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In silico Study of 1,687 FDA Approved Drugs and 612 Natural Products Reveals Benzydamine's Potential as a Direct Inhibitor of *TNF-α*

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Abstract—*1,687 FDA approved drugs and 612 natural products were screened in search of an inhibitor for TNF-α. Small molecule drugs that can directly regulate TNF-α levels or activity might provide a cost effective alternative to protein-based therapeutics. The results of the virtual screening assay reveal Benzydamine as a potential inhibitor of TNF-α. Benzydamine is a non-steroidal topical agent used to treat oral inflammatory conditions. It has also been shown to suppress the production of pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α) and interleukin 13 (IL-13). However, benzydamine has not been previously reported as a direct inhibitor or TNF-α. Herein, we describe the evolution of benzydamine into four promising analogs; Benzydam (A, B, C, D). All four analogs possessed higher interaction affinity and desirable ADME properties serving as a favorable prototype for the development of an innovative cancer drug.*

Keywords—*ADME; affinity; analogs; chirality; hydrophobic effect; magic methyl; specificity; TNF-α; trimer.*

I. INTRODUCTION

Benzydamine (N,N-dimethyl-3- [(1-benzyl- 1H-indazol3-yl)ossi]-1-propanamine) is a non-steroidal anti-inflammatory drug extensively used in clinical practice for the topical treatment of oral inflammatory conditions [1, 2]. Benzydamine has been shown to have suppressive activity on pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin 13 (IL-13) [3]. TNF-α is a unique cytokine that controls many signaling pathways involved in immunity, inflammation, cell survival (anti-apoptosis), cell death (apoptosis), and even tumorgenesis.

TNF-α is a 23 kilodalton (kDa) type II transmembrane protein arranged as a stable homotrimer [4-6]. Metalloprotease TNF-α converting enzyme (TACE) cleaves the trans-membrane form to create a soluble 51 kDa homo-trimeric cytokine [6]. Both forms of TNF-α display bioactivities via TNF-α receptors, TNFR I, and, TNFRII.

In the clinical setting, TNF-α production by tumors is correlated with unfavorable prognosis, cachexia, and loss of hormone responsiveness [7]. Pre-cancerous tumor cells with elevated expression levels of TNF-α are associated with the progression of malignant diseases like prostate cancer, Barrett's adenocarcinoma, cervical carcinoma, chronic lymphocytic leukemia, and breast cancer [8]. Five TNF-α blockers are currently approved by The USA Food and Drug Administration (FDA) to treat ankylosis, inflammatory bowel disease (IBD), osteoarthritis, and psoriasis [9, 10]. Four are antibody-based drugs while the fifth is a peptide [9, 10].

To date, there are no small molecule inhibitors of TNF-α. However, small-molecule drugs that can directly regulate TNF-α levels or activity might provide a groundbreaking economic alternative to protein-based therapeutics [9]. Herein, we reveal benzydamine's role as a direct inhibitor of TNF-α. We also evolve benzamine into four novel analogs having higher affinity and specificity.

II. MATERIALS AND METHODS

Virtual Screening

A total of 1,487 FDA approved drugs and 612 natural products, totaling 2,299 compounds, were screened against *TNF*-α (PDB: 1TNF). The natural products were selected on the basis of their structurally diverse moieties across 7 classes of natural products: phenylpropanoids, alkaloids, terpenoids, polyketides, cannabinoids, curcuminoids, and catechins. The receptor was prepared in LeadIT [11]. We identified a total of 6 pockets in the *TNF*-α homotrimer. Only the largest pocket (A) at the base containing 26 amino acids was determined to be ideal for ligand binding. The remaining cavities were infinitesimal and incapable of accommodating a small molecule with a molecular weight of up to 500 Daltons. Interestingly, *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 [4].

FLEXX Ligand Docking Algorithm

The FLEXX docking algorithm in LeadIT relies on a unique ensemble. It 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 in 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

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assigning multiple confirmations of the base fragments into the active site [12].

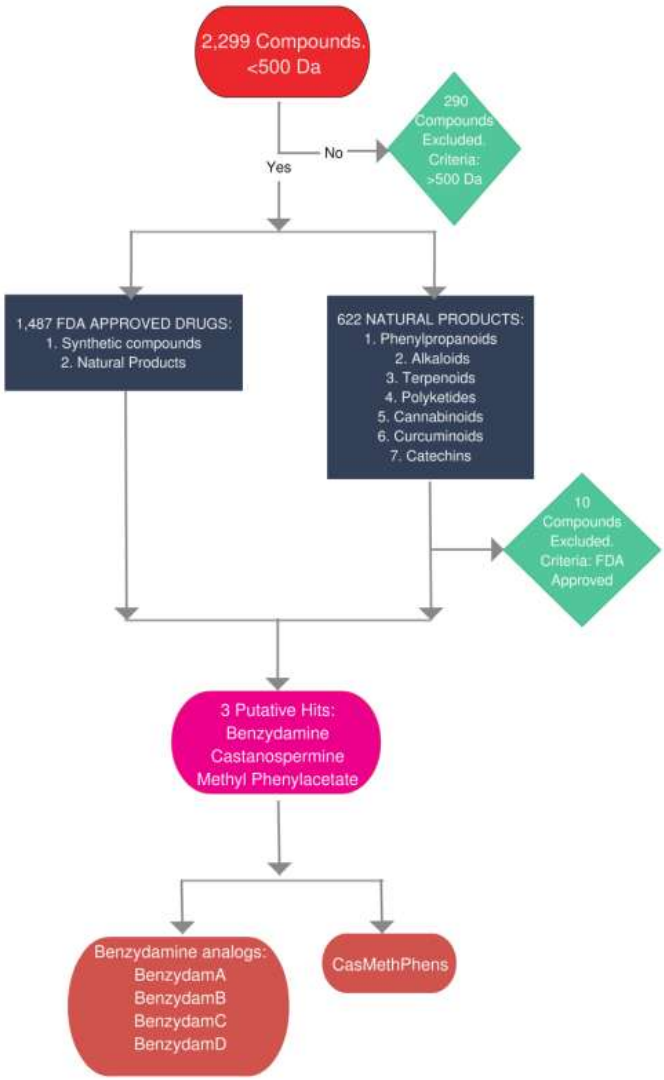


Fig. 1. Section criteria algorithm for the virtual screening of 2,299 compounds, 1487 FDA approved drugs and 612 natural products.

The HYDE Scoring Function

We employed a hybrid protocol to triumph the bottleneck of accurately estimating free energy of binding from a structure-based design approach [11, 12]. A hundred poses for each FDA approved drug were analyzed and ranked with LeadIT [11]. The HYDE scoring function relies on a strategic allocation of hydrogen bond and dehydration energies in protein–ligand complexes rather than experimental biding affinity data or protein ligand complexes. This allows for the eligibility of all known protein targets.

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 [12, 13]. 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 [12, 13]. 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 [12, 13]. 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.

III. Results and Discussion

Of the 2099 compounds screened three putative hits were identified: benzydamine, castanospermine, and methyl phenylacetate. Morgan, et al. elaborated on the docking studies of the two natural products, castanospermine, and methyl phenylacetate, in a previous study [14]. We further describe the evolution of benzydamine into four promising analogs, Benzydam (A,B,C,D) using SeeSAR. All four analogs (Table 1) possessed higher interaction affinity and desirable ADME properties.

TABLE 1. depicts the molecular structure, estimated affinity, and predicted drug likeliness for benzydamine (Benz) and four novel analogs (A,B,C,D) [15, 16].

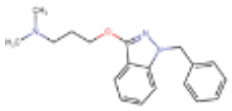
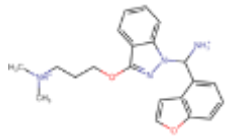
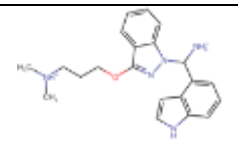
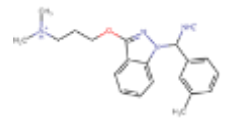
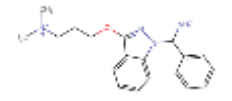
Cpd	Molecular Structure	Estimated Affinity			Predicted Drug Likeliness
		nM	uM	mM	
Benz					1.12
A					0.57
B					-0.25
C					0.95
D					0.92

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TABLE 2. ADME properties for benzydamine and four novel analogs (A,B,C,D) [15, 16].

Cpd	MW	HBA	HBD	HA	Rot. Bonds	logP	logS (ESOL)	TPSA	Synthetic Accessibility	GI Abs	BBB
Benz	309.41	3	0	23	7	3.39	-4.22	30.29	2.88	High	Yes
A	366.46	3	2	27	7	1.18	-4.32	72.27	3.95	High	No
B	365.47	2	3	27	7	0.99	-4.14	74.92	3.72	High	No
C	340.46	2	2	25	7	1.26	-4.09	59.13	3.70	High	No
D	326.44	2	2	24	7	0.90	-3.79	59.13	3.58	High	No

A-D: Benzydamine Analogs, Benz: Benzydamine, MW: molecular weight (g/mol), HBA: H-Bond acceptors, HBD: H-Bond donors, HA: heavy atom count, Rot. Bonds: rotatable bonds, logP: octanol-water partition coefficient, logS: aqueous solubility, TPSA: topological surface area (Å), GI Abs: GI absorption, BBB: blood brain barrier permeation.

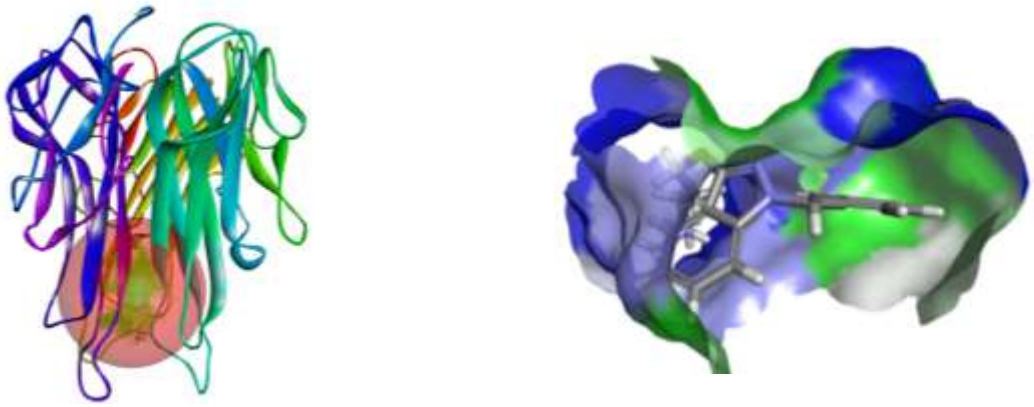
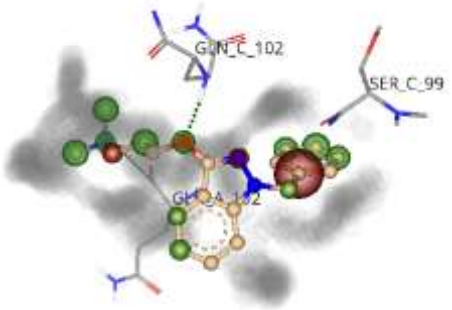
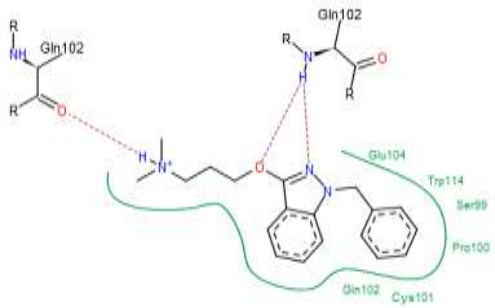
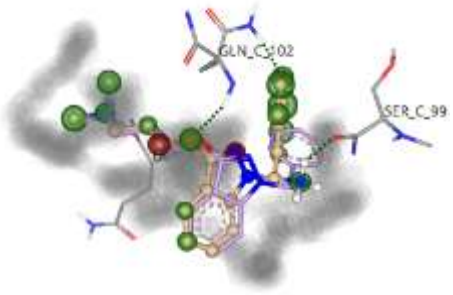
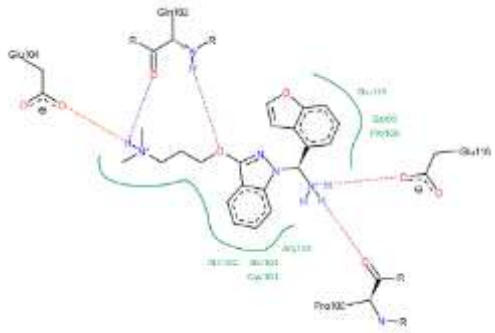


Fig. 2. (A and B) benzydamA sitting inside the binding pocket at the base of the TNF-α homotrimer, visualized in Discovery Studio 4.0.

TABLE 3. 3D and 2D ligand interaction diagram of benzydamine (Benz) and four novel analogs (A,B,C,D) inside the binding pocket of TNF-α.

Cpds	3D Corona Interaction Assessment	2D Ligand Interaction Assessment
Benz		
A		

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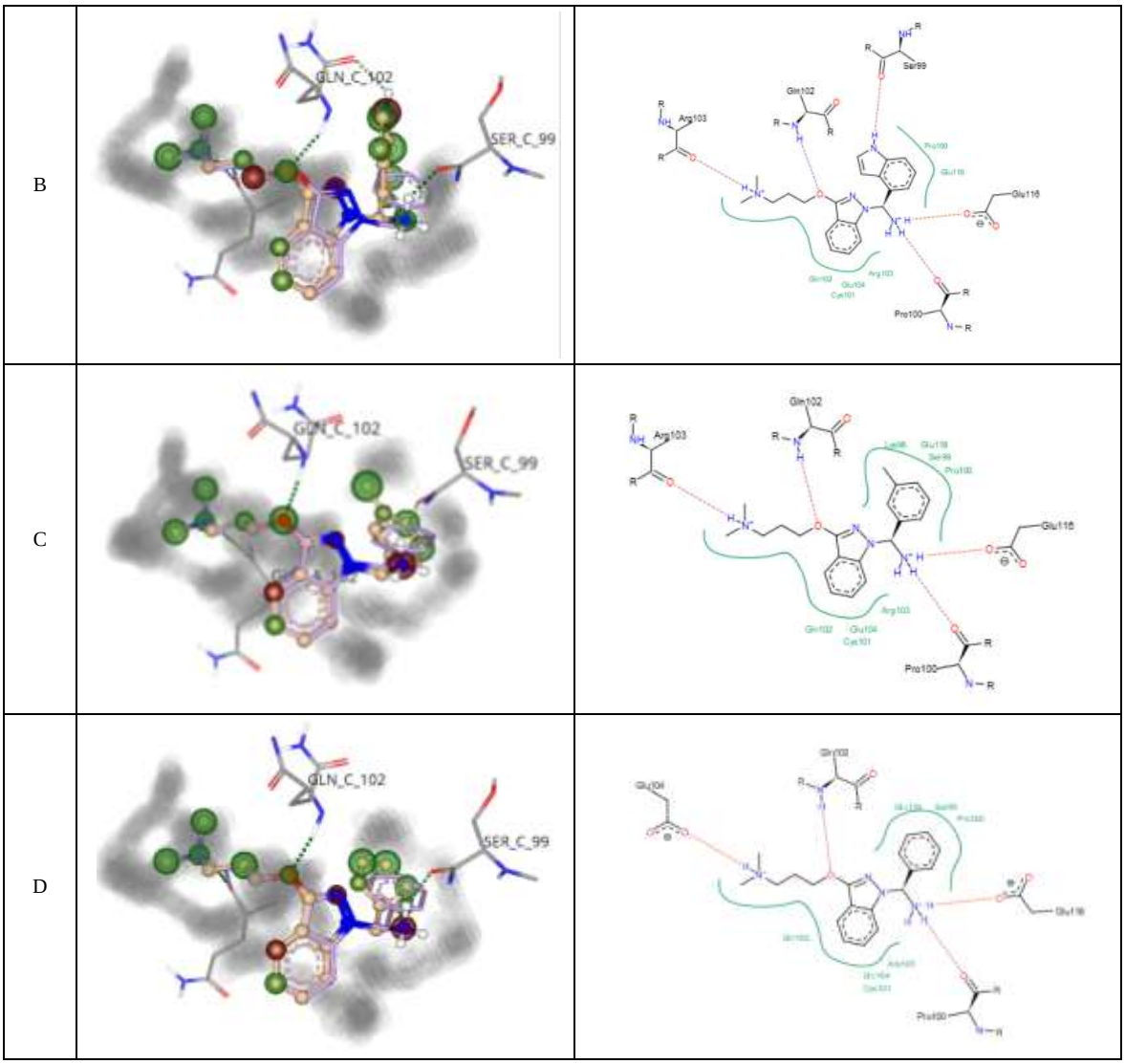

The colored coronas depict the contributions of each atom to the estimated binding affinity. The red coronas allude to unfavorable contributions while the green coronas illustrate a favorable contribution. The magnitude of the corona spheres directly correlate with effect. As such, the larger the sphere is, the stronger is the effect. The absence of spheres suggests that such an atom is not determined to have a significant impact on the binding affinity. Benzydam (A,B,C,D) are also matched against benzydamine (purple) to compare positioning inside the binding pocket [11, 16].

Benzydamine as a Direct Inhibitor of TNF-α

Many studies have demonstrated evidence that benzydamine primarily acts by inhibiting the synthesis of pro-inflammatory cytokines, (TNF-α) and interleukin-1β (IL-1β) [2, 8, 17]. However, the role of benzydamine as a direct inhibitor of TNF-α has not been previously explored and reported in literature. The results of our study introduces benzydamine and four novel analogs, Benzydam(A,B,C,D) as potentially potent inhibitors of TNF-α itself.

The structure of the TNF-α monomer is an extended, antiparallel beta pleated sheet sandwich with a "jelly-roll" topology [4, 18, 19]. Three monomers are associated intimately via a 3-fold axis of symmetry to generate a compact bell shaped trimer with a cavity of 1413 cubic Å at the center of the base [4, 20] . The very nature of this active site is atypical and may provide an explanation as to why TNF-α is a difficult target for a rational drug design approach. As such, screening of currently approved FDA drugs and bioactive natural products may be the only practical approach to developing a small molecule inhibitor against TNF-α.

Evolution of Benzydamine into Benzydam (A,B,C,D)

In table 1, the tertiary amine at the tail end of benzydamine hydrogen bonds to Gln A 102, while the backbone NH of Gln C 102 hydrogen bonds to the only ether oxygen in benzydamine. An achiral carbon bridges the indazole ring and phenyl ring at the posterior region of benzydamine. The phenyl ring stabilizes benzydamine inside the active site primarily via hydrophobic interactions with Trp A 114, Pro A 100, Ser C 99, Cys A 101. We evolved benzydmine into four novel analogs with much greater affinity and specificity by introducing only two minor structural modifications.

In BenzydamA (table 1), we increased the chirality of the molecule by adding a primary amine to the carbon between the indazole ring and the benzofuran ring [21]. This amine

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group creates a salt bridge with the carbonyl carbon of Ser C 99 increasing overall affinity and specificity. The benzofuran ring at the posterior end is designed to occupy a hydrophobic dead space within the active site. Furthermore, the hydrogen bond created between the oxygen in the benzofuran ring and amine group in the back bone of GLN C 102 significantly increases the specificity and affinity. BenzydamA is predicted to have an estimated affinity in the nanomolar range, while benzydamine is estimated at six orders of magnitude less.

In benzydamB, the benzofuran ring was substituted with an indole ring. The NH group on the indole ring hydrogen bonds with the carbonyl oxygen on the side chain of GLN C 102. However, the overall affinity is slightly reduced as compared to that of benzydamA due to slightly unfavorable desolvation of the amine group on the indole ring. As such benzydamB is predicted to have an estimated affinity in the micromolar range. BenzydamC differs from benzydamine only by the presence of a methyl group at the meta-position of the phenyl ring. Based on the corona interaction assessment, we believe this methyl group acts as a "magic methyl" siting in a largely unoccupied hydrophobic space within the active site. BenzydamB affinity is estimated to be in the micromolar range which further supports the "magic methyl" postulate [22]. BenzydamD differs from benzydamine only by the presence of the chiral carbon between the indazole ring and the benzofuran ring. As such, benzydamD has the lowest predicted affinity of all four benzydam's but nevertheless still in the micromolar range.

IV. Conclusion

We report the discovery of benzydamine as a direct inhibitor of TNF-α. Currently, there are no FDA approved small molecule inhibitors of TNF-α [10, 23]. Small-molecule drugs that can regulate TNF-α levels or activity may provide an economic alternative to antibody therapeutics [9]. Pre-cancerous tumor cells with elevated expression levels of TNF-α is closely associated with the progression of cervical carcinoma, chronic lymphocytic leukemia, prostate cancer, breast cancer, and Barrett's adenocarcinoma [8]. As such, the development of the first small molecule to therapeutically target *TNF*-α has the potential to be of major clinical significance. We also report the evolution of benzydamine into four novel analogs, benzydam (A,B,C,D) with desirable affinity, specify and ADME-Tox properties.

V. Appendix

TABLE A1. SMILES for Benzydamine (Benz) and four novel analogs(A,B,C,D).

Cpd	SMILES
Benz	CN(C)CCCOC1=NN(C2=CC=CC=C21)CC3=CC=CC=C3
A	C[NH+](C)CCCOC1=NN(C([NH3+])C2=CC=CC3=C2C=CO3)C2=CC=CC=C12
B	C[NH+](C)CCCOC1=NN(C([NH3+])C2=CC=CC3=C2C=CN3)C2=CC=CC=C12
C	C[NH+](C)CCCOC1=N[N](C([NH3+])C2=CC=CC(=C2)C)C3=CC=CC=C13
D	C[NH+](C)CCCOC1=N[N](C([NH3+])C2=CC=CC=C2)C3=CC=CC=C13

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