Master's Thesis

SAR Studies on the Inhibitors for the Treatment of Inflammatory Diseases

Min-Jeong Kim

School of Molecular Sciences

(Chemistry)

Graduate School of UNIST

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Approved by

Advisor
Cheol-Min Park

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This certifies that the thesis of Min-Jeong Kim is approved.

06. 13. 2016

Advisor: Cheol-Min Park

Tae-Hyuk Kwon

Min-Sang Kwon

Abstract

Inflammation is defensive host response that occurs from infection and injury and the inflammatory process is the pivotal physiological response of our body and essential part of the human physiology. Due to the mechanistic relationship between chronic diseases and inflammation, a better understanding for the molecular mechanism of chronic inflammation could attenuate cellular inflammation pathways. Under inflammatory pathways, the impetus of proinflammatory mediators usually caused by the increased expression of transcriptional factors which is also a potential targets in the development of novel and effective anti-inflammatory therapeutics. Among others, we are interested in the Nuclear Factor Kappa-B (NF-κB) which is reported as a major mediator that regulates inflammatory gene expression and also decrease the prevalence of inflammation responses. To suppress the inflammatory activity, inhibitors that could selectively target this protein are needed. We therefore, chose the natural product cerulenin which has been studied widely because of its antifungal and antibacterial properties, for designing inhibitors. In light of the interesting inhibitory properties displayed by cerulenin for fatty acid synthase (FASN), we were keen to explore the possible binding mode of this natural product with a view to design various derivatives that would be amicable to synthetic manipulation in order to enable SAR studies. Potent analogues of cerulenin, with various chain lengths and substitutions, are synthesized and evaluated for their ability to inhibit NF-κB enhanceosome. Taken together, by identifying target protein with constructed inhibitors derived from cerulenin might give revolutionary effect on discovering new therapeutic agents.

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Abbreviations

RA, Rheumatoid arthritis

COPD, chronic obstructive pulmonary disease

IBD, Inflammatory bowel disease

MS, Multiple sclerosis

TNFα, Tumor necrosis factor α

IL-1, Interleukin-1

TLR, Toll-like receptor

COX-2, Cyclooxygenase-2

VCAM-1, Vascular cell adhesion molecule 1

WHO, World health organization

UC, Ulcerative colitis

CD, Crohn's disease

CRP, C-reactive protein

NSAID, Non-steroidal anti-inflammatory drugs

IMID, Immune-mediated inflammatory diseases

CCR, C-C chemokine receptor

CXCR, Chemokine receptor

AA, Arachidonic acid

5-LO, 5-lipoxygenase

FLAP, 5-lipoxygenase-activating protein

S1P, Sphingosine 1-phosphate

MAPK, Mitogen-activated protein kinase

Syk, Spleen tyrosine kinase

JAK, Janus kinase

DHODH, Dihydroorotate dehydrogenase

PBE4, Phosphodiesterase-4

GC, Glucocorticoids

IκB, Inhibitory κB protein

IKK, IκB kinase

MMP, Matrix metalloproteinase

NE, Neutrophil elastase

NFAT5, Nuclear factor of activated T-cells 5

FASN, Fatty acid synthase

NF- κB, Nuclear factor κB

TonEBP, Tonicity-responsive enhancer binding protein

TBAB, Tetrabutylammonium bromide

SO_3·pyr, Sulfur trioxide pyridine complex

DMSO, Dimethyl sulfoxide

TPAP, Tetrapropylammonium perruthenate

NMO, N-methylmorpholine-N-Oxide

TMS, Tetramethylsilane

TFAA, Trifluoroacetic anhydride

DCI, N,N'-diisopropylcarbodiimide

DMAP, 4-Dimethylaminopyridine

m-CPBA, meta-Chloroperoxybenzoic acid

DMF, Dimethylformamide

NO, Nitric oxide

MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)

I. INTRODUCTION

Inflammation is defensive host response that occurs from infection and injury which are caused by foreign substances such as bacteria and viruses. Inflammation process is the pivotal physiological response of our body and essential part of the human physiology. However, inflammatory pathways dysregulation can lead to either acute or chronic inflammatory diseases. Acute inflammation is first defensive attempt of our body against injury and infectious agents. If it fails to remove exogenous stimuli or the symptoms lasts more than few weeks, it can become chronic and may serve as a genesis of various diseases. Contrast to acute inflammation, chronic inflammation which pathomechanism is less understood, persist over several months or years and eventually leading to other chronic diseases as well as cancer. Continued inflammation acts as major drivers for the development of inflammatory diseases like asthma, rheumatoid arthritis (RA), chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), multiple sclerosis (MS), and psoriasis. Moreover, inflammatory responses play roles in the progression of several permanent diseases including cancer, atherosclerosis, diabetes, obesity, and Alzheimer; and this ardent phenomenon provide clues to understand different inflammatory markers. In chronic inflammation, inflammatory cells respond to external proinflammatory factors and inflammatory stimulus like environmental pollutants, viruses, bacteria, food, stress, etc; and generate bioactive mediators such as transcription factors (NF-kB, STAT) and their gene products such as cytokines (IL-1, IL-6, TNF-α), chemokines (IL-8, VCAM-1), chemokine receptors and COX-2. Despite the essentiality of these molecules in normal cell regulation processes, uncontrolled and too high expression to those molecules can result in chronic disorders.[1] As these mediators have complex, pleiotropic effects, it interacts with many cell types to amplify the inflammatory response. Therefore, due to the mechanistic relationship between chronic diseases and inflammation, a better understanding for the molecular mechanism of chronic inflammation could attenuate cellular inflammation pathways.

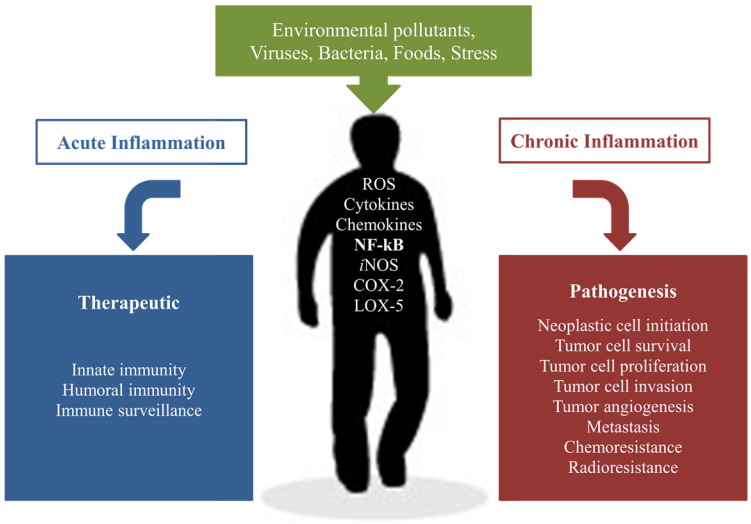


Figure 1. External/internal proinflammatory factors and their biological responses

1.1. DISEASES ASSOCIATED WITH CHRONIC INFLAMMATION

To fully understand the relationship between inflammation and the diseases, we need to know some major diseases that associate with chronic inflammation. Moreover, pathogenesis of these chronic inflammatory diseases will forest new therapeutic approaches for anti-inflammatory remedy. There are various immune-mediated inflammatory diseases but we will focus on the most common ones which are rheumatoid arthritis (RA), chronic obstructive pulmonary disease (COPD) and inflammatory bowel disease (IBDs). It is not mentioned in this paper, but other autoimmune diseases like multiple sclerosis (MS) and psoriasis also have their own distinct pathogenesis.

1.1.1. Rheumatoid arthritis (RA)

Arthritis is the typical type of disease that takes huge part in inflammatory diseases. Among 100 different forms of arthritis, Rheumatoid arthritis (RA) is a chronic systemic disorder which affects about 1% of the worldwide population, most commonly middle-aged women. Though the exact cause for RA is not yet wholly established, it is characterized by cytokine mediated chronic inflammation of the synovium inside of the joints that leads to destruction of cartilage and bone.[2] From the process of a massive infiltration of leukocytes into the synovial tissue leads to production of proinflammatory mediator, cytokines. Cytokines which maintain the inflammation and degrade proteoglycans and collagen, eventually result in tissue breakdowns and destroy the nearby articular cartilage.[3] The proximate triggers of synovitis in the affected joints still a matter of debate, but activation of toll-like receptor (TLR) on T cell is implicated.[4] It is well established that interleukin-1 (IL-1), and interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) are pivotal cytokines that starts the proinflammatory cascade in RA.[5] Currently, the treatment for RA consist of glucocorticoids and NSAIDs, but only a small portion of ongoing clinical trials for treating RA are conducted with small molecules having anti-inflammatory properties.

1.1.2. Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease which associated with an abnormal response in the lung and also with systemic inflammation caused by inhalation of noxious gas and particles. Statistic data from the World Health Organization (WHO) shows 65 million people suffer from COPD, and more than 3 million people died from it in 2005 and it is considered as a significant health burden worldwide. Now, COPD is the fourth leading cause of death in the world, but will rise to third by 2030. The pathomechanism of COPD involve with the increased systemic oxidative stress and activation of inflammatory cells from severe inflammatory process in the airways.[6] Initiators of this abnormalities are not clearly identified but neutrophils and macrophages are thought to be responsible for the release of proteases, cytokines (IL-1, IL-8, TNF-α, and LTB4) and mediators, which lead to inflammation and remodeling.[7] Therefore, from this pathway, patients with COPD suffers from chronic airflow limitation, chronic bronchitis and emphysema accompanied by mucus hypersecretion.[8] unfortunately, there is no cure for COPD and the available

medications that is in the market is unable to stop the deterioration in lung function. Since drugs that are mostly used for COPD only treat symptoms and have no progress on stopping inflammatory process, there is a large unmet need for drugs.

1.1.3. Inflammatory bowel disease (IBD)

Inflammatory bowel disease (IBD), generally characterized to two major forms, ulcerative colitis (UC) and crohn's disease (CD). IBD is a chronic intestinal inflammatory disease caused by dysregulated immune response which occurs from increased activity of the immune system in the intestinal microflora, then leads to tissue damage. Etiologies of these processes remain uncertain but both genetic and environmental factors are responsible for intestinal inflammation. Cytokines associated with IBD are IL-6, IL-1B and TNF-α which stimulate the production of CRP.[9] So far, there is no efficacious pharmacological treatment for both UC and CD. Currently, diverse strategies that have anti-inflammatory properties for treating IBD are in clinical development. Besides biological, only about 16% of recent clinical trials to manage IBD have been performed with small molecules.[10]

1.2. VARIABLE SMALL MOLECULES TO TARGET INFLAMMATORY MEDIATORS

Currently, many new pharmacological approaches to map the pathology of immune-related inflammatory diseases are ventured to minimize the inflammation. Anti-inflammatory drugs can be classified by their chemical properties into biologicals and small molecules, which can be subdivided to steroidal and non-steroidal agents. Though the biologicals cause huge influence in the autoimmune disorder and cancer market, a strong need for small molecules that are safe and potent is demanded. Practically, small molecules are much economical compared to biologics and can easily be taken orally. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most prevalent drugs for the treating inflammation, while glucocorticoids considered as strongest weapons among others against acute phases of chronic inflammation. Sadly, both type of treatment for various inflammatory diseases are not satisfying. Therefore, it is important to focus on small molecules in clinical trials for chronic inflammatory diseases that particularly belong to the field of immune-mediated inflammatory diseases (IMIDs).

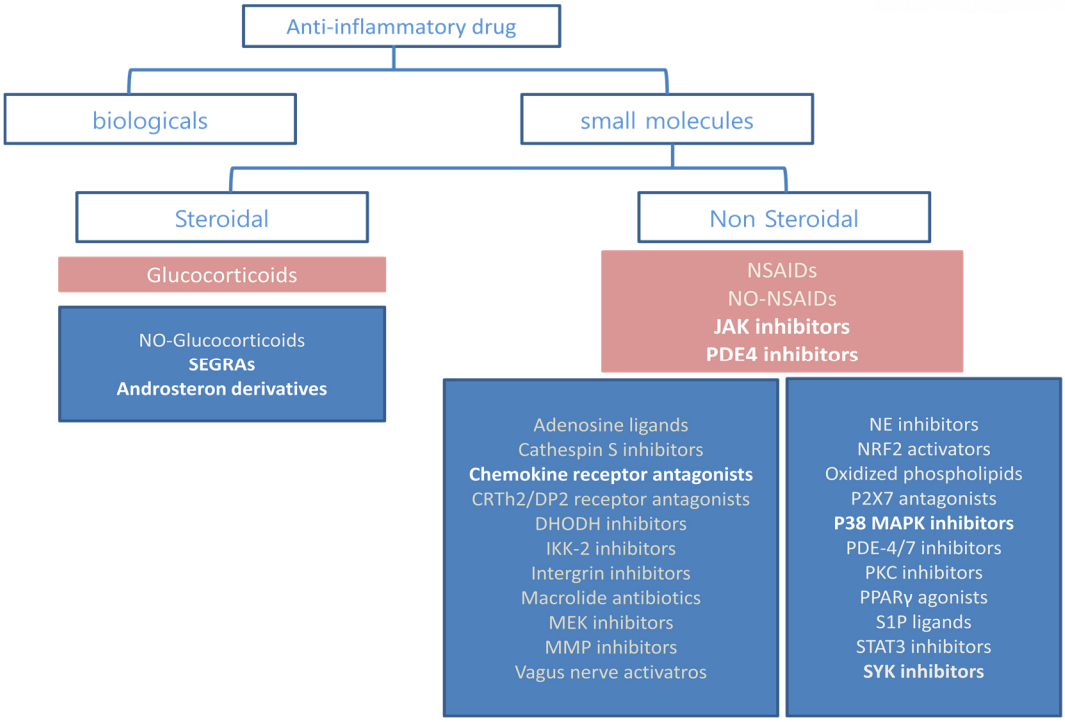


Figure 2. Category of anti-inflammatory drugs and outline of the drug classes

A wide spectrum of drugs and natural compounds has been established to be useful reducing agent for inflammation process, it is important to know about the root causes and their mechanism of inhibition. There are some drugs that are developed for one particular disease, but in most cases for new inflammatory targets are involved in broad spectrum of diseases. From these aspects, it is important for us to know better about the current clinical anti-inflammatory approaches for the targets. Approaches are distributed by their mode of action into four classes and various agents in clinical trials are also demonstrated together.

1. **Agents inhibiting the effect of mediators on the extracellular side;** Chemokine receptor, Arachidonic acid metabolites, ligands of adenosine, ligand-gated ion channel 7 (P2X7), oxidized phospholipids (OxPL), very late antigen-4 (VLA-4), S1P, chemoattractant homologous receptor expressed on Th2 cells (CRTh2), α7-nicotinic acetylcholine receptor (nAChR)

1.1. Chemokine receptor antagonists

Chemokine receptors take huge part in regulating the immune system, and therefore regarded as prospective targets for treating various inflammatory diseases. Chemokine receptors are sorted by several types. CCR1 antagonist AZD4818 which is inhalable was registered in phase II for COPD. It showed safety and efficacy but failed to give a clinical profit.[11] Another CCR1 antagonist CCX354 showed adequate safety and efficiency in randomized, placebo-controlled phase IIa with RA patients,[12] and further phase IIb is ongoing. CCR2 antagonist AZD2423 has gone through phase I and II specifically for the treatment of pain which showed little clinical efficacy but rather satisfying

safety and tolerability.[13] Interestingly, CCR5 is the only chemokine receptor that is implemented in a marketed drug which is CCR5 antagonists Maraviroc.[14] CCR9 antagonists vercirnon exhibits efficacy on crohn's disease in phase II with well tolerance and safety, but failed to meet desirable end points in phase III where it didn't show greater improvement.[15] CXCR2 antagonists danirixin has gone four phase I studies for inflammatory diseases and showed good pharmacokinetics profile in COPD. Recently, therefore a phase II clinical trial has been investigated to observe clinical benefit of oral danirixin in COPD patients. Navarixin subjected to seven clinical trials and at phase II for asthma didn't give significant improvement than phase I with healthy volunteers. Other CXCR2 antagonists AZD5069 showed safety and tolerability in a phase I and also was tested in a phase II trial for COPD and asthma.

1.2 Arachidonic acid cascade

The arachidonic acid (AA) cascade can be targeted either with NSAIDs directly or indirectly with glucocorticoids. Arachidonic acid (AA) metabolites, prostaglandins and leukotrienes are well known for significant role as proinflammatory mediators.[16] The 5-lipoxygenase (5-LO) is an important enzyme for metabolism of AA to leukotrienes. So far, zileuton is only one to be addressed to the market as direct 5-LO inhibitor to treat asthma, but no significance in RA nor IBD.[17] Another 5-LO inhibitor setileuton was evaluated in a phase II clinical trial for COPD, but it didn't show more effectiveness than placebo. 5-lipoxygenase-activating protein (FLAP) inhibitor fiboflapon showed satisfactory clinical results in preclinical and phase I clinical trial.[18] The LTB4 receptor antagonist amelubant (BIIL284) is evaluated in phase II clinical trials for COPD and phase I for RA which result is rather disappointing.[19]

1.3 Adenosine receptor ligands

Selective A3AR agonist CF101 (CanFite BioPharma's) showed a good clinical benefit on symptoms and biomarkers of RA and also for plaque psoriasis in phase II.[20] A phase III trial for psoriasis with CF101 is ongoing.

1.4 Oxidized phospholipids

VB-201 recently entered in phase II clinical trials for active plaque psoriasis. VB- 201 is a small synthetic molecule which belongs to the class of the lecinoxoids, but has no proinflammatory activity.

1.5 Integrin receptor antagonists

In phase II clinical trial, firategrast significantly reduced new lesion formation in proportion to placebo at high dosage. Moreover, firategrast displayed acceptable safety and tolerability, but could observe little adverse event.[21]

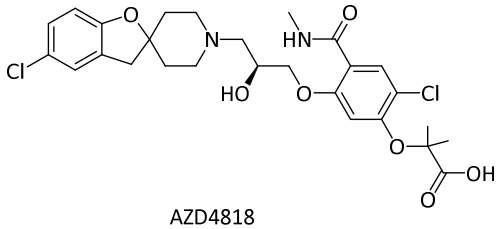
1.6 S1P receptor ligands

Fingolimod is a molecule that targets S1P signaling which has become a vital target for treating especially for MS, has recently approved in the market to treat MS. The S1PR1 and S1PR5 selective agonist siponimod and the S1PR1 agonist ponesimod are dominating the clinical network showing

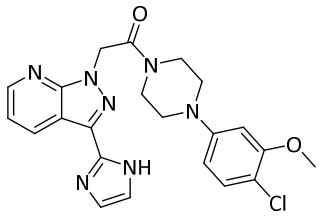
significant clinical benefit in phase II trials. [22] Two other promising S1P agonists, ceralifimod and GSK2018682 are also in MS pipline.[23]

Chemokine receptor antagonists

CCR1 antagonists

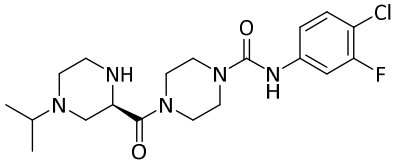


AZD4818



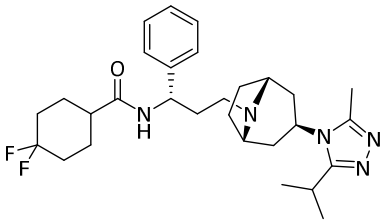
CCX354

CCR2 antagonists



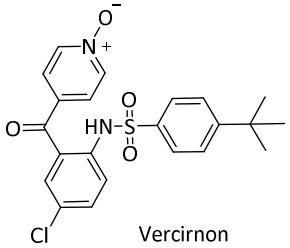
AZD2423

CCR5 antagonists



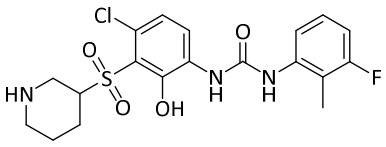
Maraviroc

CCR9 antagonists

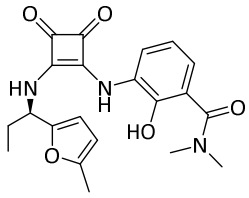


Vercirnon

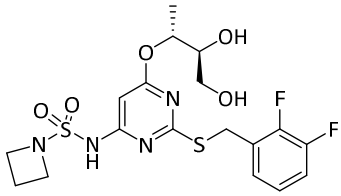
CXCR2 antagonists



Danirixin

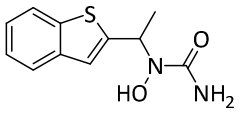


Navarixin

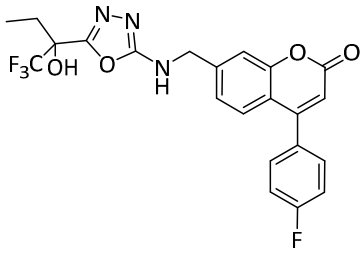


AZD5069

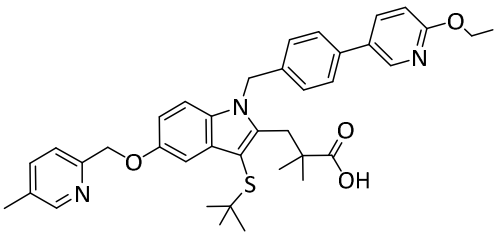
Arachidonic acid cascase



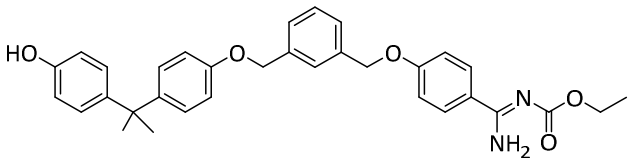
Zileuton



Setileuton (MK-0633)

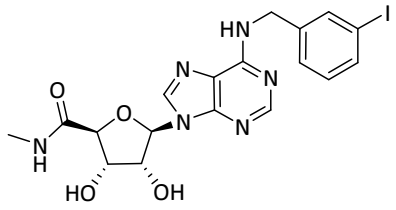


Fiboflapon (GSK2190915/AM803)



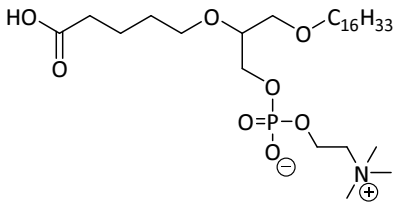
Amelubant (BIIL284)

Adenosine receptor ligands



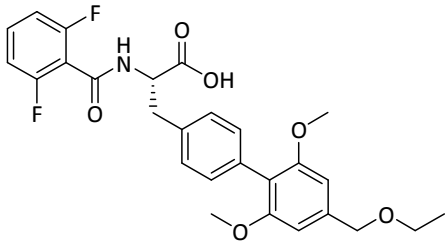
CF101

Oxidized phospholipid



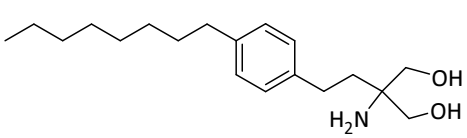
VB-201

Integrin receptor antagonists

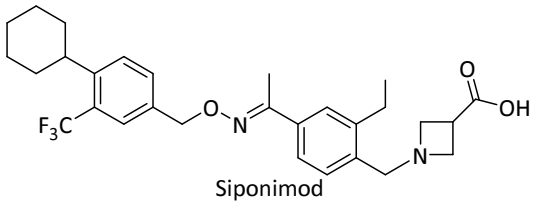


Firategrast (SB683699)

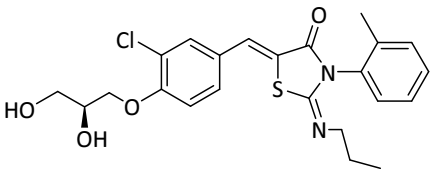
S1P receptor ligands



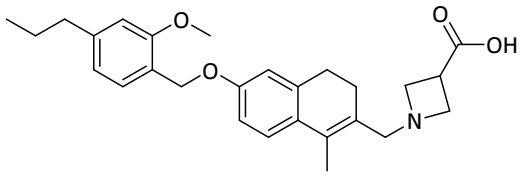
Fingolimod



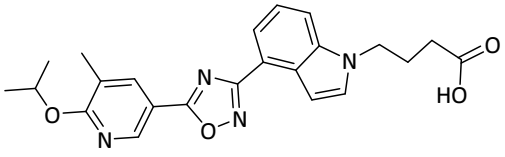
Siponimod



Ponesimod



Ceralifimod



GSK2018682

Figure 3. Agents inhibiting the effect of mediators on the extracellular side

2. Agents inhibiting the production of intracellular mediators by interfering with intracellular signaling pathways; p38, mitogen-activated protein kinase kinase 1(MEK1), MEKK1, JAK, SYK, S1P lyase, DHODH, or PDE ligands

2.1. Kinase inhibitors

Kinase inhibitors considered as attractive targets for anti-inflammatory small molecules. Especially, p38 MAPK was the first kinase targeting anti-inflammatory drug since it plays pivotal role in regulating production of cytokine.[24] Several p38 MAPK inhibitors which are talmapimod,

pamapimod, and VX-702 entered phase II clinical trials, but failed because of high toxicity and low efficacy. MEK-162, the dual MEK1/2 inhibitor exhibited good preclinical results in models of RA, and displayed well tolerance in a phase II clinical trial but did not give contentable result.[25] As dual MEK1/MEKK1 inhibitor E6201 showed good result in several preclinical for psoriasis[26], entered two phase II for psoriasis but no results have been published so far. Two tyrosine kinases, JAK and SYK are targeted for inhibiting pathogenesis of RA. The selective JAK3 inhibitor decernotinib (VX-509) showed clear efficacy in a phase IIa trial for RA and also undergoing phase II b study.[27] Recently, JAK3/JAK1 inhibitor tofacitinib (Xeljanz®) a highly potent molecule was approved by the FDA for diverse severity of RA treatment. The dual JAK1/2 inhibitor baricitinib showed improvement in a phase II trial for signs and symptoms of RA[28] and undergoing four phase III trials. SYK inhibitor, fostamatinib (Astra Zeneca's) revealed significant efficacy and achieved desired end points in most cases at phase II trials for RA.[29]

2.2. S1P Lyase inhibitor

From the fact that S1P signaling can be regulated with S1P lyase (SPL), it got attention. The SPL inhibitor LX3305 (LX2931) showed fine safety profile, but did not give satisfying result in a phase IIa study for RA. Currently, another phase II trial with higher dosage is ongoing.[30]

2.3. DHODH inhibitors

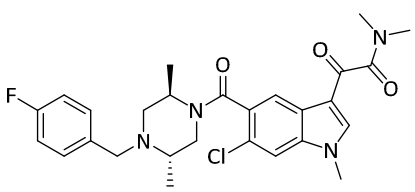
The first inhibitor of DHODH, teriflunomide (Aubagio®) has recently been approved by the FDA, for treatment of MS. Another DHODH inhibitor vidofludimus (ENTRANCE) revealed significant efficacy for IBD[31] and entered two more phase IIa trials which also showed good clinical efficiency with CD or UC. From this result, we can expect reduced steroid treatment at the end of the trials.[32]

2.4. Phosphodiesterase inhibitors

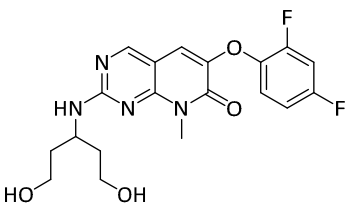
PDE4 inhibitors roflumilast is the first inhibitor that is approved for efficacy for COPD.[33] Moreover, PDE4 inhibitors came to be interested in other inflammatory diseases like IBD and psoriasis. The PDE4 inhibitor tetomilast (OPC-6535) showed rather moderate result in phase I and II and recently entered in phase III trial for IBD.[34] The inhibitor AN2728 which not only inhibit PDE4 but also downgrade activation of cytokine undergone clinical trials in phase II for psoriasis and entered phase III in sequence

Kinase inhibitors

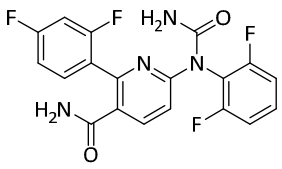
p38 MAPK inhibitors



Talmapimod (Scio-469)



Pamapimod (RO4402257)



VX-702

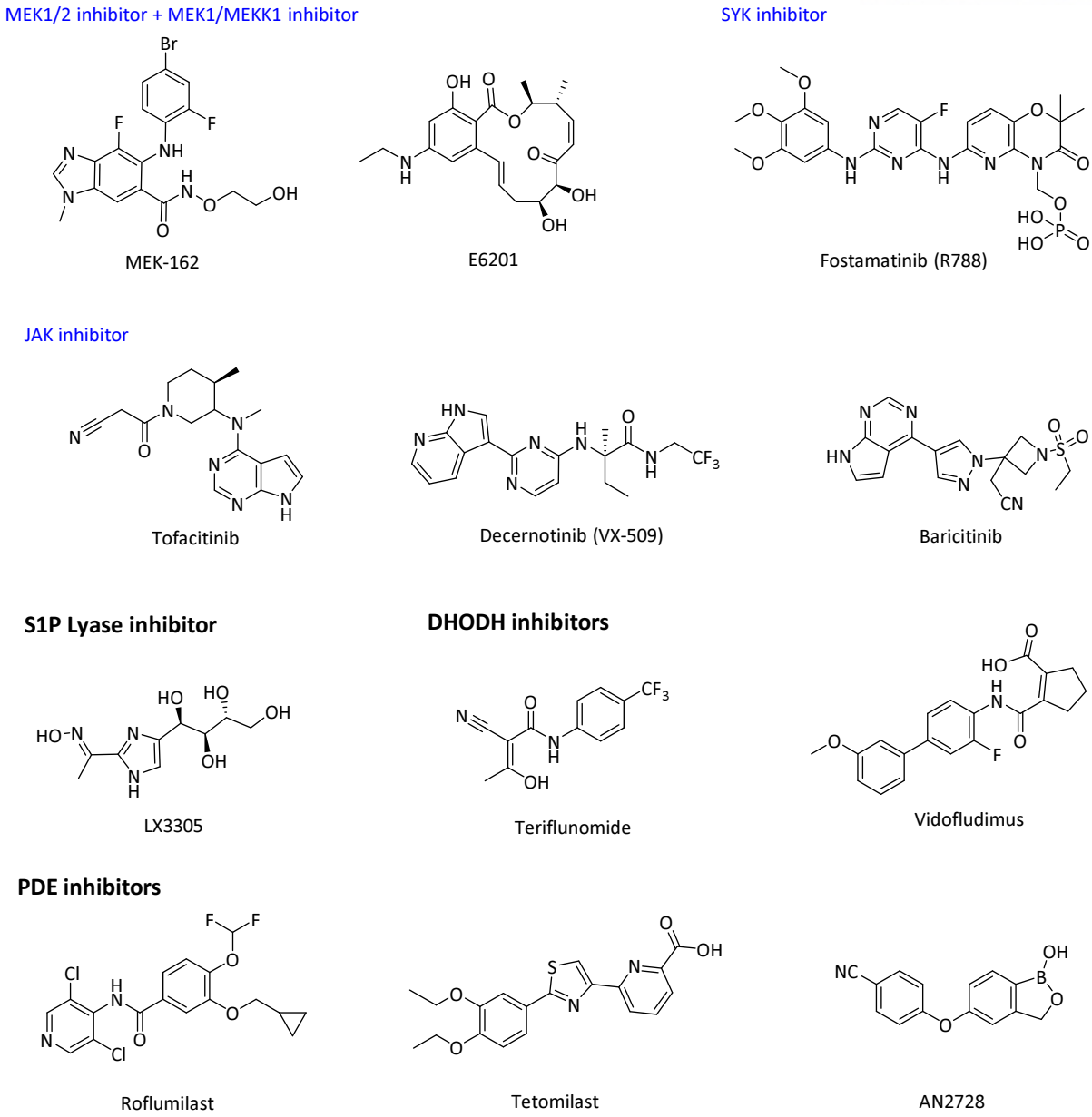


Figure 4. Agents inhibiting the production of intracellular mediators by interfering with intracellular signaling pathways

3. **Agents inhibiting the production of mediators in the cell nucleus by interfering with the transcription process;** selective glucocorticoid receptor agonists (SEGRA), PPAR, signal transducers and activators of transcription (STAT) ligands, IκB kinase 2 (IKK2) modulators, or NRF2.

3.1. SEGRA

Glucocorticoids (GCs) are most powerful tool for treating acute inflammation with their pleiotropic property. However, it is not suitable for chronic inflammation due to side effects. Both ZK216348 and a natural product compound A (CpdA) display anti-inflammatory activity in chronic diseases. Recently, AZD5423 was evaluated safety and efficacy in phase II for COPD.

3.2. PPARγ agonists

PPARγ participate in inflammation in numerous ways and consider as good drug target. PPARγ agonists troglitazone showed good result for psoriasis the in vitro trial. Another PPARγ agonist Pioglitazone also showed robust improvement in clinical trial for the psoriasis without adverse events.[35] In addition, not only the result for clinical trials for psoriasis but also RA and MS is very promising.[36]

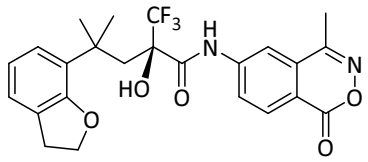
3.3. STAT3 inhibitor

The STAT3 inhibitor STA-21 showed robust efficacy in trial for psoriasis[37] and potentiality for RA treatment.[38] In addition, possibility of STAT3 inhibitor for treatment for IBD is also mentioned.[39]

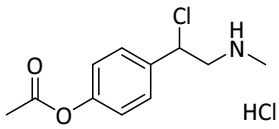
3.4. IKK-2 inhibitor

IKK-2 is subunit of IκB kinase and plays potent role in NF-κB pathway which is important for regulation of chronic inflammation. The IKK-2 inhibitor IMD-1041 is currently evaluated in a phase II clinical trial for COPD, but no results have been confirmed yet and the structure is not disclosed.

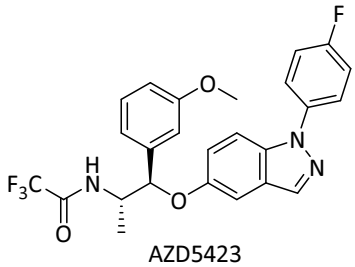
SEGRA's



ZK216348

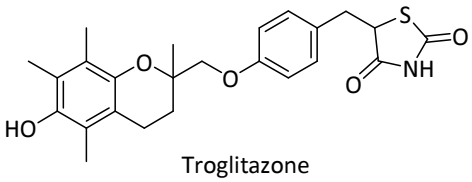


Compound A

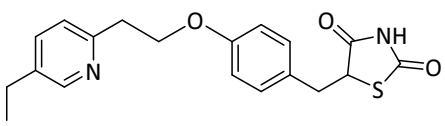


AZD5423

PPAR agonists

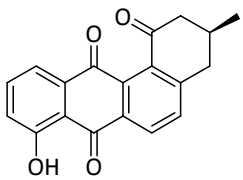


Troglitazone



Pioglitazone

STAT3 inhibitor



STA-21

IKK-2 inhibitor

structure not disclosed

IMD-1041

Figure 5. Agents inhibiting the production of mediators in the cell nucleus by interfering with the transcription process

4. **Agents directly blocking the cytotoxic effects of enzymes/proteases;** Matrix metalloproteinase (MMP), Neutrophil elastase (NE), or Cathepsin S inhibitors

4.1. MMP inhibitors

Among several MMPs, MMP-9 was shown to be potential target for MS and COPD treatment.[40] The selective MMP-9 and MMP-12 inhibitor AZD1236, displayed good safety profile but rather unsatisfying result in clinical trials for COPD. However, still hold possibility for therapy of COPD in a longer trial.[41] The dual MMP-13/TACE (TNFα-converting enzyme) inhibitor apratastat undergone clinical trial in phase II for RA, but was terminated due to a lack of efficacy.

4.2. NE inhibitor

NE inhibitor AZD9668 didn't show any benefits or effects in phase II clinical trial for COPD.[42] Therefore, it is not certain whether NE could serve as a potential target for a disease relieving agent in COPD.

4.3. Cathepsin S inhibitor

Cathepsin S serve as attractive target for treatment of autoimmune disease like COPD and arthritis.[43] Cathepsin S inhibitor RWJ 445380 entered in phase II trials for RA and psoriasis, but no results are known so far and the structure is not yet disclosed.

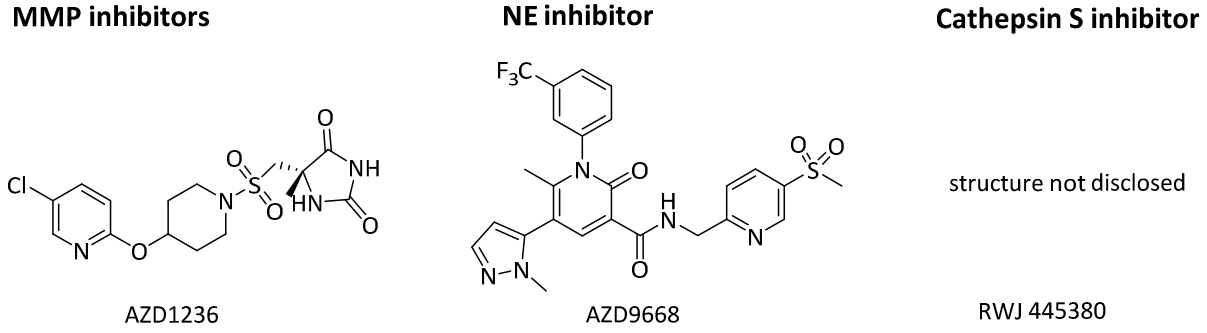


Figure 6. Agents directly blocking the cytotoxic effects of enzymes/proteases

As a demand for better design for inflammation trials and clinical parameters is imperative, tracking current clinical breakthrough for inflammatory diseases is important. So far, clinical trials for inflammatory, immune-mediated disorders which are RA, MS, IBD, and COPD, take minor part of clinical studies of small molecules. Chemokine receptors, the adenosine receptors, p38 MAPKs, JAK, PPARγ, STAT3, NRF2, and, MMPs are markedly presented by current research on each target. P2X7, S1P, JAK, STAT3 and NRF2 have got spotlighted during the last few years, while a decreasing attention can be observed for VLA-4, OxPLs, PDE4 and NE. In this milieu, various new therapeutic approaches to interfere with the inflammatory process are under investigation. New molecular targets that interfere in the inflammatory process are also being recognized and further studies needs to be establish and demonstrate the effectiveness.

1.3. A PROMISING THERAPEUTIC TARGET: NF-κB

Under inflammatory pathways, the impetus of proinflammatory mediators usually caused by the increased expression of transcriptional factors that is also a potential targets in the development of novel and effective anti-inflammatory therapeutics. The detection of transcription factors is pivotal in molecular foundation of chronic inflammatory disease in which the Nuclear Factor Kappa-B (NF-κB) is reported as a major mediator that regulates inflammatory gene expression and also decreases the prevalence of inflammation responses.[44] Therefore, understanding the pathway of controlling inflammatory gene expression by the key transcriptional regulator, NF-κB is very important. NF-κB is a diametric transcription factor belonging to Rel family with a highly conserved Rel homology domain which is responsible for DNA-binding, dimerization and interaction with the inhibitory κB protein (IκB).[45] Under physiological conditions, NF-κB dimers bind to IκB protein and remain inactive in the cytoplasm. Stimulation by inflammatory stimuli such as physical and chemical stresses, Bacteria and viruses, ROS, Microbial products (LPS, peptidoglycan) and inflammatory cytokines (IL-1, TNFα) activate IκB kinases (IKK) that consists of three subunits, IKK-α (IKK1), IKK-β(IKK2) and IKK-γ (IKK3 or NEMO). This cascade leads to IκB phosphorylation which then ubiquitinated and degraded by proteasome and release NF-κB.[46] Once NF-κB (p50 and p65 heterodimer) becomes liberated, it is not sequestered to the cytoplasm anymore but translocates into the nucleus and activates the transcription of proinflammatory genes and cell adhesion molecules.[47] As a result, NF-κB causes enhanced production of the transcriptional genes that leads inducible expression of the mediators like cytokines, chemokines, nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) which are indispensable element in inflammatory process. Clearly, targeting the activation of NF-κB shows promising avenue for suppressing inflammation and will shed a bright light on designing safe and effective drugs that are clinically useful for curing inflammatory diseases.[48]

At present, NF-κB and its signaling is one of the most exciting and extensively studied research fields since NF-κB dysregulation is associated with numerous chronic diseases including autoimmune disorders such as Rheumatoid arthritis (RA), inflammatory bowel disease (IBD) and more permanent diseases like cancer, AIDS and diabetes. Several natural and synthetic compounds, including some drugs, have been tested for their potential to inhibit NF-κB, but very few of them are suitable for anti-inflammatory and anticancer therapy. Therefore, it has been suggested that the development of novel NF-κB inhibitors with antitumor and anti-inflammatory activities is most demanded.

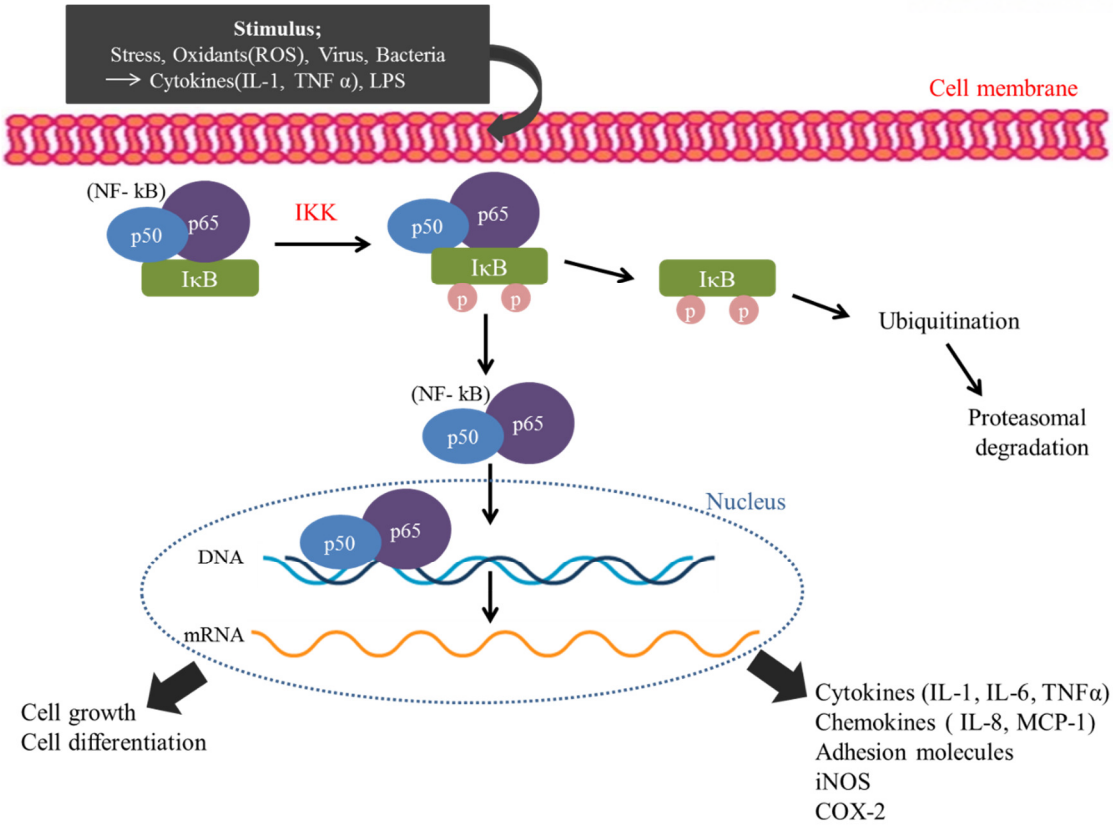


Figure 7. NF-κB activation pathway.

1.4. INHIBITION OF NF-κB SIGNALING

Current research is venturing to map the different pathways in an attempt to minimize the inflammation in chronic diseases by means of various drugs. An extensive collection of drugs that inhibit NF-κB are receiving a great deal of attention as an optimal therapeutics for treating inflammatory diseases. Drugs targeted against NF-κB complexes can be classified according to their action in NF-κB molecular signal transduction pathways and these inhibitors take a wide variety form of chemicals, synthetic compounds, metabolites, peptides, natural products, and proteins. To understand better about the molecular cascade of NF-κB inhibition, it is important to know the various stages of regulation for signaling pathway.[49] Generally, regulating NF-κB activation undergoes unique multi step procedure including whether it is blocked by incoming stimuli at an early stage of NF-κB induction; interfered at cytoplasmic stage by blockage of specific component; or blocked at later levels in the NF-κB nuclear activity. Likewise, inhibitors of NF-κB will be classified by which step of NF-κB activation is blocked according to mechanism.

1. Upstream target inhibitors

As IKK complex plays a prime role in NF-κB activation pathway, the action that block incoming signals before it activate IKK would make it possible to inhibit activation of NF-κB. From the fact that cytokines, oxidants and other foreign stimuli activate NF-κB by signaling cell-surface receptors,

it is clear that anti-TNF agents can limit NF-κB activation and consider as promising candidate for anti-inflammatory drugs. Rofecoxib is COX-2 selective inhibitor that blocks NF-κB activation in LPS-stimulated macrophage cells.[50] Similarly, Ibuprofen inhibit COX-2 expression in T cells and another inhibitor, tepoxalin blocks NF-κB activation by suppressing both 5-lipoxygenase and COX. Antioxidants are also potential therapeutic inhibitors of NF-κB.[51] However, the precise mechanism for antioxidants to block NF-κB activation is not known. Despite of the uncertainty, various antioxidant molecules are investigated. Both *N*-acetylcysteine and dithiocarbamates down regulate LPS-mediated activation towards NF-κB activity.[52] Especially, pyrrolidine dithiocarbamate is a potent molecule that inhibited the expression of ICAM-I on IL-1-stimulated fibroblasts which also showed relief of symptoms in experimental with rats.[53] Thus, vitamin C and E derivatives, and glutathione peroxidase also has inhibitory aspects NF-κB activation by inflammatory stimuli.

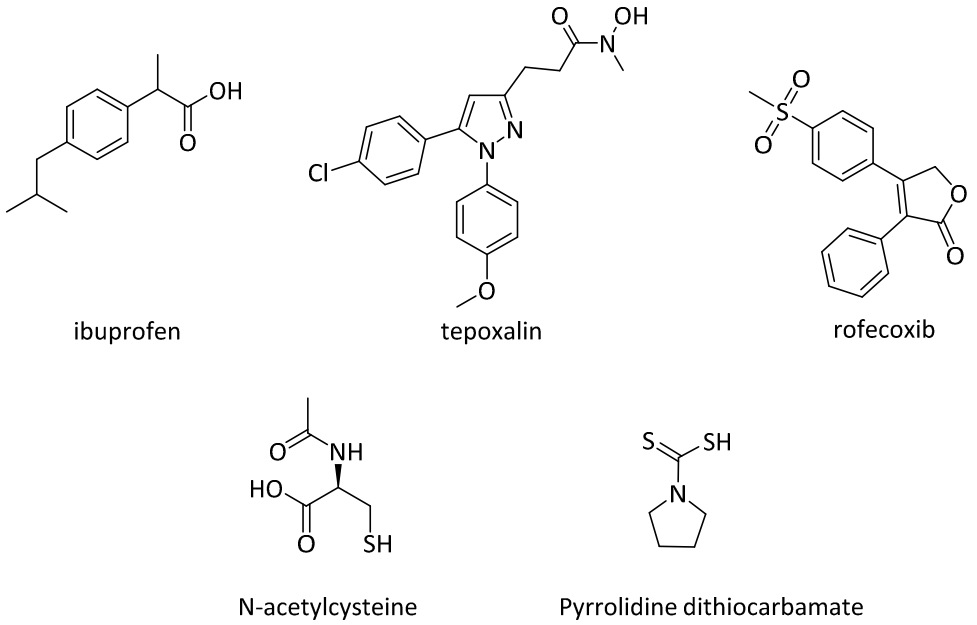


Figure 8. Upstream target inhibitors

2. IKK complex activity Inhibitors

IKK is a pivotal target for the development of NF-κB signaling inhibitors and also for kinase inhibitors for therapeutic applications because it takes significant part in NF-κB activation. Over years, diverse agents have been discovered which subdue activation at IKK step, but it is still mystery rather the inhibitors suppress IKK itself or its activation. A number of nonsteroidal anti-inflammatory drugs (NSAID) such as sodium salicylate, aspirin and sulindac inhibit the activity of IKK. Both sodium salycilate and aspirin directly bind and inhibit the ATP binding site of IKK-β.[54] However, to achieve satisfactory NF-κB inhibition, it requires relatively high concentrations because of low specificity. Similar to asprin, sulindac which is related to indomethacin structurally and pharmacologically, also reduces IKK-β kinase activity and consequently inhibits NF-κB activation.[55] In addition, some of

natural products also inhibit NF-κB activation in vitro by blocking the activity of IKK. polyphenols from green tea inhibit LPS-mediated activation of NF-κB which was confirmed by mice.[56] Resveratrol which is found in red wine and anti-inflammatory sesquiterpene lactone, parthenolide that is derived from medicinal plants, target IKK and also undergo blockage of IκB phosphorylation in specific cell types to inhibit NF-κB activity.[57] Moreover, Curcumin and capsaicin which can be easily found in natural ingredient serve as potent inhibitors of IKK activity in several cell types.[58]

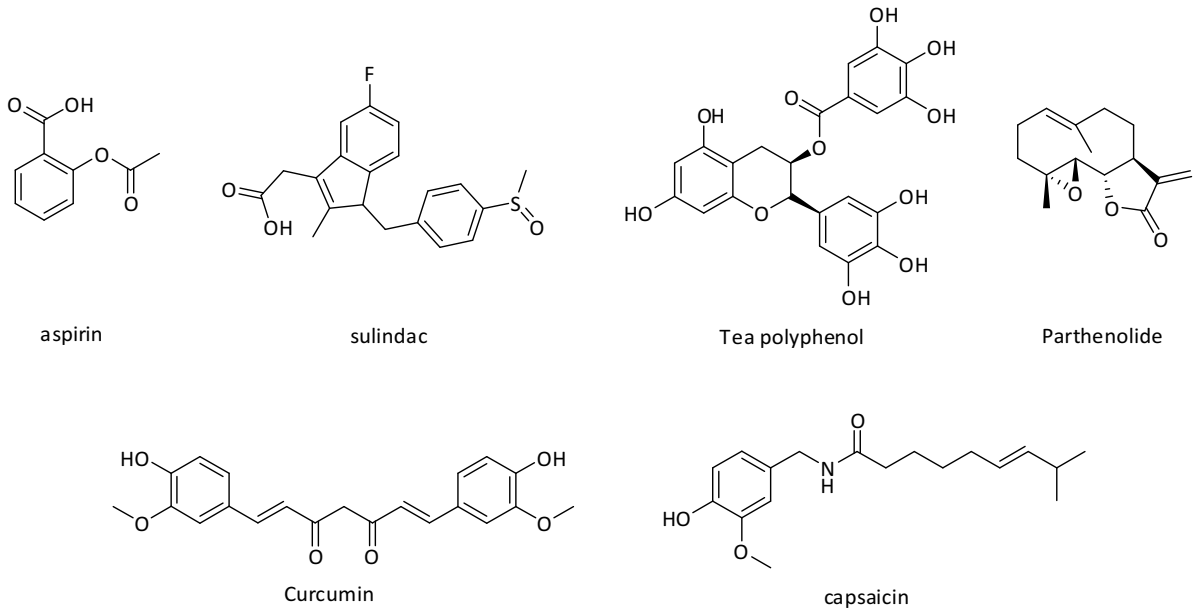


Figure 9. IKK complex activity Inhibitors

3. IκB degradation inhibitors

3.1. IκBα upregulator

Some molecules inhibit NF-κB by inducing IκB expression in high level. Glucocorticoids (GC) are the molecules that are applicable in this level inhibiting NF-κB with various methods.[59] Dexamethasone increases the level of IκBα, causing NF-κB (p50,p65) to sustain in the cytoplasm.[60] In addition, hydrocortisone induces the expression of IκBα and inhibit the translocation of NF-κB into the nucleus in human cell.[61]

3.2. IκB degradation inhibitors

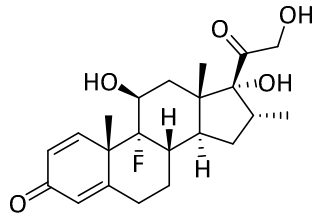
NSAIDs, sulfasalazine and leflunomide block nuclear translocation of NF-κB through inhibition of IκB-α degradation which might have been caused by a direct effect on IKK or upstream signals. Sulfasalazine suppresses nuclear translocation of NF-κB by phosphorylation and degradation of IκBα.[62] Similarly, natural product, gliotoxin from *Aspergillus* impede the phosphorylation and degradation of IκB-α that inhibit activation of NF-κB induced by LPS.[63]

3.3. Proteasome/Protease inhibitors

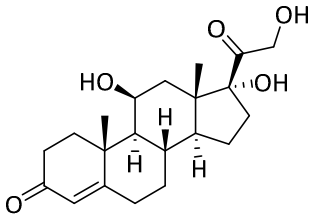
Activation of NF-κB is possible by proteasome mediated IκBα degradation which is induced by phosphorylation after IKK complex.[64] Inhibitors that block proteasome activity include several types:

peptide aldehydes, lactacystin and its precursor and peptide boronic acids. Two Peptide aldehydes MG132 and PSI are potent proteasome inhibitors. MG132 inhibit the chymotrypsin specified activity of the proteasome complex that blocks IκBα degradation and prevents radiation-induced activation of NF-κB.[65] Lactacystin is a very specific targeting inhibitor of the proteasome that reduce proteasome activity by acylating threonin residue in the active site.[66] Additionally, dipeptidyl boronate analogs also serve as proteasome activity inhibitor. Among several molecules, PS-341 and PS-519 are showing good efficacy in clinical evaluation. Especially, PS-341 which now called bortezomib is approved in U.S. to treat relapsed multiple myeloma and mantle cell lymphoma.[67] Moreover, well known immunosuppressive agents, cyclosporin A and FK-506 target activation of NF-κB.[68] Serine protease inhibitors, TLCK, TPCK and DCIC block proteases activity by suppressing IKB phosphorylation and degradation. [69] However, these serine proteases inhibitors inhibit NF-κB in all cases, but still serve as potent candidate for potent anti-inflammatory drugs.

IκBα upregulator

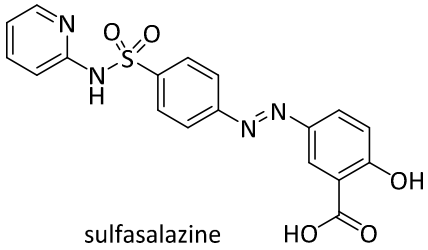


dexamethasone

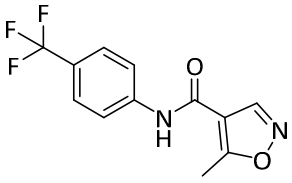


hydrocortisone

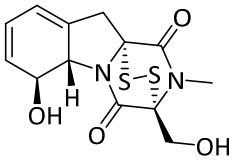
IκB degradation inhibitors



sulfasalazine

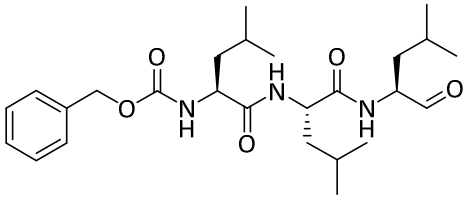


Leflunomide

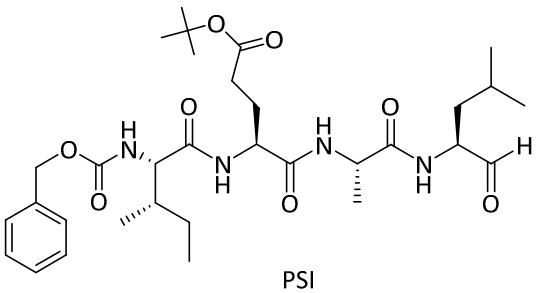


Gliotoxin

Proteasome inhibitors



MG132



PSI

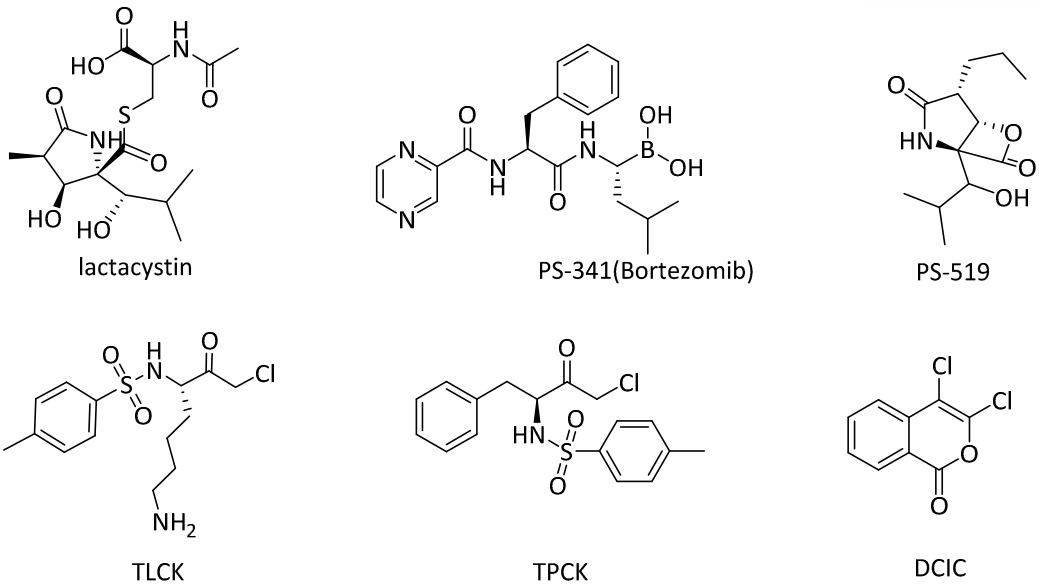


Figure 10. IκB phosphorylation/degradation inhibitors

4. NF-κB nuclear functions Inhibitors

Last step of NF-κB activation pathway include nuclear translocation, DNA binding and transcriptional activation and by blocking these steps, we can successfully inhibit NF-κB activation. Inhibitors that block nuclear translocalization are cell pervading peptides which effectively inhibit LPS- or TNFα- activated cells. SN-50 and D-amino acid cell-penetrating peptide are the commonly used kinds for treatment for inflammatory diseases.[70] Attenuating NF-κB DNA binding is possible by several sesquiterpene lactones like pathenolide. It not only inhibits DNA binding but also targets IKK activity.[71] Moreover, κB site decoy oligonucleotides plays crucial role in inhibiting NF-κB DNA binding.[72] It showed good efficacy in several clinical trials with animals having inflammation and cancer.[73] The last step in NF-κB pathway that can be blocked is transactivation of specific target genes. Natural product maealamine, wortmannin derived from fungi and LY294, 002 blocks specific genes induced transactivation of NF-κB.[74] Furthermore, particular proteins which are Bcl-2 and antithrombin are reported to inhibit transactivation by interfering interactions with RelA.[75]

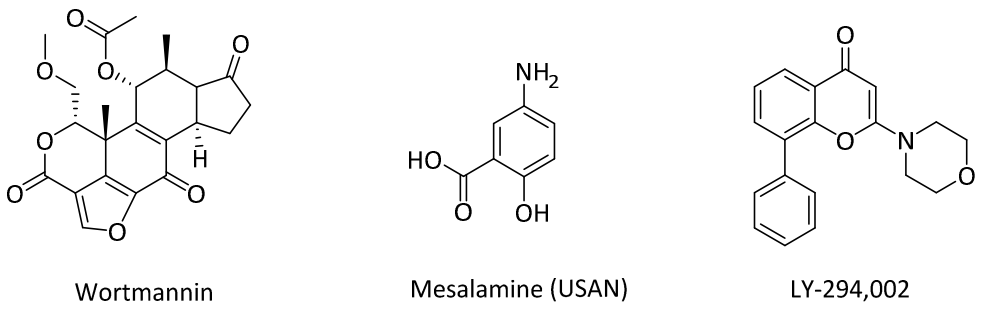


Figure 11. NF-κB nuclear functions inhibitors

Now it is clear that in chronic inflammatory disease, NF-κB takes critical part in regulating genes that induce inflammation and also suggests a promising molecular target for the production of effective and safe anti-inflammatory drugs. Although various NSAIDs, GCs and others have proven to be the potent suppresser for inflammation from intense studies for NF-κB, there are still strong eager to find molecules that can directly inhibit specific signaling pathway or molecular target of NF-κB. In addition, to eliminate unwanted side effects, complete blockade need to be done, so more efforts are needed by adjusting the mechanisms that we know so far about inhibitory actions of NF-κB. Consequently, careful consideration should be given to inhibitors of NF-κB when developing anti-inflammatory pharmaceuticals.

1.5. TonEBP, POTENTIAL TARGET FOR INFLAMMATION

Recently, possibilities for new pathogenesis of preventing inflammation is suggested from the accumulation of well known transcriptional factor NF-κB and specific protein, Tonicity-responsive enhancer binding protein (TonEBP). TonEBP has been identified to be a Rel-like transcription factor and designated as a member of the nuclear factor of activated T-cell (NFAT) family NFAT5.[76] TonEBP contains a RHD similar to that in NFAT5 proteins but lacks the translocation domain. TonEBP is known to be largely dedicated to regulating osmotic responses induced by hypertonic stress in invertebrates and mammals.[77] It protects cells from stresses that comes from high hypertonicity and acts as important role on cell growth and survival of various organs. TonEBP is known to translocate into the nucleus and induce osmoprotective gene, COX-2 enzymes in renal epithelial cells in response to hypertonicity.[78] TonEBP not only expressed in the kidney but also in various tissues, including heart, thymus and brain.[77, 79] Among others, TonEBP take huge part in tonity regulation in brain, similar to those for the kidney. Therefore, TonEBP in neurological diseases have been actively studied.[80] It is believe that TonEBP haploinsufficiency decreased NF-κB-mediated transcriptional activity and attenuated the severity of neuro inflammation.[81]

In addition to well documented classical action of TonEBP in osmoadaptation, recent studies also entail tonicity-independent functions of TonEBP might affect a wide range of pathological process like inflammation, tumor metastasis, and hepatic detoxification tonicity.[82] TonEBP was shown to enhance binding of NF-κB by interacting with p65 which is one of the NF-κB subunit and successfully induce binding of NF-κB–TonEBP complexes.[83] After NF-κB anchors to promoter's target genes and drags TonEBP to protein-protein interaction, NF-κB's activation increased. Therefore, activation rate of TonEBP proportionally determines the activation of NF-κB. As TonEBP was found to be the important modulator of NF-κB, the fact that TonEBP takes part in inflammation has come up in the surface. Especially, regulating increased expression or activation of TonEBP has alleviated inflammatory responses of chronic human diseases including diabetic nephropathy, atherosclerosis and rheumatoid arthritis.[84] These results enlightens that TonEBP plays a curial role in regulating inflammatory signaling in chronic inflammatory diseases and thus give huge impact on drug market.

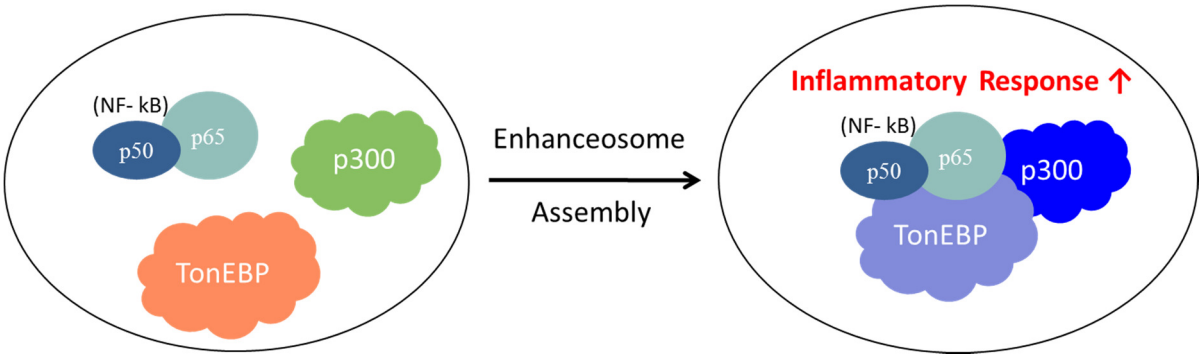


Figure 12. NF-κB enhanceosome complex

1.6. CERULENIN, ALSO REMARKABLE INHIBITOR FOR INFLAMMATION

There have been numerous approaches to find appropriate inhibitor for Fatty acid synthase (FASN) which is consider being suitable target for cancer treatment. FASN is a key metabolic enzyme involved in catalyzing the terminal steps in long chain saturated fatty acid synthesis.[85] Common in many types of human cancers, overexpression of FASN can be detected and by blocking FASN made it possible to induce apoptosis in the cancer cell, eventually inhibits growth of the cancer cells.[86] One of the most studied inhibitors of FASN is cerulenin, which has been isolated from a natural product of *Cephalosporium caerulens*.[87] The natural product cerulenin has been studied widely because of its antifungal and antibacterial properties and known to inhibit fatty acid synthases (FAS I/ FAS II) and also polyketide synthases which show efficacy against *Candida* infections.[88] Among two type of FAS which is differentiated by the fatty acid biosynthesis pathway, bacterial fatty acid synthase (type II FAS) has gained a lot of interest for a targeting antibiotic therapy including anti-cancer and anti-inflammation. So far, antibiotics, which successfully used in clinical use in vivo and vitro inhibiting FAS I pathway are triclosan, the anti-Mycobacterium tuberculosis agent and isoniazid.[89] A further pathway, several inhibitors of FASN have been elucidated and among them, cerulenin is believed to act primarily in inhibiting steps at the FAS II pathway though the inhibition of that is unselective in FAS I and FAS II system.[90] The pathway of FASN is well established in *E. coli* serves as a model to understand type II FAS systems in other bacteria and plants.[91] The β-ketoacyl-ACP synthases is a key component in type II systems by becoming covalently bound to the cysteine within the active site and carries the fatty acid intermediates from enzyme to enzyme through the cytosol. In addition, cerulenin consider as attractive drug that can defeat Malaria and tuberculosis, which are infectious disease.[92] Therefore, from this aspect, cerulenin could also give clues for the treatment against inflammatory disease.

As the mechanism of cerulenin with FASN inhibition in inflammation is not fully understood, strong need for the solution is demanded. The structure of cerulenin is composed of an α-keto-epoxycarboxamide with an octadienyl side chain. It exists in equilibrium between the open chain and a cyclized hydroxylactam form (figure 11).[93] The epoxycarboxamide moiety has been proposed as the "warhead" of the compound, which alkylates the active center cysteine residues in fatty acid synthase and palmitoyl acyltransferases, leading to irreversible inhibition. In a number of biochemical studies have demonstrated cerulenin analogues in a form of racemic and the homochiral.[94] The compound which have the 1,4-diene side chain and the *cis*-epoxy amide function was mainly featured. Furthermore, it is shown that the *cis*-epoxide flanked by two carbonyl groups is critical to Cerulenin's inhibitory activity, in which a cysteine residue opens the epoxide at C-2 of hydroxyl lactam form, thus irreversibly deactivating the *β*-ketoacyl synthase (figure 12).[94b, 95]

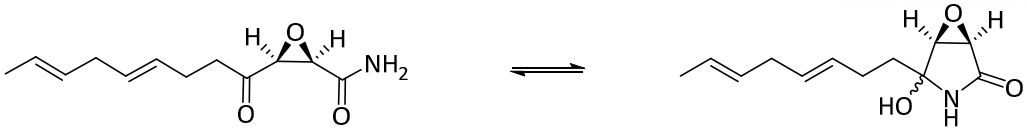


Figure 13. Open and closed form of cerulenin

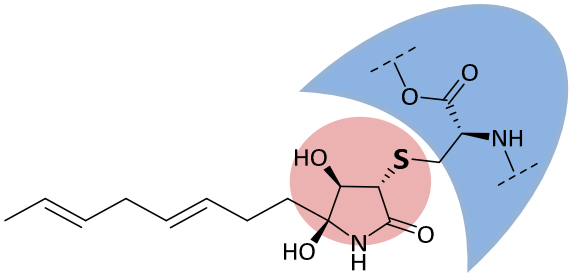


Figure 14. Cysteine opening of cerulenin's epoxide functionality

Likewise, we are interested in cerulenin analogues as a potential tool for elucidating the mechanism of protein complexes that can be derived from that of FASN. Various attempts of structure activity studies have been performed to find out which parts of the molecule are important and should be developed for a more efficient and selective inhibition towards target protein. Thus, developing chemically more stable synthetic derivatives is also important due to the cytotoxicity of cerulenin having pharmacological limitations. By constructing novel inhibitor derived from cerulenin might give revolutionary effect on drug market.

II. RESULTS AND DISCUSSION

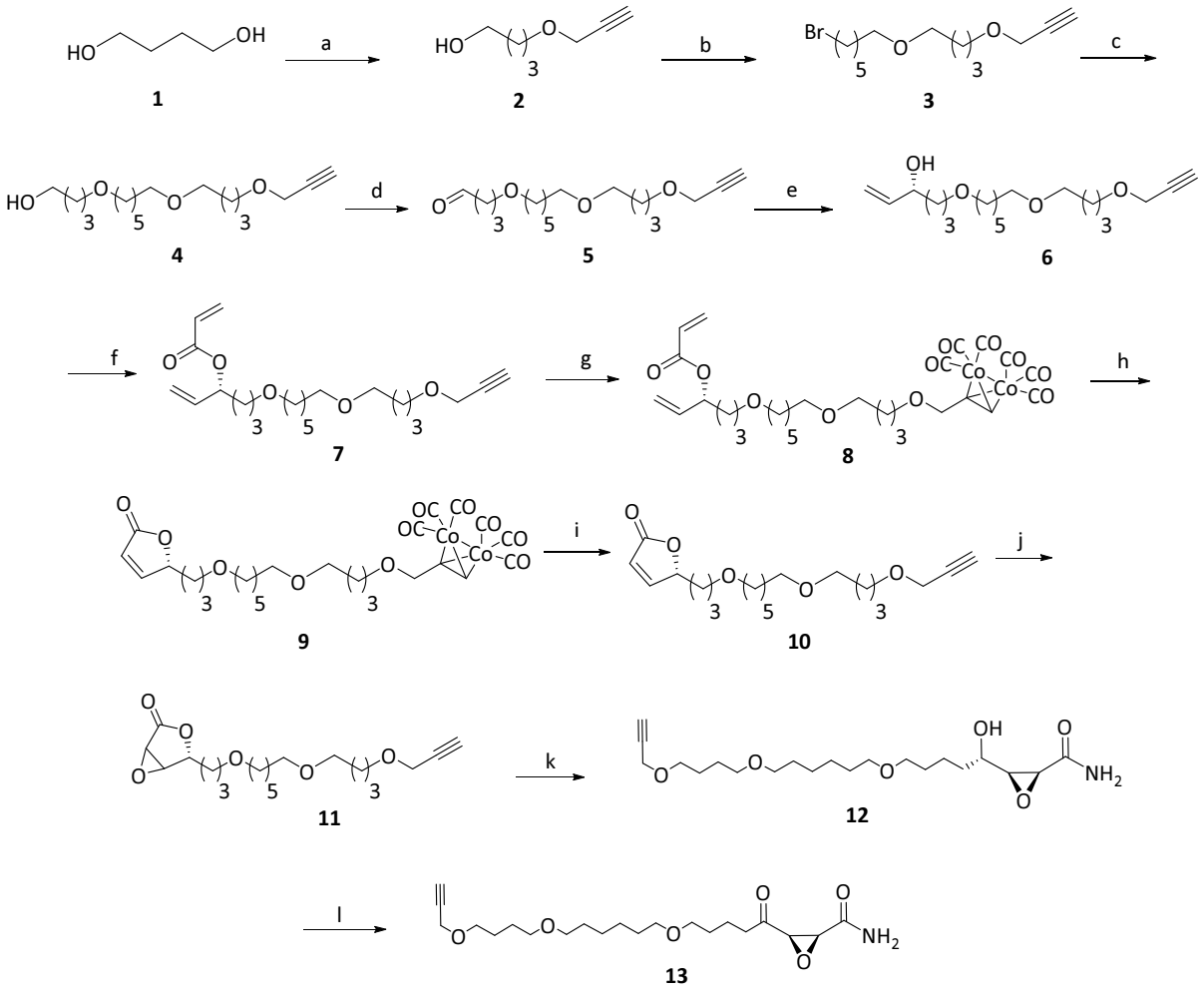
Cerulenin has previously been shown to inhibit the fatty acid synthesis and protein palmitoylatoin[96]. The epoxycarboxamide moiety of cerulenin has been proposed as the warhead of the compound, which alkylates the cysteine residues in fatty acid synthase (FASN) and palmitoyl acyltransferases, leading to irreversible inhibition.[97] In light of the interesting inhibitory properties displayed by cerulenin, we were keen to explore the possible binding mode of this natural product with a view to design various derivatives that would be amicable to synthetic manipulation in order to enable SAR studies. Therefore, assuming that a similar cysteine-based epoxide opening mechanism can be observed with that of FASN, designing cerulenin analogues could be performed for inhibiting enhanceosomes which are related to inflammation. Recent studies have shown that DNA bound NF-κB initiates the formation of distinct enhanceosomes in a target gene-specific manner.[98] When the cell is infected by viruses or other stimulus, several different DNA-binding proteins are produced, including TonEBP, transcriptional cofactor p300, and NF-κB (p65) which shown to act crucial role in inflammation.[99] Furthermore, the compelling evidence that cerulenin prevents inflammation by specifically disrupting the p65-TonEBP-p300 interaction offers a promising target for useful anti-inflammatory agents.

Not only designing analogues but also from the basis of these results, we can hypothesize that a clickable long chain analogue of cerulenin might serve as a chemical probe to investigate its cellular targets and directly label the binding proteins *in vitro* and in cells. Click chemistry reaction is the most widely employed reaction in biological studies. The copper(I)-catalyzed reaction is mild and very efficient, requiring no protecting groups, and no purification in many cases.[100]The coupling reaction of azide and alkyne groups which form a very stable triazole ring as a linker, is largely inert towards biological molecules and aqueous environments, which allows the use of the Huisgen 1,3-dipolar cycloaddition in target guided synthesis[101] and activity-based protein profiling.[102] After, by using biotin-streptavidin, we can easily fish out the target protein from protein complexes. From this effort, we can dissect new cellular targets of cerulenin analogues which are considered as potent inhibitor of inflammatory activity. We therefore, synthesized clickable analogues of cerulenin which has an alkyne group that can act as a chemical probe to explore accumulation of target proteins of interest. By using click chemistry of copper catalyzed azide-alkyne cycloaddition, we can selectively tag the target protein by a covalently bond with cerulenin epoxide group with a cysteine residue. Taken together, identification of the target protein of NF-κB enhanceosome may shed light on the detailed mechanism of inhibition by cerulenin, which could provide a basis for the discovery of new therapeutic agents.

2.1. Preparation of the click compound.

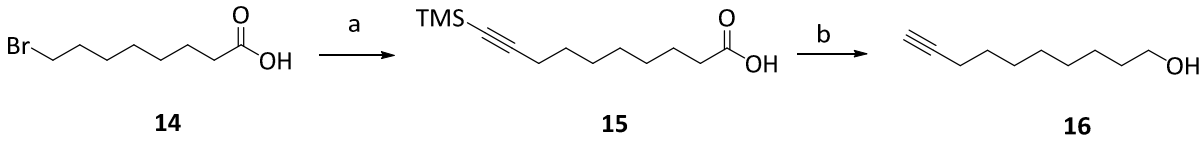
Monoalkylation of diol **1** using sodium hydride gave alkynol **2.** Alkynol **2** reacted with a excess of 1,6-dibromohexane with sodium hydride in the presence of a catalytic amount of

tetrabutylammonium bromide (TBAB) afforded the bromoether **3** in good yield.[103] Alkynol **4** was synthesized by reacting with diol on to the alkyl bromide **3**. Parikh–Doering oxidation (SO_3·pyr, DMSO) of the alcohol **4** afforded aldehyde **5**.[104] Aldehyde **5** was reacted with vinylmagnesium bromide to give secondary alcohol **6**. Reaction of the acryloyl chloride with the racemic alcohol **6** in the presence of Hunig's base gave the acryloyl ester **7**. We then tried to make lactone **10** with olefin metathesis of **7** using Grubbs' second-generation catalyst, but failed to have desired compound. We therefore decided to protect alkyne with dicobalt–octacarbonyl which forms very stable complexes with alkynes and also can be deprotected in mild oxidative condition. Fortunately, alkyne protection reaction with dicobalt–octacarbonyl[105] lead to the desired cobalt hexacarbonyl protected ester **8** in quantitative yield. Ring-closing metathesis of bis olefinic ester **8** proceeded with Grubbs' second generation catalyst [106] to furnish the *α,β*-unsaturated *γ*-lactone **9**. Subsequently, cobalt-protected-alkyne **9** was deprotected using NaOCl to regenerate the furanonic alkyne **10** in good yield. Highly efficient *sodium hypochlorite* olefin epoxidation of **10** afforded epoxy anhydride **11**, which was converted to amide **12** by reacting with ammonia. Oxidation of the resulting secondary carbinol **12** with TPAP/NMO provided ketone **13** in moderate yield.



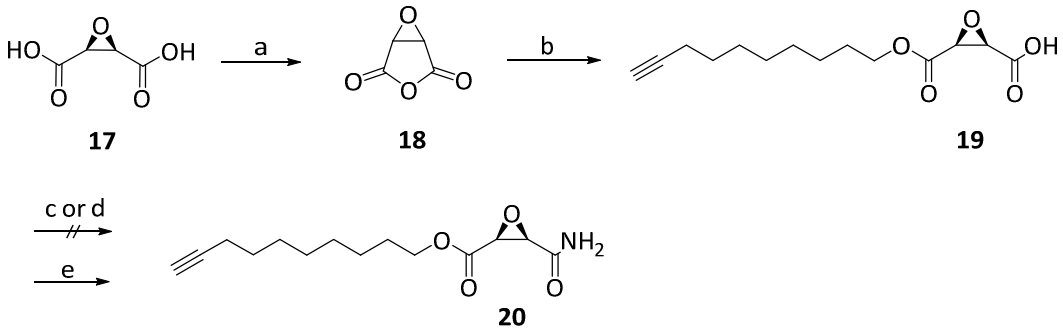
Scheme 1. Reagents and conditions: a) NaH, Propargyl bromide, THF, 81%, b) NaH, 1,6-dibromohexane, TBAB, THF, 59%, c) NaH, 1,4-butanediol, THF, 82%, d) SO_3-Pyridine, TEA, DMSO/DCM, 78%, e) Vinyl magnesium bromide, THF, 44% f) Acryloyl chloride, DIPEA, DCM, 66%, g) $Co_2(CO)_8$, DCM, 82%, h) Grubbs 2nd generation catalyst, DCM, 40%, i) NaOCl, pyridine,65%, j) NaOCl, pyridine, 55%, k) NH_4OH, Methanol, 57%, l) NMO, TPAP, 4A° ms, DCM, 40%

For the synthesis of another click compound **20**, the required alcohol **16** was prepared. Bromocarboxylic acid **14** was treated with lithium (trimethylsilyl)acetylide, which prepared by deprotection of trimethylsilylacetylene with butyllithium, to give compound **15** in 56% yield. Following reduction with lithium aluminum hydride and deprotection of the trimethylsilyl (TMS) group, desired alkynol **16** was obtained. (Scheme 2) The ester-amide **20** was prepared starting from the known epoxy maleic anhydride**.** Sodium tungstate catalyzed epoxidation of maleic acid or its anhydride gave the disodium salt in 99% yield on a molar scale, which was converted to carboxylic acid **17** via the corresponding barium salt in 75-80% overall yield.[107] The anhydride **18**[108] was formed by heating **17** with 2 equivalents of TFAA in dichloromethane. Ring opening of anhydride **18** was achieved by reacting with the alkynol **16** to give the mono substituted ester-acid **19** in good yield. Attempts to make amide **20** by employing coupling reaction such as DCI and DMAP, and oxalyl chloride did not give the desired compound. At last, the carboxamide **20** was prepared by activating the ester-acid with PCl_5 and then quenching the intermediate acid chlorides with NH_3 gas to give the ester-amide **20**.



14 **15** **16**

Scheme 2. Reagents and conditions: a) $(CH_3)_3SiC{\equiv}CH$, BuLi, THF, - 78℃ 57%, b) $LiAlH_4$, THF then TBAF, 93%



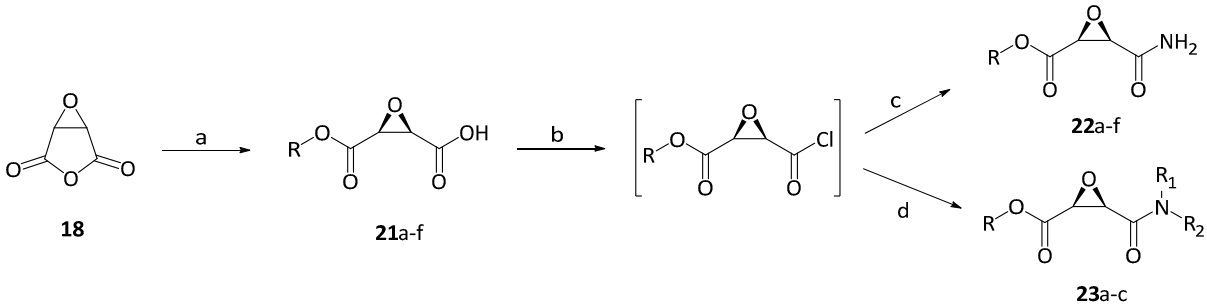
17 **18** **19** **20**

Scheme 3. Reagents and conditions: a) TFAA, DCM, quantitative, b) **16**, TEA, DCM, 79%, c) $(COCl)_2$, DMF, NH_4OH, DCM, d) DIC, DMAP, NH_3, DCM, e) PCl_5, DCM, then NH_3 (g), 83%

2.2. Preparation of the cerulenin analogues

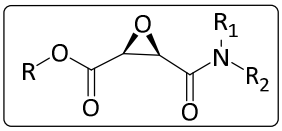
To further increase the scope of the structural diversities of cerulenin derivatives, we designed compounds containing *cis* epoxide flanked by dicarbonyl groups, while bearing various side chains and head units. As the active form of cerulenin in biological system is unknown, we aimed to resolve this question by exploring binding mode of our synthesized analogues. Therefore, we synthesized analogues which mimic cerulenin structure but replacing the ketone with ester, amide, thioester and sulfone. In order to expedite synthesis and biological evaluation of molecules corresponding to the designed inhibitors, cerulenin analogues we decided to prepare the inhibitors as racemates.

Ester acid **21** and ester amides **22, 23** were synthesized by the same procedure for ester-amide **20**. Esterification of anhydride **18** was achieved by reacting with the appropriate alcohol to give the mono substituted ester-acids **21a-f** in good yields (Scheme 4). The carboxamide series **22** was prepared by activating the ester-acids with PCl_5 and then quenching the intermediate acid chlorides with NH_3 to give the desired ester-amides **22a-f** in moderate yields. **23a-c** was prepared in same manner of **22** but with appropriate amines having different substitute at the amide function. This route is short and ideal for analogue synthesis with the potential to add more complex and various side chains as a last step.



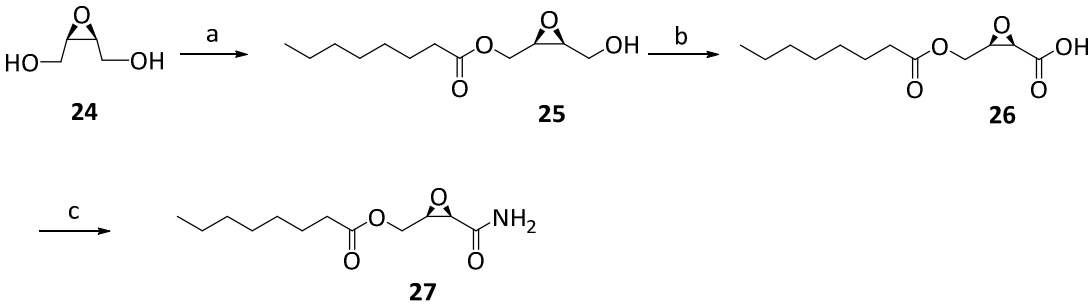
Scheme 4. Reagents and conditions: a) R-OH, TEA, DCM, 98%, b) PCl_5, DCM, c) NH_3 (g), 40-70%, d) R-NH_2, 30-90%

Tabel 1. R, R_1, R_2 group substituent on scheme 4



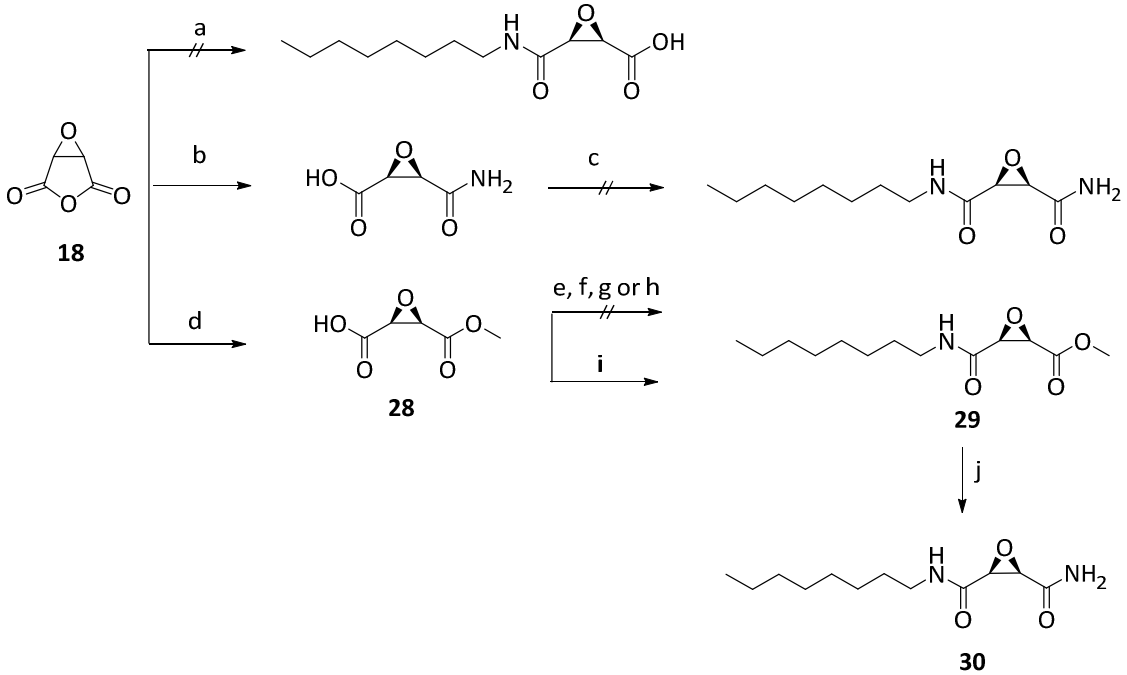
Compound	R	Compound	R	Compound	R_1	R_2
22a	C_3H_7	22d	*n*-C_8H_{17}	23a	H	CH_3
22b	*n*-C_4H_9	22e	*n*-$C_{10}H_{21}$	23b	CH_3	CH_3
22c	*n*-C_6H_{13}	22 f	$C_{10}H_{17}$	23c	H	OH

In addition, the ester alcohol **25** was readily prepared by esterifcation of the known meso-epoxy diol **24** prepared by m-CPBA epoxidation of cis-butene-1,4-diol.(Scheme 5).[109] Ester acid **26** was prepared by oxidation of the epoxy alcohol **25** with $RuCl_3$ to the acid and subsequent amide formation using gaseous ammonia to have **27** in hand.



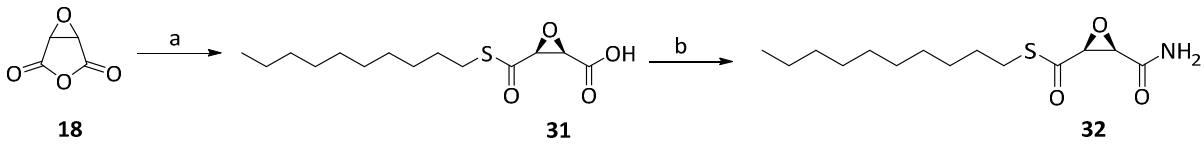
Scheme 5.Reagents and conditions: a) Octanoyl chloride, Pyridine, DMAP, DCM, 44%, b) NaIO4, $RuCl_3 \cdot H_2O$, H_2O/ACN/EA, 39%, c) PCl_5, DCM, then NH_3 (g), 77%

Another functional analogue of cerulenin which we prepared was the diamide **30**. We first tried to make amide-acid by directly reacting anhydride **18** with octylamine to prepare diamide, but did not work out. Next, we then prepared acid amide to react with octylamine to make diamide, but the acid-amide failed to react with octylamine. Because of difficulties encountered with the synthesis of structure **30**, we tried alternative route to prepare **30** which are discussed below. (Scheme 6) Thus, aliphatic amide-ester **29** was to start from the ester-acid **28**, activate with PCl_5, and quench with octylamine. Ester-acid **28** was obtained by simply heating anhydride **18** with neat methanol. Treatment of **29** with methanolic ammonia gave the diamide **30**.



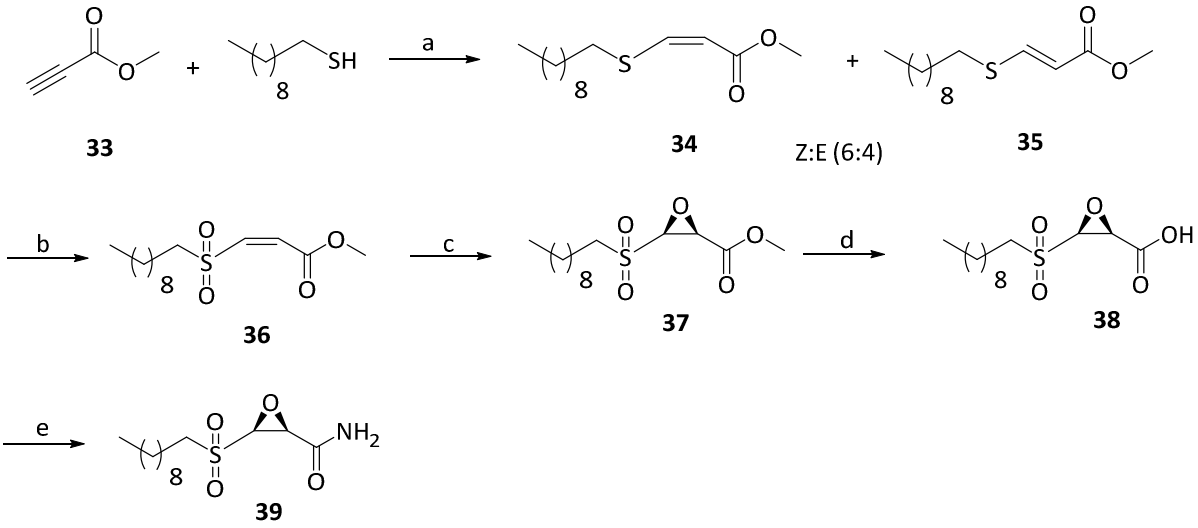
Scheme 6. Reagents and conditions: a) Octylamine, DCM, b) Hexamethyldisilazane, THF, c) Octylamine, EDCI, HOBT, DMF, d) MeOH, quantitative, e) Octylamine, DIC, DMAP, DCM, f) N-methylmorpholine, Isobutyl chloroformate, Octylamine, Ether, g) Octylamine, EDCI, HOBT, DCM, h) PCl_5, DCM, then Octylamine, TEA, RT, i) PCl_5, DCM, then octylamine, TEA, -20°C, 58%, j) NH_4OH(aq), MeOH, 85%,

The third series of target molecules were the carboxamides that have thioester and sulfone in the opposite side. Ring opening of anhydride **18** with thiol and DMAP in acetonitrile/pyridine solution successfully gives the thioester-acid **31** in moderate yield. Thioester-amide **32** was prepared in a similar manner of the ester-amide **20** in moderate yield. (Scheme 7)



Scheme 7. Reagents and conditions: a) $CH_3(CH_2)_9SH$, DMAP, ACN:Pyridine (9:1), 60%, b) PCl_5, DCM, then NH_3 (g), 62%

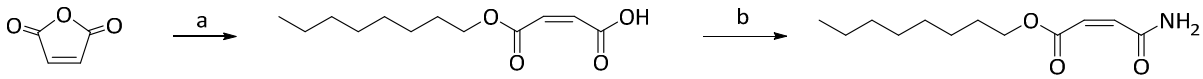
Copper-catalyzed conjugate addition of thiol to methylpropiolate **33**, which proceeds through an allenolate intermediate gave mixture of stereoselective isomers **34** and **35** in 42% and 20% yield (*Z/E* =60/40).[110] Oxidation of the Z-vinyl sulfide **34** with excess sodium perborate gave sulfone **36** in moderate yield. Stereoselective oxidation to the epoxide **37** was effected following the procedure described by Hegedus et al.[111] The methyl ester **37** was hydrolyzed by LiOH-H_2O to give acid **38** in almost quantitative yield. Amidation of **38** with same procedure using ammonia led to sulfonyl amide **39.** (Scheme 8)



Scheme 8. Reagents and conditions: a) $CH_3(CH_2)_9SH$, CuCl, K_2CO_3 DMSO, 62%, b) $NaBO_3$, AcOH,

50%, c) NaClO, Et_2O:DMF (1:1), 70%, d) $LiOH \cdot H_2O$, THF:H_2O (2:1), 68%, e) PCl_5, DCM, then NH_3(g), 60%

Lastly, we synthesized michael acceptor compound **42** having olefin, which is different from other analogues having epoxide moiety. We anticipated that cysteine residues of enhanceosome can undergo Michael additions with **42**, showing similar binding mechanism of epoxide function. Esterification of maleic anhydride **40** over alcohol gave acrylic acid **41**. Amidation of **41** with in the presence oxalyl chloride and DMF as a catalyst gave ester-amide **42.** (Scheme 9)



40 **41** **42**

Scheme 9. Reagents and conditions: a) 1-octanol, TEA, DCM, 20%, b) $(COCl)_2$, DMF, DCM, then NH_4OH, 34 %

2.3. Structure activity relationship (SAR)

Potent analogues of cerulenin, with various chain lengths and substitutions, are synthesized for use in SAR studies. The cerulenin analogues were evaluated for their ability to inhibit enhanceosome in two assays, NO (nitric oxide) assay and MTS assay in order to derive SAR. The NO assay determines quantitative nitrite (NO_2^-) production of the compounds in biological fluids. As Nitric oxide (NO) plays an important role in inducing inflammation, it has been observed that the overproduction of nitric oxide causes dysfunction of several organs and affects lactate level. Therefore, we tried this assay to see the inhibitory activity of cerulenin analogues towards nitrite production by LPS-activated macrophages quantified nitrite in the culture medium using Griess reagent. Comparing nitrite production of Cerulenin derivatives with Cerulenin could screen out inhibitory activity of inflammation. Another assay, MTS assay is a sensitive colorimetric assay for quantification of the number of viable cells in the proliferation and cytotoxicity. As cell viability is important for analysis of many pharmaceutical compounds and drugs, the MTS assay considered the most economic, reliable and convenient methods for showing cytotoxicity *in vitro* to assess for cell viability.

The effects of the cerulenin and its analogues on NO production were determined by NO assay. (Table 2) Among others, **22d**, **22e** and **42**, a chain length consisting of more than eight carbons, showed similar inhibition of NO production with that of cerulenin. This indicates that the longer hydrophobic side chain might afford better inhibition of NO production, giving positive effects on inhibiting enhanceosome. Meanwhile, the shorter analogues **22b** and **22c** give moderate inhibition but not sufficient than that of cerulenin. The other epoxysuccinamate esters **22a** and **22f** which have branched form of the side chain did not seem to inhibit NO production as well as cerulenin because of

the steric effect of the molecule with binding of the protein. Moreover, other aliphatic analogues display a rather high range of NO production depending on functionalities surrounding the epoxide moiety. Amide ester **29**, thioester **32** and sulfone **39** did affected inhibition of NO production, but not as satisfying as other analogues that were mentioned above. Furthermore, octyl ester amide analogues **23a-23c** with different substituents at the amide moiety did not give satisfying result in reducing NO formation. Other analogues **21d**, **27** and **30** also showed less inhibition of NO than cerulenin and other analogues. For the click compound **20** also did not appear to be the essential molecules for inhibiting NO production, but **13** successfully inhibit NO formation more than any other analogues including cerulenin itself. Overall, we cannot assure that which analogue is the most potent for inhibiting NO production, but most of the epoxycarboxamide analogues that we synthesized successfully inhibit the inflammatory process. Interestingly, from the results of the NO production of some analogues, it seems likely that the open straight chain form of the cerulenin analogues can be considered as active conformation when binding with cysteine residue of the protein which was an unrevealed issue.

Table 2. Inhibition of NO production in RAW 264.7 cells by cerulenin and its analogues

Compound	Inhibition of NO production(μM)	Compound	Inhibition of NO production(μM)
cerulenin	4.1	**23c**	>20
21d	>20	**27**	>20
22a	>20	**29**	13
22b	6.6	**30**	>20
22c	6.9	**39**	9.9
22d	3.8	**32**	11.8
22e	4.6	**42**	5
22f	15		
23a	>20	**20**	16
23b	>20	**13**	0.9

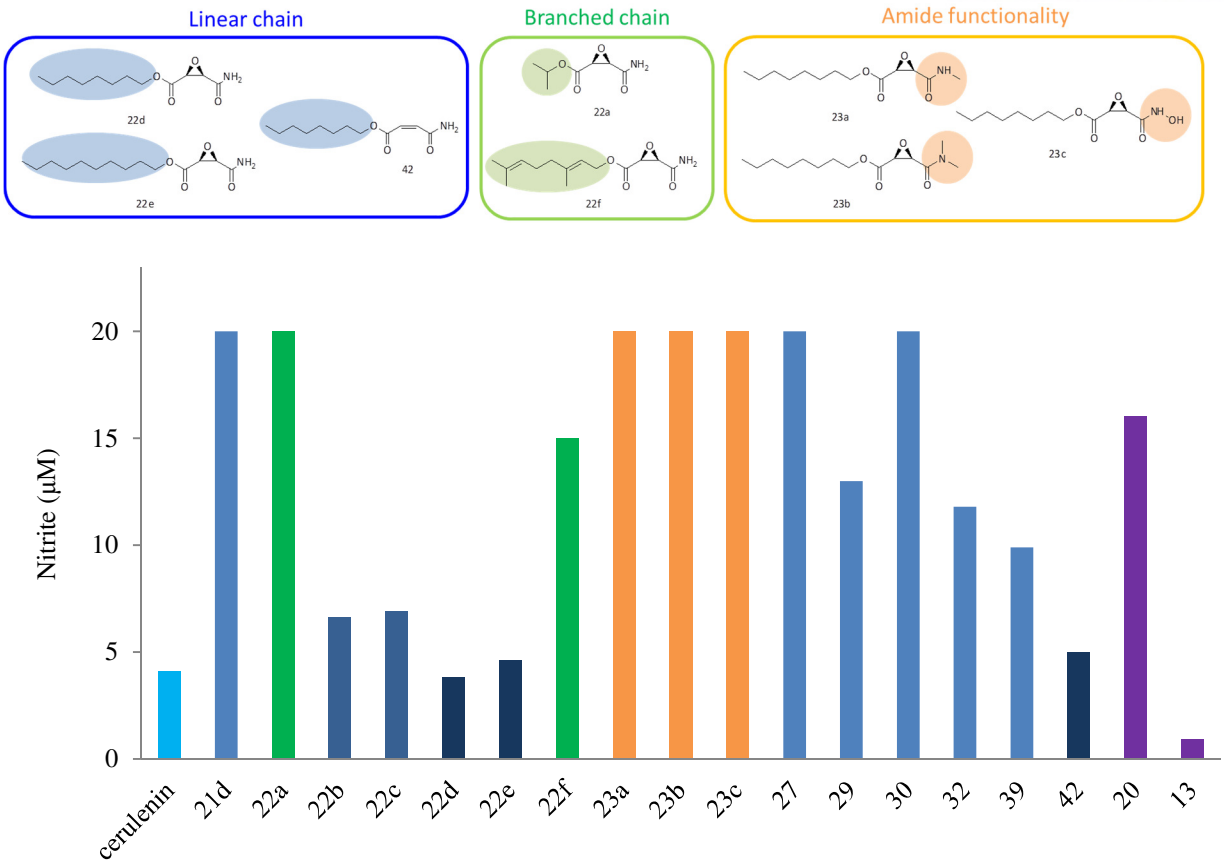


Figure 15. Inhibition of NO production in RAW 264.7 cells by cerulenin and its analogues.

Cytotoxicity testing of cerulenin and its analogues were performed using RAW 264.7 cells with LPS. The results obtained with the MTS assay (figure 2) demonstrate all of the synthesized aliphatic analogues **20-42** have similar cytotoxicity with cerulenin. Cell viability based on reduction of MTS indicates the high cell survival of analogues similar to cerulenin. From this result, the analogues that we synthesized are considered to be are essentially nontoxic. Moreover, the cytotoxicity of the analogues appears to be independent from chain length and other functionalities surrounding the epoxide moiety. However, the click compound **13** which showed greatest inhibition of NO production displayed low percentage of surviving cells, but somehow can be considered as mild survival rate.

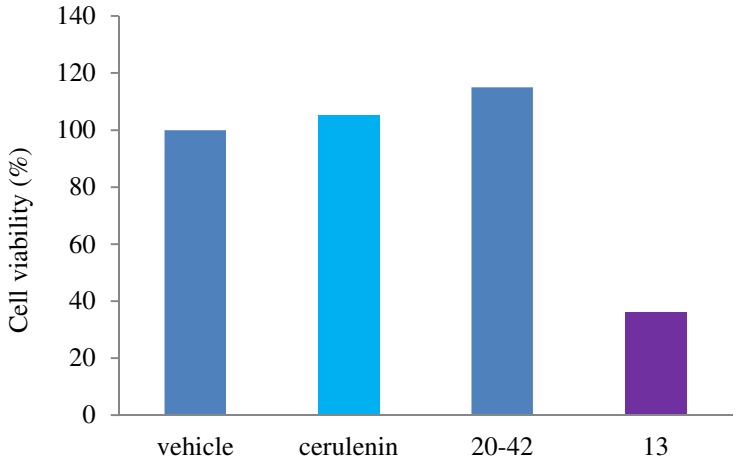


Figure 16. Effect of cerulenin and its analogues with concentration of 10 μM on the cell viability of RAW 264.7 cells

Additionally, we tried fatty acid synthase activity assay with several analogues of cerulenin including the click compound **13** to see whether the inhibitory activity towards fatty acid synthase (FASN) is same as cerulenin. Because cerulenin and certain of its analogues are known to inhibit FASN, the inhibitory activity of the synthesized compounds against FASN has been determined. (Figure 3) To compare the efficacies of various compounds towards FASN, data was indicated in percentage by which fatty acid synthesis is reduced in samples treated with 10 μM of the indicated analogues and **13** with a dose of 7.5 μM. As expected, cerulenin effectively inhibited the tumor cell fatty acid synthase activity. Greatest inhibition was occurred with **13** showing similar pattern with cerulenin. However, cerulenin analogues showing contentable inhibition of NO production had no significant inhibitory effects on fatty acid synthesis. Likewise, these compounds are essentially inactive against fatty acid synthase but inhibit inflammatory response, meaning that the target for the inhibitory activities of the cerulenin analogues is clearly distinguished from that of cerulenin. Therefore, the analogues that we synthesized are more closely associated with inhibition of other protein that is related to inflammation than with suppression in the fatty acid synthesis. More interestingly, from the result that the open side chain analogues don't inhibit FASN unlike molecules that can form hydroxylactam, we have learn that the structure of inhibitors play a crucial role in targeting specific protein related to inflammation.

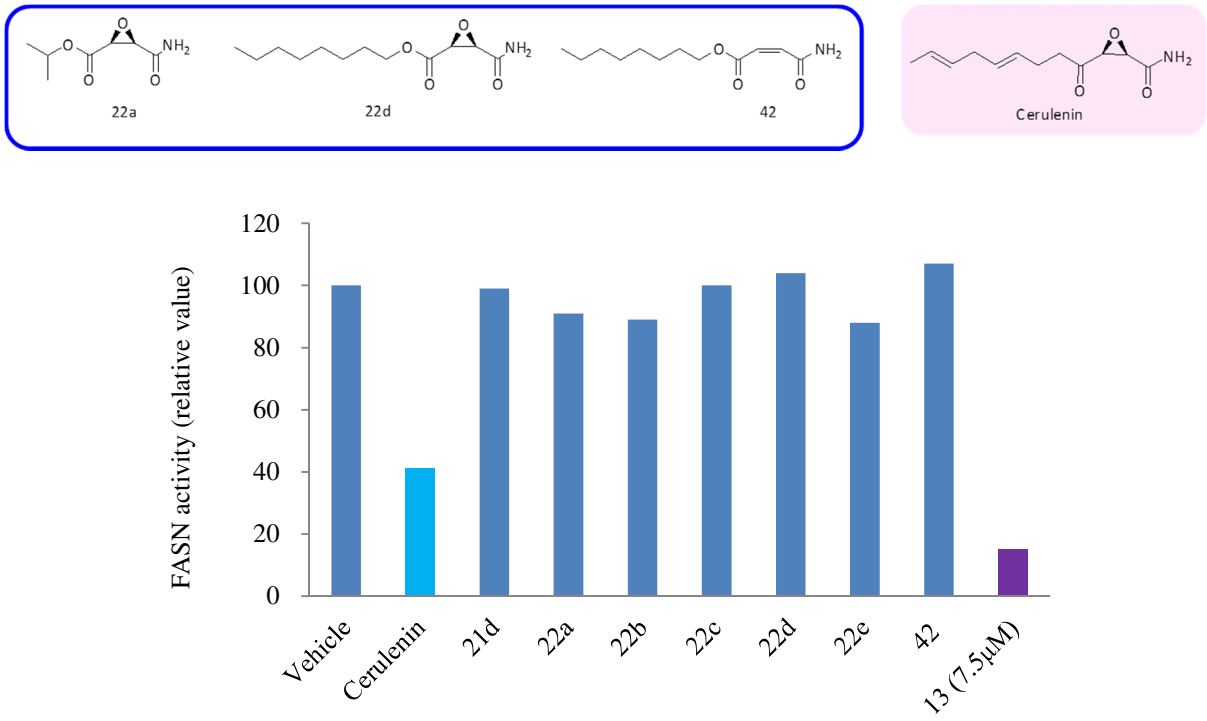


Figure 17. Inhibition of Fatty acid synthase activity in RAW 264.7 cells by cerulenin and its analogues with concentration of 10 μM

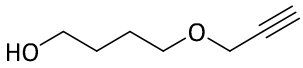
III. CONCLUSION

Cerulenin offers an attractive opportunity to develop new class of effective and safe anti-inflammatory agents. We have designed and synthesized several analogues of the natural product cerulenin with varying chain lengths and substitutions, which can be considered as promising candidates for the anti-inflammation therapeutic molecule. Assaying of these compounds revealed that a significant decrease in NO production and low cytotoxicity could be achieved by the derivatives with epoxycarboxamide moiety similar to that displayed by cerulenin. In addition, comparing the activity of cerulenin and its analogues towards FASN, we have discovered that synthesized analogues are more closely related to inhibition of other protein associated with inflammation than with suppression in the fatty acid synthesis. From these result, we have also learned that the structure of inhibitors play a crucial role in targeting specific protein related to inflammation. As such differentiation is displayed, the clickable analogue of cerulenin could be used as a chemical probe to explore the target protein. Still, more work is needed in order to optimize the structures of these inhibitors as potential anti-inflammatory drug leads.

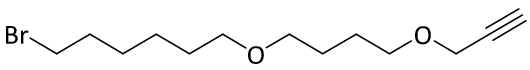
IV. EXPERIMENTAL

General methods: All the reactions were carried out in a flame or oven dried glassware under nitrogen atmosphere with freshly distilled dry solvents under anhydrous conditions unless otherwise indicated. Flash column chromatography was performed with silica gel 60 (230 – 400 mesh). Chromatograms were visualized by fluorescence quenching with UV light at 254 nm or by staining using base solution of potassium permanganate and molybdate. All reagents were obtained from commercial sources and were used without further purification. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a 300 / 75 MHz Bruker DPX300 Fourier transform spectrometer. The residual solvent signals were taken as the reference (0.0 ppm for 1H NMR spectra and 77.0 ppm for 13C NMR spectra in CDCl3) Chemical shift (δ) is reported in ppm, coupling constants (*J*) are given in Hz. The following abbreviations classify the multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublet, q = quartet and br = broad signal. Samples were prepared in deuterated chloroform ($CDCl_3$) as indicated. Solvents for this purpose were obtained from Cambridge Isotope Laboratories, Inc. LC-MS analysis was carried out using an Agilent Technologies 1200 series HPLC coupled to a Bruker HCT Ultra mass spectrometer.

Synthetic Procedures:

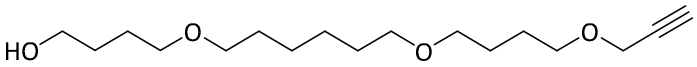


4-(prop-2-ynyloxy)butan-1-ol (2). To the solution of Sodium hydride (3.2 g, 110.96 mmol) in anhydrous THF (50 mL) was added 1,4-butanediol (10 g, 110.96 mmol) in THF dropwise at 0 °C under N_2 atmosphere. After stirring for 45 minutes, propargyl bromide (1 mL, 77.67 mmol) in THF (20 mL) was add to the mixture drop wise. After completion of reaction by TLC monitoring in 9h, reaction mixture was quenched by cold water (5 mL) and aqueous layer was extracted with Ethyl acetate (2 x 15 mL). Combined organic layer was washed with brine solution (15 mL) and dried over Na_2SO_4 filtered, concentrated under reduced pressure. Crude was Purification by Column chromatography using 20% EtOAc/Hexane. Obtained pure compound **2** in 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (d, *J* = 2.4, 2H), 3.63 (t, *J* = 6.0, 2H), 3.54 (t, *J* = 5.9, 2H), 2.42 (t, *J* = 2.4, 1H), 1.66 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 79.65, 74.35, 69.99, 62.47, 58.04, 29.69, 26.16; ESI-MS m/z calculated for $C_7H_{12}O_2$ $[M + H]^+$: 129.1; Found:129.2

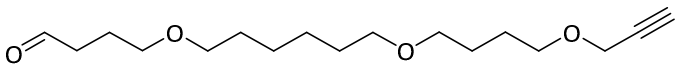


1-bromo-6-(4-(prop-2-ynyloxy)butoxy)hexane (3). To the solution of Sodium hydride (4.49 g, 112.35 mmol) in anhydrous THF (75 mL) was added 1-butanol, 4-(2-propynyloxy) (12 g, 93.62 mmol) in THF (25 mL) at 0°C under N_2 atmosphere. After stirring for 30 min, 1,6-dibromohexane (43.2 mL,

280.87 mmol) in THF (50 mL) was added dropwise, followed by catalytic TBAB (5 mol%) was added at 0 °C. The reaction mixture was stirred for 13 h. After completion of reaction by TLC monitoring, reaction was quenched by Ammonium chloride (25 mL) and aqueous layer was extracted with Ethyl acetate (2 x 50 mL). Combined organic layers was washed with brine solution (50 mL) and dried over Na_2SO_4 filtered, concentrated under reduced pressure. Crude was purified by Column chromatography using 2% EtOAc/Hexane. Obtained pure compound **3** in 70% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (dd, *J* = 2.3, 1.2, 2H), 3.53 (t, *J* = 5.7, 2H), 3.40 (m, 6H), 2.41 (t, *J* = 2.3, 1H), 1.86 (qu, *J* = 6.9 , 2H), 1.65 (m, 4H), 1.57 (q, *J* = 6.7, 2H), 1.41 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 79.98, 74.05, 70.62, 70.47, 69.91, 57.98, 33.87, 32.72, 29.55, 27.98, 26.35, 26.26, 25.40; ESI-MS m/z calculated for $C_{13}H_{23}BrO_2$ $[M + H]^+$: 291.1 ; Found: 291.2

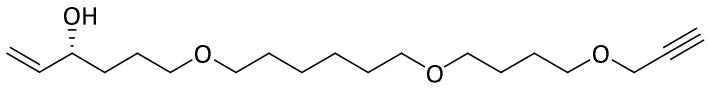


4-(6-(4-(prop-2-ynyloxy)butoxy)hexyloxy)butan-1-ol (4). To the solution of Sodium hydride (0.3 g, 12.1 mmol) in anhydrous THF (8 mL) was added drop wise 1,4-butanediol (1.1g, 12.1 mmol) at 0°C under N_2 atmosphere. After stirring reaction mixture for 30 min, bromo compound **3** (1.16 g, 4.02 mmol) in THF (3 mL) was added to the mixture dropwise. Reaction mixture was stirred for 4h at room temperature. Upon reaction completed, reaction mixture was quenched by Ammonium Chloride (2 mL) and aqueous layer was extracted was extracted with ethyl acetate (2 x 10 mL). Combined organic layer was washed with brine solution and dried over Na_2SO_4 filtered, concentrated under reduced pressure. Crude was purified by Column chromatography using 25% EtOAc/Hexane system. Obtained pure compound **4** in 82% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.14 (dt, *J* = 2.4, 0.5, 2H), 3.63 (t, *J* = 5.6, 2H), 3.54 (bt, 2H), 3.42 (m, 8H), 2.61 (bs, 1H) 2.42 (td, *J* = 2.4, 0.5, 1H), 1.66 (m, 8H), 1.57 (qu, *J* = 7.0, 4H), 1.36 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 79.93, 74.09, 70.98, 70.81, 70.75, 70.39, 69.88, 62.60, 57.93, 30.30, 29.63, 29.53, 26.88, 26.30, 26.21, 25.99, 25.96; ESI-MS m/z calculated for $C_{17}H_{32}O_4$ $[M + H]^+$: 301.2; Found:301.2

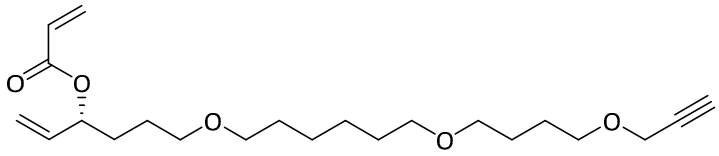


4-(6-(4-(prop-2-ynyloxy)butoxy)hexyloxy)butanal (5). To the solution of alcohol **4** (1.63 g, 5.42 mmol) in DCM (25 mL) at 0 °C was added triethylamine (1.89 mL, 13.57 mmol) followed by SO_3-Pyridine (2.16 g, 13.57 mmol) complex in DMSO (5 mL). The reaction mixture was stirred at 0 °C for 4h. Upon reaction finished cold water (5 mL) was added. The DCM was removed under reduced pressure and extracted with diethylether (2 x 15 mL). The combined organic layers were washed with 1M KH_2PO_4 (10 mL) and Brine solution (15 mL). The organic phase was dried over $MgSO_4$ filtered, concentrated under reduced pressure. Crude was purified by Column chromatography using 15% EtOAc/Hexane system. Obtained pure compound **5** in 78% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78

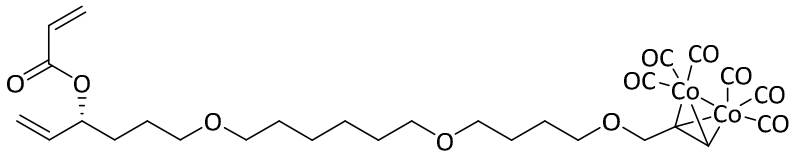
(t, *J* = 1.6, 1H), 4.13 (d, *J* = 2.4, 2H), 3.53 (t, *J* = 6.2, 2H), 3.41 (m, 8H), 2.51 (td, *J* = 7.1, 1.6, 2H), 2.41 (t, *J* =2.4, 1H), 1.91 (m, 2H), 1.65 (m, 4H), 1.55 (m, 4H), 1.34 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 202.31, 79.99, 74.05, 70.92, 70.79, 70.43, 69.92, 69.55, 57.97, 40.96, 29.70, 29.62, 26.35, 26.26, 26.03, 22.60; ESI-MS m/z calculated for $C_{17}H_{30}O_4$ $[M + H]^+$: 299.2; Found: 299.2



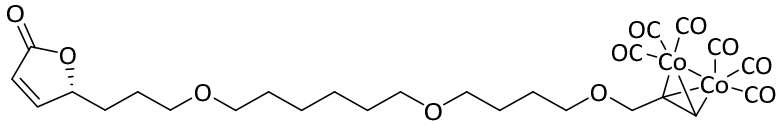
6-(6-(4-(prop-2-ynyloxy)butoxy)hexyloxy)hex-1-en-3-ol (6). To the solution of aldehyde **5** (1.24 g, 4.16 mmol) in anhydrous THF (15 mL) was added 1M solution of vinyl magnesium bromide (6.24 mL, 6.24 mmol) in THF drop wise with vigorous stirring at -78 °C under nitrogen atmosphere. The reaction mixture was stirred at -78 °C for 4h then raised temperature to room temperature. Upon completion of reaction quenched with saturated ammonium chloride (2 mL) at 0 °C. The THF was removed under reduced pressure and extracted with diethyl ether (2 x 10 mL). The organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4 filtered, concentrated under reduced pressure. Crude was purified by column chromatography using 15% EtOAc/Hexane. Obtained pure compound **6** in 44% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 (ddd, *J* = 17.2, 10.4, 5.8, 1H), 5.24 (dd, *J* = 17.2, 1.5, 1H), 5.10 (dd, *J* = 10.4, 1.5, 1H), 4.14 (d, *J* = 2.4, 2H), 3.53 (m, 2H), 3.42 (m, 9H), 2.78 (bd, 1H), 2.41 (t, *J* =2.4, 1H), 1.67 (m, 6H), 1.58 (m, 6H), 1.36 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 141.20, 114.26, 79.97, 74.06, 72.56, 71.02, 70.90, 70.78, 70.41, 69.91, 57.96, 34.69, 29.66, 29.54, 26.33, 26.24, 26.01, 25.98, 25.92; ESI-MS m/z calculated for $C_{19}H_{34}O_4$ $[M + H]^+$: 327.2; Found: 327.2



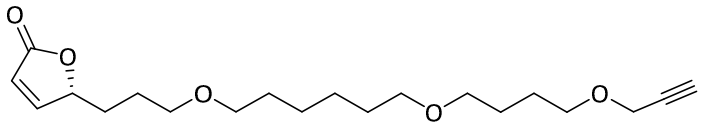
6-(6-(4-(prop-2-ynyloxy)butoxy)hexyloxy)hex-1-en-3-yl acrylate (7). To the acrylic acid (16 µl, 0.24 mmol) in dry DCM (2 mL) was added oxalyl chloride (27 µl, 0.31 mmol) and 1 drop of DMF at 0 °C. The reaction mixture was stirred for 30 min. To the acryloyl chloride reaction mixture was added allylic alcohol **6** (40 mg, 0.12 mmol) in DCM (1 mL) and DIPEA (51 µl, 0.36 mmol) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 2h at 0 °C. Upon completion of reaction, solvent was removed under reduced pressure and purified by column chromatography using 10% EtOAc/Hexane. Obtained pure compound **7** in 66% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.41 (dd, *J* = 17.3, 1.5, 1H), 6.13 (dd, *J* = 17.3, 10.4, 1H), 5.82 (dd, *J* = 10.4, 1.4, 1H), 5.34 (q, *J* = 6.3, 1H), 5.26 (dt, *J* = 17.3, 1.3, 1H), 5.18 (dt, *J* = 10.5, 1.2, 1H), 4.13 (d, *J* = 2.4, 2H), 3.53 (bt, 2H), 3.39 (m, 9H), 2.41 (t, *J* = 2.4, 1H), 1.73 (m, 2H), 1.65 (m, 4H), 1.57 (m, 6H), 1.35 (m, 4H)



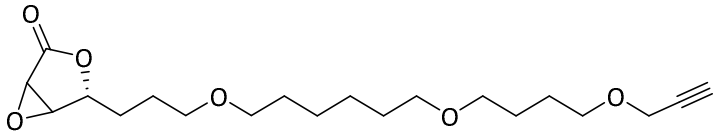
Dicobalt-octacarbonyl protected acryloyl ester (8). To the solution of **7** (225 mg, 0.59 mmol) in Dry DCM (10 mL) was added $Co_2(CO)_8$ (222 mg, 0.65 mmol) under organ atmosphere at room temperature. The reaction mixture was stirred for 2h at room temperature. Upon completion of the reaction, solvent was removed under reduced pressure and crude was purified by column chromatography using 6% EtOAc/Hexane as eluent system and obtained pure compound **8** in 82% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.41 (dd, *J* = 17.3, 1.2, 1H), 6.13 (dd, *J* = 17.3, 10.4, 1H), 6.03 (s, 1H), 5.82 (d, *J* = 10.5, 1H), 5.34 (q, *J* = 6.3, 1H), 5.26 (d, *J* = 17.2, 1H), 5.18 (d, *J* = 10.5, 1H), 4.62 (s, 2H), 3.61 (t, *J* = 5.8, 2H), 3.40 (m, 9H), 1.64 (m, 12H), 1.35 (dt, *J* = 7.0, 3.8, 4H)



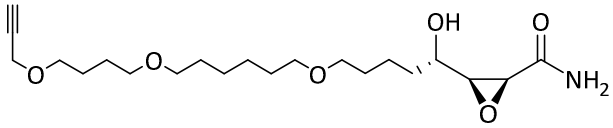
Dicobalt-octacarbonyl protected lactone (9). To the solution of **8** (210 mg, 0.32 mmol) in dry DCM (100 mL) was added Grubbs 2nd generation catalyst (32.5 mg, 20 mol%) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 3h. Upon completion of the reaction, solvent was removed under reduced pressure and crude was purified by column chromatography using 20% EtOAc/Hexane as eluent system and obtained pure compound **9** in 82% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 5.7, 1H), 6.11 (dd, *J* = 5.6, 1.7, 1H), 6.03 (s, 1H), 5.08 (bs, 1H), 4.62 (s, 2H), 3.61 (t, *J* = 5.6, 2H), 3.41 (m, 8H), 1.89 (m, 2H), 1.64 (m, 10H), 1.32 (m, 4H)



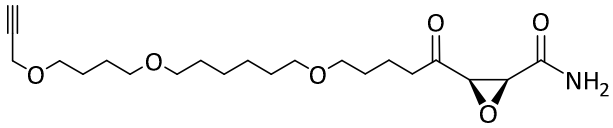
5-(3-(6-(4-(prop-2-ynyloxy)butoxy)hexyloxy)propyl)furan-2(5H)-one (10). To the solution of **9** (0.023 mmol) in dry Pyridine (1 mL) was added NaOCl (0.050 mmol) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at room temperature until the reaction was finished checked by TLC. Upon completion of reaction, solvent was removed under reduced pressure and crude was purified by column chromatography using 20% EtOAc/Hexane as eluent system and obtained pure compound **10** in 65% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 5.7, 1.5 1H), 6.11 (dd, *J* = 5.7, 2.0, 1H), 5.09 (tt, *J* = 7.0, 1.9, 1H), 4.13 (d, *J* =2.4, 2H), 3.53 (bt, 2H), 3.41 (m, 8H), 2.41 (t, *J* = 2.4, 1H), 1.73 (m, 2H), 1.65 (m, 4H), 1.55 (m, 6H), 1.35 (qu, *J* = 3.6, 4H)



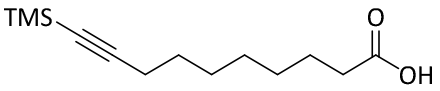
4-(3-(6-(4-(prop-2-ynyloxy)butoxy)hexyloxy)propyl)-3,6-dioxabicyclo[3.1.0]hexan-2-one (11). To the solution of **10** (0.045 mmol) in dry pyridine (1 mL) was added NaOCl (0.113 mmol) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3h. Upon completion of reaction, solvent was removed under reduced pressure and crude was purified by column chromatography using 15% EtOAc/Hexane as eluent system and obtained pure compound **11** in 55% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.62 (bt, 1H) 4.13 (d, *J* =2.4, 2H), 3.99 (d, *J* = 2.5, 1H), 3.78 (dd, *J* = 2.5, 0.8, 1H), 3.53 (bt, 2H), 3.41 (m, 8H), 2.41 (t, *J* = 2.4, 1H), 1.72 (m, 2H), 1.65 (m, 4H), 1.57 (m, 6H), 1.35 (qu, *J* = 3.5, 4H)



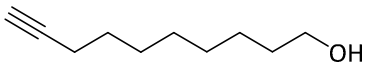
(2S,3S)-3-((S)-1-hydroxy-5-(6-(4-(prop-2-ynyloxy)butoxy)hexyloxy)pentyl)oxirane-2-carboxamide (12). To the solution of **11** (0.026 mmol) in dry MeOH (0.4 mL) was added NH_4OH (0.01 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 4h. Upon completion of reaction, solvent was removed under reduced pressure and crude was purified by column chromatography using 50% EtOAc/Hexane as eluent system and obtained pure compound **12** in 57% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14 (s, 1H) 5.61 (s, 1H), 4.14 (d, *J* = 2.5, 2H), 3.54 (m, 2H), 3.42 (m, 11H), 3.13 (dd, *J* = 7.7, 4.6, 2H), 2.42 (t, *J* = 2.4, 1H), 1.78 (m, 2H), 1.65 (m, 4H), 1.57 (m, 8H), 1.35 (dt, *J* = 7.2, 3.5, 4H)



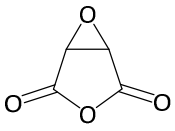
(2S,3R)-3-(5-(6-(4-(prop-2-ynyloxy)butoxy)hexyloxy)pentanoyl)oxirane-2-carboxamide (13). To the solution of **12** (0.015 mmol) in dry DCM (2 mL) was added NMO (0.045 mmol), TPAP (5 mol%) and 4A° molecular sieves (25 mg) powder at room temperature under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 4h. Upon completion of reaction, solvent was removed under reduced pressure and crude was purified by column chromatography using 50% EtOAc/Hexane as eluent system and obtained pure compound **13** in 40% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.05 (s, 1H) 5.50 (s, 1H), 4.13 (d, *J* = 2.4, 2H), 3.52 (m, 4H), 3.40 (m, 6H), 2.74 (dt, *J* = 17.4, 7.1, 1H), 2.60 (dt, *J* = 17.4, 6.8, 1H), 2.41 (t, *J* = 2.4, 1H), 1.62(m, 14H), 1.36 (m, 4H)



10-(Trimethylsilyl)dec-9-ynoic acid (15). To the solution of ethynyltrimethylsilane (0.1 mL, 0.75 mmol) in dry THF (1 mL) was added dropwise BuLi (0.4 mL, 0.78 mmol) at -78 °C. After 15 min of stirring, dry DMPU (51 µL) was added and the reaction was stirred for 1 h at -78 °C. The solution of 8-Bromooctanoic acid (67mg, 0.3 mmol) in THF (0.5 mL) was added dropwise. The reaction was slowly warm to room temperature and stirred overnight. The reaction was quenched with 1 *N* HCl to pH = 1~2 and extracted with ethyl acetate (2 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated *in vacuo.* The residue was purified *via* silica gel column chromatography (3:7 EtOAc–Hexane with 1% AcOH) to yield the title compound (41 mg, 0.17 mmol, 57%) as colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (t, *J* = 7.5, 2H), 2.21 (t, *J* = 7.1, 2H), 1.63 (m, 2H), 1.51 (qu, *J* = 6.5, 2H), 1.36 (m, 6H), 0.14 (s, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 179.74, 107.55, 84.34, 33.95, 28.87, 28.66, 28.52, 28.49, 24.57, 19.79, 0.15; ESI-MS m/z calculated for $C_{13}H_{24}O_2Si$ $[M - H]^-$: 239.2; Found: 239.2



Dec-9-yn-1-ol (16). To the suspension of LiAlH4(6.2 mg, 0.16 mmol) in THF (0.45 mL) was added dropwise the solution of alkynoic acid **6a** in THF (50 µL) at 0°C. The reaction was slowly warm to room temperature and stirred at room temperature for 3 h. After reaction completion monitored by TLC, the reaction mixture was cooled to 0 °C and slowly added H_2O (50 µL) and 15% NaOH aq.solution (50 µL). More H_2O (150 µL) was added and warm to room temperature and stirred at room temperature for 5 min. The precipitated solid was filtered through Celite and dried over Na_2SO_4 and concentrated *in vacuo*. Combined filtrate was dissolved in the solution of TBAF (1.0 M, 0.32 mmol) and stirred at room temperature for further 5 h. After the reaction was complete, the reaction mixture was concentrated *in vacuo* to dryness. The residue was purified *via* silica gel column chromatography (1:9 Acetone–DCM) to yield the title compound (23 mg, 0.15 mmol, 93%) as colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (t, *J* = 6.4, 2H), 2.18 (td, *J* = 7.1, 2.6, 2H), 1.93 (t, *J* = 2.6, 1H), 1.53 (m, 4H), 1.37 (m, 8H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 84.72, 68.06, 63.03, 32.75, 29.24, 29.03, 28.64, 28.43, 25.66, 18.36; ESI-MS m/z calculated for $C_{10}H_{18}O$ $[M + H]^+$: 155.1; Found: 155.2

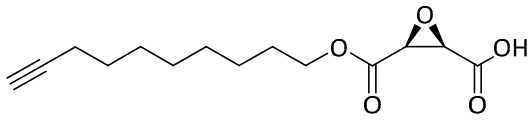


***cis*-2,3-Epoxysuccinic anhydride (18).** *cis*-2,3-Epoxysuccinic acid (1.52 g, 11.5 mmol) was added to

a mixture of trifluororacetic anhydride (3.25 mL, 23.0 mmol) and dichloromethane (12 mL) and the resulting solution was stirred at 40 °C for 3 hours. The solvents were then removed under reduced pressure to yield the title compound (1.31 g, 11.51 mmol, quantitative) as a colourless prisms; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.31 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 162.30 (1-C and 4-C), 50.97 (2-C and 3-C); ESI-MS m/z calculated for $C_4H_2O_4$ [M -H]; 113.0; Found: 113.2

General Method for Epoxysuccinic Ester-Acids 19, 21a-g.

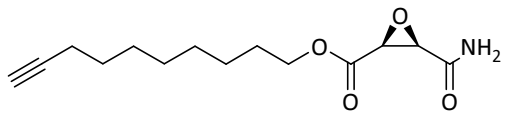
cis-2,3-Epoxysuccinic anhydride **18** (0.5 mmol, 1 eq), an appropriate distilled alcohol (0.6 mmol, 1.2 eq) and triethylamine (0.75 mmol, 1.5 eq) were stirred in dichloromethane (2.5 mL) at room temperature for 1 hour. The excess alcohol and solvents were removed by evaporation at reduced pressure. The resulting residue was purified *via* column chromatography (4:1 EtOAc–hexane using 1% AcOH) to give quantitative yields of the desired compound.



(2R,3S)-3-((dec-9-ynyloxy)carbonyl)oxirane-2-carboxylic acid (19). This compound was isolated as clear oil (79%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.25 (td, *J* = 6.8, 2.5, 2H), 3.80 (d, *J* = 4.7, 1H), 3.76 (d, *J* = 4.7, 1H), 2.19 (td, *J* = 7.0, 2.6, 2H), 1.94 (t, *J* = 2.6, 1H), 1.68 (qu, *J* = 6.8, 2H), 1.53 (dt, *J* = 14.6, 6.9, 2H), 1.34 (m, 10H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.24, 166.95, 84.66, 68.14, 37.18, 53.23, 52.87, 28.93, 28.85, 28.54, 28.35, 28.25, 25.56, 18.34 ; ESI-MS m/z calculated for $C_{14}H_{20}O_5$ [M - H]: 267.1; Found: 267.2

General Method for Epoxysuccinamate Esters 20, 22a-g.

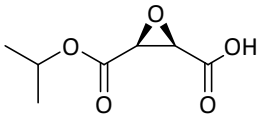
The following general method is exemplified by the procedure described below for the preparation of **20**, using the appropriate ester-acid **19** and other reagents scaled accordingly.



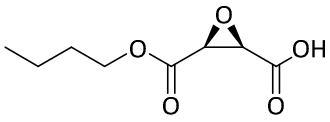
(2S,3R)-dec-9-ynyl 3-carbamoyloxirane-2-carboxylate (20). PCl_5 (136 mg, 1.08 mmol) was added in one portion to a chilled (-5 °C) solution of ester-acid **19** (220 mg, 0.9 mmol) in dichloromethane (5 ml) at room temperature for 3 hours. After this time ammonia gas was added through pipet tubing by heating excess aq. NH_4OH (10mL) in other flask with reflux condensed at 40 °C. After reaction completion monitored by TLC, the reaction mixture was filtered through a pad of Celite and washed with dichloromethane and concentrated to dryness. The resulting residue was purified *via* silica gel

column chromatography (1:1 EtOAc–Hexane) to yield the title compound (200 mg, 0.75 mmol, 83%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.41 (bs, 1H), 5.48 (bs, 1H), 4.19 (td, *J* = 6.8, 1.9, 2H), 3.73 (d, *J* = 5.0, 1H), 3.68 (d, *J* = 5.0, 1H), 2.19 (td, *J* = 7.0, 2.6, 2H), 1.94 (t, *J* = 2.6, 1H), 1.66 (qu, *J* = 6.7, 2H), 1.53 (m, 2H), 1.32 (m, 8H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.49, 166.22, 84.64, 68.15, 66.40, 54.48, 53.32, 28.95, 28.85, 28.54, 28.34, 25.58, 18.32; ; ESI-MS m/z calculated for $C_{14}H_{21}NO_4$ $[M + H]^+$: 268.1; Found: 268.2

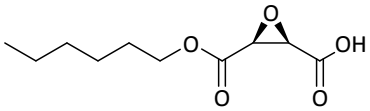
Epoxysuccinic Ester-Acids 21a-f.



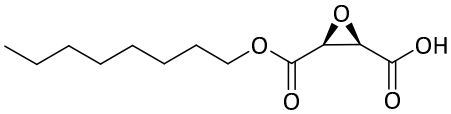
(2R,3S)-3-(isopropoxycarbonyl)oxirane-2-carboxylic acid (21a). This compound was isolated as yellowish oil (98%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (qu, *J* = 6.3, 1H), 3.77 (d, *J* = 4.8, 1H), 3.72 (d, *J* = 4.8, 1H), 1.30 (dd, *J* = 11.5, 6.3, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 168.05, 166.31, 71.33, 53.17, 52.97, 21.52; ESI-MS m/z calculated for $C_7H_{10}O_5$ $[M + H]^+$: 175.1; Found:175.2



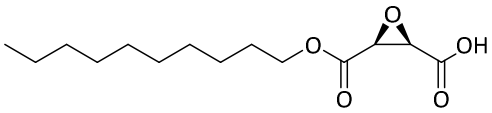
(2R,3S)-3-(butoxycarbonyl)oxirane-2-carboxylic acid (21b). This compound was isolated as yellowish oil (99%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.26 (td, *J* = 6.7, 2.2, 2H), 3.79 (d, *J* = 4.7, 1H), 3.76 (d, *J* = 4.7, 1H), 1.67 (dt, *J* = 14.7, 6.8, 2H), 1.39 (m, 2H), 0.95 (t, *J* = 7.4, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.75, 166.58, 66.69, 52.92, 52.88, 30.27, 18.86, 13.54; ESI-MS m/z calculated for $C_8H_{12}O_5$ $[M + H]^+$: 189.1; Found:189.2



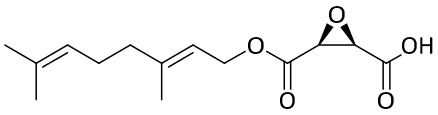
(2R,3S)-3-(hexyloxycarbonyl)oxirane-2-carboxylic acid (21c). This compound was isolated as yellowish oil (98%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.24 (td, *J* = 6.8, 1.8, 2H), 3.79 (d, *J* = 4.7, 1H), 3.76 (d, *J* = 4.7, 1H), 1.68 (qu, *J* = 6.8, 2H), 1.31 (m, 6H), 0.89 (t, *J* = 6.7, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.39, 166.70, 67.09, 53.02, 52.88, 31.26, 28.24, 25.29, 22.44, 13.93; ESI-MS m/z calculated for $C_{10}H_{16}O_5$ $[M + H]^+$: 217.1; Found: 217.2



(2R,3S)-3-(octyloxycarbonyl)oxirane-2-carboxylic acid (21d). This compound was isolated as yellowish oil (98%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 (td, *J* = 6.8, 3.0, 2H), 3.78 (d, *J* = 4.7, 1H), 3.75 (d, *J* = 4.7, 1H), 1.67 (qu, *J* = 6.8, 2H), 1.27 (m, 10H), 0.87 (br t, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.25, 166.74, 67.12, 53.05, 52.67, 31.71, 29.08, 29.07, 28.28, 25.63, 22.59, 14.04; ESI-MS m/z calculated for $C_{12}H_{20}O_5$ $[M + H]^+$: 245.1; Found: 245.2

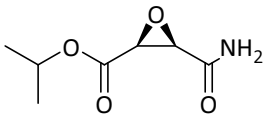


(2R,3S)-3-(decyloxycarbonyl)oxirane-2-carboxylic acid (21e). This compound was isolated as yellowish oil (96%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 (td, *J* = 6.8, 3.0, 2H), 3.78 (d, *J* = 4.7, 1H), 3.75 (d, *J* = 4.7, 1H), 1.67 (qu, *J* = 6.8, 2H), 1.28 (m, 14H), 0.88 (t, *J* = 6.6, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.18, 167.06, 67.32, 53.30, 52.87, 31.85, 29.47, 29.42, 29.25, 29.11, 28.28, 25.63, 22.65, 14.08; ESI-MS m/z calculated for $C_{14}H_{24}O_5$ $[M + H]^+$: 273.2; Found: 273.2

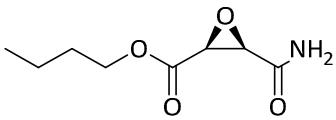


(2R,3S)-3-(((E)-3,7-dimethylocta-2,6-dienyloxy)carbonyl)oxirane-2-carboxylic acid (21f). This compound was isolated as yellowish oil (86%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (t, *J* = 7.3, 1H), 5.07 (t, *J* = 6.7, 1H), 4.74 (m, 2H), 3.78 (d, *J* = 4.7, 1H), 3.75 (d, *J* = 4.7, 1H), 2.07 (m, 4H), 1.70 (bd, *J* = 14.1, 6H), 1.60 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.76, 166.84, 144.67, 132.04, 123.50, 116.57, 63.73, 53.20, 52.91, 39.48, 26.17, 25.64, 17.68, 16.50; ESI-MS m/z calculated for $C_{14}H_{20}O_5$ $[M + H]^+$: 269.1; Found:269.2

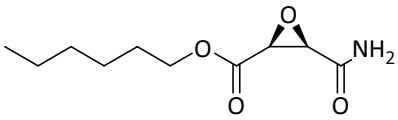
Epoxysuccinamate Esters, 22a-f



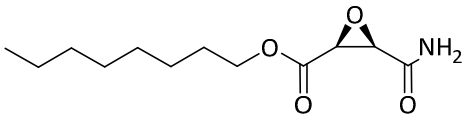
(2S,3R)-isopropyl 3-carbamoyloxirane-2-carboxylate (22a). This compound was isolated as white solid (49%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.43 (bs, 1H), 5.96 (bs, 1H), 5.08 (qu, *J* = 6.3, 2H), 3.68 (d, *J* = 5.0, 1H), 3.65 (d, *J* = 5.1, 1H), 1.26 (dd, *J* = 16.8, 6.3, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.27, 167.58, 165.66, 70.48, 54.42, 53.44, 21.61, 21.60; ESI-MS m/z calculated for $C_7H_{11}NO_4$ $[M + H]^+$: 174.1; Found:174.2



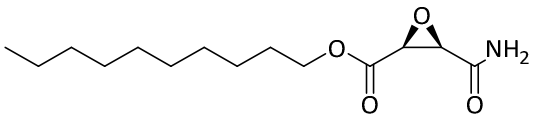
(2S,3R)-butyl 3-carbamoyloxirane-2-carboxylate (22b). This compound was isolated as white solid (43%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.42 (bs, 1H), 5.70 (bs, 1H), 4.20 (t, *J* = 6.6 , 2H), 3.72 (d, *J* = 5.0, 1H) and 3.67 (d, *J* = 5.0, 1H), 1.64 (dt, *J* = 14.7, 6.9, 2H), 1.38 (m, 2H), 0.93 (t, *J* = 7.4, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.34, 166.23, 66.19, 54.48, 53.33, 30.38, 18.90, 13.58; ESI-MS m/z calculated for $C_8H_{13}NO_4$ $[M + H]^+$: 188.1; Found:188.2



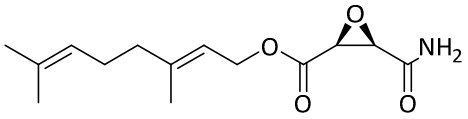
(2S,3R)-hexyl 3-carbamoyloxirane-2-carboxylate (22c). This compound was isolated as white waxy solid (61%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.41 (bs, 1H), 5.45 (bs, 1H), 4.19 (td, *J* = 6.8, 3.0, 2H), 3.73 (d, *J* = 5.0, 1H) and 3.68 (d, *J* = 5.0, 1H), 1.66 (dt, *J* = 13.9, 6.8, 2H), 1.33 (m, 6H), 0.89 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.51, 166.23, 66.49, 54.48, 53.34, 31.28, 28.33, 25.32, 22.45, 13.94; ESI-MS m/z calculated for $C_{10}H_{17}NO_4$ $[M + H]^+$: 216.1; Found:216.2



(2S,3R)-octyl 3-carbamoyloxirane-2-carboxylate (22d). This compound was isolated as white solid (72%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.42 (bs, 1H), 5.50 (bs, 1H), 4.19 (td, *J* = 6.8, 4.6, 2H), 3.73 (d, *J* = 5.0, 1H), 3.68 (d, *J* = 5.0, 1H), 1.66 (qu, *J* = 6.8, 2H), 1.29 (m, 10H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.27, 166.24, 66.51, 54.49, 53.33, 31.72, 29.09, 28.38, 25.67, 22.60, 14.05; ESI-MS m/z calculated for $C_{12}H_{21}NO_4$ $[M + H]^+$: 244.1; Found: 244.2



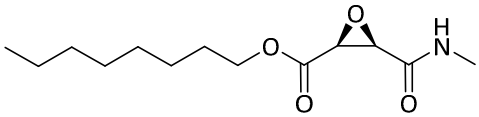
(2S,3R)-decyl 3-carbamoyloxirane-2-carboxylate (22e). This compound was isolated as white solid (50%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.41 (bs, 1H), 5.48 (bs, 1H), 4.19 (m, 2H), 3.73 (d, *J* = 5.0, 1H) and 3.68 (d, *J* = 5.0, 1H), 1.66 (qu, *J* = 6.8, 2H), 1.28 (m, 14H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.27, 166.24, 66.52, 54.50, 53.34, 31.85, 29.48, 29.44, 29.26, 29.13, 28.38, 25.67, 22.65, 14.09; ESI-MS m/z calculated for $C_{14}H_{25}NO_4$ $[M + H]^+$: 272.2; Found:272.2



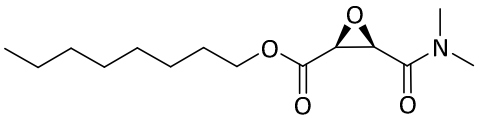
(2S,3R)-((E)-3,7-dimethylocta-2,6-dienyl) 3-carbamoyloxirane-2-carboxylate (22f). This compound was isolated as white solid (65%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.42 (bs, 1H), 5.55 (bs, 1H), 5.33 (t, *J* = 7.3, 1H), 5.07 (t, *J* = 6.1, 1H), 4.76 (dd, *J* = 12.2, 7.3, 1H), 4.66 (dd, *J* = 12.2, 7.2, 1H), 3.73 (d, *J* = 5.0, 1H), 3.67 (d, *J* = 5.0, 1H), 2.07 (m, 4H), 1.70 (bd, *J* = 8.9, 6H), 1.60 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.45, 166.17, 144.09, 131.99, 123.51, 116.91, 63.06, 54.51, 53.37, 39.47, 26.19, 25.65, 17.68, 16.50; ESI-MS m/z calculated for $C_{14}H_{21}NO_4$ $[M + H]^+$: 268.1; Found:268.2

General Method for Epoxysuccinamate Esters 23a-c.

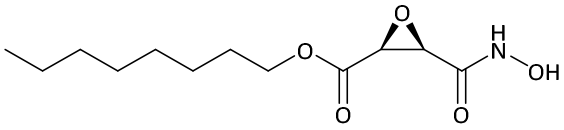
The following general method is exemplified by the procedure described below for the preparation of **23a**, using the ester-acid **21d** and appropriate amine scaled accordingly.



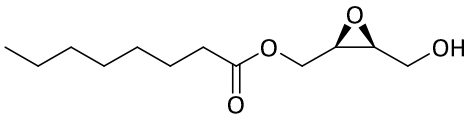
(2S,3R)-octyl 3-(methylcarbamoyl)oxirane-2-carboxylate (23a). PCl_5 (50 mg, 0.39 mmol) was added in one portion to a chilled (-5 °C) solution of ester-acid **21d** (80 mg, 0.33 mmol) in dichloromethane (3 ml) at room temperature for 3 hours. After this time methylamine hydrochloride (30 mg, 0.43 mmol) and triethylamine (0.14 mL, 0.99 mmol) were added slowly at room temperature for additional 2 hours. After reaction completion monitored by TLC, the reaction mixture was concentrated by evaporation at reduced pressure. The resulting residue was purified *via* silica gel column chromatography (1:1 EtOAc–Hexane) to yield the title compound (36mg, 0.14 mmol, 42%) as white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.44 (bs, 1H), 4.16 (m, 2H), 3.69 (bd, 2H), 2.81 (d, *J* = 5.0, 3H), 1.63 (dt, *J* = 13.9, 6.7, 2H), 1.29 (m, 10H), 0.87 (bt, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.30, 165.24, 66.37, 54.78, 53.39, 31.72, 29.10, 29.08, 28.40, 25.65, 22.59, 14.04; ESI-MS m/z calculated for $C_{13}H_{23}NO_4$ $[M + H]^+$: 258.2; Found: 258.2



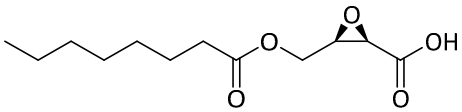
(2S,3R)-octyl 3-(dimethylcarbamoyl)oxirane-2-carboxylate (23b). This compound was isolated as white solid (95%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 (m, 2H), 3.76 (d, *J* = 4.6, 1H), 3.69 (d, *J* = 4.6, 1H), 3.17 (s, 3H), 2.94 (s, 3H), 1.63 (qu, *J* = 6.8, 2H), 1.29 (m, 10H), 0.86 (bt, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.63, 164.12, 66.06, 54.55, 52.64, 36.47, 35.31, 31.74, 29.14, 29.12, 28.40, 25.68, 22.60, 14.05; ESI-MS m/z calculated for $C_{14}H_{25}NO_4$ $[M + H]^+$: 272.2; Found: 272.2



(2S,3R)-octyl 3-(hydroxycarbamoyl)oxirane-2-carboxylate (23c).This compound was isolated as white solid (30%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (bs, 1H, OH), 4.19 (m, 2H), 3.77 (d, *J* = 4.7, 1H), 3.72 (d, *J* = 4.7, 1H), 1.66 (qu, *J* = 6.8, 2H), 1.28 (m, 10H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.19, 163.07, 66.85, 53.35, 53.03, 31.72, 29.09, 28.30, 25.66, 22.59, 14.04; ESI-MS m/z calculated for $C_{12}H_{21}NO_5$ $[M + H]^+$: 260.1; Found: 260.2

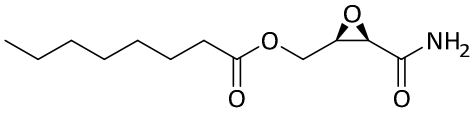


((2R,3S)-3-(hydroxymethyl)oxiran-2-yl)methyl octanoate (25). Epoxy diol (0.14 g, 1.32 mmol), pyridine (0.2 ml, 2.64 mmol) and DMAP (16 mg, 0.13 mmol) were dissolved in dichloromethan e (7 ml) and cooled to - 5°C. Octanoyl chloride (0.23 ml, 1.32 mmol) was added dropwise ove r 15 min keeping the temperature below 5°C. The reaction was allowed to warm to room temperature and stirred for 5 hours. After TLC monitoring, the reaction mixture was quenched with ice water (0.1 mL). After stirring for 2~3 hours at room temperature, the solution was diluted with dichlromethane (2 ml), washed sequentially with 1M H_2SO_4 (3 x 2 ml), saturated NaHCO 3 (2 x 2 ml), dried over Na_2SO_4 and concentrated to dryness. The crude oil was purified *via* silica gel column chromatography (1:1 EtOAc–Hexane) to yield the title compound (135 mg, 0.58 mmol, 44%) as yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.33 (dd, *J* = 12.2, 5.4, 1H), 4.15 (dd, *J* = 12.2, 5.6, 1H), 3.84 (bt, 2H), 3.23 (m, 2H), 2.36 (t, *J* = 7.5, 2H), 1.63 (qu, *J* = 7.5, 2H), 1.29 (m, 8H), 0.87 (bt, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.99, 61.45, 6020, 56.08, 53.75, 34.07, 31.60, 29.02, 28.86, 24.84, 22.56, 14.03; ESI-MS m/z calculated for $C_{12}H_{22}O_4$ $[M + H]^+$: 231.2 ; Found: 231.2

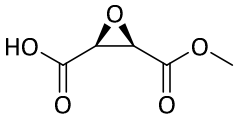


(2R,3R)-3-(octanoyloxymethyl)oxirane-2-carboxylic acid (26). *suspension A* was prepared prior to use; $NaIO_4$ (0.5 g, 2.21 mmol) and $RuCl_3 \cdot H_2O$ (1.8 mg, 9 μmol) were suspended in H_2O (1 mL) and stired at room temperature for 1 minute. Epoxy alcohol (51 mg, 0.22 mmol) was dissolved in Acetonitrile (2mL) and EtOAc (2 mL), cooled to 0°C and dropwise added *suspension A* over the course of 15 minutes. The flask containing *suspension A* was washed with H_2O (0.5 mL), which was added the mixture over the course of 5 minutes. The mixture was left to stir at 0°C for 2 h. After TLC monitoring, the mixture was filtered through a pad of Celite and diluted with EtOAc (2 x 3 mL). The

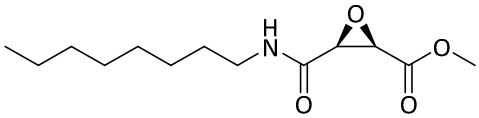
organic phases were pooled in a separation funnel and added H_2O (5 mL). After separation of the two phases the aqueous phase was extracted with EtOAc (2 x 6 mL). The pooled organic phases was washed with brine (6 mL), dried over $MgSO_4$, filtered, concentrated *in vacuo.* The resulting residue was purified *via* silica gel column chromatography (1:1 EtOAc–Hexane with 1% ^{1}AcOH) to yield the title compound (21 mg, 0.086 mmol, 39%) as colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.39 (dd, *J* = 12.4, 4.6, 1H), 4.27 (dd, *J* = 12.4, 6.4, 1H), 3.65 (d, *J* = 4.6, 1H), 3.49 (dt, *J* = 6.3, 4.6, 1H), 2.36 (t, *J* = 7.6, 2H), 1.63 (qu, *J* = 7.5, 2H), 1.29 (m, 8H), 0.87 (bt, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.72, 170.65, 60.98, 54.61, 51.38, 33.94, 31.90, 29.01, 28.85, 24.80, 22.56, 14.03; ESI-MS m/z calculated for $C_{12}H_{20}O_5$ $[M + H]^+$: 245.1; Found: 245.2



((2R,3R)-3-carbamoyloxiran-2-yl)methyl octanoate (27). This compound was isolated as white solid (77%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.24(bs, 1H), 5.62 (bs, 1H), 4.32 (dd, *J* = 12.6, 3.7, 1H), 4.23 (dd, *J* = 12.6, 7.8, 1H), 3.59 (d, *J* = 4.8, 1H), 3.47 (ddd, *J* = 8.4, 4.6, 3.8, 1H), 2.37 (t, *J* = 7.6, 2H), 1.64 (qu, *J* = 7.5, 2H), 1.29 (m, 8H), 0.87 (bt, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.55, 168.47, 61.36, 54.98, 53.66, 33.97, 31.61, 29.02, 28.87, 24.80, 22.57, 14.04; ESI-MS m/z calculated for $C_{12}H_{21}NO_4$ $[M + H]^+$: 244.1; Found:244.2

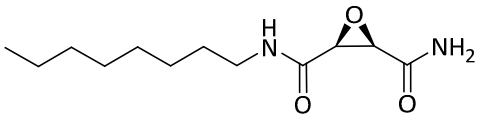


Monomethyl-cis-2,3-epoxysuccinate (28). *cis*-2,3-Epoxysuccinic anhydride **18** (93 mg, 0.82 mmol) was heated at 40°C in dry methanol (4 mL) for 17 hours. The excess alcohol was removed under reduced pressure to yield the desired compound (101 mg, 0.69 mmol, quantitative) which was used without further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (s, 3H), 3.80 (d, *J* = 4.7, 1H), 3.78 (d, *J* = 4.7, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 168.49, 166.84, 53.37, 52.82, 52.78; ESI-MS m/z calculated for $C_5H_6O_5$ $[M + H]^+$: 147.0; Found:147.2

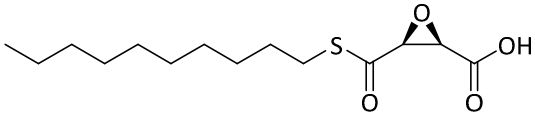


1-methyl-4-*N*-(4-octyl)-cis-2,3-epoxysuccinamate (29). PCl_5 (24 mg, 0.19 mmol) was added in one portion to a chilled (-5 °C) solution of ester-acid **28** (23 mg, 0.16 mmol) in dichloromethane (1 ml) at room temperature for 3 hours. After this time 1-Octylamine (23 µl, 0.16 mmol) and tritylamine (24 µl,

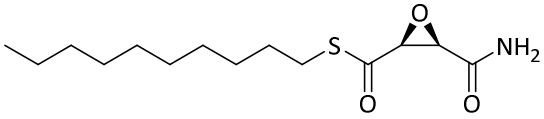
0.24 mmol) were added at -20°C and stirred for 30 minutes. After reaction completion monitored by TLC, the reaction was quenched by the addition of acetic acid (28 ul, 0.47 mmol, 3 eq) at -20°C. The resulting residue was then concentrated to dryness and purified *via* silica gel column chromatography (3:7 EtOAc–Hexane) to yield the title compound (23 mg, 0.089 mmol, 58%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.43 (bs, 1H), 3.78 (s, 3H), 3.72 (d, *J* = 5.0, 1H), 3.69 (d, *J* = 5.0, 1H), 3.24 (m, 2H), 1.48 (qu, *J* = 7.2, 2H), 1.28 (m, 10H), 0.88 (t, *J* = 6.7, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.54, 164.38, 54.70, 53.33, 52.79, 39.14, 31.76, 29.33, 29.17, 29.15, 26.78, 22.61, 14.05; ESI-MS m/z calculated for $C_{13}H_{23}NO_4$ $[M + H]^+$: 258.2; Found: 258.2



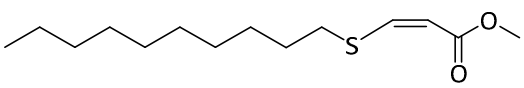
4-*N*-(4-octyl)-cis-2,3-epoxysuccinamide (30). The amide-ester **29** (15 mg, 0.058 mmol) was stirred with aqueous ammonia solution (0.1 mL, 2.56 mmol, excess) and methanol (0.5 ml) in room temperature for 3 hours. The resulting residue was then concentrated to dryness and purified *via* silica gel column chromatography (3:7 EtOAc–Hexane) to yield the title compound (12 mg, 0.049 mmol, 85%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.23 (bs, 1H), 5.51 (bs, 1H), 3.66 (d, *J* = 0.9, 2H), 3.26 (m, 2H), 1.49 (qu, *J* = 6.9, 2H), 1.27 (m, 10H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.19, 164.79, 54.79, 54.59, 39.50, 31.75, 29.32, 29.15, 26.78, 22.60, 14.06; ESI-MS m/z calculated for $C_{12}H_{22}N_2O_3$ $[M + H]^+$: 243.2;. Found: 243.2



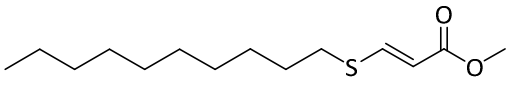
(2R,3S)-3-(decylthiocarbonyl)oxirane-2-carboxylic acid (31). To a stirred solution of *cis*-2,3-Epoxysuccinic anhydride **18** (100 mg, 0.876 mmol) and DMAP (6 mg, 0.05 mmol) in 10 ml of acetonitrile-pyridine (9:1), Decanethiol (166 ul, 0.789 mmol) was added under argon. The mixture was allowed to stir at room temperature overnight before concentrating to dryness under reduced pressure, followed by dissolving the residue in EtOAc, and then washing with 1M HCl (3 x 5mL) and H_2O (3 x 5mL) before drying over anhydrous $MgSO_4$. The solution was concentrated under reduced pressure and dried *in vacuo.* The resulting residue was purified *via* silica gel column chromatography (1:1 EtOAc–Hexane with 1% AcOH) to yield the title compound (151 mg, 0.52 mmol, 60%) as colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (d, *J* = 4.7, 1H), 3.81 (d, *J* = 4.7, 1H), 3.00 (td, *J* = 7.2, 2.1, 2H), 1.60 (qu, *J* = 7.3, 2H), 1.35 (m, 2H), 1.28 (m, 12H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 193.89, 167.78, 58.30, 54.12, 31.86, 29.49, 29.41, 29.35, 29.26, 28.99, 28.95, 28.70, 22.65, 14.09; ESI-MS m/z calculated for $C_{14}H_{24}O_4S$ $[M + H]^+$: 289.1; Found: 289.2



(2S,3R)-S-decyl 3-carbamoyloxirane-2-carbothioate (32). This compound was isolated as white solid (62%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.32(bs, 1H), 5.57 (bs, 1H), 3.89 (d, *J* = 5.0, 1H), 3.72 (d, *J* = 5.0, 1H), 2.96 (m, 2H), 1.58 (dt, *J* = 15.0, 6.6, 2H), 1.27 (m, 14H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 192.58, 166.84, 58.91, 55.64, 31.85, 29.48, 29.41, 29.25, 29.15, 29.13, 29.00, 28.72, 22.65, 14.09; ESI-MS m/z calculated for $C_{1s}H_{25}NO_3S$ $[M + H]^+$: 288.16; Found: 288.2

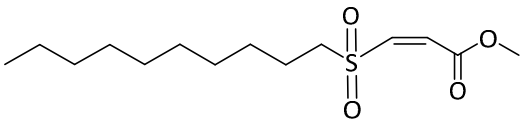


34



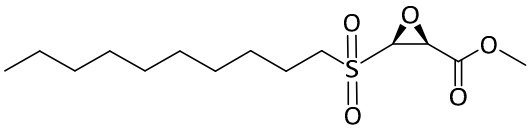
35

(Z)-methyl 3-(decylthio)acrylate(34). (E)-methyl 3-(decylthio)acrylate (35). Copper (I) chloride (59 mg, 0.6 mmol), potassium carbonate (165 mg, 1.19 mmol), DMSO (2 mL) were added to a Schlenk tube under argon atmosphere. Then Methylpropiolate (1 g, 11.89 mmol) and Decanethiol (2.2 mL,10.70 mmol) were added using syringe. The tube was sealed with an oxygen balloon, then stirred at 70^{o}C for 4h and monitored by TLC. Upon completion, the reaction mixture was cooled to room temperature and washed with 1 M hydrochloric acid (10 mL) solution, and the aqueous phase was re-extracted with ethyl acetate (2x15 mL). The combined organic extracts were dried over Na_2SO_4, concentrated in vacuum, and the resulting residue was purified by silica gel column chromatography using 2% EA/HX. Both E, Z isomers were separated and obtained compounds **34** in 42% yield, **35** in 20%; **cis** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, *J* = 10.2, 1H), 5.84 (d, *J* = 10.2, 1H), 3.74 (s, 3H), 2.75 (t, *J* = 7.4, 2H), 1.67 (qu, *J* = 7.4, 2H), 1.39 (m, 2H), 1.28 (m, 12H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.05. 150.71, 112.47, 51.18, 36.06, 31.85, 30.30, 29.50, 29.44, 29.26, 29.13, 28.43, 22.64, 14.08; ESI-MS m/z calculated for $C_{14}H_{26}O_2S$ $[M + H]^+$: 259.2; Found: 259.2
; **trans** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 15.2, 1H), 5.74 (d, *J* = 15.2, 1H), 3.72 (s, 3H), 2.78 (bt, 2H), 1.67 (qu, *J* = 7.3, 2H), 1.40 (qu, *J* = 6.2, 2H), 1.26 (m, 12H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.78, 147.28, 113.17, 51.38, 31.99, 31.85, 29.48, 29.42, 29.25, 29.06, 28.75, 28.57, 22.65, 14.08; ESI-MS m/z calculated for $C_{14}H_{26}O_2S$ $[M + H]^+$: 259.2; Found: 259.2

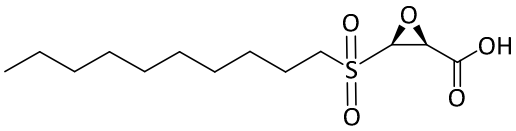


(Z)-methyl 3-(decylsulfonyl)acrylate(36). To the stirred solution of sulfide (1.2 g, 4.64 mmol) in glacial acetic acid (10 ml), sodium perborate tetrahydrate (3.57 g, 23.21 mmol) was added in one portion and maintained at 50-60°C. Stirring was continued at 50-60°C for 3-4 h, until separation of

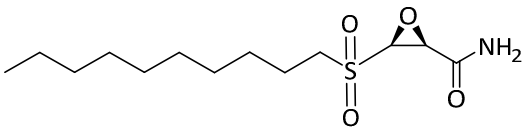
sodium borate was complete. The mixture was cooled, the inorganic salts removed by filtration with Ether (50 mL). The filtrate was concentrated under reduced pressure and purified by short column chromatography using 30% EA/HX. Obtained pure compound **36** in 50% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (d, *J* = 2.1, 1H), 3.85 (s, 3H), 3.22 (m, 2H), 1.83 (qu, *J* = 7.7, 2H), 1.43 (qu, *J* = 7.1, 2H), 1.28 (m, 12H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.90, 136.42, 133.67 55.50, 52.78, 31.82, 29.42, 29.21, 29.00, 28.38, 22.63, 21.94, 14.07; ESI-MS m/z calculated for $C_{14}H_{26}O_4S$ $[M + H]^+$: 291.2; Found: 291.2



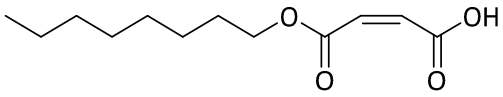
(2S,3R)-methyl 3-(decylsulfonyl)oxirane-2-carboxylate (37). A 14.5% aqueous solution of sodium hypochlorite (1.6 mL, 3.78 mmol) was added dropwise at 0°C into a solution of sulfone **36** (500 mg, 1.72 mmol) in 40 mL of a mixture of Ether/DMF (1:1). The mixture was stirred for 3 h at 0°C. Then the reaction was quenched by addition of 10% $Na_2S_2O_3$ solution (30 mL). The aqueous phase was extracted with ether (3 x 15 mL). The organic phase was dried over $MgSO_4$ filtered, concentrated under reduced pressure. Crude was purified by Column chromatography using 30% EA/HX system. Obtained pure compound **37** in 70% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.25 (d, *J* = 4.1, 1H), 3.87 (d, *J* = 4.1, 1H), 3.85 (s, 3H), 3.16 (m, 2H), 1.88 (qu, *J* = 8.8, 2H), 1.45 (qu, *J* = 7.1, 2H), 1.28 (m, 12H), 0.88 (t, *J* = 6.9, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.09, 66.02, 53.45, 53.09, 52.61, 31.80, 29.40, 29.19, 29.18, 28.94, 28.37, 22.62, 21.11, 14.06; ESI-MS m/z calculated for $C_{14}H_{26}O_5S$ $[M + H]^+$: 307.2; Found: 307.2



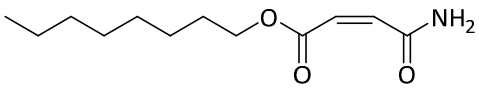
(2S,3R)-3-(decylsulfonyl)oxirane-2-carboxylic acid (38). A stirred solution of Epoxy methyl ester **37** (330 mg, 1.07 mmol) in tetrahydrofuran (8 mL) was prepared. A solution of lithium hydroxide monohydrate (91 mg, 2.15 mmol) in water (4 mL) was added. The reaction mixture was stirred at room temperature for 3 hours, then 2 M hydrochloric acid (5 mL) was added. The mixture was extracted ethyl acetate (2 x 20 mL), dried over anhydrous Na_2SO_4 filtered, concentrated under reduced pressure. Crude was purified by column chromatography using 30% ethylacetate/hexane. Obtained pure compound **38** in 68% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.13 (bs, 1H), 4.31 (d, *J* = 4.2, 1H), 3.92 (d, *J* = 4.2, 1H), 3.19 (m, 2H), 1.89 (qu, *J* = 8.4, 2H), 1.45 (qu, *J* = 7.1, 2H), 1.28 (m, 12H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.17, 66.43, 53.33, 52.77, 31.82, 29.41, 29.21, 29.20, 28.94, 28.37, 22.64, 21.17, 14.08; ESI-MS m/z calculated for $C_{13}H_{24}O_5S$ $[M + H]^+$: 293.1; Found: 293.2



(2S,3R)-3-(decylsulfonyl)oxirane-2-carboxamide (39). This compound was isolated as white solid (60%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.34 (bs, 1H), 5.81 (bs, 1H), 4.25 (d, *J* = 4.4, 1H), 3.80 (d, *J* = 4.4, 1H), 3.12 (td, *J* = 7.4, 3.9 2H), 1.88 (qu, *J* = 7.9, 2H), 1.45 (qu, *J* = 7.0, 2H), 1.28 (m, 12H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.32, 66.30, 54.28, 53.32, 31.82, 29.40, 29.20. 29.19, 28.95, 28.40, 22.63, 21.26, 14.08; ESI-MS m/z calculated for $C_{13}H_{25}NO_4S$ $[M + H]^+$: 292.2; Found: 292.2



(Z)-4-(octyloxy)-4-oxobut-2-enoic acid (41). Maleic anhydride (98 mg, 1 mmol) and 1-octanol (0.19 mL, 1.2 mmol) were dissolved in 5 mL Dichloromethane. After slowing adding triethylamine (0.21 mL, 1.5mmol) to the mixture, the mixture was stirred at room temperature for 1 h. After reaction completion monitored by TLC, the reaction mixture was quenched with citric acid. The solution was diluted with 5mL Dichloromethane, washed with 1N HCl (2 x 3mL), dried over Na_2SO_4 and concentrated to dryness. The crude oil was purified *via* silica gel column chromatography (1:1 EtOAc–Hexane) to yield the title compound (62 mg, 0.27 mmol, 27%) as colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (d, *J* = 12.8, 1H), 6.37 (d, *J* = 12.8, 1H), 4.28 (t, *J* = 6.7, 2H), 1.75 (qu, *J* = 6.7, 2H), 1.32 (m, 10H), 0.87 (m, 3H)



(Z)-octyl 4-amino-4-oxobut-2-enoate(42). Oxalyl chloride (76 ul, 0.88 mmol) was add to a stirred solution of ester acid **41** (101 mg, 0.44 mmol) in dichlorimethane (3 ml) under N_2 atmosphere. Catalytic amount of DMF (2.3 ul, 0.03 mmol) was added and the mixture was stirred at room temperature for 1h. After reaction completion monitored by TLC, ammonium hydroxide (excess, 3.3 ml) was added to reaction mixture and stirred for 5 hours. The reaction mixture was evaporated and diluted with EtOAc (10 ml), washed with brine (2 x 5 ml), dried over anhydrous MgSO4, filtered, and concentrated *in vacuo*. The resulting residue was purified *via* silica gel column chromatography (1:1 EtOAc–Hexane) to yield the title compound (35 mg, 0.15 mmol, 35%) as colorless liquid; H NMR (400 MHz, $CDCl_3$) δ 6.92 (d, *J* = 15.5, 1H), 6.83 (d, *J* = 15.5, 1H), 5.60 (bd, 2H) 3.19 (t, *J* = 6.7, 2H), 1.67 (m, 2H), 1.31 (m, 10H), 0.88 (bt, 3H)

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