative **70** in the GTPγS assay was regrettably not retained in the RPC assay. The (mesomerically) electron donating 2-methoxy group of **68** seemed to stabilize the 3-ethoxycarbonyl against metabolism in HLM, in comparison to the electron withdrawing 2-difluoromethoxy group of **69**.

Replacement of the ethyl ester functionality of **92** (Table 8) with an *i*-propyl ester (**94**) gave a moderate, five-fold lowering (to 0.53 μM) of GTPγS potency. Unfortunately, the clearance in both RLM and HLM remained high which showed that introduction of esters with branched alkyl substituents, which has been utilized successfully with other chemical series,¹⁵⁸ was not a viable strategy to reduce metabolism in the present case.

The ethyl amide **96a** showed a 200-fold lower GTPγS potency (5.2 μM) than the comparable ethyl ester **29** while the methyl amide **96b** showed no potency.¹⁵⁹ Low potency of amide analogs was also observed in the urea sub-series (vide supra). The *n*-propyl ketone **97** had a 4-fold lower potency in the GTPγS assay than **29** and was stable

Table 8.¹⁶⁰ Effects on binding affinity, potency and metabolic stability in rat liver microsomes (RLM) and human liver microsomes (HLM) by replacement of the 3-ethoxycarbonyl substituents of scaffolds D1-D4 with non-cyclic analogs and oxazoles.^a



Cpd No	R-X	Scaffold (R)	Binding IC ₅₀ (μM)	GTPγS IC ₅₀ (μM)	WPA IC ₅₀ (μM)	RLM H/M/L	HLM H/M/L	logD pH7.4	Hydrogen bond acceptor strength V _{min} (kcal/mol)
92	R-CO ₂ Et	D1	0.090	0.10	0.27	H	H	-	(=O): -45, -45
93	R-CO ₂ CH ₃	D1	0.65	3.2	-	-	-	0.90	-
94	R-CO ₂ - <i>i</i> -Pr	D1	0.25	0.53	-	H	H	1.5	-
29	R-CO ₂ Et	D2	0.011	0.025	0.009	H	L	1.4	(=O): -41, -38
95	R-CO ₂ - <i>n</i> -Pr	D2	0.065	0.014	0.018	H	M/H	3.2	-
96a	R-C(O)NH ₂ Et	D2	1.5	5.2	-	-	-	-0.10	(=O): -50, -50
96b	R-C(O)NHCH ₃	D2	13	>33 ^b	-	-	-	-0.30	(=O): -51, -49
97	R-C(O)- <i>n</i> -Pr	D2	-	0.10	-	L	L	-	(=O): -41, -38
48	R-CO ₂ Et	D3	0.18	0.22	-	-	-	-	(=O): -45, -44
98	R-O- <i>n</i> -Pr	D3	21	>33 ^b	-	-	-	-	(O): NV ^c
99		D1	6.7	5.7	-	-	M	-	(=N): -50, (O): -15
100		D1	20	20	-	H	L	1.6	(=N): -51, (O): -17
101		D1	0.32	0.87	2.5	H	M	0.80	(=N): -54, (O): -15
102		D1	0.40	0.73	1.0	H	H	2.3	(=N): -54, (O): -15
89		D2	0.18	0.11	0.20	L	L	2.3	(=N): -54, (O): -15
77		D4	0.097	0.31	0.24	-	H	-	-
103	R-CO ₂ Et	D4	0.042	0.034	0.18	H	H	2.3	-

^a L/M/H = low/moderate/high. logD was determined by a shake-flask method. V_{min} (see references for exact definition) was calculated by quantum mechanical methods and is a predictor of hydrogen bonding acceptor strength; the lower the V_{min}, the stronger is the hydrogen bond acceptor. ^bMaximum concentration tested was 33 μM. ^cNo minimum found.

in both RLM and HLM, and the ketone sub-series was investigated further (not reported here). Shape and electrostatic matching of 5-membered heterocycles with the ethoxycarbonyl functionality presented 5-methyl-oxazole as a possible replacement.¹⁶¹ An oxazole has been used previously as a bioisosteric replacement for a carboxylic ester,¹⁶² however its introduction is non-trivial.¹⁶³

The 5-methyl-oxazole **101** showed a similar affinity (0.32 μM), and a moderately lower potency in GTP γ S (0.87 μM) than the comparable ethyl ester **92**. The unsubstituted oxazole **99** and the 4-methyl-oxazole **100** had notably lower affinity and potency. Extension of the side-chain to give 5-ethyl-oxazole **102** led to a 2.5-fold higher potency in WPA (to 1.0 μM), but also increased the clearance in HLM from moderate to high.

Following a project decision to further investigate the most potent compounds, the 5-ethyl-oxazole moiety was combined with scaffolds D2 and D4 to give **89** and **77**, respectively. Compound **89** showed low metabolism in both RLM and HLM, a clear improvement of the metabolic profile in comparison to the matched-pair *n*-propyl ester **95**. However, the potency of **89** in the GTP γ S assay was 8-fold lower, and in the WPA assay 11-fold lower than for **95**.

Compound **77** showed high clearance in HLM. No matched-pair *n*-propyl ester was available for comparison. The corresponding ethyl ester **103** showed high metabolism in both RLM and HLM. It appeared unlikely that the homologous *n*-propyl ester should have lower metabolic stability; the ethyl/*n*-propyl pair of esters **29/95** seemed to indicate an increased metabolism by elongation of the unbranched alkyl group of the ester. By this reasoning, replacement of an ethyl ester (and probably also of an *n*-propyl ester) with a 5-ethyl-oxazole did not increase the metabolic stability in this case.

Further analysis¹⁶⁴ showed that the metabolism of the oxazoles (with **89** as a notable exception) was CYP450-dependent. A similar dependency was not observed for the ethyl esters.

6.4 Binding model of ethoxycarbonyl substituent and isosteres

The lack of structural information for the P2Y₁₂ receptor¹⁶⁵ made it difficult to draw definite conclusions about the nature of the bioactive conformation, in particular which specific binding interactions between the ligand and the receptor were required in the region of the ethyl ester and its bioisosteres to provide high affinity and potency. Pair-wise comparison of compounds in particular gave some hints along these lines and will be presented in the following.

The difference in binding affinity was 7-fold for ethyl/methyl ester **92/93**, 8-fold for ethyl/methyl amide **96a/96b** and 21-fold for 5-methyl-oxazole/oxazole **101/99**. This highlighted the importance of the alkyl substituent being ethyl rather than methyl (or 5-methyl rather than 5-hydrogen for the oxazoles). The 63-fold lower affinity of the 4-methyl-oxazole **100** compared to the 5-methyl-oxazole **101** underlined the importance of the alkyl substituent being correctly positioned.

The hydrogen bond acceptor (HBA) strengths (Table 8) of the esters and their replacements were quantified by quantum mechanical calculations¹⁶⁶ of the electrostatic potential at a minimum (V_{\min})¹⁶⁷ with the lowest values corresponding to the strongest HBAs. The nitrogens of the oxazoles and the carbonyls of the esters, the ketone, and the amides, respectively, were all strong HBAs, as confirmed by literature.¹⁶⁸ Of these, the ethyl amide **96a** had a surprisingly low binding affinity and potency. This may be explained by unfavourable interactions of the amide nitrogen or hydrogen, the higher hydrophilicity of the ethyl amide in comparison to the ethyl ester, or by a non-optimal positioning of the alkyl group.

The >115-fold lower affinity and potency of the *n*-propyl ether **98**, which is lacking a strong HBA, compared to the corresponding ethyl ester **48** also indicated that a strong HBA was required in this region. In the low affinity compound 4-methyl-oxazole **100** the oxazole ring must flip for the 4-methyl group to overlay with the alkyl group of the ethyl ester, which means that only the oxygen of the oxazole, which is a weak HBA, becomes available for hydrogen bonding.

Assuming that similar compounds bind in a similar fashion¹⁶⁹ it appeared that a correctly positioned alkyl group and a correctly positioned strong HBA both were required for binding. Density functional theory (B3LYP) calculations¹⁷⁰ showed that planarity between the pyridine ring and the ethoxycarbonyl of **92** was the preferred conformation. A similar result was obtained for the 5-ethyl-oxazole **102**. This is summarized in Figure 11 for compound **102**.

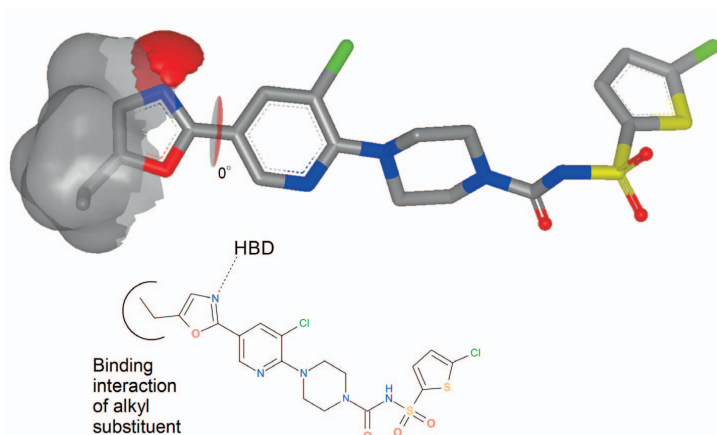
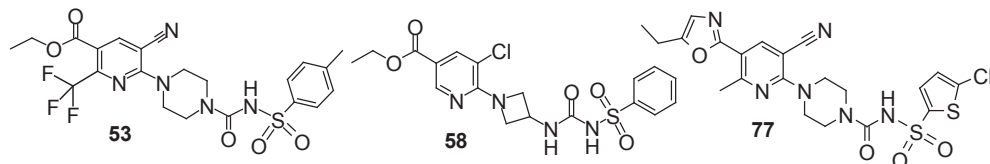


Figure 11. Suggested interactions for the 5-ethyl-oxazole moiety of **102**. It appeared that a correctly positioned alkyl group and a correctly positioned strong hydrogen bond acceptor both were necessary structural motifs for binding. HBD = hydrogen bond donor.

6.5 Pharmacokinetic evaluation in vivo

A selection of the sulfonylurea P2Y₁₂ antagonists (Table 9) were tested in vivo in rat for determination of pharmacokinetic properties.¹⁷¹ The moderate to high clearance (CL) values and the low levels of bioavailability (*F*) showed that further improvements in properties were needed. This was addressed subsequently by investigating the acyl sulfonamide series of P2Y₁₂ antagonists.¹⁷²

Table 9. Pharmacokinetic data in vivo in rat for a selection of compounds.^a

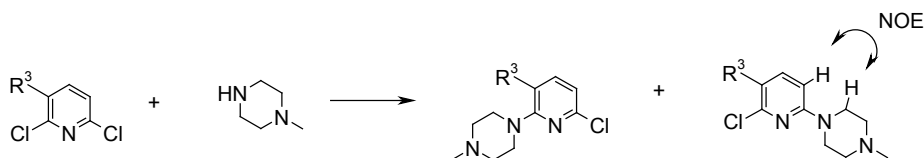


Cpd No	N, iv	CL (mL/min/kg) ± SEM	t _{1/2} iv (h)	N, po	<i>F</i> (%) ^b ± SEM	t _{1/2} po (h)	GTPγS IC ₅₀ (μM)	WPA IC ₅₀ (μM)	Caco-2 A to B (10 ⁻⁶ cm/s)	CLint RLM L/M/H
53	2	89 (±2)	1.5	2	20 (±10)	0.65	0.30	0.16	M (0.22)	H
58	2	29 (±2)	0.2	3	20 (±1)	2.1	0.38	ND	H (3.9)	-
77	1	71	0.85	2	8.9 (±0.8)	2.0	0.73	1.0	M (0.19)	H

^a In vitro data are included for reference. L/M/H = low/moderate/high. ^b The compounds were administered as solutions in TEG/EtOH/H₂O 50:5:45. CL = clearance, *F* = bioavailability.

7. Regioselectivity in the reaction of 3-substituted 2,6-dichloropyridines with 1-methylpiperazine (paper VI)

The findings in the synthesis of the 2-dimethylaminopyridine **74** (vide supra, Scheme 10) gave inspiration to a chemical design study of the regioselectivity in the S_NAr reaction of 3-substituted 2,6-dichloropyridines with 1-methylpiperazine (Scheme 14).



Scheme 14. The two possible regioisomers formed in the reaction of a 3-substituted 2,6-dichloropyridine with 1-methyl piperazine. NOE between the piperazine hydrogens and the 5-hydrogen of pyridine can be observed in the 6-isomer only.

In the literature, examples of regioselectivity for the 2-position in the S_NAr reactions between 3-substituted 2,6-dihalogenopyridines and amine nucleophiles included the treatment of 2,6-dibromo-3-trifluoroacetylaminopyridine with benzylamine in the presence of 0.1 equiv CuI and 0.2 equiv L-proline in DMSO which gave the 2-regioisomer solely.¹⁷³ Likewise, the 2-isomers were isolated as the sole products in the reaction of ammonia with 2,6-dichloro-3-hydroxymethylpyridine¹⁷⁴ (in EtOH) and 2,6-dichloronicotinic acid¹⁷⁵ (in water), respectively, however no further analysis of the regioselectivity outcomes was provided. Also the treatment of 2,6-dichloronicotinamides with amines was reported to give selectivity for the 2-isomer, exemplified by the reaction of phenethylamine with 2,6-dichloro-*N*-(2-phenoxyethyl)nicotinamide in THF.¹⁷⁶ Reaction of 2,6-dichloro-3-nitropyridine with ammonia in *i*-PrOH,¹⁷⁷ methylamine in EtOH,¹⁷⁸ allylamine in DCM,¹⁷⁹ diethylamine in MeCN,¹⁸⁰ *i*-BuNH₂, *tert*-BuCH₂NH₂, or *i*-PrSO₂NH₂ in EtOH,¹⁸¹ 4-ethoxycarbonyl-1-piperazine in CHCl₃,¹⁸² or *N-tert*-butoxycarbonyl-aniline in DMF¹⁸³ gave primarily or solely the 2-isomer. Hirokawa et al.¹⁸⁴ reported the reaction of methyl 2,6-dichloronicotinate with methylamine to give moderate selectivity for the 2-isomer (49:12 in THF at 5 °C), while reaction with benzylamine increased the selectivity for the 2-isomer (86:14 in THF at – 20 °C).

With 2,6-dichloro-3-trifluoromethylpyridine, the regioselectivity was biased towards the 6-isomer. Thus, treatment with methylamine in EtOH gave the 2-isomer and the 6-isomer in a 1:4 ratio, while treatment with the sterically more demanding *N,N*-bisbenzylamine in *N*-methyl-2-pyrrolidone gave solely the 6-regioisomer.¹⁸⁵ DABCO reacted selectively in the 6-position of methyl 2,6-dichloronicotinate in DMF-*d*₇ or THF to

form a reactive intermediate that was reacted further by treatment with phenols.¹⁸⁶ A number of other reports¹⁸⁷ described the reaction of *N*-nucleophiles with 3-substituted 2,6-dichloropyridines without mentioning the regiochemical outcome.

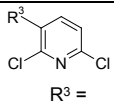
Given the differences in nucleophiles and reaction conditions, including solvents, in these literature reports it was difficult to draw general conclusions about the factors that are controlling the regioselectivity.

In the present study, three parameters that expressed different aspects of the character of the pyridine 3-substituent (R^3) were varied systematically: lipophilicity (PI), size (MR; molar refractivity) and inductive effect (σ_p).¹⁸⁸ While the size and inductive effects are usual in this context, it was decided also to include the lipophilicity as a way to represent and study the effect of the polar character of the 3-substituent. 1-Methylpiperazine was selected as nucleophile since the products could be easily detected by LC/MS.

Each value of PI, MR and σ_p were categorized as low (L), medium (M), or high (H), thus each starting material could be assigned a three letter code, e.g. LLH. Fifteen starting materials were selected, based on letter codes and synthetic feasibility, such that the starting materials **104-111** (Table 10) included the eight possible combinations of extreme (either low or high) values of the three variables, while seven other starting materials **112-117** included at least one medium value. The secondary amine **118** was included to facilitate comparison with primary amine **104** and secondary amine **107**. Reaction of starting materials **104-118** with 1-methylpiperazine (MP) would produce the 2-MP regioisomers **119a-133a** and the 6-MP regioisomers **119b-133b**, such that compound **104** would give **119a** and **119b**, compound **105** would give **120a** and **120b** etc.

Reaction of 1-methylpiperazine with reactive starting materials (i.e. those having an electron withdrawing 3-substituent) were run in acetonitrile at a concentration of 0.1M with three equivalents of *N,N*-diisopropylethylamine (DIPEA) as base. The reactions were run at room temperature or with single node heating in a microwave oven. Less reactive starting materials (i.e. those having an electron donating 3-substituents) reacted insufficiently under those conditions, and were instead reacted in neat 1-methylpiperazine (54 equivalents) as a solvent-base with single node heating in a microwave oven. To reveal any effect of the reaction medium, a selection of the reactive starting materials were also reacted in neat 1-methylpiperazine. These reactions were performed at 0 °C and to prevent formation of bis-addition products the reactions were quenched with formic acid after 20-30 seconds of reaction time.

Table 10. Chemical design of starting materials with variation of lipophilicity (PI), size (MR), and inductive effect (σ_p).

Cpd No		PI	MR	σ_p	Letter code ^a
R ³ =					
104	-NH ₂	-1.23	5.4	-0.66	LLL
105	-CH ₃	0.56	5.67	-0.17	HLL
106	- <i>p</i> -N(CH ₃) ₂ Ph	2.06	39.9	-0.56	HHL
107	morpholino	-0.77	24.6	-0.50	LHL
108	-CN	-0.57	6.3	0.66	LLH
109	-CF ₃	0.88	5.0	0.54	HLH
110	-COOPh	1.46	30.2	0.44	HHH
111	-SO ₂ N(CH ₃) ₂	-0.78	21.9	0.65	LHH
112	-COOCH ₃	-0.01	12.9	0.45	MMH
113	-OCH ₂ CH ₃	0.38	12.5	-0.24	MML
114	-COO ⁻	-4.36	6.0	0	LLM
115	-CONH ₂	-1.49	9.8	0.36	LMH
116	-Br	0.86	8.9	0.23	HMH
117	-SCH ₂ CH ₃	1.06	18.1	0.03	HHM
118	-NHPh	1.37	30.0	-0.56	HHL

^a The letter code refers to the combination (PI, MR, σ_p) with each value being either L = low, M = medium, or H = high.

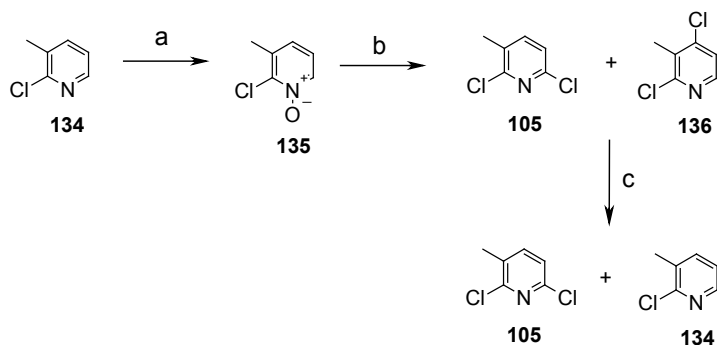
The regioselectivity R_{sel} for the pyridine 2-position was determined by ¹H NMR as the integral of the two aromatic pyridine hydrogens in the 2-isomer to the total integral of the four aromatic pyridine hydrogens in the 2-isomer and the 6-isomer. Only in the 6-MP regioisomers (Scheme 14) could NOE between the pyridine 5-hydrogen and hydrogens in MP be observed, and this was used as a diagnostic tool to identify the 6-MP regioisomers. The spectra of the pure regioisomers were compared with the spectrum of the reaction mixture, and thereby R_{sel} could be determined.

To study any effect of the solvent, the synthetically useful compound **112** was treated with 1-methylpiperazine in 21 different and separate solvents. These were selected based on differences in e.g. polarity, hydrogen bond donor and acceptor ability, molecular dipolar momentum μ , and relative static permittivity (dielectricity constant) ϵ .

7.1 Synthesis

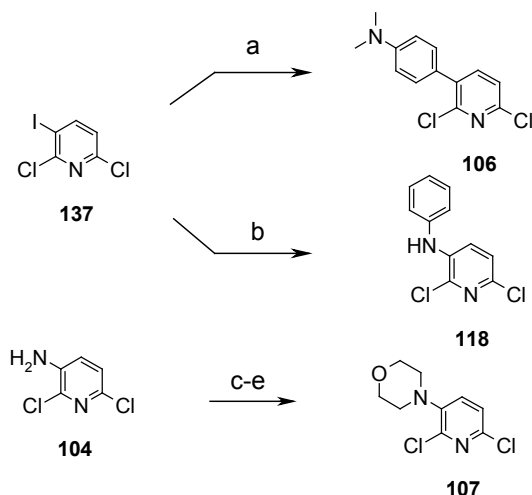
Oxidation of 2-chloro-3-methylpyridine **134** (Scheme 15) to *N*-oxide **135** followed by chlorination provided a 6:1 mixture of 2,6-dichloro-3-methylpyridine **105** and by-product 2,4-dichloro-3-methylpyridine **136**. Separation of the products by chromatography was

not possible.¹⁸⁹ This was solved by treating the mixture with Zn which selectively reduced the 4-chlorine of by-product **136** to give **134**, and compound **105** could be separated from **134** by chromatography.



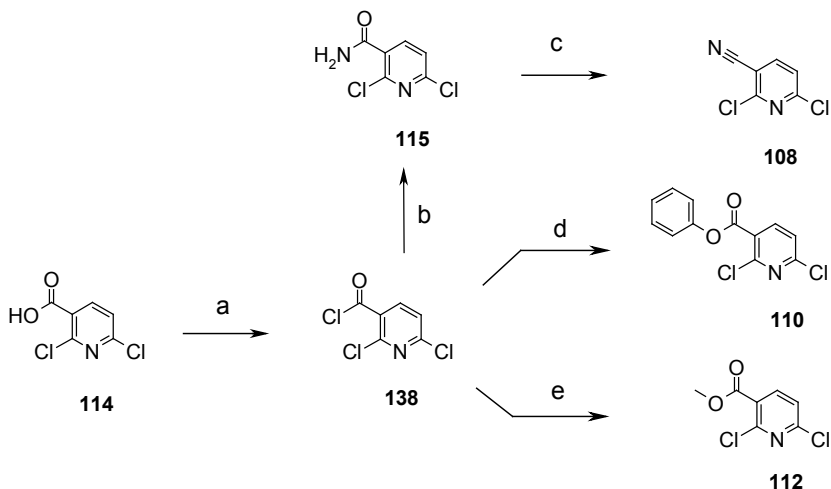
Scheme 15. Synthesis of 2,6-dichloro-3-methylpyridine **105**. Reagents and conditions: (a) *m*-CPBA, CHCl_3 , 0 °C to rt, 18 h (86%); (b) POCl_3 , DCM, 0 °C, 2 h, then rt, 3 h (18%); (c) Zn, NH_4Cl , THF/MeOH, MW, 120 °C, 1 h (62% of **105**).

Derivatization of 2,6-dichloro-3-iodopyridine **137** (Scheme 16) by Suzuki¹⁹⁰ and Buchwald-Hartwig¹⁹¹ reactions produced **106** and **118**, respectively. 2,6-Dichloro-3-morpholinopyridine **107** was synthesized from **104** in three steps.



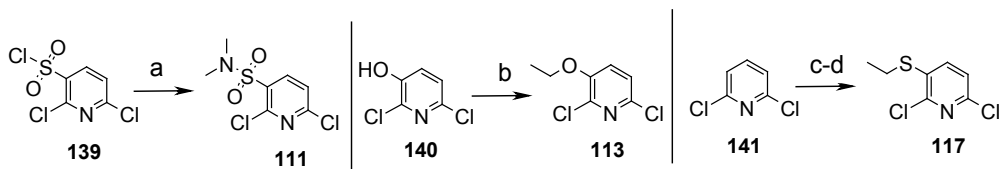
Scheme 16. Derivatizations leading to introduction of 4-dimethylaminophenyl group to give **106**, of *N*-phenyl group to give **118** and of morpholino group to give **107**. Reagents and conditions: (a) 4- $[\text{N}(\text{CH}_3)_2]\text{PhB}(\text{OH})_2$, $\text{Pd}(\text{Ph}_3\text{P})_4$, LiCl, $\text{BaOH}\cdot 8\text{H}_2\text{O}$, 1,4-dioxane/ H_2O 1:3, 95 °C, 2 h (12%); (b) PhNH_2 , *tert*-BuONa, $\text{Pd}_2(\text{dba})_3$, BINAP, 1,4-dioxane, 100 °C, 18 h (46%); (c) 1,4-dioxane-2,6-dione, MeCN, MW, 100 °C, 2 h; (d) T3P (propylphosphonic anhydride), TEA, EtOAc, rt, 16 h; (e) BH_3 , THF, 60 °C, 4 h (38%, 3 steps).

Treatment of 2,6-dichloronicotinic acid **114** (Scheme 17) with oxalyl chloride produced the acid chloride **138**.¹⁹² This was treated with aqueous ammonia to give primary amide **115** which could be dehydrated to provide 2,6-dichloronicotinonitrile **108**. In addition, acid chloride **138** was treated with phenol or methanol to give the phenyl and methyl esters **110** and **112**,¹⁹³ respectively.



Scheme 17. Derivatization of 2,6-dichloronicotinic acid **114** to give primary amide **115**, nitrile **108**, phenyl ester **110**, and methyl ester **112**. Reagents and conditions: (a) $(\text{COCl})_2$, DMF (cat.), toluene, reflux, 2 h; (b) NH_3 (aq); 1,4-dioxane, 0 °C, 75 min., 54% (2 steps); (c) POCl_3 , THF, 100 °C, 18 h (30%); (d) PhOH, pyridine, DMAP, DCM, reflux, 18 h (67%, 2 steps); (e) MeOH, rt, 18 h (65%, 2 steps).

Dimethyl sulfonamide **111** (Scheme 18) was made by treatment of commercially available sulfonyl chloride **139** with dimethylamine. 3-Hydroxy compound **140**, prepared by the method of Voisin et al.,¹⁹⁴ was O-ethylated with ethyl bromide to provide **113**. Deprotonation of 2,6-dichloropyridine **141** with LDA,¹⁹⁵ followed by reaction with elemental sulfur gave a mixture of thiols that was ethylated swiftly to avoid disulfide formation and provided **117**.



Scheme 18. Synthesis of dimethyl sulfonamide **111**, ethyl ether **113**, and ethyl thioether **117**. Reagents and conditions: (a) $\text{HN}(\text{CH}_3)_2$, TEA, THF, 0 °C to rt, 18 h (67%); (b) EtBr, K_2CO_3 , DMF, rt, 16 h (89%); (c) LDA, THF, -78 °C, 3 h; add $\text{S}^{(0)}$, THF, -78 °C to rt, 1.5 h; (d) EtI, K_2CO_3 , DMF, rt, 16 h (15%, 2 steps).

7.2 Results and discussion

The outcomes in regioselectivity by treatment of starting material **104-118** with 1-methylpiperazine are presented in Table 11.

Table 11. Regioselectivity R_{sel} for the 2-isomer in the reaction of 3-substituted 2,6-dichloropyridines with 1-methylpiperazine (MP).

Cpd No	3-substituent	B1	2-isomer Cpd No	6-isomer Cpd No	Reaction conditions ^a		
					a	b	c
					R_{sel}	R_{sel}	R_{sel}
104	-NH ₂	1.65	119a	119b	-	0.96	-
105	-CH ₃	1.7	120a	120b	0.52	0.26	-
106	- <i>p</i> -N(CH ₃) ₂ Ph	1.65	121a	121b	-	0.17	-
107	morpholino	1.76	122a	122b	-	0.43	-
108	-CN	1.7	123a	123b	0.11		0.15
109	-CF ₃	2.1	124a	124b	0.09		0.02
110	-COOPh	2.03	125a	125b	0.67		0.40
111	-SO ₂ N(CH ₃) ₂	1.95	126a	126b	0.37		0.20
112	-COOCH ₃	1.74	127a	127b	0.75		0.56
113	-OCH ₂ CH ₃	1.42	128a	128b	-	0.96	-
114	-COO ⁻	1.7	129a	129b	0.95	0.93	-
115	-CONH ₂	1.7	130a	130b	0.91	0.94	-
116	-Br	1.82	131a	131b	0.75	0.66	-
117	-SCH ₂ CH ₃	1.85	132a	132b	0.42	0.26	-
118	-NPh	1.85	133a	133b	-	0.71	-

^a Reaction conditions a-c: (a) 0.1M in MeCN, MP, 1.0 equiv, DIPEA, 3.0 equiv, stirring at rt or heating in microwave oven. (b) 0.1M in neat MP, 54 equiv, heating in microwave oven. (c) 0.1M in neat MP, 54 equiv, 0 °C, 20-30 s, quench with HCOOH.

Single variable data analysis showed no correlation between R_{sel} and the parameters used in the chemical design, however further analysis using 2D and 3D-based descriptors¹⁹⁶ showed a statistically significant correlation between R_{sel} and the Verloop steric parameter B1 (R^2 : 0.45, $p=0.006$),¹⁹⁷ as illustrated in Figure 12. Since B1 describes the smallest width of the 3-substituent, as measured perpendicular to the axis of the bond between the first atom of the substituent and the parent molecule, this implied that 3-substituents that are bulky close to the pyridine ring directed the regioselectivity towards the 6-position. The lack of correlation between R_{sel} and the inductive effect (σ_p) of the R³ substituent was very surprising.

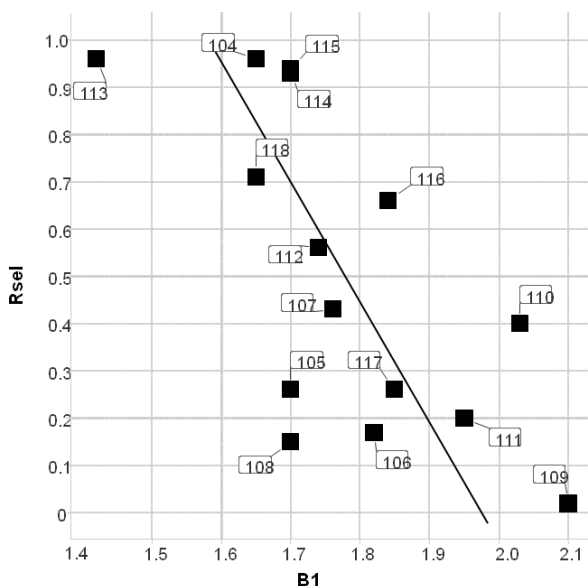


Figure 12. Relationships between the regioselectivity R_{sel} and the Verloop steric parameter $B1$ for the pyridine 3-substituent. Labels indicate compound numbers of the starting materials.

The observed differences in regioselectivities with 2,6-dichloronicotinic acid and its derivatives could be useful in the planning of synthesis. Thus, 3-carboxylate (**114**) and 3-amide (**115**) substituents were preferred to obtain the 2-isomer (9:1 ratio with the 6-isomer), while the 3-cyano (**108**) and 3-trifluoromethyl (**109**) substituents were preferred to obtain the 6-isomer (9:1 ratio with the 2-isomer). The 3-NH₂ substituent (**104**) gave a high selectivity for the 2-isomer ($R_{sel} = 0.96$), however to avoid prolonged heating at high temperatures, an electron withdrawing 3-substituent would be preferred in synthesis.

For a given starting material, it appeared that R_{sel} was higher with MeCN than with 1-methylpiperazine as reaction medium (Table 11, compare columns (a) and (c)). This apparent effect of the solvent was investigated further by reaction of **112** with 1-methylpiperazine in 21 different and separate solvents (Table 12).¹⁹⁸

Table 12.¹⁹⁹ Solvent effect on the regioselectivity R_{sel} for the 2-position in the reaction of methyl 2,6-dichloronicotinate (**112**) with 1-methylpiperazine.^a

Solvent No	Solvents	R_{sel}	Unre-acted 112 (%) ^b	Reac-tion time (h)	β	Bp (°C)	DN	α	n	ϵ	μ	π^*	$E_T(30)$
S1	DMSO	0.34	0	20	0.76	189	29.8	0	1.479	46.71	3.96	1	45.1
S2	DMF	0.47	0	20	0.69	153	26.6	0	1.430	37.06	3.79	0.88	43.8
S3	MeCN	0.74	<1	20	0.40	81	14.1	0.19	1.344	35.94	3.95	0.75	45.6
S4	Acetone	0.72	<1	20	0.43	56	17.0	0.08	1.359	21.36	2.88	0.71	42.2
S5	<i>N</i> -(CH ₃)-pyrrolidone	0.40	<1	20	0.77	203	27.3	0	1.469	32.58	3.75	0.92	42.2
S6	1,2-DCE	0.91	<1	20	0	84	0	0	1.445	10.74	1.36	0.81	41.3
S7	CH ₃ NO ₂	0.82	1	20	0.06	101	2.7	0.22	1.381	36.16	3.50	0.85	46.3
S8	DCM	0.93	6	20	0.10	40	1.0	0.13	1.424	9.02	1.60	0.82	40.7
S9	THF	0.78	21	20	0.55	66	20.0	0	1.406	7.47	1.69	0.58	37.4
S10	O=P(OEt) ₃	0.44	14	4	0.77	215	26.0	0	1.405	13.01	2.86	0.72	41.7
S11	<i>N</i> -(CH ₃)-imidazole	0.32	5	4	0.82	198	-	0	1.496	-	3.80	-	-
S12	Triethylamine	0.82	0	20	0.71	89	61.0	0	1.401	2.45	0.72	0.14	32.1
S13	Pyridine	0.57	3	20	0.64	115	33.1	0	1.509	13.22	2.21	0.87	40.5
S14	DMA	0.39	5	4	0.76	165	27.8	0	1.438	38.30	3.78	0.88	43.7
S15	Methyl formamide	0.48	7	4	0.80	199	27.0	0.62	1.432	182.4	3.83	0.90	54.1
S16	EtOAc	0.81	11	20	0.45	77	17.1	0	1.372	6.03	1.78	0.55	38.1
S17	1,4-dioxane	0.80	5	20	0.37	101	14.3	0	1.422	2.27	0.46	0.55	36.0
S18	Tetra-methylurea	0.43	5	4	0.80	177	29.6	0	1.450	24.46	3.47	0.83	41.0
S19	1,2-DME	0.73	10	20	0.41	84	20.0	0	1.380	7.20	1.61	0.53	38.2
S20	Toluene	0.94	30	20	0.11	111	1	0	1.497	2.43	0.38	0.54	33.9
S21	MeOH	0.62	13	20	0.66	65	30.0	0.98	1.326	33.0	1.70	0.60	55.4

^a Reaction conditions: 0.1M solution of **112**, add 1-methylpiperazine, 1.0 equiv, and DIPEA, 3.0 equiv, rt, 4 h or 20 h. ^b As percentage of total ¹H NMR intensity in aromate region from starting material **112**, 2-isomer **127a**, and 6-isomer **127b**.

Data analysis of the reaction outcomes showed that R_{sel} was correlated with the solvatochromic β parameter ($R^2 = 0.75$; $p = 4.6 \times 10^{-7}$), as illustrated in Figure 13. R_{sel} was also correlated with the boiling point Bp ($R^2 = 0.72$) and Gutmann's donor number DN ($R^2 = 0.68$), which is a measure for the tendency to donate electron pairs to acceptors, however both Bp and DN were cross-correlated with β ($R^2 = 0.46$ and $R^2 = 0.68$, respectively).²⁰⁰

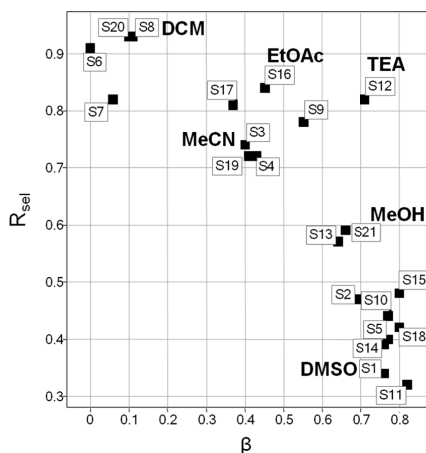


Figure 13. Regioselectivity R_{sel} for the pyridine 2-position as function of the solvatochromic β parameter in the reaction of methyl 2,6-dichloronicotinate (**112**) with 1-methylpiperazine in 21 different solvents (S1-S21). Labels indicate solvent numbers. Selected solvents frequently used in synthesis are highlighted.

Combination of α , β and π^* in a linear free energy relationship, as originally described by Kamlet and Taft,²⁰¹ gave accurate predictions of $R_{sel} = 1.28990 + 0.03992\alpha - 0.59417\beta - 0.46169\pi^*$ (RMSE = 0.047; $R^2 = 0.95$; $p = 1.9 \times 10^{-10}$), as shown in Figure 14.

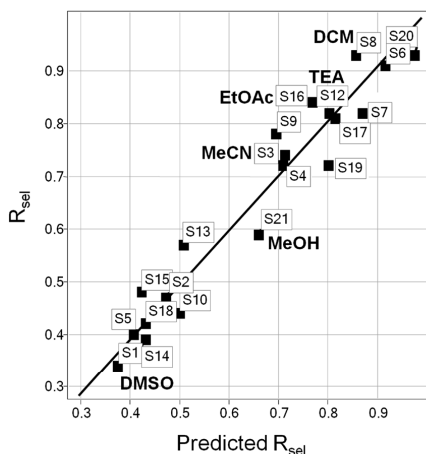


Figure 14. Prediction of the regioselectivity R_{sel} for the pyridine 2-position in the reaction of methyl 2,6-dichloronicotinate (**112**) with 1-methylpiperazine in 21 different solvents (S1-S21) based on the Kamlet-Taft equation: $R_{sel} = 1.28990 + 0.03992\alpha - 0.59417\beta - 0.46169\pi^*$ ($R^2 = 0.95$; $p = 1.9 \times 10^{-10}$).

This meant that R_{sel} could be controlled by choosing a solvent with either low or high solvatochromic β parameter. Thus, solvents with low β like 1,2-DCE and DCM gave the selectivity 16:1 for the 2-isomer while solvents with high β like DMSO gave the selectivity

2:1 for the 6-isomer, indicating that selectivity for the 2-isomer was most easily attained.²⁰² It was notable that common solvents frequently employed in synthesis like MeCN, EtOAc, acetone and ethers (1,4-dioxane, 1,2-DME, THF) gave only moderate regioselectivity.

Solvents that are the weakest hydrogen bond acceptors have the lowest β values. The regioselectivity for the 2-position in solvents with a low β parameter (like DCM) could be tentatively explained by formation of a hydrogen bond between the 3-methoxycarbonyl substituent on pyridine and the hydrogen of the secondary amine of 1-methylpiperazine. This could guide the nucleophile to reaction in the 2-position. A similar effect would be less effective in solvents (like DMSO) with a high β parameter, i.e. solvents that are strong hydrogen bond acceptors, where hydrogen bonds between the hydrogen of the secondary amine of 1-methylpiperazine and the solvent could compete with a hydrogen bond to the 3-methoxycarbonyl substituent.

8. Conclusions

Synthesis was developed to investigate the SAR of a novel series of 2-alkynylpyridine derivatives as mGluR5 antagonists. A two-step method efficiently provided terminal alkynes for use in Sonogashira coupling. It was shown that replacement of the linker oxygen changed the potency in the order $N < O < S < C$. Variation of substituents on the right-side phenyl group indicated that lipophilicity was the driving force in providing potency. The high lipophilicity was the likely cause of the high microsomal metabolism which prevented further development of the series. Nevertheless, testing of one lead compound in dog showed a 31% reduction of TLESRs which was a promising starting point compared to the standard mGluR5 antagonist MPEP.

SAR investigations was made for a series of ethyl 6-amino nicotines featured by a urea linker as antagonists of the P2Y₁₂ receptor. Most importantly it was shown that the 3-ethoxycarbonyl substituent was central to binding, since both replacement with 3-carboxylate and 3-hydrogen led to lower or no binding affinity. *N*-Methylation of the urea linker led to a significant lowering in binding affinity. The low aqueous solubility of the urea compounds was addressed by variation of the linker which led to the discovery of sulfonylureas as P2Y₁₂ antagonists with increased solubility compared to the matched-pair ureas.

The chemical stability of the sulfonylurea compounds during prolonged storage in solution was found to be related to the sulfonyl urea linker and depended on the type of solvent (acetonitrile or ethanol) and the substitution pattern of the sulfonyl urea functionality.

The 3-ethoxycarbonyl functionality was the main site of microsomal metabolism in both the urea, sulfonylurea, and acyl sulfonamide series. In order to stabilize the 3-ethoxycarbonyl functionality synthesis was developed to replace the 2-methyl substituent with more electron donating substituents. In addition, different strategies were pursued for making 5-ethyl-oxazoles as alkyl ester bioisosteres.

The 2-aminopyridines showed low to moderate microsomal clearance, however the potency in higher-order assays was generally lower than for the matched-pair 2-methylpyridine and 2-methoxypyridine. One 5-ethyl-oxazole showed low clearance in both rat and human liver microsomes, but also showed lower potency, compared to the matched-pair *n*-propyl ester.

Pair-wise comparison of compounds led to the conclusion that a correctly positioned alkyl group like in ethyl ester or in 5-methyl/ethyl oxazole and a correctly positioned strong hydrogen bond acceptor both were required for binding.

Chemical design based on the parameters PI, MR, and σ_p for the 3-substituent R^3 was used to model the factors that govern the regioselectivity R_{sel} for the 2-position in the reaction of 3-substituted 2,6-dichloropyridines with 1-methyl piperazine. Data analysis showed no correlation between R_{sel} and any of these parameters but showed a statistically significant correlation with the Verloop steric parameter B1 (R^2 : 0.45, $p = 0.006$). This implied that 3-substituents that are bulky close to the pyridine ring directed the regioselectivity towards the 6-position.

With $R^3 = -CO_2CH_3$, the regioselectivity R_{sel} found by reaction in 21 different solvents was predicted by the Kamlet-Taft equation: $R_{sel} = 1.28990 + 0.03992\alpha - 0.59417\beta - 0.46169\pi^*$ ($R^2 = 0.95$; $p = 1.9 \times 10^{-10}$). Thus R_{sel} was mainly correlated with the solvatochromic β parameter, and the 16:1 regioselectivity for the 2-isomer in DCM ($\beta = 0.10$) could be switched to a 2:1 selectivity for the 6-isomer in DMSO ($\beta = 0.76$).

Acknowledgments

I am deeply grateful to my supervisor Thomas Antonsson and co-supervisor Ruth Bylund who have supported me with advice and encouragement.

I sincerely thank Kristina Luthman for accepting me as a Ph.D. student and for all the practical help and advice when it was needed.

I am grateful to Jan Kihlberg and Kjell Andersson for giving me the freedom to pursue a Ph.D. while synchronously working as a Medicinal Chemist at AstraZeneca R&D, Mölndal, and to Michael Rowley for not interfering with that plan.

Fredrik Zetterberg, Jonas Boström and Fabrizio Giordanetto: Thank you for good discussions from which I have learnt a lot.

Thanks to all members of the former mGluR5 and P2Y₁₂ groups at AstraZeneca R&D, Mölndal.

Thanks to Michaela Marczyne who synthesized compounds for the regioselectivity study during her internship at AstraZeneca R&D, Mölndal.

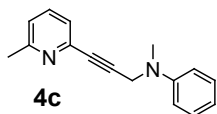
Thanks to Gunnar Grönberg, Tineke Papavoine, Richard Lewis and Marie Rydén-Landergren from the NMR-group at AstraZeneca R&D Mölndal for your help to set up experiments and with structure elucidations.

Thanks to Gustav Hulthe and Kristina Öhlén for LC/MS analysis, Britt-Marie Wissing, Pia Berntsson, and Linda Nilsson for in vitro screening and to Petter Svanberg and Marie Strimfors for in vivo experiments.

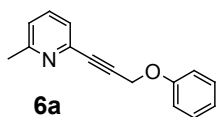
I am thankful to Laurent Knerr, Tomas Fex and Asim Ray for proof-reading of the manuscript.

Appendix

Compounds with experimentals not reported in publications or patent applications:

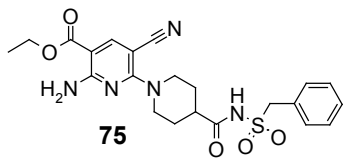


N-Methyl-N-(3-(6-methylpyridin-2-yl)prop-2-yn-1-yl)aniline (4c). Compound **3** (0.042 g, 0.15 mmol) was dissolved in TEA (0.20 mL) and *N*-methylaniline (0.040 g, 0.37 mmol) was added. The reaction mixture was stirred at rt for 21 h. The mixture was concentrated and the crude material was purified by reverse-phase chromatography on a Kromasil C8 column (250 mm length, 20 mm ID, flow 25 mL/min.) using a gradient 0→100% of MeCN in 0.1M ammonium acetate/MeCN 95:5 over 11 min. Yield: 0.004 g (8%). ¹H NMR (400 MHz, CDCl₃): δ 2.53 (3H, s), 3.05 (3H, s), 4.30 (2H, s), 6.81 (1H, app t, *J* = 7.2 Hz), 6.91 (2H, app d, *J* = 8.4 Hz), 7.06 (1H, app d, *J* = 7.9 Hz), 7.16 (1H, app d, *J* = 7.7 Hz), 7.25-7.31 (2H, m), 7.48 (1H, app d, *J* = 7.8 Hz). ¹³C NMR (101 MHz, CDCl₃) δ 158.7, 149.1, 142.2, 136.2, 129.1, 124.4, 122.5, 118.1, 114.1, 84.9, 83.7, 43.1, 38.7, 24.5. MS ^{m/z}: 237 (M+1).



2-Methyl-6-(3-phenoxyprop-1-yn-1-yl)pyridine (6a). A solution of phenol (0.941 g, 10.0 mmol) in acetone (15 mL) was cooled to 0 °C and anhydrous K₂CO₃ (1.382 g, 10.0 mmol) was added, followed by 3-bromoprop-1-yne (1.190 g, 1.34 mL, 10.0 mmol). The reaction mixture was allowed to reach rt and then heated at 50 °C for 17 h. The mixture was cooled and concentrated. The crude was extracted with DCM (3 x 15 mL) from K₂CO₃ (15 mL, saturated, aq solution). Combined organic phases were dried (Na₂SO₄) and concentrated. Yield: 1.026 g (78%) of (prop-2-yn-1-yloxy)benzene. ¹H NMR (MHz, CDCl₃): δ 2.48 (1H, t, *J* = 2.4 Hz), 4.64 (2H, d, *J* = 2.4 Hz), 6.92-7.01 (3H, m), 7.23-7.32 (2H, m). ¹³C NMR (75 MHz, CDCl₃) δ 157.3, 129.3, 121.4, 114.8, 78.6, 75.4, 55.7.

A reaction mixture of 6-methyl-2-bromopyridine (**2**, 1.055 g, 6.13 mmol), (prop-2-yn-1-yloxy)benzene (0.851 g, 6.44 mmol), Pd(PPh₃)₂Cl₂ (0.129 g, 0.184 mmol), and CuI (0.035 g, 0.184 mmol) in TEA (3.5 mL) was heated at 60 °C for 2 h. Phosphate buffer (0.20 M, pH7, 10 mL) was added and the mixture was extracted with DCM (3 x 10 mL). Combined organic phases were dried (MgSO₄), concentrated, passed through a silica plug while rinsing with Et₂O/pentane 1:1, and concentrated to give 1.491 g of a crude material which was purified by flash chromatography on silica gel (heptane/ethyl acetate 9:1, then 3:1). Yield: 1.008 g (74%). ¹H NMR (500 MHz, CDCl₃) δ 2.55 (3H, s), 4.93 (2H, s), 6.96-7.06 (3H, m), 7.10 (1H, app d, *J* = 7.7 Hz), 7.24 (1H, app d, *J* = 7.7 Hz), 7.28-7.34 (2H, m), 7.52 (1H, app t, *J* = 7.7 Hz). ¹³C NMR (126 MHz, CDCl₃) δ 158.2, 157.1, 141.0, 135.8, 128.9, 123.8, 122.5, 120.9, 114.3, 85.9, 83.2, 55.7, 23.9. MS ^{m/z}: 224 (M+1).



Ethyl 2-amino-6-(4-((benzylsulfonyl)carbamoyl) piperidin-1-yl)-5-cyanonicotinate (75). To compound **72**²⁰³ (1.45 mmol, 0.572 g) was added ammonia (5.0 mL, saturated solution in THF) and the reaction mixture was stirred at rt for 2 h. LC/MS showed complete reaction. A part of the mixture was taken out, concentrated, co-concentrated twice with THF, and continued in the next step.

To crude ethyl 2-amino-6-chloro-5-cyanonicotinate (0.047 g, 0.21 mmol) in ethanol (1.0 mL) in a microwave vial was added *N*-(benzylsulfonyl)piperidine-4-carboxamide²⁰⁴ (0.089 g, 0.32 mmol) and TEA (0.140 mL, 0.106 g, 1.05 mmol). The reaction mixture was heated in a microwave oven, single node heating, at 80 °C for 5 minutes and then at 120 °C for 20 minutes. The mixture was concentrated and the crude was purified by preparative HPLC. Yield: 0.022 g (22%, 3 steps). ¹H NMR (400 MHz, CDCl₃) δ 1.35 (3H, t, *J* = 7.10 Hz), 1.74-1.83 (4H, m), 2.34-2.46 (1H, m), 2.90-3.02 (2H, m), 3.91-4.00 (2H, m), 4.27 (2H, q, *J* = 7.10 Hz), 4.65 (2H, s), 7.30-7.40 (5H, m), 8.12 (1H, s). MS ^{m/z}: 472 (M+1).

References and notes

- ¹ Balasubramanian, M.; Keay, J. G. Pyridines and their benzo derivatives: applications. In *Comprehensive Heterocyclic Chemistry*. Vol. 5. Katritzky, A. R.; Rees, C. W., Eds. Pergamon 1997. A database search (4 April 2012) in the Thomson Reuters Integrity database (<http://www.integrity.com>) on marketed drugs containing a pyridine substructure presented 214 hits, including iso(quinolines).
- ² Joule, J. A.; Mills, K. *Heterocyclic Chemistry*. Blackwell Publishing. 4th Ed., 2000.
- ³ Even in 2010, niacin was number 45 on the Top 200 brand name drugs by US retail sales in 2010. Link to the poster by: Mack, D. J.; Weinrich, M. L.; Vitaku, E.; Njarðarson, J. T. University of Arizona: http://cbc.arizona.edu/njardarson/group/sites/default/files/Top%20200%20Brand-name%20Drugs%20by%20US%20Retail%20Sales%20in%202010sm_0.pdf
- ⁴ Gille, A.; Bodor, E. T.; Ahmed, K.; Offermans, S. Nicotinic acid: pharmacological effects and mechanism of action. *Annu. Rev. Pharmacol. Toxicol.* **2008**, *48*, 79-106.
- ⁵ Steppuhn, A.; Gase, K.; Krock, B.; Halitschke, R.; Baldwin, I. T. Nicotine's defensive function in Nature. *PLoS Biol.* **2004**, *2*(8), e217, 1074-1080.
- ⁶ Timmins G. S.; Master S.; Rusnak F.; Deretic V. Nitric oxide generated from isoniazid activation by KatG: Source of nitric oxide and activity against mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* **2004**, *48*, 3006-3009.
- ⁷ Vannelli, T. A.; Dykman, A.; Ortiz de Montellano, P. R. The antituberculosis drug ethionamide is activated by a flavoprotein monooxygenase. *J. Biol. Chem.* **2002**, *277*, 12824-12829.
- ⁸ See ref. 1, p. 278.
- ⁹ Jacoby, E.; Bouhelal R.; Gerspacher, M.; Seuwen, K. The 7TM G-protein-coupled receptor target family. *Chem. Med. Chem.* **2006**, *1*, 760-782.
- ¹⁰ Rajagopal, S.; Rajagopal, K.; Lefkowitz, R. J. Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nature Rev. Drug Discov.* **2010**, *9*, 373-386.
- ¹¹ Thomsen, W. J.; Behan, D. P. G protein-coupled receptors. Chp. 2.20, pp. 771-826 in Triggler, D.; Taylor, J. (Eds.) *Comprehensive Medicinal Chemistry II*. Elsevier, Oxford, **2007**.
- ¹² Progress in de-orphanization of GPCRs is monitored on the web-page of the International Union of Basic and Clinical Pharmacology: <http://www.iuphar-db.org/>
- ¹³ Rask-Andersen, M.; Almén, M. S.; Schiöth, H. B. Trends in the exploitation of novel drug targets. *Nature Rev. Drug Discov.* **2011**, *10*, 579-590.
- ¹⁴ Overington, J. P.; Al-Lazikani, B.; Hopkins, A. L. How many drug targets are there? *Nat. Rev. Drug Discov.* **2006**, *5*, 993-996.
- ¹⁵ Parmentier, M.-L.; Prezeau, L.; Bockaert, J.; Pin, J.-P. A model for the functioning of family 3 GPCRs. *Trends Pharmacol. Sci.* **2002**, *23*, 268-274.
- ¹⁶ Pin, J.-P.; Acher, F. The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. *Curr. Drug Targets - CNS Neurol. Disord.* **2002**, *1*, 297-317.
- ¹⁷ Bononi, A.; Agnoletto, C.; DeMarchi, E.; Marchi, S.; Patergnani, S.; Bonora, M.; Giorgi, C.; Missiroli, S.; Poletti, F.; Rimessi, A.; Pinton, P. Protein kinases and phosphatases in the control of cell fate. *Enzyme Research.* **2011**, Article ID 329098, 26 pages.
- ¹⁸ Hermans, E.; Challiss, R. A. J. Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors. *Biochem. J.* **2001**, *359*, 465-484.
- ¹⁹ Julio-Pieper, M.; Flor, P. J.; Dinan, T. G.; Cryan, J. F. Exciting times beyond the brain: metabotropic glutamate receptors in peripheral and non-neural tissues. *Pharmacol. Rev.* **2011**, *63*(1), 35-58.
- ²⁰ GORD is occasionally used and refers to the spelling GastroOesophageal Reflux Disease.
- ²¹ Vakil, N.; van Zanten, S. V.; Kahrilas, P.; Dent, J.; Jones, R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am. J. Gastroenterol.* **2006**, *101*, 1900-1920.
- ²² Holloway, R. H.; Boeckxstaens, G. E. E.; Penagini, R.; Siffrin, D. A.; Smout, A. J. P. M. Objective definition and detection of transient lower esophageal sphincter relaxation revisited: is there room for improvement? *Neurogastroenterol. Motil.* **2012**, *24*, 54-60.
- ²³ Mittal, R.K.; Holloway, R. H.; Penagini, R.; Blackshaw, L. A.; Dent, J. Transient lower esophageal sphincter relaxations. *Gastroenterol.* **1995**, *109*, 601-610.
- ²⁴ Hershcovici, T.; H. Mashimo, H.; Fass, R. The lower esophageal sphincter. *Neurogastroenterol. Motil.* **2011**, *23*, 819-830.
- ²⁵ Fox, M.; Forgacs, I. Gastro-oesophageal reflux disease. *Br. Med. J.* **2006**, *332*, 88-93.
- ²⁶ See the NIH web-page: [www://www.ncbi.nlm.nih.gov/pubmedhealth/PM00001331/](http://www.ncbi.nlm.nih.gov/pubmedhealth/PM00001331/)

- ²⁷ (a) Mainie, I.; Tutuian, R.; Shay, S.; Vela, M.; Zhang, X.; Sifrim, D.; Castell, D. O. Acid and non-acid reflux in patients with persistent symptoms despite acid suppressive therapy: a multicentre study using combined ambulatory impedance-pH monitoring. *Gut* **2006**, *55*, 398–402; (b) Zerbib, F.; Roman, S.; Ropert, A.; des Varannes, S. B.; Pouderoux P.; Chaput, U.; Mion, F.; Vérin, E.; Galmiche, J. P.; Sifrim, D. Esophageal pH-impedance monitoring and symptom analysis in GERD: a study in patients off and on therapy. *Am. J. Gastroenterol.* **2006**, *101*, 1956-1963.
- ²⁸ Blondeau, K. Treatment of gastro-esophageal reflux disease: the new kids to block. *Neurogastroenterol. Motil.* **2010**, *22*, 836-840.
- ²⁹ Claudine L.; Frisby, C. L.; Mattsson, J. P.; Jensen, J. M.; Lehmann A.; Dent, J.; Blackshaw, L. A. Inhibition of transient lower esophageal sphincter relaxation and gastroesophageal reflux by metabotropic glutamate receptor ligands. *Gastroenterol.* **2005**, *129*, 995-1004.
- ³⁰ Jensen, J.; Lehmann, A.; Uvebrandt, A.; Carlsson, A.; Jerndal, G.; Nilsson, K.; Frisby, C.; Blackshaw, L. A.; Mattson, J. P. Transient lower esophageal sphincter relaxations in dog are inhibited by a metabotropic glutamate receptor 5 antagonist. *Eur. J. Pharmacol.* **2005**, *519*, 154-157.
- ³¹ I.e. relating to or arising from both afferent and efferent impulses of the vagus nerve.
- ³² Rohof, W. O.; Aronica, E.; Beaumont, H.; Troost, D.; Boeckstaens, G. E. Localization of mGluR5, GABA_B, GABA_A, and cannabinoid receptors on the vago-vagal reflex pathway responsible for transient lower esophageal sphincter relaxation in humans: an immunohistochemical study. *Neurogastroenterol. Motil.* **2012**, *24(4)*, 383-e173.
- ³³ (a) Johnson, D. A.; Levy III, B. H. Evolving drugs in gastroesophageal reflux disease: pharmacologic treatment beyond proton pump inhibitors. *Expert Opin. Pharmacother.* **2010**, *11*, 1541-1548; (b) Zerbib, F. Medical treatment of GORD. Emerging therapeutic targets and concepts. *Best Pract. Res. Clin. Gastroenterol.* **2010**, *24*, 937-946.
- ³⁴ (a) O'Neill, M. J.; Fell, M.; J.; Svensson, K. A.; Witkin, J. M.; Mitchell, S. N. Recent developments in metabotropic glutamate receptors as novel drug targets. *Drugs Fut.* **2010**, *35*, 307-324; (b) Bird, M. K.; Lawrence, A. J. The promiscuous mGlu5 receptor - a range of partners for therapeutic possibilities? *Trends Pharmacol. Sci.* **2009**, *30*, 617-623.
- ³⁵ Fundytis, M. E. Glutamate receptors and nociception: implications for the drug treatment of pain. *CNS Drugs* **2001**, *15 (1)*, 29-58. (b) Crock, L. W.; Stemler, K. M.; Song, D. G.; Abbosh, P.; Vogt, S. K.; Qiu, C.-S.; Lai, H. H.; Mysorekar, I. U.; Gereau, R. W. Metabotropic glutamate receptor 5 (mGluR5) regulates bladder nociception. *Mol. Pain* **2012**, *8*, Paper No. 20.
- ³⁶ Liechti, M. E.; Athina, M. Role of the glutamatergic system in nicotine dependence: implications for the discovery and development of new pharmacological smoking cessation Therapies. *CNS Drugs*. **2008**, *22*, 705-724.
- ³⁷ (a) Cleva, R. M.; Olive, M. F. Positive allosteric modulators of type 5 metabotropic glutamate receptors (mGluR5) and their therapeutic potential for the treatment of CNS disorders. *Molecules* **2011**, *16*, 2097-2106; (b) Krystal, J. H.; Mathew, S. J.; D'Souza, D. C.; Garakani, A.; Gunduz-Bruce, H.; Charney, D. S. Potential psychiatric applications of metabotropic glutamate receptor agonists and antagonists. *CNS Drugs*. **2010**, *24*, 669-693; (c) Yasuhara, A.; Shigeyuki, C. Metabotropic glutamate receptors: potential drug targets for psychiatric disorders. *Open Med. Chem. J.* **2010**, *4*, 20-36.
- ³⁸ Rianza Bermudo-Soriano, C.; Perez-Rodriguez, M. M.; Vaquero-Lorenzo, C.; Baca-Garcia, E. New perspectives in glutamate and anxiety. *Pharmacol. Biochem. Behav.* **2012**, *100*, 752-774.
- ³⁹ Gaspar, P. A.; Bustamante, M. L.; Silva, H.; Aboitiz, F. Molecular mechanisms underlying glutamatergic dysfunction in schizophrenia: therapeutic implications. *J. Neurochem.* **2009**, *111*, 891-900.
- ⁴⁰ (a) Paluncha, A.; Pilc, A. The involvement of glutamate in the pathophysiology of depression. *Drug News Perspect.* **2005**, *18*, 262-268. (b) Li, X.; Need, A. B.; Baez, M.; Witkin, J. M. Metabotropic glutamate 5 receptor antagonism is associated with antidepressant-like effects in mice. *J. Pharmacol. Exp. Ther.* **2006** *319*, 254-259.
- ⁴¹ Carroll, F. I. Antagonists at metabotropic glutamate receptor subtype 5. *Ann. N. Y. Acad. Sci.* **2008**, *1141*, 221-232.
- ⁴² Ure, J.; Baudry, M.; Perassolo, M. Metabotropic glutamate receptors and epilepsy. *J. Neurol. Sci.* **2006**, *247*, 1-9.
- ⁴³ (a) Simonyi, A.; Schachtman, T. R.; Christoffersen, G. R. J. Metabotropic glutamate receptor subtype 5 antagonism in learning and memory. *Eur. J. Pharmacol.* **2010**, *639*, 17-25; (b) Gravius, A.; Pietraszek, M.; Dekundy, A.; Danysz, W. Metabotropic glutamate receptors as therapeutic targets for cognitive disorders. **2010**, *10*, 187-206; (c) Homayoun, H.; Moghaddam, B. Group 5 metabotropic glutamate receptors: Role in modulating cortical activity and relevance to cognition. *Eur. J. Pharmacol.* **2010**, *639*, 33-39.
- ⁴⁴ Dölen, G.; Bear, M. F. Role for metabotropic glutamate receptor 5 (mGluR5) in the pathogenesis of fragile X syndrome. *J. Physiol.* **2008**, *586*, 1503-1508.

- ⁴⁵ Xu, J.; Yongling, Z.; Contractor, A.; Heinemann, S. F. mGluR5 has a critical role in inhibitory learning. *J. Neurosci.* **2009**, *29*, 3676-3684.
- ⁴⁶ Search in Integrity database from Thomas Reuters, May 2012. (www.integrity.com)
- ⁴⁷ Bach, P.; Isaac, M.; Slassi, A. Metabotropic glutamate receptor 5 modulators and their potential therapeutic applications. *Expert Opin. Ther. Pat.* **2007**, *17*, 371-384.
- ⁴⁸ Schoepp, D. D.; Jane, D. E.; Monn, J. A. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. *Neuropharmacology* **1999**, *38*, 1431-1476.
- ⁴⁹ Varney, M. A.; Cosford, N. D. P.; Jachec, C.; Rao, S. P.; Sacaan, A.; Lin, F.; Bleicher, L.; Santori, E. M.; Flor, P. J.; Allgeier, H.; Gasparini, F.; Kuhn, R.; Hess, S. D.; Velicelebi, G.; Johnson, E. C. SIB-1757 and SIB-1893: Selective, noncompetitive antagonists of metabotropic glutamate receptor type 5. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 170-181.
- ⁵⁰ Rocher, J.-P.; Bonnet, B.; Bolea, C.; Lutjens, R.; Le Poul, E.; Poli, S.; Epping-Jordan, M.; Bessis, A.-S.; Ludwig, B.; Mutel, V. mGluR5 negative allosteric modulators overview: A medicinal chemistry approach towards a series of novel therapeutic agents. *Curr. Top. Med. Chem.* **2011**, *11*, 680-695.
- ⁵¹ (a) Christopoulos, A.; Kenakin, T. G protein-coupled receptor allosterism and complexing. *Pharmacol. Rev.* **2002**, *54*, 323-374; (b) May, L. T.; Christopoulos, A. Allosteric modulators of G-protein-coupled receptors. *Curr. Opin. Pharmacol.* **2003**, *3*, 551-556.
- ⁵² Gasparini, F.; Lingenhohl, K.; Stoehr, N.; Flor, P. J.; Heinrich, M.; Vranesic, I.; Biollaz, M.; Allgeier, H.; Heckendorf, R.; Urwyler, S.; Varney, M. A.; Johnson, E. C.; Hess, S. D.; Rao, S. P.; Sacaan, A. I.; Santori, E. M.; Velicelebi, G.; Kuhn, R. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology* **1999**, *38*, 1493-1503.
- ⁵³ Cosford, N. D. P.; Tehrani, L.; Roppe, J.; Schweiger, E.; Smith, N. D.; Anderson, J.; Bristow, L.; Brodtkin, J.; Jiang, X.; McDonald, I.; Rao, S.; Washburn, M.; Varney, M. A. 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine: A potent and highly selective metabotropic glutamate subtype 5 receptor antagonist with anxiolytic activity. *J. Med. Chem.* **2003**, *46*, 204-206.
- ⁵⁴ Mu, L.; Schubiger, A.; Ametamey, S. M. Radioligands for the PET imaging of metabotropic glutamate receptor subtype 5. *Curr. Top. Med. Chem.* **2010**, *10*, 1558-1568.
- ⁵⁵ Porter, R. H. P.; Jaeschke, G.; Spooren, W.; Ballard, T. M.; Büttelmann, B.; Kolczewski, S.; Peters, J.-U.; Prinssen, E.; Wichmann, J.; Vieira, E.; Mühlemann, A.; Gatti, S.; Mutel, V.; Malherbe P. Fenobam: a clinically validated nonbenzodiazepine anxiolytic is a potent, selective, and noncompetitive mGlu5 receptor antagonist with inverse agonist activity. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 711-721.
- ⁵⁶ (a) Wällberg, A.; Nilsson, K.; Österlund, K.; Peterson, A.; Elg, S.; Raboisson, P.; Bauer, U.; Hammerland, L. G.; Mattsson, J. P. Phenyl ureas of creatinine as mGluR5 antagonists. A structure-activity relationship study of fenobam analogues. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1142-1145; (b) Jaeschke, G.; Porter, R.; Büttelmann, B.; Ceccarelli, S. M.; Guba, W.; Kuhn, B.; Kolczewski, S.; Huwyler, J.; Mutel, V.; Peters, J.-U.; Ballard, T.; Prinssen, E.; Vieira, E.; Wichmann, J.; Spooren, W. Synthesis and biological evaluation of fenobam analogs as mGlu5 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1307-1311.
- ⁵⁷ Berry-Kravis, E.; Hessl, D.; Coffey, S.; Hervey, C.; Schneider, A.; Yuhas, J.; Hutchison, J.; Snape, M.; Tranfaglia, M.; Nguyen, D. V.; Hagerman, R. A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *J. Med. Genet.* **2009**, *46*, 266-271.
- ⁵⁸ Search on www.clinicaltrials.gov (10 October 2012).
- ⁵⁹ Levenga, J.; Hayashi, S.; de Vrij, F.M.S.; Koekkoek, S.K.; van der Linde, H. C.; Nieuwenhuizen, I.; Song, C.; Buijssen, R. A. M.; Pop, A. S.; GomezMancilla, B.; Nelson, D. L.; Willemsen, R.; Gasparini, F.; Oostra, B. A. AFQ056, a new mGluR5 antagonist for treatment of fragile X syndrome. *Neurobiol. Dis.* **2011**, *42* (3), 311-317.
- ⁶⁰ Keyword, C.; Wakefield, M.; Tack, J. A proof-of-concept study evaluating the effect of ADX10059, a metabotropic glutamate receptor-5 negative allosteric modulator, on acid exposure and symptoms in gastro-oesophageal reflux disease. *Gut* **2009**, *58*, 1192-1199.
- ⁶¹ Experimental procedures and a detailed description of the FLIPR assay are included in the patent applications: (a) Bach, P.; Bauer, U.; Nilsson, K.; Wällberg, A. Preparation of phenylthioalkyl or phenylaminoalkyl pyridinylalkynes for treating gastroesophageal reflux disease (GERD): WO2005044265 (A1), Oct. 2004; (b) Bach, P.; Bauer, U.; Nilsson, K.; Wällberg, A. Preparation of phenylalkyl pyridinyl alkynes for treating gastroesophageal reflux disease (GERD): WO-2005044266(A1), Oct. 2004; (c) Bach, P.; Bauer, U.; Nilsson, K.; Wällberg, A. Preparation of phenoxyalkyl pyridinyl alkynes for treating gastroesophageal reflux disease (GERD): WO-2005044267(A1), Oct. 2004.
- ⁶² It was also attempted to replace the alkynyl part of the linker, but that work is not part of the present thesis.

- ⁶³ Sonogashira, K.; Tohda, Y.; Hagihara, N. A convenient synthesis of acetylenes: substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines. *Tetrahedron Lett.* **1975**, *16*, 4467-4470.
- ⁶⁴ See appendix for synthesis description of compound **4c**.
- ⁶⁵ Luo, F.-T.; Ko, S.-L.; Liu, L.; Chen, H. Stereoselective synthesis of (Z)- α -phenoxyethylene- γ -butyrolactone and its sulfur analogues from 2-propynyloxy- or 2-propynyl-thiobenzene. *Heterocycles* **2000**, *53* (9), 2055-2066.
- ⁶⁶ See appendix for synthesis description of compound **6a**.
- ⁶⁷ Jeffery, T. Palladium-catalysed vinylation of organic halides under solid-liquid phase transfer conditions. *J. Chem. Soc., Chem. Commun.* **1984**, *19*, 1287-1289.
- ⁶⁸ Corey, E. J.; Fuchs, P. L. A synthetic method for formyl- \rightarrow ethynyl conversion (RCHO- \rightarrow RCCH or RCCR'). *Tetrahedron Lett.* **1972**, *13*, 3769-3772.
- ⁶⁹ Grandjean, D.; Pale, P.; Chucho, J. An improved procedure for aldehyde-to-alkyne homologation via 1,1-dibromoalkenes; synthesis of 1-bromoalkynes. *Tetrahedron Lett.* **1994**, *35*, 3529-3530.
- ⁷⁰ Re-screening confirmed the lack of potency. Maximum concentration tested was 900 μ M.
- ⁷¹ The screening values in the FLIPR assay were determined as the mean of 2-3 measurements with max variability of \pm 40% from the value given. As exemplified in Papers I-II, the majority of the compounds showed considerably lower variability.
- ⁷² Experimental logD values are not available for these compounds, thus calculated values are used in the discussion. ACDlogD was part of the ACD/labs program package from Advanced Chemical Development, Inc. (www.acdlabs.com)
- ⁷³ See however: Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* **2002**, *45*, 2615-2623.
- ⁷⁴ Leeson, P. D.; Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discov.* **2007**, *6*, 881-890.
- ⁷⁵ In these comparisons with racemic compounds it is assumed that both enantiomers are antagonists and show similar potencies.
- ⁷⁶ Aqueous solubility is referring to kinetic aqueous solubility throughout the thesis.
- ⁷⁷ This is an overview of the main signalling pathways that are relevant for the present discussion, however each signalling molecule, like PKC or Ca²⁺, typically have roles in several signalling pathways. The reader is referred to the referenced literature for broader discussions.
- ⁷⁸ An alternative spelling of hemostasis is haemostasis.
- ⁷⁹ Michelson, A. D. Antiplatelet therapies for the treatment of cardiovascular disease. *Nat. Rev. Drug Discov.* **2010**, *9*, 154-169.
- ⁸⁰ At low shear rate, such as in veins and larger arteries, platelets bind primarily to collagen, fibronectin and laminin, while at high shear rate, such as in small arteries and microvasculature, interactions with glucoprotein Ib α and von Willebrand factor become important. See Broos, K.; Feys, H. B.; De Meyer, S. F.; Vanhoorelbeke, K.; Deckmyn, H. Platelets at work in primary hemostasis. *Blood Rev.* **2011**, *25*, 155-167.
- ⁸¹ Jackson, S. P.; Nesbitt, W. S.; Kulkarni, S. Signaling events underlying thrombus formation. *J. Thromb. Haemost.* **2003**, *1*, 1602-1612.
- ⁸² Bennett, J. S. Platelet-fibrinogen interactions. *Ann. NY Acad. Sci.* **2001**, *936*, 340-354.
- ⁸³ Both P2Y₁ and mGlu5 receptors are G_q-coupled and thus signal via the PLC/IP₃ pathway.
- ⁸⁴ Li, Z.; Delaney, K.; O'Brien, K. A.; Du, X. Signaling during platelet adhesion and activation. *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 2341-2349.
- ⁸⁵ Wijeyeratne, Y. D.; Heptinstall, S. Anti-platelet therapy: ADP receptor antagonists. *Br. J. Clin. Pharmacol.* **2011**, *72*(4), 647-657.
- ⁸⁶ (a) Cattaneo, M. Advances in antiplatelet therapy: overview of new P2Y₁₂ receptor antagonists in development. *Eur. Heart J. Suppl.* **2008**, *10*, 133-137; (b) Storey, R. F. Biology and pharmacology of the platelet P2Y₁₂ receptor. *Curr. Pharm. Design* **2006**, *12*, 1255-1259.
- ⁸⁷ Jin, J.; Kunapuli, S. P. Coactivation of two different G protein-coupled receptors is essential for ADP-induced platelet aggregation. *Proc. Natl. Acad. Sci. USA.* **1998**, *95*, 8070-8074.
- ⁸⁸ (a) Abbracchio, M. P.; Burnstock, G.; Boeynaems, J. M.; Barnard, E. M.; Boyer, J. L.; Kennedy, C.; Knight, G. E.; Fumagalli, M.; Gachet, C.; Jacobson, K. A.; Weisman, G. A. International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology. Antagonists of platelet P2Y₁₂ receptors. *J. Med. Chem.* **2009**, *52*, 281-341.

- ⁸⁹ The gaps are due to non-mammalian P2Y receptors and to premature assignment of putative P2Y receptors that were later shown to be special homologs or completely different GPCRs.
- ⁹⁰ Mahaut-Smith, M. P.; Ennion, S. J.; Rolf, M. G.; Evans, R. J. ADP is not an agonist at P2X₁ receptors; evidence for separate receptors stimulated by ATP and ADP on human platelets. *Br. J. Pharmacol.* **2000**, *131*, 108-114.
- ⁹¹ (a) Vial, C.; Rolf, M. G.; Mahaut-Smith, M. P.; Evans, R. J. A study of P2X₁ receptor function in murine megakaryocytes and human platelets reveals synergy with P2Y receptors. *Br. J. Pharmacol.* **2002**, *135*, 363-372; (b) Vial, C.; Pitt, S. J.; Roberts, J.; Rolf, M. G.; Mahaut-Smith, M. P.; Evans, R. J. Lack of evidence for functional ADP-activated human P2X₁ receptors supports a role for ATP during haemostasis and thrombosis. *Blood* **2003**, *102*, 3646-3651.
- ⁹² Harden, T. K.; Sesma, J. I.; Fricks, I. P.; Lazarowski, E. R. Signalling and pharmacological properties of the P2Y₁₄ receptor. *Acta Physiol.* **2010**, *199* (2), 149-160.
- ⁹³ Furie, B.; Furie, B. C. Mechanisms of thrombus formation. *N. Engl. J. Med.* **2008**, *359*, 938-949.
- ⁹⁴ Adams, M. N.; Ramachandran, R.; Yau, M.-K.; Suen, J. Y.; Fairlie, D. P.; Hollenberg, M. D.; Hooper, J. D. Structure, function and pathophysiology of protease activated receptors. *Pharmacol. Therap.* **2011**, *130*(3), 248-282.
- ⁹⁵ Félétou, M.; Vanhoutte, P. M.; Verbeuren, T. J. The thromboxane/endoperoxide receptor (TP): The common villain. *Cardiovasc. Pharmacol.* **2010**, *55*, 317-332.
- ⁹⁶ Jin, J.; Quinton, T. M.; Zhang, J.; Rittenhouse, S. E.; Kunapuli S. P. Adenosine diphosphate (ADP)-induced thromboxane A₂ generation in human platelets requires coordinated signaling through integrin α IIb β 3 and ADP receptors. *Blood* **2000**, *99*, 193-198.
- ⁹⁷ Gachet, C. P2Y₁₂ receptors in platelets and other hematopoietic and non-hematopoietic cells. *Purinerg. Signal.* **2012**, *8*, 609-619 and references cited therein.
- ⁹⁸ Hollopeter, G.; Jantzen, H.-M.; Vincent, D.; Li, G.; England, L.; Ramakrishnan, V. Yang, R.-B., Nurden, P.; Nurden, A., Julius, D.; Conley, P. B. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* **2001**, *409*, 202-207.
- ⁹⁹ Wihlborg, A.-K.; Wang, L.; Braun, O. Ö.; Eyjolfsson, A.; Gustafsson, R.; Gudbjartsson, T.; Erlinge, D. ADP receptor P2Y₁₂ is expressed in vascular smooth muscle cells and stimulates contraction in human blood vessels. **2004**, *24*(10), 1810-1815.
- ¹⁰⁰ (a) Savi, P.; Pereillo, J. M.; Uzabiaga, M. F.; Combalbert, J.; Picard, C.; Maffrand, J. P.; Pascal, M.; Herbert, J. M. Identification and biological activity of the active metabolite of clopidogrel. *Thromb. Haemost.* **2000**, *84*, 891-896; (b) Yusuf, S.; Zhao, F.; Mehta, S. R.; Chrolavicius, S.; Tognoni, G.; Fox, K. K. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N. Engl. J. Med.* **2001**, *345*, 494-501.
- ¹⁰¹ Sugidachi, A.; Ogawa, T.; Kurihara, A.; Hagihara, K.; Jakubowski, J. A.; Hashimoto, M.; Niitsu, Y.; Asai, F. The greater in vivo antiplatelet effects of prasugrel as compared to clopidogrel reflect more efficient generation of its active metabolite with similar antiplatelet activity to that of clopidogrel's active metabolite. *J. Thromb. Haemost.* **2007**, *5*, 1545-1551.
- ¹⁰² Springthorpe, B.; Bailey, A.; Barton, P.; Birkinshaw, T. N.; Bonnert, R. V.; Brown, R. C.; Chapman, D.; Dixon, J.; Guile, S. D.; Humphries, R. G.; Hunt, S. F.; Ince, F.; Ingall, A. H.; Kirk, I. P.; Leeson, P. D.; Leff, P.; Lewis, R. J.; Martin, B. P.; McGinnity, D. F.; Mortimore, M. P.; Paine, S. W.; Pairedeau, G.; Patel, A.; Rigby, A. J.; Riley, R. J.; Teobald, B. J.; Tomlinson, W.; Webborn, P. J. H.; Willis, P. A. From ATP to AZD6140: The discovery of an orally active reversible P2Y₁₂ receptor antagonist for the prevention of thrombosis. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6013-6018.
- ¹⁰³ van Giezen, J. J. J.; Nilsson, L.; Berntsson, P.; Wissing, B.; Giordanetto, F.; Tomlinson, W.; Greasley, P. J. Ticagrelor binds to human P2Y₁₂ independently from ADP but antagonizes ADP-induced receptor signaling and platelet aggregation. *J. Thromb. Haemost.* **2009**, *7*, 1556-1565.
- ¹⁰⁴ Storey, R. F. Pharmacology and clinical trials of reversibly-binding P2Y₁₂ inhibitors. *Thromb. Haemost.* **2011**, *105* (Suppl 1), S75-S81.
- ¹⁰⁵ Wallentin, L.; Becker, R. C.; Budaj, A.; Cannon, C. P.; Emanuelsson, H.; Held, C.; Horrow, J.; Husted, S.; James, S.; Katus, H.; Mahaffey, K. W.; Scirica, B. M.; Skene, A.; Steg, P. G.; Storey, R. F.; Harrington, R. A. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N. Engl. J. Med.* **2009**, *361*, 1045-1057.
- ¹⁰⁶ Jacobson, K. A.; Deflorian, F.; Mishra, S.; Costanze, S. Pharmacology of the platelet purinergic receptors. *Purinergic Signal.* **2011**, *7*, 305-324.
- ¹⁰⁷ Scarborough, R. M.; Laibelman, A. M.; Clizbe, L. A.; Fretto, L. J.; Conley, P. B.; Reynolds, E. E.; Sedlock, D. M.; Jantzen H.-M. Novel tricyclic benzothiazolo[2,3-c]thiadiazine antagonists of the platelet ADP receptor (P2Y₁₂). *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1805-1808.
- ¹⁰⁸ Search in Integrity database (www.integrity.com) from Thomas Reuters, May 2012.

¹⁰⁹ (a) Parlow, J. J.; Burney, M. W.; Case, B. L.; Girard, T. J.; Hall, K. A.; Harris, P. K.; Hiebsch, R. R.; Huff, R. M.; Lachance, R. M.; Mischke, D. A.; Rapp, S. R.; Woerndle, R. S.; Ennis, M. D. Piperazinyl glutamate pyridines as potent orally bioavailable P2Y₁₂ antagonists for inhibition of platelet aggregation. *J. Med. Chem.* **2010**, *53*, 2010-2037; (b) Parlow, J. J.; Burney, M. W.; Case, B. L.; Girard, T. J.; Hall, K. A.; Harris, P. K.; Hiebsch, R. R.; Huff, R. M.; Lachance, R. M.; Mischke, D. A.; Rapp, S. R.; Woerndle, R. S.; Ennis, M. D. Part II: Piperazinyl-glutamate-pyridines as potent orally bioavailable P2Y₁₂ antagonists for inhibition of platelet aggregation. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1388-1394; (c) Parlow, J. J.; Burney, M. W.; Case, B. L.; Girard, T. J.; Hall, K. A.; Hiebsch, R. R.; Huff, R. M.; Lachance, R. M.; Mischke, D. A.; Rapp, S. R.; Woerndle, R. S.; Ennis, M. D. Piperazinyl-glutamate-pyridines as potent orally bioavailable P2Y₁₂ antagonists for inhibition of platelet aggregation. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4657-4663; (d) Parlow, J. J.; Burney, M. W.; Case, B. L.; Girard, T. J.; Hall, K. A.; Hiebsch, R. R.; Huff, R. M.; Lachance, R. M.; Mischke, D. A.; Rapp, S. R.; Woerndle, R. S.; Ennis, M. D. Piperazinyl-glutamate-pyrimidines as potent P2Y₁₂ antagonists for inhibition of platelet aggregation. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6148-6156.

¹¹⁰ Baqi, Y.; Atzler, K.; Köse, M.; Glänzel, M.; Müller, C. E. High-affinity, non-nucleotide-derived competitive antagonists of platelet P2Y₁₂ receptors. *J. Med. Chem.* **2009**, *52*, 3784-3793.

¹¹¹ Kortum, S. W.; Lachance, R. M.; Schweitzer, B. A.; Yalamanchili, G.; Rahman, H.; Ennis, M. D.; Huff, R. M.; TenBrink, R. E. Thienopyrimidine-based P2Y₁₂ platelet aggregation inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5919-5923.

¹¹² (a) Douglass, J. G.; deCamp, J. B.; Fulcher, E. H.; Jones, W.; Mahanty, S.; Morgan, A.; Smirnov, D.; Boyer, J. L.; Watson, P. S. Lipophilic modifications to dinucleoside polyphosphates and nucleotides that confer antagonist properties at the platelet P2Y₁₂ receptor. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2167-2171; (b) Douglass, J. G.; Patel, R. I.; Yerxa, B. R.; Shaver, S. R.; Watson, P. S.; Bednarski, K.; Plourde, R.; Redick, C. C.; Brubaker, K.; Jones, A. C.; Boyer, J. L. Lipophilic modifications to dinucleoside polyphosphates and nucleotides that confer antagonist properties at the platelet P2Y₁₂ receptor. *J. Med. Chem.* **2008**, *51*, 1007-1025.

¹¹³ Mackman, N. Triggers, targets and treatments for thrombosis. *Nature* **2008**, *451*, 914-918.

¹¹⁴ (a) Goodman, S. G.; Menon, V.; Cannon, C. P.; Steg, G.; Ohman, M.; Harrington, R. A. Acute ST-segment elevation myocardial infarction: American college of chest physicians evidence-based clinical practice guidelines (8th edition). *Chest* **2008**, *133*, 708S-775S; (b) Di Minno, M. N. D.; Tufano, A.; Ageno, W.; Prandoni, P.; Di Minno, G. Identifying high-risk individuals for cardiovascular disease: similarities between venous and arterial thrombosis in perspective. A 2011 update. *Intern Emerg. Med.* **2012**, *7*, 9-13.

¹¹⁵ (a) Paez Espinosa, E. V.; Murad, J. P.; Khasawneh, F. T. Aspirin: Pharmacology and clinical applications. *Thrombosis* **2012**, Article ID 173124, 15 pages; (b) Armstrong, P. C. J.; Leadbeater, P. D.; Chan, M. V.; Kirkby, N. S.; Jakubowski, J. A.; Mitchell, J. A.; Warner, T. D. In the presence of strong P2Y₁₂ receptor blockade, aspirin provides little additional inhibition of platelet aggregation. *J. Thromb. Haemost.* **2011**, *9*, 552-561.

¹¹⁶ Compounds were screened in vitro for receptor affinity using a radio-ligand binding assay and for potency in inhibiting receptor signaling using a GTP γ S assay and cell membranes expressing recombinant human P2Y₁₂ receptors. As the project developed, more advanced assays were added. These included the washed platelet assay (WPA) and the residual platelet count assay (RPC). Screening values in the binding assay and the potency assays were determined as single values or as a mean of two measurements with maximum four times difference between highest and lowest screening value. Although for some compounds the affinity and potency may have been determined with lower variability, in general a difference in binding affinity/potency between compounds of ≥ 3 times is considered significant in the SAR discussions.

¹¹⁷ The GTP γ S functional assay is based on the principle that activation of a GPCR (P2Y₁₂) by an agonist (ADP) leads to conformational changes that favors binding of the receptor to a G-protein. This leads to formation of a agonist-receptor-G-protein ternary complex which induces GDP bound on the G-protein α -subunit to exchange with GTP γ ³⁵S. GTP γ ³⁵S is not hydrolyzed by GTPases and the effect of an added antagonist can thus be determined by the fraction of unbound GTP γ ³⁵S.

¹¹⁸ Sundermeier, M.; Zapf, A.; Beller, M.; Sans, J. A new palladium catalyst system for the cyanation of aryl chlorides. *Tetrahedron Lett.* **2001**, *42*, 6707-6710.

¹¹⁹ Cottet, F.; Schlosser, M. Three chloro(trifluoromethyl)pyridines as model substrates for regioexhaustive functionalization. *Eur. J. Org. Chem.* **2004**, 3793-3798.

¹²⁰ Mosti, L.; Menozzi, G.; Schenone, P.; Dorigo, P.; Gaion, R. M.; Belluco, P. Synthesis and cardiotoxic activity of 2-substituted 5-cyano-1,6-dihydro-6-oxo-3-pyridinecarboxylic acids and their methyl or ethyl esters. *Farmaco* **1992**, *47*, 427-437.

¹²¹ Compounds that were inactive in the binding assay were not screened in other assays. Maximum concentration tested was 33 μM .

¹²² The Big Picker Program was used for selecting a diverse set of reagents, see: Blomberg, N.; Cosgrove, D. A.; Kenny, P. W.; Kolmodin, K. Design of compound libraries for fragment screening. *J. Comput. Aided. Mol. Des.* **2009**, *23*, 513-525.

¹²³ These compounds were synthesized by colleagues at AstraZeneca R&D Mölndal and have been included here to complement the SAR discussion.

¹²⁴ The lipophilicities, expressed by logD, of the ureas were determined by a chromatographic method. Of the 18 compounds with both affinity and potency lower than 0.5 μM , logD was determined for 14 compounds which showed $6.4 \geq \log D \geq 5.0$.

¹²⁵ Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3-25.

¹²⁶ Compound (initial concentration 10 μM) after 60 minutes at 1 mg compound/mL protein.

¹²⁷ Compound **54** was synthesized in analogy to **43** (Scheme 6). A large scale synthesis of **54** was published recently: Bell, S. J.; McIntyre, S.; Garcia, C. F.; Kitson, S. L.; Therkelsen, F.; Andersen, S. M.; Zetterberg, F.; Aurell, C.-J.; Bollmark, M.; Ehrl, R. Development of an efficient and practical route for the multikilogram manufacture of ethyl 5-cyano-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate and ethyl 6-chloro-5-cyano-2-methylnicotinate, key intermediates in the preparation of P2Y₁₂ antagonists. *Org. Process Res. Dev.* **2012**, *16*, 819-823.

¹²⁸ Compounds **58** and **59** were synthesized by colleagues at Array Biopharma Inc, Boulder, USA, and have been exemplified in the synthesis section since they were used in the study of compound stability.

¹²⁹ The relative hydrogen bond acceptor strengths were assumed to be analogous to the amide-thioamide pair: Lee, H.-J.; Choi, Y.-S.; Lee, K.-B.; Park, J.; Yoon, C.-J. Hydrogen bonding abilities of thioamide. *J. Phys. Chem.* **2002**, *106*, 7010-7017.

¹³⁰ (a) Uehling, D.E.; Donaldson, K. H.; Deaton, D. N.; Hyman, C. E.; Sugg E. E.; Barrett D. G.; Hughes R. G.; Reitter B.; Adkison K.K.; Lancaster M. E.; Lee F.; Hart, R.; Paulik M. A.; Sherman B. W.; True T.; Cowan, C. Synthesis and evaluation of potent and selective $\alpha 3$ adrenergic receptor agonists containing acylsulfonamide, sulfonylsulfonamide, and sulfonylurea carboxylic acid isosteres. *J. Med. Chem.* **2002**, *45*, 567-583; (b) Michael P.; Winters M. P.; Crysler C.; Subasinghe N.; Ryan D. Leong L.; Zhao S.; Donatelli R.; Yurkow E.; Mazzulla, M.; Boczon, L.; Manthey C. L.; Molloy C.; Raymond H.; Lynne, M. L.; McAlonanb, L.; Tomczuka B. Carboxylic acid bioisosteres acylsulfonamides, acylsulfamides, and sulfonylureas as novel antagonists of the CXCR2 receptor. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1926-1930.

¹³¹ clogP was calculated by the software module provided by Daylight Chemical Information Systems, Inc. (<http://www.daylight.com>)

¹³² 1.0 μM stock solutions in respectively MeCN and EtOH were made of each compound. 10 μL of each solution was pipetted into microvials that were then plastic capped. The microvials were stored at room temperature until the time for the LC/MS analysis.

¹³³ Bottari, F.; Giannaccini, B.; Nannipieri, E.; Saettone, M. F. Thermal dissociation of sulfonylureas II: four *N*- and *N'*-substituted sulfonylureas in different media. *J. Pharm. Sci.* **1972**, *59*, 602-606.

¹³⁴ Hutchby, M.; Houlden, C.; Ford, J. G.; Tyler, S. N. G.; Gagné, M.; Lloyd-Jones, G.; Booker-Milburn, K. Hindered ureas as masked isocyanates: facile carbamylation of nucleophiles under neutral conditions. *Angew. Chem. Int. Ed.* **2009**, *48*, 8721-8724.

¹³⁵ Example of the opposite: Neuvonen, H.; Neuvonen, K. Correlation analysis of carbonyl carbon ¹³C NMR chemical shifts, IR absorption frequencies and rate coefficients of nucleophilic acyl substitution. A novel explanation of the substituent dependence of reactivity. *J. Chem. Soc. Perkin Trans. 2*, **1999**, 1497-1502.

¹³⁶ Further studies to elucidate the mechanism was beyond the scope of this thesis.

¹³⁷ Only small amounts of these compounds were available and each ¹³C NMR spectrum was run over night. In addition, the ¹³C NMR spectra for solutions in *d*₆-EtOD were run at low temperature (-5 °C). Nonetheless, the chemical shift of the sulfonylurea carbon of **51** in *d*₆-EtOD could not be determined due to degradation.

¹³⁸ (a) Sarmah, A. K.; Sabadie, J. Hydrolysis of sulfonylurea herbicides in soils and aqueous solutions: A review. *J. Agric. Food Chem.* **2002**, *50*, 6253-6265; (b) Zheng, W.; Yates, S. R.; Papiernik, S. K. Transformation kinetics and mechanism of the sulfonylurea herbicides pyrazosulfuron ethyl and halosulfuron methyl in aqueous solutions. *J. Agric. Food Chem.* **2008**, *56*, 7367-7372; (c) Ehlhardt, W. J.; Woodland, J.M.; Worzalla, J. F.; Bewley, J. R.; Grindey, G. B.; Todd, G.C.; Toth, J. E.; Howbert J. J. Comparison of metabolism and toxicity to the structure of the anticancer agent sulofenur and related

- sulfonylureas. *Chem. Res. Toxicol.* **1992**, *5*, 667-673; (d) Toth, J. E.; Grindey, G. B.; Ehlhardt, W. J. Sulfonimidamide analogs of oncolytic sulfonylureas. *J. Med. Chem.* **1997**, *40*, 1018-1025.
- ¹³⁹ See e.g. Clarke, S. E.; Jeffrey, P. Utility of metabolically stable screening: comparison of in vitro and in vivo clearance. *Xenobiotica* **2001**, *31*, 591-598.
- ¹⁴⁰ Durrer, A.; Wernly-Chung, G. N.; Boss, G.; Testa, B. Enzymatic hydrolysis of nicotinic esters: comparison between plasma and liver catalysis. *Xenobiotica* **1992**, *22*(3), 273-282.
- ¹⁴¹ Ciapetti, P.; Giethlen, B. Molecular variations on isosteric replacements. In *Wermuth's The practice of medicinal chemistry*. Elsevier, **2008**, Chp. 15, p. 314-342, and references cited therein.
- ¹⁴² Synthesis procedures can be found in the patent applications: (a) Antonsson, T.; Bach, P.; Brown, D.; Bylund, R.; Giordanetto, F.; Johansson, J. Patent application WO2008/085117 (A1). Filed 12 January 2006; (b) Antonsson, T.; Bach, P.; Brown, D.; Bylund, R.; Giordanetto, F.; Jacobsson, L. Patent application WO2008/004946 (A1). Filed 4 July 2006; (c) Antonsson, T.; Bach, P.; Brickmann, K.; Bylund, R.; Giordanetto, F.; Johansson, J.; Zetterberg, F. Patent application WO2008/085118 (A1). Filed 17 July 2006 (Compound **74** is example 6).
- ¹⁴³ Honda, M.; Morita, H.; Nagakura, I. Deprotection of allyl groups with sulfonic acids and palladium catalyst. *J. Org. Chem.* **1997**, *62*, 8932-8936.
- ¹⁴⁴ This principle is used in Kenner's safety-catch linker in which sulfonylurea-based linkers are activated by alkylation prior to cleavage, see e.g. Fattori, D.; D'Andrea, P.; Porcelloni, M. Solid-phase synthesis of unsymmetrical ureas through the use of Kenner safety-catch linker. *Tetrahedron Lett.* **2003**, *44*, 811-814.
- ¹⁴⁵ McElvain, S. M.; Schroeder, J. P. Orthoesters and related compounds from malono- and succinonitriles. *J. Am. Chem. Soc.* **1949**, *71*, 40-46.
- ¹⁴⁶ Under the reaction conditions employed, about 5% (by LC/MS and ¹H NMR) of the *N*-methyl pyridine regioisomer was produced. See also Carabateas, P. M.; Brundage, R. P.; Gelotte, K. O.; Gruett, M. D.; Lorenz, R. R.; Opalka, C. J., Jr.; Singh, B.; Thielking, W. H.; Williams, G. L.; Leshar, G. Y. 1-Ethyl-1,4-dihydro-4-oxo-7-(pyridinyl)-3-quinoline-carboxylic acids. I. Synthesis of 3- and 4-(3-aminophenyl)pyridine intermediates. *J. Heterocycl. Chem.* **1984**, *21*, 1849-1856.
- ¹⁴⁷ Chen, Q.-Y.; Wu, S.-W. A simple convenient method for preparation of difluoromethyl ethers using fluorosulfonyldifluoroacetic acid as a difluorocarbene precursor. *J. Fluorine Chem.* **1989**, *44*, 433-440; (b) Ando, M.; Wada, T.; Sato, N. Facile one-pot synthesis of *N*-difluoromethyl-2-pyridone derivatives. *Org. Lett.* **2006**, *8*, 3805-3808.
- ¹⁴⁸ Prepared by modification of the procedure in Tappe, H.; Müller, R. Pyridinone derivatives. Patent application DE1980-3005069. Filed 11 February 1980.
- ¹⁴⁹ See appendix for synthesis description of compound **75**.
- ¹⁵⁰ Wipf, P.; Miller, C. P. A new synthesis of highly functionalized oxazoles. *J. Org. Chem.* **1993**, *58*, 3604-3606.
- ¹⁵¹ von Geldern, T. W.; Hutchins, C.; Kester, J. A.; Wu-Wong, J.R.; Chiou, W.; Dixon, D. B.; Opgenorth, T. J. J. Azole endothelin antagonists. Part 1. A receptor model explains an unusual structure-activity profile. *J. Med. Chem.* **1996**, *39*, 957-967.
- ¹⁵² Brown, P.; Davies, D. T.; O' Hanlon, P. J.; Wilson, J. M. Chemistry of pseudomonic acid (VIII). Part 15. Synthesis and antibacterial activity of a series of 5-alkyl, 5-alkenyl, and 5-heterosubstituted oxazoles. *J. Med. Chem.* **1996**, *39*, 446-457.
- ¹⁵³ Brain, C. T.; Paul, J. M. Rapid synthesis of oxazoles under microwave conditions. *Synlett* **1999**, 1642-1644.
- ¹⁵⁴ An alternative to conversion of the pyridone to the 2-chloropyridine could be to use Py-BOP or Py-Brop which had worked well in the activation of similar in-house pyridones for subsequent coupling with amine nucleophiles. See also Kang, F.-A.; Kodah, J.; Guan, Q.; Li, X.; Murray, W. V. Efficient conversion of Biginelli 3,4-dihydropyrimidin-2(1*H*)-one to pyrimidines via PyBroP-mediated coupling. *J. Org. Chem.* **2005**, *70*, 1957-1960.
- ¹⁵⁵ Compounds **91** and **92** were prepared by colleagues at AstraZeneca R&D Mölndal in analogy to the synthesis of **29**.
- ¹⁵⁶ Fox, S. C.; Burgess-Wilson, M.; Heptinstall, S.; Mitchell, J. R. A. Platelet aggregation in whole blood determined using the Ultra-Flo 100 Platelet Counter. *Thromb. Haemost.* **1982**, *48*(3), 327-329.
- ¹⁵⁷ Values from: Hansch, C.; Leo, A.; Hoekman, D. *Exploring QSAR, Vol 2: Hydrophobic, electronic, and steric constants*. American Chemical Society, **1995**. No value for the difluoromethoxy substituent was found, however it was assumed to be electron withdrawing like the trifluoromethoxy substituent ($\sigma_p = 0.35$; $\sigma_1 = 0.39$).
- ¹⁵⁸ See e.g. Kim, I.-H.; Nishi, K.; Kasagami, T.; Morisseau, C.; Liu, J.-Y.; Tsai, H.-J.; Hammock, B. D. Biologically active ester derivatives as potent inhibitors of the soluble epoxide hydrolase. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5889-5892.

¹⁵⁹ Maximum concentration tested was 33 μM .

¹⁶⁰ The major part of these compounds were synthesized by colleagues, but have been included here to complete the discussions related to the metabolic stability and the binding model.

¹⁶¹ Using the in-house developed program It's Electric, a ranking of fifteen selected 5-membered heterocycles was made by Jonas Boström, AstraZeneca R&D Mölndal. The ranking was used to guide prioritization of synthesis. It's Electric was based on OpenEye software, principally the ZAP toolkit. A more sophisticated implementation, EON, of this approach has been subsequently described by OpenEye. Information about EON (v.2.1.0) can be found at the web-page: <http://www.eyesopen.com/eon>

¹⁶² Kim, K. S.; Kimball, S. D.; Misra, R. N.; Rawlins, D. B.; Hunt, J. T.; Xiao, H.-Y.; Lu, S.; Qian, L.; Han, W.-C.; Shan, W.; Mitt, T.; Cai, Z.-W.; Poss, M. A.; Zhu, H.; Sack, J. S.; Tokarski, J. S.; Chang, C.; Y.; Pavletich, N.; Kamath, A.; Humphreys, W. G.; Marathe, P.; Bursuker, I.; Kristen O.; Kellar A.; Roongta, U.; Batorsky, R.; Mulheron, J. G.; Bol, D.; Fairchild, C. R.; Lee, F. Y.; Webster, K. R. Discovery of aminothiazole inhibitors of cyclin-dependent kinase 2: Synthesis, X-ray crystallographic analysis, and biological activities. *J. Med. Chem.* **2002**, *45*, 3905-3927.

¹⁶³ Dhanak, D.; Christmann, L. T.; Darcy, M. G.; Meenan, R. M.; Knight, S. D.; Lee, J.; Ridgers, L. H.; Sarau, H. M.; Shah, D. H.; White, J. R.; Zhang, L. Discovery of potent and selective phenylalanine derived CCR3 receptor antagonists. Part 2. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1445-1450.

¹⁶⁴ The metabolism in RLM and HLM respectively, was determined in assay with or without added NADPH. Since metabolism by CYP450 requires NADPH, this can be used to determine whether the metabolism is CYP-dependent.

¹⁶⁵ Deflorian, F.; Jacobsen, K. A. Comparison of three GPCR structural templates for modeling of the P2Y₁₂ nucleotide receptor. *J. Comput. Aided Mol. Des.* **2011**, *25*, 329-338.

¹⁶⁶ Calculations were made by Jonas Boström, AstraZeneca R&D Mölndal.

¹⁶⁷ (a) Kenny, P. W. Hydrogen bonding, electrostatic potential, and molecular design. *J. Chem. Inf. Model.* **2009**, *49*, 1234-1244; (b) Kenny, P. W. Prediction of hydrogen bond basicity from computed molecular electrostatic properties: Implications for comparative molecular field analysis. *J. Chem. Soc. Perkin. Trans. 2.* **1994**, 199-202; (c) Murray, J. S.; Ranganathan, S.; Politzer, P. Correlations between the solvent hydrogen bond acceptor parameter β and the calculated molecular electrostatic potential. *J. Org. Chem.* **1991**, *56*, 3734-3737.

¹⁶⁸ (a) Nobeli, I.; Price, S. L.; Lommerse, J. P. M.; Taylor, R. Hydrogen bonding properties of oxygen and nitrogen acceptors in aromatic heterocycles. *J. Comp. Chem.* **1997**, *18*, 2060-2074; (b) Böhm, H.-J. Oxygen and nitrogen in competitive situations: Which is the hydrogen-bond acceptor? *Chem. Eur. J.* **1996**, *2*, 1509-1513; (c) Laurence, C.; Brameld, K. A.; Graton, J.; Le Questel, J.-Y.; Renault, E. The pK_{BHX} database: Towards a better understanding of hydrogen-bond basicity for medicinal chemists. *J. Med. Chem.* **2009**, *52*, 4073-4086.

¹⁶⁹ Boström, J.; Hogner, A.; Schmitt, S. Do structurally similar molecules bind in a similar fashion? *J. Med. Chem.* **2006**, *49*, 6716-6725.

¹⁷⁰ Based on calculations on the model compounds ethyl 5-chloro nicotinate and 5-chloro-3-(5-methyl-1,3-oxazolyl)pyridine by Jonas Boström, AstraZeneca R&D Mölndal, using Jaguar program, version 7.8; release 109, Schrödinger, LLC: New York, NY, 2011. The relaxed coordinate scan was employed, using the basis set 6-31G** and the PBF (standard Poisson-Boltzmann Finite) solvent model.

¹⁷¹ As it is presented here, some other sulfonylureas would have been more obvious choices for in vivo PK determination, however the chronology of the project meant that not all of the most potent or most metabolically stable sulfonylureas in vitro were also tested in vivo.

¹⁷² See patent application: Andersen, S.; Bach, P.; Brickmann, K.; Giordanetto, F.; Zetterberg, F.; Österlund, K. New pyridine analogues. WO2007/008140 (A1). Filed 13 July 2005.

¹⁷³ Zou, B.; Yuan, Q.; Ma, D. Synthesis of 1,2-disubstituted benzimidazoles by a Cu-catalyzed cascade aryl amination/condensation process. *Angew. Chem. Int. Ed.* **2007**, *46*, 2598-2601.

¹⁷⁴ (a) Horn, U.; Mutterer, F.; Weis, C. D. 22. Halogenierte Pyridine III. Di- und trihalogenierte Pyridin-3-aldehyde. *Helv. Chim. Acta* **1976**, *59*, 211-221; (b) Leonard, K.; Marugan, J. J.; Raboisson, P.; Calvo, R.; Gushue, J. M.; Koblish, H. K.; Lattanze, J.; Zhao, S.; Cummings, M. D.; Player, M. R.; Maroney, A. C.; Lu, T. Novel 1,4-benzodiazepine-2,5-diones as Hdm2 antagonists with improved cellular activity. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3463-3468.

¹⁷⁵ (a) Mutterer, F.; Weis, C. D. 24. Halogenierte Pyridine V. Fluorierte und bromierte Pyridinverbindungen. *Helv. Chim. Acta* **1976**, *59*, 229-235; (b) Thomas, A. A.; Le Huerou, Y.; De Meese, J.; Gunawardana, I.; Kaplan, T.; Romoff, T. T.; Gonzales, S. S.; Condroski, K.; Boyd, S. A.; Ballard, J.; Bernat, B.; DeWolf, W.; Han, M.; Lee, P.; Lemieux, C.; Pedersen, R.; Pheneger, J.; Poch, G.; Smith, D.; Sullivan, F.; Weiler, S.; Wright, S. K.; Lin, J.; Brandhuber, B.; Vigers, G. Synthesis, in vitro and in vivo activity of thiamine antagonist transketolase inhibitors. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2206-2210.

- ¹⁷⁶ Yang, W.; Wang, Y.; Corte, J. R. Efficient synthesis of 2-aryl-6-chloronicotinamides via PXPd₂-catalyzed regioselective Suzuki coupling. *Org. Lett.* **2003**, *5*, 3131-3134.
- ¹⁷⁷ (a) Choi, Y. M.; Kucharczyk, N.; Sofia, R. D. A nine-step synthesis of [¹⁴C]flupirtine maleate labeled in the pyridine ring. *J. Labelled Compd. Radiopharm.* **1987**, *24*, 1-14; (b) Seydel, J. K.; Schaper, K.-J.; Coats, E. A.; Cordes, H. P.; Emig, P.; Engel, J.; Kutscher, B.; Polymeropoulos, E. E. Synthesis and quantitative structure-activity relationships of anticonvulsant 2,3,6-triaminopyridines. *J. Med. Chem.* **1994**, *37*, 3016-3022.
- ¹⁷⁸ Oguchi, M.; Wada, K.; Honma, H.; Tanaka, A.; Kaneko, T.; Sakakibara, S.; Ohsumi, J.; Serizawa, N.; Fujiwara, T.; Horikoshi, H.; Fujita, T. Molecular design, synthesis, and hypoglycemic activity of a series of thiazolidine-2,4-diones. *J. Med. Chem.* **2000**, *43*, 3052-3066.
- ¹⁷⁹ Schmid, S.; Schühle, D.; Steinberger, S.; Xin, Z.; Austel, V. Synthesis of 1,2,2a,3-tetrahydro-1,4,7b-triazacyclopenta[cd]indenes. *Synthesis* **2005**, *18*, 3107-3118.
- ¹⁸⁰ Jacobsen, E. J.; McCall, J. M.; Ayer, D. E.; VanDoornik, F. J.; Palmer, J. R.; Belonga, K. L.; Braugher, J. M.; Hall, E. D.; Houser, D. J.; Krook, M. A.; Runge, T. A. Novel 21-aminosteroids that inhibit iron-dependent lipid peroxidation and protect against central nervous system trauma. *J. Med. Chem.* **1990**, *33*, 1145-1151.
- ¹⁸¹ Mader, M.; de Dios, A.; Shih, C.; Bonjouklian, R.; Li, T.; White, W.; de Uralde, B. L.; Sánchez-Martínez, C.; del Prado, M.; Jaramillo, C.; de Diego, E.; Martín Cabrejas, L. M.; Domínguez, C.; Montero, C.; Shepherd, T.; Dally, R.; Toth, J. E.; Chatterjee, A.; Pleite, S.; Blanco-Urgoiti, J.; Perez, L.; Barberis, M.; Lorite, M. J.; Jambriña, E.; Nevill Jr, C. R.; Lee, P. A.; Schultz, R. C.; Wolos, J. A.; Li, L. C.; Campbell, R. M.; Anderson, B. D. Imidazolyl benzimidazoles and imidazo[4,5-*b*]pyridines as potent p38a MAP kinase inhibitors with excellent in vivo antiinflammatory properties. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 179-183.
- ¹⁸² Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y. Synthesis of fluorinated pyridines by the Balz-Schiemann reaction. An alternative route to enoxacin, a new antibacterial pyridonecarboxylic acid [1]. *J. Heterocyclic Chem.* **1984**, *21*, 673-679.
- ¹⁸³ Schmid, S.; Röttgen, M.; Thewalt, U.; Austel, V. Synthesis and conformational properties of 2,6-bis-anilino-3-nitropyridines. *Org. Biomol. Chem.*, **2005**, *3*, 3408-3421.
- ¹⁸⁴ Hirokawa, Y.; Horikawa, T.; Kato, S. An efficient synthesis of 5-bromo-2-methoxy-6-methylaminopyridine-3-carboxylic acid. *Chem. Pharm. Bull.* **2000**, *48*, 1847-1853.
- ¹⁸⁵ Horikawa, T.; Hirokawa, Y.; Kato, S. A practical preparation of methyl 2-methoxy-6-methylaminopyridine-3-carboxylate from 2,6-dichloro-3-trifluoromethylpyridine. *Chem. Pharm. Bull.* **2001**, *49*, 1621-1627.
- ¹⁸⁶ Shi, Y.-J.; Humphrey, G.; Maligres, P. E.; Reamer, R. A.; Williams, J. M. Highly regioselective DABCO-catalyzed nucleophilic aromatic substitution (S_NAr) reaction of methyl 2,6-dichloronicotinate with phenols. *Adv. Synth. Catal.* **2006**, *348*, 309-312.
- ¹⁸⁷ (a) Dropinski, J. F.; Akiyama, T.; Habulihaz, B.; Doebber, T.; Berger, J. P.; Meinke, P. T.; Shi, G. Q. Synthesis and biological activities of novel aryl indole-2-carboxylic acid analogs as PPAR γ partial agonists. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5035-5038; (b) Engstrom, K. M.; Henry, R. F.; Marsden, I. Synthesis of the glucuronide metabolite of ABT-751. *Tetrahedron Lett.* **2007**, *48*, 1359-1362; (c) Ilig, C. R.; Chen, J.; Wall, M. J.; Wilson, K. J.; Ballentine, S. K.; Rudolph, M. J.; DesJarlais, R. L.; Chen, Y.; Schubert, C.; Petrounia, I.; Crysler, C. S.; Molloy, C. J.; Chaikin, M. A.; Manthey, C. L.; Player, M. R.; Tomczuk, B. E.; Meegalla, S. K. Discovery of novel FMS kinase inhibitors as anti-inflammatory agents. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1642-1648.
- ¹⁸⁸ Values from: a) Hansch, C.; Leo, A.; Hoekman, D. *Exploring QSAR, Vol 2: Hydrophobic, electronic, and steric constants*. American Chemical Society, **1995**; Values for *N*-morpholino from: b) Seydel, J. K.; Schaper, K.-J. *Chemische Struktur und biologische Aktivität von Wirkstoffen. Methoden der Quantitativen Struktur-Wirkung-Analyse*. Verlag Chemie, **1979**; MR-values for the 3-substituents of compounds **105** and **117** were calculated by the incremental procedure in c) Wildman, S. A.; Crippen, G. M. Prediction of physicochemical parameters by atomic contributions. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 868-873.
- ¹⁸⁹ Miyazawa, S.; Harada, H.; Fujisaki, H.; Kubota, A.; Kodama, K.; Nagakawa, J.; Watanabe, N.; Oketani, K. Imidazopyridine compounds. WO 2005/103049 (A1).
- ¹⁹⁰ Nishizawa, M.; Iyenaga, T.; Kurisaki, T.; Yamamoto, H.; Sharfuddin, M.; Namba, K.; Imagawa, H.; Shizuri, Y.; Matsuo, Y. Total synthesis and morphogenesis-inducing activity of (\pm)-thallusin and its analogues. *Tetrahedron Lett.* **2007**, *48*, 4229-4233.
- ¹⁹¹ Wolfe, J. P.; Buchwald, S. Scope and limitations of the Pd/BINAP-catalyzed amination of aryl bromides. *J. Org. Chem.* **2000**, *65*, 1144-1157.
- ¹⁹² Mutterer, F.; Weis, C. D. Halogenierte Pyridine IV. Kernchlorierte Pyridin-3-carbonsäuren. *Helv. Chim. Acta* **1976**, *59*, 222-229.

- ¹⁹³ Hirokawa, Y.; Horikawa, T.; Kato, S. An efficient synthesis of 5-bromo-2-methoxy-6-methylaminopyridine-3-carboxylic acid. *Chem. Pharm. Bull.* **2000**, *48*, 1847-1853.
- ¹⁹⁴ Voisin, A. S.; Bouillon, A.; Lancelot, J.-C.; Rault, S. Efficient synthesis of halohydroxypyridines by hydroxydeboronation. *Tetrahedron* **2005**, *61*, 1417-1421.
- ¹⁹⁵ Radinov, R.; Chanev, K.; Khaimova, M. Lithiation of polychloropyrimidines and dichloropyridines. *J. Org. Chem.* **1991**, *56*, 4793-4796.
- ¹⁹⁶ Todeschini, R.; Consonni, V. *Molecular descriptors for chemoinformatics*. WILEY-VCH, Weinheim, Germany, **2009**.
- ¹⁹⁷ Verloop, A. *The sterimol approach to drug design*. Marcel Dekker, New York, USA, **1987**.
- ¹⁹⁸ In fact, 23 solvents were used, however the reactions in EtOH and *i*-PrOH gave mixtures of transesterification products and were thus excluded from the list. The outlier result with TEA as solvent may be explained by precipitation, which was observed in this solvent only.
- ¹⁹⁹ Values of n , ϵ , and μ are from (a) Abboud, J.-L. M.; Notario, R. Critical compilation of scales of solvent parameters. Part I. Pure, non-hydrogen bond donor solvents. *Pure Appl. Chem.* **1999**, *71*(4), 645-718; values of π^* , α , β , ET(30) and DN are from: (b) Marcus, Y. The properties of organic liquids that are relevant to their use as solvating solvents. *Chem. Soc. Rev.* **1993**, *22*, 409-416. Values from ref. (a) and (b) are completed by values of π^* , α , and β for *N*-methylimidazole from c) Ref. 196, by values of the refractive index n for *N*-methyl formamide and MeOH from (d) *Knovel Critical Tables*. 2nd Ed., Knovel, **2008**; and by values of ϵ and μ for *N*-methyl formamide and MeOH from (e) Speight, J. G. *Lange's Handbook of Chemistry*, 16th Ed., McGraw-Hill, **2005**.
- ²⁰⁰ R_{sel} showed less or no significant single-variable correlations with the solvatochromic α parameter ($R^2 = 0.0003$), the refractive index n ($R^2 = 0.03$), the relative static permittivity ϵ ($R^2 = 0.13$), the molecular dipolar momentum μ ($R^2 = 0.25$), the dipolarity/polarizability expressed by π^* ($R^2 = 0.02$), Dimroth's and Reichard's ET(30) ($R^2 = 0.07$), or with the derived functions $f(n) = (n^2-1)/(n^2+1)$, and $g(\epsilon) = (\epsilon^2-1)/(\epsilon^2+1)$.
- ²⁰¹ Kamlet, M. J.; Abboud, J.-L. M.; Abraham, M. H.; Taft, R. W. Linear solvation energy relationships. 23. A comprehensive collection of the solvatochromic parameters, π^* , α , and β , and some methods for simplifying the generalized solvatochromic equation. *J. Org. Chem.* **1983**, *48*, 2877-2887.
- ²⁰² It would have been interesting to test the reaction in HMPA ($\beta = 1.05$), or in a DMSO/HMPA mixture, however company safety regulations prohibited the use of HMPA.
- ²⁰³ See ref. 172 (Example 113 in the patent application).
- ²⁰⁴ See ref. 172 (Example 31 in the patent application).