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Synthesis of polysubstituted pyrimidines with potential anti-inflammatory properties

Syntéza polysubstituovaných pyrimidinů s potenciálními protizánětlivými vlastnostmi

Diploma thesis

Supervisor: Ing. Zlatko Janeba, CSc.

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze dne 9. 5. 2017 .....

Podpis

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Abstract

This thesis is engaged in the synthesis of polysubstituted pyrimidines with anti-inflammatory properties. Such molecules can inhibit production of prostaglandin E_2 (PGE_2). The aim of this study was to enhance water-solubility and anti-inflammatory efficacy of such derivatives via structural modifications of the lead scaffold. Among applied synthetic tools, the Suzuki-Miyaura cross-coupling was the prevalent reaction, however, many other synthetic procedures (Heck reaction, condensation, borylation, ozonolysis, nucleophilic substitution, etc.) were utilized as well. Overall, 43 final products were prepared. The anti-inflammatory efficacy (inhibition of PGE_2 production) was successfully increased as the most potent compound achieved three orders of magnitude higher activity compared to the current lead structure WQE-134. Furthermore, no general influence of the length of the substituent in the C5 position of pyrimidine (C5pyr) on the anti-inflammatory efficacy of synthesized compounds was observed. Significant bioavailability obstacle in future development of the current lead WQE-134 is its poor solubility which was successfully enhanced by introduction of heteroatom bearing moieties to C5pyr. The most water-soluble compound achieved two orders of magnitude higher solubility than WQE-134 while biological activity decreased only slightly. Finally, WQE-134 biotinylated in C5pyr via pegylated linker was synthesized and will be used in pulldown experiments in order to clarify the mechanism of action of studied analogues.

Keywords: polysubstituted pyrimidines, anti-inflammatory properties, prostaglandin E_2, structure-activity relationship study, cross-coupling reactions, solubility

Abstrakt

Tato práce se zabývá syntézou polysubstituovaných pyrimidinů s potenciálními protizánětlivými účinky. Tyto látky jsou schopny inhibovat produkci prostaglandinu E_2 (PGE_2). Cílem této práce bylo zvýšení rozpustnosti a protizánětlivé účinnosti připravených derivátů pomocí strukturních modifikací vedoucí struktury WQE-134. Pro přípravu látek byl nejvíce využíván Suzuki-Miyaura coupling, nicméně i řada jiných postupů byla aplikována (Heckova reakce, kondenzace, borylace, ozonolýza, nukleofilní substituce, atd.). Celkem bylo připraveno 43 finálních látek. Protizánětlivá účinnost (inhibice produkce PGE_2) byla úspěšně zvýšena u mnoha derivátů. Nejúčinnější látka dosáhla o tři řády vyšší aktivity než vedoucí struktura. Nebyl pozorován žádný obecný vliv délky substituentu v poloze C5 pyrimidinu (C5pyr) na protizánětlivou aktivitu látek. Významnou překážkou v dalším vývoji WQE-134 je nízká rozpustnost této látky a s tím spojená nízká biologická dostupnost. Rozpustnost látek byla úspěšně zvýšena pomocí zavedení skupin nesoucí heteroatom do C5pyr. Nejvíce rozpustná látka dosáhla o dva řády vyšší rozpustnosti než WQE-134 zatímco se biologická aktivita snížila pouze nepatrně. Dále byla syntetizována WQE-134 biotinylovaná v poloze C5pyr přes pegylovaný řetězec, která bude použita v tzv. pulldown experimentech za účelem objasnění mechanismu účinku studovaných derivátů.

Klíčová slova: polysubstituované pyrimidiny, protizánětlivé účinky, prostaglandin E_2, studie vztahu mezi strukturou a aktivitou, cross-couplingové reakce, rozpustnost

List of abbreviations

9-BBN	9-borabicyclo[3.3.1]nonane
Ac	acetyl
AIBN	2,2′-azobis(2-methylpropionitrile)
AP-1	activator protein 1
Ar	aryl
ARA	arachidonic acid
CAN	cerium(IV) ammonium nitrate
COSY	correlated spectroscopy
COX	cyclooxygenase
Cpyr	C position of pyrimidine
CYP450	cytochrome P450
DCM	dichloromethane
DDQ	4,5-dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DPPP	1,3-bis(diphenylphosphino)propane
DSS	dextran sodium sulfate
EDG	electron-donating group
EI	electron ionization
ESI	electrospray ionization
Et	ethyl
EWG	electron-withdrawing group
FA	formic acid
FDA	U.S. Food and Drug Administration
GR	glucocorticoid receptor
GRE	glucocorticoid response elements
HAT	histone acetyltransferase
HDAC	histone deacetylase
HMBC	heteronuclear multiple bond correlation
HPLC	high-pressure liquid chromatography
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single quantum correlation
IC_{50}	half maximal inhibitory concentration

IL	interleukin
INF-γ	interferon γ
i-Pr	isopropyl
IR	infrared
ISP	increased solubility purposes
LOX	lipoxygenase
LPS	lipopolysaccharide
Me	methyl
mPGES-1	prostaglandin E_2 microsomal synthase-1
MS	mass spectrometry
MW	microwave
NBS	*N*-bromosuccinimide
NF-κB	nuclear factor κB
NMR	nuclear magnetic resonance
NSAIDs	nonsteroidal anti-inflammatory drugs
PDA	photodiode array detector
PEG	poly(ethylene glycol)
PG	prostaglandin
Ph	phenyl
PLA_2	phospholipase A_2
PMB	p-methoxybenzyl
Pr	propyl
PRRs	pattern-recognition receptors
R_f	retention factor
RNA	ribonucleic acid
SAR	structure-activity relationship
SEM	standard error of the mean
SMC	Suzuki-Miyaura coupling
t-Bu	tert-butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TNF	tumor necrosis factor
TXA_2	tromboxane A_2
UPLC	ultra-high pressure liquid chromatography
WQE-134	lead structure

Contents

1. Introduction

About one half of currently registered compounds contain a heterocycle.[1] Very important and abundant class of heterocycles is represented by pyrimidines – six-membered aromatic heterocycles containing two nitrogen atoms in the positions 1 and 3 (Figure 1).

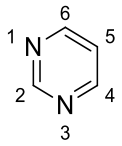


Figure 1. Pyrimidine

This structural motif of unfused pyrimidine is widely present in biological systems mainly in a glycosylated form as the essential building blocks of nucleic acids – uridine, 2´-deoxythymidine, and (2´-deoxy)cytidine but also in non-glycosylated form as vitamin B_1, non-proteogenic amino acids, toxins, and antibiotics.[2]

In addition, the pyrimidine moiety is incorporated in numerous compounds exhibiting a wide array of biological properties such as anticancer,[3–6] antiviral,[7,8] sedative,[9] anti-inflammatory,[10–12] and antiallergic.[13]

All these aspects contribute to the extensive development of chemistry around pyrimidine. Even though there are well established procedures for synthesis of pyrimidine derivatives,[1,14] new approaches are being developed as well.[15]

The reactivity of the pyrimidine core is influenced by the two nitrogen atoms which are causing electron deficiency of the ring. Thus, in general, pyrimidine ring is prone to a nucleophilic substitution and to facilitate electrophilic substitution, an activation by electron-donating group (EDG) is needed.[14,16]

The anti-inflammatory properties of pyrimidine derivatives, as well as inflammation itself, are under thorough investigation.[10–12,17–24] Inflammation is a

complex machinery that ensures the survival throughout an infection or tissue damage, however, when chronic inflammation is triggered, the major damage is done by the inflammatory condition itself.[21] Additionally, chronic inflammation was proved to be closely related to cancer,[25,26] cardiovascular,[27] metabolic,[28,29] and autoimmune diseases.[30]

Thus, an effective therapy for these chronic inflammatory conditions is needed. However, current methods for treatment of inflammation have drawbacks. Glucocorticoids are potent anti-inflammatory agents but carry a wide array of adverse effects related to endocrine, cardiovascular, musculoskeletal, and central nervous system.[31] Nonsteroidal anti-inflammatory drugs (NSAIDs) are safer but also this class of anti-inflammatory drugs possesses several adverse effects connected to cardiovascular, excretory, and gastrointestinal systems.[32,33]

Hence, the development of new molecules with anti-inflammatory properties operating through a unique mechanism of action is highly desirable.

All Figures, Schemes, and Tables presented in this thesis were prepared by me and were not copied from any literature sources.

2. Current state of knowledge

2.1. Inflammation

Inflammation is fundamentally a protective strategy in higher organisms provided by the immune system to ensure the survival throughout an infection or tissue damage.[23,34,35] However, there is a difference between acute and chronic inflammation.

The acute inflammation is the essential response provided by the immune system and as such enables the removal of the noxious stimuli.[21] The acute inflammation is thought to be a part of the innate immunity which is not completely non-specific as originally considered since it is capable to distinguish between pathogens via pattern-recognition receptors (PRRs) triggering specific signaling pathways.[23,36] Nevertheless, the processes connected to inflammatory response work in expense of normal cellular functions, thus, the longer the inflammation persists, the more damaging consequences and so it is highly desirable for the inflammation to last as brief as possible.[21]

When the abolishment of the inflammatory processes fails for any reason despite the elimination of the stimuli, the acute inflammation turns into chronic and the major damage is done by the inflammatory conditions themselves.[21] Moreover, the chronic inflammation is associated with many health disorders such as cardiovascular diseases,[27] metabolic diseases,[28,29] cancer,[25,26] and autoimmune diseases.[30] Unfortunately, for the vast majority of chronic inflammatory conditions, no specific inducer is identified, therefore, the mechanism is not fully understood yet.[21]

2.1.1. General molecular mechanism

The general molecular mechanism (Figure 2) is triggered by stimuli recognition through the pattern-recognition receptors (PRRs), these are transmembrane and cytoplasmic receptors expressed in cells of both innate and acquired immune systems.[21,23,34] The signal is subsequently transmitted into the cell nucleus, where the

proinflammatory genes are upregulated via specific transcriptional factors (e.g. nuclear factor κB (NF-κB) or activator protein 1 (AP-1)).[21,23,34]

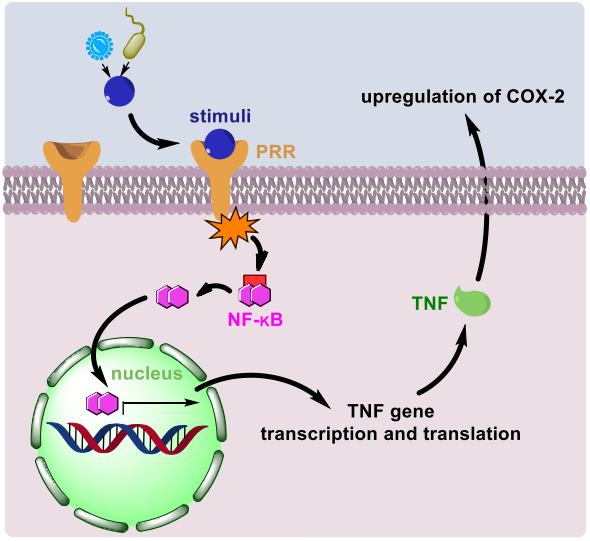


Figure 2. A simplified scheme of general molecular mechanism of inflammation[21]

This process is influenced by epigenetic factors including histone acetylation or DNA methylation. Epigenetics can be interpreted as non-DNA sequence directed variability of gene expression/phenotype and as such provides regulation of the gene transcription. For example, the histone acetylation is mediated by histone acetyltransferase (HAT) resulting in less basic histone core, lower affinity of the histone to the DNA, therefore, more relaxed chromatin which induces the transcription of inflammatory genes whereas the inhibition of the transcription is mediated by histone deacetylase (HDAC).[37,38]

After the transcription, and subsequent translation, the inflammatory cytokines (e.g. tumor necrosis factor (TNF) or interleukin 6 (IL-6)) are formed.[21,34] These pleiotropic proteins modify the vascular permeability and recruitment of blood cells into the inflamed tissue resulting in known macroscopic symptoms – redness, swelling, heat, and pain.[34] More importantly, they enhance the activity of the three inflammatory pathways (cyclooxygenase (COX), lipoxygenase (LOX), cytochrome P450 (CYP450)) which mediate production of inflammatory cytokines.[39] Only the COX pathway

(Figure 3) will be discussed further due to the fact, that this thesis deals with inhibitors of PGE_2 synthesis which is a product of the COX pathway. The issue of the arachidonic cascade including LOX and CYP450 pathway is extensively described by Hwang and co-workers.[39]

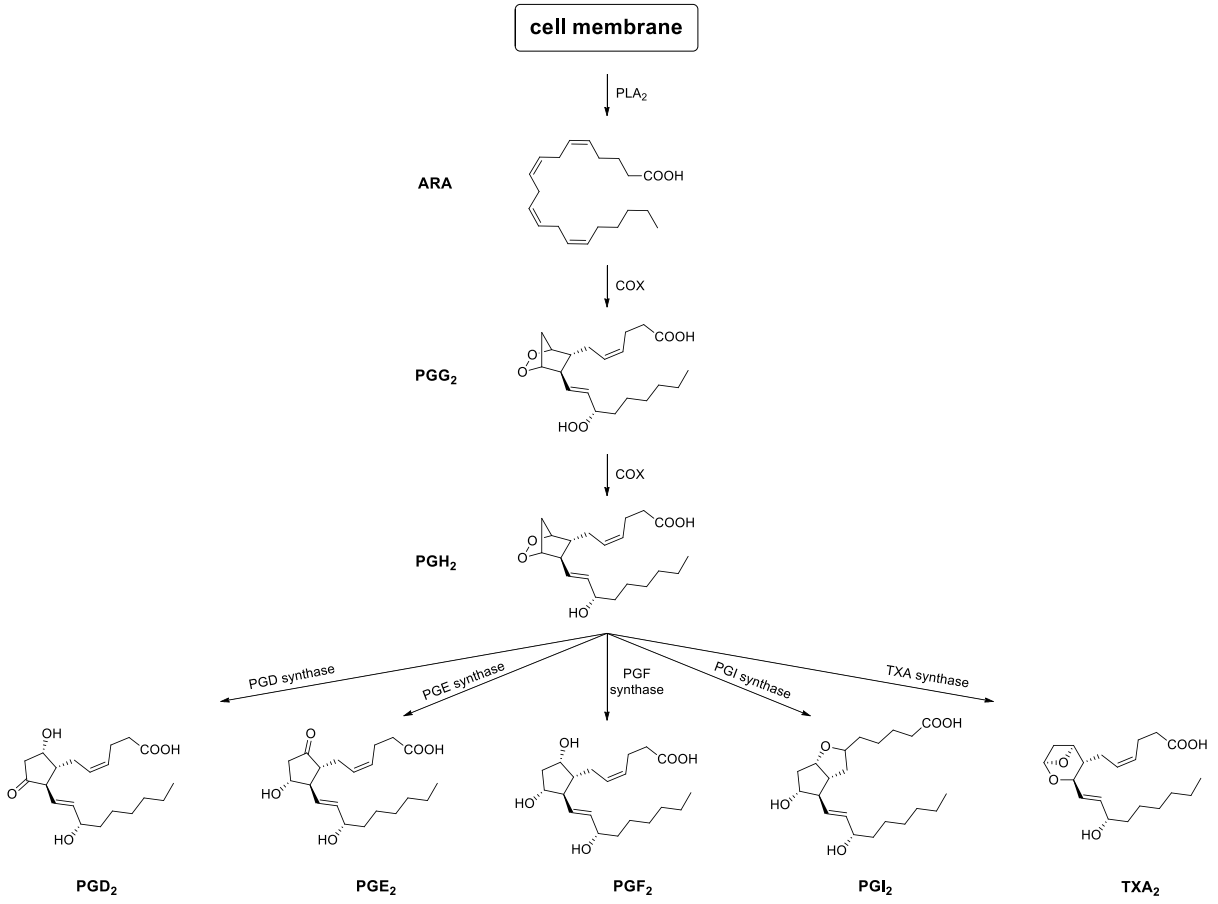


Figure 3. COX pathway[39]

The cyclooxygenase is formed by two isoforms – constitutive COX-1 and inducible COX-2. While COX-1 is involved in the maintenance of homeostatic balance, the COX-2 is implied under pathophysiological conditions and upregulated via inflammatory cytokines.[40–42] The COX enzymes transform the arachidonic acid (ARA) into biologically active prostanoids which act as local signaling agents.[43] The ARA is the most abundant unsaturated fatty acid in the membrane of cells involved in inflammatory processes and is released from the membrane in response to various

stimulants by phospholipase A_2 (PLA_2).[39,43] After displacement of the ARA from the phospholipid bilayer, the COX enzymes transform the ARA into unstable prostaglandin G_2 (PGG_2) and subsequently into more stable prostaglandin H_2 (PGH_2) which is finally converted to active prostaglandins (e.g. prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), prostaglandin I_2 (PGI_2), and thromboxane A_2 (TXA_2)) by tissue- or cell-specific synthases.[39,40,43] These bioactive prostanoids are released into the extracellular space where they bind to membrane prostanoid G-coupled receptors on target cells.[43]

2.1.2. Currently used anti-inflammatory drugs

The inflammation is quite complex process. The complexity can be nicely demonstrated on currently used anti-inflammatory drugs which are extremely diverse involving glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs) – aniline derivatives, salicylic acid derivatives, pyrazolone derivatives, coxibs, oxicams, arylalkanoic acids, etc.[9]

Glucocorticoids act via several mechanisms of action. After the glucocorticoids enter the cell, they bind the cytoplasmic glucocorticoid receptor (GR), this receptor is essential for life as shown by Cole and co-workers on GR-deficient mice.[44] After the binding, the GR dimerizes and translocates to the nucleus where it binds the DNA, specifically, the glucocorticoid response elements (GRE) and these trigger the transcription of anti-inflammatory genes.[45] Nevertheless, the main effect (inhibition of inflammatory genes transcription) is done due to the direct inhibitory interaction of inflammatory transcription factors (AP-1, NF-κB).[42,45,46] Moreover, the glucocorticoids influence the transcription through the epigenetics as well, specifically, via modifying the chromatin structure.[45,46]

The mechanism of action of the NSAIDs is generally via inhibition of the cyclooxygenase pathway preventing the formation of inflammatory prostanoids, either as non-selective, preferential, or selective COX inhibitors.[9,41] Especially selective COX-2 inhibitors were desirable since the COX-1 is involved in homeostasis maintenance including gastric, renal and cardiovascular protection.[32] However, an

increased risk of myocardial infarction and stroke was observed in case of selective COX-2 inhibitors.[33] This may be caused by the imbalance of PGI_2 and TXA_2. TXA_2 promotes vasoconstriction and platelet aggregation, whereas PGI_2 inhibits platelet aggregation and promotes vasodilatation.[47] It was observed *in vitro* on human endothelial cells that COX-1 produced mainly TXA_2 whereas the induction of COX-2 led to overproduction of PGE_2 and PGI_2 but not TXA_2.[47] Thus, selective COX-2 inhibitor causes the PGI_2/TXA_2 imbalance making the TXA_2 dominant, therefore, raising the risk of thrombosis.

The PGE_2 microsomal synthase-1 (mPGES-1) is recently drawing attention as a potential target for treatment of inflammation since it is primarily connected to COX-2 and downstream the COX, therefore, could avoid side effects provoked by inhibition of COX (cardiovascular diseases or gastric obstruction) while maintaining similar effect as other NSAIDs.[35,39]

Quite recently, the so-called biologicals have expanded dramatically among others to the field of inflammation treatment as anticytokines (inhibiting cytokines or their receptors).[42] For example, Kumar and co-workers successfully demonstrated anti-inflammatory effects of anticytokines (anti–IL-5, anti–IL-13, and anti–INF-γ) on mice chronic allergic asthma model, even though, the results do not provide a universal agent for treatment of the asthma.[48]

2.2. Biotin-streptavidin pulldown assay

Pulldown assay is widely used for studying interactions of the protein of interest with different protein, with a small molecule, or even with nucleic acid.[49–52] Streptavidin is a protein isolated from bacterium *Streptomyces avidinii* which strongly binds biotin.[52] The biotin-streptavidin (eventually avidin or neutravidin) interaction is widely used in biochemical assays due to the extraordinary specificity and strength of the interaction, ability of a biotinylated molecule to bind the target and commercial availability of both streptavidin and biotinylating reagents.[52,53] Although biotin-streptavidin pulldown

assays are used in nucleic acid hybridization[54], immunological assays[55], and other biochemical assays[56], for medicinal chemist the most valuable application is the identification of the cellular target for druggable molecules.

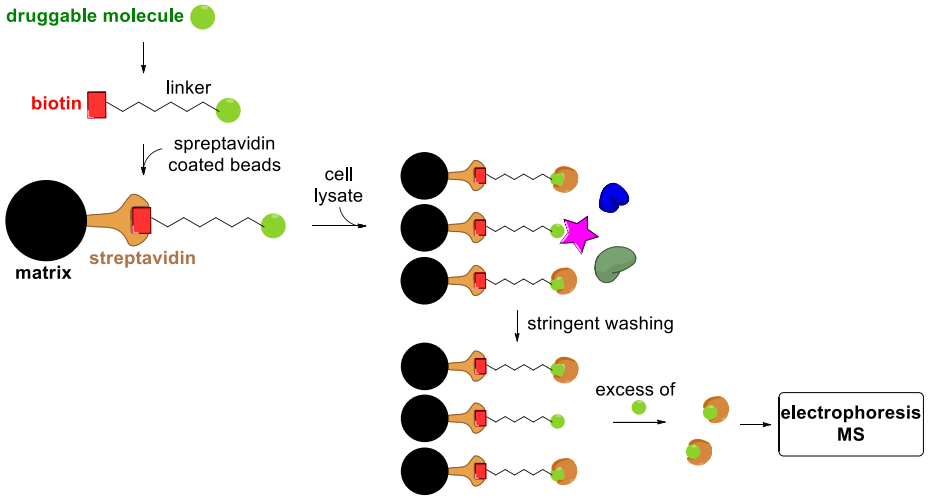


Figure 4. Standard procedure for pulldown assay[52]

The standard protocol (Figure 4) consists of attachment of biotin to the studied molecule via preferentially hydrophilic linker, immobilization of the biotinylated molecule (probe) by streptavidin coated beads, incubation of the probe with a cell lysate and finally by washing non-specifically bound proteins out.[52,57] The target protein is subsequently released e.g. by exposure to excess of the bioactive molecule and analysed using typically electrophoresis and mass spectrometry.[52,57]

2.3. Suzuki-Miyaura coupling reaction

Since the original manuscript published by Miyaura, Yamada, and Suzuki in 1979 describing the reaction of alkenyl halides with alkenylboranes in the presence of tetrakis(triphenylphosphine)palladium(0) and sodium hydroxide/ethoxide,[58] the Suzuki-Miyaura coupling (SMC) reaction has become one of the most valuable tools for C-C bond formation in organic synthesis. The main benefits include stability and general availability of wide range of boronic acids, mild reaction conditions, tolerance to various

functional groups, uncomplicated separation of the product and in general above-average yields. Additionally, the scope of the SMC reaction has been extended significantly and it is even feasible to couple highly sterically hindered substrates (using e.g. XPhos ligand, Figure 5a).[59] Certain limitation of the SMC reaction was (until the turn of the millennium) the inability to perform C-C bond formation with inactivated organic chlorides. This drawback was quite critical as organic chlorides represent cheap and abundant class among halides. The key factor in this matter is the dissociation energy of the C-halogen bond (Ph-Cl=96 kcal·mol^{-1}, compared to Ph-Br=81 kcal·mol^{-1}, Ph-I=65 kcal·mol^{-1}).[60] Ultimately, this obstacle was circumvented via the usage of suitable ligands (e.g. JohnPhos, Figure 5b).[60]

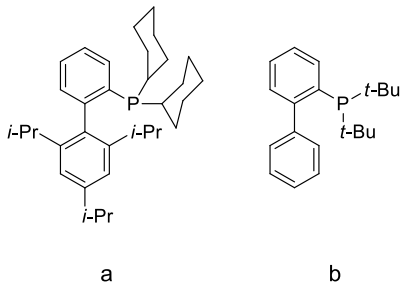


Figure 5. a) XPhos, b) JohnPhos

2.3.1. Mechanism

Even though transition-metal catalyzed reactions are not fully understood yet, the generally accepted mechanism consists of (1) oxidative addition, (2) exchange of the anion, (3) transmetallation and (4) reductive elimination (Figure 6). Oxidative addition along with reductive elimination can be influenced through a modification of the catalyst. The oxidative addition can be accelerated by increasing the electron density on the central metal atom which can be achieved when utilizing good σ-donors as ligands. On the contrary, good π-acceptors can be used to decrease the electron density on the central metal atom, therefore, accelerating the reductive elimination. The exchange of the anion and transmetallation can be affected via the selection of the base. Although Na_2CO_3 is used most commonly,[61] it is ineligible for less reactive systems. In such cases, for example, Cs_2CO_3 can be applied as caesium forms less

stable ion pair resulting in increased level of the dissociated anion, hence, accelerating the exchange of the anion and transmetallation due to quarternisation of the boron atom.[62,63]

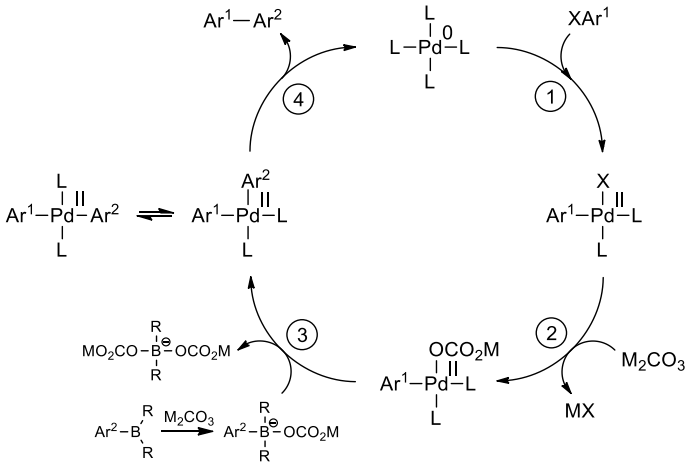


Figure 6. General molecular mechanism of Suzuki-Miyaura coupling reaction

2.3.2. Preparation of boronic reagents

The SMC reaction employes mostly organoboranes, boronic acid esters, boronic acids, and trifluoroborates. The organoboranes are frequently based on 9-borabicyclo[3.3.1]nonane (9-BBN) (Figure 7a) and obtainable via hydroboration.[64,65] Another coupling reagent, boronic acid esters (Figure 7b) are affordable either by direct hydroboration[66] or by palladium catalyzed borylation.[61,65,67] However, boronic acid esters have to be hydrolyzed (*in situ* affording boronic acid) in order to react in the SMC reaction. Boronic acids (Figure 7c) can be also obtained by the reaction of an organolithium or Grignard reagent with trialkyl borates.[61,68] Boronic acids can form cyclic trimeric anhydride – boroxine (Figure 7d), which may influence the reaction stoichiometry. This issue can be bypassed by the use of another class of nucleophilic agents, trifluoroborates, which are available through the reaction of boronic acid ester or trialkyl borates with aqueous solution of potassium bifluoride.[69–71] The trifluoroborates (Figure 7e) are suitable for multistep synthesis purposes as they act as

protected boronic acid as the quarternisation causes the loss of Lewis acid character.[65,72]

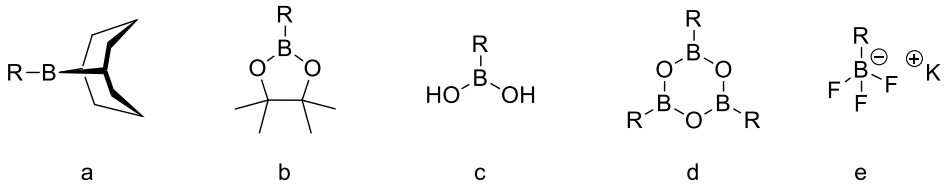


Figure 7. Generally used boronic reagents in Suzuki-Miyaura coupling reaction: a) trialkyl borate, b) boronic acid pinacol ester, c) boronic acid, d) boroxine, e) (potassium) trifluoroborate[65]

2.4. Pyrimidine

Pyrimidines are six-membered aromatic heterocycles classified as diazines with nitrogen atoms in positions 1 and 3. They represent very important and abundant class of heterocycles for the unfused pyrimidine scaffold is present in biological systems in both glycosylated and non-glycosylated forms as toxins, non-proteogenic amino acids, antibiotics, vitamin B_1, and, most importantly, nucleosides (Figure 8).[2] In addition, pyrimidine core containing molecules exhibit diverse biological activities such as antiviral,[7,8] sedative,[9] anti-inflammatory,[10–12] and antiallergic.[13] Furthermore, molecules bearing pyrimidine ring inhibit protein kinases which is used in therapy of cancer.[3–6] The various biological activities of pyrimidine are responsible for wide utilization of this moiety in drug design.

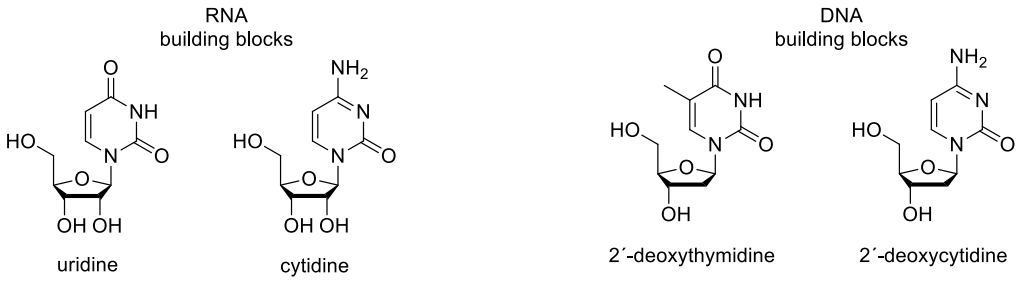
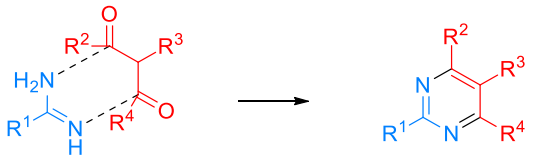


Figure 8. Unfused pyrimidine nucleosides

2.4.1. Synthesis of pyrimidine derivatives

Standardly, the N-C-N and C-C-C fragments are utilized to synthesize the pyrimidine core (Scheme 1). For example, guanidine, urea, thiourea or amidine undergoes the condensation with 1,3-diketone resulting in the formation of 2-amino,[73] 2-hydroxy,[74] 2-thio,[75] or 2-substituted[76] pyrimidine derivatives, respectively, (4,5,6-substituents depending on the diketone used). Also, esters of malonic acid can be used as substrates for the condensation in order to form corresponding 4,6-dihydroxy derivatives.[10,77]



Scheme 1. N-C-N and C-C-C building blocks for synthesis of pyrimidine derivatives

A less frequent pair of building blocks are C-N and C-C-C-N usually utilizing ethyl acetimidate.[14,78] C-N and C-C-C-N building blocks are more important in biochemistry, where they are employed in the *de novo* biosynthesis pathway of pyrimidine nucleotides incorporating aspartate and carbamoyl phosphate ultimately resulting in orotate.[79] Further, the N-C-C-C-N fragment can react with carboxylic esters or amides closing the pyrimidine ring as well.[1] The pyrimidine core is also available through the aza-Wittig reaction of α,β-unsaturated aldehyde with N-C-N-PPh_3 fragment.[1,80,81] Recently, Deibl and co-workers reported a novel Ir-catalyzed procedure for pyrimidine synthesis using up to three different alcohols with amidine or guanidin.[15]

2.4.2. Reactivity of the pyrimidine ring

Similarly to other six-membered aromatic nitrogen heterocycles, pyrimidine is electron deficient which favours a nucleophilic substitution over an electrophilic. Compared to pyridine, the additional one nitrogen atom causes a significant decrease of basicity (pK_a 1.3 compared to 5.5 at pyridine).[82]

When the unsubstituted pyrimidine is considered, the reactivity is different for the positions right next to the nitrogen atom (C2pyr, C4pyr/C6pyr) and for the position C5 of pyrimidine (C5pyr) due to different electron density which is demonstrated by the experimental ^{13}C NMR data (Table 1).[1] While C2pyr and C4pyr/C6pyr are significantly electron deficient, thus, prone to the attack of a nucleophile,[16] C5pyr is the most electron rich, however, still slightly electron deficient.[14] Nevertheless, electrophilic substitution does not occur on the unsubstituted pyrimidine. Electron-donating substituents are needed to activate C5pyr towards the electrophilic substitution. On the other hand, nucleophilic substitution can be performed on the parent pyrimidine.[83] Nevertheless, nucleophilic substitution of functionalized pyrimidines is much more frequent. For example, halogens can be readily replaced (faster at C4pyr/C6pyr than at C2pyr) for amino or thio group.[1]

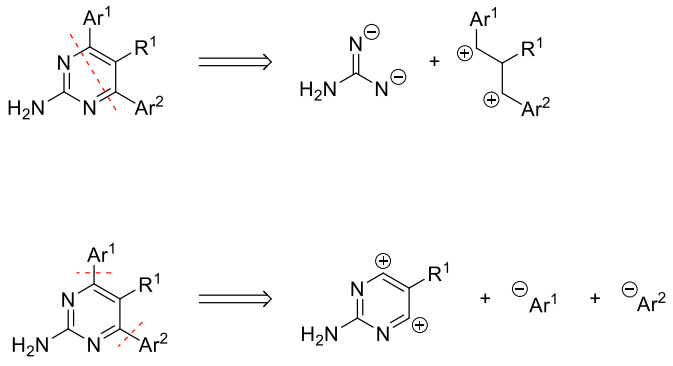
Table 1. ^{13}C NMR shifts of pyrimidine ($CDCl_3$)[1]

C2pyr	158.5 ppm
C4pyr/C6pyr	156.9 ppm
C5pyr	121.9 ppm

2.4.3. Synthesis of 2-amino-4,6-diarylpyrimidines

Further will be discussed the synthesis of 2-amino-4,6-diarylpyrimidines since this thesis is focused on such derivatives and their anti-inflammatory properties. The

retrosynthetic approach offers two main options – condensation and cross-coupling reaction (Scheme 2).



Scheme 2. Retrosynthetic approach towards 2-amino-4,6-diarylpyrimdines

One method for the synthesis of 2-amino-4,6-diarylpyrimidines is a condensation of arylaldehyde, 3-aryl-3-oxo-propanenitrile and guanidine resulting in the formation of 2-amino-4,6-diaryl-5-cyanopyrimidine. Additionally, substitution on the pyrimidine aryls can be controlled by the substitution of arylaldehyde and aryl-3-oxo-propanenitrile.[84] Furthermore, the condensation of α,β-unsaturated ketone with guanidine is possible for the preparation of polysubstituted pyrimidines, where the β-carbon and ketone substituents form the substituents in C4pyr and C6pyr and the α-carbon substituent forms the moiety in C5pyr.[85,86] The α,β-unsaturated ketone can be readily prepared in basic conditions from aryl methyl ketone and arylaldehyde.[87,88]

Besides the condensation, a convenient method for preparation of 2-amino-4,6-diarylpyrimidines is the Suzuki-Miyaura coupling (SMC) reaction using the 4,6-dichloropyrimidine derivatives as starting compounds, where the degree of arylation can be controlled via the selection of reaction conditions.[10,12,89] 4,6-Dichloropyrimidines can be generally prepared from commercially available or easily accessible 4,6-dihydroxypyrimidine precursors under several conditions. Commonly, phosphorous(V) oxychloride is used as the chlorinating reagent, either alone,[90–92] but more often, the protocol is modified. For example by the addition of an ammonium salt (applied mostly in the industry),[93–95] the addition of DMF (leading to the *in situ* formation of

(chloromethylene)dimethyliminium reagent)[96,97] or combination with phosphorous(V) pentachloride.[98] The protocol, in some cases, includes the addition of *N,N*-diethylaniline[99] in order to increase nucleophilicity of the oxygen atom. Another similar approach (applied in our laboratory) uses the (chloromethylene)dimethyliminium chloride as a chlorinating agent for the preparation of 4,6-dichloropyrimidine from 4,6-dihydroxy analogues.[10] This approach benefits from higher yields and easier work-up compared to other general methods.

2.4.4. Modification in the position C5 of pyrimidine of 2-amino-4,6-diarylpyrimidine

As it was described above, C5pyr is the most electron rich position, nevertheless, the activation by electron-donating group (EDG) is still needed for the electrophilic substitution to occur. When starting from activated 2-aminopyrimidine with hydrogen in C5pyr, there is a wide array of options. To C5pyr can be introduced for example formyl,[100] nitroso,[101] nitro,[102] thiocyanato,[103] and most importantly halogen moieties.[104–106]

However, the situation is much less explored when modifying C5pyr on 2-amino-4,6-diarylpyrimidine, possibly because the reactivity of this position towards the electrophilic substitution is relatively low. Above the electronic factors, the steric factors play a role as well.

In the case of C5pyr unsubstituted 2-amino-4,6-diarylpyrimidine, there is only one entry in the literature reporting a bromination (and subsequent cyanation).[107]

As mentioned above, it is possible to obtain 2-amino-4,6-diarylpyrimidine with diverse substituents in C5pyr either via condensations,[15,84–86] or via SMC reaction,[12,89] however, these are not afforded through the direct C5pyr modifications.

2.5. Modifications of solubility

The desired pharmacological effect exhibited by some potentially druggable compound *in vitro* is often not sufficient for successful transformation into a drug. It is necessary to concern the pharmacokinetic properties and the closely related solubility of the drug candidate. Nowadays, the poor water solubility is the more common problem as approximately 40 % of marketed drugs and up to 75 % of molecules in development are poorly water-soluble.[108] One hypothesis states that this situation could be, in part, caused by the nature of high-throughput screening as the large libraries work in nonaqueous or mixed media resulting in shifted profile of the compounds used in the screening towards more hydrophobic.[108,109]

In any case, fine tuning of the solubility is needed as both lipophilicity and hydrophilicity are important for the pharmacokinetics of the compound. Hydrophilicity is essential for the solubility in an aqueous environment and, in consequence, for the oral bioavailability. Lipophilicity is crucial for the penetration through the biomembranes. The lipophilicity is usually quantified by log *P* (Equation 1) which can be currently predicted using *in silico* calculations giving medicinal chemists some hint for the design of the molecule.[110] According to the well known Lipinski´s “rule of five”, the molecular weight should be below 500, the number of H-bond acceptors and donors should be under 10 and 5, respectively, and the log *P* value should not exceed 5 in order to achieve reasonable absorption and permeation.[111]

$$\log P = \frac{\text{solute in } n\text{-octanol}}{\text{solute in water}}$$

Equation 1. Log *P* calculation

Nevertheless, the molecular planarity and symmetry also influence the solubility. Hence, the disruption of planarity e.g. by removal of aromaticity or the disruption of symmetry e.g. by *ortho*- substitution can modify the solubility.[109]

The enhancement of aqueous solubility can be further achieved by the introduction of polar functional groups or bioisosteric moieties, the formation of salts or prodrugs. There are other methods such as determination of the best crystal polymorph, utilization of co-solvents, surfactants, or co-crystals, however, these are applied mostly in pharmaceutical industry.[108]

An example of accomplishing higher solubility through the implementation of a more polar group into the molecule are chalcones where the hydroxy group was replaced by carboxy group resulting in improved solubility and, moreover, in higher antibacterial activity.[112]

Nevertheless, the introduction of the polar functional group is often related to the formation of a prodrug, because usually, it is difficult to introduce polar group without changing the pharmacodynamic properties of the compound. The prodrug needs to be designed with regard to the subsequent requirement for degradation.[113,114] An example of the construction of more hydrophilic prodrug is the drug sulindac, a NSAID, where the inactive sulfoxide prodrug is *in vivo* reduced to the active sulfide.[115] Also, the implementation of phosphate ester prodrug is feasible obtaining active hydroxy drug after enzymatical cleavage by phosphatase placed on the surface of enterocytes.[113,114,116]

When increasing the hydrophilicity using bioisosteres, a useful transformation is the introduction of a heteroatom into the parent molecule. For example, the decrease of log *P* from 3.36 to 0.77 is achieved by replacing the CH_2 (X) group by oxygen in an RCH_2-X-CH_2R segment.[117] Furthermore, the hydrogen-fluorine bioisosteric exchange is well established in medicinal chemistry as it may improve metabolic stability and also increase lipophilicity. But interestingly, there are cases where fluorine exchanged for hydrogen decreases the lipophilicity.[118] The reason for this phenomenon remains unclear, although, structural patterns were observed in these cases revealing proximity of oxygen atom. In all cases, there was at least one low energy conformer with an O-F distance smaller than 3.1 Å.[118]

Besides the bioisosteres, the solubility can be enhanced using poly(ethylene glycol)-conjugates (PEG-conjugates). These polymers can be constructed into linear, branched or micellar structures with molecular weight up to tens of thousands.[119] Generally, they exhibit desired qualities, such as increased solubility, low immunogenicity, and increased *in vivo* circulation time, however, PEG-conjugates are successfully applied mostly for macromolecules and it is necessary to consider that in the case of small molecules so large conjugates may interfere with the target resulting in decrease of activity as in the case of chemotherapeutic agent paclitaxel.[120] This issue was solved for paclitaxel (as well as for another antitumor agent – camptothecin[121]) by connecting the PEG via a labile ester linkage, therefore, forming a PEG prodrug.[122]

Another way how to increase hydrophilicity is the formation of a salt. Among the FDA-approved drugs for the time period 1995-2006 the salts of basic drugs were mostly formed by hydrochloride followed by methanesulfonate and the salts of acidic drugs were dominantly formed by sodium followed by calcium.[123]

An interesting approach to this issue is the encapsulation of the poorly soluble molecules into a biodegradable nanoparticle.[124]

Nevertheless, both hydrophilicity and lipophilicity must be carefully balanced for they are both absolutely essential for the pharmacokinetics and pharmacodynamics of a potentially druggable molecule.

3. Aims of the study

The main goal of the study is design and synthesis of novel polysubstituted pyrimidines, derived from the current lead structure WQE-134, with improved biological properties, namely higher potency to inhibit PGE_2 production and increased solubility.

Specifically, the aims are:

a) Design and synthesis of WQE-134 analogues with modified phenyl moiety in the C4 position of pyrimidine using preferably electron-donating groups and evaluation of their biological properties
b) Design and synthesis of polysubstituted pyrimidines with hydrogen in the C5 position of pyrimidine analogous to previously prepared compounds with butyl in the C5 position of pyrimidine and evaluation of their biological properties
c) Design and synthesis of WQE-134 analogues modified in the C5 position of pyrimidine in order to increase their solubility
d) Synthesis of WQE-134 analogue biotinylated in the C5 position of pyrimidine via pegylated linker for subsequent pulldown experiments

4. Results and discussion

Biological evaluation of substituted pyrimidines revealed interesting anti-inflammatory properties of some of the compounds. An extensive structure-activity relationship (SAR) study of anti-inflammatory pyrimidines has been initiated culminating in the identification of current lead structure WQE-134. The work leading to the discovery of WQE-134 was done without my contribution, however, all subprojects investigated in this thesis were based on WQE-134, hence, the pathway leading to WQE-134 discovery will be briefly described.

In this thesis, three structure-activity relationship studies (SAR), one of which was mainly focused on the enhancement of solubility, were performed and a probe for pulldown experiments was prepared as an attempt to elucidate the mechanism of action of the studied compounds.

Anti-inflammatory potential of compounds was assessed by their inhibitory effects on the *in vitro* production of prostaglandin E_2 (PGE_2) which was induced in mouse peritoneal cells using lipopolysaccharide (LPS) from *Escherichia coli*. The viability of cells was analyzed by determination of lactate dehydrogenase activity released from damaged cells into the supernatant. All biochemical assays were provided by the group of Dr. Zdeněk Zídek from the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic and the biochemical data in this thesis are shown with approval of Dr. Zídek. The solubility measurements were performed by Ing. Eva Zborníková from the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic and these data are shown with approval of Ing. Zborníková.

4.1. Lead structure WQE-134

The lead compound WQE-134 (Figure 9) exhibits significant inhibitory activity against PGE_2 production *in vitro* and *in vivo*. 2-Amino-5-butyl-4-(4-methoxyphenyl)-6-

phenylpyrimidine was selected as the lead structure based on the previous results of our group (excluding me),[10,11] after *in vitro* screening of over 500 compounds including approximately a hundred di(hetero)arylated compounds. Even though the lead was not the best inhibitor *in vitro*, it was preferred due to the promising *in vivo* results in dextran sulfate sodium (DSS) rat colitis model. Currently, the lead is tested in the pig colitis model as well, the pharmacokinetic indicators are intensively studied, and the synthesis in large scale (over 10 kg) is being developed.

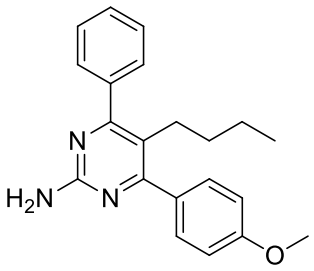


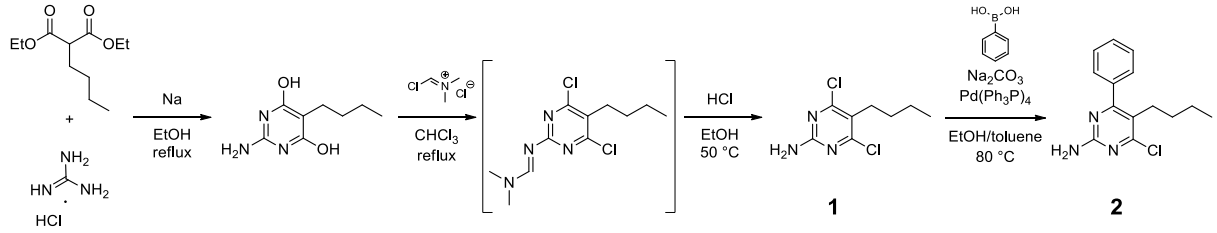
Figure 9. Lead structure WQE-134 (2-amino-5-butyl-4-(4-methoxyphenyl)-6-phenylpyrimidine)

The collected results suggest that the anti-inflammatory activity is not caused by the direct inhibition of COX-1 or COX-2, however, the mechanism of action is under investigation, and so far, remains unclear.

4.2. WQE-134 analogues with substituted phenyl moiety in the C4 position of pyrimidine

Based on the data obtained about the lead and related structures, the hypothesis was given stating that the biological activity can be increased by electron-donating substituents. This hypothesis was the foundation for the subsequent work aiming for enhancement of biological activity (inhibition of PGE_2 production) via structural modifications of the phenyl moiety in the position C4 of pyrimidine (C4pyr) by introduction of electron-donating group (EDG).

The key precursor **2** for the synthesis of diarylated compounds was prepared according to the previous work of our group (Scheme 3).[10,11]

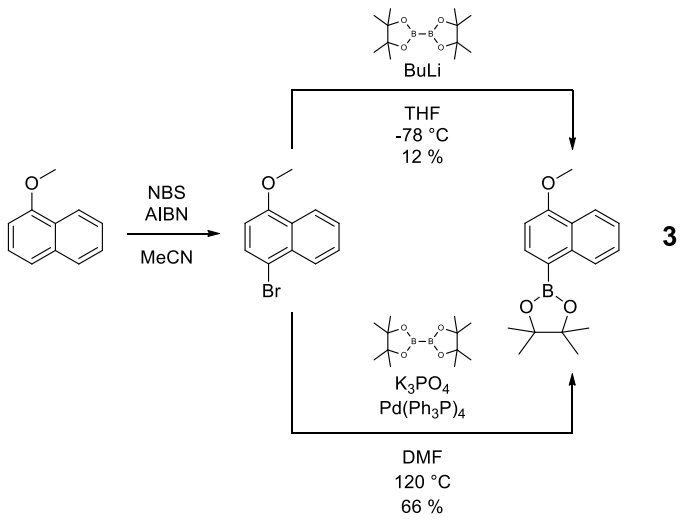


Scheme 3. Synthesis of the key precursor **2** for subsequent structure-activity relationship studies

The degree of the Suzuki-Miyaura coupling (SMC) reaction was controlled via proper selection of base, solvents, and temperature. Eligible conditions when aiming for mono-substituted product were Na_2CO_3 and toluene-EtOH mixture (3:1 ratio) at 80 °C while for diarylated product Cs_2CO_3, dioxane-water mixture (4:1 ratio) at 110 °C were suitable. However, in the case of monoarylated product preparation, the selectivity was never absolute.

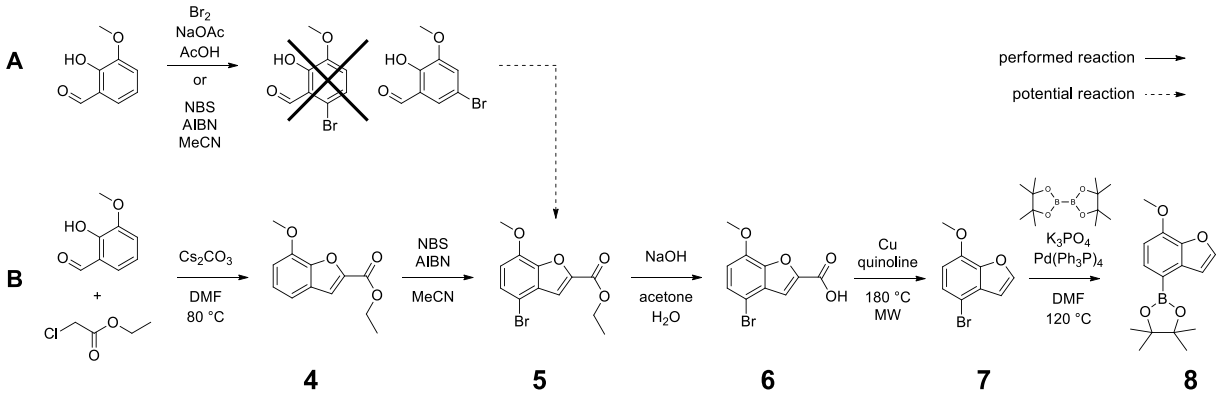
Compound **2** and suitable boronic acid or boronic acid ester were further utilized in the SMC reaction. The boronic reagents were purchased from commercial suppliers, except for boronic acid esters **3** and previously unpublished **8**, which were needed to be prepared.

To yield **3**, 1-methoxynaphatelene was selectively brominated to the para position relatively to the methoxy group using NBS.[125] Afterwards, the lithiation of 1-methoxy-4-bromonaphatelene and subsequent transmetallation by pinacol diboronate similarly to Gege and co-workers was attempted (Scheme 4).[126] Even though Gege and co-workers reported relatively low yield (29 %) other entries in the literature on slightly different substrates were reported higher yields.[127,128] However, the low yield obtained in our case (12 %) directed further effort on catalytic borylation similarly to Harada and co-workers[129] which led to higher yields (66 %) (Scheme 4).



Scheme 4. Synthesis of **3**

The preparation of compound **8** proved to be more difficult. First, it was intended to brominate *o*-vanillin to the para position relative to the methoxy group according to Saku and co-workers.[130] Surprisingly, the bromination proceeded exclusively to the meta position relative to the methoxy group, contrary to the data reported by Saku and co-workers (Scheme 5A).



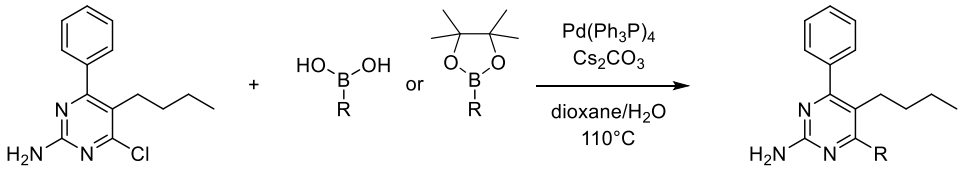
Scheme 5. Synthesis of **8**

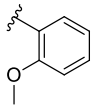
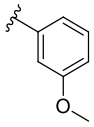
Thus, the benzofuran scaffold was assembled prior to the bromination forming the ethyl ester derivative **4**. Although Yamaguchi and co-workers[131] reported carboxylic

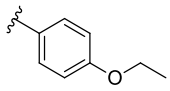
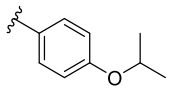
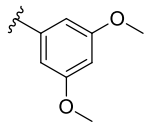
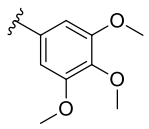
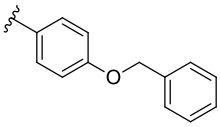
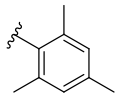
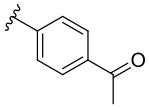
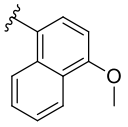
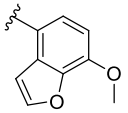
acid derivative as the product of the condensation, more entries in the literature reported the formation of the observed ethyl ester (**4**).[130,132] The ethyl ester was brominated to yield **5**. The desired para isomer relative to the methoxy group was obtained in a novel manner since the only two entries in the literature are starting from *o*-vanillin brominated in the para position relative to the methoxy group.[130,133] After hydrolysis of the ester, the decarboxylation under MW conditions was performed according to Musser and co-workers to afford **7** (Scheme 5B).[134] Afterwards, the catalytic borylation secured desired **8** suitable for use in the SMC reaction.[129]

In the first phase of this first SAR study (SAR 1.1), the SMC reaction using **2** and suitable boronic acid or boronic acid ester under standard conditions was performed to afford eleven compounds analogous to WQE-134 with modified phenyl moiety in C4pyr (Table 2).[12] Out of the final compounds, ten were modified by EDG and one was modified by electron-withdrawing group (EWG) to verify the original hypothesis. All compounds were subsequently tested *in vitro* for viability and inhibition of PGE_2 production.

Table 2. Compounds prepared for the SAR 1.1



Entry	Compound	R	Yield [%]	PGE_2 in % of LPS stimulated control response	Viability in % of control response
1	**9a**		68	18.60 ±1.37	85.00 ±14.4
2	**9b**		76	18.09 ±2.63	104.10 ±1.90

3	**9c**		63	26.35 ±0.12	104.65 ±1.30
4	**9d**		83	12.28 ±0.50	71.17 ±1.91
5	**9e**		81	16.36 ±3.77	105.15 ±0.49
6	**9f**		74	10.07 ±2.62	102.58 ±0.67
7	**9g**		71	0.33 ±0.17	105.40 ±0.23
8	**9h**		85	30.55 ±9.77	99.92 ±1.02
9	**9i**		77	25.60 ±0.11	103.99 ±0.92
10	**9j**		57	15.35 ±0.34	45.16 ±1.25
11	**9k**		30	34.62 ±1.24	35.59 ±0.84

The biochemical data of the eleven final compounds in SAR 1.1 showed that all derivatives maintained the activity similar to WQE-134 (Figure 10). Notably, even **9i** bearing electron-withdrawing substituent inhibited the production of PGE_2. The viability data showed generally no interference of prepared compounds with the viability of cells except for **9j** and **9k** which significantly decreased vitality of studied cells. However, most importantly, the obtained data revealed markedly high biological activity of **9g**.

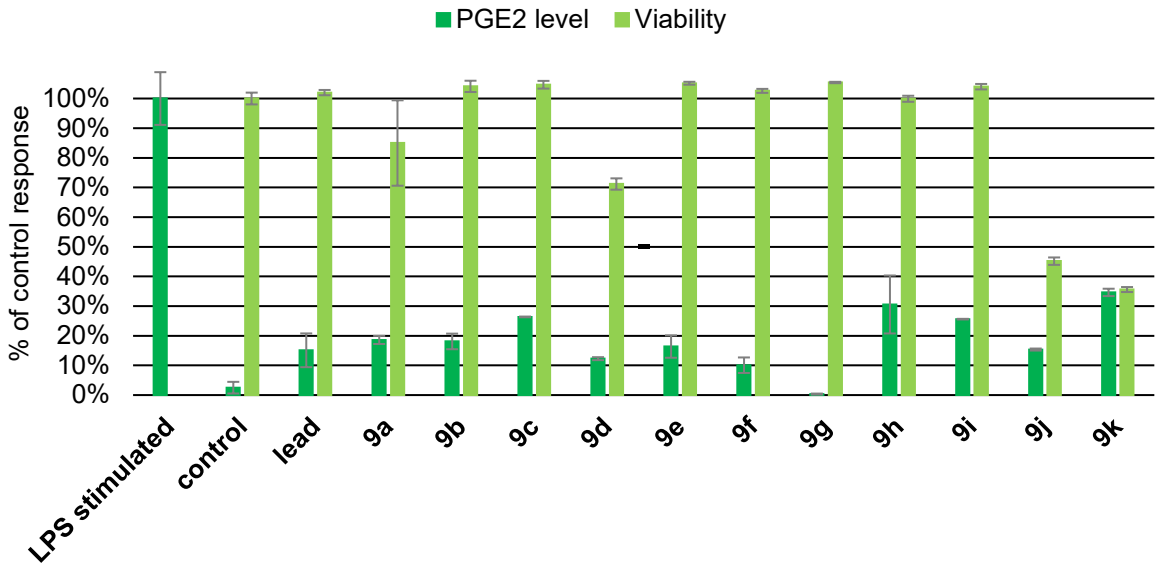
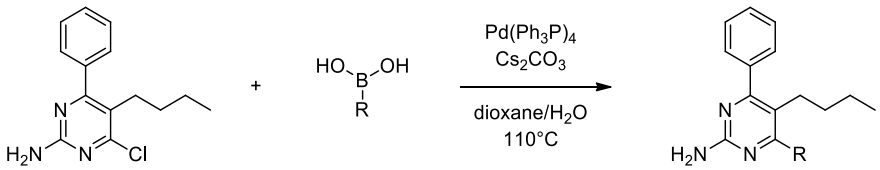
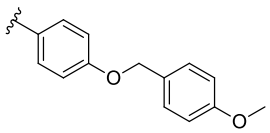


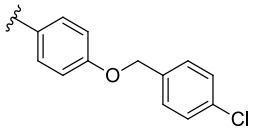
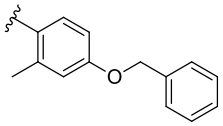
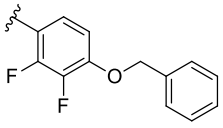
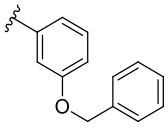
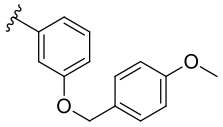
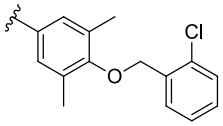
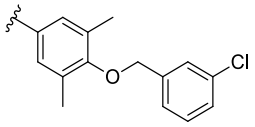
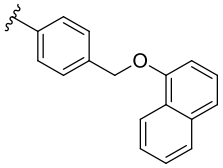
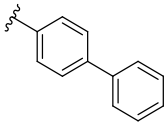
Figure 10. Effect of prepared compounds in the SAR 1.1 on *in vitro* production of PGE_2 and viability of mouse peritoneal cells. Effects are expressed as a percentage change relative to the response of LPS stimulated or unstimulated control cells. Bars are means ± SEM obtained by averaging results of two to four experiments for each compound.

Hence, in the second phase of the first SAR study (SAR 1.2), additional ten derivatives bearing benzyloxy or benzyloxy-like moiety were prepared resulting generally in very potent inhibitors of PGE_2 production (Table 3).

Table 3. Prepared compounds for the SAR 1.2



Entry	Compound	R	Yield [%]	PGE_2 in % of LPS stimulated control response	Viability in % of control response
1	**10a**		78	2.01 ±1.53	104.24 ±0.35

2	**10b**		71	0.73 ±0.53	101.25 ±0.84
3	**10c**		85	51.13 ±0.83	103.20 ±0.52
4	**10d**		25	0.04 ±0.20	103.66 ±0.44
5	**10e**		98	11.74 ±2.27	101.58 ±0.74
6	**10f**		99	20.44 ±1.30	103.57 ±0.70
7	**10g**		97	7.12 ±1.95	103.57 ±0.32
8	**10h**		94	6.54 ±0.41	102.82 ±0.46
9	**10i**		36	26.29 ±5.33	104.82 ±0.44
10	**10j**		89	2.95 ±1.86	101.83 ±0.21

The biochemical data indicate that the benzyloxy moiety is beneficial for the biological activity as all compounds bearing this moiety exhibited very good inhibitory activity against PGE_2 production (Figure 11). However, if the benzyloxy substituent was introduced in the position C3 of the phenyl (compounds **10e** and **10f**), a small decrease

of activity was observed. Interestingly, **10c** where the only difference compared to **9g** was the ortho substitution by a methyl group showed a major decrease of biological activity (155-fold) suggesting that the *o*-methyl group could be causing steric hindrance in the potential active site. Most importantly, remarkable inhibitory activity against the production of PGE_2 was exhibited by **10d**. The viability of cells treated with all compounds in this series remained unchanged fluctuating around 100 % value (Figure 11).

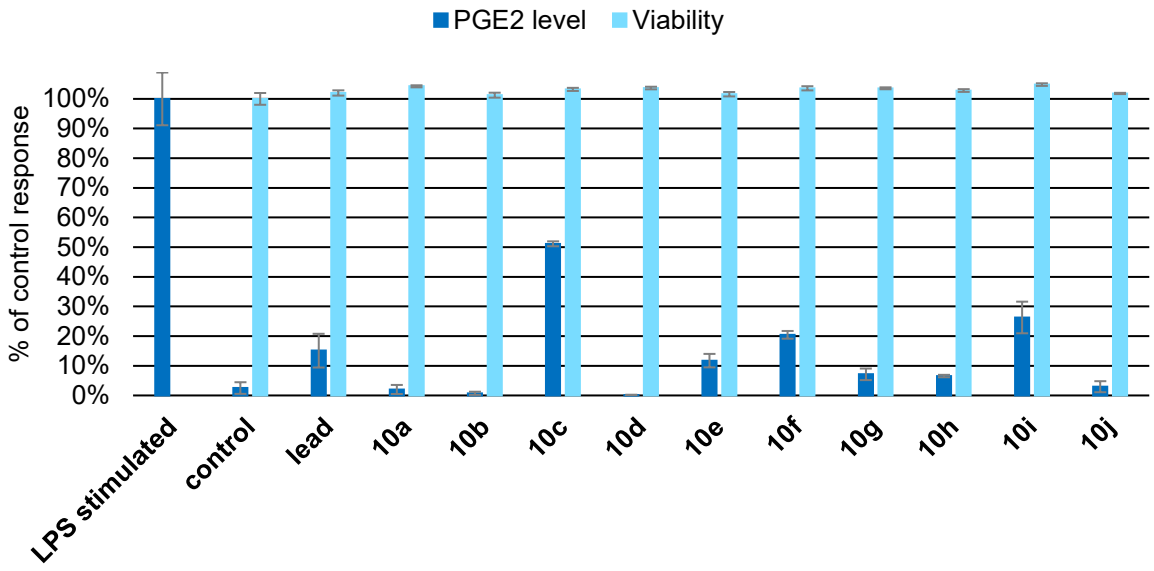
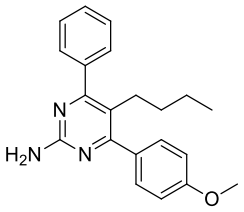
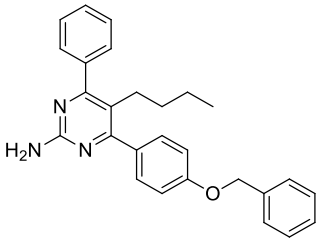
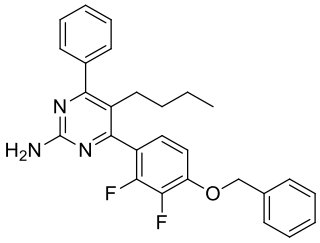


Figure 11. Effect of prepared compounds in the SAR 1.2 on *in vitro* production of PGE_2 and viability of mouse peritoneal cells. Effects are expressed as a percentage change relative to the response of LPS stimulated or unstimulated control cells. Bars are means ± SEM obtained by averaging results of two to four experiments for each compound.

Further, for the two most potent compounds, the IC_{50} values were determined implying that **10d** is more potent than the current lead structure by three orders of magnitude (Table 4).

Table 4. IC_{50} values for the two most potent molecules and the lead compound (WQE-134)

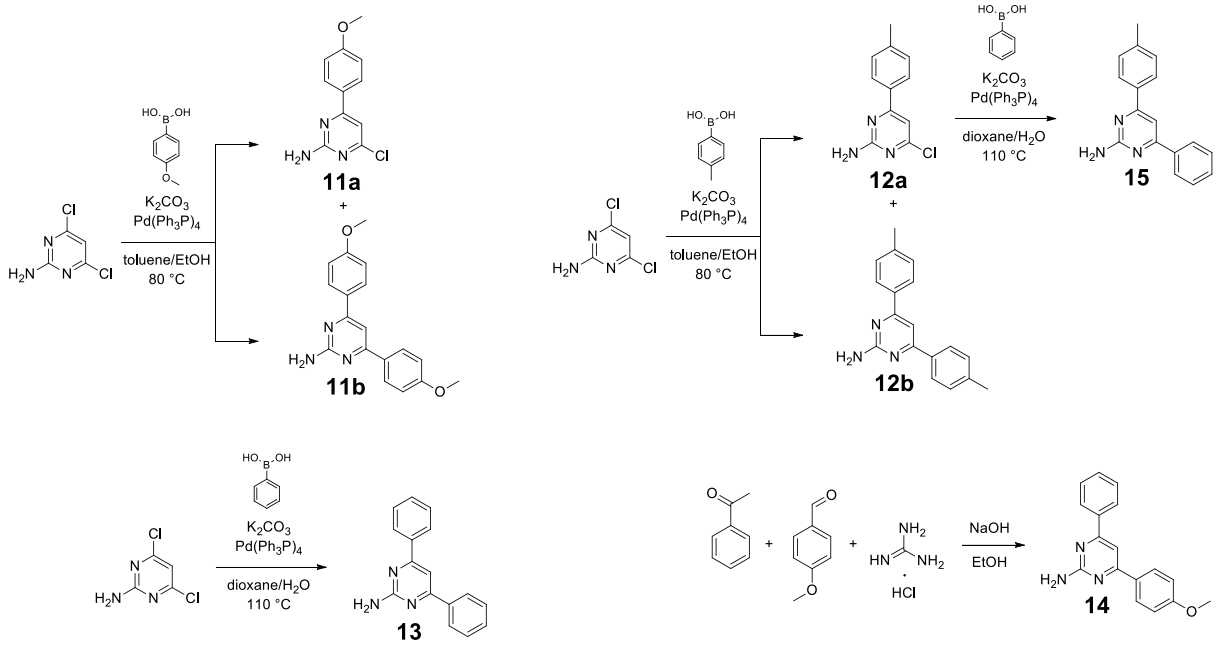
Compound	Structure	IC_{50} [μM]
WQE-134		**5.87** (4.70-7.32)
9g		**0.094** (0.045-0.197) **62 × more potent**
10d		**0.006** (0.003-0.011) **978 × more potent**

The aim of these SAR studies was to increase the biological activity. The aim was accomplished as the final most potent compound exhibited three orders of magnitude higher activity than the current lead structure (WQE-134).

4.3. Polysubstituted pyrimidines with hydrogen in the position C5 of pyrimidine

Previous work of Dr. Zídek regarding 4,6-dichloropyrimidines with various substituents in C2pyr revealed the following relationship between the length of alkyl chain in C5pyr and the inhibition of PGE_2 production. In order to exhibit the activity, the alkyl chain should be at least two carbon atoms long as hydrogen and methyl did not show substantial inhibitory activity against the production of PGE_2.[24]

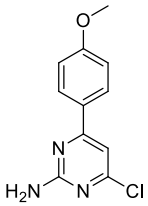
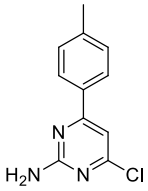
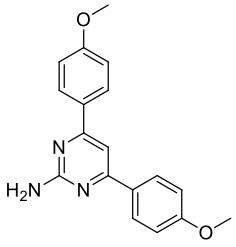
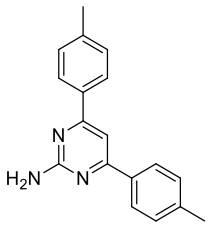
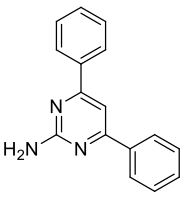
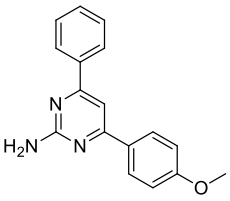
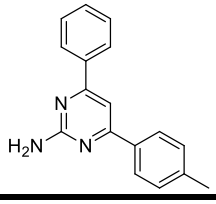
Since the 2-amino-5-butyl-4,6-diarylpyrimidines inhibited the PGE_2 production, the aim of synthesizing the corresponding 5-unsubstituted and 5-methyl analogues was to verify whether the exchange of butyl in C5pyr for hydrogen and methyl will also lead to the loss of activity in the series of polysubstituted pyrimidines bearing aryl group(s).



Scheme 6. Preparation of C5pyr unsubstituted analogues for the SAR 2.1

Hence, in the first part of this second SAR study (SAR 2.1), seven derivatives bearing hydrogen in C5pyr were prepared using standard SMC reaction conditions except for **14** prepared by one-pot condensation (Scheme 6, Table 5).[12,135] Analogous derivatives bearing butyl and methyl in C5pyr were prepared by Dr. Kolman and are shown in order to demonstrate the trend in biological activities.

Table 5. Prepared C5pyr unsubstituted analogues for the SAR 2.1

Entry	Compound	Structure	Yield [%]	PGE_2 in % of LPS stimulated control response	Viability in % of control response
1	**11a**		34	6.68 ±2.03	103.21 ±0.70
2	**12a**		41	7.52 ±1.92	102.64 ±0.40
3	**11b**		24	3.61 ±2.51	101.73 ±0.67
4	**12b**		37	3.40 ±2.89	102.85 ±1.17
5	**13**		81	1.51 ±0.70	97.42 ±0.91
6	**14**		69	7.12 ±3.30	99.92 ±1.67
7	**15**		82	2.27 ±0.94	103.13 ±0.71

Interestingly, for diarylated compounds, the replacement of butyl for methyl or hydrogen did not lead to the loss of efficacy as observed for 4,6-dichloropyrimidines. Moreover, the biological activity notably increased in order Bu<Me<H (Figure 12). The values of PGE_2 production inhibition for monoarylated molecules remained practically the same.

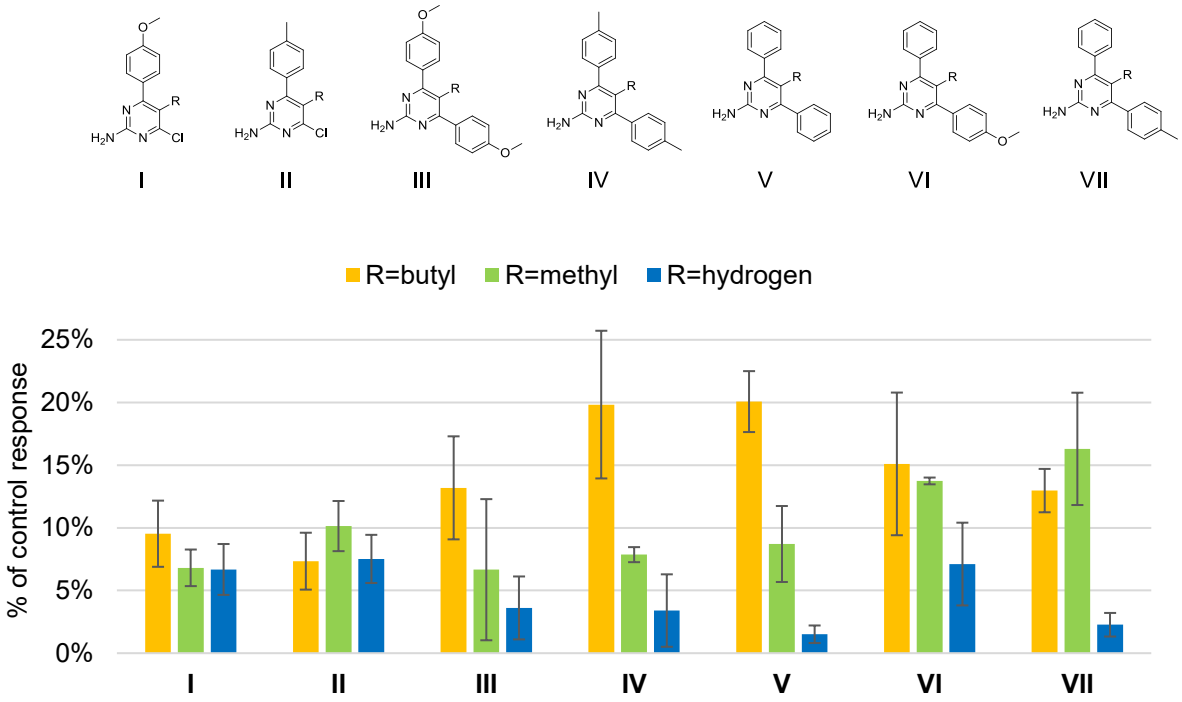
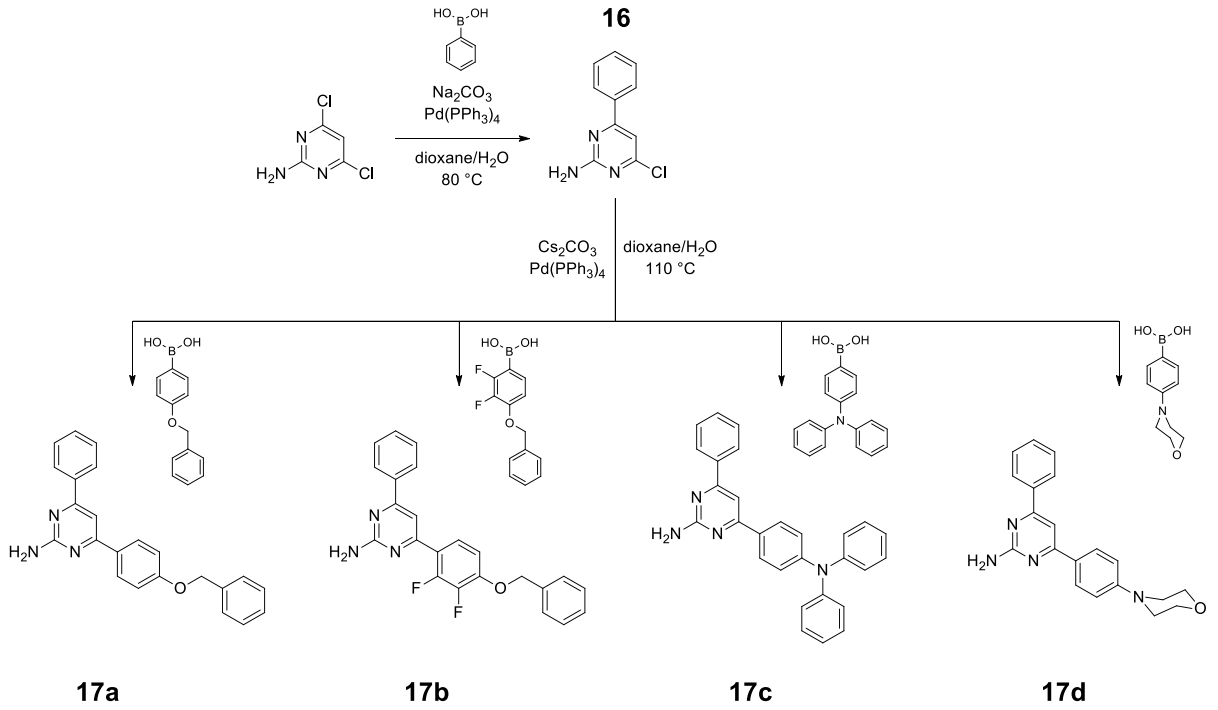


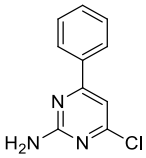
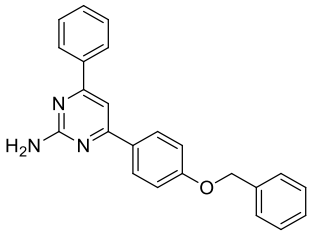
Figure 12. Effect of C5pyr substituent on *in vitro* production of PGE_2 of mouse peritoneal cells. Effects are expressed as a percentage change relative to the response of LPS stimulated cells. Bars are means ± SEM obtained by averaging results of two to four experiments for each compound.

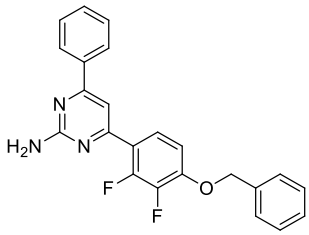
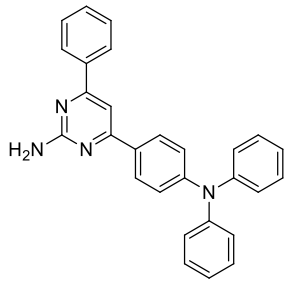
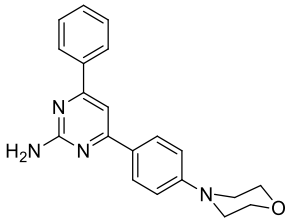
Based on the observed markedly higher efficacy of diarylated molecules with hydrogen in C5pyr compared to the corresponding compounds bearing butyl in C5pyr, seven more analogues were synthesized, four by me (Scheme 7, Table 6) and three by Dr. Kolman. The selection for the second part of the second SAR (SAR 2.2) contained three most potent, two medium, and two least potent molecules to cover a wide range of potency.



Scheme 7. Preparation of additional C5pyr unsubstituted analogues for the SAR 2.2

Table 6. Prepared C5pyr unsubstituted analogues for the SAR 2.2

Entry	Compound	Structure	Yield [%]	PGE_2 in % of LPS stimulated control response	Viability in % of control response
1	**16**		76	3.74 ±1.62	104.81 ±2.08
2	**17a**		56	0.68 ±0.86	101.23 ±0.74

3	**17b**		97	5.51 ±0.34	102.57 ±3.78
4	**17c**		57	60.99 ±0.89	99.11 ±2.20
5	**17d**		87	7.88 ±1.11	101.34 ±2.04

Interestingly, the exchange of butyl for hydrogen in the molecule did not exclusively lead to enhancement of biological activity for these additional seven derivatives as observed previously. The exchange of butyl for hydrogen resulted in both higher and lower efficacy (Figure 13).

In conclusion, the exchange of butyl for hydrogen does not cause the loss of activity against PGE_2 production in the series of analogues bearing aryl group(s) on the pyrimidine moiety. On the contrary, in some cases, this modification results in higher efficacy.

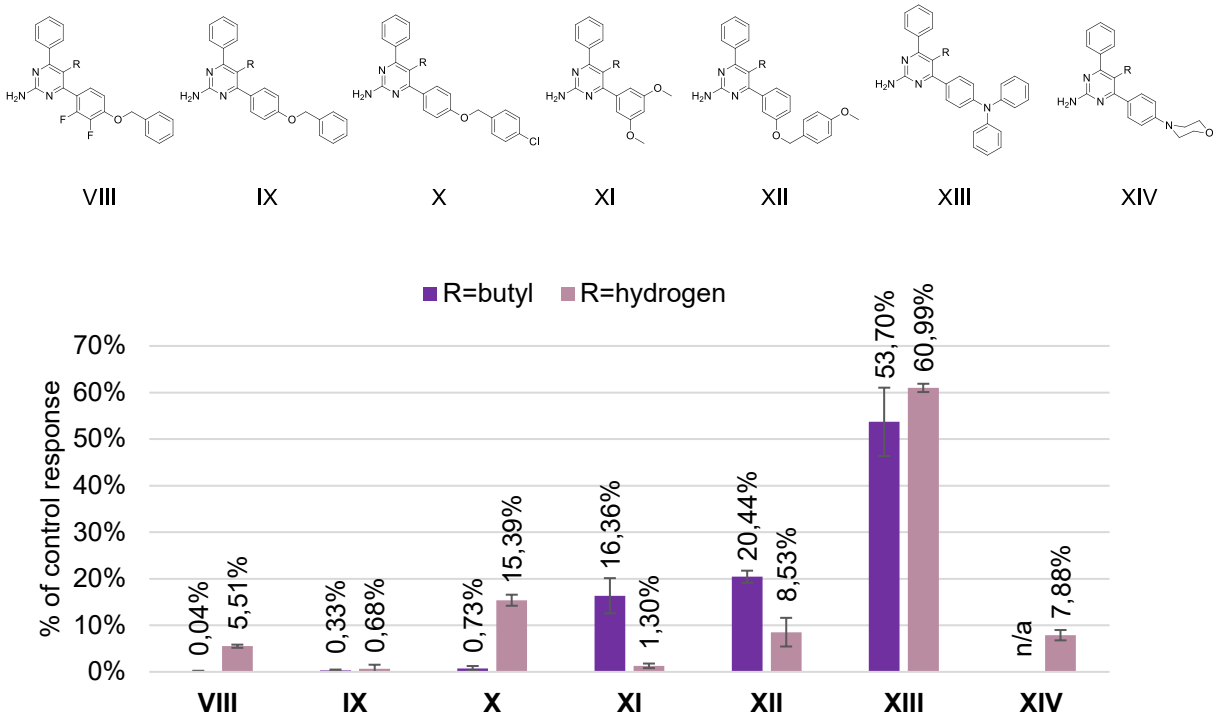


Figure 13. Effect of butyl/hydrogen exchange in C5pyr on *in vitro* production of PGE_2 of mouse peritoneal cells. Effects are expressed as a percentage change relative to the response of LPS stimulated cells. Bars are means ± SEM obtained by averaging results of two to four experiments for each compound. The compounds X-XII in the hydrogen series and compounds XIII and XIV in the butyl series were prepared by Dr. Kolman.

4.4. Modifications of solubility

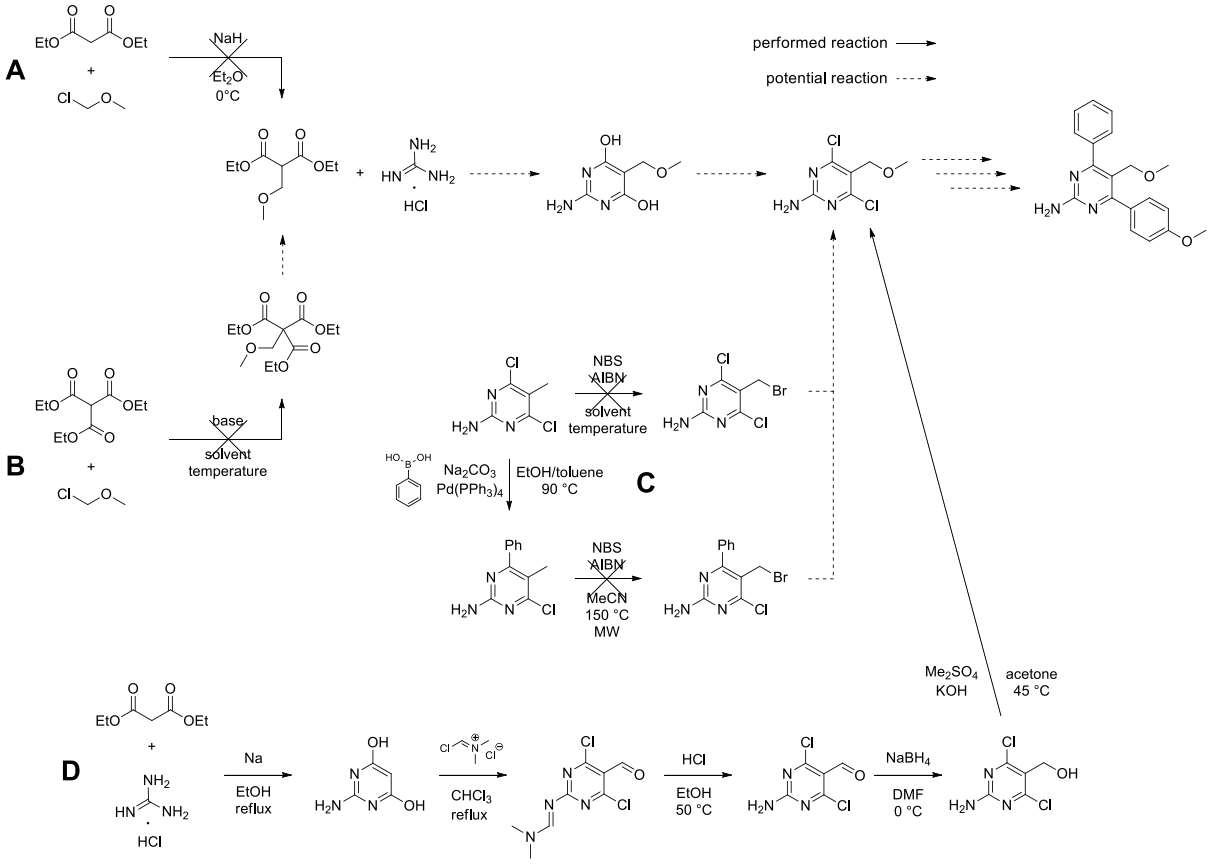
4.4.1. WQE-134 analogues modified in the position C5 of pyrimidine

Water-solubility is important pharmacokinetic property of a compound effecting bioavailability. Despite the relatively good biological activity of WQE-134, there is a significant bioavailability obstacle in the form of very poor solubility. Thus, WEQ-134 needed to be modified in order to increase the solubility.

The previous data suggest that C5pyr is most probably not the pharmacophore, therefore, should not play crucial role regarding the control of bioactivity. Therefore, it was decided to modify the solubility by introduction of a heteroatom in C5pyr as

alteration of this position was expected to have the lowest impact on the activity of the modified lead scaffold. Thus, with regard to calculated log *P* values using Virtual Computational Chemistry Laboratory (VCCLAB), ten analogues of the lead structure differing in C5pyr substituent were prepared.

The original concept for the C5pyr modified pyrimidines was considering the alkylation of diethyl malonate with chloromethyl methyl ether according to Henke and co-workers[136] (Scheme 8A) followed by standard condensation to form the pyrimidine ring, subsequent chlorination,[10] and arylation.[12] However, the dominant product of the first reaction was the dialkylated malonate. This was in conflict with the results of Henke and co-workers who obtained mono and dialkylated products in the ratio 7:3.



Scheme 8. Initial attempts to find eligible synthetic route towards 2-amino-5-methoxymethyl-4-(4-methoxyphenyl)-6-phenylpyrimidine

To avoid the undesired disubstitution, the alkylation of triethyl methanetricarboxylate according to the similar procedure with propargyl bromide by Aldegunde and co-workers[137] was tested (Scheme 8B). However, no desired product was afforded. The solvent, base, and temperature were varied (Table 7) without any success. Under standard alkylating conditions (Entry 5, Table 7), the triethyl methanetricarboxylate was successfully alkylated using butyl bromide, allyl bromide, and benzyl chloride validating its capability to be alkylated, nevertheless, chloromethyl methyl ether gave no product.

Table 7. Conditions used for unsuccessful alkylation of methanetricarboxylate using methoxymethyl chloride

Entry	Base	Solvent	Temperature (deprotonation t=0 °C)
1	EtONa	EtOH	75 °C
2	EtONa	EtOH/Et_2O	50 °C
3	NaH	Et_2O	25 °C
4	NaH	dioxane	70 °C
5	NaH	DMF	80 °C
6	DIPEA	DMF	80 °C
7	LDA	DMF	80 °C
8	*n*-BuLi	DMF	80 °C

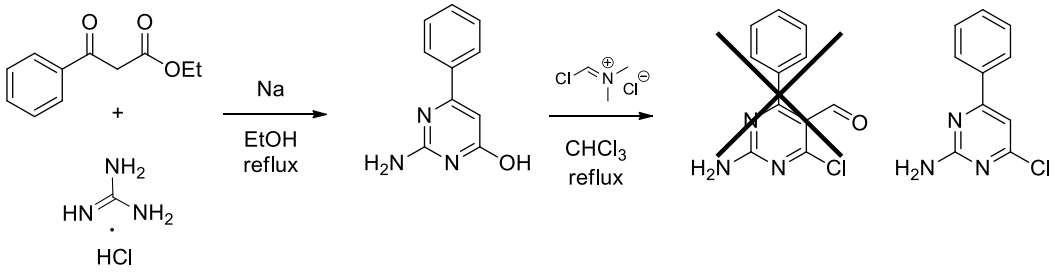
Further, the bromination of 2-amino-4,6-dichloro-5-methylpyrimidine was attempted using various solvents and temperatures (Table 8) based on the procedure of Guo and co-workers[138] on 5-methylpyrimidine yielding only the starting compound (Scheme 8C). The same result was obtained using 2-amino-4-chloro-5-methyl-6-phenylpyrimidine as the starting compound for the bromination procedure.

Table 8. Conditions used for unsuccessful bromination of 2-amino-4,6-dichloro-5-methylpyrimidine

Entry	Solvent	Temperature
1	MeCN	45 °C
2	DCM	45 °C
3	$CHCl_4$	45 °C
4	MeCN	150 °C
5	MeCN	150 °C

Hence, a different approach was necessary. 2-Amino-4,6-dihydroxypyrimidine was prepared using standard condensation of guanidine and diethyl malonate (Scheme 8D).[10] Afterwards, the formylation and chlorination were performed simultaneously based on similar procedure of Wainwright and co-workers[139] using (chloromethylene)dimethyliminium chloride. This reaction was crucial as the hydroxymethyl moiety obtained after reduction of the formyl according to Gann and Ray[140] was the key intermediate. The 5-hydroxymethyl derivative was then alkylated based on the procedure by Saadati and Meftah-Booshehri using Me_2SO_4.[141] Even though the alkylation provided only approximately 10 % conversion (according to UPLC-MS), it was sufficient for verifying the possibility of this synthetic route which would proceed by utilization of two sequential SMC reactions. However, the first SMC reaction is critical as the monoarylated product exhibits higher reactivity compared to the starting 4,6-dichloropyrimidine analogue. Even with fine tuning of the reaction conditions, the diarylated product is obtained as well complicating purification and lowering the yield of the desired monoarylated product.

Hence, further effort was devoted to bypass the first SMC reaction. Ethyl benzoylacetate was condensed with guanidine forming 2-amino-4-hydroxy-6-phenylpyrimidine.[142] Unfortunately, the subsequent formylation failed making this route ineligible (Scheme 9).



Scheme 9. Condensation of 2-amino-4-hydroxy-6-phenylpyrimidine and subsequent unsuccessful formylation

In another approach, the whole target scaffold **14** was prepared one-pot according to Nimkar and co-workers[135] and subsequently brominated identically with

the procedure published by van Veldhoven and co-workers to yield **18**.[107] The brominated derivative served as a starting compound for several other transformations. First, it was let to react with zinc cyanide affording **19** similarly to Guibourdenche and co-workers.[143] Further, the Heck reaction was utilized.[144] After optimalization of catalyst type and loadings (Table 9), the reaction with ethyl vinyl ether gave **20**.

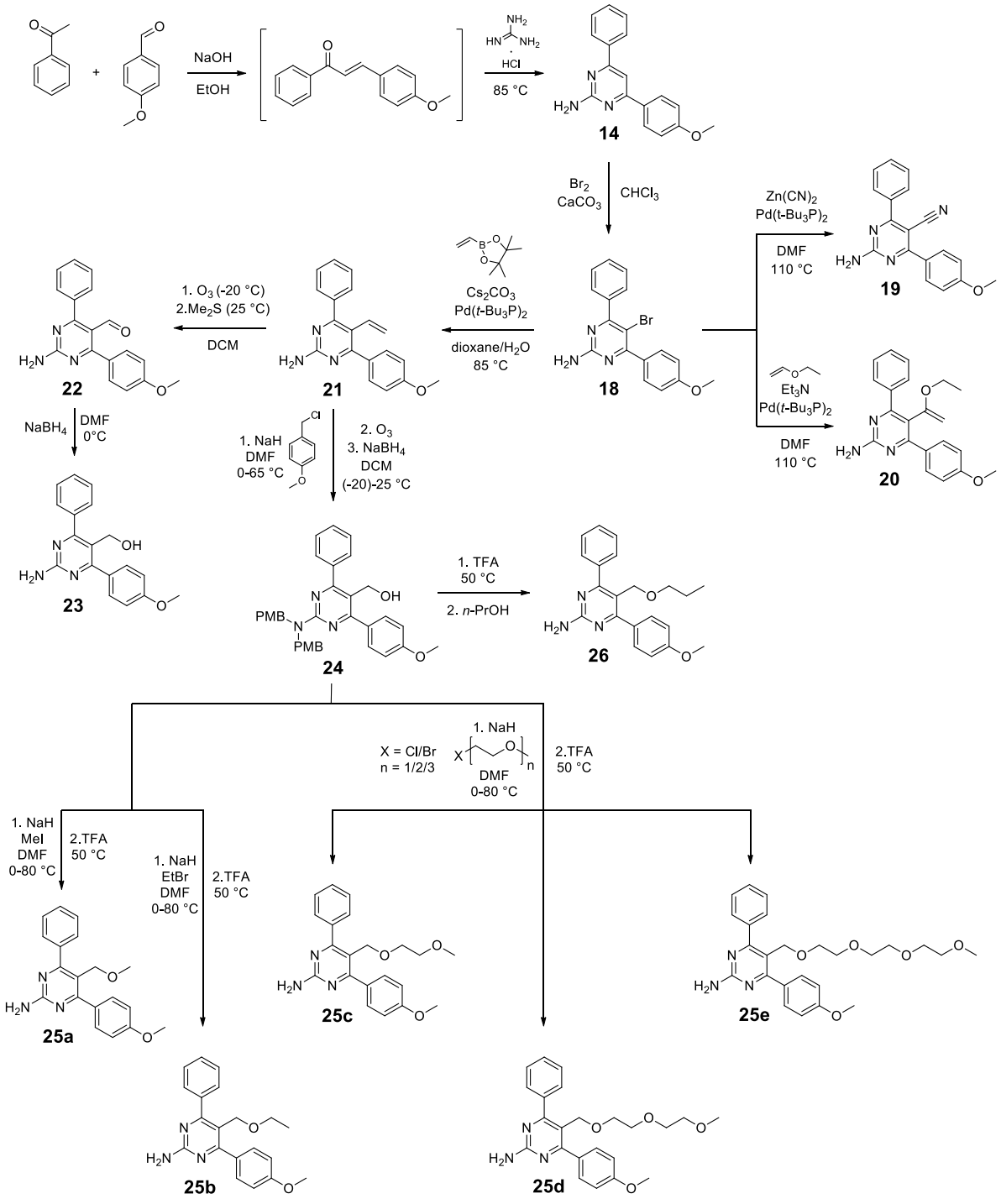
Table 9. Optimalization of catalyst type and loadings for the Heck reaction

Entry	Catalyst	mol %	Conversion
1	$Pd(t\text{-}Bu_3P)_2$	35	95 %
2	$Pd(OAc)_2$	5	1 %
3	$Pd(OAc)_2$ + DPPP	5+5	8 %
4	$Pd(t\text{-}Bu_3P)_2$	5	90 %

Further, the SMC reaction with pinacol vinyl boronate secured **21** which was subsequently ozonolysed to **22**.[145] The formyl derivative was reduced to the hydroxymethyl derivative **23** which was alkylated the same way as in the case of 4,6-dichloropyrimidine analogue with dimethyl sulfate.[140,141] The conversion was again low (15 %), nevertheless, the eligibility of this synthetic route was confirmed on the diarylated scaffold.

The low conversion was attributed to the reactive amino group in C2pyr since trialkylated compounds could be observed in the reaction mixture (according to UPLC-MS). Hence, the protection of this group was necessary for further progress. As the protecting group, benzyl was selected with regard to the required stability against electrophiles and nucleophiles. However, the standard deprotection using catalytic hydrogenation with Pd/C failed, therefore, the *p*-methoxybenzyl (PMB) group was selected to achieve easier deprotection.[146] Nevertheless, even the PMB group appeared to be relatively stable since it was resistant to catalytic (Pd/C) hydrogenation in glacial acetic acid, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and cerium(IV) ammonium nitrate (CAN).[146–148] Finally, the deprotection was achieved by trifluoroacetic acid (TFA).

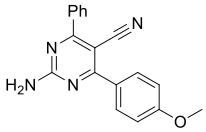
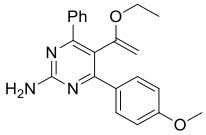
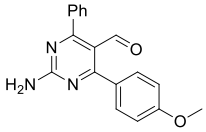
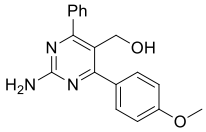
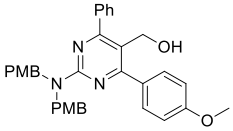
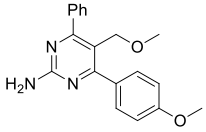
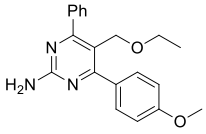
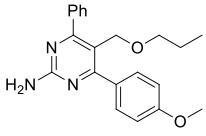
Thus, the final synthetic route backbone securing all desired products for increased solubility purposes (ISP) (Scheme 10) consisted of the one-pot diarylated scaffold condensation, bromination, vinylation, and ozonolysis.[12,107,135,145,149]

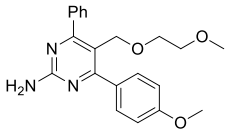
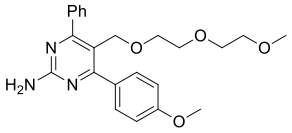
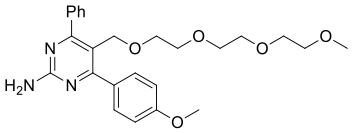


Scheme 10. The final synthetic route securing all desired products for ISP

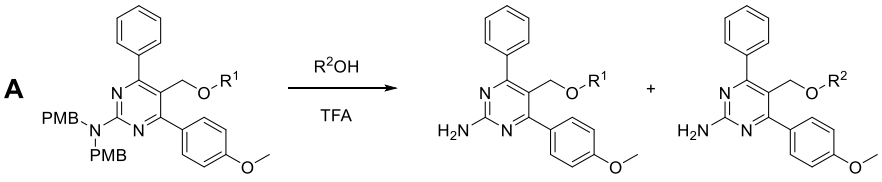
The protection was performed on the vinyl derivative according to Kang and co-workers[150] and subsequent ozonolysis afforded the key hydroxymethyl intermediate **24**.[149] The protected hydroxymethyl analogue **24** was utilized as the starting compound for subsequent nucleophilic substitutions and after the deprotection using TFA the remaining final products were afforded (Table 10).[150,151]

Table 10. Compounds prepared for ISP

Entry	Compound	Structure	Yield [%]	Calculated log *P* values	Measured solubility [µmol/L]	PGE_2 in % of LPS stimulated control response	Viability in % of control response
1	**19**		28	3.13 ±0.30	100	10.07 ±0.95	107.60 ±4.10
2	**20**		61	4.18 ±0.48	30	16.78 ±0.39	104.36 ±1.65
3	**22**		29	3.06 ±0.32	50	36.61 ±0.75	107.94 ±1.61
4	**23**		80	2.77 ±0.26	200	55.00 ±7.48	100.45 ±2.93
5	**24**		69	n/a	n/a	15.47 ±2.93	96.20 ±3.15
6	**25a**		52	3.25 ±0.28	50	20.78 ±5.15	101.01 ±2.40
7	**25b**		33	3.62 ±0.31	200	53.63 ±4.19	92.06 ±0.84
8	**26**		35	4.02 ±0.34	20	36.12 ±6.04	94.78 ±1.47

9	**25c**		37	3.04 ±0.28	15	40.99 ±5.13	104.47 ±1.66
10	**25d**		19	2.87 ±0.33	100	59.62 ±3.95	98.43 ±2.05
11	**25e**		15	2.70 ±0.41	300	21.60 ±2.98	97.54 ±1.92

An interesting situation was observed during the deprotection. Under acidic conditions of TFA, the decomposition of alkoxy chain was observed (according to UPLC-MS) giving the hydroxymethyl derivative. Furthermore, the addition of alcohol (MeOH or EtOH) resulted in partial re-etherification (Scheme 11).



Scheme 11. (Re-)etherification under acidic conditions of TFA observed in UPLC-MS

This knowledge was used in the synthesis of **26** where **24** was exposed to TFA, subsequently, *n*-propanol was added resulting in a successful transformation to propyl ether derivative (Scheme 10).

In conclusion, although many synthetic approaches failed, suitable synthetic route securing all desired products was successfully developed.

4.4.2. Solubility measurements

The reason for the synthesis of the C5pyr modified analogues of WQE-134 was to increase the solubility. Hence, the solubility of synthesized compounds was determined. For each compound, the stock solution (10 mM, eventually 20 mM for more

soluble compounds) in DMSO was prepared and 20 min sonicated. Working solutions (2, 5, 10, 20, 50, 100, and 200 µM) were prepared by dilution of appropriate stock solution amount in water, subsequently agitated for 24 h and in the case of visible cloud sonicated for 20 min.

Samples were analyzed by liquid chromatography coupled with mass spectrometer on C_{18}-reversed phase (using acidified MeCN (0.05 % FA) and acidified water (0.05 % FA) as eluents). The solubility was determined based on the linearity of response (peak surface). In the case of linearity break, the previous concentration was considered as limiting.

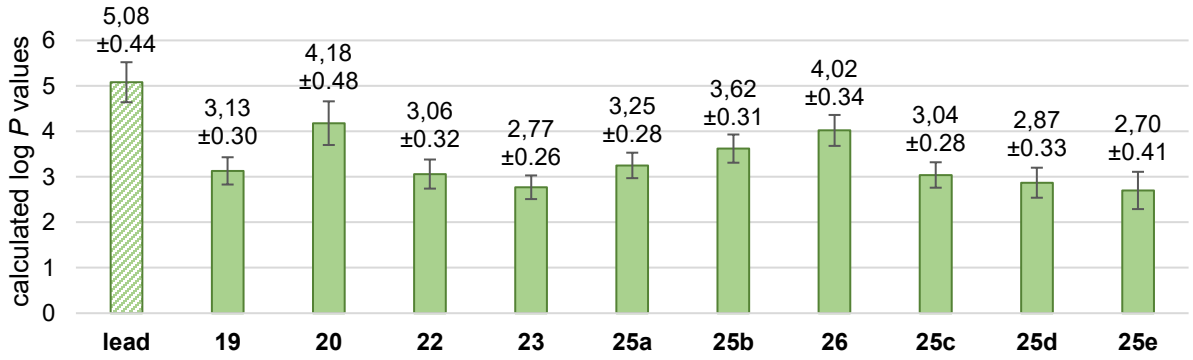


Figure 14. Log *P* values calculated for molecules prepared for ISP using VCCLAB

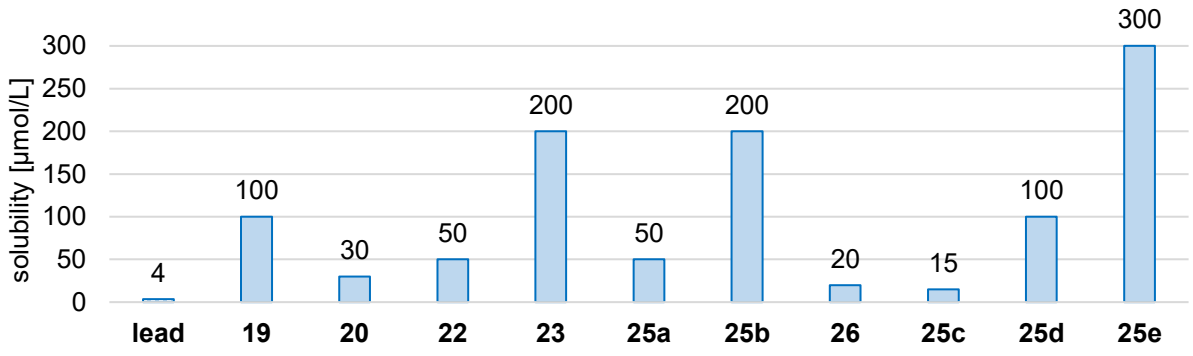


Figure 15. Experimental solubility values of molecules prepared for ISP [µmol/L]

The measured solubility data (Figure 15) were compared with calculated log *P* values (Figure 14). The experimental data correlated with the log *P* calculated values except for **19** which was slightly more soluble than expected, **25b** which was more soluble than expected and **25c** which was less soluble than expected. The most soluble

compound was found to be **25e** which is in agreement with calculated log *P* values. In addition, Figure 16 shows increased hydrophilic surface of **25e** resulting in two orders of magnitude higher solubility compared to WQE-134.

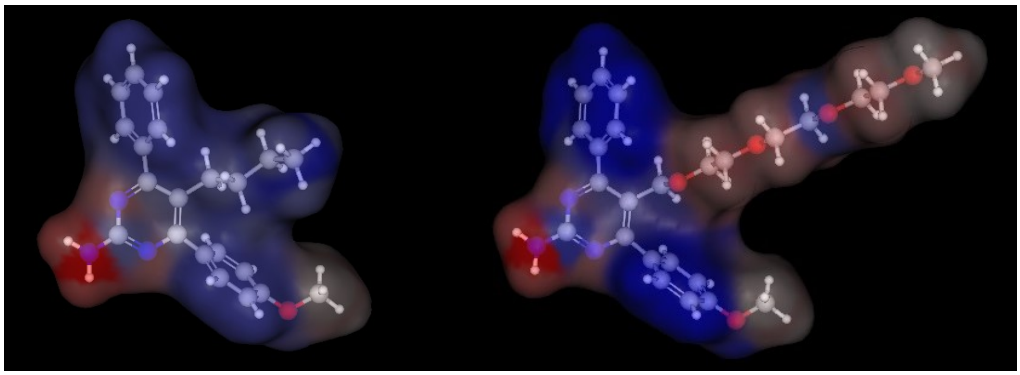


Figure 16. Polarity maps of the WQE-134 (left) and **25e** (right) (generated by MarvinSketch, ChemAxon software), blue colour indicates lipophilic areas, red colour indicates hydrophilic area

In conclusion, all modified compounds were more soluble than the lead structure. In agreement with calculated log P values, the most soluble compound proved to be **25e** achieving two orders of magnitude higher solubility compared to WQE-134.

4.4.3. Biochemical data

Molecules prepared for ISP were also tested *in vitro* for inhibition of PGE_2 production and viability (Figure 17). The data suggest that introduction of heteroatom bearing moiety into C5pyr leads to a considerable decrease of activity. Additionally, the fact that no structure-activity relationship was observed, is also noteworthy. In the sequence **25a**-**25b**-**26** or **25c**-**25d**-**25e**, there was no correlation observed.

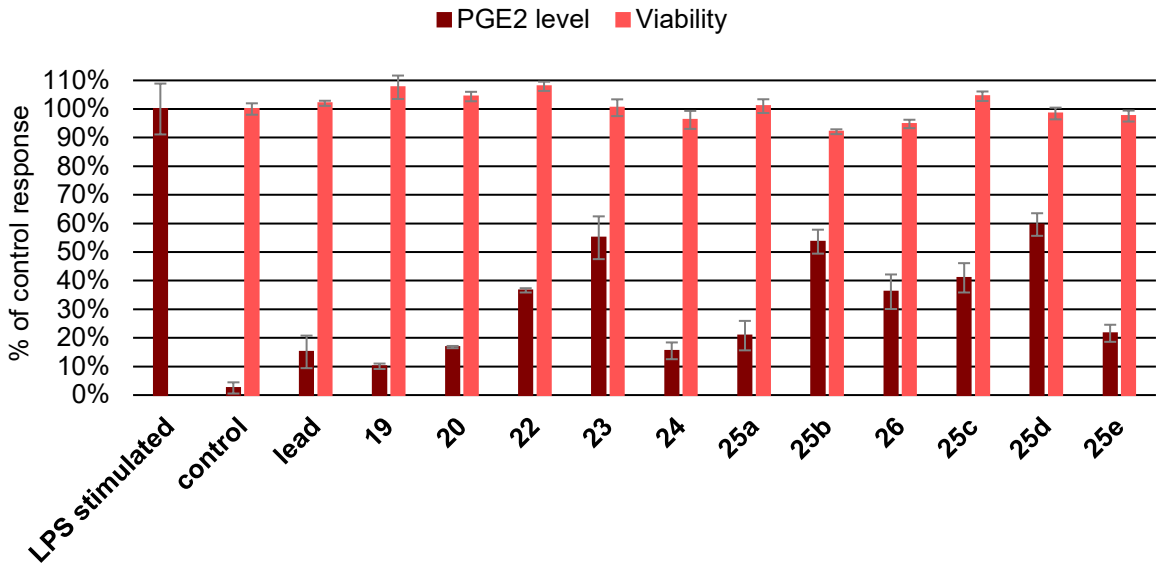


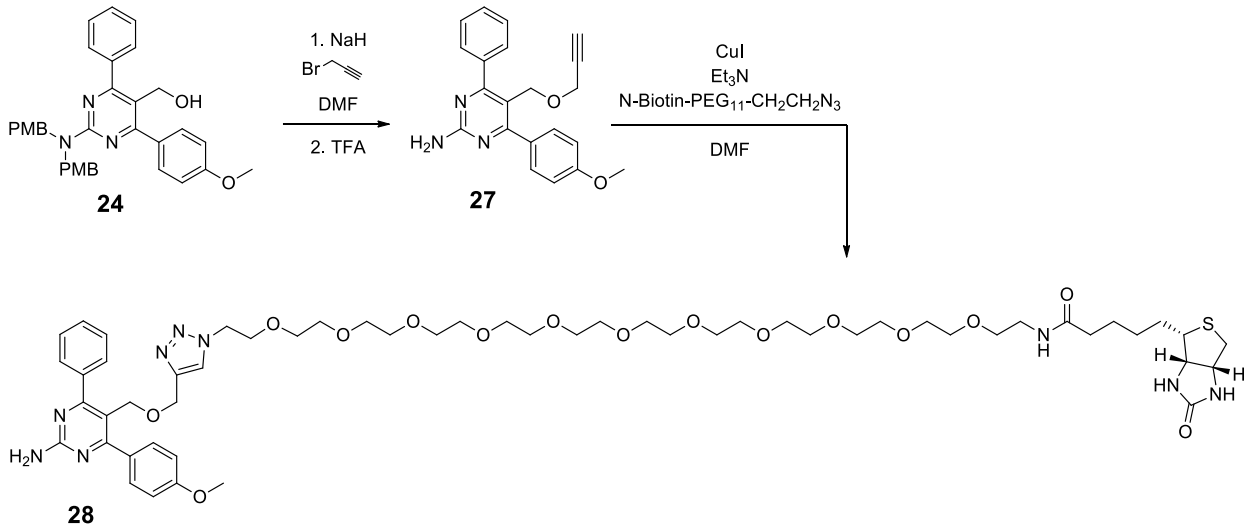
Figure 17. Effect of compounds prepared for ISP on *in vitro* production of PGE_2 and viability of mouse peritoneal cells. Effects are expressed as a percentage change relative to the response of LPS stimulated or unstimulated control cells. Bars are means ± SEM obtained by averaging results of two to four experiments for each compound.

In conclusion, it appears that the introduction of a heteroatom bearing moiety in C5pyr interferes with the biological activity.

4.5. The biotinylated probe for pulldown experiments

To clarify the mechanism of action, the pulldown experiments were designed. Therefore, it was necessary to prepare the biotinylated probes for these experiments. Because the mechanism of action is so far unclear, the pharmacophore is unknown as well, thus, biotin was attached to three different positions of WQE-134 via pegylated linker. The “click” reaction was applied to attach biotin moiety to the parent molecule.[152] The linker was bearing biotin and azide on opposite sides while the parent molecule had to be derived in order to obtain triple bond.

The derivatives biotinylated in positions C2pyr and C4pyr were prepared by Dr. Kolman. The derivative bearing biotin moiety linked to C5pyr was afforded using **24** as the starting compound for nucleophilic substitution using propargyl bromide.[151] After deprotection, **27** was obtained in excellent yield and further utilized in the "click" reaction with the biotinylated azido linker to afford **28** (Scheme 12).[150,152] The biotinylated probes will be used in pulldown experiments.



Scheme 12. Synthesis of WQE-134 biotinylated in C5pyr as probe for pulldown experiments

5. Experimental part

5.1. Materials and instruments

All used solvents and chemicals were purchased from commercial suppliers and were not further purified. Reactions were monitored using thin-layer chromatography (TLC) on silica gel 60 F254 plates (Merck KGaA, Germany) and ultra-high performance liquid chromatography with mass spectrometer (UPLC-MS Acquity Waters, USA, H-Class Core System with Waters Acquity UPLC BEH C18 1.7 µm, 2.1x100 mm column, Waters Acquity UPLC PDA detector, and mass spectrometer Waters SQD2). The column and flash chromatography were performed on 60A silica gel (Acros Organics, Belgium) or using RediSep Rf 50g HP C18 Aq column (Teledyne Isco, USA). Solvents were evaporated using rotary evaporator at 40-70 °C.

Microwave heating (MW) was performed using microwave reactor Discover (CEM, USA) with the Explorer module. The reactor frequency is 2.45 GHz and radiation power up to 300 W. Reactions were stirred in the reactor. Temperature and pressure were monitored by infrared temperature sensor (outside the reaction mixture) and CEM Explorer pressure sensor, respectively.

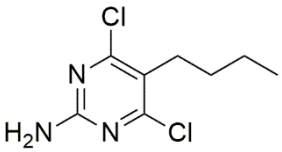
NMR spectra were measured using Bruker Avance III HD 400 MHz equipped with Prodigy cryo-probe operating at 9.39 T or Bruker Avance III HD 500 MHz equipped with Cryoprobe operation at 11.74 T in DMSO-d6 solution. At the 400 MHz spectrometer, ^{1}H and ^{13}C spectra were acquired at 401.00 MHz and 100.84 MHz, respectively. At the 500 MHz spectrometer, ^{1}H and ^{13}C spectra were acquired at 499.98 MHz and 125.73 MHz, respectively. ^{19}F spectra were measured at 377.28 MHz. Two-dimensional spectra ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC were acquired for assignment purposes. Chemical shifts (δ) are listed in ppm, interaction constants (J) in Hz.

High-resolution mass spectra were obtained using LTQ Orbitrap XL (Thermo Fisher Scientific, USA) for ESI ionization and GCT Premier (Waters, USA) for EI ionization. Infrared spectra were measured in $CHCl_3$ using Nicolet 6700 FT-IR

spectrometer (Thermo Scientific, USA). Melting points were determined using Stuart melting point apparatus SMP3.

5.2. Synthesis of WQE-134 analogues with substituted phenyl moiety in the position C4 of pyrimidine

2-Amino-5-butyl-4,6-dichloropyrimidine (1)

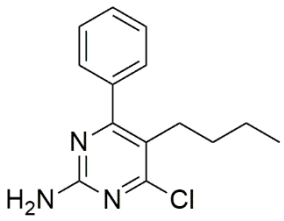


After dissolution of metallic sodium (22.76 g, 989.75 mmol, 5 eq.) in anhydrous MeOH (1 L) under argon atmosphere, guanidin hydrochloride (18.91 g, 197.95 mmol, 1 eq.) was added to the solution. Subsequently, diethyl 2-butylmalonate (43.55 mL, 197.95 mmol, 1 eq.) was added and the reaction mixture was stirred at reflux for 2 h. Volatiles were evaporated, the residue was dissolved in water and the solution neutralised with acetic acid (99%) to precipitate the product. The solid was filtered off, washed with water (3 × 200 mL) and cold EtOH (3 × 200 mL) to obtain 2-amino-5-butyl-4,6-dihydroxypyrimidine (32.40 g, 89 %) as a white solid. To the mixture of 2-amino-5-butyl-4,6-dihydroxypyrimidine (32.40 g, 176.85 mmol, 1 eq.) in $CHCl_3$ (1 L), the (chloromethylene)dimethyliminium chloride (226.56 g, 1.77 mol, 10 eq.) was added and the reaction mixture was stirred at reflux for 16 h. The mixture was let to cool to 25 °C, poured into ice and stirred while slowly adding saturated solution of $NaHCO_3$ (1.5 L). After hydrolysis of the (chloromethylene)dimethyliminium chloride, the mixture was extracted with $CHCl_3$ (3 × 1 L). Organic fractions were combined and dried over $MgSO_4$. The solid was filtered off, the filtrate was evaporated and then dissolved in EtOH. Into the solution, 37% HCl (58.64 mL, 707.40 mmol, 4 eq.) was added and the reaction mixture was stirred at 50 °C for 2 h. After that, water was added slowly to precipitate the product. The solid was filtered off and washed with water (3 × 250 mL) to afford **1** (32.05 g, 83 %) as a brownish solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.32 (s, 2H, NH_2), 2.63 – 2.57 (m, 2H, HetAr-CH_2-CH_2), 1.50 – 1.41 (m, 2H, CH_2-CH_2-CH_2), 1.41 – 1.29 (m, 2H, CH_2-CH_2-CH_3),

0.92 (t, *J* = 7.3 Hz, 3H, CH_2-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 161.23, 161.07, 117.99, 30.69, 28.78, 22.36, 14.15. **IR** ($CHCl_3$) *v*: 3538, 3424, 2962, 1608, 1579, 1516, 1465, 1356 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_8H_{11}Cl_2N_3$ 219.0330, found 219.0332. **UPLC-MS** (*m/z*) $[M+H]^+$ 219.85, t_R = 4.79 min.

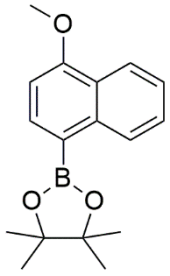
2-Amino-5-butyl-4-chloro-6-phenylpyrimidine (2)



Compound **1** (21.37 g, 97.09 mmol, 1 eq.), phenylboronic acid (17.76 g, 145.64 mmol, 1.5 eq.), Na_2CO_3 (25.73 g, 242.73 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (1.12 g, 0.97 mmol, 1 mol%) in a EtOH-toluene mixture (1:3 ratio) was stirred under argon atmosphere at 80 °C for 16 h. Solvents were evaporated and the residue was separated using silica gel column chromatography (isocratic elution 40% EtOAc in hexane). After recrystallization from hexane, compound **2** (16.63 g, 66 %) was obtained as a yellow solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.51 – 7.39 (m, 5H, Ar*H*), 6.95 (s, 2H, N*H*$_2$), 2.49 – 2.43 (m, 2H, HetAr-C*H*$_2$-CH$_2$), 1.41 – 1.31 (m, 2H, CH_2-C*H*$_2$-CH$_2$), 1.20 – 1.08 (m, 2H, CH_2-C*H*$_2$-CH$_3$), 0.72 (t, *J* = 7.3 Hz, 3H, CH_2-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 169.08, 161.55, 161.41, 139.00, 129.19, 128.54, 128.43, 118.38, 31.64, 27.97, 22.29, 13.84. **IR** ($CHCl_3$) *v*: 3533, 3422, 2961, 1603, 1568, 1526, 1495, 1458, 1328, 701 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{14}H_{16}ClN_3$ 261.1033, found 261.1031. **UPLC-MS** (*m/z*) $[M+H]^+$ 261.86, t_R = 4.94 min. **mp** = 105-107 °C.

2-(4-Methoxynaphthalen-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3)

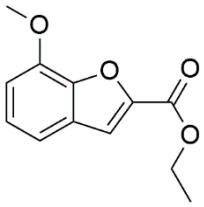


1-Methoxynaphtalene (0.92 mL, 6.32 mmol, 1 eq.), NBS (1.18 g, 6.64 mmol, 1.05 eq.) and a catalytic amount of AIBN in MeCN were stirred at 25 °C for 2.5 h. The solvent was evaporated and the residue was purified using silica gel flash chromatography (linear gradient elution 0-5 % EtOAc in hexane). The brominated intermediate (300 mg, 1.27 mmol, 1 eq.) was then allowed to react with

4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi-1,3,2-dioxaborolane (452 mg, 1.78 mmol, 1.4 eq.), K_3PO_4 (405 mg, 1.91 mmol, 1.5 eq.) and $Pd(Ph_3P)_4$ (15 mg, 12.70 µmol, 1 mol%) in anhydrous DMF and the reaction mixture was stirred at 120 °C for 1 h. The solvent was evaporated and the residue was purified using silica gel flash chromatography (linear gradient elution 0-15 % EtOAc in hexane) to afford **3** (239 mg, 66 %) as a brownish solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 8.65 (dt, *J* = 8.4, 1.1 Hz, 1H, Ar*H*), 8.19 (ddd, *J* = 8.3, 1.5, 0.7 Hz, 1H, Ar*H*), 7.96 (d, *J* = 7.8 Hz, 1H, Ar*H*), 7.58 (ddd, *J* = 8.4, 6.8, 1.5 Hz, 1H, Ar*H*), 7.50 (ddd, *J* = 8.2, 6.8, 1.3 Hz, 1H, Ar*H*), 7.00 (d, *J* = 7.9 Hz, 1H, Ar*H*), 4.01 (s, 3H, O-C*H*$_3$), 1.37 (s, 12H, C-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 158.12, 137.92, 137.57, 128.19, 127.37, 125.48, 125.11, 122.08, 104.32, 83.72, 56.15, 25.19. **IR** ($CHCl_3$) *v*: 3077, 3048, 2981, 1620, 1577, 1511, 1343, 1262, 1243, 1144, 1088, 969, 858, 822 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{17}H_{21}O_3B$ 284.1584, found 284.1582. **UPLC-MS** (*m/z*) $[M+H]^+$ 285.27, t_R = 5.55 min.

Ethyl 7-methoxybenzofuran-2-carboxylate (4)

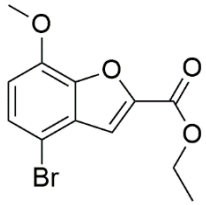


Ethyl 2-chloroacetate (0.92 mL, 8.54 mmol, 1.3 eq.), *o*-vanillin (1.00 g, 6.57 mmol, 1 eq.) and Cs_2CO_3 (4.28 g, 13.14 mmol, 2 eq.) in anhydrous DMF were stirred at 80 °C for 16 h. Volatiles were evaporated, the residue mixed with DCM and washed with water (3 × 250 mL). The organic fraction was dried over $MgSO_4$ and evaporated to yield **4** (1.22 g, 84 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.74 (s, 1H, HetAr*H*), 7.34 (dd, *J* = 7.9, 1.1 Hz, 1H, HetAr*H*), 7.28 (t, *J* = 7.8 Hz, 1H, HetAr*H*), 7.12 (dd, *J* = 7.8, 1.1 Hz, 1H, HetAr*H*), 4.37 (q, *J* = 7.1 Hz, 2H, O-C*H*$_2$-CH$_3$), 3.97 (s, 3H, O-C*H*$_3$), 1.34 (t, *J* = 7.1 Hz, 3H, O-CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 159.05, 145.85, 145.58, 144.97, 128.63, 125.26, 115.21, 114.81, 109.86, 61.69, 56.32, 14.60. **IR** ($CHCl_3$) *v*: 2842, 1725, 1624, 1596, 1581, 1571, 1494, 1325, 1307, 1273, 1231, 1199, 1096, 977 cm^{-1}. **HRMS** (EI)

m/z calcd for $C_{12}H_{12}O_4$ 220.0736, found 220.0738. **UPLC-MS** (*m/z*) $[M+H]^+$ 299.05, t_R = 5.31 min.

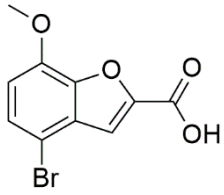
Ethyl 4-bromo-7-methoxybenzofuran-2-carboxylate (5)



Compound **4** (500 mg, 2.27 mmol, 1 eq.), NBS (424 mg, 2.38 mmol, 1.05 eq.) and a catalytic amount of AIBN in MeCN were stirred at 25 °C for 16 h. The solvent was evaporated, the residue was mixed with EtOAc-hexane (1:9 ratio) and filtered over silica gel column (3 cm high, 3.5 cm diameter). After evaporation, **5** (671 mg, 99 %) was obtained as a brown-orange solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.58 (s, 1H, HetAr*H*), 7.52 (d, *J* = 8.4 Hz, 1H, HetAr*H*), 7.12 (d, *J* = 8.5 Hz, 1H, HetAr*H*), 4.39 (q, *J* = 7.1 Hz, 2H, O-C*H*$_2$-CH$_3$), 3.98 (s, 3H, O-C*H*$_3$), 1.36 (t, *J* = 7.1 Hz, 3H, O-CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 158.58, 146.29, 145.62, 144.66, 129.28, 127.82, 113.73, 111.49, 104.99, 62.04, 56.75, 14.55. **IR** ($CHCl_3$) *v*: 2844, 1730, 1620, 1592, 1578, 1490, 1329, 1302, 1256, 1192, 1185, 1086, 915 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{12}H_{11}O_4Br$ 297.9841, found 297.9843. **UPLC-MS** (*m/z*) $[M+H]^+$ 299.05, t_R = 5.31 min.

4-Bromo-7-methoxybenzofuran-2-carboxylic acid (6)

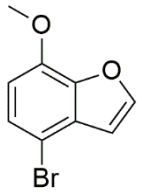


Compound **5** (500 mg, 1.67 mmol, 1 eq.) was dissolved in a mixture acetone-water (4:1 ratio), NaOH (200 mg, 5.01 mmol, 3 eq.) was then added to the solution and the mixture was sonicated for 1 min. Acetic acid (99%) was added dropwise to precipitate the product. After filtration, **6** (424 mg, 94 %) was readily obtained as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 13.84 (s, 1H, COO*H*), 7.54 – 7.43 (m, 2H, HetAr*H*), 7.08 (dd, *J* = 8.6, 1.8 Hz, 1H, HetAr*H*), 3.97 (s, 3H, O-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.94, 147.40, 145.60, 144.57, 129.45, 127.58, 113.08, 111.20, 104.91, 56.73. **IR** ($CHCl_3$) *v*: 3512, 2928, 1731, 1492, 1303, 1185, 1087 cm^{-1}. **HRMS** (ESI)

[M-H]$^-$ *m/z* calcd for $C_{10}H_6O_4Br$ 268.9455, found 268.9456. **UPLC-MS** (*m/z*) [M-H]$^-$ 268.70, t_R = 4.52 min.

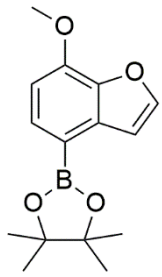
4-Bromo-7-methoxybenzofuran (7)



Compound **6** (100 mg, 0.37 mmol, 1 eq.) and metallic copper (4 mg, 55.50 µmol, 15 mol%) in quinoline (0.5 mL) were heated at 180 °C under microwave conditions for 30 min. The mixture was filtered over celite pad and the filtrate was purified using silica gel flash chromatography (linear gradient elution 0-5 % EtOAc in hexane) to afford **7** (75 mg, 89 %) as a colorless liquid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 8.10 (d, *J* = 2.1 Hz, 1H, HetAr*H*), 7.39 (d, *J* = 8.5 Hz, 1H, HetAr*H*), 6.93 (d, *J* = 8.5 Hz, 1H, HetAr*H*), 6.90 (d, *J* = 2.2 Hz, 1H, HetAr*H*), 3.94 (s, 3H, O-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 147.42, 145.37, 129.92, 126.57, 108.84, 107.24, 103.97, 56.59. **IR** ($CHCl_3$) *v*: 3036, 2966, 2845, 1620, 1588, 1484, 1398, 1335, 1284, 1201, 1183, 1091, 1034, 896 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_9H_7O_2Br$ 225.9629, found 225.9628.

2-(7-Methoxybenzofuran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (8)



Compound **7** (75 mg, 0.33 mmol, 1 eq.), 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi-1,3,2-dioxaborolane (117 mg, 0.46 mmol, 1.4 eq.), K_3PO_4 (106 mg, 0.50 mmol, 1.5 eq.) and $Pd(Ph_3P)_4$ (19 mg, 16.50 µmol, 5 mol%) in anhydrous DMF were stirred at 120 °C for 1 h. The solvent was evaporated and the residue was purified using silica gel flash chromatography (linear gradient elution 0-15 % EtOAc in hexane) to yield **8** (68 mg, 75 %) as a white solid.

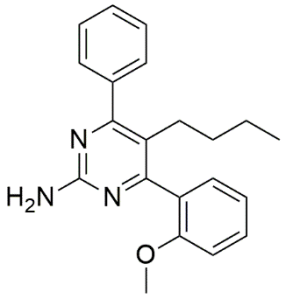
^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.98 (d, *J* = 2.1 Hz, 1H, HetAr*H*), 7.55 (d, *J* = 7.9 Hz, 1H, HetAr*H*), 7.08 (d, *J* = 2.1 Hz, 1H, HetAr*H*), 6.95 (d, *J* = 8.0 Hz, 1H, HetAr*H*), 3.96 (s, 3H, O-C*H*$_3$), 1.32 (s, 12H, C-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 148.16, 146.70, 143.27, 134.26, 132.40, 108.76, 106.82, 83.74, 56.26, 25.21. **IR** ($CHCl_3$) *v*: 2982, 1621, 1580, 1399, 1372, 1328, 1283, 1182, 1144, 1134, 1090, 1032 cm^{-1}. **HRMS** (EI) *m/z*

calcd for $C_{15}H_{19}O_4B$ 274.1376, found 274.1377. **UPLC-MS** (*m/z*) $[M+H]^+$ 275.24, t_R = 5.84 min.

General procedure for the Suzuki-Miyaura coupling reaction (A)

A water-(1,4-dioxane) mixture in the ratio 1:4 was bubbled with argon for 2 min. Starting compound **2** (1 eq.), corresponding boronic acid (1.4 eq.), Cs_2CO_3 (2.5 eq.) and $Pd(Ph_3P)_4$ (2.5 mol%) were added and the reaction mixture was stirred at 110 °C for 16 h. Solvents were evaporated, the residue was twice co-distilled with EtOH and purified using silica gel flash chromatography (linear gradient elution 0-60 % EtOAc in hexane) to afford the desired product.

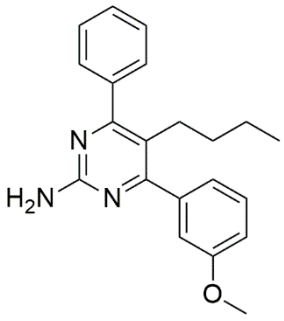
2-Amino-5-butyl-4-(2-methoxyphenyl)-6-phenylpyrimidine (9a)



The reaction of **2** (300 mg, 1.15 mmol, 1 eq.), 2-methoxyphenylboronic acid (245 mg, 1.61 mmol, 1.4 eq.), Cs_2CO_3 (938 mg, 2.88 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (33 mg, 28.75 µmol, 2.5 mol%) following the procedure A gave **9a** (260 mg, 68 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.50 – 7.38 (m, 6H, Ar*H*), 7.20 (dd, *J* = 7.4, 1.8 Hz, 1H, Ar*H*), 7.11 (dd, *J* = 8.4, 1.0 Hz, 1H, Ar*H*), 7.03 (td, *J* = 7.4, 1.0 Hz, 1H, Ar*H*), 6.45 (s, 2H, NH_2), 3.75 (s, 3H. OCH_3), 2.33 – 2.14 (m, 2H, HetAr-CH_2-CH_2), 1.07 – 0.87 (m, 2H, CH_2-CH_2-CH_2), 0.87 – 0.75 (m, 2H, CH_2-CH_2-CH_3), 0.43 (t, *J* = 7.3 Hz, 3H, CH_2-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.82, 166.74, 161.63, 156.22, 140.05, 130.07, 130.04, 128.92, 128.68, 128.59, 128.46, 120.56, 120.02, 111.53, 55.72, 32.06, 27.17, 22.14, 13.56. **IR** ($CHCl_3$) *v*: 3527, 3419, 2960, 1600, 1548, 1495, 1447, 1435, 1248, 1050, 702 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{21}H_{23}N_3O$ 333.1841, found 333.1844. **UPLC-MS** (*m/z*) $[M+H]^+$ 334.12, t_R = 4.89 min.

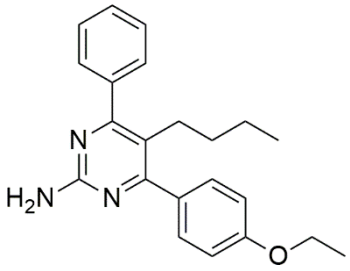
2-Amino-5-butyl-4-(3-methoxyphenyl)-6-phenylpyrimidine (9b)



The reaction of **2** (300 mg, 1.15 mmol, 1 eq.), 3-methoxyphenylboronic acid (245 mg, 1.61 mmol, 1.4 eq.), Cs_2CO_3 (938 mg, 2.88 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (33 mg, 28.75 µmol, 2.5 mol%) following the procedure A gave **9b** (290 mg, 76 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.50 – 7.35 (m, 6H, Ar*H*), 7.06 – 6.98 (m, 3H, Ar*H*), 6.53 (s, 2H, N*H*$_2$), 3.80 (s, 3H, OC*H*$_3$), 2.48 – 2.42 (m, 2H, HetAr-C*H*$_2$-CH$_2$), 1.04 – 0.93 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 0.90 – 0.78 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.46 (t, *J* = 7.3 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.08, 167.86, 161.46, 159.31, 141.45, 140.10, 129.61, 128.70, 128.61, 128.44, 120.90, 118.59, 114.40, 114.04, 55.66, 32.29, 26.98, 22.06, 13.58. **IR** ($CHCl_3$) *v*: 3527, 3420, 2961, 1601, 1547, 1497, 1448, 1430, 1257, 1042, 701 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{21}H_{23}N_3O$ 333.1841, found 333.1842. **UPLC-MS** (*m/z*) $[M+H]^+$ 333.99, t_R = 4.76 min.

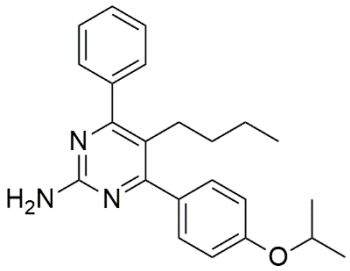
2-Amino-5-butyl-4-(4-ethoxyphenyl)-6-phenylpyrimidine (9c)



The reaction of **2** (100 mg, 0.38 mmol, 1 eq.), 4-ethoxyphenylboronic acid (88 mg, 0.53 mmol, 1.4 eq.), Cs_2CO_3 (310 mg, 0.95 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (11 mg, 9.50 µmol, 2.5 mol%) following the procedure A gave **9c** (83 mg, 63 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.50 – 7.42 (m, 7H, Ar*H*), 7.03 – 6.98 (m, 2H, Ar*H*), 6.47 (s, 2H, N*H*$_2$), 4.08 (q, *J* = 7.0 Hz, 2H, O-C*H*$_2$-CH$_3$), 1.36 (t, *J* = 7.0 Hz, 3H, O-CH$_2$-C*H*$_3$), 1.02 – 0.92 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 0.90 – 0.79 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.47 (t, *J* = 7.3 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.04, 167.60, 161.48, 158.97, 140.25, 132.30, 130.20, 128.65, 128.42, 118.57, 114.23, 63.53, 32.18, 27.02, 22.02, 15.11, 13.63. **IR** ($CHCl_3$) *v*: 3527, 3419, 2962, 1600, 1544, 1513, 1249, 1175, 702 cm^{-1}. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{22}H_{26}N_3O$ 348.2070, found 348.2071. **UPLC-MS** (*m/z*) $[M+H]^+$ 348.01, t_R = 4.84 min.

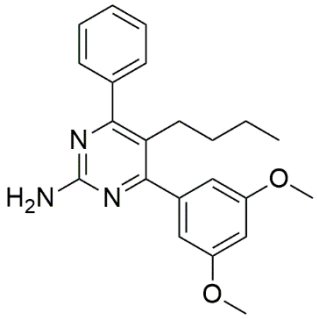
2-Amino-5-butyl-4-(4-isopropoxyphenyl)-6-phenylpyrimidine (9d)



The reaction of **2** (100 mg, 0.38 mmol, 1 eq.), 4-isopropoxyphenylboronic acid (95 mg, 0.53 mmol, 1.4 eq.), Cs_2CO_3 (310 mg, 0.95 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (11 mg, 9.50 µmol, 2.5 mol%) following the procedure A gave **9d** (114 mg, 83 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.50 – 7.40 (m, 7H, Ar*H*), 7.02 – 6.96 (m, 2H, Ar*H*), 6.46 (s, 2H, N*H*$_2$), 4.69 (p, *J* = 6.0 Hz, 1H, OC*H*-(CH$_3$)$_2$), 1.30 (d, *J* = 6.0 Hz, 6H, OCH-(C*H*$_3$)$_2$), 1.03 – 0.92 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 0.90 – 0.80 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.47 (t, *J* = 7.3 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.02, 167.61, 161.48, 157.91, 140.26, 132.17, 130.23, 128.64, 128.42, 118.57, 115.41, 69.66, 32.18, 27.00, 22.26, 22.02, 13.59. **IR** ($CHCl_3$) *v*: 3527, 3419, 2979, 2962, 1600, 1545, 1511, 1247, 1182, 702 cm^{-1}. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{23}H_{28}N_3O$ 362.2227, found 362.2227. **UPLC-MS** (*m/z*) $[M+H]^+$ 362.00, t_R = 4.99 min.

2-Amino-5-butyl-4-(3,5-dimethoxyphenyl)-6-phenylpyrimidine (9e)

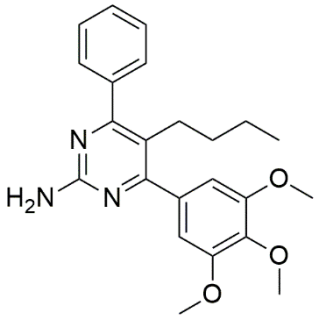


The reaction of **2** (300 mg, 1.15 mmol, 1 eq.), 3,5-dimethoxyphenylboronic acid (293 mg, 1.61 mmol, 1.4 eq.), Cs_2CO_3 (938 mg, 2.88 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (33 mg, 28.75 µmol, 2.5 mol%) following the procedure A gave **9e** (338 mg, 81 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.50 – 7.42 (m, 5H, Ar*H*), 6.60 (d, *J* = 2.3 Hz, 2H, Ar*H*), 6.56 (t, *J* = 2.3 Hz, 1H, Ar*H*), 6.53 (s, 2H, N*H*$_2$), 3.78 (s, 6H, OC*H*$_3$), 2.48 – 2.42 (m, 2H, HetAr-C*H*$_2$-CH$_2$), 1.06 – 0.95 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 0.93 – 0.80 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.48 (t, *J* = 7.3 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.08, 167.86, 161.42, 160.52, 142.05, 140.10, 128.70, 128.60, 128.43, 118.54, 106.65, 100.64, 55.81, 32.35, 27.03, 22.11, 13.61. **IR** ($CHCl_3$) *v*: 3528, 3419, 2961, 1598, 1547, 1457, 1424, 1156, 1056, 702 cm^{-1}. **HRMS** (ESI) *m/z*

[M+H]$^+$ calcd for $C_{22}H_{26}N_3O_2$ 364.2020, found 364.2020. **UPLC-MS** (*m/z*) [M+H]$^+$ 363.96, t_R = 4.81 min.

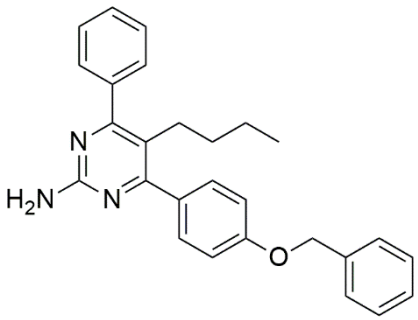
2-Amino-5-butyl-4-(3,4,5-trimethoxyphenyl)-6-phenylpyrimidine (9f)



The reaction of **2** (300 mg, 1.15 mmol, 1 eq.), 3,4,5-trimethoxyphenylboronic acid (341 mg, 1.61 mmol, 1.4 eq.), Cs_2CO_3 (938 mg, 2.88 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (33 mg, 28.75 µmol, 2.5 mol%) following the procedure A gave **9f** (336 mg, 74 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.51 – 7.39 (m, 5H, Ar*H*), 6.77 (s, 2H, Ar*H*), 6.51 (s, 2H, N*H*$_2$), 3.80 (s, 6H, *m*-OC*H*$_3$), 3.71 (s, 3H, *p*-OC*H*$_3$), 2.49 – 2.45 (m, 2H), HetAr-C*H*$_2$-CH$_2$), 1.08 – 0.97 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 0.94 – 0.83 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.49 (t, *J* = 7.3 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.10, 167.85, 161.41, 152.94, 140.16, 137.89, 135.60, 128.69, 128.58, 128.43, 118.64, 106.19, 60.63, 56.47, 32.37, 27.19, 22.17, 13.62. **IR** ($CHCl_3$) *v*: 3528, 3420, 2962, 1600, 1588, 1546, 1506, 1496, 1464, 1413, 1314, 1237, 1130, 1002, 703 cm^{-1}. **HRMS** (ESI) *m/z* [M+H]$^+$ calcd for $C_{23}H_{28}N_3O_3$ 394.2125, found 394.2126. **UPLC-MS** (*m/z*) [M+H]$^+$ 394.05, t_R = 4.58 min.

2-Amino-5-butyl-4-(4-benzyloxyphenyl)-6-phenylpyrimidine (9g)

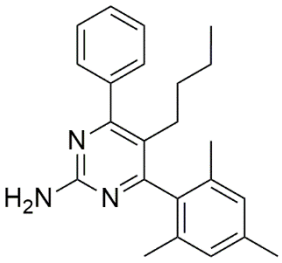


The reaction of **2** (300 mg, 1.15 mmol, 1 eq.), 4-benzyloxyphenylboronic acid (367 mg, 1.61 mmol, 1.4 eq.), Cs_2CO_3 (938 mg, 2.88 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (33 mg, 28.75 µmol, 2.5 mol%) following the procedure A gave **9g** (334 mg, 71 %) as a light-orange solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.51 – 7.31 (m, 12H, Ar*H*), 7.13 – 7.07 (m, 2H, Ar*H*), 6.47 (s, 2H, N*H*$_2$), 5.17 (s, 2H, O-C*H*$_2$-Ph), 1.02 – 0.92 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 0.90 – 0.78 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.47 (t, *J* = 7.3 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR**

(101 MHz, DMSO-d_6) δ 168.06, 167.54, 161.48, 158.78, 140.24, 137.40, 132.67, 130.20, 128.90, 128.64, 128.42, 128.32, 128.21, 118.59, 114.71, 69.71, 32.20, 27.03, 22.03, 13.63. **IR** ($CHCl_3$) *v*: 3527, 3419, 2961, 1600, 1545, 1512, 1496, 1377, 1245, 1174, 702 cm^{-1}. **HRMS** (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{28}N_3O$ 410.2227, found 410.2229. **UPLC-MS** (*m/z*) $[M+H]^+$ 410.03, t_R = 5.19 min.

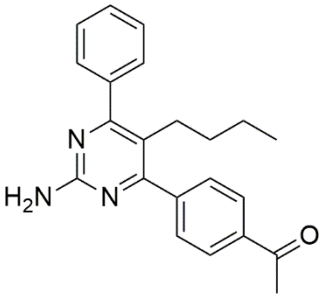
2-Amino-5-butyl-4-(2,4,6-trimethylphenyl)-6-phenylpyrimidine (9h)



The reaction of **2** (200 mg, 0.76 mmol, 1 eq.), 2,4,6-trimethylphenylboronic acid (174 mg, 1.06 mmol, 1.4 eq.), Cs_2CO_3 (619 mg, 1.90 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (22 mg, 19.00 µmol, 2.5 mol%) following the procedure A gave **9h** (223 mg, 85 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.50 – 7.40 (m, 5H, Ar*H*), 6.94 (s, 2H, Ar*H*), 6.49 (s, 2H, NH_2), 2.28 (s, 3H, Ar-(*p*-CH_3)), 2.18 – 2.11 (m, 2H, HetAr-CH_2-CH_2), 2.02 (s, 6H, Ar-*o*-CH_3), 1.00 – 0.91 (m, 2H, CH_2-CH_2-CH_2), 0.87 – 0.76 (m, 2H, CH_2-CH_2-CH_3), 0.44 (t, *J* = 7.3 Hz, 3H, CH_2-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 168.28, 167.74, 162.18, 140.09, 136.89, 136.23, 134.53, 128.71, 128.55, 128.46, 128.33, 31.64, 27.08, 22.20, 21.19, 19.98, 13.57. **IR** ($CHCl_3$) *v*: 3527, 3419, 2960, 1600, 1546, 1497, 1447, 1432, 702 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{23}H_{27}N_3$ 345.2205, found 345.2207. **UPLC-MS** (*m/z*) $[M+H]^+$ 346.32, t_R = 4.64 min.

2-Amino-5-butyl-4-(4-acetylphenyl)-6-phenylpyrimidine (9i)

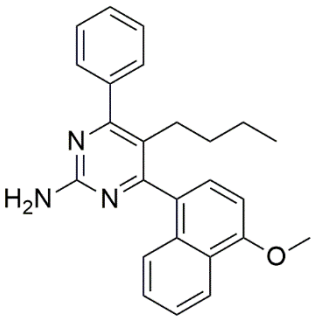


The reaction of **2** (200 mg, 0.76 mmol, 1 eq.), 4-acetylphenylboronic acid (174 mg, 1.06 mmol, 1.4 eq.), Cs_2CO_3 (619 mg, 1.90 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (22 mg, 19.00 µmol, 2.5 mol%) following the procedure A gave **9i** (202 mg, 77 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 8.09 – 8.04 (m, 2H, Ar*H*), 7.67 – 7.63 (m, 2H, Ar*H*), 7.52 – 7.43 (m, 5H, Ar*H*), 6.61 (s, 2H, NH_2), 2.64 (s, 3H C(O)-

C*H*$_3$), 2.49 – 2.42 (m, 2H, HetAr-C*H*$_2$-CH$_2$), 1.01 – 0.91 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 0.89 – 0.77 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.45 (t, *J* = 7.3 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 198.11, 168.38, 167.03, 161.52, 144.54, 139.93, 136.80, 129.10, 128.81, 128.62, 128.49, 128.46, 118.57, 32.27, 27.31, 26.88, 21.98, 13.57. **IR** ($CHCl_3$) *v*: 3529, 3420, 2963, 1684, 1601, 1544, 1448, 1437, 1268, 702 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{22}H_{23}N_3O$ 345.1841, found 345.1839. **UPLC-MS** (*m/z*) $[M+H]^+$ 346.07, t_R = 4.91 min.

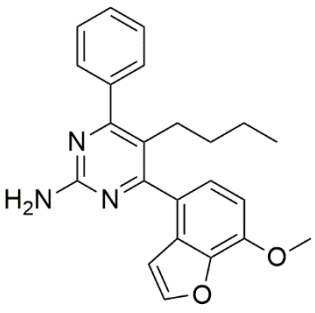
2-Amino-5-butyl-4-(4-methoxynaphthalen-1-yl)-6-phenylpyrimidine (9j)



The reaction of **2** (58 mg, 0.22 mmol, 1 eq.), (4-methoxy-1-naphthyl)boronic acid (**3**) (88 mg, 0.31 mmol, 1.4 eq.), Cs_2CO_3 (179 mg, 0.55 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (7 mg, 5.50 µmol, 2.5 mol%) following the procedure A gave **9j** (48 mg, 57 %) as a yellow solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 8.27 – 8.21 (m, 1H, Ar*H*), 7.59 – 7.40 (m, 9H, Ar*H*), 7.06 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.54 (s, 2H, N*H*$_2$), 4.03 (s, 3H, O-C*H*$_3$), 0.97 – 0.80 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 0.75 – 0.63 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.28 (t, *J* = 7.3 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 167.87, 167.70, 161.69, 155.15, 140.10, 131.99, 129.76, 128.73, 128.67, 128.47, 127.32, 126.81, 125.78, 125.00, 122.17, 120.32, 103.99, 56.16, 32.38, 27.36, 21.87, 13.45. **IR** ($CHCl_3$) *v*: 3527, 3419, 2961, 1600, 1585, 1544, 1497, 1455, 1447, 1435, 1365, 1270, 1088, 703 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{25}H_{26}N_3O$ 384.2070, found 384.2071. **UPLC-MS** (*m/z*) $[M+H]^+$ 384.13, t_R = 5.10 min.

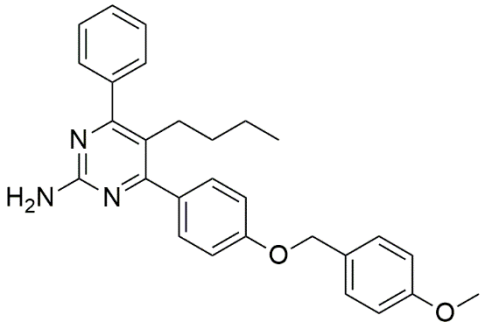
2-Amino-5-butyl-4-(7-methoxybenzofuran-4-yl)-6-phenylpyrimidine (9k)



The reaction of **2** (47 mg, 0.18 mmol, 1 eq.), **8** (68 mg, 0.25 mmol, 1.4 eq.), Cs_2CO_3 (147 mg, 0.45 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (5 mg, 4.50 µmol, 2.5 mol%) following the procedure A gave **9k** (20 mg, 30 %) as a yellow solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 8.02 (d, *J* = 2.1 Hz, 1H, HetAr*H*), 7.58 – 7.52 (m, 2H, Ar*H*), 7.52 – 7.42 (m, 3H, Ar*H*), 7.28 (d, *J* = 8.2 Hz, 1H, HetAr*H*), 7.03 (d, *J* = 8.3 Hz, 1H, HetAr*H*), 6.86 (d, *J* = 2.2 Hz, 1H, HetAr*H*), 6.53 (s, 2H, NH_2), 3.99 (s, 3H, O-CH_3), 2.48 – 2.42 (m, 2H, HetAr-CH_2-CH_2), 0.90 – 0.81 (m, 2H, CH_2-CH_2-CH_2), 0.79 – 0.68 (m, 2H, CH_2-CH_2-CH_3), 0.35 (t, *J* = 7.3 Hz, 3H, CH_2-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 161.58, 146.79, 145.56, 143.52, 140.13, 128.80, 128.75, 128.45, 128.20, 125.49, 124.35, 119.22, 107.28, 106.70, 56.37, 32.20, 27.07, 25.43, 21.75, 13.52. **IR** ($CHCl_3$) *v*: 3528, 3419, 1601, 1541, 1504, 1496, 1447, 1435, 1333, 1284, 1199, 1093, 703 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{23}H_{23}N_3O_2$ 373.1790, found 373.1788. **UPLC-MS** (*m/z*) $[M+H]^+$ 374.08, t_R = 4.72 min.

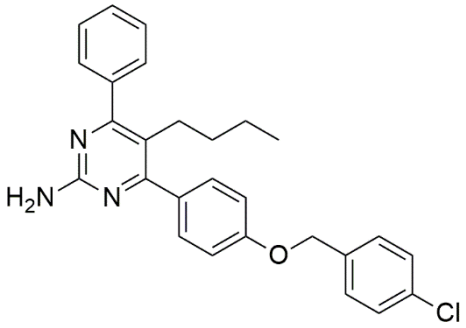
2-Amino-5-butyl-4-(4-(4-methoxybenzyloxy)phenyl)-6-phenylpyrimidine (10a)



The reaction of **2** (200 mg, 0.76 mmol, 1 eq.), 4-(4´-methoxybenzyloxy)phenylboronic acid (274 mg, 1.06 mmol, 1.4 eq.), Cs_2CO_3 (619 mg, 1.90 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (22 mg, 19.00 µmol, 2.5 mol%) following the procedure A gave **10a** (261 mg, 78 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.50 – 7.38 (m, 9H, Ar*H*), 7.11 – 7.06 (m, 2H, Ar*H*), 6.98 – 6.94 (m, 2H, Ar*H*), 6.47 (s, 2H, NH_2), 5.08 (s, 2H, , O-CH_2-Ar), 3.77 (s, 3H, OCH_3), 1.02 – 0.91 (m, 2H, CH_2-CH_2-CH_2), 0.90 – 0.79 (m, 2H, CH_2-CH_2-CH_3), 0.47 (t, *J* = 7.3 Hz, 3H, CH_2-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 168.05, 167.55, 161.46, 159.48, 158.84, 140.24, 132.54, 130.17, 130.05, 129.23, 128.64, 128.42, 118.58, 114.73, 114.28, 69.50, 55.57, 32.19, 27.03, 22.03, 13.63. **IR** ($CHCl_3$) *v*: 3528, 3419, 2962, 1609, 1600, 1545, 1512, 1497, 1377, 1243, 1174, 702 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{28}H_{29}N_3O_2$ 439.2260, found 439.2266. **UPLC-MS** (*m/z*) $[M+H]^+$ 440.18, t_R = 5.14 min.

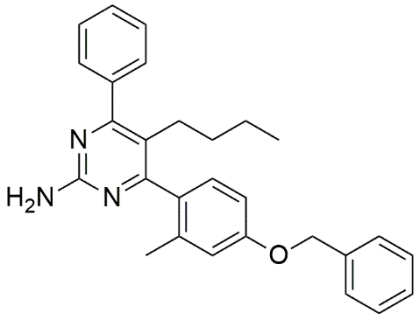
2-Amino-5-butyl-4-(4-(4-chlorobenzyloxy)phenyl)-6-phenylpyrimidine (10b)



The reaction of **2** (200 mg, 0.76 mmol, 1 eq.), 4-(4´-chlorobenzyloxy)phenylboronic acid (278 mg, 1.06 mmol, 1.4 eq.), Cs_2CO_3 (619 mg, 1.90 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (22 mg, 19.00 µmol, 2.5 mol%) following the procedure A gave **10b** (241 mg, 71 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.53 – 7.41 (m, 11H, Ar*H*), 7.12 – 7.07 (m, 2H, Ar*H*), 6.47 (s, 2H, N*H*$_2$), 5.18 (s, 2H, O-C*H*$_2$-Ar), 1.01 – 0.91 (m, 2H, CH_2-C*H*$_2$-CH_2), 0.89 – 0.78 (m, 2H, CH_2-C*H*$_2$-CH_3), 0.46 (t, *J* = 7.3 Hz, 3H, CH_2-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.06, 167.51, 161.47, 158.55, 140.22, 136.47, 132.89, 132.80, 130.21, 130.03, 128.90, 128.64, 128.43, 118.59, 114.77, 68.85, 32.18, 27.01, 22.03, 13.61. **IR** ($CHCl_3$) *v*: 3527, 3419, 2961, 1600, 1545, 1512, 1496, 1447, 1436, 1376, 1244, 1175, 1015, 702 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{27}H_{26}N_3OCl$ 443.1764, found 443.1768. **UPLC-MS** (*m/z*) $[M+H]^+$ 444.34, t_R = 5.50 min.

2-Amino-5-butyl-4-(4-benzyloxy-2-methylphenyl)-6-phenylpyrimidine (10c)

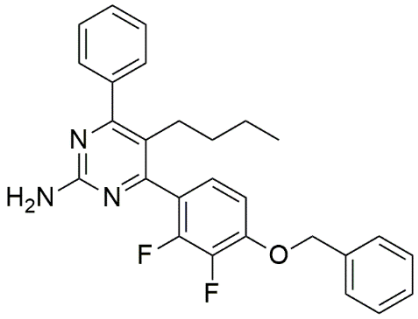


The reaction of **2** (100 mg, 0.38 mmol, 1 eq.), 4-benzyloxy-2-methylphenylboronic acid (129 mg, 0.53 mmol, 1.4 eq.), Cs_2CO_3 (310 mg, 0.95 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (11 mg, 9.50 µmol, 2.5 mol%) following the procedure A gave **10c** (137 mg, 85 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.50 – 7.31 (m, 10H, Ar*H*), 7.13 (d, *J* = 8.4 Hz, 1H, Ar*H*), 6.97 (d, *J* = 2.5 Hz, 1H, Ar*H*), 6.90 (dd, *J* = 8.4, 2.6 Hz, 1H, Ar*H*), 6.47 (s, 2H, N*H*$_2$), 5.14 (s, 2H, O-C*H*$_2$-Ph), 2.13 (s, 3H, C*H*$_3$-Ar), 1.00 – 0.89 (m, 2H, CH_2-C*H*$_2$-CH_2), 0.86 – 0.76 (m, 2H, CH_2-C*H*$_2$-CH_3), 0.44 (t, *J* = 7.3 Hz, 3H, CH_2-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.51, 167.68, 161.58, 158.25, 140.08, 137.55, 136.68, 132.29, 129.70, 128.87, 128.70, 128.62, 128.45, 128.25, 128.13, 119.42, 116.57,

112.06, 69.57, 32.04, 27.01, 22.02, 19.96, 13.59. **IR** ($CHCl_3$) *v*: 3527, 3419, 2960, 1600, 1546, 1504, 1497, 1454, 1241, 1170, 702 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{28}H_{29}N_3O$ 423.2311, found 423.2314. **UPLC-MS** (*m/z*) $[M+H]^+$ 424.16, t_R = 5.20 min.

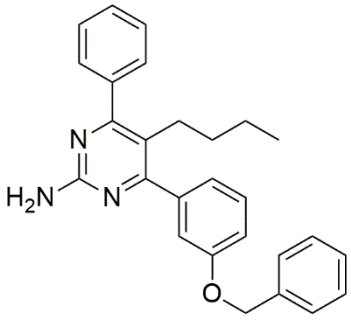
2-Amino-5-butyl-4-(4-benzyloxy-2,3-difluorophenyl)-6-phenylpyrimidine (10d)



The reaction of **2** (100 mg, 0.38 mmol, 1 eq.), 4-benzyloxy-2,3-difluorophenylboronic acid (140 mg, 0.53 mmol, 1.4 eq.), Cs_2CO_3 (310 mg, 0.95 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (11 mg, 9.50 µmol, 2.5 mol%) following the procedure A gave **10d** (42 mg, 25 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.52 – 7.34 (m, 10H, Ar*H*), 7.24 – 7.20 (m, 2H, Ar*H*), 6.64 (s, 2H, NH_2), 5.30 (s, 2H, O-CH_2-Ph), 1.01 – 0.91 (m, 2H, CH_2-CH_2-CH_2), 0.90 – 0.79 (m, 2H, CH_2-CH_2-CH_3), 0.45 (t, *J* = 7.2 Hz, 3H, CH_2-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 168.10, 162.16, 162.13, 161.69, 139.68, 136.48, 129.01, 128.90, 128.70, 128.57, 128.53, 128.41, 124.97, 124.92, 121.59, 121.45, 119.90, 111.12, 71.27, 32.12, 27.00, 21.92, 13.54. **^{19}F NMR** (377 MHz, DMSO-d_6) δ -139.80 – -140.33 (m), -159.44 – -159.92 (m). **IR** ($CHCl_3$) *v*: 3529, 3420, 2961, 1635, 1602, 1550, 1510, 1497, 1378, 1301, 1073, 702 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{27}H_{25}N_3OF_2$ 445.1966, found 445.1964. **UPLC-MS** (*m/z*) $[M+H]^+$ 446.14, t_R = 5.41 min.

2-Amino-5-butyl-4-(3-benzyloxyphenyl)-6-phenylpyrimidine (10e)

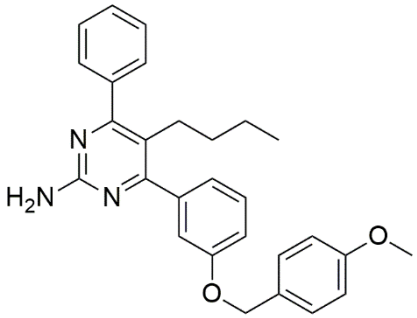


The reaction of **2** (200 mg, 0.76 mmol, 1 eq.), 3-benzyloxyphenylboronic acid (242 mg, 1.06 mmol, 1.4 eq.), Cs_2CO_3 (619 mg, 1.90 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (22 mg, 19.00 µmol, 2.5 mol%) following the procedure A gave **10e** (306 mg, 98 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.49 – 7.30 (m, 11H, Ar*H*), 7.11 – 7.02 (m, 3H, Ar*H*), 6.52 (s, 2H, NH_2), 5.17 (s, 2H, O-CH_2-Ph), 2.46 – 2.38 (m,

2H, HetAr-C*H*$_2$-CH$_2$), 1.00 – 0.88 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 0.87 – 0.75 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.45 (t, *J* = 7.3 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.06, 167.83, 161.45, 158.33, 141.63 – 141.35 (m), 140.09, 137.51, 129.66, 128.89, 128.71, 128.62, 128.44, 128.26, 128.03, 121.16, 118.61, 115.36, 115.04, 69.68, 66.82, 32.25, 26.91, 22.02, 13.57. **IR** ($CHCl_3$) *v*: 3527, 3419, 2962, 1600, 1547, 1497, 1488, 1454, 1432, 1374, 700 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{27}H_{27}N_3O$ 409.2154, found 409.2151. **UPLC-MS** (*m/z*) $[M+H]^+$ 410.01, t_R = 5.43 min.

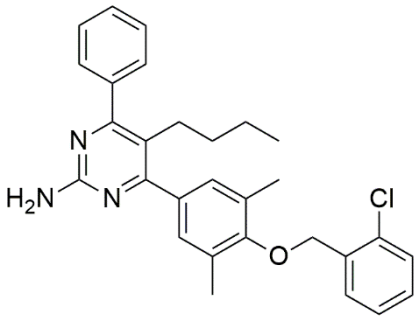
2-Amino-5-butyl-4-(3-(4-methoxybenzyloxy)phenyl)-6-phenylpyrimidine (10f)



The reaction of **2** (200 mg, 0.76 mmol, 1 eq.), 3-(4´-methoxybenzyloxy)phenylboronic acid (274 mg, 1.06 mmol, 1.4 eq.), Cs_2CO_3 (619 mg, 1.90 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (22 mg, 19.00 µmol, 2.5 mol%) following the procedure A gave **10f** (330 mg, 99 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.50 – 7.34 (m, 8H, Ar*H*), 7.09 – 7.01 (m, 3H, Ar*H*), 6.97 – 6.92 (m, 2H, Ar*H*), 6.52 (s, 2H, N*H*$_2$), 5.08 (s, 2H, O-C*H*$_2$-Ar), 3.75 (s, 3H, OC*H*$_3$), 2.46 – 2.39 (m, 2H, HetAr-C*H*$_2$-CH$_2$), 1.00 – 0.89 (m, 2H, CH$_2$-C*H*$_2$-CH$_2$), 0.87 – 0.75 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.44 (t, *J* = 7.3 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.05, 167.86, 161.44, 159.43, 158.37, 141.42, 140.10, 129.84, 129.60, 129.34, 128.71, 128.63, 128.44, 121.04, 118.61, 115.41, 115.07, 114.27, 69.47, 55.55, 55.53, 32.25, 26.92, 22.02, 13.56. **IR** ($CHCl_3$) *v*: 3527, 3419, 2962, 1601, 1546, 1516, 1497, 1488, 1447, 1432, 1373, 1247, 701 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{28}H_{29}N_3O_2$ 439.2260, found 439.2261. **UPLC-MS** (*m/z*) $[M+H]^+$ 440.06, t_R = 5.34 min.

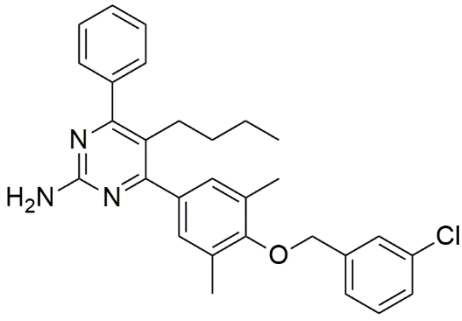
2-Amino-5-butyl-4-(4-(2-chlorobenzyloxy)-3,5-dimethylphenyl)-6-phenylpyrimidine (10g)



The reaction of **2** (200 mg, 0.76 mmol, 1 eq.), 4-(2´-chlorobenzyloxy)-3,5-dimethylphenylboronic acid (308 mg, 1.06 mmol, 1.4 eq.), Cs_2CO_3 (619 mg, 1.90 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (22 mg, 19.00 µmol, 2.5 mol%) following the procedure A gave **10g** (349 mg, 97 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.69 – 7.65 (m, 1H, Ar*H*), 7.56 – 7.51 (m, 1H, Ar*H*), 7.50 – 7.41 (m, 7H, Ar*H*), 7.19 (s, 2H, Ar*H*), 6.48 (s, 2H, N*H*$_2$), 4.96 (s, 2H, O-C*H*$_2$-Ar), 2.27 (s, 6H, Ar-C*H*$_3$), 1.05 – 0.94 (m, 2H, CH_2-C*H*$_2$-CH_2), 0.94 – 0.81 (m, 2H, CH_2-C*H*$_2$-CH_3), 0.50 (t, *J* = 7.3 Hz, 3H, CH_2-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.04, 167.66, 161.44, 155.50, 140.21, 135.85, 135.28, 133.12, 131.06, 130.69, 130.45, 129.80, 129.27, 128.66, 128.62, 128.44, 127.88, 118.57, 71.16, 32.23, 26.96, 22.02, 16.57, 13.56. **IR** ($CHCl_3$) *v*: 3527, 3419, 2961, 1600, 1546, 1483, 1447, 1438, 1376, 1183, 703 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{29}H_{31}N_3OCl$ 472.2150, found 472.2150. **UPLC-MS** (*m/z*) $[M+H]^+$ 472.14, t_R = 6.27 min.

2-Amino-5-butyl-4-(4-(3-chlorobenzyloxy)-3,5-dimethylphenyl)-6-phenylpyrimidine (10h)

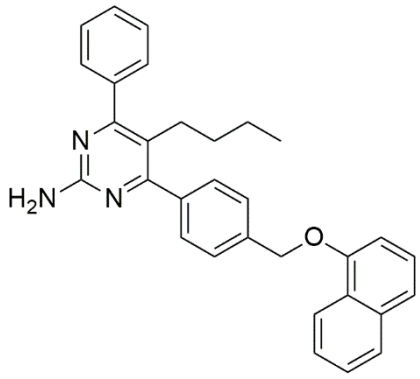


The reaction of **2** (200 mg, 0.76 mmol, 1 eq.), 4-(3´-chlorobenzyloxy)-3,5-dimethylphenylboronic acid (308 mg, 1.06 mmol, 1.4 eq.), Cs_2CO_3 (619 mg, 1.90 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (22 mg, 19.00 µmol, 2.5 mol%) following the procedure A gave **10h** (336 mg, 94 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.52 – 7.41 (m, 9H, Ar*H*), 7.20 (s, 2H, Ar*H*), 6.48 (s, 2H, N*H*$_2$), 4.89 (s, 2H, O-C*H*$_2$-Ar), 2.28 (s, 6H, Ar-C*H*$_3$), 1.06 – 0.95 (m, 2H,

CH_2-C*H*$_2$-CH_2), 0.94 – 0.82 (m, 2H, CH_2-C*H*$_2$-CH_3), 0.50 (t, *J* = 7.3 Hz, 3H, CH_2-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.05, 167.65, 161.44, 155.50, 140.48, 140.21, 135.82, 133.48, 130.81, 130.63, 129.29, 128.67, 128.61, 128.44, 128.30, 128.02, 126.93, 118.56, 72.88, 32.22, 26.96, 22.01, 16.63, 13.57. **IR** ($CHCl_3$) *v*: 3527, 3419, 2960, 1601, 1546, 1483, 1447, 1436, 1376, 1183, 1154, 703 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{29}H_{31}N_3OCl$ 472.2150, found 472.2150. **UPLC-MS** (*m/z*) $[M+H]^+$ 472.18, t_R = 6.31 min.

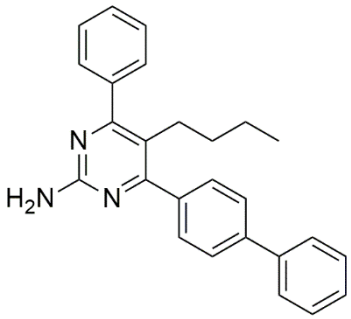
2-Amino-5-butyl-4-(4-((naphthalen-1-yloxy)methyl)phenyl)-6-phenylpyrimidine (10i)



The reaction of **2** (200 mg, 0.76 mmol, 1 eq.), 4-[(1´-naphtyloxy)methyl]phenylboronic acid (295 mg, 1.06 mmol, 1.4 eq.), Cs_2CO_3 (619 mg, 1.90 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (22 mg, 19.00 µmol, 2.5 mol%) following the procedure A gave **10i** (125 mg, 36 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 8.28 – 8.23 (m, 1H, Ar*H*), 7.92 – 7.87 (m, 1H, Ar*H*), 7.66 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.58 – 7.40 (m, 11H, Ar*H*), 7.10 (dd, *J* = 7.7, 1.0 Hz, 1H, Ar*H*), 6.53 (s, 2H, N*H*$_2$), 5.41 (s, 2H, O-C*H*$_2$-Naph), 1.03 – 0.93 (m, 2H, CH_2-C*H*$_2$-CH_2), 0.89 – 0.79 (m, 2H, CH_2-C*H*$_2$-CH_3), 0.45 (t, *J* = 7.3 Hz, 3H, CH_2-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.12, 167.79, 161.49, 154.08, 140.08, 139.61, 137.65, 134.54, 128.89, 128.72, 128.63, 128.46, 127.98, 127.48, 126.95, 126.60, 125.89, 125.52, 121.99, 120.68, 118.66, 106.41, 69.66, 32.25, 26.92, 22.02, 13.55. **IR** ($CHCl_3$) *v*: 3527, 3419, 2961, 1600, 1582, 1546, 1459, 1269, 1099, 702 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{31}H_{30}N_3O$ 460.2383, found 460.2383. **UPLC-MS** (*m/z*) $[M+H]^+$ 460.22, t_R = 5.64 min.

2-Amino-5-butyl-4-([1,1'-biphenyl]-4-yl)-6-phenylpyrimidine (10j)



The reaction of **2** (200 mg, 0.76 mmol, 1 eq.), 4-biphenylboronic acid (210 mg, 1.06 mmol, 1.4 eq.), Cs_2CO_3 (619 mg, 1.90 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (22 mg, 19.00 µmol, 2.5 mol%) following the procedure A gave **10j** (256 mg, 89 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.82 – 7.73 (m, 4H, Ar*H*), 7.65 – 7.58 (m, 2H, Ar*H*), 7.54 – 7.38 (m, 8H, Ar*H*), 6.55 (s, 2H, N*H*$_2$), 1.07 – 0.95 (m, 2H, CH_2-C*H*$_2$-CH_2), 0.93 – 0.80 (m, 2H, CH_2-C*H*$_2$-CH_3), 0.47 (t, *J* = 7.3 Hz, 3H, CH_2-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.21, 167.57, 161.54, 140.35, 140.12, 139.94, 139.17, 129.49, 129.42, 128.73, 128.65, 128.46, 128.18, 127.16, 126.66, 118.67, 116.16, 66.82, 32.28, 26.98, 22.02, 13.60 (d, *J* = 2.8 Hz). **IR** ($CHCl_3$) *v*: 3528, 3420, 2960, 2928, 1600, 1544, 1494, 1448, 701 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{26}H_{25}N_3$ 379.2048, found 379.2050. **UPLC-MS** (*m/z*) $[M+H]^+$ 380.07, t_R = 5.30 min.

5.3. Synthesis of polysubstituted pyrimidines with hydrogen in the position C5 of pyrimidine

General procedure for preparation of 11 and 12 (B)

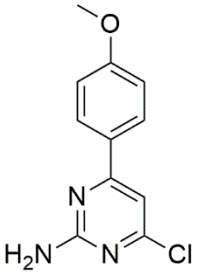
A EtOH-toluene mixture in the ratio 1:3 was bubbled with argon for 2 min. 2-Amino-4,6-dichloropyrimidine (1 eq.), corresponding boronic acid (1.5 eq.), K_2CO_3 (2.5 eq.) and $Pd(Ph_3P)_4$ (1 mol%) were added and the reaction mixture was stirred at 80 °C for 16 h. Solvents were evaporated and the residue was purified using silica gel flash chromatography to afford mono- (a) and di-arylated (b) products.

11

The reaction of 2-amino-4,6-dichloropyrimidine (400 mg, 2.44 mmol, 1 eq.), *p*-methoxyphenylboronic acid (556 mg, 3.66 mmol, 1.5 eq.), K_2CO_3 (843 mg,

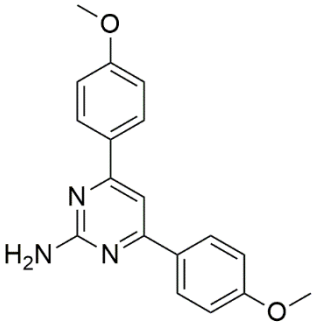
6.10 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (28 mg, 24.40 µmol, 1 mol%) following the procedure B (using linear gradient elution 0-55 % EtOAc in hexane) gave both derivatives (**11a** 197 mg, 34 %, **11b** 185 mg, 24 %) as white solids in overall yield 59 %.

2-Amino-4-chloro-6-(4-methoxyphenyl)pyrimidine (11a)



^{1}H NMR (401 MHz, DMSO-d_6) δ 8.11 – 8.06 (m, 2H, Ar*H*), 7.20 (s, 1H, HetAr*H*), 7.09 (s, 2H, NH_2), 7.08 – 7.02 (m, 2H, Ar*H*), 3.84 (s, 3H, O-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.98, 163.89, 162.17, 161.35, 129.21, 128.58, 114.55, 104.25, 55.84. **IR** ($CHCl_3$) *v*: 3538, 3424, 2842, 1604, 1559, 1511, 1425, 1257, 1175, 1033, 822 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{15}H_{18}N_3OCl$ 235.0512, found 235.0514. **UPLC-MS** (*m/z*) $[M+H]^+$ 235.83, t_R = 4.23 min.

2-Amino-4,6-bis(4-methoxyphenyl)pyrimidine (11b)

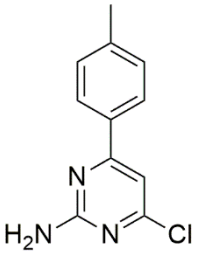


^{1}H NMR (401 MHz, DMSO-d_6) δ 8.22 – 8.17 (m, 4H, Ar*H*), 7.60 (s, 1H, HetAr*H*), 7.09 – 7.04 (m, 4H, Ar*H*), 6.58 (s, 2H, NH_2), 3.85 (s, 6H, O-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.55, 164.27, 161.59, 130.23, 128.94, 114.35, 100.71, 55.78. **IR** ($CHCl_3$) *v*: 3531, 3422, 2840, 1607, 1597, 1570, 1537, 1512, 1439, 1368, 1304, 1256, 1237, 1175, 1034, 827 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{22}H_{25}N_3O_2$ 307.1321, found 307.1320. **UPLC-MS** (*m/z*) $[M+H]^+$ 308.24, t_R = 4.04 min.

12

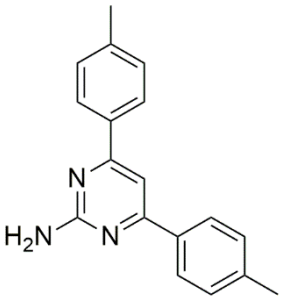
The reaction of 2-amino-4,6-dichloropyrimidine (400 mg, 2.44 mmol, 1 eq.), *p*-tolylboronic acid (498 mg, 3.66 mmol, 1.5 eq.), K_2CO_3 (843 mg, 6.10 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (28 mg, 24.40 µmol, 1 mol%) following the procedure B (using linear gradient elution 0-30 % EtOAc in hexane) gave both derivatives (**12a** 218 mg, 41 %, **12b** 249 mg, 37 %) as white solids in overall yield 78 %.

2-Amino-4-chloro-6-(4-methylphenyl)pyrimidine (12a)



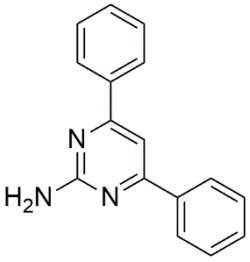
^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 8.01 (d, *J* = 8.3 Hz, 2H, Ar*H*), 7.32 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.22 (s, 1H, HetAr*H*), 7.14 (s, 2H, N*H*$_2$), 2.37 (s, 3H, Ph-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 166.36, 163.96, 161.50, 141.54, 133.56, 129.81, 127.47, 104.77, 21.44. **IR** ($CHCl_3$) *v*: 3538, 3424, 1606, 1658, 1540, 1511, 1447, 1424, 1323, 835, 813 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{11}H_{10}N_3Cl$ 219.0563, found 219.0565. **UPLC-MS** (*m/z*) $[M+H]^+$ 221.75, t_R = 4.49 min.

2-Amino-4,6-bis(4-methylphenyl)pyrimidine (12b)



^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 8.13 (d, *J* = 8.2 Hz, 4H, Ar*H*), 7.65 (s, 1H, HetAr*H*), 7.33 (d, *J* = 7.9 Hz, 4H, Ar*H*), 6.67 (s, 2H, N*H*$_2$), 2.39 (s, 6H, Ph-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 165.06, 164.40, 140.61, 135.07, 129.65, 127.34, 101.57, 21.43. **IR** ($CHCl_3$) *v*: 3523, 3423, 1597, 1580, 1569, 1538, 1512, 1436, 1366, 815 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{18}H_{17}N_3$ 275.1422, found 275.1420. **UPLC-MS** (*m/z*) $[M+H]^+$ 275.89, t_R = 4.67 min.

2-Amino-4,6-diphenylpyrimidine (13)



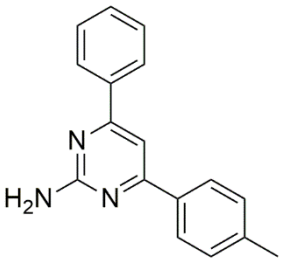
2-Amino-4,6-dichloropyrimidine (300 mg, 1.83 mmol, 1 eq.), phenylboronic acid (558 mg, 4.58 mmol, 2.5 eq.), K_2CO_3 (759 mg, 5.49 mmol, 3 eq.) and $Pd(Ph_3P)_4$ (53 mg, 45.75 µmol, 2.5 mol%) in a water-(1,4-dioxane) mixture (1:4 ratio) were stirred at 110 °C for 16 h. Solvents were evaporated and the residue was purified using silica gel flash chromatography (linear gradient elution 0-30 % EtOAc in hexane) to obtain **13** (365 mg, 81 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 8.26 – 8.19 (m, 4H, Ar*H*), 7.71 (s, 1H, HetAr*H*), 7.53 (p, *J* = 3.8, 3.3 Hz, 6H, Ar*H*), 6.76 (s, 2H, N*H*$_2$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 165.32, 164.48, 137.81, 130.90, 129.07, 127.44, 102.30. **IR** ($CHCl_3$) *v*: 3533, 3423,

1596, 1568, 1545, 1498, 1451, 1430, 1365, 694, 628 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{16}H_{13}N_3$ 247.1109, found 247.1108. **UPLC-MS** (*m/z*) $[M+H]^+$ 247.85, t_R = 4.55 min.

2-Amino-4-(4-methoxyphenyl)-6-phenylpyrimidine (14) (see Chapter 5.4)

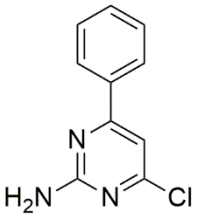
2-Amino-4-(4-methylphenyl)-6-phenylpyrimidine (15)



Compound **12a** (100 mg, 0.46 mmol, 1 eq.), phenylboronic acid (78 mg, 0.64 mmol, 1.4 eq.), K_2CO_3 (159 mg, 1.15 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (11 mg, 9.20 µmol, 2 mol%) in a water-(1,4-dioxane) mixture (1:4 ratio) were stirred at 110 °C for 16 h. Solvents were evaporated and the residue was purified using silica gel flash chromatography (linear gradient elution 0-35 % EtOAc in hexane) to yield **15** (98 mg, 82 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 8.24 – 8.19 (m, 2H, Ar*H*), 8.14 (d, *J* = 8.2 Hz, 2H, Ar*H*), 7.68 (s, 1H, HetAr*H*), 7.55 – 7.50 (m, 3H, Ar*H*), 7.33 (d, *J* = 7.7 Hz, 2H, Ar*H*), 6.71 (s, 2H, NH_2), 2.39 (s, 3H, Ph-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.21, 165.17, 164.44, 140.69, 137.88, 135.01, 130.83, 129.67, 129.05, 127.41, 127.37, 101.93, 21.44. **IR** ($CHCl_3$) *v*: 3532, 3423, 1597, 1586, 1579, 1571, 1538, 1515, 1498, 1453, 1438, 1365, 1234, 821, 696 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{17}H_{15}N_3$ 261.1266, found 261.1269. **UPLC-MS** (*m/z*) $[M+H]^+$ 261.90, t_R = 4.58 min.

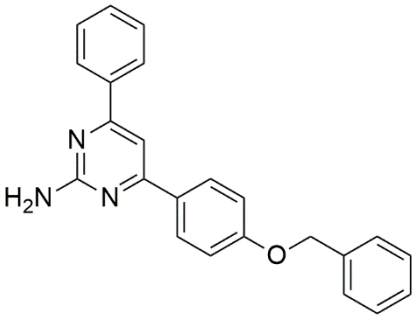
2-Amino-4-chloro-6-phenylpyrimidine (16)



2-Amino-4,6-dichloropyrimidine (5.00 g, 30.49 mmol, 1 eq.), phenylboronic acid (3.72 g, 30.49 mmol, 1 eq.), Na_2CO_3 (7.11 g, 67.08 mmol, 2.2 eq.) and $Pd(Ph_3P)_4$ (1.76 g, 1.52 mmol, 5 mol%) in a water-(1,4-dioxane) mixture (1:4 ratio) were stirred under argon atmosphere at 90 °C for 16 h. Solvents were evaporated and the residue was purified using silica gel flash chromatography (linear gradient elution 0-30 % EtOAc in hexane) to yield **16** (4.76 g, 76 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 8.13 – 8.08 (m, 2H, Ar*H*), 7.57 – 7.48 (m, 3H, Ar*H*), 7.26 (s, 1H, HetAr*H*), 7.19 (s, 2H, NH_2). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.45, 163.99, 161.60, 136.33, 131.57, 129.22, 127.52, 105.15. **IR** ($CHCl_3$) *v*: 3538, 3425, 1608, 1569, 1543, 1498, 1455, 1438, 1424, 1323, 823 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{10}H_8N_3Cl$ 205.0407, found 205.0406. **UPLC-MS** (*m/z*) $[M+H]^+$ 205.84, t_R = 4.20 min.

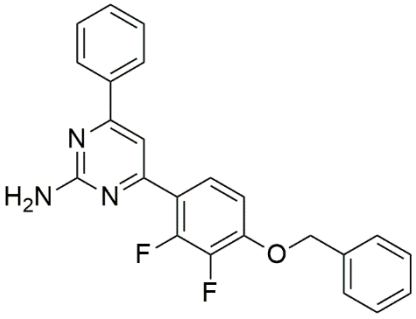
2-Amino-4-(4-benzyloxyphenyl)-6-phenylpyrimidine (17a)



The reaction of **16** (100 mg, 0.49 mmol, 1 eq.), 4-benzyloxyphenylboronic acid (157 mg, 0.69 mmol, 1.4 eq.), Cs_2CO_3 (401 mg, 1.23 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (14 mg, 12.25 µmol, 2.5 mol%) following the procedure A gave **17a** (97 mg, 56 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 8.25 – 8.18 (m, 4H, Ar*H*), 7.66 (s, 1H, HetAr*H*), 7.55 – 7.47 (m, 5H, Ar*H*), 7.45 – 7.33 (m, 3H, Ar*H*), 7.18 – 7.13 (m, 2H, Ar*H*), 6.67 (s, 2H, NH_2), 5.21 (s, 2H, O-CH_2-Ph). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.00, 164.82, 164.37, 160.78, 137.94, 137.30, 130.78, 130.29, 129.02, 128.93, 128.39, 128.25, 127.39, 115.24, 101.54, 69.82. **IR** ($CHCl_3$) *v*: 3532, 3423, 1596, 1570, 1540, 1513, 1497, 1453, 1366, 1233, 1175, 832, 697 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{23}H_{19}N_3O$ 353.1528, found 353.1526. **UPLC-MS** (*m/z*) $[M+H]^+$ 353.97, t_R = 4.90 min.

2-Amino-4-(4-benzyloxy-2,3-difluorophenyl)-6-phenylpyrimidine (17b)

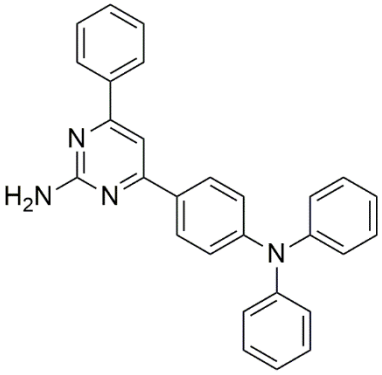


The reaction of **16** (21 mg, 0.10 mmol, 1 eq.), 4-benzyloxy-2,3-difluorophenylboronic acid (36 mg, 0.14 mmol, 1.4 eq.), Cs_2CO_3 (82 mg, 0.25 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (3 mg, 2.50 µmol, 2.5 mol%) following the procedure A gave **17b** (38 mg, 97 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 8.13 – 8.08 (m, 2H, Ar*H*), 7.79 (td, *J* = 8.7, 2.3 Hz, 1H, Ar*H*), 7.56 – 7.49 (m, 5H, Ar*H*), 7.47 – 7.35 (m, 4H, Ar*H*+HetAr*H*), 7.34 – 7.27 (m, 1H,

Ar*H*), 6.84 (s, 2H, N*H*$_2$), 5.32 (s, 2H, O-C*H*$_2$-Ph). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 165.26, 164.36, 161.10, 137.55, 136.38, 131.05, 129.21, 129.05, 128.78, 128.48, 127.31, 124.82, 110.91, 105.53, 105.45, 71.29. **IR** ($CHCl_3$) *v*: 3534, 3423, 1600, 1587, 1574, 1542, 1509, 1497, 1368, 1300, 1090, 696 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{23}H_{17}N_3OF_2$ 389.1340, found 389.1345. **UPLC-MS** (*m/z*) $[M+H]^+$ 389.97, t_R = 5.21 min.

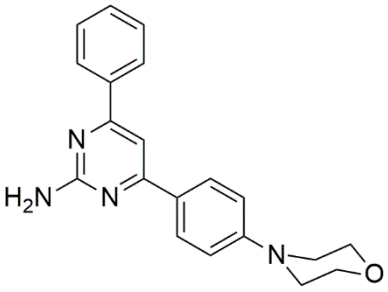
2-Amino-4-(4-diphenylaminophenyl)-6-phenylpyrimidine (17c)



The reaction of **16** (200 mg, 0.97 mmol, 1 eq.), 4-(diphenylamino)phenylboronic acid (393 mg, 1.36 mmol, 1.4 eq.), Cs_2CO_3 (792 mg, 2.43 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (28 mg, 24.25 µmol, 2.5 mol%) following the procedure A gave **17c** (229 mg, 57 %) as a yellow solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 8.24 – 8.15 (m, 2H, Ar*H*), 8.12 (d, *J* = 8.7 Hz, 2H. Ar*H*), 7.62 (s, 1H, HetAr*H*), 7.57 – 7.46 (m, 3H, Ar*H*), 7.37 (t, *J* = 7.7 Hz, 4H, Ar*H*), 7.19 – 7.06 (m, 6H, Ar*H*), 7.02 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.66 (s, 2H, N*H*$_2$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 164.98, 164.73, 164.39, 149.85, 147.10, 137.95, 130.79, 130.21, 129.05, 128.65, 127.35, 125.38, 124.43, 121.69, 101.57. **IR** ($CHCl_3$) *v*: 3533, 3423, 1592, 1586, 1577, 1532, 1510, 1492, 1366, 1332, 698 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{28}H_{22}N_4$ 414.1844, found 414.1847. **UPLC-MS** (*m/z*) $[M+H]^+$ 415.36, t_R = 5.45 min.

2-Amino-4-(4-morpholinophenyl)-6-phenylpyrimidine (17d)

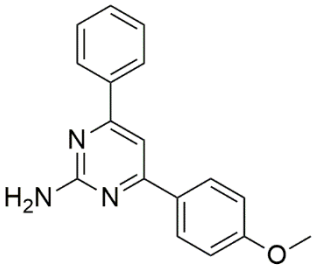


The reaction of **16** (70 mg, 0.34 mmol, 1 eq.), 4-morpholinophenylboronic acid (100 mg, 0.48 mmol, 1.4 eq.), Cs_2CO_3 (277 mg, 0.85 mmol, 2.5 eq.) and $Pd(Ph_3P)_4$ (10 mg, 8.50 µmol, 2.5 mol%) following the procedure A gave **17d** (98 mg, 87 %) as a yellowish solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 8.24 – 8.17 (m, 2H, Ar*H*), 8.14 (d, *J* = 8.6 Hz, 2H, Ar*H*), 7.61 (s, 1H, HetAr*H*), 7.55 – 7.48 (m, 3H, Ar*H*), 7.05 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.59 (s, 2H, NH_2), 3.80 – 3.73 (m, 4H, CH_2-O-CH_2), 3.28 – 3.21 (m, 4H, CH_2-N-CH_2). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.00, 164.72, 164.33, 153.03, 138.06, 130.68, 129.01, 128.48, 127.60, 127.34, 114.46, 101.11, 66.45, 47.95. **IR** ($CHCl_3$) *v*: 3532, 3422, 2971, 1595, 1577, 1533, 1519, 1450, 1366, 1122, 930, 826, 634 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{20}H_{20}N_4O$ 332.1637, found 332.1630. **UPLC-MS** (*m/z*) $[M+H]^+$ 333.29, t_R = 3.92 min.

5.4. Synthesis of WQE-134 analogues modified in the C5 position of pyrimidine

2-Amino-4-(4-methoxyphenyl)-6-phenylpyrimidine (14)

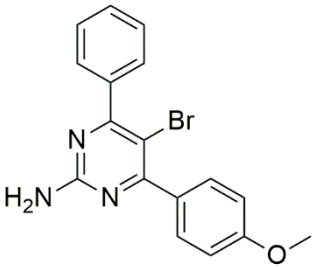


Acetophenone (8.57 mL, 73.45 mmol, 1 eq.) was added dropwise to a 10% solution of NaOH in EtOH. After 3 min, *p*-anisaldehyde (8.93 mL, 73.45 mmol, 1 eq.) was added and the solution was stirred for another 30 min. After full conversion to α,β-unsaturated ketone (monitored by TLC on silica gel with 40% solution of EtOAc in hexane, α,β-unsaturated ketone R_f = 0.5), guanidin hydrochloride (7.01 g, 73.45 mmol, 1 eq.) was added and the reaction mixture was stirred at 90 °C for 24 h. The mixture was then undercooled to 0 °C to promote the crystallization. The solid was filtered off to obtain **14** as a white solid (12.28 g, 60 %). In order to collect an additional amount of product the black-brown filtrate was evaporated, the residue mixed with EtOAc and washed with water (3 × 500 mL). Organic fraction was dried over $MgSO_4$ and purified using silica gel flash chromatography (linear gradient elution 0-50 % EtOAc in hexane) to afford additional amount of **14** (1.79 g, 9 %) as a white solid, resulting in overall yield 69 % (14.07 g).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 – 8.18 (m, 4H, Ar*H*), 7.66 (s, 1H, HetAr*H*), 7.55 – 7.47 (m, 3H, Ar*H*), 7.09 – 7.03 (m, 2H, Ar*H*), 6.68 (s, 2H, NH_2), 3.84 (s, 3H, O-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.83, 164.68, 161.53, 138.26, 130.57, 130.42,

128.95, 128.92, 127.33, 114.31, 100.21, 55.77. **IR** ($CHCl_3$) *v*: 3534, 3423, 1596, 1570, 1540, 1515, 1497, 1453, 1439, 1366, 1256, 1235, 1176, 832 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{17}H_{15}N_3O$ 277.1215, found 277.1217. **UPLC-MS** (*m/z*) $[M+H]^+$ 277.97, t_R = 3.98 min. **mp** = 167-169 °C.

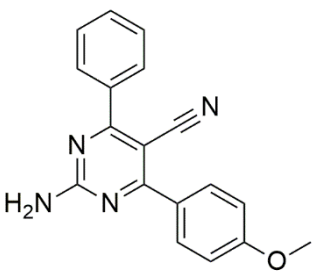
2-Amino-5-bromo-4-(4-methoxyphenyl)-6-phenylpyrimidine (18)



To a suspension of $CaCO_3$ (2.17 g, 21.63 mmol, 0.6 eq.) and **14** (10.00 g, 36.06 mmol, 1 eq.) in $CHCl_3$ was added dropwise the solution of Br_2 (2.04 mL, 39.67 mmol, 1.1 eq.) in $CHCl_3$ and the reaction mixture was stirred at 25 °C for 5 h. The mixture was washed with an aqueous solution of NaOH (2 $mol \cdot L^{-1}$) and the aqueous fraction was then extracted with $CHCl_3$ (3 × 500 mL). Organic fractions were combined, dried over $MgSO_4$ and evaporated to afford **18** (8.58 g, 67 %) as a brown-orange solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.67 – 7.63 (m, 2H, Ar*H*), 7.63 – 7.59 (m, 2H, Ar*H*), 7.50 – 7.45 (m, 3H, Ar*H*), 7.06 – 7.01 (m, 2H, Ar*H*), 3.82 (s, 3H, O-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 167.32, 166.54, 162.05, 160.48, 139.44, 131.33, 131.16, 129.54, 129.27, 128.26, 113.59, 103.33, 55.72. **IR** ($CHCl_3$) *v*: 3533, 3422, 1600, 1541, 1525, 1514, 1496, 1364, 1254, 1176, 1032, 838, 699 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{17}H_{14}N_3OBr$ 355.0320, found 355.0322. **UPLC-MS** (*m/z*) $[M+H]^+$ 355.91, t_R = 4.48 min.

2-Amino-5-carbonitrile-4-(4-methoxyphenyl)-6-phenylpyrimidine (19)

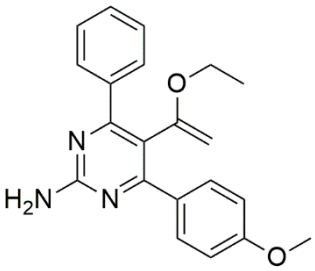


Compound **18** (70 mg, 0.20 mmol, 1 eq.), $Zn(CN)_2$ (70 mg, 0.60 mmol, 3 eq.) and Pd(*t*-$Bu_3P)_2$ (31 mg, 6.00 µmol, 30 mol%) in anhydrous DMF were stirred under argon atmosphere at 100 °C for 16 h. The solvent was evaporated and the residue was purified using silica gel flash

chromatography (linear gradient elution 0-50 % EtOAc in hexane) to obtain **19** (17 mg, 28 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.94 – 7.90 (m, 2H, Ar*H*), 7.89 – 7.85 (m, 2H, Ar*H*), 7.84 (s, 2H, NH_2), 7.62 – 7.54 (m, 3H, Ar*H*), 7.15 – 7.10 (m, 2H, Ar*H*), 3.86 (s, 3H, O-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.24, 170.14, 163.13, 161.92, 137.08, 131.19, 131.05, 129.21, 128.76, 119.38, 114.20, 90.80, 81.64, 55.89. **IR** ($CHCl_3$) *v*: 3540, 3424, 2218, 1603, 1544, 1530, 1512, 1497, 1258, 1174, 1030, 842 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{18}H_{14}N_4O$ 302.1168, found 302.1163. **UPLC-MS** (*m/z*) $[M+H]^+$ 302.98, t_R = 4.22 min.

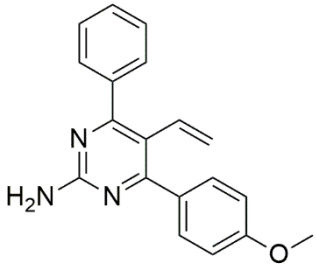
2-Amino-5-(1-ethoxyvinyl)-4-(4-methoxyphenyl)-6-phenylpyrimidine (20)



Compound **18** (100 mg, 0.28 mmol, 1 eq.), ethyl vinyl ether (126 µl, 1.40 mmol, 5 eq.), Et_3N (59 µL, 0.42 mmol, 1.5 eq.) and Pd(*t*-$Bu_3P)_2$ (7 mg, 14.00 µmol, 5 mol%) in anhydrous DMF were stirred under argon atmosphere at 110 °C for 16 h. The solvent was evaporated and the residue was purified using silica gel flash chromatography (linear gradient elution 0-50 % EtOAc in hexane) to yield **20** (59 mg, 61 %) as a orange solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.68 – 7.63 (m, 2H, Ar*H*), 7.62 – 7.58 (m, 2H, Ar*H*), 7.42 – 7.37 (m, 3H, Ar*H*), 6.99 – 6.94 (m, 2H, Ar*H*), 6.82 (s, 2H, NH_2), 4.07 (d, *J* = 2.1 Hz, 1H, HetAr-C=CH_2), 3.80 (s, 3H, O-CH_3), 3.76 (d, *J* = 2.1 Hz, 1H, HetAr-C=CH_2), 3.55 (q, *J* = 6.8 Hz, 2H, O-CH_2-CH_3), 1.06 (t, *J* = 7.0 Hz, 3H, O-CH_2-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.96, 165.95, 162.76, 160.31, 157.65, 139.75, 131.71, 130.30, 129.01, 128.54, 127.99, 117.35, 113.48, 90.42, 63.06, 55.62, 14.56. **IR** ($CHCl_3$) *v*: 3532, 3422, 1600, 1542, 1514, 1496, 1453, 1291, 1252, 1176, 1033, 839, 700 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{21}H_{21}N_3O_2$ 347.1634, found 347.1633. **UPLC-MS** (*m/z*) $[M+H]^+$ 348.02, t_R = 4.63 min.

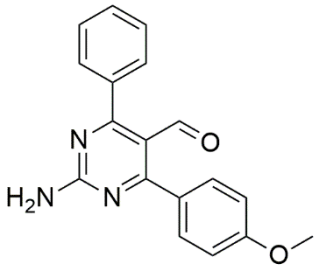
2-Amino-5-vinyl-4-(4-methoxyphenyl)-6-phenylpyrimidine (21)



A mixture water-(1,4-dioxane) in the ratio 1:4 were bubbled with argon for 2 min. Starting compound **18** (7.00 g, 19.65 mmol, 1 eq.), 2-vinyl-4,4,5,5-tetramethyl-1,3,2-dioxaoborolane (10.00 mL, 58.95 mmol, 3 eq.), K_2CO_3 (8.15 g, 58.95 mmol, 3 eq) and Pd(*t*-$Bu_3P)_2$ (501 mg, 0.98 mmol, 5 mol%) were added and the reaction mixture was stirred at 85 °C for 15 h. Solvents were evaporated, the residue was twice co-distilled with EtOH and purified using silica gel flash chromatography (linear gradient elution 0-50 % EtOAc in hexane) to obtain **21** (3.43 g, 58 %) as a orange solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.57 – 7.47 (m, 4H, Ar*H*), 7.47 – 7.37 (m, 3H, Ar*H*), 7.02 – 6.97 (m, 2H, Ar*H*), 6.76 (s, 2H, N*H*$_2$), 6.52 (dd, *J* = 17.8, 11.3 Hz, 1H, HetAr-C*H*), 5.00 (dd, *J* = 11.3, 1.8 Hz, 1H, HetAr-CH=C*H*$_{cis}$), 4.60 (dd, *J* = 17.8, 1.8 Hz, 1H, HetAr-CH=C*H*$_{trans}$), 3.81 (s, 3H, O-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 166.63, 165.99, 161.93, 160.03, 139.91, 133.02, 131.80, 131.24, 129.49, 128.82, 128.28, 119.95, 117.01, 113.67, 55.63. **IR** ($CHCl_3$) *v*: 3530, 3421, 1600, 1543, 1513, 1496, 1452, 1297, 1252, 1175, 701 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{19}H_{17}N_3O$ 303.1372, found 303.1375. **UPLC-MS** (*m/z*) $[M+H]^+$ 303.98, t_R = 4.37 min.

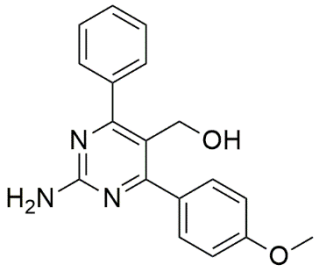
2-Amino-5-carbaldehyde-4-(4-methoxyphenyl)-6-phenylpyrimidine (22)



Compound **21** (120 mg, 0.40 mmol, 1 eq.) was dissolved in DCM and the solution was cooled to -20 °C. An O_2-O_3 mixture (1:1 ratio) was bubbled through the solution for 3 min. After no starting compound was observed (monitored by silica gel TLC with 30% solution of EtOAc in hexane, starting compound R_f = 0.4), an excess of Me_2S (1.00 mL, 13.62 mmol, 34 eq.) was added and the mixture was stirred at 25 °C for 3 h. Volatiles were evaporated and the residue was purified using C_{18}-reversed phase flash chromatography (linear gradient elution 0-100 % MeOH in water) to afford **22** (35 mg, 29 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 9.68 (s, 1H, HetAr-C*H*O), 7.72 (s, 2H, NH_2), 7.64 – 7.57 (m, 4H, Ar*H*), 7.53 – 7.44 (m, 3H, Ar*H*), 7.07 – 7.02 (m, 2H, Ar*H*), 3.84 (s, 3H, O-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 188.58, 171.73, 171.09, 162.97, 161.17, 138.41, 131.99, 130.19, 129.94, 128.22, 116.47, 113.75, 55.77. **IR** ($CHCl_3$) *v*: 3538, 3421, 1688, 1602, 1538, 1512, 1495, 1255, 1174, 839 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{18}H_{15}N_3O_2$ 305.1164, found 305.1163. **UPLC-MS** (*m/z*) $[M+H]^+$ 305.93, t_R = 4.09 min.

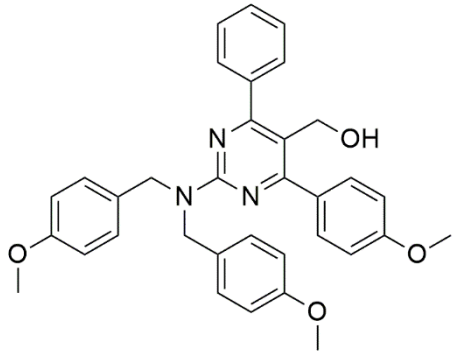
2-Amino-5-hydroxymethyl-4-(4-methoxyphenyl)-6-phenylpyrimidine (23)



A solution of **22** (185 mg, 0.61 mmol, 1 eq.) in anhydrous DMF was added dropwise to a suspension of $NaBH_4$ (23 mg, 0.61 mmol, 1 eq.) in anhydrous DMF. The reaction mixture was stirred at 0 °C for 1 h. The excessive $NaBH_4$ was hydrolyzed by addition of water. Volatiles were evaporated and the residue was purified using silica gel flash chromatography (linear gradient elution 0-70 % EtOAc in hexane) and subsequently C_{18}-reversed phase flash chromatography (linear gradient elution 0-100 % MeOH in water) to obtain **23** (149 mg, 80 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.92 – 7.87 (m, 2H, Ar*H*), 7.85 – 7.81 (m, 2H, Ar*H*), 7.50 – 7.45 (m, 3H, Ar*H*), 7.06 – 7.01 (m, 2H, Ar*H*), 6.69 (s, 2H, NH_2), 5.19 (t, *J* = 3.1 Hz, 1H, HetAr-CH_2-O*H*), 4.12 (d, *J* = 2.9 Hz, 2H, HetAr-CH_2-OH), 3.83 (s, 3H, O-CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 169.27, 168.62, 162.50, 160.49, 139.43, 131.54, 131.17, 129.47, 129.28, 128.18, 116.94, 113.62, 57.75, 55.66. **IR** ($CHCl_3$) *v*: 3606, 3533, 3422, 1601, 1546, 1514, 1497, 1374, 1253, 1177, 1033, 834, 702 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{18}H_{18}N_3O_2$ 308.1394, found 308.1394. **UPLC-MS** (*m/z*) $[M+H]^+$ 308.27, t_R = 3.55 min.

***N*,*N*-Bis(4-methoxybenzyl)-2-amino-5-hydroxymethyl-4-(4-methoxyphenyl)-6-phenylpyrimidine (24)**



A solution of **21** (3.30 g, 10.88 mmol, 1 eq.) in anhydrous DMF (cooled to 0 °C) was added under a stream of argon to a suspension of 60% NaH (2.18 g, 54.40 mmol, 5 eq.) in anhydrous DMF also cooled to 0 °C. The mixture was stirred at 0 °C for 15 min, subsequently, *p*-methoxybenzyl chloride (7.38 mL, 54.40 mmol, 5 eq.) was added dropwise. The reaction mixture was then stirred at 65 °C for 16 h. The excessive NaH was hydrolyzed by addition of water (until evolution of hydrogen obtained), volatiles were evaporated and the residue was purified using silica gel flash chromatography (linear gradient elution 0-20 % EtOAc in hexane). The isolated intermediate (yellow viscose oil, 4.50 g, 8.28 mmol, 1 eq.) was dissolved in DCM and the solution was cooled to -20 °C. An O_2-O_3 mixture (1:1 ratio) was bubbled through the solution for 10 min. After consumption of the starting compound (monitored by silica gel TLC with 20% solution of EtOAc in hexane, starting compound R_f = 0.3), an excess of $NaBH_4$ (6.26 g, 165.60 mmol, 20 eq.) was added and the mixture was stirred for 7 days at 25 °C. After the reduction of the ozonide, MeOH was slowly added to quench the reaction. Volatiles were evaporated, the residue mixed with EtOAc and washed with brine (3 × 500 mL). The organic fraction was dried over $MgSO_4$, the solid filtered off and the filtrate was purified using silica gel flash chromatography (linear gradient elution 0-30 % EtOAc in hexane) to afford desired **24** (3.13 g, 69 %) as a light-yellow viscose oil.

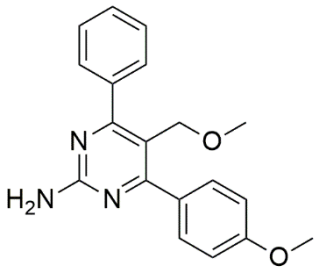
^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.99 – 7.94 (m, 2H, Ar*H*), 7.93 – 7.89 (m, 2H, Ar*H*), 7.51 – 7.46 (m, 3H, Ar*H*), 7.25 – 7.20 (m, 4H, Ar*H*), 7.06 – 7.01 (m, 2H, Ar*H*), 6.92 – 6.86 (m, 4H, Ar*H*), 5.35 (t, *J* = 3.0 Hz, 1H, CH_2-O*H*), 4.80 (s, 4H, N-C*H*$_2$-Ar), 4.20 (d, *J* = 3.0 Hz, 2H, HetAr-C*H*$_2$-OH), 3.82 (s, 3H, O-C*H*$_3$), 3.73 (s, 6H, O-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 168.98, 168.34, 160.76, 160.39, 158.77, 139.57, 131.69, 131.38, 130.94, 129.68, 129.58, 129.28, 128.34, 116.67, 114.31, 113.90, 113.81,

57.85, 55.69, 55.48 (d, *J* = 3.5 Hz), 48.64. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{34}H_{34}N_3O_4$ 548.2544, found 548.2544. **UPLC-MS** (*m/z*) $[M+H]^+$ 548.47, t_R = 5.75 min.

General procedure for nucleophilic substitution and deprotection (C)

A solution of **24** (1 eq.) in anhydrous DMF (cooled to 0 °C) was added dropwise to a suspension of 60% NaH (3 eq.) in anhydrous DMF (also cooled to 0 °C). The mixture was stirred at 0 °C for 20 min. The corresponding halide (3 eq.) was added and the reaction mixture was stirred at 80 °C for 19 h. The excessive NaH was hydrolyzed by addition of water, volatiles were evaporated, the residue mixed with EtOAc and washed with water (3 × 100 mL). The organic fraction was dried over $MgSO_4$, the solid filtered off and the filtrate evaporated. The residue was dissolved in TFA (4 mL) and the reaction mixture was stirred at 50 °C for 16 h. Volatiles were evaporated and the residue was purified using silica gel or C_{18}-reversed phase flash chromatography.

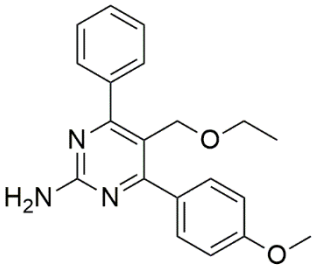
2-Amino-5-methoxymethyl-4-(4-methoxyphenyl)-6-phenylpyrimidine (25a)



The reaction of **24** (105 mg, 0.19 mmol, 1 eq.), MeI (35 µL, 0.57 mmol, 3 eq.) and 60% NaH (23 mg, 0.57 mmol, 3 eq.) following the procedure C gave after silica gel flash chromatography (linear gradient elution 0-60 % EtOAc in hexane) and subsequently after C_{18}-reversed phase flash chromatography (linear gradient elution 0-100 % MeOH in water) **25a** (32 mg, 52 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-*d*$_6$) δ 7.80 – 7.72 (m, 4H, Ar*H*), 7.52 – 7.48 (m, 3H, Ar*H*), 7.08 – 7.04 (m, 2H, Ar*H*), 6.80 (s, 2H, N*H*$_2$), 3.87 (s, 2H, HetAr-C*H*$_2$-O), 3.84 (s, 3H, Ar-O-C*H*$_3$), 3.21 (s, 3H, CH_2-O-C*H*$_3$). **^{13}C NMR** (101 MHz, DMSO-*d*$_6$) δ 169.67, 168.98, 162.81, 160.66, 139.15, 131.26, 130.91, 129.58, 129.21, 128.35, 114.18, 113.82, 69.12, 57.22, 55.70. **IR** ($CHCl_3$) *v*: 3532, 3422, 1601, 1546, 1514, 1497, 1373, 1252, 1177, 1095, 841 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{19}H_{20}N_3O_2$ 322.1550, found 322.1550. **UPLC-MS** (*m/z*) $[M+H]^+$ 322.26, t_R = 4.90 min.

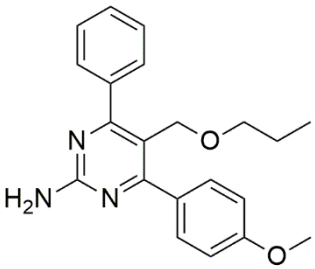
2-Amino-5-ethoxymethyl-4-(4-methoxyphenyl)-6-phenylpyrimidine (25b)



The reaction of **24** (210 mg, 0.38 mmol, 1 eq.), EtBr (85 µL, 1.14 mmol, 3 eq.) and 60% NaH (46 mg, 1.14 mmol, 3 eq.) following the procedure C gave after silica gel flash chromatography (linear gradient elution 0-60 % EtOAc in hexane) **25b** (42 mg, 33 %) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.77 – 7.72 (m, 2H, Ar*H*), 7.72 – 7.68 (m, 2H, Ar*H*), 7.47 – 7.43 (m, 3H, Ar*H*), 7.03 – 6.99 (m, 2H, Ar*H*), 6.95 (bs, 2H, N*H*$_2$), 3.85 (s, 2H, HetAr-C*H*$_2$-O), 3.77 (s, 3H, O-C*H*$_3$), 3.29 (q, *J* = 7.0 Hz, 2H, O-C*H*$_2$-CH$_3$), 1.13 (t, *J* = 7.0 Hz, 3H, O-CH$_2$-C*H*$_3$). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 160.98, 138.31, 131.12, 129.97, 129.32, 128.41, 114.47, 113.89, 66.79, 65.04, 55.75, 15.52. **IR** ($CHCl_3$) *v*: 3532, 3422, 1601, 1546, 1514, 1497, 1373, 1252, 1178, 1091, 841 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{20}H_{22}N_3O_2$ 336.1707, found 336.1707. **UPLC-MS** (*m/z*) $[M+H]^+$ 335.99, t_R = 4.59 min.

2-Amino-5-propoxymethyl-4-(4-methoxyphenyl)-6-phenylpyrimidine (26)

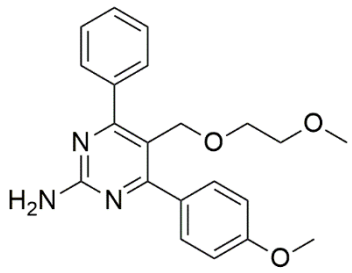


Compound **24** (105 mg, 0.19 mmol, 1 eq.) was dissolved in TFA (2 mL) and the solution was stirred at 50 °C for 5 h. Propan-1-ol (5 mL) was added dropwise and the mixture was stirred for another 5 min. Volatiles were evaporated and the residue was purified using silica gel flash chromatography (linear gradient elution 0-50 % EtOAc in hexane) to yield **26** (21 mg, 35 %) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.83 – 7.79 (m, 2H, Ar*H*), 7.79 – 7.75 (m, 2H, Ar*H*), 7.53 – 7.48 (m, 3H, Ar*H*), 7.08 – 7.04 (m, 2H, Ar*H*), 3.90 (s, 2H, HetAr-C*H*$_2$-O), 3.83 (s, 3H, O-C*H*$_3$), 3.25 (t, *J* = 6.3 Hz, 2H, O-C*H*$_2$-CH$_2$), 1.64 – 1.55 (m, 2H, CH$_2$-C*H*$_2$-CH$_3$), 0.97 (t, J = 7.4 Hz, 3H, CH$_2$-C*H*$_3$). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 169.35, 168.67, 161.41, 161.02, 138.18, 131.12, 130.27, 130.06, 129.31, 128.44, 114.50, 113.90,

71.40, 67.06, 55.76, 22.88, 11.48. **IR** ($CHCl_3$) *v*: 3532, 3422, 1601, 1546, 1514, 1497, 1253, 1178, 1092, 1034, 839 cm^{-1}. **HRMS** (EI) *m/z* calcd for $C_{21}H_{23}N_3O_2$ 349.1790, found 349.1794. **UPLC-MS** (*m/z*) $[M+H]^+$ 350.03, t_R = 4.86 min.

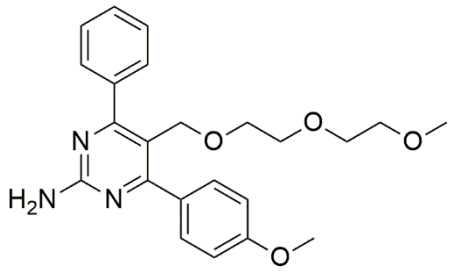
2-Amino-5-((2-methoxyethoxy)methyl)-4-(4-methoxyphenyl)-6-phenylpyrimidine (25c)



The reaction of **24** (165 mg, 0.30 mmol, 1 eq.), 2-chloroethyl methyl ether (82 µL, 0.90 mmol, 3 eq.) and 60% NaH (36 mg, 0.90 mmol, 3 eq.) following the procedure C gave after silica gel flash chromatography (linear gradient elution 0-50 % EtOAc in hexane) **25c** (40 mg, 37 %) as a white solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.85 – 7.78 (m, 4H, Ar*H*), 7.51 – 7.45 (m, 3H, Ar*H*), 7.06 – 7.01 (m, 2H, Ar*H*), 6.79 (s, 2H, NH_2), 3.96 (s, 2H, HetAr-CH_2-O), 3.83 (s, 3H, O-CH_3), 3.56 – 3.51 (m, 2H, CH_2-O-CH_3), 3.49 – 3.44 (m, 2H, HetAr-CH_2-O-CH_2). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 169.57, 168.89, 162.82, 160.66, 139.09, 131.22, 131.09, 129.58, 129.39, 128.31, 114.11, 113.75, 71.49, 68.75, 67.55, 58.62, 55.68. **IR** ($CHCl_3$) *v*: 3533, 3422, 1601, 1546, 1514, 1497, 1452, 1352, 1252, 1177, 1093, 1034, 842 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{21}H_{24}N_3O_3$ 366.1812, found 366.1813. **UPLC-MS** (*m/z*) $[M+H]^+$ 365.99, t_R = 4.25 min.

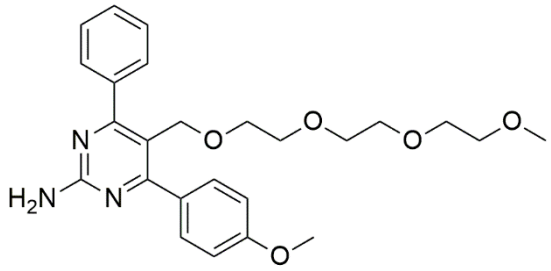
2-Amino-5-((2-(2-methoxyethoxy)ethoxy)methyl)-4-(4-methoxyphenyl)-6-phenylpyrimidine (25d)



The reaction of **24** (210 mg, 0.38 mmol, 1 eq.), 1-bromo-2-(2-methoxyethoxy)ethane (155 µL, 1.14 mmol, 3 eq.) and 60% NaH (46 mg, 1.14 mmol, 3 eq.) following the procedure C gave after silica gel flash chromatography (linear gradient elution 0-60 % EtOAc in hexane) **25d** (26 mg, 19 %) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.86 – 7.82 (m, 2H, Ar*H*), 7.82 – 7.79 (m, 2H, Ar*H*), 7.51 – 7.46 (m, 3H, Ar*H*), 7.06 – 7.02 (m, 2H, Ar*H*), 6.80 (s, 2H, NH_2), 3.95 (s, 2H, HetAr-CH_2-O), 3.83 (s, 3H, O-CH_3), 3.63 – 3.60 (m, 2H, HetAr-CH_2-O-CH_2-CH_2), 3.60 – 3.57 (m, 2H, CH_2-CH_2-O-CH_3), 3.52 – 3.48 (m, 2H, CH_2-O-CH_3), 3.48 – 3.43 (m, 2H, HetAr-CH_2-O-CH_2), 3.27 (s, 3H, CH_2-O-CH_3). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 169.57, 168.92, 162.81, 160.66, 139.07, 131.20, 131.12, 129.59, 129.40, 128.33, 114.11, 113.75, 71.91, 70.08, 70.05, 69.09, 67.59, 58.60, 55.65. **IR** ($CHCl_3$) *v*: 3532, 3422, 1601, 1546, 1514, 1497, 1452, 1252, 1177, 1097, 1033, 841 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{23}H_{28}N_3O_4$ 410.2074, found 410.2075. **UPLC-MS** (*m/z*) $[M+H]^+$ 410.00, t_R = 4.22 min.

2-Amino-5-(2,5,8,11-tetraoxadodecyl)-4-(4-methoxyphenyl)-6-phenylpyrimidine (25e)

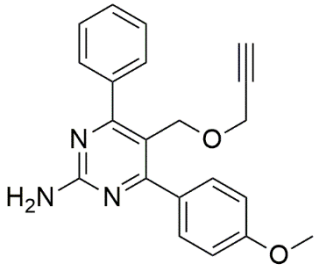


The reaction of **24** (165 mg, 0.30 mmol, 1 eq.), 1-(2-bromoethoxy)-2-(2-methoxyethoxy)ethane (223 µL, 0.90 mmol, 3 eq.) and 60% NaH (36 mg, 0.90 mmol, 3 eq.) following the procedure C gave after silica gel flash chromatography (linear gradient elution 0-70 % EtOAc in hexane) **25e** (20 mg, 15 %) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 – 7.82 (m, 2H, Ar*H*), 7.82 – 7.78 (m, 2H, Ar*H*), 7.50 – 7.47 (m, 3H, Ar*H*), 7.06 – 7.02 (m, 2H, Ar*H*), 6.79 (s, 2H, NH_2), 3.95 (s, 2H, HetAr-CH_2-O), 3.83 (s, 3H, O-CH_3), 3.64 – 3.60 (m, 2H, HetAr-CH_2-O-CH_2-CH_2), 3.58 (s, 4H, CH_2-CH_2-O-CH_2-CH_2-O-CH_3), 3.54 – 3.52 (m, 2H, CH_2-CH_2-O-CH_3), 3.47 – 3.44 (m, 2H, HetAr-CH_2-O-CH_2), 3.43 – 3.40 (m, 2H, CH_2-O-CH_3), 3.21 (s, 3H, CH_2-O-CH_3). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 169.58, 168.91, 162.83, 160.65, 139.07, 131.21, 131.10, 129.57, 129.40, 128.34, 114.13, 113.75, 71.73, 70.42, 70.29, 70.11, 70.06, 69.07, 67.58, 58.50, 55.65. **IR** ($CHCl_3$) *v*: 3532, 3422, 1601, 1546, 1541, 1497,

1452, 1252, 1177, 1095, 1033, 841 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{25}H_{32}N_3O_5$ 454.2337, found 454.2337. **UPLC-MS** (*m/z*) $[M+H]^+$ 454.33, t_R = 4.21 min.

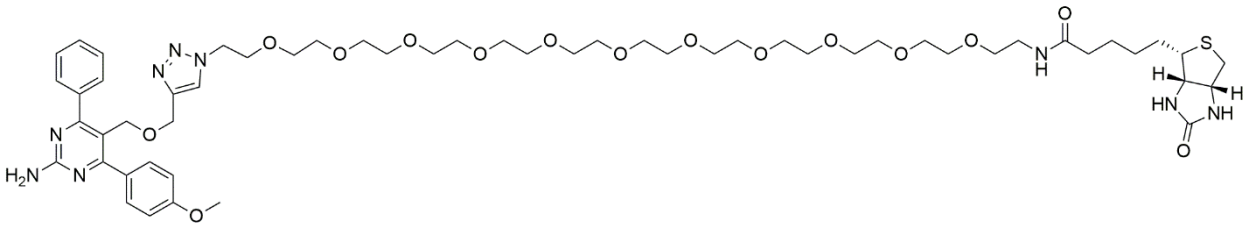
2-Amino-5-((prop-2-yn-1-yloxy)methyl)-4-(4-methoxyphenyl)-6-phenylpyrimidine (27)



The reaction of **24** (160 mg, 0.29 mmol, 1 eq.), propargyl bromide (80 wt. % in toluene) (94 µL, 0.87 mmol, 3 eq.) and 60% NaH (35 mg, 0.87 mmol, 3 eq.) following the procedure C gave after silica gel flash chromatography (linear gradient elution 0-50 % EtOAc in hexane) **27** (98 mg, 98 %) as a brownish solid.

^{1}H NMR (401 MHz, DMSO-d_6) δ 7.81 – 7.73 (m, 4H, Ar*H*), 7.54 – 7.45 (m, 3H, Ar*H*), 7.08 – 7.00 (m, 2H, Ar*H*), 6.90 (bs, 2H, NH_2), 4.12 (d, *J* = 2.4 Hz, 2H, O-CH_2-C), 4.07 (s, 2H, HetAr-CH_2-O), 3.84 (s, 3H, O-CH_3), 3.41 (t, *J* = 2.3 Hz, 1H, C≡C*H*). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 160.86, 131.13, 129.83, 129.35, 128.42, 113.86, 113.71, 80.08, 78.08, 66.72, 57.17, 55.71. **IR** ($CHCl_3$) *v*: 35322, 3423, 3307, 1607, 1546, 1513, 1498, 1252, 1180, 1033, 840 cm^{-1}. **HRMS** (ESI) $[M+H]^+$ *m/z* calcd for $C_{21}H_{20}N_3O_2$ 346.1550, found 346.1550. **UPLC-MS** (*m/z*) $[M+H]^+$ 346.28, t_R = 4.25 min.

N-(35-(4-(((2-Amino-4-(4-methoxyphenyl)-6-phenylpyrimidin-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (28)



Compound **27** (27 mg, 78.17 µmol, 1 eq.), biotin-PEG_{11}-N_3 (62 mg, 78.17 µmol, 1 eq.), CuI (3 mg, 15.63 µmol, 20 mol%) and Et_3N (22 µl, 156.34 µmol, 2 eq.) in anhydrous

DMF were stirred at 25 °C for 1 h. The solvent was evaporated and the residue was purified using C_{18}-reversed phase flash chromatography (linear gradient elution 0-100 % MeOH in acidified water (0.1 % TFA)) and subsequently using HPLC (linear gradient elution 10-100 % MeCN in water) to afford **28** (28 mg, 31 %) as a orange viscose oil.

HRMS (ESI) $[M+H]^+$ *m/z* calcd for $C_{55}H_{84}N_9O_{15}S$ 1142.5802, found 1142.5808. **UPLC-MS** (*m/z*) $[M+H]^+$ 1142.78, t_R = 3.76 min.

6. Conclusion

The thesis deals with polysubstituted pyrimidines with potential anti-inflammatory properties. Such compounds were shown to inhibit the production of prostaglandin E_2 (PGE_2). In total, 43 final products were designed and prepared out of which 21 were synthesized in an attempt to increase biological activity, 11 to investigate the influence of the substituent length in the position C5 of pyrimidine (C5pyr) on biological activity, 10 to enhance solubility, and 1 as a biotinylated probe for pulldown experiments.

The anti-inflammatory efficacy of studied compounds was successfully enhanced. In the first part of the first structure-activity relationship study (SAR 1.1), the phenyl moiety in C4pyr of the lead structure WQE-134 was modified by EDG (10 analogues) or EWG (1 analogue) resulting in the discovery of **9g**, which was 62 times better inhibitor of PGE_2 production than WQE-134. Moreover, in the second part of the first structure-activity relationship study (SAR 1.2), the lead was further modified using benzyloxy or benzyloxy-like moieties revealing compound **10d** as 978 times more potent inhibitor of PGE_2 production than WQE-134.

Furthermore, molecules with hydrogen in C5pyr, corresponding to previously prepared compounds with butyl in this position, were synthesized in order to study the influence of the substitution on biological activity. The replacement of butyl for hydrogen in C5pyr resulted in both increased and decreased anti-inflammatory efficacy. Thus, no overall SAR conclusion can be made in this regard.

The synthesis of compounds **19**, **20**, **22**, **23**, **26**, and **25a-25e** for increased solubility purposes was the most challenging part of the thesis as many approaches applied were not successful. Nevertheless, eligible synthetic route was developed and all desired final products were prepared. Moreover, all synthesized molecules proved to be more soluble than the lead compound WQE-134 and the most soluble compound achieved two orders of magnitude higher solubility than WQE-134.

Last but not least, WQE-134 biotinylated in C5pyr via pegylated linker was successfully prepared and will be used in pulldown experiments in order to clarify the mechanism of action this class of compounds.

7. Literature

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