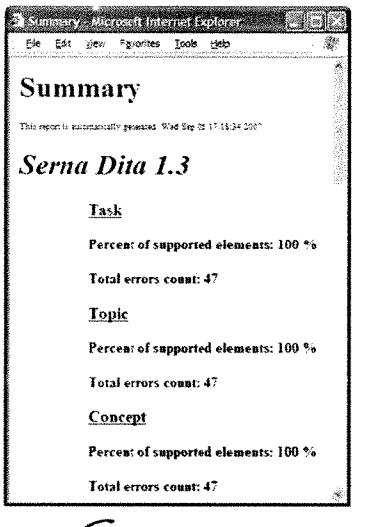
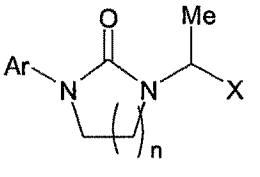

Summary - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Summary
This report is automatically generated Wed Sep 05 17:18:34 2007
Serna Dita 1.3
Task
Percent of supported elements: 100 %
Total errors count: 47
Topic
Percent of supported elements: 100 %
Total errors count: 47
Concept
Percent of supported elements: 100 %
Total errors count: 47

Summary

SUMMARY

Arthritis was once considered to be the disease of Western countries. With growing affluence more and more people are falling prey to this disease in India as well. Though arthritis does not cause morbidity, it severely affects the quality of life of the patient. It is one of the most common autoimmune inflammatory conditions, affecting approximately 1% of the worldwide adult population. It was proved beyond doubt that TNF-α plays a pivotal role in the origin and progression of the disease. One of the ways of excluding TNF-α from the biological system is to block TACE, the enzyme responsible for maturation of inactive form of TNF. So far, most of the clinical interest in TACE has remained concentrated on arthritis, but recently preclinical studies in a variety of tumor model systems have revealed that inhibition of TACE inhibits pathogenic EGFR signaling also in cancer. Hence it was speculated that TACE inhibitors might cure cancer as well.

In this piece of work, novel 2-imidazolidinones and tetrahydropyrimidin-2(1*H*)-ones (I) were synthesized as potential TACE inhibitors. As TACE is a zinc metalloproteinase, zinc chelating groups (namely, hydroxamates, carboxylates and thiols)



(II)

n = 1, 2

X = -CONHOH

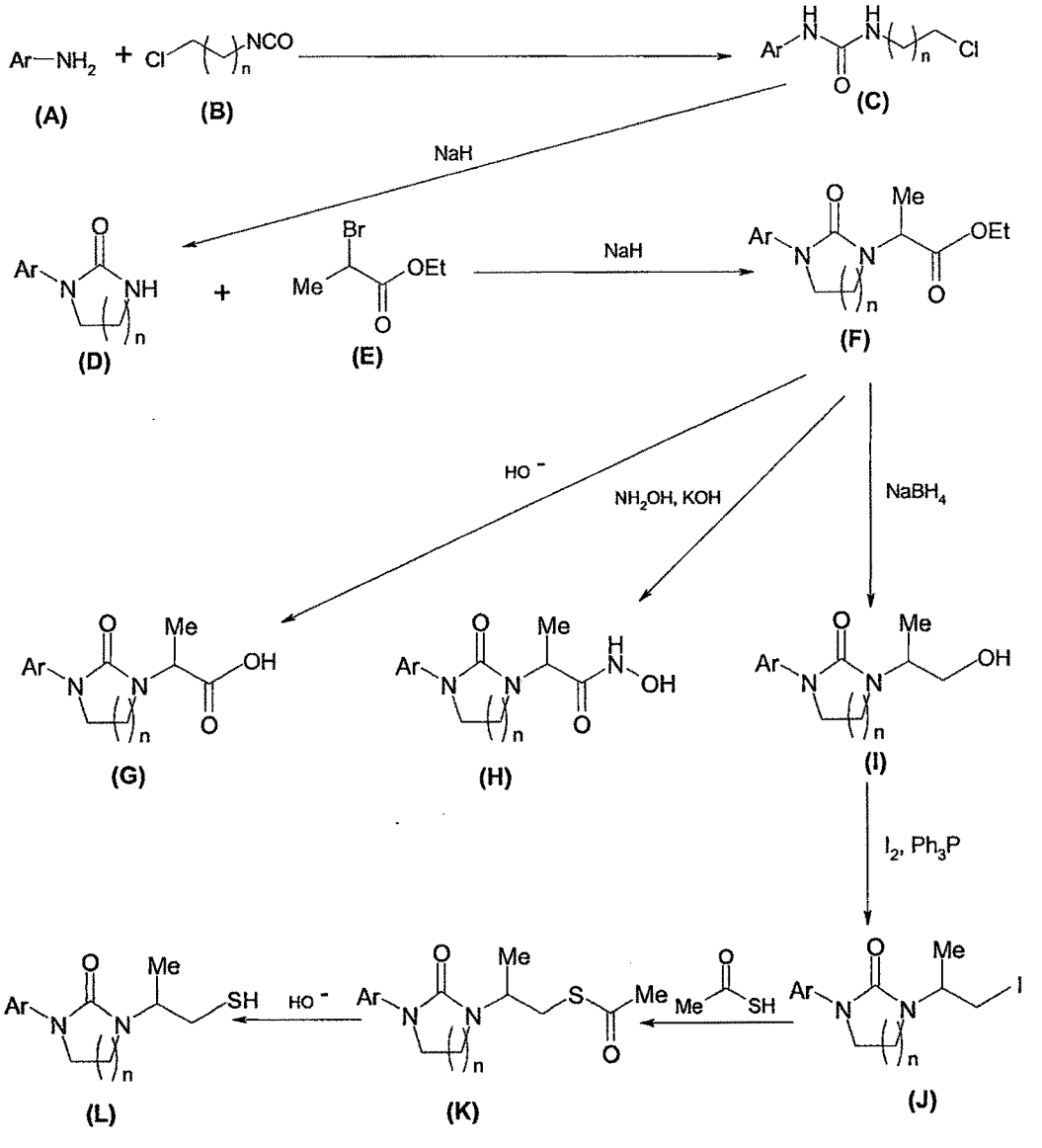
-COOH

$-CH_2SH$

were incorporated in the designed molecules as group X. It is known that S1' site of TACE is larger than that of MMPs, so it was planned to incorporate larger aromatic moieties for the group 'Ar' to increase selectivity for TACE over MMPs.

To synthesize the envisaged compounds, a scheme was planned as given in general Scheme-1, wherein arylamines (A) were reacted with chloroalkyl isocyanates (B) to obtain the expected urea derivatives (C). The urea derivatives were cyclized under strong basic conditions to obtain the desired five/six membered heterocycles (D). The required side chain was attached by reacting the cyclized products (D) with ethyl 2-

bromopropionate (E) to obtain the esters (F). The esters were converted to the three types of zinc binding ligands using three different sequence of reactions. The esters (F) were saponified to the free acids (G) using lithium/sodium hydroxide while hydroxylamine hydrochloride treatment of the esters (F) under basic conditions offered the desired hydroxamates (H). In a separate sequence of reactions the esters (F) were first reduced to alcohols (I) by sodium borohydride treatment, which on further treatment with iodine, triphenyl phosphine and imidazole offered the iodo derivatives (J). The iodo derivatives (J) were treated with thiolacetic acid to obtain the desired thioesters (K), which on hydrolysis under basic conditions yielded the desired thiols (L).



Scheme-1

The aromatic moieties attached in the final compounds (1) are given in Figure-1.

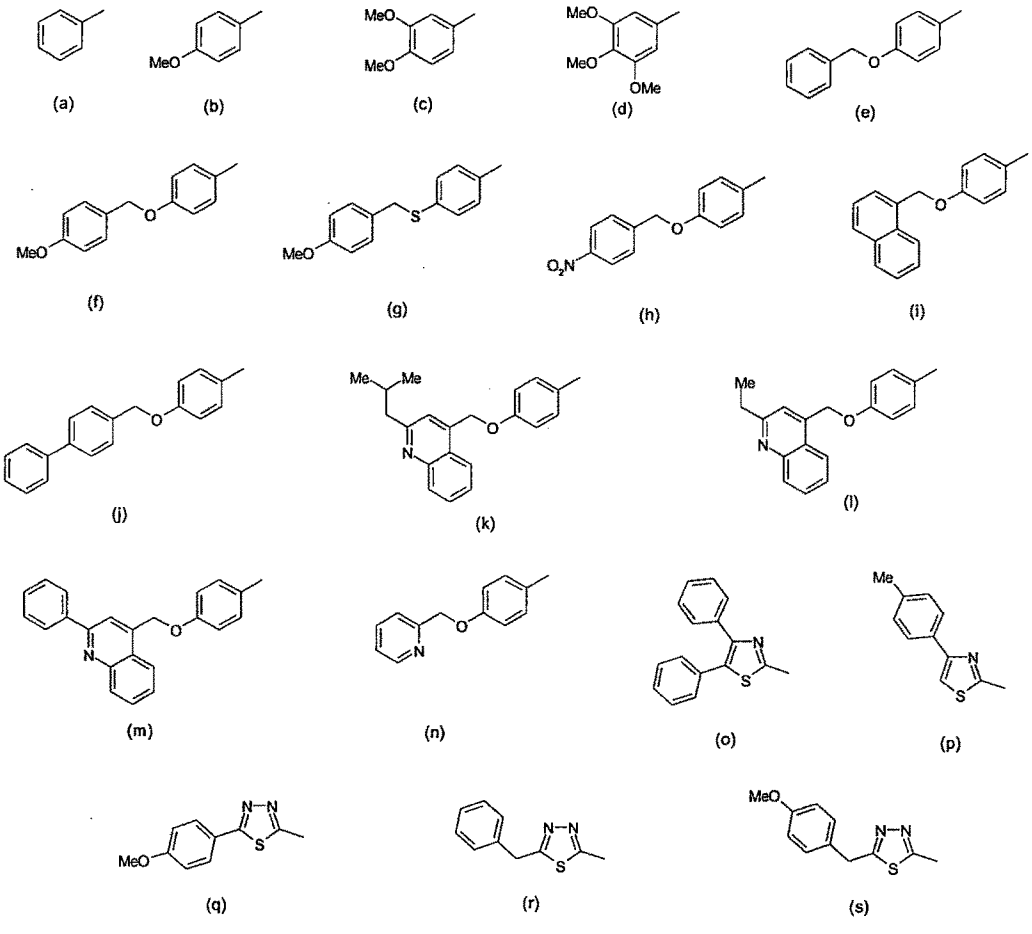


Figure-1 Aromatic moieties attached with compound (1)

All the synthesized compounds were characterized by their physical, spectral and elemental data.

Biological Studies

Some of the synthesized compounds were tested for their TACE inhibitory activity using an Innozyme™ TACE activity ELISA kit procured from Calbiochem, USA (Catalog No. CBA042).

The kit contains 96 well plate and the wells are pre-coated with a monoclonal antibody specific for human TACE that captures the enzyme from the cell lysate. The diluted cell lysate was added to the wells and incubated for 1 hour at 25 °C. After the enzyme was attached to the antibody, the wells were washed to discard the unbound

material and inhibitors were added to the designated wells. It was again incubated for 2 hours at 25 °C. In the presence of inhibitors, the enzyme would bind to the inhibitor and there would be decrease in the concentration of enzyme that was previously bound to the antibody. Upon addition of an internal fluorescent substrate (MCA-KPLGL-Dpa-AR-NH2), the free enzyme would cleave the scissile amide bond of it. Fluorescence intensity of the cleaved product, MCA-KPLG was measured at an excitation wavelength of 355 nm and emission wavelength of 405 nm. The level of fluorescence intensity is indirectly related to the inhibitory activity of the test compounds. The inhibitory activity of a test compound in a particular concentration was calculated from the following equation:

% Inhibition = [1 - (Fluorescence intensity of test/Fluorescence intensity of blank)] x 100

[The blank well contains solvent (DMSO) and the substrate]

Most of the compounds showed moderate to high TACE inhibitory activity. The most potent compound (**151**) showed 35 % inhibition at 0.1 μM/L concentration. At this concentration compound (**150**) showed 34 % inhibition of the enzyme. From the activity data (Table-11), it could be concluded that five-membered 2-imidazolidinone ring was preferred over six-membered tetrahydropyrimidin-2-(1*H*)-one ring. It was also observed that the compounds possessing hydroxamate as the zinc binding ligand were more active than carboxylates and thiols. The SAR of this series of compounds also reveals that compounds with thioether linkage are more active than the compounds possessing ether linkage at P1' site of the molecule.

In this thesis, novel 2-imidazolidinones and tetrahydropyrimidin-2(1*H*)-ones were synthesized and evaluated for their TACE inhibitory activity. Most of the synthesized compounds showed moderate to high TACE inhibitory activity. This piece of work has provided some important leads in the form of compounds (**131, 132, 148, 151** and **150**) as potential TACE inhibitors. These leads need to be exploited further in order to broaden the scope of the work.

SOME NOVEL IMIDAZOLIDINONE DERIVATIVES AS TNF CONVERTING ENZYME INHIBITORS

A Thesis Submitted to

The Maharaja Sayajirao University of Baroda

For the Degree of

DOCTOR OF PHILOSOPHY
IN
PHARMACY

BY

SHIRSHENDU DAS GUPTA

UNDER THE GUIDANCE OF

Prof. M. R. YADAV

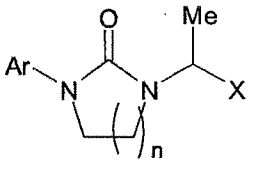
Pharmacy Department,
Faculty of Technology and Engineering,
The M. S. University of Baroda,
Vadodara-390 001

September 2008

SUMMARY

Arthritis was once considered to be the disease of Western countries. With growing affluence more and more people are falling prey to this disease in India as well. Though arthritis does not cause morbidity, it severely affects the quality of life of the patient. It is one of the most common autoimmune inflammatory conditions, affecting approximately 1% of the worldwide adult population. It was proved beyond doubt that TNF-α plays a pivotal role in the origin and progression of the disease. One of the ways of excluding TNF-α from the biological system is to block TACE, the enzyme responsible for maturation of inactive form of TNF. So far, most of the clinical interest in TACE has remained concentrated on arthritis, but recently preclinical studies in a variety of tumor model systems have revealed that inhibition of TACE inhibits pathogenic EGFR signaling also in cancer. Hence it was speculated that TACE inhibitors might cure cancer as well.

In this piece of work, novel 2-imidazolidinones and tetrahydropyrimidin-2(1*H*)-ones (I) were synthesized as potential TACE inhibitors. As TACE is a zinc metalloproteinase, zinc chelating groups (namely, hydroxamates, carboxylates and thiols)



(II)

n = 1, 2

X = -CONHOH

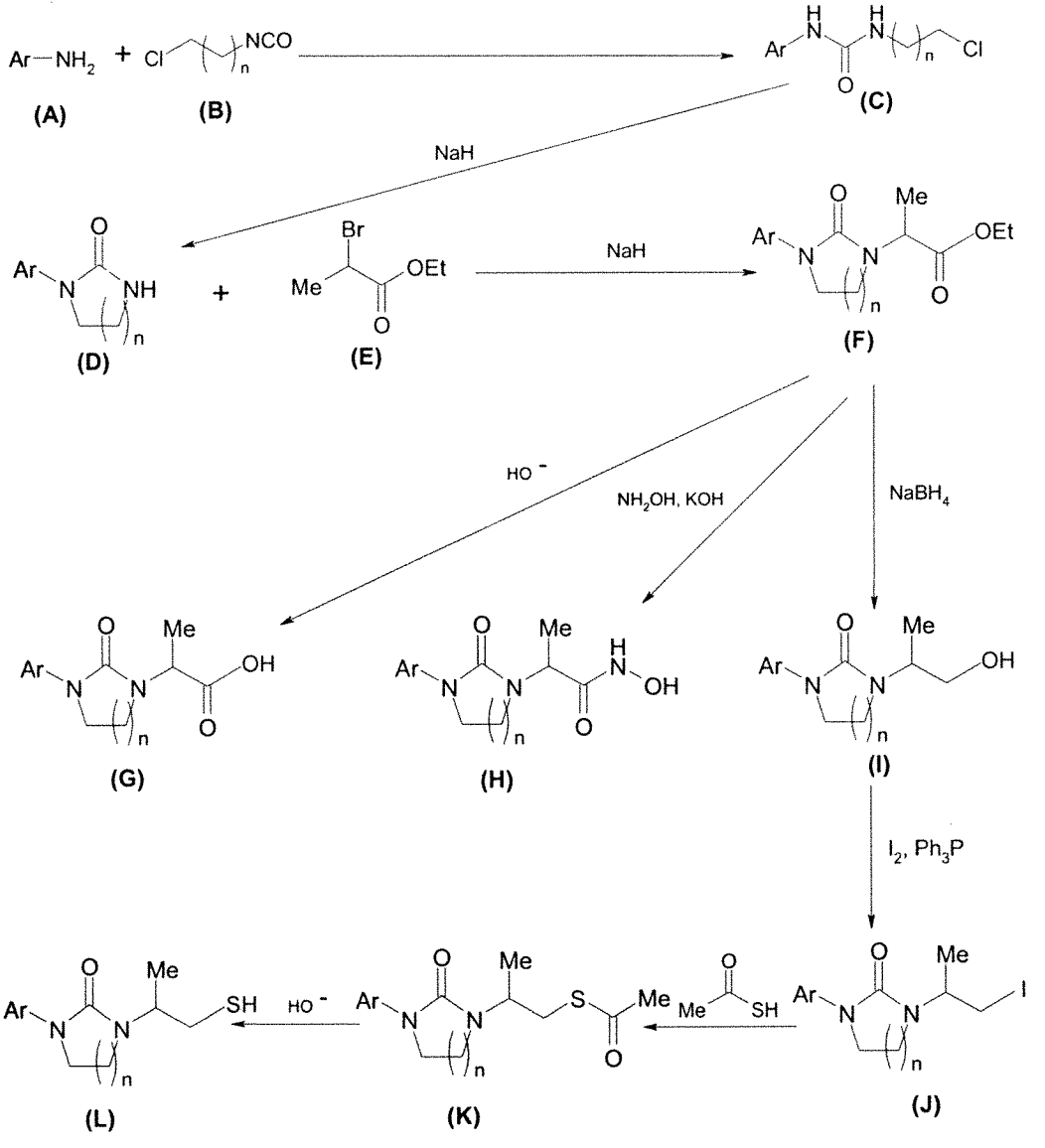
-COOH

$-CH_2SH$

were incorporated in the designed molecules as group X. It is known that S1' site of TACE is larger than that of MMPs, so it was planned to incorporate larger aromatic moieties for the group 'Ar' to increase selectivity for TACE over MMPs.

To synthesize the envisaged compounds, a scheme was planned as given in general Scheme-1, wherein arylamines (A) were reacted with chloroalkyl isocyanates (B) to obtain the expected urea derivatives (C). The urea derivatives were cyclized under strong basic conditions to obtain the desired five/six membered heterocycles (D). The required side chain was attached by reacting the cyclized products (D) with ethyl 2-

bromopropionate (E) to obtain the esters (F). The esters were converted to the three types of zinc binding ligands using three different sequence of reactions. The esters (F) were saponified to the free acids (G) using lithium/sodium hydroxide while hydroxylamine hydrochloride treatment of the esters (F) under basic conditions offered the desired hydroxamates (H). In a separate sequence of reactions the esters (F) were first reduced to alcohols (I) by sodium borohydride treatment, which on further treatment with iodine, triphenyl phosphine and imidazole offered the iodo derivatives (J). The iodo derivatives (J) were treated with thiolacetic acid to obtain the desired thioesters (K), which on hydrolysis under basic conditions yielded the desired thiols (L).



Scheme-1

The aromatic moieties attached in the final compounds (1) are given in Figure-1.

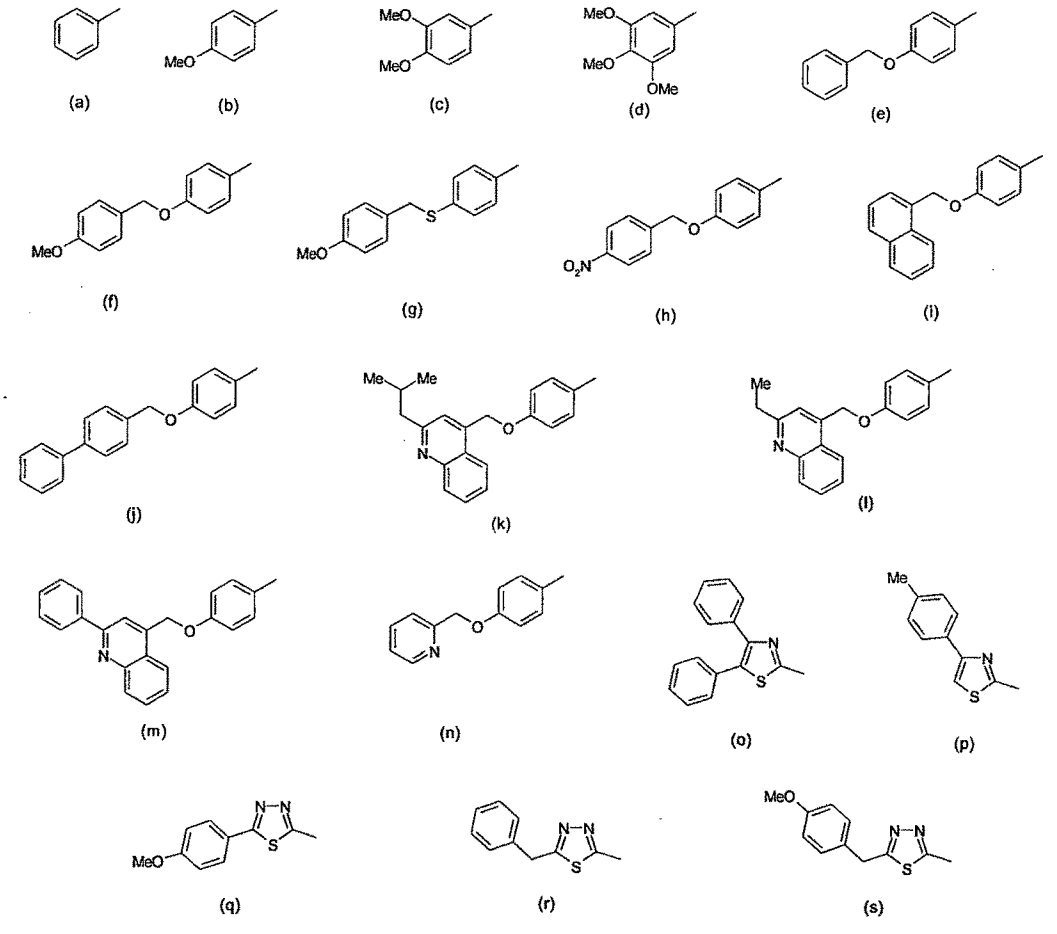


Figure-1 Aromatic moieties attached with compound (1)

All the synthesized compounds were characterized by their physical, spectral and elemental data.

Biological Studies

Some of the synthesized compounds were tested for their TACE inhibitory activity using an Innozyme™ TACE activity ELISA kit procured from Calbiochem, USA (Catalog No. CBA042).

The kit contains 96 well plate and the wells are pre-coated with a monoclonal antibody specific for human TACE that captures the enzyme from the cell lysate. The diluted cell lysate was added to the wells and incubated for 1 hour at 25 °C. After the enzyme was attached to the antibody, the wells were washed to discard the unbound

material and inhibitors were added to the designated wells. It was again incubated for 2 hours at 25 °C. In the presence of inhibitors, the enzyme would bind to the inhibitor and there would be decrease in the concentration of enzyme that was previously bound to the antibody. Upon addition of an internal fluorescent substrate (MCA-KPLGL-Dpa-AR-NH2), the free enzyme would cleave the scissile amide bond of it. Fluorescence intensity of the cleaved product, MCA-KPLG was measured at an excitation wavelength of 355 nm and emission wavelength of 405 nm. The level of fluorescence intensity is indirectly related to the inhibitory activity of the test compounds. The inhibitory activity of a test compound in a particular concentration was calculated from the following equation:

% Inhibition = [1 - (Fluorescence intensity of test/Fluorescence intensity of blank)] x 100

[The blank well contains solvent (DMSO) and the substrate]

Most of the compounds showed moderate to high TACE inhibitory activity. The most potent compound (**151**) showed 35 % inhibition at 0.1 μM/L concentration. At this concentration compound (**150**) showed 34 % inhibition of the enzyme. From the activity data (Table-11), it could be concluded that five-membered 2-imidazolidinone ring was preferred over six-membered tetrahydropyrimidin-2-(1*H*)-one ring. It was also observed that the compounds possessing hydroxamate as the zinc binding ligand were more active than carboxylates and thiols. The SAR of this series of compounds also reveals that compounds with thioether linkage are more active than the compounds possessing ether linkage at P1' site of the molecule.

In this thesis, novel 2-imidazolidinones and tetrahydropyrimidin-2(1*H*)-ones were synthesized and evaluated for their TACE inhibitory activity. Most of the synthesized compounds showed moderate to high TACE inhibitory activity. This piece of work has provided some important leads in the form of compounds (**131, 132, 148, 151** and **150**) as potential TACE inhibitors. These leads need to be exploited further in order to broaden the scope of the work.