CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

Department of Organic and Bioorganic Chemistry

Synthesis of pyrrole-based inhibitors of the macrophage infectivity potentiator proteins

DIPLOMA THESIS

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DECLARATION

I declare that this thesis is my original work. All used literature and sources are listed in the list of used literature at the end of the thesis and are properly cited. This work has not been used to gain equal or different degree.

Hradec Králové 2017 Josef Škoda

Acknowledgement

I would like to thank Prof. Dr. Ulrike Holzgrabe for the opportunity to make all my experimental part during my Erasmus internship in her workgroup at Julius-Maximilian-Universität Würzburg.

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My big thanks also belong to my Czech supervisor PharmDr. Jaroslav Roh, PhD, for all the help and consultations he provided me.

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Abstrakt

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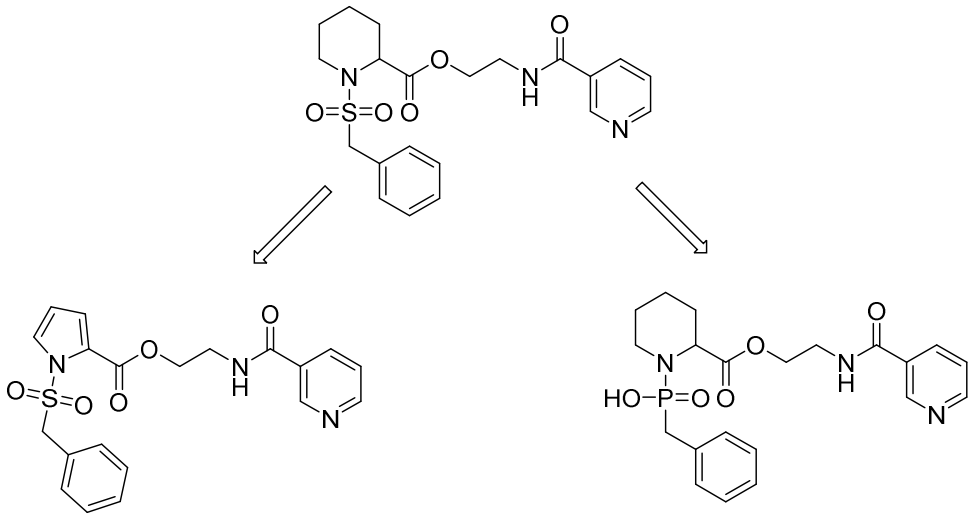
Název diplomové práce: Syntéza pyrrolových inhibitorů potenciátorů infektivity makrofágů

Bakterie *Burkholderia pseudomallei* a *Legionella pneumophila* způsobují závažná infekční onemocnění jako legionelózu (legionářskou nemoc) a melioidózu, u kterých je i dnes vysoká mortalita. Zatímco legionářská nemoc se při akutním průběhu manifestuje podobně jako zápal plic, melioidóza může mít různý průběh s nespecifickou fází následovanou multiorgánovým postižením a septickým šokem. Gramnegativní bakterie *B. pseudomallei* a *L. pneumophila* jsou citlivé pouze k některým rezervním antibiotikům, dále hrozí riziko rezistence, která by byla významným problémem. Pro tyto patogeny se jeví jako výhodný farmakoterapeutický cíl jejich virulenční faktor zvaný potenciátor infektivity makrofágů (MIP). MIP protein je peptidyl-prolyl *trans/cis* izomeráza, pro tyto patogeny je důležitým faktorem šíření a průniku tkáněmi. MIP protein patří do rodiny FK506 binding protein (FKBPs), tvořící stabilní komplexy s jeho inhibitory takrolimem a rapamycimem, které však jsou jako imunosupresiva v léčbě infekcí kontraindikovány. Efekt inhibice MIP proteinu ale dokazuje, že je tento protein vhodným farmakologickým cílem.



Obrázek 1. Analogy inhibitorů Mip proteinu

Před nedávnem bylo zjištěno, že inhibice MIP proteinu je u rapamycinu vázána na strukturu pipekolové kyseliny. Skupina prof. Holzgrabe připravila knihovnu inhibitorů MIP proteinu se strukturou pipekolové kyseliny, které nevykazují imunosupresivní aktivitu. Cílem mé práce bylo rozšířit řadu potenciálních inhibitorů o pyrrolové a fosfoamidové analogy (Obr. 1).

V první části práce jsem pracoval na syntéze analogů dříve připravených inhibitorů, u kterých byla struktura pipekolové kyseliny zaměněna za 1*H*-pyrrol-2-karboxylovou kyselinu. Tato syntéza byla problematická z důvodu nízké reaktivity a stability produktů, nicméně se podařilo připravit jeden analog. V druhé části jsem připravoval analogy, kde byla sulfonamidová skupina zaměněna za fosfoamidovou. Syntéza těchto derivátů byla neúspěšná z důvodu vysoké hydrofility, složité detekce a separace produktů. Díky mé práci bude možno připravit další pyrrolové analogy a testovat jejich aktivitu na MIP proteinu, dále vycházet z výsledků syntézy fosfoamidových analogů a případně zvolit přípravu jejich lipofilnějších analogů.

Abstract

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

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Title of diploma thesis: Synthesis of pyrrole macrophage infectivity potentiator inhibitors

Pathogens *Burkholderia pseudomallei* and *Legionella pneumophila* cause severe diseases like Legionnaire's disease and melioidosis. While Legionnaire's disease manifests as acute pneumonia, melioidosis has different clinical features and ends by multi-organ involvement and septic shock. Low sensibility of gram negative bacteria *B. pseudomallei* and *L. pneumophila* to antibiotics together with threatening resistance represent a great problem. For these pathogens their virulent factor macrophage infectivity potentiator (MIP) protein is a suitable target. MIP proteins are peptidyl/prolyl *cis/trans* isomerases, highly important factor of penetration and dissemination for *B. pseudomallei* a *L. pneumophila*. MIP protein belongs to FK506 binding protein (FKBPs) superfamily, which forms highly stable complex with its inhibitors tacrolimus and rapamycin. Due their immunosuppressive activity, these drugs are contraindicated for treatment of infection diseases. However inhibition of the protein proves that MIP protein is appropriate pharmacological target.

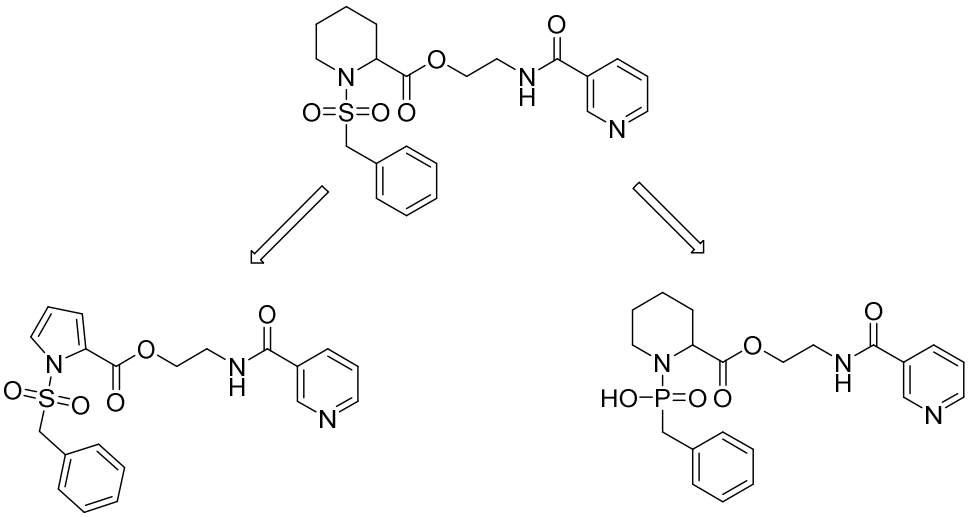


Figure 1. MIP protein inhibitor analogues

Recently it has been discovered that MIP protein inhibition is conserved to pipecolic acid part of rapamycin structure. Group of prof. Holzgrabe prepared library of MIP protein inhibitors based on pipecolic acid structure without immunosuppressive activity. The aim of my work was preparation of new analogues of potential inhibitors, namely pyrrole and phosphonamide analogues (Fig. 1).

In the first part of my work I prepared analogues of previously synthetized inhibitors with substitution of pipecolic acid by 1*H*-pyrrole-2-carboxylic acid. This synthesis was problematic due to low reactivity and stability of products, however I managed to prepare one analogue. In the second part I tried to prepare analogues with substitution of sulphonamide by phosphonamide group. Synthesis of these analogues was unsuccessful because of high hydrophilicity, complicated detection and separation of the products. Synthesis developed in my work could enable the preparation of new pyrrole analogues and to test their potential activity on MIP protein. Furthermore, results of phosphonamide synthesis can be utilized and eventually more lipophilic analogues can be prepared.

Content:

Legionnaires' disease and melioidosis

Legionnaires' disease

Pathogen *L. pneumophila*

Legionnaires' disease is one of the community or hospital acquired pneumonia caused by *Legionella pneumophila (L. pneumophila),* gram-negative aerobic bacteria. It was recognised in 1976 for the first time as pneumonia with high mortality.[1] About 80 % of infected patients had some risk factors like diabetes mellitus, were heavy smokers or older people.[2] *L. pneumophila* is transmitted by inhalation of aerosols or by consuming infected water, no evidence of person-to-person transmission exists. The most of the cases occur in North America, Oceania and Southeast Asia during summer and autumn, when infection can disseminate due to higher humidity.[3]

Diagnosis, manifestation and treatment

Culture and isolation from clinical specimens is still golden standard. Isolated strain allows epidemiological typing, which is important for further studies.[4] Some kits for urine antigen test are commercially available, but they are not reliable for non-*L. pneumophila* serogroup I strains.[5,6] Also serology is not suitable for clinical management because of low reliability. As PCR methods are evolving, real-time PCR is the method of molecular diagnosis, providing fast and specific diagnosis.[7]

Legionnaires' disease manifests by fever and organ specific symptoms like diarrhoea, rhabdomyolysis and renal failure 2 – 10 days after infection.[8] Given that, the symptoms are not specific and high mortality rate is characteristic of Legionnaires, every patient with risk factors in endemic areas should be tested and early treatment should be initiated.[9] According to the older guidelines, pneumonia resistant to β-lactams should be treated as Legionnaires' disease.

Because *L. pneumophila* is a residing intracellular pathogen, administered antibiotics should achieve sufficient intracellular concentrations in tissues.[10] Erythromycin used to be the drug of choice, nowadays azithromycin, tetracyclines of fluoroquinolones are used due to lower side effects and higher activity. Azithromycin and levofloxacin are the antibiotics of the first choice, therapy should last at least 5 to 14 days and the patients should be afebrile 48 – 72 hours before therapy is finished. There is no evidence of higher effectivity of drug combinations.[11, 12]

Melioidosis

Pathogen *Burkholderia pseudomallei*

Burkholderia pseudomallei (B. pseudomallei), one of burkholderia gram-negative species occurring in Southeast Asia and northern Australia, causes infection called melioidosis.[13] *B. pseudomallei* has one of the most complex and highly evolving

genome, which differs within a single patient, indicating high ability of the organism to evolve rapidly.[14,15] As an intracellular pathogen, bacteria can invade, survive and replicate in phagocytic cells.[16] Higher incidence in rainy months results from its way of transmission as percutaneous inoculation, inhalation or ingestion of infected water, aerosol or soil.[17] One or more risk factors such as diabetes, alcoholism, chronic pulmonary disease or immunodeficiency were presented in the most of the patients. Melioidosis is unlikely fatal for healthy persons when medical care is provided.[18]

Diagnosis, manifestation and treatment

Cultivation of clinical sample is necessary for correct diagnosis of *B. pseudomallei* infection, however misidentification is common in laboratories unfamiliar with this bacteria.[19] PCR identification assays are faster, but not so sensitive.[20] Serologic diagnosis is possible but alone inadequate, actually in the endemic regions the background seropositivity is higher than 50%.[21]

For melioidosis abscesses in infected tissue are typical, for example, imitation of tuberculosis. The most usual presenting features are pneumonia, genitourinary and skin infections.[22] In patients with risk factors, the infection can lead to septic shock after 1-21 days.[23] A delay in diagnosis can be fatal due to insufficient empirical antibiotic therapy, in endemic regions antibiotics effective to melioidosis should be used for experimental therapy.[24]

B. pseudomallei is inherently resistant to wide range of antibiotics, for example penicillin, first and second generation of cephalosporins, tigecycline and moxifloxacine, with various mechanisms of antibiotic resistance.[24, 25] Treatment begins by intensive intravenous therapy by ceftazidime, meropenem or imipenem for two weeks followed by 3 – 6 months therapy with trimethoprime – sulfametoxazole.[26] Careful search for abscesses is important because the resistance rate to trimethoprime – sulfametoxazole therapy is 13%.[27] Amoxicillin – clavulanate is used for following therapy at pregnant women and children.[28]

Mip proteins

PPIases

L. pneumophila and *B. pseudomallei* are threatening pathogens in endemic areas. *B. pseudomallei* is even considered as potential biologic weapon, thus the need of new effective treatment is urgent. As the classic antibiotics and chemotherapeutics target physiological pathways quite common for majority of pathogen and symbiotic bacteria, their effect is not specific and resistances occurs frequently. Novel antimicrobial treatment with utilization of advanced technologies can be more effective without frequent side effects. For example, bacterial virulence factors consist of difficult pathways of secretion systems, which are specific for the pathogen adapted to host. A group of studied virulence factors is a family of peptidyl-prolyl *cis/trans* isomerases (PPIases), which catalyse *cis/trans* isomerization of peptide bond next to the prolyl residue.[29] The most of the peptide bonds are at *trans* configuration because of steric hindrance and high energetic difference of *cis/trans* configuration. In case of peptidyl-prolyl bond, α-amino group is part of heterocycle, so isomerization is possible due to smaller energetic difference of *cis/trans* configuration. That allows 5% to 6% occurrence of *cis* isomers and prolongs half-live from seconds to minutes (Fig. 1).[30]

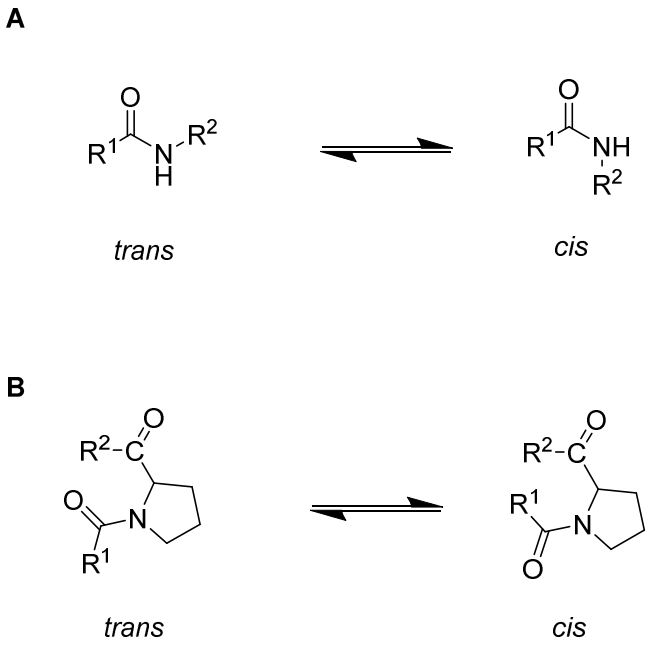


Figure 1. Conformation of peptide bonds (A) and conformation of peptidyl-prolyl bond with *cis/trans* conformation allowed by lower energetic difference (B).[30]

Family of PPIases is wide and still enigmatic. It is possible to measure enzymatic PPIase activity, however many of phenotypes seam to be independent of that activity in physiological function. Prepared PPIases with deleted or changed PPIase domain still poses effect at protein-protein interaction or chaperoning activity.[31] That leads to interpretation of PPIases as chaperones or as a part of the multiprotein

complexes. Importantly, different PPIases has different selectivity for different peptides. Human PPIases prefer to isomerise protein with leucine or phenylalanine preceding the prolyl, but bacterial PPIase of *L. pneumophila* catalyses isomerization of proteins without preference.[32]

Mip protein *L. pneumophila and B. pseudomallei*

Mip proteins are widespread PPIases virulence factors (Tab. 1), firstly identified in *L. pneumophila*. The name macrophage infectivity potentiator arises from the fact that Mip's deletion resulted in reduced intracellular replication in human macrophages.[33] Structures of Mip protein and of Mip protein-inhibitor complexes were extensively studied and were obtained by X-ray and nuclear magnetic resonance (NMR) studies. Mip protein of *L. pneumophila* (LpMip) consists of a N-terminal domain made of two α-helices, a long connecting α-helix in the center, and PPIase C-terminal binding FK506 (FK506 binding protein, FKBP) domain.[34,35] Despite the mechanism of Mip action is still unknown, LpMip is involved in dissemination and invasion in lung tissue, possibly through interaction with human collagen.[36,53] LpMip protein is PPIase conformed as homodimer localized in outer membrane.[29]

B. pseudomallei Mip protein (BpMip) quite differs from LpMip. Long helix and N-terminal dimer domain are not involved in BpMip protein, localization in outer membrane is not proven, but PPIase part is identical in 40 %, showing that PPIase domain is highly conserved in Mip proteins. However, it was found that BpMip plays a role in low pH survival and external protease activity. Both LpMip and BpMip have highly identical FKBP domain, their PPIase activity can be inhibited by rapamycine, that proves that Mip proteins are good pharmacological target.[37,29]

Table 1. Mip – like proteins as virulence factors[29]

Organism	Protein	Phenotype of mutants	Ref.
Burkholderia pseudomallei	BpML1	Severe defects in cell culture and murine infection model, flagellation (swarming) and survival affected at pHs of 5, X-ray structure suggests alternative inhibitor structures	37
Chlamydia psittaci	Mip–like protein	Immunodominant in convalescent guinea pigs	38
Legionella pneumophila	Mip	Impaired in infection of macrophages, blood monocytes, and amoebae in the initial time points of infection, significant attenuation in guinea pigs	32
Neisseria meningitidis	NmMip	Upregulated in blood stages, drastic attenuation in *ex vivo* blood-stage septicemia model, potential serogroup B vaccine candidate	39
Salmonella enterica	FkpA	Probable attenuation in Caco-2 epithelial and J774 macrophage cells, extracellular localization not confirmed	40
Aeromonas hydrophila	FkpA	Cross-reactive with L. longbeachae anti-Mip antibodies, no significant attenuation in suckling mouse model	41

Mip protein inhibitors

After the discovery of Mip proteins, their inhibition by FK506, rapamycine and their analogues was studied to obtain non-immunosuppressive inhibitor. Structure-activity relationships (SAR) studies resulted in the discovery of the small molecule inhibitors with pipecolic acid moiety. The pipecolinyl ring mimics peptidyl-prolyl bond and occupies hydrophobic cavity on PPIase (Fig. 2).[42,43] Further studies using docking analysis, combinatorial chemistry and synthetic SAR approach, led to the development of series of Mip protein inhibitors with different structures, even peptide structures, some of them with micro-molar to nanomolar activities.[29]

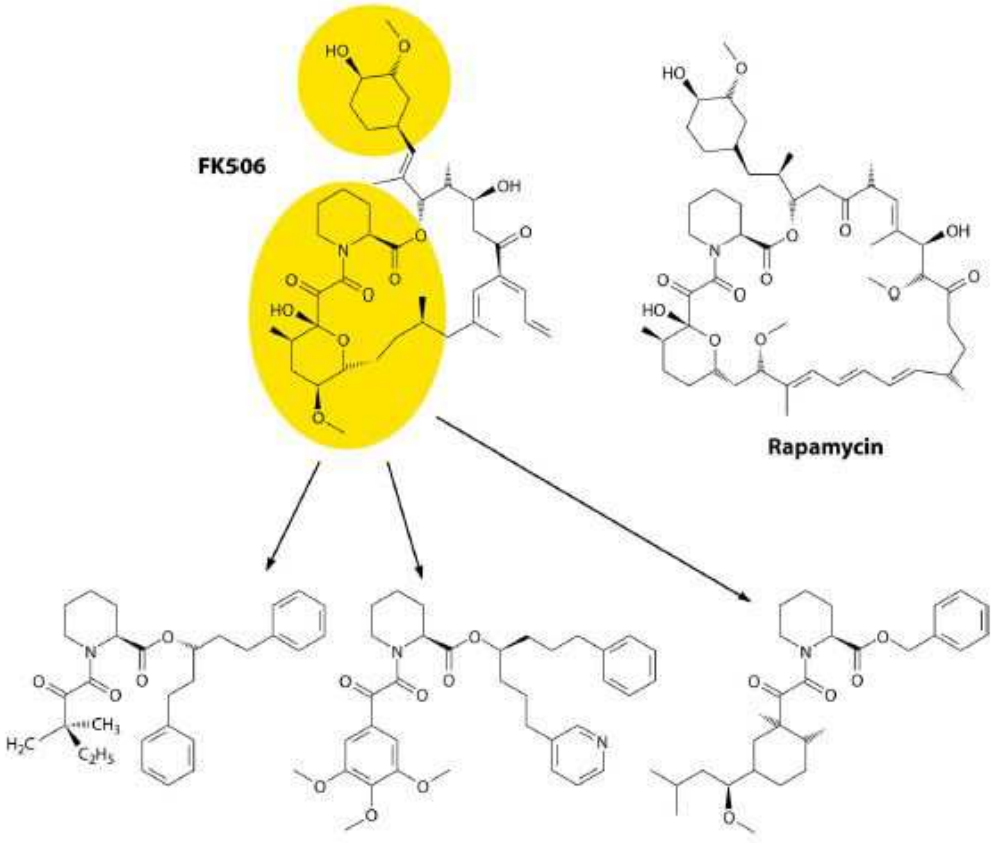


Figure 2. Macrocyclic FKBP inhibitors and their pipecolic acid analogues.[44,45,46]

Mip protein inhibitors of prof. Holzgrabe group

Group of prof. Holzgrabe used crystal structure of LpMip protein for docking analysis and developed well-clustered inhibitor structure with sulphonamide group. Synthesized new leading structure (Fig. 3) had nanomolar activity without immunosuppressive effect. It was confirmed that pipecolic acid inhibitors binds to the same cavity as rapamycine by NMR-HSQC experiments. [47]

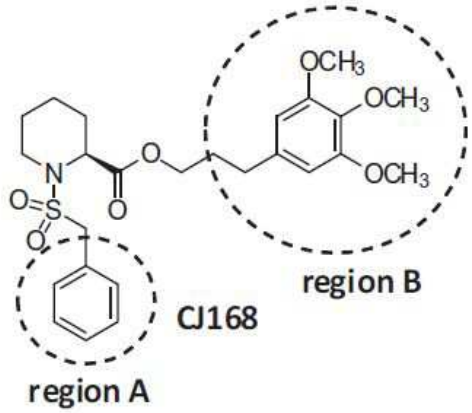
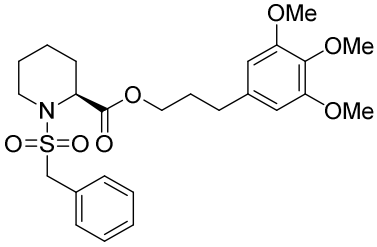
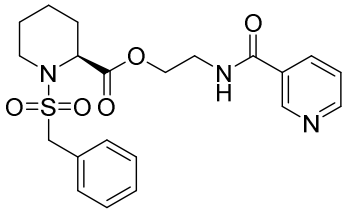
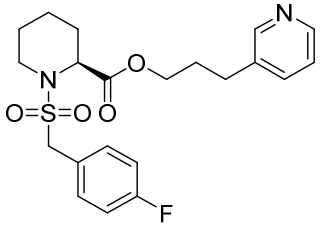
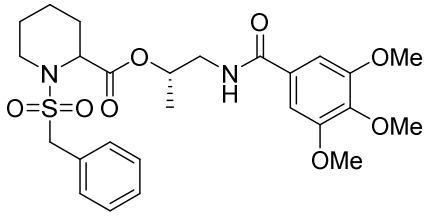


Figure 3. Juli et. al. sulphonamide Mip inhibitor.[47]

After synthesis of novel effective compounds, Seufert et. al. focused on the development of structure applicable as drug, where solubility and stability is needed. Fig. 3 illustrates the regions which were substituted. Library of new compounds (Tab. 2) was prepared and their inhibition activity, cytotoxicity and stability were evaluated. Docking analysis was used for preparation of inhibitors, showing that LpMip and BpMip protein FKBP sites are highly homologous. NMR – HSQC analysis verified that pipecolic acid inhibitors bind to FKBP site. The activity was tested by enzyme coupled PPIase assay, cytotoxicity data were measured on murine macrophage cells. Stability was monitored in buffer and in medium with macrophages.[47,53,48]

Table 2. Mip protein inhibitors of prof. Holzgrabe group.[53]

Leading structude Juli et al.[47]	Leading structure Seufert et al.
 BpMip IC_{50} = 0.1 µM; LpMip IC_{50} = 6 µM	 BpMip IC_{50} = 0.42 µM; LpMip IC_{50} = 11 µM
Best activity for BpMip	**Highest activity for LpMip**
 BpMip IC_{50} = 0.07 µM; LpMip IC_{50} = 5.7 µM	 BpMip IC_{50} = 0.58 µM; LpMip IC_{50} = 2.2 µM

From Fig 3. is obvious, that novel analogues had structure altered in the region A or B. Region A, benzyl-sulfonyl moiety, was studied previously and fits well in hydrophobic cavity of FKBP, but substitution on benzene-ring is possible in para position with small non-polar substituents like halogens. Region B offers more options. New inhibitors with aryl connected to pipecolic acid by ester or amide chain showed the best activities, nevertheless only amides had satisfying stabilities.[53]

Aim of work

Previously prepared pipecolic acid analogues (Tab. 2) are promising non-immunosuppressive inhibitors of Mip protein of *L. pneumophila* and *B. pseudomallei*. SAR of lead structure was already studied at regions A and B (Fig. 3), however, the importance and possible analogues of pipecolic acid were not studied yet. Thus the first part of my work includes the synthesis of 1*H*-pyrrole-2-carboxylic acid analogues of pipecolic acid inhibitors (**B**, Fig.4). These analogues could bring new information about the importance of piperidine heterocycle for inhibitory activity.

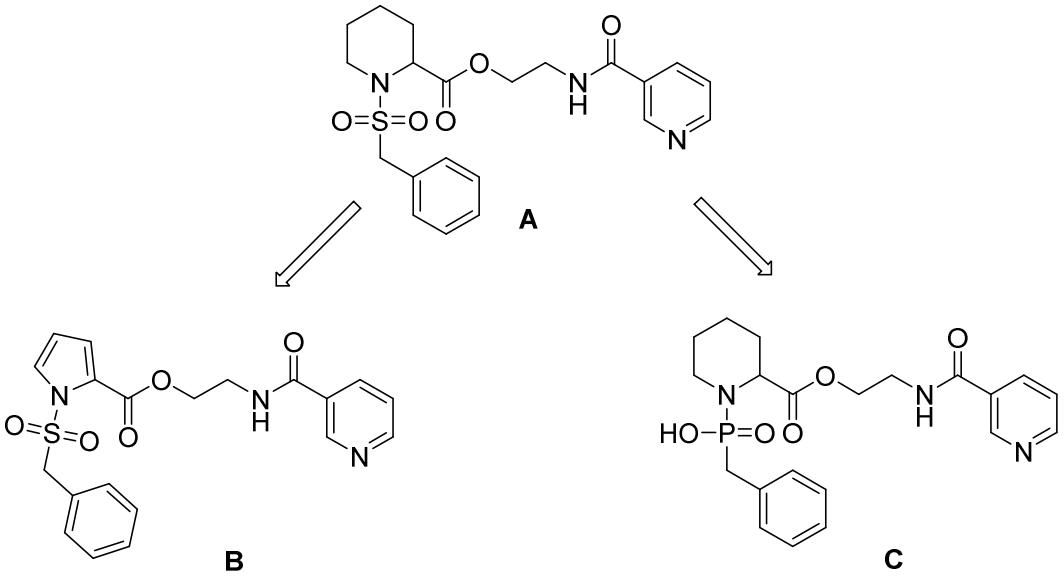


Figure 4. Lead structure (A) and designed pyrrole (B) and phosphonamide (C) analogues

In the second part of this work, the role of the sulphonamide moiety was studied. Thus, the sulphonamide was replaced with phosphonamide group as similar electronegative moiety (**C**, Fig. 4). Benzyl-sulphonamide derivatives showed the best activities in previous work, while this moiety fitted into hydrophobic cavity of FKBP.[47] Phosphonamide group has comparable electronegativity, but is not hydrophobic, which might bring new data for SAR.

In summary, some effective Mip protein inhibitors were prepared before,[53] but the physiological function of Mip protein and SAR of its inhibitors have not been clear yet. Thus the extension of Mip protein inhibitors library would help in further studies.

Plan of synthesis

Pyrrole analogues

Juli et al. developed synthesis for pipecolic acid Mip inhibitors (Fig. 5).[47] Firstly, the reaction of ethyl pipecolinate with benzylsulfonyl chloride in basic conditions of Et_3N provided benzylsulphonamide. Then ethyl ester was hydrolysed in basic conditions using LiOH. Specific ester was prepared by esterification with corresponding alcohol using *N,N'*-dicyclohexylcarbodiimide (DCC), or 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl). Possible substitutions on benzylsulphonamide followed.[53]

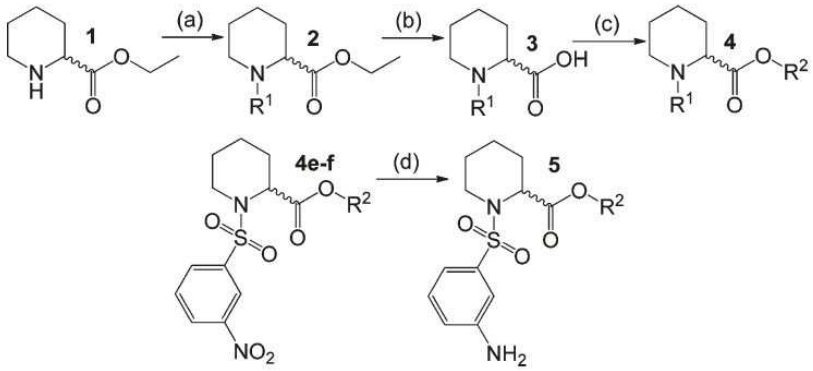


Figure 5. Synthesis of pipecolic acid inhibitors[47]

As lower reactivity of pyrrole analogues was expected due to possible mesomeric effects, three parallel preparations of *N*-benzylsulfonyl-pyrrole-2-carboxylic acid were considered (Fig. 6). First option was similar to earlier synthesis of pipecolic acid analogues,[53] ethyl ester reacts with benzylsulfonyl chloride. To enhance the reactivity of pyrrole-2-carboxylate, two bases, *N*-methylmorpholine (NMM), and sodium hydride, was used. Hydrolysis with LiOH followed. Second option used pyrrole-2-carbaldehyde and benzylsulfonyl chloride again in basic conditions and subsequent oxidation to carboxylic acid using sodium chlorite.[49] Third option used benzyl-ester and follows as first option with availability to deprotect acid by hydrogenation.

JS2 (*N*-(benzylsulfonyl)pyrrole-2-carboxilic acid) esterified using agent EDC.HCl with corresponding alcohol should provide final product (Fig. 6). Corresponding alcohols, which contain ester or amide moiety, was planned to be prepared by same coupling reaction with EDC.HCl. Preparation of alcohols and final esterification could be one-pot synthesis.[53]

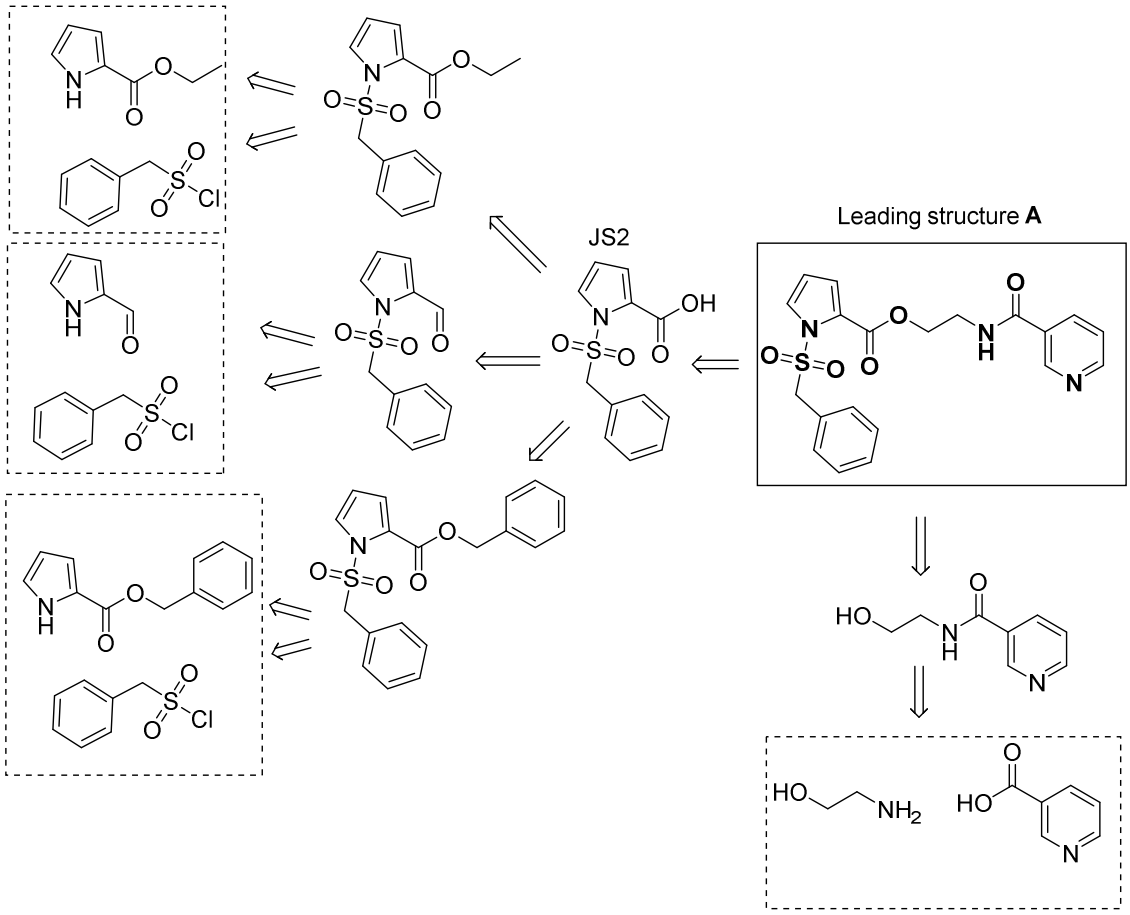


Figure 6. Retrosynthesis of pyrrole analogues

Ethyl *N*-(benzylsulfonyl)pyrrole-2-carboxylate could provide H_3BO_3-catalyzed transesterification reaction with corresponding alcohol (Fig. 7) using Dean-Stark condenser.[50]

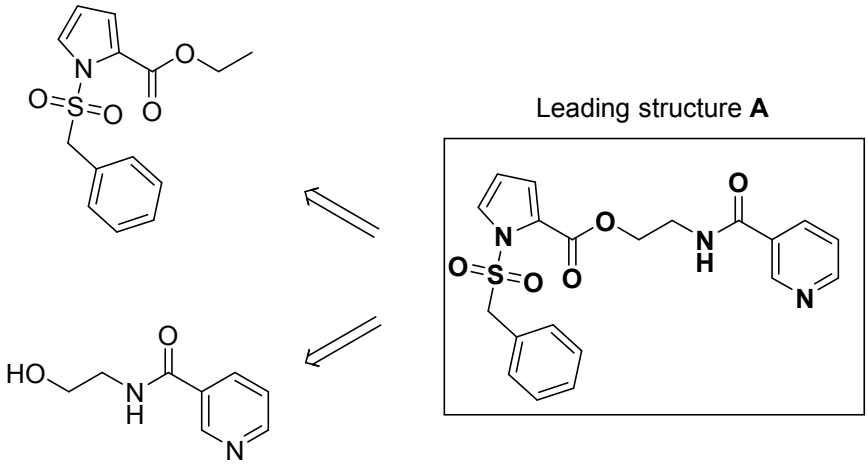


Figure 7. Transesterification of pyrrole-2-carboxylate

Phosphonamide analogues

Synthesis of phosphonamide analogues should follow the earlier synthesis.[53] As benzylphosphonic monochloride is not available at the market, I found tree different options how benzylphosphonyl-pipecolinate could be prepared. Two syntheses include preparation of benzylphosphonyl chloride, simple reflux of benzylphosphonic acid in $SOCl_2$ or secondly the reaction with $SOCl_2$ catalyzed by *N,N*-dimethylformamide (DMF).[51] Products should be transferred directly into following reaction and benzyl-phosphonyl chloride with ethyl pipecolinate should provide the phosphonamide.[53] Third option suggests phosphoamidation of ethyl pipecolinate with benzylphosphonic acid facilitated by coupling agent EDC.HCl, which should provide the corresponding phosphonamide.[52]

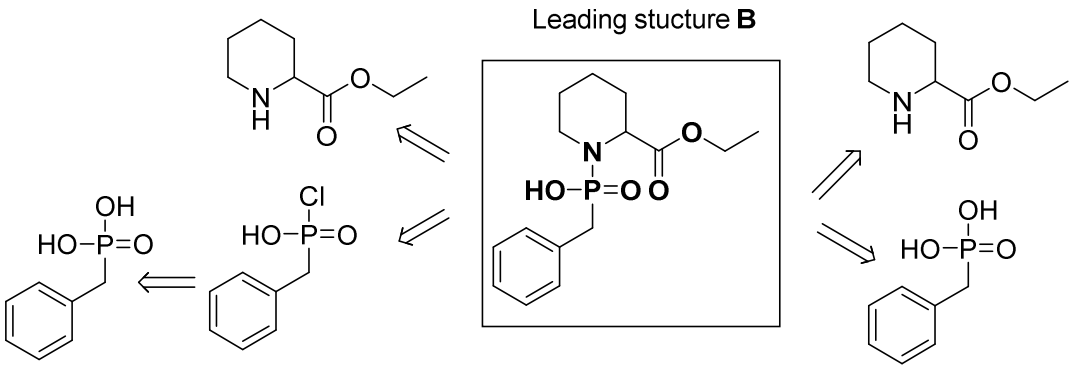
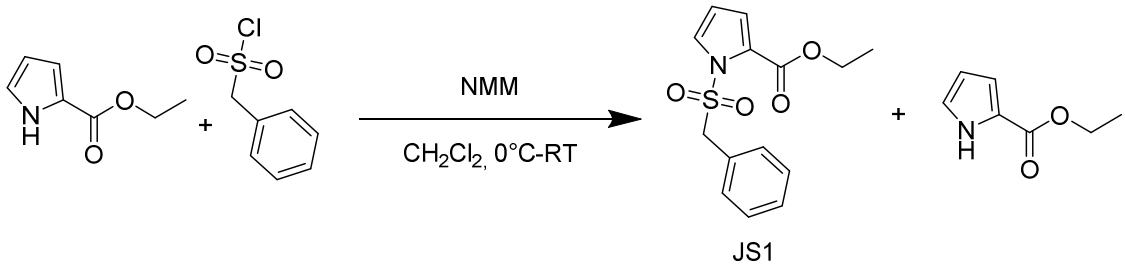


Figure 7. Retrosynthesis of phosphonamide analogues

Experimental part

JS1 – Synthesis of ethyl 1-(benzylsulfonyl)-1*H*-pyrrole-2-carboxylate

Approach 1:

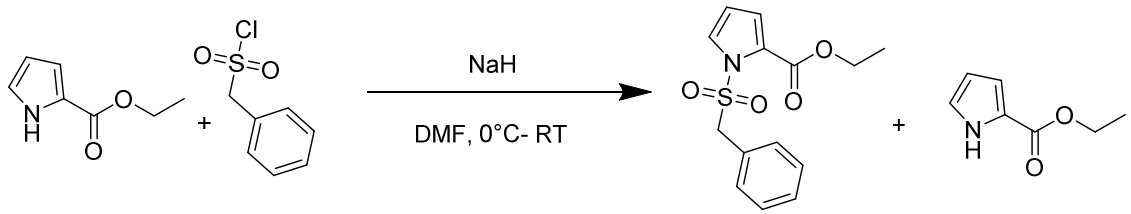


JS1

Ethyl 1*H*-pyrrole-2-carboxylate was dissolved in a dry flask in anhydrous dichloromethane (DCM, 3 mL) under argon atmosphere. The reaction mixture was cooled to 0 °C, firstly *N*-methylmorpholine (NMM) was added dropwise, then benzylsulfonyl chloride dissolved in DCM (1 mL). The reaction was stirred at room temperature (RT), monitored with thin layer chromatography (TLC), (petroleum ether (PE)/ethyl acetate (EtOAc) = 4/1, detection UV). Ethyl 1*H*-pyrrole-2-carboxylate was still detected after 24 h (R_f = 0.62). Reaction was washed with 10% HCl (2 × 5 mL), then with water (3 × 5 mL) and with brine (1 × 5 mL). DCM was dried over sodium sulphate and evaporated under reduced pressure. Column chromatography followed (on silica gel, mobile phase PE/EtOAc gradient 9/1 to 1/9) and only starting material ethyl 1*H*-pyrrole-2-carboxylate was isolated (control via TLC and NMR).

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
Ethyl 1*H*-pyrrole-2-carboxylate	1.00	139.16	0.15	21 mg	
NMM	3.00	101.15	0.45	50 µL	
Benzylsulfonyl chloride	1.00	190.65	0.15	29 mg	
JS1		293.34			0%

Approach 2:

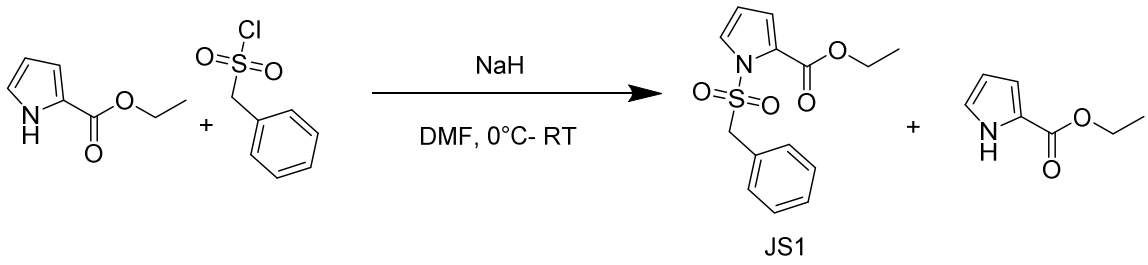


JS1

NaH was suspended in a dry flask in anhydrous DMF (3 mL) under argon atmosphere, the stirring suspension was cooled to 0 °C and ethyl 1*H*-pyrrole-2-carboxylate in dry DMF (5 mL) was added dropwise. Reaction was stirred at RT for 1.5 hours, then was cooled again to 0 °C, benzylsulfonyl chloride was added dropwise in dry DMF (3 mL). Reaction was stirred at RT overnight. After 24 h, there were detected tree spots on TLC (PE/EtOAc = 4/1, detection UV) , JS1 (R_f = 0.55), ethyl 1*H*-pyrrole-2-carboxylate (R_f = 0.62) and side product (R_f = 0.91). To enhance the yield of the reaction, another 1.2 equivalent (eq.) of NaH and 1.2 eq. of benzylsulfonyl chloride were added and reaction was heated to 70 °C for 3 hours. The reaction was monitored with TLC and no changes were detected. The reaction was quenched with water (10 mL) and products were extracted to ethyl acetate (4 × 10 mL). Combined organic extracts were washed with water (3 × 25 mL) and with brine (30 mL), dried over sodium sulphate and evaporated under reduced pressure. Brown oil was gained, column chromatography was performed (on silica gel, mobile phase PE/EtOAc = 4/1). JS2, ethyl 1*H*-pyrrole-2-carboxylate and mixture of both detected chemicals were obtained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
NaH	1.20	23.99	0.86	24 mg	
Ethyl 1*H*-pyrrole-2-carboxylate	1.00	139.15	0.72	100 mg	
Benzylsulfonyl chloride	1.20	190.65	0.86	168 mg	
2. addition of NaH	1.20	23.99	0.86	24 mg	
2. addition of benzylsulfonyl chloride	1.20	190.65	0.86	168 mg	
JS1		293.34	0,07	20 mg	9%

Approach 3:

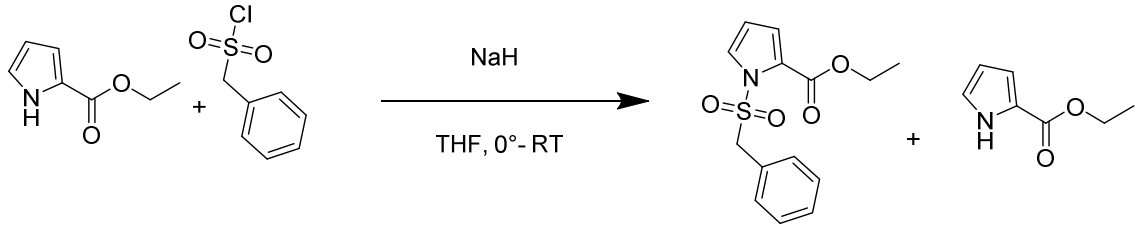


JS1

NaH was suspended in a dry flask in anhydrous DMF (3 mL) under argon atmosphere, the suspension was cooled to 0 °C and ethyl 1*H*-pyrrole-2-carboxylate in dry DMF (5 mL) was added dropwise. The reaction was stirred at RT for 1.5 hours, then was cooled again to 0 °C and benzylsulfonyl chloride was added dropwise in dry DMF (3 mL). Reaction was stirred at 0 °C for 1 hour, than at RT overnight. On TLC (PE/EtOAc = 4/1, detection UV) after 24 h were detected tree spots, JS1 (R_f = 0.55), ethyl 1*H*-pyrrole-2-carboxylate (R_f = 0.62) and side product (R_f =0.91). Reaction was quenched with water (10 mL) and the products were extracted to ethyl acetate (4 × 10 mL). Combined organics phases were washed with water (3 × 25 mL) and with brine (30 mL), dried over sodium sulphate and evaporated under reduced pressure. Brown oil was gained, column chromatography was performed (on silica gel, mobile phase PE/EtOAc = 4/1). JS2, ethyl 1*H*-pyrrole-2-carboxylate, mixture of both detected chemicals and side product were obtained. Isolated side product was more lipophilic than JS1, the signals in aromatic area were observed on NMR spectrum, but the compound was not recognized.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
NaH	1.50	23.99	1.80	28 mg	
Ethyl 1*H*-pyrrole-2-carboxylate	1.00	139.15	0.72	100 mg	
Benzylsulfonyl chloride	1.20	190.65	0.86	168 mg	
JS1		293.34	0.17	50 mg	24%
Side product				10 mg	

Approach 4:



JS1

NaH was suspended in a dry flask in anhydrous tetrahydrofurane (THF, 5 mL) under argon atmosphere, suspension was cooled to 0 °C and ethyl 1*H*-pyrrole-2-carboxylate in dry THF (7 mL) was added dropwise. Reaction was stirred at RT for 1.5 hours, than was cooled again to 0 °C and benzylsulfonyl chloride was added dropwise in dry THF (5 mL). Reaction was stirred at 0 °C for 1 hour, then at RT overnight. After 24 h were detected tree spots on TLC (PE/EtOAc = 4/1, detection UV), JS1 (R_f = 0.55), ethyl 1*H*-pyrrole-2-carboxylate (R_f = 0.62) and side product (R_f = 0.91). Reaction was quenched with water (20 mL) and products were extracted to ethyl acetate (4 × 15 mL). Combined organic phases were washed with water (3 × 40 mL) and with brine (40 mL), dried over sodium sulphate and evaporated under reduced pressure. Brown oil was gained, column chromatography was performed (on silica gel, mobile phase PE/EtOAc = 4/1). JS1, ethyl 1*H*-pyrrole-2-carboxylate and mixture of both chemicals were obtained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yeld:
NaH	1.50	23.99	2.10	56 mg	
Ethyl 1*H*-pyrrole-2-carboxylate	1.00	139.15	1.44	200 mg	
Benzylsulfonyl chloride	1.20	190.65	1.72	336 mg	
JS1		293.34	0.03	10 mg	2%

Properties:

JS1: yellow crystal solid, melting point 68.5°C

1H NMR (400 MHz, CDCl3) δ 7.33 – 7.14 (m, 5H), 7.00 (dd, J = 3.7, 1.9 Hz, 1H), 6.92 (dd, J = 3.2, 1.9 Hz, 1H), 6.04 – 5.97 (m, 1H), 5.10 (s, 2H), 4.33 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H).

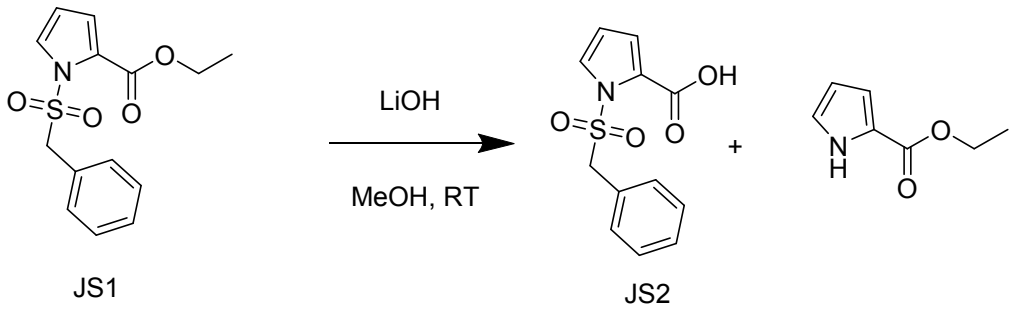
Ethyl 1*H*-pyrrole-2-carboxylate: white crystal solid

1H NMR (400 MHz, CDCl3) δ 9.01 (s, 1H), 6.89 – 6.87 (m, 1H), 6.86 – 6.83 (m, 1H), 6.22 – 6.17 (m, 1H), 4.25 (q, J = 7.1 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H).

Side product: yellow crystal solid

JS2 – Hydrolysis of ethyl-ester JS1

Approach 1:

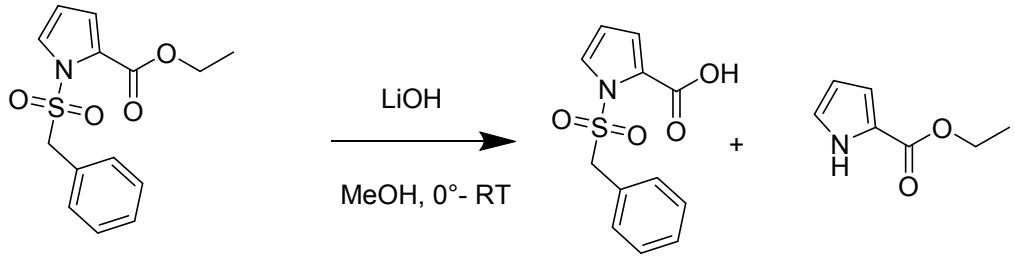


JS1 JS2

JS1 was dissolved in methanol (3 mL). LiOH (1 mL, 1M water solution) was added dropwise and the reaction was stirred at RT overnight. Reaction was controlled via TLC (PE/EtOAc = 4/1, detection UV), JS2 (R_f = 0.17), starting material (R_f = 0.55), ethyl 1*H*-pyrrole-2-carboxylate (R_f = 0.62) and side product (R_f = 0.91) were observed. HCl was added to the reaction (10% HCl water solution to pH=1), the organics were extracted with CH_2Cl_2 (4 × 5 mL), organic phase was washed with water (3 × 10 mL), subsequently with brine (10 mL) and evaporated under reduced pressure without further purification. The mixture (15 mg) of product and starting material was detected by NMR.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
JS1	1.00	293.07	0.17	50 mg	
LiOH . H_2O	6.00	41.04	1.02	42 mg/1 mL	
JS2 (mixture)		265.04		15 mg	

Approach 2:



JS1 JS2

JS1 was dissolved in methanol (10 mL) and cooled to 0 °C. LiOH (3 mL, 1M aqueous solution) was added and the reaction was stirred at RT overnight. The reaction was controlled via TLC (PE/EtOAc = 4/1, detection UV), JS2 (R_f = 0.17), starting material (R_f = 0.55), ethyl 1*H*-pyrrole-2-carboxylate (R_f = 0.62) and side product (R_f = 0.91) were detected. HCl was added to the reaction (10% HCl water solution to pH=1), organics were extracted with ethyl acetate (3 × 10 mL), organic phase was washed with water (2 × 20 mL), subsequently with brine (20 mL) and evaporated under reduced pressure. The desired product was insoluble in a small amount of methanol at RT, thus the organic material was suspended in a small amount of methanol, and the product was gained by filtration. Pure product was detected by NMR.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
JS1	1.00	293.07	0.49	145 mg	
LiOH . H_2O	6.00	41.04	2.97	122 mg/3 mL	
JS2		265.04	0.31	83 mg	63%

Properties:

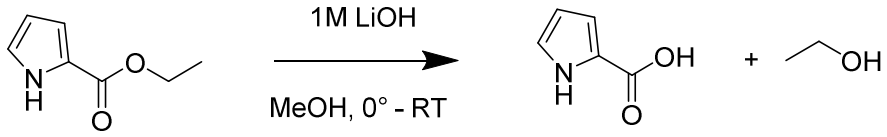
JS2: yellow crystals

1H NMR (400 MHz, DMSO) δ 7.46 – 7.37 (m, 3H), 7.30 – 7.25 (m, 2H), 7.16 (dd, J = 3.6, 1.8 Hz, 1H), 7.09 (dd, J = 3.2, 1.8 Hz, 1H), 6.32 – 6.25 (m, 1H), 5.48 (s, 2H).

Mixture: yellow crystals

JS3 – hydrolysis of ethyl 1*H*-pyrrole-2-carboxylate

Approach 1:

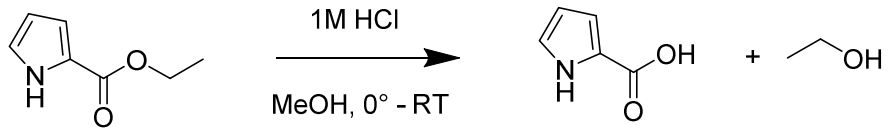


JS3

Ethyl 1*H*-pyrrole-2-carboxylate was dissolved in methanol (18 mL) and cooled to 0 °C. A solution of LiOH (6.1 mL, 1M water solution) was added dropwise. The reaction was stirred at RT and controlled via TLC (PE/EtOAc = 4/1 with drop of formic acid, detection UV). 1*H*-pyrrole-2-carboxylate (R_f = 0.62) and 1H-pyrrole-2-carboxylic acid (R_f = 0.08) were detected. According to TLC, after 24 h hydrolysis was just partial. The reaction was warmed to 90 °C and controlled via TLC, the hydrolysis was complete after 5 hours at 90 °C. The reaction was not worked up.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
Ethyl 1*H*-pyrrole-2-carboxylate	1.00	139.15	1.08	150 mg	
LiOH . H_2O	6.00	41.04	6.48	266 mg/ 6 mL	

Approach 2:

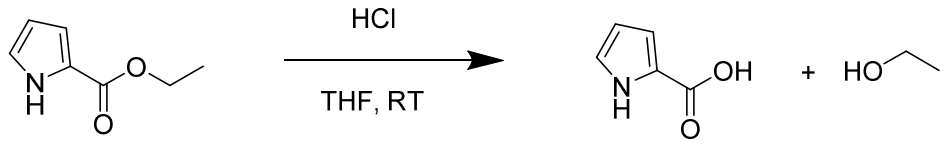


JS3

Ethyl 1*H*-pyrrole-2-carboxylate was dissolved in methanol (18 mL) and cooled to 0 °C. Water solution of HCl (6.1 mL, 1M water solution) was added dropwise. The reaction was stirred at RT and controlled via TLC (PE/EtOAc = 4/1 with drop of formic acid, detection UV). The reaction was performed analogically to approach 1, however on TLC was detected less hydrolysed product than at approach 1 (basic hydrolysis compared to acid hydrolysis). Ethyl 1*H*-pyrrole-2-carboxylate (R_f = 0.62) and 1H-pyrrole-2-carboxylic acid (R_f = 0.08) were detected on TLC. As hydrolysis catalysed with LiOH was more effective, the reaction was stopped and reaction was not worked up.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
Ethyl 1*H*-pyrrole-2-carboxylate	1.00	139.15	1.08	150 mg	
HCl	6.00	36.46	6.48	236 mg/ 6mL	

Approach 3:



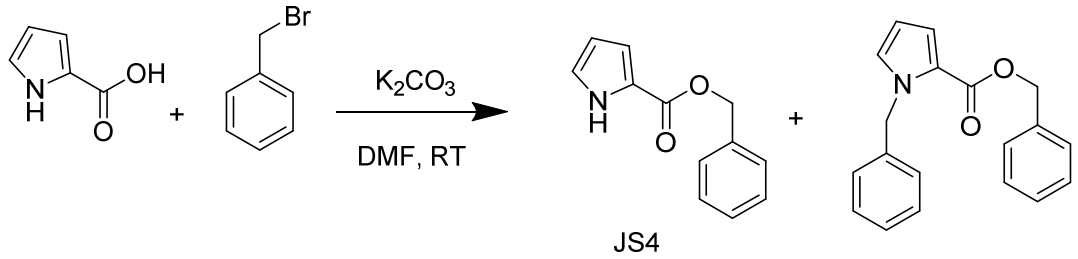
JS3

Ethyl 1*H*-pyrrole-2-carboxylate was dissolved in 1 ml of THF, hydrochloric acid was added dropwise and the reaction was stirred at RT overnight. TLC was performed (PE/EtOAc=4/1 with drop of formic acid, detection UV), the hydrolysis of ethyl 1*H*-pyrrole-2-carboxylate (R_f= 0.62) to 1H-pyrrole-2-carboxylic acid (R_f = 0.08) was minimal. The reaction was not worked up.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
ethyl 1*H*-pyrrole-2-carboxylate	1.00	139.15	0.21	29 mg	
HCl (36%)	3.00	36.46	0.63	54 µL	

JS4 – synthesis of benzyl 1*H*-pyrrole-2-carboxylate

Approach:



JS4

K_2CO_3 was suspended and 1*H*-pyrrole-2-carboxylic acid was dissolved in dry DMF (5 mL) under argon atmosphere at RT. Benzylbromide was added dropwise and the reaction was stirred overnight. On TLC (cyclohexane (CH)/EtOAc = 6/1, detection UV) there were two spots, product JS4 (R_f = 0.18) and side product benzyl 1-benzyl-1*H*-pyrrole-2-carboxylate (R_f = 0.35). The reaction was quenched with water (5 mL), extracted with ethyl acetate (3 × 10 mL), organic fraction was washed with water (3 × 15 mL) and with brine (15 mL). The solvent was evaporated under reduced pressure and a column chromatography was done in the same conditions as TLC, the products were confirmed by NMR.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
1*H*-pyrrole-2-carboxylic acid	1.00	111.1	0.90	100 mg	
Benzylbromide	1.05	171.04	0.95	180 mg/ 125 µL	
K_2CO_3	1.50	293.34	1.35	187 mg	
JS4		201.23	0.30	60 mg	33%

Properties:

JS4: white solid, melting point 54°C

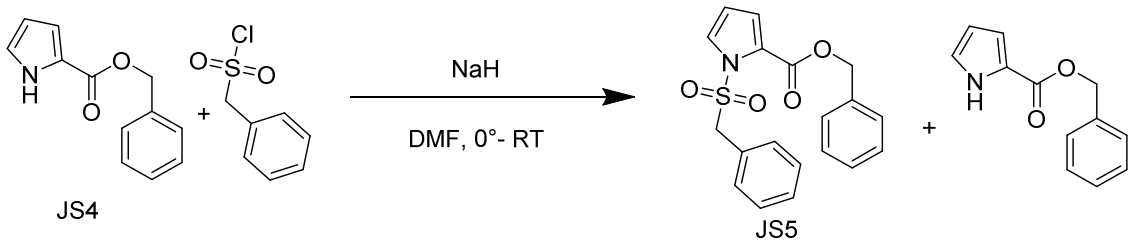
1H NMR (400 MHz, CDCl3) δ 9.17 (s, 1H), 7.37 – 7.23 (m, 5H), 6.91 – 6.88 (m, 1H), 6.88 – 6.85 (m, 1H), 6.21 – 6.15 (m, 1H), 5.23 (s, 2H)

Side product (benzyl 1-benzyl-1*H*-pyrrole-2-carboxylate):

1H NMR (400 MHz, CDCl3) δ 7.34 – 7.15 (m, 10H), 7.00 (dd, J = 4.0, 1.8 Hz, 1H), 6.81 (dd, J = 2.5, 1.9 Hz, 1H), 6.14 – 6.06 (m, 1H), 5.49 (s, 2H), 5.15 (s, 2H).

JS5 – synthesis of benzyl 1-(benzylsulfonyl)-1*H*-pyrrole-2-carboxylate

Approach:



NaH was suspended in a dry flask in anhydrous DMF (1.5 mL) under argon atmosphere, the suspension was cooled to 0 °C and JS4 in dry DMF (2.5 mL) was added dropwise. The reaction was stirred at RT for 1.5 hours, then was cooled again to 0 °C, benzylsulfonyl chloride in anhydrous DMF (1.5 mL) was added dropwise. The reaction was stirred at 0 °C for 1 hour, then at RT overnight. After 24 h were detected JS4 (R_f = 0.29), JS5 (R_f =0.52) and side product (R_f = 0.78) on TLC (CH/diethyl ether (Et_2O) = 6/1, detection UV). The reaction was quenched with water (5 mL) and products were extracted to ethyl acetate (4 × 5 mL). Combined organics phases were washed with water (3 × 15 mL) and with brine (15 mL), dried over sodium sulphate and evaporated under reduced pressure. Brown oil was gained, a column chromatography was performed (on silica gel, mobile phase CH/Et_2O = 4/1). JS5, JS4 and their mixture were obtained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
JS4	1.00	201.23	0.30	60 mg	
NaH	1.50	24.00	0.45	11 mg	
Benzylsulfonyl chloride	1.20	190.65	0.36	69 mg	
JS5		355.41	0.04	15 mg	14%

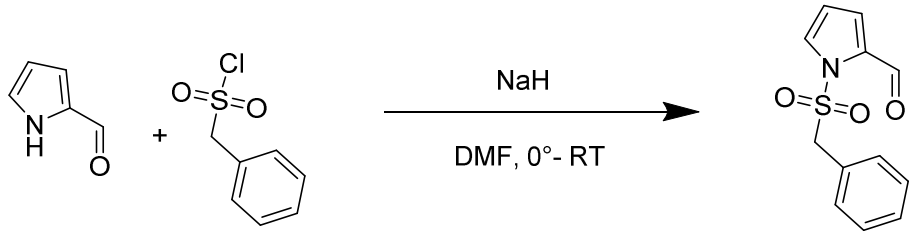
Properties:

JS5: yellow crystal solid, melting point 105 °C

1H NMR (400 MHz, CDCl3) δ 7.46 – 7.12 (m, 10H), 7.04 (dd, J = 3.6, 1.9 Hz, 1H), 6.94 (dd, J = 3.2, 1.8 Hz, 1H), 6.04 – 5.97 (m, 1H), 5.31 (s, 2H), 5.07 (s, 2H).

JS6 – Synthesis of 1-benzylsulfonyl-1*H*-pyrrole-2-carbaldehyde

Approach:



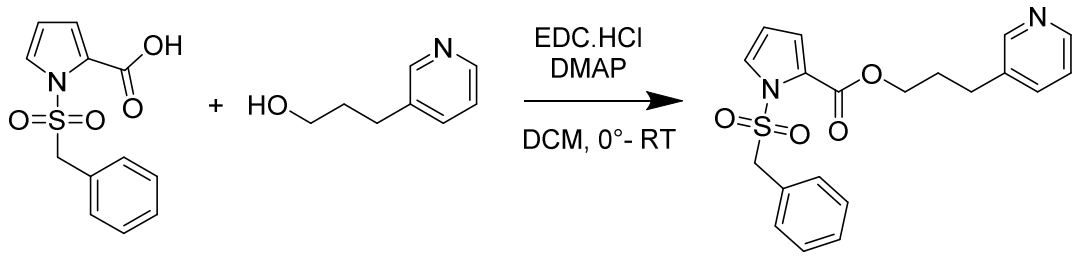
JS6

NaH was suspended in a dry flask in anhydrous DMF (1.5 mL) under argon atmosphere, the suspension was cooled to 0 °C and 1*H*-pyrrole-2-carbaldehyde in dry DMF (2.5 mL) was added dropwise. The reaction was stirred at RT for 1.5 hours, then was again cooled to 0 °C, benzylsulfonyl chloride in dry DMF (2.5 mL) was added dropwise. The reaction was stirred at 0 °C for 1 hour, then at RT overnight. After 24 h were detected two large spots on TLC (CH/Et_2O = 9/2, detection UV), probably starting material and product (R_f =0.28, R_f =0.43), and a lot of smaller spots on start, under and over the large ones. The reaction was quenched with water (10 mL) and products were extracted to ethyl acetate (4 × 10 mL). Combined organic phases were washed with water (3 × 30 mL) and with brine (30 mL), dried over sodium sulphate and concentrated under reduced pressure. Complete reaction mixture was extracted to ethyl acetate (TLC control) and the concentrated solution was triturated with n-hexane. Unfortunately, no clean substance crystallized, just slurry dark stuff, the product was not gained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
1*H*-pyrrole-2-carbaldehyde	1.00	95.10	3.15	300 mg	
NaH	1.50	24.00	4.73	113 mg	
benzylsulfonyl chloride	1.20	190.65	3.79	721 mg	
JS6		249.28			0%

JS7 – Synthesis of 3-(pyridin-3-yl)-propyl 1-(benzylsulfonyl)-1*H*-pyrrole-2-carboxylate

Approach:



JS2 JS7

JS2 was dissolved in dry DCM (5 mL) under argon atmosphere and cooled to 0 °C. To the stirring solution 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl), 4-dimethylaminopyridine (DMAP) and 3-(pyridin-3-yl)propan-1-ol were added. The reaction was stirred overnight at RT and controlled via TLC (Et_2O/EtOAc = 2/1, detection UV), two spots were observed (R_f = 0.29, R_f = 0.21). The reaction was washed with water (4 × 5 mL), DCM was dried over sodium sulphate and evaporated under reduced pressure. The product was purified by column chromatography (conditions same as TLC), JS7 (R_f = 0.29) was confirmed by NMR.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
JS2	1.00	265.28	0.31	83 mg	
EDC.HCl	1.50	191.70	0.47	90 mg	
DMAP	1.50	135.12	0.47	63 mg	
3-(pyridin-3-yl)propan-1-ol	1.50	137.18	0.47	62 µL	
JS7		384.45		94 mg	79%

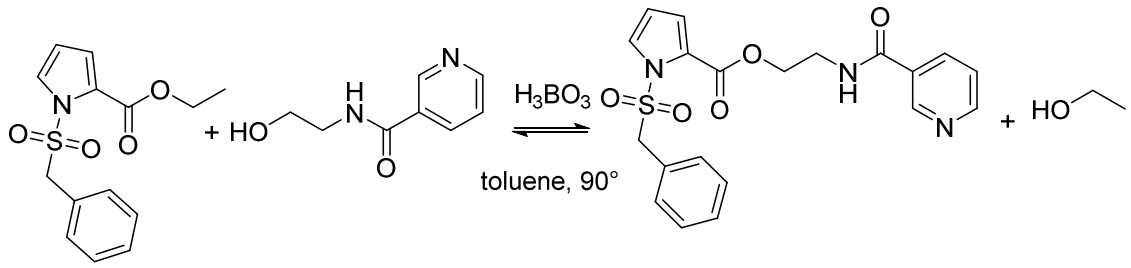
Properties:

JS7: yellow oil

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.48 (dd, *J* = 2.3, 0.8 Hz, 1H), 8.40 (dd, *J* = 4.9, 1.6 Hz, 1H), 7.83 – 7.77 (m, 1H), 7.44 – 7.23 (m, 6H), 7.10 (dd, *J* = 3.7, 1.8 Hz, 1H), 7.03 (dd, *J* = 3.2, 1.8 Hz, 1H), 6.19 – 6.16 (m, 1H), 5.29 (s, 2H), 4.40 (t, *J* = 6.3 Hz, 2H), 2.92 – 2.83 (m, 2H), 2.21 – 2.12 (m, 2H).

JS8 – transesterification of JS3 with *N*-(hydroxyethyl)nicotinamide

Approach:



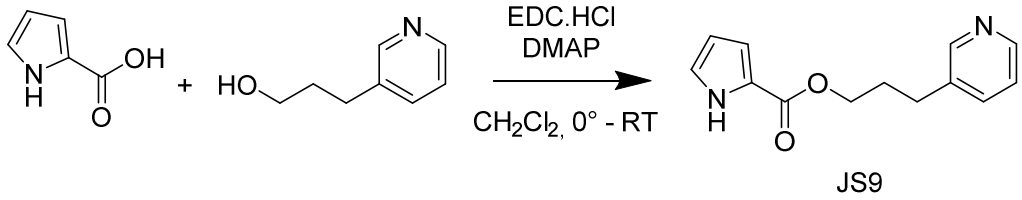
JS1 JS11 JS8

JS1, *N*-(hydroxyethyl)nicotinamide and H_3BO_3 were suspended in dry toluene under argon atmosphere and Dean-Stark condenser was filled with anhydrous toluene (25 ml of toluene together). The reaction was heated to 90 °C – 100 °C and continuously controlled by TLC (CH/Et_2O = 6/1, detection UV), JS1 was used for control (R_f = 0,44). After 30 hours no product was detected, only starting material.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
JS1	1.00	293.34	0.34	100 mg	
N-(hydroxyethyl)nicotinamide	3.00	166.18	1.02	170 mg	
H_3BO_4	0.10	61.83	0.03	2 mg	
JS8	1.50	399.42			0%

JS9 – Synthesis of 3-(pyridin-3-yl)-propyl-1*H*-pyrrole-2-carboxylate

Approach:



1*H*-pyrrole-2-carboxylic acid was dissolved in dry DCM (10 mL) under argon atmosphere and cooled to 0 °C. To the stirring solution EDC.HCl, DMAP and 3-(pyridin-3-yl)propan-1-ol were added. The reaction was stirred overnight at RT and controlled via TLC (CH/Et_2O = 2/1, detection UV), there were two spots observed (R_f = 0.57, R_f = 0.39). The reaction was extracted with water (4 × 5mL), DCM was dried over sodium sulphate and evaporated under reduced pressure. The product purified by flash column chromatography (on silica gel, $CHCl_3$/methanol (MeOH) = 1/1), JS9 was gained, but not pure enough to calculate the yield.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
1*H*-pyrrole-2-carboxylic acid	1.00	111.1	0.90	100 mg	
EDC.HCl	1.50	191.7	1.35	259 mg	
DMAP	1.50	122.17	1.35	165 mg	
3-(pyridin-3-yl)propan-1-ol	1.50	137.18	1.35	177 µL	
JS9		384.45		250 mg	

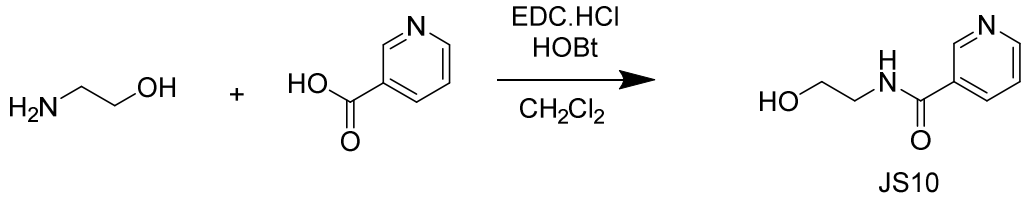
Properties:

JS9: white crystal solid

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.15 (s, 1H), 8.44 – 8.35 (m, 2H), 7.49 – 7.43 (m, 1H), 7.18 – 7.12 (m, 1H), 6.91 – 6.88 (m, 1H), 6.86 – 6.84 (m, 1H), 6.25 – 6.16 (m, 1H), 4.23 (t, *J* = 6.4 Hz, 2H), 2.75 – 2.66 (m, 2H), 2.07 – 1.94 (m, 2H).

JS10 – Synthesis of *N*-(2-hydroxyethyl)nicotinamide

Approach:



JS10

Nicotinic acid was suspended in dry DCM (30 mL) under argon atmosphere and cooled to 0 °C. To the stirring solution EDC.HCl, 1-hydroxybenzotriazole (HOBt) and 2-aminoethan-1-ol were added. After addition of 2-aminoethan-1-ol, the suspension was dissolved and later reaction turned into yellow suspension. The reaction was stirred at RT and controlled via TLC (EtOAc/MeOH = 10/1, detection UV), there were two spots observed, JS10 (R_f = 0.25) and nicotinic acid (R_f = 0.69). After the conversion of all reagents (1 hour), the reaction was washed with water (20 mL), but extraction to DCM was not successful (product was obtained in both phases), the solvents were evaporated under reduced pressure and the product was purified by column chromatography (on silica gel, EtOAc/MeOH = 5/1), JS10 was gained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
Nicotinic acid	1.00	123.11	8.00	985 mg	
EDC.HCl	1.30	191.70	10.40	1994 mg	
HOBt	0.20	135.12	1.60	217 mg	
2-aminoethan-1-ol	1.00	61.08	8.00	483 µL	
JS10		166.18		1263 mg	95%

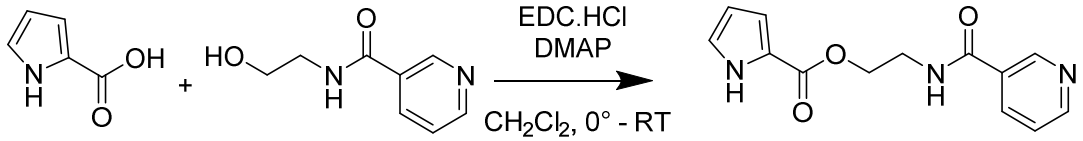
Properties:

JS10: white crystals, melting point 85.5 °C

1H NMR (400 MHz, MeOD) δ 9.01 (dd, J = 2.3, 0.9 Hz, 1H), 8.69 (dd, J = 4.9, 1.6 Hz, 1H), 8.29 – 8.25 (m, 1H), 7.58 – 7.52 (m, 1H), 3.75 (t, J = 5.8 Hz, 2H), 3.55 (dd, J = 7.2, 4.3 Hz, 2H).

JS11 – Synthesis of 2-(nicotinamido)ethyl 1*H*-pyrrole-2-carboxylate

Approach:



JS10 JS11

1*H*-pyrrole-2-carboxylic acid was dissolved in dry DCM (5 mL) under argon atmosphere and cooled to 0 °C. To the stirring solution EDC.HCl, 4-dimethylaminopyridine and JS10 were added. The reaction was stirred overnight at RT and controlled via TLC (EtOAc/acetone (A) = 1/3, detection UV), there were detected two spots (R_f = 0.57, R_f = 0.39). The reaction was washed with water (4 × 5 mL), the solvent was evaporated under reduced pressure and the product was purified by flash column chromatography (on silica gel, $CHCl_3$/MeOH = 1/1), JS11 was gained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
1*H*-pyrrole-2-carboxylic acid	1.00	111.10	0.45	50 mg	
EDC.HCl	1.50	191.70	0.68	129 mg	
DMAP	1.50	122.17	0.68	80 mg	
JS10	1.50	166.18	0.68	112 mg	
JS11		259.27		130 mg	50%

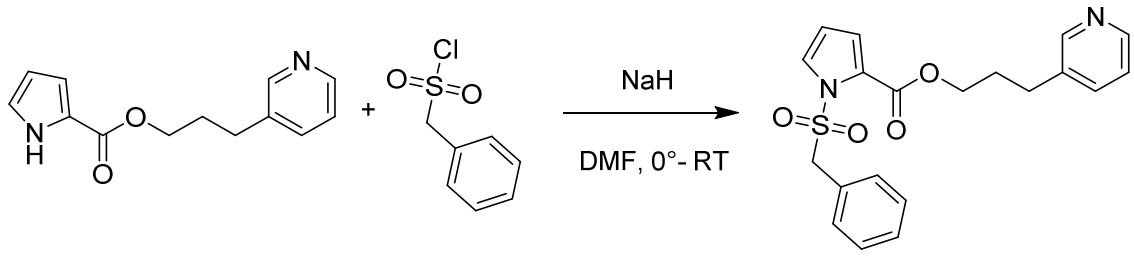
Properties:

JS11: white crystals

1H NMR (400 MHz, MeOD) δ 8.94 (dd, J = 2.3, 0.8 Hz, 1H), 8.66 (dd, J = 4.9, 1.6 Hz, 1H), 8.23 – 8.19 (m, 1.7 Hz, 1H), 7.54 – 7.49 (m, 1H), 6.96 (dd, J = 2.5, 1.5 Hz, 1H), 6.89 (dd, J = 3.7, 1.5 Hz, 1H), 6.18 (dd, J = 3.7, 2.5 Hz, 1H), 4.43 (t, J = 5.6 Hz, 2H), 3.75 (dd, J = 7.2, 3.9 Hz, 2H).

JS12 – Synthesis of 3-(pyridin-3-yl)propyl 1-(benzylsulfonyl)-1*H*-pyrrole-2-carboxylate

Approach:



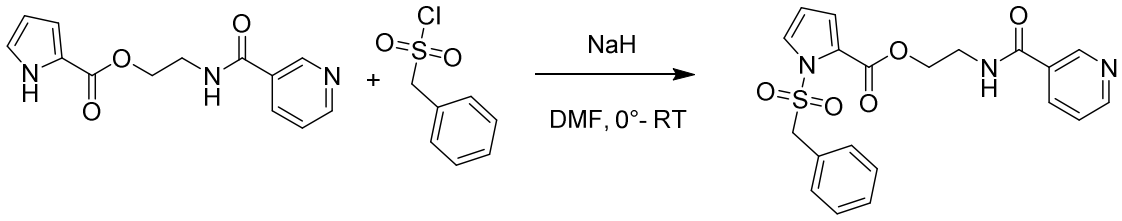
JS9 JS12

NaH was suspended in a dry flask in anhydrous DMF (1.0 mL) under argon atmosphere, the suspension was cooled to 0 °C and JS9 in dry DMF (1.5 mL) was added dropwise. The reaction was stirred at RT for 1.5 hours, then was again cooled to 0 °C, benzylsulfonyl chloride in dry DMF (1.0 mL) was added dropwise. The reaction was stirred at 0 °C for 1 hour, then at RT overnight. After 24 h were detected tree spots on TLC (EtOAc/MeOH = 4/1, detection UV). The reaction was quenched with water (5 mL) and the products were extracted to ethyl acetate (4 × 5 mL), but the products were presented in both phases. The solvents were concentrated under reduced pressure and flash column chromatography was done (concentrated product washed through silica gel by ethyl acetate and then with acetone), yellow oil was obtained. Because of lack of the time product was not gained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
JS9	1.00	137.18	0.90	123 mg	
NaH	1.50	24.00	1.35	32 mg	
benzylsulfonyl chloride	1.50	190.65	1.35	258 mg	
JS12		384.45			

JS13 – Synthesis of 2-(nicotinamido)ethyl 1-(benzylsulfonyl)-1*H*-pyrrole-2-carboxylate

Approach:



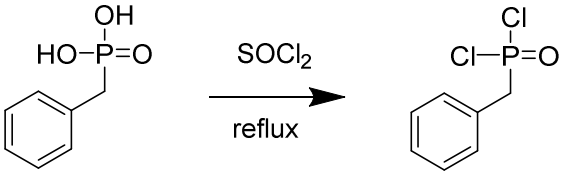
JS11 JS13

NaH was suspended in a dry flask in anhydrous DMF (1.0 mL) under argon atmosphere, the suspension was cooled to 0 °C and JS11 in dry DMF (1.5 mL) was added dropwise. The reaction was stirred at RT for 1.5 hours, then was again cooled to 0 °C, benzylsulfonyl chloride in dry DMF (1.0 mL) was added dropwise. The reaction was stirred at 0 °C for 1 hour, then at RT overnight. After 24 h were detected tree spots (R_f = 0.47; 0.70 and 0.82) on TLC (EtOAc/MeOH = 4/1, detection UV). The reaction was quenched with water (5 mL) and the products were extracted to ethyl acetate (4 × 5 mL). The most hydrophilic compound was not extracted to organic phase (R_f = 0.47). Combined organics were washed with water (3 × 15 mL) and with brine (15 mL), dried over sodium sulphate and concentrated under reduced pressure. I tried to crystalize the product from organic phase by changing polarity of solvent by adding Et_2O or toluene, but the crystallization was not successful. Because of the lack of time the product was not gained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
JS11	1.00	259.27	0.90	233 mg	
NaH	1.50	24.00	1.35	32 mg	
benzylsulfonyl chloride	1.50	190.65	1.35	258 mg	
JS13		413.45			

JS14 – Synthesis of benzylphosphonic dichloride

Approach 1:



JS14

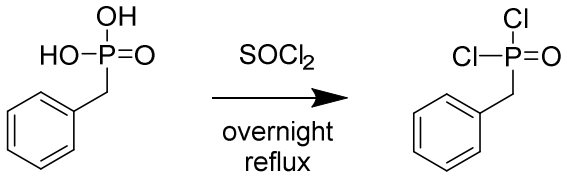
Benzylphosphonic acid and $SOCl_2$ were added into a dry flask under nitrogen atmosphere. The reaction was stirred and heated to reflux (160 °C on stirrer) overnight. The reaction was evaporated under reduced pressure, but evaporation of solvent did not work, the quality of $SOCl_2$ was probably very low. Brown concentrated liquid was gained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
benzylphosphonic acid	1.00	172.19	0.58	300 mg	
$SOCl_2$	39.44	118.97	68.71	5 mL	
JS14		209.01			

Properties:

JS14a: brown slurry liquid

Approach 2:



JS14

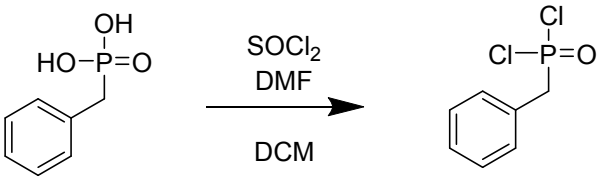
Benzylphosphonic acid and $SOCl_2$ were added into a dry flask under nitrogen atmosphere. The reaction was stirred and heated to reflux (160 °C on stirrer) overnight. The reaction was evaporated and dark slurry product was gained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
benzylphosphonic acid	1.00	172,19	0.58	100 mg	
$SOCl_2$	71.08	118,97	41.23	3 mL	
JS14		209,01			

Properties

JS14b: dark slurry solid

Approach 3:



JS14

Benzylphosphonic acid was dissolved in dry flask under argon atmosphere in anhydrous DCM (3 mL) and DMF (1 mL) was added for catalysis. The reaction was cooled to 0 °C. $SOCl_2$ was added dropwise and the reaction was stirred 2.5 hours at RT. Toluene (3 mL) was added to the reaction to use co-evaporation of $SOCl_2$ with toluene and the solvents were evaporated to dryness and the product was gained.

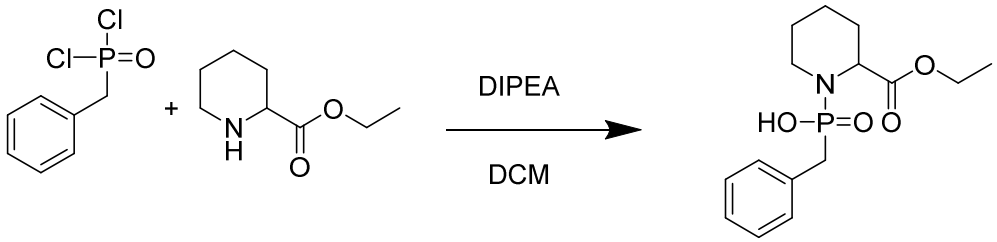
Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
benzylphosphonic acid	1.00	172.19	0.58	100 mg	
SOCl2	3.50	118.97	2.03	165 µL	
DMF				1 mL	
JS14		209.01			

Properties:

JS14c: dark solid

JS15 – Synthesis of benzyl (2-(ethoxycarbonyl)piperidin-1-yl)phosphinic acid

Approach 1:

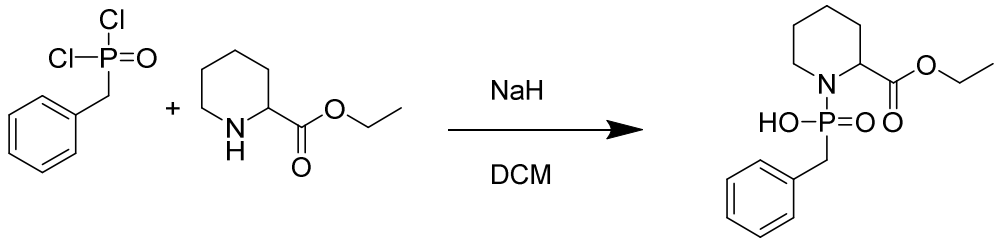


JS14a JS16 JS15a

Ethyl piperidine-2-carboxylate JS16 and *NN*-diisopropylethylamine (DIPEA) were dissolved in dry DCM (15 mL) under argon atmosphere, the reaction was stirred at 0 °C. The product from reaction JS14a was dissolved in dry DCM (15 mL) and added to the reaction, reaction turned black. The reaction was stirred at RT, TLC was performed after 24 hours (PE/EtOAc = 8/1, detection UV), there were two spots of hydrophobic products (R_f = 0.86, R_f = 0.75) and long hydrophilic spot (R_f = 0.00 - 0.32). The reaction was quenched with water (20 mL), the phases in separation funnel did not separate and a lot of dark stuff precipitated. Brine was added to separate phases, more ethyl acetate was added, but precipitate was not possible to dissolve. Isolation of the product was not successful and no product was gained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
JS14a	1.00	209.01	1.14	all reaction JS14a	
JS16	1.00	157.21	1.14	180 mg	
DIPEA	3.00	129.25	3.42	598 µL	
JS15a		311.32			

Approach 2:

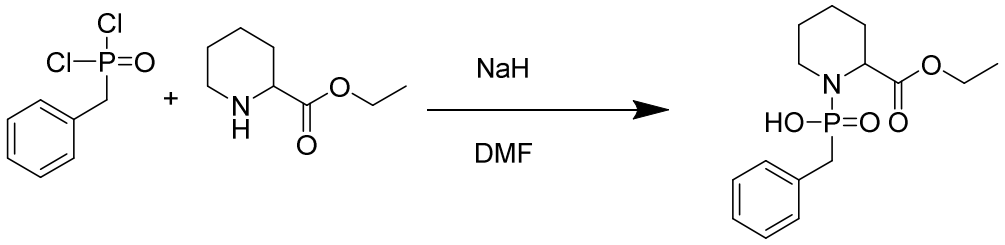


JS14b JS16 JS15b

JS16 and NaH were suspended in dry DCM (10 mL) under argon atmosphere, the reaction was stirred at 0 °C. The product from reaction JS14b was dissolved in anhydrous DCM (5 mL) and added to reaction, the reaction was yellow solution. The reaction was stirred at RT, TLC was performed after 24 hours (EtOAc/MeOH = 1/1, detection UV), there were two spots of hydrophobic products (R_f = 0.62, R_f = 0.34) and long hydrophilic spot on start. The reaction was quenched with water (10 mL) and extracted to ethyl acetate (3 × 10 mL). At that, no spots were detected on TLC and product was not gained.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
JS14b	1.00	209.01	0.58	all reaction JS14b	
JS16	0.80	157.21	0.46	73 mg	
NaH	2.00	129.25	1.17	28 mg	
JS15b		311.32			

Approach 3:

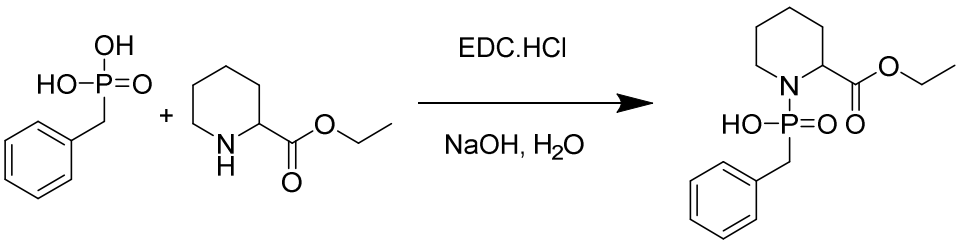


JS14c JS16 JS15c

The product from reaction JS14c was dissolved in dry DMF (5 mL) under argon atmosphere, cooled to 0 °C, JS16 and NaH were added to the reaction. The reaction was stirred at RT, TLC was performed after 24 hours (EtOAc/MeOH = 1/1, detection UV), there were two spots of hydrophobic products (R_f = 0.85, R_f = 0.62) and long hydrophilic spot on start. The reaction was quenched with water (5 mL) and organics were extracted to DCM (4 × 5 mL). Combined organic phases were washed with water (4 × 10 mL). Only one spot (R_f = 0.85) was detected in organic layer and one spot was observed on start in water layer. Both phases were evaporated under reduced pressure, but the product was not recognized by NMR.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/volume:	Yield:
JS14c	1.00	209.01	0.58	all reaction JS14c	
JS16	0.80	157.21	0.46	73 mg	
NaH	2.00	129.25	1.17	28 mg	
JS15c		311.32			0%

Approach 4:



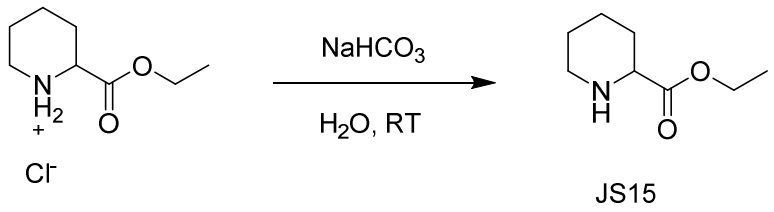
JS15d

All the reagents were dissolved in water (10 mL) and pH was adjusted (pH = 7.5, NaOH, 1 M water solution). The reaction was stirred overnight at RT, then the excess of ethyl piperidine-2-carboxylate and EDC were extracted to ethyl acetate (3 × 10 mL) and the water phase was evaporated under reduced pressure. The crude material was suspended in acetone (3 mL) and the inorganic compounds were filtrated. Acetone was evaporated under reduced pressure and the organic material gained. The product was not detected.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
benzylphosphonic acid	1.00	172.19	0.58	100 mg	
ethyl piperidine-2-carboxylate . HCl	3.00	157.21	1.74	337 mg	
EDC . HCl	3.00	191.70	1.74	334 mg	
JS15d		311.32			0%

JS16 – Preparation of ethyl pipecolinate

Scheme:



Approach:

Ethyl pipecolinate hydrochloride was dissolved in saturated solution of $NaHCO_3$ (20 mL) and more solid $NaHCO_3$ was added until gas evolving stopped. The reaction was stirred at RT for 1 hour. The reaction was extracted to DCM (4 × 20 mL), DCM was dried over sodium sulphate and evaporated under reduced pressure, JS16 was gained without further purification.

Substances:	Equivalents:	Mr:	Molar amount (mmol):	Mass/ volume:	Yield:
ethyl pipecolinate . HCl	1,00	193,67	0,57	110 mg	
JS16		157,21	0,55	86 mg	96%

Properties:

JS25: white crystal solid

Results and discussion:

The aim of the work was to synthetize the analogues of previously described Mip protein inhibitors.[53] My task was to synthesize the small molecule inhibitors of Mip protein, exactly pyrrole and phosphonamide analogues, for the treatment of *Legionella pneumophila* and *Burkholderia pseudomallei* infections.

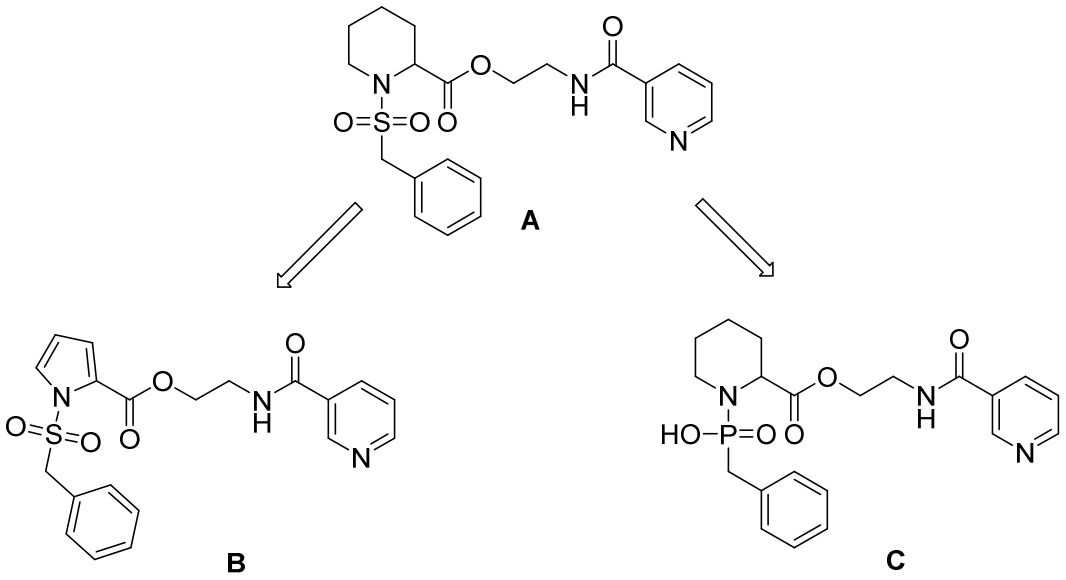
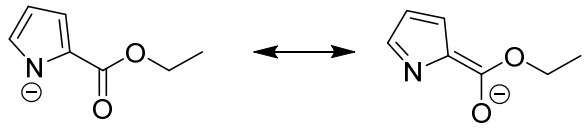
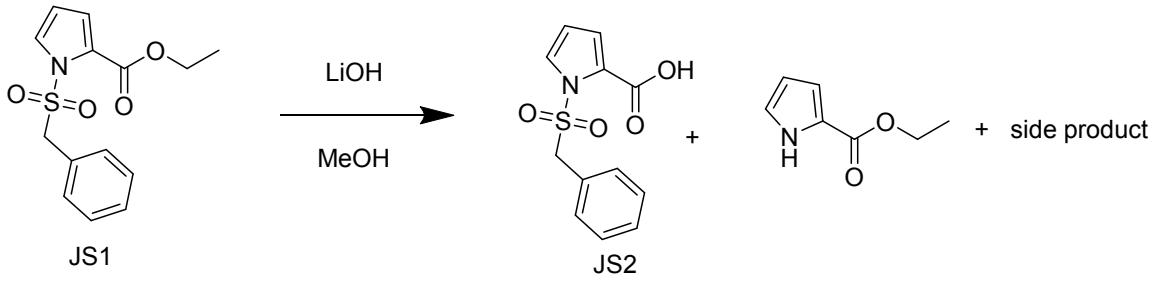


Figure 8. Lead structure (A) and designed pyrrole (B) and phosphonamide (C) analogues

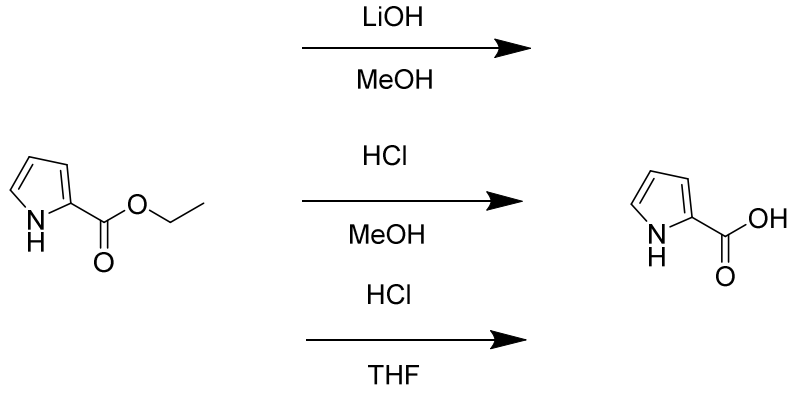
In the first part, I attempted to prepare the Mip protein inhibitor analogue with 1*H*-pyrrole-2-carboxylic acid instead of original pipecolic acid. I chose to utilize previously published synthesis.[53] The reaction of ethyl 1*H*-pyrrole-2-carboxylate with benzylsulfonyl chloride under basic conditions should provide the sulphonamide for subsequent reactions. However, the reaction using NMM and CH_2Cl_2 did not work so slightly as in the pipecolic acid case. I performed the reaction with stronger base (NaH), with different solvents (DMF, THF) and in different reaction temperatures and times. Even after optimization of the reaction, JS1 was obtained only in 24 % yield. Such a low yield of the product can be explained by the resonance structures after deprotonation. The negative charge is probably localized on oxygen atom rather than on nitrogen, lowering its reactivity towards sulfonation.



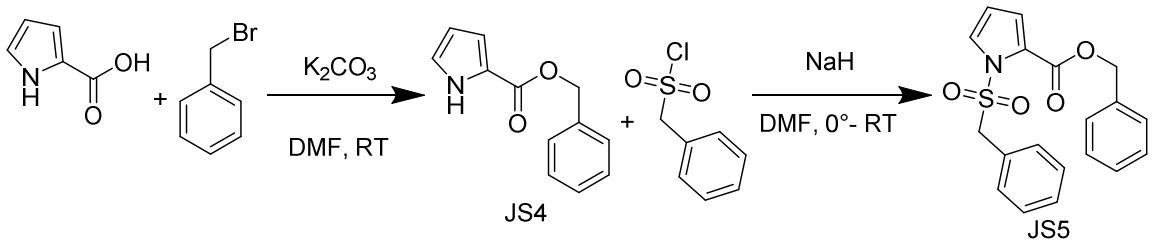
After the preparation of sulphonamide JS1 ester bond hydrolysis and preparation of the carboxylic acid JS2 follows. However, the hydrolysis provided moderate yield of JS2 (63%), because of low stability of pyrrole sulphonamide: the sulphonamide bond was also hydrolysed. The relative instability of the sulphonamide bond in JS2 can be another reason for very low yield of compound JS1 in the first reaction.



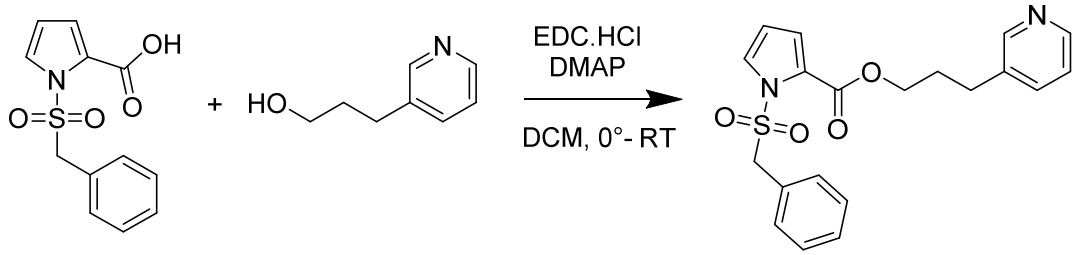
To keep low amount of JS1, I tried the hydrolysis in different conditions just with ethyl 1*H*-pyrrole-2-carboxylate. I performed the hydrolysis in methanol catalysed with water solution of LiOH or HCl and in THF catalysed by HCl. Basic hydrolysis was more successful.



I tried to further optimize the synthesis of sulphonamide JS2 using benzyl ester protecting group. Benzyl ester is cleavable by catalytic hydrogenation, so no acid/base hydrolysis would be needed. 1*H*-Pyrrole-2-carboxylic acid was protected using benzyl bromide and subsequent sulfonation provided the product JS5 in 14% yield. Further deprotection reaction was not performed due to lack of time.

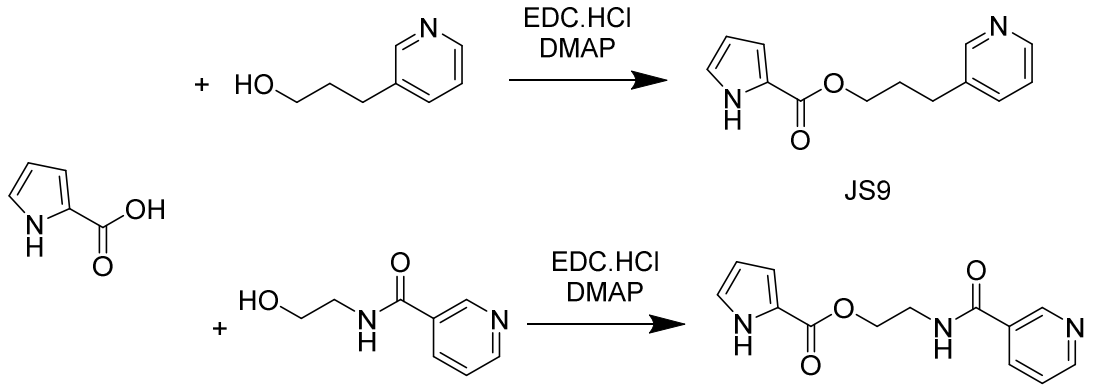


The next step was the coupling of carboxylic acid JS2 with 3-(pyridin-3-yl)propan-1-ol. The reaction was done using coupling agent EDC.HCl in basic conditions (DMAP) with high yield (79%) of product JS7.

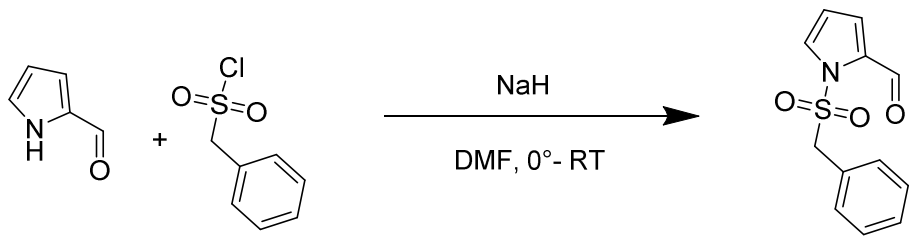


JS2 JS7

The coupling reaction was also done just with 1*H*-pyrrole-2-carboxylic acid to save low amounts of sulphonamide JS2. The coupling reactions worked well (yield over 90 %) and esters JS9 and JS11 were prepared. JS9 and JS11 could be used for synthesis of the final products by sulfonation with benzylsulfonyl chloride, but reaction was not performed due to lack of time.



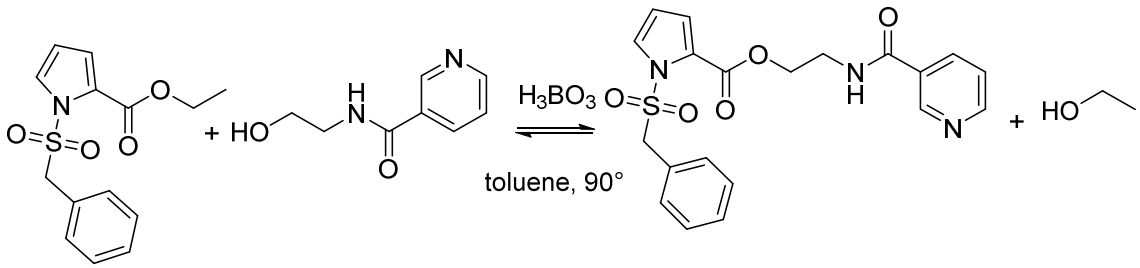
As an alternative synthetic approach, I tried to prepare benzylsulfonyl-1*H*-pyrrole-2-carbaldehyde JS6, however, this previously published reaction did not worked.[54]



JS6

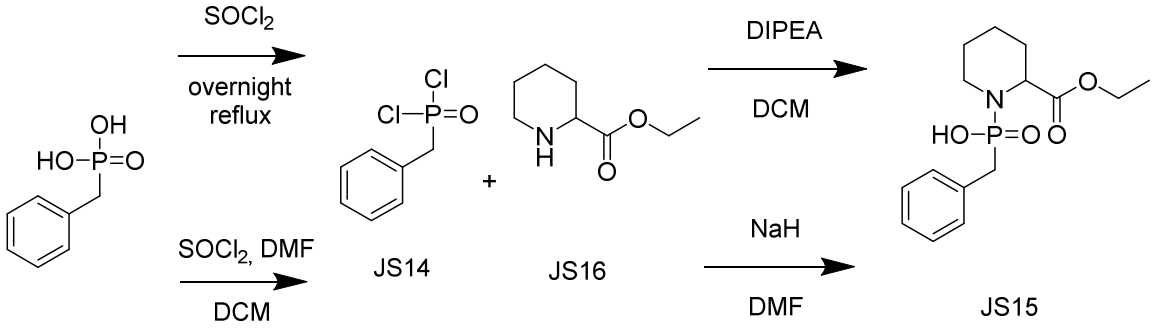
Transesterification of prepared sulphonamide JS1 with JS11 catalysed with H_3BO_3 was also performed, but no product was obtained. It was probably due to high

stability of ethyl ester and impossibility of separation of ethanol from the reaction mixture.



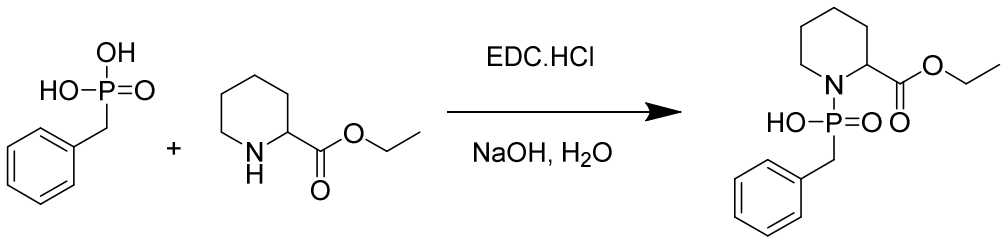
JS1 JS11 JS8

The second aim of this work was to prepare the phosphonamide analogues of pipecolic acid MIP protein inhibitors. I chose analogous approach as in the case of pipecolic acid derivatives synthesis. However, the mono-chlorides of benzyl-phosphonic acid are not commercially available. The corresponding chlorides were prepared using $SOCl_2$ and DMF as a catalyst, the crude product was used directly for phosphoamidation. However, I did not detected phosphonamides, only the mixture of very hydrophilic material, which was very difficult to separate.



JS14 JS16 JS15

I also tried a coupling of benzylphosphonic acid with ethyl pipecolinate with coupling agent EDC.HCl as published previously, but without any success.[55]



JS15

CONCLUSIONS

To conclude, I developed the synthesis of pyrrole analogues of Mip protein inhibitors and completely finished the preparation of one final product. The synthesis of pyrrole sulfonamides analogues was difficult because of low reactivity and low stability of the products. I also tried to prepare phosphonamide analogues, but the synthesis was not successful due to the difficult detection and separation of very hydrophilic products. The method developed in this work can be used in the further synthesis of pyrrole-based Mip protein inhibitors. Regarding the phosphonamide analogues, another synthetic approach using hydrophobic protective groups should be developed.

List of abbreviations:

B. pseudomallei – Burkholderia pseudomallei

DCC - *N,N'*-dicyclohexylcarbodiimide

DCM – dichloromethane

DIPEA – *N,N*-diisopropylethylamine

DMAP - 4-dimethylaminopyridine

DMF – *N,N*-dimethylformamide

EDC.HCl - 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

Et_2O – diethyl ether

EtOAc – ethyl acetate

FKBP – F506 binding protein

HOBt – 1-hydroxybenzotriazole

L. pneumophila – Legionella pneumophila

LpMip - *L. pneumophila* Mip protein

MeOH – methanol

MIP - macrophage infectivity potentiator

MpMip - *B. pseudomallei* Mip protein

NMM – *N*-methylmorpholine

PE – petroleum ether

PPIases - peptidyl-prolyl cis/trans isomerases

RT – room temperature

SAR - structure-activity relationship

THF – tetrahydrofuran

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