



약학석사 학위논문

# Design, Synthesis and SAR of Novel Analogues as Potent TRPV1 Antagonists and HIF-1a Inhibitors

효과적인 TRPV1 길항제와 HIF-1a 저해제 개발을 위한 신규 유도체의 합성 및 활성 연구

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### Abstract

### Part 1. Urea Analogues with Benzooxazinone and Quinolinone as Potent TRPV1 Antagonists

A series of N-methylated and N-ethylhydroxylated 6,6 fused heterocycles were designed combining previously identified pharmacophoric elements (BCTC, **M4** etc.) and evaluated as TRPV1 antagonists. This alkylation method was performed due to the desire of better antagonism and solubility. As a result of the FLIPR assay, the structure-activity relationship (SAR) analysis indicated that specific binding interactions of the 2-amino substituents in the C-region and the function of the secondary nitrogen on the A-region of the ligand were critical for high potency.

Although the compounds' activity dropped compared to the lead compounds, there were still some promising results. In particular, compound **111**, **166**, **193** were excellent TRPV1 antagonist (IC<sub>50</sub> [CAP] near or below 1.0) and was thus approximately 2- to 5-fold more potent, respectively, than the parent compound BCTC (IC<sub>50</sub> [CAP] = 2.5) for capsaicin antagonism. For further research, rat neuropathic model study and docking analysis is needed.

### Part 2. Simple Amide Analogues from Deguelin as Potent HIF-1α Inhibitors

A series of analogues having a pyridine ring on its A-region and an amide linker on its B-region were synthesized as ring-truncated Deguelin surrogates and evaluated for their HIF-1 $\alpha$  inhibition. Their structure-activity relationship was systematically investigated based on the variation of the linker B-region moiety. The potency of the compounds was checked by western blot assay.

Among the inhibitors, compound **22** and **44** exhibited potent HIF-1 $\alpha$  inhibition (HIF WB around 30) in a dose-dependent manner (approximately 2-fold more potent) and significant antitumor activity compared to Deguelin. To foster with these promising results, we need further docking study and characterized assays for cell viability, proliferation and migration.

Keyword : TRPV1 Antagonist, Capsaicin, HSP90, HIF-1α Inhibitor, Deguelin Student Number : 2014-21044

# **Table of Contents**

Abstract	i
Table of Contents	iii
List of Tables	vi
List of Figures	viii
List of Schemes	ix
Abbreviations	x

# **Table of Contents**

Part 1. Urea Analogues with Benzooxazinone and Quinolinone
as Potent TRPV1 Antagonists

Chapter 1. Introduction 1	
1.1. Study Background1	
1.1.1. Neuropathic Pain and Analgesics1	
1.1.2. TRPV1 Antagonists	
1.2. Purpose of Research	•
Chapter 2. Design of the Urea Analogues 5	ļ
2.1. Previous Study	
2.2. Lead Compound SAT-1251 and SAT-769 6	ļ
2.3. Design Approach of the Urea Analogues7	
Chapter 3. Synthesis of the Series	,
3.1. C-region Carbamate	)
3.1.1. Benzooxazinones	ļ
3.1.2. Quinolinones	ļ
3.2. A-region Amine	ļ
3.2.1. CF <sub>3</sub> Pyridines	ļ
3.2.2. $CF_3$ and <i>t</i> -butyl Pyrazoles	
3.2.3. Thiazoles	
3.3. B-region Urea 14	
Chapter 4. Structure-Activity Relationship	
4.1. Results of the FLIPR Assay	,
4.2. Biological Evaluation	
Chapter 5. Conclusion 26	
Experimental 27	,

# **Table of Contents**

Chapter 1. Introduction	43
1.1. Study Background	43
1.1.1. HSP90	43
1.1.2. HIF-1α Inhibitors	43
1.2. Purpose of Research	45
-	
Chapter 2. Design of the Amide Analogues	46
2.1. Previous Study	46
2.2. Lead Compound L-22	46
2.3. Design Approach of the Amide Analogues	47
Chapter 3. Synthesis of the Series	48
3.1. A-region Pyridine Amine	48
3.2. C-region Carboxylic Acid	48
3.3. B-region Amide with Various Linkers	49
Chapter 4. Structure-Activity Relationship	52
4.1. Results of the Western Blot Assay	52
4.2. Biological Evaluation	56
Chanter 5 Conclusion	58
Chapter 5. Conclusion	50
Experimental	59
Bibliography	66
zzwegrupuj	50
Abstract in Korean	69

# List of Tables

# Part 1. Urea Analogues with Benzooxazinone and Quinolinone as Potent TRPV1 Antagonists

<b>Table 1.</b> FLIPR Results of Urea Analogues with N-Methylated Benzooxazinone A-regions and Pyridine C-regions       16
<b>Table 2.</b> FLIPR Results of Urea Analogues with N-Methylated Benzooxazinone A-regions and Pyrazole, Thiazole C-regions17
<b>Table 3.</b> FLIPR Results of Urea Analogues with N-EthylhydroxylatedBenzooxazinone A-regions and Pyridine C-regions18
<b>Table 4.</b> FLIPR Results of Urea Analogues with N-EthylhydroxylatedBenzooxazinone A-regions and Pyazole, Thiazole C-regions
<b>Table 5.</b> FLIPR Results of Urea Analogues with N-Methylated Quinolinone A-regions and Pyridine C-regions20
<b>Table 6.</b> FLIPR Results of Urea Analogues with N-Methylated Quinolinone A-regions and Pyrazole, Thiazole C-regions21
<b>Table 7.</b> FLIPR Results of Urea Analogues with N-Ethylhydroxylated QuinolinoneA-regions and Pyridine C-regions22
<b>Table 8.</b> FLIPR Results of Urea Analogues with N-Ethylhydroxylated QuinolinoneA-regions and Pyrazole, Thiazole C-regions23

# List of Tables

Table 1. WB results of Simple Amides	52
Table 2. WB Results of Amides with Benzyl Linkers	52
Table 3. WB Results of Amides with Pyridine Linkers	53
Table 4. WB Results of Amides with Amine Linkers (Two Carbon	s) 54
Table 5. WB Results of Amides with Pyrrolidine, Piperidine,           Piperazine Linkers	Morpholine and
Table 6. WB Results of Amides with Oxy Linkers	55
Table 7. WB Results of Amides with Amine Linkers (Three Carbo	ons) 56

# **List of Figures**

# Part 1. Urea Analogues with Benzooxazinone and Quinolinone as Potent TRPV1 Antagonists

Figure 1. Mechanism of Neuropathic Pain    2
Figure 2. Mechanism of TRPV1 Pain Circuit    3
Figure 3. The Production of Capsazepine   5
Figure 4. TRPV1 Antagonists in Clinical Development
<b>Figure 5.</b> Structure of N-(4-tertiarybutylphenyl)-4-(3-cholorphyridin-2-l)tetrahydro pyrazine-1(2H)- carbox-amide (BCTC)
Figure 6. Deriving the Lead Compound SAT-1251 and SAT-769 7
Figure 7. Design Approach of the TRPV1 Analogues

Figure 1. Mechanism of Molecular Chaperones	44
<b>Figure 2.</b> Mechanism of HSP90 and HIF-1α	44
Figure 3. The B-C Ring Truncation of Natural Product Deguelin	46
Figure 4. The Introduction of the Polar Group on SH-42	46
<b>Figure 5.</b> Design Approach of the HIF-1α Surrogates	47

# **List of Schemes**

# Part 1. Urea Analogues with Benzooxazinone and Quinolinone as Potent TRPV1 Antagonists

Scheme 1. Synthesis of the A-region Benzooxazinone Carbamate
Scheme 2. Synthesis of the A-region Quinolinone Carbamate 10
<b>Scheme 3.</b> Synthesis of the C-region CF <sub>3</sub> Pyridine Key Intermediate 1
Scheme 4. Synthesis of the C-region CF <sub>3</sub> Pyridine Amines 1
<b>Scheme 5.</b> Synthesis of the C-region CF <sub>3</sub> Pyrazole Amines
Scheme 6. Synthesis of the C-region <i>t</i> -butyl Amines
Scheme 7. Synthesis of the C-region Thiazole Amines
Scheme 8. Synthesis of the Urea Analogues

Scheme 1. Synthesis of the A-region Pyridine Amine	48
Scheme 2. Synthesis of the C-region Carboxylic Acid	49
Scheme 3. Synthesis of the Key Intermediate	49
Scheme 4. Synthesis of the Necessary Linkers	50
Scheme 5. Synthesis of the Amide Analogues	51

# Abbreviations

- **18-C-6** 18-Crown-6, 1,4,7,10,13,16-hexaoxacyclooctadecane
- Ac Acyl group
- ACN Acetonitrile
- ATP Adenosine triphosphate
- CAP Capsaicin
- DBU 1,8-Diazabicycloundec-7-ene
- **DCM** Dichloromethane
- **DMF** Dimethylformamide
- **DPPA** Diphenylphosphoryl azide
- EDCI 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
- FLIPR Fluorometric Imaging Plate Reader
- HDMS Bis(trimethylsilyl)amine
- HIF-1 Hypoxia-inducible factors
- HOBt Hydroxybenzotriazole
- HSP90 Heat Shock Protein 90
- LAH Lithium aluminium hydride
- MC Dichloromethane
- NCS N-Chlorosuccinimide
- **PFA** Paraformaldehyde
- Ph Phenyl group
- RTX Resiniferatoxin
- TBAF Tetra-n-butylammonium fluoride
- **TBDMS***t*-butyldimethylsilyl
- TEA Triethylamine
- TEBAC Triethyl benzyl ammonium chloride
- TFA Trifluoroacetic acid
- THF Tetrahydrofuran
- TRPV1 Transient Receptor Potential cation channel subfamily V member 1
- Ts Tosyl group
- VEGF Vascular Endothelial Growth Factor
- VHL Von Hippel–Lindau tumor surpressor
- WB Western Blot

### **Chapter 1. Introduction**

#### **1.1. Study Background**

#### 1.1.1. Neuropathic Pain and Analgesics

Pain outbreaks by tissue-damaging stimuli which results in the promotion of nociceptive afferents. Pain can also arise without the pathway stimulation of its peripheral sensory systems but straightly to the nervous system.<sup>1</sup> For this type of pain, by Harold Merskey in 1964, the International Association for the Study of Pain (IASP) introduced the term neuropathic pain, defined to be the type of pain initiated or caused by a primary lesion or dysfunction in the nervous system.<sup>2</sup>

Neuropathic pain can be classified into two types which are central neuropathic pain (non-dermatomal) and peripheral (dermatomal) neuropathic pain. First, the central neuropathic pain is usually found in spinal cord injury, multiple sclerosis and some strokes.<sup>3</sup> Second, peripheral pain is caused by herpes zoster infection, HIV-related neuropathies, nutritional deficiencies, toxins, remote manifestations of malignancies, immune mediated disorders and physical trauma to the nerve trunk.<sup>4</sup>

The sensory mechanism of pain is starts from the peripheral ends of the body, when a dangerous or lethal stimuli irritates the neural sensors, the signal is sent to the nociceptor. The nociceptor is divided into two parts which are sensation-detecting branches and nerve fascicles. When the peripheral nociceptors meet noxious chemicals due to tissue damage, the signal is transferred to the dorsal horn in the spinal cord. In the spinal cord, the neurotransmitter sends signaling molecules to the brain and at last, we sense danger and 'feel painful'. Usually patients feel burning, prickling, tingling, electricshock-like or lancinating. (Figure 1)

Neuropathic pain can be treated in several ways such as antidepressants, NMDA antagonism, opioids, cannabinoids, anticonvulsants, botulinum toxin type A, neuromodulators etc. Among these, as analgesics, the most responsive

<sup>&</sup>lt;sup>1</sup> R. Treede et al. "Neuropathic pain: Redefinition and a grading system for clinical and research purposes." *Neurology* 70 (2008): 1630-1635.

<sup>&</sup>lt;sup>2</sup> H. Merskey et al. "Part III: Pain Terms, A Current List with Definitions and Notes on Usage." Classification of Chronic Pain. Seattle: IASP Press, 1994. 209-214.

<sup>&</sup>lt;sup>3</sup> P. Foley et al. "Prevalence and natural history of pain in adults with multiple sclerosis: Systematic review and meta-analysis." *Pain* 154 (2013): 632–642.

<sup>&</sup>lt;sup>4</sup> M. Wampler et al. "Case report: painful peripheral neuropathy following treatment with docetaxel for breast cancer." *Clin J Oncol Nurs* 9 (2005): 189-193.

pharmacological treatment is known to be topical agents such as lidocaine patch or capsaicin, opioids (oxycodone, tramadol, fentanyl, morphine or hydrocodone) which are also known as narcodics, TCA type antidepressants (amitriptyline, nortriptyline, desipramine, imipramine or doxepin), SNRI type antidepressants (duloxetine or venlafaxine) which are considered to be first-line in medication of neuropathic pain, and anticonvulsants (carbamazepine, valproate, lamotrigine, topiramate, oxcarbazepine, gabapentin or pregabalin).<sup>5</sup> Still, however, further investigation is needed to confirm the mechanism of the drugs and prevent side effects.



Figure 1. Mechanism of Neuropathic Pain.

#### 1.1.2. TRPV1 Antagonists

Among the painkillers stated above, the capsaicin looks pretty promising. Most of the ion channels are known to be stimulated by capsaicin heat or cations. When any of these factors stimulate the ion channel, the calcium or the sodium cations can penetrate through the neuronal membrane. As a result, the receptor activation leads to an increase in intracellular calcium cations that leads to excitation of primary sensory neurons pain. Then the signal is sent to the spinal

<sup>&</sup>lt;sup>5</sup> Treatment For Trigeminal Neuropathic Pain. 23 March 2011.

cord through depolarization and ultimately the central perception of pain.<sup>6</sup>

The official designation of this heat, cation, exogenous agents such as capsaicin induced non selective channel is called transient receptor potential V1 (TRPV1). As explained above, the TRPV1 is a molecular integrator of protons, heat or inflammatory endogenous mediators and especially, capsaicin. (**Figure 2**) This TRPV1 is located predominantly in primary sensory neurons.<sup>7</sup> So the pain-preventing strategy is to block from binding to the TRPV1 which means an antagonist is needed.



Figure 2. Mechanism of TRPV1 Pain Circuit

These days, intense organic, pharmaceutical and medicinal chemistry efforts are producing promising and potent antagonists along with better understanding of TRPV1 pharmacology. However, still, the hyperthermia issue remains while dealing with TRPV1 antagonists.<sup>8</sup> But as the field matures, this pH related obstacle is finding a way to be controlled and TRPV1 will still be one of the most attractive and potential targets for the treatment of neuropathic pain.

<sup>&</sup>lt;sup>6</sup> K. Alawi et al. "The paradoxical role of the transient receptor potential vanilloid 1 receptor in inflammation." *J. Pharmacol. Ther.* 125 (2010): 181.

<sup>&</sup>lt;sup>7</sup> A. Szallasi et al. "Vanilloid (capsaicin) receptors and mechanisms." *Pharmacol. Rev.* 51 (1999): 159-211.

<sup>&</sup>lt;sup>8</sup> A. Garami et al. "Contributions of Different Modes of TRPV1 Activation to TRPV1 Antagonist-Induced Hyperthermia." *J. Neurosci.* 30 (2010): 1435.

### **1.2.** Purpose of Research

The purpose of this research is to synthesize derivatives that mimic some natural products that can activate the TRPV1 channel. By blocking the binding site and prevent the activators, those can act as analgesics. Starting from the structural analysis of certain natural products, we investigated certain types of compounds that show great TRPV1 antagonism.

### **Chapter 2. Design of the Urea Analogues**

### 2.1. Previous Study

Starting from mimicking the structure of capsaicin and RTX, many types of antagonists has been produced. For example, there was Capsazepine (**Figure 3**) produced in 1990 as the first competitive vanilloid antagonist.<sup>9</sup>



Figure 3. The Production of Capsazepine

Moreover, the clinical development of TRPV1 antagonists in the pharmaceutical industry has been extensively improved. Many international pharmaceutical companies tried to design potent TRPV1 antagonists without any side effects. Some candidates had excellent improvement in potency such as the **SB-705498** from GSK or **MK-2295** from Merck-Neurogen. However, the previous one showed toxicity and the latter candidate failed because of the hyperthermia issue. (**Figure 4**) Especially, BCTC from Purdue Pharma showed exceptional potency but the only remaining problem was bad solubility. (**Figure 5**)

<sup>&</sup>lt;sup>9</sup> M. Gunthorpe et al. "Clinical development of TRPV1 antagonists: targeting a pivotal point in the pain pathway." *Drug Discovery Today* 14 (2009): 56-67.



Figure 4. TRPV1 Antagonists in Clinical Development



**Figure 5.** Structure of N-(4-tertiarybutylphenyl)-4-(3-cholorphyridin-2yl)tetrahydropyrazine-1(2H)- carbox-amide (BCTC)

### 2.2. Lead Compound SAT-1251 and SAT-769

Most of the compounds had a hydrogen bond interacting linker on the middle and aromatic rings on both sides. The capsaicin structure has been divided into three pharmacophoric regions.<sup>10</sup> Previously, we have produced a series of (S)-2-(3-fluoro-4-(methylsulfonamido)phenyl)-N-((2-(4-methylpiperidin-1-yl)-6-

(trifluoromethyl)pyridin-3-yl)methyl)propanamides (M4), <sup>11</sup> originally designed based on a pharmacophoric combination approach, that showed potent hTRPV1

<sup>&</sup>lt;sup>10</sup> C. Walpole et al. "Analogs of capsaicin with agonist activity as novel analgesic agents; structure-activity studies. 2. The amide bond "B-region"." *J. Med. Chem.* 36 (1993): 2373-2380.

<sup>&</sup>lt;sup>11</sup> M. Kim et al. "2(3-Fluoro-4-methylsulfonylaminophenyl)propanamides as Potent Transient Receptor Potential Vanilloid 1 (TRPV1) Antagonists." *J. Med. Chem.* 55 (2012): 8392-8408.

antagonism toward multiple activators. However, this **M4** compound needed additional modification due to its toxicity. As part of our continuing program for the discovery of new antagonists for the treatment, we have investigated a set of novel antagonists which contains a 6,6-fused heterocycle on the A-region (**SAT-1251, SAT-769**). (**Figure 6**) The 6,6-fused heterocycle on the A-region with a urea linker on the B-region's efficacy was already discussed through the Amgen's **AMG0347** and within our laboratory.<sup>12</sup>



Figure 6. Deriving the Lead Compound SAT-1251 and SAT-769

### 2.3. Design Approach of the Urea Analogues

In an effort to optimize the properties of the antagonistic template, our focus was

<sup>&</sup>lt;sup>12</sup> W. Sun et al. "6,6-Fused heterocyclic ureas as highly potent TRPV1 antagonists." *Bioorganic & Medicinal Chemistry Letters* 25 (2015): 803-806.

next directed to the property of the nitrogen atom on the end of the A-region lactam. To identify the contribution of this nitrogen, we decided to alkylate the secondary amine. We tried two methods. One was to simply methylate the nitrogen and considering the that there was a solubility issue confronting both benzooxazinone (SAT-1251) and quinolinone (SAT-769), the second plan was to alkylate with an ethylhydroxyl group to obtain better solubility. (Figure 7)



Figure 7. Design Approach of the TRPV1 Analogues

The B-region urea linker was decided to stay still. Another way for improvement was to alter the C-region with various compounds. Expecting the possibility of having a spacious binding site below the C-region amine,<sup>13</sup> we decided to substitute the C-region with various pyridines, pyrazoles or thiazoles.

<sup>&</sup>lt;sup>13</sup> H. Park et al. "Synthesis of 2-substituted-pyrrolidinethiourea derivatives and their antagonist effect on vanilloid receptor." *Bioorganic and Medicinal Chemistry Letters* 13 (2003): 197-200.

### **Chapter 3. Synthesis of the Series**

#### 3.1. C-region Carbamate

#### 3.1.1. Benzooxazinones

Starting from the commercially available compound 2-amino-4-chloro-6nitrophenol (1) and 2-chloroacetyl chloride (2), with TEBAC and 18-Crown-6, we cyclized the compound to synthesize the benzooxazinone compound (3). With cesium carbonate, we alkylated the nitrogen on the lactam to get the alkylated compounds (4, 5). With palladium and triethylamine with a quite long time, we performed reduction on the nitro group and dechlorination (6, 7). The triethylamine is used for neutralizing the hydrochloric acid which is generated after the dechlorination. A certain amount of tetrahydrofuran is used as a solvent because the cyclized nitro compound (4, 5) does not dissolve well in only methanol. At last, with the phenyl chloroformate and pyridine as a base, we get the desired A-region carbamate (8, 9).



Scheme 1. Synthesis of the A-region Benzooxazinone Carbamate. Reagents and conditions: (a) TEBAC,  $K_2CO_3$ , 18-C-6, chloroform,  $H_2O$ , 60 °C, overnight; (b)  $Cs_2CO_3$ , R-X, DMF, 60 °C, overnight; (c) 10 % Pd/C,  $H_2$ , TEA, MeOH/THF, r.t.; (d) Phenyl chloroformate, pyridine, ACN, r.t., 1 h;

#### 3.1.2. Quinolinones

The synthesis of the quinolinone A-region starts from the commercially available

2-chloroquinoline (10). After nitration on the  $5^{th}$  position using nitric acid and sulfuric acid (11), we hydrolyzed the compound and formed a lactam on the ring (12). The alkylation, reduction, carbamation steps are the same as the benzooxazinone carbamate.



Scheme 2. Synthesis of the A-region Quinolinone Carbamate. Reagents and Conditions: (a)  $HNO_3$ ,  $H_2SO_4$ , r.t., overnight; (b) HCl,  $H_2O$ , reflux, overnight; (c)  $Cs_2CO_3$ , R-X, DMF, 60 °C, overnight; (d) 10 % Pd/C,  $H_2$ , MeOH/THF, r.t.; (e) Phenyl chloroformate, pyridine, ACN, r.t., 1 h;

### 3.2. A-region Amine

#### 3.2.1. CF<sub>3</sub> Pyridines

The CF<sub>3</sub> pyridine C-region amines start from the synthesis of the key intermediate 2-chloro-6-(trifluoromethyl)nicotinonitrile (22). For the synthesis of the key intermediate (22), we started from the commercially available ethylvinyl ether (19). After acetylation to get the vinyl ketone (20), condensation to obtain the pyridine (21), and at last the chlorination, we can get the desired compound (22).



Scheme 3. Synthesis of the C-region CF<sub>3</sub> Pyridine Key Intermediate. Reagents and conditions: (a) (CF<sub>3</sub>CO)<sub>2</sub>O, pyridine, CHCl<sub>3</sub>, r.t., overnight; (b) NCCH<sub>2</sub>CONH<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>, toluene, reflux, overnight; (c) POCl<sub>3</sub>, r.t., overnight;

From this key intermediate (22), we substituted the chlorine with several kinds of linkers on the  $2^{nd}$  position using K<sub>2</sub>CO<sub>3</sub> (for amines), DBU (for alcohols), Pd(PPh<sub>3</sub>)<sub>4</sub> (for alkyls) or NaH (for thiol). After the substitution, we performed borane reduction for the production of amine (41 – 57).



Scheme 4. Synthesis of the C-region CF<sub>3</sub> Pyridine Amines. Reagents and conditions: (a) R-H,  $K_2CO_3$ , ACN, reflux, 12 h; (b) R-H, DBU, ACN, reflux, 12 h; (c) R-H, NaH, DMF, r.t., 2 h; (d) R-B(OH)<sub>2</sub>, 2M Na<sub>2</sub>CO<sub>3</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, toluene, 1,4-dioxane, reflux, 2 h;

#### 3.2.2. CF<sub>3</sub> and *t*-butyl Pyrazoles

The  $CF_3$  pyrazole C-region amine's starting material is ethyl 2,2,2-trifluoroacetate (58). After reduction and the production of the hydrzone (60 –

63), chlorination and cyclization is performed yielding the cyclized nitrile (68 – 71). At the last step, LAH reduction is performed to get the desired  $CF_3$  pyrazole (72 – 75).



Scheme 5. Synthesis of the C-region CF<sub>3</sub> Pyrazole Amines. Reagents and conditions: (a) LAH, ether, -78  $^{\circ}$ C, 5 h; (b) R-NHNH<sub>2</sub>HCl, ethanol, reflux, 2 h; (c) NCS, DMF, r.t., 2 h; (d) 2-chloroacrylonitrile, TEA, toluene, r.t., overnight; (e) LAH, THF, 0  $^{\circ}$ C to r.t., 2 h;

For *t*-butyl amines, commercially available compound 4,4-dimethyl-3-oxopentanenitrile (**76**) passes through cyclization, Sandmeyer reaction, cyanation, LAH reduction.



Scheme 6. Synthesis of the C-region *t*-butyl Amines. Reagents and conditions: (a) R-NHNH<sub>2</sub>HCl, ethanol, reflux, 12 h; (b) *t*-BuONO, CuI, ACN, 0 °C to 65 °C, 2 h; (c) Zn(CN)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, DMF, reflux, 2 h; (d) LAH, THF, 0 °C to r.t. 2 h;

#### 3.2.3. Thiazoles

Thiazole compounds required two types of intermediates. The *t*-butyl part (**86**) was synthesized by thiation starting from the commercially available pivalamide (**85**). The thioamide phenyl part (**89**) was synthesized in two steps alkylation and tosylation starting from 1-(3-chlorophenyl)ethan-1-one (**87**). After that, we cyclized the *t*-butyl intermediate (**86**) into the phenyl part (**89**), reduced it to produce an alcohol (**91**), made an azide out of it (**92**) and performed Staudinger reaction at last to provide the desired product (**93**).



Scheme 7. Synthesis of the C-region Thiazole Amines. Reagents and conditions: (a) HDMS, P<sub>2</sub>S<sub>5</sub>, toluene, r.t., overnight; (b) Dimethyl carbonate, NaH, THF, r.t., 2 h; (c) DMP, P-TsOH, ACN, r.t. 2 h; (d) MeOH, reflux, overnight; (e) LAH, THF, r.t., 2 h; (f) Diphenylphosphoryl azide, DBU, THF, reflux, 2 h; (g) Pd(PPh<sub>3</sub>)<sub>4</sub>, H<sub>2</sub>O, toluene, r.t., 2 h;

### 3.3. B-region Urea

With the C-region carbamates (8, 9, 17, 18), we add various types of A-region amines (41 - 57, 72 - 75, 83, 84, 93) go on a urea coupling using triethylamine as a base. Some of the amines were not coupled because either we did not produce a sufficient amount of amines or the compounds were already synthesized before. The ones with the ethyl hydroxyl groups needed additional silyl deprotection using TBAF.



**Scheme 8. Synthesis of the Urea Analogues.** Reagents and conditions: (a) TEA, DMF, 0 °C to r.t., 2 h; (b) TBAF, THF, 0 °C to r.t., 2 h;

### **Chapter 4. Structure-Activity Relationship**

#### 4.1. Results of the FLIPR Assay

The synthesized TRPV1 ligands were assayed in vitro for antagonism as measured by inhibition of activation by  $IC_{50}$  [CAP]. The synthesized analogues were evaluated for their ability to inhibit the effect of the given concentration of capsaicin (100 nM) on *h*TRPV1 as determined using a fluorescence imaging plate reader (FLIPR). The results of the FLIPR assay are shown below. Since the activity varies in different conditions, we set a correction value for BCTC to be  $IC_{50}$  [CAP] = 2.5 nM. The derivated compounds' activity were compared with the presynthesized compound **M4** (IC<sub>50</sub> [CAP] = 0.20 nM) and the lead compounds benzooxazinone **SAT-1251** (IC<sub>50</sub> [CAP] = 0.60 nM) and quinolinone **SAT-769** (IC<sub>50</sub> [CAP] = 0.27 nM).

**Table 1.** FLIPR Results of Urea Analogues with N-Methylated Benzooxazinone Aregions and Pyridine C-regions

	F	R <sub>1</sub> C			
	R <sub>1</sub>	IC <sub>50</sub> [CAP] nM		R <sub>1</sub>	IC <sub>50</sub> [CAP] nM
94	ξ−N	5.91	101	₽-0 <sup>-</sup>	61.84
95	₹-N	4.21	102	}–s–	9.70
96	ξ−N	4.84	103	ξ-√_F	11.13
97	₹-N_	2.23	104	Ş− <b>C</b> I	11.92

1 6



Compared to the lead compound **SAT-1251**, most of the compounds did not show better antagonism but the one with the benzylpiperidine (97) had similar activity with BCTC.

**Table 2.** FLIPR Results of Urea Analogues with N-Methylated Benzooxazinone Aregions and Pyrazole, Thiazole C-regions



Moreover, the pyrazoles on the C-region did not display good activity. Only the compound with the thiazole (111) slightly maintained the lead compound's potency.

**Table 3.** FLIPR Results of Urea Analogues with N-EthylhydroxylatedBenzooxazinone A-regions and Pyridine C-regions

$F_{3}C \xrightarrow{H} H H \xrightarrow{O} \xrightarrow{O} O$					
	R <sub>3</sub>	IC <sub>50</sub> [CAP] nM		R <sub>3</sub>	IC <sub>50</sub> [CAP] nM
134	§−N	3.81	142	,∕	8.23
135	ξ−N	3.89	143	⊷∽	22.42
136	₹-N	45.79	144	₽-0	8.21
137	ξ−N	5.96	145	₽-o-€	46.24
138	ξ−N	3.24	146	}-s-<	10.66
139	₽°	12.80	147	ξ- <b>√</b> −F	9.39
140	}0	14.80	148		1.93
141	₽-0	8.76			

Within the benzooxazinones with an alcohol on the end, none of them had better antagonism than the lead compound **SAT-1251**. Only the compound with a bulky isopropyl on the meta position (**148**) showed better potency than BCTC.



**Table 4.** FLIPR Results of Urea Analogues with N-EthylhydroxylatedBenzooxazinone A-regions and Pyazole, Thiazole C-regions

Compared to the C-region pyridines, the C-region pyrazoles had better potency however, none of them showed better antagonism than **SAT-1251** but the potency did not drop drastically either. The good part is, most of them had similar or less-dropped potency compared to BCTC. Especially when there is a bulky chlorine on the meta position (**149**, **151**, **153**, **155**) or an isopropyl (**152**), it displays higher activity compared to the meta positioned fluorine compounds (**150**, **154**).

$F_{3}C + H + H + V = N$ $N + N + N$ $R_{5} = 0$								
	R <sub>5</sub>	IC <sub>50</sub> [CAP] nM		R <sub>5</sub>	IC <sub>50</sub> [CAP] nM			
156	ξ−N	2.16	161	<u>}−o</u>	2.75			
157	ξ−N	0.99	162	₽-0 <sup>-</sup>	3.73			
158	₹-N	1.09	163	ξ−s−	3.39			
159	ξ−N	1.94	164		0.98			
160	ŧ-o	1.44	165		0.81			

**Table 5.** FLIPR Results of Urea Analogues with N-Methylated Quinolinone A-regions and Pyridine C-regions

In the quinolinone case, most of the compounds showed better analgesic activity than benzooxazinone. Although none of them were better than the lead compound **SAT-769** they still showed outstanding results. Especially, the compounds having C-region with the dipropylamine functional group (157) and the ones with the methyl (164) and isopropyl (165) on the meta position displayed great potency.

**Table 6.** FLIPR Results of Urea Analogues with N-Methylated Quinolinone A-regions and Pyrazole, Thiazole C-regions



With pyrazoles in the C-region, compound (166) with a  $CF_3$  pyrazole and a chlorine on the meta position showed great analgesic activity. However, when the  $CF_3$  changes into *t*-butyl groups (167) or into a thiazole (168), the activity drops.

**Table 7.** FLIPR Results of Urea Analogues with N-Ethylhydroxylated QuinolinoneA-regions and Pyridine C-regions

$F_{3}C \xrightarrow{H} H \xrightarrow{H} H \xrightarrow{H} O \xrightarrow{N} O \longrightarrow{N} O $								
	$R_7$	IC <sub>50</sub> [CAP] nM		<b>R</b> <sub>7</sub>	IC <sub>50</sub> [CAP] nM			
192	§−N	1.31	200	,+-ó{	1.16			
193	ξ−N	0.46	201	$\operatorname{ke}(\mathbf{x}) = \operatorname{ke}(\mathbf{x}) = $	0.81			
194	₹-N	2.49	202	₽-0	1.08			
195	ξ−N	1.23	203	Į−ó−€	1.14			
196	ξ−N	1.02	204	}−s-	1.92			
197	₽o	1.15	205	ξ <b>√</b> −F	1.45			
198	₽-0	1.30	206	Ş−−⊂⊂F	2.24			
199	<b>₽</b> −0	1.18	207		1.86			

Compounds with an alcohol type quinolinones on the A-region and a pyridine on the C-region displays a much more positive activity than BCTC. Particularly the compound with a dipropylamine on the end of the C-region (193) revealed to have excellent potency.

**Table 8.** FLIPR Results of Urea Analogues with N-Ethylhydroxylated QuinolinoneA-regions and Pyrazole, Thiazole C-regions

	<b>R</b> <sub>8</sub>	IC <sub>50</sub> [CAP] nM		R <sub>8</sub>	IC <sub>50</sub> [CAP] nM		
208	F <sub>3</sub> C N.N CI	2.63	212	N.N CI	2.85		
209	F <sub>3</sub> C NN F	4.72	213	N.N.F	6.40		
210		1.51	214	N CI	1.59		
211	F <sub>3</sub> C NN	1.34					

In the case of pyrazoles and thiazoles on the C-region, the ones with a chlorine on the meta position (**208**, **210**, **212**, **214**) and the one with an isopropyl group on the meta position (**211**) was observed to have better activity than BCTC. However, all of them did not show better potency than the lead compound **SAT-769**.

#### 4.2. Biological Evaluation

By the FLIPR assay results, first of all we are able to know that the free nitrogen atom in the cyclized lactam on the A-region contributes to the activity. Comparing the lead compounds with the benzooxazinone alkylated compounds (SAT-1251 vs. 94, 134), the activity dropped and the same result is shown in the quinolinone case (SAT-769 vs. 156, 192). Therefore, we assume the ring free nitrogen needs to be present at the distal area of the ring and blocking the nitrogen atom is detrimental to antagonism.

As another aspect, comparing the N-methylated A-regions with the Nethylhydroxylated A-regions (**Table 1, 2** vs. **Table 3, 4** or **Table 5, 6** vs. **Table 7, 8**), the ones with an N-ethylhydroxyl group had a tendency of better antagonism. This means that we can predict there is an interaction pocket where the end of the Aregion meets in the protein and the interaction strengthens when there is a polar group on the end of the A-region. Along with the interaction pocket benefit, there might be another advantage for N-ethylhydroxyled A-region surrogates because there is an alcohol group, which means better solubility leading to better absorption in the body.

Going on to the alteration of the C-region, the tendency is quite indistinct for each derivative. Still, by the trend that analogues which have a dipropylamine (95, 135, 157, 193) on the 2<sup>nd</sup> position of the pyridine turns out to be strong antagonists compared to other pyridine compounds, the three carbon length is assumed to be crucial for activity. Moreover, for N-ethylhydroxylated benzooxazinone with a meta-substituted phenyl ring (148, 149, 151, 152, 153, 155) and quinolinone compounds with a bulky meta-substituted phenyl ring (164, 165, 166, 207, 208, 210, 211, 212, 214), had slight inclination of becoming a promising antagonist. So we can suppose that there is a certain binding pocket near that place. The activity increases whether it is a halogen atom on the end or it is a simply alkylated methyl or isopropyl so this pocket is considerably related to steric effects. Nevertheless, this might be a hasty conclusion because there are some exceptions (167, 206) which are devoid of activity and the N-methylated benzooxazinone analogues do not fit in this propensity.

Regarding the C-region, along with the meta-positioned bulkiness, the choice of having either a CF<sub>3</sub> pyrazole or *t*-butyl pyrazole is also vital in interaction with the target protein. The derivatives having a *t*-butyl pyrazole showed less activity than the ones having a CF<sub>3</sub> pyrazole (**108 vs. 110** or **149 vs. 154** or **150 vs. 155** or **166 vs. 167** or **208 vs. 212** or **209 vs. 213**). Particularly in the case of N-methylated quinolinone, the potency drastically drops nearly 500 times when the CF<sub>3</sub> pyrazole changes into *t*-butyl pyrazoles (**166 vs. 167**). On most cases, the

activity bounces back when the *t*-butyl pyrazole is transferred into a *t*-butyl thiazole. We require further study to optimize the C-region.
# **Chapter 5. Conclusion**

Starting from the structure of BCTC, compound M4 was previously designed and synthesized with a high activity against TRPV1 receptors. Nevertheless, the M4 compound showed toxicity and the team needed a modification of the structure. More investigation has taken place and compounds with 6,6-fused heterocyclic rings were designed to show good potency without toxicity. From the benzooxazinone lead compound SAT-1251 and the quinolinone lead compound SAT-769, we wanted to identify the role of the secondary nitrogen on the A-region lactam ring and sought for higher solubility and antagonism by substituting the C-region amine into various kinds.

The structure-activity relationship of derivatives having a urea linker on the B-region, various pyridine, pyrazole and thiazole on the C-region and N-methylated or N-ethylhydroxylated benzooxazinone or quinolinone weas investigated for *h*TRPV1 antagonism. Total 77 surrogates were synthesized and 28 compounds turned out to be stronger antagonists than BCTC. None of them showed better values than their lead compounds, but still three compounds' (**111**, **166**, **193**) IC<sub>50</sub> value were comparable to their lead compounds.

On the A-region, the nitrogen on the lactam ring is revealed to be critical to potency. As another aspect, the introduction of the N-ethylhydroxyl groups were successful in gaining solubility (considering the log P value) and maintaining potency. So this joyful result is expected to give us another chance to improve.

The analysis indicated that among the current series the ones with a bulky functional group on the meta position of the phenyl C-region shows high antagonism which means that there should be an interacting pocket near the Cregion.

In conclusion, the three selected antagonists **111** (IC<sub>50</sub> [CAP] = 1.04), **166** (IC<sub>50</sub> [CAP] = 0.46) and **193** (IC<sub>50</sub> [CAP] = 0.46) showed excellent antagonism to multiple *h*TRPV1 activators. Moreover, we found some compounds like **201** (IC<sub>50</sub> [CAP] = 0.81) which has an alcohol group on the C-region and still retain potency. So we believe there is some room for improvement considering these four derivatives.

# **Experimental**

**Experimental Information** All chemical reagents were commercially available. Melting points were determined on a Büchi Melting Point B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh, Merck. Nuclear magnetic resonance (<sup>1</sup>H NMR and <sup>13</sup>C NMR) spectra were recorded on JEOL JNM-LA 300 [300 MHz (<sup>1</sup>H), 75 MHz (<sup>13</sup>C)] and Bruker Avance 400 MHz FT-NMR [400 MHz (<sup>1</sup>H), 100 MHz (<sup>13</sup>C)] spectrometers. Chemical shifts are reported in ppm units with Me4Si as a reference standard. Infrared (IR) spectra were recorded on a VG Trio-2 GC–MS and 6460 Triple Quad LC/MS.

**Biological Assay (FLIPR assay)** Cell lines: PrecisIONTM *h*TRPV1-HEK Recombinant Cell Line, Millipore, CYL3063 TRPV1 ligand: capsaicin (Sigma, M2028), MES (Sigma, M1317) Fluo4-NW (molecular Probes, F-36206) TRPV1 antagonist: Capsazepine (Sigma, C191) First, cells were plated at 5x104 cells per well and grown overnight. then medium was removed and add 100  $\mu$ L of the dye loading solution to each well. Incubated the plate at 37 °C, 5 % CO<sub>2</sub> for 30 min. Added test compounds to each wells and incubation for 30 min at room temperature. Capsaicin was added at a final concentration of 10 nM. At last we measured fluorescence using instrument settings appropriate for ex485/em535.

General Procedure for Alkylation To a solution of nitro compound (1.0 eq.) in ACN or DMF,  $Cs_2CO_3$  (1.2 eq.) was added at 0 °C. A solution of R-X (1.0 eq.) in ACN or DMF was added to the mixture. The temperature was increased to room temperature. The mixture was stirred for overnight. Then the mixture was extracted with EtOAc, washed by water several times, dried over MgSO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford the corresponding alkylated product.

**General Procedure for Carbamation** To a solution of amine (1.0 eq.) in pyridine (1.0 eq.) was added at 0 °C. A solution of Phenyl chloroformate (1.0 eq.) in ACN was added to the mixture. The temperature was increased to room temperature. The mixture was stirred for at least 2 h. Then the mixture was quenched by water, extracted with EtOAc, washed again by water several times, dried over MgSO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford the corresponding carbamate product.

General Procedure for Urea Coupling To a solution of amine (1.0 eq.) in DMF, TEA (1.2 eq.) was added at 0 °C. A solution of carbamate (1.0 eq.) in DMF was added to the mixture. The temperature was increased to room temperature. The mixture was stirred for at least 2 h. Then the mixture was extracted with EtOAc, washed by water several times, dried over MgSO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford the corresponding urea product.

**General Procedure for Silyl Deprotection** To a solution of silyl protected starting material (1.0 eq.) in MC, TBAF 1M in THF (1.1 eq.) was added at 0 °C and warmed to room temperature. The reaction mixture was stirred for 2 h. Then the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over MgSO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford the corresponding compound.

**1-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)-3-((2-(4-methylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (94).** Yield 69 %, white solid, mp 90–91 °C. <sup>1</sup>H NMR (DMSO, 400 MHz)  $\delta$  8.24 (s, 1H), 7.81 (d, *J* = 8.2 Hz, 1H), 7.79 (d, *J* = 7.6 Hz, 1H), 7.44 (d, *J* = 7.7 Hz, 1H), 7.38 (t, *J* = 5.4 Hz, 1H), 6.94 (t, *J* = 8.2 Hz, 1H), 6.75 (d, *J* = 7.9 Hz, 1H), 4.70 (s, 2H), 4.32 (m, 2H), 3.42 (m, 2H), 3.26 (s, 3H), 2.78 (m, 2H), 1.71 (m, 2H), 1.65–1.50 (br-s, 1H), 1.32 (m, 2H), 0.96 (d, *J* = 6.4 Hz, 3H). MS (FAB) *m/z* 478 (MH<sup>+</sup>).

**1-((2-(dipropylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (95).** Yield 67 %, white solid, mp 95–96 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.24 (s, 1H), 7.82 (dd, J = 8.2, 1.0 Hz, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.36 (m, 2H), 6.94 (t, J = 8.2 Hz, 1H), 6.75 (dd, J = 8.0, 1.0 Hz, 1H), 4.70 (s, 2H), 4.31 (m, 2H), 3.26 (s, 3H), 3.18 (m, 4H), 1.51 (m, 4H), 0.82 (t, J = 7.3 Hz, 6H). MS (FAB) m/z 480 (MH<sup>+</sup>).

**1-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)-3-((2-(piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (96).** Yield 61 %, white solid, mp 92–93 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.25 (s, 1H), 7.80 (m, 2H), 7.43 (d, *J* = 7.6 Hz, 1H), 7.38 (m, 1H), 6.93 (t, *J* = 7.8 Hz, 1H), 6.74 (d, *J* = 8.2 Hz, 1H), 4.69 (s, 2H), 4.31 (m, 2H), 3.25 (s, 3H), 3.08 (m, 4H), 1.65 (m, 6H). MS (FAB) *m/z* 464 (MH<sup>+</sup>).

1-((2-(4-benzylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-

**methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (97).** Yield 66 %, white solid, mp 93–94 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.26 (s, 1H), 7.82 (d, J = 8.2 Hz, 1H), 7.78 (d, J = 7.3 Hz, 1H), 7.44 (d, J = 7.6 Hz, 1H), 7.39 (m, 1H), 7.35–7.15 (br-m, 5H), 6.95 (t, J = 8.0 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 4.71 (s, 2H), 4.31 (m, 2H), 3.39 (m, 2H), 3.26 (s, 3H), 2.74 (m, 2H), 2.57 (m, 2H), 1.68 (m, 3H), 1.36 (m, 2H). MS (FAB) m/z 554 (MH<sup>+</sup>).

**1-((2-butoxy-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-methyl-3-oxo-3,4dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (98).** Yield 66 %, white solid, mp 91– 92 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.74 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 7.5 Hz, 1H), 7.17 (d, J = 7.4 Hz, 1H), 6.97 (t, J = 6.1 Hz, 1H), 6.75 (s, 1H), 6.64 (d, J = 8.1 Hz, 1H), 5.36 (t, J = 5.7 Hz, 1H), 4.57 (s, 2H), 4.40 (m, 4H), 3.32 (s, 3H), 1.75 (m, 2H), 1.43 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H). MS (FAB) *m/z* 453 (MH<sup>+</sup>).

**1-((2-isobutoxy-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (99).** Yield 60 %, white solid, mp 92–93 °C. <sup>1</sup>H NMR (DMSO, 400 MHz)  $\delta$  8.28 (s, 1H), 7.95 (s, 1H), 7.81 (m, 2H), 7.47 (d, J = 7.4 Hz, 1H), 7.31 (t, J = 5.7 Hz, 1H), 6.94 (t, J = 8.2 Hz, 1H), 6.74 (dd, J = 8.0, 0.8 Hz, 1H), 4.70 (s, 2H), 4.30 (m, 2H), 4.12 (m, 2H), 3.26 (s, 3H), 2.08 (m, 1H), 1.00 (d, J = 6.6 Hz, 6H). MS (FAB) *m/z* 453 (MH<sup>+</sup>).

**1-((2-(cyclopentylmethoxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (100).** Yield 63 %, white solid, mp 92–93 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.74 (dd, J = 8.5, 0.9 Hz, 1H), 7.69 (d, J = 7.4 Hz, 1H), 7.16 (d, J = 7.3 Hz, 1H), 6.96 (t, J = 8.2 Hz, 1H), 6.85–6.75 (br-s, 1H), 6.63 (d, J = 8.0 Hz, 1H), 5.50–5.40 (br-s, 1H), 4.56 (s, 2H), 4.41 (m, 2H), 4.26 (m, 2H), 3.32 (s, 3H), 2.34 (m, 1H), 1.78 (m, 2H), 1.59 (m, 4H), 1.33 (m, 2H). MS (FAB) *m/z* 479 (MH<sup>+</sup>).

**1-((2-(benzyloxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (101).** Yield 62 %, white solid, mp 92–93 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.30 (s, 1H), 7.82 (m, 2H), 7.52 (m, 3H), 7.35 (m, 4H), 6.94 (t, *J* = 8.2 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 5.44 (s, 2H), 4.70 (s, 2H), 4.33 (m, 2H), 3.26 (s, 3H). MS (FAB) *m/z* 487 (MH<sup>+</sup>).

**1-((2-(cyclohexylthio)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (102).** Yield 62 %, white solid, mp 94–95 °C. <sup>1</sup>H NMR (DMSO, 400 MHz)  $\delta$  8.33 (s, 1H), 7.80 (dd, *J* = 8.0, 0.7 Hz, 1H), 7.75 (d, *J* = 7.7 Hz, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.43 (t, *J* = 5.6 Hz,

1H), 6.94 (t, J = 8.2 Hz, 1H), 6.75 (dd, J = 8.0, 0.8 Hz, 1H), 4.70 (s, 2H), 4.23 (m, 2H), 3.91 (m, 1H), 3.26 (s, 3H), 2.10–2.00 (br-m, 2H), 1.80–1.70 (br-m, 2H), 1.50 (m, 6H). MS (FAB) m/z 495 (MH<sup>+</sup>).

**1-((2-(4-fluorophenyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (103).** Yield 62 %, white solid, mp 91–92 °C. <sup>1</sup>H NMR (DMSO, 400 MHz)  $\delta$  8.23 (s, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.77 (dd, J = 8.4, 0.7 Hz, 1H), 7.66 (m, 2H), 7.44 (t, J = 5.6 Hz, 1H), 7.37 (m, 2H), 6.92 (t, J = 8.2 Hz, 1H), 6.74 (dd, J = 8.1, 0.7 Hz, 1H), 4.70 (s, 2H), 4.39 (m, 2H), 3.25 (s, 3H). MS (FAB) *m/z* 475 (MH<sup>+</sup>).

**1-((2-(3-chlorophenyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (104).** Yield 69 %, white solid, mp 91–92 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.25 (s, 1H), 8.10 (d, J = 7.8 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 8.2 Hz, 1H), 7.65 (m, 1H), 7.58 (m, 3H), 7.45 (t, J = 5.8 Hz, 1H), 6.93 (t, J = 8.2 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 4.70 (s, 2H), 4.40 (m, 2H), 3.26 (s, 3H). MS (FAB) m/z 491 (MH<sup>+</sup>).

**1-((2-(3-chloro-4-fluorophenyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (105).** Yield 61 %, white solid, mp 91–92 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.22 (s, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.82 (dd, J = 7.1, 2.0 Hz, 1H), 7.75 (d, J = 8.2 Hz, 1H), 7.60 (m, 2H), 7.42 (t, J = 5.6 Hz, 1H), 6.91 (t, J = 8.2 Hz, 1H), 6.73 (d, J = 8.2 Hz, 1H), 4.69 (s, 2H), 4.39 (m, 2H), 3.24 (s, 3H). MS (FAB) *m/z* 509 (MH<sup>+</sup>).

**1-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)-3-((2-(m-tolyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (106).** Yield 62 %, white solid, mp 93–94 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.25 (s, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.78 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.37 (m, 5H), 6.93 (t, *J* = 8.2 Hz, 1H), 6.74 (dd, *J* = 8.0, 1.2 Hz, 1H), 4.70 (s, 2H), 4.39 (m, 2H), 3.25 (s, 3H), 2.40 (s, 3H). MS (FAB) *m/z* 471 (MH<sup>+</sup>).

**1-((2-(3-isopropylphenyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (107).** Yield 63 %, white solid, mp 91–92 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.25 (s, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.44 (m, 5H), 6.93 (t, *J* = 8.2 Hz, 1H), 6.74 (d, *J* = 7.3 Hz, 1H), 4.70 (s, 2H), 4.39 (m, 2H), 3.26 (s, 3H), 2.99 (m, 1H), 1.25 (d, *J* = 6.7 Hz, 6H). MS (FAB) *m/z* 499 (MH<sup>+</sup>).

**1-((1-(3-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)-3-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (108).** Yield 64 %, white solid, mp 91–92 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.18 (s, 1H), 7.77 (m, 2H), 7.62 (m, 3H), 7.35 (t, J = 5.3 Hz, 1H), 6.94 (t, J = 8.2 Hz, 1H), 6.84 (s, 1H), 6.75 (d, J = 6.9 Hz, 1H), 4.69 (s, 2H), 4.42 (m, 2H), 3.25 (s, 3H). MS (FAB) m/z 480 (MH<sup>+</sup>).

**1-((1-(3-chloro-4-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)-3-**(**4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea** (109). Yield 66 %, white solid, mp 100–101 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.15 (s, 1H), 7.96 (m, 1H), 7.76 (d, J = 8.2 Hz, 1H), 7.65 (m, 2H), 7.31 (t, J = 5.4 Hz, 1H), 6.94 (t, J = 8.2 Hz, 1H), 6.84 (s, 1H), 6.75 (d, J = 8.2 Hz, 1H), 4.69 (s, 2H), 4.41 (m, 2H), 3.26 (s, 3H). MS (FAB) *m/z* 498 (MH<sup>+</sup>).

**1-((3-(tert-butyl)-1-(3-chlorophenyl)-1H-pyrazol-5-yl)methyl)-3-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (110).** Yield 63 %, white solid, mp 94–95 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.15 (s, 1H), 7.82 (d, J = 8.2 Hz, 1H), 7.61 (m, 1H), 7.51 (m, 3H), 7.28 (t, J = 5.1 Hz, 1H), 6.95 (t, J = 8.2 Hz, 1H), 6.74 (d, J = 8.2 Hz, 1H), 6.33 (s, 1H), 4.69 (s, 2H), 4.40 (m, 2H), 3.26 (s, 3H), 1.28 (s, 9H). MS (FAB) *m/z* 468 (MH<sup>+</sup>).

**1-((2-(tert-butyl)-4-(3-chlorophenyl)thiazol-5-yl)methyl)-3-(4-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (111).** Yield 62 %, white solid, mp 100–101 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.17 (s, 1H), 7.85 (d, *J* = 8.2 Hz, 1H), 7.54 (m, 5H), 6.96 (t, *J* = 8.2 Hz, 1H), 6.76 (d, *J* = 8.2 Hz, 1H), 4.70 (s, 2H), 4.55 (m, 2H), 3.26 (s, 3H), 1.39 (s, 9H). MS (FAB) *m/z* 485 (MH<sup>+</sup>).

**1-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)-3-((2-(4-methylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea** (134). Yield 62 %, white solid, mp 180–181 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.28 (s, 1H), 7.79 (m, 2H), 7.43 (m, 2H), 6.90 (m, 2H), 4.89 (m, 1H), 4.69 (s, 2H), 4.32 (m, 2H), 3.94 (t, *J* = 6.2 Hz, 2H), 3.56 (m, 2H), 3.43 (m, 2H), 2.78 (m, 2H), 1.72 (m, 2H), 1.65–1.50 (br-s, 1H), 1.29 (m, 2H), 0.96 (d, *J* = 6.2 Hz, 3H). MS (FAB) *m/z* 508 (MH<sup>+</sup>).

1-((2-(dipropylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea(135).Yield 62 %, white solid, mp 184–185 °C. <sup>1</sup>H NMR (DMSO, 300 MHz) δ 8.22 (s,1H), 7.76 (m, 2H), 7.36 (m, 2H), 6.88 (m, 2H), 4.85 (t, J = 5.4 Hz, 1H), 4.67 (s,

2H), 4.29 (m, 2H), 3.93 (t, J = 5.8 Hz, 2H), 3.55 (m, 2H), 3.16 (m, 4H), 1.49 (m, 4H), 0.81 (t, J = 7.1 Hz, 6H). MS (FAB) m/z 510 (MH<sup>+</sup>).

**1-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)-3-((2-(pyrrolidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (136).** Yield 59 %, white solid, mp 97–98 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.22 (s, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 7.5 Hz, 1H), 7.27 (t, J = 5,3 Hz, 1H), 7.10 (d, J = 7.5 Hz, 1H), 6.90 (m, 2H), 4.87 (t, J = 5.6 Hz, 1H), 4.68 (s, 2H), 4.40 (m, 2H), 3.94 (t, J = 6.2 Hz, 2H), 3.56 (m, 6H), 1.89 (m, 4H). MS (FAB) m/z 480 (MH<sup>+</sup>).

**1-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)-3-((2-(piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (137).** Yield 62 %, white solid, mp 174–175 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.26 (s, 1H), 7.79 (m, 2H), 7.45 (d, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 5.3 Hz, 1H), 6.90 (m, 2H), 4.87 (t, *J* = 5.6 Hz, 1H), 4.69 (s, 2H), 4.33 (m, 2H), 3.94 (t, *J* = 6.4 Hz, 2H), 3.56 (m, 2H), 3.09 (m, 4H), 1.63 (m, 6H). MS (FAB) *m/z* 494 (MH<sup>+</sup>).

**1-((2-(4-benzylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea** (138). Yield 64 %, white solid, mp 192–193 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.25 (s, 1H), 7.79 (m, 2H), 7.42 (m, 2H), 7.29 (m, 2H), 7.19 (m, 3H), 6.90 (m, 2H), 4.87 (t, *J* = 5.6 Hz, 1H), 4.69 (s, 2H), 4.31 (m, 2H), 3.94 (t, *J* = 6.0 Hz, 2H), 3.56 (m, 2H), 3.39 (m, 2H), 2.74 (t, *J* = 11.8 Hz, 2H), 2.57 (m, 2H), 1.68 (m, 3H), 1.37 (m, 2H). MS (FAB) *m/z* 584 (MH<sup>+</sup>).

**1-((2-butoxy-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (139).** Yield 60 %, white solid, mp 172–173 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.27 (s, 1H), 7.77 (m, 2H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.31 (t, *J* = 5.3 Hz, 1H), 6.88 (m, 2H), 4.85 (t, *J* = 5.4 Hz, 1H), 4.67 (s, 2H), 4.33 (t, *J* = 6.4 Hz, 2H), 4.27 (m, 2H), 3.93 (t, *J* = 6.0 Hz, 2H), 3.54 (m, 2H), 1.73 (m, 2H), 1.44 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H). MS (FAB) *m/z* 483 (MH<sup>+</sup>).

**1-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)-3-((2-(pentyloxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (140).** Yield 62 %, white solid, mp 141–142 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.29 (s, 1H), 7.78 (m, 2H), 7.47 (d, J = 7.5 Hz, 1H), 7.33 (t, J = 5.6 Hz, 1H), 6.90 (m, 2H), 4.87 (t, J = 5.6 Hz, 1H), 4.68 (s, 2H), 4.34 (t, J = 6.5 Hz, 2H), 4.28 (m, 2H), 3.94 (t, J = 6.2 Hz, 2H), 3.56 (m, 2H), 1.75 (m, 2H), 1.35 (m, 4H), 0.88 (t, J = 7.1 Hz, 3H). MS (FAB)

*m*/*z* 497 (MH<sup>+</sup>).

#### 1-((2-(hexyloxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-(2-

hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (141). Yield 58 %, white solid, mp 172–173 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.29 (s, 1H), 7.78 (m, 2H), 7.47 (d, J = 7.5 Hz, 1H), 7.33 (t, J = 6.0 Hz, 1H), 6.90 (m, 2H), 4.87 (t, J = 5.4 Hz, 1H), 4.68 (s, 2H), 4.34 (t, J = 6.3 Hz, 2H), 4.28 (m, 2H), 3.94 (t, J = 6.2 Hz, 2H), 3.56 (m, 2H), 1.76 (m, 2H), 1.43 (m, 2H), 1.29 (m, 4H), 0.86 (t, J = 6.9 Hz, 3H). MS (FAB) m/z 511 (MH<sup>+</sup>).

**1-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)-3-((2-isobutoxy-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (142).** Yield 61 %, white solid, mp 179–180 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.29 (s, 1H), 7.78 (m, 2H), 7.48 (d, J = 7.5 Hz, 1H), 7.33 (t, J = 5.4 Hz, 1H), 6.90 (m, 2H), 4.86 (t, J = 5.8 Hz, 1H), 4.68 (s, 2H), 4.31 (m, 2H), 4.12 (d, J = 6.3 Hz, 2H), 3.94 (t, J = 6.2 Hz, 2H), 3.56 (m, 2H), 2.09 (m, 1H), 1.01 (d, J = 6.7 Hz, 6H). MS (FAB) *m/z* 483 (MH<sup>+</sup>).

**1-((2-(cyclobutylmethoxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea** (143). Yield 59 %, white solid, mp 79–80 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.30 (s, 1H), 7.78 (m, 2H), 7.48 (d, *J* = 7.5 Hz, 1H), 7.33 (t, *J* = 5.4 Hz, 1H), 6.90 (m, 2H), 4.87 (t, *J* = 5.6 Hz, 1H), 4.68 (s, 2H), 4.31 (m, 4H), 3.94 (t, *J* = 6.0 Hz, 2H), 3.56 (m, 2H), 2.73 (m, 1H), 2.04 (m, 2H), 1.88 (m, 4H). MS (FAB) *m/z* 495 (MH<sup>+</sup>).

**1-((2-(cyclopentylmethoxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea** (144). Yield 62 %, white solid, mp 140–141 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.30 (s, 1H), 7.78 (m, 2H), 7.47 (d, J = 7.3 Hz, 1H), 7.33 (t, J = 5.8 Hz, 1H), 6.90 (m, 2H), 4.87 (t, J = 5.6 Hz, 1H), 4.68 (s, 2H), 4.29 (m, 2H), 4.23 (d, J = 6.7 Hz, 2H), 3.94 (t, J = 6.0 Hz, 2H), 3.55 (m, 2H), 2.34 (m, 1H), 1.77 (m, 2H), 1.58 (m, 4H), 1.38 (m, 2H). MS (FAB) m/z 509 (MH<sup>+</sup>).

# **1-((2-(benzyloxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea** (145). Yield 60 %, white solid, mp 175–176 °C. <sup>1</sup>H NMR (DMSO, 300 MHz) $\delta$ 8.29 (s, 1H), 7.78 (m, 2H), 7.51 (m, 3H), 7.35 (m, 4H), 6.88 (m, 2H), 5.43 (s, 2H), 4.86 (t, J = 4.9 Hz, 1H), 4.66 (s, 2H), 4.32 (m, 2H), 3.92 (t, J = 5.8 Hz, 2H), 3.54 (m, 2H). MS (FAB) m/z 517 (MH<sup>+</sup>).

# **1-((2-(cyclohexylthio)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea** (146). Yield 66 %, white solid, mp 167–168 °C. <sup>1</sup>H NMR (DMSO, 300 MHz) $\delta$ 8.35 (s, 1H), 7.77 (m, 2H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.45 (m, 1H), 6.90 (m, 2H), 4.87 (t, *J* = 5.6 Hz, 1H), 4.69 (s, 2H), 4.23 (m, 2H), 3.92 (m, 3H), 3.57 (m, 2H), 2.06 (m, 2H), 1.73 (m, 2H), 1.44 (m, 6H). MS (FAB) *m/z* 525 (MH<sup>+</sup>).

#### 1-((2-(4-fluorophenyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(4-(2-

hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (147). Yield 61 %, white solid, mp 100–101 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.23 (s, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.64 (m, 2H), 7.44 (m, 1H), 7.36 (m, 2H), 6.87 (m, 2H), 4.86 (t, J = 5.6 Hz, 1H), 4.66 (s, 2H), 4.38 (m, 2H), 3.92 (t, J = 6.0 Hz, 2H), 3.55 (m, 2H). MS (FAB) m/z 505 (MH<sup>+</sup>).

**1-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)-3-((2-(3-isopropylphenyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (148).** Yield 58 %, white solid, mp 174–175 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.25 (s, 1H), 8.07 (d, *J* = 8.2 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.44 (m, 5H), 6.89 (m, 2H), 4.87 (t, *J* = 5.6 Hz, 1H), 4.68 (s, 2H), 4.39 (m, 2H), 3.94 (t, *J* = 6.2 Hz, 2H), 3.56 (m, 2H), 2.99 (m, 1H), 1.25 (d, *J* = 6.9 Hz, 6H). MS (FAB) *m/z* 529 (MH<sup>+</sup>).

**1-((1-(3-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea** (149). Yield 65 %, white solid, mp 185–186 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.17 (s, 1H), 7.75 (m, 2H), 7.63 (m, 3H), 7.36 (t, J = 5.3 Hz, 1H), 6.92 (m, 2H), 6.84 (s, 1H), 4.87 (t, J = 5.8 Hz, 1H), 4.67 (s, 2H), 4.42 (m, 2H), 3.94 (t, J = 6.2 Hz, 2H), 3.55 (m, 2H). MS (FAB) m/z 510 (MH<sup>+</sup>).

**1-((1-(3-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea** (150). Yield 58 %, white solid, mp 179–180 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.16 (s, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.59 (m, 2H), 7.42 (m, 2H), 7.35 (t, J = 5.4 Hz, 1H), 6.87 (m, 3H), 4.86 (t, J = 6.0 Hz, 1H), 4.66 (s, 2H), 4.42 (m, 2H), 3.92 (t, J = 6.2 Hz, 2H), 3.54 (m, 2H). MS (FAB) m/z 494 (MH<sup>+</sup>).

1-((1-(3-chloro-4-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (151). Yield 59 %, white solid, mp 212–213 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.12 (s, 1H), 7.95 (m, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.63 (m, 2H), 7.30 (t, *J* = 5.3 Hz, 1H), 6.90 (m, 2H), 6.82 (s, 1H), 4.86 (t, *J* = 5.8 Hz, 1H), 4.65 (s, 2H), 4.39 (m, 2H), 3.92 (t, *J* = 6.0 Hz, 2H), 3.54 (m, 2H). MS (FAB) *m/z* 528 (MH<sup>+</sup>).

## 1-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)-3-((1-(3-isopropylphenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)urea (152). Yield 59 %, white solid, mp 87–88 °C. <sup>1</sup>H NMR (DMSO, 300 MHz) $\delta$ 8.18 (s, 1H),

7.77 (d, J = 7.8 Hz, 1H), 7.45 (m, 5H), 6.90 (m, 2H), 6.81 (s, 1H), 4.87 (t, J = 6.0 Hz, 1H), 4.67 (s, 2H), 4.38 (m, 2H), 3.94 (t, J = 6.0 Hz, 2H), 3.56 (m, 2H), 3.00 (m, 1H), 1.24 (d, J = 6.7 Hz, 6H). MS (FAB) m/z 518 (MH<sup>+</sup>).

# **1-((3-(tert-butyl)-1-(3-chlorophenyl)-1H-pyrazol-5-yl)methyl)-3-(4-(2-hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea** (153). Yield 60 %, white solid, mp 93–94 °C. <sup>1</sup>H NMR (DMSO, 300 MHz) $\delta$ 8.14 (s, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.51 (m, 4H), 7.27 (t, J = 5.1 Hz, 1H), 6.89 (m, 2H), 6.32 (s, 1H), 4.86 (t, J = 5.8 Hz, 1H), 4.66 (s, 2H), 4.39 (m, 2H), 3.92 (t, J = 6.2 Hz, 2H), 3.54 (m, 2H), 1.26 (s, 9H). MS (FAB) m/z 498 (MH<sup>+</sup>).

# 1-((3-(tert-butyl)-1-(3-fluorophenyl)-1H-pyrazol-5-yl)methyl)-3-(4-(2-

hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (154). Yield 62 %, white solid, mp 133–134 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.15 (s, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.55 (m, 1H), 7.39 (m, 2H), 7.26 (m, 2H), 6.90 (m, 2H), 6.33 (s, 1H), 4.87 (t, J = 5.8 Hz, 1H), 4.67 (s, 2H), 4.40 (m, 2H), 3.91 (m, 2H), 3.55 (m, 2H), 1.27 (s, 9H). MS (FAB) m/z 482 (MH<sup>+</sup>).

#### 1-((2-(tert-butyl)-4-(3-chlorophenyl)thiazol-5-yl)methyl)-3-(4-(2-

hydroxyethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)urea (155). Yield 59 %, white solid, mp 178–179 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.14 (s, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.68 (s, 1H), 7.52 (m, 4H), 6.90 (m, 2H), 4.85 (t, J = 5.6 Hz, 1H), 4.66 (s, 2H), 4.53 (m, 2H), 3.93 (t, J = 6.2 Hz, 2H), 3.54 (m, 2H), 1.38 (s, 9H). MS (FAB) m/z 516 (MH<sup>+</sup>).

# **1-(1-methyl-2-oxo-1,2-dihydroquinolin-5-yl)-3-((2-(4-methylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (156).** Yield 63 %, white solid, mp 200–201 °C. <sup>1</sup>H NMR (DMSO, 300 MHz) $\delta$ 8.85 (s, 1H), 8.05 (d, *J* = 10.0 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.60–7.40 (br-m, 2H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.09 (t, *J* = 5.6 Hz, 1H), 6.64 (d, *J* = 9.9 Hz, 1H), 4.36 (m, 2H), 3.61 (s, 3H), 3.39 (m, 2H), 2.79 (m, 2H), 1.72 (m, 2H), 1.65–1.50 (br-s, 1H), 1.33 (m,

2H), 0.96 (d, *J* = 6.3 Hz, 3H). MS (FAB) *m*/*z* 474 (MH<sup>+</sup>).

**1-((2-(dipropylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-methyl-2-oxo-1,2-dihydroquinolin-5-yl)urea (157).** Yield 62 %, white solid, mp 170–171 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.78 (s, 1H), 8.05 (d, *J* = 9.9 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.52 (t, *J* = 8.2 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 7.02 (m, 1H), 6.65 (d, *J* = 9.7 Hz, 1H), 4.35 (m, 2H), 3.61 (s, 3H), 3.18 (m, 4H), 1.51 (m, 4H), 0.83 (t, *J* = 7.3 Hz, 6H). MS (FAB) *m/z* 476 (MH<sup>+</sup>).

#### 1-(1-methyl-2-oxo-1,2-dihydroquinolin-5-yl)-3-((2-(piperidin-1-yl)-6-

(trifluoromethyl)pyridin-3-yl)methyl)urea (158). Yield 66 %, white solid, mp 210–211 °C. <sup>1</sup>H NMR (DMSO, 300 MHz) δ 8.85 (s, 1H), 8.07 (d, *J* = 9.6 Hz, 1H), 7.84 (d, *J* = 7.5 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.50 (m, 2H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.09 (m, 1H), 6.64 (d, *J* = 9.9 Hz, 1H), 4.37 (m, 2H), 3.61 (s, 3H), 3.20–3.00 (br-m, 4H), 1.70–1.60 (br-m, 6H). MS (FAB) *m/z* 460 (MH<sup>+</sup>).

**1-((2-(4-benzylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-methyl-2-oxo-1,2-dihydroquinolin-5-yl)urea (159).** Yield 63 %, white solid, mp 223–224 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.81 (s, 1H), 8.05 (d, *J* = 10.0 Hz, 1H), 7.83 (d, *J* = 7.7 Hz, 1H), 7.64 (d, *J* = 8.2 Hz, 1H), 7.50 (m, 2H), 7.29 (m, 2H), 7.20 (m, 5H), 7.01 (m, 1H), 6.64 (d, *J* = 9.8 Hz, 1H), 4.36 (m, 2H), 3.61 (s, 1H), 3.44 (m, 2H), 2.74 (m, 2H), 2.57 (m, 3H), 1.69 (m, 3H), 1.35 (m, 2H). MS (FAB) *m/z* 550 (MH<sup>+</sup>).

**1-((2-butoxy-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-methyl-2-oxo-1,2-dihydroquinolin-5-yl)urea (160).** Yield 64 %, white solid, mp 195–196 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.84 (s, 1H), 8.06 (d, *J* = 9.9 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.52 (m, 2H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.99 (m, 1H), 6.64 (d, *J* = 9.9 Hz, 1H), 4.34 (m, 4H), 3.60 (s, 3H), 1.75 (m, 2H), 1.45 (m, 2H), 0.93 (t, *J* = 7.2 Hz, 3H). MS (FAB) *m/z* 449 (MH<sup>+</sup>).

**1-((2-isobutoxy-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-methyl-2-oxo-1,2-dihydroquinolin-5-yl)urea (161).** Yield 62 %, white solid, mp 189–190 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.85 (s, 1H), 8.06 (d, J = 9.8 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.65 (d, J = 8.2 Hz, 1H), 7.50 (m, 2H), 7.18 (d, J = 8.2 Hz, 1H), 6.99 (t, J = 5.7 Hz, 1H), 6.64 (d, J = 9.5 Hz, 1H), 4.35 (m, 2H), 4.12 (d, J = 6.2 Hz, 2H), 3.60 (s, 3H), 2.09 (m, 1H), 1.01 (d, J = 6.2 Hz, 6H). MS (FAB) m/z 449 (MH<sup>+</sup>).

**1-((2-(cyclopentylmethoxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1methyl-2-oxo-1,2-dihydroquinolin-5-yl)urea (162).** Yield 61 %, white solid, mp 195–196 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.84 (s, 1H), 8.07 (d, *J* = 9.8 Hz, 1H), 7.82 (d, *J* = 7.5 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.50 (m, 2H), 7.19 (d, *J* = 8.6 Hz, 1H), 6.97 (t, *J* = 5.7 Hz, 1H), 6.64 (d, *J* = 9.9 Hz, 1H), 4.34 (m, 2H), 4.23 (d, *J* = 6.9 Hz, 2H), 3.61 (s, 3H), 2.35 (m, 1H), 1.77 (m, 2H), 1.58 (m, 4H), 1.38 (m, 2H). MS (FAB) *m/z* 475 (MH<sup>+</sup>).

**1-((2-(cyclohexylthio)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-methyl-2-oxo-1,2-dihydroquinolin-5-yl)urea (163).** Yield 63 %, white solid, mp 193–194 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.89 (s, 1H), 8.07 (d, *J* = 10.0 Hz, 1H), 7.78 (d, *J* = 7.7 Hz, 1H), 7.64 (m, 2H), 7.50 (t, *J* = 8.4 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.11 (m, 1H), 6.63 (d, *J* = 9.8 Hz, 1H), 4.26 (m, 2H), 3.91 (m, 1H), 3.59 (s, 3H), 2.05 (m, 2H), 1.72 (m, 2H), 1.46 (m, 6H). MS (FAB) *m/z* 491 (MH<sup>+</sup>).

#### 1-(1-methyl-2-oxo-1,2-dihydroquinolin-5-yl)-3-((2-(m-tolyl)-6-

(trifluoromethyl)pyridin-3-yl)methyl)urea (164). Yield 67 %, white solid, mp 248–249 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.81 (s, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 8.02 (d, *J* = 9.8 Hz, 1H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.43 (m, 5H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.09 (m, 1H), 6.63 (d, *J* = 9.9 Hz, 1H), 4.43 (m, 2H), 3.60 (s, 3H), 2.40 (s, 3H). MS (FAB) *m*/*z* 467 (MH<sup>+</sup>).

**1-((2-(3-isopropylphenyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-methyl-2-oxo-1,2-dihydroquinolin-5-yl)urea (165).** Yield 67 %, white solid, mp 223–224 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.79 (s, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 9.8 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.46 (m, 5H), 7.19 (d, *J* = 8.2 Hz, 1H), 7.08 (t, *J* = 5.8 Hz, 1H), 6.64 (d, *J* = 9.6 Hz, 1H), 4.43 (m, 2H), 3.60 (s, 3H), 2.99 (m, 1H), 1.25 (d, *J* = 6.9 Hz, 6H). MS (FAB) *m/z* 495 (MH<sup>+</sup>).

**1-((1-(3-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)-3-(1-methyl-2-oxo-1,2-dihydroquinolin-5-yl)urea (166).** Yield 62 %, white solid, mp 193–194 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.76 (s, 1H), 7.98 (d, J = 9.8 Hz, 1H), 7.76 (m, 1H), 7.60–7.45 (br-m, 5H), 7.19 (d, J = 7.7 Hz, 1H), 7.08 (m, 1H), 6.89 (s, 1H), 6.61 (d, J = 9.9 Hz, 1H), 4.46 (m, 2H), 3.60 (s, 3H). MS (FAB) *m/z* 476 (MH<sup>+</sup>).

1-((3-(tert-butyl)-1-(3-chlorophenyl)-1H-pyrazol-5-yl)methyl)-3-(1-methyl-2oxo-1,2-dihydroquinolin-5-yl)urea (167). Yield 61 %, white solid, mp 182–183 <sup>o</sup>C. <sup>1</sup>H NMR (DMSO, 300 MHz) δ 8.68 (s, 1H), 7.83 (m, 2H), 7.58 (m, 6H), 6.66 (s, 1H), 6.58 (d, *J* = 9.3 Hz, 1H), 6.37 (s, 1H), 4.43 (m, 2H), 3.58 (s, 3H), 1.28 (s, 9H). MS (FAB) *m/z* 464 (MH<sup>+</sup>).

#### 1-((2-(tert-butyl)-4-(3-chlorophenyl)thiazol-5-yl)methyl)-3-(1-methyl-2-oxo-

**1,2-dihydroquinolin-5-yl)urea (168).** Yield 67 %, white solid, mp 98–99 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.72 (s, 1H), 8.01 (d, *J* = 9.9 Hz, 1H), 7.71 (s, 1H), 7.64 (m, 2H), 7.50 (m, 3H), 7.22 (m, 2H), 6.62 (d, *J* = 9.9 Hz, 1H), 4.59 (m, 2H), 3.61 (s, 3H), 1.40 (s, 9H). MS (FAB) *m/z* 482 (MH<sup>+</sup>).

#### 1-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)-3-((2-(4-

**methylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (192).** Yield 62 %, white solid, mp 181–182 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.80 (s, 1H), 7.40 (d, J = 10.2 Hz, 1H), 7.83 (d, J = 7.5 Hz, 1H), 7.50 (m, 3H), 7.32 (d, J = 8.4 Hz, 1H), 7.04 (t, J = 5.7 Hz, 1H), 6.62 (d, J = 9.6 Hz, 1H), 4.96 (t, J = 6.3 Hz, 1H), 4.35 (m, 2H), 4.30 (t, J = 6.9 Hz, 1H), 3.61 (m, 2H), 2.77 (m, 2H), 1.71 (m, 2H), 1.70–1.50 (br-s, 1H), 1.30 (m, 2H), 0.95 (d, J = 6.3 Hz, 3H). MS (FAB) *m/z* 504 (MH<sup>+</sup>).

#### 1-((2-(dipropylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-(2-

**hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea** (193). Yield 60 %, white solid, mp 174–175 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.76 (s, 1H), 8.04 (d, *J* = 9.9 Hz, 1H), 7.81 (d, *J* = 7.5 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.50 (t, *J* = 8.2 Hz, 1H), 7.34 (m, 2H), 7.02 (m, 1H), 6.63 (d, *J* = 9.6 Hz, 1H), 4.91 (m, 1H), 4.31 (m, 4H), 3.62 (m, 2H), 3.18 (m, 4H), 1.51 (m, 4H), 0.83 (t, *J* = 7.3 Hz, 6H). MS (FAB) *m/z* 506 (MH<sup>+</sup>).

# **1-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)-3-((2-(pyrrolidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (194).** Yield 61 %, white solid, mp 192–193 °C. <sup>1</sup>H NMR (DMSO, 300 MHz) $\delta$ 8.73 (s, 1H), 8.04 (d, *J* = 9.9 Hz, 1H), 7.69 (d, *J* = 7.5 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.49 (t, *J* = 8.4 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 6.95 (m, 1H), 6.62 (d, *J* = 9.7 Hz, 1H), 4.92 (t, *J* = 5.3 Hz, 1H), 4.44 (m, 2H), 4.30 (t, *J* = 6.4 Hz, 2H), 3.58 (m, 6H), 1.92 (m, 4H). MS (FAB) *m/z* 476 (MH<sup>+</sup>).

**1-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)-3-((2-(piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea (195).** Yield 59 %, white solid, mp 184–185 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.83 (s, 1H), 8.06 (d, *J* = 10.0 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.48 (m, 2H), 7.32 (d, *J* = 8.6 Hz,

1H), 7.11 (m, 1H), 6.63 (d, J = 9.7 Hz, 1H), 4.93 (t, J = 5.6 Hz, 1H), 4.37 (m, 2H), 4.30 (t, J = 6.3 Hz, 2H), 3.61 (m, 2H), 3.10 (m, 4H), 1.80–1.60 (br-m, 6H). MS (FAB) m/z 490 (MH<sup>+</sup>).

**1-((2-(4-benzylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (196).** Yield 61 %, white solid, mp 126–127 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.78 (s, 1H), 8.04 (d, *J* = 9.8 Hz, 1H), 7.83 (d, *J* = 7.8 Hz, 1H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.50 (m, 2H), 7.30 (m, 3H), 7.19 (m, 3H), 7.03 (m, 1H), 6.63 (d, *J* = 9.9 Hz, 1H), 4.92 (t, *J* = 5.8 Hz, 1H), 4.35 (m, 2H), 4.30 (t, *J* = 6.9 Hz, 2H), 3.62 (m, 2H), 3.45 (m, 2H), 2.74 (t, *J* = 11.7 Hz, 2H), 2.57 (m, 2H), 1.68 (m, 3H), 1.37 (m, 2H). MS (FAB) *m/z* 580 (MH<sup>+</sup>).

**1-((2-butoxy-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (197).** Yield 63 %, white solid, mp 174–175 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.80 (s, 1H), 8.05 (d, *J* = 9,8 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.50 (m, 2H), 7.31 (d, *J* = 8.4 Hz, 1H), 6.96 (t, *J* = 5.6 Hz, 1H), 6.63 (d, *J* = 9.7 Hz, 1H), 4.93 (t, *J* = 5.8 Hz, 1H), 4.33 (m, 6H), 3.61 (m, 2H), 1.75 (m, 2H), 1.45 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 3H). MS (FAB) *m/z* 479 (MH<sup>+</sup>).

#### 1-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)-3-((2-(pentyloxy)-6-

(trifluoromethyl)pyridin-3-yl)methyl)urea (198). Yield 63 %, white solid, mp 174–175 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.82 (s, 1H), 8.07 (d, *J* = 9,8 Hz, 1H), 7.81 (d, *J* = 7,5 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.50 (m, 2H), 7.30 (d, *J* = 8.6 Hz, 1H), 6.99 (t, *J* = 5.8 Hz, 1H), 6.63 (d, *J* = 9.6 Hz, 1H), 4.93 (t, *J* = 5.6 Hz, 1H), 4.32 (m, 6H), 3.61 (m, 2H), 1.77 (m, 2H), 1.37 (m, 4H), 0.88 (t, *J* = 7.1 Hz, 3H). MS (FAB) *m*/z 493 (MH<sup>+</sup>).

#### 1-((2-(hexyloxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-(2-

**hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (199).** Yield 62 %, white solid, mp 180–181 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.80 (s, 1H), 8.06 (d, *J* = 9.9 Hz, 1H), 7.81 (d, *J* = 7.5 Hz, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.50 (m, 2H), 7.31 (d, *J* = 8.4 Hz, 1H), 6.96 (t, *J* = 5.8 Hz, 1H), 6.63 (d, *J* = 9.6 Hz, 1H), 4.93 (t, *J* = 5.8 Hz, 1H), 4.33 (m, 6H), 3.61 (m, 2H), 1.76 (m, 2H), 1.43 (m, 2H), 1.28 (m, 4H), 0.85 (t, *J* = 6.9 Hz, 3H). MS (FAB) *m/z* 507 (MH<sup>+</sup>).

#### 1-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)-3-((2-isobutoxy-6-

(trifluoromethyl)pyridin-3-yl)methyl)urea (200). Yield 62 %, white solid, mp 163–164 °C. <sup>1</sup>H NMR (DMSO, 400 MHz)  $\delta$  8.76 (s, 1H), 8.05 (d, *J* = 9.8 Hz, 1H),

7.82 (d, J = 7.4 Hz, 1H), 7.59 (d, J = 7.9 Hz, 1H), 7.49 (m, 2H), 7.31 (d, J = 8.5 Hz, 1H), 6.93 (t, J = 5.8 Hz, 1H), 6.62 (d, J = 9.9 Hz, 1H), 4.89 (t, J = 5.8 Hz, 1H), 4.35 (m, 2H), 4.30 (t, J = 6.6 Hz, 2H), 4.13 (d, J = 6.4 Hz, 2H), 3.62 (m, 2H), 2.08 (m, 1H), 1.00 (d, J = 6.7 Hz, 6H). MS (FAB) m/z 479 (MH<sup>+</sup>).

**1-((2-(cyclobutylmethoxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (201).** Yield 61 %, white solid, mp 187–188 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.81 (s, 1H), 8.06 (d, *J* = 9.9 Hz, 1H), 7.81 (d, *J* = 7.5 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.50 (m, 2H), 7.31 (d, *J* = 8.6 Hz, 1H), 6.96 (t, *J* = 5.8 Hz, 1H), 6.63 (d, *J* = 9.9 Hz, 1H), 4.93 (t, *J* = 5.6 Hz, 1H), 4.31 (m, 6H), 3.62 (m, 2H), 2.77 (m, 1H), 2.05 (m, 2H), 1.91 (m, 4H). MS (FAB) *m/z* 491 (MH<sup>+</sup>).

**1-((2-(cyclopentylmethoxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (202).** Yield 62 %, white solid, mp 181–182 °C. <sup>1</sup>H NMR (DMSO, 400 MHz)  $\delta$  8.76 (s, 1H), 8.05 (d, *J* = 9.8 Hz, 1H), 7.82 (d, *J* = 7.4 Hz, 1H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.49 (m, 2H), 7.31 (d, *J* = 8.5 Hz, 1H), 6.92 (t, *J* = 5.8 Hz, 1H), 6.62 (d, *J* = 9.9 Hz, 1H), 4.89 (t, *J* = 5.7 Hz, 1H), 4.32 (m, 4H), 4.24 (m, 2H), 3.62 (m, 2H), 2.35 (m, 1H), 1.79 (m, 2H), 1.70–1.45 (br-m, 4H), 1.37 (m, 2H). MS (FAB) *m/z* 505 (MH<sup>+</sup>).

#### 1-((2-(benzyloxy)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-(2-

**hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (203).** Yield 64 %, white solid, mp 198–199 °C. <sup>1</sup>H NMR (DMSO, 400 MHz)  $\delta$  8.77 (s, 1H), 8.04 (d, *J* = 9.8 Hz, 1H), 7.86 (d, *J* = 7.4 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.52 (m, 4H), 7.38 (m, 2H), 7.32 (m, 2H), 6.98 (t, *J* = 5.8 Hz, 1H), 6.62 (d, *J* = 9.8 Hz, 1H), 5.45 (s, 2H), 4.89 (t, *J* = 5.7 Hz, 1H), 4.37 (m, 2H), 4.30 (d, *J* = 6.6 Hz, 2H), 3.61 (m, 2H). MS (FAB) *m/z* 513 (MH<sup>+</sup>).

#### 1-((2-(cyclohexylthio)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-(2-

**hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (204).** Yield 63 %, white solid, mp 169–170 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.89 (s, 1H), 8.08 (d, J = 9.8 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.62 (m, 2H), 7.50 (t, J = 8.4 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 7.12 (t, J = 5.8 Hz, 1H), 6.63 (d, J = 9.9 Hz, 1H), 4.93 (t, J = 5.8 Hz, 1H), 4.29 (m, 4H), 3.61 (m, 2H), 2.10–2.00 (br-m, 2H), 1.80–1.70 (br-m, 2H), 1.70–1.25 (br-m, 7H), 0.93 (t, J = 7.1 Hz, 1H). MS (FAB) m/z 521 (MH<sup>+</sup>).

1-((2-(4-fluorophenyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-(2hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (205). Yield 62 %, white solid, mp 217–218 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.76 (s, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 7.98 (m, 2H), 7.66 (m, 2H), 7.54 (m, 2H), 7.40 (m, 3H), 7.08 (t, *J* = 5.3 Hz, 1H), 6.62 (d, *J* = 9.8 Hz, 1H), 4.92 (t, *J* = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, *J* = 6.5 Hz, 2H), 3.61 (m, 2H). MS (FAB) *m/z* 501 (MH<sup>+</sup>).

# **1-((2-(3-chloro-4-fluorophenyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-3-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (206).** Yield 64 %, white solid, mp 220–221 °C. <sup>1</sup>H NMR (DMSO, 300 MHz) $\delta$ 8.77 (s, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 7.99 (m, 2H), 7.84 (dd, *J* = 7.3, 2.0 Hz, 1H), 7.70–7.45 (br-m, 4H), 7.32 (m, 2H), 7.09 (t, *J* = 5.6 Hz, 1H), 6.62 (d, *J* = 9.9 Hz, 1H), 4.92 (t, *J* = 5.8 Hz, 1H), 4.45 (m, 2H), 4.30 (t, *J* = 6.6 Hz, 2H), 3.61 (m, 2H). MS (FAB) *m/z* 535 (MH<sup>+</sup>).

#### 1-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)-3-((2-(3-

**isopropylphenyl)-6-(trifluoromethyl)pyridin-3-yl)methyl)urea** (207). Yield 63 %, white solid, mp 192–193 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.77 (s, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 9.9 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.60–7.35 (br-m, 6H), 7.31 (d, *J* = 8.2 Hz, 1H), 7.08 (t, *J* = 5.7 Hz, 1H), 6.62 (d, *J* = 9.9 Hz, 1H), 4.91 (t, *J* = 5.8 Hz, 1H), 4.43 (m, 2H), 4.30 (t, *J* = 6.5 Hz, 2H), 3.61 (m, 2H), 2.99 (m, 1H), 1.25 (d, *J* = 6.7 Hz, 6H). MS (FAB) *m/z* 525 (MH<sup>+</sup>).

**1-((1-(3-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)-3-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (208).** Yield 64 %, white solid, mp 209–210 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.69 (s, 1H), 7.96 (d, *J* = 9.9 Hz, 1H), 7.76 (m, 1H), 7.63 (m, 4H), 7.50 (m, 2H), 7.34 (m, 1H), 7.01 (m, 1H), 6.88 (s, 1H), 6.60 (d, *J* = 9.9 Hz, 1H), 4.92 (t, *J* = 5.7 Hz, 1H), 4.46 (m, 2H), 4.30 (t, *J* = 6.4 Hz, 2H), 3.61 (m, 2H). MS (FAB) *m/z* 506 (MH<sup>+</sup>).

**1-((1-(3-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)-3-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (209).** Yield 61 %, white solid, mp 198–199 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.68 (s, 1H), 7.96 (d, *J* = 9.9 Hz, 1H), 7.63 (m, 2H), 7.51 (m, 4H), 7.32 (m, 1H), 7.01 (t, *J* = 5.6 Hz, 1H), 6.88 (s, 1H), 6.60 (d, *J* = 9.9 Hz, 1H), 4.92 (t, *J* = 5.8 Hz, 1H), 4.47 (m, 2H), 4.30 (t, *J* = 6.5 Hz, 2H), 3.61 (m, 2H). MS (FAB) *m/z* 490 (MH<sup>+</sup>).

**1-((1-(3-chloro-4-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)-3-**(**1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (210).** Yield 61 %, white solid, mp 186–187 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.66 (s, 1H), 7.96 (m, 2H), 7.69 (m, 2H), 7.49 (m, 2H), 7.32 (t, J = 4.7 Hz, 1H), 6.97 (t, J = 5.4 Hz, 1H), 6.88 (s, 1H), 6.60 (d, J = 9.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J) = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J) = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1H), 4.44 (m, 2H), 4.30 (t, J) = 5.8 Hz, 1H), 4.92 (t, J = 5.8 Hz, 1 J = 6.5 Hz, 2H), 3.61 (m, 2H). MS (FAB) m/z 524 (MH<sup>+</sup>).

#### 1-(1-(2-hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)-3-((1-(3-

**isopropylphenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)methyl)urea (211).** Yield 65 %, white solid, mp 169–170 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.68 (s, 1H), 7.98 (d, *J* = 9.8 Hz, 1H), 7.55–7.35 (br-m, 6H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.02 (t, *J* = 5.4 Hz, 1H), 6.85 (s, 1H), 6.60 (d, *J* = 9.9 Hz, 1H), 4.91 (t, *J* = 5.8 Hz, 1H), 4.42 (m, 2H), 4.30 (t, *J* = 6.6 Hz, 2H), 3.61 (m, 2H), 3.00 (m, 1H), 1.23 (d, *J* = 6.9 Hz, 6H). MS (FAB) *m/z* 514 (MH<sup>+</sup>).

#### 1-((3-(tert-butyl)-1-(3-chlorophenyl)-1H-pyrazol-5-yl)methyl)-3-(1-(2-

hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (212). Yield 63 %, white solid, mp 176–177 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.62 (s, 1H), 7.97 (d, *J* = 9.9 Hz, 1H), 7.70–7.40 (m, 6H), 7.31 (d, *J* = 8.2 Hz, 1H), 6.94 (t, *J* = 5.4 Hz, 1H), 6.60 (d, *J* = 9.9 Hz, 1H), 6.38 (s, 1H), 4.92 (t, *J* = 5.6 Hz, 1H), 4.44 (m, 2H), 4.30 (t, *J* = 6.6 Hz, 2H), 3.61 (m, 2H), 1.29 (s, 9H). MS (FAB) *m/z* 494 (MH<sup>+</sup>).

#### 1-((3-(tert-butyl)-1-(3-fluorophenyl)-1H-pyrazol-5-yl)methyl)-3-(1-(2-

hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (213). Yield 65 %, white solid, mp 166–167 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.65 (s, 1H), 7.98 (d, *J* = 9.8 Hz, 1H), 7.65–7.20 (br-m, 7H), 6.97 (t, *J* = 5.4 Hz, 1H), 6.64 (d, *J* = 9.8 Hz, 1H), 6.37 (s, 1H), 4.92 (t, *J* = 5.6 Hz, 1H), 4.45 (m, 2H), 4.30 (t, *J* = 6.6 Hz, 2H), 3.61 (m, 2H), 1.29 (s, 9H). MS (FAB) *m/z* 478 (MH<sup>+</sup>).

#### 1-((2-(tert-butyl)-4-(3-chlorophenyl)thiazol-5-yl)methyl)-3-(1-(2-

**hydroxyethyl)-2-oxo-1,2-dihydroquinolin-5-yl)urea (214).** Yield 61 %, white solid, mp 166–167 °C. <sup>1</sup>H NMR (DMSO, 300 MHz)  $\delta$  8.70 (s, H), 8.00 (d, *J* = 9.8 Hz, 1H), 7.80–7.45 (br-m, 6H), 7.33 (d, *J* = 8.2 Hz, 1H), 7.22 (t, *J* = 5.8 Hz, 1H), 6.61 (d, *J* = 9.8 Hz, 1H), 4.92 (t, *J* = 5.8 Hz, 1H), 4.58 (m, 2H), 4.30 (t, *J* = 6.5 Hz, 2H), 3.61 (m, 2H), 1.40 (s, 9H). MS (FAB) *m/z* 512 (MH<sup>+</sup>).

# **Chapter 1. Introduction**

#### 1.1. Study Background

#### 1.1.1. HSP90

HSP90 (Heat Shock Protein 90) is one of the many molecular chaperone that regulates the client protein's conformation to become a functional protein. It usually assists the stability of the proteins when they are struck by heat and prevents degradation. Reasonably, scientists are expecting HSP90 investigation to be a stunning drug target related to cancer. Structurally, the HSP90 protein contains three functional domains such as the ATP-binding, client-binding, and dimerizing domain. So when the ATP and the misfolded protein binds to the HSP90 in an open state, it becomes a closed state. And by co-activator binding, the misfolded protein becomes a folded, functional protein. (**Figure 1**)

#### 1.1.2. HIF-1a Inhibitors

Along with HSP90, HIF-1 $\alpha$  is one of the signaling protein which is dependent to molecular chaperone HSP90. HIF-1 $\alpha$  is known to be strongly related to cell survival and angiogenesis. HIF-1 $\alpha$  is a transcription factor that regulates the expression level of target genes to induce angiogenesis, growth, metastasis, etc. under hypoxic condition.<sup>14</sup> So, cancer cell could survive under hypoxic condition through this HIF-1 $\alpha$  pathway. And structurally, this HIF-1 $\alpha$  protein is a heterodimer that consists of a constitutively expressed subunit and an oxygen-regulated subunit.

Especially in normoxia, HIF-1 $\alpha$  is degradable by prolyl hydroxylation, VHL complex mediated polyubiquitination and proteasome degradation pathway. In contrast, in hypoxia, HIF-1 $\alpha$  is stabilized by HSP90. Through phosphorylation, it moves into the nucleus and then transcripts the genes such as VEGF to induce angiogenesis to help the cancer cell's survival.<sup>15</sup> But, our compounds will inhibit this HSP90 mediated HIF-1 $\alpha$  stabilization, so, finally it is known to block the angiogenic pathway. (**Figure 2**)

<sup>&</sup>lt;sup>14</sup> Q. Ke et al. "Hypoxia-inducible factor-1 (HIF-1)." *Mol. Pharmacol.* 70 (2006): 1469-1480.

<sup>&</sup>lt;sup>15</sup> G. Semenza et al. "Targeting HIF-1 for cancer therapy." *Nature Reviews* 3 (2003): 721-732.



Figure 1. Mechanism of Molecular Chaperones



Figure 2. Mechanism of HSP90 and HIF-1 $\alpha$ 

## **1.2.** Purpose of Research

Our purpose of this research was to find an analogue that inhibits the HIF-1 $\alpha$  signaling protein so the cells would reach angiogenesis and cell apoptosis which can eventually become a sufficient candidate of cancer remedy.

# **Chapter 2. Design of the Amide Analogues**

#### 2.1. Previous Study

In this study, we referred to the study of destabilization of HIF-1 $\alpha$  induced by novel HSP90 inhibitors from the natural parental compound, Deguelin.<sup>16</sup> Deguelin has strong potency against HIF-1 $\alpha$  and its potency was checked by western blot assay. By this previous research, the team proved that Deguelin inhibits the functions of the ATP binding site of HSP90 and therefore bothers the stability of HIF-1 $\alpha$ . The ring-truncated compound **SH-42** was synthesized. (Figure 3)



Figure 3. The B-C Ring Truncation of Natural Product Deguelin

#### 2.2. Lead Compound L-22

By the ring truncated molecule **SH-42**, our team added a polar pyridine ring instead of a plain phenyl ring and got **L-22** with a slightly better potency. (**Figure 4**)



Figure 4. The Introduction of the Polar Group on SH-42

<sup>&</sup>lt;sup>16</sup> D. Chang et al. "Design, synthesis, and biological evaluation of novel deguelin-based heat shock protein 90 (HSP90) inhibitors targeting proliferation and angiogenesis." *J. Med. Chem.* 55 (2012): 10863–10884.

Further research has been taken incorporating the polar A-region such as adding a fluorine atom on the phenyl ring.<sup>17</sup> The process was quite successful so we started to focus on the B-region ketone.

## 2.3. Design Approach of the Amide Analogues

To derivate the compounds as HIF-1 $\alpha$  inhibitors, with the representative compound **SH-42** and **L-22**, we divided their structure into 3 regions and applied several modifications for each region. We maintained the chromene ring on the C-region and the dimethoxy phenyl ring on the A-region. On the B-region, we applied bioisosteric replacements and incorporated heterocycles. (**Figure 5**) Especially, the amide looked like a good choice due to its convenience in synthesizing and its stability throughout the chemical. Moreover, the elimination of the chiral center next to the ketone is expected to give us much more convenience in synthesis.



Figure 5. Design Approach of the HIF-1 $\alpha$  Surrogates

<sup>&</sup>lt;sup>17</sup> H. Kim et al. "Ring-truncated deguelin derivatives as potent Hypoxia Inducible Factor-1a (HIF-1 $\alpha$ ) inhibitors." *European Journal of Medicinal Chemistry* 104 (2015): 157-164.

# **Chapter 3. Synthesis of the Series**

#### 3.1. A-region Pyridine Amine

The A-region pyridine amines were prepared by 5 simple steps starting from the commercially available 2-bromopyridin-3-ol (1). Methylation on the alcohol with  $K_2CO_3$  and  $CH_3I$ , nitration on the 6<sup>th</sup> position with KNO<sub>3</sub> and  $H_2SO_4$ , methoxylation with NaOMe and at last, palladium reduction was performed to obtain the desired A-region compound (5).



Scheme 1. Synthesis of the A-region Pyridine Amine. Reagents and conditions:
(a) K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>I, acetone, reflux, 2 h; (b) KNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, 0 °C to 70 °C, overnight;
(c) NaOMe, DMF, 0 °C to r.t., 2 h; (d) 10 % Pd/C, H<sub>2</sub>, MeOH, r.t., 2 h;

## 3.2. C-region Carboxylic Acid

The C-region carboxylic acid was synthesized starting with the commercially available 2,4-dihydroxybenzaldehyde (6) and 3-methylbut-2-enal (7). By heating the two starting materials with pyridine over 120  $^{\circ}$ C, we were able to get the aldehyde compound (8) which has a chromene ring. After the simple methylation which yields the methoxy compound (9), with the presence of the buffer solution, with sodium hypochlorite, oxidation occurs and finally we were able to get the expected C-region carboxylic acid (10).



Scheme 2. Synthesis of the C-region Carboxylic Acid. Reagents and conditions: (a) Pyridine, acetone, 120  $^{0}$ C, overnight; (b) CH<sub>3</sub>I, K<sub>2</sub>CO<sub>3</sub>, acetone, r.t., 3 h; (c) H<sub>2</sub>O<sub>2</sub>, NaO<sub>2</sub>Cl, NaHPO<sub>4</sub>, ACN, r.t., 2 h;

## 3.3. B-region Amide with Various Linkers

With the pre-synthesized 5,6-Dimethoxypyridin-2-amine (5) and 5-methoxy-2,2dimethyl-2H-chromene-6-carboxylic acid (10), with EDC coupling with the HOBt intermediate and triethylamine as a base, we were able to obtain the key amide intermediate with a pyridine ring on the A-region and chromene ring on C-region (11).



Scheme 3. Synthesis of the Key Intermediate. Reagents and conditions: EDC.HCl, HOBt, TEA, r.t. to 50 °C, overnight;

Most of the alkyl halide linkers were commercially available while some of them went through a couple of simple reactions such as boc protection, silyl protection, bromination etc.



Scheme 4. Synthesis of the Necessary Linkers. Reagents and conditions: (a)  $Boc_2O$ , MC, r.t., 2 h; (b)  $CBr_4$ , PPh<sub>3</sub>, THF, 0 °C to r.t., 2 h; (c) TBDMSCl, Imidazole, DMF, 0 °C to r.t., 2 h; (d)  $Ac_2O$ , TEA, MC, 0 °C to r.t., 2 h;

For the derivation from the key intermediate (11), we used sodium hydride as a base for the alkylation on the nitrogen of the amide. We anticipated the sodium hydride to easily deprotonate the proton, but unfortunately, the reaction did not occur without a certain amount of heat.



Scheme 5. Synthesis of the Amide Analogues. Reagents and conditions: (a) R-X, NaH, DMF, 60 °C, overnight; (b) i) DPPA, DBU, DMF, 90 °C, 2 h; ii) Lindlar's catalyst, H<sub>2</sub>, MeOH, r.t. 2 h; (c) Ac<sub>2</sub>O, Pyridine, MC, 0 °C to r.t., 2 h; (d) Boc<sub>2</sub>O, MC, 0 °C to r.t., 2 h; (e) TBAF, MC, 0 °C to r.t., 2 h; (f) NaH, CH<sub>3</sub>I, DMF, 0 °C to r.t., 2 h; (g) TFA, MC, 0 °C to r.t., 2 h; (h) i) PFA, ZnCl<sub>2</sub>, MC, r.t., 2 h; ii) NaBH<sub>4</sub>, MeOH, r.t., 2 h;

# **Chapter 4. Structure-Activity Relationship**

## 4.1. Results of the Western Blot Assay

The results of the western blot assay are shown below. The derivated compounds' activity were compared with the natural product Deguelin and the ring truncated compound **SH-42** along with the lead compound **L-22** which has a pyridine ring on the A-region.

	<u>^</u>		
	R	HIF WB (100 nM)	HIF WB (10 nM)
11	ξ−H	88.4	N/A
21	ξ−CH₃	62.3	90.4

Table 1. WB results of Simple Amides

The amide without any linkers (11) did not display any better activity compared to Deguelin or the lead compound. However, when a methyl functional group was introduced on the nitrogen (21), the activity increased even better than Deguelin (Table 1).

	R	HIF WB (100 nM)	HIF WB (10 nM)
22		38.6	83.4
23	₽ F	63.1	83.3
24	₹ CI	57.6	103.8

Table 2. WB Results of Amides with Benzyl Linkers



The next approach was to add a bulky benzyl ring on the linker (**Table 2**). The bulky benzyl ring showed proficient activity. The ones with the electron withdrawing halogen group on the para position of the benzyl had better activity compared to Deguelin. On the other hand, other type of electron donating groups like methoxy (**25**) and methyl (**26**) did not show satisfying results. The position of the additional group mattered as we see the benzyl compounds with a halogen group on the meta position (**27, 28**).

	R	HIF WB (100 nM)	HIF WB (10 nM)
29	N N	97.0	N/A
30		95.8	N/A
31		55.2	108.2

Table 3. WB Results of Amides with Pyridine Linkers

Since the bulky benzyl group gave us some promising results, we started some other modifications besides the benzyl group and we decided to transfer the benzyl ring to a pyridine ring. In this case, the position of the nitrogen mattered again. Surrogates with pyridines (**Table 3**) did not show good potency except the one with the nitrogen on the 4<sup>th</sup> position (**31**).

	R	HIF WB (100 nM)	HIF WB (10 nM)
44	₹~~NH <sup>2</sup>	33.3	54.0
49	₹~~~H~	41.1	93.0
34	*N	37.9	75.5
35		64.4	N/A
45	₹ N	58.1	66.0
46	<sup>₹</sup> <sup>↓</sup> <sup>↓</sup> <sup>↓</sup> <sup>0</sup> <sup>↓</sup>	57.4	N/A

Table 4. WB Results of Amides with Amine Linkers (Two Carbons)

On our next trial, we expected the length of the linker would matter. Assuming that the pocket exists on the end of the two carbons, we made more analogues with two carbon length. As a result, the amine linkers with two carbons (**Table 4**) showed quite interesting results. Amine linkers with various groups such as methyl (**49**), dimethyl (**34**), diethyl (**35**), acyl (**45**), boc protected (**46**) groups added showed competent results. Among those, the amide analogue with the free amine linker (**44**) showed the best results.

**Table 5.** WB Results of Amides with Pyrrolidine, Piperidine, Morpholine and

 Piperazine Linkers

	R	HIF WB (100 nM)	HIF WB (10 nM)
36		66.0	N/A
37	₹~~N~	79.7	N/A



Bulky cyclized groups with a nitrogen atom in the cycle (**Table 5**) were synthesized later. Only the ones with a nitrogen on the end of the linker (**50**, **51**, **53**) showed better activity. But the ones without a nitrogen on the end did not show good results (**36**, **37**, **39**).

	R	HIF WB (100 nM)	HIF WB (10 nM)
47	₹OH	87.9	N/A
48	₽ <u>~~</u> 0_	52.5	N/A
32		57.2	49.3

Table 6. WB Results of Amides with Oxy Linkers

Besides nitrogen, we needed another type of polar group added on the end to see whether the potency will occur with any polar group or only nitrogen. So as the next step, we added linkers with oxy groups (**Table 6**). Contrary results were shown compared to the amine linkers. The free alcohol (47) displayed less potency compared to methylated (48) and benzylated (41) linkers. However, none of them was observed to have better potency than the free amine (44).

	R	HIF WB (100 nM)	HIF WB (10 nM)
52	₹~NH2	45.3	111.0
43	₹ <u></u> N	43.9	60.0
54		76.6	N/A

Table 7. WB Results of Amides with Amine Linkers (Three Carbons)

At last, we tried longer amine linkers with three carbon length (**Table 7**). The results were quite complicated because the ones without any group attached (52) and dimethylated groups (43) showed good potency while acylated groups (54) did not.

#### 4.2. Biological Evaluation

With the results of the increase of potency with the methyl group (21) from the free amide (11), we decided to elongate the linker with the benzyl group (22) and obtained a substantial increase in potency. We assume that this means a pi-interaction will exist on the end of the linker.

Then we added various functional groups to check the electronic effect or the steric effect of the linker. As the electron withdrawing group derivatives on the benzyl para position (23, 24) and the pyridine ring with the nitrogen on the para position (31) had good activity, we assume that there will be an electronic pocket on the end of the linker. This assumption is in the line with the positive results of the amine linkers with two carbons (34, 35, 44, 45, 46, 49).

However, considering the steric effect, still there are some questions remaining. On the benzyl group (22) with some functional groups added, generally the potency decreased on those derivatives (23, 24, 25, 26, 27, 28). Knowing that the size of fluorine and hydrogen is not really different, the phenyl ring with fluorine atom (23) did not show any better effect than the phenyl ring linker without any functional group (22). We believe that there must be more complex interactions on the end of the linker besides the steric effect.

Generally, the analogues which have an amide bond on the B-region, the

ones with a two carbon linker with a functional group on the end, whether it is a non-polar or polar group, will show a better potency than their parent compound without any linkers (11) because we assume that there will be a pocket near the end of the B-region linker. For the non-polar groups, there will be a pi interaction with the pocket. On the other hand, in the case of polar groups, there are two possibilities of action in the pocket. First, due to the influence of body pH, the protons will be protonated to have a pi-cationic bonding with the pocket. The other possibility is to have a different structural orientation compared to the non-polar group linked derivatives to have an ionic bonding with the other side of pocket.

# **Chapter 5. Conclusion**

We rationally designed and synthesized possible HIF-1 $\alpha$  inhibitors based on the previous study which gave us an idea of ring-truncated analogues of natural product Deguelin. Compounds were distributed into three regions and we modified the regions one by one. After the rational design, we wanted to effectively manage producing a substantial amount of analogues so we synthesized a key intermediate first (11) and then attached various types of linkers for surrogates. The produced compounds' potency was examined by western blot assay so we can see the expression level of HIF-1 $\alpha$  in hypoxia condition.

We manufactured 30 analogues with a pyridine ring on the A-region and an amide linker on the B-region. We observed inhibition in most of the analogues. Among the 30 analogues, 18 Compounds with a linker showed better potency than the parent compound Deguelin (HIF WB = 64.0). Especially, compound 22 with a simple benzyl linker (HIF WB = 38.6) and compound 44 with an ethyl amino linker (HIF WB = 33.3) had great results approximately doubled the potency compared to Deguelin. Unfortunately, from these effective compounds, slightly substituting the functional groups did not help in further activity.

By the results of the excellent activity of the phenyl and benzyl surrogates (22 - 31), we assume a certain hydrophobic pocket existing near to the end of the B-region linker. Along with that, most of the linkers with two carbons with a lipophilic or polar group on the end showed good inhibition against HIF-1 $\alpha$  in hypoxia condition. However, the one with the hydroxyl group (47) turned out to show a negative activity and this signifies the pi-cationic interaction on the end of the B-region linker.

Overall, compound 22 and 44 is a potential anticancer and antiangiogenic agent with an HIF-1 $\alpha$  inhibition mechanism. In addition, we need further study such as molecular modeling or *in vivo* examinations to confirm the structure activity relationship of the potent compounds.

# **Experimental**

**Experimental Information** All chemical starting material reagents were commercially available. Melting points were determined on a melting point Buchi B-540 apparatus. Silica gel column chromatography was performed on Silica Gel 60, 230–400 mesh, Merck. Proton NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz and Bruker Analytik, DE/AVANCE Digital 400 at 400 MHz or Bruker AMX-500 (500 MHz) spectrometer. Chemical shifts are reported in ppm units with tetramethylsilane as a reference standard. Optical rotations were measured using JASCO DIP-1000 digital polarimeter at ambient temperature using a 100 mm cell of 2 mL capacity. Mass spectra and HRMS results were recorded on VG Trio-2 GC–MS instrument and JEOL JMS-AX instrument, respectively.

General Procedure for Amide Coupling To a solution of carboxylic acid (1.0 eq.) in 1,4-Dioxane, EDCI (1.4 eq.), HOBt (1.4 eq.) was added. A solution of aniline compound (1.0 eq.) in 1.4-Dioxane was added to the mixture. TEA (1.3 eq.) was dropped to the resulting mixture at room temperature. The temperature was increased to 50 to 60 °C The mixture was stirred overnight. Then the mixture was extracted with EtOAc, washed by water several times, dried over MgSO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford the corresponding amide product.

**General Procedure for Alkylation** To a solution of amide compound (1.0 eq.) in DMF was added NaH (3.0 eq.) at 0 °C. After being stirred for 30 min, alkylhalide (3.0 eq.) was added dropwise at 0 °C. The reaction mixture was stirred for an additional 2 h and warmed to 60 °C. The reaction mixture was quenched with water and extracted with EtOAc, washed with brine several times, dried over MgSO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford the corresponding alkylated product.

**General Procedure for Silyl Deprotection** To a solution of silyl protected starting material (1.0 eq.) in MC, TBAF 1M in THF (1.1 eq.) was added at 0 °C and warmed to room temperature. The reaction mixture was stirred for 2 h. Then the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over MgSO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford the corresponding compound.

General Procedure for Boc Deprotection To a solution of silvl protected starting material (1.0 eq.) in DCM, TFA (1.2 eq.) was added dropwise at 0  $^{\circ}$ C and warmed to room temperature. The reaction mixture was stirred for 1 h. Then the reaction mixture was quenched with saturated sodium bicarbonate solution and extracted with EtOAc. The organic layer was washed with brine, dried over MgSO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford the corresponding compound.

#### N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-2H-chromene-6-

**carboxamide (11).** Yield 55 %, pale yellow solid, mp 127–128 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  9.99 (s, 1H), 7.96 (d, J = 8.6 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 6.71 (d, J = 8.6 Hz, 1H), 6.61 (d, J = 10.1 Hz, 1H), 5.73 (d, J = 9.9 Hz, 1H), 3.99 (s, 3H), 3.91 (s, 3H), 3.88 (s, 3H), 1.47 (s, 6H). HRMS (FAB) calcd for C<sub>20</sub>H<sub>23</sub>N<sub>2</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 371.1607, found 371.1609.

**N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-N,2,2-trimethyl-2H-chromene-6-carboxamide (21).** Yield 61 %, pale yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  6.87 (m, 2H), 6.50 (m, 2H), 6.40 (d, *J* = 8.4 Hz, 1H), 5.60 (d, *J* = 9.9 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.80 (s, 3H), 3.47 (s, 3H), 1.39 (s, 6H). HRMS (FAB) calcd for C<sub>21</sub>H<sub>25</sub>N<sub>2</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 385.1763, found 385.1768.

#### N-benzyl-N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-2H-

**chromene-6-carboxamide (22).** Yield 62 %, pale yellow solid, mp 71–72 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.40 (m, 2H), 7.22 (m, 4H), 6.88 (m, 1H), 6.70 (m, 1H), 6.50 (m, 1H), 6.47 (m, 1H), 5.62 (m, 1H), 5.19 (s, 2H), 3.91 (s, 3H), 3.88 (s, 3H), 3.75 (s, 3H), 1.45 (s, 6H). HRMS (FAB) calcd for C<sub>27</sub>H<sub>29</sub>N<sub>2</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 461.2076, found 461.2079.

**N-(5,6-dimethoxypyridin-2-yl)-N-(4-fluorobenzyl)-5-methoxy-2,2-dimethyl-2H-chromene-6-carboxamide (23).** Yield 61 %, white solid, mp 45–46 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  7.34 (m, 1H), 6.96 (d, J = 8.6 Hz, 1H), 6.93 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.2 Hz, 1H), 6.71 (d, J = 7.5 Hz, 1H), 6.49 (d, J = 10.0 Hz, 1H), 6.37 (d, J = 8.6 Hz, 1H), 6.40–6.15 (br-m, 1H), 5.61 (d, J = 10.0 Hz, 1H), 5.15 (s, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 3.75 (s, 3H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>27</sub>H<sub>28</sub>FN<sub>2</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 479.1982, found 479.1982.

N-(4-chlorobenzyl)-N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-2H-chromene-6-carboxamide (24). Yield 59 %, white solid, mp 45–46 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  7.32 (m, 2H), 7.26–7.20 (br-m, 2H), 6.86 (d, J = 8.2 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 6.50 (d, J = 10.0 Hz, 1H), 6.37 (d, J = 8.2 Hz, 1H), 6.37–6.22 (br-m, 1H), 5.61 (d, J = 9.9 Hz, 1H), 5.15 (s, 2H), 3.89 (s, 3H), 3.85 (s, 3H), 3.75 (s, 3H), 1.38 (s, 6H). HRMS (FAB) calcd for  $C_{27}H_{28}CIN_2O_5^+$  [M + H]<sup>+</sup>: 495.1687, found 495.1687.

**N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-N-(4-methoxybenzyl)-2,2-dimethyl-2H-chromene-6-carboxamide (25).** Yield 62 %, white solid, mp 44–45 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  7.35–7.25 (br-m, 2H), 6.87 (d, J = 8.4 Hz, 1H), 6.79 (m, 2H), 6.71 (d, J = 7.1 Hz, 1H), 6.50 (d, J = 10.0 Hz, 1H), 6.36 (d, J = 8.4 Hz, 1H), 6.36–6.25 (br-m, 1H), 5.60 (d, J = 9.8 Hz, 1H), 5.13 (s, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 3.76 (s, 3H), 3.74 (s, 3H), 1.37 (s, 6H). HRMS (FAB) calcd for C<sub>28</sub>H<sub>31</sub>N<sub>2</sub>O<sub>6</sub><sup>+</sup> [M + H]<sup>+</sup>: 491.2182, found 491.2182.

**N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(4-methylbenzyl)-2H-chromene-6-carboxamide (26).** Yield 62 %, white solid, mp 44–45 °C. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz)  $\delta$  7.22 (m, 2H), 7.07 (m, 2H), 6.91 (d, *J* = 10.5 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.48 (d, *J* = 9.9 Hz, 1H), 6.36 (d, *J* = 8.7 Hz, 1H), 5.68 (d, *J* = 10.0 Hz, 1H), 5.12 (s, 2H), 3.82 (s, 3H), 3.79 (s, 3H), 3.69 (s, 3H), 2.27 (s, 3H), 1.34 (s, 6H). HRMS (FAB) calcd for C<sub>28</sub>H<sub>31</sub>N<sub>2</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 475.2233, found 475.2233.

**N-(5,6-dimethoxypyridin-2-yl)-N-(3-fluorobenzyl)-5-methoxy-2,2-dimethyl-2H-chromene-6-carboxamide (27).** Yield 61 %, white solid, mp 43–44 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  7.20 (m, 3H), 6.89 (m, 2H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.50 (d, *J* = 10.0 Hz, 1H), 6.38 (d, *J* = 8.4 Hz, 1H), 6.39–6.15 (br-m, 1H), 5.61 (d, *J* = 9.9 Hz, 1H), 5.18 (s, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 3.75 (s, 3H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>27</sub>H<sub>28</sub>FN<sub>2</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 479.1982, found 479.1982.

N-(3-chlorobenzyl)-N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-

**2H-chromene-6-carboxamide (28).** Yield 64 %, white solid, mp 44–45 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  7.49 (s, 1H), 7.21 (m, 3H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.50 (d, *J* = 10.0 Hz, 1H), 6.39 (d, *J* = 8.4 Hz, 1H), 6.41–6.30 (br-m, 1H), 5.62 (d, *J* = 9.9 Hz, 1H), 5.15 (s, 2H), 3.89 (s, 3H), 3.85 (s, 3H), 3.75 (s, 3H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>27</sub>H<sub>28</sub>ClN<sub>2</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 495.1687, found 495.1687.

#### N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(pyridin-2-

ylmethyl)-2H-chromene-6-carboxamide (29). Yield 66 %, pale yellow oil.  $^{1}$ H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  8.61 (m, 1H), 7.64 (m, 1H), 7.52 (m, 1H), 7.27 (m, 1H),
7.14 (m, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.79 (m, 1H), 6.52 (d, J = 9.6 Hz, 1H), 6.39 (d, J = 8.4 Hz, 1H), 5.62 (d, J = 10.0 Hz, 1H), 5.33 (s, 2H), 3.87 (s, 3H), 3.76 (s, 3H), 3.75 (s, 3H), 1.39 (s, 6H). HRMS (FAB) calcd for  $C_{26}H_{28}N_3O_5^+$  [M + H]<sup>+</sup>: 462.2029, found 462.2037.

### N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(pyridin-3-

**ylmethyl)-2H-chromene-6-carboxamide (30).** Yield 64 %, pale yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  8.62 (m, 1H), 8.47 (d, *J* = 4.0 Hz, 1H), 7.80 (d, *J* = 7.6 Hz, 1H), 7.26 (m, 2H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.72 (d, *J* = 7.6 Hz, 1H), 6.52 (d, *J* = 10.0 Hz, 1H), 6.41 (d, *J* = 8.3 Hz, 1H), 5.61 (d, *J* = 10.0 Hz, 1H), 5.22 (s, 2H), 3.90 (s, 3H), 3.83 (s, 3H), 3.76 (s, 3H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>26</sub>H<sub>28</sub>N<sub>3</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 462.2029, found 462.2032.

### N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(pyridin-4-

**ylmethyl)-2H-chromene-6-carboxamide (31).** Yield 57 %, pale yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  8.62 (m, 1H), 7.78 (m, 1H), 7.27 (m, 1H), 7.24 (m, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.71 (d, *J* = 8.2 Hz, 1H), 6.48 (m, 2H), 5.61 (m, 2H), 5.20 (s, 2H), 3.90 (s, 3H), 3.83 (s, 3H), 3.76 (s, 3H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>26</sub>H<sub>28</sub>N<sub>3</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 462.2029, found 462.2037.

### N-(2-(benzyloxy)ethyl)-N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-

**dimethyl-2H-chromene-6-carboxamide (32).** Yield 61 %, pale yellow solid, mp 69–70 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.39 (m, 1H), 7.32 (m, 5H), 6.83 (m, 2H), 6.50 (d, J = 10.0 Hz, 1H), 6.36 (d, J = 8.2 Hz, 1H), 5.59 (d, J = 10.4 Hz, 1H), 4.49 (m, 2H), 4.26 (m, 2H), 3.85 (s, 3H), 3.82 (s, 3H), 3.76 (s, 3H), 3.61 (m, 2H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>29</sub>H<sub>33</sub>N<sub>2</sub>O<sub>6</sub><sup>+</sup> [M + H]<sup>+</sup>: 505.2339, found 505.2334.

#### N-(5,6-dimethoxypyridin-2-yl)-N-(2-(dimethylamino)ethyl)-5-methoxy-2,2-

**dimethyl-2H-chromene-6-carboxamide (34).** Yield 55 %, pale yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.24 (m, 1H), 6.84 (m, 2H), 6.47 (m, 1H), 6.38 (d, *J* = 8.3 Hz, 1H), 5.60 (m, 1H), 4.18 (m, 2H), 3.91 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H), 2.89 (m, 2H), 2.45 (s, 6H), 1.43 (s, 6H). HRMS (FAB) calcd for C<sub>24</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 442.2342, found 442.2350.

### N-(2-(diethylamino)ethyl)-N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-

**dimethyl-2H-chromene-6-carboxamide (35).** Yield 57 %, yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  6.97 (br-m, 2H), 6.49–6.38 (m, 3H), 5.70 (d, *J* = 10.1 Hz, 1H), 4.09 (m, 2H), 3.85 (s, 3H), 3.82 (s, 3H), 3.74 (s, 3H), 2.90 (m, 2H), 2.68 (m, 4H), 1.35 (s, 6H), 1.09–1.00 (br-m, 6H). HRMS (FAB) calcd for C<sub>26</sub>H<sub>36</sub>N<sub>3</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>:

### 470.2655. found 470.2649.

### N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(2-(pyrrolidin-1-

**yl)ethyl)-2H-chromene-6-carboxamide (36).** Yield 66 %, pale yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  6.85 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 6.45 (m, 2H), 6.40 (d, J = 8.3 Hz, 1H), 5.62 (d, J = 10.0 Hz, 1H), 4.46 (t, J = 7.2 Hz, 2H), 3.87 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.43 (t, J = 7.6 Hz, 2H), 2.14 (m, 4H), 2.00 (m, 2 H), 1.93 (m, 2H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>26</sub>H<sub>34</sub>N<sub>3</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 468.2498, found 468.2488.

#### N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(2-(piperidin-1-

**yl)ethyl)-2H-chromene-6-carboxamide (37).** Yield 58 %, pale yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.27 (m, 1H), 6.85 (m, 2H), 6.49 (d, J = 10.0 Hz, 1H), 6.38 (d, J = 8.3 Hz, 1H), 5.60 (d, J = 9.6 Hz, 1H), 4.13 (m, 2H), 3.91 (s, 3H), 3.87 (s, 3H), 3.75 (s, 3H), 2.72 (m, 2H), 2.52 (m, 4H), 1.58 (m, 4H), 1.54 (m, 2H), 1.38 (s, 6H). HRMS (FAB) calcd for C27H36N3O5+ [M + H]+: 482.2655, found 482.2648.

#### N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(2-

**morpholinoethyl)-2H-chromene-6-carboxamide (39).** Yield 67 %, pale yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.27 (m, 1H), 6.84 (m, 2H), 6.49 (d, *J* = 10.0 Hz, 1H), 6.38 (d, *J* = 8.3 Hz, 1H), 5.61 (d, *J* = 10.0 Hz, 1H), 4.12 (m, 2H), 3.91 (s, 3H), 3.87 (s, 3H), 3.75 (s, 3H), 3.67 (m, 2H), 2.59 (m, 4H), 1.65 (m, 4H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>26</sub>H<sub>34</sub>N<sub>3</sub>O<sub>6</sub><sup>+</sup> [M + H]<sup>+</sup>: 484.2448, found 484.2442.

**N-(5,6-dimethoxypyridin-2-yl)-N-(3-(dimethylamino)propyl)-5-methoxy-2,2dimethyl-2H-chromene-6-carboxamide (43).** Yield 64 %, pale yellow oil. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz)  $\delta$  7.04 (d, *J* = 7.9 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 1H), 6.59 (m, 1H), 6.48 (d, *J* = 9.9 Hz, 1H), 6.36 (d, *J* = 8.4 Hz, 1H), 5.70 (d, *J* = 10.1 Hz, 1H), 4.02 (m, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 3.74 (s, 3H), 2.71 (m, 2H), 2.44 (s, 6H), 2.03–1.89 (br-m, 2H), 1.35 (s, 6H). HRMS (FAB) calcd for C<sub>25</sub>H<sub>34</sub>N<sub>3</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 456.2498, found 456.2495.

**N-(2-aminoethyl)-N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-2Hchromene-6-carboxamide (44).** Yield 62 %, pale yellow solid, mp 50–51 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.78 (m, 1H), 7.58 (m, 1H), 7.17 (m, 1H), 6.93 (m, 1H), 6.40 (d, J = 8.2 Hz, 1H), 5.86 (m, 1H), 4.60 (s, 2H), 4.34 (m, 2H), 3.91 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 3.45 (m, 2H), 1.45 (s, 6H). HRMS (FAB) calcd for  $C_{22}H_{28}N_4O_5^+$  [M + H]<sup>+</sup>: 414.2029, found 414.2026. **N-(2-acetamidoethyl)-N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-2H-chromene-6-carboxamide (45).** Yield 62 %, yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  6.81 (t, *J* = 8.6 Hz, 2H), 6.70 (m, 1H), 6.48 (d, *J* = 9.5 Hz, 1H), 6.43 (m, 1H), 6.37 (d, *J* = 8.4 Hz, 1H), 5.62 (d, *J* = 9.9 Hz, 1H), 4.15 (m, 2H), 3.92 (s, 3H), 3.85 (s, 3H), 3.78 (s, 3H), 3.53 (m, 2H), 1.99 (s, 3H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>24</sub>H<sub>30</sub>N<sub>3</sub>O<sub>6</sub><sup>+</sup> [M + H]<sup>+</sup>: 456.2135, found 456.2135.

#### tert-butyl(2-(N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-2H-

**chromene-6-carboxamido)ethyl)carbamate (46).** Yield 63 %, pale yellow solid, mp 64–65 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  8.09 (s, 1H), 7.74 (m, 1H), 7.32 (m, 1H), 7.14 (m, 1H), 6.75 (m, 1H), 6.46 (d, *J* = 8.2 Hz, 1H), 5.67 (m, 1H), 4.34 (m, 2H), 3.91 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H), 3.35 (m, 2H), 1.45 (s, 6H), 1.26 (s, 9H). HRMS (FAB) calcd for C<sub>27</sub>H<sub>36</sub>N<sub>3</sub>O<sub>7</sub><sup>+</sup> [M + H]<sup>+</sup>: 514.2553, found 514.2548.

**N-(5,6-dimethoxypyridin-2-yl)-N-(2-hydroxyethyl)-5-methoxy-2,2-dimethyl-2H-chromene-6-carboxamide (47).** Yield 60 %, pale yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  6.84 (m, 2H), 6.62 (m, 1H), 6.50 (d, *J* = 10.0 Hz, 1H), 6.37 (d, *J* = 8.3 Hz, 1H), 5.60 (d, *J* = 9.6 Hz, 1H), 4.09 (m, 2H), 3.90 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>22</sub>H<sub>26</sub>N<sub>2</sub>O<sub>6</sub><sup>+</sup> [M]<sup>+</sup>: 414.1791, found 414.1792.

**N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-N-(2-methoxyethyl)-2,2-dimethyl-2H-chromene-6-carboxamide (48).** Yield 62 %, pale yellow solid, mp 60–61 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.27 (m, 1H), 6.87 (m, 3H), 6.47 (d, *J* = 8.2 Hz, 1H), 5.59 (m, 1H), 4.19 (m, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 3.68 (m, 2H), 3.45 (s, 3H), 1.44 (s, 6H). HRMS (FAB) calcd for C<sub>23</sub>H<sub>29</sub>N<sub>2</sub>O<sub>6</sub><sup>+</sup> [M + H]<sup>+</sup>: 429.2026, found 429.2018.

#### N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(2-

(methylamino)ethyl)-2H-chromene-6-carboxamide (49). Yield 57 %, pale yellow solid, mp 88–89 °C. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz)  $\delta$  7.03 (d, J = 7.9 Hz, 1H), 6.91 (d, J = 8.2 Hz, 1H), 6.59 (m, 1H), 6.47 (d, J = 9.9 Hz, 1H), 6.38 (d, J = 8.2 Hz, 1H), 5.69 (d, J = 9.9 Hz, 1H), 4.10 (t, J = 6.6 Hz, 2H), 3.84 (s, 3H), 3.81 (s, 3H), 3.74 (s, 3H), 2.88 (t, J = 6.6 Hz, 2H), 2.43 (s, 3H), 1.35 (s, 6H). HRMS (FAB) calcd for C<sub>23</sub>H<sub>30</sub>N<sub>3</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 428.2185, found 428.2186.

N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(2-(piperidin-4-yl)ethyl)-2H-chromene-6-carboxamide (50). Yield 66 %, pale yellow solid, mp

46–47 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  7.03 (d, J = 7.1 Hz, 1H), 6.82 (d, J = 7.1 Hz, 1H), 6.55–6.35 (br-m, 3H), 5.69 (d, J = 10.1 Hz, 1H), 3.99 (m, 2H), 3.83 (s, 3H), 3.81 (s, 3H), 3.74 (s, 3H), 3.04 (m, 2H), 2.63–2.56 (br-m, 2H), 1.74 (m, 2H), 1.51 (m, 3H), 1.35 (s, 6H), 1.18 (m, 2H). HRMS (FAB) calcd for C<sub>27</sub>H<sub>36</sub>N<sub>3</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 482.2655, found 482.2664.

### N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(2-(piperazin-1-

**yl)ethyl)-2H-chromene-6-carboxamide (51).** Yield 61 %, pale yellow solid, mp 53–54 °C. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz)  $\delta$  7.03 (d, J = 7.9 Hz, 1H), 6.87 (d, J = 8.0 Hz, 1H), 6.56 (m, 1H), 6.50 (d, J = 9.9 Hz, 1H), 6.37 (d, J = 8.2 Hz, 1H), 5.69 (d, J = 9.9 Hz, 1H), 4.11 (m, 2H), 3.83 (s, 6H), 3.74 (s, 3H), 2.81 (m, 4H), 2.66 (m, 2H), 2.51 (m, 4H), 1.35 (s, 6H). HRMS (FAB) calcd for C<sub>26</sub>H<sub>35</sub>N<sub>4</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 483.2607, found 483.2604.

**N-(3-aminopropyl)-N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-2H-chromene-6-carboxamide (52).** Yield 63 %, yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  6.78 (m, 2H), 6.46 (d, *J* = 10.0 Hz, 1H), 6.41 (d, *J* = 8.0 Hz, 1H), 6.33 (d, *J* = 8.4 Hz, 1H), 5.61 (d, *J* = 10.0 Hz, 1H), 4.16 (m, 2H), 3.87 (s, 3H), 3.83 (s, 3H), 3.77 (s, 3H), 3.16 (m, 2H), 2.00 (m, 4H), 1.37 (s, 6H). HRMS (FAB) calcd for C<sub>23</sub>H<sub>30</sub>N<sub>3</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 428.2185, found 428.2185.

#### N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-dimethyl-N-(2-(4-

methylpiperazin-1-yl)ethyl)-2H-chromene-6-carboxamide (53). Yield 62 %, pale yellow oil. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz) δ 6.87–6.79 (br-m, 2H), 6.50 (d, J = 9.9 Hz, 1H), 6.49–6.39 (br-m, 1H), 6.37 (d, J = 8.0 Hz, 1H), 5.61 (d, J = 9.9 Hz, 1H), 4.08 (m, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.79 (s, 3H), 2.70 (t, J = 6.8 Hz, 2H), 2.65–2.33 (br-m, 8H), 2.30 (s, 3H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>27</sub>H<sub>37</sub>N<sub>4</sub>O<sub>5</sub><sup>+</sup> [M + H]<sup>+</sup>: 497.2764, found 497.2763.

#### N-(3-acetamidopropyl)-N-(5,6-dimethoxypyridin-2-yl)-5-methoxy-2,2-

**dimethyl-2H-chromene-6-carboxamide (54).** Yield 59 %, pale yellow solid, mp 45–46 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  6.99 (m, 1H), 6.79 (d, J = 8.4 Hz, 1H), 6.51 (d, J = 10.1 Hz, 1H), 6.44 (m, 1H), 6.35 (d, J = 8.3 Hz, 1H), 5.63 (d, J = 9.9 Hz, 1H), 4.11 (m, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 3.79 (s, 3H), 3.89 (m, 2H), 2.05 (s, 3H), 1.72 (m, 2H), 1.38 (s, 6H). HRMS (FAB) calcd for C<sub>25</sub>H<sub>32</sub>N<sub>3</sub>O<sub>6</sub><sup>+</sup> [M + H]<sup>+</sup>: 470.2291, found 470.2291.

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## **Abstract in Korean**

파트 1. TRPV1 길항제로서 Benzooxazinone 및 Quinolinone을 갖는 Urea 유도체의 합성과 구조 활성 연구

TRPV1은 신경병증성 통증 유발인자에 의해서 활성화되는 양이온 채널이다. 이 TRPV1의 효능제를 이용하여 탈감작 시키거나 길항제를 이용하여 통증신호를 차단하는 기전을 이용한 통증 치료제 개발이 활발히 진행되고 있다. 특히 TRPV1 길항제는 다른 통증 치료제와는 달리 작용기전이 밝혀진바 있어 여러 유수 연구소로부터 활발히 연구되고 있다.

본 연구실은 Capsaicin, RTX, BCTC 그리고 과거에 합성된 바 있고 매우 활성이 좋았던 M4 화합물의 구조에서 기인한 바, 지금까지 설계된 TRPV1 길항제들의 공통점을 찾아 3부분으로 나누어 수년간 최적화를 진행해오고 있다. 그리고 얼마 전 최적화 연구를 통해 6-6 fused heterocycle을 가지는 선도화합물을 설계 및 합성한 바 있다 (SAT-1251, SAT-769). 본 연구에서는 이 선도화합물의 A-region Benzooxazinone과 Quinolinone에 비극성 혹은 극성 잔기 도입을 통해 물성 및 효능을 개선하고자 하였다. 추가적인 최적화와 Binding site를 알아보기 위하여 C-region의 CF<sub>3</sub> pyridine도 그전에 다른 화합물들에서 활성을 띠었던 pyrazole이나 thiazole 등 여러가지 구조들로 치환해보았다.

이러한 진통효과는 FLIPR assay를 이용하여 세포 내에 유입된 Ca2<sup>+</sup> 양이온의 양을 측정하여 확인하였다. 77 가지의 합성된 유도체 중 28 가지가 BCTC보다 활성이 좋았지만 그 어느 것도 선도화합물들보다는 활성이 좋지 못했다. 우리는 이를 통해 heterocycle 안에 있는 2차 아민이 활성에 영향을 미친다는 것을 확인하였다. 그렇지만 선도화합물의 유도체화를 통해 강력한 진통효과는 어느정도 유지하면서 물성 개선의 여지가 있는 화합물을 다수 얻을 수 있었다. 이 중 3 가지 화합물 (111, 166, 193)들은 특히나 좋은 TRPV1 길항제로서의 가능성을 엿볼 수 있었다.

6 9

# 파트 2. HIF-1α 저해제로서 Deguelin의 단순 Amide 유도체의 합성과 구조 활성 연구

HIF-1은 저산소상태에서 세포의 성장 및 혈관 신생에 관계된 유전자들의 발현을 활성화하는 전사조절인자이다. 이 HIF-1을 구성하며 주변 산소 농도에 의해 조절 받는 subunit인 HIF-1α는 정상 산소 상태에서는 자연스럽게 분해되지만 저산소상태에서 HSP90의 도움을 받아 안정화되어 암세포의 생존에 필요한 신호를 발생시키는 역할을 한다.

선행연구를 통해 천연물인 Deguelin으로부터 B-C 고리를 절단한 단순화합물이 효능을 가짐을 확인한 바 있으며 (SH-42), 그로부터 효과적인 설계를 위해 우리는 선도화합물을 3부분으로 나누어 최적화를 시행하였다. 먼저 본 연구실에서 A-region에 극성이 있는 Pyridine을 도입하였고 이 선도화합물은 (L-22) 성공적인 활성을 띠었다. 그래서 Aregion에는 Pyridine Ring이, 그리고 물성과 안정성을 개선하고자 Bregion의 Ketone을 좀 더 구조적으로 안정하며 합성이 용이한 Bioisostere인 Amide로 치환한 화합물을 설계 및 합성하였다. 더불어 Bregion에 다양한 Linker들을 도입함으로써 부가적인 Pharmacophore를 찾고자 하였다.

합성된 Amide 유도체가 저산소상태에서 HIF-1α의 발현을 줄이는 것을 Western Blot Assay를 통해 1차적으로 확인하였다. 30 가지의 화합물들 중 2 가지 화합물에서 (22, 44) Deguelin보다 약 두 배 가량 높은 활성을 띠는 것을 확인하였고, 활성이 있는 화합물은 현재 추가적으로 독성 및 항암 활성 실험을 준비 중에 있다. 이에 따라 효능 있는 HIF-1α 저해제 연구를 통해 혈관신생질환의 치료제 및 항암제 후보물질 개발에 도움을 줄 수 있을 것이라 본다.

주요어 : TRPV1 길항제, Capsaicin, HSP90, HIF-1α 저해제, Deguelin 학번 : 2014-21044