Original Research Paper

Volume-9 | Issue-2 | February-2019 | PRINT ISSN - 2249-555X

Pharmacology

LIGAND-BASED PHARMACOPHORE MODELING STUDIES OF TNF-ALPHA- CONVERTING ENZYME INHIBITORS

Thangavel Muthusamy	Associate Professor, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education, Chettinad Medical University, Kelambakkam, Chennai, Tamil Nadu, India-603103
Devaprasad Markandeyan*	Associate Professor, Department of Neurology, Chettinad Academy of Research and Education, Chettinad Health City, Kelambakkam, Chennai-603103, Tamil Nadu, India. *Corresponding Author
Mariappan Andi	Lecturer, Department of Gunapadam, National Institute of Siddha, Tambaram Sanatorium, Chennai- Tamil Nadu, India. 600047.
Abhinand.P.A	Research Fellow, Department Of Bioinformatics, Sri Ramachandra University , Porur, Chennai 600116.
Subramaniyan Kannaian	Associate Professor, Department of Neurology, Chettinad Academy of Research and Education, Chettinad Health City, Kelambakkam, Chennai-603103, Tamil Nadu, India.

ABSTRACT

Aim: Tumor necrosis factor-α converting enzyme (TACE) is a metalloproteinase which cleaves Pro-TNF-α protein at its Ala76-Val77 amide bond, thereby releasing a soluble 17kDa ectodomain which mediates various proinflammatory activity of cytokine Tumor necrosis factor-α (TNF-α). TACE is promising target for treatment of various autoimmune diseases like Multiple Sclerosis, Psoriasis, Rheumatoid arthritis etc. We have built ligand-based pharmacophore and 3D-QSAR models based on TACE inhibitors with known IC50 values using PHASE software of Schroedinger.

Methods: We found that model AADDR.144 has highest R2 value (0.9921), Pearson R value of 0.6698 and a low Standard Deviation (SD) of 0.0761 and Root-Mean- Square-Error value (RMSE) of 0.6433. Validation using training and test set compounds was done.

Results: The generated 3D-QSAR model was found to be useful in predicting activities of new ligands. Screening of Phytochemicals was done and phytochemicals which are similar to the AADDR.144 model were further docked to TACE protein GLIDE software.

Conclusion: Phytochemical compounds Delphinidin, Canthin 6-one glucoside, Malvidin, Myrecetin, Amentoflavone, Hesperidin have good structural similarity to QSAR model built based on known TACE inhibitors and these compounds also have good binding affinity to TACE protein in docking.

KEYWORDS : TNF-α converting enzyme, pharmacophore, Schroedinger, Molecular Docking, Phytochemical compounds.

Introduction

Tumor Necrosis factor-α (TNF-α), was discovered in 1975 by Old and coworkers, as a macrophages cell product responsible for LPS induced apoptotic death of tumour cells [1]. It was initially named as "cachectin", a protein which in mice caused hyperthermia and death, when infected with parasites [2]. Later studies showed that TNF-α was same as lymphotoxin (LT-α)([3,4].TNF-α is implicated in the pathogenesis of chronic inflammatory diseases, like Psoriasis, Rheumatoid arthritis , Crohn's disease and ankylosing spondylitis [5]. The soluble form of TNF- α is a 157 amino acid protein originating from the trans-membrane precursor protein Pro-TNF-a through proteolytic cleavage that leaves the transmembrane and intracellular portions untouched..The catalytic enzyme mediating the cleavage is called TNF-α converting enzyme (TACE). Recently, TACE was also found to be a important factor contributing to radiotherapy resistance of cancer cells [7]. TACE is also a chief regulator of MAP kinase and Notch signaling pathways [8]. TACE also mediates the release of L-selectin which is useful for cell to cell adhesion [9]. TNF-α/ TACE are involved in the pathogenisis of multiple sclerosis (MS). In a longitudinal study on 11 relapsing-remitting MS patients, where mRNA expression of TNF-a and TACE was estimated , Patients who had high quantity of TACE mRNA in PBMCs showed a significantly higher disease activity [10].

Experimental Dual MMP-TACE inhibitors reduced inflammation in collagen and adjuvant-induced arthritis models in animals [11]. However, preclinical studies were halted as liver toxicity was observed .

TACE inhibitor BMS-561392 in twice daily doses was equally efficacious as Enbrel and was more effective than Remicade in reducing inflammation in collagen-induced arthritis model in animals [12]. Two TACE inhibitors TMI-005 ,BMS-561392 were evaluated in Phase II clinical trial. The trials were stopped due to liver toxicity and lack of clinical improvement [13].

In the present study, pharmacophore model was generated for Human TACE inhibitors using Phase module 4 (Schrödinger Module2016) [14,15]. Subsequently we built a atom based 3D-QSAR model was and did database screening in search of phytochemical compounds which matched the QSAR model. The matched compounds were then docked against TACE protein using glide to study its binding affinity interaction with the protein.

Methodology:

Dataset

A total of 165 TACE inhibitors were recognized from the literature [16, 17, 18 ,19, 20, 21, 22, 23, 24] and the pIC50 (pIC50 = -logIC50) values were calculated. The dataset contains different chemical classes, namely, dihydropyridine, imidazolinedione, dicarboxamide, phenylpyrrolidine,benzamide,carbamoyl imidazole, sulfanylidene imidazolidinone . The structures were downloaded as sdf files in Maestro suite and prepared using the LigPrep module. Different conformer was generated using confgen. Energy minimization was doneusing OPLS 2005 with an implicit distance-dependent dielectric solvation treatment.

The Generation of Pharmacophore hypothesis and 3D-QSAR model building and Validation of QSAR model

The pharmacophore model was generated using Phase version 4, Schrödinger suite 11 using a set of pharmacophore features to generate sites for all the compounds [25]. There are six built inpharmacological features in Phase, namely hydrogen bond receptor (A), hydrogen bond donor (D), hydrophobic group (H), negatively ionisable (N), positively ionisable (P) and aromatic ring

Ligand-based Pharmacophore Modeling Studies of TNF- alpha-converting enzyme Inhibitors

®. The 165 compounds were divided as active inactive and partially

active based on PIC50 values. Among the 165 compounds 6 had PIC50 values > 9.5 and were designated as actives. The 165 compounds yielded 26 variants of pharmacophores of which 12 variants matched all the 6 active compounds. The alignment of 12 variants was measured using survival scores [26] and the default values have were used for the hypothesis generation. The top 5 variants with high survival scores (AADDR-144, AAADR.334, AAADR.566, ADDHR.2, AADDR.104) were used to develop QSAR model.

Every 5th compound was chosen as a test set, so 33 were selected as a test set and remaining were used as training set to generate atom-based QSAR models. This type of test set selection procedure was employed to represent the range of biological activities similar to the training set molecule. To encompass the space occupied by the aligned training set molecules the rectangular grid was generated with the spacing of 1.2Å. Each model contains seven or more partial least square (PLS) factors tend to fit the pIC50 values beyond their experimental uncertainty. The statistical parameters R^2 (coefficient of determination) and SD (standard deviation of regression) were calculated to evaluate the overall significance of the model. . Based on statistical values QSAR model built using AADDR.144 was found to be the best QSAR model and was validated by predicting activities of 33 test set compounds. Regression was constructed for a series of model with the increasing number of Partial Least-Square (PLS factor). As the number of PLS factors increased, the statistical significance and predictive ability of the model was also incrementally increased up to 8.

The pharmacophore hypothesis AADDR.144 included the following features **(Figure 1):** the hydrogen bond acceptor (sphere with arrow A4 and A5), hydrogen bond donors (D6,D7 blue spheres) and the aromatic ring (R13 circle).

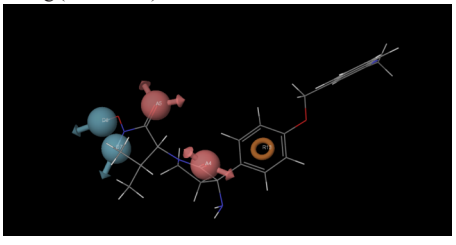


Figure 1. AADDR.144

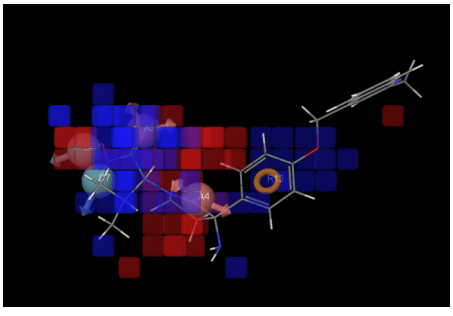
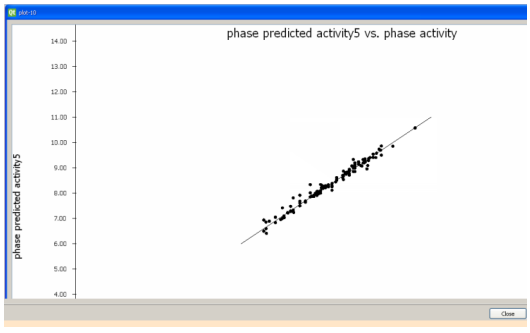


Figure 2. AADDR.144 – QSAR



AADDR.144 QSAR ACTIVITY SCATTER PLOT

It was found that AADDR.144 has highest R^2 value (0.9921), Pearson R value of 0.6698 and a low Standard Deviation (SD) of 0.0761 . Larger valueof F (416) with the smaller value of p (7.42e-66) indicates a statistically significant regression model with high degree of confidence. The small value of SD of 0.0761 and Root-Mean- Square-Error value (RMSE) of 0.6433 indicates that the data used for the analysis of the QSAR model was good even though QSAR model was generated using the different set of chemical class compounds.

Virtual screening

1200 phytochemical compounds structures were downloaded from MAPS phytochemical database [27, 28]. The structures were downloaded as sdf files in Maestro suite and prepared using LigPrepmodule. Energy minimization was done using OPLS 2005 with an implicit distance-dependent dielectric solvation treatment. Matches for the AADDR.144 were searched from

downloaded structures. 102 matches were found for AADDR model among the downloaded phytochemical compounds. These matched compounds were further docked to the TACE structure using Glide software.

Protein Preparation for docking

The X-ray crystallographic structure of human TACE was downloaded from the PDB database (PDB ID:2M2F [29] and was optimised using the protein preparation wizard of Schrödinger Module. Energy minimization was done using OPLS-2005 force field. Hydrogen atoms were added in appropriate sites of protein to optimize ionization and tautomeric states of amino acid residues. The receptor grid was generated with the help of Receptor Grid Generation Panel. The receptor site was made of residues Glu406, Leu348, LY346, Gly349, Thr347, Gly346, Gly349, Val402, Val 439, Leu401, Tyr436, Tyr433, Lys432, Asn447, Val440, Ser441, His415, Glu398, Leu350, Pro437, Ile438. The matches obtained for pharmacopore AADDR-144 were docked using Standard Precision mode.The docking scores were validated by comparing with docking scores of compound Marimastat [30] which is a TACE and Matrix metalloproteinase inhibitor.

Results and discussion:

	Compound	Predicted activity	Docking score	Glide Score	Glide Energy	Hydrogen bonds
	Marinastat	-9.954	-8.234	-8.234	-60.341	His415, Glu406, Leu348, Pro437, Gly346, Ala439
1	Delphinidin	8.534	-11.029	-11.035	-65.489	Pro437, Gly349, Glu406,Ser441, Tyr433
2	Canthin-6-one 9-glucoside	8.744	-10.664	-10.730	-60.126	Asn389, Val434, Glu398,Ser441,His405
	-3-glucoside	8.158	-10.254	-10.261	-65.458	His409, His405, Val434,
3	Myrecetin	8.227	-9.824	-9.824		Gly349,Glu406, Pro437, Ser441, Tyr433
4.	Amentoflavone	8.315	-8.805	-8.807	-71.789	Gly349
5.	Hesperidin	8.540	-8.769	-8.769	-73.353	Glu406, Gly349, Tyr433, Thr347, Met345
6.	Calyxin -l	8.838	-8.721	-8.721	-71.777	Gly349, Asp 313, Glu 406

Matches were found to AADDR.144 pharmacopore among compounds belonging to Flavinoids, Flavones, diarylheptanoids, anthocyanidins groups. These compounds also showed good binding properties and are possibly good inhibitors of TACE

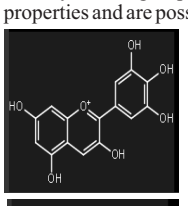


Figure 4 Delphinidin

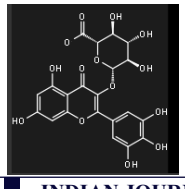


Figure 5 Myricetin

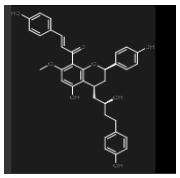


Figure 6 Calyxin I

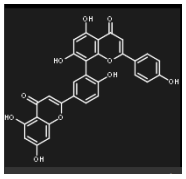


Figure 7 Amentofalvone

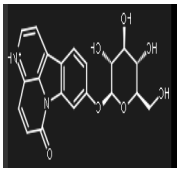


Figure 8 Canthin 6 one- 9 glucoside

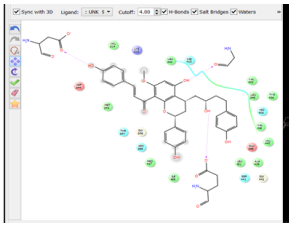


Figure 9 Calyxin I Hydrogen bonds Interaction

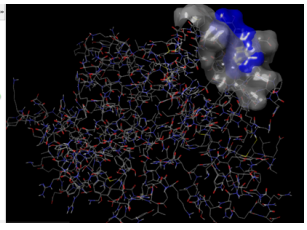


Figure 10 Delphinidin docked to TACE active site

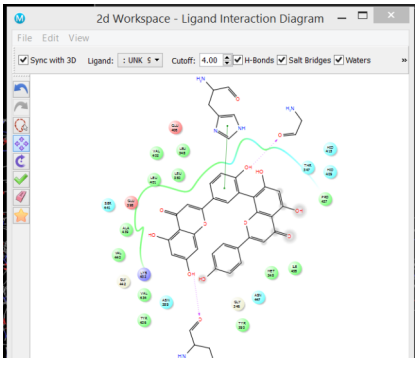


Figure 11. Amentoflavone interaction with TACE

Delphinidin (Dp)which is a anthocyanidin present in Hibiscus, showed good binding activity to TACE in our study s reduced the levels of inflammatory mediators including iNOS, NO, IL-6, and TNF-α induced by Lipopolysaccharide in in-vitro and in-vivo studies. Cellular signaling analysis revealed that Delphinidin downregulated NF-κB pathway and MEK1/2-ERK1/2 signaling.Its action may be accentuated if it possess TACE inhibiting property also[30]. Amentoflavone, a biflavonoid is shown to be inhibitory to NF-kappa-B, IkappaB-alpha degradation and translocation of NF-kappa-B into nucleus in TNFalpha-activated A549 cells [31]. The TACE inhibiting potential of these compounds need to be explored.

Conclusion

Some of the phytochemical compounds have good structural similarity to QSAR model built based on known TACE inhibitors and these compounds also have good binding affinity to TACE protein in docking.

Acknowledgment

The authors thank Schroedinger India for providing free trial and Dr.Vinod Devaraji for his technical support and guidance

Conflict of Interest

The Authors have no conflict of interest

REFERENCES:

(1) Carswell E, Old L, Kassel L, Green S, Fiore N and Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci. (1975) 72: 3666-3670.
(2) Beutler B, Greenwald D, Hulmes D, Chang M, Pan J, Mathison R and Ulevitch A. Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. Nature. (1985) 316: 552-554.
(3) Pennica D, Nedwin E, Hayflick JS, Seeburg PH and Derynck R. (1984) Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature. (1984) 312: 724-729.
(4) Agarwal B, Kohr J, Hass E, Moffat B and Spencer A. Human tumor necrosis factor. Production, purification, and characterization. J Biol Chem. (1985) 260: 2345-2354.
(5) Maini RN and Taylor PC. Anti-cytokine therapy for rheumatoid arthritis. Annu Rev Med. (2000) 51: 207-229.
(6) Moss ML, Jin JD, Becherer DM, Bickett W, Burkhart WJ, Chen D, Hassler MT, Leesnitzer G, McGeehan M, Milla M, Moyer W, Rocque T, Seaton F, Schoenen J and Warner D. Structural features and biochemical properties of TNF-alpha converting enzyme (TACE). J Neuroimmunol. (1997) 72: 127-129.
(7) Sharma A, Bender S, Zimmermann M, Riesterer O, Broggini-Tenzer A and Pruschy MN. Secretome Signature Identifies ADAM17 as Novel Target for Radiosensitization of Non-Small Cell Lung Cancer. Clinical Cancer Research. (2016) 22: 4428–4439.
(8) Sternlicht MD, Sunnarborg SW, Kouros-Mehr H, Yu Y, Lee DC and Werb Z. Mammary ductal morphogenesis requires paracrine activation of stromal EGFR via ADAM17-dependent shedding of epithelial amphiregulin. Development. (2005) 132: 3923–3933.
(9) Li Y, Brazzell J, Herrera A and Walcheck B. ADAM17 deficiency by mature neutrophils has differential effects on L-selectin shedding. Blood. (2006) 108: 2275–2279.
(10) Seifert T, Kieseier BC, Ropele S, Strasser-Fuchs S and Quehenberger F. TACE mRNA expression in peripheral mononuclear cells precedes new lesions on MRI in multiple sclerosis. Multiple Sclerosis Journal. (2002) 8: 447-451.
(11) Moss ML, Tavron LS and Nudelman R. (2008) Drug Insight: Tumor necrosis factor converting enzyme as a pharmaceutical target for rheumatoid arthritis. Nat Rev. (2008) 4: 300-309.
(12) Grootveld M, McDermott MF. BMS-561392. Bristol-Myers Squibb. Curr Opin Investig Drugs (2003) 4: 598-602.
(13) Thabet MM, Huizinga TW. Drug evaluation: Apratastat, a novel TACE/MMP inhibitor for rheumatoid arthritis. Curr Opin Investig Drugs. (2006) 7:1014-1019.
(14) Schrodinger Release (2016) 4: Phase, Schrodinger, LLC, New York, NY.
(15) Dixon SL, Smondyrev AM, Knoll EH, Rao SN, Shaw DE and Friesner RA. PHASE: A New Engine for Pharmacophore Perception, 3D QSAR Model Development, and 3D Database Screening. 1. Methodology and Preliminary Results. J. Comput. Aided Mol. Des. (2006) 20: 647-671.
(16) Girijavallabhan VM, Chen L and Dai C. Novel TNF-a converting enzyme (TACE) inhibitors as potential treatment for inflammatory diseases. Bioorg Med Chem Lett. (2010) 20: 7283-7287.
(17) Rosner KE, Guo Z and Orth P. The discovery of novel tartrate-based TNF-alpha converting enzyme (TACE) inhibitors. Bioorg Med Chem Lett. (2010) 20: 1189-1193.
(18) Chun K, Park SK and Kim HM. Chromen-based TNF-alpha converting enzyme (TACE) inhibitors: design, synthesis, and biological evaluation. Bioorg Med Chem (2008) 16: 530-535.
(19) Xue CB, He X and Corbett RL. Discovery of macrocyclic hydroxamic acids containing biphenylmethyl derivatives at P1, a series of selective TNF-alpha converting enzyme inhibitors with potent cellular activity in the inhibition of TNF-alpha release. J Med Chem. (2001) 44: 3351-3354.
(20) Ott GR, Asakawa N and Lu Z. Alpha, beta-cyclic-beta-benzamido hydroxamic acids: novel templates for the design, synthesis, and evaluation of selective inhibitors of TNF-alpha converting enzyme (TACE). Bioorg Med Chem Lett. (2008) 18: 694-699.
(21) Xue CB, He X and Roderick J. Rational design, synthesis and structure-activity relationships of a cyclic succinate series of TNF-alpha converting enzyme inhibitors. Part 1: lead identification. Bioorg Med Chem Lett. (2003) 13: 4293-4297.
(22) Yang JS, Chun K and Park JE. Structure based optimization of chromen-based TNF-a converting enzyme (TACE) inhibitors on S1 pocket and their quantitative structure-activity relationship (QSAR) study. Bioorg Med Chem. (2010) 18: 8618-8629.
(23) Gilson MK, Liu T, Baitaluk M, Nicola G, Hwang L and Chong J. Binding DB in 2015: A public database for medicinal chemistry, computational chemistry and systems pharmacology. Nucleic Acids Research. (2015) 44: 1045-1053.
(24) Schrodinger Release (2016) Maestro, Schrodinger 4, LLC, New York, NY.
(25) Dixon SL. J Comput Aided Mol Des. (2006) 20: 647.
(26) Ashfaq UA, Mumtaz A, Qamar TU, Fatima T. (2013) MAPS Database: Medicinal plant Activities, Phytochemical and Structural Database. Bioinformation. (2013) 9: 993-995.
(27) Dusterhoft S, Jung S and Hung CW. Membrane-proximal domain of a disintegrin and metalloprotease-17 represents the putative molecular switch of its shedding activity operated by protein-disulfide isomerase. J.Am.Chem.Soc. (2013) 135: 5776-5781.
(28) Schafer M, Granato DC and Krossa S. GRP78 protects a disintegrin and metalloprotease 17 against protein-disulfide isomerase A6 catalyzed inactivation. FEBS Lett. (2017) 591: 3567-3587.
(29) Millar AW, Brown PD and Moore J. (1998) Results of single and repeat dose studies of the oral matrix metalloproteinase inhibitor marimastat in healthy male volunteers. Br J Clin Pharmacol. 45: 21–26.
(30) Sogo T, Terahara N and Hisanaga A. (2015) Anti-inflammatory activity and molecular mechanism of delphinidin 3-sambubioside, a Hibiscus anthocyanin. Biofactors. (2015) 41:58-65.
(31) Banerjee T, Valacchi G, Ziboh VA. Inhibition of TNFalpha-induced cyclooxygenase-2 expression by amentoflavone through suppression of NF-kappaB activation in A549 cells. Mol Cell Biochem. (2002) 238:105-110.