SUMMARY

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Human life is continuously threatened by ailments. Hence, drugs are always in great demand for the prevention and treatment of diseases. To meet the challenges of discovery of safe drugs, techniques/methods of drug discovery and development are in great demand. The process of drug discovery and development is an expensive and time-consuming affair. To introduce a chemical entity as a new drug in the market requires not less than 10-15 years of hard work and a huge amount of money. Hence, several new technologies have been developed and applied in drug research to cut short the research cycle and to reduce the expenses. Computer-aided drug design (CADD) is one of such evolutionary technologies. Advent of computers in the drug discovery process has revolutionized our understanding of the drug-enzyme/receptor binding interactions. Hence, both ligand and structure based techniques have been applied for the development of inhibitors of enzymes like TACE, neuraminidase and aromatase which are involved in various diseased states.

Rheumatoid arthritis (RA) is one of the most common autoimmune inflammatory conditions, affecting approximately 1% of the world's population. It has been 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. While a variety of TACE inhibitors have been reported in the literature, a vast majority of these compounds are peptidic and peptide-like compounds that are expected to have bioavailability and pharmacokinetic problems, common to such compounds, limiting their clinical effectiveness. Low molecular mass, long acting, orally bioavailable inhibitors of TACE are therefore, highly desirable for the treatment of this chronic disease. Hence, TACE was one of our choices for performing *in silico* experiments.

Due to current surge of cases of patients suffering from swine flu, neuraminidase became our second choice of target for the study. Advances in understanding of the molecular and cellular biology of influenza have led to identification of several molecular targets for the design of potential drug molecules against this disease. The most advanced clinically used agents described to date for its therapy are inhibitors of neuraminidase, a glycoprotein found on the surface of the influenza virus. The 2009 swine flu virus

(H1N1pdm), which can be transmitted from human to human, has become a threat to the global health and economy. The current seasonal flu vaccine, which targets a different H1N1 strain, provides little or no protection against H1N1pdm. In terms of medication, the two FDA approved antiviral drugs, Tamiflu (oseltamivir) and Relenza (zanamivir), are effective against this new type of virus. Unfortunately, such virus types are known for their quick mutations and gene assortments which enable them to escape the host immune system and resist drugs. A case of swine flu resistant to Tamiflu was observed in Denmark on June 29, 2009 for the first time, and soon after that in Japan and Hong Kong. Developing new vaccinations and antiviral drugs for this new H1N1pdm virus is a matter of extreme urgency. Hence neuraminidase was one of our choices of target for *in silico* drug design.

Aromatase became our next choice of targets as a synthetic programme on the development of steroidal aromatase inhibitors was already running in our laboratory. Worldwide, breast cancer is the most common forms of cancer in women after skin cancer, representing 16% of all female cancers. Expression of aromatase is highest in or near breast tumor sites. A large number of aromatase inhibitors have been developed and utilized in clinical studies over the last 20 years. Treatment with aromatase inhibitors is generally well tolerated with low incidence of serious side effects. However short-term events like hot flushes, vaginal dryness, musculoskeletal pain and headache have been observed. Accordingly there is need for new, potent, more selective and less toxic CYP19 inhibitor. Hence aromatase was one of our choice of targets for *in silico* drug design. Molecular modeling studies have been carried out for some in house synthesized steroidal aromatase inhibitors. These studies revealed some interesting interactions between these compounds and the aromatase enzyme.

1 Studies on TACE Inhibitors

1.1 3D-Quantitative structure–activity relationship studies on benzothiadiazepine hydroxamates as inhibitors of TACE

A series of 2,3,4,5-tetrahydrobenzo[f][1,2,5]thiadiazepine-1,1-dioxide (benzothiadiazepine) derivatives (**Figure 1.1.1**) having selective TACE ihibitory activity were used to compare the quality and predictive power of 3D-quantitative structure-activity relationship (3D QSAR),[comparative molecular field analysis (CoMFA), and compara-

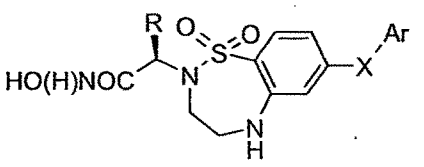
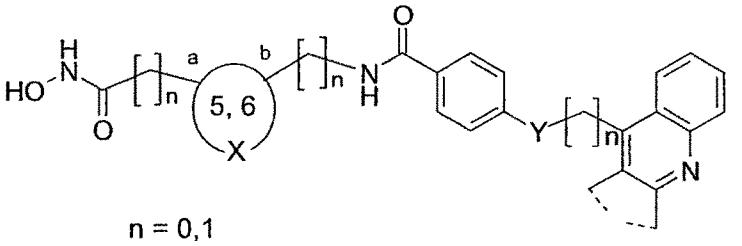


Figure 1.1.1 General structure of benzothiadiazepine hydroxamates used in 3D QSAR studies

tive molecular similarity indices (CoMSIA]) models for the atom-based, centroid/atom-based, data-based, and docked conformer-based alignments. Removal of outliers from the initial training set of molecules improved the predictivity of models. Among the 3D-QSAR models developed using the four alignments, the database alignment provided the best predictive CoMFA model for the training set. The CoMSIA models exhibited better external predictivity as compared to that of CoMFA model. CoMSIA technique provided better statistical models than CoMFA, which points to the significance of hydrophobic and hydrogen bond donor fields in the activity of these ligands in addition to steric and electrostatic fields. Some of the best compounds with increased TACE inhibitory profiles have been designed and reported in this study.

1.2 Development of predictive 3D-QSAR CoMFA and CoMSIA models for β-aminohydroxamic acid-derived TACE inhibitors

A 3D-QSAR study was performed on a series of β-aminohydroxamic acid-derived TACE inhibitors employing CoMFA and CoMSIA techniques to investigate the structu-



n = 0,1
Five membered ring, X = N-R
Six membered ring, X = CH_2,O,NH,N-R
Y = CH_2,O,NH
a,b = vicinal/geminal locations

Figure 1.2.1 General structure for the five series of compounds under study

ral requirements for the inhibitors, and derive a predictive model that could be used for the design of novel TACE inhibitors. The compounds pooled for these studies belonged to the general structure as depicted in **Figure 1.2.1.**

CLog P was used as an additional descriptor in the CoMFA to study the effects of lipophilic parameters on activity. Inclusion of CLog P did not improve the models significantly. The statistically significant model was established with 45 molecules, which was validated by a test set of 11 compounds. Docked conformer-based alignment yielded the best predictive CoMFA model having r^2_{cv} = 0.673, predictive r^2 = 0.642 while the CoMSIA model yielded r^2_{cv} = 0.635, r^2_{ncv} = 0.858 and predictive r^2 = 0.441. The developed COMFA model was validated by predicting externally the activity of IK-682, a well-known hydroxamate TACE inhibitor.

1.3 Development of predictive pharmacophore model for *in silico* screening and 3D-QSAR CoMFA and CoMSIA studies for lead optimization, and designing of potent tumor necrosis factor alpha converting enzyme inhibitors

Different pharmacophore hypotheses were developed for TACE inhibitors using PHASE. A five-point pharmacophore model with two hydrogen bond acceptors (A), one hydrogen bond donor (D) and two aromatic rings (R) as pharmacophoric features was associated with high predictive power. Pharmacophore mapping studies provided an insight into the inhibitory potential of different chemotypes as TACE inhibitors. The developed pharmacophoric model was utilized for *in silico* screening of compounds from a data base for potential TACE inhibitors. Reliability of the model got confirmed when four active and two inactive compounds grafted into the data base from outside, were correctly identified. Furthermore, the alignment based on the best pharmacophore hypothesis (CPH1) was used as the input for the development of CoMFA and CoMSIA 3DQSARs. The CoMSIA model with steric, electrostatic, acceptor and hydrophobic fields combination was considered to be the best model as it has got the best internal as well as external predictive ability. The ligand-based pharmacophore model and the 3D-CoMSIA model obtained from this study could be very useful for the *in silico* data base screening and designing of more potent TACE inhibitors.

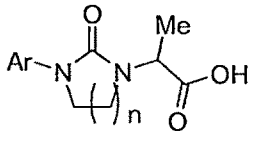
1.3.1 3D Database Screening

In order to discover unknown biological activities of existing compounds in corporate or public databases and subsequently to identify new structures for TACE inhibitory activity the ASINEX database made up of 45,533 molecules was screened with the CPH1 hypothesis. The *in silico* screening process retrieved 453 positive hits along with the four grafted active molecules and filtered out the two grafted inactives. In order to decrease the number of hits provided by 3D database query of CPH1, docking and

Lipinski's rule of five were used as filters. Finally twenty one hits were identified to have TACE inhibitory activity.

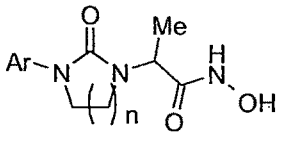
1.4 Molecular modeling studies of novel 2-imidazolidinones and tetrahydropyrimidin-2(1*H*)-ones as potential TACE inhibitors

Novel compounds belonging o the class of 2-imidazolidinones and tetrahydropyrimidin-2(1*H*)-ones (**1-14**) were designed and molecular modeling studies were performed on them. All of the designed compounds were docked in the active site



(1-6)

n = 1, 2



(7-14)

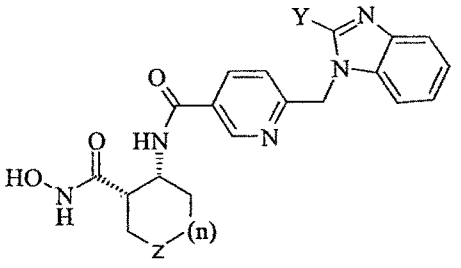
of human TACE. This study proved that the orientation of the P1' group was one of the most important factors for the biological activity of TACE inhibitors, particularly in imidazolidin-2-one and tetrahydropyrimidin-2(1*H*)-one scaffold-containing compounds.

The designed compounds (**1-14**) were synthesized in the laboratory by other researchers followed by *in vitro* biological evaluation for the binding affinity for TACE enzyme. Broadly, a good match was observed between the G-scores and % inhibition of the TACE inhibitory activity. This work proves that these two classes of molecules could be used as potential leads for the development of new TACE inhibitors.

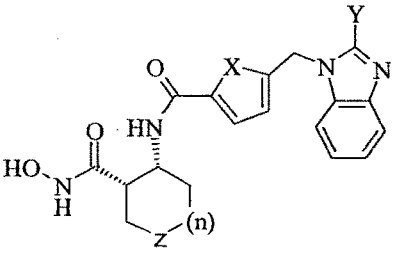
1.5 Molecular modeling studies of some new heterocyclic inhibitors of TACE

It was envisaged to make changes in the unexplored central phenyl ring of BMS-566394. The phenyl ring was replaced with monocyclic five or six-membered aromatic heterocyclic rings viz. pyridine, thiophene and furan. Tetrahydropyran ring of the lead compound BMS-566394 was also replaced with five or six-membered carbocyclic rings.

The designed compounds (**1-14**) were synthesized by one of our collaborating groups and the prepared compounds were evaluated in whole blood assay for inhibition



(1) $Y = CH_3$, $Z = O$, $n = 1$
(2) $Y = CF_3$, $Z = O$, $n = 1$
(3) $Y = CH_3$, $Z = CH_2$, $n = 1$
(4) $Y = CF_3$, $Z = CH_2$, $n = 1$
(5) $Y = CH_3$, $Z = CH_2$, $n = 0$
(6) $Y = CF_3$, $Z = CH_2$, $n = 0$



(7) $X = S$, $Y = CH_3$, $Z = O$, $n = 1$
(8) $X = S$, $Y = CF_3$, $Z = O$, $n = 1$
(9) $X = S$, $Y = CH_3$, $Z = CH_2$, $n = 0$
(10) $X = S$, $Y = CF_3$, $Z = CH_2$, $n = 0$
(11) $X = O$, $Y = CH_3$, $Z = CH_2$, $n = 0$
(12) $X = O$, $Y = CF_3$, $Z = CH_2$, $n = 0$
(13) $X = S$, $Y = CH_3$, $Z = CH_2$, $n = 1$
(14) $X = O$, $Y = CH_3$, $Z = CH_2$, $n = 1$

of TACE enzyme activity. The outcome of the study was quite surprising. Substitution of phenyl with pyridyl group afforded potent compounds while its substitution with five-membered heterocyclic rings like thiophenyl or furyl afforded almost inactive derivatives.

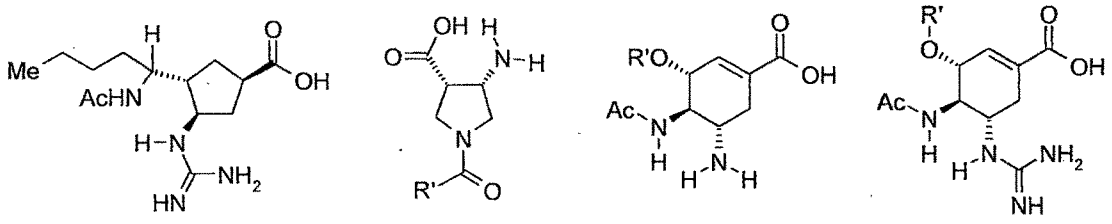
An alteration in the presumed dormant central ring system of the molecule leading to significant change in the inhibitory activity was quite unexpected. Molecular modeling studies were carried out using crystal structure of human TACE on the designed compounds (**1-14**) so as to gather insight into their binding affinity for the enzyme. Compound (**5**) having the highest activity was found to have an extra binding to the active site in the form of hydrogen bond between the pyridine nitrogen and the Ala439. Compounds (**7-14**) possessing central five-membered heterocyclic rings were shown to have a "different pose" which disallowed interaction between the zinc-binding hydroxamate group and the catalytic zinc ion site in these modeling studies. This study throws new light on the unexplored and unreported role played by the central aromatic ring in benzamidohydroxamate series of compounds belonging to BMS-566394.

2. Studies on Neuraminidase Inhibitors

2.1 Determining of structural requirements of influenza neuraminidase type A inhibitors and binding interaction analysis with the active site of A/H1N1 by 3D-QSAR CoMFA and CoMSIA modeling

In the present study, sixty one neuraminidase type A inhibitors, were selected for the development of 3D-QSAR CoMFA and CoMSIA models. The representative structures I-IV of the series under study for 3D-QSAR analysis are shown in **Figure**

2.1.1. Three different 3D-QSAR CoMFA predictive models, one with atom-fit/centroid atom-fit model and two docked conformation-based ones, were established for the neuraminidase type A inhibitors by using three different molecular alignment strategies.



(I) (II) (III) (IV)

Figure 2.2.1 The representative structures **I-IV** of the series under study for 3D-QSAR analysis.

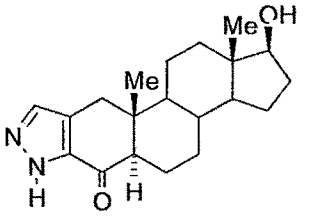
Homology modelled conformation of A/H1N1 structure was used for docking purpose. The model developed by considering the docked structure-based conformers having database RMS fitting was found to perform better than the one developed by atom-fit based-alignment, with excellent cross-validated r^2 and predictive r^2 values. The CoMFA model generated using docked conformer-based alignment having database RMS fitting served for alignment of compounds for CoMSIA studies also. CoMSIA model with a combination of steric, electrostatic and acceptor fields yielded the highest cross-validated r^2. CoMSIA model showed better predictive ability than COMFA model. COMFA steric, electrostatic and CoMSIA donor contour maps were mapped in the active site of homology modelled A/H1N1 structure. The active site of homology model of A/H1N1 and the contour maps generated by the CoMFA/CoMSIA study involving the Alignment-III were found to be complementary and in consonance with each other.

3. Studies on Aromatase Inhibitors

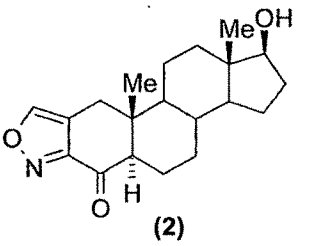
3.1 Molecular modeling studies on some novel androstanes as potential aromatase inhibitors

Novel pyrazole (1), isoxazole (2 and 3) and nitrile (4) derivatives were designed. Molecular docking studies were carried out for the designed compounds showing good docking scores for compound (4) followed by compound (1) while, compound (3) afforded poorest of the score. Aromatase inhibitory activity for compound (1) having pyrazole ring at 2,3-position was found to be the highest followed by the nitrile derivative

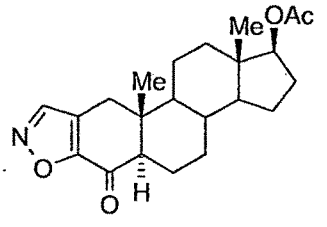
(4). Isomeric forms of isoxazole (2 and 3) showed very poor activity compared to fadrozole and aminoglutethimide. The modeling studies showed that compounds (1 and 4) slightly acidic protons attached to position 3 of the A-ring bind to a site in the aroma-



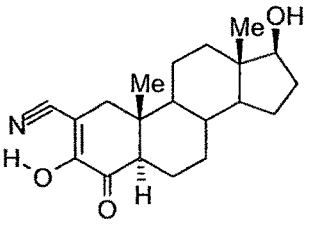
(1)



(2)



(3)



(4)

tase enzyme that acts as a hydrogen bond acceptor thereby increasing the stability of the enzyme-inhibitor complex, whereas this feature lacked in compounds (2 and 3) showing poor binding with the active site of the enzyme. Thus, docking study was found to be in agreement with the biological data for the designed compounds.

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