Original Article

Fragment-Based Design of a Potential TNF-α Inhibitor Inspired by Castanospermine and Methyl Phenylacetate

Paul M. Morgan[1], Meghan Mangal[1], Velabo Mdluli[2]

Objective: We describe the fragment-based design of CasMethPhens - novel potential small molecule inhibitors of Tumor Necrosis Factor Alpha (TNF-α) derived from castanospermine and methylphenyl acetate.

Methods: A virtual library of 612 natural products was screened against TNF-α using a hybrid docking approach where ligand binding is driven by enthalpy and entropy. The compounds docked were selected on the basis of their structurally diverse moieties across 7 classes of natural products: phenylpropanoids, alkaloids, terpenoids, polyketides, cannabinoids, curcuminoids, and catechins. Molecular docking was employed to predict their potential binding modes and estimate binding strength. Each compound was ranked on the basis of HYdrogen bonding energy and DEydration energy (HYDE) scoring function algorithm which assesses compounds using three parameters-hydrogen bonding, the hydrophobic effect and dehydration.

Results: Of the 612 compounds, two hits were identified. Both castanospermine and methyl phenylacetate had low molecular weight (MW) and favorable ligand-lipophilicity efficiency (LLE), ligand efficiency (LE), octanol/water partition coefficient (log P) and topological polar surface area (TPSA) with high mM predicted activity. Both compounds had mutually exclusive scaffolds and occupied different locations in the TNF-α binding pocket. We evolved these two natural products into new molecules having higher interaction affinity and desirable absorption, distribution, metabolism and excretion (ADME) properties.

Conclusions: We report the computational design of a potential small molecule inhibitor of TNF-α derived from two low-cost natural products. Our compound designs provide a favorable point of reference for the development of an innovative cancer drug.

Key words: trimer, analogs, hydrophobic effect, HYDE, ADME

Introduction

Tumor necrosis factor alpha (TNF-α) is a versatile cytokine that controls a vast

From the [1]School of Medicine for International Students, I-Shou University, Kaohsiung, Taiwan, and [2]Department of Chemistry, Carnegie Mellon University, Pittsburgh, U.S.A.
Received: March 5, 2018 Accepted: June 13, 2018
Address reprint request and correspondence to: Paul M. Morgan, School of Medicine for International Students, I-Shou University,, No. 8, Yida Road, Jiaosu Village, Yanchao District, Kaohsiung City 82445, Taiwan.
Tel: +886-966543608, E-mail: buddymorgan@gmail.com; isu10557002A@cloud.isu.edu.tw

array of signaling pathways involved in immunity, inflammation, cell survival (anti-apoptosis), cell death (apoptosis), and even tumorgenesis. TNF-α gene was cloned in 1984 and since then widespread research has unraveled a variety of roles for TNF-α under physiological conditions. TNF-α play critical roles in body development and immunity, transplant rejection, septic shock, tumor growth, and in pathological responses like inflammation.[1,2] Macrophages are the primary producers of TNF-α.[3] However TNF-α is also produced by T lymphocytes, NK cells, smooth muscle cells, and fibroblasts.[3] TNF-α is a 23 kilodalton (kDa) type II transmembrane protein arranged as a stable homotrimer.[3-5] Metalloprotease TNF-α converting enzyme (TACE) cleaves the transmembrane form to create a soluble 51 kDa soluble homotrimeric cytokine. Both forms of TNF-α display bioactivities via TNF-α receptors, TNFR I and TNFRII.[3,6]

Clinically, TNF-α production by tumors is correlated with unfavorable prognosis, cachexia and loss of loss of hormone responsiveness.[2] Pre-cancerous tumor cells with elevated expression levels of TNF-α is intimately associated with the progression of malignant diseases such as cervical carcinoma, chronic lymphocytic leukemia, prostate cancer, breast cancer, and Barrett's adenocarcinoma.[1] Five TNF-α blockers are currently approved by The USA Food and Drug Administration (FDA), for the treatment of ankylosis, inflammatory bowel disease, osteoarthritis, and psoriasis.[4,7] Four are antibody-based drugs while the fifth is a peptide.[4,7]

The majority of pharmacologically active substances are derived from natural products, from which drugs are essentially developed.[8] TNF-α inhibitors from natural origins are currently in its infancy stages of development for the treatment of inflammatory disorders. However, small-molecule drugs that can regulate TNF-α levels or activity might provide an economic alternative to protein-based therapeutics.

Herein, we describe the very first reported computer-aided design of a novel potential small molecule TNF-α inhibitor, CasMeth-Phen, derived from the structures of two low-cost natural products, castanospermine and methyl phenylacetate. Castanospermine is a tetrahydroxylated indolizidine alkaloid isolated from the seeds of Castanospermum austral.[9] Castanospermine is a potent inhibitor of β-glucosidase and β-glucocerebrosidase. While, methyl phenylacetate is a naturally occurring ester with a strong odor similar to that of honey commonly found in coffee, honey, brandy, capsicum, and wines.[10]

Methods

The drug discovery process has now integrated computational methods as a mainstay standard during the drug discovery process.[11] New bioactive compounds for protein targets are typically done via virtual screening techniques, which is the first step in the drug design and development process.[11] The binding mode and binding strength for thousands of compounds are evaluated via molecular docking.[11]

The initial step of the docking process involves selecting potential bioactive confirmations from a cohort of docking poses.[11] Then, non-binders are discriminated from true binders. In the final step, compound affinities are accurately predicted.[11] To date, one of the major limitations in current computational docking and screening assays is the precise estimation of free energy of binding.[11,12]

The FLEXX docking algorithm in LeadIT relies on a peculiar assembly. It essentially consists of three stages: (1) Base selection: the algorithm selects the most suitable base fragment of the ligand.[12] (2) Base Placement: the base fragment is docked into the active site of the protein irrespective of the remaining parts of the ligand.[12] (3) Complex Construction: in

the third stage the ligand is built incrementally and initiated by assigning multiple confirmations of the base fragments into the active site.[12] It is worthwhile noting that the FLEXX algorithm base selection is an interactive process.

Virtual Screening of Natural Products and Optimization of Hits

A virtual library of 612 natural products was screened against TNF-α (PDB: 1TNF). 322 phenylpropanoids, 167 alkaloids, 57 terpenoids, 40 polyketides, 14 cannabinoids, 7 curcuminoids, and 5 catechins were selected for docking based on the structural diversity of moieties present in each class of compounds in an attempt to increase the probability of hits. The receptor was prepared in LeadIT.[13] We identified a total of 6 pockets in the TNF-α homotrimer. However, only the largest pocket (A) at the base containing 26 amino acids was deemed viable. All the other cavities were miniscule and incapable of accommodating a small molecule with a molecular weight near 500 Daltons. It is also reported that TNF-α binds to its receptor as a trimer where the general site of interaction is at the base of the trimer making pocket A an ideal target.[6]

The HYDE scoring function

To overcome the challenges in estimating free energy in structure-based design the docking strategy employed was a hybrid approach where ligand binding is driven by enthalpy and entropy.[13,14] Around 100 poses for each molecule were analyzed and ranked with LeadIT.[13] The HYDE scoring function relies on a steady delineation of hydrogen bond and dehydration energies in protein-ligand complexes. All known protein targets can be appropriately evaluated as it is not dependent on protein-ligand complexes or experimental binding affinity data.[11,12]

The HYDE scoring function is based on a rather novel concept: in the unbound state, both molecules, ligand and protein, are solvated in aqueous solution.[11,12] Water molecules that surround the ligand are stripped off and those that are inside the active site are essentially squeezed out by ligand placement during the binding simulation.[11,12] The ligand and protein to water molecule hydrogen bonds are then broken creating an unfavorable enthalpic contribution. The protein and ligand form new hydrogen bonds to counter balance the loss in energy. In addition, a hydrophobic region of the protein or ligand interacting with water molecules creates incoherence in the water hydrogen bond network which ultimately results in unfavorable energy.[11,12]

The stripping of these water molecules from the hydrophobic surfaces and their discharge to the bulk water prompts an increase in energy which is termed the hydrophobic effect. Schneider et al. in 2013 proposes that these processes constituted the major contributions to the binding energy and these contributions (i.e., hydrophobic effect, hydrogen bonding, and dehydration) are mainstay pillars of the HYDE scoring function (Table 1).

Of the 612 compounds two putative hits were identified: castanospermine and methyl phenylacetate.[13] Both compounds had low molecular weight and favorable LLE, LE, logP and TPSA with high mM predicted activity as indicated by the HYDE affinity parameter making them ideal starting fragments for optimization (Table 3).[15,16] Also, both lead compounds possessed mutually exclusive scaffolds and occupied different locations in the TNF-α binding pocket. In SeeSAR, we evolved both compounds into new molecules (Table 2) having higher interaction affinity and desirable ADME properties leading to our novel compounds - CasMethPhen(s).[16]

Results

Table1. Shows the HYDE scoring function and its derivatives - dehydration energy and hydrogen bond energy. The HYDE scoring function is proposed to be the driving force for predicting binding energies in SeeSAR and LeadIT.

	Function	Description
HYDE scoring function[11]	$\Delta G_{HYDE} = \sum_{atoms\ i} (\Delta G^{i}_{dehydration} + \Delta G^{i}_{H}$	The change in dehydration ($\Delta G_{dehydration}$) and hydrogen bond ($\Delta G_{H\text{-}bonds}$) energy for each atom i in the protein-ligand interface.[11]
Dehydration energy[11]	$\Delta G^{i}\frac{hydrophobic}{dehydration} = -2.3RT * plogP^{i} * (acc^{i}_{unbound} - acc^{i}_{bound})$ $\Delta G^{i}\frac{hydrophobic}{dehydration} = \sum_{H-Bond\ functions\ j}^{-2.3RT * plogP^{i} * f^{i}_{bur} * f^{i}_{water}} w^{j} * p^{j}_{dehyd}$	The major contributor to overall binding energy is dehydration (desolvation), while, unfavorable binding energies typically comes from the dehydration of hydrophilic groups.[11]
Hydrogen bond energy[11]	$\Delta G^{i}_{H-bonds} = \frac{2.3RT}{F_{sat}(T)} * plogP^{i} * f^{i}_{bur} \sum_{atoms\ i} w^{j} * f^{i}_{dev}$	The concept of the hydrogen bond contribution arises from the notion that not all hydrogen bonds in the web of bulk water are ideally appreciated and as such the energy required to disrupt these hydrogen bonds is lower than that of an ideal hydrogen bond.[11]

Table2. Shows the molecular structure of castanospermine, methyl phenylacetate and six novel analogs of Casmethphen. All six compounds occupy the same binding pocket with slightly different interactions and torsions resulting in significant differences in estimated affinity.[16]

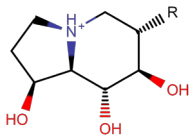
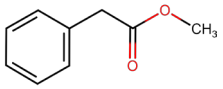
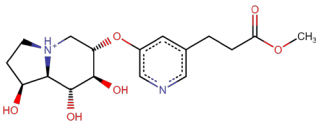
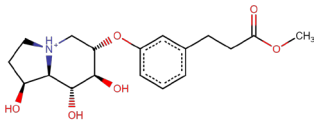
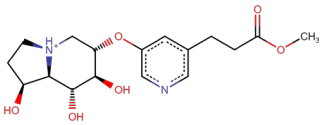
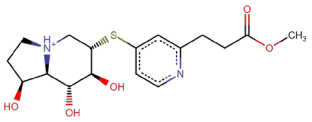
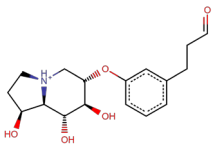
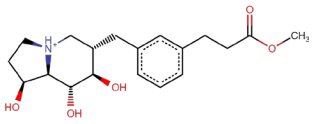
Compound	Molecular structure	Estimated affinity[16]	Predicted drug likeliness[15]
Castanospermine		mM	-0.73
Methyl phenylacetate		mM	-1.22
CasMethPhen1		nM	0.87
CasMethPhen2		nM	0.59
CasMethPhen3		µM	0.55
CasMethPhen4		µM	0.82
CasMethPhen5		µM	0.48
CasMethPhen6		µM	0.58

Table3. Shows a few ADME properties for castanospermine, methyl phenylacetate and six novel CasMethPhen analogs (1-6). All six CasMethPhens are predicted to have good oral bioavailability and low cardiac toxicity.[15,16] In addition, compounds 1-2 are predicted to have excellent LLE and LE.[16] The ADME evaluation encompasses physiochemical properties, lipophilicity, water solubility, pharmacokinetics, drug likeliness, and medicinal chemistry properties.[15]

Cpd	MW	HBA	HBD	HA	Rot. Bonds	logP	logS (ESOL)	TPSA	Synthetic Accessibility	GI Abs	BBB
Cas	190.22	4	5	13	0	0.78	0.39	85.36	3.4	low	no
Met	150.17	2	0	11	3	2.02	-2.13	26.30	1.18	high	yes
1	353.39	7	4	25	6	2.82	-1.29	113.55	4.37	low	no
2	352.40	6	4	25	6	2.76	-1.93	100.66	4.17	high	no
3	368.47	5	4	25	6	2.35	-2.37	116.73	4.56	high	no
4	369.46	6	4	25	6	2.73	-1.72	129.62	4.53	low	no
5	322.38	5	4	23	5	2.38	-1.58	91.43	4.02	high	no
6	350.43	5	4	25	6	1.82	-2.22	91.43	3.95	high	no

1-6: CasMethPhen Analogs, Cas: Castanospermine, Met: methyl phenylacetate, MW: molecular weight (g/mol),[15,16] HBA: H-Bond acceptors,[15,16] HBD: H-Bond donors,[15,16] HA: heavy atom count,[15,16] Rot. Bonds: rotatable bonds,[15,16] logP: octanol-water partition coefficient,[15,16] logS: aqueous solubility,[15,16] TPSA: topological surface area (Å),[15,16] GI Abs: GI absorption,[15] BBB: blood brain barrier permeation.[15]

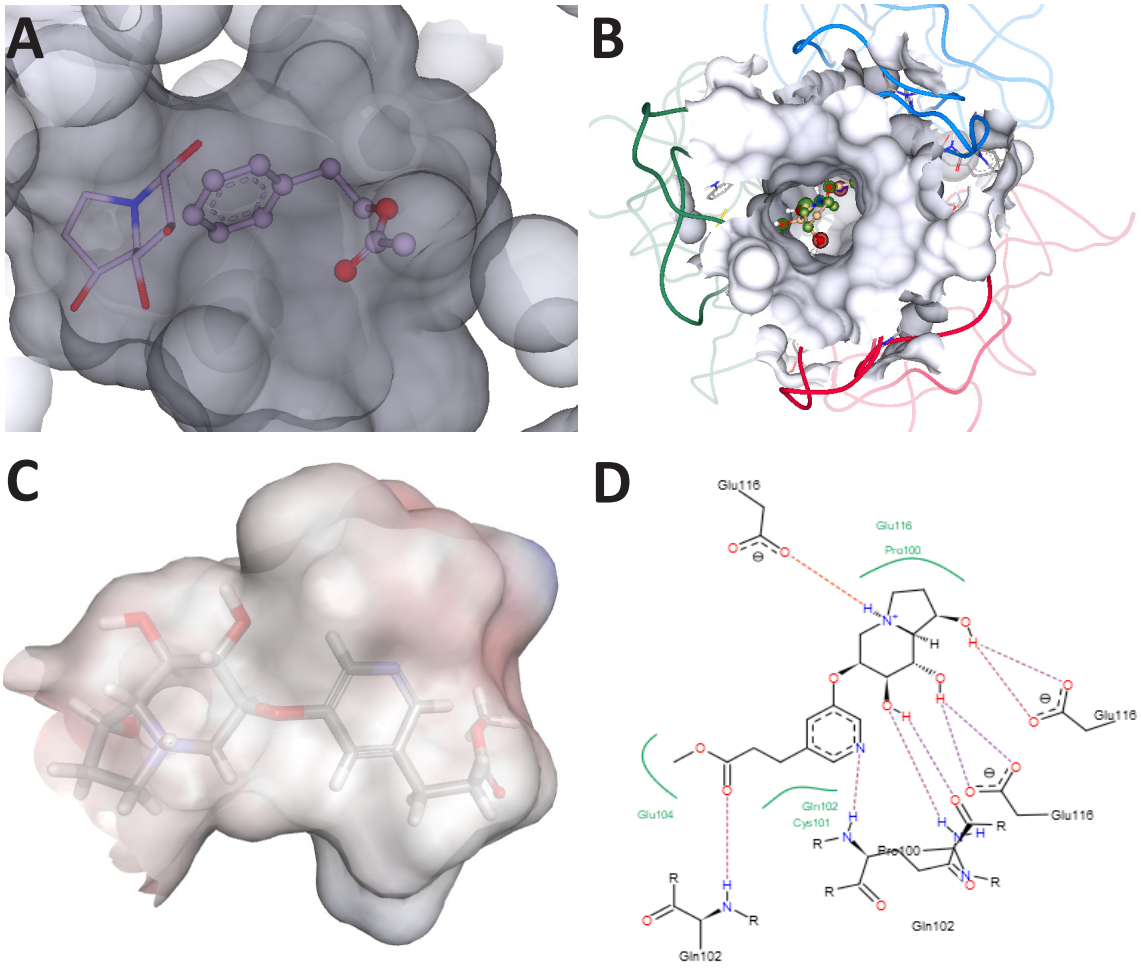


Fig. 1 (A) Shows Castanospermine and methyl phenylacetate occupying different locations inside the TNF-α binding pocket.[16] Our optimization strategy aimed at joining these two fragments using several common functional groups (ether, sulfide, alkane, alkene, and amide). (B) Depicts CathMethPhen1 sitting inside the binding pocket at the base of the TNF-α homotrimer.[16] (C) Shows CathMethPhen1 occupying the TNF-α binding pocket with a depth of 18.3 Å. We also estimate that the mouth of the pocket has a diameter of 9.97 Å.[17] (D) 2-D ligand integration diagram of CathMethPhen1 inside the binding pocket.[13] Critical Interacting residues for CasMethPhen(1-6): ARG B 103, GLN B 102, GLU C 104, PRO B 100, GLN A 102, SER C 99,CYS and TYR A 115.

Discussion

TNF-α inhibitors: a universe of opportunities for novel cancer therapies

Chronic inflammation is a major promoter of tumor growth and development.[1] TNF-α has the potential to act as an endogenous tumor promoter linking carcinogenesis and inflammation as it is by nature a major pro-inflammatory cytokine.[1] TNF-α is a largely implicated target for solid tumor therapy.[2] Many patients with advanced cancer responded positively to TNF-α antagonist therapy. However, patients who had elevated serum levels of TNF-α responded poorly to the antibody infliximab.[2] Yu M et al. 2013, suggests that a possible explanation is as a consequence of the consumption of the antibody in the circulation by soluble serum TNF-α, and so the antibody reaching the tumor site diminishes.[2] This bottleneck can potentially be remedied by administering the antibody in adjuvant with a small molecule inhibitor such as CasMethPhen1. The CasMethPhens may also be of therapeutic use in diseases like psoriasis, ulcerative colitis and rheumatoid arthritis.

TNF-α binding pocket: potential implications of a CasMethPhen drug

The structure of the TNF-α monomer is an extended, antiparallel beta pleated sheet sandwich with a "jelly-roll" topology.[6] Three monomers are associated intimately via a 3-fold axis of symmetry to generate a compact bell shaped trimer.[6] All six CasMethPhens sits inside the trimeric "pseudo-cavity" of about 1,413 cubic Å formed by the association of the three monomers.

Our In Silico studies demonstrate that the TNF-α cavity is not a conventional protein pocket but is certainly still exploitable by the universe of small molecule inhibitors. Based on its Solvent Accessible Surface Topology (SAS), much of the anterior and inferior regions of the pocket is polar while the inferior and superior regions are largely nonpolar.[17] This starling computational observation from our study may provide an explanation as to why TNF-α is a difficult target for a rational drug design approach. And therefore, large scale screening of bioactive natural products might currently be the most receptive approach for developing a small molecule inhibitor against TNF-α.

In Figure 1D, the nitrogen that forms the indolizidine ring of CasMethPhen1 hydrogen bonds to the carbonyl oxygen of Gln C 102 while the NH portion hydrogen bonds to the ether oxygen which connects castanospermine from the anterior portion of the pocket to methyl phenylacetate at the posterior end.

On the opposite side of the indolizidine ring, the three hydroxyls (OH) on castanospermine forms multiple hydrogen bonds with the carbonyl oxygen of Gln B 102, Arg B 103, Pro B 100, and Glu B 104. The pyridine ring of CaMethPhen1 in the middle of the pocket is adequately desolvated by Trp A 114, Pro A 100, and Cys A 101, while the nitrogen in the pyridine ring interacts with Glu A 116 primarily by van der Waals forces. The alkane region of methyl phenylacetate is stabilized by hydrophobic interactions. The carbonyl oxygen of methyl phenylacetate hydrogen bonds with Tyr A 115 and Serine C 99 at the posterior end of the pocket. There are a few regions in the binding pocket that is currently unoccupied by the CasMethPhen moiety and as such may provide infinite enumerations for further optimization and design of other novel scaffolds with higher affinity and selectivity.

In conclusion, we are the first to report the computational design of a potential small molecule inhibitor of TNF-α from two low-cost natural products. The development of the first small molecule to therapeutically target TNF-α derived from two inexpensive natural products has the potential to be of major clinical significance. Our team is currently working on simple synthetic pathways to access these

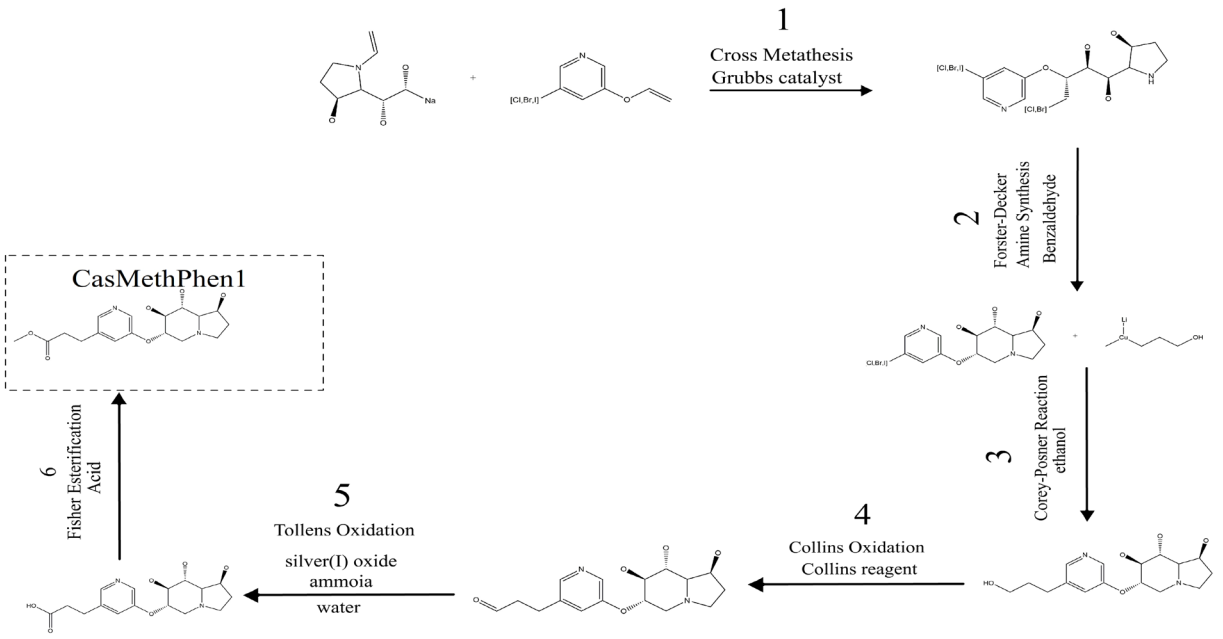


Fig. 2 We propose a simple six step retrosynthetic pathway for the synthesis of CasMethPhen1 from two inexpensive, commercially available, organic compounds. Step 1 involves the augmentation of these two organic compounds via cross metathesis using Grubbs catalyst. Step 2 involves a Forster-Decker Amine Synthesis in benzaldehyde. Step 3 involves a Corey Posner reaction in ethanol from which the product is oxidized in a one-step reaction using Collins reagent. In step 5 the aldehyde form of CasMethPhen1 is further oxidized to the carboxylic acid form via Tollens Oxidation in silver (I) oxide, ammonia and water. In the 6th and final step CasMethPhen1 is formed via Fisher Esterification.

novel CasMethPhens (Fig. 2). Our compounds are predicted to have desirable ADME properties which provide a great point of reference for the development of an innovative TNF-α inhibitor. In our future reports we hope to elaborate on total synthesis, molecular dynamics simulations, and In vitro cell culture assays to evaluate the efficacy of the CasMethPhens. Equally important, we also hope to report on the therapeutic potential of the CasMethPhens in terms of whether they are full agonists, partial agonists, neutral antagonists or inverse agonists.

Acknowledgment

The authors thank I-Shou University School of Medicine for International Students, Republic of China, Taiwan, for the opportunity to continue developing as a physicians in training scientists. The author also thanks Paul Morgan Sr. for editing the manuscript.

FUNDING SOURCES: The research was supported by the E-Da Healthcare Group Research Scholarship grant EDAHS107003, BioSolveIT Winter 2017 Scientific Challenge Software Grant, and Professor Lin Chih-Wen Research Group.

Disclosure

The authors declare no conflict of interest concerning the materials and methods used in this study or the findings specified in this manuscript.

References

1. Wang X, Lin Y: Tumor necrosis factor and cancer, buddies or foes? Acta pharmacologica Sinica 2008;29:1275-88.
2. Yu M, Zhou X, Niu L, et al: Targeting transmembrane TNF-α suppresses breast cancer

growth. Cancer Res 2013;73:4061-74.
3. Roberts NJ, Zhou S, Diaz LA Jr, et al: Systemic use of tumor necrosis factor alpha as an anticancer agent. Oncotarget 2011;2:739-51.
4. Lee JU, Shin W, Son JY, et al: Molecular basis for the neutralization of tumor necrosis factor α by certolizumab pegol in the treatment of inflammatory autoimmune diseases. Int J Mol Sci 2017;18:228.
5. Hu S, Liang S, Guo H, et al: Comparison of the inhibition mechanisms of adalimumab and infliximab in treating tumor necrosis factor α-associated diseases from a molecular view. J Biol Chem 2013;288:27059-67.
6. Eck MJ, Sprang SR The structure of tumor necrosis factor-α at 2.6 Å resolution. Implications for receptor binding. J Biol Chem 1989;264:17595-605.
7. Aggarwal BB, Gupta SC, Sung B: Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. Br J Pharmacol 2013;169:1672-92.
8. Gulcubuk A, Altunatmaz K, Sonmez K, et al: Effects of curcumin on tumour necrosis factor-α and interleukin-6 in the late phase of experimental acute pancreatitis. J Vet Med A Physiol Pathol Clin Med 2006;53:49-54.
9. Saul R, Chambers JP, Molyneux RJ, et al: Castanospermine, a tetrahydroxylated alkaloid that inhibits β-glucosidase and β-glucocerebrosidase. Arch Biochem Biophys 1983;221:593-7.
10. Cao Y, Marra M, Anderson BD: Predictive relationships for the effects of triglyceride ester concentration and water uptake on solubility and partitioning of small molecules into lipid vehicles. J Pharm Sci 2004;93:2768-79.
11. Schneider N, Lange G, Hindle S, et al: A consistent description of hydrogen bond and dehydration energies in protein–ligand complexes: methods behind the HYDE scoring function. J Comput Aided Mol Des 2013;27:15-29.
12. Rarey M, Kramer B, Lengauer T, et al: A fast flexible docking method using an incremental construction algorithm. J Mol Biol 1996;261:470-89.
13. LeadIT software package version 2.3.2 (BioSolveIT GmbH, St Augustin, Germany).
14. Reddy MR, Reddy CR, Rathore RS, et al: Free energy calculations to estimate ligand-binding affinities in structure-based drug design. Current Pharmaceutical Design 2014;20:3323-37.
15. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:42717.
16. SeeSAR version 8.0; BioSolveIT GmbH, Sankt Augustin, Germany, 2018, www.biosolveit.de/SeeSAR.
17. ault Systèmes BIOVIA SD. DassDiscovery Studio Visualizer v4.0. 2018.
18. Huard K, Ahn K, Amor P, et al: Discovery of fragment-derived small molecules for in vivo inhibition of ketohexokinase (KHK). J Med Chem 2017;60:7835-49.
19. Han W, Ding Y, Xu Y, et al: Discovery of a selective and potent inhibitor of mitogen-activated protein kinase-interacting kinases 1 and 2 (MNK1/2) utilizing structure-based drug design. J Med Chem 2016;59:3034-45.
20. Li X, Zuo Y, Tang G, et al: Discovery of a series of 2,5-diaminopyrimidine covalent irreversible inhibitors of bruton's tyrosine kinase with in vivo antitumor activity. J Med Chem 2014;57:5112-28.
21. Liu Z, He W, Gao J, e al: Computational prediction and experimental validation of a novel synthesized pan-PIM inhibitor PI003 and its apoptosis-inducing mechanisms in cervical cancer. Oncotarget 2015;6:8019-35.
22. Watanabe S, Rathore APS, Sung C, et al: Dose- and schedule-dependent protective efficacy of celgosivir in a lethal mouse model for dengue virus infection informs dosing regimen for a proof of concept clinical trial. Antiviral Res 2012;96:32-5.
23. Smith CR, Dougan DR, Komandla M, et al: Fragment-based discovery of a small molecule inhibitor of bruton's tyrosine kinase. J Med Chem2015;58:5437-44.
24. Li Y, Martin RC 2nd: Herbal medicine and hepatocellular carcinoma: applications and challenges. Evid Based Complement Alternat Med 2011;2011:541209.
25. Ahmed MI, Salahy EE, Fayed ST, et al: Human papillomavirus infection among Egyptian females with cervical carcinoma: relationship to spontaneous apoptosis and TNF-α. Clin Biochem 2001;34:491-8.
26. Vorontsov II, Wu Y, DeLucia M, et al: Mechanisms of allosteric activation and inhibition of the deoxyribonucleoside triphosphate triphosphohydrolase from enterococcus faecalis. J Biol Chem 2014;289:2815-24.
27. Brethon A, Chantalat L, Christin O, et al: New Caspase-1 inhibitor by scaffold hopping into bio-inspired 3D-fragment space. Bioorg Med Chem Lett 2017;27:5373-7.
28. Govinda Rao B, Bandarage UK, Wang T, et al: Novel thiol-based TACE inhibitors: Rational design, synthesis, and SAR of thiol-containing aryl sulfonamides. Bioorg Med Chem Lett 2007;17:2250-3.
29. Cherkasov A, Muratov EN, Fourches D, et al: QSAR modeling: where have you been? Where are you going to? J Med Chem 2014;57:4977-5010.
30. Ehlers S: Role of tumour necrosis factor (TNF) in host defence against tuberculosis: implications for immunotherapies targeting TNF. Ann Rheum Dis 2003;62:ii37-42.
31. Michalaki V, Syrigos K, Charles P, et al: Serum levels of IL-6 and TNF-α correlate with clinicopathological features and patient survival in patients with prostate cancer. Br J Cancer

2004;90:2312-6.
32. Mukai Y, Nakamura T, Yoshikawa M, et al: Solution of the structure of the TNF-TNFR2 complex. Sci Signal 2010;3:ra83.
33. Liang S, Dai J, Hou S, et al: Structural basis for treating tumor necrosis factor α (TNFα)-associated diseases with the therapeutic antibody infliximab. J Biol Chem 2013;288:13799-807.
34. Mukai Y, Shibata H, Nakamura T, et al: Structure–function relationship of tumor necrosis factor (TNF) and its receptor interaction based on 3D structural analysis of a fully active TNFR1-selective TNF mutant. J Mol Biol 2009;385:1221-9.
35. van Horssen R, Ten Hagen TL, Eggermont AM: TNF-α in cancer treatment: molecular insights, antitumor effects, and clinical utility. Oncologist 2006;11:397-408.