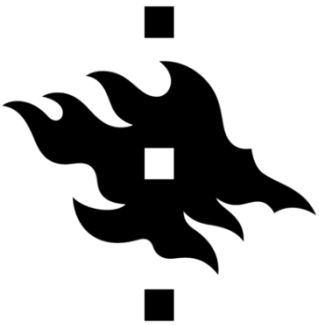
CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis

UNIVERSITY OF HELSINKI
FACULTY OF PHARMACY

Division of Pharmaceutical Chemistry and Technology



Synthesis of novel 5,6-disubstituted derivatives of uracil as potential drugs

(Diploma thesis)

Lien Phuong Vu

Supervisors:
PharmDr. Marta Kučerová, Ph.D.
Tanja Bruun, M.Sc.
Prof. Jari Yli-Kauhaluoma

Hradec Králové 2020

AUTHOR'S DECLARATION:

" I declare that this thesis is my original author's work, which has been composed solely by myself (under the guidance of my consultant). All the literature and other resources from which I drew information are cited in the list of used literature and are quoted in the paper. The work has not been used to get another or the same title."

In Hradec Králové, 30th August 2020

_____

ACKNOWLEDGEMENT:

With this, I would like to express my greatest gratitude to prof. Jari Yli- Kauhaluoma and his research group for giving me the opportunity to work in the Division of Pharmaceutical Chemistry and Technology at University of Helsinki. I would like to thank every member of JYK group for amazing and very friendly working environment. Everyone's willingness to help was humbling. I would like to also thank Nina Sipari for HRMS measurements of final compounds. In addition, a very special and most sincere thanks belong to my supervisor Tanja Bruun, M.Sc. (Pharm.) for her guidance, infinite patience, optimism and unwavering support.

I would also like to deliver endless thanks to PharmDr. Marta Kučerová, Ph.D. for her wonderful and caring support, understanding and helpful guidance.

Last but not least, I would like to thank my dearest family and my fantastic friends who supported and motivated me till the end of my study.

This work was supported by SVV 260 547.

ABSTRACT

Charles University
Faculty of Pharmacy in Hradec Králové
Department of Pharmaceutical Chemistry and Pharmaceutical Analysis

Candidate: Vu Lien Phuong
Supervisors: PharmDr. Marta Kučerová, Ph.D.
Tanja Bruun, M.Sc. (Pharm.)
Prof. Jari Yli-Kauhaluoma

Title of diploma thesis: Synthesis of novel 5,6-disubstituted derivatives of uracil as potential drugs

In this thesis, uracil was used as the core structure given its many biological activities that were reported such as antitumor, antiviral, antibiotic, hypoglycemic, diuretic and many others. The work was focused on the preparation of new 5,6- disubstituted uracil derivatives as potential biologically active agents.

2,4,6-Trichloropyrimidine was used for the preparation of 6-chlorouracil that was condensed with phenols or anilines to give the respective 6-phenoxyuracils and 6- phenylaminouracils. These intermediates were then modified in position 5 to give the final products. For this very challenging last step, various alkylating and acylating agents were used, *e.g.* Vilsmeier reagent, alkylchlorides, chloroacetyl chloride, ethyl chlorooxoacetate and ethyl bromoacetate. In the end, ethyl bromoacetate gave the most promising results affording four novel 5,6-disubstituted uracil derivatives.

During the experimental work it was found that pH of water used for the work up was critical aspect for NMR spectra identification of all signals that were expected. In addition, given the hydrophilic nature of the compounds, all of them had strong binding ability to water.

ABSTRAKT

Univerzita Karlova
Farmaceutická fakulta v Hradci Králové
Katedra farmaceutické chemie a farmaceutické analýzy

Kandidát: Vu Lien Phuong
Školitelé: PharmDr. Marta Kučerová, Ph.D.
Tanja Bruun, M.Sc. (Pharm.)
Prof. Jari Yli-Kauhaluoma

Název diplomové práce: Synthesis of novel 5,6-disubstituted derivatives of uracil as potential drugs
(Syntéza nových 5,6-disubstituovaných derivátů uracilu jako potenciálních léčiv)

V této diplomové práci je uracil použit jako základní struktura především kvůli svým mnoha popsaným biologickým aktivitám jako je aktivita protinádorová, antivirová, antibiotická, hypoglykemická, diuretická a další. Hlavním cílem bylo připravit nové 5,6- disubstituované deriváty uracilu jako potenciální biologicky aktivní látky.

2,4,6-Trichlorpyrimidin byl použit k přípravě 6-chloruracilu, který byl kondenzován s fenoly nebo aniliny za vzniku příslušných 6-fenoxyuracilů a 6- phenylaminouracilů. Tyto meziprodukty pak byly použity k substituci v poloze 5 za účelem získání konečných produktů. Pro tento poslední a velmi obtížný krok byla použita různá alkylační a acylační činidla jako např. Vilsmeierovo činidlo, alkylchloridy, chloracetylchlorid, ethyl-chloroxoacetát a ethyl-bromacetát. Ethyl-bromacetát byl nejefektivnější a byly z něj připraveny čtyři nové 5,6-disubstituované deriváty uracilu.

Během experimentální práce bylo zjištěno, že pH vody použité při zpracování bylo kritickým aspektem, které ovlivňovalo správnou identifikaci signálů v NMR spektrech. Vzhledem k hydrofilní povaze sloučenin, byla objevena jejich silná tendence vázat vodu.

CONTENTS:

1. LIST OF ABBREVIATIONS

2,4,6-TCP – 2,4,6-trichloropyrimidine

5-FU – 5-fluorouracil

5-HT – 5-hydroxytryptamine (serotonin)

ACN – acetonitrile

APCI – atmospheric pressure chemical ionization

ASAP – atmospheric solids analysis probe

CMS – content management system

DCM – dichloromethane

DIPEA – *N,N*-diisopropylethylamine

DMAc – *N,N*-dimethylacetamide

DMF – *N,N*-dimethylformamide

DMSO – dimethylsulfoxide

DNA – deoxynucleic acid

DPP-4 – dipeptidyl peptidase 4

EC_{50} – half maximal effective concentration

EMAU – (3-ethyl-4-methylphenyl)aminouracil

eq. – equivalent

ESP – electrostatic potential

ESI – electrospray ionization

Et_2O – diethyl ether

EtOAc – ethyl acetate

FCC – flash column chromatography

HDMS – high definition mass spectrometer

HEPT – 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine

HIV – human immunodeficiency virus

HRMS – high resolution mass spectrometry

IC_{50} – half maximal inhibitory concentration

LC-MS – liquid chromatography-mass spectroscopy

MeOH – methanol

MEP – molecular electrostatic potential

MS – mass spectroscopy

M_w – molecular weight

MW – microwave irradiation

m/z – mass-to-charge ratio

NMR – nuclear magnetic resonance spectroscopy

pH – potential of hydrogen

ppm – parts per million

Q-TOF – quadrupole time-of-flight

rpm – rotations per minute

RNA – ribonucleic acid

RSV – respiratory syncytial virus

RT – room temperature

SAR – structure-activity relationship

t-BuOK – potassium *tert*-butoxide

THF – tetrahydrofuran

TLC – thin layer chromatography

t_R – retention time

UPLC – ultra performance liquid chromatography

UV – ultraviolet

2. INTRODUCTION AND THE AIM OF WORK

Uracil is considered one of privileged structures given its wide range of biological activities such as antitumor, antiviral, antibiotic, hypoglycaemic, diuretic and many others[1]. Considering its big potential for many biological active derivatives, the aim of this thesis was the synthesis of new 6-phenoxyuracil and 6- phenylaminouracil derivatives substituted in position 5 for future evaluation of biological activity (target was not disclosed) in the Division of Pharmaceutical Chemistry and Technology at Faculty of Pharmacy at University of Helsinki.

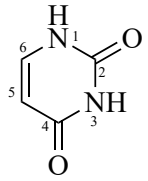
Our synthetic approach started with the synthesis of 6-chlorouracil from 2,4,6- trichloropyrimidine followed by condensation with phenols and anilines to synthetize corresponding 6-phenoxyuracils or 6-phenylaminouracils. Compounds of the highest interest were 6-(4-chlorophenoxy)uracil derivatives as they were suggested during molecular docking (detailed information was not provided by the external supervisor). Thus, big part of this thesis was focused on optimization of the synthesis of 6-(4-chlorophenoxy)uracil. The prepared intermediates were then used for alkylation or acylation in position 5.

The first part of this thesis should be general introduction to uracil, its reactivity and properties. Due to the many active derivatives, the emphasis should be put on derivatives with substitution in position 5 or/and 6. The second part should be dedicated to experimental part with above mentioned objective.

3. THEORETICAL PART

3.1. Uracil

Uracil **1** (Fig. 1) is a pyrimidine derivative with two oxo groups in positions 2 and 4. It is a common and naturally occurring substance present in living organisms. In human body, aside from being fundamental structure for RNA and being involved in protein synthesis reactions, it also plays an important role in many biochemical reactions as allosteric regulator or coenzyme *via* bonding with ribose and phosphates.[2] Given it being one of basic nucleobases and its vast involvement in metabolic pathways, uracil derivatives are promising molecules for drug development.



1

Fig. 1 Uracil with numbering of atoms

3.2 Uracil reactivity and synthesis

Synthetic accessibility and chemical reactivity are other reasons, apart from broad spectrum of pharmacological activities, why uracil is attractive to pharmaceutical chemistry.[1] Furthermore, it is a good substrate for pteridine and purine syntheses which are other two very promising structures in regards to pharmacological activity.[3]

There are many ways how to prepare uracil[4]. The condensation of maleic acid with urea in fuming sulfuric acid is the most common one (Fig. 2).

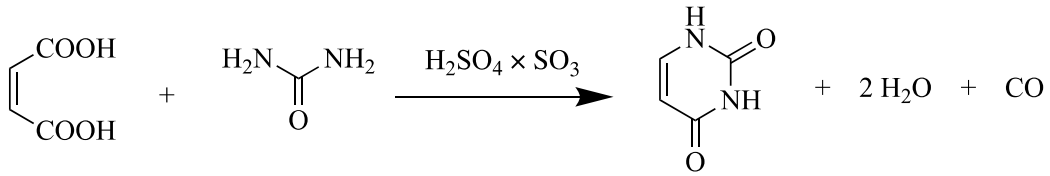
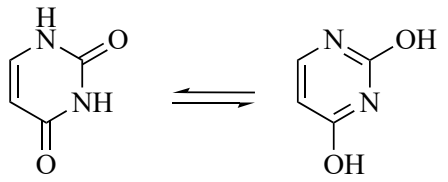


Fig. 2 Synthesis of uracil from maleic acid and urea

Uracil undergoes prototrophic tautomeric shifts. There are eighteen tautomer-rotamer forms in total when rational isomerism of *exo*-OH is considered.[5] The most prominent pair is lactam-lactim (amide-imide) forms (Fig. 3). These tautomeric forms are predominant at pH 7 where lactam is the most common and stable form of uracil.[1,5] The rest of tautomeric forms are not that stable but were studied as they are most likely reason for the mutations.[6]



amide - lactam imide - lactim

Fig. 3 Uracil lactam-lactim tautomers

Uracil is a weak acid and many studies were published focused on acid-base relation to hydrogen-binding that occurs in organisms.[7,8] Uracil has four acidic sites N(1), N(3), C(5) and C(6), where C(6) is found to be slightly more acidic than C(5) site. In addition, it was found that N(1) and N(3) has the same acidity which is a surprising revelation as glycosylation of nucleobase is specifically at N(1).[9,10] O(2) and O(4) are sites where proton binding of uracil in lactam form can occur.[11]

To predict and understand the reactivity of uracil, visualization of electrostatic potential (ESP) can be used. In the picture (Fig. 4) we can see ESP (electrostatic potential surface) of uracil created by Matondo A. *et al.*[12] using the method of numerical analysis of molecular electrostatic potential (MEP) and respective calculation of charges on nuclei. This "map" indicates the distribution of the charge within the molecule. Red color signifies the most negative electrostatic potential that attracts positively charged parts most strongly. Blue is used for the most positive electrostatic area and indicates regions that attract negatively charged parts.[13]

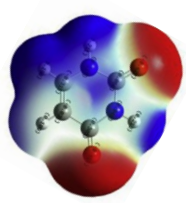


Fig. 4 ESP of uracil created by Matondo A. *et al.*[12]

As nitrogen atoms in positions 1 and 3 both have a free electron pair, they can be 1-alkylated or 1,3-dialkylated depending on reaction conditions (Fig. 5). The double bond in uracil can be reduced to 5,6-dihydrouracil. Uracil can also undergo oxidative halogenation[14] and nitration. Reacting with diphosphorus pentosulfide, 4-thiouracil can be obtained. With phosphoryl chloride it can be converted into 2,4- dichloropyrimidine. Uracil reacts with hydrazine affording pyrazol-3(2*H*)-one and urea. The same reaction with *N*-methyl- and *N,N*-dimethyl-hydrazine gave a 2-methyl and 1,2-dimethyl derivatives of pyrazole.[3]

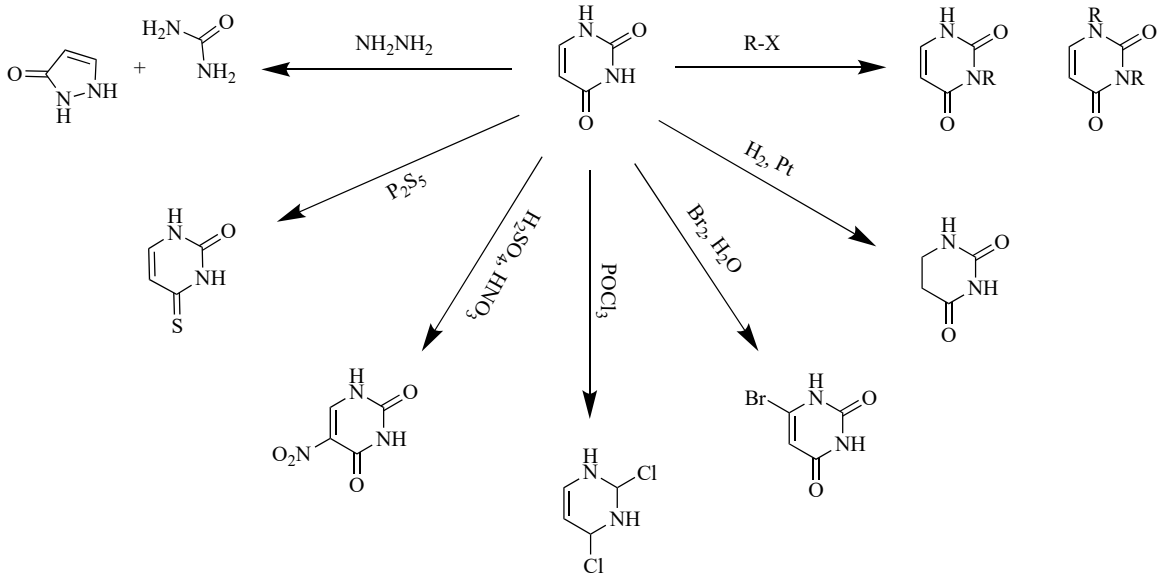


Fig. 5 Scheme of uracil reactivity

In addition, it has an interesting ability to undergo photodimerization (Fig. 6). Uracil, thymine, their derivatives substituted in position 5 and/or 6, *N*-alkylated derivatives and their respective nucleosides and nucleotides can dimerize in solution catalyzed by the UV light.[15,16,17] This reaction happens very quickly and also occurs in living tissues resulting most frequently to cyclobutene-like dimers that are one of the reasons for lesions in DNA and RNA strands and can cause inactivation of nucleotides.[18]

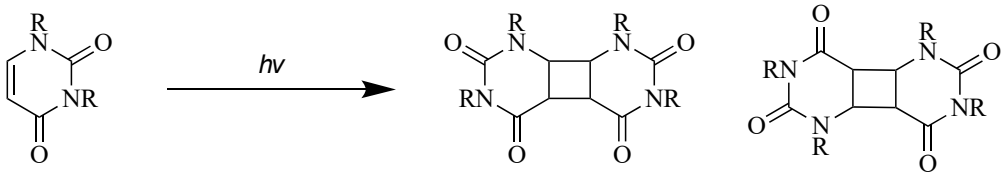


Fig. 6 Photodimerization of uracil

3.3. Uracil derivatives

As the most frequent approach to development of new antitumor and antiviral drugs is to use analogues of existing structures that take part in DNA and RNA synthesis, it is not surprising that those are the most reported activities of uracil derivatives. Nevertheless, antibacterial[19], antiparasitic[20], herbicidal[21], insecticidal[22], anti-inflammatory[23,24,25] and hypoglycemic and many other activities were also published.[1]

3.3.1 Antitumor activity

Generally, it was observed that substitution of uracil in the position 5 by halogen leads to derivatives with antibacterial and anticancer activities (Fig. 7).[26] The most known derivative is 5-fluorouracil (5-FU), antitumor agent that is widely used for treatment of multiple solid tumors such as breast and colorectal cancer.[27,28] Even though 5-FU was clinically successful, its toxicity and low selectivity initiated research of its conjugates and derivatives with better properties.[29,30]

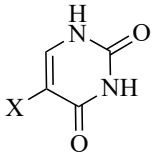
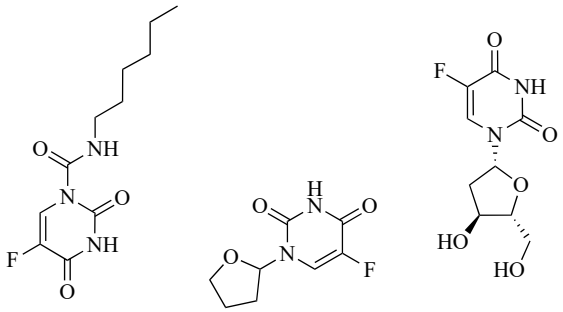


Fig. 7 Structure of 5-halouracils (X= F, Cl, Br, I)

In clinical practice there are already 5-FU analogs with anticancer activity *e.g.* carmofur **2,** chemotherapeutic drugs used for treatment of colorectal cancer [31], tegafur **3**, 5-FU prodrug or floxuridine **4**, antimetabolite used for treatment of colorectal, kidney and stomach cancer (Fig. 8).

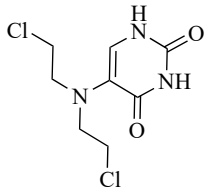


2 carmofur **3** tegafur **4** fluroxidine

Fig. 8 Examples of 5-FU analogs with antitumor activity

Antitumor and antibacterial activities of N^1-mono or N^1,N^3-disubstitued 5-fluoro, 5-bromo- and 5-iodouracils were tested and it was concluded that 5-fluoro and 5- iodouracils showed more significant antitumor activity than 5-bromouracils.[19] Aside from antiviral and antitumor activities, 5-halouracils are interesting as it was reported that 5-chlorouracil and 5-bromouracil are produced during inflammation in a human tissue.[32] 5- Chlorouracil was found in human atherosclerotic tissues and is believed to be a possible reason for mutation of phagocytes.[33]

5-Aminouracils are another molecules with promising biological activity. 5- Aminouracil alone is often used as a substitute of thymine in the chemistry of DNA oligonucleotides. It exhibits antitumor, antibacterial and antiviral activity.[34] Its derivative, uramustine (uracil mustard) **5** (Fig. 9) is a DNA-alkylating agent used for treatment of lymphosarcoma, chronic lymphatic leukemia and thrombocythemia.[35]

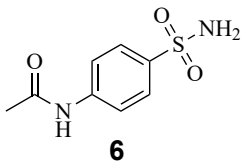


5

uramustine

Fig. 9 Structure of 5-aminouracil derivative: uramustine

Newly 5-aminosulfonyl uracil derivatives were prepared by the reaction of 5- aminouracils with variously substituted aromatic sulfonyl chlorides. The products showed promising antiproliferative reactivity especially on lymphoma and leukemia cells. *N*-(4-sulfamoylphenyl)acetamide **6** (Fig. 10) had the best results with IC_{50} = 57.2 ± 13.6 μM against lymphoma cells and IC_{50} = 79.9 ± 12.1 μM against leukemia cells. 5- FU was used as positive control (IC_{50} > 100 μM against lymphoma cells, IC_{50} = 76.3 ± 11.4 μM against leukemia cells).[36]



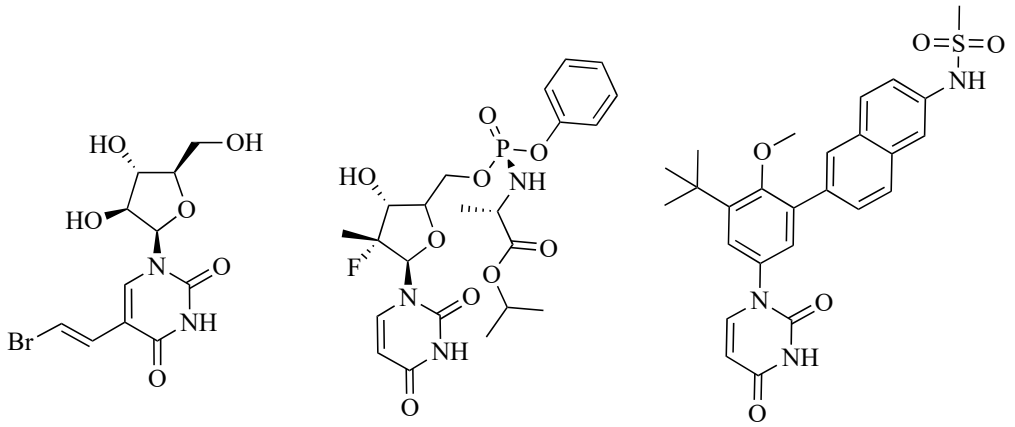
6

N-(4-sulfamoylphenyl)acetamide

Fig. 10 Structure of 5-aminosulfonyl uracil derivative showing the best antiproliferative activity in the series

3.3.2 Antiviral activity

Many antiviral agents containing uracil used in clinical practice possess character of nucleosides and their analogs *e.g.* sorivudine **7,** antiviral drug with potent effect against varicella zoster virus, Epstein- Barr virus and herpes simplex type 1 virus[37] or sofosbuvir **8** and dasabuvir **9** that are used for treatment of hepatitis C virus infections (Fig. 11).



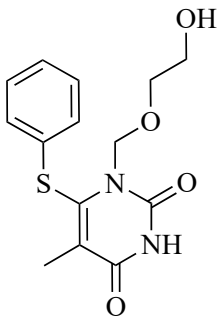
7 sorivudine

8 sofosbuvir

9 dasabuvir

Fig. 11 Examples of nucleosides and their analogs with antiviral activity

In 1989, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine known as HEPT **10** (Fig. 12) was the first non-nucleoside reverse transcriptase inhibitor described[38] and it quickly became the leading structure for new antiviral molecules.

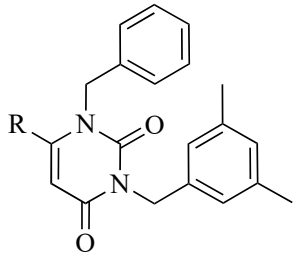


10

HEPT

Fig. 12 Structure of (1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine)

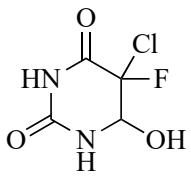
Inspired by HEPT, series of uracil analogs were synthetized (Fig. 13). 6-Azido-1- benzyl-3-(3,5-dimethylbenzyl)pyrimidine-2,4(1*H*,3*H*)-dione **11** and 6-amino-1-benzyl-3-(3,5-dimethylbenzyl)pyrimidine-2,4(1*H*,3*H*)-dione **12** had the highest inhibitory effect with EC_{50} = 0.067 ± 0.011 μM and EC_{50} = 0.069 ± 0.006 μM respectively. In this study, nevirapine was used as positive control with EC_{50} = 0.061 ± 0.007 μM.[39]



R: - N_3 **(11)**
- NH_2 **(12)**

Fig. 13 Structures of 3-(3,5-dimethylbenzyl)uracil analogs with most potent anti- HIV activity

Recently, 5-chloro-5-fluoro-6-alkoxypyrimidines were synthesized and tested for antiviral activity. It was found that 5-chloro-5-fluoro-6-hydroxy-5,6-dihydrouracil **13** (Fig. 14) had the highest antiviral activity against respiratory syncytial virus (RSV) (EC_{50} = 0.124 ± 0.016 mM) and human influenza A viruses H3N2 (EC_{50} = 0.228 ± 0.016 mM) and H1N1(EC_{50}=0.186 ± 0.01 mM). Oseltamivir (EC_{50} = 0.004 mM) was used as standard for influenza A H1N1. Umifenovir (EC_{50} = 0.011 mM) was used as standard for RSV and influenza A H3N2.[40]



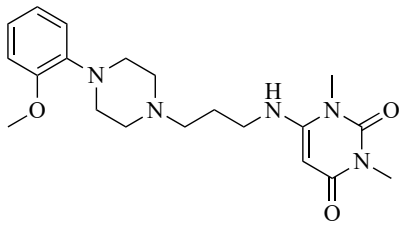
13

5-chloro-5-fluoro-6-hydroxy-5,6-dihydrouracil

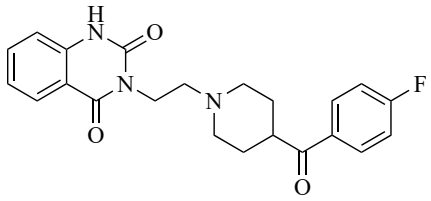
Fig. 14 Structure of 5-chloro-5-fluro-6-alokoxypyrimidine with the highest activity against RSV and human influenza A virus

3.3.3. Antihypertensive activity

Urapidil **14** and ketanserin **15** (Fig. 15) are the uracil derivatives with antihypertensive activity. Urapidil is an α_1-adrenoceptor antagonist and a weak 5-HT_{1A} receptor agonist. It shows many advantages in therapy *e.g.* good tolerance and reduction of peripherical vascular resistance without causing significant tachycardia. Given its central activity, it is not a drug of the first choice for the treatment of hypertension, but it still has importance in hypertension crisis management.[41] Ketanserin is an antagonist of 5-HT_2 that inhibits serotonin-dependent vasoconstriction and platelet activity.[42] It was investigated for treatment of Raynaud's syndrome, septic states and diabetic foot ulcers. Nowadays, it is more important in research of 5-HT_2 receptors given its selectivity[43] like in the study[44] where [^{3}H] ketanserin was used for mapping 5-HT_2 receptors in a human brain.



14
urapidil

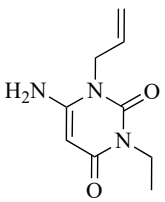


15
ketanserin

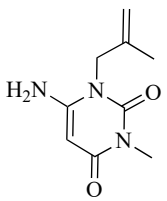
Fig. 15 Uracil derivatives with antihypertensive activity

3.3.4 Diuretic agents

Aminometradine **16** and amisometradine **17** (Fig. 16) are alkyl-substituted uracil derivatives used for the treatments of edema and heart failures.[45,46] Nowadays, their use is obsolete given their higher toxicity in comparison with other diuretics.



16
aminometradine

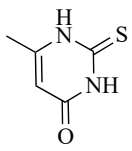


17
amisometradine

Fig. 16 Uracil derivatives with diuretic activity

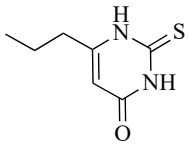
3.3.5 Thyroidal inhibitors

Methylthiouracil **18** and propylthiouracil **19** (Fig. 17) are well known drugs used for the treatment of hyperthyroidism. Their mechanism of action is the inhibition of thyroid peroxidase which is responsible for conversion of iodine (I^-) to iodine (I^0). They also inhibit incorporation of iodine to thyreoglobuline which results in the reduction of thyroxine level. In addition, they lower iodine level in thyroid gland and block binding of iodized thyrosyl residues to thyreoglobulines. In peripheral tissue they inhibit tetraiodothyronine 5'deiodinase that converts thyroxine to biologically active triiodothyronine. Propylthiouracil is especially important as it is used as a medication of the first choice for the treatment of hyperthyroidism during pregnancy.



18

methythiouracil



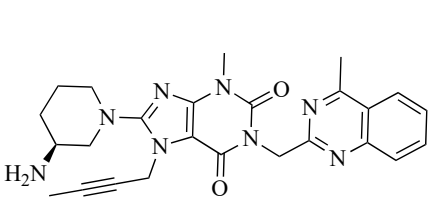
19

propylthiouracil

Fig. 17 Thiouracil derivatives

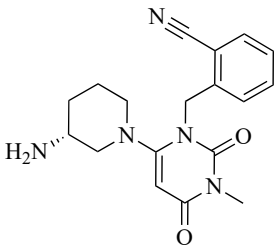
3.3.6 Hypoglycemic activity

Uracil is also present in some molecules (Fig. 18) known as gliptins that inhibit dipeptidyl peptidase-4 (DPP-4) and are used in therapy of diabetes mellitus 2 namely: linagliptin **20**, alogliptin **21** and trelagliptin **22**. Due to some undesirable side effects in class of DPP-4 inhibitors, the research of new molecule targeting DPP-4 is still on-going.



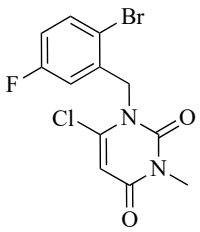
20

linagliptin



21

alogliptin

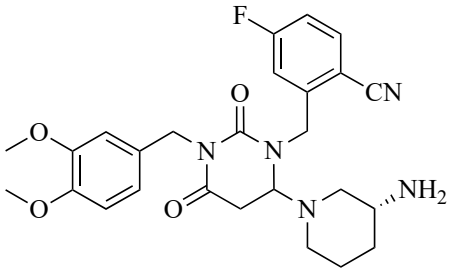


22

trelagliptin

Fig. 18 Gliptins containing uracil moiety in molecules

Recently, a series of *N*,*N*-disubstituted uracils were prepared and tested. As results, a very promising DPP-4 inhibition activity was revealed. It was found that 2- {[6- ((*R*)- 3- aminopiperidin-1-yl)-3-(3,4-dimethoxybenzyl)-2,4- dioxotetrahydro-pyrimidin-1(2*H*)-yl]methyl}-4-fluorobenzonitrile **23** (Fig. 19) showed the most potent inhibitory activity of DPP-4 at 100 nM with inhibitory rate of (100 ± 0.54)% with IC_{50} = 64.47 nM. In this study, sitagliptin was used as control with IC_{50} = 37.96 nM.



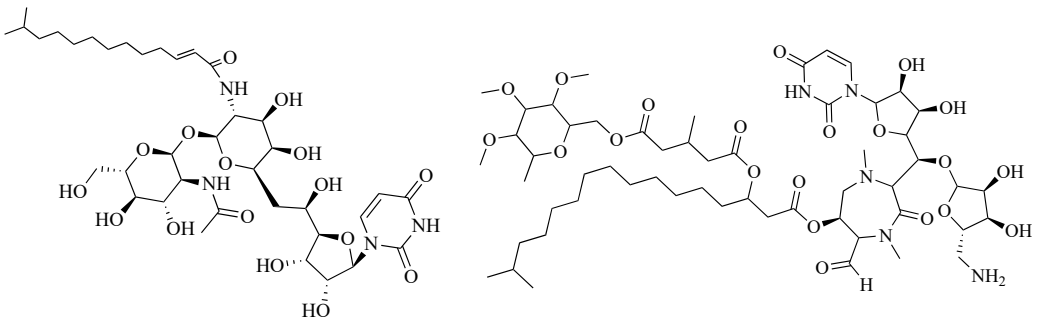
23

Fig. 19 Structure of new potential inhibitor of DPP-4

Furthermore, it was reported that 5-alkynyl and alkynylfurano[2,3-*d*]pyrimidine glucopyranonucleosides have also hypoglycemic activity, targeting glycogen phosphorylase, enzyme that catalyzes the first step of intra-cellular degradation of glycogen to glucose-1-phophate.[47]

3.3.7 Antibacterial activity

Some natural antibiotics (Fig. 20) also have uracil moiety in their molecules *e.g.* tunicamycin **24**, an uracil derivative studied mostly for its antitumor activity[48] or caprazamycin B **25**.



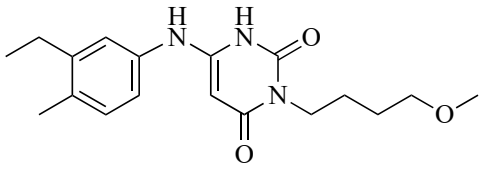
24
tunicamycin

25
caprazamycin B

Fig. 20 Structure of natural antibiotics with uracil moiety

Caprazamycin B was studied by Igarashi M. *et al*. and showed great anti-mycobacterial activity *in vitro* against *Mycobacterium tuberculosis* H37Rv and Kurono strains (MIC = 3.13 μg/l), *Mycobacterium bovis* Ravenel strain (MIC = 3.13 μg/l) and multi drug-resistant *Mycobacterium tuberculosis* (MIC = 6.25 – 12.5 μg/l).

Also, some substituted 6-(benzylamino)uracils and 6-phenylaminouracils have antibacterial properties. It was found that they are potent and selective inhibitors of Gram + DNA polymerase IIIC during SAR studies.[49] Zhi *et al*.[50] synthetized a series of N^3-substituted uracils that showed good inhibition activity against some Gram + organisms *e.g.* 6-(3-ethyl-4-methylphenylamino)-3- (4- methoxybutyl)uracil **26** (Fig. 21)with MIC: 5 μg/ml against *Bacillus subtillis*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Enterococcus fecalis*, MIC = 2.5 μg/ml against *Enterococcus fecium*, vancomycin-resistant *Enterococcus* and MIC > 2.5 μg/ml against *Escherichia coli.*



26

6-(3-ethyl-4-methylphenylamino)-3-(4-methoxybutyl)uracil

Fig. 21 Structure of 6-phenylaminouracil with antibacterial properties

4. EXPERIMENTAL PART

4.1 Chemicals and instruments

All chemicals and solvents used were purchased from Fluorochem, Sigma-Aldrich, and Honeywell.

Reaction progress was monitored by TLC. TLC plates Silica gel 60 F_{254} (Merck KGaA, Germany) or NH_2-modified silica TLC plates (Biotage, Sweden) were used using detection with UV light (254 nm and 366 nm). Mobile phase DCM-MeOH 9:1 was used if not stated otherwise. Additionally, if possible, MS Advion expression ® CMS with ASAP and Plate Express TLC reader (Advion Inc., USA) with APCI was used with MassExpress program for the interpretation of results.

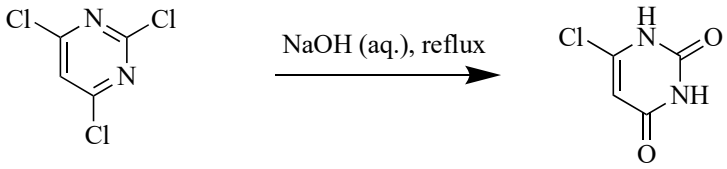
Chemical structures of reaction products were verified by NMR spectroscopy using Bruker Ascend 400 MHz, Avance III HD NMR spectrometer (Bruker, USA). MestreNova software was used for the interpretation of the results. Chemical shifts are in ppm and were referred to the signal of protons of tetramethylsilane. In addition, LC- MS Waters Acquity UPLC mass spectrometer with Waters Synapt G2 HDMS Q- TOF detector (Waters, Milford, MA, USA) using ESI ion source was used for HRMS measurement and purity testing. Eluent used was: water-ACN with 0.1% formic acid, column: Acquity UPLC BEH C18 (particle size 1.7 µm, 2.1 mm x 50 mm), flow rate 0.6 ml/min, 10 min run.

To purify the products, flash column chromatography (FCC) using Biotage Isolera™ Spektra Systems with ACI™ and Assist (Biotage, Sweden) was implemented for purification of products utilizing silica gel cartridges from Biotage: Sfar Silica D Duo (60 µm silica with pore size 60 Å), SNAP KP-Sil (50µm with pore size 60 Å) , SNAP Ultra (25 µm spherical silica) Sfar KP Amino D (50 µm pore size 100 Å).

Microwave Biotage Initiator+ (Biotage, Sweden) was used for some reaction.

The structures were designed in ChemOffice (PerkinElmer Informatics, Inc., USA, version 19.0.1.32).

4.2 General procedure for synthesis of 6-chlorouracil



Scheme 1. Preparation of 6-chlorouracil

1 eq. of 2,4,6-trichloropyrimidine (2,4,6-TCP) and 3.8–4 eq. of 2M aqueous solution of sodium hydroxide were stirred and refluxed (110 °C). At the beginning of the reaction, white solid droplets were formed. After completion of the reaction, there was white powder in the solution. The mixture was cooled and then acidified with 2M HCl (till pH = 2–3) while more white precipitate was formed. Suspension was stored overnight in the refrigerator. The precipitated product was collected, then washed by acidified distillated water (using 2 M HCl to obtain solution with pH = 3) and let dry by air pump. For completion the drying it was then put into desiccator (drying agents used: phosphorus pentoxide and silica). NMR spectra were measured for the verification of the product and was compared to NMR spectra measured of purchased 6-chlorouracil (M_w = 146.53 g/mol). The reaction was repeated seven times with different amount of substances (Tab.1).

4.2.1 6-chlorouracil; 6-chloropyrimidine-2,4(1*H*,3*H*)-dione, VLP-1

Appearance: white powder

Tab. 1: Summary of all attempts with yields

	$V_{2,4,6\text{- TCP}}$ (ml)	$n_{2,4,6\text{- TCP}}$ (mmol)	$V_{2M\ NaOH}$ (ml)	$n_{2M\ NaOH}$ (mmol)	$m_{VLP\text{- }1}$ (g)	yield
1st attempt	0.30	2.610	5.0	10	0.3076	**80%**
2nd attempt	0.20	1.828	3.7	7.4	0.2211	**83%**
3rd attempt	0.32	2.783	5.5	11	0.1010	**25%**
4th attempt	0.60	5.220	10.0	20	0.2830	**37%**
5th attempt	0.90	7.826	15.0	30	0.8870	**77%**
6th attempt	0.90	7.826	15.0	30	0.9155	**80%**
7th attempt	1.50	13.04	26.0	52.18	1.411	**74%**

1st attempt (VLP-1): time of reflux: 16 h

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 12.03 (s, 1H), 11.27 (s, 1H), 5.73 (s, 1H).

^{13}C NMR (101 MHz, DMSO) δ 162.70, 150.42, 144.90, 99.78.

2nd attempt (VLP-1b): time of reflux: 15 h

NMR spectra after work up without acidification:

^{1}H NMR (400 MHz, DMSO) δ 11.22 (s, 1H), 5.71 (d, J = 1.6 Hz, 1H)

^{13}C NMR (101 MHz, DMSO) δ 162.82, 150.64, 145.38, 99.60

NMR spectra after acidification:

^{1}H NMR (400 MHz, DMSO) δ 12.06 (s, 1H), 11.28 (s, 1H), 5.74 (d, J = 1.8 Hz, 1H)

^{13}C NMR (101 MHz, DMSO) δ 162.71, 150.38, 144.83, 99.84

3rd attempt (VLP-1c): time of reflux: 1 h

NMR spectra after work up without acidification:

^{1}H NMR (400 MHz, DMSO) 6.09 (s, 1H)

NMR spectra after acidification:

^{1}H NMR (400 MHz, DMSO) δ 11.13 (s, 1H), 6.42 (s, 1H), 5.67 (d, J = 1.4 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 163.42, 151.48, 146.67, 99.67.

4th attempt (VLP-1d): time of reflux: 16 h

NMR spectra after work up without acidification:

^{1}H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 6.24 (s, 1H), 5.61 (d, J = 1.4 Hz, 1H).

NMR spectra after acidification:

^{1}H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 6.24 (s, 1H), 5.61 (d, J = 1.4 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 163.22, 159.77, 151.70, 98.56.

5th attempt (VLP-1e): time of reflux: 16 h

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 12.05 (s, 1H), 11.29 (s, 1H), 5.74 (d, J = 1.8 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 162.66, 150.33, 144.71, 99.86.

6th attempt (VLP-1f): time of reflux: 16 h

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 12.05 (s, 1H), 11.29 (s, 1H), 5.75 (d, J = 1.8 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 162.65, 150.32, 144.69, 99.87.

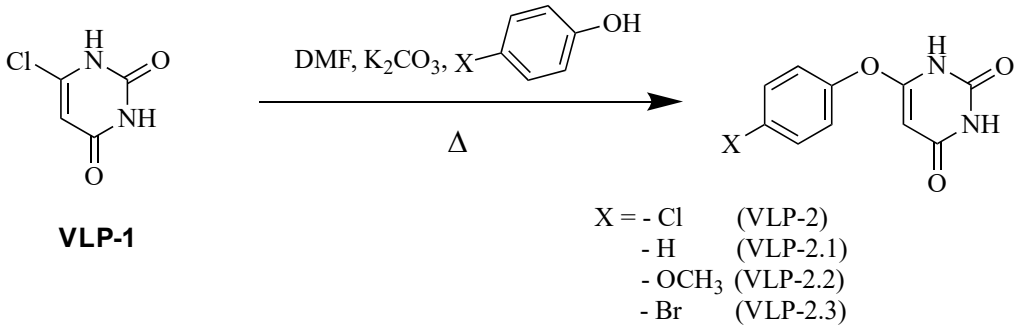
7th attempt (VLP-1g): time of reflux: 16 h

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 12.05 (s, 1H), 11.29 (s, 1H), 5.74 (d, J = 1.8 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 162.66, 150.33, 144.73, 99.85.

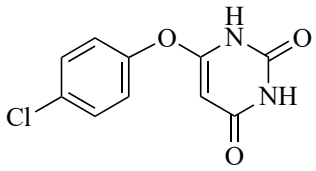
4.3 General procedure for synthesis of 6-phenoxyuracils



Scheme 2. Synthesis of 6-phenoxyuracils

1 eq. of 6-chlorouracil (VLP-1), 3 eq. of phenol or its substituted derivative and 5 eq. of potassium carbonate in dry DMF (5ml) were stirred and refluxed (160 °C). Not all components were directly dissolved after DMF was added. After refluxing the mixture was evaporated with rotary evaporator and then additionally dried by air pump. Then, it was washed with Et_2O (40 ml), filtered and dried. The solid was suspended in water (20 ml) and acidified with concentrated acetic acid (till pH = 4 of the solution). During the acidification we could see gas evolving as potassium carbonate in excess was hydrolyzed and carbonic acid was decomposed and in addition brownish precipitate was formed. The crude product was then filtered and dried overnight by air pump. NMR spectra were measured for the verification of the product.

4.3.1 6-(4-chlorophenoxy)uracil; 6-(4-chlorophenoxy)pyrimidine-2,4(1*H*,3*H*)-dione, VLP-2



M_w: 238.63 g/mol

Appearance: brown powder

1st attempt (VLP-2): time of reflux: 14 h

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.1490	146.53	1.017		
4-chlorophenol	3	0.3981	128.56	3.097		
K_2CO_3	5	0.7066	138.205	5.113		
DMF			73.095		0.948	5.00
VLP-2		0.0311	238.63			

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 11.91 (s, 1H), 10.91 (s, 1H), 7.55 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.3 Hz, 2H), 4.30 (s, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.27, 162.69, 150.44, 150.19, 130.91, 130.30, 123.11, 79.68.

In addition, sample (0.053 g) from NMR was used for ASAP measurement. Before the analysis, the sample was diluted in DCM (5 drops) and EtOAc (6 drops). The exact mass measured corresponded to our product. In addition, 2D-NMR spectra were measured for confirmation of the structure.

Yield = **13 %**

2nd attempt (VLP-2a):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.1463	146.53	0.998		
4-chlorophenol	3	0.3987	128.56	3.101		
K_2CO_3	12.5	1.7730	138.205	12.50		
DMF			73.095		0.948	5.00
VLP-2a		0.0203	238.63			

In this attempt, we changed the amount of potassium carbonate so it would correspond to the ratio in DMF in the article[51]. The mixture of VLP-1, 4- chlorophenol, potassium carbonate and DMF was stirred and refluxed (160 °C) for 16 h. The mixture was orange at the beginning and during refluxing white "foam" on the surface became visible. The stirring was fastened to 770 rpm. When the reflux was finished the mixture was white-brown. The mixture was evaporated on rotary evaporator and the residue was washed with Et_2O (40 ml). During removal of the solid from flask was broken. The flask fragments were washed with EtOAc (30 ml) but it was not dissolved, therefore DCM was tried but unsuccessfully. It was dissolved in MeOH, filtered and the yellow-brown solution was evaporated with rotary evaporator. The solid was suspended in water (20 ml) and neutralized with concentrated acetic acid (3–4 drops), precipitate started to form. The extraction was done with EtOAc (3×30 ml). The phases were not clear, on the interface it was misty and cloudy. The EtOAc solution was then washed with brine (30 ml), dried with anhydrous sodium sulfate and filtered. Then the solution was evaporated on rotary evaporator. TLC was done. Tested with mobile phases: Heptane-EtOAc (1:1), Heptane- EtOAc (3:7), DCM–MeOH (20:1) were not successful. The most suitable mobile phase for TLC performance was: DCM–MeOH (9:1). NMR spectra were measured. Acetone was tried for dissolving but unsuccessfully, therefore DMSO was used.

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 11.90 (s, 1H), 10.92 (s, 1H), 7.60–7.51 (m, 2H), 7.40– 7.31 (m, 2H), 4.30 (d, J = 1.7 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.24, 162.60, 150.36, 150.16, 130.94, 130.31, 123.11, 79.70.

VLP-2a HRMS (ESI^+): calculated 239.0223 ($C_{10}H_8ClN_2O_3$), found 239.0223.
LC-MS: $[M+H]^+$ m/z 239 (t_R = 2.06 min). Purity 100%.

Yield = **9**%

3rd attempt (VLP-2b):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.1913	146.53	1.306		
4-chlorophenol	3	0.5322	128.56	4.140		
K_2CO_3	12.5	1.751	138.205	12.50		
THF			72.11		0.889	15.00
VLP-2b		0.300	238.63			

In this attempt, the reaction solvent was changed to THF. The mixture was stirred and refluxed (65 °C) overnight (16 h). The components were not instantly dissolved when THF (5 ml) was added. At the beginning of the reaction the mixture was pinkish. The solvent was rapidly evaporated as the glass joint between cooler and flask did not fit tightly so another 10 ml of THF were added and we used sealer for better isolation. The white solid was formed. The mixture was put on rotary evaporator to get rid of THF. Then the dry white solid was washed with Et_2O (40 ml) and filtered off. The white solid was suspended in water, part of it dissolved in the water. The solution was neutralized with concentrated acetic acid. The water was extracted with EtOAc (3×30 ml). There was a small part of white solid that was dissolved neither in water, nor in EtOAc. The solution of EtOAc was dried with anhydrous sodium sulfate and then filtered off. The EtOAc solution of reaction mixture was then evaporated with rotary evaporator, white product was resulted. Mass spectrum was measured and did not correspond to the desired product (majority peak was at 226 g/mol). NMR spectra were measured and revealed starting material. The residue from the reaction therefore was used for setting the reaction again.

Yield = **0%**

4th attempt (VLP-2b$_1$):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d	V (ml)
VLP-1	1	0.0300	146.53	0.2047		
4-chlorophenol	3	0.5264	128.56	0.6301		
K_2CO_3	12.5	1.7726	138.205	12.50		
DMF			72.11		0.889	5.00
VLP-2b$_1$		0.0065				

In this attempt, VLP-2b is VLP-1 in this reaction. To put VLP-1 to another flask, EtOAc was used. The mixture was stirred and refluxed (165 °C) for 10 h. The components were not instantly dissolved when DMF was added. At the beginning of the reaction the mixture was orange. At the end of the reaction a white solid was visible and solution was colored to yellow-brown. The mixture was evaporated with rotary evaporator to get rid of DMF. Then the dry light brown-white solid was washed with Et_2O (30 ml) and filtered off. The solid was suspended in water (20 ml) and it was dissolved. The solution was neutralized with concentrated acetic acid (pH = 8 at the beginning), no precipitate was formed. The water was extracted by EtOAc (3×20 ml). The EtOAc solution with the reaction mixture was dried with anhydrous sodium sulfate and then filtered. The filtered solution was evaporated with rotary evaporator and additionally dried with air pump. The results of NMR did not match the expected product.

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 8.56 (s, 1H), 7.00 (d, J = 8.6 Hz, 1H), 6.62 (d, J = 8.3 Hz, 1H), 2.89 (s, 1H), 2.73 (d, J = 0.7 Hz, 1H), 1.54 (s, 12H), 1.24 (s, 1H).

^{13}C NMR (101 MHz, DMSO) δ 26.14.

Yield = **0%**

5th attempt (VLP-2c):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.5008	146.53	3.418		
4-chlorophenol	3	1.3245	128.56	10.3		
K_2CO_3	12.5	3.455	138.205	25		
DMF			72.11		0.889	10.00
VLP-2c		0.0537	238.63			

In this attempt, we used purchased 6-chlorouracil instead of VLP-1. The mixture was stirred and refluxed (165 °C). The components were not instantly dissolved when DMF was added. At the beginning of the reaction, the mixture was slightly pink, during refluxing the gas was evolved. The mixture turned to green color and later it turned to yellow. After 6 h, TLC was conducted. The results were not specific, so the mixture was refluxing and stirred for 23 h in total. At the end of the reaction, the white solid was visible and brown solution was present. The mixture was evaporated with rotary evaporator and then washed with Et_2O (40 ml). The mixture was filtered, some parts were very hard solid. Then the precipitate was suspended in water (40 ml), neutralized with concentrated acetic acid and filtered off. NMR spectra was measured and did not confirm the desired product. After acidification with concentrated acetic acid it did.

NMR spectra before the acidification:

^{1}H NMR (400 MHz, DMSO) δ 8.89 (s, 1H), 7.40–7.32 (m, 2H), 7.07–6.98 (m, 2H), 4.43 (d, J = 1.6 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 172.05, 167.37, 152.89, 128.75, 127.37, 123.29, 77.15.

NMR spectra after acidification:

^{1}H NMR (400 MHz, DMSO) δ 11.90 (s, 1H), 10.87 (s, 1H), 7.59–7.51 (m, 2H), 7.39– 7.30 (m, 2H), 4.30 (d, J = 1.6 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.32, 162.84, 150.58, 150.23, 130.85, 130.28, 123.11, 79.64

Yield = **7%**

6th attempt (VLP-2d):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.4997	146.53	3.41	-	-
4-chlorophenol	3	1.3259	128.56	10.31	-	-
K_2CO_3	5	2.348	138.205	16.99	-	-
DMF			73.095	-	0.948	5.00
VLP-2d		0.0758	238.63			

In this attempt, pure 6-chlorouracil was used instead of VLP-1. In addition, smaller amount of DMF (5 ml) was used and ratio of VLP-1, 4-chlorophenol and potassium carbonate was back to 1:3:5 respectively.

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 11.91 (s, 1H), 10.89 (s, 1H), 7.59–7.51 (m, 2H), 7.39– 7.31 (m, 2H), 4.30 (d, J = 1.7 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.30, 162.76, 150.50, 150.21, 130.88, 130.29, 123.11, 79.66.

Yield = **9%**

7th attempt (VLP-2e):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.0773	146.53	0.5275		
4-chlorophenol	3	0.1972	128.56	1.5339		
K_2CO_3	5	0.3543	138.205	2.5636		
DMF			73.095		0.948	1.50
VLP-2e		0.029	238.63			

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 11.90 (s, 1H), 10.95–10.91 (m, 1H), 7.60–7.51 (m, 2H), 7.40–7.31 (m, 2H), 4.30 (t, J = 1.6 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.32, 162.84, 150.58, 150.23, 130.85, 130.28, 123.11, 79.64

Yield = **23%**

8th attempt (VLP-2f):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.5017	146.53	3.4239		
4-chlorophenol	3	1.3276	128.56	10.327		
K_2CO_3	5	2.3516	138.205	17.015		
DMF			73.095		0.948	7.00
VLP-2f		0.0865	238.63			

In the next attempt, purchased 6-chlorouracil was used. Firstly, DMF (5ml) was put into flask. 4- Chlorophenol was added to the flask and let to be completely dissolved. Then 6- chlorouracil and potassium carbonate were added. The mixture was stirred and

refluxed. At the beginning, the mixture was white and more like a suspension after 1 h DMF (2 ml) was added. The solution turned to brown. After 3 h, the suspension became thick again. On the surface of the mixture green color was visible. The mixture was refluxed for 21 h in total. During the work up, the solution was acidified with concentrated acetic acid till pH reached 4. Then it was refrigerated for 2 h. The brown solid was filtered and dried with air pump. The NMR spectrum of the solid was measured and the structure was confirmed.

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 11.90 (s, 1H), 10.92 (s, 1H), 7.56 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 4.30 (s, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.25, 162.63, 150.38, 150.17, 130.93, 130.31, 123.11, 79.70.

Yield = **11%**

9th attempt (VLP-2g): time of reflux: 15 h

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.5014	146.53	3.422		
4-chlorophenol	3	1.323	128.56	10.29		
K_2CO_3	5	2.3534	138.205	17.03		
DMF			73.095		0.948	5.00
VLP-2g		0.1067	238.63			

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 11.91 (s, 1H), 11.00–10.83 (m, 1H), 7.62–7.48 (m, 2H), 7.39–7.31 (m, 2H), 4.30 (d, J = 1.7 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.26, 162.67, 150.42, 150.18, 130.92, 130.30, 123.11, 79.68.

Yield = **13%**

10th attempt (VLP-2h):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.1534	146.53	1.0469		
4-chlorophenol	3	0.4022	128.56	3.1285		
K_2CO_3	5	2.3534	138.205	17.028		
DIPEA			129.25		0.742	5.00
VLP-2h		0	238.63			

In the next attempt, DIPEA was used as reaction solvent. The mixture was stirred and refluxed (160 °C) for 28 h. The brown suspension was evaporated with rotary evaporator and suspended in water (40 ml). Then, it was acidified with concentrated acetic acid (pH = 4), no precipitates were formed. The solution was extracted with EtOAc (3×40 ml) and TLC was conducted but no spot was visible. The reaction most probably went overboard and it burned.

Yield = **0%**

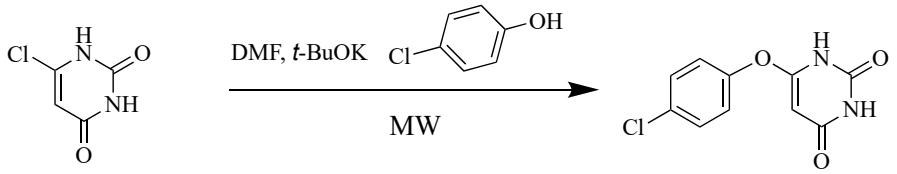
11th attempt (VLP-2l):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.1501	146.53	1.024		
4-CP	3	0.3865	128.56	3.006		
t-BuOK	3.3	0.3787	112.22	3.375		
DMF (dry)			73.095		0.948	5.00
VLP-2l		0	238.63			

In the next attempt, *t*-BuOK was used as a base. The reaction was stirred and refluxed (165 °C) for 7 h until the starting material was not visible on TLC plate anymore. Mass spectrum was measured with detection 273 g/mol which did not correspond to our desired product. The mixture was evaporated with rotary evaporator and suspended in the water (40 mol). Then, it was acidified by concentrated acetic acid (till pH = 3). No precipitate was formed. Given measured mass spectrum it was concluded that reaction did not work out.

Yield = **0%**

4.3.1.1 General procedure for synthesis of 6-(4-chlorophenoxy)uracil under microwave irradiation



VLP-1 VLP-2

Scheme 3. Synthesis of 6-6-(4-chlorophenoxy)uracil under microwave irradiation

1 eq. of 6-chlorouracil, 3 eq. of 4-chlorophenol, 3.3 eq of *t*-BuOK and dry DMF (2 ml) were put into 2–5 ml vial and it was placed into a microwave reactor (10 s pre-stirring, high absorption, 130 °C, P = 400 W) for the bellow mentioned time period. The mixture was cooled and acidified with 1M HCl (pH = 4). The formed precipitate was collected.

1st attempt (VLP-2i):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.0313	146.53	0.2136		
4-chlorophenol	3	0.0813	128.56	0.6324		
t-BuOK	3.3	0.0793	112.22	0.7066		
DMF (dry)			73.095		0.948	2.00
VLP-2i		0	238.63			

Time: 9 + 15 min

After microwave irradiation for 9 min, we got a green suspension that is usually visible at the beginning of the regular reaction in flask while refluxing. On the TLC plate there were not any corresponding spots for the product therefore the mixture was irradiated for another 15 mins but it went overboard and a polymerized solid appeared.

Yield = **0%**

2nd attempt (VLP-j):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.0306	146.53	0.2088		
4-CP	3	0.0824	128.56	0.6409		
t-BuOK	3.3	0.0894	112.22	0.7966		
DMF (dry)			73.095		0.948	2.00
VLP-2j		0	238.63			

Time: 15 min

After the irradiation the mixture looked similar to the one from the first attempt (after 9 min irradiation). TLC was conducted and mass spectrum was measured with detection of 238 g/mol on positive side. After acidification with 1 M HCl no precipitate was formed.

Yield = **0%**

3rd attempt (VLP-k):

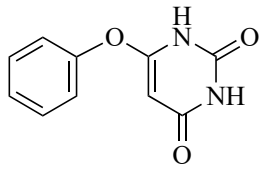
substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1g	1	0.0300	146.53	0.2047		
4-CP	3	0.0791	128.56	0.6153		
t-BuOK	3.3	0.1043	112.22	0.9294		
DMF (dry)			73.095		0.948	2.00
VLP-2k		0	238.63			

Time: 18 min

With suspicion that the reaction needed more time, we irradiated the mixture for 18 min but the reaction polymerized again and was burnt.

Yield = **0%**

4.3.2 6-Phenoxyuracil; 6-phenoxypyrimidine-2,4(1*H*,3*H*)-dione, VLP-2.1



Mw: 204.19 g/mol

Appearance: dark brown powder

1st attempt (VLP-2.1):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1e	1	0.3001	146.53	2.048		
phenol	3	0.5890	94.113	6.258		
K_2CO_3	5	1.4166	138.205	10.249		
DMF			73.095		0.948	5.00
VLP-2.1		0.0282	204.19			

After 18 h the mixture was orange-red and the temperature was raised (165ºC) as no product was detected. After 20 h in total TLC was done again but looked very similar to the previous one. The mixture was evaporated with rotary evaporator to get rid of DMF and additionally dried with air pump. The red solid was suspended in water (20 ml) and acidified with concentrated acetic acid (pH = 4). Then it was cooled down with an ice-bath for 30 min. The solid was filtered off and dried in desiccator. NMR spectra were measured, but no product was found. The water filtrate from filtration was extracted with EtOAc (3×40 ml). The obtained brown solution was evaporated and additionally dried in desiccator. NMR spectra were measured, but according to the results starting material and phenol were still present.

Yield = **7%** (crude product)

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 11.89 (s, 2H), 10.87 (s, 1H), 7.56–7.44 (m, 2H), 7.40–7.33 (m, 1H), 7.32–7.25 (m, 2H), 4.22 (s, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.29, 162.97, 151.31, 150.42, 130.39, 126.88, 121.15, 79.36.

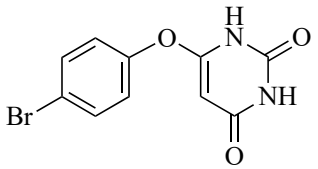
2nd attempt (VLP-2.1a):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1e	1	0.3004	146.53	2.050		
phenol	3	0.5890	94.113	6.258		
K_2CO_3	5	1.4166	138.205	10.249		
DMF			73.095	-	0.948	5.00
VLP-2.1a		0.1754	204.19			

At the beginning the solution was blue-green. After 18 h of reflux the mixture was orange-red. We added dry DMF (2 ml) and stirred under reflux over the weekend. The reaction was terminated after 3 days and evaporated with rotary evaporator. The dark brown solid was additionally dried by air pump. Then it was washed with Et_2O (40 ml), suspended in water (100 ml) and acidified with concentrated acetic acid (pH = 4). Formed precipitate was filtered off and dried in desiccator. Crude product weighed 0.1754 g. NMR spectra of the solid were measured, but it was very impure. The recrystallization from concentrated acetic acid was done. NMR spectra were measured but they did not correspond the expected structure. The solution of acetic acid from filtration was evaporated and NMR spectra were measured, the product was present but it was not pure. Recrystallisation from ethanol was tried but with no success. Purification with diisopropylether was tried but without any success. The evaporated residue was suspended in water and the precipitate filtered off. NMR spectra were measured but the results did not correspond to the desired product.

Yield = **42%** (crude product)

4.3.3 6-(4-bromophenoxy)uracil; 6-(4-bromophenoxy)pyrimidine-2,4(1*H*,3*H*)-dione, VLP-2.3



Mw: 283.08 g/mol

Appearance: brown powder

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1f	1	0.4969	146.53	3.391		
4-bromophenol	3	1.7721	173.02	10.242		
K_2CO_3	5	2.3863	138.205	17.266		
DMF			73.095		0.948	5.00
VLP-2.3		0.4952	283.08			

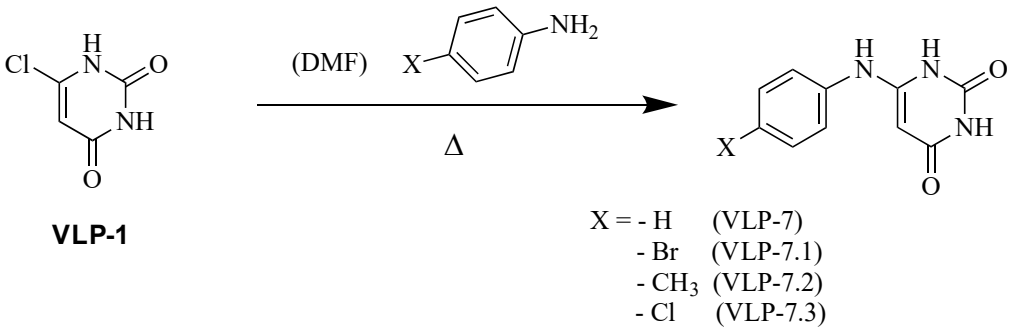
NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 11.90 (s, 1H), 10.95–10.90 (m, 1H), 7.73–7.64 (m, 2H), 7.35–7.25 (m, 2H), 4.31 (d, J = 1.7 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.24, 162.49, 150.67, 150.35, 133.27, 132.01, 123.47, 119.13, 117.53, 79.76.

Yield = **52%**

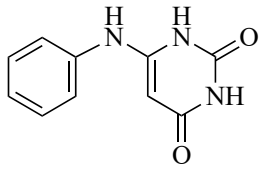
4.4 General procedure for synthesis of 6-phenylaminouracils



Scheme 4. Synthesis of 6-phenylaminouracils

1 eq. of 6-chlorouracil and 20 eq. of aniline or 3 eq. of substituted aniline in presence of DMF (3–5 ml, not in case of aniline) were stirred and heated (160 °C) under argon for 2– 6 h. The mixture was cooled down with an ice-bath and dry MeOH (6 ml) was added and the mixture was stirred at a room temperature (RT) for 15 min. The precipitate was filtered off and washed with Et_2O (2×10 ml) and MeOH (10 ml). The solid was dried with air pump and additionally with oil pump. The structure of the desired product was confirmed using NMR and comparison with the published NMR spectra[52].

4.4.1 6-phenylaminouracil; 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, VLP-7



Mw: 203.07 g/mol

Appearance: white powder

1st attempt (VLP-7):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1f	1	0.5	146.53	3.412		
aniline	20		93.13	66. 339	1.0297	6.00
VLP-7		0.664	203.07			

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 10.45 (s, 1H), 10.16 (s, 1H), 8.25 (s, 1H), 7.42–7.35 (m, 2H), 7.22–7.17 (m, 3H), 7.17–7.13 (m, 1H), 4.69 (d, J = 1.6 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.37, 152.19, 150.86, 137.92, 129.42, 124.66, 122.70, 75.86.

VLP-7 HRMS (ESI$^+$): calculated 204.0773 ($C_{10}H_{10}N_3O_2$), found 204.0777.

LC-MS: $[M+H]^+$ m/z 204 (t_R = 1.20 min). Purity 100%.

Yield = **96%**

2nd attempt (VLP-7a):

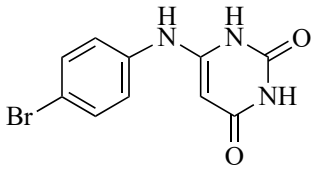
substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1f	1	0.5772	146.53	3.939		
aniline	20		93.13	84.03	1.0297	7.60
VLP-7a		0.6972	203.07			

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 10.45 (s, 1H), 10.16 (s, 1H), 8.26 (s, 1H), 7.43–7.34 (m, 2H), 7.21 (t, J = 1.6 Hz, 1H), 7.20–7.12 (m, 2H), 4.69 (d, J = 1.6 Hz, 1H).

Yield = **87%**

4.4.2 6-(4-bromophenyl)aminouracil; 6-((4-bromophenyl)amino)pyrimidine-2,4(1*H*,3*H*)-dione, VLP-7.1



Mw: 282.1 g/mol

Appearance: pink crystals

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.5014	146.53	3.422		
4-bromoaniline	3	1.6626	172.02	9.665		
DMF			73.095		0.948	5.00
VLP-7.1		0.8637	282.1			

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 11.90 (s, 1H), 11.00–10.87 (m, 1H), 7.79–7.60 (m, 2H), 7.33–7.26 (m, 2H), 4.31 (d, J = 1.7 Hz, 1H).

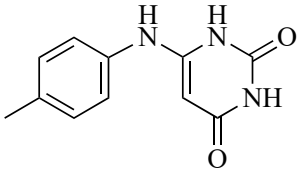
^{13}C NMR (101 MHz, DMSO) δ 164.24, 162.49, 150.67, 150.35, 133.27, 123.47, 79.76.

VLP-7.1 HRMS (ESI$^+$): calculated 281.9878 ($C_{10}H_9BrN_3O_2$), found 281.9876.

LC-MS: $[M+H]^+$ m/z 284 (t_R = 2.04 min). Purity 100%

Yield = **90%**

4.4.3 6-(4-methylphenyl)aminouracil; 6-(*p*-tolylamino)pyrimidine-2,4(1*H*,3*H*)-dione, VLP-7.2



Mw: 217.23 g/mol

Appearance: grey crystals

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.4990	146.53	3.4054		
p-toluidine	3	1.1962	107.16	11.16		
DMF			73.095		0.948	3.00
VLP-7.2		0.6498	217.23			

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 10.40 (s, 1H), 10.11 (s, 1H), 8.13 (s, 1H), 7.26–7.16 (m, 2H), 7.13–7.03 (m, 2H), 4.59 (s, 1H), 2.28 (s, 3H).

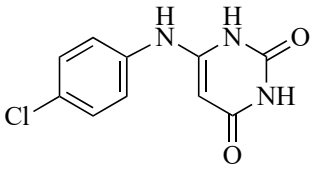
^{13}C NMR (101 MHz, DMSO) δ 164.38, 152.58, 150.89, 135.14, 134.19, 129.87, 123.14, 75.33, 20.48.

VLP-7.2 HRMS (ESI$^+$): calculated 218.0930 ($C_{11}H_{12}N_3O_2$), found 218.0933.

LC-MS: $[M+H]^+$ m/z 218 (t_R = 1.71 min). Purity 100%.

Yield = **88%**

4.4.4 6-(4-chlorophenyl)aminouracil; 6-((4-chlorophenyl)amino)pyrimidine-2,4(1*H*,3*H*)-dione, VLP-7.3



Mw: 237.64 g/mol

Appearance: yellow crystals

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-1	1	0.5028	146.53	3.431		
4-chloroaniline	3	1.3108	127.545	10.27		
DMF			73.095		0.948	5.00
VLP-7.2		0.6521	237.64			

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 10.50 (s, 1H), 10.27 (s, 1H), 8.39 (s, 1H), 7.46–7.38 (m, 2H), 7.26–7.18 (m, 2H), 4.71 (d, J = 1.3 Hz, 1H).

^{13}C NMR (101 MHz, DMSO) δ 164.34, 151.91, 150.89, 137.13, 129.30, 128.25, 124.11, 76.59.

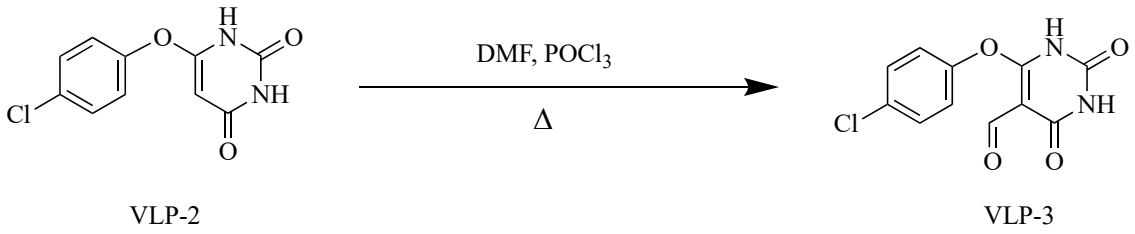
VLP-7.3HRMS (ESI^{+}): calculated 238.0383 ($C_{10}H_9ClN_3O_2$), found 238.0384.

LC-MS: $[M+H]^{+}$ m/z 238 (t_R = 1.88 min). Purity 100%.

Yield = **80%**

4.5 Formylation, acylation and alkylation of 6-phenoxyuracils and 6- phenylaminouracils at position 5

4.5.1 Reaction with Vilsmeier reagent



VLP-2 VLP-3

Scheme 5. Formylation of 6-(4-chlorophenoxy)uracil with Vilsmeier reagent

1st attempt (VLP-3):

substance	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-2	0.02	238.63	0.0838		
$POCl_3$		155.33	1.694	1.645	0.016
DMF		73.095		0.948	5.00
VLP-3	0	266.64			

DMF (2 ml) and $POCl_3$ (16 µl) were added into flask. DMF (2 ml) was used to dissolve VLP-2 and added to the mixture of DMF and $POCl_3$. Then, DMF (1 ml) was used to get the residue of VLP-2 into flask. The mixture of DMF, $POCl_3$ and VLP-2 was then stirred and refluxed (150 °C) for 2 h. The solution was yellow at the beginning, then turned to orange, when the temperature lowered it was slightly pink. The solution was then poured into ice water (40 ml) and the precipitate was filtered off. The pinkish powder was dried

overnight. NMR spectra were measured, the solid was not soluble in $CDCl_3$ but it was dissolving in DMSO.

NMR spectra:
^{1}H NMR (400 MHz, DMSO) δ 11.90 (s, 1H), 10.93 (s, 1H), 7.60–7.51 (m, 2H), 7.40– 7.31 (m, 2H), 4.30 (d, J = 1.7 Hz, 1H).
^{13}C NMR (101 MHz, DMSO) δ 164.20, 162.56, 150.22 (d, J = 19.5 Hz), 130.90, 130.28, 123.07, 79.66.

Mass spectrum was measured with the majority peak (239.1 g/mol) on positive side. For the negative side there were peaks 237.1 g/mol and 239.1 g/mol detected which could correspond to pattern of chlorine. We conclude that the desired product was not resulted.

Yield = **0%**

2nd attempt (VLP-3a):

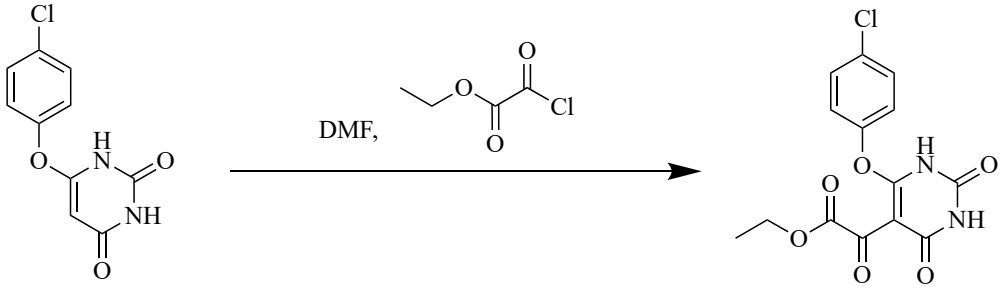
Substance	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-2	0.1737	238.63	0.7279		
$POCl_3$		155.33	1.482	1.645	0.14
DMF		73.095		0.948	5.00
VLP-3a	0	266.64			

DMF and $POCl_3$ were put into flask. The solution was yellow after adding VLP-2 it turned to orange-brown. The mixture was stirred and refluxed (160 °C) for 2 h, then TLC_1 was done. Starting material was still present, it was stirred and refluxed for other 2 h and temperature was lowered (90 °C). TLC_{2-3} was done again. Upon the results small sample of reaction mixture was poured into iced water and no precipitate resulted. Then the water phase was extracted with EtOAc. Starting material was still present in EtOAc phase so $POCl_3$ was added (0.14 ml) and the mixture was stirred and refluxed overnight (22 h). After 26 h in total, the reaction was terminated. The mixture was suspended in ice water (10 ml). No precipitates were observed so the water was filtered and some dark brown solid remained on the filtration paper. The solid was collected but the amount was too small for NMR testing. TLC_6 was done with residual water from the filtration. Then we extracted it with EtOAc (3×80 ml). During the extraction, the interface was not

completely clear. There were some brown spots. The solution of EtOAc was extracted again with water (20 ml) to get rid of residual DMF, dried with anhydrous sodium sulfate and filtered off. The solvent was evaporated and put to dry by air pump over the weekend. NMR spectrum did not confirm the desired product.

Yield = **0%**

4.5.2 Acylation with ethyl chlorooxoacetate



VLP-2 VLP-6

Scheme 6. Acylation of 6-(4-chlorophenoxy)uracil with ethyl chlorooxoacetate

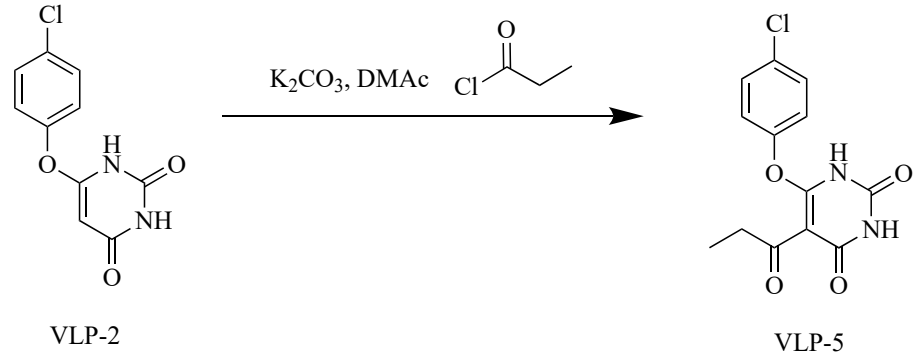
substance	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-2	0.0700	238.63	0.2933		
ethyl chlorooxoacetate	0.0400	153.33	0.2896	1.22	0.033
DMF		73.095		0.948	3.00
VLP-6	0.0418	266.64			

DMF (3 ml) was used to dissolve the VLP-2. Then, ethyl chlorooxoacetate was added. The mixture was stirred and heated (60 °C) for 30 mins. TLC was done and acetone (3 ml) was added. The solution was then stirred and heated (60 °C) for another 1 h. TLC was done but no spots were visible, except for one on the base line. The mixture was evaporated in vacuo and additionally dried in desiccator overnight. NMR spectra were measured for the solid but it was not pure. We tested several mobile phases with NMR sample (DMSO, each TLC plate was dried with hairdryer and then dried by air pump.): Hexane- EtOAc (1:1) and Hexane-EtOAc (3:7), but the spots did not moved at all. The spots moved in mobile phases: DCM-MeOH (9:1), EtOAc-EtOH (1:3), DCM-MeOH (3:7). The sample was then purified with FCC. Cartridge: Sfar Silica D Duo 25g, normal phase, gradient elution with solvents DCM-MeOH (3–30%). Fractions containing the

same compound were combined, evaporated and dried. NMR spectra were measured using DMSO (not soluble in $CDCl_3$). The fraction $F_{16\text{-}26}$ had light red colour and was not completely pure. The sample was purified by FCC again. Cartridge: Sfar Silica D Duo 5g, normal phase, gradient elution, solvent DCM-MeOH (2–40%). FII(2-4) was evaporated, dried and the NMR spectra were measured. The product was visible but it still was not pure m = 14.8 mg. The residue was too small for further purification.

4.5.3 Acylation with acyl chlorides

4.5.3.1 Acylation of 6-(4-chlorophenoxy)uracil with propionyl chloride



VLP-2 VLP-5

Scheme 7. Acylation of 6-(4-chlorophenoxy)uracil with propionyl chloride

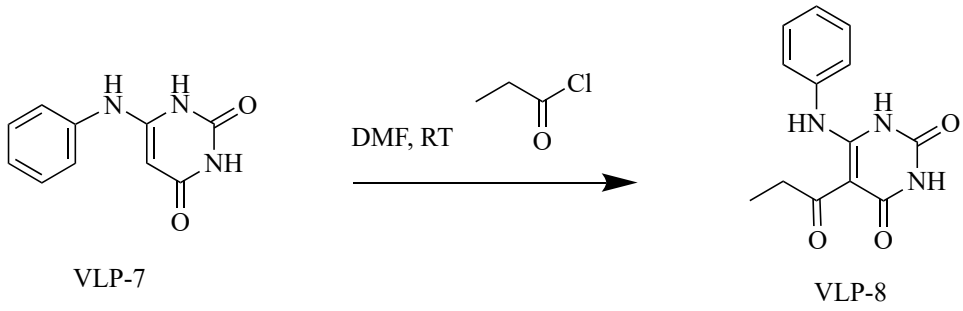
substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-2	1	0.1080	238.63	0.4191		
propionyl chloride	3	0.0575	92.52	1.5649	1.0646	0.136
K_2CO_3	2	0.1166	138.205	0.8437		
DMAc			87.122		0.937	4.00
VLP-5		0	294.69			

The mixture of VLP-2, potassium carbonate, propionyl chloride (0.027 ml) and DMAc was stirred at RT. After 4 h, propionyl chloride (0.027 ml) and potassium carbonate (0.033 g) were added. After 14 h in total, propionyl chloride (0.012 ml) was added and mixture was stirred and reaction temperature was raised (60 °C). After 17 h in total TLC was conducted. The starting material was still visible. Mass spectra were measured with detection of mass possibly corresponding to the product (292.9 g/mol and 292.8 g/mol on positive side). The mixture was let to react further, temperature was raised (100 °C) and propionyl chloride (0.007 ml) was added. After 19 h TLC was conducted again with the still visible starting material. The mixture was let to stir and react over the weekend. TLC

was conducted and mass spectra were measured (highest peak: 268.2 and 271.2 g/mol). The solution was poured into the ice water (20 ml). The precipitate was formed, pH of the solution was 1. The brown solid was filtered off and dried by air pump. NMR spectra were measured and the results corresponded to the starting material.

Yield = **0%**

4.5.3.2 Acylation of 6-phenylaminouracil with propionyl chloride



VLP-7 VLP-8

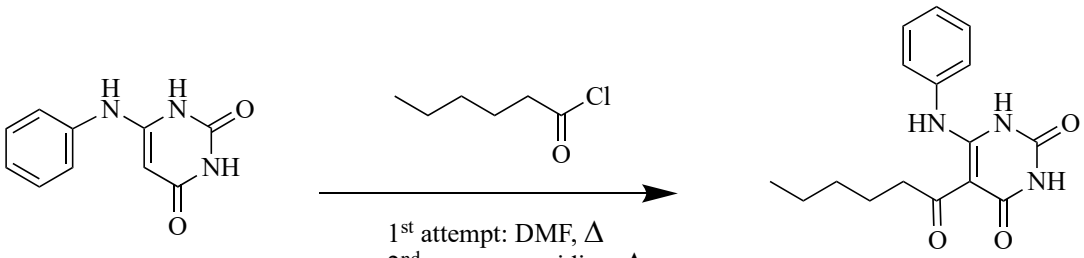
Scheme 8. Acylation of 6-phenylaminouracil with propionyl chloride

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-7	1	0.2084	203.05	1.026		
propionyl chloride	1.5		92.52	1.421	1.0646	0.14
DMF			73.095		0.948	3.00
VLP-8		0	259.27			

VLP-7 was dissolved in dry DMF. Then, propionyl chloride was added and the white suspension was let to stir. After 1 h TLC_1 was conducted and mass spectra were measured and the desired product was detected (results: 259.1 g/mol, 260.1 g/mol on positive side). The suspension was filtered off and yellow liquid was evaporated with rotary evaporator. Then it was additionally dried by air pump. NMR spectra were measured but results showed it was a mixture. We attempted to crystalize the product with chloroform, but unsuccessfully. The product was not dissolving neither acetone, DCM, MeOH nor THF. It was partly soluble in ACN. There was some yellow solid, ASAP was performed for the solution of ACN with positive results (exact mass of product: 259.01g/mol, mass detected 259.1; 260. g/mol). The suspension was filtered off. The TLC of solution was done. Next day the solid was dissolved by ACN but it was not soluble anymore. The solid was filtered off and ASAP and mass spectra were measured. With ASAP we detected the product

(259.1; 260.1 g/mol) but with TLC mass was 287 g/mol. The solution was purified by FCC: Cartridge Sfar Silica D Duo 10g, normal phase, gradient elution with solvents: MeOH-DCM 2–30%. F(2-4) was too small to measure NMR spectra.

4.5.3.3 Acylation of 6-phenylaminouracil with hexanoyl chloride



VLP-7 VLP-8.1

Scheme 9. Acylation of 6-phenylaminouracil with hexanoyl chloride

1st attempt (VLP-8.1):

substance	eq.	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-7	1	0.2064	203.05			
hexanoyl chloride	1.5		92.52	1.421	1.0646	0.23
DMF			73.095	-	0.948	3.00
VLP-8.1			301.35			

VLP-7 was dissolved in dry DMF. Then hexanoyl chloride was added and the mixture was stirred for 2 h. According to the TLC_1 results, the starting material was still visible, so the mixture was heated (60 °C). After another 2 h temperature was raised again (100 °C). The reaction was terminated after 6 h in total and was evaporated with rotary evaporator. Then, it was suspended in water and the solid was filtered off. NMR spectra were measured and results showed a lot of other impurities. The solid was attempted to be dissolved in DCM (more soluble) and EtOAc (less soluble), but unsuccessfully. TLC was conducted from both solutions. Mass spectra were measured with sample of EtOAc solution (with detection of mass: 301 g/mol, which corresponded to the desired product – exact mass = 301.14 g/mol). NH_2 modified silica TLC plate was used for TLC performance to compare the separation with usual silica TLC plate. Firstly, DCM-MeOH 5% was used but even after several (three times) goes the spots were together and moved only slightly. The yellow solid was dissolved in DMF for FCC. Mass spectra from NH_2

modified silica TLC plate were measured and the mass corresponded to the product (302.14 g/mol). FCC: Cartridge Sfar KP Amino D 11g, normal phase, solvents: MeOH- DCM 3–30%. F(1): was evaporated and NMR were measured. Product was detected but it was not pure (m = 0.0057 g).

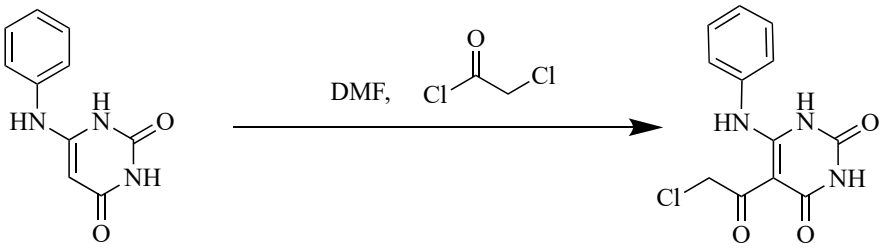
2nd attempt (VLP-8.1a):

substance	eq.	m (g)	M (g/mol)	n (mmol)	d	V (ml)
VLP-7	1	0.1080	203.05	0.5319		
hexanoyl chloride	2		134.60	1.1768	0.9900	0.16
pyridine			79.10		0.982	1.00
VLP-8.1a			301.35			

To the mixture of VLP-7 and pyridine, hexanoyl chloride was added while stirring. The mixture was stirred for 2 h at 90 °C and then it was evaporated with rotary evaporator with heptane to get rid of pyridine entirely. The solid was then suspended in the water (3 ml) and filtered, pH of mixture was 6. Crystallization from ethanol was not successful, so the solid was collected and dried in desiccator. NMR spectra were measured, revealing the starting material.

Yield = **0%**

4.5.3.4 Acylation of 6-phenylaminouracil with chloroacetyl chloride



VLP-7 VLP-10

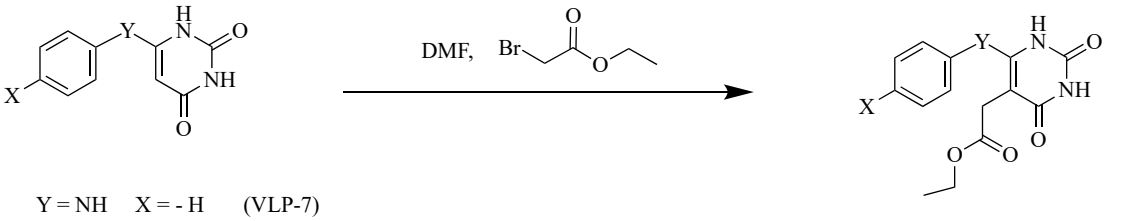
Scheme 10. Acylation of 6-phenylaminouracil with chloroacetyl chloride

substance	eq.	m (g)	M (g/mol)	n (mmol)	d	V (ml)
VLP-7	1	0.1258	203.05	0.6196		
chloroacetyl chloride	3		112.94	2.012	1.42	0.16
DMF			73.095		0.948	1.00
VLP-10		0	279.68			

VLP-7, chloroacetyl chloride and DMF were stirred at RT for 30 mins. The mixture was then poured into acetone (25 ml) and stirred for another 30 mins. The precipitate was filtered off and dried. NMR was measured and results revealed starting material.

Yield = **0%**

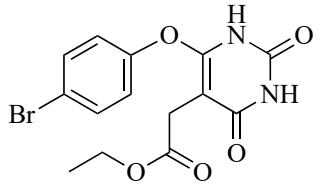
4.5.4 General procedure for alkylation of 6-phenylaminouracils and 6- phenoxyuracils with ethyl bromoacetate



Scheme 11. General procedure for alkylation of 6-phenylaminouracils and 6-phenoxyuracils

To 1 eq. of 6-(4-subst.)phenylaminouracil or 6-phenoxyuracil, dry DMF (3–5 ml) was added and then 2 eq. of ethyl bromoacetate were added. The mixture was stirred and heated (160 ºC). The reaction was ended once the starting material was not visible anymore or there were no changes on TLC plates. The mixture was evaporated with rotary evaporator and additionally dried by air pump. The dried mixture was then suspended in water (40 ml) which resulted in formation of solid of amorphous consistency. The extraction was then performed with EtOAc (5×40ml). The EtOAc solution was washed with brine (60 ml) and dried with anhydrous sodium sulfate. The suspension was filtered and then the solution was evaporated. To purify the product FCC was performed and structure then verified with NMR.

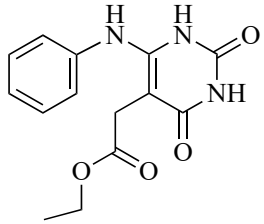
4.5.4.1 Ethyl 2-(6-(4-bromophenoxy)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5- yl)acetate (VLP-14)



substance	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-2.3	0.2731	283.03	0.9647		
ethyl bromoacetate		167.002	1.989	1.51	0.22
DMF		73.095		0.948	5.00
VLP-14		368.17			

Yield = **0%**

4.5.4.2 Ethyl 2-(2,4-dioxo-6-phenylamino-1,2,3,4-tetrahydropyrimidin-5-yl)acetate (VLP-9)



Mw: 289.29 g/mol

Appearance: yellow crystals

substance	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-7	0.2384	203.07	1.005		
ethyl bromoacetate		167.002	1.989	1.51	0.22
ACN		73.095		0.948	2.50
VLP-9		289.29			

Purification FCC: Cartridge Sfar Silica D Duo 10g, normal phase, gradient elution with solvents DCM-MeOH 3–40%.

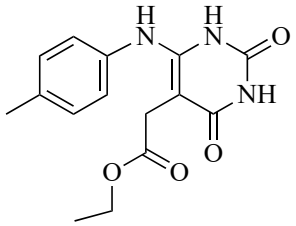
NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 10.71 (s, 1H), 10.50–10.26 (m, 1H), 8.41 (s, 1H), 7.34–7.26 (m, 2H), 7.07–6.96 (m, 3H), 4.00 (q, J = 7.1 Hz, 2H), 3.26 (s, 2H), 1.14 (t, J = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, DMSO) δ 164.58, 150.43, 148.81, 139.84, 129.14, 122.94, 120.82, 87.72, 59.98, 28.76, 14.09.

VLP-9 HRMS (ESI^{+}): calculated 290.1141 ($C_{14}H_{16}N_3O_4$), found 290.1144.
LC-MS: $[M+H]^{+}$ m/z 290 (t_R = 1.85 min). Purity 100%.

Yield = **3%**

4.5.4.3 Ethyl 2-(2,4-dioxo-6-(*p*-tolylamino)-1,2,3,4-tetrahydropyrimidin-5- yl)acetate (VLP-11)



Mw: 303.32 g/mol

Appearance: yellow crystals

substance	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-7.2	0.2839	282.10	1.0064		
ethyl bromoacetate		167.002	1.989	1.51	0.22
DMF		73.095		0.948	5
VLP-11	0.00381	303.32			

1st purification FCC: Cartridge Sfar Silica D Duo 50g, normal phase, gradient elution with solvents DCM-MeOH 3–25%.
2nd purification FCC: Cartridge Sfar Silica D Duo 25g, normal phase, gradient elution with solvents DCM-MeOH 3–18%.

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 10.63 (s, 1H), 10.17 (s, 1H), 8.32 (s, 1H), 7.16 – 7.09 (m, 2H), 6.94 – 6.88 (m, 2H), 4.00 (q, J = 7.1 Hz, 2H), 3.25 (s, 2H), 1.14 (t, J = 7.1 Hz, 3H).

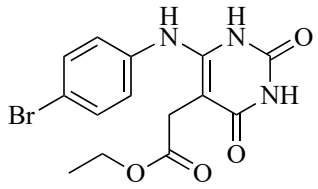
^{13}C NMR (101 MHz, DMSO) δ 171.47, 164.95, 150.82, 149.70, 137.19, 133.03, 130.08, 122.28, 86.60, 60.40, 29.13, 20.88, 14.57.

VLP-11 HRMS (ESI^{+}): calculated 304.1297 ($C_{15}H_{18}N_3O_4$), found 304.1297. LC-MS: $[M+H]^{+}$ m/z 304 (t_R = 2.33 min). Purity >96%.

One proton signal (for -CH_3) is missing in ^{1}H NMR, but mass spectrum corresponded to the desired product.

Yield = **1%**

4.5.4.4 Ethyl 2-{6-[(4-bromophenyl)amino]-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl}acetate (VLP-12)



Mw: 368.19 mol/mol
Appearance: yellow crystals

substance	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-7.1	0.2839	282.10	1.0064		
ethyl bromoacetate		167.002	1.989	1.51	0.22
DMF		73.095		0.948	5.00
VLP-12	0.00194	368.19			

1st purification FCC: Cartridge Sfar Silica D Duo 25g, normal phase, gradient elution with solvents DCM-MeOH 3–35%.

2nd purification FCC: Cartridge SNAP Ultra 25g, normal phase, gradeint elution with solvents DCM-MeOH 5–20%.

NMR spectra:

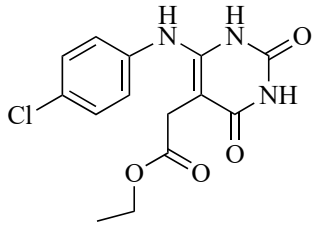
^{1}H NMR (400 MHz, DMSO) δ 10.75 (s, 1H), 10.55 (s, 1H), 8.51 (s, 1H), 7.50–7.42 (m, 2H), 6.97–6.89 (m, 2H), 4.00 (q, J = 7.1 Hz, 2H), 3.26 (s, 2H), 1.15 (q, J = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 171.33, 165.02, 150.96, 148.90, 139.99, 132.29, 122.99, 114.90, 89.17, 60.50, 29.22, 14.55.

VLP-12 HRMS (ESI^{+}): calculated 368.0246 ($C_{14}H_{15}BrN_3O_4$), found 368.0246. LC-MS: $[M+H]^{+}$ m/z 370 (t_R = 2.68 min). Purity >94%.

Yield = **0.5%**

4.5.4.5 Ethyl 2-(6-((4-chlorophenyl)amino)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acetate (VLP-13)



Mw: 323.73 g/mol

Appearance: yellow crystals

substance	m (g)	M_w (g/mol)	n (mmol)	d (g/cm^3)	V (ml)
VLP-7.3	0.2384	237.64	1.003		
ethyl bromoacetate		167.002	1.989	1.51	0.22
DMF		73.095		0.948	3
VLP-13	0.00155	323.73			

1st purification FCC: Cartridge SNAP KP-Sil 100g, normal phase, graduent elution with solvents DCM-MeOH 3–20%.

2nd purification FCC: Cartridge Sfar Duo 25g, normal phase, isocratic elution with solvents Hexane-EtOAc (4:6) with 1% of acetic acid

NMR spectra:

^{1}H NMR (400 MHz, DMSO) δ 10.75 (s, 1H), 10.55 (s, 1H), 8.51 (s, 1H), 7.50–7.42 (m, 2H), 6.97–6.89 (m, 2H), 4.00 (q, J = 7.1 Hz, 2H), 3.26 (s, 2H), 1.15 (q, J = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 171.33, 165.02, 150.96, 148.90, 139.99, 132.29, 122.99, 114.90, 89.17, 60.50, 29.22, 14.55.

VLP-13 HRMS (ESI^{+}): calculated 324.0751 ($C_{14}H_{15}ClN_3O_4$), found 324.0752.

LC-MS: $[M+H]^{+}$ m/z 324 (t_R = 2.53 min). Purity 100%.

Yield = **0.5 %**

5. DISCUSSION

5.1. Synthesis of starting material: 6-chlorouracil (VLP-1)

Even though the first step was a simple reaction and it was published in many articles with high yield (up to 71–98%) it did not go as smoothly as predicted (Tab. 1).

Tab.2 Summary of all syntheses of 6-chlorouracil with yields

	time of reflux	pH of the work up*	yield
1st attempt	16 h	~2–3	80%
2nd attempt	15 h	~1–2	83%
3rd attempt	1h	~2–3	25%
4th attempt	15 h	~2–3	37%
5th attempt	16 h	~1–2	77%
6th attempt	16 h	~1–2	80%
7th attempt	16 h	~1–2	74%

* - pH during the acidification with 2 M HCl

After the first successful attempt (yield: 80%) that was done following the article by R. M. Cresswell and H. C. Wood who used 6-chlorouracil as intermediate to synthesize riboflavins[53], the next attempts did not bring the correct ^{1}H-NMR spectra. One signal of N-H was missing or there were three signals (see Appendix 1), which did not correspond to NMR spectrum of 6- chlorouracil. It was suspected that the reaction was not completed and the white solid was an intermediate. Nevertheless, the ^{13}C-NMR spectra (see Appendix 2) contained four signals so it indicated that it should be the correct product. In the third attempt, procedure from another article[52] was used. It had a shorter time of refluxing and specified pH during work up (pH = 2–3). The yield was rather low (25%). The reason was the most probably because of the short time of reflux. With another attempt the mixture was refluxed overnight but ^{1}H-NMR spectrum missed one signal. In meanwhile VLP-1 that was synthetized in the first experiment and its NMR-spectra corresponded, was used in the reaction with 4- chlorophenol in multiple attempts and some products (VLP-2, 6- (4- chlorophenoxy)uracil) also missed one signal N-H (see Appendix 3). In addition, similarly to VLP -1, ^{13}C-NMR spectra did correspond to ones of VLP- 2 (see Appendix 4). That proved that the white solids whose NMR spectra were

missing one signal were our desired product because for the second reaction we used only VLP-1 with NMR spectra that corresponded to our desired product. It was concluded that the different spectra were either hydrate of VLP-1 given its polar nature or have some relation to pH. Through retrospection of all experiments, pH was the most probable cause of missing/additional signals in the spectra of the products, as after the filtration the product was washed with distillated water and its amount varied. Therefore, acidification of the product that did not have corresponding ^{1}H-NMR spectrum was done and after drying the spectra corresponded. It is also noteworthy to mention the binding of water to the compound and its' relation to the broadness of second N-H peak. It seems that with the lower amount of water, the peak was more pointed. This peak is usually described as broad and in a particular study[54] its phenomenon was described to be bigger with higher concentration of acetate ionic liquid. Nevertheless, it should be also noted that DMSO was used as solvent in the experiment and given its nature to bind water it could affect the broadness of the peak in addition that the peaks of the water were not included in results of the mentioned study. With this revelation, the following work ups were done with acidified distillated water (pH = 3) that worked very well. The ^{1}H-NMR spectra in Appendix 1, VLP-1d after acidification, were measured after drying with air pump over weekend and even then the water signal was very high so it is recommendable using oil pump in combination with desiccator for effective drying.

5.2. Condensation of 6-chlorouracil with phenols and anilines

In general, there were many experiments done using 6-phenylthiouracils and 6- phenoxyaminouracils. The most known 6-(arylsulfanyl)uracil derivative is HEPT described above. As for the 6-phenylaminouracils, some of them have antibacterial properties[50] or they are used to synthetize 5- deazaflavins[52,55]. In contrast there were not many references to the synthesis of 6- phenoxyuracils, except for one article by Yoneda[51] that described synthesis of several 6-phenoxyuracils as intermediates to prepare 10-oxo-5-deazaflavins. If we compare the yields of syntheses of 6-phenylaminouracils (around 85% and more) and 6- phenoxyuracils (around 7–50%), the experiments with anilines were more successful as they are stronger nucleophiles.

5.2.1 Synthesis of 6-phenoxyuracils

In overall, the condensation with 4-bromophenol gave the highest yield (around 52 %), it can be explained that 4-bromophenol is stronger nucleophile that 4- chlorophenol. The other reactions afforded quite low yields and condensation with non-substituted phenol did not proceed as successfully (experimental yield 7%) as reported in the article by Yoneda with the yield 53%.

5.2.1.1. Synthesis of 6-(4-chlorophenoxy)uracil

As our main interest was preparation of 6-(4-chlorophenoxy)uracil derivatives, we tried to optimize the procedure to get higher yield as it was around 10% only. (Tab. 2)

Tab. 3 Optimization of condensation of 6-chlorouracil with 4-chlorophenol

	Base	eq.	Solvent	work up	yield
VLP-2	K_2CO_3	5	DMF	filtration	13%
VLP-2a (!)	K_2CO_3	12.5	DMF	extraction	9%
VLP-2b	K_2CO_3	12.5	THF	filtration	0%
VLP-2b2	K_2CO_3	12.5	DMF	extraction	0%
VLP-2c	K_2CO_3	12.5	DMF	filtration	7%
VLP-2d*	K_2CO_3	5	DMF dry	filtration	9%
VLP-2e*	K_2CO_3	5	DMF dry	filtration	23%
VLP-2f	K_2CO_3	5	DMF dry	filtration	11%
VLP-2g	K_2CO_3	5	DMF dry	filtration	13%
VLP-2h	K_2CO_3	5	DIPEA	filtration	0%
VLP-2l	*t*-BuOK	3.3	DMF	filtration	0%

(!) – flask broke during work up
* - pure 6-chlorouracil was used as starting material

We tried to change the amount of potassium carbonate so it would correspond to ratio that was described in the article from F. Yoneda[51]. Nevertheless, the yields were even lower in comparison with the case, when the ratio 1:3:5 (VLP-1, 4-chlorophenol and potassium carbonate) was used. From the reason, that DMF is rather difficult to evaporate with rotary evaporator, use of different reaction solvent was tested, but without any success. In addition we used the procedure from the article in ref.[56] that had very good

yield (around 90%) using microwave irradiation to condensate 1,3-dimethyl-6- chlorouracil and 6-chlorouracil with various nucleophiles. For 6-chlorouracil and phenol, the authors used NMP as solvent with 0% yield. For this reaction we decided to use dry DMF as solvent, but without any success. Due to lack on time, more experiments with variable conditions weren't accomplished, therefore it can be one of proposals for further investigation as the mixture is sort of suspension that needs a lot of time for refluxing and the stirring needs to be more intensive.

5.2.2 Synthesis of 6-phenylaminouracils

For the synthesis of 6-phenylaminouracils procedures from two articles affording very good results (yield around 90%)[52,57] were used. In article from J. Daďová[57] condensation was done under nitrogen atmosphere. The mixture was then cooled down and MeOH was added. The precipitate was then filtrated and washed with Et_2O and MeOH. In contrast to article from J. M. Wilson[52] where work up was to cool the mixture, add Et_2O and sonicated it. The suspension was then filtrated and washed with water, MeOH and Et_2O. In our experiments we let the mixture to react under argon. For synthesis of 6- phenylaminouracil we used equivalents from article J. Daďová but for the condensation with substituted anilines we used ratio from article J.M. Wilson. As aniline is liquid but other substituted anilines were solids, DMF was used as solvent to ensure better homogenization and avoiding sonification. For the work up we used procedure from J. Daďová to avoid work up with water because of compounds' high tendency to bind water. In overall condensation with anilines were much more feasible and with very nice yield (more than 90%) . It is important to highlight the importance of using either oil pump or desiccator for long time as the compounds tends to bind water a lot.

5.3. Formylation, acylation and alkylation of 6-phenoxyuracils and 6- phenylaminouracils

The article by F. Yoneda[51] described formylation of the position 5 in uracil using Vilsmeier reagent. The products could be good intermediates for preparation of many derivatives, so we carried out two attempts. The reaction did not turn out to be successful for the first time. In the second attempt, we raised the reaction temperature, so it was suspected that it was overdone and the possible product was decomposed as the reaction time was also long. In addition, it was reported in the article that the product is not stable

and cannot be purified by crystallization, so the focus was shifted to other ways to introduce substitution in position 5 of uracil.

Acylation reactions in position 5 were carried out with acyl chlorides (propionyl chloride, hexanoyl chloride, chloroacetyl chloride[58] and ethyl chloroxoacetate). First attempt with 6-(4-chlorophenoxy)uracil in DMAc was not successful but it is mostly suspected that reaction went over as it was let to react over weekend. The acylation reactions of 6-phenylaminouracil with propionyl chloride or hexanoyl chloride in DMF were concluded as unsuccessful, but as the mass spectra of the desired product were detected. The most probable reason for the failure is use of not optimal mobile phase for FCC. Reaction with ethyl chloroxoacetate[59] was tested but due to purification using FCC a lot of product was lost and the optimization of FCC conditions remains the challenge for future work.

Alkylation with ethyl bromoacetate[60,61] was the most successful approach. Using ACN as solvent did not bring any product, but using DMF brought positive results. The problematic step is still work up that needs to be improved. As described above, during the suspension of evaporated reaction mixture in the water, “amorphous” solid was formed and extraction with EtOAc or DCM was not optimal neither as the emulsion was misty so the extraction was difficult. A lot of product was lost during purification with FCC, the mobile phase composition also needs optimization as the purification required more than one FCC. Overall alkylation of 6-phenylaminouracils gave the highest yield (2%), surprisingly in case of 6-(4-methylphenyl)aminouracil we obtained lower yields than expected and halogen-substituted 6- phenylaminouracils were the least reactive starting materials in our series. Mass spectra were measured for all four final products verifying the product and purity was tested.

6. CONCLUSION

In this thesis we focused on obtaining of new 5,6-disubstituted uracil derivatives that required three-step synthesis. (Fig. 20)

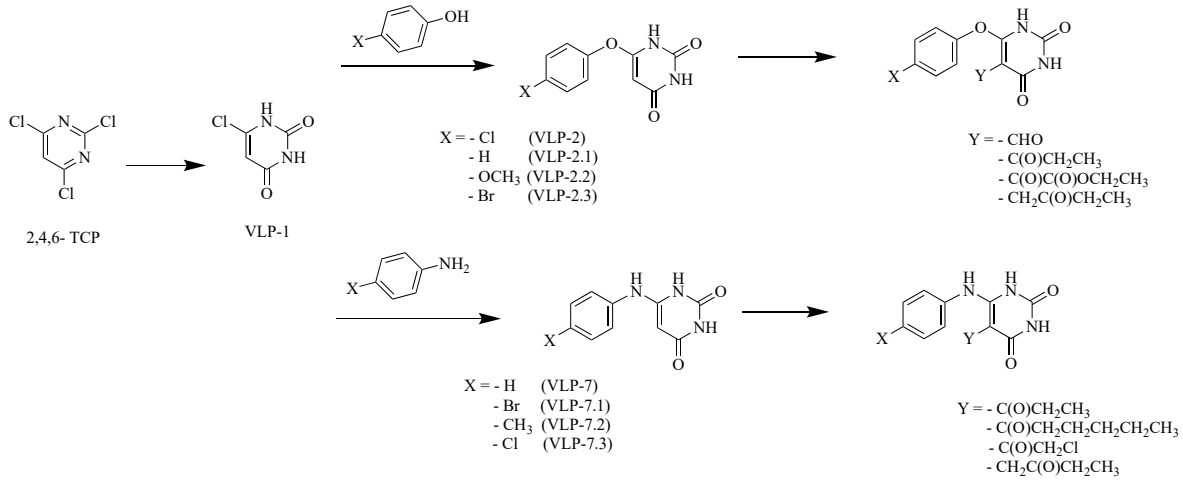


Fig. 22 Scheme of synthesis of 5,6-disubstituted uracils

In the first step 6-chlorouracil from 2,4,6-trichloropyrimidine was prepared. In the second step condensation with phenols and anilines was performed. During the first and the second step we found interesting effects of pH during work up and the amount of water that had on NMR spectra of uracil compounds. We tried to optimize the condensation with phenols as their yields were rather low in comparison to article F. Yoneda[51] and in addition they don't have any other references than this one. Two new 6-phenoxyuracils were prepared:

-6- (4- chlorophenoxy)uracil
- 6-(4-bromophenoxy)uracil

In the third step, experiments were carried out to substitute position 5 of prepared 6-phenoxyuracils and 6-phenylaminouracils. Firstly, formylation with Vilsmeier reagent was tested described also in article by F. Yoneda. It did not go well, therefore alkylation and acylation was carried out with: propionyl chloride, hexanoyl chloride, ethyl chlorooxoacetate, ethyl bromoacetate and chloroacetyl chloride.

Reaction with ethyl bromoacetate brought four new molecules:

- ethyl 2-(2,4-dioxo-6-phenylamino-1,2,3,4-tetrahydropyrimidin-5-yl)acetate (VLP-9)
- ethyl 2-{6-[(4-bromophenyl)amino]-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5- yl}acetate (VLP-12),
- ethyl 2-[2,4-dioxo-6-(*p*-tolylamino)-1,2,3,4-tetrahydropyrimidin-5-yl]acetate (VLP-11)
- ethyl 2-{6-[(4-chlorophenylamino]-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5- yl}acetate (VLP-13)

Their mass spectra were measured with HRMS and purity was tested with LC-MS revealing good purity (more than 94%) for future testing for biological activity in the Division of Pharmaceutical Chemistry and Technology at University of Helsinki.

The experimental work in overall was challenging given poor solubility of compounds in other solvents than DMSO and DMF (most suitable solvent for reactions with our compounds), pH sensitivity, water binding tendency and their hard purification. For further research it is recommended to improve the work up method and to find optimal mobile phase for FCC that could lead to higher yields.

7. REFERENCES:

(1) Pałasz, A.; Cież, D. In Search of Uracil Derivatives as Bioactive Agents. Uracils and Fused Uracils: Synthesis, Biological Activity and Applications. *Eur. J. Med. Chem.* **2015**, *97*, 582–611. https://doi.org/10.1016/j.ejmech.2014.10.008.

(2) Anderson, C. M.; Parkinson, F. E. Potential Signalling Roles for Utp and Udp: Sources, Regulation and Release of Uracil Nucleotides. *Trends Pharmacol. Sci.* **1997**, *18* (10), 387–392. https://doi.org/10.1016/S0165-6147(97)01106-1.

(3) Brown, D. J. 2.13 - Pyrimidines and Their Benzo Derivatives. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, 1984; pp 57–155. https://doi.org/10.1016/B978-008096519-2.00035-7.

(4) Cassis, R.; Tapia, R.; Valderrama, J. A. New Synthesis of Uracil and 2-Thiouracil. *Synth. Commun.* **2006**. https://doi.org/10.1080/00397918408063767.

(5) Raczyńska, E. D.; Zientara, K.; Stepniewski, T. M.; Kolczynska, K. Stability, Polarity, Intramolecular Interactions and Π-Electron Delocalization for All Eighteen Tautomers Rotamers of Uracil. Dft Studies in the Gas Phase. *Collect. Czechoslov. Chem. Commun.* **2009**, *74* (1), 57–72. https://doi.org/10.1135/cccc2008149.

(6) Raczyńska, E. D.; Zientara, K.; Kolczyńska, K.; Stępniewski, T. Change of Tautomeric Equilibria, Intramolecular Interactions and π-Electron Delocalization When Going from Phenol to Uracil. *J. Mol. Struct. THEOCHEM* **2009**, *894* (1), 103–111. https://doi.org/10.1016/j.theochem.2008.10.025.

(7) Miller, T. M.; Arnold, S. T.; Viggiano, A. A.; Stevens Miller, A. E. Acidity of a Nucleotide Base: Uracil. *J. Phys. Chem. A* **2004**, *108* (16), 3439–3446. https://doi.org/10.1021/jp037367l.

(8) Wolken, J. K.; Tureček, F. Proton Affinity of Uracil. A Computational Study of Protonation Sites. *J. Am. Soc. Mass Spectrom.* **2000**, *11* (12), 1065–1071. https://doi.org/10.1021/jasms.8b01424.

(9) Whittleton, S. R.; Hunter, K. C.; Wetmore, S. D. Effects of Hydrogen Bonding on the Acidity of Uracil Derivatives. *J. Phys. Chem. A* **2004**, *108* (38), 7709–7718. https://doi.org/10.1021/jp048318r.

(10) Kurinovich, M. A.; Lee, J. K. The Acidity of Uracil and Uracil Analogs in the Gas Phase: Four Surprisingly Acidic Sites and Biological Implications. *J. Am. Soc. Mass Spectrom.* **2002**, *13* (8), 985–995. https://doi.org/10.1021/jasms.8b01830.

(11) Di Laudo, M.; Whittleton, S. R.; Wetmore, S. D. Effects of Hydrogen Bonding on the Acidity of Uracil. *J. Phys. Chem. A* **2003**, *107* (48), 10406–10413. https://doi.org/10.1021/jp035496w.

(12) Matondo, A.; Thomas, R.; Tsalu, P. V.; Mukeba, C. T.; Mudogo, V. α-Methylation and α-Fluorination Electronic Effects on the Regioselectivity of Carbonyl Groups of Uracil by H and Triel Bonds in the Interaction of U, T and 5FU with HCl and TrH3 (Tr = B, Al). *J. Mol. Graph. Model.* **2019**, *88*, 237–246. https://doi.org/10.1016/j.jmgm.2019.02.006.

(13) Paula Yurkanis Bruice. *Organic Chemistry*, 6th edition.; Pearson Education, Inc.: US, 2010.

(14) Rewcastle, G. W. 8.02 - Pyrimidines and Their Benzo Derivatives. In *Comprehensive Heterocyclic Chemistry III*; Katritzky, A. R., Ramsden, C. A., Scriven, E. F. V., Taylor, R. J. K., Eds.; Elsevier: Oxford, 2008; pp 117–272. https://doi.org/10.1016/B978-008044992-0.00702-1.

(15) Jennings, B. H.; Pastra-Landis, S.; Lerman, J. W. Photosensitized Dimerization of Uracil. *Photochem. Photobiol.* **1972**, *15* (5), 479–491.

https://doi.org/10.1111/j.1751-1097.1972.tb06259.x.
(16) Undheim, K.; Benneche, T. 6.02 - Pyrimidines and Their Benzo Derivatives. In *Comprehensive Heterocyclic Chemistry II*; Katritzky, A. R., Rees, C. W., Scriven, E. F. V., Eds.; Pergamon: Oxford, 1996; pp 93–231. https://doi.org/10.1016/B978-008096518-5.00118-0.
(17) Wagner, P. J.; Bucheck, D. J. Photodimerization of Thymine and Uracil in Acetonitrile. *J. Am. Chem. Soc.* **1970**, *92* (1), 181–185. https://doi.org/10.1021/ja00704a030.
(18) González-Ramírez, I.; Roca-Sanjuán, D.; Climent, T.; Serrano-Pérez, J.; Merchán, M.; Serrano-Andrés, L. On the Photoproduction of DNA/RNA Cyclobutane Pyrimidine Dimers. *Theor. Chem. Acc. Theory Comput. Model.* **2011**, *128* (4–6), 705–711. https://doi.org/10.1007/s00214-010-0854-z.
(19) Arutyunyan, A. A.; Mamyan, S. S.; Stepanyan, H. M.; Paronikyan, R. V. Synthesis and Antitumor and Antibacterial Properties of New N-Alkylated Pyrimidines. *Pharm. Chem. J.* **2013**, *47* (6), 303–306. https://doi.org/10.1007/s11094-013-0948-8.
(20) Pérez-Moreno, G.; Sánchez-Carrasco, P.; Ruiz-Pérez, L. M.; Johansson, N. G.; Müller, S.; Baragaña, B.; Hampton, S. E.; Gilbert, I. H.; Kaiser, M.; Sarkar, S.; Pandurangan, T.; Kumar, V.; González-Pacanowska, D. Validation of Plasmodium Falciparum DUTPase as the Target of 5′-Tritylated Deoxyuridine Analogues with Anti-Malarial Activity. *Malar. J.* **2019**, *18* (1), 392. https://doi.org/10.1186/s12936-019-3025-2.
(21) Kozak, W.; Demkowicz, S.; Daśko, M.; Rachon, J.; Rak, J. Modifications at the C(5) Position of Pyrimidine Nucleosides. *Russ. Chem. Rev.* **2020**, *89* (3), 281. https://doi.org/10.1070/RCR4919.
(22) Yagi, K.; Akimoto, K.; Mimori, N.; Miyake, T.; Kudo, M.; Arai, K.; Ishii, S. Synthesis and Insecticidal/Acaricidal Activity of Novel 3-(2,4,6-Trisubstituted Phenyl)Uracil Derivatives. *Pest Manag. Sci.* **2000**, *56* (1), 65–73. https://doi.org/10.1002/(SICI)1526-4998(200001)56:1<65::AID-PS90>3.0.CO;2-S.
(23) Goto, Y.; Watanabe, N.; Kogawa, N.; Tsuchiya, M.; Takahashi, O.; Uchi, H.; Furue, M.; Hayashi, H. CX-659S: A Novel Diaminouracil Derivative That Has Antioxidative and Acute Anti-Inflammatory Activities. *Eur. J. Pharmacol.* **2002**, *438* (3), 189–196. https://doi.org/10.1016/S0014-2999(02)01340-7.
(24) Tobe, M.; Isobe, Y.; Goto, Y.; Obara, F.; Tsuchiya, M.; Matsui, J.; Hirota, K.; Hayashi, H. Synthesis and Biological Evaluation of CX-659S and Its Related Compounds for Their Inhibitory Effects on the Delayed-Type Hypersensitivity Reaction. *Bioorg. Med. Chem.* **2000**, *8* (8), 2037–2047. https://doi.org/10.1016/S0968-0896(00)00126-7.
(25) Grabovskii, S. A.; Andriyashina, N. M.; Grabovskaya, Y. S.; Antipin, A. V.; Kabal'nova, N. N. Reactivity of 5-Aminouracil Derivatives towards Peroxyl Radicals. *J. Phys. Org. Chem.* **2020**, *33* (8), e4065. https://doi.org/10.1002/poc.4065.
(26) Selvam, T. P.; James, C. R.; Dniandev, P. V.; Valzita, S. K. A Mini Review of Pyrimidine and Fused Pyrimidine Marketed Drugs. *Res. Pharm.* **2012**.
(27) Ardalan, B.; Luis, R.; Jaime, M.; Franceschi, D. Biomodulation of Fluorouracil in Colorectal Cancer. *Cancer Invest.* **1998**, *16* (4), 237–251. https://doi.org/10.3109/07357909809039773.
(28) Fluorouracil. In *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*; National Institute of Diabetes and Digestive and Kidney Diseases: Bethesda (MD), 2012.

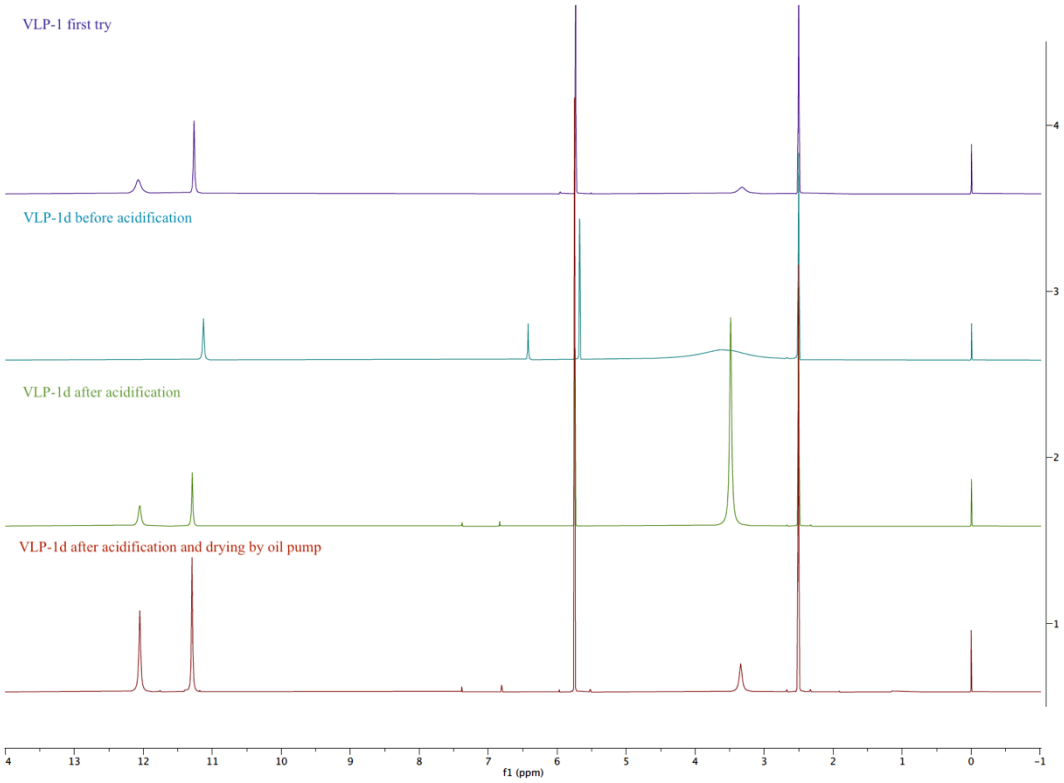
(29) Kim, T.; Park, S. Y.; Lee, M.-H.; Kim, D.-H.; Chung, I. Syntheses of Polyrotaxane Conjugated with 5-Fluorouracil and Vitamins with Improved Antitumor Activities. *J. Bioact. Compat. Polym.* **2019**, *34* (1), 25–38. https://doi.org/10.1177/0883911518813617.
(30) Zhao, L.-M.; Zhang, L.-M.; Liu, J.-J.; Wan, L.-J.; Chen, Y.-Q.; Zhang, S.-Q.; Yan, Z.-W.; Jiang, J.-H. Synthesis and Antitumor Activity of Conjugates of 5-Fluorouracil and Emodin. *Eur. J. Med. Chem.* **2012**, *47*, 255–260. https://doi.org/10.1016/j.ejmech.2011.10.050.
(31) Sakamoto, J.; Oba, K.; Matsui, T.; Kobayashi, M. Efficacy of Oral Anticancer Agents for Colorectal Cancer. *Dis. Colon Rectum* **2006**, *49* (1), S82. https://doi.org/10.1007/s10350-006-0601-7.
(32) Henderson, J. P.; Byun, J.; Takeshita, J.; Heinecke, J. W. Phagocytes Produce 5-Chlorouracil and 5-Bromouracil, Two Mutagenic Products of Myeloperoxidase, in Human Inflammatory Tissue. *J. Biol. Chem.* **2003**, *278* (26), 23522–23528. https://doi.org/10.1074/jbc.M303928200.
(33) Takeshita, J.; Byun, J.; Nhan, T. Q.; Pritchard, D. K.; Pennathur, S.; Schwartz, S. M.; Chait, A.; Heinecke, J. W. Myeloperoxidase Generates 5-Chlorouracil in Human Atherosclerotic Tissue - A Potential Pathway for Somatic Mutagenesis by Macrophages. *J. Biol. Chem.* **2006**, *281* (6), 3096–3104. https://doi.org/10.1074/jbc.M509236200.
(34) Shaker, R. M.; Elrady, M. A.; Sadek, K. U. Synthesis, Reactivity, and Biological Activity of 5-Aminouracil and Its Derivatives. *Mol. Divers.* **2016**, *20* (1), 153–183. https://doi.org/10.1007/s11030-015-9595-1.
(35) Baraldi, P. G.; Romagnoli, R.; Guadix, A. E.; Pineda de las Infantas, M. J.; Gallo, M. A.; Espinosa, A.; Martinez, A.; Bingham, J. P.; Hartley, J. A. Design, Synthesis, and Biological Activity of Hybrid Compounds between Uramustine and DNA Minor Groove Binder Distamycin A. *J. Med. Chem.* **2002**, *45* (17), 3630–3638. https://doi.org/10.1021/jm011113b.
(36) Ismaili, H.; Ban, Ž.; Matić, J.; Saftić, D.; Jukić, M.; Glavaš-Obrovac, L.; Žinić, B. An Efficient Synthesis and In Vitro Cytostatic Activity of 5-Aminosulfonyl Uracil Derivatives. *Croat. Chem. Acta* **2019**, *92* (2), 269–277. https://doi.org/10.5562/cca3567.
(37) Diasio, R. B. Sorivudine and 5-Fluorouracil; a Clinically Significant Drug-Drug Interaction Due to Inhibition of Dihydropyrimidine Dehydrogenase. *Br. J. Clin. Pharmacol.* **1998**, *46* (1), 1–4. https://doi.org/10.1046/j.1365-2125.1998.00050.x.
(38) Puig-de-la-Bellacasa, R.; Giménez, L.; Pettersson, S.; Pascual, R.; Gonzalo, E.; Esté, J. A.; Clotet, B.; Borrell, J. I.; Teixidó, J. Diverse Combinatorial Design, Synthesis and in Vitro Evaluation of New HEPT Analogues as Potential Non-Nucleoside HIV-1 Reverse Transcription Inhibitors. *Eur. J. Med. Chem.* **2012**, *54*, 159–174. https://doi.org/10.1016/j.ejmech.2012.04.038.
(39) Isono, Y.; Sakakibara, N.; Ordonez, P.; Hamasaki, T.; Baba, M.; Ikejiri, M.; Maruyama, T. Synthesis of 1-Benzyl-3-(3,5-Dimethylbenzyl)Uracil Derivatives with Potential Anti-HIV Activity. *Antivir. Chem. Chemother.* **2011**, *22* (2), 57–65. https://doi.org/10.3851/IMP1844.
(40) Chernikova, I. B.; Khursan, S. L.; Eropkin, M. Yu.; Yunusov, M. S. Synthesis of C5-C6 Derivatives of 1,3-Dimethyl-5-Fluorouracil and 5-Fluorouracil. Screening for Antiviral Activity. *Pharm. Chem. J.* **2019**, *53* (2), 108–112. https://doi.org/10.1007/s11094-019-01962-9.
(41) Salkic, S.; Brkic, S.; Batic-Mujanovic, O.; Ljuca, F.; Karabasic, A.; Mustafic, S. Emergency Room Treatment of Hypertensive Crises. *Med. Arch.* **2015**, *69* (5), 302–306. https://doi.org/10.5455/medarh.2015.69.302-306.

(42) NCI Thesaurus https://ncit.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary=NCI_Thesaurus&ns=NCI_Thesaurus&code=C604 (accessed May 24, 2020).
(43) Davie, M.; Wilkinson, L. S.; Roberts, M. H. Evidence for Excitatory 5-HT2-Receptors on Rat Brainstem Neurones. *Br. J. Pharmacol.* **1988**, *94* (2), 483–491.
(44) Pazos, A.; Probst, A.; Palacios, J. M. Serotonin Receptors in the Human Brain—IV. Autoradiographic Mapping of Serotonin-2 Receptors. *Neuroscience* **1987**, *21* (1), 123–139. https://doi.org/10.1016/0306-4522(87)90327-7.
(45) Ford, R. V.; Moyer, J. H.; Handley, C. A.; Spurr, C. L.; Rochelle, J. B. 3rd. Laboratory and Clinical Observations on Three Similar Diuretic Agents: Aminophylline, Aminometradine (Mictine) and Aminoisometradine (Rolicton). *J. Med. Sci.* **1957**, *234* (6), 640–655.
(46) Platts, M. M.; Hanley, T.; PLATTS; HANLEY. Aminometradine in Treatment of Congestive Heart Failure. *BMJ* **1956**, *1* (4975), 1078–1080. https://doi.org/10.1136/bmj.1.4975.1078.
(47) Kantsadi, A. L.; Manta, S.; Psarra, A.-M. G.; Dimopoulou, A.; Kiritsis, C.; Parmenopoulou, V.; Skamnaki, V. T.; Zoumpoulakis, P.; Zographos, S. E.; Leonidas, D. D.; Komiotis, D. The Binding of C5-Alkynyl and Alkylfurano[2,3-d]Pyrimidine Glucopyranonucleosides to Glycogen Phosphorylase b: Synthesis, Biochemical and Biological Assessment. *Eur. J. Med. Chem.* **2012**, *54*, 740–749. https://doi.org/10.1016/j.ejmech.2012.06.029.
(48) Wang, Y.; Zhang, L.; He, Z.; Deng, J.; Zhang, Z.; Liu, L.; Ye, W.; Liu, S. Tunicamycin Induces ER Stress and Inhibits Tumorigenesis of Head and Neck Cancer Cells by Inhibiting N-Glycosylation. *Am. J. Transl. Res.* **2020**, *12* (2), 541–550.
(49) Trantolo, D. J.; Wright, G. E.; Brown, N. C. Inhibitors of Bacillus Subtilis DNA Polymerase III. Influence of Modifications in the Pyrimidine Ring of Anilino- and (Benzylamino)Pyrimidines. *J. Med. Chem.* **1986**, *29* (5), 676–681. https://doi.org/10.1021/jm00155a016.
(50) Zhi, C.; Long, Z.-Y.; Gambino, J.; Xu, W.-C.; Brown, N. C.; Barnes, M.; Butler, M.; LaMarr, W.; Wright, G. E. Synthesis of Substituted 6-Anilinouracils and Their Inhibition of DNA Polymerase IIIC and Gram-Positive Bacterial Growth. *J. Med. Chem.* **2003**, *46* (13), 2731–2739. https://doi.org/10.1021/jm020591z.
(51) Yoneda, F.; Hirayama, R.; Yamashita, M. Synthesis of 2H-Chromeno[2,3-d]Pyrimidine-2,4-(3H)-Diones (10-Oxa-5-Deazaflavins) and Their Use in the Oxidation of Benzyl Alcohol. *J. Heterocycl. Chem.* **1982**, *19* (2), 301–304. https://doi.org/10.1002/jhet.5570190216.
(52) Wilson, J. M.; Henderson, G.; Black, F.; Sutherland, A.; Ludwig, R. L.; Vousden, K. H.; Robins, D. J. Synthesis of 5-Deazaflavin Derivatives and Their Activation of P53 in Cells. *Bioorg. Med. Chem.* **2007**, *15* (1), 77–86. https://doi.org/10.1016/j.bmc.2006.10.011.
(53) Cresswell, R. M.; Wood, H. C. S. 928. The Biosynthesis of Pteridines. Part I. The Synthesis of Riboflavin. *J. Chem. Soc. Resumed* **1960**, No. 0, 4768–4775. https://doi.org/10.1039/JR9600004768.
(54) Araújo, J. M. M.; Pereiro, A. B.; Canongia Lopes, J. N.; Rebelo, L. P. N.; Marrucho, I. M. Hydrogen-Bonding and the Dissolution Mechanism of Uracil in an Acetate Ionic Liquid: New Insights from NMR Spectroscopy and Quantum Chemical Calculations. *J. Phys. Chem. B* **2013**, *117* (15), 4109–4120. https://doi.org/10.1021/jp400749j.
(55) Dickens, M. P.; Roxburgh, P.; Hock, A.; Mezna, M.; Kellam, B.; Vousden, K. H.; Fischer, P. M. 5-Deazaflavin Derivatives as Inhibitors of P53 Ubiquitination by

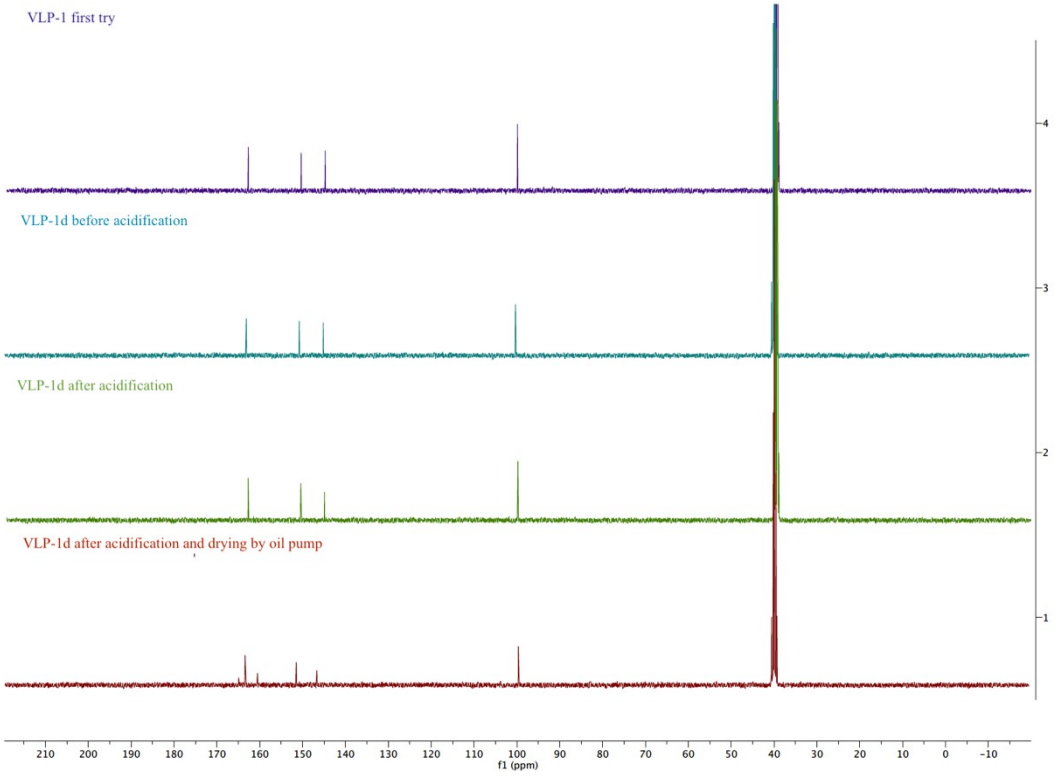
HDM2. *Bioorg. Med. Chem.* **2013**, *21* (22), 6868–6877. https://doi.org/10.1016/j.bmc.2013.09.038.
(56) Fang, W.-P.; Cheng, Y.-T.; Cheng, Y.-R.; Cherng, Y.-J. Synthesis of Substituted Uracils by the Reactions of Halouracils with Selenium, Sulfur, Oxygen and Nitrogen Nucleophiles under Focused Microwave Irradiation. *Tetrahedron* **2005**, *61* (12), 3107–3113. https://doi.org/10.1016/j.tet.2005.01.085.
(57) Daďová, J.; Kümmel, S.; Feldmeier, C.; Cibulková, J.; Pažout, R.; Maixner, J.; Gschwind, R. M.; König, B.; Cibulka, R. Aggregation Effects in Visible-Light Flavin Photocatalysts: Synthesis, Structure, and Catalytic Activity of 10-Arylflavins. *Chem. - Eur. J.* **2013**, *19* (3), 1066–1075. https://doi.org/10.1002/chem.201202488.
(58) Aromaticity in heterocyclic systems. II. the application of N.M.R. in a study of the synthesis and structure of certain lmidazo[1,2-c]pyrimidines and related pyrrolo[2,3-d]pyrimidines - Noell - 1964 - Journal of Heterocyclic Chemistry - Wiley Online Library https://onlinelibrary.wiley.com/doi/abs/10.1002/jhet.5570010108 (accessed Aug 5, 2020).
(59) Gasparyan, S. P.; Alexanyan, M. V.; Arutyunyan, G. K.; Martirosyan, A. H.; Paronikyan, R. V.; Stepanyan, H. M. N-Substituted 2-(6-Amino-2,4-Dioxo-1,2,3,4-Tetrahydropyrimidin-5-Yl)-2-Oxoacetamides. *Russ. J. Org. Chem.* **2016**, *52* (8), 1179–1182. https://doi.org/10.1134/S1070428016080145.
(60) Ogura, H.; Sakaguchi, M.; Takeda, K. Synthesis of Pyrrolopyrimidines and Thienopyrimidines. *Chem. Pharm. Bull. (Tokyo)* **1972**, *20* (2), 404–408. https://doi.org/10.1248/cpb.20.404.
(61) Abdou, W. M.; Fahmy, A. F. M.; Kamel, A. A. A Facile Synthesis of Pyrrolo[3,2-d]Pyrimidines from 6-Azidouracils and Ylide Phosphoranes. *Heteroat. Chem.* **2002**, *13* (4), 357–365. https://doi.org/10.1002/hc.10048.

8. APPENDICES:

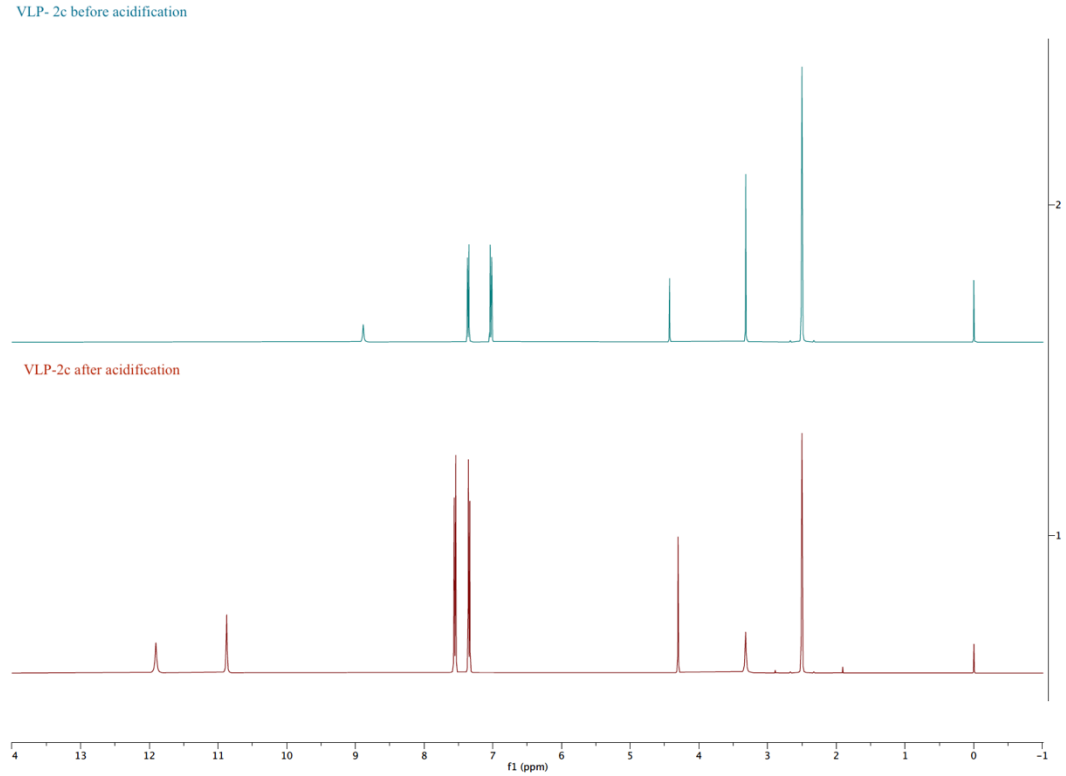
Appendix 1: ^{1}H-NMR spectra of VLP-1 before and after acidification



Appendix 2: ^{13}C-NMR spectra of VLP-1 before and after acidification



Appendix 3: ^{1}H-NMR spectra of VLP-2 before and after acidification



Appendix 4: ^{13}C-NMR spectra of VLP-2 before and after acidification