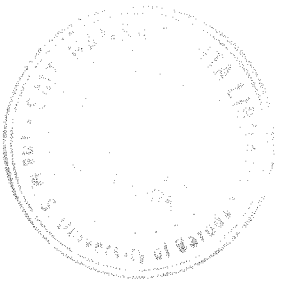
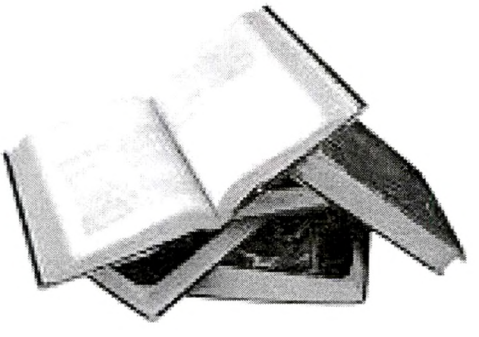


It's not that I'm so smart, it's just

that I stay with problems longer.

Albert Einstein

CHAPTER-1



INTRODUCTION

1 INTRODUCTION

Autoimmune diseases have become the main focus in pharmaceutical research as proper understanding of immune and inflammatory processes is still incomplete. Rheumatoid Arthritis (RA) is one of the most common autoimmune inflammatory conditions, affecting approximately 1% of the worldwide adult population[1-3]. One in three patients with RA is likely to be disabled severely[4,5].

1.1 Definition

RA is a chronic and usually progressive inflammatory disorder of unknown etiology characterized by polyarticular symmetrical joint involvement and systemic manifestations[6]. RA is manifested by formation of hypertrophied synovia known as pannus. Pannus formation mirrors the destruction of articular and peri-articular structures, with or without generalized manifestations. The inflamed joint lining the synovium, can invade and damage bone and cartilage. Inflammatory cells release enzymes that may digest bone and cartilage. The involved joint can lose its shape and alignment, resulting in pain and loss of movement[7-12].

1.2 Etiology

No single factor responsible for RA has been identified till date, although there are abundant evidences that indicate that RA is an autoimmune disease. RA results from the irregular function of humoral and cell mediated components of the immune system. There is an increased chemotactic and immuno-stimulatory activity within the joints of sufferers resulting in an influx of inflammatory cells. The presence of activated immune cells increases local levels of cytokines (e.g. IL-1, TNF-α) and other inflammatory mediators propagating this process and supporting pannus formation, proliferation and neovasculaturization, cartilage and bone erosion and eventually joint destruction[7].

1.3 Pathophysiology[2,5]

The pathogenesis of RA is likely to be multifactorial, involving autoimmunity and genetic factors; infectious agents are also suspected of having a role in it. Further details are provided below.

1.3.1 Genetic Factors

Family studies reveal that RA has a genetic component and the risk for RA is thought to be associated with a sequence of amino acids within the genetic code of certain individuals. Human Leukocyte Antigen (HLA) is an important genetic factor in the genesis of RA.

1.3.2 Autoimmunity

Macrophage-derived cytokines appear to be involved in the induction and perpetuation of the chronic inflammatory processes of the joints seen in RA. High titers of serum rheumatoid factors or binding of auto-antibodies to the Fc portion of the immunoglobulin G (IgG) molecules are associated with more severe disease and with extra-articular manifestations.

1.3.3 Infectious Agents

Viral infections such as rubella, Ross River virus and parvovirus are associated with the development of acute polyarthritis. *Chlamydia pneumoniae* has been detected in some individuals with RA. However, the cause and connection between RA and infectious agents has not been demonstrated beyond doubt.

1.4 Clinical Features

Symptoms of the RA disease include inflammation of joints, swelling, pain and difficulty in moving. The elbows, shoulders, hips, knees and ankles are the major joints which are affected by it. But the disease may also involve small joints of fingers of hands and feet, and wrist[12]. Other symptoms include:

- Loss of appetite
- Fever
- Loss of energy (weakness)
- Anemia
- Myalgia

Joints stiffness typically is worse in the morning and usually lasts at least one hour prior to maximal improvement is seen for the day. Chronic joint deformities commonly involve subluxations of the wrists, Metocarpophalangeal (MCP) and Proximal Inter Phalangeal (PIP) joints. Extra-articular involvement may include subcutaneous nodules, vasculitis, pleural effusions, pulmonary fibrosis, ocular manifestations,

pericarditis, cardiac conduction abnormalities and bone marrow suppression[4-6]. Stiffness and myalgias may precede development of synovitis.

1.5 Diagnosis

It is important to diagnose RA early in the course of development of the disease because with the use of disease modifying drugs, the condition can be controlled from worsening in many cases. The diagnosis of RA is based on the overall pattern of symptoms, medical history, physical examination, x-rays and lab tests including the test for rheumatoid factor. Rheumatoid factor is an antibody found in the blood of about 80 % of adults with RA[4,6].

The laboratory test abnormalities that may be seen include normocytic and normochromic anemia, thrombocytosis or thrombocytopenia, leucopenia, elevated Erythrocyte Sedimentation Rate (ESR), positive Anti-Nuclear Antibodies (ANA), C-reactive protein (CRP) and Plasma Viscosity (PV). Radiographs, mainly of the hands and feet, are used to establish the diagnosis of RA and to follow its progression[5].

Prognosis of the disease may be poor in those cases where, the onset of the disease occurs prior to the age of 50 years in males, the disease state is more than five years old, the number of affected joints are more than twenty, there is extra-articular involvement, there occurs functional disability within one year of onset of the disease and there occurs several co-morbidities in the patient. Lack of formal education and lower socioeconomic status also leads to poor prognosis[5].

1.6 Management

There is no known cure for RA. To date the key goals of management of RA are:

- Relief of pain and inflammation
- Preventing joints' destruction
- Conservation and restoration of affected joints' function, and
- Preserving or improving the functional ability and normalizing life style of the patient.

Treatment of RA should begin as soon as possible, as there is evidence that most of the patients develop joint destruction within first 2 years of the onset of the disease[9,10].

A multidisciplinary approach is important for treating RA patients. Physiotherapist, occupational therapists, podiatrists, social workers and pharmacists, all have crucial roles in successful treatment of the disease. Educating the patient regarding the disease process and its management also helps in improving the outcome of the treatment[4,5].

1.7 Current Treatment

Treatment of patients suffering from the disease may involve the followings:

1.7.1 Non-Pharmacological Approaches[4]

Non-pharmacological methods such as physiotherapy, occupational therapy and electrotherapy not only play a vital role in acute flares and in chronic stage but are also helpful in relieving symptoms and protecting joints from further damage. Patients may benefit from surgical procedures such as tenosynovectomy, tendon repair and joint replacements in severe conditions.

1.7.2 Pharmacological Approaches[13,14]

Presently, three categories of drugs are used for the treatment of RA.

I. Non-steroidal anti-inflammatory drugs (NSAIDs)

This class of drugs blocks the production of prostaglandins by inhibiting the enzyme, cyclooxygenase (COX). Apart from causing gastric irritation, this class of drugs also causes renal and cardiac disturbances. These drugs are useful for symptomatic treatments only[1].

II. Corticosteroids

This class of drugs acts by lowering the activity of white blood cells and inhibits the production of prostaglandins. When used for a long time, these drugs become less effective and also cause major side effects like osteoporosis, bone cell death, onset of diabetes, artery hardening etc.

III. Disease modifying anti-rheumatic drugs (DMARDs)

DMARD is a general heading for a diverse class of drugs that slow and stop the progression of RA by suppressing the immune response. The most commonly used drug in this class is methotrexate, which leads to significant amelioration of symptoms but it does not stop joint destruction[15].

1.8 Cytokines: Role in RA

Cytokines are extra-cellular protein messenger molecules or cell surface signaling molecules involved in processes like inflammation, immunity, differentiation, cell division, fibrosis, repair, apoptosis etc[16].

All cytokines are proteins with different physical characteristics. Some are single chain proteins (e.g. IL-1), some are homo-dimers (e.g. IFN-γ) while the members of TNF family form trimers, mostly homo-trimers, except for LT which forms hetero-trimer. Many of these cytokines are glycosylated proteins.

Virtually, all cells can produce cytokines in response to diverse stimuli. Which cytokine the cell would make from its potential repertoire, depends on factors like nature of the stimuli, its duration and intensity as well as on certain other factors like presence or absence of other cytokines or hormones. Macrophages, T cells and mast cells can produce a wide variety of cytokines.

A distinctive feature of cytokines is that they are usually not produced constitutively, but are generated in response to some stimuli. Typically, their production cycle lasts for a few hours to few days in the normal state, but if the stimuli persist, as in a diseased state, cytokine production can be prolonged[17].

Cytokines are bioactive at very low concentrations, often in the range of 10^{-10} to 10^{-3} mol/L. This can be attributed to the fact that they have high affinity towards the receptors and that signaling does not require high receptor occupancy.

1.8.1 Cytokines and Rheumatoid Arthritis

Cytokines are critical in the induction and resolution of inflammatory responses. There are numerous so called 'pro-inflammatory cytokines' but considerably fewer 'anti-inflammatory cytokines', known so far. The classic 'pro-inflammatory cytokines' include Inerleukin (IL)-1, 6 and 12, GM-CSF, tumor necrosis factor-α (TNF-α) and IFN-γ, whereas the 'anti-inflammatory cytokines' include TGF-β and IL-10[16-18].

Abundant laboratory and clinical evidences suggest that pro-inflammatory cytokines play a major role in the pathogenesis of RA[18]. Several studies have indicated that cytokines, especially tumor necrosis factor-α (TNF-α), are present in high

concentrations in serum, synovial fluid, synovial fluid cells and synovial tissue specimens of patients with RA[19].

Although the precise mechanism of bone and cartilage destruction in RA is not completely understood, the cytokines, especially IL-1 and TNF-α, play an important role in the process. These cytokines:

- Are abundant in inflamed joints and promote the influx of inflammatory neutrophils and monocytes into the joints,
- Stimulate the cells in the inflamed synovium to produce proteolytic enzymes, including collagenase and stromelysin, that can degrade tissue and
- Cause systemic manifestations such as malaise and fatigue.

Thus, although the initial cause of RA remains unknown, the maintenance and propagation of the disease appear to be related to immunologically mediated inflammatory processes. Hence, interfering with key steps in the inflammatory process would be expected to provide symptomatic relief and to slow down the disease progression.

The earliest change observed in the joints of arthritis patients is swelling and congestion of the synovial membrane and the underlying connecting tissues, which get infiltrated with lymphocytes (especially CD4 T-cells), plasma cells and macrophages. In the initial cell-mediated response, macrophages engulf and process antigens and present them to T-lymphocytes. The processed antigens are recognized by the Major Histocompatibility Complex (MHC) proteins on the lymphocyte surface, resulting in T-cell activation and the production of cytokines and cytotoxins that ultimately results in joint damage[7,8].

Activated B-lymphocytes produce plasma cells from antibodies, in combination with their complements, resulting in accumulation of polymorphonuclear leukocytes (PMNs). PMNs release cytotoxins, free oxygen radicals and hydroxyl radicals that cause cellular damage to synovium and bone.

During active phases of the disease, effusion of synovial fluid takes place into joint space and hypertrophy of synovial membrane occurs resulting into formation of lymphoid follicles, resembling an immunologically active lymph node. Inflammatory

granulation tissue, known as pannus, spreads over and under the articular cartilage, which is progressively eroded and destroyed. Later, fibrous or bony ankylosis may occur.

Vasoactive substances (histamine, kinins, PGs) are released at the site of inflammation thereby increasing the blood flow and vascular permeation that cause edema, warmth and pain[4-6]. Around 30 cytokines are found to be present in the tissues and synovial fluid of active RA affected joints[20,21]. Many of these appear to mediate closely related functions, like induction of cartilage destruction mediated by IL-1, TNF-α and LT- α. There is considerable evidence to suggest that in an inflammatory site there is a cytokine network/cascade, where the actions of certain cytokines are regulated by certain others. The rheumatoid synovium produces significant levels of IL-1α, IL-1β, TNF-α, IL-6, IL-8, GM-CSF and other pro-inflammatory cytokines[17]. Evidence for the cytokine network was obtained by the use of neutralizing antibodies for TNF-α and IL-1 and its receptor antagonist (IL-1Ra) in dissociated cultures of rheumatoid synovium. It was found that anti-TNF-α antibodies reduce the production of IL-1, IL-6, IL-8 and GM-CSF, whereas IL-1Ra reduces the production of IL-6, IL-8 and GM-CSF, but not TNF-α[22]. This experiment led to the notion of a network in rheumatoid joints with TNF-α at the apex followed by IL-1, IL-6, IL-8 and GM-CSF downstream[20,21].

Another important question to be answered is whether such a network really operates *in vivo*. Confirmation of this has come from clinical trials of anti-TNF-α antibodies in patients with active rheumatoid arthritis, in whom the levels of IL-1, IL-6, IL-8, and GM-CSF all got diminished after the treatment[23].

1.8.2 Tumor Necrosis Factor- α

In 1975, TNF was defined as an endotoxin-induced serum factor which produced necrosis of tumors both *in vitro* and *in vivo* experiments[24]. In 1984, Goeddel and colleagues successfully cloned and sequenced human TNF-α[25]. In the late 1980s, TNF-α was included in the class of cytokines because of its striking similarities in activities with IL-1[25-27].

The predominant source of TNF-α is cells of the monocyte/macrophage lineage, but other important sources are neutrophils, T cells, mast cells, epithelial cells, osteoblasts and dendritic cells. In macrophages, TNF-α synthesis is induced by biological, chemical and physical stimuli including virus, bacterial and parasitic products, ischemia, trauma and irradiation[28-30]. Transcription of TNF-α gene is regulated in a complex manner and there are multiple transcription factors like NF-κB, AP-1, NFIL-6,

and NFAT. The p38 MAPK signaling pathway also plays a critical role in the production of TNF-α[26,28].

The biological responses to TNF-α are mediated through two distinct receptors, namely TNF-R1 and TNF-R2. Both of them are transmembrane glycoproteins. Although their extracellular domains share structural and functional homology, their intracellular domains are distinct. TNF-R1 is constitutively expressed in most tissues while TNF-R2 is typically found in cells of immune system. It has been experimentally proved that under physiological conditions, signaling through TNF-R1 seems to be primarily responsible for pro-inflammatory properties of TNF-α[31]. Much information is not available about TNF-R2, but it has been speculated that it acts as ligand passer, at least in some cells[32].

It has been experimentally proved that TNF-α plays a pivotal role in the origin and progression of RA[19-23,26,27]. TNF-α is responsible for causing a variety of inflammatory effects in inflamed joint[16,33-37]. Some of the major effects are listed below:

- It releases vasodialatory molecules e.g. bradykinin, histamine, prostacyclin
- It stimulates bone resorption
- It inhibits bone collagen synthesis
- It stimulates matrix metalloproteinase release, which in turn plays an important role in the destruction of connective tissue
- It acts as osteoclast activating factor
- It stimulates production of COX and PGE_2
- It produces reactive oxygen species (ROS) which in turn cause tissue destruction
- It induces neutrophil production by ROS
- It causes neoangiogenesis which leads to the formation of pannus tissue
- It induces production of other pro-inflammatory cytokines e.g. IL-1, IL-6, IL-8, GM-CSF etc.

Once the role of TNF-α was clearly elucidated in RA, efforts were made to develop inhibitors of TNF-α. Three protein based drugs have been approved by USFDA for their use in the treatment of RA[38]. Etanercept is genetically engineered version of TNF-R2 that binds and inactivates TNF-α, preempting its role in RA. Infliximab (Mouse-human chimeric anti-human TNF-α antibody) and Adalimumab (Human anti-human TNF-α antibody) have also been approved for the treatment of RA[28,39]. These TNF-α blockers are

effective for the treatment of RA and show significant, documentable improvement in symptoms, signs and laboratory parameters within 8-12 weeks of treatment[15,40].

The success of these biological agents proved that inhibition of TNF-α could result in effective control of RA. Despite the marked activity of the biological agents that neutralize TNF-α, an orally administered, selective small molecule inhibitor of TNF-α would be desirable from the stand point of ease of administration, reduced cost of treatment, patient compliance and potential for more precise control of TNF-α levels[39, 41, 42]. Newton *et al* demonstrated that inhibition of TACE by small molecular weight orally bioavailable drug would be more effective than the biological agents in blocking downstream cytokine production[41].

Attempts to disable TNF-α are targeted at different levels. TNF-α can be inhibited at two stages[42]:

1. **Inhibition of Pro-TNF-α processing**

TNF-α is produced in the body from its precursor, pro-TNF-α. It is proteolytically cleaved by a zinc metalloproteinase, called TNF-α converting enzyme (TACE), to the active and soluble form, s-TNF-α. Inhibition of this enzyme would automatically reduce the active TNF-α levels in the blood.

2. **Inhibition of Pro-TNF-α synthesis**

It has been seen that NF-κB and PDE, especially PDE4, are involved n the production of TNF-α. NF-κB and PDE inhibitors could inhibit the production of pro-TNF-α. Thalidomide and some of its analogs also inhibit TNF-α production in the body by some unknown mechanism.

It has been hypothesized that small size orally bioavailable TACE inhibitors would have the potential to effectively treat RA by limiting the levels of soluble TNF-α[26,27,43,44]. Based on this hypothesis, several research groups world-wide are actively pursuing for small molecule orally bioavailable TACE inhibitors[45].

1.9 TNF Converting Enzyme (TACE)

TNF-α is produced in the body as pro-TNF-α, the inactive form of the cytokine as 233 aa membrane anchored precursor. It is transformed into the active and soluble form by controlled proteolysis at the Ala76 and Val77 bond. The proteinase responsible

for this cleavage, called TACE, has been cloned by two research groups at the same time[46,47].

TACE (ADAM17, CD156b, EC 3.4.24) belongs to the ADAM (A Disintegrin And Metalloproteinase Domain) family of proteins that belongs to the super-family of metzincin. There are 39 distinct ADAM family members deposited in public databases[48]. The structure of TACE closely resembles other ADAM family members.

1.9.1 Structure of TACE

TACE is a type I transmembrane protein synthesized as a zymogen. It contains a pro-domain, a catalytic domain, a disintegrin cysteine-rich region, a transmembrane segment and a cytoplasmic tail as shown in Figure 1. The pro-domain segment is thought to act as an inhibitor of the protease activity. The free cysteine residue present in the pro-domain coordinates with the zinc in the active site of TACE and thus prevents its activity. Pro-domain removal is therefore regarded as pre-requisite for TACE activity[49]. The potential furin cleavage site localized between pro- and the catalytic domains is responsible for the generation of the active TACE by removal of the pro-domain by furin[49,50].

The catalytic domain, as shown in Figure 2, contains the zinc-binding motif which is involved in coordinating zinc with histidine residue and creating the active site of the enzyme. The crystal structure of the catalytic domain is well documented in literature[48]. Analysis of the amino acid sequence of TACE indicates the presence of an EGF-like domain and a crambin-like domain, unique for TACE, within the cysteine-rich domain. The cysteine rich domain is responsible for the substrate recognition and also for the process of TACE maturation[49,52].

The cytoplasmic tail of TACE contains potential sites for interaction with Src-homology-2 and Src-homology-3 and a potential tyrosine phosphorylation site as well as potential MAPK phosphorylation site[53].

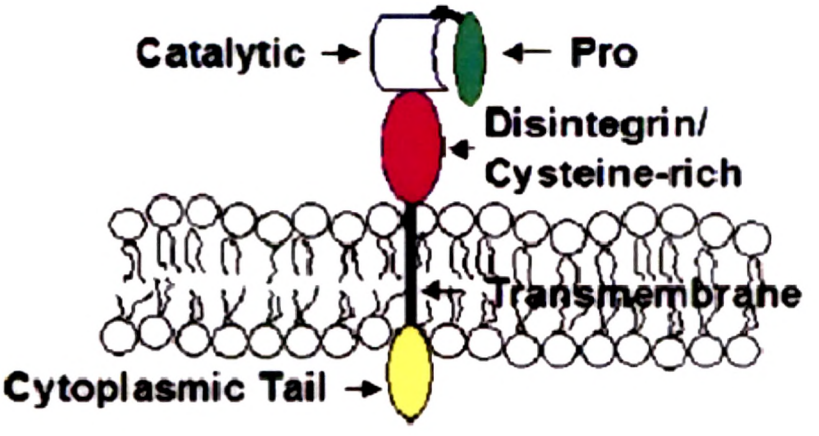


Fig 1. Domain Structure of TACE[48]

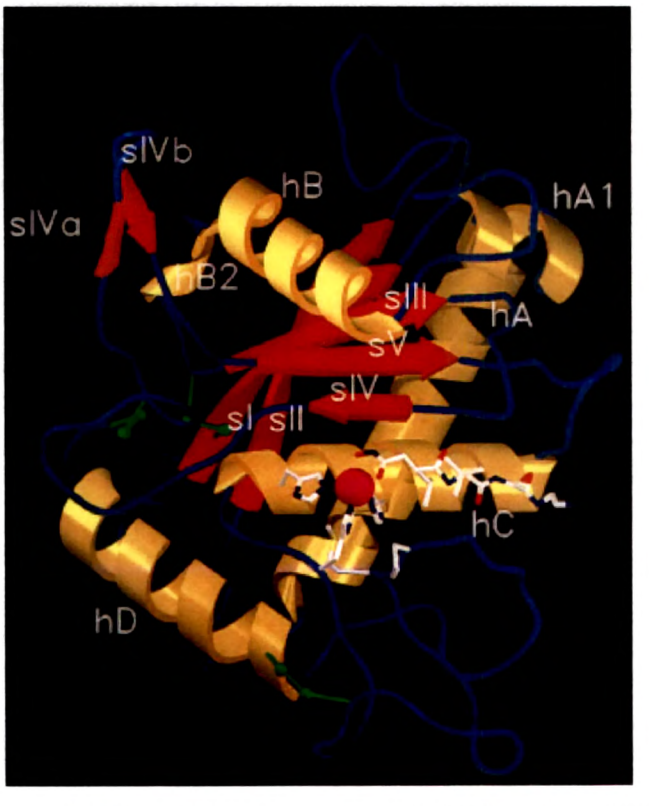


Fig 2. Ribbon Diagram of Catalytic Domain of TACE[50]
(The disulfide bonds are shown in green, the zinc atom is shown as pink ball and the inhibitor is shown in white).

1.9.2 Functions of TACE

The role of TACE in shedding TNF has been confirmed by *in vitro* experiments. Both T-cells and monocytes derived from 'TACE $^{\Delta Zn/\Delta Zn}$' transgenic mice, are deficient in releasing TNF[46]. Apart from this, these mice were also shown to be deficient of Transmembrane Growth Factor-α (TGF-α). Hence, most 'TACE $^{\Delta Zn/\Delta Zn}$' mice show developmental defects in the embryos such as failure of eyelids to fuse and specific hair and skin defects that closely resemble the defects characteristic for TGF-α deficiency. It proves that TACE is also responsible for the release of active TGF- α from its inactive precursor form[54].

Apart from TGF-α, TACE is responsible for the shedding of a number of proteins, such as, L-selectin, TNF-R2, Amyloid Precursor Protein (APP), Type II IL-1 receptor etc. But, it has also been shown that TACE is not a universal sheddase[55].

Hence the question arises, what makes a given protein a TACE substrate. The sequences cleaved by TACE in various substrates are highly variable. Moreover, the structure of the substrate-binding cleft of the enzyme does not suggest a strong interaction between the substrate to be cleaved and TACE. It has been suggested that interactions distant to the cleavage site are required and in some cases the substrate and the enzyme both have to anchor to cell membrane[55].

1.9.3 Regulation of TACE

The regulation of TACE activity is poorly understood. It has been observed that the shedding rate of TACE increases within minutes of addition of cell activators like phorbol ester. Inhibitors of Mitogen Activated Protein Kinase (MAPK) cascade block the increase in shedding rate in a number of cases where TACE is a primary sheddase[56]. However, the mechanism of action of MAPK cascade is not clear. Interestingly, a small protein TIMP-3, that inhibits most matrix metalloproteases[57], also inhibits TACE. But whether TIMP-3 acts as a physiological regulator of the enzyme is unknown.

1.10 Matrix Metalloproteinases (MMPs)

MMPs are an important class of zinc metalloproteinases. MMPs are a collection of 24 zinc-containing endopeptidases that include the gelatinases, stromelysins and collagenases, released as inactive zymogens. They become active after the pro-peptide is cleaved, like TACE.

TACE and MMPs both belong to the same metzincin family of enzymes. There is considerable similarity in structure and amino acid sequence, especially that of the

active site, between them. The structural homology within the catalytic sites of these two has been shown to be 35-44 %[58]. Though this is quite a low homology, but there is very high sequence similarity in the active sites of the enzymes. Figure 3 shows the overlapped structures of the catalytic domains near the metal atom of TACE and MMP-2. It can be seen that the core structures of these two enzymes are very similar, the only variation being in the peripheral loop.

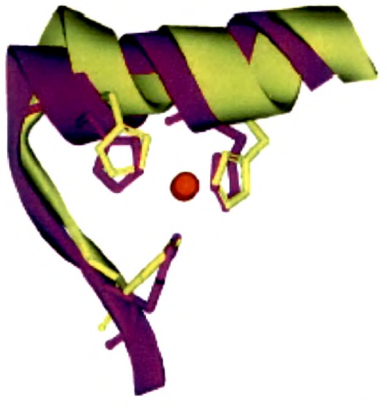


Fig 3. Catalytic zinc atom of TACE (pink) and MMP-2 (gold) and the proximal environment[58]

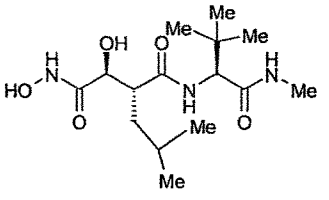
It is believed that MMPs and TACE assume parallel roles in RA[59]. Certain MMPs found in excess in arthritic joints have been implicated in RA[60]. The list of MMPs, believed to play a major role in RA, is given in Table 1.

Table 1. List of MMPs implicated in RA[61]

MMP Group	Group Name	Common Nomenclature
Collagenases		
MMP-1		Fibroblast collagenase
MMP-8		Neutrophil collagenase
MMP-13		Collagenase-3
Stromelysins		
MMP-3		Stromelysin-1
MMP-10		Stromelysin-2
MMP-11		Stromelysin-3
MMP-7		Matrilysin
Gelatinases		
MMP-2		Gelatinase-A
MMP-9		Gelatinase-B

1.11 TACE and MMP Inhibitors

As the catalytic sites of TACE and MMPs are identical and both of them are zinc endopeptidases, some previously identified MMP inhibitors have been found to inhibit TACE as well, e.g. marimastat (1I), prinomastat (2I), and CGS 27023A (3I). But they failed in clinical trials as they showed dose-limiting musculoskeletal side effects[62-64].



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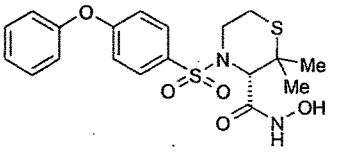

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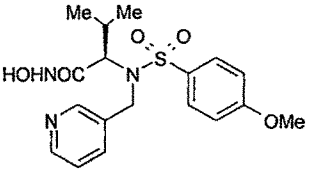


(3I)

Though the exact reason for this side effect is unknown, according to some researchers the efficacy of these molecules is due to their ability to inhibit TACE and their ability to inhibit MMP-1 and/or MMP-14 is the cause for their toxicity[65-70].

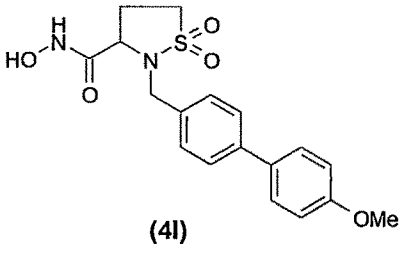
Hence, according to some researchers, it is desirable to develop selective TACE inhibitors devoid of any MMP activity[41]. Some researchers argue that compounds which are active against TACE as well as MMPs may be more effective than a selective TACE inhibitor only, as MMPs are also over-expressed in RA. Various research groups are engaged in developing a dual TACE and MMP-13 inhibitor which does not inhibit MMP-1[71,72]. On the other hand, TACE and MMPs are involved in various normal physiological processes, hence selective inhibitors might present fewer side effects. The optimal MMP selectivity profile for a TACE inhibitor in the treatment of RA is still unknown[73,74].

Because of the structural similarities between TACE and MMPs, identification of selective TACE inhibitors proved elusive until recently[75]. The differences in the shape and size of the S1' pocket of TACE and MMPs might be exploited to design selective TACE inhibitors devoid of any MMP activity[65].

Duan *et al* reported a selective inhibitor of TACE having 200 to 2000 fold higher selectivity for TACE over most of the MMPs. They achieved this level of selectivity based on the fact that most of the MMPs have a shallow and straight S1' pocket, whereas the S1' pocket of TACE is "L" shaped and deep[65,75]. Cherney *et al* transformed novel sultamhydroxamates with potent MMP activity into potent specific TACE inhibitors

devoid of MMP activity by incorporating into the molecule 2-methyl-4-quinolinylmethoxyphenyl group, which interacts with the S1' site of the enzyme[76].

Researchers at Bristol-Myers-Squibb reported a sulphonamide hydroxamate (**4I**) as MMP inhibitor which lacked TACE activity. This compound has Ki value of 3.8, 46.7 and 55 nM for MMP-2, 9 and 13 respectively, while the IC_{50} value for TACE is >1000 nM[77]. To convert a non-selective MMP inhibitor into a selective TACE inhibitor, the



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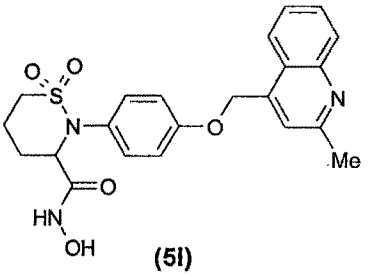
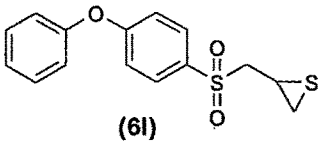


(5I)

same group of scientists replaced the 4-methoxyphenyl group of **4I** with a quinolinyl moiety. It was argued that the quinolinyl moiety would fit into the larger S1' pocket of TACE while it would clash with the smaller S1' poicket of MMPs. Introduction of the quinolinyl group and small changes in the structure of the molecule provided compound (**5I**), which has an IC_{50} value of 3.7 nM for TACE and the compound is more than 1000 fold more selective for TACE over MMP-1, 2, 9 and 13[76].

This difference in the shape and size of the S1' pocket was exploited by Levin to develop selective TACE inhibitors. Incorporation of butynyloxy group at the P1' position of the molecule instead of methoxy group converted various dual (TACE as well as MMP) inhibitors into selective TACE inhibitors[78].

Solomon *et al* found that the molecule SB-3CT (**6I**), binds differently to MMP-2 and TACE. Although there exists high three-dimensional structural similarity among the active sites of TACE and MMPs, the TACE conformational structure around the catalytic zinc ion and the total effective charge of this metal ion is very different from MMPs. They also discovered that active site of TACE is more polar than MMPs. Hence,



(6I)

they argued that the differences in electronic, structural and kinetic behavior observed between TACE and MMPs, might allow designing of specific inhibitors of TACE[58].

Lukacova *et al* extensively compared the binding sites of TACE and MMPs. According to them, specificity for TACE in comparison to MMPs can be achieved by placing a negatively charged ligand at the bottom of S2 and at the entrance of S1' sub-sites[59].

Zhao *et al* analyzed and compared the properties of catalytic domain of TACE, MMP-1, MMP-2, MMP-3 and MMP-9. They reported that the conformations and molecular surface hydrophobicity of all of them are very similar, although there are substantial differences in electrostatic potentials of catalytic domain[79].

1.12 TACE Inhibitors – Concerns and Clarifications

Some researchers doubt the role of TACE inhibitors in RA and are of the opinion that TACE inhibitors would not help in curing RA[80]. Following are some of their concerns:

1. Not only the soluble form, but the membrane bound pro-TNF-α is also biologically active and shows similar biological activity. Upon inhibition of TACE there would be increase in the level of pro-TNF-α[81].
2. TACE sheds TNF-R2. Hence, a TACE inhibitor would lead to increase in the number of TNF-R2, which stores active TNF-α[82].
3. TNF-α is not only processed by TACE but some MMPs are also capable of producing active TNF-α from pro-TNF-α[83].

It was proved experimentally that upon TACE inhibition there was a transient increase in the levels of pro-TNF-α, more than 85 % of which was rapidly degraded (half life 4-6 hours) by the cells[41,84]. It is also known that apart from TNF-α, there are a wide variety of sheddases that sheds TNF-R2. It has also been proved experimentally that in the presence of TACE inhibitors, TNF-R2 expression is not increased. This means that a cell has its own mechanism to degrade TNF-R2[41].

Although there are some MMPs that cleave pro-TNF-α, the specificity constants for TNF-α cleavage by the MMPs are approximately 100-1000 times slower relative to TACE. MMP-1 cleaves not only Ala76-Val77 but also Ala74-Gln75; whereas MMP-9 cleaves solely Ala74-Gln75[85]. Hence, it has been proved that TACE plays the central role in the conversion of pro-TNF-α.

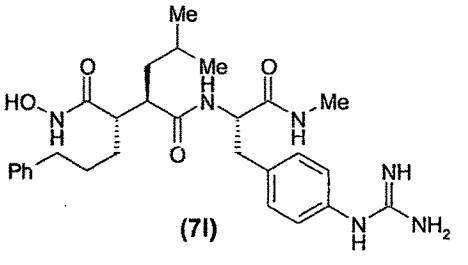
Hence, it has been proved that the ultimate result of inhibition of TACE would be anti-inflammatory. This is illustrated beyond doubt in a mouse model of collagen induced arthritis[41].

1.13 Selective/Dual TACE Inhibitors

1.13.1 Succinate-Based Dual TACE-MMP Inhibitors

As previously stated, the early MMP inhibitors were all TACE inhibitors like marimastat (1I), prinomastat (2I), CGS 27023A (3I) etc. All of them showed good activity against TACE also. The IC_{50} values of marimastat and prinomastat against TACE were found to be 3.8 nM and 22 nM, respectively[86]. They are used as the lead molecules in the designing of newer TACE inhibitors.

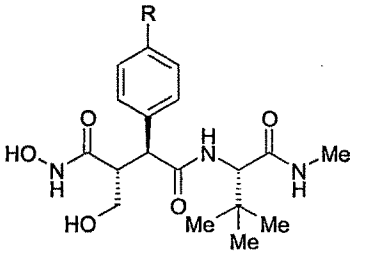
Compound (7I), developed by Daiichi Fine Chemical Co. has a phenyl guanidine group to enhance its water solubility. Its IC_{50} value for inhibition of TNF- α synthesis in



(7I)

LPS stimulated THP1 cells is 130 nM. This compound is also an inhibitor of MMP-1 (IC_{50} = 6 nM) and MMP-13 (IC_{50} = 0.1 nM)[87].

PKF-242-484 (8I) and PKF-241-466 (9I) developed by Novartis are broad spectrum TACE inhibitors. In human peripheral blood mononuclear cells the IC_{50} values

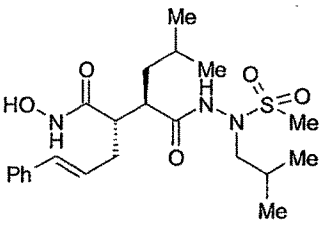


(8I) PKF-242-484: R = OMe

(9I) PKF-241-466: R = Me

of PKF-242-484 (8I) and PKF-241-466 (9I) for TACE are 56 and 141 nM, respectively. They are also potent inhibitors of MMP-1 and MMP-2. These compounds were shown to be effective in various models of lung inflammation[88].

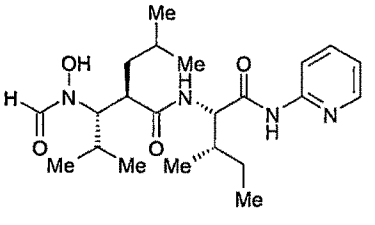
Ro-32-7315 (10I) has around 100-fold higher selectivity for TACE over most of



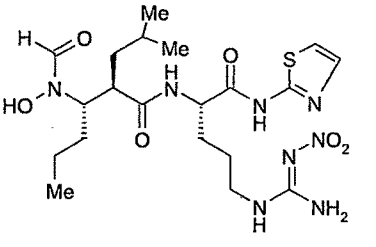
(10I) Ro-32-7315

the MMPs. Structurally, it has the same succinate hydroxamic acid motif of MMP inhibitors with P2' amino acid replaced by a hydrazide group. It has shown excellent potency against TACE in *in vitro* model (IC_{50} = 3 nM); but its oral bioavailability was very poor. Hence, its further development was stopped[89].

N-Hydroxyformamide analogs of succinate hydroxamates have also afforded potent inhibitors of TACE, like GW-3333 (11I) and GW-4459 (12I). They have shown IC_{50} values of 40 and 4.3 nM in cell free TACE. Both the compounds are broad spectrum



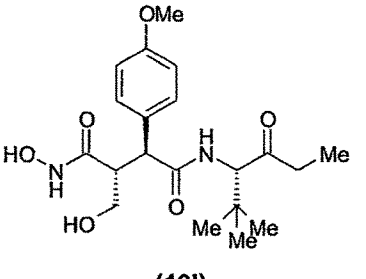
(11I) GW3333



(12I) GW-4459

TACE/MMP inhibitors. GW-3333 was shown to be exceptionally potent (EC_{50} = 1 mg/Kg, po) and long lasting ($T_{1/2}$ > 12 h) in rat LPS model. The only problem of this compound was its poor oral bioavailability. Hence, an elaborate series based on this compound was developed and studied by scientists at Glaxo Smith Kline Inc to obtain a molecule with same or increased potency which was orally bioavailable. But none of the molecules of this series showed good oral absorption[90,91].

Kottrisch *et al* have synthesized some β-arylsuccinic acid hydroxamates as dual TACE and MMP inhibitors. The best compound (13I) of the series shows inhibition of



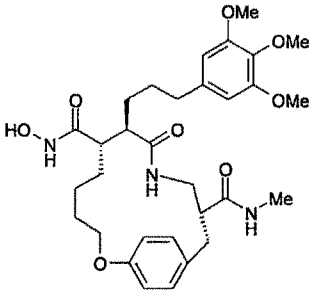
(13I)

TACE, MMP-1, MMP-2, MMP-3 at nano-molar level. Oral administration of 13I to rats inhibits LPS-induced plasma TNF levels with an ED_{50} of 1 mg/Kg[92].

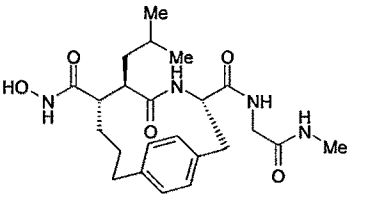
1.13.2 Macrocyclic Derivatives

In this type of compounds the P1' and P2' groups of succinate TACE inhibitors are joined together to form a cycle. The reason for the synthesis of the cyclic system was to reduce the potency of the compounds towards MMP-1[93].

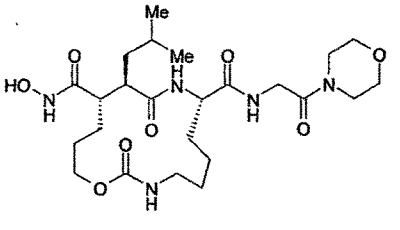
The trimethoxyphenyl group has been used as P1' moiety in the construction of macrocyclic hydoxamate TACE inhibitor (**14I**). Glycine N-methylamide analog SL-422 (**15I**) is a 0.22 μM inhibitor of TNF-α in human whole blood. It was observed that longer P1' group would reduce the potency of the compound. However, this compound is a broad spectrum inhibitor of TACE[94]. Dramatic improvement in potency was seen in the macrocyclic carbamate compound, SP057 (**16I**). The terminal glycine residue of (**15I**) is extremely important for the *in vivo* activity of the compound as its deletion from the compound reduces the oral bioavailability. Compounds (**15I**) and (**16I**) both have short half-lives and moderate bioavailability in dogs. When P1' group i.e. isopropyl group of (**16I**) is replaced with biphenyl moiety, the resulting compound (**17I**) has the same TACE inhibitory potency, but interestingly this change increases its selectivity for TACE. Compound (**17I**) was more than 100 fold more selective towards TACE over most of the MMPs[94].



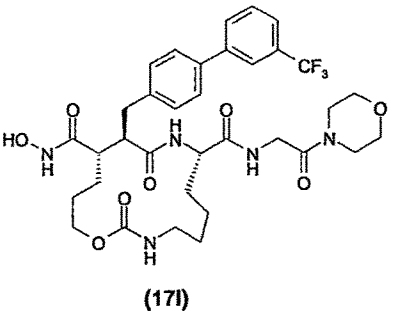
(**14I**)



(**15I**) **SL-422**



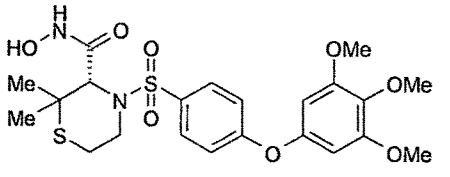
(**16I**) **SP-057**



(**17I**)

1.13.3 Sulphonamide Inhibitors

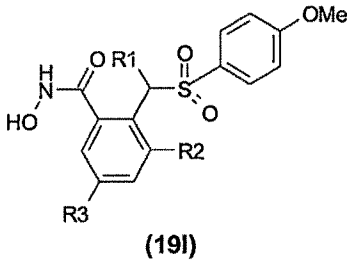
Some sulphonamide hydroxamate-bearing novel P1' groups are also reported as TACE inhibitors. Compound (**18I**) having a trimethoxyphenyl group as P1'



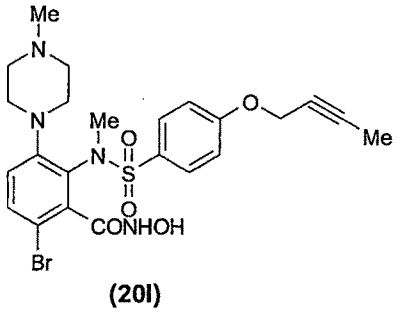
(18I)

moiety is a potent TACE inhibitor. Its IC_{50} value is reported to be 550 nM in THP-1 cells[43].

Some aryl hydroxamates were also reported as TACE/MMP inhibitors. The aryl groups were either single or fused. Among these, anthranilate derivatives (**19I**) showed the most promising *in vitro* TACE inhibitory activity[95]. The SAR reveals that presence of halogen at R_3 is extremely important for TACE inhibitory activity. Removal of halogen at



(19I)



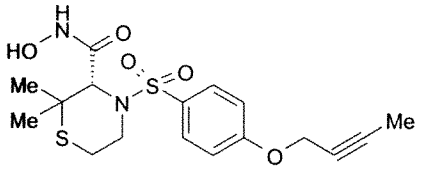
(20I)

5-position of the ring decreases the activity towards TACE dramatically by six times. Replacement of bromo group at this position by methyl group decreases the activity by two times. The most potent compound of the series has bromo group at R_3 and methyl groups both at R_1 and R_2. The IC_{50} values of the best compound of the series against TACE and MMP-13 are 32 and 11 nM; while against MMP-1 it is 114 nM. It has also been observed that although this series of compounds showed very good *in vitro* activity, they were not active in *in vivo* models because of their low oral bioavailability[78,95].

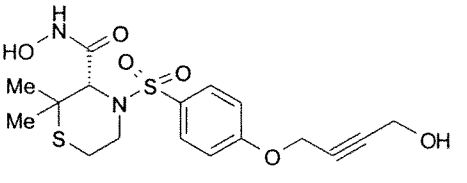
Researchers have sought further improvement in activity, selectivity and pharmacokinetic profile of anthranilate TACE inhibitors. The fact that S1' site of TACE is narrower and longer than that of the MMPs[75], is utilized in the designing of selective

TACE inhibitors. It was postulated that a carbon-carbon triple bond attached to a –O-CH_2- group would fit perfectly with the S1' site of TACE, while it would not allow effective binding with MMPs[78]. Several new molecules were synthesized based on the above theory and screened for their TACE as well as MMP inhibitory activity. The most potent and selective compound (**20I**) has butynyloxy group at P1'. Its IC_{50} value for TACE is equal to 25 nM. It is more than 10 times more selective towards TACE over MMP-13 and almost 500 times more selective over MMP-1[96]. The oral activity of **20I** got improved due to the attachment of basic piperazine moiety at 3-position[97]. Compound (**20I**) has shown 100 % inhibition of TNF-α in mouse (50 mg/Kg) after 1 hour of oral dosing[98].

Thiomorpholine hydroxamates bearing propargylic ether at P1' site were also explored as TACE inhibitors[99,100]. Among the compounds developed, the most promising was TMI-1 (**21I**). This compound is currently in the Phase-II of clinical trials. This is one of the best dual acting compounds discovered so far. Its IC_{50} value for TACE, MMP-1, MMP-2 and MMP-13 are 8.4, 6.6, 4.7 and 3.0 nM respectively. This compound has also shown high activity in various *in vivo* models[74,99,100]. Apratastat, commonly known as TMI-05 (**22I**), developed by this group is a broad spectrum TACE inhibitor. It is also a



(21I) TMI-1

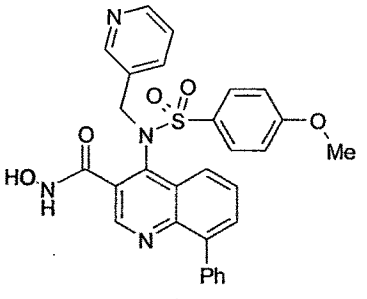


(22I) Apratastat, TMI-05

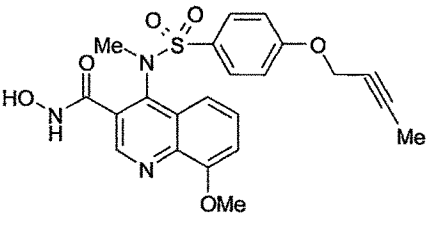
thiomorpholine derivative, like TMI-1 (**21I**). The P1' group is slightly different from that of TMI-1 (**21I**). In the structure of the compound free hydroxyl group is present. The compound has excellent *in vitro* activity against TACE. Its IC_{50} value is 0.44 μM. It also showed *in vivo* activity in mouse collagen induced arthritis model[101]. Though the compound (**22I**) did not show any side effect in Phase I clinical trials, it was withdrawn from the Phase II of clinical trials due to lack of required level of efficacy[102].

All quinoline hydroxamates are moderately active TACE inhibitors. In fact, they are selective inhibitors of MMP-13 over MMP-1 and TACE. The most potent quinoline derivative (**23I**) has IC_{50} value of 120 nM towards TACE while its IC_{50} value for MMP-13

is only 4 nM[103]. The concept of attaching butynyloxy group at P1' site to make the molecule selective TACE inhibitor was extended to the quinoline moiety as well. It was found that compound (24I) was more than 7 times more potent towards TACE. It has



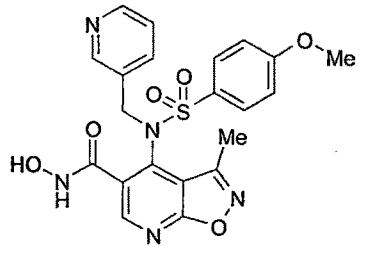
(23I)



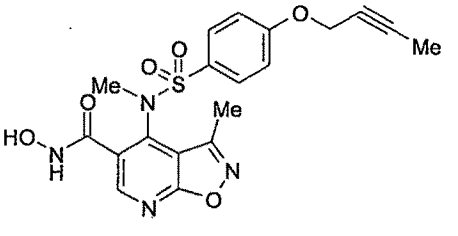
(24I)

IC_{50} value of 17 nM towards TACE and 50 times higher selectivity over MMP-1[103].

Some heteroaryl-fused pyridinehydroxamates were also developed as moderately active TACE inhibitors. The *in vitro* studies revealed that actually this class of compounds selectively inhibited MMP-13 over TACE. Compound (25I) is 15 times more potent towards MMP-13 over TACE (IC_{50} values for MMP-13 and TACE are 10 and 147 nM, respectively)[103]. Some heteroaryl-fused pyridine derivatives were synthesized with



(25I)

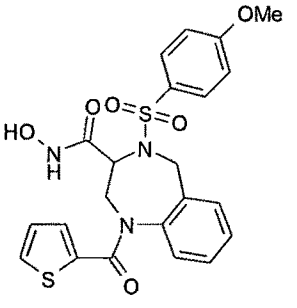


(26I)

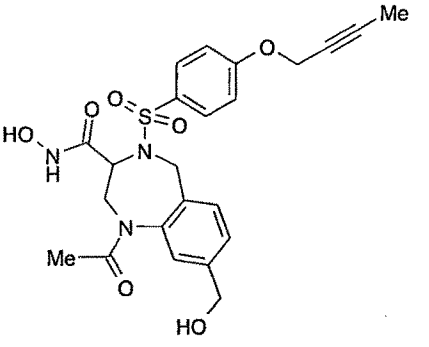
butynyloxy group at P1'. The best compound (26I) of the series shows IC_{50} value equal to 6 nM and about 10 times more potency towards TACE over MMP-13. It inhibited 95 % production of TNF-α in mouse model at a dose of 100 mg/Kg after 1 hour of oral dosing[98].

Some analogs of benzodiazepines were synthesized as dual TACE and MMP inhibitors. The compound having thiophine nucleus at N1 of benzodiazepine moiety (27I) showed most potent *in vitro* activity. The compound is a broad spectrum inhibitor; it inhibited MMP-1, MMP-2, MMP-9, MMP-13 and TACE in nanomolar range[104]. Incorporation of butynyloxy group at P1' of the benzodiazepine analogs did not afford

selective TACE inhibitors. The best compound (28I) in the series has IC_{50} value of 12 nM



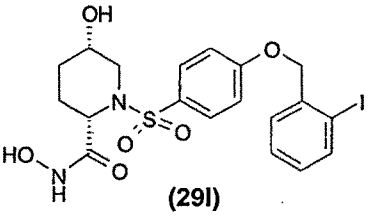
(27I)



(28I)

for TACE and 20 nM for MMP-13. This compound was found to be 40 times more selective towards TACE over MMP-1[105].

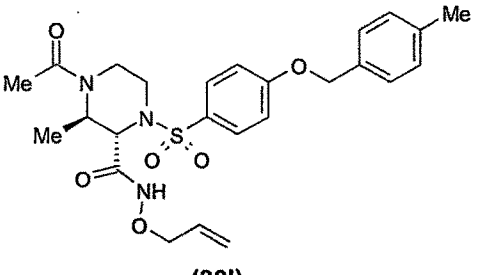
It was also reported that substituted benzylic ether as P1' group can also produce nano-molar range TACE inhibitors. One of these active compounds is (29I), which has



(29I)

IC_{50} value equal to 110 μM[106]. The 2-position of the terminal phenyl group of 5-hydroxypipecolic acid has to be substituted with alkyl or halo group for the TACE inhibitory activity.

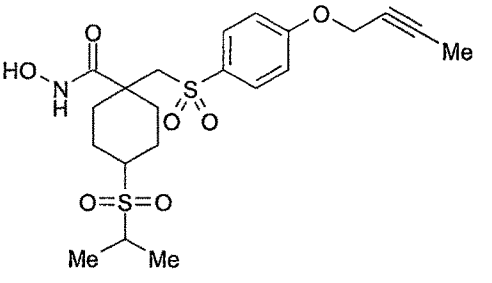
Letavic *et al* have developed a piperazine-based dual inhibitor of TACE and MMP-13 which possessed minimal MMP-1 activity. The best compound (30I) of the series has IC_{50} values of 1, 3 and 1600 against TACE, MMP-13 and MMP-1 respectively.



(30I)

Although this compound has shown moderate *in vivo* activity (ED_{50} = 17 mg/kg), may be due to short half-life of the compound in rat, search for a better compound in terms of activity both *in vivo* and *in vitro* still continues[107].

Researchers at Wyeth Research Lab reported some β-sulphonepiperidine hydroxamates, bearing butynyloxy group at P1' site, as TACE inhibitors. The compounds were not only excellent TACE inhibitors, but also showed selectivity over MMPs 1, 2, 9 and 13. The best compound (31I) has IC_{50} value equal to 1.5 nM against

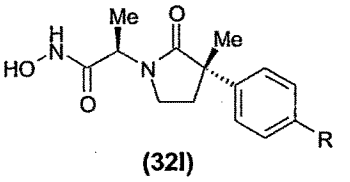


(31I)

TACE. This compound showed 150 times higher selectivity towards TACE over MMP-13 and 230 times over MMP-2[108-110].

1.13.4 γ-Lactam-based Inhibitors

Scientists at Bristol-Myers-Squibb have designed and developed γ-lactam hydroxamates (32I) as selective TACE inhibitors. Docking studies of this series of compounds have revealed that hydroxamate group binds with zinc atom present in the active site of TACE as a bidentate ligand. The aromatic moiety of the molecule occupies the S1' site of the enzyme. The oxygen atom of the pyrrolidinone ring forms hydrogen bond with Val163 and Leu164. The methyl group near the hydoxamate group interacts with a small hydrophobic pocket of the enzyme, known as S2' site.

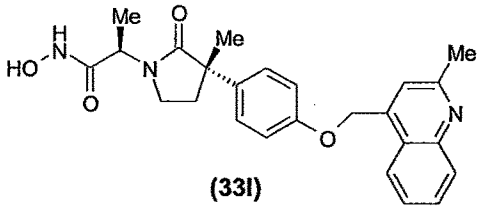


(32I)

As the S1' site of TACE is larger than most of the MMPs, it has been seen that more the bulk of the P1' group the higher is the potency as well as selectivity of the molecules towards TACE over MMPs. Hence, the molecule possessing isobutyl group at R is 250 times less potent than the compound having benzyloxy group (IC_{50} values are 1000 nM and 4 nM, respectively). It has also been observed that an ether linkage at this position should be present in the molecule. The molecule having biphenyl moiety at R has IC_{50} value of 13000 nM, while it is 185 nm for the molecule having phenoxy group at this position. Mono-substitution of the phenyl group did not improve the selectivity or

potency of the compounds towards TACE. But the effect of di-substitution was dramatic. The compound possessing 3,5-bistrifluromethylbenzyloxy group at R showed IC_{50} value of 2 nM. This compound is found to be more than 1000 times more selective for TACE over MMP-1, MMP-2 and MMP-9.

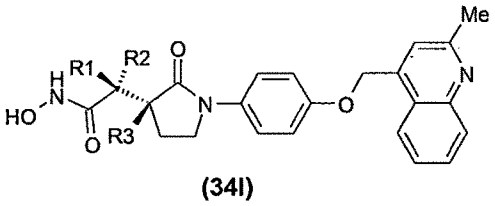
Although, these compounds were highly active *in vitro*, they were found to possess low to moderate activity in human whole blood assay. The reason for the low activity was high protein binding. Some of the compounds were found to be so highly protein bound that less than 1 % of them were available in free fraction (e.g. **32I**; R = 3,5-dimethoxybenzyloxy). To increase the free fraction, several polar bioisosteres of benzyloxy group were tried. Several pyridyl and quinolinyl derivatives were synthesized and their activity and selectivity profiles were checked. It was observed that the compound IK-682, (**33I**) possessing (2-methylquinolin-4-yl)methoxy as R is the molecule of choice[75]. *In vitro* IC_{50} value of IK-682 (**33I**) is equal to 1 nM and in whole



(33I)

blood assay it is 0.35 μM. It is at least 1000 times more selective towards TACE over most of the MMPs. The free fraction of **33I** in human serum is relatively high (3.6 %). The compound was found to be 32 % bioavailable in dog and 41 % in rat[75].

To broaden the TACE inhibitory portfolio, the researchers at Bristol-Myers-Squibb have designed and synthesized N-hydroxy-2-(2-oxo-3-pyrrolidinyl)acetamide scaffold (**34I**) as selective TACE inhibitor. As the (2-methyl-4-quinolinyl)methoxy P1'

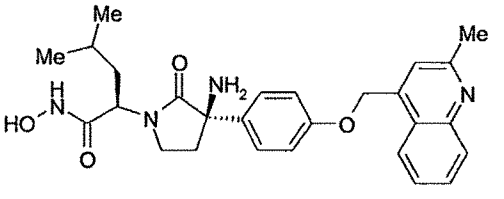


(34I)

group has already been optimized for potency and selectivity, this group was kept constant. It has been observed that any other group than hydrogen at R2 clashes with the backbone of the protein; whereas R1 group is projected towards the solvent and hence is expected to tolerate variations. The compound having methyl group at R3 and hydrogen atoms at R1 and R2, is an excellent inhibitor of TACE (IC_{50} value is equal to 1 nM). But, its IC_{50} value

in whole blood assay is poor (IC_{50} = 1.57 μM). The compound possessing Boc protected amino group at R1 and hydrogen atoms at R2 and R3 shows activity in sub-micromolar level in whole blood assay (IC_{50} = 0.42 nM). The compound is 700 fold more selective for TACE over MMP-1 and about 300 fold more selective over MMP-2 and MMP-9[111].

Another excellent selective TACE inhibitor (**35I**), known as DPC-333 or BMS-561392, has been developed at Bristol-Myer-Squibb. *In vitro* IC_{50} value of the compound (**35I**) is 0.20 nM and in whole blood assay it is 90 nM. It is more than 100 fold more selective against TACE over MMPs. After oral administration to mice, the compound

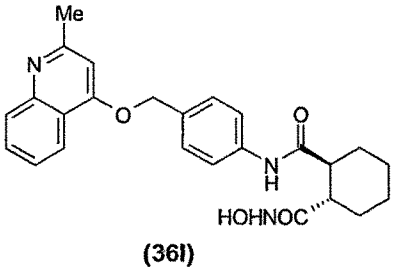


(35I) BMS-561392, DPC-333

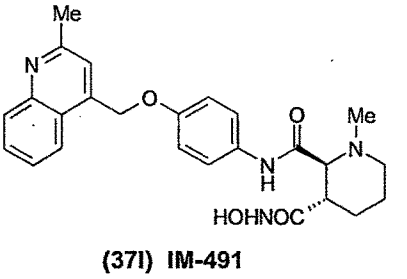
inhibited soluble TNF production following LPS challenge with an ED_{50} value of 6 mg/kg. This compound has shown good (54 %) bioavailability in dogs and reasonable bioavailability in rats (16 %). In Phase I of clinical studies, it was observed that the compound was well tolerated among healthy human volunteers at a dose range of 15 to 530 mg. It was also noted that the half life of the molecule in humans is 3 – 6 hours[112-114].

1.13.5 Succinate Derivatives as Selective TACE Inhibitors

Researchers at Bristol-Myers-Squibb prepared some succinate hydroxamates as selective TACE inhibitors. They identified that compound (**36I**) is a nono-molar range inhibitor of TACE *in vitro* with excellent selectivity (IC_{50} = 8 nM; almost 5000 fold more



(36I)



(37I) IM-491

selective over MMPs). But, the compound offered very poor whole blood assay (IC_{50} > 50 μM) indicating that the compound would be inactive *in vivo*[115].

Next aim of the scientists at Bristol-Myers-Squibb was to improve the *in vivo* activity of the compound (**36I**). After suitable modification of the P1' cyclohexyl ring, compound (**37I**) was obtained. Although its *in vitro* TACE inhibitory activity (IC_{50} = 6.2

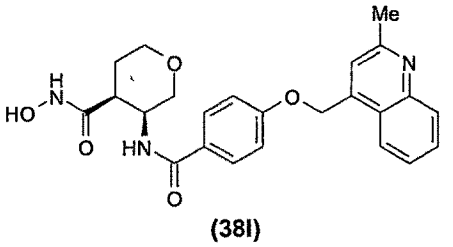
nM) was in the same range as that of the lead compound (36I), the *in vivo* (whole blood assay IC_{50} = 0.020 μM) activity increased many fold. The compound (37I), also known as IM-491, has around 2000 fold higher selectivity for TACE over most of the MMPs[116].

Although the compound IM-491 (37I) has shown very promising *in vitro* and *in vivo* activities and very good bioavailability, it was unfortunately found to be unsuitable for development in the pre-clinical trials because of the mutagenic property of 4-(2-methylquinolin-4-ylmethoxy)aniline moiety[117].

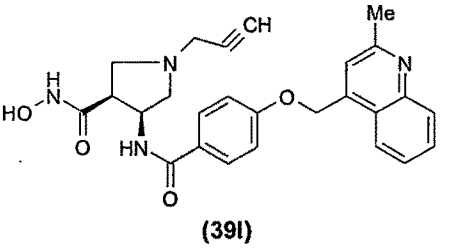
1.13.6 β-Benzamidohydroxamates as TACE Inhibitors

To overcome the toxicity of IM-491 (37I), scientists at Bristol-Myers-Squibb examined the possibility of reversing the central amide moiety to obtain a series of β-benzamidohydroxamates. This could serve two purposes; it could eliminate the toxicity problems of 4-(2-methylquinolin-4-ylmethoxy)aniline moiety present in IM-491 (37I) and the new series of compounds could have the same binding interactions with TACE as that of IM-491 (37I).

After synthesizing and evaluating the biological activities of various derivatives of β-benzamidohydroxamates, it was found that compound (38I) was a very potent and selective TACE inhibitor. The *in vitro* IC_{50} value of the compound (38I) is less than 1 nM; the IC_{50} value of whole blood assay is 130 nM. The compound was found to be more than 30,000 fold more selective over MMP-1, 2, 13, 14, 15 and 16. This compound has shown much better bioavailability in rats (58 %) and has shown oral ED_{50} value equal to 3.0 mg/kg in rats[117]. In search for a more potent and selective TACE inhibitor, Ott *et al*



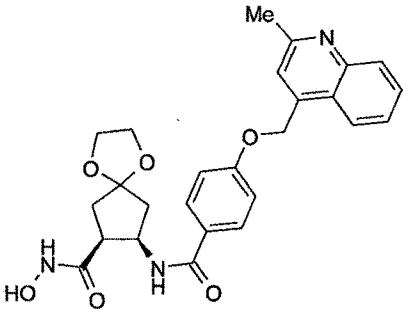
(38I)



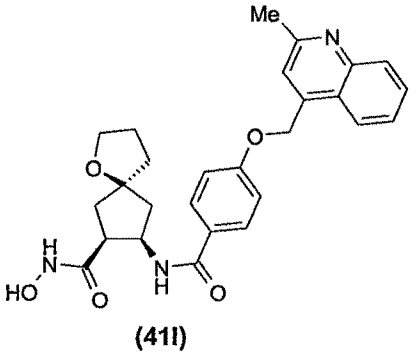
(39I)

evaluated various 5-membered carbocyles and heterocycles in place of tetrahydropyran ring of 38I. The best compound (39I) of the series has *in vitro* IC_{50} value equal to 0.14 nM and whole blood assay IC_{50} value equal to 109 nM. The compound is 1,000 fold more selective for TACE over MMP-1, 2, 7, 8, 9, 10, 13, 14 and 15. Although the oral bioavailability of the compound in rat is a modest 25 %, the ED_{50} value is 3.3 mg/kg[118].

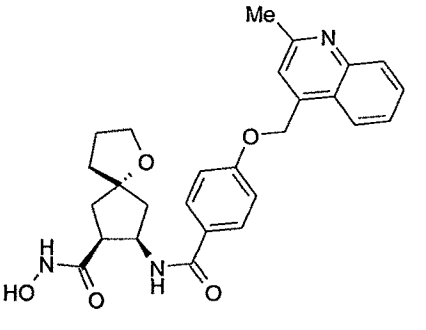
In the quest for a better molecule, scientists at Bristol-Myers-Squibb synthesized and biologically evaluated the 1,3-dioxalane moiety at 4-position of β-benzamidohydroxamic acid scaffold to arrive at compound (40I). This change has decreased the IC_{50} value in whole blood assay dramatically to 24 nM. But the molecule was not chosen for development because of its acid sensitive nature[119]. Hence, one oxygen atom of the dioxalane species was changed to carbon and compounds (41I) and (42I) were synthesized and screened for their TACE inhibitory activities. It was observed that both the compounds (41I) and (42I) have comparable activities; the IC_{50} value of 41I in whole blood assay is 108 nM and that of 42I is 143 nM. Both the compounds are more than 2,000 fold more selective for TACE over MMP-1, 2 and 9. The oral bioavailabilities for (41I) and (42I) are 31 and 35 %, respectively in dogs[119].



(40I)



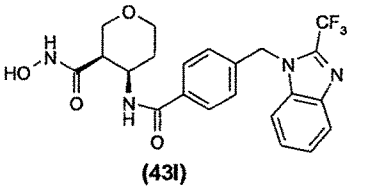
(41I)



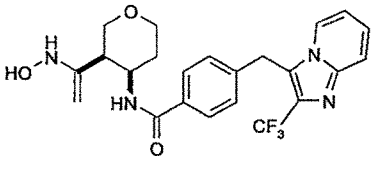
(42I)

To obtain a more potent and selective TACE inhibitor, researchers at Bristol-Myers-Squibb changed the 4-(2-methylquinolinylmethoxy)phenyl P1' group to 2-substituted benzimidazolemethylphenyl group in β-benzamidohydroxamic acid scaffold. After the biological evaluation of a number of synthesized compounds, it was observed that compound (43I) showed IC_{50} value of 1.4 nM. This compound is found to have more than 10,000 fold higher selectivity for TACE over MMP-1, 2, 3, 7, 8, 9, 10, 13, 14,

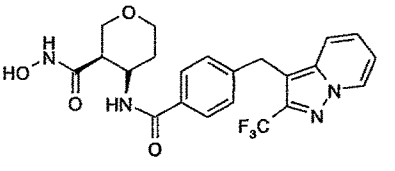
15 and 16. Also, it is 8,000 fold more selective for TACE over MMP-12. The compound (43I) has half-life of about 5 hours and oral bioavailability of 99 % in dogs[120].



(43I)



(44I)

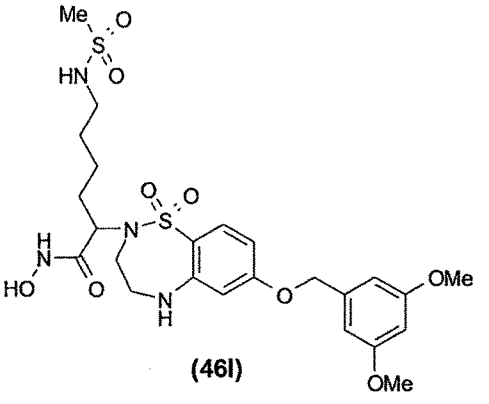


(45I)

As it is known that P1' group of TACE inhibitors, occupying the S1' site of the enzyme, is the most critical determinant for TACE selectivity, researchers at Bristol-Myers-Squibb wanted to optimize the P1' group in β-benzamido hydroxamic acids. Compounds possessing indole and benzofuran rings as P1' group did not show good selectivity over MMP-12 (only 75 fold). The trifluromethyl analog 44I of imidazopyridine P1' group showed good selectivity over most of the MMPs, but this compound (44I) was found to be inactive *in vivo* because of its poor oral absorption. Interestingly, the trifluromethyl analog (45I) of pyrazolopyridine P1' group showed not only very good selectivity over all MMPs but also its whole blood assay IC_{50} value was found to be equal to 171 nM. The compound (45I) showed oral bioavailability of more than 90 % in rats[121].

1.13.7 Benzothiadiazepine Derivatives

Scientists at Bristol-Myers-Squibb also designed and developed some benzothiadiazepine derivatives as selective TACE inhibitors. Most of the compounds of this class not only showed very good *in vitro* inhibition of TACE but were also selective over MMP-1, 2 and 9. The most potent compound (46I) of the series showed Ki value of 5 nM and over 75 fold higher selectivity for TACE over MMPs. Most of the compounds of this series were inactive in whole blood assay indicating that the compounds might not be active *in vivo* [122].



(46l)

Molecular modeling studies of this series of TACE inhibitors reveal the significance of hydrophobic and hydrogen bond donor fields apart from electrostatic field and steric contributions for the TACE inhibitory activities in the structures of these compounds. It was also predicted that an aliphatic substitution (n-butyl or n-pentyl) at 4-position of 3, 5-dimethoxyphenyl ring could improve the TACE inhibitory activity[123].

1.13.8 Non-hydroxamate TACE Inhibitors

It is a well known fact that hydroxamic acids are the most potent motif for zinc binding. It is postulated that the oxygen of the carbonyl group and the hydroxyl group both share their lone pairs with the zinc atom in the enzyme and thus forms a five membered stable cyclic system. At the same time the hydrogen atom of the hydroxyl group forms hydrogen bond with the oxygen atom of Glu406 of TACE [124]. As hydroxamic acid

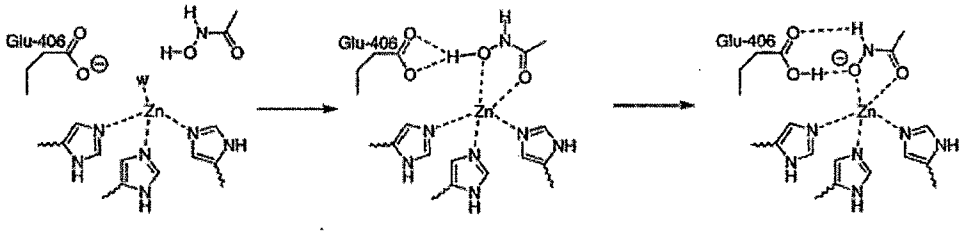
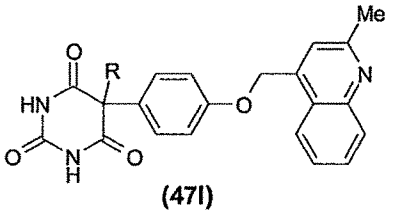


Fig 4.Interaction of hydroxamic acid and zinc atom present in catalytic site of TACE

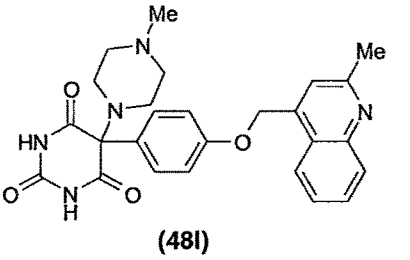
is excellent zinc binding motif, most of the TACE and MMP inhibitors, reported in the literature, rely on hydroxamate or reverse hydroxamate moieties as the zinc binding ligands. But there are many problems with compounds possessing hydroxamic acid group. Often, the bioavailabilities of these molecules are very low because of very high renal clearance. They also carry potential metabolic liabilities. Hydroxamic acids undergo O-glucuronidation and hydsrolysis *in vivo* to give corresponding acid and toxic

hydroxylamine[125]. Hence, the researchers all over the world are interested to develop non-hydroxamate TACE inhibitors[45].

Bristol-Myers-Squibb group replaced the hydroxamate moiety with 5-phenylpyrimidine-2,4,6-trione. As 4-(2-methylquinolin-4-ylmethoxy)phenyl moiety was previously designed for selective TACE inhibitory activity, this group was kept intact in the molecule. The compound (47I) possessing methyl group at R showed IC_{50} value of



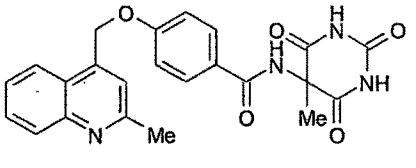
(47I)



(48I)

1.0 μM indicating that the pyrimidinetrione is an effective zinc binding motif. To further increase the potency of the molecule, different aliphatic and aromatic moieties were introduced in the molecule. It was found that introduction of the piperazine group at R increases the potency of the compound. The compound (48I) showed inhibition of TACE (IC_{50} = 0.091 μM)[92] at sub-micromolar level.

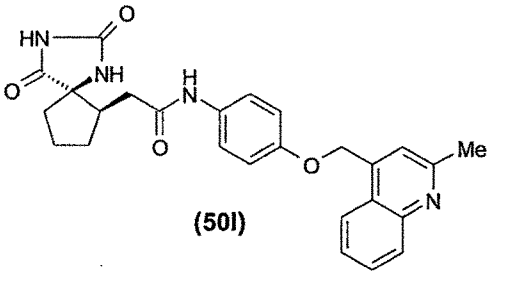
To further increase the potency of the pyrimidinetrione derivatives against TACE, the same group of scientists introduced a spacer in between pyrimidine and benzene rings. Several modifications were tried and the best compound of the series was found to be **49I** which had IC_{50} value equal to 0.026 μM. The compound also showed very good selectivity over most of the MMPs (almost 200 fold). But, the compound was found to be inactive in whole blood assay (IC_{50} >50 μM)[127]. Various other groups were



(49I)

also tried at 5-position of pyrimidine nucleus in order to increase the potency of the molecule in whole blood assay. But, none of the compounds in this series were found to be effective in that particular assay indicating that this series of compounds although very potent *in vitro*, might be inactive *in vivo*[127].

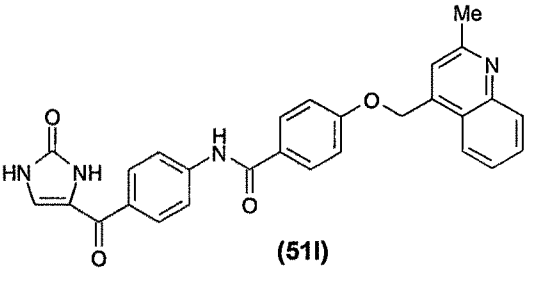
Bristol-Myers-Squibb researchers also examined hydantions as zinc binding ligands. Molecular modeling analysis revealed that although hydantoin moiety is a good



(50I)

zinc binding motif, it is intrinsically less potent than hydroxamic acid[128]. Compound **(50I)** shows IC_{50} value equal to 11 nM against TACE and is found to be more selective for TACE by at least 200 fold over most of the MMPs. It has been observed that the stereochemistry of the hydantoin ring plays a very critical role in the potency of the compound as a wrong stereochemistry would not project the quinolinyl P1' group into the S1' pocket of the enzyme. Hence, the above compound in (5R, 6S)-*trans* form shows IC_{50} value of 11 nM, while in (5S, 6R)-*trans* form, its IC_{50} value becomes 900 nM[129].

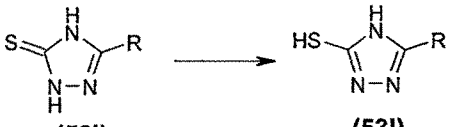
The same group of researchers at Bristol-Myers-Squibb also developed triazolone



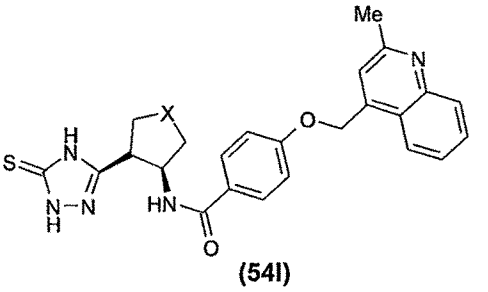
(51I)

and imidazolone ring systems as potential zinc binding groups. The P1' group, namely 4-[(2-methyl-4-quinolinyl)methoxy]benzoyl, selected on the basis of activity and selectivity for hydantoin derivatives was kept as such in these compounds as well. Compounds possessing triazolone and imidazolone rings were found to be active, hence it was argued that these two ring systems could also be regarded as zinc binding ligands. All the compounds synthesized so, showed very good selectivity for TACE over MMPs. The choice of the P1' group proved to be ideal. The most potent compound **(51I)** of the series has IC_{50} value equal to 9 nM. The compound is at least 350 fold more selective for TACE over MMP-2, 3, 7, 12 and 13[130].

The group at Bristol-Myers-Squibb also tested 1,3,4-triazole-2-thione scaffold (**52I**) as non-hydroxamate zinc binding ligand. Molecular modeling studies showed that the thiocarbonyl group interacts with zinc in the active site of the enzyme. One of the NH



(52I) **(53I)**



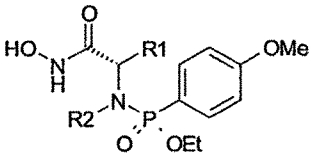
(54I)

groups of thiourea moiety can tautomerize to thiol (**53I**) thus creating a strong hydrogen bond to the active site Glu406[97]. The most potent compound of the series (**54I, X = O**) was reported to have IC_{50} value equal to 1.5 nM and more than 3000 fold higher selectivity for TACE over MMPs[131].

Although molecular modeling studies suggest that triazolethiones have less number of interactions (i.e. two) than hydroxamates[128], they are potent and selective TACE inhibitors. Moreover, triazolethiones appear to have intrinsically higher potency than substituted 5-phenylpyrimidine-2,4,6-triones, hydantoins and triazolones, discussed above[131].

1.13.9 Miscellaneous TACE Inhibitors

Sawa *et al* have designed and synthesized phosphonamide derivatives as TACE inhibitors. It has been shown that the compound (**55I**) possessing isobutyl group at R1

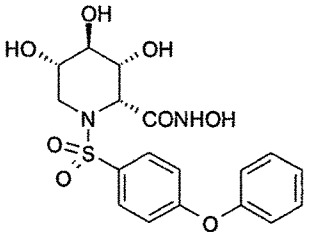


(55I)

and hydrogen at R2 is the most selective TACE inhibitor in the series. The compound also showed promising TACE inhibition. Its IC_{50} value is 76 nM. It has also been

reported that the *S*-configuration at carbon attached to nitrogen favors strong binding of the hydroxamate group with the zinc atom present in the enzyme[132].

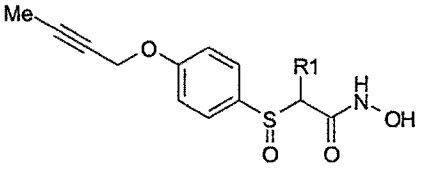
Moriyama *et al* have synthesized some azasugar based TACE and MMP inhibitors. These compounds possess improved water solubility over most of the reported



(56I)

TACE and MMP inhibitors due to the presence of azasugar in the molecule. Hence, they have better bioavailability. It was reported that the stereochemistry of 2, 3, 4, 5 positions of the azasugar scaffold is crucial for the selectivity profile of these compounds. The best compound **(56I)** of the series has Ki value of 2.3 nM and 0.061 nM for TACE and MMP-9, respectively[133,134].

Venkatasen *et al* have synthesized a series of 4-alkynyloxyphenylsulfanyl, sulfinyl and sulfonyl-alkyl hydroxamates **(e.g. 57I)**. Their SAR revealed that the oxidation state of sulfur atom decides their selectivity. The sulfanyl and sulfonyl derivatives are predominantly MMP inhibitors with little or no TACE inhibitory activity while the sulfinyl derivatives are selective TACE inhibitors. The compound possessing n-hexyl

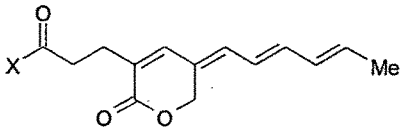


(57I)

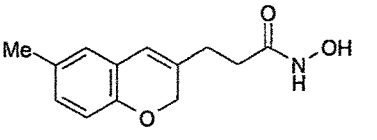
group at R1 is the most potent TACE inhibitor. Its IC_{50} value for TACE is 4 nM. Although this compound showed good *in vitro* activity, its *in vivo* activity was not encouraging. Replacement of 4-piperidine ring at R1 instead of n-hexyl group increases the cellular potency of the compound to a minor degree[73].

Gelastatin **(58I)** and its hydroxamate **(59I)** were prepared from the known methyl ester of gelastatin by Park *et al.* Both the compounds inhibited TACE as well as MMPs. The hydroxamate of gelastatin was more active. Its IC_{50} values for the enzymes

were found to be 0.028, 0.006 and 0.023 μM for TACE, MMP-2 and MMP-9, respectively [135]. The same research group has also developed chromen based TACE inhibitors. They have synthesized these chromen analogs as these compounds showed similar docking pattern as that of gelastatins. The most active compound (**60I**) in the



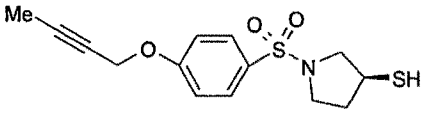
(58I) X = OH
(59I) X = NHOH



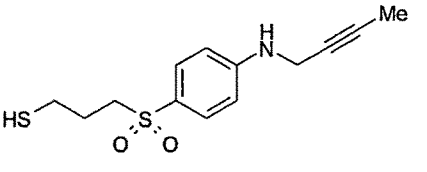
(60I)

series has IC_{50} value of 0.06 μM. This compound was found to be 4 times more selective for TACE over MMP-2 and 50 times more selective over MMP-9[136].

The fact, that the S1' site of TACE is broader than MMPs, has been exploited by many a researchers to design and develop selective TACE inhibitors. Another approach might be to use a non-hydroxamate zinc binding group[137]. Govind Rao *et al* have applied a combination of these two approaches to develop selective TACE inhibitors. The designed



(61I)



(62I)

molecule possesses butynyloxy group, which would interact with the S1' site of TACE. Thiol group is taken as the zinc binding motif. One of the potent compounds (**61I**) of the series has Ki value equal to 28 nM. This compound was shown to possess 200 fold higher selectivity for TACE over MMP-2 and MMP-7 while as much as 40 fold selectivity over MMP-8 and MMP-13[138]. The same group has also developed some thiol containing arylsulphones as selective TACE inhibitors. It has been shown that in this series of compounds, replacement of butynyloxy tail of P1' group with bytynylamino group produces more potent TACE inhibitors. The most potent compound (**62I**) of the series has Ki value of 2 nM[139].